nature research

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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 our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒ A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry data were acquired on LSR Fortessa cytometers with FACSDiva software v8.0 (BD Biosciences) . Infinite 200 (Tecan) for Elisa. ChIP-seq data,RNA-seq data, Single-cell RNA-seq data and Single-cell ATAC-seq data are acquired on NextSeq sequencer (Illumina).Microbiome sequencing data was acquired on Illumina MiSeq Platform(Illumina).
Data analysis	GraphPad Prism v7.0 and Flowjo v10.5.0 for FACS data analysis were used. Cell Ranger v1.3.1, v5.0.0 & v6.0.0 (10x Genomics), Seurat v4.0.4 & v4.0.5 (https://satijalab.org/seurat/) and R v4.1.1 (https://www.r-project.org) were used for scRNA-seq analysis. Cell Ranger v1.2.0, Seurat v4.0.4,Signac v1.4.0 and R v4.1.1 for scATAC-seq analysis. BBDuk v38.34 (BBMap-Bushnell B.), DADA2 v1.10 for Microbiome sequencing analysis. Trimmomatic v4.1.4, STAR v. 2.4.0h, HTseq v0.10.0, DESeq2 v1.20 for RNA-seq analysis. Bowtie2 (v2.3.4), Samtools (v1.6), Macs2 (v2.2.5), Homer (V4.1.0),IntersectBed (2.27.0) for ChIP-seq analysis. CellOracle v0.7.1 for gene regulatory network inference. No custom codes have been developed in the study.

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 and reviewers. 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

In vitro, in vivo, flow cytometry data are included in the published article and its supplementary information. Accession numbers for the sequence data reported in

this paper: GEO: GSE149993; GSE149943; GSE149985 and GSE186164; GSE186291 for LRF ChIP-seq, RNA-seq, and single-cell RNA-seq, single-cell ATAC-seq, respectively. All the data are publicly available on Gene Expression Omnibus (GEO).

Field-specific reporting

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

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications The exact sample sizes of experiments are provided in the figure legends.
Data exclusions	No data was excluded.
Replication	All animal experiments and in vitro cell-based assays were repeated at least twice. The exact repeat times of experiments are provided in the figure legends.
Randomization	No randomization was performed in this study because the study design involved genotyping of the mice. The mice's gender and age were matched in each experiments.
Blinding	No blinded experiments were conducted in this study because the study design involved genotyping of the mice.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Flow Cytometry antibody

LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit, Invitrogen L23105, (1:1000)
APC-eFluor 780 Rat anti-mouse CD8a (53-6.7), eBioscience 47-0081-82, (1:200)
BV786 Mouse Anti-Mouse CD45.2 (104),BD Bioscience 563686, (1:200)
BV650 Mouse Anti-Mouse CD45.1(A20), BD Bioscience 563754, (1:200)
BUV395 Rat Anti-Mouse CD8b (H35-17.2), BD Bioscience 740278, (1:200)
BUV737 Rat Anti-Mouse CD4 (GK1.5), BD Bioscience 612761, (1:200)
FITC Mouse Anti-Mouse CD45.2 (104), eBioscience 11-0454-82, (1:200)
eFluor 450 Rat Anti-Mouse CD8b (H35-17.2), eBioscience 48-0083-82, (1:200)
V500 Rat anti-Mouse CD8a(53-6.7), BD Bioscience 560778, (1:200)
PE-Cyanine7 Rat Anti-Mouse CD4 (GK1.5), eBioscience 25-0041-82, (1:200)
PE Rat anti-mouse LPAM-1 (Integrin α4β7) Antibody (DATK32), Biolegend 120605, (1:200)
PE Rat Anti-Mouse CD49d (Integrin alpha 4, R1-2), eBioscience 12-0492-82, (1:200)
PE Rat anti-Mouse Integrin b7 chain(M293), BD Bioscience BDB557498, (1:200)
APC Rat Anti-Mouse CD8a (53-6.7), BD Bioscience 553035, (1:200)
PE Hamster Anti-Mouse TCR beta (H57-597), PE, Invitrogen 12-5961-82, (1:200)
eFluor™ 660 Rat anti-Mouse CD4 (GK1.5), eBioscience 50-164-28, (1:200)
PerCP-Cy5.5 Mouse anti-Mouse CD45.2, (104), BD Bioscience BDB552950, (1:200)
FITC Rat anti-Mouse CD8b (H35-17.2), eBioscience 11-0083-82, (1:200)

APC-eFluor 780 Mouse Anti-Mouse CD45.1 (A20), Invitrogen 47-0453-82 (1:200)
PE Rat anti-Mouse CD8a (53-6.7), Invitrogen 12-0081-82, (1:200)
eFluor 660 Hamster anti-Human Pokemon (LRF) Monoclonal Antibody (13E9), eBioscience 50-3309-80, (1:200)
Alexa Fluor 700 Rat anti-Mouse CD4 (GK1.5) , eBioscience 56-0041-82, (1:200)
PE-Cyanine7 Rat anti-Mouse CD8a (53-6.7), eBioscience 25-0081-82, (1:200)
BV711 Hamster Anti-Mouse TCR β Chain (H57-597) BD Bioscience 563135, (1:200)
eFluor 450 Hamster anti-Mouse CD279 (PD-1) (J43), eBioscience 48-9985-82, (1:200)
PE Mouse anti-Mouse T-bet (eBio4B10 (4B10)), eBioscience 12-5825-82, (1:100)
APC CD1d PBS-57, NIH Tetramer Core Facility, (1:100)
BV510 Mouse Anti-Mouse NK-1.1(PK136), BD Bioscience 563096, (1:200)
PerCP-Cyanine5.5 Rat anti-Mouse CD5 (53-7.3), Invitrogen A15859, (1:200)
PE-Cyanine7 Mouse Anti-Mouse MHC Class I (H-2Kb) (AF6-88.5.5.3), Invitrogen 25-5958-82, (1:200)
Alexa Fluor 700Rat anti-Mouse CD44 (IM7), Invitrogen 56-0441-82, (1:200)
FITC Rat anti-Mouse CD122 (TM-beta1), eBioscience 50-974-6, (1:200)
PE Rabbit anti-Mouse Bim (C34C5), Cell Signaling12186, (1:20)
PE Hamster Anti-Mouse Bcl-2 Set, BD Bioscience 563096, (5ul/test)
Pacific Blue Rabbit Anti-Mouse Cleaved Caspase-3 (Asp175) (D3E9), Cell Signaling 8788, (1:10)
Rat anti-Mouse S1P1/EDG-1 Antibody (713412), R&D systems MAB7089, (2ug/1x106 cells)
PE Mouse anti-Rat IgG1 Secondary Antibody (R1-12D10), eBioscience 12-4812-82, (1:50)
Normal Rat Serum, eBioscience 24-5555, (1:100)
Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™, 2.4G2), BD Bioscience 553142, (1:100)

Cell culture antibody
InVivoMAb anti-mouse CD3ε (145-2C11), BioXcell, Cat: BE0001-1 (1 µg/mL),
InVivoMAb anti-mouse CD28 (37.51), BioXcell, Cat: BE0015-1 (3 µg/mL)

Validation

The antibodies commercially available were validated by the manufacturers.
LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit, Invitrogen L23105, (1:1000)
(App: Fc; React: Mammalian cells)

APC-eFluor 780 Rat anti-Mouse CD8a (53-6.7), eBioscience 47-0081-82, (1:200)
(App: Fc, FN; React: Mouse)

BV786 Mouse Anti-Mouse CD45.2 (104),BD Bioscience 563686, (1:200)
(App: Fc; React: Mouse)

BV650 Mouse Anti-Mouse CD45.1(A20), BD Bioscience 563754, (1:200)
(App: Fc; React: Mouse)

BUV395 Rat Anti-Mouse CD8b (H35-17.2), BD Bioscience 740278, (1:200)
(App: Fc; React: Mouse)

BUV737 Rat Anti-Mouse CD4 (GK1.5), BD Bioscience 612761, (1:200)
(App: Fc; React: Mouse)

FITC Mouse Anti-Mouse CD45.2 (104), eBioscience 11-0454-82, (1:200)
(App: Fc; React: Mouse)

eFluor 450 Rat Anti-Mouse CD8b (H35-17.2), eBioscience 48-0083-82, (1:200)
(App: Fc; React: Mouse)

V500 Rat anti-Mouse CD8a(53-6.7), BD Bioscience 560778, (1:200)
(App: Fc; React: Mouse)

PE-Cyanine7 Rat Anti-Mouse CD4 (GK1.5), eBioscience 25-0041-82, (1:200)
(App: Fc; React: Mouse)

PE Rat anti-Mouse LPAM-1 (Integrin α4β7) Antibody (DATK32), Biolegend 120605, (1:200)
(App: Fc; React: Mouse)

PE Rat Anti-Mouse CD49d (Integrin alpha 4, R1-2), eBioscience 12-0492-82, (1:200)
(App: Fc; React: Mouse)

PE Rat anti-Mouse Integrin b7 chain(M293), BD Bioscience BDB557498, (1:200)
(App: Fc; React: Murine)

APC Rat Anti-Mouse CD8a (53-6.7), BD Bioscience 553035, (1:200)
(App: Fc; React: Murine)

PE Hamster Anti-Mouse TCR beta (H57-597), PE, Invitrogen 12-5961-82, (1:200)
(App: Fc; React: Mouse)

eFluor™ 660 Rat anti-Mouse CD4 (GK1.5), eBioscience 50-164-28, (1:200)
(App: Fc; React: Mouse)

PerCP-Cy5.5 Mouse anti-Mouse CD45.2, (104), BD Bioscience BDB552950, (1:200)
(App: Fc; React: Murine)

FITC Rat anti-Mouse CD8b (H35-17.2), eBioscience 11-0083-82, (1:200)
(App: Fc; React: Mouse)

APC-eFluor 780 Mouse Anti-Mouse CD45.1 (A20), Invitrogen 47-0453-82 (1:200)

(App: Fc; React: Mouse)

PE Rat anti-Mouse CD8a (53-6.7), Invitrogen 12-0081-82, (1:200)
(App: Fc; React: Mouse)

eFluor 660 Hamster anti-Human Pokemon (LRF) Monoclonal Antibody (13E9), eBioscience 50-3309-80, (1:200)
(App: Fc; React: Human, Mouse)

Alexa Fluor 700 Rat anti-Mouse CD4 (GK1.5) , eBioscience 56-0041-82, (1:200)
(App: Fc; React: Mouse)

PE-Cyanine7 Rat anti-Mouse CD8a (53-6.7), eBioscience 25-0081-82, (1:200)
(App: Fc; React: Mouse)

BV711 Hamster Anti-Mouse TCR β Chain (H57-597) BD Bioscience 563135, (1:200)
(App: Fc; React: Mouse)

eFluor 450 Hamster anti-Mouse CD279 (PD-1) (J43), eBioscience 48-9985-82, (1:200)
(App: Fc; React: Mouse)

BV510 Mouse Anti-Mouse NK-1.1(PK136), BD Bioscience 563096, (1:200)
(App: Fc React: Mouse)

PerCP-Cyanine5.5 Rat anti-Mouse CD5 (53-7.3), Invitrogen A15859, (1:200)
(App: Fc React: Mouse)

PE-Cyanine7 Mouse Anti-Mouse MHC Class I (H-2Kb) (AF6-88.5.5.3), Invitrogen 25-5958-82, (1:200)
(App: Fc; React: Mouse)

Alexa Fluor 700 Rat anti-Mouse CD44 (IM7), Invitrogen 56-0441-82, (1:200)
(App: Fc; React: Human, Mouse)

FITC, Rat anti-Mouse CD122 (TM-beta1), eBioscience 50-974-6, (1:200)
(App: Fc; React: Mouse)

PE Rabbit anti-Mouse Bim (C34C5), Cell Signaling12186, (1:20)
(App: Fc; React: Mouse, Human, Rat)

PE Hamster Anti-Mouse Bcl-2 Set, BD Bioscience 563096, (5ul/test)
(App: Fc; React: Mouse,)

Pacific Blue Rabbit Anti-Mouse Cleaved Caspase-3 (Asp175) (D3E9), Cell Signaling 8788, (1:10)
(App: Fc; React: Mouse, Human)

Rat anti-Mouse S1P1/EDG-1 Antibody (713412), R&D systems MAB7089, (2ug/1x106 cells)
(App: Fc; Immunohistochemistry, CyTOF-ready; React: Mouse)

PE Mouse anti-Rat IgG1 Secondary Antibody (R1-12D10), eBioscience 12-4812-82, (1:50)
(App: Fc; React: Rat)

Normal Rat Serum, eBioscience 24-5555, (1:100)
(App: blocking)

Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™, 2.4G2), BD Bioscience 553142, (1:100)
(App: Blocking, Fc, Immunohistochemistry-frozen, Immunoprecipitation; React: Mouse)

Animals and other organisms

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

Laboratory animals	CD45.1, CD45.2 C57BL/6 mice were purchased from Charles River Laboratories. NOD-scid IL2RGammanull (NSG) animals were

Laboratory animals	obtained from the National Cancer Institute (Frederick, MD). Rag2-Il2rg double knockout mice were purchased from Envigo. Mice carrying Rosa26BirA were obtained from Ming Li, Memorial Sloan Kettering Cancer Center). Mice carrying floxed alleles for Lrf were obtained from P.P. Pandolfi. Mice carrying floxed alleles for Zbtb7b were generated by our lab. Cd4-cre mice were from Taconic. Mice were housed in a specific pathogen-free facility under a 12h light/dark cycle at 22±2°C and ±30% of humidity, and analyzed between 6 and 20 weeks of age unless described otherwise. Age and sex matched mice from both sexes were used in the experiments.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field-collected samples were used in the study
Ethics oversight	All animal experiments were approved by the NCI Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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.*	GEO: GSE149993
Files in database submission	LRF_CHIP1 LRF_CHIP2 control_CHIP1 LRF_Input1 LRF_Input2 control_Input1
Genome browser session (e.g. UCSC)	No longer applicable for final submission documents

Methodology

Replicates	Two replicates
Sequencing depth	All the samples were sequenced on NextSeq runs using TruSeq Chip Samples Prep Kit (IP-202-1012) and paired end sequencing. All the samples have percent of Q30 bases above 89%. All the samples have yields between 54 and 271 million pass filter reads.
Antibodies	M280 Streptavidin beads (Invitrogen 11205D)
Peak calling parameters	macs2 callpeak -t -c -n --format=BEDPE --nomodel --extsize 300 -q 0.05 --keep-dup all --g mm
Data quality	Samples were aligned with Mouse - mm10 reference using Bowtie2 alignment. Overall alignment percentage for the samples is 95%. Unique alignment is above 52%. There are 2 to 7% of unmapped reads. Library complexity is measured by uniquely aligned reads using picard's markduplicate utility. All the samples have library complexity with percent non-duplicated reads ranging from 52 to 89%.
Software	Bowtie2 (v2.3.4), Samtools (v1.6), Macs2 (v2.2.5), Homer (V4.1.0), IntersectBed (2.27.0)

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	Single cell suspension were prepared from mouse thymus, spleens,mLN, and small intestine. Thymus, spleens, mLN were processed by mechanical disruption. Splenocytes were treated with red blood lysis buffer. Small intestines were treated with DTT and EDTA as described in the online methods. Small intestine lamina propria was digested by Liberase as described in the

	online methods.
Instrument	Flow cytometry data were acquired on LSR Fortessa cytometers (BD Biosciences). Cell sorting was performed on a FACSAria or FACS Fusion (BD Biosciences).
Software	FACSDiva software v8.0, FlowJo software v10.5.0 (BD Bioscience)
Cell population abundance	Relevant cell population abundance is noted in each figure. Post-sort purities were routinely over 90% and were tested by flow cytometry on appropriate numbers of sorted cells
Gating strategy	All analyses begin with live cells>singlet (FSC-W vs FSC-H)>singlet (SSC-W vs SSC-H). Further gates identifying cell sub-population are shown in the manuscript and described in the figure legends.

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