Stromal cell contact induced PI3K signalling and APRIL induced NF-κB signalling synergistically maintain memory plasma cells by inducing IRF4 and preventing endoplasmic reticulum stress

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ABSTRAKT

Langlebige Gedächtnis-Plasmazellen überleben im Knochenmark in engem Kontakt mit mesenchymalen Stromazellen, bieten eine humorale Immunität gegen längst verschwundene Krankheitserreger und bilden somit die Grundlage für ein schützendes humorales Gedächtnis. Gedächtnisplasmazellen, die pathogene (Auto-)Antikörper sezernieren, stellen eine therapeutische Herausforderung für die Behandlung von chronisch-rheumatischen Entzündungen dar, da sie gegenüber herkömmlichen immunsuppressiven Therapien refraktär sind. Die molekularen Mechanismen, die es diesen hochspezialisierten und einzigartigen Zellen ermöglichen, Jahrzehnte zu überleben, sind jedoch noch unklar. Ein detaillierteres Verständnis kann zum Impfstoffdesign sowie zur Behandlung von Antikörper-vermittelten Krankheiten beitragen.

Anhand einer *In-vitro*-Nische, die die Umgebung des Knochenmarks nachahmt, zeigen wir, dass der direkte Kontakt zu Stromazellen und die Aktivierung des BCMA-Rezeptors, z. B. durch seinen Liganden APRIL, notwendig und ausreichend sind, um den Caspase-vermittelten Tod von Plasmazellen zu verhindern. Stromalzellkontakt und APRIL verhindern synergistisch die Aktivierung der mit dem endoplasmatischen Retikulum assoziierten Caspase 12 und regulieren den Master-Transkriptionsfaktor IRF4 hoch. IRF4 reguliert verschiedene ER-assoziierte Gene, die die Proteinproduktion, -faltung und -glykosylierung kontrollieren, wie durch Einzelzell-RNA-Sequenzierung gezeigt wurde. Darüber hinaus behält IRF4 die Plasmazellidentität bei und kontrolliert die Expression des anti-apoptotischen Proteins BCL2.

BCMA induziert überlebensfördernde Signalwege durch Aktivierung des NF-κB-Signalwegs. Die Persistenz von Plasmazellen erfordert zusätzlich eine durch Zellkontakt induzierte PI3K-Signalgebung. Wir zeigen weiter, dass der siRNA-vermittelte Knock-down von FoxO1/3 Plasmazellen in Abwesenheit von Stromazellen am Leben erhält. Es wurden nur geringfügige Transkriptionsunterschiede nach dem FoxO1/3-Knock-down festgestellt, und die aktive Caspasen 3 und 7 wurden weiterhin detektiert. Unerwarteterweise wurde das anti-apoptotische Protein MCL1 durch FoxO1/3-Knockdown verringert.

Diese Ergebnisse identifizieren PI3K/FoxO1/3 als einen neuen, lebenswichtigen Signalweg und als potenzielles therapeutisches Ziel für die Ablation pathogener langlebiger Plasmazellen bei rheumatischen Erkrankungen.

ABSTRACT

Long-lived memory plasma cells are maintained in the bone marrow in close contact to mesenchymal stromal cells, and provide humoral immunity against long gone pathogens, thus constituting the basis for protective humoral memory. Memory plasma cells secreting pathogenic (auto-) antibodies pose a therapeutic challenge for the treatment of chronic rheumatic inflammation as they are refractory to conventional immunosuppressive therapies. However, the molecular mechanisms enabling these highly specialised and unique cells to survive for up to decades remain unclear. More detailed understanding may contribute to vaccine design as well as treatment of antibody-mediated diseases.

Using an *in vitro* niche mimicking the bone marrow environment, we demonstrate that direct contact to stromal cells and activation of the BCMA receptor, e.g. by its ligand APRIL, are necessary and sufficient to prevent caspase-mediated death of plasma cells. Stromal cell contact and APRIL synergistically prevent activation of the endoplasmic reticulum associated caspase 12 and upregulate the master transcription factor IRF4. IRF4 regulates various ER associated genes controlling protein production, folding and glycosylation as revealed by single cell RNA sequencing. Moreover, IRF4 maintains plasma cell identity and controls the expression of the anti-apoptotic protein BCL2.

BCMA induces pro-survival signalling by activating the NF-κB pathway. Persistence of plasma cells additionally requires cell contact induced PI3K signalling. We further show that siRNA mediated knock-down of PI3K downstream targets FoxO1/3 maintains plasma cells in absence of stromal cells. Only minor transcriptional differences after FoxO1/3 knock-down were detected, and active levels of caspases 3 and 7 remained high. Unexpectedly, the anti-apoptotic protein MCL1 was decreased by FoxO1/3 knock-down.

These results identify PI3K/FoxO1/3 as a novel, vital signalling pathway and as a potential therapeutic target for the ablation of pathogenic long-lived plasma cells in rheumatic diseases.

List of Abbreviations

Apaf-1
Apoptotic Protease Activation Factor-1
APRIL
A Proliferation-Inducing Ligand
ATF6
Activating Transcription Factor
ATG
Autophagy-Related Genes
BAD
BCL2 Associated Agonist of Cell Death
BAFF
B Cell Activating Factor
BAK
BCL2-Antagonist/Killer 1
BAX
Bcl2-Associated X Protein
BCL2
B Cell Lymphoma 2
BCL6
B-Cell Lymphoma 6 Protein
BCL-w
Bcl-2-Like Protein 2
BCL-xL
B-Cell Lymphoma Extra-Large
BCMA
B Cell Maturation Antigen
BCR
B Cell Receptor
BIM
Bcl-2-Like Protein 11
BiP
Binding Immunoglobulin Protein
BLIMP-1
B lymphocyte-induced maturation protein-1
CCCP
Carbonylcyanid-m-chlorphenylhydrazon
CD
Cluster of Differentiation
CXCL12
C-X-C Chemokine Receptor Type 12
CXCR4
C-X-C Chemokine Receptor Type 4
DAPI
4',6-Diamidino-2-phenylindol
EDTA
Ethylenediaminetetraacetic Acid
EF
Extrafollicular Foci
eIF2α
Eukaryotic Initiation Factor 2α
ELISA
Enzyme-linked Immunosorbent Assay
ER
Endoplasmic Reticulum
FAK
Focal Adhesion Kinase
FO
Follicular
FoxO1
Forkhead-Box-Protein O1
FoxO3
Forkhead-Box-Protein O3
GC
Germinal Centre
GFP
Green Fluorescent Protein
GLUT1
Glucose Transporter 1
HLA-DR
Human Leukocyte Antigen – DR Isotype
ICAM-1
Intercellular Adhesion Molecule 1
IFA
Incomplete Freund`s Adjuvants
Ig
Immunoglobulin
IL-6
Interleukin 6
IRE1
Inositol-Requiring Protein 1
IRF4
Interferon regulatory factor 4
JNK
c-Jun N-terminal Kinases
LFA-1
Lymphocyte Function-Associated Antigen 1
LPS
Lipopolysaccharide
MACS
Magnetic Cell Sorting
MCL1
Myeloid Cell Leukemia 1
MHCII
Major histocompatibility complex
Mitf
Microphthalmia-Associated Transcription Factor
MOMP
Mitochondrial Outer Membrane Permeabilisation
mRNA
Messenger Ribonucleic Acid
mTOR
Mammalian Target Of Rapamycin
MZ
Marginal Zone
NF-κB
Nuclear Factor kappa-Light-Chain-enhancer of Activated B Cells
NOXA
Phorbol-12-myristate-13-acetate-induced protein 1
NP-CGG
4-Hydroxy-3-Nitropheylacetyl Hapten Coupled Chicken Gamma Globulin
PAX5
Paired Box gene 5
PDK1
Phosphoinositide Dependent Kinase 1

PERK
PKR-Like Endoplasmic Reticulum Kinase
PI3K
Phosphoinositide 3-Kinases
PIP2
Phosphatidylinositol (4,5)-bisphosphate
PUMA
P53 Upregulated Modulator of Apoptosis
ROS
Reactive Oxygen Species
siRNA
small interfering RNA
TCR
T Cell Receptor
TMRM
Tetramethylrhodamin-Methylester
t-SNE
t-distributed stochastic neighbour embedding
TNFR
Tumor Necrosis Factor Receptor
TRAF
TNF Receptor Associated Factor
UPR
Unfolded Protein Response
VCAM-1
Vascular Cell Adhesion Molecule 1
VLA-4
Very Late Antigen 4
WT
wild type
XBP-1
X-Box Binding Protein 1
ZBTB20
Zinc Finger And BTB Domain-Containing Protein 20

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In loving memory of Paula.

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1 INTRODUCTION

1.1 THE IMMUNE SYSTEM

The immune system is a complex network of cells that can differentiate “self” from “non-self” and thereby defend the body against infections caused by bacteria, fungi, parasites, and virus particles. It monitors the entire body for the emergence of tumour cells or infected cells while keeping a delicate balance between being protective and pathogenic. A misbalance can result either in infections or autoimmune diseases. The vertebrate immune system has been traditionally separated into the innate and the adaptive arms. From an evolutionary perspective, the innate arm of the immune system is older and composed of physical barriers such as the skin, small molecules, and cells. The adaptive immune system is a more recent evolutionarily development that is specific for vertebrates. B cells and T cells develop in the bone marrow or thymus respectively and represent the cellular part of the adaptive immune system. Contrary to the innate immune system, the adaptive immune system can deploy from an enormous variety of receptors making it a powerful tool. This is possible due to somatic recombination of BCR (B cell receptor) and TCR (T cell receptor) genes producing high numbers of unique receptors[1].

1.2 IMMUNOLOGICAL MEMORY

Immunological memory is a hallmark of the adaptive immune system and provides the body with long-term protection against reinfection. It describes the ability of immune cells to memorise previously encountered antigen even after an infection is cleared. Memory T and B cells are maintained as quiescent circulating or tissue-resident cells and can be found in blood, lymph or organs like skin, spleen, or bone marrow. They possess TCRs and BCRs that recognise their antigen more efficiently upon reencounter, allowing them to rapidly clear the pathogen[2].

Although plasma cells were initially thought to be exclusively short-lived, the existence of long-lived memory plasma cells as important components of immune memory is now widely accepted[2].

Memory plasma cells are unable to recirculate and can be found in various organs like the spleen, lymphoid tissues, and the bone marrow. They are quiescent in terms of proliferation and secret high-affinity antibodies after the infection is cleared providing the body with humoral memory[2].

The protective mechanism of vaccines relies on the formation of immunological memory, especially of memory plasma cells. Therefore, the success of vaccination is predominantly defined by the long-term detection of specific antibodies in the blood. However, the immunological memory can also play a detrimental role by facilitating pathogenesis in the context of autoimmune diseases. In patients suffering from rheumatoid arthritis or systemic lupus erythematosus, cells of the immunological memory that recognise self-antigens can be detected. Especially pathogenic memory plasma cells can harm the tissue as they continuously secrete antibodies directed against self-antigens that result in inflammation and are refractory to most treatments. Depletion of plasma cells result in decreased disease activity in systemic lupus erythematosus patients and other antibody-mediated autoimmune diseases[3,4]. However, these treatments affect both pathogenic and protective plasma cells making patients vulnerable towards infections after treatment[2].

Thus, a more profound understanding of the biology of memory plasma cells is fundamental for the design of efficient vaccines and the treatment of autoimmune diseases.

1.3 PLASMA CELLS

1.3.1 PLASMA CELL GENERATION

Plasma cells can be generated in either T-dependent or T-independent reactions from MZ (marginal zone), FO (follicular) and memory B cells. However, most of them are short-lived and it remains elusive if all of them have the potential to become long-lived[5,6]. Most memory plasma cells are derived from a GC response which is evident by their highly mutated antibodies[7,8]. B cells can respond to antigen recognition either by massive proliferation in an extrafollicular response yielding low affinity antibodies produced by plasmablasts or they take part in a GC response, where they undergo rounds of somatic hypermutations and affinity maturation, resulting in plasma cells producing high affinity antibodies. These plasma cells are generated late in an immune response and mainly contribute to the pool of memory plasma cells[8] (Figure 1). The transformation from B cell to plasma cell is accompanied by radical changes in morphology and cell biology as the ER (endoplasmic reticulum) massively expands transforming the cell into an antibody factory. This conversion is initiated by the activation of the plasma cell-specific transcriptional program of plasma cells defined by BLIMP-1 (B lymphocyte-induced maturation protein-1), IRF4 (Interferon regulatory

factor 4) and XBP-1 (X-box binding protein 1) and the suppression of the B cell transcriptional program PAX5 (Paired box gene 5), BCL6 (B-cell lymphoma 6) and Mitf (Microphthalmia-associated transcription factor)[9,10]. Plasma cells can be identified by expression of the cell surface marker CD138, which is upregulated already at the plasmablast stage. Additionally, plasma cells lose the expression of the CD45 isoform B220 and HLA-DR (Human Leukocyte Antigen – DR isotype). Contrary to B cells, plasma cells express lower levels of surface IgA and IgM, and IgG is only expressed in secreted form[10]. CD19 is differentially expressed in plasma cells and its loss is associated with longevity[11].

Plasma cells exiting the GC express the chemokine receptor CXCR4 (C-X-C chemokine receptor type 4) and migrate through the body following a CXCL12 (C-X-C motif chemokine 12) gradient[12]. CXCL12 is highly expressed in the bone marrow by $CXCL12^{+}$-abundant reticular stromal cells. Memory plasma cells can be found in numerous organs, including the spleen, gut-associated lymphoid organs, lymph nodes and inflamed tissues, but the majority resides in the bone marrow[11,13–15]. Once they have entered the bone marrow, they find a survival niche and lose their migratory capacity[12].

Plasma cells display a special and unique cell biology among immune cells. They represent the end stage of differentiation and are maintained for up to decades. Although they are quiescent in terms of proliferation, they produce enormous amounts of antibodies with one cell secreting antibodies in the range of 10^8 molecules/hour[16]. Transcriptomes comparing B cells and plasma cells have revealed that unlike other long-lived cells, plasma cells are not metabolically quiescent but very active[17–19]. How does a plasma cell persist so long while being constantly active? Is the survival of plasma cells an intrinsic property or is every plasma cell able to become a memory plasma cell if it finds the right niche?[20]

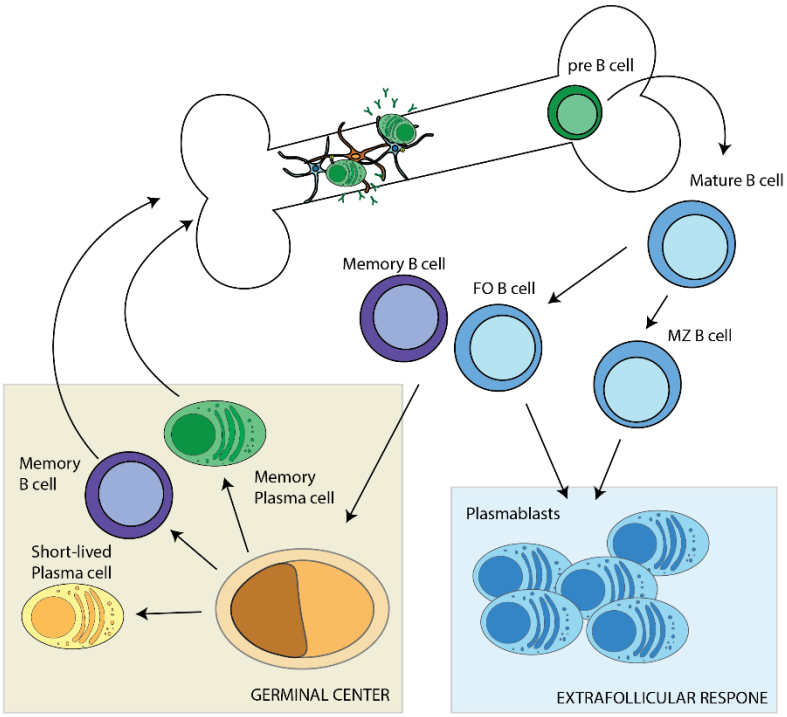

Figure 1: Generation of plasma cells.
Mature naïve B cells originate from pre-B cells in the bone marrow. They can be separated into MZ (marginal zone) B cells giving rise to short-lived plasmablasts after antigen encounter in an extrafollicular response or FO (follicular) B cell capable of initiating a germinal centre response producing high-affinity plasma cells, and memory B cells. Memory B cells can upon reencounter of antigen enter a GC (Germinal centre) response. Memory plasma cells and memory B cells can migrate to the bone marrow where they survive in dedicated niches.

1.3.2 PLASMA CELL TRANSCRIPTION FACTORS

Plasma cell identity is orchestrated by the three transcription factors BLIMP-1, XBP-1 and IRF4[9]. They initiate the differentiation of B cells into plasmablast/plasma cells, yielding cells that are fundamentally different from their precursor B cells regarding morphology, metabolism, and mRNA (messenger ribonucleic acid) transcription. The changes initiated by these transcription factors are multifaceted and while some downstream targets are overlapping, they exert distinct functions[21].

1.3.2.1 BLIMP-1

BLIMP-1 is crucial for the generation of plasma cells but dispensable for the long-term survival of memory plasma cells. While BLIMP-1 does not interfere with cell cycle regulation or known survival mediators of plasma cells, it is associated with metabolism and immunoglobulin secretion. BLMP-1 was demonstrated to control the UPR (unfolded protein response), a part of the ER stress response, and BLIMP-1-deficient plasma cells downregulate the amino acid transporter CD98 and decrease mTOR (mammalian target of rapamycin) activity[22,23].

1.3.2.2 XBP-1

XBP-1 is a direct target of BLIMP-1. Mice deficient in XBP-1 maintain memory plasma cell numbers comparable to wild type controls. However, the memory plasma cells display severe functional defects regarding their immunoglobulin production. The observed phenotype is comparable to that of BLIMP-1-deficient mice, but transcriptome analysis revealed that the genetic targets of XBP-1 and BLIMP-1 have only minimal overlap. Contrary to BLIMP-1, XBP-1 controls very specific functions of the UPR, which mainly regulate protein folding and targeting to the ER. XBP-1 directly regulates all *Igh* transcripts, whereas BLIMP-1 deficiency only decreased *Ighm* and *Ighg3* transcripts[22,24].

1.3.2.3 IRF4

IRF4 is the master transcription factor of plasma cells that controls their differentiation as well as long-term survival[22,25]. Deletion of IRF4 in plasma cells results in rapid cell death, making it challenging to decipher how IRF4 regulates plasma cell survival. Using a mouse model with inducible IRF4 deletion and BCL2 (B cell lymphoma 2) overexpression, Low et al elucidated IRF4-dependent survival mechanisms in plasma cells: IRF4-induced cell death was prevented using caspase-inhibitors indicating that IRF4 signalling prevents the activation of caspases. The authors further described that IRF4 impacts mitochondrial homeostasis and deletion of IRF4 results in increased levels of ROS (reactive oxygen species) as well as a higher metabolic activity. Additionally, loss of IRF4 downregulates BLIMP-1, and thus XBP-1, and thereby impacts ER organisation[21,26]. IRF4 has been shown to control the expression of CD98 and GLUT1 (Glucose transporter 1) in T cells[27].

1.4 THE PLASMA CELL NICHE

Plasma cells in the bone marrow of mice individually dock onto non-proliferating VCAM-1$^+$ (vascular cell adhesion molecule 1) CXCL12$^+$ mesenchymal bone marrow stromal cells[28]. The role of stromal cells in memory cell maintenance remained elusive for a long time as they were merely regarded as physical organisers of the niche. The niche microenvironment additionally contains a variety of cells of hematopoietic origin that provide plasma cells with diverse and mainly redundant pro-survival signals, from cell-cell contact to soluble secreted cytokines (Figure *2*). The individual micro-environment of plasma cells underlies some heterogeneity and dynamic as the vicinity to blood vessels and the composition of accessory cells differs[29]. This might explain

the redundancy of many extrinsic factors for plasma cell survival. Until now, only two factors have been described as essential and sufficient for memory plasma cell survival: the cytokine APRIL (A proliferation-inducing ligand) and cell-cell contact[30].

1.4.1 SOLUBLE SURVIVAL FACTORS

In vitro plasma cell survival can be readily enhanced by the soluble factors IL-6 (Interleukin 6), CXCL12, BAFF (B-cell activating factor) and APRIL[31,32]. However, IL-6 and CXCL12 are not essential for memory plasma cell maintenance *in vivo*[33,34]. APRIL-deficient mice develop a normal immune system and comparable levels of serum immunoglobulins indicating that APRIL is dispensable for memory plasma cell survival[35]. However, another study showed a clear decrease in survival of plasmablasts homing to the bone marrow in APRIL but not BAFF-deficient mice[36]. Additionally, a recently identified APRIL-deficient patient displayed impaired immunoglobulin secretion suggesting that APRIL is essential for memory plasma cell maintenance[37]. The importance of APRIL signalling was unambiguously proven by deletion of the APRIL/BAFF receptor BCMA (B-cell maturation antigen) ablating almost all plasma cells in the bone marrow[38]. BCMA is a member of the TNFR (tumour necrosis factor receptor) superfamily and its activation results in strong pro-survival signalling. BCMA associates with TRAF1 (TNF receptor associated factor), TRAF2 and TRAF3 and activates Elk-1, JNK (c-Jun N-terminal kinases) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells)[39]. Atacicept, a recombinant fusion protein binding APRIL and BAFF, is effectively used in autoimmune diseases to target plasma cells, but initial studies using atacicept had to be halted after patients developed serious infections and severe reduction of serum antibodies, highlighting the significance of this signalling pathway[40]. The plasma cell surface marker CD138 improves survival by amplifying IL-6 and APRIL signalling[41].

1.4.2 CELL-CELL-CONTACT

Receptors involved in cell-cell interactions, such as CD44[31,42], CD28[43] and CD93[44] were reported as beneficial or essential for memory plasma cell maturation and long-term persistence *in vivo* and *in vitro*. These receptors enable plasma cells to interact with various accessory cells in their microenvironment. *In vitro* studies using stromal cell supernatant to enhance plasma cell survival revealed the impact of stromal cells on plasma cell maintenance[31]. More precisely two factors produced by stromal cells, fibronectin and the YWHAZ protein, were identified that maintained plasma cells

together with APRIL *in vitro*[45]. Additionally, a study showed that extracellular vesicles produced by mesenchymal stromal cells enabled plasma cell survival *in vitro* possibly by activating integrin signalling[46]. Inhibiting the binding of integrins VLA-4 (very late antigen 4, $\alpha 4\beta 1$) and LFA-1 (Lymphocyte function-associated antigen 1, $\alpha L\beta 2$) to their respective partners VCAM-1 and ICAM-1 (Intercellular Adhesion Molecule 1) on stromal cells ablated plasma cells from the bone marrow indicating that integrin signalling is an essential survival signal[47]. Furthermore, mice deficient in CD37, a tetraspanin that activates PI3K (Phosphoinositide 3-kinases) signalling by mediating VLA-4 clustering, also displayed decreased memory plasma cell survival[48]. A study in mice infected with Salmonella suggested that the interaction between laminin $\beta 1$ and a so far unknown integrin binding partner is essential in the maintenance of IgG^+ plasma cells in the bone marrow[49].

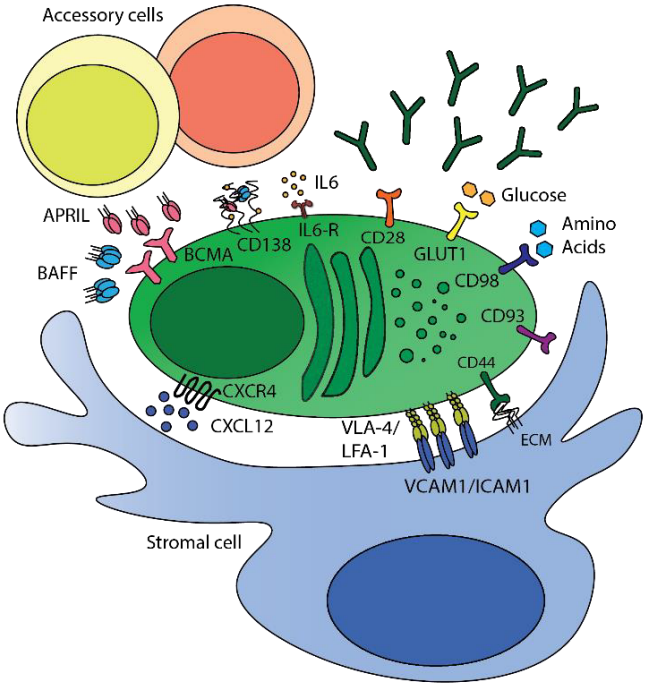

Figure 2: The plasma cell microenvironment in the bone marrow.
Memory plasma cells survive in niches organised by bone marrow stromal cells. They receive a variety of survival signals in the form of cell-cell contacts and soluble cytokines as well as nutrients enabling their persistence and continuous antibody secretion. Modified from[50].

1.4.2.1 INTEGRIN SIGNALLING via PI3K-AKT-FOXO

Integrin signalling is essential for memory plasma cell survival and activates the PI3K signalling pathway via the FAK (focal adhesion kinase)[51]. Integrins are heterodimers consisting of a α and β subunit and presented on the cell surface in different ligand affinity conformations: low, intermediate, and high. CXCL12:CXCR4 binding and signalling can activate integrin VLA-4[34]. VLA-4 ($\alpha 4\beta 1$) interacts with VCAM-1 and can

activate PI3K-AKT signalling. The tetraspanin CD37 was shown to specifically co-localise with VLA-4 but not LFA-1 and to enable the phosphorylation and activation of AKT resulting in survival of plasma cells[48]. The serine-threonine kinase AKT is activated by PI3K, a family of kinases that phosphorylate the hydroxyl group on the third position of the inositol ring of phosphatidylinositol (PIP2 (phosphatidylinositol (4,5)-bisphosphate) to PIP3). AKT binds PIP3 on the cell membrane and can subsequently be phosphorylated and activated by PDK1 (phosphoinositide dependent kinase 1) and mTORC2 (mammalian target of rapamycin complex 2). Active AKT regulates cell cycle, cell metabolism and overall cell survival by activating or deactivating downstream targets through phosphorylation. For example, AKT can promote cell survival by directly phosphorylating and deactivating the pro-apoptotic protein BAD (BCL2 associated agonist of cell death) and by activating the NF-κB pathway. Furthermore, AKT regulates cell metabolism by activating mTOR and deactivating glycogen synthase kinase 3. A substantial part of pro-survival signalling of AKT results from phosphorylation of FoxO1 (Forkhead-Box-Protein O1) and FoxO3 (Forkhead-Box-Protein O3) leading to their inactivation and degradation[52,53].

FOXO transcription factors were first identified in the nematode *Caenorhabditis elegans*. Mammals express four different FOXO proteins: FoxO1, FoxO3, FoxO4 and FoxO6. FOXO proteins have numerous downstream targets involved in cell cycle regulation, cell metabolism, autophagy, cell stress and survival[53]. They positively influence the pro-apoptotic proteins BIM (Bcl-2-like protein 11) and PUMA (P53 Upregulated Modulator of Apoptosis) and can initiate the intrinsic apoptotic pathway[54,55]. Therefore, FOXO proteins are tightly regulated by a variety of post-translational modifications including phosphorylation, acetylation, methylation, or ubiquitination. These modifications control their intracellular localisation and activation status and can result in their degradation. For example, phosphorylation by AKT promotes nuclear export and ultimately degradation of FOXOs whereas JNK phosphorylates FoxO3 causing nuclear translocation and activation of FoxO3 and initiation of apoptosis[56,57].

1.5 SOURCES FOR CELL STRESS IN PLASMA CELLS

Plasma cells, being professional secretors, experience high level of cell stress. Synthesis, folding and trafficking of proteins goes hand in hand with metabolic stress as high amounts of amino acids and energy are consumed. Additionally, the cells

experience endoplasmic reticulum stress in form of the UPR, proteasome stress as well as oxidative stress (Figure 3). Therefore, memory plasma cells must find ways to cope with the burden of extensive protein production. Surprisingly, when comparing short-lived PC and memory PC on a transcriptional level, no clear differences in their biology were found[19]. Instead, the ability to cope with cell stress appears to distinguish memory and short-lived plasma cells[19,58].

1.5.1 METABOLIC STRESS

Memory plasma cells and short-lived plasma cells can be divided according to the expression of the cell surface receptor GLUT1 and correspondingly, the amount of imported glucose. Memory plasma cells import more glucose that is primarily used for the glycosylation of antibodies. However, under metabolic stress memory plasma cells but not short-lived plasma cells can also use glucose to generate pyruvate[59]. Additionally, plasma cells express high amounts of the amino acid transporter CD98, as they need amino acids to produce immunoglobulins[19]. The intracellular domain of CD98 does not only form complexes with amino acid transporters, but has also been reported to support integrin signalling[60].

1.5.2 AUTOPHAGY

Autophagy is a stress-induced mechanism to recycle damaged and old intracellular organs, like mitochondria or the endoplasmic reticulum, to generate energy. The group of ATG (autophagy-related genes) control this process and ATG5 was reported to be essential for the survival of memory plasma cells[61]. Using ATG5-deficient mice, Pengo et al described that plasma cells lacking this key regulator of autophagy displayed an enlarged ER, amplified ER stress signalling and higher expression of BLIMP-1, ultimately resulting in increased immunoglobulin secretion and cell death. In plasma cells, autophagy is essential to preserve energy in form of ATP by negatively regulating antibody synthesis[61]. Notably, in plasma cells autophagy is focused on recycling of the ER and not of mitochondria or ribosomes. Stromal cells may be regulators of autophagy in plasma cells since stromal cell derived factors fibronectin1 and YWHAZ protein were shown to downregulate mTORC1 (mammalian target of rapamycin complex 1)[45]. mTORC1 controls protein translation and is a cellular sensor that gives feedback when adequate amounts of energy are present to allow the production of proteins, thereby negatively regulating autophagy.

1.5.3 PROTEASOME STRESS

In addition to autophagy, cells utilise the ubiquitin-proteasome system to recycle unwanted short-lived and misfolded proteins. Proteins are tagged for degradation by a chain of small ubiquitin proteins that are abundant in the cell cytoplasm. Subsequently, ubiquitinylated proteins are directed to the proteasome, a protease complex dependent on ATP. The proteasome degrades proteins into small peptide fragments. Hence, recycling of misfolded and unwanted proteins generates amino acids for the production of new proteins. In activated B cells, the capacity to degrade proteins using the ubiquitin-proteasome system decreases, contrary to the need of the cell to cope with accumulation of misfolded proteins due to increased protein production. Thus, plasma cells are extremely vulnerable towards proteasome inhibition. The proteasome inhibitor bortezomib efficiently depletes all plasma cells *in vivo*[3,62].

1.5.4 ENDOPLASMIC RETICULUM STRESS

The endoplasmic reticulum serves many important functions in the cell, such as protein production, folding and transport. Additionally, it is the main calcium storage of the cell. When continuous, high levels of protein synthesis exceed the capacity of the ER to correctly fold and process proteins, the ER stress response is activated. More precisely, three independent signalling pathways controlled by ER stress sensors can be induced that are summarised as the UPR: IRE1 (inositol-requiring protein 1), PERK (PKR-like endoplasmic reticulum kinase), and ATF6 (activating transcription factor 6). Under normal conditions, these sensors are bound to chaperones, predominantly BiP (Binding immunoglobulin protein). When misfolded proteins accumulate, they bind to the chaperones, releasing and thereby activating the ER stress sensors. Upon activation, the stress sensors can either promote cell survival or cell death, depending on the intensity and duration of the ER stress[63].

While plasma cells express phosphorylated eIF2α (eukaryotic initiation factor 2α), the downstream target of PERK, PERK-deficient mice still developed memory plasma cells in comparable numbers to wild type animals, suggesting that PERK signalling is not essential for plasma cell maintenance[19,64]. ATF6 is detectable very early in plasmablasts after LPS (lipopolysaccharide) stimulation[65]. However, ATF6-deficient and WT (wild type) mice have comparable antibody responses *in vitro* and *in vivo*, indicating that ATF6 is also dispensable for memory plasma cell function and survival[66]. Although the arms of the UPR individually are not essential in memory

plasma cell survival, the loss of XBP-1 severely diminishes antibody secretion[24]. The transcription factor XBP-1, important for the generation of plasma cells, is specifically spliced following IRE1 activation. XBP-1's genetic targets reduce ER stress by increasing the capacity of the ER to correctly fold new proteins and degrade misfolded proteins. When cells experience prolonged ER stress, the IRE1-XBP-1 pathways can induce pro-apoptotic signalling. IRE1 associates with TRAF2, which can result in the induction of JNK and in the activation of caspase 12 and, ultimately in ER-stress induced cell death[67,68].

ATF6 and XBP-1 are both regulated by the plasma cell master transcription factor BLIMP-1, and ATF6 additionally promotes transcription of XBP-1[22,69]. Considering that the UPR is upregulated prior to immunoglobulin synthesis, it does not represent a stress response but a general feature of memory plasma cell biology[63].

1.5.5 APOPTOSIS

Apoptosis, or programmed cell death, is a controlled way of cell suicide mediated by caspases, a family of cysteine proteases. Apoptosis can be triggered by extrinsic or intrinsic factors. The extrinsic pathway is initiated by binding of a ligand to a death receptor, resulting in activation of the effector caspase 8, which, in turn, activates the caspase cascade. In the mitochondria, the linchpin of the intrinsic apoptosis pathway, MOMP (mitochondrial outer membrane permeabilisation) occurs through oligomerisation of BAX (Bcl2-associated X protein) and BAK (BCL2-antagonist/killer 1), followed by release of cytochrome C. As a result, the apoptosome, a complex of Apaf-1 (apoptotic protease activation factor-1) and caspase 9, activates the caspase cascade. The caspase cascade comprises effector caspases 3, 6 and 7, and has numerous downstream targets to ensure the orderly degradation of the cell[70,71].

MOMP represents the "point of no return" in apoptosis, and its initiation is therefore tightly controlled by the BCL2 family. The BCL2 family consists of over 20 members that are separated into pro-and anti-apoptotic proteins. Anti-apoptotic proteins such as BCL2, BCL-w (Bcl-2-like protein 2), BCL-xL (B-cell lymphoma extra-large) and MCL1 (myeloid cell leukaemia 1) prevent MOMP by binding to BAX and BAK. Pro-apoptotic proteins such as BIM, PUMA and NOXA (Phorbol-12-myristate-13-acetate-induced protein 1) compete in binding to the anti-apoptotic proteins, thereby allowing the oligomerisation of BAX and BAK and MOMP. Consequently, the balance of pro-and anti-apoptotic proteins plays a pivotal role in apoptosis initiation[70].

In plasma cells, the extrinsic apoptotic pathway was reported to be negligible as the first caspase detectable in apoptotic human plasma cells is the ER-associated caspase 4[72]. In mice, caspase 12 is associated with the ER.

Studies regarding the intrinsic apoptosis pathway were predominantly focused on mitochondria as key players, but recently the ER has emerged as a platform coordinating and integrating signals that control autophagy, UPR and apoptosis. The ER and mitochondria can be found in proximity to one another, and they interact in a dynamic and complex manner to regulate diverse cellular functions. Being the main calcium storage of the cell, the ER communicates with the mitochondria via Ca^{2+} release, inducing either mitochondrial metabolism or apoptosis. The outcome of this communication is determined by the duration and intensity of ER stress and calcium release as well as by BCL2 family members. The crosstalk between the ER and BCL2 family members is bidirectional as ER stress regulates the expression of pro-apoptotic proteins and pro- and anti-apoptotic proteins regulate diverse ER-associated functions. Thus, the multifaceted role of BCL2 family members must be considered[73,74].

1.5.5.1 BCL2 family members MCL1 and BCL2 in plasma cell survival

The anti-apoptotic proteins BCL2 and Bcl-xL were found to be essential for the generation of plasma cells. Bcl-w, BCL2 and MCL1 are highly upregulated in bone marrow plasma cells, contrary to Bcl-xL[33,75].

MCL1 is the master survival factor of plasma cells and MCL1 deletion results in ablation of almost all memory plasma cells. BCMA, the receptor for APRIL and BAFF, has been shown to induce MCL1 expression[33]. Furthermore, the transcription factor ZBTB20 (Zinc finger and BTB domain-containing protein 20) that is transcriptionally controlled by IRF4 was linked to MCL1 expression in plasma cells[76,77]. MCL1 has a very short half-life, and its regulation is highly complex with steps at transcriptional, post-transcriptional and post-translational levels[78]. In multiple myeloma, the balance between NOXA and MCL1 as well as MCL1 and BIM was reported to be decisive for cell survival[79,80].

The role of BCL2 in plasma cell survival remains elusive, as treatment with the drug ABT-737, specifically targeting BCL2 and Bcl-xL but with a preference for BCL2, did not decrease plasma cell numbers *in vivo*[81]. However, the same group later presented evidence that Blimp1^{high} memory plasma cells of spleen and bone marrow are decreased following ABT-737 treatment *in vivo* and *in vitro*. Additionally, the treatment

of BCMA$^{-/-}$ mice, which exhibit decreased MCL1 levels and very low plasma cell numbers, with ABT-737 resulted in cell death of the remaining plasma cells, suggesting a synergistic effect of MCL1 and BCL2 with non-overlapping targets[75]. Furthermore, BCL2 overexpression in mice rescued plasma cells from cell death following IRF4 deletion[21].

Although BCL2 family members are primarily known as regulators of apoptosis, many of them have been implicated in regulation of autophagy. BCL2 can be located at the mitochondria, where is binds to BAX and inhibits MOMP, but it can also be found at the ER, where it binds to the autophagy regulating protein Beclin1. Additionally, Bcl-xL was shown to interact with Beclin1[82–84]. MCL1 can also negatively regulate autophagy and lower levels of MCL1 induce autophagy without triggering the initiation of apoptosis[85].

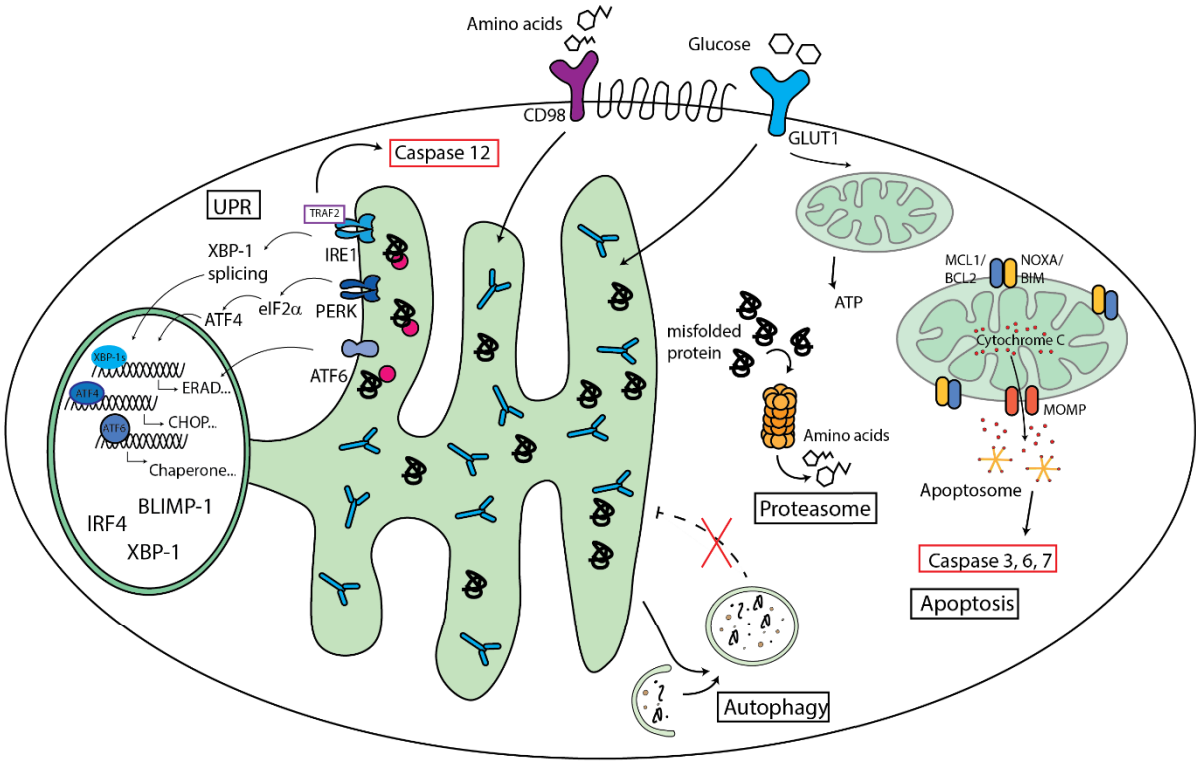

Figure 3: Overview of intracellular mechanisms in memory plasma cells.
Memory plasma cells produce high amounts of antibodies in the ER (endoplasmic reticulum). The ER and immunoglobulin production are controlled by autophagy and misfolded proteins are degraded by proteasomes. Amino acids and glucose are primarily utilised for antibody generation. Under stress conditions, autophagy is blocked leading to an expansion of the ER, amplified antibody production and accumulation of misfolded proteins. The UPR (unfolded protein response) is activated to enable cell survival, but upon prolonged ER stress caspase 12 is activated and the cell undergoes apoptosis. Additionally, pro-apoptotic proteins (e.g. BIM, NOXA) bind to anti-apoptotic proteins (e.g. MCL1, BCL2) resulting in MOMP (mitochondrial outer membrane permeabilisation). Cytochrome c release and formation of the apoptosome activates effector caspases 3, 6 and 7.

A complex network of extracellular and intracellular signals influences the memory plasma cell phenotype, function, and survival. It remains elusive whether the property to survive for decades is intrinsically imprinted in the plasma cells, possibly during their generation, or predominantly determined by the supportive microenvironment. It is likely a combination of both factors. Cell stress elicits diverse signalling pathways that should not be regarded as isolated events since they are highly dynamic and interconnected. All previously mentioned signalling pathways have been shown to interact either directly or indirectly depending on the context, adding complexity to the molecular mechanism of plasma cell survival.

1.6 RESEARCH AIM

The aim of this thesis was to gain more insight into how the supportive microenvironment of memory plasma cells in the bone marrow enables their long-term survival. The influence of stromal cells was investigated to understand if they are merely physical organisers of the memory plasma cell niche or actively contribute to the survival. Furthermore, we wanted to elucidate which signalling pathways are essential for memory plasma cell maintenance.

2 MATERIAL AND METHODS

2.1 MATERIAL

2.1.1 BUFFERS AND MEDIA

Name	Composition	Source
PBS pH 7.2 (Phosphate buffered saline)	137 mM NaCl 2.7 mM KCL 1.5mM KH2PO4 7.9mM NaHPO4 x 2H2O	Carl Roth Carl Roth Carl Roth Honeywell Riedel-de Haen
PBS/BSA pH 7.2	PBS 0.2% (w/V) BSA (bovine serum albumin)	PAN Biotech
PBS/BSA/EDTA pH 7.2	PBS/BSA 2 mM EDTA (ethylenediaminetetraacetic acid)	Invitrogen by Life technologies
MACS Buffer	PBS 1% FCS (fetal calf serum) 2 mM EDTA	Thermo Fisher Scientific
Complete cell culture medium	RPMI (Roswell Park Memorial Institute) 1640 Glutamax 10% FSC 10 U/mL Penicillin 10 µg/mL streptomycin 0.1 % (v/V) b-Mercaptoethanol	Gibco Biowest Gibco Gibco Gibco
Cell culture medium for siRNA	RPMI (Roswell Park Memorial Institute) 1640 Glutamax 5% FSC 20 U/mL Penicillin 20 µg/mL streptomycin 0.2 % (v/V) b-Mercaptoethanol	Gibco Biowest Gibco Gibco Gibco
50x TAE (pH 8,5)	50 mM EDTA 2 M Tris 1 M glacial acid in ultrapure water	

2.1.2 REAGENTS; CHEMICALS, RECOMBINANT PROTEINS

Name	Source
4-hydroxy-3-nitropheylacetyl hapten coupled chicken gamma globulin (NP-CGG)	Biomol, DRFZ
Incomplete Freud's Adjuvant	Sigma-Aldrich Chemie GmbH
Paraformaldehyde (PFA), 20 % (w/v)	Electron Microscopy Sciences, 20%
Trypsin-EDTA	Gibco
Recombinant multimeric APRIL	Adipogene
HEPES (4-(2-hydroxyethyl)-1-Piperazineethanesulfonic acid)	Merck
EDTA (0,5 M)	Thermo Fisher Scientific

Cell Signalling Buffer Set A	Miltenyi Biotec
Wortmannin	Selleckchem
Pan Caspase Inhibitor Z-VAD-FMK	Santa Cruz Biotechnology
FCgR block	Miltenyi Biotec
Tunicamycin	Sigma-Aldrich Chemie GmbH
4',6-Diamidin-2-phenylindol (DAPI)	Thermo Fisher Scientific
Tetramethylrhodamine, Methyl Ester, Perchlorat (TMRM)	Life Technologies
Carbonyl cyanide m-chlorophenyl hydrazine (CCCP)	abcam
Antimycin A	Sigma-Aldrich Chemie GmbH
Agarose	Biozym Scientific GmbH
Gel Red	Biotium
Isoflurane	Baxter
DNA Polymerase Dream Taq, 10 x Dream Taq Green Buffer	Therme Fisher Scientific
dNTPs 10 mM	Therme Fisher Scientific
GeneRuler^{TM} 1kb Plus DNA Ladder	Therme Fisher Scientific

2.1.3 STAINING KITS

Name	**Source**
CaspGLOW™Fluorescein ActiveCaspase-12 Staining Kit	BioVision
CaspGLOW™Fluorescein Active Caspase-3 Staining Kit	BioVision
CaspGLOW™Fluorescein Active Caspase-8 Staining Kit	BioVision
CaspGLOW™Fluorescein Active Caspase-9 Staining Kit	ThermoFisher Scientific
CellROX™ Green Flow Cytometry Assay	ThermoFisher Scientific
MitoSOX™ Red	ThermoFisher Scientific

2.1.4 ANTIBODIES

Specificity	**Clone**		**Source**	**Identifier**
Anti-mouse active caspase 3	D3E9	pure	Cell Signaling Technology	Catalog # 8788
Anti-mouse active caspase 7	D6H1	pure	Cell Signaling Technology	Catalog # 8438T
Anti-mouse BCL2	REA356	APC	Miltenyi biotec	Catalog # 130-105-474
Anti-mouse BIM		APC	Cell Signaling Technology	
Anti-mouse NOXA	114C307	A405	Abcam, DRFZ	Catalog # ab13654

Anti-mouse MCL1	Y37	A488, pure	Abcam	Catalog # ab32087
Anti-mouse FoxO1	C29H4	Pure	Cell Signaling Technology	Catalog # 2880
Anti-mouse FoxO3	D19A7	pure	Cell Signaling Technology	Catalog # 12829
Anti-mouse IRF4	REA201	APC	Miltenyi biotec	Catalog # 130-100-913
Anti-mouse CD138	REA104	PE, PE-Vio770	Milentyi biotec	Catalog # 130-102-318
Anti-mouse B220	RA3-6B2	Biotin	Miltenyi biotec	Catalog # 130-101-928
Anti-mouse CD19	6D5	APC	Miltenyi biotec	Catalog # 130-112-036
Anti-mouse CD29	REA-1074	PE	Miltenyi biotec	Catalog # 130-119-165
Anti-mouse CD49b	R1-2	Biotin	Miltenyi biotec	Catalog # 130-101-912
Anti-mouse CD98	4F2	APC	BioLegend	Catalog #128212

2.1.5 MAGNETIC BEADS

Specificity	**Manufacturer**
Streptavidin	Miltenyi Biotec
CD138	Miltenyi Biotec

2.1.6 OLIGONUCLEOTIDES

FoxO1	SMARTPool	Catalog # E-041127-00-0010
FoxO3	SMARTPool	Catalog # E-040728-00-0010
ITGB1	Individual siRNA	Catalog # A-040783-13-0020
IRF4	Individual siRNA	Catalog # A-043796-13-0010
Non-Targeting	Individual siRNA	Catalog # D-001910-04-20

2.1.7 PRIMERS

Primer	**Sequence**
Oligo 1 (-5`)	GGCAAGATCAAGTATGAGTGC
Oligo 2 (-5`)	TGAGTAGTCACAGAGTACCCA
Oligo 3 (-5`)	GCGGAATTCATTTAATCACCCA

2.1.8 KITS

Name	Source	Cat number
PureLink™ Genomic DNA Mini kit	Thermo fisher scientific	K182002
IgG (total) mouse uncoated ELISA kit with plates	Thermo fisher scientific	88-50400-22
IgA ELISA mouse uncoated ELISA kit with plates	Thermo fisher scientific	88-50450-22
IgM ELISA mouse uncoated ELISA kit with plates	Thermo fisher scientific	88-50470-22
NextGem5'version2	**1**0xGenomics	

2.1.9 CONSUMABLES

Name	Source
Cell Culture Flasks Cellstar®, 25/75 cm^2	Greiner Bio-one GmbH
Cell culture plates (96 well)	Greiner Bio-one GmbH
MACS® LS- und MS-Columns	Miltenyi Biotech
MACS® Separation Filter, 30 µm	Miltenyi Biotech
MACS® Separator and MultiStand	Miltenyi Biotech
Single-use needles	B. Braun Melsungen AG

2.1.10 TECHNICAL EQUIPMENT

Biometra Thermocycler T-Gradient ThermoBlock	Analytik Jena
Centrifuge 5810 R	Eppendorf
ChemiDoc™ MP System	Bio-Rad Laboratories GmbH
Gel Electrophoresis System	Peqlab
Heraeus Fresco 21 Centrifuge	Thermo Fisher Scientific
MACSQuant Analyzer	Miltenyi Biotech
Illumina nextseq2000	10x Genomics

2.1.11 SOFTWARE

Name	Source
Loupe Browser v5.0	10x Genomics
FlowJo10 v7.1	FlowJo LLC
GraphPad Prism 9	GraphPad Software
Microsoft Office	Microsoft Corporation

2.2 METHODS

2.2.1 MICE

2.2.1.1 Mice

All used mice were maintained under specific pathogen free (SPF) conditions at the experimental animal facility of the “Deutsches Rheuma Forschungszentrum” (DRFZ, Berlin, Germany). Experiments were performed according to institutional guidelines and German Federal laws on animal protection and with permission of the “Landesamt für Gesundheit und Soziales Berlin” (LAGeSo Berlin, Germany). Animals are housed in IVC cages with a maximum of 5 animals per cage. The animals are provided with enrichment in form of nesting material and wood. Special, autoclaved food as well as autoclaved water is supplied ad libitum. Cages are equipped with wood chip bedding material and a shelter made from red plastics. Animals have a settling-in-period of five days after arrival from breeding facilities. Animals are exposed to light for a cycle of 12 hours, followed by 12 hours of darkness

Following mouse strains were used for experiments described in this work:

1) **C57BL/6J**: Wild type strain purchased from Charles River Laboratories or Janvier Labs.

2) **Blimp-1:GFP**: Antibody secreting cells of heterozygous mice express the GFP (green fluorescent protein) under the control of the Blimp-1 regulatory elements (Kallies et al., 2004). The mice were donated by S. Nutt (Walter and Eliza Hall Institute, Melbourne, Australia). The genetic background of this mouse line is C57BL/6J. The mice were bred and maintained under SPF conditions at the “Bundesinstitut für Risikobewertung” (BfR, Berlin, Germany)

2.2.1.2 Immunisation

Mice were primed and challenged twice with 100µg NP-CGG (4-hydroxy-3-nitropheylacetyl hapten coupled chicken gamma globulin) in IFA (incomplete Freud`s Adjuvants) intraperitoneally (i.p.) in a total volume of 200 µL (100 µg NP-CGG in PBS + 100 µL IFA). The period in between injections was 21 days. 30 days after the last injection, mice were ready for the experiment.

2.2.1.3 Genotyping

Ear punches or tail cuts were used for genotyping. Samples were processed using the PureLink™ Genomic DNA Mini kit according to manufacturer’s instructions.

After DNA Isolation, following primers were used for the polymerase chain reaction (PCR)

Oligo 1 (-5`): GGCAAGATCAAGTATGAGTGC
Oligo 2 (-5`): TGAGTAGTCACAGAGTACCCA
Oligo 3 (-5`): GCGGAATTCATTTAATCACCCA

The mastermix was prepared after following protocol:

Component	**1x [µL]**
10x PCR reaction buffer	1.8
dNTPs (1,25 mmol/L)	1
Oligo 1 (20 µM)	0.5
Oligo 2 (20 µM)	0.5
Oligo 3 (20 µM)	0.5
ddH_2O	13.5
DreamTaq Green DNA 5U/mL polymerase	0.2

18 µL Mastermix was added to 1 µL DNA of the respective sample in 0.2 µL sample tubes.

PCR was performed with following cycles:

1. Step	94°C	300s	
2. Step	94°C	20s	
	60°C	30s	34x
	72°C	60s	
3. Step	72°C	600s	
4. Step	4°C	∞	

The PCR product was loaded on a 2 % agarose gel. It was run at 120V for 60 min. The wild-type product had a size of 611 bp and for the heterozygous an additional product at 531 bp was detected.

2.2.2 CELL CULTURE

2.2.2.1 ST2 cell culture

The stromal cell line ST2, originally isolated from bone marrow of a BALB/c mice, was used as feeder cell line in the plasma cell *in vitro* niche. ST2 cells were cultured in cell culture flasks in the presence of RPMI1640 Medium supplemented with 10% FCS, 10U/mL Penicillin, and 10 µg/mL streptomycin at 37°C and 5% CO_2. The cells were split when confluency was observed, approximately twice per week. Splitting of cells was performed by taking off the culture supernatant, washing with PBS and incubation with trypsin. Trypsin was incubated for 5 min and the reaction was stopped with RPMI1640 media. Cells were washed, counted, and plated in a new culture flask. For the *in vitro* niche, cells were trypsinised one day before the experiment. Cells were

counted using the MACSQuant Analyser and 2500 cells in 100 µL were plated into wells of a 96 well plate.

2.2.2.2 Isolation of plasma cells by magnetic enrichment

Plasma cells were isolated from bone marrow in a two-step protocol consisting of a depletion step followed by an enrichment. Tibia, femur, and hip bones were used. The bone marrow was isolated by cleaning the bones from flesh, then cutting them on one end. Cut bones were placed in 0.2 mL reaction tubes with a hole in the bottom. These tubes were placed into 1.5 mL reaction tubes containing 0.2 mL MACS Buffer (PBS, 1% FCS, 2 mM EDTA). Tubes were centrifuged at 300xg for 15 seconds. If bone marrow remained in the bones, the procedure was repeated. Afterwards, bone marrow from the tubes was pooled and counted using anti-CD138 antibody and DAPI. The cell concentration was set to $1*10^8$ total cells per mL and samples were stained with biotinylated anti-CD49b and anti-B220 antibodies at 4°C. The samples were washed with MACS buffer and centrifuged at 300xg for 8 min with low break. Subsequently, cells were resuspended in MACS buffer, magnetic streptavidin beads were added, and the samples were incubated for 15 min at 4°C. Cells were washed and resuspended in buffer. LS columns with 30 µM filters were attached to MACS magnets and primed with buffer. Cells were added to the filters and to reduce the flow rate, e a 26G cannula was attached to the bottom of the column. Columns and filters were washed 3 times with 1 mL MACS buffer. The negative collection was collected, washed, and counted. Cells were resuspended in MACS buffer and incubated with magnetic anti-CD138 Microbeads for 15 min at 4°C. Cells were washed and added to a pre-wetted MS column placed in a MACS magnet. The column was washed three times with 0.5 mL MACS buffer. The cells were eluted on a second MS column using 1 mL MACS buffer. The column was washed three times and the cells were eluted using 2mL RPMI1640 media or PBS. Cells were counted using DAPI and anti-CD138 antibody[30].

2.2.2.3 Memory plasma cell in vitro niche

Isolated plasma cells were cultured in RPMI1640 media (10%FCS, 10U/mL Penicillin, 10 µg/mL streptomycin, 0.1% β-Mercaptoethanol, 25 mM HEPES buffer) in the presence or absence of 50 ng/mL multimeric APRIL. Plasma cells were seeded on top of ST2 cells in a ratio of 1:1, 5000 plasma cells on 5000 stromal cells. If culture conditions without stromal cells were used, 5000 plasma cells were seeded into empty wells. The total culture volume was 200 µL. If cells were cultured longer than 3 days,

the media was changed on day 3. 100 µL media was carefully taken off without disturbing the cells and 100 µL fresh media with or without 50 ng/mL APRIL was added to the culture. Cultures were performed at hypoxic conditions 4.2 % O_2, 5% CO_2, 37°C[30].

2.2.2.4 siRNA treatment

If plasma cells were treated with siRNA (small interfering RNA), the cells were eluted with PBS and washed following the isolation protocol. The Accell self-delivery system was used. Cells were resuspended in Accell medium in the presence of 100 ng/mL multimeric APRIL at a concentration of 100.000 cells/mL. siRNAs were added to obtain a concentration of 2 µM. If multiple siRNAs were used, the concentration of all used siRNAs was 2 µM. Cells were incubated for an hour at 37°C, 5% CO_2. 50 µL RPMI1640 media with 5% FCS, 20U/mL Penicillin, 20 µg/mL streptomycin, 0.2% b-Mercaptoethanol, 50 mM HEPES buffer was added to stromal cells or empty wells. After incubation, 50 µL plasma cells were added to the respective wells and cultures were performed under hypoxic conditions.

2.2.2.5 ELISA

Total IgG, IgA and IgM ELISAs were performed using IgM, IgG or IgA mouse uncoated ELISA kits according to manufacturer's instructions. First, cell culture supernatants were titrated to identify the correct dilution of the samples. In brief, plates were coated overnight with capture antibodies in blocking buffer at 4°C. Plates were washed and blocked with blocking buffer. Standard was prepared, and standard and diluted samples were added to the plates and incubated for 2 hours at room temperature. Subsequently, plates were washed and incubated with detection antibodies for 1 hour at room temperature. Plates were washed again, and Substrate solution was added and incubated for 15min. Finally, stop solution was added and plates were read at 450nm using a plate reader.

2.2.3 FLOW CYTOMETRY

2.2.3.1 Plasma cell counts

Viable plasma cells were counted on different days of the culture using the MACSQuant Analyser. The cell culture supernatant was carefully removed without disturbing the cells. 100 µL anti-CD138 PE antibody (diluted 1:100) in PBE was added to the wells and incubated for 5 min on ice. Using a multichannel pipet, cells were resuspended and scraped off the plate. Cells were transferred into a 96 well-v-bottom

plate. The MACSQuant Analyser was programmed to add DAPI (1:100) automatically to each well before measuring. Viable plasma cells were defined as $CD138^+$/DAPI.

2.2.3.2 Cell surface staining

Single cell suspension was prepared in PBE buffer. Cells were incubated with FcγR blocking reagent for 10 min on ice before the antibody mastermix was added. Cells were stained for 10 min on ice, washed and measured on a flow cytometer.

2.2.3.3 Intracellular staining

The cell culture supernatant was carefully removed and 50 µL 4% PFA was added to the wells. Cells were incubated at 37°C for 10 min and washed with PBE. Cells were either stored in the fridge or 50 µL pre-chilled (-20°C) methanol was added to the wells and incubated in the fridge for 30 min. Afterwards, cells were washed with PBE and resuspended in 10 µL FcγR blocking reagent buffer. Cells were incubated for 10 min at room temperature (RT). The antibody mastermix was added to the well plates and incubates for 60 min at RT in the dark. The cells were washed and if necessary incubated with secondary antibody for 30 min at RT. Cells were washed and measured on a flow cytometer.

2.2.3.4 Active Caspase staining

Active caspase stainings were performed using a fluorescence-coupled substrate against the respective caspase. Cells were cultured with or without ST2 cells and with or without APRIL. On the day of analysis, 150 µL of cell culture supernatant was carefully removed from the well plate. 50 µL RPMI1640 with or without APRIL containing the fluorescence-coupled caspase substrate was added and cells were incubated at 37°C for 30 min. As a negative staining control, a pan caspase inhibitor was added according to manufacturer's instruction 2 hours before the staining. After incubation, cells were washed twice using wash buffer. Cells were resuspended in anti-CD138 antibody mastermix in PBE (diluted 1:100 or 1:50) and measured directly. DAPI was used to discriminate between life and dead cells. As negative control, cells were incubated for 24 hours with 10 mM panCaspase Inhibitor Z-VAD-FMK. As positive controls, cells were incubated with 10 mM wortmannin or 2.5 mM tunicamycin for 2 hours prior to staining.

2.2.3.5 TMRM staining

Mitochondrial membrane depolarisation was assessed using TMRM dye. Cells were incubated with 250 nM TMRM in pre-warmed HBSS for 30 min at 37°C protected from light. Next, cells were washed and resuspended in anti-CD138 antibody mastermix in PBE. As a negative control, cells were incubated with 5µM CCCP 30min prior to staining. Cells were measured on a MACSQuant Analyser and DAPI was added directly before acquisition.

2.2.3.6 Detection of reactive oxygen species (ROS)

Total cellular ROS was detected using CellROX™ dye. CellROX™ was diluted in DMSO and added to cells in cell culture medium to achieve a final concentration of 500 µM. Samples were incubated for 30 min at 37°C, protected from light. Subsequently, samples were washed with PBE and stained with anti-CD138 antibodies. Samples were measured on a MACSQuant Analyser and DAPI was added directly prior to the sample. As negative control, 3000 µM NAC (N-acetylcysteine*)* to increase the antioxidant capability of the cell was added one hour before CellROX™ dye and as positive control, 150 µM TBHP (tert-butyl hydroperoxide*)* was added 30 min before CellROX™ dye to induce oxidative stress.

Mitochondria ROS was assessed using MitoSOX™ dye. Cells were incubated with 3µM MitoSOX™ in pre-warmed HBSS for 15min at 37°C protected from light. Next, cells were washed, and surface stained with anti-CD138 antibodies. Samples were measured on a MACSQuant Analyser and DAPI was added directly prior to the sample. As positive control, 20 µM Antimycin A was added 30 min prior to staining to the cells. As negative control, 5 µM CCCP was added 30 min prior to staining to the cells

2.2.4 SINGLE CELL RNA SEQUENCING

For single cell RNA sequencing, plasma cells were isolated and cultured with siRNAs against FoxO1, FoxO3, IRF4 or the non-targeting scrambled control siRNA. After 3 days, cells were sorted for $CD138^{++}$ and $DAPI^{-}$ cells. During preparation, no buffer containing EDTA was used as that can interfere with the single cell sequencing protocol. Cells were counted and adjusted to the cell concentration needed for the 10X genomics protocol. The NextGem5'version2 kit was used according to manufacturer's instruction. In short, the first step was the Gel Beads-in-emulsion (GEM) generation and barcoding where barcoded single cell VDJ 5' Ged beads, a master mix with cell surface protein labelled cells and partitioning oil were combined in a chromium next

GEM Chip K. That was followed by a post GEM-RT clean-up and cDNA Amplification. V(D)Js were amplified from full-length cDNA using primers specific to BCR constant regions and the V(D)J library was constructed. Afterwards, the 5' Gene Expression Library was constructed, and samples were sequenced. (Flow cell P3; 200 cycles; Read1: 26nt; Read2: 90nt; Index1: 10nt; Index2: 10nt).

2.2.4.1 Analysis of single cell data

Illumina output was demultiplexed and mapped to the mm10 reference genome by cellranger-5.0.0 (10x Genomics Inc.) using refdata-cellranger-mm10-1.2.0 in default parameter setting and 3000 expected cells. Raw UMI-counts were further analysed using R 4.1.2 with Seurat package [86], as proposed by Butler and colleagues [87], including log-normalisation of UMI counts, detection of variable genes and scaling. T-distributed Stochastic Neighbour Embedding (t-SNE) and the underlying Principle Component Analysis (PCA) was performed based on 30 components using variable genes and a perplexity of 30 as set by default. Data were analysed using 38,243 plasma cells

2.2.5 STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad prism. Datasets were tested for normal distribution. Subsequently, the appropriate test for parametric or non-parametric data was chosen. Respective analysis is indicated in figure legends.

3 RESULTS

3.1 APRIL and ST2 cell contact prevent cell death of *ex vivo* memory plasma cells by inhibiting specific caspases

Memory plasma cells can persist for decades in dedicated niches in the bone marrow while continuously secreting antibodies. However, taken out of their microenvironment they die fast in the absence of survival factors, indicating that their persistence is determined by external factors[88]. Using an *in vitro* niche, we aimed to elucidate the molecular signalling network enabling plasma cell survival.

We previously reported that memory plasma cells require two essential survival signals *in vitro*: **(1)** stromal cell contact provided by the cell line **ST2** and **(2)** the cytokine **APRIL** (**ST2+A**). Additionally, bone marrow memory plasma cells were cultured under physiological oxygen conditions (4.2 % O_2). We isolated *ex vivo* memory plasma cells from the bone marrow of mice immunised and challenged twice in 21-day intervals with NP-CGG/IFA using a two-step magnetic cell sorting (MACS) protocol which routinely resulted in a purity of ~90%. Plasma cells were identified as $CD138^{++}$, $B220^{-}$, HLA-DR^{-} and $Ki67^{-}$ and cultured in a 1:1 ratio with cells of the stromal cell line ST2 and recombinant APRIL[30].

Using the *in vitro* culture system, we demonstrated that a pan-caspase inhibitor prevented cell death if memory plasma cells were cultured only with either APRIL or ST2 cells, suggesting that memory plasma cells die in a caspase-dependent way[30]. The caspase-network can be activated by numerous stimuli, some being extrinsic and other intrinsic. Caspase 8 can be activated by FAS/FAS-L interaction whereas caspases 3, 7, and 12 are activated intrinsically by the release of mitochondrial apoptogenic factors or prolonged ER stress[68].

To examine the activation of different caspases and the contribution of ST2 and APRIL to prevent their activation, memory plasma cells were cultured with or without APRIL and with or without ST2 cells for one day. Subsequently, activation of caspases 3, 7, 8 and 12 was measured in viable plasma cells using flow cytometry (S 1) (Figure 4). Active caspase 7 was detected using intracellular antibodies whereas active caspases 3, 8 and 12 were detected using a caspase-specific fluorescence-coupled substrate.

Caspase 8 activation was not detected in any condition (Figure 4C) (APRIL 130.5 ± 9.5; ST2 117.8 ± 8.4; ST2+A 115.8 ± 8.3; panC Inhib 117 ± 11.6). Caspases 3 and 7 were highly activated in cells cultured only with APRIL and activation was significantly downregulated in co-culture with ST2 cells (Figure 4 A,B) (caspase 3 APRIL 742 ± 38; ST2 573.7 ± 47; ST2+A 478.8 ± 76; panC Inhib 473 ± 45; caspase 7 APRIL 1296 ± 114; ST2 1088 ± 133; ST2+A 1194 ± 150). Memory plasma cells cultured with APRIL alone displayed lower levels of active caspase 12 compared to plasma cells cultured only with ST2 cells. Together with ST2 cells, the activation of caspase 12 was completely inhibited (APRIL 1988 ± 590.7; ST2 2590 ± 288; ST2+A 1354 ± 258; panC Inhib 1162 ± 355.7). As controls for the caspase stainings a pan-caspase inhibitor, wortmannin to block PI3K signalling, or tunicamycin as inducer of the unfolded protein response were used (S 2).

Based on these results, we conclude that the extrinsic pathway of apoptosis does not contribute to plasma cell death via activation of caspase 8 in line with previous work[72]. However, the intrinsic apoptotic pathway defined by caspases 3, 7 and 12 was activated in plasma cells when cultured only with APRIL. While activation of caspases 3 and 7 was very low in memory plasma cells cultured with ST2 cells alone, cells of this condition expressed the highest levels of the ER-associated caspase 12. Thus, the data suggest that ST2 cells are sufficient to prevent activation of caspases 3 and 7 but both signals are required to fully prevent activation of caspase 12.

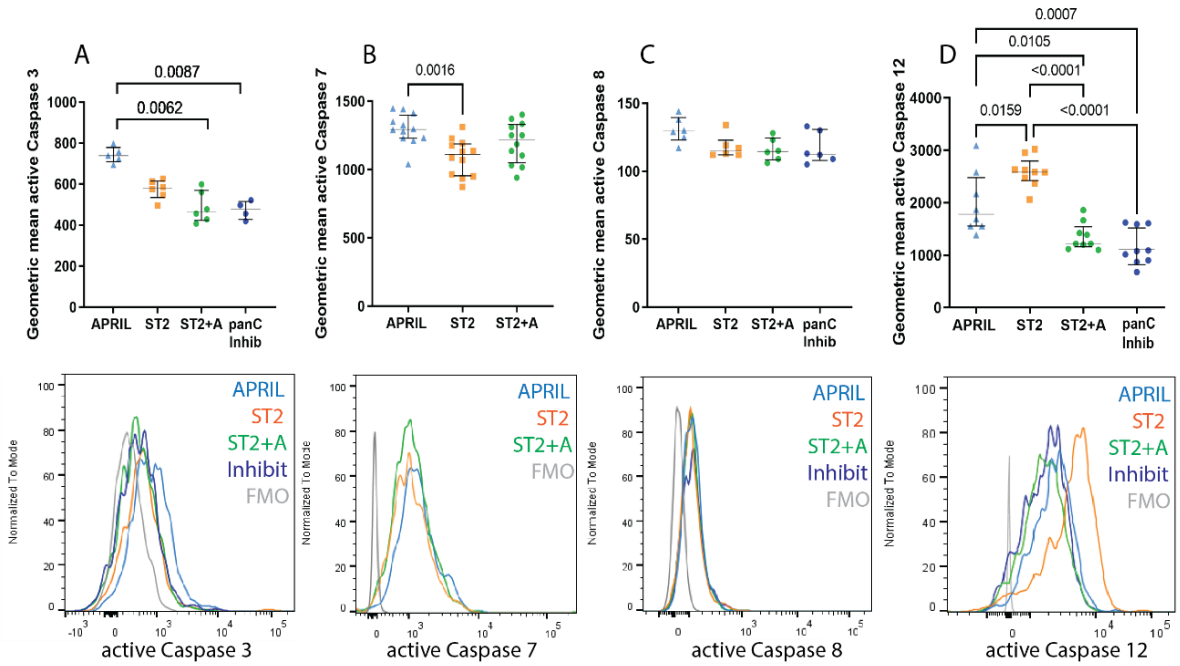

Figure 4: ST2 cells inhibit activation of caspases 3 and 7, and APRIL together with ST2 cells prevent the activation of caspase 12. Geometric mean expression of (A) active caspase 3, (B) active caspase 7, (C) active caspase 8, and (D) active caspase 12 in *ex vivo* memory plasma cells cultured with 50 ng/mL APRIL (blue), ST2 stromal cells (orange) or ST2+A (green) for one day measured by flow cytometry. Pan-caspase Inhibitor (panC Inhib) was used as control. Data is pooled from a minimum of two independent biological experiments with n = 6–12 technical replicates for each group. Statistics: Kruskal-Wallis (caspases 3, 8), Ordinary one-way ANOVA (caspases 7, 12). Significance is determined as p≤0.05; not significant is not indicated.

3.2 ST2 cell contact prevents formation of cellular ROS in *ex vivo* memory plasma cells

Since mitochondria depolarisation is the main event leading to activation of caspases 3 and 7, we examined the influence of APRIL- and ST2 cell-signalling on mitochondria in memory plasma cells. Memory plasma cells were cultured with or without ST2 cells in the presence or absence of APRIL for one day and mitochondrial membrane potential, mitochondrial ROS levels as well as total cellular ROS formation were analysed (Figure 5).

Mitochondrial membrane potential was analysed by TMRM staining, a non-cytotoxic positively charged dye that selectively localises in negatively charged mitochondria. Upon mitochondria depolarisation, the TMRM staining is reduced indicating the onset of apoptosis. As a staining control, cells were incubated with CCCP, an uncoupling agent that induces mitochondria depolarisation and decreases TMRM staining.

Mitochondrial ROS production was analysed using the MitoSOX™ reagent, a cell-permeable, mitochondria-specific dye that is oxidated by superoxides. Superoxides are

classified as ROS and are generated as a by-product of mitochondrial respiration. As a positive control Antimycin A, an inhibitor of oxidative phosphorylation, was used. CCCP was used as a negative control.

To analyse total cellular ROS production the cell-permeable dye CellROX was used. It emits fluorescence after oxidation by ROS and does not discriminate between cellular compartments or different ROS species. Positive and negative controls were treated with the oxidant TBHP (tert-butyl hydroperoxide) and the antioxidant NAC (N-acetylcysteine), respectively.

No differences between the conditions were observed in mitochondrial membrane potential (APRIL 729 ± 91; ST2 751 ± 129; ST2+A 742.8 ± 37) or mitochondrial ROS production (APRIL 28.7% ± 9.6; ST2 32.9% ± 7.2; ST2+A 27% ± 6; neg ctrl 26.7% ± 4; pos ctrl 54% ± 14). However, total cellular ROS levels (APRIL 785 ± 179; ST2 559 ± 109; ST2+A 556 ± 130.8; neg ctrl 511 ± 131.5; pos ctrl 1184 ± 168.7) were significantly increased in plasma cell cultured only with APRIL.

These results imply that mitochondria are not predominantly responsible for increased cell stress or onset of apoptosis due to mitochondria membrane depolarisation. Additionally, CellROX staining suggests that ST2 cell contact is sufficient to prevent formation of cellular ROS and that, as evident by MitoSOX staining, cellular ROS does not originate from mitochondria but might originate from other cellular compartments such as the ER.

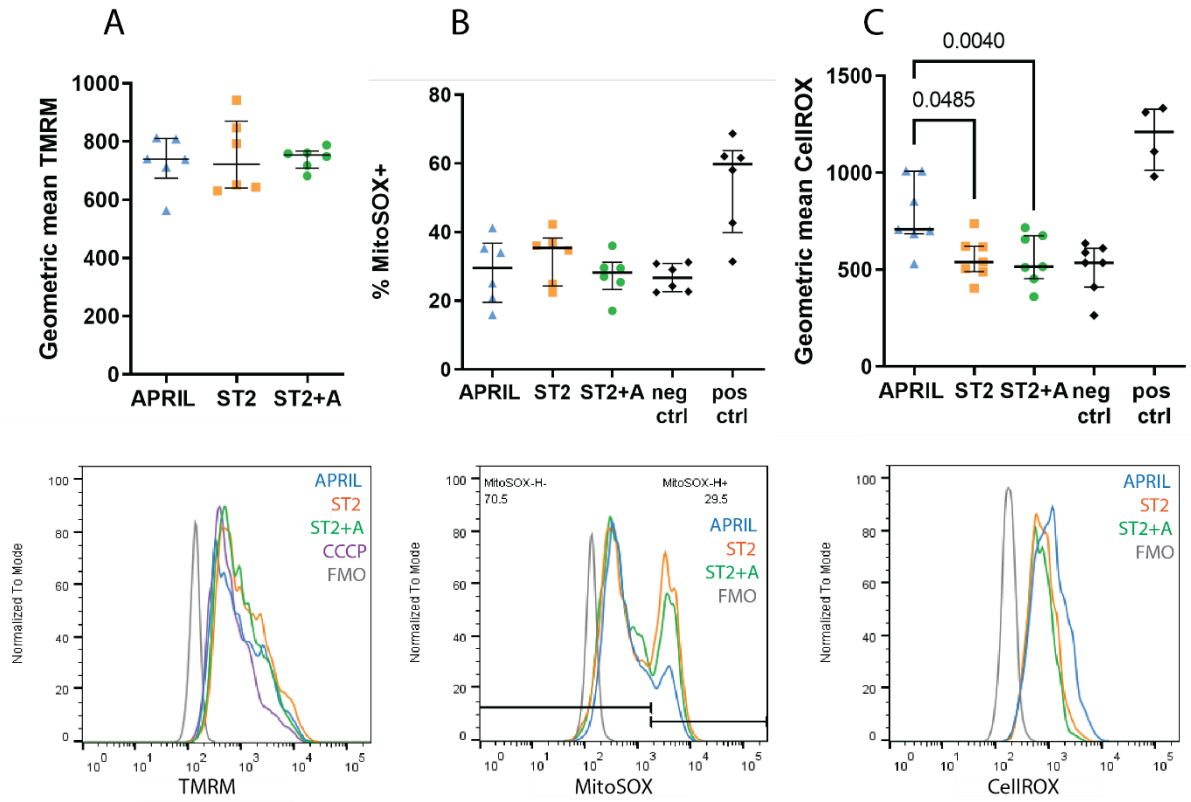

Figure 5: ST2 cell contact dampens cellular ROS formation in memory plasma cells. Geometric mean expression of (A) TMRM, percentage of (B) MitoSOX positive cells and geometric mean expression of (C) CellROX in *ex vivo* memory plasma cells cultured with APRIL (blue), ST2 cells (orange) or ST2+A (green) for one day measured by flow cytometry. Data is pooled from two independent biological experiments with n = 6 technical replicates for each group. Statistics: Friedman test. Data was generated by Lena Peter and Rebecca Cornelis. Significance is determined by p≤0.05; not significant (NS) is not shown.

3.3 Integrin β1 is important for *ex vivo* memory plasma cell survival *in vitro*

While signalling induced by APRIL via BCMA is well understood[39], it remains elusive how contact with stromal cells supports memory plasma cell survival. Work by DiLillo et al implies that the integrins LFA-1 and VLA-4 retain memory plasma cells in the bone marrow of mice *in vivo*, suggesting that cell-cell contact plays a vital role[89]. Integrins are heterodimers that bind to their respective binding partners, LFA-1 to ICAM-1 and VLA-4 to VCAM-1. Since we detected only VCAM-1 expression in our ST2 cells, we focused on the role of VLA-4 (α4β1) signalling in memory plasma cell survival.

Hence, we used a specific siRNA targeting integrin β1 (CD29, ITGB1) in the *in vitro* culture system. Plasma cells were cultured with ST2+A and either scrambled control (SCR) siRNA (ST2+A SCR) or siRNA targeting ITGB1 (ST2+A ITGB1), and cells were counted by flow cytometry on day one and three of culture (Figure 6). ITGB1 protein expression on the plasma cells was reduced by 30% by the ITGB1 siRNA compared to scrambled control (ST2+A SCR 100.6% ± 8.2; ITGB1 68.9% ± 11.5). The survival

was significantly reduced in cells with ITGB1 knock-down (ST2+A SCR: day 1, 103% ± 17.6, day 3, 87.6% ± 26.6; ST2+A ITGB1: day 1, 99.9% ± 12.8; day 3, 74% ± 24.8), indicating that ITGB1 is important for the survival of memory plasma cells.

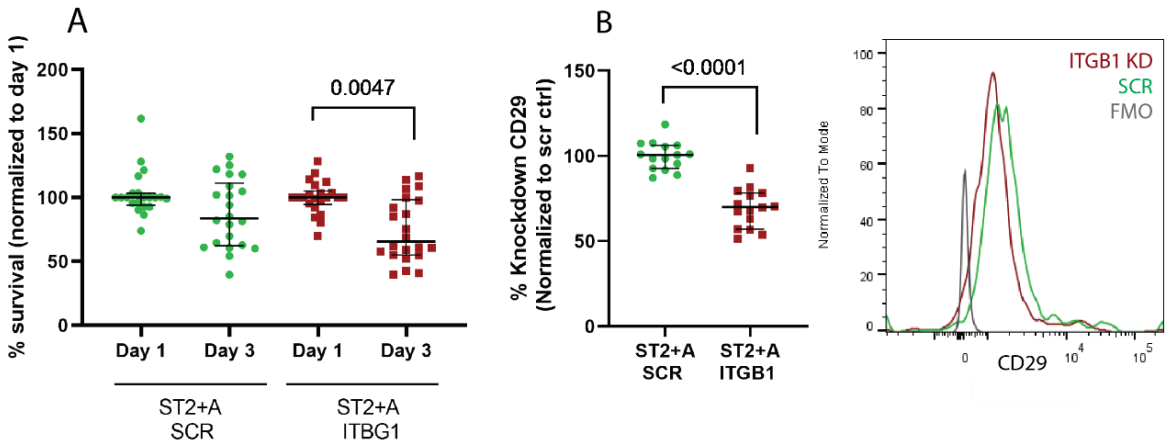

Figure 6: ITGB1 is important for memory plasma cell survival in the *in vitro* niche.
(A) Percentage of survival of *ex vivo* memory plasma cells cultured with ST2 cells and 50 ng/mL APRIL with siRNA against ITGB1 (red) or a scrambled control siRNA (green) on day one and three of culture. (B) Knock-down efficiency of ITGB1 knock-down (red) compared to scrambled control siRNA (green) measured by CD29 expression using flow cytometry. For all experiments, plasma cells were counted as $CD138^{++}$, $DAPI^{-}$ using flow cytometry. Data is pooled from eight (survival) or four (CD29 expression) independent biological experiments with n = 15-21 technical replicates for each group. Statistics: Kruskal-Wallis test (survival), unpaired t test (ITGB1 KD). Significance is determined as $p \leq 0.05$; not significant is not indicated.

Integrins are known to signal via PI3K activation and we previously demonstrated that ST2 cell contact induces PI3K signalling in plasma cells which is essential for memory plasma cell survival *in vitro* and *in vivo*[30]. Therefore, targets downstream of PI3K, FoxO1 and FoxO3, were analysed after ITGB1 knock-down. Moreover, activation of caspase 3 was measured using flow cytometry to assess apoptosis (Figure 7).

As expected, expression of FoxO1 (ST2+A ITGB1 871.6 ± 194.6; APRIL SCR 1430 ± 270, ST2+A SCR 906 ± 259) and FoxO3 (ST2+A SCR 1102 ± 217.8; APRIL SCR 1693 ± 263, ST2+A ITGB1 1071 ± 222;) was significantly increased in plasma cells cultured without ST2 cells (APRIL SCR) compared to plasma cells cultured in the presence of ST2 cells (ST2+A SCR). However, FoxO1 and FoxO3 expression was not increased in ST2+A ITGB1 cells compared to ST2+A SCR control suggesting that PI3K signalling in the surviving plasma cells was not impacted by ITGB1 knock-down. APRIL SCR cells also expressed higher levels of active caspase 3 compared to both ST2+A conditions, but no increase in ST2+A ITGB1 cells compared to ST2+A SCR control was observed (ST2+A ITGB1 778.4 ± 225.9; APRIL SCR 1450 ± 365.8; ST2+A SCR

761.8 ± 227.8), indicating that cells did not undergo increased caspase-dependent cell death due to ITGB1 knock-down.

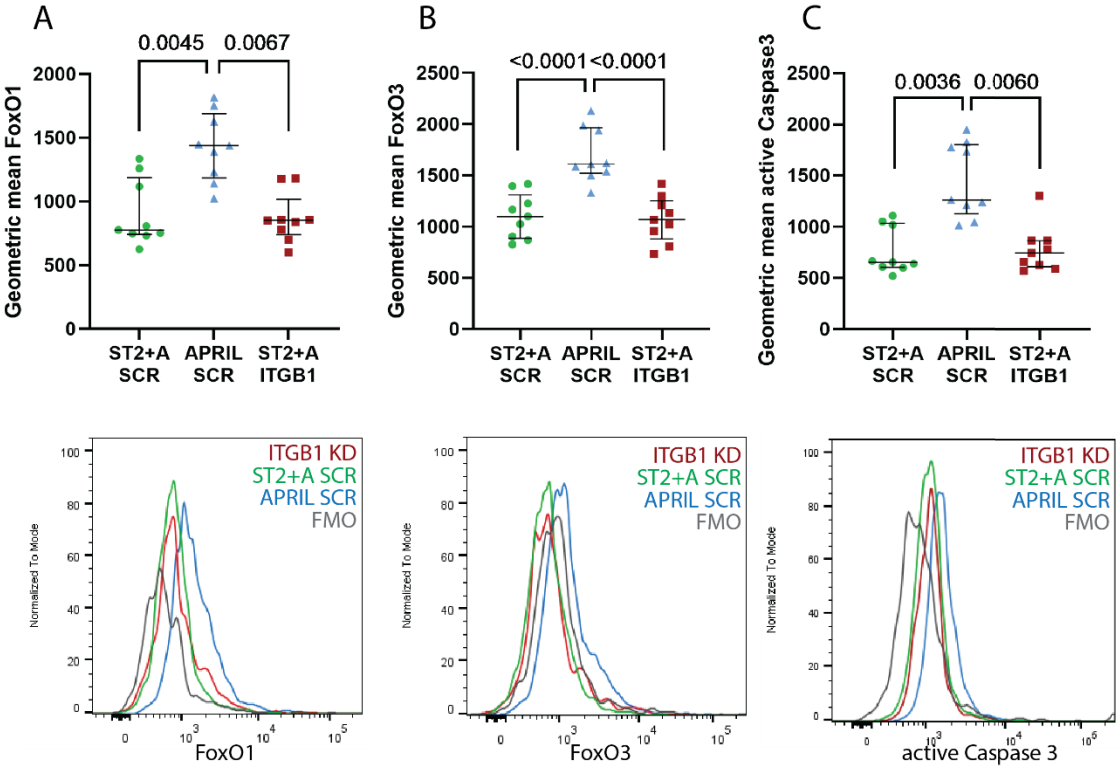

Figure 7: ITGB1 knock-down does not increase FoxO1 or FoxO3 expression or activation of caspase 3 in memory plasma cells. Geometric mean expression of (A) FoxO1, (B) FoxO3 and (C) active caspase 3 in *ex vivo* memory plasma cells cultured with APRIL and scrambled siRNA (blue), ST2+A ITGB1 (red) and ST2+A SCR (green) for three days measured by flow cytometry. Data is pooled from three independent biological experiments with n = 9 technical replicates for each group Statistics: Kruskal-Wallis test (FoxO1, Casp3), Ordinary one-way ANOVA (FoxO3). Significance is determined as p≤0.05; not significant is not indicated.

3.4 FoxO1/3 downregulation rescues *ex vivo* memory plasma cell survival *in vitro* in the absence of stromal cells

The PI3K signalling pathway is crucial for memory plasma cell maintenance and the downstream targets FoxO1 and FoxO3, inhibited by active PI3K signalling, were upregulated in the absence of ST2 cell contact and thus PI3K activation (Figure 7A, B)[30]. To understand if the downregulation of FoxO1 and FoxO3 is essential for memory plasma cell survival, we used specific siRNA targeting FoxO1 and FoxO3.

Memory plasma cells were cultured with APRIL and with or without ST2 cells. Scrambled control siRNA was used for plasma cells cultured with only APRIL (APRIL SCR) and ST2+A (ST2+A SCR). Individual FoxO1 and FoxO3 targeting siRNAs were used for plasma cells cultured with APRIL (APRIL FOXO). Survival was analysed over three days and the knock-down efficiency was evaluated by flow cytometry (Figure 8).

FoxO1 (ST2+A SCR 991.3 ± 129.4; APRIL SCR 1238 ± 166.5; APRIL FOXO 971.9 ± 114.7) and FoxO3 (ST2+A SCR 842 ± 103.7; APRIL SCR 960 ± 137.5; APRIL FOXO 763 ± 68.9) protein levels were successfully reduced by the knock-down to similar levels as in the ST2+A SCR control (Figure 8C,D). Regarding cell survival, knock-down of FoxO1 and FoxO3 could completely compensate for the absence of ST2 cell contact until day three of culture (ST2+A SCR: day1 100% ± 19.7%, day 3 79% ± 22%; APRIL SCR: day 1 100.7% ±9.2%, day 3 73% ± 27%; APRIL FOXO: day 1 99.7% ± 11.7%, day 3 90% ± 16%) (Figure 8A). Plasma cells continued to secret antibodies as determined by ELISA on day one and three of culture (Figure 8B).

Thus, stromal cell-contact induced PI3K signalling and subsequent downregulation of FoxO1 and FoxO3 is critical for memory plasma cell survival.

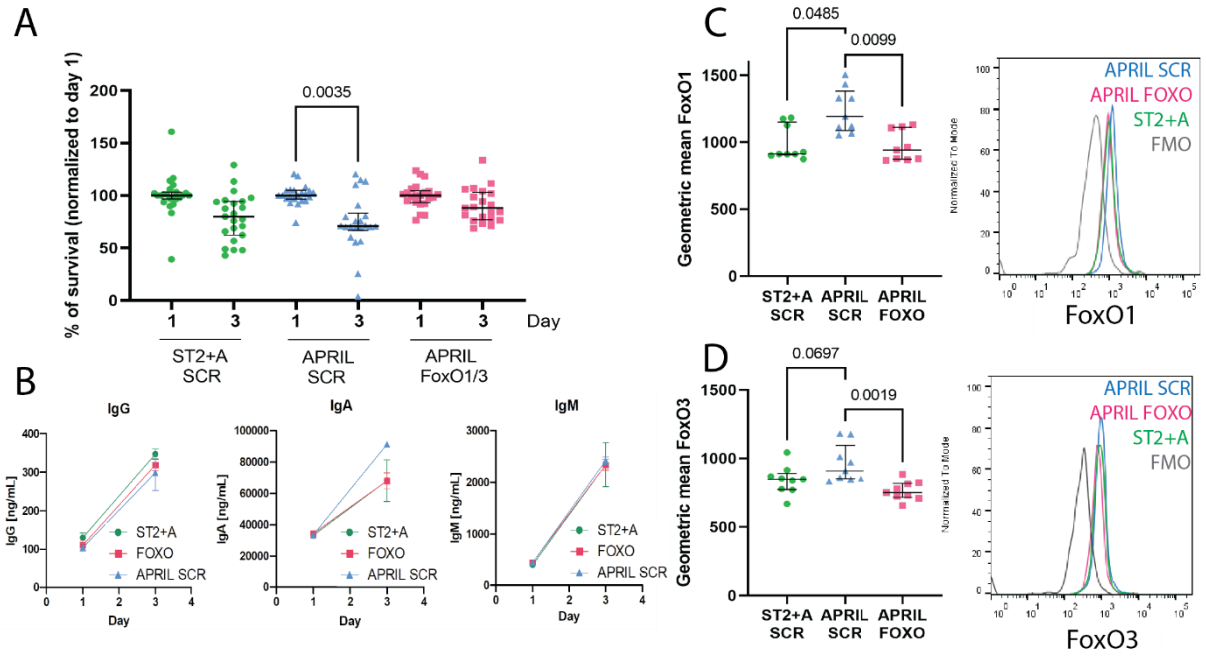

Figure 8: FoxO1/3 downregulation maintains memory plasma cells in the absence of stromal cells. *Ex vivo* memory plasma cells were cultured with 50 ng/mL APRIL and siRNA targeting FoxO1/3 (pink) or scrambles control (blue) or with ST2+A in the presence of scrambled control siRNA (green). (A) Percentage of survival of plasma cells normalised to day one. Data pooled from nine (survival) or three (FoxO1, FoxO3) independent biological experiments with n = 9-23 technical replicates for each group. Statistics: Ordinary one-way ANOVA (survival, FoxO3), Kruskal-Wallis test (FoxO1). (B) Concentration of IgG, IgM, and IgA in cell culture supernatant until day three of culture measured by ELISA. (C) FoxO1 and (D) FoxO3 protein expression levels of plasma cells cultured as in (A) at day 3. Significance is determined as p≤0.05; not significant is not shown.

3.4.1 siRNA induced FoxO1/3 knock-down does not induce transcriptional changes in *ex vivo* memory plasma cells

With the aim to identify FoxO1/3 regulated signalling pathways, single-cell RNA sequencing of *ex vivo* isolated memory plasma cells after siRNA-induced FoxO1/3 knock-down was performed. As in the previous experiments, *ex vivo* memory plasma cells were cultured with ST2+A (ST2+A SCR) or only APRIL with scrambled control siRNA (APRIL SCR), and only APRIL with FoxO1- and FoxO3-specific siRNAs (APRIL FOXO). On day three of culture, plasma cells were sorted as live $CD138^{++}$ cells and sequenced using 10X Genomics-based droplet sequencing.

8,942 cells of ST2+A SCR were sequenced with 23,065 mean reads per cell and 1,249 median genes per cell. 9,298 cells were sequenced for the condition APRIL SCR with 22,555 mean reads per cell and 1,262 median genes per cell. 9,642 cells of APRIL FOXO were sequenced with 26,075 mean reads per cell and 1,288 median genes per cell. The sequencing saturation ranged from approximately 28% - 34%. Additionally, the sequences of their antibody heavy and light chain were determined.

Most cells expressed the genes associated with plasma cell identity: *Xbp1, Tnfrsf17* (BCMA)*, Tnfrsf13b* (TACI), *Sdc1* (CD138)*,* and *Prdm1* (BLIMP-1)*. Mki67* and *Cd19* were expressed in few cells at low levels. Cells with low UMI counts and high expression of mitochondrial genes were excluded from the analysis as they were assumed to be of low quality. On a transcriptional level, expression of *Foxo1* and *Foxo3* genes was low (Figure 9) and no differences in *Foxo1* or *Foxo3* gene expression could be detected between the three samples (Figure 10E), possibly due to technical limitations of single cell RNA sequencing or insufficient sequencing depth. However, knock-down was verified on protein level using flow cytometry (S 3).

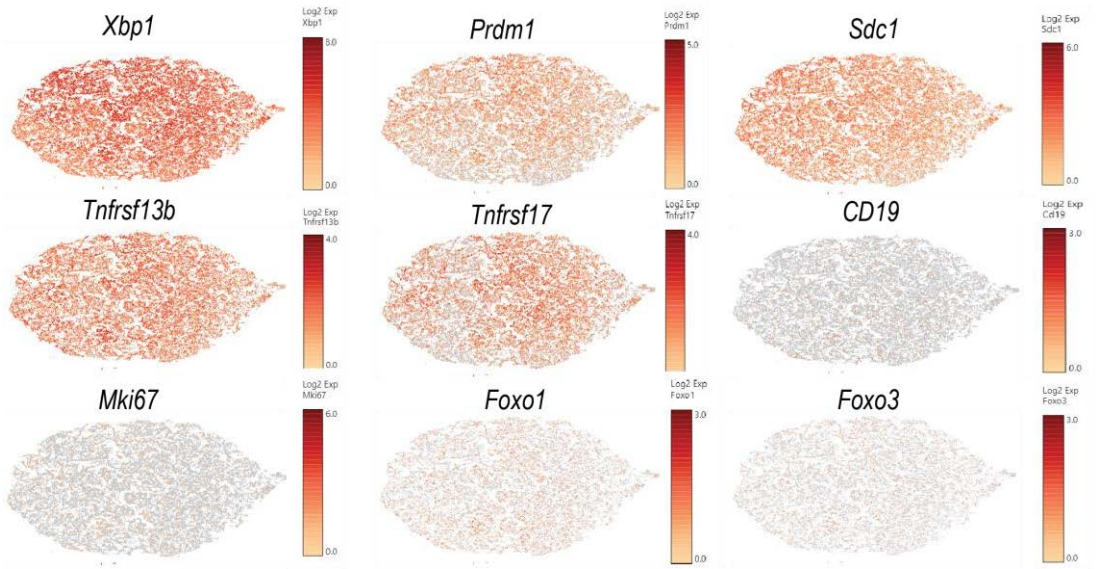

Figure 9: Expression of genes defining plasma cell identity and *Foxo1/Foxo3* in memory plasma cells *in vitro*. *Ex vivo* memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNAs targeting FoxO1/FoxO3 for three days. Cells were sorted as DAPI^{-}CD138^{++} and subsequently sequenced using 10X Genomics-based droplet sequencing. t-SNE coordinates, and clustering was computed for 38,243 cells. Expression of representative genes defining the plasma cell phenotype as well as *Foxo1* and *Foxo3* in combined samples (ST2+A SCR, APRIL SCR, and APRIL FOXO) mapped on t-SNE are depicted.

According to their transcriptomes, memory plasma cells could be subdivided into 12 clusters that where equally present in the three analysed conditions and a clear separation into different immunoglobulin isotypes was observed (Figure 10A-D). Plasma cells expressing *Igha* clustered away from plasma cells expressing *Ighm* or *Ighg1*, *Ighg2b*, *Ighg2c* and *Ighg3* (Figure 10D)

Five genes were significantly upregulated in APRIL FOXO cells (*mt-Atp8, Mt1, Rpl27, Slpi, Stat1*), two genes were significantly upregulated in APRIL SCR cells (*Serpina3f, Stat1*) and six genes in ST2+A SCR cells (*Cd52, Ccr10, Ifi27l2a, Il2rg, Laptm5, Tnfrsf17*) (Figure 10E). Differentiation into immunoglobulin subsets IgA (*Igha* expressing), IgM (*Ighm* expressing) and IgG (*Ighg1, Ighg2b, Ighg2c, Ighg3* expressing) did not reveal more differences between the culture conditions (S 5).

To conclude, transcriptional differences between the three analysed samples were most distinct between the groups cultured without ST2 cells (APRIL SCR and ARIL FOXO) and ST2+A SCR cells. ST2+A SCR and APRIL FOXO cells did not share a transcriptomic signature that could explain the observed survival advantage of these cells compared to APRIL SCR cells.

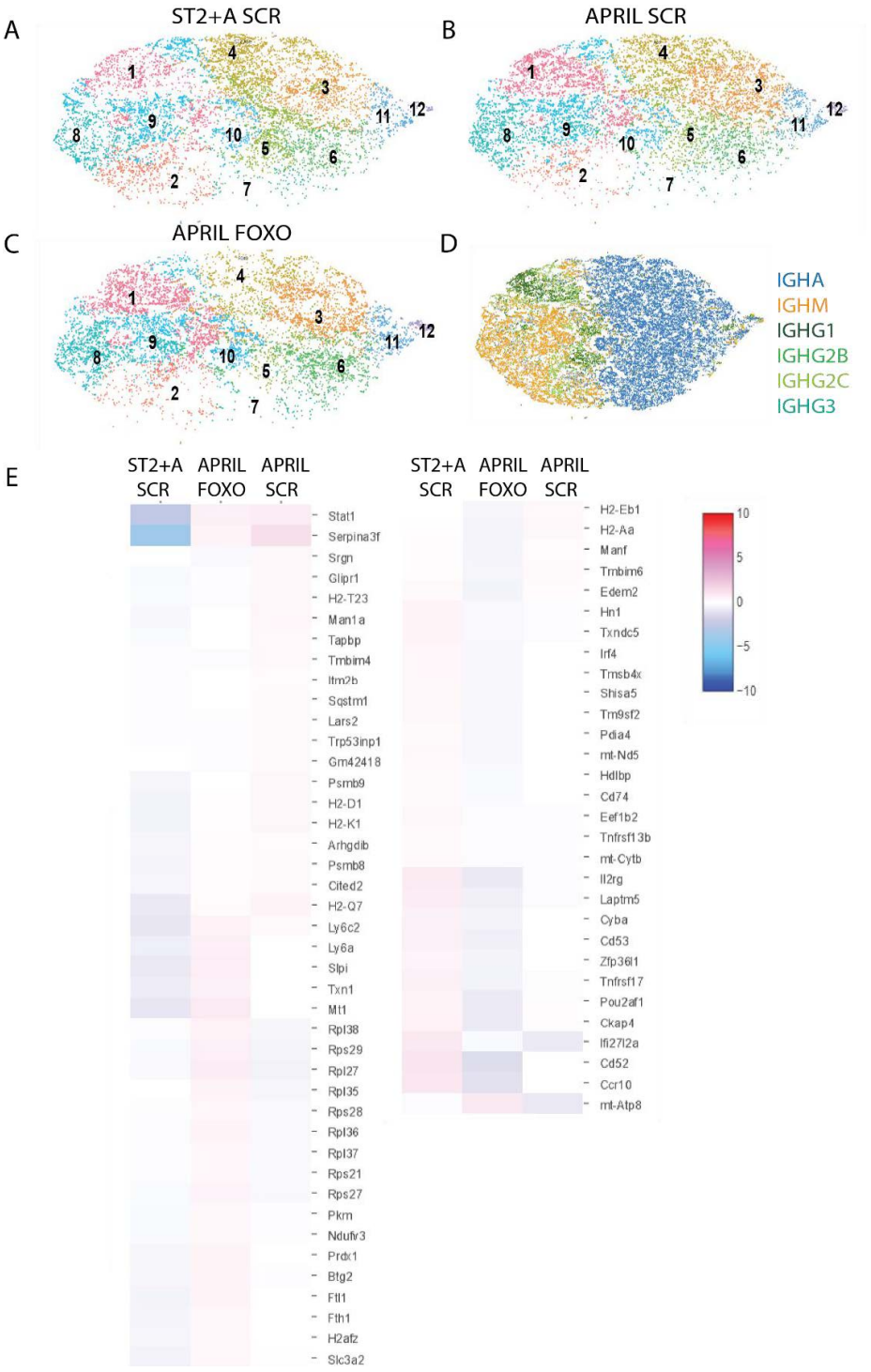

Figure 10: Differential gene expression of memory plasma cells *in vitro* after FoxO1/3 knock-down. *Ex vivo* memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNAs targeting FoxO1/FoxO3 for three days. Cells were sorted as DAPI^{-}CD138^{++} and subsequently sequenced using 10X Genomics-based droplet sequencing. (A, B, C) 12 transcriptionally defined clusters were identified by shared nearest neighbour (SNN) modularity optimisation-based clustering algorithm mapped to t-SNE representation of memory plasma cells. t-SNE coordinates, and clustering was computed for 38,243 cells, presentation is separated by sample. (D) Distribution of immunoglobulin gene expression mapped on t-SNE are depicted. (E) Heatmap of differentially expressed genes between the samples APRIL FOXO, ST2+A SCR, APRIL SCR.

3.4.2 FoxO1 and FoxO3 modulated MCL1 expression in *ex vivo* memory plasma cells

FoxO1 and FoxO3 have numerous downstream targets and were previously described to upregulate pro-apoptotic proteins, e.g. BIM[54,55]. Single cell RNA sequencing did not reveal differential expression of mRNA of pro- and anti-apoptotic proteins after FoxO1/3 knock-down compared to controls. However, this could be due to insufficient sequencing depth.

Thus, to investigate how FoxO1 and FoxO3 regulate apoptosis in memory plasma cells, the pro- and anti-apoptotic proteins BIM, NOXA, MCL1 and BCL2 as well as activation of caspases 3, 7 and 12 were analysed using flow cytometry.

Ex vivo memory plasma cells were, as described before, cultured with APRIL, with or without ST2 cells and treated either with scrambled control siRNA (APRIL SCR; ST2+A SCR) or siRNAs targeting FoxO1 and FoxO3 (APRIL FOXO). After three days, memory plasma cells were analysed for expression of BCL2, BIM, NOXA, MCL1, active caspases 3, 7, and 12, using flow cytometry (Figure 11).

Activation of caspase 3 (ST2+A SCR 1589 ± 215.5; APRIL SCR 2316 ± 289.5; APRIL FOXO 2333 ± 387;), caspase 7 (ST2+A SCR 1608 ± 130; APRIL SCR 2117 ± 284.3; APRIL FOXO 1900 ± 231;) and caspase 12 (ST2+A 4571 ± 1736; APRIL SCR 13000 ± 2553; APRIL FOXO 11624 ± 3514) was not prevented by FoxO1/3 knock-down but remained at a similar level to the APRIL SCR control, whereas all caspases where significantly inhibited in ST2+A SCR control (Figure 11A-C).

BCL2 was equally expressed in all analysed conditions (ST2+A SCR 140 ± 23; APRIL SCR 154 ± 20; APRIL FOXO 135 ± 14) (Figure 11E). Co-culture with ST2 cells prevented increased expression of the pro-apoptotic proteins NOXA and BIM. However, FoxO1/3 knock-down did not alter NOXA (ST2+A SCR 336 ± 32.7; APRIL SCR 417 ± 36.6; APRIL FOXO 384.9 ± 38.6) or BIM (ST2+A SCR 177 ± 36; APRIL SCR 254.8 ± 33.8; APRIL FOXO 238 ± 17.6) expression compared to APRIL SCR control (Figure 11F, G).

The anti-apoptotic protein MCL1 was significantly upregulated in APRIL SCR cells compared to ST2+A SCR cells. APRIL FOXO cells did not significantly upregulate MCL1 compared to ST2+A SCR control and expressed reduced levels of MCL1

(*p=0.0622*) compared to APRIL SCR cells (ST2+A SCR 425 ± 66; APRIL SCR 485 ± 27.6; APRIL FOXO 436.7 ± 20.9) (Figure 11D).

The results demonstrate that knock-down of FoxO1/3 in memory plasma cells neither prevented the activation of caspases 3, 7 and 12 nor dampened the expression of the pro-apoptotic proteins BIM and NOXA. Surprisingly, plasma cells cultured with ST2+A SCR and APRIL FOXO expressed lower levels of MCL1.

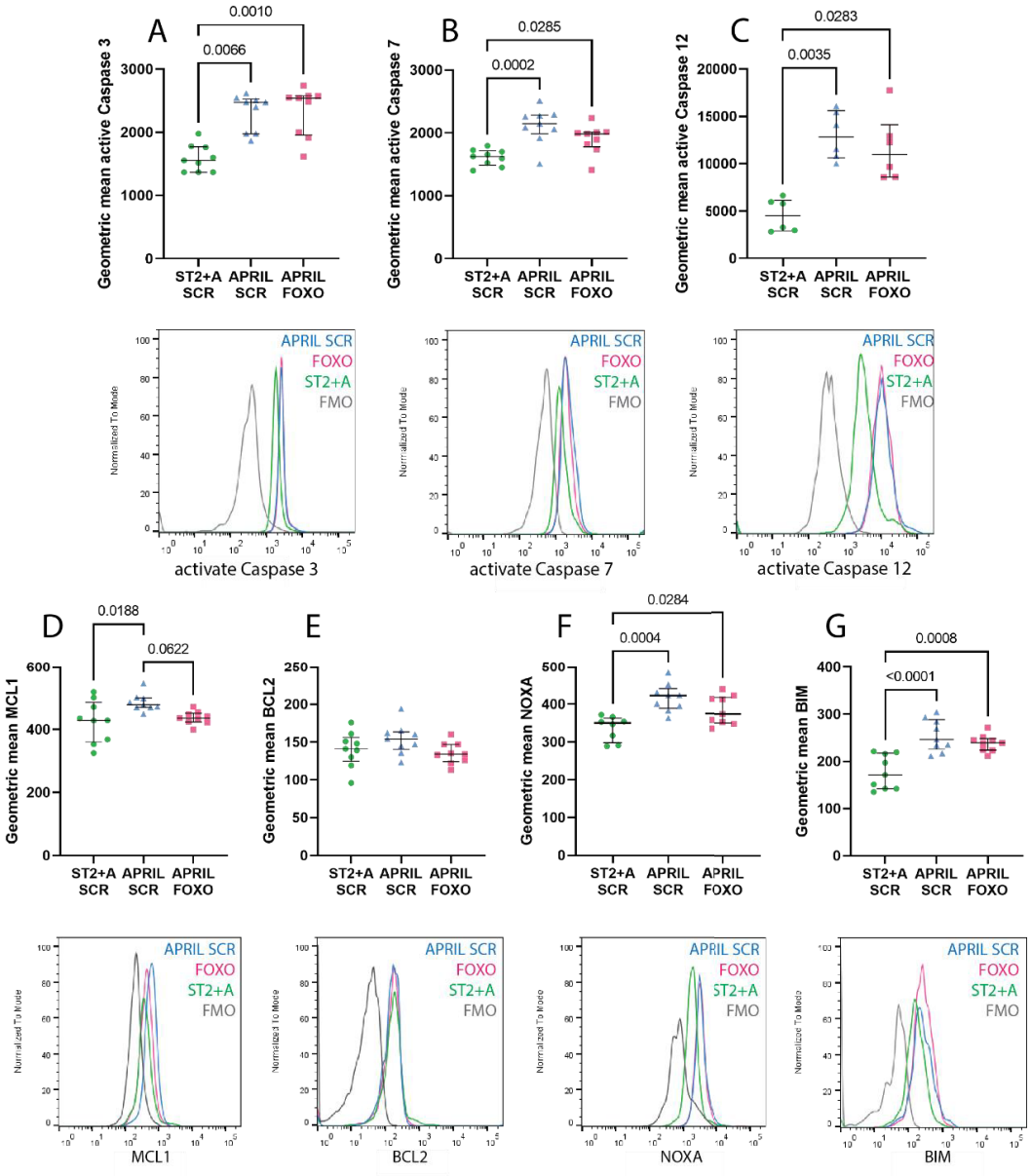

Figure 11: FoxO1/3 regulate MCL1 but not BCL2, NOXA, BIM expression in bone marrow memory plasma cells. Geometric mean expression of (A) active caspase 3 and (B) active caspase 7 and (C) active caspase 12, (D) MCL1, (E), BCL2, (F) NOXA, and (G) BIM in *ex vivo* memory plasma cells cultured with APRIL and siRNAs targeting FoxO1/3 or scrambled control or cultured with ST2+A and scrambled control siRNA on day 3 of culture measured using flow cytometry. Data is pooled from three independent biological experiments with n = 9 technical replicates for each group. Statistic Kruskal-Wallis test (caspase 12), ordinary one-way ANOVA (MCL1, BCL2, BIM, NOXA, caspase 3, caspase 7). Significance is determined by p≤0.05; not significant is not shown.

3.5 IRF4 is synergistically upregulated by APRIL and ST2 cells and essential for *ex vivo* memory plasma cell survival

IRF4 has been demonstrated to be essential for memory plasma cell survival[22,25]. To gain a more detailed understanding of how this central transcription factor is regulated, we analysed IRF4 expression in bone marrow derived memory plasma cells cultured with stromal cells and with or without APRIL (ST2+A, ST2) and only APRIL (Figure 12).

Plasma cells cultured with either stromal cells or APRIL alone expressed low levels of IRF4 that were significantly upregulated in plasma cells receiving both signals (ST2+A 92 ± 12; ST2 78.5 ±11.9; APRIL 75.7 ±16.8), indicating that both ST2 and APRIL induce signalling pathways that are required for full IRF4 expression.

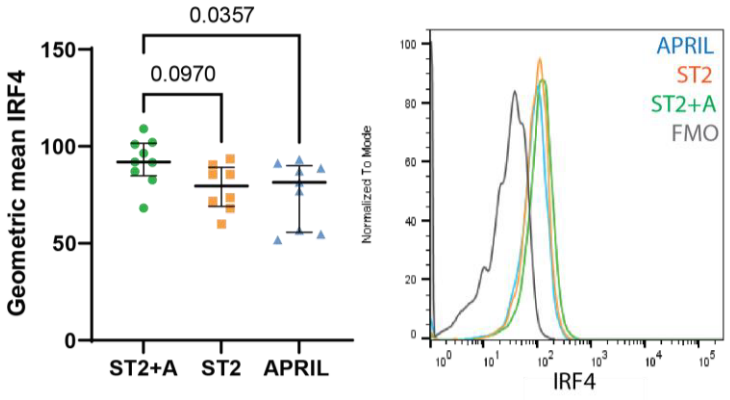

Figure 12: IRF4 is synergistically upregulated by ST2 cells and APRIL in memory plasma cells *in vitro*. *Ex vivo* memory plasma cells were cultured for one day with ST2 cells (orange) in the presence of 50 ng/mL APRIL (green) or with APRIL alone (blue). Plasma cells were identified as $CD138^{++}$ cells and IRF4 expression was analysed using flow cytometry. Pooled from three independent experiments with n=9 technical replicates per group. Statistics: Ordinary one-way ANOVA. Significance is determined as $p \leq 0.05$.

Next, we used specific siRNA targeting IRF4 to analyse its role in plasma cell maintenance. Memory plasma cells were cultured with ST2 stromal cells with either scrambled control siRNA (ST2+A SCR) or siRNA targeting IRF4 (ST2+A IRF4). Survival was analysed over three days and protein levels of IRF4 were measured after three days of culture using flow cytometry. Additionally, immunoglobulin levels of IgG, IgA and IgM in the cell culture supernatant were assessed using ELISA (Figure 13).

ST2+A IRF4 cells displayed significantly decreased survival compared to ST2+A SCR cells (ST2+A IRF4: day 1 96.5% ± 16 day 3 64% ± 23.7; ST2+A SCR: day 1 100% ± 18.9, day 3 86 ± 18.7) and surviving cells continued to secret immunoglobulins of all subclasses. IRF4 siRNA significantly decreased protein expression of IRF4 by 30% compared to scrambled control (ST2+A SCR 100% ± 11.6; ST2+A IRF4 71% ± 13.5).

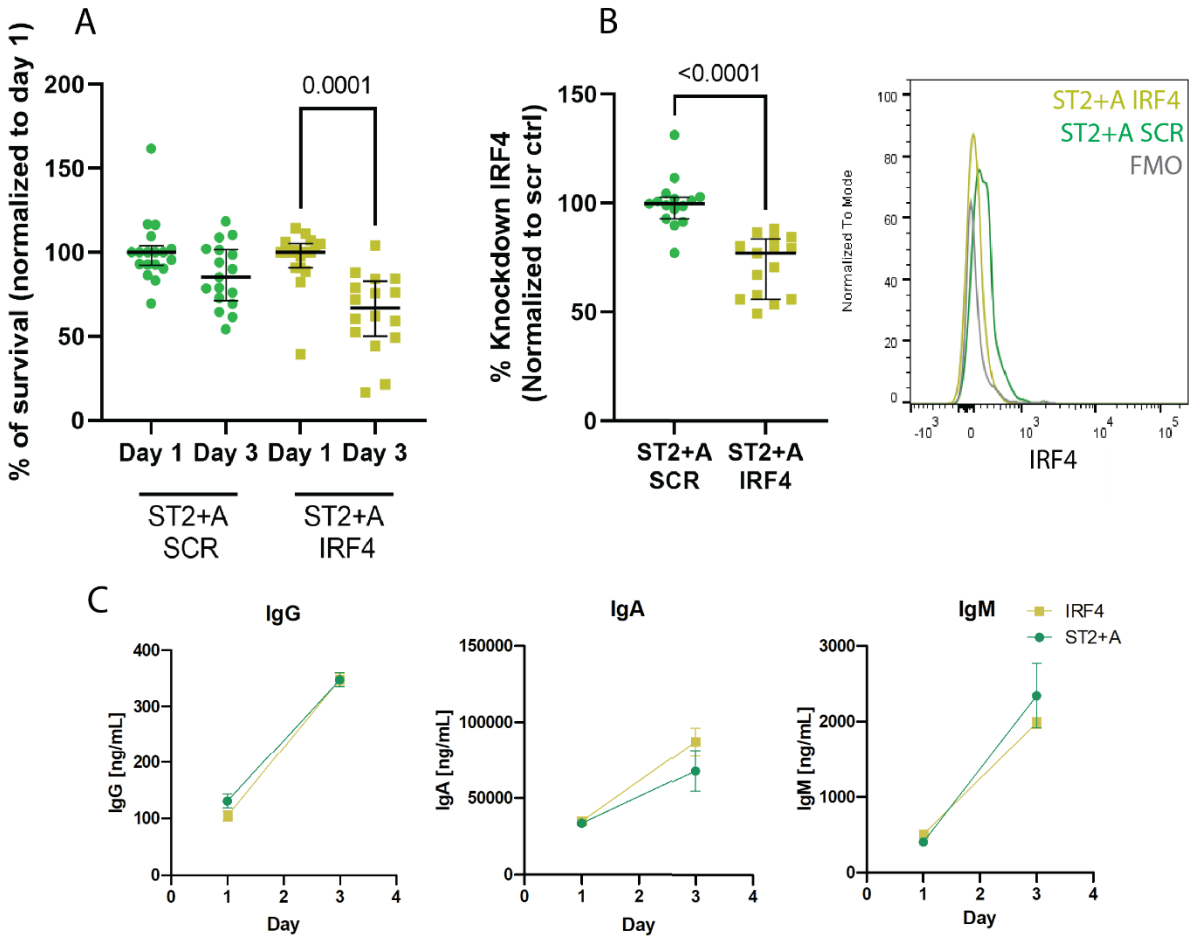

Figure 13: IRF4 is essential for plasma cell survival *in vitro*.
(A) Percentage of survival of *ex vivo* memory plasma cells cultured with ST2+A, and scrambled siRNA or siRNA targeting IRF4 for three days. B) Knock-down efficiency of IRF4 compared to scrambled control on day 3 of culture. Data is pooled from at least four independent biological experiments with n= 15-17 technical replicates. (C) Concentration of IgG, IgM and IgA antibodies on day 3 of culture from plasma cells cultured as in (A) measured by ELISA. Statistics: Kruskal-Wallis test (survival), Mann-Whitney test (IRF4 KD). Significance is determined as p≤0.05; not significant is not shown

3.5.1 IRF4 knock-down downregulated BCL2 expression in *ex vivo* memory plasma cells

IRF4 has been previously reported to regulate plasma cell survival by controlling mitochondrial homeostasis, ROS production and cell metabolism. Moreover, the authors showed that BCL2 overexpression rescued IRF4-deficient plasma cells from cell death[21]. IRF4 has also been implied in controlling MCL1 expression via the transcription factor ZBTB20[77].

Our goal was to examine the influence of IRF4 on the expression of pro- and anti-apoptotic proteins BCL2, MCL1, BIM and NOXA using our *in vitro* culture system. We cultured memory plasma cells in the presence of ST2 cells and APRIL with either IRF4-specific siRNA or a scrambled control. After three days of culture, we analysed the pro- and anti-apoptotic proteins using flow cytometry (Figure 14).

MCL1 (ST2+A SCR 967.8 ± 451; ST2+A IRF4 1145 ± 309), NOXA (ST2+A SCR 1436 ± 273.8; ST2+A IRF4 1618 ± 559.5) and BIM (ST2+A SCR 177 ± 36; ST2+A IRF4 162 ± 40) expression were not affected by IRF4 knock-down. BCL2 expression was significantly decreased by IRF4 knock-down (ST2+A SCR 278.8 ± 20; ST2+A IRF4 208 ± 11) (Figure 14B).

The results show that IRF4 might promote survival by regulating the anti-apoptotic protein BCL2 in memory plasma cells.

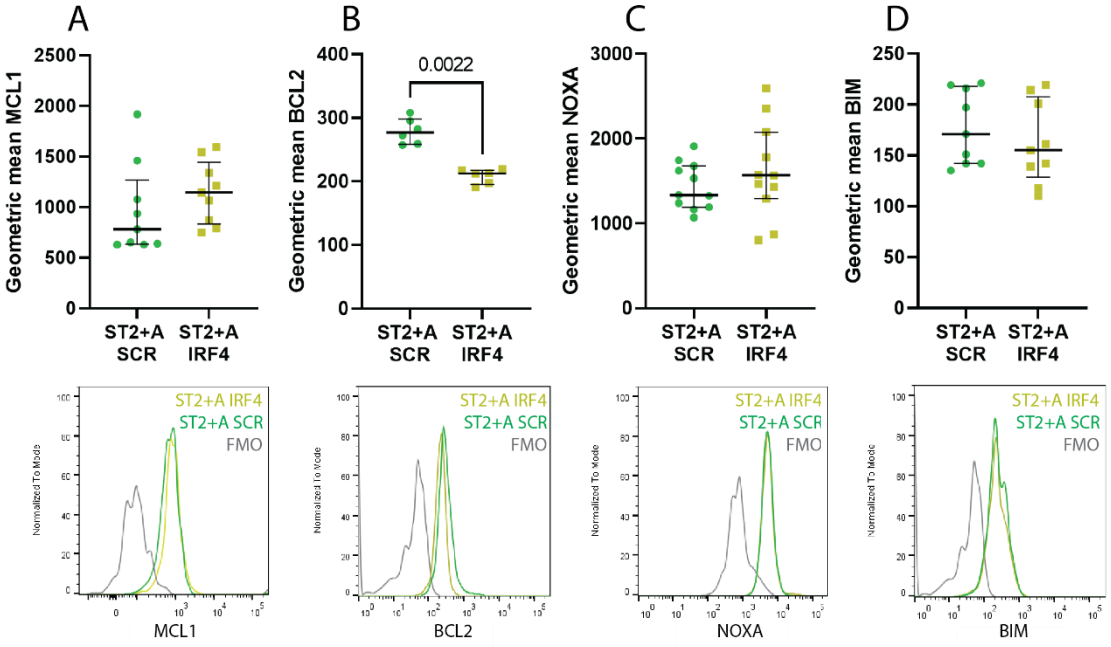

Figure 14: IRF4 regulates expression of BCL2 in memory plasma cells.
Geometric mean expression of (A) MCL1, (B) BCL2, (C) NOXA and (D) BIM in *ex vivo* memory plasma cells cultured with ST2+A and scrambled siRNA or siRNA targeting IRF4 for three days and measured using flow cytometry. Pooled from two-three independent biological experiments with n= 6-9 technical replicates per group. Statistics: Unpaired t test. Significance is determined as p≤0.05; not significant is not shown.

3.5.2 IRF4 regulates genes defining the plasma cell identity and ER-associated genes in *ex vivo* memory plasma cells

With the aim to identify IRF4 downstream targets in memory plasma cells, single cell sequencing of IRF4 knock-down plasma cells was performed. Plasma cells were cultured with ST2 cells and APRIL with scrambled control siRNA (ST2+ APRIL SCR) or IRF4 specific siRNA (ST2+A IRF4). On day three of culture, plasma cells were sorted as live, $CD138^{++}$ cells and sequenced using 10X Genomics-based droplet sequencing.

For the condition ST2+A SCR 8,942 cells were sequenced with 23,065 mean reads per cells and 1,249 median genes per cell and a sequencing saturation of 30%. 10,361 cells were sequences for ST2+A IRF4 with 23,129 mean reads per cells and 1,274 median genes per cell and a sequencing saturation of 32.4%. Additionally, BCR sequencing of the samples was performed. Cells with low UMI counts and high expression of mitochondrial genes were excluded from the analysis as they were assumed to be low quality.

Xbp1, Tnfrsf17, Tnfrsf13b, Sdc1, and *Prdm1* were expressed in memory plasma cells, however apart from *Tnfrsf13b* they were downregulated by IRF4 knock-down (S 6*).*

Memory plasma cells clustered into 12 population according to their transcriptomes (Figure 15A, B). ST2+A SCR cells were predominantly assigned to clusters 1, 3 ,4, 8 and 9 whereas ST2+A IRF4 cells were mostly in clusters 5 and 6 as well as 2 and 7 that were enriched for ribosomal genes (S 7).

34 genes were significantly upregulated in ST2+A IRF4 cells with the majority encoding for ribosomal genes (*Rps* and *Rpl* genes, *Prg2, Cd79b, mt-Atp8, Laptm5, H2afj, Tmsb4x, Fau, Limd2, Rac2, Capg*). 27 genes were significantly upregulated in ST2+A SCR cells (*Ccr10, Tnfrsf17, Ly6c2, Edem1, Sdc1, Eaf2, Clptm1l, Ergic1, Tmem176a, Ctsb, Ifi27l2a, Bst2, Irf4, Tmem176b, H13, Slc3a2, Tm9sf, Txndc5, Ssr2, Ell2, Rexo2, Edem2, Stt3a, Lman1, Grn, Sec61a1, Ckap4)* (Figure 15D).

The data illustrates that IFR4 knock-down results in downregulation of typical plasma cell markers such as *Tnfrsf17, Prdm1, Xbp1* and *Sdc1*, which could explain the reduced survival. Ribosomal, lysosomal, and mitochondrial genes were upregulated, possibly as a result of elevated cell stress. Some of the differential regulated genes are indirect targets of IRF4, as IRF4 regulates BLIMP-1 expression. Among BLIMP-1 target genes are *Xbp1, Ell2, H2-Aa, Sdc1* and *Cd79b*[22]*.*

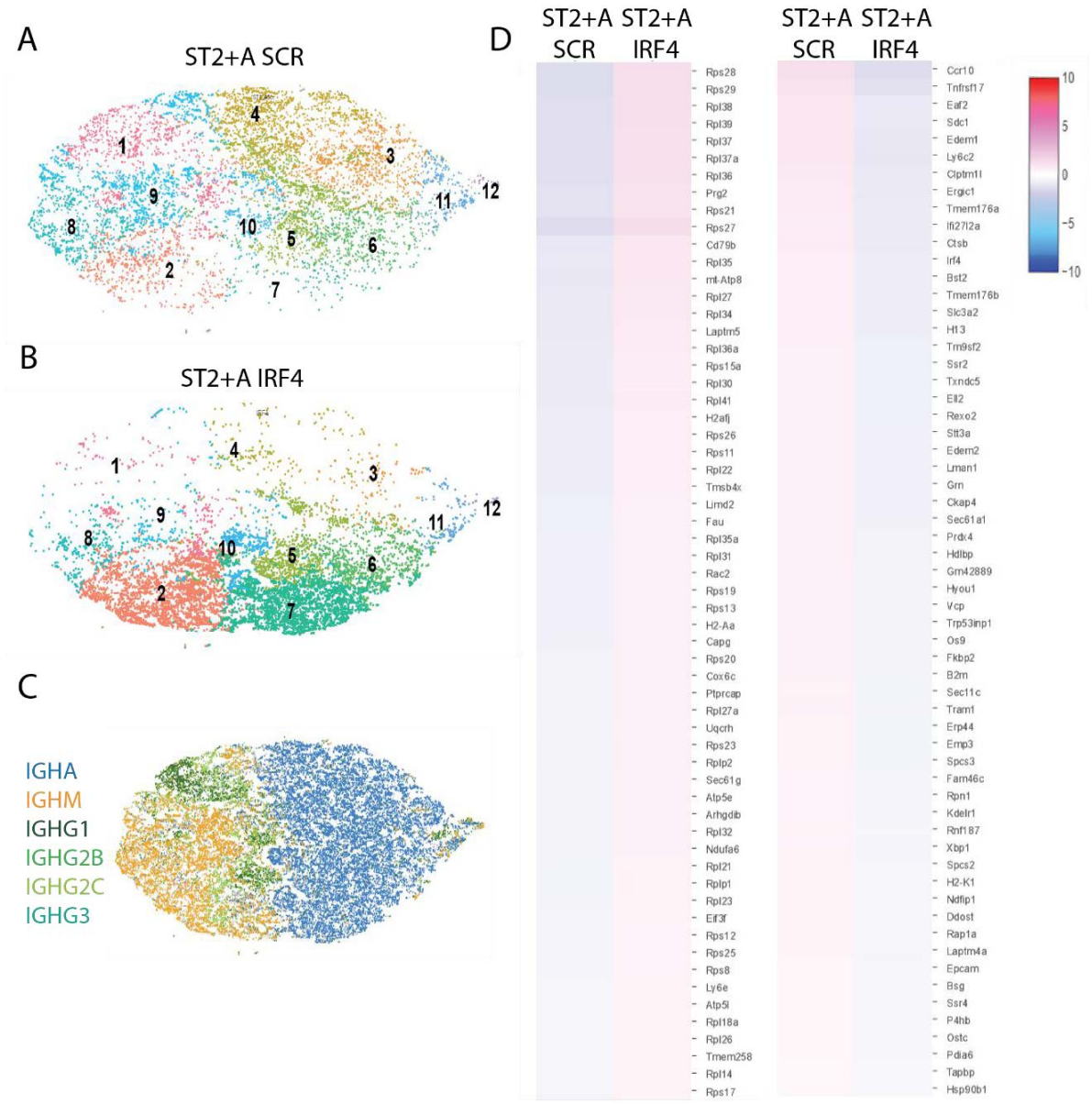

Figure 15: Differential gene expression of memory plasma cells *in vitro* after IFR4 knock-down. Memory plasma cells were cultured with 50 ng/mL APRIL and ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNA targeting IRF4 for three days. Cells were sorted as DAPI^{-}CD138^{++} and subsequently sequenced using 10X Genomics-based droplet sequencing. (A, B) 12 transcriptionally defined clusters were identified by shared nearest neighbour (SNN) modularity optimisation-based clustering algorithm mapped to tSNE representation of memory plasma cells. tSNE coordinates and clustering was computed for 38,243 cells, presentation is separated by sample. (C) Distribution of immunoglobulin gene expression as determined by BCR sequencing mapped on tSNE are depicted. (D) Heatmap of differentially expressed genes between the samples ST2+A IRF4 and ST2+A SCR.

Since IRF4 has been described to regulate the amino acid transporter CD98 and the glucose transporter GLUT1 in T cells, thereby influencing their metabolism[27], we analysed the expression of these transporters in memory plasma cells. GLUT1 was not detected in single cell RNA sequencing, probably due to technical limitations, as it is reported to be expressed by plasma cells[90]. CD98 is a heterodimer of SLC3A2 and SLC7A5. *Slc3a2* was significantly downregulated in memory plasma cells after IRF4

knock-down and is a direct target of IRF4 according to a published ChIP seq databank[91]. Reduced expression of CD98 as well as CD138 (*Sdc1*) and BLIMP-1GFP due to IRF4 knock-down could be verified using flow cytometry (Figure 16).

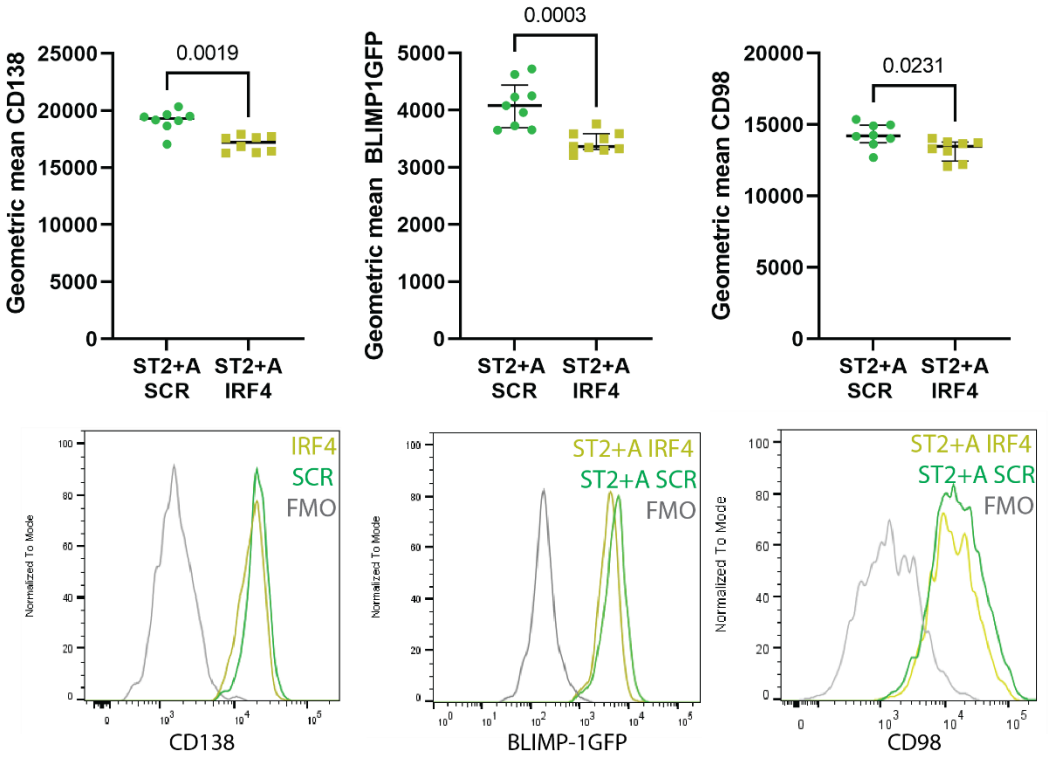

Figure 16: Blimp1GFP, CD138 and CD98 expression of memory plasma cells after siRNA induced IRF4 knock-down *in vitro*. Memory plasma cells were cultured with ST2+A, and scrambled siRNA or siRNA targeting IRF4 for three days. Cells were analysed for CD98 using flow cytometry. Pooled from two independent biological experiments with n=8 technical replicates per group. Statistic: Unpaired t-test. Significance is determined by p≤0.05.

Taken together the results indicate that IRF4 regulates the anti-apoptotic protein BCL2, as well as a variety of genes associated to ER functions such as protein production and protein folding, and cellular metabolism. Genes defining the typical plasma cell identify are reduced in IRF4 knock-down cells, potentially decreasing cell survival.

4 DISCUSSION

Durable protective immunity is provided by memory plasma cells that can survive years to decades in dedicated niches while continuously secreting antibodies. The basis of their longevity remains poorly understood owing to their low frequency and rapid cell death once isolated from tissue. Thus, various *in vitro* culture systems for memory plasma cells have been described using *in vitro* generated memory plasma cells, differentiated either from B cells or from antibody-secreting cells in the blood after booster vaccination[32,45,92]. It is questionable that the survival requirements of these

cells are identical to memory plasma cells that migrated to and reside in the bone marrow.

Therefore, we isolated *ex vivo* memory plasma cells from the bone marrow of mice immunised and challenged twice in 21-day intervals with NP-CGG/IFA for our *in vitro* culture system. We have identified two essential survival factors of memory plasma cells that are sufficient to maintain *ex vivo* memory plasma cells *in vitro*: (1) direct cell contact to stromal cells of the cell line ST2 and (2) recombinant multimeric APRIL. Additionally, we previously showed that memory plasma cells cultured *in vitro* maintained the transcriptional program of *ex vivo* bone marrow plasma cells[30].

Memory plasma cells in the bone marrow can be found in direct contact to mesenchymal stromal cells[93]. These stromal cells organise the survival niche of memory plasma cells where they receive different and majorly redundant survival signals from accessory cells[31,42,94,95]. Signalling via BCMA, the receptor of APRIL and BAFF, is essential for memory plasma cells as BCMA deficient mice display severely reduced numbers of bone marrow memory plasma cells[95]. Downstream of BCMA, the NF-κB pathway is activated and induces pro-survival signalling, resulting in expression of the anti-apoptotic protein MCL1[39,95]. Contrary to BCMA, the role of stromal cells and the survival signalling pathways they induce in memory plasma cells remains largely unknown. Thus, we aimed to elucidate how stromal cells promote memory plasma cell maintenance in the bone marrow using our *in vitro* culture system.

4.1 Endoplasmic reticulum stress is the main driver of caspase activation and cell death in *ex vivo* bone marrow memory plasma cells

Ex vivo plasma cells lacking survival signals rapidly die *in vitro*. We previously demonstrated that the essential survival signals (**1**) ST2 stromal cells and (**2**) the cytokine APRIL protect plasma cells from caspase-mediated apoptosis[30]. Caspases are serine/cysteine proteases with numerous downstream targets to ensure orderly degradation of the cell. The caspase-cascade is activated either by extrinsic or intrinsic factors. Indicative for the extrinsic apoptotic pathway is caspase 8 activation. The intrinsic apoptotic pathway is activated by mitochondria or the ER. ER stress can activate caspase 12 whereas activation of caspases 3, 7 and 9 is associated with mitochondria[71]. To gain a deeper understanding of how the individual survival signals protect memory plasma cells from cell death, we analysed the activation of caspases

3, 7, 8 and 12 in *ex vivo* memory plasma cells cultured with APRIL alone, with ST2 cells alone or the combination of both (ST2+A).

In line with previous reports, we did not observe activation of caspase 8, the mediator of the extrinsic pathway of apoptosis[96]. Moreover, memory plasma cells cultured with ST2+A did not display activation of any of the other analysed caspases, corresponding to cell survival in this condition.

As professional secretory cells, plasma cells experience high ER stress, which is counteracted by the UPR, enabling the cells to cope with amplified protein production. As a result of excessive and prolonged ER stress, the stress sensor IRE1 associates with TRAF2 and activates caspase 12. Activation of the ER-associated caspase 12 was detected in memory plasma cells cultured only with either APRIL or ST2 cells. Activation of caspases 3 and 7 is preceded by mitochondria depolarisation, cytochrome c release and caspase 9 activation[70,71]. Memory plasma cells cultured with APRIL, but not ST2 cells, expressed high levels of active caspases 3 and 7. Unexpectedly, memory plasma cells cultured with APRIL alone did not display mitochondria membrane depolarisation, as measured by TMRM staining. Reports demonstrated that caspase 12 can activate caspase 9 in a cytochrome c independent way. Thus, ER stress-induced activation of caspases 3 and 7 does not always depend on mitochondrial membrane depolarisation[97].

While memory plasma cells co-cultured with ST2 cells also expressed active caspase 12, activation of caspases 3 and 7 was not detected in this condition. This observed discrepancy might be explained with the fact that caspase 9 can be regulated by Ras kinase, protein kinase C as well as AKT[98,99]. PI3K/AKT signalling induced by stromal cell contact appears to prevent caspase 9 activation and subsequently activation of caspases 3 and 7. Indeed, inhibition of PI3K/AKT signalling using wortmannin resulted in activation of caspases 3 and 7 in memory plasma cells co-cultured with ST2 cells.

We suggest that plasma cell death is mediated by excessive ER stress and activation of caspase 12. Downstream of caspase 12, caspases 3 and 7 are activated, presumably by caspase 9 which is prevented by stromal cell induced PI3K/AKT signalling. However, caspase 12 activation alone is sufficient to induce cell death and APRIL and stromal cell contact are both necessary to protect plasma cells from ER stress-induced cell death. Future studies will explore caspase 9 activation and evaluate if specific inhibition of caspase 12 is sufficient to rescue plasma cells from apoptosis.

As an additional indicator of cell stress, we analysed mitochondrial ROS formation in *ex vivo* memory plasma cells. Since ROS is produced during oxidative phosphorylation and utilised as secondary messenger, e.g. to amplify the NF-κB signalling pathway, basal levels of ROS are expected[100]. However, high levels of ROS are a source of cell stress and can result in apoptosis. *Ex vivo* memory plasma cells in all culture conditions displayed a basal level of mitochondrial ROS.

Apart from mitochondrial ROS production, ROS can also be generated during increased protein folding in the ER of plasma cells with approximately 10^5 H_2O_2 molecules generated per second in IgM producing cells[101]. Accordingly, we evaluated total cellular ROS levels in *ex vivo* memory plasma cells. Interestingly, memory plasma cells cultured with APRIL expressed high ROS levels indicating oxidative stress, while ST2 cells protected plasma cells from excessive ROS production. A recent study suggests that the PI3K signalling pathway is important to generate a robust UPR and could consequently impact ROS production by preventing protein stress[102].

Collectively, our data highlights that uncontrolled ER stress is the initiator of memory plasma cell death, and stromal cells and APRIL synergistically prevent excessive ER stress. It would be interesting to determine if the secretory capacity of memory plasma cells correlates with ER stress and differs depending on the respective culture condition. Potentially, both survival signals are required for memory plasma cells to establish a tolerable amount of immunoglobulin production. Further investigations are necessary to understand how ST2 cell contact prevents the formation of ROS and if it impacts redox homeostasis of memory plasma cells.

It should be noted that in the present study memory plasma cells were analysed after one day in culture, and while the data indicates that the ER is the initiator of cell stress, mitochondria likely play a role at later time points. It is to be expected that prolonged ER stress and Ca^{2++} release from the ER will ultimately result in mitochondrial membrane depolarisation[101].

4.2 ITGB1 mediated cell-cell contact to ST2 stromal cells is important for *ex vivo* memory plasma cell survival

Bone marrow memory plasma cells are found in proximity to stromal cells, which are heterogeneous and express cell adhesion molecules e.g., VCAM-1 and secret soluble factors (e.g., CXCL12, BAFF) relevant for plasma cell maintenance[93,103]. We

previously illustrated that ST2 cell contact is essential for memory plasma cell survival *in vitro*[30]. Moreover, the importance of integrin mediated cell-cell contact *in vivo* was demonstrated by targeting the integrins LFA-1 ($\alpha L\beta 2$) and VLA-4 ($\alpha 4\beta 1$) with antibodies ablating memory plasma cells from the bone marrow of mice[89]. Integrins can activate the PI3K signalling pathway resulting in pro-survival signalling[104].

To elucidate whether VLA-4 is important for memory plasma cell survival and activates the PI3K signalling pathway *in vitro*, we treated *ex vivo* memory plasma cells with specific siRNA against ITGB1. VLA-4 is a heterodimer consisting of ITGB1 and ITGA4 and the binding partner of VCAM-1 on stromal cells.

siRNA mediated knock-down of ITGB1 reduced ITGB1 protein levels by 30% and significantly decreased cell survival of plasma cells compared to control cells. Unexpectedly, the PI3K/AKT downstream targets FoxO1 and FoxO3 were not upregulated and activation of caspase 3 which would be suggestive of apoptosis was not detected in ITGB1 knock-down memory plasma cells. Collectively, this indicated that ST2 cell contact still induced pro-survival PI3K/AKT signalling in memory plasma cells after ITGB1 knock-down which prevented caspase activation.

Hence, the observed survival disadvantage of memory plasma cells following ITGB1 knock-down might be a consequence of weaker attachment to ST2 stromal cells. Conceivably, memory plasma cells are more easily detached from stromal cells and die as a result of absent stromal cell contact. Memory plasma cells that successfully attach to ST2 stromal cells still receive survival signals, most likely through other cell-cell contacts.

Of note, single cell RNA sequencing data of *ex vivo* memory plasma cells (S 8) revealed gene expression of *Itga4* and *Itgb7*, encoding for the receptor for MAdCAM-1, and *Itgal* and *Itgb2*, encoding for the receptor for ICAM-1. Although we previously excluded ICAM-1 expression on ST2 stromal cells, it is yet to be determined if ST2 cells express MAdCAM-1. Additionally, as ITGB1 expression was only decreased by 30%, residual VLA-4 could induce PI3K signalling. Other cell-cell contacts such as via CD44 have also been reported to induce pro-survival signalling in memory plasma cells[31,42]. To assess the impact of integrin mediated signalling in *ex vivo* plasma cells, siRNAs targeting all expressed integrins should be used in the future. It would be interesting to investigate if memory plasma cells expressing different immunoglobulin

subtypes utilise distinct cell-cell contacts to ST2 stromal cells *in vitro*. For *in vivo* plasma cells it was suggested that laminin β1 binding to a so far unknown integrin is important for the maintenance of IgG^+ plasma cells in the bone marrow[49].

4.3 ST2 cell contact induced downregulation of FoxO1/3 is essential for *ex vivo* memory plasma cell survival and modulates MCL1 expression

Using our *in vitro* culture system, we previously demonstrated that stromal cells are not only organisers of the plasma cell survival niche but induce PI3K signalling in memory plasma cells. PI3K activates AKT that has a variety of downstream targets, among them FoxO1 and FoxO3[30]. FoxO1 and FoxO3 are transcription factors regulating numerous signalling pathways such as cell survival, autophagy, and cell stress[53]. We demonstrate here that FoxO1 and FoxO3 downregulation is essential for plasma cell survival and induced by stromal cell contact. Knock-down of FoxO1 and FoxO3 using specific siRNAs sufficed to maintain plasma cells in the absence of stromal cells for three days *in vitro*.

Single cell RNA sequencing of plasma cells revealed very few transcriptional differences between plasma cells with siRNA induced FoxO1/3 knock-down compared to scrambled control. Notably, although verified on protein level by flow cytometry, siRNA induced downregulation of FoxO1 and FoxO3 was not detected on transcript level. As close to 70% of transcripts encode for immunoglobulins, plasma cells are challenging to sequence. Thus the sequencing depth was possibly not sufficient to detect subtle changes in mRNA levels[23].

The observed transcriptional differences predominantly distinguished memory plasma cells cultured with or without stromal cell contact (ST2+A SCR vs APRIL SCR and APRIL FOXO). Further separation into immunoglobulin subtypes only revealed transcriptional differences according to their respective immunoglobulin expression but not due to FoxO1/3 downregulation. *Stat1* was among the significantly upregulated genes in memory plasma cells cultured with APRIL, independent of FoxO1/3 knock-down. As BLIMP-1 is described to directly antagonise *Stat1* expression it would be interesting to determine BLIMP-1 expression in plasma cells cultured with and without stromal cells[105]. Potentially, *Stat1* expression is increased as a result of lower BLIMP-1 expression.

Unexpectedly, activation of caspases 3, 7 and 12 was not prevented by FoxO1/3 knock-down. On a transcriptional level, plasma cells cultured in the absence of stromal cells upregulated *Serpina3f,* encoding for the serine protease inhibitor A3F. Serpins are serine protease inhibitors, but some were shown to also inhibit cysteine proteases, such as caspases. In T cells, serpins exhibit anti-apoptotic properties and are important for cell survival[106]. One might speculate that in *ex vivo* memory plasma cells the upregulation of *Serpin* genes results in higher tolerance of caspase activation. However, *Serpina3f* expression was not exclusive to memory plasma cells after FoxO1/3 knock-down (APRIL FOXO) but also expressed in scrambled control cells (APRIL SCR). Thus, the mechanism enabling memory plasma cells to survive despite caspase activation remains to be elucidated.

Since FoxO1 and FoxO3 can positively regulate pro-apoptotic proteins such as BIM[54,55], we determined the expression of pro- and anti-apoptotic proteins in memory plasma cells after FoxO1/3 knock-down. Although expression of BIM and NOXA was decreased in memory plasma cells co-cultured with stromal cells compared to plasma cells cultured without stromal cells, this was independent of FoxO1/3 expression. Instead, we observed that expression of the anti-apoptotic protein MCL1 was modulated by FoxO1/3. Memory plasma cells cultured with APRIL+SCR, i.e., high FoxO1/3 expression, displayed significantly higher expression of MCL1 compared to memory plasma cells co-cultured with stromal cells, i.e., low FoxO1/3 expression. Conversely, *Mcl1* transcript expression was elevated in memory plasma cells cultured with stromal cells, indicating posttranscriptional regulation of MCL1.

MCL1 expression is essential for memory plasma cell survival and induced by NF-κB signalling downstream of BCMA[95]. In addition to inhibiting apoptosis, MCL1 regulates autophagy and mitophagy, the recycling of damaged and old mitochondria, modulates calcium oscillations from the ER and positively regulates mitochondrial Ca^{2+} voltage-dependent anion channels (VDAC), which is reported to induce ROS production[107].

Collectively, the data suggests that stromal cell contact induced FoxO1/3 downregulation ensures that the optimal protein concentration of MCL1 is expressed in memory plasma cells thereby promoting survival. In line with that, suppression of FoxO1/3 by AKT in multiple myeloma cell lines enhances cell survival and stabilises MCL1 expression[108].

Mechanistically there is evidence that FoxO3 activates the NF-κB pathway through BCL10 (B cell lymphoma 10)[109]. Thus, in the absence of stromal cell contact, memory plasma cells can potentially upregulate the NF-κB signalling pathway through BCMA and FoxO3, resulting in high expression of MCL1. In the presence of stromal cell contact, FoxO3 is inhibited, and NF-κB signalling is only induced by BCMA, resulting in adequate MCL1 expression.

Of note, FoxO1/3 additionally regulate other signalling pathways, such as metabolism and redox homeostasis. Thus, more in depth analysis will be necessary to understand how FoxO1/3 downregulation enhances memory plasma cell survival.

4.4 IRF4 is induced by ST2 cells and APRIL and regulates *ex vivo* memory plasma cell survival by maintaining a functional endoplasmic reticulum and BCL2 expression.

IRF4 is the master transcription factor of memory plasma cells and essential for their generation and survival. Genetic deletion of IRF4 results in loss of memory plasma cells[22]. IRF4 is a target of NF-κB and plasma cells expressing only one IRF4 allele displayed reduced uptake of glucose, reduced mitochondrial mass, and expressed elevated levels of ROS[100], suggesting that IRF4 controls metabolism in memory plasma cells. Moreover, Low and colleagues showed that IRF4-deficiency resulted in caspase-dependent cell death of plasma cells due to mitochondria depolarisation and indicated that IRF4 controls mitochondrial homeostasis. However, previous studies have concentrated on splenic plasma cells, and the experimental model was based on observations in cell lines. Thus, the authors disregarded potential differences between *ex vivo* plasma cells and cell lines[110].

We aimed to elucidate the signalling pathways regulating IRF4 expression as well as downstream targets of IRF4 in *ex vivo* bone marrow plasma cells using our *in vitro* culture system. Our data demonstrates that the two essential survival signals, stromal cell contact, and APRIL are required for full expression of IRF4, corresponding to the survival of *ex vivo* memory plasma cells in the respective *in vitro* conditions. IRF4 expression in plasma cells cultured with ST2+A was not significantly upregulated compared to plasma cells cultured with only ST2 cells ($p=0.09$). However, this was most likely due to low intensity of IRF4 staining. We have previously shown that this difference is reliable and significant[30].

Using specific siRNA we assessed the impact of IRF4 on the regulation of pro-and anti-apoptotic proteins, as it was previously suggested that IRF4 induces MCL1 expression[111]. However, neither MCL1, nor the pro-apoptotic proteins BIM and NOXA were regulated by IRF4. Surprisingly, we found BCL2 expression significantly decreased in memory plasma cells with IRF4 knock-down. This observation is contrary to previous results demonstrating that BCL2 is not a target of IRF4. However, as the study was limited to mouse and human plasma cell lines it is likely that *ex vivo* plasma cells differ from cell lines in this aspect. Accordingly, the authors demonstrated that IRF4-deficient plasma cells can be rescued *in vivo* by BCL2 overexpression, which, with regards to our data, highlights IRF4-dependent BCL2 regulation in bone marrow memory plasma cells[21].

Single cell RNA sequencing of plasma cells comparing IRF4 knock-down and scrambled control cells illustrated that IRF4 regulates almost all genes defining plasma cell identity, as described before[21]. Downregulation of *Cd98, Prdm1* (BLIMP-1) and *Sdc1* (CD138) was confirmed on protein level in IRF4 knock-down cells. As IRF4 controls BLIMP-1 expression, it is challenging to differentiate between direct targets of IRF4 and genes which are indirectly affected by altered BLIMP-1 expression.

IRF4-deficient plasma cells display a disorganised ER structure which was ascribed to downregulation of XBP-1 and not as a direct effect of IRF4[21]. However, siRNA-mediated IRF4 knock-down predominantly downregulated genes associated to functions in the ER such as ribosomal genes, UPR genes and genes encoding for protein folding, glycosylation, transport to Golgi and redox metabolism. One interesting example is the apoptosis suppressor *Tmbim6 (*encoding for BI1 (Bax Inhibitor 1)) identified as a target of IRF4 in published ChIP (Chromatin immunoprecipitation) seq dataset[91]. BI1 is predominantly located at the ER where it can interfere with all three UPR signalling pathways and directly interacts with IRE1, BAX, BCL2, and BCL-xL. Additionally, BI1 is described to regulate ROS production and Ca^{2+} efflux from the ER[112]. The TMBIM family and the BCL-2 family closely interact at the ER to integrate cellular pathways, such as UPR, calcium signalling, and autophagy and the ER directly impacts mitochondria homeostasis and cell survival[73]. Thus, we suggest that IRF4 possibly impacts ER function independently of regulating XBP-1 expression. The effect on mitochondrial homeostasis is most likely a direct result of a dysregulated ER as these organelles are highly interconnected.

Ckap4, encoding for the ER protein Ckap4, is upregulated by IRF4. Ckap4 can also localise to the cell surface membrane where it forms the receptor for DKK1 (Dickkopf1). DKK1 is produced by bone marrow stromal cells[113] and can induce PI3K and NF-κB signalling by binding to Ckap4[114]. Thus, IRF4 deficiency might impact pro-survival signalling in plasma cells induced by stromal cell contact.

Interestingly, *Ifnar2,* a direct target of IRF4 based on ChIP seq data, was significantly decreased[91]. *Ifnar2* encodes for IFNAR2 (Interferon Alpha/Beta Receptor 2) and is highly expressed in IgG^+ plasma cells compared to IgA^+ and IgM^+ plasma cells based on single cell sequencing data (S 9). A pilot experiment using IFNAabR (interferon-α/β receptor) deficient mice did not display any differences in plasma cell survival *in vitro* between knock-out and wild type mice (S 10). As the mice used for this experiment were not immunised, the isolated plasma cells expressed presumably predominantly IgA and IgM. Hence, future experiment should assess the survival of IFNAabR knock-out IgG^+ memory plasma cells *in vitro* and *in vivo*.

Collectively, our data illustrates that the master transcription factor IRF4 is synergistically induced by stromal cell contact and APRIL in *ex vivo* memory plasma cells. IRF4 knock-down predominantly affected genes associated with diverse functions in the ER, mostly ribosomal genes. Additionally, IRF4 controls genes defining the plasma cell identity, and influences metabolism by regulating CD98. The higher expression of ribosomal genes could result in higher transcription in IRF4 knock-down cells, thus in the future it should be determined if IRF4 knock-down cells produce more immunoglobulins compared to control cells. To gain deeper insight into IRF4-dependent ER regulation, it would be interesting to analyse Ca^{2+} signalling in memory plasma cells *in vitro* using the FRET-reporter YellowCaB mice, expressing an intracellular calcium indicator[115]. As the ER is the biggest Ca^{2+} storage in the cell and high levels of cytoplasmic calcium indicate cell stress, this could be helpful in determining if IRF4 influences calcium signalling in plasma cells and how this might impact plasma cell survival.

Mathematic modelling of survival data as well as protein expression of the analysed pro-and anti-apoptotic proteins and active caspases in *in vitro* cultured memory plasma cells supports that these parameters are essential and can explain the survival of the memory plasma cells *in vitro*[116]. Thus, it is of interest to gain deeper understanding of

how ST2 cells and APRIL influence the expression of these parameters and how they interact in the context of memory plasma cell survival.

5 CONCLUSION

Stromal cell contact and APRIL prevent caspase-dependent cell death in *ex vivo* memory plasma cells. They synergistically prevent activation of caspase 12 and induce expression of the essential master transcription factor IRF4. IRF4 expression is required to maintain the memory plasma cell identity and a functional ER.

While APRIL induces NF-κB signalling, stromal cell contact induces PI3K signalling. Downstream of PI3K, activation of caspases 3 and 7 is prevented. However, this is not sufficient for plasma cell survival most likely due to high caspase 12 activation Moreover, PI3K activation downregulates FoxO1/3, which is essential for memory plasma cell survival, potentially by modulating MCL1.

Thus, we propose a model in which IRF4, and FoxO1/3 control independent and essential survival signalling pathways in memory plasma cells that regulate BCL2 and MCL1 respectively.

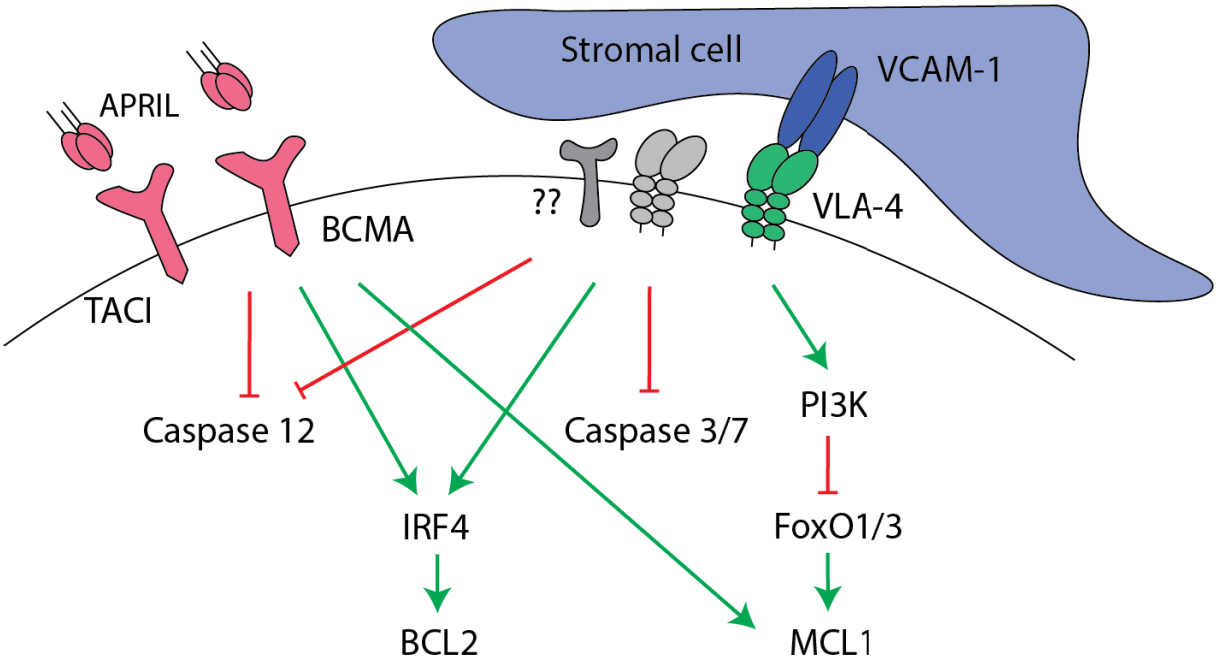

Figure 17: Graphical summary of survival signalling pathways in memory plasma cell. Stromal cells prevent activation of caspases 3 and 7 and together with APRIL inhibit activation of caspase 12. APRIL activates the NF-κB pathway and stromal cells activate PI3K resulting in inactivation of FoxO1/3. Stromal cells and APRIL synergistically regulate IRF4 expression. BCL2 and MCL1 are regulated by IRF4 and FoxO1/3 respectively.

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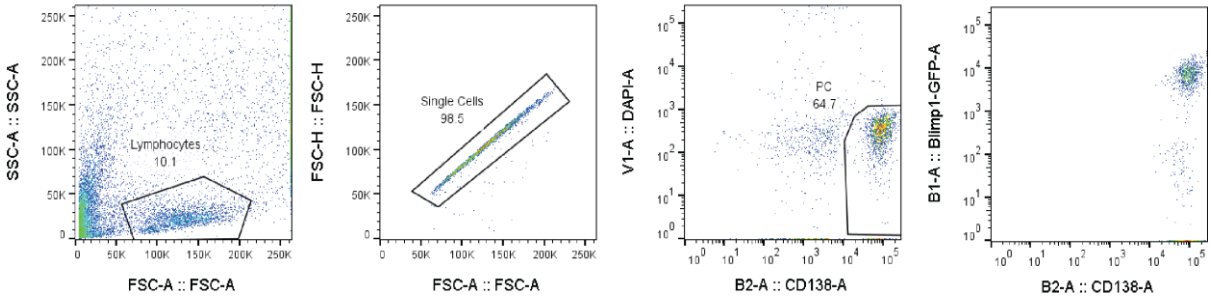
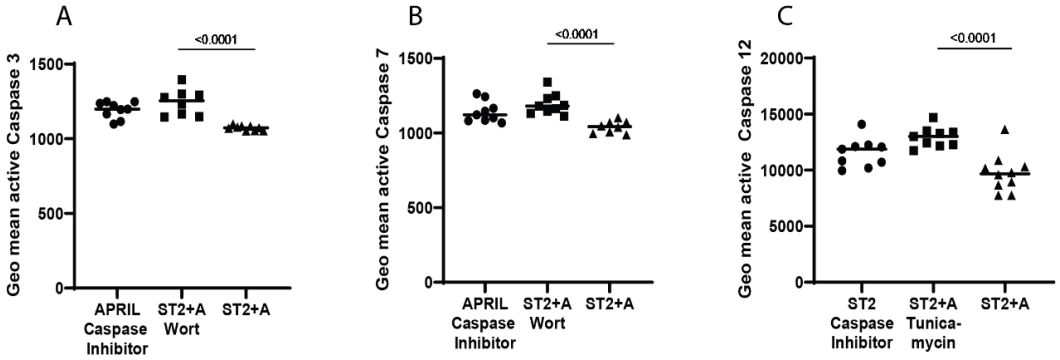
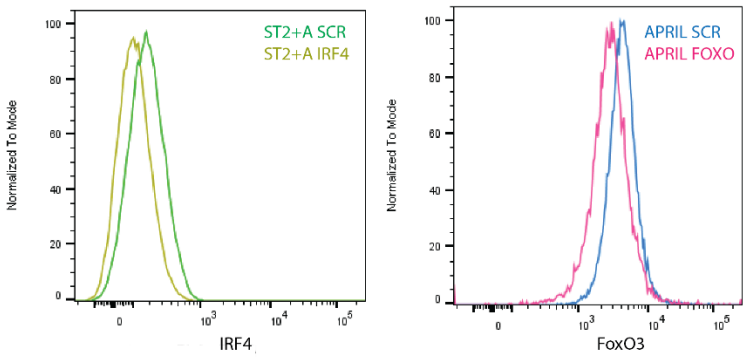
APPENDIX



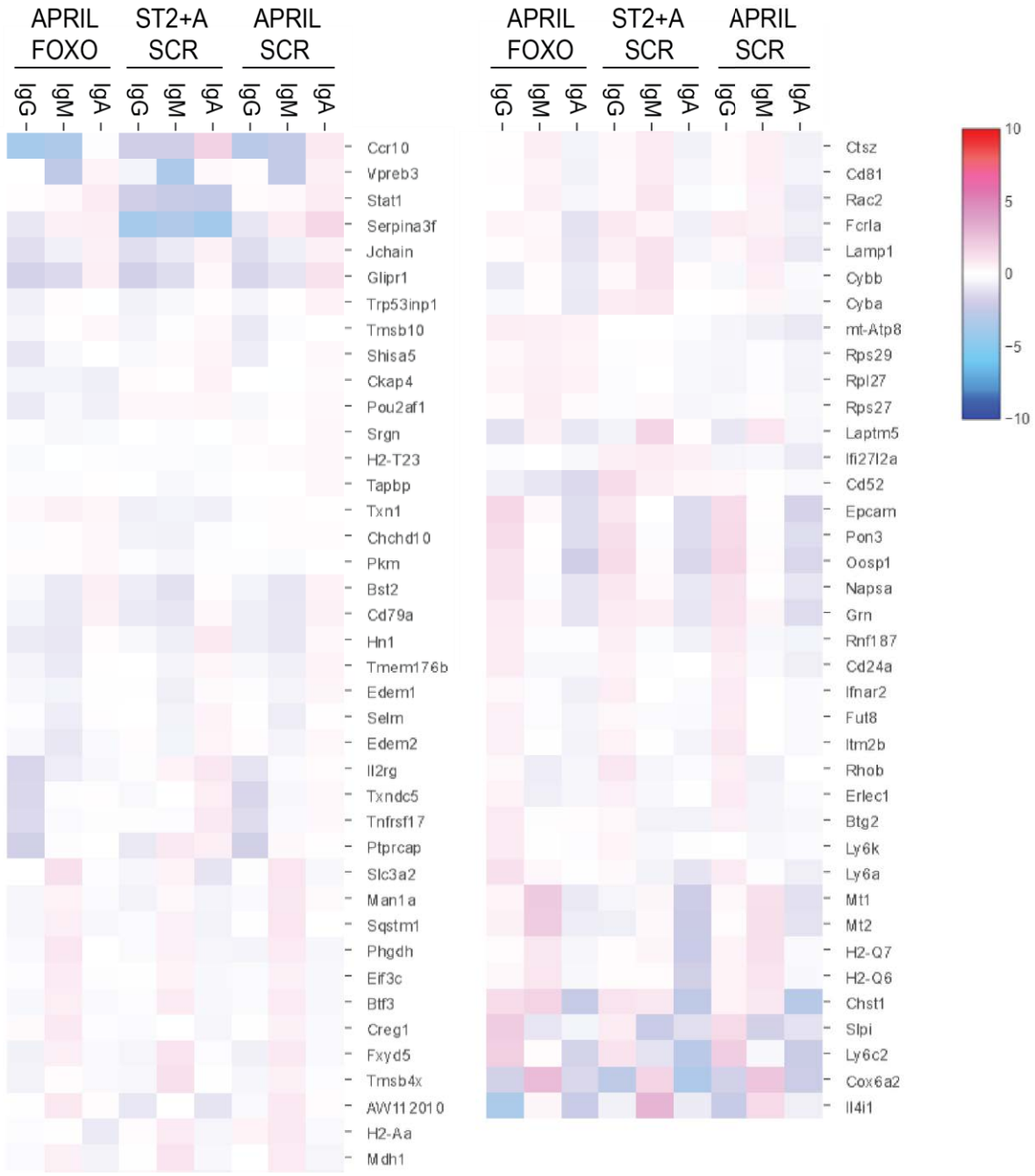
S 1: Exemplary gating of memory plasma cell staining after *in vitro* culture using flow cytometry. Plasma cells were identified by big scatter gate, DAPI^{-} CD138^{++} cells expressing high levels of BlimpGFP.



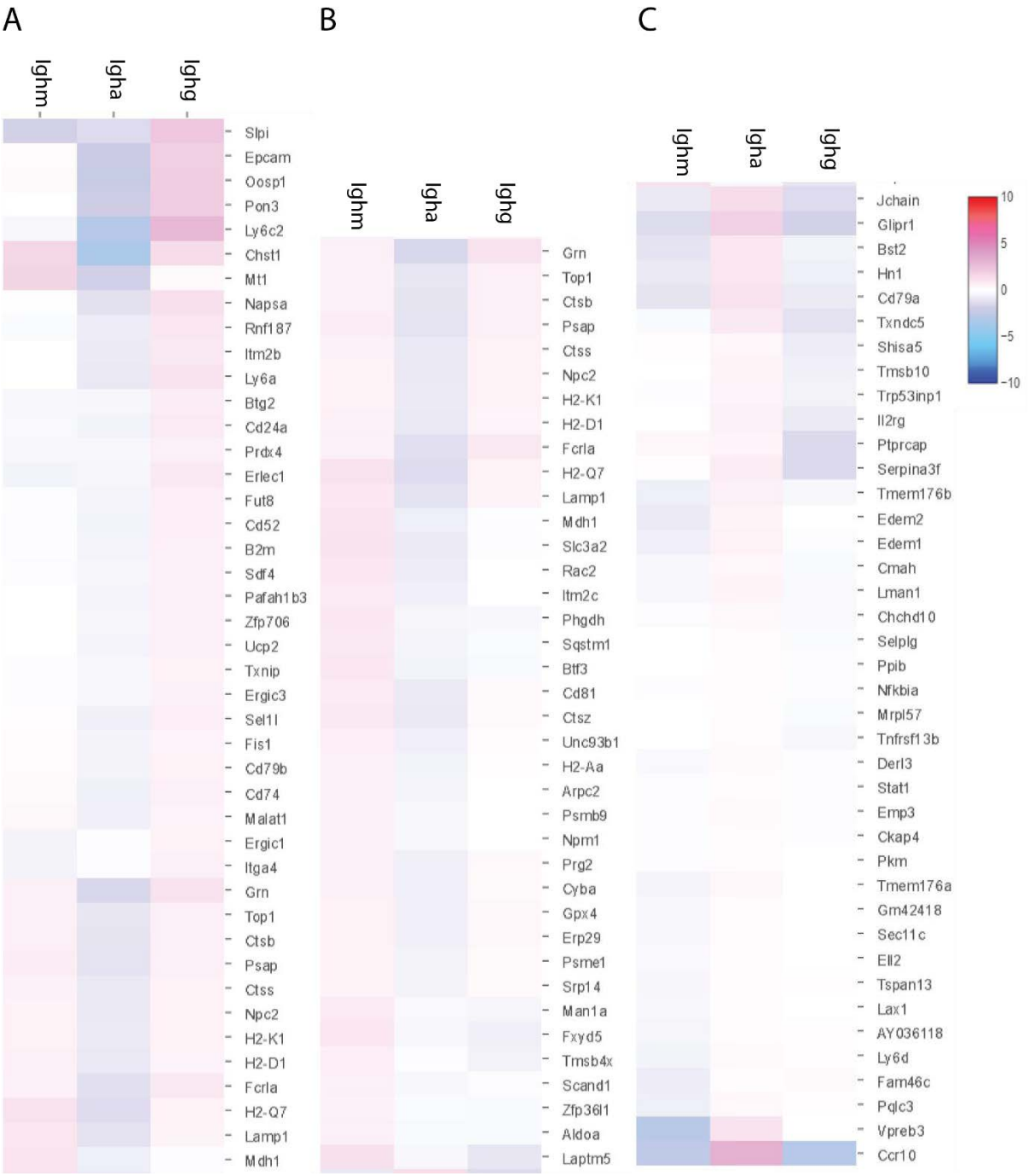
S 2: Controls of active caspases 3, 7 and 12 flow cytometric staining in memory plasma cells after *in vitro* culture. Active caspases (A) 3 and (7) stainings were validated using a pan Caspase Inhibitor that decreased staining or the PI3K Inhibitor Wortmannin (Wort, 10 µM) to trigger caspase activation. (C) Active caspase 12 staining was validated using a pan Caspase Inhibitor and Tunicamycin to trigger ER stress.



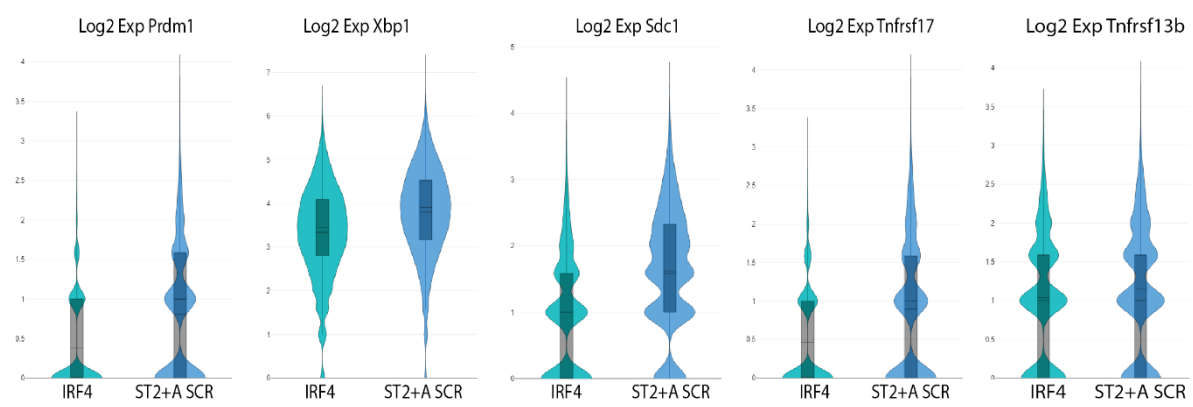
S 3: Validation of IRF4 and FoxO3 protein knock-down of cells used in single cell sequencing. Memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells for three days with scrambled control siRNA, IRF4 siRNA or FoxO1/3 siRNA. Cells were sorted as $DAPI^{-}CD138^{++}$ and used for single cell sequencing, left over cells were used to assess protein expression of IRF4 (left) and FoxO3 (right) using flow cytometry.



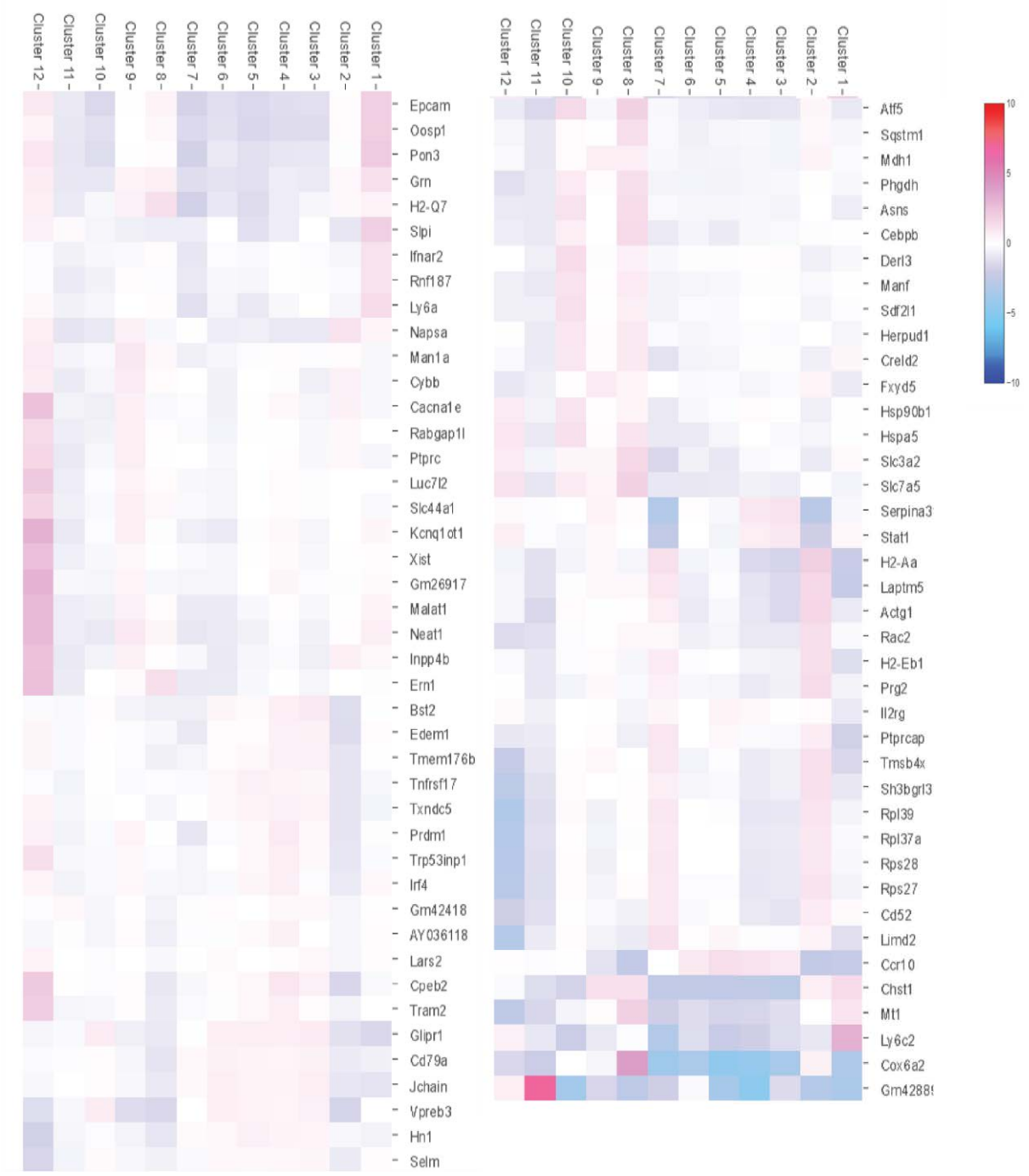
S 4: Differential gene expression of memory plasma cells *in vitro* after FoxO1/3 knock-down. Memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNA targeting FoxO1/FoxO3 for three days. Cells were sorted as $DAPI^{-}CD138^{++}$. Subsequently, cells were sequenced using 10X Genomics-based droplet sequencing. Depicted is a heatmap of differentially expressed genes between the samples APRIL FOXO, ST2+A SCR, APRIL SCR further divided in the expression of IgA, IgM or IgG as defined by BCR sequencing.



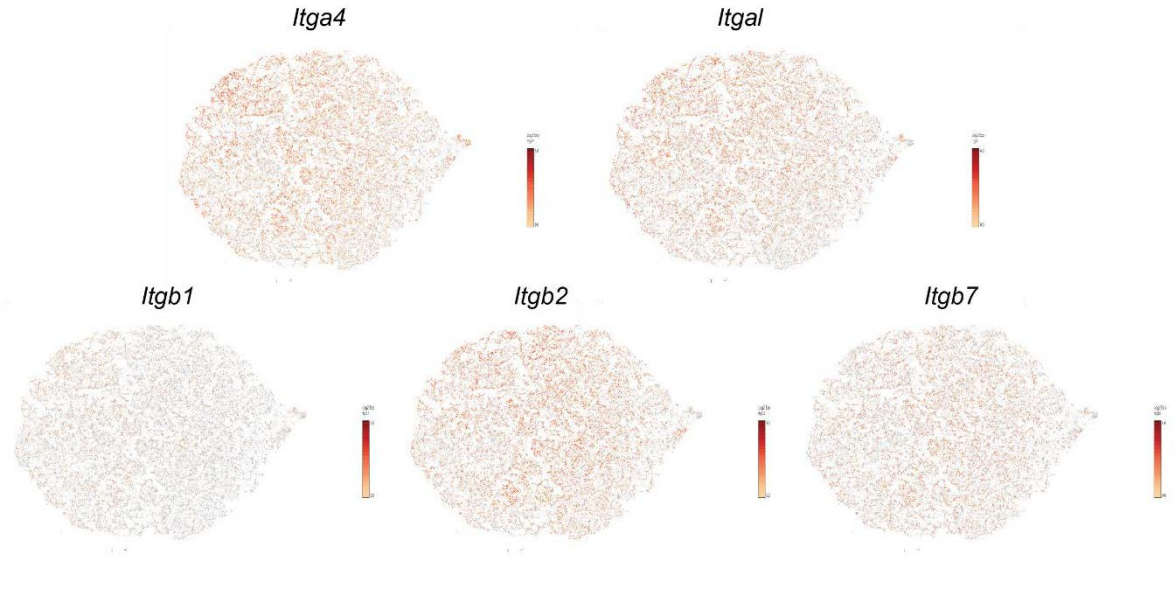
S 5: Differential gene expression of *Ighg, Ighm* and *Igha* expressing memory plasma cells *in vitro*. Memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNA targeting FoxO1/FoxO3 or IRF4 for three days. Cells were sorted as $DAPI^{-}CD138^{++}$. Subsequently, cells were sequenced using 10X Genomics-based droplet sequencing. Depicted is a heatmap of differentially expressed genes between the samples APRIL FOXO, ST2+A SCR, APRIL SCR generated using the Cell loupe browser.



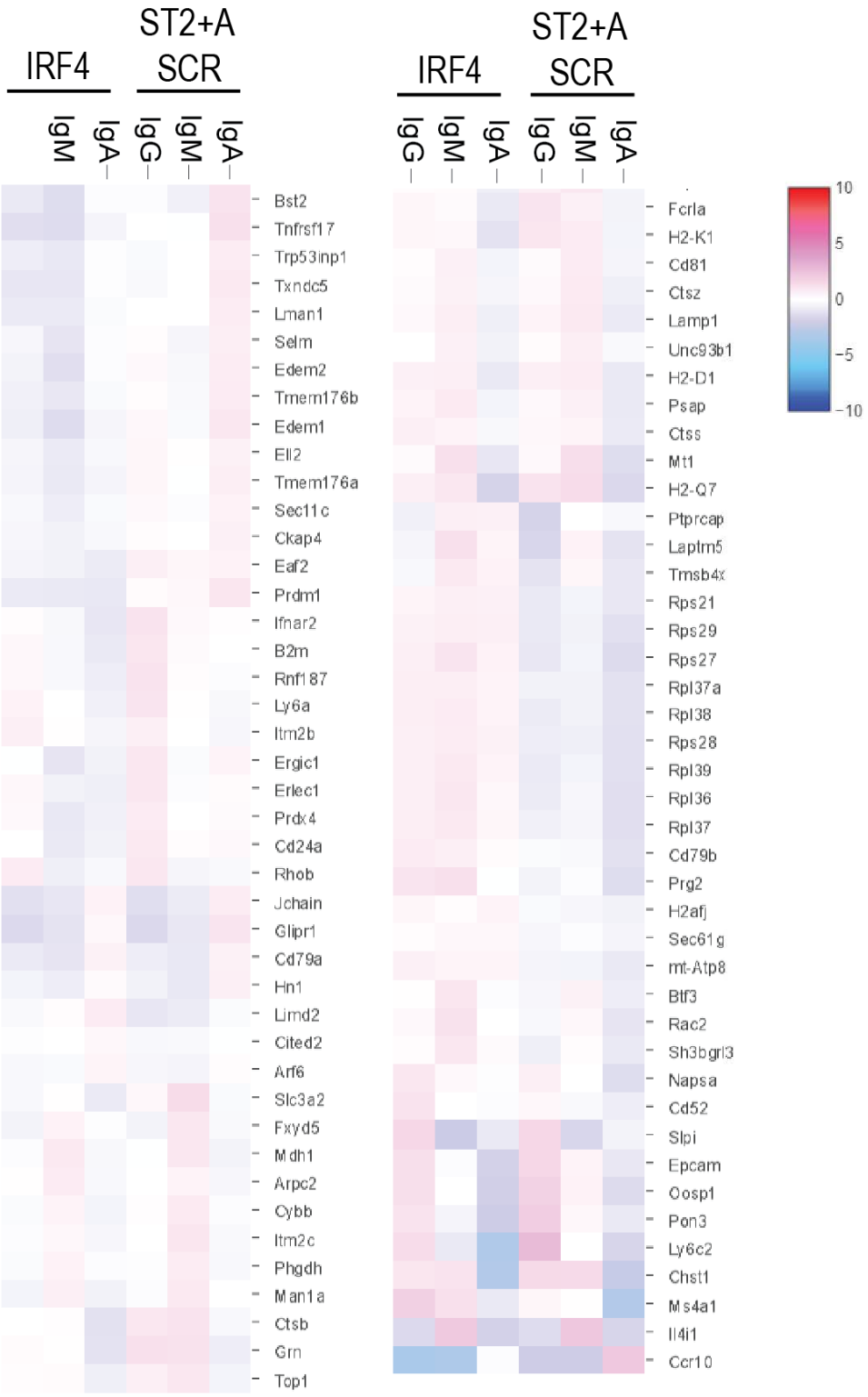
S 6: Expression of *Prdm1, Xbp1, Sdc1* and *Tnfrsf17* but not *Tnfrsf13b* is reduced in memory plasma cells after IRF4 siRNA treatment *in vitro*. *Ex vivo* memory plasma cells were cultured with ST2+A and scrambled siRNA (ST2+A SCR) or siRNA targeting IRF4 (ST2+A IRF4) for three days. Cells were sorted as $DAPI^{-}CD138^{++}$ and subsequently sequenced using 10X Genomics-based droplet sequencing. Expression of selected genes (*Xbp1, Tnfrsf17, Sdc1, Prdm1, Tnfrsg13b*) defining plasma cell identity after IRF4 knock-down is depicted.



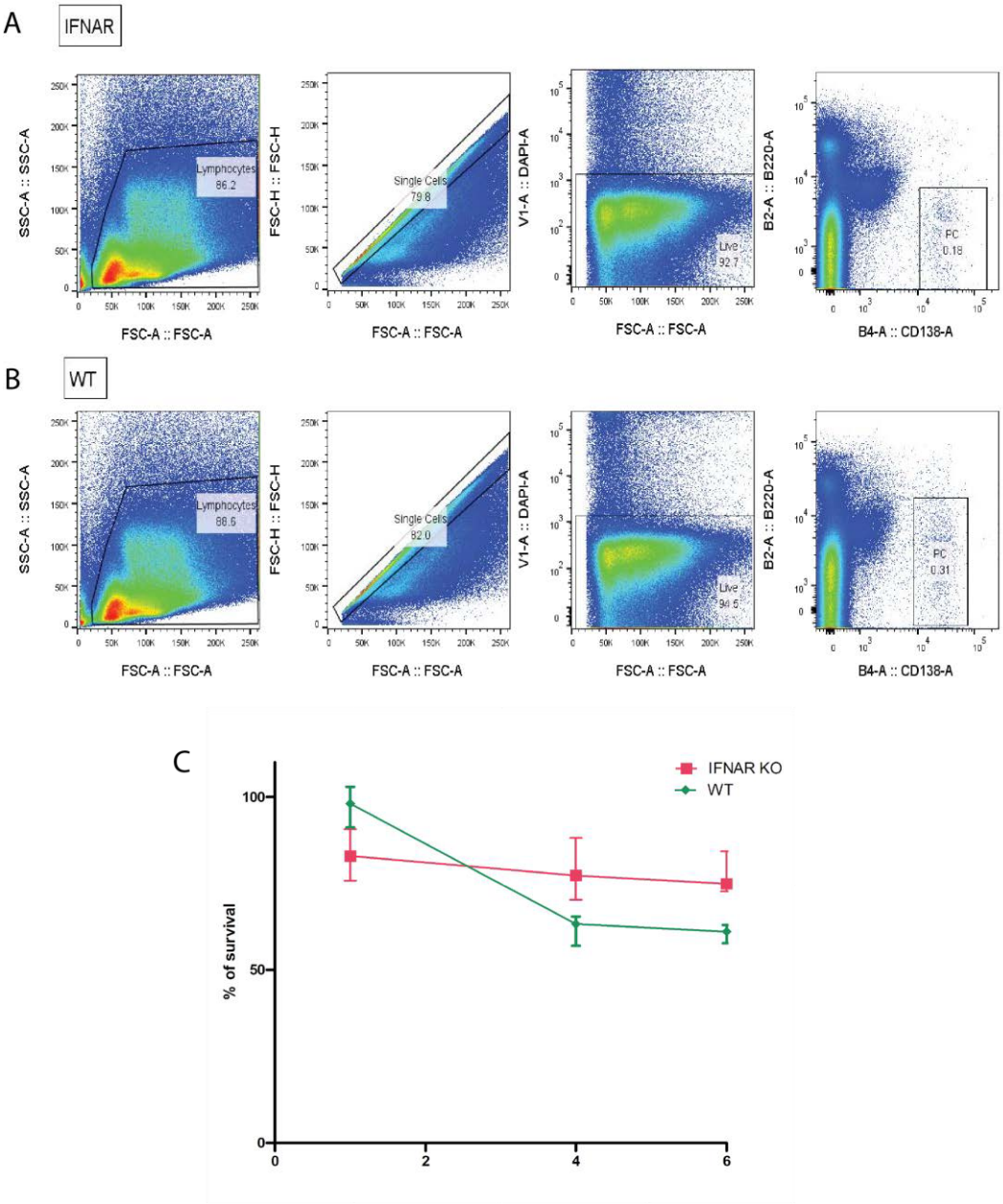
S 7: Differential gene expression of 12 different clusters of memory plasma cells after *in vitro* culture. Memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNA targeting FoxO1/FoxO3 or IRF4 for three days. Cells were sorted as DAPI^{-}CD138^{++}. Subsequently, cells were sequenced using 10X Genomics-based droplet sequencing. Depicted is a heatmap of differentially expressed genes between the samples APRIL FOXO, ST2+A SCR, APRIL SCR generated using the Cell loupe browser.



S 8: Gene expression of different Integrin genes expressed in ex vivo plasma cells. *Ex vivo* memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNAs targeting FoxO1/FoxO3 or IRF4 for three days. Cells were sorted as $DAPI^{-}CD138^{++}$ and subsequently sequenced using 10X Genomics-based droplet sequencing. tSNE coordinates and clustering was computed for 38,243 cells.



S 9: Differential gene expression of memory plasma cells *in vitro* after IRF4 knock-down. Memory plasma cells were cultured with 50 ng/mL APRIL and with or without ST2 cells. Additionally, plasma cells were treated with scrambled control siRNA or siRNA targeting IRF4 for three days. Cells were sorted as DAPI^{-}CD138^{++}. Subsequently, cells were sequenced using 10X Genomics-based droplet sequencing. Depicted is a heatmap of differentially expressed genes between the samples ST2+A SCR and ST2+A IRF4 further divided in the expression of IgA, IgM or IgG as defined by BCR sequencing.



S 10: IFNAR deficient mice express bone marrow plasma cells that can be maintained *in vitro.* Plasma cells were identified by big scatter gate, DAPI^{-} B220^{-}CD138^{++} cells in (A) IFNAabR knock-out and (B) wild-type (WT) mice. (C) Survival of bone marrow plasma cells isolated from IFNAR KO (pink) and WT control (green) mice. Cells were identified as DAPI^{-} CD138^{++} and counted on day one, four and six on culture by flow cytometry.

Table 1: FeatureID table for single cell sequencing of ST2+A+ SCR, APRIL SCR and APRIL FOXO. Displayed are Feature ID, Feature name, Log2FolgChange, p-value and Average expression for the samples APRIL SCR (Ascr), APRIL FOXO (FOXO) and ST2+A+SCR (ST2_AScr).

FeatureID	FeatureName	Ascr Average	Ascr Log2 Fold Change	Ascr P-Value	FOXO Average	FOXO Log2 Fold Change	FOXO P-Value	ST2_Ascr Average	ST2_Ascr Log2 Fold Change	ST2_Ascr P-Value
ENSMUSG00000000682	Cd52	5,17139	-0,03846	1,00000	2,62373	-1,33800	0,00002	8,18153	1,07158	0,00011
ENSMUSG00000044052	Ccr10	0,89823	-0,06022	1,00000	0,50456	-1,17687	0,00034	1,39799	0,99658	0,00049
ENSMUSG00000079017	Ifi27l2a	0,71856	-0,77029	0,14857	0,92898	-0,26795	1,00000	1,54258	0,90423	0,00268
ENSMUSG00000031304	Il2rg	0,98018	-0,15623	1,00000	0,68545	-0,86165	0,02238	1,52692	0,87550	0,00384
ENSMUSG00000028581	Laptm5	0,85440	-0,18739	1,00000	0,66766	-0,68068	0,14779	1,29902	0,77204	0,01982
ENSMUSG00000022496	Tnfrsf17	0,78279	-0,17174	1,00000	0,65632	-0,53003	0,43522	1,12259	0,64211	0,10095
ENSMUSG00000040747	Cd53	0,80662	-0,06101	1,00000	0,60345	-0,65001	0,18633	1,09576	0,63693	0,10752
ENSMUSG00000006519	Cyba	3,86153	-0,06703	1,00000	3,01249	-0,57454	0,33124	5,14805	0,58351	0,18805
ENSMUSG00000046841	Ckap4	1,36128	0,14647	1,00000	0,86966	-0,77118	0,05894	1,61461	0,53474	0,28843
ENSMUSG00000032053	Pou2af1	1,63098	0,15828	1,00000	1,03139	-0,78054	0,05385	1,92091	0,53031	0,30013
ENSMUSG00000021127	Zfp36l1	1,24653	0,03007	1,00000	0,91901	-0,59748	0,28342	1,54320	0,51202	0,35276
ENSMUSG00000038991	Txndc5	9,12670	-0,17746	1,00000	8,54463	-0,31479	1,00000	12,21838	0,46775	0,46070
ENSMUSG00000020737	Hn1	0,93408	-0,16159	1,00000	0,86722	-0,31644	1,00000	1,23450	0,45507	0,50161
ENSMUSG00000021356	Irf4	1,27903	-0,07729	1,00000	1,10078	-0,38969	0,83557	1,61503	0,44109	0,55006
ENSMUSG00000049775	Tmsb4x	1,20634	-0,05483	1,00000	1,01820	-0,40780	0,78362	1,50395	0,43569	0,57118
ENSMUSG00000025647	Shisa5	2,59028	0,00340	1,00000	2,13403	-0,40250	0,79691	3,06490	0,37623	0,79855
ENSMUSG00000025544	Tm9sf2	0,89813	-0,02862	1,00000	0,77653	-0,33464	0,94807	1,06527	0,34775	0,85936
ENSMUSG00000025967	Eef1b2	2,18603	-0,16476	1,00000	2,16120	-0,18944	1,00000	2,75944	0,34425	0,87204
ENSMUSG00000064367	mt-Nd5	2,78620	0,03241	1,00000	2,32714	-0,34807	0,90613	3,14842	0,30082	1,00000
ENSMUSG00000038312	Edem2	4,71829	0,21992	1,00000	3,27322	-0,55169	0,37963	4,88194	0,28995	1,00000
ENSMUSG00000025823	Pdia4	4,69692	0,04069	1,00000	3,92974	-0,33696	0,94087	5,24598	0,28285	1,00000
ENSMUSG00000064370	mt-Cytb	8,46319	-0,11588	1,00000	8,26093	-0,16763	1,00000	10,14243	0,27847	1,00000
ENSMUSG00000034088	Hdlbp	2,18179	-0,02654	1,00000	1,95861	-0,25556	1,00000	2,50387	0,27464	1,00000
ENSMUSG00000024610	Cd74	55,16610	0,00781	1,00000	47,97432	-0,28856	1,00000	62,22762	0,27134	1,00000
ENSMUSG00000010142	Tnfrsf13b	1,22131	-0,10430	1,00000	1,18424	-0,17015	1,00000	1,45029	0,27003	1,00000
ENSMUSG00000037805	Rpl10a	3,45925	-0,10660	1,00000	3,36293	-0,16697	1,00000	4,11055	0,26911	1,00000
ENSMUSG00000064357	mt-Atp6	6,44639	0,12324	1,00000	5,01879	-0,40748	0,78036	6,87928	0,26371	1,00000
ENSMUSG00000105650	Gm42889	39,20512	-0,01013	1,00000	34,85145	-0,26023	1,00000	44,42366	0,26296	1,00000
ENSMUSG00000032116	Stt3a	1,03327	0,14462	1,00000	0,79060	-0,42322	0,72694	1,08866	0,25628	1,00000
ENSMUSG00000038642	Ctss	2,87041	-0,15734	1,00000	2,94984	-0,09989	1,00000	3,46944	0,25348	1,00000
ENSMUSG00000028757	Ddost	2,15008	0,03428	1,00000	1,84085	-0,29578	1,00000	2,37588	0,25213	1,00000
ENSMUSG00000001576	Ergic1	1,49337	0,00721	1,00000	1,31578	-0,26217	1,00000	1,66765	0,24800	1,00000
ENSMUSG00000022498	Txndc11	1,75253	0,12006	1,00000	1,38272	-0,38351	0,84708	1,85807	0,24585	1,00000

ENSMUSG00000023367	Tmem176a	4,30015	-0,00572	1,00000	3,84687	-0,24294	1,00000	4,81941	0,24283	1,00000
ENSMUSG00000044533	Rps2	3,55736	-0,22567	1,00000	3,91469	-0,02272	1,00000	4,42113	0,24258	1,00000
ENSMUSG00000057113	Npm1	0,88956	-0,05803	1,00000	0,83712	-0,18767	1,00000	1,02111	0,24230	1,00000
ENSMUSG00000038612	Mcl1	1,44826	0,01617	1,00000	1,26965	-0,26406	1,00000	1,60563	0,24098	1,00000
ENSMUSG00000027073	Prg2	8,17744	0,11819	1,00000	6,48369	-0,37529	0,86856	8,65581	0,24033	1,00000
ENSMUSG00000034994	Eef2	6,26052	0,05577	1,00000	5,28265	-0,30566	1,00000	6,81361	0,23987	1,00000
ENSMUSG00000065947	mt-Nd4l	3,58464	-0,34593	1,00000	4,41191	0,09494	1,00000	4,70958	0,23556	1,00000
ENSMUSG00000067274	Rplp0	9,09715	-0,02324	1,00000	8,31224	-0,21567	1,00000	10,24036	0,23458	1,00000
ENSMUSG00000024516	Sec11c	8,45866	0,11307	1,00000	6,76417	-0,36264	0,88109	8,94938	0,23418	1,00000
ENSMUSG00000071644	Eef1g	0,98816	-0,07998	1,00000	0,95517	-0,15272	1,00000	1,13992	0,23047	1,00000
ENSMUSG00000008668	Rps18	2,75635	0,13085	1,00000	2,17078	-0,37750	0,86493	2,88743	0,22966	1,00000
ENSMUSG00000032518	Rpsa	7,68789	-0,03658	1,00000	7,14901	-0,19181	1,00000	8,67116	0,22524	1,00000
ENSMUSG00000021025	Nfkbia	2,02675	-0,02348	1,00000	1,86304	-0,20335	1,00000	2,27033	0,22340	1,00000
ENSMUSG00000025935	Tram1	1,86521	0,08035	1,00000	1,55149	-0,31228	1,00000	1,99128	0,22157	1,00000
ENSMUSG00000029580	Actb	4,07705	-0,10989	1,00000	4,08023	-0,10865	1,00000	4,74069	0,21694	1,00000
ENSMUSG00000020321	Mdh1	1,47692	0,00349	1,00000	1,32907	-0,22186	1,00000	1,62745	0,21437	1,00000
ENSMUSG00000032399	Rpl4	1,77922	-0,00093	1,00000	1,60817	-0,21690	1,00000	1,96424	0,21404	1,00000
ENSMUSG00000022283	Pabpc1	1,57966	0,11007	1,00000	1,28206	-0,33527	0,94454	1,65752	0,21270	1,00000
ENSMUSG00000016559	H3f3b	4,63713	-0,01447	1,00000	4,24949	-0,20109	1,00000	5,14753	0,21243	1,00000
ENSMUSG00000060036	Rpl3	3,89295	0,01888	1,00000	3,45508	-0,23603	1,00000	4,25577	0,21234	1,00000
ENSMUSG00000030062	Rpn1	1,52972	-0,08654	1,00000	1,50224	-0,12563	1,00000	1,75461	0,21091	1,00000
ENSMUSG00000028495	Rps6	3,07530	-0,02215	1,00000	2,84283	-0,19028	1,00000	3,42184	0,20979	1,00000
ENSMUSG00000032575	Manf	4,22135	0,25131	1,00000	2,97428	-0,49458	0,53013	4,15753	0,20953	1,00000
ENSMUSG00000036908	Unc93b1	1,37655	0,17858	1,00000	1,04586	-0,40738	0,78243	1,39830	0,20798	1,00000
ENSMUSG00000028788	Ptp4a2	1,18497	-0,03983	1,00000	1,11534	-0,16948	1,00000	1,32794	0,20751	1,00000
ENSMUSG00000064351	mt-Co1	11,45063	-0,02049	1,00000	10,59061	-0,18758	1,00000	12,70706	0,20557	1,00000
ENSMUSG00000020592	Sdc1	2,43642	0,17709	1,00000	1,86069	-0,39834	0,80727	2,46921	0,20139	1,00000
ENSMUSG00000059291	Rpl11	5,40090	0,04211	1,00000	4,71965	-0,24628	1,00000	5,80690	0,19887	1,00000
ENSMUSG00000041355	Ssr2	1,23491	0,17382	1,00000	0,94774	-0,39132	0,82654	1,25173	0,19857	1,00000
ENSMUSG00000024353	Mzb1	20,01794	0,13919	1,00000	15,93365	-0,34852	0,90528	20,57154	0,19517	1,00000
ENSMUSG00000041571	Sepw1	2,97482	0,21557	1,00000	2,19638	-0,43238	0,70104	2,95226	0,19212	1,00000
ENSMUSG00000023010	Tmbim6	2,37702	0,26305	1,00000	1,67208	-0,48780	0,54921	2,31053	0,19156	1,00000
ENSMUSG00000038900	Rpl12	2,16160	-0,07021	1,00000	2,11136	-0,12088	1,00000	2,43810	0,19054	1,00000
ENSMUSG00000029632	Ndufa4	1,36256	-0,00347	1,00000	1,25157	-0,18561	1,00000	1,48787	0,18713	1,00000
ENSMUSG00000062683	Atp5g2	1,49968	-0,00420	1,00000	1,37910	-0,18390	1,00000	1,63748	0,18622	1,00000
ENSMUSG00000058558	Rpl5	2,39761	-0,01934	1,00000	2,23733	-0,16776	1,00000	2,63562	0,18574	1,00000
ENSMUSG00000012405	Rpl15	2,88351	-0,00073	1,00000	2,64416	-0,18653	1,00000	3,14205	0,18522	1,00000

ENSMUSG00000021939	Ctsb	2,65056	-0,12881	1,00000	2,74706	-0,05259	1,00000	3,05927	0,18070	1,00000
ENSMUSG00000029810	Tmem176b	6,93781	0,10541	1,00000	5,75632	-0,29446	1,00000	7,18840	0,18004	1,00000
ENSMUSG00000021610	Clptm1l	1,39073	0,12262	1,00000	1,13880	-0,30571	1,00000	1,42544	0,17308	1,00000
ENSMUSG00000041891	Lman1	1,84847	0,10761	1,00000	1,53673	-0,28834	1,00000	1,90661	0,17225	1,00000
ENSMUSG00000025289	Prdx4	2,52211	0,06184	1,00000	2,19462	-0,23658	1,00000	2,65295	0,17015	1,00000
ENSMUSG00000064358	mt-Co3	8,25358	-0,08213	1,00000	8,23582	-0,08709	1,00000	9,27186	0,16933	1,00000
ENSMUSG00000031320	Rps4x	8,53372	-0,05404	1,00000	8,29787	-0,11446	1,00000	9,45852	0,16852	1,00000
ENSMUSG00000037706	Cd81	2,24178	-0,00681	1,00000	2,08527	-0,16232	1,00000	2,43079	0,16820	1,00000
ENSMUSG00000061983	Rps12	3,62995	-0,11271	1,00000	3,73575	-0,05140	1,00000	4,12673	0,16403	1,00000
ENSMUSG00000020048	Hsp90b1	16,71047	0,02684	1,00000	15,08803	-0,19258	1,00000	17,80521	0,16356	1,00000
ENSMUSG00000031770	Herpud1	3,19783	-0,04460	1,00000	3,09203	-0,11712	1,00000	3,51830	0,16189	1,00000
ENSMUSG00000005583	Mef2c	1,22338	0,03463	1,00000	1,09931	-0,19519	1,00000	1,29589	0,15842	1,00000
ENSMUSG00000028452	Vcp	1,86245	0,00318	1,00000	1,72466	-0,16213	1,00000	2,00109	0,15814	1,00000
ENSMUSG00000033487	Fndc3a	1,39901	0,12686	1,00000	1,15218	-0,29004	1,00000	1,41908	0,15430	1,00000
ENSMUSG00000027808	Serp1	5,17612	0,09676	1,00000	4,39384	-0,25537	1,00000	5,31697	0,15249	1,00000
ENSMUSG00000038393	Txnip	1,15266	-0,08192	1,00000	1,15932	-0,06984	1,00000	1,28482	0,15240	1,00000
ENSMUSG00000002778	Kdelr1	1,20053	-0,02077	1,00000	1,14134	-0,12970	1,00000	1,29975	0,15070	1,00000
ENSMUSG00000020719	Ddx5	2,92242	0,08569	1,00000	2,51096	-0,24056	1,00000	3,01323	0,14983	1,00000
ENSMUSG00000060743	H3f3a	1,54410	-0,09404	1,00000	1,57260	-0,05504	1,00000	1,72872	0,14959	1,00000
ENSMUSG00000044468	Fam46c	2,87977	0,13551	1,00000	2,35890	-0,29328	1,00000	2,90204	0,14838	1,00000
ENSMUSG00000020571	Pdia6	2,90213	0,06025	1,00000	2,55777	-0,21145	1,00000	3,02429	0,14803	1,00000
ENSMUSG00000030082	Sec61a1	1,89466	0,15088	1,00000	1,52911	-0,30974	1,00000	1,89596	0,14791	1,00000
ENSMUSG00000012848	Rps5	5,93665	-0,04349	1,00000	5,77587	-0,10280	1,00000	6,48371	0,14685	1,00000
ENSMUSG00000047215	Rpl9	5,42553	-0,09439	1,00000	5,53507	-0,05170	1,00000	6,06675	0,14654	1,00000
ENSMUSG00000090841	Myl6	0,97664	-0,18303	1,00000	1,08084	0,03457	1,00000	1,13856	0,14603	1,00000
ENSMUSG00000029614	Rpl6	3,82154	-0,06699	1,00000	3,80221	-0,07818	1,00000	4,21756	0,14586	1,00000
ENSMUSG00000019188	H13	3,70570	0,11281	1,00000	3,11324	-0,26195	1,00000	3,76186	0,14233	1,00000
ENSMUSG00000014867	Surf4	1,05160	-0,01716	1,00000	1,00051	-0,12454	1,00000	1,13219	0,14214	1,00000
ENSMUSG00000027523	Gnas	2,38283	-0,06874	1,00000	2,38118	-0,07051	1,00000	2,62497	0,14007	1,00000
ENSMUSG00000040462	Os9	1,48677	0,06127	1,00000	1,31724	-0,19952	1,00000	1,53997	0,13572	1,00000
ENSMUSG00000031708	Tecr	1,10784	-0,19297	1,00000	1,24336	0,05511	1,00000	1,29098	0,13472	1,00000
ENSMUSG00000020372	Gnb2l1	3,07756	-0,04174	1,00000	3,00711	-0,09186	1,00000	3,33947	0,13445	1,00000
ENSMUSG00000041084	Ostc	0,91428	-0,15414	1,00000	0,99094	0,01887	1,00000	1,04565	0,13420	1,00000
ENSMUSG00000025130	P4hb	2,02961	-0,06463	1,00000	2,02780	-0,06681	1,00000	2,22377	0,13240	1,00000
ENSMUSG00000031167	Rbm3	2,20593	0,02879	1,00000	2,01920	-0,16189	1,00000	2,31512	0,13229	1,00000
ENSMUSG00000079523	Tmsb10	1,36906	-0,29665	1,00000	1,68791	0,15350	1,00000	1,67485	0,13129	1,00000
ENSMUSG00000064363	mt-Nd4	2,58200	0,16765	1,00000	2,06895	-0,30929	1,00000	2,54406	0,13019	1,00000

ENSMUSG00000105646	Gm43291	1,68111	0,13846	1,00000	1,38692	-0,27589	1,00000	1,67715	0,12920	1,00000
ENSMUSG00000000740	Rpl13	9,38418	-0,10872	1,00000	9,78085	-0,01979	1,00000	10,47869	0,12879	1,00000
ENSMUSG00000032383	Ppib	3,34223	0,08567	1,00000	2,90548	-0,21622	1,00000	3,41004	0,12680	1,00000
ENSMUSG00000053565	Eif3k	0,96147	-0,00536	1,00000	0,91168	-0,12017	1,00000	1,02205	0,12615	1,00000
ENSMUSG00000021484	Lman2	1,17157	0,10782	1,00000	0,99758	-0,23870	1,00000	1,18293	0,12567	1,00000
ENSMUSG00000061315	Naca	1,85191	-0,06318	1,00000	1,85522	-0,05958	1,00000	2,01978	0,12384	1,00000
ENSMUSG00000020460	Rps27a	6,49850	-0,11940	1,00000	6,85974	-0,00303	1,00000	7,27171	0,12241	1,00000
ENSMUSG00000032115	Hyou1	0,99791	0,03786	1,00000	0,91100	-0,15883	1,00000	1,03729	0,12033	1,00000
ENSMUSG00000005610	Eif4g2	1,22968	-0,05686	1,00000	1,22753	-0,06087	1,00000	1,33420	0,11893	1,00000
ENSMUSG00000026864	Hspa5	7,21883	-0,04380	1,00000	7,11989	-0,07379	1,00000	7,78409	0,11865	1,00000
ENSMUSG00000039236	Isg20	0,97102	0,04636	1,00000	0,88031	-0,16535	1,00000	1,00441	0,11806	1,00000
ENSMUSG00000032042	Srpr	2,17529	0,01236	1,00000	2,03679	-0,12976	1,00000	2,28474	0,11763	1,00000
ENSMUSG00000030744	Rps3	5,25010	-0,07647	1,00000	5,34197	-0,03928	1,00000	5,74260	0,11667	1,00000
ENSMUSG00000016756	Cmah	0,99289	0,03171	1,00000	0,91344	-0,14841	1,00000	1,03312	0,11644	1,00000
ENSMUSG00000053477	Tcf4	1,16743	0,00321	1,00000	1,10342	-0,11867	1,00000	1,23043	0,11607	1,00000
ENSMUSG00000021917	Spcs1	5,66439	-0,04730	1,00000	5,61296	-0,06721	1,00000	6,10934	0,11562	1,00000
ENSMUSG00000062328	Rpl17	6,72111	-0,03213	1,00000	6,56930	-0,08165	1,00000	7,19581	0,11484	1,00000
ENSMUSG00000037742	Eef1a1	15,00001	0,02919	1,00000	13,84819	-0,14342	1,00000	15,60764	0,11392	1,00000
ENSMUSG00000042079	Hnrnpf	1,05071	-0,00006	1,00000	0,99778	-0,11183	1,00000	1,10734	0,11268	1,00000
ENSMUSG00000074227	Spint2	1,33902	-0,09228	1,00000	1,38516	-0,01939	1,00000	1,47263	0,11245	1,00000
ENSMUSG00000030432	Rpl28	5,46532	-0,10335	1,00000	5,71166	-0,00842	1,00000	6,04117	0,11218	1,00000
ENSMUSG00000027642	Rpn2	1,89023	0,09488	1,00000	1,64169	-0,20954	1,00000	1,90682	0,11108	1,00000
ENSMUSG00000054408	Spcs3	2,02449	0,10101	1,00000	1,74841	-0,21558	1,00000	2,03617	0,11056	1,00000
ENSMUSG00000041736	Tspo	0,94768	-0,04322	1,00000	0,93836	-0,06477	1,00000	1,01725	0,10934	1,00000
ENSMUSG00000043716	Rpl7	3,25910	-0,03722	1,00000	3,20960	-0,07048	1,00000	3,48792	0,10888	1,00000
ENSMUSG00000028639	Ybx1	1,25658	-0,09945	1,00000	1,31177	-0,00683	1,00000	1,38306	0,10688	1,00000
ENSMUSG00000064341	mt-Nd1	3,32785	-0,09178	1,00000	3,45030	-0,01395	1,00000	3,64890	0,10641	1,00000
ENSMUSG00000021877	Arf4	1,42294	0,00622	1,00000	1,34812	-0,11066	1,00000	1,49017	0,10516	1,00000
ENSMUSG00000056201	Cfl1	1,41152	-0,09754	1,00000	1,47282	-0,00591	1,00000	1,55020	0,10406	1,00000
ENSMUSG00000031029	Eif3f	1,64467	0,01566	1,00000	1,54670	-0,11724	1,00000	1,71254	0,10210	1,00000
ENSMUSG00000075706	Gpx4	2,30423	-0,02245	1,00000	2,24583	-0,07813	1,00000	2,44123	0,10175	1,00000
ENSMUSG00000036594	H2-Aa	1,19777	0,37718	1,00000	0,78894	-0,52224	0,49179	1,06454	0,10102	1,00000
ENSMUSG00000038179	Slamf7	2,62367	-0,02473	1,00000	2,56666	-0,07241	1,00000	2,77823	0,09835	1,00000
ENSMUSG00000023175	Bsg	1,84906	0,11087	1,00000	1,59224	-0,21248	1,00000	1,84042	0,09747	1,00000
ENSMUSG00000022365	Derl1	1,61059	0,01356	1,00000	1,52129	-0,10994	1,00000	1,67475	0,09704	1,00000
ENSMUSG00000006333	Rps9	5,56008	-0,05567	1,00000	5,60153	-0,03980	1,00000	5,96778	0,09663	1,00000
ENSMUSG00000027828	Ssr3	1,14882	-0,05506	1,00000	1,15746	-0,03903	1,00000	1,23200	0,09539	1,00000

ENSMUSG00000074129	Rpl13a	5,10057	-0,03471	1,00000	5,05378	-0,05483	1,00000	5,40706	0,09078	1,00000
ENSMUSG00000071866	Ppia	5,93675	-0,05057	1,00000	5,97005	-0,03864	1,00000	6,33871	0,09039	1,00000
ENSMUSG00000037072	Sep15	1,74435	-0,03197	1,00000	1,72525	-0,05598	1,00000	1,84554	0,08927	1,00000
ENSMUSG00000026238	Ptma	1,96677	-0,11477	1,00000	2,09953	0,02642	1,00000	2,16166	0,08819	1,00000
ENSMUSG00000078812	Eif5a	1,76317	-0,00337	1,00000	1,69915	-0,08358	1,00000	1,84001	0,08804	1,00000
ENSMUSG00000017404	Rpl19	8,02033	-0,03802	1,00000	7,98857	-0,04678	1,00000	8,49650	0,08601	1,00000
ENSMUSG00000068328	Aup1	1,37074	0,10584	1,00000	1,19352	-0,19396	1,00000	1,35946	0,08487	1,00000
ENSMUSG00000025794	Rpl14	3,38390	-0,11680	1,00000	3,62483	0,03194	1,00000	3,71645	0,08452	1,00000
ENSMUSG00000064354	mt-Co2	7,37594	-0,13878	1,00000	8,05991	0,05303	1,00000	8,18498	0,08444	1,00000
ENSMUSG00000025362	Rps26	3,83533	0,00758	1,00000	3,66803	-0,08918	1,00000	3,97222	0,08248	1,00000
ENSMUSG00000028081	Rps3a1	8,63163	-0,04296	1,00000	8,65105	-0,03825	1,00000	9,14971	0,08239	1,00000
ENSMUSG00000071076	Jund	3,03934	0,06236	1,00000	2,76895	-0,13969	1,00000	3,06208	0,07661	1,00000
ENSMUSG00000022769	Sdf2l1	2,36264	-0,01254	1,00000	2,31326	-0,05849	1,00000	2,45794	0,07221	1,00000
ENSMUSG00000024608	Rps14	6,59384	0,01387	1,00000	6,30056	-0,08493	1,00000	6,77613	0,07185	1,00000
ENSMUSG00000008682	Rpl10	9,47854	-0,06387	1,00000	9,73483	-0,00615	1,00000	10,09128	0,07085	1,00000
ENSMUSG00000029390	Tmed2	2,13402	-0,05020	1,00000	2,16941	-0,01464	1,00000	2,25238	0,06587	1,00000
ENSMUSG00000034634	Ly6d	10,60470	-0,24650	1,00000	12,86321	0,17203	1,00000	12,27757	0,06471	1,00000
ENSMUSG00000020738	Sumo2	1,26357	-0,09953	1,00000	1,34480	0,03555	1,00000	1,36343	0,06389	1,00000
ENSMUSG00000003970	Rpl8	5,69227	-0,06324	1,00000	5,86294	0,00076	1,00000	6,03700	0,06321	1,00000
ENSMUSG00000055447	Cd47	1,16448	0,14195	1,00000	0,99035	-0,20958	1,00000	1,12478	0,06252	1,00000
ENSMUSG00000055681	Cope	2,06960	0,04427	1,00000	1,93164	-0,10561	1,00000	2,08753	0,06150	1,00000
ENSMUSG00000002014	Ssr4	13,79120	-0,10637	1,00000	14,79534	0,04611	1,00000	14,89962	0,05966	1,00000
ENSMUSG00000041453	Rpl21	5,06560	-0,08564	1,00000	5,33708	0,02762	1,00000	5,41594	0,05811	1,00000
ENSMUSG00000003814	Calr	2,30965	0,02559	1,00000	2,19785	-0,08229	1,00000	2,34498	0,05734	1,00000
ENSMUSG00000064345	mt-Nd2	2,40421	-0,00702	1,00000	2,35939	-0,04801	1,00000	2,47621	0,05609	1,00000
ENSMUSG00000007815	Rhoa	1,05514	-0,09139	1,00000	1,11866	0,03548	1,00000	1,13000	0,05592	1,00000
ENSMUSG00000018293	Pfn1	1,69471	-0,16208	1,00000	1,91464	0,10278	1,00000	1,87644	0,05588	1,00000
ENSMUSG00000060586	H2-Eb1	1,05130	0,33096	1,00000	0,74565	-0,41386	0,77469	0,93206	0,05392	1,00000
ENSMUSG00000018567	Gabarap	2,00558	0,10291	1,00000	1,77782	-0,15905	1,00000	1,96320	0,05382	1,00000
ENSMUSG00000028343	Erp44	1,13335	0,07086	1,00000	1,03648	-0,12340	1,00000	1,12447	0,05190	1,00000
ENSMUSG00000003429	Rps11	5,52708	-0,09035	1,00000	5,86597	0,03888	1,00000	5,90337	0,05122	1,00000
ENSMUSG00000048758	Rpl29	3,32903	-0,28219	1,00000	4,19027	0,21724	1,00000	3,89622	0,05076	1,00000
ENSMUSG00000042747	Krtcap2	4,49341	0,04557	1,00000	4,21177	-0,09523	1,00000	4,50496	0,04976	1,00000
ENSMUSG00000040212	Emp3	1,64792	-0,04549	1,00000	1,68049	-0,00304	1,00000	1,72225	0,04937	1,00000
ENSMUSG00000021660	Btf3	1,49977	-0,05332	1,00000	1,54172	0,00656	1,00000	1,57171	0,04744	1,00000
ENSMUSG00000005873	Reep5	2,01602	-0,00795	1,00000	1,98998	-0,03631	1,00000	2,06676	0,04520	1,00000
ENSMUSG00000046364	Rpl27a	4,95745	-0,03801	1,00000	5,03130	-0,00594	1,00000	5,15191	0,04470	1,00000

ENSMUSG00000004460	Dnajb11	1,41211	-0,05620	1,00000	1,45758	0,01264	1,00000	1,47952	0,04412	1,00000
ENSMUSG00000061787	Rps17	1,20329	-0,13300	1,00000	1,33161	0,08729	1,00000	1,30654	0,04351	1,00000
ENSMUSG00000005881	Ergic3	1,02224	0,05534	1,00000	0,95233	-0,09883	1,00000	1,01735	0,04348	1,00000
ENSMUSG00000040592	Cd79b	2,29379	0,16276	1,00000	1,93086	-0,21185	1,00000	2,17523	0,04278	1,00000
ENSMUSG00000061904	Slc25a3	1,11336	0,01811	1,00000	1,07537	-0,05751	1,00000	1,12520	0,04017	1,00000
ENSMUSG00000004980	Hnrnpa2b1	1,44698	-0,06127	1,00000	1,50507	0,02435	1,00000	1,51470	0,03718	1,00000
ENSMUSG00000057841	Rpl32	7,32837	-0,16843	1,00000	8,39296	0,12668	1,00000	8,06732	0,03698	1,00000
ENSMUSG00000054452	Aes	1,16625	0,01311	1,00000	1,13391	-0,04819	1,00000	1,17896	0,03585	1,00000
ENSMUSG00000037563	Rps16	6,97849	0,01281	1,00000	6,79524	-0,04522	1,00000	7,04610	0,03298	1,00000
ENSMUSG00000034708	Grn	1,82601	0,01347	1,00000	1,77851	-0,04401	1,00000	1,84157	0,03117	1,00000
ENSMUSG00000047675	Rps8	10,94423	-0,11902	1,00000	12,02571	0,08618	1,00000	11,73449	0,03067	1,00000
ENSMUSG00000079435	Rpl36a	1,87555	-0,15073	1,00000	2,12035	0,11639	1,00000	2,04118	0,03049	1,00000
ENSMUSG00000025508	Rplp2	2,81456	-0,18639	1,00000	3,28514	0,15023	1,00000	3,11449	0,02976	1,00000
ENSMUSG00000022136	Dnajc3	3,47275	0,04284	1,00000	3,29394	-0,07240	1,00000	3,45337	0,02965	1,00000
ENSMUSG00000022174	Dad1	2,56112	-0,01146	1,00000	2,55445	-0,01721	1,00000	2,61036	0,02931	1,00000
ENSMUSG00000015092	Edf1	1,38788	0,02064	1,00000	1,34470	-0,04828	1,00000	1,39319	0,02819	1,00000
ENSMUSG00000001542	Ell2	1,67648	0,03358	1,00000	1,60524	-0,06112	1,00000	1,67276	0,02787	1,00000
ENSMUSG00000003072	Atp5d	1,24968	-0,04679	1,00000	1,29027	0,02285	1,00000	1,29150	0,02414	1,00000
ENSMUSG00000001175	Calm1	2,39071	-0,06097	1,00000	2,50089	0,03722	1,00000	2,48633	0,02353	1,00000
ENSMUSG00000052146	Rps10	8,35780	-0,10888	1,00000	9,12951	0,08362	1,00000	8,88768	0,02325	1,00000
ENSMUSG00000058569	Tmed9	1,12222	-0,02650	1,00000	1,13801	0,00392	1,00000	1,14837	0,02307	1,00000
ENSMUSG00000067149	Jchain	497,36808	-0,01491	1,00000	499,11561	-0,00731	1,00000	506,13852	0,02260	1,00000
ENSMUSG00000024425	Ndfip1	1,39152	0,00481	1,00000	1,37207	-0,02594	1,00000	1,40269	0,02167	1,00000
ENSMUSG00000059070	Rpl18	5,56569	-0,10887	1,00000	6,08390	0,08521	1,00000	5,91391	0,02159	1,00000
ENSMUSG00000062647	Rpl7a	3,63655	-0,01662	1,00000	3,65777	-0,00398	1,00000	3,70079	0,02098	1,00000
ENSMUSG00000025393	Atp5b	1,40117	0,05909	1,00000	1,31460	-0,08005	1,00000	1,37732	0,02065	1,00000
ENSMUSG00000056629	Fkbp2	3,56839	0,11587	1,00000	3,17579	-0,13838	1,00000	3,41798	0,01978	1,00000
ENSMUSG00000024014	Pim1	1,89988	0,11143	1,00000	1,69818	-0,13340	1,00000	1,82320	0,01954	1,00000
ENSMUSG00000035227	Spcs2	4,20008	0,01890	1,00000	4,09313	-0,03741	1,00000	4,20096	0,01880	1,00000
ENSMUSG00000063457	Rps15	1,53031	-0,10570	1,00000	1,67071	0,08575	1,00000	1,62088	0,01796	1,00000
ENSMUSG00000032026	Rexo2	2,91149	0,01088	1,00000	2,86052	-0,02769	1,00000	2,92052	0,01715	1,00000
ENSMUSG00000090862	Rps13	4,55468	-0,04494	1,00000	4,71095	0,02864	1,00000	4,68547	0,01614	1,00000
ENSMUSG00000030104	Edem1	7,41908	0,15735	1,00000	6,36095	-0,17837	1,00000	6,96332	0,01564	1,00000
ENSMUSG00000030824	Nucb1	1,57188	-0,06097	1,00000	1,65049	0,04550	1,00000	1,62818	0,01497	1,00000
ENSMUSG00000001289	Pfdn5	2,05768	0,05529	1,00000	1,94317	-0,06971	1,00000	2,01988	0,01409	1,00000
ENSMUSG00000073421	H2-Ab1	1,46806	0,20428	1,00000	1,20456	-0,22726	1,00000	1,34861	0,01376	1,00000
ENSMUSG00000025381	Cnpy2	1,15817	0,16030	1,00000	0,99172	-0,17831	1,00000	1,08406	0,01262	1,00000

ENSMUSG00000034868	Myl12b	1,62832	-0,06600	1,00000	1,72173	0,05579	1,00000	1,68612	0,00934	1,00000
ENSMUSG00000036781	Rps27l	1,41792	0,06168	1,00000	1,33737	-0,06613	1,00000	1,38118	0,00384	1,00000
ENSMUSG00000060938	Rpl26	5,37598	-0,02896	1,00000	5,51094	0,02521	1,00000	5,45686	0,00341	1,00000
ENSMUSG00000045128	Rpl18a	7,29773	-0,05233	1,00000	7,64243	0,04853	1,00000	7,48635	0,00294	1,00000
ENSMUSG00000027248	Pdia3	2,35447	0,02292	1,00000	2,30290	-0,02548	1,00000	2,33266	0,00248	1,00000
ENSMUSG00000029076	Sdf4	1,76405	0,07397	1,00000	1,64599	-0,07743	1,00000	1,70774	0,00246	1,00000
ENSMUSG00000020577	Tspan13	2,40313	0,13089	1,00000	2,12690	-0,13596	1,00000	2,26699	0,00156	1,00000
ENSMUSG00000020484	Xbp1	14,97134	0,02618	1,00000	14,65022	-0,02124	1,00000	14,75618	-0,00534	1,00000
ENSMUSG00000049517	Rps23	3,84193	-0,04194	1,00000	4,00029	0,04643	1,00000	3,90687	-0,00542	1,00000
ENSMUSG00000051695	Pcbp1	1,26623	0,08673	1,00000	1,17261	-0,08131	1,00000	1,21341	-0,00692	1,00000
ENSMUSG00000022354	Ndufb9	1,40177	0,00467	1,00000	1,40138	0,00409	1,00000	1,39277	-0,00912	1,00000
ENSMUSG00000039218	Srrm2	1,70170	0,03595	1,00000	1,65713	-0,02215	1,00000	1,66264	-0,01444	1,00000
ENSMUSG00000071415	Rpl23	7,42864	-0,11598	1,00000	8,30510	0,12829	1,00000	7,77783	-0,01681	1,00000
ENSMUSG00000018770	Atp5g3	1,99445	-0,06688	1,00000	2,13657	0,08397	1,00000	2,03826	-0,01940	1,00000
ENSMUSG00000058600	Rpl30	4,01233	-0,14625	1,00000	4,61401	0,15984	1,00000	4,25462	-0,02018	1,00000
ENSMUSG00000009013	Dynll1	0,99909	-0,00294	1,00000	1,01117	0,02342	1,00000	0,99042	-0,02130	1,00000
ENSMUSG00000048076	Arf1	1,25904	0,06194	1,00000	1,20193	-0,03978	1,00000	1,21059	-0,02339	1,00000
ENSMUSG00000022890	Atp5j	1,08193	-0,07126	1,00000	1,16587	0,09255	1,00000	1,10557	-0,02394	1,00000
ENSMUSG00000031059	Ndufb11	1,19255	-0,01681	1,00000	1,22362	0,03962	1,00000	1,18825	-0,02397	1,00000
ENSMUSG00000075701	Vimp	1,69096	0,01650	1,00000	1,68547	0,00942	1,00000	1,65700	-0,02695	1,00000
ENSMUSG00000031818	Cox4i1	2,72404	-0,09395	1,00000	3,00076	0,11827	1,00000	2,80735	-0,02834	1,00000
ENSMUSG00000026353	Ubxn4	1,39832	0,03540	1,00000	1,37109	-0,00768	1,00000	1,35727	-0,02883	1,00000
ENSMUSG00000009927	Rps25	1,80769	-0,01693	1,00000	1,86030	0,04608	1,00000	1,79553	-0,03069	1,00000
ENSMUSG00000026511	Srp9	2,01907	-0,06881	1,00000	2,17723	0,09669	1,00000	2,05402	-0,03092	1,00000
ENSMUSG00000027620	Rbm39	1,05160	-0,13769	1,00000	1,20583	0,16254	1,00000	1,10463	-0,03151	1,00000
ENSMUSG00000042682	Selk	4,37777	0,00731	1,00000	4,41182	0,02437	1,00000	4,29533	-0,03311	1,00000
ENSMUSG00000006699	Cdc42	1,10144	-0,17270	1,00000	1,30512	0,19964	1,00000	1,17353	-0,03661	1,00000
ENSMUSG00000045679	Pqlc3	1,10863	0,13258	1,00000	0,99729	-0,09967	1,00000	1,02623	-0,03680	1,00000
ENSMUSG00000030057	Cnbp	1,51406	-0,04502	1,00000	1,60495	0,08310	1,00000	1,51617	-0,04085	1,00000
ENSMUSG00000009092	Derl3	1,74593	-0,02478	1,00000	1,81760	0,06366	1,00000	1,73185	-0,04114	1,00000
ENSMUSG00000098274	Rpl24	4,01155	-0,09270	1,00000	4,44739	0,13403	1,00000	4,09625	-0,04653	1,00000
ENSMUSG00000031812	Map1lc3b	1,45574	0,05871	1,00000	1,40862	-0,01354	1,00000	1,38598	-0,04723	1,00000
ENSMUSG00000062997	Rpl35	2,23508	-0,47357	1,00000	3,43710	0,46870	0,53062	2,74701	-0,04730	1,00000
ENSMUSG00000021248	Tmed10	3,31052	0,03566	1,00000	3,27283	0,01058	1,00000	3,18339	-0,04828	1,00000
ENSMUSG00000021967	Mrpl57	1,08972	-0,04125	1,00000	1,15580	0,08829	1,00000	1,08448	-0,05021	1,00000
ENSMUSG00000028234	Rps20	9,77207	-0,06559	1,00000	10,59901	0,11314	1,00000	9,82788	-0,05187	1,00000
ENSMUSG00000022838	Eaf2	1,05997	-0,04481	1,00000	1,12892	0,09390	1,00000	1,05546	-0,05251	1,00000

ENSMUSG00000003380	Rabac1	3,12750	0,07578	1,00000	2,98639	-0,02566	1,00000	2,94704	-0,05275	1,00000
ENSMUSG00000019505	Ubb	9,61309	-0,02033	1,00000	10,03797	0,07496	1,00000	9,44057	-0,05786	1,00000
ENSMUSG00000014769	Psmb1	1,20998	0,00612	1,00000	1,23408	0,04962	1,00000	1,17364	-0,05836	1,00000
ENSMUSG00000007892	Rplp1	6,27185	-0,03970	1,00000	6,66693	0,09487	1,00000	6,21113	-0,05901	1,00000
ENSMUSG00000053317	Sec61b	3,32224	-0,05118	1,00000	3,56835	0,10622	1,00000	3,30721	-0,05927	1,00000
ENSMUSG00000008683	Rps15a	4,70046	-0,16296	1,00000	5,57895	0,21419	1,00000	4,92412	-0,06237	1,00000
ENSMUSG00000073702	Rpl31	0,91921	-0,11030	1,00000	1,04254	0,16697	1,00000	0,93853	-0,06401	1,00000
ENSMUSG00000022205	Sub1	2,53827	-0,00291	1,00000	2,61777	0,06515	1,00000	2,46378	-0,06552	1,00000
ENSMUSG00000022956	Atp5o	1,06026	0,00574	1,00000	1,08514	0,05696	1,00000	1,02498	-0,06573	1,00000
ENSMUSG00000035242	Oaz1	2,29694	-0,06942	1,00000	2,51653	0,13183	1,00000	2,29643	-0,06799	1,00000
ENSMUSG00000022255	Mtdh	3,31258	0,05077	1,00000	3,25856	0,01467	1,00000	3,13318	-0,06852	1,00000
ENSMUSG00000016319	Slc25a5	1,06332	0,03240	1,00000	1,06452	0,03504	1,00000	1,01318	-0,07050	1,00000
ENSMUSG00000040952	Rps19	3,81159	-0,20854	1,00000	4,72747	0,26588	1,00000	4,05887	-0,07367	1,00000
ENSMUSG00000024991	Eif3a	1,02687	0,02560	1,00000	1,03589	0,04503	1,00000	0,97988	-0,07393	1,00000
ENSMUSG00000006304	Arpc2	0,92295	-0,07291	1,00000	1,01762	0,14250	1,00000	0,92079	-0,07581	1,00000
ENSMUSG00000028936	Rpl22	2,42765	-0,06902	1,00000	2,66751	0,13887	1,00000	2,41733	-0,07603	1,00000
ENSMUSG00000036835	Psenen	1,02707	0,01130	1,00000	1,05065	0,06150	1,00000	0,98530	-0,07642	1,00000
ENSMUSG00000020267	Hint1	0,98826	0,01087	1,00000	1,01175	0,06283	1,00000	0,94783	-0,07737	1,00000
ENSMUSG00000061477	Rps7	2,86115	-0,04860	1,00000	3,08979	0,12110	1,00000	2,81946	-0,07797	1,00000
ENSMUSG00000027447	Cst3	5,23768	0,02151	1,00000	5,31578	0,05432	1,00000	4,99354	-0,07969	1,00000
ENSMUSG00000036372	Tmem258	1,40108	-0,08781	1,00000	1,56791	0,16048	1,00000	1,40467	-0,08008	1,00000
ENSMUSG00000015656	Hspa8	2,43110	0,07805	1,00000	2,34707	0,00058	1,00000	2,25593	-0,08268	1,00000
ENSMUSG00000019054	Fis1	1,06716	0,04343	1,00000	1,06364	0,03631	1,00000	1,00545	-0,08354	1,00000
ENSMUSG00000070544	Top1	2,34787	-0,01909	1,00000	2,47597	0,09834	1,00000	2,27555	-0,08416	1,00000
ENSMUSG00000020964	Sel1l	2,24966	0,02600	1,00000	2,27964	0,05540	1,00000	2,13441	-0,08553	1,00000
ENSMUSG00000020077	Srgn	6,80158	0,38290	1,00000	4,92947	-0,32629	0,97297	5,52180	-0,08628	1,00000
ENSMUSG00000098178	Gm42418	52,17138	0,25678	1,00000	42,78607	-0,18078	1,00000	44,59968	-0,08976	1,00000
ENSMUSG00000028277	Ube2j1	1,69806	0,07741	1,00000	1,64892	0,01270	1,00000	1,56701	-0,09479	1,00000
ENSMUSG00000062006	Rpl34	4,57369	-0,17505	1,00000	5,55794	0,25556	1,00000	4,74215	-0,09587	1,00000
ENSMUSG00000022285	Ywhaz	1,38285	0,05967	1,00000	1,36571	0,03229	1,00000	1,28524	-0,09662	1,00000
ENSMUSG00000006057	Atp5g1	1,43703	0,01992	1,00000	1,47214	0,07347	1,00000	1,35873	-0,09843	1,00000
ENSMUSG00000060126	Tpt1	13,23792	0,02427	1,00000	13,50781	0,06909	1,00000	12,49158	-0,09848	1,00000
ENSMUSG00000003379	Cd79a	6,54893	0,04049	1,00000	6,58582	0,05311	1,00000	6,13409	-0,09855	1,00000
ENSMUSG00000028211	Trp53inp1	2,99502	0,28271	1,00000	2,40669	-0,20021	1,00000	2,52099	-0,09885	1,00000
ENSMUSG00000105361	AY036118	7,40697	0,06301	1,00000	7,30811	0,03350	1,00000	6,85725	-0,10162	1,00000
ENSMUSG00000040128	Pnrc1	1,10715	0,15762	1,00000	1,00227	-0,06226	1,00000	0,98217	-0,10251	1,00000
ENSMUSG00000028618	Tmem59	1,29371	0,11167	1,00000	1,22225	-0,01381	1,00000	1,17071	-0,10357	1,00000

ENSMUSG00000035202	Lars2	4,96563	0,27685	1,00000	4,03841	-0,18027	1,00000	4,16254	-0,11258	1,00000
ENSMUSG00000022587	Ly6e	12,08832	-0,01172	1,00000	12,81982	0,11862	1,00000	11,50962	-0,11402	1,00000
ENSMUSG00000084786	Ubl5	1,24426	-0,09514	1,00000	1,42191	0,20061	1,00000	1,22991	-0,11661	1,00000
ENSMUSG00000034566	Atp5h	1,47022	-0,04458	1,00000	1,60778	0,15378	1,00000	1,41866	-0,11774	1,00000
ENSMUSG00000035530	Eif1	6,86305	-0,04610	1,00000	7,51968	0,15658	1,00000	6,62194	-0,11934	1,00000
ENSMUSG00000063882	Uqcrh	1,19068	-0,12758	1,00000	1,40363	0,23724	1,00000	1,19086	-0,12382	1,00000
ENSMUSG00000036199	Ndufa13	1,40945	-0,12896	1,00000	1,66554	0,24133	1,00000	1,40843	-0,12699	1,00000
ENSMUSG00000004207	Psap	1,94963	0,03758	1,00000	1,99106	0,08449	1,00000	1,80159	-0,12927	1,00000
ENSMUSG00000023944	Hsp90ab1	3,50476	0,01921	1,00000	3,64086	0,10400	1,00000	3,26326	-0,13091	1,00000
ENSMUSG00000068798	Rap1a	1,91702	-0,03081	1,00000	2,08546	0,15639	1,00000	1,82257	-0,13519	1,00000
ENSMUSG00000074884	Serf2	2,88952	-0,12721	1,00000	3,42274	0,24886	1,00000	2,87062	-0,13733	1,00000
ENSMUSG00000062397	Zfp706	1,92372	-0,00556	1,00000	2,05136	0,13741	1,00000	1,80263	-0,14109	1,00000
ENSMUSG00000079641	Rpl39	2,48784	-0,17967	1,00000	3,08783	0,30007	1,00000	2,52913	-0,14116	1,00000
ENSMUSG00000024121	Atp6v0c	1,44058	-0,23836	1,00000	1,88102	0,35369	0,87123	1,50531	-0,14260	1,00000
ENSMUSG00000093674	Rpl41	9,62797	-0,23780	1,00000	12,57453	0,35493	0,86856	10,04753	-0,14471	1,00000
ENSMUSG00000027422	Rrbp1	1,76041	0,06254	1,00000	1,77069	0,07577	1,00000	1,59488	-0,14671	1,00000
ENSMUSG00000046330	Rpl37a	3,13361	-0,23764	1,00000	4,10144	0,36027	0,86444	3,26002	-0,15094	1,00000
ENSMUSG00000092341	Malat1	49,13893	0,11860	1,00000	47,07505	0,02347	1,00000	43,31861	-0,15121	1,00000
ENSMUSG00000041841	Rpl37	2,89632	-0,30716	1,00000	4,02267	0,42213	0,67189	3,11418	-0,15269	1,00000
ENSMUSG00000003363	Pld3	1,18191	0,19104	1,00000	1,06091	-0,04887	1,00000	1,00796	-0,15356	1,00000
ENSMUSG00000014313	Cox6c	0,90749	-0,12057	1,00000	1,07557	0,25748	1,00000	0,89156	-0,15379	1,00000
ENSMUSG00000048163	Selplg	1,20427	0,07116	1,00000	1,20652	0,07561	1,00000	1,08198	-0,15584	1,00000
ENSMUSG00000038690	Atp5j2	1,24150	-0,13987	1,00000	1,49764	0,27748	1,00000	1,22928	-0,15645	1,00000
ENSMUSG00000078974	Sec61g	2,03641	-0,22275	1,00000	2,63976	0,35434	0,87123	2,09495	-0,15919	1,00000
ENSMUSG00000064356	mt-Atp8	1,10163	-0,94880	0,01792	2,57419	0,91305	0,00462	1,64823	-0,15946	1,00000
ENSMUSG00000067288	Rps28	1,60635	-0,32494	1,00000	2,27143	0,44470	0,60015	1,73509	-0,16123	1,00000
ENSMUSG00000038274	Fau	5,07673	-0,13210	1,00000	6,09983	0,27680	1,00000	4,99020	-0,16389	1,00000
ENSMUSG00000038803	Ost4	2,17529	-0,07031	1,00000	2,47763	0,21975	1,00000	2,07688	-0,16413	1,00000
ENSMUSG00000069744	Psmb3	0,96935	0,02007	1,00000	1,02064	0,13526	1,00000	0,88686	-0,16612	1,00000
ENSMUSG00000022108	Itm2b	7,89977	0,24295	1,00000	6,80472	-0,08912	1,00000	6,53675	-0,16925	1,00000
ENSMUSG00000041697	Cox6a1	1,28189	-0,09289	1,00000	1,49354	0,24789	1,00000	1,23210	-0,17203	1,00000
ENSMUSG00000057863	Rpl36	2,04370	-0,32147	1,00000	2,90196	0,45922	0,56096	2,18180	-0,18167	1,00000
ENSMUSG00000025290	Rps24	18,71960	-0,03584	1,00000	20,84279	0,20426	1,00000	17,42353	-0,18347	1,00000
ENSMUSG00000004285	Atp6v1f	0,98077	-0,06662	1,00000	1,12189	0,23365	1,00000	0,92590	-0,18365	1,00000
ENSMUSG00000053398	Phgdh	1,00382	-0,16163	1,00000	1,25010	0,32839	0,92232	0,98687	-0,19212	1,00000
ENSMUSG00000049751	Rpl36al	6,65383	-0,08345	1,00000	7,74748	0,25673	1,00000	6,30446	-0,19225	1,00000
ENSMUSG00000061518	Cox5b	1,16989	-0,09693	1,00000	1,37832	0,26951	1,00000	1,11538	-0,19241	1,00000

ENSMUSG00000026223	Itm2c	1,15039	0,02135	1,00000	1,22274	0,15799	1,00000	1,03813	-0,19298	1,00000
ENSMUSG00000020225	Tmbim4	1,68308	0,31302	1,00000	1,37324	-0,14091	1,00000	1,33504	-0,19435	1,00000
ENSMUSG00000036751	Cox6b1	0,90759	-0,05812	1,00000	1,03482	0,23530	1,00000	0,84886	-0,19460	1,00000
ENSMUSG00000000088	Cox5a	1,02037	0,07349	1,00000	1,03755	0,11121	1,00000	0,89719	-0,19773	1,00000
ENSMUSG00000033685	Ucp2	2,04970	0,03618	1,00000	2,15582	0,14943	1,00000	1,83124	-0,19964	1,00000
ENSMUSG00000017707	Serinc3	5,31580	0,10130	1,00000	5,27776	0,08564	1,00000	4,61051	-0,20023	1,00000
ENSMUSG00000005447	Pafah1b3	1,55198	0,06313	1,00000	1,59683	0,12728	1,00000	1,36656	-0,20435	1,00000
ENSMUSG00000039195	11100008P14Rik	1,24347	0,17267	1,00000	1,15990	0,01743	1,00000	1,04262	-0,20465	1,00000
ENSMUSG00000051998	Lax1	1,82325	0,17953	1,00000	1,69026	0,01050	1,00000	1,52400	-0,20486	1,00000
ENSMUSG00000015837	Sqstm1	2,96566	0,27592	1,00000	2,51692	-0,09059	1,00000	2,37713	-0,20521	1,00000
ENSMUSG00000039001	Rps21	2,34964	-0,26116	1,00000	3,19934	0,42853	0,65058	2,40615	-0,20641	1,00000
ENSMUSG00000038717	Atp5l	0,95831	-0,07082	1,00000	1,10987	0,25796	1,00000	0,89615	-0,20695	1,00000
ENSMUSG00000020496	Rnf187	1,22121	0,00687	1,00000	1,32203	0,18476	1,00000	1,10150	-0,20743	1,00000
ENSMUSG00000057322	Rpl38	1,56301	-0,42190	1,00000	2,43463	0,56638	0,27757	1,72997	-0,20967	1,00000
ENSMUSG00000014294	Ndufa2	0,88739	-0,15110	1,00000	1,10225	0,33412	0,90263	0,86055	-0,20977	1,00000
ENSMUSG00000044894	Uqcrq	1,51839	-0,07621	1,00000	1,77167	0,26953	1,00000	1,41814	-0,21457	1,00000
ENSMUSG00000035885	Cox8a	2,00696	0,00680	1,00000	2,18250	0,19510	1,00000	1,79981	-0,21918	1,00000
ENSMUSG00000021242	Npc2	1,37970	-0,06725	1,00000	1,60143	0,26703	1,00000	1,27897	-0,22130	1,00000
ENSMUSG00000016427	Ndufa1	1,59887	-0,17425	1,00000	2,03670	0,36820	0,83764	1,55563	-0,22539	1,00000
ENSMUSG00000029759	Pon3	1,11759	0,07078	1,00000	1,15169	0,13862	1,00000	0,97048	-0,22554	1,00000
ENSMUSG00000021282	Eif5	1,25579	-0,05156	1,00000	1,44282	0,26024	1,00000	1,15046	-0,23040	1,00000
ENSMUSG00000009549	Srp14	0,93192	0,00413	1,00000	1,02201	0,21170	1,00000	0,83017	-0,23513	1,00000
ENSMUSG00000028648	Ndufs5	0,95979	-0,07257	1,00000	1,12531	0,28484	1,00000	0,88540	-0,23615	1,00000
ENSMUSG00000002379	Ndufa11	0,89449	-0,06204	1,00000	1,03999	0,27667	1,00000	0,82036	-0,23802	1,00000
ENSMUSG00000023272	Creld2	3,37651	0,19060	1,00000	3,14305	0,03017	1,00000	2,76142	-0,23921	1,00000
ENSMUSG00000090733	Rps27	1,84472	-0,32383	1,00000	2,67748	0,51092	0,41299	1,91517	-0,24063	1,00000
ENSMUSG00000067212	H2-T23	3,81021	0,39880	1,00000	2,93157	-0,18900	1,00000	2,84536	-0,24383	1,00000
ENSMUSG00000008348	Ubc	3,80923	0,12614	1,00000	3,76497	0,10036	1,00000	3,19665	-0,24455	1,00000
ENSMUSG00000024308	Tapbp	2,52576	0,32110	1,00000	2,09670	-0,09680	1,00000	1,94159	-0,25093	1,00000
ENSMUSG00000060636	Rpl35a	5,03458	-0,10666	1,00000	6,11341	0,33014	0,91213	4,68066	-0,25261	1,00000
ENSMUSG00000016252	Atp5e	1,55336	-0,13919	1,00000	1,94200	0,36318	0,85799	1,46355	-0,25662	1,00000
ENSMUSG00000034892	Rps29	1,87024	-0,50297	1,00000	3,16142	0,67218	0,10375	2,10434	-0,25916	1,00000
ENSMUSG00000068523	Gng5	1,65068	0,00849	1,00000	1,82356	0,23327	1,00000	1,44591	-0,26502	1,00000
ENSMUSG00000046718	Bst2	2,53453	0,03056	1,00000	2,74843	0,21356	1,00000	2,19600	-0,26673	1,00000
ENSMUSG00000056888	Glipr1	2,77467	0,37481	1,00000	2,21133	-0,13568	1,00000	2,06331	-0,27231	1,00000
ENSMUSG00000055839	Tceb2	1,13118	-0,11839	1,00000	1,39796	0,35901	0,86856	1,04627	-0,27399	1,00000

ENSMUSG00000032294	Pkm	1,15098	-0,13567	1,00000	1,44438	0,37626	0,81859	1,07227	-0,27591	1,00000
ENSMUSG00000030695	Aldoa	2,13599	0,10400	1,00000	2,18915	0,16006	1,00000	1,77184	-0,28760	1,00000
ENSMUSG00000024038	Ndufv3	0,90463	-0,12726	1,00000	1,13205	0,37906	0,81141	0,83424	-0,28829	1,00000
ENSMUSG00000063316	Rpl27	0,99722	-0,61411	0,56038	1,86705	0,79308	0,02466	1,16247	-0,30271	1,00000
ENSMUSG00000060032	H2afj	1,04588	0,01839	1,00000	1,16645	0,26597	1,00000	0,88968	-0,31450	1,00000
ENSMUSG00000027566	Psma7	1,27746	0,20794	1,00000	1,21522	0,09526	1,00000	0,98927	-0,33310	0,97220
ENSMUSG00000028484	Psip1	1,17275	0,18786	1,00000	1,13645	0,11716	1,00000	0,91536	-0,33487	0,96433
ENSMUSG00000032330	Cox7a2	1,20279	0,02997	1,00000	1,34578	0,28593	1,00000	0,99888	-0,35162	0,90927
ENSMUSG00000003746	Man1a	1,67974	0,41676	1,00000	1,33952	-0,09731	1,00000	1,17144	-0,36526	0,86708
ENSMUSG00000045394	Epcam	1,28514	-0,03552	1,00000	1,53038	0,36276	0,86493	1,08939	-0,37037	0,85488
ENSMUSG00000060802	B2m	29,76726	0,15661	1,00000	30,08420	0,18148	1,00000	23,09284	-0,37405	0,82810
ENSMUSG00000020585	Laptm4a	2,94399	0,10434	1,00000	3,12428	0,24066	1,00000	2,32639	-0,38340	0,79872
ENSMUSG00000020423	Btg2	1,12813	-0,10343	1,00000	1,44976	0,47156	0,52745	0,95795	-0,42885	0,68886
ENSMUSG00000037894	H2afz	1,33971	-0,01043	1,00000	1,60846	0,41054	0,70104	1,07268	-0,45910	0,57118
ENSMUSG00000096727	Psmb9	1,26840	0,35420	1,00000	1,11319	0,05490	1,00000	0,86305	-0,46399	0,55803
ENSMUSG00000028691	Prdx1	1,48106	-0,05435	1,00000	1,85024	0,45827	0,56557	1,20423	-0,46861	0,54135
ENSMUSG00000049422	Chchd10	2,45208	0,14254	1,00000	2,60341	0,28145	1,00000	1,81318	-0,47953	0,50619
ENSMUSG00000030220	Arhgdib	1,50125	0,25372	1,00000	1,44810	0,17158	1,00000	1,05723	-0,47998	0,50619
ENSMUSG00000039910	Cited2	3,11430	0,27055	1,00000	2,96431	0,15769	1,00000	2,17293	-0,48399	0,49767
ENSMUSG00000024338	Psmb8	3,05136	0,25549	1,00000	2,95649	0,18354	1,00000	2,12825	-0,49712	0,45674
ENSMUSG00000024661	Fth1	5,48138	0,02994	1,00000	6,45643	0,40899	0,70104	4,20649	-0,50537	0,44416
ENSMUSG00000050708	Ftl1	11,11700	-0,07840	1,00000	14,43484	0,52672	0,36929	8,85573	-0,52955	0,37924
ENSMUSG00000061232	H2-K1	16,07396	0,38220	1,00000	14,13208	0,08485	1,00000	10,41000	-0,53657	0,36528
ENSMUSG00000073411	H2-D1	8,07776	0,34022	1,00000	7,37994	0,13176	1,00000	5,31290	-0,54056	0,35562
ENSMUSG00000010095	Slc3a2	9,83137	0,08991	1,00000	11,25377	0,40535	0,71688	7,08243	-0,57436	0,27737
ENSMUSG00000079197	Psme2	1,60970	0,19258	1,00000	1,69876	0,31923	0,94678	1,09847	-0,59080	0,23865
ENSMUSG00000075602	Ly6a	19,66215	-0,08749	1,00000	27,10524	0,66997	0,10743	14,25131	-0,71539	0,07573
ENSMUSG00000028367	Txn1	2,29280	0,04857	1,00000	2,86541	0,57790	0,25262	1,52400	-0,75965	0,04622
ENSMUSG00000060550	H2-Q7	1,51209	0,46083	1,00000	1,37617	0,23733	1,00000	0,80209	-0,84804	0,01643
ENSMUSG00000017002	Slpi	66,00651	-0,04979	1,00000	91,41144	0,72881	0,05819	43,65436	-0,85140	0,01648
ENSMUSG00000031765	Mt1	2,33999	-0,06024	1,00000	3,40573	0,85151	0,01432	1,40770	-1,03021	0,00156
ENSMUSG00000022584	Ly6c2	13,64867	0,27880	1,00000	15,20051	0,54260	0,34421	7,02930	-1,03745	0,00137
ENSMUSG00000026104	Stat1	4,74242	0,76859	0,08245	4,65251	0,72109	0,06141	0,78716	-2,57701	0,00000
ENSMUSG00000066363	Serpina3f	2,16485	1,28880	0,00002	1,60573	0,45575	0,59416	0,11713	-4,00654	0,00000

Table 2: FeatureID table for single cell sequencing of ST2+A+SCR, ST2+A+IRF4. Displayed are Feature ID, Feature name, Log2FolgChange, p-value and Average expression for the samples ST2+A+IRF4 (IRF4) and ST2+A+SCR (ST2_AScr).

FeatureID	FeatureName	IRF4 Average	IRF4 Log2 Fold Change	IRF4 P-Value	ST2_AScr Average	ST2_AScr Log2 Fold Change	ST2_AScr P-Value
ENSMUSG00000044052	Ccr10	0,540692	-1,34292	7,00E-07	1,371686	1,342923	7,00E-07
ENSMUSG00000022496	Tnfrsf17	0,484432	-1,18491	1,87E-05	1,101467	1,184911	1,87E-05
ENSMUSG00000022584	Ly6c2	3,431863	-1,00695	0,000901	6,897048	1,00695	0,000901
ENSMUSG00000030104	Edem1	3,506	-0,96251	0,001181	6,83231	0,962512	0,001181
ENSMUSG00000020592	Sdc1	1,253407	-0,95073	0,001486	2,422756	0,950732	0,001486
ENSMUSG00000022838	Eaf2	0,539553	-0,94052	0,001848	1,035602	0,94052	0,001848
ENSMUSG00000021610	Clptm1l	0,75732	-0,88495	0,004166	1,398626	0,884953	0,004166
ENSMUSG00000001576	Ergic1	0,901388	-0,86012	0,005808	1,636272	0,860122	0,005808
ENSMUSG00000023367	Tmem176a	2,6543	-0,83309	0,008308	4,728737	0,833086	0,008308
ENSMUSG00000021939	Ctsb	1,71672	-0,80608	0,012523	3,001709	0,806084	0,012523
ENSMUSG00000079017	Ifi27l2a	0,874835	-0,79079	0,017636	1,513556	0,790792	0,017636
ENSMUSG00000046718	Bst2	1,28811	-0,74217	0,03004	2,154687	0,742172	0,03004
ENSMUSG00000021356	Irf4	0,947658	-0,74166	0,030649	1,584645	0,741661	0,030649
ENSMUSG00000029810	Tmem176b	4,239922	-0,7342	0,031933	7,053157	0,734201	0,031933
ENSMUSG00000019188	H13	2,230772	-0,72646	0,035396	3,691086	0,726464	0,035396
ENSMUSG00000010095	Slc3a2	4,201188	-0,72602	0,036545	6,949187	0,726017	0,036545
ENSMUSG00000025544	Tm9sf2	0,632444	-0,72473	0,037025	1,045231	0,724733	0,037025
ENSMUSG00000038991	Txndc5	7,303115	-0,71504	0,040189	11,9885	0,715041	0,040189
ENSMUSG00000041355	Ssr2	0,748644	-0,7141	0,041758	1,228177	0,714096	0,041758
ENSMUSG00000001542	Ell2	1,005583	-0,70674	0,044922	1,641291	0,706742	0,044922
ENSMUSG00000032026	Rexo2	1,766671	-0,69775	0,048666	2,865575	0,69775	0,048666
ENSMUSG00000038312	Edem2	2,985288	-0,68215	0,057629	4,790095	0,682151	0,057629
ENSMUSG00000032116	Stt3a	0,666708	-0,67995	0,060595	1,068176	0,67995	0,060595
ENSMUSG00000041891	Lman1	1,171383	-0,67535	0,062749	1,870742	0,675347	0,062749
ENSMUSG00000034708	Grn	1,136155	-0,66933	0,06792	1,806926	0,669327	0,06792
ENSMUSG00000030082	Sec61a1	1,182951	-0,65309	0,077562	1,860294	0,653092	0,077562
ENSMUSG00000046841	Ckap4	1,01049	-0,64868	0,082191	1,584236	0,648679	0,082191
ENSMUSG00000025289	Prdx4	1,681404	-0,63049	0,100171	2,603038	0,63049	0,100171
ENSMUSG00000034088	Hdlbp	1,594035	-0,62404	0,106734	2,456764	0,624036	0,106734
ENSMUSG00000105650	Gm42889	28,83923	-0,59587	0,3615	43,58788	0,595872	0,3615
ENSMUSG00000040462	Os9	1,005232	-0,58792	0,149325	1,510996	0,587922	0,149325
ENSMUSG00000028452	Vcp	1,31142	-0,58221	0,155002	1,963444	0,582214	0,155002
ENSMUSG00000032115	Hyou1	0,679853	-0,58207	0,158614	1,017779	0,582068	0,158614

ENSMUSG00000028211	Trp53inp1	1,653011	-0,58146	0,157578	2,473563	0,581456	0,157578
ENSMUSG00000056629	Fkbp2	2,247598	-0,57732	0,160915	3,353671	0,577324	0,160915
ENSMUSG00000060802	B2m	15,23359	-0,57277	0,167156	22,65838	0,572766	0,167156
ENSMUSG00000025935	Tram1	1,31659	-0,56945	0,173205	1,953815	0,569446	0,173205
ENSMUSG00000024516	Sec11c	5,927898	-0,56684	0,174956	8,781004	0,566839	0,174956
ENSMUSG00000028343	Erp44	0,74733	-0,56197	0,185605	1,103311	0,561966	0,185605
ENSMUSG00000040212	Emp3	1,151315	-0,55356	0,194923	1,689844	0,553564	0,194923
ENSMUSG00000054408	Spcs3	1,380737	-0,53298	0,23044	1,997862	0,532979	0,23044
ENSMUSG00000030062	Rpn1	1,201441	-0,51894	0,259513	1,721599	0,518942	0,259513
ENSMUSG00000044468	Fam46c	1,990484	-0,51652	0,262141	2,847445	0,516516	0,262141
ENSMUSG00000002778	Kdelr1	0,89797	-0,50605	0,287038	1,275296	0,506047	0,287038
ENSMUSG00000020484	Xbp1	10,30155	-0,49103	0,312141	14,47856	0,491033	0,312141
ENSMUSG00000020496	Rnf187	0,772831	-0,48379	0,334438	1,080775	0,483794	0,334438
ENSMUSG00000035227	Spcs2	2,976174	-0,46983	0,357205	4,121921	0,46983	0,357205
ENSMUSG00000024425	Ndfip1	0,99866	-0,46268	0,374031	1,376296	0,462685	0,374031
ENSMUSG00000061232	H2-K1	7,425975	-0,45989	0,379226	10,21415	0,459893	0,379226
ENSMUSG00000068798	Rap1a	1,303445	-0,45621	0,388522	1,788283	0,456209	0,388522
ENSMUSG00000028757	Ddost	1,699281	-0,4561	0,388199	2,33118	0,456104	0,388199
ENSMUSG00000020585	Laptm4a	1,666068	-0,45421	0,391745	2,282627	0,454215	0,391745
ENSMUSG00000045394	Epcam	0,78545	-0,44448	0,427571	1,068893	0,444482	0,427571
ENSMUSG00000023175	Bsg	1,330173	-0,44099	0,424568	1,805799	0,440989	0,424568
ENSMUSG00000002014	Ssr4	10,8099	-0,4355	0,434134	14,6193	0,435498	0,434134
ENSMUSG00000041084	Ostc	0,763454	-0,42634	0,457523	1,025973	0,426337	0,457523
ENSMUSG00000025130	P4hb	1,627948	-0,42252	0,467573	2,181935	0,422522	0,467573
ENSMUSG00000020571	Pdia6	2,22753	-0,41372	0,488631	2,967394	0,413724	0,488631
ENSMUSG00000020048	Hsp90b1	13,17282	-0,40731	0,505501	17,47023	0,407311	0,505501
ENSMUSG00000024308	Tapbp	1,43691	-0,40683	0,506355	1,905057	0,406833	0,506355
ENSMUSG00000014867	Surf4	0,839694	-0,40374	0,515307	1,110891	0,403742	0,515307
ENSMUSG00000026353	Ubxn4	1,009526	-0,3996	0,528681	1,331737	0,399596	0,528681
ENSMUSG00000075701	Vimp	1,237108	-0,39417	0,542612	1,625823	0,394167	0,542612
ENSMUSG00000025823	Pdia4	3,931192	-0,38882	0,555157	5,14728	0,388818	0,555157
ENSMUSG00000004460	Dnajb11	1,11819	-0,37653	0,588785	1,451687	0,376532	0,588785
ENSMUSG00000023010	Tmbim6	1,747742	-0,3753	0,588917	2,267057	0,3753	0,588917
ENSMUSG00000055681	Cope	1,580802	-0,37371	0,594745	2,048259	0,373712	0,594745
ENSMUSG00000022498	Txndc11	1,411847	-0,36879	0,608404	1,82311	0,368789	0,608404
ENSMUSG00000021248	Tmed10	2,424089	-0,3657	0,615408	3,123503	0,365696	0,615408
ENSMUSG00000067212	H2-T23	2,170481	-0,36317	0,623784	2,791823	0,363166	0,623784

ENSMUSG0 0000070544	Top1	1,737226	-0,362	0,627802	2,232742	0,362003	0,627802
ENSMUSG0 0000003746	Man1a	0,895341	-0,36034	0,632777	1,149406	0,360345	0,632777
ENSMUSG0 0000024121	Atp6v0c	1,15079	-0,36	0,632282	1,476988	0,360003	0,632282
ENSMUSG0 0000024338	Psmb8	1,629438	-0,35786	0,632777	2,088208	0,357863	0,632777
ENSMUSG0 0000021917	Spcs1	4,686935	-0,35495	0,63559	5,994405	0,354948	0,63559
ENSMUSG0 0000042682	Selk	3,303131	-0,35151	0,644717	4,214521	0,35151	0,644717
ENSMUSG0 0000022136	Dnajc3	2,662274	-0,34792	0,649441	3,388396	0,347918	0,649441
ENSMUSG0 0000020225	Tmbim4	1,0338	-0,34149	0,669011	1,309919	0,341489	0,669011
ENSMUSG0 0000056888	Glipr1	1,597803	-0,34144	0,670723	2,024494	0,341445	0,670723
ENSMUSG0 0000074227	Spint2	1,148161	-0,33164	0,695397	1,444926	0,331642	0,695397
ENSMUSG0 0000030824	Nucb1	1,272949	-0,32766	0,703387	1,597552	0,32766	0,703387
ENSMUSG0 0000023272	Creld2	2,168465	-0,32131	0,710321	2,709467	0,321309	0,710321
ENSMUSG0 0000003380	Rabac1	2,314987	-0,32084	0,708609	2,891593	0,320836	0,708609
ENSMUSG0 0000051695	Pcbp1	0,957823	-0,31381	0,726548	1,190584	0,313809	0,726548
ENSMUSG0 0000028618	Tmem59	0,925136	-0,31222	0,730294	1,148689	0,312221	0,730294
ENSMUSG0 0000027642	Rpn2	1,511572	-0,30769	0,744147	1,870947	0,307692	0,744147
ENSMUSG0 0000026511	Srp9	1,632155	-0,30425	0,753155	2,015378	0,304247	0,753155
ENSMUSG0 0000038393	Txnip	1,025125	-0,29834	0,766244	1,260648	0,298339	0,766244
ENSMUSG0 0000058569	Tmed9	0,916636	-0,29774	0,766244	1,126768	0,297742	0,766244
ENSMUSG0 0000027828	Ssr3	0,983762	-0,29719	0,767194	1,208817	0,297187	0,767194
ENSMUSG0 0000029076	Sdf4	1,366278	-0,29441	0,772075	1,675606	0,294407	0,772075
ENSMUSG0 0000068328	Aup1	1,087782	-0,29422	0,772566	1,333888	0,294222	0,772566
ENSMUSG0 0000021877	Arf4	1,194606	-0,29152	0,77664	1,462135	0,291516	0,77664
ENSMUSG0 0000032042	Srpr	1,834148	-0,2895	0,780881	2,241756	0,289495	0,780881
ENSMUSG0 0000041571	Sepw1	2,373788	-0,2872	0,787873	2,896715	0,287203	0,787873
ENSMUSG0 0000033487	Fndc3a	1,142727	-0,28504	0,795559	1,392378	0,285043	0,795559
ENSMUSG0 0000025381	Cnpy2	0,874309	-0,28281	0,795559	1,063669	0,282807	0,795559
ENSMUSG0 0000032383	Ppib	2,75201	-0,28188	0,795559	3,345886	0,281879	0,795559
ENSMUSG0 0000039195	1110008P14 Rik	0,841885	-0,28109	0,797964	1,023003	0,281088	0,797964
ENSMUSG0 0000092341	Malat1	35,03467	-0,27878	0,804015	42,50363	0,278781	0,804015
ENSMUSG0 0000079197	Psme2	0,890346	-0,27563	0,804015	1,077805	0,275632	0,804015
ENSMUSG0 0000031708	Tecr	1,047208	-0,27449	0,804015	1,266692	0,274492	0,804015
ENSMUSG0 0000062397	Zfp706	1,462498	-0,27425	0,804015	1,768718	0,274245	0,804015
ENSMUSG0 0000038179	Slamf7	2,258113	-0,27162	0,804015	2,725959	0,271623	0,804015
ENSMUSG0 0000026223	Itm2c	0,845829	-0,26812	0,807014	1,018598	0,268122	0,807014
ENSMUSG0 0000020964	Sel1l	1,743536	-0,2644	0,814583	2,094252	0,264396	0,814583
ENSMUSG0 0000020577	Tspan13	1,857808	-0,25975	0,82323	2,224342	0,259754	0,82323

ENSMUSG0 0000005873	Reep5	1,704539	-0,25056	0,838783	2,027875	0,250564	0,838783
ENSMUSG0 0000075602	Ly6a	11,80514	-0,24426	0,84671	13,98319	0,244256	0,84671
ENSMUSG0 0000105361	AY036118	5,698564	-0,23961	0,850861	6,728238	0,239609	0,850861
ENSMUSG0 0000022354	Ndufb9	1,158414	-0,23838	0,854085	1,366564	0,238381	0,854085
ENSMUSG0 0000022205	Sub1	2,050424	-0,23753	0,855736	2,417429	0,237529	0,855736
ENSMUSG0 0000018770	Atp5g3	1,696652	-0,23722	0,856823	1,99991	0,237223	0,856823
ENSMUSG0 0000022956	Atp5o	0,855906	-0,23264	0,866925	1,005691	0,232641	0,866925
ENSMUSG0 0000006057	Atp5g1	1,136418	-0,23035	0,871031	1,333171	0,230347	0,871031
ENSMUSG0 0000037072	Sep 15	1,54645	-0,22766	0,876148	1,810818	0,22766	0,876148
ENSMUSG0 0000029390	Tmed2	1,889969	-0,22566	0,880274	2,210001	0,225663	0,880274
ENSMUSG0 0000055447	Cd47	0,945116	-0,22366	0,887799	1,103618	0,223656	0,887799
ENSMUSG0 0000021127	Zfp36l1	1,301605	-0,21822	0,899701	1,514171	0,218216	0,899701
ENSMUSG0 0000032294	Pkm	0,905331	-0,21673	0,903072	1,052094	0,216726	0,903072
ENSMUSG0 0000010142	Tnfrsf13b	1,225803	-0,21519	0,906629	1,423005	0,215193	0,906629
ENSMUSG0 0000016559	H3f3b	4,352617	-0,21457	0,906858	5,050685	0,214575	0,906858
ENSMUSG0 0000098178	Gm42418	37,79299	-0,21149	0,914302	43,76059	0,211492	0,914302
ENSMUSG0 0000034868	Myl12b	1,432528	-0,20773	0,916903	1,654402	0,207727	0,916903
ENSMUSG0 0000035202	Lars2	3,54263	-0,20522	0,922123	4,084226	0,205221	0,922123
ENSMUSG0 0000003814	Calr	2,006696	-0,19733	0,930403	2,30086	0,197331	0,930403
ENSMUSG0 0000016756	Cmah	0,891573	-0,18516	0,930403	1,013681	0,185162	0,930403
ENSMUSG0 0000036908	Unc93b1	1,207488	-0,18425	0,930403	1,371993	0,184247	0,930403
ENSMUSG0 0000021484	Lman2	1,023898	-0,18087	0,930403	1,160673	0,180873	0,930403
ENSMUSG0 0000071076	Jund	2,667007	-0,17187	0,930403	3,004475	0,171872	0,930403
ENSMUSG0 0000005610	Eif4g2	1,162532	-0,17129	0,930403	1,309099	0,171287	0,930403
ENSMUSG0 0000020737	Hn1	1,078931	-0,16691	0,930403	1,211275	0,166908	0,930403
ENSMUSG0 0000021967	Mrpl57	0,949848	-0,16382	0,930403	1,064079	0,16382	0,930403
ENSMUSG0 0000053317	Sec61b	2,911589	-0,15639	0,937969	3,244989	0,156388	0,937969
ENSMUSG0 0000025393	Atp5b	1,21371	-0,15502	0,939862	1,351404	0,15502	0,939862
ENSMUSG0 0000032053	Pou2af1	1,6949	-0,15318	0,943263	1,884775	0,153175	0,943263
ENSMUSG0 0000015092	Edf1	1,231587	-0,15045	0,946549	1,366974	0,150452	0,946549
ENSMUSG0 0000028639	Ybx1	1,224839	-0,14785	0,946549	1,357038	0,147853	0,946549
ENSMUSG0 0000004980	Hnrnpa2b1	1,352519	-0,13597	0,958743	1,486207	0,13597	0,958743
ENSMUSG0 0000022365	Derl1	1,495448	-0,13595	0,958743	1,643237	0,135947	0,958743
ENSMUSG0 0000067149	Jchain	452,7609	-0,13336	0,961755	496,6161	0,13336	0,961755
ENSMUSG0 0000022285	Ywhaz	1,152455	-0,12991	0,96796	1,261058	0,129911	0,96796
ENSMUSG0 0000054452	Aes	1,062193	-0,12306	0,978155	1,156781	0,123057	0,978155
ENSMUSG0 0000051998	Lax1	1,373814	-0,12226	0,980017	1,495323	0,122256	0,980017

ENSMUSG00000037706	Cd81	2,195807	-0,11926	0,98261	2,38506	0,119258	0,98261
ENSMUSG00000022174	Dad1	2,362747	-0,11636	0,982994	2,561246	0,116364	0,982994
ENSMUSG00000039218	Srrm2	1,505088	-0,11621	0,982994	1,631355	0,116208	0,982994
ENSMUSG00000021282	Eif5	1,041862	-0,11563	0,982994	1,128816	0,115634	0,982994
ENSMUSG00000021242	Npc2	1,161919	-0,11106	0,984861	1,254912	0,111064	0,984861
ENSMUSG00000033685	Ucp2	1,66379	-0,11093	0,984861	1,796785	0,110929	0,984861
ENSMUSG00000020738	Sumo2	1,242365	-0,10674	0,991114	1,337781	0,106739	0,991114
ENSMUSG00000017002	Slpi	39,98617	-0,0992	0,996873	42,83305	0,099202	0,996873
ENSMUSG00000050708	Ftl1	8,117658	-0,09813	0,996873	8,689121	0,098127	0,996873
ENSMUSG00000027248	Pdia3	2,143665	-0,09448	1	2,288773	0,094479	1
ENSMUSG00000022769	Sdf2l1	2,263371	-0,09156	1	2,411693	0,091557	1
ENSMUSG00000026864	Hspa5	7,222405	-0,08063	1	7,637642	0,080628	1
ENSMUSG00000007815	Rhoa	1,051502	-0,07646	1	1,108739	0,076461	1
ENSMUSG00000038612	Mcl1	1,494922	-0,07566	1	1,575426	0,07566	1
ENSMUSG00000035242	Oaz1	2,138232	-0,07556	1	2,253228	0,07556	1
ENSMUSG00000078812	Eif5a	1,71821	-0,07139	1	1,805389	0,071391	1
ENSMUSG00000049422	Chchd10	1,697791	-0,06745	1	1,779064	0,067446	1
ENSMUSG00000027523	Gnas	2,469395	-0,06073	1	2,575586	0,060728	1
ENSMUSG00000053477	Tcf4	1,159202	-0,05862	1	1,20728	0,058621	1
ENSMUSG00000008348	Ubc	3,011665	-0,05858	1	3,136512	0,058583	1
ENSMUSG00000032575	Manf	3,918924	-0,05785	1	4,079309	0,05785	1
ENSMUSG00000031812	Map1lc3b	1,308791	-0,05526	1	1,359906	0,055263	1
ENSMUSG00000027808	Serp1	5,02248	-0,05478	1	5,216935	0,054784	1
ENSMUSG00000024610	Cd74	58,91347	-0,05154	1	61,05688	0,051535	1
ENSMUSG00000073411	H2-D1	5,031593	-0,05106	1	5,21294	0,051064	1
ENSMUSG00000030057	Cnbp	1,438837	-0,04811	1	1,487641	0,048113	1
ENSMUSG00000019505	Ubb	8,990741	-0,04301	1	9,262953	0,043013	1
ENSMUSG00000042747	Krtcap2	4,293816	-0,04184	1	4,420208	0,041837	1
ENSMUSG00000020077	Srgn	5,268201	-0,04041	1	5,417909	0,040408	1
ENSMUSG00000028788	Ptp4a2	1,269707	-0,03728	1	1,302953	0,037282	1
ENSMUSG00000001175	Calm1	2,379923	-0,03569	1	2,439555	0,035689	1
ENSMUSG00000006519	Cyba	4,945275	-0,03056	1	5,051198	0,030557	1
ENSMUSG00000090841	Myl6	1,093828	-0,03042	1	1,117139	0,030418	1
ENSMUSG00000048163	Selplg	1,040636	-0,0288	1	1,06162	0,028799	1
ENSMUSG00000003379	Cd79a	5,902134	-0,02819	1	6,018681	0,028192	1
ENSMUSG00000006699	Cdc42	1,129232	-0,02811	1	1,151454	0,02811	1
ENSMUSG00000020719	Ddx5	2,915445	-0,02018	1	2,956536	0,020177	1

ENSMUSG0 0000031304	Il2rg	1,479324	-0,01828	1	1,498191	0,018276	1
ENSMUSG0 0000022890	Atp5j	1,072621	-0,01625	1	1,08477	0,016245	1
ENSMUSG0 0000048076	Arf1	1,176554	-0,01374	1	1,187818	0,013743	1
ENSMUSG0 0000042079	Hnrnpf	1,081034	-0,00729	1	1,086511	0,007289	1
ENSMUSG0 0000027447	Cst3	4,874907	-0,00727	1	4,899596	0,007271	1
ENSMUSG0 0000025967	Eef1b2	2,701621	-0,00313	1	2,707521	0,003133	1
ENSMUSG0 0000022283	Pabpc1	1,622953	-0,003	1	1,626336	0,002995	1
ENSMUSG0 0000003072	Atp5d	1,266727	-0,00054	1	1,267204	0,000538	1
ENSMUSG0 0000031765	Mt1	1,38284	0,001705	1	1,381212	-0,00171	1
ENSMUSG0 0000038803	Ost4	2,040873	0,002177	1	2,037811	-0,00218	1
ENSMUSG0 0000022108	Itm2b	6,424336	0,002393	1	6,413767	-0,00239	1
ENSMUSG0 0000040747	Cd53	1,078843	0,00496	1	1,075141	-0,00496	1
ENSMUSG0 0000056201	Cfl1	1,531465	0,009867	1	1,521034	-0,00987	1
ENSMUSG0 0000041697	Cox6a1	1,218617	0,01153	1	1,208919	-0,01153	1
ENSMUSG0 0000031059	Ndufb11	1,180146	0,017527	1	1,165897	-0,01753	1
ENSMUSG0 0000027422	Rrbp1	1,5879	0,021079	1	1,564876	-0,02108	1
ENSMUSG0 0000079523	Tmsb10	1,670801	0,023916	1	1,64334	-0,02392	1
ENSMUSG0 0000036199	Ndufa13	1,405712	0,024622	1	1,381929	-0,02462	1
ENSMUSG0 0000028367	Txn1	1,527434	0,030658	1	1,495323	-0,03066	1
ENSMUSG0 0000031818	Cox4i1	2,830353	0,039184	1	2,754537	-0,03918	1
ENSMUSG0 0000064367	mt-Nd5	3,176064	0,040026	1	3,089188	-0,04003	1
ENSMUSG0 0000014769	Psmb1	1,185492	0,0419	1	1,151557	-0,0419	1
ENSMUSG0 0000018567	Gabarap	1,983473	0,042234	1	1,926261	-0,04223	1
ENSMUSG0 0000034994	Eef2	6,885108	0,042479	1	6,685421	-0,04248	1
ENSMUSG0 0000060743	H3f3a	1,749056	0,044281	1	1,696195	-0,04428	1
ENSMUSG0 0000020321	Mdh1	1,647227	0,04483	1	1,596835	-0,04483	1
ENSMUSG0 0000025647	Shisa5	3,118927	0,052622	1	3,007241	-0,05262	1
ENSMUSG0 0000036835	Psenen	1,002691	0,052631	1	0,966767	-0,05263	1
ENSMUSG0 0000028277	Ube2j1	1,603324	0,060459	1	1,537526	-0,06046	1
ENSMUSG0 0000009092	Derl3	1,774207	0,062266	1	1,699268	-0,06227	1
ENSMUSG0 0000071644	Eef1g	1,172435	0,067977	1	1,118471	-0,06798	1
ENSMUSG0 0000060126	Tpt1	12,86848	0,070305	1	12,25657	-0,07031	1
ENSMUSG0 0000071866	Ppia	6,545444	0,073721	1	6,219451	-0,07372	1
ENSMUSG0 0000035530	Eif1	6,8384	0,073824	1	6,497353	-0,07382	1
ENSMUSG0 0000034566	Atp5h	1,467406	0,076143	1	1,391968	-0,07614	1
ENSMUSG0 0000022255	Mtdh	3,243365	0,077278	1	3,074232	-0,07728	1
ENSMUSG0 0000026238	Ptma	2,257588	0,090054	1	2,120987	-0,09005	1

ENSMUSG00000021025	Nfkbia	2,383778	0,097755	0,999655	2,22762	-0,09776	0,999655
ENSMUSG00000024014	Pim1	1,914769	0,098105	0,998326	1,788898	-0,0981	0,998326
ENSMUSG00000028691	Prdx1	1,266552	0,100198	0,997017	1,18157	-0,1002	0,997017
ENSMUSG00000075706	Gpx4	2,589627	0,112544	0,985096	2,395303	-0,11254	0,985096
ENSMUSG00000044894	Uqcrq	1,509031	0,117026	0,982994	1,391456	-0,11703	0,982994
ENSMUSG00000031770	Herpud1	3,744973	0,11749	0,982994	3,452109	-0,11749	0,982994
ENSMUSG00000037894	H2afz	1,148949	0,126481	0,976244	1,052504	-0,12648	0,976244
ENSMUSG00000004285	Atp6v1f	1,001814	0,14107	0,956558	0,908482	-0,14107	0,956558
ENSMUSG00000024353	Mzb1	22,33366	0,145991	0,946549	20,18451	-0,14599	0,946549
ENSMUSG00000029632	Ndufa4	1,616644	0,147149	0,946549	1,459881	-0,14715	0,946549
ENSMUSG00000045679	Pqlc3	1,116438	0,14894	0,946549	1,006921	-0,14894	0,946549
ENSMUSG00000053565	Eif3k	1,114334	0,152102	0,946549	1,002823	-0,1521	0,946549
ENSMUSG00000061904	Slc25a3	1,23886	0,166227	0,930403	1,104028	-0,16623	0,930403
ENSMUSG00000030695	Aldoa	1,960426	0,173325	0,930403	1,7385	-0,17332	0,930403
ENSMUSG00000062647	Rpl7a	4,106808	0,177597	0,930403	3,631163	-0,1776	0,930403
ENSMUSG00000012405	Rpl15	3,493556	0,180399	0,930403	3,082939	-0,1804	0,930403
ENSMUSG00000004207	Psap	2,003629	0,180748	0,930403	1,767694	-0,18075	0,930403
ENSMUSG00000031167	Rbm3	2,596462	0,192866	0,930403	2,271564	-0,19287	0,930403
ENSMUSG00000032399	Rpl4	2,2079	0,196107	0,930403	1,927285	-0,19611	0,930403
ENSMUSG00000027620	Rbm39	1,243417	0,198134	0,930403	1,083848	-0,19813	0,930403
ENSMUSG00000038642	Ctss	3,905779	0,198317	0,930403	3,40417	-0,19832	0,930403
ENSMUSG00000015837	Sqstm1	2,677522	0,199083	0,930403	2,332409	-0,19908	0,930403
ENSMUSG00000020372	Gnb2l1	3,767057	0,20123	0,929095	3,276641	-0,20123	0,929095
ENSMUSG00000008682	Rpl10	11,41132	0,204776	0,92145	9,901421	-0,20478	0,92145
ENSMUSG00000034634	Ly6d	13,93041	0,209632	0,916903	12,04658	-0,20963	0,916903
ENSMUSG00000057113	Npm1	1,160517	0,21201	0,916059	1,001901	-0,21201	0,916059
ENSMUSG00000005447	Pafah1b3	1,558193	0,216714	0,909096	1,340854	-0,21671	0,909096
ENSMUSG00000038690	Atp5j2	1,401944	0,217004	0,908412	1,206154	-0,217	0,908412
ENSMUSG00000018293	Pfn1	2,142877	0,218951	0,902221	1,841139	-0,21895	0,902221
ENSMUSG00000058558	Rpl5	3,010088	0,21907	0,901599	2,586034	-0,21907	0,901599
ENSMUSG00000067274	Rplp0	11,72338	0,222542	0,895169	10,0477	-0,22254	0,895169
ENSMUSG00000028495	Rps6	3,920326	0,223613	0,892014	3,357461	-0,22361	0,892014
ENSMUSG00000037805	Rpl10a	4,712786	0,224662	0,889475	4,033214	-0,22466	0,889475
ENSMUSG00000030744	Rps3	6,614323	0,231306	0,871818	5,634556	-0,23131	0,871818
ENSMUSG00000005583	Mef2c	1,494922	0,233522	0,870121	1,271506	-0,23352	0,870121
ENSMUSG00000060036	Rpl3	4,916181	0,235532	0,864693	4,175699	-0,23553	0,864693
ENSMUSG00000032330	Cox7a2	1,154821	0,236673	0,863515	0,980083	-0,23667	0,863515

ENSMUSG0 0000003970	Rpl8	6,982117	0,237249	0,860985	5,923418	-0,23725	0,860985
ENSMUSG0 0000025290	Rps24	20,18071	0,23936	0,856964	17,09573	-0,23936	0,856964
ENSMUSG0 0000016427	Ndufa1	1,811275	0,246904	0,84671	1,526361	-0,2469	0,84671
ENSMUSG0 0000031320	Rps4x	11,02416	0,248401	0,845448	9,280572	-0,2484	0,845448
ENSMUSG0 0000015656	Hspa8	2,62985	0,248664	0,845448	2,213484	-0,24866	0,845448
ENSMUSG0 0000064341	mt-Nd1	4,256484	0,24961	0,845448	3,580253	-0,24961	0,845448
ENSMUSG0 0000061518	Cox5b	1,303883	0,25266	0,842521	1,094399	-0,25266	0,842521
ENSMUSG0 0000064357	mt-Atp6	8,048516	0,253882	0,8373	6,749851	-0,25388	0,8373
ENSMUSG0 0000073421	H2-Ab1	1,581941	0,257616	0,835829	1,323235	-0,25762	0,835829
ENSMUSG0 0000000740	Rpl13	12,33059	0,262201	0,82323	10,28155	-0,2622	0,82323
ENSMUSG0 0000049751	Rpl36al	7,424836	0,263401	0,821714	6,185853	-0,2634	0,821714
ENSMUSG0 0000023944	Hsp90ab1	3,853287	0,267186	0,813502	3,201864	-0,26719	0,813502
ENSMUSG0 0000020267	Hint1	1,122046	0,270815	0,807147	0,929993	-0,27082	0,807147
ENSMUSG0 0000064370	mt-Cytb	12,01205	0,271497	0,804195	9,951613	-0,2715	0,804195
ENSMUSG0 0000068523	Gng5	1,717772	0,275956	0,804015	1,418703	-0,27596	0,804015
ENSMUSG0 0000064363	mt-Nd4	3,023495	0,276488	0,804015	2,4962	-0,27649	0,804015
ENSMUSG0 0000044533	Rps2	5,265923	0,279683	0,804015	4,337954	-0,27968	0,804015
ENSMUSG0 0000043716	Rpl7	4,163068	0,282689	0,79873	3,422301	-0,28269	0,79873
ENSMUSG0 0000105646	Gm43291	2,002139	0,282929	0,804015	1,645593	-0,28293	0,804015
ENSMUSG0 0000017404	Rpl19	10,14688	0,283512	0,796714	8,336648	-0,28351	0,796714
ENSMUSG0 0000047215	Rpl9	7,250973	0,284666	0,795559	5,952612	-0,28467	0,795559
ENSMUSG0 0000084786	Ubl5	1,475205	0,289751	0,790881	1,206768	-0,28975	0,790881
ENSMUSG0 0000029622	Arpc1b	1,09453	0,293361	0,781102	0,893117	-0,29336	0,781102
ENSMUSG0 0000061477	Rps7	3,392866	0,29449	0,776065	2,76642	-0,29449	0,776065
ENSMUSG0 0000039910	Cited2	2,619334	0,29696	0,774316	2,13205	-0,29696	0,774316
ENSMUSG0 0000029614	Rpl6	5,086451	0,297664	0,770553	4,138208	-0,29766	0,770553
ENSMUSG0 0000059291	Rpl11	7,010159	0,299092	0,767756	5,697655	-0,29909	0,767756
ENSMUSG0 0000001289	Pfdn5	2,444771	0,302826	0,75921	1,981882	-0,30283	0,75921
ENSMUSG0 0000059070	Rpl18	7,168161	0,304905	0,75451	5,802649	-0,3049	0,75451
ENSMUSG0 0000052146	Rps10	10,78309	0,306309	0,753344	8,720466	-0,30631	0,753344
ENSMUSG0 0000036106	Prr5	1,044491	0,308766	0,75241	0,843232	-0,30877	0,75241
ENSMUSG0 0000064351	mt-Co1	15,4539	0,309759	0,744993	12,46799	-0,30976	0,744993
ENSMUSG0 0000048758	Rpl29	4,757917	0,315665	0,727697	3,822918	-0,31567	0,727697
ENSMUSG0 0000074129	Rpl13a	6,622999	0,320053	0,718078	5,305335	-0,32005	0,718078
ENSMUSG0 0000038900	Rpl12	2,986427	0,320066	0,718503	2,39223	-0,32007	0,718503
ENSMUSG0 0000028081	Rps3a1	11,21625	0,32121	0,713617	8,977574	-0,32121	0,713617
ENSMUSG0 0000029580	Actb	5,829487	0,325683	0,708609	4,651502	-0,32568	0,708609

ENSMUSG00000062683	Atp5g2	2,01467	0,326464	0,708002	1,606668	-0,32646	0,708002
ENSMUSG00000036781	Rps27l	1,700596	0,327527	0,705639	1,355194	-0,32753	0,705639
ENSMUSG00000074884	Serf2	3,535006	0,327756	0,705057	2,816612	-0,32776	0,705057
ENSMUSG00000055762	Eef1d	1,217916	0,32796	0,705434	0,970249	-0,32796	0,705434
ENSMUSG00000064345	mt-Nd2	3,067312	0,336247	0,692192	2,429619	-0,33625	0,692192
ENSMUSG00000055839	Tceb2	1,301079	0,341826	0,680921	1,026588	-0,34183	0,680921
ENSMUSG00000006333	Rps9	7,462167	0,349814	0,652421	5,855505	-0,34981	0,652421
ENSMUSG00000012848	Rps5	8,155077	0,358293	0,637359	6,361731	-0,35829	0,637359
ENSMUSG00000036751	Cox6b1	1,071219	0,363024	0,632777	0,832886	-0,36302	0,632777
ENSMUSG00000008668	Rps18	3,647789	0,364643	0,628509	2,833104	-0,36464	0,628509
ENSMUSG00000037563	Rps16	8,902845	0,364856	0,627283	6,91354	-0,36486	0,627283
ENSMUSG00000021660	Btf3	2,003278	0,377422	0,598879	1,542135	-0,37742	0,598879
ENSMUSG00000061315	Naca	2,577534	0,379191	0,590607	1,98178	-0,37919	0,590607
ENSMUSG00000035885	Cox8a	2,299125	0,380631	0,588228	1,765952	-0,38063	0,588228
ENSMUSG00000024038	Ndufv3	1,067013	0,382402	0,586089	0,818546	-0,3824	0,586089
ENSMUSG00000014294	Ndufa2	1,102154	0,384358	0,578648	0,844359	-0,38436	0,578648
ENSMUSG00000098274	Rpl24	5,259	0,387896	0,566011	4,019181	-0,3879	0,566011
ENSMUSG00000060586	H2-Eb1	1,196709	0,387942	0,58225	0,914526	-0,38794	0,58225
ENSMUSG00000022312	Eif3h	1,195219	0,390838	0,563419	0,911555	-0,39084	0,563419
ENSMUSG00000017707	Serinc3	5,951471	0,395731	0,546852	4,523768	-0,39573	0,546852
ENSMUSG00000032518	Rpsa	11,25709	0,403954	0,524597	8,508019	-0,40395	0,524597
ENSMUSG00000020460	Rps27a	9,465008	0,407725	0,513049	7,134898	-0,40772	0,513049
ENSMUSG00000065947	mt-Nd4l	6,136551	0,40924	0,510304	4,620977	-0,40924	0,510304
ENSMUSG00000064354	mt-Co2	10,67732	0,410915	0,505665	8,030986	-0,41091	0,505665
ENSMUSG00000037742	Eef1a1	20,36351	0,411152	0,504679	15,314	-0,41115	0,504679
ENSMUSG00000024661	Fth1	5,498762	0,413899	0,499002	4,12735	-0,4139	0,499002
ENSMUSG00000062328	Rpl17	9,40866	0,414247	0,497813	7,060429	-0,41425	0,497813
ENSMUSG00000063457	Rps15	2,130433	0,421762	0,47955	1,590382	-0,42176	0,47955
ENSMUSG00000000682	Cd52	10,77967	0,425286	0,475321	8,027606	-0,42529	0,475321
ENSMUSG00000041881	Ndufa7	1,0416	0,446043	0,425941	0,764563	-0,44604	0,425941
ENSMUSG00000030432	Rpl28	8,100307	0,450561	0,410432	5,927516	-0,45056	0,410432
ENSMUSG00000064358	mt-Co3	12,44407	0,451945	0,408436	9,097421	-0,45194	0,408436
ENSMUSG00000024608	Rps14	9,133143	0,458064	0,393428	6,648647	-0,45806	0,393428
ENSMUSG00000061787	Rps17	1,76115	0,45815	0,394177	1,281954	-0,45815	0,394177
ENSMUSG00000025794	Rpl14	5,033959	0,465176	0,379217	3,646528	-0,46518	0,379217
ENSMUSG00000036372	Tmem258	1,911615	0,471943	0,363775	1,378242	-0,47194	0,363775
ENSMUSG00000045128	Rpl18a	10,19919	0,473536	0,360476	7,345502	-0,47354	0,360476

ENSMUSG00000060938	Rpl26	7,441486	0,474932	0,358119	5,354196	-0,47493	0,358119
ENSMUSG00000038717	Atp5l	1,227468	0,481233	0,348779	0,879289	-0,48123	0,348779
ENSMUSG00000022587	Ly6e	15,84912	0,48898	0,330695	11,29308	-0,48898	0,330695
ENSMUSG00000047675	Rps8	16,22839	0,495182	0,315196	11,51372	-0,49518	0,315196
ENSMUSG00000031029	Eif3f	2,392366	0,50969	0,290293	1,680318	-0,50969	0,290293
ENSMUSG00000061983	Rps12	5,769546	0,510867	0,287038	4,049091	-0,51087	0,287038
ENSMUSG00000009927	Rps25	2,51733	0,514871	0,279995	1,761753	-0,51487	0,279995
ENSMUSG00000007892	Rplp1	8,821435	0,53357	0,240197	6,094277	-0,53357	0,240197
ENSMUSG00000071415	Rpl23	11,09287	0,539608	0,228694	7,631496	-0,53961	0,228694
ENSMUSG00000041453	Rpl21	7,851168	0,563107	0,190311	5,314042	-0,56311	0,190311
ENSMUSG00000057841	Rpl32	11,76142	0,571315	0,179758	7,915544	-0,57131	0,179758
ENSMUSG00000022450	Ndufa6	1,009877	0,571905	0,182364	0,679339	-0,57191	0,182364
ENSMUSG00000016252	Atp5e	2,150764	0,582756	0,164524	1,436014	-0,58276	0,164524
ENSMUSG00000030220	Arhgdib	1,554775	0,583773	0,164524	1,037343	-0,58377	0,164524
ENSMUSG00000078974	Sec61g	3,119102	0,601609	0,141796	2,055532	-0,60161	0,141796
ENSMUSG00000063882	Uqcrh	1,778676	0,606164	0,137428	1,168458	-0,60616	0,137428
ENSMUSG00000049517	Rps23	5,84158	0,607751	0,133619	3,833366	-0,60775	0,133619
ENSMUSG00000025508	Rplp2	4,665815	0,610534	0,130337	3,055897	-0,61053	0,130337
ENSMUSG00000046364	Rpl27a	7,757927	0,617972	0,122439	5,054988	-0,61797	0,122439
ENSMUSG00000014313	Cox6c	1,349189	0,625044	0,115086	0,874782	-0,62504	0,115086
ENSMUSG00000045826	Ptprcap	1,405011	0,629	0,111259	0,908482	-0,629	0,111259
ENSMUSG00000028234	Rps20	15,07603	0,64472	0,09176	9,642981	-0,64472	0,09176
ENSMUSG00000056737	Capg	1,049837	0,649582	0,090478	0,669198	-0,64958	0,090478
ENSMUSG00000090862	Rps13	7,224596	0,652132	0,084928	4,597315	-0,65213	0,084928
ENSMUSG00000036594	H2-Aa	1,643722	0,654092	0,102801	1,044514	-0,65409	0,102801
ENSMUSG00000040952	Rps19	6,331358	0,668841	0,073271	3,98251	-0,66884	0,073271
ENSMUSG00000073702	Rpl31	1,468983	0,673685	0,070752	0,920877	-0,67368	0,070752
ENSMUSG00000033220	Rac2	1,242716	0,674672	0,070935	0,778494	-0,67467	0,070935
ENSMUSG00000060636	Rpl35a	7,384262	0,685148	0,061789	4,592603	-0,68515	0,061789
ENSMUSG00000040699	Limd2	1,06009	0,723564	0,042921	0,64195	-0,72356	0,042921
ENSMUSG00000038274	Fau	8,086198	0,723771	0,040997	4,896318	-0,72377	0,040997
ENSMUSG00000049775	Tmsb4x	2,449853	0,731312	0,040252	1,475656	-0,73131	0,040252
ENSMUSG00000003429	Rps11	9,626865	0,732938	0,036975	5,792303	-0,73294	0,036975
ENSMUSG00000028936	Rpl22	3,944776	0,733924	0,036975	2,371846	-0,73392	0,036975
ENSMUSG00000060032	H2afj	1,459519	0,741481	0,034399	0,872938	-0,74148	0,034399
ENSMUSG00000025362	Rps26	6,525114	0,743461	0,032699	3,89749	-0,74346	0,032699
ENSMUSG00000008683	Rps15a	8,361364	0,79128	0,017709	4,831478	-0,79128	0,017709

ENSMUSG0 0000058600	Rpl30	7,257546	0,797857	0,016109	4,174572	-0,79786	0,016109
ENSMUSG0 0000093674	Rpl41	17,28515	0,810107	0,013495	9,858501	-0,81011	0,013495
ENSMUSG0 0000079435	Rpl36a	3,56708	0,832724	0,010121	2,002779	-0,83272	0,010121
ENSMUSG0 0000028581	Laptm5	2,333302	0,872312	0,006031	1,274579	-0,87231	0,006031
ENSMUSG0 0000063316	Rpl27	2,112468	0,889093	0,004681	1,140596	-0,88909	0,004681
ENSMUSG0 0000062006	Rpl34	8,647747	0,894187	0,00431	4,652936	-0,89419	0,00431
ENSMUSG0 0000062997	Rpl35	5,216147	0,952513	0,001848	2,695331	-0,95251	0,001848
ENSMUSG0 0000064356	mt-Atp8	3,177553	0,974371	0,001317	1,617219	-0,97437	0,001317
ENSMUSG0 0000040592	Cd79b	4,260953	0,997393	0,000905	2,134303	-0,99739	0,000905
ENSMUSG0 0000039001	Rps21	5,201951	1,139708	6,49E-05	2,360886	-1,13971	6,49E-05
ENSMUSG0 0000027073	Prg2	18,88874	1,153198	6,18E-05	8,492961	-1,1532	6,18E-05
ENSMUSG0 0000057863	Rpl36	5,071466	1,24426	8,49E-06	2,140756	-1,24426	8,49E-06
ENSMUSG0 0000041841	Rpl37	7,281732	1,252821	7,23E-06	3,055589	-1,25282	7,23E-06
ENSMUSG0 0000046330	Rpl37a	7,638922	1,25588	6,81E-06	3,198689	-1,25588	6,81E-06
ENSMUSG0 0000057322	Rpl38	4,125649	1,281239	4,12E-06	1,697424	-1,28124	4,12E-06
ENSMUSG0 0000079641	Rpl39	6,086688	1,294397	3,02E-06	2,481552	-1,2944	3,02E-06
ENSMUSG0 0000067288	Rps28	4,266825	1,32552	1,66E-06	1,702444	-1,32552	1,66E-06
ENSMUSG0 0000034892	Rps29	5,228504	1,340404	1,23E-06	2,064751	-1,3404	1,23E-06
ENSMUSG0 0000090733	Rps27	5,057533	1,428328	1,62E-07	1,879141	-1,42833	1,62E-07

7 List of Publications

Burt P, **Cornelis R**, Geissler G, Hahne S, Radbruch A, Chang H-D, Thurley K. (2022). Mathematical modeling reveals a complex network of signaling and apoptosis pathways in the survival of memory plasma cells. Cells. 11.

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Männe, Christian & Takaya, Akiko & Yamasaki, Yuzuru & Mursell, Mathias & Hojyo, Shintaro & Wu, Tsungyen & Sarkander, Jana & McGrath, Mairi & **Cornelis, Rebecca** & Hahne, Stefanie & Cheng, Qingyu & Kawamoto, Tadafumi & Hiepe, Falk & Kaufmann, Stefan & Yamamoto, Tomoko & Radbruch, Andreas & Tokoyoda, Koji. (2019). Salmonella SiiE prevents an efficient humoral immune memory by interfering with IgG + plasma cell persistence in the bone marrow. Proceedings of the National Academy of Sciences of the United States of America. 116. 7425-7430.

Martrus G, Niehrs A, **Cornelis R**, et al. Kinetics of HIV-1 latency reversal quantified on the single cell level using a novel flow-based technique. *J Virol*. 2016;90:JVI.01448-16.