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Supplemental Information

Modest Declines in Proteome Quality Impair Hematopoietic Stem Cell Self-Renewal

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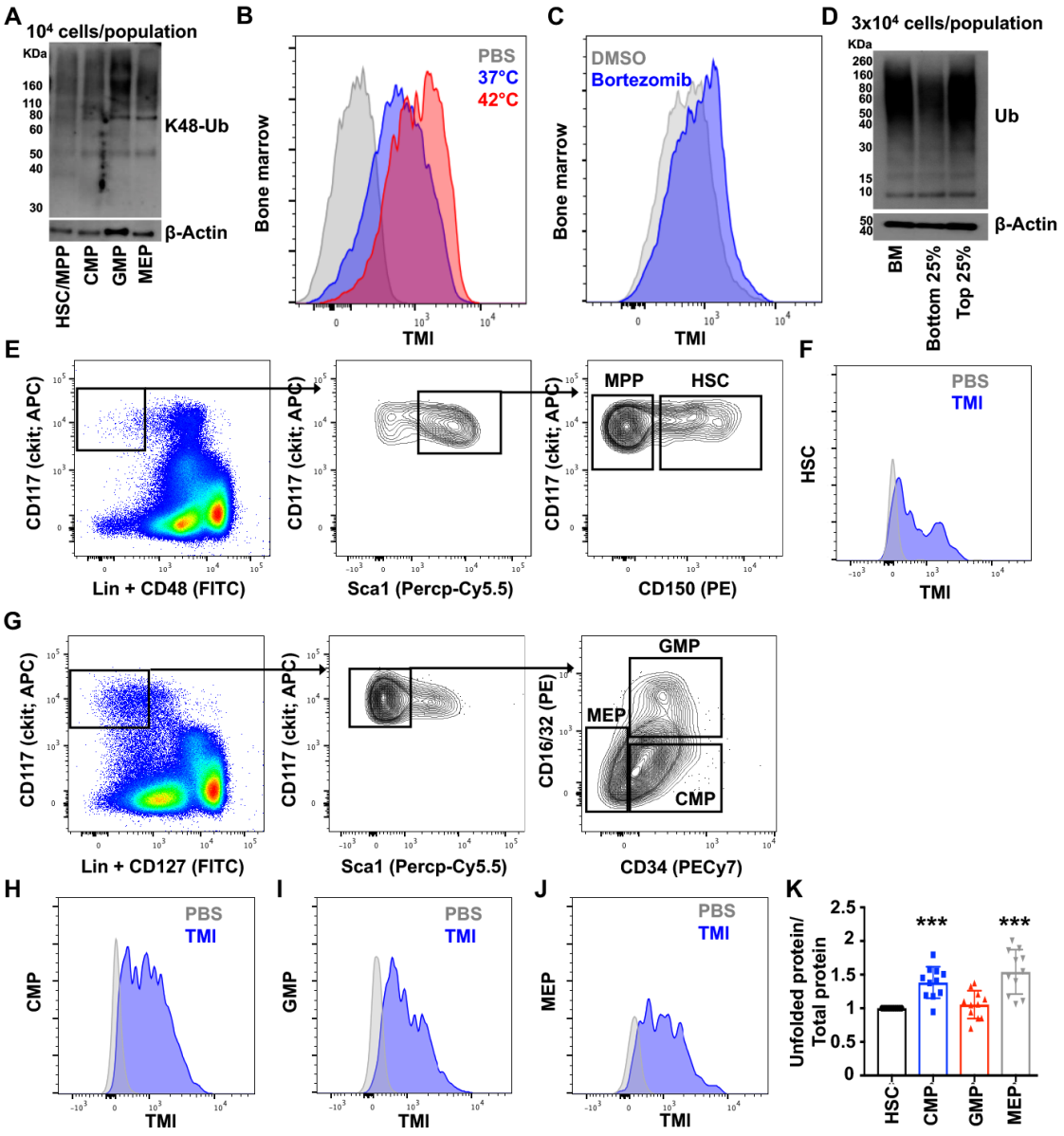

Figure S1. Quantifying unfolded protein abundance within hematopoietic stem and progenitor cells with TMI. Related to Figure 1.

(A) Western blot examining LysK48-ubiquitylated protein in 10^4 HSCs/MPPs, CMPs, GMPs and MEPs (one of two representative blots). (B) Representative histogram showing TMI fluorescence in bone marrow cells after a 4 h incubation at 37°C or 42°C. (C) Representative histogram showing TMI fluorescence in bone marrow cells from mice treated 18 h earlier with bortezomib (BZ) or vehicle (DMSO). (D) Western blot analysis showing ubiquitylated protein in $3x10^4$ unfractionated bone marrow cells, as well as bone marrow cells with low (lowest quartile, bottom 25%) and high (highest quartile, top 25%) of TMI fluorescence. (E) Representative flow cytometry plots showing gating strategies for analysis and isolation of HSCs and MPPs. (F) Representative histograms showing TMI fluorescence in HSCs. The background from PBS treated controls is shown in gray. (G) Representative flow cytometry plots showing gating strategies for analysis and isolation of CMPs, GMPs and MEPs. (H-J) Representative histograms showing TMI fluorescence in CMPs (H), GMPs (I) and MEPs (J). The background from PBS treated controls is shown in gray. (K) TMI fluorescence relative to total protein content in HSCs, CMPs, GMPs and MEPs (data from Fig. 1F,L). Data in K represent mean ± standard deviation. Statistical significance was assessed using an ANOVA followed by Dunnett's multiple comparisons test relative to HSCs. ***P<0.001.

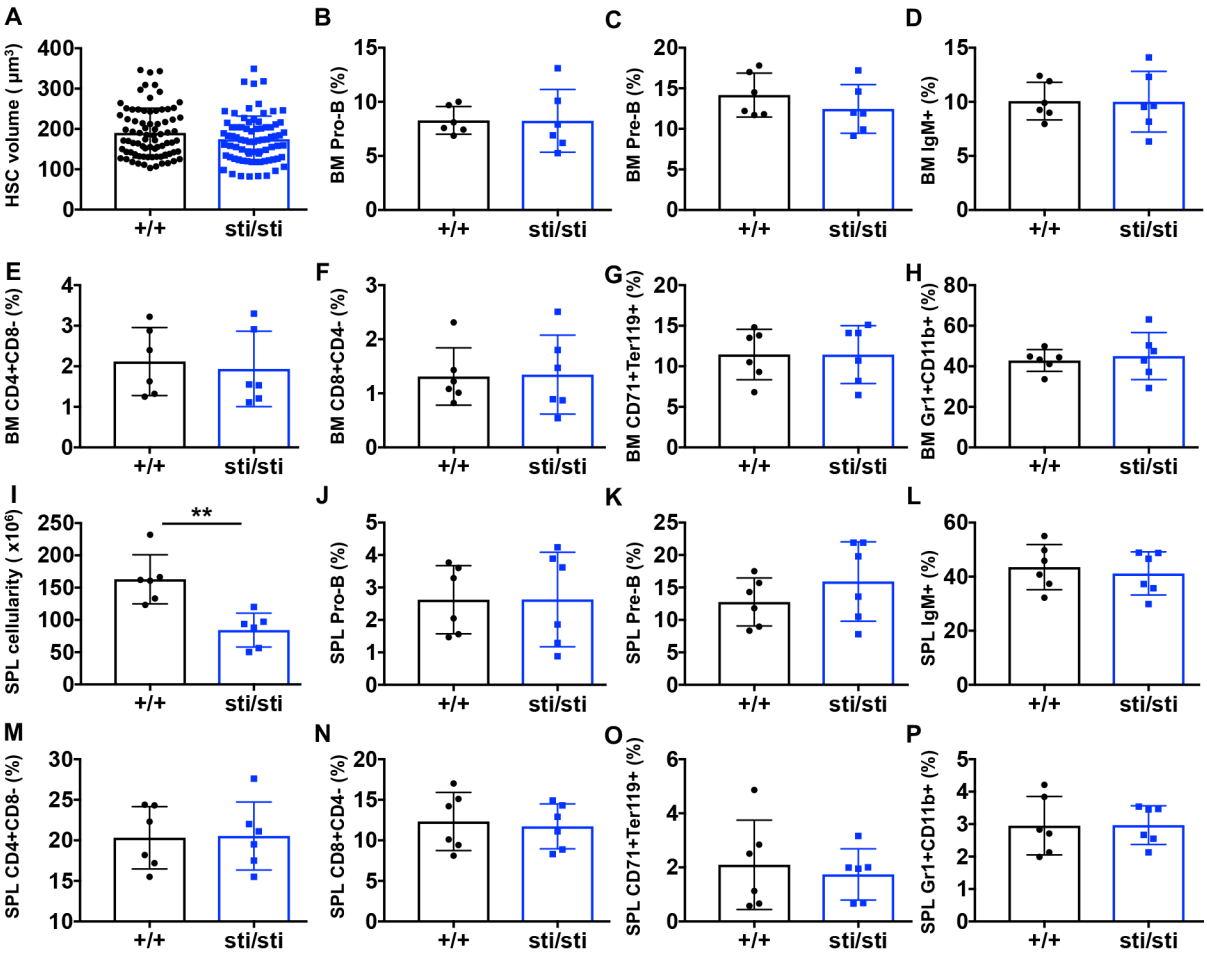

Figure S2. *Aars*$^{sti/sti}$ mice have normal frequencies of lymphoid, myeloid and erythroid lineage cells. Related to Figure 2.

(A) Cell volume of wild-type (+/+) and *Aars*$^{sti/sti}$ (sti/sti) HSCs (n ≥ 78 cells/population). (B-P) Frequency of IgM$^-$ CD45R(B220)$^+$CD43$^+$ pro-B cells (Hardy et al., 1991), IgM$^-$CD45R(B220)$^+$CD43$^-$ pre-B cells (Hardy et al., 1991), IgM$^+$CD45R(B220)$^+$ B cells (D, L), CD4$^+$CD8$^-$ T cells, (E, M) CD4$^-$CD8$^+$ T cells, (F, N) CD71$^+$Ter119$^+$ erythroid cells (Zhang et al., 2003) and CD11b$^+$Gr1$^+$ myeloid cells (H, P) in the bone marrow (B-H) and spleen (J-P) of wild-type (+/+) and *Aars*$^{sti/sti}$ mice (n = 6 mice per genotype). (I) Spleen (SPL) cellularity in wild-type (+/+) and *Aars*$^{sti/sti}$ (sti/sti) mice (n = 6 mice per genotype). Data represent mean ± standard deviation. Statistical significance was assessed using a two-tailed Student's t-test. **P<0.01.

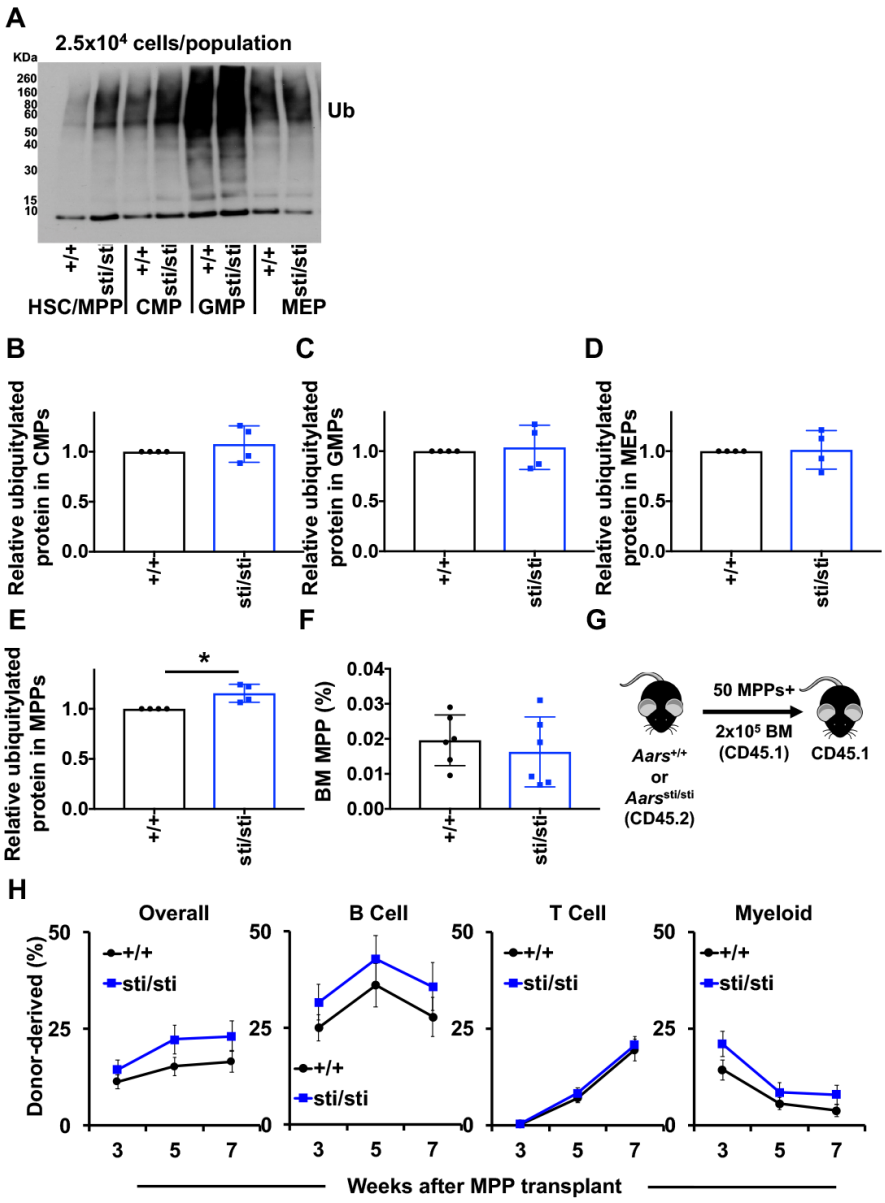

Figure S3. *Aars*$^{sti/sti}$ MPPs have normal capacity to reconstitute irradiated mice in short-term transplantation. Related to Figure 2. (A-D) Flow cytometry analysis showing relative ubiquitylated protein content in *Aars*$^{sti/sti}$ (sti/sti) relative to wild-type (+/+) CMPs (A), GMPs (B), MEPs (C) and MPPs (D) (n = 4 mice per genotype). (E) Frequency of MPPs in the bone marrow of wild-type (+/+) and *Aars*$^{sti/sti}$ mice (n = 6 mice per genotype). (F) Diagram showing MPP transplantation strategy. (G) Donor cell engraftment when 50 CD150^{-}CD48^{-}LSK MPPs from wild-type (+/+) and *Aars*$^{sti/sti}$ (sti/sti) mice were transplanted along with 2x10^{5} recipient bone marrow cells into irradiated mice (n = 3 donors and 15 recipients per genotype). Data represent mean ± standard deviation (A-E) or standard error of the mean (G). Statistical significance was assessed using a two-tailed Student's t-test. *P<0.05.

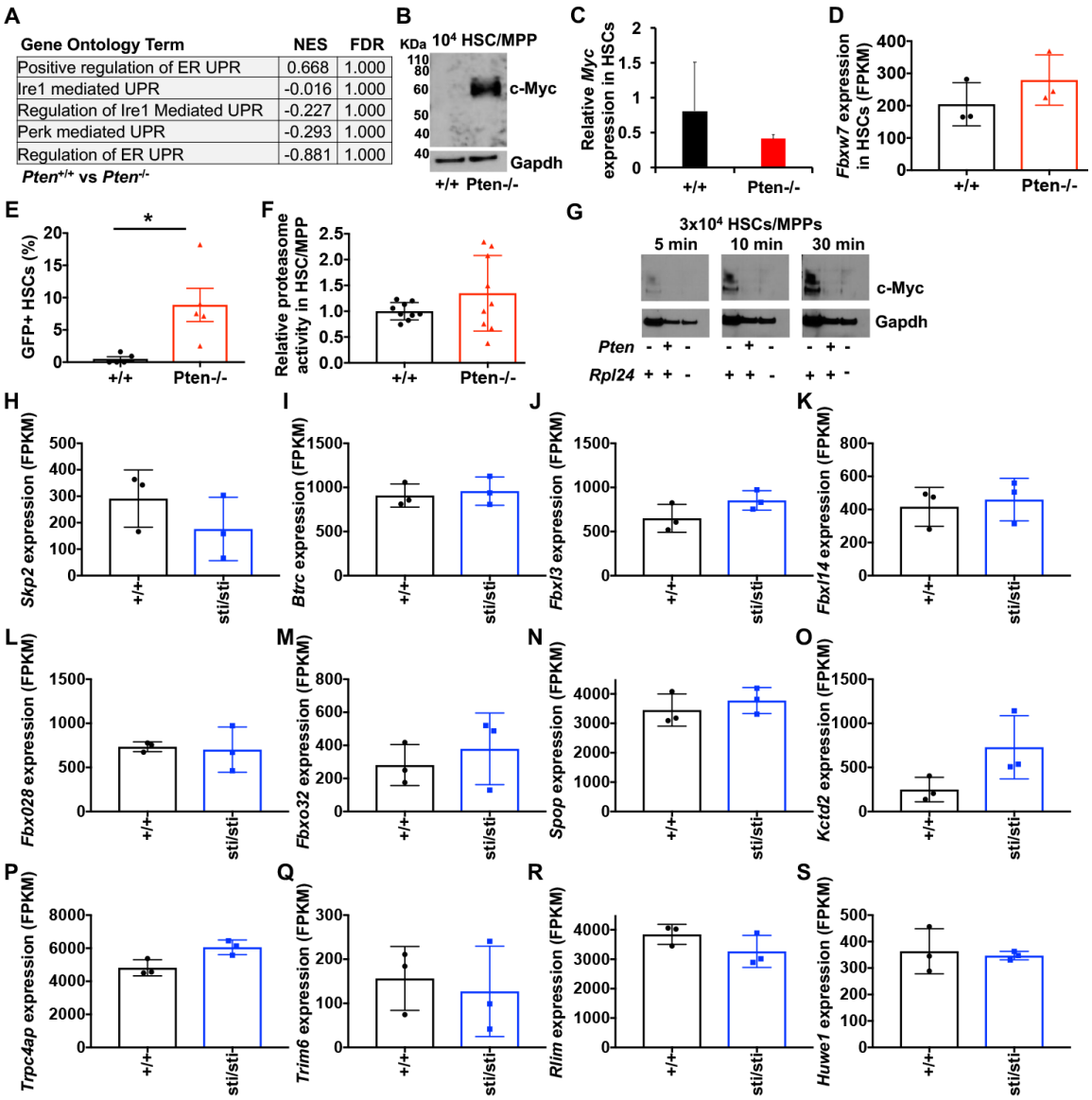

Figure S4. Accumulation of ubiquitylated protein overwhelms the proteasome leading to c-Myc stabilization in HSCs. Related to Figure 3.

(A) Gene set enrichment analysis demonstrating no significant activation of the UPR^{ER} in *Pten*$^{fl/fl}$ (Pten+/+) as compared to Mx1-*Cre*$^{+}$;*Pten*$^{fl/fl}$ (Pten-/-) HSCs. (B) Western blot examining c-Myc protein in 10^4 HSCs/MPPs isolated from *Pten*$^{fl/fl}$ (+/+) and Mx1-*Cre*$^{+}$;*Pten*$^{fl/fl}$ (Pten-/-) mice (one of three representative blots). (C) *Myc* mRNA expression normalized to β-Actin in *Pten*$^{fl/fl}$ (+/+) and Mx1-*Cre*$^{+}$;*Pten*$^{fl/fl}$ (Pten-/-) HSCs (n = 3 per genotype). (D) *Fbxw7* expression in *Pten*$^{fl/fl}$ (+/+) and Mx1-*Cre*$^{+}$;*Pten*$^{fl/fl}$ (Pten-/-) HSCs (n = 3 per genotype). (E) Frequency of HSCs that are GFP^{+} in the bone marrow of Ub^{G76V}-*GFP* (+/+) and Mx1-*Cre*$^{+}$;Ub^{G76V}-*GFP*;*Pten*$^{fl/fl}$ (Pten-/-) mice (n = 4-5 mice per genotype). (F) Relative proteasome activity in *Pten*$^{fl/fl}$ (+/+) and Mx1-*Cre*$^{+}$;*Pten*$^{fl/fl}$ (Pten-/-) HSCs HSCs/MPPs (n = 9 replicates per genotype in 3 experiments). (G) Western blot examining ubiquitylated protein in 3x10^4 wild-type (*Pten*$^{fl/fl}$;*Rpl24*$^{+/+}$), *Pten*-deficient mice (*Mx1-Cre*$^{+}$*Pten*$^{fl/fl}$;*Rpl24*$^{+/+}$) and in *Mx1-Cre*$^{+}$;*Pten*$^{fl/fl}$;*Rpl24*$^{Bst/+}$ HSCs/MPPs. Exposure times of 5, 10 and 30 minutes are shown. (H-S) Expression of E3 ubiquitin ligases that target c-Myc is not reduced in *Aars*$^{sti/sti}$ HSCs**.** Expression of (H) *Skp2*, (I) *Btrc*, (J) *Fbxl3*, (K) *Fbxl14*, (L) *Fbxo28*, (M) *Fbxo32*, (N) *Spop*, (O) *Kctd2*, (P) *Trpc4ap*, (Q) *Trim6*, (R) *Rlim* and (S) *Huwe1* in wild-type (+/+) and *Aars*$^{sti/sti}$ (sti/sti) HSCs based on RNA sequencing (n = 3 per genotype). Data represent mean ± standard deviation. Statistical significance was assessed using a Student's t-test (C-F; *P<0.05). Adjusted P values (H-S) did not reach the threshold of statistical significance for any of these genes.