Supplemental Information

Inventory of Supplemental Information

Supplementary Table 1: Antibodies used for flow-cytometry, related to Figure 1

Antibody	Clone	Conjugated	Company
CD2	RM2-5	Biotin	eBioscience
CD3ε	145-2C11	PE	BioLegend
CD3ε	145-2C11	Biotin	eBioscience
CD4	GK1.5	PE	BioLegend
CD5	53-7.3	Biotin	eBioscience
CD8α	53-6.7	Biotin	eBioscience
Mac-1/CD11b	M1/70	APC-eFluor 780	eBioscience
Fcγr/CD16/32	93	Alexa Fluor 700	eBioscience
CD34	RAM34	FITC	eBioscience
CD34	RAM34	Alexa Fluor 647	eBioscience
CD41	MWReg30	PE	BioLegend
CD41	eBioMWReg30	PE-Cy7	eBioscience
CD41	eBioMWReg30	Biotin	eBioscience
CD45.1	A20	PE-Cy7	BioLegend
CD45.1	A20	APC-eFluor 780	eBioscience
CD45.2	104	Pacific Blue	BioLegend
B220/CD45R	RA3-6B2	APC	BioLegend
B220/CD45R	RA3-6B2	Biotin	eBioscience
CD48/Slamf2	HM48-1	FITC	BioLegend
CD48/Slamf2	HM48-1	PerCP-Cy5.5	BioLegend
CD48/Slamf2	HM48-1	Alexa Fluor 700	eBioscience
CD48/Slamf2	HM48-1	Pacific Blue	BioLegend
CD49b	HMa2	FITC	eBioscience
CD61	2C9.G2	PE	BD Pharmingen
CD71	R17217	Biotin	eBioscience
CD84/Slamf5	mCD84.7	PE	BioLegend
Thy1.1/CD90.1	OX-7	FITC	BioLegend
Thy1.1/CD90.1	OX-7	APC	BioLegend
Thy1.2/CD90.2	53-2.1	APC	eBioscience
c-Kit/CD117	2B8	APC-eFluor 780	eBioscience
IL-7Rα/CD127	A7R34	PE	eBioscience
Flt3/CD135	A2F10	PE-Cy5	eBioscience

CD150/Slamf1	TC15-12F12.2	PE-Cy7	BioLegend
EPCR/CD201	eBiomRCR-16	Alexa Fluor 647	eBioscience
CD229/Slamf3	Ly9ab3	PE	BioLegend
CD229/Slamf3	Ly9ab3	APC	eBioscience
CD244.2/Slamf4	2B4	FITC	BD Pharmingen
CD244.2/Slamf4	2B4	PE	BD Pharmingen
Sca-1/Ly-6A/E	D7	PerCP-Cy5.5	eBioscience
Sca-1/Ly-6A/E	E13-161.7	PE-Cy7	BioLegend
Sca-1/Ly-6A/E	E13-161.7	APC	BioLegend
Sca-1/Ly-6A/E	D7	Alexa Fluor 700	eBioscience
Gr-1/Ly-6G	RB6-8C5	PE-Cy7	BioLegend
Gr-1/Ly-6G	RB6-8C5	Pacific Orange	Invitrogen
Gr-1/Ly-6G	RB6-8C5	Biotin	eBioscience
Ter119/Ly-76	TER-119	APC	eBioscience
Ter119/Ly-76	TER-119	Biotin	eBioscience
Ly-108/Slamf6	330-AJ	PE	BioLegend
Ly-108/Slamf6	eBio13G3-19D	PE	eBioscience
ESAM	1G8	PE	eBioscience
Streptavidin		PE	Invitrogen
Streptavidin		PE-Texas Red	Invitrogen
Streptavidin		APC	Invitrogen
Streptavidin		APC-Cy7	Invitrogen

Figure S1

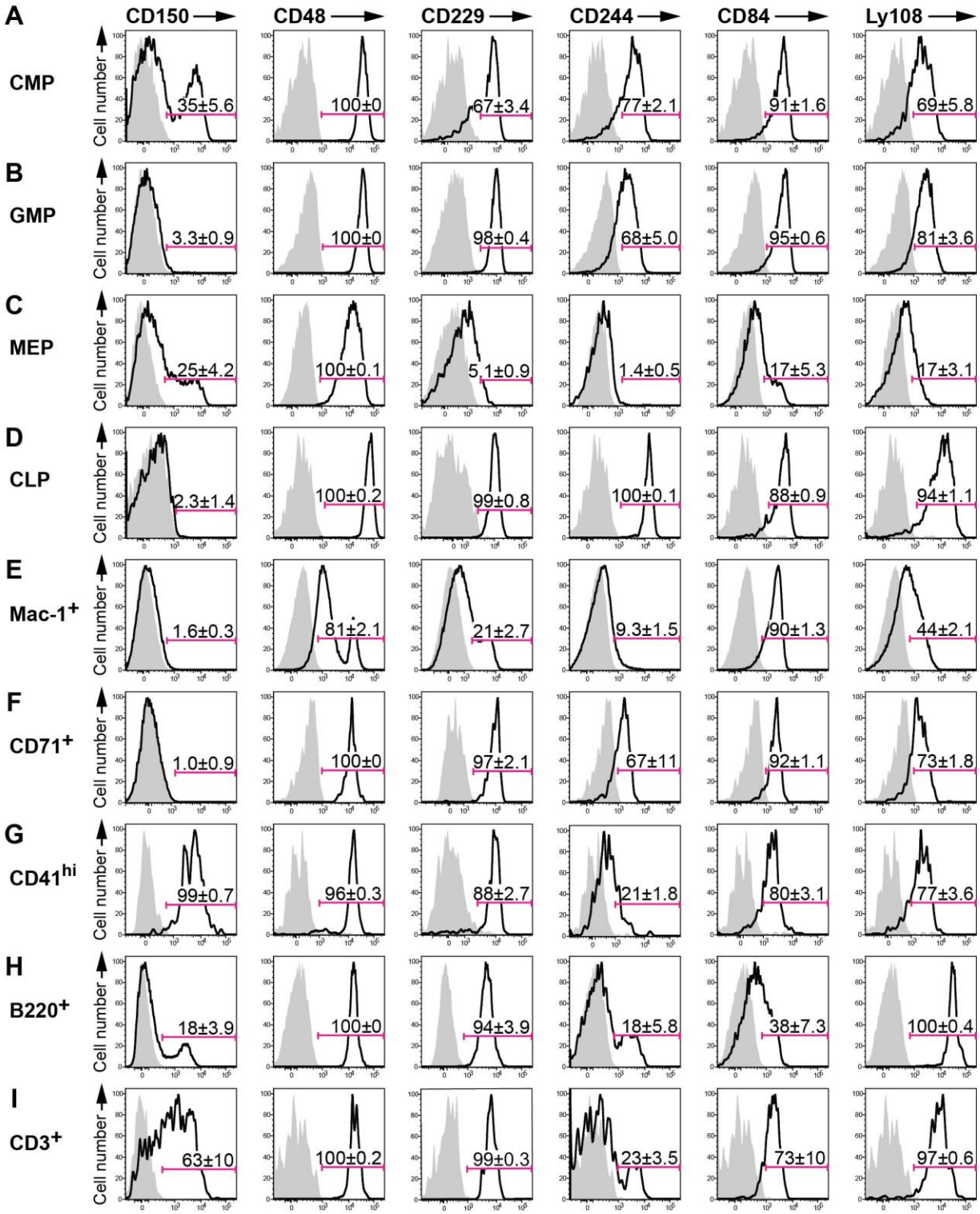

A
CMP
B
GMP
C
MEP
D
CLP
E
Mac-1+
F
CD71+
G
CD41hi
H
B220+
I
CD3+
CD150
CD48
CD229
CD244
CD84
Ly108
Cell number
35±5.6
100±0
67±3.4
77±2.1
91±1.6
69±5.8
3.3±0.9
100±0
98±0.4
68±5.0
95±0.6
81±3.6
25±4.2
100±0.1
5.1±0.9
1.4±0.5
17±5.3
17±3.1
2.3±1.4
100±0.2
99±0.8
100±0.1
88±0.9
94±1.1
1.6±0.3
81±2.1
21±2.7
9.3±1.5
90±1.3
44±2.1
1.0±0.9
100±0
97±2.1
67±11
92±1.1
73±1.8
99±0.7
96±0.3
88±2.7
21±1.8
80±3.1
77±3.6
18±3.9
100±0
94±3.9
18±5.8
38±7.3
100±0.4
63±10
100±0.2
99±0.3
23±3.5
73±10
97±0.6

Figure S1. Expression of SLAM family markers on restricted progenitor and differentiated cell fractions, related to Figure 1

Cell-surface expression of SLAM family markers on restricted progenitor and differentiated cell fractions from the bone marrow. Solid lines indicate staining with antibodies against the indicated SLAM family markers, and shaded areas indicate background fluorescence in control cells stained with all antibodies except the antibody against the indicated SLAM family marker. Data are shown as mean ± S.D. for the percentage of cells that stained above background in 3 independent experiments. The sources of all antibodies are listed in Supplementary Table 1.

Figure S2

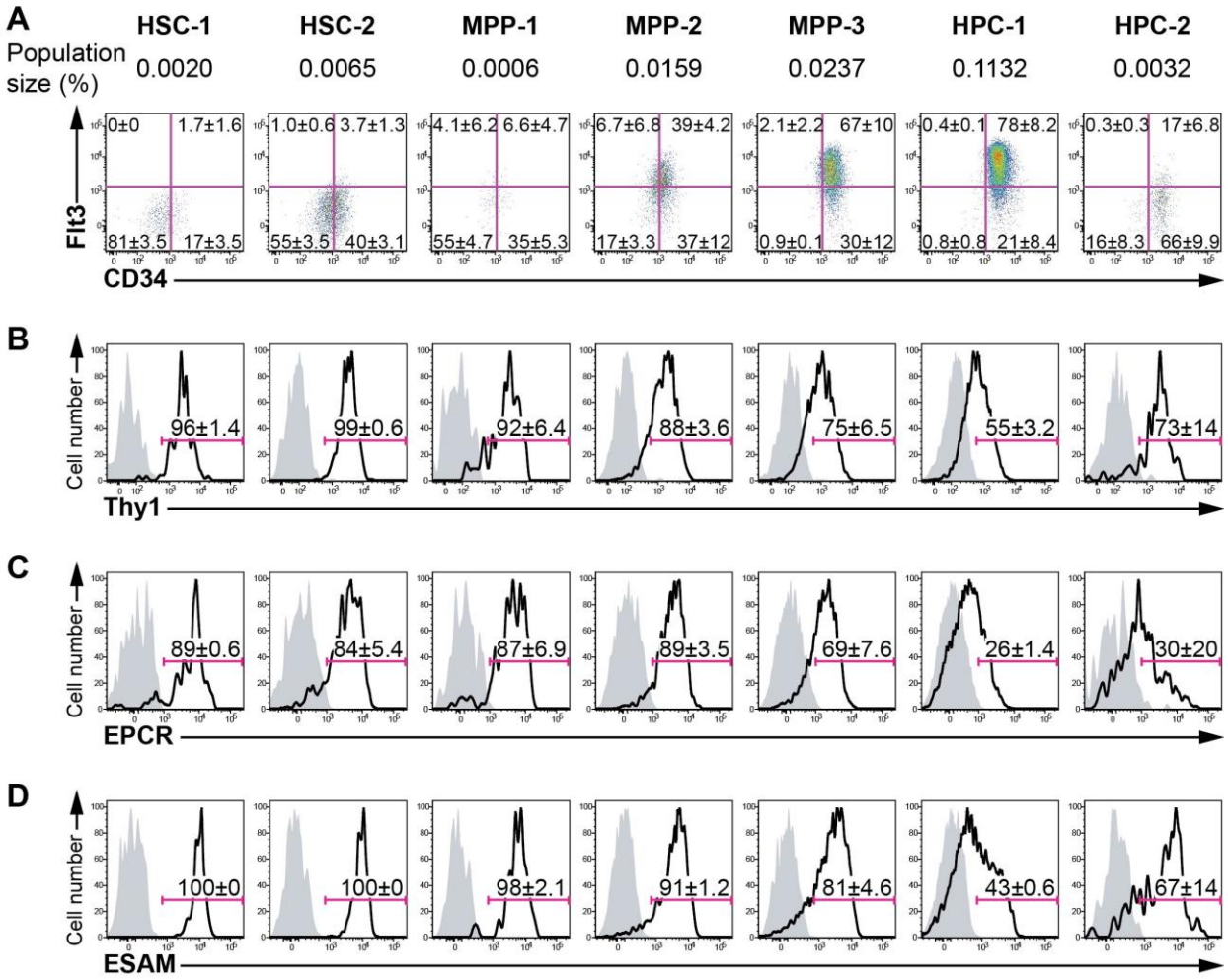

A
HSC-1
HSC-2
MPP-1
MPP-2
MPP-3
HPC-1
HPC-2
Population size (%)
0.0020
0.0065
0.0006
0.0159
0.0237
0.1132
0.0032
0±0
1.7±1.6
81±3.5
17±3.5
1.0±0.6
3.7±1.3
55±3.5
40±3.1
4.1±6.2
6.6±4.7
55±4.7
35±5.3
6.7±6.8
39±4.2
17±3.3
37±12
2.1±2.2
67±10
0.9±0.1
30±12
0.4±0.1
78±8.2
0.8±0.8
21±8.4
0.3±0.3
17±6.8
16±8.3
66±9.9
Flt3
CD34
B
Cell number
96±1.4
99±0.6
92±6.4
88±3.6
75±6.5
55±3.2
73±14
Thy1
C
Cell number
89±0.6
84±5.4
87±6.9
89±3.5
69±7.6
26±1.4
30±20
EPCR
D
Cell number
100±0
100±0
98±2.1
91±1.2
81±4.6
43±0.6
67±14
ESAM

Figure S2. Expression of HSC markers by stem/progenitor cell populations distinguished using SLAM family markers, related to Figure 1

(A-D) Cell-surface expression of CD34 and Flt3 (A), Thy1 (B), EPCR (C), and ESAM (D) on HSC-1, HSC-2, MPP-1, MPP-2, MPP-3, HPC-1, and HPC-2 cells. Solid lines indicate staining with antibodies against indicated the antigens and shaded areas indicate background fluorescence in control cells stained with all antibodies except the antibody against the indicated marker (B-D). The expression level of Thy1.1 on the cells in (B) was lower than from T cells in the same samples (data not shown). Data represent mean ± S.D. from at least 3 independent experiments with 1 mouse per experiment.

Figure S3

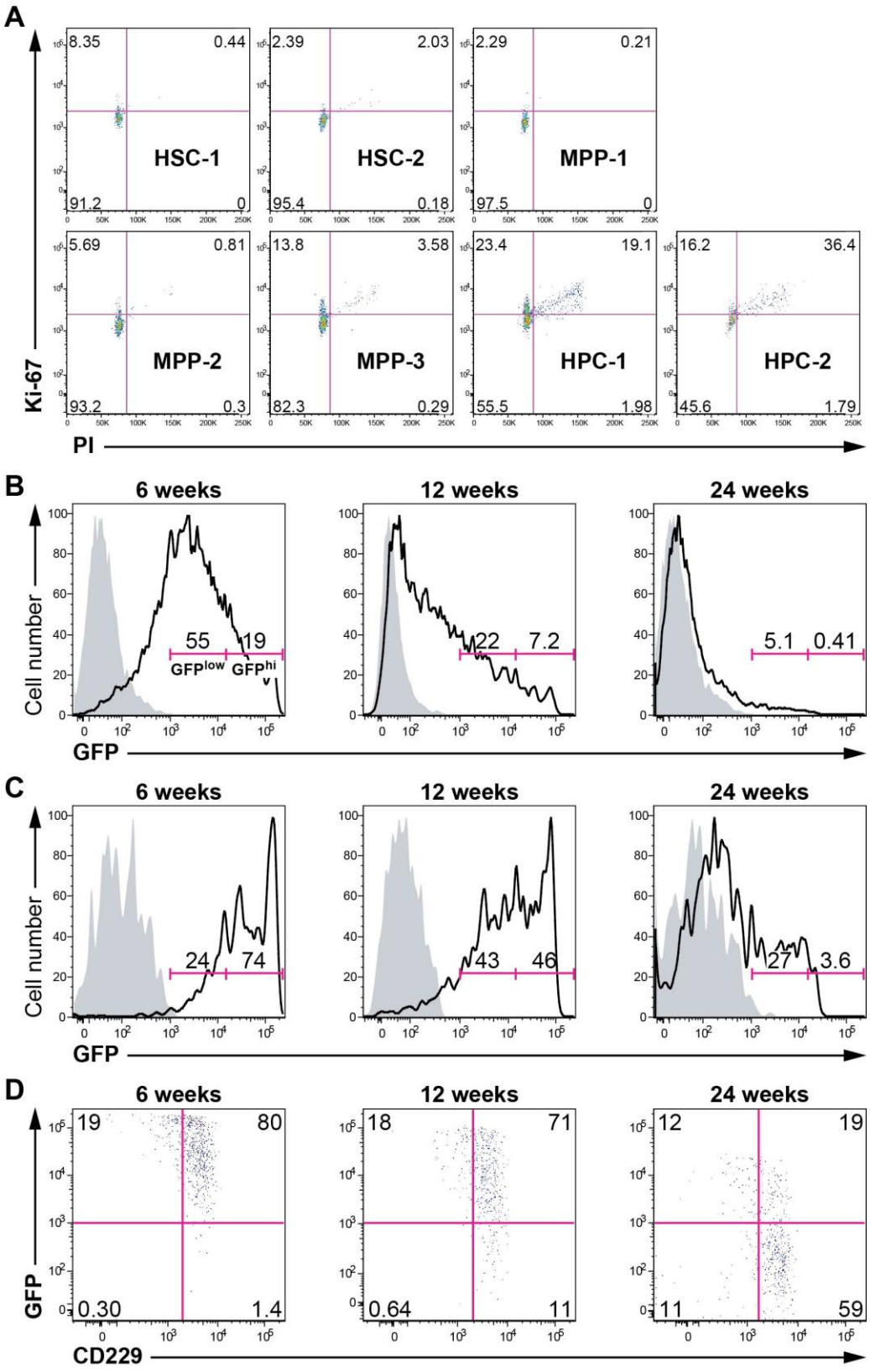

A
8.35
0.44
HSC-1
91.2
0
2.39
2.03
HSC-2
95.4
0.18
2.29
0.21
MPP-1
97.5
0
5.69
0.81
MPP-2
93.2
0.3
13.8
3.58
MPP-3
82.3
0.29
23.4
19.1
HPC-1
55.5
1.98
16.2
36.4
HPC-2
45.6
1.79
Ki-67
PI
B
6 weeks
12 weeks
24 weeks
Cell number
55
19
GFPlow
GFPhi
22
7.2
5.1
0.41
GFP
C
6 weeks
12 weeks
24 weeks
Cell number
24
74
43
46
27
3.6
GFP
D
6 weeks
12 weeks
24 weeks
19
80
0.30
1.4
18
71
0.64
11
12
19
11
59
GFP
CD229

Figure S3. Retention of H2B-GFP after the removal of doxycycline, related to Figure 2

(A) Representative plots showing Ki-67/PI staining for cell cycle analysis.

(B and C) Representative histograms of H2B-GFP fluorescence intensity (solid line) in LSK cells (B) and $CD150^{+}CD48^{-/low}$LSK cells (C) after chasing for 6, 12, and 24 weeks without doxycycline. The gates used to distinguish the GFP^{hi} and GFP^{lo} fractions are shown in each histogram. The shaded area indicates background fluorescence in control cells stained with all antibodies but negative for H2B-GFP (*Col1A1-H2B-GFP; Rosa26-M2-rtTA* mice without doxycycline treatment).

(D) GFP versus CD229 staining for $CD150^{+}CD48^{-}$LSK cells after chasing for 6, 12, and 24 weeks without doxycycline. $CD229^{-/low}$ HSCs retained more GFP^{+} cells than $CD229^{+}$ HSCs.

Figure S4

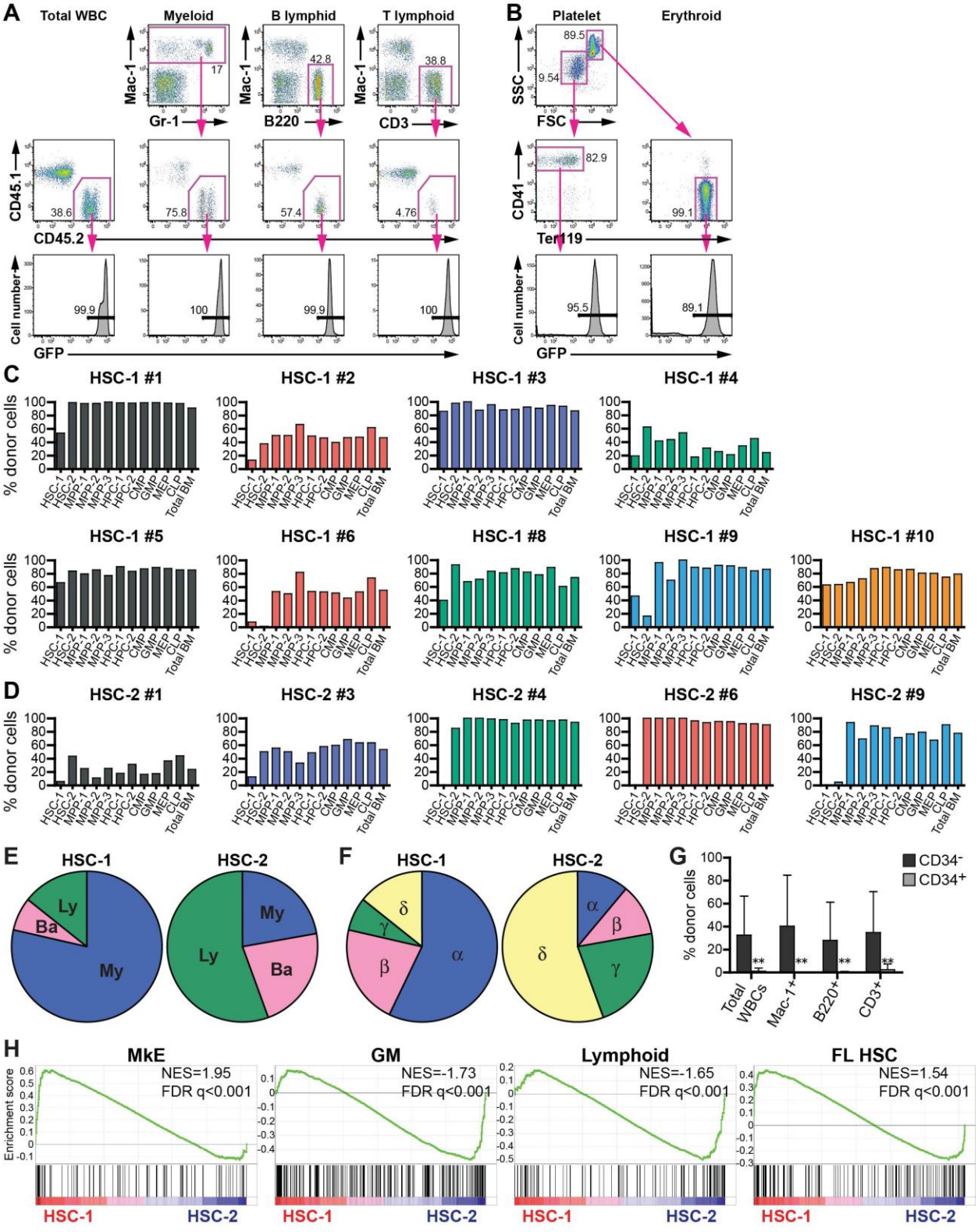

A
Total WBC
Myeloid
B lymphid
T lymphoid
Mac-1
Gr-1
B220
CD3
17
42.8
38.8
CD45.1
CD45.2
38.6
75.8
57.4
4.76
Cell number
GFP
99.9
100
99.9
100
B
Platelet
Erythroid
SSC
FSC
89.5
9.54
CD41
Ter119
82.9
99.1
95.5
89.1
C
HSC-1 #1
HSC-1 #2
HSC-1 #3
HSC-1 #4
HSC-1 #5
HSC-1 #6
HSC-1 #8
HSC-1 #9
HSC-1 #10
% donor cells
HSC-1 HSC-2 MPP-1 MPP-2 MPP-3 HPC-1 HPC-2 CMP GMP MEP CLP Total BM
D
HSC-2 #1
HSC-2 #3
HSC-2 #4
HSC-2 #6
HSC-2 #9
E
HSC-1
HSC-2
Ly
Ba
My
F
δ
γ
β
α
G
CD34⁻
CD34⁺
Total WBCs
Mac-1⁺
B220⁺
CD3⁺
H
MkE
GM
Lymphoid
FL HSC
Enrichment score
NES=1.95
FDR q<0.001
NES=-1.73
FDR q<0.001
NES=-1.65
FDR q<0.001
NES=1.54
FDR q<0.001
HSC-1
HSC-2

Figure S4. Donor-derived hematopoietic stem/progenitor cell populations in the bone marrow of primary recipient mice reconstituted by HSC-1 or HSC-2 cells, related to Fig. 3

(A and B) Gating strategy for flow cytometric analysis of white blood cells (A) and platelets/erythrocytes (B). Doublets, dead cells, and $CD45^-$ cells were excluded prior to analysis.

(C and D) Frequencies of donor-derived hematopoietic stem/progenitor cell populations in the bone marrow of individual recipient mice that were long-term reconstituted by 5 HSC-1 (C) or 5 HSC-2 (D) cells at 17 to 25 weeks after transplantation.

(E and F) Classification of lineage-biased differentiation patterns of single HSC-1 and HSC-2 cells in Figure 3F and 3G using the criteria described by (Muller-Sieburg et al., 2002) (E) or (Dykstra et al., 2007) (F). My, myeloid-biased HSCs; Ba, balanced HSCs; Ly, lymphoid-biased HSCs.

(G) Five $CD45.1^+$ $CD34^{-/low}$ HSC-2 or $CD34^+$ HSC-2 donor cells were transplanted into irradiated $CD45.1^+CD45.2^+$ recipient mice along with 200,000 $CD45.2^+$ competitor bone marrow cells. Data represent mean ± S.D. of the frequency of donor-derived cells in the peripheral blood of recipients at 16 weeks after transplantation from one experiment with 12 recipients for each population. **p<0.01 by student *t*-test.

(H) Gene set enrichment analysis comparing HSC-1 cells versus HSC-2 cells for megakaryocyte/erythrocyte lineage (MkE), granulocyte/macrophage lineage (GM), lymphoid lineage, and fetal liver HSC gene sets. NES, Normalized enrichment score; FDR, False discovery rate.

Figure S5

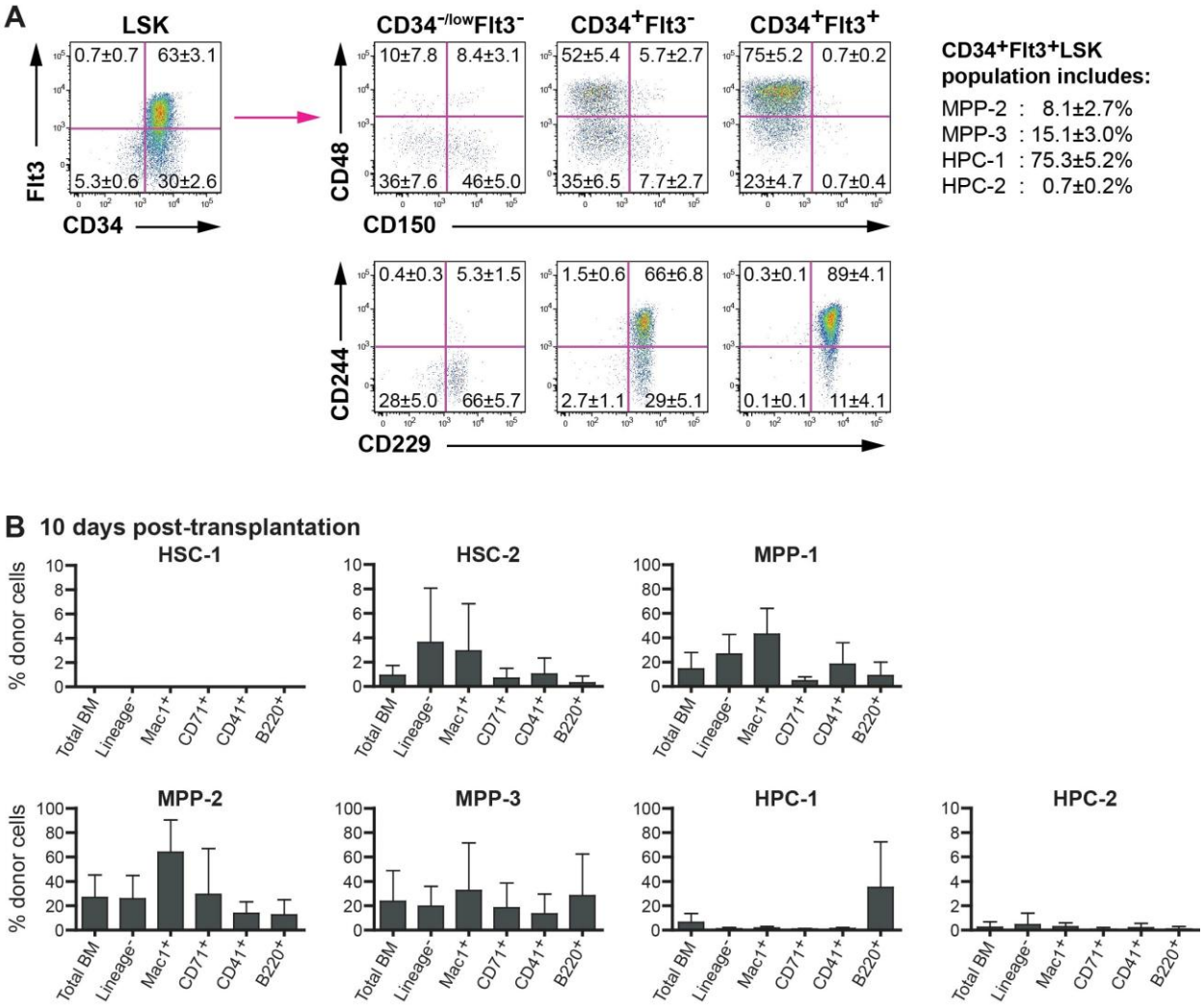

A
LSK
CD34-/lowFlt3-
CD34+Flt3-
CD34+Flt3+
0.7±0.7
63±3.1
5.3±0.6
30±2.6
Flt3
CD34
10±7.8
8.4±3.1
36±7.6
46±5.0
52±5.4
5.7±2.7
35±6.5
7.7±2.7
75±5.2
0.7±0.2
23±4.7
0.7±0.4
CD48
CD150
0.4±0.3
5.3±1.5
28±5.0
66±5.7
1.5±0.6
66±6.8
2.7±1.1
29±5.1
0.3±0.1
89±4.1
0.1±0.1
11±4.1
CD244
CD229
CD34+Flt3+LSK
population includes:
MPP-2 : 8.1±2.7%
MPP-3 : 15.1±3.0%
HPC-1 : 75.3±5.2%
HPC-2 : 0.7±0.2%
B 10 days post-transplantation
HSC-1
HSC-2
MPP-1
MPP-2
MPP-3
HPC-1
HPC-2
% donor cells
Total BM
Lineage-
Mac1+
CD71+
CD41+
B220+

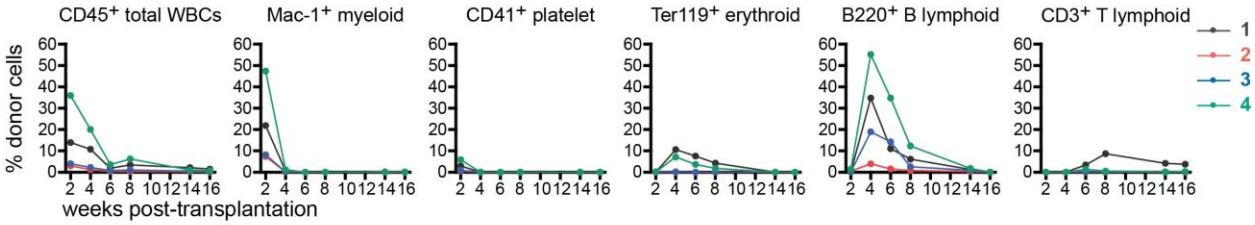
Figure S5. The Flt3$^+$LSK population is heterogeneous, including MPP-2, MPP-3, HPC-1, and HPC-2 cells, related to Figure 6.

(A) CD150, CD48, CD229, and CD244 expression in CD34$^{-/low}$Flt3$^-$LSK, CD34$^+$Flt3$^-$LSK, and CD34$^+$Flt3$^+$LSK cell populations in young adult bone marrow. Data represent mean ± S.D. from 3 independent experiments with 1 mouse per experiment.

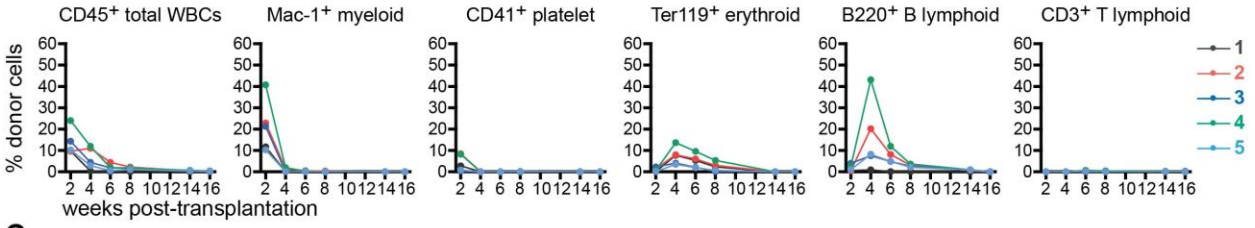
(B) Thirty GFP$^+$CD45.2$^+$ donor cells from each HSC, MPP, and HPC fraction were transplanted into irradiated CD45.1$^+$ recipient mice. The percentages of donor-derived cells in the indicated bone marrow fractions were analyzed 10 days after transplantation. Data reflect mean ± S.D. from 3 to 4 independent experiments with one recipient mouse per experiment. HSC-1 cells did not give rise to detectable progeny within 10 days after transplantation. HSC-2 cells reconstituted 2 of 4 recipient mice with more than 1% donor cells in multiple bone marrow fractions. MPP-1, MPP-2, and MPP-3 cells reconstituted nearly all recipient mice with more than 1% donor-derived cells in multiple bone marrow fractions. HPC-1 and HPC-2 cells each reconstituted 1 to 3 out of 4 recipient mice with more than 1% of donor-derived cells in certain bone marrow cell fractions.

Figure S6

A Flt3$^+$MPP-2 (Flt3$^+$CD150$^-$CD48$^{-/low}$CD229$^+$CD244$^-$LSK) primary transplantation (25 cells)



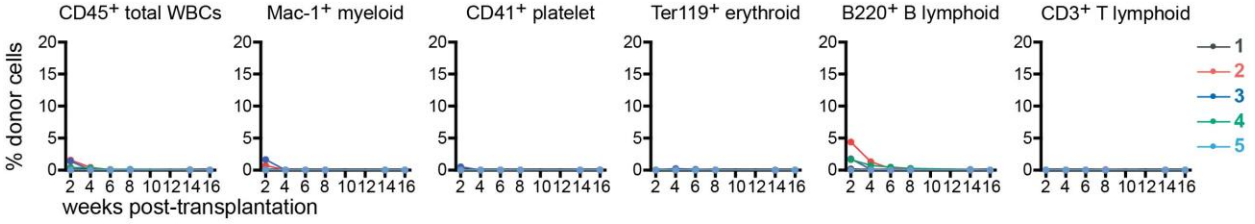
B Flt3$^+$MPP-3 (Flt3$^+$CD150$^-$CD48$^{-/low}$CD229$^+$CD244$^+$LSK) primary transplantation (25 cells)



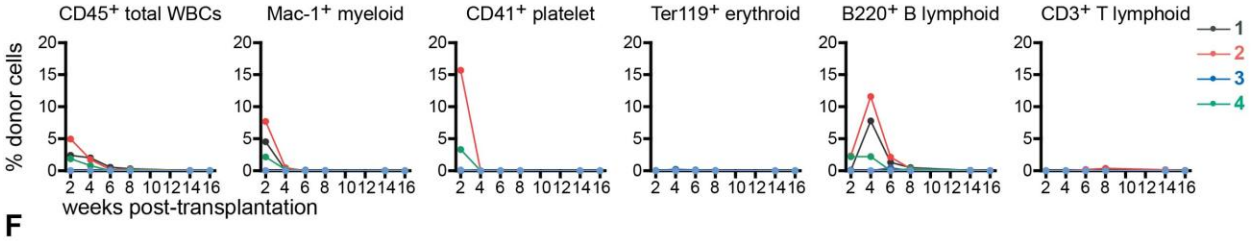
C

Fraction	Cell dose	# total mice	Long-term	Transient					no recon-stitution	Platelet potential	Erythroid potential
				M+B+T	M+B	B+T	M	B			
MPP-2	25	4	0 (0%)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)
MPP-3	25	5	0 (0%)	0 (0%)	4 (80%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	3 (60%)	5 (100%)

D Flt3$^+$HPC-1 (Flt3$^+$CD150$^-$CD48$^+$LSK) primary transplantation (25 cells)



E Flt3$^+$HPC-2 (Flt3$^+$CD150$^+$CD48$^+$LSK) primary transplantation (25 cells)



F

Fraction	Cell dose	# total mice	Long-term	Transient					no recon-stitution	Platelet potential	Erythroid potential
				M+B+T	M+B	B+T	M	B			
HPC-1	25	5	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	2 (40%)	2 (20%)	0 (0%)	0 (0%)
HPC-2	25	5	0 (0%)	0 (0%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	2 (40%)	2 (40%)	2 (40%)

Figure S6 Flt3$^+$ MPP-2, Flt3$^+$ MPP-3, and Flt3$^+$ HPC-2 cells were able to make erythrocytes and platelets while Flt3$^+$ HPC-1 cells were not, related to Figure 6.

(A and B) Twenty-five GFP$^+$CD45.2$^+$ Flt3$^+$MPP-2 (A) or Flt3$^+$MPP-3 (B) cells were transplanted into irradiated CD45.1$^+$ recipient mice along with 200,000 CD45.1$^+$ recipient bone marrow cells. Each line represents the frequency of donor-derived blood cells in a single recipient.

(C) Summary of the reconstitution results from Figures S6A and S6B.

(D and E) Twenty-five GFP$^+$CD45.2$^+$ Flt3$^+$HPC-1 (D) or Flt3$^+$HPC-2 (E) donor cells were transplanted into irradiated CD45.1$^+$ recipient mice along with 200,000 CD45.1$^+$ recipient bone marrow cells. Each line represents the frequency of donor-derived blood cells in one recipient.

(F) Summary of the reconstitution results from Figures S6D and S6E.

Figure S7

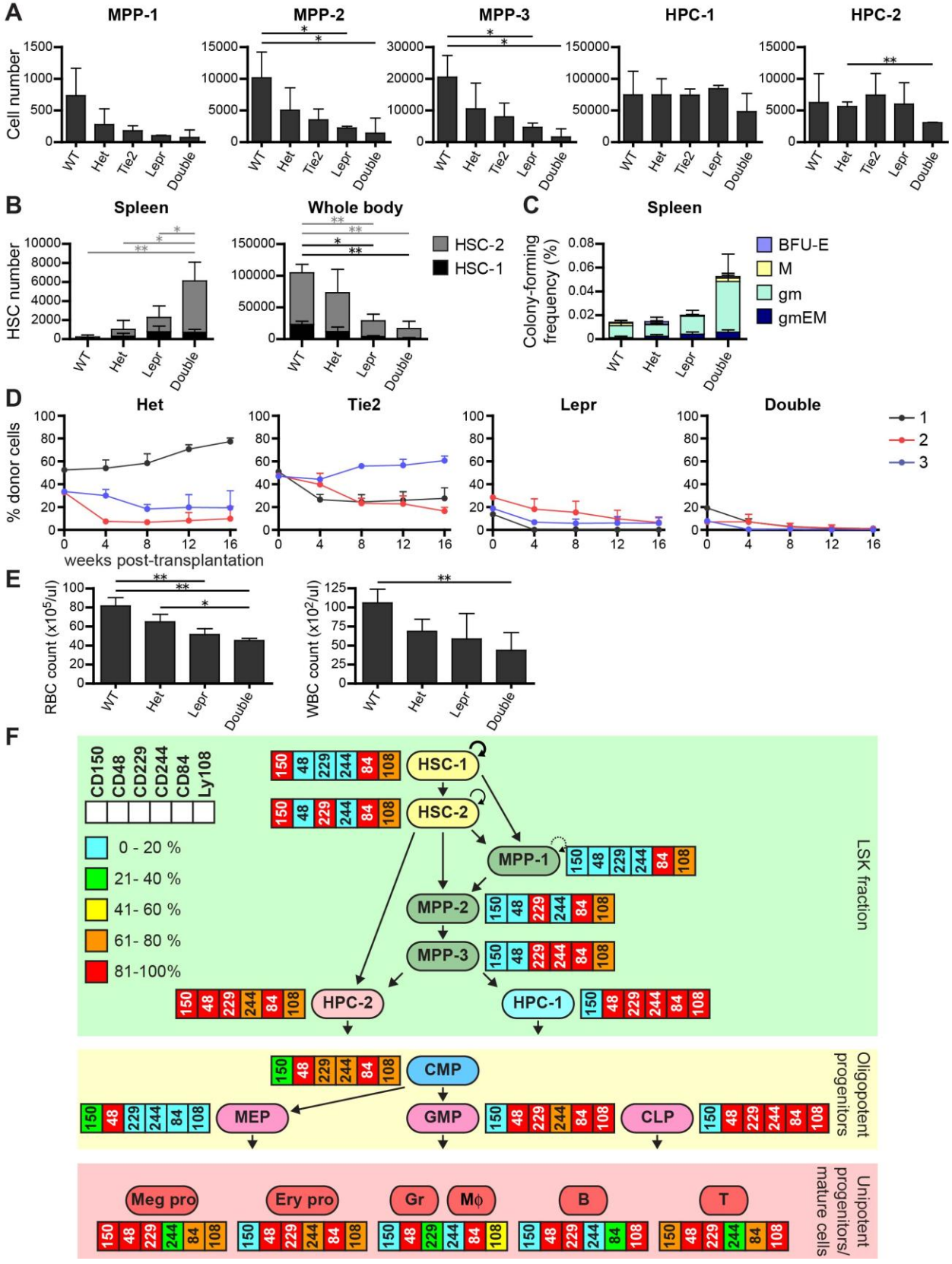

A
MPP-1
MPP-2
MPP-3
HPC-1
HPC-2
Cell number
WT
Het
Tie2
Lepr
Double
B
Spleen
Whole body
HSC number
HSC-2
HSC-1
C
Spleen
Colony-forming frequency (%)
BFU-E
M
gm
gmEM
D
Het
Tie2
Lepr
Double
% donor cells
weeks post-transplantation
1
2
3
E
RBC count (x10^5/ul)
WBC count (x10^2/ul)
F
CD150
CD48
CD229
CD244
CD84
Ly108
0 - 20 %
21- 40 %
41- 60 %
61- 80 %
81-100%
HSC-1
HSC-2
MPP-1
MPP-2
MPP-3
HPC-2
HPC-1
LSK fraction
CMP
MEP
GMP
CLP
Oligopotent progenitors
Meg pro
Ery pro
Gr
Mϕ
B
T
Unipotent progenitors/ mature cells

Figure S7. Summary of SLAM family marker expression by restricted hematopoietic progenitors, related to Figure 7

(A) The number of MPPs and HPCs in femurs and tibias from 5-10 month old adult wild-type (WT), *Scf*$^{fl/-}$ (Het), *Tie2-cre*; *Scf*$^{fl/-}$ (Tie2), *Lepr-cre*; *Scf*$^{fl/-}$ (Lepr), and *Tie2-cre*; *Lepr-cre*; *Scf*$^{fl/-}$ (Double) mice. Data represent mean ± S.D. from 3 independent experiments. Note that these are the same mice as shown in Figure 7C. *p<0.05 by student *t*-test.

(B) The number of HSC-1 and HSC-2 cells in spleens (left panel) and whole bodies (bone marrow plus spleen; right panel). The number of HSCs in a whole body was calculated by assuming that femurs and tibias contain 15% of bone marrow cells in the mouse. Statistical significance was separately analyzed for HSC-1 cells (black bars) and HSC-2 cells (gray bars). Data represent mean ± S.D. from 3 independent experiments. *p<0.05, **p<0.01 by student's *t*-test.

(C) Myelo-erythroid colony-forming progenitors in the spleen. Splenocytes were plated into semi-solid culture medium (MethoCult M3434 from Stem Cell Technology supplemented with 10 ng/ml Thrombopoietin and 10 ng/ml Flt-3 ligand from Peprotech). Colonies were counted and their composition was morphologically analyzed after 14 days of culture. g, granulocyte; m, macrophage; E, erythroblasts; M, megakaryocyte; BFU-E, burst-forming unit-erythroid. Data represent mean ± S.D. from 3 independent experiments.

(D) Secondary transplantation assay. Five million bone marrow cells from primary recipients that were long-term reconstituted by bone marrow cells from the indicated mutant mice were transplanted into secondary recipient mice. Each line represents the frequency of donor-derived $CD45^+$ white blood cells. The time point at 0 weeks post-transplantation represents the percentage of donor-derived cells at 16 weeks post-transplantation of the primary recipients (which are shown in Figure 7D). Data represent mean ± S.D. from one experiment with 3-4 secondary recipients per primary donor.

(E) The number of red blood cells (RBCs) and white blood cells (WBCs) in the peripheral blood

of 19-month old mice of the indicated genetic backgrounds. Data represent mean ± S.D. from 3 independent experiments. *p<0.05, **p<0.01 by student's *t*-test.

(F) A schematic summary of changes in SLAM family marker expression during hematopoietic differentiation. Boxes indicate expression levels of CD150, CD48, CD229, CD84, and Ly108 on each stem and progenitor cell population, based on the data in Figure 1 and Figure S1. Meg pro, megakaryocyte progenitor; Ery pro, erythroid progenitor; Gr, granulocyte; Mϕ, macrophage; B, B cell; T, T cell. These data summarize the lineage relationships we observed in transplantation experiments performed in irradiated mice but do not address the progeny each population is fated to form in non-irradiated mice. Specific lineage relationships between HPC-1 and HPC-2 cells and oligopotent progenitors are not shown because our transplantation experiments did not have the resolution to carefully assess this.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Colony formation assays

Cells were sorted into individual wells of 96-well round bottom plates containing 200 µl SF-O3 medium (Sanko Junyaku) supplemented with 50 µM 2-mercaptoethanol (Sigma), 2 mM L-glutamine (Invitrogen), 100 units/ml penicillin (Invitrogen), 100 µg/ml streptomycin (Invitrogen), 20 ng/ml mouse Stem cell factor (PeproTech), 20 ng/ml mouse Thrombopoietin (PeproTech), 20 ng/ml mouse Interleukin-3 (PeproTech), 5 units/ml human Erythropoietin (Amgen), and 10% heat-inactivated fetal bovine serum (Invitrogen). Large (>2 mm in diameter), medium (1-2 mm in diameter), and small (<1 mm in diameter) colonies were counted and their composition was analyzed by morphology using Giemsa staining of cytospun samples after 14 days of culture.

Cell cycle analysis

For Ki-67 and PI staining, cells in each fraction were sorted into 70% ethanol and kept at -20°C for at least 12 hours. Ki-67 staining was performed using the FITC mouse anti-human Ki-67 kit (BD Biosciences), followed by staining with 50 µg/ml propidium iodide (Molecular Probes) and analyzed using a FACSCanto II.

For analysis of BrdU incorporation mice were given an intraperitoneal injection of 1 mg BrdU (Sigma) per 6 g of body mass in phosphate buffered saline (PBS) and maintained on 1 mg/ml of BrdU in the drinking water for the indicated periods of time. Cells in each LSK fraction were sorted and cytospun onto a slide. Slides were fixed with cold methanol for 5 minutes at -20°C, then washed with PBS containing 0.01% NP-40 and treated with 2N HCl for 30 minutes, followed by incubating in 0.1M sodium borate (pH 8.5) for 10 minutes. Slides were blocked in PBS containing 4% goat serum, 4 mg/ml bovine serum albumin, and 0.1% NP-40 followed by staining overnight at 4°C with an antibody against BrdU (IU-4, Invitrogen) diluted in blocking buffer. Primary antibody staining was developed with Alexa Fluor 488 conjugated goat anti-mouse IgG_1 (Invitrogen) together with 2 µg/ml DAPI. Slides were analyzed on an Olympus

IX81 fluorescence microscope. For H2B-GFP label retention analysis, 2 mg/ml doxycycline (Research Products International) and 10 mg/ml sucrose (Sigma) were added to the drinking water for 6 weeks as described (Foudi et al., 2009).

Competitive repopulation assays

Adult recipient mice were irradiated using a Cesium 137 Gammacell 40 Exactor (MDS Nordia) delivering 1.1 Gy/minute in two equal doses of 5.4 Gy, delivered at least 4 hours apart. Donor cells were injected into the tail vein of recipient mice along with 200,000 recipient bone marrow cells. Cells were counted using Turk blood diluting fluid (Ricca Chemical) to determine total mononucleated cells. For single cell transplants, cells were sorted into individual wells of a round-bottom 96-well plate, and visually confirmed under a microscope before transplantation. At the indicated times, peripheral blood was collected from the tail veins of recipient mice and subjected to ammonium-chloride potassium red cell lysis, followed by staining with antibodies against CD45.1, CD45.2, Gr-1, Mac-1, B220, and CD3 to monitor donor chimerism. Donor chimerism in bone marrow stem and progenitor cell fractions was analyzed one week after the final peripheral blood analysis. The sources of all antibodies are listed in Supplementary Table 1.

Microarray analysis

Gene expression profiling was performed in three independent experiments. Total RNA was isolated from 5,000 cells and extracted using Trizol LS Reagent (Ambion). The extracted RNA was treated with Recombinant DNase I (Ambion) and the DNA-free RNA samples were purified using RNeasy MinElute Cleanup Kit (Qiagen). 500 pg of the purified RNA was amplified and labeled using TargetAmp 2-Round Biotin-aRNA Amplification Kit 3.0 (Epicentre) following the manufacture’s instructions. The Biotin-aRNA was hybridized to Mouse WG-6 v2.0 BeadChips (illumina). Expression values for all probes were normalized by quantile normalization using GenomeStudio Software (illumina). For GSEA, normalized expression data were assessed by

GSEA v2.08 software (Broad Institute).

SUPPLEMENTARY REFERENCES

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