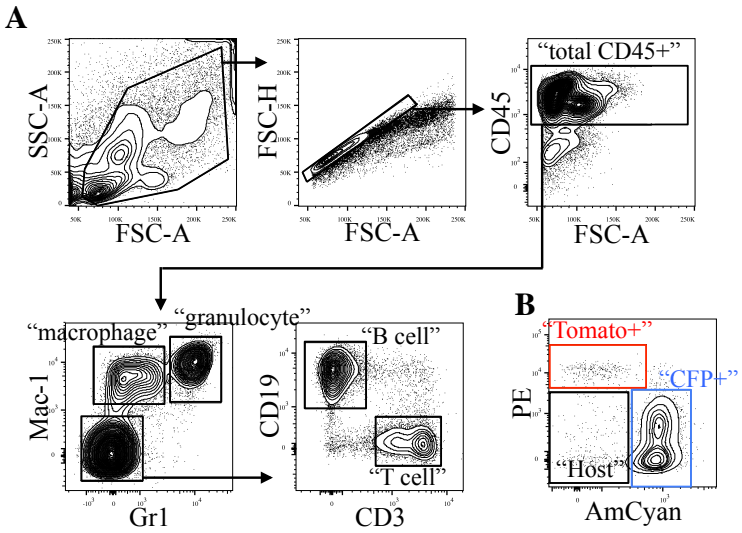
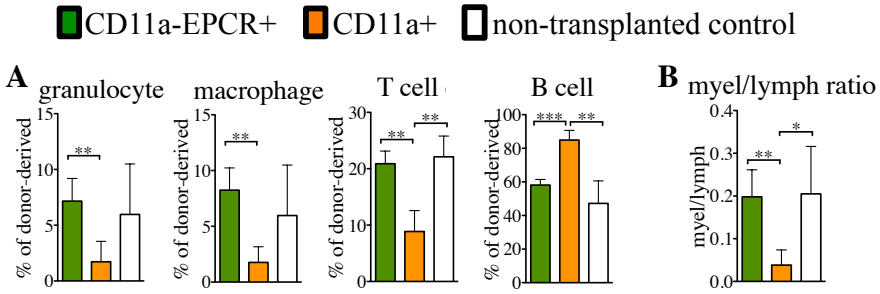
SUPPORTING INFORMATION

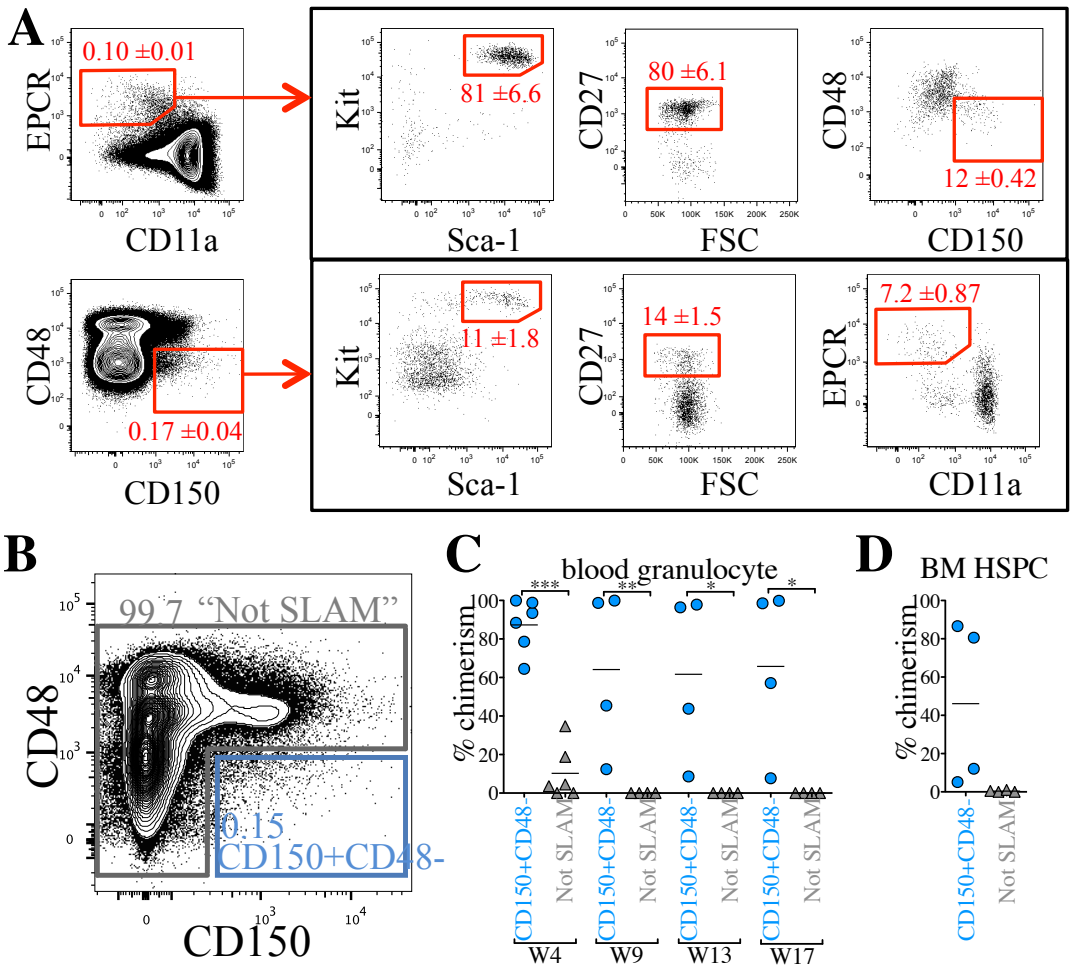
Figures & Legends



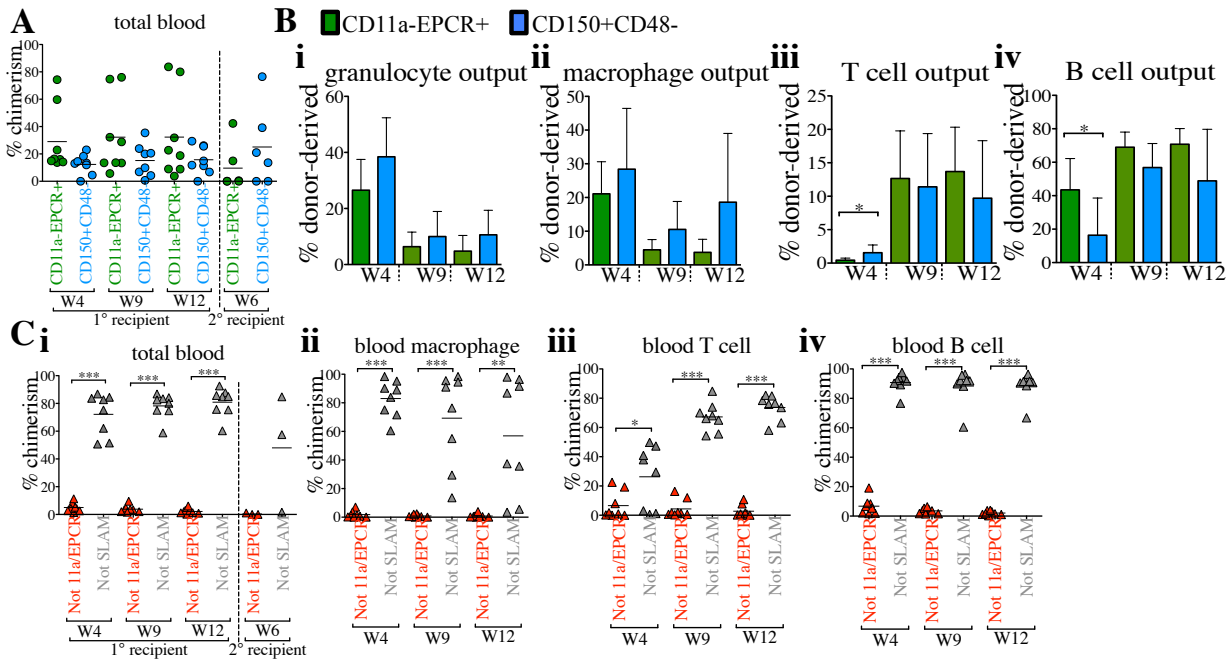
Supporting Information Figure S1. Representative blood analysis to determine donor chimerism. A) Representative blood analysis of recipient mice. After gating based on size and granularity, single cells are gated on based on CD45 expression. Within CD45+ fraction, macrophages, granulocytes, T cells, 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.



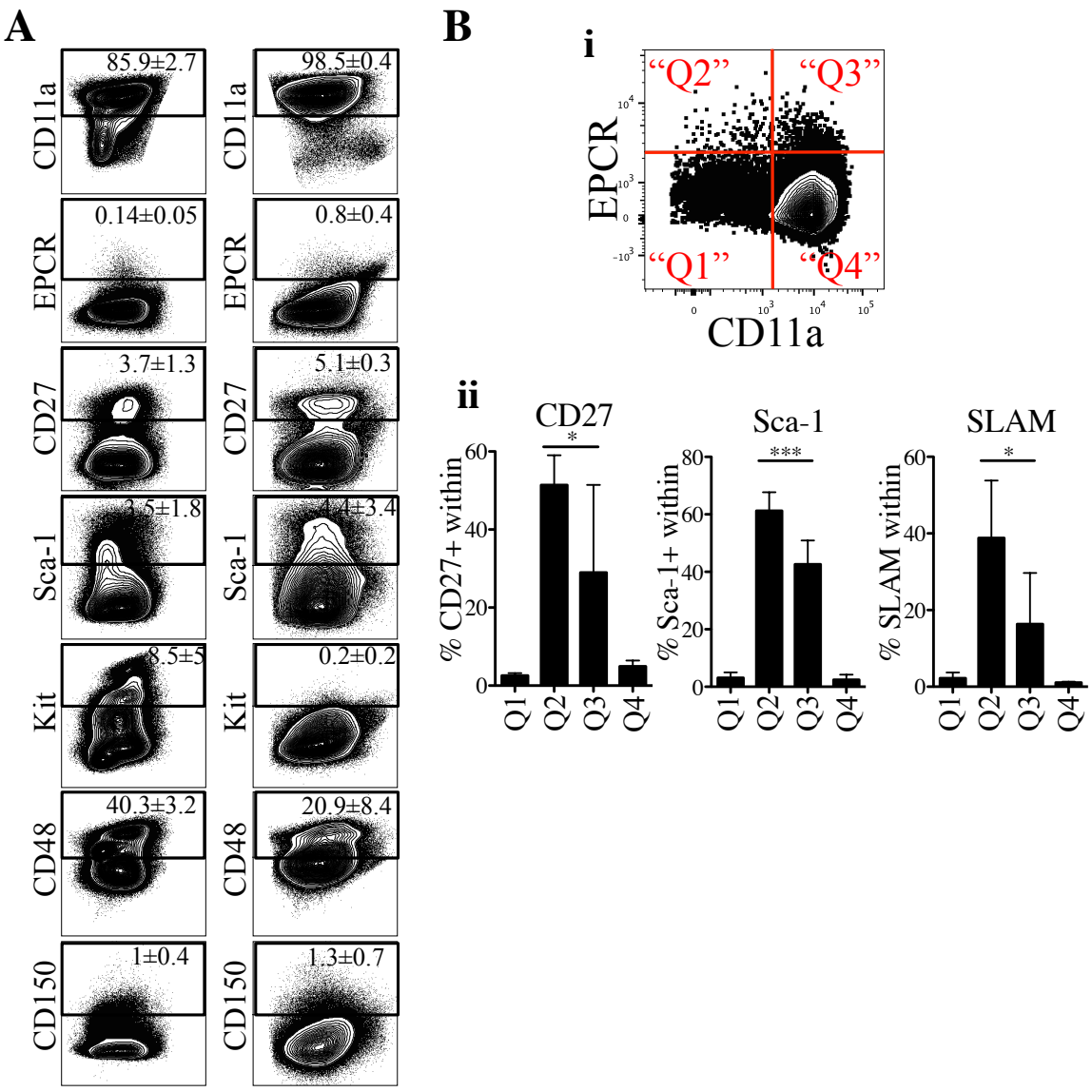
Supporting Information Figure S2. Lineage output from CD11a- EPCR+ KLS and CD11a+ KLS in competitively transplanted recipients. A) Lineage distribution of granulocytes (Mac-1+ Gr1+), Macrophages (Mac-1+ Gr1-) T cells (CD3+) and B cells (CD19+) in the peripheral blood of recipients from CD11a- EPCR+ (green) 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).*



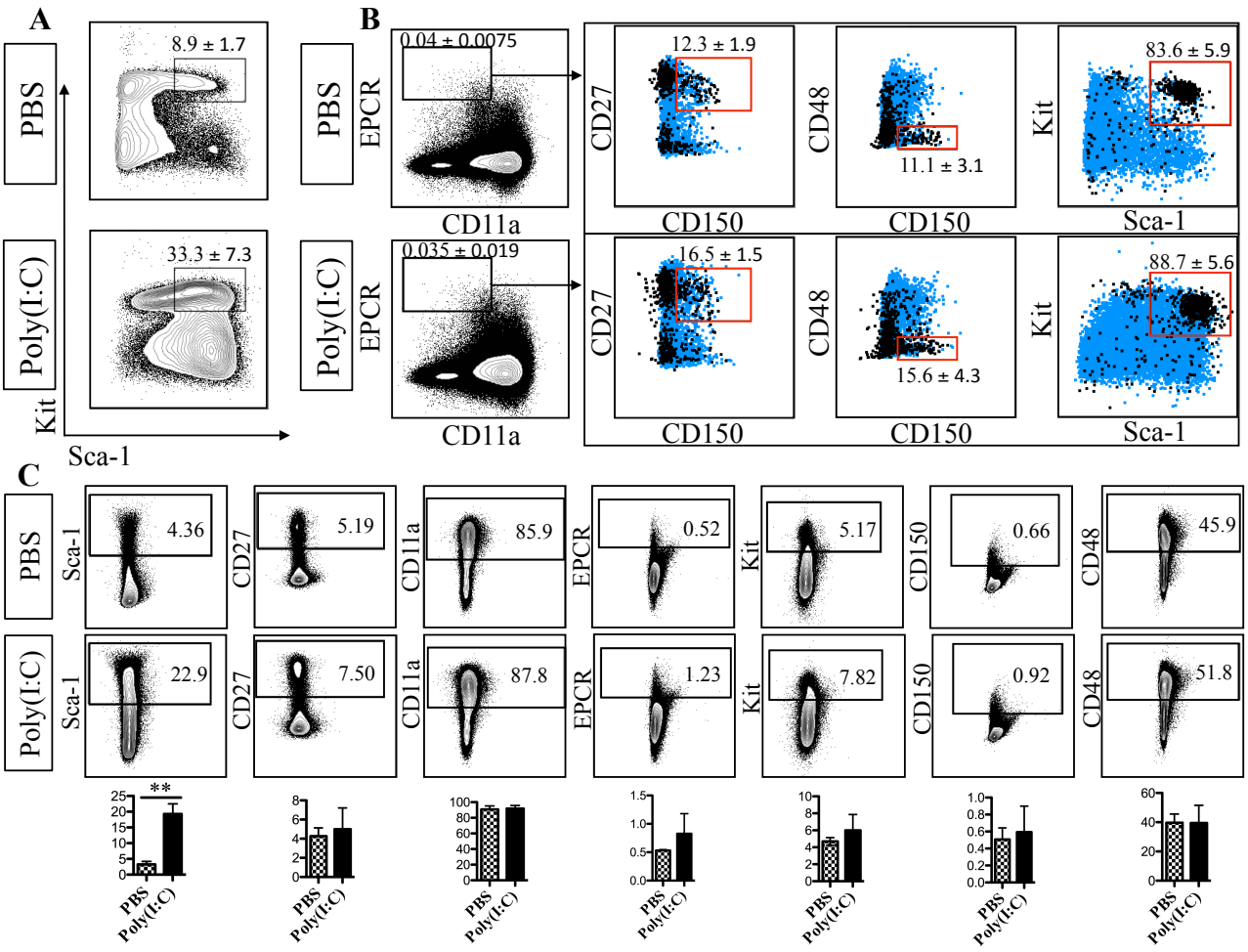
Supporting Information Figure S3. "SLAM" 2-marker sorting method. **A)** Analysis of HSC marker expression in CD11a- EPCR+ cells (top row) and CD150+ CD48- cells (bottom row). CD11a/EPCR and CD48/CD150 plots are gated on live, singlet, non-autofluorescent, Ter119- cells. Plots in the black boxes are gated on either CD11a- EPCR+ (top row) or CD150+ CD48- (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+ CD150+ CD48- and Tomato+ "Not SLAM" (not CD150+ CD48-) cells (and vice versa) were sorted and co-transplanted in a competitive setting and at the physiological ratios shown. Approximately 750 CD150+ CD48- and 500,000 "Not SLAM" were transplanted into each recipient. **C)** Time-course analysis of blood granulocyte chimerism from CD150+ CD48- 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+ CD48- 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+CD48-.



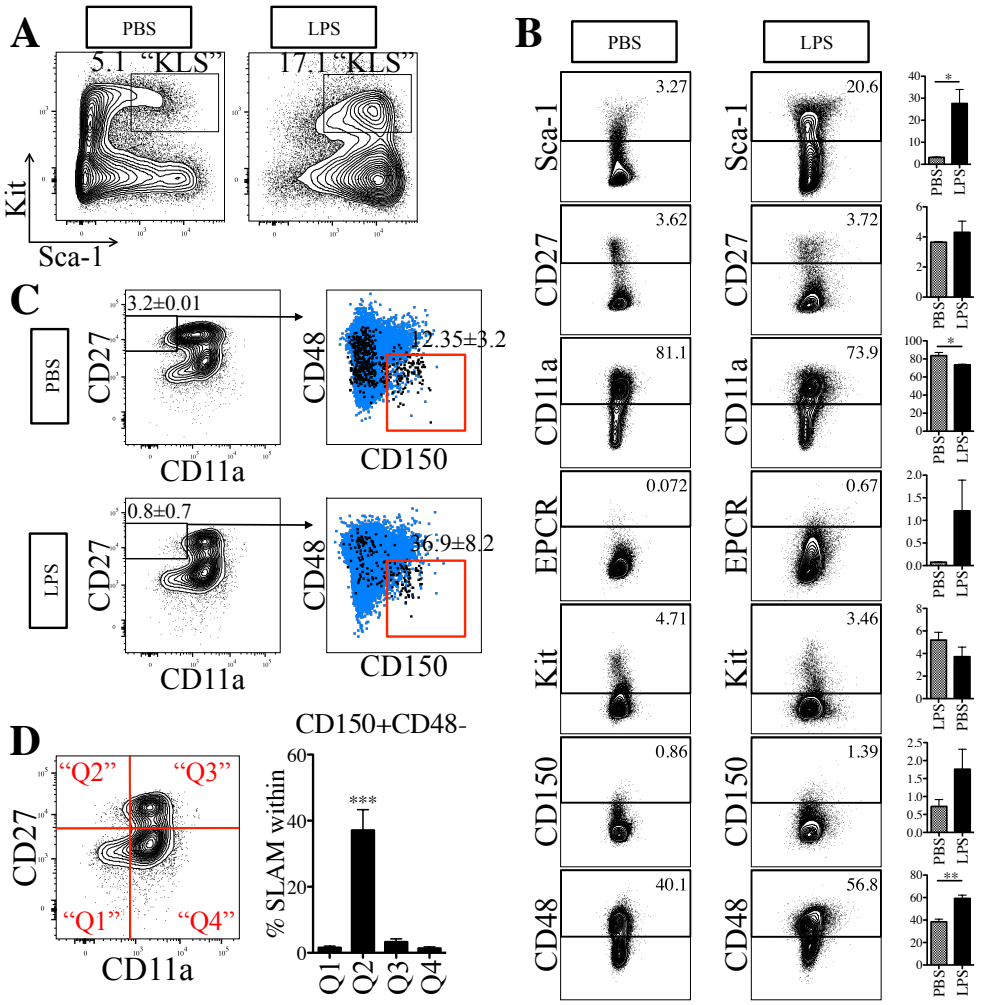
Supporting Information Figure S4. Comparison of total blood chimerism and lineage output between two-color sorting methods. **A)** Time-course analysis of total blood chimerism from CD11a- EPCR+ and CD150+ CD48- sources in primary recipients 4, 9, and 12 weeks post-transplant, and in secondary recipients (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=8, 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. 11a/EPCR staining identifies phenotypic HSCs during poly(I:C)-induced inflammation. A) Expression of Sca-1 and Kit on Ter119- CD27+ BM cells 24 hours after injection with PBS (top plot; n=3) or poly(I:C) (bottom plot; n=3). Numbers are percentages of KLS cells in each condition. **B)** Expression of HSC markers on Ter119- CD11a- EPCR+ in PBS (top panel) and poly(I:C) (bottom panel). Numbers are percentage of gated cells ±SD. Boxed plots show Ter119- CD11a- EPCR+ gated cells (black) and total Ter119- cells (blue). Red gates show phenotypic HSCs and the percentage of Ter119- CD11a- EPCR+ cells that fall within those gates; each condition, n=3. **C)** Expression of HSC markers on BM leukocytes of animals 24 hours after injection with PBS (top row) or poly(I:C) (bottom row), and quantification of percentages in each condition (bar graphs, error bars SD, n=3). FACS plots are gated on Ter119- BM cells. Numbers are percentages of cells positive for each marker. Empty channels AmCyan and Qdot605, which were not used for antibody detection, are the X-axes of FACS plots. Bar graphs are representative of two independent experiments. ***p ≤ 0.01 (Student's unpaired t test).*



Supporting Information Figure S7. 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 24 hours after injection with PBS (left FACS plots; n=2) or PBS (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

Table S1. Antibodies Table				
Antigen	**Clone**	**Conjugate**	**Source**	**Catalogue #**
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
Secondary antibodies				
		Qdot 655-Streptavidin	Life Technologies	Q10121MP
		Qdot 605-Streptavidin	Life Technologies	Q10103MP

Table S2. Marker definitions of populations analyzed	
Population	*Markers used*
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+ or Ter119- CD27+ Kit+ Sca-1+ CD34- CD150+ CD48-
SLAM	CD150+ CD48-