

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Data collection code was not used in this study
Data analysis	Computational analysis of single-cell RNA sequencing data used Python v. 3.9.2 and 3.9.11; CellRanger v6.0.1 and v7.0.1; and R v. 4.0.3 for q-value pathway analysis. Non-single cell RNA sequencing graphs were plotted using Prism v.9, and edited for appearance using Adobe Illustrator 27.1.1. Statistical tests were performed in Prism v.9. Flow cytometry data was analyzed using FlowJo v10. Structural modeling of the Thy1-G5:mSrtA interaction was performed using Alphafold 2; COOT v. 0.8.9.2; the GalaxyWEB server (https://galaxy.seoklab.org/); CHARMM-GUI (https://www.charmm-gui.org/); ChimeraX v. 1.4; and PyMOL v. 2.4.2.

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Processed scRNA-seq data, python code and Jupyter notebooks used for data analysis are available at <https://github.com/pritykinlab/ulipstic-analysis>. Single-cell RNA-seq data are available at GEO under accession number GSE253000.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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](https://www.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 determine sample size. Numbers of mice per group within each independent experiment were limited to numbers typically used in the field. The cell line experiment was performed twice with triplicate technical replicates, as is the standard for such assays.
Data exclusions	No data points were excluded from the analysis.
Replication	Experiments were performed multiple times independently, as described in the figure legends, with the exception of scRNA-seq experiments, where all biological replicates were performed simultaneously to prevent batch effects, and the XXXXXXXXXX experiment, which was performed only once.
Randomization	Littermate mice were used to control for litter, cage, and age effects. Mice were divided stochastically (but without a specific randomization procedure) between experimental groups. Randomization is not relevant for cell line experiments.
Blinding	Experimenters were not blinded to experimental group, since most readouts (e.g. FACS fluorescence intensities) are not subjective.

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

Methods

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

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

Antibodies

Antibodies used

Rat monoclonal anti-MHC-II BV421 (clone M5/114.15.2) BioLegend 107632
 Rat monoclonal anti-MHC-II FITC (clone M5/114.15.2) BioLegend 107606
 Rat monoclonal anti-MHC-II AF700 (clone M5/114.15.2) Invitrogen 56-5321-82
 Rat monoclonal anti-MHC-II BUV395 (clone 2G9)v BD Biosciences 743876
 Hamster monoclonal anti-CD11c BUV496 (clone N418) BD Biosciences 750450
 Armenian hamster monoclonal anti-CD11c APC (clone N418) BioLegend 117310
 Rat monoclonal anti-CD11b BV711 (clone M1/70) BioLegend 101242
 Mouse monoclonal anti-XCR1 PerCP-Cy5.5 (clone ZET) BioLegend 148208
 Mouse monoclonal anti-biotin PE (clone Bio3-18E7) Miltenyi Biotec 130-090-756
 Mouse monoclonal anti-biotin APC (clone Bio3-18E7) Miltenyi Biotec 130-113-288
 Rat monoclonal anti-DYKDDDDK (FLAG) APC (clone L5) BioLegend 637308
 Rat monoclonal anti-DYKDDDDK (FLAG) BV421 (clone L5) BioLegend 637321
 Mouse monoclonal anti-CD90.1 (Thy-1.1) BV421 (clone OX-7) BioLegend 202529
 Mouse monoclonal anti-CD90.1 (Thy-1.1) PE-Cy7 (clone OX-7) BioLegend 202518
 Armenian hamster monoclonal anti-CD3? BV785 (clone 145-2C11) BioLegend 100355
 Mouse monoclonal anti-CD19 BV605 (clone H1B19) BioLegend 302244
 Rat monoclonal anti-B220 BUV395 (clone RA3-6B2) BD Biosciences 563793
 Rat monoclonal anti-B220 FITC (clone RA3-6B2) BioLegend 103206
 Rat monoclonal anti-B220 BV785 (clone RA3-6B2) BioLegend 103245
 Rat monoclonal anti-NK1.1 BV785 (clone PK136) BioLegend 108749
 Rat monoclonal anti-F4/80 PE-Cy7 (clone BM8) BioLegend 123114
 Rat monoclonal anti-F4/80 BUV805 (clone T45-2342) BD Biosciences 749282
 Rat monoclonal anti-CD4 BV785 (clone RM4-5) BioLegend 100551
 Rat monoclonal anti-CD4 PE-Cy7 (clone GK1.5) Invitrogen 25-0041-81
 Rat monoclonal anti-CD4 BUV496 (clone GK1.5) BD Biosciences 612952
 Rat monoclonal anti-CD4 AF700 (clone RM4-5) BD Biosciences 557956
 Rat monoclonal anti-CD8 BUV805 (clone 53-6.7) BD Biosciences 612898
 Rat monoclonal anti-CD8 BV711 (clone H35-17.2) BD Biosciences 740761
 Rat monoclonal anti-CD45 FITC (clone 30-F11) BD Biosciences 553080
 Rat monoclonal anti-CD45 AF700 (clone 30-F11) BioLegend 103128
 Rat monoclonal anti-CD45 BUV395 (clone 30-F11) BD Biosciences 564279
 Mouse monoclonal anti-CD45.1 PE-Cy7 (clone A20) BioLegend 110730
 Mouse monoclonal anti-CD45.2 FITC (clone 104) BioLegend 109816
 Mouse monoclonal anti-CD45.2 PE-Cy7 (clone 104) BioLegend 109830
 Rat monoclonal anti-CD86 BV605 (clone GL1) BioLegend 105037
 Rat monoclonal anti-CD86 AF488 (clone GL1) BioLegend 105018
 Rat monoclonal anti-CD40 BV421 (clone 3/23) BD Biosciences 562846
 Armenian hamster monoclonal anti-CD69 FITC (clone H1.2F3) Invitrogen 11-0691-85
 Armenian hamster monoclonal anti-CD80 PerCP-Cy5.5 (clone 16-10A1) BioLegend 104722
 Armenian hamster monoclonal anti-TCR APC-eFluor780 (clone H57-597) Invitrogen 47-5961-82
 Hamster monoclonal anti-TCR BUV395 (clone H57-597) BD Biosciences 742485
 Armenian hamster monoclonal anti-CD103 BV785 (clone 2E7) BioLegend 121439
 Rat monoclonal anti-FOXP3 APC (clone FJK-16s) Invitrogen 17-5773-82
 Rat monoclonal anti-FOXP3 PerCP-Cy5.5 (clone FJK-16s) Invitrogen 45-5773-82
 Rat monoclonal anti-FOXP3 FITC (clone FJK-16s) Invitrogen 11-5773-82
 Armenian hamster monoclonal anti-JAML AF647 (clone 4E10) BioLegend 128506
 Armenian hamster monoclonal anti-TCR / PerCP-eFluor710 (clone GL3) Invitrogen 46-5711-82
 Rat monoclonal anti-CD326 (Ep-CAM) BV605 (clone G8.8) BioLegend 118227
 Rat monoclonal anti-CD8a Biotin (clone 53-6.7) BioLegend 100704
 Rat monoclonal anti-CD11b Biotin (clone M1/70) BioLegend 101204
 Mouse monoclonal anti-NK1.1 Biotin (clone PK136) BioLegend 108704
 Rat monoclonal anti-CD25 Biotin (clone 7D4) BD Biosciences 553070
 Armenian hamster monoclonal anti-CD11c Biotin (clone N418) BioLegend 117304
 Rat monoclonal anti-TER-119 Biotin (clone TER-119) BD Biosciences 553672
 Rat monoclonal anti-B220 Biotin (clone RA3-6B2) BD Biosciences 553086
 Rat monoclonal anti-CD4 Biotin (clone GK1.5) BioLegend 100404
 Armenian hamster monoclonal anti-CD40L (clone MR1) BioXCell BE0017-1
 Rat monoclonal anti-MHC-II (clone M5/114) BioXCell BE0108

Armenian hamster monoclonal anti-JAML (clone 4E10) BioLegend 128502
 Rat monoclonal anti-Ly-6G/Ly-6C FITC (clone RB6-8C5) Invitrogen 11-5931-82
 Rat monoclonal anti-Ly-6G APC (clone 1A8) BioLegend 127614
 Rat monoclonal anti-Ly-6C FITC (clone HK1.4) BioLegend 128005
 Rat monoclonal anti-CD279 (PD-1) PE-Cy7 (clone RMP1-30) BioLegend 109109
 Rat monoclonal anti-CD279 (PD-1) APC-Cy7 (clone 29F.1A12) BioLegend 135224
 Rat monoclonal anti-CD185 (CXCR5) BV650 (clone L138D7) BioLegend 145517
 Mouse monoclonal anti-CX3CR1 APC (clone SA011F11) BioLegend 149007
 TotalSeq-C0301 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 1 BioLegend 155861
 TotalSeq-C0302 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 2 BioLegend 155863
 TotalSeq-C0303 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 3 BioLegend 155865
 TotalSeq-C0304 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 4 BioLegend 155867
 TotalSeq-C0305 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 5 BioLegend 155869
 TotalSeq-C0306 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 6 BioLegend 155871
 TotalSeq-C0307 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 7 BioLegend 155873
 TotalSeq-C0308 Rat monoclonal anti-CD45 (clone 30-F11) and anti-MHC-I (clone M1/42) Hashtag 8 BioLegend 155875
 TotalSeq-C0436 Mouse monoclonal anti-biotin (clone 1D4-C5) BioLegend 409011
 TotalSeq-C1129 Rat monoclonal anti-DYKDDDDK (FLAG) (clone L5) BioLegend 637335

Validation

All antibodies validated on the manufacturers' websites.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

293T adherent cells (ATCC Cat# CRL-3216) were used for protein expression and binding assays. LCMV strains were produced in BHK-21 cells (ATCC, Cat# CCL-10) and infectious viral titers were assessed by plaque assays on Vero E6 (ATCC, Cat# CRL-1586 VERO C1008) monolayers. All lines were obtained directly from ATCC.

Authentication

Cell lines were only used for protein expression and virus production only, which were confirmed successful as per the experimental data. No further authentication was performed beyond that provided by the vendor.

Mycoplasma contamination

293T lines tested negative for mycoplasma. BHK-21 and Vero E6 lines were not tested after receipt from the vendor but are were used at low passage numbers (3-4 passages).

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

5-12 week old adult male and female mice on the C57BL/6J background were used. See 'mice' section in the methods for further details. Mice were housed at 72 °F (22.2 °C) and 30–70% humidity in a 12-h light/dark cycle with ad libitum access to food and water.

Wild animals

The study did not involve wild animals.

Reporting on sex

Both sexes of mice were used throughout the study. No significant differences were noted between sexes.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All protocols were approved by the Rockefeller University and Mount Sinai School of Medicine Institutional Animal Care and Use Committees (protocol numbers 22058-H and IACUC-2018-0018/PROTO201900609, respectively).

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

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

For flow cytometry of lymph node cells, lymph nodes were collected into microfuge tubes with 500 µl HBSS (Gibco) supplemented with CaCl₂, MgCl₂, and collagenase D at 400 U ml⁻¹ (Roche). pLNs were cut into small pieces and incubated for 30 min at 37 °C. After digestion, tissue was forced 5 times through a 21-gauge needle and filtered through a 70 µm strainer into a 15 ml falcon tube with PBS supplemented with 0.5% BSA and 2 mM EDTA (PBE).

For flow cytometry of immune cells from other tissues:

Intraepithelial leukocytes: Small intestines were harvested and washed in PBS. Peyer's patches were surgically removed and the intestine was segmented in ~1 cm pieces prior to incubation with 1 mM dithiothreitol for 10 min at room temperature followed by addition of 30 mM EDTA and incubation for 30 min at 37°C. Intraepithelial cells were recovered from the supernatant of dithiothreitol and EDTA washes and mononuclear cells were isolated by collecting the middle ring after 40% and 80% gradient Percoll centrifugation.

Bone marrow cells: Punctured tibiae and femurs were centrifuged at up to 10,000 x G for 10 s, then treated with ACK red blood cell lysing buffer.

Immune cells from the kidney, lungs, spleen, thymus and liver: Tissue was cut into pieces and incubated in 1.5 ml HBSS supplemented with collagenase D at 400 U ml⁻¹, 0.1 mg ml⁻¹ DNase1 and 0.8 mg ml⁻¹ dispase 1 for 30 min at 37°C. After digestion, tissue was forced 5 times through a 21-gauge needle and filtered through a 70 µm strainer into a 15 ml falcon tube with PBE. Red-blood cells were lysed with ACK buffer and the resulting cell suspensions were filtered through a 70-µm mesh into PBE.

Immune cells from the brain: Mice were anesthetized and perfused transcardially with 10 ml ice-cold HBSS without Ca²⁺ and Mg²⁺ and the brains were removed and kept in ice-cold HBSS before further process. To stain and discard CD45+ cells from blood vessels for downstream analysis, anti-CD45 antibodies were retro-orbitally injected 15 min before perfusion. The entire brain was minced by mashing through a 150 µm cell strainer and the strainer was washed thoroughly by ice-cold HBSS to collect as many cells as possible. Minced tissues were spun down at 290 g for 5 min at 4°C to discard the supernatant and digested in 2 ml of Digestion solution (2 mg ml⁻¹ collagenase D, 25 mM HEPES, 1 mM sodium pyruvate, 14 µg ml⁻¹ DNase 1 in HBSS) for 20 min at 37°C without shaking. Digestion was stopped by adding 2 ml ice-cold HBSS and the tissues were homogenized with syringes fitted with 21G, 25G, and 27G needles, sequentially. The homogenates were filtered through a 70 µm mesh and spun down at 420 g for 7 min at 4°C to discard the supernatant. The pellets were resuspended in 37% Percoll solution in HBSS and centrifuged at 500 g for 10 min at room temperature to discard supernatant with a myelin layer. The cells in pellets were washed and resuspended in HBSS for further analysis.

Instrument

BD FACSymphony A5 cytometer for analysis and BD FACSAria II/III and FACSymphony S6 for cell sorting.

Software

FlowJo v.10 software

Cell population abundance

Cell sorting was performed for Chromium single cell RNA sequencing. Cell population abundance was confirmed by quantification of hashtag oligos used to identify different samples and cell types.

Gating strategy

Gating strategy for main populations are shown in Extended Data Figure 1C and Extended Data Figure 6A. Gating strategy for cell sorting for single cell RNA sequencing is shown in Extended Data Figure 6C and Extended Data Figure 9C.

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