Supporting Information

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SI Materials and Methods

ChIP-Seq Analyses. ChIP-Seq for Notch1 and CSL was performed as described (26) with sheared chromatin from T6E cells. Data are available through the Gene Expression Omnibus database (accession no. GSE29600). For Tcf-1 ChIP-Seq, 10^8 WT thymocytes were fixed with formaldehyde and sonicated to an average size of 300 bp. Tcf-1 antibodies [a kind gift of Hiroshi Kawamoto (Riken Yokohama Institute, Yokohama, Japan)] coupled to Protein G Dynabeads (Life Technologies) were incubated overnight with sheared chromatin. Immunoprecipitation and input samples were de-cross-linked overnight at 65 °C in elution buffer [50 mM Tris·HCl (pH 8)/10 mM EDTA/1% SDS/0.3 M NaCl]. DNA was extracted with phenol/chloroform, quantified, and quality-checked for enrichment of known target regions of Tcf-1 by real-time PCR. ChIP-Seq libraries were prepared from 10 ng of immunoprecipitated material according to an Illumina ChIP DNA library preparation kit. After deep sequencing on an Illumina Genome Analyzer II, 32-bp reads were mapped to mouse genome version mm9 with the efficient local alignment of nucleotide data (ELAND) alignment tool in GApipeline, accepting no more than two mismatches. Uniquely aligned tags were selected, converted to .bed files, and after read normalization were analyzed by the peak-calling software Model-based Analysis of ChIP-Seq (MACS). (1). The algorithm was applied using a 2*d* sliding window across the genome to find candidate peaks with significant tag enrichment according to Poisson distribution at a default *P* value of 10^{-5} with input control data.

EMSA. Nuclear extracts were prepared according to standard protocols. In brief, cell homogenates were centrifuged and resuspended in a hypotonic buffer containing 0.4% (vol/vol) Nonidet P-40 followed by a buffer with a higher salt concentration before the supernatant (nuclear proteins) was collected. Before the addition of biotin-labeled DNA probe, 5 μg of nuclear extract or 0.5 μg of purified CSL protein was incubated for 20 min on ice in 20 μL of reaction buffer containing 1× binding buffer, 1 μg of double-stranded poly(dI:dC), 2.5% (vol/vol) glycerol, 0.05% (vol/vol) Nonidet P-40, and 1 μg of BSA. Samples were incubated for 20 min at room temperature with the following biotinylated probes (20 fmol each): WT, 5′-CCGAGACGTAGTATTCCCACCACACGCCACCTTC-3′; mutant, 5′-CCGAGACGTAGTATTAAAACCACACGCCACCTTC-3′. Underlined base pairs represent the consensus Notch binding motif and the engineered mutation. For competition experiments, a 100-fold molar excess of unlabeled oligonucleotides was added. Protein–DNA complexes were separated by electrophoresis through 6% nondenaturing TBE gels (Life Technologies) and were visualized with a LightShift Chemiluminescent EMSA kit (Pierce).

1. Zhang Y, et al. (2008) Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 9:R137.

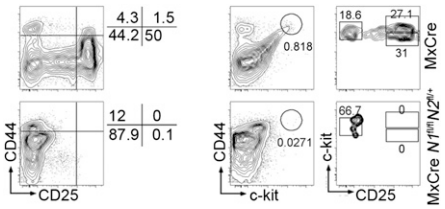

Fig. S1. Thymic profile of Notch1-deficient thymocytes. (*Left*) Plots show the CD44 versus CD25 profile of gated Lin$^-$ thymocytes from MxCre and MxCre Notch1$^{fl/fl}$ Notch2$^{fl/+}$ (MxCre*N1*$^{fl/fl}$*N2*$^{fl/+}$) mice as indicated. Numbers to the right of the plots are quadrant frequencies. (*Right*) CD44 versus c-kit plots show the electronic gating for pro-T cells in the indicated mice. c-kit versus CD25 plots are gated pro-T cells and depict the early thymic progenitor (ETP), double-negative 2a (DN2a), and DN2b subsets for the indicated mice. Numbers in the plots indicate gate frequencies.

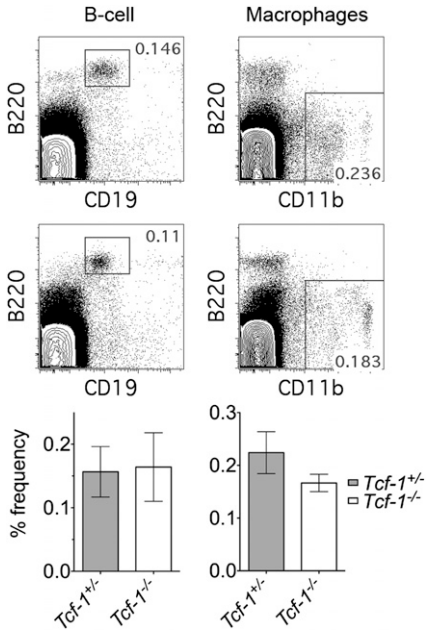

Fig. S2. B cells and macrophages in Tcf-1$^{-/-}$ thymi. Thymocyte suspensions from Tcf-1$^{-/-}$ or Tcf-1$^{+/-}$ controls were stained with antibodies to B220, CD19, and CD11b. (*Upper*) Plots show B220 versus CD19 and B220 versus CD11b thymocyte profiles. Gates in B220 versus CD19 plots depict B cells, and gates in B220 versus CD11b plots depict macrophages. (*Lower*) Histogram bars are average frequencies of B cells and macrophages as defined by the gates in the plots from three independent mice. Error bars are SD.

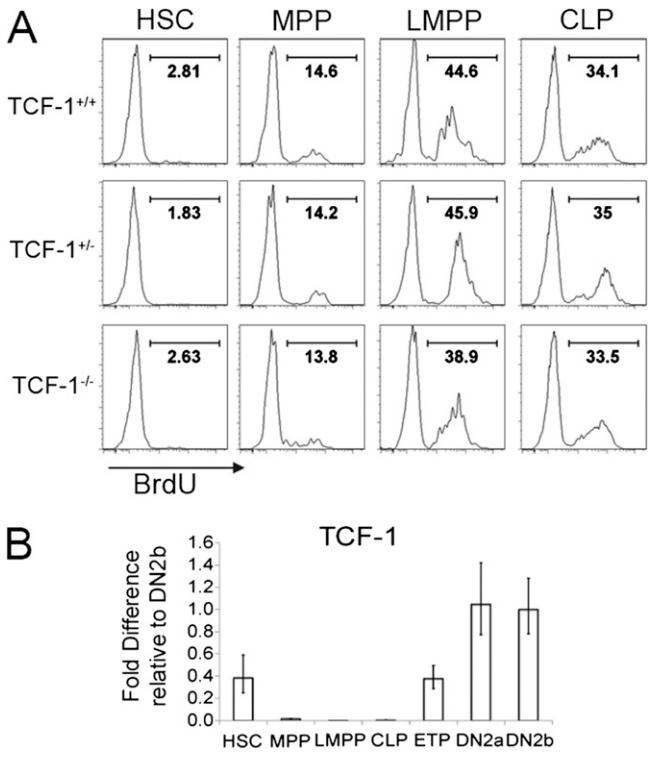

Fig. S3. Cycling of *Tcf-1*$^{-/-}$ bone marrow (BM) progenitors and their expression of Tcf-1. (*A*) Cycling of the indicated progenitors was compared in the indicated mouse strains by staining for BrdU. All mice were injected i.p. with BrdU at 2 h before harvesting the cells for surface markers followed by BrdU staining. Histogram plots show BrdU staining in the indicated gated populations and mouse strains. Numbers in the plots represent the fraction of BrdU^{+} cells in each population. Data are from cells harvested at 2 h after BrdU injection. This is one representative experiment out of three. (*B*) Hematopoietic stem cells (HSCs), multipotent progenitors (MPPs), lymphoid-primed MPPs (LMPPs), and common lymphoid progenitors (CLPs), as defined in Fig. 1, were sorted from the BM of WT mice. Similarly ETP, DN2a, and DN2b cells were sorted from the thymi of WT mice. RNA was extracted and analyzed by quantitative PCR. Tcf-1 expression was normalized to GAPDH. Histograms show relative expression levels in the various subsets normalized to the levels of DN2b.

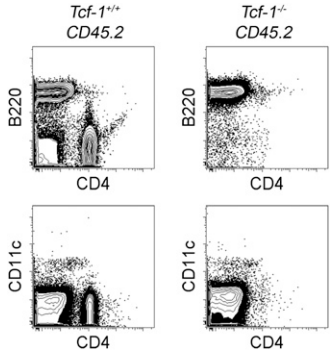

Fig. S4. Competitive reconstitution of peripheral blood lineages. BM progenitors from Tcf-1$^{-/-}$ (CD45.2) mice were isolated by sorting and mixed with their WT CD45.1 counterparts before injecting WT lethally irradiated (CD45.1) recipients. At 10 wk after injection, splenocytes were stained with the indicated surface markers and analyzed. (*Upper*) B220 versus CD4 are the profiles of gated *Tcf-1*$^{-/-}$ (CD45.2^{+}) or *Tcf-1*$^{+/+}$ (CD45.2^{-}) cells isolated from the recipients. (*Lower*) CD11c versus CD4 are the profiles of gated *Tcf-1*$^{-/-}$ (CD45.2^{+}) or *Tcf-1*$^{+/+}$ (CD45.2^{-}) cells isolated from the recipients.