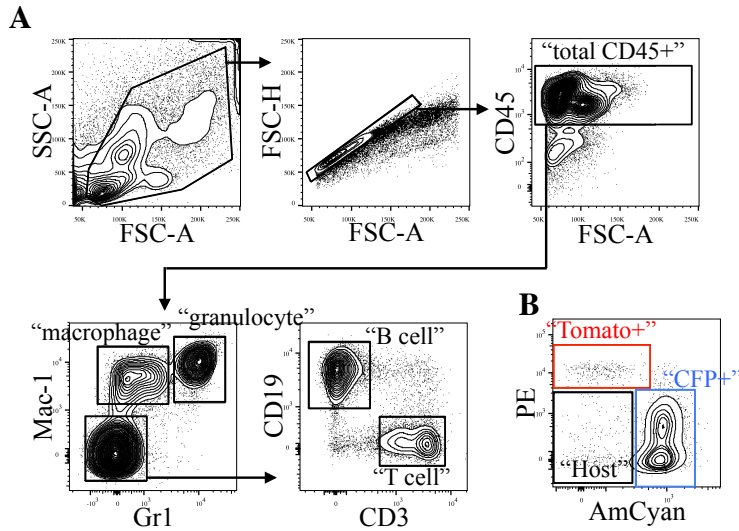
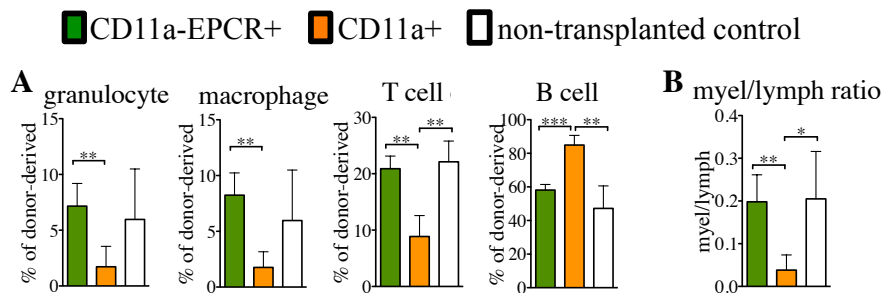


## SUPPORTING INFORMATION

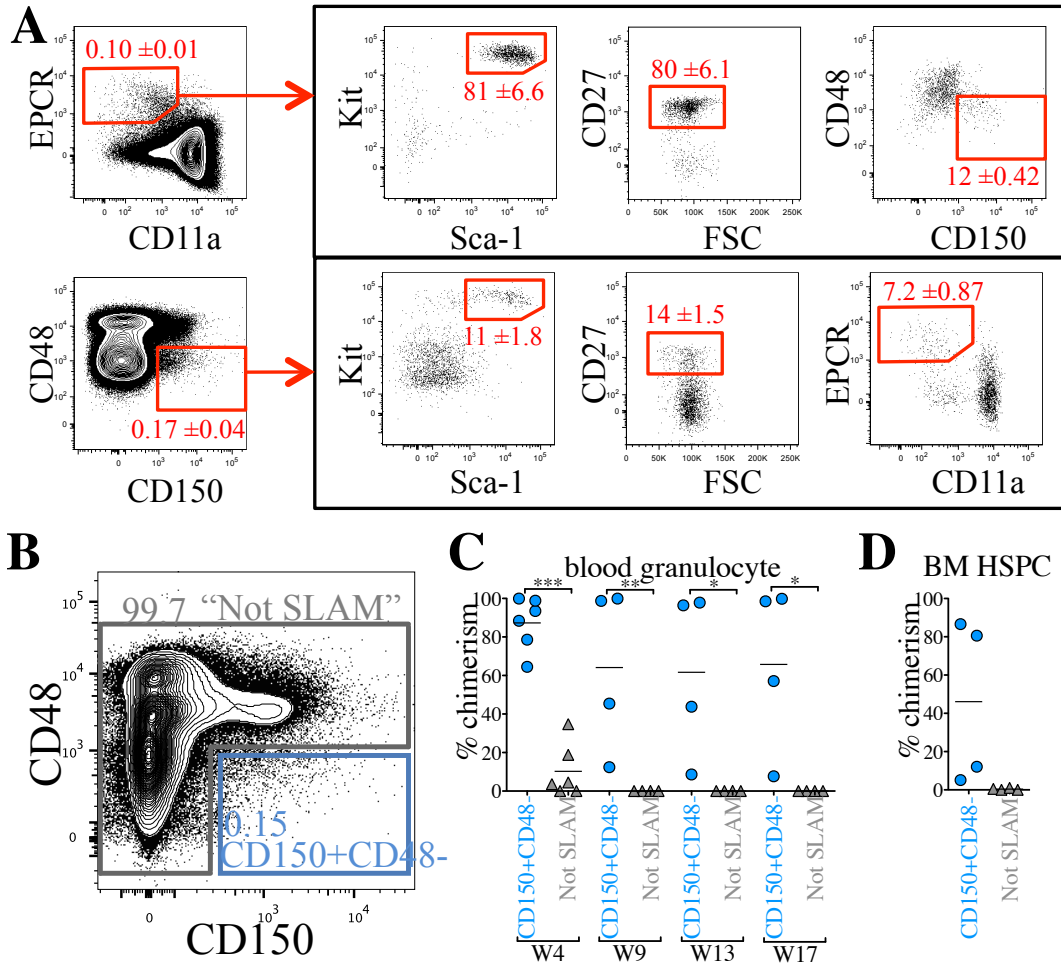
### Figures & Legends



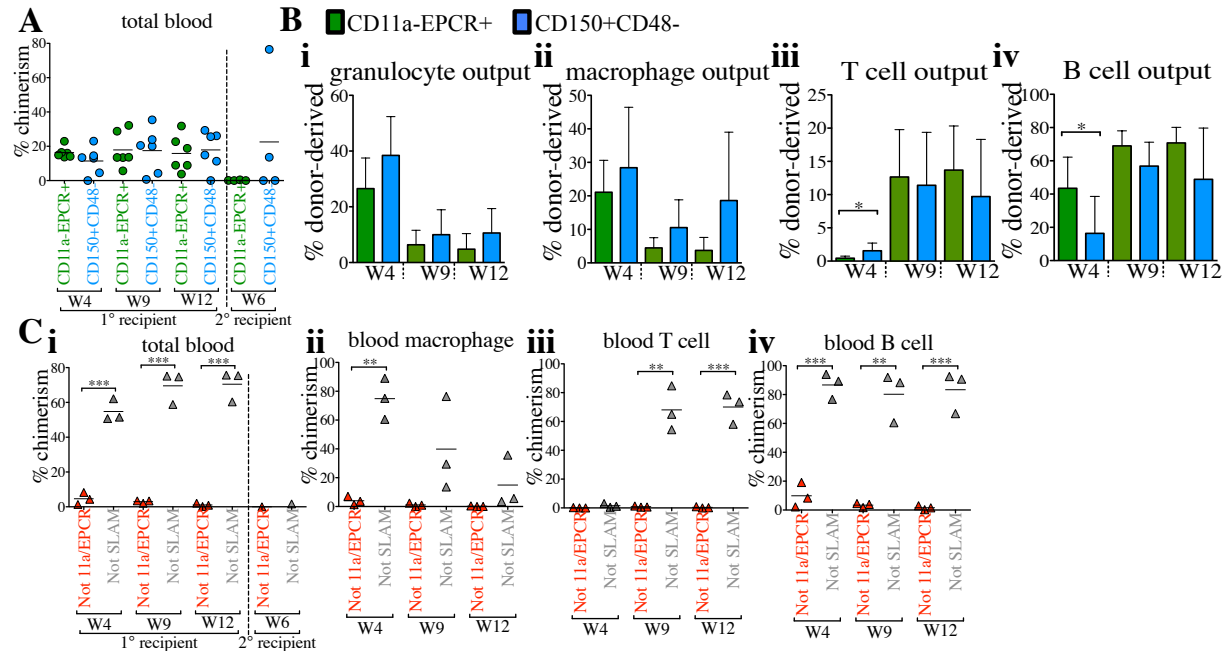
**Supporting Information Figure S1. Representative blood analysis to determine donor chimerism.** **A)** Representative blood analysis of recipient mice. After gating based on size and granularity, single cells are gated on based on CD45 expression. Within CD45+ fraction, macrophages, granulocytes, T cells, and B cells can be identified as distinct populations. Macrophages are defined as CD45+ Mac-1+ Gr1-, granulocytes as CD45+ Mac-1+ Gr1+, T cells as CD45+ CD3+, and B cells as CD45+ CD19+. **B)** Representative analysis of T cells for donor chimerism. CFP and Tomato expression can be detected in AmCyan and PE channels, respectively. “Host” cells are defined by the lack of CFP/Tomato expression. Donor chimerism within each cell type is analyzed similarly.



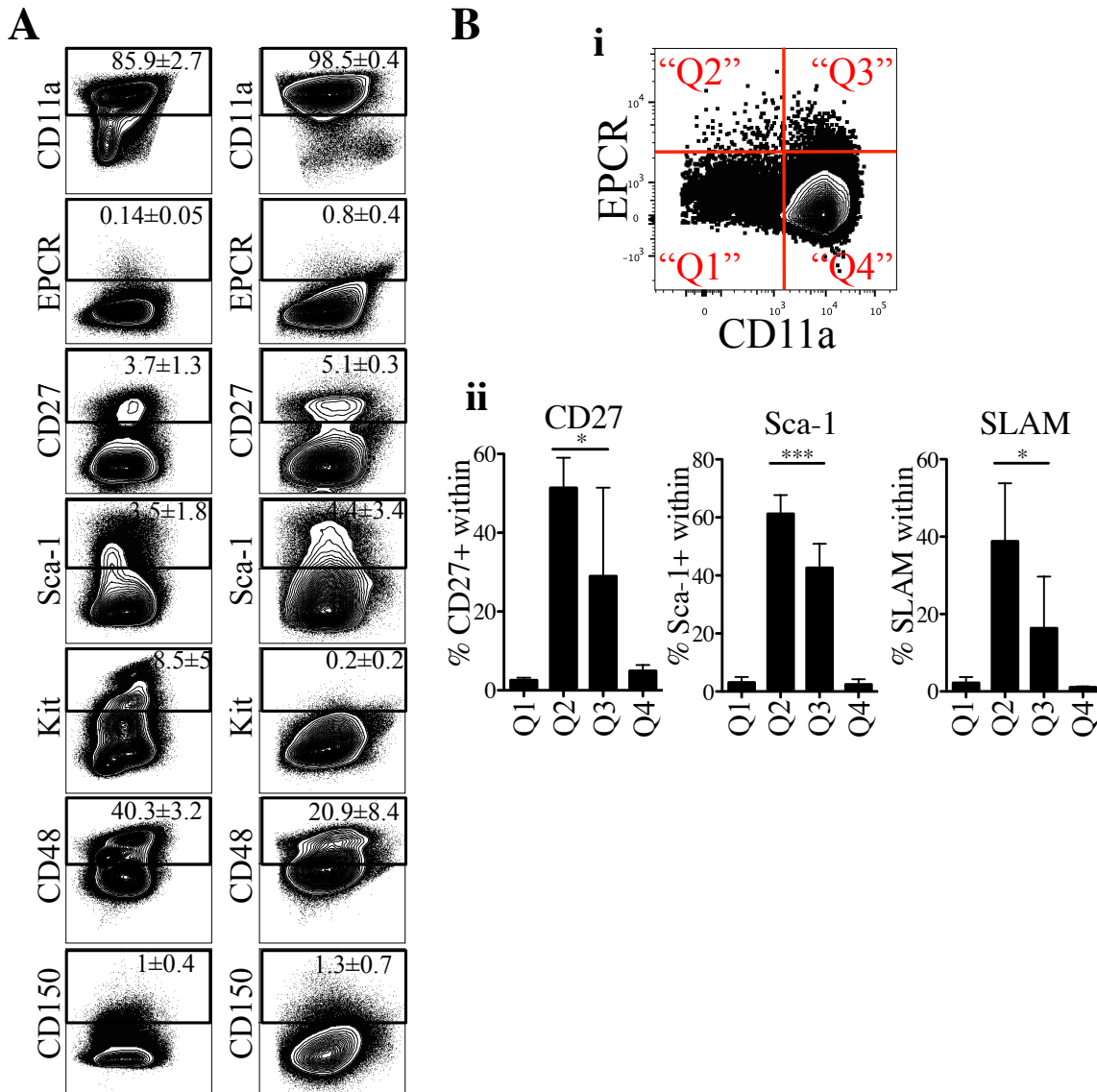
**Supporting Information Figure S2. Lineage output from CD11a- EPCR+ KLS and CD11a+ KLS in competitively transplanted recipients.** **A)** Lineage distribution of granulocytes (Mac-1+ Gr1+), Macrophages (Mac-1+ Gr1-) T cells (CD3+) and B cells (CD19+) in the peripheral blood of recipients from CD11a- EPCR+ (green) or CD11a+ (orange) KLS cells 12 weeks post-transplant, and in non-transplanted controls (n=4, error bars SD). **B)** Ratio of myeloid to lymphoid cells. Myeloid cells are defined as CD45+, Mac-1+ or Gr1+, and lymphoid cells as CD45+, CD3+ or CD19+. CD11a- EPCR+ KLS cells show similar lineage distribution and myeloid/lymphoid ratios as the non-transplanted control, indicating they can reconstitute peripheral immune cells at physiologic ratios, indicative of HSCs. Conversely, CD11a+ KLS cells show a distinct B lineage bias and skewed myeloid/lymphoid ratios, suggesting they are more differentiated than HSCs. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$  (Student's unpaired t test).



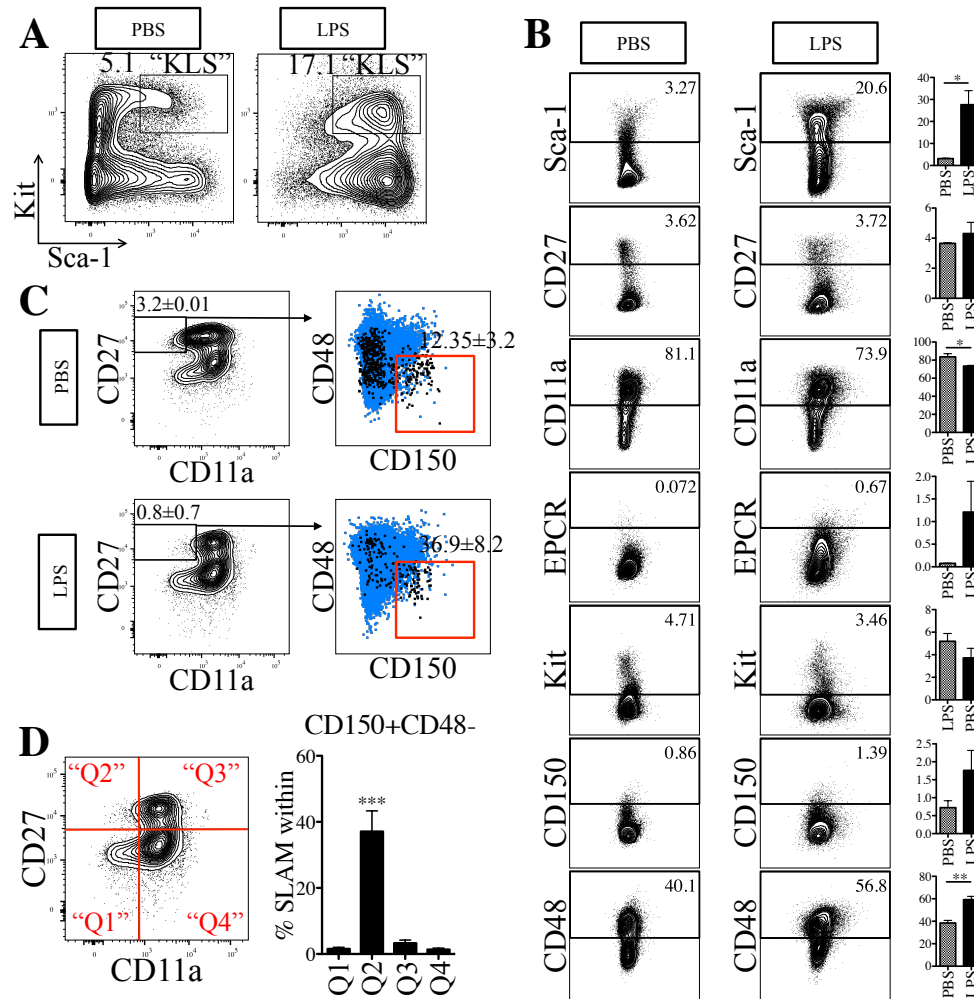
**Supporting Information Figure S3. "SLAM" 2-marker sorting method.** **A)** Analysis of HSC marker expression in CD11a- EPCR<sup>+</sup> cells (top row) and CD150<sup>+</sup> CD48<sup>-</sup> cells (bottom row). CD11a/EPCR and CD48/CD150 plots are gated on live, singlet, non-autofluorescent, Ter119<sup>-</sup> cells. Plots in the black boxes are gated on either CD11a- EPCR<sup>+</sup> (top row) or CD150<sup>+</sup> CD48<sup>-</sup> (bottom row). Percentages of cells in each gate are listed as the average of two animals ± SD. FACS plots are representative of two independent experiments. **B)** Sorting strategy using only CD150 and CD48 as HSC markers. CFP<sup>+</sup> CD150<sup>+</sup> CD48<sup>-</sup> and Tomato<sup>+</sup> "Not SLAM" (not CD150<sup>+</sup> CD48<sup>-</sup>) cells (and vice versa) were sorted and co-transplanted in a competitive setting and at the physiological ratios shown. Approximately 750 CD150<sup>+</sup> CD48<sup>-</sup> and 500,000 "Not SLAM" were transplanted into each recipient. **C)** Time-course analysis of blood granulocyte chimerism from CD150<sup>+</sup> CD48<sup>-</sup> and "Not SLAM" sources in primary recipients 4, 9, 13, and 17 weeks (W) post-transplant. **D)** Donor chimerism of HSPCs in the BM of primary recipients transplanted with CD150<sup>+</sup> CD48<sup>-</sup> and "Not SLAM" sorted cells 18 weeks post-transplant. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$  (Student's unpaired *t* test). "Not SLAM" = not CD150<sup>+</sup> CD48<sup>-</sup>.



**Supporting Information Figure S4. Comparison of total blood chimerism and lineage output between two-color sorting methods.** **A)** Time-course analysis of total blood chimerism from CD11a- EPCR+ and CD150+ CD48- sources in primary recipients 4, 9, and 12 weeks post-transplant, and in secondary recipients (separated by dashed line) at week 6 following secondary transplant. **B)** Lineage output analysis from CD11a- EPCR+ and CD150+ CD48- sources. Granulocyte (i), macrophage (ii), T cell (iii), and B cell (iv) lineage output are shown from donor sources over time (n=6, error bars SD). **C)** Time-course analysis of total blood (i), blood macrophage (ii), T cell (iii), and B cell (iv) chimerism from “Not 11a/EPCR” and “Not SLAM” sources in primary recipients 4, 9, and 12 weeks post-transplant. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$  (Student’s unpaired *t* test). “Not 11a/EPCR”=not CD11a- EPCR+; “Not SLAM”=not CD150+ CD48-.



**Supporting Information Figure S5. Expression of HSC markers post-irradiation. A)** Expression of HSC markers on BM leukocytes without irradiation (0 Gy, left FACS plots; n=3) and 48 hours post-irradiation (6 Gy, right FACS plots; n=5). FACS plots are gated on live Ter119- BM cells. Numbers are percentage of cells positive for each marker. PE/Cy5.5 (empty channel), which was not used for antibody detection, is plotted on the X-axes of FACS plots. FACS plots are representative of two independent experiments. **B) i)** Representative gating on Ter119- BM populations (“Q1”-“Q4”) with differential CD11a/EPCR expression profiles. **ii)** Quantification (n=5, error bars SD) of percentages of CD27+ (“CD27”), Sca-1+ (“Sca-1”), and CD150+ CD48- (“SLAM”) cells within each gate from (i). Percentages are representative of two independent experiments. \*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001 (One-way ANOVA with Turkey’s multiple comparison test ).



**Supporting Information Figure S6. CD11a can help identify phenotypic HSCs post LPS-induced inflammation in the BM.** **A)** Expression of Sca-1 and Kit on Ter119- CD27+ BM cells 24 hours after PBS-injection (left plot) or LPS-injection (2 mg/Kg; right plot) of animals. Numbers are percentages of KLS cells in each condition. **B)** Expression of HSC markers on BM leukocytes of animals 24 hours after injection with PBS (left FACS plots; n=2) or LPS (right FACS plots; n=3), and quantification of percentages in each condition (bar graphs, error bars SD). FACS plots are gated on Ter119- BM cells. Numbers are percentages of cells positive for each marker. Qdot605 (empty channel), which was not used for antibody detection, is plotted on the X-axes of FACS plots. Bar graphs are representative of two independent experiments. \* $p \leq 0.05$ , \*\* $p \leq 0.01$  (Student's unpaired *t* test). **C)** Expression of CD150 and CD48 ("SLAM") on Ter119- Kit+ CD27+ CD11a- BM cells from PBS-injected (top plots; n=2) and LPS-injected (bottom plots; n=3). Kit and CD27 were selected due to their unchanged expression after LPS-induced BM stress. Plots on the right show Ter119- Kit+ CD27+ CD11a- cells in black and total Ter119- cells in blue from BM of PBS- and LPS-injected animals. **D)** Representative gating on Ter119- Kit+ populations ("Q1"- "Q4") with differential CD11a/ CD27 expression profile, and quantification (n=3, error bars SD) of percentages of CD150+ CD48- ("SLAM") cells within each gate from (i). Data are representative of two independent experiments. \*\*\* $p \leq 0.001$  (One-way ANOVA with Turkey's multiple comparison test).

## Supplementary Tables

<b>Table S1. Antibodies Table</b>				
<b>Antigen</b>	<b>Clone</b>	<b>Conjugate</b>	<b>Source</b>	<b>Catalogue #</b>
TER119	TER119	PE/Cy5	Biolegend	116210
SCA1 (Ly-6A/E)	E13-161.7	FITC	Biolegend	122506
	E13-161.7	PE/Cy7	eBioscience	122514
	D7	Alexa Fluor 700	eBioscience	56-5981-82
	E13-161.7	PE	Biolegend	122507
KIT (CD117)	ACK2	APC	Biolegend	135107
	2B8	APC-eFluor 780	eBioscience	47-1171-82
	2B8	PE/Cy7	eBioscience	25-1171-81
	2B8	BV421	Biolegend	105828
CD27	LG.7F9	eFluor 780	eBioscience	47-0271-82
	LG.7F9	APC	eBioscience	17-0271-82
CD11A	M17/4	PE/Cy7	eBioscience	25-0111-30
	M17/4	Biotin	Biolegend	101103
	M17/4	APC	Biolegend	101119
	M17/4	PE	Biolegend	101107
EPCR (CD201)	eBio1560	PerCP-eFluor 710	eBioscience	46-2012-82
	eBio1560	APC	eBioscience	17-2012-82
GR1 (Ly-6G/Ly-6C)	RB6-8C5	Alexa Fluor 700	eBioscience	108422
MAC1 (CD11b)	M1/70	BV650	Biolegend	101239
	M1/70	APC	Biolegend	101212
	M1/70	FITC	Biolegend	101205
CD19	6D5	APC	Biolegend	115512
	eBio1D3	PerCP-Cy5.5	eBioscience	45-0193-82
CD45	30-F11	APC/Cy7	Biolegend	103116
CD3ε	17A2	PerCP-eFluor 710	eBioscience	46-0032-82
	17A2	PE/Cy7	Biolegend	100220
CD150 (SLAMf1)	TC15-12F12.2	PE/Cy7	Biolegend	115914
	TC15-12F12.2	BV421	Biolegend	115925
	TC15-12F12.2	BV650	Biolegend	115931
	HM48-1	FITC	eBioscience	11-04781-82
NK-1.1	PK136	APC	Biolegend	108709
MHCII	M5/114.15.2	PE/Cy7	Biolegend	107629
<b>Secondary antibodies</b>				
		Qdot 655-Streptavidin	Life Technologies	Q10121MP
		Qdot 605-Streptavidin	Life Technologies	Q10103MP

<b>Table S2. Marker definitions of populations analyzed</b>	
<i>Population</i>	<i>Markers used</i>
Total blood	CD45+
Granulocyte	CD45+ Gr1+ Mac-1+
Macrophage	CD45+ Gr1- Mac-1+
T cell	CD45+ CD3+
B cell	CD45+ CD19+
KLS/HSPC	Ter119- CD27+ Kit+ Sca-1+
HSC	Ter119- CD27+ Kit+ Sca-1+ CD11a- EPCR+
SLAM	CD150+ CD48-