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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 (*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	Complete TCRa sequence data was obtained using the Amp2Seq service from iRepertoire, a commercial platform based on semi-quantitative multiplex PCR coupled with Illumina sequencing. Flow cytometry data was collected using an LSRFortessa running FACSDiva version 8.0.2 (BD Biosciences), or using an Aurora running SpectroFlo version 2.2.0.4 (Cytek Biosciences). Cell sorting was performed using a FACSAria running FACSDiva version 8.0.2 (BD Biosciences). RNA sequencing data was collected using the NovaSeq 6000 System (Illumina). Immunofluorescence microscopy images were obtained using a SP8 laser scanning confocal microscope running Leica Application Suite X (Leica Microsystems).
Data analysis	Flow cytometry data were analyzed using FlowJo version 9.6.2 or newer (Tree Star, Inc). Summary plots and associated statistics were generated using Prism (versions 8 and 9; GraphPad Software). Following QC and alignment to mm10 using STAR aligner 2.7.5a, RNA sequencing data (paired-end 100-bp reads) were analyzed and visualized using R version 3.5.2 and the following packages: FeatureCounts, edgeR. Immunofluorescence microscopy image analysis was performed using Fiji version 2.3.0/1.53q. For cloning, DNA sequence alignment was assessed using Snapgene 4.0.8. No custom code or software was used in this 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 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

The RNA sequencing data are available at the National Institutes of Health Gene Expression Omnibus (GEO) repository under accession number GSE190127. All other source data, including TCR sequencing data, are provided with this 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

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 predetermine sample sizes. All experiments used sample sizes consistent with those reported in previous publications (see manuscript references 25, 69, 70). For each experiment, group numbers are reported in the corresponding figure legend and Source Data.
Data exclusions	Mice displaying signs of pathology unrelated to experimental approaches were excluded from analysis to ensure that any immunological changes were appropriately attributed to experimental manipulations. Samples for which one or more flow cytometry antibodies were inadvertently omitted from the staining mix were excluded from analysis due to an inability to property gated on cells of interest. In Fig. 5, one outlier was excluded from analysis per the Grubbs' test. Otherwise, all collected data were included in analyses.
Replication	Biological and technical replicates were included for each experiment. Information on replicates is included in each figure legend. Unless indicated otherwise, experiments in each primary figure were replicated at least twice, and all attempts at data replication for these experiments were successful.
Randomization	For generation of TCRrg mice, donor mice of the appropriate genotype were randomly selected for bone marrow isolation and retroviral transduction with a Group 1 or Group 3 TCR. Recipient mice were age-matched to donor mice but were otherwise randomized to TCRrg bone marrow treatment. Unless otherwise noted, all mice were used at 6 to 24 weeks of age. Both female and male mice were used, as noted in figured legends. Experiments involving FACS isolation and transfer or stimulation of TCRrg cells did not require randomization, as cells were harvested from all available TCRrg mice and pooled by TCR. Recipient mice in adoptive transfer studies were randomized to treatment condition. In all other settings, including Treg cell depletion and B cell depletion, mice in each cage were randomly assigned to each treatment group. Littermate controls were used whenever possible to control for age, potential effects of the microbiota, and rare polymorphisms in various mouse lines on the B6 background.
Blinding	For the RNA sequencing experiments in Fig. 3 and Extended Data Fig. 5, sequencing data acquisition and quality control were conducted by researchers blinded to sample identity. In Fig. 3, immunofluorescence microscopy (IF) images were collected, processed, and analyzed by researchers blinded to sample identity. Data resulting from IF were unblinded at time of analysis by the co-first authors. For all other experiments, data collection and analysis were not performed blind to the conditions of the experiments, as investigators used mouse ages and genotypes to establish experimental groups.

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 antibodies:
[*1:300, all others 1:100 unless otherwise indicated]

From BioLegend
-B220* (clone RA3-6B2, cat# 103206, 103239)
-CD3e* (clone 145-2C11, cat# 100301)
-CD4* (clone GK1.5, cat # 100447)
-CD5 (clone 53-7.3, cat# 100625)
-CD8a* (clone 53-6.7, cat# 100730, 100741)
-CD8b* (clone YTS156.7.7, cat# 126631)
-CD11b* (clone M1/70, cat# 101206)
-CD11c* (clone N418, cat# 117308)
-CD38 (clone 90, cat# 102718)
-CD44 (clone IM7, cat# 103026)
-CD45.1 (clone A20, cat# 110708)
-CD45.2 (clone 104, cat# 109816)
-CD69 (clone H1.2F3, cat# 104512, 104541)
-CD73 (TY/11.8, cat# 127215)
-CD200 (clone OX-90, cat# 123819)
-CXCR5 (clone L138D7, cat# 145511, 145529)
-F4/80* (clone BM8, cat# 123108)
-FR4 (clone 12A5, cat# 125014)
-GL7 (clone GL7, cat# 144610)
-ICOS (clone C398.4A, cat# 313536)
-IL-17 (clone TC11-18H10.1, cat# 506928)
-Neuropilin-1 (clone 12C2, cat# 145205)
-NK1.1* (clone PK136, cat# 108723)
-PD-1 (clone RMP1-30, cat# 109103, 109110, 109112)
-TCRb* (clone H57-597, cat# 109220)
-Tbet; 1:20 (clone 4B10, cat# 644816)
-Thy1.1 (clone OX-7, cat# 202526, 202529)
-Thy1.2 (clone 53-2.1, cat# 140303, 140305)

From eBioscience/Invitrogen
-CD45.2 (clone 104, cat# 58-0454-82)
-Eomes (clone Dan11mag, cat# 48-4875-82, 17-4875-82)
-Foxp3 (clone FJK-16s, cat# 17-5773-82, 25-5773-82, 11-5773-82, 61-5773-82)
-GATA3 (clone TWAJ, cat# 12-9966-41)
-IL-21 (clone mhalx21, cat# 12-7213-82)
-Ki67 (clone SolA15, cat# 12-5698-82)
-TCRb* (clone H57-597, cat# 58-5961-82)

From BD Biosciences
-B220* (clone RA3-6B2, cat# 612950)
-Bcl6; 1:50 (clone K112-91, cat# 561522, 561525)
-CD4* (clone GK1.5, cat# 563331, 565974, 612900)
-CD25 (clone PC61, cat# 563061)
-CD62L (clone MEL-14, cat# 740218)
-CD69 (clone H1.2F3, cat# 612793)
-IgG2b, κ isotype control antibody (clone A95-1, cat# 553986)
-IL-4 (clone 11B11, cat# 564004)
-IL-10 (clone JES5-16E3, cat# 564082)
-IFN-γ (clone XMG1.2, cat# 562018)
-MHC Class II blocking antibody (clone M5/114.15.2, cat# 556999)
-RORγt (clone Q31-378, cat# 564723)
-TCRb* (clone H57-597, cat# 748405)

Validation	All antibodies were obtained from commercial vendors and validated by their respective manufacturers. Validation entailed comparison of antibody staining with that of a corresponding isotype control.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Plat-E Retroviral Packaging Cell Line (Cell Biolabs)
Authentication	The Plat-E cell line is commercially available and authenticated. Transfection of Plat-E cells was confirmed via fluorescent microscopy of GFP expression.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals	Strains used include C57BL/6J (B6), B6.SJL-Ptprca Pepcb/BoyJ (B6 CD45.1/.1), B6.129S7-Rag1tm1Mom/J (Rag1-/-), B6.129S2-Tcratm1Mom/J (Tcrα-/-), B6.129(Cg)-Foxp3tm3(DTR/GFP)Ayr/J (Foxp3DTR-EGFP), B6.Cg-Foxp3tm2Tch/J (Foxp3EGFP), B6.129S2-H2dlAb1-Ea/J (MHCII-/-), B6.C(Cg)-Cd79atm1(cre)Reth/EhobJ (Mb1Cre), and C57BL/6-Gt(ROSA)26Sortm1(HBEGF)Awai/J (Rosa26LSL-DTR), B6.CgTg(CD4-cre)1Cwi (Cd4Cre), H2-DM-/-, and TCR Vβ3 transgenic (TCRβtg) mice expressing a fixed TCRβ chain of sequence Vβ3-(TRBV26-ASSLGSSYEQY). All mice were housed in rooms with a humidity range of 30% to 70%, a temperature between 20°C and 23°C, and a 12-h light / 12-h dark cycle. Unless otherwise noted, all mice were used at 6 to 24 weeks of age. Both female and male mice were used, as noted in figured legends.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago.

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

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	Murine secondary lymphoid organs and non-lymphoid tissues were processed to create single cell suspensions. Cells were stained for 20 minutes on ice in staining buffer [phosphate-buffered saline with 2% FBS, 0.1% NaN3, 5% normal mouse serum, 5% normal mouse serum, 5% normal rabbit serum (all sera from Jackson Immunological Labs), and 10ug/mL 2.4G2 antibody]. Intracellular staining was performed using fixation and permeabilization reagents and protocols from eBioscience. For assessment of cytokine production, cells were cultured in RPMI containing 10% FBS, 50 ng/mL PMA, and 500 ng/mL Ionomycin in U-bottom 96-well plates (Corning) for 1 hr at 37°C, followed by addition of 2 µM monensin for another 4 hr at 37°C prior to intracellular staining.
Instrument	Flow cytometry was performed on an LSR Fortessa (BD Biosciences) or on an Aurora (Cytek Biosciences). Cell sorting was performed using a FACSAria (BD Biosciences).
Software	FACSDiva software was used to acquire data on an LSR Fortessa or FACSAria (BD Biosciences). SpectroFlo was used to acquire data on an Aurora (Cytek Biosciences). All flow cytometry data was analyzed using FlowJo (Tree Star, Inc.) and summarized using Prism (GraphPad Software).

Cell population abundance	For experiments involving cell sorting, the purity of sorted T cell subsets was ≥99% as determined by purity check on the same cell sorter.
Gating strategy	For all experiments, cells were first gated through a lymphocyte gate, followed by a doublet discriminating gate. The gating strategy for each experiment is indicated in parentheses under each figure and/or in the figure legend.

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