Figure S1. Hhex expression and gene structure of the conditional allele. (A) bar graph shows qRT-PCR of *Hhex* expression in various hematopoietic stem and progenitor cells. (B) schematic shows the gene structure of *Hhex*. Blue boxes show the 4 exons of *Hhex*, all coding, with triangles denoting the positions of *loxP* sites. Cre-mediated recombination induces deletion of the floxed allele resulting in the gene structure shown. (C) shows a screenshot of our RNA-seq study loaded on to the Integrated Genome Viewer for hematopoietic stem cells (HSC) and multipotent progenitor cells (MPP) sorted from WT or *Hhex* cKO mice. Gray bars show the reads corresponding to *Hhex* mRNA which is present in
WT HSC and MPPs but not detectable in *Hhex* cKO cells.

Figure S2. Hhex expression across multiple stem and T-cell progenitor cell types. Stem and T- cell progenitor populations of interest were selected within the My GeneSet browser to display the Immunological Genome Project's gene expression data as a W plot as previously described (www.Immgen.org)[11,12]. The y-axis shows relative expression Hhex mRNA; the expression value for the given cell type was divided by the mean value of all samples. The description of the sorted cell types ,
1-26, are shown.

Figure S3. Hhex cKO mice show normal blood counts at steady state. Peripheral blood cells and hemoglobin were analyzed by an automated Hemavet (cell counter) machine for WT (n=5) and *Hhex* cKO (n=6) mice at steady state. Bar graphs show the means ± SEM for RBC, red blood cells; WBC, white blood cells; Hb, Hemoglobin; PLT, platelets; NE, neutrophils; and, LY, lymphocytes. White bars are WT and black bars are Hhex cKO samples.

Figure S4. Comparison of hematopoietic cell populations in WT and *Hhex* cKO mice. (A) Representative flow cytometry plots are shown for CD4 and CD8 stained cells in bone marrow, spleen and thymus isolated from WT and *Hhex cKO* mice. The right panel shows bar graphs representing the proportions and absolute numbers of each T-cell population. Total cells were calculated based on the total number of cells multiplied by the percentage of each population and represented as mean ± SEM, n=9-10 for WT and cKO. (B) Representative flow cytometry plots for Mac-1 (or CD11b) and Gr-1 are shown for stained cells from the bone marrow and spleen of WT and *Hhex cKO* mice. Bar graphs show the proportions and absolute number of each myeloid population in the bone marrow, and spleen. Total cells were calculated based on the total number of cells multiplied by the percentage of each population and represented as means ± SEM, n=9-10. (C) Histograms show flow cytometry analysis for Ter119 staining done on bone marrow, spleen and thymus cells isolated from WT and *Hhex cKO* mice. Bar graphs show the proportion and absolute number of erythroid cells in the bone marrow and spleen.
Total cells were calculated based on the total number of cells multiplied by the percentage of each population and represented as means ± SEM, n=9-10. P values were generated by Student t test. Asterisks denote significance level: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$;

****, P ≤ 0.0001.

Figure S5. *Hhex* cKO LSKs are unable to upregulate B-cell specific transcriptional program in OP9-driven differentiation *in vitro*. (E) RNA-seq analysis of LSK cells after enrichment from bone marrow and at passage 1 (7 days of co-culture with OP9 cells). Genes shown are important for lymphoid priming. Values shown are reads per kilobase of gene per million reads (RPKM).

Figure S6. Gating schemes for B-cell subset analyses and IL7R-expressing cells. (A) Representative flow plots of B-cell subsets of the bone marrow and the spleen of WT and *Hhex* cKO mice. (B) Histogram depicting the mean fluorescence intensity (MFI) of IL7R staining in the bone

marrow of WT and *Hhex* cKO mice. (C) Graph representing MFI of IL7R in bone marrow of WT and *Hhex* cKO mice. Bars represent mean of n=9. P value was generated by Student t test.

Figure S7. *Igh* gene rearrangements in *Hhex* cKO and WT mice. Nested PCR analysis of DJ recombination was used to amplify four possible junctions between D-Q25 and JH regions from genomic DNA. Igh rearrangement was analyzed in $B220^{+}$ (lanes 1,3,5,7,9 and 11) and $B220^{-}$ (lanes

2,4,6,8,10 and 12) sorted splenocytes from WT and *Hhex* cKO mice. Both cKO and WT gDNA showed

the same pattern of DJ recombination.

Figure S8. Contribution of GFP^{+} and GFP^{-} grafts in *Hhex* cKO bone marrow transplants. Figure

3A shows the outline of the BMT experiment. As shown in Figure 3G, Hhex cKO bone marrow did not compete with WT bone marrow and contributed very little to post-transplant hematopoiesis. Here, (A) shows supplemental information on the content of the bone marrow, spleen, and thymus of host mice receiving Hhex cKO bone marrow retrovirally transduced with MIG-Hhex. First, CD45.2+ population were gated followed by GFP+ and GFP-. The bar graphs show the proportion of cells in that specific gate that are Mac-1+ or Gr-1+ etc. The GFP+ cells are shown in black and the GFP- cells are shown in gray. For bone marrow and spleen, the GFP+ and GFP- gated cells were stained for Gr-1 and Cd11b (or Mac-1). For the thymus, the various progenitor populations are, DP: double positive; DN: double negative. Two tailed pairwise comparisons and P values were generated by Student t test, with "****" denoting P<0.0001. Bracket and asterisks are not shown for pairwise comparisons without significance. In (B), the same gating hierarchy was used with respect to donor and GFP to show the proportions of LK (Lin-Kit+), LSK (Lin-Sca-1+Kit+), LSKFlt3-, LSKFlt3+. In this case, the y-axis shows the % of parent population since LSK=% of LK that are positive for Sca-1; and LSKFlt3+/- =% of LSK that are positive

or negative for Flt3.

Figure S9. Normal homing and engraftment of *Hhex* cKO lymphocytes. (A) In vivo homing

assay: colony forming units were quantified from bone marrow of lethally irradiated mice 16 hours after injection with WT or *Hhex* cKO bone marrow. (B) In vivo engraftment assay: bar graphs represent percent donor and host cells in bone marrow, spleen, thymus and peripheral blood of host mice 7 days post-transplant. Values are means ± SEM.

Figure S10. Lin-Sca-1+Kit+ (LSK) cells in the bone marrow of Hhex cKO and WT mice 3 weeks and 6 weeks after sublethal irradiation. Representative FACS plots of LSK cells in WT or Hhex cKO mice after sublethal irradiation. Time points shown are for 3, 6, 9 weeks after 650 cGy of radiation. FACS analyses were all done side by side between WT and cKO mice with the same gates.

Figure S11. In vitro differentiation of T-cells from DN thymocytes. (A) graph shows population doublings of DN thymocytes co-cultured with OP9-DL1 cells. DN thymocytes were isolated from WT mice (solid square), Hhex cKO (solid triangle), Hhex cKO transduced with MIG-Hhex (solid circle), Hhex cKO transduced with empty MIG (open triangle). (B) bar graph shows proportion of maturing T- cell progenitors derived from the same 4 groups shown in panel A at passage 5. (C) graph shows the proportion of GFP+ cells in co-cultures of WT DN cells on OP9-DL1. Solid circle denotes WT thymocytes transduced with MIG-Hhex and solid squares denote WT thymocytes transduced with empty MIG. (D) bar graph shows proportion of various T-cell progenitor populations at passage 4. Passage numbers correspond to weeks in co-culture. For panel D, ** denotes P<.01 for pairwise comparisons of MIG-Hhex versus MIG transduced cells. All values are the mean of 3 independent experiments ± SEM.

Figure S12. Gating on Flt3 positive and negative populations for HSPC characterization. In the main paper, we refer to LSKFlt3 gating to analyze hematopoietic stem and progenitor populations after sublethal irradiation and for transcriptomic analysis. In Figure 7, BrdU labeling is shown for LSK cells negative for Flt3, intermediate for Flt3, or positive for Flt3. (A) shows these representative FACS plots. (B) comparison of WT and Hhex cKO for LK and LSK absolute numbers. Each point is a mouse sampled and analyzed by FACS. P values were generated by two-tailed Student t test.