

**Faculté de pharmacie
et des sciences biomédicales**

UNDERSTANDING THE ROLE OF SLC38A2 IN HEMATOPOIESIS

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Résumé

L'absorption de la glutamine constitue un élément majeur du métabolisme cellulaire à travers les compartiments hématopoïétiques et immunitaires, néanmoins le rôle *in vivo* des transporteurs individuels de la glutamine dans le développement des cellules sanguines est encore mal défini. *SLC38A2* (*SNAT2*) est un transporteur d'acides aminés neutre exprimé de manière ubiquitaire et présente une expression élevée à travers le compartiment des cellules souches hématopoïétiques (CSH), les cellules progénitrices et immunitaires différenciées, suggérant une fonction potentielle dans l'équilibre hématopoïétique. Cette étude a été conçue pour évaluer les conséquences de l'ablation restreinte hématopoïétique de *Slc38a2* chez les souris.

À l'aide d'un modèle murin hématopoïétique Vav-Cre *Slc38a2* knockout, des paramètres hématologiques combinés à la cytométrie en flux multiparamétrique ont été réalisés pour examiner les compartiments de cellules souches et progénitrices, les populations myéloïdes et lymphoïdes progénitrices engagées dans la lignée, ensemble avec des sous-ensembles de cellules immunitaires matures dans la moelle osseuse, le thymus et la circulation sanguine. La perte de *Slc38a2* a eu des effets mineurs sur les CSH et les compartiments des cellules progénitrices multipotentes, avec un changement non significatif croissant des LMPP à biais lymphoïde. Alternativement, les populations de progéniteurs engagés étaient significativement altérées, caractérisées par des proportions accrues de GMP et CLP, des populations de MEP plus faibles ainsi qu'une redistribution des CLP favorisant les progéniteurs biaisés Ly6D⁺ B au détriment de T/NK-cellules biaisées, pointant une asymétrie de la différenciation des lignées vers des lignées myéloïdes-granulocytaires et lymphoïdes B.

Malgré les altérations dans les progéniteurs en amont, le développement des lymphocytes T dans le thymus est resté largement non affecté et les proportions de cellules B, T et NK en périphérie n'ont montré aucun changement majeur. Néanmoins, les cellules T CD4⁺ naïves étaient nettement réduites, accompagnées d'une augmentation correspondante des cellules T CD4⁺ effectrices-mémoire et les proportions de cellules dendritiques périphériques étaient significativement plus faibles, soutenant les observations précédentes suggérant que transport *Slc38a2*-dépendant du de la glutamine soutient la survie et le fonctionnement de cDC1.

Dans l'ensemble, *Slc38a2* joue un rôle limité pour la production hématopoïétique en conditions basales, mais contribue à l'équilibre des pools de progéniteurs engagés ainsi qu'au maintien des sous-ensembles de DC et de cellules T CD4⁺ et pourrait révéler le contexte-capacités métaboliques spécifiques en cas de stress inflammatoire ou immunitaire.

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III. ABBREVIATIONS

AA: Amino acid

ABC: ATP-binding cassette transporter

AML: Acute myeloid leukemia

APC: Antigen-presenting cell

ATP: Adenosine triphosphate

BM: Bone marrow

CBC: Complete blood count

CLP: Common lymphoid progenitor

CMP: Common myeloid progenitor

DC: Dendritic cell

DN: Double negative (CD4⁻CD8⁻ thymocyte)

DP: Double positive (CD4⁺CD8⁺ thymocyte)

EDTA: Ethylenediaminetetraacetic acid

ETP: Early thymic progenitor

FACS: Fluorescence-activated cell sorting / flow cytometry

FBS: Fetal bovine serum

FAO: Fatty acid oxidation

FMO: Fluorescence-minus-one control

GMP: Granulocyte–monocyte progenitor

HGB: Hemoglobin

HSC: Hematopoietic stem cell

HSPC: Hematopoietic stem and progenitor cell

HCT: Hematocrit

KO: Knockout

LAT1: L-type amino acid transporter 1 (SLC7A5)

LSK: Lineage⁻ Sca-1⁺ c-Kit⁺ cells

LMA: Acute Myeloid Leukemia (AML)

LMPP/MPP4: Lymphoid-primed multipotent progenitor

Ly6D⁺/Ly6D⁻ CLP: B-biased / T/NK-biased common lymphoid progenitors

MCH: Mean corpuscular hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

MCV: Mean corpuscular volume

MEP: Megakaryocyte–erythroid progenitor

MPP: Multipotent progenitor

NK: Natural killer (cell)

NOX: NADPH oxidase

OXPHOS: Oxidative phosphorylation

PBS: Phosphate-buffered saline

PCR: Polymerase chain reaction

RBC: Red blood cell

ROS: Reactive oxygen species

SD: Standard deviation

SEM: Standard error of the mean

SLC: Solute carrier (transporter)

SLAM: Signalling Lymphocytic Activation Molecule

SNAT: Sodium-coupled neutral amino acid transporter (SLC38 family)

SPF: Specific pathogen-free

TCA: Tricarboxylic acid cycle

Treg: Regulatory T cell

WBC: White blood cell

IV. INTRODUCTION

In the mammalian hematopoietic organization, hematopoietic stem cells (HSCs) sit at the top of the differentiation hierarchy of mature blood cells. In the adult, HSCs are located primarily in the bone marrow (BM), where they support life-long blood production, called hematopoiesis, through their dual capacity to self-renew and to give rise to progenitor cells that undergo proliferation and differentiation into terminally differentiated blood cells (Pinho and Frenette, 2019) (Seita and Weissman, 2010) (figure 1). In adult BM, these cells consist of functionally distinct subsets including long-term HSCs (LT-HSCs) that ensure durable multilineage reconstitution, and short-term HSCs (ST-HSCs) with limited self-renewal and transient output. Downstream, HSCs differentiate into multipotent progenitors (MPPs), which have mostly lost durable self-renewal but preserve multilineage potential. MPPs are classified into MPP1-4 based on their lineage inclination: MPP2 and MPP3 mainly generate respectively megakaryocyte-erythroid and granulocyte-monocyte lineages, whereas MPP4, also called lymphoid-primed multipotent progenitor (LMPP) favors lymphoid lineages but preserve the ability to generate granulocyte-monocyte cells. Interestingly, MPP1 designates the LSK CD150⁻ (Slamf1⁻) CD48⁻ Flt3⁻ fraction, corresponding to multipotent progenitors that are intrinsically linked to HSCs but lack long-term self-renewal capacity, as they are located immediately downstream of HSCs (Pietras et al., 2015).

In contrast, committed progenitors have limited self-renewal ability, exhibit restricted lineage differentiation potential and exhaust within a few weeks after transplantation. At steady state, most HSCs are quiescent and are thereby protected from genotoxic insults, whereas downstream progenitors ensure most of the hematopoiesis (Pinho and Frenette, 2019). Within this pool of committed precursor cells, common myeloid progenitors (CMPs) preserve the capacity to generate various myeloid cell types and may subsequently mature into granulocyte-monocyte progenitors (GMPs) leading to the formation of granulocytes and monocytes, or into megakaryocyte-erythroid progenitors (MEPs), responsible for generating platelets and red blood cells (Pietras et al., 2015).

Initially represented as a simple hierarchical branching structure, differentiation of hematopoietic cells is now recognized as involving heterogeneous progenitor populations with lineage-

specific tendencies. Single-cell transplantation and in vitro differentiation assays have uncovered the existence of lineage-restricted progenitors (restricted to one or two lineages) and can be generated directly from HSCs, bypassing the classically defined multipotent intermediates (Pinho and Frenette, 2019).

To ensure hematopoietic homeostasis throughout life, the balance between self-renewal and differentiation needs to be tightly regulated: excessive differentiation or insufficient self-renewal depletes the HSC pool, whereas insufficient differentiation or unrestrained self-renewal favors myeloproliferative diseases or leukemia (Testa et al., 2022). HSC activity is regulated by an intricate interplay of cell-intrinsic factors, including transcriptional, epigenetic and metabolic regulators, and cell-extrinsic cues, from systemic factors and the bone marrow microenvironment, collectively referred to as the “stem cell niche” (Chambers et al., 2007). Within this niche, various stromal, endothelial, and neural elements integrate environmental cues to control HSCs quiescence, activation and lineage commitment.

Ageing significantly affects hematopoiesis, leading to preferential output of myeloid over lymphoid cells and an increased occurrence of myeloid malignancies in comparison with lymphoid leukemias. Single-cell RNA sequencing of HSCs from young (8-12 weeks) and aged (18-24 months) mice revealed that glutamine synthetase (glul), which converts glutamate to glutamine, is significantly upregulated in old HSCs, yet intracellular glutamine levels are decreased whereas glutamate levels remain unaffected. These findings suggest that rather than a simple upregulation of glutamine synthesis, its metabolism is perturbed in aged HSCs of mice and in human CD34⁺ cells, potentially connecting metabolic rewiring with age-related functional decline (Hellmich et al., 2023).

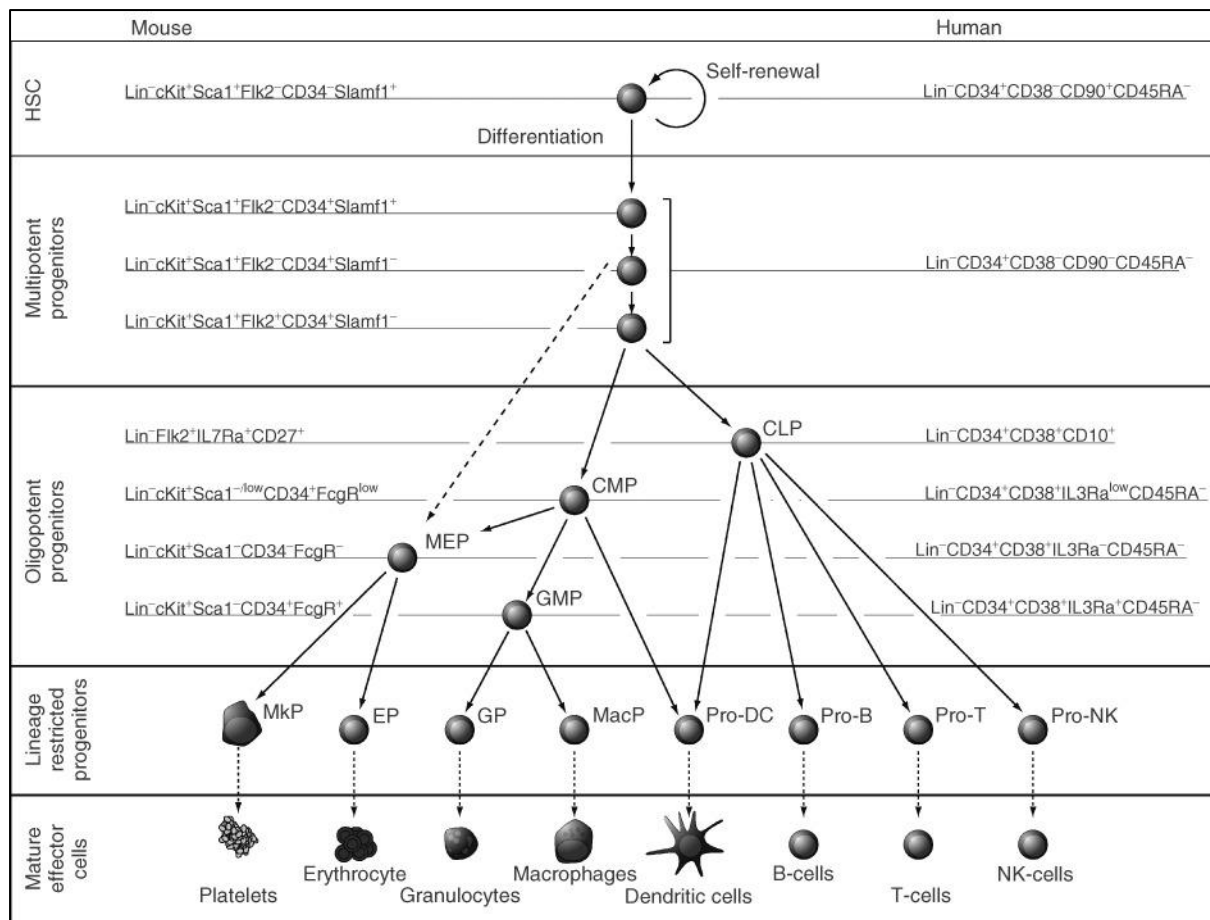


Figure 1: Hematopoietic stem cell differentiation hierarchy in human and mouse systems (Seita and Weissman, 2010). As HSCs undergo differentiation, they progressively lose self-renewal capability, ultimately becoming mature committed blood cells. There are distinct cell surface markers profiles of hematopoietic compartment pool in murine and human systems. In mice, the heterogeneity of multipotent progenitors is defined by proteins at the membrane and functional characteristics. Abbreviations: Lin (lineage markers); Hematopoietic stem cell (HSCs); MPP (Multipotent Progenitor); CLP (Common Lymphoid Progenitor); CMP (Common Myeloid Progenitor); MEP (Megakaryocyte-Erythroid Progenitor); GMP (Granulocyte-Monocyte Progenitor); MkP (Megakaryocyte Progenitor); EP (Erythrocyte Progenitor); GP (Granulocyte Progenitor); MacP (Macrophage Progenitor); DC (Dendritic Cell); NK (Natural Killer).

Throughout the blood-forming hierarchy, common lymphoid progenitors (CLPs) constitute the branching point from which major lymphoid lineages emerge. CLPs possess the potential to generate B cells, T cells and natural killer (NK) cells, thereby seeding the initial supply for both the innate and adaptative immune cell compartments. B cells mediate humoral

immunity through antibody production, CD4⁺ and CD8⁺ T cells orchestrate helper and cytotoxic responses (Kondo et al., 1997), and NK cells provide immediate cytotoxic protective response against infected or transformed cells (Vivier et al., 2011). Thus, qualitative or quantitative changes in CLP function can significantly affect immune functionality across several lymphoid lineages.

1. METABOLIC REGULATION OF HEMATOPOIESIS

Quiescent hematopoietic stem cells (HSCs) reside in a hypoxic bone marrow niche where they rely predominantly on glycolysis, supplemented with fatty acid oxidation to fulfill metabolic demands while preserving low levels of mitochondrial oxidative phosphorylation and preventing reactive oxygen species (ROS) accumulation to sustain their long-term self-renewal capacity (Morganti et al., 2022).

As HSCs transition to an activated state and progress toward proliferative progenitors and differentiated lineages, they undergo a metabolic reprogramming characterized by increased mitochondrial oxidative phosphorylation and enhanced glycolytic flux, reflecting the elevated bioenergetic and biosynthetic demands of cell division and lineage specification. These metabolic shifts are orchestrated by nutrient-sensing and signaling pathways that couple metabolism to cell fate, thereby regulating the balance between quiescence and proliferation (Li et al., 2023).

Among the main nutrients that fuel these pathways (glucose, fatty acids and amino acids), glutamine plays a central role by providing both carbon and nitrogen source for the synthesis of nucleotides, amino acids, lipids, and by serving as an anaplerotic substrate for the tricarboxylic acid cycle (Li et al., 2023).

2. Glutamine metabolism in hematopoiesis and immune cells

Although classified as a nonessential amino acid, glutamine can become conditionally essential during catabolic states when cellular demands are elevated. It is the second most consumed nutrient after glucose in rapidly dividing cells, such as immune cells, enterocytes, and

cancer cells(Cluntun et al., 2017). Glutamine plays crucial roles as a nitrogen and carbon donor and is a substrate for various biosynthetic pathways, including neurotransmitters, glutathione, proteins, nucleotides, and amino sugars(Menchini and Chaudhry, 2019).

Hematopoietic stem cells

Glucose and glutamine metabolism intricately regulate the fate of hematopoietic stem cells (HSCs), particularly in the context of erythropoiesis, the red blood cell production process. HSCs, under physiological conditions, take up glucose via the Glut1 transporter and glutamine via the Slc1a5 (also known as ASCT2) transporter to support nucleotide biosynthesis, a critical process for cell proliferation and differentiation. Disruption of these metabolic pathways, such as through glucose or glutamine transport inhibition, results in impaired nucleotide biosynthesis, redirecting HSC differentiation towards myelomonocytic lineages instead of the desired erythroid lineage(Oburoglu et al., 2014).

T cells

The immune system plays a significant role in eliminating cancer cells and foreign antigens like bacteria and viruses, thus maintaining the system's homeostasis. These functions of the immune system are elicited as innate or adaptive immunity. T cells are key players in the adaptive immunity(Kumar et al., 2018). Activation of naive T-cells by antigenic stimuli results in differentiation and expansion of the effector T-cells. This process is accompanied by dynamic switching between glycolysis and mitochondrial metabolism(Buck et al., 2016). Naïve T cells depend on oxidative phosphorylation and fatty acid oxidation for ATP production, and upon activation they lose their quiescence and become CD4⁺ /CD8⁺ effector T cells switching to glycolysis and glutaminolysis for their survival(Zhang and Romero, 2018) (figure 2).

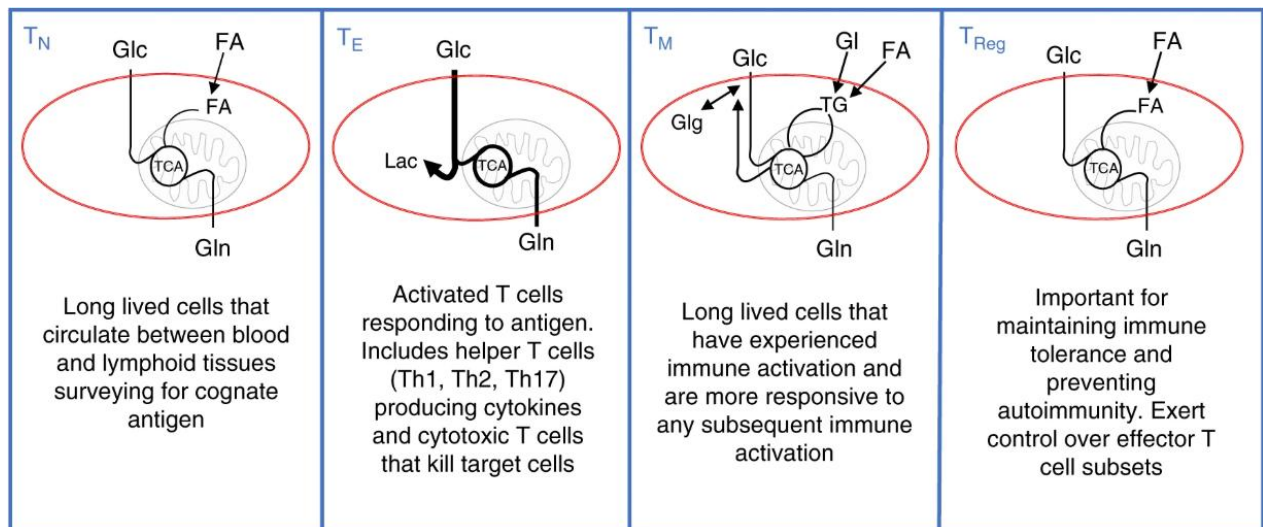


Figure 2: Metabolic changes during T-cell differentiation and expansion post anti-genic stimuli. T_N (Naïve T cells); T_E (Effector T cells); T_M (Memory T cells); T_{Reg} (Regulatory T cells); Glc (Glucose); Gln (Glutamine); TCA (tricarboxylic acid cycle, Krebs Cycle); Lac (Lactate); FA (fatty acid); TG (Triglycerides); GI (Glycerol); Glg (Glycogen), (Kedia-Mehta and Finlay, 2019).

Lack of glutamine transporters such as Slc7a1 has been reported to impact the transition of central memory T cells to effector memory (Huang et al., 2021). However, the lack of Slc1a5 did not affect the innate or adaptive immune cells (Masle-Farquhar et al., 2017). In vivo, studies using stable isotope labelled glutamine (^{13}C) revealed that the $CD8^+$ T cells primarily utilize glutamine as an oxidizable source and for aspartate synthesis via Got1 for their energy and proliferation. However, at the peak of expansion, they reduce their reliance on glucose and glutamine and predominantly use acetate to fuel the electron transport chain (Ma et al., 2024).

Neutrophils

Glutamine also plays an important role in neutrophils, where its metabolism supports reactive oxygen species (ROS) production and helps delay apoptosis, both of which are crucial for the immune response. A low glutamine level in plasma is associated with impaired immune defense functions. Within neutrophils, glutamine is metabolized to produce glutamate, aspartate, alanine and lactate, essential for diverse metabolic processes. Notably, the activity of

NADPH oxidase (NOX) and the production of superoxide are significantly elevated in the presence of glutamine. This increase compared to neutrophils cultured without glutamine underscores this amino acid's role in amplifying the oxidative burst necessary for pathogen killing. Alpha-ketoglutarate, a metabolite of glutamine, further stimulates the production of superoxide and hydrogen peroxide, increasing myeloperoxidase activity in neutrophils. Extracellular glutamine levels also regulate neutrophil superoxide production and cytolytic activity, contributing to effective immune response. Clinical studies have demonstrated that glutamine supplementation boosts bactericidal activity, phagocytosis and ROS production in neutrophils from postoperative and burn patients, enhancing their immune function. Consequently, glutamine is essential for the optimal performance of neutrophils and the overall immune defense system (Curi et al., 2020).

B cells and NK cells

Upon activation, B cells undergo proliferative expansion and maturation into immunoglobulin-secreting plasma cells, mechanisms that depend upon robust rises in both glycolytic pathway activity and mitochondrial oxidative phosphorylation (OXPHOS) to sustain the increased synthesis-related and secretory needs of immunoglobulins. Glutamine contributes to these metabolic processes by supplying carbon and nitrogen for nucleotide and amino acid synthesis and by fueling the TCA cycle, thereby promoting proliferation, survival and adequate antibody secretion (Waters et al., 2018).

Natural killer (NK) cells also rely on proper nutrient levels to perform effective killing responses. Metabolic reprogramming upon NK- cell activation implicates increased absorption and utilization of glucose and amino acids, together with glutamine, which is crucial to support cytokine production and elimination of target cells, though these mechanisms are less broadly described in T cells (Keppel et al., 2015).

Overall, along with the functions of glutamine in hematopoietic stem and progenitor cells and in myeloid effector cells such as neutrophils, these observations support that glutamine

metabolism plays a role in modulating both commitment to myeloid, erythroid and lymphoid lineages and the effector activities in innate and adaptative immunity.

3. Solute carrier (SLC) nutrient transporters

Nutrients are primarily transported into cells via specific proteins known as solute carrier (SLC) transporters and play a key role in deciding the fate of HSCs. The SLC superfamily and the ATP-binding cassette family constitute major groups of cell membrane transporters (Chen and Chen, 2022). While amino acids are renowned for their role in protein synthesis, they also serve as vital sources of energy, carbon, nitrogen and metabolic intermediates. Moreover, they act as precursors for synthesizing crucial macromolecules such as hormones, hemoglobin and cytochromes (Chen and Chen, 2022). Of particular importance is glutamine, not only as an energy source but also as a provider of carbon and especially nitrogen necessary for nucleotide and amino acid biosynthesis. This multifaceted role of glutamine underscores its potential significance in hematopoiesis. Several glutamine transporters, including SLC1A5, SLC7A5, SLC38A1 and SLC38A2, are intricately involved in facilitating this process, highlighting the complexity of studying nutrient regulation in hematopoietic stem cell biology.

SLC transporters in hematopoietic cells

The SLC superfamily consists of various classes of transporters that facilitate the uptake of important nutrients, including glucose transporters of the SLC2/GLUT family and multiple amino acid transporters of SLC1, SLC7 and SLC38 families, along with other nutrients and ions. In the hematopoietic and immune cells, these proteins carefully govern uptake of key nutrients and metabolites. This regulated nutrient uptake determines cell viability, growth and cell fate in line with metabolic need and microenvironmental conditions (Song et al., 2020).

Glutamine Transporters

Within the SLC family, various transporters are mediating glutamine transport, notably SLC1A5 (ASCT2), SLC7A5 (LAT1) and members of the SLC38 family such as SLC38A1 and

SLC38A2, which carry out sodium-driven uptake of neutral amino acids, particularly glutamine. Through control of cellular glutamine import and its exchange with other key amino acids, they modulate the supply of nutrients required for anabolic processes and trigger nutrient-responsive signaling cascades, like mTORC1 in various cellular contexts (Menchini and Chaudhry, 2019).

4. Glutamine transporters in normal hematopoiesis and AML

Normal hematopoiesis and immune cells

Diverse amino acid transporters are found in HSCs and progenitor populations as well as in different immune cell lineages, where they support glutamine acquisition for macromolecule synthesis and ATP generation. Knockout mouse models have shown that genetic ablation of SLC1A5 does not necessarily affect lymphopoiesis and immune function, indicating compensatory mechanisms among amino acid transport pathways in steady-state hematopoiesis and immunity. Altogether, glutamine uptake is controlling expansion, survival and differentiation of blood cell populations but exclusive roles of individual glutamine transporters to baseline hematopoiesis remain largely uninvestigated (Masle-Farquhar et al., 2017).

Glutamine addiction

Cancer cells exhibit a high demand for glutamine, termed "glutamine addiction" (Yoo et al., 2020), which reflects glutaminolysis (conversion of glutamine to alpha-ketoglutarate) to fuel the tricarboxylic acid (TCA) cycle. Glutamine transporters are often upregulated in cancer cells to meet their cellular demands and metabolic response to therapy (van Gastel et al., 2020). Several glutamine metabolism inhibitors are evaluated in the clinic to target glutamine addiction in cancer cells. However, most of these agents had a significant target effect on the normal cells.

Glutamine transporters - Slc38a2 in particular - are involved in sensing amino acid availability and regulating cellular processes and have been reported to be overexpressed in multiple cancer types.

Focus on Slc38a2

Transcriptomic and metabolomic profiling indicate that hematopoietic stem and progenitor cells show elevated levels of diverse transporters, particularly the glutamine transporters SLC1A5 (ASCT2), SLC7A5 (LAT1), SLC38A1 and SLC38A2, supporting their elevated nutrient requirements. In malignant hematopoiesis, especially acute myeloid leukemia (AML), these proteins are transcriptionally enhanced and promote nutrient-driven pathways that drive hyperproliferation and drug resistance. Van Gastel et al. (2020) describes that Slc1a5, Slc38a1, Slc38a2, Slc7a5 and Slc7a6 are upregulated at the mRNA level in post-chemotherapy AML populations. Slc38a1 has been validated at the protein level and functionally characterized. Whereas Slc38a2 presented a moderate transcription upregulation, its specific transcription in normal hematopoiesis remains poorly understood, emphasizing the necessity to investigate its function in immune function and healthy hematopoietic settings.

5. Slc38a2 (SNAT2): function and regulatory mechanisms

SLC38A2, commonly called SNAT2, is a member of sodium-coupled neutral amino acid transporters, the solute carrier transporter 38 family. As a sodium-dependent transporter, it utilizes the sodium gradient across the cell membrane to import amino acids into the cell. It transports glutamine, serine, alanine, glycine, cysteine, asparagine, histidine and methionine, making it crucial for protein synthesis, nitrogen balance and energy metabolism (Zhang and Grewer, 2007). SLC38A1 is very similar to SLC38A2 in terms of the binding order, stoichiometry and substrate specificity, with both being ubiquitously expressed. Structurally, SLC38A2, like other transporters in the SLC38 family, is thought to have multiple transmembrane domains, facilitating the translocation of substrates and sodium ions (Du et al., 2023).

Beyond nutrient uptake, plasma membranes amino acid transporters (AATs) function as nutrient sensors that regulate signaling pathways such as mTORC1, and the amino acid deprivation-induced GCN2/ATF4 pathway. In this setting, transporters including Slc38a2 and Slc38a9, have thus been considered to function as “transceptors”, coupling amino acid transport to intracellular signaling that regulate growth and stress responses (Jung et al., 2015).

Slc38a2 expression is highly sensitive to amino acid availability. Amino acid depletion induces an amino acid response (AAR) including GCN2 and ATF4, which promote *Slc38a2* transcription and enhance protein stability whereas adequate amino acid supply upholds low basal expression. At the post-translational level, Slc38a2 abundance is further regulated by ubiquitination and proteasome degradation, and unsaturated fatty acids have been reported to facilitate Slc38a2 turnover, linking lipid status to transport stability (Taylor, 2014) (Nardi et al., 2015) (Menchini and Chaudhry, 2019). Through these controlled mechanisms, Slc38a2 modifies intracellular pool of neutral amino acids, including glutamine, thus supporting mTORC1 activation and cellular stress responses across different cell types (Menchini and Chaudhry, 2019) (Jung et al., 2015).

Through its dual role as an amino acid transporter and a metabolic sensor, Slc38a2 associates extracellular nutrient availability with intracellular growth and stress signaling pathways, such as mTORC1 and the GCN2/ATF4 amino acid starvation response. In the hematopoietic system, where metabolic state fundamentally shapes stem and progenitor cell behavior, changes in Slc38a2 expression or activity may affect the hematopoietic stem and progenitor cell function and influence lymphoid versus myeloid lineage output.

6. Aims of the thesis

Considering the key role of glutamine and amino acid transport in immune cell metabolism, and the high expression of the glutamine transporter *Slc38a2* across hematopoietic and lymphoid compartments, this Master thesis investigates the function of *Slc38a2* in steady-state hematopoiesis. The aim is to assess whether *Slc38a2* is essential to support normal hematopoietic maintenance and differentiation and to preserve a balanced lymphoid versus myeloid output.

The main challenge lies in the fact that the hematopoietic stem and progenitor cells can adapt fuel utilization to environmental conditions and can activate compensatory pathways upon loss of a nutrient transporter. This metabolic adaptability indicates that the knockout of a single transporter such as *Slc38a2* may have subtle or cell type specific effects, and that alternative transporters or pathways might compensate for its loss. Thus, both progenitor

populations and mature immune compartments need to be analyzed in depth to uncover potential lineage biases or compensatory adaptations that may occur following loss of this transporter.

The objectives of my research project are:

- a) To generate a hematopoietic compartment-specific *Slc38a2*-KO transgenic mouse model.
- b) To determine, under steady state conditions, the consequences of hematopoietic-specific *Slc38a2* deletion on stem and progenitor populations, with a particular focus on common lymphoid progenitors (CLPs).
- c) To assess how loss of *Slc38a2* influences mature lymphoid compartments (B, cells, T cells, and NK cells) and myeloid cells, to reveal possible shifts in lymphoid versus myeloid lineage output.

V. MATERIALS AND METHODS

1. Ethical Approval, animal housing and experimental design

All animal experiments were conducted in conformity with FELASA recommendations and were approved by the Animal Ethics Committee of UCLouvain. Mice were housed and bred under specific pathogen-free (SPF) conditions in the Laboratory Animal Facility (LAF) of the de Duve Institute (UCLouvain, Brussels).

2. Generation of hematopoietic-specific *Slc38a2* knockout mice

Using a Cre-lox system model, we generated a conditional knockout of *Slc38a2* in the hematopoietic system. Cre-Lox recombination is a site-specific recombinase technology used to carry out deletions, insertions, translocations and inversions at specific sites in the DNA of cells. Cre recombinase recognises the loxP sites and cleaves to delete the sequences between the loxP sites, as illustrated in Figure 3.

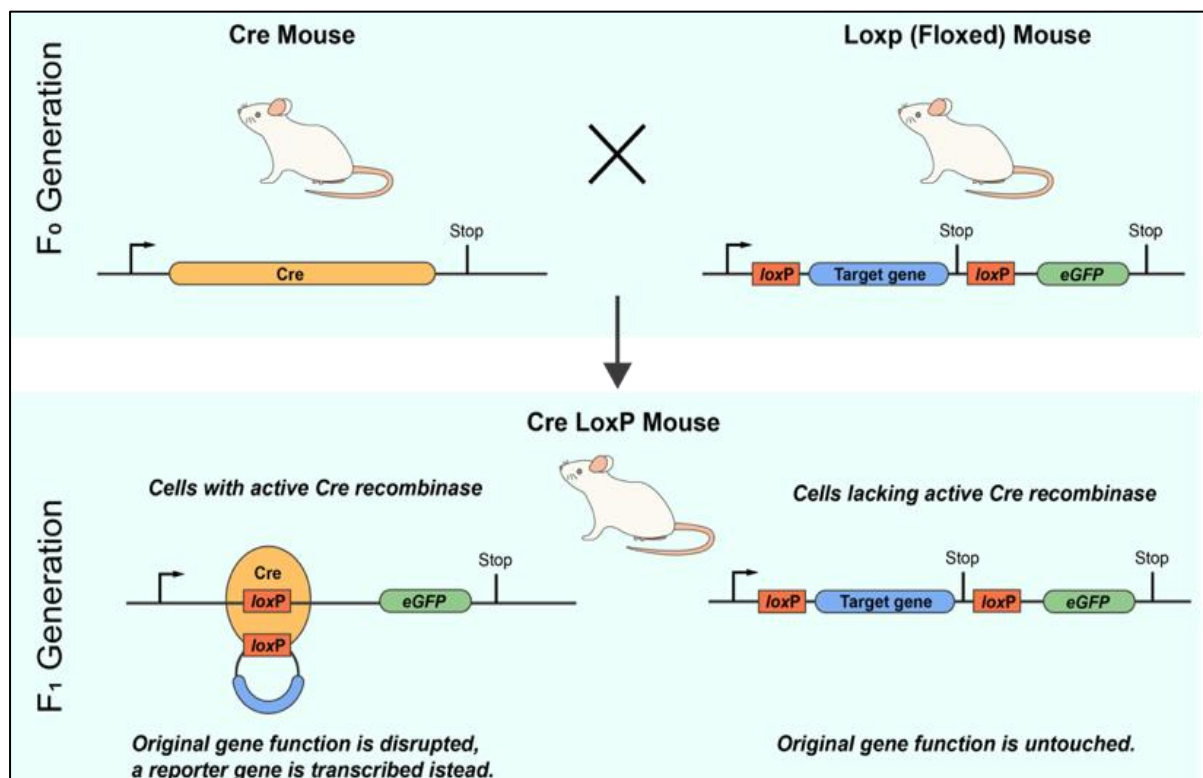


Figure 3: Illustrates the general scheme for generating a conditional mouse model system using the Cre-lox system.

In the present study, we generated a hematopoietic compartment-specific knockout of the *Slc38a2* gene in the C57BL6 mouse strain. *Slc38a2*^{tm1c(KOMP)Wtsi} mice were used, in which exon 2 of the *Slc38a2* gene is flanked by loxP sites (*Slc38a2*^{fl/fl} mice). We then crossed Vav-cre transgenic mice with *Slc38a2*^{fl/fl} mice to generate a knockout of *Slc38a2* in the hematopoietic compartment. As Cre recombinase is under the control of the regulatory elements of the human Vav 1 oncogene (VAV1, Vav Guanine Nucleotide Exchange Factor 1), a guanine nucleotide exchange factor specifically involved in hematopoiesis, Cre activity will result in the deletion of exon 2 of *Slc38a2* in blood cells only (figure 4).

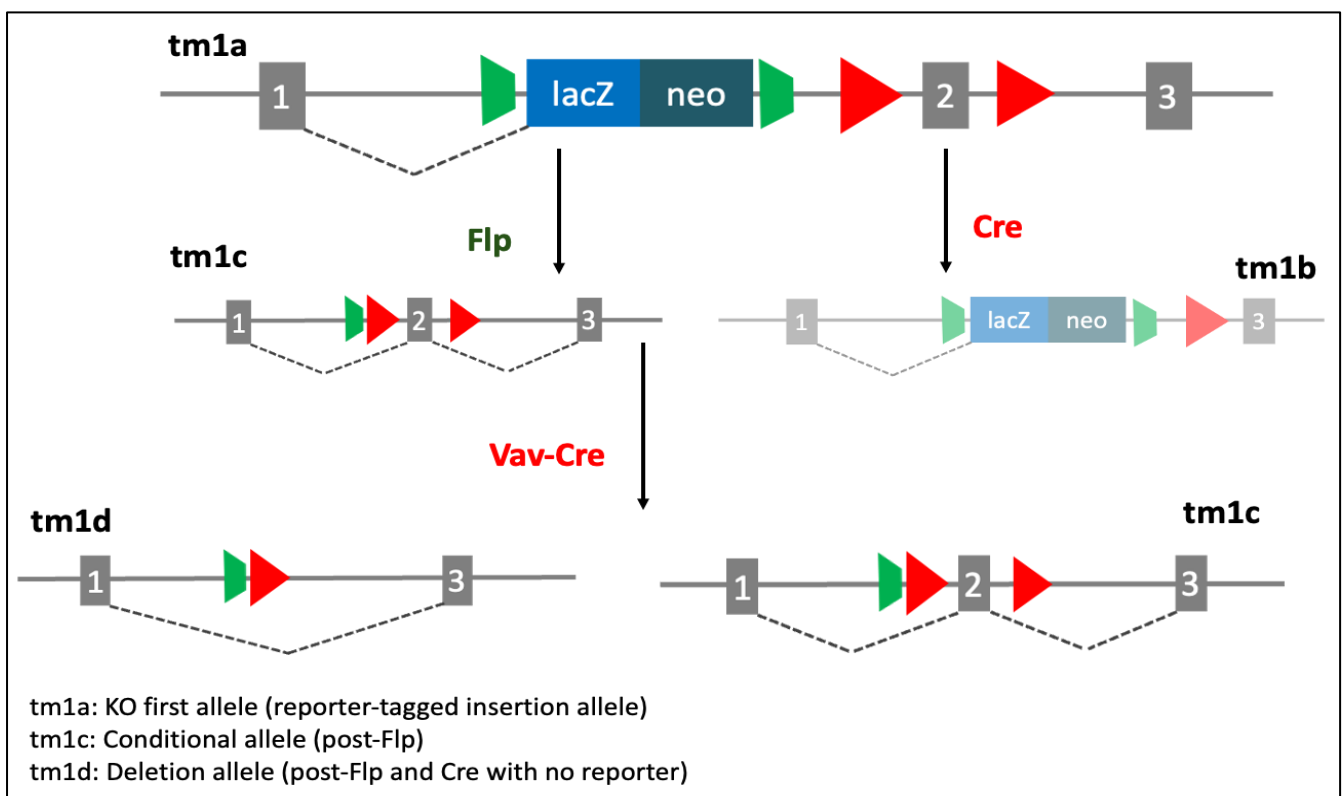


Figure 4: Illustrates the steps carried out to generate *Slc38a2* homozygous floxed (tm1c) and Vav-Cre specific *Slc38a2* gene deletion (tm1d) mice used in this study. <https://elifesciences.org/articles/76963>

3. Genotyping procedure and PCR validation

The pups were genotyped using gene-specific primers to confirm the floxed allele of *Slc38a2* and to identify Cre-Positive and Cre-Negative pups for further experiments (figure 5).

Genotyping DNA was isolated from ear snips using proteinase K digestion followed by precipitation with isopropanol. Samples were incubated overnight at 55°C in lysis buffer supplemented with proteinase K in a shaking heating block. After centrifugation, the supernatant was mixed with an equal volume of isopropanol to precipitate DNA. The resulting DNA pellet was resuspended in TE buffer to dissolve it at 55°C before storage at -20°C.

PCR reactions for *Slc38a2* and Vav-Cre genotyping were performed in a 25µl volume including DreamTaq master mix (ThermoFisher Scientific) and genetic-specific forward and reverse primers. Primer used for genotyping were as follows:

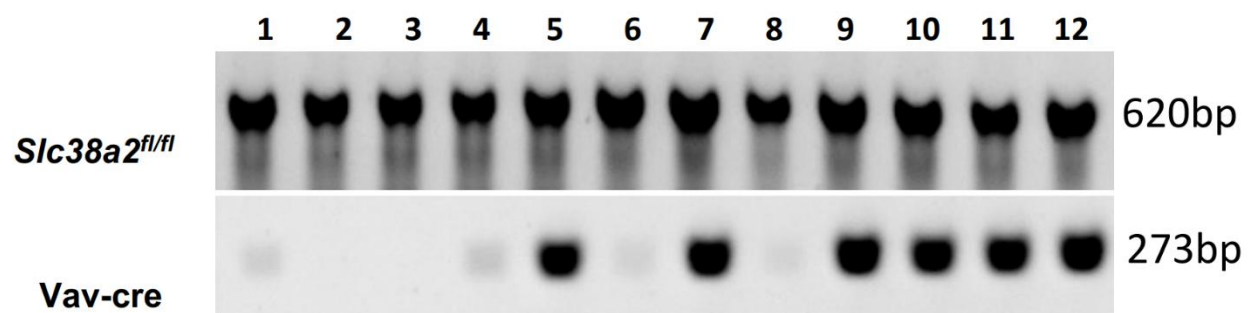
For *Slc38a2* :

forward 5'-CAGTGTCACCCCAGAACCAG'-3 and reverse 5'-AAGGAAGGCAGGTAGAGGTGG'-3.

For *Vav-Cre* :

forward 5'-AGA-TGC-CAG-GAC-ATC-AGG-AAC-CTG-3' and reverse 5'-ATC-AGC-CAC-ACC-AGA-CAC-AGA-GAT-C'-3.

The PCR program consisted of 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 45 seconds, and final extension at 72°C for 5 minutes. For Vav-Cre, conditions were 94°C for 5 minutes, then 36 cycles of 94°C for 30 seconds, 65°C for 40 seconds, followed by a final extension at 72°C for 7 minutes, before holding at 12-14°C.



Samples -1 to 12, *Slc38a2* floxed – 620bp; Vav-cre – 273bp

Figure 5: Illustrates agarose gel electrophoresis of gene-specific primer-based PCR amplified product DNA samples of the pups. The upper panel represents *Slc38a2* floxed 620bp, and the lower panel represents Vav-cre 273bp. PC, positive control; NTC, negative control

4. Peripheral blood sampling and complete blood counts (CBC)

Peripheral blood was collected at the time of euthanasia by cardiac puncture in EDTA-coated tubes to prevent coagulation. Complete Blood Counts (CBCs) were conducted on fresh samples using an automated hematology analyser (Sysmex), providing red and white blood cell parameters, including platelets for Cre-Positive and Cre-Negative mice.

5. Flow cytometry sample preparation and immunostaining

Peripheral blood, bone marrow, spleen, and thymus were harvested from 8-11 weeks old Cre-Negative and Cre-Positive mice for immunostaining. Prior to tissue collection, mice were sacrificed by isoflurane anesthesia followed by cervical dislocation, in accordance with FELASA guidelines. Blood was obtained by cardiac puncture into EDTA-coated tubes, while bone marrow cells were collected by crushing femurs and tibias, and single cell suspension from thymus were prepared by mechanical dissociation through 40µm strainers in iced cold flow buffer (PBS, 5mM EDTA, 2% FBS). Spleens were photographed to record organ dimensions and morphology to compare between both groups.

Red blood cells in the bone marrow suspension were lysed on ice using ACK buffer, followed by washes in flow buffer and centrifugation (500xg, 10 minutes, 4°C). Cell pellets from bone marrow and thymus were suspended in flow buffer, and viable cells were counted by trypan blue exclusion. Specified numbers of cells, typically 5×10^6 per tube, were then transferred into FACS tubes for staining with antibody panels targeting hematopoietic and immune compartments.

Surface immunolabeling was conducted by treating cells with optimized antibody cocktails for 30 minutes at 4°C in the dark. Subsequently they were washed in flow buffer, pelleted at 500xg for 10 minutes at 4°C, and diluted in 450µl of flow buffer for acquisition. For progenitor

panels including biotinylated antibodies, secondary staining step with streptavidin-APC/Fire750 (Biolegend, cat.405250) was applied for 15 minutes at 4°C.

6. Spectral acquisition and gating strategy

Cells were analyzed by spectral flow cytometry and were carried out using a Cytex Aurora instrument. FlowJo was used to analyze the data using a conventional gating strategy to exclude debris and doublets and identify live single cells.

7. Statistical analysis

All quantitative data are expressed as mean $\pm$ standard deviation SD, unless specified. Statistical analyses comparing Cre-Positive and Cre-Negative mice were conducted using unpaired two-sided t-tests with Welch's correction in GraphPad Prism, and P values <0.05 were interpreted as indicative of a significant difference.

VI. RESULTS

1. Expression profile of *Slc38a2* in normal murine hematopoiesis

To investigate glutamine transporter expression in normal murine hematopoiesis, we utilised the public domain microarray dataset from the Gene Expression Commons database (<https://gexc.riken.jp/>). This open-access tool defines, for each probeset, a gene-specific dynamic range based on many microarrays to sort expression as active or inactive above a baseline. It revealed that while multiple glutamine transporters are expressed in most hematopoietic cell types, *Slc38a2* shows high expression across all populations (Figure 6). All these proteins were detectable in various hematopoietic cell types. However, *Slc38a2* displayed the most uniformly elevated activity throughout hematopoietic and early progenitor compartments with the largest dynamic range above baseline (8.87) among the transporters evaluated.

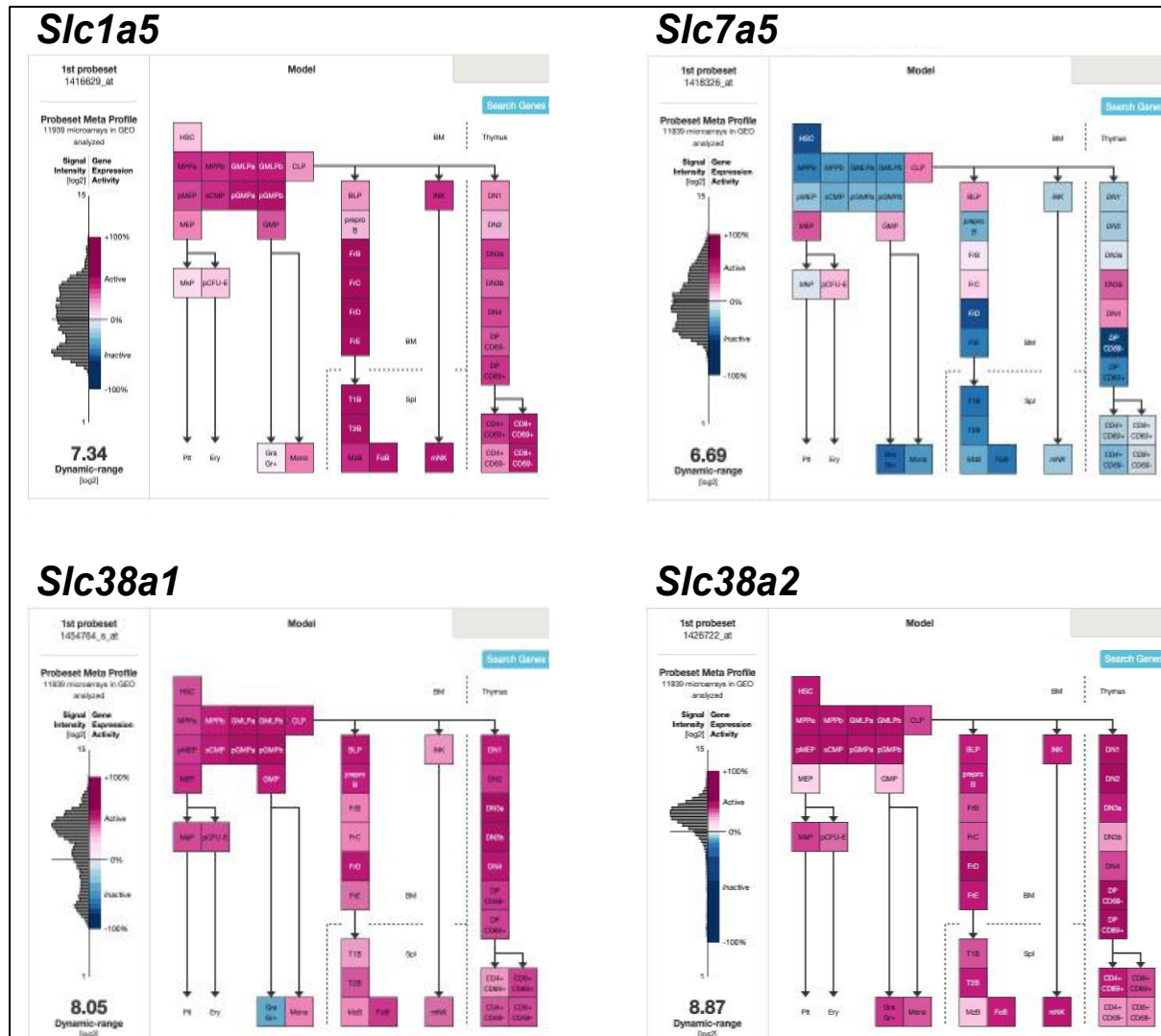


Figure 6: Expression of glutamine transporters in the mouse hematopoietic system. Data was extracted from the Gene Expression Commons database (<https://gexc.riken.jp/>). This figure illustrates the expression levels of glutamine transporters within the mouse hematopoietic system. Of the transporters examined (*Slc1a5*, *Slc7a5*, *Slc38a1*, *Slc38a2*), *Slc38a2* showed the highest expression in the hematopoietic and early progenitor cells, with a dynamic range value above the baseline expression.

While expressed in all hematopoietic cell types, we observed that *Slc38a2* shows some differential gene expression patterns. Besides HSCs and early myeloid progenitors, mature immune cells such as lymphoid cells (T-cells, B-cells and NK cells), granulocytes and monocytes have the highest expression of *Slc38a2* compared to other cell types (Figure 6). This was confirmed by examining a different dataset based on RNA sequencing retrieved from the Blood Spot database (Figure 7).

This high expression suggests that *Slc38a2* may play a key role in the maintenance or differentiation of hematopoietic cells.

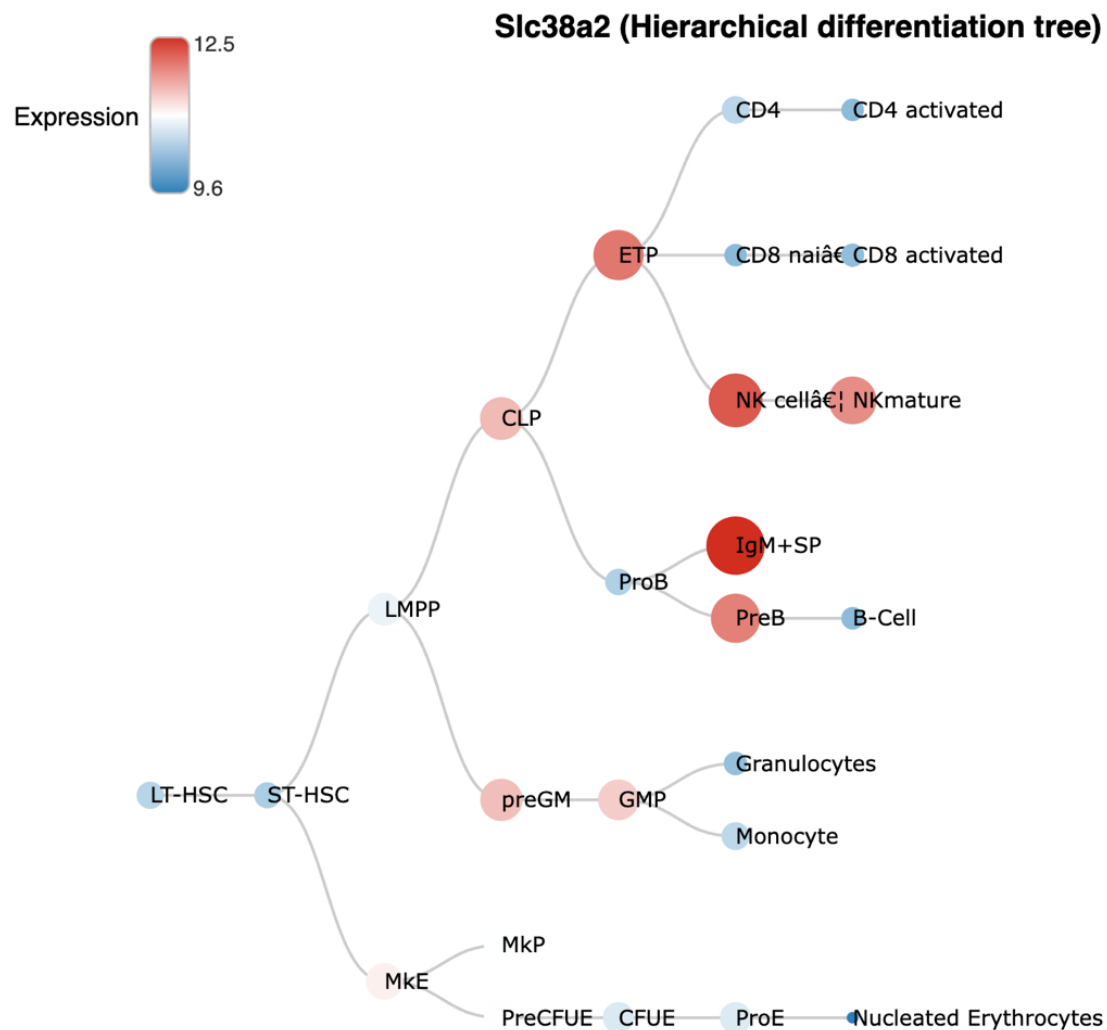


Figure 7: *Slc38a2* transcript levels in normal hematopoiesis (mouse). (Data from [GSE14833](#) and [GSE6506](#)).

Based on these observations, we decided to generate hematopoietic cell-specific *Slc38a2* knockout (KO) mice to investigate the importance of this transporter for normal hematopoiesis.

2. Steady-state hematological parameters in *Slc38a2*^{Δ/Δ} mice

Slc38a2 does not have any effect on hematological parameters at steady state:

To assess the role of *Slc38a2* on the steady state hematopoietic system, we first performed a complete blood count analysis of peripheral blood collected from the vav-Cre Negative (*Slc38a2^{fl/fl}*) and vav-Cre Positive animals (*Slc38a2^{Δ/Δ}*) using an automated cell counter (SYS-MEX hematology analyser). As shown in Table 1, we observed no significant differences between these two groups of animals.

Haematological Parameters	Cre negative (n=15)	Cre Positive (n=13)	p-value
WBC (10 ³ /μL)	9.93 ± 3.68	8.60 ± 2.60	0.29
RBC (10 ⁶ /μL)	10.46 ± 0.77	9.90 ± 1.12	0.13
HGB (g/dL)	15.07 ± 1.18	14.41 ± 1.84	0.26
HCT (%)	46.69 ± 2.67	46.28 ± 4.82	0.78
MCV (fL)	44.74 ± 2.45	46.95 ± 3.95	0.08
MCH (pg)	14.43 ± 0.81	14.54 ± 0.70	0.72
MCHC (g/dL)	32.33 ± 2.34	31.23 ± 3.31	0.32
PLT (10 ³ /μL)	1417.47 ± 216.47	1267.23 ± 303.32	0.14
NEUT# (10 ³ /μL)	0.866 ± 0.87	0.93 ± 0.29	0.58
LYMPH# (10 ³ /μL)	8.53 ± 3.35	7.19 ± 2.27	0.23
MONO# (10 ³ /μL)	0.29 ± 0.12	0.29 ± 0.13	0.73
EO# (10 ³ /μL)	0.23 ± 0.12	0.18 ± 0.15	0.32
BASO# (10 ³ /μL)	0.016 ± 0.01	0.01 ± 0.01	0.23

Table 1: Comparison of hematological parameters between Cre-Negative and Cre-Positive mice. White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Hemoglobin Concentration (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (PLT), Neutrophil Count (NEUT#), Lymphocyte Count (LYMPH#), Monocyte Count (MONO#), Eosinophil Count (EO#), Basophil Count (BASO#). (Mean ± SD, n=15 and n=13 animals per group, the p-value was obtained by Student's t-test with Welch's correction).

3. Limited impact of *Slc38a2* deletion on HSCs and multipotent progenitors

To determine whether *Slc38a2* deletion alters hematopoietic stem cell compartment and early multipotent progenitors, hematopoietic stem and progenitor compartments were characterized and quantified by flow cytometry using conventional marker sets. Flow cytometry is a technology that analyses large numbers of single cell in suspension in a rapid manner by measuring light scatter and fluorescence as each individual cell passes sequentially through one or more lasers. The optical signals obtained are then converted into digital data (.fcs files), enabling cell populations to be characterised, quantified, and using sorters, physically isolated according to their light-scattering and fluorescent traits (McKinnon, 2018).

We defined these populations by using combinations of lineage depletion and antibodies recognizing cell surface antigens. Stem and multipotent progenitors were initially distinguished within LSK compartment, representing lineage-negative (Lin⁻) Sca-1⁺ c-Kit⁺ cells, a population known to comprise all HSCs and multipotent progenitors. LSK cells were further divided, using SLAM family markers CD150 and CD48, into long-term HSCs (CD150⁺ CD48⁻, also called SLAM positive cells), multipotent progenitors (CD150⁻ CD48⁻, also named MPP) and more differentiated progenitors (CD48⁺ subpopulations MPP2 and MPP3) (Figure 8 a-b).

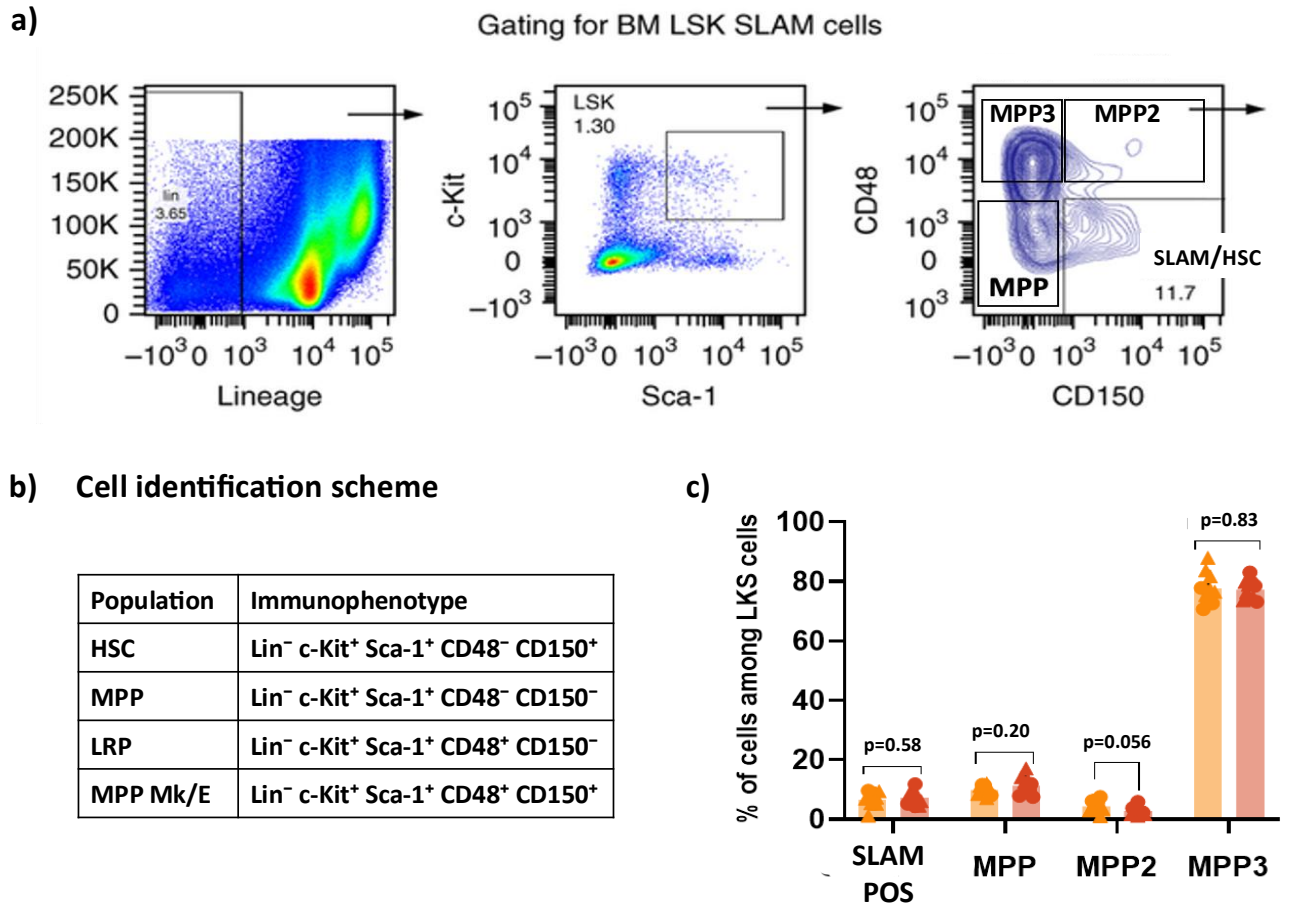


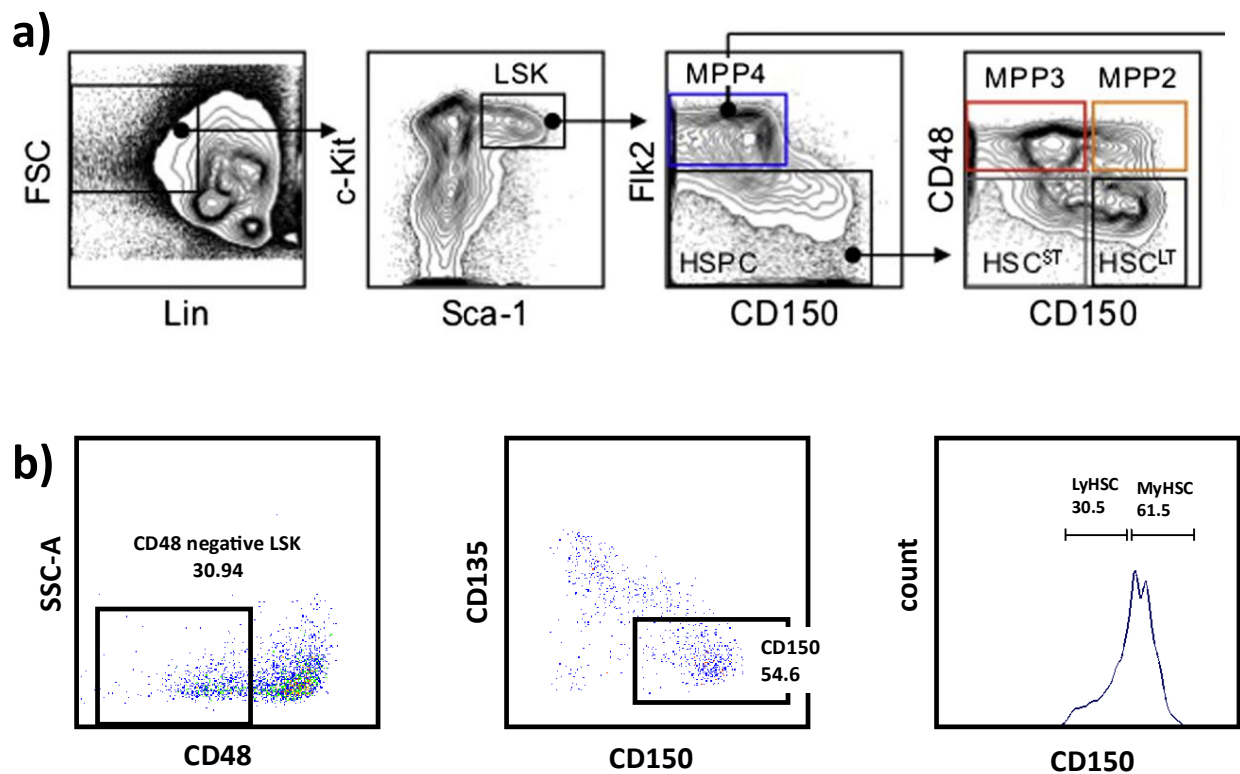
Figure 8: a) Gating strategy used to identify HSC and stem populations; b) Cell phenotype of the different cell populations; c) Bar graph representing the proportion of HSC and MPPs among LSK cells in Cre-Negative (light color) and Cre-Positive (dark color) animals. (n=10) Data bars are presented as mean $\pm$ SEM, with a two-tailed unpaired t-test.

To distinguish functionally distinct multipotent progenitor subsets, Flk2/Flt3/CD135 expression was coupled with SLAM markers to identify MPP2, MPP3 and MPP4 (also designated as long-primed multipotent progenitors, LMPP) (Figure. 9a-b). MPP2 and MPP3 correspond to myeloid-biased MPP populations, with MPP2 predominantly giving rise to megakaryocytic-erythroid lineage and MPP3 contributing to granulocyte-monocyte output. In contrast, MPP4/LMPP exhibits lymphoid priming and is actively involved in lymphoid and granulocyte-monocyte formation (Pietras et al., 2015).

Using the gating strategy in Figure 8, HSCs and MPPs within LSK population remained largely unchanged between Cre-Negative (*Slc38a2^{fl/fl}*) and Cre-Positive (*Slc38a2^{Δ/Δ}*) mice (Figure 8c-

d). Consistently, when Flk2 was included to separate MPP2, MPP3, and MPP4 compartments, HSC, MPP2, and MPP3 proportions did not differ between both groups, whereas MPP4/LMPP number showed a trend towards an increase in the Cre-Positive animals ($p = 0.083$) (Figure 9a-d).

Overall, *Slc38a2* deletion did not result in major shifts in HSC abundance, and only a non-significant tendency for elevated MPP4/LMPP frequencies was noted within the multipotent progenitor compartment. This finding led us to examine whether the phenotypic changes observed at this stage were reflected in the composition of more differentiated and fate-committed myeloid and lymphoid lineage progenitors.



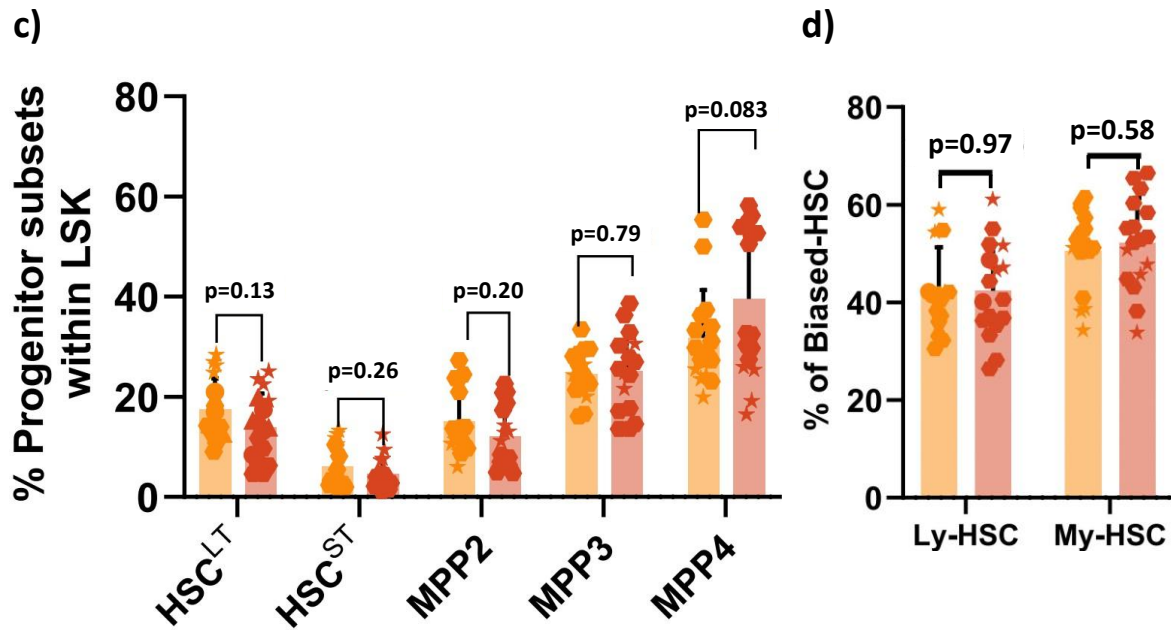


Figure 9: a) Gating strategy used to distinguish different MPPs and HSC with the addition of Flk2 (CD135) marker, resulting in HSPC compartment (Hematopoietic Stem and Progenitor cells); b) Gating strategy to identify lymphoid and myeloid biased HSC; c) Bar graph illustrating the proportion of long/short term HSC and MPP2, MPP3 and MPP4 (LMPPs) between the Cre-Negative (light colored bars) and Cre-Positive (dark colored bars) (n=17); d) Proportion of lymphoid and biased HSC's among CD150 positive cells based on the intensity of CD150 (n=17). Data bars are presented as mean $\pm$ SEM, with a two-tailed unpaired t-test.

4. Alterations in committed progenitor composition in *Slc38a2*^{Δ/Δ} mice (myeloid and lymphoid progenitors)

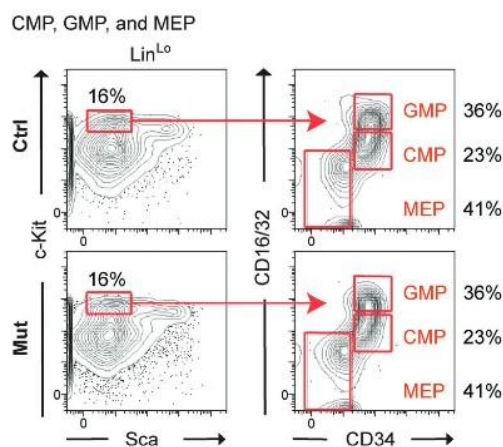
Lack of Slc38a2 affects committed progenitor cells composition in bone marrow

We next assessed bone marrow committed progenitors that differentiate into mature myeloid and lymphoid lineages. Common Myeloid Progenitors (CMPs), Granulocyte-Macrophage Progenitors (GMPs) and Megakaryocyte-Erythroid Progenitors (MEPs) which are myeloid progenitors, were gated within c-Kit⁺ Sca-1^{low} cells. In contrast, Common Lymphoid Progenitors (CLPs) were identified among Lin⁻ IL7Ra⁺ (CD127) c-Kit^{low} Sca-1^{low}. Interestingly, Ly6D expression was utilized to discriminate CLPs into early B cell-specified CLPs (Ly6D⁺) and CLPs that retain T and NK cell potential (Ly6D⁻) (Figure 10a-b) (Inlay et al., 2009).

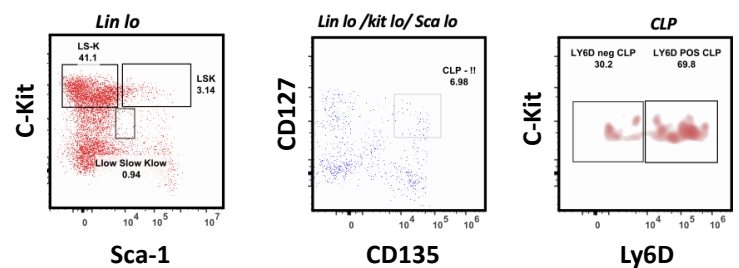
Further analysis of downstream lineage-committed progenitors showed that GMPs (figure 10c) and CLPs (figure 10d) were significantly increased in the Cre-Positive animals, corroborating the increase in MPP4 cells that are thought to give rise to both GMPs and CLPs. We also noted a significant decrease in the MEPs which gives rise to megakaryocytes and erythrocytes (figure 10c). When we further investigated CLP subpopulations that give rise to B cells or T/NK cells using the expression of Ly6D, there was a significant increase in the Ly6D⁺ CLPs (B cell biased) and decreased Ly6D⁻ CLPs (T/NK cell biased) in the Cre-Positive animals in comparison to Cre-Negative animals (Figure 10e).

Slc38a2^{Δ/Δ} mice thus revealed changes in committed progenitor composition in bone marrow, with enriched GMPs and CLPs proportions, reduced MEP frequencies, and a shift within CLPs towards Ly6D⁺ B-biased population. These changes led to us to further our investigation to mature myeloid and lymphoid subsets in peripheral blood.

a) Myeloid Progenitors



b) Lymphoid Progenitors



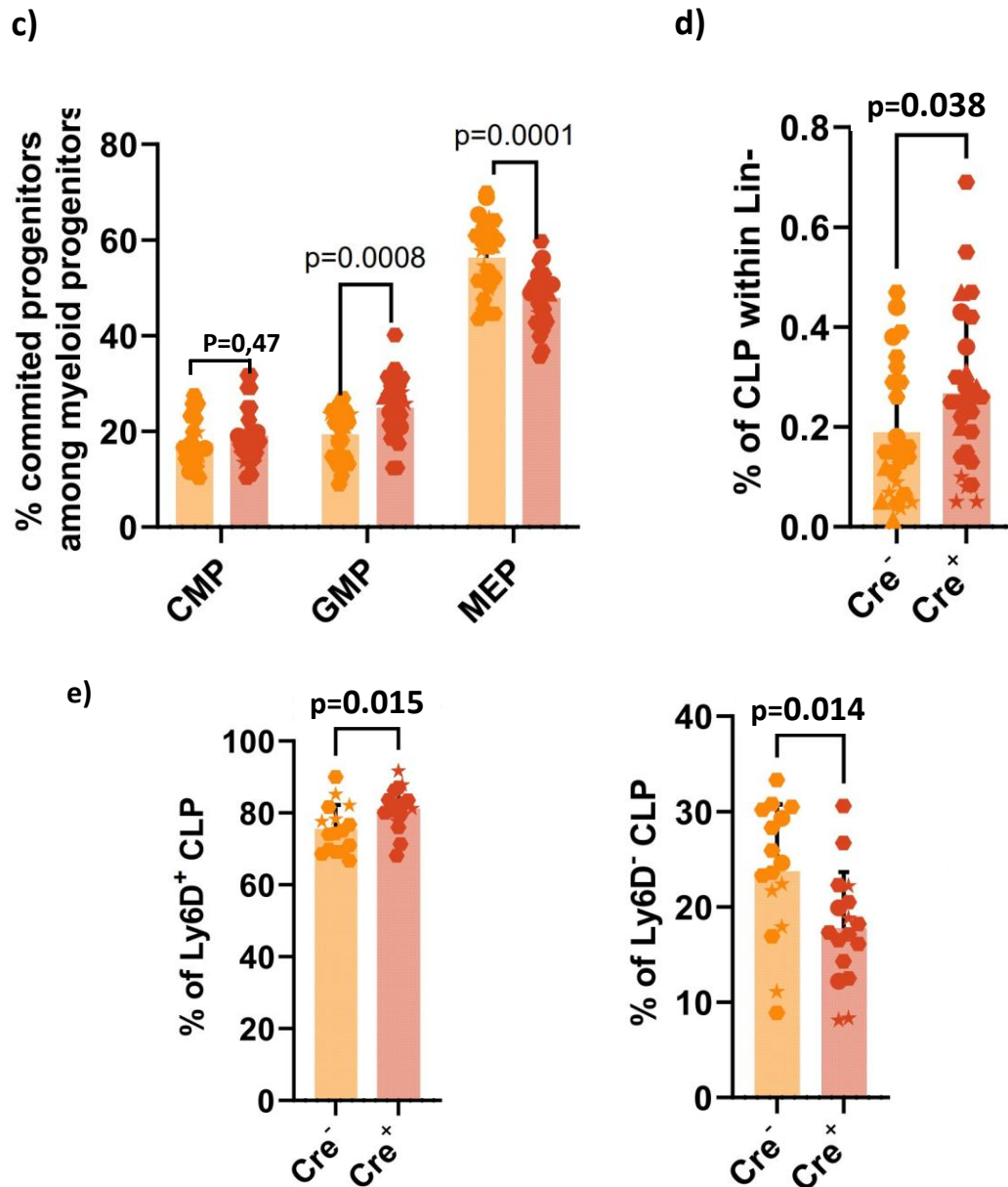
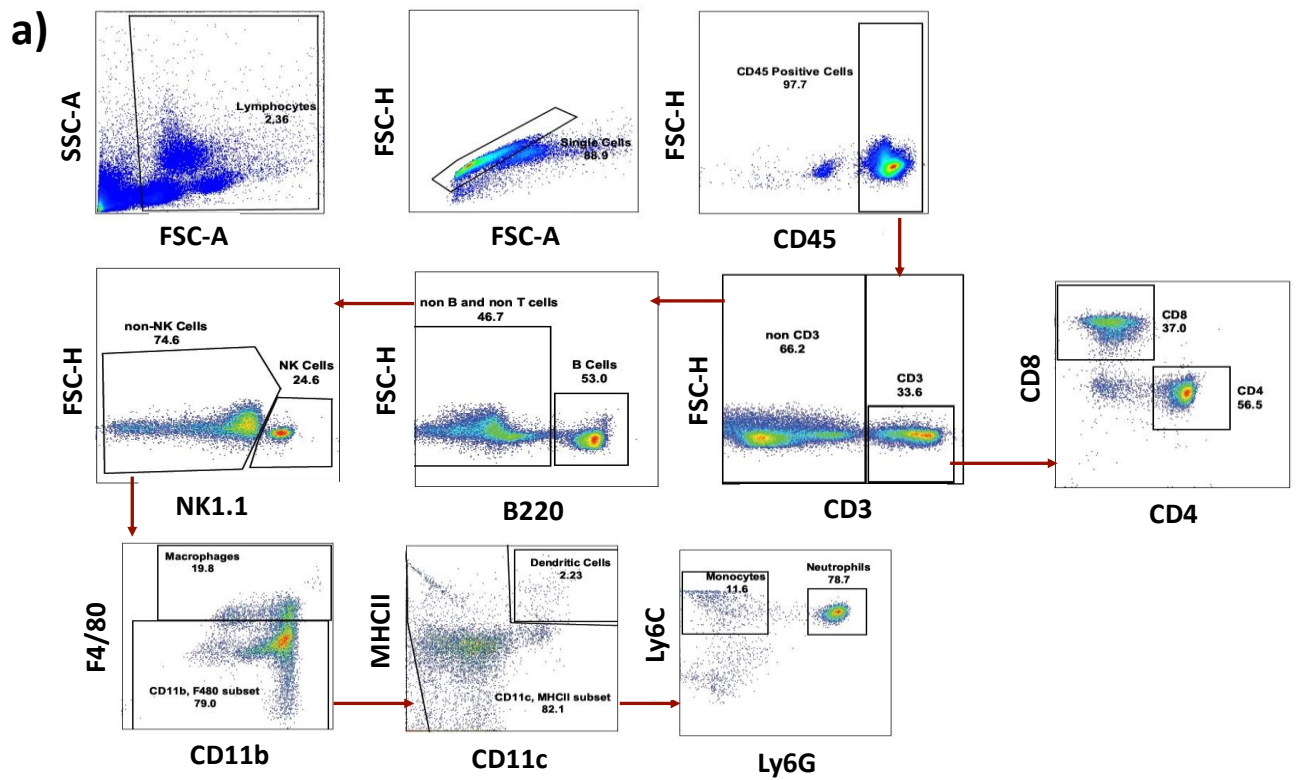


Figure 10: (a-b) Gating strategy used to identify myeloid progenitor and lymphoid progenitors; (c,d,e) bar graph illustrating the proportions of committed myeloid progenitors among myeloid progenitors (*c-Kit*⁺ *Sca-1*^{low}) (n=23); Proportion of CLP's among lineage negative cells (n=23); Proportion of Ly6D cells among CLP's Figure 10: (a-b) Gating strategy used to identify myeloid progenitor and lymphoid progenitors; (c,d,e) bar graph illustrating the proportions of committed myeloid progenitors among myeloid progenitors (*c-Kit*⁺ *Sca-1*^{low}) (n=23); Proportion of CLP's among lineage negative cells (n=23); Proportion of Ly6D cells among CLP's population (n=17). Data bars are presented as mean ± SEM. Statistical analysis was done with a two-tailed unpaired t-test.

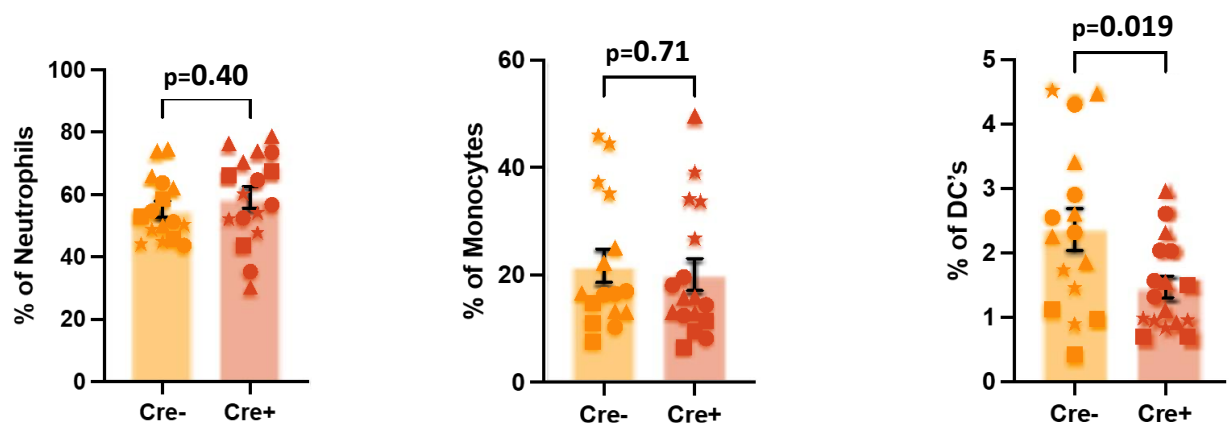
5. Perturbations in innate immune cell subsets following *Slc38a2* deletion

Given the alterations detected in committed progenitor populations, we subsequently analysed mature myeloid and lymphoid cells in circulation by flow cytometry. Figure 11a depicts the gating strategy used to determine major innate and adaptative immune cell subsets. Lymphocytes were first isolated based on forward and side-scatter, CD45⁺ leukocytes were then identified. CD3 and B220 enabled to identify T cells, B cells (B220⁺) and non-T/non-NK cells. CD4 and CD8 further selected CD4⁺ and CD8⁺ T cells. NK1.1 gated NK cells, and within CD11b⁺ myeloid cells, F4/F80, CD11c, MHC-II, Ly6G and Ly6C defined macrophages, dendritic cells, neutrophils and monocytes.

Among innate immune cells, dendritic cell (antigen presenting cell of the innate immune system) proportions were significantly diminished in Cre-Positive mice, whereas no significant differences were observed in circulating neutrophil and monocyte distributions between both groups (Figure 11b). Despite the notable increase in the Ly6D⁺ CLP population, which gives rise to B-cells, and a decrease in the Ly6D⁻ CLP population, responsible for generating T cells and NK cells, no significant differences were detected in the levels of mature lymphoid cells in the circulation under steady-state conditions (Figure 11c). These data prompted a more-in depth analysis of lymphoid differentiation in the thymus and peripheral blood T-cell subsets.



b) Mature myeloid cells



c) Mature lymphoid cells

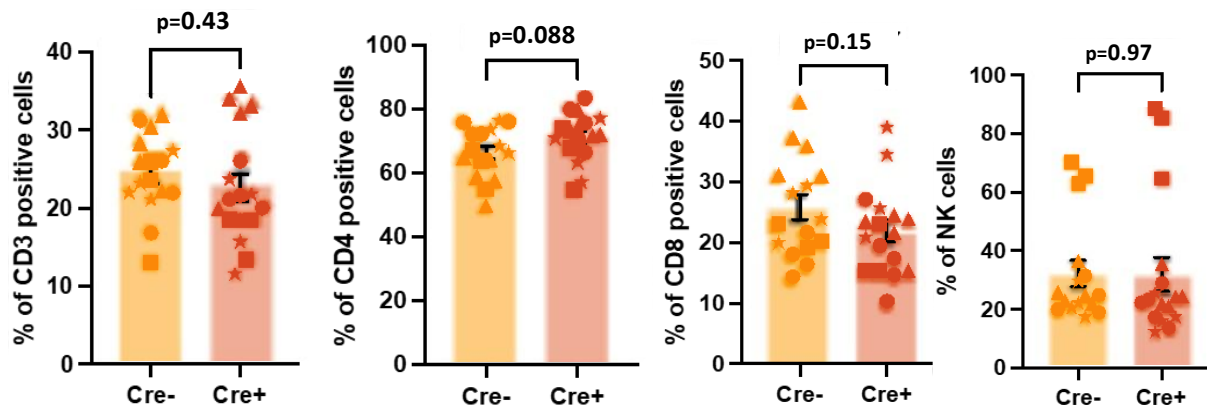


Figure 11: a) Gating strategy used to identify innate and adaptive immune cells in blood; (b-c) bar graph illustrating the proportions of committed myeloid lineage (Neutrophils, Monocytes and DC's) and proportions of committed lymphoid lineage (CD3 positive T cells, CD4 and CD8 positive cells (% of CD3 positive T cells), NK cells). Relative percentages of cell types are represented as bar graphs (n=17). Data bars are presented as mean $\pm$ SEM. Statistical analysis was done with a two-tailed unpaired t-test.

6. T-cell and lymphoid subsets in the blood and thymus of *Slc38a2*^{Δ/Δ} mice

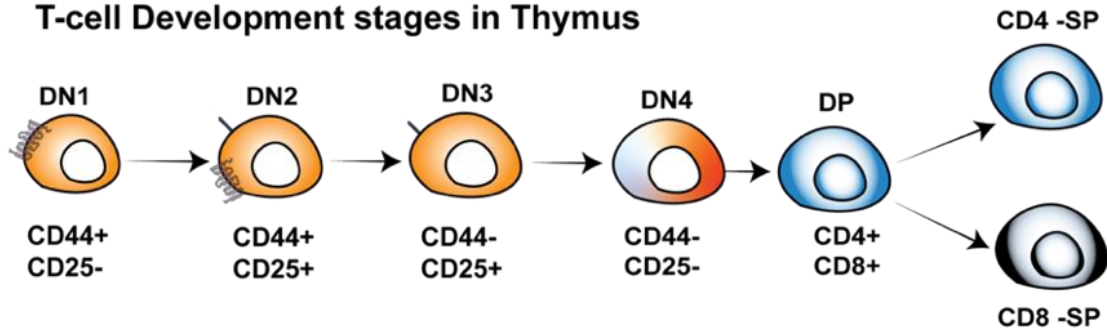
Thymic T cell development

The above analyses indicated that both multipotent and lineage-restricted lymphoid progenitors were altered in the absence of *Slc38a2* expression, but this was not reflected in the circulating mature lymphocyte distribution. On this basis, we next assessed T cell differentiation along the thymic axis.

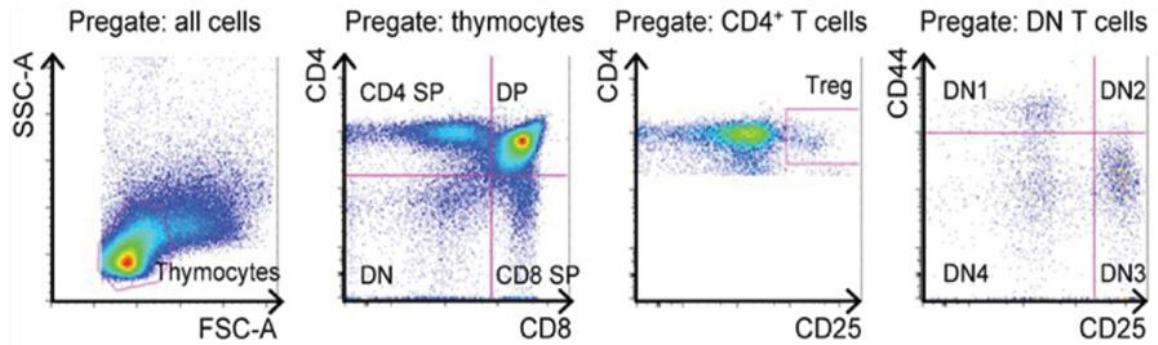
T cells stem from early thymic progenitors (ETPs) derived from HSCs in bone marrow, which migrate to the thymus and colonize it. There, they progress through a precisely orchestrated maturation process defined by sequential double negative (DN), double-positive (DP) single-positive (SP) CD4⁺ and CD8⁺ stages, which can be characterized by differential expression of cell surface markers, including CD4, CD8, CD25 and CD44 (Figure 12a). Following positive and negative selection, most developing thymocytes undergo apoptosis, while surviving DP cells progress into naive CD4⁺ or CD8⁺ SP T cells that subsequently migrate from thymus to peripheral blood circulation (Bao et al., 2022).

To assess whether *Slc38a2* loss alters T cell development, thymocytes from both animal groups were studied through a defined flow cytometric gating strategy to identify DN, DP, SP CD4⁺, SP CD8⁺ and CD4⁺ CD25⁺ regulatory T cells (Figure 12b). Quantification of these thymocyte subsets showed comparable frequencies between both genotypes, indicating that T cell development and thymic selection were largely unaffected by the loss of *Slc38a2* (Figure 12c-g).

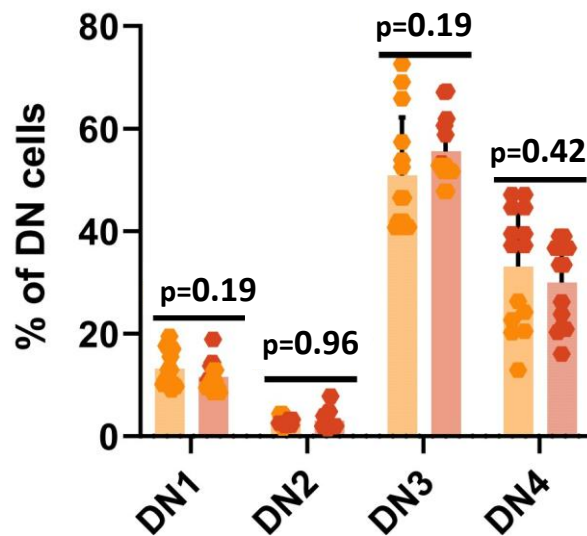
a) T-cell Development stages in Thymus



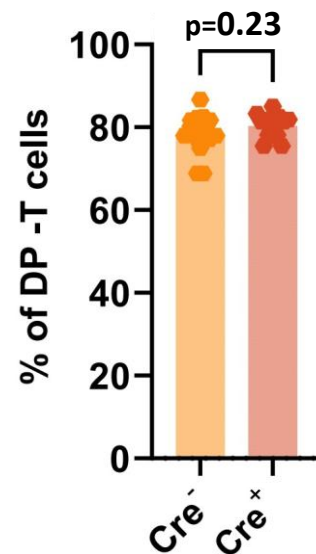
b)



c)



d)



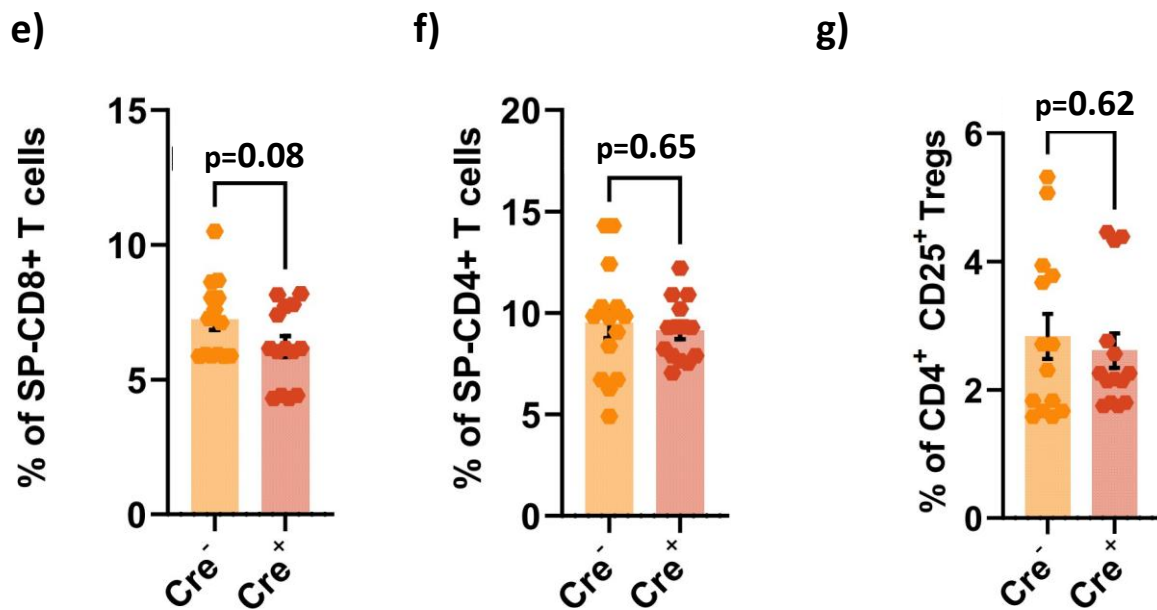


Figure 12: a) T cell development in thymus. b) A representative gating strategy was used for flow cytometry analysis to identify thymocyte populations at different developmental stages. Bar graphs represent the c) Percentage of DN T cells based on their expression of CD25 and CD44, d) Single positive (SP) CD8⁺ T cells, e) DN (double negative) T cells, f) SP-CD4⁺ T cells, g) CD4⁺CD25⁺ (T regs) cells. Data bars are presented as mean ± SEM. Statistical analysis was done with a two-tailed unpaired t-test (n=14).

Naïve CD4 T cells are affected by the lack of Slc38a2

T cell activation and differentiation require dynamic metabolic shifts, including alterations in glutamine uptake. *Slc38a2* and other glutamine transporters support this process, implying the potential that *Slc38a2* deletion may influence distinct peripheral T cell subsets. CD4⁺ and CD8⁺ T cells in peripheral blood were profiled for CD44 and CD62L expression to define naïve (Tn), central memory (Tcm) and effector-memory (Teff) populations (Gerberick et al., 1997) (Figure 13a).

Cre-Positive mice exhibited a shift in the composition of CD4⁺ T cell subsets, with lower naïve T cells and increased frequencies of effector-memory T cells (Figure 13b). By comparison, proportions of naïve, central memory and effector-memory CD8⁺ T cell subset were similar in both groups (Figure 13c). These results indicate that, despite largely normal thymic T cell

development, *Slc38a2* contributes to peripheral CD4⁺ T cell subset homeostasis, likely by regulating their metabolism during activation and differentiation.

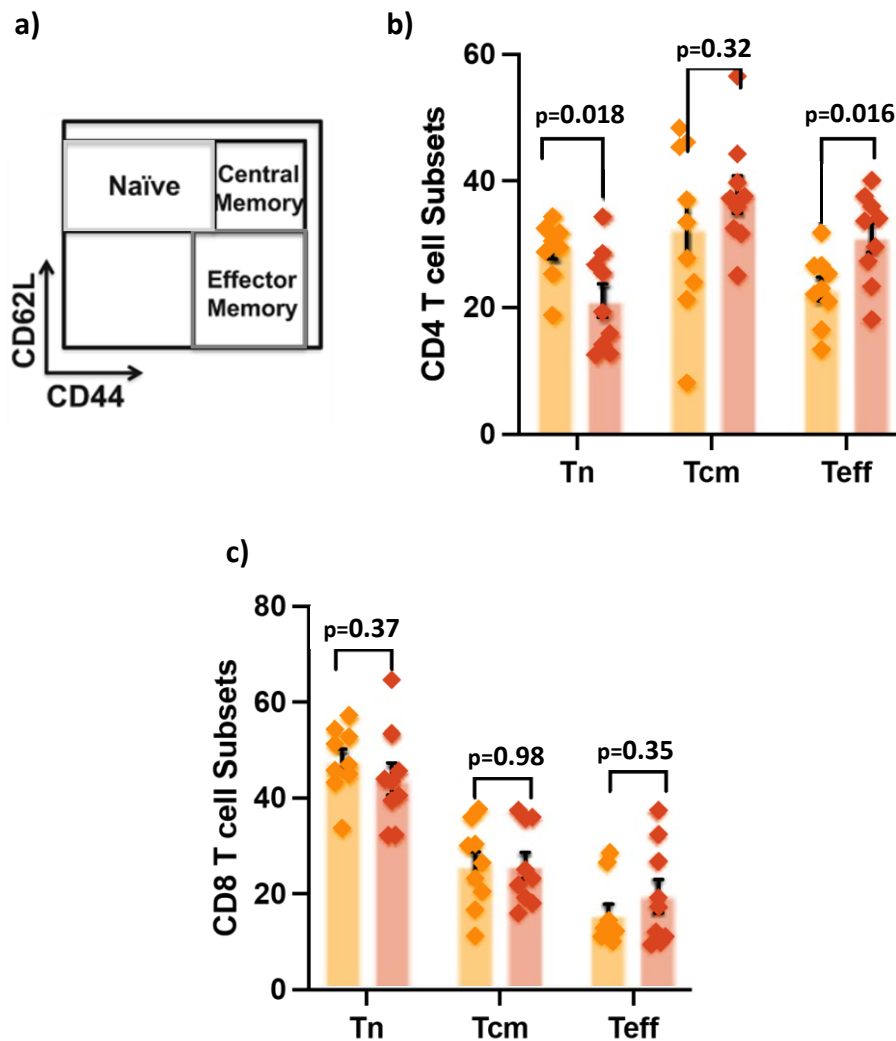


Figure 13: a) Identification of naïve, central memory and effector memory cells strategy based on the expression of surface marker CD62L and CD44; (b-c) Proportion of CD44 and CD62L surface markers Teff (CD44⁺CD62L⁻) and Tcm (CD44⁺CD62L⁺) cell and Tn (CD44⁻CD62L⁺) on CD4 and CD8 cells in the peripheral blood. Data bars are presented as mean $\pm$ SEM. Statistical analysis was done with a two-tailed unpaired t-test. (n=9).

VII. DISCUSSION

1. Methodological considerations and limitations

Focusing on steady-state hematopoiesis in vivo, this study offered insights on homeostatic cellular processes but does not depict stress-induced scenarios, including infection, cytotoxic treatments or inflammatory challenges. Therefore, the data shown here are most relevant to basal hematopoietic function and may not capture the contribution of *Slc38a2* when metabolic requirements and glutamine dependency are heightened, such as during hematopoietic stress following infection or transplantation.

This limitation is particularly pertinent given literature linking altered *Slc38a2* expression to malignant or regenerative contexts including AML relapse, where metabolic reprogramming and upregulated nutrient transporter expression frequently act as a compensatory mechanism. Future studies using stress-inducing models will be critical to clarify whether *Slc38a2* becomes essential for hematopoietic or immune effector functions beyond homeostasis.

2. *Slc38a2* modulation in progenitors and lineage bias (myeloid vs lymphoid)

Expression profiling indicated that *Slc38a2* was broadly expressed along the hematopoietic hierarchy, with elevated levels in HSCs, early progenitors and mature immune cells. Consistent with this expression landscape, deletion of *Slc38a2* in hematopoietic cells caused only limited effects on HSC and multipotent progenitor abundance, including a subtle enrichment in MPP4, but led to more pronounced alterations in committed progenitor subsets.

Within the committed progenitor compartment, Cre-Positive mice exhibited elevated GMP and CLP frequencies and a decline in MEP lineage, together with the preferential shift of Ly6D⁺ B-biased cells at the expense of Ly6D⁻ T/NK-biased CLPs. These variations denote that loss of *Slc38a2* appears to bias progenitor lineage priming toward myeloid-granulocytic and B-lymphoid differentiation pathways over erythroid and T/NK-biased outputs, without affecting steady-state peripheral blood counts.

3. Nutrient sensing and lymphoid/immune cell differentiation via *Slc38a2*

Glutamine metabolism is a major contributor of hematopoietic and immune cell biology, providing critical carbon and nitrogen components for anabolic pathways and maintaining mitochondrial function and anaplerotic reactions essential for cell expansion and immune/effector responses. In accordance with this, several glutamine transporters, including *Slc1a5*, *Slc7a5* and *Slc38* family members, are broadly expressed within hematopoietic cells. Our analyses highlight *Slc38a2* as one of the most uniformly and robustly expressed transporters in both progenitor compartments and mature lymphoid cells. Considering this metabolic landscape, *Slc38a2* ablation revealed a clear perturbation at the progenitor level, whereas circulating B, T and NK cells remained largely preserved under steady state. This pattern demonstrates that transporter redundancy among glutamine transporters and nutrient-sensing pathways endow metabolic robustness, enabling lymphoid and myeloid populations to partially compensate for shift in progenitor bias and maintain peripheral homeostasis in basal conditions. Within this framework, the selective skewing of peripheral CD4⁺ T cell population in Cre-Positive animals, marked by diminished naive and increased effector memory CD4⁺ T cell under with thymic development remaining intact, points to a subtle role of *Slc38a2* in fine-tuning lymphoid differentiation and effector maturation rather than acting as a primary determinant of lineage commitment.

Interestingly, the reduction in circulating dendritic cells noted in *Slc38a2*^{Δ/Δ} aligns with recent reports identifying *SLC38A2*-dependent glutamine uptake as necessary for c-DC1 fitness, IL-12 secretion and cross-presentation in tumor models. This convergence of evidence indicates that DCs represent a particularly *Slc38a2*-sensitive lineage, with possible impacts on T cell priming that would become more noted under infectious or tumoral challenge (Guo et al., 2023) (2023).

4. Metabolic adaptability in steady-state hematopoiesis

Despite the widespread and elevated expression of the *Slc38a2* transporter in HSCs, progenitors and mature immune cells at baseline, the relatively mild hematopoietic changes in Cre-Positive mice reflect the exceptional metabolic flexibility of the hematopoietic system to adapt. Redundant glutamine transporters and the reliance on alternative nutrient pathways,

such as glucose or fatty acid metabolism, are thought to buffer *Slc38a2* deficiency, supporting hematopoietic cells to fulfil their energy production and biosynthetic needs at baseline.

However, the interplay of disrupted progenitor distribution and slight yet consistent alterations in peripheral CD4⁺ T cell subpopulation implies that this compensatory response is not fully neutral. This adaptative capacity may potentially become even more limited under challenging conditions, when glutamine requirement and transporter load are heightened, as previously described for other glutamine transporters, which are nonessential at baseline but become critical under regenerative or leukemic conditions. Taken together, *Slc38a2* does not significantly impact overall hematopoietic output in steady state yet may reflect an underlying metabolic vulnerability in certain lineages, potentially exposed by infection, immune stimulation, chemotherapy or leukemic transformation(Wang et al., 2024) (van Gastel et al., 2020).

VIII. CONCLUSION

This master thesis was undertaken to characterize the effects of hematopoietic compartment specific *Slc38a2* knockout in murine steady-state hematopoiesis.

Slc38a2 deletion produced minimal effects on HSCs and multipotent progenitors with a non-significant trend toward expansion of LMPPs compartment. More pronounced alterations emerged within restricted progenitor populations in which knockout mice exhibited elevated GMP and CLP proportions, decreased MEP abundance, and a preferential redistribution of CLPs toward Ly6D⁺ B-biased cells over Ly6D⁻ T/NK-biased progenitors, suggesting that in the bone marrow, progenitor fate decisions are biased towards myeloid-granulocytic and B-lymphoid outputs.

These upstream progenitor changes did not significantly impair thymic T cell developmental subsets, as DN, DP and SP CD4⁺ thymocytes were largely preserved with only a slight non-significant trend towards diminished SP CD8⁺ cells. In the periphery, B, T and NK cells populations remained relatively constant, yet naïve CD4⁺ T cells were decreased significantly accompanied by a rise in CD4⁺ effector/effector-memory cells, implying that *Slc38a2* plays a role in regulating peripheral CD4⁺ T cell outside the thymus. Importantly, the significant decline in dendritic cells in *Slc38a2*-deficient mice mirrors recent work identifying *Slc38a2*-dependent glutamine uptake for cDC1 fitness and effector function.

Collectively, these findings indicate that *Slc38a2* is mostly non-essential for global hematopoietic function output in homeostasis while supporting balanced committed progenitor pools and regulating discrete aspects of immune cells homeostasis, most prominently in circulating dendritic cells and CD4⁺ T cells subsets. The simultaneous presence of altered progenitor populations and selective DCs decrease, coexisting with maintained total lymphocyte numbers, underscores metabolic plasticity underpinned by compensatory transporter mechanisms and alternative nutrient pathways, while pointing that *Slc38a2* may become indispensable in response to stress under elevated glutamine demand. Subsequent research conducted with experimental models related to inflammation, infection or tumor-associated stress are pivotal for a full understanding of the involvement of *Slc38a2* in hematopoietic process and immune function beyond baseline physiological conditions.

IX. FUTURE PERSPECTIVES

1) Stress hematopoiesis and infection models (LPS, poly(I:C))

As the current study was carried out in homeostatic conditions, an important subsequent step will consist of evaluating whether *Slc38a2* proves to be critical when hematopoiesis and immune functions are perturbed. In living organisms, challenge of mice lacking *Slc38a2* using classical inflammatory stimuli e.g., LPS (bacterial analog) and poly(I:C) (viral analog) (Zhao and Baltimore, 2015) would allow assessment of hematopoietic stress, encompassing HSC activation, emergency myeloid cell production and lymphoid lineage output in states of increased metabolic requirement. Targeted focus should be directed towards DCs (Guo et al., 2023) and CD4⁺ T lymphocyte populations, which already display quantitative and qualitative deviations at steady state, to examine if these impairments are amplified during pathogen-induced or inflammatory stress and whether they modulate T cell activation and downstream effector responses.

2) Metabolic characterization of *Slc38a2*-dependent pathways

To progress from cellular phenotype to underlying mechanisms, it will be necessary to clarify how *Slc38a2* deficiency alters amino acid utilisation and related metabolic networks in disrupted hematopoietic lineages. *Slc38a2* functions as a sodium-coupled transporter of neutral amino acid that enables cellular uptake of various neutral amino acids such as glutamine, alanine, serine and proline and participates in amino acid sensing. Further experiments ought to evaluate amino acid absorption and metabolic pathways in cell populations showing greatest shifts in Cre-Positive mice, for example CLPs, DCs and peripheral CD4⁺ T lymphocytes in baseline and challenged conditions. These investigations may reveal whether other glutamine transport mechanisms or alternative nutrient sources restore function in the absence of *Slc38a2* or if defined metabolic constraints arise in specific blood cell compartments under regenerative, inflammatory or leukemic challenges.

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Glutamine uptake constitutes a major regulator of cellular metabolism across hematopoietic and immune compartments, nonetheless the *in vivo* role of individual glutamine transporters to blood cell development is still poorly defined. *SLC38A2* (*SNAT2*) is a ubiquitously expressed neutral amino acid transporter and displays elevated expression across the hematopoietic stem cell (HSC) compartment, progenitor and differentiated immune cells, suggesting a potential function in hematopoietic balance. This study was designed to assess the consequences of hematopoietic-restricted ablation of *Slc38a2* in mice.

Using a hematopoietic Vav-Cre *Slc38a2* knockout murine model, hematological parameters combined with multiparametric flow cytometry were performed to examine stem and progenitor cell compartments, lineage-committed myeloid and lymphoid progenitor populations, together with mature immune cell subsets in bone marrow, thymus and blood circulation. Loss of *Slc38a2* produced minor effects on HSCs and multipotent progenitor compartments, exhibiting a non-significant shift towards growth of lymphoid-biased LMPPs. Alternatively, committed progenitor populations were significantly impaired, characterized by increased GMP and CLP proportions, lower MEP populations along with a redistribution of CLPs favoring Ly6D⁺ B-biased progenitors at the expense of T/NK-biased cells, pointing a skewing of lineage differentiation towards myeloid-granulocytic and B-lymphoid outputs.

Despite the alterations in upstream progenitors, T cell development in the thymus largely remained unaffected and bulk B, T and NK cell proportions in the periphery showed no major alterations. Nevertheless, naïve CD4⁺ T cells were markedly reduced accompanied by a corresponding rise in effector-memory CD4⁺ T cells and peripheral dendritic cell proportions were significantly lower, supporting previous observations suggesting that *Slc38a2*-depending glutamine transport supports cDC1 survival and function.

Overall, *Slc38a2* plays a limited role for hematopoietic output in basal conditions yet contributes to the equilibrium of committed progenitor pools as well as the maintenance of DCs and CD4⁺ T cell subsets and may uncover context-specific metabolic abilities in settings of inflammatory or immune stress.