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Supplemental Information

**Proteinase 3 Limits the Number of Hematopoietic Stem and Progenitor
Cells in Murine Bone Marrow**

Kutay Karatepe, Haiyan Zhu, Xiaoyu Zhang, Rongxia Guo, Hiroto Kambara, Fabien Loison, Peng Liu, Hongbo Yu, Qian Ren, Xiao Luo, John Manis, Tao Cheng, Fengxia Ma, Yuanfu Xu, and Hongbo R. Luo

Supplemental Figures and Associated Legends (Figures S1-S6)

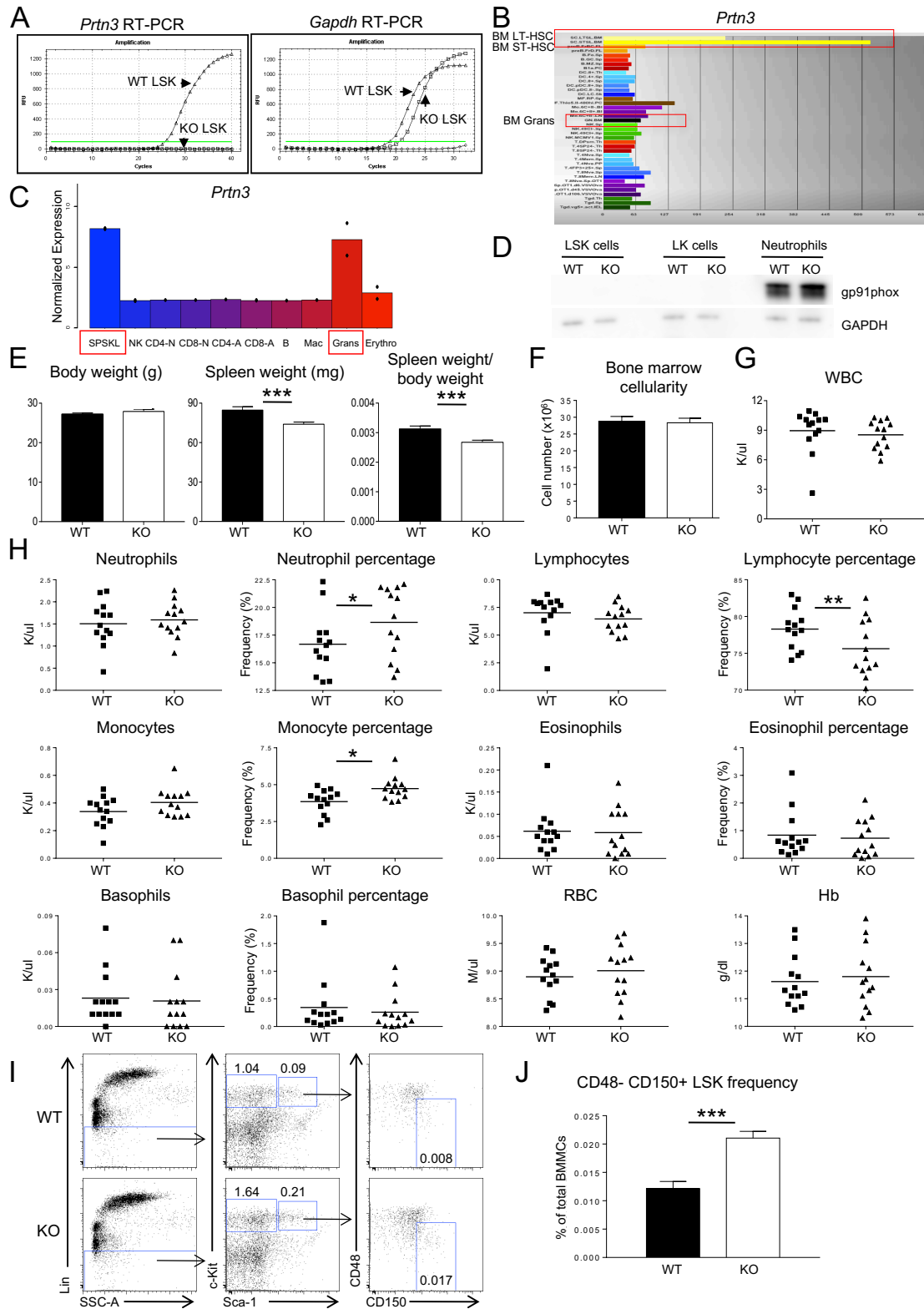


Figure S1. *Prtn3* expression in HSPCs and systemic parameters in *Prtn3*^{-/-} mice. Related to Figure 1.

- (A) Representative plots for *Prtn3* and *Gapdh* mRNA expression in sorted WT and *Prtn3*^{-/-} LSK cells from three independent experiments.
- (B) *Prtn3* mRNA expression in hematopoietic stem cells and neutrophils from the ImmGen Database. HSC and neutrophil populations are highlighted in red.
- (C) *Prtn3* mRNA expression in hematopoietic stem cells and neutrophils from the Gene Expression Across Multiple Hematopoietic Lineages Database. HSC and neutrophil populations are highlighted in red.
- (D) gp91phox was used to rule out neutrophil contamination in sorted LSK and LK cells and GAPDH was used as a loading control.
- (E) Body weights, spleen weights, and spleen weight/body weight ratios of WT and *Prtn3*^{-/-} mice (n=28).
- (F) The number of BM mononuclear cells per one femur and one tibia in WT and *Prtn3*^{-/-} mice (n=18).
- (G) Total white blood cell counts in WT and *Prtn3*^{-/-} mice (n=13).
- (H) Numbers and percentages of different blood cell lineages and hemoglobin levels in WT and *Prtn3*^{-/-} mice (n=13).
- (I) Representative flow cytometry plots for CD48- CD150+ LSK cells in WT and *Prtn3*^{-/-} mice. Numbers denote the frequency of each population among live singlets.
- (J) Quantification of the frequency of CD48- CD150+ LSK cells in WT and *Prtn3*^{-/-} mice (n=17). All values shown are mean ± SEM. *p < 0.05, ***p < 0.001, by unpaired, 2-tailed Student's t test.

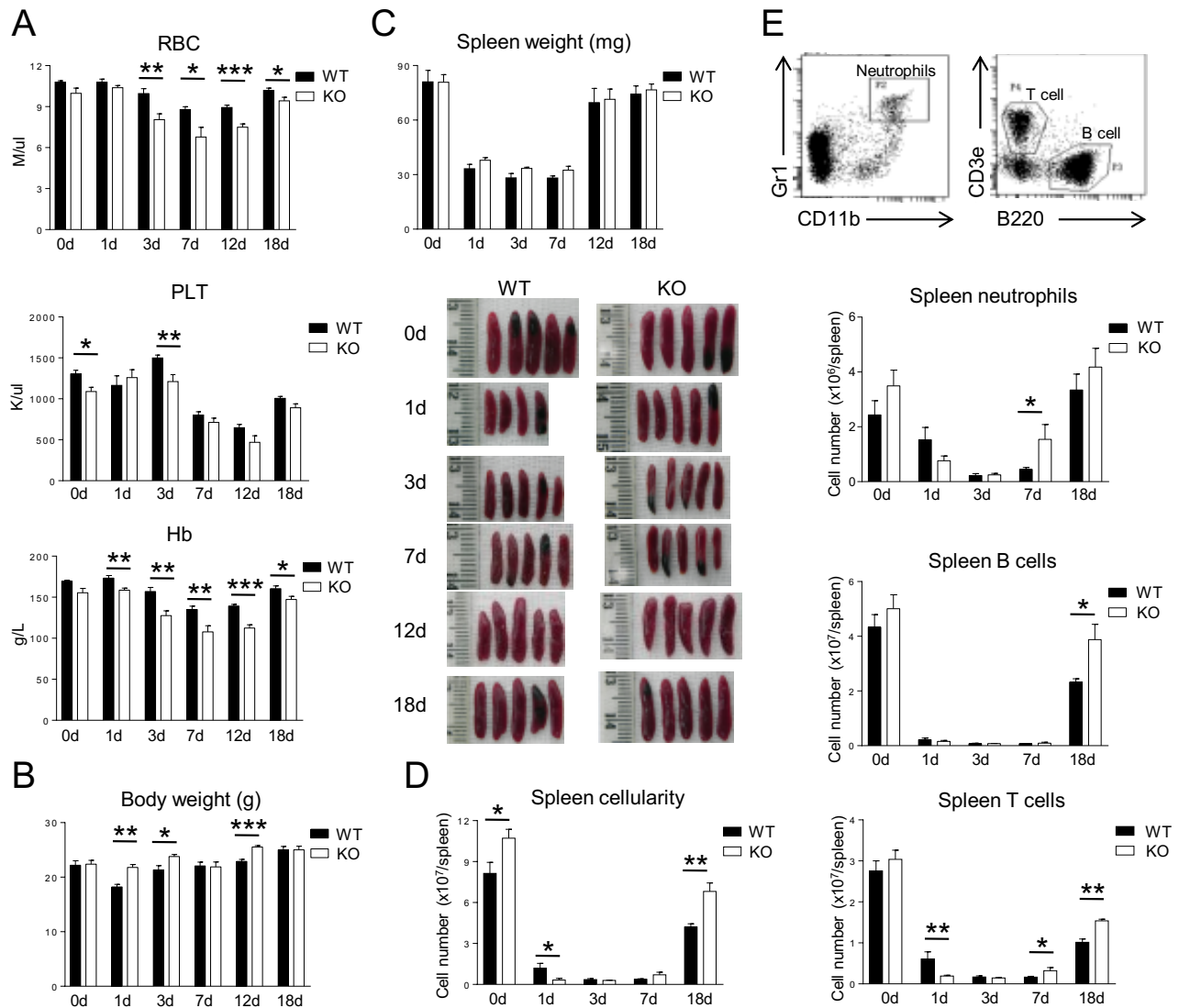


Figure S2. Additional information on the hematopoietic recovery of mice after sublethal irradiation. Related to Figure 3. (A) Red blood cell, hemoglobin, and platelet counts in the peripheral blood of WT and *Prtn3*^{-/-} mice before and after sublethal irradiation (n=5-9).

(B-C) Body weights and spleen weights of WT and *Prtn3*^{-/-} mice before and after sublethal irradiation (n=5-9).

(D-E) Quantitative analysis of total mononuclear cells, neutrophils, B cells, and T cells in the spleen before and after sublethal irradiation (n=5-9). All values shown are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, by unpaired, 2-tailed Student's t test.

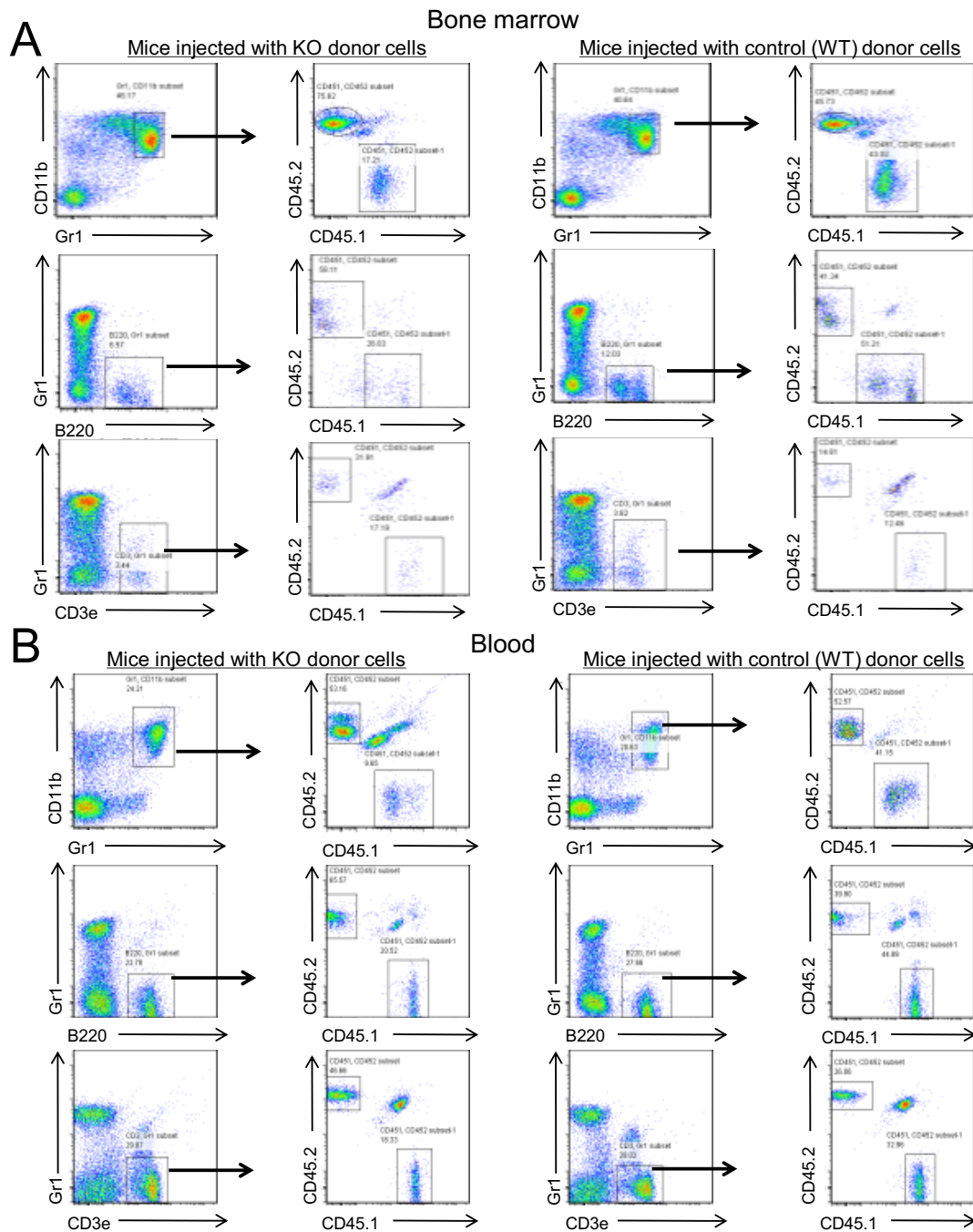


Figure S3. Gating strategy used in the transplantation study. Related to Figure 4.

(A) Gating strategy to analyze the chimerism of different donors of myeloid cells, B cells, and T cells in the BM of recipient mice.

(B) Gating strategy to analyze the chimerism of different donors of myeloid cells, B cells, and T cells in the peripheral blood of recipient mice.

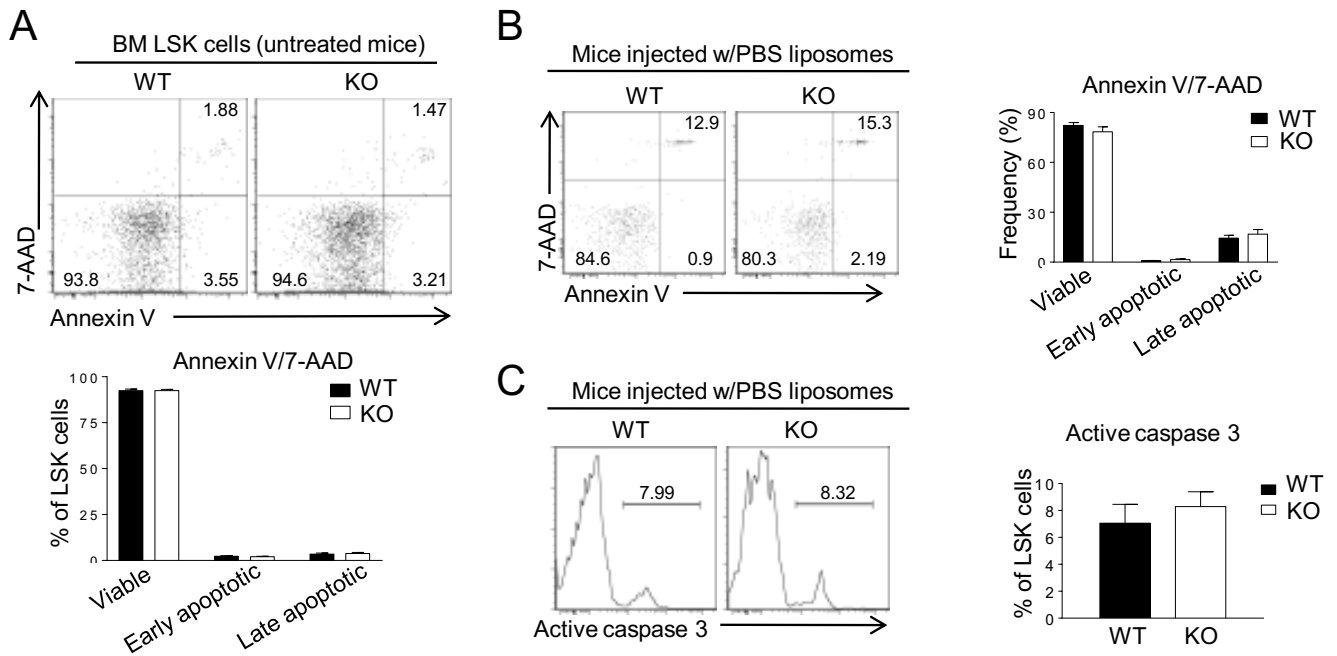


Figure S4. Additional information on the apoptosis of LSK cells from WT and *Prtn3*^{-/-} mice. Related to Figure 5. (A) Analysis of the frequency of viable (Annexin V⁻ 7-AAD⁻), early apoptotic (Annexin V⁺ 7-AAD⁻), and late apoptotic (Annexin V⁺ 7-AAD⁺) LSK cells in untreated WT and *Prtn3*^{-/-} mice (n=12-15). (B) Analysis of the frequency of viable (Annexin V⁻ 7-AAD⁻), early apoptotic (Annexin V⁺ 7-AAD⁻), and late apoptotic (Annexin V⁺ 7-AAD⁺) BM LSK cells in WT and *Prtn3*^{-/-} mice after injection of PBS liposomes. Numbers denote the frequency of cells among LSK cells (left panel). Quantification of LSK viability is shown in the right panel (n=5). (C) Representative FACS plots showing the frequency of BM LSK cells with active caspase 3 after treatment with PBS liposomes (left panel). Quantitative analysis of the frequency of LSK cells with active caspase 3 is shown in the right panel (n=5). All values shown are mean ± SEM. Statistical analysis was performed by unpaired, 2-tailed Student's t test.

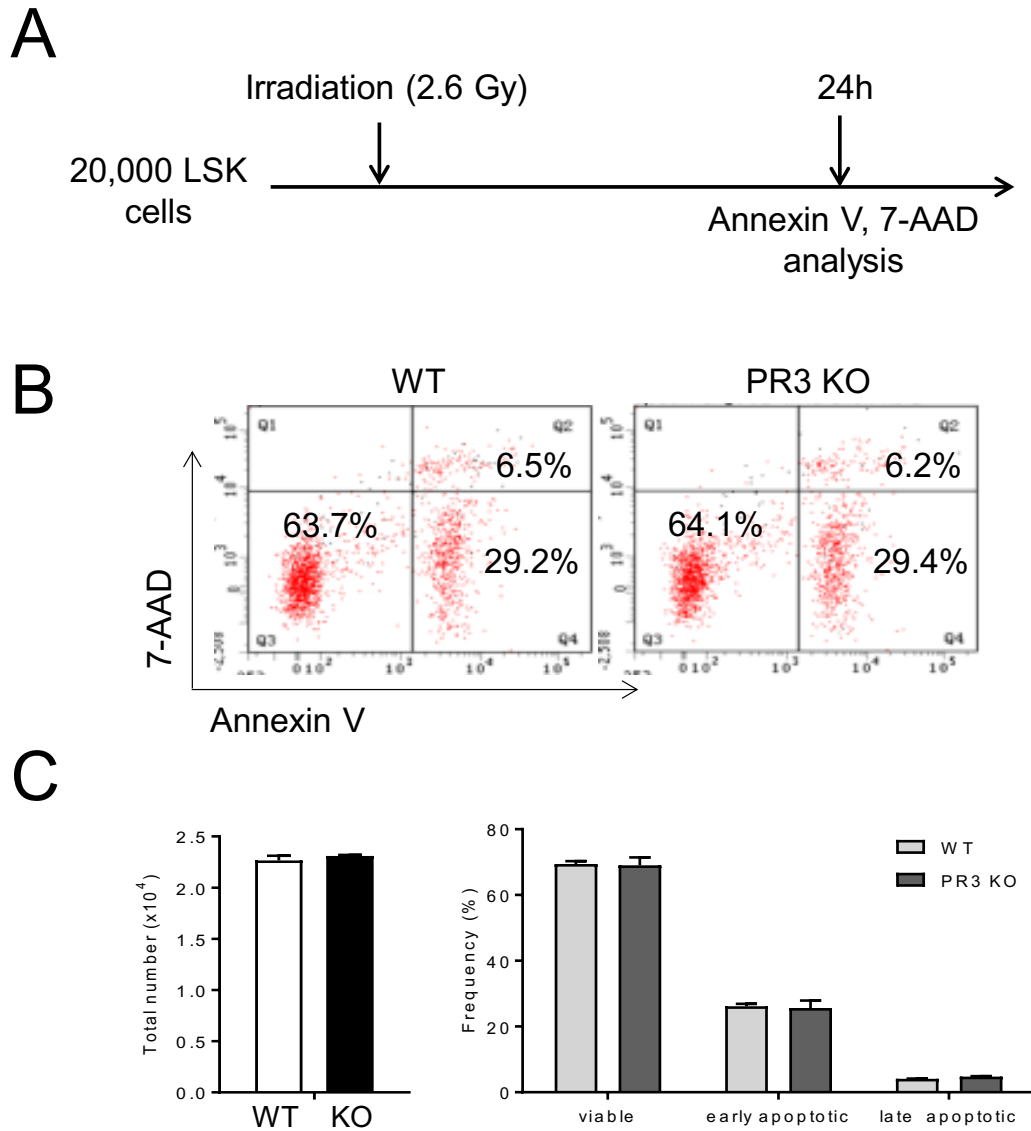


Figure S5. Irradiated WT and *Prtn3*^{-/-} LSK cells displayed similar rate of apoptosis. Related to Figure 6.

(A) Scheme of the experimental setup.

(B) Gating strategy to identify viable (AnnexinV⁻/7-AAD⁻), early apoptotic (AnnexinV⁺/7-AAD⁻), and late apoptotic (AnnexinV⁺/7-AAD⁺) cells.

(C) Number of viable cells was measured using Annexin V⁻ 7-AAD⁻ cellular events (n=4). Total cell numbers and the percentages of viable, early apoptotic, and late apoptotic cells in cultured WT and *Prtn3*^{-/-} LSK populations were measured 24 hr after the irradiation. All values shown are mean \pm SEM (n=4). This experiment was repeated twice and shown is a representative result of the two experiments performed.

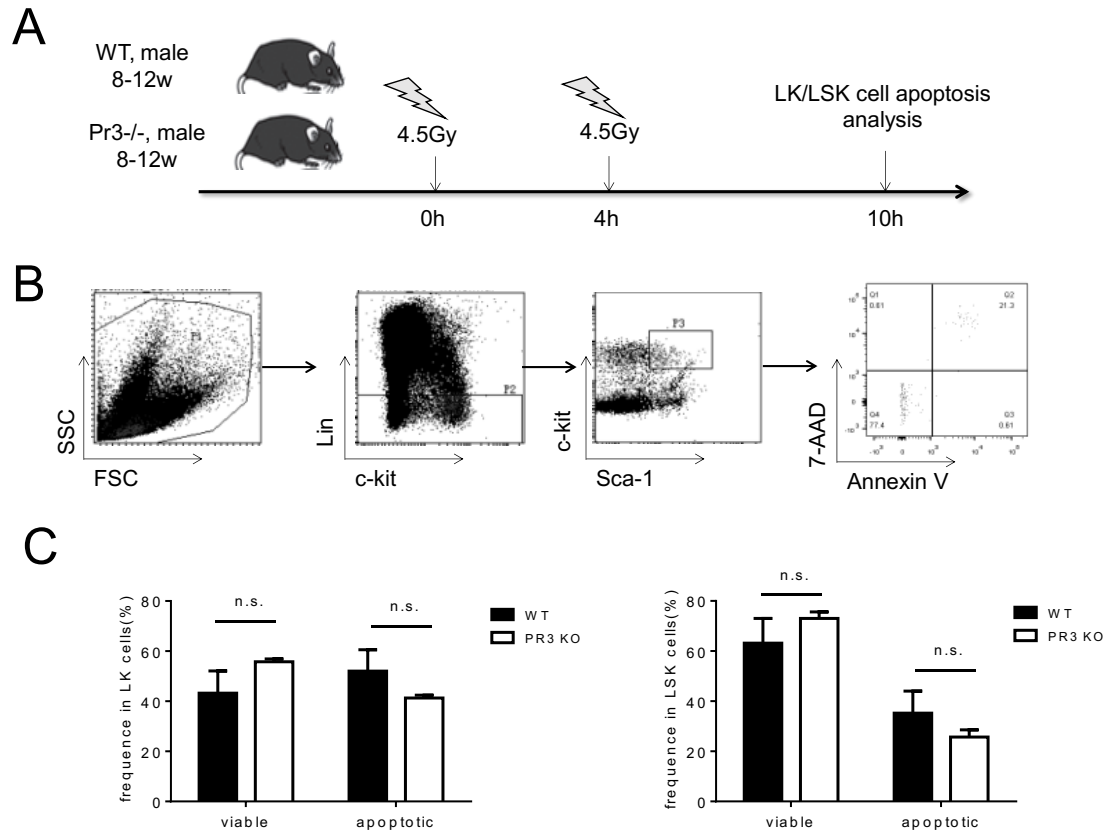


Figure S6. WT and *Prtn3*^{-/-} HSPCs displayed similar rate of apoptosis in irradiated mice. Related to Figure 6. (A) The experimental setup used to analyze HSPC apoptosis after challenge with sublethal irradiation. The dose of irradiation was chosen based on previous studies (Shao et al., 2010; Yu et al., 2010). (B) Gating strategy to identify viable (AnnexinV⁻/7-AAD⁻) and apoptotic (AnnexinV⁺ or 7-AAD⁺) cells. (C) The percentages of viable and apoptotic cells in LK and LSK cell populations. All values shown are mean ± SD (n=3). n.s., not statistically significant (P<0.05) based on Student's t test.