

Stem Cell Reports, Volume 13

Supplemental Information

CCR5 Signaling Promotes Murine and Human Hematopoietic Regeneration following Ionizing Radiation

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SUPPLEMENTARY DATA

Figure S1

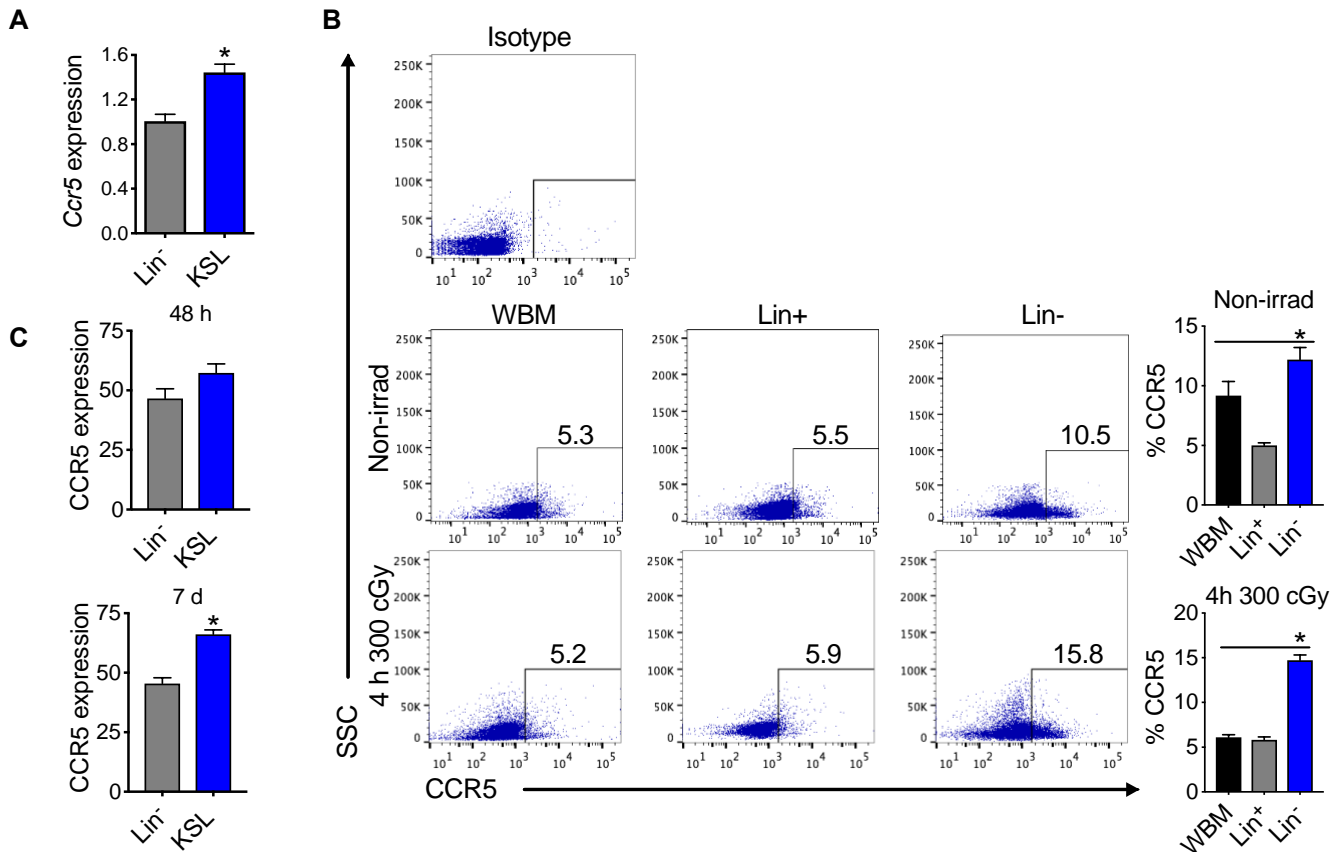


Figure S1. CCR5 expression in hematopoietic cell subsets following irradiation. (A) *Ccr5* mRNA expression of C57BL/6 KSL cells compared to Lin⁻ cells at 2 h following 500 cGy. $n=4$ per group, $*p=0.004$. **(B)** Representative flow cytometric analysis and quantification of CCR5 expression in subsets of non-irradiated hematopoietic cells and at 4 h following 300 cGy. $n=5$ per group. $*p=0.0004$ and <0.0001 by Anova analysis for non-irradiated and irradiated comparisons, respectively. **(C)** Percentage of CCR5 by flow cytometric analysis in Lin⁻ or KSL cells at 48 h and at 7 days following 300 cGy. $n=6$ per group. $*p<0.0001$ at 7 days, respectively. Student's t test (two-tailed with unequal variance) were used in these analyses, except as noted in **(B)**.

Figure S2

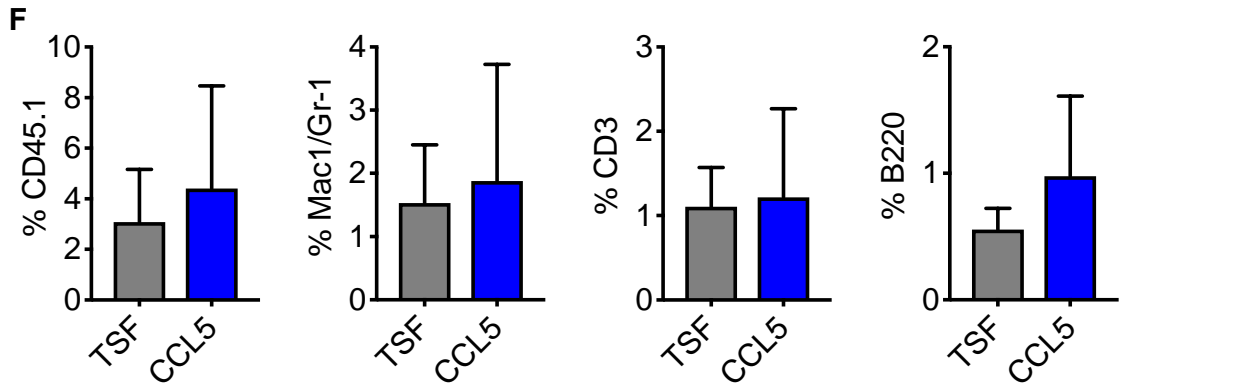
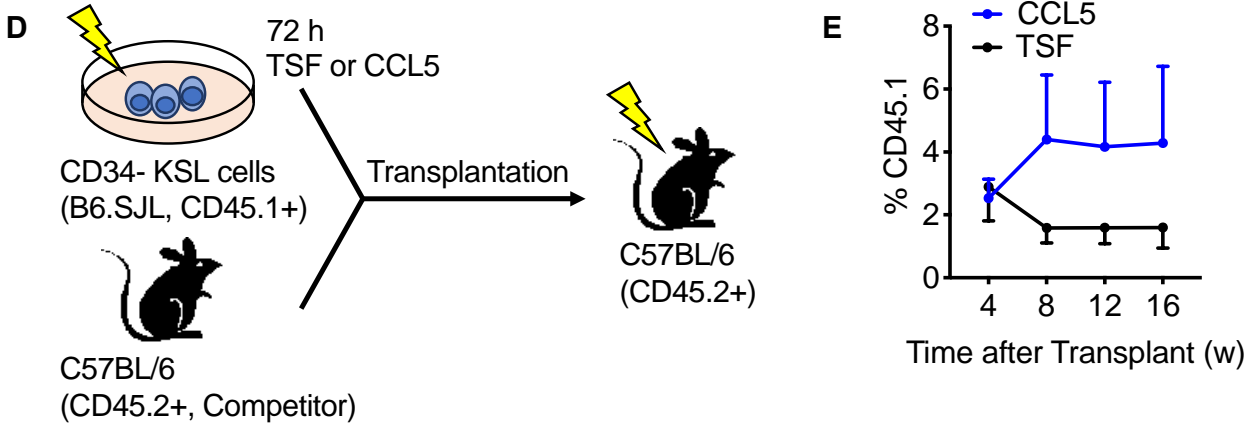
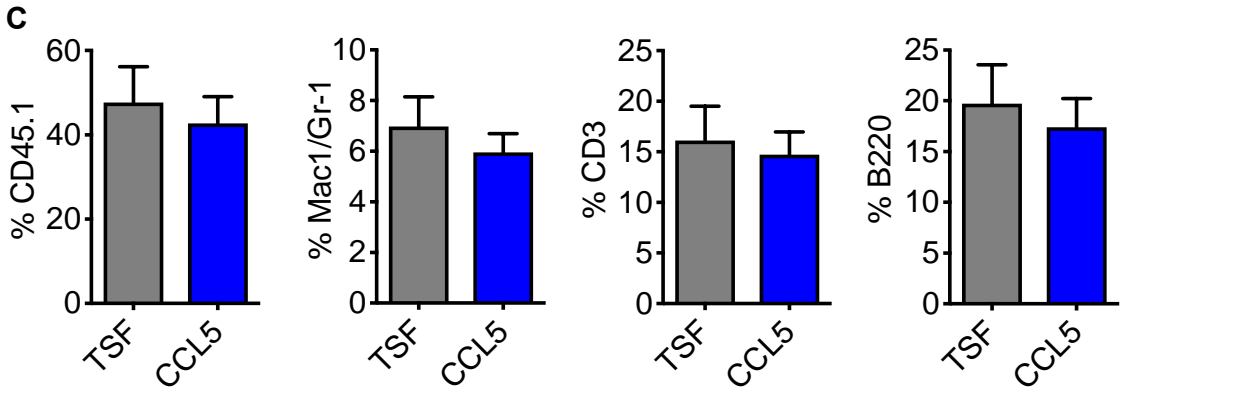
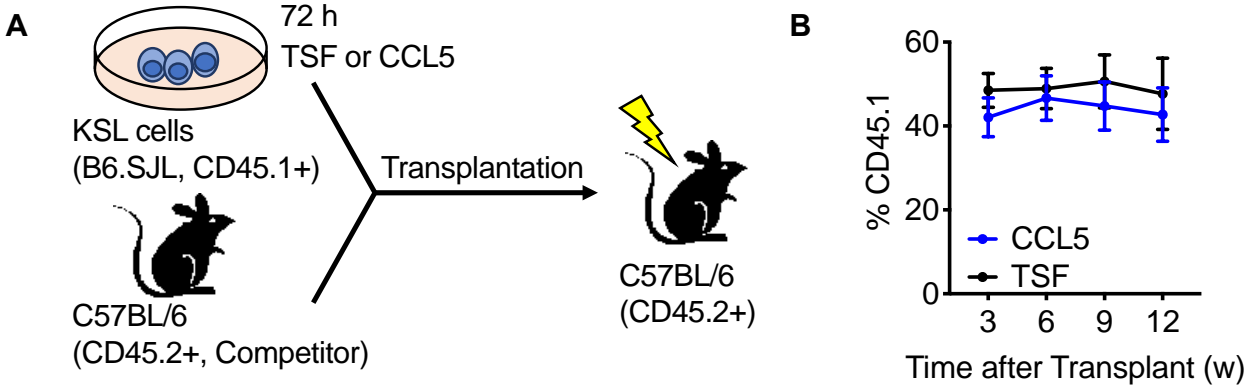


Figure S2. Competitive transplantation following CCL5 treatment in vitro and in vivo. (A)

Schematic diagram of competitive transplantation assay of non-irradiated KSL cells and progeny (B6.SJL, CD45.1+) cultured for 7 days with 30 ng/ml CCL5 and TSF or TSF alone. Recipient mice (C57BL/6, CD45.2+) were conditioned with 900 cGy total body irradiation and then transplanted with 1.5×10^3 non-irradiated KSL cells and progeny along with 2×10^5 competing host bone marrow (CD45.2+). **(B)** Total CD45.1 peripheral blood donor engraftment over time in recipient mice transplanted as outline in **(A)**. **(C)** Total and multi-lineage engraftment in the peripheral blood of recipient mice at 12 weeks following transplantation. $n= 8-9$ mice per group. **(D)** Schematic diagram of study design for competitive transplantation following in vitro treatment with CCL5. CD34-KSL cells were isolated from B6.SJL donor mice (CD45.1) and irradiated with 300 cGy. One thousand CD34-KSL cells per well were cultured with either 30 ng/ml CCL5 + TSF or TSF alone. At 72 h, CD34-KSL cells and progeny were transplanted into lethally-irradiated (900-cGy) C57BL/6 recipient mice along with 2×10^5 competing host bone marrow cells. One well of cultured cells was transplanted into 1 animal. Mice were followed for peripheral blood and marrow engraftment over time. **(E)** Total CD45.1 peripheral blood donor engraftment over time of recipient mice. $n= 11-12$ mice per group. **(F)** Total and multi-lineage engraftment in the bone marrow of recipient mice at 16 weeks following transplantation. Student's t test (two-tailed with unequal variance) were used in these analyses. All comparisons were $p > 0.05$.

Figure S3

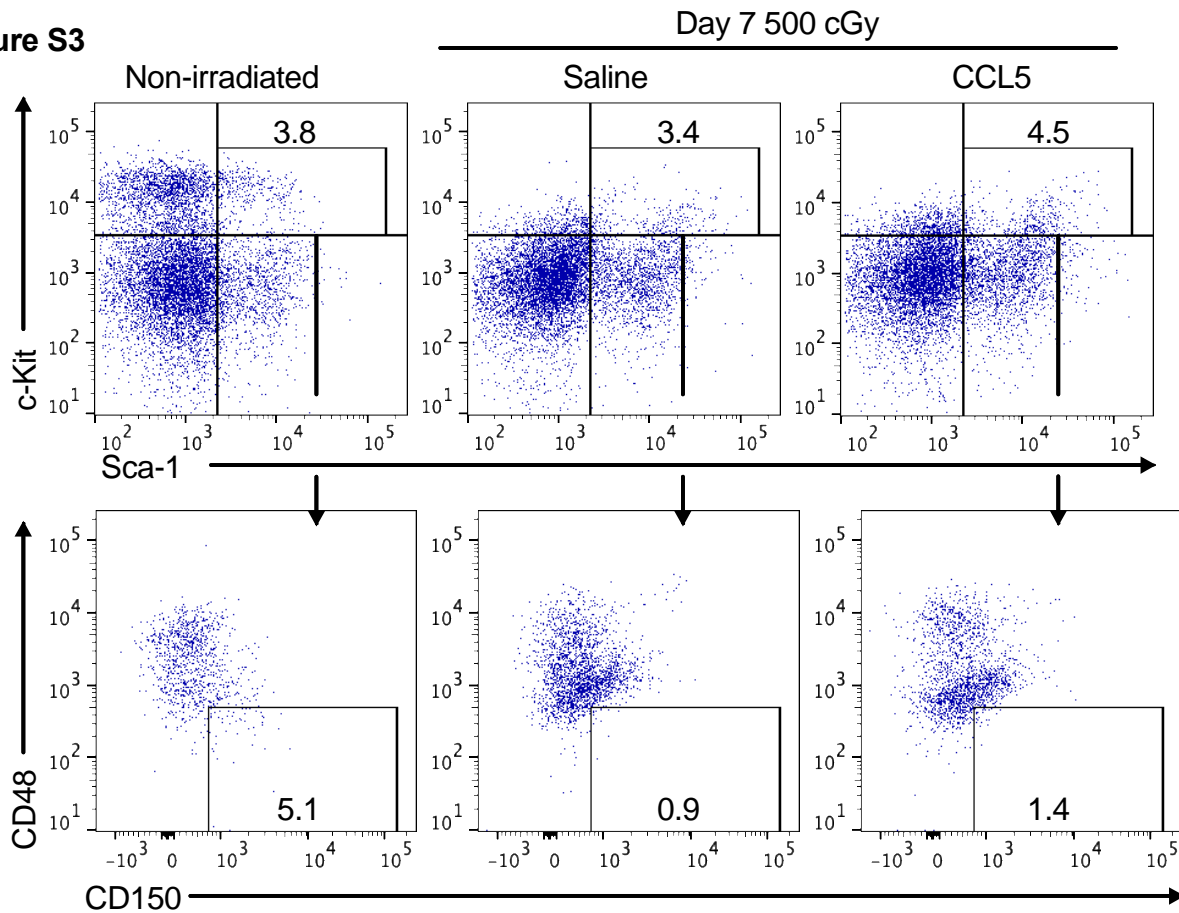


Figure S3. Representative flow cytometric analysis for SLAMF6⁺KSL. From Lineage- cells, ckit+Sca-1⁺ cells are gated (top row). From this KSL gate, CD150⁺CD48⁻ KSL cells are gated (bottom row).

Figure S4

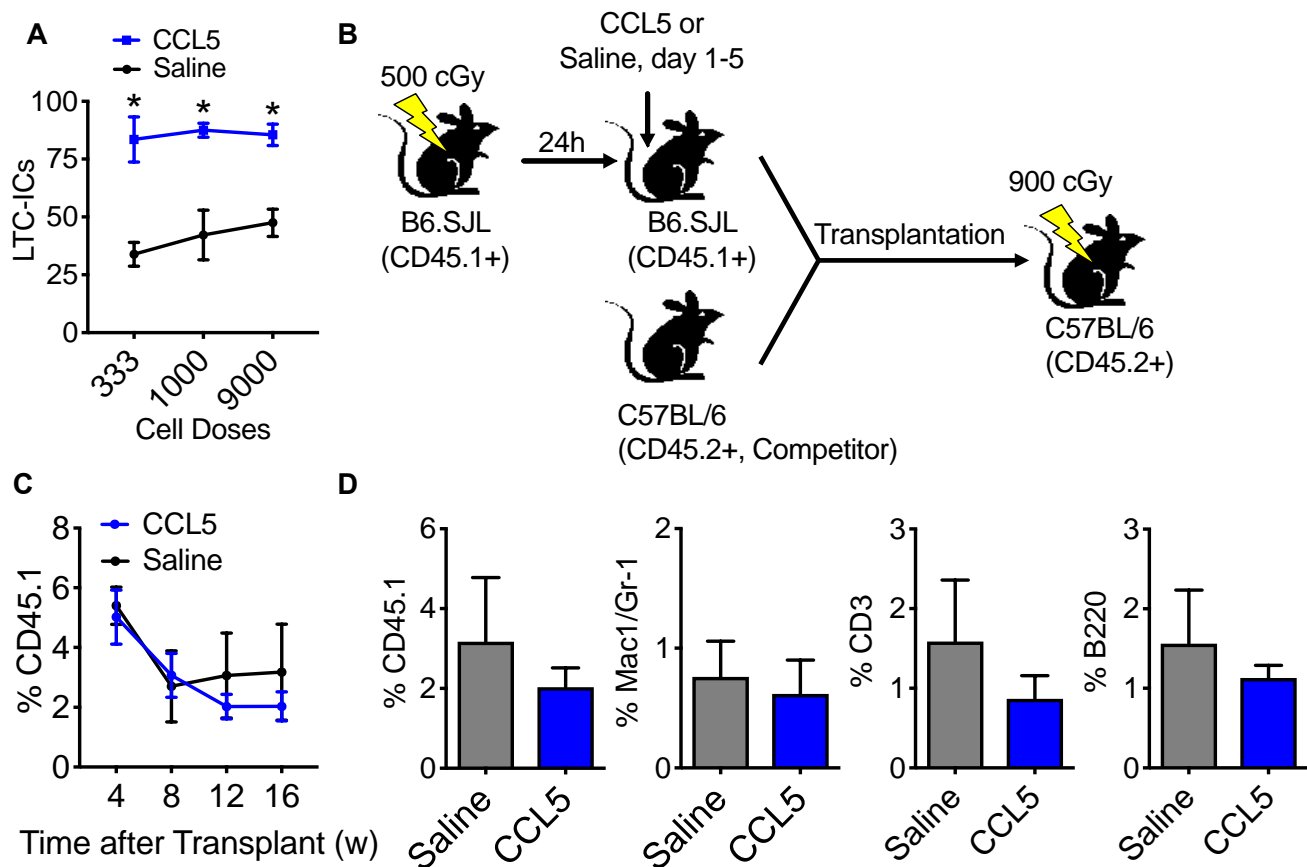


Figure S4. HSC content in C57BL/6 mice following 500 cGy TBI and treatment with CCL5. (A) Levels of long-term colony-initiating cells (LTC-ICs) on day 7 from whole bone marrow (WBM) of irradiated (500cGy) C57BL/6 mice treated with 0.1 $\mu\text{g}/\text{gram}$ body weight CCL5 or Saline subcutaneously starting 24 h after irradiation for 5 doses. $n= 4-7$ per group. * $p= 0.001, 0.002, \text{ and } 0.002$ for cell doses $3.3 \times 10^2, 10^3, \text{ and } 9 \times 10^3$ respectively. **(B)** Schematic diagram of competitive transplantation of donor cells from irradiated (500 cGy) B6.SJL mice treated with 0.1 $\mu\text{g}/\text{gram}$ body weight CCL5 or Saline subcutaneously starting 24 h after irradiation for 5 doses. On day 7 post-irradiation, 10^6 donor cells and 2×10^5 competing host cells are transplanted to irradiated (900 cGy) mice. **(C)** Total peripheral blood donor engraftment of recipient mice transplanted with CCL5- or Saline-treated donor mice. $n= 8-10$ mice per group. **(D)** Total and multi-lineage engraftment in the bone marrow of recipient mice at 16 weeks following transplantation. $n= 8-10$ mice per group. Student's t test

(two-tailed with unequal variance) were used in these analyses. All comparisons in Fig. S4C, D were $p > 0.05$.

Figure S5

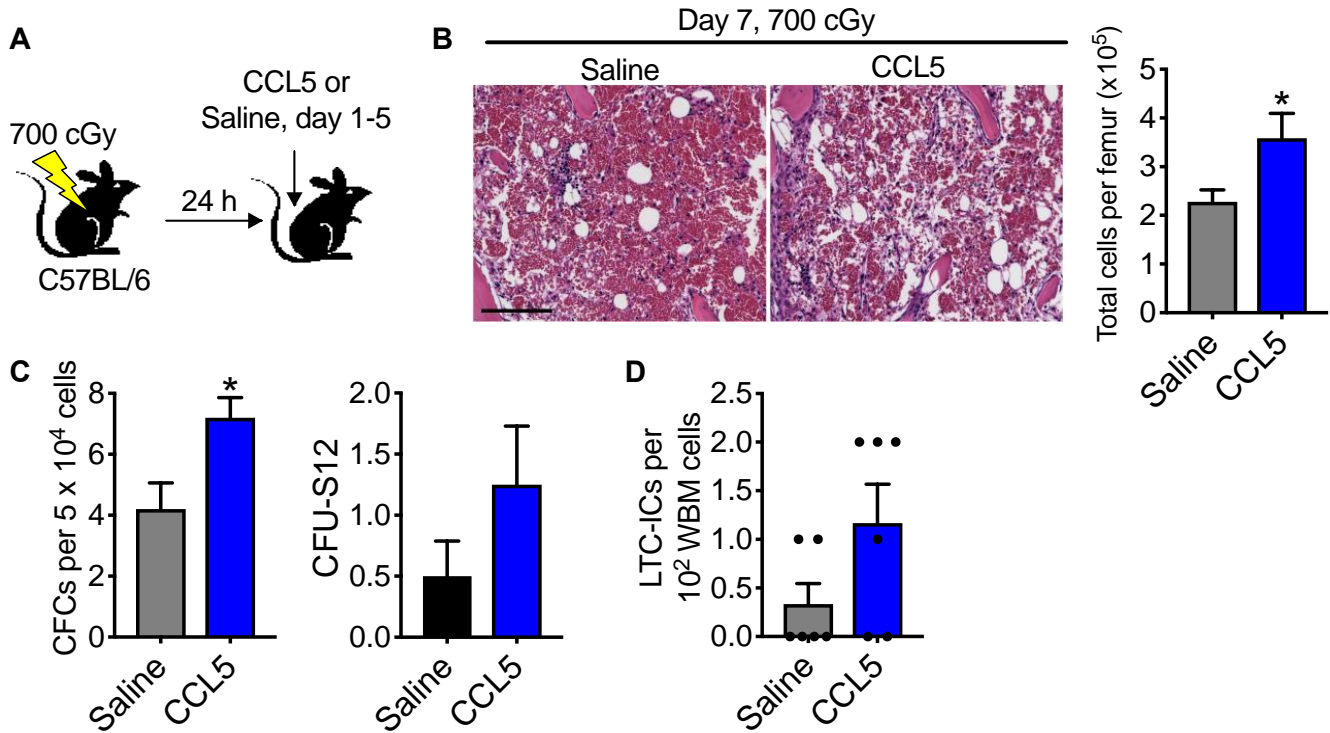


Figure S5. HSPC content in C57BL/6 mice following 700 cGy TBI and treatment with CCL5. (A) Schematic diagram of study design. Irradiated (700 cGy) C57BL/6 mice are treated with 0.1 μg/gram body weight CCL5 or Saline subcutaneously starting 24 h after irradiation for 5 doses. Hematopoietic stem/progenitor assays are performed on day 7 post-irradiation. (B) Left, Representative femurs stained with hematoxylin and eosin. Scale bar 100 μm. Right, total cells per femur. $n = 6-10$ mice per group. * $p = 0.02$. (C) CFCs and CFU-S12. $n = 5$ and 4 per group for CFCs and CFU-S12, respectively. * $p = 0.02$ for CFCs. $p > 0.05$ for CFU-S12. (D) Levels of LTC-ICs as shown. $n = 6$ per group. $p > 0.05$. Student's t test (two-tailed with unequal variance) were used in these analyses.

Figure S6

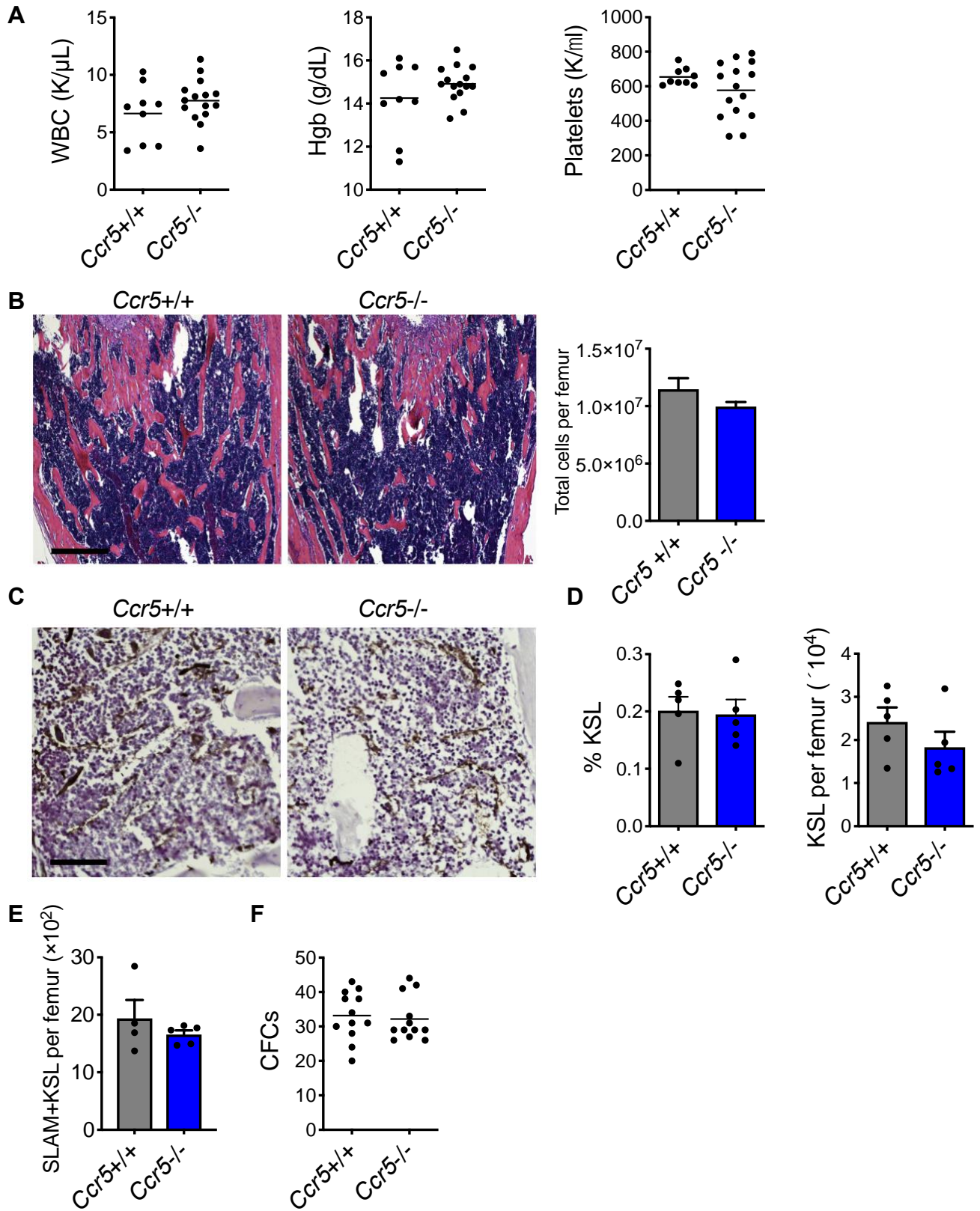


Figure S6. Hematopoietic phenotype of non-irradiated *Ccr5*^{+/+} and *Ccr5*^{-/-} mice. (A) White blood cell count (WBC), Hemoglobin (Hgb), and platelets are shown at baseline in indicated genotypes. *n*= 9-15 per group. (B) Left, representative femur sections stained with hematoxylin and eosin. Scale bar 100 μ m. Right, quantification of total cells per femur. *n*= 5 per group. (C) MECA-32 and hematoxylin-stained femur sections. Scale bar 100 μ m. (D) Percentage KSL and total KSL per femur. *n*= 5 per group. (E) Total SLAMF6⁺KSL cells per femur. *n*= 4-5 per group. (F) Total CFCs per 1×10^4 cells. *n*= 12 per group. Student's t test (two-tailed with unequal variance) were used in these analyses. All comparisons were *p*> 0.05.

Figure S7

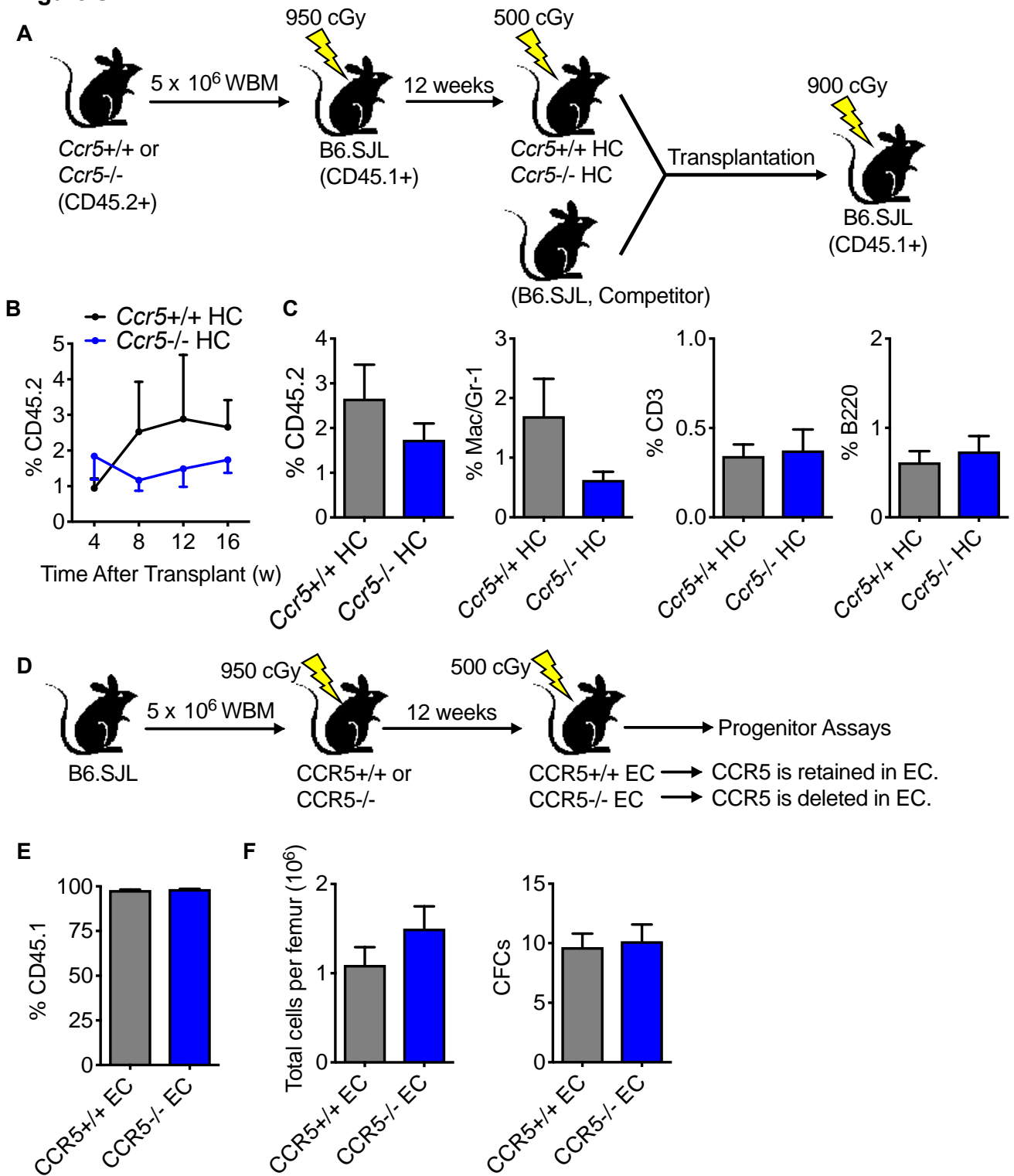


Figure S7. Deletion of CCR5 in hematopoietic cells or in the marrow microenvironment. (A)

Schematic diagram of study design. *Ccr5*^{-/-} hematopoietic cell (HC) and *Ccr5*^{+/+} HC control mice are

generated by transplanting 5×10^6 donor cells from corresponding genotypes into B6.SJL recipients. At 12 weeks following transplantation, *Ccr5*^{-/-} HC and controls are irradiated with 500 cGy. At day 7 following irradiation, 10^6 irradiated donor cells and 2×10^5 competing host marrow cells are transplanted into irradiated recipient mice (B6.SJL). Peripheral blood engraftment is measured over time. **(B)** Total CD45.2 donor engraftment in the peripheral blood of recipients of *Ccr5*^{+/+} (HC) or *Ccr5*^{-/-} HC marrow on day 7 following 500 cGy. $n= 7-8$ per group. **(C)** Total CD45.2+ peripheral blood donor engraftment and multi-lineage engraftment at 16 weeks post-transplantation. **(D)** Schematic diagram for the isolation of *Ccr5* deficiency in the marrow microenvironment. *Ccr5*^{+/+} or *Ccr5*^{-/-} mice were transplanted with WBM cells from B6.SJL mice following 950 cGy total body irradiation to generate *Ccr5*^{-/-} EC mice, which bear a deficiency of *Ccr5* in endothelial cells and other cells within the marrow microenvironment, and also control *Ccr5*^{+/+} EC mice, which retain *Ccr5*. At 12 weeks post-transplantation, mice were exposed to 500 cGy and hematopoietic response was determined. **(E)** RT-PCR analysis of peripheral blood donor chimerism of *Ccr5*^{+/+} EC and *Ccr5*^{-/-} EC mice. $n= 12$ per group. **(F)** Total cells per femur and CFCs at day 7 following 500 cGy total body irradiation. $n= 4-6$ per group. Student's t test (two-tailed with unequal variance) were used in these analyses. All comparisons were $p > 0.05$.