Supplemental Material

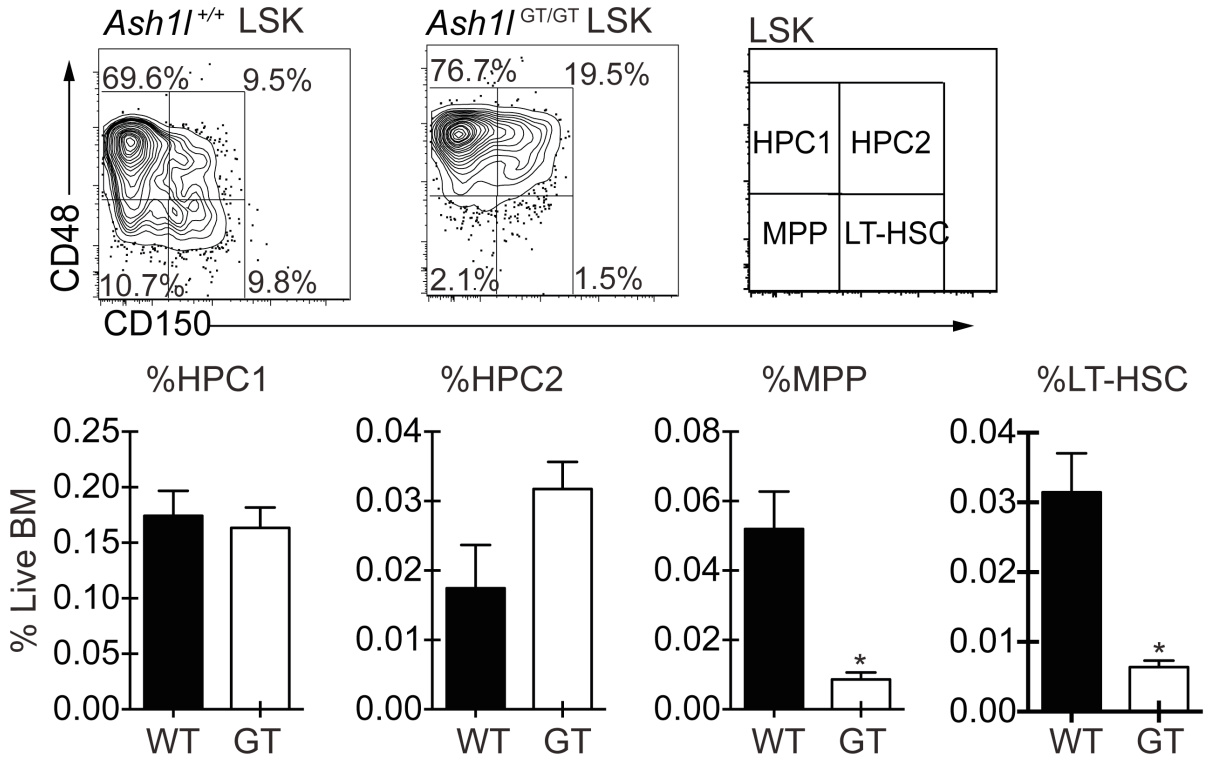

Figure S1. Bone marrow HPC1/2 hematopoietic progenitors are maintained in adult *Ash1l*-deficient mice, while both multipotent progenitors and long-term HSCs become depleted.

Reduced numbers of LSK $CD150^{+}CD48^{-}$ long-term hematopoietic stem cells (LT-HSC) and $CD150^{-}CD48^{-}$ multipotent progenitors (MPP), but not $CD150^{-}CD48^{+}$ (HPC1) and $CD150^{+}CD48^{+}$ hematopoietic progenitor cells (HPC2) in adult GT mice (n=3-5/genotype, mean +/- SEM, *$p<0.05$).

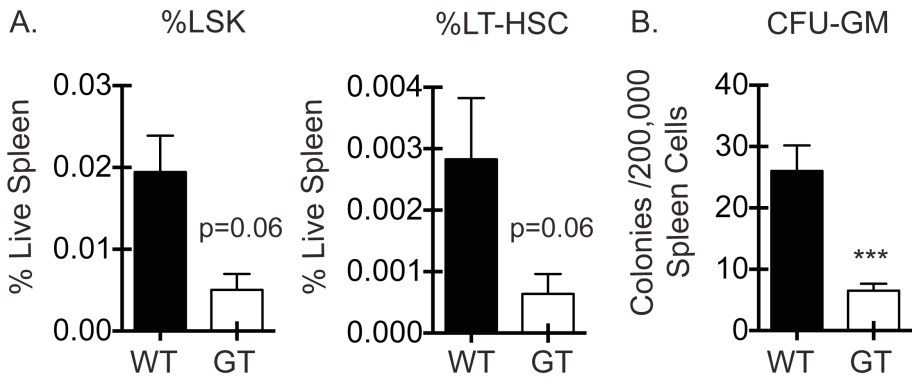

Figure S2. Frequency of hematopoietic stem and progenitor cells and colony formation are reduced in the *Ash1l*-deficient spleen.

(A) Flow cytometric analysis of splenic LSK and LT-HSCs in wild-type (WT) and *Ash1l*$^{GT/GT}$ (GT) in adult (13-21 weeks) mice (n=4/genotype; mean +/- SEM); (**B**) Myeloid colony formation by GT spleen in CFU-GM assays (n=4/genotype; mean +/- SEM).

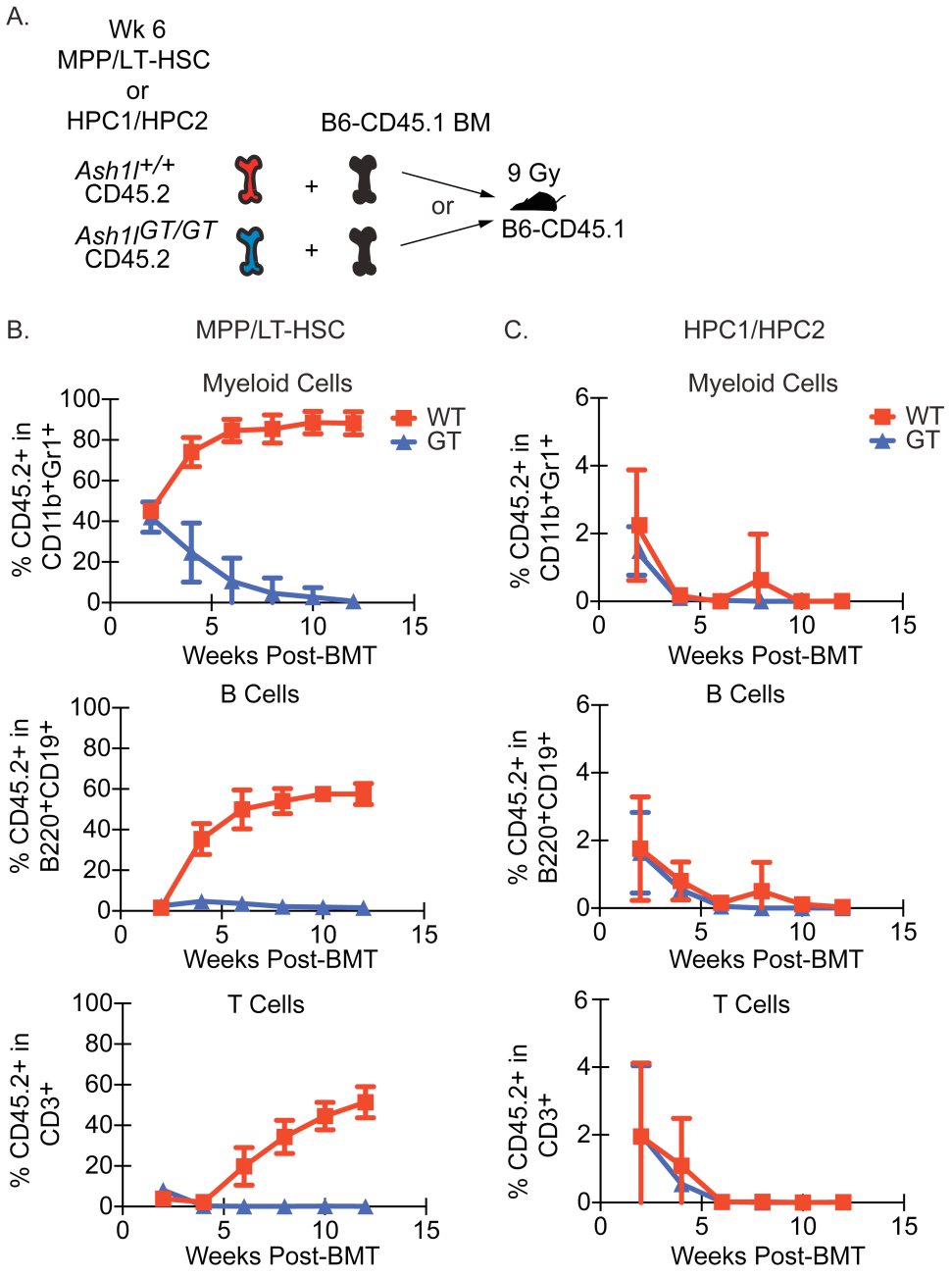

Figure S3. *Ash1l*-deficient CD48^{-}LSK progenitors provide transient hematopoietic output at two weeks, but do not sustain long-term reconstitution.
(A) Experimental strategy: 500 sort-purified *Ash1l*$^{+/+}$ or *Ash1l*$^{GT/GT}$ B6-CD45.2 CD48^{-}LSK cells (containing LT-HSCs and multipotent progenitors) or 500 CD48^{+}LSK cells (containing HPC1/2 progenitors) were transplanted into irradiated (9 Gy) B6-CD45.1 recipients, together with 2x10^{5} B6-CD45.1 BM competitor cells (5 mice/group); **(B)** *Ash1l*-deficient CD48^{-}LSK progenitors (LT-HSC/MPP) provided transient reconstitution of myeloid cells at 2 weeks, but failed to sustain long-term hematopoiesis; **(C)** *Ash1l*$^{+/+}$ and *Ash1l*$^{GT/GT}$ HPC1/HPC2 cells provided only minimal transient contribution to hematopoietic output as assessed in the peripheral blood.

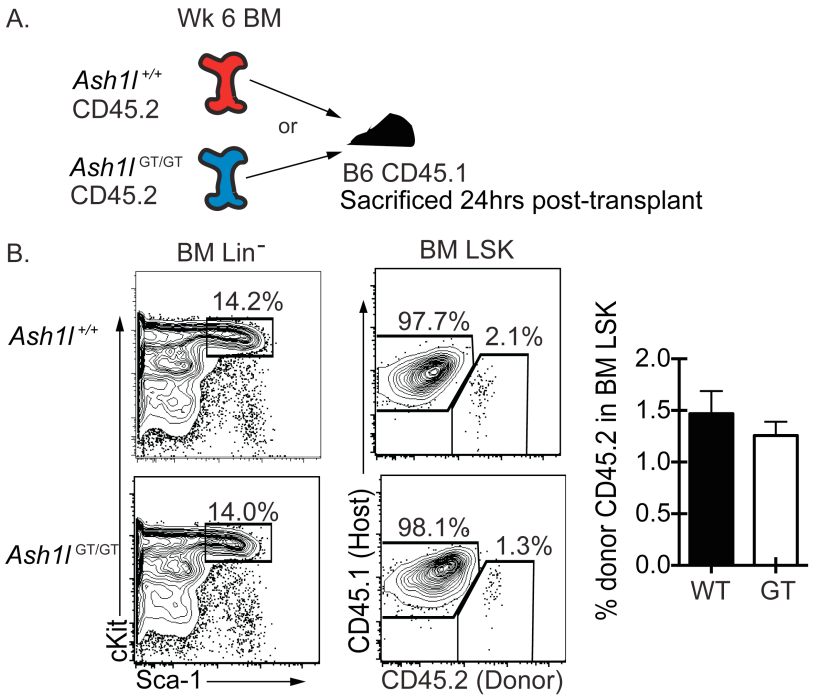

Figure S4. *Ash1l*-deficient HSCs can home to the bone marrow.

(A) Experimental strategy: *Ash1l*$^{+/+}$or *Ash1l*$^{GT/GT}$ B6-CD45.2 BM cells (25 x 10^6) were injected into B6-CD45.1 recipients; **(B)** Percentage of CD45.2$^+$ donor-derived cells among BM LSK progenitors 24 hours after transplantation (n=4/genotype). Bar graph shows data corrected by the % LSK cells in donor BM (mean +/- SEM).

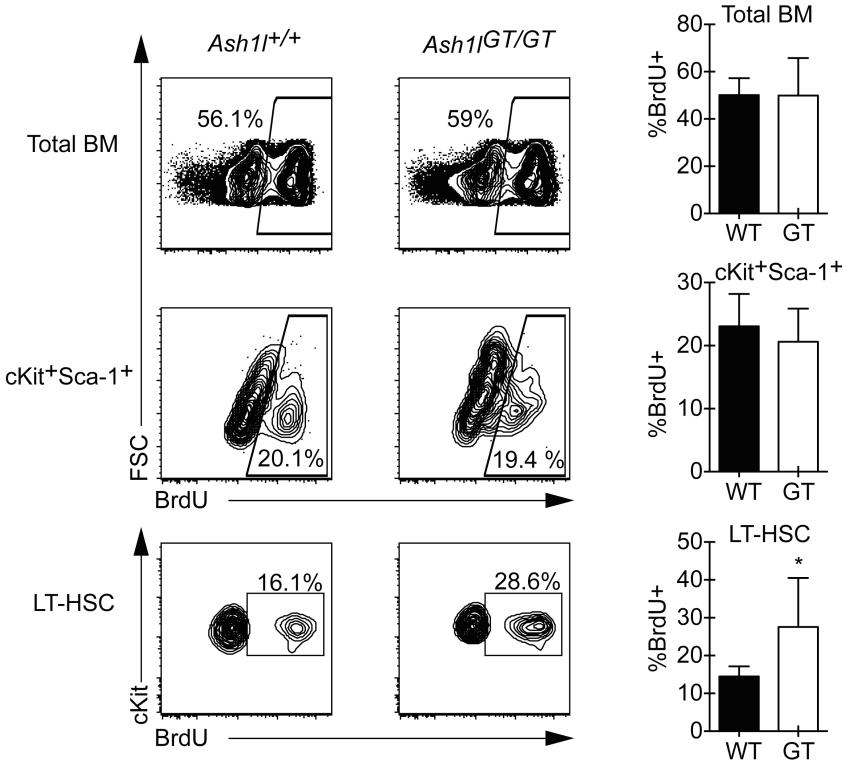

Figure S5. Increased BrdU incorporation in phenotypically defined *Ash1l*-deficient bone marrow LT-HSCs.

Flow cytometric analysis of BrdU incorporation in P19 total BM, c-Kit$^+$Sca-1$^+$ cells and LT-HSCs (CD150$^+$CD48$^-$LSK) (BrdU 0.5 mg i.p. 12 hours before analysis). There was no significant difference between WT and GT in total BM and c-Kit$^+$Sca-1$^+$ cells, but increased uptake in GT LT-HSCs (n≥4/genotype from 3 independent experiments; mean +/- 2SEM).

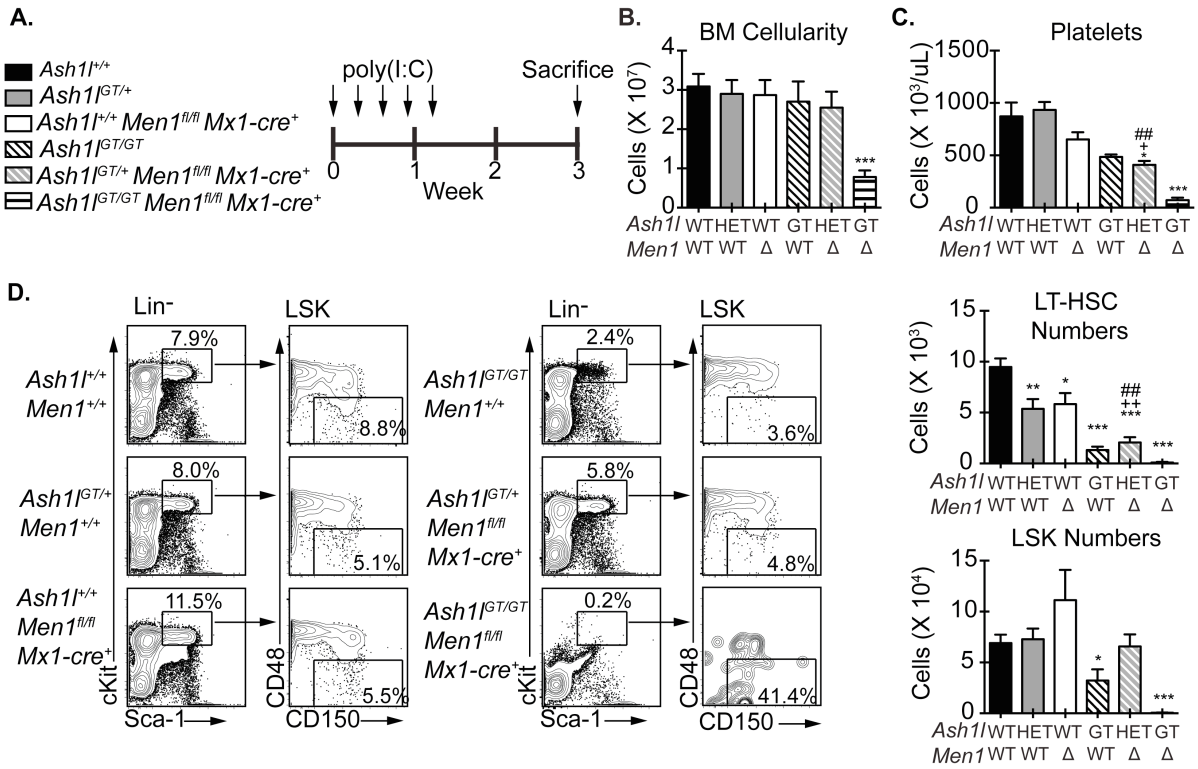

Figure S6. Combined *Ash1l* and *Men1* deficiency induces overt hematopoietic failure and profound depletion of LT-HSCs and LSK progenitors. (**A**) Experimental strategy: mice of indicated genotypes were injected with poly(I:C) (20 μg every 2 days x5); (**B**) BM cellularity 3 weeks after initiation of poly(I:C) (≥5 mice/genotype; mean +/- SEM); (**C**) Platelet count 3 weeks after initiation of poly(I:C); **(D)** Representative flow cytometry plots (left) and bar graphs (right) quantifying BM LT-HSC and LSK progenitors. LT-HSC and LSK frequencies and absolute cell numbers reflected a profound defect in *Ash1l*$^{GT/GT}$ *Men1*$^{fl/fl}$*Mx1-Cre*$^{+}$ mice and reduced LT-HSC numbers in *Ash1l*$^{GT/+}$ *Men1*$^{fl/fl}$*Mx1-cre*$^{+}$mice (≥5 mice/genotype; mean +/- SEM). *p<0.05, **p<0.01, ***p<0.001 compared to wild-type. For comparisons between *Ashl*$^{GT/+}$ *Men1*$^{fl/fl}$*Mx-1-Cre*$^{+}$and *Ashl*$^{GT/+}$ or *Men1*$^{fl/fl}$*Mx1-Cre*$^{+}$: ## p<0.01 compared to *Ash1l*$^{GT/+}$; ++ p<0.01 compared to *Men1*$^{fl/fl}$*Mx1-Cre*$^{+}$.