# Supporting Information

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SI Materials and Methods

Antibodies, Reagents, and Plasmids. The anti-Ubc13 antibody was from Zymed, and the antibodies for ERK1 (K-23) and β-catenin (H-102) were from Santa Cruz Biotechnology Inc. Fluorescence-labeled antibodies for mCD4-Pacific Blue (L3T4), mCD8-APC-Cy7 (53–6.7), mCD25-PE (PC61.5), CD44-FITC (IM7), B220-PE-Cy5.5 (RA3–6B2), CD43-PE (S7), CD19-PE-Cy7 (1D3), CD93-APC (AA4.1), Mac-1-APC-Cy7 (M1/70), Gr-1-PerCP-Cy5.5 (RB6–8C5), c-Kit$^-$APC-Cy7 (2B8), Sca-1$^-$APC (D7), CD45.1-FITC (A20), CD45.2-PE$^-$ (104), β-catenin-FITC were from eBioscience. The streptavidin-PerCP-Cy5.5 and mouse lineage panel (559971) antibodies and the Annexin-V FITC Apoptosis Detection Kit I were from BD Biosciences.

**Flow Cytometry Analyses and Cell Sorting.** Single-cell suspensions were prepared from the thymus, peripheral blood, spleen, lymph nodes, and bone marrow. After lysis of RBCs, the cell suspensions were filtered through nylon mesh, stained with fluorescence-labeled antibodies, and subjected to flow cytometry essentially as described (36) using a LSR II flow cytometer and the FlowJo analyzing software (Treestar). For β-catenin intracellular staining, the surface-stained cells were fixed and permeabilized using the Cytofix/Cytoperm buffer (BD Biosciences) and then stained with β-catenin-FITC, followed by washing with the Perm/Wash buffer (BD Biosciences). For cell sorting, total bone marrow cells were stained with mouse lineage mixture (BD Biosciences) followed by incubation with anti-biotin microbeads (Miltenyi Biotec). Lineage negative cells were first purified by MACS sorting, and then stained for IL-7R, Sca-1, and c-Kit. LSK (Lin$^-$IL-7R$^-$Sca-1$^+$c-Kit$^+$), B220$^+$, and myeloid (Gr-1$^+$Mac-1$^+$) cells were sorted using BD FACS Aria.
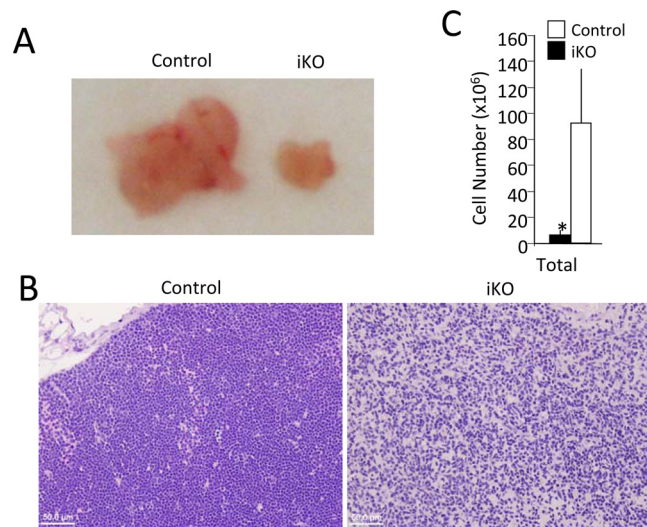
Fig. S1. Thymic atrophy in Ubc13 iKO mice. Control and Ubc13 iKO mice were prepared by poly(I:C) injection, as described in *Materials and Methods*. (*A*) Appearance of freshly isolated thymus from control and iKO mice showing the reduced size of the Ubc13 iKO thymus. (*B*) H&E staining of thymus sections (magnification, ×20). Data are representative of three control and three iKO mice. (*C*) Total thymocyte numbers in control and iKO mice (*n* = 3 each). Data are presented as mean ± SD. *, $P < 0.05$; **, $P < 0.01$.
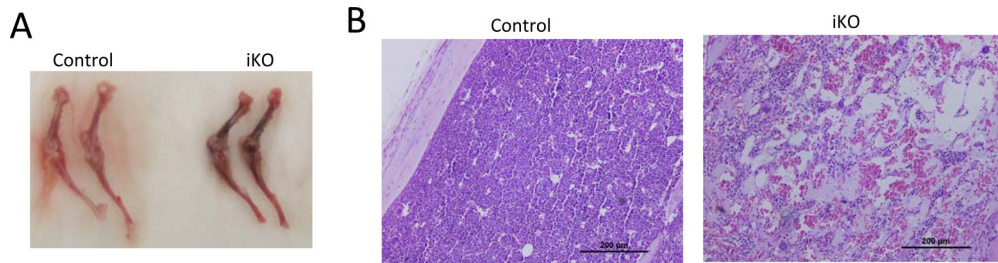
Fig. S2. Bone marrow atrophy in Ubc13 iKO mice. Control and Ubc13 iKO mice were prepared as described in *Materials and Methods*. (*A*) Appearance of bones (containing the femur and tibia) from age-matched control and Ubc13 iKO mice [9 days after initial poly(I:C) injection]. Data are representative of three control and three iKO mice. (*B*) H&E staining of femur sections derived from age-matched control and Ubc13 iKO mice, showing the loss of nucleated cells in the iKO bone marrow. Data are representative of three control and three iKO mice.
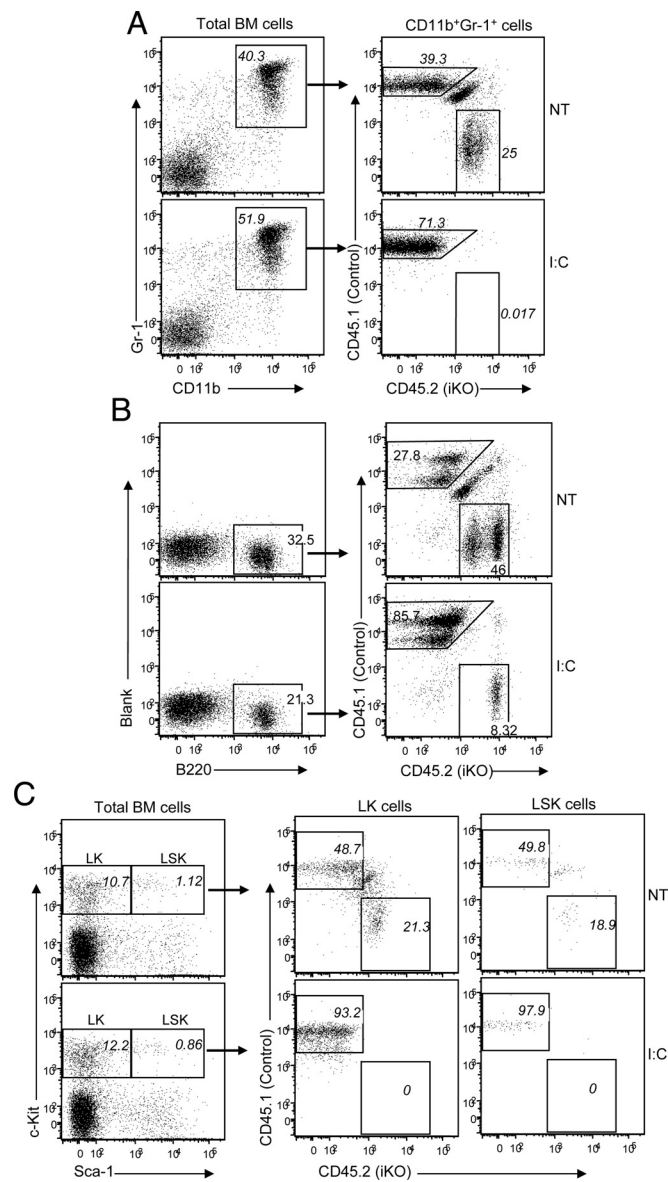
Fig. S3. Cell-intrinsic function of Ubc13 in hematopoiesis. Bone marrow cells from Ubc13$^{+/+}$ control (CD45.1^{+}) and Ubc13$^{fl/fl}$Mx1-Cre^{+} (CD45.2^{+}) mice were mixed in a 1:1 ratio and adoptively transferred into lethally irradiated Ubc13$^{+/+}$ recipient mice (CD45.1^{+}CD45.2^{+}) as described in *Materials and Methods*. After 6 weeks, the chimeric mice were either not treated (NT) or subjected to three consecutive poly(I:C) injections [Poly(I:C)] and subjected to bone marrow flow cytometry assays 9 days after the initial poly(I:C) injection. (*A*) Flow cytometry analysis of bone marrow myeloid cells based on expression of Gr-1 and Mac-1. Numbers indicate percentages of myeloid cells (Gr-1^{+}Mac-1^{+}) within total bone marrow cells (left). The gated myeloid cells were further analyzed based on their expression of CD45 congenital markers to separate into control and Ubc13 iKO populations (right). (*B*) Percentage of bone marrow B cells was analyzed based on B220 expression (Left) and further separated into CD45.1 (control) and CD45.2 (Ubc13 iKO) populations (right). (*C*) Percentage of LK and LSK bone marrow progenitor cells were analyzed as described in Fig. 4*A* (left) and further separated into CD45.1^{+} (control) and CD45.2^{+} (iKO) populations (right). Data are representative of three nontreated and three poly(I:C)-treated chimeric mice.
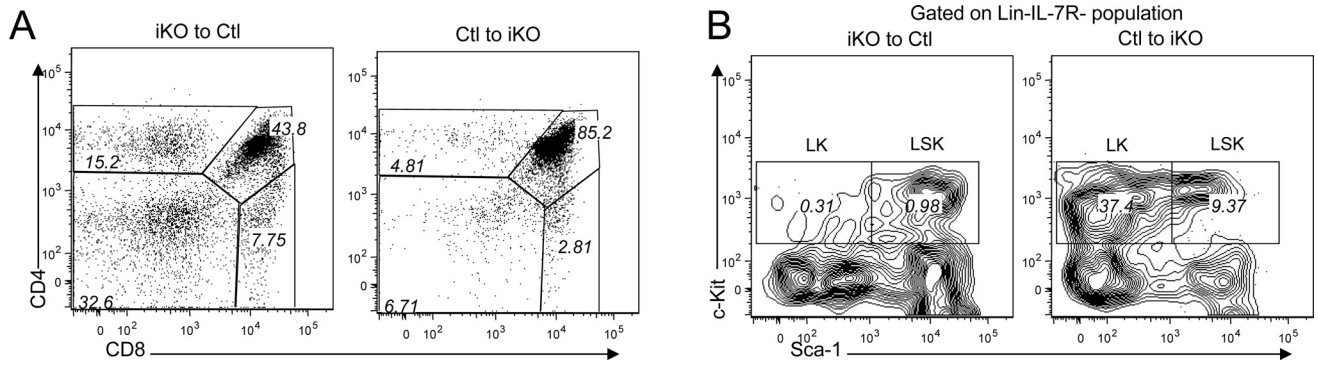
Fig. S4. Reciprocal bone marrow adoptive transfer. Ubc13^{fl/fl}Mx1-Cre^− (control) and Ubc13^{fl/fl}Mx1-Cre^+ (iKO) mice (five mice/group) were lethally irradiated twice at the dose of 600cGy with 6-h interval and used as recipients. After 12 h, the irradiated mice were adoptively transferred with 5 millions of bone marrow cells from nonirradiated control or iKO mice. Six weeks later, both groups of the recipient mice were subjected to three consecutive intra-peritoneal polyIC injections. At day 9 after the first polyIC injection, thymocytes and bone marrow cells were isolated and stained with appropriate antibodies to analyze thymocyte development (*A*) and hematopoiesis phenotypes (*B*). The numbers in the squares indicate the percentages of different cell subsets. LK and LSK are defined as Lin^−IL-7R^−Sca-1^−c-Kit^+ and Lin^−IL-7R^−Sca-1^+c-Kit^+ progenitor cells, respectively.
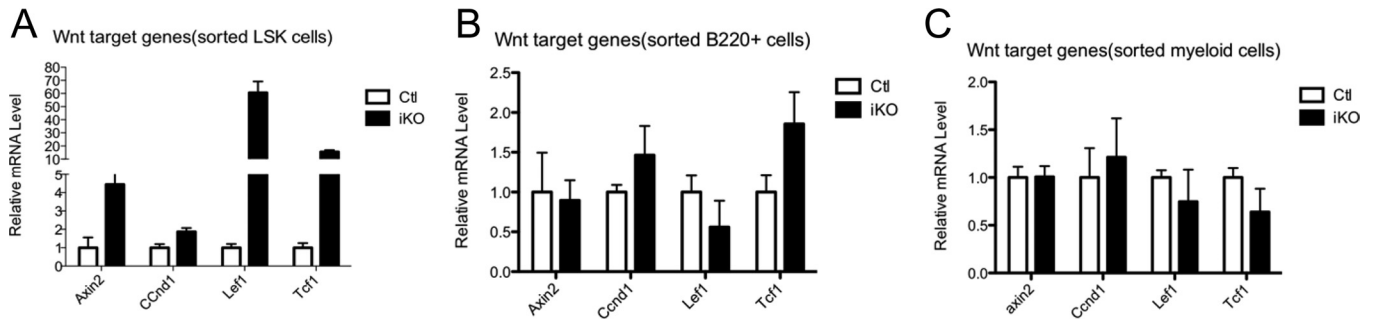
Fig. S5. Real-time RT-PCR analyses of Wnt target gene expression in different populations of bone marrow cells. Control (Ctl) and Ubc13 iKO mice were generated by polyIC injection, as described in *Materials and Methods*, and used for bone marrow cell preparation. LSK cell (*A*), B cell (*B*), and myeloid cell (*C*) populations were isolated by flow cytometric cell sorting and subjected to RNA preparation and real-time RT-PCR assays. Data are presented as fold relative to the control samples (arbitrarily set to 1) and are representative of three independent experiments.
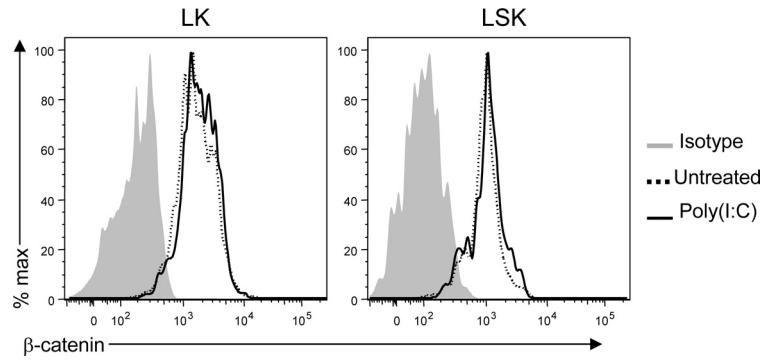
Fig. S6. Flow cytometry analysis of intracellular β-catenin level. Control mice (Ubc13$^{+/fl}$Mx1-Cre^{+}) were either left untreated or injected with PolyIC as described in *Materials and Methods*. Bone marrow cells were intracellularly stained with anti-β-catenin or an isotype control as well as with antibodies for surface markers. Flow cytometry was performed to detect β-catenin intracellular level in gaited LK and LSK cell populations. Comparison was visualized by overlay of histogram of untreated and polyIC-treated mice. Data are representative of three independent experiments.