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Examining how PSMB11 orchestrates T cell development

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Résumé

Les lymphocytes T CD8 jouent un rôle majeur dans l'immunosurveillance de l'organisme contre les pathogènes de même que les cellules pré-cancéreuses. Afin d'induire une réponse cytotoxique efficace contre ces événements anormaux, les lymphocytes T CD8 doivent exprimer à leur surface un récepteur des cellules T (TCR) qualifié et capable de reconnaître les molécules étrangères. Cette qualification s'effectue au sein du thymus, qui représente l'unique organe se spécialisant dans l'éducation des lymphocytes T CD4 et CD8. Dans le cortex thymique, les thymocytes exprimant un TCR fonctionnel sont sélectionnés positivement par les cellules épithéliales corticales thymiques (cTECs). Ceux-ci transitent ensuite vers la médulla thymique où les thymocytes autoréactifs sont éliminés afin de prévenir l'autoimmunité.

PSMB11 est une sous-unité catalytique du thymoprotéasome, qui est exprimé de façon exclusive par les cTECs, des cellules spécialisées dans la production et la présentation des peptides associés au CMH de classe I (MIPs). Ainsi, une déficience en PSMB11 altère sévèrement le développement des thymocytes CD8. Étant donné que les thymoprotéasomes présentent une faible activité chymotrypsique, leur rôle présumé est de produire des peptides ayant une faible affinité pour le CMH de classe I pour ainsi assurer la sélection positive des thymocytes CD8 exprimant un TCR de faible affinité. À ce jour, aucune étude n'a toutefois réussi à élucider le rôle fondamental de PSMB11 dans la sélection positive.

Puisque les protéasomes peuvent être impliqués dans plusieurs processus cellulaires, nous avons vérifié si PSMB11 1) orchestre des activités peptide-indépendantes dans les cTECs, et 2) son impact sur le développement des thymocytes. Nous avons montré que les cTECs de souris *Psmb11*$^{-/-}$ présentent une expression différentielle de gènes impliqués dans l'adhésion cellule-cellule ainsi que dans la signalisation des cytokines, deux processus étroitement liés à la sélection positive. Nous avons également observé des niveaux élevés de CXCR4 à la surface des thymocytes 8SM des souris *Psmb11*$^{-/-}$, suggérant que les thymocytes CD8 sont retenus plus longtemps dans le cortex comparativement aux souris WT. Des gènes pro-inflammatoires sont aussi surexprimés dans les cTECs déficientes en PSMB11, menant à la détection d'une augmentation du stress dans les thymocytes. Enfin, nous avons montré que PSMB11 altère l'expression de Cd83 et Prss16, deux gènes ayant un rôle essentiel dans le développement des lymphocytes T CD4. En conclusion, notre étude dévoile un tout nouveau rôle de PSMB11 dans le développement des lymphocytes CD4 et CD8, soit l'orchestration de la transcription des gènes au sein des cTECs.

Mots-clés: Thymoprotéasome, PSMB11, cellules épithéliales thymiques, thymocytes, sélection positive.

Abstract

CD8 T cells are central to the body's immunosurveillance against pathogens and pre-cancerous cells. To achieve a cytotoxic response to abnormal events, CD8 T cells must bear a competent T cell receptor (TCR). The thymus is unique for "educating" developing CD4 and CD8 T cells to recognize foreign material. In the thymic cortex, thymocytes displaying functional TCRs are positively-selected by cortical thymic epithelial cells (cTECs). Upon arrival to the medulla, T cells which recognize self-antigens presented by medullary thymic epithelial cells (mTECs) receive the "death sentence" to prevent autoimmunity.

PSMB11 is a catalytic subunit of the thymoproteasome expressed exclusively in cTECs, which mediates the generation of major histocompatibility complex class I-associated peptides (MIPs). In consequence, PSMB11-deficiency severely impairs the development of CD8 thymocytes. Since thymoproteasomes display a low chymotrypsin activity, it was inferred that they produce peptides with low MHC class I affinity, and by similarity, that they produce low TCR affinity. However, indirect studies failed to elucidate the fundamental role of PSMB11 in positive selection.

Proteasomes regulate essentially all cellular processes, therefore we investigated 1) if PSMB11 orchestrates peptide-independent processes in cTECs, and 2) the impact on thymocyte development. We found that cTECs in *Psmb11*$^{-/-}$ mice show differential expression of genes involved in cell-cell adhesion and cytokine signaling, which are at the core of positive selection. Elevated levels of CXCR4 at the cell surface of 8SM thymocytes in *Psmb11*$^{-/-}$ mice suggest that CD8 thymocytes have a longer retention time in the cortex compared to the WT. Pro-inflammatory genes were upregulated in cTECs lacking PSMB11, which might underlie the increased stress detected in medullary thymocytes. Finally, we found that PSMB11 alters the expression of *Cd83* and *Prss16*, genes which have an essential role in CD4 T cell development. In conclusion, our study describes novel peptide-independent means of PSMB11 to regulate CD4 and CD8 thymocyte development, by orchestrating the transcriptome in cTECs.

Keywords: Thymoproteasome, PSMB11, thymic epithelial cells, thymocytes, positive selection

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List of Acronyms

7-AAD	7-amino-actinomycin D
APC	Antigen-presenting cell
β_2m	β_2-microglobulin
BCR	B cell receptor
CMJ	Cortico-medullary junction
cTEC	Cortical thymic epithelial cell
CTL	Cytotoxic T cell
DC	Dendritic cell
DEG	Differentially expressed gene
DNA	Deoxyribonucleic acid
DP	Double positive
ECM	Extracellular matrix
e.g.	for example
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
FACS	Fluorescence activated cell sorting
FPKM	Fragments per kilobase million
GMFI	Geometric mean fluorescence intensity
GO	Gene-ontology
HLA	Human leukocyte antigen
i.e.	for instance
IFNg	Interferon-γ
IL-2	Interleukin-2
iNKT	Invariant natural killer T cell
M1; M2	Mature 1; mature 2
MHC	Major histocompatibility complex
MIIC	MHC class II compartment

MIP	MHC class I-associated peptide
mTEC	Medullary thymic epithelial cell
PCD	Programmed cell death
PWM	Position weight matrix
RNA	Ribonucleic acid
RNA-Seq	RNA-Sequencing
SM	Semi-mature
TAP	Transporter associated with antigen processing
TCR	T cell receptor
TEC	Thymic epithelial cell
TF	Transcription factor
TGFβ	Tumor growth factor β
Tfh	Follicular T helper
Th	T helper
TNFa	Tumor necrosis factor α
Treg	Induced regulatory T cell
nTreg	Natural regulatory T cell
TSSP	Thymus specific serine protease

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Chapter 1 – Introduction

1.1 The adaptive immune system

There are two major threats to the immune system, foreign materials (viruses and pathogenic bacteria) and abnormal cellular processes in stressed and neoplastic cells. The immune system has evolved in vertebrates to recognize these threats, fight them and develop long-term memory for antigen re-exposure [1].

1.1.1 Antigen recognition by immune cells

The adaptive immune system is defined by B and T cell lineages, which recognize foreign antigens via surface B cell receptors (BCRs) and T cell receptors (TCRs), respectively. Genetic rearrangements and somatic hypermutations of the DNA regions encoding lymphocyte receptors allow the recognition of a diverse antigen repertoire [2]. In the peripheral lymphoid tissues, the binding of an antigen to the BCR on B cells has a two-fold function: it triggers signal transduction in B cells and the internalization of the antigen. The antigen is degraded intracellularly and presented at the cell surface via major histocompatibility complex (MHC) class II molecules (discussed in 1.1.2) for recognition by helper T cells. Helper T cells that recognize the antigenic peptides on B cells and receive co-stimulatory signals become activated. The activation of helper T cells allows the activation of B cells through the interaction between the CD40 ligand and the CD40 protein on B cells, and the production of cytokines which stimulate the proliferation and differentiation of B cells into secreting cells. The release of about 2000 antibodies with the same binding site per second mediates target neutralization [3].

Targets that are not accessible to antibodies, i.e. intracellular pathogens which multiply in the cytoplasm of infected cells or molecules expressed by abnormal cells, can be eliminated solely via proteolytic destruction by the respective antigen-presenting cells (APCs). Subject APCs load peptides resulting from the internal antigen degradation onto MHC class I or class II complexes for surface presentation, which activates a T cell response [4]. Recognition of peptide/MHC class I molecules on APCs by the TCR is necessary for a cytotoxic immune response by CD8 T cells. Cytotoxic T cells (CTLs) release granzymes, perforins and cytokines like interferon-γ (IFNg) into the intercellular space, which induce the programmed-cell death (PCD) of the infected cells. CTLs are also thought to detect quantitative and qualitative antigenic differences at the cell surface. In this regard, cancerous or stressed cells alter their intracellular protein homeostasis and thus the repertoire of peptide/MHC class I at the cell surface, allowing CD8 T cells to scan for cellular alterations and induce apoptosis, a form of PCD [5].

Recognition of surface MHC class II complexes loaded with macromolecular parts of pathogens is the role of CD4 T cells. The initial activation of a naïve CD4 T cell signals its differentiation into effector T helper (Th) cells, including Th1, Th2, Th17, Th9, Tfh (follicular helper T cells) and iTreg (induced regulatory T cells). The task of Th1 cells is to aid CTLs in their immune response by secreting IFNg and tumor necrosis factor α (TNFa), or to stimulate B cells to produce antibodies. Th2 cells are identified by the production of interleukins and mainly priming of B cells to produce immunoglobulin E for the defense against extracellular pathogens [3]. In addition to common cytokines produced by more than one Th cell type, e.g. IL-4 and IL-13, Th17 and Th9 cells produce the signature cytokines IL-17 and IL-9, respectively. In contrast, Tfh cells are essential for germinal center formation and the development of memory B cells with the highest antigen affinity, whereas iTregs are suppressive cells involved in tolerance that are not generated in the thymus [6].

1.1.2 Antigen presentation

Whereas B cells are capable of recognizing native antigens in their intact form, TCRs recognize antigen-derived peptides in complex with an MHC molecule. Both MHC class I and MHC class II complexes have the purpose of presenting antigen-derived peptides on the cell surface of T cells. What makes the difference between these complexes is the origin of the pathogen. In the classic pathway described below, class I molecules load peptides derived from intracellular sources, whereas class II molecules from extracellular sources. Other non-classical pathways are termed cross-presentation, where the extracellular-derived antigens are presented by class I molecules, and cytosolic antigens by class II molecules via autophagy [7,8].

1.1.3 MHC class I presentation

MHC class I presentation starts with the degradation of pathogen-derived peptides into 8-10 amino-acid long peptides by cytosolic proteasomes. The resulting peptides are translocated by the transporter associated with antigen presentation (TAP) to the endoplasmic reticulum (ER) where they are loaded onto MHC class I molecules, or further trimmed by the ER-associated degradation (ERAD) machinery to fit the binding groove of the MHC. The trimeric complex formed by the MHC I heavy chain, the β2-microglobulin light chain and the peptide are transported to the cell surface via the Golgi apparatus, where they are accessible for interaction with the TCR on CD8 T cells (Figure 1) [8,9].

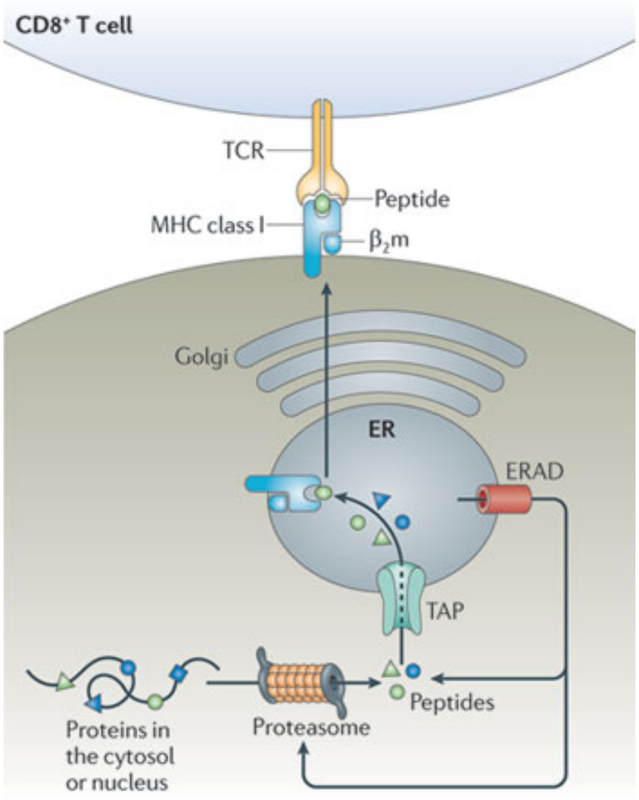

Figure 1. MHC class I presentation pathway. Intracellular proteins are degraded into 8-10 amino-acid-long peptides by the proteasome and translocated to the endoplasmic reticulum (ER) via the transporter associated with antigen presentation (TAP) complex. Peptides are loaded onto MHC class I complexes forms by a heavy chain and the light chain β2-microglobulin. Peptides of inappropriate length are further trimmed by the ER-associated degradation (ERAD) machinery to fit the binding groove of the MHC. Peptide/MHC complexes are now ready for transport to the plasma membrane via the Golgi for recognition by CD8 T cells. Figure adapted from [8].

1.1.4 MHC class II presentation

Whereas MHC class I molecules are present on all nucleated cells, MHC class II complexes are only found on professional APCs, i.e. dendritic cells (DCs), B cells and macrophages, or on non-APCs induced with IFNg, which constitutively process protein antigens and present them to the cell surface for immune surveillance in the form of peptide/MHC molecules [8].

The α and β chains of the MHC II complex are assembled in the ER with the Ii chain which is involved in transport and stability, promoting an open conformation of the binding groove. Upon transport to the endosomal compartments, H2-M facilitates the exchange of the CLIP chain, resulted from the proteolytic fragmentation of the Ii chain in the endosome, with the antigenic peptides. The peptides suitable for MHC II presentation are derived from exogenous proteins and cleaved by proteases in early endosomes which fuse with the endosomal compartments in APCs. Stable peptide/MHC class II complexes are translocated to the plasma membrane of APCs and recognized by the TCR on CD4 T cells (Figure 2) [8].

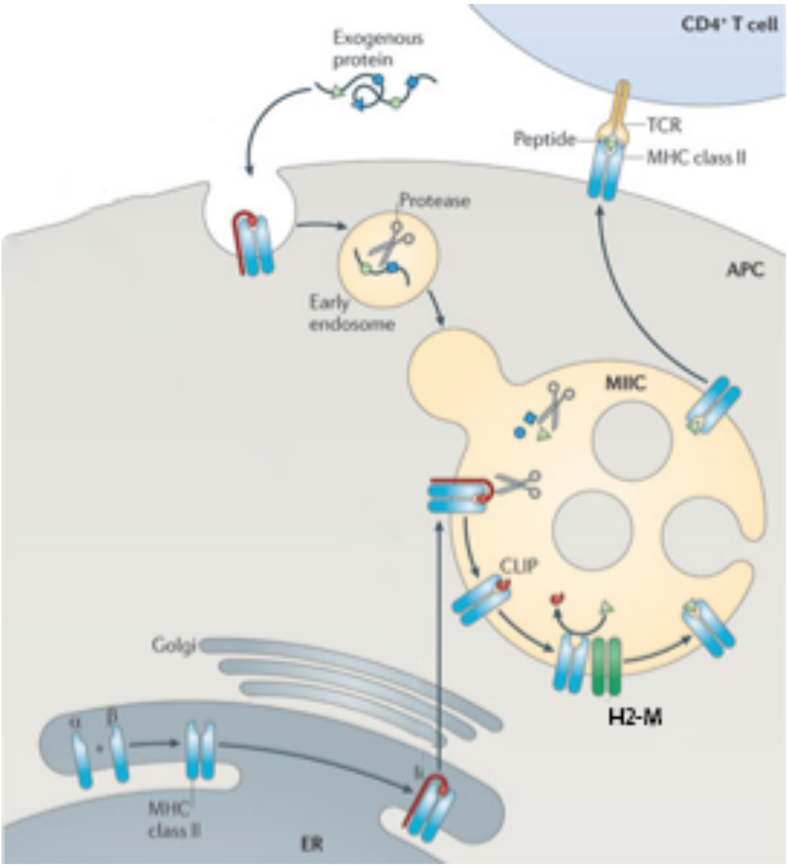

Figure 2. MHC class II presentation pathway. Extracellular proteins are endocytosed and cleaved by proteases in early endosomes that fuse use the endosomal compartments. The α and β chains of the MHC II complex are assembled in the ER lumen and transported to the MHC II compartment (MIIC) via Golgi. With the help from H2-M, the CLIP peptide derived from the surrogate Ii chain is exchanged for an antigenic peptide. Peptide/MHC II complexes are now ready for translocation to the plasma membrane for interactions with CD4 T cells. Figure adapted from [8].

1.1.5 Human HLA molecules

In humans, the MHC molecules are called human leukocyte antigens (HLA), and there are six major types of HLA molecules. Similar to MHC class I in mice, CD8 T cells interact with HLA-A, HLA-B and HLA-C, whereas HLA-DP, -DQ and –DR are recognized by the TCRs on CD4 T cells [10].

1.1.6 Antigen processing

The stability of peptide/MHC complexes is essential for translocation to the cell surface, and is conferred by peptide binding into the cleft of MHC molecules. The peptides resulted from proteolytic degradation have two particularities that allow them to fit into the MHC binding cleft: proper length and anchor residues [9].

The ubiquitously expressed 26S proteasome, also called the constitutive proteasome, degrades cytosolic proteins for MHC class I presentation. Constitutive proteasomes generate peptides with hydrophobic C-termini which serve as anchors to the binding groove of the MHC I molecules. Two alternative proteasomes have been described, the immunoproteasome and the thymoproteasome. Immunoproteasomes are expressed by immune cells, stressed and IFNg-exposed cells, and display an increased activity compared to constitutive proteasomes, thus efficiently cleaving proteins that cannot be handled by constitutive proteasomes. Thymoproteasomes are thymus-specific, and play a role in the development of CD8 T cells [8]. The core of the proteasome is composed of two outer rings with 7 α (α1-α7) subunits and two inner rings with 7 β (β1-β7) subunits. The proteolytic subunits of constitutive proteasomes are PSMB6, PSMB7 and PSMB5 (or β1, β2 and β5, respectively), whereas immunoproteasomes assemble PSMB9, PSMB10 and PSMB8 (also known as β1i, β2i and β5i). Lastly, only one catalytic subunit is different between immunoproteasomes and thymoproteasomes: PSMB8 is replaced by PSMB11 (or β5t) (Figure 3). The assembly of different catalytic subunits alters the protein cleavage preferences and characteristics of the resulting peptides [11].

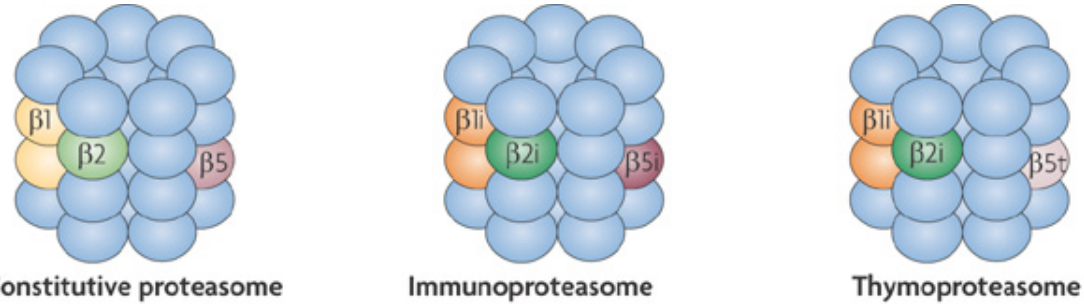

Figure 3. Different forms of proteasomes. Proteolytic subunits of the constitutive proteasomes PSMB6, PSMB7 and PSMB5 (or β1, β2 and β5, respectively), immunoproteasomes assemble PSMB9, PSMB10 and PSMB8 (also known as β1i, β2i and β5i). Lastly, only one catalytic subunit is different between immunoproteasomes and thymoproteasomes, PSMB8 is replaced by PSMB11 (or β5t). Figure adapted from [11].

Proteolytic cleavage of MHC class II-associated peptides is performed by lysosomal proteases residing in the endosomal compartment. Among them, cathepsin S and cathepsin L are the two known proteases with non-redundant roles in endosomal presentation. Whereas cathepsin S has essential roles in B cells and DCs, cathepsin L is specifically expressed in the thymus [12].

1.2 T cell development

1.2.1 The thymus and the thymic journey of lymphocytes

The thymus is unique for its ability to produce immunocompetent T cells [13]. Located in the pericardial mediastinum, it is formed by two lobes linked by a connective tissue called isthmus. Each lobe is divided into interconnecting lobules in most species, but not in the mouse. The thymic lobes of mice are subdivided into cortical and medullary areas separated by the corticomedullay junction (CMJ) predominant in blood vessels (Figure 1). Being an epithelial organ, the thymic stroma is formed by cortical thymic epithelial cells (cTECs) in the cortex, medullary thymic epithelial cells (mTECs) in the medulla, endothelial cells forming the vasculature, fibroblasts and connective tissue. Other types of cells functionally important for T cell development in the thymus are B cells, DCs and macrophages. Lymphoid precursors travel from the bone-marrow (BM) to the thymus as a prerequisite for becoming mature T cells, and emigrate into the periphery as naïve T cells upon completion of the thymic "education" [14].

Lymphoid precursors enter the thymus through the vasculature at the CMJ and progress through 4 different stages of maturation characterized by the surface expression of the CD4 and CD8 coreceptors. Initially, double-negative thymocytes reside in the cortex and receive cues to differentiate into double-positive (DP) cells expressing both CD4 and CD8 coreceptors. DP thymocytes represent around 80% of total thymocytes and express a diverse repertoire of TCR specificities which must be selected for their immunocompetence, i.e. for their ability to bind the limited MHC repertoire to insure instant reaction in the case of pathogenic threats. Each T cell expresses a unique cell surface TCR resulted from the rearrangement of the TCRβ and then TCRα transmembrane chains. Successful engagement of the TCR with MHC class I or class II molecules on professional APCs signals thymocytes to differentiate into CD8 or CD4 single-positive thymocytes, respectively, which migrate to the medulla. Further interactions with the medullary stroma prepare thymocytes for entry into the peripheral circulation. Mature thymocytes can be identified by changes in cell size, proliferation competence and expression of differentiation antigens and interleukin receptors [15,16].

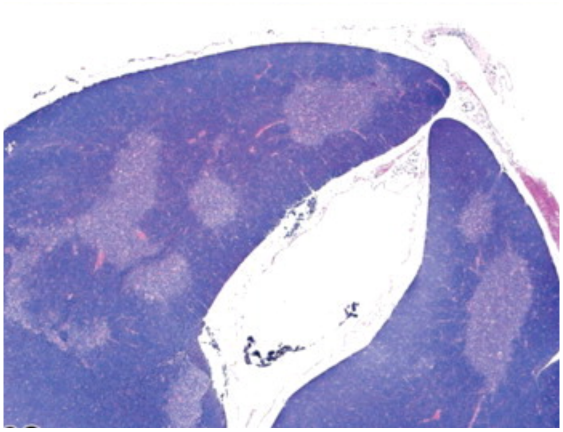

Figure 4. Thymus morphology in the mouse. Hematoxylin and eosin staining of a normal thymus from a 3-month-old B6C3F1 mouse showing the cortex in dark blue and the medulla in light blue. Occasional pink areas inside the organ are stained blood vessels. Figure adapted from [16].

1.2.2 Antigen presentation in the thymus

All thymic epithelial cells, namely cTECs and mTECs, are professional APCs and capable to present both MHC class I and class II molecules loaded with peptides. In the thymus, antigen presentation serves a different purpose than described in **1.1**: it prepares T cells for the encounter of a diverse repertoire of antigens in the periphery and prevents an inappropriate recognition of the host. Thus, the cortex is specialized in selecting the T cells with functional TCRs via a process called positive selection, whereas in the medulla the T cells with high affinity to the host are "sentenced to death" through negative selection [17].

1.2.3 Positive selection

The positive selection of DP T cells is mediated by cTECs which exclusively express thymoproteasomes, and the proteases cathepsin L and thymus-specific serine protease (TSSP). cTECs express unique peptide/MHC complexes at the cell surface for TCR engagement, and the TCR signaling strength determines the fate of T cells. A too low interaction results in "death by neglect", whereas excessive signaling can cause apoptosis. The intermediate affinity of the TCR to the peptide/MHC on cTECs signals the decrease in the CD8 coreceptor expression on DP cells. CD8 thymocytes require a timely (18-53 h) termination of the TCR signaling which allows cytokines to restore surface CD8 levels and induce expression of the transcription factors (TFs) specifying assignment to the CD8 lineage, e.g. Runx3. Since the TCR intensities may vary, the cytokine signaling is used as a "compensatory" mechanism to ensure correct commitment to the CD8 lineage (that is, stronger TCR signals accelerate cytokine responsiveness). If TCR signaling continues, it results in the expression of the TF ThPOK which prevents induction of Runx3 and determines commitment to the CD4 lineage [18]. cTECs express the CD83 receptor on the cell surface, which limits the MHC II turnover and allows longer signaling times of CD4 T cells. Thus CD4 T cells are selected based on stronger TCR signals than CD8 T cells [19].

Whereas the extracellular domains of the CD4 and CD8 coreceptors couple to the MHC II and MHC I molecules respectively, the intracellular tails associate with the Src family protein tyrosine kinase Lck, which is needed for transduction of the TCR signals. CD8 coreceptors inherently attach to low amounts of Lck molecules, limiting the MHC I signaling intensity.

Thus, the strength of TCR signaling determines lineage decision in positive selection, and it is defined by the persistence or cessation of the CD4 and CD8 coreceptor expression [18].

1.2.4 Negative selection

Following positive selection, thymocytes migrate to the medulla. mTECs and medullary DCs that are responsible for the negative selection of self-recognizing TCRs constitutively express immunoproteasomes and constitutive proteasomes. As a safeguard against autoimmunity, mTECs ectopically express a myriad of antigens from peripheral tissues, named tissue-restricted antigens, for presentation to T cells. This phenomenon is controlled by the autoimmune regulator AIRE, and perhaps the transcription factor Fezf2 [20]. High-affinity TCR interactions with the self-peptide/MHC induces thymocyte apoptosis. Therefore, immunoproteasomes facilitate the cleavage of an otherwise exhausting pool of proteins, but also shape the TCR repertoire of CD8 T cells [21].

Similar to cTECs, mTECs display a high rate of macroautophagy. TECs inefficiently use the classical endocytic pathway for MHC II loading, and instead fuse autophagosomes loaded with intracellular proteins with the endosomal compartments. Cell-surface presentation of self-antigen/MHC II complexes shapes the repertoire of CD4 T cells [21].

1.2.5 Agonist selection and unconventional T cells

Approx. 98% of DP thymocytes die an apoptotic death due to failing positive selection, or, much less often, due to negative selection [4]. Nonetheless, in exceptional cases T cells receiving strong TCR signals evade apoptosis and develop into unconventional T cells, i.e. natural regulatory T cells (nTregs), invariant natural killer T (iNKT) cells. This process is called agonist selection [22,23].

iNKT cells are αβ T cells that recognize CD1 non-polymorphic molecules loaded with lipid antigens on the surface of DCs. iNKT cells branch off from the DP stage of thymocyte development in the thymus and lose the CD4 and CD8 coreceptors to become DN cells. iNKT cells originate preferentially from DP cells with high-affinity self-ligands and development of this T cell lineage is dependent on the interaction between the CD28 and B7 costimulatory molecules [23,24].

Finally, nTreg development results from the strong reactivity of CD4 thymocytes to self and are identified by expression of Foxp3. The availability of interleukin-2 (IL-2) and transforming growth factor β (TGFβ) in the thymic medulla is favorable and limiting for the commitment to the nTreg fate. Paradoxically, the role of nTreg in the periphery is to promote T cell tolerance to self-antigens and to prevent autoimmune diseases, and it differs from iTregs in that nTregs develop in the thymus, whereas iTregs in the periphery (section 1.1.1) [23].

Another unconventional T cell lineage are γδ T cells, which arise from the DN stage. TCR rearrangements during early T cell development result in αβ or γδ TCR T cells. DN thymocytes rearrange the β, γ and δ TCR genes. The successful in-frame rearrangement of the TCRβ is necessary for the expression of the pre-TCR, a prerequisite to the αβ TCR. This induces the silencing of the TCRγ gene and initiation of the TCRα gene rearrangement. Transition to the DP stage is a hallmark of αβ T cell commitment, whereas γδ T cells with successfully rearranged γ and δ TCRs egress to the periphery as DN cells [22].

1.3 Research context

1.3.1 The thymoproteasome

The proteasome activity is known to be required for MHC class I but not for class II antigen presentation [25]. Since the identification of β5t in 2007, scientists have been trying to understand how its specific activity in cTECs influences the positive selection of CD8 T cells. It has been shown that absence of the thymoproteasome exclusively depletes and affects the repertoire of CD8 T cells ($CD4^-CD8^=TCR\beta^+$ cells) and not the CD4 population ($CD4^+CD8^-$ $TCR\beta^+$ cells), based on cell numbers and distribution of a set of TCR specificities. CD8 T cells developed in the absence of PSMB11 show impaired allogeneic and antiviral responses, despite normal thymic morphology [26,27]. Thymoproteasomes display decreased chymotrypsin activity compared to constitutive proteasomes and immunoproteasomes, theoretically resulting in MHC I-associated peptides (MIPs) with hydrophilic C-termini and low MHC affinity. However, evidence of similar half-life of peptide/MHC complexes between cTECs and mTECs went against this hypothesis [28].

It has also been proposed that the role of proteasomes is to solely produce a different repertoire of peptides relative to the other intrathymic APCs. However, replacement of the β5t locus with the β5i gene in β5i-deficient mice did not rescue the CD8 thymocyte numbers. The role of PSMB11 is also not to prevent excessive thymocyte deletion during positive selection, since the inactivation of the cell-death mediator Bim did not restore the number of CD8 thymocytes [17].

Since the nature of MIPs cannot be directly assessed with the current mass spectrometry technology which requires 10^5 times more cells than can be isolated from one mouse (approx. 10^4 cTECs) [26], researchers examined 100 MIPs on the surface of fibroblasts with transgenic expression of β5t or β5i. This indirect approach unveiled a differential pattern of the amino-acids involved in TCR recognition, and it was inferred that thymoproteasomes generate low TCR-affinity peptides [29]. It is known that CD8 thymocytes in PSMB11-deficient thymi receive a stronger TCR signaling compared to cells in WT mice based on two widely-accepted readout molecules, CD5 and Nur77 [30]. However, 100 MIPs are not enough to draw a strong conclusion and the precise role of thymoproteasomes in positive selection has not been elucidated to date.

1.3.2 Objectives

None of the studies mentioned in **1.3.1** examined a crucial aspect of proteasomes, the regulation of essentially all cellular processes. The cross talk between thymocytes and the cortical microenvironment is critical for the development of T lymphocytes during positive selection (Robey and Fowlkes, 1994). Therefore, we hypothesized that PSMB11 orchestrates cellular functions in cTECs that are peptide-independent, yet essential for thymocyte development. More precisely, we wondered whether a distinct proteolytic activity in PSMB11-deficient cTECs might change the levels of activation and survival-inducing molecules central to thymocyte development. This work had two main objectives:

1) to understand how thymoproteasomes regulate the cTEC biology using a genome-wide RNA-Sequencing (RNA-Seq) approach

2) to evaluate the impact of 1) on thymocyte development. We employed RNA-Seq, flow cytometry and microscopy to examine the cell numbers and the phenotype of CD4 and CD8 thymocytes, including proliferation ability, resistance to apoptosis and cytokine responsiveness.

Chapter 2 addresses these questions in the form of a manuscript in preparation.

Chapter 2 – PSMB11 shapes the transcriptome of cTECs and has pervasive effects on both CD4 and CD8 thymocyte population

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This article is in preparation as of August 2017.

2.1 ABSTRACT

How thymoproteasomes contribute to the positive selection of CD8 T cells is still not completely clear. Indirect evidence led to the dogma that PSMB11 generates a unique peptide repertoire with low T cell receptor (TCR) affinity for major histocompatibility complex (MHC) class I presentation. However, the role of proteasomes in cellular functions has been largely disregarded. Herein, we aimed to investigate if PSMB11 might regulate the cortical thymic epithelial cell (cTEC) biology with an effect on T cell development. We found that thymoproteasomes have an overall repressive activity on the transcriptome of cTECs, principally on the genes encoding extracellular matrix (ECM) components, cell-cell adhesion molecules and tumor-necrosis factor (TNF)-associated chemokines. We show that post-selection T cells of both CD4 and CD8 lineages undergo significant stress in the PSMB11-deficient thymus, likely due to extended interactions with cTECs. CD8 thymocytes appear to be reprogrammed to commit to apoptosis or unconventional T cell lineages, whereas CD4 thymocytes show transcriptional stimulation of recovery and survival genes. Although it remains possible that CD4 thymocytes are affected by the decline in the CD8 cell number, our data suggest a direct effect of PSMB11-deficiency on the selection of CD4 T cells through a decrease in the transcription of *Prss16* and *Cd83*. In summary, our study describes novel means of PSMB11 to orchestrate CD4 and CD8 thymocyte development, by controlling the matricellular and chemokine homeostasis in the thymic cortex.

2.2 INTRODUCTION

Vertebrates express two main types of proteasomes: constitutive proteasomes and immunoproteasomes (Kniepert and Groettrup, 2014). Via proteolysis of cellular proteins, both types of proteasomes regulate many basic cellular processes including cell cycle progression and division, differentiation and development, morphogenesis and response to stress (Glickman and Ciechanover, 2002; de Verteuil et al., 2014). In addition, degradation of proteins by proteasomes generates peptides that are presented by MHC class I proteins (Kincaid et al., 2012; de Verteuil et al., 2010). Quite remarkably, Murata and colleagues reported in 2007 that cTECs expressed a third type of proteasome that they named the thymoproteasome (Murata et al., 2007). Thymoproteasomes share two catalytic subunits with immunoproteasomes (PSMB9 and PSMB10) and contain a third catalytic chain which is unique to cTECs, PSMB11. *Psmb11*$^{-/-}$ mice display defective positive selection of CD8 thymocytes in the thymus, and their CD8 T cells show impaired antigen responsiveness (Nitta et al., 2010; Takada et al., 2015; Xing et al., 2013).

Given that MHC I peptides (MIPs) regulate the positive selection of CD8 thymocytes, it was logical to posit that the key role of thymoproteasomes might be to generate a unique MIP repertoire (Nitta et al., 2010). Indeed, since they are shown to have a lower chymotryptic activity than other types of proteasomes, it was suggested that thymoproteasomes might generate peptides with hydrophilic C-termini and thereby a low MHC binding affinity (Florea et al., 2010). However, indirect approaches failed to support the idea that thymoproteasomes generate unstable peptide-MHC I complexes: the cell surface density and half-life of MHC I-peptide complexes is similar in wild-type (WT) and *Psmb11*$^{-/-}$ cTECs (Nitta et al., 2010; Xing et al., 2013). To gain some insights into the impact of PSMB11 on the MIP repertoire, Sasaki et al. therefore sequenced about 100 MIPs extracted from mouse embryonic fibroblasts transfected with PSMB8 or PSMB11 and treated with IFN-γ (Sasaki et al., 2015). MIPs from the two cell types showed similar MHC I binding affinity, hydrophobic C-termini and anchor residues. However, analysis of 48 MIPs unique to PSMB8- or PSMB11-transfected cells revealed some discrepancies in residues located in the center of MIPs (P3 and P4). These provocative results suggest that Psmb11 generates MIPs with a relatively low T-cell receptor binding affinity (Sasaki et al., 2015). Nevertheless, they must be considered with some reserve for two reasons: i) it is difficult to estimate whether 100 MIPs extracted from transfected embryonic fibroblasts are representative of the 10,000 MIPs present at the surface of primary TECs (Granados et al., 2015) and ii) mechanistically, it is difficult to envision how PSMB11-mediated cleavage might affect the center rather than the extremities of MIPs (de Verteuil et al., 2010).

It is important to consider that the non-redundant functions of different types of proteasomes go well beyond MIP production (Kniepert and Groettrup, 2014). Accordingly, replacement of constitutive proteasomes by immunoproteasomes has pleiotropic effects on cell functions including proliferation, survival and differentiation (de Verteuil et al., 2014) and may even enhance organismal lifespan (Pickering et al., 2015). These considerations prompted us to evaluate whether PSMB11 might have some MIP-independent effect on cTEC biology.

Our RNA-Sequencing (RNA-Seq) analyses indicate an overall repressive activity of thymoproteasomes on the transcriptome of cTECs. In the absence of PSMB11, cTECs upregulate expression of extracellular matrix (ECM) organization molecules and cytokines, which potentially extends the contact time with thymocytes and impairs the thymocyte migration patterns. Our data show that lack of PSMB11 has a more pervasive effect on thymocyte development than the changes at the peptide level described in other studies (Nitta et al., 2010; Xing et al., 2013), which appears to distress both helper and cytotoxic T cells developing in the absence of PSMB11. Thus, our study introduces a model of transcriptional regulation by which PSMB11 orchestrates CD4 and CD8 T cell development.

2.3 RESULTS

2.3.1 PSMB11 regulates genes involved in chemokine-mediated signaling and cell adhesion-related processes

The concept that proteasomes regulate the level of transcriptional regulators by ubiquitin-mediated proteolysis is well established (Geng et al., 2012). Therefore, we performed RNA-Sequencing (RNA-Seq) to assess the overall impact of thymoproteasomes on the transcriptome of cTECs from 10-week-old mice. cTECs were defined as thymic epithelial cells (TECs) (Epcam+CD45-) with a Ly51+UEA1- staining pattern (Supplementary Figure 9A). By comparing cTECs from PSMB11-deficient mice to WT control mice in three replicates, we found that among the genes with FPKM higher than 1, 851 were differentially expressed genes (DEGs) with an adjusted p-value lower than 0.1 and a fold change of at least 1.4. Of these, 681 and 170 DEGs were over- and under-expressed in the *Psmb11*$^{-/-}$ condition, respectively (Figure 5A).

Gene ontology (GO) term and KEGG-pathway enrichment analyses of the 851 DEGs highlighted processes linked to the interaction between TECs and thymocytes: chemotaxis, cell-

cell adhesion, ECM organization (Figure 5B). The TNF-induced genes *Cxcl2*, *Cxcl9* and *Ccl5*, which serve as chemoattractants for hematopoietic progenitors to the thymus or for T cell emigration (Bichele et al., 2016) were upregulated in cTECs from *Psmb11*$^{-/-}$ mice (Figure 5C). Notably, previous comparative analyses of the transcriptome between cTECs and mTECs have shown that *Cxcl2*, *Cxcl9*, *Ccl5* and *Ccl11* are primarily expressed by mTECs (Bunting et al., 2010; Gotter et al., 2004). In line with this, the transcripts of CCL25 and CXCL12, which are the major chemokines secreted by cTECs (Gameiro et al., 2010), decreased in the absence of PSMB11. These results imply an altered chemokine gradient with potential slow-down of thymocytes in their cortex-to-medulla migration. Notably, *Ccl2* was also upregulated in the *Psmb11*$^{-/-}$ cTECs. Even though CCL2 can recruit peripheral dendritic cells (DCs) to the cortex (Lopes et al., 2015), we found no increase in the abundance of DCs in *Psmb11*$^{-/-}$ thymi (data not shown).

Positive selection is accompanied by high-speed migration interrupted by brief migratory pauses (Ross et al., 2014). Acting in synergy with chemokines, the ECM components facilitate thymocyte migration and compartmentalization, and also support thymocyte adhesion to TECs, with the thymic medulla containing a denser laminin network than the cortex (Emre et al., 2013; Gameiro et al., 2010; Lannes-Vieira et al., 1991). The integrin subunit alpha m gene (*Itgam*), and components of the fibronectin (*Fndc1*, *Fndc7*) and collagen (16 genes) families were collectively upregulated in cTECs in the absence of PSMB11 (Figure 5C). Additionally, a group of laminin and laminin-associated genes had elevated expression in the *Psmb11*$^{-/-}$ cTECs, *Lama1*, *Lamb2*, *Nid1*, *Ntn4*. By similarity to the laminin-related protein *Ntn1*, the products of these genes may increase thymocyte adhesion in the cortex (Guo et al., 2013). Akin to laminins, the expression of *HpsE*, *Vit*, *Parvb* and *Mmp3* was elevated in cTECs lacking thymoproteasomes, which could promote cell-adhesion by degradation of the ECM (Uniprot). Thus, we infer that a denser ECM in the cortex of *Psmb11*$^{-/-}$ mice can affect the migration pattern of thymocytes and their communication with cTECs.

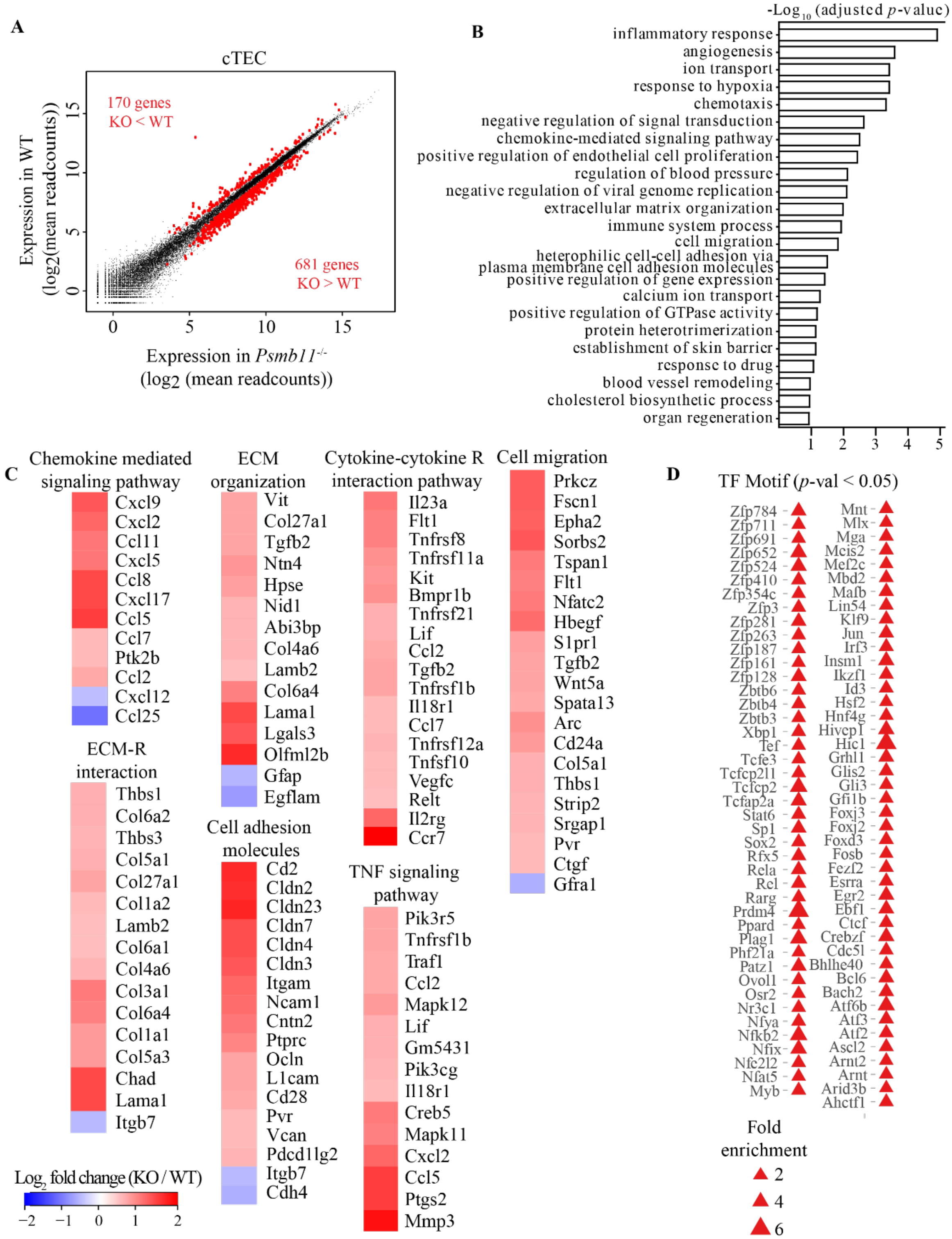

Figure 5. PSMB11 is a master regulator of the transcriptome in cTEC. (A) Differential gene expression from cTECs between WT and *Psmb11*$^{-/-}$ mice at 10 weeks of age (mean of 3 replicates per genotype). Differentially expressed genes (DEGs) in red, with

an adjusted p-value < 0.1, FPKM > 1, fold-change between WT and *Psmb11*$^{-/-}$ > 1.4. (B) GO term analysis of the DEGs in cTECs showing the most significant biological processes identified. (C) Heatmap of the DEGs involved in the biological processes and KEGG pathways associated with cell adhesion, cell migration and cytokine signaling from (B). (D) Transcription factors (TFs) identified as enriched in the DEG dataset for cTECs, based on the most common motifs in the promoter regions. The size of the triangles increases with the fold enrichment. Related to Figure S9.

Lastly, several members of the TNF (*Tnfrsf21*, *Tnfrsf8*, *Tnfsf10*, *Lif*) and tumor growth factor (TGF) (*Tgfb2*, *Gdf7*) families were also among the upregulated DEGs in cTECs (Figure 5C). The cytokine-dependent activation of NF-κB is necessary for thymocyte survival and transition to mature stages (Xing et al., 2016). Thus, although these signaling events occur in the medulla of WT thymi, we found that lack of PSMB11 might influence the secretion of these cytokines in the cortex and therefore affect thymocyte co-stimulation and the timing of maturation. Overall these results indicate that PSMB11 balances the expression of several chemokines and adhesion molecules involved in guiding post-selection T cells through their thymic development.

2.3.2 *Psmb11*$^{-/-}$ cTECs resemble mTECs in their transcription factor activity

In order to identify possible transcriptional regulators of the DEGs in cTECs, we employed two types of analyses of their promoter regions. Motif enrichment analysis using CIS-BP (Catalog of Inferred Sequence Binding Preferences) (Weirauch et al., 2014) with a 10% false discovery rate (FDR) revealed 118 transcription factor (TF) binding motifs enriched 1.5 to 4 times in the promoters of the downregulated genes, whereas only 19 possible TFs were found for the upregulated genes. In parallel, we performed multiple sequence alignments of the promoter regions of the DEGs using the motif-based sequence analysis tool MEME (Bailey and Elkan, 1994), and examined the top 4 consensus motifs identified (Supplementary Figure 9B). These motifs were similar to the binding sites for 10 TFs in the Tomtom database (meme-suite.org) which were found to be enriched in our dataset, namely RARG, EGR2, FOXJ3, FOXD3, FOXJ2, SP1, IRF3, ELF3, RREB1 and GLIS2 (Figure 5D). The retinoic acid receptor γ (RARG) has been shown to preferentially restrict cTEC cellularity, but also to modulate T lymphopoiesis (Joseph et al., 2016; Sitnik et al., 2012). The ubiquitous TF SP1 is implicated in a number of essential cellular processes, including proliferation, apoptosis, DNA damage response, differentiation, and additionally in inflammation and epigenetic silencing, being strongly associated to the *Prdm1*-mediated autoimmunity in mTECs (Roberts et al., 2017). Moreover, the TF ELF3 is one of the top 50 targets of HDAC3 in the thymic medulla (Goldfarb et al., 2016). In addition, RREB1 activation was also found to be induced by TNF signaling in the thymic stroma, and might be responsible for the upregulation of mTEC-related cytokines in *Psmb11*$^{-/-}$ thymi (Bichele et al., 2016). Altogether, these data suggest that PSMB11 provides a unique cellular program to cTECs by controlling the homeostasis of TFs, whereas the absence of thymoproteasomes skews the transcriptional activity to an mTEC phenotype.

Of note, the *Psmb11* deletion had no impact on the transcriptome of mTEC^{hi}, while 26 genes were differentially expressed in mTEC^{lo} between WT and *Psmb11*$^{-/-}$ mice (Supplementary Figure 9C). PSMB11^{+} cTEC precursors are thought to also give rise to the mTEC^{lo} subset which then differentiate into mTEC^{hi} [31]. Thus, our results show that the DEGs identified in cTECs are not spurious differences, but they are induced specifically by lack of PSMB11.

2.3.3 *Psmb11* deletion affects CD4 and CD8 thymocyte maturation irrespective of age

In order to assess the impact of *Psmb11* deletion on αβ T cell development, we examined the cell number of post-selection thymocytes at the semi-mature, mature 1 and mature 2 (SM, M1, M2) developmental subsets defined by Hogquist and colleagues (Hogquist et al., 2015) (Figure 6A). We found that in *Psmb11*$^{-/-}$ mice, CD8 T cells had a strong decrease in numbers and percentages at all three stages of maturation (Figure 6B and Supplementary Figure 10A). Moreover, the development of CD8 T cells was similarly impaired in 7-month-old *Psmb11*$^{-/-}$ mice as those at 4-5 weeks of age.

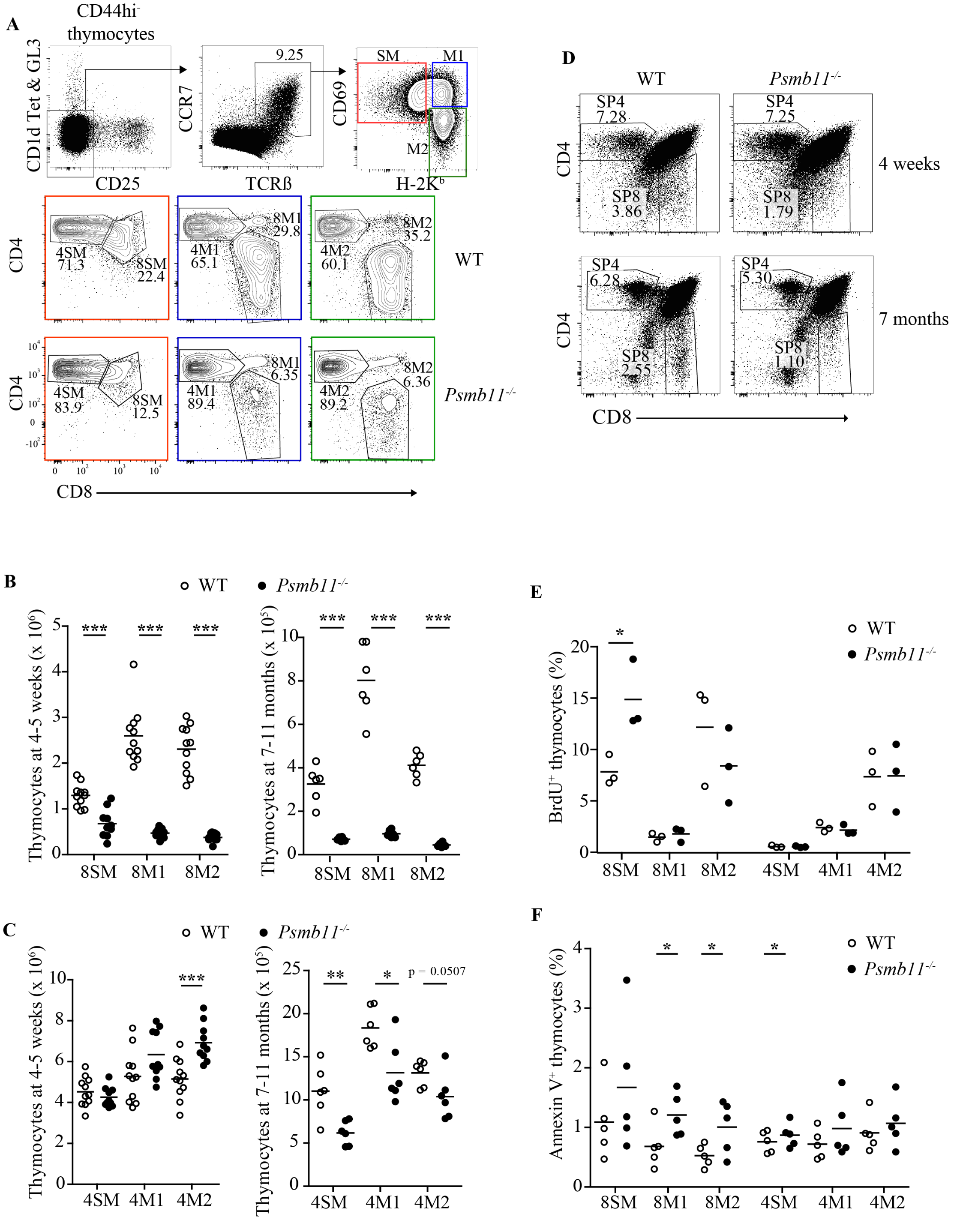

Figure 6. Impact of *Psmb11*-deletion on CD4 and CD8 thymocyte numbers. (A) Flow cytometry analysis of thymocytes from WT and *Psmb11*$^{-/-}$ mice. CD1d–α-GalCer tetramers as well as antibodies against CD25 and TCRγδ. Contour plots show the percentages of SM, M1 and M2 elements among post-selection CD4 and CD8 thymocytes (CCR7^{+}TCRβ^{+}). Numbers adjacent to the outlined areas indicate percentages of the parent population. Gating representative for at least 20 experiments. (B) Absolute numbers of CD8 thymocytes in the

SM, M1 and M2 subsets in mice at 4-5 weeks and 7 months of age. (C) Absolute numbers of CD4 thymocytes in the SM, M1 and M2 subsets in mice at 4-5 weeks and 7 months of age. (D) Representative gating of total thymocytes in WT vs. *Psmb11*$^{-/-}$ mice showing the percentages of CD4 and CD8 thymocytes. (E) Flow cytometry analysis of BrdU incorporation into WT and *Psmb11*$^{-/-}$ CD4 and CD8 thymocytes after 24 h of chase. Each dot represents the proportion of BrdU+ cells in one mouse. (F) Annexin V staining of WT and *Psmb11*$^{-/-}$ thymocytes. Each dot represents the percentage of AnnexinV^{+} thymocytes in one mouse. 7-ADD^{+} thymocytes were excluded from the analysis. Related to Figure S2.

Strikingly, the number of CD4 thymocytes in the thymus of 4-5-week-old mice lacking PSMB11 was significantly increased in the 4M2 subset (Figure 6C). The effect of PSMB11 deletion on the CD4 lineage was masked by population averaging in young mice, yet at 7 months old the total CD4 single-positive T cell population as well as discrete 4SM, 4M1 and 4M2 subsets show a slight decline in the percentage and absolute numbers (Figure 6C and D, and Supplementary Figure 10B). This indicates that absence of PSMB11 affects the commitment of thymocytes to both CD4 and CD8 lineages. Although we observed an age-dependent effect on the CD4 subset, we decided to focus on the 4-5-week-old group for further analysis.

2.3.4 CD8 thymocytes selected in *Psmb11*$^{-/-}$ mice show increased susceptibility to apoptosis

We considered two non-mutually exclusive explanations for the altered cellularity in the CD4 and CD8 cell subsets in *Psmb11*$^{-/-}$ mice, proliferation and apoptosis. To examine the proliferation ability, we performed in vivo 5-bromo-2’-deoxyuridine (BrdU) labeling in young adult mice. BrdU was pulsed twice at 2-hour intervals, and was chased for 24 hours to allow incorporation into newly synthesized DNA. The percentage of BrdU^{+} 8SM cells was greater in *Psmb11*$^{-/-}$ mice compared to the WT, suggesting a compensatory reaction to the abnormal decrease in the CD8 cell number (Figure 6E and Supplementary Figure 10C). The proportion of BrdU^{+} cells in all other CD8 and CD4 subsets was similar to the WT control.

The early apoptosis status was assessed using Annexin V staining. We found that a higher percentage of 8SM, 8M1 and 8M2 thymocytes were early apoptotic in the absence of PSMB11 (Figure 6F and Supplementary Figure 10D). Previous research has shown that splenic CD8 T cells developed in *Psmb11*$^{-/-}$ mice are functionally capable to survive in lymphopenic environments and undergo TCR-mediated proliferation (Nitta et al., 2010). Our results indicate that CD8 thymocytes selected in the absence of PSMB11 do not lose their proliferation ability, however these cells are more susceptible to apoptosis. Nonetheless, these experiments did not elucidate the changes identified in the cellularity of the CD4 subsets.

2.3.5 Lack of thymoproteasomes induces stress in developing thymocytes

To gain a global understanding of the commitment of thymocytes to the CD4 and CD8 lineages in the absence of PSMB11, we carried out RNA-Seq on discrete thymocyte subsets and used the DESeq2 package in the R software to extract DEGs between the WT and *Psmb11*$^{-/-}$ conditions. Differential gene expression was defined as genes with a fold change expression higher than 2 and an adjusted *p*-value lower than 0.1. We found a total of 304 DEGs in the CD8 thymocytes and 610 DEGs in the CD4 thymocytes between the WT and *Psmb11*$^{-/-}$ conditions, with little overlap between the maturation subsets (Figure 11A and Appendix 1-6).

Our RNA-Seq results showed a 20-80-fold upregulation of the *Hspa1a* and *Hspa1b* genes, which encode for the stress-responsive HSP70 chaperone, in all CD4 and CD8 thymocyte subsets from *Psmb11*$^{-/-}$ mice (Figure 7A). This prompted us to perform immunofluorescence staining for HSP70 on thymic slices from 4-week-old mice. By comparing *Psmb11*$^{-/-}$ vs. WT thymi for the areas of medulla and cortex with an HSP70 intensity at least 3 times higher than the isotype (that is, pixels above a threshold of 12, Supplementary Figure 11A and B), we detected a significant increase in the HSP70 levels in the medulla, but not in the cortex of thymi deficient in PSMB11 (Figure 7B and C). These results demonstrate that the cellular stress was elevated in thymocytes selected in the absence of PSMB11.

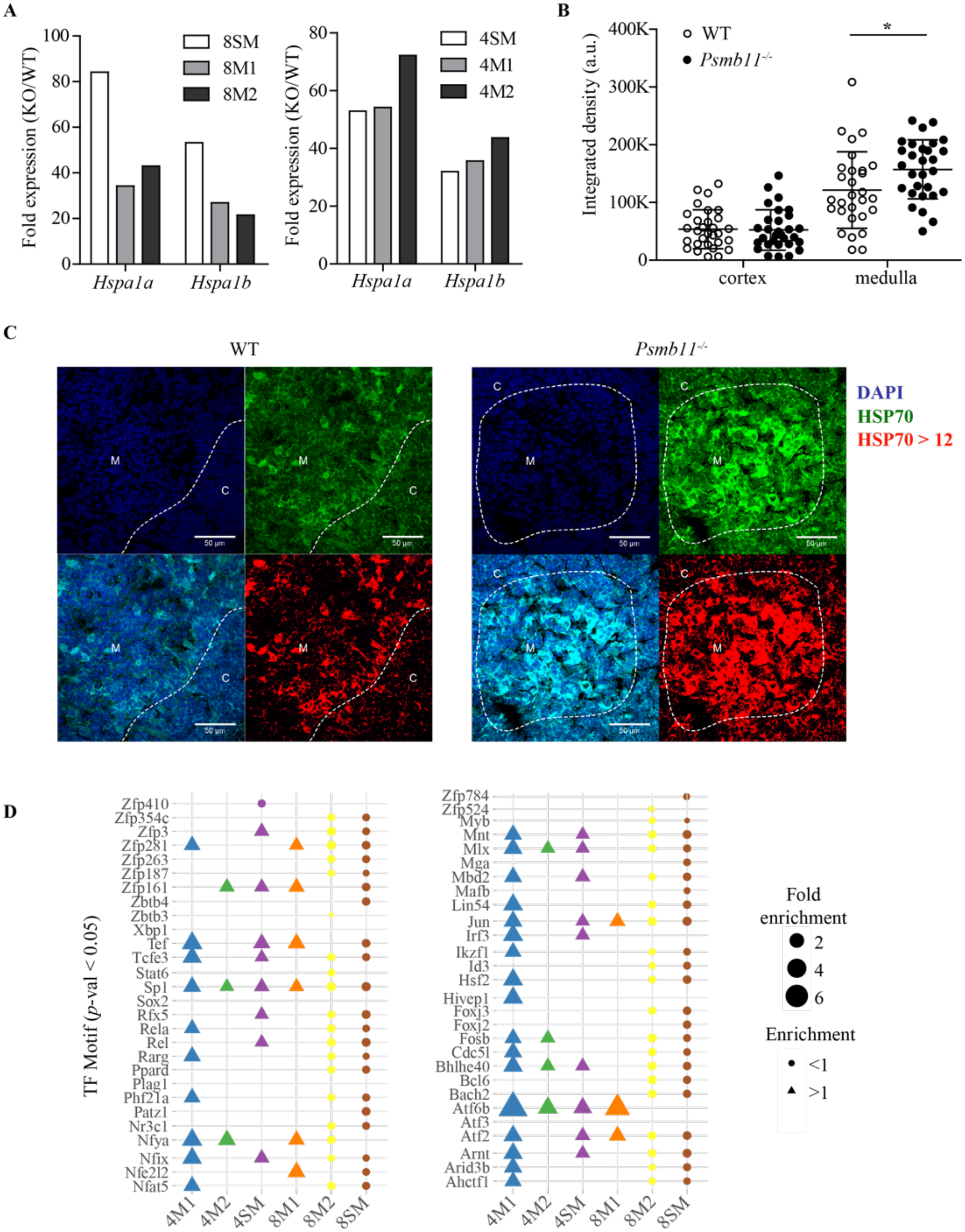

Figure 7. Loss of PSMB11 induces stress in developing thymocytes. (A) Bar chart showing the fold-change upregulation of *Hspa1a* and *Hspa1b* transcripts expressed as a ratio between the readcounts in *Psmb11*$^{-/-}$ and WT thymocytes. Data obtained by RNA-Seq. (B) Immunofluorescence quantification of the HSP70 intensity in the cortex and medulla of WT vs. *Psmb11*$^{-/-}$ mice (n = 2 mice per genotype). The tissue from 4-week-old mice was snap-

frozen and cut into 8 µm slices. Five areas of cortex and medulla were analyzed per tissue section, and three sections were chosen randomly at different Z-planes in the organ. (C) Representative images from immunofluorescence staining for HSP70 on thymic slices from WT vs. *Psmb11*$^{-/-}$ mice. The cortex and medulla areas were defined based on the nuclei abundance using DAPI. From left to right, the upper images represent DAPI (blue) and HSP70 (green), and the lower images show the merged channels and HSP70 quantification of pixels > 12 (red). Scale bar 50 µm. (D) Motif comparison on the promoter regions of the DEG in each thymocyte maturation subset, showing transcription factors (TFs) identified as enriched.

2.3.6 CD8 thymocytes show patterns of agonist selection, whereas CD4 thymocytes upregulate stress-responsive genes

Overall the CD8 cell subsets from *Psmb11*$^{-/-}$ thymi showed a gene expression pattern with signatures of CD4 and unconventional T cell lineages. To exemplify, 8SM and 8M1 thymocytes displayed a lag in the downregulation of *Zbtb7b* (Table 1). We found that 8M1 and 8M2 subsets upregulated transcripts restricted to regulatory T cells (Tregs), namely *Tigit* and *Foxp3*, and to γδ T cells, namely *Sox13*. Thus, this transcription pattern is suggestive of an agonist selection in response to stronger TCR signaling (Oh-hora et al., 2013). These data are consistent with the higher expression of negative selection-associated genes in cTECs, including *Pvr* (CD155, which has high affinity to TIGIT) (Qiu et al., 2010). Correspondingly, the expression level of *Ctla4* was also higher in 8M1 thymocytes from mice lacking PSMB11. Moreover, the upregulation of genes involved in apoptosis, *Faslg*, *Pdcd1*, *Gadd45b*, are in line with the increased Annexin V levels in the CD8 maturation subsets from *Psmb11*$^{-/-}$ mice (Table 1 and Figure 6F). Likewise, the increased expression of *Cldn10* and *Itga9* genes suggest an induction of negative selection signals which promote cell-cell adhesion (Schmitz et al., 2003). In conclusion, CD8 thymocytes appear to receive stronger TCR signals in the cortex of *Psmb11*$^{-/-}$ thymi and skew their expression pattern to unconventional T cell lineages.

Unlike the CD8 lineage, we found that CD4 thymocytes upregulate a group of genes encoding co-chaperones of HSPA1A and HSPA1B, suppressors of apoptosis (*Areg*, *Serpine1*, *Ppp1r15l*) and molecules involved in the shut-off of protein synthesis in response to stress (*Ppp1r15a*) (Table 2). We reason that the 4SM, 4M1 and 4M2 subsets in *Psmb11*$^{-/-}$ mice use stress-responsive mechanisms to rescue their fate from a stress-induced apoptosis and the initial decline in the percentage of 4SM cells (Supplementary Figure 10B).

In an effort to understand what signals trigger the transcriptomic changes in thymocytes, we again searched for enriched motifs in the promoter regions. Interestingly, all thymocyte subsets except 8M2 showed strong enrichment for the binding site of ATF6β (Figure 7D). The processing of ATF6β, an endoplasmic reticulum (ER) transmembrane TF, is activated in response to stress and appears to regulate the strength and duration of the unfolded protein response (UPR) mediated by the homologous protein ATF6α (Thuerauf et al., 2004). Although it is unexpected that lack of PSMB11 in cTECs would cause ER stress in thymocytes and we did not find differential expression of the UPR-specific genes, as stated above, we observed a general response to stressful stimuli in the CD4 subsets marked by the upregulation of HSP70-associated genes (Table 2).

The zinc finger protein Hivep1 was another possible upstream regulator of the genes with increased expression in the 8SM subset (Figure 7D), and its transient expression has been linked to the TCR-induced apoptosis (Schmitz et al., 2003). Similarly, upregulation of apoptosis genes in 8M1 might be induced by the aryl hydrocarbon receptor (AhR), which has been demonstrated to regulate FasL and NF-κB in stromal cells, but also cell motility genes in thymocytes (Camacho et al., 2005). These data suggest that CD8 thymocytes receive longer exposure to TCR signals in *Psmb11*$^{-/-}$ mice.

The SP1 TF was also identified as a putative regulator of the transcriptional changes in all thymocyte subsets (Figure 7D). However, since SP1 is a versatile partner for many TFs and is implicated in a wide array of cellular functions, it is difficult to clearly characterize its function in our model. In a similar situation is TEF, which is associated with DNA damage repair actions and the circadian clock rhythm (Siddiqui et al., 2017). Other TF identified, but with minimal annotation available were Zkscan1, Zkscan5, Cenpb, Hmbox1, Nfya and Smarcc2.

Table 1. **CD8 thymocytes in *Psmb11*$^{-/-}$ mice show aberrant expression of genes specific to unconventional T cell lineages.**

Subset	Gene	Log_2fold change (KO/WT)	Protein function
8SM	*Cxcr4*	1.25	Lymphoid progenitor localization to the cortex [32]
	Faslg	1.17	Initiator of apoptosis mediated by caspase activation [33]
	Zbtb7b	1.37	Necessary and sufficient for commitment of CD4 lineage [18]
8M1	*Cd4*	1.39	Co-receptor for MHC class II antigen/TCR interaction [34]
	Ctla4	1.03	Antagonistic homolog of CD28 [35]
	Zbtb7b	2.30	Necessary and sufficient for commitment of CD4 lineage.
	Fos	1.45	Members of the AP-1 DNA-binding complex [36]
	Fosb	1.57	
	c-Jun	1.54	AP-1 components [36]
	Junb	1.06	
	Tigit	1.59	Highly expressed and upregulated on Tregs after activation [37]
	Cldn10	1.04	Tight junction-specific obliteration of the intercellular space (UniProt ID: Q9Z0S6)
8M2[a]	*Itga9*	1.34	Laminin receptor involved in controlling thymocyte migration in the thymus [38]
	Pdcd1	1.02	Inhibitory cell surface receptor, associated to negative selection [39]

	Gadd45b	1.13	Negative selection- and stress response-associated, regulator of apoptosis [39]
	Sox13	1.28	γδ-T-cell lineage-restricted transcription factor that opposes development of αβ T cells [40]
	Foxp3	1.07	Transcriptional regulator crucial for the development and inhibitory function of Tregs [41]

Select DEGs from the 8SM, 8M1 and 8M2 thymocyte subsets showing genes whose expression is associated with unconventional T cells lineage, apoptosis or cell-cell adhesion. [a] means that all DEGs in 8M1, except *Zbtb7b*, are also present in the 8M2 subset.

Table 2. CD4 thymocytes in *Psmb11*$^{-/-}$ mice upregulate expression of stress-responsive and rescue genes.

Subset	Gene	Log_2fold change (KO/WT)	Protein function
4SM	*Hsph1*	1.53	Nucleotide-exchange factor for chaperones HSPA1A and HSPA1B (UniProt ID: Q61699)
	Dnaja1	1.00	Co-chaperone for HSPA1B, protects cells against apoptosis (UniProt ID: P63037)
	Dnajb4	1.34	Stimulates ATP hydrolysis and the folding of unfolded proteins mediated by HSPA1A/B (UniProt ID: Q9D832)
	Dnajb9	1.27	Co-chaperone for HSP70 (UniProt ID: Q9QYI6)
	Fzd4	3.48	Receptors for Wnt ligands, important for thymus development [42]
	Fzd5	1.58	
	Fzd7	1.00	
	Fosl1	2.29	AP-1 components (UniProt ID: P48755 and P47930)
	Fosl2	1.31	

	Areg	1.58	Promotes cell proliferation and inhibits apoptosis [43]
	Serpine1	1.04	Activated in stressed thymic tissue, role in the inhibition of apoptotic external signals and to the monocyte/macrophage chemotaxis [44]
4M1	*Areg*	2.47	Promotes cell proliferation and inhibits cell apoptosis.
	Dnajb4	1.17	Stimulates ATP hydrolysis and the folding of unfolded proteins mediated by HSPA1A/B.
4M2	*Hspa1*	1.30	Nucleotide-exchange factor for chaperone proteins HSPA1A and HSPA1B.
	Ppp1r15a	1.28	Reverses the shut-off of protein synthesis initiated by stress-inducible kinases and facilitates recovery of cells from stress [45]
	Ppp1r15l	1.47	Suppresses the activation of apoptosis (UniProt ID: Q5I1X5)

Select DEGs from the 8SM, 8M1 and 8M2 thymocyte subsets showing genes whose expression is associated with unconventional T cells lineage, apoptosis or cell-cell adhesion.

2.3.7 Data suggest impaired cytokine responsiveness and migration of CD8 thymocytes in *Psmb11*$^{-/-}$ thymi

A comprehensive analysis of MHC I positive selection by Kimura et al unveiled two phases of surface CD8 expression (Kimura et al., 2016). In phase 1, the TCR signaling intensity determines the extent of CD8 loss. Thus, the higher the TCR signaling intensity, the stronger the decline in surface CD8 at the intermediate maturation stage (CD4+CD69+CCR7+). Phase 2 of positive selection corresponds to cytokine signaling, which fully restores CD8 expression independently of the phase I levels.

Given the evidence that the role of PSMB11 in positive selection is to promote a weak TCR signaling intensity reflected by the CD5 levels on maturing thymocytes (Klein et al., 2014, Xing et al., 2013), we checked both CD5 and CD8β levels on post-selection thymocytes. We found that 8SM, 8M1 and 8M2 thymocytes had increased CD5 levels in the absence of PSMB11 (Figure 8A), consistent with bone-marrow (BM) chimera studies on total CD8 T cells (Xing et al., 2013). In addition, the CD8 coreceptor did not reach normal levels on these subsets in *Psmb11*$^{-/-}$ mice, indicating that phase 2 differentiation was also affected (Figure 8B). Although a longer phase 1 signaling is expected to increase cytokine responsiveness of the MHC I-restricted thymocytes, prolongation of TCR signaling results in lineage errors (Kimura et al., 2016). We found that 8M1 and 8M2 thymocytes, but not CD4 thymocytes, in the *Psmb11*$^{-/-}$ thymus showed slightly lower CD127 levels on the cell surface, causing CD8 cells to lose full cytokine response potential and aberrantly express transcripts specific to the CD4 lineage (Figure 8C and Table 1). We suspect that prolonged interactions with cTECs due to a different peptide/class I repertoire and abundance of matricellular molecules in the *Psmb11*$^{-/-}$ thymus predispose MHC I-restricted thymocytes to lineage errors. Correspondingly, we found that significantly more 8SM and 8M1 thymocytes in the *Psmb11*$^{-/-}$ condition are positive for CXCR4 relative to the WT, which supports the assumption that CD8 thymocytes have a longer retention in the cortex (Figure 8D).

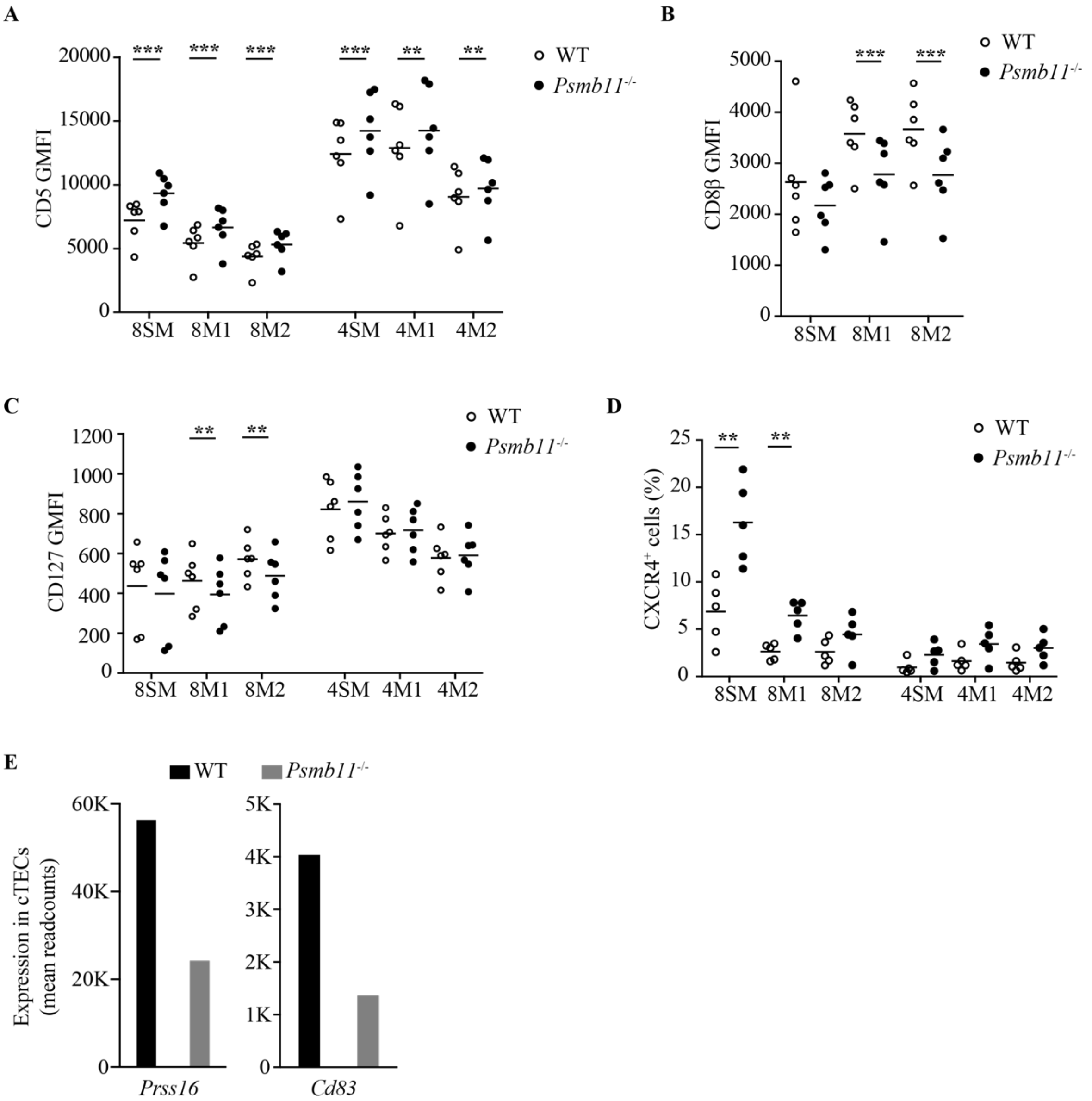

Figure 8. Impact of *Psmb11*-deletion on CD4 and CD8 thymocytes. (A) Geometric mean fluorescence intensity (GMFI) of CD5 on the surface of SM, M1 and M2 thymocytes in the CD4 and CD8 lineages from WT and *Psmb11*$^{-/-}$ mice. (B) GMFI of CD8β on the surface of 8SM, 8M1 and 8M2 thymocytes from WT and *Psmb11*$^{-/-}$ mice. (C) GMFI of CD127 on the surface of SM, M1 and M2 thymocytes in the CD4 and CD8 lineages from WT and *Psmb11*$^{-/-}$ mice. (A-C) Statistical analysis was determined using a paired Student's t-test, with $*P < 0.05$, $** P < 0.01$, $***P < 0.001$. (D) Percentages of CXCR4+ thymocytes. Each data point from WT is paired with a data point from the Psmb11-/- condition. (E) Expression level of *Prss16* and *Cd83* in WT and *Psmb11*$^{-/-}$ cTECs expressed in mean readcounts between 3 replicates. Data extracted from RNA-Seq (padj < 0.05).

2.3.8 Data suggest a direct impact of PSMB11 on the CD4 lineage

A notable characteristic of cTECs is constitutive macroautophagy and the expression of specific enzymes for MHC class II-peptide production, namely cathepsin L and the thymus-specific enzyme protease (TSSP, encoded by Prss16) (Klein et al., 2009; Mizushima et al., 2004). To our surprise, CD5 levels on the 4SM, 4M1 and 4M2 subsets were slightly but reproducibly higher in *Psmb11*$^{-/-}$ mice (Figure 8A). Together with the significantly elevated cellularity of the 4M2 subset and the extensive amount of DEGs identified at all CD4 maturation stages assessed, it was tempting to infer that an altered pool of endogenous proteins in the *Psmb11*$^{-/-}$ cTECs become substrates for lysosomal degradation and change the MHC II ligandome. Although this hypothesis remains valid, we found a more than two-fold downregulation in the expression of *Prss16* and *Cd83* in cTECs (Figure 8E), which have both been shown to control the positive selection of CD4 T cells (Klein et al., 2009; Liu et al., 2016; Rohrscheidt et al., 2016).

We conclude that several factors regulated by PSMB11 in cTECs might be responsible for the changes in the CD4 lineage. These factors include the MHC II levels and ligandome, the cytokine gradient and the ECM composition in the cortex (Luckheeram et al., 2012; Robey, 2016), as suggested by our RNA-Seq data from cTECs.

2.4 DISCUSSION

Our study provides strong evidence that PSMB11 is a master regulator of the transcriptome in cTECs, which is essential for the normal positive selection of both CD4 and CD8 T cells in the thymus. We surmise that population averaging has eluded other researchers from identifying an effect of *Psmb11* deletion on the CD4 lineage. Nonetheless, Nitta et al. have reported a significant increase in the number of CD4 T cells in the lymph nodes of *Psmb11*$^{-/-}$ mice (Nitta et al., 2010). We show here that this increase cannot be a mere consequence of the loss in CD8 T cells and thus an expansion of the CD4 T cell pool. Instead, it is likely a direct effect of lack of PSMB11 in cTECs at the antigen presentation and cell motility levels, and correlated with the greater abundance of the 4M1 and 4M2 subsets detected in the present study. Moreover, FACS analysis of thymocytes from *MHCII*$^{-/-}$*Psmb11*$^{-/-}$ mice confirmed that this expansion was not a result of CD8 thymocytes mistakenly engaging to a CD4 cell-fate (data not shown).

Although total single-positive cells appeared to have equal intensities of Nur77 and CD5 between WT and *Psmb11*$^{-/-}$ mice in another study (Xing et al., 2013), our data suggest that 4SM, 4M1 and 4M2 thymocytes receive slightly stronger TCR signaling in the absence of PSMB11 (Figure 8). We infer that longer interactions with cTECs would account for the elevated stress in thymocytes positively-selected in the absence of PSMB11. Considering that thymocytes migrate to the medulla within 12-24 h after positive selection, these data may reflect a tuned response of post-DP thymocytes to the changes in the cortical microenvironment resulted from lack of PSMB11 (that is, different peptide/MHC repertoire on cTECs [26], upregulation of pro-inflammatory cytokines and cell-adhesion molecules), and/or an increased sensitivity to the negative selection signals in the thymic medulla. The different responses elicited by CD4 vs. CD8 thymocytes at the transcriptome level may reflect the threshold of TCR signaling that these cells receive in a WT condition. In other words, CD4 thymocytes are accustomed to longer TCR signals in the WT (Kimura et al., 2016), whereas CD8 thymocytes seem to undergo agonist selection. It is also interesting to mention that the intensity of TCR signaling of different transgenic TCRs only weakly correlates with the independency of β5t-mediated peptides (Nitta et al., 2010). Our DEG dataset for cTECs contains genes encoding members of the TGF-β and TNF-α families, whereas post-selection CD8 thymocytes show impaired cytokine responsiveness which is independent of the TCR signaling intensity. Since the cytokine-dependent activation of NF-κB is essential for the completion of positive selection by CD8 thymocytes (Xing et al., 2016), we conclude that the dependency on β5t cannot be explained merely by the changes at the peptide level. Additionally, the fact that cytokine signaling is not required for commitment to the CD4 lineage (Kimura et al., 2016) might in part explain why CD4 thymocytes are less strongly affected by lack of PSMB11.

Surprisingly, we observe a seemingly paradoxical upregulation of mTEC-associated genes in *Psmb11*$^{-/-}$ cTECs. However, this may be explained by the fact that immunoproteasomes become the major form of proteasomes expressed in β5t-deficient cTECs (Nitta et al., 2010), reinforcing the importance of proteasomes in establishing a unique cellular program. We show here that cTECs lacking thymoproteasomes increase the expression of costimulatory and cell-cell adhesion genes, which contradicts a model in which the PSMB11-mediated peptide production is the sole stimulus for the positive selection of thymocytes. In the same direction goes our evidence that absence of a thymoproteasome-generated MIP repertoire directly affects the CD4 lineage as well. We favor the hypothesis that the cell-cell communication between cTECs and thymocytes is enhanced in the *Psmb11*$^{-/-}$ condition, and we believe that live-cell microscopy experiments could give a definitive insight into this possibility.

In summary, this study enhances the understanding of the fundamental role of thymoproteasomes in positive selection. Our results have revealed that PSMB11 provides the optimal microenvironment for the positive selection of both CD4 and CD8 thymocytes by determining the expression level of costimulatory and cell-cell adhesion molecules. Thus, the duration of TCR signaling and cytokine signaling in thymocytes are regulated by PSMB11 through the cytokine gradient and ECM composition.

2.5 EXPERIMENTAL PROCEDURES

2.3.9 Mice

C57BL/6 (B6), B6.B6CB-Psmb11^{tm1Smta} (*Psmb11*$^{-/-}$) and B6.129S2-H2$^{dlAb1\text{-}Ea}$/J (*MHCII*$^{-/-}$) mice were purchased from the Jackson Laboratory and maintained under specific-pathogen free conditions at the Institute for Research in Immunology and Cancer. All procedures were in accordance with the regulations of the Canadian Council on Animal Care and the University of Montreal.

2.3.10 Flow cytometry and cell sorting

Thymi were isolated and stromal cell enrichment was performed as previously described (Jain and Gray, 2014; St-Pierre et al., 2015), whereas thymocytes were extracted by mechanical force. CCR7 staining on thymocytes was performed for 20 min at 37 degrees with anti-CCR7 PE (4B12, eBioscience) or PE-Cy5 (4B12, BioLegend), followed by surface marker staining for 25 min with the following antibodies: anti-CD4 AF700 (RM4-5, BD Biosciences), anti-CD8α V500 (53-6.7, BD Horizon) or BUV395 (53-6.7, BD Biosciences), anti-TCRβ PE/Dazzle594 (H57-597, BioLegend), anti-MHCI APC (AF6-88.5.53, eBioscience) or PE (AF6-88.5, BD Biosciences), anti-CD25 APC-Cy7 (PC61, BioLegend), CD1d–α-GalCer tetramers BV421

(NIH), anti-TCRγδ BV421 (GL3, BioLegend), anti-CD44 BV650 (IM7, BioLegend), anti-CD69 PE-Cy7 (H1.2F3, BD Biosciences), anti-CD5 PE (53-7.3, BD Biosciences), anti-CD8β PE (H35-17.2, BD Biosciences), anti-CD127 PE (A7R34, eBioscience), and anti-Thy1.1 PE-Cy5 (HIS51, ThermoFisher) for cell sorting of thymocytes from BM chimera. Staining with anti-CXCR4 PE (2B11, eBioscience) was performed for 40 min at 4 degrees C in staining buffer (phosphate-buffered saline with 0.1% BSA). Cell viability and early apoptosis were assessed using 7-amino-actinomycin D (7-AAD) (BD Biosciences) and Annexin V AF350 (ThermoFisher). cTECs were stained for 25 min at 4 degrees C with APC-Cy7 anti-EPCAM (BioLegend), A700 anti-CD45 (BD), A647 anti-Ly51 (BioLegend), Biotinylated *UEA1* (Vector Laboratories), Streptavidin PE-Cy7 (BD), PE anti-I-A^b (BD). Flow cytometry was performed on a ZE5 (Bio-Rad) for thymocytes, and on an LSR-II (BD Biosciences) for stromal cells. Cell-sorting was performed using a three-laser FACSAria (BD Biosciences) and all data were analyzed using the FACS Diva or FlowJo softwares.

2.3.11 Immunofluorescence analyses

Frozen tissues were embedded in OCT compound (Sakura Finetek) and sliced into 8 µm-thick sections. Sections were stained with rabbit anti-HSP70 (polyclonal, Novus Biologicals), anti-rabbit PE/Texas Red and 4’-6-diamidino-2-phenylindole (DAPI, Life Technologies). Images were acquired with an LSM700 confocal microscope (Zeiss) and analyzed using ImageJ.

2.3.12 BrdU administration

Mice received two doses of 1 mg of BrdU (BD Biosciences) 2 hours apart by intraperitoneal injection. BrdU incorporation was detected 24 h after the first injection using a BrdU Flow Kit (BD Biosciences) following manufacturer’s instructions.

2.3.13 RNA sequencing

Total RNA was isolated using TRIzol (Invitrogen) and purified using RNeasy Micro columns (Qiagen) following the manufacturers’ instructions. Sample quality was assessed using the 2100 Bioanalyzer RNA Nano chips (Agilent technologies). Transcriptome libraries were generated from 75 ng total RNA for thymocytes using the TruSeq RNA Sample Prep Kit v2 (Illumina) following the manufacturer’s protocol. Paired-end (2 x 100 bp) sequencing was performed using

Illumina HiSeq200 sequencer for thymocytes and the Next-Generation Sequencer for cTECs by the staff at IRIC's genomics platform. Sequences were trimmed for sequencing adapters and low quality 3' bases using Trimmomatic version 0.35 and aligned to the reference mouse genome version GRCm38 (or mm10) using STAR version 2.5.1b by the personnel at IRIC's bioinformatics platform.

2.3.14 Promoter region analysis

Differentially expressed genes (DEGs) were analyzed for enriched transcription factor (TF) motifs using the R package RTFBSDB [46]. To detect possible motifs, an upstream region of 1kb was extracted from all DEGs based on their gencode annotated TSS (gencode.vM14.basic). For each dataset the repertoire of transcription factor motifs was adjusted only to those TFs deemed "on" in the dataset based on a 0.5 RPKM filter. Motifs for the resulting TFs were then extracted from CisBP and clustered by similarity (See RTFBSDB for details). Enriched motifs were then searched within the set of DEGs using gencode M14 basic annotations minus the DEGs as background for enrichment. GC correction, clustering by motif position weight matrixes (as per [46]), and a false discovery rate of 0.1 was applied in all enrichment tests.

De novo motif searching was done using MEME (meme-suite.org) using parameters: -dna -nmotifs 4 -minw 5 -maxw 15 -revcomp -mod zoops -maxsize 1000000 [47]. The TFs overlapping between the two analyses were considered for further discussion

2.3.15 Statistical test

Statistical significance was determined using the GraphPad Prism software, and calculated using a two-tailed unpaired Student's t-test unless stated otherwise. *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

2.6 AUTHOR CONTRIBUTIONS

A.A. designed the study, carried out experiments, analyzed data, discussed results and wrote the first draft of the manuscript. S.B. designed the study, carried out experiments, analyzed data and discussed results. A.A., S.B. and A.R. performed differential gene expression analysis of RNA-

Seq data. G.V. performed bioinformatics analysis of the promoters of the DEGs from cTECs and thymocytes. M. P. H. carried out experiments.

2.7 CONFLICT OF INTEREST

The authors have no financial conflict of interests.

2.8 ACKNOWLEDGMENTS

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2.10 Supplementary Figures

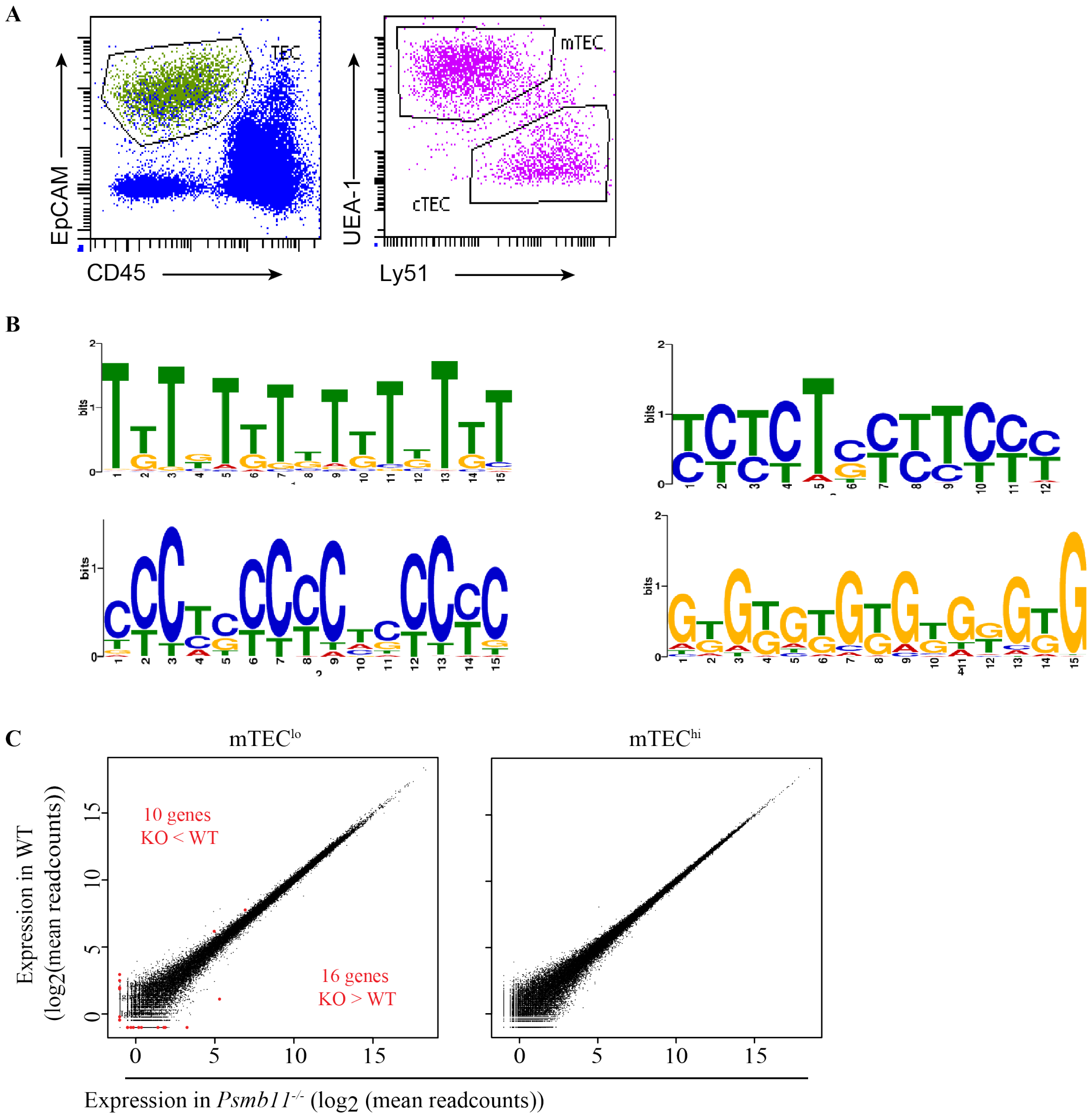


Figure 9. Analysis of TEC populations in WT and *Psmb11*$^{-/-}$ mice. (A) Flow cytometry analysis of cTECs and mTECs, representative from WT. TECs were defined as CD45- and EpCAM+, whereas cTECs were gated on Ly51+UEA-1- cells, and mTECs Ly51-UEA-1+ cells. (B) MEME analysis on the promoter regions of the DEGs in cTECs, showing the 4 most significant consensus motifs (in Position Weight Matrix representation) identified. (C) Differential gene expression analysis in mTEClo and mTEChi subsets (MHCII^{lo} and MHCII^{hi} respectively) between WT and *Psmb11*$^{-/-}$ mice. Red datapoints represent DEGs based on the criteria described in Figure 5A.

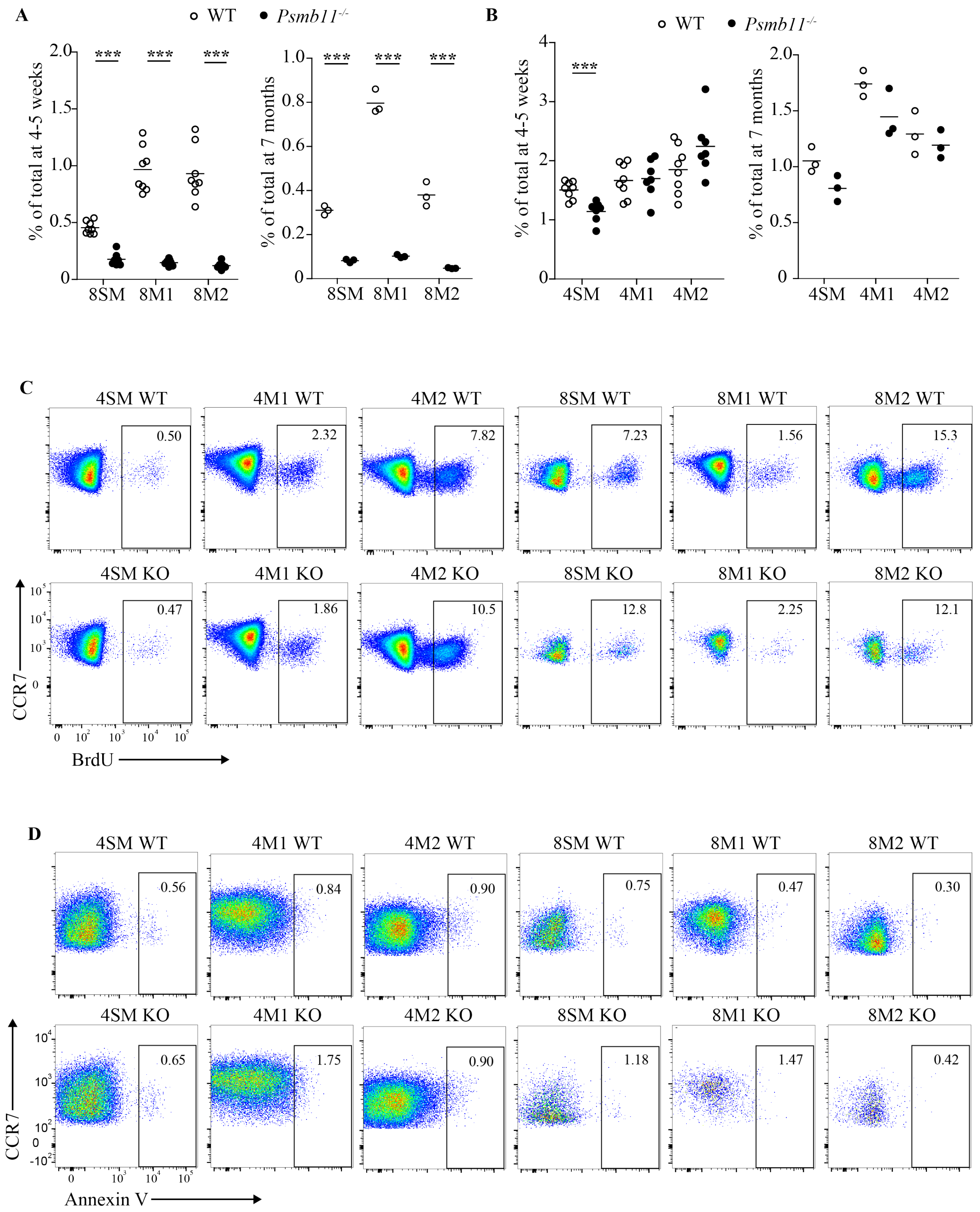
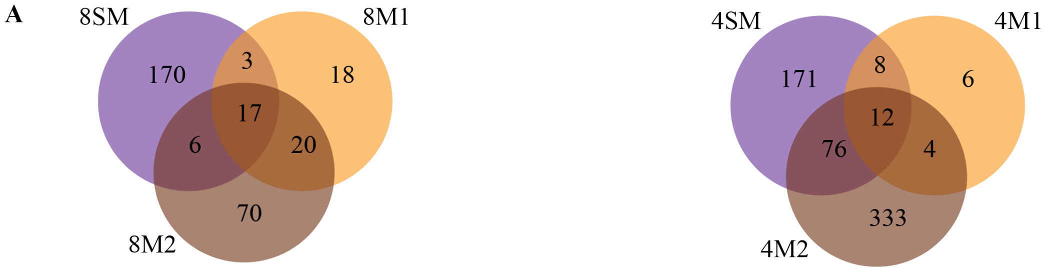


Figure 10. Flow cytometry analysis of the thymocyte maturation subsets. (A) Percentages of CD8 thymocytes in the SM, M1 and M2 subsets in mice at 4-5 weeks and 7 months of age. (B) Percentages of CD4 thymocytes in the SM, M1 and M2 subsets in mice

at 4-5 weeks and 7 months of age. (C) Representative flow cytometry analysis of BrdU incorporation into CD4 and CD8 thymocytes at the maturation stages SM, M1 and M2. Gate for BrdU+ cells was set using the PBS control. (D) Flow cytometry analysis of Annexin V staining between WT and *Psmb11*$^{-/-}$ mice. Each dot represents the percentage of Annexin V+ thymocytes at each maturation stage SM, M1 and M2 from the CD4 and CD8 lineages. 7-ADD+ thymocytes were excluded from the analysis. Gate for Annexin V+ cells was set using the flurescence-minus-one (FMO) control for the Annexin V channel.



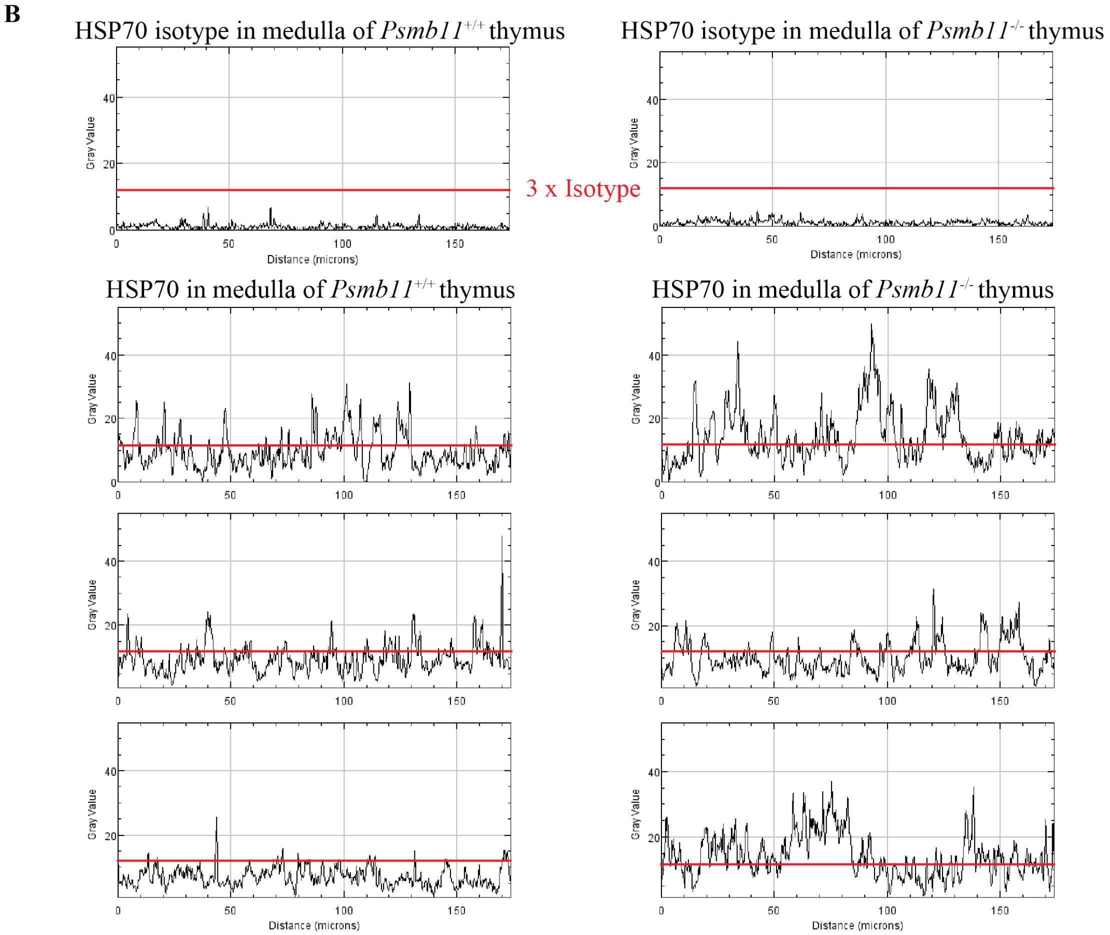


Figure 11. Immunofluorescence analysis of HSP70. (A) Representative images of the DAPI (blue) and HSP70 (green) stainings on WT and *Psmb11*$^{-/-}$ thymic slices showing the strategy for cortex and medulla delineation. Scale bar 100 µm. (B) Plot profiles of the intensity of HSP70 and the isotype in the medulla of WT and *Psmb11*$^{-/-}$ thymi. The red line on each graph was set at a threshold of 12 (3 times the intensity of the isotype) used for quantification of the HSP70 integrated density in Figure 7B.

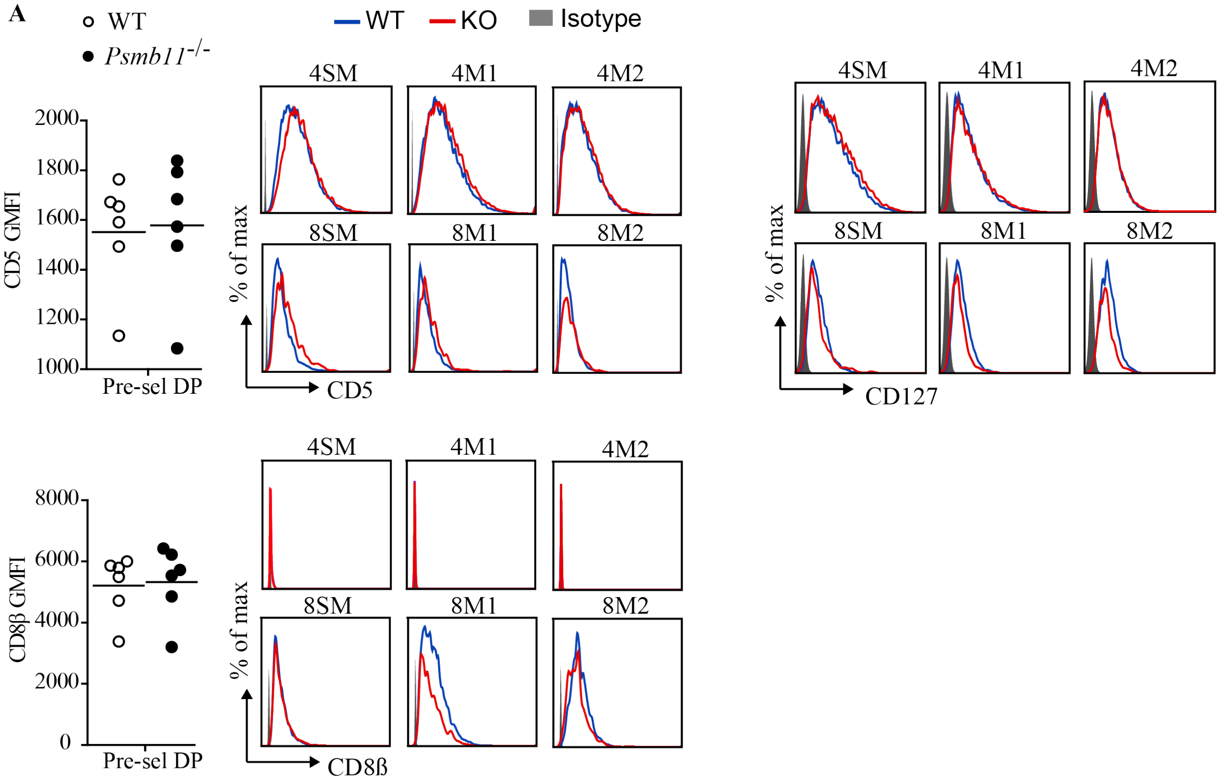


Figure 12. Impact of Psmb11 deletion on the phenotype of CD4 and CD8 subsets. (A) Flow cytometry profiles of the intensities of surface CD5, CD8β and CD127 on thymocytes from the SM, M1 and M2 maturation subsets in the CD4 and CD8 lineages. Gray shaded areas represent the isotype for each antibody, whereas the WT and *Psmb11*$^{-/-}$ conditions are shown in blue and red, respectively. Statistical analysis was determined using a paired Student's t-test, with $^{*}P < 0.05$, $^{**} P < 0.01$, $^{***}P < 0.001$.

Chapter 3 – Discussion & Perspectives

The present results help us understand the role of the cTEC-specific expression of thymoproteasomes. Our RNA-Seq data suggest that cTECs lacking thymoproteasomes increase their costimulatory capacity and the density of ECM components in the cortical microenvironment. We believe that previous studies have underestimated the importance of thymoproteasomes for the cellular homeostasis. Lack of immunoproteasomes triggers proteotoxic stress in mTECs [48], therefore by similarity we expected that absence of thymoproteasomes would induce upregulation of the ER stress-responsive genes *Xbp1*, *Ddit3*, *Hsph1* and others. However, the fact that we did not detect any significant changes in these transcripts goes in line with the finding that immunoproteasomes are the dominant form of proteasomes in PSMB11-deficient cTECs [26] and likely compensate for the decreased proteolytic activity. Herein, we introduce the concept that the transcriptome of *Psmb11*$^{-/-}$ cTECs is a footprint of the immunoproteasome, since several upregulated transcripts specific for mTECs reach a readcount level similar to the mTEC^{lo} subset, whereas the expression of genes specific to cTECs (e.g. *Cxcl12, Prss16*) are downregulated in our model organism. This finding implies that the cortical microenvironment resembles the medulla, including possibly higher levels of TNFa and TGFβ, thus the presence of a pattern of negative selection and increased TCR intensities become less surprising. Nevertheless, since the RNA cannot be taken as a surrogate for protein levels, the next step would be to check these findings at the proteomic level.

In this regard, it would also be interesting to understand how the assembly of the different forms of proteasomes is regulated and what induces the increased expression of β5i in β5t-deficient cTECs [26]. IFNg administration or systemic infection induce expression of immunoproteasome subunits in cTECs [11], but how and when this expression happens in *Psmb11*$^{-/-}$ mice is not known.

It is necessary to mention that lack of PSMB11 does not change the morphology of the thymus, and no difference was found in the number of DN and DP thymocytes, or in other professional APCs compared to the WT [26,27]. Although the cellular composition appears similar between WT and *Psmb11*$^{-/-}$ mice, no study has assessed the migration pattern of thymocytes in the PSMB11-deficient thymus. We feel that such experiments could answer two main fundamental questions:

1. How do post-selection thymocytes from WT mice react to the cortical microenvironment in *Psmb11*$^{-/-}$ mice? Would a possibly altered chemokine gradient change the cortex-to-medulla migration? An approach using cytokine-responsive thymocytes at the SM, M1 and M2 stages could answer how PSMB11 influences phase 2 of positive selection.
2. Inversely, these experiments could examine if 8SM and 8M1 thymocytes developed in the absence of PSMB11 are retained longer in the WT or *Psmb11*$^{-/-}$ cortex. A recent article reported that the expression of CXCR4 correlates with the stages of positive selection, and is dispensable for thymocytes after the pre-DP stage [32]. Nevertheless, the study did not examine the impact of transgenic CXCR4 expression on positive selection. We suspect that the delayed downregulation in CXCR4 at the 8SM and 8M1 stages reflects a change in the timing of positive selection in *Psmb11*$^{-/-}$ thymi.

Current limitations for live-cell microscopy on thymic slices are the low number of cells in each CD8 maturation subset per mouse, but also the lack of reporter mice crossed with *Psmb11*$^{-/-}$ mice. Therefore, the use of TCR-transgenic mice is regularly employed in these experiments, which leads to another unresolved question in the PSMB11 field. It appears that selection of some CD8 TCR transgenic clones is strongly dependent on thymoproteasomes, whereas others are independent of the PSMB11 expression. In general, high-affinity TCRs do not require PSMB11, yet their efficiency of positive selection does not have a perfect correlation with the TCR intensity in PSMB11-deficient mice [26]. Although it was suggested that thymoproteasomes favor the production of low-affinity peptides [17], these results may also mean that thymoproteasome deficiency disturbs other processes necessary for the positive selection of high-affinity TCRs. We believe that our data bring forward changes at the cell-cell communication level that should be considered in further studies on PSMB11.

Lastly, a striking finding in our study was the effect of PSMB11-deficiency on the CD4 thymocyte population. How did other scientists miss this effect? We found that the TSSP and CD83-encoding genes had diminished expression in *Psmb11*$^{-/-}$ mice. Mice deficient in TSSP show normal CD4 cell numbers but an altered TCR repertoire, whereas mice deficient in CD83 show impaired positive selection of CD4 T cells [21,49]. We believe that population averaging and the rather small spectrum of TCR specificities examined for MHC class II selection by others did not allow the identification of more subtle changes. Notably, only 30% of the TCRs tested by Nitta and colleagues had a significantly altered distribution in CD8 T cells between WT and *Psmb11*$^{-/-}$ mice [26]. The increased CD5 levels on the surface of 4SM, 4M1 and 4M2 thymocytes identified here suggest a direct effect of PSMB11 on the MHC II-dependent CD4 lineage development. A possible explanation for this effect is the high rate of macroautophagy displayed by cTECs which can alter the peptide repertoire loaded on MHC II molecules, or the decreased expression of *Cd83* and *Prss16* in thymoproteasome-deficient cTECs. However, we presume that the cortex-to-medulla directionality could be impaired in the CD8 lineage and not in the CD4 lineage due to the cytokine signaling being a prerequisite for the former, and not for CD4 thymocytes. We do not know to what extend the cell adhesion molecules play a role in this relocalization, but we expect that a denser ECM could affect the speed of migration and duration of cell-cell communication.

In conclusion, our study does not exclude the possibility that PSMB11 promotes peptides optimal for the positive selection of CD8 T cells. Instead, our results show that the role of thymoproteasomes in positive selection is has been reduced to the peptide level, disregarding the impact of proteasomes on the cell biology.

Conclusion

We believe that our study stands out for the genome-wide analysis of different thymocyte maturation subsets and TEC populations between WT and *Psmb11*$^{-/-}$ mice. Our results answer a previously unaddressed question about the role of thymoproteasomes on cTEC biology, and put a basis for the abnormal positive selection of thymocytes in the absence of PSMB11.

We conclude that thymoproteasomes are likely to maintain the optimal chemokine gradient and extracellular network for normal migration and cell-cell adhesion processes. Lack of PSMB11 in cTECs disturbed the expression levels of these genes, whereas post-selection thymocytes underwent increased stress. Cellular recovery appeared to restore the percentage of CD4 cells, whereas CD8 thymocytes showed signatures of unconventional T cell lineages despite upregulation of the stress-responsive HSP70 chaperones.

We propose that future studies on positive selection should take into account that absence of PSMB11 does not have consequences solely on antigen-presentation. The present results leave open several questions that are central to the PSMB11-mediated T cell development:

1. What events are the cause of stress in WT and *Psmb11*$^{-/-}$ mice?
2. Is a faster differentiation underlying the increased cellularity in 4M1 and 4M2 thymocytes?
3. Is the migration pattern of thymocytes and the interaction with cTECs altered in the *Psmb11*$^{-/-}$ thymus?
4. What triggers the expression of β5i in β5t-deficient cTECs?

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Appendix 1 – DEGs identified in the 8SM subset using the DESeq2 package (p-adjusted < 0.1, fold-change > 2)

	log2FoldChange	pvalue	padj	X8SM_WT_mean	X8SM_KO_mean	symbol
ENSMUSG00000091971.3	6.393838265	6.57E-05	0.000969141	21.34878951	1791.153273	Hspa1a
ENSMUSG00000090877.3	5.734537121	1.75E-06	5.14E-05	67.09461755	3568.852119	Hspa1b
ENSMUSG00000089762.3	2.544898025	2.18E-07	8.90E-06	10.79871202	62.67885036	Ier5l
ENSMUSG00000022528.7	2.540032736	7.58E-13	1.56E-10	20.5187765	119.741674	Hes1
ENSMUSG00000056749.7	2.279283239	0.000724141	0.006582457	51.36603201	249.0411702	Nfil3
ENSMUSG00000056501.3	2.169940469	3.67E-05	0.000602032	88.14097244	396.1930467	Cebpb
ENSMUSG00000040289.8	2.057618535	2.78E-05	0.000485629	134.6377455	560.1454324	Hey1
ENSMUSG00000061048.7	1.778890933	2.81E-06	7.59E-05	20.07203919	68.23971677	Cdh3
ENSMUSG00000022114.4	1.778265124	1.89E-11	2.63E-09	43.18830483	147.6482155	Spry2
ENSMUSG00000017737.2	1.749187476	4.93E-10	4.63E-08	47.24573393	158.6489307	Mmp9
ENSMUSG00000021701.7	1.660146185	0.003189281	0.019686458	91.05060803	287.5827687	Plk2
ENSMUSG00000005413.7	1.592089085	5.21E-05	0.000803224	24.72291856	74.60447034	Hmox1
ENSMUSG00000052684.4	1.58788116	0.00805854	0.039263641	1719.647242	5169.210676	Jun
ENSMUSG00000024232.1	1.559491398	1.06E-05	0.000228402	231.5442621	682.3380768	Bambi
ENSMUSG00000018102.4	1.502731627	3.07E-05	0.000525146	26.66022664	75.65265728	Hist1h2bc
ENSMUSG00000068246.5	1.494208234	0.000288973	0.003206492	17.14550637	48.81307928	Apol9b
ENSMUSG00000028214.13	1.467474974	4.82E-06	0.000119244	64.07603572	176.9507987	Gem
ENSMUSG00000017493.12	1.434436539	1.25E-17	8.45E-15	149.7406475	405.2741826	Igfbp4

ENSMUSG00000055148.7	1.420225145	0.002935398	0.018420693	442.9671419	1185.573678	Klf2
ENSMUSG00000106333.1	1.417012111	0.001552026	0.011418273	17.23154956	46.15242004	4930428O21Rik
ENSMUSG00000027875.12	1.405096209	7.82E-06	0.000175374	31.53892627	82.86486463	Hmgcs2
ENSMUSG00000046546.3	1.387361556	1.48E-09	1.18E-07	99.71664584	260.98261	Fam43a
ENSMUSG00000020592.14	1.377484785	0.001187662	0.009351726	17.12522954	45.03497113	Sdc1
ENSMUSG00000028042.15	1.372658832	1.25E-24	5.05E-21	2673.278493	6922.148273	Zbtb7b
ENSMUSG00000049649.8	1.370251331	0.008558913	0.040991443	106.4408156	275.0456625	Gpr3
ENSMUSG00000003032.8	1.341469796	8.17E-07	2.72E-05	102.2192941	259.0518087	Klf4
ENSMUSG00000075330.3	1.311663815	5.40E-08	2.63E-06	51.11589643	127.1676361	A930003A15Rik
ENSMUSG00000042821.7	1.304528729	0.005563243	0.02982735	22.77026952	56.01401317	Snai1
ENSMUSG00000051212.7	1.279459638	1.23E-18	1.14E-15	647.6451869	1572.090645	Gpr183
ENSMUSG00000059430.14	1.270972866	0.005191178	0.028321255	19.73387634	47.37427612	Actg2
ENSMUSG00000024521.7	1.264315844	8.25E-05	0.001163212	60.3077948	145.360977	Pmaip1
ENSMUSG00000053310.11	1.260988228	2.74E-15	1.19E-12	159.3747743	380.9035849	Nrgn
ENSMUSG00000021136.13	1.260410846	5.84E-18	4.29E-15	188.8171263	453.0755719	Smoc1
ENSMUSG00000033350.7	1.257951457	1.33E-09	1.08E-07	195.0009527	465.9327644	Chst2
ENSMUSG00000078202.3	1.255745261	2.82E-05	0.000490819	47.85323925	114.4871857	Nrarp
ENSMUSG00000045382.6	1.249796454	9.06E-20	1.47E-16	1114.134985	2648.540131	Cxcr4
ENSMUSG00000042364.10	1.2398095	0.022610611	0.08156379	1026.899709	2425.121469	Snx18
ENSMUSG00000097604.1	1.236939197	0.002256416	0.015039047	19.66220966	46.72833682	Gm17322
ENSMUSG00000022500.14	1.234360291	1.47E-05	0.000297984	58.02116621	136.8760787	Litaf

ENSMUSG00000045092.8	1.226178066	0.001174503	0.009277351	28.53750792	67.16284002	S1pr1
ENSMUSG00000074466.12	1.214194562	2.65E-08	1.42E-06	81.87410583	190.2194893	Gm15417
ENSMUSG00000036181.2	1.206269812	0.004656337	0.026111532	401.6652367	926.6559474	Hist1h1c
ENSMUSG00000028041.17	1.204046978	1.42E-11	2.03E-09	459.4024655	1058.79393	Adam15
ENSMUSG00000070306.2	1.192950119	5.32E-05	0.000815652	43.15735255	98.77229728	Ccdc153
ENSMUSG00000037211.12	1.185331148	7.53E-08	3.54E-06	119.7243142	271.7304809	Spry1
ENSMUSG00000044927.6	1.179158775	1.29E-05	0.000265502	65.43174936	148.5793462	H1fx
ENSMUSG00000068101.10	1.175193848	0.000122867	0.001623528	31.29131139	70.73365494	Cenpm
ENSMUSG00000000817.10	1.171716847	0.001404137	0.010600575	24.54107004	55.44264272	Fasl
ENSMUSG00000036858.8	1.164254891	5.30E-07	1.89E-05	70.97846333	159.3073906	Ptcra
ENSMUSG00000026360.9	1.162576562	0.000504219	0.004959104	5675.862681	12705.62901	Rgs2
ENSMUSG00000104557.1	1.159088486	0.015519097	0.062556519	19.24100653	42.83781729	Gm36520
ENSMUSG00000036381.13	1.154857112	1.16E-05	0.000245808	47.8131678	106.5136771	P2ry14
ENSMUSG00000090698.2	1.150929453	0.000788884	0.007049345	25.96603517	57.74667396	Apold1
ENSMUSG00000021256.5	1.147911448	0.00696494	0.035340945	16.59164534	37.08744655	Vash1
ENSMUSG00000093798.1	1.144445023	0.000846586	0.007412737	27.21982724	59.98595107	Gm8355
ENSMUSG00000032501.8	1.143849605	3.32E-14	1.06E-11	1441.826136	3186.507389	Trib1
ENSMUSG00000049036.7	1.139842894	0.011983741	0.052402376	15.68604862	34.75726425	Tmem121
ENSMUSG00000020323.14	1.139840005	0.002621686	0.017042362	23.22119013	51.5947476	Prss57
ENSMUSG00000021250.13	1.136928352	0.00019666	0.002343094	2063.410397	4537.395649	Fos
ENSMUSG00000020205.8	1.123142317	9.71E-20	1.47E-16	431.8011381	940.8809653	Phlda1

ENSMUSG00000043099.4	1.114066597	0.000926259	0.007875098	31.72973322	68.94009433	Hic1
ENSMUSG00000045591.5	1.100721316	0.001550446	0.011413582	28.66984434	61.06960883	Olig3
ENSMUSG00000071552.4	1.098578094	8.71E-05	0.001220711	49.98837975	107.4087735	Tigit
ENSMUSG00000045005.9	1.097826889	0.000156637	0.001950633	56.00495004	119.8003692	Fzd5
ENSMUSG00000041075.8	1.093689564	0.002958432	0.018535326	27.13394478	58.16640645	Fzd7
ENSMUSG00000031749.12	1.091667397	3.45E-19	4.64E-16	1951.974099	4158.561412	St3gal2
ENSMUSG00000041046.7	1.088823638	1.97E-05	0.000369767	108.9971081	231.3109768	Ramp3
ENSMUSG00000021453.2	1.076546334	1.12E-09	9.30E-08	131.7511786	278.0958726	Gadd45g
ENSMUSG00000002104.11	1.074722026	1.29E-07	5.62E-06	172.7959722	362.7861053	Rapsn
ENSMUSG00000032698.15	1.071003792	0.00187625	0.013179434	28.321649	59.64371415	Lmo2
ENSMUSG00000006362.15	1.064380916	1.40E-14	5.29E-12	219.031025	458.1212543	Cbfa2t3
ENSMUSG00000074622.4	1.060416259	1.79E-05	0.000344115	116.552205	242.7324507	Mafb
ENSMUSG00000037169.14	1.056310452	4.99E-06	0.000122245	64.45464671	134.5316724	Mycn
ENSMUSG00000085609.1	1.047799319	0.000976265	0.008157287	37.5838185	78.22821089	1700016P03Rik
ENSMUSG00000024899.6	1.038947743	1.30E-05	0.000266995	72.01365981	147.1081421	Papss2
ENSMUSG00000027654.2	1.035740798	3.53E-08	1.83E-06	181.8794024	373.0009286	Fam83d
ENSMUSG00000043017.9	1.034813858	1.05E-13	2.82E-11	322.6058679	660.8568385	Ptgir
ENSMUSG00000074607.11	1.026042811	6.46E-19	7.11E-16	439.7218608	895.6978525	Tox2
ENSMUSG00000042622.13	1.024924033	7.10E-07	2.40E-05	190.1283141	386.5353336	Maff
ENSMUSG00000045662.16	1.024104903	0.005155646	0.028165447	25.09477033	50.86815048	Henmt1
ENSMUSG00000048332.13	1.022809498	0.002273195	0.015107818	35.50087153	72.07453636	Lhfp

ENSMUSG00000058385.7	1.016612805	0.002127277	0.014408168	36.13809433	73.18774299	Hist1h2bg
ENSMUSG00000087651.2	1.014532903	0.005282389	0.028702557	23.70542298	48.04804614	1500009L16Rik
ENSMUSG00000027718.8	1.011857032	0.030565449	0.099989618	17.1850956	34.92346034	Il21
ENSMUSG00000027459.16	1.005717444	1.18E-13	2.98E-11	302.5899425	606.8728555	Fam110a
ENSMUSG00000078521.2	1.004329633	0.023470137	0.083668033	17.68418719	35.39889277	Aunip
ENSMUSG00000030847.8	1.000756821	0.016538691	0.065383138	22.76452996	45.39730015	Bag3
ENSMUSG00000053541.3	-1.004873839	5.71E-11	7.13E-09	820.9492659	408.5123605	Gm4759
ENSMUSG00000034684.12	-1.005851946	2.64E-06	7.16E-05	170.563255	85.31074295	Sema3f
ENSMUSG00000052563.13	-1.006506089	6.45E-08	3.11E-06	251.9916431	125.8042224	D930048N14Rik
ENSMUSG00000055228.7	-1.008286243	1.13E-06	3.60E-05	192.2536421	95.37272592	Zfp935
ENSMUSG00000097103.1	-1.010163007	0.000567155	0.005424004	80.36357971	40.06270906	Gm2885
ENSMUSG00000063605.5	-1.011412869	1.06E-08	6.44E-07	380.7356127	189.0460511	Ccdc102a
ENSMUSG00000061723.17	-1.014406297	1.03E-08	6.34E-07	307.8980512	152.8415145	Tnnt3
ENSMUSG00000028017.7	-1.017626974	0.000102536	0.001391293	101.4301562	50.08829362	Egf
ENSMUSG00000051037.15	-1.019254288	0.000370236	0.003914612	83.79703739	41.20173267	Zfp455
ENSMUSG00000074829.10	-1.019996357	3.97E-11	5.12E-09	407.1104544	200.8159879	2010315B03Rik

ENSMUSG00000102780.1	-1.02005103	0.001269722	0.009843393	105.4286837	52.01622827	Gm38253
ENSMUSG00000002617.14	-1.023019584	1.18E-07	5.18E-06	371.6153776	182.6740215	Zfp40
ENSMUSG00000109015.1	-1.023224497	0.002585013	0.016822018	58.41393777	28.81840067	RP24-128P19.3
ENSMUSG00000056290.15	-1.030272401	1.14E-13	2.98E-11	1214.766041	594.5845632	Ms4a4b
ENSMUSG00000106717.1	-1.030319789	0.000733586	0.006648366	72.66702725	35.47032937	Gm42798
ENSMUSG00000105699.1	-1.037629335	0.004174178	0.024004993	50.63643872	24.63811337	Gm43703
ENSMUSG00000089525.1	-1.037867567	0.017012748	0.066758255	37.05071651	17.97933514	Gm23833
ENSMUSG00000023266.11	-1.039409063	0.001269158	0.009843393	69.33844296	33.63550456	Frs3
ENSMUSG00000024677.13	-1.042646504	2.47E-18	2.00E-15	3187.48706	1546.914018	Ms4a6b
ENSMUSG00000107120.1	-1.042850029	6.39E-06	0.000148814	133.9495647	64.98232658	Gm43059
ENSMUSG00000103527.1	-1.04517912	0.015498039	0.062492424	293.0777949	142.0367412	Gm37261
ENSMUSG00000107201.1	-1.045653752	0.015005854	0.061035896	41.78173878	20.39386253	5930420M18Rik
ENSMUSG00000029360.3	-1.046530523	0.001901505	0.013295176	75.96911292	37.12729666	Gm9754
ENSMUSG00000019852.6	-1.047012114	0.013385452	0.05669005	38.69679792	18.61071631	Arfgef3

ENSMUSG00000103388.1	-1.047073522	0.000537996	0.005193279	90.3456356	43.57878164	Gm37581
ENSMUSG00000029298.15	-1.047354684	5.07E-14	1.46E-11	6964.239576	3369.774135	Gbp9
ENSMUSG00000107215.1	-1.049950235	1.09E-08	6.59E-07	325.960579	157.290633	Gm43197
ENSMUSG00000025787.5	-1.051453208	0.011013704	0.049317463	53.30516343	25.87771987	Tgm4
ENSMUSG00000030782.15	-1.052275244	6.67E-05	0.000977323	102.7216045	49.40619147	Tgfb1i1
ENSMUSG00000060131.11	-1.055308573	0.001147876	0.009138508	88.90135807	43.02183231	Atp8b4
ENSMUSG00000047632.10	-1.055624422	0.002222356	0.014893965	93.22244448	44.79513378	Fgfbp3
ENSMUSG00000031200.16	-1.058421671	1.85E-07	7.73E-06	255.7155749	122.8729984	Mtcp1
ENSMUSG00000085603.2	-1.059106465	1.03E-12	2.07E-10	599.1962553	287.6252791	Gm11346
ENSMUSG00000057132.15	-1.059367726	9.83E-08	4.41E-06	275.670673	132.3947263	Rpgrip1
ENSMUSG00000103558.1	-1.066709239	0.001772633	0.012657038	75.71892212	36.16086218	Gm38220
ENSMUSG00000104835.4	-1.071298227	4.22E-07	1.53E-05	180.755642	86.01537741	Gm5547
ENSMUSG00000019737.14	-1.07437919	0.002089258	0.014198285	61.64081643	29.4443464	Syne4
ENSMUSG00000106099.1	-1.080932537	3.42E-06	9.06E-05	167.4263324	79.49482752	Gm42664

ENSMUSG00000109096.1	-1.081032133	0.000754341	0.006816066	70.597222	33.34556975	RP24-165E9.2
ENSMUSG00000040663.8	-1.081841226	5.85E-05	0.000884488	142.7109257	67.50320638	Clcf1
ENSMUSG00000024049.14	-1.082637796	0.00033992	0.003648189	84.18406938	39.61761608	Myom1
ENSMUSG00000090086.7	-1.083464466	2.85E-14	9.59E-12	1033.758103	488.3337961	AI480526
ENSMUSG00000065663.1	-1.084344032	0.008298051	0.040067622	40.99174199	19.39119669	Gm22579
ENSMUSG00000025163.6	-1.084507041	1.16E-12	2.21E-10	894.2182263	421.4518305	Cd7
ENSMUSG00000103126.2	-1.087019936	0.000428933	0.004382278	77.6184132	36.51923837	Gm37387
ENSMUSG00000001986.16	-1.096938837	1.30E-06	4.07E-05	174.2389094	80.94473711	Gria3
ENSMUSG00000091183.5	-1.104464157	0.000113607	0.00151587	101.3631039	47.15304826	Gm5141
ENSMUSG00000032373.14	-1.105987749	2.08E-05	0.000385461	117.698176	54.65310816	Car12
ENSMUSG00000105703.1	-1.107039761	0.025546005	0.088490836	684.0936018	317.3886135	Gm43305
ENSMUSG00000108465.1	-1.107192223	9.59E-05	0.001316605	91.96854279	42.38525126	RP23-4H17.4
ENSMUSG00000106962.1	-1.108587685	0.009879927	0.045536354	43.40818631	20.21633909	Gm43633
ENSMUSG00000074867.3	-1.110850782	0.004308728	0.024586071	48.23469241	22.48097669	Zfp808

ENSMUSG00000084788.1	-1.113006062	0.007181305	0.03615117	46.2624095	21.54974746	Gm11342
ENSMUSG00000106087.1	-1.124860702	0.006028897	0.031684775	47.05385292	21.64513046	Gm43609
ENSMUSG00000089788.1	-1.126907018	0.001593127	0.01167105	1063.511042	486.9562715	Gm16586
ENSMUSG00000033022.7	-1.143106331	2.22E-05	0.000408643	115.6463931	52.40839561	Cdo1
ENSMUSG00000089769.1	-1.166940013	0.000193768	0.00232464	74.87142335	33.15101795	Gm16574
ENSMUSG00000035509.17	-1.168247654	0.012182234	0.053021598	34.88172635	15.38316444	Fbxl21
ENSMUSG00000032021.13	-1.175608953	1.07E-10	1.25E-08	256.2691688	113.6084942	Crtam
ENSMUSG00000107749.1	-1.179985321	3.14E-05	0.000533564	99.10329543	43.68022361	Gm44321
ENSMUSG00000097088.1	-1.18401002	0.006646852	0.034083751	36.26023752	15.93223813	Gm26615
ENSMUSG00000023052.7	-1.187028242	0.001971857	0.01361349	49.09715955	21.60669444	Npff
ENSMUSG00000072763.2	-1.195174854	0.001015464	0.008358949	67.91272524	29.47372823	5430403G16Rik
ENSMUSG00000105140.1	-1.206766784	0.000142195	0.001819406	115.6339495	50.14296744	Gm43127
ENSMUSG00000090659.8	-1.208364447	0.000214914	0.002516049	75.18169206	32.50814239	Zfp493
ENSMUSG00000040724.5	-1.211408031	1.49E-18	1.29E-15	1834.589584	791.7011378	Kcna2

ENSMUSG00000032826.16	-1.21233173	0.007971511	0.038992933	39.67915793	16.84512344	Ank2
ENSMUSG00000091575.2	-1.215429575	3.07E-09	2.23E-07	462.6129851	199.393521	2010016I18Rik
ENSMUSG00000069892.9	-1.21836476	5.81E-10	5.23E-08	239.7885643	103.1864315	9930111J21Rik2
ENSMUSG00000106547.1	-1.22789022	8.37E-05	0.001177344	97.07624308	41.44858659	B230303O12Rik
ENSMUSG00000096795.4	-1.230621836	0.002121298	0.014383751	74.06828153	31.5407503	Zfp433
ENSMUSG00000024186.15	-1.232080173	2.15E-05	0.000396144	103.1751011	44.20376976	Rgs11
ENSMUSG00000069893.10	-1.263808691	1.05E-05	0.000226698	97.20557659	40.3896658	9930111J21Rik1
ENSMUSG00000001558.5	-1.26978208	0.005146621	0.028141518	36.6173913	15.37308414	Klhl10
ENSMUSG00000043833.6	-1.271773275	0.001843871	0.013012337	51.80021614	21.49059581	2900005J15Rik
ENSMUSG00000067916.9	-1.280064237	0.000938176	0.007921869	51.85450337	21.35984052	Gm13139
ENSMUSG00000049044.16	-1.283831492	9.64E-23	2.92E-19	1446.490379	594.4126576	Rapgef4
ENSMUSG00000081739.3	-1.28732175	0.00535534	0.028994932	36.69785566	14.86261351	Mdm4-ps
ENSMUSG00000038540.14	-1.288561227	0.000216468	0.002531793	58.18815599	23.9113492	Tmc3
ENSMUSG00000105981.1	-1.290184543	0.008855782	0.042031142	36.48553709	14.99942776	2810428J06Rik

ENSMUSG00000092021.8	-1.297481472	2.61E-07	1.04E-05	278.9716302	113.5435491	Gbp11
ENSMUSG00000017713.9	-1.307462715	0.000125381	0.001647761	66.2769978	26.72522754	Tha1
ENSMUSG00000087445.1	-1.316499296	0.002512927	0.01645899	41.1277795	16.39521855	Gm14286
ENSMUSG00000090248.1	-1.321270763	0.00447961	0.025281528	35.88742131	14.20587185	Gm14027
ENSMUSG00000097751.1	-1.353588965	0.003504973	0.021097744	38.68467584	15.12017126	Gm26690
ENSMUSG00000073491.10	-1.354005983	3.93E-11	5.12E-09	527.7590356	206.5315175	Pydc4
ENSMUSG00000105382.1	-1.354144126	9.20E-07	3.02E-05	96.41793976	37.79569975	Gm43339
ENSMUSG00000067931.5	-1.357625116	2.63E-05	0.000465728	71.87976712	28.13550795	Zfp948
ENSMUSG00000000386.14	-1.359874076	6.13E-06	0.000144278	98.38260536	38.31562716	Mx1
ENSMUSG00000031425.15	-1.361369999	5.81E-06	0.000138504	95.68641351	37.57720155	Plp1
ENSMUSG00000004612.9	-1.366829491	1.47E-14	5.39E-12	524.6481912	203.3257997	Nkg7
ENSMUSG00000032238.17	-1.384496218	2.45E-25	1.49E-21	1621.455163	620.4288498	Rora
ENSMUSG00000070605.4	-1.390043022	0.000102993	0.001395938	60.43809274	22.9117471	Gm13251
ENSMUSG00000029138.4	-1.395255185	0.001306556	0.010090211	40.64000631	15.28093191	4930548H24Rik

ENSMUSG00000028362.2	-1.412511365	6.72E-06	0.000154612	83.74548681	31.35541972	Tnfsf8
ENSMUSG00000097250.1	-1.433735386	9.05E-05	0.001263037	63.18647812	23.25240289	Gm26771
ENSMUSG00000032494.12	-1.45140161	1.30E-08	7.64E-07	139.5710035	50.8927204	Tdgf1
ENSMUSG00000105607.1	-1.545672366	0.000304216	0.003338937	42.49749297	14.5664527	Gm43513
ENSMUSG00000061577.11	-1.548727784	2.23E-15	1.00E-12	305.2568999	104.4075955	Adgrg5
ENSMUSG00000045903.8	-1.567941546	0.000176928	0.002154616	45.79571683	15.52076926	Npas4
ENSMUSG00000020926.16	-1.59469545	3.39E-15	1.42E-12	293.399119	97.68004182	Adam11
ENSMUSG00000107468.1	-1.594701711	0.000130493	0.001696545	43.92659029	14.51494115	5730507A11Rik
ENSMUSG00000000617.14	-1.627840187	6.85E-06	0.000156693	82.03567752	26.50146094	Grm6
ENSMUSG00000074158.9	-1.63506884	0.000308109	0.003378599	52.40852514	16.78896703	9830147E19Rik
ENSMUSG00000079419.4	-1.697871567	8.84E-09	5.64E-07	106.3690434	32.51357782	Ms4a6c
ENSMUSG00000045441.5	-1.725665476	1.77E-12	3.19E-10	171.1100864	51.40721237	Gprin3
ENSMUSG00000020263.14	-1.750077882	1.91E-16	1.05E-13	225.0684095	66.85716855	Appl2
ENSMUSG00000005947.11	-1.787296942	1.73E-17	1.11E-14	335.4842107	97.0828544	Itgae

ENSMUSG00000099757.1	-1.850082982	5.05E-05	0.000782022	41.91793702	11.64270174	BE692007

Appendix 2 – DEGs identified in the 8M1 subset using the DESeq2 package (p-adjusted < 0.1, fold-change > 2)

	log2FoldChange	pvalue	padj	X8M1_WT_mean	X8M1_KO_mean	symbol
ENSMUSG00000091971.3	5.095605155	0.000108666	0.001529059	28.39854254	969.7500546	Hspa1a
ENSMUSG00000090877.3	4.747046547	4.93E-05	0.00081303	96.10234651	2579.622528	Hspa1b
ENSMUSG00000028042.15	2.300547607	4.04E-11	4.01E-09	19.97057618	98.57959482	Zbtb7b
ENSMUSG00000054364.4	2.148821492	0.002020414	0.016526471	39.29439178	174.3489154	Rhob
ENSMUSG00000003032.8	1.97589092	0.0001189	0.001646338	70.8058812	278.4927037	Klf4
ENSMUSG00000045573.9	1.914816654	8.79E-09	4.50E-07	24.62519163	93.37646411	Penk
ENSMUSG00000032220.9	1.901013215	4.82E-12	5.85E-10	31.12777234	115.7202104	Myo1e
ENSMUSG00000056749.7	1.832753795	1.12E-07	4.34E-06	29.69862338	106.09407	Nfil3
ENSMUSG00000035042.2	1.753257938	3.61E-07	1.22E-05	21.34390574	71.94146464	Ccl5
ENSMUSG00000021701.7	1.651197512	1.75E-11	1.84E-09	84.92590823	267.1470441	Plk2
ENSMUSG00000085148.1	1.605223282	1.19E-09	7.75E-08	42.67365095	130.1109686	Mir22hg
ENSMUSG00000071552.4	1.588625708	1.16E-05	0.000242847	24.33912014	74.00098767	Tigit
ENSMUSG00000003545.3	1.569529959	0.000103105	0.001473615	243.4545996	723.0428922	Fosb
ENSMUSG00000053310.11	1.550260317	2.05E-24	1.77E-21	179.8277275	528.4726744	Nrgn
ENSMUSG00000052684.4	1.537987855	0.003253324	0.023687476	1312.727337	3812.014895	Jun

ENSMUSG00000028214.13	1.530152923	0.000953333	0.009239053	79.91564925	231.0464975	Gem
ENSMUSG00000029581.14	1.52780135	2.13E-08	1.00E-06	34.82844972	100.912761	Fscn1
ENSMUSG00000021250.13	1.44918091	0.000377835	0.004251209	1439.572007	3930.889142	Fos
ENSMUSG00000023274.14	1.388993604	1.93E-08	9.14E-07	356.393739	933.0666574	Cd4
ENSMUSG00000030124.2	1.360956739	1.38E-05	0.00027978	32.76214322	84.37032732	Lag3
ENSMUSG00000022468.11	1.321182459	5.38E-09	2.89E-07	99.12351605	249.0215771	Endou
ENSMUSG00000076752.2	1.314076282	0.000105388	0.001492241	24.78161981	61.91150747	Tcrg-C2
ENSMUSG00000085903.1	1.301258075	1.34E-06	3.82E-05	44.93269334	110.6367308	Gm15340
ENSMUSG00000000078.6	1.282990568	0.002402922	0.018926158	2286.680854	5564.582198	Klf6
ENSMUSG00000026360.9	1.25134078	1.27E-07	4.85E-06	2287.400501	5445.610273	Rgs2
ENSMUSG00000024232.1	1.250394142	1.32E-05	0.000269794	73.16618774	175.1600407	Bambi
ENSMUSG00000023132.7	1.225280787	2.43E-06	6.50E-05	47.95694229	112.5831572	Gzma
ENSMUSG00000097296.1	1.21116357	7.59E-05	0.00115421	41.52041733	97.04099957	Gm26532
ENSMUSG00000090582.1	1.203056673	0.000214301	0.002704803	32.90537187	76.07967948	Gm17024
ENSMUSG00000058672.6	1.201764681	6.10E-05	0.000958077	993.4024907	2285.219933	Tubb2a
ENSMUSG00000040435.11	1.186742032	0.000208542	0.002643954	3644.656333	8296.677666	Ppp1r15a
ENSMUSG00000026435.15	1.16943126	1.61E-05	0.000317291	40.36877801	91.02168006	Slc45a3
ENSMUSG00000032501.8	1.161955209	2.33E-06	6.28E-05	667.080405	1493.049123	Trib1
ENSMUSG00000026358.13	1.128475889	1.82E-13	2.89E-11	310.3493686	677.5325775	Rgs1
ENSMUSG00000022114.4	1.116601084	2.10E-07	7.50E-06	92.60196984	201.2844369	Spry2
ENSMUSG00000028328.13	1.104899473	3.51E-05	0.000611221	55.14116429	118.8863276	Tmod1

ENSMUSG00000024521.7	1.103921497	4.74E-05	0.000790325	64.88935649	140.1195722	Pmaip1
ENSMUSG00000026475.7	1.08454234	1.73E-22	1.09E-19	867.3783675	1841.911256	Rgs16
ENSMUSG00000034765.6	1.069582061	0.00039962	0.004459525	3126.869312	6562.781871	Dusp5
ENSMUSG00000031749.12	1.063252361	5.02E-09	2.71E-07	123.1561722	258.7206952	St3gal2
ENSMUSG00000052837.5	1.061956087	0.000199488	0.002552127	12387.71047	25862.46992	Junb
ENSMUSG00000024190.6	1.045573628	6.17E-09	3.28E-07	3082.083064	6361.81852	Dusp1
ENSMUSG00000024042.6	1.04217247	0.001326063	0.011796418	3267.469926	6729.131367	Sik1
ENSMUSG00000022132.15	1.035743956	5.23E-06	0.000124878	67.65656849	139.4991024	Cldn10
ENSMUSG00000026011.13	1.0289731	0.007371286	0.044957667	269.1901285	549.3147312	Ctla4
ENSMUSG00000078851.4	1.024517587	0.002246866	0.017897119	28.31338298	57.72687754	Hist3h2a
ENSMUSG00000040249.15	1.021619687	0.002076288	0.016896635	28.88920264	58.67308446	Lrp1
ENSMUSG00000090958.2	1.012547659	0.001678986	0.01433625	212.0981113	428.1282169	Lrrc32
ENSMUSG00000031004.7	-1.011086094	1.46E-12	2.01E-10	17083.45452	8477.003864	Mki67
ENSMUSG00000047632.10	-1.019155618	0.000200814	0.002560376	89.93361192	44.20251601	Fgfbp3
ENSMUSG00000060131.11	-1.05900882	1.36E-27	1.92E-24	2127.011957	1021.727666	Atp8b4
ENSMUSG00000025912.16	-1.084906548	6.33E-05	0.000986071	93.5944653	43.8879307	Mybl1
ENSMUSG00000044966.4	-1.191787722	3.47E-07	1.18E-05	124.3318807	54.20944919	Fbxo48
ENSMUSG00000026605.14	-1.196088979	1.12E-49	1.27E-45	2501.19012	1093.270082	Cenpf

ENSMUSG00000051804.3	-1.199015477	0.000746103	0.007600637	73.82285698	32.67454055	Adam6b
ENSMUSG00000025461.10	-1.317632213	2.71E-07	9.41E-06	175.3052526	70.44167314	Cd163l1
ENSMUSG00000054672.12	-1.534091736	2.97E-05	0.000532532	676.43195	233.6538152	5830411N06Rik
ENSMUSG00000105703.1	-1.568201699	7.83E-05	0.001176573	1950.176313	657.2210608	Gm43305

Appendix 3 – DEGs identified in the 8M2 subset using the DESeq2 package (p-adjusted < 0.1, fold-change > 2)

	log2FoldChange	pvalue	padj	X8M2_WT_mean	X8M2_KO_mean	symbol
ENSMUSG00000091971.3	5.424837555	1.46E-05	0.000785785	27.80376844	1195.873888	Hspa1a
ENSMUSG00000090877.3	4.422591688	7.23E-05	0.002613691	165.5245556	3550.33264	Hspa1b
ENSMUSG00000030361.16	3.241596287	5.83E-11	1.98E-08	6.872009652	63.977596	Klrb1a
ENSMUSG00000054364.4	3.053904191	0.000164234	0.004555818	37.07419219	307.9729543	Rhob
ENSMUSG00000076754.2	3.00711492	1.45E-17	1.72E-14	12.88279301	103.0046362	Trgv2
ENSMUSG00000058715.11	2.956677448	5.62E-12	2.23E-09	11.77474722	91.41770872	Fcer1g
ENSMUSG00000062372.13	2.871829182	1.56E-10	5.15E-08	7.999658301	58.9204621	Otof
ENSMUSG00000022114.4	2.729986641	2.60E-31	1.55E-27	43.84751329	291.6963443	Spry2
ENSMUSG00000026418.16	2.727358682	1.13E-16	1.23E-13	19.05134321	125.2629958	Tnni1
ENSMUSG00000032220.9	2.708618447	1.93E-18	2.86E-15	21.65127815	142.0383785	Myo1e
ENSMUSG00000053310.11	2.704635214	1.14E-40	1.36E-36	62.40053443	406.7551147	Nrgn

ENSMUSG00000047293.6	2.558460635	2.10E-10	6.24E-08	16.65948793	97.3670264	Gpr15
ENSMUSG00000035042.2	2.516187918	1.54E-21	3.05E-18	35.10766623	198.802295	Ccl5
ENSMUSG00000076752.2	2.334646198	4.89E-25	1.16E-21	64.87267687	327.5456819	Tcrg-C2
ENSMUSG00000061769.2	2.165326839	1.03E-05	0.000602477	9.636000517	43.44095236	Klra6
ENSMUSG00000004709.14	2.12816306	1.78E-09	3.86E-07	18.97721033	82.47791306	Cd244
ENSMUSG00000050335.16	2.035502377	2.70E-19	4.59E-16	56.25855043	227.5911103	Lgals3
ENSMUSG00000003032.8	1.932079459	3.22E-05	0.001401534	72.59594585	276.7972644	Klf4
ENSMUSG00000021701.7	1.925612495	3.48E-16	3.45E-13	74.21429122	280.7574837	Plk2
ENSMUSG00000030124.2	1.827965984	1.71E-08	2.44E-06	27.07221037	96.17799026	Lag3
ENSMUSG00000017167.6	1.825918412	0.000269839	0.006496346	11.84506741	41.40899735	Cntnap1
ENSMUSG00000032841.15	1.801904783	5.54E-08	7.09E-06	23.62188651	83.32679958	Prr5l
ENSMUSG00000043102.2	1.77446676	0.000153293	0.004382488	12.30331337	41.25935441	Qrfp
ENSMUSG00000059994.10	1.773933692	0.000103599	0.003339039	18.56720531	62.89740703	Fcrl1
ENSMUSG00000085903.1	1.684660435	0.000225102	0.005754866	13.48301563	42.98220167	Gm15340
ENSMUSG00000025089.15	1.673542197	0.000306317	0.00710141	13.47177374	43.44208362	Gfra1
ENSMUSG00000024754.12	1.647368719	1.68E-07	1.87E-05	29.72506932	93.28391117	Tmem2
ENSMUSG00000033350.7	1.644993117	2.05E-06	0.000158777	26.34914806	82.18582473	Chst2
ENSMUSG00000003545.3	1.643183692	0.00151869	0.021999739	188.3892742	588.2297098	Fosb
ENSMUSG00000052684.4	1.6318621	0.000723182	0.012783077	1524.992233	4726.033675	Jun
ENSMUSG00000030268.17	1.62629636	1.55E-05	0.000809901	21.55051772	65.99496511	Bcat1
ENSMUSG00000032501.8	1.5793323	6.56E-13	3.39E-10	165.5706636	495.4063692	Trib1

ENSMUSG00000021250.13	1.568913046	0.000141426	0.004196874	1745.793088	5179.219865	Fos
ENSMUSG00000028214.13	1.554818146	5.60E-10	1.45E-07	73.71028073	216.1033615	Gem
ENSMUSG00000031749.12	1.548824852	3.32E-08	4.58E-06	38.35157779	113.0257521	St3gal2
ENSMUSG00000032348.5	1.547369105	1.02E-12	5.07E-10	71.03654399	208.1982674	Gsta4
ENSMUSG00000025511.14	1.533112409	4.61E-06	0.000315368	27.0014243	77.73941934	Tspan4
ENSMUSG00000022468.11	1.52687363	1.94E-08	2.74E-06	83.36193663	239.957542	Endou
ENSMUSG00000056749.7	1.519046496	1.59E-07	1.82E-05	58.51023119	167.962543	Nfil3
ENSMUSG00000084128.10	1.502946448	2.48E-07	2.64E-05	40.12561812	112.6731212	Esrp2
ENSMUSG00000031442.20	1.48931699	0.000175344	0.004744371	16.62161849	46.53303387	Mcf2l
ENSMUSG00000001444.2	1.488761095	1.48E-08	2.25E-06	46.17473679	129.9236163	Tbx21
ENSMUSG00000045928.2	1.469509086	7.15E-09	1.18E-06	45.16992783	125.7589095	4933440M02Rik
ENSMUSG00000074063.9	1.395817074	0.000435739	0.008919526	20.91536794	55.66262804	Osgin1
ENSMUSG00000021636.14	1.385209675	1.78E-05	0.000873807	38.52720669	101.8381684	Marveld2
ENSMUSG00000024812.9	1.366463298	8.25E-10	1.96E-07	65.52448724	168.1933543	Tjp2
ENSMUSG00000023132.7	1.35962601	9.03E-08	1.10E-05	87.11361001	223.262179	Gzma
ENSMUSG00000039115.13	1.342084592	0.001506842	0.021908163	15.68712972	39.54908263	Itga9
ENSMUSG00000049892.7	1.340734679	0.006950422	0.058692905	18.3282809	46.15515779	Rasd1
ENSMUSG00000022469.16	1.337063088	0.000803589	0.013802002	20.55025704	52.53152221	Rapgef3
ENSMUSG00000030257.16	1.293169506	4.64E-09	8.12E-07	97.53683874	237.4898651	Srgap3
ENSMUSG00000004633.17	1.284616392	3.40E-30	1.35E-26	350.7282613	854.4482623	Chn2
ENSMUSG00000090958.2	1.277020956	0.003673029	0.0397163	20.80918883	50.3988472	Lrrc32

ENSMUSG00000070643.11	1.276692475	0.005402946	0.050397833	16.0696368	38.86136	Sox13
ENSMUSG00000026475.7	1.262487544	4.23E-10	1.14E-07	178.9020053	428.9911822	Rgs16
ENSMUSG00000058297.16	1.257107593	1.02E-11	3.90E-09	158.0733484	375.9209752	Spock2
ENSMUSG00000026826.13	1.228798349	1.56E-08	2.31E-06	185.9396033	435.872734	Nr4a2
ENSMUSG00000049630.6	1.227624304	0.00174873	0.024127195	25.25482057	58.27773145	C1ql3
ENSMUSG00000053158.10	1.219529086	0.000398408	0.00841611	34.98937483	80.76594658	Fes
ENSMUSG00000054871.4	1.212095564	0.000412175	0.008615105	26.39578299	60.80351299	Tmem158
ENSMUSG00000042622.13	1.204458353	0.000281304	0.006700487	47.12675654	108.7072324	Maff
ENSMUSG00000024451.8	1.203641882	2.24E-13	1.27E-10	157.5601679	362.1231026	Arap3
ENSMUSG00000001025.8	1.201037216	4.34E-08	5.73E-06	77.54765996	177.316093	S100a6
ENSMUSG00000076749.2	1.198778158	1.32E-06	0.000109972	54.01616238	123.3568855	Tcrg-C1
ENSMUSG00000011267.8	1.196656764	0.002766891	0.032645467	20.14901751	46.19965869	Zfp296
ENSMUSG00000076757.9	1.189183932	3.06E-05	0.001348611	49.90727319	113.6709119	Tcrg-C4
ENSMUSG00000040751.12	1.164733666	9.22E-05	0.003090165	38.55845597	86.06752622	Lat2
ENSMUSG00000015312.8	1.132621599	1.66E-07	1.87E-05	97.98852588	214.2628954	Gadd45b
ENSMUSG00000071552.4	1.123015845	6.58E-06	0.000411788	60.38710382	132.0110297	Tigit
ENSMUSG00000023274.14	1.118022022	1.03E-07	1.22E-05	108.4479446	235.9492308	Cd4
ENSMUSG00000004562.16	1.116432226	0.005427557	0.050508676	23.69468311	51.06441583	Arhgef40
ENSMUSG00000030653.16	1.114680537	1.06E-14	8.44E-12	415.3416018	900.267835	Pde2a
ENSMUSG00000028337.14	1.107273115	1.69E-27	5.02E-24	1083.115276	2329.877679	Coro2a
ENSMUSG00000052117.5	1.099968624	0.005972837	0.053855156	20.32799321	43.74554381	D630039A03Rik

ENSMUSG00000034936.2	1.097138618	0.00307078	0.035088443	26.25220032	56.02680174	Arl4d
ENSMUSG00000058672.6	1.096700855	1.79E-12	7.90E-10	410.5383619	878.5410349	Tubb2a
ENSMUSG00000034765.6	1.09115558	0.000325119	0.007407358	920.0295534	1960.685113	Dusp5
ENSMUSG00000026883.17	1.074025858	0.001568646	0.022315681	30.24821679	63.14104161	Dab2ip
ENSMUSG00000039521.12	1.073690908	2.07E-06	0.000158777	148.3017217	312.3190981	Foxp3
ENSMUSG00000024232.1	1.070983214	8.37E-05	0.002844098	82.42175944	173.5651537	Bambi
ENSMUSG00000024521.7	1.044634124	5.30E-06	0.000348515	112.501372	231.7189061	Pmaip1
ENSMUSG00000026011.13	1.039703913	1.08E-07	1.28E-05	286.848465	591.9494627	Ctla4
ENSMUSG00000015437.4	1.037860663	0.004421186	0.044148758	26.39578299	54.00120683	Gzmb
ENSMUSG00000085148.1	1.037836654	0.012339185	0.08390505	22.17080908	46.03228379	Mir22hg
ENSMUSG00000026573.7	1.036304874	6.36E-09	1.08E-06	127.3983926	262.1266764	Xcl1
ENSMUSG00000052837.5	1.03217586	0.000663117	0.011949166	7807.593272	15967.8818	Junb
ENSMUSG00000026285.7	1.021151631	5.37E-13	2.90E-10	244.3749662	495.8511786	Pdcd1
ENSMUSG00000026360.9	1.011663445	5.61E-14	3.71E-11	1665.712467	3360.266578	Rgs2
ENSMUSG00000035547.14	1.010914991	0.001322282	0.02026533	35.33534875	71.09198497	Capn5
ENSMUSG00000032369.13	1.010708608	0.015346503	0.09545814	19.36105369	39.46071665	Plscr1
ENSMUSG00000058385.7	1.008506	0.008735669	0.067638876	25.24853149	49.99719479	Hist1h2bg
ENSMUSG00000033863.1	1.007125081	0.001488453	0.021772169	38.39118803	77.27413319	Klf9
ENSMUSG00000034675.17	1.005920356	0.012946967	0.086622195	24.23789931	48.79155357	Dbn1
ENSMUSG00000035863.13	1.004280949	1.59E-13	9.47E-11	294.5282619	590.3810444	Palm
ENSMUSG00000105449.1	-1.001707253	0.006685347	0.057448581	50.11842992	25.18765929	Gm43379

ENSMUSG00000107017.1	-1.023090476	0.012295789	0.08365779	48.96488933	23.99685171	Gm43196
ENSMUSG00000099375.1	-1.023518066	0.004503943	0.044675056	57.8287162	28.33956641	Gm28187
ENSMUSG00000105429.1	-1.041883683	0.002968443	0.034209009	57.74668824	28.07326484	Gm43692
ENSMUSG00000109096.1	-1.045977371	0.010166084	0.074449038	47.78853387	22.93527823	RP24-165E9.2
ENSMUSG00000103285.1	-1.053346346	0.005142809	0.049087818	58.08517159	27.76189458	Gm37274
ENSMUSG00000053541.3	-1.068627653	0.000233472	0.005895283	2450.328008	1167.963	Gm4759
ENSMUSG00000096746.3	-1.069696499	0.002129064	0.027552736	59.28855912	28.4830707	Trav7d-3
ENSMUSG00000035954.9	-1.078905425	5.13E-06	0.000338645	148.4144596	69.8906167	Dock4
ENSMUSG00000104693.1	-1.089315072	0.016236386	0.098570362	42.5604918	19.99009042	Gm42941
ENSMUSG00000072763.2	-1.137410713	9.48E-05	0.003148872	119.672782	54.2221196	5430403G16Rik
ENSMUSG00000025461.10	-1.165889135	0.004114248	0.042291056	350.4051267	156.1114011	Cd163l1
ENSMUSG00000103839.1	-1.231909837	0.002366659	0.029667052	58.62457511	24.69803335	Gm37607
ENSMUSG00000103509.1	-1.285984347	7.39E-05	0.002634136	76.55837144	31.56684967	Gm38372
ENSMUSG00000097294.1	-1.348137281	0.001403859	0.021027826	44.92920127	17.56557044	Gm26888

ENSMUSG00000054672.12	-1.419799979	0.00103872	0.016876355	2084.69341	779.2333596	5830411N06Rik
ENSMUSG00000107546.1	-1.46276857	0.001222943	0.019137444	39.49836343	14.17720002	Gm44031
ENSMUSG00000105703.1	-1.535523698	0.000350742	0.00773909	3145.579533	1084.924084	Gm43305
ENSMUSG00000051804.3	-1.651533177	3.04E-14	2.13E-11	212.8965382	67.42127573	Adam6b

Appendix 4 – DEGs identified in the 4SM subset using the DESeq2 package (p-adjusted < 0.1, fold-change > 2)

	log2FoldChange	pvalue	padj	X4SM_WT_mean	X4SM_KO_mean	symbol
ENSMUSG00000095280.1	7.235560274	0.002911965	0.028228547	0.332347312	49.04135935	Gm21738
ENSMUSG00000075015.3	6.447545452	0.008383979	0.057409864	0.664694623	57.33121661	Gm10801
ENSMUSG00000091971.3	5.725025309	3.25E-05	0.001368952	46.6452094	2466.133285	Hspa1a
ENSMUSG00000090877.3	4.999404355	0.000462795	0.008120058	130.0625169	4159.588681	Hspa1b
ENSMUSG00000075014.1	4.959407517	0.015484119	0.086831701	9.598691807	298.2910594	Gm10800
ENSMUSG00000064357.1	3.480402343	0.000147495	0.003722041	13.62147045	152.2011134	mt-Atp6
ENSMUSG00000049791.4	3.475017546	1.88E-09	8.10E-07	5.73850767	63.68608733	Fzd4
ENSMUSG00000076137.1	3.462636837	0.003388162	0.031444126	5.42958147	59.87853223	Gm26384
ENSMUSG00000083863.1	3.18917532	0.00054567	0.00899953	25.4170549	231.9329848	Gm13341
ENSMUSG00000067929.3	3.150652109	7.88E-07	0.00010392	8.56040185	76.32279211	Gm10226
ENSMUSG00000105814.1	3.053582623	0.002480915	0.025365809	37.96344385	315.2425027	Mir703

ENSMUSG00000064346.1	2.768862666	0.002064047	0.022348165	29.13349631	198.5904444	mt-Tw
ENSMUSG00000077737.1	2.614310256	2.55E-05	0.001175766	10.41613803	64.10722473	Snord72
ENSMUSG00000054626.11	2.466054476	0.000227327	0.004956588	7.333194457	40.62239168	Xlr
ENSMUSG00000024521.7	2.358821142	6.87E-17	2.90E-13	43.27281739	221.8613281	Pmaip1
ENSMUSG00000108676.1	2.339984163	0.001237038	0.015531024	5.71933443	29.07582452	RP23-353F16.3
ENSMUSG00000024912.5	2.285986589	0.001225769	0.015481595	7.90213602	38.46603008	Fosl1
ENSMUSG00000084592.1	2.13323569	0.000399246	0.007394214	8.010633528	35.15018054	Mir1898
ENSMUSG00000026826.13	2.114644722	2.22E-05	0.001076613	346.1175655	1498.77553	Nr4a2
ENSMUSG00000077167.1	2.08443729	6.99E-10	3.85E-07	25.65115565	108.3589553	Gm24119
ENSMUSG00000108500.1	2.081265489	0.000345895	0.006665333	9.510601007	40.40311579	RP23-335G1.5
ENSMUSG00000021701.7	2.071837936	1.43E-05	0.000821502	125.8527673	528.8450897	Plk2
ENSMUSG00000102349.1	2.043019966	2.46E-05	0.001144699	16.62958885	68.65525118	Gm37376
ENSMUSG00000028214.13	2.009467284	9.64E-08	1.89E-05	46.68147977	187.5123333	Gem
ENSMUSG00000084350.1	1.974098635	2.21E-06	0.000217509	32.64454025	128.4399516	Znf41-ps
ENSMUSG00000040289.8	1.939095376	0.000110728	0.003076098	226.6253965	868.7102355	Hey1
ENSMUSG00000102496.1	1.855724504	9.31E-05	0.002731163	19.6444489	71.39377869	Gm36989
ENSMUSG00000056501.3	1.834415302	0.001965644	0.021596507	77.49453926	276.1173931	Cebpb
ENSMUSG00000091509.7	1.832167256	0.010287164	0.066795382	97.98823894	348.9829169	Gm17066
ENSMUSG00000078851.4	1.830872403	1.16E-07	2.10E-05	37.84717094	134.7095287	Hist3h2a
ENSMUSG00000088929.1	1.826339968	0.005081684	0.040950874	9.375154858	33.22766675	Gm24299
ENSMUSG00000100131.1	1.819578457	0.000746097	0.011156504	18.59496943	65.74517122	Gm28439

ENSMUSG00000103593.1	1.80592391	0.008211402	0.056669805	8.306815004	28.88470267	Gm37352
ENSMUSG00000090582.1	1.793948095	2.00E-07	3.34E-05	36.39757349	125.8981564	Gm17024
ENSMUSG00000100394.1	1.738443004	0.000604703	0.009623597	13.31575892	44.66642133	Gm28791
ENSMUSG00000081603.1	1.716922218	3.09E-05	0.001327437	20.24074715	66.51830537	Gm14681
ENSMUSG00000046168.8	1.699509447	0.000367248	0.006974951	15.17967855	49.01797006	Kcnrg
ENSMUSG00000050921.12	1.694621473	5.32E-11	3.55E-08	89.32824392	288.9586621	P2ry10
ENSMUSG00000052684.4	1.689887363	0.003806701	0.033864671	929.6921486	2999.225289	Jun
ENSMUSG00000105572.1	1.688947429	4.39E-08	1.03E-05	83.56756628	270.1219538	Gm43300
ENSMUSG00000073427.3	1.680698671	0.001640237	0.018923975	21.38919407	68.69167176	Gm4924
ENSMUSG00000000078.6	1.670895594	7.75E-07	0.00010383	1670.767982	5319.579957	Klf6
ENSMUSG00000097990.1	1.664121707	1.74E-05	0.000928409	29.14023795	92.44515345	Gm19557
ENSMUSG00000095868.1	1.653412367	0.000538594	0.008989338	19.00814471	59.77329518	Gm23136
ENSMUSG00000074794.9	1.64933306	0.001142813	0.014787698	584.0595369	1832.097763	Arrdc3
ENSMUSG00000104145.1	1.645435301	0.000256407	0.005377754	23.94931796	75.25438983	D130019J16Rik
ENSMUSG00000084284.1	1.640308558	0.000171609	0.004154592	25.12087342	78.52966842	Tpt1-ps6
ENSMUSG00000078495.10	1.631700798	0.014656587	0.083861627	167.0622883	517.8311989	Gm13157
ENSMUSG00000042622.13	1.618997911	0.00325122	0.030492541	130.8705203	401.8209662	Maff
ENSMUSG00000064358.1	1.602567721	0.000625315	0.009864863	38.53559981	117.2295463	mt-Co3
ENSMUSG00000064602.1	1.592264092	4.82E-05	0.001772774	24.72469074	74.7736008	Snora41
ENSMUSG00000107461.1	1.590160767	0.001978994	0.021686761	15.54322265	46.88985397	Gm44045
ENSMUSG00000098274.7	1.58785361	9.39E-05	0.002734801	25.33404629	76.51780832	Rpl24

ENSMUSG00000098188.1	1.576978779	0.006009164	0.04550155	9.667088185	28.65527823	Sowahc
ENSMUSG00000045005.9	1.576075293	0.000298993	0.00603842	33.57567931	99.75067333	Fzd5
ENSMUSG00000029378.5	1.574878389	0.000198952	0.004565814	19.15001965	56.95439234	Areg
ENSMUSG00000073448.1	1.558610044	0.000645471	0.01011983	17.37978559	51.48550146	Gm10509
ENSMUSG00000064493.1	1.541326059	8.74E-06	0.000585583	45.49603297	132.8053787	Snora28
ENSMUSG00000105818.1	1.540438976	0.005857629	0.044820724	10.69636066	31.34976094	Gm43256
ENSMUSG00000029657.15	1.527006739	0.005875019	0.044888265	1917.217738	5524.953214	Hsph1
ENSMUSG00000026358.13	1.525824658	2.67E-12	2.60E-09	203.9831847	587.3820651	Rgs1
ENSMUSG00000024042.6	1.52384674	0.004737809	0.039151055	5545.265896	15945.64544	Sik1
ENSMUSG00000102466.1	1.515500491	0.000481955	0.008317993	17.56704356	50.25937394	Gm38200
ENSMUSG00000065089.1	1.512436017	0.010508672	0.067756192	9.639418647	27.51235756	Gm26493
ENSMUSG00000071862.2	1.507760493	0.002604727	0.02622948	19.57077062	55.41403076	Lrrtm2
ENSMUSG00000106190.1	1.494977614	0.003150407	0.029829171	12.67551944	35.91352453	Gm20768
ENSMUSG00000055148.7	1.492568589	0.003450562	0.031836646	496.7024501	1397.441758	Klf2
ENSMUSG00000031266.6	1.490955991	3.02E-12	2.74E-09	287.3290818	806.952854	Gla
ENSMUSG00000103303.1	1.487563342	3.20E-05	0.001353951	37.79742662	106.3413421	Gm37802
ENSMUSG00000028524.21	1.482842125	1.72E-05	0.000921869	43.03487653	120.0340029	Sgip1
ENSMUSG00000104641.1	1.482406442	0.000964388	0.013221713	18.15091021	50.83568432	Gm43290
ENSMUSG00000104078.1	1.46985469	0.010944589	0.069479604	10.20617957	28.38660167	Gm37023
ENSMUSG00000071267.11	1.454802523	0.011430045	0.071539431	459.9780648	1261.023795	Zfp942
ENSMUSG00000023892.7	1.450693117	0.002173503	0.023215798	275.6403448	753.6585172	Zfp51

ENSMUSG00000105655.1	1.448686922	3.26E-08	8.25E-06	110.7201492	302.9912781	Gm42659
ENSMUSG00000025981.13	1.442313564	1.93E-13	3.05E-10	258.3493795	701.7252574	Coq10b
ENSMUSG00000082179.1	1.437185669	0.005673331	0.044118939	14.79624031	40.10235054	Gm11407
ENSMUSG00000021879.12	1.430816029	0.015948724	0.08838077	10.49811289	28.44215412	Dnah12
ENSMUSG00000103821.1	1.426482102	2.99E-07	4.56E-05	69.72339292	187.762289	D430013B06Rik
ENSMUSG00000067591.12	1.421924485	0.008202331	0.056656015	12.10979228	32.57795201	Klra3
ENSMUSG00000067058.7	1.420799667	0.003535455	0.03238405	18.7769455	50.1992848	Rps15a-ps5
ENSMUSG00000105137.1	1.413043185	0.000448643	0.007948829	24.36860918	64.97083156	Gm42869
ENSMUSG00000037370.13	1.408661865	0.000313521	0.006239172	22.0453924	58.40690798	Enpp1
ENSMUSG00000045382.6	1.407166295	0.00292542	0.028289477	437.1934913	1159.295592	Cxcr4
ENSMUSG00000108064.1	1.401702253	0.00052543	0.008863044	34.69998395	91.76067208	Gm44423
ENSMUSG00000083899.2	1.400721405	0.000238684	0.005116163	26.36372681	69.46820063	Gm12346
ENSMUSG00000078502.10	1.392728183	0.015351047	0.08640234	159.5479339	419.0348597	Gm13212
ENSMUSG00000085148.1	1.386891733	2.27E-08	6.13E-06	96.86197993	252.7987581	Mir22hg
ENSMUSG00000022528.7	1.386348176	5.14E-06	0.000412098	62.56689263	163.3850916	Hes1
ENSMUSG00000102973.1	1.383089195	0.000295418	0.006026333	1548.011054	4037.870973	E430014B02Rik
ENSMUSG00000003032.8	1.370765247	4.43E-05	0.001679485	85.04618848	219.4383023	Klf4
ENSMUSG00000069755.5	1.368854309	0.00012701	0.003394908	55.28011496	142.9937229	Zfp125
ENSMUSG00000044751.5	1.361431913	0.01897633	0.099376665	10.98476674	28.18301173	Gm12231
ENSMUSG00000046546.3	1.348854323	6.19E-06	0.000463801	71.98142772	183.0609826	Fam43a
ENSMUSG00000073008.11	1.346315335	0.001598227	0.018523644	1650.574055	4196.922781	Gpr174

ENSMUSG00000042364.10	1.345608363	0.00225713	0.023807932	869.6168563	2209.812575	Snx18
ENSMUSG00000061544.13	1.339679823	0.001194232	0.015301459	26.74322975	67.84099382	Zfp229
ENSMUSG00000028035.13	1.337078343	1.13E-19	1.43E-15	691.9410763	1747.389495	Dnajb4
ENSMUSG00000053347.14	1.331446334	0.016728951	0.091280157	263.4203955	663.0428326	Zfp943
ENSMUSG00000104488.1	1.329511678	5.82E-05	0.001966695	38.59093888	97.11012939	Gm38062
ENSMUSG00000045414.7	1.324750594	5.71E-13	6.57E-10	530.7759042	1329.72328	1190002N15Rik
ENSMUSG00000021136.13	1.324582414	0.003771401	0.033645146	21.53003526	53.91958507	Smoc1
ENSMUSG00000022114.4	1.324267845	0.000187977	0.004409791	40.10127045	100.1063378	Spry2
ENSMUSG00000084899.1	1.316146495	2.17E-05	0.001064881	35.94708548	89.55282299	Gm15344
ENSMUSG00000097375.2	1.310699506	7.72E-05	0.002396318	35.00538262	87.01252955	6720427I07Rik
ENSMUSG00000029135.10	1.308238553	0.017682779	0.094637658	1163.288818	2880.654727	Fosl2
ENSMUSG00000104969.1	1.305576495	0.014304017	0.082556212	11.12021289	27.48011527	Gm43445
ENSMUSG00000049516.9	1.305444511	7.97E-12	6.31E-09	3205.292094	7921.381314	Spty2d1
ENSMUSG00000023951.16	1.3032449	0.000616	0.009766563	58.51239602	144.2353145	Vegfa
ENSMUSG00000109539.1	1.302443494	1.50E-06	0.00016623	93.39714444	230.6680613	RP23-413C19.2
ENSMUSG00000097468.1	1.30107069	0.012213031	0.074669248	15.54850455	38.48363832	2310010G23Rik
ENSMUSG00000100455.1	1.300802829	3.57E-05	0.001446839	48.63038032	120.2451426	Gm29170
ENSMUSG00000030178.15	1.30053787	0.004194922	0.036142943	18.66554645	46.16055982	Klra13-ps
ENSMUSG00000024232.1	1.295143322	0.008813243	0.059481177	221.635331	543.6759699	Bambi
ENSMUSG00000035868.8	1.294975046	1.43E-06	0.000160539	117.997066	289.9439366	3110052M02Rik
ENSMUSG00000104118.1	1.294690078	2.63E-05	0.001197446	47.26098513	116.1602581	Gm37298

ENSMUSG00000026360.9	1.294478158	2.67E-13	3.38E-10	5971.625729	14646.67834	Rgs2
ENSMUSG00000033107.5	1.293669831	1.15E-07	2.10E-05	263.2298186	645.0844801	Rnf125
ENSMUSG00000103076.1	1.291025481	0.000826925	0.01191751	21.73750023	53.18580543	Gm37902
ENSMUSG00000102691.1	1.286337488	2.02E-06	0.000204423	124.3682461	303.167316	Gm37780
ENSMUSG00000020653.11	1.280708177	0.006101011	0.04600453	198.8034628	482.7585889	Klf11
ENSMUSG00000091183.5	1.279004808	0.002651217	0.026536477	44.88024863	109.1479181	Gm5141
ENSMUSG00000104973.1	1.276269691	0.000595948	0.009532159	27.69342891	67.24739472	A530041M06Rik
ENSMUSG00000014905.3	1.273233645	9.63E-11	6.10E-08	756.2619399	1827.825932	Dnajb9
ENSMUSG00000096433.1	1.267984262	0.00017788	0.004236347	89.92475983	216.9409566	Gm4944
ENSMUSG00000065434.1	1.267015281	4.89E-05	0.00177316	37.68436811	90.77622138	Mir7-1
ENSMUSG00000027132.3	1.265447928	4.69E-06	0.000385522	166.6665137	401.1370322	Katnbl1
ENSMUSG00000042271.13	1.264184374	0.00599628	0.045431147	18.0484155	43.25532375	Nxt2
ENSMUSG00000030641.10	1.262083301	9.72E-08	1.89E-05	100.6300248	241.6382156	Ddias
ENSMUSG00000034640.9	1.259647165	7.61E-13	8.04E-10	570.877574	1366.959783	Tiparp
ENSMUSG00000098206.1	1.250950849	8.92E-05	0.002646083	56.28524515	134.3455232	A430106G13Rik
ENSMUSG00000104383.1	1.249272037	1.98E-05	0.001006793	70.26579388	167.1414555	Gm37553
ENSMUSG00000035235.12	1.246534979	1.12E-16	3.56E-13	495.4393211	1175.135962	Trim13
ENSMUSG00000064918.2	1.230878571	0.001685762	0.019278154	24.5626088	57.74689109	Gm22571
ENSMUSG00000094411.1	1.226109418	0.005257308	0.041908006	18.57579619	43.49589746	Snord16a
ENSMUSG00000104107.1	1.225070792	0.008691473	0.058753241	26.26797374	61.52147619	Gm37879
ENSMUSG00000058331.14	1.222520141	2.64E-05	0.001199069	103.6204297	242.1824653	Zfp85

ENSMUSG00000053581.13	1.218674844	0.003628428	0.032864989	1366.401368	3179.87204	Zfand2a
ENSMUSG00000069727.5	1.218586629	0.000562069	0.009128579	37.67897307	87.82890165	Gm5595
ENSMUSG00000085334.7	1.215945129	6.72E-17	2.90E-13	538.9061209	1252.650872	Gm12940
ENSMUSG00000066613.14	1.215180317	0.008465035	0.057653243	271.2037565	629.8683708	Zfp932
ENSMUSG00000101335.1	1.208361207	0.000533698	0.008939339	40.06323682	92.89279095	Gm28229
ENSMUSG00000102258.1	1.207841861	6.17E-07	8.59E-05	89.65490129	207.3674303	Gm38257
ENSMUSG00000109005.1	1.207382227	1.39E-07	2.45E-05	342.6235055	791.6095099	RP24-344N22.5
ENSMUSG00000055480.6	1.206723508	0.002577846	0.026041593	40.66803137	94.00673838	Zfp458
ENSMUSG00000062931.15	1.205849177	0.000236962	0.005094313	85.37583397	197.2892462	Zfp938
ENSMUSG00000042417.4	1.20292918	0.003849105	0.03406769	34.25343123	78.77712163	Ccno
ENSMUSG00000029054.8	1.202798852	0.017562978	0.094285682	16.11713326	36.96133343	Gabrd
ENSMUSG00000093734.1	1.198420612	0.005190639	0.041617101	22.70397108	52.27457756	Gm25082
ENSMUSG00000107756.1	1.193463466	4.44E-09	1.56E-06	414.3041908	948.0997121	Gm44164
ENSMUSG00000103192.1	1.190632971	0.005853521	0.044820724	19.60506867	44.84245926	Gm37645
ENSMUSG00000026011.13	1.185555923	6.70E-09	2.18E-06	240.5925758	547.3744632	Ctla4
ENSMUSG00000102672.1	1.185430635	7.11E-06	0.000507731	71.52875907	162.9811932	Gm37105
ENSMUSG00000085063.1	1.184023217	0.018362518	0.097288325	15.45772053	34.95163928	Gm16024
ENSMUSG00000072761.10	1.183189906	0.000574359	0.009240302	46.83816666	106.674196	Gm6712
ENSMUSG00000074622.4	1.183167814	0.009291429	0.062080074	31.56563659	71.40746322	Mafb
ENSMUSG00000104649.1	1.182483279	0.010147922	0.066264887	27.48803145	62.71299069	Gm43712
ENSMUSG00000085609.1	1.171580217	0.000187188	0.004399427	69.98278285	157.2578099	1700016P03Rik

ENSMUSG00000031196.13	1.165235869	0.010712843	0.068679296	18.31371305	41.07407715	F8
ENSMUSG00000062101.12	1.154747753	0.006068742	0.04584307	25.7781055	57.59406093	Zfp119b
ENSMUSG00000065663.1	1.152016163	0.007318759	0.05229218	29.30013925	65.47296961	Gm22579
ENSMUSG00000085241.7	1.149796892	3.83E-05	0.0015217	100.6086709	223.5066086	Snhg3
ENSMUSG00000079555.2	1.148489169	0.001528587	0.017892187	718.8252166	1593.695895	Haus3
ENSMUSG00000107801.1	1.147776972	0.016006809	0.088497755	16.57218227	36.80875926	Gm44086
ENSMUSG00000069184.2	1.147354381	0.01209078	0.074244307	27.41185967	60.65181997	Zfp72
ENSMUSG00000049536.5	1.146948122	0.016417986	0.089958065	17.42662837	38.63863443	Tceal1
ENSMUSG00000073062.3	1.146068843	0.002603468	0.02622948	54.56578915	121.1419924	Zxdb
ENSMUSG00000098905.1	1.143201474	9.95E-05	0.002852095	127.2618555	281.5724876	Zfp953
ENSMUSG00000109157.1	1.140642166	0.005256746	0.041908006	22.70687262	50.1275344	RP24-439I22.8
ENSMUSG00000062743.9	1.139525126	1.15E-05	0.00069917	139.5240866	307.9401046	Zfp677
ENSMUSG00000103388.1	1.139278721	4.44E-05	0.001679485	52.58620438	115.9639339	Gm37581
ENSMUSG00000019850.11	1.137783955	0.001706731	0.019460722	9703.903295	21352.51233	Tnfaip3
ENSMUSG00000108291.1	1.137155093	8.05E-07	0.000105151	102.3604706	225.0412901	Gm44292
ENSMUSG00000070605.4	1.136670585	0.006229506	0.04658523	35.81401969	79.08519298	Gm13251
ENSMUSG00000106022.1	1.134649859	3.30E-05	0.001381727	52.80974133	115.926899	Gm42929
ENSMUSG00000090110.8	1.134461786	0.00339134	0.031450587	32.1643152	70.80188475	Cmc4
ENSMUSG00000026049.11	1.133879998	3.86E-09	1.44E-06	190.4325955	418.4321734	Tex30
ENSMUSG00000106287.1	1.130952141	0.004658521	0.038850655	23.19383933	50.93059129	Gm43585
ENSMUSG00000090031.2	1.130835903	0.004891346	0.039976494	29.14345235	64.02504186	4732440D04Rik

ENSMUSG00000095990.1	1.130797172	0.004967433	0.040305247	37.93049241	83.22527155	Zfp97
ENSMUSG00000092887.1	1.128685306	0.0067183	0.049251982	20.34002746	44.57867778	Snord53
ENSMUSG00000049649.8	1.128427756	0.000605764	0.009628385	76.84611634	167.6507679	Gpr3
ENSMUSG00000017418.13	1.11497371	2.19E-11	1.63E-08	1208.796005	2618.759052	Arl5b
ENSMUSG00000102929.1	1.11161097	0.000380198	0.007135332	49.62171436	107.4128707	Gm37154
ENSMUSG00000059897.4	1.111212853	0.00054915	0.00899953	118.5800986	256.4924729	Zfp930
ENSMUSG00000041633.14	1.108938942	5.41E-05	0.001875058	88.16426414	190.2267841	Kctd12b
ENSMUSG00000023905.14	1.104816074	0.000342508	0.006634391	120.7672435	259.1715707	Tnfrsf12a
ENSMUSG00000069114.8	1.097761654	0.00199471	0.021802401	41.90488195	89.5026264	Zbtb10
ENSMUSG00000098557.1	1.097618186	4.17E-09	1.51E-06	613.0907828	1311.234822	Kctd12
ENSMUSG00000106093.1	1.086731646	0.006758419	0.049460224	29.45341203	62.79468484	Gm42722
ENSMUSG00000102235.1	1.085099137	0.014908383	0.084956993	20.84398675	44.35672395	Gm37886
ENSMUSG00000104235.1	1.084779096	0.007950807	0.055708423	29.27465035	62.35052119	Gm37589
ENSMUSG00000081219.1	1.083871994	0.010510019	0.067756192	24.27285612	51.25708865	Bambi-ps1
ENSMUSG00000109429.1	1.080100574	5.26E-05	0.001851991	124.5977951	263.9622249	RP24-344N22.3
ENSMUSG00000034317.14	1.077544003	1.62E-06	0.000178291	383.0698498	808.9301768	Trim59
ENSMUSG00000107165.1	1.077151549	0.00645634	0.047718152	44.3329738	93.91290659	Gm43747
ENSMUSG00000064647.1	1.074381858	0.001915609	0.021175336	35.96832622	75.69472124	Gm23301
ENSMUSG00000103823.1	1.073288424	0.001399574	0.016837418	33.29327603	69.97214623	Gm7899
ENSMUSG00000071252.5	1.068380288	0.001652406	0.019047027	33.76428389	70.95502211	2210408I21Rik
ENSMUSG00000101970.6	1.068351967	5.37E-09	1.79E-06	3222.644929	6758.614791	1810026B05Rik

ENSMUSG00000038174.14	1.061970385	6.54E-08	1.38E-05	470.8151621	983.4712756	Fam126b
ENSMUSG00000084433.1	1.061229638	0.014110081	0.081918655	21.58744184	45.21018223	Gm25945
ENSMUSG00000106475.1	1.058644968	0.001077289	0.014200928	39.98126196	83.24294193	Gm43011
ENSMUSG00000047141.5	1.05420862	0.002699019	0.026774605	1093.53035	2271.027839	Zfp654
ENSMUSG00000072762.9	1.05039469	0.000665093	0.010325236	53.42977382	110.814633	4930522L14Rik
ENSMUSG00000027654.2	1.04814756	0.001680221	0.019245059	118.0580084	243.7374951	Fam83d
ENSMUSG00000031327.10	1.042540095	0.005483798	0.042974583	28.88768486	59.43382866	Chic1
ENSMUSG00000105677.1	1.042040824	4.08E-07	6.01E-05	356.0957516	733.6833786	Gm43328
ENSMUSG00000106251.1	1.041318156	0.000143844	0.003682341	83.34589712	172.0272822	Gm42658
ENSMUSG00000037411.10	1.041133391	0.003717305	0.033326836	69.05672598	141.6328049	Serpine1
ENSMUSG00000106962.1	1.037972042	0.004643637	0.038777584	54.29219503	111.8928766	Gm43633
ENSMUSG00000105135.1	1.035102263	0.01842808	0.09751333	33.02269659	67.78075108	Gm43667
ENSMUSG00000105176.1	1.029211335	0.003919358	0.034503425	29.8815124	61.15668716	Gm43668
ENSMUSG00000025326.12	1.027166029	0.007360499	0.052531155	1890.589401	3853.204833	Ube3a
ENSMUSG00000086920.1	1.025791098	6.10E-05	0.002032762	77.97631064	158.9347928	Gm12207
ENSMUSG00000057835.7	1.022411641	0.013326194	0.078944239	23.92983187	48.62168345	Zfp119a
ENSMUSG00000015243.4	1.021821501	3.40E-08	8.44E-06	502.6336287	1019.6608	Abca1
ENSMUSG00000060149.6	1.02011266	1.32E-06	0.000153988	149.8482071	304.3239103	BC002059
ENSMUSG00000079470.8	1.018015176	2.05E-08	5.64E-06	445.1978965	901.366119	Utp14b
ENSMUSG00000024524.16	1.017618116	0.000321365	0.006329278	102.206885	207.1487176	Gnal
ENSMUSG00000026761.12	1.017354349	0.010803298	0.069014717	1000.535999	2025.456203	Orc4

ENSMUSG00000051341.5	1.00950021	0.000233816	0.005045968	226.8516181	457.0067499	Zfp52
ENSMUSG00000062082.16	1.007686375	0.003625668	0.032864989	34.88474842	70.17331423	Cd200r4
ENSMUSG00000038702.6	1.007206786	0.00046217	0.008120058	115.4340405	232.4568535	Dsel
ENSMUSG00000105895.1	1.005382739	0.001071182	0.014138743	58.97666223	118.3568555	Gm42829
ENSMUSG00000026064.15	1.005357411	1.96E-10	1.18E-07	1291.055993	2591.539161	Ptp4a1
ENSMUSG00000041075.8	1.003729568	0.013514136	0.079751493	23.78402163	47.85627591	Fzd7
ENSMUSG00000085903.1	1.002807152	5.00E-05	0.001793769	91.2027453	182.5974946	Gm15340
ENSMUSG00000035958.2	1.001581694	6.17E-08	1.35E-05	698.0826307	1398.194452	Tdp2
ENSMUSG00000028410.13	1.001082032	1.95E-14	4.12E-11	6343.48396	12696.27237	Dnaja1
ENSMUSG00000059901.12	-1.000755058	0.010509859	0.067756192	52.34432823	26.08304969	Adamts14
ENSMUSG00000080374.1	-1.00705161	0.003579997	0.03267896	85.77150409	42.64233678	Gm22953
ENSMUSG00000097174.1	-1.014534835	0.017322014	0.093416465	44.33338185	21.96309135	Gm4890
ENSMUSG00000077394.1	-1.018077531	0.001040093	0.01381121	142.9769164	70.71496039	Gm24339
ENSMUSG00000045775.15	-1.022244783	2.09E-09	8.54E-07	452.2163353	222.6810204	Slc16a5
ENSMUSG00000085651.1	-1.063008651	0.009236524	0.061778399	48.4508798	23.19385796	Gm11695
ENSMUSG00000087633.1	-1.077455684	0.002049264	0.022226092	68.23554418	32.19428709	Gm14455
ENSMUSG00000039670.2	-1.098882825	0.000898351	0.012554043	81.90987815	38.35638667	Oxld1

ENSMUSG00000006587.5	-1.122249745	4.90E-05	0.00177316	132.9929007	60.92373766	Snai3
ENSMUSG00000095288.1	-1.144651187	0.005286816	0.042095153	45.71417487	20.60543148	Gm7618
ENSMUSG00000001420.13	-1.145549717	0.012908265	0.077169375	41.26380843	18.64835586	Tmem79
ENSMUSG00000058966.13	-1.149797366	0.006782803	0.049522475	45.51405931	20.56592337	Fam57b
ENSMUSG00000034684.12	-1.160992103	0.003013304	0.028853014	73.42677702	32.57164663	Sema3f
ENSMUSG00000080006.4	-1.166534409	0.001289322	0.015872817	61.00909258	27.07066705	Rps19-ps7
ENSMUSG00000052565.6	-1.182892165	0.003035549	0.029022137	53.30064332	23.39966505	Hist1h1d
ENSMUSG00000097057.1	-1.185638939	0.002120683	0.022747507	60.51041518	26.71182501	Gm17638
ENSMUSG00000095174.1	-1.199665803	3.68E-08	8.97E-06	256.9949187	112.0335847	Gm20069
ENSMUSG00000028678.13	-1.211847216	0.007447275	0.05297141	44.06196837	19.00225162	Kif2c
ENSMUSG00000068601.2	-1.214140979	0.014414645	0.082889114	36.33829912	15.74085144	Gm10244
ENSMUSG00000020475.3	-1.229856074	0.015406861	0.086551714	35.8970283	15.18578767	Pgam2
ENSMUSG00000028068.14	-1.232583861	0.013598852	0.080086166	33.75050569	14.27213786	Iqgap3
ENSMUSG00000097407.1	-1.24482647	0.010329413	0.067001029	33.37556375	14.0291934	4933408J17Rik

ENSMUSG00000049608.8	-1.245654987	0.01180036	0.073157145	34.05943161	14.27213786	Gpr55
ENSMUSG00000031750.15	-1.255582099	0.004392308	0.037393657	54.28453277	22.56477545	Il34
ENSMUSG00000042010.16	-1.265850855	2.13E-06	0.000212803	126.7407905	52.59391149	Acacb
ENSMUSG00000091133.1	-1.267861195	0.012052558	0.074153375	33.02797849	13.61979638	Gm17108
ENSMUSG00000058290.3	-1.271237479	0.000526555	0.008870205	80.55902188	33.33004434	Espl1
ENSMUSG00000108428.1	-1.297032379	0.011552286	0.072055322	29.64813255	12.0352876	RP24-576D1.5
ENSMUSG00000062758.6	-1.368895695	0.013936071	0.081280913	31.54324896	12.093262	Gm16477
ENSMUSG00000050179.3	-1.399832638	0.000114344	0.003135314	76.07197703	28.71057469	A930002I21Rik
ENSMUSG00000097620.7	-1.454232228	0.002939198	0.028395226	37.57368995	13.63836507	4921514A10Rik
ENSMUSG00000045777.14	-1.454696163	0.006276142	0.046803434	33.47877936	12.07469332	Ifitm10
ENSMUSG00000052374.14	-1.497164863	2.84E-07	4.43E-05	163.5516524	57.61116811	Actn2
ENSMUSG00000097573.2	-1.554875532	0.006670265	0.048956499	29.94006587	10.08078751	G730003C15Rik
ENSMUSG00000004612.9	-2.970586463	0.004719896	0.039073202	73.17100953	9.280357355	Nkg7
ENSMUSG00000069516.7	-3.07771762	0.000430765	0.007762342	31.61724009	3.707934577	Lyz2

ENSMUSG00000098343.1	-5.308354159	0.002072402	0.022400329	66.95428503	1.677045005	Mir6240

Appendix 5 – DEGs identified in the 4M1 subset using the DESeq2 package (p-adjusted < 0.1, fold-change > 2)

	log2FoldChange	pvalue	padj	X4M1_WT_mean	X4M1_KO_mean	symbol
ENSMUSG00000092609.1	5.778595426	0.00067929	0.081909115	0.926457323	51.64944403	Gm20481
ENSMUSG00000091971.3	5.759798238	1.96E-06	0.001325797	47.83947918	2593.115793	Hspa1a
ENSMUSG00000090877.3	5.154970124	2.58E-05	0.009981299	149.3893899	5323.560685	Hspa1b
ENSMUSG00000029378.5	2.468534845	0.000119483	0.032868322	8.809042147	49.09221094	Areg
ENSMUSG00000028214.13	2.345014362	2.69E-08	6.65E-05	36.53464157	186.6465923	Gem
ENSMUSG00000003032.8	2.17473823	0.000418722	0.059578839	85.85726241	387.8642786	Klf4
ENSMUSG00000042622.13	2.071228252	0.00071889	0.082293594	109.8619634	462.0054838	Maff
ENSMUSG00000054364.4	1.955005533	2.14E-06	0.001325797	41.53555452	161.2181727	Rhob
ENSMUSG00000026826.13	1.776797688	0.000349148	0.054026229	226.5254677	776.5886755	Nr4a2
ENSMUSG00000021701.7	1.758756899	0.000806134	0.088310884	213.8745017	723.8753539	Plk2
ENSMUSG00000064493.1	1.693966421	0.000382998	0.057818698	19.71889226	63.62051054	Snora28
ENSMUSG00000024521.7	1.645751164	1.61E-05	0.006863984	67.97925363	213.088993	Pmaip1
ENSMUSG00000102461.1	1.629450537	0.000168499	0.033811552	34.63808205	107.7752033	Gm37166
ENSMUSG00000000078.6	1.599749297	0.000669039	0.081909115	2215.748322	6715.900423	Klf6
ENSMUSG00000040289.8	1.55840179	9.50E-07	0.000890001	75.49772554	222.8397052	Hey1
ENSMUSG00000028524.21	1.549426001	0.000363858	0.055607377	42.82158325	125.764418	Sgip1

ENSMUSG00000064427.1	1.451914949	0.000979949	0.097341634	24.27368041	65.98706913	Gm22748
ENSMUSG00000024232.1	1.292942105	0.000143093	0.033059051	102.3856328	251.378211	Bambi
ENSMUSG00000041649.13	1.273972491	0.000169345	0.033811552	38.72044042	93.77927811	Klf8
ENSMUSG00000056501.3	1.272039509	0.000612619	0.078995999	75.02040288	180.9583918	Cebpb
ENSMUSG00000028035.13	1.174264304	0.000122223	0.032891315	567.3315335	1280.834668	Dnajb4
ENSMUSG00000035235.12	1.119175272	1.01E-06	0.000890001	262.1362487	570.5321812	Trim13
ENSMUSG00000102258.1	1.116319788	0.000841457	0.090577342	54.68272022	119.0560914	Gm38257
ENSMUSG00000026360.9	1.10270543	0.000226777	0.040103816	3544.108048	7611.632095	Rgs2
ENSMUSG00000031266.6	1.094399027	4.66E-05	0.014800896	187.7132241	401.9174691	Gla
ENSMUSG00000064368.1	-1.048303697	6.29E-08	0.000111314	549.0106009	265.6439649	mt-Nd6
ENSMUSG00000046380.3	-1.112938698	2.79E-06	0.001571184	199.5558773	92.23485427	Jrk
ENSMUSG00000020900.15	-1.142752124	1.24E-05	0.005885606	215.9940127	97.70943971	Myh10
ENSMUSG00000097000.1	-1.43328824	0.000564784	0.075176959	76.7442687	28.398387	Gm17435
ENSMUSG00000047632.10	-1.779445578	0.000266827	0.04485497	88.94846335	25.76898282	Fgfbp3

Appendix 6 – DEGs identified in the 4M2 subset using the DESeq2 package (p-adjusted < 0.1, fold-change > 2)

	log2FoldChange	pvalue	padj	X4M2_WT_mean	X4M2_KO_mean	symbol

ENSMUSG00000092609.1	10.02445888	0.000243368	0.002116707	0	38.6271845	Gm20481
ENSMUSG00000091971.3	6.173514205	5.21E-07	1.39E-05	23.25521334	1675.504495	Hspa1a
ENSMUSG00000090877.3	5.446427467	8.89E-06	0.00014507	94.31614904	4110.715286	Hspa1b
ENSMUSG00000056749.7	2.227450285	7.56E-07	1.87E-05	25.86441637	120.5595837	Nfil3
ENSMUSG00000003032.8	2.209393833	0.000490794	0.003670247	63.86620831	295.0141705	Klf4
ENSMUSG00000097125.1	2.187995096	0.000906095	0.006015053	6.657697551	30.61312613	Gm26885
ENSMUSG00000003545.3	2.160727976	0.001026704	0.006632301	98.66925148	441.2953253	Fosb
ENSMUSG00000054364.4	2.132475055	0.002242672	0.012480324	58.08160983	254.3267767	Rhob
ENSMUSG00000021136.13	2.073339967	0.000247252	0.00214758	13.12584999	55.38413685	Smoc1
ENSMUSG00000105482.1	1.977942173	0.009513575	0.038567331	5.93520021	23.62459509	Gm42596
ENSMUSG00000028214.13	1.929876771	6.65E-06	0.000113679	37.09645074	140.9161537	Gem
ENSMUSG00000080885.1	1.899410146	0.000517007	0.003810592	14.56147252	54.43727583	Rpl10-ps6
ENSMUSG00000042622.13	1.881156228	0.002291965	0.012672349	101.1033946	372.1566399	Maff
ENSMUSG00000091842.2	1.760569985	0.005653709	0.02585501	8.001308775	27.30775935	Gm16490
ENSMUSG00000097407.1	1.727308621	0.000556422	0.004041142	13.06490204	43.42095007	4933408J17Rik
ENSMUSG00000104309.1	1.70565992	0.009641878	0.038955138	7.602884871	24.58521261	Gm5846
ENSMUSG00000022615.7	1.663178263	0.007030136	0.03066989	7.731482032	24.52594202	Tymp
ENSMUSG00000021701.7	1.64919151	6.94E-10	4.65E-08	184.0704039	576.4239622	Plk2
ENSMUSG00000052684.4	1.645902763	1.58E-05	0.000233632	2212.534171	6923.539222	Jun
ENSMUSG00000097233.1	1.636831972	0.012168094	0.046504285	8.454568756	26.22849934	Gm17552
ENSMUSG00000064371.1	1.616131089	0.000515578	0.003802497	28.03131901	85.8944643	mt-Tt

ENSMUSG00000046408.13	1.612200882	0.00252801	0.013712055	13.89055099	42.43699242	1700067K01Rik
ENSMUSG00000082424.4	1.605205303	0.023421387	0.076806615	8.373697742	25.62389405	Gm13292
ENSMUSG00000101875.1	1.566479605	0.026135532	0.083533631	7.430002548	22.02164192	Gm6028
ENSMUSG00000070167.1	1.53171849	0.009257891	0.037829048	9.696207139	28.13061126	Snora57
ENSMUSG00000082978.1	1.477432848	0.01389989	0.051375239	11.06033081	30.99344203	Rpsa-ps11
ENSMUSG00000034758.12	1.476329608	0.018980937	0.065468192	9.226873752	25.45144891	Tle6
ENSMUSG00000060198.7	1.474006644	0.024252231	0.078628609	8.142538358	22.58204298	Gm11353
ENSMUSG00000038550.10	1.473255697	1.64E-11	1.84E-09	80.39376541	223.2442098	Ciart
ENSMUSG00000040734.14	1.47249505	0.00047366	0.003577495	20.31767854	56.32209038	Ppp1r13l
ENSMUSG00000083429.2	1.462522187	0.01291641	0.04860669	9.818103042	27.06961215	Gm15198
ENSMUSG00000079681.2	1.44698862	0.021459153	0.07186497	10.65864663	29.29262569	Zglp1
ENSMUSG00000101122.1	1.434183173	0.011016353	0.043199697	13.15691335	35.55044023	Gm17971
ENSMUSG00000024521.7	1.43011929	5.00E-06	8.94E-05	55.20738051	148.3587038	Pmaip1
ENSMUSG00000095288.1	1.396476943	0.000135671	0.001336438	36.3628131	95.69036726	Gm7618
ENSMUSG00000040289.8	1.39380549	0.001185519	0.007481517	20.10436071	52.90297424	Hey1
ENSMUSG00000024042.6	1.383877753	0.000328197	0.002694107	2315.820431	6043.302198	Sik1
ENSMUSG00000026826.13	1.382761393	1.58E-05	0.000233632	117.069883	304.6664825	Nr4a2
ENSMUSG00000082481.1	1.377048279	0.023481504	0.076956997	10.90855031	28.59034333	Rps12-ps19
ENSMUSG00000002012.13	1.372137648	0.016357544	0.058345235	10.82038866	28.03008598	Pnck
ENSMUSG00000081402.4	1.370729035	0.031400108	0.09569663	9.135451825	23.68173598	Gm15455
ENSMUSG00000103119.1	1.362262346	0.019664431	0.067301985	10.99001071	28.43122359	Gm37583

ENSMUSG00000046546.3	1.324013591	0.027929254	0.088019259	15.25408527	38.02151438	Fam43a
ENSMUSG00000046341.5	1.307959063	0.006371472	0.028478884	16.63695324	41.09900851	Gm11223
ENSMUSG00000029657.15	1.303067233	0.011239485	0.043809454	1598.658192	3944.49271	Hsph1
ENSMUSG00000047394.7	1.301535472	0.026145594	0.08354502	12.2422	30.24045025	Odf3b
ENSMUSG00000061390.6	1.29930562	0.000262164	0.002257278	36.31793856	89.46875579	Gm7866
ENSMUSG00000028655.11	1.291347272	0.025181146	0.081015136	16.37708803	39.78455083	Mfsd2a
ENSMUSG00000040435.11	1.28419391	1.89E-15	5.93E-13	5854.864252	14257.66331	Ppp1r15a
ENSMUSG00000021250.13	1.279622301	0.000937625	0.006169758	1748.153814	4243.682689	Fos
ENSMUSG00000107950.1	1.279346828	0.008218264	0.034581273	19.36076154	47.11185567	Gm6375
ENSMUSG00000077323.1	1.269778297	0.015667553	0.056527194	12.77515223	30.87731795	Rnu11
ENSMUSG00000023905.14	1.26794829	1.25E-07	4.07E-06	81.90849081	197.0055796	Tnfrsf12a
ENSMUSG00000030847.8	1.267840468	0.000671281	0.004687302	44.3720019	106.7391967	Bag3
ENSMUSG00000022415.12	1.262954429	0.002949191	0.015524918	22.60262742	54.06623961	Syngr1
ENSMUSG00000086016.1	1.250668938	0.00300816	0.015770756	22.43210263	53.55814524	1700084C06Rik
ENSMUSG00000024232.1	1.241045553	0.000151505	0.001459965	90.42699177	213.1628584	Bambi
ENSMUSG00000096768.7	1.229710103	0.007284067	0.031513813	655.2672327	1536.762799	Erdr1
ENSMUSG00000092505.1	1.221833114	0.001670217	0.009844404	26.46116815	61.89573977	Gm19246
ENSMUSG00000045382.6	1.203024098	0.000233237	0.002050097	1145.866358	2637.593831	Cxcr4
ENSMUSG00000103034.1	1.200590684	0.027000856	0.085829874	12.75522917	29.43387325	Gm8797
ENSMUSG00000038264.7	1.184929555	0.027875032	0.087891473	12.88649722	29.21669147	Sema7a
ENSMUSG00000077563.1	1.180703927	0.002198299	0.012281242	27.20476732	61.69405546	Snora68

ENSMUSG00000006356.10	1.174873651	0.019630181	0.067238471	16.27570457	36.65201791	Crip2
ENSMUSG00000107211.1	1.167805678	0.016996998	0.060158702	16.07352703	36.26662279	Gm5864
ENSMUSG00000037032.15	1.165167059	0.005669672	0.025872786	25.09881263	56.23293208	Apbb1
ENSMUSG00000031824.14	1.161324357	0.006937407	0.030385133	23.46368346	52.40493699	6430548M08Rik
ENSMUSG00000080006.4	1.157322024	0.003672387	0.018455251	34.11997256	76.34148381	Rps19-ps7
ENSMUSG00000073409.12	1.143914005	6.58E-05	0.0007458	168.1071864	370.8560918	H2-Q6
ENSMUSG00000034936.2	1.133835947	0.012659272	0.047905488	30.92624149	67.86704481	Arl4d
ENSMUSG00000065750.1	1.130642092	0.011460336	0.044468043	21.89394127	47.69254601	Gm23346
ENSMUSG00000109208.1	1.126127715	0.001592783	0.009444295	34.1572431	74.43806202	Cd37
ENSMUSG00000000078.6	1.120566419	0.007538603	0.032367651	3963.676383	8618.039012	Klf6
ENSMUSG00000089542.1	1.117364972	0.001308855	0.008077453	38.79044636	84.13818911	Gm25835
ENSMUSG00000024347.16	1.108419049	0.004543364	0.021784759	41.33584984	88.95206892	Psd2
ENSMUSG00000012819.15	1.101120716	0.001737645	0.010181142	34.34678821	73.64057861	Cdh23
ENSMUSG00000037211.12	1.088800182	0.00042558	0.003295679	69.04517693	146.3555766	Spry1
ENSMUSG00000082474.3	1.08172269	0.028937835	0.090290259	16.44532662	34.92530064	Gm8213
ENSMUSG00000053581.13	1.080548369	3.07E-08	1.19E-06	919.996628	1945.068198	Zfand2a
ENSMUSG00000043801.6	1.07502573	0.009061704	0.037204793	29.15776275	61.51876211	Oaz1-ps
ENSMUSG00000090439.1	1.07349762	0.00140807	0.008562319	39.03858189	82.27583593	Gm17455
ENSMUSG00000082436.1	1.065038974	0.008595	0.03567605	27.79925689	57.95549127	Gm11688
ENSMUSG00000029720.9	1.062281682	8.32E-05	0.000892923	80.25430398	167.787407	Gm20605
ENSMUSG00000090582.1	1.060757457	0.002612373	0.014104181	49.18401865	102.1242802	Gm17024

ENSMUSG00000091845.1	1.05815257	0.00056284	0.004083134	83.76208898	174.6234948	Gm4604
ENSMUSG00000052837.5	1.049485441	1.11E-11	1.31E-09	14434.01091	29873.74776	Junb
ENSMUSG00000081640.1	1.04708163	0.021574046	0.072184894	24.85887048	51.61221781	Rplp0-ps1
ENSMUSG00000076495.1	1.037916205	0.005952062	0.026960452	38.30553364	78.87808873	Trbj2-4
ENSMUSG00000020893.17	1.03791071	2.81E-10	2.11E-08	2687.753386	5518.944486	Per1
ENSMUSG00000044628.4	1.02908798	0.02718989	0.086281422	18.84162049	38.55079159	Rnf208
ENSMUSG00000032501.8	1.017184115	5.80E-07	1.51E-05	375.1266572	759.1236269	Trib1
ENSMUSG00000021636.14	1.014484219	0.002631363	0.014194783	48.77491118	98.68900523	Marveld2
ENSMUSG00000059033.7	1.013682615	0.02430016	0.078740529	24.49644305	49.68768691	Rpl18a-ps1
ENSMUSG00000080779.1	1.012176217	0.029145232	0.090739153	25.77910632	52.27140319	Gm8731
ENSMUSG00000032177.16	1.010128393	0.000518444	0.003817066	59.22322423	119.4655485	Pde4a
ENSMUSG00000094955.1	1.005278761	0.012679548	0.047943998	56.68976847	114.0138799	Gm3699
ENSMUSG00000063894.14	-1.000191708	3.08E-07	8.78E-06	1077.41986	538.2500325	Zkscan8
ENSMUSG00000108542.1	-1.00192136	0.029697346	0.091918857	43.7646992	21.95850066	RP23-185A21.1
ENSMUSG00000047648.13	-1.002109291	0.000480906	0.003619462	120.7115696	60.11298884	Fbxo30
ENSMUSG00000079489.2	-1.003797188	0.00387254	0.019280212	78.87559903	39.39272424	C030013D06Rik
ENSMUSG00000101609.1	-1.00408152	7.15E-10	4.75E-08	1913.687229	953.7606678	Kcnq1ot1
ENSMUSG00000076483.1	-1.005597293	0.000420947	0.003271631	118.1514522	58.94919105	Trbj1-1

ENSMUSG00000072704.6	-1.007623305	4.49E-11	4.44E-09	761.6076267	378.9959943	Smim10l1
ENSMUSG00000025597.13	-1.007790188	0.01925046	0.066220141	52.29896021	26.06589764	Klhl4
ENSMUSG00000026097.9	-1.009878876	3.38E-08	1.29E-06	769.077332	382.4817649	Ormdl1
ENSMUSG00000031197.11	-1.010741981	1.49E-14	3.62E-12	1361.147709	675.4330513	Vbp1
ENSMUSG00000063253.10	-1.012531122	7.68E-07	1.89E-05	296.1478319	146.5405697	Scoc
ENSMUSG00000034587.8	-1.014683754	0.000495733	0.003687878	127.5066471	62.86051536	8430429K09Rik
ENSMUSG00000103358.1	-1.01692883	0.000458926	0.003499108	357.7094649	176.67861	Gm37593
ENSMUSG00000057137.9	-1.017965365	0.003603226	0.018193035	75.82939013	37.35086071	Tmem140
ENSMUSG00000001833.16	-1.019037284	1.58E-26	3.39E-23	9886.011908	4877.727264	7-Sep
ENSMUSG00000035293.13	-1.020043249	8.97E-07	2.17E-05	682.4101036	336.2328608	G2e3
ENSMUSG00000040321.3	-1.020375546	6.41E-09	3.11E-07	589.4944726	290.4537089	Zfp770
ENSMUSG00000030469.10	-1.022569567	8.03E-05	0.000871347	158.4969641	77.80913085	Zfp719
ENSMUSG00000037075.6	-1.022816553	4.73E-20	5.52E-17	1949.832491	959.7775331	Rnf139
ENSMUSG00000026775.9	-1.02334769	4.35E-19	3.73E-16	5494.512347	2702.626553	Yme1l1

ENSMUSG00000035898.13	-1.024311809	2.99E-10	2.22E-08	694.6521503	341.7955908	Uba6
ENSMUSG00000102153.1	-1.024566536	0.001576771	0.009366634	93.58559178	45.88655628	Gm37474
ENSMUSG00000061778.10	-1.025361576	1.12E-05	0.000177619	262.2253218	128.485897	Mospd2
ENSMUSG00000064351.1	-1.026609476	7.63E-17	3.07E-14	231111.9994	113444.0771	mt-Co1
ENSMUSG00000065434.1	-1.027035728	0.018107172	0.063217732	48.74977898	23.82578936	Mir7-1
ENSMUSG00000025104.13	-1.029808631	3.93E-08	1.47E-06	285.3567757	139.8801216	Hdgfrp3
ENSMUSG00000104369.1	-1.03044763	0.006548895	0.029120045	76.53345654	37.68895838	Gm38082
ENSMUSG00000085241.7	-1.032006393	0.0011778	0.007440109	170.8370693	83.24597815	Snhg3
ENSMUSG00000024259.8	-1.034299972	5.75E-14	1.21E-11	1446.380859	706.2776481	Slc25a46
ENSMUSG00000031176.8	-1.034372482	1.01E-07	3.39E-06	502.8279151	244.9642021	Dynlt3
ENSMUSG00000031931.3	-1.034705897	2.05E-09	1.20E-07	501.8989446	245.321598	Ankrd49
ENSMUSG00000024766.14	-1.036171172	4.57E-09	2.39E-07	384.0828014	187.3146198	Lipo1
ENSMUSG00000086503.3	-1.037691445	7.34E-10	4.81E-08	47646.80067	23208.816	Xist
ENSMUSG00000091998.1	-1.038535969	0.006942945	0.030399032	65.76346492	32.1795151	Gm17072

ENSMUSG00000087175.7	-1.041443449	0.006675032	0.029517554	90.37823023	43.68717027	Gm15133
ENSMUSG00000076752.2	-1.041476341	0.026415716	0.084276093	43.70719224	21.17999667	Tcrg-C2
ENSMUSG00000095387.5	-1.042144676	0.004297781	0.02087192	68.33114899	33.04429783	Trav6d-4
ENSMUSG00000104125.1	-1.047652295	0.000820469	0.005527842	100.3168697	48.35347236	Gm37488
ENSMUSG00000020191.11	-1.047796084	0.013815934	0.051123728	55.33212801	26.68958366	4930415F15Rik
ENSMUSG00000040651.8	-1.051533545	4.08E-09	2.18E-07	4002.266639	1930.924278	Fam208a
ENSMUSG00000032328.12	-1.05530892	3.95E-14	9.06E-12	3390.133343	1631.072793	Tmem30a
ENSMUSG00000103192.1	-1.057187248	0.013210771	0.049367575	75.73570598	36.37314844	Gm37645
ENSMUSG00000045827.10	-1.058557724	4.88E-09	2.52E-07	342.505698	164.4255754	Serpinb9
ENSMUSG00000055660.8	-1.060238639	1.09E-09	6.75E-08	590.908622	283.739718	Mettl4
ENSMUSG00000025766.14	-1.061899109	0.00015806	0.001510682	239.4350981	114.2893797	D3Ertd751e
ENSMUSG00000072972.3	-1.063127192	0.012156671	0.046504285	50.29230747	24.04417969	Adam4
ENSMUSG00000109429.1	-1.064024401	0.000286682	0.002416585	225.2801575	107.9062628	RP24-344N22.3
ENSMUSG00000031095.15	-1.067105174	1.06E-16	4.04E-14	2058.141376	981.7684637	Cul4b

ENSMUSG00000067931.5	-1.068619535	0.000825102	0.005553238	105.9757226	50.33649641	Zfp948
ENSMUSG00000059142.15	-1.068820189	9.32E-09	4.32E-07	1075.852332	512.6111458	Zfp945
ENSMUSG00000109206.1	-1.07179188	0.002309821	0.01274914	85.63468004	40.83655766	RP24-417N13.4
ENSMUSG00000042460.5	-1.071829457	7.55E-17	3.07E-14	2993.926298	1423.984051	C1galt1
ENSMUSG00000024293.15	-1.072504233	1.65E-18	1.24E-15	2857.477283	1358.290189	Esco1
ENSMUSG00000049630.6	-1.072962336	0.009661593	0.039019721	49.68056573	23.52503333	C1ql3
ENSMUSG00000021886.6	-1.073678813	5.99E-15	1.71E-12	1325.907535	629.6331184	Gpr65
ENSMUSG00000025912.16	-1.074572478	0.006525226	0.029034882	77.71157139	36.80755439	Mybl1
ENSMUSG00000030291.12	-1.075073689	2.59E-08	1.02E-06	483.5285956	228.9807907	Med21
ENSMUSG00000073664.11	-1.076202425	1.89E-10	1.51E-08	1319.454634	625.6108081	Nbeal1
ENSMUSG00000103373.1	-1.076868498	0.024369012	0.078915092	54.14591509	25.82639819	Gm37238
ENSMUSG00000033578.7	-1.077167855	0.000680955	0.004741971	107.0591926	50.47866511	Tmem35
ENSMUSG00000021712.14	-1.077316727	2.72E-09	1.53E-07	708.9643665	335.840732	Trim23
ENSMUSG00000035868.8	-1.079481406	5.40E-07	1.43E-05	344.8206848	163.0699644	3110052M02Rik

ENSMUSG00000078851.4	-1.079504784	0.008456949	0.035296781	59.70723421	28.25408795	Hist3h2a
ENSMUSG00000021211.3	-1.07954275	0.033224119	0.099882145	39.56324543	18.83414019	Akr1c12
ENSMUSG00000030031.14	-1.080433377	1.15E-05	0.000180623	213.1242183	100.8869377	Kbtbd8
ENSMUSG00000079317.10	-1.080661681	5.74E-05	0.000667886	201.2306719	94.78536333	Trappc2
ENSMUSG00000107165.1	-1.08124407	0.008958798	0.036859332	53.75360306	25.38763153	Gm43747
ENSMUSG00000107549.1	-1.083059462	0.001561081	0.009286303	93.26328649	44.16022774	Gm43961
ENSMUSG00000027115.14	-1.086484135	3.63E-07	1.02E-05	293.4760355	138.019163	Kif18a
ENSMUSG00000073131.12	-1.088289175	9.00E-13	1.36E-10	694.9893983	326.5640601	Vma21
ENSMUSG00000056260.15	-1.091435102	2.77E-08	1.09E-06	1647.194615	773.0030674	Lrif1
ENSMUSG00000100615.1	-1.091887539	2.21E-07	6.59E-06	266.0172968	124.4842058	Gm5511
ENSMUSG00000086429.9	-1.092366701	7.34E-05	0.000812695	237.9630803	111.4924261	Gt(ROSA)26Sor
ENSMUSG00000064797.1	-1.092700362	0.000182675	0.00169429	119.2148665	55.96540201	Gm24357
ENSMUSG00000003929.10	-1.093749215	8.74E-14	1.73E-11	851.9546502	398.7101972	Zfp81
ENSMUSG00000029276.13	-1.09393977	2.18E-09	1.27E-07	377.7270401	177.1153482	Glmn

ENSMUSG00000034459.8	-1.094045493	1.05E-20	1.50E-17	4914.962757	2301.98119	Ifit1
ENSMUSG00000060149.6	-1.094514371	2.06E-05	0.000288887	399.2366073	186.4961397	BC002059
ENSMUSG00000015882.17	-1.094696002	5.68E-07	1.49E-05	585.8736698	274.2367983	Lcorl
ENSMUSG00000055560.7	-1.096635955	0.008423351	0.035233329	60.92392111	28.49237885	Zfp459
ENSMUSG00000079339.6	-1.105872834	7.93E-20	8.50E-17	2022.408123	939.5090252	Ifit1bl1
ENSMUSG00000025893.7	-1.106567141	5.25E-05	0.00062272	147.2321936	68.60753103	Kbtbd3
ENSMUSG00000027550.14	-1.108761224	3.27E-17	1.68E-14	1331.117362	617.013611	Lrrcc1
ENSMUSG00000014956.15	-1.10938567	3.94E-18	2.53E-15	8183.11219	3792.979863	Ppp1cb
ENSMUSG00000108216.1	-1.110562954	0.004468322	0.021505159	55.7443631	25.8886183	Gm44153
ENSMUSG00000108752.1	-1.113654135	0.000102071	0.001049697	152.1563254	70.33705411	RP23-385F8.3
ENSMUSG00000108621.1	-1.11402861	7.41E-11	6.86E-09	1135.216653	524.2396321	Gm37494
ENSMUSG00000003198.9	-1.115013407	4.13E-09	2.19E-07	415.1331264	191.3486434	Zfp959
ENSMUSG00000055480.6	-1.117623181	0.000575492	0.004154176	88.7607619	40.83684507	Zfp458
ENSMUSG00000044583.13	-1.12506118	6.06E-12	8.15E-10	1337.576568	612.5207286	Tlr7

ENSMUSG00000077394.1	-1.126560023	0.017455438	0.061442952	80.96230544	37.38525283	Gm24339
ENSMUSG00000064493.1	-1.132935166	0.000840174	0.005645811	121.1026655	55.20743233	Snora28
ENSMUSG00000020290.14	-1.136470565	1.33E-17	7.76E-15	8075.397537	3672.899177	Xpo1
ENSMUSG00000074165.10	-1.136601452	4.02E-08	1.50E-06	691.9211574	314.3219026	Zfp788
ENSMUSG00000031200.16	-1.137818383	0.00015149	0.001459965	307.6587724	139.7823008	Mtcp1
ENSMUSG00000031802.8	-1.138537703	0.006829229	0.030040743	88.76044854	40.39856844	Phxr4
ENSMUSG00000103779.1	-1.140494227	3.12E-06	6.00E-05	685.8540616	311.0063791	Gm36931
ENSMUSG00000080237.2	-1.145120679	0.027282429	0.08652659	30.75689546	13.84664909	Gm14239
ENSMUSG00000098274.7	-1.148220303	0.028008366	0.088132758	47.7958089	21.40399865	Rpl24
ENSMUSG00000059423.13	-1.153786877	8.67E-08	2.96E-06	376.9879379	169.5177075	Zfp933
ENSMUSG00000102780.1	-1.15956156	0.000330562	0.00270661	176.9312552	79.18953561	Gm38253
ENSMUSG00000104706.1	-1.163977443	0.001370388	0.008400731	76.43266246	34.22500428	Gm43421
ENSMUSG00000076904.2	-1.165712433	0.000461567	0.003515073	85.16090761	37.87107192	Traj25
ENSMUSG00000057842.13	-1.169747871	2.47E-08	9.89E-07	429.5054625	191.0072812	Zfp595

ENSMUSG00000071281.10	-1.172869465	5.51E-07	1.45E-05	619.8876666	274.4653306	Zfp65
ENSMUSG00000054293.10	-1.174371916	1.46E-12	2.11E-10	533.509074	236.4398184	A630033H20Rik
ENSMUSG00000042659.15	-1.176593994	0.008800276	0.036340361	55.87680992	24.82282738	Arrdc4
ENSMUSG00000031246.14	-1.176609673	6.70E-13	1.05E-10	798.0873172	352.9807471	Sh3bgrl
ENSMUSG00000102850.1	-1.177448683	0.002902968	0.015344429	112.9431884	50.15235449	Gm37082
ENSMUSG00000078862.10	-1.180077232	2.53E-05	0.000343094	122.7400943	54.10120654	Gm14326
ENSMUSG00000108365.1	-1.181509159	0.011071681	0.043345722	45.1367029	19.77833909	RP24-174G2.2
ENSMUSG00000074024.5	-1.183685331	5.26E-05	0.000622725	131.2673406	57.96402485	4632427E13Rik
ENSMUSG00000104910.1	-1.186527286	0.009134095	0.037430282	45.48069939	19.85949624	Gm43331
ENSMUSG00000102875.1	-1.189970024	0.021413861	0.071742294	32.11658009	14.14619656	Gm37139
ENSMUSG00000069206.13	-1.193669044	3.61E-09	1.98E-07	477.8511044	209.4168589	Zfp874a
ENSMUSG00000106962.1	-1.194593952	0.002539872	0.013750224	138.7683106	60.75237496	Gm43633
ENSMUSG00000023882.15	-1.198522546	1.74E-07	5.39E-06	251.7144253	109.8795595	Zfp54
ENSMUSG00000105572.1	-1.20142409	0.000433333	0.003343637	187.5087175	81.55166702	Gm43300

ENSMUSG00000106959.1	-1.201687078	0.001400696	0.008533622	72.5565562	31.51955228	Gm42548
ENSMUSG00000064339.1	-1.205097134	6.71E-25	1.23E-21	41230.09367	17883.3516	mt-Rnr2
ENSMUSG00000025326.12	-1.205398005	3.45E-18	2.33E-15	4087.721732	1772.3307	Ube3a
ENSMUSG00000104235.1	-1.205616354	0.001389895	0.008487934	117.0719645	50.4830682	Gm37589
ENSMUSG00000069208.8	-1.206591111	1.99E-05	0.000281627	151.8340201	65.94425488	Zfp825
ENSMUSG00000102973.1	-1.206593681	0.001981185	0.011289066	1124.378946	487.0866126	E430014B02Rik
ENSMUSG00000075271.4	-1.210362229	0.002990514	0.015697451	52.57281732	22.72261441	Ttc30a1
ENSMUSG00000072066.6	-1.211733105	0.029717213	0.091918857	29.43646757	12.66391426	6720489N17Rik
ENSMUSG00000056019.12	-1.212001087	7.53E-11	6.91E-09	456.4599174	196.6825483	Zfp709
ENSMUSG00000102930.1	-1.214771416	0.011276918	0.043915413	38.08610393	16.31250032	Gm38115
ENSMUSG00000067942.5	-1.217549249	1.97E-13	3.61E-11	924.7348571	397.1422534	Zfp160
ENSMUSG00000080810.1	-1.220037281	0.00312035	0.016246295	55.07552307	23.56401463	Gm11737
ENSMUSG00000032740.16	-1.220288487	6.01E-06	0.000104269	145.185238	62.27619937	Ccdc88a
ENSMUSG00000103657.1	-1.221392231	0.005307875	0.024635229	54.35687539	23.226407	Gm37204

ENSMUSG00000036676.14	-1.222372424	0.002379122	0.013042052	72.09156654	30.66255327	Tmtc3
ENSMUSG00000102844.1	-1.223058968	1.58E-05	0.000233632	156.2126999	66.96786996	Gm38034
ENSMUSG00000064337.1	-1.225706697	2.35E-19	2.32E-16	17011.64333	7274.124542	mt-Rnr1
ENSMUSG00000101452.1	-1.229417614	0.003483557	0.017749158	52.89571199	22.60073499	Gm28530
ENSMUSG00000062743.9	-1.236942283	1.05E-11	1.27E-09	524.4126609	222.3404881	Zfp677
ENSMUSG00000090015.8	-1.240011247	1.31E-10	1.10E-08	490.7543224	207.7671093	Gm15446
ENSMUSG00000058900.4	-1.240536826	1.32E-07	4.30E-06	259.0376073	109.5218487	Rsl1
ENSMUSG00000101111.1	-1.240945844	0.001534432	0.009183018	79.06722565	33.50441862	Gm28437
ENSMUSG00000073236.4	-1.241463768	0.005136357	0.024001405	58.46147015	24.84258425	2500004C02Rik
ENSMUSG00000064367.1	-1.242173407	4.66E-29	1.50E-25	46961.00352	19852.04297	mt-Nd5
ENSMUSG00000076920.1	-1.24273341	0.019224954	0.066150102	38.10199663	15.97169814	Traj8
ENSMUSG00000027132.3	-1.244400125	5.11E-09	2.60E-07	339.4499942	143.01759	Katnbl1
ENSMUSG00000064341.1	-1.246398725	8.34E-32	5.36E-28	52942.24443	22315.14821	mt-Nd1
ENSMUSG00000095362.7	-1.247900476	4.23E-05	0.000523345	130.5864576	54.76919809	Gm14325

ENSMUSG00000103839.1	-1.248989091	0.000682245	0.004748383	92.56515125	38.99105421	Gm37607
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ENSMUSG00000064370.1	-1.258088809	1.18E-27	3.02E-24	110120.3147	46040.99609	mt-Cytb
ENSMUSG00000107402.1	-1.259346004	0.00085926	0.005747029	72.1630654	30.2138732	4732416N19Rik
ENSMUSG00000068130.11	-1.259681058	0.026277183	0.083923772	30.68716474	12.86254773	Zfp442
ENSMUSG00000033972.9	-1.260450761	7.47E-09	3.53E-07	636.5091191	265.6774752	Zfp944
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ENSMUSG00000095432.8	-1.274749548	1.49E-12	2.13E-10	700.6723266	288.9956713	Zfp748
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ENSMUSG00000089417.1	-1.288201045	0.004627145	0.02209582	513.9725115	210.6590669	Gm22009
ENSMUSG00000071291.10	-1.290686493	1.15E-05	0.000180623	135.6913891	55.24510376	Zfp58
ENSMUSG00000071266.13	-1.295203742	6.63E-05	0.000748371	106.5813525	43.59982668	Zfp946
ENSMUSG00000082705.1	-1.296538883	0.006860259	0.030108786	40.55212853	16.4518632	Gm15616

ENSMUSG00000078546.8	-1.300589058	1.82E-10	1.47E-08	498.0876566	202.028325	2210404O09Rik
ENSMUSG00000057396.6	-1.303058236	2.16E-05	0.000301675	145.1282251	58.51937428	Zfp759
ENSMUSG00000076899.1	-1.303157022	0.005840481	0.026520445	59.91878389	24.44130293	Traj30
ENSMUSG00000107205.1	-1.304645921	0.003625415	0.018276355	46.91247227	18.87713586	Gm42576
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ENSMUSG00000052155.5	-1.308425599	1.69E-05	0.000246873	136.8252188	54.98773213	Acvr2a
ENSMUSG00000105703.1	-1.309744527	0.020212815	0.068648806	764.5833836	308.4207047	Gm43305
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ENSMUSG00000093384.1	-1.31509879	0.018488692	0.064062569	32.92556625	13.30373149	Gm20689
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ENSMUSG00000078870.9	-1.366750509	8.88E-06	0.00014507	119.4738663	46.17142611	Gm14410
ENSMUSG00000021714.14	-1.368648333	3.31E-05	0.000431505	94.72199705	36.5122663	Cenpk
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ENSMUSG00000062101.12	-1.414372044	4.42E-07	1.21E-05	136.0877315	50.91970517	Zfp119b
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ENSMUSG00000107197.1	-1.419375375	0.02531131	0.081302602	24.22109381	8.997844742	Gm43312
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ENSMUSG00000103672.1	-1.424871899	0.009484078	0.038514189	45.85132021	16.89706133	Gm37621
ENSMUSG00000021510.10	-1.426291031	5.32E-17	2.36E-14	921.2528683	342.7920375	Zfp729a
ENSMUSG00000090494.1	-1.431273904	0.000502127	0.003724099	61.3652707	22.86076874	Traj36
ENSMUSG00000108211.1	-1.434017057	0.012911921	0.048604026	42.49584712	15.60900941	Gm44130
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ENSMUSG00000041540.16	-1.455336522	0.008831706	0.036435039	34.55330947	12.56300026	Sox5
ENSMUSG00000048280.17	-1.458073792	1.61E-13	3.01E-11	661.6923184	240.6301559	Zfp738
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ENSMUSG00000064602.1	-1.462989433	0.015184733	0.05504787	65.18768231	23.47098566	Snora41
ENSMUSG00000050714.9	-1.470300675	9.13E-08	3.10E-06	268.0128298	96.54463043	Zbtb26
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ENSMUSG00000087556.1	-1.476955294	0.020714421	0.069975672	26.16412771	9.35680651	Gm15764

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ENSMUSG00000078502.10	-1.488568859	6.69E-10	4.50E-08	531.4811967	189.8186049	Gm13212
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ENSMUSG00000106946.1	-1.489392855	0.008460093	0.035298441	37.75247764	13.30721344	Gm42856
ENSMUSG00000047632.10	-1.490581648	0.00017506	0.001641428	87.46147318	31.02122763	Fgfbp3
ENSMUSG00000103869.1	-1.494541352	0.000188062	0.001731756	69.21656712	24.46628272	Gm37420
ENSMUSG00000002617.14	-1.496744832	3.44E-14	8.03E-12	424.6580386	150.5851717	Zfp40
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ENSMUSG00000016833.14	-1.504593166	9.10E-15	2.31E-12	319.8622638	112.6547279	Mrps18c
ENSMUSG00000083909.1	-1.51378964	0.003393598	0.017394221	35.52077739	12.46247497	Gm15842
ENSMUSG00000094942.7	-1.514650347	0.000924483	0.006103866	49.98086645	17.43209388	Gm3604
ENSMUSG00000073062.3	-1.520667895	2.50E-05	0.000340144	143.3095399	50.20683327	Zxdb
ENSMUSG00000079083.2	-1.520855858	4.26E-19	3.73E-16	583.6047264	203.0658403	Jrkl
ENSMUSG00000098905.1	-1.523191325	2.35E-15	7.21E-13	385.1949026	133.9515248	Zfp953

ENSMUSG00000104432.1	-1.534203669	0.008465379	0.035309036	29.74464831	10.18004714	A430027C01Rik
ENSMUSG00000100213.1	-1.535388743	0.013431228	0.050002444	26.93611935	9.219040896	Gm28151
ENSMUSG00000107083.1	-1.539033854	3.43E-06	6.52E-05	138.576132	47.8294918	Gm43313
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ENSMUSG00000027171.10	-1.545998259	0.001305913	0.008069423	56.35936511	19.19551905	Prrg4
ENSMUSG00000103697.1	-1.547304606	0.006510224	0.0289882	45.1695344	15.38834568	Gm38020
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ENSMUSG00000030165.16	-1.548091539	0.008449101	0.035275477	37.38470842	12.68381483	Klrd1
ENSMUSG00000088929.1	-1.553385973	0.027750689	0.087585343	33.20535452	11.18284051	Gm24299
ENSMUSG00000098715.6	-1.554763755	0.000532076	0.003895335	45.7572274	15.54959512	Gm28053
ENSMUSG00000103525.1	-1.556259502	0.022846742	0.075230111	24.92633898	8.436378836	Gm37262
ENSMUSG00000063383.5	-1.557584514	0.001296324	0.008031864	46.24096135	15.75035828	Zfp947
ENSMUSG00000102992.1	-1.558639505	0.00014499	0.001404554	84.67980721	28.67512613	Gm37766
ENSMUSG00000064345.1	-1.566420041	9.94E-39	1.28E-34	33614.96817	11349.94951	mt-Nd2

ENSMUSG00000103348.1	-1.575688133	0.010352461	0.041112417	26.67566475	9.036149917	Gm37053
ENSMUSG00000035133.8	-1.576303531	7.48E-16	2.60E-13	363.9503243	122.2065423	Arhgap5
ENSMUSG00000102426.1	-1.581993819	1.15E-05	0.000180531	114.3596579	38.03420603	Kantr
ENSMUSG00000107201.1	-1.58373826	0.008492065	0.0353798	27.67853975	9.296327373	5930420M18Rik
ENSMUSG00000067916.9	-1.597292579	5.03E-05	0.000603053	78.45725206	25.98846746	Gm13139
ENSMUSG00000023892.7	-1.598314949	1.43E-29	6.15E-26	1049.398148	346.9250056	Zfp51
ENSMUSG00000054626.11	-1.606806599	0.0111555	0.043587827	23.57620721	7.694971474	Xlr
ENSMUSG00000090110.8	-1.609506114	3.25E-05	0.000425366	113.2205818	37.20680733	Cmc4
ENSMUSG00000069755.5	-1.613412693	4.04E-08	1.50E-06	181.6083723	59.34781025	Zfp125
ENSMUSG00000071267.11	-1.62194738	7.61E-06	0.000127627	1390.138831	451.739314	Zfp942
ENSMUSG00000078435.5	-1.633000436	3.72E-07	1.03E-05	113.1344063	36.34980831	AU041133
ENSMUSG00000091509.7	-1.637871343	0.004365681	0.021096704	320.7989856	103.1132895	Gm17066
ENSMUSG00000107120.1	-1.639886142	1.59E-05	0.000234126	91.36621059	29.33310295	Gm43059
ENSMUSG00000107054.1	-1.644152413	0.013857861	0.051244739	28.72393176	9.05992115	C730045M19Rik

ENSMUSG00000106341.1	-1.646500313	0.002835885	0.015070401	40.74670206	12.84733766	Gm43330
ENSMUSG00000072762.9	-1.647774503	5.62E-12	7.69E-10	206.4935911	65.76460083	4930522L14Rik
ENSMUSG00000059897.4	-1.661542656	5.85E-14	1.21E-11	295.8732901	93.1990738	Zfp930
ENSMUSG00000064358.1	-1.679431755	3.08E-07	8.78E-06	122.0593065	38.09159194	mt-Co3
ENSMUSG00000074867.3	-1.679543848	1.02E-05	0.000163384	112.0089606	34.9654905	Zfp808
ENSMUSG00000102642.1	-1.681181408	1.08E-08	4.92E-07	162.3057925	50.55953485	A130048G24Rik
ENSMUSG00000053347.14	-1.685006325	8.31E-15	2.23E-12	705.6391013	219.3350576	Zfp943
ENSMUSG00000102277.1	-1.699104178	0.003881806	0.019303915	28.99049823	9.015860632	A130050O07Rik
ENSMUSG00000064368.1	-1.702202664	5.45E-13	9.34E-11	745.3880871	229.4546421	mt-Nd6
ENSMUSG00000044501.17	-1.702277712	1.66E-12	2.34E-10	482.5560925	148.5722324	Zfp758
ENSMUSG00000074865.10	-1.712714142	0.001552219	0.009247179	41.64878298	12.86240403	Zfp934
ENSMUSG00000076474.3	-1.726874897	5.59E-17	2.39E-14	359.6058148	108.3495173	Trbv17
ENSMUSG00000078495.10	-1.727191967	1.10E-15	3.61E-13	802.0027588	242.2522911	Gm13157
ENSMUSG00000103996.1	-1.728767463	0.010942458	0.042990615	24.41792956	7.315187996	4833421G17Rik

ENSMUSG00000108500.1	-1.739982784	0.000347346	0.002820677	45.34943134	13.50691176	RP23-335G1.5
ENSMUSG00000105135.1	-1.749666072	1.16E-05	0.000181205	79.89160052	23.74235882	Gm43667
ENSMUSG00000104011.1	-1.772868041	0.021796313	0.072693849	27.20535671	7.894281072	Gm32391
ENSMUSG00000076890.1	-1.773773852	0.000915657	0.006061158	42.5376421	12.32591791	Traj40
ENSMUSG00000073427.3	-1.781379795	2.83E-07	8.15E-06	103.8994718	30.15847327	Gm4924
ENSMUSG00000069743.4	-1.793131689	0.000304299	0.002528613	86.89742025	24.81390508	Zfp820
ENSMUSG00000109255.1	-1.796048644	0.000453124	0.00346721	39.3171914	11.28148112	RP24-215A14.6
ENSMUSG00000101249.1	-1.797323767	1.08E-09	6.72E-08	185.3696926	53.02354379	Gm29216
ENSMUSG00000107468.1	-1.802708393	0.000800755	0.00542631	36.01062322	10.25960702	5730507A11Rik
ENSMUSG00000095990.1	-1.804009904	1.60E-07	5.01E-06	113.6997333	32.65982386	Zfp97
ENSMUSG00000067928.6	-1.805828473	9.19E-05	0.000963024	60.38870666	17.1346761	Zfp760
ENSMUSG00000032261.16	-1.812337606	0.004341809	0.021022204	38.93986933	11.07844456	Sh3bgrl2
ENSMUSG00000096433.1	-1.817193671	5.84E-13	9.63E-11	381.3967784	108.0316665	Gm4944
ENSMUSG00000072763.2	-1.839478803	1.99E-09	1.17E-07	175.7275995	48.83818421	5430403G16Rik

ENSMUSG00000100131.1	-1.841749684	0.005568643	0.025560004	54.7937486	15.34641485	Gm28439
ENSMUSG00000108522.1	-1.843579877	0.000572669	0.004140418	41.85363141	11.58223715	RP23-385F8.2
ENSMUSG00000053164.6	-1.853925418	0.011146206	0.043565688	28.58270218	7.795496759	Gpr21
ENSMUSG00000105179.1	-1.855155693	0.002521066	0.013680163	28.07026239	7.715260759	Gm42866
ENSMUSG00000081642.1	-1.857740876	0.003002863	0.015751513	27.67736098	7.733809072	Gm13532
ENSMUSG00000103283.2	-1.862110382	8.04E-06	0.000133008	62.38806876	17.07313212	Gm37276
ENSMUSG00000062931.15	-1.865700416	1.64E-11	1.84E-09	241.8008326	66.09125778	Zfp938
ENSMUSG00000083645.1	-1.892662807	0.010270222	0.040912209	32.88916111	8.776792291	Gm14086
ENSMUSG00000076895.1	-1.89674188	5.36E-06	9.47E-05	67.11998458	18.09273282	Traj34
ENSMUSG00000102157.1	-1.89817299	0.014378548	0.052689917	28.90084445	7.617684997	Gm37470
ENSMUSG00000073448.1	-1.926225173	8.33E-05	0.000892923	54.66120648	14.2479304	Gm10509
ENSMUSG00000102555.1	-1.940680776	0.002926815	0.015445075	33.97467527	8.89625461	6430511E19Rik
ENSMUSG00000102349.1	-1.985479427	0.000142941	0.00138994	57.21014625	14.36753642	Gm37376
ENSMUSG00000081603.1	-2.02952933	2.60E-06	5.18E-05	64.16668974	15.57109295	Gm14681

ENSMUSG00000103162.1	-2.032404701	0.001418132	0.008602355	26.58483221	6.492479787	Gm38147
ENSMUSG00000102496.1	-2.038693295	7.01E-05	0.000781645	88.45698286	21.4417714	Gm36989
ENSMUSG00000082179.1	-2.048269916	0.002776355	0.014796867	32.56078128	7.737291018	Gm11407
ENSMUSG00000103443.1	-2.08182369	0.004013537	0.019782984	24.09534825	5.591952679	Gm37132
ENSMUSG00000009628.14	-2.106143277	0.004114773	0.02013206	28.35005067	6.454174612	Tex15
ENSMUSG00000104118.1	-2.163680591	5.91E-05	0.000684932	47.48169699	10.52031689	Gm37298
ENSMUSG00000084497.1	-2.179476258	0.000639268	0.004520239	42.8001782	9.299276896	Gm22107
ENSMUSG00000070605.4	-2.182773907	1.36E-08	6.03E-07	118.6390358	26.30564211	Gm13251
ENSMUSG00000106099.1	-2.193896287	0.00037151	0.002968152	317.9844774	69.45337673	Gm42664
ENSMUSG00000084350.1	-2.206270454	1.67E-10	1.36E-08	153.1213984	33.18191974	Znf41-ps
ENSMUSG00000048521.7	-2.227562664	0.000753046	0.005151894	31.25937371	6.553491348	Cxcr6
ENSMUSG00000097751.1	-2.29077286	0.000264443	0.002273857	49.49949003	9.979816401	Gm26690
ENSMUSG00000105077.1	-2.332822446	0.000304179	0.002528613	29.85363577	5.970527606	Gm4859
ENSMUSG00000108101.1	-2.359142822	6.80E-05	0.000762513	39.74292432	7.69443905	RP23-14P17.5

ENSMUSG00000093954.8	-2.402404081	0.002346045	0.012899232	26.839669	5.009665063	Gm16867
ENSMUSG00000054945.3	-2.415116475	0.000225047	0.001992409	29.4715613	5.530941118	Gm9958
ENSMUSG00000061544.13	-2.470766236	0.000491553	0.003672603	31.40789394	5.532149668	Zfp229
ENSMUSG00000076908.1	-2.577243763	4.83E-13	8.50E-11	123.2605094	20.75828237	Traj21
ENSMUSG00000104571.1	-2.592411802	0.001495413	0.008970385	26.36481312	4.250098108	Gm43010
ENSMUSG00000086240.4	-2.625293822	0.000209231	0.001880888	26.13424312	4.227535426	Gm7846
ENSMUSG00000083863.1	-2.630068162	7.23E-10	4.77E-08	177.8952822	28.85265048	Gm13341
ENSMUSG00000103427.1	-2.632832522	6.28E-05	0.000718005	37.41459301	5.911933145	Gm37534
ENSMUSG00000076137.1	-2.762773895	1.86E-06	3.95E-05	71.23730707	10.69784125	Gm26384
ENSMUSG00000064357.1	-3.03456649	2.00E-10	1.57E-08	152.4557814	18.63603914	mt-Atp6
ENSMUSG00000067929.3	-3.084453737	1.45E-06	3.22E-05	53.68120145	6.19293231	Gm10226
ENSMUSG00000104925.1	-3.231787934	8.95E-05	0.000944991	26.7366127	2.746317971	Gm43061
ENSMUSG00000064346.1	-3.237052836	1.32E-06	2.98E-05	43.19425838	4.608916173	mt-Tw
ENSMUSG00000081376.1	-3.624708783	2.04E-06	4.28E-05	35.78331349	2.845102284	Gm11694

ENSMUSG00000066009.8	-5.762485736	7.14E-07	1.78E-05	35.27816435	0.64102578	Gm13051