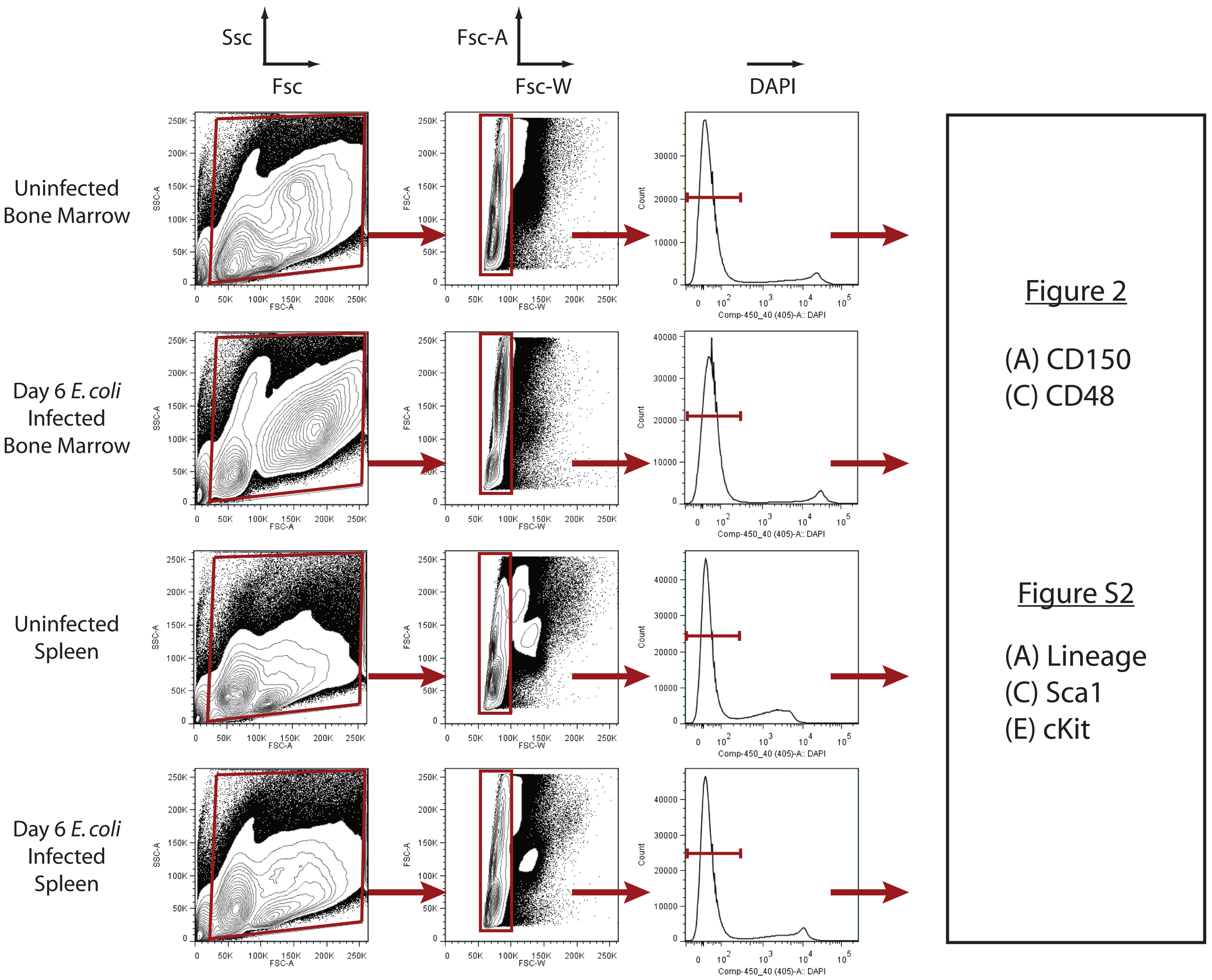


Figure S1 | Pre-gating details, related to Figure 2 and Figure S2

Representative flow cytometry plots showing pre-gating details of sorting experiments described in Figure 2 and Figure S2.

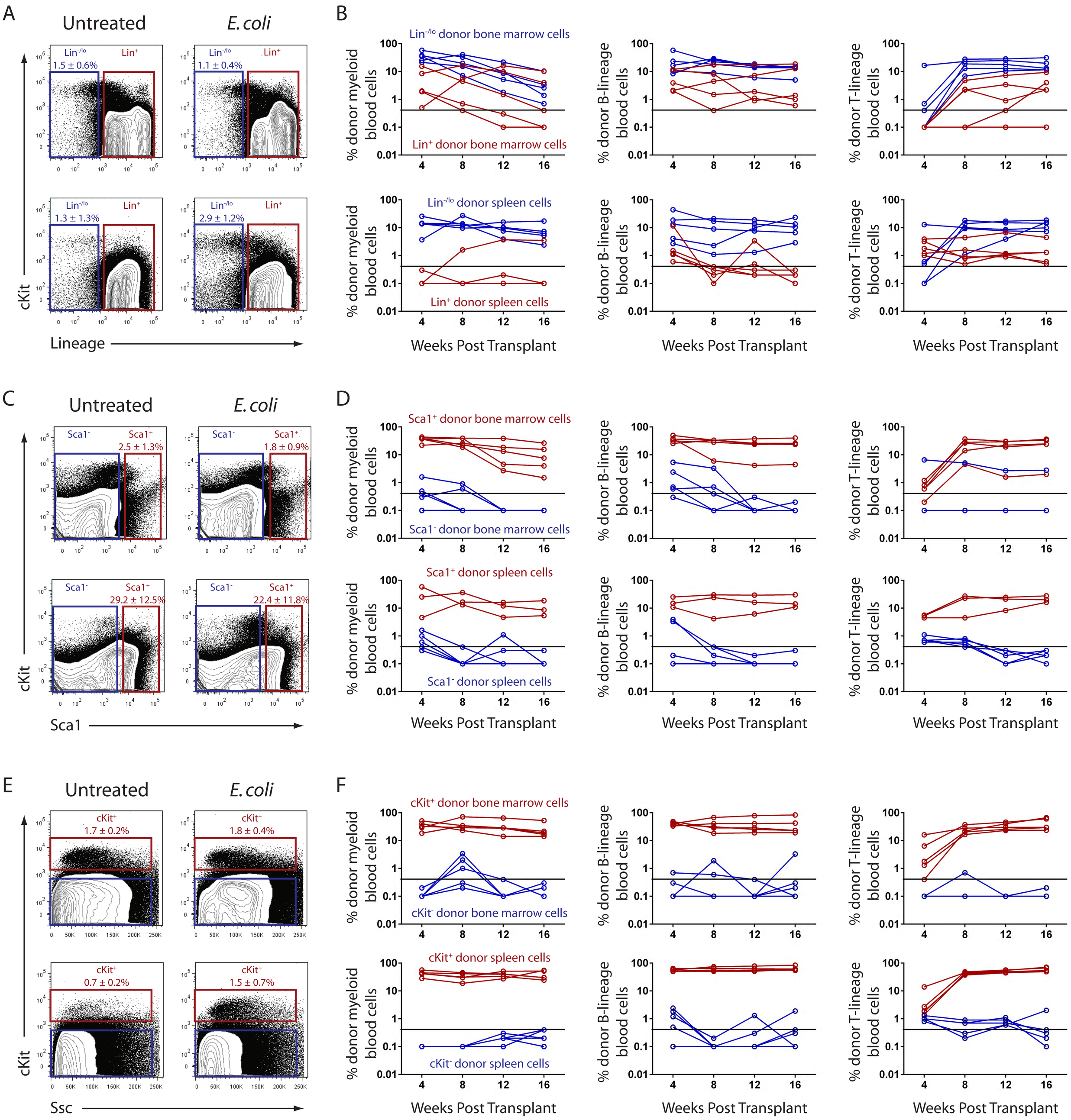
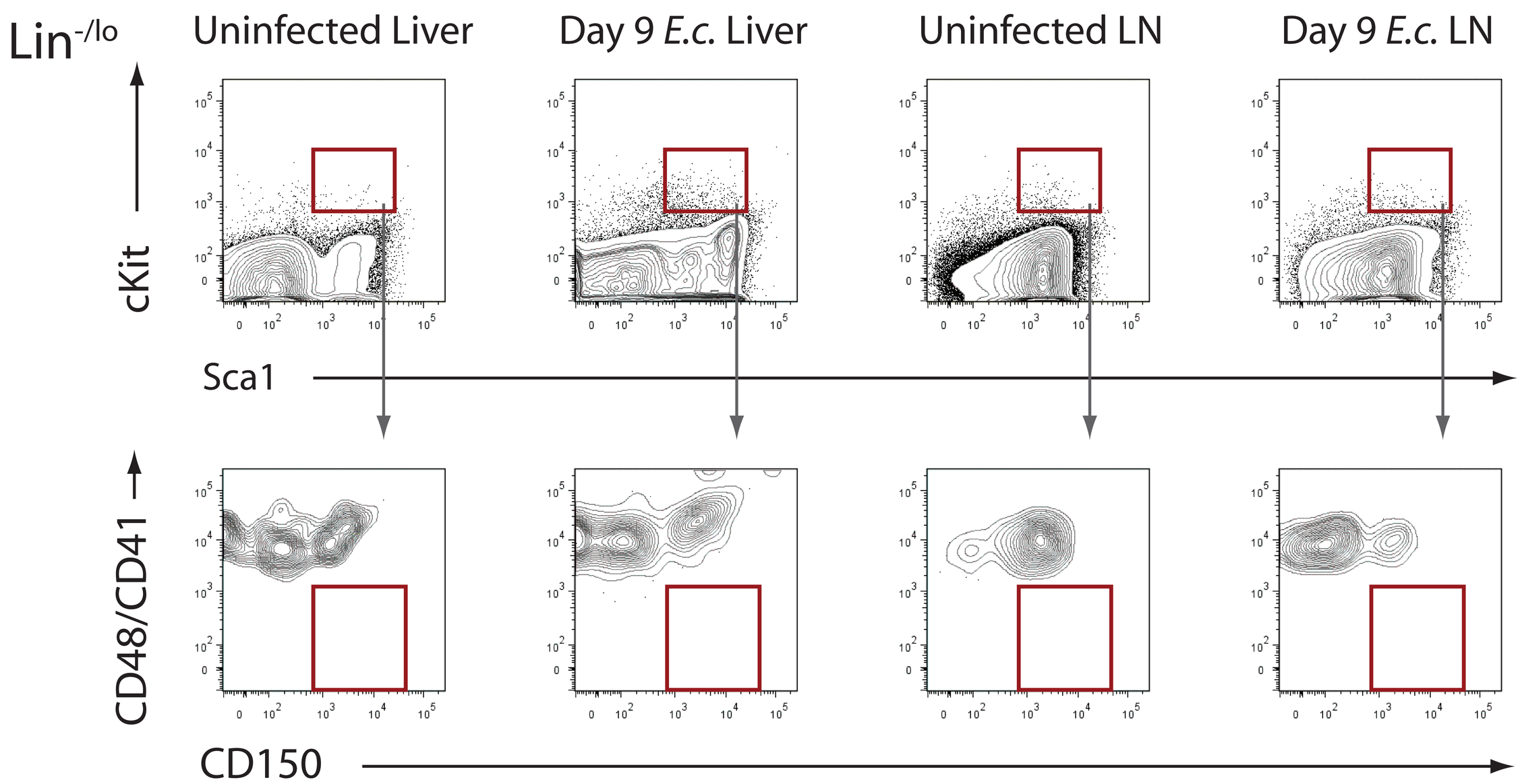


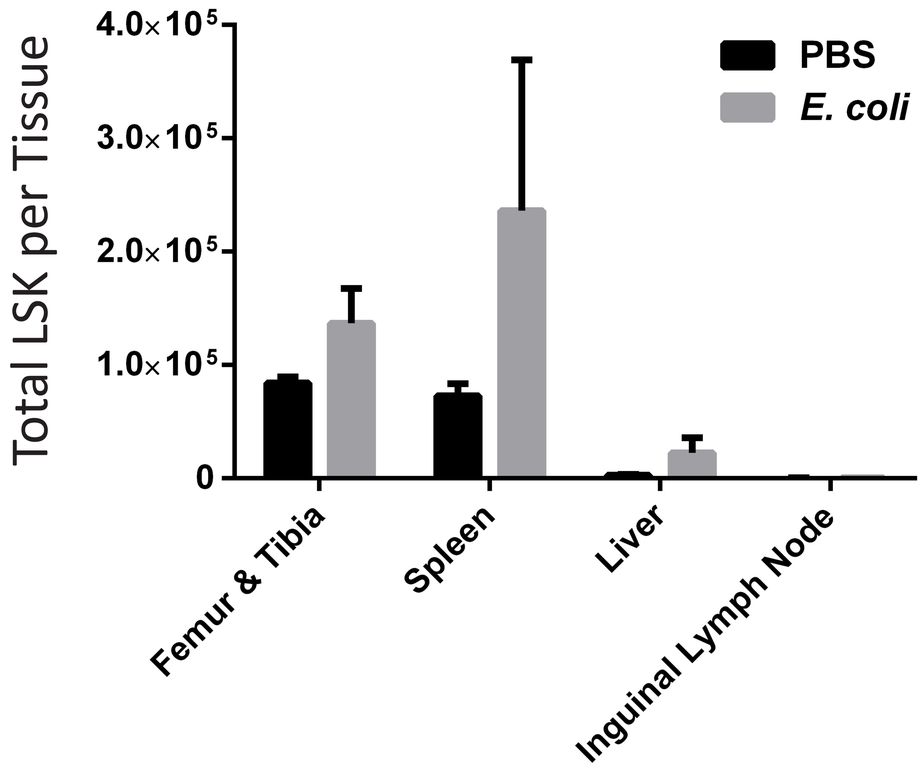
Figure S2 | All HSC activity exists in the cKit+ Sca1+ fraction of bone marrow and spleen after infection, related to Figure 2
(A-F) Wild type (CD45.1) mice were injected with ~1x10^8 E. coli K12 i.p and after 6 days, (top) bone marrow or (bottom) spleen cells were sorted and resorted based on positive or negative expression of (A, B) Lineage (CD3, CD4, CD8, B220, Ter119, Gr-1, Mac1), (C, D) Sca1 or (E,F) cKit. (B, D, F) Positive or negative fractions from (top) bone marrow or (bottom) spleen were mixed with 3x10^5 whole bone marrow from WT (CD45.2) mice and transplanted into lethally irradiated (CD45.2) recipients. Peripheral blood chimerism of Myeloid (Mac1+), B-lineage (B220+) and T-lineage (CD3+) cells was analyzed by flow cytometry. Connected data points represent individual mice bled over successive 4 week intervals. See also Figure S1, Table S1 and Table S2.

Burberry et al Figure S2

A



B



C

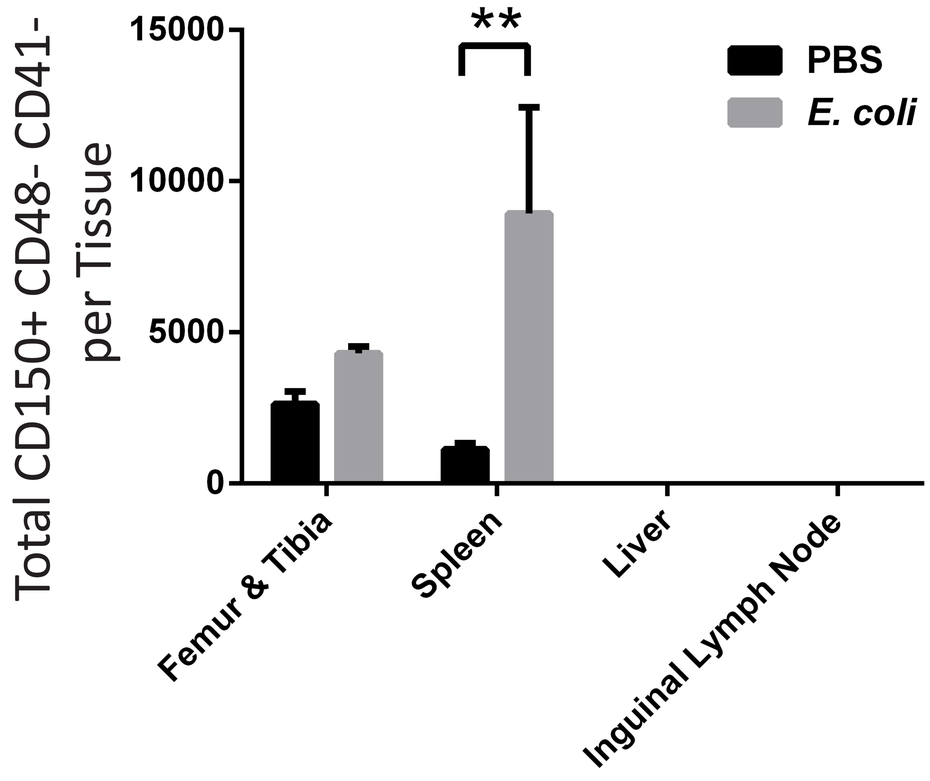


Figure S3 | HSCs do not accumulate in liver or inguinal lymph nodes during infection, related to Figure 3

(A-C) Wild type mice were injected with PBS or ~1x10^8 E. coli K12 i.p. and after 9 days, femur and tibia, spleen, liver and inguinal lymph nodes were isolated. (A) Representative flow plots of liver and inguinal lymph nodes. See Figure 3A and 3B for representative flow plots of bone marrow and spleen. Quantification of (B) total LSK cells and (C) total HSCs (CD150+ CD48- CD41- LSK) per tissue. n = 4 mice per condition. Error bars indicate SEM. **p<0.01 by One way ANOVA.

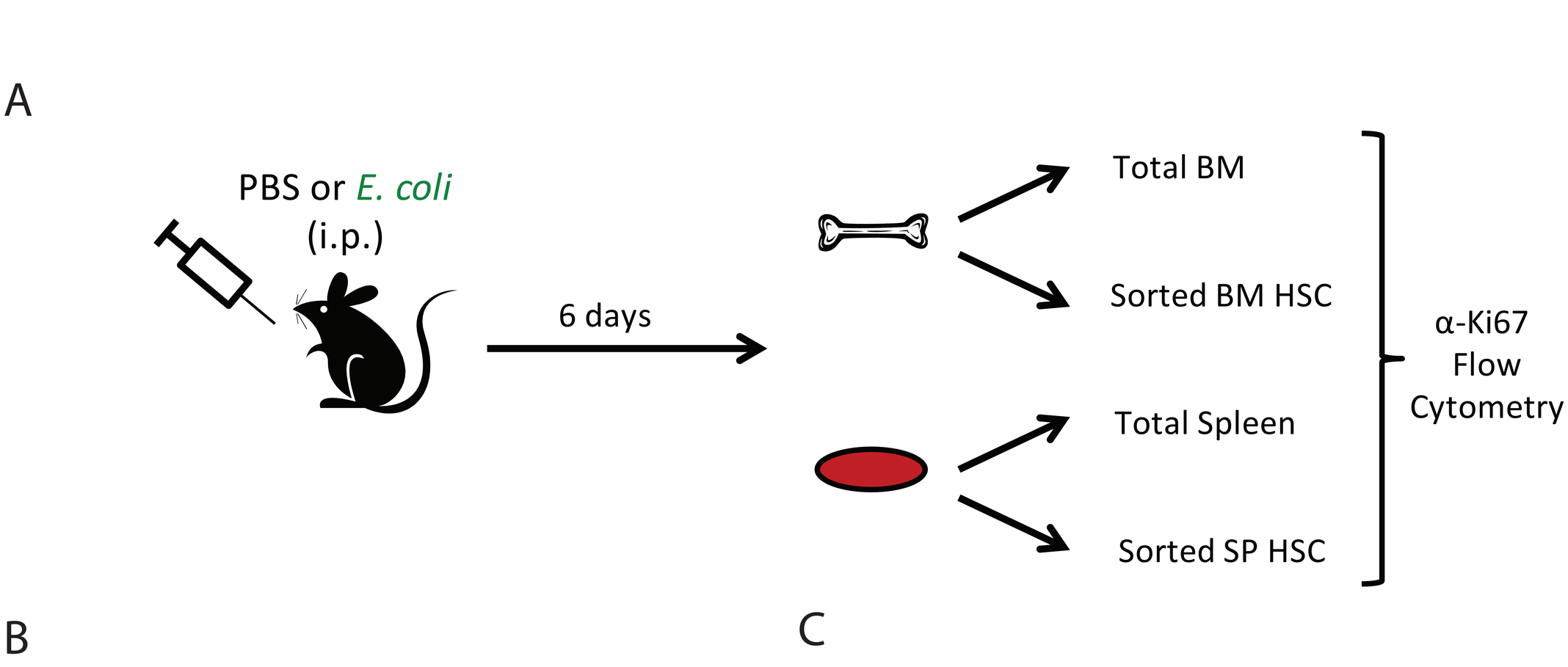


Figure S4 | Splenic HSC accumulation is largely due to mobilization, related to Figure 3

(A) Schematic of experiments in (B, C). Wild type mice were injected with PBS or ~1x10^8 E. coli K12 i.p. and after six days, bone marrow and spleens were harvested for staining and sorting as in described in Supplemental Experimental Procedures. (B) Representative flow plots of intracellular Ki67 staining from total bone marrow, total splenocytes, or sorted HSCs (CD150+ CD48- LSK). There were too few HSCs in individual PBS treated spleens to recover for Ki67 staining. Isotype control cells were stained with Rat IgG2a κ APC. (C) Quantification of Ki67 positivity in each population. n = 6-7 mice per condition. Error bars represent SEM. **p<0.01 ***p<0.001 by one way ANOVA. (ns) not significant.

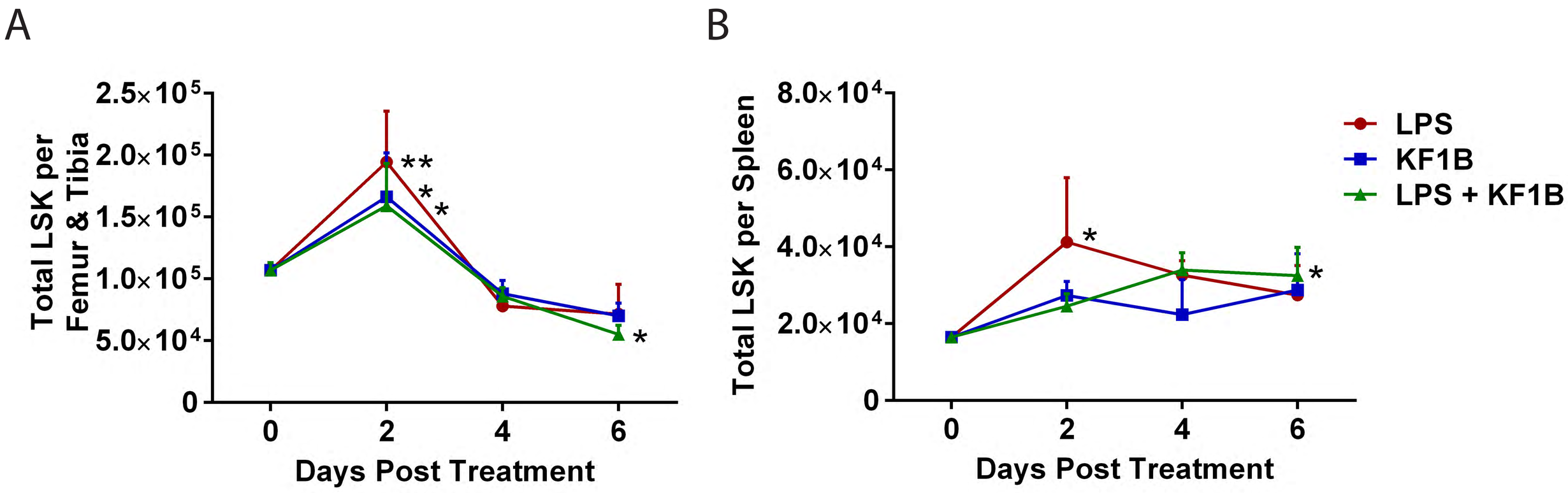


Figure S5 | LSK cell kinetics after NOD1 and TLR4 stimulation, related to Figure 4

Wild type mice were injected with the TLR4 agonist LPS (100 ug), the NOD1 agonist KF1B (50 ug) or both i.p. and LSK were quantified in (A) bone marrow and (B) spleen at indicated time points. n = 3-6 mice per condition. *p<0.05 **p<0.01 by Two way ANOVA versus PBS treated mice.

Burberry et al Figure S5

INVENTORY

Main Figures

Supplemental Figures and Tables

Table S1 | Summary of cells transplanted, related to Figure 2 and Figure S2

Table S2 | Summary of peripheral blood reconstitution, related to Figure 2 and Figure S2

Table S1 | Summary of cells transplanted, related to Figure 2 and Figure S2

	Avg. percentage within infected tissue				Number of cells injected			
	Bone marrow		Spleen		Bone marrow		Spleen	
Population	+	-	+	-	+	-	+	-
CD150	4	94	60	36	1×10^4	2.8×10^5	1.2×10^6	7×10^5
CD48	33	54	81	14	1×10^5	1.6×10^5	1.6×10^6	3×10^5
Lineage	98	1.1	96	3	2.9×10^5	3×10^3	1.9×10^6	6×10^6
Sca1	1.8	78	22	53	5×10^3	2×10^5	4×10^5	1×10^6
cKit	1.8	96	1.5	98	5×10^3	2.9×10^5	3×10^4	1.9×10^6

Table S1 | Summary of cells transplanted, related to Figure 2 and Figure S2

Percentages represent the average of each gated population in bone marrow or spleen of mice that were infected six days prior with *E. coli*. The number of cells to inject (along with $3x10^5$ congenic WBM) was calculated by dividing the percentage of the gated population by 100 and then multiplying by $3x10^5$ for bone marrow or by $2x10^6$ for spleen.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Preparation of hematopoietic cells from liver and inguinal lymph nodes

After sacrificing mice, the liver was perfused with 10 mL HBSS + 2% FBS (buffer) by inserting a syringe with 30.5 G needle into the portal vein. After perfusion, the entire liver was ground over a 100 μm filter into a conical tube. Liver cells were pelleted by centrifugation and supernatant was aspirated. Liver cells were brought up in 5 mL of 40% Percoll (GE Healthcare, salt balanced with buffer) and pipetted gently on top of 6 mL of 60% Percoll (salt balanced with buffer). Tubes were centrifuged for 35 mins at 800 x g with slow ramp up and ramp down. Top layer was aspirated and hematopoietic cells were pipetted from the interphase layer, washed in buffer and stained as described for bone marrow and spleen. Inguinal lymph nodes were isolated, mashed between two microscope slides and stained as above.

HSC sorting and Ki67 staining

Mice were injected with PBS or ~1E8 E. coli i.p. as described in Experimental Procedures. After six days, mice were sacrificed and bulk bone marrow was obtained by crushing two femurs, two tibias and one spine using a mortar and pestle. Spleens were processed as described in Experimental Procedures. Bulk bone marrow and splenocytes were stained with anti-cKit Biotin (Biolegend, clone 2B8) followed by staining with anti-Biotin Microbeads (Miltenyi). cKit+ cells were enriched using AutoMACS (Miltenyi) and stained for HSC markers as described in Experimental Procedures with addition of Streptavidin APC/Cy7 (BioLegend) to visualize cKit. Phenotypically defined HSCs were

sorted for yield and then re-sorted for purity using BD FACSAria II. Cells were fixed in 2% paraformaldehyde (Affymetrix), permeabilized in PBS + 2% BSA + 0.1% Saponin and stained for anti-Ki67 APC (Biolegend clone 16A8).