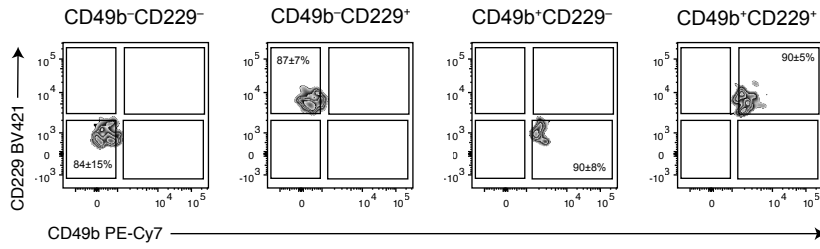


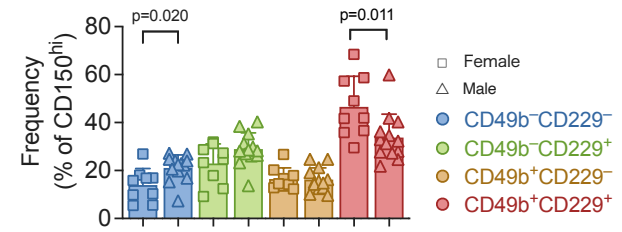
Figure S1, related to Figure 1-2

A

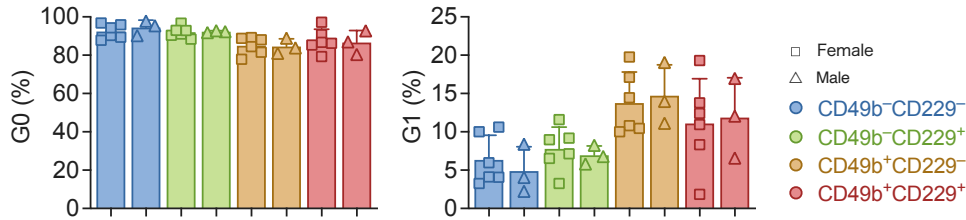
Pre-gated on: Lineage<sup>-</sup>Sca-1<sup>+</sup>c-kit<sup>+</sup>CD48<sup>-</sup>CD34<sup>-</sup>CD150<sup>hi</sup>



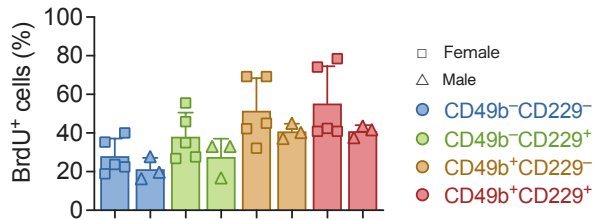
B



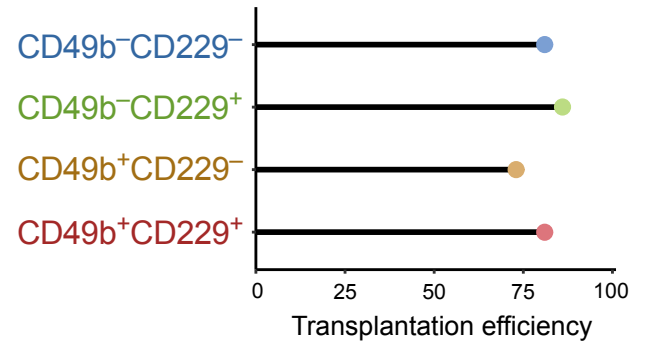
C



D



E

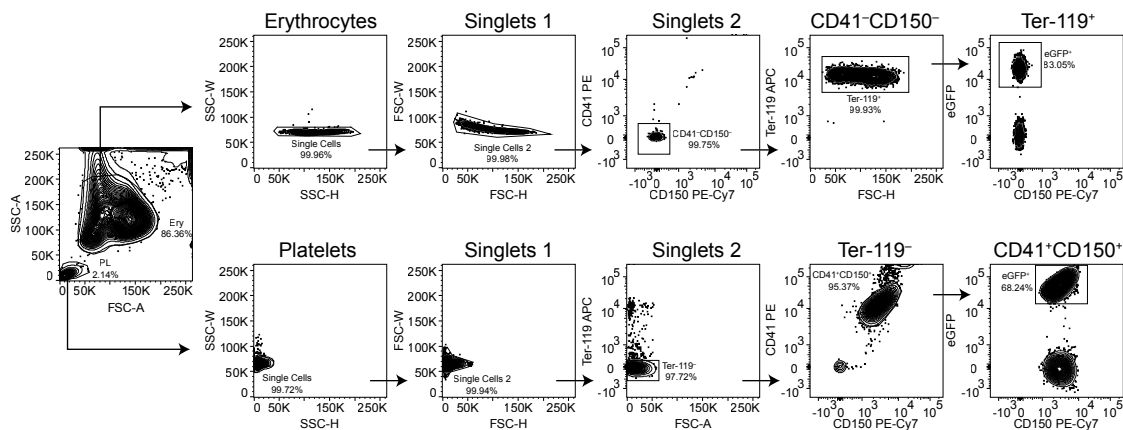


**Supplementary Figure S1, related to Figure 1-2.** CD49b and CD229 subfractionated subsets in female and male mice. **(A):** Sort purity analysis of CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> populations pre-gated on Lineage<sup>-</sup>Sca-1<sup>+</sup>c-kit<sup>+</sup>CD48<sup>-</sup>CD34<sup>-</sup>CD150<sup>hi</sup> (CD150<sup>hi</sup>). Sort purity is represented as mean  $\pm$  SD for each subset, from 4 experiments **(B):** Frequency of CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> subsets within the phenotypic HSC compartment (CD150<sup>hi</sup>) of young adult mice, separated into females and males. n = 9 females, 13 males from 9 experiments **(C):** Cell cycle analysis of CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> subsets by Ki-67 and DAPI staining in young adult mice, separated into females and males. Frequency of cells in G0 (left) and G1 (right) phases are shown. n = 6 females, 3 males from 2 experiments. **(D):** Cell proliferation analysis by BrdU incorporation in young adult mice, separated into females and males. Frequencies of BrdU<sup>+</sup> CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> cells are shown. n = 5 females, 3 males from 2 experiments. **(E):** Transplantation success rate of transplanted CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> cells.

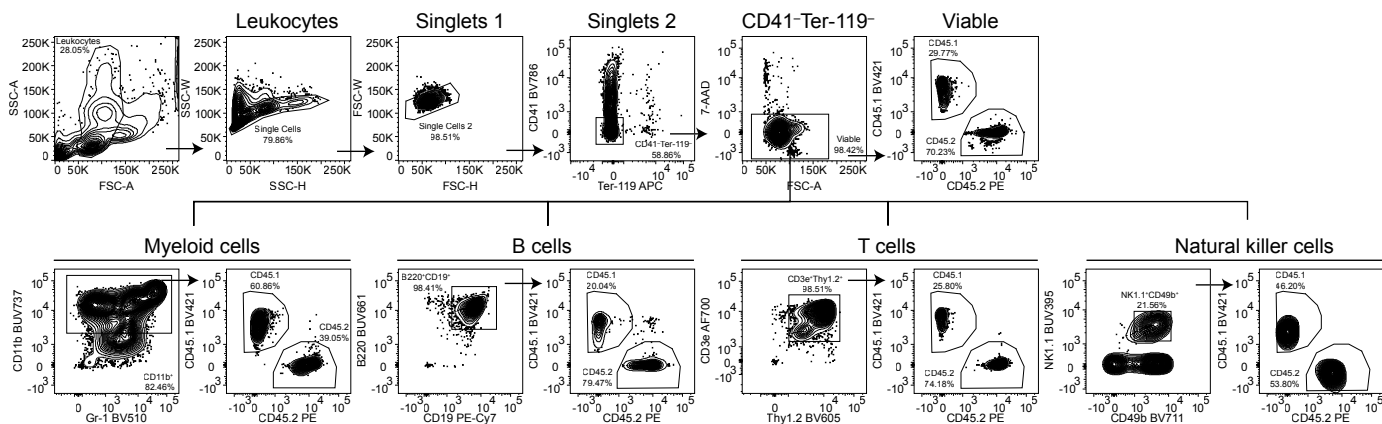
Statistical analysis was performed with the Mann-Whitney test in (B-D) between female and male groups within the same subset. All data are represented as mean  $\pm$  SD. Abbreviations: BrdU, Bromodeoxyuridine.

Figure S2, related to Figure 2

A

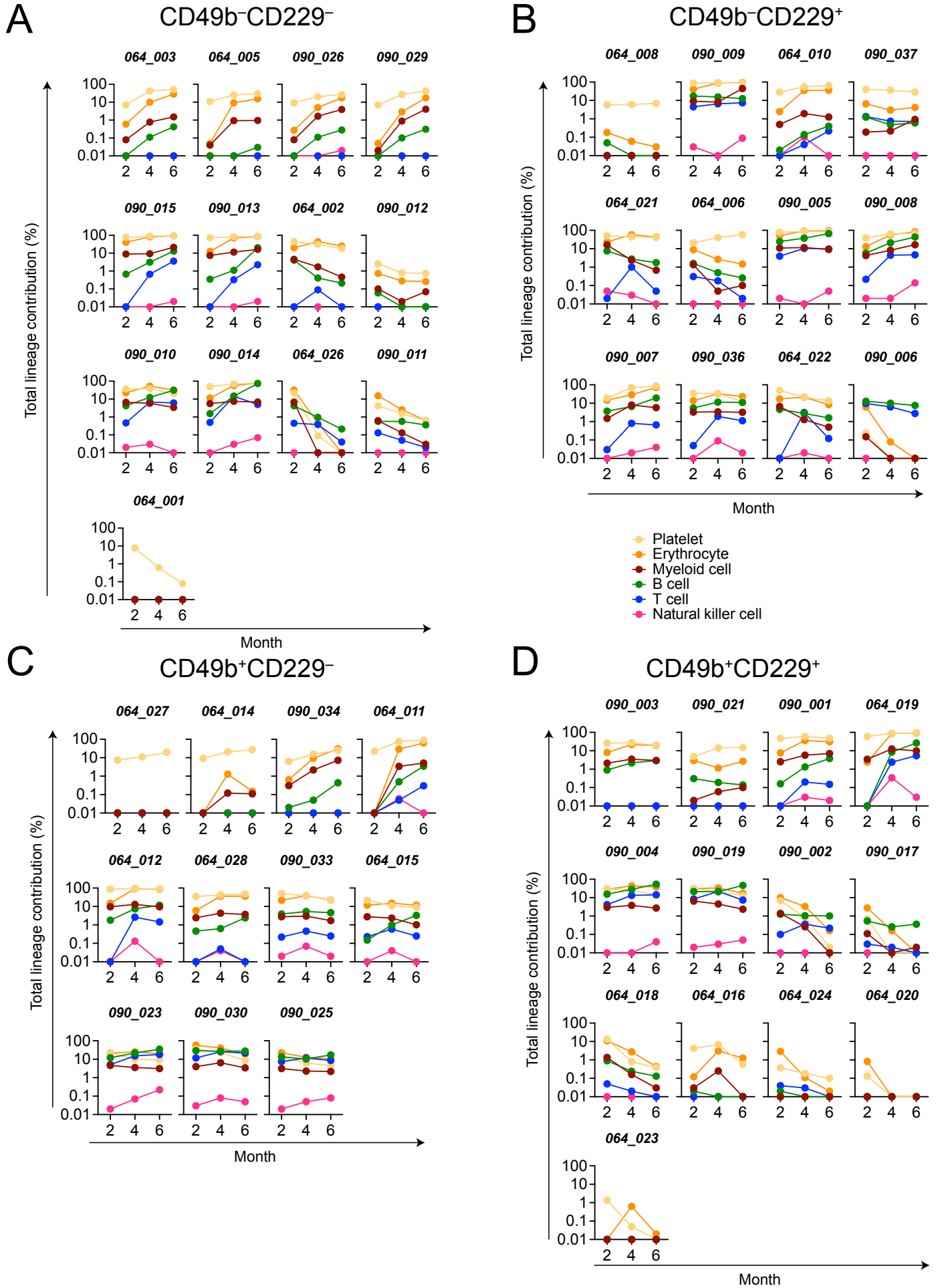


B



**Supplementary Figure S2, related to Figure 2.** Flow cytometry analysis and gating strategies. **(A):** Representative FACS profile and gating strategy of platelet and erythrocyte lineages in the peripheral blood. Platelet:  $CD41^+CD150^+Ter-119^-$ ; Erythrocyte:  $Ter-119^+CD41^-CD150^-$ . **(B):** Representative FACS profile and gating strategy of myeloid, B, T, and natural killer (NK) cell lineages in the peripheral blood. Donor leukocytes:  $CD45.2^+$ ; myeloid cell:  $CD11b^+B220^-CD19^-NK1.1^-CD49b^-CD3e^-Thy1.2^-CD41^-Ter-119^-$ ; B cell:  $B220^+CD19^+CD11b^-Gr-1^-NK1.1^-CD49b^-CD3e^-Thy1.2^-CD41^-Ter-119^-$ ; T cell:  $CD3e^+Thy1.2^+CD11b^-Gr-1^-NK1.1^-CD49b^-B220^-CD19^-CD41^-Ter-119^-$ ; NK cell:  $NK1.1^+CD49b^+CD3e^-Thy1.2^-CD11b^-Gr-1^-B220^-CD19^-CD41^-Ter-119^-$ . Frequency of parent gates are shown in all FACS plots.

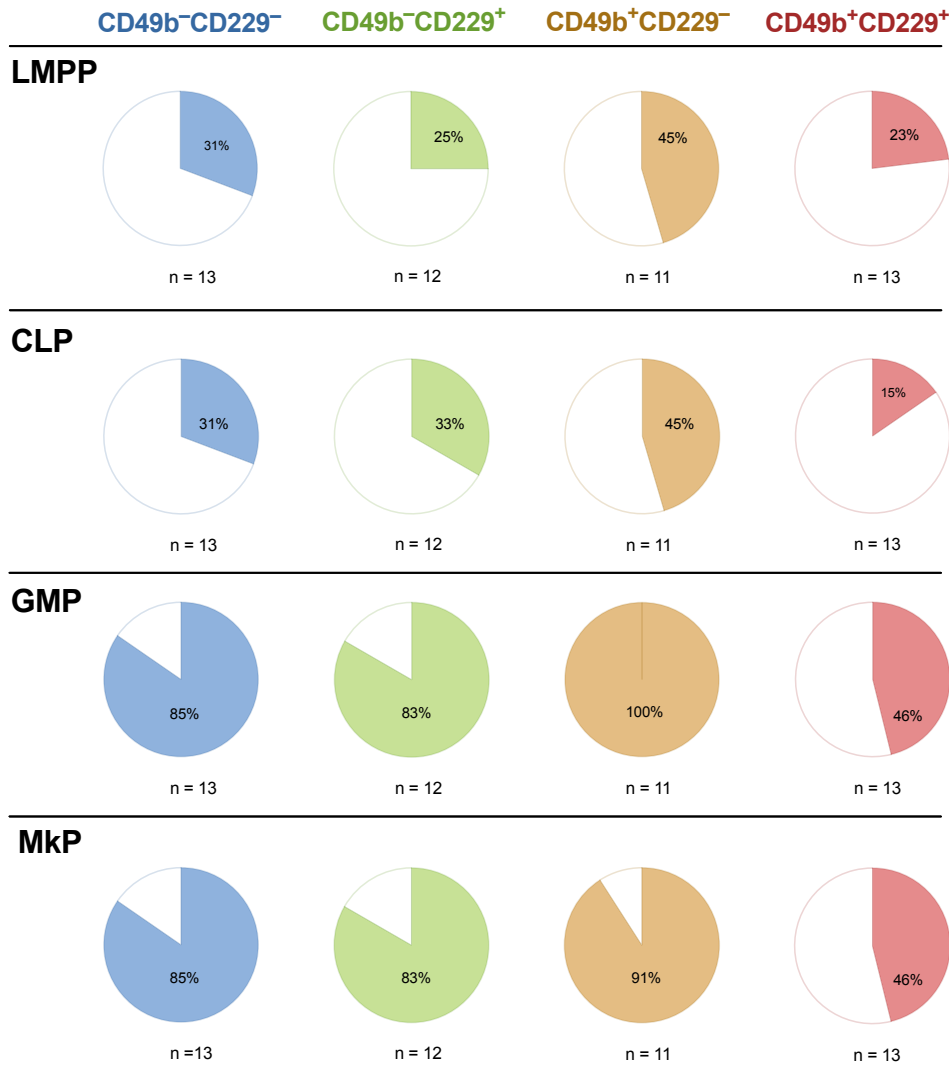
Figure S3, related to Figure 2



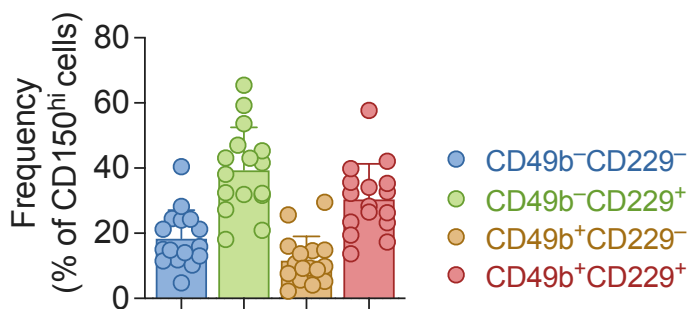
**Supplementary Figure S3, related to Figure 2.** Blood repopulation patterns of primary transplanted mice. **(A-D)**: Frequency of mature lineage repopulation in peripheral blood 2-, 4-, and 6-months post-transplantation. Individual mice, each transplanted with 5 cells from the CD49b<sup>-</sup>CD229<sup>-</sup> subset in (A), CD49b<sup>-</sup>CD229<sup>+</sup> subset in (B), CD49b<sup>+</sup>CD229<sup>-</sup> subset in (C), and CD49b<sup>+</sup>CD229<sup>+</sup> subset in (D) are shown. Data are represented as mean  $\pm$  SD.

Figure S4, related to Figure 2

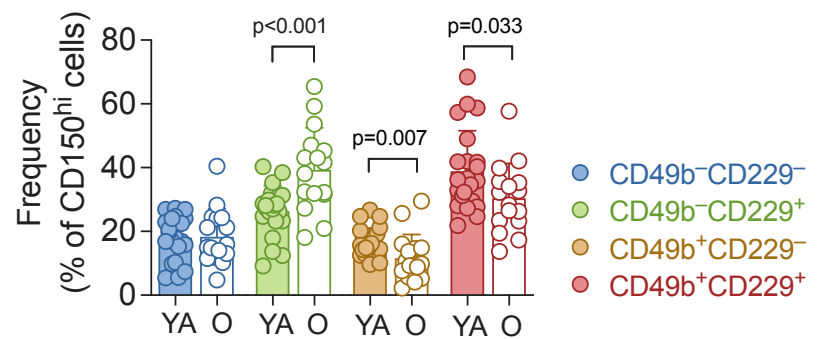
A



B



C



**Supplementary Figure S4, related to Figure 2.** Repopulation of the bone marrow progenitor compartment and CD49b and CD229 subfractionation in aging. **(A):** Proportion of mice with LMPP, CLP, GMP, and MkP repopulation 6 months post-transplantation.  $n_{CD49b^-CD229^-} = 13$  mice,  $n_{CD49b^-CD229^+} = 12$  mice,  $n_{CD49b^+CD229^-} = 11$  mice, and  $n_{CD49b^+CD229^+} = 13$  mice, 2 experiments. **(B):** Frequency of CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> subsets within the phenotypic HSC compartment (Lineage<sup>-</sup>Sca-1<sup>c</sup>-kit<sup>+</sup>CD48<sup>-</sup>CD34<sup>-</sup>CD150<sup>hi</sup>) of old mice. n = 16 biological replicates, 10 experiments. **(C):** Comparison of the frequency of CD49b<sup>-</sup>CD229<sup>-</sup>, CD49b<sup>-</sup>CD229<sup>+</sup>, CD49b<sup>+</sup>CD229<sup>-</sup>, and CD49b<sup>+</sup>CD229<sup>+</sup> subsets in young adult and old mice from Fig. 1B and Fig. S4B.

Statistical analysis was performed with the Mann-Whitney test in (C). Data are represented as mean  $\pm$  SD in (B-C). Abbreviations: LMPP (Lymphoid-primed multipotent progenitor): Lineage<sup>-</sup>Sca-1<sup>c</sup>-kit<sup>+</sup>Flt-3<sup>hi</sup>; CLP (Common lymphoid progenitor): Lineage<sup>-</sup>B220<sup>low</sup>Sca-1<sup>low</sup>c-kit<sup>low</sup>Flt-3<sup>hi</sup>IL-7Ra<sup>+</sup>; MkP (Megakaryocyte progenitor): Lineage<sup>-</sup>Sca-1<sup>c</sup>-kit<sup>+</sup> (LK) CD150<sup>+</sup>CD41<sup>+</sup>; GMP (Granulocyte-monocyte progenitor): LK CD41<sup>-</sup>CD150<sup>-</sup>CD16/32<sup>+</sup>; YA, young adult; O, old.



**Supplementary Table S1. Antibody list**

Antigen	Clone	Supplier	Order number
<b>Donor and recipient</b>			
Anti-mouse CD45.2 (Ly5.2) PE	104	BioLegend	109808
Anti-mouse CD45.1 (Ly5.1) BV421	A20	BD Biosciences	563983
Anti-mouse CD45.1 (Ly5.1) BUV395	A20	BD Biosciences	565212
<b>Live/Dead, cell proliferation and cell cycle</b>			
7-AAD		BD Biosciences	559925
DAPI		ThermoFisher Scientific	D3571
BrdU PE		BD Biosciences	556029
Anti-mouse/Human Ki-67 PE		BD Biosciences	567719
Ki-67 FITC		BD Biosciences	556026
<b>Hematopoietic cells</b>			
Anti-mouse CD19 PE-Cy7	6D5	BioLegend	115520
Anti-mouse CD45R/B220 BUV395	RA3-6B2	BD Biosciences	563793
Anti-mouse CD45R/B220 BUV661	RA3-6B2	BD Biosciences	565077
Anti-mouse/Human CD45R/B220 PE-Dazzle 594	RA3-6B2	BioLegend	103258
Anti-mouse/Human CD45R/B220 BV510	RA3-6B2	BioLegend	103248
Anti-mouse CD11b (Mac-1) BUV395	M1/70	BD Biosciences	563553
Anti-mouse CD11b (Mac-1) BUV737	M1/70	BD Biosciences	564443
Anti-mouse/Human CD11b (Mac-1) BV510	M1/70	BioLegend	101263
Anti-mouse F4/80 APC	BM8	BioLegend	123116
Anti-mouse F4/80 APC	BM8	ThermoFisher Scientific	17-4801-82
Anti-mouse Gr-1 BV510	RB6-8C5	BioLegend	108437
Anti-mouse Gr-1 BUV395	RB6-8C5	BD Biosciences	563849
Anti-mouse Gr-1 (Ly-6G/Ly-6C) PE	RB6-8C5	BD Biosciences	553128
Anti-mouse Thy1.2 BV605	53-2.1	BioLegend	140318
Anti-mouse CD3e AF700	500A2	BD Biosciences	557984

Antigen	Clone	Supplier	Order number
Anti-mouse CD3e BV510	145-2C11	BioLegend	100353
Anti-mouse CD3e BUV395	1451-2C11	BD Biosciences	563565
Anti-mouse CD4 BUV395	RM4-5	BD Biosciences	740208
Anti-mouse CD5 BV510	53-7.3	BioLegend	100627
Anti-mouse CD5 BUV395	53-7.3	BD Biosciences	740206
Anti-mouse CD8a BUV395	53-6.7	BD Biosciences	563786
Anti-mouse NK1.1 BUV395	PK136	BD Biosciences	564144
Anti-mouse CD41 BV786	MWRReg30	BD Biosciences	740903
Anti-mouse CD41 PE	eBioMWRReg30	ThermoFisher Scientific	12-0411-83
Anti-mouse Ter-119 BV510	TER-119	BD Biosciences	563995
Anti-mouse Ter-119 BV650	TER-119	BD Biosciences	747739
Anti-mouse Ter-119 BUV395	TER-119	BD Biosciences	563827
Anti-mouse Ter-119 APC	TER-119	Proteintech	APC-65149
Anti-mouse CD229 (Ly9) Biotin	Ly9ab3	BioLegend	122903
Anti-mouse/Human/Rat Streptavidin BV421		BioLegend	405226
Anti-mouse CD229 (Ly9) BV421	Ly9.7.144	BD Biosciences	748251
Anti-mouse CD229 (Ly9) BUV805	Ly9.7.144	BD Biosciences	749390
Anti-mouse CD49b PE-Cy7	HMA2	BioLegend	103518
Anti-mouse CD49b AF647	HMA2	BioLegend	103511
Anti-mouse CD49b BV711	HMA2	BD Biosciences	740704
Anti-mouse CD150 (SLAM) BV785	TC15-12F12.2	BioLegend	115937
Anti-mouse CD150 (SLAM) PE-Cy7	TC15-12F12.2	BioLegend	115914
Anti-mouse CD34 FITC	RAM34	ThermoFisher Scientific	11-0341-85
Anti-mouse CD34 AF647	RAM34	BD Biosciences	560230
Anti-mouse CD48 AF700	HM48-1	BioLegend	103426
Anti-mouse CD48 APC	HM48-1	BioLegend	103412
Anti-mouse CD117 (c-kit) APC-eF780	2B8	ThermoFisher Scientific	47-1171-82
Anti-mouse Sca-1 (Ly-6A/E) BV605	D7	BioLegend	108134
Anti-mouse Sca-1 (Ly-6A/E) BV650	D7	BD Biosciences	740450

Antigen	Clone	Supplier	Order number
Anti-mouse CD105 (Endoglin) BV650	MJ7/18	BD Biosciences	740609
Anti-mouse CD135 (Flt-3) PE	A2F10	BioLegend	135306
Anti-mouse CD135 (Flt-3) BV421	A2F10.1	BD Biosciences	562898
Anti-mouse CD127 (IL7-Ra) BV711	A7R34	BioLegend	135035
Anti-mouse CD16/32 AF700	93	ThermoFisher Scientific	56-0161-82
Anti-mouse CD16/32 BUV737	AB93	BD Biosciences	751697
Anti-mouse CD16/32 (Fc-block) Purified	2.4G2	BD Biosciences	553142