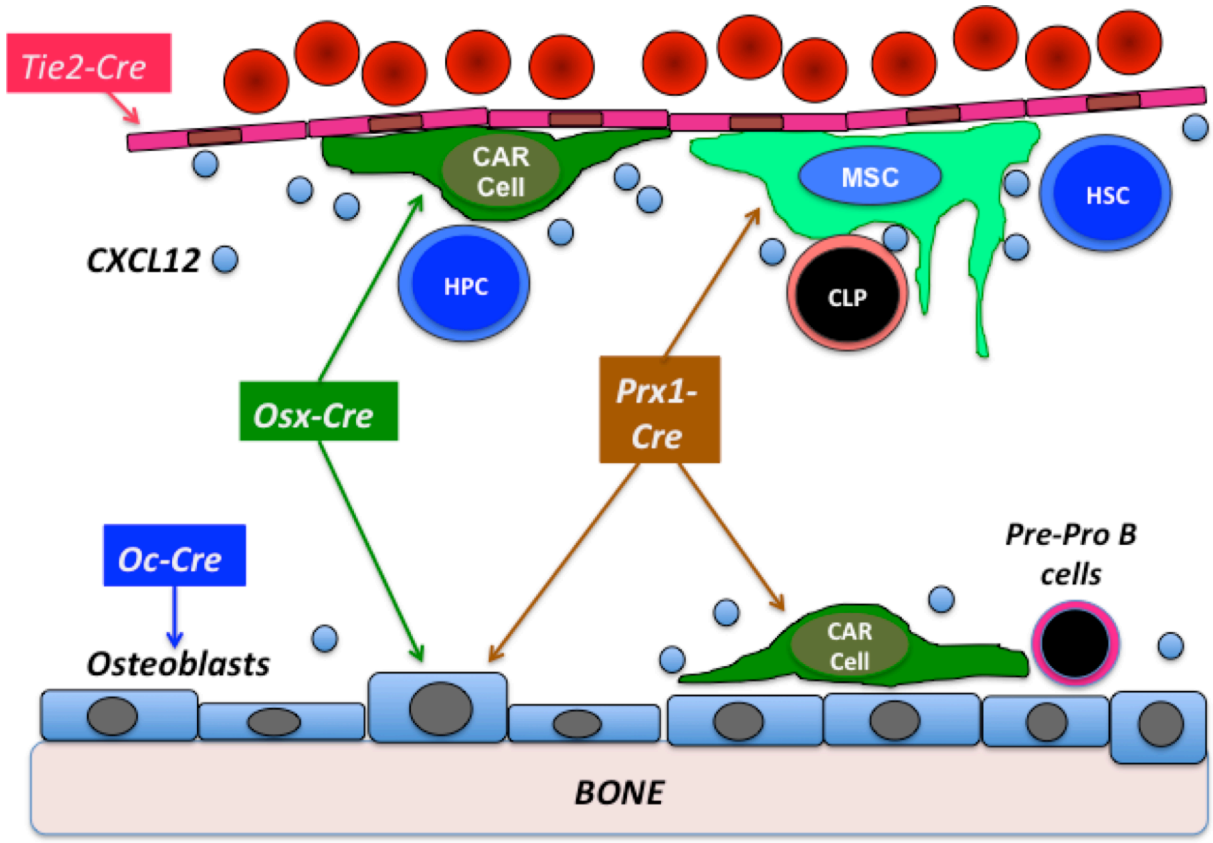
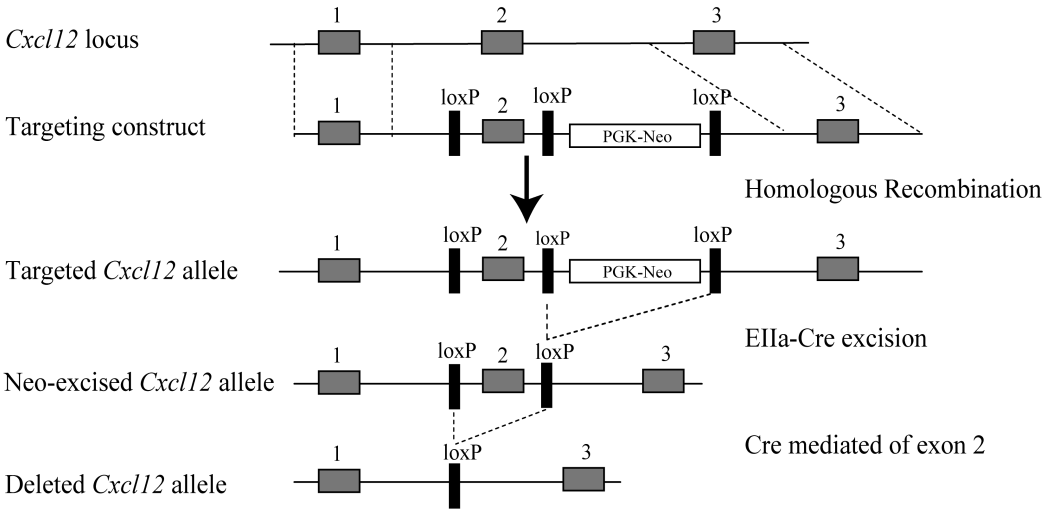
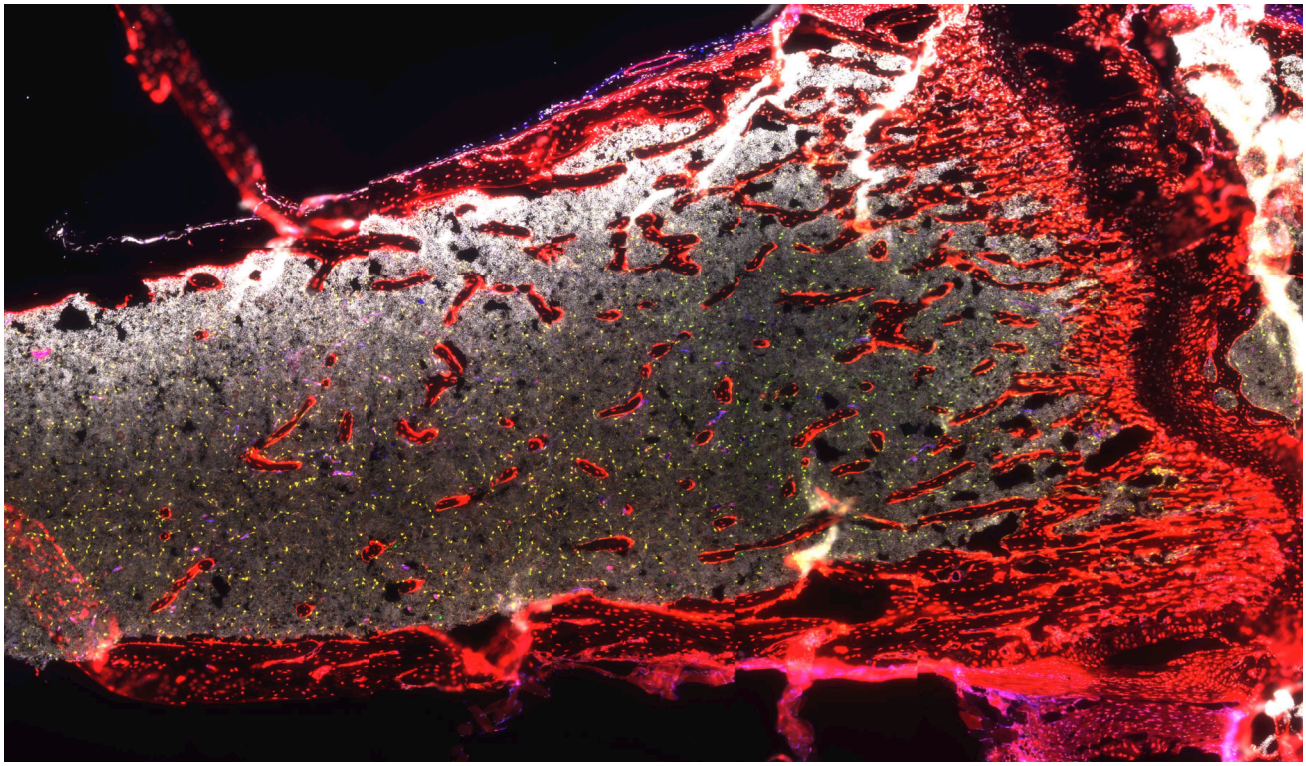


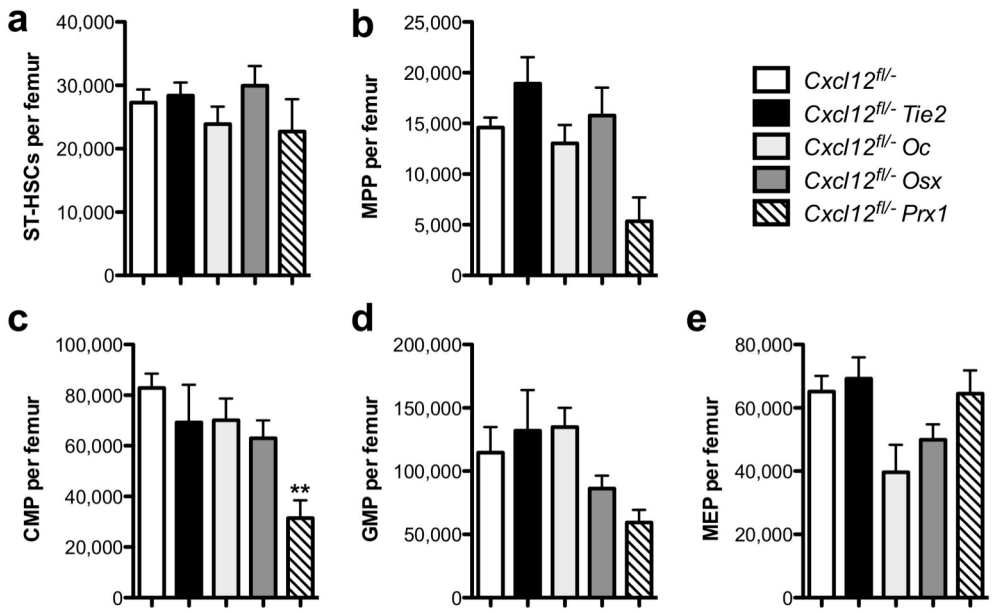
Supplementary Figure 1. Targeted stromal cell populations and model. *Cxcl12* was conditionally deleted using the following transgenes expressing *Cre*-recombinase: 1) *Osteocalcin-Cre* (*Oc-Cre*), which targets recombination in mineralizing osteoblasts; 2) *Osterix-Cre (Osx-Cre*), which targets CXCL12-abundant reticular (CAR) cells and osteoblasts; 3) *Prx1-Cre*, which targets mesenchymal stem cells (MSCs), as well as CAR cells and osteoblasts; and 4) *Tie2-Cre*, which targets endothelial cells. Our studies of hematopoiesis in mice with deletion of *Cxcl12* in these different bone marrow stromal cell populations support the following conclusions. HSC maintenance is dependent on CXCL12 expression from MSCs and, to a lesser extent, endothelial cells. Common lymphoid progenitos (CLPs) are dependent on CXCL12 expression from MSCs, but not other stromal cell populations. In contrast, maintenance of committed B cell precursors (e.g., pre-pro-B cells) is dependent on CXCL12 expression from CAR cells and/or osteoblasts. Finally, retention of hematopoeitic progenitor cells (HPCs) in the bone marrow is dependent on CXCL12 expression from CAR cells. No role for CXCL12 expression from mineralizing osteoblasts in HPC maintenance was observed.



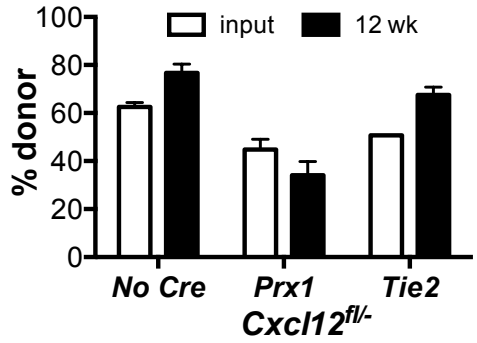
Supplementary Figure 2. Generation of Cxcl12^{fl} allele. The murine *Cxcl12* locus is shown with exons 1-3 depicted as shaded boxes. Homologous recombination in embryonic stem cells with the indicated targeting vector generated the *Cxcl12* targeted allele. Mice carrying this targeted allele were crossed with *EIIa-Cre* mice, and offspring carrying the Neo-excised *Cxcl12* allele (*Cxcl12^{fl}*) were identified. Finally, *Cxcl12^{fl}* mice were crossed with mice carrying tissue-specific *Cre-recombinase* transgenes to generate the deleted *Cxcl12* (null) allele lacking exon 2.



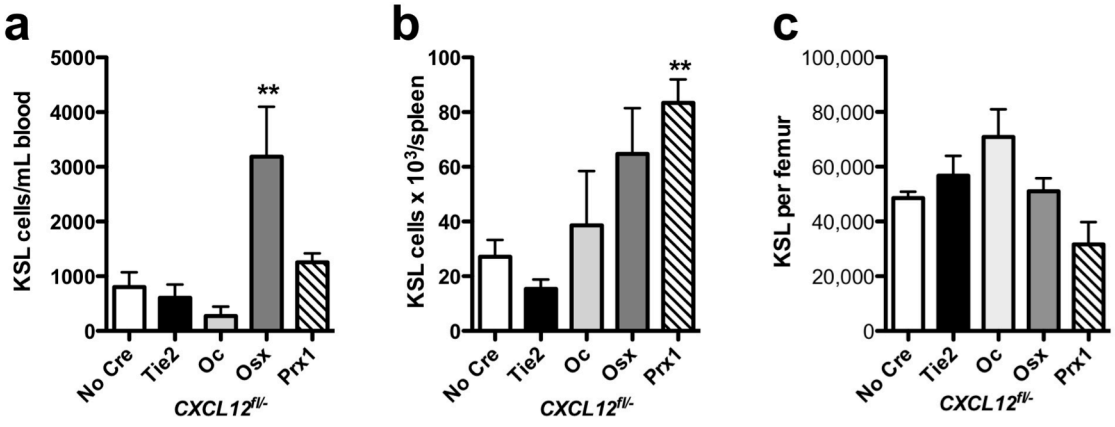
Supplementary Figure 3. Prx1-Cre lineage mapping. Shown is a composite image of the ephiphyseal region of the femur of a *Prx1-Cre ROSA26*$^{Ai9/+}$ *Cxcl12*$^{gfp/+}$ mouse. Cells that had undergone *Cre*-mediated recombination express tdTomato (red). Cells that express CXCL12 also express GFP (green). Cells that express Sca1 antigen are blue. Counterstaining with DAPI highlights nuclei (white).



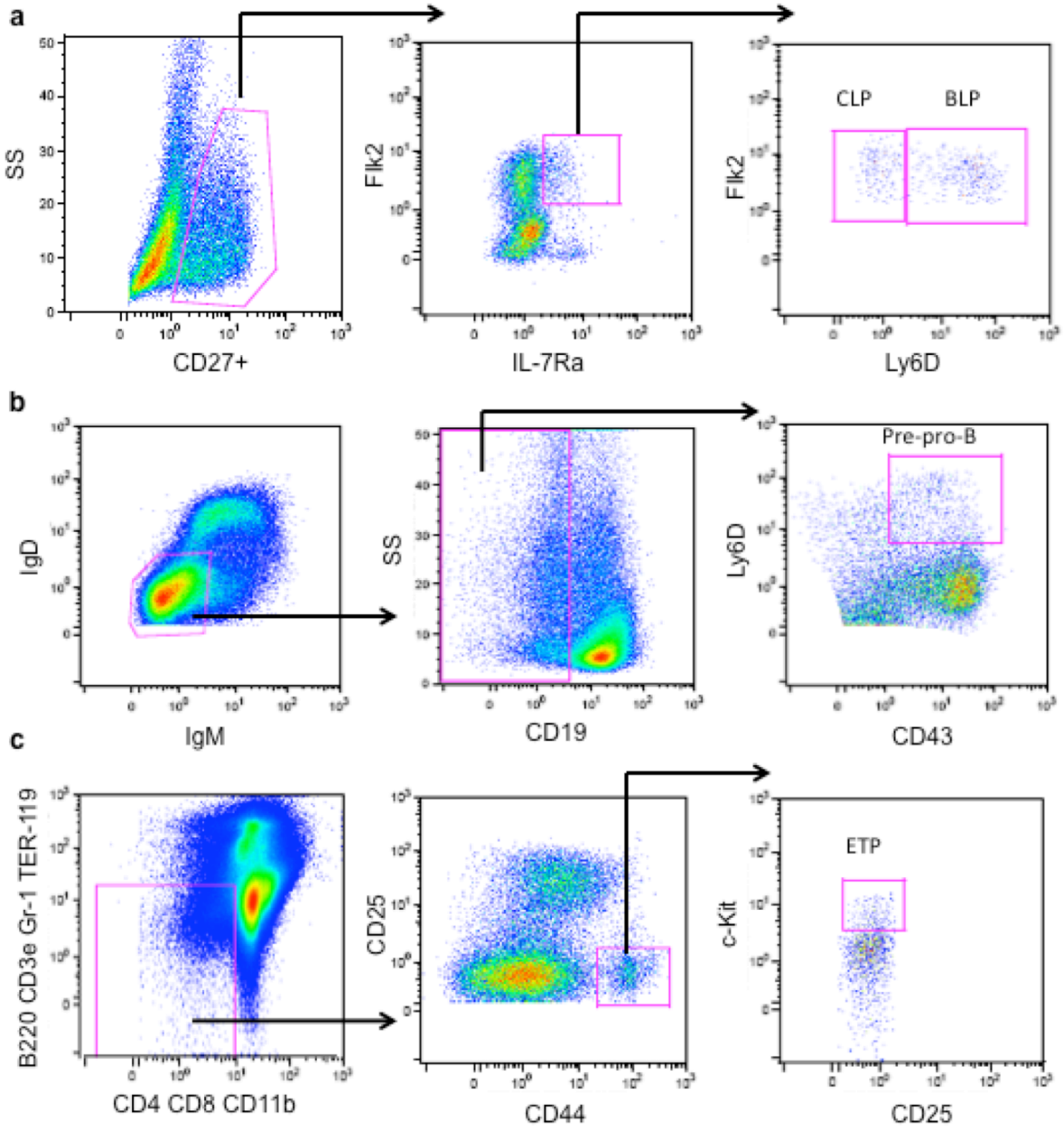
Supplementary Figure 4. Effect of selective Cxcl12 deletion on myeloid progenitors. Bone marrow from the femur of the indicated mice was analyzed by flow cytometry as described in Methods to quantify (**a**) short term-HSCs (ST-HSCs, KSL CD34+ Flk2- cells), (**b**) multipotent progenitors (MPP, KSL CD34+ Flk2+ cells), (**c**) common myeloid progenitors (CMP), (**d**) granulocyte-macrophage progenitors (GMP), and (**e**) megakaryocyte-erythroid progenitors (MEP). **P < 0.01.



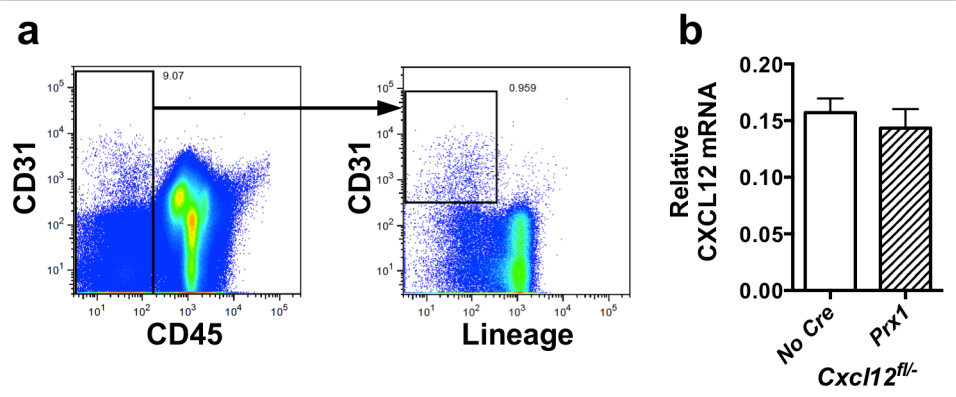
Supplementary Figure 5. Serial transplantation of HPCs from* Prx1-Cre *and Tie2-Cre Cxcl2$^{fl/-}$ mice. Bone marrow from primary recipients competitively repopulated with bone marrow from the indicated mice was transplanted into secondary recipients. Shown is the percentage of donor at the time of secondary transplantation (input) and 12 weeks after transplantation. Data represent the mean ± SEM of 8-12 mice.



S*upplementary Figure 6. HPC mobilization.* Shown is the number of Kit^+ Sca^+ $lineage^-$ (KSL) cells in the blood (**a**), spleen (**b**), and femoral bone marrow (**c**) of mice of the indicated genotype. Data represent the mean ± SEM of 4-8 mice. **P < 0.01.



Supplementary Figure 7. Lymphoid progenitor analysis. (**a**) Representative dot plots showing the gating strategy used to identify common lymphoid progenitors (CLP) and B lymphoid progenitors (BLP) in the bone marrow; data are gated on lineage$^-$ cells. (**b**) Representative dot plots showing the gating strategy to identify pre-pro-B cells in the bone marrow; data are gated on B220$^+$ CD3e$^-$ CD11c$^-$ NK1.1$^-$ cells. (**b**) Representative dot plot showing the gating strategy used to identify earliest thymic progenitors (ETP) in the thymus.



Supplementary Figure 8. CXCL12 mRNA expression in sorted CD31+ endothelial cells.
(**a**) Representative dot plots showing the gating strategy used to sort $CD31^+$ $CD45^-$ $lineage^-$ endothelial cells. (**b**) CXCL12 mRNA expression relative to β-actin in sorted $CD31^+$ $CD45^-$ $lineage^-$ cells from control (*Cxcl12*$^{fl/-}$) and *Prx1-Cre*-targeted (*Prx1-Cre Cxcl12*$^{fl/-}$) mice is shown. Data represent the mean ± SEM of 3-4 mice.

Supplementary Table 1. Cell counts

Organ	*Parameter*	*Unit*	*Cxcl12*$^{fl/-}$	*Cxcl12*$^{fl/-}$ Tie2-Cre	*Cxcl12*$^{fl/-}$ Oc-Cre	*Cxcl12*$^{fl/-}$ Osx-Cre	*Cxcl12*$^{fl/-}$ Prx1-Cre
Blood	WBC	x 10^3 / µL	12.2 ± 1.1	12.6 ± 1.6	9.27 ± 0.59	13.5 ± 1.9	9.63 ± 1.03
	RBC	x 10^6 / µL	9.15 ± 0.63	8.19 ± 0.30	8.83 ± 0.36	9.73 ± 0.50	8.75 ± 0.44
	MCV	fL	45.2 ± 0.3	46.7 ± 0.5	46.0 ± 0.3	45.0 ± 0.8	45.7 ± 0.4
	Plt	x 10^3 / µL	783 ± 64	587 ± 47	681 ± 54	505 ± 90	656 ± 55
	$B220^+$	x 10^3 / µL	5.48 ± 0.65	5.46 ± 0.73	2.99 ± 0.253*	5.05 ± 1.50	3.47 ± 0.60
	$CD3e^+$	x 10^3 / µL	2.02 ± 0.27	2.63 ± 0.54	2.29 ± 0.40	3.80 ± 0.5848*	1.94 ± 0.44
	$Gr1^{hi}$	x 10^3 / µL	1.01 ± 0.20	1.75 ± 0.25	1.03 ± 0.09	1.69 ± 0.29	2.15 ± 0.81
	$CD115^+$ $Gr1^{int}$	x 10^3 / µL	0.79 ± 0.11	0.87 ± 0.15	0.45 ± 0.07	1.32 ± 0.18	0.92 ± 0.31
Bone Marrow	Cellularity	x 10^6 / femur	26.1 ± 1.05	25.8 ± 1.76	22.7 ± 2.27	12.9 ± 0.61**	15.2 ± 0.68***
	$B220^+$	x 10^6 / femur	7.91 ± 1.20	5.48 ± 0.47	5.03 ± 0.38	2.6 ± 0.47***	0.94 ± 0.30***
	$Gr1^{hi}$	x 10^6 / femur	6.18 ± 0.77	8.44 ± 0.88	6.41 ± 0.75	4.53 ± 0.47	4.65 ± 0.75
Spleen	Cellularity	x 10^6	115.90 ± 19.75	113.40 ± 6.07	103.30 ± 17.61	87.17 ± 17.55	96.93 ± 10.35

WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; Plt = platelets.

Bold values are significant relative to *Cxcl12*$^{fl/-}$ mice by one-way ANOVA with Bonferroni post-testing. *$p < 0.05$. ***$p < 0.001$.

Data represent mean ± SEM

Supplementary Table 2. Gene Expression Profiling

Gene Symbol	Gene Title	TdT+ PaS	CAR
Vcam1	Vascular cell adhesion molecule 1	178	1,271
Cxcl12	Chemokine (C-X-C motif) ligand 12	216	2,087
Lepr	Leptin receptor	20	1,065
Nes	Nestin	6	5
MCAM	CD146	28	24
Kitl	Kit ligand	34	484
Thpo	Thrombopoietin	14	12
Angpt1	Angiopoietin 1	10	203
Spp1	Osteopontin	46	628
Sp7	Osterix	16	59
Runx2	Runt related transcription factor 2	12	59
Pparg	Peroxisome proliferator activated receptor gamma	61	133
Ibsp	Bone siaoloprotein	112	2,583
Bglap	Osteocalcin	117	76
Alpl	Aklaline phosphatase	21	71
Sparc	Osteonectin	1,731	3,087
Prg4	Proteocglycan 4	1,411	34
Actb	Beta-actin	2,901	3,030
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	3,678	2,276

Tdt+ PaS: TdTomato+ PDGFRa+Sca+lineage-CD45- cells sorted from *Prx1-Cre ROSA26*$^{Ai9/+}$ mice (n=1).

CAR: CXCL12-bright lineage- CD45+ cells sorted from *Cxcl12*$^{GFP/+}$ mice (n=2, mean is shown).