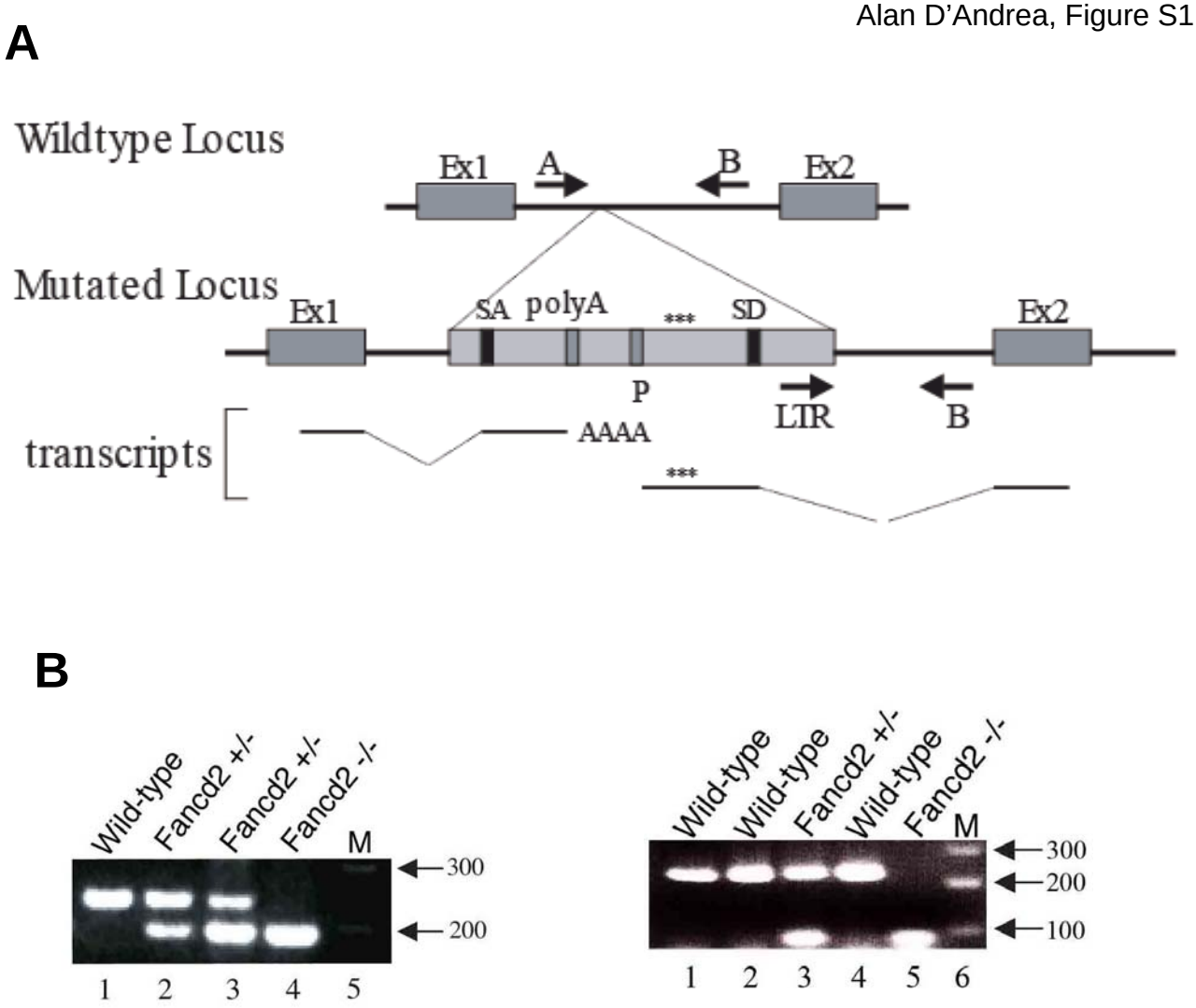
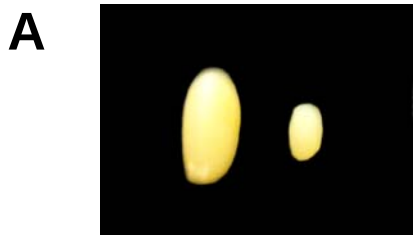
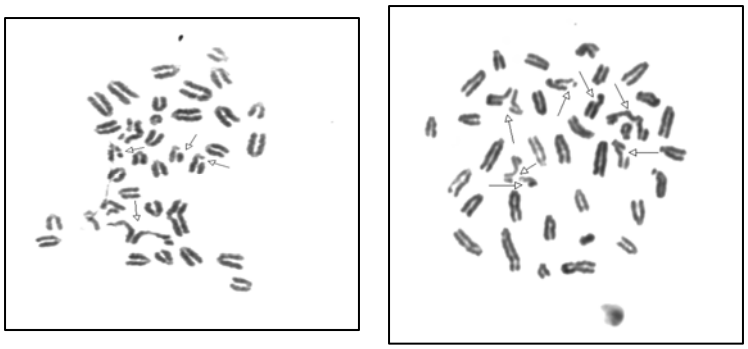
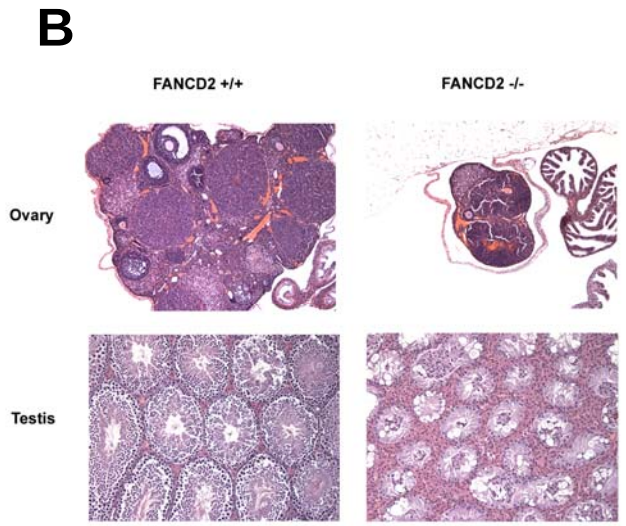
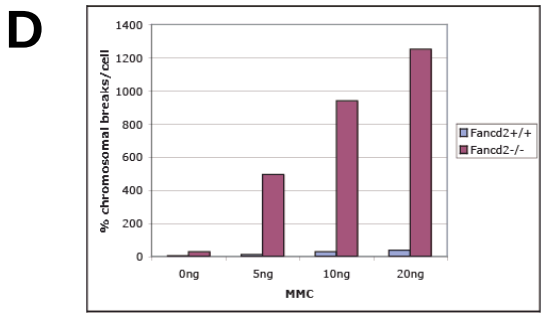


Figure S1. Generation of Fancd2-deficient mice using gene trap insertional mutagenesis of the murine *Fancd2* gene. (A) Schematic representation of the murine *Fancd2* gene carrying a VICTR insertion within intron 1. Two independently derived ES cell lines (OST2 and OST5), both carrying retroviral insertions at different sites within intron 1, were used to generate Fancd2-/- mice. Ex: exon, SA: splice acceptor, SD: splice donor. Asterisks depict the stop codons in the reading frames. Arrows illustrate the positions of the PCR primers used to genotype the mice. (B) PCR genotyping of wild type, Fancd2+/- and Fancd2-/- mice, left panel for OST2 and right panel for OST5 mouse lines.









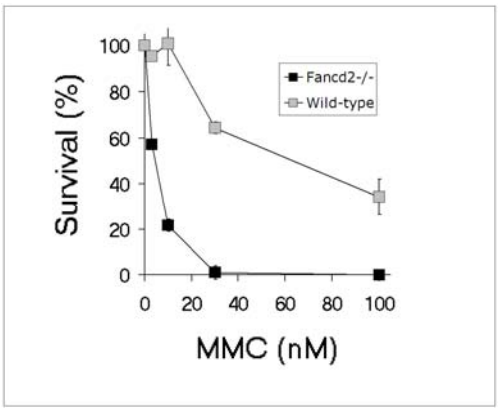


Figure S2. Fancd2-deficient mice exhibit hallmark features of Fanconi anemia. (A) Reduced testes size in *Fancd2-/-* mice (right) (B) Histology of ovaries and testes from wild-type (*Fancd2+/+)* and *Fancd2-/-* mice. H & E staining of the tissues is shown, Magnification 200X (C) Metaphase spreads of splenocytes from Fancd2-/- mice after 72 hours of in vitro culturing with 5ng/ml MMC (left panel) or 10 ng/ml MMC (right panel). Arrows indicate radial forms or breaks (D) Chromosomal aberrations per cell were compared between wild-type (*Fancd2+/+*) and *Fancd2-/-* mouse splenocytes treated with the indicated concentrations of MMC for 48 hours. The values in the y-axis of the plot denote the number of aberrations per 100 cells. (E) Clonogenic survival assay of bone marrow cells from wild-type (gray squares) and *Fancd2-/- (*filled squares) mice. Bone marrow cells were plated in methylcellulose in the presence of the indicated concentration of MMC. After 7-10 days in culture, hematopoietic colonies were enumerated.

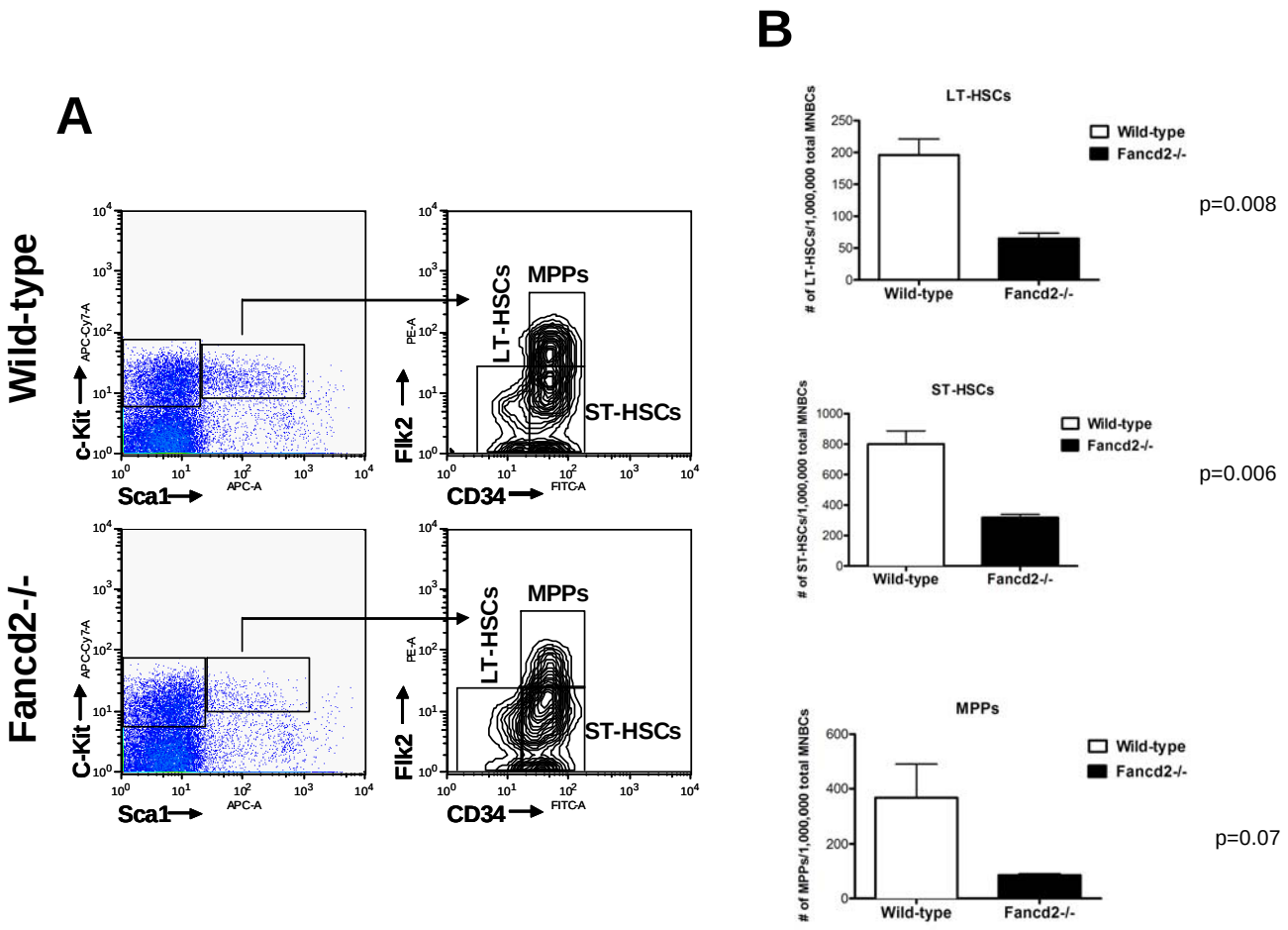


Figure S3. *Fancd2-/-* bone marrow contains low numbers of MPPs, ST-HSCs and LT-HSCs. Bone marrow cells were harvested from wild-type and *Fancd2-/-* mice and stained for multipotent progenitors (MPPs), short-term HSCs (ST-HSCs) and long-term HSCs (LT-HSCs). (A) Representative flow cytometric plots (upper plots from wild-type mouse and lower plots from Fancd2-/- mouse) pregated on Lineage negative cells are shown. MPPs were gated as Lin-Sca+c-Kit+Flk2+CD34+, ST-HSCs were gated as Lin-Sca+c-Kit+Flk2-CD34+, and LT-HSCs were gated as Lin-Sca+c-Kit+Flk2-CD34- cells. (B) The numbers of LT-HSCs, ST-HSCs and MPPs in wild-type and Fancd2-/- mice are shown. Data are average from 3 mice per group. Error bars indicate SEM..

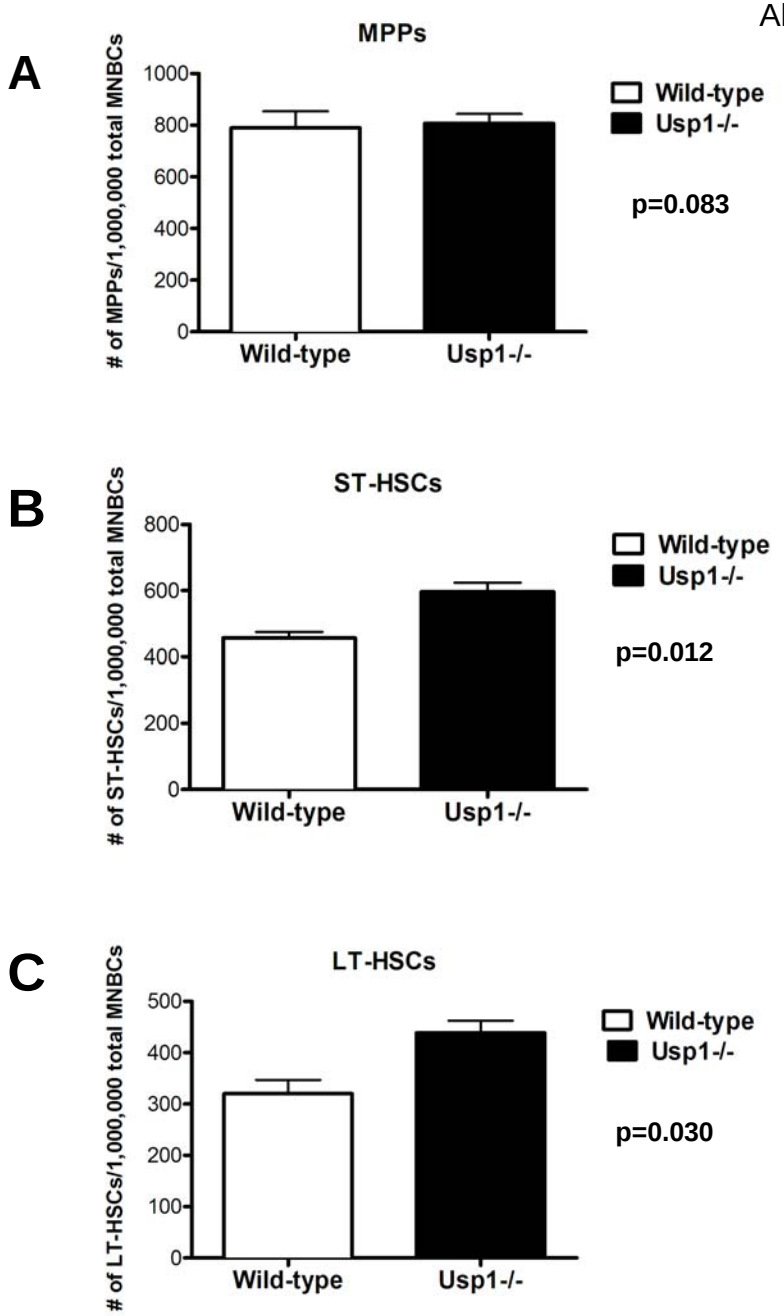


Figure S4. Increased numbers of ST-HSCs and LT-HSCs in *Usp1-/-* mice. Bone marrow cells were harvested from wild-type and *Usp1-/-* mice and stained for multipotent progenitors (MPPs), short-term HSCs (ST-HSCs) and long-term HSCs (LT-HSCs). The numbers of (A) MPPs (Lin-Sca+c-Kit+Flk2+CD34+ cells), (B) ST-HSCs (Lin-Sca+c-Kit+Flk2-CD34+ cells), and (C) LT-HSCs (Lin-Sca+c-Kit+Flk2-CD34- cells) in wild-type and *Usp1-/-* mice are shown. Data are average from 3 mice per group in panels A-C. Error bars indicate SEM..

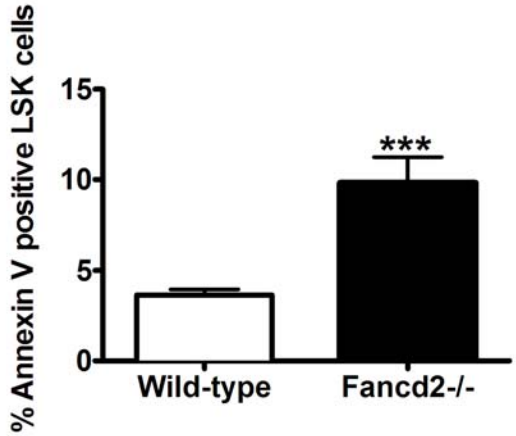


Figure S5. Loss of Fancd2 leads to increased apoptosis in bone marrow HSCs. Bone marrow cells were harvested from wild-type or *Fancd2-/-* mice and stained with antibodies against lineage markers (lin), Sca-1, and c-Kit. The cells were further stained with Annexin-V. Cells were then analyzed by flow cytometry to detect Lin-Sca-1+c-Kit+ (LSK) fractions. Frequency of apoptotic cells in HSC fraction was determined by analyzing Annexin-V staining on LSK cells. Data are from n=9 wild-type or *Fancd2-/-* mice and mean values ± SEM are shown. Asterisks represent statistically significant difference in the Fancd2-/- mice compared to the wild-type mice with p values =0.0007.

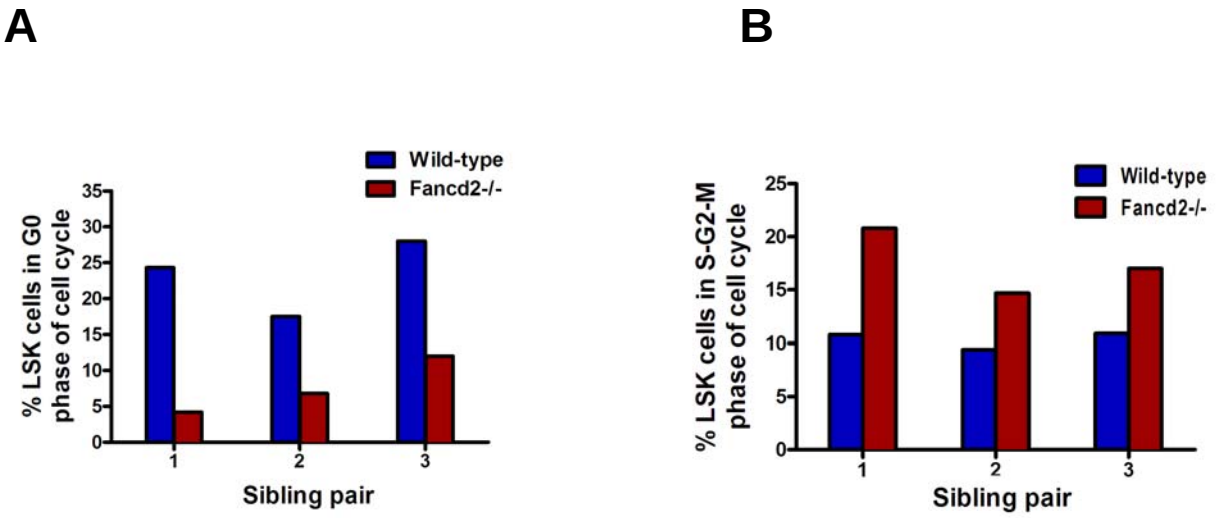


Figure S6. Loss of Fancd2 leads to decreased quiescence and increased cycling of bone marrow HSCs. Bone marrow cells were harvested from wild-type or *Fancd2-/-* mice, lineage depleted, and stained with anti-Sca-1 and anti-c-Kit antibodies. Following fixation and permeabilization, cells were stained with anti-Ki67 and 7-AAD and analyzed by flow cytometry to separate cells in different phases of the cell cycle, based on DNA content (7-AAD) and expression of Ki67. The frequencies of Lin-Sca-1+c-Kit+ (LSK) cells in G0 phase of cell cycle (A) and S/G2/M phase of cell cycle (B) are shown. Data are from three individual wild-type or *Fancd2-/-* mouse sibling pairs.