Supplemental Figures

Supplemental Figure 1. Supplemental data for Figure 1. A mixture of 5x10^6 total BM cells (A) or two thousand FACS-purified Lin^− Sca-1^+ c-Kit^− CD48^− CD34^− CD150^− HSCs (B) from two donor strains with different Cxcr4 genotypes (~1:1 ratio) were transferred via tail vein injection into recipient mice that had undergone lethal irradiation (900 rads) 8 hours prior to transplantation. (A) and (B) Left panels: Experimental design; Right panels: Representative flow cytometry plots corresponding to the experimental design to the left demonstrating the relative contributions of CD45 congenic markers in mixed donor cells prior to transplantation (Donors) and in recipient blood (Recipients) 300 days after BM transplantation (A) or 16 days after HSC transplantation (B) in a single mouse. Percentages of total donor-derived cells for each Cxcr4 genotype are shown in red adjacent to the corresponding gate. R, residual recipient cells post radiation; +/o, +/+ and +/w, cells derived from the Cxcr4^+/o, Cxcr4^+/+ and Cxcr4^+/w donors, respectively.
Supplemental Figure 2.
Isolation of HSCs from lineage-CD34-CD48- BM cells using a sorting cytometer. (A) Gating strategy; (B) Post-sorting verification.
Supplemental Figure 3.
Selective advantage of Cxcr4+/w over Cxcr4+/o donor bone marrow for engraftment is independent of CD45 genotype background: The same competitive repopulation experiments as for Figure 1A were performed with BM from donor mice in which the CD45 genetic background was switched between the Cxcr4+/w and Cxcr4+/o donors. BM cells from donors with a Cxcr4+/w genotype on a CD45.2 homozygous and Cxcr4+/o on a CD45.1/45.2 heterozygous background were mixed (58:42 ratio) and then 5 million BM cells were injected intravenously into each CD45.1 recipient mouse that had been lethally irradiated 8 hours prior. Serial blood draws at various time for flow cytometry were then performed to determine which mouse BM could engraft more readily. (upper left) Experimental design; (upper right) Representative flow cytometry plots demonstrating the relative contributions of CD45 congenic markers in mixed donor bone marrow prior to transplantation (left panel) and in blood after bone marrow transplantation (right panel) in a single mouse; (lower panels) Donor-derived cell frequencies for each Cxcr4 genotype for the leukocyte subsets indicated at the top of each panel. Data are presented as the mean ± SEM percentage (%) of total donor-derived cells for each subset (n=5 mice per data point). SEM was < 5% of the mean in all cases, and therefore is not visible for most data points.
Supplemental Figure 4.
Donor-derived absolute leukocyte counts after non-competitive BM transplantation.
Experimental design is to the left. Data are absolute numbers of donor-derived cells per ml (mean ± SEM) of the subset indicated at the top of each graph. Dashed lines mark the average absolute blood counts of naive Cxcr4+/- (black, n=58) or Cxcr4-/- (blue, n=38) mice from our colony. Each recipient was transplanted with 5 million BM cells. N was at least 5 mice for all conditions. SEM was < 5% of the mean at all timepoints lacking visible error bars. Genotype color codes are to the right of the corresponding graphs. +/-o, +/-+ and +/w denote Cxcr4-/-, Cxcr4+/- and Cxcr4+/- donors, respectively.
Supplemental Figure 5.
Gating scheme for quantitation of BrdU+ HSCs (data summarized in Figure 2D). Cxcr4− total donor BM cells on a homozygous CD45.2 background were mixed with Cxcr4− total donor BM cells on a heterozygous CD45.1/45.2 background (final donor ratio before transplantation = 38:62, Cxcr4−: Cxcr4−) and then injected intravenously into a lethally irradiated CD45.1 recipient mouse. Six days after transplantation, each mouse was given 1.25 mg of BrdU intraperitoneally. Twenty hours later, the mice were euthanized for HSC proliferation analysis. Bone marrow cells were first gated with CD45.2 (Cxcr4+/o) and CD45.1/2 (Cxcr4+/w), then HSCs were gated as Flt3− Lin− Sca1+ c-Kit+ (Flt3−LSK), which includes long-term and short-term HSCs, and BrdU+ cells were quantitated.
Supplemental Figure 6.

**Absolute leukocyte counts.** Total white blood cell counts were measured and leukocyte subset cell counts were calculated from FACS analysis for the mice from the competitive HSC transplantation experiment shown in Figure 1B at day 465 after transplantation.
Supplemental Figure 7 (Supplemental data for Figure 3A). Three types of competitive BM transplantation experiments were performed in non-irradiated congenic Cxcr4+/w recipients: Cxcr4+/o vs. Cxcr4+/+ (51:49 donor ratio of 10^7 total cells), Cxcr4+/+ vs. Cxcr4+/w (41:59 donor ratio of 10^7 total cells) and Cxcr4+/o vs. Cxcr4+/w (47:53 donor ratio of 2x10^7 total cells). Left panels: Experimental design; Right panels: Representative flow cytometry plots demonstrating the relative contributions of CD45 congenic markers in mixed donor BM cells prior to transplantation (Donors) and in the blood of a recipient mouse 180 days after BM transplantation (Recipients). Percentages of total donor cells for each Cxcr4 genotype are shown in red adjacent to the corresponding gate. R, recipient cells; +/o, +/+ and +/w, cells derived from the Cxcr4+/o, Cxcr4+/+ and Cxcr4+/w donors, respectively.
Methods

Mice

Cxcr4 floxed mice (Strain 008767, B6.129P2-\textit{Cxcr4}\textsuperscript{-/-}/J) and EIIa promoter driven Cre recombinase transgenic mice (Strain 003724, B6.FVB-Tg(EIIa-cre)C5379Lmgd/J) (The Jackson Laboratory, Bar Harbor, ME) were crossed to generate \textit{Cxcr4}\textsuperscript{+/+} mice on a homozygous CD45.2 background. \textit{Cxcr4}\textsuperscript{-/-} mice (The Jackson Laboratory) were on homozygous CD45.1 and homozygous CD45.2 backgrounds. \textit{Cxcr4}\textsuperscript{-/-} mice have been previously described (1, 2). Both \textit{Cxcr4}\textsuperscript{+/-} and \textit{Cxcr4}\textsuperscript{-/-} mice on a homozygous CD45.2 background were crossed to \textit{Cxcr4}\textsuperscript{-/-} mice on a homozygous CD45.1 background to produce an F1 generation of \textit{Cxcr4}\textsuperscript{-/-} and \textit{Cxcr4}\textsuperscript{-/-} mice on a heterozygous CD45.1/CD45.2 background. \textit{Cxcr4}\textsuperscript{-/-} mice on a heterozygous CD45.1/CD45.2 background were further crossed to \textit{Cxcr4}\textsuperscript{-/-} mice on a homozygous CD45.1 background to produce \textit{Cxcr4}\textsuperscript{-/-} mice on a homozygous CD45.1 background. Mouse strains were expanded in a specific pathogen free facility and were used as donors and recipients in transplantation experiments at 6-8 weeks of age.

Transplantation Experiments

5-50 million total BM cells (from a single donor strain or as a 1:1 mixture of cells from two donor strains) were transferred via tail vein injection into recipient mice that had undergone lethal irradiation (900 rads) 8 hours prior to transplantation, or into unconditioned \textit{Cxcr4}\textsuperscript{-/-} mice. The donor ratio for mixed BM cells in each competitive transplantation was determined by FACS. In each experiment, donors and recipients
were sex-matched. Results were similar in both male and female mice. Irradiated mice were given water containing neomycin for four weeks post irradiation. All animal experiments were performed using an NIAID Animal Care and Use Committee-approved protocol in approved and certified facilities.

**Blood cell counts**

Blood was collected from mandibular veins of recipient mice using EDTA as anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Total leukocyte counts were measured with a Cellometer Auto 2000 Cell Viability Counter (Nexcelom Bioscience, Lawrence, MA). Absolute leukocyte subset counts and frequencies were quantitated by flow cytometry.

**Flow cytometry analysis**

100 µl of mouse blood was collected, then 2 µl of Fc block (BioLegend, San Diego, CA) was added and incubated for 10 min prior to incubation with specific antibodies on ice for 30 min. The monoclonal Abs used were CD45.1-PECy7 and CD45.2-eFlour450 (catalog #25-0453-82 and #48-0454-82, eBioscience, San Diego, CA), and Ly6G-APC-Cy7, CD11b-PerCP-Cy5.5, CD19-FITC, and CD3-APC (catalog #127624, #101228, #115506, and #100312, Biolegend). Erythrocytes were lysed with 1 ml ACK lysis buffer (Quality Biologicals, Inc., Gaithersburg, MD) for 2 min at room temperature before centrifugation. Cells were then washed twice with FACS buffer, fixed with 1% paraformaldehyde, stored on ice, and analyzed using an LSRII FACS cytometer (BD Biosciences, San Jose, CA) and FlowJo software (TreeStar Inc., Ashland, OR). A similar procedure was followed for staining BM cells isolated from femurs and tibiae of
sacrificed mice. Additional monoclonal Abs used for HSC and HPC analysis were: Lin-FITC (FITC-conjugated antibodies against lineage markers including B220, CD3, CD4, CD8, CD11b, Gr1, and Ter119), Sca1-APC-Cy7, c-Kit-APC, Flt3-PE-Cy5, and IL-7Rα-Violet 605 (catalog #133302, #108126, #105812, #135312, and #135025, Biolegend) and biotinylated CD34-streptavidin-PE (clone RAM) from eBioscience. Lineage depletion of mouse BM cells was performed with a Lineage Depletion Kit from Miltenyi Biotec (San Diego, CA) following instructions of the manufacturer.

**HSC purification**

HSC purification involved first mixing BM from mice with different Cxcr4 and CD45 genotypes in equal amounts, then negatively selecting the population using magnetic bead technology and the Lineage Depletion Kit with antibodies for CD34 (clone RAM, eBioscience), CD48 (catalog #103410, Biolegend) and lineage markers (catalog #130-090-858, Miltenyi Biotec), then positively sorting (FACS Aria II, BD Biosciences) with antibodies directed against Sca-1-BV605, c-Kit-APC and CD150-BV421 (catalog #108133, #105812, #115926, Biolegend). The relative contributions of the two donors were verified post-sorting with CD45 congenic markers. Two thousand FACS-purified LinSca-1-c-KitCD48CD34CD150- HSCs, approximately half from each donor mouse, were then transplanted into each lethally irradiated Cxcr4+/+ (CD45.1) recipient mouse. HSC frequency was not significantly different among the donor mouse strains (2).

**Bone marrow homing analysis**

3.6 million Cxcr4−/− and Cxcr4+/− lineage-negative BM cells were mixed (49:51 ratio) and injected intravenously into lethally-irradiated congenic recipients. Four hours later, the
mice were euthanized and BM was harvested and analyzed by flow cytometry for homing of transplanted HSCs and HPCs.

HSC proliferation analysis

Cxcr4<sup>−/−</sup> BM cells on a homozygous CD45.2 background were mixed with Cxcr4<sup>−/−</sup> BM cells on a heterozygous CD45.1/CD45.2 background, and then 5 million BM cells were injected intravenously into each Cxcr4<sup>−/−</sup> recipient mouse on a CD45.1 background that had been lethally irradiated 8 hours prior. Six days after BM transplantation, each mouse was given 1.25 mg of BrdU I.P., and mice were euthanized 20 hours later. For intracellular staining of BM cells, BD Cytofix/Cytoperm (BD Biosciences) was used according to the manufacturer’s instructions before staining the cells.

Longevity study

Survival of Cxcr4<sup>−/−</sup> (n=15) and Cxcr4<sup>−/−</sup> (n=27) mice was studied using cohoused littermates from multiple breeders.

Statistical analysis

Data are expressed as means ± SEM. Two-tailed student’s t test for single comparisons and 2-way ANOVA for multiple comparisons were used. P less than 0.05 was considered significant.

REFERENCES