UNIVERSITÄT ZU LÜBECK

From the Lübeck Institute of Experimental Dermatology
of the University of Lübeck
Director: Prof. Dr. Ralf Ludwig

Exploration of the autoimmune pre-disease in lupus-prone mouse models

Dissertation
for Fulfillment of
Requirements
for the Doctoral Degree

of the University of Lübeck

from the Department of Natural Sciences

Submitted by
Sarah Stenger
from Preetz
Lübeck 2025

First referee: Prof. Dr. Jennifer Elisabeth Hundt

Second referee: Prof. Dr. Rudolf Manz

Date of oral examination: 03.11.2025

Approved for printing. Lübeck, 04.11.2025

Contents

List of abbreviations

Abbreviation	Full Term
°C	Degree Celsius
ACR	American College of Rheumatology
Alox5	Arachidonate 5-lipoxygenase
am	*Ante meridien*
ANA	Anti-nuclear antibodies
ANCA	Antineutrophil cytoplasmic antibody
APRIL	A proliferation-inducing ligand
aqua dest.	Destilled water
ATP	Adenosine triphosphate
BAFF	B-cell activating factor
BSA	Bovine serum albumin
C1q, C3, C4	Complement proteins
C8a	Complement component 8 alpha chain
CAR	Chimeric antigen receptor
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
Ciita	Class II Transactivator
CLE	Cutaneous lupus erythematosus
cm^2	Squarecentimetre
CO_2	Carbon dioxide
Csf1	Colony stimulating factor 1
CTLA-4	Cytotoxic T lymphocyte antigen 4 (CD152)
CXCL	CXC-chemokine ligand
CXCR	CXC-chemokine receptor
DAPI	4',6-diamidino-2-phenylindole
Defb3	Defensin beta 3
DIL	Drug-induced lupus
dsDNA	Double-stranded deoxyribonucleic acid
Dusp26	Dual specificity phosphatase 26
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid

Abbreviation	Full Term
ENT	Ears, Nose, and Throat Clinics
EULAR	European League Against Rheumatism
f	Female
F7	Coagulation factor VII
Faah	Fatty acid amide hydrolase
FAS	First apoptosis signal
FasL	Fatty acid synthase ligand
Fc	Crystalline fraction
Fcgr3	Fc gamma receptor III
FCS file	Flow cytometry standard file
FDR	False discovery rate
Fli-1	FMS-like tyrosine kinase 3 receptor-interacting protein 1
FMO	Fluorescence minus one
FoxP3	Forkhead box protein 3
Fut2	Fucosyltransferase 2
g	Gram / gravitational force
GAGE	Generally applied geneset enrichment
GCs	Glucocorticoids
Gna14	G protein subunit alpha 14
GSVA	Geneset variation analysis
GZMB	Granzyme B
h	Hour(s)
H&E	Haematoxillin and eosin
H2-ea	Histocompatibility 2 class II antigen A
Hist1h1c	Histone cluster 1 H1c
Hivep2	Human immunodeficiency virus type I enhancer binding protein 2
HLA	Human leukocyte antigen
ICAD	Inhibitor of caspase-activated DNase
Ifi202b	Interferon-activated gene 202B
IFN	Interferon
Ifnb1	Interferon beta 1
Ig	Immunoglobulin
IL	Interleukin

Abbreviation	Full Term
Il7r	Interleukin-7 receptor
Irf1	Interferon regulatory factor 1
IRF5	Interferon regulatory factor 5
ISEF	Institute for Systemic Inflammation Research
KEGG	Kyoto Encyclopedia of Genes and Genomes
Ki-67	Kiel-67
Klra17	Killer cell lectin-like receptor subfamily a member 17
Krt1	Keratin 1
LDH	Lactate dehydrogenase
LIED	Lübeck Institute for Experimental Dermatology
LIMMA	Linear models for microarray data
Ly6G	Lymphocyte antigen 6 complex
m	Male
mAh	Milliampere-hour(s)
Masp2	Mannan-binding lectin serine peptidase 2
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MHC	Major histocompatibility complex
min	Minute(s)
mJ	Millijoule
ml	Millilitre
MLL	Medical Laser Center Lübeck
mm	Millimetre
Mmp8	Metallopeptidase 8
MPV	Mean platelet volume
mRNA	Messenger ribonucleic acid
NAD+	Nicotinamide adenine dinucleotide
NADH	Reduced form of nicotinamide adenine dinucleotide
NET	Neutrophil extracellular trap
Nfkb1	Nuclear factor kappa B subunit 1
NK cell	Natural killer cell
NLE	Neonatal lupus erythematosus

Abbreviation	Full Term
nm	Nanometre
Nod2	Nucleotide-binding oligomerisation domain containing 2
NPSLE	Neuropsychiatric systemic lupus erythematosus
NZB	New Zealand Black mouse
NZW	New Zealand White mouse
Oas1	2'-5'-Oligoadenylate synthethase 1
oLED	Organic light-emitting diode
PARP	Poly(ADP-ribose) polymerase
PAS	Periodic acid–Schiff
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCA	Principal component analysis
Pcsk9	Proprotein convertase subtilisin/kexin type 9
pDC	Plasmacytoid dendritic cell
Pdcd4	Programmed cell death 4
PIR sensor	Passive infrared sensor
pm	*Post meridiem*
RAD	Rodent activity detector
Raver2	Ribonucleoprotein PTB binding 2
RBC	Red blood cell
RDW	Red cell distribution width
RIN	Ribonucleic acid integrating number
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT	Room temperature
S100a9	S100 calcium binding protein A9
sec	Second(s)
SEM	Standard error of the mean
Slc11a1	Solute carrier family 11 member 1
SLE	Systemic lupus erythematosus
SLE-DAS	Systemic lupus erythematosus disease activity score
SLE123	B6;NZM-Sle1NZM2410/AegSle2NZM2410/AegSle3NZM2410/Aeg/LmoJ
SLEDAI-2K	Systemic lupus erythematosus disease activity index 2000

Abbreviation	Full Term
SOP	Standard operating procedure
ssDNA	Single-stranded deoxyribonucleic acid
STAT4	Signal transducer and activator of transcription 4
TBS	Tris(hydroxymethyl)aminomethane buffered saline
TCA cycle	Tricarboxylic acid cycle
TCE	Trichlorethylene
Tfh	T follicular helper cell
TGF	Tumour growth factor
Th	T helper cell
TLR	Toll-like receptor
TNF	Tumour necrosis factor
Tregs	Regulatory T cells
TYK2	Tyrosine kinase 2
UKSH	University Hospital Schleswig-Holstein
UV light	Ultra violet light
Zfx	Zinc finger protein X-linked
µl	Microlitre
µm	Micrometre

List of figures

List of tables

Zusammenfassung

Der systemische Lupus erythematodes (SLE) ist eine systemische Autoimmunerkrankung, an der hauptsächlich Frauen im gebärfähigen Alter erkranken. Sie ist zwar behandelbar, aber nicht heilbar. Auch die Entstehung ist nur teilweise bekannt. Es gibt genetische Varianten, die eine Entstehung begünstigen, aber dennoch benötigt es zum Auslösen des SLE meist noch zusätzliche Reize. Die Symptome der Krankheit sind im Menschen vielfältig und gut untersucht. Sie reichen von Gelenkschmerzen bis hin zum Nierenversagen. Alle Organe und Zelltypen können betroffen sein. Erste Symptome können Müdigkeit oder Haarausfall sein. Da die Krankheitsentstehung nicht vollständig geklärt ist, befasst sich diese Doktorarbeit damit, insbesondere mit der Phase, in der noch keine klinischen SLE-Symptome feststellbar sind. Dazu werden zwei Mausmodelle verwendet, die aufgrund von genetischer Manipulation Lupussymptome ab einem Alter von etwa 22 Wochen entwickeln. Diese Mauslinien sind die NZM2410 und die SLE123 Mäuse. Hinzu werden noch C57BL/6J Mäuse, die als Wildtypen bekannt sind und keine Autoimmunität entwickeln, als Vergleich betrachtet. Alle Tiere wurden im Alter von 8-21 Wochen regelmäßig untersucht. Hierzu wurden das Gewicht und Milzgewicht gemessen, Blutbilder erstellt und T-Zellen des Blutes, der Milz und der Lymphknoten mittels Durchflusszytometrie gemessen. Es wurde zwischen CD4+ T Helferzellen, CD4+ regulatorischen T-Zellen und CD8+ T Killerzellen unterschieden. Deren jeweiliger Differenzierungsstatus wurde ermittelt, sowie bei den regulatorischen T-Zellen auch deren Funktionalität. Des weiteren wurde die Bewegungsaktivität von NZM2410 und C57BL/6J Mäusen betrachtet, da chronische Erschöpfung zu frühen Symptomen von SLE zählt. Signifikante Nierenschäden konnten wir in dieser Prä-SLE Phase auch ausschließen.

Dennoch konnten wir bereits in der Phase vor Entstehung der Organschäden durch die Autoimmunerkrankung feststellen, dass einige Blutwerte der SLE-Tiere von den gesunden Vergleichstieren abwichen. Zudem wurde festgestellt, dass die SLE-Mäuse höhere Frequenzen von CD4+ T-Zellen, insbesondere der naiven CD4+ T-Zellen aufwiesen. Gleiches galt für die CD8+ T-Zellen. In dem Alter, in dem die naiven T-Zellen abnahmen, nahmen die Effektor T-Zellen zu, was ein Hinweis auf beginnende Immunreaktionen ist. Zudem waren die Frequenzen der regulatorischen T-Zellen in den SLE-prädispositionierten Tieren geringer und wiesen auch in geringeren Frequenzen die Expression von funktionalen Markern wie CD39, Ki-67, Helios und CTLA-4 auf. Dies weist auf eine gestörte Toleranz hin, die im weiteren Verlauf die Erkrankung verstärken kann. Zudem wurde bei NZM2410 Tieren im Alter von 18 Wochen für verschiedenste Marker eine kurzzeitig andere Ptoteinexpression mittels Durchflusszytometrie festgestellt, was auf einen symptomfreien ersten Schub von SLE hindeutet. Gleiches wurde bei den SLE123-Tieren im Alter von 10-12

Wochen festgestellt. Ein Set an differentiell regulierten Markern wurde ermittelt, was in zukünftige Untersuchungen von Patientenproben die Feststellung der Erkrankung in frühen Stadien erleichtern könnte.

Ein Faktor, der als zusätzliche Begünstigung für SLE gilt, ist (ultraviolettes, UV) Licht. Diesem ist man in Form von Tageslicht regelmäßig ausgesetzt, besonders im Bereich von UVA und UVB. Blaulicht erreicht uns zusätzlich durch Bildschirme und künstliche Beleuchtung in Innenräumen. UVC wird derzeit als potenzielle Dekontaminationsmöglichkeit in belebten Räumen untersucht. Gesunden Menschen droht in Dosen, die keine Hautschäden (Sonnenbrand) verursachen in der Regel keine Gefahr, allerdings kann UV Licht SLE Schübe bei bereits bestehender Erkrankung auslösen. Wir haben nun untersucht, wie UVA, UVB, UVC und Blaulicht die Signalwege in der Haut von NZM2410 und C57BL/6J Mäusen beeinträchtigt. Die Dosis wurde so gering gewählt, dass sie keine Gewebsschäden auslöst. Dennoch konnten wir mit Hilfe von Transkriptomanalysen feststellen, dass die SLE-prädispositionierten Tiere auch vor Ausbruch der Krankheit schon mit stärkeren Entzündungsreaktionen auf die Lichtreize reagierten. Insbesondere apoptotische Signalwege waren durch UVB und UVC verstärkt aktiviert. Auch die anderen Wellenlängen waren in der Lage SLE-spezifische Kaskaden zu aktivieren. Dies ist von besonderer Bedeutung für die Prävention der Krankheitsentstehung von prädispositionierten Menschen, wie zum Beispiel Verwandten von bereits bekannten SLE-Erkrankten.

Abstract

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease which mostly affects women in childbearing age. Even though it is treatable, it is not yet curable, and the development is rather unclear. Certain genetic predispositions are known; however, often secondary triggers cause the outbreak of the disease. The symptoms in human SLE are diverse and well-studied. They range from joint pain to kidney failure, with the ability to affect all organs and cell types. First symptoms can be as nonspecific as fatigue or hair loss. Since the development of SLE is not fully elucidated, this doctoral thesis aims to explore the pre-disease phase before any clinical symptoms arise. Therefore, two mouse models, which develop SLE spontaneously after an age of 22 weeks due to genetic manipulations, were chosen. These are NZM2410 and SLE123 mice. Additionally, C57BL/6J mice were used as a healthy comparison. All animals were regularly investigated at the age of 8-21 weeks. To do so, haemograms, body weight, spleen weight, and T cells of the blood, spleens, and lymph nodes were examined using flow cytometry. It was distinguished between CD4+ T helper cells, CD4+ regulatory T cells, and CD8+ cytotoxic T cells. Their state of differentiation was measured, as well as the functionality of the regulatory T cells. Furthermore, the physical activity of NZM2410 as well as C57BL/6J mice was investigated, as fatigue is one of the early known but nonspecific symptoms. We could exclude significant kidney damage during this pre-disease phase. Nevertheless, we found that even before the onset of organ damage due to autoimmunity, several blood values of the SLE-prone mice differed from the healthy ones. Additionally, SLE-prone mice exhibited higher frequencies of CD4+ T cells, especially naive CD4+ T cells. The same accounts for CD8+ T cells. At the age when the naive T cell frequencies dropped, the effector ones were rising, indicating an upcoming immune reaction. Moreover, the frequencies of regulatory T cells were lower in SLE-prone animals, and they also showed reduced frequencies of the expression of functional markers such as CD39, Ki-67, helios, and CTLA-4. This indicates a disturbed tolerance, which could enhance further autoimmunity. Additionally, NZM2410 mice at the age of 18 weeks showed in a variety of flow cytometric markers short-term changes in the expression, leading to the assumption of an early immunological flare before the manifestation of other symptoms. The same was seen for SLE123 mice at the age of 10-12 weeks. A set of differentially regulated markers was established, potentially facilitating future examinations of patient samples in order to detect SLE in early stages.

Another factor influencing the development of SLE is (ultraviolet, UV) light. Exposure occurs regularly from sunlight, especially regarding UVA and UVB light. Blue light reaches the skin additionally through emission from digital screens and artificial room light. UVC is currently

being researched as a potential source of indoor decontamination while humans are in the rooms. Healthy people can usually tolerate light dosages that do not cause direct physical damage to the skin (sunburns). However, UV light is known to be able to cause flares in SLE patients. We investigated the influence of UVA, UVB, UVC, and blue light of low, non-damaging dosages on the skin of NZM2410 and C57BL/6J mice. By transcriptome analysis, we could observe that the skin of SLE-prone animals responded with stronger inflammatory reactions to the light stimuli than the skin of C57BL/6J mice. Especially, apoptotic pathways were increasingly activated by UVB and UVC light in NZM2410 mice. The other wavelengths were also able to activate SLE-specific cascades. This is of special importance for predisposed people, such as relatives of SLE patients, to prevent the potential outbreak of the disease.

1 Introduction

1.1 Autoimmunity and autoimmune diseases

Autoimmunity describes the recognition of the body's own cells by the immune system. This process itself is a physiological process, and it is also needed to train the body to distinguish between self- and non-self (Cohen and Lohse 2008; Huetz *et al.* 1988). The term autoimmune disease describes the mistaken attack of the body's own tissues (Lleo *et al.* 2010). This is often mediated by autoantibodies, which bind to self-proteins or nucleic acids. This self-destruction can occur in every tissue or organ and also be systemic (JohnsHopkinsUniversity 2025). Therefore, autoimmune diseases can be separated into **systemic or rheumatic autoimmune diseases**, which affect the entire organism and are themselves divided into arthritides, connective tissue diseases, and vasculitis-associated autoimmune diseases. Among the systemic autoimmune diseases, the most prominent ones are systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, and systemic sclerosis (SSc) (Cooper and Stroehla 2003). Besides the systemic autoimmune diseases, it can also be differentiated into **organ-specific autoimmune diseases** and those affecting specific tissues or cell types. Among the organ-specific ones, it can be differentiated between endocrine, gastrointestinal, dermatologic, neurologic, haematologic, renal, and pulmonary autoimmune diseases. The **autoimmune diseases affecting specific tissues or cells** are divided into autoimmune auto-inflammatory syndromes and reproductive and pregnancy-related autoimmune diseases. However, the transitions here are fluent, as some systemic autoimmune diseases can also occur or flare pregnancy-related (Wilder 1998) (**Figure 1**).

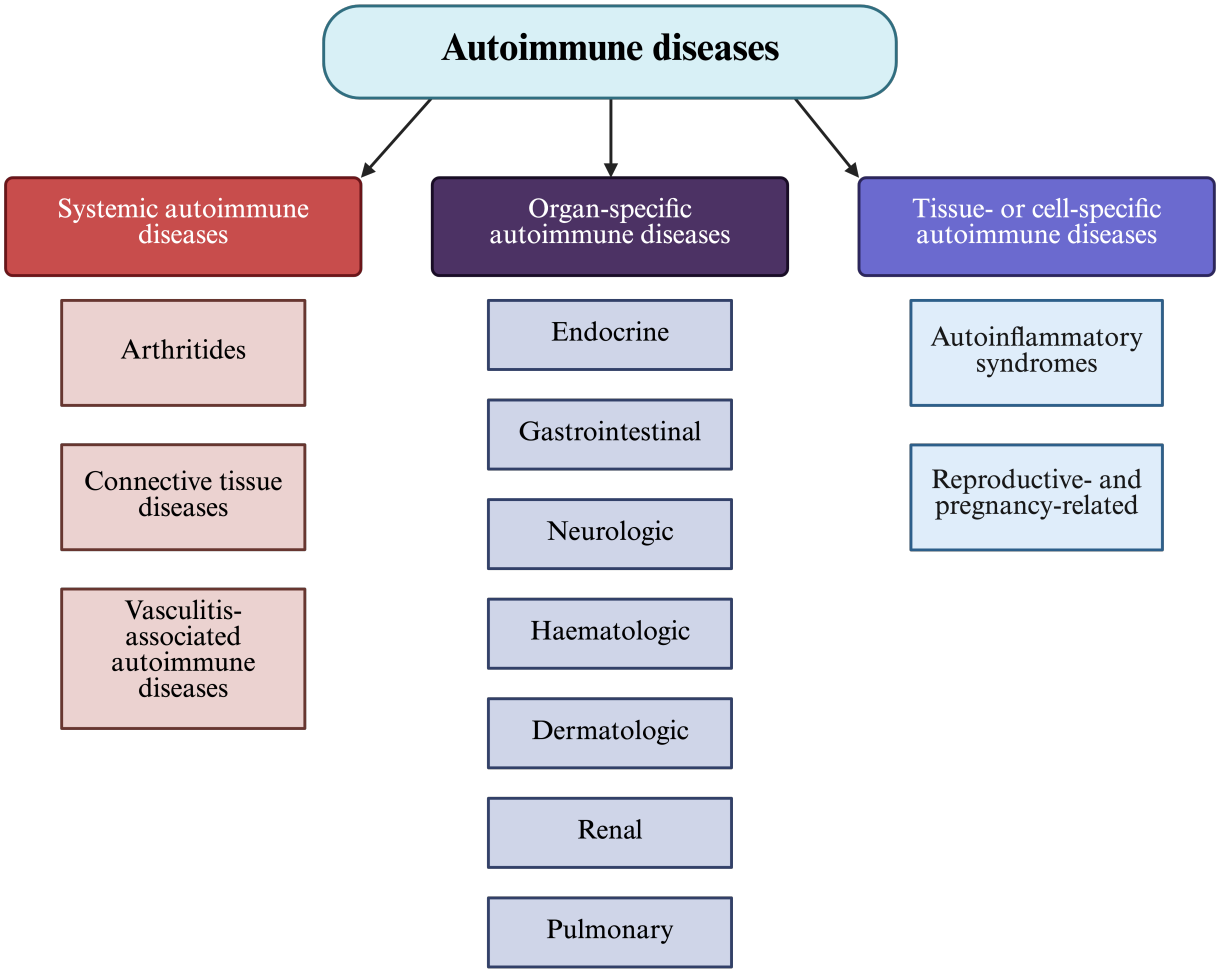


Figure 1: Autoimmune disease classifications
Autoimmune diseases can be classified into three main groups. These are systemic, organ-specific, and tissue- or cell-specific autoimmune diseases. Thereafter, they are further differentiated into subgroups. The systemic autoimmune diseases are split into arthritides, connective tissue diseases, and vasculitis-associated diseases. The organ-specific autoimmune diseases can be divided into endocrine, gastrointestinal, neurologic, haematologic, dermatologic, renal, and pulmonary autoimmune diseases. The tissue- or cell-specific autoimmune diseases are either autoinflammatory syndromes or reproductive- and pregnancy-related. The illustration was generated using BioRender.com.

The above-described separation of autoimmune diseases is just one approach to cluster them. In total, more than 80 autoimmune diseases are classified by Hayter and Cook 2012. However, this number varies. This discrepancy relies on issues regarding the exact classification of diseases and the clinical heterogeneity of symptoms (Miller 2023). Many diseases present a multitude of vague symptoms, which are difficult to relate to one specific disease and symptoms often overlap between diseases (Mackintosh *et al.* 2021, Ramos-Casals, Pilar Brito-Zerón, and Font 2007).

The global prevalence of autoimmune diseases is rising, and the reasons for this are not fully understood. Potential causes are nutrition, stress and personal lifestyle, contact with xenobiotics, air pollution, infections, and even climate change (Miller 2023). One argument is that the healthcare system and diagnostic tools have improved, and therefore, more autoimmune diseases have been detected. However, it was also found that the general presence of anti-nuclear antibodies (ANAs) in the population increased since 1988-1991 with 11.0 % of the population showing them to 16.1 % in 2011-2012 (Miller 2023; Dinse *et al.* 2022) (**Figure 2**). This finding demonstrates that it is not the healthcare development alone but also other underlying factors.

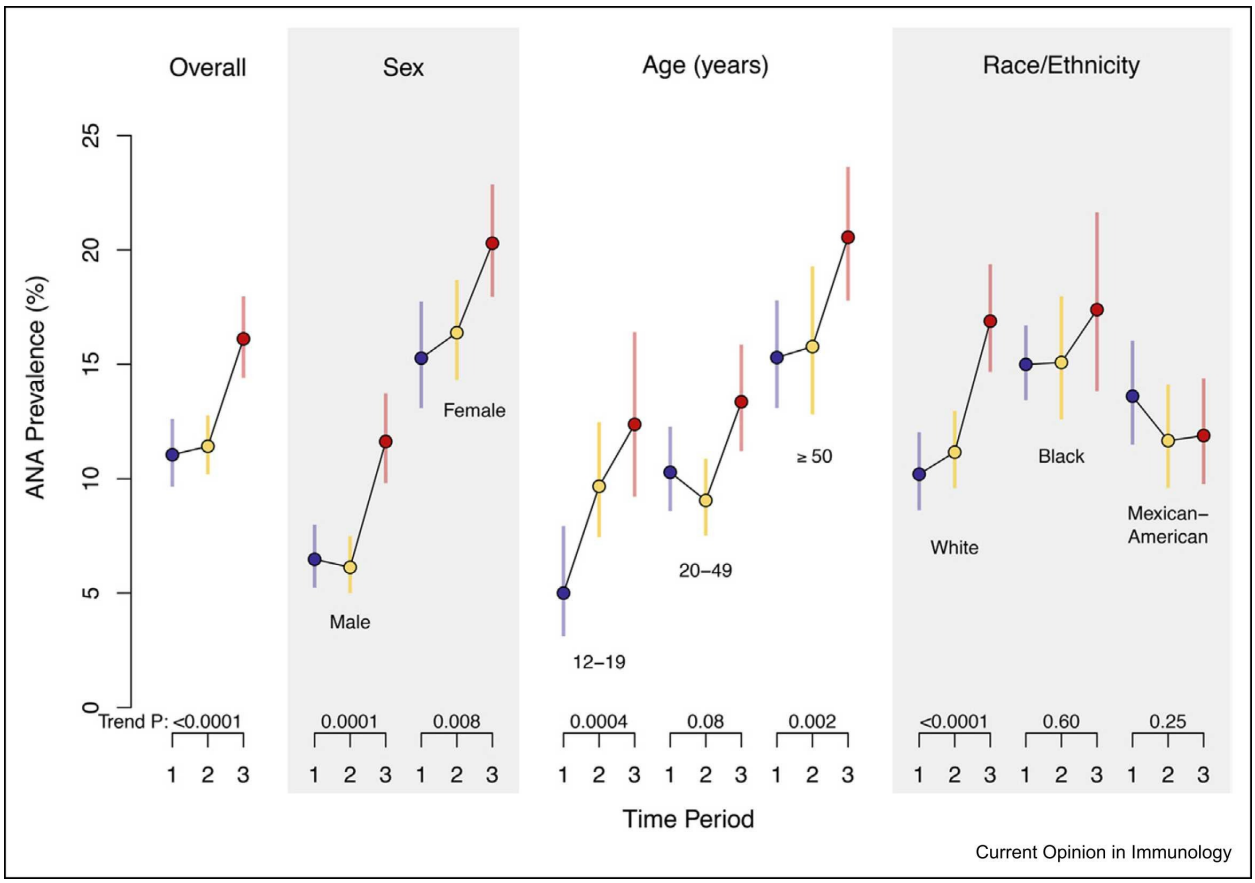


Figure 2: Anti-nuclear antibody-increase in the population of the United States
Since 1988-1991 to 2011-2012, more anti-nuclear antibodies have been detected in the United States population. 1, 2, and 3 are the periods of investigation with 1 = 1988-1991 (blue), 2 = 1999-2004 (yellow), and 3 = 2011-2012 (red). Vertical lines display the 95 % confidence interval. These antibodies are seen as one marker for the increase of autoimmunity over time. This development has been seen overall, in males and females, in three age groups and predominantly in the white population. Figure taken from Dinse *et al.* 2022.

ANA-measurement is just one option guiding the finding of rising prevalences in general autoimmunity and autoimmune disease. To identify an autoimmune disease, autoantibodies

are measured more specifically, for example by identifying antibodies against double-stranded deoxyribonucleic acid (dsDNA) (Arbuckle *et al.* 2001). This rising trend was also investigated for a variety of autoimmune diseases in the United States and Canada (Miller 2023) (**Figure 3**).

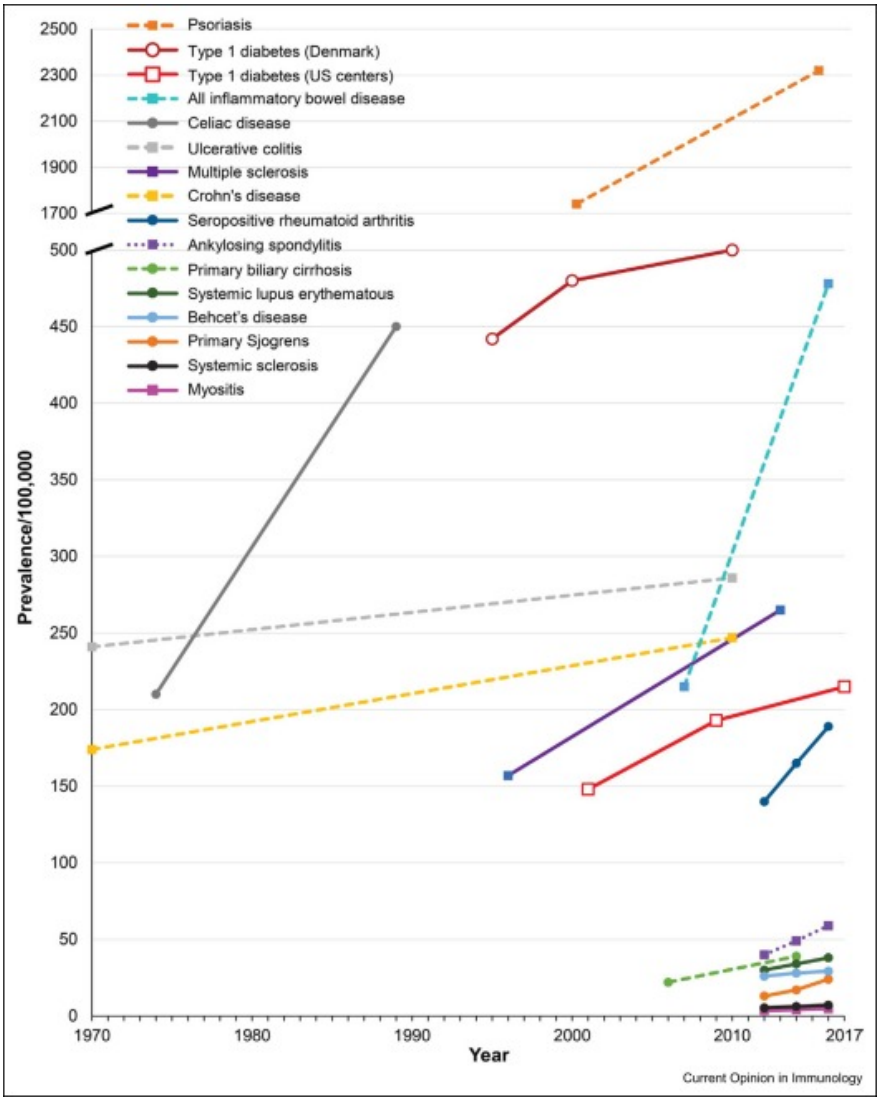


Figure 3: Increasing prevalence of autoimmune diseases
An increase in the prevalence of autoimmune diseases per 100,000 inhabitants could be demonstrated for 16 investigated common autoimmune diseases in the United States and Canada. All of these diseases showed rising numbers in different time intervals. Figure taken from Miller 2023.

Ethnicities that tend to have more autoimmune diseases vary depending on the disease. It was found for SLE that South-Asian, Afro-Caribbean and mixed-race/others had increased risks of developing SLE compared to white people. A similar trend was found for Sjögren's, vitiligo,

myasthenia gravis and autoimmune thyroid disease. Psoriasis, multiple sclerosis, and coeliac disease, in contrast, showed the highest prevalence in white people (A. Subramanian *et al.* 2021).

Lifestyle factors that can interfere with health include, for example, nutrition, sleep patterns, stress, social aspects, nicotine, and other drugs. This will be discussed to a greater extent in section 1.3.

In addition, the prevalence of most autoimmune diseases is higher in women. The reason for this is not fully understood yet. However, it is known that the hormone oestrogen is playing a role. Oestrogen is able to act on all subsets of immune cells via oestrogen receptor-dependent and -independent mechanisms (Khan and Ansar Ahmed 2016). Estradiol, the active form of oestrogen acts pro-inflammatory (Vázquez-Martínez *et al.* 2018). Thus, in biologically females, the immune system is better capable of fighting infections (Vázquez-Martínez *et al.* 2018), and preventing fetal organ dysfunctions in septic challenges after trauma (Oberholzer *et al.* 2000). Even though cancer has a higher incidence in women, men showed a lower survival rate (M. Dong *et al.* 2020). Besides this hormonal influence, the women's immune system might also be influenced by mosaicism of the X-chromosome or X-chromosomal escape. Females naturally have two X-chromosomes of which one is inactivated by Xist. During early embryonic development of the female embryo, one of the two X-chromosomes is randomly chosen for this inactivation (Loda and Heard 2019). This process occurs multiple times independently in each cell, causing in some to silence the maternal X chromosome and in others the paternal one (mosaic-like) (Migeon 2007). The silencing itself is mediated by the coating with Xist RNA. If one of the X-chromosomes has a genetic defect, if might be expressed in some of the cells, causing disease. The other mechanism, the X-chromosomal escape, occurs when not all genes of the inactivated X-chromosome are silenced but some remain active. This is causing a gene-dosage effect, in which some genes are translated to proteins more than usual (Posynick and Brown 2019).

Many components of the immune system are coded on the X-chromosome, such as toll-like receptor (TLR)7, TLR8, Forkhead box protein 3 (FoxP3), Bruton's Tyrosine Kinase, and cluster of differentiation (CD)40 Ligand. An overexpression of TLR7 due to X-chromosomal escape, for example, is linked to the development of SLE (Souyris *et al.* 2019). An escape and overexpression of CD40L is linked to Sjögren's syndrome and SLE (Pucino, Gardner, and B. A. Fisher 2020; Youness, Miquel, and Guéry 2021).

Moreover, the protein Xist itself, which is used to form an envelope for the X-chromosome inactivation, has been found to have immunologic influences by acting as an autoantigen, against which autoantibodies have been detected (Dou *et al.* 2024).

General genetic predispositions for autoimmune diseases are mostly polygenic, meaning that

there is usually not a single-locus variant that is inherited in a Mendelian way, but multiple small differences in the genes in combination with other triggers cause the disease.

1.2 Systemic lupus erythematosus in humans

The autoimmune disease that this doctoral thesis focuses on is SLE, which is one of the autoimmune diseases with a very high female-to-male ratio (up to 9:1) (M. R. W. Barber *et al.* 2021).

It is surprisingly difficult to describe the worldwide epidemiology of SLE, as 79.8 % of countries do not provide any statistical data, and the prevalence and incidence vary strongly between countries and varying social-economic status (Tian *et al.* 2023), and environmental exposures (M. R. W. Barber *et al.* 2021). The highest incidences were seen in central Europe and the lowest in central Asia (**Figure 4**).

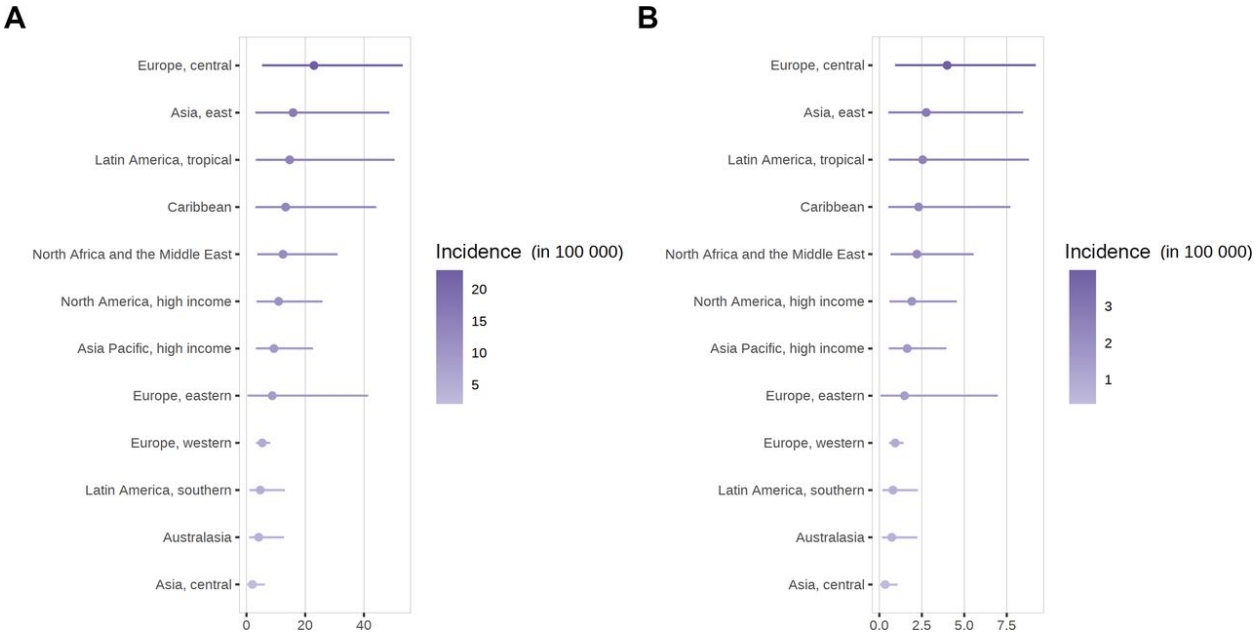


Figure 4: Incidence of systemic lupus erythematosus in males and females
The incidence of systemic lupus erythematosus (SLE) varies strongly depending on the country/ethnicity and socioeconomic status. The incidence in females (**A**) is generally higher than in males (**B**). Figure taken from Tian *et al.* 2023.

1.2.1 Symptoms and diagnostics

SLE is a systemic disease, symptoms can occur in every organ. Most prominently, however, are renal inflammation, pain in joints, central nervous symptoms, fever, and rashes (Al-Gahtani 2021). Depending on the symptoms, SLE can be sub-grouped. The most common is SLE with multiple

organ involvement. However, if a patient shows mainly or only skin involvement, this can be called cutaneous lupus erythematosus (CLE). If the organ damage is mainly restricted to the kidneys, it is called SLE with lupus nephritis. Most prominent symptoms of seizures, psychosis, headache and cognitive dysfunction are called neuropsychiatric SLE (NPSLE). If SLE was triggered by drugs, it is called drug-induced lupus (DIL). This usually resolves after withdrawal from the causing medication. A passive transfer of SLE from the mother to the baby is called neonatal lupus (NLE) (Maidhof and Hilas 2012) (**Figure 5**).

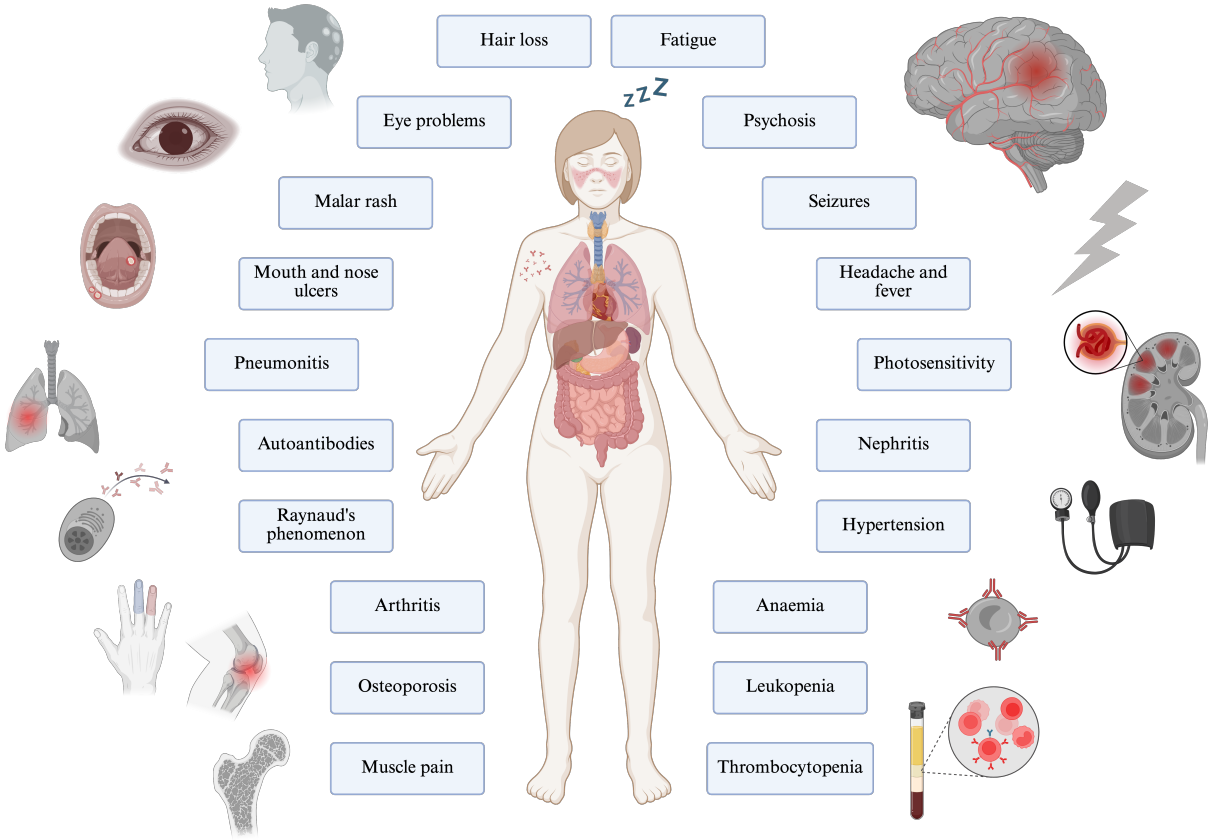


Figure 5: Common symptoms of human systemic lupus erythematosus symptoms
Systemic lupus erythematosus (SLE) is known for a variety of symptoms throughout the body. Autoantibodies and otherwise induced inflammation are able to cause damage in almost all tissues. Commonly involved symptoms are nephritis, arthritis and other joint pain. Neurological symptoms such as fatigue, psychosis, seizures, and headache are also highly prominent. Other symptoms are hair loss, eye problems, rashes, ulcers in the mouth and nose, pneumonitis, Raynaud's phenomenon, osteoporosis, muscle pain, photosensitivity, hypertension, anaemia, leukopenia, and thrombocytopenia. The illustration was generated using BioRender.com.

To diagnose SLE, the European League Against Rheumatism (EULAR) and American College of

Rheumatology (ACR) developed classification criteria. The latest update on these is from 2019 and states that SLE requires ANA-positivity (more than 1:80 on HEp2 cells) as an entry criterion, and thereafter a multitude of symptoms, which are ranked in a point system. A total of at least 10 points needs to be reached to be classified for SLE. The items are also only counted if there is no other potential diagnosis. These criteria are on purpose called classifying criteria and not diagnosing criteria to avoid the lack of adequate care for SLE patients not fulfilling all diagnosis criteria, as the disease has so many facets. The EULAR/ACR criteria achieve a sensitivity of 96 % and a specificity of 93 % (Aringer 2019) (**Figure 6**).

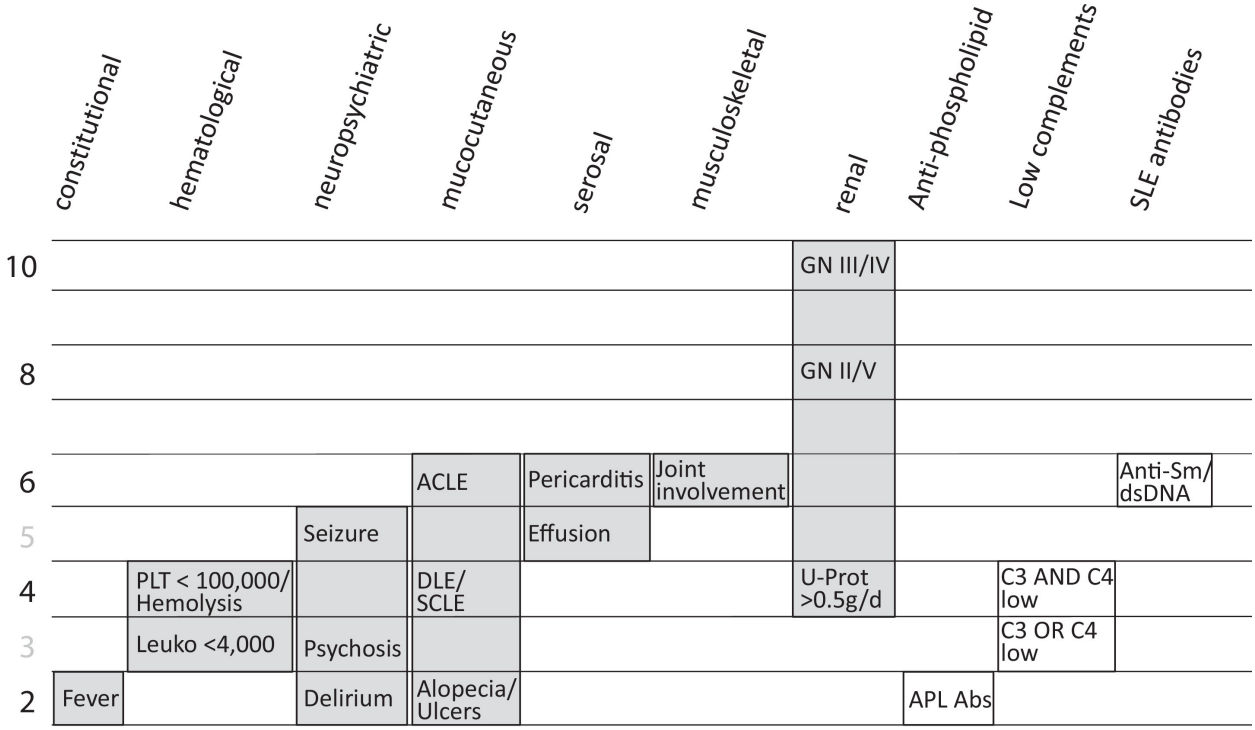


Figure 6: Current European League Against Rheumatism criteria for systemic lupus erythematosus
The European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) criteria for classifying systemic lupus erythematosus (SLE) are based on a point system, based on severity and likelihood. The positivity for anti-nuclear antibodies is an entry-criterion for this. PLT = platelets; ACLE = acute cutaneous lupus erythematosus; DLE = discoid lupus erythematosus; SCLE = subacute cutaneous lupus erythematosus; GN = glomerulonephritis; LEUKO = leukocytes; U-Prot = urinary protein; APL Abs = antiphospholipid antibodies; Anti-Sm/ dsDNA = anti-Smith/double-stranded DNA. A patient needs to reach at least 10 points to be classified for SLE. Figure taken from Aringer 2019.

To assess the severity and activity of the disease, scoring systems such as the systemic lupus erythematosus disease activity index 2,000 (SLEDAI-2K) or the SLE disease activity score (SLE-DAS) are used. While the SLEDAI-2K is widely used and validated, it might miss smaller

changes in symptoms. The newer SLE-DAS is more sensitive and better at tracking mild to moderate disease, but not yet as widely used as the SLEDAI-2K. The SLEDAI-2K includes 24 clinical and laboratory items over the past 10 days, resulting in a score with 0 meaning no disease activity and >20 meaning a very severe flare. Included items are for example seizures, psychosis, vasculitis, arthritis, proteinuria, haematuria, leukopenia, thrombocytopenia, and the presence of new rash, alopecia, or mucosal ulcers. The SLE-DAS focuses on continuous scoring and also includes a global physical assessment. A study from Lai *et al.* 2021 and colleagues compared the SLEDAI-2K, and SLE-DAS, and came to the conclusion that both are highly effective tools.

Not all patients develop all symptoms, which makes the diagnosis and differentiation into subtypes difficult. The diagnosis often relies (after the symptom-dependent suspicion) on autoantibody tests. However, even if these tests cover about 13 different types of autoantibodies and are helpful for the majority of patients, not all SLE patients show detectable autoantibodies in their blood (Diaz-Gallo *et al.* 2022). Therefore, several attempts were made to cluster patients. Diaz-Gallo *et al.* 2022 and colleagues aimed to cluster SLE into four subtypes. They used clustering algorithms depending on human leukocyte antigen (HLA)-type and autoantibodies in association with the most prevalent symptoms. The HLA-DRB1 is the major histocompatibility complex (MHC) II, which is involved in presenting antigen to T helper cells, which is needed to distinguish self from non-self. Autoreactive lymphocytes are usually deleted during maturation processes, which is an impaired mechanism in autoimmune diseases (Medzhitov and Janeway Jr 2000). Diaz-Gallo *et al.* 2022 and colleagues could find that in subtype 1, anti-SSA/Ro60/Ro52/SSB autoantibodies and HLA-DRB1*03 were predominant, together with discoid lesions. 29.3 % of patients fit into this cluster. The second subgroup was classified by anti-nucleosome/SmRNP/DNA/RNPA autoantibodies, an HLA-DRB1*15 type, and the most often occurring nephritis and the highest SLEDAI scores of all subtypes. The third subgroup had anti-ß2 GPI-IgG/anti-CL-IgG/IgM autoantibodies and a higher frequency of HLA-DRB1*04. Regarding the symptoms, vascular events were more common in this group. The fourth group, to which 18.2 % of all patients belong, was negative for all investigated autoantibodies and was not associated with any variant of HLA-DRB1 (Diaz-Gallo *et al.* 2022) (**Figure 7**).

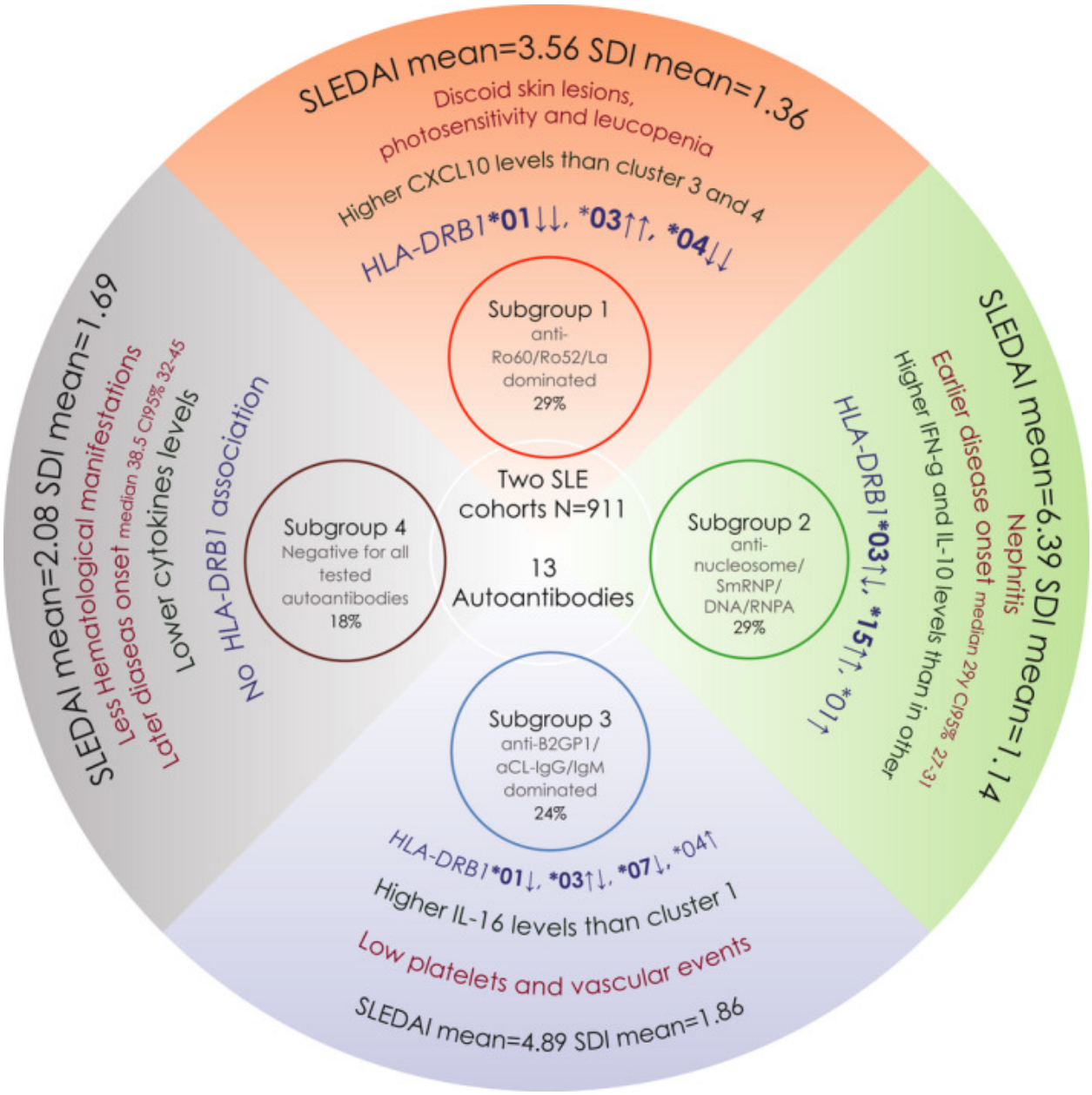


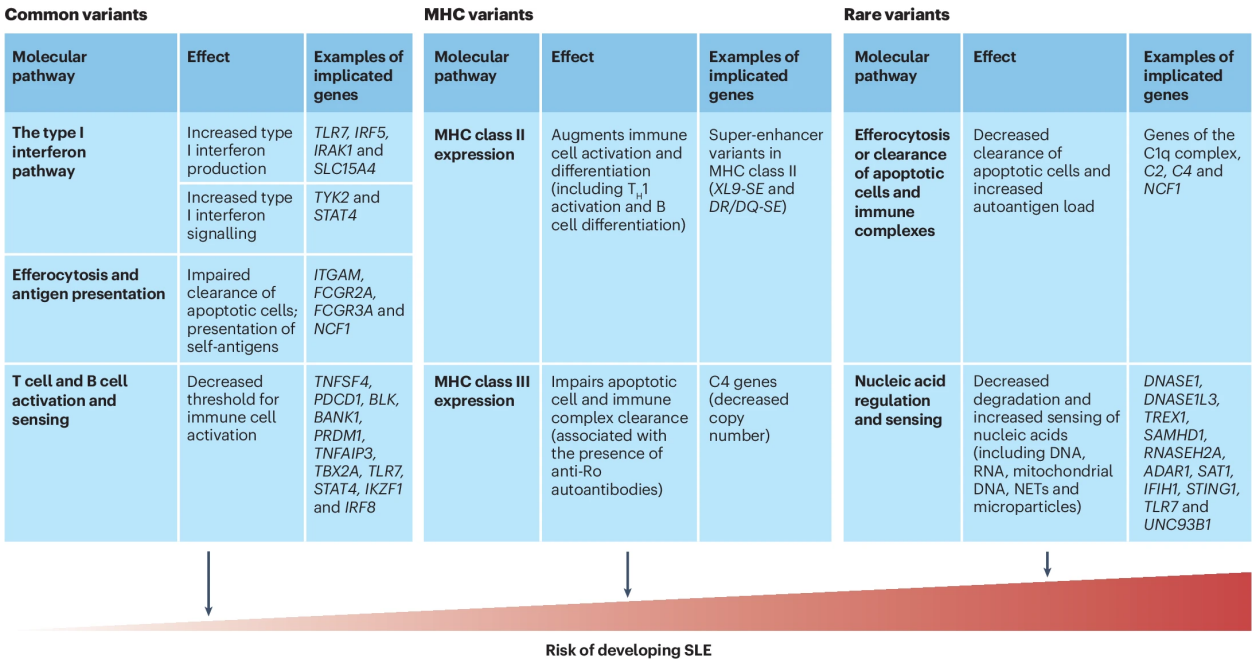
Figure 7: Subtypes of human systemic lupus erythematosus
Four subtypes of human systemic lupus erythematosus (SLE) were described depending on their symptoms, auto-antibody profile, cytokine expression, SLE disease activity index (SLEDAI), SDI= systemic lupus international collaborating clinics (SLICC) damage index (measuring the organ damage), and human leukocyte antigen (HLA)-type. The first subtype describes 29 % of the patients, and the main symptoms are discoid lesions, photosensitivity and leukopenia. The second group also describes 29 % of the patients, but this group is rather classified by nephritis and early onset of the disease. The third group describes 24 % of the patients, and those show low platelet counts and vascular events. The fourth subtype describes 18 % of the patients. These patients are negative for the tested auto-antibodies and have fewer haematological manifestations and later disease onset. Figure taken from Diaz-Gallo *et al.* 2022.

As this disease is so heterogeneous, it is a valuable approach to cluster the patients. This might also aid in choosing the correct treatment option. Many other approaches were made to cluster subtypes of SLE, not only depending on symptoms, but on immunologic properties, which might have caused the symptoms but might also help to predict the treatment success of drugs (Z. Yu *et al.* 2024; M. Cui *et al.* 2021; Sean J. Bradley *et al.* 2015).

1.2.2 Genetic components

By genome-wide association studies, more than 200 SLE-associated risk loci were identified. Strongly associated variants were often found in genome super enhancers, which are responsible for MHC II gene regulation (Ghodke-Puranik, Olferiev, and Crow 2024). Common genetic variants that mildly increase the risk of SLE are, for example, genes of the type I interferon (IFN) pathway, such as *TLR7*, IFN regulatory factor 5 (*IRF5*), tyrosine kinase 2 (*TYK2*), or signal transducer and activator of transcription 4 (*STAT4*). Also, common variants with mild effects are found in the efferocytosis and antigen presentation pathways. This causes impairments with clearance processes after cell death and also the presentation of self-antigens. Moreover, mild effects can also be caused by genetic variants leading to a decreased threshold for immune cell activation (Ghodke-Puranik, Olferiev, and Crow 2024).

MHC variants cause an intermediate risk increase for SLE. All three MHC classes were associated with different risk aspects for SLE (D. L. Morris *et al.* 2014). MHC class I presents intracellular antigens to CD8+ T cells. In SLE, gene polymorphisms of MHC class I were associated with the risk of SLE (Gambelunghe *et al.* 2005). Changes in MHC class II cause changes in immune cell activation and differentiation. Variations of this MHC class have the clearest SLE-association (D. L. Morris *et al.* 2014). Changes in MHC class III influence clearance mechanisms due to impaired complement factors (Relle and Schwarting 2012). Even though SLE is mostly a polygenic disease, some rare genetic single-gene variants are able to cause SLE. These are variants of complement genes *C1q* and C2, which influence clearance processes. If the nucleic acid sensing and regulation is impaired, for example by a variant in *DNASE1*, this is also leading to a highly increased SLE risk (Ghodke-Puranik, Olferiev, and Crow 2024) (**Figure 8**).



Common variants

Molecular pathway	Effect	Examples of implicated genes
The type I interferon pathway	Increased type I interferon production	*TLR7, IRF5, IRAK1* and *SLC15A4*
	Increased type I interferon signalling	*TYK2* and *STAT4*
Efferocytosis and antigen presentation	Impaired clearance of apoptotic cells; presentation of self-antigens	*ITGAM, FCGR2A, FCGR3A* and *NCF1*
T cell and B cell activation and sensing	Decreased threshold for immune cell activation	*TNFSF4, PDCD1, BLK, BANK1, PRDM1, TNFAIP3, TBX2A, TLR7, STAT4, IKZF1* and *IRF8*

MHC variants

Molecular pathway	Effect	Examples of implicated genes
MHC class II expression	Augments immune cell activation and differentiation (including T_H1 activation and B cell differentiation)	Super-enhancer variants in MHC class II (*XL9-SE* and *DR/DQ-SE*)
MHC class III expression	Impairs apoptotic cell and immune complex clearance (associated with the presence of anti-Ro autoantibodies)	C4 genes (decreased copy number)

Rare variants

Molecular pathway	Effect	Examples of implicated genes
Efferocytosis or clearance of apoptotic cells and immune complexes	Decreased clearance of apoptotic cells and increased autoantigen load	Genes of the C1q complex, *C2, C4* and *NCF1*
Nucleic acid regulation and sensing	Decreased degradation and increased sensing of nucleic acids (including DNA, RNA, mitochondrial DNA, NETs and microparticles)	*DNASE1, DNASE1L3, TREX1, SAMHD1, RNASEH2A, ADAR1, SAT1, IFIH1, STING1, TLR7* and *UNC93B1*

Figure 8: Genetic predispositions increasing the risk of systemic lupus erythematosus development
Genetic predispositions for systemic lupus erythematosus (SLE) are manifold. Many common gene variants are known; however, the most common ones only show a minor contribution to the risk of developing SLE. Certain major histocompatibility complex (MHC) variants result in an intermediately increased SLE risk, and rare variants, especially influencing apoptosis, efferocytosis, or nucleic acid sensing, result in a high risk of SLE development (Ghodke-Puranik, Olferiev, and Crow 2024).

Besides gene variants, epigenetic changes have been associated as a risk factor for SLE. Epigenetics regulate DNA methylation, histone modifications, and microRNA expressions. Dysregulations in these areas are associated with abnormal adaptive and innate immune functions, contributing to the pathology of SLE (Araki and Mimura 2024).

1.2.3 Treatment options

The treatment options are broadening with every year of research and drug development, yet there is no cure. Even if a wide range of chemicals and biological agents have been developed, the side effects of these medications are strong, and the quality of life often remains unsatisfying. Nevertheless, the life expectancy has extended with several breakthroughs during the past century. The most recent treatment recommendation was also developed from EULAR, with the latest update in 2023 (Fanouriakis *et al.* 2024). They agreed on five overarching treatment principles and 13 recommendations. Hydroxychloroquine, first used as an antimalarial drug, is recommended for all lupus patients. It has low side-effects and high efficiency as it blocks TLRs, mainly TLR7 and TLR9,

which reduces the overall B cell activation ('t Veld *et al.* 2021). A blockage of TLR7 and TLR9 also prevents the immune activation by UV light (Marshak-Rothstein 2006) and CXCR4 (Smith *et al.* 2019). Additionally, hydroxychloroquine decreases IFN signalling and inhibits antigen presentation, which lowers T cell activation (Schmidt *et al.* 2017; S. A. Richard *et al.* 2020). It also interferes with autoantigen processing and reduces the risk of blood clotting (Gies *et al.* 2020; Arachchillage, Laffan, and Pericleous 2023). All these effects reduce autoimmune activity and lower inflammation. However, this is often not enough to force SLE into remission.

Glucocorticoids (GCs) are highly effective in reducing inflammation, but the side effects are dangerous. GCs are able to cause irreversible organ damage, including kidney failure and even death (Ruiz-Irastorza, Danza, and Khamashta 2012; Bultink *et al.* 2021), thus contributing a significant proportion of the organ damage in SLE (Gladman *et al.* 2003). Therefore, the treatment should be done with caution and only as a bridging therapy to gain control of the inflammation. For maintenance treatment, it should be of a low dose and, if possible, left out. Instead of GCs, immunosuppressive drugs such as methotrexate, azathioprine and mycophenolate should be considered, as well as biologicals, meaning antibodies directed against cell types that are commonly overactive in SLE. The most prominent ones here are belimumab, anifrolumab, and rituximab. Belimumab targets the B cell-activating factor (BAFF)-receptor, which is required for B cells to survive and mature. Rituximab targets CD20 on B cells, directly destroying mature B cells. Without B cells, no auto-antibodies can be produced, lowering the disease activity. Anifrolumab blocks type I IFN, which is often over-reactive in SLE, thereby reducing inflammation (Basta *et al.* 2020; S. N. Liossis and Staveri 2021) (**Figure 9**).

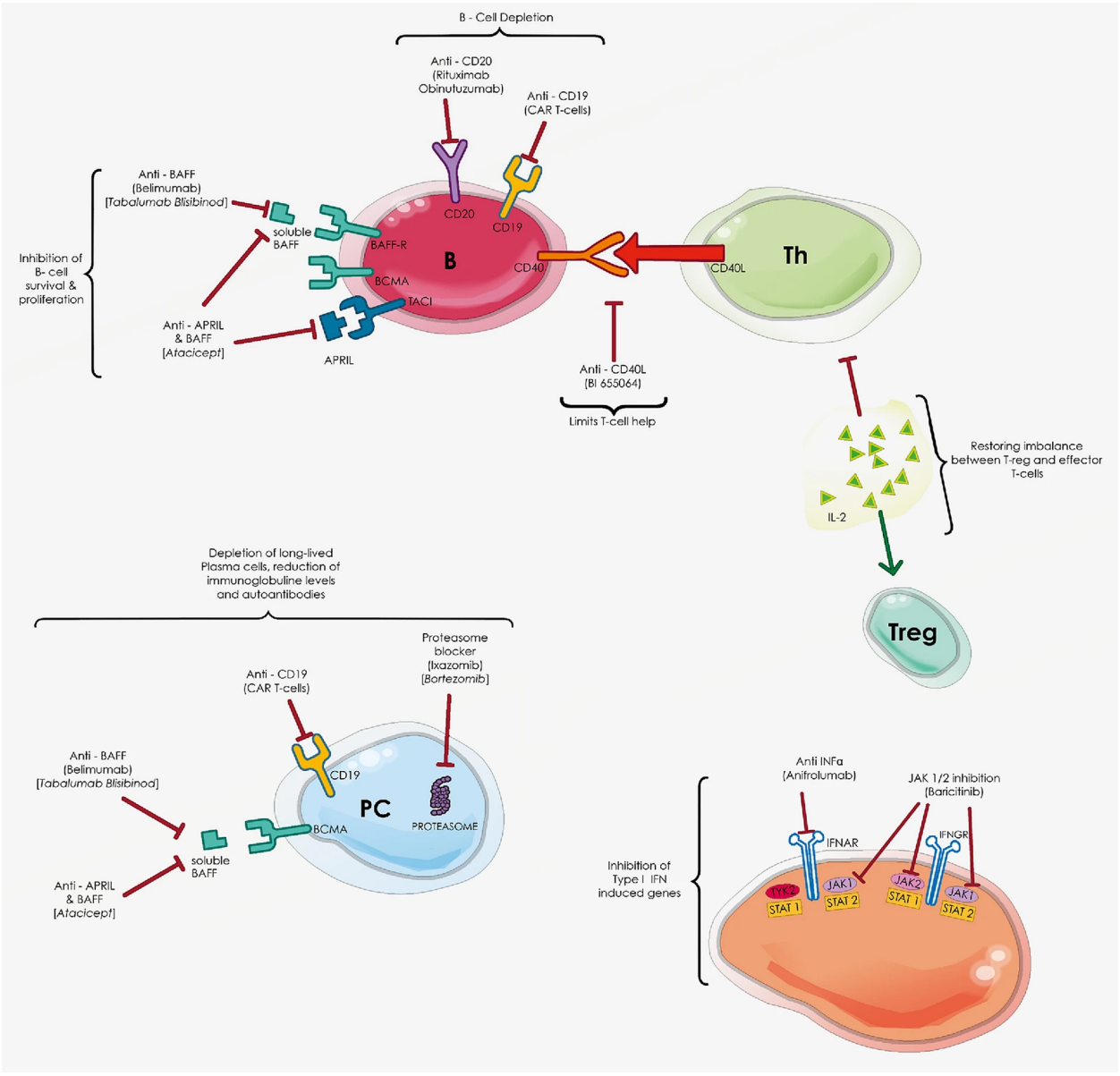


Figure 9: Overview of systemic lupus erythematosus drug treatment targets
Several medication strategies are used to treat systemic lupus erythematosus using biologicals. B cells can be inhibited using anti-BAFF treatments or a combination of anti-APRIL and anti-BAFF treatments. A depletion of B cells can be achieved by targeting CD20 or CD19. The communication between B and T helper (Th) cells can be blocked by anti-CD40 ligand treatment. Another approach is to increase immunologic balance by restoring regulatory T cells (Tregs) with interleukin (IL)-2. Plasma cells (PCs) can similarly be inhibited or depleted as B cells. Additionally, proteasome blockers can be used. Type I interferon (IFN) is also targeted by biologicals aiming to reduce overall inflammation. Figure taken from Basta *et al.* 2020.

The latest treatment attempts, which are not yet widely available, are chimeric antigen receptor (CAR) T cell therapies. These are genetically modified T cells that specifically target and destroy

immune cells of choice. To do so, T cells are taken from the patient's blood and are genetically modified to express a CAR, which targets CD19. CD19 is found on B cells. Before the CAR T cells are reintroduced in the patient, the immune cells are reduced by low-dose chemotherapy to improve the success of the CAR T cells. These CAR T cells are only infused once, causing the destruction of all B cells. This is promoting a rapid drop in autoantibodies, allowing the immune system to exit the vicious circle of autoimmunity and reset. Only a few patients have been treated this way so far, but most achieved long-term remission and did not require further immunosuppressive drugs. The B cells regenerate but are thought to be less autoreactive after this therapy. More investigation is urgently needed, as this is a highly promising therapy, especially for all patients suffering from severe flares and low quality of life (Abdalhadi, Chatham, and Alduraibi 2024).

Concluding, many treatment options are available, however, prevention or cure could not yet be reached, neither is the disease development fully understood.

1.3 External triggers and influences on systemic lupus erythematosus

Not only within the body, predispositions are occurring, but these can also be found in the external world. Factors that influence health are the healthcare system in the country you live in, the doctors that treat you, and your lifestyle (Mosca *et al.* 2024; Vordenbäumen *et al.* 2023). Among this, the socio-economic status plays a role because this is associated with chronic stress, poor nutrition, limited access to health care, and higher exposures to pollution and infections. Moreover, nutrition influences health and the immune system of all socio-economic layers in society. In rich countries, highly processed foods, high amounts of sugar, saturated fatty acids, and meat are consumed, leading to a pro-inflammatory environment in the body (Christ, Lauterbach, and Latz 2019). Mostly, these kinds of diets are also low in fibre, disturbing the gut microbiome (Kundi *et al.* 2021).

Next, abiotic factors such as light and temperature also play a role. Light-exposure has on the one side the positive effect of enhancing the vitamin D3 production in the body. Many immune cell types respond to vitamin D3 by enhancing the innate immune system and regulating the adaptive one (Ghaseminejad-Raeini *et al.* 2023). Multiple different modes of action are known, and regarding autoimmunity, especially anti-inflammatory effects are of importance. A deficiency of vitamin D3 is associated with an increased incidence of multiple autoimmune diseases (Adorini and Penna 2008). Notwithstanding, sunlight exposure is also capable of damaging the skin and causing autoimmune flares (Fernandez and Kirou 2016). Temperature can have positive and negative effects. Cold is able to reduce inflammation. This is why cold therapy is used to relieve pain and inflammation in patients with arthritis (Nam *et al.* 2007). However, cold is also able to cause stress to the body, which might trigger flares in SLE by intensifying hypercoagulation (Matsuda *et al.* 1992). The

EULAR recommendations also suggest preventing cold exposure to avoid Raynaud's phenomenon (Parodis *et al.* 2024). Heat, on the other hand, is improving circulation and relaxation, reducing pain and fatigue (Heinonen *et al.* 2011; Abdelaziz, Elmetwaly, and Maged 2020). At the same time, it might support inflammatory processes (Mirsanei *et al.* 2024).

1.3.1 Circadian rhythm and interaction with the immune system

Environmental changes in light and temperature are anticipated by the circadian system. It is guiding not only the behaviour of all organisms, separating also our 24 h rhythms into active and inactive phases, but it also orchestrates all organs in their specific tasks and each cell within them. Therefore, a dysbalance of this machinery is influencing the whole system, including the immune system. It is suggested that shift work is a risk factor for autoimmune diseases (Stenger, Grasshoff, *et al.* 2023). However, shift work is not the only factor affecting the endogenous rhythms. Jet lags, due to travelling across time zones, and social jet lags (due to work- and leisure time schedules, which do not match natural sleep- and wake times) are also causing disturbances. The same accounts for the timing of meals as well as physical activity and exposure to light (Healy, A. R. Morris, and A. C. Liu 2021). Immune cells underlie the circadian rhythm as well. In the blood, the numbers are mostly peaking during the rest phase (nights for humans, days for rodents), and decrease during the active phase (Scheiermann, Kunisaki, and P. S. Frenette 2013). An exception are effector CD8+ T cells, which are peaking during the active phase (Dimitrov *et al.* 2009). The symptom severity for many diseases is also fluctuating with the circadian rhythm (Scheiermann, Kunisaki, and P. S. Frenette 2013).

1.3.2 Influence of light on the immune system

Light is not only acting on the body as a *zeitgeber* for the entrainment and stabilisation of circadian rhythms (Roenneberg *et al.* 2013), but it can also cause damage to light-exposed body surfaces. Ultraviolet (UV) light is powerful enough to burn the skin, directly causing apoptosis. Besides this damage, it is also influencing cell-cell communication. The work of Skopelja-Gardner and colleagues showed that irradiation of the skin of healthy C57BL/6 mice with low doses of UVB light leads neutrophils to traffic first to the skin and then to the kidneys and cause inflammation, resembling a lupus phenotype (Skopelja-Gardner *et al.* 2021). These effects of UV light are dose-dependent but also wavelength-dependent. The different wavelengths have different energetic properties. The shorter the wavelength, the more powerful it is, but the longer the wavelength, the deeper the penetration into the skin. Thus, UVA (320-400 nm) is able to penetrate the skin deeply,

reaching the dermis, the second layer of the skin, but as it has a long wavelength, it is not as powerful as UVB (280-320 nm) or UVC light (100-280 nm). UVA light is known for different effects on the skin, such as ageing and skin damage, but also for anti-inflammatory effects (Yang *et al.* 2023). UVB light, with an intermediate wavelength and penetration depth (reaching the epidermis), is the main causative agent of sunburns and skin damage, leading to skin cancer (Ichihashi *et al.* 2003). UVC light is mostly blocked by the ozone layer of the atmosphere. It also has a minimal penetration depth. Far-UVC (200-235 nm) is thought to be useful as germicidal disinfecting lamps in open spaces while people use them (Yamano *et al.* 2020; Buonanno *et al.* 2017). However, it is not fully known how this may damage the skin, especially in photosensitive people. Blue light is not part of the UV spectrum but of the visible light. It has a longer wavelength (380-500 nm) and is known for its effect on the circadian master clock (K. Dong *et al.* 2019). However, little is known about blue light effects on skin. It is thought not to be powerful enough to cause any damage. Nevertheless, dependent on the dose, it is well competent in disinfecting surfaces due to its antimicrobial activity (J. Cabral and Ag 2019).

SLE-predisposed people might react differently to light than healthy, immune-competent individuals. Therefore, it is important to investigate the effects of light also in these cohorts.

1.3.3 Influence of physical activity on the immune system

Physical activity has several influences on the immune system, some are immediate, some are developing long-term. Among the immediate effects, the increased heart pumping rate leads to faster circulation of blood and lymphatic fluids and therefore also faster trafficking of immune cells. This acts as a booster for defence mechanisms, while stress hormones do not peak in short acute exercises. This is then allowing a faster detection and elimination of intruders (Nieman and Wentz 2019). Other fast effects are the reduction of pro-inflammatory cytokines (Stewart *et al.* 2005; Gleeson, McFarlin, and Flynn 2006) and the stimulation of interleukin (IL)-10 release (Nieman, Henson, *et al.* 2006) and Treg activation (Fernandes *et al.* 2019). TLRs (especially TLR1, TLR2, and TLR4 on monocytes), responsible for antigen presentation, and cells expressing them, are also reduced for a couple of hours after exercising (Gleeson, McFarlin, and Flynn 2006; Lancaster *et al.* 2005; Stewart *et al.* 2005). Even the stress hormone cortisol is reduced upon low-intensity training (but increased after high-intensity training) (Hill *et al.* 2008). Inflammatory immune dysfunctions can thereby be reduced. For the long-term effects, physical activity cannot only have beneficial effects but can also be harmful when overly intense. Among the positive effects are a reduced infection risk, improved vaccine responses (Chastin *et al.* 2021), and lowered autoimmunity-related inflammation (Sharif *et al.* 2018). Additionally, movement can act as *zeitgeber* and thereby stabilise the circadian

rhythm (Lewis *et al.* 2018), which is also beneficial for the immune system (Chen Wang *et al.* 2022). When it comes to autoimmunity, exercise might be especially helpful to regulate the immune system and thereby reduce inflammation-related symptoms. Muscle training might also reduce the muscle loss mediated by inflammation and steroidal treatments (Bouredji, Argaw, and J. Frenette 2022). Detrimental effects of over-exercising are increased oxidative stress and inflammation, as well as a short-term immune suppression with decreased white blood cell function (Pedersen and Toft 2000; Gleeson 2007). Too high training intensities also increase the cortisol levels and may therefore have opposite effects to low exercise intensities (Hill *et al.* 2008).

1.3.4 Influence of mental health, stress, and quality of life on the immune system

Health is often separated into mental and physical health, even though they are tightly linked. Mental health is seen as the well-being of the mind, the state of happiness and satisfaction of a person. The definition of mind is still difficult. It is important to know that perceived well-being is biologically mediated but influenced by life events and the reprocessing of them. Traumatic events can cause post-traumatic stress disorder and also measurable inflammation (Y. Sun, Qu, and Zhu 2021). It is also known that this is able to trigger autoimmune diseases, including SLE (Katrinli *et al.* 2022; Bookwalter *et al.* 2020). On the other hand, mindfulness training has been shown to be an effective tool in reducing inflammation and improving the overall state of mind (Gardi *et al.* 2022). This process can also function in the other direction: Infections, chronic diseases and cancer are linked to reduced mental health, potentially by mediating inflammation, which is rendering the brain's neurotransmitter release (Jeon and Y.-K. Kim 2017; Moulton *et al.* 2019; Pelgrim *et al.* 2019). This has been mechanistically shown in lipopolysaccharide-induced inflammation and depression (O'Connor *et al.* 2009). For autoimmune diseases, this may also play a role. However, stress is a broad expression, used for different terms. It can be divided into physical and mental stress as well as acute and chronic stress. The aforementioned lifestyle factors serve all these definitions: UV light can be an acute physical stress, but a lack of daylight can also lead to mental stress. Mental stress can be a traumatic event, but also an excessive demand on daily tasks. Stress means a release of corticosteroids and catecholamines (Knezevic *et al.* 2023). Acute stress is only short-term and can have positive effects such as a boost of energy, immune cell enhancement and allowing high performance in situations. Chronic stress, however, is leading to immunosuppression, autoimmune disorders, dysregulation of T cells and a chronic low-grade increase in pro-inflammatory factors (Dhabhar and Mcewen 1997; Dhabhar 2013; Rohleder 2019) (**Figure 10**).

Quality of life describes the perceived well-being and contentment with life. As a medical improvement of patients' haematologic values does not necessarily mean that a patient is feeling

better, it is important to also target the overall well-being. Many questionnaires are available to assess this measure; however, for SLE, there are specific ones. The most commonly used one is the Lupus Quality of Life (LupusQoL) questionnaire (McElhone *et al.* 2007). It addresses the feelings of the last four weeks with questions about physical health, emotional health, body image, pain, planning, fatigue, intimate relationships, and burden to others.

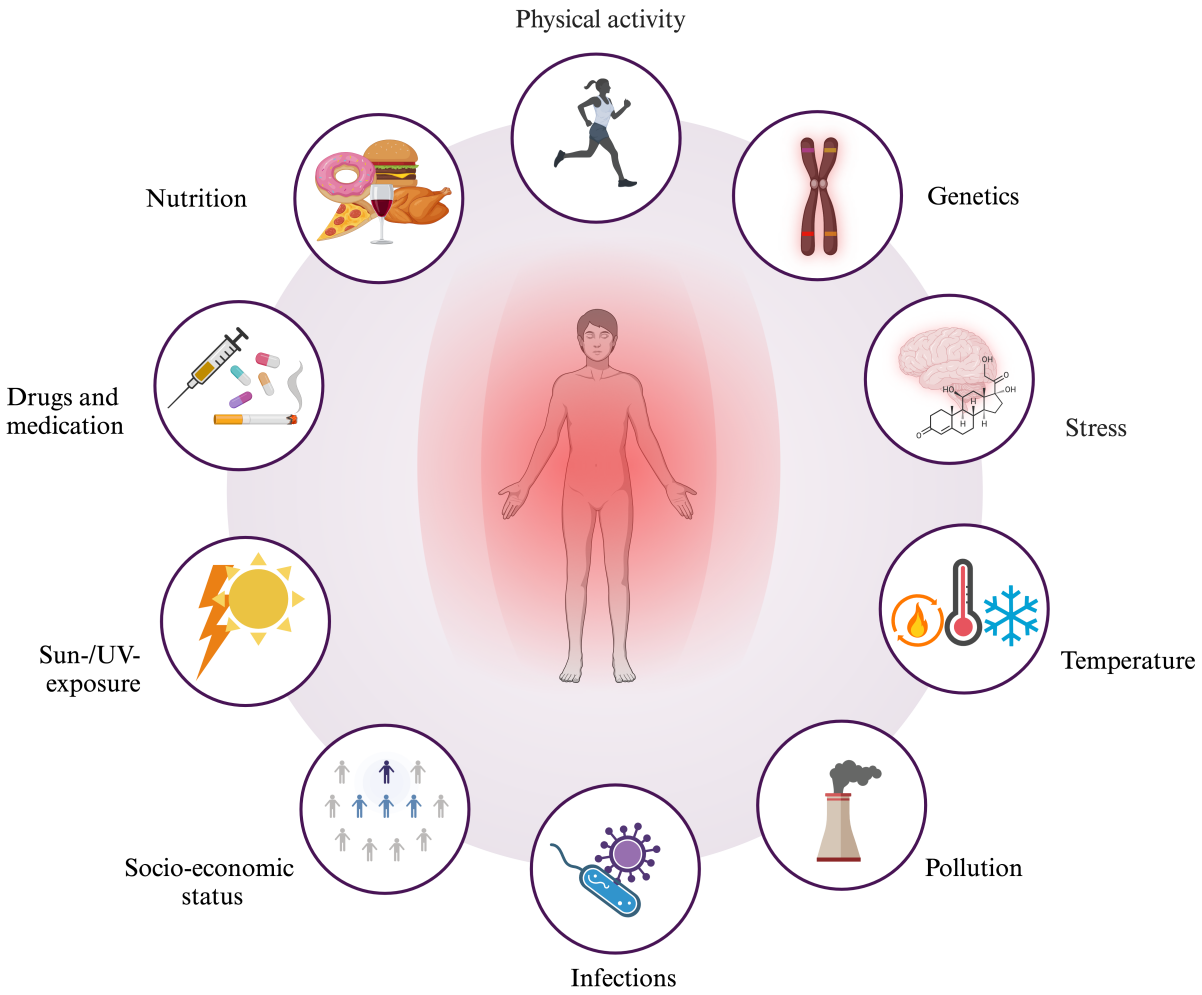


Figure 10: Triggers of systemic lupus erythematosus
Outbreaks and flares of systemic lupus erythematosus can be caused by various factors. Genetic variants and mutations are common, but stress, temperature extremes, pollution and infections can also influence. Additionally, the socio-economic status, sun-/ or ultraviolet (UV) light exposures, drugs, medication, nutrition, and physical activity have an influence. Illustration generated using BioRender.com.

1.4 Immunology of systemic lupus erythematosus

During the development and progression of SLE, complex interactions of immune cells, receptors and other factors are involved. Disturbed or misguided actions and interactions cause a cascade of consequences, fostering inflammation and feeding more into a vicious cycle. Dissecting the individual roles and contributions of each cell type and receptor is intricate and challenging, as not only one single factor is responsible and could be inhibited to resolve the disease.

1.4.1 Pre-disease phase

As the disease is not only diverse but also influenced by many factors, the pre-disease phase is not yet fully understood. Patients are looking for medical care as soon as symptoms occur, which is already past the pre-disease phase, and this is why the human pre-disease phase is scarcely investigated. However, there is data from relatives of autoimmune patients stating to develop increased levels of fatigue, ANAs, TNF, IFN-γ, and other inflammatory mediators as well as increased levels of IL-10. This shows that there is a time frame in the pre-disease in which increased inflammation is controlled by increased regulation (Munroe *et al.* 2022). Moreover, data from SLE-relatives also showed that a reduced sleep duration (less than 7 h per night) and insufficient vitamin D3 levels lead to the transition to SLE (K. A. Young *et al.* 2018; Kendra A. Young *et al.* 2017).

One of the discussed starting points for SLE is interfered clearance after regular apoptosis (Kuhn, Wenzel, and Weyd 2014). This programmed cell death is naturally occurring, but is also elevated after certain triggers such as sunburns or infections. After apoptosis, the dead cell particles are usually cleared soon by the immune system. If this process is not working efficiently or fast enough, cell particles, including nuclear fragments and DNA, are visible to immune cells. This acts as damage-associated molecular patterns, which are recognised by TLRs and other pattern recognition receptors, causing a loss of self-tolerance (Poon *et al.* 2014). Both, the innate and the adaptive immune system contribute to the development and progression of SLE.

1.4.2 The role of the innate immune system

The innate immune system is composed of macrophages, mast cells, monocytes, antimicrobial peptides, dendritic cells, the complement system, natural killer (NK) cells, platelets, and granulocytes, including neutrophils, basophils and eosinophils. It is able to respond to potential threats such as pathogens - or in autoimmunity - self-antigens. These responses are rather unspecific but therefore fast.

The **complement system** is usually also involved in clearance mechanisms of dead cells, debris

and immune complexes. Genetic deficiencies of key components of the complement system, such as complement 1q (C1q), are associated with an accumulation of immune complexes (Santer *et al.* 2010). Further, complement activation is also leading to increased inflammation and tissue, feeding into vicious cycles. Low levels of C3 and C4 are seen as a sign of active SLE flares under the assumption that these factors are depleted by intense complement activation (Sturfelt and Truedsson 2012).

TLRs were already described above in other aspects but especially TLR3, TLR7, TLR8, and TLR9 on innate immune cells are recognizing self-DNA and RNA. This is increasing the IFN production and also the B cell activation and autoantibody production (Farrugia and Baron 2017).

Monocytes are usually also involved in phagocytosis and clearance. These are classically highly expressing CD14 but not CD16. In SLE, these showed, among other defects, a reduced phagocytic activity. Therefore, monocytes, which also express CD16 are increased in SLE. These are known to be highly pro-inflammatory, releasing respective cytokines, increasing inflammation (Katsiari, S.-N. C. Liossis, and Sfikakis 2010). Moreover, during SLE, monocytes are more likely to differentiate into the pro-inflammatory M1 macrophages than the anti-inflammatory M2 macrophages. Anti-inflammatory M2 fail to resolve inflammation due to impaired efferocytosis (dead cell clearance) and persistent immune complex deposition in organs (Ahamada, Jia, and X. Wu 2021). Monocytes were also found to infiltrate kidneys and increase nephritis by supporting inflammation and fibrosis, therefore, higher monocyte counts in the blood correlate with the severity of kidney involvement and when found in the urine, these are also typical biomarkers of active lupus nephritis (Cheng *et al.* 2024; Rosa *et al.* 2012).

Macrophages, derived from monocytes, are among the cells responsible for clearance after apoptosis. Defective macrophage function then leads to an accumulation of freely accessible autoantigens.

Dendritic cells, especially **plasmacytoid dendritic cells** (pDCs), are found in the blood (in contrast to classical dendritic cells, which are tissue resident), lymphoid, and inflamed tissues. The pDCs are hyperactive in SLE, releasing excessive amounts of type I IFNs. This is fostering the B and T cell activation and inflammation (X. Huang *et al.* 2015; Panda, Kolbeck, and Sanjuan 2017).

Neutrophils are undergoing increased neutrophil extracellular trap (NET)osis formation in SLE. The NETs contain nuclear DNA, histones and antimicrobial proteins, which stimulate IFN production, increasing the inflammation. The NETs also trigger the autoantibody production (Y. Yu and Su 2013).

Eosinophils do not play a major role in the pathology of SLE, however, they can contribute to inflammation and organ damage (Diny, Rose, and Čiháková 2017). **Basophils** are also not key

players in SLE, but they contribute to inflammation by releasing cytokines and interacting with other immune cells. They also contribute to vascular involvement (Pellefigues and N. Charles 2013).

NK cells usually destroy autoreactive immune cells. If their function or number is reduced, the autoimmunity is less controlled (Spada, Rojas, and D. F. Barber 2015).

Platelets are not only required for blood clotting but also for releasing cytokines. In SLE, they are hyper-activated, contributing to the anti-phospholipid syndrome (Tohidi-Esfahani *et al.* 2024). Moreover, they promote the formation of NETs by releasing platelet-derived CXCL4 (Matsumoto *et al.* 2021; Lood *et al.* 2017) (**Figure 11**).

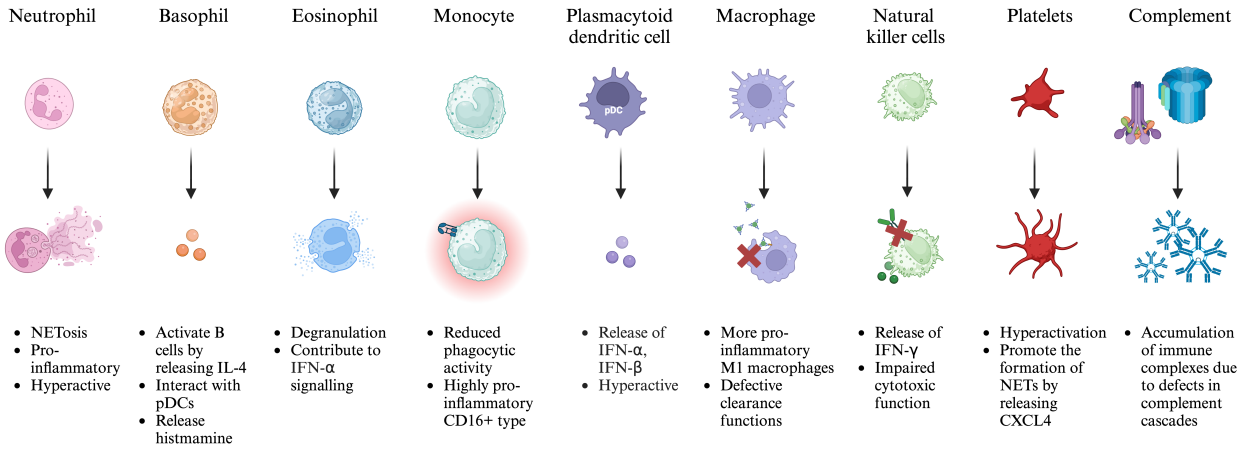


Figure 11: The role of innate immune cells in systemic lupus erythematosus
The innate immune system consists of various cell types and particles, which are all involved in the immune reactions of systemic lupus erythematosus (SLE). Neutrophils undergo the formation of neutrophil extracellular traps (NETosis), which contributes to inflammation, tissue damage, and autoimmune responses by releasing chromatin and antimicrobial proteins into the extracellular space. Moreover, in SLE, they are especially pro-inflammatory and hyperactive. Basophils play a rather minor role during SLE, however, they contribute to inflammation by activating B cells, releasing interleukin (IL-) 4, interacting with plasmacytoid dendritic cells (pDCs), and by releasing histamine. Eosinophils are dysregulated and contribute to the interferon (IFN)-α signalling. Monocytes show during SLE a reduced phagocytic activity, while predominantly the highly pro-inflammatory CD16+ type is found. pDCs are able to release IFNs and are also hyperactive. Macrophages display defective clearance functions and additionally, more pro-inflammatory M1 macrophages are formed. Natural killer cells release IFN-γ and show impaired cytotoxic functions. Platelets are cell particles with immunologic functions, such as the promotion of the formation of NETs. In SLE, they are also hyper-activated. The complement system is causing an accumulation of immune complexes due to defects in the complement cascades. The illustration was generated using BioRender.com.

1.4.3 The role of the adaptive immune system

The adaptive immune system is trained to react very specifically to pathogens (or autoantigens). Therefore, these responses are slower but highly tailored to the exact target. This system is also forming long-lasting memory. The adaptive immune system is composed of B cells, plasma cells, and T cells.

B cells, which become autoreactive, form antibodies against self-antigens, leading to the formation of immune complexes. These complexes are then deposited in several tissues, including skin, joints, and kidneys, causing inflammation and tissue damage (rashes, arthritis and nephritis) (Jacobi *et al.* 2009; Pirkle, Freedman, and Fogo 2013; Hamilton, Hsu, and Mountz 2019). Additionally, the increased IFN levels found in SLE promote survival and activation of B cells (M. Liu *et al.* 2019). B cells were found to be hyperactive in SLE, potentially due to the dysregulated activation by T helper (Th) cells (Suárez-Fueyo, Sean J Bradley, and George C Tsokos 2016; Sakane *et al.* 1988). B cells can differentiate after activation by antigen encounter. These antigens are presented to the B cells by antigen-presenting cells, such as Th cells and monocytes. Activated B cells then move towards secondary lymphoid organs to migrate to germinal centres, where they undergo proliferation and affinity maturation. During affinity maturation, the B cell receptor is somatically hyper-mutated to increase affinity and specificity to the antigens, in the case of SLE, self-antigens. The class of antibody of the B cells is also modulated by class switching, meaning that the type of immunoglobulin produced by the B cells is changed to target that specific antigen more effectively (Nashi, YingHua Wang, and Diamond 2010).

After these processes, B cells can differentiate into **plasma cells**. These are producing and releasing high amounts of (auto-)antibodies. Some of these highly specific plasma cells are moving to the bone marrow to become long-lived plasma cells, surviving in niches up to decades. This is especially difficult when it comes to B cell-directed SLE medication, as these long-lived plasma cells still remember self-antigens and can restart the production of high amounts of auto-antibodies, even after depletion of the B cells (Z. Liu, Zou, and Davidson 2011).

T cells are separated into several subtypes, most importantly into CD8+ cytotoxic T cells and CD4+ Th cells. Th cells are required for the sensing of (self-)antigens and the activation of B cells. Cytotoxic T cells, which are dysregulated in SLE, start attacking own cells and tissues. They also produce pro-inflammatory cytokines, increasing the inflammatory processes. Their cytotoxic ability by perforin and granzyme release is also impaired in SLE (P.-M. Chen and George C. Tsokos 2021). Th cells assist other immune cells by presenting antigens to them. This is, for example, activating B cells and stimulating them to produce antibodies against this self-antigen. Depending on the Th subtype (Th1, Th2 or Th17), different cytokines are released, contributing to different

aspects of inflammation (Suárez-Fueyo, Sean J Bradley, and George C Tsokos 2016; Raphael *et al.* 2015). Th1 cells release large amounts of pro-inflammatory IFN-γ (Y.-Y. Tang *et al.* 2023). In later stages of SLE, often a shift from Th1 dominance to Th2 dominance is seen (Xiang *et al.* 2022; Dai, Y. Fan, and X. Zhao 2025). Th2 cells activate B cells, causing autoantibody production, and release cytokines such as IL-4, IL-5 and IL-13 (Dai, Y. Fan, and X. Zhao 2025). Th17 cells produce mainly IL-17, which recruits neutrophils and macrophages, leading to tissue inflammation and damage, for example, in the kidneys (Paquissi and Abensur 2021). T follicular helper (Tfh) cells have a special function in SLE, as these cells are located in secondary lymphoid tissues and are essential for the formation of germinal centres and the activation and further maturation and differentiation of B cells. In SLE, the number of Tfh cells is not only expanded, but these cells are also hyperactive, supporting the rapid activation and differentiation of self-attacking B cells. Tfh cells are also resistant to suppression by Tregs and circulate in increased numbers in the blood of patients. The circulating Tfh cells are provoking extra-follicular B cell activation, a known phenomenon in autoimmune flares (Gensous *et al.* 2018).

Among the CD4+ T cells, regulatory T cells (Tregs) are found as well. These have a special role in autoimmunity. In healthy people, these cells are able to counteract pro-inflammatory stimuli and start resolution of inflammation by the release of anti-inflammatory cytokines such as IL-10 and tumour growth factor (TGF)-β. Tregs also promote tolerance to self-antigens by inhibiting self-reactive T cells that escaped the thymus during negative selection of T cell maturation. They also suppress B cell maturation and antibody production by Tfh cells in the germinal centres. Tregs are even able to modulate other antigen-presenting cells such as dendritic cells, which helps keeping type I IFN responses under control. The dysregulation of Tregs can be manifold in SLE. They were not only found to be reduced in patients, but also their suppressive functions can be impaired (La Cava 2018). Clinical studies are targeting Tregs with the idea of resolving lupus by restoring correct immunological balances and tolerance, using the Treg-inducing cytokine IL-2 (J. Y. Humrich, Spee-Mayer, *et al.* 2019) (**Figure 12**).

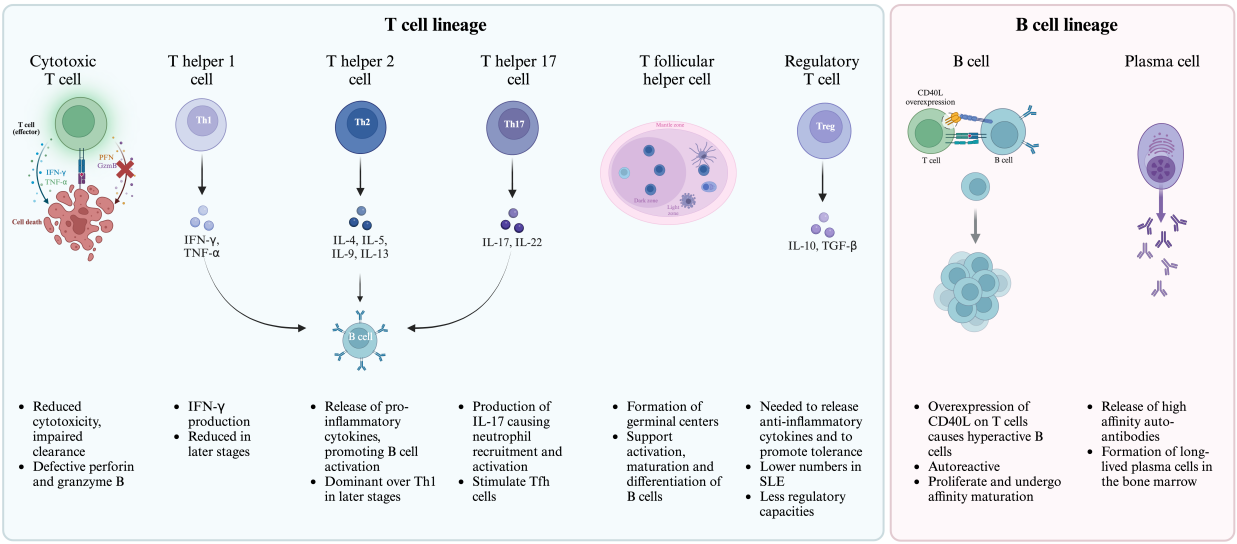


Figure 12: The role of adaptive immune cells in systemic lupus erythematosus
The adaptive immune cells can be separated into cells from the B cell- and from the T cell-lineage. Among the T cells, it can be differentiated between the cytotoxic, CD8+ T cells, which have reduced cytotoxicity during systemic lupus erythematosus (SLE) as well as defective perforin and granzyme B releases, and CD4+ T cells. Among the CD4+ T cells, T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) cells all release different kinds of cytokines, interacting with B cells. Th1 cells release interferon (IFN) -γ and are reduced in later stages of the disease. Th2 cells release pro-inflammatory cytokines and are in later stages dominant over Th1 cells. Th17 cells produce interleukin (IL)-17, which causes the activation and recruitment of neutrophils, leading to further inflammation. They also stimulate T follicular helper (Tfh) cells. The Tfh cells are needed to form germinal centres and also support the activation, maturation, and differentiation of B cells. Regulatory T cells reveal reduced numbers in SLE but are needed to promote tolerance and to reduce inflammation. B cells are hyperactive and autoreactive in SLE and can become plasma cells, releasing large amounts of autoantibodies. The illustration was generated using BioRender.com.

1.4.4 The specific role of T cells investigated in this thesis

In this thesis, the adaptive immune cells, T cells, were investigated using flow cytometry. Fluorescently labelled antibodies were used to distinguish CD4+ T helper cells and CD8+ cytotoxic T cells. CD62L and CD44 were used to differentiate between naive, effector and central memory T cells. The intranuclear transcription factor FoxP3 and the activation marker CD25 defined the activated Tregs (later only referred to as Tregs). Helios, Ki-67, CD39, and CTLA-4 were used to further characterise the Tregs. The intranuclear marker helios is also classifying Tregs as functional, as it stabilises FoxP3 (Getnet *et al.* 2010). Moreover, helios+ Tregs were shown to not produce pro-inflammatory cytokines (Golding *et al.* 2013; Y. C. Kim *et al.* 2012; Elkord, Abd Al Samid, and Chaudhary 2015). Ki-67 is a proliferation marker that is only positively expressed in the active

phases of the cell cycle (Uxa *et al.* 2021). CD39 characterises the suppressive potential of the Tregs as CD39+ Tregs can release adenosine and break down extracellular adenosine triphosphate (ATP). CTLA-4 is also needed for the immunosuppressive capacities of Tregs as it competes with CD28 for its ligands, providing inhibitory downstream signals. Moreover, CXC-chemokine receptor (CXCR)4 and CX3CR1 were used to investigate the trafficking of the T cells. CXCR4 is not only a target for hydroxychloroquine (J. Kim *et al.* 2012) and is dysregulated in SLE (L.-d. Zhao *et al.* 2017), but it is also expressed for many purposes in health and disease. One of them is for the migration towards the bone marrow and injured tissues (Arieta Kuksin, Gonzalez-Perez, and Minter 2015; Kohli, Pillarisetty, and T. S. Kim 2022; Contento *et al.* 2008). CX3CR1 has several purposes and is expressed, for example, for migrating into the endothelium or towards inflamed tissue (Nanki *et al.* 2002; Blaschke *et al.* 2003). It is also an important chemokine receptor in cardiovascular disease (Apostolakis *et al.* 2009), which is a common problem in SLE (Frostegård 2023). Moreover, in a lupus mouse model, CX3CR1 was found to contribute to nephritis and cardiovascular disease (Cabana-Puig *et al.* 2023).

1.5 Systemic lupus erythematosus in mice

Studying SLE in mice is a great option to investigate, dissect and analyse each organ that is involved. From humans, mostly blood, urine and, if needed, kidney biopsies are taken for medical purposes. Even though mice are not humans and differ in many aspects, including the proportions of immune cell types, they still provide valuable insights into disease mechanisms. They can be used to investigate changes in the bone marrow, thymus, brain, spleen and more. Moreover, mice can be (genetically) modified as needed. Depending on the research aim, many mouse models have been generated. Mice are nocturnal animals, meaning that many circadian rhythms known from human cell types are inverted due to their nighttime active phase. Laboratory mice are living a special life with different stress factors than humans or wild mice. The mice are kept in small groups in rather small cages, not allowing normal family systems and territorial behaviour, which can cause tensions in the animals that influence the immune system (Vogt *et al.* 2024). Moreover, their nutrition in the lab is different from natural eating habits (Graham 2021). However, it can also be discussed how natural human life is nowadays regarding shift work, and stress factors such as junk food, social media and general digitalisation. Therefore, as the mice's behaviour and environment can be widely controlled, confounders are known and calculated in these models. As SLE is such a heterogeneous systemic disease with patients suffering from a multitude of varying symptoms, it is important to dissect the different aspects or subtypes of the disease.

1.5.1 Mouse models available for research

A wide variety of mouse models is available for researching SLE. The options do not only differ in genetics but also in the symptoms and aspects of the disease, which are aimed to investigate (M. L. Richard and Gilkeson 2018). **Spontaneous models** are widely used as they are thought to display a rather natural progression of the disease. NZBW/F1 mice have been known for decades. They are the filial generation one (F1) breed of New Zealand White (NZW) and New Zealand Black (NZB) mice. These mice display autoantibodies, immune-complex glomerulonephritis and mild vasculitis (Dixon *et al.* 1978). From these mice, by breeding many generations, the NZM strains were developed (U H Rudofsky *et al.* 1993). Mostly studied is the NZM2410 strain (Morel and Wakeland 2000). It also develops autoantibodies and nephritis, but no vasculitis. However, females and males develop it to a similar extent, whereas NZBW/F1 mice show a female bias (Syrett, Sierra, *et al.* 2020). The NZM2410 strain was used to study the genetics of SLE in detail. This model was further refined by backcrossing it on C57BL/6 mice to allow the use of these commonly used wild-type mice as controls. NZM2410 mice had the difficulty that no true control exists, as it is a homogenous breed and the ancestors (NZB and NZW mice) also each showed traits of autoimmunity and were not completely healthy. This cross-breed of NZM2410 and C57BL/6 mice was generated by Morel, Mohan and Wakeland to further characterise the roles of the susceptibility loci *Sle1*, *Sle2* and *Sle3* (Morel, Croker, *et al.* 2000). All these models have a long incubation time until first symptoms develop (about 5 months, depending on genotype, facility, sex, and other influences) (Perry *et al.* 2011, M. L. Richard and Gilkeson 2018). This renders them ideal for studying the pre-disease phase. However, no skin involvement is known in these strains and also no cerebral involvement is described.

Another spontaneous model, which is commonly used, is the MRL/lpr mouse. This was generated by crossing LG, C57BL/6, AKR and C3H mice. Due to these multiple strains involved, the genetics are complex, but the lpr gene is known to have a single mutation in the first apoptosis signal (FAS) receptor gene, causing similar effects to a complete FAS deficiency in mice. Defects in the FAS receptor cause survival of self-reactive lymphocytes (Watson *et al.* 1992). However, if humans have a FAS deficiency, they show immune abnormalities but no SLE-specific symptoms (Yamada *et al.* 2017; Ramenghi *et al.* 2000). The MRL/lpr mice develop SLE symptoms much faster and show central nervous system involvement as well as skin symptoms. A large amount of autoantibodies is also seen in this strain. The onset of the disease starts at approximately 8–10 weeks of age (S. Williams, Stafford, and Hoffman 2014).

The last spontaneous model to describe here, even though there are many more, are the BXSB mice, as these show a disease type linked to another known factor influencing human SLE: TLR7.

In these mice, the *Yaa* region of the X-chromosome was translocated to the Y-chromosome. Thus, disease symptoms are only found in males and are characterised by glomerulonephritis due to overexpression of TLR7, leading to increased type I IFN signalling. This model also supports the idea of X-chromosomal escape mechanism, causing autoimmunity in humans (Perry *et al.* 2011).

Another option to receive SLE mouse models is to **induce the disease** in otherwise healthy mice. This can, for example, be done by injecting the drug pristane in BALB/c mice, which causes immune complex glomerulonephritis, autoantibodies and mild nephritis after several months. This is due to pristane-dependent overproduction of type 1 IFN. Similarly, the mouse model using resiquimod cream (TLR7 ligand) on the ears is also causing nephritis, autoantibody production and splenomegaly by inducing TLR7-dependent type 1 IFN pathways (M. L. Richard and Gilkeson 2018).

Trichlorethylene (TCE) is a suspected threat of environmental pollution, thought to be causing autoimmunity. In this model, autoimmunity is only accelerated by exposure in genetically predisposed models and not induced in healthy mice (M. L. Richard and Gilkeson 2018).

Graft versus host-disease can also be used as a model for murine lupus nephritis. This is caused by injections of a mixture of spleen, lymph node, and thymus cells from DBA/2 donor mice into (C57BL/10xDBA/2) F1 mice or by transplantation of bone marrow cells. This model shows autoantibodies against nuclear antigens and severe nephritis after 12-14 weeks (Bruijn *et al.* 1988).

A very straightforward approach is to inject antibodies against the glomerular basement membranes of the kidneys, causing a rapid onset and progression of nephritis, allowing to study the details of renal inflammation and immune complex deposition (Cruz *et al.* 2024).

SLE can also be studied in **transgenic models**, for example, by incorporating genes of the immunoglobulin (Ig)G heavy and light chains coding for autoantibodies. Overexpressions of FMS-like tyrosine kinase 3 receptor-interacting protein 1 (Fli-1), TLR7, TGF-β, and IL-10 were also used to cause SLE-like diseases (M. L. Richard and Gilkeson 2018).

Another option for genetic manipulation is the **knockout of genes**. As defects in certain TLRs are known from human SLE and other mouse models to cause disease, knockouts of TLR7 and 9 were studied in lupus-prone MRL/lpr mice, to show the specific effect of these receptors on SLE. The lack of TLR7 reduced the SLE symptoms and prolonged survival, while the lack of TLR9 in MRL/lpr mice prevented the production of anti-dsDNA antibodies but had even stronger disease symptoms. As the double knockouts of both TLR receptors were protected from SLE, receptor interactions were investigated. It is suggested that the exacerbation of SLE in TLR9 deficient mice is type I IFN dependent (Nickerson, Cullen, *et al.* 2013) and independent from the *Fas* deficiency in the MRL/lpr mice (Nickerson, Yujuan Wang, *et al.* 2017).

Moreover, defects in the complement system are also known to be tightly linked to a high risk predisposition in humans, therefore, knockout models of C1q and C4 were developed (Botto 1999; Paul *et al.* 2002).

As described above, mice were often characterised to be very different from humans as a species. To overcome this problem, humanised mouse models were developed, aiming to imitate the human immune system by injecting human SLE patients' peripheral blood mononuclear cells (PBMCs) into immune-deficient mice. Another way is to take haematopoietic stem cells from a human fetus to inject these together with pristane into mice. These models are still limited by their variability and the limited human B cells immune response. Future studies aim to extend the life span of the mice to allow studying the development of autoimmunity in more detail. Furthermore, the models need to be more standardised to allow a broader use of them (J. Chen *et al.* 2022) (**Figure 13**).

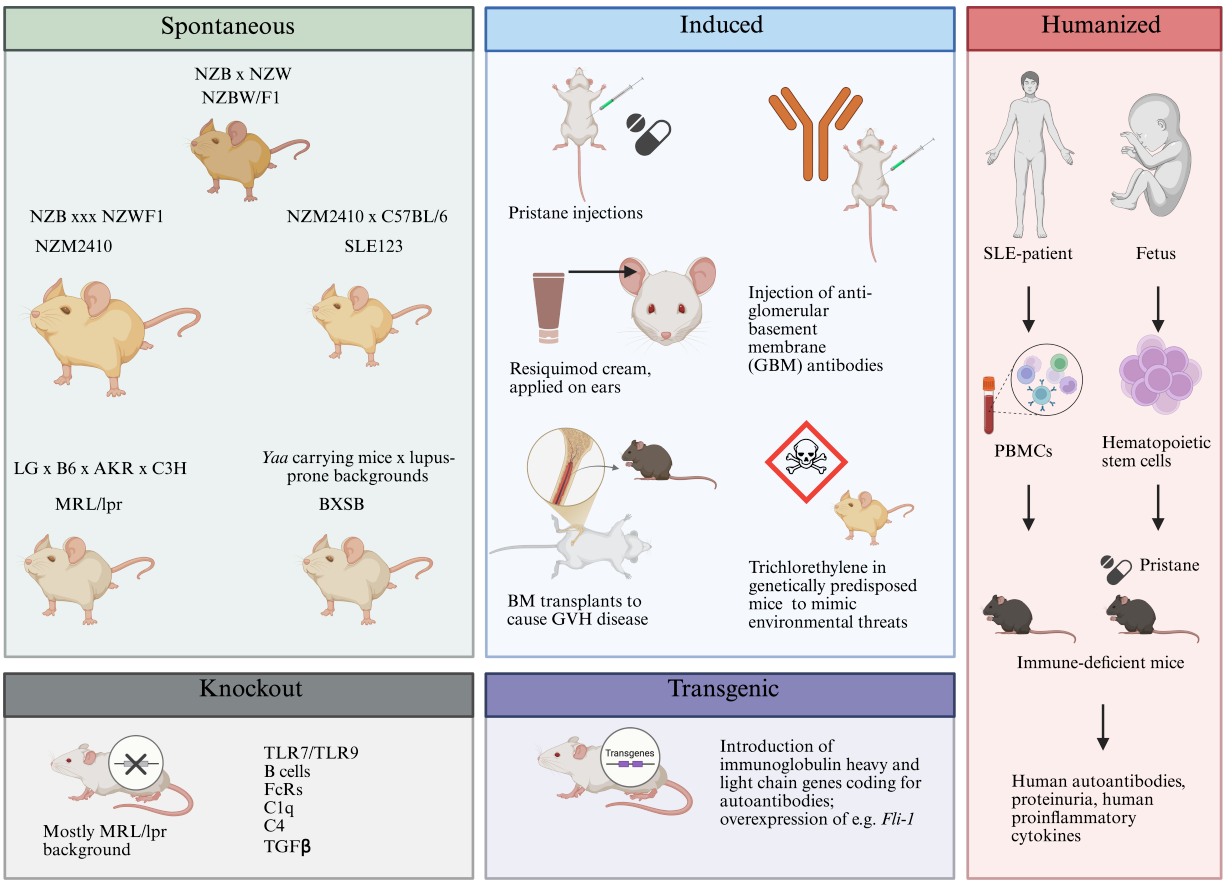


Figure 13: Examples of mouse models available for lupus research
Mouse models available for research can be divided into spontaneous models, which develop the disease due to their heritage, induced models, which receive a certain kind of treatment to cause systemic lupus erythematosus (SLE) or SLE-like symptoms, or humanized mice, in which immune-deficient mice receive human patient peripheral blood mononuclear cells (PBMCs) or haematopoietic stem cells and pristane to mimic human SLE. Besides, knock-out and transgenic models of certain genes are available to investigate their specific influence on the diseases. BM= bone marrow; GBM= ; GVH disease = graft versus host disease; TLR= toll-like receptor; TGF= tumour growth factor; Fli-1= FMS-like tyrosine kinase 3 receptor-interacting protein 1. The illustration was generated using BioRender.com.

1.5.2 Mouse models chosen

For our experiments to explore the SLE pre-disease phase, we decided to use two spontaneous SLE-prone mouse lines as well as one healthy mouse line. The first SLE-prone mouse is the **NZM2410 mouse**. This spontaneous line is known to have a long phase of pre-disease before the first symptoms occur. Moreover, the disease phase is well studied, and it is known that they develop glomerulonephritis and a variety of autoantibodies, including anti-dsDNA and anti-glomerular

basement membrane antibodies (Mathenia *et al.* 2010). The genetic background is also widely studied, and the combination of the three susceptibility alleles *Sle1*, *Sle2*, and *Sle3* leads to high similarity to human SLE. *Sle1* is responsible for the loss of tolerance towards nuclear antigens, while *Sle2* lowers the activation threshold of B cells, and *Sle3* causes the dysregulation of CD4+ T cells (Morel, Croker, *et al.* 2000). Spontaneous models present the difficulty that you cannot be certain when exactly the disease develops in each mouse, however, this can be seen as a more natural model than the induced ones. We see this variance as part of the natural process and try to understand its importance to include in our results and interpretation. We also chose the NZM2410 mice above the knockout or transgene models, as we would like to study this complex disease with as many facets as a model can represent and not the influence of a single gene or receptor. NZM2410 mice also have the advantage that males and females develop disease at a similar rate and degree, which is different from the human disease, where it is more frequent in women. Nevertheless, human men also develop it, and this model lets us study both sexes. The **SLE123 mice** also express the susceptibility alleles *Sle1*, *Sle2*, and *Sle3* but they are backcrossed on C57BL/6J mice. They are also spontaneously SLE-developing mice and are frequently studied. They represent certain differences to the NZM2410 mice, such as a delayed disease onset and improved breeding and survival (TheJacksonLaboratory 2025d). Yet, we chose them as they allow the direct control of the disease with C57BL/6J mice. As a healthy mouse model, we chose the **C57BL/6J mice**, because they act as true controls for the SLE123 mice and still as a comparison to the NZM2410 mice. We decided not to use BALB/c mice, which are also often used as healthy controls, because BALB/c mice have a different genetic background from both SLE-prone mouse lines. Additionally, some immunologic imbalances are known in BALB/c mice, such as a skewness towards Th2 responses. Moreover, these mice also differ in cytokine responses, antibody production and immune cell composition from C57BL/6J mice (Qidi Zhang *et al.* 2023; Watanabe *et al.* 2004). Other common control animals for NZM2410 mice would have been NZB or NZW mice, but these already show some autoimmune features (Dixon *et al.* 1978). This is why we do not see them as a healthy comparison.

We chose these spontaneous mouse models and C57BL/6J mice as a healthy comparison but we purposely avoid calling them controls for the NZM2410 mice, as these mice have a genetically different background from C57BL/6J mice. However, they can be called controls for the SLE123 mice. As we were kindly gifted all these mice by different cooperating working groups at the University of Lübeck, the distribution of experimental animals over the pre-disease phase is not always balanced, but therefore, we were able to make the best use of these animals, even though they were not bred for my experiments.

1.6 Aims of this doctoral thesis

This thesis aims to explore the SLE pre-disease phase in mouse models with a focus on changes in blood cell composition, T cells, and the influence of UV-light. Therefore, **the first hypothesis** is that we can find distinct differences between lupus-prone mice before the onset of nephritis compared to healthy age-matched C57BL/6J mice in physical activity, blood cell composition, and T cells in blood, spleens and lymph nodes.

The physical activity serves as a measure of the general health constitution because fatigue and reduced movement are common early SLE symptoms in humans.

The blood cell composition is of great interest as this is a standard measure in human medical care, which requires minimal procedure and delivers multiple valuable measures. These can identify various immunologic imbalances, especially when seen in the correct combination and context.

The focus on T cells was chosen as these cells help B cells to become activated and differentiate. Without this help, autoreactive B cells would not produce and release high-affinity auto-antibodies. We investigated both CD4+ and CD8+ T cells, as both have different roles in SLE. Among these, we divided for the expressions of CD44 and CD62L to see how and when these cells differentiated from naive T cells, to memory or effector T cells.

Central as well as peripheral tolerance is also widely mediated by T cells, especially the regulatory ones. As tolerance is one of the main deciding points of whether autoimmunity will break through or be inhibited, we analysed Tregs in detail. We included the marker helios as a marker of stability. Moreover, we also included CD25 as an activation marker and CD39, and cytotoxic T-lymphocyte antigen 4 (CTLA-4) to investigate their repressive capacities. Kiel-67 (Ki-67) was also included to see how their proliferative behaviour develops during the pre-disease phase. For Tregs, we also investigated the differentiated state by CD44 and CD62L as we did for the CD4+ and CD8+ T cells.

The second hypothesis is that UV light will already affect the skin of lupus-prone NZM2410 mice in the pre-disease phase differently than C57BL/6J mice, causing more inflammation and activating SLE-typical pathways. Additionally, we expect wavelength-dependent differences.

The effect of UV light and blue light is a lifestyle factor that we found especially interesting, as it affects most patients, due to daily sunlight and artificial light exposure in their everyday lives. In SLE patients, it is already known that UV irradiation can cause flares. However, we wanted to see the effects here on SLE-prone mouse skin during the pre-disease phase and not the active disease phase. Even though NZM2410 and SLE123 mice are not known for their skin involvement during active SLE, it is not clear how they react to UV light at all, as the illumination in animal facilities is restricted to the visible range of regular artificial light. We aimed to see if the SLE-prone mouse skin of NZM2410 mice was more sensitive to light exposure than healthy age-matched C57BL/6J

mouse skin. To gain the best insight, this was investigated on the transcriptome level as well as the protein expression during apoptosis and actual skin damage using microscopy.

2 Material and methods

2.1 Animals and ethical approvals

Three mouse lines were used to explore the pre-disease phase of murine lupus. Thereof, NZM2410 and SLE123 mice are spontaneous lupus-prone mouse models, and C57BL/6J mice serve as healthy comparison (**Figure 14**).

2.1.1 C57BL/6J mice

C57BL/6J mice are so-called wild-type mice. It is the most widely used inbred mouse strain, and its genomics, immune system, metabolism, and developmental biology are well-characterised. We considered them as healthy and used them therefore as a comparison to both lupus-prone mouse strains, even though their genetic background is only close to the SLE123, but not the NZM2410 mice. These mice were kindly gifted by the Ehlers laboratory, Nutritional Medicine, University of Lübeck, Lübeck, Germany. The sacrificing and organ harvest of the C57BL/6J mice was ethically approved under the number 5.5_2023-11-22_Hundt.

2.1.2 NZM2410 mice

NZM2410 mice are lupus-prone mice. These mice are bred from New Zealand Black and New Zealand White mice, inheriting the three susceptibility alleles, called *Sle1*, *Sle2*, and *Sle3*. While *Sle1* causes the loss of tolerance towards self-antigens, *Sle2* leads to a decreased activation threshold and thereby to over-activation of B cells, and *Sle3* is responsible for the dysregulation of CD4+ T cells (Morel, Croker, *et al.* 2000). Both sexes of this mouse strain are known to develop early onset (at several months of age) of lupus nephritis (TheJacksonLaboratory 2025c). This mouse strain was used in numerous publications to study murine lupus, especially because of its systemic effects on several organs. Due to its heritage, there is no ideal mouse strain that can be used as a control. This is why we compare them to C57BL/6J mice, and we also worked with SLE123 mice.

The mice were kindly gifted by the Ludwig laboratory, Lübeck Institute of Experimental Dermatology (LIED), University of Lübeck, Lübeck, Germany. The Ludwig laboratory purchased the breeding pairs from Jackson Laboratory. The sacrificing and organ harvest of the NZM2410 mice was ethically approved under 5(22-3/22)_Ludwig.

2.1.3 SLE123 mice

B6;NZM-Sle1NZM2410/Aeg Sle2NZM2410/Aeg Sle3NZM2410/Aeg/LmoJ (here referred to as SLE123) mice are generated by backcrossing NZM2410 mice on C57BL/6J mice. Thereby, the three susceptibility alleles are retained, but these mice are better comparable to C57BL/6J mice as controls. Moreover, these mice are expected to form the bridge for comparison between NZM2410 mice and C57BL/6J mice. According to Jackson Laboratory, the SLE123 mice have a slightly delayed disease onset compared to NZM2410 mice and die before the age of twelve months from kidney failure (TheJacksonLaboratory 2025d). These animals were kindly received from the Manz laboratory, Institute for Systemic Inflammation Research (ISEF), University of Lübeck, Lübeck, Germany. The sacrificing and organ harvest of the SLE123 mice was ethically approved under 39.1(66-8/22)Manz_ZUCHT.

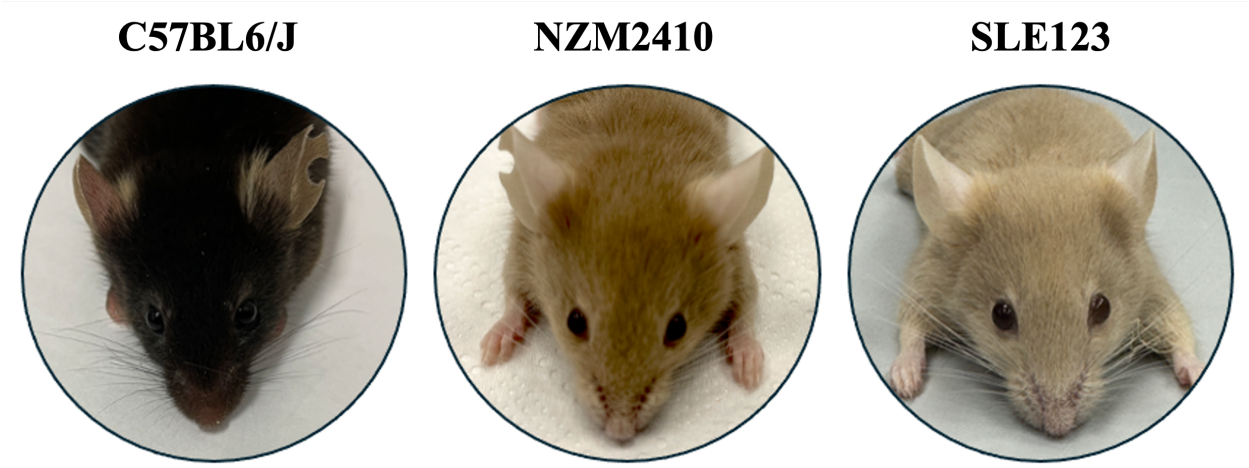


Figure 14: Mouse lines used to investigate the lupus pre-disease phase
The mice chosen for exploring the lupus pre-disease phase are C57BL/6J mice as healthy controls, NZM2410 mice as lupus-prone mice and SLE123 mice as a second lupus-prone line, which is backcrossed on C57BL/6J mice.

2.2 Weight and spleen weight assessment

All mice were weighed and thereafter sacrificed. Next, the spleen was removed and also weighed on a scale (KERN KB, Balingen-Frommern, Germany) with an accuracy of 0.001 g.

2.3 Activity measurement

The activity of mice was measured with self-constructed rodent activity detectors (RADs), following the instructions of B. A. Matikainen-Ankney *et al.* 2019. The detection is based on passive infrared (PIR) sensors placed on the mouse cages' feeding grid (**Figure 15**). The sensors detect warm moving objects in light and dark but cannot measure through glass or plastic, which avoids bias from neighbouring cages. They neither emit light nor sound and are not reachable for the animals, preventing any kind of interference with their regular behaviours in the cages. It detects and counts the times that an animal passes under the sensor, coupled with the time of day. This construction is ideal for measuring individual animals' behaviour but can also count the movements of mouse groups. However, it is not possible to distinguish between the individual animals. B. A. Matikainen-Ankney *et al.* 2019 validated the functionality and reliability by video monitoring.

Figure 15: Rodent activity detector
A rodent activity detector (RAD) is placed on the feeding grid of a mouse cage and can count the movement under the detector, coupling it to the time of day. It does not emit sound or light and is not reachable by animals. Moreover, the RAD is unable to measure through glass or plastic, restricting the measurement to the cage. Picture from B. A. Matikainen-Ankney *et al.* 2019.

2.3.1 Material and construction of activity sensors

The RAD comprises the PIR sensors (MSP430-PIR, Olimex), connected to a microprocessor, which has a micro secure digital (microSD) card logger (M0 AdaLogger, Adafruit, #2796), and an organic light-emitting diode (oLED) screen showing the counts, the time, and the date (Feather-Wing, Adafruit, #2900). A rechargeable 6600 milliampere hours (mAh) battery (Adafruit, #353) allows continuous measurements for more than two weeks (**Figure 16**). The sensor logs the movement counts every minute. The code to read out the data as well as detailed instructions to build the

sensors are available at hackaday.io/project/160742-rad-rodent-activity-detector (Kravitz and B. Matikainen-Ankney 2025).

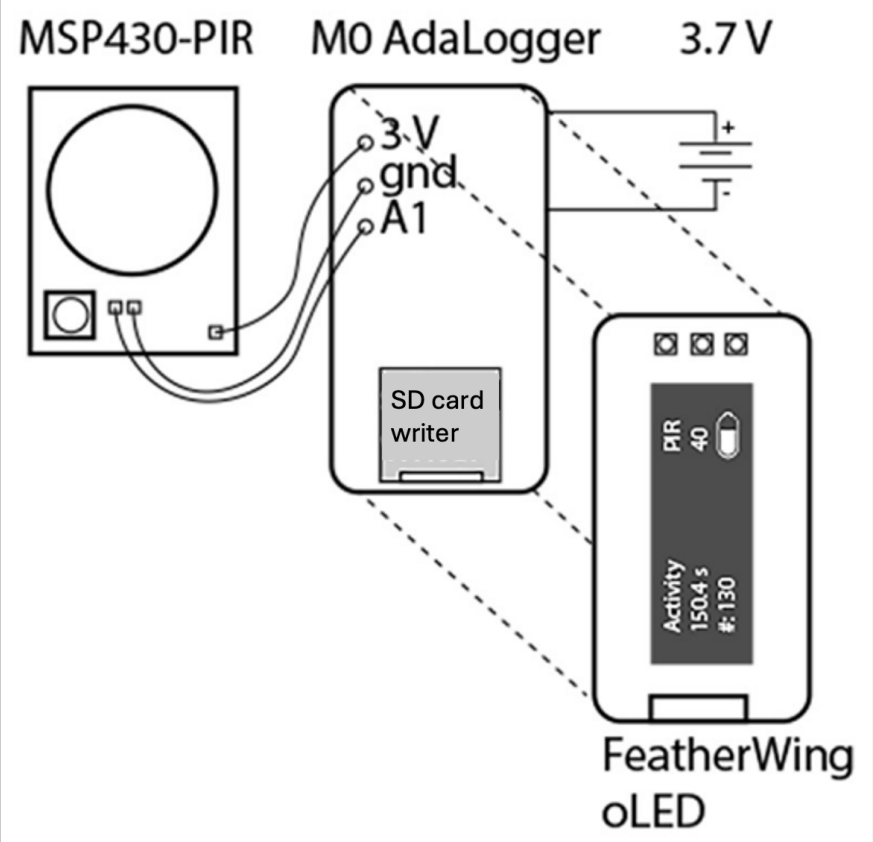


Figure 16: Setup of rodent activity detector
A Rodent activity detector (RAD) is composed of a passive infrared (PIR) sensor (MSP430-PIR), and M0 AdaLogger with a secure digital (SD) card writer, an SD card, and a FeatherWing organic light-emitting diode (oLED). This setup is connected to a battery. Picture adapted from B. A. Matikainen-Ankney *et al.* 2019.

2.3.2 Measurement and analysis of data

Two NZM2410 and two C57BL/6J mice were placed in standard green line cages, and the RAD was positioned on the feeding grid. The cages were placed in areas of the room where they were as undisturbed as possible. The mice had access to standard chow and water *ad libitum*, and the light was on from 7 *ante meridiem* (am) to 7 *post meridiem* (pm) and switched off during the night. Due to legal restrictions, the measurement was limited to 24 hours (h). However, this time was sufficient to control the movement of the mice over one light-dark cycle.

2.4 Haemograms

Haemograms are a standard medical tool to gain an overview of the blood composition. This can indicate the basics of the health and disease state of an organism.

2.4.1 Material for haemogram measurements

Haemograms were generated with the ScilVet ABC Plus+ using 10 µl of ethylenediaminetetraacetic acid (EDTA) mouse blood. The blood was taken from the heart of the mice directly after death with a 26-gauge x 1-inch needle (100 Sterican Braun, #4657683) and a 1 ml syringe (Injekt-F Braun, #9166017V) and collected in EDTA-coated microvettes 500K3E (Sarstedt, #20.1341). The blood must be shaken regularly after sampling and kept at room temperature (RT) until measuring. The maximum period between sampling and measuring with reliable results is 8 h according to company experience.

2.4.2 Use of ScilVet ABC Plus+ device, measurement, and output

After switching on the device, the correct species was chosen, and the blood was taken up by a nozzle and immediately processed and measured using impedance measurement (Coulter principle). Thereby, cells are counted and classified by size and granulation. The cells pass through a small aperture. The passing of each cell causes a temporal change in electrical resistance. By counting the changes in resistance, the cell numbers are determined. The volume, granularity, and size are measured using the same method to identify the cell type (erythrocytes, leukocytes (WBC), or platelets (PLT)). Impedance measurement is also able to further classify the leukocytes into granulocytes, eosinophils, lymphocytes, and monocytes. By measuring the mean corpuscular volume (MCV) and the red blood cell (RBC) count, the device is also able to calculate the haematocrit (HCT), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) (**Table 2**).

The measurement takes approximately 60 seconds per sample and provides the following results:

Table 2: Haemogram data gained by the ScilVet ABC Plus+.

Parameter	Unit
White blood cells (WBC)	10^9/l
Lymphocytes (LYM)	% and 10^9/l
Monocytes (MON)	% and 10^9/l
Granulocytes (GRA)	% and 10^9/l
Eosinophils (EOS)	% and 10^9/l
Red blood cells (RBC)	10^{12}/l
Haemoglobin (HGB)	g/dl
Haematocrit (HCT)	%
Mean corpuscular volume (MCV)	μm^3
Mean corpuscular haemoglobin (MCH)	pg
Mean corpuscular haemoglobin concentration (MCHC)	g/dl
Red cell distribution width (RDW)	%
Platelets (PLT)	10^9/l
Mean platelet volume (MPV)	μm^3

$$\text{HCT} = \frac{\text{RBC count} \times \text{MCV}}{10}$$

$$\text{MCH} = \frac{\text{HGB}}{\text{RBC count}}$$

$$\text{MCHC} = \frac{\text{HGB}}{\text{HCT}}$$

2.5 Flow cytometry

Flow cytometry is a method to distinguish cell types or expression patterns of cellular molecules based on labelling with fluorescent dyes. The dyes are bound to specific antibodies or other molecules that bind to the respective receptor or are incorporated otherwise. For the following experiments, we used antibodies, coupled with fluorescent dyes, which bind to surface- and intranuclear molecules. Flow cytometry makes use of laser light scattering of cells when they are singly flown through a thin nozzle. The scattering is detected from several sides, including forward and side scatters.

Laser light shines on the single cells when they pass the flow chamber, and detectors directly behind the light source measure the scattering of the light. The larger the cell, the larger the scattering; therefore, the cells appear further on the right on the forward scatter (x-axis). Detectors on the side measure the scattering of the light to the sides. This scattering is caused by dense particles in the cells (granules) that do not allow the light to pass through. Therefore, the side scatter provides information about the granularity of cells (y-axis). The more granulation, the more scatters the light to the sides. These basic measurements already allow for distinguishing certain cell types such as lymphocytes, granulocytes, and RBCs (**Figure 17**).

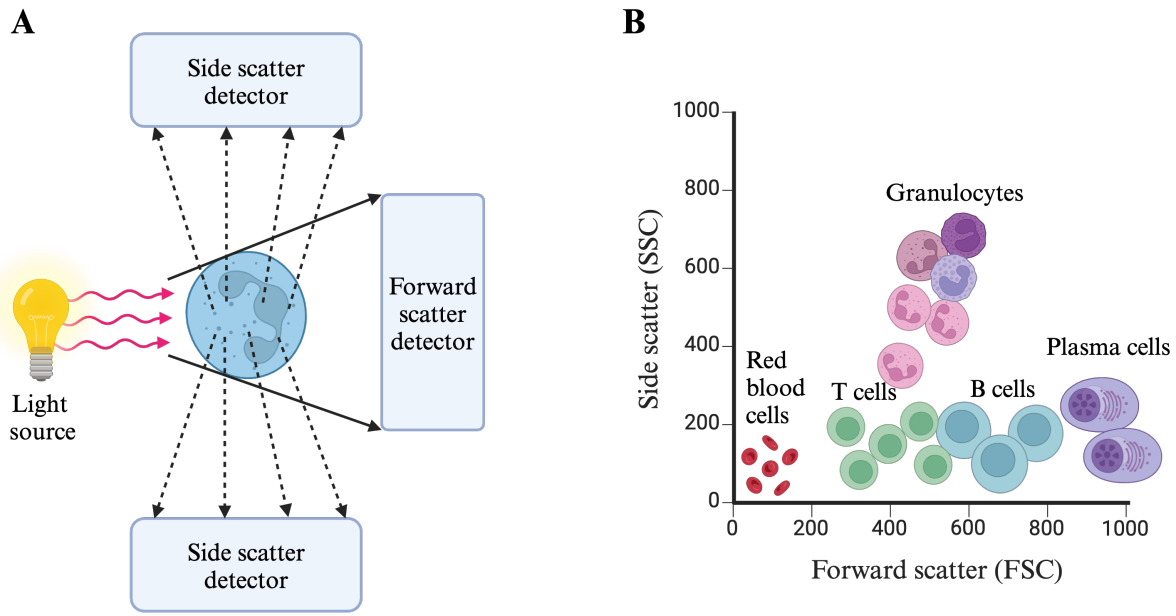


Figure 17: Schematic illustration of flow cytometry
A light source in a flow cytometry device is illuminating single cells. The light is scattered and then detected. The forward scatter detector measures the gap of light created by the cell passing through. This indicates the size of the cell. The side scatter detectors measure light that is scattered and shed to the sides due to granules in the cell, that the light can not pass (**A**). This state of granulation and the size allow basic discrimination of cell types when plotted on an X/Y scheme (**B**). Red blood cells are the smallest cells with the lowest granularity. T cells are larger but low in granularity, while granulocytes are similar in size to T cells but have more granules. B cells and plasma cells are large cell types with low granularity. Illustration created with BioRender.com.

All other discriminations are detected by the fluorescent signals they were labelled with. The fluorophores are excited by laser light and emit light in another wavelength, which then passes filters and is measured by several photodetectors, each measuring different spectra of wavelengths. The configuration of the flow cytometry device determines the number of distinguishable colours. The

device we used was the Beckman Coulter Cytoflex S from the Department of Rheumatology and Clinical Immunology, University Hospital Schleswig-Holstein (UKSH), Lübeck, Germany (**Figure 18**).

Laser		Filter LP/BP	Fluorophore
blue	488 nm	525/40	FITC
		690/50	PerCP/Cy5.5
yellow-green	561 nm	585/42	PE
		610/20	PE-Dazzle594
		690/50	PE-Cy5.5
		780/60	PE-Cy7
red	638 nm	660/10	APC
		712/25	Alexa700
		780/60	APC-Cy7
violet	405 nm	450/45	BV421
		525/40	BV510
		610/20	BV605
		660/10	BV650

Figure 18: Laser configuration of the Cytoflex S
The Beckman Coulter flow cytometer Cytoflex S has four separate lasers and additional filters, to measure a maximum of 13 colours simultaneously.

2.5.1 Staining panel for flow cytometry measurements

The antibodies were chosen in a colour combination with minimal laser light spillover. This describes the problem that the light emissions of several fluorophores can overlap and therefore lead to false results. By choosing fluorophores with separate emission peaks, most spillover is avoided. However, it cannot be fully prevented. Therefore, the device offers a compensation calculation, a mathematical option to further minimise false measurements. In the chosen panel, the emission spectra of APC-Cy7 and PE-Cy7 overlap almost fully, however, they are excited by separate lasers and can therefore be well distinguished (**Figure 19**). To subtract the potential leftover laser light spillover, the chosen panel was compensated in the Cytoflex S using VersaComp Antibody Capture Bead Kit (Beckman Coulter, #B22804). Moreover, markers that are only expressed in a minority of cell types were labelled with a bright colour to ensure sufficient detection.

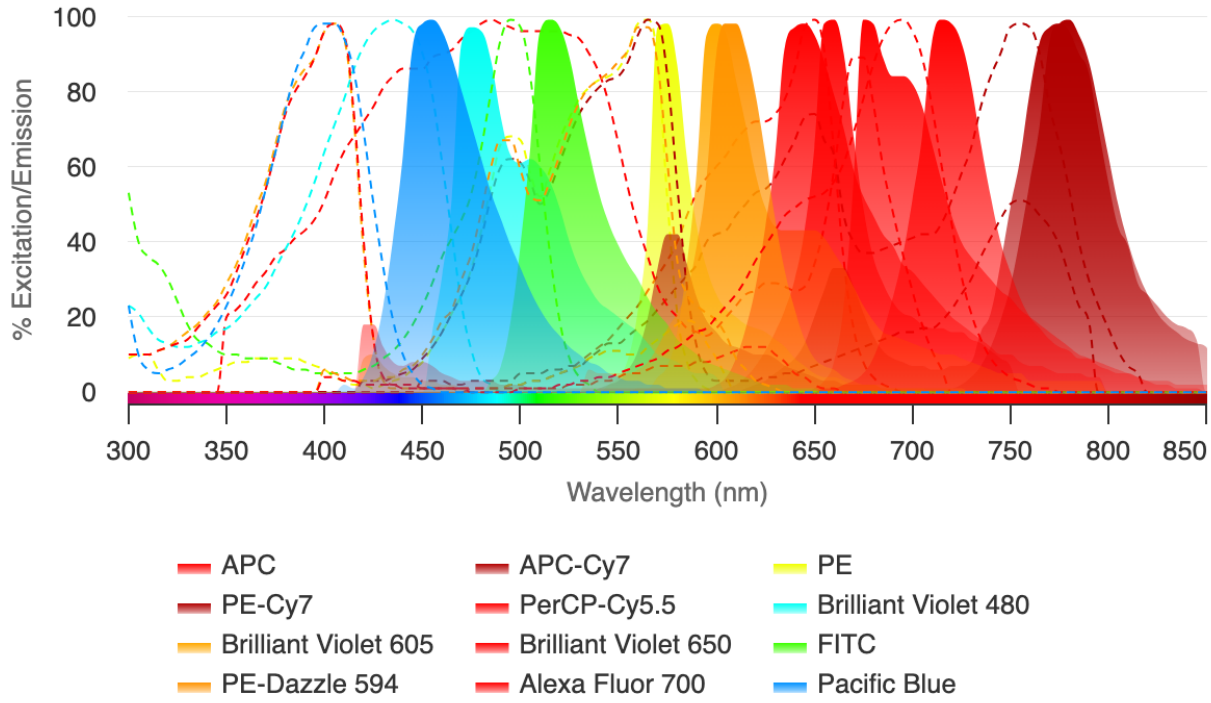


Figure 19: Excitations and emissions of fluorophores in the chosen panel
The dotted lines represent the excitation spectra, while the filled peaks indicate the emission spectra. These signals are detected by the flow cytometry device. The figure was created using the Beckman Coulter Fluorescence Spectrum Analyzer.

2.5.2 Flow cytometry material and sample preparation

Inguinal lymph nodes, spleens, and blood were dissected from sacrificed animals. Single-cell suspensions of splenocytes were gained by gently rubbing the spleens between frosted glass slides until the cells detached from the organ. The cells were filtered through a 70 µm cell strainer (Labsolute, #7696768). Lymph nodes were placed on a membrane of a 70 µm cell strainer and smashed using the plunger of a syringe. Thereafter, the plunger and filter were flushed with 5 ml flow cytometry buffer [phosphate buffered saline (PBS) with 0,5 % bovine serum albumin (BSA) (Roth, #0163.2)]. Cell suspensions and blood were centrifuged at 350 g for 6 minutes (min) at RT. The supernatant was removed, and 50 µl of flow cytometry buffer was added. To block unspecific binding, fragment crystallizable (Fc)-receptors (which can bind to Fc-parts of the antibodies) of cells such as monocytes, macrophages, and dendritic cells were blocked with TrueStain FcX Plus (Biolegend, #156604). To ensure the best distribution of the antibody, samples were vortexed and then incubated at RT for 5 to 10 min. This was followed by the addition of all surface antibodies from

the below-mentioned staining panel (**Table 3**). To access the cell nucleus to stain for FoxP3, Kiel-67 (Ki-67), and helios, the cells were fixed with the True Nuclear buffer kit (Biolegend, #424401) for 1 h, followed by centrifugation at 350 g for 6 min at RT. The cells were permeabilised with the perm buffer from the True Nuclear buffer kit three times for 6 min of centrifugation at 350 g and RT. The intranuclear binding antibodies were mixed in permeabilisation buffer and then pipetted on the individual samples (**Table 4**). This was incubated for 30 min at RT in the dark. To wash off excess antibodies, the samples were topped up with 1 ml FACS buffer and spun down at 350 g, for 6 min at RT. The samples were then resuspended in 300 µl FACS buffer and acquired in the Cytoflex S (Beckman Coulter).

To enable correct gating in the downstream analysis, fluorescence minus one (FMO) controls were prepared. For each organ, samples were taken and processed as previously described but for each control, one respective antibody was left out. This allows the correct determination of positive and negative populations in further data processing.

Table 3: Flow cytometry surface marker panel

Marker	Fluorophore	Clone	Per sample [µl]	Manufacturer and ordering number	Function
CD44	PerCP-Cy5.5	IM7	2	Biolegend #103032	T cell differentiation marker
CD4	BV650	GK1.5	2	Biolegend #100469	T helper cell marker
CD184 (CXCR4)	BV480	2B11	2	BD Biosciences #746781	Promigratory molecule
CX3CR1	PE-Dazzle594	SA011F11	2	Biolegend #149014	Migration marker
CD25	APC	3C7	2	Biolegend #101910	Part of the IL-2 receptor, functionality of Tregs
CD8	AF700	53-6.7	2	Biolegend #100730	Cytotoxic T cell marker
CD62L	APC-Cy7	MEL-14	2	Biolegend #104428	T cell Differentiation marker
CD39	PE-Cy7	Duha59	2	Biolegend #143806	Immuno-suppressive capacity
CTLA-4 (CD152)	BV605	UC10-4B9	2	Biolegend #106323	Immuno-suppressive capacity

Table 4: Flow cytometry intranuclear marker panel

Marker	Fluorophore	Clone	Per sample [µl]	Manufacturer and ordering number	Function
FoxP3	PE	MF14	2	Biolegend #126404	Transcription factor for Tregs
Helios	Pacific Blue	22F6	2	Biolegend #137220	Stability of Tregs
Ki-67	FITC	11F6	2	Biolegend #151212	Proliferation marker

2.6 Ultraviolet light irradiation of murine skin

These experiments were performed in collaboration with Zuzana Penxova from the Ears, Nose and Throat (ENT) clinic of the UKSH, Lübeck, and with the help of the internship student Steffen Pichlo.

As ultraviolet (UV) light is a known trigger for autoimmunity, we aimed to explore the influence of several UV wavelengths on the murine skin of C57BL/6J and lupus-prone NZM2410 mice during the pre-disease phase. Therefore, two separate experiments were conducted. In the first experiment, the influence of UVB (310 nm), UVC (233 nm) and blue light (405 nm) was investigated, whereas in the second experiment, the effects of UVA (375 nm), UVA and UVB in combination, and blue light (445 nm) were of interest (**Figure 20**).

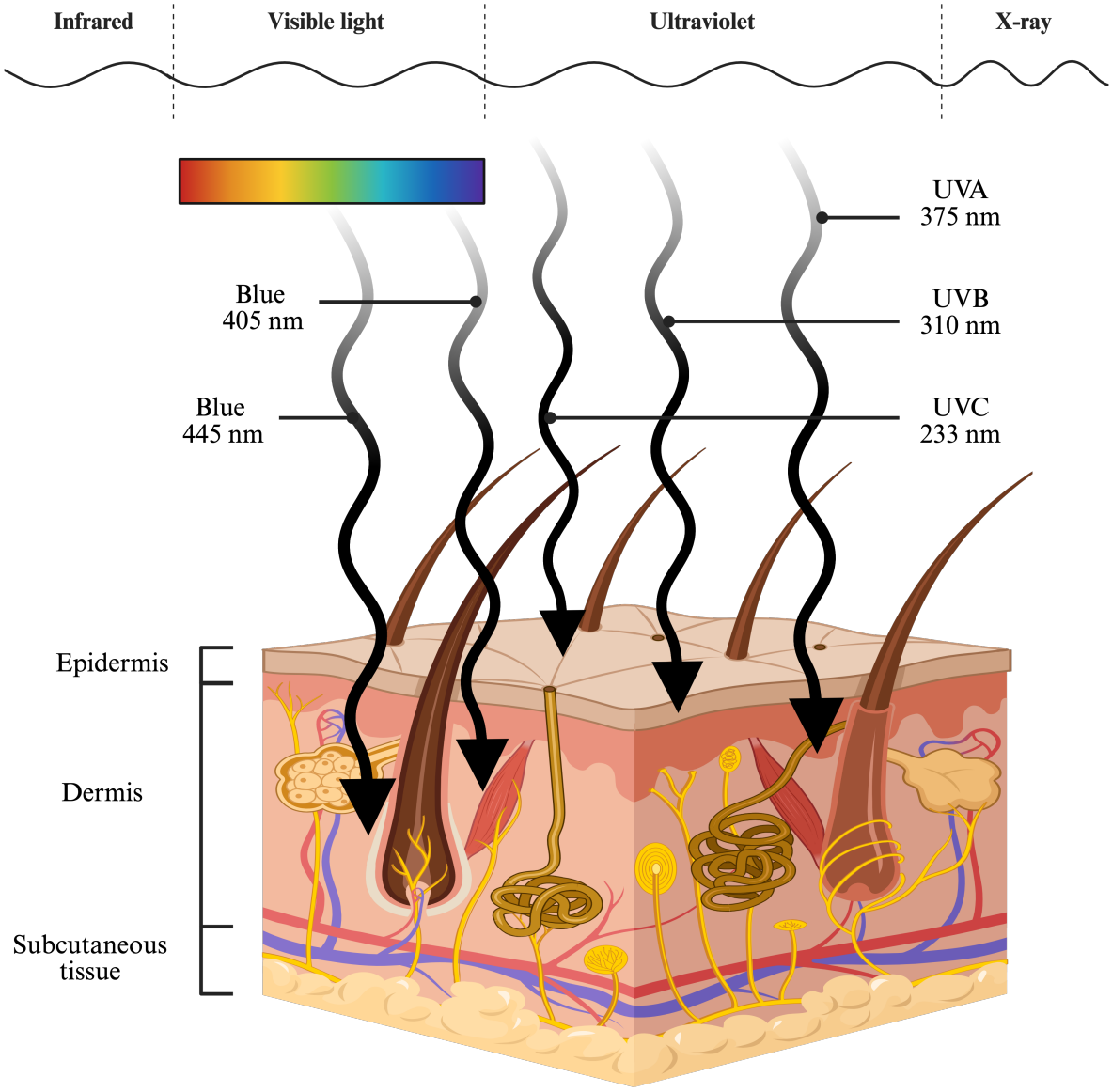


Figure 20: Penetration of light in skin
Different wavelengths of light have different penetration depths in the skin and are therefore causing different biological effects. Ultraviolet (UV) light has a shorter wavelength than visible light. In the experiments, UVA (375 nm), UVB (310 nm), far-UVC (233 nm), and blue light (405 nm, as well as 445 nm) were used to irradiate murine skin. The illustration was created using BioRender.com.

2.6.1 Material for ultraviolet light experiments

For these experiments, NZM2410 mice at the age of 16-18 weeks were chosen as haemogram data indicated already some abnormal changes at this age. However, this age is still before the occurrence

of kidney inflammation and therefore provides a robust pre-disease age. The C57BL/6J mice were age- and sex-matched as well as possible (**Tables 5** and **6**).

Table 5: Experimental mice of ultraviolet light experiment 1

Mouse number	Genotype	Sex	Age in weeks rounded
1	NZM2410	m	16
2	NZM2410	m	16
3	NZM2410	m	16
4	NZM2410	f	16
5	NZM2410	f	16
6	NZM2410	f	16
7	C57BL/6J	m	18
8	C57BL/6J	m	18
9	C57BL/6J	m	18
10	C57BL/6J	f	14
11	C57BL/6J	f	10
12	C57BL/6J	f	10

Table 6: Experimental mice of ultraviolet light experiment 2

Mouse number	Genotype	Sex	Age in weeks rounded
13	NZM2410	f	18
14	NZM2410	f	18
15	NZM2410	f	18
16	NZM2410	m	18
17	NZM2410	m	18
18	NZM2410	m	18
19	C57BL/6J	m	16
20	C57BL/6J	f	16
21	C57BL/6J	f	16
22	C57BL/6J	f	17
23	C57BL/6J	m	17
24	C57BL/6J	m	17
25	C57BL/6J	m	17

2.6.2 Light sources and calculation of intensities

In this table are the setups of the UV light sources described. Most of them were constructed by the Medical Laser Centre Lübeck (MLL) and kindly provided for these experiments (**Table 7**).

Table 7: Light sources, settings, and light intensities for experiments

MLL = Medical Laser Center Lübeck; mJ/cm^2 = millijoule per square centimetre; sec = seconds

Wavelength	**Device**	**Company /setup**	**Dose (mJ/cm^2)**	**Duration (sec)**
UVA 375 nm	Diode laser and optical lens system	Prototype @A.R.C. Laser GmbH, Nürnberg; optical lens system @MLL	500	14
UVB 310 nm	Prototype with light emitting diodes (LEDs)	MLL	500	60
UVC 233 nm	Multi λ Flood Lamp	Prototype @Ferdinand-Braun- Institute GmbH, Leibnitz-Intitut für Höchstfrequenz -technik, Berlin (LED-array)	40	87
Blue 405 nm	405 nm laser module	Robot arm (Rotrics)	6,839	124
Blue 445 nm	Diode laser and optical lens system	Fox IV 445 nm and Wolf 445 nm (A.R.C. Laser GmbH Nürnberg) with optical lens system from MLL	20,000	33

We chose low doses for the irradiation of the skin as we did not want to primarily cause tissue damage, but instead we wanted to investigate wavelength-specific effects on healthy murine skin from C57BL/6J mice, as well as on lupus-prone skin from NZM2410 mice. For UVB 310 nm,

we chose the dose used by Skopelja-Gardner *et al.* 2021, who described it as the lowest dose with effects on murine skin. For UVA, we chose the same dose as for UVB. The same accounts for the combination of UVA and UVB irradiation in equal shares. We chose the 50:50 distribution; however, this changes in natural sunlight with seasons (Nishimura *et al.* 2021). For the UVC light, we used a dose that was able to cause a 5 log reduction of Methicillin-resistant *Staphylococcus aureus* (MRSA) (Zwicker *et al.* 2022). For the blue light irradiations, we chose for blue light 405 nm a lifestyle calculation resulting in a dose that is (roughly) equivalent to 8 h use of a PC-screen in a room with artificial room light, 1.5 h of smartphone use, and 1 h of daylight (Suitthimeathegorn *et al.* 2022). For blue light 445 nm, we chose a dose able to treat acne vulgaris (Nakayama *et al.* 2023), inhibit TGF-β signalling (Ge *et al.* 2023), and adapt inflammatory responses and tissue formation (Magni *et al.* 2022).

2.6.3 Ultraviolet light experimental procedure

The NZM2410 and C57BL/6J mice were sacrificed, weighed, and shaved with a veterinarian's electric shaver to avoid skin lesions from shaving with a blade. Next, the skin was taken off the mouse, and 6 millimetre (mm) biopsy punches were taken (Pfm medical, #48801). At this time point the first control samples (time 0 controls) were already embedded in cryomatrix (Epredia, #6769006), or fixed in embedding cassettes (Leica Biosystems, #39LL-500-3) in histofix (Roth Industries GmbH & Co. KG, #P087.3), or stored in RNA protect (Qiagen, #1017980). These other punches were placed on transwell inserts (Sarstedt, #83.3930.041) in 6-well plates (Sarstedt, #83.3290500) in a medium composed of William's E medium (Bio&SELL, #BS.F1115) with 1 % L-glutamine (BIOCHROM, #K0283) and 1 % penicillin-streptomycin (Gibco, #15140122). This was following the skin organ culture method published by Burmester *et al.* 2019. However, we left out hydrocortisone and insulin as these substances would have altered the skin's reactions to UV light that we wanted to measure. The skin samples were incubated for 24 h at 37°C with 5 % carbon dioxide (CO_2). Next, the 24 h control samples were harvested in the same way as the time 0 controls, and additionally, medium samples were collected. The other samples were then irradiated as described in **Table 7**. 30 h control samples, which are the direct controls for the irradiated samples, were taken out of the incubator for the same time as the irradiated samples. Thereby, we aimed to reduce any bias of temperature changes in the samples. All samples were after irradiation or control time further incubated for 6 h to allow the development of the biological changes caused by the irradiation. This time was also used in the work of Skopelja-Gardner *et al.* 2021 to be able to see significant transcriptomic effects. Thereafter, all samples were harvested as described above. The samples in RNA-protect were stored overnight at 4°C and thereafter at

-20°C until further processing. The cryomatrix-embedded samples were stored at -20°C until they were sectioned for immunofluorescent stainings in 6 µm sections by Steffen Pichlo with technical assistance from Sylva Dürkop using the cryostat (Leica CM3050S). The histofix-stored samples were fixed at least overnight at RT until they were paraffin-embedded and cut into 4 µm sections using the microtome (Reichert Jung model 1140). These samples were thereafter haematoxylin and eosin (H&E) stained. The medium was stored at 4°C until the cytotoxicity was measured by a lactate dehydrogenase (LDH) assay (**Figure 21**).

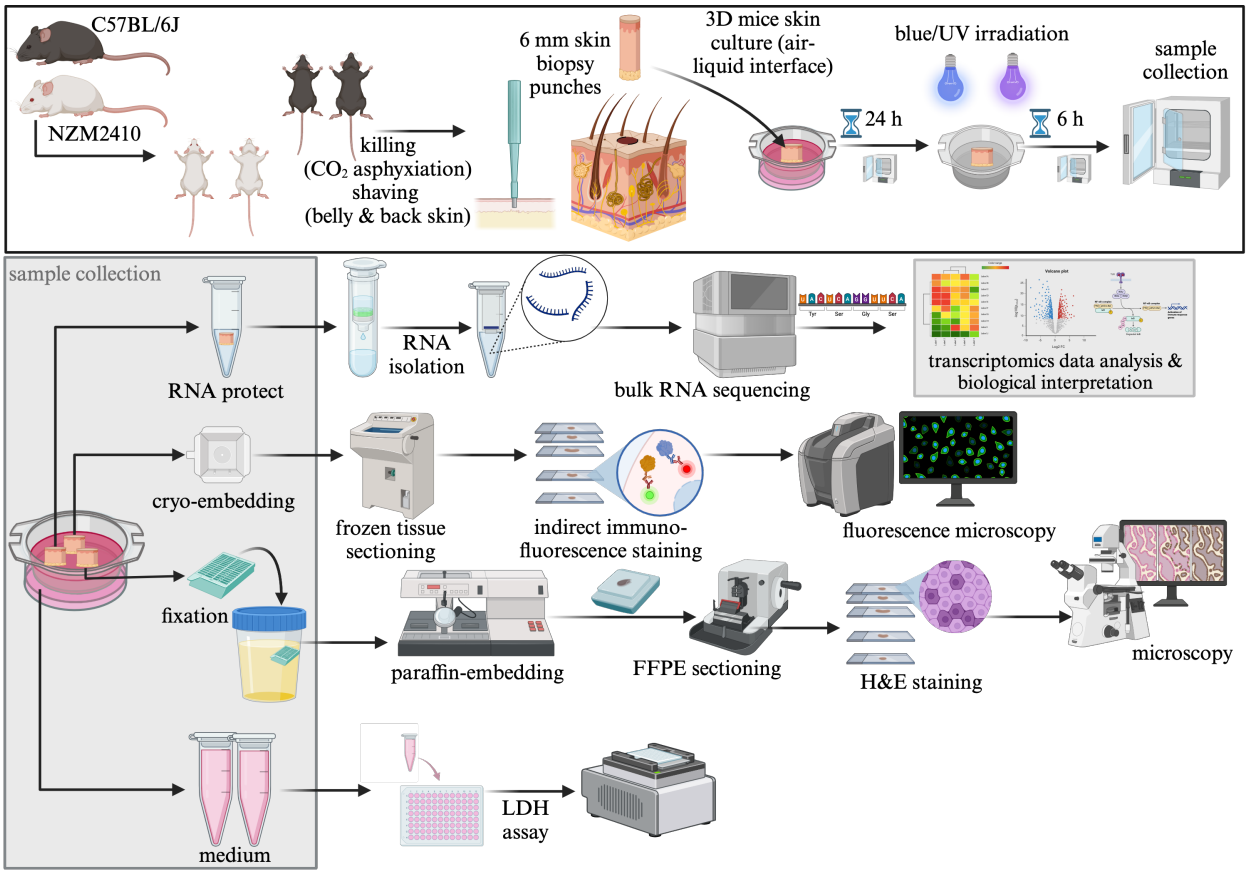


Figure 21: Experimental design of ultraviolet light experiments
C57BL/6J mice and NZM2410 mice were sacrificed and shaved. Thereafter, the skin was removed, and 6 mm biopsy punches were made. The punches were placed on transwell inserts and incubated for 24 hours (h) at 37 degrees Celsius (°C) and 5 % carbon dioxide (CO_2). This was followed by the respective irradiation. The controls were taken out of the incubator for the same time as the treated samples. Next, the samples were incubated for another 6 h. The skin punches were then harvested and either stored in ribonucleic acid (RNA) protect, cryomatrix or histofix. The medium was collected to investigate the cytotoxicity with a lactate dehydrogenase (LDH) assay. RNA was isolated for RNA bulk sequencing, cryomatrix-embedded tissues were cut for immunofluorescence staining and histofix-fixated samples were paraffin-embedded and sectioned (FFPE sectioning) for haematoxylin and eosin (H&E) staining. The picture was created with BioRender.com and adapted from Zuzana Penxova, Ear, Nose, and Throat clinic, University Hospital Schleswig-Holstein.

2.7 Assessment of cell cytotoxicity using lactate dehydrogenase assay

The lactate dehydrogenase (LDH) assay was used to quantify cell damage or cell death by measuring the release of LDH into the culture medium. LDH is released from damaged or lysed cells, catalysing the conversion of lactate to pyruvate. During this process, nicotinamide adenine dinucleotide (NAD+) is reduced to nicotinamide adenine dinucleotide (NADH/ H+). The free proton is then

used to convert iodotetrazolium via diaphorase into formazan (**Figure 22**).

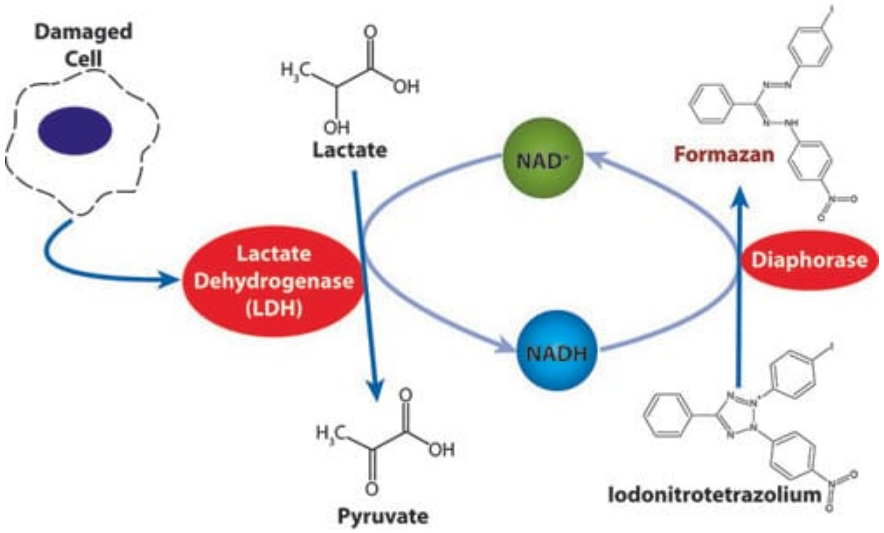


Figure 22: Chemical reactions in a lactate dehydrogenase assay
Damaged cells release the enzyme lactate dehydrogenase (LDH). This converts lactate to pyruvate. This process converts nicotinamide adenine dinucleotide (NAD+) to NADH, which is used by the assay's iodonitrotetrazolium to form formazan and thereby provoke a substrate-dependent colour switch. The picture was taken from G-Bioscience (Man 2025).

We collected the medium after the above-described cultivation of the murine skin samples of the UV experiments to get insight into the potential effects on the samples of the cultivation conditions and the treatments. Control samples were harvested before cultivation (time 0), after 24 h of the un-irradiated controls, and after 30 h of the un-irradiated controls and each treatment condition. The cytotoxicity detection kit (Roche, #11644793001, version 10) contains a catalyst, a dye solution, and a stop solution. The assay components were thawed at RT before use. For the blank, 200 µl of culture medium was pipetted into three wells. 50 µl of the medium samples of the UV experiments were pipetted in three technical replicates on the plate. The reaction solution was prepared by mixing the catalyst with the dye solution in a ratio of 1:46 and was protected from light. 100 µl of

the reaction solution was added to the medium samples and incubated in the dark for 30 min at RT. Thereafter, the reaction was stopped with a stop solution, and the optical density was measured in a plate reader (Glomax Discovery, Promega).

2.8 Investigation of the murine skin transcriptome upon ultraviolet light treatment

The investigation of the murine skin transcriptome after irradiation with different UV light and blue light wavelengths was performed to find out how light influences signalling pathways in the skin and how it therefore might influence the development and progression of lupus.

2.8.1 Ribonucleic acid isolation from murine skin samples

The murine skin samples were stored in RNA protect (Qiagen, #1017980) at 4°C overnight and then until further processing at -20°C. Before starting any work with RNA, all equipment and gloves were cleaned with RNAse Away (MolecularBioProducts, #7000) to avoid unwanted enzymatic digestion of the RNA. Additionally, only quiet times in the lab were used to ensure that frequent door opening and closing do not cause contaminating airflows. The kit used for RNA extraction was innuPREP RNA Mini Kit 2.0 (Analytik Jena, #845-KS-2040050). To access the RNA, the tissue needed to be disrupted. Therefore, the samples were cut in half and transferred into lysis buffer of the innuPREP RNA Mini Kit 2.0 in innuSPEED lysis tubes P (Analytik Jena, #845-CS-1020050), and then disrupted with a tissue disruptor (OMNI INTERNATIONAL INNC., Bead Ruptor 12) for 30 seconds at medium speed. The skin's RNA was then further processed according to the manufacturer's instructions.

2.8.2 Photometric determination of ribonucleic acid concentration

To assess the quantity and purity of the RNA samples, 1 µl was pipetted on a nanodrop [NanoDrop One (VWR)] and measured. The quantity is provided in ng per µl, while two ratios indicate the purity. The first ratio is the 260/280 ratio: While nucleic acids have an absorbance peak at 260nm, proteins such as tryptophan and tyrosine residues from the RNA extraction have absorbance peaks around 280 nm. For pure RNA, the value should be around 2.0. Lower values might indicate protein contamination, and higher values might indicate phenol contamination. The other ratio is the 260/230 ratio, which is used to detect contaminations with organic compounds or salts such as phenol, carbohydrates, EDTA, or guanidine. For pure RNA, the value should be between 2.0 and 2.2. As clean RNA is required for further processing, whenever contaminations were measured,

the RNA was additionally purified with the Kit RNA Clean & Concentrator -25 (Zymo Research, #ZRC202056) following the manufacturer's instructions.

2.8.3 Messenger ribonucleic acid bulk sequencing

RNA sequencing was performed to understand molecular functionality by exploring gene expression profiles, transcription factor regulations, as well as pathway analysis to identify the effects of UV light treatments on the murine skin of NZM2410 and C57BL/6J mice. RNA exists in different varieties with different functions. The major separation, which further also decides the kind of sequencing method, is the separation between coding and non-coding RNA. Coding RNA (messenger RNA, mRNA) codes for proteins. Non-coding RNA is not translated into proteins but has other structural and related roles in cellular processes. mRNA forms only about 3-7 % of the total RNA mass (Palazzo and E. Lee 2015). However, we decided to sequence the mRNA as it codes for proteins important for a cell's function and composition.

The RNA was sent on dry ice to the company Novogene, which sequenced the samples for us, using the next-generation sequencing Illumina NovaSeq platforms. Upon the arrival of the extracted RNA, Novogene first performed a sample quality check using 1 % agarose gel electrophoresis, nanodrop to check the quantity and purity, and an Agilent2100 Bioanalyzer to control the RNA quality by measuring the RNA integrating number (RIN). Next, the polyA enrichment library was constructed, resulting in complementary deoxyribonucleic acid (cDNA) with a length of 250-300 base pairs. This was followed by the paired-end 150 base pairs sequencing method (Novogene 2025).

2.9 Kidney collection to investigate nephritis

As nephritis is one of the hallmarks of active lupus disease, the kidneys of lupus-prone mice and C57BL/6J mice were collected after sacrificing and stored in histofix. Thereafter, the samples were embedded in paraffin and 4 µm microtome sections were prepared by the students Steffen Pichlo and Leo Li. To investigate the health state of the kidneys and the glomeruli. Periodic acid-Schiff (PAS) stainings were done as described below.

2.10 Histology

Histologic examinations of murine skin sections were used to identify sunburned cells and the existence and intensity of apoptosis. The murine kidneys were investigated for the development of glomerulonephritis, examined on PAS-stained sections.

2.10.1 Paraffin sectioning

Paraffin sections of the skin were specifically used to stain thereafter with H&E. Paraffin sections of the kidneys were specifically used to stain thereafter with PAS. To do so, the samples were collected in cassettes and stored and fixed in histofix until further processing, but at least overnight. This process cross-links proteins. Thereafter, the samples were dehydrated by an ascending ethanol row and xylene baths and embedded in paraffin. After pre-cooling the paraffin blocks, the samples were trimmed in 20 µm steps with a Reichert Jung Microtome (model 1140) until the 6 mm biopsy punches were cut at full length (this was regularly controlled by light microscopy), thereafter samples were cut in 4 µm sections and transferred to a 37°C water bath. After the samples were stretched out, they were collected on Superfrost Plus Adhesion slides (Epredia, #J1800AMNZ). These sections were dried overnight at 37 °C and then handed to the routine laboratory (Clinic of Allergology and Venerology, UKSH, Lübeck, Germany), which did the H&E and periodic acid-Schiff (PAS) stainings for us.

The H&E staining consists of haematoxylin (H), an alkaline reagent that stains acidic structures such as nuclei (rich in DNA and RNA), ribosomes (rich in RNA), the endoplasmic reticulum, and chromatin during cell division in blue/purple, and eosin (E), which does the opposite. It is an acidic dye that binds to alkaline structures, such as proteins, in pink. Eosin helps to identify the cytoplasmic components, muscle fibres, connective tissue fibres, and RBCs.

For the H&E staining, the sections were first incubated for 20 min at 80°C to get rid of excessive paraffin. Thereafter the samples were transferred to an Autostrainer XL (Leica) to be deparaffinised using a xylene bath for 20 min and then rehydration by quick bathes in a descending alcohol row (2x xylene, 2x 100 % ethanol, 2x 96 % ethanol, 2x 70 % ethanol, aqua dest.). Next, the robot continued with bathing the slides in haematoxylin for 5 min and a washing step in water to remove excess haematoxylin. Unspecific background staining was removed with a bath in acetic acid and ethanol. Thereafter, a washing step with water followed and a bath in ammonia water to blue the haematoxylin. After another washing step, the slices were counter-stained with eosin for 30 to 90 sec. To dehydrate the samples again, washing in an ascending alcohol row (96 % and twice 100 %) was used. Lastly, the samples were washed in xylene to render them transparent. A Leica CV 5030 automat sealed the object slides.

Periodic acid-Schiff (PAS) staining can detect carbohydrates and carbohydrate-rich structures in tissues. The periodic acid cleaves the carbon-carbon bonds between two adjacent hydroxyl groups of carbohydrate molecules. This results in aldehyde groups, which react with Schiff's reagent and turn pink. PAS staining can highlight the thickening of the glomerular basement membrane. This allows us to identify nephritis. This staining was performed by the routine laboratory.

To do so, the slides were deparaffinised and rehydrated as described above and incubated for 8 min with 0,5 % periodic acid. This was followed by a washing step in water and a 15 min incubation in Schiff's reagent. Next, the slides were washed three times for 2 min each in sodium bisulfite solution. Another washing step for 10 min in water followed and thereafter an incubation with haematoxylin for 1 min. Another 10 min washing step in water, as well as an ascending alcohol row for dehydration, followed. The slides were washed with xylene before embedding.

2.10.2 Cryosectioning

Cryosectioning is not only faster than paraffin sectioning but is also able to preserve delicate features such as enzymes, lipids, and antigens. The samples were placed in a Cryomatrix medium (Epredia, #6769006) and frozen. The freezing preserves molecular integrity without the need for fixation. For cutting, the cryostat's chamber was cooled to -22°C and the object table to -20°C. The samples were fixed with Cryomatrix on the object table. Next, the samples were trimmed in 20 µm steps until the 6 mm skin punches were in full-length cut (regularly checked by a light microscope). Then, slides with a thickness of 6 µm were collected.

Cleaved caspase-3 staining detects programmed cell death, also called apoptosis. Staining for cleaved caspase-3 allowed us to identify the intensity of cell death in a tissue. Cleaved caspase-3 is cleaving structural proteins such as actin and nuclear lamins. It also degrades DNA repair enzymes and causes DNA fragmentation by activating caspase-activated DNase. Caspase-3 is activated (or cleaved) in two main pathways. In the intrinsic pathway, the mitochondria release cytochrome c upon cell stress, activating caspase-9, which then cleaves caspase-3. Extrinsic signals can also trigger this cascade by binding death ligands such as fatty acid synthase ligand (FasL) or tumour necrosis factor (TNF) to surface receptors. This activates caspase-8, which then cleaves caspase-3. This two-day staining protocol started with the preparation of the TritonX100 washing buffer by adding 0,3 % TritonX100 (Roth, #3051) to PBS (pH 7.2). Next, the blocking buffer was prepared by adding 5 % normal goat serum (DAKO, #X0907) to TritonX100 washing buffer. Two slices of the same samples were placed on each slide, so one could be fully stained and one could serve as a negative control. For the negative control, the primary antibody is left out. This enabled us to see if the blocking antibody and the secondary antibody generate unspecific background staining. The staining began with the drying of the slides. Therefore, the slides were taken out of the -20°C freezer and placed in RT for 10 min. For the following 10 min, the slides were fixed in acetone at -20°C. Thereafter, three washing steps of 5 min at RT in PBS followed. To avoid excessive use of antibodies, the samples were encircled with a fat pen (DAKO, #S2002). Then, 50 µl of blocking solution was added to the fat pen circles. The samples were then incubated for 1 h at RT. Thereafter,

the blocking solution was removed, and the primary antibody (rabbit anti-mouse cleaved caspase-3 (Invitrogen, #700182) was added in a 1:400 dilution in the TritonX100 washing buffer. This was distributed (50 µl per sample) on the slides. For the negative controls, the primary antibody was left out. This was then incubated overnight at 4°C in the dark. On the second day, the slides were washed three times with PBS for 5 min at RT before they were incubated for 1h at RT with the secondary antibody (Alexa Flour 488 goat anti-rabbit, Invitrogen, #A-11070) in a dilution of 1:800 in TritonX100 washing buffer with 2 % normal goat serum. Three washing steps with PBS at RT for 5 min were performed before the slides were embedded with 4',6-diamidino-2-phenylindole (DAPI) Fluoromount-G (Southern Biotech, #0100-20). The DAPI Fluoromount stains the nuclei of each cell so the staining of the cleaved caspase-3 can be located to the individual cells. The slides were stored in folders at -20°C until the microscopic pictures were taken.

2.11 Statistical data analysis

The statistical analysis was done separately for each experiment. The overall goal is to explore the lupus pre-disease phase in as many aspects as possible, and robust yet careful statistics enabled us to do so. We expected high variations for all measured values because the animals developed the disease due to genetic modifications, and the beginning of the symptoms or measurable changes are individual. To accommodate this, we did not remove outliers but instead also used the variance itself as a measure.

All data was first tested for normal distribution by a Shapiro-Wilk test, which is suitable for small sample sizes. The normality was tested not only for each week and each genotype separately but also for each measured value separately. The data was not normally distributed in all weeks for all genotypes. Therefore, we used non-parametric Mann-Whitney tests, followed by a correction for multiple comparisons using a false discovery rate (FDR) of 1 % (two stage step-up method of Benjamini, Kriger, and Yekutieli (Benjamini and Yekutieli 2005) to compare weeks and genotypes. The p-value describes the probability that the null-hypothesis is correct, the q-value describes the FDR, thus q-values of less than 0.01 are criteria to accept p-values < 0.05 as significant results. Due to limited animal numbers, not all ages could be compared as there is for example only one animal of SLE123 at the age of 15 weeks. Significant discoveries are indicated with an asterisk in the respective colours (red for the comparison of NZM2410 mice with C57BL/6J mice and turquoise for the comparison of SLE123 mice with C57BL/6J mice). Only one asterisk is shown, no matter if the p-values are 0.05, 0.001, or less. The standard error of the mean (SEM) is shown as shadowed bands over time.

2.11.1 Programs

Raw data was collected in Excel spreadsheets. All mice received consecutive numbering. Statistical testing was performed in GraphPad Prism 10 and Jamovi, an open-source statistical program.

2.11.2 Analysis of haemogram data

The haemogram data was collected in an Excel table. After finishing data collection, the individual animals were ordered according to their age and sex. Next, the data were transferred to GraphPad Prism 10, plotted, and statistically analysed. Each parameter of the haemogram measurement was tested for normality before choosing the accurate statistical test.

2.11.3 Analysis of flow cytometry data

The flow cytometry data was acquired in a Cytoflex S (Beckman Coulter) and saved as flow cytometry standard (FCS) files. These were analysed with KALUZA 2.1.1. (Beckman Coulter). For the gating, first, FSC and SSC were chosen as axes, and the lymphocyte gate was set. Thereafter, doublets were excluded in an FSC area/ FSC height gate, as single cells should correlate in their height and size. If the measured events have a larger size but similar height to the single cells, these cells are sticking together, forming doublets. Next, CD4 and CD8 were chosen to determine the helper and cytotoxic T cells, respectively. Following the CD4+ gate, these cells were separated for CD44 and CD62L. These markers are used to differentiate naive cells (CD44-, CD62L+) from memory (CD44+, CD62L+) and effector cells (CD44+, CD62L-). Tregs are classified by the co-expression of CD4, CD25, and FoxP3. Thereafter, the Tregs were gated for their expression of helios, Ki-67, and Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4). Moreover, Tregs were also separated for their expressions of CD44 and CD62L, as the T helper and cytotoxic T cells (**Figure 23**). For all T cell subtypes, the median fluorescence intensities (MFIs) of C-X-C chemokine receptor type 4 (CXCR4) and C-X3-C chemokine receptor 1 (CX3CR1) were measured. A gating scheme fitting all genotypes could be created and applied to all samples of all experiments. Only minor adjustments were made where needed. Frequently included FMO stainings allowed for controlling for the correct gating regularly. Even though the gating strategy could be used for all genotypes, the schemes were separately improved and adapted for each organ (blood, spleen, and lymph node).

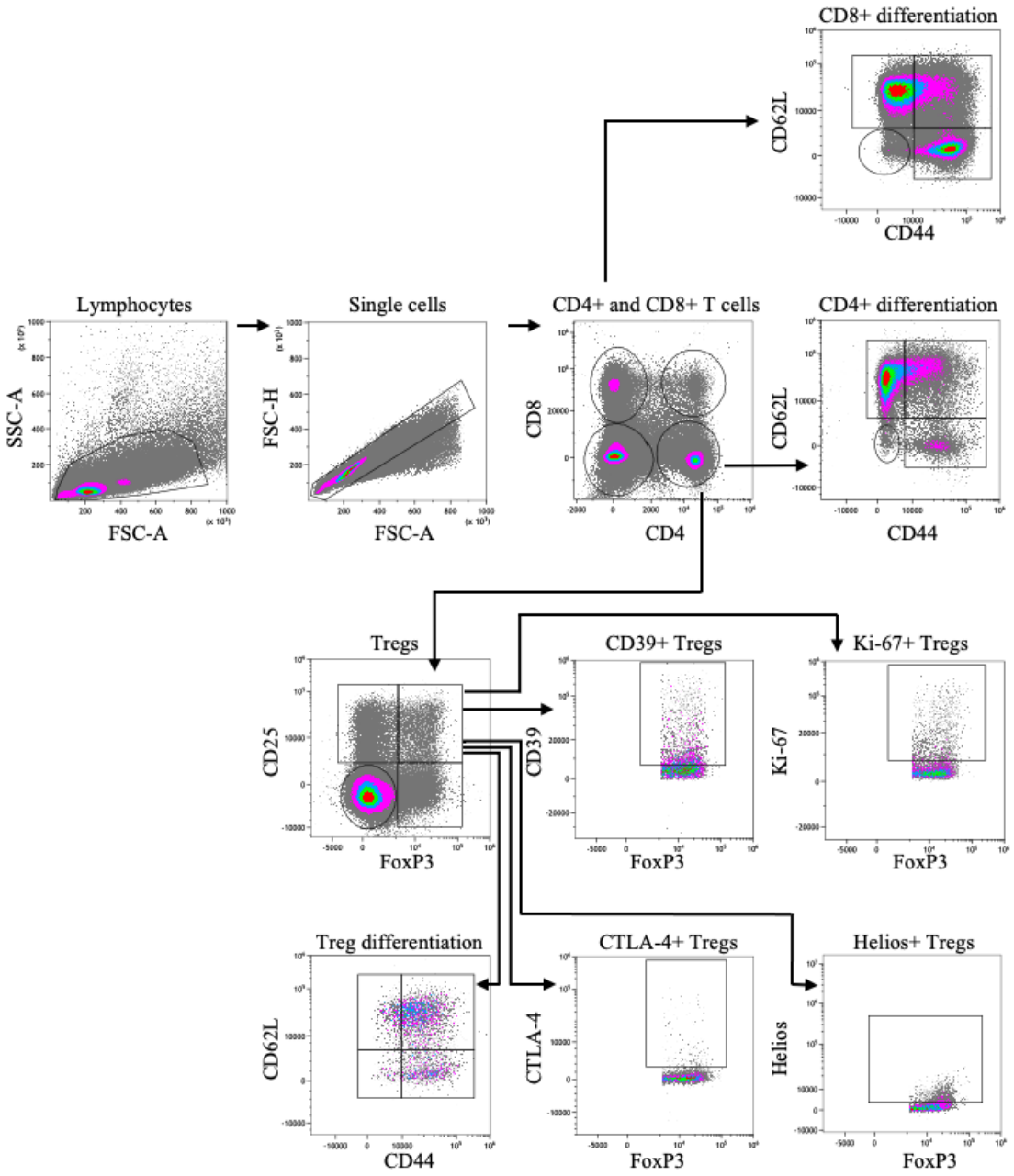


Figure 23: Gating strategy to analyse flow cytometry data
To analyse the flow cytometry data, the software KALUZA 2.1.1 (Beckman Coulter) was used. First, lymphocytes were gated using a forward scatter area (FSC-A) and a side scatter area (SSC-A) setting. Next, doublets were excluded by gating for the FSC-A and FSC-Height (FSC-H). Thereafter, T cells were gated by cluster of differentiation 4 (CD4) and CD8. To analyse the differentiation state, CD4+ and CD8+ T cells were gated for CD44 and CD62L. Regulatory T cells (Tregs) were gated from the CD4+ T cells by gating for FoxP3 and CD25. These double-positive cells were further classified for their differentiation state by CD44 and CD62L, and for their expression of helios, Kiel-67 (Ki-67), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and CD39.

2.11.4 Analysis of kidney histology

Kidneys of NZM2410 and C57BL/6J mice of different ages were PAS-stained and microscopically investigated as described above. The analysis was performed by Hiroshi Nishi and colleagues from Tokyo University, who are trained nephrologists and have experience with nephritis in these mouse lines. Mouse nephritis is different from human nephritis but three major kinds of inflammatory signatures can be seen: mesangial cell proliferation and matrix accumulation (or sclerotic lesions), which leads to pink fibrotic lesions in the glomeruli; cellular crescent formation, which surround the glomerulus; and intracapillary hyaline thrombosis, seen as pink capillaries in the stainings. Of each kidney, four microscopic pictures were taken at a magnification of 100x of different regions. Additionally, 400x magnification pictures of each glomerulus in these areas were taken to facilitate the analysis. The pictures were taken by the bachelor student Leo Li under my supervision. In these regions, the number of glomeruli and the number of abnormal glomeruli were counted. Thereof, the ratio was calculated and served as a readout of nephritis severity (**Figure 24**).

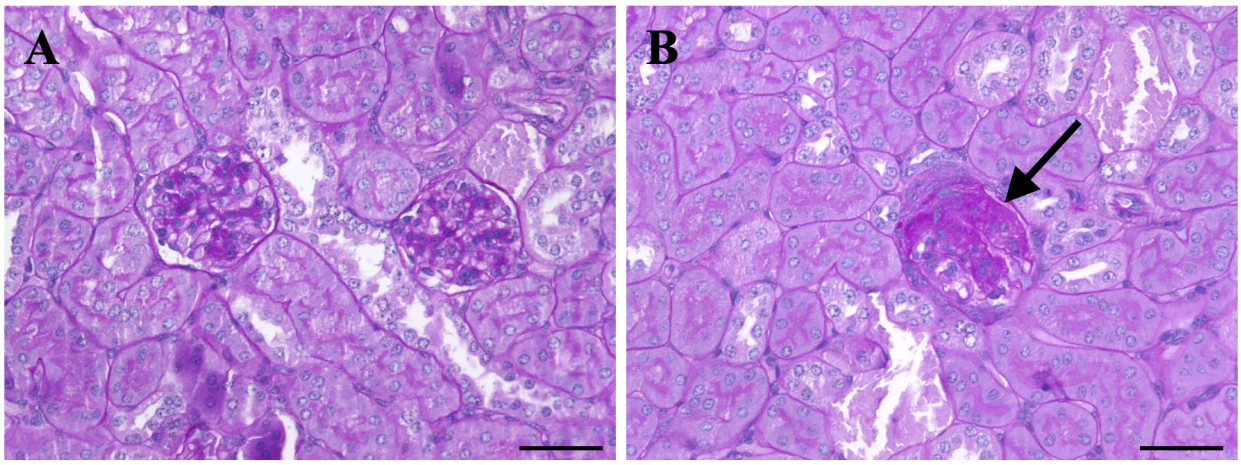


Figure 24: Examples of healthy and abnormal glomeruli in NZM2410 kidneys to evaluate nephritis
Examples of haematoxylin and eosin-stained murine glomeruli from lupus-prone NZM2410 mice. The left image shows two healthy glomeruli (**A**), while the right image displays a glomerulus with sclerotic lesions, indicated with an arrow (**B**). Pictures taken in 400x magnification, scale: 50 µm.

2.11.5 Analysis of messenger ribonucleic acid bulk sequencing data

The sequencing data were received from Novogene and analysed by Sen Guo and Dr. Axel Künstner from the LIED (Systems Biology, Busch laboratory). They used several publicly available R packages. First, the data is pseudoaligned to a reference genome with the R-package Kallisto (Bray *et al.* 2016). Next, the downstream analysis followed. Therefore, either differentially expressed

genes were analysed using the package Sleuth (Pimentel *et al.* 2017), or, gene set variation analysis (GSVA) was used to investigate expression levels of biological pathways. Sleuth uses likelihood ratio tests and Wald tests to identify differentially expressed genes across all tested groups. By modelling the variability in the estimated counts, it is able to account for technical and biological variances. GSVA works slightly differently. It calculates the geneset enrichment scores per sample and not per group, which helps to explore the variability in pathway activity across samples in a non-parametric approach. The package linear models for microarray data (Limma) was then used to either calculate differentially expressed pathways between the experimental groups, or generally applied geneset enrichment (GAGE) was used to calculate this from the differentially expressed genes data. It detects up- and downregulated pathways using two-sample t-tests or rank-based tests. Moreover, decoupleR (Badia-i-Mompel *et al.* 2022) was used to investigate the expression levels of transcription factors (**Figure 25**). As the RNA was gained from murine skin, without separation for the skin layer or cell type, a deconvolution was also performed to see which cell types provided the most signals. For this, the work of Joost *et al.* 2020 was used as a reference.

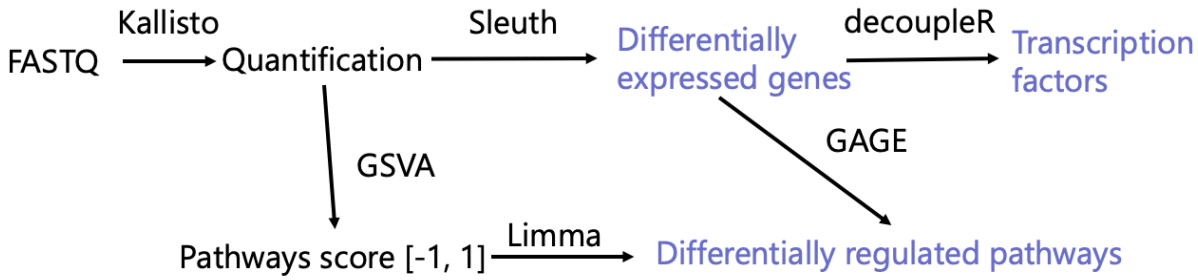


Figure 25: Workflow for sequencing data
The sequencing data was processed in several steps using R packages. The first step is the pseudoalignment of the data from the FASTQ-files using Kallisto. Next, Sleuth is used to identify differentially expressed genes. After that, decoupleR is used to identify significant transcription factors. Generally applied geneset enrichment (GAGE) was used to calculate differentially regulated pathways from the differentially expressed genes. Geneset variation analysis (GSVA) and linear models for microarray data (Limma) are other approaches to investigate and quantify pathway regulation. Sen Guo, Systems Biology, LIED, University of Lübeck, Lübeck, Germany, generated the picture.

2.11.6 Analysis of histologic stainings

Microscopy pictures were taken with a Keyence BZ-X810 microscope to analyse the histological sections.

Microscopy and analysis of H&E staining was done by Zuzana Penxova and me by

live-microscopy of H&E stained paraffin sections in 20x magnification to gain an overview and 100x magnification to see details of suspected sunburned cells in the epidermis and dermis. Sunburned/apoptotic cells showed a shrunk, pycnotic nucleus and a reddish staining around it (**Figure 26**).

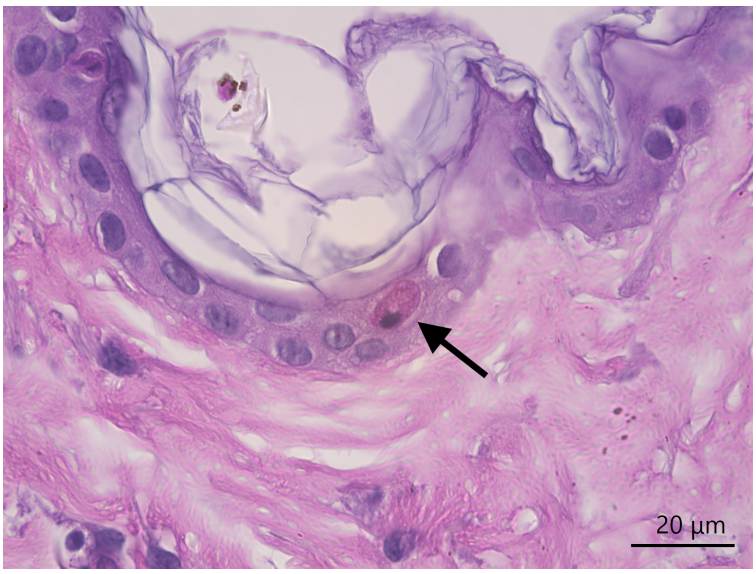


Figure 26: Apoptotic cell in haematoxylin and eosin stained murine skin
Apoptotic cells are characterised by a pycnotic nucleus and red cytoplasm, indicated here with an arrow. Image taken with 100x magnification, scale = 20 µm.

Fluorescence microscopy and **analysis of cleaved caspase-3 staining** was done by photographing cleaved caspase-3 stained paraffin sections in 200x magnification. The slide was placed upside down on the sample holder, and the autofocus was used to gain a quick, sharp focus. Next, the navigation tool created a stitched overview picture for orientation. To take the final picture, the frame of three pictures in the x-direction and two pictures in the y-direction was set to have a straight epidermis on top without too much of a background, without a sample. Z-stacks of five pictures were generated to generate an in-depth picture with optimal sharpness. For analysis, the extra module "hybrid cell count" was used in the analyser program. Here, the conditions were set once and used for all pictures. This program measures the fluorescent area and the fluorescence intensity, which provides information about the intensity of apoptosis in the samples (**Figure 27**).

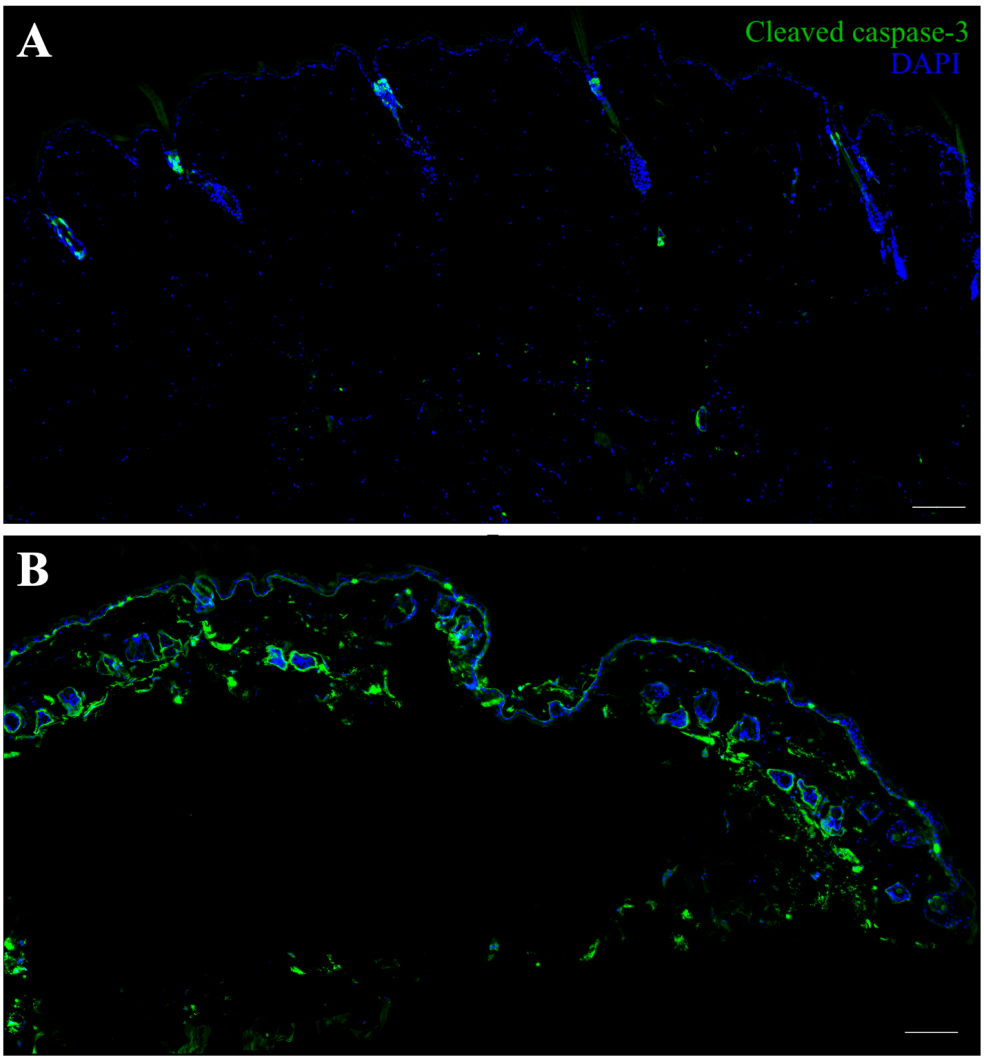


Figure 27: Apoptosis in cleaved caspase-3 stained murine skin
Nuclei are stained with DAPI (blue), and cleaved caspase-3 with Alexa Fluor 488 (green). Example of immunofluorescence image of murine skin after 30 hours of incubation (**A**), and after 30 hours of incubation with additional UVB irradiation (**B**). Magnification 200x, with 3x2 pictures stitched. Scale: 200 µm. Images taken by Zuzana Penxova, Steffen Pichlo, and me.

2.12 Storage of material and data

As large amounts of material and data are generated, organised storage is more and more important. Moreover, not all materials were exhausted. To enable future experiments with these, correct storage is essential. The University of Lübeck requires the storage of material for 15 years and the storage of data for 15 years.

2.12.1 Storage of material

Animals received individual numbers in the order of appearance. All paraffin sections are stored at RT, cryosections were also stored after microscopy at -80°C. Snap-frozen organs are stored in 1.5 ml cups at -80°C. Organs in RNA protect were stored in RNA protect at -20°C. Paraffin blocks were stored after cutting at RT ordered for the project and mouse number. Paraffin blocks that were not yet cut are stored at -20°C.

2.12.2 Storage of data

All five lab books are stored in the Hundt laboratory. They are scanned, and the scans are stored on the SSD hard drive together with my other data. Standard operating procedures (SOPs) were generated with the Hundt laboratory's template and updated and improved when required. They are also stored on the lab's online server and the SSD hard drive. The raw data from the ScilVet ABC Plus+ is in an Excel table on the SSD hard drive, the processed data can be found in the same folder as a GraphPad Prism file. The flow cytometry data is saved in individual folders with the dates of the experiments. Each sample had to be analysed in KALUZA individually, so all analysed files are in the folder "FACS analysis", organised in sub-folders for mouse strain, organ, and date of experiment. These were in the next step, all collected in three Excel tables according to the strains. Next, the data were further processed in GraphPad Prism files. All digital data is saved on the SSD hard drive and is extra secured on a second one. This also accounts for the transcriptome data, which can be found in the folder "UV-experiments" in the sub-folder "sequencing".

3 Results

3.1 Lupus pre-disease phase

The lupus pre-disease phase is investigated in this thesis in mice with a special focus on T cells and also the influence of UV light on its development and progression.

We define the pre-disease phase as a phase in which the mice behave and look physically unobtrusive, meaning, no hunching or other signs of pain or discomfort and also no other visual signs of sickness such as rough fur, lack of hygiene, hair loss or similar signs. We also specifically chose the age before these mice are by law classified as a burdened breed. This line was set due to the known onset of proteinuria at the age of 24.0 +/- 2.7 weeks under normal diet in NZM2410 mice (Vorobyev *et al.* 2019). For SLE123 mice, it is known that the mortality from nephritis is lower than NZM2410 mice until the age of 8 months and thereafter even higher (Morel, Croker, *et al.* 2000). Autoantibodies are also classically used to define the start of autoimmunity. For NZM2410 mice and SLE123, the known start of autoantibody production is not precisely known, as most research focused on the lupus disease state rather than the pre-disease phase. For NZM2410 mice and SLE123, it is known that they definitely show anti-dsDNA-autoantibodies at the age of 5 months (Morel, Ulrich H. Rudofsky, *et al.* 1994). However, even C57BL/6 mice are known to produce autoantibodies by the age of 8-12 months, even though these mice remained healthy later on in life (Nusser *et al.* 2014). Therefore, we did not use autoantibodies as a sign of beginning autoimmunity. Even if it is a classical diagnostic test, we rather focused on the development of abnormalities in glomeruli and used blood measurements and flow cytometry analysis for several T cell populations of blood, spleens, and lymph nodes to describe in short intervals the distinct changes in these aspects. Moreover, we also investigated the general parameters of body weight, spleen weight and physical movement as part of the overall health assessment.

3.1.1 NZM2410 mice have higher body weight but SLE123 developed higher normalised spleen weight than C57BL/6J mice

C57BL/6J mice increased less in **body weight** than NZM2410 mice. The mean weight in NZM2410 mice at week 8 was 33.4 g, which increased to 40.8 g mean weight at week 21. SLE123 mice started with 20.6 g mean weight in week 8. At week 18, the last week measured, they weighed 28.1 g. C57BL/6J mice weighed 24.1 g at week 8 and increased to a mean of 26.9 g in week 21. This body weight gain in NZM2410 mice was significantly different from C57BL/6J mice throughout all tested weeks (week 8 p= 0,019; week 9 $p \leq 0.001$; week 12 $p \leq 0.001$; week 16 $p \leq 0.001$; week 18 $p \leq 0.001$;

week 19 p= 0.002; week 20 p≤0.001; week 21 p≤0.001), except for week 10 and week 17. SLE123 mice do not show differences in weight development compared to C57BL/6J mice at any age tested (**Figure 28 A**).

The **spleen weight** was assessed to identify potential enlargements due to inflammatory reactions. NZM2410 mice had only significantly different spleen weights at week 9 (p≤0.001) and week 18 (p≤0.001) compared to C57BL/6J. When comparing SLE123 mice to C57BL/6J mice, SLE123 mice had a peak in spleen weight at week 14. The spleens were significantly larger in SLE123 mice from the age of 14 weeks (mean of 0.115 g in SLE123, and 0.074 g in C57BL/6J mice) to the last tested age of 18 weeks (week 14 p≤0.001; week 16 p= 0.002; week 17 p≤0.001; week 18 p≤0.001) (**Figure 28 B**).

As NZM2410 mice had significantly higher body weight than C57BL/6J mice, the **spleen weight was normalised** to the weight to control for this difference. The only significant difference between these mice was found at the age of 20 weeks (p≤0.001). Comparing the normalised spleen weights of SLE123 mice to C57BL/6J mice, significantly higher spleen weights of SLE123 mice were found at the ages of 14 weeks (p= 0.004), 16 weeks (p= 0.005), 17 weeks (p≤0.001), and 18 weeks (p≤0.001) (**Figure 28 C**).

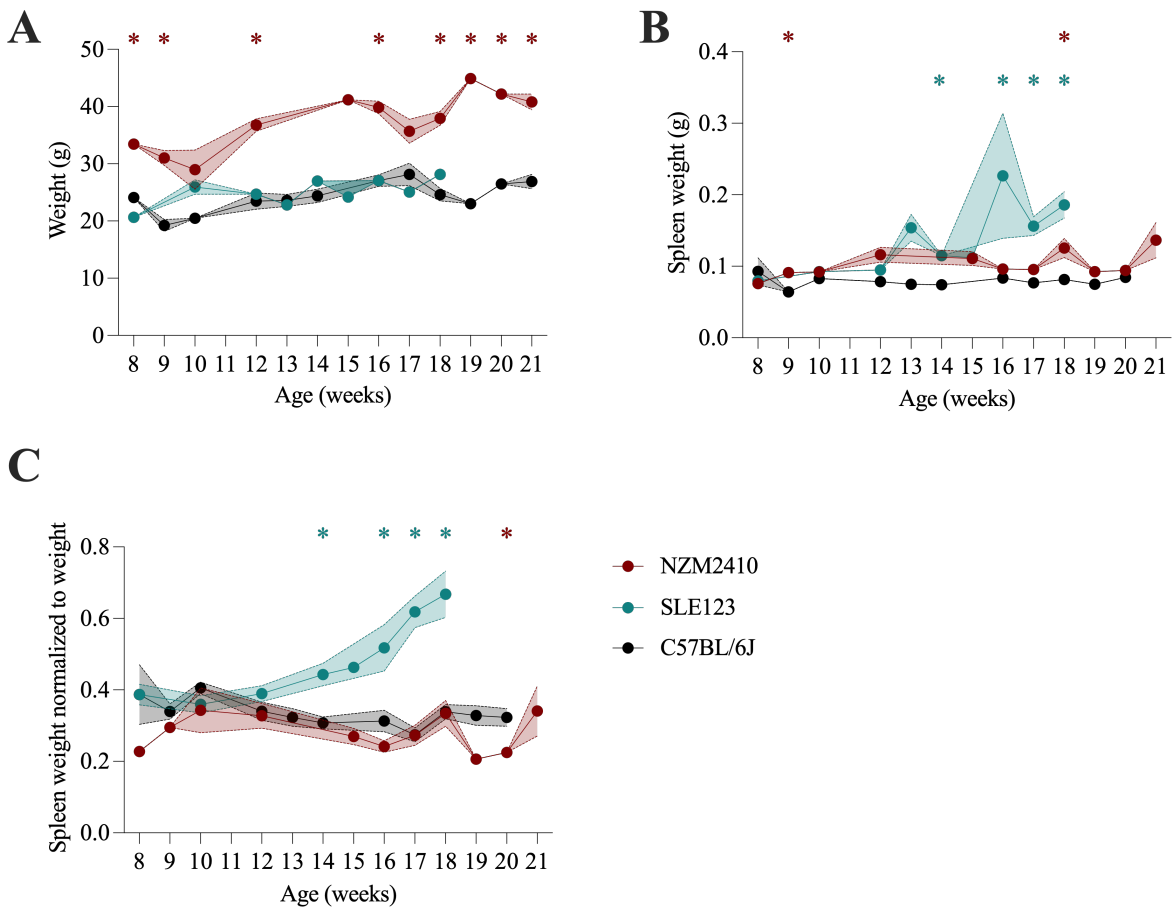


Figure 28: NZM2410 mice have higher body weight but SLE123 mice exhibit increased normalised spleen weight compared to C57BL/6J mice from 14 weeks onwards
NZM2410 mice have higher body weights than C57BL/6J mice (**A**). Their spleen weight was only elevated compared to C57BL/6J mice at the age of 9 weeks and 18 weeks. SLE123 mice have higher spleen weight than C57BL/6J mice from the age of 14 weeks onwards (**B**). When normalising the spleen weight to the body weight, NZM2410 mice show only at week 20 a significant difference, while SLE123 mice have increased normalised spleen weights compared to C57BL/6J mice from week 14 onwards (**C**). Data presented as mean +/- SEM, gained from 42 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

3.1.2 NZM2410 mice show less physical activity during the pre-disease phase compared to C57BL/6J mice

The physical activity of two NZM2410 and two C57BL/6J mice was measured over 24 h with RAD sensors. These sensors detect movements through the cage. C57BL/6J mice moved more in the night (accumulated mean of 5,040 counts) and less during the day (accumulated mean of 3,179

counts). This accounted also for NZM2410 mice (accumulated mean of 466 counts during the day and 2,583 counts during the night), however, these showed overall less movement than C57BL/6J mice (C57BL/6J mice: mean 8,220 counts; NZM2410 mice: mean 3,050 counts). According to the cosinor.online tool from Molcan 2023, both, NZM2410 and C57BL/6J mice depicted a rhythm ($p<0.00001$), however, the mesor (average activity counted per minute over the full 24 h) in NZM2410 mice was less than half as much as in C57BL/6J mice (2.12 for NZM2410 and 5.71 for C57BL/6J mice) (**Figure 29**).

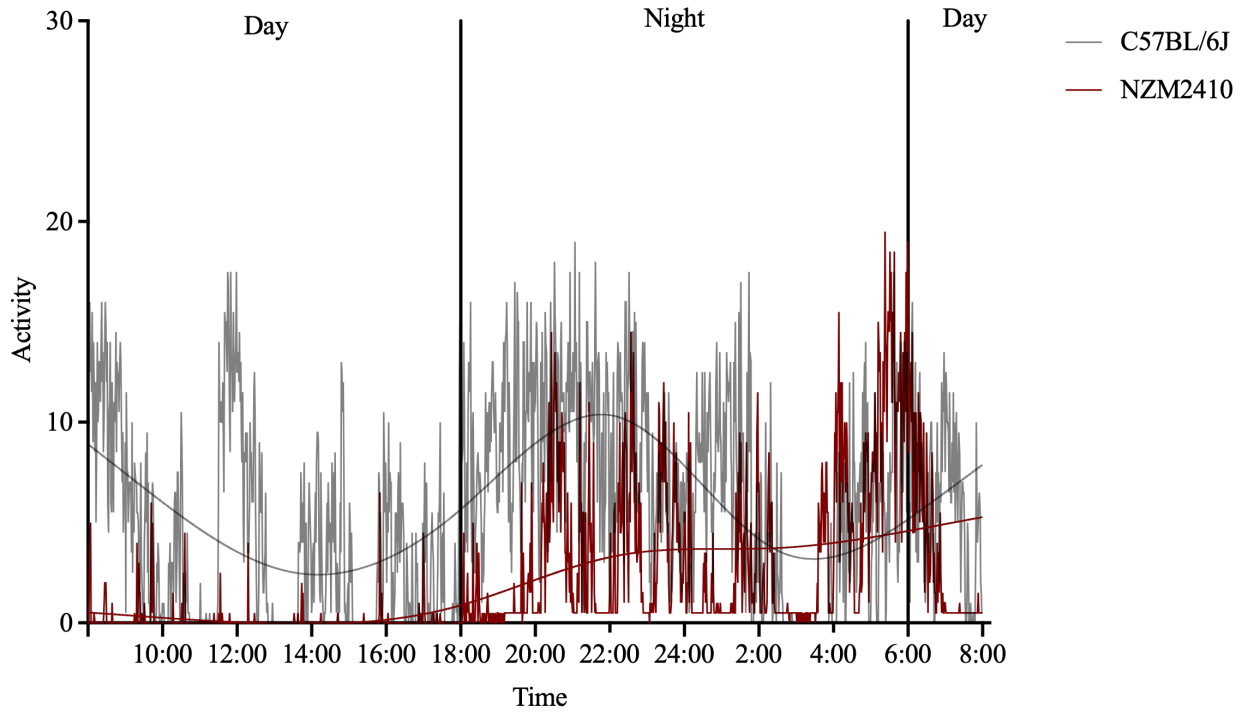


Figure 29: NZM2410 mice move less than C57BL/6J mice
Rodent activity detectors (RAD) were placed on the feeding grids of standard mouse cages. The movement of two NZM2410 and two C57BL6/J mice was counted in individual cages over 24 h. The mean of the results is displayed in this graph. Due to the low number of mice, no further statistics were applied.

3.1.3 NZM2410 mice did not develop nephritis during the pre-disease phase

Nephritis is one of the hallmarks in the development of lupus. It is not expected in the pre-disease phase, however, we investigated this to avoid missing out on the onset of it. Kidneys were fixated, paraffin embedded, cut in 4 µm sections, and PAS-stained. Abnormal glomeruli were counted and a ratio of abnormal of total glomeruli was calculated. This was done for 64 NZM2410 and 24 C57BL/6J mice.

The values in C57BL/6J mice are 0 most of the weeks but increase up to 0.02 in week 21. For NZM2410 mice, the variance is higher. Two peaks could be seen. A smaller one at week 10 and a higher one at week 18 (0.09). No significant difference was found at any time point compared (**Figure 30**).

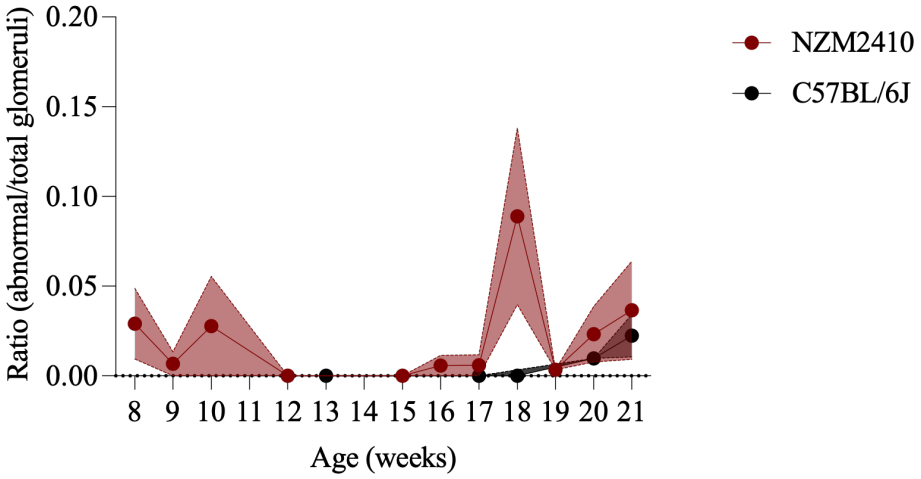


Figure 30: Until the age of 21 weeks, NZM2410 mice do not develop significantly more nephritis than C57BL/6J mice
Kidneys of NZM2410 and C57BL/6J were paraffin-embedded and sectioned in 4 µm thickness. In total, 24 C57BL/6J kidneys and 64 NZM2410 kidneys were analysed for abnormal glomeruli. Per kidney, the healthy and abnormal glomeruli were counted in four pictures of different parts of the kidney. The ratio of abnormal and total glomeruli serves as readout. Neither of the tested mouse lines showed normally distributed data, so a Mann-Whitney test was used to compare the data and no significant differences ($p<0.05$) were found.

3.1.4 NZM2410 mice showed elevated counts of monocytes, granulocytes, and platelets in the blood at the age of 17-20 weeks

WBC fluctuations of NZM2410 and SLE123 mice between the ages of 8 weeks and 21 weeks were compared to the WBC counts of C57BL/6J mice of the same age. However, these changes were not significantly different. NZM2410 mice started at week 8 with 5.14 x $10^9/l$ and end with 3.23 x $10^9/l$ in week 21. The highest values were seen in week 19 with 7.63 x $10^9/l$. In SLE123 mice, the lowest values were found in week 9 with 5.60 $10^9/l$, the highest values in week 13 with 7.45 $10^9/l$ and ended with 5.06 $10^9/l$ in week 18. C57BL/6J mice revealed less in- and decreases but started with a mean of 4.00 $10^9/l$ in week 8 and end with 6.55 $10^9/l$ in week 21 (**Figure 31 A**).

Significant differences became apparent when WBCs were divided into their cell types. **Lymphocyte** counts decreased significantly in NZM2410 compared to C57BL/6J mice only

at the age of 21 weeks ($p \leq 0.001$) with mean values of 2.20 x $10^9/l$ for NZM2410 and 5.29 x $10^9/l$ for C57BL/6J mice (**Figure 31 B**).

Monocyte counts markedly increased in NZM2410 at the age of 19 ($p = 0.001$, mean 0.46 x $10^9/l$) and 20 weeks ($p \leq 0.001$, mean 0.41 x $10^9/l$) compared to C57BL/6J mice (week 19 mean 0.22 x $10^9/l$ and week 20 0.12 x $10^9/l$. SLE123 mice showed a similar increase earlier at the age of 14 weeks ($p \leq 0.001$, mean 0.37 x $10^9/l$) and 16 weeks ($p \leq 0.001$, mean 0.47 x $10^9/l$) (**Figure 31 C**).

Although similar patterns were observed for **granulocyte** counts, this was not significant for SLE123 but only for NZM2410 mice at the ages of 10 ($p = 0.003$, mean 2.38 x $10^9/l$), 19 ($p = 0.001$, mean 2.536 x $10^9/l$) and 20 weeks ($p \leq 0.001$, mean 2.39 x $10^9/l$) (**Figure 31 D**).

Until the age of 20 weeks, the **eosinophil** counts were higher in NZM2410 mice (mean 0.95 x $10^9/l$) than in C57BL/6J mice (mean 0.17 x $10^9/l$), however, this observation was liable to high variances and was not significant (**Figure 31 E**).

Neutrophil and basophil counts were increased with a significant peak at week 19 with a mean of 1.74 x $10^9/l$ ($p \leq 0.001$) (**Figure 31 F**). In this figure, recurring increases in cell counts in the NZM2410 mice were indicated with red arrows and can be seen at the age of 10 weeks and 18-20 weeks. Even if not all increases are significantly different from C57BL/6J mice, we still want to highlight this repeating pattern.

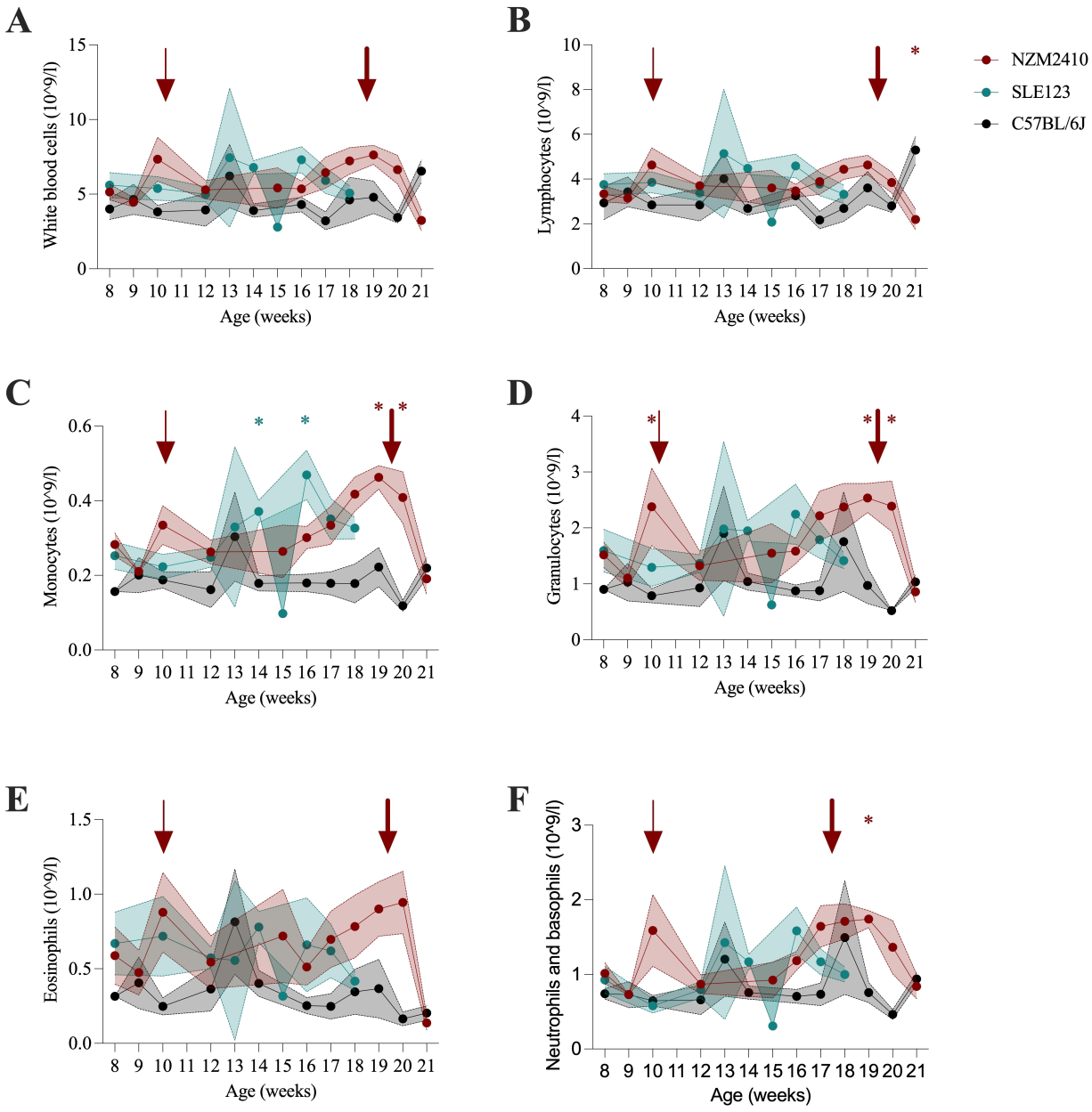


Figure 31: Counts of monocytes, granulocytes, neutrophils and basophils peak in NZM2410 mice between 17 and 20 weeks

Blood of NZM2410, SLE123, and C57BL/6J mice was investigated using a ScilVet ABC Plus+ device. No significant differences could be detected in the white blood cell counts (**A**). At the age of 21 weeks, NZM2410 mice showed fewer lymphocytes (**B**). Monocyte counts were increased in NZM2410 mice compared to C57BL/6J mice at the ages of 19 and 20 weeks and at 14 and 16 weeks for SLE123 mice (**C**). Granulocytes showed an increased number for NZM2410 mice at ages 10, 19, and 20 weeks (**D**). No significant changes could be detected for eosinophil numbers (**E**), but neutrophils and basophils were increased in NZM2410 mice at the age of 19 weeks (**F**). Red arrows indicate recurring patterns in cell counts of NZM2410 mice compared to C57BL/6J mice. Data presented as mean +/- SEM, gained from 46 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

The **RBCs** were also investigated using the ScilVet ABC Plus+ device. No significant differences in **RBC counts**, **haemoglobin** or **haematocrit** could be detected when comparing NZM2410 and SLE123 mice to C57BL/6J mice (**Figure 32 A-C**).

However, the **MCV** was significantly decreased in NZM2410 mice at the ages of 8 ($p= 0.010$), 9 ($p\leq0.001$), 10 ($p= 0.009$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.012$), 19 ($p= 0.005$), and 20 weeks ($p\leq0.001$). In SLE123 mice, this could also be detected at the ages of 10 ($p= 0.007$), 12 ($p= 0.007$), 14 ($p\leq0.001$), 16 ($p= 0.006$), and 17 ($p\leq0.001$) weeks (**Figure 32 D**).

No significant changes could be seen in the **MCHC** but the **RDW** was significantly lower in NZM2410 mice at the ages of 9 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 18 ($p= 0.010$), 19 ($p= 0.002$), and 20 weeks ($p\leq0.001$). This could not be detected for SLE123 mice (**Figure 32 E-F**).

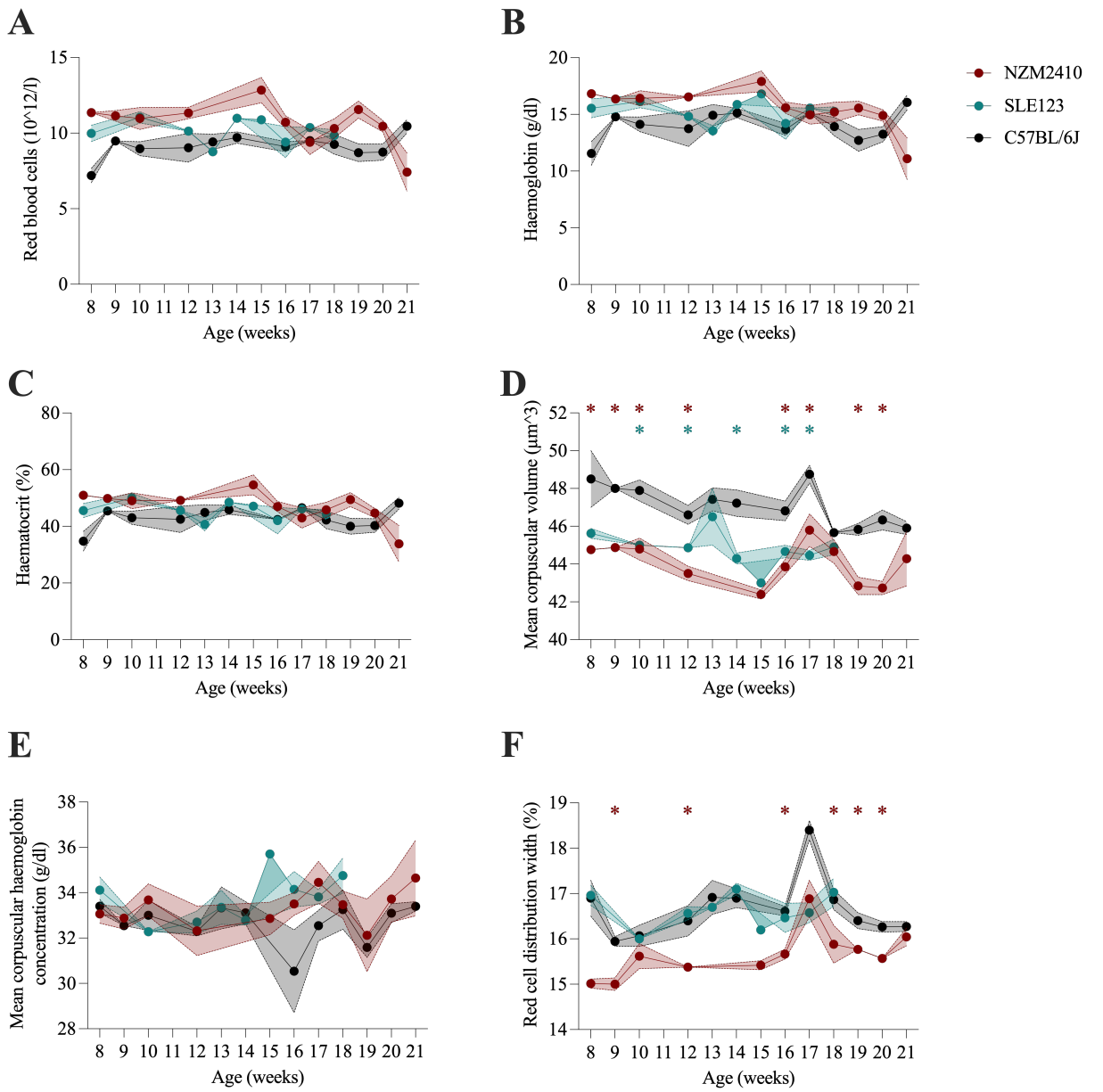


Figure 32: Lupus-prone mice have a reduced mean corpuscular volume compared to C57BL/6J mice Red blood cell (RBC) counts are similar in NZM2410, SLE123, and C57BL/6J mice at all investigated ages (**A**). This was also detected for the haemoglobin levels (**B**) and the haematocrit (**C**). The mean corpuscular volume of NZM2410 and SLE123 mice is lower than the mean corpuscular volume of C57BL/6J mice at all ages. A rise can be detected in NZM2410 mice between the ages of 16 and 19 weeks (**D**). The mean corpuscular haemoglobin concentration shows the highest variances of all measured red blood cell values but no significant differences (**E**). The red cell distribution width of NZM2410 mice is consistently lower than in C57BL/6J mice. SLE123 mice show similar values to the C57BL/6J mice throughout (**F**). Data presented as mean +/- SEM, gained from 46 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

The measurement of platelets and mean platelet volume in the blood of the mouse strains revealed that neither the **platelet counts** of SLE123 and C57BL/6J mice nor the MPV were different at any measured age. However, NZM2410 mice had higher platelet counts than C57BL/6J mice at the ages of 10 (p= 0.001, mean NZM2410 1029.00 x $10^9/l$, mean C57BL/6J 308.90 x $10^9/l$), 18 (p= 0.002, mean NZM2410 1484 x $10^9/l$, mean C57BL/6J 341.17 x $10^9/l$), and 20 weeks (p= 0.001, mean NZM2410 1140.23 x $10^9/l$, mean C57BL/6J 475.56 x $10^9/l$). As for the WBC counts and their cell types, a peak could be seen in NZM2410 mice between the ages of 17 and 20 weeks (**Figure 33 A**).

The **MPV** was significantly elevated at the ages of 17 ($p \leq 0.001$, NZM2410 mean 6.84 μm^3, C57BL/6J mean 5.53 μm^3), 19 (p= 0.002, NZM2410 mean 6.76 μm^3, C57BL/6J mean 6.13 μm^3), and 21 weeks ($p \leq 0.001$, NZM2410 mean 6.50 μm^3, C57BL/6J 5.49 μm^3) (**Figure 33 B**).

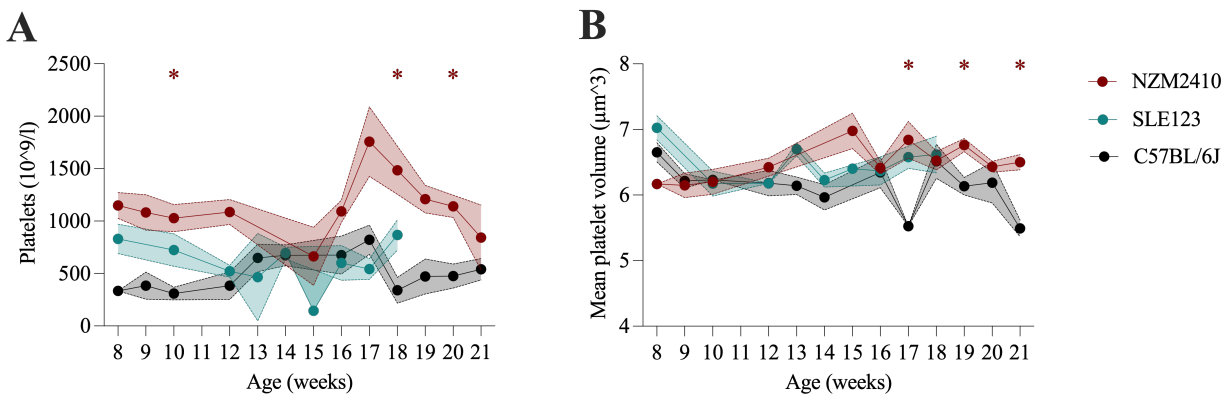


Figure 33: NZM2410 mice have more platelets than C57BL/6J mice
The platelet counts were higher in the blood of NZM2410 mice compared to C57BL/6J mice. A peak in platelet counts was measured for NZM2410 mice at week 17, thereafter the numbers dropped again (**A**). The mean platelet volume is increased in NZM2410 mice at 17, 19, and 21 weeks of age (**B**). Data presented as mean +/- SEM, gained from 46 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

3.1.5 Differences in T cells were found in NZM2410 and SLE123 mice compared to C57BL/6J mice in the blood

The blood was investigated as it flows between all organs, immunologic ones and those which are potentially inflamed during lupus disease and pre-disease. The mean **CD4+ T cell** frequencies were ranging from 9.7 % in C57BL/6J, 25.8 % in NZM2410 % to 18.1 % in SLE123 at week 8. In week 21, NZM2410 mice had a mean value of 16.9 % and C57BL/6J 9.9 %. At week 18, SLE123 mice had a mean CD4+ T cell frequency of 8.1 %. NZM2410 mice had significantly higher CD4+ T cell frequencies at all tested ages [week 9 (p= 0.002), 10 (p= 0.002), 12 (p= 0.002), 16 ($p \leq 0.001$), 17

(p= 0.033), 18 (p= 0.001), 19 (p= 0.023), 20 (p≤0.001), 21 weeks (p≤0.001)]. SLE123 mice have higher CD4+ cell frequencies than C57BL/6J mice (significant at the age of 14 weeks (p= 0.001) but constantly lower frequencies than NZM2410 mice (**Figure 34 A**).

When separating the CD4+ T cells into naive, effector, and central memory T cells, NZM2410 mice had significantly increased **naive CD4+ T cells** at the age of 9 (p= 0.002), 10 (p= 0.002), 16 (p≤0.001), and 18 weeks (p≤0.001). SLE123 mice only had a significantly higher frequency at the age of 14 weeks (p≤0.001). C57BL/6J mice started with a frequency of 59.4 % in week 8 and increased to 73.0 % in week 21. NZM2410 mice exhibited a naive CD4+ T cell frequency of 79.0 % at week 8, which dropped to 48.5 % in week 21. SLE123 mice had a mean frequency of 44.7 % in week 8 and it decreased to 27.7 % in week 21 (**Figure 34 B**).

In contrast, the **effector CD4+ T cells** in NZM2410 mice are significantly lower at the age of 9 weeks (p= 0.003) but significantly higher than in C57BL/6J mice at 20 (p≤0.001) and 21 weeks (p≤0.001). SLE123 mice differ here from the NZM2410 mice, as they show a higher frequency of effector CD4+ T cells throughout all measured ages, with a significantly higher value than in C57BL/6J mice at the age of 18 weeks (p≤0.001). C57BL/6J mice started with a frequency of 7.0 % in week 8 and decreased to 5.3 % in week 21. NZM2410 mice had a mean effector CD4+ T cell frequency of 8.6 % at week 8, which rose to 19.5 % in week 21. SLE123 mice had a mean frequency of 34.6 % in week 8 and it increased to 43.9 % in week 21 (**Figure 34 C**).

Central memory CD4+ T cells were lower in NZM2410 mice than C57BL/6J mice but this difference was not significant. In SLE123 mice the central memory CD4+ T cells were also decreased compared to C57BL/6J mice but this was only significant at the age of 17 weeks (p≤0.001, SLE123 mean frequency of 18.0 %, C57BL/6J mean frequency of 42.2 %), in which the C57BL/6J mice showed a peak in their central memory CD4+ T cell frequencies (**Figure 34 D**).

Double positive T cells (CD4+ and CD8+) were significantly higher in NZM2410 at the age of 21 weeks (p≤0.001, mean frequency NZM2410 0.7 %, mean frequency C57BL/6J 0.1 %) and significantly lower in SLE123 mice at the age of 14 weeks (p≤0.001, mean frequency SLE123 0.3 %, mean frequency C57BL/6J 0.4 %) (**Figure 34 E**).

Activated CD4+ T cells, which are not Tregs, peaked at the age of 18 weeks (p≤0.001) in NZM2410 mice. At the other measured time points, however, the frequency was lower in NZM2410 mice than in C57BL/6J mice [significant at the age of 12 (p≤0.001), 16 (p≤0.001), and 17 weeks (p= 0.003)]. In SLE123 mice, the frequency of activated CD4+ T cells is higher until the age of 14 weeks with a peak at the age of 10 weeks (28.0 %). After the age of 14 weeks, the frequency dropped below the one of C57BL/6J mice (SLE123 at week 18 3.4 % and C57BL/6J 5.8 %). Due to high variance, this peak in SLE123 mice was not significant (**Figure 34 F**).

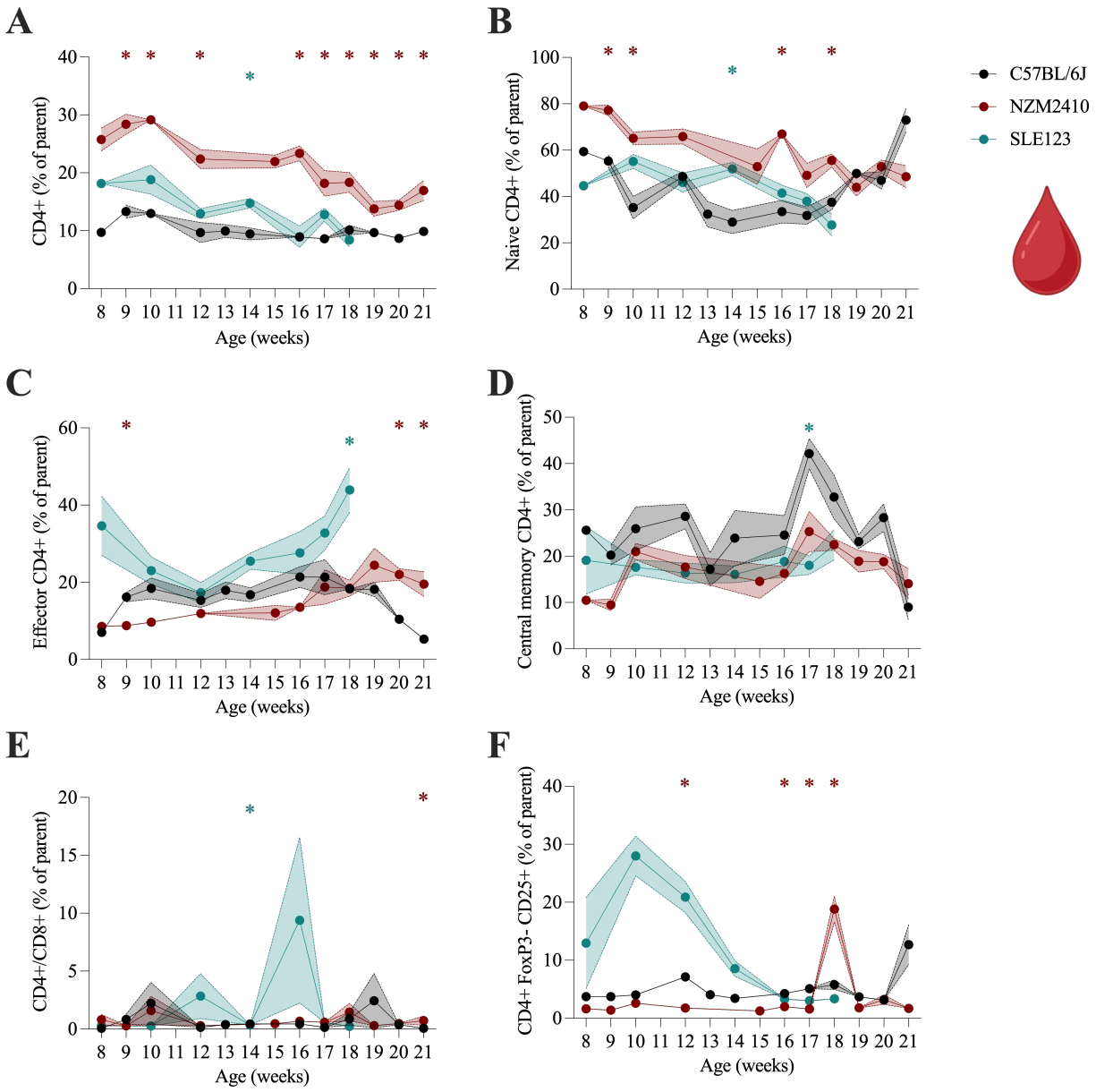


Figure 34: NZM2410 mice demonstrate complex changes in CD4+ T cells and subsets in the lupus pre-disease phase

CD4+ T cells were measured in the blood of lupus-prone NZM2410 and SLE123 mice compared to C57BL/6J mice using flow cytometry at the ages of 8-21 weeks (**A**). The CD4+ T cells were further classified into naive CD4+ T cells (**B**), effector CD4+ T cells (**C**), and central memory CD4+ T cells (**D**). Further, CD4+CD8+ T cells were analysed (**E**) and activated CD4+ T cells, which also express CD25 (**F**). The parent gate of naive, effector, memory, and CD4+ FoxP3- CD25+ cells is the CD4+ gate. The parent gate of the CD4+ as well as the CD4+/CD8+ cells is the single cell gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

Frequencies of **CD8+ T cells** in the blood were higher in NZM2410 mice compared to C57BL/6J at the ages of 10 (p= 0.002, mean NZM2410 11.1 %, C57BL/6J 8.0 %) and 16 weeks (p= 0.003, mean NZM2410 8.3 %, C57BL/6J 6.1 %) but significantly dropped at the age of 20 weeks (p= 0.002, mean NZM2410 5.3 %, C57BL/6J 8.2 %). The differences of SLE123 mice compared to C57BL/6J mice were at no measured age significantly different (**Figure 35 A**).

The frequencies of **naive CD8+ T cells** are at most time points higher in NZM2410 mice compared to C57BL/6J mice, ranging between 88.2 % in week 8 to 61.6 % in week 19 for NZM2410 and 45.5 % at week 13 and 69.1 % in week 21 for C57BL/6J mice (significantly higher in NZM2410 mice at the ages of 9 (p= 0.002), 10 (p= 0.002), 12 (p= 0.002), 16 ($p \leq 0.001$), and 18 weeks ($p \leq 0.001$). Naive CD8+ T cell frequencies of SLE123 mice are until the age of 14 weeks (64.9 %) also higher than in C57BL/6J mice (48.3 %), but they drop at the age of 17 and 18 weeks to 39.6 %. These changes are at no measured age significant (**Figure 35 B**).

Effector CD8+ T cells were liable to high variances. No measured differences at any time in any genotype were significantly different. However, the frequencies were rising in both NZM2410 mice and SLE123 mice, exceeding the ones of C57BL/6J mice at the ages of 16 weeks (SLE123, mean frequency 11.3 %) and 19 weeks (NZM2410, mean frequency 6.8 %) (**Figure 35 C**).

NZM2410 mice had throughout lower **central memory CD8+ T cell** frequencies, with significant differences at the ages of 9 (p= 0.002), 10 (p= 0.002), 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), 18 ($p \leq 0.001$), and 20 weeks ($p \leq 0.001$). The central memory CD8+ T cell frequencies of SLE123 mice underlied several increases and decreases but were significantly lower than in C57BL/6J mice at the age of 12 weeks (p= 0.001). The frequencies were ranging from 20.9 % at week 9 to 33.5 % in week 18 in C57BL/6J mice. In NZM2410 mice, the highest values were seen in week 19 with 24.1 % and the lowest in week 8 with 8.9 %. For SLE123 mice, the highest frequencies were found in week 18 with 38.7 % and the lowest in week 12 with 19.2 % (**Figure 35 D**).

The frequencies of **activated CD8+ T cells** were in C57BL/6J mice and NZM2410 mice low most of the time (e.g. 0.3 % at week 8 in C57BL/6J mice and 0.7 % in NZM2410 mice at week 8) with a sharp and significant peak in NZM2410 mice at the age of 18 weeks ($p \leq 0.001$) to about 70.1 %. At the ages of 12 ($p \leq 0.001$), 16 (p= 0.012), 17 (p= 0.003), 20 ($p \leq 0.001$), and 21 weeks ($p \leq 0.001$) the frequencies of NZM2410 mice were significantly lower than in C57BL/6J mice. For SLE123 mice, the frequencies were higher until the age of 16 weeks (mean frequency at 16 weeks 0.5 %), with a peak at the age of 10 weeks (p= 0.004) with a mean value of 87.0 %. Other significant differences were seen at the ages of 12 ($p \leq 0.001$), 14 (p= 0.004), 17 (p= 0.010), and 18 weeks ($p \leq 0.001$) (**Figure 35 E**).

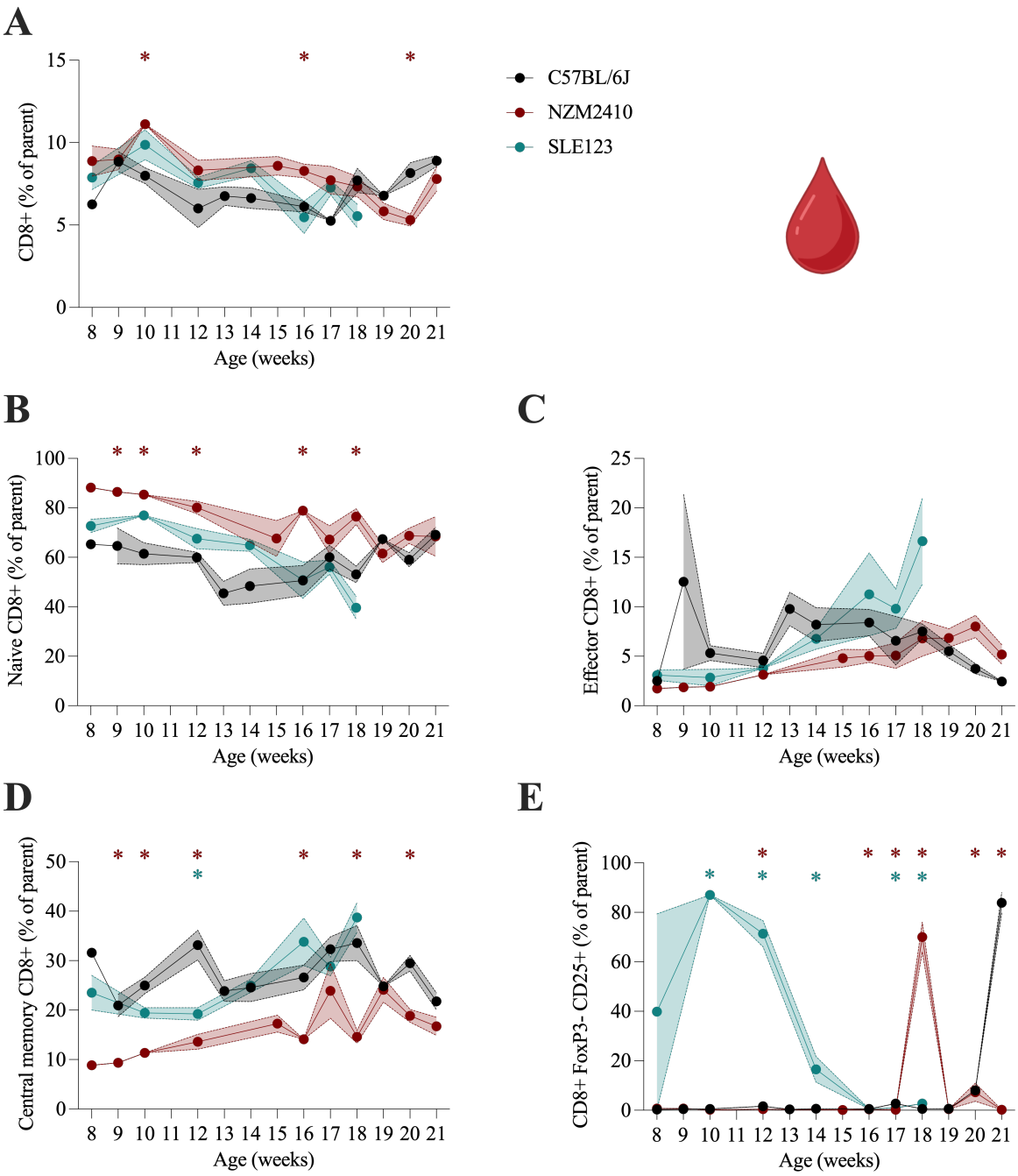


Figure 35: NZM2410 mice had more naive CD8+ T cells but reduced central memory CD8+ T cell frequencies in the blood

CD8+ T cells in the blood were measured by flow cytometry for NZM2410, SLE123, and C57BL/6J mice from the age of 8 weeks to 21 weeks (**A**). The CD8+ T cells were further separated into naive CD8+ T cells (**B**), effector CD8+ T cells (**C**), central memory CD8+ T cells (**D**), and activated CD8+ T cells (**E**). The parent gate of naive, effector, memory, and CD8+ FoxP3- CD25+ cells is the CD8+ gate. The parent gate of the CD8+ cells is the single cell gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

Active CD4+ Tregs (in the figure only referred to as CD4+ Tregs) in the blood are characterised by the expression of both, FoxP3 and CD25. This cell type was generally lowly found in the blood with frequencies of up to 1.2 % of the CD4+ T cells in C57BL/6J, 0.3 % in NZM2410 and 0.6 % in SLE123 mice. CD4+ Tregs were lower in NZM2410 than C57BL/6J mice at all measured ages, which was significant at the age of 9 weeks ($p \leq 0.001$) (**Figure 36 A**).

Naive CD4+ Tregs showed similar frequencies among NZM2410 mice, SLE123, and C57BL/6J mice. The values were ranging from 4.3 % to 36.7 % in NZM2410, 10.3 % to 33.0 % in SLE123 and 5.2 % to 53.5 % in C57BL/6J mice. Only in week 16, NZM2410 mice had significantly higher frequencies ($p \leq 0.001$, mean NZM2410 36.6 %, C57BL/6J 13.3 %) (**Figure 36 B**).

Effector CD4+ Tregs had higher frequencies in the blood of NZM2410 mice than in C57BL/6J mice. This was significant in weeks 9 ($p = 0.002$, mean NZM2410 25.5 %, C57BL/6J 3.3 %), 19 ($p \leq 0.001$, mean NZM2410 44.6 %, C57BL/6J 6 %), and 21 ($p = 0.002$, NZM2410 34.1 %, C57BL/6J 5.0 %). SLE123 mice increased in effector CD4+ Tregs from 0.0 % in week 8 to 23.1 % in week 18, but this was never significantly different from C57BL/6J mice (**Figure 36 C**).

Among the frequencies of **central memory CD4+ Tregs**, there was no significant difference between the genotypes at any measured week. Yet, the frequencies were varying in NZM2410 mice from 31.9 % to 71.4 %, in SLE123 from 43.0 % to 71.1 %, and in C57BL/6J mice from 12.6 % to 67.3 % (**Figure 36 D**).

Inactive CD4+ Tregs, which do not express CD25, increased in C57BL/6J mice from week 12 (0.4 %) to week 13 (8.8 %) and week 14 (13.2 %). They decreased in week 16 to 2.8 %. NZM2410 mice were not showing any peaks in inactive CD4+ Tregs and ranged from 0.6 % to 2.0 %. However, NZM2410 mice had significantly lower frequencies of inactive Tregs at the ages of 9 weeks ($p = 0.002$, mean NZM2410 0.6%, C57BL/6J 2.1 %) and 16 weeks ($p = 0.002$, mean NZM2410 0.6 %, C57BL/6J 2.8 %). SLE123 mice showed a peak of 6.2 % in week 16, otherwise, the frequencies ranged from 0.8 % to 4.2 %. SLE123 mice only demonstrated a significant increases in the inactive Tregs at week 12 ($p = 0.003$, mean SLE123 1.4 %, C57BL/6J 0.4 %), and a decreased frequency at week 17 ($p \leq 0.001$, mean SLE123 1.2 %, C57BL/6J 4.6 %) (**Figure 36 E**).

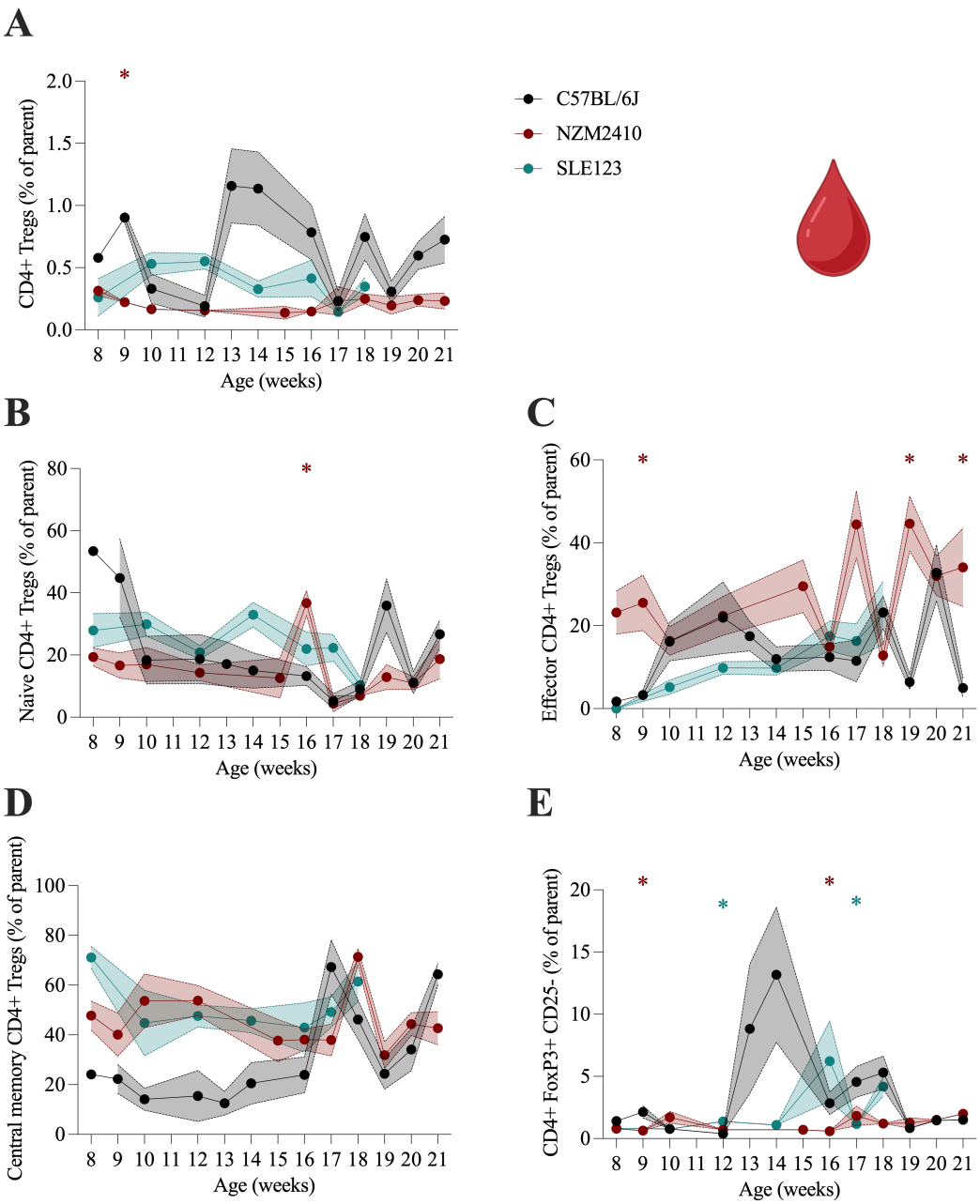


Figure 36: NZM2410 mice have less regulatory T cells in the blood than C57BL/6J mice but more effector regulatory CD4+ T cells

CD4+ regulatory T cells (Tregs) were measured in the blood by flow cytometry for lupus-prone NZM2410 and SLE123 mice compared to C57BL/6J mice. Tregs are characterised by expression of FoxP3 and CD25 among CD4+ T cells (**A**). The Tregs were further subdivided into naive CD4+ Tregs (**B**), effector CD4+ Tregs (**C**), and central memory CD4+ Tregs (**D**). Inactive CD4+ Tregs, which express FoxP3 but not CD25 were also analysed (**E**). The parent gate of naive, effector, and memory Tregs is the CD4+ Treg gate. The parent gate of the CD4+ Tregs as well as the CD4+ FoxP3+ CD25- cells is the CD4+ gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

CD4+ Tregs in the blood were further investigated for their expression of helios, the proliferation marker Ki-67, and the markers CTLA-4 and CD39, which characterise their repressive capabilities. High variances but no significant differences among genotypes were found for the expression of **helios**. The frequencies of helios+ CD4+ Tregs were ranging from 13.5 % to 32.3 % in C57BL/6J mice, from 12.6 % to 28.4 % in NZM2410 mice, and from 9.5 % to 22.5 % in SLE123 mice (**Figure 37 A**).

SLE123 mice had higher frequencies of **Ki-67+ Tregs** than C57BL/6J mice until the age of 16 weeks (mean week 16 SLE123 40.5 %, C57BL/6J 6.4 %). This effect was significant at the ages of 10 ($p= 0.016$), 12 ($p\leq0.001$), 14 ($p\leq0.001$), and 16 weeks ($p= 0.001$). NZM2410 mice showed two peaks in the expression of Ki-67 at the ages of 10 weeks (mean 41.6 %) and 18 weeks (mean 70.5 %) as for other white blood cell numbers measured by the ScilVet ABC Plus+. This was significantly higher than in C57BL/6J mice at the age of 18 ($p\leq0.001$, mean C57BL/6J 32.9 %), and 20 weeks ($p\leq0.001$, mean NZM2410 37.6 %, C57BL/6J 6.0 %) (**Figure 37 B**).

The above-described peaks in expressions of the blood in NZM2410 mice could also be seen in the CTLA-4+ and CD39+ Treg frequencies. This was significant for **CTLA-4** at both, the age of 10 weeks ($p= 0.002$, mean NZM2410 16.8 %, C57BL/6J 1.3 %) and 18 weeks ($p\leq0.001$, mean NZM2410 44.6 %, C57BL/6J 5.5 %). SLE123 mice had higher CTLA-4+ Tregs frequencies at the age of 12 weeks ($p\leq0.001$, mean SLE123 20.8 %, C57BL/6J 0.5 %) (**Figure 37 C**).

For **CD39**, it was significant at the age of 18 weeks in NZM2410 mice ($p\leq0.001$, mean NZM2410 75.1 %, C57BL/6J 42.2 %). For SLE123 mice, it ranged from 41.7 % to 62.9 %, but there were no detectable significant differences in the frequencies of CD39+ in Tregs of SLE123 compared to C57BL/6J mice (**Figure 37 D**).

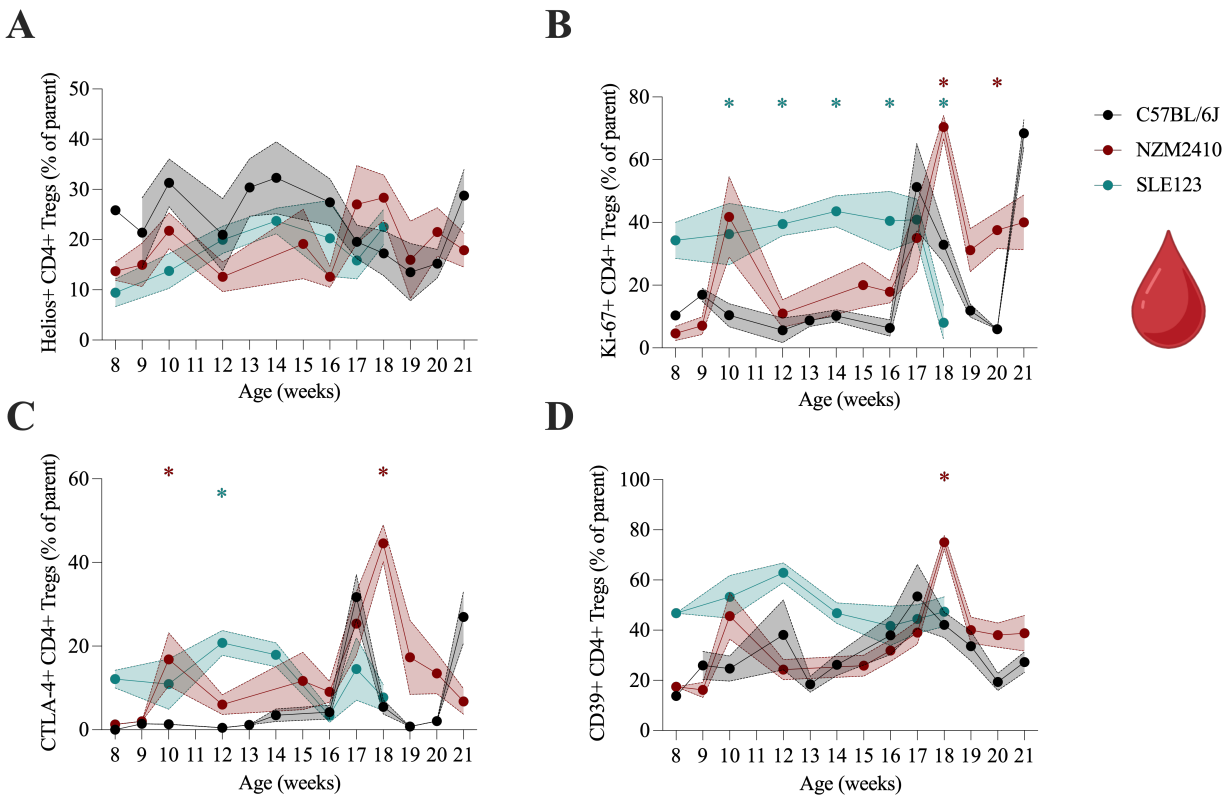


Figure 37: Lupus-prone mice reveal a higher Ki-67 expression in CD4+ regulatory T cells during the pre-disease phase in the blood
Regulatory T cells (Tregs) were measured in the blood by flow cytometry and investigated for their expression of helios (**A**), Ki-67 (**B**), CTLA-4 (**C**), and CD39 (**D**). Parent gate for all these markers was the Treg gate. Analysed mouse lines were the lupus-prone SLE123 and NZM2410 mice, which were compared to C57BL/6J mice. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

CD8+ Treg frequencies of all CD8+ T cells in the blood were low in all genotypes and ranged from 0.0 % to 1.7 % in C57BL/6J, 0.2 % to 0.4 % in NZM2410, and 0.1 % to 0.7 % in SLE123 mice. However, SLE123 mice had higher frequencies of CD8+ Tregs compared to C57BL/6J mice at the ages of 10 (p= 0.002, mean SLE123 0.7 %, C57BL/6J 0.0 %), 12 (p= 0.001, mean SLE123 0.7 %, C57BL/6J 0.0 %), and 18 weeks (p= 0.004, mean SLE123 0.6 %, C57BL/6J 0.21 %). At 17 weeks, the frequency decreased significantly below the one of C57BL/6J mice (p= 0.003, mean SLE123 0.1 %, C57BL/6J 0.6 %). NZM2410 mice had only lower CD8+ Tregs frequencies at the age of 21 weeks ($p \leq 0.001$, mean NZM2410 0.1 %, C57BL/6J 1.7 %) (**Figure 38 A**).

Inactive CD8+ Tregs, which expressed FoxP3 but lacked CD25, were significantly higher in NZM2410 mice at the age of 21 weeks only ($p \leq 0.001$, mean NZM2410 1.4 %, C57BL/6J 0.1 %).

SLE123 mice did not significantly differ from C57BL/6J mice. (**Figure 38 B**).

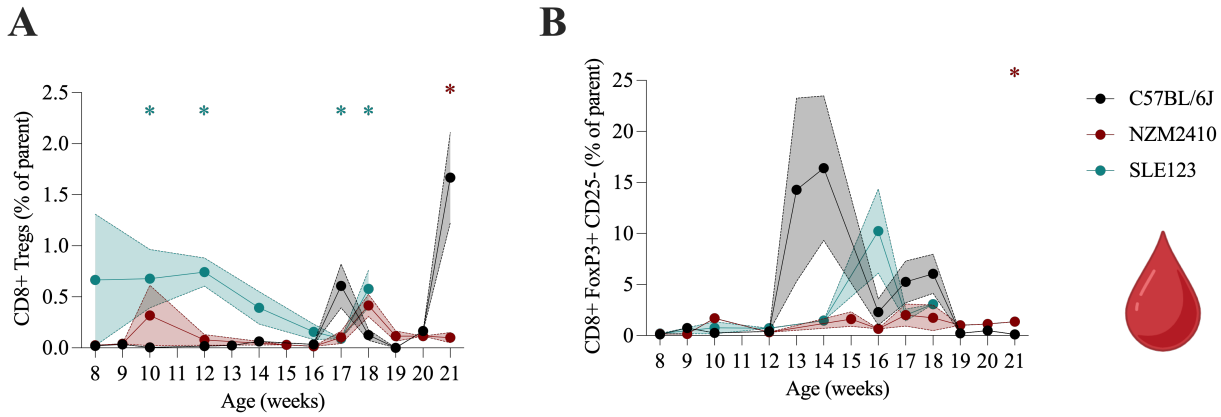


Figure 38: SLE123 mice have more CD8+ regulatory T cells in their blood than C57BL/6J mice
CD8+ regulatory T cells (Tregs) were measured by flow cytometry in the blood of lupus-prone NZM2410 and SLE123 mice during the pre-disease phase from the age of 8 weeks to 21 weeks. This was compared to C57BL/6J mice. CD8+ Tregs are characterized by the expression of CD8, FoxP3 and CD25 (**A**). In-active CD8+ Tregs do not express CD25 (**B**). Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

The chemokine receptors CXCR4 and CX3CR1 were chosen as both have been recognised to contribute to SLE. CXCR4 is known to be dysregulated in this disease (L.-d. Zhao *et al.* 2017), and CR3CR1 has been found to contribute to the development of nephritis (Cabana-Puig *et al.* 2023). Less CD4+ T cells expressed **CX3CR1** most of the measured times in NZM2410 mice compared to C57BL/6 mice. This was significant at the ages of 9 (p= 0.006, mean NZM2410 1.6 %, C57BL/6J 4.8 %), 12 (p= 0.007, mean NZM2410 4.3 %, C57BL/6J 15.2 %), 16 ($p \leq 0.001$, mean NZM2410 5.7 %, C57BL/6J 21.1 %), and 18 weeks (p= 0.002, mean NZM2410 6.7 %, C57BL/6J 21.3 %). However, the expression had also two peaks, exceeding the frequencies of C57BL/6J mice at the ages of 10 weeks (p= not significant, mean NZM2410 14.7 %, C57BL/6J 12.3 %) and 21 weeks (p= 0.002, NZM2410 23.3 %, C57BL/6J 3.0 %) (**Figure 39 A**). Similar peaks could also be seen for **CXCR4** in CD4+ T cells at the ages of 10 (mean NZM2410 0.5 %) and 17 weeks (mean NZM2410 1.5 %) but this finding was not significant. The expression of CXCR4 in CD4+ T cells of NZM2410 mice was significantly lower at the ages of 9 weeks (p= 0.006, mean NZM2410 0.1 %, C57BL/6J 0.6 %) and 16 weeks (p=<0.001, mean NZM2410 0.4 %, C57BL/6J 1.3 %) (**Figure 39 B**). For SLE123 mice, the expressions of CXCR4 and CX3CR1 was similar in CD4+ T cells, compared to those of C57BL/6J mice.

NZM2410 mice showed lower expression frequencies of CX3CR1 on CD8+ T cells in the blood at

most tested ages ranging from 5.4 % to 63.1 % in NZM2410 and from 6.0 % to 62.8 % in C57BL/6J mice (significant at the age of 12 weeks, $p \leq 0.001$, mean NZM2410 5.4 %, C57BL/6J 62.8 %), only exceeding the ones of C57BL/6J mice at the ages of 18 (NZM2410 63.1 %, C57BL/6J 38.7 %) and 21 weeks (NZM2410 46.0 %, C57BL/6J 6.0 %). The frequencies of CX3CR1+ CD8+ T cells in SLE123 mice was ranging from 22.3 % to 58.3 %. However, this was never significantly different from C57BL/6J mice (**Figure 39 C**).

For CXCR4 on CD8+ T cells, the variances were high in NZM2410 and SLE123 mice, but showed significant peaks for SLE123 mice at the age of 16 weeks ($p \leq 0.001$, mean SLE123 3.1 %, C57BL/6J 0.4 %) and for NZM2410 mice at 18 weeks ($p \leq 0.001$, mean NZM2410 1.2 %, C57BL/6J 1.1 %) (**Figure 39 D**).

No differences could be found in the expression of CX3CR1 on Tregs in any genotype. However, Tregs expressed higher frequencies of CXCR4 (up to 47.2 % in C57BL/6J, 65.4 % in NZM2410, and 57.0 % in SLE123 mice) compared to CXCR4+ frequencies in CD4+ (up to 2.3 % in C57BL/6J, 1.5 % in NZM2410, and 1.7 % in SLE123 mice) and CD8+ T cells (up to 1.3 % in C57BL/6J, 2.1 % in NZM2410, and 3.1 % in SLE123 mice). SLE123 mice showed a significant peak in CXCR4 expression on Tregs at the age of 12 weeks ($p \leq 0.001$, SLE123 27.9 %, C57BL/6J 2.0 %) (**Figure 39 E and F**).

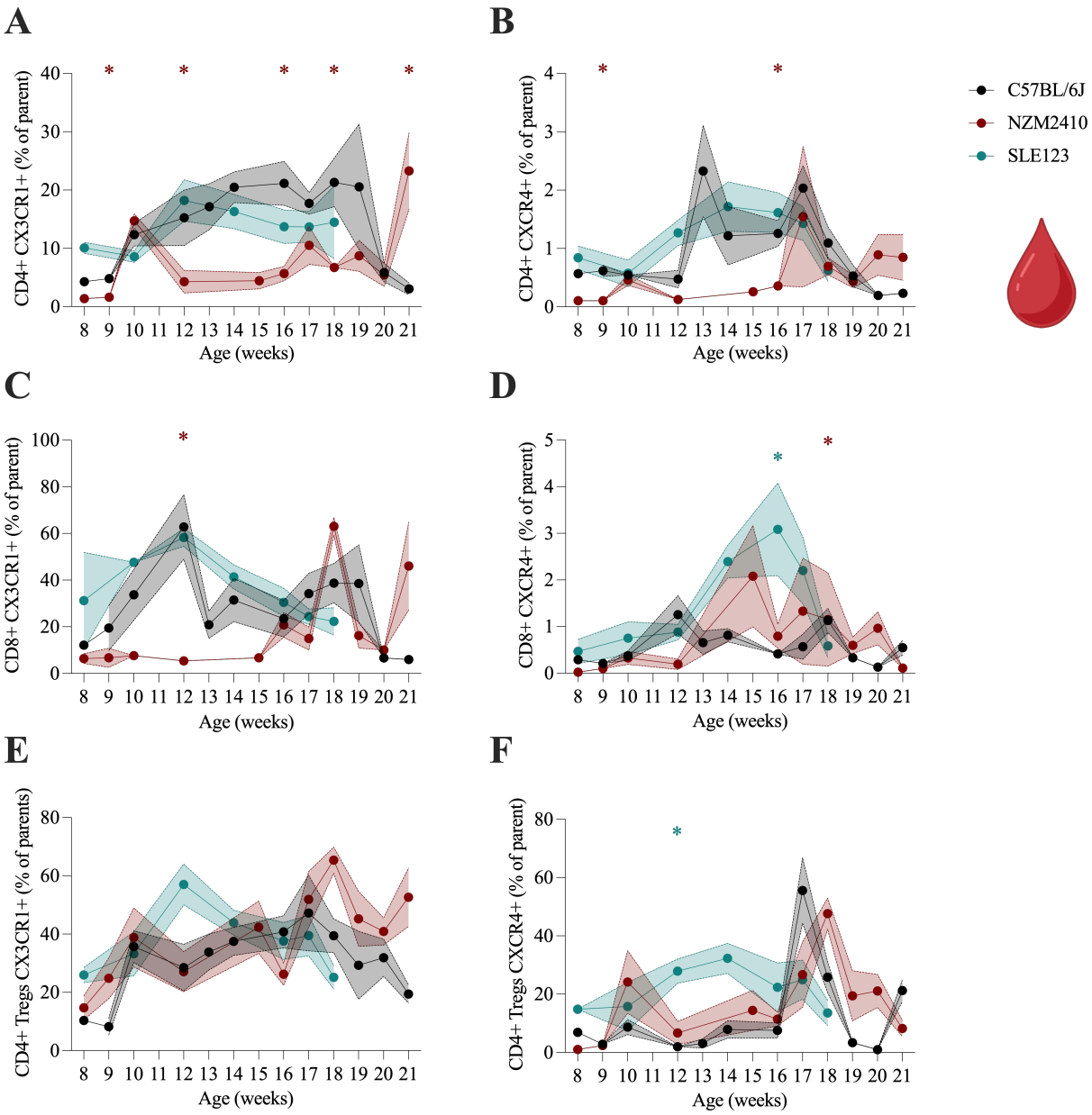


Figure 39: Lupus-prone mice express complex patterns of expression frequencies of CX3CR1 and CXCR4 on T cells in the blood

The trafficking markers CXCR4 and CX3CR1 were measured using flow cytometry in the blood of lupus-prone mouse lines NZM2410 and SLE123 compared to C57BL/6J mice between the ages of 8 and 21 weeks. CX3CR1 was measured in CD4+ T cells (**A**), CD8+ T cells (**C**), and CD4+ regulatory T cells (Tregs) (**E**). CXCR4 was also measured in CD4+ T cells (**B**), CD8+ T cells (**D**), and CD4+ Tregs (**F**). Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

3.1.6 Differences in T cells were found in NZM2410 and SLE123 mice compared to C57BL/6J mice in the spleen

As for the blood, the same flow cytometric measurements of T cells were also performed with the spleens of lupus-prone NZM2410 and SLE123 mice, and then each was compared to C57BL/6J mice. The spleen was chosen as it is a major lymphatic organ, orchestrating the activation of naive T cells by their interaction with antigen-presenting cells.

CD4+ T cells in the spleens had frequencies varying from 12.8 % to 19.1 % in C57BL/6J, 21.8 % to 26.8 % in NZM2410, and 19.1 % to 23.2 % in SLE123 mice. NZM2410 mice had significantly higher frequencies at all tested ages [age 8 ($p= 0.019$), 9 ($p\leq0.001$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p\leq0.001$), 18 ($p\leq0.001$), 19 ($p= 0.007$), 20 ($p\leq0.001$), and 21 weeks ($p\leq0.001$)]. For SLE123 mice this was significant at the ages of 10 ($p= 0.002$), 12 ($p= 0.014$), 14 ($p\leq0.001$), 16 ($p\leq0.001$), and 18 weeks ($p\leq0.001$) (**Figure 40 A**).

The frequencies of **naive CD4+ T cells** of NZM2410 were above the ones of C57BL/6J mice until the age of 17 weeks (mean NZM2410 17 weeks old 51.7 %, C57BL/6J 39.3 %). This was significant at the ages of 9 weeks ($p= 0.001$) and 16 weeks ($p\leq0.001$). Thereafter the frequency decreased and was at the age of 21 weeks significantly below the one of C57BL/6J mice ($p= 0.002$, mean NZM2410 41.2 %, C57BL/6J 53.8 %). The frequency of naive CD4+ T cells was also higher in SLE123 mice compared to C57BL/6J mice until the age of 17 weeks. This was significant at the age of 10 weeks ($p= 0.002$, mean SLE123 60.3 %, C57BL/6J 41.1 %). After 17 weeks, it dropped and was at the age of 18 weeks significantly below the frequency observed in C57BL/6J mice ($p\leq0.001$, mean SLE123 30.0 %, C57BL/6J 46.9 %) (**Figure 40 B**).

Effector CD4+ T cell frequencies were first similar to those of C57BL/6J mice and started rising just before the naive T cell frequencies dropped. They rose in C57BL/6J mice from week 8 to week 21 from 20.1 % to 21.9 %, in NZM2410 mice from 17.0 % to 30.4 %, and in SLE123 17.8 % to 47.1 % until week 18. The frequencies in NZM2410 mice were significantly above those of C57BL/6J mice at the ages of 17 ($p= 0.003$), 18 ($p= 0.001$), and 21 weeks ($p= 0.002$), and for SLE123 mice at the ages of 14 ($p= 0.002$), 17 ($p\leq0.001$), and 18 weeks ($p\leq0.001$) (**Figure 40 C**).

Central memory CD4+ T cell frequencies in the spleens of NZM2410 and SLE123 mice were lower than in C57BL/6J mice. The frequencies were ranging from 18.1 % to 34.6 % in C57BL/6J, from 8.7 % to 21.1 % in NZM2410, and from 12.6 % to 22.7 % in SLE123 mice. This was significant for NZM2410 mice at the ages of 8 ($p= 0.019$), 9 ($p\leq0.001$), 10 ($p= 0.019$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.006$), 19 ($p\leq0.001$), and 20 weeks ($p\leq0.001$). For SLE123 mice, this was significant at the ages of 10 ($p= 0.002$), 12 ($p= 0.001$), 14 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p\leq0.001$), and 18 weeks ($p\leq0.001$). Only at the age of 21 weeks, NZM2140 mice had a similar frequency of

central memory CD4+ T cells than C57BL/6J mice (mean C57BL/6J 18.1 %, NZM2410 21.1 % (**Figure 40 D**).

Low frequencies of **CD4+CD8+ T cells** in the spleen were measured at all ages (up to 0.6 % in C57BL/6J, 1.0 % in NZM2410, and 0.8 % in SLE123 mice). These values were comparably stable in C57BL/6J mice (ranging between 0.2 % and 0.6 %), while in NZM2410 mice it peaks to 1.0 % at the age of 15 weeks but this is not significant. SLE123 mice had significantly elevated CD4+CD8+ T cell frequencies at the age of 16 weeks ($p \leq 0.001$) with a value of 0.8 % (**Figure 40 E**).

Activated CD4+ T cells, which co-expressed CD25, ranged in C57BL/6J mouse spleens between 1.4 % and 6.3 %, in NZM2410 mice between 2.0 % and 9.4 %, and in SLE123 mice between 3.8 % and 15.0 %. Especially in weeks 10 ($p = 0.002$, mean SLE123 14.0 %, mean C57BL/6J 4.4 %) and 12 ($p \leq 0.001$, mean SLE123 15.0 %, mean C57BL/6J 6.0 %), they were strongly increased in SLE123 mice compared to C57BL/6J mice. Also in weeks 14 ($p = 0.014$, mean SLE123 5.9 %, mean C57BL/6J 3.5 %) and 17 ($p \leq 0.001$, mean SLE123 4.8 %, mean C57BL/6J 2.6 %), they were significantly higher in SLE123 mice compared to C57BL/6J mice. In week 16, SLE123 mice had significantly lower frequencies of activated CD4+ T cells ($p = 0.003$, mean SLE123 4.0 %, mean C57BL/6J 6.3%). The activated CD4+ T cells were significantly elevated in NZM2410 mice only at the age of 18 weeks ($p \leq 0.001$) and 21 weeks ($p = 0.002$). At the other tested weeks, the frequencies were either comparable to the ones of C57BL/6J mice or below at the ages of 9 ($p \leq 0.001$), 12 ($p \leq 0.001$), and 16 weeks ($p \leq 0.001$) (**Figure 40 F**).

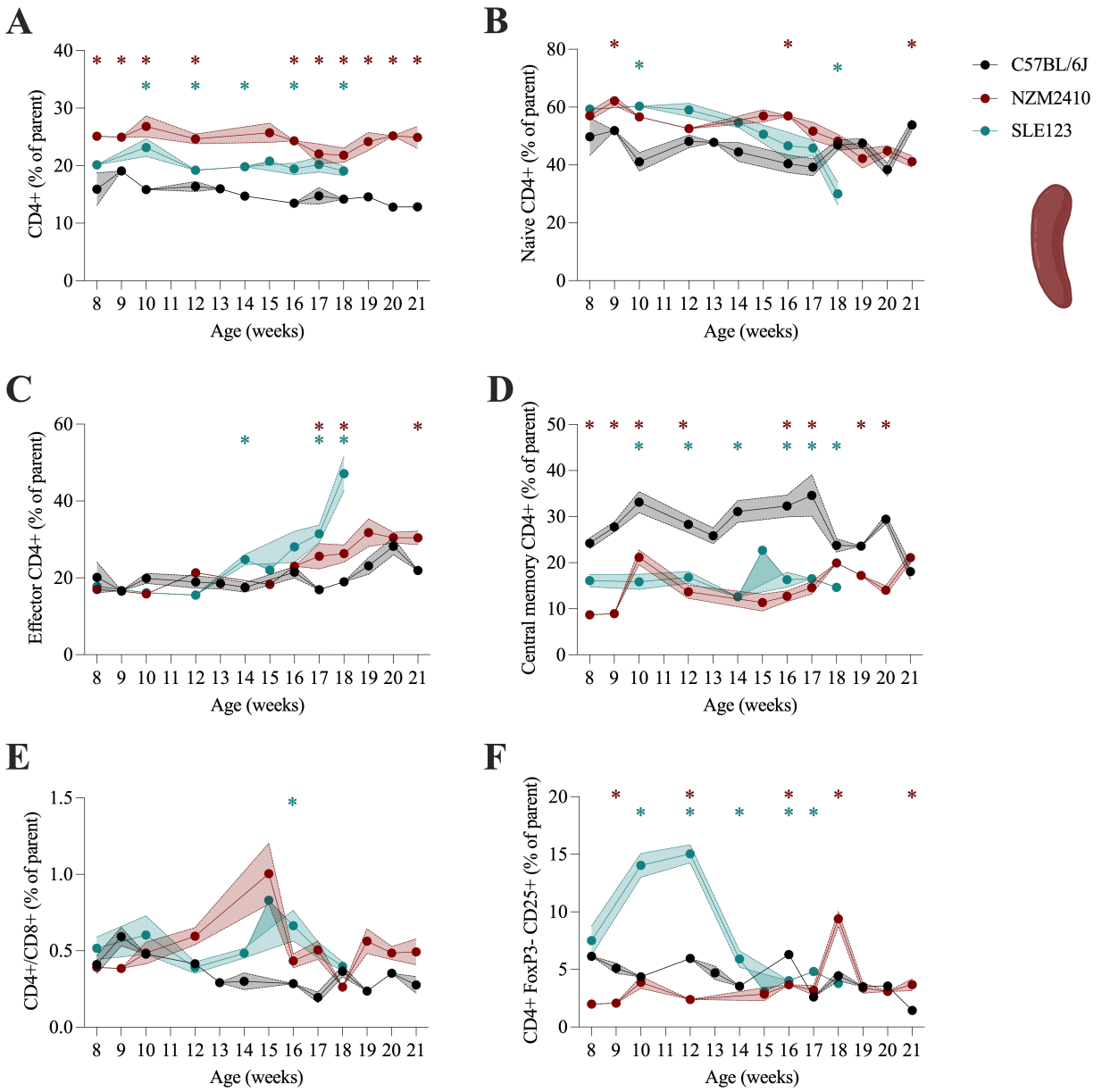


Figure 40: Lupus-prone mice had higher CD4+ T cell frequencies but lower central memory CD4+ T cell frequencies in the spleen

CD4+ T cells were measured using flow cytometry in the spleen of lupus-prone NZM2410 and SLE123 mice compared to C57BL/6J mice at the ages of 8-21 weeks (**A**). The CD4+ T cells were further classified into naive CD4+ T cells (**B**), effector CD4+ T cells (**C**), and central memory CD4+ T cells (**D**). Further, CD4+/CD8+ T cells were analysed (**E**) and activated CD4+ T cells, which also express CD25 (**F**). The parent gate of naive, effector, memory, and CD4+ FoxP3- CD25+ cells is the CD4+ gate. The parent gate of the CD4+ as well as the CD4+/CD8+ cells is the single cell gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

Frequencies of **CD8+ T cells** in spleens of NZM2410 mice were lower than in C57BL/6J mice at the ages of 9 (p= 0.002), 19 (p≤0.001), and 20 weeks (p≤0.001). The variance over the age from 8 weeks to 21 weeks was between 7.7 % and 12.7 % for C57BL/6J and from 7.8 % to 10.7 % in NZM2410 mice. In SLE123 mice, this was different as the frequencies were at all measured ages above those of C57BL/6J mice, ranging from 10.6 % to 15.5 %. This effect was significant at the ages of 10 (p≤0.001), 12 (p≤0.001), 14 (p≤0.001), and 16 weeks (p≤0.001) (**Figure 41 A**).

NZM2410 mice revealed higher frequencies of **naive CD8+ T cells** in the spleens at the all ages, ranging between 65.3 % and 76.4 %, with significant differences compared to C57BL/6J mice at the ages of 9 (p= 0.001), 16 (p≤0.001), 18 (p≤0.001), and 20 weeks (p= 0.005). For C57BL/6J mice, the frequencies were stable over time (similar to NZM2410 mice), spanning from 57.7 % to 69.2 %. Naive CD8+ T cells in SLE123 mice were first above the ones of C57BL/6J mice [maximum of 79.3 %; significant at week 10, (p= 0.002), 12 (p≤0.001), and 14 (p= 0.004)] and then dropped to 48.1 % in week 18, which was significantly below the one's of C57BL/6J mice (p≤0.001, mean C57BL/6J 65.5 %) (**Figure 41 B**).

Effector CD8+ T cells in the spleen started with similar frequencies of all genotypes at the age of 8 weeks (3.8 % in C57BL/6J, 3.3 % in NZM2410, and 3.2 % in SLE123). Thereafter, the lupus-prone mice demonstrated significantly lower effector CD8+ T cell frequencies at the age of 10 weeks (NZM2410 p≤0.001, mean NZM2410 2.3 %; SLE123 p= 0.002, mean SLE123 1.7 %, and C57BL/6J 4.3 %). This difference was still significant for SLE123 mice at the age of 12 weeks (p≤0.001). Thereafter, the frequencies in the lupus-prone mice rose. At the age of 18 weeks, SLE123 mice showed significantly higher frequencies than C57BL/6J mice (p≤0.001, mean SLE123 9.2 %, C57BL/6J 3.9 %). In the NZM2410 mice, this was increasing as well, exceeding the frequencies of C57BL/6J mice at the age of 15 weeks, however, this was not significant (**Figure 41 C**).

The frequencies of **central memory CD8+ T cells** covered values from 26.3 % to 37.1 % in C57BL/6J, from 9.3 % to 20.6 % in NZM2410, and from 17.3 % to 39.1 % in SLE123 mice. Throughout all measured time points, NZM2410 mice had significantly lower central memory CD8+ T cell frequencies [8 weeks (p= 0.019), 9 (p≤0.001), 10 (p= 0.012), 12 (p≤0.001), 16 (p≤0.001), 17 (p= 0.011), 18 (p≤0.001), 19 (p= 0.009), 20 (p≤0.001), 21 (p= 0.001)]. SLE123 mice started also with significantly lower frequencies at the ages of 10 (p= 0.002), 12 (p≤0.001), and 14 weeks (p= 0.001), but then increased to significantly higher levels than C57BL/6J mice at the age of 18 weeks (p= 0.001) (**Figure 41 D**).

The **activated CD8+ T cells**, which also express CD25, were low in NZM2410 and C57BL/6J mice with frequencies below 2.4 %. NZM2410 mice only demonstrated one sharp peak at the

of 18 weeks with a frequency of 64.8 % ($p \leq 0.001$). However, even during the other weeks, the values were significantly lower than in C57BL/6J mice at the ages of 9 ($p \leq 0.001$), 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), 17 ($p = 0.003$), 19 ($p \leq 0.001$), 20 ($p \leq 0.001$), and 21 weeks ($p \leq 0.001$). SLE123 mice had high frequencies of activated CD8+ T cells until the age of 12 weeks with frequencies up to 74.4 %. This was significantly higher than in C57BL/6J mice in weeks 10 ($p = 0.002$), and 12 ($p \leq 0.001$). Thereafter it decreased to 7.9 % in week 14 and lower until 0.3 % in week 18 (**Figure 41 E**).

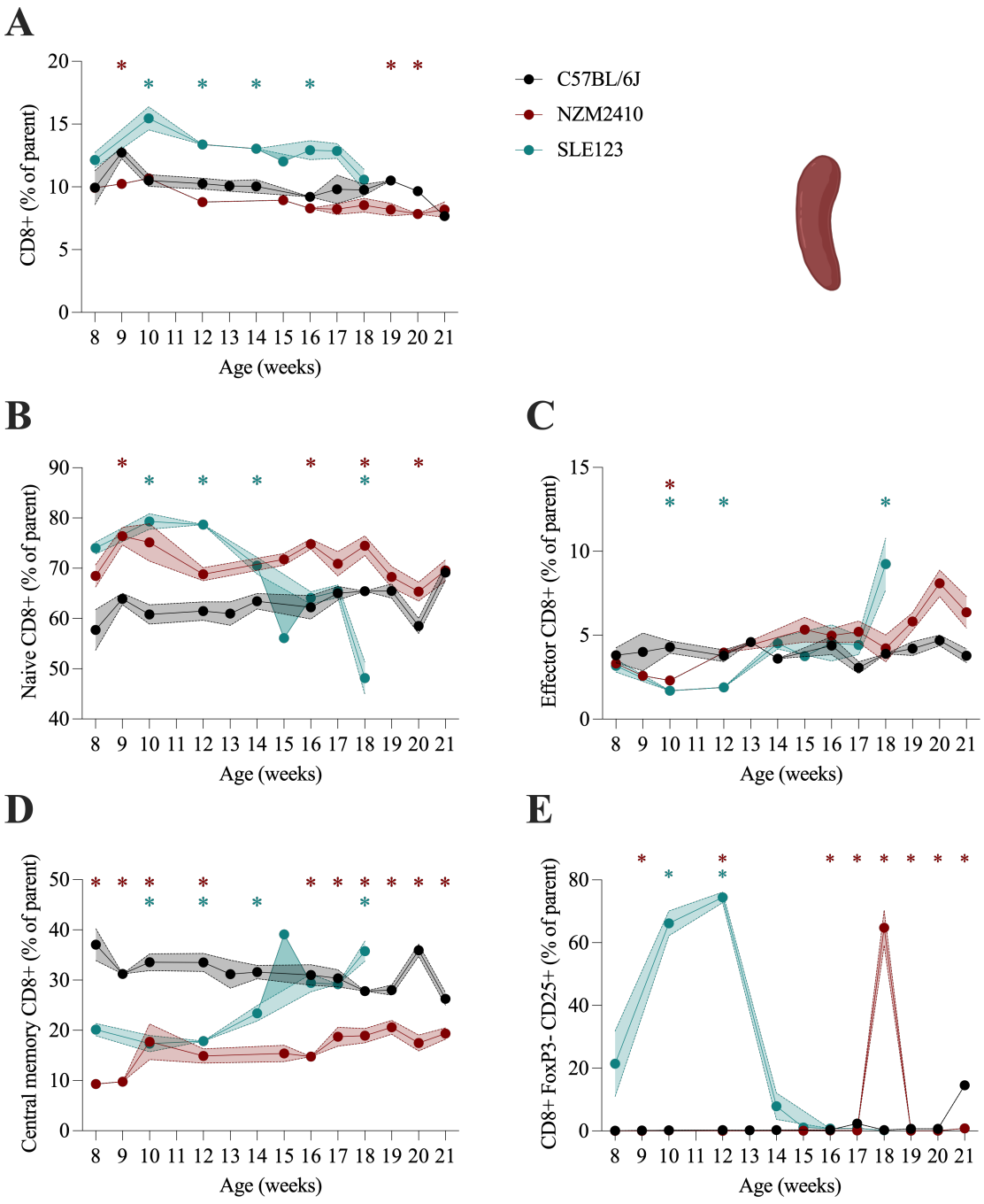


Figure 41: While naive CD8+ T cell frequencies decrease in spleens of SLE123 mice, the effector CD8+ T cells rise

CD8+ T cells in the spleen were measured by flow cytometry for NZM2410, SLE123, and C57BL/6J mice from the age of 8 weeks to 21 weeks (**A**). The CD8+ T cells were further separated into naive CD8+ T cells (**B**), effector CD8+ T cells (**C**), central memory CD8+ T cells (**D**), and activated CD8+ T cells (**E**). The parent gate of naive, effector, memory, and CD8+ FoxP3- CD25+ cells is the CD8+ gate. The parent gate of the CD8+ cells is the single cell gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

CD4+ Tregs in the spleens of NZM2410 mice varied from 0.6 % to 3.2 %, in C57BL/6J mice from 1.1 % to 5.8 %, in SLE123 mice from 1.9 % to 4.3 %. NZM2410 mice had lower frequencies of CD4+ Tregs than C57BL/6J mice at the ages of 10 ($p \leq 0.001$), 12 ($p \leq 0.001$), 19 ($p \leq 0.001$), 20 ($p \leq 0.001$), and 21 weeks ($p \leq 0.001$). SLE123 mice showed a peak in the frequency at the age of 12 weeks ($p \leq 0.001$) (**Figure 42 A**).

Inactivated CD4+ Tregs in the spleens increased with age in all mouse strains. In C57BL/6J mice the frequency at week 8 was 1.4 %, which was rising to 11.7 %. In NZM2410 mice, it started with 4.1 %, rising to 6.6 %, and in SLE123 inactivated CD4+ Tregs started with a frequency of 2.2 % and rose to 9.7 % in week 18. However, at weeks 14 ($p = 0.005$) they were lower, and in weeks 16 ($p \leq 0.001$), and 18 ($p = 0.002$), the frequency was significantly higher in SLE123 mice than in C57BL/6J mice. In NZM2410 mice, it was at week 21 significantly lower than in C57BL/6J mice ($p \leq 0.001$) (**Figure 42 B**).

Naive CD4+ Treg frequencies in C57BL/6J mice ranged from 13.0 % to 39.6 %, in NZM2410 from 10.5 % to 35.2 %, and in SLE123 mice from 14.7 % to 27.3 %. Naive CD4+ Tregs showed decreases in their frequency in NZM2410 mice at the age 10 ($p = 0.005$), 19 ($p \leq 0.001$), and 21 weeks ($p \leq 0.001$). At week 9, the frequency was higher than in C57BL/6J mice ($p \leq 0.001$, mean NZM2410 32.6 % and C57BL/6J 17.9 %). SLE123 mice did not show a significantly different naive Treg frequency at any measured time point (**Figure 42 C**).

At similar times, when naive Tregs dropped in NZM2410 mice, **central memory Tregs** showed significant peaks [week 10 ($p = 0.003$, mean NZM2410 65.7 %, C57BL/6J 50.7 %), week 18 ($p \leq 0.001$, mean NZM2410 61.5 %, C57BL/6J 47.1 %)]. In week 9, when naive Treg frequencies were high in NZM2410 mice, the central memory Tregs were significantly below those of C57BL/6J mice ($p \leq 0.001$, mean NZM2410 45.5 %, C57BL/6J 58.6 %). SLE123 mice did not show any significant difference but the frequencies dropped from 60.0 % in week 10 to 40.7 % in week 18 (**Figure 42 D**).

At the age of 12 weeks, the frequency of **effector CD4+ Tregs** in SLE123 mice was significantly below the one of C57BL/6J mice ($p \leq 0.001$, mean SLE123 10.2 %, C57BL/6J 19.2 %). Effector CD4+ Tregs started rising at the age of 14 weeks (22.0 %) and were above the levels of C57BL/6J mice at the age of 17 weeks (SLE123 28.6 %, C57BL/6J 26.1 %). NZM2410 mice showed significantly higher effector Treg frequency at the age of 9 weeks ($p = 0.001$, mean NZM2410 15.4 %, C57BL/6J 11.7 %), even though this percent difference was low. At the age of 18 weeks, a significant drop was measured ($p \leq 0.001$, mean NZM2410 16.8 %, C57BL/6J 28.1 %). Thereafter, it was rising again, exceeding the frequencies of C57BL/6J mice significantly at the age of 21 weeks ($p \leq 0.001$, mean NZM2410 33.5 %, C57BL/6J 12.4 %) (**Figure 42 E**).

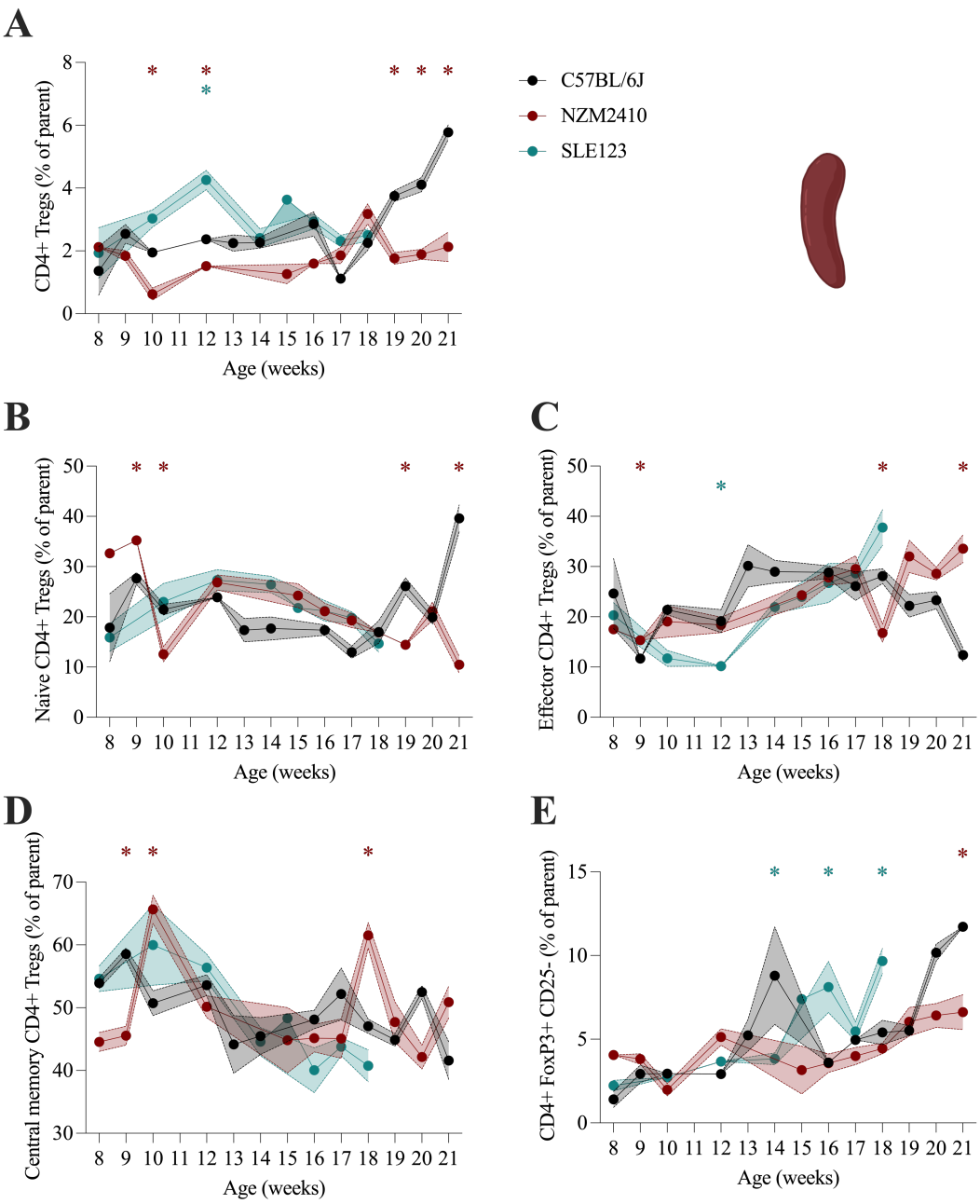


Figure 42: NZM2410 mice show lower CD4+ regulatory T cell frequencies in the spleen than C57BL/6J mice

CD4+ regulatory T cells (Tregs) were measured in the spleen by flow cytometry for lupus-prone NZM2410 and SLE123 mice compared to C57BL/6J mice. Tregs are characterised by expression of FoxP3 and CD25 among CD4+ T cells (**A**). The Tregs were further subdivided into naive CD4+ Tregs (**B**), effector CD4+ Tregs (**C**), and central memory CD4+ Tregs (**D**). CD4+ T cells were also gated for inactive CD4+ Tregs, which express FoxP3 but not CD25 (**E**). The parent gate of naive, effector, and memory Tregs is the CD4+ Treg gate. The parent gate of the CD4+ Tregs as well as the CD4+ FoxP3+ CD25- cells is the CD4+ gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

When going into more detail with the CD4+ Tregs, **helios+ Tregs** were similar in NZM2410 mice compared to C57BL/6J mice, except for week 18, when NZM2410 mice showed significantly lower frequencies ($p \leq 0.001$, NZM2410 23.0 %, C57BL/6J 45.7 %). SLE123 mice started with similar frequencies at week 8 compared to the two other strains (C57BL/6J 25.7 %, NZM2410 32.1 %, SLE123 27.5 %). Thereafter, the frequencies decreased to significantly lower values in SLE123 mice in weeks 10 ($p= 0.002$), 12 ($p \leq 0.001$), and 14 ($p= 0.002$). These were rising again, however, in week 17, they were still significantly below the values of C57BL/6J mice ($p= 0.002$, mean SLE123 35.1 %, C57BL/6J 46.5 %) (**Figure 43 A**).

The **Ki-67** expression on CD4+ Tregs in the spleen changed a lot with the different ages, also in C57BL/6J mice (frequencies ranging from 4.9 % to 33.7 %), which showed a peak at week 17 and 18. However, NZM2410 mice, demonstrated two peaks in Ki-67 expression over time. The first was at week 10 ($p \leq 0.001$, mean NZM2410 24.8 %, C57BL/6J 13.5 %), the second one was at week 18, but this one was not significantly different from the C57BL/6J mice. However, in week 21, NZM2410 mice had again significantly higher frequencies than C57BL/6J mice ($p \leq 0.001$, mean NZM2410 26.3 %, C57BL/6J 4.9 %). SLE123 mice started with comparably high frequencies of 38.2 %, which is significantly above those of C57BL/6J mice at the ages of 10 ($p= 0.002$, mean SLE123 36.5 %, C57BL/6J 13.5 %) and 16 weeks ($p \leq 0.001$, mean SLE123 26.5 %, C57BL/6J 13.3 %). After week 17, it dropped down to 5.4 % in week 18, which was below the levels of C57BL/6J mice ($p \leq 0.001$) (**Figure 43 B**).

The expression of **CTLA-4** was low in C57BL/6J and NZM2410 mice with frequencies from 1.7 % to 25.1 % in C57BL/6J and 3.3 % to 37.1 % in NZM2410 mice. However, both mouse strains showed a peak in early adulthood at the ages of 17 weeks (C57BL/6J, 25.1 %) and 18 weeks (NZM2410 mice, 37.2 %). Therefore, the NZM2410 mice differed from C57BL/6J mice significantly at the age of 17 ($p= 0.003$), 18 ($p \leq 0.001$), and 19 weeks ($p \leq 0.001$). SLE123 mice started with higher frequencies of about 22.0 %, which was above the ones of C57BL/6J mice at the ages of 10 ($p= 0.002$) and 12 weeks ($p \leq 0.001$). Afterwards, the frequencies decreased, which was significantly lower than those of C57BL/6J mice at the of 17 weeks ($p \leq 0.001$, mean SLE123 5.5 %, C57BL/6J 25.1 %) (**Figure 43 C**).

CD39 expression also started higher in SLE123 mice than in the two other mouse strains (C57BL/6J 46.1 %, NZM2410 10.5 %, SLE123 61.8 %). This was significant for SLE123 at the weeks 10 ($p= 0.002$, mean SLE123 81.5 %, C57BL/6J 32.5 %) and 12 ($p \leq 0.001$, mean SLE123 82.7 %, C57BL/6J 45.7 %). Next, it dropped below the frequency of C57BL/6J mice at the age of 15 weeks, which became significant at the ages of 16 ($p= 0.002$, mean SLE123 39.5 %, C57BL/6J 51.3 %) and 17 weeks ($p= 0.006$, mean SLE123 39.5 %, C57BL/6J 61.3 %). NZM2410 mice

showed again a small peak in expression at the age of 10 weeks, which was not significant, but also another peak at the age of 18 weeks, which was ($p \leq 0.001$, mean NZM2410 72.6 %, C57BL/6J 51.1 %). At other measured time points, the frequency was below the one of C57BL/6J mice, which spanned values from 9.3 % to 40.4 %. This was a significant difference at the ages of 9 ($p \leq 0.001$), 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), 17 ($p = 0.003$), and 20 weeks ($p \leq 0.001$) (**Figure 43 D**).

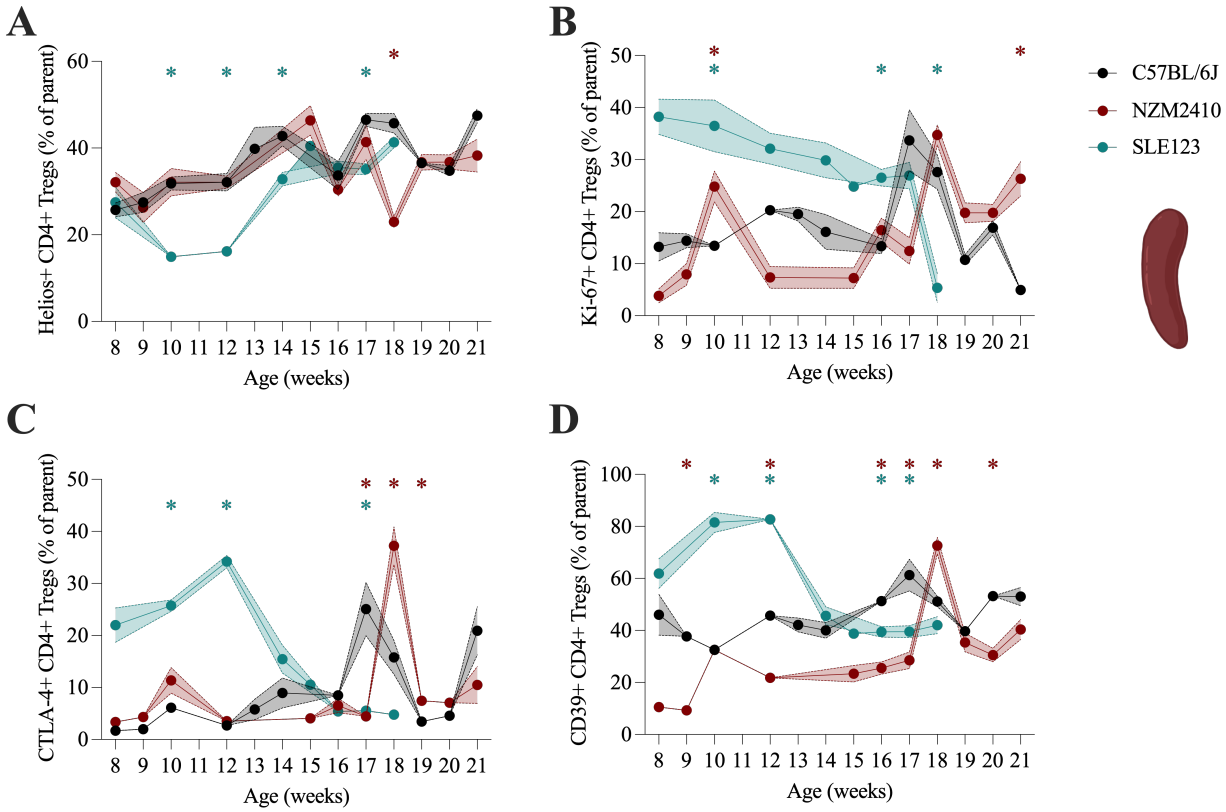


Figure 43: Regulatory T cells of SLE123 mice have lower helios+ but higher Ki-67+, CTLA-4+, and CD39+ frequencies in the spleen before the age of 16 weeks
Regulatory T cells (Tregs) were measured in the spleen by flow cytometry and investigated for their expression of helios (**A**), Ki-67 (**B**), CTLA-4 (**C**), and CD39 (**D**). Parent gate for all these markers was the Treg gate. Analysed mouse lines were the lupus-prone SLE123 and NZM2410 mice, which were compared to C57BL/6J mice. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

CD8+ Tregs were low in C57BL/6J and NZM2410 mice with frequencies below 1.4 %, except for week 18 in NZM2410 mice. At that time, NZM2410 mice showed one peak with values rising up to 3.3 % ($p \leq 0.001$). However, even in the very low ranges the NZM2410 mice had significantly lower frequencies than the C57BL/6J mice at weeks 8 ($p = 0.010$), 9 ($p \leq 0.001$), 10 (p= 0,030636), 12

(p≤0.001), 16 (p≤0.001), 17 (p= 0.001), 19 (p= 0.018), 20 (p≤0.001), 21 (p≤0.001). SLE123 mice demonstrated higher values of 0.1 % to 4.2 %, which were significantly above those of C57BL/6J mice at weeks 10 (p= 0.002) and 12 (p≤0.001). Thereafter, it decreased but was still significantly higher than in C57BL/6J mice at the ages of 16 (p= 0.001, mean SLE123 0.2 %, C57BL/6J 0.1 %). At week 17, SLE123 mice had significantly lower CD8+ Tregs than C57BL/6J mice (p= 0.004, mean SLE123 0.2 %, C57BL/6J 0.4 %) (**Figure 44 A**).

Inactive CD8+ Tregs were low in SLE123 mice until the age of 12 weeks, which was significantly lower than in C57BL/6J mice at the ages of 10 (p= 0.002, mean SLE123 0.3 %, C57BL/6J 1.0 %) and 12 weeks (p≤0.001, mean SLE123 0.3 %, C57BL/6J 1.5 %). Thereafter, the frequency rose and was significantly above C57BL/6J values at the ages of 16 (p≤0.001), 17 (p= 0.006), and 18 weeks (p≤0.001), with a maximum of 9.3 % at week 18. Inactive CD8+ Tregs in NZM2410 mice stayed between a 1.0 % and 2.9 % of all CD8+ T cells, which was at week 20 below C57BL/6J mice (p≤0.001, NZM2410 2.5 %, C57BL/6J 7.4 %) (**Figure 44 B**).

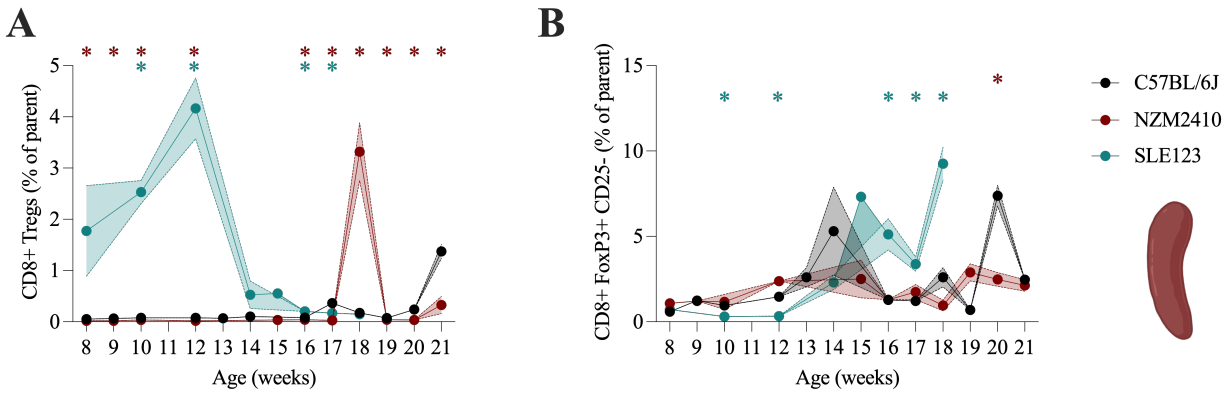


Figure 44: SLE123 mice have higher CD8+ regulatory T cell frequencies in the spleen than C57BL/6J mice
CD8+ regulatory T cells (Tregs) were measured by flow cytometry in the spleens of lupus-prone NZM2410 and SLE123 mice during the pre-disease phase from the age of 8 weeks to 21 weeks. This was compared to C57BL/6J mice. CD8+ Tregs are characterised by the expression of CD8, FoxP3 and CD25 (**A**). In-active CD8+ Tregs do not express CD25 (**B**). Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

The **CX3CR1** expression showed peaks in NZM2410 mice at the ages of 15 and 17 weeks in CD4+, CD8+ T cells and in CD4+ Tregs. This was never significant. SLE123 mice did not peak in CX3CR1 expression at any measured week. At weeks 10 (p= 0.002, mean SLE123 16.2 %, C57BL/6J 45.3 %), 14 (p≤0.001, mean SLE123 22.7 %, C57BL/6J 42.1 %) and 18 (p≤0.001, mean

SLE123 16.4 %, C57BL/6J 40.5 %) the frequency of CX3CR1 in CD4+ T cells of SLE123 mice was below the one of C57BL/6J mice. In CD8+ T cells of SLE123 mice, this was only significantly lower at week 18 (p= 0.001, mean SLE123 27.3 %, C57BL/6J 49.9 %). For CD4+ Tregs of SLE123 mice, it was lower at week 18 ($p \leq 0.001$, mean SLE123 37.6 %, C57BL/6J 63.3 %) but significantly higher at week 12 (p= 0.003, mean SLE123 73.0 %, C57BL/6J 58.4 %) (**Figure 45 A, C, E**).

CXCR4 expression was low in all strains in CD4+ and CD8+ T cells with expression levels of 0.4 % to maximum 5.8 %. For CD4+ T cells in SLE123 mice, the CXCR4 expression was significantly higher at week 12 ($p \leq 0.001$, mean SLE123 4.1 %, C57BL/6J 1.8 %) but significantly lower at week 18 (p= 0.003, mean SLE123 2.4 %, C57BL/6J 5.1 %). For CD8+ T cells in SLE123 mice, it was the opposite with significantly lower frequencies at week 10 (p= 0.002, mean SLE123 0.9 %, C57BL/6J 2.0 %), and significantly higher ones at week 17 (p= 0.002, mean SLE123 2.5 %, C57BL/6J 0.9 %). NZM2410 mice only differed from C57BL/6J mice significantly at weeks 12 (p= 0.001, mean NZM2410 0.4 %, C57BL/6J 1.8 %) and 18 ($p \leq 0.001$, mean NZM2410 0.8 %, C57BL/6J 2.1 %) (**Figure 45 B, D**).

CD4+ Tregs exhibited higher expressions of CXCR4 in all tested mouse lines compared to CD4+ and CD8+ T cells. Tregs of SLE123 mice started with high frequencies of 29.3 % in week 8, which was significantly higher than in C57BL/6J mice at weeks 10 (p= 0.002, mean SLE123 42.0 %, C57BL/6J 11.1 %) and 12 weeks ($p \leq 0.001$, SLE123 48.7 %, C57BL/6J 7.9 %). Thereafter, it dropped below the expression of C57BL/6J, which was significant at weeks 17 ($p \leq 0.001$, mean SLE123 14.0 %, C57BL/6J 33.6 %) and 18 (p= 0.001, mean SLE123 6.0 %, C57BL/6J 22.6 %). NZM2410 mice showed a small insignificant peak at week 10, and a larger, significant peak at week 18 ($p \leq 0.001$, mean NZM2410 44.0 %, C57BL/6J 22.6 %). As C57BL/6J also demonstrated a peak at week 17, they differed significantly from NZM2410 mice (p= 0.003, mean NZM2410 5.3 %, C57BL/6J 33.6 %). Moreover, NZM2410 mice showed significantly lower expressions at weeks 9 (p= 0.001, mean NZM2410 1.7 %, C57BL/6J 4.6 %) and 12 ($p \leq 0.001$, mean NZM2410 3.4 %, C57BL/6J 7.9 %) (**Figure 45 E**).

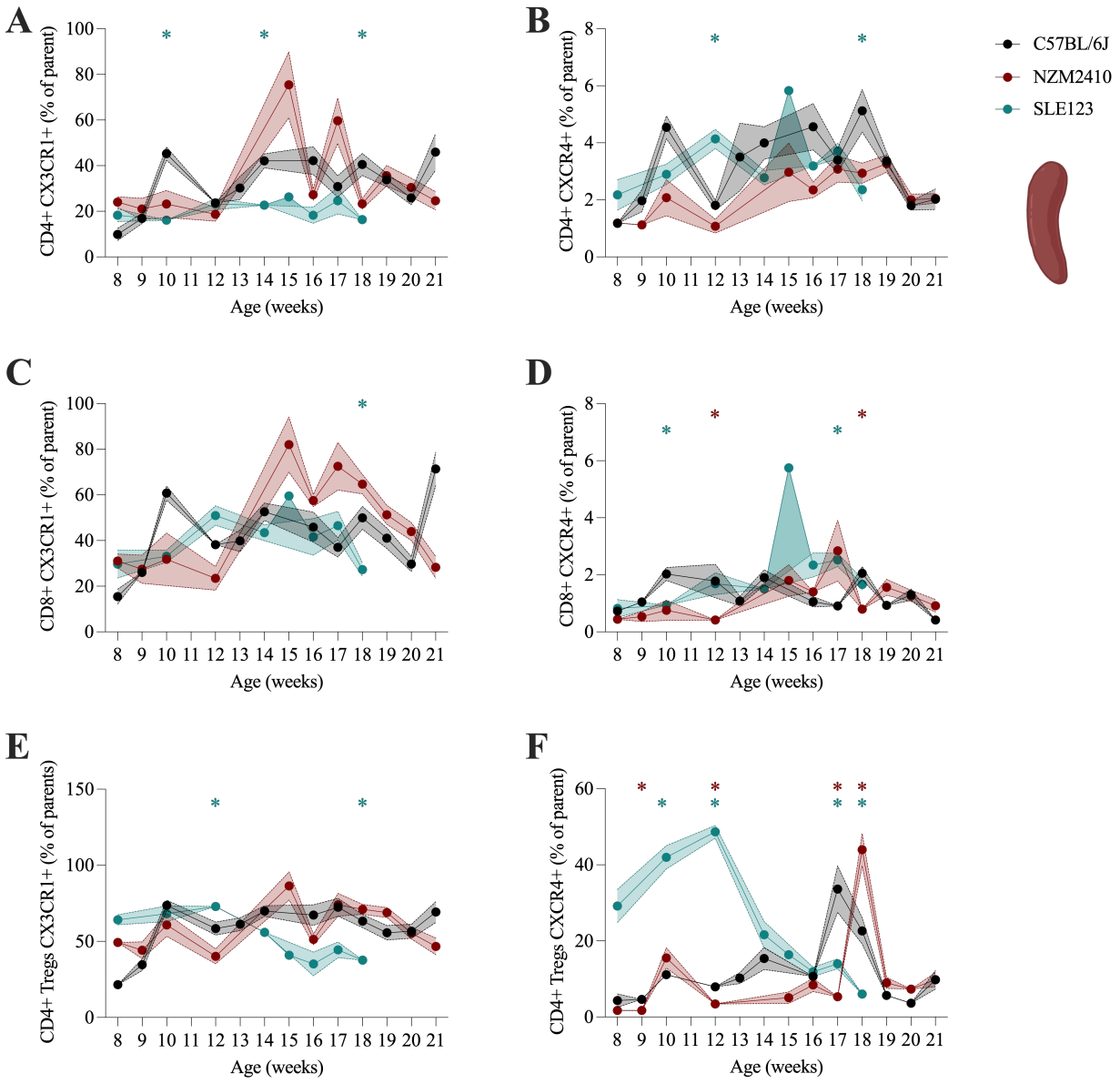


Figure 45: CXCR4 exhibits higher frequencies among CD4+ regulatory T cells than CD4+ and CD8+ T cells in the spleen

The trafficking markers CXCR4 and CX3CR1 were measured using flow cytometry in the spleens of lupus-prone mouse lines NZM2410 and SLE123 compared to C57BL/6J mice between the ages of 8 and 21 weeks. CX3CR1 was measured in CD4+ T cells (**A**), CD8+ T cells (**C**), and CD4+ regulatory T cells (Tregs) (**E**). CXCR4 was also measured in CD4+ T cells (**B**), CD8+ T cells (**D**), and CD4+ Tregs (**F**). Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

3.1.7 Differences in T cells of NZM2410 and SLE123 mice were found during the pre-disease phase in the lymph nodes compared to C57BL/6J mice

The lymph nodes are a draining and distributing organ for immune cells. In those, T and B cells interact with each other, germinal centres are formed and especially Tregs can be studied well in the lymph nodes because these are the primary site of peripheral tolerance by being a main spot for T cell activation and interaction with other cell types such as dendritic cells, other T cells, and B cells. Lymph nodes are also draining surrounding tissues, making them additionally interesting to the spleens.

CD4+ T cells demonstrated higher frequencies in the lymph nodes of NZM2410 mice, ranging from 37.2 % to 60.7 %, compared to C57BL/6J mice, which ranged from 19.1 % to 28.7 %. This was a significant difference at the age of 8 ($p= 0.019$), 9 ($p= 0.002$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 18 ($p\leq0.001$), 20 ($p\leq0.001$), and 21 weeks ($p\leq0.001$). At week 19, there was a reduction, but this underlied high variance. SLE123 mice demonstrated similar CD4+ T cell frequencies in the lymph nodes compared to C57BL/6J mice (**Figure 46 A**).

The **naive CD4+ T cells** were also increased in the NZM2410 mice, significantly exceeding the frequency of C57BL/6J mice at weeks 9 ($p= 0.006$), 10 ($p\leq0.001$), 16 ($p\leq0.001$), 18 ($p\leq0.001$), and 20 ($p\leq0.001$). The frequencies in NZM2410 mice spanned from 65.5 % to 84.8 %, with the lowest value in week 19. C57BL/6J mice varied from 59.7 % to 77.4 %. SLE123 mice started with a low frequency of 59.3 % and then increased to 81.5 % in week 10, and 81.0 % in week 16. In week 18, this reduced again to 62.4 %. However, the difference between SLE123 and C57BL/6J mice was not significant at any measured time point (**Figure 46 B**).

Effector CD4+ T cell frequencies in the lymph nodes varied in C57BL/6J mice from 4.9 % to 10.8 %, in NZM2410 from 3.5 % to 7.8 % and in SLE123 mice from 5.2 % to 17.8 %. They were significantly lower in NZM2410 mice than in C57BL/6J mice at the age of 9 ($p= 0.009$), 10 ($p\leq0.001$), 12 ($p= 0.008$), 16 ($p\leq0.001$), 18 ($p= 0.003$), 20 ($p\leq0.001$), and 21 weeks ($p= 0.003$). Effector CD4+ T cells of SLE123 mice looked opposing to the naive CD4+ T cell frequency. At the age of 8 weeks, it was high with a frequency of 17.8 %, lowering to 5.1 % in week 10, and increasing again at the age of 18 weeks to 16.3 %. This difference was never significantly different from C57BL/6J mice (**Figure 46 C**).

Central memory CD4+ T cell frequencies in the lymph nodes were lower in lupus-prone mice at all measured ages except for week 8 in SLE123 mice. The values fluctuated from 12.6 % to 25.4 % in C57BL/6J, from 4.8 % to 13.8 % in NZM2410, and from 8.0 % to 54.6 % in SLE123 mice. This was significant for NZM2410 at all compared weeks [week 8 ($p= 0.019$), 9 ($p\leq0.001$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.003$), 18 ($p\leq0.001$), 19 ($p= 0.002$), and 20

($p \leq 0.001$)] except for week 21. In SLE123 mice, this difference was significant at weeks 10 ($p = 0.003$), 12 ($p \leq 0.001$), 14 ($p \leq 0.001$), 16 ($p \leq 0.001$), and 17 ($p \leq 0.001$) (**Figure 46 D**).

CD4+CD8+ T cells in the lymph nodes were at most 1.3 % in C57BL/6J mice. In NZM2410 mice, the values reached up to 3.0 % at week 15. Two more peaks could be seen at week 17 and week 20. In-between at week 16 and 18, the values decreased to 1.3 % and 0.9 %. The difference between NZM2410 and C57BL/6J mice was significant at the ages of 12 ($p = 0.004$), 16 ($p \leq 0.001$), 17 ($p = 0.003$), 19 ($p \leq 0.001$), and 21 weeks ($p \leq 0.001$). Even though SLE123 mice showed a peak with values of 2.3 % at week 16, there was no significant difference between them and C57BL/6J mice (**Figure 46 E**).

Activated CD4+ T cells in the lymph nodes were significantly lower in NZM2410 mice at the ages of 9 ($p \leq 0.001$) and 16 weeks ($p \leq 0.001$). SLE123 mice had higher frequencies of activated CD4+ T cells at the ages of 10 ($p = 0.003$), 12 ($p \leq 0.001$), 14 ($p \leq 0.001$), and 17 weeks ($p = 0.009$), but lower ones at 18 weeks ($p \leq 0.001$). For C57BL/6J and NZM2410 mice the values varied from 0.5 % to 5.0 %. SLE123 mice reached values of 16.5 % at week 10 and decreased to 1.8 % at week 18 (**Figure 46 F**).

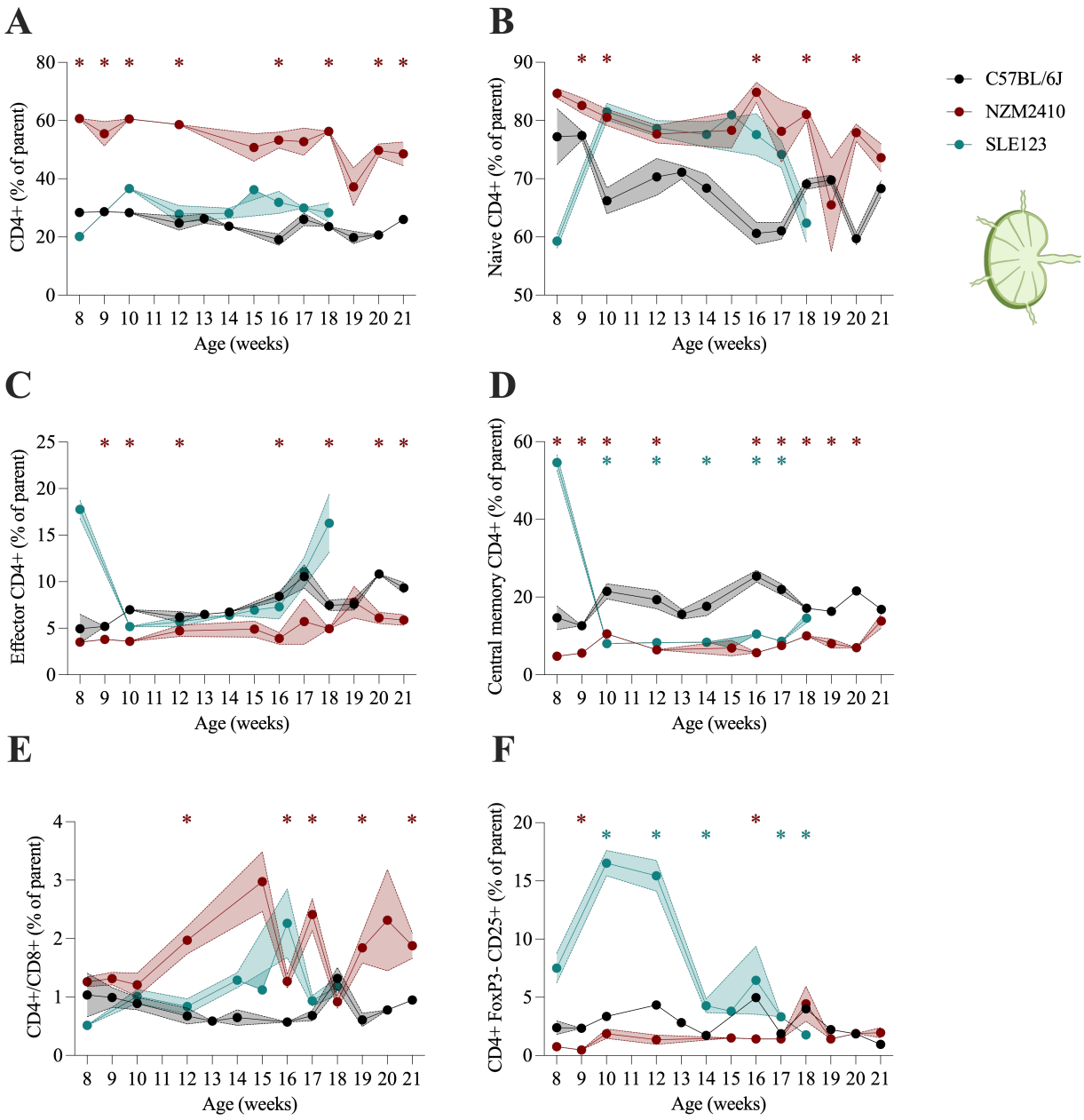


Figure 46: NZM2410 mice reveal higher frequencies of CD4+ T cells but lower central memory T cell frequencies in the lymph nodes

CD4+ T cells were measured using flow cytometry in the lymph nodes of lupus-prone NZM2410 and SLE123 mice compared to C57BL/6J mice at the ages of 8-21 weeks (**A**). The CD4+ T cells were further classified into naive CD4+ T cells (**B**), effector CD4+ T cells (**C**), and central memory CD4+ T cells (**D**). Further, CD4+/CD8+ T cells were analysed (**E**) and activated CD4+ T cells, which also express CD25 (**F**). The parent gate of naive, effector, memory, and CD4+ FoxP3- CD25+ cells is the CD4+ gate. The parent gate of the CD4+ as well as the CD4+/CD8+ cells is the single cell gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

CD8+ T cells in C57BL/6J mice spanned frequencies from 17.5 % to 28.0 %, in NZM2410 mice from 13.8 % to 19.5 %, and in SLE123 mice from 12.1 % to 29.7 %. NZM2410 mice demonstrated significantly less CD8+ T cells than C57BL/6J mice in the lymph nodes at the age of 9 ($p= 0.002$), 12 ($p\leq0.001$), 17 ($p= 0.006$), 20 ($p\leq0.001$), and 21 weeks ($p\leq0.001$). There was no measured significant difference between SLE123 and C57BL/6J mice (**Figure 47 A**).

Naive CD8+ T cells showed higher frequencies in NZM2410 mice at the ages of 8 ($p= 0.010$), 9 ($p\leq0.001$), 10 ($p\leq0.001$), 12 ($p= 0.004$), 16 ($p\leq0.001$), 18 ($p\leq0.001$), 20 ($p\leq0.001$), and 21 weeks ($p= 0.002$). The spectrum of values spanned from 78.3 % to 88.2 % in NZM2410 mice. The frequency of naive CD8+ T cells of C57BL/6J mice was ranging between 69.9 % and 78.5 %. For SLE123 mice, the vales were between 67.1 % and 87.6 %. SLE123 mice showed an increase in weeks 10 and 12, which was significantly higher than in C57BL/6J mice at week 12 ($p\leq0.001$) (**Figure 47 B**).

The values of **effector CD8+ T cells** in C57BL/6J and NZM2410 mice were varying between 0.6 % and 1.9 %, while for SLE123 mice, 3.2 % was measured as highest value in week 8. Effector CD8+ T cells of NZM2410 mice were at no measured time point significantly different from C57BL/6J mice. SLE123 mice had significantly lower frequencies at the ages of 10 ($p= 0.003$), and 12 weeks ($p\leq0.001$). (**Figure 47 C**).

Frequencies of **central memory CD8+ T cells** were consistently low, spanning values from 6.5 % to 10.9 % in NZM2410 mice. In C57BL/6J mice the values were between 18.4 % and 28.0 %. This was significantly different between NZM2410 and C57BL/6J mice at all measured time points [8 ($p= 0.019$), 9 ($p\leq0.001$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.003$), 18 ($p\leq0.001$), 19 ($p\leq0.001$), 20 ($p\leq0.001$), and 21 weeks ($p= 0.001$)]. SLE123 mice were also showing significantly lower frequencies than C57BL/6J mice at the ages of 10 ($p= 0.003$, mean SLE123 11.3 %, C57BL/6J 23.7 %) and 12 weeks ($p\leq0.001$, mean SLE123 10.8 %, C57BL/6J 24.0 %). Thereafter, the frequencies increased in SLE123 mice, reaching a value of 29.9 % at week 18 (**Figure 47 D**).

The values of **activated CD8+ T cells** in NZM2410 mice ranged from 0.0 % to 20.1 % with a peak at week 18 of 20.1 %. SLE123 mice had significantly higher frequencies at the ages of 10 ($p= 0.003$), 12 ($p\leq0.001$), and 14 weeks ($p\leq0.001$), reaching a maximum of 80.1 % at week 10. Thereafter, the values decreased to 0.2 % in week 18. Activated CD8+ T cells in NZM2410 mice were significantly lower than those of C57BL/6J mice at the ages of 8 ($p= 0,010$), 9 ($p= 0.018$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.001$), 20 ($p\leq0.001$), and 21 weeks ($p\leq0.001$) (**Figure 47 E**).

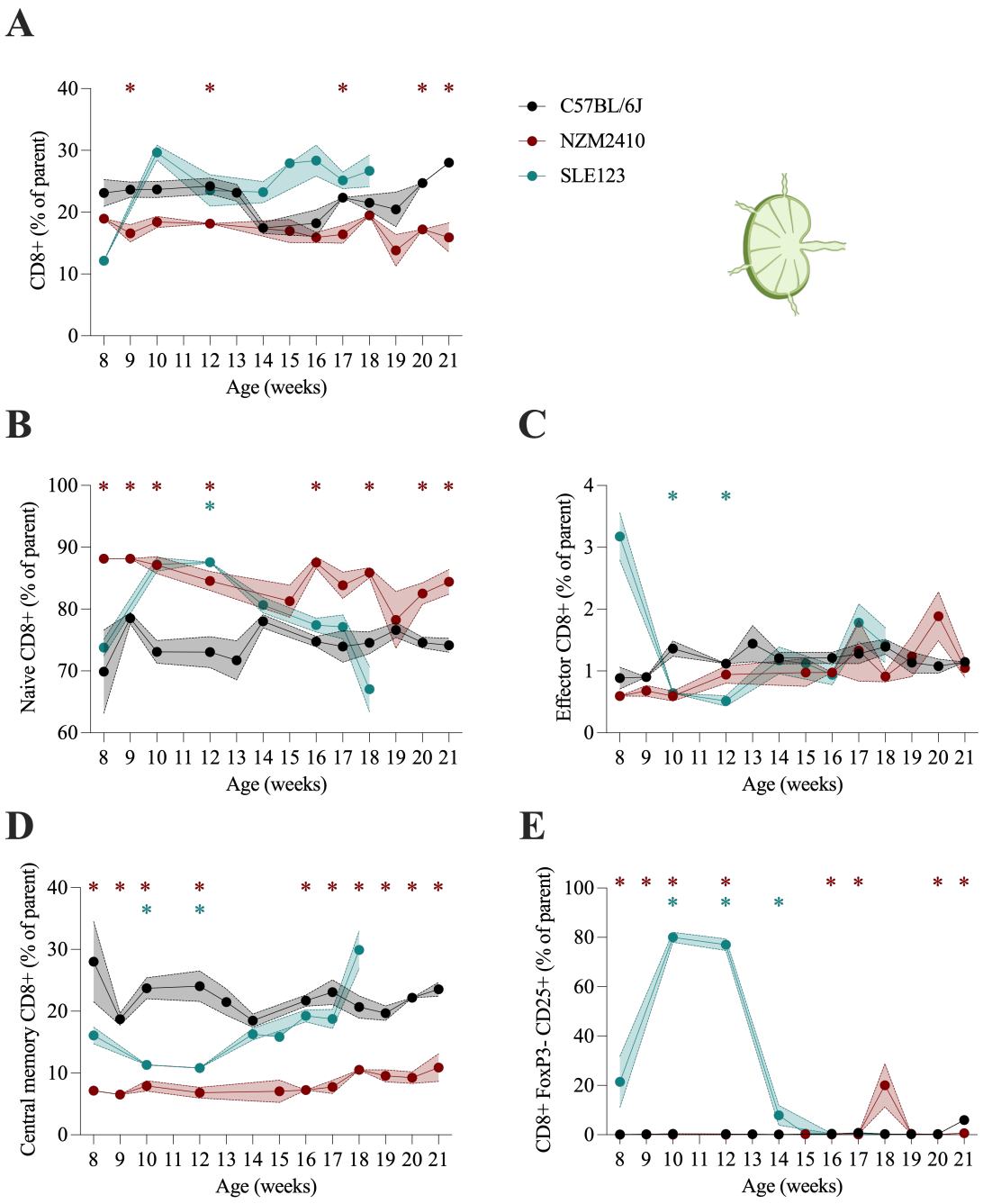


Figure 47: NZM2410 mice demonstrate significantly lower central memory CD8+ T cell frequencies than C57BL/6J mice in the lymph nodes

CD8+ T cells in the lymph nodes were measured by flow cytometry for NZM2410, SLE123, and C57BL/6J mice from the age of 8 weeks to 21 weeks (**A**). The CD8+ T cells were further separated into naive CD8+ T cells (**B**), effector CD8+ T cells (**C**), central memory CD8+ T cells (**D**), and activated CD8+ T cells (**E**). The parent gate of naive, effector, memory, and CD8+ FoxP3- CD25+ cells is the CD8+ gate. The parent gate of the CD8+ cells is the single cell gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

The frequencies of **CD4+ Tregs** in the lymph nodes of C57BL/6J mice were ranging from 3.8 % to 6.9 %, while NZM2410 mice had frequencies of 1.1 % to 2.7 %, and SLE123 of 1.9 % to 6.4 %. The values were lower in NZM2410 mice compared to C57BL/6J mice at all measured time points [week 8 ($p= 0.02$), 9 ($p\leq0.001$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.003$), 18 ($p= 0.003$), 19 ($p\leq0.001$), 20 ($p\leq0.001$), and 21 weeks ($p\leq0.001$)]. SLE123 mice did not differ from C57BL/6J mice (**Figure 48 A**).

Frequencies of **inactive CD4+ Tregs** spanned values from 1.7 % to 3.6 % in NZM2410 mice. C57BL/6J mice demonstrated a peak at week 14, therefore, the values varied from 2.2 % to 22.0 %. SLE123 mice had values from 2.2 % to 8.8 %. Frequencies of inactive Tregs were significantly lower in NZM2410 mice at the ages of 10 ($p= 0.007$), 16 ($p= 0.014$), 17 ($p= 0.003$), 18 ($p\leq0.001$), 19 ($p= 0.003$), 20 ($p\leq0.001$) and 21 weeks ($p\leq0.001$). SLE123 mice had significantly higher frequencies at the age of 12 weeks ($p= 0.005$), and significantly lower values of the ages of 14 ($p\leq0.001$) and 17 weeks ($p\leq0.001$) (**Figure 48 B**).

Naive CD4+ Tregs in the lymph nodes revealed week-dependent differences in all genotypes. NZM2410 mice started with a frequency of 36.3 % at the age of 8 weeks, which was similar to the values of C57BL/6J mice (41.0 %). In these following weeks, the values decreased in C57BL/6J mice to 21.2 % in week 17, showing a small peak in week 19 (36.6 %). In NZM2410 mice, the values dropped in week 10 (18.3 %), causing a significant difference compared to C57BL/6J mice ($p\leq0.001$). Thereafter, the values slightly increased again to be significantly above those of C57BL/6J mice at weeks 16 ($p\leq0.001$) and 17 ($p= 0.003$). Then the frequencies in NZM2410 mice decreased again, with significantly lower ones at week 21 ($p\leq0.001$). In SLE123 mice, the mice started with a low frequency of 15.9 % in week 8, rose to 48.0 % and 49.6 % in weeks 10 and 12, which was significantly above the levels of C57BL/6J mice [($p= 0.003$) and ($p\leq0.001$)]. Then the frequency reduced again to 34.9 % in week 17, which was still a significantly higher frequency than in C57BL/6J mice ($p= 0.002$). This decrease continued in week 18 (23.2 %) (**figure 48 C**).

Central memory CD4+ Tregs in the lymph nodes varied between 20.1 % and 42.1 % and are similar in all genotypes. Only at week 12, SLE123 mice have significantly lower frequencies than C57BL/6J mice ($p\leq0.001$) (**Figure 48 D**).

Effector CD4+ Tregs increased from weeks 8 to 21 in all genotypes. The NZM2410 mice started with a frequency of 23.5 % and ended with a frequency of 35.5 %. This was above the ones of C57BL/6J mice at the ages of 9 ($p= 0.004$), 10 ($p\leq0.001$), 12 ($p\leq0.001$), and 21 weeks ($p\leq0.001$). SLE123 mice did not significantly differ from C57BL/6J mice at any measured time point (**Figure 48 E**).

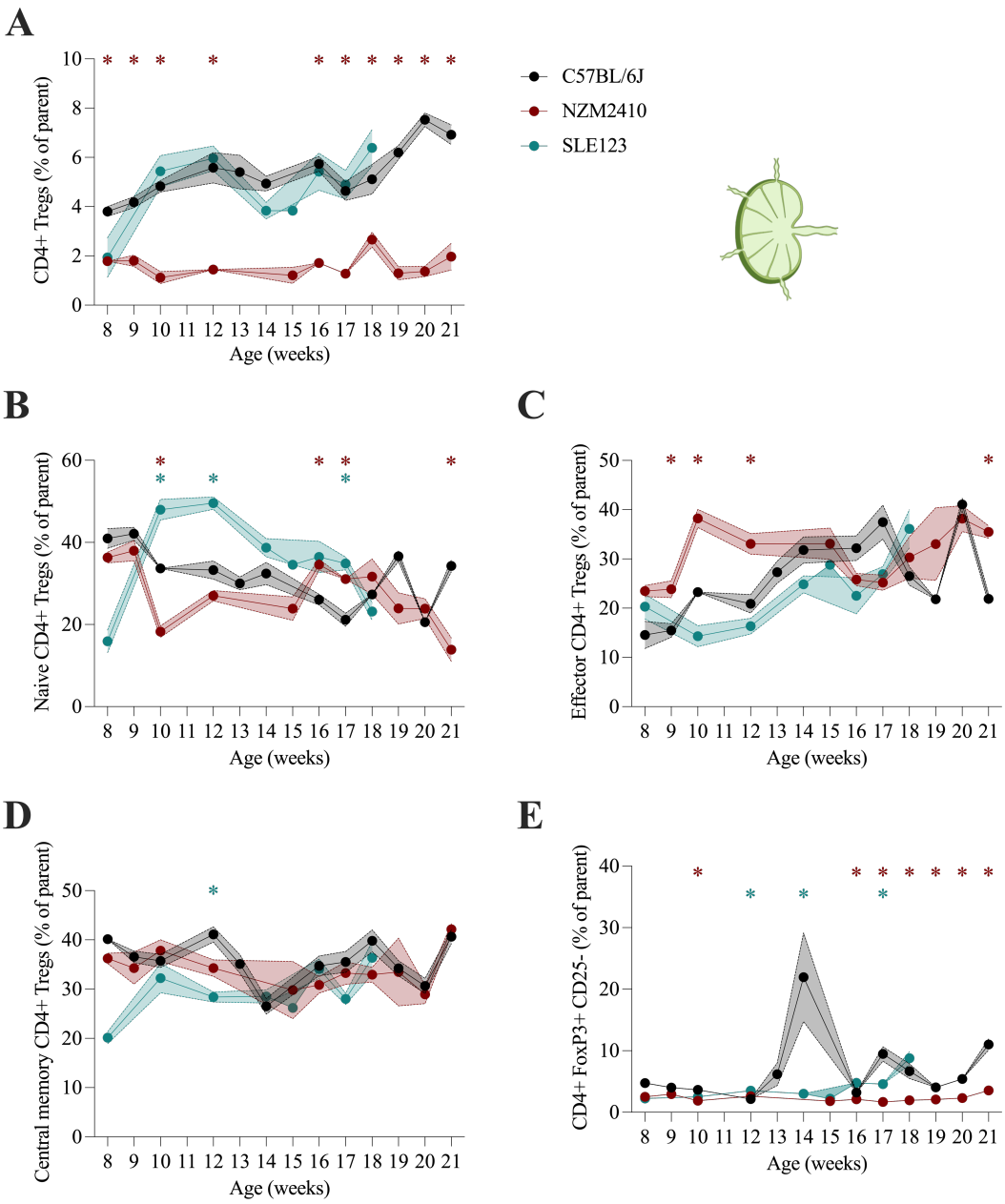


Figure 48: NZM2410 mice exhibit significantly lower CD4+ regulatory T cell frequencies at all measured time points in the lymph nodes

CD4+ regulatory T cells (Tregs) were measured in the lymph nodes by flow cytometry for lupus-prone NZM2410 and SLE123 mice compared to C57BL/6J mice. Tregs are characterised by expression of FoxP3 and CD25 among CD4+ T cells (**A**). The Tregs were further subdivided into naive CD4+ Tregs (**B**), effector CD4+ Tregs (**C**), and central memory CD4+ Tregs (**D**). Among CD4+ T cells, inactive CD4+ Tregs, which express FoxP3 but not CD25 (**E**). The parent gate of naive, effector, and memory Tregs is the CD4+ Treg gate. The parent gate of the CD4+ Tregs as well as the CD4+ FoxP3+ CD25- cells is the CD4+ gate. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

When investigating further markers of the CD4+ Tregs, which define their functionality, it became apparent that NZM2410 mice had significantly lower frequencies of **helios+ Tregs** at the ages of 16 ($p \leq 0.001$), 17 ($p = 0.003$), and 20 weeks ($p \leq 0.001$). This was shown earlier in SLE123 mice at the ages of 10 ($p = 0.003$), 12 ($p \leq 0.001$), 14 ($p \leq 0.001$), 16 ($p = 0.005$), and 17 weeks ($p \leq 0.001$). The values were spanning a range of 28.3 % to 46.4 % in C57BL/6J mice, 27.0 % to 41.2 % in NZM2410, and 17.7 % to 40.7 % in SLE123 mice (**Figure 49 A**).

The frequency of **Ki-67** expressing Tregs is reduced in SLE123 mice from the age of 8 weeks with a value of 38.2 % to 2.3 % in week 18. This was significantly different from C57BL/6J mice at the ages 10 ($p = 0.003$), 14 ($p = 0.002$), and 18 weeks ($p \leq 0.001$). The values of C57BL/6J mice varied over the weeks with a peak of 25.9 % in week 17. NZM2410 mice also varied with minimum values of 2.8 % in week 8 and a maximum percentage of 21.2 % in week 18. NZM2410 mice were significantly different from C57BL/6J mice at the age of 9 ($p \leq 0.001$), 10 ($p = 0.002$), and 20 weeks ($p = 0.001$) (**Figure 49 B**).

CTLA-4+ Treg frequencies peaked in C57BL/6J mice at week 17 with values of 12.9 %. The highest values in NZM2410 mice were seen at week 19 (10.9 %), however, the variance was high. Significantly lower frequencies were only seen in NZM2410 mice in week 16 ($p \leq 0.001$). SLE123 mice started with the highest value at week 8 (22.0 %). This dropped to 1.2 % in week 18. SLE123 mice differed significantly from C57BL/6J mice at weeks 10 ($p = 0.003$), 12 ($p \leq 0.001$), 16 ($p = 0.003$), 17 ($p \leq 0.001$) and 18 ($p \leq 0.001$). NZM2410 mice differed significantly only with lower frequencies at week 16 ($p \leq 0.001$) (**Figure 49 C**).

The **CD39+ CD4+ Treg** frequency in NZM2410 mice varied from 8.4 % to 47.4 %. In C57BL/6J mice, the values ranged from 30.7 % to 68.0 %, and in SLE123 from 23.0 % in week 18 to 80.2 % in week 10. The CD39+ CD4+ Treg frequency was significantly lower in NZM2410 mice at all measured time points compared to C57BL/6J mice. This was significant at weeks 8 ($p = 0.019$), 9 ($p \leq 0.001$), 10 ($p \leq 0.001$), 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), 17 ($p = 0.003$), 19 ($p = 0.003$), 20 ($p \leq 0.001$), and 21 ($p = 0.007$). SLE123 mice started with significantly higher frequencies than C57BL/6J mice at the ages of 10 ($p = 0.003$), and 12 weeks ($p \leq 0.001$). This dropped below the frequencies of C57BL/6J mice with significantly lower values at weeks 16 ($p \leq 0.001$), 17 ($p \leq 0.001$), and 18 ($p \leq 0.001$). (**Figure 49 D**).

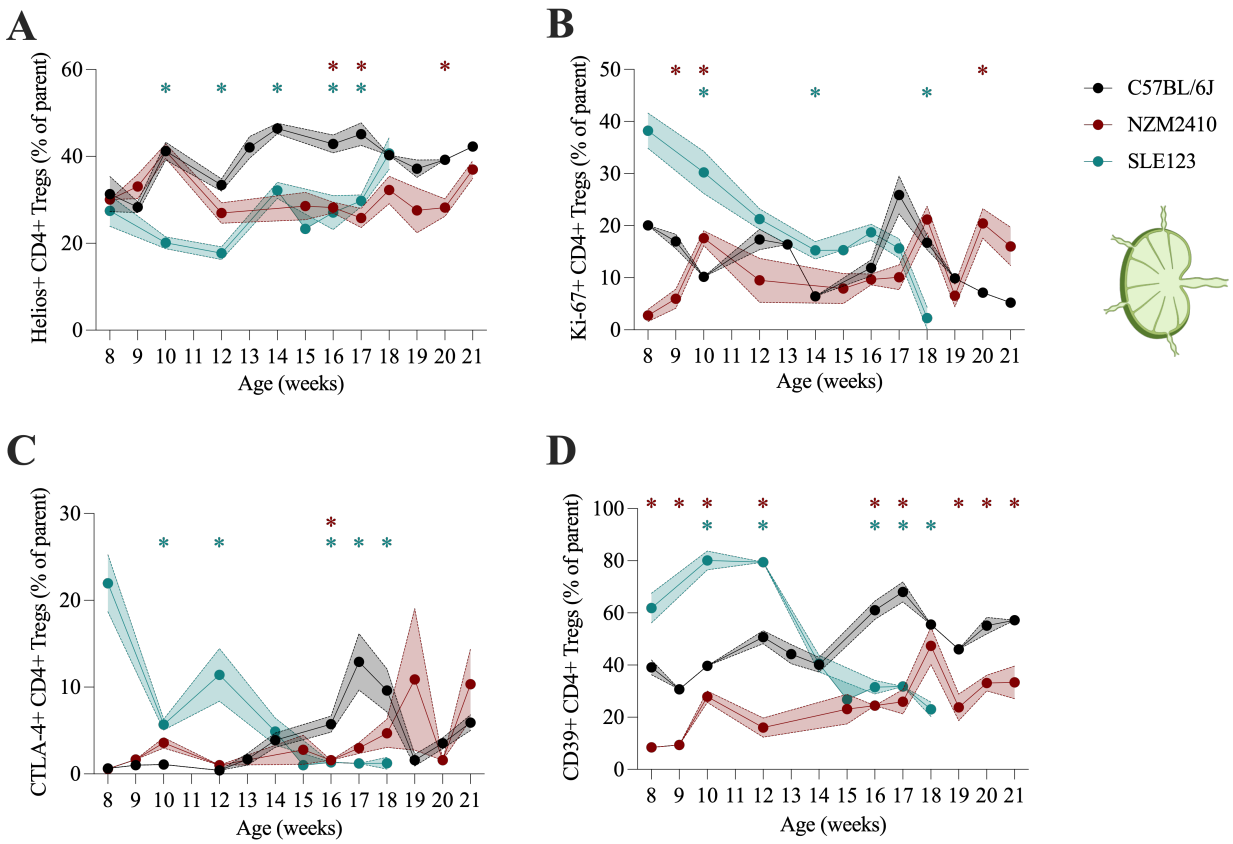


Figure 49: CD39+ regulatory T cell frequencies are reduced in NZM2410 mice compared to C57BL/6J mice and in SLE123 mice the frequencies, which express Ki-67, CTLA-4, and CD39 decrease with age in the lymph nodes
Regulatory T cells (Tregs) were measured in the lymph nodes by flow cytometry and investigated for their expression of Helios (**A**), Ki-67 (**B**), CTLA-4 (**C**), and CD39 (**D**). Parent gate for all these markers was the Treg gate. Analysed mouse lines were the lupus-prone SLE123 and NZM2410 mice, which were compared to C57BL/6J mice. Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * $p<0.05$. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

CD8+ Tregs in the lymph nodes showed a frequency up to 0.5 % in C57BL/6J mice until the age of 20 weeks. Thereafter, it rose to 3.7 %. NZM2410 mice had even lower frequencies of maximum 0.9 %, with a significant difference at the ages of 10 ($p\leq0.001$), 12 ($p\leq0.001$), 16 ($p\leq0.001$), 17 ($p= 0.003$), 19 ($p= 0.001$), 20 ($p\leq0.001$), and 21 weeks ($p\leq0.001$). At the age of 18 weeks, a small peak became apparent, however, this was not a significant difference from C57BL/6J mice. SLE123 mice started with higher values already in week 8 (1.8 %), 10 ($p= 0.001$), and 12 ($p\leq0.001$). The variance in week 12 was high, nevertheless, the values reached a mean of 2.7 % in SLE123 mice. Thereafter, the frequencies decreased to a significantly lower value than in C57BL/6J mice in week

17 (p= 0.002, 0.2 %) (**Figure 50 A**).

Inactive CD8+ Tregs showed a peak with high variance in C57BL/6J mice at the age of 14 weeks with 25.1 %. In all other weeks, the values were varying between 0.4 % and 6.3 %. NZM2410 mice did not significantly differ from C57BL/6J mice at any measured point in time. SLE123 mice had significantly lower frequencies in week 10 (p= 0.003, mean SLE123 0.1 %, C57BL/6J 0.8 %) and 12 ($p \leq 0.001$, mean SLE123 0.1 %, C57BL/6J 0.9 %) but higher ones in week 16 ($p \leq 0.001$, mean SLE123 6.3 %, C57BL/6J 0.8 %) (**Figure 50 B**).

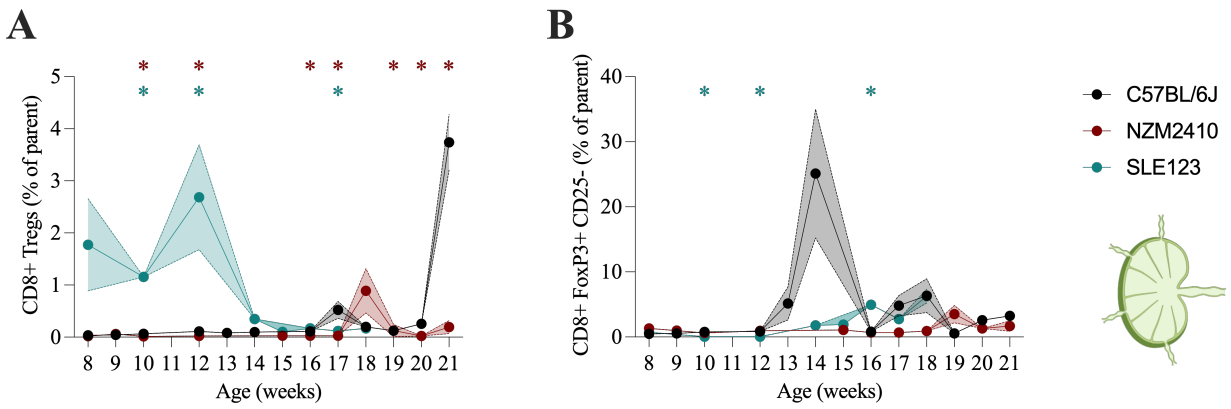


Figure 50: Young SLE123 mice demonstrate higher CD8+ regulatory T cell frequencies than C57BL/6J mice in the lymph nodes
CD8+ regulatory T cells (Tregs) were measured by flow cytometry in the lymph nodes of lupus-prone NZM2410 and SLE123 mice during the pre-disease phase from the age of 8 weeks to 21 weeks. This was compared to C57BL/6J mice. CD8+ Tregs are characterized by the expression of CD8, FoxP3 and CD25 (**A**). In-active CD8+ Tregs do not express CD25 (**B**). Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

The C57BL/6J mice showed a bow over time in the expression of **CX3CR1** in CD4+ T cells. They started with a frequency of 20.7 % in week 8, followed by an increase up to 80.7 % in week 14, and a decrease down to 15.8 % in week 21. SLE123 and NZM2410 started with similar frequencies but thereafter underlyed several increases and decreases but NZM2410 mice demonstrated a significantly lower frequency than C57BL/6J mice at the ages of 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), and 18 weeks ($p \leq 0.001$). SLE123 mice revealed significantly lower frequencies than C57BL/6J mice at the ages of 10 (p= 0.003), 14 (p= 0.004), 16 (p= 0.002) and 18 weeks ($p \leq 0.001$) (**Figure 51 A**).

The expression shape of CX3CR1 in C57BL/6J mice was similar in CD8+ T cells, with a minimum of 23.2 % in week 21 and a maximum of 87.6 % in week 18. NZM2410 and SLE123 mice also underlyed several in- and decreases over time. For CD8+ T cells of SLE123 mice, none

of these changes were significantly different from C57BL/6J mice. For NZM2410 mice, week 12 ($p \leq 0.001$, mean NZM2410 14.2 %, C57BL/6J 66.1 %) and week 18 ($p \leq 0.001$, mean NZM2410 55.8 %, C57BL/6J 87.6 %) showed significantly lower frequencies (**Figure 51 C**).

In CD4+ Tregs, the CX3CR1+ frequencies were lower in NZM2410 and C57BL/6J mice at the weeks 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), and 18 ($p = 0.002$). For SLE123 mice, this was the case in weeks 16 ($p = 0.002$) and 18 ($p \leq 0.001$). The frequencies were spanning from 24.3 % to 85.8 % in C57BL/6J, from 13.2 % to 67.5 % in NZM2410, and from 36.8 % to 77.5 % in SLE123 mice (**Figure 51 E**).

The frequencies of **CXCR4+** T cells in the lymph nodes were below 7.4 % for CD4+ and CD8+ T cells in all genotypes. For CD4+ Tregs, the CXCR4+ frequency was higher, in SLE123 mice up to 29.2 %.

In CD4+ T cells, the CXCR4+ frequency was except for week 21 lower in NZM2410 mice than in C57BL/6J mice. This was significant in the weeks 9 ($p = 0.006$), 12 ($p \leq 0.001$), 16 ($p \leq 0.001$), 17 ($p = 0.006$), and 18 ($p \leq 0.001$). In week 21, NZM2410 mice revealed significantly higher frequencies ($p \leq 0.001$). SLE123 mice showed a peak in week 14 (7.4 %), which was significantly above the levels of C57BL/6J mice ($p = 0.010$). At the ages of 16 ($p \leq 0.001$), 17 ($p = 0.002$), and 18 weeks ($p \leq 0.001$), significantly lower frequencies of CXCR4 were measured in SLE123 mice in the CD4+ T cells (**Figure 51 B**).

In CD8+ T cells, the CXCR4+ T cell frequency was even lower than in CD4+ T cells and ranged for C57BL/6J mice all the time below 1.1 %. NZM2410 mice showed a peak with high variance in week 19 (5.4 %), but this was not significant compared to C57BL/6J mice. However, at weeks 12 ($p \leq 0.001$) and 18 ($p \leq 0.001$), NZM2410 mice revealed significantly lower frequencies. SLE123 mice did not show any peaks over time, however, they demonstrated a significantly lower frequency than C57BL/6J mice at the age of 18 weeks ($p \leq 0.001$) (**Figure 51 D**).

Tregs showed peaks in expression of CXCR4 at the age of 17 weeks in C57BL/6J (16.0 %) and 18 weeks in NZM2410 mice (14.1 %). NZM2410 mice had significantly lower frequencies in weeks 9 ($p \leq 0.001$), 16 ($p \leq 0.001$), and 17 ($p = 0.003$). SLE123 mice started with the highest frequency of 29.2 % in week 8 and decreased in week 12 from 26.9 % to 6.4 % in week 14. This decrease continued until the last measured week, 18 (0.8 %). In weeks 10 ($p = 0.001$) and 12 ($p \leq 0.001$) SLE123 mice had significantly higher frequencies of CXCR4 and in weeks 16 ($p = 0.002$), 17 ($p \leq 0.001$), and 18 ($p \leq 0.001$), it was significantly lower (**Figure 51 E**).

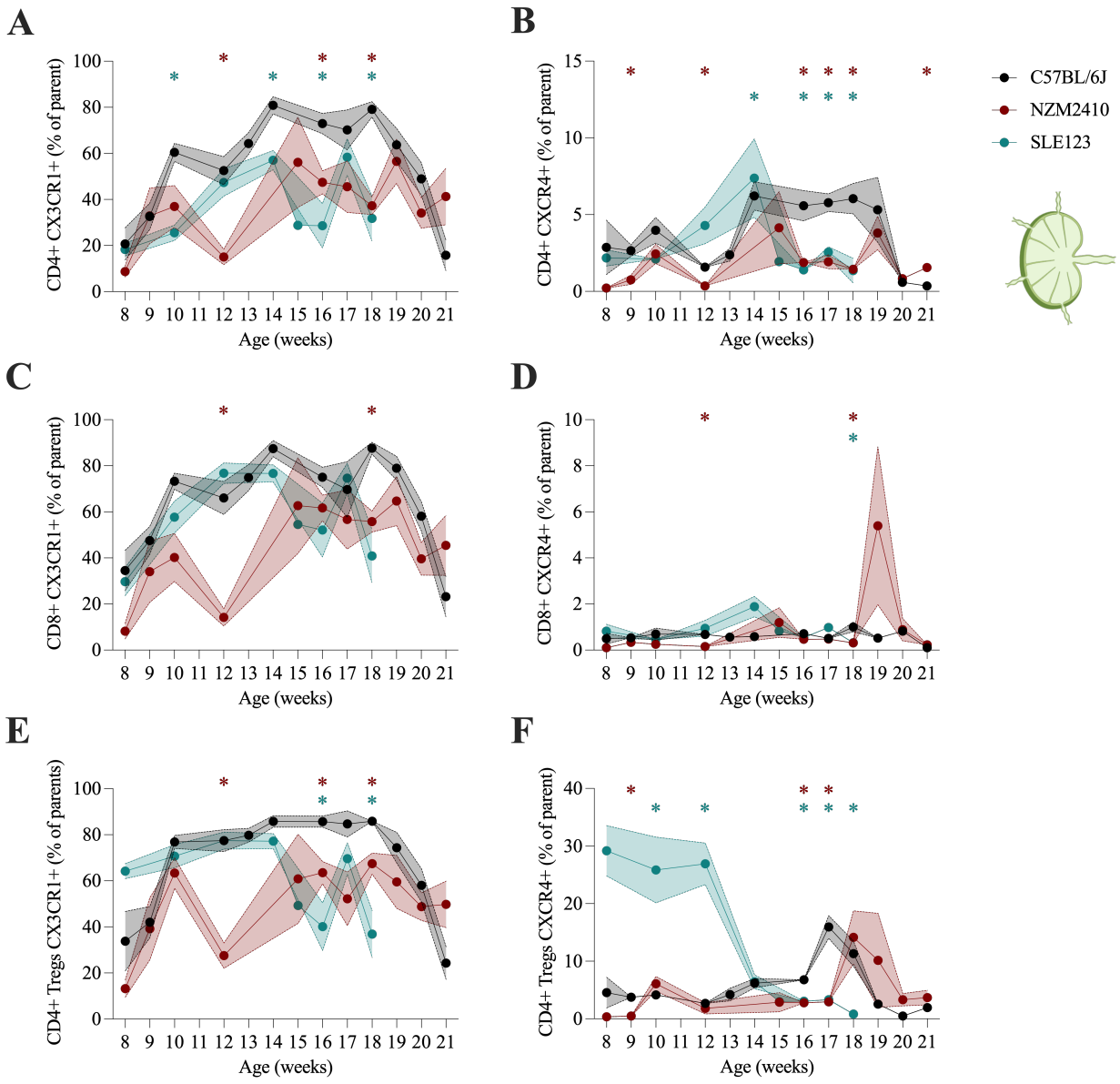


Figure 51: The frequency of CXCR4+ T cells is the highest in regulatory T cells compared to CD4+ and CD8+ T cells in lymph nodes

The trafficking markers CXCR4 and CX3CR1 were measured using flow cytometry in the lymph nodes of lupus-prone mouse lines NZM2410 and SLE123 compared to C57BL/6J mice between the ages of 8 and 21 weeks. CX3CR1 was measured in CD4+ Tcells (**A**), CD8+ T cells (**C**), and CD4+ regulatory T cells (Tregs) (**E**). CXCR4 was also measured in CD4+ T cells (**B**), CD8+ T cells (**D**), and CD4+ Tregs (**F**). Data presented as mean +/- SEM, gained from 44 independent experiments. Mann-Whitney test with a FDR of 1 %, * p<0.05. Red * = NZM2410 vs C57BL/6J, turquoise * = SLE123 vs C57BL/6J.

3.2 Ultraviolet light causes different effects in NZM2410 mice compared to C57BL/6J mice

We hypothesised that UV light is causing different effects in lupus-prone NZM2410 mice compared to healthy C57BL/6J mice. As UV light spans a wavelength spectrum of 10-400 nm, with different energy intensities, we tested different wavelengths. The penetration depth in the skin is also depending on the wavelength. Therefore, we chose UVB, which is known to cause sunburn, as well as UVC, which is more powerful but cannot penetrate the skin deeply. Even though blue light does not belong to the UV spectrum, it is still powerful and has certain biological effects. Blue light is not known to cause skin damage in healthy people, however, it has been reported that it is able to kill bacteria (J. Cabral and Ag 2019) and also to alter the circadian rhythm (K. Dong *et al.* 2019). This is why we want to investigate if it might have an effect on the skin of lupus-prone mice, even if it has no known effect on healthy skin. In a second experiment with another set of mice, we tested UVA light, a combination of UVA and UVB light, as well as another wavelength of blue light. UVA is known to have damaging but also anti-inflammatory effects (Yang *et al.* 2023). We wondered if this might even be beneficial for lupus-prone skin. Sunlight consists of a spectrum of light, therefore, we wanted to test a mixture of UVA and UVB light to find out if the effects can neutralise each other. Experimentally, cytotoxicity, the mRNA transcriptome, and histological changes due to (UV) treatments were investigated.

3.2.1 Increased cytotoxicity in NZM2410 mouse skin after 30 h of cultivation, UVC, and blue light 405 nm treatment

Cytotoxicity between the samples of the UV experiments was compared using LDH asays of the medium in which the samples were cultivated. After 24 h of tissue culture, no significant difference in the absorbance could be measured. However, after 30 h, NZM2410 mice showed significantly higher cytotoxicity than C57BL/6J mice ($p = 0.004$). The same accounts for the comparison of UVC ($p = 0.002$) and blue light-treated samples ($p = 0.015$) (**Figure 52**).

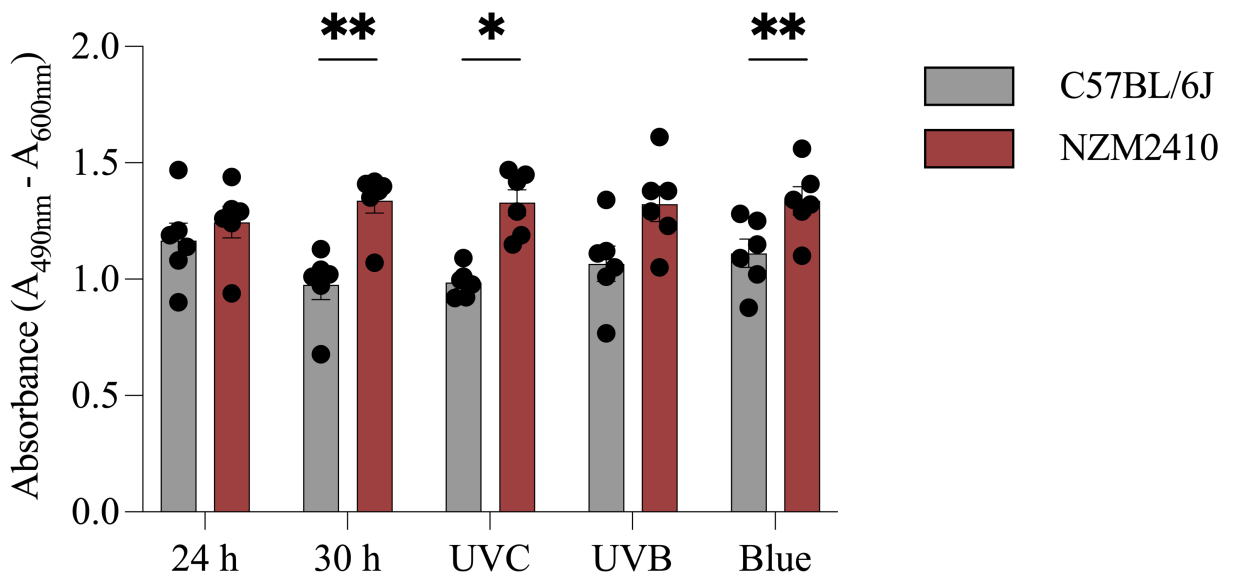


Figure 52: NZM2410 mice exhibited greater cytotoxicity compared to C57BL/6J mice following 30 hours of cultivation and exposure to UVC and blue light irradiation
Cytotoxicity in the samples of the first UV light experiment was compared using lactate dehydrogenase (LDH) assays. After 24 hours (h) and exposure to UVB radiation, no significant difference in LDH activity was observed between the two groups of mice. After 30 h, a significant increase in LDH activity was observed in NZM2410 mice compared to C57BL/6J mice. This increase was also significant following blue light irradiation and UVC irradiation. The biostatistics were prepared using the Mann-Whitney test. $* \leq p = 0.05$, $** \leq p = 0.01$. n = 6 per group. Data presented as mean ± SEM.

In the second UV experiment, other wavelengths were compared in another set of mice. Details about the mice can be found in the methods section. The wavelengths and intensities tested in the second experiment were UVA, a combination of UVA and UVB irradiation and blue light 445 nm. In the cytotoxicity assays we could see that NZM2410 mice showed significantly less LDH after 24 h of incubation (p= 0.005) and upon UVA irradiation (p= 0.002). No further significant differences between the genotypes and the conditions were found (**Figure 53**).

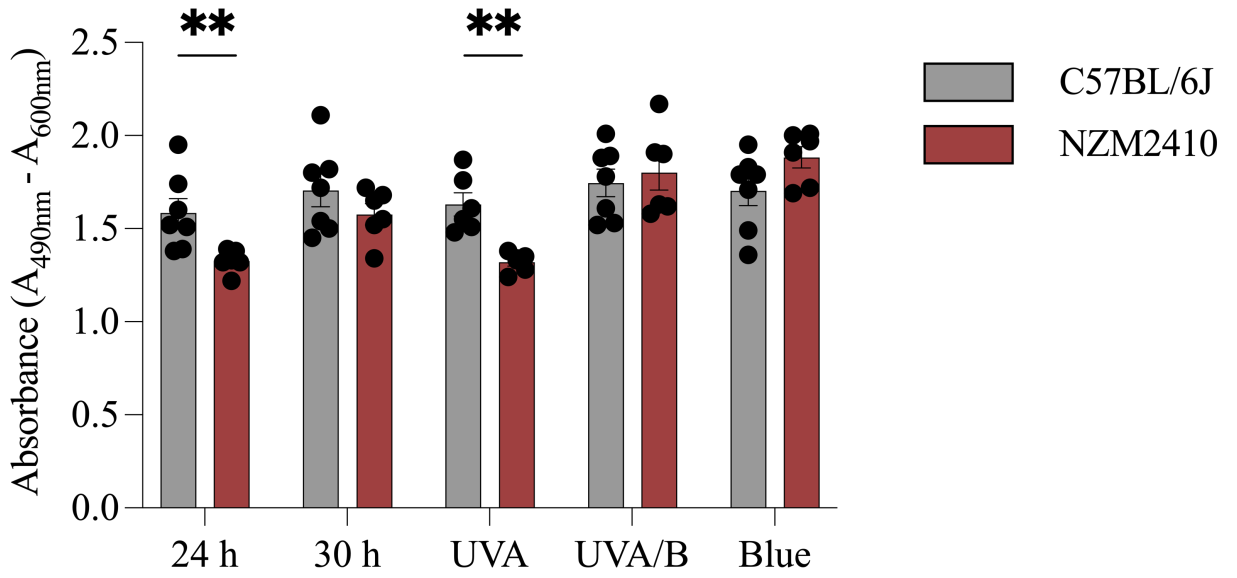


Figure 53: NZM2410 mice exhibit lower cytotoxicity compared to C57BL/6J mice following 24 hours of cultivation or exposure to UVA light irradiation
Cell cytotoxicity in the samples of the second UV light experiment was compared using lactate dehydrogenase (LDH) assays. After 30 hours (h) and exposure to UVA/B radiation, no significant difference in LDH activity was observed between the two groups of mice. After 24 h, a significant increase in LDH activity was observed in C57BL/6J mice compared to NZM2410 mice. This increase was also significant following UVA light irradiation. The biostatistics were prepared using the Mann-Whitney test. $* \leq p = 0.05$, $** \leq p = 0.01$. n= 6-7 per group. Data presented as mean ± SEM.

3.2.2 Transcriptomic analysis of the differential effects of (UV) light treatment in NZM2410 and C57BL/6J mice

Transciptomics of the mRNA were performed to identify early changes in the mouse skin upon UV light irradiation, as we harvested the samples 6 h after the treatments. Potential genotype and disease-related differences between NZM2410 and C57BL/6J mice were investigated. The chosen age was 16-18 weeks as at this time of the pre-disease, several differences already occurred according to the haematology and flow cytometry analysis.

Principal component analysis reveals genotype-dependent clustering

Principal component analyses (PCAs) were used to identify the clustering of samples based on the variation. In both experiments, the samples clustered well together mainly depending on their genotype (**Figures 54** and **55**).

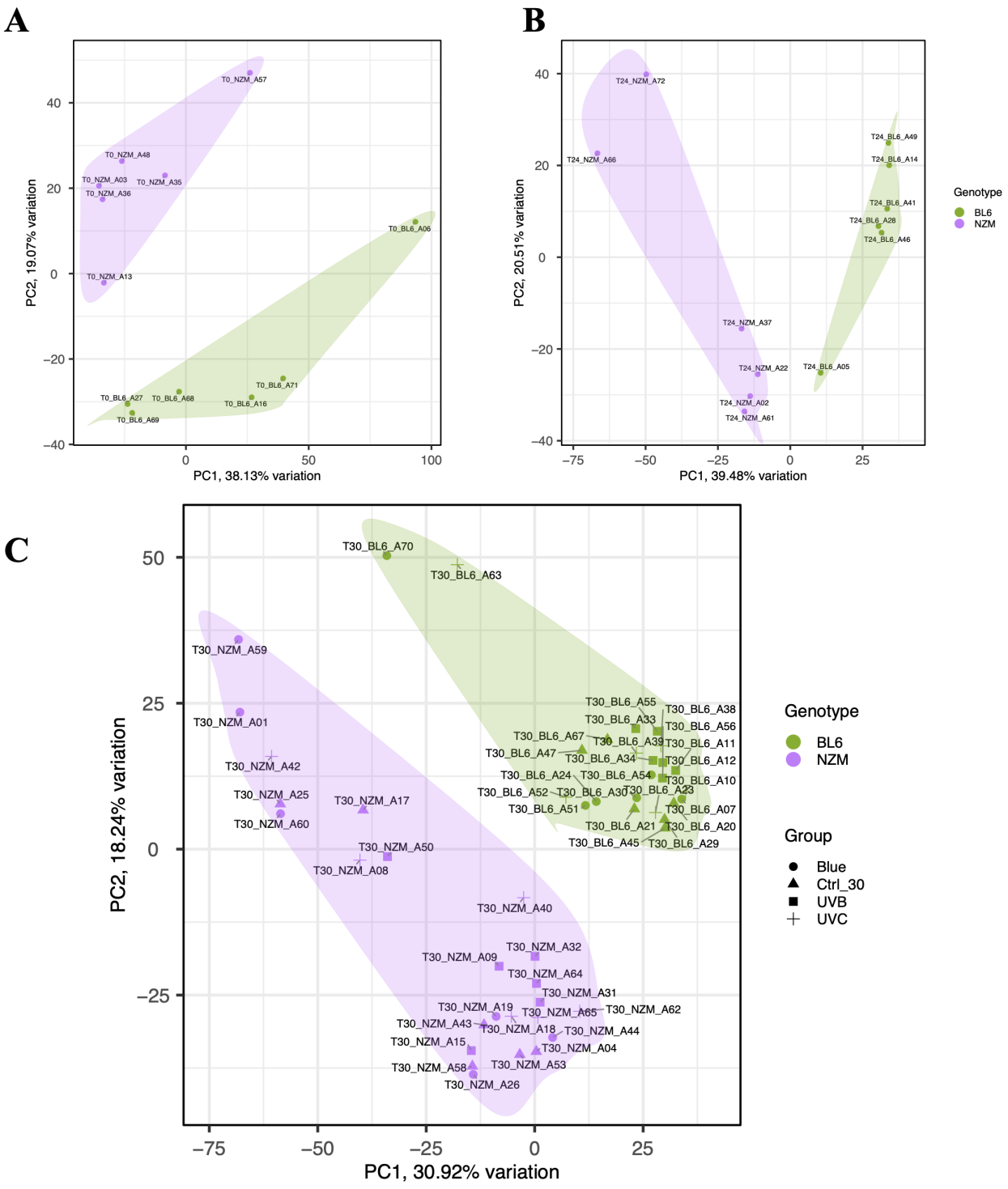


Figure 54: Principal component analysis shows genotype dependent separation of samples in UV experiment 1

Principal component analysis (PCA) of transcriptomic samples of UV experiment 1 shows separations dependent on the genotype, NZM2410 (NZM, purple) or C57BL/6J (BL6, green). This is already detectable in the time 0 controls (**A**), but also in the time 24 controls (**B**), and all samples at time 30 (**C**).

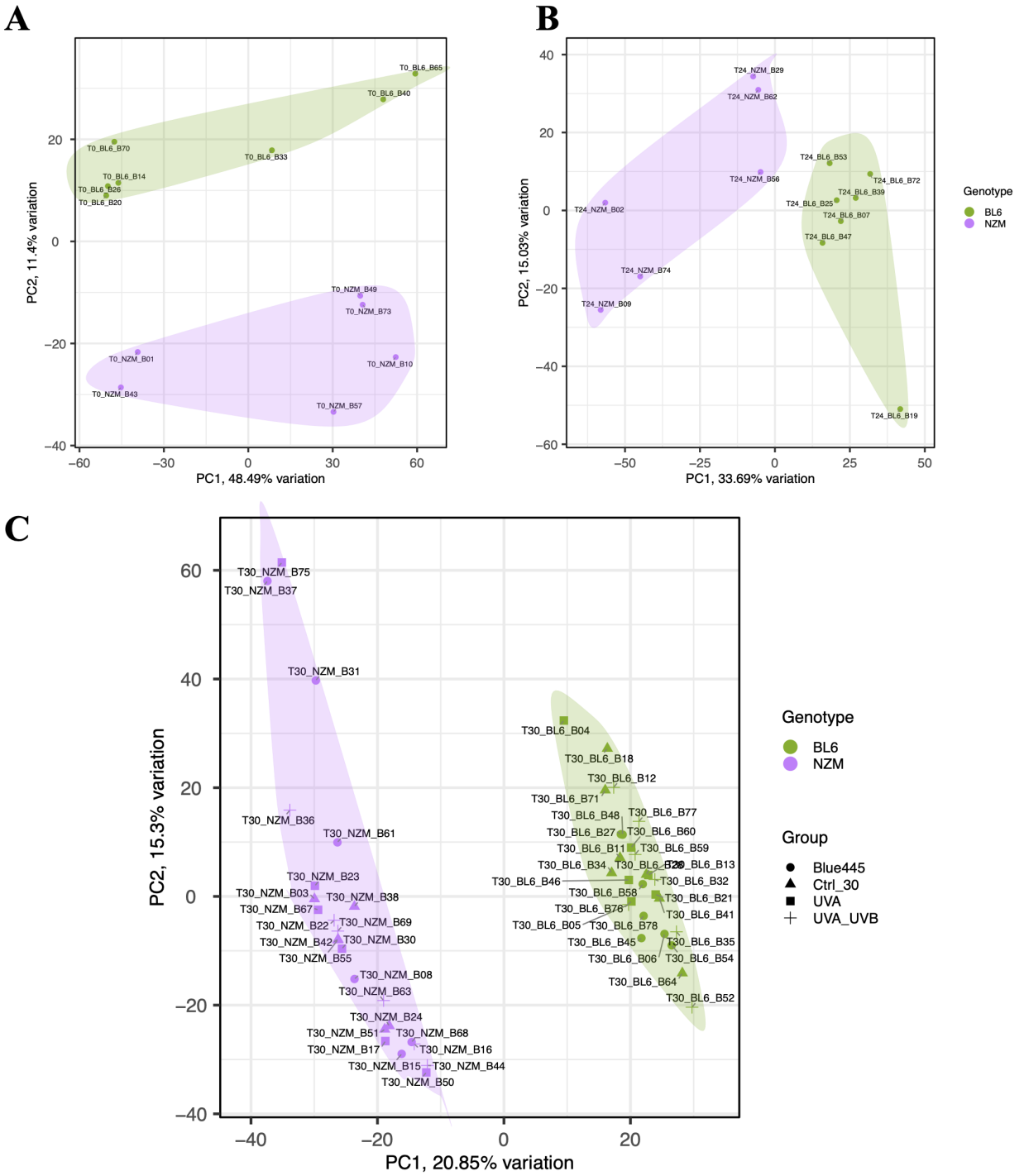


Figure 55: Principal component analysis shows genotype dependent separation of samples in UV experiment 2

Principal component analysis (PCA) of transcriptomic samples of experiment 2 shows separations dependent on the genotype, NZM2410 (NZM, purple) or C57BL/6J (BL6, green). This is already detectable in the time 0 controls (**A**), but also in the time 24 controls (**B**), and all samples at time 30 (**C**).

3.2.3 Deconvolution of transcriptomics data reveals that most signals are from epidermal cells

Deconvolution is a method to trace the origin of the cell types in the samples. As a reference, Joost *et al.* 2020 was used. It is not able to identify expressions on a single cell level but we could see that most signals at the endpoint (30 h after starting the experiment) stem from epidermal keratinocytes (**Figures 56** and **57**).

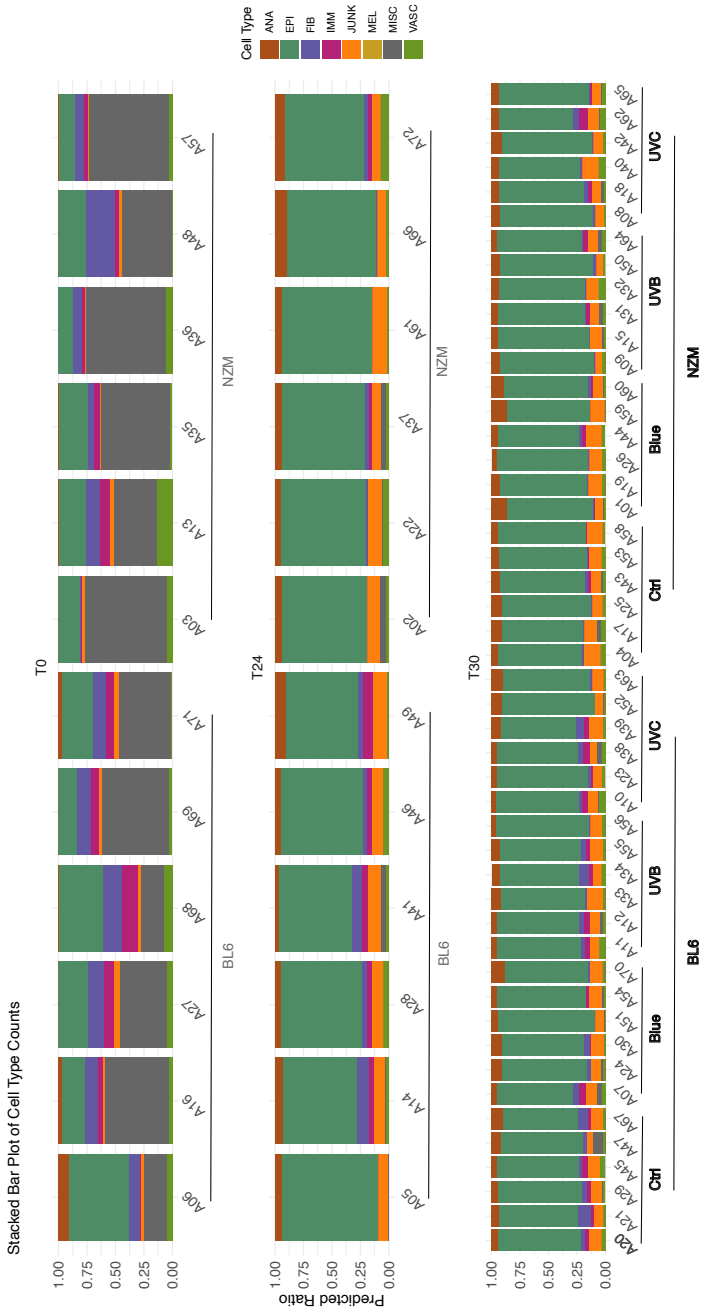


Figure 56: Deconvolution confirms signals originate from epidermal keratinocytes in experiment 1
Transcriptomic data was deconvoluted to confirm origin of the signals. The signals were calculated as predicted ratios over all signals of the individual samples. The upper row shows the time 0 controls (T0) of C57BL/6J (BL6) and NZM2410 (NZM) mice. The middle row shows the controls after 24 h of incubation (T24). The third row shows all time 30 control samples (Ctrl), blue light 405 nm irradiated samples, UVB light irradiated, and UVC light irradiated samples. The codes A01 to A72 stand for the individual samples. A sample list can be found in the supplements (Table S3). ANA= anagen hair follicle keratinocytes, EPI= permanent epidermal keratinocytes, FIB= fibroblast-like cells, IMM= immune cells, JUNK= junk signals, MEL= melanocytes, MISC= miscellaneous cells, VASC= vascular cells.

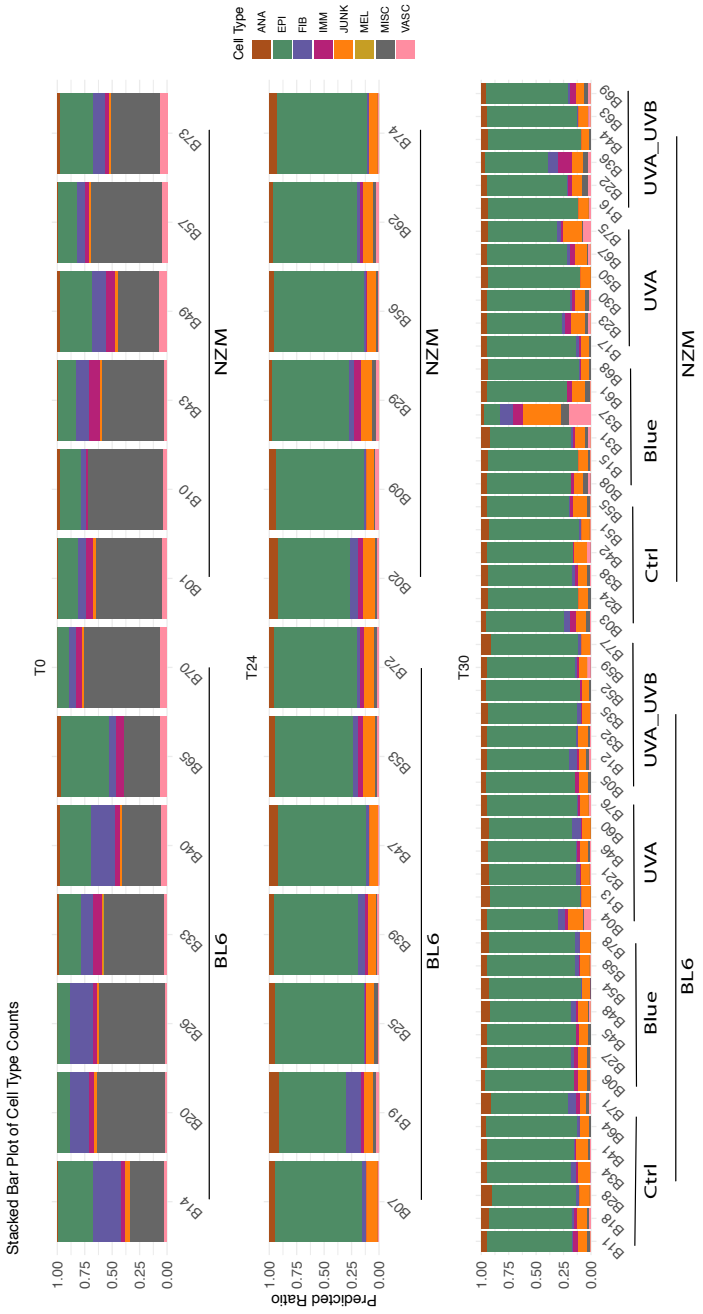


Figure 57: Deconvolution confirms signals originate from epidermal keratinocytes in experiment 2
Transcriptomic data was deconvoluted to gain insight about the origin of the signals. The signals were calculated as predicted ratios over all signals of the individual samples. The upper row shows the time 0 controls (T0) of C57BL/6J (BL6) and NZM2410 (NZM) mice. The middle row shows the controls after 24 h of incubation (T24). The third row shows all time 30 control samples (Ctrl), blue light 445 nm irradiated samples, UVA light irradiated, and UVA/B light irradiated samples. The codes B01 to B78 stand for the individual samples. A sample list can be found in the supplements (Table S4). ANA= anagen hair follicle keratinocytes, EPI= permanent epidermal keratinocytes, FIB= fibroblast-like cells, IMM= immune cells, JUNK= junk signals, MEL= melanocytes, MISC= miscellaneous cells, VASC= vascular cells.

3.2.4 Overview of differentially expressed genes and shared up- and downregulated ones between the treatment groups

Upset plots were generated with the R tool UpSetR and provide information about commonly and individually significantly up- or downregulated gene numbers between the experimental groups. For UV experiment 1, the controls after 30 h of incubation (Ctrl 30) were compared with UVB, UVC, and blue light (405 nm) irradiation. The intersection size describes the number of differentially regulated genes, comparing NZM2410 mice with C57BL/6J mice. Most downregulated genes (120) could be seen in the Ctrl 30 NZM2410 vs C57BL/6J mice. 117 genes were commonly downregulated in NZM2410 Ctrl 30 and UVB irradiated samples. 105 genes were also commonly downregulated in NZM2410 mice over all experimental groups. Within the experimental groups, most genes were downregulated in the NZM2410 Ctrl 30 samples, thereafter, UVB followed in which samples of NZM2410 mice had 90 downregulated genes, that were not in other groups differentially regulated. Thereafter, UVC irradiated samples with 29 genes and blue light irradiated samples with 17 genes followed, meaning that the least treatment-dependent downregulated genes between NZM2410 and C57BL/6J were found upon blue light (405 nm) irradiated samples. The most different, regarding the downregulated genes among the groups were the UVC and blue light treated samples, which only shared three commonly downregulated genes in NZM2410 mice (**Figure 58 A**).

Among the upregulated genes (comparing NZM2410 vs C57BL/6J mice), the highest number of genes was shared with all groups (76 genes). Thereafter, the second most genes (68 genes) were upregulated in the Ctrl 30 samples. In UVB-treated samples, 33 genes were upregulated in NZM2410 mice, which were not significantly upregulated in any other group. However, also 33 genes were commonly upregulated in Ctrl 30, UVB, and UVC samples comparing NZM2410 with C57BL/6J mice, which were not differentially regulated in blue light (405 nm) treated samples. Also, among all treatments, the fewest genes were only upregulated in the blue light-treated samples (21 genes). Overall, the least shared upregulated genes were seen between UVB and blue light samples (6 genes) as well as between Ctrl 30 and blue light samples (also 6 genes) (**Figure 58 B**). A list of the specific gene names that were up- or downregulated can be found in the supplements (**Table S5** and **Table S6**).

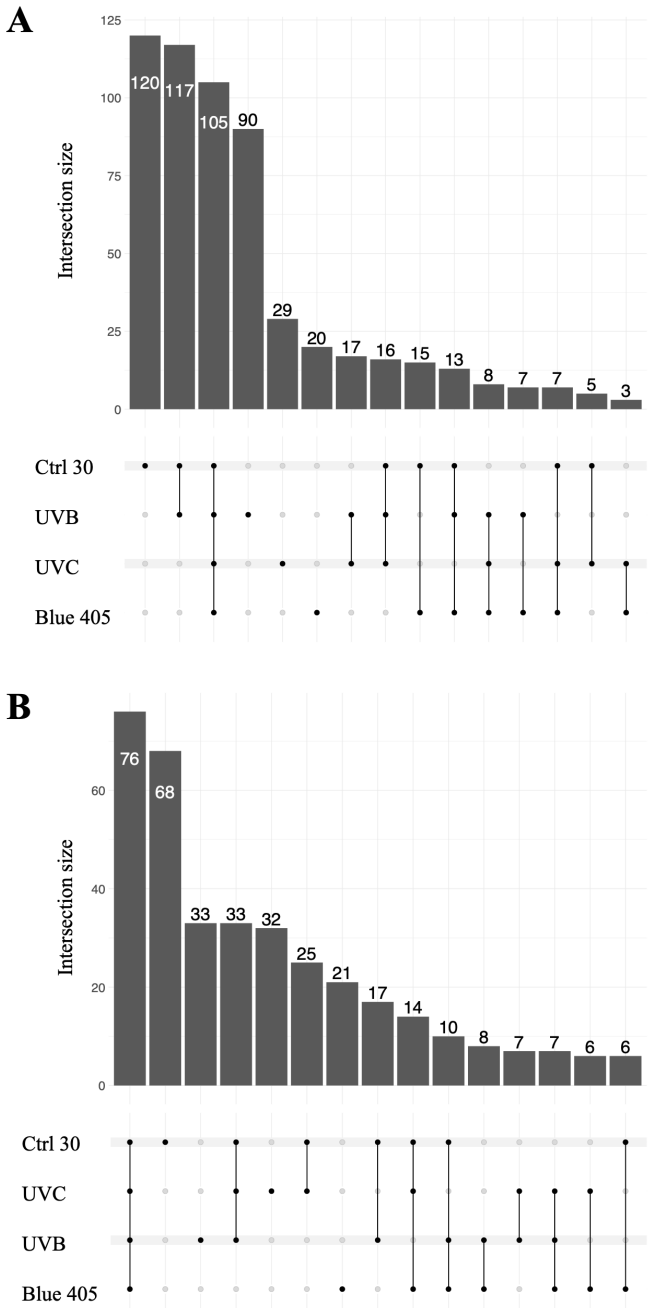


Figure 58: Overview of differentially regulated genes in UV experiment 1
Upset plots demonstrate shared downregulated (**A**) and commonly upregulated genes (**B**) between the experimental groups of UV experiment 1. The intersection size describes the number of differentially regulated genes when comparing NZM2410 mice with C57BL/6J mice. Each dot represents that this experimental group is involved, and the vertical lines mean shared differentially genes between the respective groups. Compared were the un-irradiated samples (Ctrl 30), UVB, UVC, and blue light (405 nm) irradiated samples. Data was calculated using the R tool UpSetR and a lrt qval < 0.05 and |b| >1.

In the second UV experiment, upset plots were generated for comparing UVA, UVA/B, and blue light (445 nm) irradiation with controls after 30 h of incubation but without irradiation. Most genes were commonly downregulated in all conditions (81 genes) in NZM2410 compared to C57BL/6J mice. 38 genes were specifically downregulated in NZM2410 mice upon UVA/B irradiation, while 32 downregulated genes were specific for the Ctrl 30 controls. Specifically for blue light (445 nm) treatment, 24 genes were downregulated in NZM2410 mice compared to C57BL/6J mice. UVA treatment showed with 17 genes the least downregulated genes that were specific for only one group. The comparison of UVA and blue light irradiation showed the least shared downregulated genes (2 genes) of all group comparisons (**Figure 59 A**). The upregulated genes in NZM2410 compared to C57BL/6J mice in UV experiment 2 were also investigated, showing the most genes (52 genes) were commonly upregulated in all treatment groups. UVA/B treatment caused an upregulation of 51 genes in NZM2410 mice, which were specific for this treatment. 47 genes were specifically in the Ctrl 30 samples upregulated. After UVA treatment, this were 31 genes and upon blue light (445 nm) only 11 genes were specifically upregulated in NZM2410 mice by this treatment. The least shared upregulated genes were found comparing Ctrl 30 samples with blue light treated samples (3 genes) (**Figure 59 B**). A list of the gene names that were specifically differentially regulated can be found in the supplements (**Table S7** and **Table S8**).

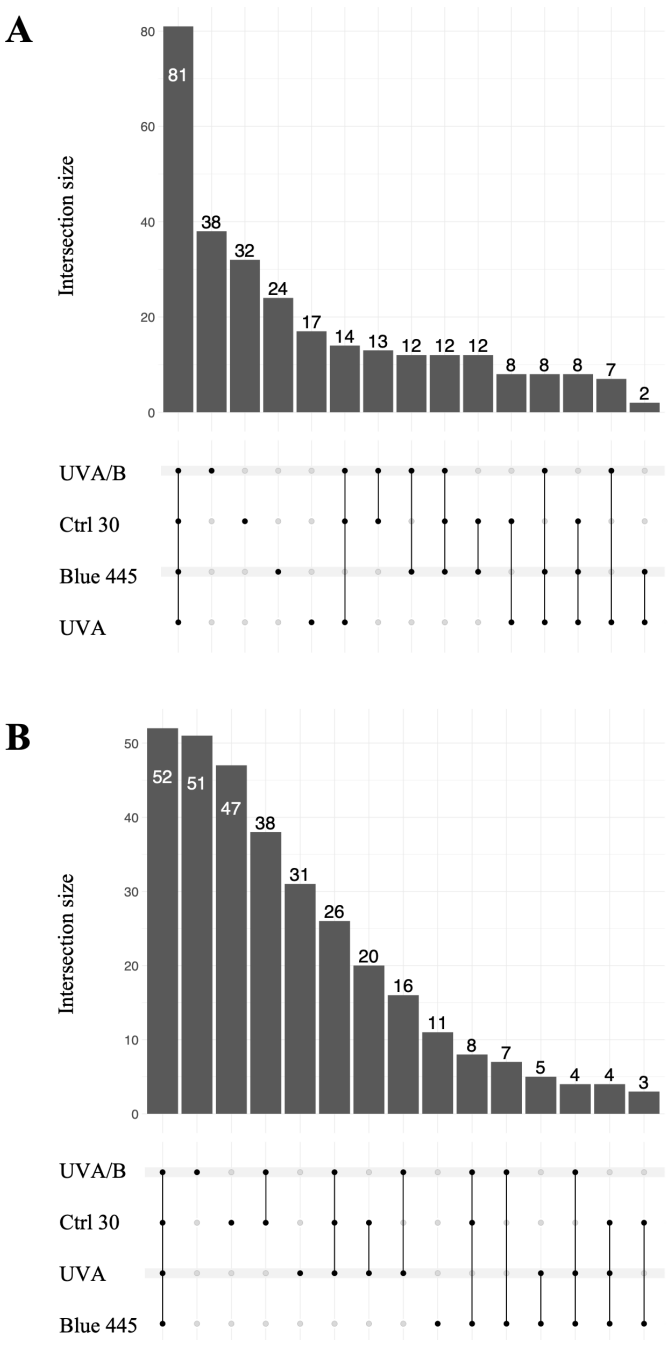


Figure 59: Overview of differentially regulated genes in UV experiment 2
Upset plots demonstrate shared downregulated (**A**) and commonly upregulated genes (**B**) between the experimental groups of UV experiment 2.The intersection size describes the number of differentially regulated genes when comparing NZM2410 mice with C57BL/6J mice. Each dot represents that this experimental group is involved, and the vertical lines mean shared differentially genes between the respective groups. Compared were the un-irradiated samples (Ctrl 30), UVA/B, UVA and blue light (445 nm) irradiated samples. Data was calculated using the R tool UpSetR and a lrt qval < 0.05 and |b| >1.

3.2.5 UVB irradiation causes the most differentially expressed pathways in UV experiment 1

Differentially expressed pathways were investigated using the R-tool GAGE ($q \leq 0.05$). The main findings were summarised in **tables 8** and **9**.

In experiment 1, when looking at both sexes, no pathways were significantly differently regulated between NZM2410 and C57BL/6J mice after 30 h of incubation without any irradiation. The same accounts for blue light (405 nm) irradiation, and UVC irradiation. However, when the mouse skin was irradiated with UVB light, NZM2410 mice had significantly upregulated pathways for translation, rRNA processing, citric acid TCA cycle, and mitotic cell cycle. When splitting the results for the sex of the mice, it could be seen that in male mice after 30 h, blue light (405 nm), and UVC irradiation, the keratinisation was significantly upregulated. Moreover, pathways for the extracellular matrix were downregulated after 30 h of incubation in male NZM2410 mice. After blue light (405 nm) irradiation, IFN signalling and collagen-related pathways were downregulated. UVB light did not cause any upregulation in pathways in male mice, however, in male NZM2410 mice, TLR and complement-related pathways were downregulated. In female mice, no differentially regulated pathways between NZM2410 and C57BL/6J mice were found in the 30 h controls. Upon blue light irradiation, ribosome pathways were significantly upregulated in female NZM2410 mice. After UVB treatment, oxidative phosphorylation and Alzheimer‘s disease-related pathways were upregulated. UVC treatment caused only in female NZM2410 compared to female C57BL/6J mice an upregulation of interleukin pathways (**Table 8**).

Table 8: Pathway regulation of NZM2410 compared to C57BL/6J mice for each sex of UV experiment 1

	30 h ctrl	Blue 405 nm	UVB	UVC
Both sexes	NA	NA	Up: Translation rRNA processing, Citric acid TCA cycle, Cell cycle mitotic	NA
Male mice	Up: Keratinisation Down: Extracellular matrix	Up: Keratinisation Down: Interferon, collagen	Down: Toll-like receptor, Complement	Up: Keratinisation
Female mice	NA	Up: Ribosome	Up: Oxidative phosphorylation, Alzheimer's disease	Up: Interleukins

3.2.6 The combination of UVA and UVB causes the most differentially regulated pathways in female mice of experiment 2

In experiment 2, most effects were seen upon UVA/B irradiation. When it was calculated for both sexes, only the cytokine-cytokine-receptor interaction pathways were significantly upregulated in NZM2410 mice compared to C57BL/6J mice. The investigation of male mice separately demonstrated that no pathways were differentially regulated between the two mouse strains after any of the tested treatments. Female mice showed differentially regulated pathways only after UVA and UVA/B irradiation. Upon UVA treatment, it was an upregulation of cytokine-cytokine-receptor interactions in female NZM2410 mice. After UVA/B treatment, it was the same but additionally also the upregulation of complement cascades, extracellular matrix (ECM) receptor interaction, and pathways specific for SLE were upregulated in NZM2410 mice compared to C57BL/6J mice (this detailed Kyoto Encyclopedia of Genes and Genomes (KEGG) plot can be found in the supplements (**Figure S1**)). Here, specifically complement factors were upregulated in NZM2410 mice in comparison to C57BL/6J mice, as well as Fc γ receptor and MHC II (**Table 9**).

Table 9: Pathway regulation of NZM2410 compared to C57BL/6J mice for each sex of UV experiment 2

	30 h ctrl	Blue 445 nm	UVA	UVA/B
Both sexes	NA	NA	NA	Up: Cytokine cytokine receptor interaction
Male mice	NA	NA	NA	NA
Female mice	NA	NA	Up: Cytokine cytokine receptor interaction	Up: Cytokine cytokine receptor interaction, Complement cascades, ECM receptor interaction, Systemic lupus erythematosus

3.2.7 Transcription factor regulation upon UV light treatment showed commonly downregulated *Zfx*, *Hivep2*, and *Ciita* over all treatments comparing NZM2410 to C57BL/6J mice

The differential regulation of transcription factors was calculated with the R tool decoupleR. Transcription factors regulate the expression of genes and thereby also indirectly the expression of proteins. This way they regulate cellular responses and processes in diseases. If irradiation with (UV) light is able to cause changes in transcription factors, these might then further orchestrate other processes in the response to the treatment as well as the lupus progression. It could be found that depending on the light, several transcription factors were differentially regulated, whereby most of them were significantly downregulated in NZM2410 mice. Common downregulated transcription factors (no matter the treatment) were *Zfx* and *Hivep2*, as well as *Ciita* and *Irf1* in experiment 1. No common upregulated transcription factors were found in experiment 1 (**Figure 60**). When splitting for sexes, only *Zfx* and *Hivep2* were commonly downregulated (**Figures S10**, **S11**, **S12**, and **S13**).

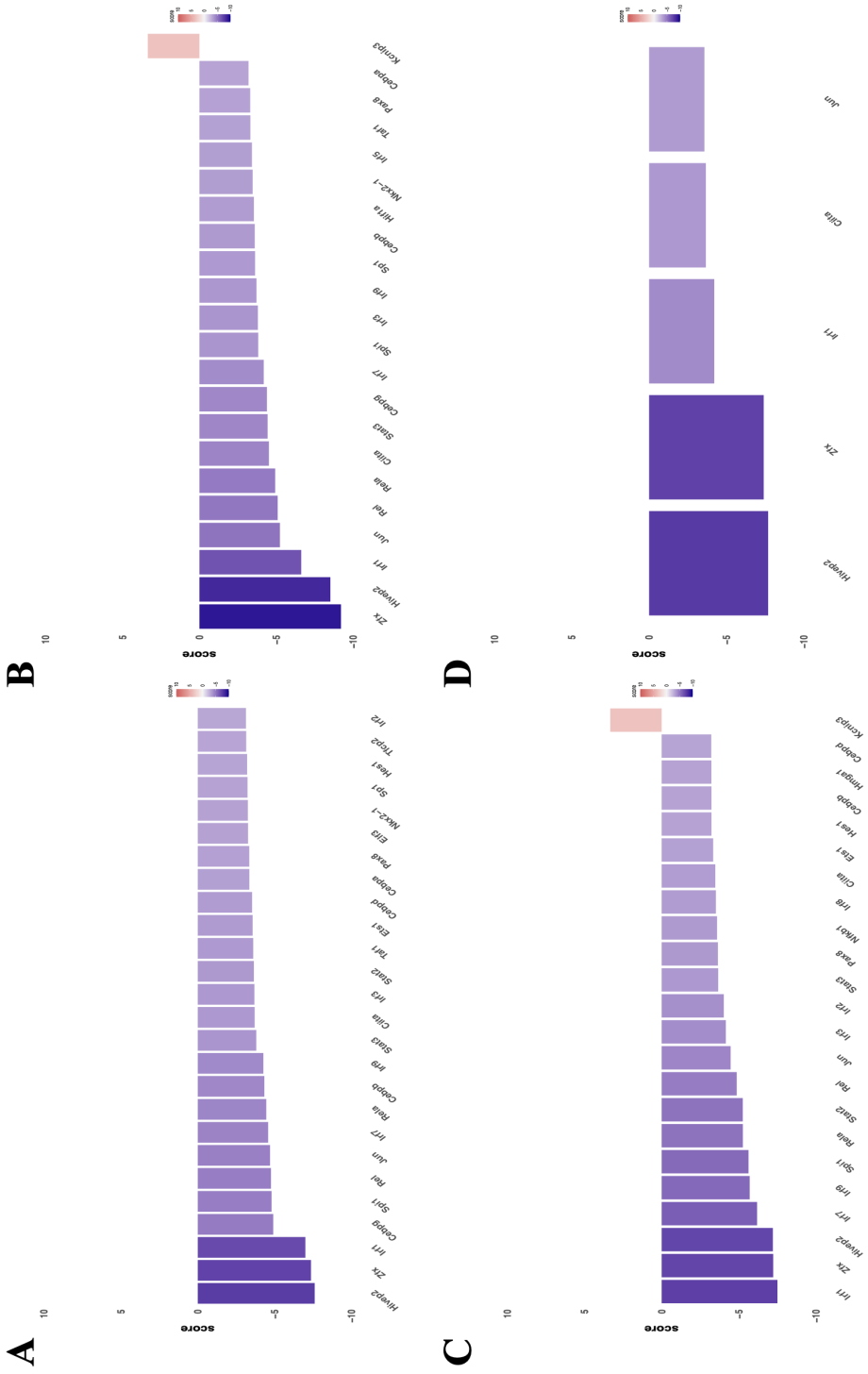


Figure 60: *Zfx*, *Hivep2*, *Ciita*, and *Irf1* are commonly downregulated transcription factors in all treatment conditions of UV experiment 1
Transcription factor analysis compared their expressions in NZM2410 mice and C57BL/6J mice in un-irradiated controls after 30 h of incubation (**A**) and the treatment conditions blue light 405 nm (**B**), UVB light (**C**), and UVC light (**D**). The shown data represents only significantly differentially regulated transcription factors, identified with the R tool decoupleR.

In UV experiment 2, common downregulated transcription factors were *Zfx*, *Hivep2*, and *Ciita*. A common upregulated transcription factor in all samples that were treated with light was *Nfkb1*. This was not upregulated in NZM2410 mice vs C57BL/6J mice that were not irradiated with any light (**Figure 61**). When splitting for sexes, only *Zfx* was commonly downregulated (**Figures S14**, **S15**, **S16**, and **S17**).

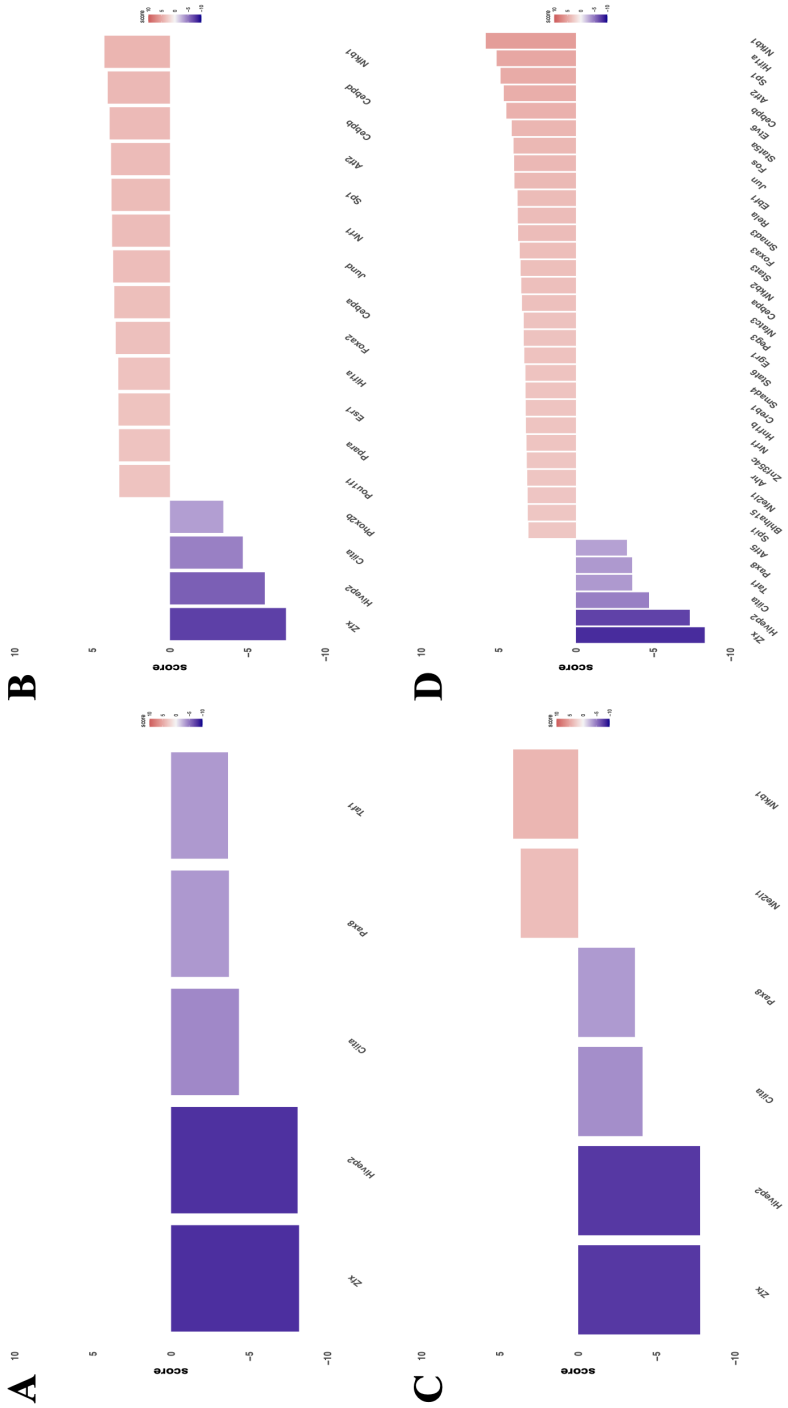


Figure 61: *Nfkb1* is a commonly upregulated transcription factor in NZM2410 mice compared to C57BL/6J mice when treated with light, while *Zfx*, *Hivep2*, and *Ciita* were commonly downregulated in UV experiment 2

Transcription factor analysis compared their expressions in NZM2410 mice and C57BL/6J mice in un-irradiated controls after 30 h of incubation (**A**) and the treatment conditions blue light 445 nm (**B**), UVA (**C**), and UVA/B (**D**). The shown data represents only significantly differentially regulated transcription factors, identified with the R tool decoupleR.

3.2.8 UV light treatments induce apoptosis in NZM2410 mice

The programmed cell death, apoptosis, is a known consequence of UV light-induced skin damage. Apoptosis can be investigated in several different stages and approaches. We decided to study it not only on transcriptomic level but also using fluorescent staining of cleaved caspase-3 and light microscopic investigation of sunburned cells on H&E stained sections. On mRNA level, we were able to see several changes on KEGG plots. After 30 h of incubation without irradiation, *Caspase-3* was already slightly upregulated in NZM2410 compared to C57BL/6J mice. At the same time, *Granzyme B* (*Gzmb*) was downregulated in NZM2410 mice. This lead in the end to a detectable decrease in the substrate cleavage for *Actin* and *Poly(ADP-ribose) polymerase* (*Parp*), as well as an increase in an *Inhibitor of caspase-activated DNase* (*Icad*). UVB irradiation caused in addition a higher upregulation in *Caspase-3* in NZM2410 mice and also an upregulation in *Caspase-7*. As an outcome, the cleavage of the substrates for *Actin* and *Parp* was downregulated, while it was upregulated for α*-Tubulin*, *Lamin* and *Icad*. Upon UVC irradiation, *Caspase-3* was also more upregulated in NZM2410 mice than after 30 h in un-irradiated controls. *Gzmb* and *Caspase-7* were similarly regulated as in the 30 h controls. In the end, the cleavage of the substrates for α*-tubulin* and *Actin* were downregulated in NZM2410 mice compared to C57BL/6J mice, while *Lamin* and *Icad* were upregulated. Blue light (405 nm) also caused the downregulation of *Gzmb* and a slight upregulation of *Caspase-3*. This resulted in a downregulated cleavage of α*-tubulin*, *Actin*, and *Parp*, as well as an upregulation of *Lamin* and *Icad* (**Figure 62 A**).

Next, we investigated cleaved caspase-3, the active protein of caspase-3, on cryosections using fluorescence staining. The stained area was compared in irradiated and control NZM2410 and C57BL/6J mouse skin sections. It could be seen that during the controls for the incubation times and conditions (0 h, 24 h, and 30 h), no significant difference between the genotypes could be detected. However, after UVB ($p= 0.026$), UVC ($p= 0.009$), and blue light (405 nm) ($p= 0.009$) treatment, a significantly larger area of cleaved caspase-3 could be detected in NZM2410 mice (**Figure 62 B**).

As UV light is able to cause sunburns, we decided to investigate it even though the treatments were of low intensity, as lupus-prone skin might potentially develop sun burns easier. The sunburned cells were counted in H&E stained 4 µm paraffin sections of the 6 mm skin punches. In H&E staining detectable sunburned cells demonstrate a late stage of apoptosis, not necessarily caused by sunburns, but used as a standard measure in dermatology after known UV light exposure. In general, only very few apoptotic cells could be found, and no difference between the genotypes could be found at any time or condition (**Figure 62 C**).

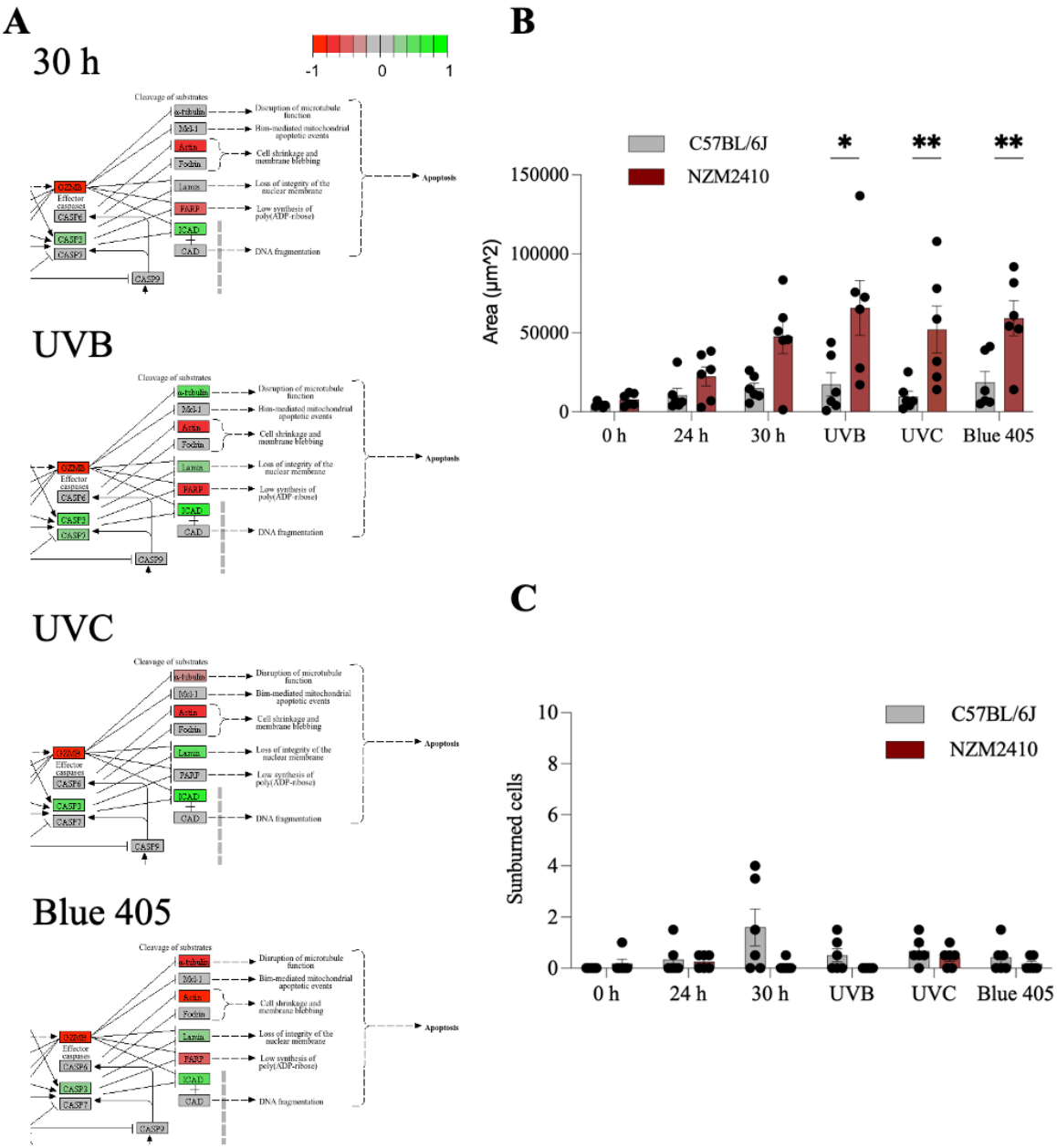


Figure 62: UV light-treatments of experiment 1 induce more apoptosis in NZM2410 mice than in C57BL/6J mice

Apoptosis in UV-treated skin samples of NZM2410 and C57BL/6J was investigated in three different ways. Firstly, via transcriptomic analysis of mRNA, demonstrating an upregulation of *Caspase-3* NZM2410 mice compared to C57BL/6J mice (**A**). Secondly, via fluorescence staining of cleaved caspase-3 on cryosections of irradiated skin samples, demonstrating significantly increased fluorescent areas in UVB, UVC, and blue light 405 nm treated NZM2410 skin samples compared to C57BL/6J samples (**B**). Thirdly, it was also investigated in H&E-stained skin sections by counting sunburned cells as a late stage of apoptosis. No differences between genotypes or conditions could be found in the number of sunburned cells (**C**). Transcriptomic results are shown as extracts of KEGG plots of murine apoptosis. Full KEGG plots can be found in the supplements (**Figures S2, S3, S4, S5**). Cleaved caspase-3 data and sunburned cells represented as mean +/- SEM, Mann-Whitney test with * $p<0.05$, ** $p<0.01$.

As presented for UV experiment 1, the same was performed for experiment 2. It could be demonstrated here as well in the transcriptomic data that *Caspase-3* and *Caspase-7* were upregulated in NZM2410 mice in all measured conditions. Regarding the different light-treatments, UVA is causing more downregulation of *Actin* in the NZM2410 mice than in the 30 h controls. Additionally, *Lamin* was not upregulated. The combination of UVA and UVB lead to a strong downregulation of *Caspase activated DNase* (*Cad*) and an upregulation of α*-Tubulin* in the NZM2410 mice. Actin was not differentially expressed in the mouse lines upon this treatment. Blue light 445 nm lead to a strong downregulation of *Granzyme B* in the NZM2410 mice. This is accompanied by a slight downregulation of α*-Tubulin* and *Actin*. Otherwise, as in the 30 h controls, *Caspase-3* and *Caspase-7* were upregulated in the NZM2410 mice compared to the C57BL/6J mice (**Figure 63 A**).

The staining for cleaved caspase-3 revealed that in the second UV experiment only after 24 h the NZM2410 mice had significantly more apoptosis. The controls directly at the punching time (0 h) showed no difference and only minimal cleaved caspase-3. After 30 h and for all the treatments, the spread was larger but for the UVA/B irradiation and the blue light 445 nm treatment, the trend is also visible that NZM2410 mice had more cleaved caspase 3 (**Figure 63 B**).

The counting of the sunburned cells showed as in experiment 1 that only very few cells were found. For none of the investigated samples a significant difference was found (**Figure 63 C**).

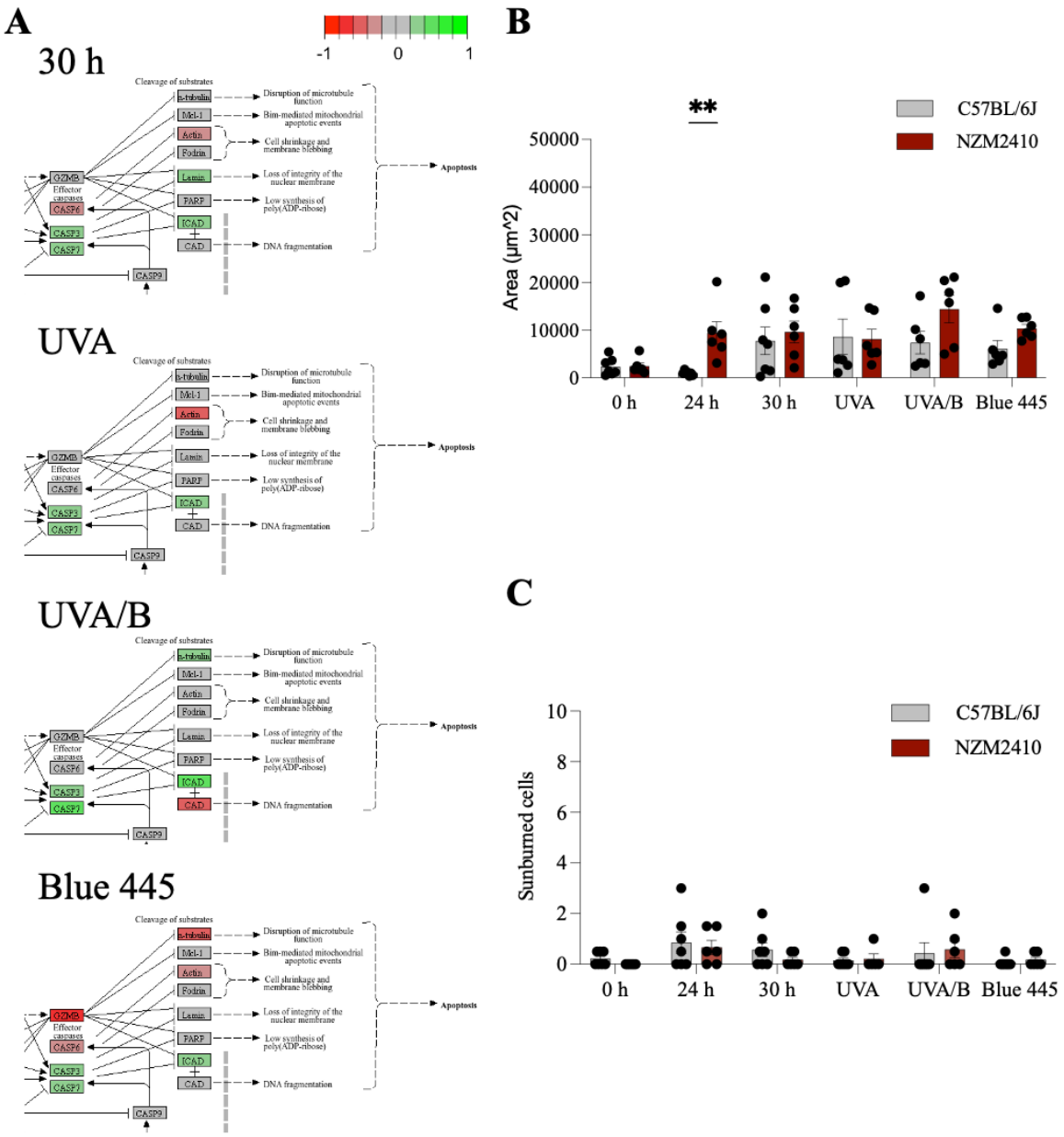


Figure 63: UV light-treatments of experiment 2 did not induce apoptosis in NZM2410 mice
Apoptosis in UV-treated skin samples of NZM2410 and C57BL/6J was investigated in three different ways. Firstly, via transcriptomic analysis of mRNA, demonstrating an upregulation of *Caspase-3* in NZM2410 mice compared to C57BL/6J mice (**A**). Secondly, via fluorescence staining of cleaved caspase-3 on cryosections of irradiated skin samples, demonstrating significantly increased fluorescent areas in 24 h control NZM2410 skin samples compared to C57BL/6J samples (**B**). Thirdly, apoptosis was also investigated in H&E-stained skin sections by counting sunburned cells as a late stage of apoptosis. No differences between genotypes or conditions could be found in the number of sunburned cells (**C**). Transcriptomic results are shown as extracts of KEGG plots of murine apoptosis. Full KEGG plots can be found in the supplements (**Figures S6, S7, S8, S9**). Cleaved caspase-3 data and sunburned cells represented as mean +/- SEM, Mann-Whitney test with * $p<0.05$, ** $p<0.01$.

4 Discussion

4.1 Comparison of lupus-prone mouse lines during the pre-disease phase

Two lupus-prone mouse lines (NZM2410 and SLE123 mice) were investigated from the age of 8 weeks to 21 weeks and compared to healthy C57BL/6J mice. This phase is known not to show clinical symptoms of SLE yet, so our aim is to explore the immunologic events preceding the full-blown disease in order to characterise it and potentially enable earlier diagnosis for patients.

4.1.1 Physical appearances

Firstly, the overall appearance of the lupus-prone NZM2410 and SLE123 mice as well as the healthy C57BL/6J mice was investigated at the ages between 8 and 21 weeks. Therefore, the **body weight**, and **spleen weight** as well as the **normalised spleen weight** were compared. The NZM2410 mice showed higher body weights throughout all measured time points, however, neither the spleen weight nor the normalised spleen weight differed majorly from the healthy C57BL/6J mice. The spleen weight was only significantly higher in NZM2410 mice at weeks 9 and 18. The normalised spleen weight was never significantly above the one of C57BL/6J mice but even significantly below at week 20. This looked different in SLE123 mice. Their body weight never differed from the ones of C57BL/6J mice, however, their spleen weight increased after the age of 12 weeks, reaching a significantly higher one from week 14 onwards. This is even clearer when the normalised spleen weight is seen, also with a continuous significant rise from week 14 onwards. An increased spleen weight can mean a splenomegaly and therefore an ongoing immune system activation, and inflammation (Qian Zhang *et al.* 2020; M. L. Richard and Gilkeson 2018). In the case of SLE, this is a hint for continuous B and T cell activation and the expansion of lymphoid follicles in the spleen (Chan and J. Shlomchik 1998). Additionally, splenomegaly is linked to an increased formation of germinal centres and plasma cells, which is driven by IgG (found in MRL/lpr mice) (Qian Zhang *et al.* 2020). Splenomegaly is known for many lupus mouse models during the active phase of the disease (Perry *et al.* 2011), also the SLE123 mice from an age of 9-10 months (Yin *et al.* 2015); however, it has before not been described at this early age. Nevertheless, potentially similar trends will appear at later ages in the NZM2410 mice, with the first hints in week 18. Since the NZM2410 mice showed higher body weight but not spleen expansion, these first stages of inflammation should not be linked to the excessive weight and fat depots in the NZM2410 mice, even though obesity is causing inflammation and the activation of immune cells as well (Heredia, Gómez-Martínez, and Marcos 2012). Especially here, the SLE123 mice confirm that at this early age, weight-independent

changes in the spleen already occur. Nevertheless, the increased weight in NZM2410 mice might have enhanced the disease onset after the described pre-disease flare as the developing team of the SLE123 mouse strain (Laurence Morel and colleagues) described that SLE123 mice (which have according to our data normal weight) demonstrate a later disease onset characterised by nephritis and autoantibodies than the NZM2410 mice (Morel, Croker, *et al.* 2000). This is hard to follow up though, as our experiments ended after 21 weeks and their investigations started thereafter because they were interested in the active disease phase. Moreover, a higher body weight in the NZM2410 mice, might also be induced by fatigue or pain and resulting reduced physical movement (as discussed below), but probably not a reduced caloric intake. Vorobyev and colleagues were able to show that the development of lupus in NZM2410 mice was largely abolished by calorie-restricted diet (Vorobyev *et al.* 2019). However, that study did not take the physical movement into account.

The assessment of the **physical movement** using the RAD detectors demonstrated that the NZM2410 mice moved in a period of a day (24 h), only about half as much as the corresponding C57BL/6J mice. Unfortunately, we do not have data for the SLE123 mice here. The reduced movement might explain the higher body weight of the NZM2410 mice and might at the same time be a hint for fatigue. Fatigue is a very common SLE symptom in humans (Dey, Parodis, and Nikiphorou 2021; Arnaud *et al.* 2019). It was also seen in lupus-prone MRL/lpr mice before by increased floating time in the forced swim test and reduced running wheel activity (Meeks and Larson 2012). This fatigued condition is not only impairing physical performance but also memory and mood. This was shown in the MRL/lpr mice, where it was mediated by infiltration of T cells into the brains (Mackay 2015). This is interesting to keep in mind when interpreting the T cell data later on, which are already dysregulated at this early age. Therefore, fatigue might explain why these young and unobtrusive NZM2410 mice did not move as much as the C57BL/6J mice and weighed more. They might already have inflammatory processes without an enlarged spleen.

4.1.2 Control of the development of nephritis

As nephritis is one of the hallmarks of active lupus in the NZM2410 and SLE123 mice, mediated by the locus *Sle3* (Chandra Mohan *et al.* 1999; Morel, Croker, *et al.* 2000; McGaha and Madaio 2014), we controlled for this factor to be sure to explore the pre-disease phase. The nephritis was investigated using PAS staining to evaluate lesions. So far, only kidneys from NZM2410 mice and C57BL/6J mice have been investigated. No significant difference between the genotypes could be found at any age, however, the NZM2410 mice showed peaks at weeks 10 and 18. These ages are discussed later in more detail, as many simultaneous changes occurred. The peaks are confirming that SLE is already acting systemically, not only involving the immune system, but it is already

starting to cause organ damage. This early damage seems to be able to heal again, as the values at older ages are lower again. As nephritis can have many forms, we talked to Dr. Hiroshi Nishi and colleagues from Tokyo University and they confirmed that fibrotic types of crescent formation in the kidneys can heal as causing immune cells might egress again.

4.1.3 Haematologic changes during the pre-disease phase

Next, blood values were assessed. As this **blood examination** is a regular process in human healthcare, we wanted to include it for our mice as well, as this might facilitate translational interpretation of results later on. Leukocyte counts could either indicate when too high (leukocytosis) that there is an infection, inflammation, or stress (Chmielewski and Strzelec 2018), or, when the numbers are too low (leukopenia), autoimmune diseases, problems with the leukocyte generation in the bone marrow or certain infections. As this is rather broad, the differential WBC count can provide more detail.

High lymphocyte counts may suggest infections or chronic inflammation (Hamad and Mangla 2023), while low counts might point to immunodeficiency and are linked to autoimmunity, as well as SLE (Rivero, Díaz-Jouanen, and Alarcón-Segovia 1978; Schulze-Koops 2004). In the mouse, 60-90 % of the circulating leukocytes are lymphocytes (Hedrich 2004). Monocytes are known for their tissue repair functions and are also involved in certain disease defence mechanisms (Ogle *et al.* 2016; Serbina *et al.* 2008). High levels indicate chronic infections or inflammations (Kapellos *et al.* 2019; Lichtmann 2012). Eosinophils fight parasitic infections and are involved in allergic reactions, thus high levels indicate such (L. Huang and Appleton 2016; Gigon *et al.* 2023). In the ScilVet ABC Plus+, it cannot be differentiated between neutrophils and basophils. However, both cell types are involved in inflammation, which is especially important to measure in lupus disease. In human SLE for example, elevated neutrophil counts (together with decreased lymphocyte counts) are a known measure (B. K. Han *et al.* 2020), whereas the counts of basophils were found to be significantly lower in SLE patients, and among them lower during active disease phases (P. Liang *et al.* 2015). This is, unfortunately, a limitation of the ScilVet ABC Plus+, nevertheless, the proportion of neutrophils outweighs the one of basophils in humans (Orfanakis *et al.* 1970), and in the mouse, 5-20 % of the circulating leukocytes are neutrophils, while basophils are very rarely observed (Hedrich 2004). This mix of neutrophils and basophils is potentially still delivering an insight into the role of the neutrophils in the pre-disease phase, even though peaks might not be detectable to the full extent.

Even though the **WBC counts** did not show any significant differences in SLE123 or NZM2410 mice from the C57BL/6J mice, the NZM2410 mice showed two peaks at week 10 and 19. These peaks were also seen in the counts of lymphocytes, monocytes, granulocytes, eosinophils, and neutrophils and basophils. In monocytes, granulocytes, basophils and neutrophils, the peak at weeks

19 and/or 20 was also statistically significantly higher in NZM2410 compared to C57BL/6J mice. Only for monocytes, the peak at week 10 was also significant, but a rise could be seen in all cell populations. This consistent rise and thereafter drop at an age when no significant kidney symptoms or proteinuria are measured yet could indicate an interesting phase in which the immune system tries to adapt to or fight against an immune activation event, such as an encounter with self-antigens. As this is seen in many animals in independent experiments, it should not be linked to external triggers such as infections, medication (they did not receive any), nutrition or similar. It might rather indicate early auto-reactivity, maybe influenced by the hormonal development of maturing animals. The fact that the peak decreases after a couple of weeks again could show that this is not the full disease onset yet, but an early phase with a recovery at week 20-21. What might be interpreted as a recovery at weeks 20-21 can also be discussed for the lymphocytes as lymphopenia, as these values of the NZM2410 mice were significantly lower than those of the C57BL/6J mice. As mentioned above, this might point towards autoimmunity. This idea of an early flare is also supported by a human study, showing that monocytes are elevated in early SLE flares even if SLE patients often show a reduced monocyte count (R. Lu *et al.* 2019).

The **RBCs** seem not to play a major role in the early development of SLE. For most of the measured times in NZM2410 and SLE123 mice, the RBC counts are slightly but not significantly above the values of the healthy C57BL/6J mice. This is similar for the haemoglobin and haematocrit. For later stages, it is known that about 50 % of human patients develop anaemia with low RBC counts, low haemoglobin and low haematocrit (Kunireddy *et al.* 2018). This is usually caused by one of three different kinds of anaemia. Firstly, it could be anaemia of chronic disease, which is mediated by an impaired erythropoietin function, leading to a decreased RBC production (S. A. Ahmed *et al.* 2019). Secondly, it could be an iron deficiency anaemia, which is often caused by medications such as long-term use of corticosteroids and thus increased gastrointestinal blood loss and menorrhagia (Voulgarelis *et al.* 2000). Thirdly, it could be an autoimmune haemolytic anaemia in which antibodies directly attack RBCs (Michalak *et al.* 2020). As it has not yet been seen in the mice, this supports the idea that we are still in the pre-disease phase.

However, the **MCV**, describing the average size of RBCs, differed in NZM2410 mice largely from the C57BL/6J mice. It was significantly lower at all measured ages except for week 21. Due to iron deficiency, which might cause anaemia, the MCV is often low (Wincup and Rahman 2018), whereas large red blood cells are seen in vitamin B12 or folate deficiency, or certain bone marrow disorders (Green and Mitra 2017). This effect was also seen at most tested ages in SLE123 mice.

The **MCHC** was at no time differing in NZM2410 or SLE123 mice from C57BL/6J mice and is therefore probably not associated with the development of SLE. It was also found for human SLE

patients that the MCHC was not significantly differing from healthy people (Jonny *et al.* 2023). However, another cross-sectional study was able to detect a correlation between lowered MCHC and increases in IL-6 and TNF (Aldakheel *et al.* 2023).

The **RDW** describes the variation in size of RBCs. A high RDW would support the idea of the iron deficiency (Evans and Jehle 1991), and is also seen as a marker for SLE (Mercader-Salvans *et al.* 2024) but here the RDW is lower not higher in NZM2410 mice than in C57BL/6J mice. A lower RDW was described in literature as infrequent and clinically meaningless (Salvagno *et al.* 2015). Even though we could not find a biological explanation for the decreased RDW, we would not call it clinically meaningless if it could be included in a panel of SLE-predictor values. Another interpretation could be that the low RDW in NZM2410 mice is caused by stress, as a significantly reduced RDW has also been found in physically stressed lambs (Clarke and Inglis 2019). As the NZM2410 mice also did not move as much as the C57BL/6J mice, potentially due to fatigue or joint pain or the cage-condition, this difference might also be a sort of physical stress. Taken together, we see a decreased RDW in lupus-prone mice, even though it is known to be increased in human SLE patients. We cannot fully explain this, but only speculate. Hence, the decreased RDW might just be a mechanism of overcompensation during the pre-disease phase, as human lupus patients often have higher RDW values, which correlate with disease activity scores (Moreno-Torres *et al.* 2022). It could also be associated with the young age of the lupus-prone mice we investigated here, as in humans the RDW was shown to vary with age (Bukhari, Zahid, and Zafar 2019). Unfortunately, no reliable references for healthy mouse blood measurements with the Scil vet ABC Plus+ exist, so our only reference is our measured healthy C57BL/6J mice. However, in week 17, a rise in the RDW was seen in NZM2410 mice, pointing towards the development of active SLE. In SLE123 mice, the RDW did not differ from C57BL/6J mice.

The **platelet counts** in NZM2410 mice were always higher than in C57BL/6J mice except for week 15. This difference was significant at weeks 10, 18, and 20. The increased numbers between week 17 and 20 fit to the phase, in which the WBCs were also increased. Platelets are released by megakaryocytes and are mostly known for their role in promoting blood clotting. However, platelets have more attributes, such as the ability to release inflammatory mediators such as P-selectin, non-neuronal serotonin, and they express CD40L (Linge *et al.* 2018). Moreover, complement activation occurs on the platelet surface, and a deposition of complement results also in platelet activation. Regarding lupus, both the involvement in inflammation as well as the direct connection to complement activation are of great importance to understand the disease progression (Scherlinger *et al.* 2018). In SLE, platelet activation causes inflammation and vice versa, thus, it is not easy to identify the starting point but platelets are commonly dysregulated (Scherlinger *et al.* 2018; Boilard,

Blanco, and Nigrovic 2012). In human SLE, reduced platelet counts are more frequent and also part of the classification criteria (Hochberg 1997). However, active inflammation could also be a reason for higher platelet numbers as inflammatory cytokines such as IL-6, IL-1α and TNF are able to stimulate platelet production in the bone marrow (Couldwell and Machlus 2019). Also, in mice, it was seen that platelets can be rapidly produced when needed (Hedrich 2004). Another reason for high platelet counts can be iron deficiency anaemia, which was also indicated by the low MCV. In this case, the bone marrow tries to compensate for this anaemia by an increased platelet production (Akan *et al.* 2000). Lastly, it could again be a mechanism of overcompensation as part of the dysbalance during the pre-disease phase. This could be described by the hidden Markov model (P. Chen and Y. Li 2016). The model demonstrates three phases, the healthy phase in which the system has a high consistence score and is able to respond to challenges with low dynamical changes. The second phase is the pre-disease stage. The duration in this stage underlies high individual variance. During this stage already rather small triggers or challenges can cause high dynamic changes. The last stage is the disease stage, which is stationary, stable and not dynamic. At this point, the body is not able to regulate inflammation in SLE any longer.

The high platelet counts were only visible in the NZM2410 mice and not in the SLE123 mice. So this may be a trait of the immune system that is not caused by the susceptibility genes *Sle1*, *Sle2*, or *Sle3* but is otherwise genotype-mediated. In humans, it was also found that obesity (and the NZM2410 mice had increased body weight) leads to increased platelet counts (L. E. Charles *et al.* 2007).

The **platelet volume** is usually indicative of the activation state of the platelets as they increase in volume upon activation (Thompson *et al.* 1983). Larger platelets tend to be younger and more active, whereas smaller ones are older and less active. In human SLE, low MPV values are often seen during active SLE phases and flares (Khadra, Drie, and Kudsi 2023). Low platelet counts and high volumes could also point to the body's approach to compensate for the loss of platelets with the production of new, larger, and younger ones (Celkan 2020). A high platelet volume could also be due to increased platelet production. As the platelet volume in NZM2410 mice is only slightly increased compared to C57BL/6J mice between weeks 17 and 21 this should not be over-interpreted but might support the idea of a phase of immune activation as seen in other cell types.

Since there was more than one independent experiment in every tested age in NZM2410 mice, except for week 10 (5 animals), the recurrent peaks in NZM2410 mice, especially between the ages of 16 and 21 weeks can be seen as true values. For SLE123 mice, also only weeks 10 (4 animals) and 15 (1 animal) consisted of only one independent experiment, whereby at week 15, only one animal was measured. Therefore, the drastically differing values in week 15 in SLE123 mice need

to be interpreted with caution.

4.1.4 T helper cells and their state of differentiation

The pre-disease phase in NZM2410 and SLE123 mice was further explored in T cells and their subsets in blood, spleens, and lymph nodes using flow cytometry.

In the **blood**, the frequencies of **CD4+ T cells** of both lupus-prone mouse lines were above those of C57BL/6J mice, more prominently in NZM2410 mice, though. Multiple reasons are potentially responsible for the elevated CD4+ T cell frequencies. Firstly, the known loss of tolerance in SLE might allow auto-reactive T cells to expand without being regulated by Tregs (Ohmes *et al.* 2022; Sakaguchi *et al.* 2008; J. Humrich, Kamradt, and Riemekasten 2015). Another potential reason could be defects in apoptosis (Kaplan 2004), luring T cells to tissues. This is supported by studies that found CD4+ T cells primed to nucleosomal antigens before the onset of disease in lupus-prone mice (Mohan *et al.* 1993; Amoura *et al.* 1994; Fournel *et al.* 2003). In humans, T cells primed for nuclear antigens were also found in healthy people but were expanded in SLE patients. Whether this priming precedes the onset of the disease is not yet clear (Abdirama *et al.* 2021). Besides, in human SLE patients, lymphopenia is often reported (Sobhy, Niazy, and Kamal 2020).

Next, the CD4+ cells were split for their differentiation state by CD44 and CD62L. CD44 is important for the activation and trafficking of T cells. The expression is upregulated upon activation of the cells and allows trafficking to the sites of inflammation as CD44 binds to hyaluronic acid, which is upregulated in inflamed tissues in mice and humans (Johnson and Ruffell 2009; Puré and Cuff 2001; Misra *et al.* 2015). It is also involved in the entry into secondary lymphoid organs and inflamed tissues, where the T cells interact with other immune cells (DeGrendele *et al.* 1996; Baaten *et al.* 2012; Zahalka *et al.* 1995). The adhesion molecule CD62L (L-selectin) is necessary for T cell trafficking and homing. It is needed for the initial adhesion of T cells to the endothelium (Savage *et al.* 2002). Next, they roll along the endothelium until they can enter through high endothelial venules to migrate into lymphoid tissues or sites of inflammation. This marker is also useful to distinguish stages of differentiation of T cells as T cells downregulate CD62L when they become effector T cells in non-lymphoid tissues (R. Ahmed *et al.* 2009). This has the purpose that naive T cells and memory T cells can recirculate throughout the body until they get activated. Dysregulation of CD44 or CD62L not only impairs the optimal fighting of pathogens but can also exacerbate autoimmune reactions. The frequencies of **naive T cells** (CD62L+, CD44-) in the **blood** were higher in NZM2410 mice until the age of 18 weeks. Then they dropped below the values of C57BL/6J mice, while at the same time, the frequencies of effector CD4+ T cells (CD44+, CD62L-) were rising, showing significantly higher values than C57BL/6J mice at weeks 20 and 21. The **central memory**

CD4+ T cells (CD44+, CD62L+) were also showing a small rise in NZM2410 mice at this time, however, they never exceeded the frequencies of C57BL/6J mice. The higher frequencies of naive T cells could be explained by the recruitment of naive T cells as part of the onset of active disease (Gordon *et al.* 1996). However, in human patients, mostly a reduced number of naive T cells and an increase in memory T cells is seen (Yuan *et al.* 2022). Even though this is the opposite of our data, it makes sense in the scheme of the pre-disease. This might be the result of expanding autoreactive T cells, while Tregs are already impaired but try to regulate the upcoming autoimmunity with varying success, leading to ups and downs of inflammation/dysregulation (Ohmes *et al.* 2022). The shift towards **effector T cells** at the age of 18 weeks supports the peaks seen in the haemogram data. A phase of more effector and memory T cells points towards an active immunologic process, like a "pre-SLE flare", as CD4+ T cells start differentiating upon encountering (auto-) antigens, such as dsDNA (and antibodies against it), which is accumulating already before the onset of clinical symptoms (Arbuckle *et al.* 2001). It could not be expected that this is due to facing real pathogens or other antigens, as the animals were kept all the time in the animal facility of the University of Lübeck, which is specific pathogen-free. It could also be seen that this effect will probably decrease again in effector and memory T cells after week 21. Here, longer observation of the pre-disease phase would have been beneficial. The changes in CD4+ T cell subtypes in SLE123 mice were not as prominent as in NZM2410 mice. However, SLE123 mice showed more naive CD4+ T cells in week 14, whereby this frequency decreased afterwards. As in the NZM2410 mice, the effector T cells were also rising in the SLE123 mice, significantly exceeding the frequencies of C57BL/6J mice in week 18. The interpretation of the CD4+ naive and effector T cells of SLE123 mice is the same as for the NZM2410 mice.

CD4+CD8+ T cells were also investigated as they have been described in literature several times to be an increased population in autoimmunity with rather undefined and controversial functions (Overgaard *et al.* 2015). However, rather recent research suggests that increased frequencies of double-positive T cells are associated with an increased risk of lupus nephritis in SLE patients (Chang *et al.* 2023). As this association does not tell anything about their function, Wu and colleagues investigated this and suggested a suppressive role in the production of autoantibodies (Y. Wu *et al.* 2014). Nevertheless, they also state that much more research is needed to fully identify their functions.

The expression of **CD25 on non-Treg CD4+ T cells** was also investigated as this might provide insight into their activation state. CD25 is the α-chain of the IL-2 receptor whose upregulation allows IL-2 signalling. This promotes the proliferation and differentiation into Th1, Th2 effectors and CD8+ memory T cells (Létourneau *et al.* 2009; M. A. Williams, Tyznik, and Bevan 2006). The

opposite effect is seen on Tfh and Th17 cells (Johnston *et al.* 2012; Laurence *et al.* 2007). This is especially interesting regarding SLE, as Tfh and Th17 cells contribute significantly to inflammation and worsening of autoimmunity (Suárez-Fueyo, Sean J Bradley, and George C Tsokos 2016). Even further, it was found in SLE, that Tfh cells can become T follicular regulatory cells by IL-2 (Hao *et al.* 2021). As IL-2 is not directly reliably measurable using flow cytometry, we have to rely on the positive correlation with CD25 expression (Spee-Mayer *et al.* 2016; Létourneau *et al.* 2009; Boyman and Sprent 2012; Boyman, Kovar, *et al.* 2006).

Therefore, it is not surprising to see a peak in the frequency of CD25+ CD4+ T cells in NZM2410 mice at week 18, the phase, which has already before found to be immunologically very active. These high values could only be found in week 18, however, as for this age, several independent experiments were performed, we take this short and sudden peak seriously. SLE123 mice showed a rather high (but not significant) frequency of CD25+ CD4+ T cells at younger ages (before 14 weeks). This might either indicate that SLE progression is earlier in these mice compared to NZM2410 mice, or that the T cells already become exhausted and lose the CD25 expression after the age of 14 weeks.

In the **spleens**, NZM2410 mice had at all measured ages higher **CD4+ T cell** frequencies than C57BL/6J mice. Most of the time, SLE123 mice had higher CD4+ T cell frequencies than C57BL/6J mice, but they were throughout still lower than those of NZM2410 mice. As in the blood, NZM2410 mice had for a rather long period higher **naive CD4+ T cell** frequencies than C57BL/6J mice and as they dropped, the **effector CD4+ T cell** frequencies were rising. Most prominently, the NZM2410 mice had lower **central memory CD4+ T cell** frequencies than the C57BL/6J mice at all ages except for week 21. This is pointing towards a lupus-mediated altered T cell differentiation (Paredes, Fernandez-Ruiz, and Niewold 2021). This looked similar in SLE123 mice, however, their naive CD4+ T cells dropped earlier, and the **effector cells** rose earlier, leading towards an earlier disease progression. SLE123 mice showed a significant peak in the frequency of **CD4+CD8+ T cells** at the age of 16 weeks, however, the actual frequency was still very low (about 0.5 %). No significant differences regarding CD4+CD8+ T cells were found in the spleens of NZM2410 mice. When checking the **active CD4+ T cells** (expressing CD25) in the spleens, it becomes apparent, that SLE123 mice had early on (ages 10-12 weeks) significantly higher frequencies of these activated CD4+ T cells, whereas NZM2410 mice had only a peak at week 18 and otherwise even lower frequencies than C57BL/6J mice. This might mean again that NZM2410 mice had a "pre-SLE flare" at week 18, while SLE123 mice had such an immunologic activation earlier. In the **lymph nodes**, the frequencies of **CD4+ T cells** were also significantly higher throughout all measured time points, except for week 19 (about 60 % of live cells in NZM2410 mice, about 30 % in C57BL/6J mice). Potentially, the drop in week 19 can be explained by increased apoptosis and reduced T cell

expansion right after the "pre-SLE flare" from week 18 (Miyara *et al.* 2005). SLE123 mice did not differ significantly from C57BL/6J mice regarding CD4+ T cell frequencies in the lymph nodes. The **naive CD4+ T cells** in the lymph nodes of NZM2410 mice had a trough at week 19 but were otherwise higher than in C57BL/6J mice. In SLE123 mice, they were starting lower, increasing to week 10 and dropping again after week 17, which is directly opposing the **effector CD4+ T cells**. This means that at very early ages (8 weeks) large amounts of effector T cells were needed, probably due to facing auto-antigens during their "pre-SLE flare". Both NZM2410 and SLE123 mice had lower **central memory CD4+ T cell** frequencies, potentially again due to altered T cell differentiation processes. **CD4+ CD8+ T cells** were in NZM2410 mice at several weeks significantly higher than in C57BL/6J mice, which is highly interesting as this was not found in the other tested organs. Yet, their function is unclear, and it can only be speculated why they were increased in the NZM2410 mice. SLE123 mice showed -as in the spleens- a small rise at week 16, but in the lymph nodes, this was not significant. The **active CD4+ T cells** were much higher until the age of 14 weeks in SLE123 mice compared to C57BL/6J mice, again pointing towards the "pre-SLE flare" at weeks 10-12. NZM2410 mice had, most of the time, lower frequencies but also showed a rise in week 18. This was not significant but supports their "pre-SLE flare". To our knowledge, these changes in the T cells in the pre-disease phase of NZM2410 and SLE123 mice have not been reported before.

Comparing the **CD4+ T cells in all three organs**, similar trends can be seen in NZM2410 mice, even though the frequency was much higher in the lymph nodes. For SLE123 mice, significant differences from C57BL/6J mice could only be seen in the spleen at several ages. This effect would have been lost when only investigating the blood. Regarding the naive CD4+ T cells, all three organs showed higher frequencies in lupus-prone mice (not always significant) with decreases over the weeks. The simultaneous rise in effector CD4+ T cells could only be seen for NZM2410 mice in blood and spleen, but not in the lymph nodes. For SLE123 mice, this was detectable in all three organs. The reduced frequencies of central memory CD4+ T cells were found in the spleens and lymph nodes of both lupus-prone mouse lines. In the blood, this effect is not detectable. This indicates a difficulty in transitioning the findings to human studies, as they are mostly limited to blood examinations. Differences in double-positive T cells of NZM2410 mice were most prominently seen in the lymph nodes. For SLE123 mice, the choice of organ would not make a difference for this cell type. When investigating the active CD4+ T cells, differences between NZM2410 and C57BL/6J mice can be best seen in blood and spleens, but not in lymph nodes. For SLE123 mice, the differences cannot be detected in the blood but in the other two organs. Taken together, it remains important to investigate this systemic pre-disease phase in multiple organs.

4.1.5 Cytotoxic T cells

CD8+ T cells in the **blood** were also investigated. There is a trend towards higher **CD8+ T cell** frequencies in NZM2410 mice compared to the other mouse lines; however, this was only significant in weeks 10, 16, and 20. CD8+ T cells are needed for their cytotoxic function. In human and murine SLE, CD8+ T cells are often found to be dysregulated (P.-M. Chen and George C. Tsokos 2021), increased in circulation (Xiong *et al.* 2023; Ciurtin *et al.* 2022), or impaired in functionality (P.-M. Chen and George C. Tsokos 2021; Suárez-Fueyo, Sean J Bradley, and George C Tsokos 2016). We did not investigate the functionality, even though this would have been highly interesting, but we also checked blood, spleens, and lymph nodes. The elevated frequencies in the blood of lupus-prone mice might be due to high activation, which caused homeostatic expansion in the peripheral blood (Ciurtin *et al.* 2022). Another potential mechanism could be the impaired retention in the lymphoid organs, due to altered chemokine receptor expressions such as CCR7 and CXCR5 (Haynes *et al.* 2007; Wiener *et al.* 2016; L. Han and L. Zhang 2023), which would also be interesting to investigate in future studies. CD8+ T cells might also have been actively recruited to the blood by circulatory chemokines such as RANTES (CCL5), binding to CXCR5, which is more highly expressed on CD8+ T cells of SLE patients (Sengupta *et al.* 2023). The CD8+ T cells might also have been recruited to inflamed tissues such as the kidneys (Winchester *et al.* 2012). This is, however, not supported by our data as it does not show significant nephritis. The frequencies of **naive CD8+ T cells** were also higher in NZM2410 mice compared to C57BL/6J mice. As the frequencies for both NZM2410 and SLE123 mice dropped below the values of C57BL/6J mice, the **effector CD8+ T cells** of both lupus-prone mouse lines rose (non-significantly). This is similar to the CD4+ T cells, also occurring at similar ages, leading to the same interpretation, even if the changes were not significant.

In the **spleens**, the changes in **naive**, **effector**, and **central memory CD8+ T cells** indicated again that the immune activation in SLE123 mice occurred earlier than in the NZM2410 mice.

In the spleens, the frequency of **activated CD8+ T cells** (of all CD8+ T cells) was much higher during the peak times (NZM2410 week 18 and SLE123 weeks 10-12) with up to 80 % than for the activated CD4+ T cells (of all CD4+ T cells) with frequencies up to 15 %. Thus, CD8+ T cells were more activated than CD4+ T cells during this "pre-SLE flare". This could mean that CD8+ T cells were of more relevance for this process than the CD4+ T cells, as during acute inflammatory reactions, the cytotoxic capacities are needed. This difference between CD4 and CD8 T cells was also seen in the blood.

In the **lymph nodes**, the **CD8+ T cells** of SLE123 mice were very similar to those of C57BL/6J mice. The CD8+ T cells of NZM2410 mice were even lower in many weeks. Interestingly, the **naive CD8+ T cells** of NZM2410 mice remained rather constant (but higher than in C57BL/6J mice),

only with a small drop in week 19 after the "pre-SLE flare". In the lymph nodes, in contrast to the spleens, the **effector CD8+ T cells** of NZM2410 mice did not increase. One potential reason is the aforementioned impaired trafficking by CXCR5. Another reason could be the choice of lymph nodes, as these often only drain the neighbouring organs. We chose the inguinal lymph nodes, which drain mainly the lower extremities, skin, and subcutaneous regions as well as mammary glands (Harrell, Iritani, and Ruddell 2008). Hence, these organs are potentially not tissues of an active immunologic process in these early phases of lupus. The effector CD8+ T cells were neither rising in SLE123 mice. Instead, when the naive CD8+ T cells of SLE123 mice were decreasing, the **central memory CD8+ T cells** were rising. This was the same in the spleens. This is likely due to immune activation and antigen-driven differentiation (Blanco *et al.* 2005). In NZM2410 mice, the central memory CD8+ T cells seemed impaired, with constantly significantly lower frequencies. The **active CD8+ T cells** (CD25+) in the lymph nodes were very high in weeks 10-12 in SLE123 mice, their "pre-SLE flare". The same is seen in NZM2410 mice at week 18, their "pre-SLE flare".

Comparing the three organs, we can see in NZM2410 mice elevated CD8+ T cell frequencies in the blood, but reduced frequencies compared to C57BL/6J mice in the spleens and lymph nodes. Unfortunately, we cannot precisely name the reason for this CD8+ T cell distribution between the organs, as we lack mechanistic markers here, but it supports the notion of early changes in the immune cells in the pre-disease phase. This finding was only seen in NZM2410 mice, not the SLE123 mice. For NZM2410 mice, the naive CD8+ T cells were higher than in C57BL/6J mice in all three organs. For SLE123 mice, significant differences of naive CD8+ T cells were not detectable in the blood, but were well detectable in the spleens. The same accounts for the rise of effector CD8+ T cells of SLE123 mice. Here, the organ does not make a difference for NZM2410 mice. The reduced frequency of central memory CD8+ T cells of NZM2410 mice was measurable in all organs. For SLE123 mice, changes of the central memory CD8+ T cells were better identified in the spleens than in the lymph nodes or the blood. Changes in the state of activity were visible in all organs of NZM2410 mice. The highly active states of CD8+ T cells of SLE123 mice at the ages of 10 and 12 weeks were also detectable in all organs, but minor later changes were only significant in the blood. This is again leading to the assumption that each organ is differently involved in the immunologic processes in the disease, which might make the transition to human studies only possible for some markers.

4.1.6 CD4+ regulatory T cell frequencies and their differentiation

From the CD4+ gate, the **Tregs** were found by setting the axes to FoxP3 and CD25. The intranuclear transcription factor FoxP3 is specific for the development, function, and maintenance of Tregs.

FoxP3 helps, for example, to upregulate immunosuppressive molecules such as CTLA-4 and IL-10. CD25 is a high-affinity receptor for IL-2, which is crucial for the survival and function of T cells. IL-2 itself cannot be well detected, as mentioned before, so we used CD25. This acts as a functional marker for Tregs. CD4+, FoxP3+ T cells can be divided into functional (CD25+) and non-functional (CD25-) Tregs (Horwitz *et al.* 2008; Horwitz 2010; Zelenay *et al.* 2005). We classified Tregs as CD4+, FoxP3+, and CD25+ cells (the active form).

While the frequencies in the **blood** of all mice were consistently low (below 2 %), the NZM2410 mice still show a (mostly non-significant) trend towards even lower Treg frequencies than the C57BL/6J mice. Reduced active Treg frequencies are a common feature in human and murine SLE (W. Li *et al.* 2019; Mizui and George C. Tsokos 2018; Okamoto *et al.* 2011) and it is impressive to see this even before the onset of any symptoms. This leads to the assumption that insufficient frequencies of active Tregs in the blood lead to defective tolerance, allowing auto-reactivity (Barreto *et al.* 2009; W. Li *et al.* 2019). Due to the low frequency of Tregs in the blood, the analysis of **naive**, **effector**, and **central memory Tregs** is difficult to interpret. Nevertheless, at 19 and 21 weeks of age, higher effector CD4+ Tregs were found compared to C57BL/6J mice. This might indicate the approach of the immune system to regulate the seen "pre-SLE flare". SLE123 mice did not seem to differ in any of these categories at any measured age.

The frequencies of **CD4+ FoxP3+ but CD25- Tregs** (inactive ones) were in the blood not much different between the different mouse lines. This could simply indicate that in the blood, the Tregs were not yet affected by the reduction of CD25 expression that comes with (pre-)disease. Differences here might be seen in older ages. Another explanation could be that the blood is not the place to detect these differences, as immunologic processes and mechanisms occur mainly in tissues.

In the **spleens**, the **CD4+ Treg** frequencies were reaching about 6 % in C57BL/6J mice, however, in NZM2410 mice, they were even during the peak at week 18 below 4 %. The frequencies in the spleen are higher than in the blood, which is expected because only about 0.3 % of overall Tregs are circulating in the blood. The majority is located in tissues (Burton *et al.* 2023). This peaking value in the pre-disease phase is interpreted as described before as "pre-SLE flare" even if the frequency remained below the one of the C57BL/6J mice, as it is still a peak in this time course. In SLE123 mice, the Treg frequencies were higher at younger ages and dropped later on. At the age of 12 weeks, they were even significantly exceeding the frequency of C57BL/6J mice. This can be seen as an attempt to counter-regulate upcoming autoimmunity. Lower frequencies of CD4+ Tregs are also known from human disease (but measured in blood) (Barreto *et al.* 2009; W. Li *et al.* 2019) and nowadays an important target for therapy (J. Y. Humrich, Spee-Mayer, *et al.* 2019; J. Y. Humrich and Riemekasten 2016). The **CD25- (inactive) CD4+ Tregs** are rising over time in both lupus-prone

mouse lines, supporting the notion of the loss of tolerance already at an early age. The frequency of **naive CD4+ Tregs** in NZM2410 was already dropping early, with a trough at week 10 and another one at week 18, exactly the ages at which occurring immune reactions were seen before, and probably at this time, the CD4+ Tregs differentiate. According to this data, they most likely differentiated into **central memory CD4+ Tregs** as this cell type is peaking at the same time. The formation of Treg memory is especially important and needed at these stages, because the cells are here potentially memorising auto-aggressive cells, which would allow increased reaction time and intensity during future flares. It is known that Tregs differentiate upon specific antigen encounter (Z. Xu *et al.* 2021; Sennikov *et al.* 2020), but how their long-term survival in the absence of antigens (as between SLE-flares) is promoted is rather unclear (Khantakova, Bulygin, and Sennikov 2022). Moreover, memory Tregs are required to avoid constant immunosuppression but only allow adequate regulation when needed (Khantakova, Bulygin, and Sennikov 2022). That the **effector CD4+ Tregs** dropped at the age of 18 weeks in NZM2410 mice in the spleen might either mean that they are dysregulated and not capable of reacting, or simply that they have been recruited to another tissue. SLE123 mice showed an increase in inactive CD4+ Tregs in the spleen with age, while at the same time memory Tregs and naive Tregs dropped. The effector CD4+ Tregs were first lower in SLE123 mice compared to the C57BL/6J mice but increased potentially due to the aim of regulating the autoimmunity.

In the **lymph nodes**, the frequency of **CD4+ Tregs** was even higher in C57BL/6J mice, reaching almost 8 %. The SLE123 mice demonstrated similar numbers and did not differ significantly from C57BL/6J mice. The NZM2410 mice, however, differed strongly and significantly at all measured ages. They showed lower Treg frequencies, mostly below 2 % of all CD4+ T cells. This is a difference between the SLE123 and the NZM2410 mice, meaning that this effect might not be due to one of the SLE-gene loci, but rather to other traits of the NZM background. Another explanation could be that this effect is only appearing later in the SLE123 (after the measured time points here). This is likely as reduced Treg frequencies in blood and lymph nodes are a known feature of human SLE as well (W. Li *et al.* 2019; X. Liu *et al.* 2017; Tsai *et al.* 2023; Miyara *et al.* 2005). In literature, it is also reported that SLE123 mice at the age of 5 months demonstrated Treg frequencies, which were not different from C57BL/6 mice in the spleens (Stocks *et al.* 2016). At week 18, there is a small peak for NZM2410 mice, potentially the aim to counteract the "pre-SLE flare". For several inflammatory conditions, an increase in Tregs in the spleen could already be shown in mice (J. H. Lee, Chuanwu Wang, and C. H. Kim 2009; Romano *et al.* 2016). Interestingly, even though the NZM2410 mice had already reduced active Treg frequencies, the **inactive ones** were not increased but also reduced. This means that there is a general reduction in Tregs, and they are not

only inactivated. The **naive Tregs** were in SLE123 mice higher at the ages of 10-12 weeks and decreased thereafter. As the **effector Tregs** rose at the same time, they potentially differentiated as a measure after the "pre-SLE flare". In NZM2410 mice, a drop of naive Tregs is also seen after week 18, as well as an increase of effectors, pointing to the same interpretation as for the SLE123 mice.

When comparing **all three organs**, it becomes apparent that changes in CD4+ Treg frequencies are best detectable in the lymph nodes of NZM2410 mice. Similar trends can be seen in the spleen, but this is not always significant. Changes in CD4+ Treg frequencies would be missed in the blood. For SLE123 mice, the choice of organ does not make a difference regarding the frequencies of CD4+ Tregs. The differences in naive CD4+ Treg frequencies were for SLE123 mice only detectable in the lymph nodes, and also for NZM2410 mice, they would have been missed in the blood. Differences in effector Tregs could be detected for NZM2410 mice in all organs for week 9. At the other ages, the frequency varied depending on the organ. For SLE123 mice, the organ does not make a difference regarding the effector CD4+ Tregs. Differences in central memory CD4+ Tregs could not be well detected in blood or lymph nodes for both lupus-prone mouse lines. The frequency of inactive Tregs varied depending on the organ. For Treg investigations in mice, the blood can be used but due to the low frequencies, a certain volume of blood needs to be processed and analysed to gain reliable results. This requires additional time and dissection skills. Moreover, it limits the experimental options, as often not enough blood can be gained to analyse serum and blood. In humans, it is easier to draw larger blood volumes, which compensates for this limitation. Nevertheless, the Tregs have different functions and purposes depending on the organ (Sjaastad *et al.* 2021; Rothstein and Camirand 2015). Human studies should consider this when evaluating their results.

4.1.7 Functional CD4+ regulatory T cell analysis

The active Tregs were then further investigated for their expression of CTLA-4, CD39, Helios, and Ki-67.

Helios is an intranuclear marker linked to functionality and stability of Tregs. The break of tolerance in lupus may be associated with a reduced expression of helios, resulting in a lower suppressive capacity and stability. In the **blood**, the expression of helios seemed to underlie high variances. At no measured age did any of the mouse lines differ significantly.

In the **spleens**, SLE123 mice showed lower frequencies of helios+ CD4+ Tregs at almost all measured ages, even though their frequency was increasing over time. This could mean that this mouse line had early on less stable Tregs, but they tried to increase this as a counter-regulation of the upcoming autoimmunity. In human SLE, similar trends are seen as helios is upregulated during active disease compared to healthy controls or patients in remission (Golding *et al.* 2013;

T. Alexander *et al.* 2013). The helios expression also correlates with the disease activity when measured with the SLEDAI score (Golding *et al.* 2013). The NZM2410 mice showed a drop in helios+ Tregs at the typical age of 18 weeks, potentially the Tregs were recruited to another tissue at this age because helios-expression is stabilising the Tregs and can therefore also be seen as a functional marker (H.-J. Kim *et al.* 2015; Getnet *et al.* 2010). Most likely, primarily functional Tregs are recruited to the sites of inflammation.

In the **lymph nodes**, both lupus-prone mouse lines showed at most ages reduced frequencies of helios+ Tregs. As helios is not only stabilising the FoxP3 expression, but a knockdown also eliminates their repressive capabilities (Getnet *et al.* 2010), reduced helios+ Treg frequencies in the lymph nodes, an immunologically active site during inflammation, is most likely pointing out that this impairment is already fostering the lupus development. The frequency-reduction of helios+ Tregs was larger in NZM2410 mice than in SLE123 mice.

Another feature of helios expression in Tregs is that those Tregs do not produce pro-inflammatory cytokines such as IFN-γ, IL-2, or IL-17 but instead IL-10 (Golding *et al.* 2013; Y. C. Kim *et al.* 2012; Elkord, Abd Al Samid, and Chaudhary 2015). It could even be shown in a tumour setting that a loss of helios is able to convert Tregs into effector T cells (Nakagawa *et al.* 2016). The reduction of the frequency of helios+ Tregs during or right after the "pre-SLE flares" could be explained by a migration to sites of inflammation, such as kidneys, and it is also supporting the idea that flare frequency correlates with disease severity (Hammond *et al.* 2021; Katz *et al.* 2020). Thus, the inflammation of the flares is feeding into the vicious cycle, worsening the disease. As IL-2 signalling is required to maintain Tregs (Barron *et al.* 2010), it is likely that the IL-2 signalling is also at this early age already impaired. Future experiments should include IL-2 measurements. Due to the local secretion and short half-life of IL-2 (Rokade *et al.* 2024), these experiments are ideally in vitro experiments of isolated and stimulated T cells of the mice to identify their capacities in production. Moreover, helios was initially thought to allow differentiation between central and peripherally induced Tregs because thymus-derived Tregs were found to be helios+ (Thornton, Korty, *et al.* 2010; Petzold *et al.* 2014). However, others found that helios expression could be stimulated by antigen-presenting cells, hence, leading to the assumption that helios is expressed in Tregs depending on the context of stimulation during FoxP3 induction (Gottschalk, Corse, and Allison 2012; Thornton and Shevach 2019). Therefore, we would not like to use helios expression to identify a loss of central or peripheral tolerance, but rather as a marker of functionality as described above.

Ki-67 is a proliferation marker. It is expressed during the active phases of the cell cycle (G1, S, G2, and M phase but not G0), which means that Ki-67+ cells are actively proliferating (S.-W. Huang *et*

al. 2024; Gerdes *et al.* 1984). The expansion of Tregs can indicate the attempt of the immune system to compensate for autoimmune processes. At the same time, a reduced expression of Ki-67 on Tregs means a reduced immunosuppressive potential, thus the inability to control autoimmune processes. This marker can be especially interesting in the autoimmune pre-disease. For this marker, in the **blood**, higher expression frequencies were seen in NZM2410 mice at the ages of 18 and 20 weeks. This can again be seen as an attempt to compensate for the preceding immune dysregulation, which was seen in other immune cells. The Tregs aimed to proliferate to be able to counteract the "pre-SLE flare". Interestingly, SLE123 mice showed continuously higher Ki-67 expression than C57BL/6J mice, except for weeks 17 and 18, supporting the idea that the pre-disease phase in SLE123 mice is setting on earlier than in NZM2410 mice. In C57BL/6J mice, there is also a peak in Ki-67 expression in weeks 17 and 18, and 21. This might be due to a specific developmental stage, as usually, Ki-67 expression reduces with ageing (Tomasetti *et al.* 2019). Of course, these developmental stages might also cause the peaks in the NZM2410 mice, thus, this marker should be evaluated with caution. Hormones might also play a specific role in certain maturation stages in all mouse lines. It would not be surprising if this is interfering with the development of autoimmunity as SLE in humans is also known to develop mostly in young women in the childbearing ages with a peak between 20-25 years of age (Brinks *et al.* 2016), which also show high levels of oestrogen, progesterone and fertility (Lipson and Ellison 1992; Lephart 2018). For future experiments, the detection of certain hormones such as oestrogen would be interesting to correlate with other immunologic changes.

In the **spleens**, the expression patterns of Ki-67 are very similar to the Tregs found in the blood. Nevertheless, the expression of Ki-67 during the 18-week peak in NZM2410 mice was almost twice as high in the blood as in the spleen, indicating a higher need for proliferation in the periphery than in the spleen.

In the **lymph nodes**, this peak in Ki-67 expression in NZM2410 Tregs is also seen at week 18, but not that prominently. This might mean that the Tregs in the spleens proliferated more upon the "pre-SLE flare" than the Tregs from the lymph nodes. Reasons therefore could be that the spleen is filtering blood-borne antigens (Bronte and Pittet 2013), which can include immune complexes and apoptotic cell remains. This means a constant exposure to antigens from the whole body. Especially during a flare, more auto-antigens are present in the spleens than in the lymph nodes. Lymph nodes are often only draining neighbouring tissues (Null, Arbor, and Agarwal 2025). The spleen might also have a higher availability of IL-2 for the Tregs, which they need to maintain and function. A reason for this is antigen-presenting cells, including (plasmacytoid) dendritic cells, which produce IL-2 (Naranjo-Gómez *et al.* 2007) and also IL-10 (Simpson *et al.* 2016), which promotes the development and function of Tregs (Carlini *et al.* 2023).

A mouse study also suggested that Tregs from lupus-prone mice are hindered more from egress from the lymph nodes than the spleens due to higher CD69 expression in the lymph nodes. This in turn, is mediated by IFN-signalling (Zhou *et al.* 2022). In SLE123 mice, Ki-67+ Treg frequencies decrease from about 40 % in week 8 to less than 5 % in week 18. This strong loss of proliferation indicates the strong and long-enduring impact of the "pre-SLE flare" on the tolerance, potentially facilitating more new flares.

CTLA-4 is a surface marker that is constitutively expressed on Tregs and is important for the immunosuppressive function of Tregs (Tai *et al.* 2012; Tekguc *et al.* 2021). CTLA-4 and CD28 (expressed on other T cells) have the same ligands on antigen-presenting cells (CD80, CD86). Whereas CD28 promotes activating signals in these cells, CTLA-4 is inhibitory. CTLA-4 also has a higher affinity (100-1,000 fold) to its ligands than CD28 and can thereby outcompete it and prevent T-cell activation (X. Zhang *et al.* 2003).

In the **blood**, the frequency of CTLA-4 expressing Tregs is higher in NZM2410 mice at most of the measured ages, but this is only significant at the peaks at age 10 and 18 weeks. The peak at 18 weeks is again especially interesting as this "pre-SLE flare" seems now to be counteracted by Tregs upregulating their CTLA-4 and thereby their immunosuppressive capacities. This reaction might especially prevent CD4+ and CD8+ T cells from getting further over-activated due to its ability to outcompete CD28. SLE123 mice showed again this trade earlier, but this is not significant.

In the **spleens**, the expression of CTLA-4 was only significantly exceeding the frequencies of C57BL/6J mice at the age of 18 weeks. At weeks 17 and 19, it was even significantly below. As this looks different in the blood, it might mean that the anergic capacities mediated by CTLA-4 were more needed in the periphery for most of the time, only during this "pre-SLE flare" in week 18 it was also highly needed in the spleens of NZM2410 mice. In SLE123 mice, the earlier high expression of CTLA-4, which was lost after the age of 14 weeks, was also visible in the spleen, and here it was even significant at weeks 10 and 12. This is potentially the age of the "pre-SLE flare" in this mouse line.

In the **lymph nodes**, CTLA-4+ Tregs never showed significantly higher frequencies in NZM2410 mice than in C57BL/6J mice. Yet, a peak with high variance among the animals is seen at week 19, right after the "pre-SLE flare". SLE123 mice showed a continuous decline in CTLA-4 expressing Treg frequencies with a short peak at week 12 before the frequency dropped below the one of C57BL/6J mice. The interpretation is the same as for the spleen values. The almost complete loss of CTLA-4 expression in SLE123 mice older than 15 weeks is another important feature leading to increased autoimmunity.

CD39 is a measure of immunosuppressive capacity in Tregs (Álvarez-Sánchez *et al.* 2019; Timperi

and Barnaba 2021). CD39 helps reduce immune activation and inflammation by hydrolysing extracellular ATP, which generates immunosuppressive adenosine (Schuler *et al.* 2014). This also inhibits natural killer cells (H. Zhao *et al.* 2017), promotes stronger stability and function of more Tregs (Gu *et al.* 2017), and promotes tissue repair and resolution of inflammation (Lei *et al.* 2015).

In the **blood**, CD39+ Tregs of NZM2410 mice showed again peaks at weeks 10 and 18. Besides these weeks, there was no difference between them and the C57BL/6J mice. The significant peak in week 18 supports the previous discussion by actively upregulating suppressive abilities. SLE123 mice were again expressing higher frequencies in earlier ages, but this was not significant at any measured age.

In the **spleens**, the frequency of CD39+ Tregs was lower at all ages in NZM2410 mice compared to C57BL/6J mice except for weeks 10 and 18. The usually lower expression demonstrates the dysregulation of Tregs, which might be genetic (Rissiek *et al.* 2015; Timperi and Barnaba 2021). However, they aimed to compensate for this at the "pre-SLE flare". The same accounts for SLE123 mice, just earlier.

In the **lymph nodes**, CD39 in SLE123 mice was decreasing drastically, as CTLA-4 did. This means that the Tregs lost their regulatory capacity at the early age of approximately 15 weeks. This might facilitate further flares (Romo-Tena, Gómez-Martín, and Alcocer-Varela 2013). Besides, the interpretation of the lymph node data on CD39 is the same as for the spleens.

Comparing the expression of the functional markers **in all three organs**, only changes Ki-67 can also be reliably detected in the blood. Differences in frequencies of CTLA-4+, helios+, and CD39+ Tregs could only be partly seen in NZM2410 mice and barely at all in SLE123 mice. The spleens and lymph nodes, however, showed strong differences between the genotypes with low variances in the frequencies of CTLA-4+, helios+, and CD39+ Tregs, which supports their specificity and importance.

4.1.8 Frequencies of CD8+ regulatory T cells

CD8+ Tregs are a subset of CD8+ T cells which are not cytotoxic but immunosuppressive. Similarly to CD4+ Tregs they were gated on the expressions of FoxP3 and CD25 (Cosmi *et al.* 2003). However, various other CD8+ Tregs - natural and induced - were identified with other markers (R. K. Dinesh *et al.* 2010; Niederlova *et al.* 2021), which we did not include in our panel. The CD8+ FoxP3+ Tregs are less common than CD4+ Tregs (according to literature), with expression frequencies of about 0.07 % to 0.4 % of the CD8+ T cells in blood, spleens, and lymph nodes of C57BL/6 mice (Churlaud *et al.* 2015; Lerret *et al.* 2012). We could measure similar values in C57BL/6J mice. Nevertheless, the CD8+ Tregs have a known role in SLE by being reduced or functionally impaired, yet the data is

inconsistent (Mak and Kow 2014). It could be shown that in active SLE, the suppressive abilities of CD8+ Tregs are reduced, while in inactive SLE, they are as potent as in healthy individuals (Filaci *et al.* 2001). These FoxP3+ CD8+ Tregs in the mouse are able to suppress the production of IFN-γ of conventional T cells, as well as their proliferation and cytokine production (Lerret *et al.* 2012). CD8+ Tregs were also found to be able to reduce the numbers of Th17 cells and to increase the numbers of CD4+ Tregs in mice (J. Sun *et al.* 2019). Low-dose IL-2 therapy is also able to replenish CD8+ Tregs, even more than CD4+ Tregs in mice and humans (Churlaud *et al.* 2015).

In the **blood**, the frequencies of CD8+ Tregs in all mouse lines were low, however, NZM2410 mice showed a small peak in weeks 10 and 18. Even though this was not significant, it supports the previous interpretation of the data. In SLE123 mice, the frequencies of CD8+ T cells were again higher in the younger ages and significantly above those of C57BL/6J mice. Even though in human SLE, the frequencies are known to drop, opposite effects in the pre-disease phase are not uncommon, as the immune system still tries to counter-regulate the upcoming disease and has more capabilities to do so than during the active, irreversible phase. This effect is also described by the high dynamics of the system's state during the pre-disease phase in the hidden-Markov-model (P. Chen and Y. Li 2016). The inactive CD8+ Tregs were also investigated, and the NZM2410 mice had significantly more inactive CD8+ Tregs in week 21 compared to C57BL/6J mice, indicating the continuing loss of tolerance.

In the **spleens**, the peak at week 18 in NZM2410 mice was very prominent with frequencies over 3 %, which is high for CD8+ Tregs (Churlaud *et al.* 2015; Lerret *et al.* 2012), but it is known that numbers and frequencies vary depending on the health state (Niederlova *et al.* 2021). At the other ages, the frequencies in NZM2410 mice were significantly below those of C57BL/6J mice, showing a strong dysregulation in tolerance from a very early age. In SLE123 mice, the peak at week 10-12 was also very strong with values over 4 %. The inactive CD8+ Tregs are similar in NZM2410 and C57BL/6J mice. In SLE123 mice, they started rising after the age of 12 weeks, indicating further loss of tolerance after the first "pre-SLE flare". Moreover, another study found that CD8+ Tregs from the spleens of lupus-prone SLE123 mice were not capable of suppressing CD4+ effector T cells and B cells (Stocks *et al.* 2016). This demonstrates that the immune system aims to regulate the upcoming inflammation but fails in many different ways.

In the **lymph nodes**, the prominent peak of CD8+ Tregs of the spleens of NZM2410 mice was much smaller, only about 1 % of all CD8+ T cells. This indicates that the need for CD8+ Tregs was higher in the spleen than in the lymph nodes, pointing again to a more systemic problem. Other lymph nodes, such as renal lymph nodes, might have been more appropriate as well, as a study using NZM2328 mice could show at later ages more immunologic changes in renal lymph nodes

than inguinal ones (Bagavant *et al.* 2006). For SLE123 mice, the values peaked at week 12 with about 3 %, which is still remarkable but smaller than in the spleen. The reasons could be the same as for the NZM2410 mice. There was, as in the spleens, no difference between the inactive CD8+ Tregs of NZM2410 mice and the C57BL/6J mice. The SLE123 mice started with significantly lower inactive Tregs until the age of 12 weeks and thereafter increased to significantly higher values of inactive CD8+ Tregs than C57BL/6J mice. This might mean that the CD8+ Tregs that were high in the young ages of SLE123 mice lost their CD25 and became inactive after the "pre-SLE flare". A similar but not significant trend has been seen in the NZM2410 mice, which showed a very small peak at week 19, right after the "pre-SLE flare" of week 18.

In all three organs, a steep rise at week 21 in the frequency of CD8+ Tregs could be detected for C57BL/6J mice. This might be explained by an age-related effect, as Srinivasan and colleagues were able to demonstrate fluctuations during the lifetime of C57BL/6J mice (Srinivasan *et al.* 2024). Differences in CD8+ Treg frequencies in SLE123 mice could be detected similarly in all three organs. The differences between NZM2410 and C57BL/6J mice can only be seen in the spleens and lymph nodes. The differences in inactive CD8+ Tregs in SLE123 mice were most prominently seen in the spleens and were not detectable in the blood. Again, this supports the importance of investigating several organs. As this is not feasible in human studies, our findings underscore the importance of animal experiments, which allow for the dissection of all organs of choice.

4.1.9 Changes of CXCR4 and CX3CR1 on T cells during the pre-disease phase

CXCR4 and CX3CR1 are trafficking markers which were chosen to be included in this panel not only because CXCR4 is needed for the migration of T cells into various tissues (Arieta Kuksin, Gonzalez-Perez, and Minter 2015; Kohli, Pillarisetty, and T. S. Kim 2022; Contento *et al.* 2008), but also because it serves as a target for hydroxy chloroquine (J. Kim *et al.* 2012) and is known to be dysregulated in SLE patients (L.-d. Zhao *et al.* 2017). CX3CR1 is a receptor needed to guide T cells towards inflamed tissues, where its ligand is upregulated (Nanki *et al.* 2002; Blaschke *et al.* 2003). Additionally, it is an important receptor in cardiovascular disease (Apostolakis *et al.* 2009), which is an important complication in SLE (Frostegård 2023). Moreover, in lupus-prone MRL/lpr mice, CX3CR1 was found to contribute to nephritis (Cabana-Puig *et al.* 2023). Thus, these two markers indicate a certain activity of the disease but also the T cells' attempts to react to it.

In the **blood**, CX3CR1 is at most ages expressed less on CD4+ T cells of NZM2410 mice than in the other mouse lines. Only at week 21, the frequency of CD4+ CX3CR1+ T cells of NZM2410 mice exceeds the ones of C57BL/6J mice, potentially guiding the T cells to inflamed tissues at this age. Interestingly, a similar trend is seen in CD8+ T cells with an extra peak at week 18, meaning

that CD8+ T cells were recruited to inflamed tissues during the "pre-SLE flare", while CD4+ T cells were not guided to the inflamed tissues but rather to lymphoid organs. This makes sense as cytotoxic T cells are needed on the site of inflammation, while T helper cells are more crucial in the lymphoid organs to orchestrate and pass on signals to other cell types, such as B cells. CD4+ Tregs of NZM2410 mice showed an increase in CX3CR1 expression over time. Even though this was not significant compared to C57BL/6J mice, it is still interesting, and the peak at week 18 supports previous notions. SLE123 mice had similar frequencies of CX3CR1-expressing T cells compared to C57BL/6J mice at all ages.

Generally, the CXCR4 expression patterns were surprising, as human CD4+ T cells are known to express CXCR4 in very high frequencies (80-100 %) (Niu *et al.* 2017), and this is similar for CD8+ T cells in humans, but with high variation (Kobayashi *et al.* 2004). In the mice we only saw values up to 6 %. Which is partly supported by literature but those values were about 17.8 % on average (Ramonell *et al.* 2017) and varying depending on the age (Aboumrad, Madec, and Thivolet 2007). One reason for the low expression we found in mice could be the timing of the experiments. A study showed in mice spleens and lymph nodes that the expression of CXCR4 was higher in the evenings (tested in spleens and lymph nodes) (Hand *et al.* 2020) and we conducted all experiments in the mornings. The opposite was found for humans, meaning that their CXCR4 expression was higher in the mornings than the evenings (Dimitrov *et al.* 2009), potentially because humans are diurnal and mice are nocturnal animals. Only the Tregs expressed CXCR4 in much higher frequencies (up to 60 %). Also for human Tregs, it is known that they highly express CXCR4 (Santagata *et al.* 2017). For murine Tregs, we could not find data in the literature that would be comparable to ours. The higher frequencies could be explained by the high need for Treg recruitment, especially in the spleen. Moreover, fluctuations in CXCR4 expression also in the wild types could be explained by variances in oestrogen levels. This hormone was shown to cause a downregulation of CXCR4 in murine Tregs (X.-L. Fan *et al.* 2015). Here, future analyses, which separate for sex-specific differences are needed.

In our experiments, lower frequencies of CD4+ T cells of NZM2410 mice expressed CXCR4 compared to C57BL/6J mice. Only at weeks 10 and 18, peaks were seen again (but this was not significant). This might mean that CD4+ T cells were generally trafficking less to tissues in NZM2410 mice compared to C57BL/6J mice. Only during active times of inflammation, such as the "pre-SLE flare", CD4+ T cells increased their levels of CXCR4. The same is seen for CD4+ Tregs. This might be due to trafficking but also due to changes in the differentiation state (Kobayashi *et al.* 2004). In CD8+ T cells, the picture is rather difficult, with high variance, potentially meaning various diffuse responses. SLE123 mice showed again higher frequencies in the younger ages with a significant peak for CD8+ T cells at week 16 and for CD4+ Tregs at week 12. Potentially, their

"pre-SLE flare" is not as distinct as the one in NZM2410 mice but earlier, likely between weeks 10 and 12.

In the **spleens**, the expression of CX3CR1 was not lower in NZM2410 mice than in C57BL/6J mice. At some weeks, this was even higher, but it was never significant. SLE123 mice showed here a reduced frequency of CX3CR1+ CD4+ T cells compared to C57BL/6J. In CD8+ T cells, the expression frequency of CX3CR1 varied largely depending on the week of age, but when comparing the mouse lines, only week 18 in SLE123 mice was significantly different from the C57BL/6J mice. The CD4+ Tregs in NZM2410 and C57BL/6J mice had similar expression frequencies of CX3CR1. In SLE123 mice, the expression decreased with age, leading to significantly lower values at the age of 18 weeks. This could mean an impaired capability of Tregs to invade inflamed tissues.

CXCR4 expression frequencies in CD4+ T cells were underlying high variances in all three mouse lines. NZM2410 mice showed tendentially lower values, but this difference was never significant. SLE123 mice revealed significantly higher values at week 12, pointing out their "pre-SLE flare", while they were significantly lower at week 18, potentially showing a loss of functionality in the trafficking. CD8+ CXCR4+ T cells showed a peak in NZM2410 mice at week 17 (not significant) and a small drop at week 18 (significant). This could indicate an attempt by the immune system to recruit the CD8+ T cells to certain tissues to fight upcoming inflammation, which was seen as "pre-SLE flare" one week later. In week 18, this was potentially no longer required. SLE123 mice, in turn, showed a significantly lower value than C57BL/6J mice at week 10, which is during their "pre-SLE flare", which would be similar to the NZM2410 mice, where CXCR4 was also reduced during the active inflammation phase. Usually, CXCR4 is known to be upregulated during inflammation to guide the cells to inflamed tissues (L. Lu *et al.* 2024), so our finding of low values during times of inflammation might either mean that the measured site is not inflamed and/or the cells were needed elsewhere. On the other hand, if we knew the distribution of the CD8+ T cells throughout the entire organism, we could also speculate whether the expression of CXCR4 or its ligand CXCL12 is disturbed in SLE-prone mice. For human SLE-patients, it was already found that both CXCR4 and CXCL12, on B and T cells, are dysregulated, and on B cells, this was even correlating with disease activity (Guilpain *et al.* 2011). CD4+ Tregs showed the clearest expression frequencies of CXCR4, compared to the CD4+ and CD8+ T cells. Here, the frequencies were also much higher, reaching almost 50 %. In NZM2410 mice, we saw the typical peaks at 10 and 18 weeks. In this cell type, it was now expected to be upregulated during the "pre-SLE flare" and not afterwards, because the Tregs are then recruited to regulate the inflammation as soon as it happened. In SLE123 mice, this is similar with a significant peak at weeks 10-12.

In the **lymph nodes**, both lupus-prone mouse strains showed reduced CX3CR1 expression in

CD4+ T cells at several time points. For NZM2410 mice, this was also the case in CD8+ T cells and CD4+ Tregs. SLE123 mouse CD8+ T cells and Tregs started with similar frequencies of CXCR4 expression compared to C57BL/6J mice but revealed decreased values after the age of week 12. Lower values in lupus-prone mice could indicate an impaired guiding of the cells towards inflamed tissues. If immune cells, especially Tregs, do not reach their target region, it might prevent the resolution of inflammation. Here, the expression levels of other cell types, such as monocytes, would be interesting as well.

The frequencies of CXCR4+ CD4+ T cells is mostly lower in NZM2410 mice and in SLE123 mice after the age of 14 weeks. CD8+ T cells showed an increase in CXCR4+ frequencies in NZM2410 mice at the age of 19 weeks, so right after the "pre-disease flare". A similar pattern is seen in the Tregs, with the peak in NZM2410 mice lasting from week 18-19. This could indicate an increased need for migrating into the lymph nodes, potentially to regulate and cooperate with the inflammatory processes of the "pre-disease flare". SLE 123 mice demonstrated this peak earlier in Tregs, lasting from week 8-12, but leading to the same explanation as their "pre-disease flare" was also earlier.

4.1.10 Comparison of NZM2410 and SLE123 mice and the "pre-disease flare"

The lupus-prone mouse strains show some differences, such as the higher body weight of the NZM2410 mice or the increasing normalised spleen weight of the SLE123 mice. The reduced RDW was also only seen in NZM2410 mice. Potential reasons for this could be the different genetic background.

Nevertheless, these two mouse lines also have similarities, such as the "pre-SLE flares". Even though this took place at different ages (SLE123 weeks 10-12, NZM2410 week 18), they show the same properties such as the shift from naive T cells to effectors and the increases in active CD4+ and CD8+ T cells during these times. The increases in Ki-67+, CTLA-4+ and CD39+ Tregs during these times are similar hallmarks of the "pre-SLE flares". This accounts for the blood, spleens, and lymph nodes. Thus, these changes can be linked to beginning autoimmunity rather than to other genetic or metabolic differences of NZM2410 mice.

4.1.11 Definition of the pre-disease phase and markers to use to identify the pre-disease state reliably

The exploration of the pre-disease phases of the NZM2410 and SLE123 mice showed that even if the genetic background had some similarities, their pre-disease phases looked different, especially

regarding the timing. We found that every week had distinct features, therefore, we could not merge several weeks of age. The fast changes and development are, of course, interesting, but make it rather difficult to find markers that work throughout the entire pre-disease phase. This is needed as human patients would not have complaints or clinical symptoms during the pre-disease phase, and therefore, we would not know "where" in this process they currently are. Humans show even more variance than inbred mouse strains due to ethnicity and simply genetic variation (Ernst and Carvunis 2018; Masopust, Sivula, and Jameson 2017). Thus, identifying a pre-disease phase in them is even harder. Indicators that work throughout the entire pre-disease phase in the tested mouse lines, and even later on, according to our experiments, are reduced Treg frequencies, low central memory CD4+ and CD8+ T cells, as well as high CD4+ T cell frequencies. The naive CD4+ and CD8+ T cells work only during the early pre-disease phase as markers, thus not as a general pre-disease marker, but they might aid in clarifying the state of pre-disease when seen together with the effector T cells. To transition these findings into human clinical studies, the markers have to give reliable, significant results in the blood. This was only found for the increased CD4+ T cell frequency and increased naive CD8+ T cell frequency in NZM2410 mice. In SLE123 mice, Ki-67+ CD4+ Tregs as well as CD8+ Tregs could be used as well. Since human patients show high individuality, and we do not know whether they resemble NZM2410 or SLE123 mice more, we would suggest using all four cell types to identify the SLE pre-disease phase.

From the analysis of the peripheral blood by haematology, only the MCV was decreased throughout the entire pre-disease phase in both lupus-prone mouse lines. However, the RDW was also decreased in the NZM2410 mice, and the platelets were increased, thus, these two markers could be seen as second-line indicators if other values were already pointing towards autoimmunity. Other haemogram data showed peaks during the pre-SLE flare but not before or after. Hence, they could not be used to identify the pre-disease phase, but rather, if this had been confirmed already, they might be used as identifiers of the "pre-SLE flare".

Moreover, the RAD-detectors suggested a form of fatigue early on in the NZM2410 mice and fatigue is also known as an early symptom of autoimmunity (Leuchten *et al.* 2018). Since it is not ideally measurable and not well defined yet, it is hard to evaluate. Nevertheless, it would be valuable to collect questionnaires of patients such as the Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT-Fatigue) scale (Kosinski *et al.* 2013) and analyse movement data of wearables (such as smartwatches). In animal experiments, it would be useful to measure the physical activity throughout the disease progression.

4.1.12 Limitations and outlook for the exploration of the pre-disease phase in lupus-prone mice

The exploration of the pre-disease phase in NZM2410 and SLE123 mice is liable to several **limitations**. As the animals were kindly gifted to us due to structural changes at the university, the numbers per age were varying. No more animals could be ordered from professional breeders to overcome this because these lines are complicated to breed, and even Jackson Laboratories lost their strain of NZM2410 mice for a while. Due to this difficulty and due to the fact that both sexes of these breeds develop SLE (M. L. Richard and Gilkeson 2018), we did not differentiate between male and female individuals, even though this would have been of high importance for human patients. In humans, the sex difference in SLE is very strong, with mostly women developing it (9:1 cases) (M. R. W. Barber *et al.* 2021) and lower testosterone levels leading to higher disease burdens. Moreover, in men, a later age of onset but a more rapid progression with organ damage is observed (J.-W. Kim *et al.* 2022). In our experiments, not every week could be compared across all mouse lines. To still gain the best possible and statistically reliable results, we decided to use non-parametric tests throughout all weeks, even if some weeks showed normal distributions, which would have allowed the use of parametric tests. Even though statistical tests and calculated significances are the standard for interpreting data, in such a complex disease development system, it is also important to describe trends that are not significant. We aimed to describe and explain the data as detailed as possible without over-interpreting the results. Another potential limitation is the comparison of NZM2410 mice with C57BL/6J mice, because these mice have different genetic backgrounds. Therefore, we did not call them controls but only a healthy comparison. There is no perfect control for NZM2410 mice, as this breed consists of NZW and NZB mice, which both already show autoimmune traits. NZB mice develop autoantibodies, circulating immune complexes causing nephritis, and haemolytic anaemia (TheJacksonLaboratory 2025a), while NZW mice also develop nephritis, anti-DNA-antibodies and high serum levels of retroviral gp70 antigen (TheJacksonLaboratory 2025b). We could overcome this problem with the additional use of SLE123 mice, which are NZM2410 mice backcrossed on C57BL/6J mice. Traits of autoimmunity seen in both mouse lines can clearly be linked to SLE and not to other genetic differences of the NZM2410 mice. The NZM2410 mice are also showing higher body weight from early ages on, which might lead to the assumption that obesity is one of the reasons for their changes in the immune system and not the development of SLE. By seeing the same trends in the SLE123 mice, which are not differing in weight from the healthy C57BL/6J mice, we could show that the increased weight in NZM2410 mice plays a minor role. Some effects might be increased due to this fact, such as the increased frequency of CD4+ T cells. A study in humans could show that circulating CD4+ T cell frequencies

increase with rising body mass index (Pecht *et al.* 2014). This is not surprising, as obesity leads to low-grade systemic inflammation (Y. Zhao *et al.* 2018; Heredia, Gómez-Martínez, and Marcos 2012; Suren Garg *et al.* 2023), but we also did not asses the grade of adiposity, as these animals are not only weighing more but are also larger. However, SLE123 mice showed similar trends, and their "pre-SLE flare" occurred even at younger ages.

In the **future**, professional statisticians will also use prediction models to gain more output from this data. This will also involve the analysis of the sex influence. FoxP3, the transcription factor of Tregs is X-linked (Gavin *et al.* 2007), suggesting one mechanism why females could be affected differently than males in mice and humans. X-linked immunologic features, such as FoxP3 or TLR7 can contribute to autoimmune dominance in females because of X-chromosomal escape of certain genes, causing a gene-dosage effect (Syrett and Anguera 2019) or X-chromosome reactivation in immune cells. The latter identified unusual maintenance of X chromosome inactivation specifically in lymphocytes of females as an enhancer for autoimmunity (J. Wang *et al.* 2016; Huret *et al.* 2024).

Moreover, the current data can also be explored for the expression of the activation marker CD25 on naive, effector, and central memory T cells. Moreover, the frequencies of Ki-67+ T cell subsets (not only Tregs) could be interesting because they might help to understand changes in frequency and capability of these cells. CD39 would also be interesting to investigate in CD4+ non-Tregs, as CD39-expressing conventional T cells were associated with Th17 functions (Timperi and Barnaba 2021). This is an important cell type in SLE involved in the development of nephritis (Paquissi and Abensur 2021), and we did not include any other marker that would allow us to identify Th17 T cells among the T cells. Additionally, CD39 on CD8+ T cells was found to counteract CD8+ Tregs (Parodi *et al.* 2013). This would be especially interesting to see in SLE123 mice, as after week 12, the CD8+ Tregs decreased suddenly. For NZM2410 mice, it would be interesting to check for the CD39+ CD8+ T cells around the "pre-disease flare" in week 18.

In future experiments, it would also be great to be able to differentiate between the different CD4+ T cells, such as Th1, Th2, and Th17, as these have different properties. It would be interesting to see how their relations and proportions change during the pre-disease phase. Another now-known cell type of interest in SLE is the double-negative T cell. These T cells neither express CD4 nor CD8 but are known to be an expanded cell population causing kidney dysfunction in active SLE, also in mouse models (J. J. Alexander *et al.* 2020). Their development in the pre-disease phase would contribute to the full picture of T cell development during this critical phase. Unfortunately, we cannot analyse them as we did not include CD3 in our panel due to technical limitations and compromises in colour and marker choice.

An important next step would be to measure the iron and ferritin in the mice to find out about the

state of anaemia, as iron is used by neutrophils and T cells for their effector functions (Morel and Scindia 2024) and a dysregulated iron metabolism can lead to lupus nephritis (Morel and Scindia 2024). As iron stimulates IL-2 production in T cells (Z. Wang *et al.* 2018), and this is required for Treg maintenance, it adds another level of interest for this factor. Moreover, the reduced MCV values in both lupus-prone mouse lines indicate already an iron-deficiency anaemia.

Furthermore, glycolysis and mitochondrial function should be explored in more detail as Yin and colleagues demonstrated in SLE123 mice that CD4+ T cells have increased glycolysis and mitochondrial oxidative metabolism, which is required for inflammatory effector functions and chronic activation (Yin *et al.* 2015).

We would also like to gain a detailed insight into cytokine patterns of these mice of all ages, ideally not only from the blood but also from other organs. Some organs are still frozen, so future projects are already planned. Of major interest here is IL-2 as it is required for Treg survival, maintenance and function as well as IFN, a major driver of inflammation in SLE.

Not only are the cytokine patterns of interest, but also the development of a set of autoantibodies. For both mouse lines, it is known that they develop anti-dsDNA antibodies and glomerular-basement membrane antibodies (Mathenia *et al.* 2010; Morel, Croker, *et al.* 2000), and we would like to test if and when the productions start already during the pre-disease phase.

The analysis of the kidneys of the SLE123 mice is ongoing. Not only will the nephritis be evaluated by PAS staining, but sections will also be investigated for the infiltration of neutrophils and for the depositions of IgG and C3.

We would also like to check human blood for the same T cell markers and T cell subtypes to improve the translational ideas for the identification of the pre-disease phase and the pre-SLE flare. Of course, this is only possible in the blood, but therefore no less valuable. Ideal subjects would be relatives of SLE patients or a general screening and follow-up of young people, especially women. This could be included in the regular blood donations. A follow-up of donors fulfilling the above-mentioned pre-disease criteria would be required. In addition to movement by wearables, sleep should be investigated. In human SLE, the central nervous system involvement is well-known. With it, potentially, insomnia is occurring. This itself can drive autoimmunity (Stenger, Vorobyev, *et al.* 2025).

4.1.13 Summary and conclusion of the exploration of the pre-disease phase

The pre-disease in SLE is an unstable phase in which the immune system transitions from healthy to chronically diseased. This cannot be described as a single step or a switch but rather as a struggle, which is ongoing for a certain time period and is influenced by a variety of factors. Firstly, a

pre-requisite to develop the disease is needed, such as a genetic predisposition. In the case of this mouse study, the susceptibility alleles *Sle1*, *Sle2*, and *Sle3*. Thereafter, another trigger is needed to cause a dysbalance. This can be an infection (Doria *et al.* 2008), UV-light (Bijl and Kallenberg 2006), nutrition (Aparicio-Soto, Sánchez-Hidalgo, and Alarcón-de-la-Lastra 2017), hormonal changes during maturation or pregnancy (J.-W. Kim *et al.* 2022; Roussou *et al.* 2013), shift work (Stenger, Grasshoff, *et al.* 2023) and more. As an imbalance is caused, the body aims to come back to the healthy state, however, some regulatory processes also foster dysregulatory ones. IL-10, to name just one example, has anti-inflammatory properties but at the same time might enhance autoimmunity by supporting extrafollicular antibody response (Biswas, Bieber, and Manz 2022). This is why the balance is further disturbed. Even overshooting regulatory (anti-inflammatory) processes are seen in this phase. This is a critical and fragile phase in which the immune system is highly dynamic. Hence, not all disease-indicating measures are as established as in the disease state; some are potentially even going in the complete opposite direction, due to overcompensation (Munroe *et al.* 2022). This is described and can even be calculated by the hidden Markov model during the dynamic pre-disease state (P. Chen and Y. Li 2016) as described above. Only the last stage of human SLE, the active disease, has been widely investigated. The dynamic pre-disease stage in human SLE is barely described, as in this stage the body is still responding to the upcoming inflammation in various ways, which either do not cause clinical symptoms or are difficult to classify. This is also the reason why the diagnosis of many autoimmune diseases might take years after the onset of discomfort in the patients, with a median delay between symptom-onset and diagnosis of 47 months (Mitchell 2024). In our experiments, we could identify several differences between genotypes of lupus-prone and healthy mice, as well as certain phases which we characterised as "pre-SLE-flares". This collection of haematologic, physiologic and T cell data allowed us to unravel certain autoimmune characteristics before the onset of clinically known symptoms such as nephritis (**Figure 64**). This is valuable for the future of patients as it might enable earlier diagnosis, earlier treatments or even preventive strategies.

If each flare leads to more loss of tolerance and damage (Jackson and Davidson 2019; Katz *et al.* 2020), it is especially important to prevent flares, not only to prevent further organ damage but also to ensure that the disease is not spiralling up further. Remission or a steady state can only be achieved if flares are prevented. One way to prevent flares is to reduce their triggers, but this might not be doable in some cases, as life happens and with it comes stress and specific adventures, which require strength and resilience of the body.

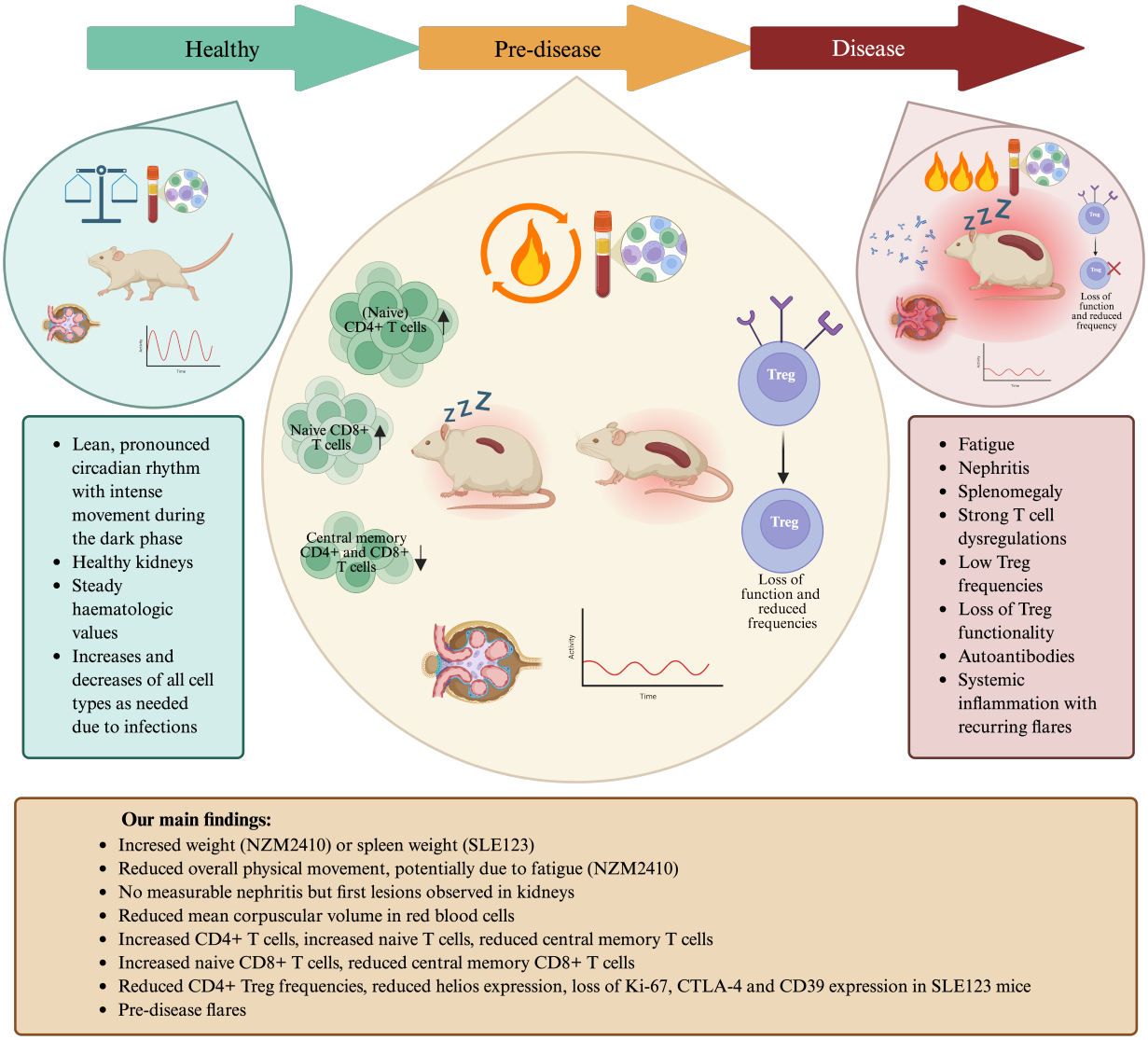


Figure 64: Summary of findings of the exploration of the pre-disease phase
The exploration of the pre-disease phase of systemic lupus erythematosus in NZM2410 and SLE123 mice revealed multiple changes. We found that NZM2410 mice moved less than C57BL/6J mice of the same age, assuming fatigue. At the same time, NZM2410 mice had significantly increased weight but no splenomegaly. While SLE123 mice stayed lean, they developed enlarged spleens during the pre-disease phase. SLE-prone mice also showed T cell dysregulations by increased CD4+ T cell frequencies, especially naive CD4+ T cells, while central memory CD4+ T cells were reduced. This was similarly seen in CD8+ T cells. CD4+ regulatory T cells (Tregs) were found to be reduced in frequency and also to lose their repressive capacities, measured by the frequencies of helios+, CD39+, CTLA-4+, and Ki-67+ Tregs. Among the red blood cells, a reduced mean corpuscular volume was found throughout the entire pre-disease phase. Many (other) measured values showed distinct changes at the age of 18 weeks for NZM2410 mice and the age of 10-12 weeks for SLE123 mice, which we defined as "pre-SLE flares". Nephritis was not found during the pre-disease state in NZM2410 mice, however, the first small lesions in the kidneys were detected. The illustration was generated using BioRender.com.

4.2 Investigation of ultraviolet light as a disease-trigger

In former times, light was used as one of the first treatments for lupus. Niels Ryberg Finsen even received 1903 the Nobel Prize in Medicine "in recognition of the treatment of lupus vulgaris by means of concentrated light rays" (Finsen and Forchhammer 1904; Hönigsmann 2013). Since then, a lot has been learned about the different kinds of light, the influence of the wavelength on the penetration depth in the skin and its effects on different cell types of the skin. Especially in SLE, sensitivity to UV light is known and associated with IFN-driven inflammation (Sarkar *et al.* 2018; Niewold *et al.* 2008; Y.-F. Wang *et al.* 2021; Stannard *et al.* 2017). Keratinocytes, the main cell type in the skin, are a major source of type I IFN in the skin (Psarras, Wittmann, and Vital 2022). Thus, this UV-enhanced IFN production triggers SLE flares (Sarkar *et al.* 2018) by the upregulation of CXCL9 and CXCL10, which attracts CXCR3+ lymphocytes (Psarras, Wittmann, and Vital 2022). Moreover, a study with healthy C57BL/6 mice was able to show that UV light-activated neutrophils migrate to the kidneys and caused a lupus phenotype with transient proteinuria (Skopelja-Gardner *et al.* 2021). Whether these neutrophils would then exacerbate or enhance the progression of the disease in lupus-prone animals, such as NZM2410 mice, is not known. Nowadays, we even know that light - depending on the spectrum - is especially in SLE able to cause flares and enhance the disease (Fernandez and Kirou 2016). So far, mostly MRL/lpr mice with known skin involvement have been used (Ghoreishi and Dutz 2009) or other lupus-prone mouse models without known skin involvement, such as NZM2328 mice, but here only systemic effects of UVB were investigated (Wolf *et al.* 2019). In mouse lines such as NZM-related strains, no skin involvement is reported. This means that they do not form skin lesions under regular housing conditions, but these do only expose the mice to dim artificial light and never natural sunlight, which includes a wide UV-spectrum and is more intense. Thus, details about the light intensities, wavelengths, and their effects during the pre-disease phase remain to be investigated.

The UV light experiments aimed to find out about the differential influences of UV light wavelengths on skin of healthy and lupus-prone mice during their pre-disease phase. We therefore chose the age of 16-18 weeks in NZM2410 mice, as this age already showed immunologic changes - aforementioned as "pre-SLE flare" - and hence might be especially vulnerable towards such triggers, even though the disease has not fully clinically broken out. We split these experiments into two separate ones, which are mainly compared within themselves, as different mice were used, different light wavelengths, and the RNA was sequenced in separate runs. The chosen dosages of all wavelengths were low in order to prevent direct tissue damage and sunburn-associated changes by the UV-light to only investigate the effect of the light on the lupus-prone skin.

UVB light is known to be able to cause sunburns, tissue damage, trigger inflammation, and

increase the risk of developing skin cancer. Moreover, this powerful wavelength can cause apoptosis in the skin and might thus trigger flares in SLE patients (Mak and Tay 2014). We used a wavelength of 310 nm.

UVC light has an even shorter wavelength (we used 233 nm) and is therefore more powerful. However, this light is usually unable to pass the ozone layer and is consequently not known to cause skin damage. Even when this light reaches the skin or the eye, it is not able to pass the stratum corneum of the skin or the cornea of the eye. Nevertheless, bacteria are killed by this wavelength (Zwicker *et al.* 2022) and this is why recent publications propose to use it as a potent decontamination source (Yamano *et al.* 2020; Buonanno *et al.* 2017). It is suggested to be used even indoors with people in rooms like in schools, care facilities, and more (Hugenroth 2020). Even if the UVC light is not able to cause cancer in healthy humans, it is uncertain how immunocompromised people such as those with autoimmune diseases react to this light. We proposed that it might still trigger inflammatory or disease-related pathways and can not be broadly used in public places.

UVA light has a longer wavelength (we used 375 nm). This means it is less powerful but can penetrate the skin deeper. It is able to pass through the epidermis into the dermis. This is also why it is associated with more photoaging effects and long-term skin damage (Battie *et al.* 2014). However, UVA light has also immuno-regulatory effects. It might reduce some inflammatory reactions in the skin, as a study was able to show that the induction of erythema was lower in skin irradiated by UVA and UVB than by UVB alone (Yang *et al.* 2023). Hence, it is of high interest to us, how it modulates immune reactions in the skin of lupus-prone mice and how it might counteract inflammatory reactions caused by UVB light. It was even used in low doses to treat some forms of cutaneous lupus, with the effect that anti-dsDNA antibodies decreased significantly (McGrath, Martínez-Osuna, and F. A. Lee 1996). As these studies are from the 90s, not followed up long-term, were not standardised, and were done with small cohort sizes, this form of therapy should be seen with caution, especially since UVA-light therapy is able to cause erythema as well (Kurz *et al.* 2023).

Blue light has known effects on the circadian rhythm of human keratinocytes by reducing the expression of *Per1*, a core clock gene (K. Dong *et al.* 2019). Other potential effects range from the induction of pigmentation, over inflammation, up to the promotion of wound healing, but are highly debated (Uzunbajakava *et al.* 2023; Prado *et al.* 2023). Moreover, blue light is also used to irradiate skin due to germicidal effects (J. Cabral and Ag 2019). We chose two blue light wavelengths (405 nm and 445 nm).

4.2.1 Cytotoxicity during skin cultures

The LDH-assays were performed to find out if the culturing conditions were appropriate and if the light treatments caused already measurable cytotoxicity. In the first experiment, it could be seen that NZM2410 mice reacted with more cytotoxicity to the culture after 30 h. The treatment with UVB and blue light caused the same effects. This is potentially not due to the light but due to the culturing conditions because the measured absorbance was not higher than after 30 h incubation without light treatment. It is good to see that the light treatments did not cause cytotoxicity themselves, as the chosen intensities were low. We did not aim to cause skin damage, we aimed instead to understand processes triggered by light, not the pure damage.

In the second experiment, NZM2410 mice showed even slightly less cytotoxicity after 24 h of incubation than C57BL/6J mice. This effect is not seen any longer after 30 h of incubation, thus, it might have been due to inflammatory responses upon the punching of the skin samples. Interestingly, upon UVA-irradiation, NZM2410 mice showed significantly reduced cytotoxicity. This could be due to potentially anti-inflammatory capabilities of UVA (Yang *et al.* 2023). The treatment of UVA in combination with UVB light and the treatment with blue light led to non-significantly increased cytotoxicity in NZM2410 mice. It follows from the foregoing that the irradiations did not cause cytotoxicity, and only upon UVA, the genotypes reacted differently.

4.2.2 Transcriptomic analysis of light-treated murine skin

RNA of the skin punches was sent for transcriptomic analysis in order to identify genes, pathways and transcription factors which are differentially regulated upon light treatments and between the genotypes. The **PCA** displayed that the main separation of the data is due to the genotype. This is expected as NZM2410 and C57BL/6J mice have different genetic backgrounds.

As a next step, **deconvolution** was performed. This allowed to identify the cell types behind this data. The deconvolution confirmed that after 24 h and 30 h of incubation the majority of signals resulted from keratinocytes. It was important to clarify, as the skin harbours also other cell types such as fibroblasts, melanocytes, Merkel cells, mast cells, endothelial cells, adipocytes, and also immune cells (Leyva-Castillo *et al.* 2022). We could demonstrate here the influence of different light treatments, mostly on keratinocytes and their response to it. Directly after taking punch biopsies of the skin, the samples were too irritated to be identified by their origin. Therefore, a majority of signals at time 0 is from "miscellaneous". This reveals how important it is to cultivate the samples for 24 h before doing any treatments. This ensures that the influence of the treatment, not the skin punching, is measured.

Thereafter, **differentially expressed genes** were investigated. Firstly, upset plots were used to identify the largest similarities and differences between the treatment groups and genotypes. The largest difference in the first experiment in the upregulated genes was seen in the control 30 h group, in which 120 genes were upregulated in NZM2410 mice compared to C57BL/6J mice. These genes were not upregulated by any other treatment. This is pointing to a stronger effect of the culturing of the skin in NZM2410 than in C57BL/6J mice. However, the upset plots are only investigating the number of genes and not the genes themselves. The quantity of differentially regulated genes is not necessarily more meaningful than the impacts of single genes, which are causing direct effects on the development and progression of SLE. It rather just means that the culturing has broader effects, which might even be unspecific, compared to the individual light treatments. This makes sense, as the culturing is also handling-wise a more intense intervention than the short treatments. Since the treated samples were also cultured, we can still very well evaluate the effects of the individual light treatments, without being influenced by the culturing system. Of the individual light treatments, UVB led to the highest number of differentially upregulated genes in NZM2410 mice (90), next UVC light with 29 genes and then blue light with 20 genes. Regarding the downregulation, most genes were commonly downregulated over all treatments and the control, indicating a genotype difference between NZM2410 and C57BL/6J mice. Thereafter, the second-most genes were downregulated in the 30 h control samples, again pointing to broad effects of the culturing on NZM2410 mouse skin. Of the light treatments, it was again UVB that also caused the most downregulation with 33 genes, then UVC with 32 genes, and then blue light with 21 genes. It could be seen as an indicator that the used UVB dose had the strongest or broadest effects of all light treatments and the chosen blue light dosages had the least strong effects on NZM2410 mice compared to C57BL/6J mice, even though we do not know which genes are influenced at this point of analysis.

In the second experiment, the highest number of upregulated genes was seen commonly over all conditions when comparing NZM2410 mice to C57BL/6J mice, meaning that the major difference is the genotype. Thereafter, UVA/B treatment also led to 38 upregulated genes, which is a treatment-dependent effect between NZM2410 and C57BL/6J mice. Next, the control 30 h samples followed, meaning that the culturing itself has a differential effect on the two genotypes. The blue light (445 nm) led to an upregulation of 24 genes, which were not upregulated by any other treatment. UVA lead to an upregulation of 17 genes. Regarding the downregulation, most genes (52) were also commonly downregulated over all treatments, again supporting the PCA analysis, showing the genotype difference. Yet, UVA treatment also led to 51 genes that were downregulated specifically by this wavelength in NZM2410 mice compared to C57BL/6J mice. The control showed a downregulation of 47 genes, UVA of 31 genes and blue light of 11 genes. This indicates again that

blue light and UVA light treatment caused less differential gene regulation than UVA/B treatment, comparing the two mouse strains. Nevertheless, the specific genes are of importance to identify if the treatments cause SLE-specific genetic triggering.

When checking the **individually upregulated and downregulated genes**, some were found that are specifically associated with SLE. A full list of all up- and downregulated genes on both UV light experiments can be found in the supplements (**Tables S5; S6; S7; and S8**). After **UVB** irradiation, some genes in NZM2410 mice were downregulated that were not differentially regulated by other treatments and have a known role in SLE. One of those is Fc-γ receptor III (*Fcgr3*). A reduced copy number of *Fcgr3* was strongly associated with an increased risk of SLE, as it encodes for a low-affinity IgG receptor on neutrophils, needed for clearance processes (Willcocks *et al.* 2008). This could be a clear indicator of how UVB light is able to cause SLE flares even in doses that do not cause sunburns. Solute carrier family 11 member 1 (*Slc11a1*) (Pedroza *et al.* 2011) and IFNβ1 (*Ifnb1*) (Postal *et al.* 2020) are also SLE-associated. Usually, *Ifnb1* is upregulated in SLE (Catalina *et al.* 2019), so a downregulation by the low dose of UVB light might be a beneficial effect or simply again an overcompensation during the pre-disease phase due to effects described by the hidden Markov model (described above). Complement component 8 α chain (*C8a*), needed for the formation of the membrane attack complex (Jasin 1977), IL-7 receptor (*Il7r*) (Meyer, Parmar, and Shahrara 2022), colony stimulating factor 1 (*Csf1*) (Menke *et al.* 2015), *Tlr5* (Y.-w. Wu, W. Tang, and Zuo 2015), 2'-5'-Oligoadenylate synthethase 1 (*Oas1*) (Yingyu Wang *et al.* 2022), and *Cxcl9* (S. Wang and Y. Cui 2024) were also found to be downregulated and SLE associated. Cxcl9 is usually upregulated in active SLE (S. Wang and Y. Cui 2024), accordingly, a downregulation by UVB light might either indicate a beneficial effect of this treatment or (as discussed before) mean that the skin of NZM2410 mice tries to overcompensate for the caused harm by the light. *Il7r* is especially interesting as this receptor is located on precursor B cells in close proximity to CXCR4. These two molecules interact to coordinate the proliferation, differentiation and migration of B cells (Abdelrasoul *et al.* 2020). Consequently, if UVB light dysregulates *Il7r*, this is a potential way in which CXCR4 is dysregulated in SLE and also might add to B cell abnormalities. Moreover, as CXCR4 is a target for the standard SLE-treatment drug hydroxychloroquine, UVB light could be able of rendering the effect of the drug.

Regarding the upregulated genes, UVB light caused the upregulation of fatty acid amide hydrolase (*Faah*). It is located on the *Sle2* locus (Morel 2017) and inhibition of *Faah* was even found to prevent from polyreactive autoantibodies in SLE mouse models (Pathak *et al.* 2016). Dual specificity phosphatase 26 (*Dusp26*) (H. Li *et al.* 2021), and keratin 1 (*Krt1*) (Luo *et al.* 2017) were also upregulated by UVB light in NZM2410 mice compared to C57BL/6J mice.

Upon **UVC** light, downregulated genes were IFN-activated gene 202B (*Ifi202b*) and *Il27*. *Ifi202b* was found to control CD8+ T cell-mediated suppression in NZB/NZW mice (R. Dinesh *et al.* 2011). As a consequence of downregulation by UVC light, CD8+ T cell immunity is impaired, and the effects thereof can be manifold. IL-27 was shown to promote pro-inflammatory activity of monocytes and is able to do both inhibit and promote the function of macrophages and dendritic cells (W.-D. Xu, D.-C. Wang, *et al.* 2024). Furthermore, IL-27 has an anti-inflammatory role in neutrophils, promotes NK cell function, enhances Th1 and Th2, but inhibits Th17, Th9 and Tfh cells (W.-D. Xu, D.-C. Wang, *et al.* 2024). Due to this dual role, its effects are also going in several directions regarding SLE (W.-D. Xu, D.-C. Wang, *et al.* 2024). UVC light caused the specific upregulation of programmed cell death 4 (*Pdcd4*) (Kamil Alhassbalawi *et al.* 2023), *Cd96* (Law *et al.* 2024), *Il20ra* (J. Wu *et al.* 2019), and arachidonate 5-lipoxygenase (*Alox5*). *Alox5* was also shown to promote pyroptosis and tissue inflammation in RA (Cai *et al.* 2024) and to play a key role in lupus nephritis (W. Chen *et al.* 2025). Thus, this upregulation might be a direct link to how UVC light causes kidney damage in SLE patients or predisposed individuals.

Upon **blue light 405 nm** irradiation, these downregulated and SLE-associated ones are *Il33* (Sarrand and Soyfoo 2022), *Il17b* (Xiao *et al.* 2025), and fucosyltransferase 2 (*Fut2*) (Yao *et al.* 2023). IL-17B is involved in the activation and differentiation of B cells, and higher levels can mitigate SLE symptoms (Xiao *et al.* 2025). The downregulation by blue light could be an indicator for worsening effects on SLE. However, a neutralisation of IL-33 was even found to ameliorate SLE in MRL/lpr mice via the expansion of Tregs and inhibition of Th17 cells (P. Li, W. Lin, and Zheng 2014), pointing out a potentially beneficial effect of blue light. Blue light 405 nm caused the upregulation of ribonucleoprotein PTB binding 2 (*Raver2*), which is also SLE-associated (Bouzid *et al.* 2012).

In the **second UV experiment**, **UVA** light caused the specific downregulation of proprotein convertase subtilisin/kexin type 9 (*Pcsk9*), which is associated with SLE, but the function is unclear (Ministrini and Carbone 2022). Regarding the upregulated genes, UVA light caused in NZM2410 mice specifically the upregulation of *Tnf*, which has pro- and anti-inflammatory roles in SLE (Richter *et al.* 2023). Further, CD4 (Frangou, Bertsias, and Boumpas 2013), histocompatibility 2 class II antigen A (*H2-ea*) (S. Subramanian *et al.* 2005), defensin β 3 (*Defb3*) (Pagenkopf and Y. Liang 2020), mannan-binding lectin serine peptidase 2 (*Masp2*) (W.-D. Xu, X.-Y. Liu, *et al.* 2020), coagulation factor VII (*F7*) (Adams *et al.* 2011), killer cell lectin-like receptor subfamily a member 17 (*Klra17*) (Swiecki and Colonna 2015), matrix metallopeptidase 8 (*Mmp8*) (D.-C. Wang *et al.* 2024), and histone cluster 1 H1c (*Hist1h1c*) (Tyagi and Gupta 2021) were also upregulated in NZM2410 mice compared to C57BL/6J mice by UVA light.

UVA/B treatment caused in the NZM2410 mice the downregulation of *Hist1h1c* in lupus-prone mice, and this gene is associated with SLE (Tyagi and Gupta 2021). The treatment of the mouse skin with UVA/B together caused the upregulation of S100 calcium binding protein A9 (*S100a9*) (Davison *et al.* 2021), and *Il23a*. Overexpression of *Il23a* has recently been used as a lupus mouse model (Christodoulou-Vafeiadou *et al.* 2024). Moreover, nucleotide-binding oligomerization domain containing 2 (*Nod2*) (Esmaeilzadeh *et al.* 2021), G protein subunit α 14 (*Gna14*) (Y.-J. Zhao *et al.* 2022), and *Il19* (J. R. Lin *et al.* 2016) have also been found to be upregulated.

Blue light 445 nm caused (as the other blue light treatment) in NZM2410 mice the downregulation of *Fut2* (Yao *et al.* 2023). Blue light 445 nm led in NZM2410 mice to the specific upregulation of no directly SLE-associated genes.

Of note, some genes were commonly up- or downregulated that are already known or typical SLE-associated genes, however, we focused here on the specific effects of each wavelength. Moreover, it needs to be mentioned again that the lupus-prone mice were still in the pre-disease phase, and the chosen light doses were low, so during full-blown disease or with other doses, other or stronger effects could also be visible. Moreover, as research progresses, some of the differentially regulated genes might not yet have been found to be associated with SLE. Nevertheless, we could show here that each tested wavelengths had certain characteristics that may influence the development and progression of lupus. Many of the SLE-associated genes listed here were only found to be associated, but their function has not yet been fully elucidated. It could be worth re-evaluating this data at a later point, as science constantly progresses.

Next, the **pathway regulation** was investigated. This is of major interest as the pathways triggered by the treatments shed light on the full processes, which would then be later passed on to other tissues and cause systemic effects. For **experiment 1**, when having both sexes together, in NZM2410 mice, UVB light caused a significant upregulation of translational ribosomal RNA (rRNA) processing. Notably, ANAs of SLE patients are able to recognise rRNA (Tsuzaka *et al.* 1996). Antibodies against ribosomal proteins are also known to be involved in neuropsychiatric and liver effects in SLE (Dema and N. Charles 2016). Moreover, UVB light is known to cause DNA damage, and this upregulation of translational rRNA processing is most likely indicating DNA repair mechanisms (Powley *et al.* 2009). Interestingly, this is significantly increased in NZM2410 mice compared to C57BL/6J mice, indicating that their skin had more damage to repair upon UVB irradiation. DNA damage causes the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts (S.-i. Kim, Jin, and Pfeifer 2013), which we will investigate in the future. This DNA damage could have activated oncogenes such as *Ras* or *Myc* stronger in NZM2410 mice, which is increasing rRNA transcription, hence boosting the translational capacity as well (Ehrhart *et al.* 2003; Grandori *et al.*

2005). Enhanced rRNA translation could also point to activated immunologic processes, especially the augmentation of sensing dsDNA (Bianco and Mohr 2019). Free self-dsDNA can also be caused by UV-induced damage, and its sensing is a key mechanism in SLE, known to lead to the formation of autoantibodies (Bai *et al.* 2018). Thus, UVB light in a dose not causing any sunburns is still able to trigger SLE pathogenesis-associated mechanisms in lupus-prone mice more than in healthy mice.

The citric acid (TCA) cycle was also influenced, as well as the mitotic cell cycle. This is not surprising as UVB is known to cause metabolic reprogramming in keratinocytes (De Leo *et al.* 1984). It is interesting in the context of SLE, however, because the NZM2410 mice showed this stronger than the C57BL/6J mice. An altered skin metabolism is also a characteristic of lupus-affected tissues -here UVB induced- with downstream immunologic processes (Kingsmore *et al.* 2021). It could also be an indirect effect of the aforementioned DNA damage and cyclobutane pyrimidines, which induce a hypermetabolic state in keratinocytes (Hegedűs *et al.* 2021).

The other light treatments or the cultivation did not lead to any significant up- or downregulated pathways. This picture looks different, though, when separating the sexes. It could be seen that in the male mice, 30 h control, blue light 405 nm, and UVC light caused an upregulation of keratinisation in NZM2410 mice compared to C57BL/6J mice. This could be a protection mechanism, called the cornified envelope, to strengthen and thicken the stratum corneum of the skin (Schäfer and Werner 2011; K.-F. Huang *et al.* 2017; A. Cabral *et al.* 2001), and it is not seen in female mice. Moreover, the extracellular matrix-pathways have been downregulated in the male 30 h controls. This could mean that the regular skin integrity is not actively maintained any longer (Cole *et al.* 2018; K. Wang *et al.* 2023). This is not surprising as the skin is not within its natural context of the organism any longer and thus is not nurtured by it. Blue light in NZM2410 males caused a significant downregulation of IFN and collagen. This could be interpreted as an anti-inflammatory effect of blue light in NZM2410 mice. A similar effect was seen in a phototherapy for acne treatment. This effect was only the first phase of the reaction and thereafter, pro-inflammatory effects of blue light developed (Bonnans *et al.* 2020). UVB light led to the downregulation of TLRs and complement pathways in NZM2410 males compared to C57BL/6J males. This is surprising as this indicates anti-inflammatory effects in the lupus-prone males. As this is only seen in males, it might be a counter-regulatory mechanism to this low dose that females are not capable of and thus might suffer more from UVB irradiation. UVC light treatment did not cause any specific downregulations in the male NZM2410 mice.

In the female mice, the 30 h controls did not show any specific up- or downregulated pathways when comparing the genotypes. Upon blue light 405 nm treatment, ribosome-related pathways were upregulated in female NZM2410 compared to female C57BL/6J mice. This could be a stress

response to cope with changing environmental conditions. This is a known mechanism in plants and fungi (Akagi *et al.* 2023; D. Chen *et al.* 2017) but might be conserved as a stress or light-sensing function in mammals. The interesting fact is that lupus-prone mice are more sensitive to this, and also that females are more sensitive than males. UVB light caused in the females an upregulation of pathways for oxidative phosphorylation. As the UVB light is able to cause DNA damage, which needs repairments and causes stress responses, the upregulation of oxidative phosphorylation might be one mechanism to synthesise ATP in mitochondria (Senior 1988). Additionally, here, pathways for Alzheimer's disease seemed to be upregulated in the NZM2410 mice, compared to the C57BL/6J mice. It could be argued that this is an effect of age, as the NZM2410 mice were older than the C57BL/6J females. Still, it is surprising that only upon UVB light treatment this effect is seen, therefore, another reason could be that UVB is accelerating the amyloid precursor protein processing, which is linked to Alzheimer's disease development and progression (Almenar-Queralt *et al.* 2014). As seen for other gene regulations, the female mice might be more sensitive towards light treatments and therefore it is not seen in the males. Upon UVC irradiation, interleukin-related pathways were upregulated in the NZM2410 females. This is also an indicator that the female lupus-prone mice are reacting more sensitively and with more inflammatory processes to UVC light than the healthy C57BL/6J mice and also more than the male mice. This is especially important regarding the aim to use UVC light as a decontamination method. SLE-prone women might thereby unknowingly be triggered to develop inflammation and potentially SLE. As SLE in humans is also a disease affecting mainly females of reproductive age, this finding is especially noticeable.

In the **second experiment**, only the combination of UVA and UVB caused a significant upregulation for both sexes of pathways related to cytokine-cytokine-receptor interactions in NZM2410 mice compared to C57BL/6J mice. This means that cytokines will be released by keratinocytes, or receptors for binding of cytokines will be upregulated. This could result in inflammatory signalling as well, meaning again that the skin of the NZM2410 mice is more sensitive to light than the skin of C57BL/6J mice. When dividing the results of experiment 2 for sex, no significantly up- or downregulated pathways between the males of both genotypes were found, meaning that the male mice are rather resistant towards the treatments. This accounted for both, NZM2410 and C57BL/6J mice. In the females, no effects were seen for the 30 h controls and the blue light 445 nm. UVA caused an upregulation of cytokine-cytokine receptor interactions in NZM2410 females compared to C57BL/6J females, indicating immunologic processes. This was also found for the UVA/B treatment. Here, also pathways for complement cascades, ECM receptor interaction and specifically SLE were upregulated in the female NZM2410 mice. This means that the NZM2410 females reacted more sensitively to the treatments than the males and also more

sensitively than the healthy mice. As these treatments were of low intensity, not causing any visible sunburns or increased cytotoxicity, female lupus-prone individuals have to be especially careful with light. Not only does skin damage trigger flares, but apparently also lower non-damaging doses of light. Moreover, this sex-specific effect of NZM2410 mice was so far unknown. This genotype was thought to develop SLE in both sexes to similar extents (TheJacksonLaboratory 2025b). Thus, we could not only show that the skin is involved in this genotype in light reactions, but also found a sex difference.

As a further step, the expression of **transcription factors** was investigated. Transcription factors regulate gene expression and thereby control cellular responses. The analysis of these revealed in experiment 1 that *Zfx*, *Hivep2*, *Ciita*, and *Irf1* were commonly downregulated in NZM2410 mice vs. C57BL/6J mice. This gives another insight into the genotype differences. The function of *Zfx* is involved in the maintenance and self-renewal of haematopoietic stem cells and lymphocytes (Smith-Raska *et al.* 2018; Krause 2007; Galan-Caridad *et al.* 2007). A dysregulation in this transcription factor is causing a dysregulation in early immune processes (Smith-Raska *et al.* 2018) and thus might cause effects that are visible from a very early age onwards. Dysregulated lymphocytes can be both B and T cells, thus, the entire adaptive immune response is impaired. Studies suggest that it is required for B cell development and survival (Arenzana, Smith-Raska, and Reizis 2009), thus, a lower expression might explain the self-recognition of B cells in the NZM2410 mice. The IFN signalling might also be regulated by *Zfx*, so a dysregulation might feed again into the vicious cycle. Since *Zfx* is X-linked (Shepherdson *et al.* 2024), it could also explain the stronger sensitivity of the female NZM2410 skin, which is more prone to inflammation. *Hivep2* is also involved in immunity by encoding for Schnurri-2, which is required for positive selection of thymocytes (Takagi, Harada, and Ishii 2001). This indicates a very early dysregulation of T cells in the lupus-prone mice, potentially leading to auto-reactivity. Another study was able to show *Hivep2*'s importance in Treg immunosuppressive function (Schumann *et al.* 2020). It is also known to be dysregulated in human SLE, even though it has been found to be upregulated in active disease patients (Shi *et al.* 2014). *Ciita* is the master regulator of the MHC II gene expression (León Machado and Steimle 2021). The MHC II is essential for antigen presentation (Wieczorek *et al.* 2017). A dysregulation here might influence the presentation of self-antigens to the adaptive immune system, fostering the autoimmunity. *Irf1* is known as IFN regulatory factor 1, hence playing a role in the IFN signalling (Feng *et al.* 2021). Impaired IFN signalling is playing a key role in SLE (Rönnblom, Alm, and Eloranta 2009). It is surprising that this transcription factor is downregulated (as some of the other ones as well) but this might be due to the pre-disease phase in which the immune system still has some regulatory capacities, trying to suppress auto-immune processes.

In human SLE, it is known to be upregulated (J. Liu, Berthier, and Kahlenberg 2017). *Irf1* is also interacting with *Ciita* by inducing it's expression (Tur *et al.* 2021). It also influences T cell differentiation by driving Th1 differentiation (Kröger 2017). These are pro-inflammatory and known to release IFN in SLE (Y.-Y. Tang *et al.* 2023). *Irf1* is even involved in apoptotic processes by being an essential gene in DNA-damage induced cell death (Zander *et al.* 2022). Its downregulation in the NZM2410 mice might indicate both, impaired immunologic processes and impaired apoptotic processes, which in turn might trigger further autoimmune development.

In the second UV experiment, *Zfx*, *Hivep2* and *Ciita* were again commonly downregulated in the NZM2410 mice compared to the C57BL/6J mice. This confirms the previous findings of the autoimmune dysregulations in the NZM2410 mice during the pre-disease phase. Additionally, in this experiment, *Nfkb1* was commonly upregulated. In human SLE, the NF-kB pathway has already been found to be involved in the pathology of SLE and other autoimmune diseases (Q. Li and Verma 2002). NF-kB is activated by many receptors such as TNF receptor, IL-1 receptor, TLRs, B cell receptor, BAFF receptor and CD40. This is demonstrating that NF-kB is working as a connecting link between many different immune signalling pathways (Moynagh 2005). This would explain a prominent role in the immune dysregulation of SLE and the conservation of this effect between the species mouse and humans.

To our knowledge, all these specific dysregulations in NZM2410 mice upon light treatments and skin culturing are novel findings, potentially aiding future protection of SLE-prone individuals.

4.2.3 Support of transcriptomic findings by stainings for cleaved caspase-3 and sunburned cells

Apoptosis and thereafter impaired clearance are key mechanisms in the development of SLE (Kaplan 2004) and the flare-triggering function of sunlight by causing DNA damage which starts the coordinated cascade of apoptotic cell death (C.-H. Lee *et al.* 2013). This is why apoptosis was controlled in these experiments in three ways. We chose transcriptomics as they offer a very detailed insight into the mechanisms and the genes involved but we controlled for the results on the protein-layer by staining for cleaved caspase-3. This is the active form of pro-caspase-3, which is needed for the cleavage of structural proteins and DNA fragmentation. It is an early process in apoptosis that is already measurable after 5 minutes (Tyas *et al.* 2000). Additionally, we aimed to see if late stages of apoptosis, clinically known as sunburned cells, were already detectable.

The **transcriptome data** of **UV experiment 1** revealed after 30 h of cultivation, caspase-3 was already slightly upregulated in the skin of NZM2410 mice compared to the C57BL/6J mouse skin. Upon UVB irradiation, this was even increased, and additionally, caspase-7 was upregulated

in NZM2410 mice. UVC was also able to further upregulate the expression of caspase-3 in the lupus-prone mice. Blue light (405 nm) caused a similar upregulation of caspase-3 as the 30 h control samples. Additionally, α-tubulin was downregulated by blue light treatment. This can also enhance apoptotic processes by destabilising microtubules and impairing mitochondrial function (Cho *et al.* 2021; Shen *et al.* 2018). Hence, the light treatments did cause stronger activations of certain apoptotic processes in lupus-prone mice than in C57BL/6J mice.

The stainings of **cleaved caspase-3** support the findings of the transcriptome data. UVB, UVC, and blue light (405 nm) did cause a significantly stronger activation of caspase-3 in NZM2410 mice compared to C57BL/6J mice, concluding higher rates of apoptosis, which might trigger lupus.

However, the number of **sunburned cells** was extremely low. This can have two reasons. On the one hand, the chosen intensities were low, so we do not expect such an extent of skin damage, and on the other hand, sunburns need time to develop. According to the literature, sunburned cells start developing 8 h after irradiation, and the fully developed sunburned cells can be found after 48 h (Danno and Horio 1980). We only waited 6 h after the irradiation before we harvested the samples.

In **UV experiment 2**, it was also found that NZM2410 mice reacted more sensitively to the treatments, resulting in altered apoptotic pathway regulations seen in the **transcriptomic data**. The downregulation of actin by UVA has been described in literature before as a causative agent and enhancer for apoptosis (Kulms, Düßmann, *et al.* 2002). Adding UVB to UVA light may reduce CAD levels, possibly impairing caspase-3 activity, which is required to cleave ICAD (Bell and Megeney 2017). It might also indicate that DNA-repair mechanisms are activated, which delay apoptosis because they reduce the cleavage of caspase-3 (Kulms, Pöppelmann, *et al.* 1999). Most interestingly, the irradiation with blue light 445 nm caused a strong downregulation of granzyme B in NZM2410 mice compared to the C57BL/6J mice. This suggests a disturbed cell death cascade (Perl *et al.* 2012), which might lead to extended exposure of cell particles to the immune system, thereby potentially triggering autoimmunity. A study already showed that lupus mice had decreased granzyme B functions in regulatory B cells, which enhanced the disease (Xue *et al.* 2023).

In the stainings for **cleaved caspase-3**, only in the 24 h control samples of the NZM2410 mice, a significant increase in apoptosis could be measured. A similar trend (but not significant) was measured for the blue light 445 nm treatment and the UVA/B treatment.

The number of **sunburned cells** was low in all conditions, as in the first UV experiment. Taken together, in both UV experiments, we could demonstrate that NZM2410 mice reacted to most light treatments more sensitively with increased apoptosis.

4.2.4 Limitations and outlook

Limitations that need to be mentioned in the UV experiments are that NZM2410 mice were compared to C57BL/6J mice, which are as explained before, not ideal controls but serve rather as a healthy comparison. The NZM2410 mice have light fur, whereas the C57BL/6J mice have dark fur. The skin of both strains, however, was light. As the mice were shaved, we expect only minimal influence of the fur colour. Moreover, humans also differ in their skin colour and this is not the main determinant of the SLE-outcome (Hasan, Fike, and Hasni 2022) or reaction to the UV-treatments. As stated before, the light intensities were also chosen to be of very low doses to ensure not only to investigate the effects of sunburns but rather to explore the different wavelength effects. This is why we do not expect the fur colour to have an influence on the meaningfulness of our results. Nevertheless, it would have been interesting to also investigate SLE123 mice or other lupus-prone mouse models with other fur colours. Another interesting model would be the MRL/lpr mouse, as this model is known for its skin involvement. NZM2410 mice have so far not been shown to have a skin involvement in SLE (Chandra Mohan *et al.* 1999). Yet, we expect the NZM2410 mice to be a valuable model for these experiments, as this strain has never before even been exposed to UV light and thus the effects are unknown. The light in the animal facilities is usually dim, and depending on the row in the shelves, barely there. It then also has to pass through the plastic of the cages, so the animals were never really exposed to light. Humans are even in nowadays lifestyles way more often exposed to (UV) light, as they leave their homes to go to work, the supermarkets, sports, social events and so on. The skin of the face is also exposed to blue light from screens and artificial room lights. This has already been shown to have effects on the circadian rhythm (Wahl *et al.* 2019), and we could show that it has effects on lupus-prone individuals regarding keratinisation, ribosome-related pathways, as well as IFN and collagen-related pathways.

In UV experiment 1, the mice were not perfectly age-matched, however, this was controlled for in the analysis of the data. Thus, we do not expect this to be a limitation.

Moreover, we only investigated the skin, but it could be expected that the effects of the irradiation are systemic (M. S. Fisher and Kripke 1977; Slominski *et al.* 2018). Consequently, -as an **outlook**- it would be enlightening to trace these seen effects on cells of the skin to other organs. This would require irradiation of living animals and also labelling of several cell types in them to be able to follow their trafficking throughout the organism. Even without the cell labelling, it would be insightful to investigate inflammatory reactions in, e.g. kidneys, brain, and joints upon irradiation.

Another effect that has not been addressed here is the production of vitamin D induced by UV light (Rivas *et al.* 2015). Moreover, UVA light is also able to cause the release of nitric oxide. This has not only antimicrobial properties but also may act neurotransmitter (Juzeniene and Moan

2012). Both vitamin D and nitric oxide are able to positively influence the mood (W. Liu *et al.* 2020; Lansdowne and Provost 1998), and this in turn is known to improve the quality of life and reduce the disease burden. By investigation of systemic effects, neurotransmitters could also be measured in the brain or cerebrospinal fluid upon irradiation.

The circadian rhythm is able to influence almost all activities in an organism, including inflammatory reactions (Cutolo, Straub, and Buttgereit 2008), which is why it would be interesting to see in future experiments if and how it differentially affects lupus-prone skin compared to healthy skin.

In the existing samples of UV experiments 1 and 2, we will also investigate DNA damage by measuring the amount of 4-6 photoproducts as well as cyclobutane pyrimidine dimers. This will give an indication of whether the lupus-prone mice develop more tissue damage or if only the clearance mechanisms are affected.

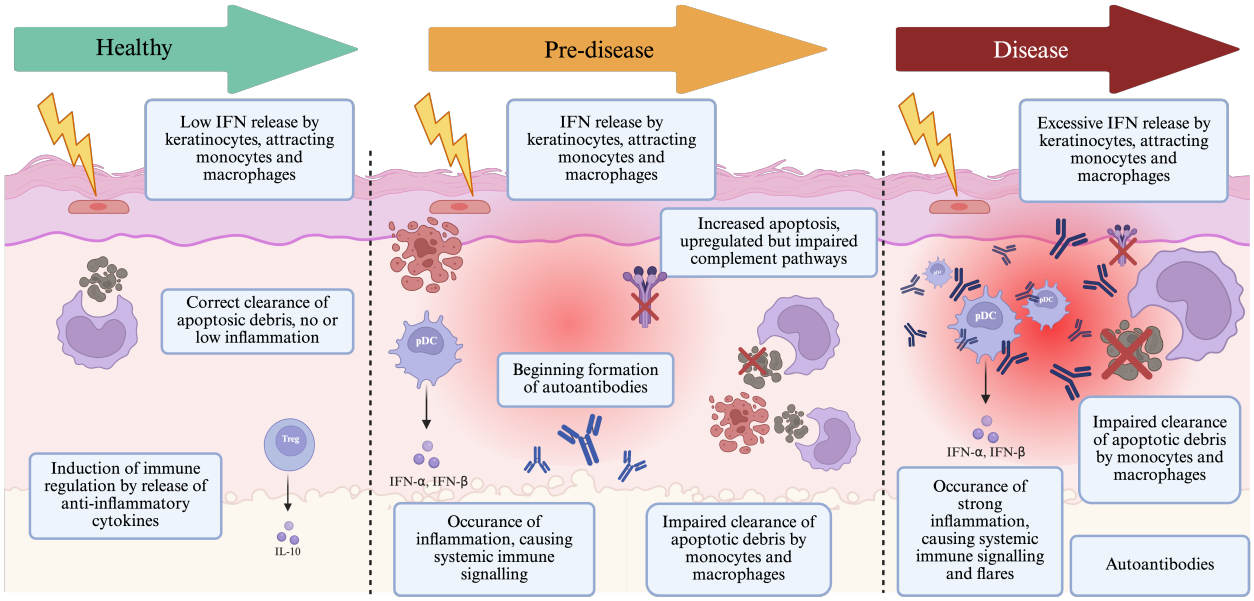
Also, human skin would be very interesting to investigate, and this is an ongoing project of my colleague Zuzana Penxova that follows up on these mouse experiments. Ideally, not only skin from healthy subjects is irradiated, but also skin from lupus patients and their relatives.

Our experiments focused on an SLE model, but there are more autoimmune diseases that could be triggered by UV light or show photosensitivity. Such are dermatomyositis (Love *et al.* 2009; Parks *et al.* 2020), Sjögren syndrome (Estadt *et al.* 2022; Brito-Zerón *et al.* 2014; Tsukazaki *et al.* 2002), and autoimmune blistering diseases (Jappe *et al.* 2000; Kano *et al.* 2000; Igawa, Matsunaga, and Nishioka 2004). In multiple sclerosis, an opposing effect of sunlight is suggested by direct effects of too little sun exposure as well as a lack of vitamin D (Hedström *et al.* 2020). This indicates the need to extend the experiments further, also investigating the influence of UV light on the pre-disease phases of them. In systemic sclerosis, phototherapy is often used to improve the skin lesions; however, known side effects are also erythema, pruritus, and xerosis, which indicates immunologic activity upon treatment (Chaowattanapanit *et al.* 2017).

4.2.5 Summary, statement of significance, and conclusion of the effects of ultraviolet light on lupus-prone skin

This data is extremely valuable to society and science because there is a need to understand that skin from autoimmune-prone individuals differs from healthy skin, even if they do not yet show any signs of skin- or systemic disease. UV light not only reaches the skin as natural daylight, which might already cause damage and trigger SLE. It also serves or is planned to serve different technical purposes. Several wavelengths and forms of UV light have the ability to decontaminate surfaces and is used for this for decades (McDonald *et al.* 2000). However, since the COVID-19 pandemic, a

growing demand for decontamination of rooms, even with people in them, has arisen. Airports, doctors' offices, care centres, and more would benefit from an ongoing sterilisation of pathogens to avoid the spread of infectious diseases. Far-UVC light is thought not to cause cancer as it is of short wavelength and cannot penetrate the skin deeply (Hugenroth 2020; Buonanno *et al.* 2017). We could, however, show in the skin of lupus-prone animals that UVC light is able to trigger several processes, which are related to disease development and progression. Thus, it should not be broadly used due to the fact that humans often do not yet know of their predisposition to autoimmunity and might be more sensitive to disease triggers. Also, people with already ongoing disease might worsen their symptoms by UVC exposure. A protection from outbreak or prevention of autoimmunity is better for the body than medical treatment with severe side effects. Even blue light with 405 nm is upregulating certain genes and pathways in the skin of NZM2410 mice, suggesting that already extended exposure to artificial room-light and screens is not only influencing their circadian cycles but also directly their immune system. The chosen dose was respective of a common regular amount of blue light received from 8 h PC- and smartphone screens, artificial room light and 1 h of sunlight and is therefore not an unusual amount. Awareness of screen use is needed to avoid harm. The detrimental effects of UVB light on the skin are long known, but we could show that lupus-prone animals during their pre-disease phase responded more sensitively to the low-dose treatment that did not cause sunburns. Even with half of that dose of UVB in addition to UVA, we could demonstrate that SLE-specific pathways were significantly more triggered in these mice compared to healthy ones. UVA light alone also promotes immunomodulatory effects in the NZM2410 mice. These experiments enabled us to understand the influence of UV light on autoimmune-prone skin, even in doses that do not cause physical damage. We also learned differences between the wavelengths and concluded that this is an important finding for disease-prevention in the future (**Figure 65**).



Supportive findings of NZM2410 skin during the pre-disease phase, depending on the wavelengths:

UVB:	Changes in translation, rRNA processing, TCA cycle, mitotic cell cycle; downregulated TLR and complement (males); upregulated oxidative phosphorylation and Alzheimer's pathways (females); increased apoptosis
UVC:	Increased cytotoxicity; upregulated keratinisation (males); upregregulated interleukins (females); increased apoptosis, upregulation of *Alox5*
UVA:	Reduced cytotoxicity; upregulated cytokine-cytokine receptor interaction (females)
UVA/B:	Upregulated cytokine-cytokine receptor interaction; upregulated complement cascades, ECM receptor interaction and systemic lupus erythematosus (females)
Blue 405 nm:	Increased cytotoxicity; upregulated keratinisation (males); upregulated ribosomal pathways (females); downregulated interferon and collagen pathways (males); increased apoptosis
Blue 445 nm:	Downregulation of granzyme B; no specific differential regulation of SLE-related genes

Figure 65: Summary of findings of the UV experiments
In healthy skin, low doses of ultraviolet (UV) or blue light do not cause significant harm to the skin, but some cells undergo apoptosis. The keratinocytes react to the stimulus by attracting monocytes and macrophages, which clear up the apoptotic debris. Additionally, immune regulation is induced by the release of IL-10. In SLE pre-disease skin, our experiments demonstrated that even low doses of light caused more apoptosis in the skin of NZM2410 mice than in healthy skin. The clearance processes are most likely disturbed, causing extended presentation of self-antigens, which induces the production of autoantibodies. Inflammation occurs, potentially causing systemic signalling. We found during the pre-disease phase of NZM2410 mice distinct changes depending on the wavelength of low-dose light treatments. UVB (310 nm)= 500 mJ/cm^2; UVC (233 nm)= 40 mJ/cm^2; blue light (405 nm)= 6,839 mJ/cm^2; UVA (375 nm)= 500 mJ/cm^2; UVA/B (310 nm and 375 nm) = 500 mJ/cm^2 in equal shares; blue light (445 nm)= 20,000 mJ/cm^2. During the active disease, UV light causes exacerbated reactions of the ones seen during the pre-disease phase, leading to systemic signalling, inflammation, and disease flares. *Alox5*= Arachidonate 5-lipoxygenase; ECM= extracellular membrane; IFN= interferon; pDC= plasmacytoid dendritic cell; rRNA; ribosomal ribonucleic acid; SLE= systemic lupus erythematosus; TCA cycle= citric acid cycle; TLR= toll-like receptor; Treg= regulatory T cell. The illustration was generated using BioRender.com.

4.3 Concluding remarks on the pre-disease phase and the effects of UV light

We investigated in lupus-prone mice physiologic and immunologic changes during the pre-disease phase in order to improve the understanding of the disease development. However, as genes are not the only determining factor for the SLE disease, severity and progression, we also investigated the effects of UV light, a known trigger, by exposure of the mouse skin during the pre-disease phase. Even though the chosen mouse model (NZM2410 mice) is not known for cutaneous involvement as a factor of the disease, we could still demonstrate multiple changes upon each tested treatment in NZM2410 mice regarding gene expression, pathway regulation and transcription factor expression. These findings demonstrate that the genetic vulnerability leads to elevated responses to low-dose triggers in a phase in which the mice are officially regarded as unburdened, as no clinical symptoms were detectable by that age. The upregulation of interleukin-related pathways by UVC light, for example, might contribute to stronger inflammatory reactions by the UV light trigger, which might be harder to resolve in NZM2410 mice, as we could already detect lower frequencies of Tregs and also impairments in their capabilities. UVB light caused a downregulation in TLR and complement pathways, which might cause disturbed apoptotic processes, leading to extended exposure of the immune cells to cellular debris. This in turn, might amplify proinflammatory reactions, which again cannot be easily resolved by regulatory mechanisms in lupus-prone mice, even at the young and healthy tested ages. The combined treatment of UVA and UVB light even caused a direct upregulation of SLE-associated pathways in females. Together with the aforementioned changes in T cells, this might foster a vicious cycle. Especially the reduced frequencies of Tregs and their impaired regulatory capabilities would not be able to contain upcoming autoimmunity. The increased CD4+ T cell numbers might even further promote inflammatory processes, which were caused by external triggers such as UV light. Even blue light, which we are exposed to even indoors, turned out to affect lupus-prone skin differently than healthy skin. Moreover, if such triggers hit individuals during a "pre-disease flare", effects might be unforeseeable, even stronger, than in other phases of the pre-disease.

Our findings underscore the existence and relevance of this fragile phase in mice and emphasise its need for extension to future human studies. Our work contributed to the understanding of immunologic changes in T cells and haematologic measures, combining it with the effects of a trigger that everyone is facing daily. Nevertheless, we are excited for more upcoming research and experiments in this area, as many aspects are unclear and remain to be elucidated.

References

Abdalhadi, Haneen M., Walter W. Chatham, and Fatima K. Alduraibi (Jan. 2024). "CAR-T-Cell Therapy for Systemic Lupus Erythematosus: A Comprehensive Overview". In: *International Journal of Molecular Sciences* 25.19. Number: 19 Publisher: Multidisciplinary Digital Publishing Institute, p. 10511. ISSN: 1422-0067. DOI: 10.3390/ijms251910511. URL: https://www.mdpi.com/1422-0067/25/19/10511 (visited on 03/29/2025).

Abdelaziz, Salwa, Ola Elmetwaly, and Lobna Maged (May 1, 2020). "Effect of Using Warm Shower and Warm Water Footbath with and Without Adding Epsom Salt on Fatigue Level in Systemic Lupus Patients". In: *Indian Journal of Public Health Research and Development*.

Abdelrasoul, Hend *et al.* (June 24, 2020). "Synergism between IL7R and CXCR4 drives BCR-ABL induced transformation in Philadelphia chromosome-positive acute lymphoblastic leukemia". In: *Nature Communications* 11.1, p. 3194. ISSN: 2041-1723. DOI: 10.1038/s41467-020-16927-w.

Abdirama, Dimas *et al.* (Jan. 1, 2021). "Nuclear antigen–reactive CD4+ T cells expand in active systemic lupus erythematosus, produce effector cytokines, and invade the kidneys". In: *Kidney International* 99.1. Publisher: Elsevier, pp. 238–246. ISSN: 0085-2538. DOI: 10.1016/j.kint.2020.05.051. URL: https://www.kidney-international.org/article/S0085-2538(20)30704-3/fulltext (visited on 05/03/2025).

Aboumrad, E, A M Madec, and C Thivolet (June 2007). "The CXCR4/CXCL12 (SDF-1) signalling pathway protects non-obese diabetic mouse from autoimmune diabetes". In: *Clinical and Experimental Immunology* 148.3, pp. 432–439. ISSN: 0009-9104. DOI: 10.1111/j.1365-2249.2007.03370.x. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1941939/ (visited on 05/04/2025).

Adams, MJ *et al.* (Dec. 1, 2011). "Impaired control of the tissue factor pathway of blood coagulation in systemic lupus erythematosus". In: *Lupus* 20.14. Publisher: SAGE Publications Ltd STM, pp. 1474–1483. ISSN: 0961-2033. DOI: 10.1177/0961203311418267. URL: https://doi.org/10.1177/0961203311418267 (visited on 04/06/2025).

Adorini, Luciano and Giuseppe Penna (Aug. 2008). "Control of autoimmune diseases by the vitamin D endocrine system". In: *Nature Clinical Practice Rheumatology* 4.8. Publisher: Nature Publishing Group, pp. 404–412. ISSN: 1745-8390. DOI: 10.1038/ncprheum0855. URL: https://www.nature.com/articles/ncprheum0855 (visited on 03/29/2025).

Ahamada, Mariame Mohamed, Yang Jia, and Xiaochuan Wu (Dec. 20, 2021). "Macrophage Polarization and Plasticity in Systemic Lupus Erythematosus". In: *Frontiers in Immunology* 12,

p. 734008. ISSN: 1664-3224. DOI: 10.3389/fimmu.2021.734008. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8721097/ (visited on 04/10/2025).

Ahmed, Rafi *et al.* (Sept. 2009). "The precursors of memory: models and controversies". In: *Nature Reviews Immunology* 9.9. Publisher: Nature Publishing Group, pp. 662–668. ISSN: 1474-1741. DOI: 10.1038/nri2619. URL: https://www.nature.com/articles/nri2619 (visited on 03/11/2025).

Ahmed, Sahar A. *et al.* (July 1, 2019). "Pathogenic role of érythropoïétine in Egyptian systemic lupus erthymatosus anemic patients: Prevalence of different types of anemia in SLE". In: *Revista Colombiana de Reumatología* 26.3. Publisher: Elsevier, pp. 151–159. ISSN: 0121-8123. DOI: 10.1016/j.rcreu.2019.05.003. URL: http://www.elsevier.es/en-revista-revista-colombiana-reumatologia-374-articulo-pathogenic-role-erythropoietine-in-egyptian-S0121812319300465 (visited on 03/23/2025).

Akagi, Chika *et al.* (Mar. 2023). "Translational activation of ribosome-related genes at initial photoreception is dependent on signals derived from both the nucleus and the chloroplasts in Arabidopsis thaliana". In: *Journal of Plant Research* 136.2, pp. 227–238. ISSN: 1618-0860. DOI: 10.1007/s10265-022-01430-8.

Akan, H. *et al.* (2000). "Thrombopoietic cytokines in patients with iron deficiency anemia with or without thrombocytosis". In: *Acta Haematologica* 103.3, pp. 152–156. ISSN: 0001-5792. DOI: 10.1159/000041038.

Aldakheel, Fahad M. *et al.* (Dec. 28, 2023). "Clinical Assessment of Cytokine Profiles and Haematological Parameters in Patients with Systemic Lupus Erythematosus: A Cross-Sectional Study from Saudi Arabia". In: *Frontiers in Bioscience-Landmark* 28.12. Number: 12 Publisher: IMR Press, p. 358. ISSN: 2768-6701. DOI: 10.31083/j.fbl2812358. URL: https://www.imrpress.com/journal/FBL/28/12/10.31083/j.fbl2812358 (visited on 04/30/2025).

Alexander, Jessy J *et al.* (Mar. 25, 2020). "Double negative T cells, a potential biomarker for systemic lupus erythematosus". In: *Precision Clinical Medicine* 3.1, pp. 34–43. ISSN: 2096-5303. DOI: 10.1093/pcmedi/pbaa001. URL: https://doi.org/10.1093/pcmedi/pbaa001 (visited on 03/17/2025).

Alexander, Tobias *et al.* (Sept. 1, 2013). "Foxp3+ Helios+ regulatory T cells are expanded in active systemic lupus erythematosus". In: *Annals of the Rheumatic Diseases* 72.9, pp. 1549–1558. ISSN: 1468-2060. DOI: 10.1136/annrheumdis-2012-202216.

Almenar-Queralt, Angels *et al.* (Feb. 26, 2014). "UV Irradiation Accelerates Amyloid Precursor Protein (APP) Processing and Disrupts APP Axonal Transport". In: *The Journal of Neuroscience*

34.9, pp. 3320–3339. ISSN: 0270-6474. DOI: 10.1523/JNEUROSCI.1503-13.2014. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3935090/ (visited on 03/24/2025).

Álvarez-Sánchez, Nuria *et al.* (Feb. 19, 2019). "Peripheral CD39-expressing T regulatory cells are increased and associated with relapsing-remitting multiple sclerosis in relapsing patients". In: *Scientific Reports* 9.1. Publisher: Nature Publishing Group, p. 2302. ISSN: 2045-2322. DOI: 10.1038/s41598-019-38897-w. URL: https://www.nature.com/articles/s41598-019-38897-w (visited on 03/20/2025).

Amoura, Zahir *et al.* (1994). "Nucleosome-restricted antibodies are detected before anti-dsdna and/or antihistone antibodies in serum of mrl-mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria". In: *Arthritis & Rheumatism* 37.11. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.1780371118, pp. 1684–1688. ISSN: 1529-0131. DOI: 10.1002/art.1780371118. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.1780371118 (visited on 03/03/2025).

Aparicio-Soto, Marina, Marina Sánchez-Hidalgo, and Catalina Alarcón-de-la-Lastra (June 2017). "An update on diet and nutritional factors in systemic lupus erythematosus management". In: *Nutrition Research Reviews* 30.1, pp. 118–137. ISSN: 0954-4224, 1475-2700. DOI: 10.1017/S0954422417000026. URL: https://www.cambridge.org/core/journals/nutrition-research-reviews/article/an-update-on-diet-and-nutritional-factors-in-systemic-lupus-erythematosus-management/992C22937FC53A6B1D3D30BC97B331E9 (visited on 03/23/2025).

Apostolakis, Stavros *et al.* (Nov. 1, 2009). "Genetic diversity of CX3CR1 gene and coronary artery disease: New insights through a meta-analysis". In: *Atherosclerosis* 207.1, pp. 8–15. ISSN: 0021-9150. DOI: 10.1016/j.atherosclerosis.2009.03.044. URL: https://www.sciencedirect.com/science/article/pii/S0021915009002561 (visited on 04/17/2025).

Arachchillage, Deepa J., Mike Laffan, and Charis Pericleous (Jan. 2023). "Hydroxychloroquine as an Immunomodulatory and Antithrombotic Treatment in Antiphospholipid Syndrome". In: *International Journal of Molecular Sciences* 24.2. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute, p. 1331. ISSN: 1422-0067. DOI: 10.3390/ijms24021331. URL: https://www.mdpi.com/1422-0067/24/2/1331 (visited on 04/16/2025).

Araki, Yasuto and Toshihide Mimura (Jan. 2024). "Epigenetic Dysregulation in the Pathogenesis of Systemic Lupus Erythematosus". In: *International Journal of Molecular Sciences* 25.2. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute, p. 1019. ISSN: 1422-0067.

DOI: 10.3390/ijms25021019. URL: https://www.mdpi.com/1422-0067/25/2/1019 (visited on 03/29/2025).

Arbuckle, M. R. *et al.* (2001). "Development of Anti-dsDNA Autoantibodies Prior to Clinical Diagnosis of Systemic Lupus Erythematosus". In: *Scandinavian Journal of Immunology* 54.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-3083.2001.00959.x, pp. 211–219. ISSN: 1365-3083. DOI: 10.1046/j.1365-3083.2001.00959.x. URL: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-3083.2001.00959.x (visited on 04/16/2025).

Arenzana, Teresita L., Matthew R. Smith-Raska, and Boris Reizis (June 4, 2009). "Transcription factor Zfx controls BCR-induced proliferation and survival of B lymphocytes". In: *Blood* 113.23, pp. 5857–5867. ISSN: 1528-0020. DOI: 10.1182/blood-2008-11-188888.

Arieta Kuksin, Christina, Gabriela Gonzalez-Perez, and Lisa M. Minter (Mar. 26, 2015). "CXCR4 expression on pathogenic T cells facilitates their bone marrow infiltration in a mouse model of aplastic anemia". In: *Blood* 125.13, pp. 2087–2094. ISSN: 0006-4971. DOI: 10.1182/blood-2014-08-594796. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4375106/ (visited on 03/23/2025).

Aringer, Martin (Dec. 1, 2019). "EULAR/ACR classification criteria for SLE". In: *Seminars in Arthritis and Rheumatism*. Advances in Targeted Therapies: Proceedings of the 2019 Meeting 49.3, S14–S17. ISSN: 0049-0172. DOI: 10.1016/j.semarthrit.2019.09.009. URL: https://www.sciencedirect.com/science/article/pii/S0049017219306444 (visited on 02/04/2025).

Arnaud, Laurent *et al.* (June 1, 2019). "Predictors of fatigue and severe fatigue in a large international cohort of patients with systemic lupus erythematosus and a systematic review of the literature". In: *Rheumatology* 58.6, pp. 987–996. ISSN: 1462-0324. DOI: 10.1093/rheumatology/key398. URL: https://doi.org/10.1093/rheumatology/key398 (visited on 02/27/2025).

Baaten, Bas *et al.* (Feb. 27, 2012). "Regulation of Antigen-Experienced T Cells: Lessons from the Quintessential Memory Marker CD44". In: *Frontiers in Immunology* 3. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2012.00023. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2012.00023/full (visited on 03/03/2025).

Badia-i-Mompel, Pau *et al.* (Jan. 1, 2022). "decoupleR: ensemble of computational methods to infer biological activities from omics data". In: *Bioinformatics Advances* 2.1, vbac016. ISSN: 2635-0041. DOI: 10.1093/bioadv/vbac016. URL: https://doi.org/10.1093/bioadv/vbac016 (visited on 11/03/2024).

Bagavant, Harini *et al.* (Dec. 1, 2006). "Role for Nephritogenic T Cells in Lupus Glomerulonephritis: Progression to Renal Failure Is Accompanied by T Cell Activation and Expansion in Regional Lymph Nodes1". In: *The Journal of Immunology* 177.11, pp. 8258–8265. ISSN: 0022-1767. DOI: 10.4049/jimmunol.177.11.8258. URL: https://doi.org/10.4049/jimmunol.177.11.8258 (visited on 05/04/2025).

Bai, Y. *et al.* (Jan. 2018). "Self-dsDNA in the pathogenesis of systemic lupus erythematosus". In: *Clinical and Experimental Immunology* 191.1, pp. 1–10. ISSN: 1365-2249. DOI: 10.1111/cei.13041.

Barber, Megan R. W. *et al.* (Sept. 2021). "Global epidemiology of systemic lupus erythematosus". In: *Nature Reviews Rheumatology* 17.9. Publisher: Nature Publishing Group, pp. 515–532. ISSN: 1759-4804. DOI: 10.1038/s41584-021-00668-1. URL: https://www.nature.com/articles/s41584-021-00668-1 (visited on 03/28/2025).

Barreto, Marta *et al.* (Jan. 27, 2009). "Low frequency of CD4+CD25+ Treg in SLE patients: a heritable trait associated with CTLA4 and TGFβ gene variants". In: *BMC Immunology* 10, p. 5. ISSN: 1471-2172. DOI: 10.1186/1471-2172-10-5. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2656467/ (visited on 03/18/2025).

Barron, Luke *et al.* (Dec. 1, 2010). "Mechanisms of IL-2–dependent maintenance of functional regulatory T cells". In: *Journal of Immunology (Baltimore, Md. : 1950)* 185.11, pp. 6426–6430. ISSN: 0022-1767. DOI: 10.4049/jimmunol.0903940. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3059533/ (visited on 03/20/2025).

Basta, Fabio *et al.* (Sept. 1, 2020). "Systemic Lupus Erythematosus (SLE) Therapy: The Old and the New". In: *Rheumatology and Therapy* 7.3, pp. 433–446. ISSN: 2198-6584. DOI: 10.1007/s40744-020-00212-9. URL: https://doi.org/10.1007/s40744-020-00212-9 (visited on 03/29/2025).

Battie, Claire *et al.* (2014). "New insights in photoaging, UVA induced damage and skin types". In: *Experimental Dermatology* 23 (s1). _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/exd.12388, pp. 7–12. ISSN: 1600-0625. DOI: 10.1111/exd.12388. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/exd.12388 (visited on 11/03/2024).

Bell, Ryan A. V. and Lynn A. Megeney (Aug. 2017). "Evolution of caspase-mediated cell death and differentiation: twins separated at birth". In: *Cell Death & Differentiation* 24.8. Publisher: Nature Publishing Group, pp. 1359–1368. ISSN: 1476-5403. DOI: 10.1038/cdd.2017.37. URL: https://www.nature.com/articles/cdd201737 (visited on 03/26/2025).

Benjamini, Yoav and Daniel Yekutieli (Oct. 2005). "Quantitative trait Loci analysis using the false discovery rate". In: *Genetics* 171.2, pp. 783–790. ISSN: 0016-6731. DOI: 10.1534/genetics.104.036699.

Bianco, Christopher and Ian Mohr (Dec. 16, 2019). "Ribosome biogenesis restricts innate immune responses to virus infection and DNA". In: *eLife* 8. Ed. by Nahum Sonenberg, Tadatsugu Taniguchi, and Daphne C Avgousti. Publisher: eLife Sciences Publications, Ltd, e49551. ISSN: 2050-084X. DOI: 10.7554/eLife.49551. URL: https://doi.org/10.7554/eLife.49551 (visited on 03/24/2025).

Bijl, Marc and Cees GM Kallenberg (Nov. 1, 2006). "Ultraviolet light and cutaneous lupus". In: *Lupus* 15.11. Publisher: SAGE Publications Ltd STM, pp. 724–727. ISSN: 0961-2033. DOI: 10.1177/0961203306071705. URL: https://doi.org/10.1177/0961203306071705 (visited on 03/23/2025).

Biswas, Swayanka, Katja Bieber, and Rudolf Armin Manz (Aug. 1, 2022). "IL-10 revisited in systemic lupus erythematosus". In: *Frontiers in Immunology* 13. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2022.970906. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.970906/full (visited on 03/23/2025).

Blanco, Patrick *et al.* (2005). "Increase in activated CD8+ T lymphocytes expressing perforin and granzyme B correlates with disease activity in patients with systemic lupus erythematosus". In: *Arthritis & Rheumatism* 52.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.20745, pp. 201–211. ISSN: 1529-0131. DOI: 10.1002/art.20745. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.20745 (visited on 03/18/2025).

Blaschke, Sabine *et al.* (Sept. 2003). "Proinflammatory role of fractalkine (CX3CL1) in rheumatoid arthritis". In: *The Journal of Rheumatology* 30.9, pp. 1918–1927. ISSN: 0315-162X.

Boilard, Eric, Patrick Blanco, and Peter A. Nigrovic (Sept. 2012). "Platelets: active players in the pathogenesis of arthritis and SLE". In: *Nature Reviews Rheumatology* 8.9. Publisher: Nature Publishing Group, pp. 534–542. ISSN: 1759-4804. DOI: 10.1038/nrrheum.2012.118. URL: https://www.nature.com/articles/nrrheum.2012.118 (visited on 03/02/2025).

Bonnans, Magali *et al.* (Nov. 1, 2020). "Blue light: Friend or foe ?" In: *Journal of Photochemistry and Photobiology B: Biology* 212, p. 112026. ISSN: 1011-1344. DOI: 10.1016/j.jphotobiol.2020.112026. URL: https://www.sciencedirect.com/science/article/pii/S1011134420304760 (visited on 03/24/2025).

Bookwalter, Deborah Boggs *et al.* (Jan. 15, 2020). "Posttraumatic stress disorder and risk of selected autoimmune diseases among US military personnel". In: *BMC Psychiatry* 20, p. 23. ISSN:

1471-244X. DOI: 10.1186/s12888-020-2432-9. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6964079/ (visited on 04/16/2025).

Botto, Marina (Mar. 10, 1999). "C1q Knock-Out Mice for the Study of Complement Deficiency in Autoimmune Disease". In: *Experimental and Clinical Immunogenetics* 15.4, pp. 231–234. ISSN: 0254-9670. DOI: 10.1159/000019076. URL: https://doi.org/10.1159/000019076 (visited on 04/01/2025).

Bouredji, Zineb, Anteneh Argaw, and Jérôme Frenette (Nov. 23, 2022). "The inflammatory response, a mixed blessing for muscle homeostasis and plasticity". In: *Frontiers in Physiology* 13. Publisher: Frontiers. ISSN: 1664-042X. DOI: 10.3389/fphys.2022.1032450. URL: https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2022.1032450/full (visited on 03/30/2025).

Bouzid, D. *et al.* (July 1, 2012). "Association of the *RAVER2* gene with increased susceptibility for ulcerative colitis". In: *Human Immunology* 73.7, pp. 732–735. ISSN: 0198-8859. DOI: 10.1016/j.humimm.2012.04.018. URL: https://www.sciencedirect.com/science/article/pii/S0198885912001115 (visited on 04/06/2025).

Boyman, Onur, Marek Kovar, *et al.* (Mar. 31, 2006). "Selective Stimulation of T Cell Subsets with Antibody-Cytokine Immune Complexes". In: *Science* 311.5769. Publisher: American Association for the Advancement of Science, pp. 1924–1927. DOI: 10.1126/science.1122927. URL: https://www.science.org/doi/10.1126/science.1122927 (visited on 03/18/2025).

Boyman, Onur and Jonathan Sprent (Feb. 17, 2012). "The role of interleukin-2 during homeostasis and activation of the immune system". In: *Nature Reviews. Immunology* 12, pp. 180–90. DOI: 10.1038/nri3156.

Bradley, Sean J. *et al.* (June 11, 2015). "T Cell Transcriptomes Describe Patient Subtypes in Systemic Lupus Erythematosus". In: *PloS One* 10.11. Publisher: Public Library of Science, e0141171. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0141171. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0141171 (visited on 02/04/2025).

Bray, Nicolas L. *et al.* (May 2016). "Near-optimal probabilistic RNA-seq quantification". In: *Nature Biotechnology* 34.5. Publisher: Nature Publishing Group, pp. 525–527. ISSN: 1546-1696. DOI: 10.1038/nbt.3519. URL: https://www.nature.com/articles/nbt.3519 (visited on 11/03/2024).

Brinks, Ralph *et al.* (Nov. 25, 2016). "Age-specific and sex-specific incidence of systemic lupus erythematosus: an estimate from cross-sectional claims data of 2.3 million people in the German statutory health insurance 2002". In: *Lupus Science & Medicine* 3.1, e000181. ISSN: 2053-8790.

DOI: 10.1136/lupus-2016-000181. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5133401/ (visited on 03/20/2025).

Brito-Zerón, P *et al.* (Feb. 1, 2014). "Annular erythema in primary Sjögren's syndrome: description of 43 non-Asian cases". In: *Lupus* 23.2. Publisher: SAGE Publications Ltd STM, pp. 166–175. ISSN: 0961-2033. DOI: 10.1177/0961203313515764. URL: https://doi.org/10.1177/0961203313515764 (visited on 03/22/2025).

Bronte, Vincenzo and Mikael J Pittet (Nov. 14, 2013). "The spleen in local and systemic regulation of immunity". In: *Immunity* 39.5, pp. 806–818. ISSN: 1074-7613. DOI: 10.1016/j.immuni.2013.10.010. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3912742/ (visited on 03/20/2025).

Bruijn, J. A. *et al.* (Mar. 1988). "Murine chronic graft-versus-host disease as a model for lupus nephritis." In: *The American Journal of Pathology* 130.3, pp. 639–641. ISSN: 0002-9440. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1880687/ (visited on 02/01/2025).

Bukhari, Kiran, Mudassira Zahid, and Humaira Zafar (Aug. 4, 2019). "Establishment of reference ranges and values for red cell distribution width amongst infants". In: *ISRA Medical Journal*.

Bultink, Irene E M *et al.* (Jan. 5, 2021). "Mortality, causes of death and influence of medication use in patients with systemic lupus erythematosus vs matched controls". In: *Rheumatology* 60.1, pp. 207–216. ISSN: 1462-0324. DOI: 10.1093/rheumatology/keaa267. URL: https://doi.org/10.1093/rheumatology/keaa267 (visited on 03/29/2025).

Buonanno, Manuela *et al.* (Feb. 22, 2017). "Germicidal Efficacy and Mammalian Skin Safety of 222-nm UV Light". In: *Radiation Research* 187.4, p. 483. DOI: 10.1667/RR0010CC.1. URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC5552051/ (visited on 03/26/2025).

Burmester, Imke A. K. *et al.* (June 2019). "Translational Use of a Standardized Full Human Skin Organ Culture Model in Autoimmune Blistering Diseases." In: *Current Protocols in Pharmacology* 85.1. Place: United States, e56. ISSN: 1934-8290 1934-8282. DOI: 10.1002/cpph.56.

Burton, Oliver *et al.* (Aug. 14, 2023). "The tissue-resident regulatory T cell pool is shaped by transient multi-tissue migration and a conserved residency program". In: *Preprint*. Pages: 2023.08.14.553196 Section: New Results. DOI: 10.1101/2023.08.14.553196. URL: https://www.biorxiv.org/content/10.1101/2023.08.14.553196v1 (visited on 03/20/2025).

Cabana-Puig, Xavier *et al.* (May 1, 2023). "CX3CR1 modulates SLE-associated glomerulonephritis and cardiovascular disease in MRL/lpr mice". In: *Inflammation Research* 72.5, pp. 1083–1097. ISSN: 1420-908X. DOI: 10.1007/s00011-023-01731-1. URL: https://doi.org/10.1007/s00011-023-01731-1 (visited on 04/17/2025).

Cabral, A. *et al.* (Nov. 2001). "SPRR4, a novel cornified envelope precursor: UV-dependent epidermal expression and selective incorporation into fragile envelopes". In: *Journal of Cell Science* 114 (Pt 21), pp. 3837–3843. ISSN: 0021-9533. DOI: 10.1242/jcs.114.21.3837.

Cabral, João and Rodrigues Ag (June 2019). "Blue Light Disinfection in Hospital Infection Control: Advantages, Drawbacks, and Pitfalls". In: *Antibiotics* 8.2. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute, p. 58. ISSN: 2079-6382. DOI: 10.3390/antibiotics8020058. URL: https://www.mdpi.com/2079-6382/8/2/58 (visited on 03/30/2025).

Cai, Hao *et al.* (Feb. 27, 2024). "ALOX5 drives the pyroptosis of CD4+ T cells and tissue inflammation in rheumatoid arthritis". In: *Science Signaling* 17.825. Publisher: American Association for the Advancement of Science, eadh1178. DOI: 10.1126/scisignal.adh1178. URL: https://www.science.org/doi/abs/10.1126/scisignal.adh1178 (visited on 04/06/2025).

Carlini, Valentina *et al.* (June 8, 2023). "The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions". In: *Frontiers in Immunology* 14. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2023.1161067. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2023.1161067/full (visited on 03/20/2025).

Catalina, Michelle D. *et al.* (2019). "Gene expression analysis delineates the potential roles of multiple interferons in systemic lupus erythematosus". In: *Communications Biology* 2, p. 140. ISSN: 2399-3642. DOI: 10.1038/s42003-019-0382-x.

Celkan, Tülin Tiraje (June 19, 2020). "What does a hemogram say to us?" In: *Turkish Archives of Pediatrics/Türk Pediatri Arşivi* 55.2, p. 103. DOI: 10.14744/TurkPediatriArs.2019.76301. URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC7344121/ (visited on 11/02/2024).

Chan, Owen and Mark J. Shlomchik (Jan. 1, 1998). "A New Role for B Cells in Systemic Autoimmunity: B Cells Promote Spontaneous T Cell Activation in MRL-lpr/lpr Mice1". In: *The Journal of Immunology* 160.1, pp. 51–59. ISSN: 0022-1767. DOI: 10.4049/jimmunol.160.1.51. URL: https://doi.org/10.4049/jimmunol.160.1.51 (visited on 02/27/2025).

Chang, Kai *et al.* (Jan. 2023). "Peripheral CD4+CD8+ double positive T cells: A potential marker to evaluate renal impairment susceptibility during systemic lupus erythematosus". In: *Journal of Biomedical Research* 37.1, pp. 59–68. ISSN: 1674-8301. DOI: 10.7555/JBR.36.20220094. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9898043/ (visited on 03/18/2025).

Chaowattanapanit, Suteeraporn *et al.* (2017). "Phototherapy in systemic sclerosis: Review". In: *Photodermatology, Photoimmunology & Photomedicine* 33.6. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/phpp.12331, pp. 296–305. ISSN: 1600-0781.

DOI: 10.1111/phpp.12331. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/phpp.12331 (visited on 03/22/2025).

Charles, Luenda E. *et al.* (Nov. 2007). "Obesity, white blood cell counts, and platelet counts among police officers". In: *Obesity (Silver Spring, Md.)* 15.11, pp. 2846–2854. ISSN: 1930-7381. DOI: 10.1038/oby.2007.338.

Chastin, Sebastien F. M. *et al.* (2021). "Effects of Regular Physical Activity on the Immune System, Vaccination and Risk of Community-Acquired Infectious Disease in the General Population: Systematic Review and Meta-Analysis". In: *Sports Medicine (Auckland, N.z.)* 51.8, pp. 1673–1686. ISSN: 0112-1642. DOI: 10.1007/s40279-021-01466-1. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8056368/ (visited on 03/30/2025).

Chen, Di *et al.* (July 17, 2017). "The molecular mechanisms of Monascus purpureus M9 responses to blue light based on the transcriptome analysis". In: *Scientific Reports* 7.1. Publisher: Nature Publishing Group, p. 5537. ISSN: 2045-2322. DOI: 10.1038/s41598-017-05990-x. URL: https://www.nature.com/articles/s41598-017-05990-x (visited on 03/24/2025).

Chen, Jiaxuan *et al.* (Jan. 18, 2022). "Humanized Mouse Models of Systemic Lupus Erythematosus: Opportunities and Challenges". In: *Frontiers in Immunology* 12. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2021.816956. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.816956/full (visited on 01/29/2025).

Chen, Pei and Yongjun Li (Aug. 1, 2016). "The decrease of consistence probability: At the crossroad of catastrophic transition of a biological system". In: *BMC Systems Biology* 10, p. 50. DOI: 10.1186/s12918-016-0295-y.

Chen, Ping-Min and George C. Tsokos (Nov. 1, 2021). "The role of CD8+ T-cell systemic lupus erythematosus pathogenesis: an update". In: *Current Opinion in Rheumatology* 33.6, pp. 586–591. ISSN: 1040-8711. DOI: 10.1097/BOR.0000000000000815. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8567317/ (visited on 03/18/2025).

Chen, Wei *et al.* (Apr. 1, 2025). "Identification of Cellular Senescence-related genes as biomarkers for lupus nephritis based on bioinformatics". In: *Frontiers in Genetics* 16. Publisher: Frontiers. ISSN: 1664-8021. DOI: 10.3389/fgene.2025.1551450. URL: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2025.1551450/abstract (visited on 04/06/2025).

Cheng, Yaqian *et al.* (Nov. 14, 2024). "Roles of macrophages in lupus nephritis". In: *Frontiers in Pharmacology* 15. Publisher: Frontiers. ISSN: 1663-9812. DOI: 10.3389/fphar.2024.

1477708. URL: https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2024.1477708/full (visited on 03/31/2025).

Chmielewski, P. P. and B. Strzelec (2018). "Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: a review". In: *Folia Morphologica* 77.2. Number: 2, pp. 171–178. ISSN: 1644-3284. DOI: 10.5603/FM.a2017.0101. URL: https://journals.viamedica.pl/folia_morphologica/article/view/FM.a2017.0101 (visited on 02/27/2025).

Cho, Min Jeong *et al.* (Dec. 20, 2021). "Microtubule Integrity Is Associated with the Functional Activity of Mitochondria in HEK293". In: *Cells* 10.12, p. 3600. ISSN: 2073-4409. DOI: 10.3390/cells10123600. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8700340/ (visited on 03/25/2025).

Christ, Anette, Mario Lauterbach, and Eicke Latz (Nov. 19, 2019). "Western Diet and the Immune System: An Inflammatory Connection". In: *Immunity* 51.5, pp. 794–811. ISSN: 1097-4180. DOI: 10.1016/j.immuni.2019.09.020.

Christodoulou-Vafeiadou, Eleni *et al.* (2024). "A Novel Human Interleukin-23A Overexpressing Mouse Model of Systemic Lupus Erythematosus". In: *Arthritis & Rheumatology* 76.7. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.42830, pp. 1085–1095. ISSN: 2326-5205. DOI: 10.1002/art.42830. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.42830 (visited on 04/06/2025).

Churlaud, Guillaume *et al.* (Apr. 15, 2015). "Human and Mouse CD8+CD25+FOXP3+ Regulatory T Cells at Steady State and during Interleukin-2 Therapy". In: *Frontiers in Immunology* 6. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2015.00171. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2015.00171/full (visited on 03/11/2025).

Ciurtin, Coziana *et al.* (June 21, 2022). "CD8+ T-Cells in Juvenile-Onset SLE: From Pathogenesis to Comorbidities". In: *Frontiers in Medicine* 9. Publisher: Frontiers. ISSN: 2296-858X. DOI: 10.3389/fmed.2022.904435. URL: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2022.904435/full (visited on 03/18/2025).

Clarke, Sandra T and G. Douglas Inglis (May 1, 2019). "Non-therapeutic administration of antimicrobial growth promoters to interrogate suppression mechanisms of innate and adaptive immune responses using an ovine model of stress". In: *The Journal of Immunology* 202.1, p. 73.15. ISSN: 0022-1767. DOI: 10.4049/jimmunol.202.Supp.73.15. URL: https://doi.org/10.4049/jimmunol.202.Supp.73.15 (visited on 03/02/2025).

Cohen, Irun R. and Ansgar W. Lohse (Apr. 16, 2008). "Physiology and Pathophysiology of Autoimmunity". In: *Seminars in Liver Disease* 11. Publisher: © 1991 by Thieme Medical Publishers, Inc., pp. 183–186. ISSN: 0272-8087. DOI: 10.1055/s-2008-1040435. URL: https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2008-1040435 (visited on 04/16/2025).

Cole, Megan A. *et al.* (Mar. 2018). "Extracellular matrix regulation of fibroblast function: redefining our perspective on skin aging". In: *Journal of Cell Communication and Signaling* 12.1, pp. 35–43. ISSN: 1873-9601. DOI: 10.1007/s12079-018-0459-1. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5842211/ (visited on 03/24/2025).

Contento, Rita Lucia *et al.* (July 22, 2008). "CXCR4–CCR5: A couple modulating T cell functions". In: *Proceedings of the National Academy of Sciences* 105.29. Publisher: Proceedings of the National Academy of Sciences, pp. 10101–10106. DOI: 10.1073/pnas.0804286105. URL: https://www.pnas.org/doi/10.1073/pnas.0804286105 (visited on 03/23/2025).

Cooper, Glinda S and Berrit C Stroehla (May 1, 2003). "The epidemiology of autoimmune diseases". In: *Autoimmunity Reviews* 2.3, pp. 119–125. ISSN: 1568-9972. DOI: 10.1016/S1568-9972(03)00006-5. URL: https://www.sciencedirect.com/science/article/pii/S1568997203000065 (visited on 04/16/2025).

Cosmi, Lorenzo *et al.* (Dec. 1, 2003). "Human CD8+CD25+ thymocytes share phenotypic and functional features with CD4+CD25+ regulatory thymocytes". In: *Blood* 102.12, pp. 4107–4114. ISSN: 0006-4971. DOI: 10.1182/blood-2003-04-1320. URL: https://www.sciencedirect.com/science/article/pii/S0006497120440212 (visited on 03/11/2025).

Couldwell, Genevieve and Kellie R. Machlus (July 2019). "Modulation of megakaryopoiesis and platelet production during inflammation". In: *Thrombosis Research* 179, pp. 114–120. ISSN: 1879-2472. DOI: 10.1016/j.thromres.2019.05.008.

Cruz, Abigail De la *et al.* (Nov. 21, 2024). "Immune complex deposition promotes NK cell accumulation in the kidney". In: *PloS One* 19.11. Publisher: Public Library of Science, e0312141. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0312141. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0312141 (visited on 04/01/2025).

Cui, Mintian *et al.* (2021). "Blood Genomics Identifies Three Subtypes of Systemic Lupus Erythematosus: "IFN-High," "NE-High," and "Mixed"". In: *Mediators of Inflammation* 2021.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2021/6660164, p. 6660164.

ISSN: 1466-1861. DOI: 10.1155/2021/6660164. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2021/6660164 (visited on 02/04/2025).

Cutolo, Maurizio, Rainer H. Straub, and Frank Buttgereit (July 2008). "Circadian rhythms of nocturnal hormones in rheumatoid arthritis: translation from bench to bedside". In: *Annals of the Rheumatic Diseases* 67.7, pp. 905–908. ISSN: 1468-2060. DOI: 10.1136/ard.2008.088955.

Dai, Xiaofeng, Yuting Fan, and Xing Zhao (Mar. 17, 2025). "Systemic lupus erythematosus: updated insights on the pathogenesis, diagnosis, prevention and therapeutics". In: *Signal Transduction and Targeted Therapy* 10.1. Publisher: Nature Publishing Group, pp. 1–51. ISSN: 2059-3635. DOI: 10.1038/s41392-025-02168-0. URL: https://www.nature.com/articles/s41392-025-02168-0 (visited on 04/01/2025).

Danno, KIICHIRO and TAKESHI Horio (May 1, 1980). "Histochemical staining of sunburn cells for sulphhydryl and disulphide groups: a time course study". In: *British Journal of Dermatology* 102.5, pp. 535–539. ISSN: 0007-0963. DOI: 10.1111/j.1365-2133.1980.tb07651.x. URL: https://doi.org/10.1111/j.1365-2133.1980.tb07651.x (visited on 03/25/2025).

Davison, Laura M. *et al.* (June 17, 2021). "S100a9 Protects Male Lupus-Prone NZBWF1 Mice From Disease Development". In: *Frontiers in Immunology* 12. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2021.681503. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.681503/full (visited on 04/06/2025).

De Leo, Vincent A. *et al.* (Nov. 1, 1984). "Ultraviolet Radiation Induces Changes in Membrane Metabolism of Human Keratinocytes in Culture". In: *Journal of Investigative Dermatology* 83.5, pp. 323–326. ISSN: 0022-202X. DOI: 10.1111/1523-1747.ep12264114. URL: https://www.sciencedirect.com/science/article/pii/S0022202X15435365 (visited on 03/24/2025).

DeGrendele, H C *et al.* (Mar. 1, 1996). "CD44 and its ligand hyaluronate mediate rolling under physiologic flow: a novel lymphocyte-endothelial cell primary adhesion pathway." In: *Journal of Experimental Medicine* 183.3, pp. 1119–1130. ISSN: 0022-1007. DOI: 10.1084/jem.183.3.1119. URL: https://doi.org/10.1084/jem.183.3.1119 (visited on 03/03/2025).

Dema, Barbara and Nicolas Charles (Jan. 4, 2016). "Autoantibodies in SLE: Specificities, Isotypes and Receptors". In: *Antibodies* 5.1, p. 2. ISSN: 2073-4468. DOI: 10.3390/antib5010002. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6698872/ (visited on 05/05/2025).

Dey, Mrinalini, Ioannis Parodis, and Elena Nikiphorou (Jan. 2021). "Fatigue in Systemic Lupus Erythematosus and Rheumatoid Arthritis: A Comparison of Mechanisms, Measures and Management". In: *Journal of Clinical Medicine* 10.16. Number: 16 Publisher: Multidisciplinary

Digital Publishing Institute, p. 3566. ISSN: 2077-0383. DOI: 10.3390/jcm10163566. URL: https://www.mdpi.com/2077-0383/10/16/3566 (visited on 02/27/2025).

Dhabhar, Firdaus S. (Feb. 2013). "Psychological stress and immunoprotection versus immunopathology in the skin". In: *Clinics in Dermatology* 31.1, pp. 18–30. ISSN: 1879-1131. DOI: 10.1016/j.clindermatol.2011.11.003.

Dhabhar, Firdaus S. and Bruce S. Mcewen (Dec. 1, 1997). "Acute Stress Enhances while Chronic Stress Suppresses Cell-Mediated Immunity*in Vivo:* A Potential Role for Leukocyte Trafficking". In: *Brain, Behavior, and Immunity* 11.4, pp. 286–306. ISSN: 0889-1591. DOI: 10.1006/brbi.1997.0508. URL: https://www.sciencedirect.com/science/article/pii/S0889159197905080 (visited on 03/30/2025).

Diaz-Gallo, Lina-Marcela *et al.* (2022). "Four Systemic Lupus Erythematosus Subgroups, Defined by Autoantibodies Status, Differ Regarding HLA-DRB1 Genotype Associations and Immunological and Clinical Manifestations". In: *ACR Open Rheumatology* 4.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/acr2.11343, pp. 27–39. ISSN: 2578-5745. DOI: 10.1002/acr2.11343. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/acr2.11343 (visited on 01/22/2025).

Dimitrov, Stoyan *et al.* (May 21, 2009). "Cortisol and epinephrine control opposing circadian rhythms in T cell subsets". In: *Blood* 113.21, pp. 5134–5143. ISSN: 0006-4971. DOI: 10.1182/blood-2008-11-190769. URL: https://doi.org/10.1182/blood-2008-11-190769 (visited on 02/18/2023).

Dinesh, R *et al.* (July 2011). "Interferon-inducible gene 202b controls CD8+ T cell-mediated suppression in anti-DNA Ig peptide-treated (NZB × NZW) F1 lupus mice". In: *Genes and Immunity* 12.5, pp. 360–369. ISSN: 1466-4879. DOI: 10.1038/gene.2011.4. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3149980/ (visited on 04/03/2025).

Dinesh, Ravi K. *et al.* (June 1, 2010). "CD8+ Tregs in lupus, autoimmunity, and beyond". In: *Autoimmunity Reviews* 9.8, pp. 560–568. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2010.03.006. URL: https://www.sciencedirect.com/science/article/pii/S1568997210000625 (visited on 03/11/2025).

Dinse, Gregg E. *et al.* (2022). "Increasing Prevalence of Antinuclear Antibodies in the United States". In: *Arthritis & Rheumatology* 74.12. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.42330, pp. 2032–2041. ISSN: 2326-5205. DOI: 10.1002/art.42330. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.42330 (visited on 02/04/2025).

Diny, Nicola L., Noel R. Rose, and Daniela Čiháková (Apr. 27, 2017). "Eosinophils in Autoimmune Diseases". In: *Frontiers in Immunology* 8. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2017.00484. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2017.00484/full (visited on 03/31/2025).

Dixon, Frank J. *et al.* (1978). "Etiology and pathogenesis of a spontaneous lupus-like syndrome in mice". In: *Arthritis & Rheumatism* 21 (S1). _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.1780210909, S64–S67. ISSN: 1529-0131. DOI: 10.1002/art.1780210909. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.1780210909 (visited on 01/31/2025).

Dong, K. *et al.* (2019). "Blue light disrupts the circadian rhythm and create damage in skin cells". In: *International Journal of Cosmetic Science* 41.6. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ics.12572, pp. 558–562. ISSN: 1468-2494. DOI: 10.1111/ics.12572. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/ics.12572 (visited on 11/26/2023).

Dong, Michelle *et al.* (July 1, 2020). "Sex Differences in Cancer Incidence and Survival: A Pan-Cancer Analysis". In: *Cancer Epidemiology, Biomarkers & Prevention* 29.7, pp. 1389–1397. ISSN: 1055-9965. DOI: 10.1158/1055-9965.EPI-20-0036. URL: https://doi.org/10.1158/1055-9965.EPI-20-0036 (visited on 01/22/2025).

Doria, A *et al.* (Oct. 1, 2008). "Infections as triggers and complications of systemic lupus erythematosus". In: *Autoimmunity Reviews*. Special Issue on Infections Rheumatism and Autoimmunity 8.1, pp. 24–28. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2008.07.019. URL: https://www.sciencedirect.com/science/article/pii/S1568997208001328 (visited on 03/23/2025).

Dou, Diana R. *et al.* (Feb. 1, 2024). "Xist ribonucleoproteins promote female sex-biased autoimmunity". In: *Cell* 187.3. Publisher: Elsevier, 733–749.e16. ISSN: 0092-8674, 1097-4172. DOI: 10.1016/j.cell.2023.12.037. URL: https://www.cell.com/cell/abstract/S0092-8674(24)00002-3 (visited on 02/06/2025).

Ehrhart, Jean-Claude *et al.* (Aug. 2003). "UVB-induced mutations in human key gatekeeper genes governing signalling pathways and consequences for skin tumourigenesis". In: *Photochemical & Photobiological Sciences* 2.8, pp. 825–834. ISSN: 1474-905X, 1474-9092. DOI: 10.1039/b302281a. URL: https://link.springer.com/10.1039/b302281a (visited on 03/24/2025).

Elkord, Eyad, May Abd Al Samid, and Belal Chaudhary (July 30, 2015). "Helios, and not FoxP3, is the marker of activated Tregs expressing GARP/LAP". In: *Oncotarget* 6.24, pp. 20026–20036.

ISSN: 1949-2553. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4652984/ (visited on 03/20/2025).

Ernst, Peter B. and Anne-Ruxandra Carvunis (May 2018). "Of mice, men and immunity: a case for evolutionary systems biology". In: *Nature Immunology* 19.5, pp. 421–425. ISSN: 1529-2908. DOI: 10.1038/s41590-018-0084-4. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6168288/ (visited on 03/28/2025).

Esmaeilzadeh, Emran *et al.* (Nov. 1, 2021). "Strong association of common variants in the miRNA-binding site of NOD2 gene with clinicopathological characteristics and disease activity of systemic lupus erythematosus". In: *Clinical Rheumatology* 40.11, pp. 4559–4567. ISSN: 1434-9949. DOI: 10.1007/s10067-021-05812-6. URL: https://doi.org/10.1007/s10067-021-05812-6 (visited on 04/06/2025).

Estadt, Shannon N. *et al.* (Mar. 2022). "Mechanisms of Photosensitivity in Autoimmunity". In: *The Journal of Investigative Dermatology* 142.3, pp. 849–856. ISSN: 0022-202X. DOI: 10.1016/j.jid.2021.05.007. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8688579/ (visited on 03/22/2025).

Evans, Timothy C. and Dietrich Jehle (Jan. 1, 1991). "The red blood cell distribution width". In: *The Journal of Emergency Medicine* 9, pp. 71–74. ISSN: 0736-4679. DOI: 10.1016/0736-4679(91)90592-4. URL: https://www.sciencedirect.com/science/article/pii/0736467991905924 (visited on 03/02/2025).

Fan, X.-L. *et al.* (May 8, 2015). "Lack of estrogen down-regulates CXCR4 expression on Treg cells and reduces Treg cell population in bone marrow in OVX mice". In: *Cellular and Molecular Biology* 61.2. Number: 2, pp. 13–17. ISSN: 1165-158X. DOI: 10.14715/cmb/23. URL: https://www.cellmolbiol.org/index.php/CMB/article/view/644 (visited on 03/23/2025).

Fanouriakis, Antonis *et al.* (Jan. 1, 2024). "EULAR recommendations for the management of systemic lupus erythematosus: 2023 update". In: *Annals of the Rheumatic Diseases* 83.1. Publisher: BMJ Publishing Group Ltd Section: Recommendation, pp. 15–29. ISSN: 0003-4967, 1468-2060. DOI: 10.1136/ard-2023-224762. URL: https://ard.bmj.com/content/83/1/15 (visited on 02/04/2025).

Farrugia, Mark and Byron Baron (2017). "The Role of Toll-Like Receptors in Autoimmune Diseases through Failure of the Self-Recognition Mechanism". In: *International Journal of Inflammation* 2017.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2017/8391230, p. 8391230. ISSN: 2042-0099. DOI: 10.1155/2017/8391230. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2017/8391230 (visited on 03/31/2025).

Feng, Hui *et al.* (Jan. 21, 2021). "Interferon regulatory factor 1 (IRF1) and anti-pathogen innate immune responses". In: *PLoS Pathogens* 17.1, e1009220. ISSN: 1553-7366. DOI: 10.1371/journal.ppat.1009220. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7819612/ (visited on 03/24/2025).

Fernandes, Paula *et al.* (May 16, 2019). "Physical Exercise Induces Immunoregulation of TREG, M2, and pDCs in a Lung Allergic Inflammation Model". In: *Frontiers in Immunology* 10. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2019.00854. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2019.00854/full (visited on 03/30/2025).

Fernandez, David and Kyriakos A. Kirou (Mar. 7, 2016). "What Causes Lupus Flares?" In: *Current Rheumatology Reports* 18.3, p. 14. ISSN: 1534-6307. DOI: 10.1007/s11926-016-0562-3. URL: https://doi.org/10.1007/s11926-016-0562-3 (visited on 04/02/2025).

Filaci, Gilberto *et al.* (May 15, 2001). "Impairment of CD8+ T Suppressor Cell Function in Patients with Active Systemic Lupus Erythematosus1". In: *The Journal of Immunology* 166.10, pp. 6452–6457. ISSN: 0022-1767. DOI: 10.4049/jimmunol.166.10.6452. URL: https://doi.org/10.4049/jimmunol.166.10.6452 (visited on 03/11/2025).

Finsen, Niels R and H Forchhammer (1904). "Resultate der Lichtbehandlung bei unseren ersten 800 F{\"a}llen von Lupus vulgaris.[Results of light therapy in our first 800 cases of lupus vulgaris]". In: *Mitt Fins Med Lichtinst* 5.6, pp. 1–48.

Fisher, Michael S. and Margaret L. Kripke (Apr. 1, 1977). "Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis." In: *Proceedings of the National Academy of Science* 74. ADS Bibcode: 1977PNAS...74.1688F, pp. 1688–1692. ISSN: 0027-8424. DOI: 10.1073/pnas.74.4.1688. URL: https://ui.adsabs.harvard.edu/abs/1977PNAS...74.1688F (visited on 03/23/2025).

Fournel, Sylvie *et al.* (July 15, 2003). "CD4+ T Cells from (New Zealand Black × New Zealand White)F1 Lupus Mice and Normal Mice Immunized Against Apoptotic Nucleosomes Recognize Similar Th Cell Epitopes in the C Terminus of Histone H31". In: *The Journal of Immunology* 171.2, pp. 636–644. ISSN: 0022-1767. DOI: 10.4049/jimmunol.171.2.636. URL: https://doi.org/10.4049/jimmunol.171.2.636 (visited on 03/03/2025).

Frangou, Eleni A., George K. Bertsias, and Dimitrios T. Boumpas (2013). "Gene expression and regulation in systemic lupus erythematosus". In: *European Journal of Clinical Investigation* 43.10. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/eci.12130, pp. 1084–1096. ISSN: 1365-2362. DOI: 10.1111/eci.12130. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/eci.12130 (visited on 02/27/2025).

Frostegård, Johan (2023). "Systemic lupus erythematosus and cardiovascular disease". In: *Journal of Internal Medicine* 293.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/joim.13557, pp. 48–62. ISSN: 1365-2796. DOI: 10.1111/joim.13557. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/joim.13557 (visited on 04/17/2025).

Al-Gahtani, Sarah N. (Sept. 16, 2021). "A Review of Systemic Lupus Erythematosus (SLE): Symptoms, Risk Factors, Treatment, and Health Related Quality of Life Issues". In: *Open Journal of Rheumatology and Autoimmune Diseases* 11.4. Number: 4 Publisher: Scientific Research Publishing, pp. 115–143. DOI: 10.4236/ojra.2021.114014. URL: https://www.scirp.org/journal/paperinformation?paperid=111950 (visited on 03/29/2025).

Galan-Caridad, Jose M. *et al.* (Apr. 20, 2007). "Zfx controls the self-renewal of embryonic and hematopoietic stem cells". In: *Cell* 129.2, pp. 345–357. ISSN: 0092-8674. DOI: 10.1016/j.cell.2007.03.014. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1899089/ (visited on 03/24/2025).

Gambelunghe, G. *et al.* (Mar. 1, 2005). "Contribution of MHC class I chain-related A (MICA) gene polymorphism to genetic susceptibility for systemic lupus erythematosus". In: *Rheumatology* 44.3, pp. 287–292. ISSN: 1462-0324. DOI: 10.1093/rheumatology/keh459. URL: https://doi.org/10.1093/rheumatology/keh459 (visited on 04/16/2025).

Gardi, Concetta *et al.* (Jan. 1, 2022). "A short Mindfulness retreat can improve biological markers of stress and inflammation". In: *Psychoneuroendocrinology* 135, p. 105579. ISSN: 0306-4530. DOI: 10.1016/j.psyneuen.2021.105579. URL: https://www.sciencedirect.com/science/article/pii/S0306453021004534 (visited on 03/30/2025).

Gavin, Marc A. *et al.* (Feb. 2007). "Foxp3-dependent programme of regulatory T-cell differentiation". In: *Nature* 445.7129. Publisher: Nature Publishing Group, pp. 771–775. ISSN: 1476-4687. DOI: 10.1038/nature05543. URL: https://www.nature.com/articles/nature05543 (visited on 03/19/2025).

Ge, Ge *et al.* (Aug. 1, 2023). "Induced skin aging by blue-light irradiation in human skin fibroblasts *via* TGF-β, JNK and EGFR pathways". In: *Journal of Dermatological Science* 111.2, pp. 52–59. ISSN: 0923-1811. DOI: 10.1016/j.jdermsci.2023.06.007. URL: https://www.sciencedirect.com/science/article/pii/S0923181123001500 (visited on 04/12/2025).

Gensous, Noémie *et al.* (July 17, 2018). "T Follicular Helper Cells in Autoimmune Disorders". In: *Frontiers in Immunology* 9. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2018.01637. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2018.01637/full (visited on 03/31/2025).

Gerdes, J *et al.* (Oct. 1, 1984). "Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67." In: *The Journal of Immunology* 133.4, pp. 1710–1715. ISSN: 0022-1767. DOI: 10.4049/jimmunol.133.4.1710. URL: https://doi.org/10.4049/jimmunol.133.4.1710 (visited on 03/20/2025).

Getnet, Derese *et al.* (Apr. 1, 2010). "A role for the transcription factor Helios in human CD4+CD25+ regulatory T cells". In: *Molecular Immunology* 47.7, pp. 1595–1600. ISSN: 0161-5890. DOI: 10.1016/j.molimm.2010.02.001. URL: https://www.sciencedirect.com/science/article/pii/S0161589010000398 (visited on 03/20/2025).

Ghaseminejad-Raeini, Amirhossein *et al.* (July 14, 2023). "Immunomodulatory actions of vitamin D in various immune-related disorders: a comprehensive review". In: *Frontiers in Immunology* 14. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2023.950465. URL: https://www.frontiersin.orghttps://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2023.950465/full (visited on 04/16/2025).

Ghodke-Puranik, Yogita, Mikhail Olferiev, and Mary K. Crow (Oct. 2024). "Systemic lupus erythematosus genetics: insights into pathogenesis and implications for therapy". In: *Nature Reviews Rheumatology* 20.10. Publisher: Nature Publishing Group, pp. 635–648. ISSN: 1759-4804. DOI: 10.1038/s41584-024-01152-2. URL: https://www.nature.com/articles/s41584-024-01152-2 (visited on 01/28/2025).

Ghoreishi, Mehran and Jan P. Dutz (May 1, 2009). "Murine models of cutaneous involvement in lupus erythematosus". In: *Autoimmunity Reviews*. Special Issue on The 2nd International Conference on Cutaneous Lupus Erythematosus 8.6, pp. 484–487. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2009.02.028. URL: https://www.sciencedirect.com/science/article/pii/S1568997209000627 (visited on 05/04/2025).

Gies, Vincent *et al.* (July 2, 2020). "Beyond Anti-viral Effects of Chloroquine/Hydroxychloroquine". In: *Frontiers in Immunology* 11, p. 1409. ISSN: 1664-3224. DOI: 10.3389/fimmu.2020.01409. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7343769/ (visited on 04/16/2025).

Gigon, Lea *et al.* (2023). "Eosinophils from A to Z". In: *Allergy* 78.7. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/all.15751, pp. 1810–1846. ISSN: 1398-9995. DOI: 10.1111/all.15751. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/all.15751 (visited on 03/20/2025).

Gladman, Dafna D. *et al.* (Sept. 2003). "Accrual of organ damage over time in patients with systemic lupus erythematosus". In: *The Journal of Rheumatology* 30.9, pp. 1955–1959. ISSN: 0315-162X.

Gleeson, Michael (Aug. 2007). "Immune function in sport and exercise". In: *Journal of Applied Physiology* 103.2. Publisher: American Physiological Society, pp. 693–699. ISSN: 8750-7587. DOI: 10.1152/japplphysiol.00008.2007. URL: https://journals.physiology.org/doi/full/10.1152/japplphysiol.00008.2007 (visited on 02/06/2025).

Gleeson, Michael, Brian McFarlin, and Michael Flynn (2006). "Exercise and Toll-like receptors". In: *Exercise Immunology Review* 12, pp. 34–53. ISSN: 1077-5552.

Golding, Amit *et al.* (Nov. 2013). "The Percentage of FoxP3+Helios+ Treg Cells Correlates Positively With Disease Activity in Systemic Lupus Erythematosus". In: *Arthritis and Rheumatism* 65.11, pp. 2898–2906. ISSN: 0004-3591. DOI: 10.1002/art.38119. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3891045/ (visited on 03/20/2025).

Gordon, C. *et al.* (Mar. 1, 1996). "Active Systemic Lupus Erythematosus is Associated with the Recruitment of Naive/Resting T cells". In: *Rheumatology* 35.3, pp. 226–230. ISSN: 1462-0324. DOI: 10.1093/rheumatology/35.3.226. URL: https://doi.org/10.1093/rheumatology/35.3.226 (visited on 03/17/2025).

Gottschalk, Rachel A., Emily Corse, and James P. Allison (Feb. 1, 2012). "Expression of Helios in Peripherally Induced Foxp3+ Regulatory T Cells". In: *The Journal of Immunology* 188.3, pp. 976–980. ISSN: 0022-1767. DOI: 10.4049/jimmunol.1102964. URL: https://doi.org/10.4049/jimmunol.1102964 (visited on 03/20/2025).

Graham, Andrea L. (Feb. 2021). "Naturalizing mouse models for immunology". In: *Nature Immunology* 22.2. Publisher: Nature Publishing Group, pp. 111–117. ISSN: 1529-2916. DOI: 10.1038/s41590-020-00857-2. URL: https://www.nature.com/articles/s41590-020-00857-2 (visited on 04/01/2025).

Grandori, Carla *et al.* (Mar. 2005). "c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I". In: *Nature Cell Biology* 7.3, pp. 311–318. ISSN: 1465-7392. DOI: 10.1038/ncb1224.

Green, Ralph and Ananya Datta Mitra (Mar. 1, 2017). "Megaloblastic Anemias: Nutritional and Other Causes". In: *Medical Clinics* 101.2. Publisher: Elsevier, pp. 297–317. ISSN: 0025-7125, 1557-9859. DOI: 10.1016/j.mcna.2016.09.013. URL: https://www.medical.theclinics.com/article/S0025-7125(16)37364-3/abstract (visited on 03/23/2025).

Gu, Jian *et al.* (June 2017). "Human CD39hi regulatory T cells present stronger stability and function under inflammatory conditions". In: *Cellular & Molecular Immunology* 14.6. Publisher: Nature Publishing Group, pp. 521–528. ISSN: 2042-0226. DOI: 10.1038/cmi.2016.30. URL: https://www.nature.com/articles/cmi201630 (visited on 03/20/2025).

Guilpain, Philippe *et al.* (Nov. 23, 2011). "The expression of CXCR4/CXCL12 determines subsets of patients in systemic lupus erythematosus". In: *Journal of Translational Medicine* 9 (Suppl 2), P60. ISSN: 1479-5876. DOI: 10.1186/1479-5876-9-S2-P60. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3242291/ (visited on 05/04/2025).

Hamad, Hussein and Ankit Mangla (July 17, 2023). "Lymphocytosis". In: *StatPearls [Internet]*. URL: https://www.ncbi.nlm.nih.gov/sites/books/NBK549819/ (visited on 03/20/2025).

Hamilton, Jennie A., Hui-Chen Hsu, and John D. Mountz (2019). "Autoreactive B cells in SLE, villains or innocent bystanders?" In: *Immunological Reviews* 292.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/imr.12815, pp. 120–138. ISSN: 1600-065X. DOI: 10.1111/imr.12815. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/imr.12815 (visited on 03/31/2025).

Hammond, Edward R *et al.* (Sept. 23, 2021). "Frequency, severity and costs of flares increase with disease severity in newly diagnosed systemic lupus erythematosus: a real-world cohort study, United States, 2004–2015". In: *Lupus Science & Medicine* 8.1, e000504. ISSN: 2053-8790. DOI: 10.1136/lupus-2021-000504. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8461688/ (visited on 03/20/2025).

Han, Bobby Kwanghoon *et al.* (May 21, 2020). "Neutrophil and lymphocyte counts are associated with different immunopathological mechanisms in systemic lupus erythematosus". In: *Lupus Science & Medicine* 7.1. Publisher: Lupus Foundation of America. ISSN: 2053-8790. DOI: 10.1136/lupus-2020-000382. URL: https://lupus.bmj.com/content/7/1/e000382 (visited on 02/27/2025).

Han, Le and Lingling Zhang (Aug. 1, 2023). "CCL21/CCR7 axis as a therapeutic target for autoimmune diseases". In: *International Immunopharmacology* 121, p. 110431. ISSN: 1567-5769. DOI: 10.1016/j.intimp.2023.110431. URL: https://www.sciencedirect.com/science/article/pii/S1567576923007543 (visited on 03/18/2025).

Hand, L. E. *et al.* (Apr. 3, 2020). "Regulatory T cells confer a circadian signature on inflammatory arthritis". In: *Nature Communications* 11.1. Publisher: Nature Publishing Group, p. 1658. ISSN: 2041-1723. DOI: 10.1038/s41467-020-15525-0. URL: https://www.nature.com/articles/s41467-020-15525-0 (visited on 05/04/2025).

Hao, He *et al.* (2021). "Conversion of T Follicular Helper Cells to T Follicular Regulatory Cells by Interleukin-2 Through Transcriptional Regulation in Systemic Lupus Erythematosus". In: *Arthritis & Rheumatology* 73.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.41457, pp. 132–142. ISSN: 2326-5205. DOI:

10.1002/art.41457. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.41457 (visited on 03/18/2025).

Harrell, Maria. I., Brian M. Iritani, and Alanna Ruddell (Mar. 20, 2008). "Lymph Node Mapping in the Mouse". In: *Journal of Immunological Methods* 332.1, pp. 170–174. ISSN: 0022-1759. DOI: 10.1016/j.jim.2007.11.012. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2342937/ (visited on 05/03/2025).

Hasan, Bilal, Alice Fike, and Sarfaraz Hasni (2022). "Health disparities in systemic lupus erythematosus—a narrative review". In: *Clinical Rheumatology* 41.11, pp. 3299–3311. ISSN: 0770-3198. DOI: 10.1007/s10067-022-06268-y. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9340727/ (visited on 03/25/2025).

Haynes, Nicole M. *et al.* (Oct. 15, 2007). "Role of CXCR5 and CCR7 in Follicular Th Cell Positioning and Appearance of a Programmed Cell Death Gene-1High Germinal Center-Associated Subpopulation1". In: *The Journal of Immunology* 179.8, pp. 5099–5108. ISSN: 0022-1767. DOI: 10.4049/jimmunol.179.8.5099. URL: https://doi.org/10.4049/jimmunol.179.8.5099 (visited on 03/18/2025).

Hayter, Scott M. and Matthew C. Cook (Aug. 2012). "Updated assessment of the prevalence, spectrum and case definition of autoimmune disease". In: *Autoimmunity Reviews* 11.10, pp. 754–765. ISSN: 1873-0183. DOI: 10.1016/j.autrev.2012.02.001.

Healy, Kelly L., Andrew R. Morris, and Andrew C. Liu (Oct. 2021). "Circadian Synchrony: Sleep, Nutrition, and Physical Activity". In: *Frontiers in Network Physiology* 1, p. 732243. ISSN: 2674-0109. DOI: 10.3389/fnetp.2021.732243.

Hedrich, Hans (Aug. 21, 2004). "The Laboratory Mouse". In: *Academic Press*. Google-Books-ID: J4Ip20dvFEsC.

Hedström, Anna Karin *et al.* (Apr. 2020). "Low sun exposure increases multiple sclerosis risk both directly and indirectly". In: *Journal of Neurology* 267.4, pp. 1045–1052. ISSN: 1432-1459. DOI: 10.1007/s00415-019-09677-3.

Hegedűs, Csaba *et al.* (Jan. 1, 2021). "Cyclobutane pyrimidine dimers from UVB exposure induce a hypermetabolic state in keratinocytes via mitochondrial oxidative stress". In: *Redox Biology* 38, p. 101808. ISSN: 2213-2317. DOI: 10.1016/j.redox.2020.101808. URL: https://www.sciencedirect.com/science/article/pii/S2213231720310132 (visited on 03/24/2025).

Heinonen, Ilkka *et al.* (Sept. 2011). "Local heating, but not indirect whole body heating, increases human skeletal muscle blood flow". In: *Journal of Applied Physiology* 111.3, pp. 818–824. ISSN:

8750-7587. DOI: 10.1152/japplphysiol.00269.2011. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3174790/ (visited on 04/02/2025).

Heredia, Fátima Pérez de, Sonia Gómez-Martínez, and Ascensión Marcos (May 2012). "Obesity, inflammation and the immune system". In: *Proceedings of the Nutrition Society* 71.2, pp. 332–338. ISSN: 1475-2719, 0029-6651. DOI: 10.1017/S0029665112000092. URL: https://www.cambridge.org/core/journals/proceedings-of-the-nutrition-society/article/obesity-inflammation-and-the-immune-system/BBA951027B413AEE76E3DA11A81173F1 (visited on 03/03/2025).

Hill, E. E. *et al.* (July 1, 2008). "Exercise and circulating Cortisol levels: The intensity threshold effect". In: *Journal of Endocrinological Investigation* 31.7, pp. 587–591. ISSN: 1720-8386. DOI: 10.1007/BF03345606. URL: https://doi.org/10.1007/BF03345606 (visited on 02/06/2025).

Hochberg, M C (Sept. 1, 1997). "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus". In: *Arthritis and Rheumatism* 40.9, p. 1725. ISSN: 1529-0131. DOI: 10.1002/art.1780400928. URL: https://doi.org/10.1002/art.1780400928 (visited on 03/02/2025).

Hönigsmann, Herbert (Jan. 1, 2013). "History of phototherapy in dermatology". In: *Photochemical & Photobiological Sciences* 12.1, pp. 16–21. ISSN: 1474-9092. DOI: 10.1039/c2pp25120e. URL: https://doi.org/10.1039/c2pp25120e (visited on 03/22/2025).

Horwitz, David A. (Jan. 20, 2010). "Identity of mysterious CD4+CD25-Foxp3+cells in systemic lupus erythematosus". In: *Arthritis Research & Therapy* 12.1, p. 101. ISSN: 1478-6354. DOI: 10.1186/ar2894. URL: https://doi.org/10.1186/ar2894 (visited on 05/03/2025).

Horwitz, David A. *et al.* (2008). "Critical role of IL-2 and TGF-β in generation, function and stabilization of Foxp3+CD4+ Treg". In: *European Journal of Immunology* 38.4. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eji.200738109, pp. 912–915. ISSN: 1521-4141. DOI: 10.1002/eji.200738109. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.200738109 (visited on 05/03/2025).

Huang, Kuo-Feng *et al.* (2017). "Ultraviolet B irradiation increases keratin 1 and keratin 10 expressions in HaCaT keratinocytes via TRPV1 activation and ERK phosphorylation". In: *Experimental Dermatology* 26.9. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/exd.13292, pp. 832–835. ISSN: 1600-0625. DOI: 10.1111/exd.13292. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/exd.13292 (visited on 04/15/2025).

Huang, Lu and Judith A. Appleton (Oct. 2016). "Eosinophils in helminth infection: defenders and dupes". In: *Trends in Parasitology* 32.10, pp. 798–807. ISSN: 1471-4922. DOI: 10.1016/j.pt.2016.05.004. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5048491/ (visited on 03/20/2025).

Huang, Sai-Wei *et al.* (Oct. 23, 2024). "Systemic longitudinal immune profiling identifies proliferating Treg cells as predictors of immunotherapy benefit: biomarker analysis from the phase 3 CONTINUUM and DIPPER trials". In: *Signal Transduction and Targeted Therapy* 9.1. Publisher: Nature Publishing Group, pp. 1–13. ISSN: 2059-3635. DOI: 10.1038/s41392-024-01988-w. URL: https://www.nature.com/articles/s41392-024-01988-w (visited on 03/20/2025).

Huang, Xinfang *et al.* (Oct. 12, 2015). "Predominant Role of Plasmacytoid Dendritic Cells in Stimulating Systemic Autoimmunity". In: *Frontiers in Immunology* 6. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2015.00526. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2015.00526/full (visited on 03/31/2025).

Huetz, François *et al.* (Dec. 1, 1988). "Autoimmunity: the moving boundaries between physiology and pathology". In: *Journal of Autoimmunity*. Proceedings of the First International Meeting on Immunointervention in Autoimmune Diseases 1.6, pp. 507–518. ISSN: 0896-8411. DOI: 10.1016/0896-8411(88)90044-3. URL: https://www.sciencedirect.com/science/article/pii/0896841188900443 (visited on 04/16/2025).

Hugenroth, Gundula (Apr. 16, 2020). "Universität Kobe und Ushio Inc.: 222 nm UV-C nicht krebserregend". In: *Ushio Europe B.V.* URL: https://www.ushio.eu/de/222nm-uni-kobe-ushio/ (visited on 11/01/2024).

Humrich, J.Y., T. Kamradt, and G. Riemekasten (Feb. 1, 2015). "Regulatorische T-Zellen und rheumatische Erkrankungen". In: *Zeitschrift für Rheumatologie* 74.1, pp. 26–32. ISSN: 1435-1250. DOI: 10.1007/s00393-014-1446-4. URL: https://doi.org/10.1007/s00393-014-1446-4 (visited on 03/03/2025).

Humrich, Jens Y. and G. Riemekasten (Dec. 2016). "The rise of IL-2 therapy — a novel biologic treatment for SLE". In: *Nature Reviews Rheumatology* 12.12. Publisher: Nature Publishing Group, pp. 695–696. ISSN: 1759-4804. DOI: 10.1038/nrrheum.2016.173. URL: https://www.nature.com/articles/nrrheum.2016.173 (visited on 03/18/2025).

Humrich, Jens Y., Caroline von Spee-Mayer, *et al.* (Sept. 1, 2019). "Low-dose interleukin-2 therapy in refractory systemic lupus erythematosus: an investigator-initiated, single-centre phase 1 and 2a clinical trial". In: *The Lancet Rheumatology* 1.1. Publisher: Elsevier, e44–e54. ISSN:

2665-9913. DOI: 10.1016/S2665-9913(19)30018-9. URL: https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(19)30018-9/abstract (visited on 03/18/2025).

Huret, Christophe *et al.* (May 3, 2024). "Altered X-chromosome inactivation predisposes to autoimmunity". In: *Science Advances* 10.18. Publisher: American Association for the Advancement of Science, eadn6537. DOI: 10.1126/sciadv.adn6537. URL: https://www.science.org/doi/10.1126/sciadv.adn6537 (visited on 03/22/2025).

Ichihashi, M. *et al.* (July 15, 2003). "UV-induced skin damage". In: *Toxicology*. Environmental and Nutritional Interactions Antioxidant Nutrients and Environmental Health, Part C 189.1, pp. 21–39. ISSN: 0300-483X. DOI: 10.1016/S0300-483X(03)00150-1. URL: https://www.sciencedirect.com/science/article/pii/S0300483X03001501 (visited on 04/16/2025).

Igawa, K., T. Matsunaga, and K. Nishioka (Mar. 2004). "Involvement of UV-irradiation in pemphigus foliaceus". In: *Journal of the European Academy of Dermatology and Venereology* 18.2, pp. 216–217. ISSN: 0926-9959. DOI: 10.1111/j.1468-3083.2004.00900.x.

Jackson, Shaun W and Anne Davidson (2019). "BAFF inhibition in SLE—Is tolerance restored?" In: *Immunological Reviews* 292.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/imr.12810, pp. 102–119. ISSN: 1600-065X. DOI: 10.1111/imr.12810. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/imr.12810 (visited on 03/28/2025).

Jacobi, Annett M. *et al.* (Feb. 6, 2009). "Phenotypic Characterization of Autoreactive B Cells—Checkpoints of B Cell Tolerance in Patients with Systemic Lupus Erythematosus". In: *PloS One* 4.6. Publisher: Public Library of Science, e5776. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0005776. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0005776 (visited on 03/31/2025).

Jappe, U. *et al.* (Mar. 2000). "Epidermolysis bullosa acquisita with ultraviolet radiationsensitivity". In: *The British Journal of Dermatology* 142.3, pp. 517–520. ISSN: 0007-0963. DOI: 10.1046/j.1365-2133.2000.03368.x.

Jasin, Hugo E. (Sept. 1, 1977). "Absence of the Eighth Component of Complement in Association with Systemic Lupus Erythematosus-Like Disease". In: *The Journal of Clinical Investigation* 60.3. Publisher: American Society for Clinical Investigation, pp. 709–715. ISSN: 0021-9738. DOI: 10.1172/JCI108823. URL: https://www.jci.org/articles/view/108823 (visited on 04/06/2025).

Jeon, Sang Won and Yong-Ku Kim (Dec. 15, 2017). "Inflammation-induced depression: Its pathophysiology and therapeutic implications". In: *Journal of Neuroimmunology* 313, pp. 92–98. ISSN: 0165-5728. DOI: 10.1016/j.jneuroim.2017.10.016. URL: https://www.sciencedirect.com/science/article/pii/S0165572817303119 (visited on 03/30/2025).

JohnsHopkinsUniversity (2025). *Classification of Autoimmune Diseases - Autoimmune Disease | Johns Hopkins Pathology*. Johns Hopkins Medicine Pathology. URL: https://pathology.jhu.edu/autoimmune/classification (visited on 04/17/2025).

Johnson, Pauline and Brian Ruffell (July 1, 2009). "CD44 and its Role in Inflammation and Inflammatory Diseases". In: *Inflammation & Allergy - Drug Targets (Formerly Current Drug Targets - Inflammation & Allergy)* 8.3, pp. 208–220. DOI: 10.2174/187152809788680994.

Johnston, Robert J. *et al.* (Feb. 13, 2012). "STAT5 is a potent negative regulator of TFH cell differentiation". In: *The Journal of Experimental Medicine* 209.2, pp. 243–250. ISSN: 1540-9538. DOI: 10.1084/jem.20111174.

Jonny *et al.* (Sept. 13, 2023). "Significant improvement of systemic lupus erythematosus manifestation in children after autologous dendritic cell transfer: a case report and review of literature". In: *Therapeutic Advances in Vaccines and Immunotherapy* 11, p. 25151355231186005. ISSN: 2515-1355. DOI: 10.1177/25151355231186005. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10501061/ (visited on 04/30/2025).

Joost, Simon *et al.* (Mar. 5, 2020). "The Molecular Anatomy of Mouse Skin during Hair Growth and Rest". In: *Cell Stem Cell* 26.3, 441–457.e7. ISSN: 1934-5909. DOI: 10.1016/j.stem.2020.01.012. URL: https://www.sciencedirect.com/science/article/pii/S1934590920300126 (visited on 11/28/2024).

Juzeniene, Asta and Johan Moan (Apr. 1, 2012). "Beneficial effects of UV radiation other than via vitamin D production". In: *Dermato-Endocrinology* 4.2, pp. 109–117. ISSN: 1938-1972. DOI: 10.4161/derm.20013. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3427189/ (visited on 03/22/2025).

Kamil Alhassbalawi, Nibras *et al.* (July 2023). "Circulating miR-21 Overexpression Correlates with PDCD4 and IL-10 in Systemic Lupus Erythematosus (SLE): A Promising Diagnostic and Prognostic Biomarker". In: *Reports of Biochemistry & Molecular Biology* 12.2, pp. 220–232. ISSN: 2322-3480. DOI: 10.61186/rbmb.12.2.220. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10838585/ (visited on 04/06/2025).

Kano, Y. *et al.* (2000). "Pemphigus foliaceus induced by exposure to sunlight. Report of a case and analysis of photochallenge-induced lesions". In: *Dermatology (Basel, Switzerland)* 201.2, pp. 132–138. ISSN: 1018-8665. DOI: 10.1159/000018456.

Kapellos, Theodore S. *et al.* (Aug. 30, 2019). "Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases". In: *Frontiers in Immunology* 10. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2019.02035. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2019.02035/full (visited on 03/20/2025).

Kaplan, Mariana J (Sept. 1, 2004). "Apoptosis in systemic lupus erythematosus". In: *Clinical Immunology* 112.3, pp. 210–218. ISSN: 1521-6616. DOI: 10.1016/j.clim.2004.04.007. URL: https://www.sciencedirect.com/science/article/pii/S1521661604001408 (visited on 03/03/2025).

Katrinli, Seyma *et al.* (Aug. 4, 2022). "The role of the immune system in posttraumatic stress disorder". In: *Translational Psychiatry* 12.1. Publisher: Nature Publishing Group, pp. 1–14. ISSN: 2158-3188. DOI: 10.1038/s41398-022-02094-7. URL: https://www.nature.com/articles/s41398-022-02094-7 (visited on 03/30/2025).

Katsiari, Christina G., Stamatis-Nick C. Liossis, and Petros P. Sfikakis (June 1, 2010). "The Pathophysiologic Role of Monocytes and Macrophages in Systemic Lupus Erythematosus: A Reappraisal". In: *Seminars in Arthritis and Rheumatism* 39.6, pp. 491–503. ISSN: 0049-0172. DOI: 10.1016/j.semarthrit.2008.11.002. URL: https://www.sciencedirect.com/science/article/pii/S0049017208002011 (visited on 03/31/2025).

Katz, Patricia *et al.* (Mar. 2020). "Patient-Reported Lupus Flare Symptoms Are Associated with Worsened Patient Outcomes and Increased Economic Burden". In: *Journal of Managed Care & Specialty Pharmacy* 26.3. Publisher: Academy of Managed Care Pharmacy, pp. 275–283. ISSN: 2376-0540. DOI: 10.18553/jmcp.2020.26.3.275. URL: https://www.jmcp.org/doi/10.18553/jmcp.2020.26.3.275 (visited on 03/20/2025).

Khadra, Mariam, Tasneem S. Drie, and Maysoun Kudsi (Oct. 20, 2023). "The mean platelet volume (MPV) in patients with systemic lupus erythematosus (SLE) and its correlation with disease activity: a cross-sectional/case–control study". In: *Annals of Medicine and Surgery* 85.12, pp. 5919–5925. ISSN: 2049-0801. DOI: 10.1097/MS9.0000000000001432. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10718361/ (visited on 04/30/2025).

Khan, Deena and S. Ansar Ahmed (Jan. 6, 2016). "The Immune System Is a Natural Target for Estrogen Action: Opposing Effects of Estrogen in Two Prototypical Autoimmune Diseases". In: *Frontiers in Immunology* 6. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2015.00635.

URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2015.00635/full (visited on 01/22/2025).

Khantakova, Julia N., Aleksey S. Bulygin, and Sergey V. Sennikov (Jan. 2022). "The Regulatory-T-Cell Memory Phenotype: What We Know". In: *Cells* 11.10. Number: 10 Publisher: Multidisciplinary Digital Publishing Institute, p. 1687. ISSN: 2073-4409. DOI: 10.3390/cells11101687. URL: https://www.mdpi.com/2073-4409/11/10/1687 (visited on 03/19/2025).

Kim, Hye-Jung *et al.* (Oct. 16, 2015). "Stable inhibitory activity of regulatory T cells requires the transcription factor Helios". In: *Science* 350.6258. Publisher: American Association for the Advancement of Science, pp. 334–339. DOI: 10.1126/science.aad0616. URL: https://www.science.org/doi/full/10.1126/science.aad0616 (visited on 03/20/2025).

Kim, Joseph *et al.* (Mar. 2, 2012). "Identification of Anti-Malarial Compounds as Novel Antagonists to Chemokine Receptor CXCR4 in Pancreatic Cancer Cells". In: *PloS One* 7.2. Publisher: Public Library of Science, e31004. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0031004. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0031004 (visited on 04/17/2025).

Kim, Sang-in, Seung-Gi Jin, and Gerd P. Pfeifer (Aug. 2013). "Formation of cyclobutane pyrimidine dimers at dipyrimidines containing 5-hydroxymethylcytosine". In: *Photochemical & Photobiological Sciences* 12.8, pp. 1409–1415. ISSN: 1474-905X. DOI: 10.1039/c3pp50037c. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3731422/ (visited on 05/05/2025).

Kim, Ji-Won *et al.* (Aug. 11, 2022). "Sex hormones affect the pathogenesis and clinical characteristics of systemic lupus erythematosus". In: *Frontiers in Medicine* 9. Publisher: Frontiers. ISSN: 2296-858X. DOI: 10.3389/fmed.2022.906475. URL: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2022.906475/full (visited on 03/23/2025).

Kim, Yong Chan *et al.* (Mar. 22, 2012). "Oligodeoxynucleotides stabilize Helios-expressing Foxp3+ human T regulatory cells during in vitro expansion". In: *Blood* 119.12, pp. 2810–2818. ISSN: 0006-4971. DOI: 10.1182/blood-2011-09-377895. URL: https://doi.org/10.1182/blood-2011-09-377895 (visited on 03/20/2025).

Kingsmore, Kathryn M. *et al.* (July 20, 2021). "Altered expression of genes controlling metabolism characterizes the tissue response to immune injury in lupus". In: *Scientific Reports* 11.1. Publisher: Nature Publishing Group, p. 14789. ISSN: 2045-2322. DOI: 10.1038/s41598-021-

93034-w. URL: https://www.nature.com/articles/s41598-021-93034-w (visited on 03/24/2025).

Knezevic, Emilija *et al.* (Jan. 2023). "The Role of Cortisol in Chronic Stress, Neurodegenerative Diseases, and Psychological Disorders". In: *Cells* 12.23. Number: 23 Publisher: Multidisciplinary Digital Publishing Institute, p. 2726. ISSN: 2073-4409. DOI: 10.3390/cells12232726. URL: https://www.mdpi.com/2073-4409/12/23/2726 (visited on 03/30/2025).

Kobayashi, Naoki *et al.* (2004). "Down-regulation of CXCR4 expression on human CD8+ T cells during peripheral differentiation". In: *European Journal of Immunology* 34.12. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eji.200425587, pp. 3370–3378. ISSN: 1521-4141. DOI: 10.1002/eji.200425587. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.200425587 (visited on 05/04/2025).

Kohli, Karan, Venu G. Pillarisetty, and Teresa S. Kim (Jan. 2022). "Key chemokines direct migration of immune cells in solid tumors". In: *Cancer Gene Therapy* 29.1. Publisher: Nature Publishing Group, pp. 10–21. ISSN: 1476-5500. DOI: 10.1038/s41417-021-00303-x. URL: https://www.nature.com/articles/s41417-021-00303-x (visited on 03/23/2025).

Kosinski, M *et al.* (Apr. 1, 2013). "Qualitative validation of the FACIT-Fatigue scale in systemic lupus erythematosus". In: *Lupus* 22.5. Publisher: SAGE Publications Ltd STM, pp. 422–430. ISSN: 0961-2033. DOI: 10.1177/0961203313476360. URL: https://doi.org/10.1177/0961203313476360 (visited on 03/28/2025).

Krause, Diane (July 1, 2007). "Is There a Common Pathway for Maintenance of Adult and Embryonic Stem Cells?" In: *The Hematologist* 4.4. ISSN: 1551-8779. DOI: 10.1182/hem.V4.4.6056. URL: https://doi.org/10.1182/hem.V4.4.6056 (visited on 03/24/2025).

Kravitz, Lex and Bridget Matikainen-Ankney (2025). *RAD (Rodent Activity Detector)*. RAD (Rodent Activity Detector). URL: https://hackaday.io/project/160742-rad-rodent-activity-detector (visited on 04/17/2025).

Kröger, Andrea (Aug. 2017). "IRFs as competing pioneers in T-cell differentiation". In: *Cellular and Molecular Immunology* 14.8, pp. 649–651. ISSN: 1672-7681. DOI: 10.1038/cmi.2017.37. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5549608/ (visited on 03/24/2025).

Kuhn, Annegret, Jörg Wenzel, and Heiko Weyd (Oct. 1, 2014). "Photosensitivity, Apoptosis, and Cytokines in the Pathogenesis of Lupus Erythematosus: a Critical Review". In: *Clinical Reviews in Allergy & Immunology* 47.2, pp. 148–162. ISSN: 1559-0267. DOI: 10.1007/s12016-013-8403-x. URL: https://doi.org/10.1007/s12016-013-8403-x (visited on 03/31/2025).

Kulms, D., H. Düßmann, *et al.* (June 2002). "Apoptosis induced by disruption of the actin cytoskeleton is mediated via activation of CD95 (Fas/APO-1)". In: *Cell Death & Differentiation* 9.6. Publisher:

Nature Publishing Group, pp. 598–608. ISSN: 1476-5403. DOI: 10.1038/sj.cdd.4401002. URL: https://www.nature.com/articles/4401002 (visited on 03/26/2025).

Kulms, D., B. Pöppelmann, *et al.* (July 6, 1999). "Nuclear and cell membrane effects contribute independently to the induction of apoptosis in human cells exposed to UVB radiation". In: *Proceedings of the National Academy of Sciences of the United States of America* 96.14, pp. 7974–7979. ISSN: 0027-8424. DOI: 10.1073/pnas.96.14.7974.

Kundi, Zuzanna Maria *et al.* (2021). "Dietary Fiber from Oat and Rye Brans Ameliorate Western Diet–Induced Body Weight Gain and Hepatic Inflammation by the Modulation of Short-Chain Fatty Acids, Bile Acids, and Tryptophan Metabolism". In: *Molecular Nutrition & Food Research* 65.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/mnfr.201900580, p. 1900580. ISSN: 1613-4133. DOI: 10.1002/mnfr.201900580. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/mnfr.201900580 (visited on 03/29/2025).

Kunireddy, Neeraja *et al.* (Oct. 1, 2018). "Hepcidin and Ferritin: Important Mediators in Inflammation Associated Anemia in Systemic Lupus Erythematosus Patients". In: *Indian Journal of Clinical Biochemistry* 33.4, pp. 406–413. ISSN: 0974-0422. DOI: 10.1007/s12291-017-0702-1. URL: https://doi.org/10.1007/s12291-017-0702-1 (visited on 04/09/2025).

Kurz, Bernadett *et al.* (2023). "Phototherapy: Theory and practice". In: *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* 21.8. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ddg.15126, pp. 882–897. ISSN: 1610-0387. DOI: 10.1111/ddg.15126. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/ddg.15126 (visited on 03/22/2025).

La Cava, Antonio (Feb. 21, 2018). "Tregs in SLE: an Update". In: *Current Rheumatology Reports* 20.2, p. 6. ISSN: 1534-6307. DOI: 10.1007/s11926-018-0714-8. URL: https://doi.org/10.1007/s11926-018-0714-8 (visited on 03/31/2025).

Lai, Ning-Sheng *et al.* (Jan. 2021). "A Comparison of the Correlation of Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) and Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS) with Health-Related Quality of Life". In: *Journal of Clinical Medicine* 10.10. Number: 10 Publisher: Multidisciplinary Digital Publishing Institute, p. 2137. ISSN: 2077-0383. DOI: 10.3390/jcm10102137. URL: https://www.mdpi.com/2077-0383/10/10/2137 (visited on 02/04/2025).

Lancaster, Graeme I. *et al.* (Mar. 15, 2005). "The physiological regulation of toll-like receptor expression and function in humans". In: *The Journal of Physiology* 563 (Pt 3), pp. 945–955. ISSN: 0022-3751. DOI: 10.1113/jphysiol.2004.081224.

Lansdowne, Allen T. G. and S. C. Provost (Feb. 1, 1998). "Vitamin D3 enhances mood in healthy subjects during winter". In: *Psychopharmacology* 135.4, pp. 319–323. ISSN: 1432-2072. DOI: 10.1007/s002130050517. URL: https://doi.org/10.1007/s002130050517 (visited on 03/23/2025).

Laurence, Arian *et al.* (Mar. 23, 2007). "Interleukin-2 Signaling via STAT5 Constrains T Helper 17 Cell Generation". In: *Immunity* 26.3. Publisher: Elsevier, pp. 371–381. ISSN: 1074-7613. DOI: 10.1016/j.immuni.2007.02.009. URL: https://www.cell.com/immunity/abstract/S1074-7613(07)00176-8 (visited on 03/18/2025).

Law, Calvin *et al.* (July 10, 2024). "Interferon subverts an AHR–JUN axis to promote CXCL13+ T cells in lupus". In: *Nature*. Publisher: Nature Publishing Group, pp. 1–10. ISSN: 1476-4687. DOI: 10.1038/s41586-024-07627-2. URL: https://www.nature.com/articles/s41586-024-07627-2 (visited on 07/15/2024).

Lee, Chih-Hung *et al.* (Mar. 20, 2013). "Molecular Mechanisms of UV-Induced Apoptosis and Its Effects on Skin Residential Cells: The Implication in UV-Based Phototherapy". In: *International Journal of Molecular Sciences* 14.3, pp. 6414–6435. ISSN: 1422-0067. DOI: 10.3390/ijms14036414.

Lee, Jee H., Chuanwu Wang, and Chang H. Kim (Nov. 15, 2009). "FoxP3+ Regulatory T cells Restrain Splenic Extramedullary Myelopoiesis via Suppression of Hematopoietic Cytokine-Producing T Cells". In: *Journal of Immunology (Baltimore, Md. : 1950)* 183.10, pp. 6377–6386. ISSN: 0022-1767. DOI: 10.4049/jimmunol.0901268. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2792034/ (visited on 03/19/2025).

Lei, Hong *et al.* (Sept. 2, 2015). "Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners". In: *Frontiers in Pharmacology* 6. Publisher: Frontiers. ISSN: 1663-9812. DOI: 10.3389/fphar.2015.00184. URL: https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2015.00184/full (visited on 03/20/2025).

León Machado, Jorge Alfonso and Viktor Steimle (Jan. 22, 2021). "The MHC Class II Transactivator CIITA: Not (Quite) the Odd-One-Out Anymore among NLR Proteins". In: *International Journal of Molecular Sciences* 22.3, p. 1074. ISSN: 1422-0067. DOI: 10.3390/ijms22031074.

Lephart, Edwin D (2018). "A review of the role of estrogen in dermal aging and facial attractiveness in women". In: *Journal of Cosmetic Dermatology* 17.3. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/jocd.12508, pp. 282–288. ISSN: 1473-2165. DOI: 10.1111/jocd.12508. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/jocd.12508 (visited on 03/20/2025).

Lerret, N. M. *et al.* (Sept. 1, 2012). "Donor-Specific CD8+Foxp3+ T Cells Protect Skin Allografts and Facilitate Induction of Conventional CD4+Foxp3+ Regulatory T Cells". In: *American Journal of Transplantation* 12.9, pp. 2335–2347. ISSN: 1600-6135. DOI: 10.1111/j.1600-6143.2012.04120.x. URL: https://www.sciencedirect.com/science/article/pii/S1600613522276047 (visited on 03/11/2025).

Létourneau, Sven *et al.* (Apr. 1, 2009). "IL-2– and CD25-dependent immunoregulatory mechanisms in the homeostasis of T-cell subsets". In: *Journal of Allergy and Clinical Immunology* 123.4, pp. 758–762. ISSN: 0091-6749. DOI: 10.1016/j.jaci.2009.02.011. URL: https://www.sciencedirect.com/science/article/pii/S0091674909003352 (visited on 03/18/2025).

Leuchten, N *et al.* (Aug. 1, 2018). "Early symptoms of systemic lupus erythematosus (SLE) recalled by 339 SLE patients". In: *Lupus* 27.9. Publisher: SAGE Publications Ltd STM, pp. 1431–1436. ISSN: 0961-2033. DOI: 10.1177/0961203318776093. URL: https://doi.org/10.1177/0961203318776093 (visited on 03/28/2025).

Lewis, Philip *et al.* (Dec. 5, 2018). "Exercise time cues (zeitgebers) for human circadian systems can foster health and improve performance: a systematic review". In: *BMJ Open Sport & Exercise Medicine* 4.1. Publisher: BMJ Publishing Group Ltd. ISSN: 2055-7647. DOI: 10.1136/bmjsem-2018-000443. URL: https://bmjopensem.bmj.com/content/4/1/e000443 (visited on 03/30/2025).

Leyva-Castillo, Juan Manuel *et al.* (Aug. 2022). "Single cell transcriptome profile of mouse skin undergoing antigen driven allergic inflammation recapitulates findings in atopic dermatitis skin lesions". In: *The Journal of Allergy and Clinical Immunology* 150.2, pp. 373–384. ISSN: 0091-6749. DOI: 10.1016/j.jaci.2022.03.002. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9378429/ (visited on 05/05/2025).

Li, Haiyang *et al.* (Dec. 1, 2021). "Dual-Specificity Phosphatases and Kidney Diseases". In: *Kidney Diseases* 8.1, pp. 13–25. ISSN: 2296-9381. DOI: 10.1159/000520142. URL: https://doi.org/10.1159/000520142 (visited on 04/06/2025).

Li, Pin, Wei Lin, and Xiangxiong Zheng (June 1, 2014). "IL-33 Neutralization Suppresses Lupus Disease in Lupus-Prone Mice". In: *Inflammation* 37.3, pp. 824–832. ISSN: 1573-2576. DOI: 10.1007/s10753-013-9802-0. URL: https://doi.org/10.1007/s10753-013-9802-0 (visited on 04/06/2025).

Li, Qiutang and Inder M. Verma (Oct. 2002). "NF-χB regulation in the immune system". In: *Nature Reviews Immunology* 2.10. Publisher: Nature Publishing Group, pp. 725–734. ISSN: 1474-1741.

DOI: 10.1038/nri910. URL: https://www.nature.com/articles/nri910 (visited on 02/27/2025).

Li, Wenli *et al.* (Feb. 18, 2019). "The Regulatory T Cell in Active Systemic Lupus Erythematosus Patients: A Systemic Review and Meta-Analysis". In: *Frontiers in Immunology* 10, p. 159. ISSN: 1664-3224. DOI: 10.3389/fimmu.2019.00159. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6387904/ (visited on 05/03/2025).

Liang, Peifen *et al.* (May 1, 2015). "Basophil count, a marker for disease activity in systemic lupus erythematosus". In: *Clinical Rheumatology* 34.5, pp. 891–896. ISSN: 1434-9949. DOI: 10.1007/s10067-014-2822-9. URL: https://doi.org/10.1007/s10067-014-2822-9 (visited on 02/27/2025).

Lichtmann (Jan. 12, 2012). "Chapter 77 Monocytosis and Monocytopenia". In: *Free Medical Textbook*. URL: https://medtextfree.wordpress.com/2012/01/12/chapter-77-monocytosis-and-monocytopenia/ (visited on 03/20/2025).

Lin, J R *et al.* (Apr. 1, 2016). "Analysis of interleukin 19 serum levels and single nucleotide polymorphisms in systemic lupus erythematosus". In: *Genetics and Molecular Research* 15.2. ISSN: 1676-5680. DOI: 10.4238/gmr.15028007. URL: https://doi.org/10.4238/gmr.15028007 (visited on 04/06/2025).

Linge, Petrus *et al.* (Apr. 2018). "The non-haemostatic role of platelets in systemic lupus erythematosus". In: *Nature Reviews Rheumatology* 14.4. Publisher: Nature Publishing Group, pp. 195–213. ISSN: 1759-4804. DOI: 10.1038/nrrheum.2018.38. URL: https://www.nature.com/articles/nrrheum.2018.38 (visited on 03/22/2024).

Liossis, Stamatis Nick and Chrysanthi Staveri (Mar. 5, 2021). "What's New in the Treatment of Systemic Lupus Erythematosus". In: *Frontiers in Medicine* 8. Publisher: Frontiers. ISSN: 2296-858X. DOI: 10.3389/fmed.2021.655100. URL: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2021.655100/full (visited on 03/29/2025).

Lipson, S. F. and P. T. Ellison (Apr. 1992). "Normative study of age variation in salivary progesterone profiles". In: *Journal of Biosocial Science* 24.2, pp. 233–244. ISSN: 0021-9320. DOI: 10.1017/s0021932000019751.

Liu, Jianhua, Celine C. Berthier, and J. Michelle Kahlenberg (2017). "Enhanced Inflammasome Activity in Systemic Lupus Erythematosus Is Mediated via Type I Interferon–Induced Up-Regulation of Interferon Regulatory Factor 1". In: *Arthritis & Rheumatology* 69.9. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.40166, pp. 1840–1849. ISSN: 2326-5205.

DOI: 10.1002/art.40166. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.40166 (visited on 03/24/2025).

Liu, Mei *et al.* (Apr. 2019). "Type I interferons promote the survival and proinflammatory properties of transitional B cells in systemic lupus erythematosus patients". In: *Cellular & Molecular Immunology* 16.4, pp. 367–379. ISSN: 2042-0226. DOI: 10.1038/s41423-018-0010-6.

Liu, Wei *et al.* (Sept. 3, 2020). "Repeated Nitrous Oxide Exposure Exerts Antidepressant-Like Effects Through Neuronal Nitric Oxide Synthase Activation in the Medial Prefrontal Cortex". In: *Frontiers in Psychiatry* 11. Publisher: Frontiers. ISSN: 1664-0640. DOI: 10.3389/fpsyt.2020.00837. URL: https://www.frontiersin.org/journals/psychiatry/articles/10.3389/fpsyt.2020.00837/full (visited on 03/23/2025).

Liu, X. *et al.* (June 1, 2017). "OP0043 The number of circulating regulatory t cells is reduced and low-dose il-2 selectively stimulates its proliferation in patients with systemic lupus erythematosus". In: *Annals of the Rheumatic Diseases* 76 (Suppl 2). Publisher: BMJ Publishing Group Ltd Section: Oral Presentations, pp. 69–69. ISSN: 0003-4967, 1468-2060. DOI: 10.1136/annrheumdis-2017-eular.4221. URL: https://ard.bmj.com/content/76/Suppl_2/69.1 (visited on 03/19/2025).

Liu, Zheng, Yongrui Zou, and Anne Davidson (Mar. 2011). "Plasma cells in systemic lupus erythematosus: the long and short of it all". In: *European Journal of Immunology* 41.3, pp. 588–591. ISSN: 1521-4141. DOI: 10.1002/eji.201041354.

Lleo, Ana *et al.* (Mar. 1, 2010). "Definition of human autoimmunity — autoantibodies versus autoimmune disease". In: *Autoimmunity Reviews*. Special Issue on The Environment Geoepidemiology and Autoimmune Diseases 9.5, A259–A266. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2009.12.002. URL: https://www.sciencedirect.com/science/article/pii/S1568997209002018 (visited on 04/16/2025).

Loda, Agnese and Edith Heard (Sept. 19, 2019). "Xist RNA in action: Past, present, and future". In: *PLoS Genetics* 15.9, e1008333. ISSN: 1553-7390. DOI: 10.1371/journal.pgen.1008333. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6752956/ (visited on 03/29/2025).

Lood, Christian *et al.* (Mar. 1, 2017). "Decreased platelet size is associated with platelet activation and anti-phospholipid syndrome in systemic lupus erythematosus". In: *Rheumatology* 56.3, pp. 408–416. ISSN: 1462-0324. DOI: 10.1093/rheumatology/kew437. URL: https://doi.org/10.1093/rheumatology/kew437 (visited on 03/31/2025).

Love, Lori A. *et al.* (Aug. 2009). "Ultraviolet radiation intensity predicts the relative distribution of dermatomyositis and anti-Mi-2 autoantibodies in women". In: *Arthritis and Rheumatism* 60.8, pp. 2499–2504. ISSN: 0004-3591. DOI: 10.1002/art.24702.

Lu, Liuxin *et al.* (2024). "CXCR4/CXCL12 axis: "old" pathway as "novel" target for anti-inflammatory drug discovery". In: *Medicinal Research Reviews* 44.3. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/med.22011, pp. 1189–1220. ISSN: 1098-1128. DOI: 10.1002/med.22011. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/med.22011 (visited on 03/28/2025).

Lu, Rufei *et al.* (June 13, 2019). "Immunologic findings precede rapid lupus flare after transient steroid therapy". In: *Scientific Reports* 9.1. Publisher: Nature Publishing Group, p. 8590. ISSN: 2045-2322. DOI: 10.1038/s41598-019-45135-w. URL: https://www.nature.com/articles/s41598-019-45135-w (visited on 04/30/2025).

Luo, Weiguang *et al.* (Oct. 13, 2017). "Polymorphism of keratin 1 associates with systemic lupus erythematosus and systemic sclerosis in a south Chinese population". In: *PloS One* 12.10. Publisher: Public Library of Science, e0186409. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0186409. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0186409 (visited on 04/06/2025).

Mackay, Meggan (Dec. 1, 2015). "Lupus brain fog: a biologic perspective on cognitive impairment, depression, and fatigue in systemic lupus erythematosus". In: *Immunologic Research* 63.1, pp. 26–37. ISSN: 1559-0755. DOI: 10.1007/s12026-015-8716-3. URL: https://doi.org/10.1007/s12026-015-8716-3 (visited on 02/27/2025).

Mackintosh, John A. *et al.* (Dec. 22, 2021). "Interstitial pneumonia with autoimmune features: challenges and controversies". In: *European Respiratory Review* 30.162. Publisher: European Respiratory Society Section: Reviews. ISSN: 0905-9180, 1600-0617. DOI: 10.1183/16000617.0177-2021. URL: https://publications.ersnet.org/content/errev/30/162/210177 (visited on 01/21/2025).

Magni, Giada *et al.* (Oct. 2022). "Blue-LED-Light Photobiomodulation of Inflammatory Responses and New Tissue Formation in Mouse-Skin Wounds". In: *Life* 12.10. Number: 10 Publisher: Multidisciplinary Digital Publishing Institute, p. 1564. ISSN: 2075-1729. DOI: 10.3390/life12101564. URL: https://www.mdpi.com/2075-1729/12/10/1564 (visited on 04/12/2025).

Maidhof, William and Olga Hilas (Apr. 2012). "Lupus: An Overview of the Disease And Management Options". In: *Pharmacy and Therapeutics* 37.4, pp. 240–249. ISSN: 1052-1372. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3351863/ (visited on 03/29/2025).

Mak, Anselm and Nien Yee Kow (2014). "The Pathology of T Cells in Systemic Lupus Erythematosus". In: *Journal of Immunology Research* 2014.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2014/419029, p. 419029. ISSN: 2314-7156. DOI: 10.1155/2014/419029. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2014/419029 (visited on 03/11/2025).

Mak, Anselm and Sen Hee Tay (Sept. 2014). "Environmental Factors, Toxicants and Systemic Lupus Erythematosus". In: *International Journal of Molecular Sciences* 15.9. Number: 9 Publisher: Multidisciplinary Digital Publishing Institute, pp. 16043–16056. ISSN: 1422-0067. DOI: 10.3390/ijms150916043. URL: https://www.mdpi.com/1422-0067/15/9/16043 (visited on 11/05/2024).

Man, The Protein (2025). *LDH Cytotoxicity Assay FAQs*. G-Bioscience. URL: https://info.gbiosciences.com/blog/ldh-cytotoxicity-assay-faqs (visited on 04/17/2025).

Marshak-Rothstein, Ann (2006). "Toll-like receptors in systemic autoimmune disease". In: *Nature Reviews. Immunology* 6.11, pp. 823–835. ISSN: 1474-1733. DOI: 10.1038/nri1957. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7097510/ (visited on 04/16/2025).

Masopust, David, Christine P. Sivula, and Stephen C. Jameson (July 15, 2017). "Of Mice, Dirty Mice, and Men: Using Mice to Understand Human Immunology". In: *Journal of Immunology (Baltimore, Md. : 1950)* 199.2, pp. 383–388. ISSN: 0022-1767. DOI: 10.4049/jimmunol.1700453. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5512602/ (visited on 03/28/2025).

Mathenia, J *et al.* (Nov. 2010). "Impact of Fli-1 transcription factor on autoantibody and lupus nephritis in NZM2410 mice". In: *Clinical and Experimental Immunology* 162.2, pp. 362–371. ISSN: 0009-9104. DOI: 10.1111/j.1365-2249.2010.04245.x. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2996603/ (visited on 01/26/2025).

Matikainen-Ankney, Bridget A. *et al.* (July 2019). "Rodent Activity Detector (RAD), an Open Source Device for Measuring Activity in Rodent Home Cages". In: *eNeuro* 6.4, ENEURO.0160–19.2019. ISSN: 2373-2822. DOI: 10.1523/ENEURO.0160-19.2019. URL: https://www.eneuro.org/lookup/doi/10.1523/ENEURO.0160-19.2019 (visited on 10/24/2024).

Matsuda, J. *et al.* (1992). "Effect of total-body cold exposure on plasma concentrations of von Willebrand factor, endothelin-1 and thrombomodulin in systemic lupus erythematosus patients with or without Raynaud's phenomenon". In: *Acta Haematologica* 88.4, pp. 189–193. ISSN: 0001-5792. DOI: 10.1159/000204684.

Matsumoto, Kotaro *et al.* (Jan. 8, 2021). "Platelet CXCL4 mediates neutrophil extracellular traps formation in ANCA-associated vasculitis". In: *Scientific Reports* 11.1. Publisher: Nature

Publishing Group, p. 222. ISSN: 2045-2322. DOI: 10.1038/s41598-020-80685-4. URL: https://www.nature.com/articles/s41598-020-80685-4 (visited on 03/31/2025).

McDonald, K.F. *et al.* (Oct. 2000). "A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces". In: *IEEE Transactions on Plasma Science* 28.5. Conference Name: IEEE Transactions on Plasma Science, pp. 1581–1587. ISSN: 1939-9375. DOI: 10.1109/27.901237. URL: https://ieeexplore.ieee.org/abstract/document/901237 (visited on 03/26/2025).

McElhone, Kathleen *et al.* (2007). "Development and validation of a disease-specific health-related quality of life measure, the LupusQol, for adults with systemic lupus erythematosus". In: *Arthritis Care & Research* 57.6. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.22881, pp. 972–979. ISSN: 1529-0131. DOI: 10.1002/art.22881. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.22881 (visited on 03/31/2025).

McGaha, Tracy L and Michael P. Madaio (2014). "Lupus Nephritis: Animal Modeling of a Complex Disease Syndrome Pathology". In: *Drug Discovery Today. Disease Models* 11, pp. 13–18. ISSN: 1740-6757. DOI: 10.1016/j.ddmod.2014.08.002. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4337231/ (visited on 04/30/2025).

McGrath, H, P Martínez-Osuna, and F A Lee (Aug. 1, 1996). "Ultraviolet-A1 (340-400 nm) irradiation therapy in systemic lupus erythematosus". In: *Lupus* 5.4, pp. 269–274. ISSN: 1477-0962. DOI: 10.1177/096120339600500405. URL: https://doi.org/10.1177/096120339600500405 (visited on 03/22/2025).

Medzhitov, Ruslan and Charles A. Janeway Jr (June 1, 2000). "How does the immune system distinguish self from nonself?" In: *Seminars in Immunology* 12.3, pp. 185–188. ISSN: 1044-5323. DOI: 10.1006/smim.2000.0230. URL: https://www.sciencedirect.com/science/article/pii/S1044532300902306 (visited on 04/16/2025).

Meeks, Allison and Susan J. Larson (Jan. 1, 2012). "Evaluating fatigue in lupus-prone mice: Preliminary assessments". In: *Pharmacology Biochemistry and Behavior* 100.3, pp. 392–397. ISSN: 0091-3057. DOI: 10.1016/j.pbb.2011.09.013. URL: https://www.sciencedirect.com/science/article/pii/S0091305711003200 (visited on 02/27/2025).

Menke, Julia *et al.* (Feb. 2015). "Colony-Stimulating Factor-1: A Potential Biomarker for Lupus Nephritis". In: *Journal of the American Society of Nephrology* 26.2, p. 379. ISSN: 1046-6673. DOI: 10.1681/ASN.2013121356. URL: https://journals.lww.com/jasn/abstract/2015/02000/colony_stimulating_factor_1__a_potential_biomarker.17.aspx (visited on 04/06/2025).

Mercader-Salvans, Julia *et al.* (Sept. 2024). "Red blood cell distribution width as a surrogate biomarker of damage and disease activity in patients with systemic lupus erythematosus". In: *Clinical and Experimental Rheumatology* 42.9, pp. 1773–1780. ISSN: 0392-856X. DOI: 10.55563/clinexprheumatol/f0jnnm.

Meyer, Anja, Prashant J. Parmar, and Shiva Shahrara (July 1, 2022). "Significance of IL-7 and IL-7R in RA and autoimmunity". In: *Autoimmunity Reviews* 21.7, p. 103120. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2022.103120. URL: https://www.sciencedirect.com/science/article/pii/S1568997222000908 (visited on 04/06/2025).

Michalak, Sylwia Sulimiera *et al.* (Nov. 20, 2020). "Autoimmune hemolytic anemia: current knowledge and perspectives". In: *Immunity & Ageing* 17.1, p. 38. ISSN: 1742-4933. DOI: 10.1186/s12979-020-00208-7. URL: https://doi.org/10.1186/s12979-020-00208-7 (visited on 03/23/2025).

Migeon, Barbara R. (June 1, 2007). "Why females are mosaics, x-chromosome inactivation, and sex differences in disease". In: *Gender Medicine* 4.2, pp. 97–105. ISSN: 1550-8579. DOI: 10.1016/S1550-8579(07)80024-6. URL: https://www.sciencedirect.com/science/article/pii/S1550857907800246 (visited on 03/29/2025).

Miller, Frederick W (Feb. 1, 2023). "The increasing prevalence of autoimmunity and autoimmune diseases: an urgent call to action for improved understanding, diagnosis, treatment, and prevention". In: *Current Opinion in Immunology* 80, p. 102266. ISSN: 0952-7915. DOI: 10.1016/j.coi.2022.102266. URL: https://www.sciencedirect.com/science/article/pii/S0952791522001133 (visited on 02/04/2025).

Ministrini, Stefano and Federico Carbone (Feb. 1, 2022). "PCSK9 and Inflammation: Their Role in Autoimmune Diseases, with a Focus on Rheumatoid Arthritis and Systemic Lupus Erythematosus". In: *Current Medicinal Chemistry* 29.6. Publisher: Bentham Science Publishers, pp. 970–979. ISSN: 0929-8673, 1875-533X. DOI: 10.2174/0929867328666210810150940. URL: https://www.benthamdirect.com/content/journals/cmc/10.2174/0929867328666210810150940 (visited on 04/06/2025).

Mirsanei, Zahra *et al.* (June 5, 2024). "The influence of occupational heat stress on serum inflammatory cytokines among traditional bakery workers in Iran". In: *PloS One* 19.5. Publisher: Public Library of Science, e0302847. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0302847. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0302847 (visited on 04/02/2025).

Misra, Suniti *et al.* (May 6, 2015). "Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer". In: *Frontiers in Immunology* 6,

p. 201. ISSN: 1664-3224. DOI: 10.3389/fimmu.2015.00201. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4422082/ (visited on 05/03/2025).

Mitchell, Jordan L. (Nov. 2024). "Understanding the impact of delayed diagnosis and misdiagnosis of systemic lupus erythematosus (SLE)". In: *Journal of Family Medicine and Primary Care* 13.11, pp. 4819–4823. ISSN: 2249-4863. DOI: 10.4103/jfmpc.jfmpc_1177_24. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11668484/ (visited on 03/28/2025).

Miyara, Makoto *et al.* (Dec. 15, 2005). "Global Natural Regulatory T Cell Depletion in Active Systemic Lupus Erythematosus1". In: *The Journal of Immunology* 175.12, pp. 8392–8400. ISSN: 0022-1767. DOI: 10.4049/jimmunol.175.12.8392. URL: https://doi.org/10.4049/jimmunol.175.12.8392 (visited on 05/03/2025).

Mizui, Masayuki and George C. Tsokos (Apr. 17, 2018). "Targeting Regulatory T Cells to Treat Patients With Systemic Lupus Erythematosus". In: *Frontiers in Immunology* 9. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2018.00786. URL: https://www.frontiersin.orghttps://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2018.00786/full (visited on 05/03/2025).

Mohan, C *et al.* (May 1, 1993). "Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus." In: *Journal of Experimental Medicine* 177.5, pp. 1367–1381. ISSN: 0022-1007. DOI: 10.1084/jem.177.5.1367. URL: https://doi.org/10.1084/jem.177.5.1367 (visited on 03/03/2025).

Mohan, Chandra *et al.* (June 15, 1999). "Genetic dissection of lupus pathogenesis: a recipe for nephrophilic autoantibodies". In: *The Journal of Clinical Investigation* 103.12. Publisher: American Society for Clinical Investigation, pp. 1685–1695. ISSN: 0021-9738. DOI: 10.1172/JCI5827. URL: https://www.jci.org/articles/view/5827 (visited on 01/26/2025).

Molcan, Lubos (Jan. 18, 2023). "Time distributed data analysis by Cosinor. Online application". In: *Preprint*. DOI: 10.1101/805960. URL: https://www.biorxiv.org/content/10.1101/805960v2 (visited on 01/26/2025).

Morel, Laurence (May 2017). "Immunometabolism in systemic lupus erythematosus". In: *Nature Reviews Rheumatology* 13.5. Publisher: Nature Publishing Group, pp. 280–290. ISSN: 1759-4804. DOI: 10.1038/nrrheum.2017.43. URL: https://www.nature.com/articles/nrrheum.2017.43 (visited on 04/06/2025).

Morel, Laurence, Byron P. Croker, *et al.* (June 6, 2000). "Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains". In: *Proceedings of the National Academy of Sciences* 97.12. Publisher: Proceedings of the National Academy of

Sciences, pp. 6670–6675. DOI: 10.1073/pnas.97.12.6670. URL: https://www.pnas.org/doi/10.1073/pnas.97.12.6670 (visited on 02/01/2024).

Morel, Laurence, Ulrich H. Rudofsky, *et al.* (June 1994). "Polygenic control of susceptibility to murine systemic lupus erythematosus". In: *Immunity* 1.3, pp. 219–229. ISSN: 10747613. DOI: 10.1016/1074-7613(94)90100-7. URL: https://linkinghub.elsevier.com/retrieve/pii/1074761394901007 (visited on 01/26/2025).

Morel, Laurence and Yogesh Scindia (May 1, 2024). "Functional consequence of Iron dyshomeostasis and ferroptosis in systemic lupus erythematosus and lupus nephritis". In: *Clinical Immunology* 262, p. 110181. ISSN: 1521-6616. DOI: 10.1016/j.clim.2024.110181. URL: https://www.sciencedirect.com/science/article/pii/S152166162400072X (visited on 03/22/2025).

Morel, Laurence and Edward K. Wakeland (Jan. 1, 2000). "Lessons from the NZM2410 Model and Related Strains". In: *International Reviews of Immunology* 19.4. Publisher: Taylor & Francis _eprint: https://doi.org/10.3109/08830180009055506, pp. 423–446. ISSN: 0883-0185. DOI: 10.3109/08830180009055506. URL: https://doi.org/10.3109/08830180009055506 (visited on 04/17/2025).

Moreno-Torres, Víctor *et al.* (Jan. 1, 2022). "Usefulness of the hemogram as a measure of clinical and serological activity in systemic lupus erythematosus". In: *Journal of Translational Autoimmunity* 5, p. 100157. ISSN: 2589-9090. DOI: 10.1016/j.jtauto.2022.100157. URL: https://www.sciencedirect.com/science/article/pii/S2589909022000181 (visited on 11/02/2024).

Morris, D. L. *et al.* (June 2014). "MHC associations with clinical and autoantibody manifestations in European SLE". In: *Genes & Immunity* 15.4. Publisher: Nature Publishing Group, pp. 210–217. ISSN: 1476-5470. DOI: 10.1038/gene.2014.6. URL: https://www.nature.com/articles/gene20146 (visited on 04/16/2025).

Mosca, Marta *et al.* (July 1, 2024). "Challenges and opportunities in access to care for systemic lupus erythematosus patients across Europe and worldwide". In: *Rheumatology (Oxford, England)* 63.7, pp. 1772–1778. ISSN: 1462-0324. DOI: 10.1093/rheumatology/keae227. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11250230/ (visited on 03/29/2025).

Moulton, C D *et al.* (Sept. 1, 2019). "Depressive symptoms in inflammatory bowel disease: an extraintestinal manifestation of inflammation?" In: *Clinical and Experimental Immunology* 197.3, pp. 308–318. ISSN: 0009-9104. DOI: 10.1111/cei.13276. URL: https://doi.org/10.1111/cei.13276 (visited on 03/30/2025).

Moynagh, Paul N. (Oct. 15, 2005). "The NF-κB pathway". In: *Journal of Cell Science* 118.20, pp. 4589–4592. ISSN: 0021-9533. DOI: 10.1242/jcs.02579. URL: https://doi.org/10.1242/jcs.02579 (visited on 02/27/2025).

Munroe, Melissa E. *et al.* (June 3, 2022). "Pre-Clinical Autoimmunity in Lupus Relatives: Self-Reported Questionnaires and Immune Dysregulation Distinguish Relatives Who Develop Incomplete or Classified Lupus From Clinically Unaffected Relatives and Unaffected, Unrelated Individuals". In: *Frontiers in Immunology* 13. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2022.866181. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.866181/full (visited on 03/31/2025).

Nakagawa, Hidetoshi *et al.* (May 31, 2016). "Instability of Helios-deficient Tregs is associated with conversion to a T-effector phenotype and enhanced antitumor immunity". In: *Proceedings of the National Academy of Sciences* 113.22. Publisher: Proceedings of the National Academy of Sciences, pp. 6248–6253. DOI: 10.1073/pnas.1604765113. URL: https://www.pnas.org/doi/abs/10.1073/pnas.1604765113 (visited on 03/20/2025).

Nakayama, Eiko *et al.* (Feb. 1, 2023). "Optimal blue light irradiation conditions for the treatment of acne vulgaris in a mouse model". In: *Journal of Photochemistry and Photobiology B: Biology* 239, p. 112651. ISSN: 1011-1344. DOI: 10.1016/j.jphotobiol.2023.112651. URL: https://www.sciencedirect.com/science/article/pii/S1011134423000052 (visited on 04/12/2025).

Nam, Ki-San *et al.* (2007). "The Effect of Cold Air Therapy in Release of Inflammation on Rheumatoid Arthritis". In: *Journal of the Korean Academy of Clinical Electrophysiology* 5.2. Publisher: The Korean Academy of Clinical Electrophysiology, pp. 85–93. ISSN: 1738-0472. URL: https://koreascience.kr/article/JAKO200727463414167.page (visited on 04/02/2025).

Nanki, Toshihiro *et al.* (Nov. 2002). "Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis". In: *Arthritis and Rheumatism* 46.11, pp. 2878–2883. ISSN: 0004-3591. DOI: 10.1002/art.10622.

Naranjo-Gómez, Mar *et al.* (July 2007). "Expression and function of the IL-2 receptor in activated human plasmacytoid dendritic cells". In: *European Journal of Immunology* 37.7, pp. 1764–1772. ISSN: 0014-2980. DOI: 10.1002/eji.200636980.

Nashi, Emil, YingHua Wang, and Betty Diamond (Apr. 1, 2010). "The role of B cells in lupus pathogenesis". In: *The International Journal of Biochemistry & Cell Biology*. Novel Concepts in Inflammation 42.4, pp. 543–550. ISSN: 1357-2725. DOI: 10.1016/j.biocel.2009.10.011.

URL: https://www.sciencedirect.com/science/article/pii/S135727250900288X (visited on 03/31/2025).

Nickerson, Kevin M., Jaime L. Cullen, *et al.* (Apr. 15, 2013). "Exacerbated Autoimmunity in the Absence of TLR9 in MRL.Faslpr Mice Depends on Ifnar1". In: *The Journal of Immunology* 190.8, pp. 3889–3894. ISSN: 0022-1767. DOI: 10.4049/jimmunol.1203525. URL: https://doi.org/10.4049/jimmunol.1203525 (visited on 02/01/2025).

Nickerson, Kevin M., Yujuan Wang, *et al.* (Sept. 3, 2017). "Toll-like receptor 9 suppresses lupus disease in Fas-sufficient MRL Mice". In: *PloS One* 12.3. Publisher: Public Library of Science, e0173471. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0173471. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0173471 (visited on 04/01/2025).

Niederlova, Veronika *et al.* (2021). "CD8+ Tregs revisited: A heterogeneous population with different phenotypes and properties". In: *European Journal of Immunology* 51.3. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eji.202048614, pp. 512–530. ISSN: 1521-4141. DOI: 10.1002/eji.202048614. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.202048614 (visited on 03/11/2025).

Nieman, David C., Dru A. Henson, *et al.* (Sept. 2006). "Blood Leukocyte mRNA Expression for IL-10, IL-1Ra, and IL-8, but Not IL-6, Increases After Exercise". In: *Journal of Interferon & Cytokine Research* 26.9, pp. 668–674. ISSN: 1079-9907, 1557-7465. DOI: 10.1089/jir.2006.26.668. URL: http://www.liebertpub.com/doi/10.1089/jir.2006.26.668 (visited on 02/06/2025).

Nieman, David C. and Laurel M. Wentz (May 2019). "The compelling link between physical activity and the body's defense system". In: *Journal of Sport and Health Science* 8.3, pp. 201–217. ISSN: 2095-2546. DOI: 10.1016/j.jshs.2018.09.009. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6523821/ (visited on 03/30/2025).

Niewold, Timothy B. *et al.* (2008). "Association of the IRF5 risk haplotype with high serum interferon-α activity in systemic lupus erythematosus patients". In: *Arthritis & Rheumatism* 58.8. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.23613, pp. 2481–2487. ISSN: 1529-0131. DOI: 10.1002/art.23613. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.23613 (visited on 03/22/2025).

Nishimura, Kazuki *et al.* (2021). "Seasonal Differences in the UVA/UVB Ratio of Natural Sunlight Influence the Efficiency of the Photoisomerization of (6-4) Photoproducts into their Dewar Valence Isomers". In: *Photochemistry and Photobiology* 97.3, pp. 582–588. ISSN: 0031-8655.

DOI: 10.1111/php.13361. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8246833/ (visited on 04/12/2025).

Niu, Qian *et al.* (Aug. 22, 2017). "Expression of CXCR4 on T-cell subsets and Plasma IL-17 Concentrations in Patients with Aplastic Anaemia". In: *Scientific Reports* 7.1. Publisher: Nature Publishing Group, p. 9075. ISSN: 2045-2322. DOI: 10.1038/s41598-017-08699-z. URL: https://www.nature.com/articles/s41598-017-08699-z (visited on 05/04/2025).

Novogene (2025). *A basic guide to RNA-sequencing*. Novogene. URL: https://www.novogene.com/eu-en/resources/blog/a-basic-guide-to-rna-sequencing/ (visited on 04/17/2025).

Null, Manda, Tafline C. Arbor, and Manuj Agarwal (2025). "Anatomy, Lymphatic System". In: *StatPearls*. URL: http://www.ncbi.nlm.nih.gov/books/NBK513247/ (visited on 03/20/2025).

Nusser, Anja *et al.* (2014). "The development of autoimmune features in aging mice is closely associated with alterations of the peripheral CD4+ T-cell compartment". In: *European Journal of Immunology* 44.10. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eji.201344408, pp. 2893–2902. ISSN: 1521-4141. DOI: 10.1002/eji.201344408. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.201344408 (visited on 01/26/2025).

O'Connor, J.C. *et al.* (May 2009). "Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice". In: *Molecular Psychiatry* 14.5, pp. 511–522. ISSN: 1359-4184. DOI: 10.1038/sj.mp.4002148. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2683474/ (visited on 04/16/2025).

Oberholzer, Andreas *et al.* (May 2000). "Incidence of Septic Complications and Multiple Organ Failure in Severely Injured Patients Is Sex Specific". In: *Journal of Trauma and Acute Care Surgery* 48.5, p. 932. ISSN: 2163-0755. URL: https://journals.lww.com/jtrauma/abstract/2000/05000/incidence_of_septic_complications_and_multiple.19.aspx (visited on 01/22/2025).

Ogle, Molly E *et al.* (May 2016). "Monocytes and macrophages in tissue repair: Implications for immunoregenerative biomaterial design". In: *Experimental Biology and Medicine* 241.10, pp. 1084–1097. ISSN: 1535-3702. DOI: 10.1177/1535370216650293. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4898192/ (visited on 03/20/2025).

Ohmes, Justus *et al.* (Oct. 28, 2022). "Dysregulation and chronicity of pathogenic T cell responses in the pre-diseased stage of lupus". In: *Frontiers in Immunology* 13. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2022.1007078. URL: https://www.frontiersin.org/

journals/immunology/articles/10.3389/fimmu.2022.1007078/full (visited on 03/03/2025).

Okamoto, Akiko *et al.* (2011). "Regulatory T-Cell-Associated Cytokines in Systemic Lupus Erythematosus". In: *Journal of Biomedicine and Biotechnology* 2011, p. 463412. ISSN: 1110-7243. DOI: 10.1155/2011/463412. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3247013/ (visited on 05/03/2025).

Orfanakis, N. G. *et al.* (May 1, 1970). "Normal Blood Leukocyte Concentration Values". In: *American Journal of Clinical Pathology* 53.5, pp. 647–651. ISSN: 0002-9173. DOI: 10.1093/ajcp/53.5.647. URL: https://doi.org/10.1093/ajcp/53.5.647 (visited on 02/27/2025).

Overgaard, Nana H. *et al.* (Jan. 1, 2015). "CD4+/CD8+ double-positive T cells: more than just a developmental stage?" In: *Journal of Leukocyte Biology* 97.1. Publisher: Oxford Academic, pp. 31–38. ISSN: 0741-5400. DOI: 10.1189/jlb.1RU0814-382. URL: https://dx.doi.org/10.1189/jlb.1RU0814-382 (visited on 03/18/2025).

Pagenkopf, Adam and Yun Liang (2020). "Immunometabolic function of the transcription cofactor VGLL3 provides an evolutionary rationale for sexual dimorphism in autoimmunity". In: *FEBS Letters* 594.20. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/1873-3468.13911, pp. 3371–3383. ISSN: 1873-3468. DOI: 10.1002/1873-3468.13911. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/1873-3468.13911 (visited on 04/06/2025).

Palazzo, Alex and Eliza Lee (Jan. 26, 2015). "Non-coding RNA: what is functional and what is junk?" In: *Frontiers in Genetics* 6, p. 2. DOI: 10.3389/fgene.2015.00002.

Panda, Santosh K, Roland Kolbeck, and Miguel A Sanjuan (Feb. 1, 2017). "Plasmacytoid dendritic cells in autoimmunity". In: *Current Opinion in Immunology*. Innate immunity 44, pp. 20–25. ISSN: 0952-7915. DOI: 10.1016/j.coi.2016.10.006. URL: https://www.sciencedirect.com/science/article/pii/S095279151630125X (visited on 03/31/2025).

Paquissi, Feliciano Chanana and Hugo Abensur (Sept. 3, 2021). "The Th17/IL-17 Axis and Kidney Diseases, With Focus on Lupus Nephritis". In: *Frontiers in Medicine* 8. Publisher: Frontiers. ISSN: 2296-858X. DOI: 10.3389/fmed.2021.654912. URL: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2021.654912/full (visited on 04/01/2025).

Paredes, Jacqueline L., Ruth Fernandez-Ruiz, and Timothy B. Niewold (Aug. 2021). "T Cells in Systemic Lupus Erythematosus". In: *Rheumatic Diseases Clinics of North America* 47.3, pp. 379–393. ISSN: 0889-857X. DOI: 10.1016/j.rdc.2021.04.005. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262037/ (visited on 02/04/2025).

Parks, Christine G. *et al.* (Nov. 2020). "Association of Ultraviolet Radiation Exposure with Dermatomyositis in a National Myositis Patient Registry". In: *Arthritis Care & Research* 72.11, pp. 1636–1644. ISSN: 2151-464X. DOI: 10.1002/acr.24059. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7051886/ (visited on 03/22/2025).

Parodi, Alessia *et al.* (May 1, 2013). "CD39 is highly involved in mediating the suppression activity of tumor-infiltrating CD8+ T regulatory lymphocytes". In: *Cancer Immunology, Immunotherapy* 62.5, pp. 851–862. ISSN: 1432-0851. DOI: 10.1007/s00262-013-1392-z. URL: https://doi.org/10.1007/s00262-013-1392-z (visited on 05/07/2025).

Parodis, Ioannis *et al.* (June 1, 2024). "EULAR recommendations for the non-pharmacological management of systemic lupus erythematosus and systemic sclerosis". In: *Annals of the Rheumatic Diseases* 83.6. Publisher: BMJ Publishing Group Ltd Section: Recommendation, pp. 720–729. ISSN: 0003-4967, 1468-2060. DOI: 10.1136/ard-2023-224416. URL: https://ard.bmj.com/content/83/6/720 (visited on 04/02/2025).

Pathak, Simanta *et al.* (Feb. 15, 2016). "Fatty Acid Amide Hydrolase Regulates Peripheral B Cell Receptor Revision, Polyreactivity, and B1 Cells in Lupus". In: *The Journal of Immunology* 196.4, pp. 1507–1516. ISSN: 0022-1767. DOI: 10.4049/jimmunol.1500291. URL: https://doi.org/10.4049/jimmunol.1500291 (visited on 04/06/2025).

Paul, Elahna *et al.* (2002). "Anti-DNA autoreactivity in C4-deficient mice". In: *European Journal of Immunology* 32.9, pp. 2672–2679. ISSN: 1521-4141. DOI: 10.1002/1521-4141(200209)32:9<2672::AID-IMMU2672>3.0.CO;2-X. (Visited on 04/01/2025).

Pecht, T. *et al.* (2014). "Peripheral blood leucocyte subclasses as potential biomarkers of adipose tissue inflammation and obesity subphenotypes in humans". In: *Obesity Reviews* 15.4. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/obr.12133, pp. 322–337. ISSN: 1467-789X. DOI: 10.1111/obr.12133. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/obr.12133 (visited on 03/03/2025).

Pedersen, Bente Klarlund and Anders Dyhr Toft (Aug. 1, 2000). "Effects of exercise on lymphocytes and cytokines". In: *British Journal of Sports Medicine* 34.4. Publisher: British Association of Sport and Excercise Medicine Section: Review, pp. 246–251. ISSN: 0306-3674, 1473-0480. DOI: 10.1136/bjsm.34.4.246. URL: https://bjsm.bmj.com/content/34/4/246 (visited on 02/06/2025).

Pedroza, LSRA *et al.* (Mar. 1, 2011). "Systemic lupus erythematosus: Association with KIR and SLC11A1 polymorphisms, ethnic predisposition and influence in clinical manifestations at onset revealed by ancestry genetic markers in an urban Brazilian population". In: *Lupus* 20.3. Publisher: SAGE Publications Ltd STM, pp. 265–273. ISSN: 0961-2033. DOI: 10.1177/

0961203310385266. URL: https://doi.org/10.1177/0961203310385266 (visited on 04/03/2025).

Pelgrim, Charlotte E. *et al.* (Jan. 5, 2019). "Psychological co-morbidities in COPD: Targeting systemic inflammation, a benefit for both?" In: *European Journal of Pharmacology* 842, pp. 99–110. ISSN: 0014-2999. DOI: 10.1016/j.ejphar.2018.10.001. URL: https://www.sciencedirect.com/science/article/pii/S0014299918305788 (visited on 03/30/2025).

Pellefigues, Christophe and Nicolas Charles (Dec. 1, 2013). "The deleterious role of basophils in systemic lupus erythematosus". In: *Current Opinion in Immunology*. Autoimmunity, Allergy and Hypersensitivity 25.6, pp. 704–711. ISSN: 0952-7915. DOI: 10.1016/j.coi.2013.10.003. URL: https://www.sciencedirect.com/science/article/pii/S0952791513001805 (visited on 03/31/2025).

Perl, Mario *et al.* (2012). "Granzyme B: A New Crossroad of Complement and Apoptosis". In: *Current Topics in Innate Immunity II*. Ed. by John D. Lambris and George Hajishengallis. New York, NY: Springer, pp. 135–146. ISBN: 978-1-4614-0106-3. DOI: 10.1007/978-1-4614-0106-3_8. URL: https://doi.org/10.1007/978-1-4614-0106-3_8 (visited on 03/26/2025).

Perry, Daniel *et al.* (2011). "Murine Models of Systemic Lupus Erythematosus". In: *BioMed Research International* 2011.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2011/271694, p. 271694. ISSN: 2314-6141. DOI: 10.1155/2011/271694. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2011/271694 (visited on 03/03/2025).

Petzold, Cathleen *et al.* (2014). "Fluorochrome-based definition of naturally occurring Foxp3+ regulatory T cells of intra- and extrathymic origin". In: *European Journal of Immunology* 44.12. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eji.201444750, pp. 3632–3645. ISSN: 1521-4141. DOI: 10.1002/eji.201444750. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/eji.201444750 (visited on 03/20/2025).

Pimentel, Harold *et al.* (July 2017). "Differential analysis of RNA-seq incorporating quantification uncertainty". In: *Nature Methods* 14.7, pp. 687–690. ISSN: 1548-7105. DOI: 10.1038/nmeth.4324.

Pirkle, James L., Barry I. Freedman, and Agnes B. Fogo (July 2013). "Immune complex disease with a lupus-like pattern of deposition in an antinuclear antibody-negative patient". In: *American Journal of Kidney Diseases: The Official Journal of the National Kidney Foundation* 62.1, pp. 159–164. ISSN: 1523-6838. DOI: 10.1053/j.ajkd.2013.02.353.

Poon, Ivan K. H. *et al.* (Mar. 2014). "Apoptotic cell clearance: basic biology and therapeutic potential". In: *Nature Reviews Immunology* 14.3. Publisher: Nature Publishing Group, pp. 166–180. ISSN:

1474-1741. DOI: 10.1038/nri3607. URL: https://www.nature.com/articles/nri3607 (visited on 03/31/2025).

Postal, Mariana *et al.* (Dec. 2020). "Type I interferon in the pathogenesis of systemic lupus erythematosus". In: *Current Opinion in Immunology* 67, pp. 87–94. ISSN: 0952-7915. DOI: 10.1016/j.coi.2020.10.014. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8054829/ (visited on 04/03/2025).

Posynick, Bronwyn J. and Carolyn J. Brown (Oct. 22, 2019). "Escape From X-Chromosome Inactivation: An Evolutionary Perspective". In: *Frontiers in Cell and Developmental Biology* 7. Publisher: Frontiers. ISSN: 2296-634X. DOI: 10.3389/fcell.2019.00241. URL: https://www.frontiersin.org/journals/cell-and-developmental-biology/articles/10.3389/fcell.2019.00241/full (visited on 03/29/2025).

Powley, Ian R. *et al.* (May 15, 2009). "Translational reprogramming following UVB irradiation is mediated by DNA-PKcs and allows selective recruitment to the polysomes of mRNAs encoding DNA repair enzymes". In: *Genes & Development* 23.10, pp. 1207–1220. ISSN: 0890-9369. DOI: 10.1101/gad.516509. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2685536/ (visited on 03/24/2025).

Prado, Thais P. *et al.* (Feb. 2023). "Photobiomodulation with Blue Light on Wound Healing: A Scoping Review". In: *Life* 13.2. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute, p. 575. ISSN: 2075-1729. DOI: 10.3390/life13020575. URL: https://www.mdpi.com/2075-1729/13/2/575 (visited on 11/03/2024).

Psarras, Antonios, Miriam Wittmann, and Edward M. Vital (Oct. 2022). "Emerging concepts of type I interferons in SLE pathogenesis and therapy". In: *Nature Reviews Rheumatology* 18.10. Number: 10 Publisher: Nature Publishing Group, pp. 575–590. ISSN: 1759-4804. DOI: 10.1038/s41584-022-00826-z. URL: https://www.nature.com/articles/s41584-022-00826-z (visited on 08/15/2023).

Pucino, Valentina, David H. Gardner, and Benjamin A. Fisher (May 1, 2020). "Rationale for CD40 pathway blockade in autoimmune rheumatic disorders". In: *The Lancet Rheumatology* 2.5. Publisher: Elsevier, e292–e301. ISSN: 2665-9913. DOI: 10.1016/S2665-9913(20)30038-2. URL: https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30038-2/abstract (visited on 03/29/2025).

Puré, Ellen and Carolyn A. Cuff (May 1, 2001). "A crucial role for CD44 in inflammation". In: *Trends in Molecular Medicine* 7.5. Publisher: Elsevier, pp. 213–221. ISSN: 1471-4914, 1471-499X. DOI: 10.1016/S1471-4914(01)01963-3. URL: https://www.cell.com/trends/molecular-medicine/abstract/S1471-4914(01)01963-3 (visited on 03/03/2025).

Ramenghi, Ugo *et al.* (May 15, 2000). "Deficiency of the Fas apoptosis pathway without Fas gene mutations is a familial trait predisposing to development of autoimmune diseases and cancer". In: *Blood* 95.10, pp. 3176–3182. ISSN: 0006-4971. DOI: 10.1182/blood.V95.10.3176. URL: https://doi.org/10.1182/blood.V95.10.3176 (visited on 04/01/2025).

Ramonell, Kimberly M. *et al.* (Dec. 12, 2017). "CXCR4 blockade decreases CD4+ T cell exhaustion and improves survival in a murine model of polymicrobial sepsis". In: *PloS One* 12.12. Publisher: Public Library of Science, e0188882. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0188882. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0188882 (visited on 03/23/2025).

Ramos-Casals, Manuel, Pilar Brito-Zerón, and Josep Font (Feb. 1, 2007). "The Overlap of Sjögren's Syndrome with Other Systemic Autoimmune Diseases". In: *Seminars in Arthritis and Rheumatism* 36.4, pp. 246–255. ISSN: 0049-0172. DOI: 10.1016/j.semarthrit.2006.08.007. URL: https://www.sciencedirect.com/science/article/pii/S004901720600120X (visited on 01/21/2025).

Raphael, Itay *et al.* (July 2015). "T cell subsets and their signature cytokines in autoimmune and inflammatory diseases". In: *Cytokine* 74.1, pp. 5–17. ISSN: 1096-0023. DOI: 10.1016/j.cyto.2014.09.011.

Relle, Manfred and Andreas Schwarting (2012). "Role of MHC-Linked Susceptibility Genes in the Pathogenesis of Human and Murine Lupus". In: *Clinical and Developmental Immunology* 2012, p. 584374. ISSN: 1740-2522. DOI: 10.1155/2012/584374. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3385965/ (visited on 04/16/2025).

Richard, Mara Lennard and Gary Gilkeson (Jan. 21, 2018). "Mouse models of lupus: what they tell us and what they don't". In: *Lupus Science & Medicine* 5.1. Publisher: Lupus Foundation of America. ISSN: 2053-8790. DOI: 10.1136/lupus-2016-000199. URL: https://lupus.bmj.com/content/5/1/e000199 (visited on 01/29/2025).

Richard, Seidu A. *et al.* (2020). "Elucidating the Pivotal Immunomodulatory and Anti-Inflammatory Potentials of Chloroquine and Hydroxychloroquine". In: *Journal of Immunology Research* 2020.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2020/4582612, p. 4582612. ISSN: 2314-7156. DOI: 10.1155/2020/4582612. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2020/4582612 (visited on 04/16/2025).

Richter, Patricia *et al.* (Jan. 2023). "Cytokines in Systemic Lupus Erythematosus—Focus on TNF-α and IL-17". In: *International Journal of Molecular Sciences* 24.19. Number: 19 Publisher: Multidisciplinary Digital Publishing Institute, p. 14413. ISSN: 1422-0067. DOI:

10.3390/ijms241914413. URL: https://www.mdpi.com/1422-0067/24/19/14413 (visited on 04/06/2025).

Rissiek, Anne *et al.* (Apr. 2015). "The expression of CD39 on regulatory T cells is genetically driven and further upregulated at sites of inflammation". In: *Journal of Autoimmunity* 58, pp. 12–20. ISSN: 1095-9157. DOI: 10.1016/j.jaut.2014.12.007.

Rivas, Miguel *et al.* (Oct. 1, 2015). "Ultraviolet light exposure, skin cancer risk and vitamin D production". In: *Oncology Letters* 10.4. Publisher: Spandidos Publications, pp. 2259–2264. ISSN: 1792-1074. DOI: 10.3892/ol.2015.3519. URL: https://www.spandidos-publications.com/10.3892/ol.2015.3519 (visited on 03/23/2025).

Rivero, Santiago J., Efraín Díaz-Jouanen, and Donato Alarcón-Segovia (1978). "Lymphopenia In Systemic Lupus Erythematosus". In: *Arthritis & Rheumatism* 21.3. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/art.1780210302, pp. 295–305. ISSN: 1529-0131. DOI: 10.1002/art.1780210302. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/art.1780210302 (visited on 03/20/2025).

Roenneberg, Till *et al.* (2013). "Light and the Human Circadian Clock". In: *Circadian Clocks*. Ed. by Achim Kramer and Martha Merrow. Berlin, Heidelberg: Springer, pp. 311–331. ISBN: 978-3-642-25950-0. DOI: 10.1007/978-3-642-25950-0_13. URL: https://doi.org/10.1007/978-3-642-25950-0_13 (visited on 04/16/2025).

Rohleder, Nicolas (July 1, 2019). "Stress and inflammation – The need to address the gap in the transition between acute and chronic stress effects". In: *Psychoneuroendocrinology*. Festschrift for Dirk Hellhammer 105, pp. 164–171. ISSN: 0306-4530. DOI: 10.1016/j.psyneuen.2019.02.021. URL: https://www.sciencedirect.com/science/article/pii/S0306453018306954 (visited on 03/30/2025).

Rokade, Sushama *et al.* (July 24, 2024). "IL-2 based cancer immunotherapies: an evolving paradigm". In: *Frontiers in Immunology* 15. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2024.1433989. URL: https://www.frontiersin.orghttps://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2024.1433989/full (visited on 05/03/2025).

Romano, Audrey *et al.* (May 1, 2016). "FOXP3+ Regulatory T Cells in Hepatic Fibrosis and Splenomegaly Caused by Schistosoma japonicum: The Spleen May Be a Major Source of Tregs in Subjects with Splenomegaly". In: *PLOS Neglected Tropical Diseases* 10.1. Publisher: Public Library of Science, e0004306. ISSN: 1935-2735. DOI: 10.1371/journal.pntd.0004306. URL: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004306 (visited on 03/19/2025).

Romo-Tena, Jorge, Diana Gómez-Martín, and Jorge Alcocer-Varela (Oct. 1, 2013). "CTLA-4 and autoimmunity: New insights into the dual regulator of tolerance". In: *Autoimmunity Reviews* 12.12, pp. 1171–1176. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2013.07.002. URL: https://www.sciencedirect.com/science/article/pii/S1568997213001250 (visited on 03/23/2025).

Rönnblom, Lars, Gunnar V. Alm, and Maija-Leena Eloranta (Sept. 2009). "Type I interferon and lupus". In: *Current Opinion in Rheumatology* 21.5, p. 471. ISSN: 1040-8711. DOI: 10.1097/BOR.0b013e32832e089e. URL: https://journals.lww.com/co-rheumatology/abstract/2009/09000/type_i_interferon_and_lupus.6.aspx (visited on 03/24/2025).

Rosa, Renata Ferreira *et al.* (Sept. 1, 2012). "Monocyte Chemoattractant-1 as a Urinary Biomarker for the Diagnosis of Activity of Lupus Nephritis in Brazilian Patients". In: *The Journal of Rheumatology*. Publisher: The Journal of Rheumatology Section: Article. ISSN: 0315-162X, 1499-2752. DOI: 10.3899/jrheum.110201. URL: https://www.jrheum.org/content/early/2012/08/29/jrheum.110201 (visited on 03/31/2025).

Rothstein, David M. and Geoffrey Camirand (Aug. 2015). "New insights into the mechanisms of Treg function". In: *Current Opinion in Organ Transplantation* 20.4, pp. 376–384. ISSN: 1087-2418. DOI: 10.1097/MOT.0000000000000212. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4575588/ (visited on 05/04/2025).

Roussou, E. *et al.* (May 1, 2013). "Stress as a trigger of disease flares in SLE". In: *Rheumatology International* 33.5, pp. 1367–1370. ISSN: 1437-160X. DOI: 10.1007/s00296-011-2292-1. URL: https://doi.org/10.1007/s00296-011-2292-1 (visited on 03/23/2025).

Rudofsky, U H *et al.* (Apr. 1, 1993). "Differences in expression of lupus nephritis in New Zealand mixed H-2z homozygous inbred strains of mice derived from New Zealand black and New Zealand white mice. Origins and initial characterization". In: *Laboratory Investigation* 68.4, pp. 419–426. ISSN: 1530-0307.

Ruiz-Irastorza, Guillermo, Alvaro Danza, and Munther Khamashta (July 1, 2012). "Glucocorticoid use and abuse in SLE". In: *Rheumatology* 51.7, pp. 1145–1153. ISSN: 1462-0324. DOI: 10.1093/rheumatology/ker410. URL: https://doi.org/10.1093/rheumatology/ker410 (visited on 03/29/2025).

Sakaguchi, Shimon *et al.* (May 30, 2008). "Regulatory T Cells and Immune Tolerance". In: *Cell* 133.5. Publisher: Elsevier, pp. 775–787. ISSN: 0092-8674, 1097-4172. DOI: 10.1016/j.cell.2008.05.009. URL: https://www.cell.com/cell/abstract/S0092-8674(08)00624-7 (visited on 03/03/2025).

Sakane, T. *et al.* (Jan. 1988). "B cell hyperactivity and its relation to distinct clinical features and the degree of disease activity in patients with systemic lupus erythematosus". In: *Arthritis and Rheumatism* 31.1, pp. 80–87. ISSN: 0004-3591. DOI: 10.1002/art.1780310112.

Salvagno, Gian Luca *et al.* (Mar. 4, 2015). "Red blood cell distribution width: A simple parameter with multiple clinical applications". In: *Critical Reviews in Clinical Laboratory Sciences* 52.2. Publisher: Taylor & Francis _eprint: https://doi.org/10.3109/10408363.2014.992064, pp. 86–105. ISSN: 1040-8363. DOI: 10.3109/10408363.2014.992064. URL: https://doi.org/10.3109/10408363.2014.992064 (visited on 03/02/2025).

Santagata, Sara *et al.* (Aug. 19, 2017). "Targeting CXCR4 reverts the suppressive activity of T-regulatory cells in renal cancer". In: *Oncotarget* 8.44, pp. 77110–77120. ISSN: 1949-2553. DOI: 10.18632/oncotarget.20363. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5652768/ (visited on 05/04/2025).

Santer, Deanna M. *et al.* (Oct. 15, 2010). "C1q Deficiency Leads to the Defective Suppression of IFN-α in Response to Nucleoprotein Containing Immune Complexes". In: *Journal of Immunology (Baltimore, Md. : 1950)* 185.8, pp. 4738–4749. ISSN: 0022-1767. DOI: 10.4049/jimmunol.1001731. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065655/ (visited on 03/31/2025).

Sarkar, Mrinal K. *et al.* (Nov. 1, 2018). "Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa". In: *Annals of the Rheumatic Diseases* 77.11. Publisher: BMJ Publishing Group Ltd Section: Basic and translational research, pp. 1653–1664. ISSN: 0003-4967, 1468-2060. DOI: 10.1136/annrheumdis-2018-213197. URL: https://ard.bmj.com/content/77/11/1653 (visited on 11/01/2024).

Sarrand, Julie and Muhammad Soyfoo (Jan. 2022). "Involvement of IL-33 in the Pathophysiology of Systemic Lupus Erythematosus: Review". In: *International Journal of Molecular Sciences* 23.6. Number: 6 Publisher: Multidisciplinary Digital Publishing Institute, p. 3138. ISSN: 1422-0067. DOI: 10.3390/ijms23063138. URL: https://www.mdpi.com/1422-0067/23/6/3138 (visited on 04/06/2025).

Savage, Nigel D. L. *et al.* (2002). "Inhibition of TCR-mediated shedding of L-selectin (CD62L) on human and mouse CD4+ T cells by metalloproteinase inhibition: analysis of the regulation of Th1/Th2 function". In: *European Journal of Immunology* 32.10, pp. 2905–2914. ISSN: 1521-4141. DOI: 10.1002/1521-4141(2002010)32:10<2905::AID-IMMU2905>3.0.CO;2-6. (Visited on 03/03/2025).

Schäfer, Matthias and Sabine Werner (July 1, 2011). "The Cornified Envelope: A First Line of Defense against Reactive Oxygen Species". In: *Journal of Investigative Dermatology*

131.7, pp. 1409–1411. ISSN: 0022-202X. DOI: 10.1038/jid.2011.119. URL: https://www.sciencedirect.com/science/article/pii/S0022202X15353410 (visited on 04/15/2025).

Scheiermann, Christoph, Yuya Kunisaki, and Paul S. Frenette (Mar. 2013). "Circadian control of the immune system". In: *Nature Reviews. Immunology* 13.3, pp. 190–198. ISSN: 1474-1741. DOI: 10.1038/nri3386.

Scherlinger, Marc *et al.* (June 2018). "Systemic lupus erythematosus and systemic sclerosis: All roads lead to platelets". In: *Autoimmunity Reviews* 17.6, pp. 625–635. ISSN: 15689972. DOI: 10.1016/j.autrev.2018.01.012. URL: https://linkinghub.elsevier.com/retrieve/pii/S1568997218300910 (visited on 07/10/2024).

Schmidt, Ralf L. J. *et al.* (Feb. 7, 2017). "Chloroquine inhibits human CD4+ T-cell activation by AP-1 signaling modulation". In: *Scientific Reports* 7.1. Publisher: Nature Publishing Group, p. 42191. ISSN: 2045-2322. DOI: 10.1038/srep42191. URL: https://www.nature.com/articles/srep42191 (visited on 04/16/2025).

Schuler, P J *et al.* (Aug. 1, 2014). "Human CD4+CD39+ regulatory T cells produce adenosine upon co-expression of surface CD73 or contact with CD73+ exosomes or CD73+ cells". In: *Clinical and Experimental Immunology* 177.2, pp. 531–543. ISSN: 0009-9104. DOI: 10.1111/cei.12354. URL: https://doi.org/10.1111/cei.12354 (visited on 03/20/2025).

Schulze-Koops, Hendrik (June 22, 2004). "Lymphopenia and autoimmune diseases". In: *Arthritis Research and Therapy* 6.4, p. 178. ISSN: 1478-6354. DOI: 10.1186/ar1208. URL: https://doi.org/10.1186/ar1208 (visited on 03/20/2025).

Schumann, Kathrin *et al.* (Nov. 2020). "Functional CRISPR dissection of gene networks controlling human regulatory T cell identity". In: *Nature Immunology* 21.11. Publisher: Nature Publishing Group, pp. 1456–1466. ISSN: 1529-2916. DOI: 10.1038/s41590-020-0784-4. URL: https://www.nature.com/articles/s41590-020-0784-4 (visited on 03/24/2025).

Sengupta, Soumya *et al.* (Jan. 2023). "IL-21, Inflammatory Cytokines and Hyperpolarized CD8+ T Cells Are Central Players in Lupus Immune Pathology". In: *Antioxidants* 12.1. Number: 1 Publisher: Multidisciplinary Digital Publishing Institute, p. 181. ISSN: 2076-3921. DOI: 10.3390/antiox12010181. URL: https://www.mdpi.com/2076-3921/12/1/181 (visited on 03/18/2025).

Senior, A. E. (Jan. 1988). "ATP synthesis by oxidative phosphorylation". In: *Physiological Reviews* 68.1. Publisher: American Physiological Society, pp. 177–231. ISSN: 0031-9333. DOI: 10.1152/physrev.1988.68.1.177. URL: https://journals.physiology.org/doi/abs/10.1152/physrev.1988.68.1.177 (visited on 03/24/2025).

Sennikov, Sergey V. *et al.* (2020). "Dendritic Cells Transfected with MHC Antigenic Determinants of CBA Mice Induce Antigen-Specific Tolerance in C57Bl/6 Mice". In: *Journal of Immunology Research* 2020.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2020/9686143, p. 9686143. ISSN: 2314-7156. DOI: 10.1155/2020/9686143. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2020/9686143 (visited on 03/19/2025).

Serbina, Natalya V. *et al.* (2008). "Monocyte-Mediated Defense Against Microbial Pathogens". In: *Annual Review of Immunology* 26, pp. 421–452. ISSN: 0732-0582. DOI: 10.1146/annurev.immunol.26.021607.090326. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2921669/ (visited on 03/20/2025).

Sharif, Kassem *et al.* (Jan. 1, 2018). "Physical activity and autoimmune diseases: Get moving and manage the disease". In: *Autoimmunity Reviews* 17.1, pp. 53–72. ISSN: 1568-9972. DOI: 10.1016/j.autrev.2017.11.010. URL: https://www.sciencedirect.com/science/article/pii/S1568997217302835 (visited on 03/30/2025).

Shen, Jing *et al.* (Jan. 23, 2018). "Mitochondria are transported along microtubules in membrane nanotubes to rescue distressed cardiomyocytes from apoptosis". In: *Cell Death & Disease* 9.2. Publisher: Nature Publishing Group, pp. 1–12. ISSN: 2041-4889. DOI: 10.1038/s41419-017-0145-x. URL: https://www.nature.com/articles/s41419-017-0145-x (visited on 03/25/2025).

Shepherdson, James L. *et al.* (Mar. 7, 2024). "Variants in ZFX are associated with an X-linked neurodevelopmental disorder with recurrent facial gestalt". In: *American Journal of Human Genetics* 111.3, pp. 487–508. ISSN: 0002-9297. DOI: 10.1016/j.ajhg.2024.01.007. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10940019/ (visited on 03/24/2025).

Shi, Lihua *et al.* (May 5, 2014). "The SLE Transcriptome Exhibits Evidence of Chronic Endotoxin Exposure and Has Widespread Dysregulation of Non-Coding and Coding RNAs". In: *PloS One* 9.5. Publisher: Public Library of Science, e93846. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0093846. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0093846 (visited on 03/24/2025).

Simpson, Joanne *et al.* (Dec. 14, 2016). "Plasmacytoid Dendritic Cells Respond Directly to Apoptotic Cells by Secreting Immune Regulatory IL-10 or IFN-α". In: *Frontiers in Immunology* 7. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2016.00590. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2016.00590/full (visited on 03/20/2025).

Sjaastad, Louisa E. *et al.* (Sept. 23, 2021). "Phenotypic and Functional Diversity in Regulatory T Cells". In: *Frontiers in Cell and Developmental Biology* 9. Publisher: Frontiers. ISSN: 2296-634X. DOI: 10.3389/fcell.2021.715901. URL: https://www.frontiersin.orghttps://www.frontiersin.org/journals/cell-and-developmental-biology/articles/10.3389/fcell.2021.715901/full (visited on 05/04/2025).

Skopelja-Gardner, Sladjana *et al.* (Jan. 19, 2021). "Acute skin exposure to ultraviolet light triggers neutrophil-mediated kidney inflammation". In: *Proceedings of the National Academy of Sciences* 118.3. Publisher: Proceedings of the National Academy of Sciences, e2019097118. DOI: 10.1073/pnas.2019097118. URL: https://www.pnas.org/doi/10.1073/pnas.2019097118 (visited on 09/27/2023).

Slominski, Andrzej T *et al.* (May 1, 2018). "How UV Light Touches the Brain and Endocrine System Through Skin, and Why". In: *Endocrinology* 159.5, pp. 1992–2007. ISSN: 0013-7227. DOI: 10.1210/en.2017-03230. URL: https://doi.org/10.1210/en.2017-03230 (visited on 03/23/2025).

Smith, Nikaïa *et al.* (July 10, 2019). "Control of TLR7-mediated type I IFN signaling in pDCs through CXCR4 engagement—A new target for lupus treatment". In: *Science Advances* 5.7. Publisher: American Association for the Advancement of Science, eaav9019. DOI: 10.1126/sciadv.aav9019. URL: https://www.science.org/doi/10.1126/sciadv.aav9019 (visited on 04/16/2025).

Smith-Raska, Matthew R. *et al.* (July 4, 2018). "The Transcription Factor Zfx Regulates Peripheral T Cell Self-Renewal and Proliferation". In: *Frontiers in Immunology* 9. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2018.01482. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2018.01482/full (visited on 03/24/2025).

Sobhy, Nesreen, Marwa H. Niazy, and Asmaa Kamal (Jan. 1, 2020). "Lymphopenia in systemic lupus erythematosus patients: Is it more than a laboratory finding?" In: *The Egyptian Rheumatologist* 42.1, pp. 23–26. ISSN: 1110-1164. DOI: 10.1016/j.ejr.2019.04.003. URL: https://www.sciencedirect.com/science/article/pii/S1110116419300493 (visited on 05/03/2025).

Souyris, Mélanie *et al.* (Mar. 2019). "Female predisposition to TLR7-driven autoimmunity: gene dosage and the escape from X chromosome inactivation". In: *Seminars in Immunopathology* 41.2, pp. 153–164. ISSN: 1863-2300. DOI: 10.1007/s00281-018-0712-y.

Spada, Roberto, José M Rojas, and Domingo F Barber (Oct. 1, 2015). "Recent findings on the role of natural killer cells in the pathogenesis of systemic lupus erythematosus". In: *Journal of*

Leukocyte Biology 98.4, pp. 479–487. ISSN: 0741-5400. DOI: 10.1189/jlb.4RU0315-081RR. URL: https://doi.org/10.1189/jlb.4RU0315-081RR (visited on 03/31/2025).

Spee-Mayer, Caroline von *et al.* (July 1, 2016). "Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus". In: *Annals of the Rheumatic Diseases* 75.7. Publisher: BMJ Publishing Group Ltd Section: Basic and translational research, pp. 1407–1415. ISSN: 0003-4967, 1468-2060. DOI: 10.1136/annrheumdis-2015-207776. URL: https://ard.bmj.com/content/75/7/1407 (visited on 03/18/2025).

Srinivasan, Saranya *et al.* (May 1, 2024). "Age-related dynamics of CD8+ regulatory T cells: Implications of NKG2D in Immune Aging". In: *The Journal of Immunology* 212.1, 0200_4627. ISSN: 0022-1767. DOI: 10.4049/jimmunol.212.supp.0200.4627. URL: https://doi.org/10.4049/jimmunol.212.supp.0200.4627 (visited on 05/04/2025).

Stannard, Jasmine N. *et al.* (Jan. 1, 2017). "Lupus Skin Is Primed for IL-6 Inflammatory Responses through a Keratinocyte-Mediated Autocrine Type I Interferon Loop". In: *Journal of Investigative Dermatology* 137.1, pp. 115–122. ISSN: 0022-202X. DOI: 10.1016/j.jid.2016.09.008. URL: https://www.sciencedirect.com/science/article/pii/S0022202X16323806 (visited on 03/23/2025).

Stenger, Sarah, Hanna Grasshoff, *et al.* (2023). "Potential effects of shift work on skin autoimmune diseases". In: *Frontiers in Immunology* 13. ISSN: 1664-3224. URL: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1000951 (visited on 02/28/2023).

Stenger, Sarah, Artem Vorobyev, *et al.* (Apr. 10, 2025). "Insomnia increases the risk for specific autoimmune diseases: a large-scale retrospective cohort study". In: *Frontiers in Network Physiology* 5. Publisher: Frontiers, p. 1499297. ISSN: 2674-0109. DOI: 10.3389/fnetp.2025.1499297. URL: https://www.frontiersin.orghttps://www.frontiersin.org/journals/network-physiology/articles/10.3389/fnetp.2025.1499297/full (visited on 04/10/2025).

Stewart, Laura K. *et al.* (Sept. 1, 2005). "Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4". In: *Brain, Behavior, and Immunity*. Physical Activity, Behavior, Immunity and Health 19.5, pp. 389–397. ISSN: 0889-1591. DOI: 10.1016/j.bbi.2005.04.003. URL: https://www.sciencedirect.com/science/article/pii/S088915910500070X (visited on 02/06/2025).

Stocks, B. T. *et al.* (Jan. 2016). "Lupus-Prone Mice Resist Immune Regulation and Transplant Tolerance Induction". In: *American Journal of Transplantation* 16.1, pp. 334–341. ISSN: 1600-6135. DOI: 10.1111/ajt.13449. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4718751/ (visited on 03/19/2025).

Sturfelt, Gunnar and Lennart Truedsson (Aug. 2012). "Complement in the immunopathogenesis of rheumatic disease". In: *Nature Reviews Rheumatology* 8.8. Publisher: Nature Publishing Group, pp. 458–468. ISSN: 1759-4804. DOI: 10.1038/nrrheum.2012.75. URL: https://www.nature.com/articles/nrrheum.2012.75 (visited on 03/31/2025).

Suárez-Fueyo, Abel, Sean J Bradley, and George C Tsokos (Dec. 1, 2016). "T cells in Systemic Lupus Erythematosus". In: *Current Opinion in Immunology*. Autoimmunity 43, pp. 32–38. ISSN: 0952-7915. DOI: 10.1016/j.coi.2016.09.001. URL: https://www.sciencedirect.com/science/article/pii/S0952791516300905 (visited on 03/18/2025).

Subramanian, Anuradhaa *et al.* (Jan. 2021). "Ethnicity-based differences in the incident risk of allergic diseases and autoimmune disorders: A UK-based retrospective cohort study of 4.4 million participants". In: *Clinical & Experimental Allergy* 51.1, pp. 144–147. ISSN: 0954-7894, 1365-2222. DOI: 10.1111/cea.13741. URL: https://onlinelibrary.wiley.com/doi/10.1111/cea.13741 (visited on 02/04/2025).

Subramanian, Srividya *et al.* (July 15, 2005). "Epistatic Suppression of Systemic Lupus Erythematosus: Fine Mapping of Sles1 to Less Than 1 Mb1". In: *The Journal of Immunology* 175.2, pp. 1062–1072. ISSN: 0022-1767. DOI: 10.4049/jimmunol.175.2.1062. URL: https://doi.org/10.4049/jimmunol.175.2.1062 (visited on 04/06/2025).

Suitthimeathegorn, Orawan *et al.* (Aug. 31, 2022). "Direct and Indirect Effects of Blue Light Exposure on Skin: A Review of Published Literature". In: *Skin Pharmacology and Physiology* 35.6, pp. 305–318. ISSN: 1660-5527. DOI: 10.1159/000526720. URL: https://doi.org/10.1159/000526720 (visited on 04/12/2025).

Sun, Juan *et al.* (2019). "Efficient Therapeutic Function and Mechanisms of Human Polyclonal CD8+CD103+Foxp3+ Regulatory T Cells on Collagen-Induced Arthritis in Mice". In: *Journal of Immunology Research* 2019.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2019/8575407, p. 8575407. ISSN: 2314-7156. DOI: 10.1155/2019/8575407. URL: https://onlinelibrary.wiley.com/doi/abs/10.1155/2019/8575407 (visited on 03/11/2025).

Sun, Yajing, Yuanyuan Qu, and Jianwei Zhu (Aug. 11, 2021). "The Relationship Between Inflammation and Post-traumatic Stress Disorder". In: *Frontiers in Psychiatry* 12, p. 707543. ISSN: 1664-0640. DOI: 10.3389/fpsyt.2021.707543. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8385235/ (visited on 03/30/2025).

Suren Garg, Sourbh *et al.* (June 1, 2023). "Association between obesity, inflammation and insulin resistance: Insights into signaling pathways and therapeutic interventions". In: *Diabetes Research and Clinical Practice* 200, p. 110691. ISSN: 0168-8227. DOI: 10.1016/j.diabres.

2023.110691. URL: https://www.sciencedirect.com/science/article/pii/S0168822723004539 (visited on 03/03/2025).

Swiecki, Melissa and Marco Colonna (Aug. 2015). "The multifaceted biology of plasmacytoid dendritic cells". In: *Nature Reviews Immunology* 15.8. Publisher: Nature Publishing Group, pp. 471–485. ISSN: 1474-1741. DOI: 10.1038/nri3865. URL: https://www.nature.com/articles/nri3865 (visited on 04/06/2025).

Syrett, Camille M. and Montserrat C. Anguera (Oct. 2019). "When the balance is broken: X-linked gene dosage from two X chromosomes and female-biased autoimmunity". In: *Journal of Leukocyte Biology* 106.4, pp. 919–932. ISSN: 0741-5400. DOI: 10.1002/JLB.6RI0319-094R. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7206452/ (visited on 03/22/2025).

Syrett, Camille M., Isabel Sierra, *et al.* (Feb. 2020). "Loss of epigenetic modifications on the inactive X chromosome and sex-biased gene expression profiles in B cells from NZB/W F1 mice with lupus-like disease". In: *Journal of Autoimmunity* 107, p. 102357. ISSN: 0896-8411. DOI: 10.1016/j.jaut.2019.102357. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7237307/ (visited on 04/17/2025).

't Veld, Aliede E. in *et al.* (Mar. 9, 2021). "Hydroxychloroquine Effects on TLR Signalling: Underexposed but Unneglectable in COVID-19". In: *Journal of Immunology Research* 2021, p. 6659410. ISSN: 2314-8861. DOI: 10.1155/2021/6659410. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7949870/ (visited on 04/16/2025).

Tai, Xuguang *et al.* (May 31, 2012). "Basis of CTLA-4 function in regulatory and conventional CD4+ T cells". In: *Blood* 119.22, pp. 5155–5163. ISSN: 0006-4971. DOI: 10.1182/blood-2011-11-388918. URL: https://doi.org/10.1182/blood-2011-11-388918 (visited on 03/20/2025).

Takagi, Tsuyoshi, Jun Harada, and Shunsuke Ishii (Nov. 2001). "Murine Schnurri-2 is required for positive selection of thymocytes". In: *Nature Immunology* 2.11. Publisher: Nature Publishing Group, pp. 1048–1053. ISSN: 1529-2916. DOI: 10.1038/ni728. URL: https://www.nature.com/articles/ni728 (visited on 03/24/2025).

Tang, Yang-Yang *et al.* (Dec. 18, 2023). "Th1-related transcription factors and cytokines in systemic lupus erythematosus". In: *Frontiers in Immunology* 14. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2023.1305590. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2023.1305590/full (visited on 04/01/2025).

Tekguc, Murat *et al.* (July 27, 2021). "Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells". In: *Proceedings of the National Academy of*

Sciences 118.30. Publisher: Proceedings of the National Academy of Sciences, e2023739118. DOI: 10.1073/pnas.2023739118. URL: https://www.pnas.org/doi/10.1073/pnas.2023739118 (visited on 03/20/2025).

TheJacksonLaboratory (2025a). *000684 - NZB Strain Details*. The Jackson Laboratory. URL: https://www.jax.org/strain/000684 (visited on 04/17/2025).

— (2025b). *001058 - NZW Strain Details*. The Jackson Laboratory. URL: https://www.jax.org/strain/001058 (visited on 04/17/2025).

— (2025c). *002676 - NZM2410/J Strain Details*. The Jackson Laboratory. URL: https://www.jax.org/strain/002676 (visited on 04/17/2025).

— (2025d). *007228 - B6.NZMSle1/Sle2/Sle3 Strain Details*. The Jackson Laboratory. URL: https://www.jax.org/strain/007228 (visited on 04/17/2025).

Thompson, Craig B. *et al.* (Feb. 1, 1983). "Platelet size as a determinant of platelet function". In: *The Journal of Laboratory and Clinical Medicine* 101.2. Publisher: Elsevier, pp. 205–213. ISSN: 0022-2143, 1532-6543. DOI: 10.5555/uri:pii:0022214383901804. URL: https://www.translationalres.com/article/0022-2143(83)90180-4/abstract (visited on 03/23/2025).

Thornton, Angela M., Patricia E. Korty, *et al.* (Apr. 1, 2010). "Expression of Helios, an Ikaros Transcription Factor Family Member, Differentiates Thymic-Derived from Peripherally Induced Foxp3+ T Regulatory Cells". In: *The Journal of Immunology* 184.7, pp. 3433–3441. ISSN: 0022-1767. DOI: 10.4049/jimmunol.0904028. URL: https://doi.org/10.4049/jimmunol.0904028 (visited on 03/20/2025).

Thornton, Angela M. and Ethan M. Shevach (Nov. 2019). "Helios: still behind the clouds". In: *Immunology* 158.3, pp. 161–170. ISSN: 0019-2805. DOI: 10.1111/imm.13115. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6797934/ (visited on 03/20/2025).

Tian, Jingru *et al.* (Mar. 1, 2023). "Global epidemiology of systemic lupus erythematosus: a comprehensive systematic analysis and modelling study". In: *Annals of the Rheumatic Diseases* 82.3. Publisher: BMJ Publishing Group Ltd Section: Systemic lupus erythematosus, pp. 351–356. ISSN: 0003-4967, 1468-2060. DOI: 10.1136/ard-2022-223035. URL: https://ard.bmj.com/content/82/3/351 (visited on 02/04/2025).

Timperi, Eleonora and Vincenzo Barnaba (July 28, 2021). "CD39 Regulation and Functions in T Cells". In: *International Journal of Molecular Sciences* 22.15, p. 8068. ISSN: 1422-0067. DOI: 10.3390/ijms22158068. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8348030/ (visited on 03/20/2025).

Tohidi-Esfahani, Ibrahim *et al.* (Jan. 27, 2024). "Platelets and Thrombotic Antiphospholipid Syndrome". In: *Journal of Clinical Medicine* 13.3, p. 741. ISSN: 2077-0383. DOI: 10.3390/jcm13030741. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10856779/ (visited on 04/17/2025).

Tomasetti, Cristian *et al.* (Oct. 8, 2019). "Cell division rates decrease with age, providing a potential explanation for the age-dependent deceleration in cancer incidence". In: *Proceedings of the National Academy of Sciences* 116.41, pp. 20482–20488. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/pnas.1905722116. URL: https://pnas.org/doi/full/10.1073/pnas.1905722116 (visited on 03/20/2025).

Tsai, Yi-Giien *et al.* (Sept. 12, 2023). "Pathogenesis and novel therapeutics of regulatory T cell subsets and interleukin-2 therapy in systemic lupus erythematosus". In: *Frontiers in Immunology* 14, p. 1230264. ISSN: 1664-3224. DOI: 10.3389/fimmu.2023.1230264. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10522836/ (visited on 03/19/2025).

Tsukazaki, N. *et al.* (Dec. 2002). "Photoprovocation test and immunohistochemical analysis of inducible nitric oxide synthase expression in patients with Sjögren's syndrome associated with photosensitivity". In: *The British Journal of Dermatology* 147.6, pp. 1102–1108. ISSN: 0007-0963. DOI: 10.1046/j.1365-2133.2002.04995.x.

Tsuzaka, K. *et al.* (1996). "Autoantibodies to double-stranded (ds)DNA immunoprecipitate 18S ribosomal RNA by virtue of their interaction with ribosomal protein S1 and suppress protein synthesis". In: *Clinical & Experimental Immunology* 106.3. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2249.1996.d01-869.x, pp. 504–508. ISSN: 1365-2249. DOI: 10.1046/j.1365-2249.1996.d01-869.x. URL: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2249.1996.d01-869.x (visited on 05/05/2025).

Tur, Juan *et al.* (Sept. 1, 2021). "Induction of CIITA by IFN-γ in macrophages involves STAT1 activation by JAK and JNK". In: *Immunobiology* 226.5, p. 152114. ISSN: 0171-2985. DOI: 10.1016/j.imbio.2021.152114. URL: https://www.sciencedirect.com/science/article/pii/S0171298521000620 (visited on 03/24/2025).

Tyagi, Neetu and Dinesh Gupta (Sept. 8, 2021). *Integrative analysis of transcriptomic profiles reveals shared gene signatures of Rheumatoid Arthritis and Systemic Lupus Erythromatosus*. DOI: 10.21203/rs.3.rs-869926/v1. URL: https://www.researchsquare.com/article/rs-869926/v1 (visited on 04/06/2025).

Tyas, Lorraine *et al.* (Sept. 2000). "Rapid caspase-3 activation during apoptosis revealed using fluorescence-resonance energy transfer". In: *EMBO reports* 1.3. Num Pages: 270 Publisher: John

Wiley & Sons, Ltd, pp. 266–270. ISSN: 1469-221X. DOI: 10.1093/embo-reports/kvd050. URL: https://www.embopress.org/doi/full/10.1093/embo-reports/kvd050 (visited on 03/25/2025).

Uxa, Sigrid *et al.* (Dec. 2021). "Ki-67 gene expression". In: *Cell Death & Differentiation* 28.12. Publisher: Nature Publishing Group, pp. 3357–3370. ISSN: 1476-5403. DOI: 10.1038/s41418-021-00823-x. URL: https://www.nature.com/articles/s41418-021-00823-x (visited on 03/31/2025).

Uzunbajakava, Natallia E. *et al.* (2023). "Highlighting nuances of blue light phototherapy: Mechanisms and safety considerations". In: *Journal of Biophotonics* 16.2. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jbio.202200257, e202200257. ISSN: 1864-0648. DOI: 10.1002/jbio.202200257. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/jbio.202200257 (visited on 11/01/2024).

Vázquez-Martínez, Edgar Ricardo *et al.* (June 20, 2018). "Sexual dimorphism in bacterial infections". In: *Biology of Sex Differences* 9.1, p. 27. ISSN: 2042-6410. DOI: 10.1186/s13293-018-0187-5. URL: https://doi.org/10.1186/s13293-018-0187-5 (visited on 01/22/2025).

Vogt, Caleb C. *et al.* (Feb. 14, 2024). "Female behavior drives the formation of distinct social structures in C57BL/6J versus wild-derived outbred mice in field enclosures". In: *BMC Biology* 22.1, p. 35. ISSN: 1741-7007. DOI: 10.1186/s12915-024-01809-0. URL: https://doi.org/10.1186/s12915-024-01809-0 (visited on 04/01/2025).

Vordenbäumen, Stefan *et al.* (June 1, 2023). "Beneficial effects associated to a healthy lifestyle in systemic lupus erythematosus: A cross-sectional study". In: *Lupus* 32.7. Publisher: SAGE Publications Ltd STM, pp. 855–863. ISSN: 0961-2033. DOI: 10.1177/09612033231175289. URL: https://doi.org/10.1177/09612033231175289 (visited on 03/29/2025).

Vorobyev, Artem *et al.* (Sept. 10, 2019). "Gene-diet interactions associated with complex trait variation in an advanced intercross outbred mouse line". In: *Nature Communications* 10.1. Publisher: Nature Publishing Group, p. 4097. ISSN: 2041-1723. DOI: 10.1038/s41467-019-11952-w. URL: https://www.nature.com/articles/s41467-019-11952-w (visited on 01/26/2025).

Voulgarelis, Michalis *et al.* (Mar. 1, 2000). "Anaemia in systemic lupus erythematosus: aetiological profile and the role of erythropoietin". In: *Annals of the Rheumatic Diseases* 59.3, pp. 217–222. ISSN: 0003-4967. DOI: 10.1136/ard.59.3.217. URL: https://www.sciencedirect.com/science/article/pii/S0003496724384845 (visited on 03/23/2025).

Wahl, Siegfried *et al.* (Dec. 2019). "The inner clock—Blue light sets the human rhythm". In: *Journal of Biophotonics* 12.12. ISSN: 1864-063X. DOI: 10.1002/jbio.201900102. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7065627/ (visited on 03/24/2025).

Wang, Chen *et al.* (June 3, 2022). "The circadian immune system". In: *Science Immunology* 7.72. ISSN: 2470-9468. DOI: 10.1126/sciimmunol.abm2465.

Wang, Da-Cheng *et al.* (Jan. 5, 2024). "Neuropeptide Y, a potential marker for lupus, promotes lupus development". In: *International Immunopharmacology* 126, p. 111272. ISSN: 1567-5769. DOI: 10.1016/j.intimp.2023.111272. URL: https://www.sciencedirect.com/science/article/pii/S1567576923015990 (visited on 04/06/2025).

Wang, Jianle *et al.* (Apr. 5, 2016). "Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X". In: *Proceedings of the National Academy of Sciences* 113.14. Publisher: Proceedings of the National Academy of Sciences, E2029–E2038. DOI: 10.1073/pnas.1520113113. URL: https://www.pnas.org/doi/10.1073/pnas.1520113113 (visited on 03/22/2025).

Wang, Kang *et al.* (Mar. 8, 2023). "Extracellular matrix stiffness—The central cue for skin fibrosis". In: *Frontiers in Molecular Biosciences* 10. Publisher: Frontiers. ISSN: 2296-889X. DOI: 10.3389/fmolb.2023.1132353. URL: https://www.frontiersin.org/journals/molecular-biosciences/articles/10.3389/fmolb.2023.1132353/full (visited on 03/24/2025).

Wang, Shuo and Yanhui Cui (2024). "Clinical significance of serum CXCL9, CXCL10, and CXCL11 in patients with lupus nephritis". In: *Immunity, Inflammation and Disease* 12.8. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/iid3.1368, e1368. ISSN: 2050-4527. DOI: 10.1002/iid3.1368. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/iid3.1368 (visited on 04/06/2025).

Wang, Yingyu *et al.* (Oct. 19, 2022). "The shared biomarkers and pathways of systemic lupus erythematosus and metabolic syndrome analyzed by bioinformatics combining machine learning algorithm and single-cell sequencing analysis". In: *Frontiers in Immunology* 13. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2022.1015882. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1015882/full (visited on 04/06/2025).

Wang, Yong-Fei *et al.* (Feb. 3, 2021). "Identification of 38 novel loci for systemic lupus erythematosus and genetic heterogeneity between ancestral groups". In: *Nature Communications* 12.1. Publisher: Nature Publishing Group, p. 772. ISSN: 2041-1723. DOI: 10.1038/s41467-021-21049-y. URL: https://www.nature.com/articles/s41467-021-21049-y (visited on 03/22/2025).

Wang, Zhizhang *et al.* (July 17, 2018). "Iron Drives T Helper Cell Pathogenicity by Promoting RNA-Binding Protein PCBP1-Mediated Proinflammatory Cytokine Production". In: *Immunity* 49.1. Publisher: Elsevier, 80–92.e7. ISSN: 1074-7613. DOI: 10.1016/j.immuni.2018.05.008. URL: https://www.cell.com/immunity/abstract/S1074-7613(18)30244-9 (visited on 03/22/2025).

Watanabe, Hiroyuki *et al.* (Nov. 2004). "Innate immune response in Th1- and Th2-dominant mouse strains". In: *Shock (Augusta, Ga.)* 22.5, pp. 460–466. ISSN: 1073-2322. DOI: 10.1097/01.shk.0000142249.08135.e9.

Watson, M L *et al.* (Dec. 1, 1992). "Genetic analysis of MRL-lpr mice: relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci." In: *Journal of Experimental Medicine* 176.6, pp. 1645–1656. ISSN: 0022-1007. DOI: 10.1084/jem.176.6.1645. URL: https://doi.org/10.1084/jem.176.6.1645 (visited on 04/01/2025).

Wieczorek, Marek *et al.* (Mar. 17, 2017). "Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation". In: *Frontiers in Immunology* 8. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2017.00292. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2017.00292/full (visited on 03/24/2025).

Wiener, A *et al.* (July 1, 2016). "CXCR5 is critically involved in progression of lupus through regulation of B cell and double-negative T cell trafficking". In: *Clinical and Experimental Immunology* 185.1, pp. 22–32. ISSN: 0009-9104. DOI: 10.1111/cei.12791. URL: https://doi.org/10.1111/cei.12791 (visited on 03/18/2025).

Wilder, Ronald L. (1998). "Hormones, Pregnancy, and Autoimmune Diseases". In: *Annals of the New York Academy of Sciences* 840.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1749-6632.1998.tb09547.x, pp. 45–50. ISSN: 1749-6632. DOI: 10.1111/j.1749-6632.1998.tb09547.x. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1998.tb09547.x (visited on 04/16/2025).

Willcocks, Lisa C. *et al.* (July 7, 2008). "Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake". In: *The Journal of Experimental Medicine* 205.7, pp. 1573–1582. ISSN: 0022-1007. DOI: 10.1084/jem.20072413. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2442635/ (visited on 04/03/2025).

Williams, Matthew A., Aaron J. Tyznik, and Michael J. Bevan (June 15, 2006). "Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells". In: *Nature* 441.7095, pp. 890–893. ISSN: 1476-4687. DOI: 10.1038/nature04790.

Williams, Stephanie, Phillip Stafford, and Steven A. Hoffman (June 7, 2014). "Diagnosis and early detection of CNS-SLE in MRL/lpr mice using peptide microarrays". In: *BMC Immunology* 15.1, p. 23. ISSN: 1471-2172. DOI: 10.1186/1471-2172-15-23. URL: https://doi.org/10.1186/1471-2172-15-23 (visited on 04/01/2025).

Winchester, Robert *et al.* (May 2012). "Immunologic Characteristics of Intrarenal T cells: Trafficking of Expanded CD8 T Cell β-Chain Clonotypes In Progressive Lupus Nephritis". In: *Arthritis and Rheumatism* 64.5, pp. 1589–1600. ISSN: 0004-3591. DOI: 10.1002/art.33488. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3297718/ (visited on 03/18/2025).

Wincup, C. and A. Rahman (Mar. 21, 2018). "PS8:157 Identifying the rates of iron deficiency and anaemia in patients with systemic lupus erythematosus". In: *Lupus Science & Medicine* 5 (Suppl 1). Publisher: Lupus Foundation of America. ISSN: 2053-8790. DOI: 10.1136/lupus-2018-abstract.200. URL: https://lupus.bmj.com/content/5/Suppl_1/A114.2 (visited on 04/30/2025).

Wolf, Sonya J. *et al.* (Sept. 1, 2019). "Ultraviolet light induces increased T cell activation in lupus-prone mice via type I IFN-dependent inhibition of T regulatory cells". In: *Journal of Autoimmunity* 103, p. 102291. ISSN: 0896-8411. DOI: 10.1016/j.jaut.2019.06.002. URL: https://www.sciencedirect.com/science/article/pii/S0896841119301647 (visited on 05/04/2025).

Wu, Jianfeng *et al.* (Feb. 2019). "CRISPR/cas9 mediated knockout of an intergenic variant rs6927172 identified IL-20RA as a new risk gene for multiple autoimmune diseases". In: *Genes & Immunity* 20.2. Publisher: Nature Publishing Group, pp. 103–111. ISSN: 1476-5470. DOI: 10.1038/s41435-018-0011-6. URL: https://www.nature.com/articles/s41435-018-0011-6 (visited on 04/06/2025).

Wu, Yan-wei, Wei Tang, and Jian-ping Zuo (Dec. 2015). "Toll-like receptors: potential targets for lupus treatment". In: *Acta Pharmacologica Sinica* 36.12. Publisher: Nature Publishing Group, pp. 1395–1407. ISSN: 1745-7254. DOI: 10.1038/aps.2015.91. URL: https://www.nature.com/articles/aps201591 (visited on 04/06/2025).

Wu, Yongkang *et al.* (Oct. 2014). "Double positive CD4+CD8+ T cells: key suppressive role in the production of autoantibodies in systemic lupus erythematosus". In: *Indian Journal of Medical Research* 140.4, p. 513. ISSN: 0971-5916 (Print). URL: https://journals.lww.com/ijmr/

fulltext/2014/40040/double_positive_cd4_cd8__t_cells__key_suppressive.10.aspx (visited on 03/18/2025).

Xiang, Shate *et al.* (Sept. 29, 2022). "Imbalance of helper T cell type 1, helper T cell type 2 and associated cytokines in patients with systemic lupus erythematosus: A meta-analysis". In: *Frontiers in Pharmacology* 13. Publisher: Frontiers. ISSN: 1663-9812. DOI: 10.3389/fphar.2022.988512. URL: https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2022.988512/full (visited on 04/01/2025).

Xiao, Yucai *et al.* (2025). "IL-17B alleviates the pathogenesis of systemic lupus erythematosus by inhibiting FASN-mediated differentiation of B cells". In: *JCI Insight* 9.18 (), e181906. ISSN: 2379-3708. DOI: 10.1172/jci.insight.181906. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11457847/ (visited on 04/06/2025).

Xiong, Hui *et al.* (Apr. 1, 2023). "Cytotoxic CD161−CD8+ TEMRA cells contribute to the pathogenesis of systemic lupus erythematosus". In: *eBioMedicine* 90. Publisher: Elsevier. ISSN: 2352-3964. DOI: 10.1016/j.ebiom.2023.104507. URL: https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(23)00072-5/fulltext (visited on 03/18/2025).

Xu, Wang-Dong, Xiao-Yan Liu, *et al.* (2020). "Association of MASP2 levels and MASP2 gene polymorphisms with systemic lupus erythematosus". In: *Journal of Cellular and Molecular Medicine* 24.18. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/jcmm.15656, pp. 10432–10443. ISSN: 1582-4934. DOI: 10.1111/jcmm.15656. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/jcmm.15656 (visited on 04/06/2025).

Xu, Wang-Dong, Da-Cheng Wang, *et al.* (Mar. 19, 2024). "An updated advancement of bifunctional IL-27 in inflammatory autoimmune diseases". In: *Frontiers in Immunology* 15, p. 1366377. ISSN: 1664-3224. DOI: 10.3389/fimmu.2024.1366377. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10985211/ (visited on 04/03/2025).

Xu, Zhipeng *et al.* (June 2021). "Rescue of maternal immune activation-induced behavioral abnormalities in adult mouse offspring by pathogen-activated maternal Treg cells". In: *Nature Neuroscience* 24.6. Publisher: Nature Publishing Group, pp. 818–830. ISSN: 1546-1726. DOI: 10.1038/s41593-021-00837-1. URL: https://www.nature.com/articles/s41593-021-00837-1 (visited on 03/19/2025).

Xue, Jimeng *et al.* (July 27, 2023). "Impaired regulatory function of granzyme B-producing B cells against T cell inflammatory responses in lupus mice". In: *Lupus Science & Medicine* 10.2. Publisher: Lupus Foundation of America. ISSN: 2053-8790. DOI: 10.1136/lupus-2023-000974. URL: https://lupus.bmj.com/content/10/2/e000974 (visited on 03/26/2025).

Yamada, Akiko *et al.* (Apr. 5, 2017). "Dual Role of Fas/FasL-Mediated Signal in Peripheral Immune Tolerance". In: *Frontiers in Immunology* 8, p. 403. ISSN: 1664-3224. DOI: 10.3389/fimmu.2017.00403. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5380675/ (visited on 04/01/2025).

Yamano, Nozomi *et al.* (2020). "Long-term Effects of 222-nm ultraviolet radiation C Sterilizing Lamps on Mice Susceptible to Ultraviolet Radiation". In: *Photochemistry and Photobiology* 96.4. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/php.13269, pp. 853–862. ISSN: 1751-1097. DOI: 10.1111/php.13269. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/php.13269 (visited on 11/01/2024).

Yang, Jing-Wen *et al.* (June 27, 2023). "The role and safety of UVA and UVB in UV-induced skin erythema". In: *Frontiers in Medicine* 10. Publisher: Frontiers. ISSN: 2296-858X. DOI: 10.3389/fmed.2023.1163697. URL: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2023.1163697/full (visited on 03/22/2025).

Yao, Minhao *et al.* (2023). "Disentangling the common genetic architecture and causality of rheumatoid arthritis and systemic lupus erythematosus with COVID-19 outcomes: Genome-wide cross trait analysis and bidirectional Mendelian randomization study". In: *Journal of Medical Virology* 95.2. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jmv.28570, e28570. ISSN: 1096-9071. DOI: 10.1002/jmv.28570. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.28570 (visited on 04/06/2025).

Yin, Yiming *et al.* (Feb. 11, 2015). "Normalization of CD4+ T cell metabolism reverses lupus". In: *Science Translational Medicine* 7.274. Publisher: American Association for the Advancement of Science, 274ra18–274ra18. DOI: 10.1126/scitranslmed.aaa0835. URL: https://www.science.org/doi/abs/10.1126/scitranslmed.aaa0835 (visited on 02/21/2025).

Youness, Ali, Charles-Henry Miquel, and Jean-Charles Guéry (Jan. 2021). "Escape from X Chromosome Inactivation and the Female Predominance in Autoimmune Diseases". In: *International Journal of Molecular Sciences* 22.3. Number: 3 Publisher: Multidisciplinary Digital Publishing Institute, p. 1114. ISSN: 1422-0067. DOI: 10.3390/ijms22031114. URL: https://www.mdpi.com/1422-0067/22/3/1114 (visited on 03/29/2025).

Young, K. A. *et al.* (Aug. 2018). "Less than 7 hours of sleep per night is associated with transitioning to systemic lupus erythematosus". In: *Lupus* 27.9, pp. 1524–1531. ISSN: 1477-0962. DOI: 10.1177/0961203318778368.

Young, Kendra A. *et al.* (Jan. 2017). "Combined role of vitamin D status and CYP24A1 in the transition to systemic lupus erythematosus". In: *Annals of the Rheumatic Diseases* 76.1, pp. 153–158. ISSN: 1468-2060. DOI: 10.1136/annrheumdis-2016-209157.

Yu, Yangsheng and Kaihong Su (Apr. 2013). "Neutrophil Extracellular Traps and Systemic Lupus Erythematosus". In: *Journal of Clinical & Cellular Immunology* 4, p. 139. ISSN: 2155-9899. DOI: 10.4172/2155-9899.1000139. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826916/ (visited on 03/31/2025).

Yu, Zheng *et al.* (Dec. 1, 2024). "Characterization of systemic lupus erythematosus subtypes using cluster analysis: insights from lymphocyte subpopulations". In: *Clinical Rheumatology* 43.12, pp. 3679–3688. ISSN: 1434-9949. DOI: 10.1007/s10067-024-07152-7. URL: https://doi.org/10.1007/s10067-024-07152-7 (visited on 02/04/2025).

Yuan, Shiwen *et al.* (June 22, 2022). "Phenotypical changes and clinical significance of CD4 CD8 T cells in SLE". In: *Lupus Science & Medicine* 9.1, e000660. ISSN: 2053-8790. DOI: 10.1136/lupus-2022-000660. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9226979/ (visited on 03/17/2025).

Zahalka, M A *et al.* (May 15, 1995). "Lymph node (but not spleen) invasion by murine lymphoma is both CD44- and hyaluronate-dependent." In: *The Journal of Immunology* 154.10, pp. 5345–5355. ISSN: 0022-1767. DOI: 10.4049/jimmunol.154.10.5345. URL: https://doi.org/10.4049/jimmunol.154.10.5345 (visited on 03/03/2025).

Zander, David Y. *et al.* (Apr. 18, 2022). "Cooperative effects of RIG-I-like receptor signaling and IRF1 on DNA damage-induced cell death". In: *Cell Death & Disease* 13.4. Publisher: Nature Publishing Group, pp. 1–10. ISSN: 2041-4889. DOI: 10.1038/s41419-022-04797-7. URL: https://www.nature.com/articles/s41419-022-04797-7 (visited on 03/24/2025).

Zelenay, Santiago *et al.* (Mar. 15, 2005). "Foxp3+ CD25– CD4 T cells constitute a reservoir of committed regulatory cells that regain CD25 expression upon homeostatic expansion". In: *Proceedings of the National Academy of Sciences of the United States of America* 102.11, pp. 4091–4096. ISSN: 0027-8424. DOI: 10.1073/pnas.0408679102. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC554795/ (visited on 05/03/2025).

Zhang, Qian *et al.* (Jan. 24, 2020). "Predominant Role of Immunoglobulin G in the Pathogenesis of Splenomegaly in Murine Lupus". In: *Frontiers in Immunology* 10. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2019.03020. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2019.03020/full (visited on 02/27/2025).

Zhang, Qidi *et al.* (July 2023). "A Preliminary Study in Immune Response of BALB/c and C57BL/6 Mice with a Locally Allergic Rhinitis Model". In: *American Journal of Rhinology & Allergy* 37.4, pp. 410–418. ISSN: 1945-8932. DOI: 10.1177/19458924231157619.

Zhang, Xuewu *et al.* (Mar. 4, 2003). "Crystal structure of the receptor-binding domain of human B7-2: Insights into organization and signaling". In: *Proceedings of the National Academy of Sciences* 100.5. Publisher: Proceedings of the National Academy of Sciences, pp. 2586–2591. DOI: 10.1073/pnas.252771499. URL: https://www.pnas.org/doi/10.1073/pnas.252771499 (visited on 03/20/2025).

Zhao, Li-dan *et al.* (Oct. 2017). "Contribution and underlying mechanisms of CXCR4 overexpression in patients with systemic lupus erythematosus". In: *Cellular & Molecular Immunology* 14.10. Publisher: Nature Publishing Group, pp. 842–849. ISSN: 2042-0226. DOI: 10.1038/cmi.2016.47. URL: https://www.nature.com/articles/cmi201647 (visited on 04/17/2025).

Zhao, Hai *et al.* (June 22, 2017). "What Else Can CD39 Tell Us?" In: *Frontiers in Immunology* 8. Publisher: Frontiers. ISSN: 1664-3224. DOI: 10.3389/fimmu.2017.00727. URL: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2017.00727/full (visited on 03/28/2025).

Zhao, Ying-Jie *et al.* (Feb. 18, 2022). "G-protein subunit α-14 is dysregulated in human placentas from systemic lupus erythematosus pregnancies". In: *Archives of Medical Science*. ISSN: 1734-1922, 1896-9151. DOI: 10.5114/aoms/146404. URL: https://www.archivesofmedicalscience.com/G-protein-subunit-14-is-dysregulated-in-human-placentas-from-systemic-lupus-erythematosus,146404,0,2.html (visited on 04/06/2025).

Zhao, Yueshui *et al.* (Oct. 1, 2018). "CD4+ T cells in obesity and obesity-associated diseases". In: *Cellular Immunology* 332, pp. 1–6. ISSN: 0008-8749. DOI: 10.1016/j.cellimm.2018.08.013. URL: https://www.sciencedirect.com/science/article/pii/S0008874918302077 (visited on 03/03/2025).

Zhou, Xian *et al.* (May 11, 2022). "mTORC2 contributes to murine systemic autoimmunity". In: Pages: 2021.03.27.437347. DOI: 10.1101/2021.03.27.437347. URL: https://www.biorxiv.org/content/10.1101/2021.03.27.437347v3 (visited on 03/20/2025).

Zwicker, Paula *et al.* (Feb. 16, 2022). "Application of 233 nm far-UVC LEDs for eradication of MRSA and MSSA and risk assessment on skin models". In: *Scientific Reports* 12.1. Publisher: Nature Publishing Group, p. 2587. ISSN: 2045-2322. DOI: 10.1038/s41598-022-06397-z. URL: https://www.nature.com/articles/s41598-022-06397-z (visited on 11/01/2024).

Supplements

Suppl. Table S1. Numbers of animals per week of age and genotype

Age in weeks	Number of NZM2410	Number of SLE123	Number of C57BL/6J
8	13	8	2
9	8	0	7
10	5	4	10
11	0	0	0
12	16	14	5
13	0	0	7
14	0	31	9
15	5	1	0
16	20	5	11
17	15	13	4
18	15	11	14
19	13	0	6
20	26	0	9
21	7	0	11

Suppl. Table S2. Numbers of individual experiments

	C57BL/6J	NZM2410	SLE123	Total
Flow cytometry	13	20	11	44
ScilVet ABC Plus+	12	22	12	46
Weight	13	17	12	42

Suppl. Table S3. Table of mouse IDs of UV experiment 1

Sample ID	Mouse ID	Group	Sample ID	Mouse ID	Group
A01	3	Blue	A37	2	Ctrl_24
A02	5	Ctrl_24	A38	10	UVC
A03	6	Ctrl_0	A39	7	UVC
A04	4	Ctrl_30	A40	6	UVC
A05	8	Ctrl_24	A41	12	Ctrl_24
A06	7	Ctrl_0	A42	3	UVC
A07	10	Blue	A43	2	Ctrl_30
A08	1	UVC	A44	5	Blue
A09	2	UVB	A45	11	Ctrl_30
A10	12	UVC	A46	10	Ctrl_24
A11	11	UVB	A47	8	Ctrl_30
A12	10	UVB	A48	2	Ctrl_0
A13	5	Ctrl_0	A49	7	Ctrl_24
A14	9	Ctrl_24	A50	1	UVB
A15	3	Blue	A51	11	Blue
A16	8	Ctrl_0	A52	8	UVC
A17	1	Ctrl_30	A53	5	Ctrl_30
A18	2	UVC	A54	12	Blue
A19	6	Blue	A55	9	UVB
A20	12	Ctrl_30	A56	12	UVB
A21	9	Ctrl_30	A57	3	Ctrl_0
A22	4	Ctrl_24	A58	6	Ctrl_30
A23	11	UVC	A59	2	Blue
A24	7	Blue	A60	1	Blue
A25	3	Ctrl_30	A61	6	Ctrl_24
A26	4	Blue	A62	4	UVC
A27	12	Ctrl_0	A63	9	UVC
A28	11	Ctrl_24	A64	4	UVB
A29	10	Ctrl_30	A65	5	UVC
A30	9	Blue	A66	3	Ctrl_24
A31	5	UVB	A67	7	Ctrl_30
A32	6	UVB	A68	10	Ctrl_0
A33	7	UVB	A69	11	Ctrl_0
A34	8	UVB	A70	8	Blue
A35	4	Ctrl_0	A71	9	Ctrl_0
A36	1	Ctrl_0	A72	1	Ctrl_24

Suppl. Table S4. Table of mouse IDs of UV experiment 2

Sample ID	Mouse ID	Group	Sample ID	Mouse ID	Group
B01	13	Ctrl_0	B40	23	Ctrl_0
B02	14	Ctrl_24	B41	20	Ctrl_30
B03	15	Ctrl_30	B42	14	Ctrl_30
B04	19	UVA	B43	16	Ctrl_0
B05	20	UVA_UVB	B44	18	UVA_UVB
B06	21	Blue445	B45	20	Blue445
B07	25	Ctrl_24	B46	22	UVA
B08	15	Blue445	B47	24	Ctrl_24
B09	17	Ctrl_24	B48	25	Blue445
B10	18	Ctrl_0	B49	15	Ctrl_0
B11	21	Ctrl_30	B50	16	UVA
B12	23	UVA_UVB	B51	17	Ctrl_30
B13	24	UVA	B52	21	UVA_UVB
B14	25	Ctrl_0	B53	22	Ctrl_24
B15	16	Blue445	B54	24	Blue445
B16	17	UVA_UVB	B55	13	Ctrl_30
B17	18	UVA	B56	16	Ctrl_24
B18	22	Ctrl_30	B57	17	Ctrl_0
B19	23	Ctrl_24	B58	19	Blue445
B20	24	Ctrl_0	B59	22	UVA_UVB
B21	25	UVA	B60	23	UVA
B22	14	UVA_UVB	B61	13	Blue445
B23	13	UVA	B62	15	Ctrl_24
B24	16	Ctrl_30	B63	16	UVA_UVB
B25	20	Ctrl_24	B64	19	Ctrl_30
B26	19	Ctrl_0	B65	22	Ctrl_0
B27	22	Blue445	B67	14	UVA
B28	25	Ctrl_30	B68	18	Blue445
B29	13	Ctrl_24	B69	15	UVA_UVB
B30	15	UVA	B70	20	Ctrl_0
B31	17	Blue445	B71	24	Ctrl_30
B32	19	UVA_UVB	B72	21	Ctrl_24
B33	21	Ctrl_0	B73	14	Ctrl_0
B34	23	Ctrl_30	B74	18	Ctrl_24
B35	25	UVA_UVB	B75	17	UVA
B36	13	UVA_UVB	B76	21	UVA
B37	14	Blue445	B77	24	UVA_UVB
B38	18	Ctrl_30	B78	23	Blue445
B39	19	Ctrl_24			

Suppl. Table S5. Downregulated genes in UV experiment 1

Condition	Number of genes	Genes
Blue 405 downregulated; Ctrl 30 downregulated; UVB downregulated; UVC downregulated	105	*Lyve1, Gstm2, Klk12, Gm14436, Tgtp2, Kcnh2, Psrc1, Gm13157, Lgalsl, Gm6548, Stac2, Kcnc4, Ovgp1, Mmrn1, Rab6b, Star, Myo7a, Gm14308, Psg18, 3110045C21Rik, Fmn2, Pirb, BC117090, Slc52a3, Trim12a, Il3ra, Gm4951, D930016D06Rik, Acox2, H2-Q9, Lce1f, Gm14430, Rpph1, Cyp2a5, C430002N11Rik, Gsta4, Trim34a, Hist2h2aa1, Gm14431, Stfa1, H2-Q8, Gm14288, Pcsk9, Kdelr3, Saa1, C920006O11Rik, Lpo, Pglyrp4, Rsl1, Gm3636, Hand1, Nppb, Slfn8, Gas2, Gm21188, Gpr176, Gstm6, Gm20744, Tpd52l1, Znf41-ps, 4732490B19Rik, BC021614, 2610507I01Rik, Gucy1a3, Slc6a17, Slfn10-ps, Gm3893, Ccdc109b, H2-K1, 1190007I07Rik, Myl7, AA467197, Pttg1, Klk9, 2810047C21Rik1, Gm8923, Zfp429, Halr1, Gm8898, 2210039B01Rik, Ndufs5, Gm14434, 2010005H15Rik, Lhx5, Zfp951, Defb14, Fbxo44, Clybl, Ttpa, Calm5, Trim30d, Defb4, Gm14305, Gipr, Cck, Gnb5, Ppp1r3e, Tceanc2, Atp6v0c, 2310002F09Rik, Prdm16, H2-Q7, Pnp2, Slfn2, Gm4724*
Blue 405 downregulated; UVB downredulated; UVC downregulated	8	*Cma2, Serpina9, Mthfsl, Muc2, Eml2, Fbxw10, Psg26, Degs2*

Condition	**Number of genes**	**Genes**
Blue 405 downregulated; Ctrl 30 downregulated; UVB downregulated	13	*Myo1h, Nnmt, Mcpt1, 5730480H06Rik, Mcpt-ps1, Aldh3b1, Trim30b, Rps4l, Unc5a, Trim40, Ifi203, Gm18853, F830016B08Rik*
Blue 405 downregulated; Ctrl 30 downregulated; UVC downregulated	7	*Gm5615, Hs3st1, Mup2, Slc15a2, Kansl1l, Tmem132d, D730005E14Rik*
Ctrl 30 downregulated; UVB downregulated; UVC downregulated	16	*Cox4i2, Cep19, Lce3a, Lce3b, 3000002C10Rik, Lce3c, Rundc3a, Lce3d, Wdr78, Cd300ld, Cyp24a1, Sprr2g, Lce3f, Ugt1a7c, Cetn4, Sprr2f*
Blue 405 downregulated; UVB downregulated	7	*Elf3, Gls2, Ccdc13, Sprr2a1, Sprr2a2, Nxpe2, Syt5*
Blue 405 downregulated; UVC downregulated	3	*Zfp772, Clec2e, Adrb2*
Blue 405 downregulated; Ctrl 30 downregulated	15	*Apobec3, Clic5, Zfp456, 4933431E20Rik, Lyz1, Klf12, Mup19, Jakmip3, Aox4, Bmpr1b, Fut10, Prr5, Lingo4, Adgrg7, Fam132b*
UVB downregulated; UVC downregulated	17	*Dsc2, Hist1h2bc, Hist1h4m, 2310003N18Rik, Adamts13, 2810029C07Rik, 4933409K07Rik, Pon1, Rnf222, Hist1h4i, Tinagl1, Fscn2, Mir1938, Ptgs2os2, Dlx1as, Slc22a21, Arg1*

Condition	**Number of genes**	**Genes**
Ctrl 30 downregulated; UVB downregulated	117	*Cd69, Htr1d, Arsj, B3gnt3, Ifi44, Treml2, Gucy2c, Phf11d, Ly86, Gbp7, Ptpn5, Tph1, Pik3ap1, B430306N03Rik, Zfpm2, Ptgis, Pydc4, Mnda, Vcan, Ms4a4c, Apol9a, Gzmb, 6430562O15Rik, Cpa3, Gbp6, C3, Fgf7, Oasl2, Irgm2, Mndal, Clec4e, Ddx60, Rtn4rl2, Trim30a, Sulf1, Csf3r, Shisa7, Ifit3b, Fcgr1, Ifit1, Ccr5, Slc13a3, Isg15, Pstpip1, Herc6, Plekha4, Ssc5d, Loxl3, Cd180, Isg20, BC147527, Gabra3, C3ar1, Zbp1, Serping1, Sell, Slfn4, Ccl19, Mx2, Ms4a6c, Gm4070, Cybb, Usp18, Ifit3, Htr2a, Fcrlb, Rsad2, 9130409J20Rik, Slfn3, Gm1966, 4930599N23Rik, Gvin1, Aldh1a2, Clec4d, Slc28a2, Slamf7, Ifit2, C5ar1, Igtp, Ms4a4d, Tmem132c, Ly6a, Batf2, Nlrp3, AI607873, Gbp2, Il1rl1, Ccr1, 9930111J21Rik2, Themis2, Tlr7, Fgf23, Irg1, Irf7, Pydc3, Tnfsf14, Iigp1, Cd300lf, Ifi204, Slfn1, Gbp5, Oas2, Ms4a6b, Epsti1, Elfn1, Apol9b, Olfr56, Mpeg1, Rtp4, Mx1, Spic, Cxcl10, Mefv, Pira2, Ifi205, Tlr8, Pyhin1*
Ctrl 30 downregulated; UVC downregulated	5	*Col26a1, Gm2083, Shroom1, Sprr2i, Ccdc85a*
Blue 405 downregulated	20	*Krt6b, Fut2, Ccdc163, 9830147E19Rik, Ncmap, Saxo2, Rgs17, Klk14, Synpr, Uox, Crtac1, Cited1, Gm10677, Creb3l1, Ofcc1, Il33, Il17b, Piezo2, Insm1, Trem2*

Condition	**Number of genes**	**Genes**
UVB downregulated	90	*Adap2, Ankrd66, Fcgr3, Tmem8, Clec2f, Tcea3, Slc11a1, H2-Q5, Uba7, Fbxo2, Gdf9, Slfn5, Ifit1bl1, Gm853, D830031N03Rik, Lilrb4a, Ifnb1, Ccl4, Phox2a, Clec4a2, Isoc2b, Slc22a12, Sema3e, Slc22a13b-ps, Gm12250, S100a7a, Lilr4b, C8a, C130036L24Rik, Mal, Fcgr4, Naalad2, Irgm1, Gm16793, Hk3, Maoa, Sp140, Tmem17, Vtn, Fam69c, Il7r, Ccl3, Ly6i, Csf1, Qrich2, Dnm3, Hck, Tarm1, Gm14440, Slc2a3, Foxf1, Clec10a, Oas1a, Myo1f, Bmp8a, Ms4a6d, Ly9, Tlr5, Sp110, Phf11b, Gm27162, Hoxa5, Oas3, Phf11a, Sfmbt2, Cxcl3, Oas1b, A530064D06Rik, Hist1h1c, Nacad, Myh7b, Dpf1, Sp100, Xaf1, Oas1g, 9930111J21Rik1, Samd11, Csf2rb2, Uchl1, Dhx58, Arhgdib, Sgtb, Cd200r1, Il15, Slamf1, Ticam2, Col28a1, Siglec1, Hoxa1, Cxcl9*
UVC downregulated	29	*4930590L20Rik, Stfa2, Lgi3, Pbp2, Fggy, Pla2g4e, Clcf1-pold4, Vmn2r29, Catsper4, Sprr2k, Fbxl22, Sprr2d, 1700031A10Rik, Abcc3, Lrit2, Ugt1a6a, Tmem108, Kcnj12, Chp2, 1810044D09Rik, A930006I01Rik, Lce3e, Ifi202b, Gm10400, Il27, Sec31b, Clcf1, Guca1b, Msantd1*

Condition	**Number of genes**	**Genes**
Ctrl 30 downregulated	120	*Tmprss2, C1ra, Serpina3f, Gm13177, Ip6k3, Islr, Cyp7b1, Cfb, 4933433G19Rik, C1ql3, Rnase2b, Tubb4a, Arl11, Esr1, Scrn1, Mobp, Cyp2f2, Hsf2bp, Cma1, Coch, Slc47a1, Sfrp4, Mfap5, Zfp273, Zc3h12b, Ifi27l2b, C4b, Plac8, Lrrc25, Lbp, C1s1, Tnfaip6, Sectm1b, Aldh1b1, Timp1, Olfml3, Ly6c1, Enpep, Aldh1a1, Lacc1, E030003E18Rik, Trpc3, Tlr1, Cd248, Cpxm1, Tmem132b, Lrrc48, Chst5, Igfbp6, Vcam1, Cyp2e1, Zfp36l3, Gm8979, Aknad1, Tmc3, Nphp1, Rec8, Rgag4, Sdk1, Sox11, Cd209f, Slc7a3, Sfrp2, Eif3j2, Dpep1, Cep112, Fam26f, Nr2f1, Hgf, Adra1d, 1110051M20Rik, Misp, Serpinf1, Ltf, Hyal6, Ccbe1, Dbx2, Rnf128, Saa2, Efemp1, AW551984, Hacd4, Dpyd, Epha3, Sh3gl3, Kcnn3, Muc1, Ccno, Cfh, Fpr2, Ccdc80, Tmem200a, Folr2, Prtg, Ly6c2, Gpr63, Pcolce, Saa3, Mmp23, Epdr1, Pcdhga3, Cpq, Fam161a, Ms4a4b, Pdgfd, Gfpt2, Ifi27l2a, Apol10b, Zcchc5, Gm13251, Slc2a13, BC024386, Chrdl1, Srpx, Lyz2, Ccdc122, Armcx4, Agt, C77370, Slc26a4*

Suppl. Table S6. Upregulated genes in UV experiment 1

Condition	Number of genes	Genes
Blue 405 upregulated; UVB upregulated; UVC upregulated; Ctrl 30 upregulated	76	*Lyrm7, Popdc3, 2410017I17Rik, Cilp2, Serpinb9g, Gm8615, Adh6a, Lgals6, Slc5a11, Akap6, Adh1, Gsta2, 5430427M07Rik, Gm11127, Pianp, Sfi1, Mycl, Gfap, 4930525G20Rik, Abhd1, Tgm7, Gm1821, Acbd4, Gm266, Gm5478, Skint2, Abca5, H2-Bl, Trim34b, 2300005B03Rik, Ak1, Kmo, Park2, Pcdhgb8, Atp6v0c-ps2, Morf4l1-ps1, H2-Q2, Pglyrp1, Atp10d, Gstm4, Eno1b, Klra5, Emid1, A530050N04Rik, Lgals4, Pramef12, Gm5414, Lypd2, Ano7, Gm8801, H2-Q1, Sebox, Eno2, H2-Ea-ps, Serpinb13, Cftr, Gm12338, Flg2, Gjc2, Gli2, Prom1, Rab15, Zfp473, E2f2, Fbp2, Klra17, Mrgprb3, Ren1, Pcdh18, Gm12866, C130079G13Rik, Cttnbp2, 2610305D13Rik, Gm14124, Snph, Hddc3*
UVB upregulated; UVC upregulated; Ctrl 30 upregulated	33	*Npl, Ache, Cyp17a1, Serpinb3a, Tcaf2, Rab27a, C1s2, Skint9, Serpinb3b, Postn, Rab3b, Slc6a4, Bbox1, A530016L24Rik, Pira1, Depdc1b, Ccl17, Ttk, 9530077C05Rik, Rsph1, Paqr5, Tmprss7, Pcdhb11, Cd163l1, Sectm1a, Prob1, Skint10, Fgf21, Ifitm6, Ccl22, F830045P16Rik, Kif26b, Tdh*
Blue 405 upregulated; UVC upregulated; Ctrl 30 upregulated	14	*Accsl, Ppm1n, H2-Ob, Ankrd63, Gm11128, Spns3, Pyy, Dixdc1, Kif23, Chst8, Tescl, Itln1, A530072M11Rik, Masp2*

Condition	**Number of genes**	**Genes**
Blue 405 upregulated; UVB upregulated; Ctrl 30 upregulated;	10	*Gm10324, G6b, 1500011B03Rik, Skint5, Igsf23, Gm3435, Dapl1, Otop3, Baiap2l2, Alox12e*
Blue 405 upregulated; UVB upregulated; UVC upregulated	7	*Il1f10, Ugt1a1, Fut1, P2ry4, Rnase1, Gchfr, Hdhd3*
UVC upregulated; Ctrl 30 upregulated	25	*Ccdc113, Prr15, Cacna1e, Selenbp2, Mir8116, Oas1f, H2-M5, Jakmip2, Epor, Lrrc4, Prkcq, Ntrk1, Lama1, Galnt14, BC018473, 1700012P22Rik, Pcdhb3, Creg2, Abcc8, Grik3, Gm13003, Rnase2a, 3110009F21Rik, Colgalt2, Slc30a10*
UVB upregulated; Ctrl 30 upregulated	17	*Krt10, Gm2115, Ctse, Clec9a, Rbm46, 2310007B03Rik, Ly6k, Gm16938, Pls1, Tesc, Them5, Serpina3h, Nnt, Tmem117, Calr3, 1700029M20Rik, Hmgcs2*
Blue 405 upregulated; Ctrl 30 upregulated	6	*Pramel7, Gpr37, Fam129c, Slc26a2, AA465934, Ceacam16*
UVB upregulated; UVC upregulated	7	*Skint11, Sv2c, 4933413L06Rik, Gprin2, 1700023F06Rik, Ifitm2, Atp12a*
Blue 405 upregulated; UVC upregulated;	6	*Gpha2, Lpar3, Lgi2, Slc6a19, Syt9, Nsg2*
Blue 405 upregulated; UVB upregulated	8	*Hadh, Acyp2, Jrk, Syngr1, Tnfsf18, Tmem177, Tmem56, Abhd3*

Condition	**Number of genes**	**Genes**
Ctrl 30 upregulated	68	*Fam184b, Scgb1a1, Ryr2, Lax1, Siglech, Krt12, Mthfs, Eif3j1, Gm6623, Il18r1, Irx6, Car6, Klrb1c, F2rl2, Ubash3a, Dab1, Tmem139, Comp, Serpinb10, LOC105245869, BC030867, Lor, 4930412C18Rik, Trim15, Fgf22, Ganc, Grin2c, Flrt1, Wipf3, Ucp3, B430319G15Rik, Hoxc13, Gm5577, Wnt10b, Capn3, Frem2, Rassf6, Abcb1b, Hba-a2, Mmp9, Cpn1, Nuak1, Nkx2-2, 4930579P08Rik, Pinlyp, Fam179a, Robo2, Gm17757, Vipr1, Gm6639, Cux2, Slc38a3, Kcng4, Serpinb12, Fxyd7, Snora44, Corin, Mup9, A330033J07Rik, Porcn, Mesp1, Fam229a, Mef2b, Pik3c2g, AU018091, Muc5b, Nanos3, Wnt2*
UVC upregulated	32	*Fendrr, Eml5, Pdcd4, Cd96, Htr7, Mlip, Sept3, l7Rn6, Rps19-ps3, Apol7c, Il20ra, Cml3, Vmn1r63, Mak, Gcm2, Tnfaip8l3, Car12, Gpr151, Gsdmc2, Rnase6, Ffar2, Gadl1, Alox5, Trim17, Nr5a2, Hpcal4, Ednra, Gna14, H2-DMa, Sult5a1, Tmod4, Cxcr1*
UVB upregulated	33	*Gm3219, Faah, Zdhhc15, Gal3st4, Snap91, Fabp6, Gm960, Skint1, Adamts15, Rwdd3, Pcdhb9, Shisa2, Slc5a9, Dusp26, Snurf, Krt77, Krt1, Prg2, Kcnj9, Inpp4b, Gm4477, Cyp21a1, D030028A08Rik, Pm20d1, Prdm8, H2-Q6, Clca3a2, Tjp3, Atp4a, Il1f6, Duox2, Lyplal1, Tmem229a*

Condition	Number of genes	Genes
Blue 405 upregulated	21	*Mapt, Atp8a2, Efcab7, Cnnm1, Tnfrsf19, Hist2h2bb, Sucnr1, Sox21, Raver2, Ccnb1ip1, Xcl1, Catsperg2, Tmem171, Ssx2ip, Dlg2, BC022687, Dnajc6, Spats1, Serpina11, Gng4, Zfp791*

Suppl. Table S7. Downregulated genes in UV experiment 2

Condition	Number of genes	Genes
Blue 445 downregulated; Ctrl 30 downregulated; UVA downregulated; UVA/B downregulated	81	*Nppb, Htr1d, Lyve1, Slfn8, Gstm2, Gm14440, Gm14436, Wdr78, Gstm6, Gm20744, Tpd52l1, Ccbe1, Clec2f, Hist1h2bc, Znf41-ps, Kcnh2, Psrc1, Hist1h4i, Gm13157, Lgalsl, BC021614, Cep19, 2610507I01Rik, Stac2, Sprr2a3, Slfn10-ps, Kcnc4, Gm3893, Ovgp1, Ccdc109b, Rab6b, Eml2, Ccdc163, Gls2, Trim40, Psg18, 3110045C21Rik, Pttg1, Klk9, Sema3a, Isoc2b, Trim12a, Fbxw10, Zfp429, Halr1, D930016D06Rik, Cma2, Gm8898, Acox2, H2-Q9, 2210039B01Rik, Lhx5, Zfp951, Cntn2, Mcpt1, Fbxo44, Serpina9, Ttpa, Clybl, Calm5, 5730480H06Rik, Shisa7, Mcpt-ps1, Gm14305, Gsta4, Gm14431, Stfa1, H2-Q8, Kdelr3, Saa1, Gnb5, Ppp1r3e, Rsph9, Lpo, Tceanc2, Pglyrp4, Atp6v0c, Rsl1, H2-Q7, 4933409K07Rik, Pnp2*
Blue 445 downregulated; UVA downregulated; UVA/B downregulated	8	*Cox4i2, A230056P14Rik, Mmrn1, 1190007I07Rik, Fbxl22, Rec8, Pcolce2, Gstm5*

Condition	**Number of genes**	**Genes**
Blue 445 downregulated; Ctrl 30 downregulated; UVA/B downregulated	12	*Tgtp2, Tcea3, Gucy2c, Tph1, Clec2e, Star, Ptpn22, Pirb, Pdk1, Lrrc48, D730005E14Rik, Hand1*
Blue 445 downregulated; Ctrl 30 downregulated; UVA downregulated	8	*Kansl1l, 9830147E19Rik, Fmn2, Tmem132c, Vmn2r29, Klf12, Tmem132d, Prdm16*
Ctrl 30 downregulated; UVA downregulated; UVA/B downregulated	14	*Tmprss2, Klk12, Gpr176, Slc6a17, 9130409J20Rik, Gm5615, Muc2, 2810047C21Rik1, Lce1f, Cyp2a5, Rundc3a, Cck, C920006O11Rik, Gm3636*
Blue 445 downregulated; UVA/B downregulated	12	*Cryaa, Inmt, Slc52a3, Bcl2a1d, 2010005H15Rik, Ryr1, C430002N11Rik, Tmc3, Bcl2a1a, Notum, Rab7b, Hoxa1*
Blue 445 downregulated; UVA downregulated	2	*Gas2, Slc22a21*
Blue 445 downregulated; Ctrl 30 downregulated	12	*Ip6k3, Rnf152, Fbxo2, Gm6548, Htatip2, Sema3e, Trim30d, Mthfsl, Trim34a, Gipr, Psg26, Aox4*
UVA downregulated; UVA/B downregulated	7	*Rps4l, Zfp772, Myo7a, Nudt1, Defb14, C130036L24Rik, Aknad1*
Ctrl 30 downregulated; UVA/B downregulated	13	*Gabrp, B3gnt3, Apobec3, Krt84, Lrat, Myl7, Zfp456, Prr9, Shroom1, Prr5, Elfn1, Fam132b, Slc9b2*
Ctrl 30 downregulated; UVA downregulated	8	*Ggh, Mmp10, Gm7334, 4933431E20Rik, Sord, Tmem132b, Dppa5a, Gm13251*
Blue 445 downregulated	24	*Srd5a1, Gm13305, Nfatc4, Mybpc2, Nyap1, Scd2, 5033406O09Rik, Myl1, Fut2, Alppl2, H2-K1, Ear6, Cbr3, Ncmap, Srrm4, 3000002C10Rik, Ndufs5, Gm10400, Cys1, Chst5, Arhgdib, Jakmip3, Mall, Pira2*

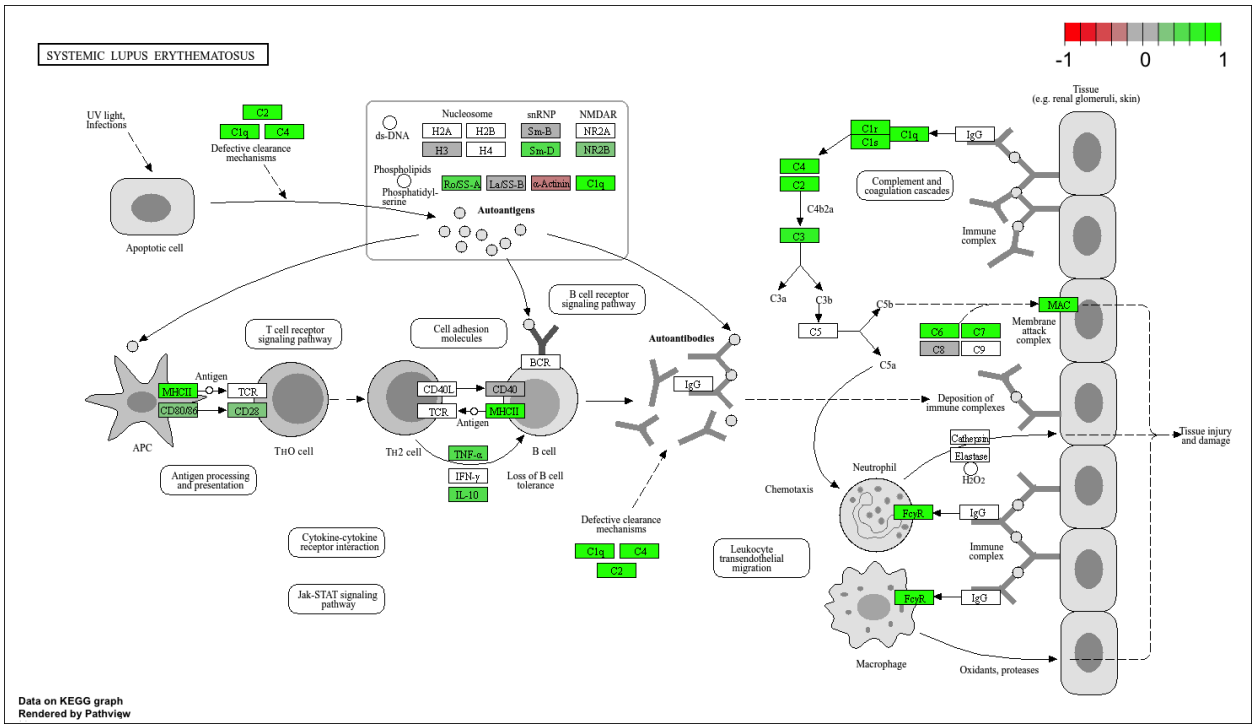
Condition	**Number of genes**	**Genes**
UVA/B downregulated	38	*Dsc2, Defb5, 1700029B22Rik, Mmp8, Nrxn2, Olfr920, Wscd1, Slc45a3, Sspn, Pbp2, AI987944, Gm853, D830031N03Rik, Cyp1a1, Ccdc106, Mmp24, Hoxa5, Ccno, Idua, S100a3, Ypel4, Hist1h1c, Morn2, Gpr63, Gpr55, Slc22a13b-ps, Dlx1as, Zfp708, Gm13546, Sec31b, Dhfr, Zfp36l3, Krt7, Mir30d, Nupr1l, Art4, Gm12359, Alyref2*
UVA downregulated	17	*Fut10, Tmem198, H2-Eb2, Rpl29, 2810002D19Rik, Myo1h, Tinagl1, Serpinb6c, Gm14308, Fam171b, Gm14327, Gm14434, Gm14430, Pcsk9, Lce3d, Gm4724, 1110051M20Rik*
Ctrl 30 downregulated	32	*Gm21188, 5830444B04Rik, 1700028J19Rik, Tchh, Unc5a, Syt17, Nckap5, Eya2, Fggy, Krt75, AA467197, Il3ra, 2810468N07Rik, Gm4951, Plac9b, Cbs, 4930557K07Rik, Myh7b, Mir1938, Klrc1, Piezo2, Cyp24a1, Lhfpl1, F830016B08Rik, Aldh3b1, Hist2h2aa1, Nphp1, Epsti1, Ugt1a7c, Maoa, Adrb2, Cited1*

Suppl. Table S8. Upregulated genes in UV experiment 2

Condition	Number of genes	Genes
Blue 445 upregulated; Ctrl 30 upregulated; UVA upregulated; UVA/B upregulated	52	*Lyrm7, Cilp2, Adh6a, Serpinb3a, Tcaf2, Akap6, 1500011B03Rik, Gsta2, Adh1, Chil1, Gm11127, Pianp, Sfi1, Hadh, Gm5088, Abhd1, Ankrd33b, Gm8909, Ctse, Gm5478, Lgi2, Ak1, Icam2, Pyy, Pcdhgb8, Morf4l1-ps1, H2-Q2, Pglyrp1, Atp10d, Gstm4, A530050N04Rik, Tmem171, Lgals4, Dixdc1, Gm5414, Lypd2, Ano7, Eno2, Gm12338, E130008D07Rik, Ifitm2, Prom1, Rab15, Olr1, Ifitm6, Cacna1b, Gsta1, Muc5b, 2610305D13Rik, Snph, Hddc3, Mgl2*
Blue 445 upregulated; UVA upregulated; UVA/B upregulated	4	*Ly6k, Pls1, Zfand2a, H2-Q1*
Blue 445 upregulated; Ctrl 30 upregulated; UVA upregulated	4	*Eml5, Abca5, Pcdh18, Wnt2*
Blue 445 upregulated; Ctrl 30 upregulated; UVA/B upregulated;	8	*Nav3, Trim15, Vmn1r63, Emid1, Serpinb13, Bckdhb, Mup9, Tcf24*
Ctrl 30 upregulated; UVA upregulated; UVA/B upregulated	26	*2410017I17Rik, Mapt, Skint5, Serpinb3b, Mgarp, Slc6a4, Timd4, Tgm7, Alox8, Pira1, Skint2, Gm2518, Gm7367, 9530077C05Rik, Tnfsf18, Fut1, Col6a5, Skint10, Dusp13, Gjc2, Il1f6, Gm12866, Ccl22, Il22ra2, Atp12a, Kif26b*
Blue 445 upregulated; UVA upregulated	5	*Gm4432, Jrk, Pramef12, Tmem117, A530072M11Rik*
Blue 445 upregulated; UVA/B upregulated	7	*Adamts15, F5, Acyp2, Eno1b, Nnt, Steap1, Lyplal1*

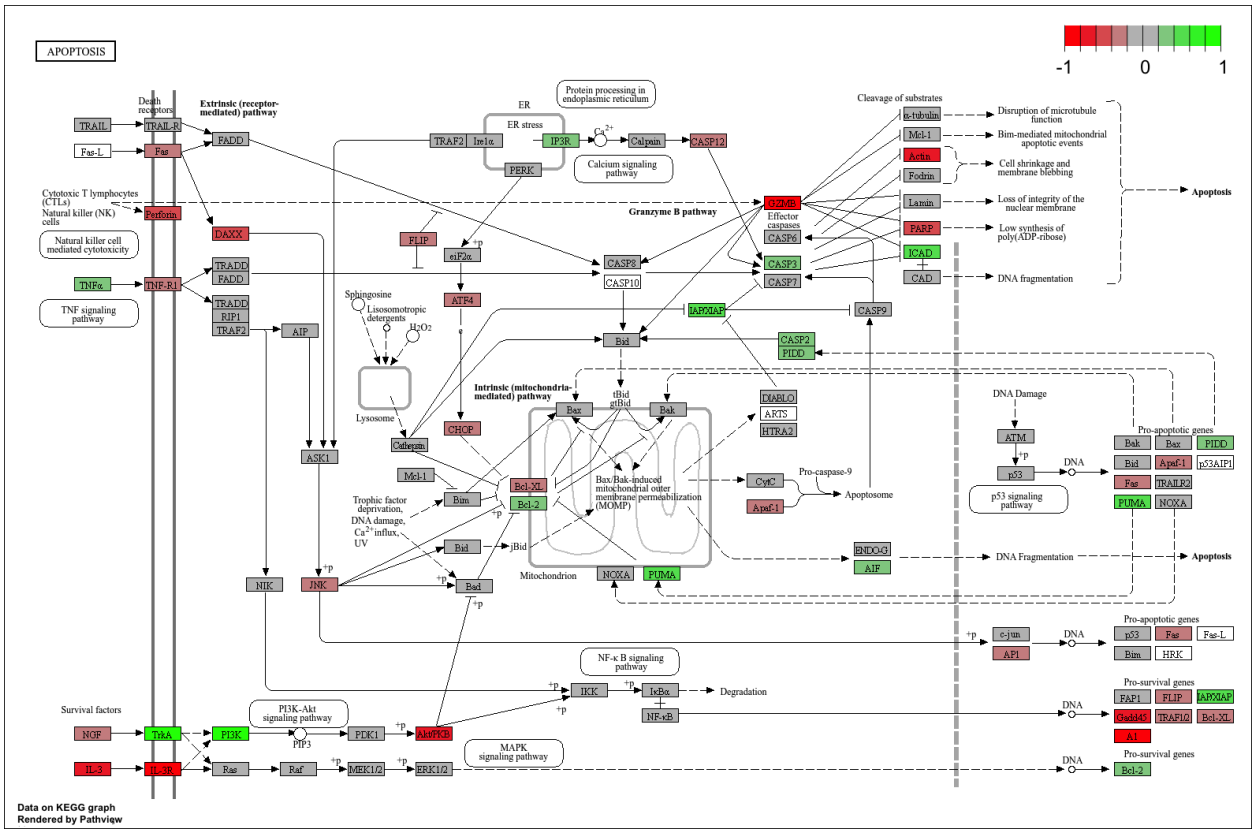
Condition	**Number of genes**	**Genes**
Blue 445 upregulated; Ctrl 30 upregulated	3	*C4a, Spns3, Gbp10*
UVA upregulated; UVA/B upregulated	16	*Col8a1, Asb16, Ankrd1, Mrap2, Accsl, Pla2g4b, Slc1a1, Capn12, Ccl24, Cd209b, 2010016I18Rik, Tnip3, Irgc1, Fgf21, Wfdc21, C130079G13Rik*
Ctrl 30 upregulated; UVA upregulated	20	*Dgki, G6b, Lgals6, 4930525G20Rik, Psca, A530016L24Rik, 4930562F07Rik, Gm266, Slc26a2, Jakmip2, Gm8580, Park2, Rsph1, Tmprss7, Smpdl3b, Ssx2ip, Them6, BC022687, Cftr, Serpina3g*
Ctrl 30 upregulated; UVA/B upregulated	38	*Lta, Ppfia4, Ache, Il1f10, Ugt1a6b, Skint9, Postn, 4930578C19Rik, Sprr2b, Sncg, Rbm46, Ccl20, 2300005B03Rik, Depdc1b, Rab26, Slc39a11, Slc6a19, Ntrk1, Kcnj9, Has3, Slurp1, Sebox, Car12, Sidt1, Abcc8, Kif23, Prdm8, Hebp1, Hamp2, Clca3a2, Gli2, Gpr182, Hdhd3, Fbp2, Mrgprb3, Fa2h, Cc2d2a, Tdh*
Blue 445 upregulated	11	*Ryr2, Hmga2-ps1, Rps19-ps3, Cd320, Cpn1, Ttc8, BC037704, Rnase1, Gdpd5, Decr1, Glis1*
UVA upregulated	31	*Popdc3, Serpinb9g, Gm10324, Slc5a11, Stra6, Igsf23, Gfap, Tnf, Pcdhga1, Fam129c, H2-Bl, Wipf3, Stk10, Trim34b, Gm11128, Aox3, F7, Cd4, Slc37a2, Gm8801, H2-Ea-ps, Tescl, Itln1, E2f2, Klra17, Atp1b1, Defb3, Slc30a10, Masp2, Pnmt, Gm14124*

Condition	**Number of genes**	**Genes**
UVA/B upregulated	51	*Npl, Mmp1a, Atl1, Abcg4, Lcn2, Spc24, Gm6623, Cacna1e, F2rl2, Gm3435, Htr7, Dab1, Comp, Slc5a9, Slc2a9, Prl2c3, H2-Ob, l7Rn6, D17Ertd648e, Hba-a1, Tpk1, Snrpn, 2610203C22Rik, Pi16, Gm10639, Fetub, Them5, Izumo1, Inpp4b, Pcdhb11, Il23a, Adgrg5, Clcn6, Dlg2, Il19, Gm12185, Gm6034, Igfbp5, Xpnpep2, P2ry4, S100a9, Il24, Hpcal4, Ednra, Gna14, Nod2, Eva1a, Snx10, Cttnbp2, Ces1d, Ctsw*
Ctrl 30 upregulated	47	*Foxd3, Siglech, Hivep3, Wnt9b, Gm3776, Upk3b, 5430427M07Rik, Ubash3a, Ugt1a1, Bbox1, Tmem45a2, Gal, Serpinb10, Pcdhb12, Ppargc1b, Acbd4, Gp5, Mir8116, Dapl1, Ppm1n, Syngr1, 4933404O12Rik, Pla2g3, Grin2c, Adamtsl1, Sv2c, Ccl17, Slc19a3, Ttk, Chst7, 1700012P22Rik, Sectm1a, Ceacam16, Gprin2, Tmem177, Cd59a, Slc16a14, Flg2, Pglyrp2, Nr5a2, Pdzrn4, Aldh3a1, Chit1, Adh7, Col23a1, Ghrh, Hnf4a*

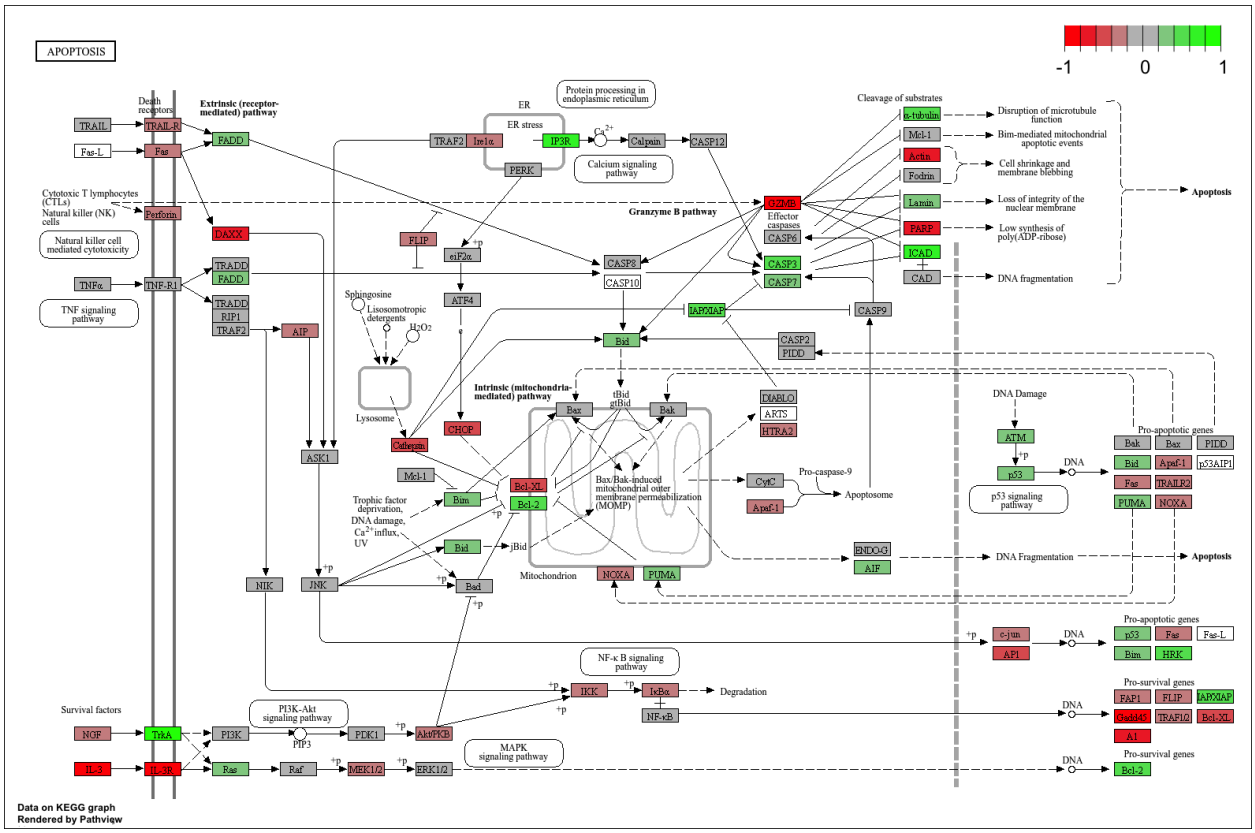


Suppl. Figure S1. KEGG pathway for systemic lupus erythematosus in females of UV experiment 2 after UVA/B irradiation

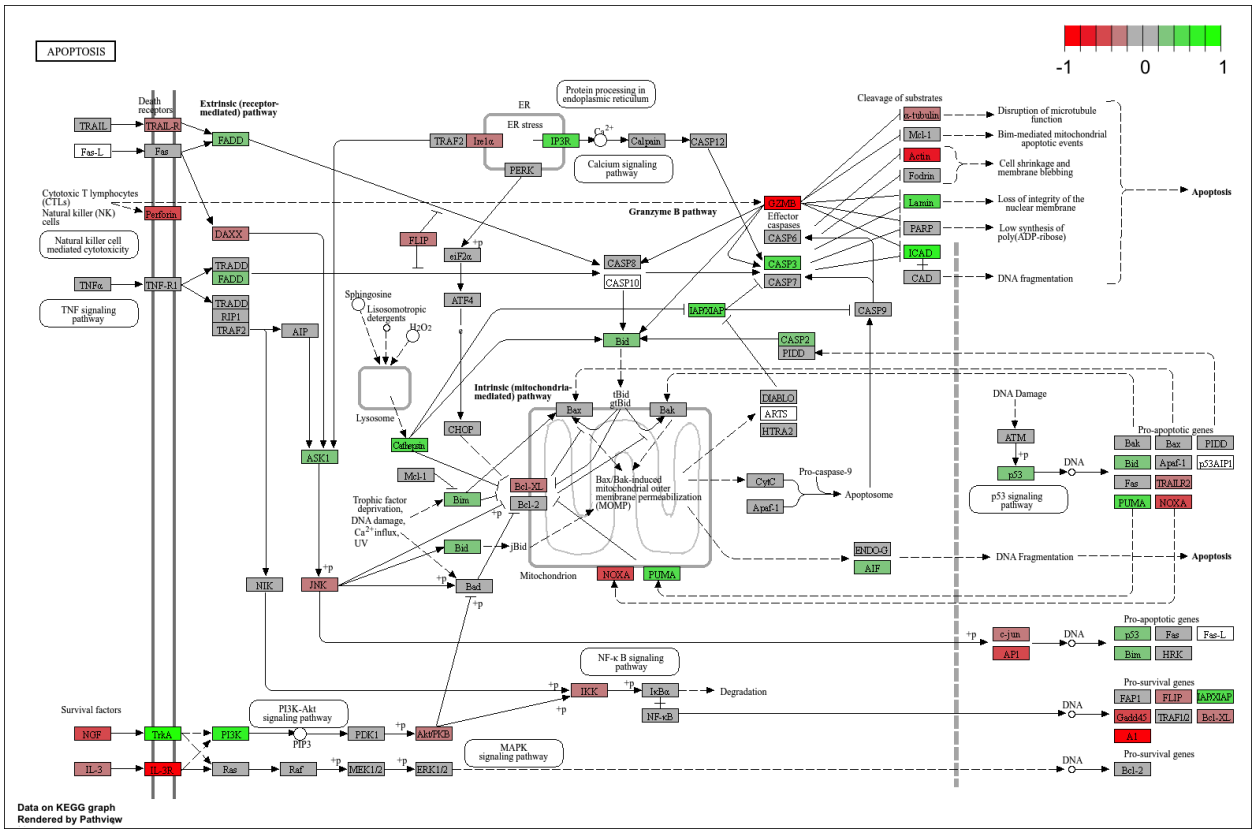
Murine systemic lupus erythematosus KEGG pathway plot for female NZM2410 mice in comparison to female C57BL/6J mice after UVA/B irradiation in UV experiment 2.



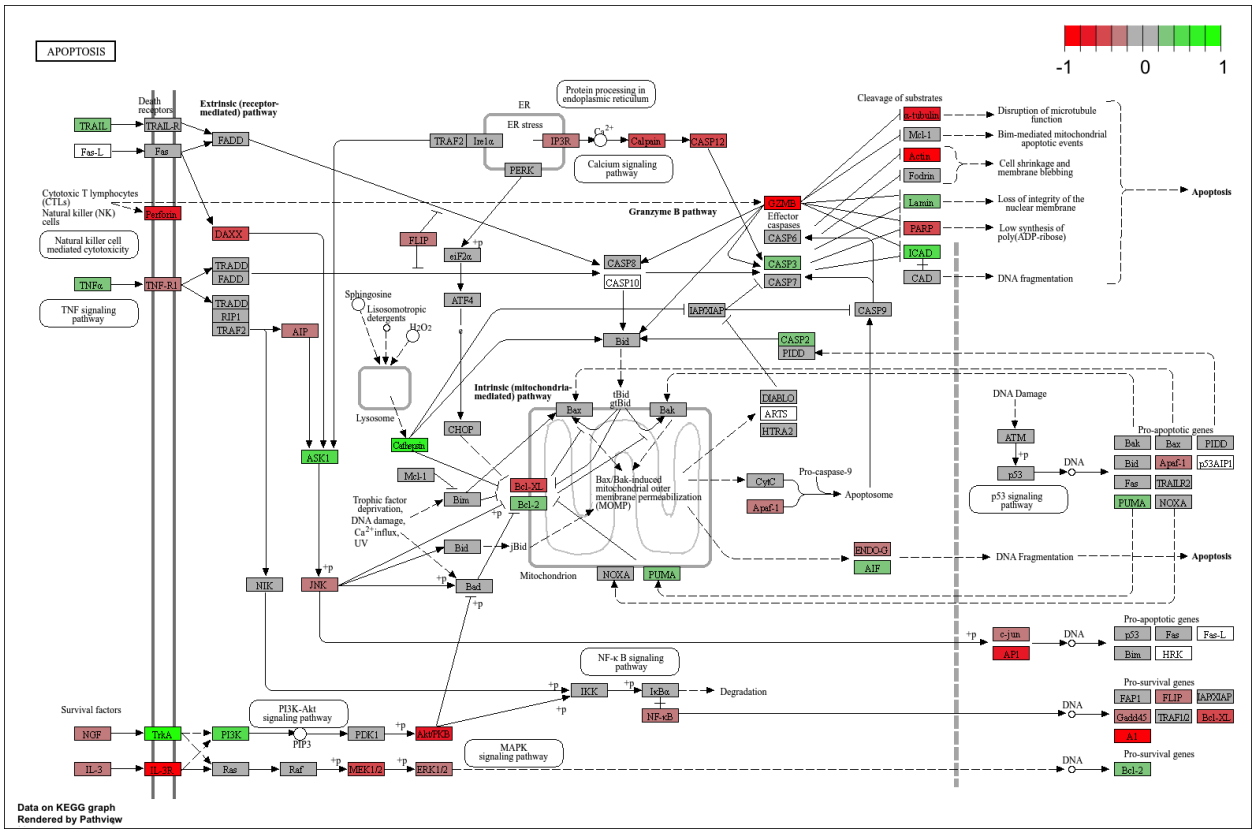
Suppl. Figure S2. Apoptosis KEGG pathway plot for control 30 h samples of UV experiment 1
Murine apoptosis KEGG pathway plot for control 30 h samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. Male and female mice were analysed together.



Suppl. Figure S3. Apoptosis KEGG pathway plot for UVB treated samples of UV experiment 1
Murine apoptosis KEGG pathway plot for UVB irradiated samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. Male and female mice were analysed together.

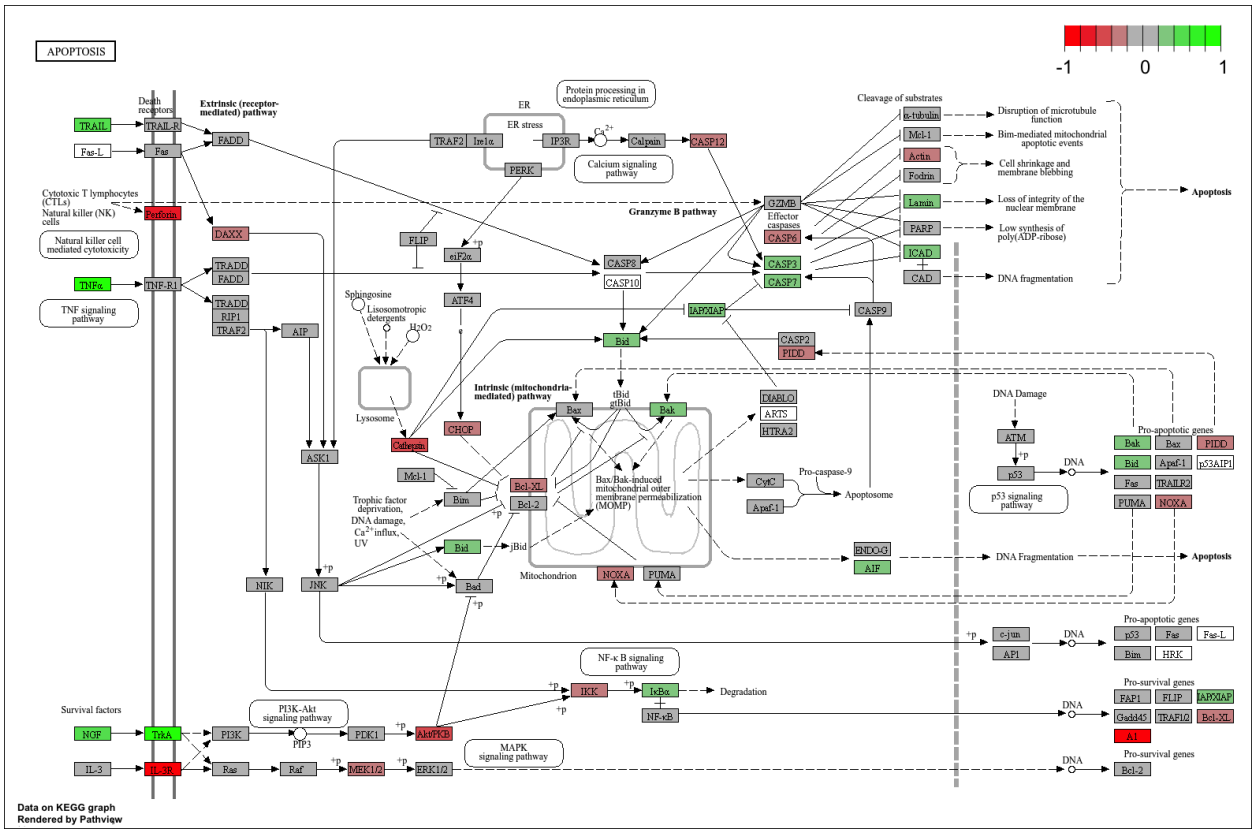


Suppl. Figure S4. Apoptosis KEGG pathway plot for UVC treated samples of UV experiment 1
Murine apoptosis KEGG pathway plot for UVC irradiated samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. Male and female mice were analysed together.

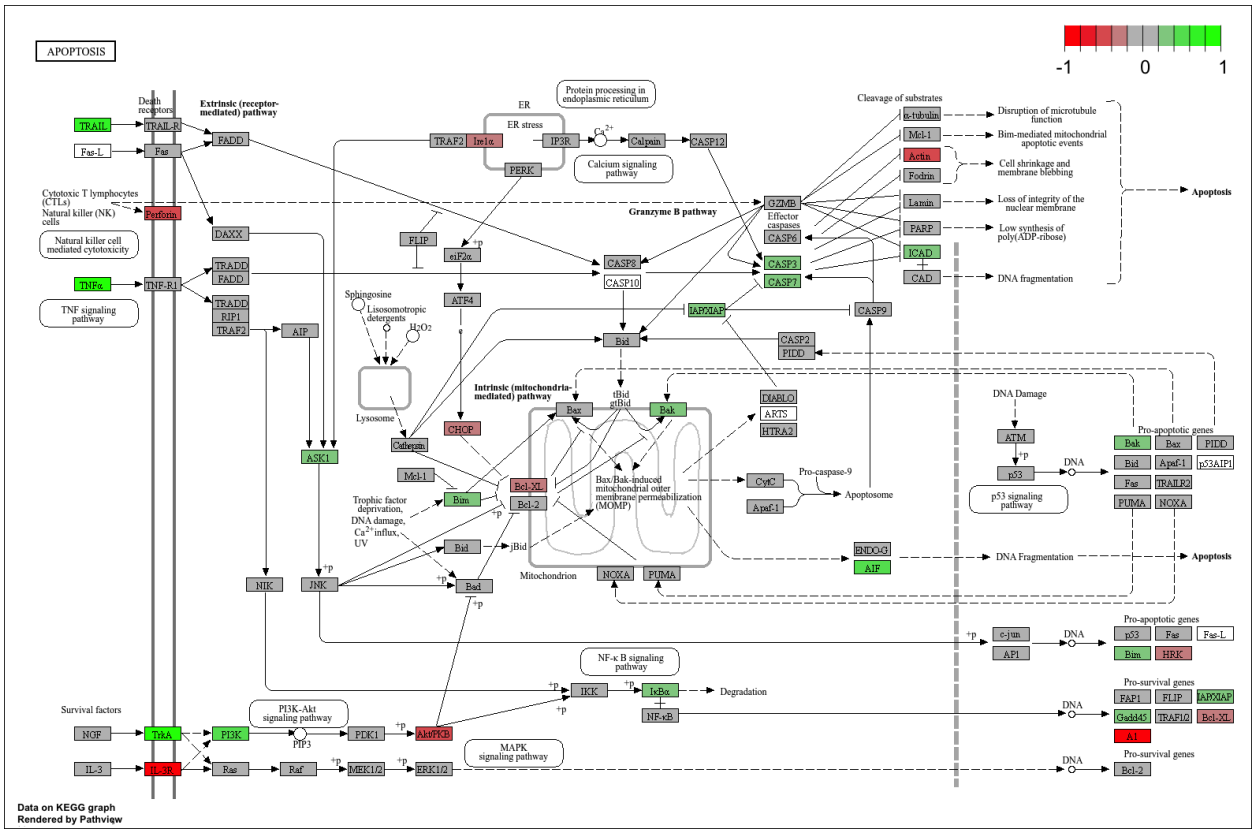


Suppl. Figure S5. Apoptosis KEGG pathway plot for blue light 405 nm treated samples of UV experiment 1

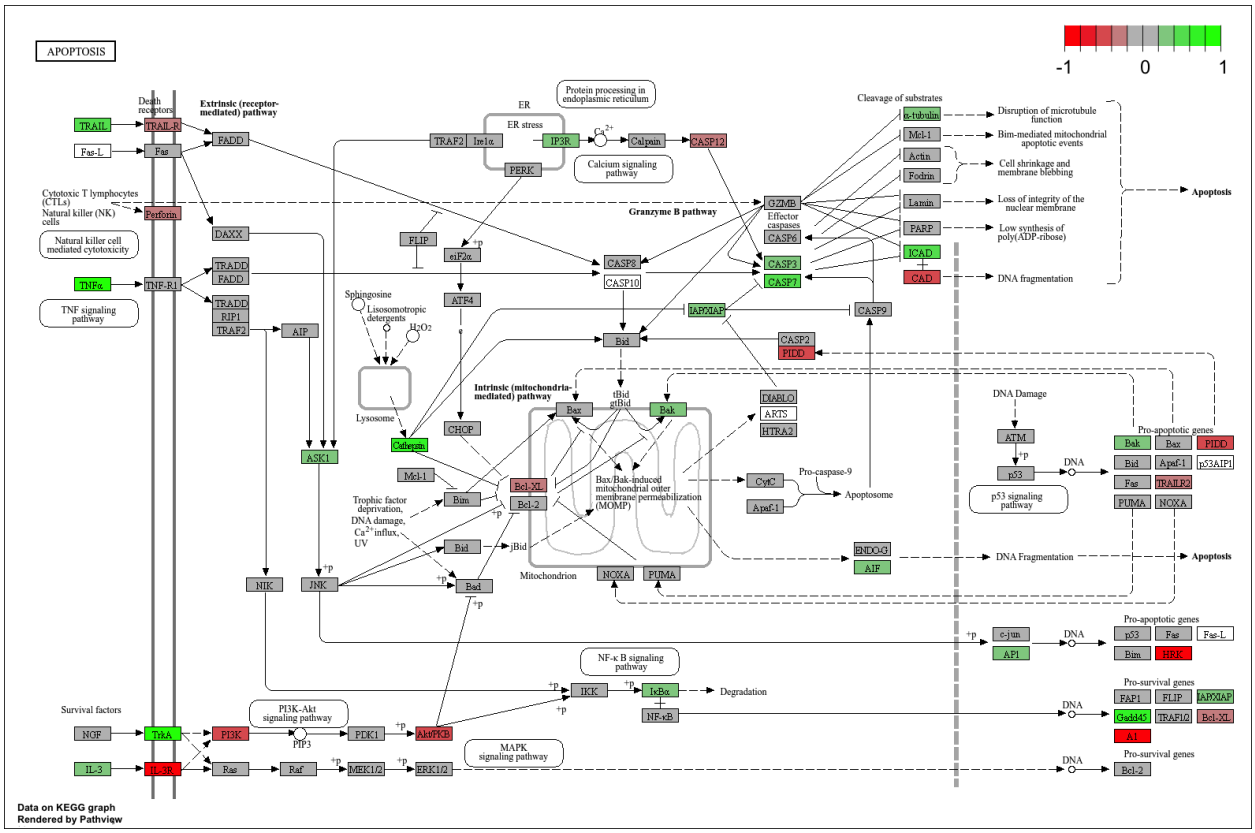
Murine apoptosis KEGG pathway plot for blue light 405 nm irradiated samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. Male and female mice were analysed together.



Suppl. Figure S6. Apoptosis KEGG pathway plot for control 30 h samples of UV experiment 2
Murine apoptosis KEGG pathway plot for unirradiated control 30 h samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. Male and female mice were analysed together.

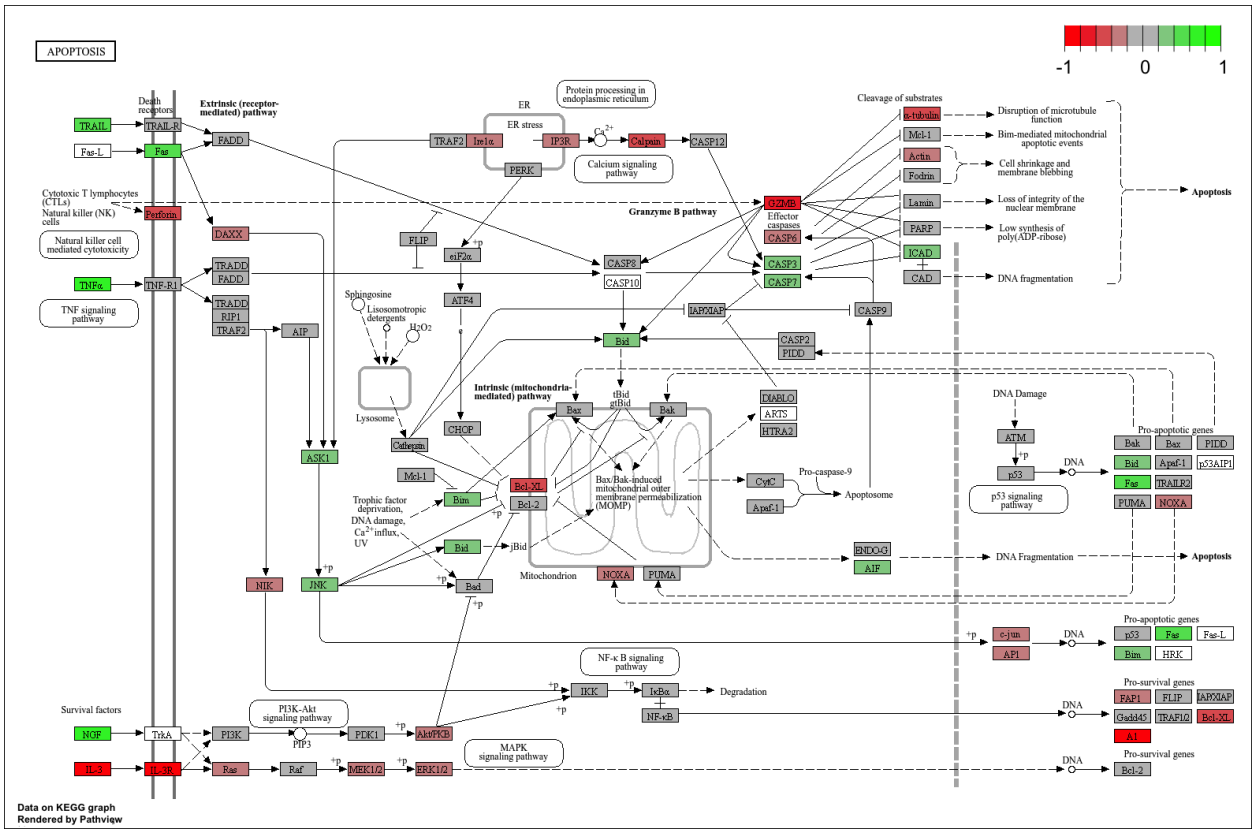


Suppl. Figure S7. Apoptosis KEGG pathway plot for UVA treated samples of UV experiment 2
Murine apoptosis KEGG pathway plot for UVA irradiated samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. Male and female mice were analysed together.



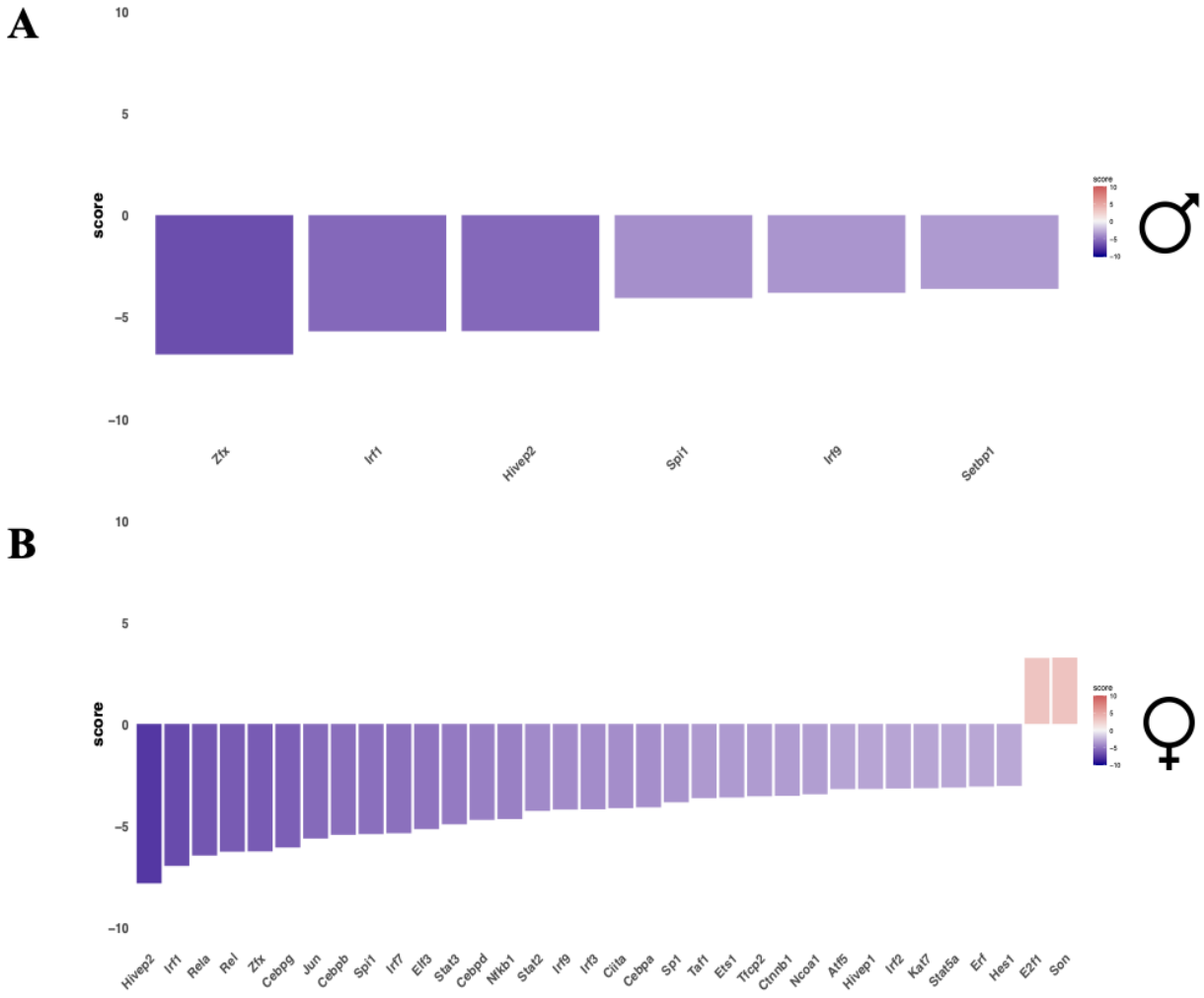
Suppl. Figure S8. Apoptosis KEGG pathway plot for UVA and UVB treated samples of UV experiment 2

Murine apoptosis KEGG pathway plot for UVA and UVB irradiated samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. Male and female mice were analysed together.

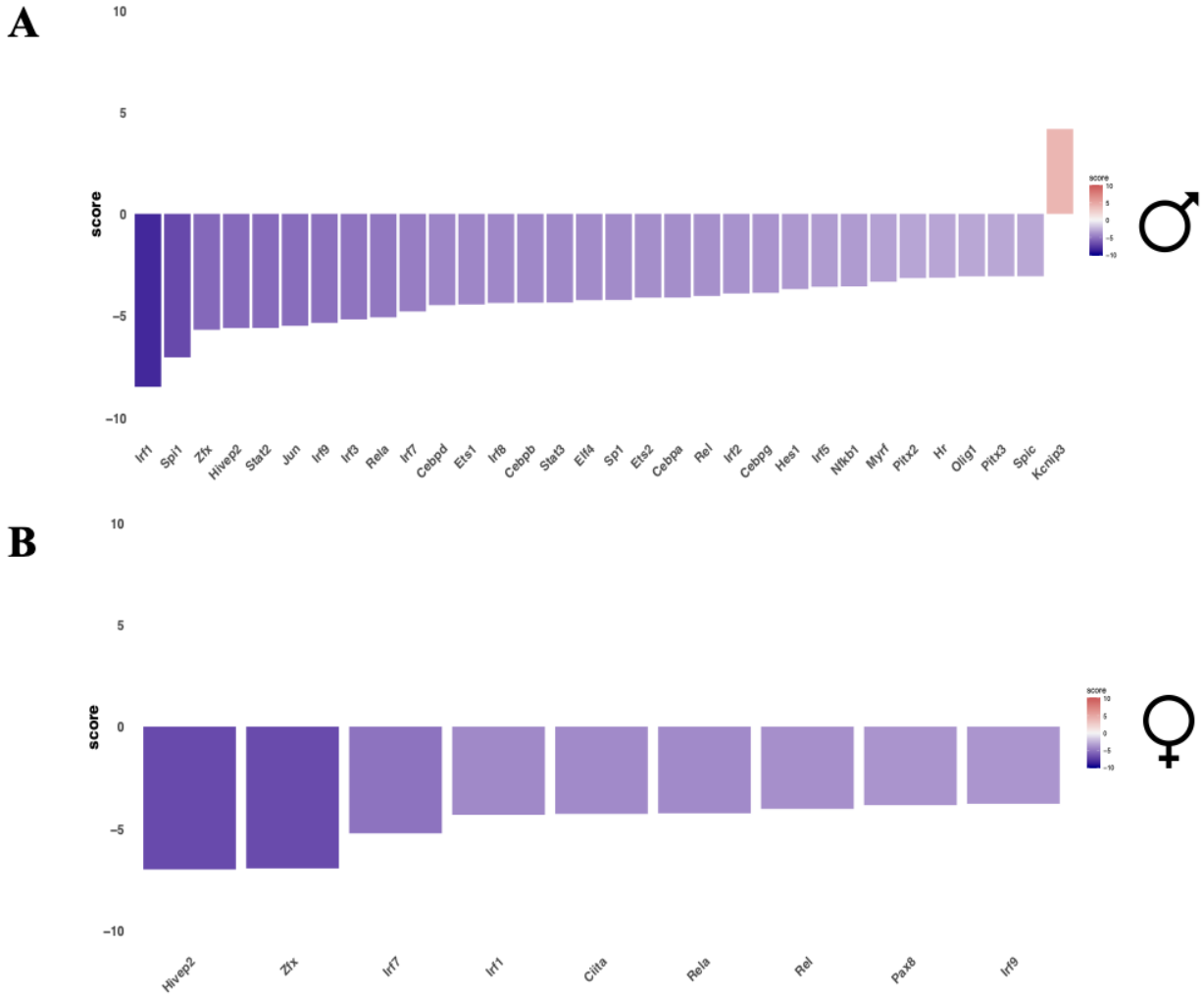


Suppl. Figure S9. Apoptosis KEGG pathway plot for blue light 445 nm treated samples of UV experiment 2

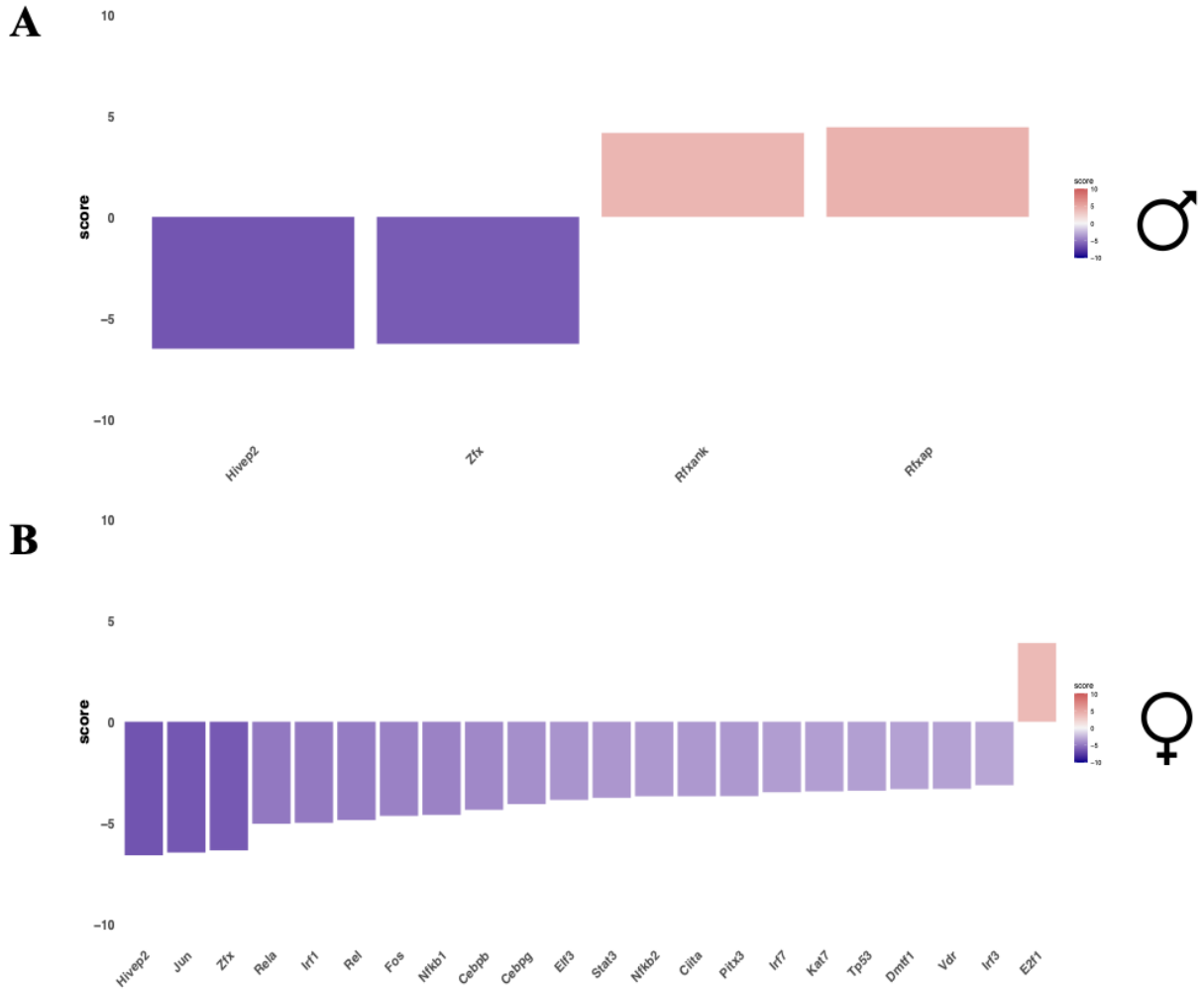
Murine apoptosis KEGG pathway plot for blue light 445 nm treated samples of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. Male and female mice were analysed together.



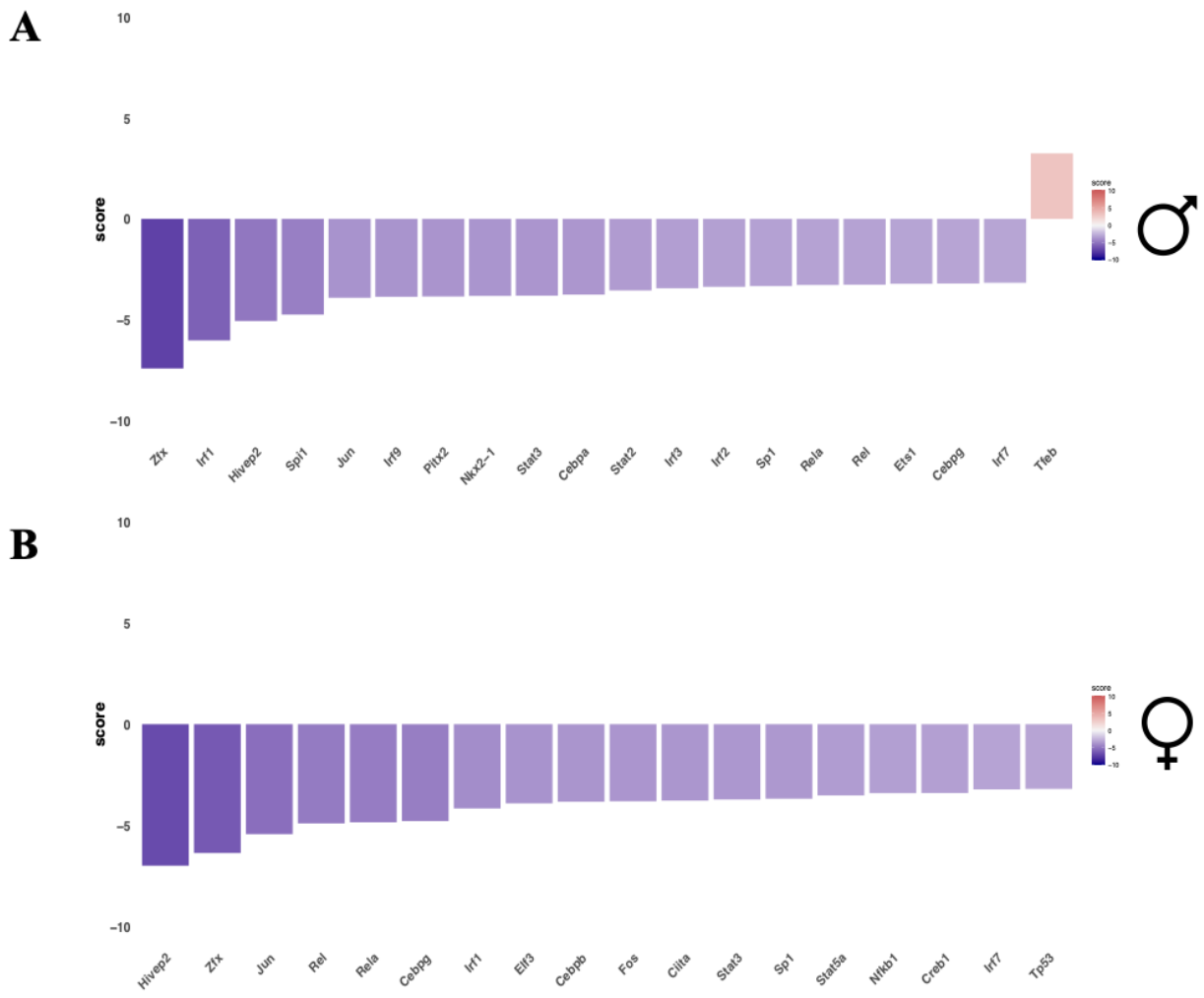
Suppl. Figure S10. Differentially regulated transcription factors in 30 h control samples for male and female mice of UV experiment 1
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. 30 h control samples were not irradiated but otherwise treated as the irradiated samples.



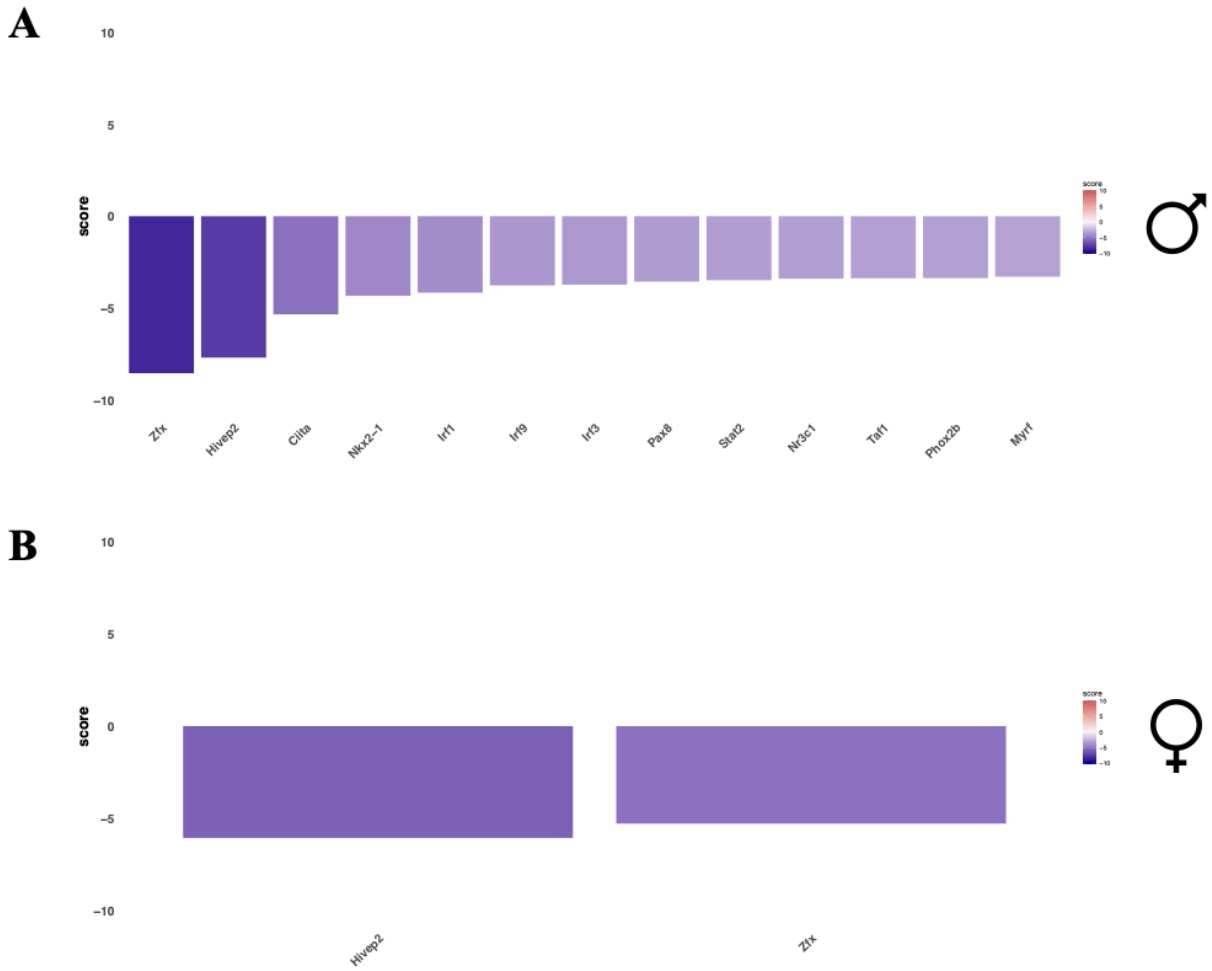
Suppl. Figure S11. Differentially regulated transcription factors in UVB irradiated samples for male and female mice of UV experiment 1
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. UVB treated samples were irradiated with 500 mJ/cm^2 UVB light with a wavelength of 310 nm.



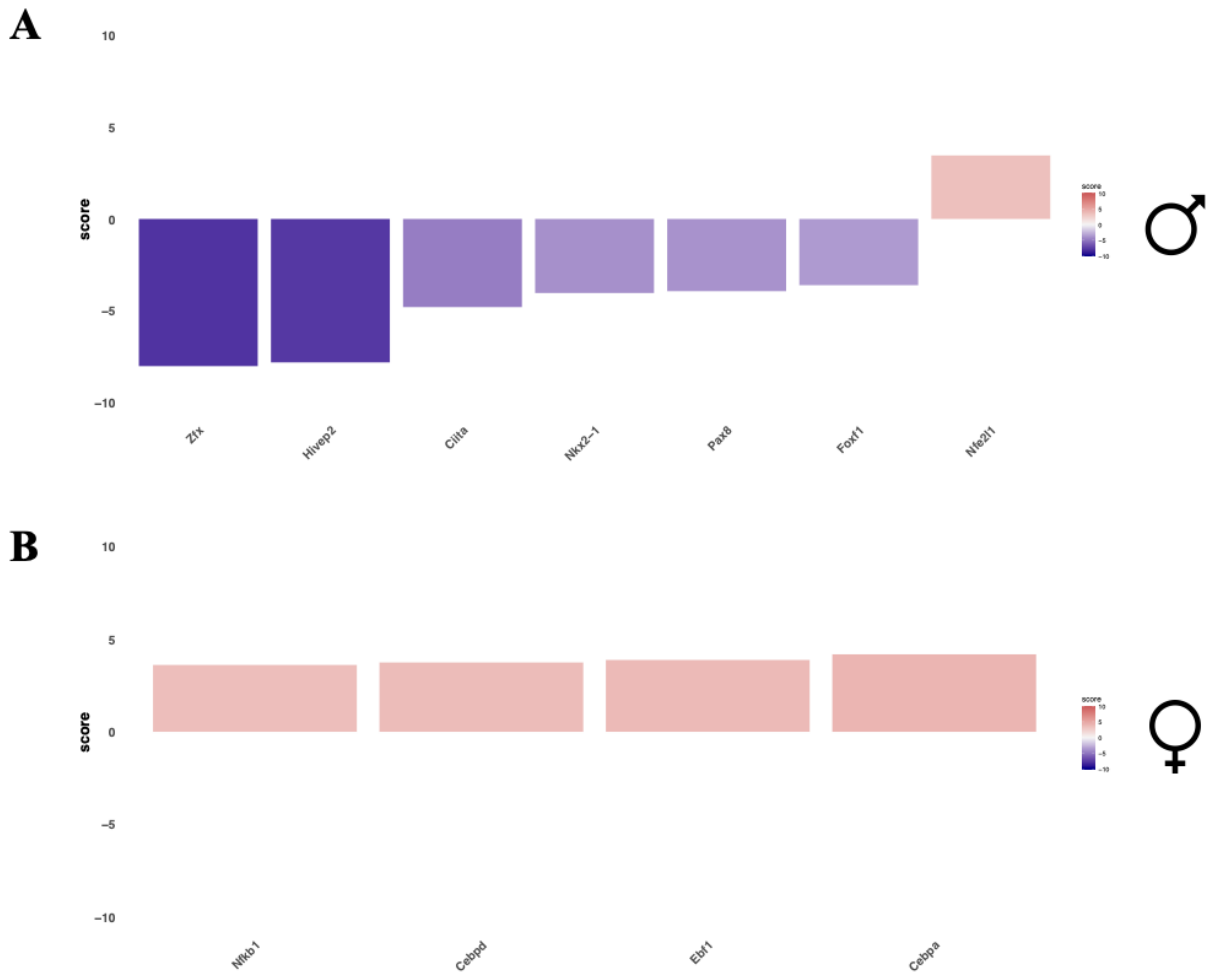
Suppl. Figure S12. Differentially regulated transcription factors in UVC irradiated samples for male and female mice of UV experiment 1
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. UVC treated samples were irradiated with 40 mJ/cm^2 UVC light with a wavelength of 233 nm.



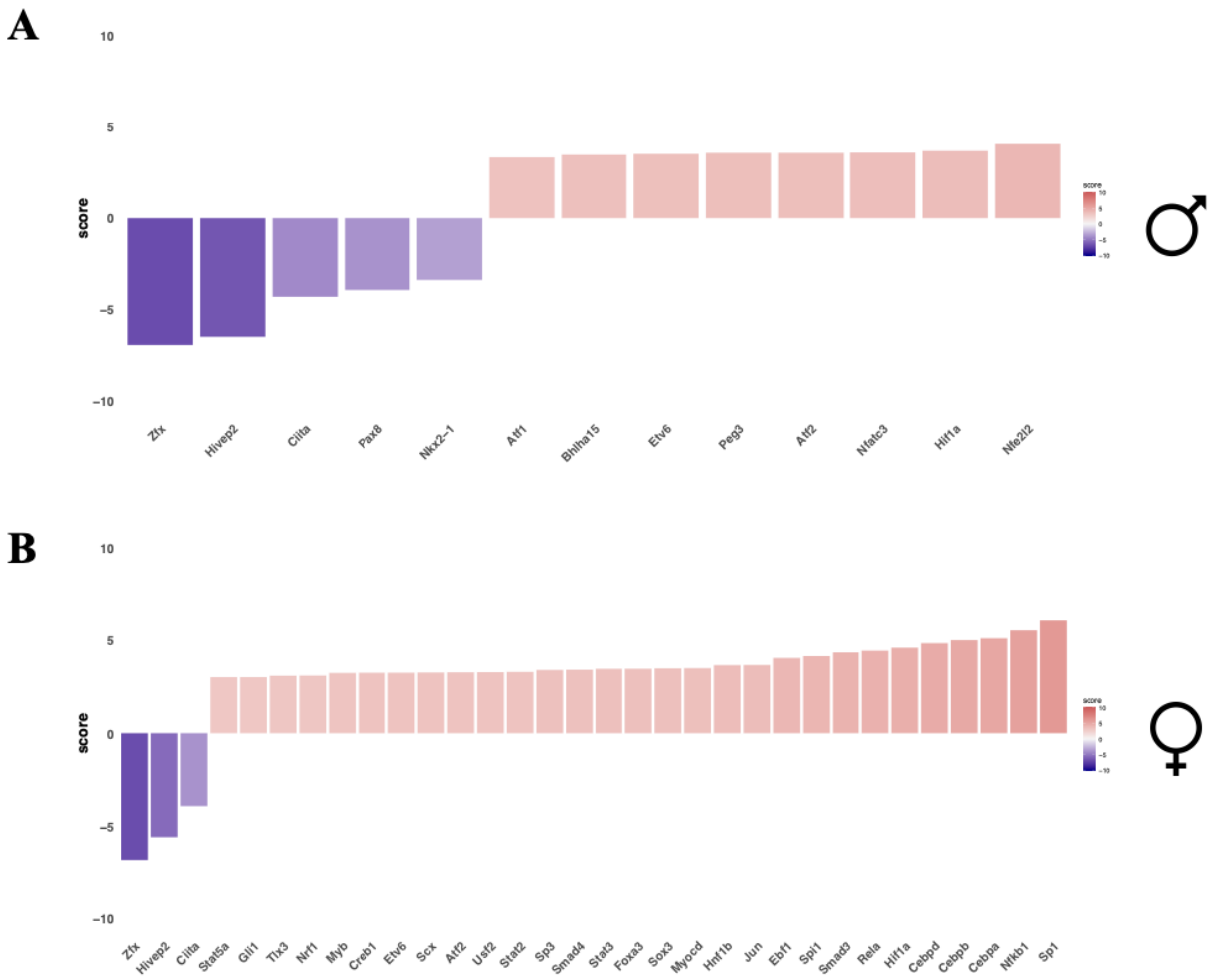
Suppl. Figure S13. Differentially regulated transcription factors in blue light 405 nm irradiated samples for male and female mice of UV experiment 1
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 1. Blue 405 treated samples were irradiated with 6,839 mJ/cm^2 blue light with a wavelength of 405 nm.



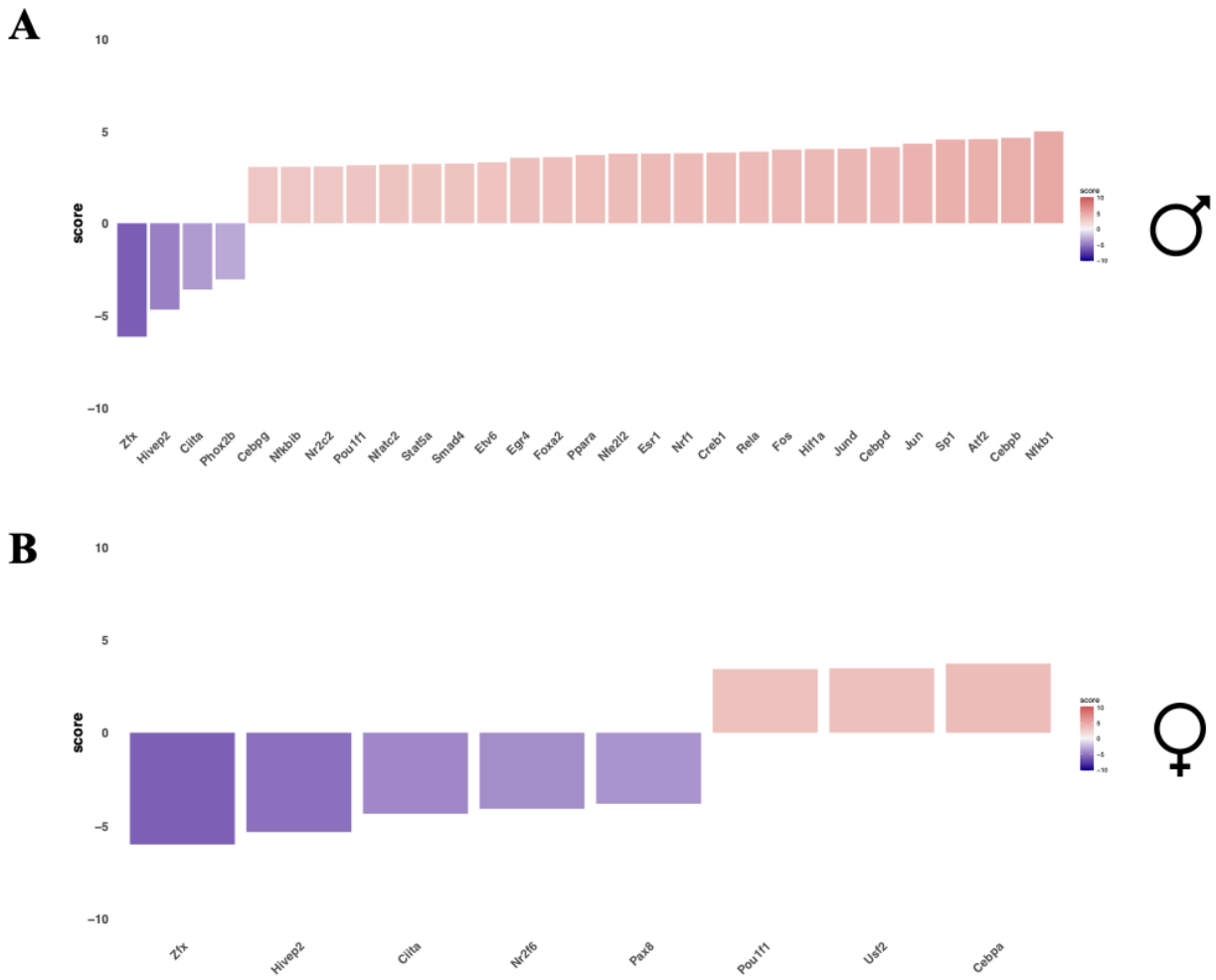
Suppl. Figure S14. Differentially regulated transcription factors in 30 h control samples for male and female mice of UV experiment 2
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. 30 h control samples were not irradiated but otherwise treated as the irradiated samples.



Suppl. Figure S15. Differentially regulated transcription factors in UVA irradiated samples for male and female mice of UV experiment 2
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. UVA treated samples were irradiated with 500 mJ/cm^2 UVA light with a wavelength of 375 nm.



Suppl. Figure S16. Differentially regulated transcription factors in UVA/B irradiated samples for male and female mice of UV experiment 2
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. UVA/B treated samples were irradiated with 500 mJ/cm^2 UVA and UVB light in equal shares with wavelengths of 375 nm and 310 nm.



Suppl. Figure S17. Differentially regulated transcription factors in blue light 445 nm irradiated samples for male and female mice of UV experiment 2
Differentially regulated transcription factors of male (**A**) and female skin samples (**B**) of NZM2410 mice in comparison to C57BL/6J mice in UV experiment 2. Blue 445 treated samples were irradiated with 20,000 mJ/cm^2 blue light with a wavelength of 445 nm.

Acknowledgements

First and foremost, I would like to thank **Prof. Dr. Jennifer Hundt** for offering me this project and supervising me throughout all the ups and downs of it. Your open ears and guidance enabled me to do this. It is a miracle to me how you can handle a group working on so many different topics and connecting all of them! You never forgot any details about any project of ours, even when we were almost 20 people. I cannot thank you enough for making so many of my sudden, crazy ideas possible and supporting them in the project design and finding funding for them. Without this support, there would never have been the UV-light project or the self-built activity sensors. Overall, I am grateful that I could work on so many different projects. Even though this was challenging, I gained so much from it. I did not only learn various techniques, and learn a lot about the immunology of various diseases, but also learned how to design projects from the beginning to the end. In this thesis, only a few of them are shown, but we make sure together that everything else will not end up in a drawer but will be published as well. The lab parties, pub nights, picnics, canoeing tours, and so many more activities kept the motivation in all of us up and reminded us that we are not only lab rats but also social, creative human beings. Thank you!

I would also like to thank my second supervisor, **Prof. Dr. Tanja Lange**. Your brain is amazing, and I wish to gain such broad knowledge as yours. You impressed me so many times with how you could draw conclusions and relate very different topics in a way that created a big picture. Your way of giving presentations is also remarkable. You can keep the audience following through long trains of thought on highly specific processes. You and Jenny were a wonderful combination for supervision. Thank you for teaching me. I learned a lot!

Axel Künstner, you were a great mentor, and I appreciated so much that you always made time for me when I had questions! Moreover, it was great working together with you and Sen on the UV experiments -wonderful teamwork. I am looking forward to continuing to work on the mouse data and hopefully publishing the paper soon.

To the dearest **Hundt lab team**:

Tasja, in the Hundt lab team, you are the one I shared the most lab time and experiments with. I am so thankful that you not only allowed me to get involved in your exciting project but also helped me with my experiments. I think we are a great team, and I love your spirit and your pragmatic problem-solving mindset. Together we achieved so much and had great fun! Thank you for also being a lovely friend who also listened to whatever was going on in my private life and even went on small adventures abroad with me.

Zuzana, even though you are not yet officially a Hundt lab-member on paper, our shared work on

the UV experiments is definitely making you a team-member in my opinion. I enjoyed working with you and learning a lot about lasers, ear infections, and the effects of UV light on bacteria. You are an impressive woman, and I look up to you because you have already achieved so much under such difficult conditions, and you did not give up. I admire your critical thinking and the questions that always pop up in your mind. You are a wonderful friend to me, and I will miss you!

Caren, you paved the way for future PhD students in the Hundt lab, and you set the bar high. I learned from you not only a lot about human skin and wound healing, but you also sharpened my mind in psychology and human interactions. You were so patient with all of us and always ready to answer questions or brainstorm for solutions. Thank you! I hope to continue meeting you for knitting and strolls through the local forests!

Vero, we have known each other since the first day of the infection biology master's program, and we are still working together. This is really cool, and I wish you all the best for your future. You mastered so much, and we had great times together. Thank you!

Maja, I am proud that it is you taking over my projects, I am sure you will do great. I wish you the best of health, success, luck, and a lot of patience with all the structural delays and difficulties. As soon as you can fully dive into it, you will strive and enjoy the spirit of science. I enjoyed your temper in the office, please stay like this!

Nadine, when you look at me, you already know how I am feeling, and I want to thank you for helping me and having an open heart and ear for me and everything that was going on in my experiments and in life. Your zodiac sign saved all of us many hours searching for stuff, thanks for being so tidy and even keeping the biggest groups as organised as possible.

Sylva, you are impressive! Always patient and ready to support all of us. Thank you for being so kind, and I always enjoy talking to you and hope to become such a good knitter as you are one day.

Sina, you are not only a secretary but also a warm-hearted woman who cares about the others in the group. You also had hard times while I was part of the group, and I wish you all the best for the future!

Laura and **Skadi**, your coordination of the research training group 2633 was great. We students felt safe and well taken care of. Thank you for all the effort!

Marieke, thank you for your organisation of so many fun events and lunch breaks. You definitely know how to celebrate a party.

Over the years, many **MD candidates** worked in the lab, and all of you brought new insights, perspectives, and ideas to us. Thanks to Chiara, Lilly, Luise, Max, Luca, Julia, Nora, Danial, Lennart, and Marie!

I would also like to thank Bety, Laura T., and Luna for bringing a lot of joy to the lab and office.

My students, were a special joy! I had so much fun, and I am so thankful for the experience I gained from training and teaching you. I could see you growing, and I felt myself growing. Thank you, Jade, Linus, Leo, Jelena, and Natalie. I need to express a special thank you to Steffen, who not only spent one internship with me but two and also worked as a student assistant in the lab! Even after this time, you offered help when needed, and I am impressed by your project-planning and learning capabilities. Without you, the UV experiments would not have worked so smoothly and many other experiments either!

The **rheumatology lab** allowed me not only to use their equipment but also offered me advice, help, and a lovely working atmosphere. I would like to especially express my gratitude to Justus Ohmes, who was always there to give me a hand when needed and also to talk to. Antje Müller, thank you for treating me like a regular lab member and for caring for me when I was working late or at weekends. Sara Comdühr helped me develop my FACS panels and thereby avoided for sure some extra days of trial and error, thank you! Silke Pitann and Gabi Marschner, you are great technical assistants, and I always enjoyed working around you. Maike Winziers, I loved seeing you in the lab, your happy attitude always improved my day, and you brought an amazing spirit to the group. Anja Stähle, thanks for letting me participate in the Pint of Science and for scientific advice from time to time.

Sen, thank you not only for analysing the transcriptomics data for me but also for having patience with all the changes that I wanted and special thanks for explaining the code to me and for taking the time for all the meetings. You did great, and I wish you the best of success for your future.

I would also like to thank the **Manz lab**, especially Swayanka Biswas and Rudi Manz, for breeding and sharing the SLE123 mice, as well as for including me in some of their lab events. I had a great time, and without you, my thesis would not be the same!

Thank you, **Ehlers lab**, for sharing the C57BL/6J mice and also for all the teamwork. I am grateful to Jana Buhre and Selina Lehrian, who shared skills, resources and knowledge, as this facilitates science so much! It was also a pleasure to help you with dissections in turn.

Another big thank you goes to the **Ludwig lab** and Katja Bieber for breeding the NZM2410 mice and for advice with the animal proposals.

Of course, I would also like to thank the **research training group 2633** for accepting me as a member of the program and also for being a student speaker for the entire time. I am grateful for everyone's trust and the good times that we had together!

I would also like to thank **Prof. Dr. Thomas Walther**, for including me in Tasja‘s projects. I learned a lot about epidermolysis bullosa acquisita and also about angiotensin. These are highly promising experiments, and it felt fulfilling to see our results from mouse experiments being of

direct interest and use for patients. Thank you for the meetings and for the research stay in Cork.

To **my lovely friends and family**: Thank you for your love, happiness, patience, distraction, and all the fun and joy we had during the past couple of years. Many of you are peer PhD students in Lübeck, whom I mentioned before, but some are even from far away. Here I want to place a small "thank you" for Emma Haberman and David Grainger, who spent weekends, college balls, parties, evenings with card games, sightseeing tours in different cities, and much more with me. As you two are also scientists, it was a special broadening of my mind to discuss ideas and results with you. Thank you for everything! Your little hidden note of "you can do it" was such a sweet motivation for the last couple of months. I also need to thank my parents, especially **my lovely mother**, who always listened to me and my thoughts throughout the years and where I could spend some "recovery weekends" to regain energy for upcoming tasks.

To the best husband: Thank you, **Jeff**, for your patience, emotional support and all the meals that you cooked and prepared for me to make sure I will survive in times I did not even have time for the shortest breaks. I love you and know that your support is beyond everything one could expect. Thank you!

This PhD was a prime example of collaboration and teamwork. All these experiments (and there were even many more than described in this thesis) would not have been possible without the support, advice, assistance, and help of each of you! THANK YOU for all the wonderful memories throughout the past years! I really hope to experience this again in my upcoming jobs and to pass on this spirit to the next generations of students.


CAFE & BAR
CELONA
50. ADF TAGUNG
06.-09. MÄRZ 2024
DÜSSELDORF