Supplementary Information

Methods

Administration of polyI:polyC.

β-catenin$^{f/f}$MxCre$^{+/-}$ were induced as described previously[32], polyI:polyC (Sigma-Aldrich) was resuspended in Dulbecco PBS at 2 mg/ml. Mice received intraperitoneal injections of 0.2mg polyI:polyC every other day for 2 weeks. Notch1$^{f/f}$MxCre^{+}Rosa$^{YFP/+}$ mice received two intraperitoneal injections of 0.2mg polyI:polyC one week apart and were rested for one week.

Intravenous transfers and intrathymic injections

For intravenous transfers of transduced progenitors, wt LSK progenitors were transduced with TCF-1, ICN1, or control virus and transferred into sublethally irradiated mice. Mice were analyzed 2-8 weeks post reconstitution for donor chimerism in BM, spleen, and thymus.

For intrathymic injection of TCF-1$^{-/-}$ or TCF-1$^{+/+}$ progenitors, fresh LSK progenitors were isolated by cell sorting and injected intrathymically. Mice were analyzed after 10 days for thymic reconstitution.

Plasmids

MSCV-IRES-GFP (MIGR1), MIGR1-ICN1 retroviral vectors were obtained from Dr. Warren Pear (University of Pennsylvania). MSCV-VEX (VEX) vector was provided by Dr Christopher Klug (University of Alabama, Birmingham). MigR1 and VEX vectors were converted to Gateway compatible vectors and full length TCF-1 cDNA was cloned into VEX according to Gateway clonase manual (Invitrogen). The mouse TCF-1 promoter

[~1.5 Kb insert containing TCF-1 promoter activity based on Promoter Prediction 2.0[33]] was cloned into pGL3 basic promoter vector. A ~1.3 kb insert containing the -31kb CSL binding site of TCF-1 (in relation to the full-length TCF-1 translational start site) was cloned into pGL3 promoter vector (Promega). Mutation of the TCF-1 binding site in pGL3 basic-mouse TCF-1 promoter or the -31kb CSL binding site in the pGL3 promoter vector was achieved with site-directed mutagenesis.

Cell Preparations, flow cytometry and cell sorting

BM and thymocytes were prepared as previously described[1]. Cell preparations were stained with optimized antibody (Ab) dilutions. Antibodies used in the lineage cocktail (Lin) include antibodies to B220 (RA3-6B2), CD19 (1D3), CD11b/Mac1 (M1/70), Gr1 (8C5), CD11c (HL3), NK1.1 (PK136), TER119 (TER-119), CD3ε (2C11), CD8α (53-6.7), CD8β (53-5.8), TCRβ (H57), $\gamma\delta$TCR (GL-3). Additional Abs used included antibodies to $CD45^{B6}$ (104), $CD45^{SJL}$ (A20), Sca1 (E13-161.7), Kit (2B8), Flt3 (A2F10.1), CD90.1/Thy1.1 (HIS51), Gr1 (RB6-8C5), CD19 (ID3) and CD25 (PC61.5). Antibodies were directly conjugated to FITC, PE, PeCy5, PeCy5.5, PerCP-Cy5.5, PeCy7, APC, APC-Cy5.5 (or Alexa 700), APC-Cy7 (or APCeFluor780), or biotin. Biotinylated Abs were revealed with Streptavidin PE-TexasRed. All Abs were purchased from eBiosciences, Biolegend, or BD Pharmingen. Cell sorting was on a FACSAria II (BD Biosciences) and flow cytometric analysis was performed on a LSR-II (BD Biosciences). Dead cells were excluded through 4,6 diamidino-2-phenylindole (DAPI) uptake. Doublets were excluded through forward scatter–height by forward scatter–width and side scatter–height by side scatter–width parameters. Data were analyzed using FlowJo (Tree Star). The LSK population was isolated as $Lin^{-}Sca1^{+}Kit^{+}$. HSCs were sorted as $Lin^{-}Sca1^{+}Kit^{+}Flt3^{-}CD150^{+}$ BM cells; LMPPs (the "lymphoid primed" subset of MPPs) sorted as Lin^{-}

$Sca1^{+}Kit^{+}Flt3^{hi}$ BM cells. Thymocyte populations were defined and cell sorted as ETP ($Lin^{-/lo}$ Kit^{+} $CD25^{-}$), DN2 ($Lin^{-/lo}$ Kit^{+} $CD25^{+}$), DN3 ($Lin^{-/lo}$ Kit^{-} $CD25^{+}$). Total thymocytes were stained and sorted as immature ISP ($CD4^{-}CD8^{+}$ $TCR\beta^{-}$), DP ($CD4^{+}CD8^{+}$), CD4 SP ($CD4^{+}CD8^{-}$), and CD8 SP ($CD8^{+}$ $CD4^{-}$ $TCR\beta^{+}$).

Retroviral Transduction

Retroviral packaging was performed as previously described[26], with the exceptions of packaging cell line 293T cells and transfection reagant Fugene 6 (Roche) in place of $CaPO_4$. Hematopoietic progenitors were transduced using Retronectin (Takara). Briefly, 24 or 12-well plates were coated with 20-100 μg/ml RetroNectin according to the manufacturer's instructions. High-titer retroviral supernatants were added into wells, centrifuged at 25 ^{o}C for 1-2 hours, following which viral supernatant was removed. Cell sorted progenitor cells were resuspended in the stimulation cocktails including DMEM-complete medium, 1% pen/strep, 15% Fetal Calf Serum (FCS), L-glutamate (2mM), IL-3 (10ng/ml), IL-6 (10 ng/ml), SCF (20 ng/ml), Flt3-ligand (20 ng/ml), Polybrene (4ug/ml) and added to virus-bound RetroNectin-coated plates. Transduced BM progenitors were sorted 36-48 hr post-infection.

Luciferase Gene Reporter Assay

For Luciferase reporter assays, 293T cells were seeded 1 day prior to transfection to reach 80% confluency. 293T cells were transiently cotransfected with Fugene6 (Roche) following instructions according to manufactures' protocol. Constructs used include: pGL3 vector (300ng/well) containing the TCF-1 promoter with a TCF-1 binding site or a mutated TCF-1 binding site, the pGL3 promoter vector containing the wild-type -31kb CSL binding site in TCF-1 locus or a mutated version, the TOPFLASH TCF-1 reporter, and with either empty vector MigR1, MigR1-ICAT, MigR1-TCF-1, or MigR1-ICN1

(300ng/well). Renilla was added at 50ng/well to control for transfection efficiency. DMEM containing 10% L-glut, 10% pen/strep was added 24 hours post transfection and cells were harvested 40-48 hours after transfection and analyzed with a Dual Assay Reporter Kit (Promega). Data were analyzed by comparing Luciferase activity to Renilla activity and adjusted to the fold increase over background.

Quantitative RT-PCR

RNA was purified from indicated cell types with the RNaEasy MicroKit (Qiagen) and reverse transcribed to cDNA, using SuperScript II Kit (Invitrogen). Real-Time PCR was performed with PCR Master Mix, using Taqman probes specific for indicated genes (Applied Biosystems), and analyzed on ABI Prism 7900 system (Applied Biosystems). Relative transcript abundance was determined by using the ΔΔCt or ΔCt method after normalization with 18S, or GAPDH. All samples were run in triplicate. Error bars represent s.e.m.

ChIP

ChIP was performed with the ChIP assay kit (Millipore, #17-295), all procedures have been described[27]. In brief, CD4/CD8 depleted (DN) thymocytes or Scid-adh cells were fixed and immunoprecipitated with IgG control antibody (rabbit IgG; Santa Cruz Biotechnologies, Inc. #sc-3888), Notch1 TAD/PEST-specific antiserum[34], or anti–TCF-1 (C63D9) (Cell Signaling, #2204). DNA was purified using a PCR purification kit (Qiagen) and eluted by water. QRT-PCR was performed using the SYBR Green primers that flank putative TCF-1 or CSL binding sites. All genomic distances greater than 2kb away from the translational start site were rounded to the nearest kb. All distances are relative to the translational start site. Primer sequences are listed in Supplemental Table 1.The relative DNA amount was calculated using the standard curve method. The input DNA

was defined as an aliquot of sheared chromatin prior to immunoprecipitation, and was used to normalize the sample to the amount of chromatin added to each ChIP. All results are the average of triplicate PCR amplifications and results were confirmed for reproducibility in separate experiments.

Gene expression analysis

All protocols were conducted as described in the Affymetrix GeneChip Expression Analysis Technical Manual. RNA was extracted from sorted cells by the UPENN Microarray Core Facility, and the quality and quantity of the RNA was tested on a bioanalyzer. This was followed by the Affymetrix WT Terminal Labeling kit for fragmentation and biotinylation according to the manufactors instructions. Biotinylated targets were heated at 99C for 5 min and hybridized for 16 h at 45C. The microarrays were then washed at low (6X SSPE) and high (100mM MES, 0.1M NaCl) stringency and stained with streptavidin-phycoerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycoerythrin stain. GeneChips were scanned using the GeneArray Scanner 3000 7G. The data were analyzed using Partek Genomics Suite, version 6.5 (Partek Inc., St. Charles, MO). RMA with default settings was used to normalize data. Gene signal values for the arrays were log2 transformed and heat maps represent the log2 transformed normalized signals values or fold change values compared to a reference population. Heat maps were generated using Matrix2png, a publicly available software[35].

Statistical analysis

The means of each data set were analyzed using Student's t-test, with a two-tailed distribution assuming equal sample variance.

Supplementary References

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