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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	LSR II and Fortessa cytometers were used to collect flow cytometry data; 10X Genomics system was used to capture and create libraries for single cell RNA-seq; FACS Aria II sorters were used for all cell sorting
Data analysis	Prism (8), FlowJo (10.5.3); Seurat R Package (version 1.4.0.12)

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

Single cell RNA-seq data will be deposited in public repositories prior to publication and made available to reviewers/editors upon request

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 ☐ Ecological, evolutionary & environmental 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 sized were not calculated prior to performing experiments but conform to standard practices; Sample sizes were sufficient to detect differences between groups and all comparisons had a minimum of 3 data points
Data exclusions	The only data excluded from the manuscript is from cells in single-cell RNA-seq data sets which did not meet pre-established, standard quality thresholds (i.e., cells with fewer than 5 genes per cells)
Replication	All replicates successfully reproduced similar results
Randomization	Animals were randomly selected for experiments
Blinding	Investigators were not blinded to genotypes prior to data collection as non-subjective measures were used to understand the phenotypes observed (i.e. abundance of a population); Bioinformaticists were initially blinded to sample sort origins during single-cell RNA-seq analysis; Investigators were not blinded during colitis experiments as non-subjective measures were used to track disease (weight); A blinded investigator was used for measuring EAE clinical score for 1 experiment and the majority of data points for the other experiment

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

anti-mouse CD4 (GK1.5) BV786, BD Biosciences, 563331, 1:100
anti-mouse CD4 (RM4-5) BV510, BD Biosciences, 563106, 1:100
anti-mouse CD4 (RM4-5) PE, eBioscience, 12-0042-83, 1:300
anti-mouse CD4 (GK1.5) eF450, eBioscience, 48-0041-82, 1:100
anti-mouse CD4 (GK1.5) APC, eBioscience, 17-0041-83, 1:300
anti-mouse CD4 (RM4-5) FITC, Tonbo Biosciences, 35-0042-U500, 1:100
anti-mouse CD4 (GK1.5) PE-Cy7, Biolegend, 100422, 1:300
anti-mouse CD4 (RM4-5) BV605, Biolegend, 100548, 1:100
anti-mouse CD8a (53-6.7) BV786, BD Biosciences, 563332, 1:100
anti-mouse CD8a (53-6.7) APC-eF780, eBioscience, 47-0081-82, 1:100
anti-mouse CD8a (53-6.7) Biotin, Tonbo Biosciences, 30-0081-U500, 1:200
anti-mouse CD8a (53-6.7) PerCP-Cy5.5, Biolegend, 100734, 1:100
anti-mouse CD3 (145-2C11) PE-Cy7, eBioscience, 25-0031-82, 1:100
anti-mouse CD25 (PC61.5) PerCP-Cy5.5, Tonbo Biosciences, 65-0251-U100, 1:100
anti-mouse CD25 (PC61.5) BV421, Biolegend, 102043, 1:100
anti-mouse CD25 (PC61.5) PE-Cy7, eBioscience, 25-0251-82
anti-mouse CD25 (PC61.5) APC, eBioscience, 17-0251-82, 1:250 (IHC)
anti-mouse FOXP3 (FJK-16s) PE, eBioscience, 12-5773-82, 1:100
anti-mouse FOXP3 (FJK-16s) APC, eBioscience, 17-5772-82, 1:100
anti-mouse FOXP3 (FJK-16s) FITC, eBioscience, 320112, 1:100
anti-mouse FOXP3 (FJK-16s) AF700, eBioscience, 56-5773-82, 1:100 (IHC)

anti-mouse CD45.1 (A20) BV650, BD Biosciences, 563754, 1:200
anti-mouse CD45.2 (104) APC, eBioscience, 17-0454-82, 1:200
anti-mouse CD90.1 (HIS51) eF450, eBioscience, 57-0900-82, 1:300
anti-mouse CD90.2 (53-2.1) APC-eF780, eBioscience, 47-0902-82, 1:300
anti-mouse CD73 (eBioTY/11.8) eF450, eBioscience, 48-0731-82, 1:100
anti-mouse CD73 (TY/11.8) PE-Cy7, Biolegend 127224, 1:100
anti-mouse CD73 (TY/11.8) BV605, Biolegend, 127215, 1:200
anti-mouse CD73 (TY/11.8) PE, Biolegend, 127206, 1:100
anti-mouse TCRb (H57-597) APC, eBioscience, 17-5961-82, 1:100
anti-mouse TCRb (H57-597) APC-eF780, eBioscience, 47-5961-82, 1:200
anti-mouse TCRb (H57-597) Pacific Blue, Biolegend, 109226, 1:100
anti-mouse TCRb (H57-597) BV421, Biolegend, 109230, 1:1000 (IHC)
anti-mouse Ter119 (Ter-119) Biotin, BD Biosciences, 553672, 1:200
anti-mouse Ter119 (Ter119) APC-eF780, eBioscience, 47-5921-82, 1:100
anti-mouse CD44 (IM7) PE, eBioscience, 12-0411-83, 1:100
anti-mouse CD44 (IM7) BV786, BD Biosciences, 563736, 1:100
anti-mouse CD45RB (C363.16A), PE, eBioscience, 12-0455-81, 1:100
anti-mouse CD11c (N418) APC-eF780, eBioscience, 47-0114-82, 1:100
anti-mouse CD11b (M1/70) APC-eF780, eBioscience, 47-0112-82, 1:100
anti-mouse NK1.1 (PK136) APC-eF780, eBioscience, 47-5941-82, 1:100
anti-mouse F4/80 (BM8) APC-eF780, eBioscience, 47-4801-82, 1:100
anti-mouse B220 (RA3-6B22) APC-eF780, eBioscience, 47-0452-82, 1:100
anti-mouse Qa2 (69H1-9-9) FITC, eBioscience, 11-5996-82, 1:100
anti-mouse HSA (M1/69) PE, eBioscience, 12-0242-83, 1:100
anti-mouse CD69 (H1.2F3) PE, BD Biosciences, 553237, 1:100
anti-mouse CD69 (H1.2F3) APC, Biolegend, 104514, 1:100
anti-mouse RORγT (Q31-378) PE-CF594, BD Biosciences, 562684, 1:500 (IHC)
anti-mouse MHC-1 H2Kb (AF6-88.5.5.3), eBioscience, 17-5958-82, 1:100
anti-cleaved caspase 3 Asp175 (D3E9) Alexa 647, Cell Signaling Technology, 9602S, 1:50
anti-cleaved caspase 3 Asp175 (D3E9) PE, Cell Signaling Technology, 12768S, 1:50
anti-GFP FITC, Rockland, 600-402-215, 1:400

Multiple lots of antibodies were used which did not affect results or reproducibility.

Validation

Validation data for all antibodies are available on the manufacturers website

Animals and other organisms

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

Laboratory animals

Mice were housed in specific pathogen–free facilities at the University of Minnesota, Cornell University, Salk Institute or University of California San Francisco, and experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee. The one exception are mice with a normalized microbial experience which were housed in the University of Minnesota's mouse vivarium. Pet store mice were purchased from various pet stores in the greater Minneapolis-St. Paul metropolitan area. Information about the age of the pet store mice was not available from the vendor. Co-housing of SPF mice with sex-matched pet store partner was performed as described (Beura, et al., 2016) within the University of Minnesota BSL-3 facility. Conversion efficiency was confirmed by assessing the conversion of naïve CD8+T cells into CD8+memory T cells; effective conversion correlated with ~30-60%CD8+CD44hiT cells. All relevant ethical guidelines have been followed. Foxp3-GFPmice (006772), Foxp3-RFPmice (008374) were from the Jackson Laboratory.CD45.1+(B6.SJL) mice were from the US National Cancer Institute. Nur77-GFPBAC reporter mice, Rag2-GFP reporter mice, Cns3-/- and Foxp3-GFPKIN, CD28-/-, Nfkb1-/-, Itk-/-, Itk-/-x Il4Ra-/-, Adap-/-, Il2ra EDEL (and littermate NOD controls), Itgal-/-, Rag2-/-, Cd1d-/- and Tcliβ x TCRα+/- have been described previously (Boursalian et al., 2004; Fontenot et al., 2005; Hsieh et al., 2004; Huang et al., 2014a; Liao and Littman, 1995; Moran et al., 2011; Peterson et al., 2001; Sha et al., 1995; Shahinian et al., 1993; Shinkai et al., 1992; Simeonov et al., 2017; Sonoda et al., 1999; Zheng et al., 2010). Age of mice was generally between six to eight weeks but the range over experiments was four to sixteen weeks old. Mice were randomly selected for experiments, in age-matched cohorts. EAE experiments were performed with female donors and recipients however other experiments used male or female mice. The investigators were not 'blinded' to genotype during data acquisition.

Wild animals

Pet store mice were purchased from various pet stores in the greater Minneapolis-St. Paul metropolitan area. Information about the age of the pet store mice was not available from the vendor. Co-housing of SPF mice with sex-matched pet store partner was performed as described (Beura et al., 2016) within the University of Minnesota BSL-3 facility.

Field-collected samples

No field-collected samples were used in this study

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	Sample preparation is described in detail within the methods section.
Instrument	LSR II, Fortessa, and Aria II
Software	FlowJo (10.5.3)
Cell population abundance	Representative post-sort data is presented in figure 1
Gating strategy	Gating strategy is presented in figures 1 and 8 for thymic TRP/Treg and tetramer staining respectively. Briefly, lymphocytes were identified by SSC-A vs FSC-A then singlets by SSC-A by SSC-W; CD4 single positive cells were identified as CD8- CD4+, either bulk CD4 single positive or CD73- CD4 single positive cells were analyzed for CD25+FOXP3-, CD25-FOXP3lo or CD25+FOXP3+ cells.

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