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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry data: Operating system software for Miltenyi Biotech MACSQuant 10 Flow Cytometer, BD FACS Aria II Cell Sorter, iCyt Mission Technology Reflection Cell Sorter. Sequencing acquisition: operating system software for HiSeq2500; RTA 1.13.48.0; bcl2fastq 1.8.4
Data analysis	bedGraphToBigWig Bedtools (v.2.17.0) Bioconductor (v3.4) Bowtie (v1.1.1) Cluster3 (v1.52) EdgeR (v.3.16.5) EaSeq FlowJo (v10.0.8) HOMER (v4.8) HOMER-IDR Limma (v.3.30.11) MatLab (R2016a) R (v3.3.2) RSEM (v1.2.25)

March 2018

Rstudio (v1.0.136)
Samtools (v0.1.19-96b5f2294a)
STAR (v2.4.0)
TreeView (v1.1.6r4)
Mascot

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

GEO: GSE110305, GSE110882 and GSE115744.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined empirically, at least two independent experiments were performed.
Data exclusions	No data were excluded, except for two RNA-seq samples with very small library size and symptoms of amplification artifacts.
Replication	The experimental findings were reliably reproduced.
Randomization	No randomization in this study.
Blinding	No blinding test in this study.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Antibodies

Antibodies used

Anti-human/mouse CD44 PE, eBioscience, Cat#12-0441-83, Clone IM7, Lot#4312996, 1:300
Anti-mouse CD117 (cKit) APC, eBioscience, Cat#17-1171-82, Clone 2B8, Lot#4299769, 1:100
Anti-mouse CD117 (cKit) PE, eBioscience, Cat#12-1171-82, Clone 2B8, Lot#4276731, 1:100
Anti-mouse CD25 APCe780, eBioscience, Cat#47-0251-82, Clone PC61.5, Lot#1942453, 1:200
Anti-mouse CD45 PECy7, eBioscience, Cat#25-0451-82, Clone 3O-F11, Lot#4329704, 1:600
Anti-mouse NK1.1 Biotin, Biolegend, Cat#108704, Clone PK136, Lot#B234365, 1:300
Anti-mouse NK1.1 APC, Biolegend, Cat#108710, Clone PK136, Lot#B191787, 1:100
Anti-mouse B220 Biotin, eBioscience, Cat#13-0452-85, Clone RA3-6B2, Lot#4273327, 1:300
Anti-mouse CD19 Biotin, eBioscience, Cat#13-0193-85, Clone eBio1D3, Lot#4300688, 1:300
Anti-mouse Ter119 Biotin, eBioscience, Cat#13-5921-85, Clone Ter-119, Lot#4300555, 1:300

Anti-mouse Sca1 PE, eBioscience, Cat#12-5981-82, Clone D7, Lot#E01976-1631, 1:300
Anti-mouse Gr-1 Ly-6G Biotin, eBioscience, Cat#13-5931-86, Clone RB6-8C5, Lot#4330079, 1:300
Anti-mouse CD11b Biotin, Biolegend, Cat#101204, Clone MI/70, Lot#B241116, 1:300
Anti-mouse CD11c Biotin, eBioscience, Cat#13-0114-85, Clone N418, #4272690, 1:300
Anti-mouse CD11c APCe780, eBioscience, Cat#47-0114-82, Clone N418, #E10192-1635, 1:300
Anti-mouse CD8a Biotin, eBioscience, Cat#13-0081-86, Clone 53-6.7, #E02387-1632, 1:300
Anti-mouse TCRγδ Biotin, eBioscience, Cat#13-5711-85, Clone eBioGL3, Lot#4335132, 1:300
Anti-mouse TCRβ Biotin, eBioscience, Cat#13-5961-85, Clone H57-597, Lot#E03095-1632, 1:300
Streptavidin PerCP-Cy5.5, eBioscience, Cat#45-4317-82, Lot#E08374-1637, 1:200
7AAD, eBioscience, Cat#00-6993-50, Lot#1910559, 1:50
Anti-mouse hNGFR PE, Biolegend, Cat#345106, Clone ME20.4, Lot#B175123, 1:500
Anti-mouse PLZF (Zbtb16) AF647, BD, Cat#563490, Clone R17-809, Lot#05578, 1:20
Anti-Chd4, Bethyl, Cat#A301-081A, Lot#A301-081A-3, 1:1000
Anti-Mta2, Santa Cruz, Cat#sc-9447, Lot#J0107, Clone C-20, 1:200
Anti-HDAC2, Abcam, Cat#ab12169, Lot#GR231997-2, Clone HDAC2-62, 1:2400
Anti-Rest, Caltech Protein Expression Center, Cat#12C11-1B11, Lot#20100929CG, Clone 12C11-1B11, 1:2000
Anti-Ring1b, Bethyl, Cat#A302-869A, Lot#1, 1:1000
Anti-LSD1, Abcam, Cat#ab17721, Lot#GR193411-1, 1:1000
Anti-Runx1, Abcam,, Cat#ab23980, Lot#GR201678-1, 1:1000
Anti-Bcl11b, Abcam, Cat#ab18465, Lot#GR87349-1, 1:1000
Anti-Bcl11b, Bethyl, Cat#A300-383A, Lot#2, 1:1000
Anti-Bcl11b, Bethyl, Cat#A300-385A, Lot#2, 1:1000
Anti-Bcl11b, CST, Cat#12120, Lot#1, Clone D6F1, 1:1000
Anti-H3K27Ac, Abcam, Cat#ab4729, Lot#GR3216173-1
Anti-LaminB, Santa Cruz, Cat#sc-6217, Lot#J1314, Clone M-20, 1:200
Anti-Myc, MBL, Cat#M192-3, Lot#004, Clone My3, 1:5000
Anti-Flag, Sigma, Cat#MF1804, Lot#SLBL1237V, Clone M2, 1:1000

Validation

Antibodies were chosen based on the validation statements for species (mouse) and application (IB, ChIP or FACS) on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293T (obtained from ATCC), Scid.adh.2c2 (previously established in our lab, Dionne et al., 2005, Devel Biol), OP9-DL1 & OP9-Mig (created and sent to us by Schmitt & Zuniga-Pflucker, 2002, Immunity). Scid.adh.2c2 have been used by our lab subsequently in Del Real and Rothenberg 2013 Development, Scripture-Adams et al 2014 J Immunol, and Champhekar et al 2015 Genes Dev. Cocultures with OP9-DL1 and OP9-Mig were also used by us in Taghon et al 2005 Genes Dev, Franco et al 2006 PNAS, Taghon et al 2006 Immunity, Taghon et al 2007 Nat Immunol, Li et al Science 2010, Yui et al 2010 J Immunol, Del Real and Rothenberg 2013 Development, Scripture-Adams et al 2014 J Immunol, Champhekar et al 2015 Genes Dev, Kueh et al 2016 Nat Immunol, and Longabaugh et al 2017 PNAS).

Authentication

Functionally in repeated tests; by cell surface phenotype; and in cases of Scid.adh.2c2, OP9-DL1, & OP9-Mig, by RNA-seq.

Mycoplasma contamination

All cell lines were negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials

C57BL/6 (referred to as B6), B6.Cg-Tg(BCL2)25Wehi/J (Bcl2-tg) and B6.Gt(ROSA)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J (Cas9) mice were purchased from the Jackson Laboratory. Vav1-iCre mice (B6N.Cg-Commd10Tg(Vav1-icre)A2Kio/J) were purchased from Jackson Laboratories and pLck-Cre mice developed by Christopher Wilson's group (B6.Cg-Tg(Lck-cre)1Cwi N9) were purchased from Taconic Laboratories. The Cre activity reporter allele ROSA26R-eYFP was also used. Except for Vav1-iCre, which was maintained in heterozygotes, the indicated transgenes were bred to homozygosity alone or in combinations on the B6 background. Bcl11bfl/fl-Rosa26-Cre-ERT2 mice were derived from stock kindly provided by Pentao Liu (Cambridge, UK), and maintained as a separate line. All animals were bred and maintained in the California Institute of Technology Laboratory Animal Facility, under specific pathogen free conditions, and the protocol supporting animal breeding for this work was reviewed and approved by the Institute Animal Care and Use Committee of the California Institute of Technology.

Method-specific reporting

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	To review GEO accession GSE110305: Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110305 Enter token czaneoukpxcptmn into the box To review GEO accession GSE110882: Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110882 Enter token gzazmmocdtsbnwl into the box To review GEO accession GSE115744: Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE115744 Enter token mfirhplmwqrtsvf into the box
Files in database submission	ChIP-seq data WT_DN3_aChd4 HOMER IDR peaks.csv Bcl11bKO_aChd4 HOMER IDR peaks.csv WT_DN3_aMta2 HOMER IDR peaks.csv Bcl11bKO_aMta2 HOMER IDR peaks.csv WT_DN3_aHdac2 HOMER IDR peaks.csv Bcl11bKO_aHdac2 HOMER IDR peaks.csv WT_DN3_aRest HOMER IDR peaks.csv Bcl11bKO_aRest HOMER IDR peaks.csv WT_DN3_aRing1b HOMER IDR peaks.csv Bcl11bKO_aRing1b HOMER IDR peaks.csv WT_DN3_aLDS1 HOMER IDR peaks.csv Bcl11bKO_aLSD1 HOMER IDR peaks.csv WT_DN3_aRunx1 HOMER IDR peaks.csv Bcl11bKO_aRunx1 HOMER IDR peaks.csv WT_DN3_aBcl11b HOMER IDR peaks.csv Bcl11bKO_aBcl11b HOMER IDR peaks.csv Scid.adh.2c2_shControl_aChd4 HOMER peaks.csv Scid.adh.2c2_shBcl11b_aChd4 HOMER peaks.csv Scid.adh.2c2_shControl_aHdac2 HOMER peaks.csv Scid.adh.2c2_shBcl11b_aHdac2 HOMER peaks.csv Scid.adh.2c2_shControl_aMta2 HOMER peaks.csv Scid.adh.2c2_shBcl11b_aMta2 HOMER peaks.csv Scid.adh.2c2_shControl_aRest HOMER peaks.csv Scid.adh.2c2_shBcl11b_aRest HOMER peaks.csv WT_DN3_aChd4-ChIP-rep1.fastq.gz WT_DN3_aChd4-ChIP-rep2.fastq.gz Bcl11bKO_aChd4-ChIP-rep1.fastq.gz Bcl11bKO_aChd4-ChIP-rep2.fastq.gz WT_DN3_aMta2-ChIP-rep1.fastq.gz WT_DN3_aMta2-ChIP-rep2.fastq.gz Bcl11bKO_aMta2-ChIP-rep1.fastq.gz Bcl11bKO_aMta2-ChIP-rep2.fastq.gz WT_DN3_aHdac2-ChIP-rep1.fastq.gz WT_DN3_aHdac2-ChIP-rep2.fastq.gz Bcl11bKO_aHdac2-ChIP-rep1.fastq.gz Bcl11bKO_aHdac2-ChIP-rep2.fastq.gz WT_DN3_aRest-ChIP-rep1.fastq.gz WT_DN3_aRest-ChIP-rep2.fastq.gz Bcl11bKO_aRest-ChIP-rep1.fastq.gz Bcl11bKO_aRest-ChIP-rep2.fastq.gz WT_DN3_aRing1b-ChIP-rep1.fastq.gz WT_DN3_aRing1b-ChIP-rep2.fastq.gz Bcl11bKO_aRing1b-ChIP-rep1.fastq.gz Bcl11bKO_aRing1b-ChIP-rep2.fastq.gz WT_DN3_aLSD1-ChIP-rep1.fastq.gz WT_DN3_aLSD1-ChIP-rep2.fastq.gz Bcl11bKO_aLSD1-ChIP-rep1.fastq.gz Bcl11bKO_aLSD1-ChIP-rep2.fastq.gz WT_DN3_aRunx1-ChIP-rep1.fastq.gz WT_DN3_aRunx1-ChIP-rep2.fastq.gz Bcl11bKO_aRunx1-ChIP-rep1.fastq.gz Bcl11bKO_aRunx1-ChIP-rep2.fastq.gz WT_DN3_aBcl11b-ChIP-rep1.fastq.gz

WT_DN3_aBcl11b-ChIP-rep2.fastq.gz
Bcl11bKO_aBcl11b-ChIP-rep1.fastq.gz
Bcl11bKO_aBcl11b-ChIP-rep2.fastq.gz
WT_DN3_1%_input-rep1.fastq.gz
WT_DN3_1%_input-rep2.fastq.gz
Bcl11bKO_1%_input-rep1.fastq.gz
Bcl11bKO_1%_input-rep2.fastq.gz
WT_DN3_aH3K27Ac-ChIP-rep1.fastq.gz
WT_DN3_aH3K27Ac-ChIP-rep2.fastq.gz
Bcl11bKO_aH3K27Ac-ChIP-rep1.fastq.gz
Bcl11bKO_aH3K27Ac-ChIP-rep2.fastq.gz
WT_DN3_1%_input-rep1.fastq.gz
WT_DN3_1%_input-rep2.fastq.gz
Bcl11bKO_1%_input-rep1.fastq.gz
Bcl11bKO_1%_input-rep2.fastq.gz
Scid.adh.2c2_shControl_aChd4.fastq.gz
Scid.adh.2c2_shBcl11b_aChd4.fastq.gz
Scid.adh.2c2_shControl_aHdac2.fastq.gz
Scid.adh.2c2_shBcl11b_aHdac2.fastq.gz
Scid.adh.2c2_shControl_aMta2.fastq.gz
Scid.adh.2c2_shBcl11b_aMta2.fastq.gz
Scid.adh.2c2_shControl_aRest.fastq.gz
Scid.adh.2c2_shBcl11b_aRest.fastq.gz
Scid.adh.2c2_shControl_1%_input.fastq.gz
Scid.adh.2c2_shBcl11b_1%_input.fastq.gz

RNA-seq data
Bcl11b_cofactorsKO_RNA-seq_RPKM_table.txt
DN3_sgBcl11b_sgId2_sgZbtb16_RNA-seq_RPKM_table.txt
Lck_Bcl11b_KO_RNA-seq_RPKM_Table
Vav_Bcl11b_KO_RNA-seq_RPKM_Table
sgControl_RNA-rep1.fastq.gz
sgControl_RNA-rep2.fastq.gz
sgBcl11b_RNA-rep1.fastq.gz
sgBcl11b_RNA-rep2.fastq.gz
sgChd4_RNA-rep1.fastq.gz
sgChd4_RNA-rep2.fastq.gz
sgMta1_2_RNA-rep1.fastq.gz
sgMta1_2_RNA-rep2.fastq.gz
sgRest_RNA-rep1.fastq.gz
sgRest_RNA-rep2.fastq.gz
sgRing1a_b_RNA-rep1.fastq.gz
sgRing1a_b_RNA-rep2.fastq.gz
sgLSD1_RNA-rep1.fastq.gz
sgLSD1_RNA-rep2.fastq.gz
sgRunx1_RNA-rep1.fastq.gz
sgRunx1_RNA-rep2.fastq.gz
sgControl_RNA-rep3.fastq.gz
sgControl_RNA-rep4.fastq.gz
sgBcl11b_RNA-rep3.fastq.gz
sgBcl11b_RNA-rep4.fastq.gz
sgId2_RNA-rep1.fastq.gz
sgId2_RNA-rep2.fastq.gz
sgZbtb16_RNA-rep1.fastq.gz
sgZbtb16_RNA-rep2.fastq.gz
sgBcl11b_Id2_RNA-rep1.fastq.gz
sgBcl11b_Id2_RNA-rep2.fastq.gz
sgBcl11b_Zbtb16_RNA-rep1.fastq.gz
sgBcl11b_Zbtb16_RNA-rep2.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep1.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep2.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep3.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep4.fastq.gz
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Lck_Bcl11b_WT_DN3_RNA-rep5.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep1.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep2.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep3.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep4.fastq.gz
Lck_Bcl11b_WT_DN2_RNA-rep5.fastq.gz
Lck_Bcl11b_HET_DN3_RNA-rep1.fastq.gz
Lck_Bcl11b_HET_DN3_RNA-rep2.fastq.gz

Lck_Bcl11b_HET_DN3_RNA-rep3.fastq.gz
Lck_Bcl11b_HET_DN3_RNA-rep4.fastq.gz
Lck_Bcl11b_HET_DN3_RNA-rep5.fastq.gz
Lck_Bcl11b_HET_DN3_RNA-rep6.fastq.gz
Lck_Bcl11b_HOM_RNA-rep1.fastq.gz
Lck_Bcl11b_HOM_RNA-rep2.fastq.gz
Lck_Bcl11b_HOM_RNA-rep3.fastq.gz
Lck_Bcl11b_HOM_RNA-rep4.fastq.gz
Lck_Bcl11b_HOM_RNA-rep5.fastq.gz
Vav_Bcl11b_WT_RNA-rep1.fastq.gz
Vav_Bcl11b_WT_RNA-rep2.fastq.gz
Vav_Bcl11b_HET_RNA-rep1.fastq.gz
Vav_Bcl11b_HET_RNA-rep2.fastq.gz
Vav_Bcl11b_HET_RNA-rep3.fastq.gz
Vav_Bcl11b_HET_RNA-rep4.fastq.gz
Vav_Bcl11b_HET_RNA-rep1.fastq.gz
Vav_Bcl11b_HOM_RNA-rep2.fastq.gz
Vav_Bcl11b_HOM_RNA-rep3.fastq.gz

Genome browser session
(e.g. UCSC)

ChIP-seq data were mapped to the mouse genome build NCBI37/mm9 using Bowtie (v1.1.1; http://bowtie-bio.sourceforge.net/index.shtml) with "-v 3 -k 11 -m 10 -t --best –strata" settings and HOMER tagdirectories were created with makeTagDirectory and visualized in the UCSC-genome browser (http://genome.ucsc.edu).

Methodology

Replicates

Data are based on reproducible ChIP-seq peaks in two replicate samples

Sequencing depth

ChIP-seq libraries were sequenced on Illumina HiSeq2500 in single read mode with the read length of 50 nt. Base calls were performed with RTA 1.13.48.0 followed by conversion to FASTQ with bcl2fastq 1.8.4 and produced approximately 30 million reads per sample.

Antibodies

Anti-Chd4 Bethyl Cat#A301-081A
Anti-Mta2 Santa Cruz Cat#sc-9447
Anti-Hdac2 Abcam Cat#ab12169
Anti-Rest Caltech Protein Expression Center Cat#12C11-1B11
Anti-Ring1b Bethyl Cat#A302-869A
Anti-LSD1 Abcam Cat#ab17721
Anti-Runx1 Abcam Cat#ab23980
Anti-Bcl11b Abcam Cat#ab18465
Anti-Bcl11b Bethyl Cat#A300-383A
Anti-Bcl11b Bethyl Cat#A300-385A
Anti-Bcl11b CST Cat#12120
Anti-H3K27Ac Abcam Cat#ab4729

Peak calling parameters

ChIP peaks were identified with findPeaks.pl against a matched control sample using the settings "-P .1 -LP .1 -poisson .1 -style factor". The identified peaks were annotated to genes with the annotatePeaks.pl command against the mm9 genomic build in the HOMER package.

Data quality

Peak reproducibility was determined by a HOMER adaptation of the IDR (Irreproducibility Discovery Rate) package according to ENCODE guidelines (https://sites.google.com/site/anshulkundaje/projects/idr). Only reproducible high quality peaks, with a normalized peak score ≥ 15, were considered for further analysis.

Software

bedGraphToBigWig
Bedtools (v.2.17.0)
Bowtie (v1.1.1)
EdgeR (v.3.16.5)
HOMER (v4.8)
HOMER-IDR

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Thymuses from 4-6wk old Bcl11b+/+, Bcl11bfl/+, and Bcl11bfl/fl ROSA26R-YFP mice with Vav1-iCre or Lck-Cre were removed, and single-cell suspensions were made. Lineage-positive cells were depleted by staining with biotinylated antibodies to CD8α (53-6.7), TCRγδ (GL3), TCRβ (H57597), Ter119 (Ter119), NK1.1 (PK136), Dx5, and CD11c (N418), CD11b (M1/70), after which the cells were incubated with streptavidin-coated magnetic beads and then passed through an LS magnetic column in accordance with the manufacturer's instructions (Miltenyi Biotec). For in vitro differentiation of pro-T cells, bone marrow hematopoietic progenitors were used for input. Bone marrow (BM) was removed from the femurs and tibiae of 2-3 month-old mice. Suspensions of BM cells were prepared and stained for lineage markers using biotin-conjugated lineage antibodies (CD11b, CD11c, Gr1, TER-119, NK1.1, CD19, CD3ε, B220), then incubated with streptavidin-coated magnetic beads (Miltenyi Biotec), and passed through a magnetic column (Miltenyi Biotec). Then, Lin-Sca1+Kit+ (LSK) cells were sorted on a FACSAria (BD Bioscience). LSK cells were cultured on OP9-DL1 monolayers using OP9 medium (α-MEM, 20% FBS, 50 μM β-mercaptoethanol, Pen-Step-Glutamine) supplemented with 10 ng/ml of IL-7 (Pepro Tech Inc) and 10 ng/ml of Flt3L (Pepro Tech Inc). On day 7, cultured cells were disaggregated, filtered through 40-μm nylon mesh, and re-cultured on new OP9-DL1 monolayers with medium containing 5 ng/ml of IL-7 and 5 ng/ml of Flt3L. In cultures that were continued for longer times, cells were passaged onto fresh OP9-DL1 monolayers at day 10 and maintained up to day 14 in 1 ng/ml each of IL-7 and Flt3L.
Instrument	Miltenyi Biotech MACSQuant 10 Flow Cytometer BD FACS Aria II Cell Sorter iCyt Mission Technology Reflection Cell Sorter
Software	FlowJo (v10.0.8)
Cell population abundance	The abundance of the post-sort fractions were higher than 98%.
Gating strategy	Doublets were excluded using forward light-scatter gating followed by gating on lymphocytes based on FSC/SSC. Dead cells were excluded by gating on 7AAD negative cells. These cells were further gated as indicated in Supplementary Figures.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.