Fig S1. Generation of Mx-Cre/Pofut1^{F/F} mice and genotyping. (A) Shown is exon 2 of Pofut1 flanked by loxP sites (solid arrows), the positions of genotyping primers, and the expected product sizes. (B) PCR genotyping results of Mx-Cre/Pofut1^{F/F} and control WT mice marrow cells (Mx-Cre/Pofut1^{+/+}, or Pofut1^{F/F} without Cre) using primers PS644 and PS645 (PS644: 5’-GGGTACCTTCTAGTACAGTACACAGTGTG-3’; PS645: 5’-ACCCACAGGTGCTCAGTCTTTTG-3’). F, floxed allele; Δ, deleted allele. (C) PCR genotyping results for thymocytes, thymus stroma and bone marrow stromal cells.
Fig S2. Myeloid hyperplasia and lymphoid defects in Vav-Cre/Pofut1F/F mouse. Vav-Cre transgenic mice were provided by T. Graf (Albert Einstein College of Medicine, Bronx, NY). Two pups born from Vav-Cre/Pofut1F/+ X Pofut1F/F breedings were confirmed to be Pofut1−/− by PCR-genotyping (Vav-Cre forward primer: 5’-GAA CCT GAT GGA CAT GTT CAG GGA-3’; reverse primer: 5’-CAG AGT CAT CCT TAG CGC CGT AAA-3’). Vav-Cre/Pofut1+/F mice were used as controls. The hematopoietic organs were analyzed when mice were 4 months old as described in Fig 1-2. Data shown are representatives from two mice in each group. (A) FACS analysis of the granulocytes and monocytes in the PB. (B-C) FACS analysis of bone marrow mature granulocytes (Gr-1+), T cells (CD4/CD8+) (B) and myeloid progenitors (C). (D-E) FACS analysis of CD4 versus CD8 on total thymocytes (D) and DN1-DN4 subpopulations defined by expression of CD44 and CD25 gated on DN (CD4+CD8−) cells (E). (F-G) FACS analysis of spleen infiltration by granulo-monocytic cells (Gr1 and CD11b) (F) and MZB cell populations defined by B220−CD23lowCD21high (G).
Figure S3

Fig S3. PCR genotyping of hematopoietic cells from transplanted mice. (A) PCR analysis of Pofut1 deletion in PB and bone marrow (A) from Mx-Cre/Pofut1^{+/+} (wt) or Mx-Cre/Pofut1^{FF} (Δ) donor reconstituted mice in different donor-recipient pairs. (B) PCR analysis of Pofut1 deletion in WT recipient total spleen cells from Mx-Cre/Pofut1^{+/+} (wt) donors, and in sorted recipient follicular B (FL) and MZB cells from Mx-Cre/Pofut1^{FF} (Δ) donors. (C) PCR analysis of sorted DP and DN thymocytes from Mx-Cre/Pofut1^{+/+} (wt) or Mx-Cre/Pofut1^{FF} (Δ) reconstituted recipient thymi.
Fig S4. *Mx-Cre/PF/F* LSKs had suppressed Notch activation and elevated expression of *PU.1* and *C/EBPα* in OP9-coculture assays. *Mx-Cre/PF/F* or control (WT) LSK cells were cultured briefly (4 days) with OP9-Dll4 or OP9-controls in the presence of IL-7 (5 ng/ml) and Flt3L (5 ng/ml), and then analyzed for *Deltex1, Gata3, Hes1, PU.1,* and *C/EBPα* expression by qRT-PCR (n=3). The bar graph represents the mean ± SD. Results were standardized for GAPDH levels and expressed as fold changes relative to the levels detected in cells cultured with OP9-controls.
Fig S5. Mx-Cre/Pofut1F/F (Mx-Cre/PF/F) thymocytes and marrow LSKs have mildly reduced cell surface or intracellular Notch expression. (A). A representative FACS profile of Mx-Cre/PF/F and control DN thymocytes from 12-week-old mice (8 weeks after plpC injection) expressing Notch1 receptor detected by PE-conjugated antibodies against mouse Notch 1, or isotype control. (B). A representative FACS profile of intracytoplasmic Notch receptor 1-3 expression was shown in Mx-Cre/PF/F and control LSKs after cell permeabilization using Fixation/Permeabilization solution (BD Biosciences, San Jose, CA), followed by PE-conjugated antibodies against mouse Notch 1, Notch2, Notch3, or isotype control.
Fig S6. Recombinant Dll1 and Dll4 show Notch-specific binding with cell surface Notch receptors.

(A) Recombinant Dll1 and Dll4 were made in HEK 293T cells as described (Zhou et al, Blood, 2008;112:308-319). Western blot of Dll1 and Dll4 were run under either non-reducing or reducing conditions. Both Dll1 and Dll4 display the dimerized forms besides the monomers on non-reducing gels.

(B) Embryonic stem cells (ES) were induced to differentiate toward hematopoietic lineages as described (Yan et al, AJP, 2010; 176:2921-2934). Isolated CD34+ hematopoietic progenitors (ES-HPC) were seeded onto OP9 cells in the presence of rmFlt3 ligand (5mg/ml) and rmIL-7 (5mg/ml). Cells were recovered from day 15 co-culture and analyzed by FACS binding with recombinant Dll1 and Dll4 in the presence of 1mM Ca++. Compared to WT ES-HPCs, Notch1-/− ES-HPC binding with Dll1 and Dll4 were decreased by 83% and 95%, respectively. The residual binding above the background was likely contributed by other Notch isoform(s) expressed on these cells. In comparison, ES-HPCs null for Pofut2, an enzyme that fucosylates thrombospondin type 1 repeats (TSR) (Luo et al, JBC, 2006; 281: 9393-9399), had normal binding with Dll1 and Dll4.
Figure S7

**A**

Defective T cell development and myeloid hyperplasia in marrow are rescued by activated Notch1 (ICN1). Marrow cells from Mx-Cre/Pofut1<sup>F/F</sup> mice after 5-FU treatment were infected with pMig-EGFP-vector or pMig-EGFP-ICN1, and injected into lethally-irradiated WT recipients. Marrow granulocytes (Gr-1<sup>+</sup>) (A) and T cells (CD4/CD8<sup>+</sup>) (B) were analyzed by FACS 3-4 wks after transplantation in GFP<sup>+</sup> compartment.

**B**

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Fig S7. Defective T cell development and myeloid hyperplasia in marrow are rescued by activated Notch1 (ICN1). Marrow cells from Mx-Cre/Pofut1<sup>F/F</sup> mice after 5-FU treatment were infected with pMig-EGFP-vector or pMig-EGFP-ICN1, and injected into lethally-irradiated WT recipients. Marrow granulocytes (Gr-1<sup>+</sup>) (A) and T cells (CD4/CD8<sup>+</sup>) (B) were analyzed by FACS 3-4 wks after transplantation in GFP<sup>+</sup> compartment.
Figure S8

**Fig S8. Normal thymocyte numbers and subpopulation distribution in Pofut1<sup>+/−</sup> mice.** Absolute numbers of total thymocytes, CD4<sup>+</sup>, CD8<sup>+</sup>, DP, and DN subsets in 8~12-week-old WT (Pofut1<sup>+/+</sup>) or Pofut1<sup>+/−</sup> mice (n=6 in each group). The bar graph represents the mean ± SD. Student t-test was performed to compare the absolute number of cells from Pofut1<sup>+/−</sup> mice with those of Pofut1<sup>+/+</sup> mice. p > 0.05 in all groups.