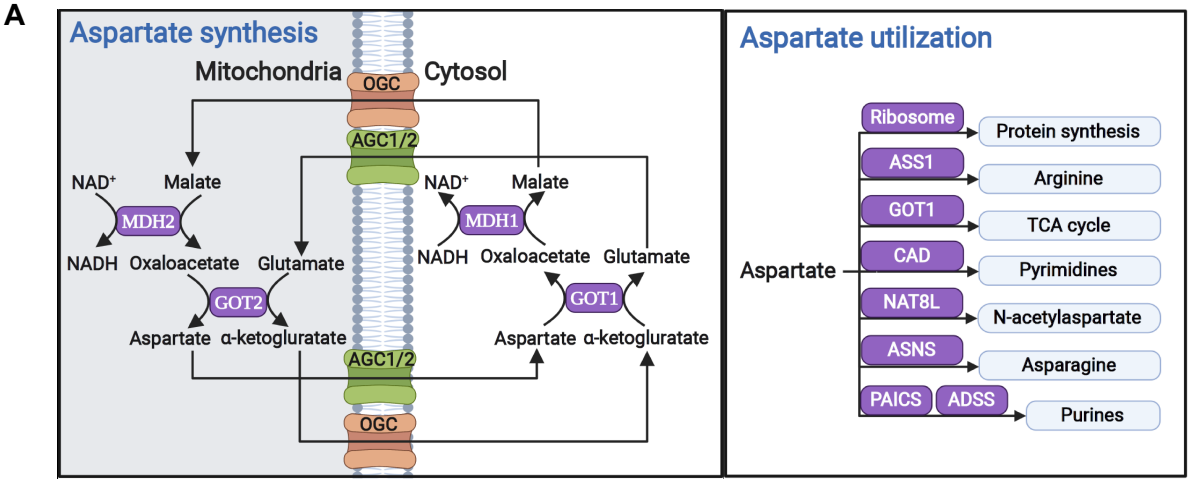
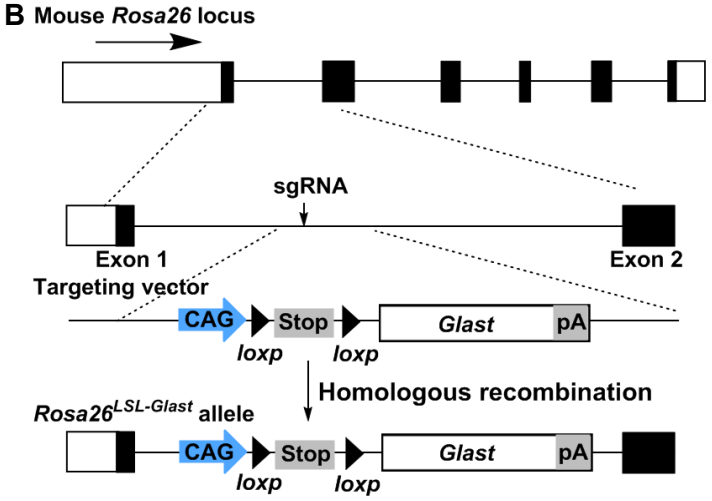
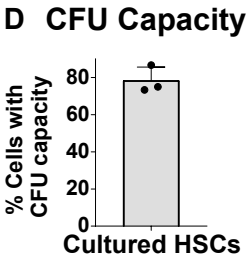
**Figure S1:**

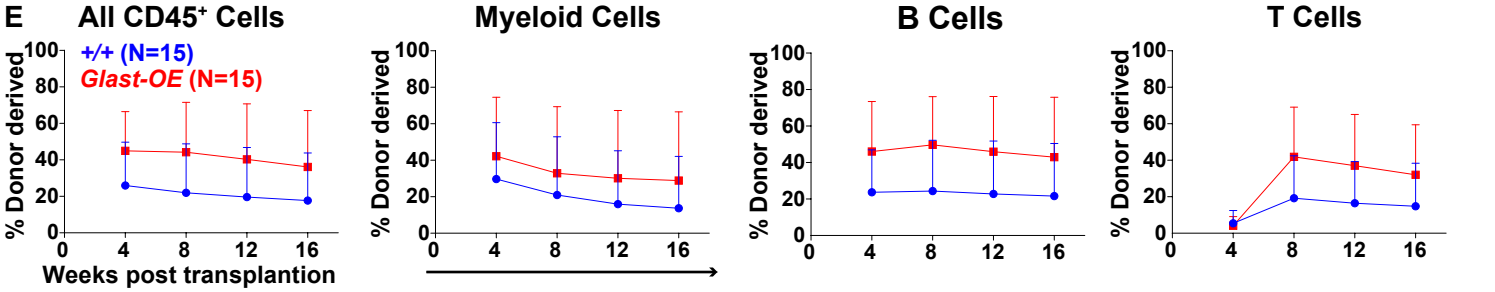


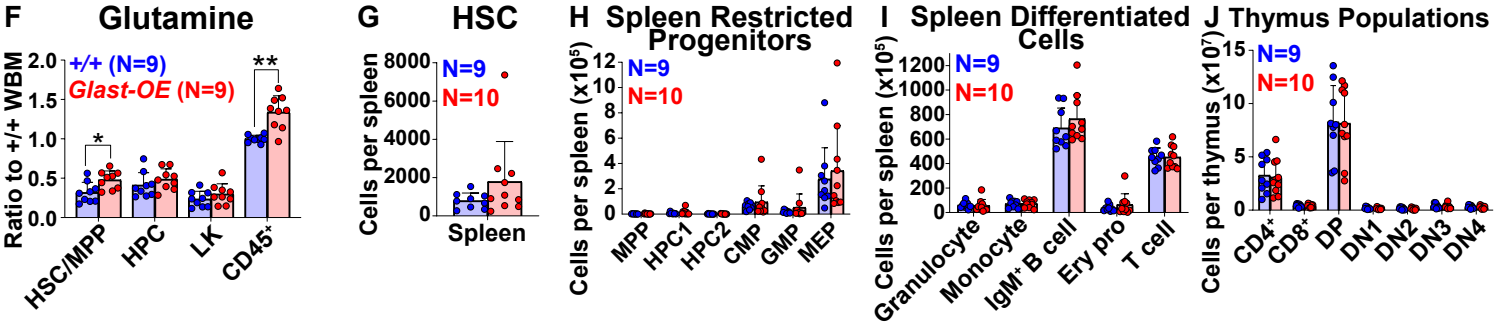




C *Rosa26*$^{LSL\text{-}Glast}$ x *Vav1-cre* progeny

| Genotype | Number of mice |
|---|---|
| +/+ | 43 |
| *Vav1-cre* | 30 |
| *Rosa26*$^{LSL\text{-}Glast}$ | 43 |
| *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ | 48 |



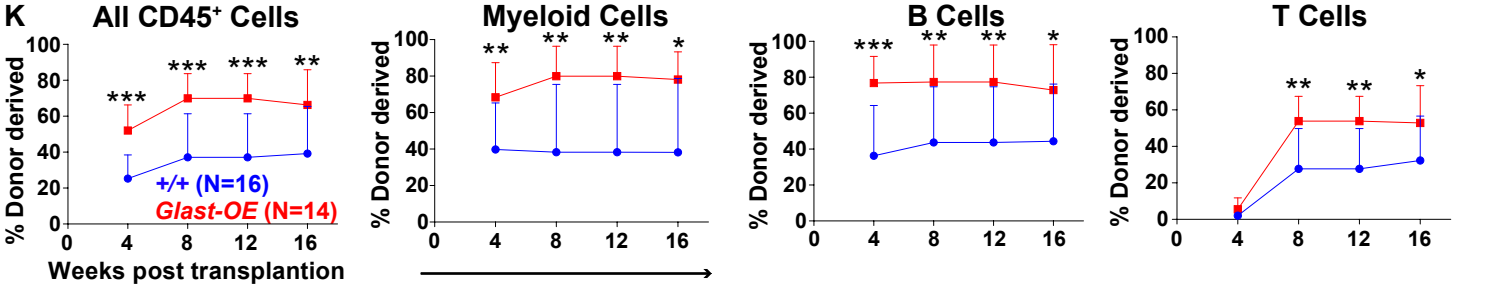



**Figure S1, Related to Figure 1: Effects of GLAST over-expression on hematopoietic stem and progenitor cell numbers.** (A) Aspartate synthesis and utilization pathways. (AGC1/2: aspartate/glutamate carrier 1 or 2; MDH2: malate dehydrogenase 2; OGC: 2-oxoglutarate carrier; ASS1: argininosuccinate synthase 1; CAD: carbamoyl-phosphate synthetase 2, aspartate transcarbamylase and dihydrooratase; PAICS: phosphoribosylaminoimidazole carboxylase; ADSS: adenylosuccinate synthetase) (B) Targeting strategy to generate the *Rosa26*$^{LSL\text{-}Glast}$ allele. The targeting vector was modified from the pR26 CAG AsiSI/MluI plasmid (Chu et al., 2016), in which the CAG promoter is followed by a *loxp-Stop-loxp* sequence (the puromycin resistance cassette was removed from the original vector) and mouse Glast coding sequence was inserted between AsiSI and MluI restriction sites. Crispr/Cas9-mediated recombination was done by injecting the targeting vector, Cas9 protein, tracrRNA and sgRosa26-1 sgRNA into C57BL/6 zygotes. Correctly targeted founder mice were identified by long-range PCR using R26F3 and SAR primers (Chu et al., 2016) and sanger sequencing. The sgRNA and primers used for genotyping are listed in Table S4. (C) *Rosa26*$^{LSL\text{-}Glast}$ and *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ mice were born at expected mendelian frequencies, survived into adulthood in normal numbers, and appeared to be developmentally normal. The statistics reflect mice genotyped at 8–12 weeks of age. (D) 78±7.2% of cells in 7-day HSC cultures formed colonies after sorting into methylcellulose. (E) Donor CD45$^{+}$, myeloid, B, and T cells in the blood of mice competitively transplanted with *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ or control donor cells derived from 7 day HSC cultures. We initiated the cultures with 10 sorted HSCs per well, then 7 days later we competitively transplanted the cells into irradiated mice (one well per recipient). Among the wells of wild-type cells, 7 of 15 gave long-term multilineage reconstitution and 8 of 15 gave transient multilineage reconstitution. Among the wells of GLAST over-expressing cells, 9 of 15 gave long-term multilineage reconstitution, 4 of 15 gave transient multilineage reconstitution, and 1 gave no donor cell reconstitution (n=15 recipient mice per genotype, total, from 3 independent experiments with 3 donors per genotype). *Glast-OE* refers to cells from *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ mice throughout the figure. (F) Glutamine levels in HSCs/MPPs, HPCs, LK cells, and CD45$^{+}$ cells from *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ or control bone marrow. (G-I) Number of HSCs (G), restricted progenitors (H) and differentiated cells (I) in the spleen. (J) Number of T cell progenitors in the thymus. (K) Donor-derived CD45$^{+}$, myeloid, B, and T cells in the blood of secondary transplant recipients of *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ or control donor bone marrow cells (n=14-16 recipient mice per genotype, total, from 4 independent experiments with 14-16 donors per genotype). All data represent mean ± standard deviation (* $P< 0.05$; ** $P<0.01$; *** $P<0.001$). The number of mice analyzed per genotype is shown in each panel. The image in panel A was created with Biorender.

## Figure S2:

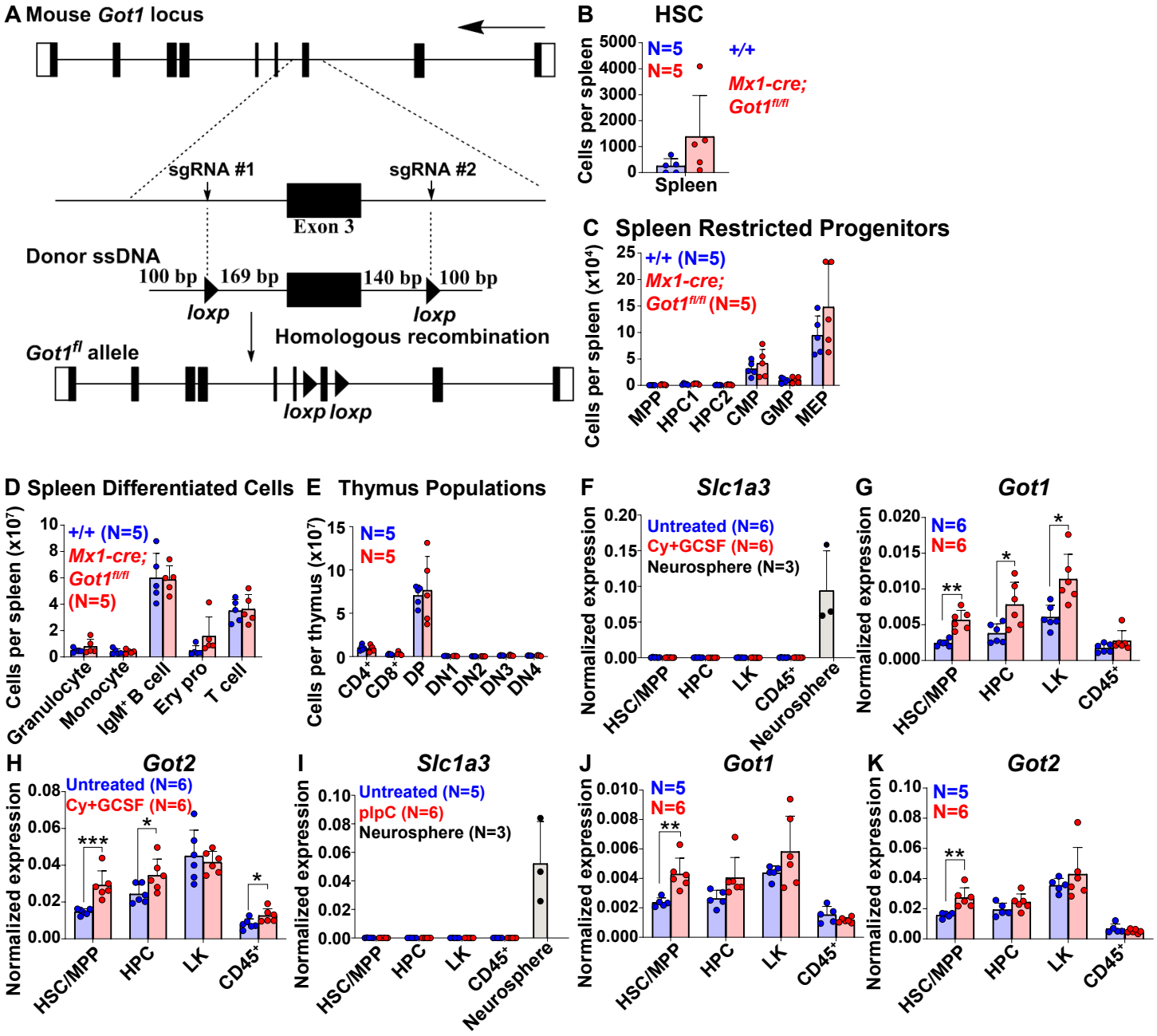
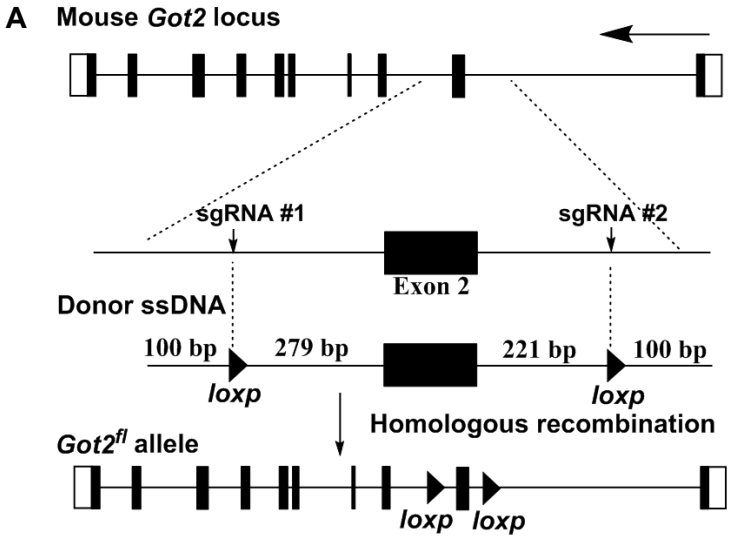

A Mouse Got1 locus
sgRNA #1
sgRNA #2
Exon 3
Donor ssDNA
100 bp
169 bp
140 bp
100 bp
loxp
loxp
Homologous recombination
Got1fl allele
loxp loxp
B HSC
Cells per spleen
N=5
N=5
+/+
Mx1-cre; Got1fl/fl
Spleen
C Spleen Restricted Progenitors
Cells per spleen (x10⁴)
+/+ (N=5)
Mx1-cre; Got1fl/fl (N=5)
MPP
HPC1
HPC2
CMP
GMP
MEP
D Spleen Differentiated Cells
Cells per spleen (x10⁷)
+/+ (N=5)
Mx1-cre; Got1fl/fl (N=5)
Granulocyte
Monocyte
IgM+ B cell
Ery pro
T cell
E Thymus Populations
Cells per thymus (x10⁷)
N=5
N=5
CD4+
CD8+
DP
DN1
DN2
DN3
DN4
F Slc1a3
Normalized expression
Untreated (N=6)
Cy+GCSF (N=6)
Neurosphere (N=3)
HSC/MPP
HPC
LK
CD45+
Neurosphere
G Got1
Normalized expression
N=6
N=6
HSC/MPP
HPC
LK
CD45+
H Got2
Normalized expression
Untreated (N=6)
Cy+GCSF (N=6)
HSC/MPP
HPC
LK
CD45+
I Slc1a3
Normalized expression
Untreated (N=5)
pIpC (N=6)
Neurosphere (N=3)
HSC/MPP
HPC
LK
CD45+
Neurosphere
J Got1
Normalized expression
N=5
N=6
HSC/MPP
HPC
LK
CD45+
K Got2
Normalized expression
N=5
N=6
HSC/MPP
HPC
LK
CD45+

**Figure S2, Related to Figure 2: Effect of *Got1* deficiency on hematopoietic stem and progenitor cell numbers.** (A) Targeting strategy to generate the *Got1$^{fl}$* allele. The donor ssDNA contained *loxp* sequences inserted on both sides of exon 3. The *loxp* insertion sites were chosen to avoid disrupting sequences conserved among species and to cause a frame-shift upon Cre-mediated recombination. Crispr/Cas9-mediated recombination was done by injecting donor ssDNA, Cas9 protein, tracrRNA and 2 sgRNAs targeting the endogenous sequences where loxp sites were inserted into C57BL/6 zygotes. Correctly targeted founder mice were identified by long-range PCR with primers flanking the whole region targeted by donor ssDNA followed by Sanger sequencing. The sequence of the donor ssDNA, sgRNAs and primers used for genotyping are shown in Table S4. (B-D) Numbers of HSCs (B), restricted hematopoietic progenitors (C) and differentiated hematopoietic cells (D) in the spleen. (E) Number of T cell progenitors in the thymus. (F-K) qPCR analysis of *Slc1a3* (F), *Got1* (G) and *Got2* (H) transcript levels normalized to *β-actin* transcript levels in hematopoietic stem/progenitor cell populations isolated from mice treated with cyclophosphamide/G-CSF (F-H) or pIpC (I-K) as compared to untreated control mice. Neurospheres were a positive control for *Slc1a3* expression in panels F and I. All data represent mean ± standard deviation (* $P< 0.05$; ** $P<0.01$; *** $P<0.001$). The number of mice analyzed per genotype is shown in each panel.

## Figure S3:




**B** *Got2*$^{fl/fl}$ x *Mx1-cre; Got2*$^{fl/+}$ progeny

| Genotype | Number |
|---|---|
| *Got2*$^{fl/+}$ | 27 |
| *Got2*$^{fl/fl}$ | 27 |
| *Mx1-cre; Got2*$^{fl/+}$ | 31 |
| *Mx1-cre; Got2*$^{fl/fl}$ | 28 |

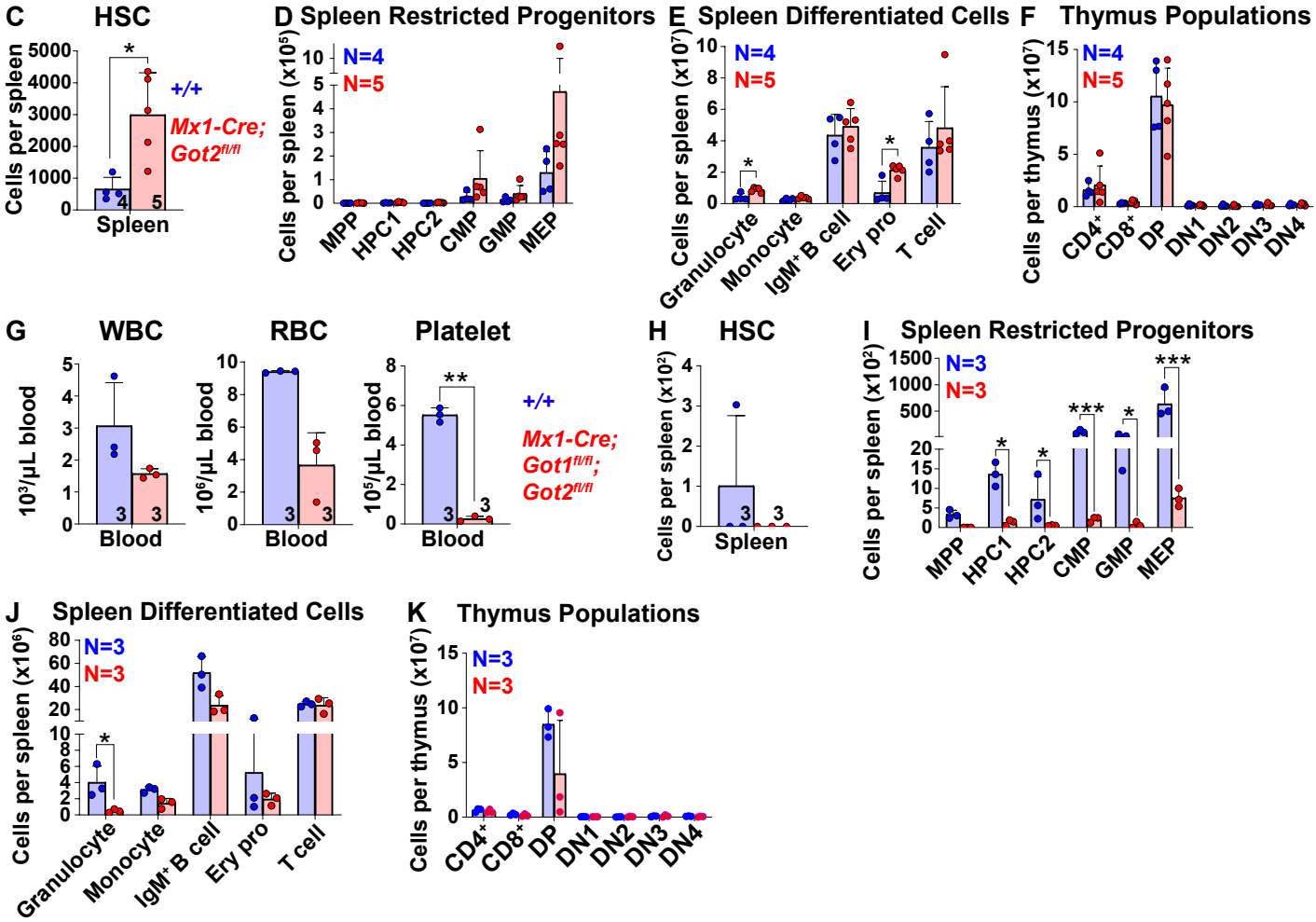


**Figure S3, Related to Figure 3: Effect of *Got2* deficiency on hematopoietic stem and progenitor cell numbers.** (A) Targeting strategy to generate the *Got2$^{fl}$* allele. The donor ssDNA contained *loxp* sequences on both sides of exon 2. The insertion sites were chosen to avoid disrupting sequences conserved among species and to cause a frame-shift upon Cre-mediated recombination. Crispr/Cas9-mediated recombination was performed by injecting the donor ssDNA and 2 sgRNAs against endogenous sequences where the loxp sites were inserted. Correctly targeted founder mice were identified by long-range PCR with primers flanking the whole region targeted by donor ssDNA followed by Sanger sequencing. The sequence of the donor ssDNA, sgRNAs and primers used for genotyping are shown in Table S4. (B) *Got2$^{fl/fl}$* and *Mx1-cre; Got2$^{fl/fl}$* mice were born at mendelian frequencies and survived into adulthood in normal numbers. The statistics reflect mice genotyped at 8–12 weeks of age. (C-E) Numbers of HSCs (C), restricted hematopoietic progenitors (D) and differentiated hematopoietic cells (E) in the spleen. (F) Number of T cell progenitors in the thymus. (G) White blood cell, red blood cell, and platelet counts in the blood of *Mx1-cre; Got1$^{fl/fl}$; Got2$^{fl/fl}$* and control mice. (H-J) Numbers of HSCs (H), restricted hematopoietic progenitors (I) and differentiated hematopoietic cells (J) in the spleen. (K) Number of T cell progenitors in the thymus. All data represent mean ± standard deviation (* $P$< 0.05; ** $P$<0.01; *** $P$<0.001). The number of mice analyzed per genotype is shown in each panel.

# Figure S4:

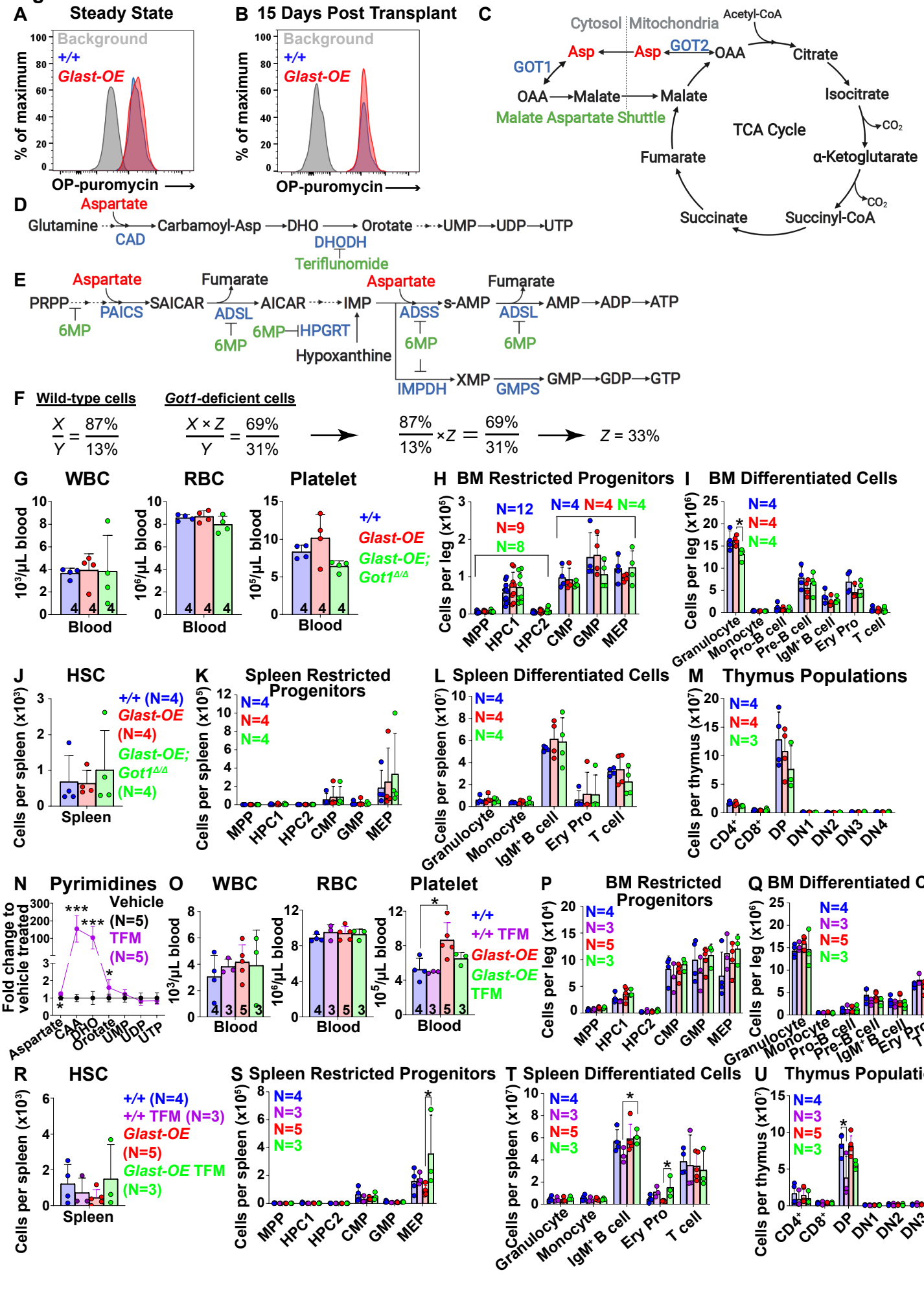

A Steady State
B 15 Days Post Transplant
Background
+/+
Glast-OE
% of maximum
OP-puromycin
C
Cytosol
Mitochondria
Acetyl-CoA
Asp
GOT1
GOT2
OAA
Citrate
Malate
Malate Aspartate Shuttle
TCA Cycle
Isocitrate
α-Ketoglutarate
Fumarate
Succinate
Succinyl-CoA
CO2
D
Aspartate
Glutamine
Carbamoyl-Asp
DHO
Orotate
UMP
UDP
UTP
CAD
DHODH
Teriflunomide
E
PRPP
PAICS
SAICAR
ADSL
AICAR
IMP
ADSS
s-AMP
AMP
ADP
ATP
6MP
HPGRT
Hypoxanthine
XMP
IMPDH
GMPS
GMP
GDP
GTP
F
Wild-type cells
Got1-deficient cells
X/Y = 87%/13%
X × Z/Y = 69%/31%
87%/13% × Z = 69%/31%
Z = 33%
G WBC
RBC
Platelet
Blood
+/+
Glast-OE
Glast-OE; Got1Δ/Δ
H BM Restricted Progenitors
Cells per leg (x10⁵)
MPP
HPC1
HPC2
CMP
GMP
MEP
N=12
N=9
N=8
N=4
I BM Differentiated Cells
Cells per leg (x10⁶)
Granulocyte
Monocyte
Pro-B cell
Pre-B cell
IgM+ B cell
Ery Pro
T cell
J HSC
Cells per spleen (x10³)
Spleen
+/+ (N=4)
Glast-OE (N=4)
Glast-OE; Got1Δ/Δ (N=4)
K Spleen Restricted Progenitors
Cells per spleen (x10⁵)
L Spleen Differentiated Cells
Cells per spleen (x10⁷)
M Thymus Populations
Cells per thymus (x10⁷)
CD4+
CD8+
DP
DN1
DN2
DN3
DN4
N Pyrimidines
Fold change to vehicle treated
Vehicle (N=5)
TFM (N=5)
Aspartate
CAD
DHO
Orotate
UMP
UDP
UTP
O WBC
RBC
Platelet
+/+ TFM
Glast-OE TFM
P BM Restricted Progenitors
Q BM Differentiated Cells
R HSC
+/+ TFM (N=3)
Glast-OE (N=5)
Glast-OE TFM (N=3)
S Spleen Restricted Progenitors
T Spleen Differentiated Cells
U Thymus Populations

**Figure S4, Related to Figure 4: Effect of *Got1* deficiency on hematopoietic stem and progenitor cell numbers in GLAST over-expressing mice.** (A and B) Representative histograms of O-propargyl-puromycin (OP-puromycin) incorporation by GLAST over-expressing or control HSCs either in normal adult bone marrow (A) or 15 days after transplantation into irradiated recipient mice (B). Background signal was from HSCs isolated from mice not treated with OP-puro (A) or HSCs isolated from OP-puro treated mice without the click reaction (B). (C) Schematic of the malate-aspartate shuttle and the TCA cycle. (OAA: oxaloacetate). (D) Schematic of de novo pyrimidine synthesis. (DHODH: dihydroorotate dehydrogenase) (E) Schematic of purine synthesis (PRPP: 5-phosphoribosyl-1-pyrophosphate; SAICAR: succinyl-5-aminoimidazole-4-carboxamide-ribonucleotide; ADSL: Adenylosuccinase; AICAR: 5-Aminoimidazole-4-carboxamide ribonucleotide; HGPRT: hypoxanthine-guanine phosphoribosyltransferase; s-AMP: adenylosuccinate; XMP: xanthosine monophosphate). (F) *X* is the rate at which malate arises from labelled aspartate in wild-type cells and *Y* is the rate at which malate arises from other sources (1-*X*). We observed a fractional enrichment of malate from labelled aspartate of 87% in wild-type cells and 69% in *Got1*-deficient cells (Figure 4J). From these data, we can estimate the fold-reduction (*Z*) in the rate at which malate arises from labelled aspartate in *Got1*-deficient as compared to wild-type cells. (G) White blood cell, red blood cell, and platelet counts in the blood of *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ (*Glast-OE*), *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$*; Got1*$^{fl/fl}$ (*Glast-OE; Got1*$^{\Delta/\Delta}$) and control mice. (H and I) Numbers of restricted hematopoietic progenitors (H) and differentiated hematopoietic cells (I) in the bone marrow from one femur and one tibia. (J-L) Numbers of HSCs (J), restricted hematopoietic progenitors (K) and differentiated hematopoietic cells (L) in the spleen. (M) Numbers of T cell progenitors in the thymus. (N) Aspartate and intermediates of pyrimidine synthesis levels in unfractionated wild-type bone marrow cells 16 weeks after transplantation into irradiated recipients, either treated with vehicle or TFM. (O) White blood cell, red blood cell, and platelet counts in the blood of *Glast-OE* or control mice treated with vehicle or TFM. (P and Q) Numbers of restricted hematopoietic progenitors (P) and differentiated hematopoietic cells (Q) in the bone marrow from one femur and one tibia. (R-T) Numbers of HSCs (R), restricted hematopoietic progenitors (S) and differentiated hematopoietic cells (T) in the spleen. (U) Numbers of T cell progenitors in the thymus. All data represent mean ± standard deviation (* $P< 0.05$; ** $P<0.01$; *** $P<0.001$). The number of mice analyzed per genotype is shown in each panel. Images in panels C-E were created with BioRender.

**Figure S5:**

**A** *Nat8l*$^{fl/fl}$ x *Vav1-cre; Nat8l*$^{fl/+}$ progeny

| Genotype | Number of mice |
|---|---|
| *Nat8l*$^{fl/+}$ | 20 |
| *Nat8l*$^{fl/fl}$ | 20 |
| *Vav1-cre; Nat8l*$^{fl/+}$ | 22 |
| *Vav1-cre; Nat8l*$^{fl/fl}$ | 21 |

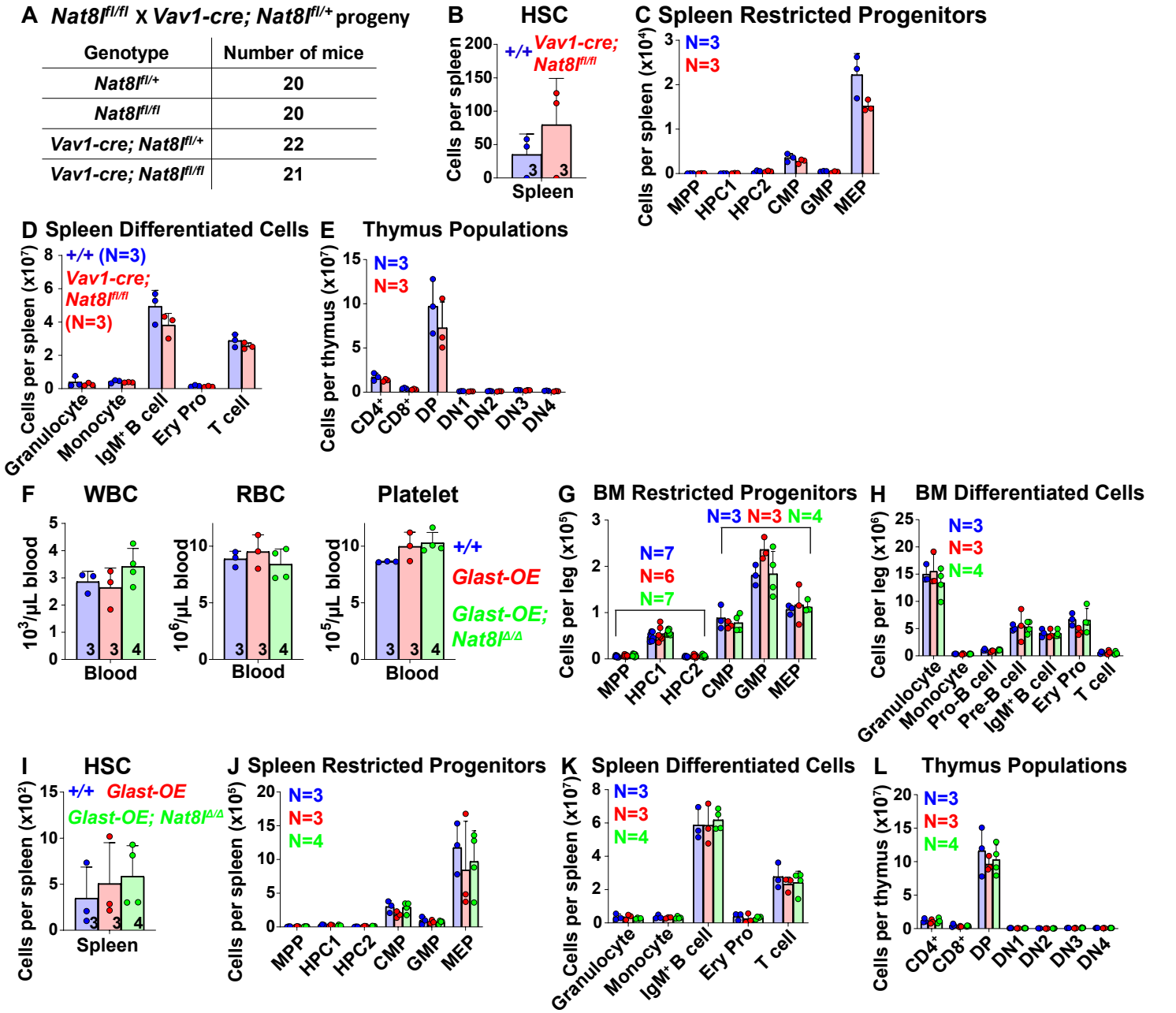
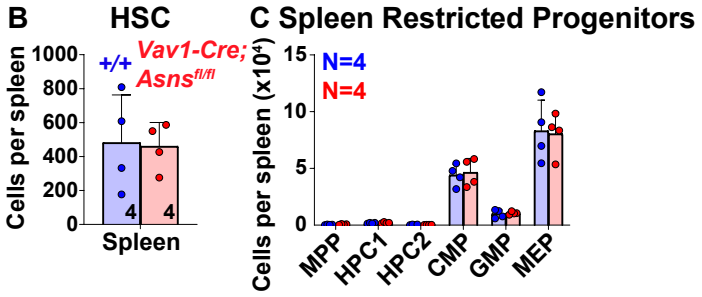


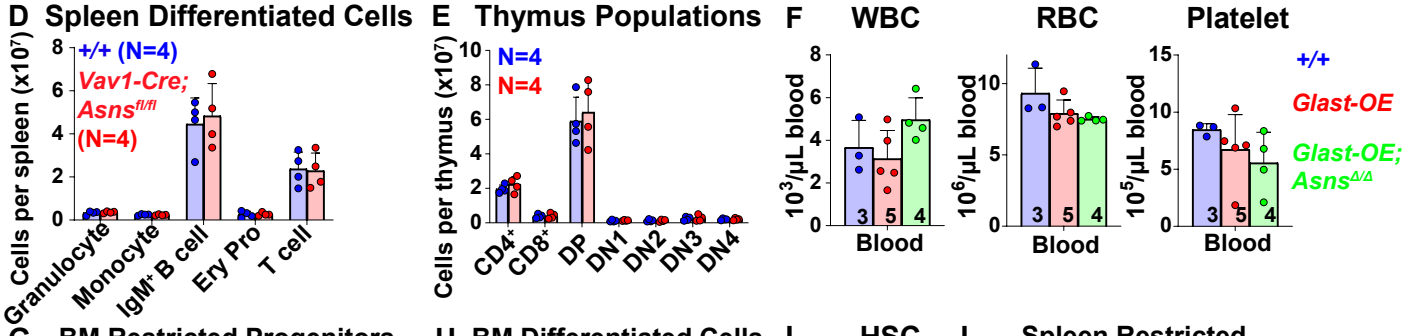

**Figure S5, Related to Figure 5: Effect of *Nat8l* deficiency on hematopoietic stem and progenitor cell numbers in wild-type or GLAST over-expressing mice.** (A) *Nat8l*$^{fl/fl}$ and *Vav1-cre; Nat8l*$^{fl/fl}$ mice were born at mendelian frequencies and survived into adulthood in normal numbers. The statistics reflect mice genotyped at 8–12 weeks of age. (B-D) Numbers of HSCs (B), restricted hematopoietic progenitors (C) and differentiated hematopoietic cells (D) in the spleen. (E) Number of T cell progenitors in the thymus. (F) White blood cell, red blood cell, and platelet counts in the blood of *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ (*Glast-OE*), *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$*; Nat8l*$^{fl/fl}$ (*Glast-OE; Nat8l*$^{\Delta/\Delta}$), and control mice. (G and H) Numbers of restricted hematopoietic progenitors (G) and differentiated hematopoietic cells (H) in the bone marrow from one femur and one tibia. (I-K) Numbers of HSCs (I), restricted hematopoietic progenitors (J) and differentiated hematopoietic cells (K) in the spleen. (L) Numbers of T cell progenitors in the thymus. All data represent mean ± standard deviation (* $P< 0.05$; ** $P<0.01$; *** $P<0.001$). The number of mice analyzed per genotype is shown in each panel.

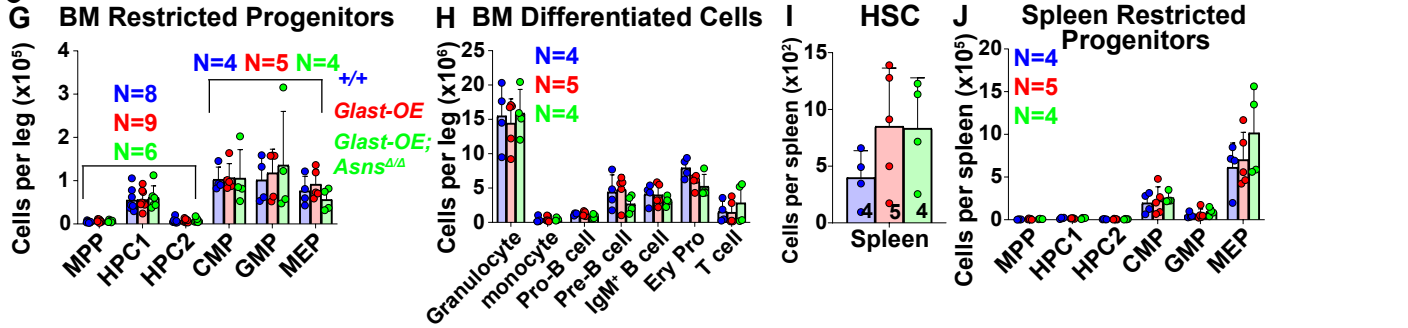
# Figure S6:

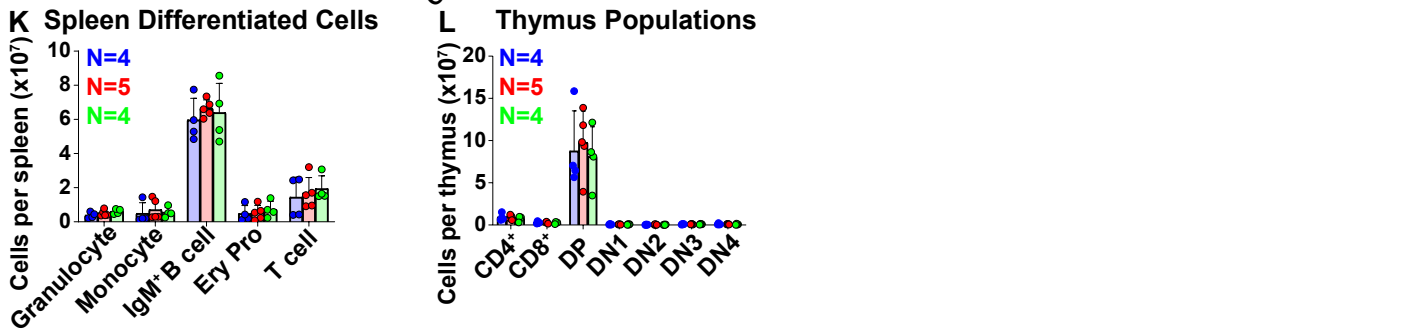
**A** *Asns*$^{fl/fl}$ x *Vav1-cre; Asns*$^{fl/+}$ progeny

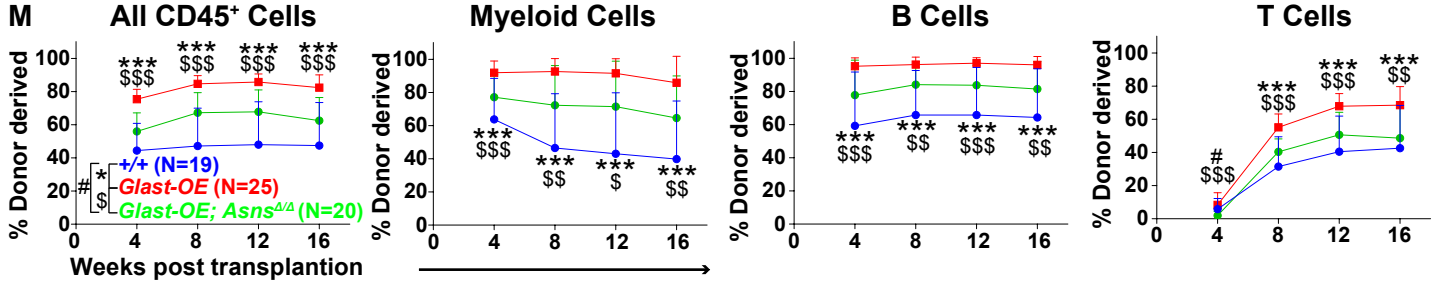
| Genotype | Number |
|---|---|
| *Asns*$^{fl/+}$ | 16 |
| *Asns*$^{fl/fl}$ | 15 |
| *Vav1-cre; Asns*$^{fl/+}$ | 15 |
| *Vav1-cre; Asns*$^{fl/fl}$ | 10 |

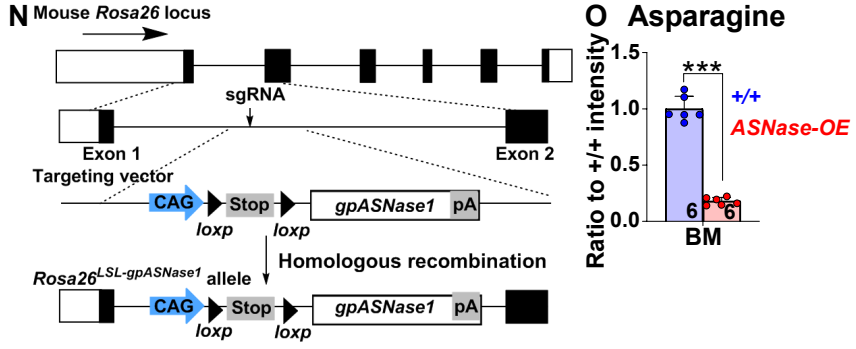


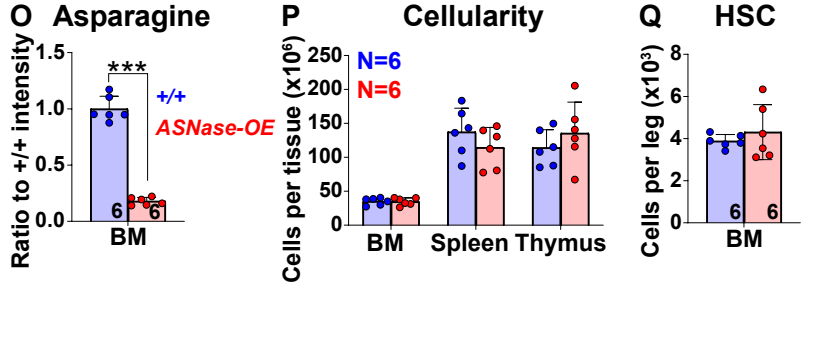



**Figure S6, Related to Figure 6: Effect of *Asns* deficiency on hematopoietic stem and progenitor cell numbers in wild-type or GLAST over-expressing mice.** (A) *Asns*$^{fl/fl}$ and V*av1-cre; Asns*$^{fl/fl}$ mice were born at mendelian frequencies and survived into adulthood. The statistics reflect mice genotyped at 8–12 weeks of age. (B-D) Numbers of HSCs (B), restricted progenitors (C) and differentiated cells (D) in the spleen of V*av1-cre; Asns*$^{fl/fl}$ or control mice. (E) Numbers of T cell progenitors in the thymus. (F) White blood cell, red blood cell, and platelet counts in the blood of *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$ (*Glast-OE*), *Vav1-cre; Rosa26*$^{LSL\text{-}Glast}$*; Asns*$^{fl/fl}$ (*Glast-OE; Asns*$^{\Delta/\Delta}$), or control mice. (G and H) Numbers of restricted hematopoietic progenitors (G) and differentiated hematopoietic cells (H) in the bone marrow from one femur and one tibia. (I-K) Numbers of HSCs (I), restricted hematopoietic progenitors (J) and differentiated hematopoietic cells (K) in the spleen. (L) Numbers of T cell progenitors in the thymus. (M) Donor-derived CD45$^{+}$, myeloid, B, and T cells in the blood of secondary transplant recipients of *Glast-OE*, *Glast-OE; Asns*$^{\Delta/\Delta}$ or control donor bone marrow cells (n=19-25 recipient mice per genotype, total, from 4 independent experiments with 19-24 donors per genotype). (N) Targeting strategy to generate the *Rosa26*$^{LSL\text{-}gpASNase1}$ allele. The sgRNA, coding sequence for guinea pig asparaginase 1,and primers used for genotyping are listed in Table S4. (O) Asparagine was severely depleted in *Vav1-cre; Rosa26*$^{LSL\text{-}gpASNase1}$ (*ASNase-OE*) as compared to control bone marrow cells. (P) Cellularity of the bone marrow from one femur and one tibia, the spleen, and the thymus. (Q) Number of HSCs in the bone marrow from one femur and one tibia. All data represent mean ± standard deviation (* $P< 0.05$; ** $P<0.01$; *** $P<0.001$). The number of mice analyzed per genotype is shown in each panel.

**Figure S7:**

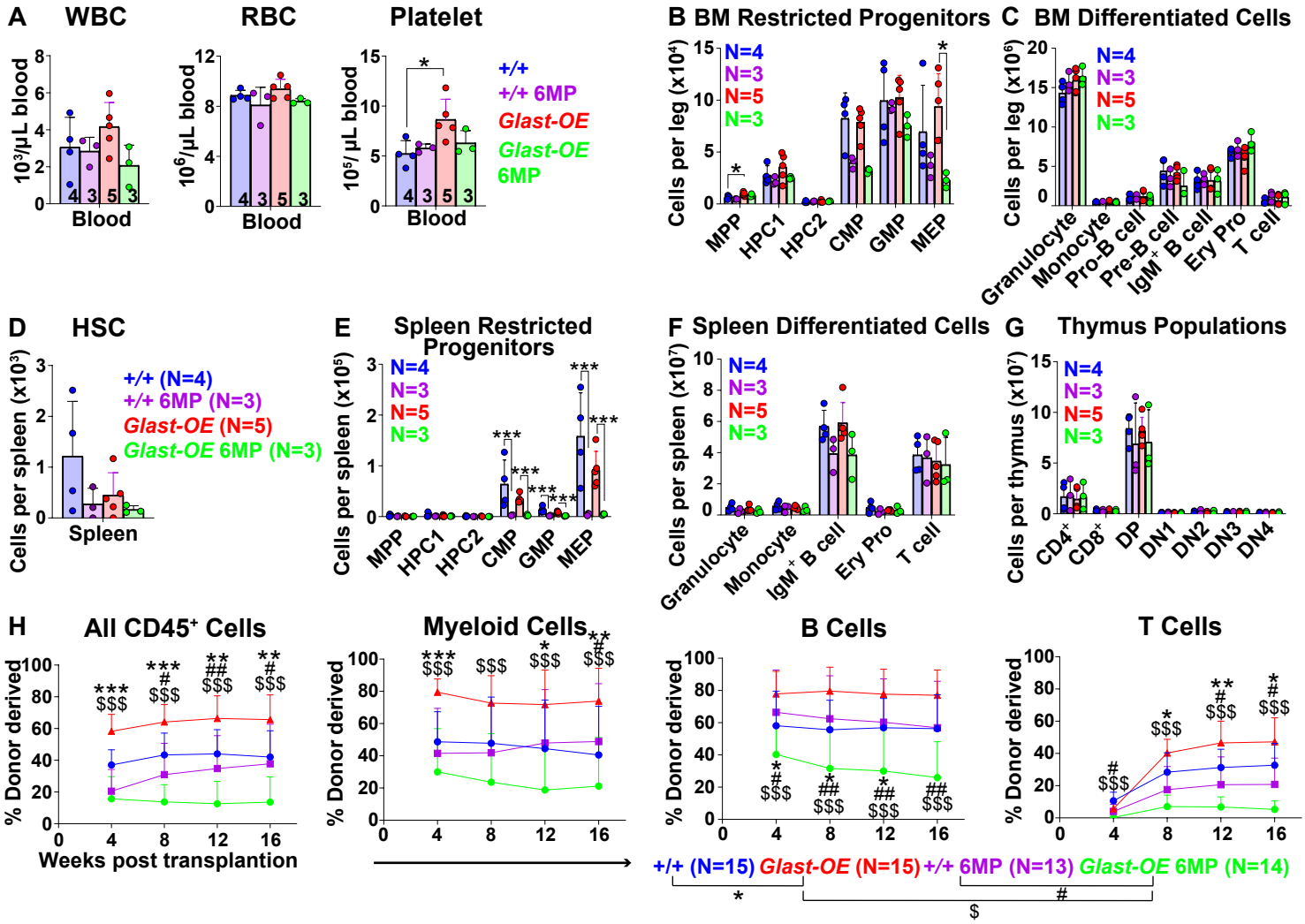



**Figure S7, Related to Figure 7: Effect of 6MP treatment on hematopoietic stem and progenitor cell numbers in wild-type or GLAST over-expressing mice.** (A) White blood cell, red blood cell, and platelet counts in *Glast-OE* or control mice treated with vehicle or 6MP. Data in panels A-G are from the same experiment as in Figures S4N-S4T. (B and C) Numbers of restricted progenitors (B) and differentiated cells (C) in the bone marrow from one femur and one tibia. (D-F) Numbers of HSCs (D), restricted progenitors (E) and differentiated hematopoietic cells (F) in the spleen. (G) Numbers of T cell progenitors in the thymus. (H) Donor-derived CD45$^+$, myeloid, B, and T cells in the blood of secondary transplant recipients of *Glast-OE* or control donor bone marrow cells. Both primary and secondary recipient mice were treated with 6MP or untreated (n=13-15 recipient mice per treatment, total, from 3 independent experiments with 13-15 donors per treatment). All data represent mean ± standard deviation (* $P$< 0.05; ** $P$<0.01; *** $P$<0.001). The number of mice analyzed per genotype is shown in each panel.

**Table S1. Related to Figures 1-7 and S1-S7. Cell populations analyzed by flow cytometry in this study.**

| Cell population | Abbreviation | Markers | Reference |
|---|---|---|---|
| Hematopoietic Stem Cells | HSC | $CD150^+$ $CD48^-$ $Lin^-$ $Sca1^+$ $c\text{-}kit^+$ | Kiel et al., 2005 |
| Multipotent progenitors | MPP | $CD150^-$ $CD48^-$ $Lin^-$ $Sca1^+$ $c\text{-}kit^+$ | Kiel et al., 2005 |
| Pooled HSCs and MPPs | HSC/MPP | $CD48^-$ $Lin^-$ $Sca1^+$ $c\text{-}kit^+$ | |
| HPC1 progenitors | HPC1 | $CD150^-$ $CD48^+$ $Lin^-$ $Sca1^+$ $c\text{-}kit^+$ | Oguro et al., 2013 |
| HPC2 progenitors | HPC2 | $CD150^+$ $CD48^+$ $Lin^-$ $Sca1^+$ $c\text{-}kit^+$ | Oguro et al., 2013 |
| Pooled HPC1 and HPC2 | HPC | $CD48^+$ $Lin^-$ $Sca1^+$ $c\text{-}kit^+$ | |
| Common Myeloid Progenitors | CMP | $Lin^-$ $Sca1^-$ $c\text{-}kit^+$ $CD34^+$ $CD16/32^-$ | Akashi et al., 2000 |
| Granulocyte-Macrophage Progenitors | GMP | $Lin^-$ $Sca1^-$ $c\text{-}kit^+$ $CD34^+$ $CD16/32^+$ | Akashi et al., 2000 |
| Megakaryocyte-Erythrocyte Progenitors | MEP | $Lin^-$ $Sca1^-$ $c\text{-}kit^+$ $CD34^-$ $CD16/32^-$ | Akashi et al., 2000 |
| Myeloid progenitors | LK | $Lin^-$ $Sca1^-$ $c\text{-}kit^+$ | |
| Pro-B cells | | $B220^+$ $IgM^-$ $CD43^+$ | Hardy et al., 1991 |
| Pre-B cells | | $B220^+$ $IgM^-$ $CD43^-$ | Hardy et al., 1991 |
| $IgM^+$ B cells | | $B220^+$ $IgM^+$ | Hardy et al., 1991 |
| Granulocytes | | $Gr1^+$ $CD11b^+$ | |
| Monocytes | | $Gr1^-$ $CD11b^+$ | |
| Myeloid cells | | $CD11b^+$ | |
| T cells | | $CD3^+$ | |
| Erythroid Progenitors | Ery Pro | $CD71^+$ $Ter119^+$ | |
| $CD4^+$ single positive thymocytes | $CD4^+$ | $CD4^+$ $CD8^-$ | |
| $CD8^+$ single positive thymocytes | $CD8^+$ | $CD8^+$ $CD4^-$ | |
| $CD4^+CD8^+$ double positive thymocytes | DP | $CD4^+$ $CD8^+$ | |
| Double negative 1 thymocytes | DN1 | $CD4^-$ $CD8^-$ $CD44^{hi}$ $CD25^-$ | Godfrey et al., 1993 |
| Double negative 2 thymocytes | DN2 | $CD4^-$ $CD8^-$ $CD44^{hi}$ $CD25^+$ | Godfrey et al., 1993 |
| Double negative 3 thymocytes | DN3 | $CD4^-$ $CD8^-$ $CD44^{lo}$ $CD25^+$ | Godfrey et al., 1993 |
| Double negative 4 thymocytes | DN4 | $CD4^-$ $CD8^-$ $CD44^-$ $CD25^-$ | Godfrey et al., 1993 |

**Table S2. Related to Figure 2. Metabolites that significantly changed between *Mx1-cre; Got1$^{fl/fl}$* and +/+ mice** (* $P$< 0.05; ** $P$<0.01; *** $P$<0.001).

| | Fold change (mutant/WT) | | | |
|---|---|---|---|---|
| | **HSCs/MPPs** | **HPCs** | **LK cells** | **CD45$^+$ cells** |
| Aspartate | 2.1 (**) | 1.9 (*) | 1.3 | 2.1 |
| AMP | 0.72 (*) | 0.39 (*) | 1.3 | 0.8 |
| Carnitine (14:0) | 1.9 (*) | 1.3 | 1.4 | 1.0 |
| Carnitine (16:0) | 1.8 (*) | 1.4 | 1.3 | 1.1 |
| Carnitine (16:1) | 2.2 (*) | N.A. | 1.1 | 0.9 |
| Glutamine | 0.60 (*) | 0.60 (*) | 0.78 | 0.82 |
| Hydroxy-2,4-pentadienoate | 0.22 (**) | 5.6 | 0.88 | 1.3 |
| Hydroxycitric acid | 0.35 (**) | 2.2 | 0.83 | 0.72 |
| Lysophosphotidylcholine (22:4) | 2.5 (**) | 0.85 | 1.1 | 2.3 |
| Methyl-erythritol-phosphate | 0.37 (*) | 1.8 (*) | 0.84 | 1.2 |
| Dimethylaminoethanol phosphate | 0.33 (**) | 1,8 | 0.7 (**) | 0.67 (**) |
| Phosphotidylcholine (18:1/18:1) | 1.7 (*) | 1.3 (*) | 1.2 (*) | 1.2 |
| Sphingomyelin (d18:1/16:1) | 1.6 (*) | 1.2 | 1.3 | 1.0 |
| UDP-Hexose | 0.55 (*) | 1.4 (*) | 0.99 | 0.99 |
| Aminocyclopropane-carboxylate | 1.5 | 0.62 (**) | 0.79 | 0.71 (*) |
| Cholinephosphate | 0.88 | 0.35 (*) | 0.59 (*) | 0.75 (*) |
| Cysteinylglycine | 0.85 | 0.53 (*) | 0.74 (*) | 0.74 |
| Glutathione (reduced) | 0.98 | 0.60 (*) | 0.77 (*) | 0.79 |
| Oxoproline | 0.95 | 0.65 (*) | 0.81 | 0.59 |
| Phosphonoacetaldehyde | 0.83 | 0.32 (*) | 0.48 (**) | 0.62 |
| Glycerol 3-phosphate | 0.46 | 1.4 | 1.63 (**) | 1.3 |
| NADH | N/A | 7.6 | 4.1 (*) | 1.0 |
| Carnitine | 0.68 | 0.87 | 0.97 | 1.3 (***) |
| CDP-ethanolamine | 0.70 | 1.5 | 1.0 | 0.83 (*) |
| Platelet-activating factor | 1.4 | 1.2 | 1.1 | 1.2 (**) |

**Table S3. Related to Figure 3. Metabolites significantly changed between *Mx1-cre; Got2$^{fl/fl}$* and +/+ mice** (* *P*< 0.05; ** *P*<0.01; *** *P*<0.001).

| | Fold change (mutant/WT) | | | |
|---|---|---|---|---|
| **Fold change (significance)** | **HSCs/MPPs** | **HPCs** | **LK cells** | **CD45$^+$ cells** |
| Aspartate | 0.62 (*) | 0.90 | 0.58 (*) | 0.61 (**) |
| Acetylcarnitine | 0.87 (*) | 0.76 (**) | 0.80 | 0.79 |
| Cholinephosphate | 0.55 (***) | 0.43 (***) | 0.42 (***) | 0.57 (***) |
| Dimethylaminoethanol phosphate | 0.58 (***) | 0.45 (***) | 0.41 (*) | 0.51 (**) |
| Proline | 1.4 (**) | 1.3 (**) | 0.73 | 1.2 |
| Phosphonoacetaldehyde | 0.44 (*) | 0.22 (***) | 0.17 (**) | 0.15 (**) |
| Carnitine (18:2) | 2.5 | 2.2 (**) | 1.8 | 1.4 (***) |
| Argininosuccinic acid | 1.8 | 2.6 (*) | 1.6 (*) | 1.5 |
| Beta-Alanine | 0.89 | 0.38 (**) | 0.31 (*) | 0.35 (**) |
| Carnitine (14:2) | N/A | 3.6 (***) | 1.3 | 1.1 |
| Carnitine (16:2) | 3.1 | 3.4 (*) | 1.9 | 1.1 |
| Carnitine (18:1) | 1.4 | 1.5 (*) | 1.2 | 1.3 (*) |
| Carnitine (3-hydroxy-C12:0) | 0.59 | 0.67 (*) | 0.94 | 0.82 (*) |
| Dihydrothymidine | 1.0 | 0.18 (**) | 0.39 (**) | 0.22 (**) |
| Fumarate | 1.6 | 0.30 (**) | 0.39 (**) | 1.0 |
| IMP | 1.2 | 2.5 (*) | 2.3 | 0.96 |
| Methylhistidine | 1.9 | 1.7 (**) | 2.9 (**) | 0.95 |
| Phosphotidylcholine (16:0/16:0) or Phosphotidylcholine (18:0/14:0) | 1.3 | 1.6 (**) | 0.87 | 1.4 |
| Phosphotidylcholine (16:0/18:1) | 1.1 | 1.5 (*) | 0.94 (*) | 1.4 (*) |
| Phosphotidylcholine (16:0/18:2) | 1.6 | 2.0 (**) | 1.5 (*) | 1.7 (***) |
| Phosphotidylcholine (18:1/18:1) | 1.3 | 1.6 (**) | 1.3 (**) | 1.4 (*) |
| S-adenosylhomocysteine | 1.1 | 1.3 (**) | 1.2 | 1.2 |
| UDP-Hexose | 0.88 | 0.27 (**) | 0.17 (**) | 1.5 |
| UDP-N-acetyl-hexosamine | 0.76 | 0.19 (**) | 0.16 | 1.4 |
| Deoxy-glucose-phosphate | 2.2 | 1.4 | 2.8 (*) | 1.8 (*) |
| Guanidineaceticacid | 0.77 | 0.83 | 0.23 (***) | 1.1 |
| Lysophosphoethanolamine (16:0) | 0.72 | 1.4 | 1.2 (**) | 1.6 (**) |
| Methylsulfolene | 1.5 | 1.5 | 3.1 (*) | 1.0 |
| Phosphotidylcholine (18:0/18:1) | 1.0 | 1.3 | 1.1 (*) | 1.3 |
| Acetylcholine | 1.2 | 1.1 | 1.1 | 1.3 (**) |
| Aminoacrylate | 0.84 | 0.74 | 0.35 | 0.49 (*) |
| Glutamate | 1.1 | 1.4 | 1.3 | 1.7 (**) |
| Lysophosphoethanolamine (18:1) | 1.5 | 1.8 | 1.5 | 1.8 (*) |

**Table S4. Related to STAR Methods. Primers, sgRNAs, donor oligos and cloning templates used in this study.**

| Primer | Application | Sequences |
|---|---|---|
| Cre F | Genotyping | ATTGCTGTCACTTGGTCGTGGC |
| Cre R | Genotyping | GAAAATGCTTCTGTCCGTTTGC |
| Vav1-iCre F | Genotyping | AGATGCCAGGACATCAGGAACC |
| Vav1-iCre R | Genotyping | ATCAGCCACACCAGACACAGAG |
| Glast KI F | Genotyping | AGAGATTGCAGCAAGGGGTC |
| Glast KI R | Genotyping | ATAGACTACAGCGCGCATCC |
| R26F3 | Genotyping | CTGCCCGAGCGGAAACGCCACTGAC |
| SAR | Genotyping | CCTGGACTACTGCGCCCTACAGA |
| Rosa WT F | Genotyping | GGAGTGTTGCAATACCTTTCTGGGAGTTC |
| Rosa WT R | Genotyping | TGTCCCTCCAATTTTACACCTGTTCAATTC |
| ASNase KI F | Genotyping | ATGTCGCTCTGCTGAGACTG |
| ASNase KI R | Genotyping | CCGGCTAAAGATGTGGCGTA |
| Got1 5'loxp F | Genotyping | CCGAGATAGGTGGGAGTCAA |
| Got1 3'loxp F | Genotyping | GGTGAGCCTGCTAGCCATAG |
| Got1 3'loxp R | Genotyping | TGTCCACTGGGCGTCGTAGA |
| Got2 5'loxp F | Genotyping | GAGCTAGGGAGAGAACATGCA |
| Got2 3'loxp F | Genotyping | TCTCCTGCCAGCTAGGATGT |
| Got2 3'loxp R | Genotyping | CAACAATAGCTTATCAATCACCGAATACA |
| Asns flox A | Genotyping | GAACTTCGGAATAGGAACTTCG |
| Asns flox B | Genotyping | ACAAGGGTCAGGCATCAGAG |
| Asns flox C | Genotyping | GCATTTAAGTGCACAGGAGGA |
| Nat8l flox A | Genotyping | TCCCTGTGTCCCATGCCCGT |
| Nat8l flox B | Genotyping | TCCCAGCCCTTGGTCTGCCA |
| Nat8l flox C | Genotyping | TGGGGCAAGTGTGGAGGGTGG |
| Slc1a3_qPCR_F | qPCR | TCATCTCCAGTCTCGTCACA |
| Slc1a3_qPCR_R | qPCR | CACCACAGCAATGATGGTAGTA |
| Got1_qPCR_F | qPCR | GAGCGTACCGCACAGATGAAT |
| Got1_qPCR_R | qPCR | GGCTGTTGTCGTTAGCAATCTT |
| Got2_qPCR_F | qPCR | GGACCTCCAGATCCCATCCT |
| Got2_qPCR_R | qPCR | GGTTTTCCGTTATCATCCCGGTA |
| Actb_qPCR_F | qPCR | GCTCTTTTCCAGCCTTCCTT |
| Actb_qPCR_R | qPCR | CTTCTGCATCCTGTCAGCAA |
| sgRosa26-1 | sgRNA | ACTCCAGTCTTTCTAGAAGA |
| Got1 5'sgRNA | sgRNA | ATAATAGGATCGTCCGGCAA |
| Got1 3'sgRNA | sgRNA | GCTACTGCTCCAATTTCAAC |
| Got2 5'sgRNA | sgRNA | AAGGCGTGGCTCATATACAT |
| Got2 3'sgRNA | sgRNA | TAATCCTGAGGGGGCACAAG |

| | | |
|---|---|---|
| Got1 exon3 donor | Donor oligo | AACTCCAGACTTGGAGCCTACGACTGGGCAGTCTGC<br>CTCTTATATCCACCATCATGGAACCCTAAGGATAGAA<br>TACAACGGAGTCTTGGGCAAAGGTGCTCCCAGTTATA<br>ACTTCGTATAATGTATGCTATACGAAGTTATGGATCC<br>GAAATTGGAGCAGTAGCTTTCTCTGGCCGCAGCTAG<br>AAGCCAGTGCTCAATGACTCCCCAAAACGCACCCTC<br>CCAGGGATAGGTTACATCTTCCACGCAAGTCACCAAG<br>TGTCACAAGGACAGCCCTCCCAGATGCTTGGCTCGC<br>CCCCTCTATGGCTAGCAGGCTCACCCCAGGTTGGTG<br>ATGATACGTAGATTGGTGTGTTCTTGTTATCTGTACCA<br>TTGTACCATCGCCCTAAGAAGTCAGCTCCAATCCGAA<br>GAGCGCCTGTCCCTCCCAAAGACTGCACCCCTCCAA<br>CCTGAAAGAGAAGATTTCGGGTCACAGGTTGTTGACA<br>ATGGTGAGATCGGCTAATATTCGAAAGAGTAAGACAA<br>TCATGGTAACATACTTTGTCCTCTTTCTGTCTAAAGCC<br>CCTTAAGTGTATGAGTTCCTCAAACCATTGATAACTTC<br>GTATAATGTATGCTATACGAAGTTATGGATCCCCGGA<br>CGATCCTATTATGTGGGTAAACCGAGAGACAGGAAG<br>GCAAGAAGAGACGAAGTCCCTGCTCAGTCACCAGTG<br>AGCAGGACAGTCAGGTCTCCTCTGACTCCAGGAGGA<br>ACCT |
| Got2 exon2 donor | Donor oligo | TGTTTCTGGGTCCGAGTTACCCCACTCATAACACTTA<br>CTGGTGATGTCCGCTTACCTGAAACTTTAATTTTCACT<br>TTTCTTTACAGCTGGATAACGTGCCCCGATGGGATCC<br>ATAACTTCGTATAATGTATGCTATACGAAGTTATTATA<br>TGAGCCACGCCTTCATTATTCATTAATAAGCTGAAGG<br>GAAAAAAATGTTTTAAAGCTGTGGTTGTACCCTATCCT<br>ACCCAAAATAACTTGGTGCTATCTTTCTGCATCTTTTC<br>ATGTTTGTGCTGATCTCCAGTCTTCTGAAGGCTTTTGT<br>GCTATTGGATAGCTGGTGCCTGTTCTGTGATACACAC<br>ATGCCAACATGGATCTTTGGCAGAGCAGAGACATACC<br>TGGCTGTAGGAATCGAGCTCATGGCTGGGGTAACCT<br>ATTTCTCATTTCAGCTCCTGGTGGACCCATGTTGAAA<br>TGGGACCTCCAGATCCCATCCTGGGCGTTACCGAAG<br>CCTTCAAGAGAGATACCAACAGCAAGAAGATGAACCT<br>GGGAGTTGGTGCCTACCGGGATGATAACGGAAAACC<br>TTACGTGCTCCCCAGTGTCCGGAAGGTGAGCTTGGC<br>ACTCGTCTCCTGCCAGCTAGGATGTGGAACCTGAGC<br>CAAAAGGAGTGCTGGACCAGGACTCCGAGACCCCGC<br>CCCCCTAGGTCCTGGCTCTGGCTTTGTGAATCACCTG<br>GCTTTTCTAGACCATGATTTTCCTTCCCTGAGAAAGC<br>AGATTAAAACCACAAGGCCTGTAAGATTATAAGTCAG<br>GTGTGGTGCCTAATCCTGAGGGGGCACATAACTTCG<br>TATAATGTATGCTATACGAAGTTATGGATCCAAGGGG<br>ATGACAATTTTAACATCCTCTCTTAAGGAGAGAAATAG<br>CCAGCTGCGGGTGCAGCTACTCTTAGTTCCATTTCTC<br>TGGTGATTGCTGTGGTGAAATCCTC |

| | | |
|---|---|---|
| Guinea pig asparaginase coding sequence | Cloning template | ATGGCTAGAGCCAGCGGATCCGAGAGGCATTTACTG<br>CTGATCTACACCGGAGGCACTTTAGGCATGCAGTCC<br>AAGGGAGGAGTGCTGGTGCCCGGTCCCGGTCTGGT<br>GACACTGCTGAGGACCCTCCCTATGTTCCACGACAA<br>GGAGTTCGCCCAAGCTCAAGGTCTGCCCGATCATGC<br>TCTCGCCCTCCCTCCCGCTTCCCATGGACCTAGGGT<br>GCTGTATACCGTGCTGGAGTGCCAGCCTCTGCTGGA<br>TTCCAGCGACATGACCATCGATGACTGGATTAGGATC<br>GCCAAGATCATCGAGAGGCACTACGAGCAGTACCAA<br>GGTTTCGTGGTCATTCACGGCACAGACACCATGGCC<br>AGCGGAGCTAGCATGCTGAGCTTCATGCTGGAGAAC<br>CTCCACAAGCCCGTGATTTTAACTGGTGCTCAAGTCC<br>CCATTCGTGTGCTCTGGAATGACGCTCGTGAGAATTT<br>ACTGGGAGCTTTACTCGTGGCTGGACAGTACATCATC<br>CCCGAGGTGTGTCTCTTCATGAACTCCCAGCTGTTTA<br>GGGGCAATCGTGTGACCAAGGTGGATTCCCAGAAGT<br>TCGAAGCCTTTTGCAGCCCCAATCTGAGCCCTTTAGC<br>TACCGTGGGAGCCGACGTGACCATTGCTTGGGATTT<br>AGTGAGGAAGGTGAAGTGGAAGGATCCTCTGGTGGT<br>GCATAGCAACATGGAACACGATGTCGCTCTGCTGAG<br>ACTGTATCCCGGCATCCCCGCCTCTTTAGTGAGAGC<br>CTTTCTGCAGCCTCCCCTCAAGGGAGTGGTGCTGGA<br>GACATTCGGCAGCGGAAACGGCCCCTCCAAACCCGA<br>TTTACTGCAAGAACTCAGAGCTGCCGCCCAGAGGGG<br>ACTGATCATGGTGAACTGTAGCCAGTGCCTCAGAGG<br>CTCCGTCACCCCCGGTTACGCCACATCTTTAGCCGG<br>AGCTAACATCGTGAGCGGTTTAGACATGACAAGCGAA<br>GCCGCTTTAGCTAAGCTGAGCTATGTGCTCGGCCTC<br>CCCGAGCTCTCCCTCGAAAGGAGGCAAGAACTGCTC<br>GCTAAGGATTTAAGGGGCGAGATGACCCTCCCTACC<br>GCCGATTTACATCAGAGCTCCCCTCCCGGTAGCACA<br>CTGGGACAAGGTGTGGCTCGTCTGTTCTCCCTCTTTG<br>GCTGTCAAGAAGAGGACTCCGTGCAAGATGCTGTGA<br>TGCCTTCCCTCGCTTTAGCTCTGGCCCATGCTGGCG<br>AACTGGAAGCTTTACAAGCTTTAATGGAGCTCGGATC<br>CGATTTAAGGCTCAAGGACTCCAACGGCCAGACTTTA<br>CTCCATGTGGCCGCCAGAAACGGCAGAGACGGAGTG<br>GTCACAATGCTGCTGCATAGGGGCATGGACGTCAAC<br>GCTAGAGATAGGGACGGTTTAAGCCCTCTGCTGCTG<br>GCTGTGCAAGGTAGGCACAGAGAGTGCATTCGTCTG<br>TTACGTAAGGCCGGCGCTTGTCTGAGCCCTCAAGATT<br>TAAAGGACGCCGGCACAGAGCTGTGCAGACTGGCTT<br>CTCGTGCCGACATGGAAGGCCTCCAAGCTTGGGGAC<br>AAGCCGGCGCCGATCTGCAGCAACCCGGATACGACG<br>GAAGATCCGCTTTATGCGTGGCTGAGGCTGCTGGCA<br>ACCAAGAAGTGCTGGCTCTGCTGAGAAATCTGGCTTT<br>AGTGGGCCCCGAAGTGCCTCCCGCTATTTGA |