

SUPPLEMENTAL MATERIAL

Morita et al., <http://www.jem.org/cgi/content/full/jem.20091318/DC1>

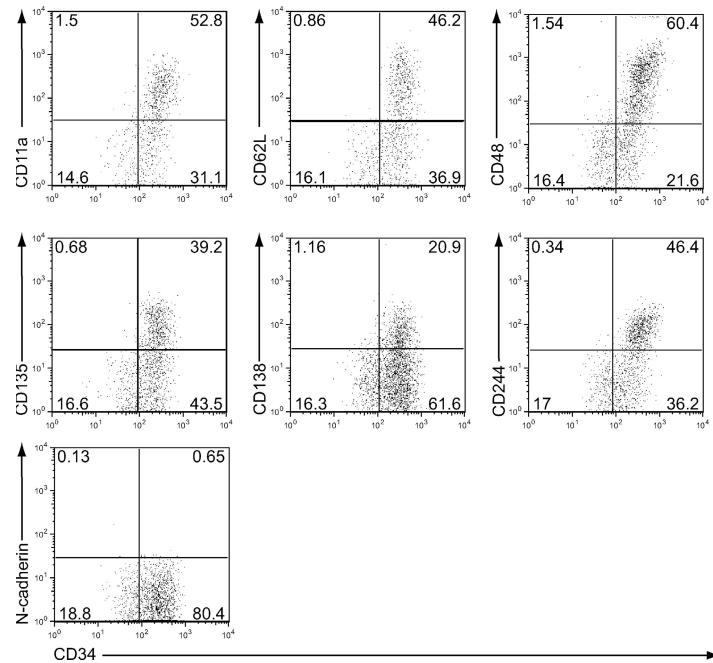


Figure S1. Negative markers for CD34⁻KSL cells. Flow cytometric profiles show the markers that were expressed by CD34⁺KSL cells but not by CD34⁻KSL cells (percentages are shown).

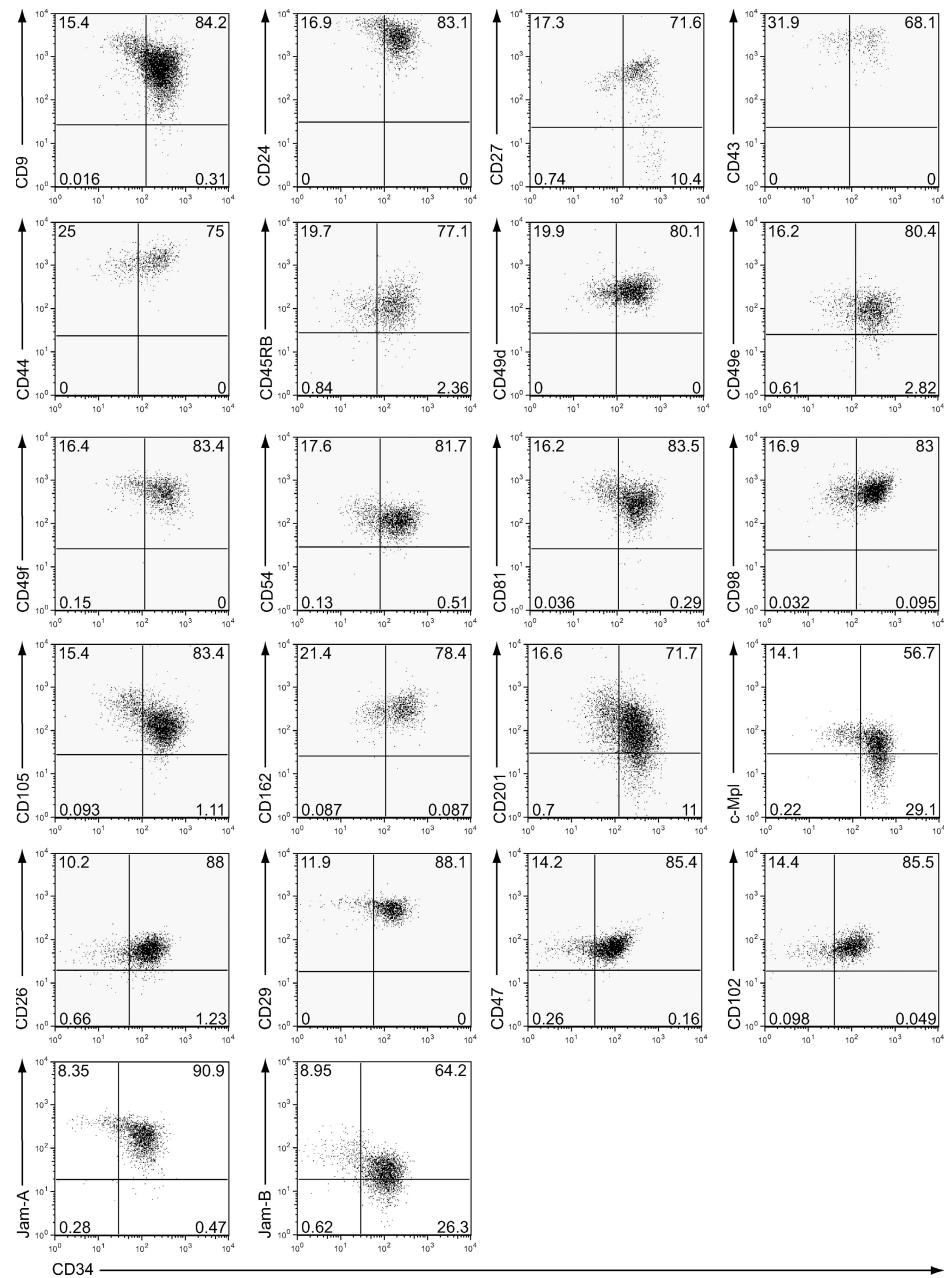


Figure S2. Positive markers for CD34⁻KSL cells. Flow cytometric profiles show the markers that were expressed by CD34⁻KSL cells (percentages are shown).

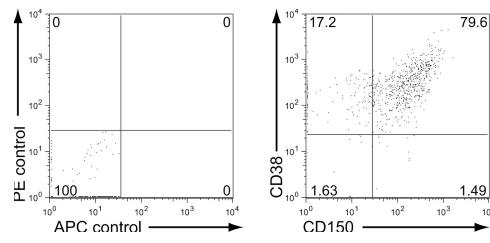


Figure S3. Relationship between CD150 and CD38 in CD34⁻KSL cells. CD34⁻KSL cells were stained with PE-conjugated anti-CD38 and APC-conjugated anti-CD150 antibodies. The flow cytometric profile shows CD150 and CD38 coexpression in CD34⁻KSL cells (percentages are shown).

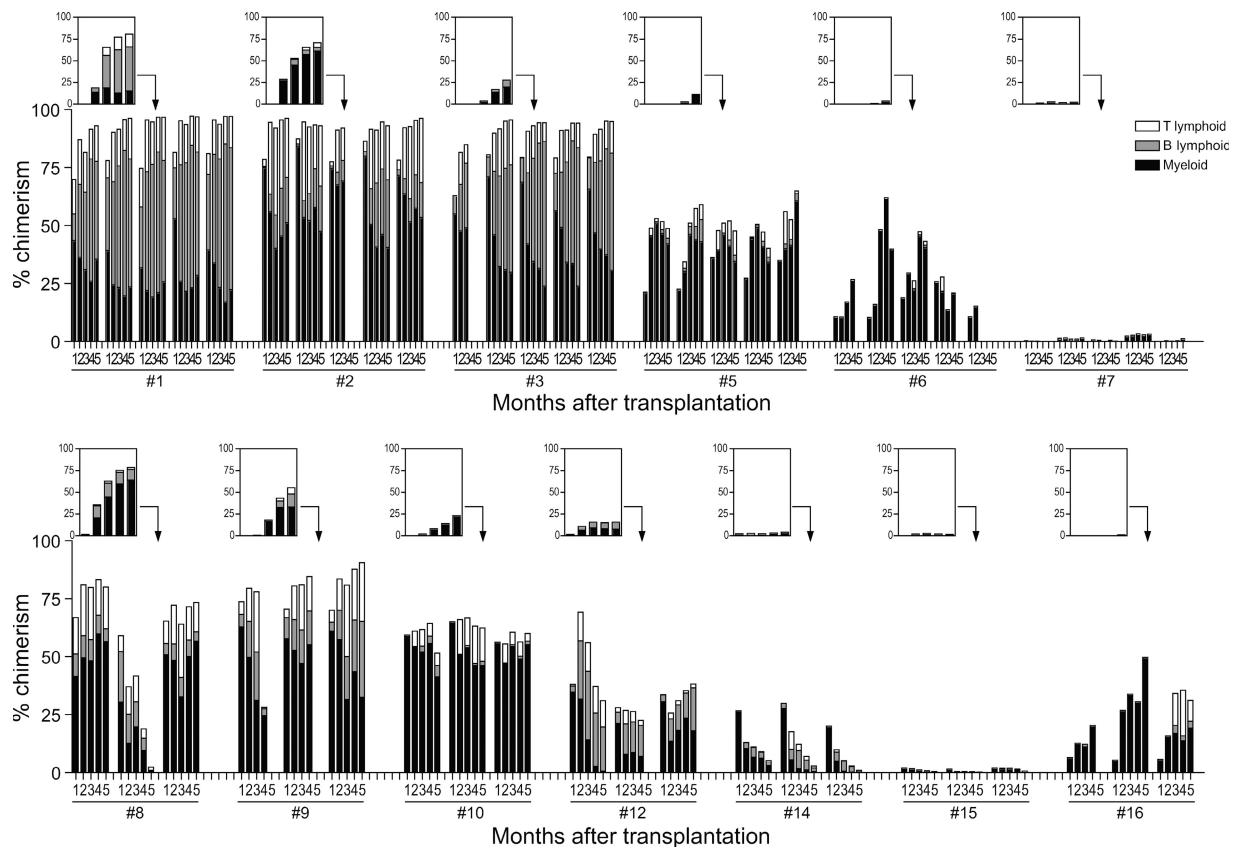


Figure S4. Repopulation kinetics of CD150^{high}CD34⁻KSL cells in individual secondary-recipient mice. Percentages of chimerism are shown for individual mice after secondary transplantation of BM from recipients of single CD150^{high}CD34⁻KSL cells. The top windows show the repopulation kinetics of primary-recipient mice.

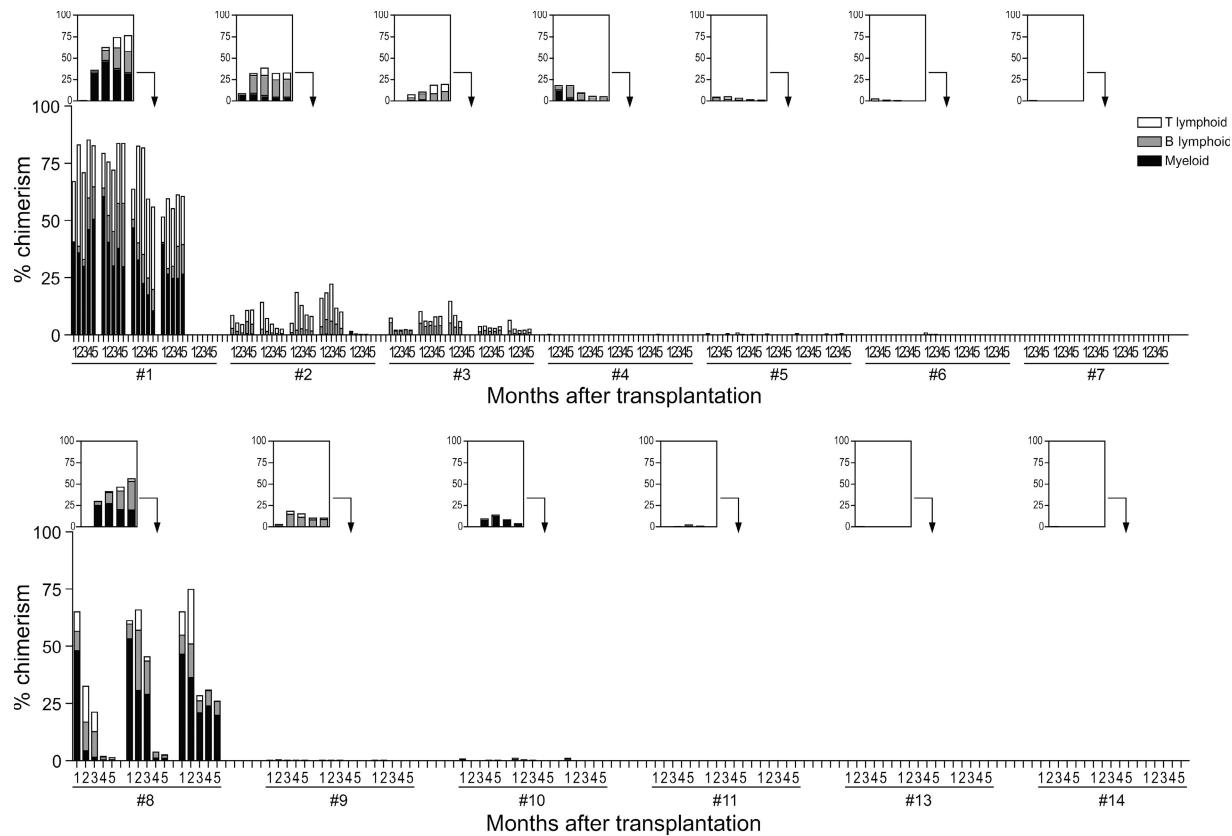


Figure S5. Repopulation kinetics of CD150^{med}CD34⁻KSL cells in individual secondary-recipient mice. Percentages of chimerism are shown for individual mice after secondary transplantation of BM from recipients of single CD150^{med}CD34⁻KSL cells. The top windows show the repopulation kinetics of primary-recipient mice.

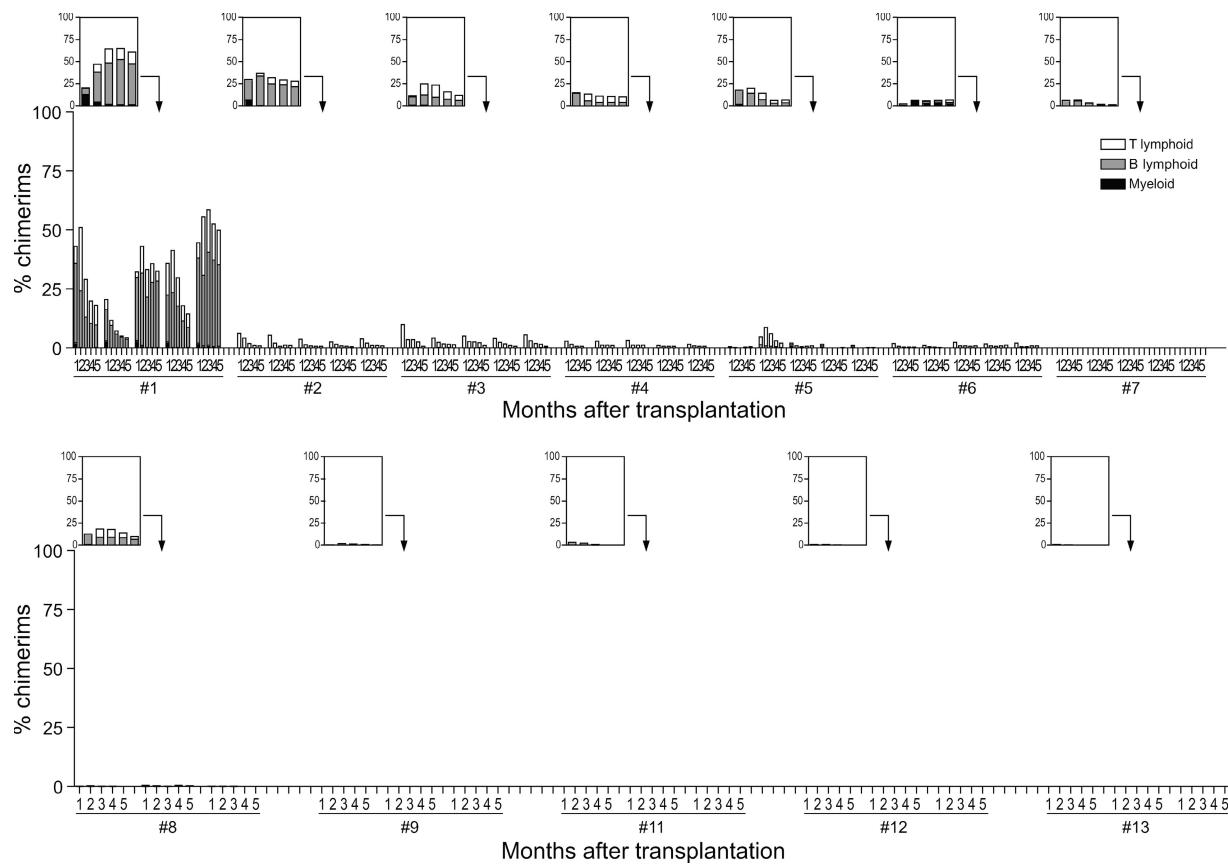


Figure S6. Repopulation kinetics of CD150^{neg}CD34⁻KSL cells in individual secondary-recipient mice. Percentages of chimerism are shown for individual mice after secondary transplantation of BM from recipients of single CD150^{neg}CD34⁻KSL cells. The top windows show the repopulation kinetics of primary-recipient mice.

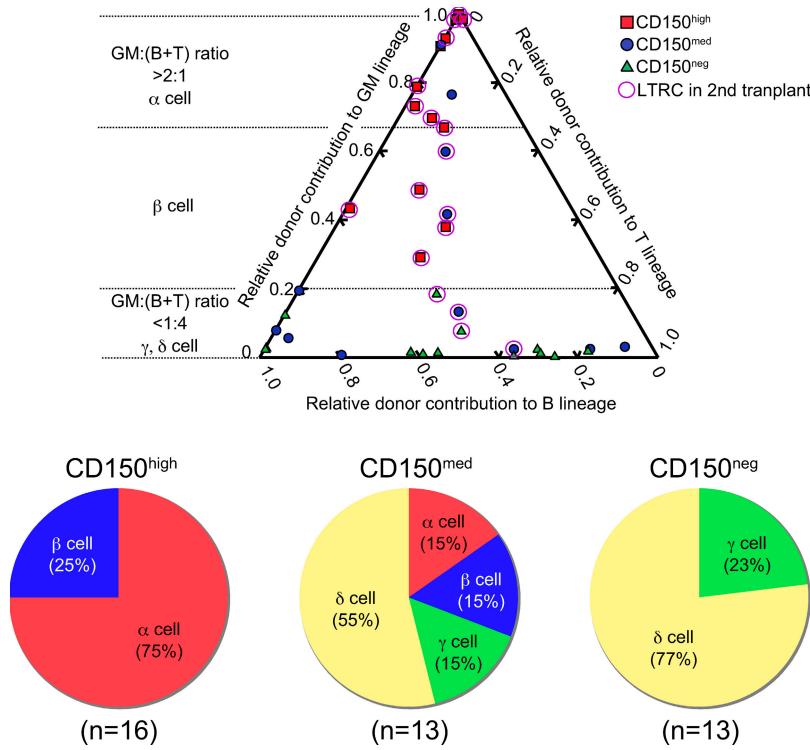


Figure S7. Dykstra et al. classification in primary-recipient mice. LTRCs from CD150^{high}, CD150^{med}, or CD150^{neg}CD34⁻KSL cells in primary-recipient mice were qualified as α, β, γ, or δ cells using the criteria given by Dykstra et al. (2007). LTRCs in secondary transplantation are indicated by circles (top).

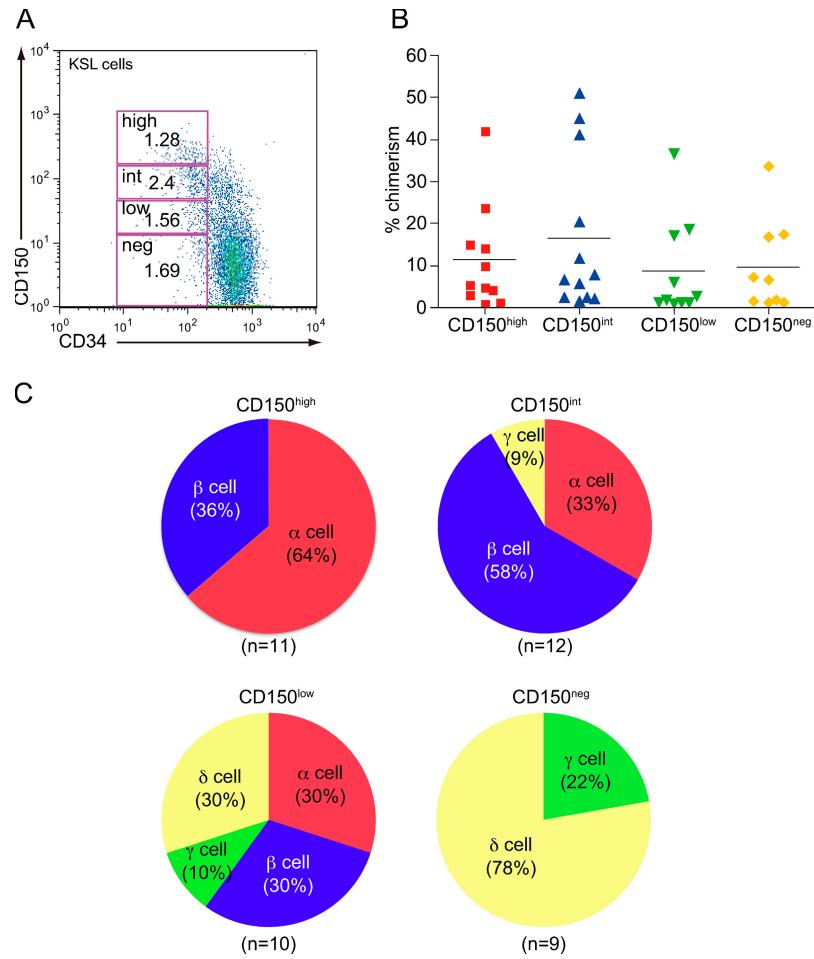


Figure S8. CD150^{int}CD34⁻KSL cells are enriched in β cells. (A) CD34⁻KSL cells were subdivided into CD150^{high}, CD150^{int}, CD150^{low}, and CD150^{neg} fractions. (B) Single CD150^{high}, CD150^{int}, CD150^{low}, and CD150^{neg}CD34⁻KSL cells were transplanted into 30 lethally irradiated mice together with 2×10^5 competitor cells. Percentage of chimerism of peripheral blood from reconstituted mice at 4 mo after transplantation is shown. Horizontal lines represent means. (C) LTRCs were qualified as α, β, γ, or δ cells.

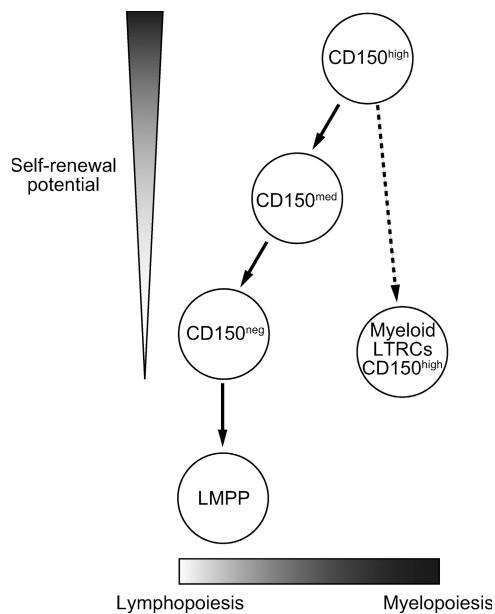


Figure S9. HSC early differentiation model. The HSC compartment resides at the top of the hematopoietic hierarchy. CD150^{high} cells give rise to CD150^{med} cells. CD150^{med} cells then give rise to CD150^{neg} cells. LMPPs are derived from CD150^{neg} cells. CD150^{high} myeloid-limited LTRCs are derived from CD150^{high} latent HSCs. Reduced expression of CD150 is associated with reduction of self-renewal potential and loss of erythroblast/megakaryocyte differentiation potential, leading to neutrophil/macrophage and lymphoid-lineage specification.

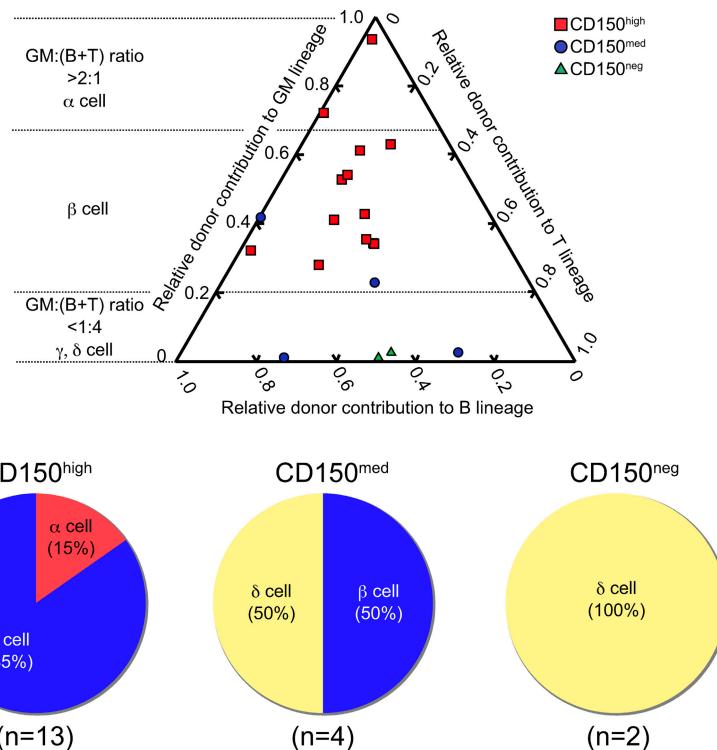


Figure S10. Dykstra et al. classification in secondary transplantation. LTRCs in secondary-recipient mice received BM cells reconstituted with CD150^{high}, CD150^{med}, or CD150^{neg}CD34⁻KSL cells were qualified as α, β, γ, or δ cells (Dykstra et al., 2007).

REFERENCE

Dykstra, B., D. Kent, M. Bowie, L. McCaffrey, M. Hamilton, K. Lyons, S.J. Lee, R. Brinkman, and C. Eaves. 2007. Long-term propagation of distinct hematopoietic differentiation programs in vivo. *Cell Stem Cell*. 1:218–229. doi:10.1016/j.stem.2007.05.015

Table S1. Antibodies used in this study

Classification	Antibody	Clone	Label	Company
Positive markers	CD9	KMC8	Biotin	BD
	CD24 (HSA)	M1/69	PE	eBioscience
	CD26 (THAM)	H194-112	FITC	BD
	CD27	LG.3A10	PE	BD
	CD29 (integrin β 1)	Ha2/5	FITC	BD
	CD43	S7	PE	BD
	CD44	IM7	PE	BD
	CD45	30-F11	PE	eBioscience
	CD45RB	C363.14A	PE	eBioscience
	CD47 (IAP)	miap301	FITC	BD
	CD49d (integrin α 4)	R1-2	PE	eBioscience
	CD49e (integrin α 5)	5H10-27	PE	BD
	CD49f (integrin α 6)	GoH3	PE	BD
	CD54 (ICAM-1)	3E2	PE	BD
	CD81 (TAPA-1)	Eat2	PE	BD
	CD98	RL388	PE	eBioscience
	CD102 (ICAM-2)	mIC2/4	FITC	BD
	CD105 (Endoglin)	MJ7/18	Biotin	eBioscience
	CD162 (PSGL-1)	2PH1	PE	BD
	CD201 (EPCR)	eBio1560	Biotin	eBioscience
	CD321 (Jam-A)	H202-106	Alexa Fluor 488	AbD Serotec
	CD322 (Jam-B)	CRAM-19 H36	FITC	AbD Serotec
Positive/negative markers	AA4.1	AA4.1	PE	eBioscience
	CD1d	1B1	PE	eBioscience
	CD11b (Mac-1)	M1/70	PE	BD
	CD18 (integrin β 2)	C71/16	PE	BD
	CD31 (PECAM-1)	MEC13.3	PE	BD
	CD38	90	PE	eBioscience
	CD49b (integrin α 2)	HM α 2	PE	BD
	CD51 (integrin α V)	RMV-7	PE	eBioscience
	CD61 (integrin β 3)	2C9.G2	PE	BD
	CD86 (B7-2)	GL1	PE	eBioscience
	CD103 (integrin α IEL)	2E7	PE	eBioscience
	CD147	RL73	PE	eBioscience
	CD150	TC15-12F12.2	PE	BioLegend
	Fc γ R	2.4G2	PE	BD
	Tie2	TEK4	PE	eBioscience
	BP-1	6C3	PE	eBioscience
Negative markers	CD2 (LFA-2)	RM2-5	PE	eBioscience
	CD3 ϵ	145-2C11	PE	BD
	CD4	RM4-5	PE	BD
	CD5	53-7.3	PE	BD
	CD8a	53-6.7	PE	BD
	CD11a (integrin α M)	M17/4	PE	eBioscience
	CD11c (integrin α X)	HL-3	PE	BD
	CD13	R3-242	PE	BD
	CD14	rmC5-3	PE	BD
	CD21/CD35 (Cr2/Cr1)	7G6	FITC	BD
	CD22.2	Cy34.1	PE	BD
	CD23 (Fc ϵ RII)	B3B4	PE	eBioscience
	CD25 (IL-2R)	PC61	PE	eBioscience
	CD28	37.51	PE	eBioscience

CD30	mCD30.1	PE	eBioscience
CD40	1C10	PE	eBioscience
CD45R (B220)	RA3-6B2	PE	BD
CD45RA	14.8	PE	BD
CD45RC	DNL-1.9	PE	BD
CD48 (BCM1)	HM48-1	PE	BD
CD62E (E-selectin)	10E9.6	PE	BD
CD62L (L-selectin)	MEL-14	PE	BD
CD62P (P-selectin)	RB40.34	FITC	BD
CD69	H1.2F3	PE	eBioscience
CD70	FR70	PE	eBioscience
CD71 (TransferrinR)	R17217	PE	eBioscience
CD72b,c	JY/93	PE	BD
CD73	TY/23	PE	BD
CD74 (B7-1)	16-10A	PE	BD
CD79a	HM47	PE	BD
CD79b	HM79b	FITC	BD
CD83	Michel17	PE	eBioscience
CD90.2	53-2.1	PE	eBioscience
CD94	18d3	PE	eBioscience
CD95 (Fas)	15A7	PE	eBioscience
CD107a	1D4B	FITC	BD
CD107b (LAMP-2)	ABL-93	FITC	BD
CD115 (M-CSFR)	AFS98	PE	eBioscience
CD120b (TNFR)	TR75-89	PE	BD
CD121a (IL-1R)	35F5	PE	BD
CD122	5H4	PE	eBioscience
CD123 (IL-3R)	5B11	PE	eBioscience
CD124 (IL-4R)	miL4R-M1	PE	BD
CD126 (IL-6R)	D7715A7	PE	BD
CD127 (IL-7R)	A7R34	PE	eBioscience
CD131	JORO50	PE	BD
CD132	4G3	PE	BD
CD133	13A4	PE	eBioscience
CD135 (Flk-2/Flt-3)	A2F10.1	PE	BD
CD137	17B5	PE	eBioscience
CD138 (Syndecan-1)	281-2	PE	BD
CD153	RM153	PE	eBioscience
CD154 (CD40L)	MR1	PE	BD
CD180	RP/14	PE	eBioscience
CD184 (CXCR4)	2B11	PE	eBioscience
CD195 (CCR5)	C34-3448	PE	BD
CD197 (CCR7)	4B12	PE	eBioscience
CD210 (IL-10R)	1B1.3a	PE	BD
CD212 (IL-12R)	114	PE	BD
CD223	C9B7W	PE	BD
CD244.2	2B4	PE	BD
CD252	RM134L	PE	eBioscience
CXCR5	2G8	PE	BD
Fc ϵ RI	MAR-1	PE	eBioscience
Flk-1 (VEGFR2)	Avas12a1	PE	eBioscience
Integrin β 7	M293	PE	BD
LPAM-1	DATK32	PE	BD
Ly-49A&D	12A8	PE	BD
Ly-49F	HBF-719	PE	BD
Ly-6G&C (Gr-1)	RB6-8C5	PE	BD

Mac-3	M3/84	PE	BD
N-cadherin	YS	Purified	IBL
NK1.1	PK136	PE	BD
NKG2	20d5	FITC	BD
Notch1	mN1A	PE	BD
Panendothelial cell antigen	MECA-32	Biotin	BD
PCLP1	10B9	PE	MBL
PD-1	J43	PE	BD
Siglec-F	E50-2440	PE	BD
Syndecan-4	KY/8.2	PE	BD

CD34⁺ KSL cells were stained with the indicated antibodies. Antibodies were classified into three groups: those antibodies that stained all CD34⁺ KSL cells (positive), those that stained some CD34⁺ KSL cells (positive/negative), and those that did not stain CD34⁺ KSL cells (negative).