Supplemental Data

Murine Erythroid Short Term Radioprotection Requires a BMP4 Dependent , Self Renewing Population of Stress Erythroid Progenitors.

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Supplemental methods

Immunofluorescence Microscopy

Paraffin embedded spleen sections were deparaffinized and rehydrated through an ethanol series as described previously (3, 7). Sections were incubated for 1 hour each at room temperature with the primary antibody and then washed with PBS for several times. BMP4 (Novocastra Laboratories/Vector Laboratories, Burlingame, CA) and SCL (Santa Cruz Biotech, CA) and CD45.2 (BD-Pharmingen, San Diego, CA) antibodies were used at the manufacturer's recommended dilutions. Alexa Fluor (Molecular Probes, Eugene, OR) fluorescent secondary antibodies were then added. For negative controls, appropriate isotype controls were used. Sections were mounted in Slowfade (Molecular Probes, Eugene, OR) and analyzed by digital microscopy (Olympus BX-60 Epi-Fluorescence Digital digital microscope). Fluorescence quantification was analyzed by ImagePro (Media Cybernetics, Bethesda, MD) and images were processed with Adobe Photoshop 8.0 software (Adobe Systems, San Jose, CA).

Figure S1, Harandi *et al.*

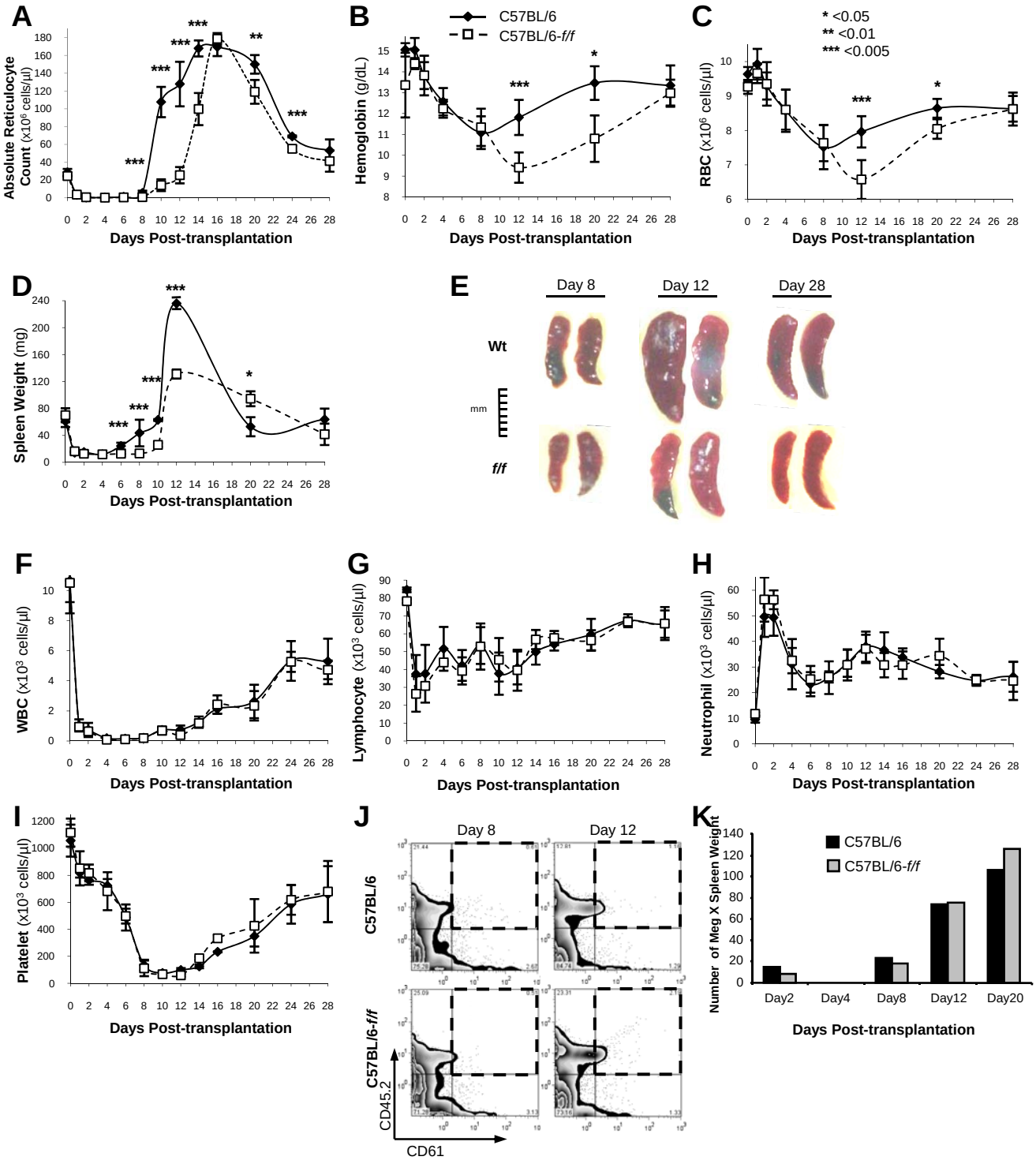

Figure S1. Mice transplanted with *f* mutant bone marrow exhibit a defect in erythroid short-term radioprotection. C57BL/6 mice were irradiated and transplanted with 5x10^5 cells of unfractionated bone marrow of C57BL/6-*f/f* mutant or C57BL/6 control bone marrow. (A) Absolute Reticulocyte Count, (B) Hemoglobin, (C) RBC, (D) Spleen Weight, (E) Representative photos of spleens isolated from mice transplanted with mutant or control donor cells on the indicated days after transplant. A ruler (mm) is shown to the left for comparison.

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Continue Figure 1. Mice transplanted with *f* mutant bone marrow exhibit a defect in erythroid short-term radioprotection. (F) WBC count, (G) Lymphocyte, (H) Neutrophil, (I) Platelet count, (J) Flow cytometry analysis of donor derived megakaryocytes (CD45.2+CD61+) in the spleens on mutant and control transplanted mice on Day 8 and 12 after transplant. (K) On the indicated days spleens were isolated, fixed and paraffin sections generated. The spleen sections were stained with Hematoxylin and Eosin. The number of megakaryocytes were counted in each section. The total number of megakaryocytes was normalized to spleen weight. The values for day 0 indicate non-transplanted mice. For each figure, each time point represents 4-18 recipients from at least 3 independent experiments. *$p<0.05$, **$p<0.01$, ***$p<0.005$.

Figure S2, Harandi *et al.*

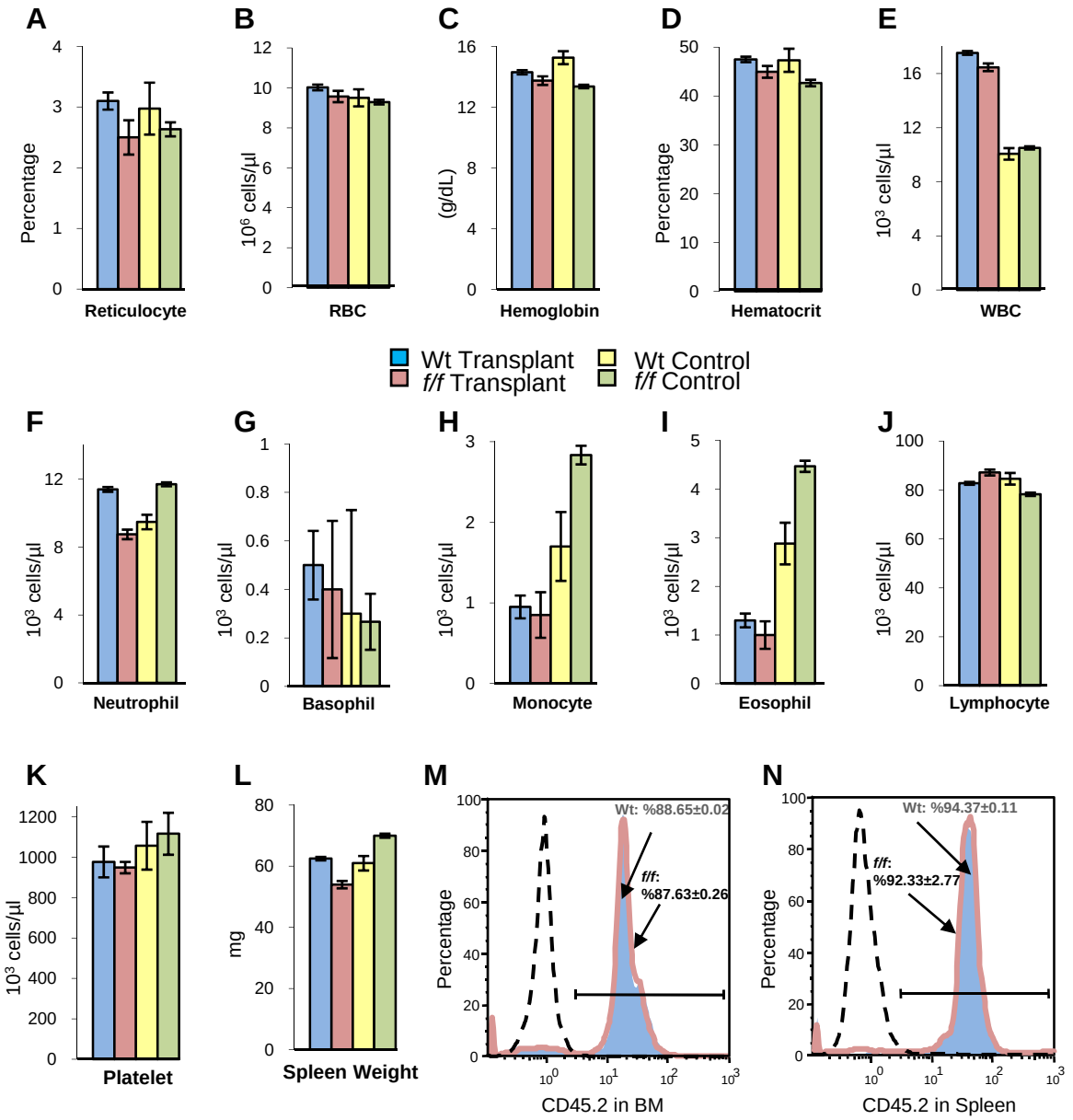


Figure S2. *f/f* mutant donor cells do not exhibit a defect in long term reconstitution. CD45.1+ recipient mice were transplanted with 5x105 f/f or control bone marrow cells (CD45.2+). Analysis of reconstitution was done 128 days after transplant. (A) Reticulocytes, (B) RBC counts, (C) Hemoglobin concentration, (D) Hematocrit, (E) WBC counts; Differential count: (F) Neautrophils, (G) Basophils, (H) Monocytes, (I) Eosophils, (J) Lymphocytes, and (K) Platelet count were done using the Advia 120 hematology autoanalyzer with Veterinary software. (L) Spleen weight. Contribution of donor derived (CD45.2+) cells was measure by flow cytometry analysis of (M) Bone marrow cells and (N) spleen cells. The dashed line is the unstained control cells. There was no significant differences between mice transplanted with *f/f* or control wildtype bone marrow or control untransplanted wildtype and f/f mice.

Figure S3, Harandi *et al.*

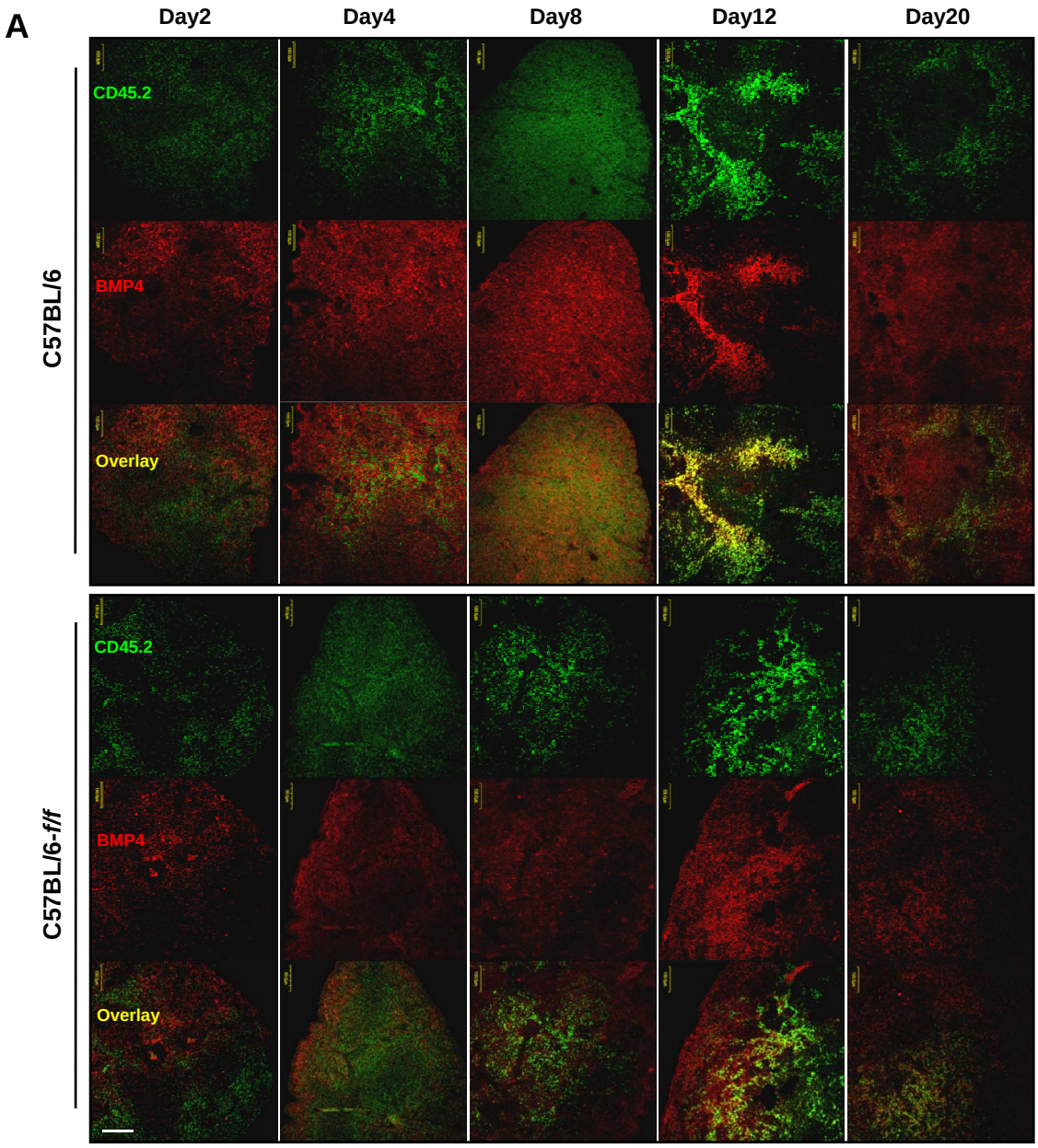


Figure S3. Expression of BMP4 is induced in the spleen during recovery from bone marrow transplant. Paraffin sections of spleens isolated on the indicated day after transplant with 5x105 cells of C57BL/6-*f/f and C57BL/6* control donor cells into CD45.1 recipients. The sections were double immunostained with antibodies to BMP4 (red) and CD45.2 (green). (A) The sections were examined by confocal microscopy at 20X magnification. Scale bar is 100µm. (B) 40X magnification. (C) Co-localization of BMP4 and CD45.2 was determined by Pearson's correlation. Scale bar is 20µm. (D) Expression BMP4 and donor derived cells on day 8 fater transplant is limited to the red pulp (RP) and excluded from the white pulp (WP).

Figure S3, Harandi *et al.* Continued

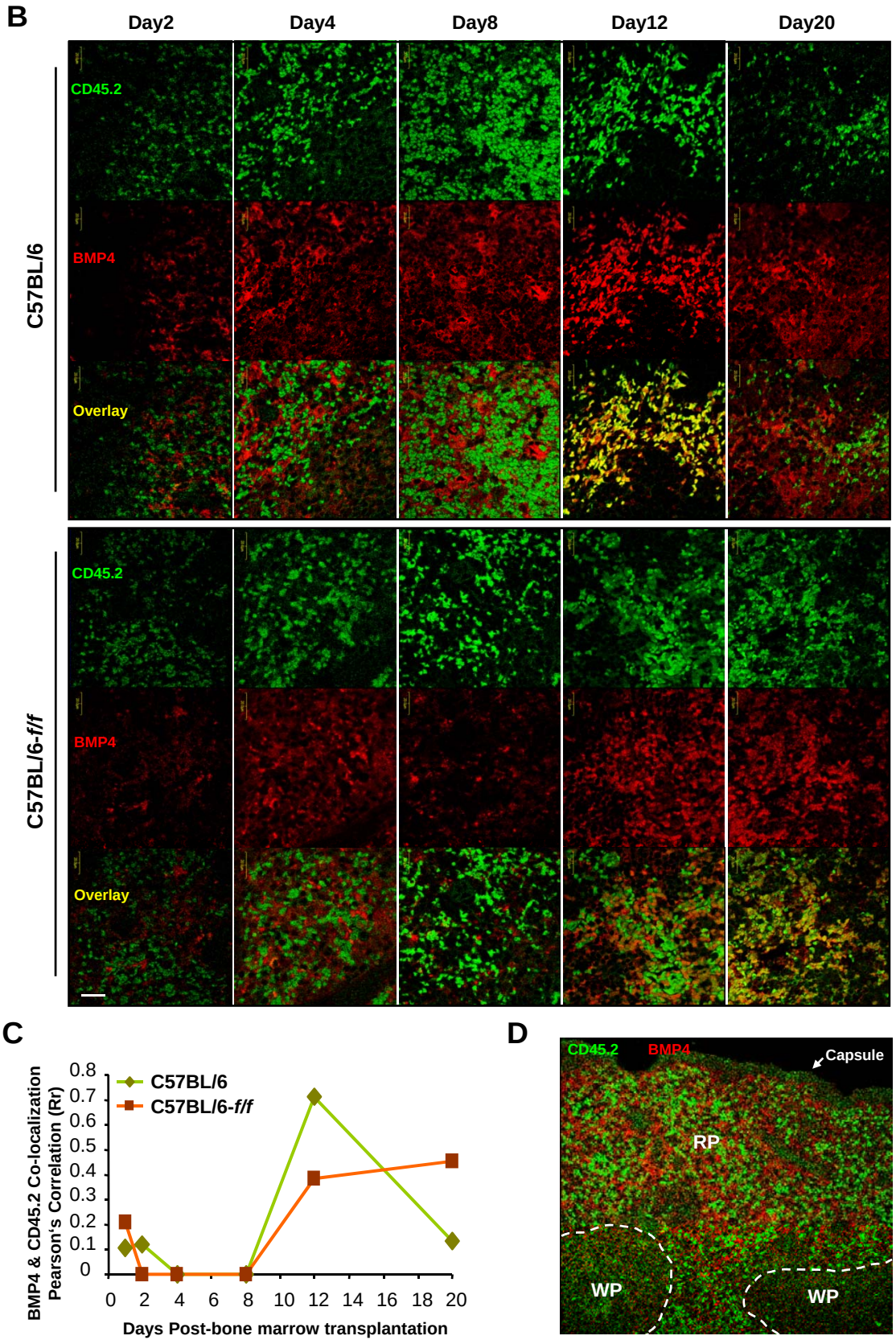


Figure S4, Harandi *et al.*

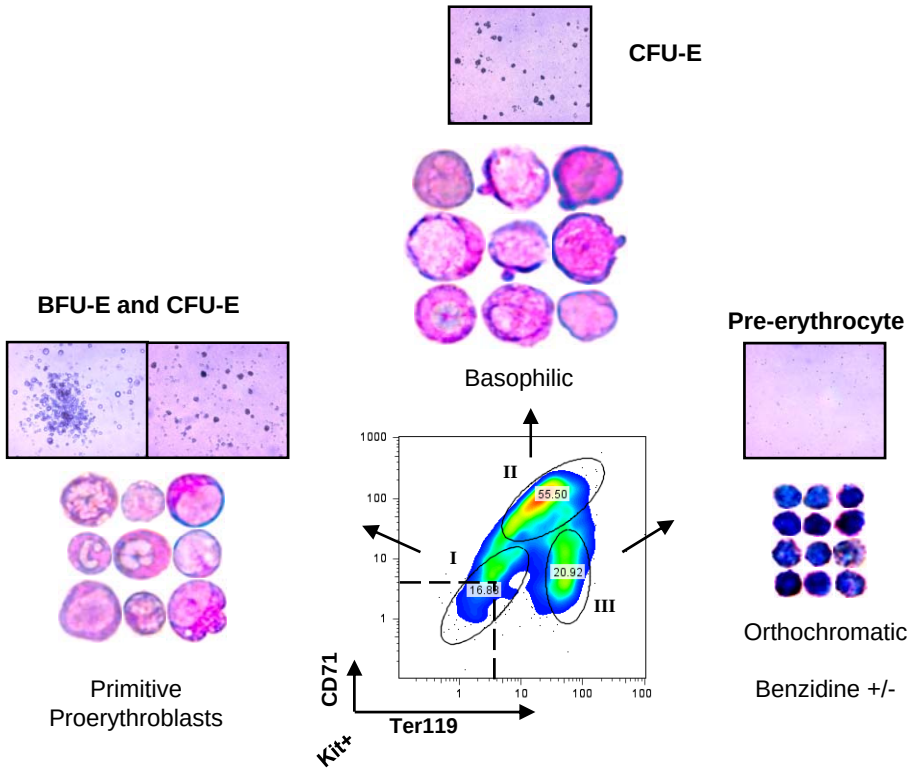


Figure S4. Functional and morphological analysis of the stress erythroid populations in spleen at day 8 post-transplantation. (center) Flow diagram of Populations I, II and III in spleen at day 8 (early recovery stage) gated on Kit+ cells analyzed for CD71 and Ter119 expression. The three populations were sorted, subjected to colony assays to evaluate their erythroid potential in vitro and Spun onto slides and stained with Hematoxylin and eosin to evaluate their morphology. (left) Population I cells exhibited primitive "blast" morphology and formed BFU E and CFU E colonies. (top) Population-II cells exhibited basophilic erythroblast morphology and formed CFU-E only. (right) Population-III cells exhibited orthochromic erythroblast morphology and formed no colonies. Only Population-III stained positive for hemoglobin by benzidine staining, with approximately 50% benzidine positive.

Figure S5, Harandi *et al.*

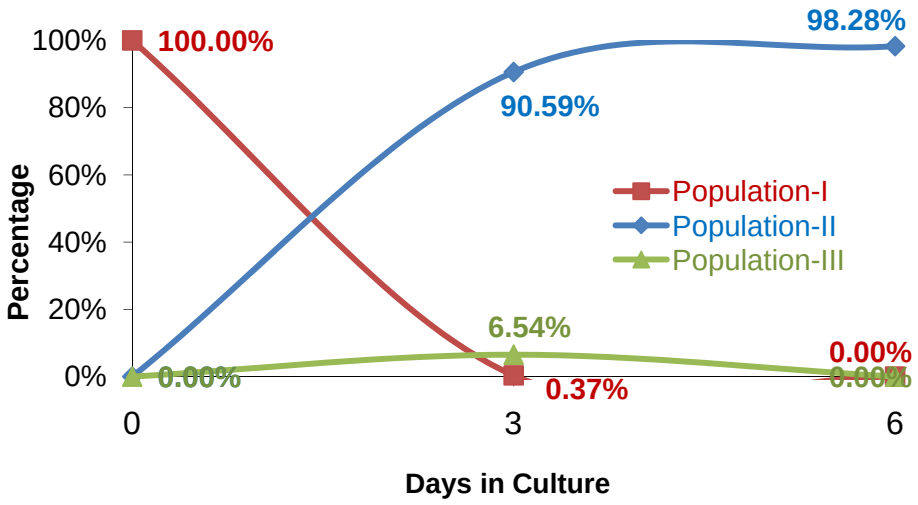
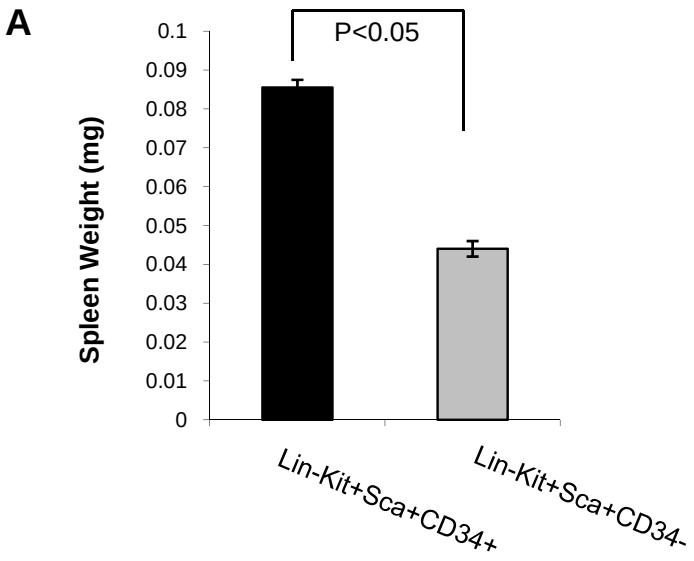
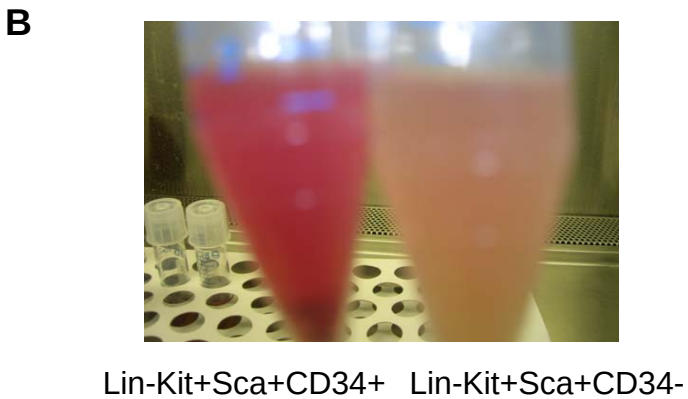


Figure S5. Population-I progenitors develop into population II and III progenitors in vitro. Donor derived Population-I cells purified by FACS from the spleens of mice on day 8 after transplant with control bone marrow. The cells were cultured in the presence of BMP4, SCF, Epo, SHH and hypoxia (2% O2) and analyzed on days 3 and 6 for the development to other Populations II and III by flow cytometry. Population-I gave rise to population II and III at day3 and Population-II cells were predominant at day6. This experiment is representative of two independent experiments done with cells isolated from independent transplants.

Figure S6, Harandi *et al.*





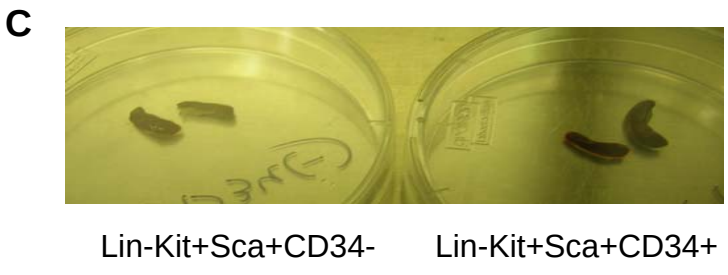


Figure S6.

Figure S7, Harandi *et al.*

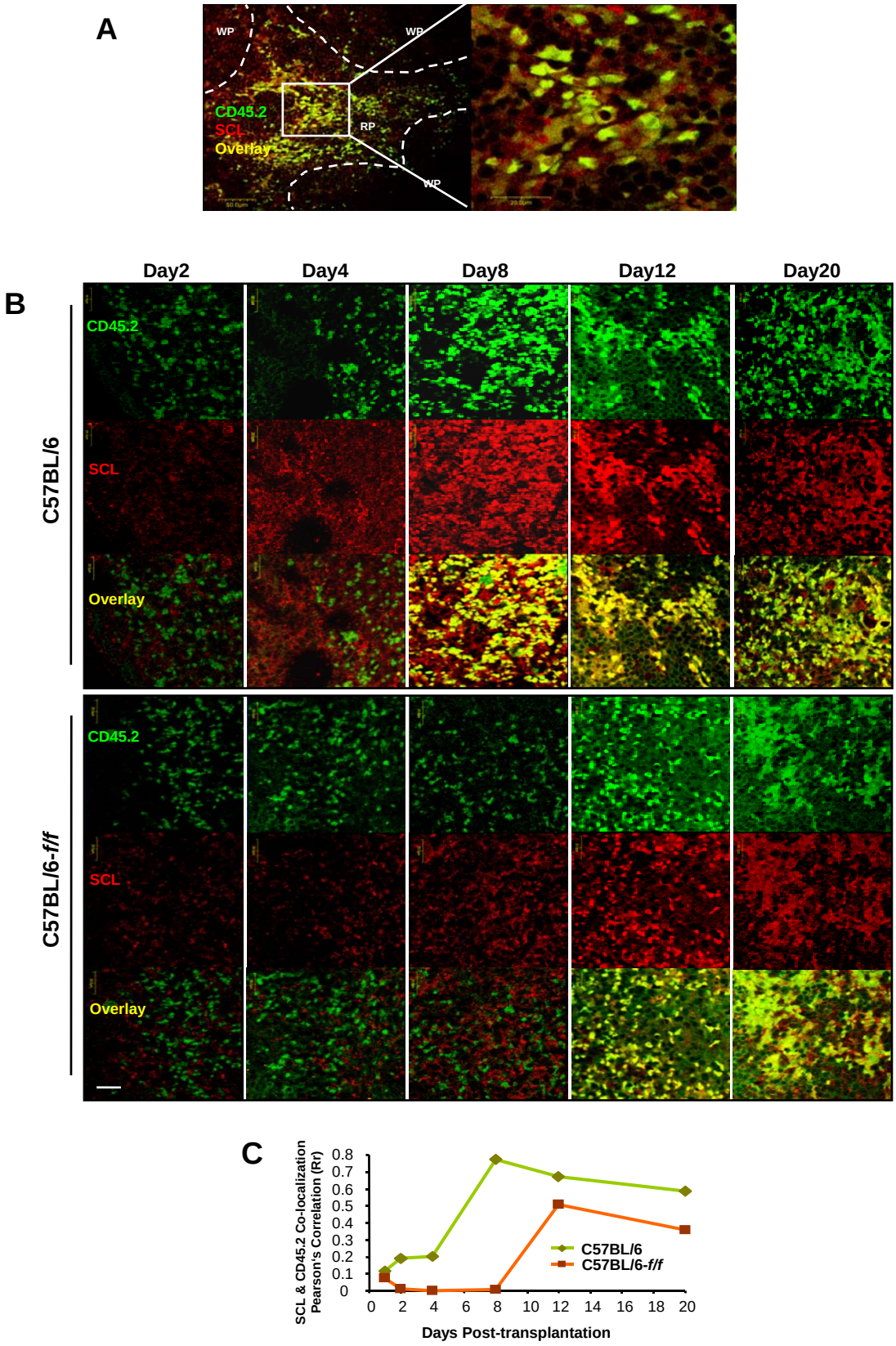


Figure S7, Harandi *et al.* Continued

Figure S7. SCL expression is defective in mice transplanted with C57BL/6-*f/f* mutant donor cells. *(A)* paraffin section of spleen isolated on day 12 after transplant with $5x10^5$ of C57BL/6 donor cells were stained with antibodies to SCL (red) and CD45.2 (green). The section was examined by confocal microscopy. The donor derived CD45.2+ cells overlaid (yellow) with SCL expression and both localized to the red pulp areas in spleen. (B) Analysis of SCL protein expression during the post transplant recovery in spleen sections from mice transplanted with 5x105 mutant (C57BL/6-*f/f*) or control (C57BL/6) bone marrow cells. SCL expression is indicated in red and CD45.2 in green. Scale bar is 20µm. (C) Analysis of colocalization of CD45.2 and SCL in spleen sections by Pearson's correlation (Rr).

Figure S8, Harandi *et al.*

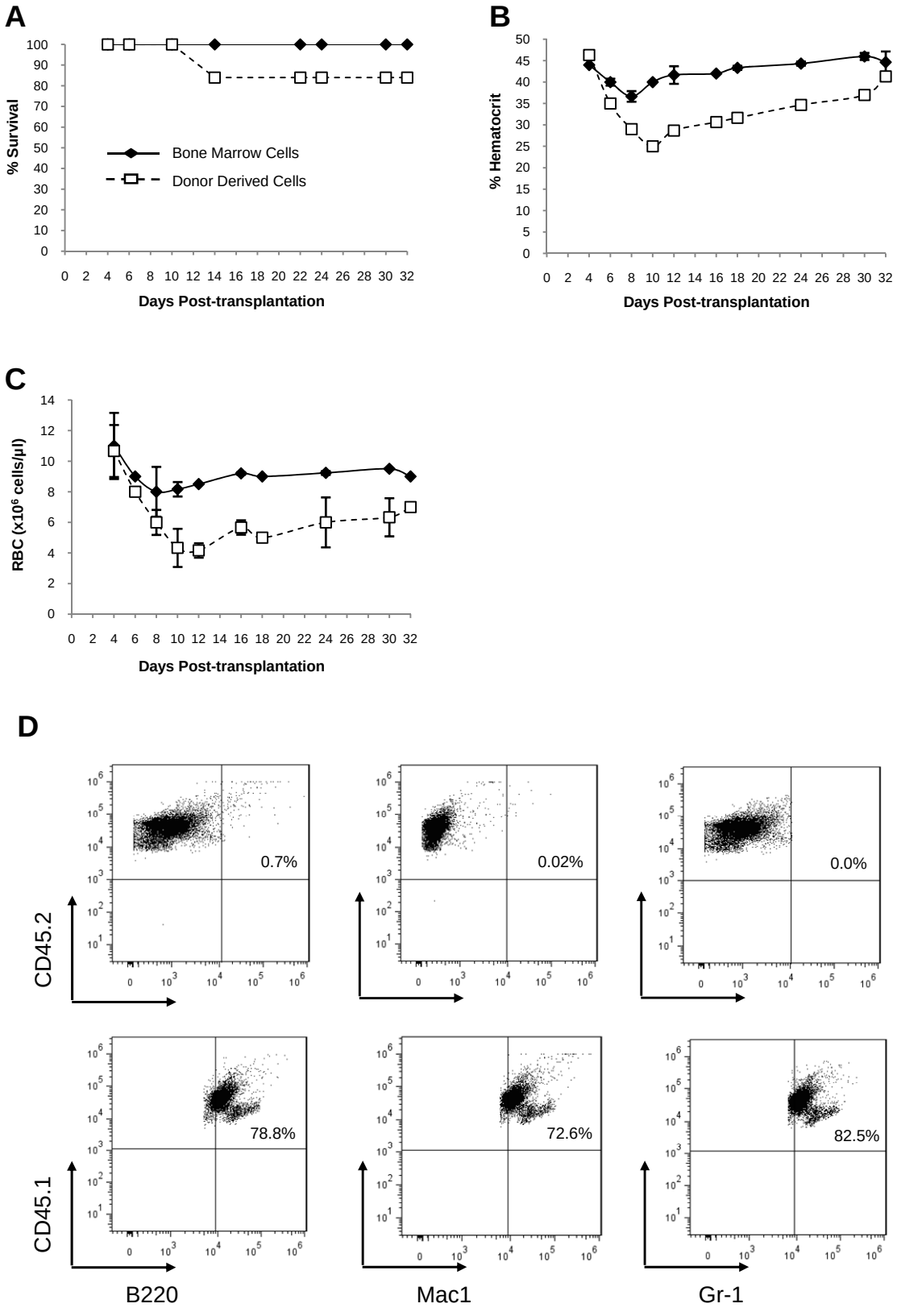


Figure S8. Analysis of tertiary transplants. CD45.1;HbbS mice were irradiated with a split dose of radiation (two doses of 475rads) and transplanted with donor derived spleen cells sorted from secondary transplanted mice. (A) Survival of tertiary transplanted mice. (B) Hematocrit analysis, (C) RBC counts. (D) Analysis of peripheral blood mononuclear cells 50 Days after tertiary transplant.

Figure S9, Harandi *et al.*

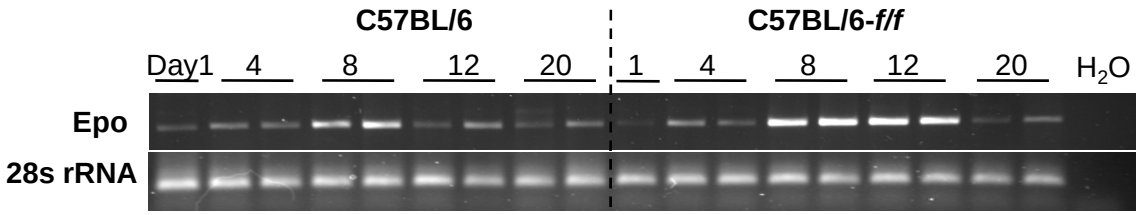


Figure S9. Expression of Epo in the kidney of CD45.1 mice transplanted with 5x105 control (left) or mutant (right) bone marrow cells on the indicated days after transplant. 28S rRNA was used as RNA loading control.