nature portfolio

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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	Flow cytometric data were collected using a Ze5 cell analyzer (BD Biosciences) or a Cytek Aurora analyzer (Cytek Biosciences). Microbiome data from feces and ileal contents were collected using the MiSeq Illumina sequencing platform. In vivo images were acquired using IVIS Lumina Living Image Software(v.4.5.5). Metabolomics data were analyzed with SCIEX Triple QuadTM 7500 LC-MS/MS- QTRAP system coupled with ExionLCTM system. Single-cell RNA sequencing was conducted by the University of Michigan's Advanced Genomics Core.
Data analysis	Flow-cytometry analysis was done in BD FACSDiva and FlowJo (v.10.5) (Tree Star). Microbiome analysis was done in the community-supported software program mothur and by the steps in the MiSeq SOP (http://mothur.org/wiki/MiSeq_SOP). In vivo images were analysed using IVIS Lumina Living Image (v.4.5.5) Software. Metabolomics was analyzed using Metaboanalyst (V. 5.0). Statistical analysis was done in GraphPad Prism 8.0.

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 authors declare that data supporting the findings of this study are available within the article and its Supplementary Information files. All relevant data are available from the authors. The scRNA sequencing data have been deposited in the NCBI Sequence Read Archive (accession number: PRJNA1074271). The bacterial 16S rRNA sequencing data have been deposited in the NCBI Sequence Read Archive (accession number: PRJNA1073670).

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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

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.
Data exclusions	No data were excluded.
Replication	All experiments were performed with multiple replicates, and standard statistical methods were used to accept. Technical replicates were used for in vitro experiments where biological factors were not present, whereas biological replicates were used for the in vivo studies.
Randomization	Mice were assigned randomly to the experimental groups.
Blinding	The investigators were not blinded to allocation during experiments and outcome assessment since our data analyses are based on objectively measurable data.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Anti-CD16/CD32 (93, 14016186, eBioscience), 1:20 dilution;
CD45 Rat anti-Mouse (APC-efluor780, Clone: 30 F11), 1:100 dilution;
CD3e Armenian Hamster anti-Mouse (PerCP-Cy5.5, Clone: 145 2C11, eBioscience), 1:100 dilution;
CD4 Rat anti-Mouse (PE-Cy7, Clone: GK1.5, eBioscience), 1:100 dilution;
Foxp3 Monoclonal Antibody (APC, Clone: FJK-16s, eBioscience), 1:50 dilution;
IL-4 Rat anti-Mouse (PE, Clone: 11B11, eBioscience), 1: 100 dilution;
IL-10 Rat anti-Mouse (FITC, Clone: JES5-16E3, eBioscience), 1: 100 dilution;
anti-mouse IFN-γ (BV421, Clone: XMG1.2, Biolegend), 1: 100 dilution;
T-bet Mouse anti-Human, Mouse (PE, Clone: 4B10, eBioscience), 1: 100 dilution;
GATA-3 Rat anti-Mouse (Alexa Fluor™ 488, Clone: TWAJ, eBioscience), 1: 100 dilution;
RORγt Mouse anti-Mouse (BV421, Clone: Q31-378, BD), 1:100 dilution;
CD11c Armenian Hamster Monoclonal Antibody (PE-Cy7, clone: N418, Biolegend), 1:250 dilution;
anti-mouse CX3CR1 (BV605, Clone: SA011F11, Biolegend), 1:100 dilution;
CD103 Rat anti-Mouse (PE,Clone: M290, BD), 1:100 dilution;
anti-mouse I-A/I-E (FITC, Clone: M5/114.15.2, Biolegend), 1:200 dilution;
F4/80 Rat anti-Mouse F4/80 (BV711, Clone: T45 2342, BD Biosciences), 1:250 dilution.

BV785-Anti-CD11c Armenian Hamster Monoclonal Antibody (Clone: N418, BioLegend), 1:250 dilution;
PerCP/Cy5.5-anti-mouse/human CD11b (Clone: M1/70, BioLegend), 1:100 dilution;
AF594-anti-mouse CD80 (Clone: B7-1), 1:100 dilution;
APC-anti-mouse CD86 (Clone: B7-2, BioLegend), 1:100 dilution;
BV421-CD45 Rat Monoclonal Antibody (Clone: 30-F11, BioLegend), 100 dilution;
Pacific Blue-CD19 Rat Monoclonal Antibody (Clone: 6D5, BioLegend), 100 dilution;
BV510-anti-mouse I-A/I-E antibody (Clone: M5/114.15.2, BioLegend), 1:200 dilution;
BV711-Anti-Ly6C Rat Monoclonal Antibody (Clone: HK1.4, BioLegend), 100 dilution;
FITC-anti-mouse/human CD11b (Clone: M1/70, BioLegend), 1:100 dilution;
PE-Anti-CD64 Mouse Monoclonal Antibody (Clone: X54-5/7.1, BioLegend), 1:100 dilution;
PE/Cy7-anti-mouse Ly6G (Clone: 1A8, BioLegend), 1:100 dilution;
AF647-Siglec-F Rat anti-mouse (Clone: E50 2440, BD), 1:100 dilution;
AF700-anti-CD3 Rat Monoclonal Antibody (Clone: 17A2, BioLegend), 1:100 dilution.

Cd28 Hamster anti-Mouse (NA/LE, Unlabeled, Clone: 37.51, BD), 1:200 dilution;
Cd3ε Hamster anti Mouse (NA/LE, Unlabeled, Clone: 145 2C11, BD),1:200 dilution;
anti-IL-4 antibody (clone 11B11, BioXCell), used in vivo without dilution;
anti-IFN-γ (clone XMG1.2; BioXcell), used in vivo without dilution;
anti-IL-10 (clone JES5-2A5; BioXcell), used in vivo without dilution;
Rat IgG1 isotype control (clone HRPN; BioXcell), used in vivo without dilution.

TotalSeq™-C0301 anti-mouse Hashtag 1 antibody (BioLegend), 1:50 dilution;
TotalSeq™-C0302 anti-mouse Hashtag 2 antibody (BioLegend), 1:50 dilution;
TotalSeq™-C0303 anti-mouse Hashtag 3 antibody (BioLegend), 1:50 dilution.

Validation

Antibody validation was provided by manufacture's website (cell images) and/or data is provided in the paper.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The Foxp3(GFP) CD4 T cell line was obtained from spleen in Foxp3(GFP) reporter mice. The CD4 T cell line was obtained from spleen in the wide-type C57BL/6 mice. The OT-II CD4 T cell line was obtained from spleen in OT-II mice.

Authentication

CD4 T cells isolated from spleen in wide-type or transgenic mice, while these mice were authenticated by the provider (The Jackson Laboratory). No further authentication of the isolated CD4 T cells was performed.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidenified 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	For the in vivo studies, 5-6 weeks-old female BALB/c mice (Jackson Laboratory, 18–20 g), 5-6 weeks-old female C3H/HeJ mice (Jackson Laboratory, 18–20 g), 5-7 weeks-old female C57BL/6 mice (Jackson Laboratory, 18–20 g) were used. OT-II mice and Foxp3(GFP) reporter transgenic mice (7 weeks-old female, 15–20 g) were obtained from the Jackson Laboratory. Mice were housed in 12-hour light–dark cycle, at 72 F, and about 30% humidity.
Wild animals	The study did not involve wild animals.
Reporting on sex	Female mice were used based on the previously published results.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All work performed on animals was in accordance with, and approved by, the Institutional Animal Care & Use Committee (IACUC) at University of Michigan, Ann Arbor.

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	The sample preparation is described in Methods.
Instrument	Ze5 cell analyzer (BD Biosciences), Cytek Aurora analyzer (Cytek Biosciences).
Software	FlowJo (v.10.5) (Tree Star) were used for data analysis.
Cell population abundance	Data on the abundance of relevant cell populations are provided in the paper.
Gating strategy	Cells were gated first by morphology to exclude cell debris, doublets were then gated out by FSC-H/FSC-W, SSC-H/SSC-W, followed by exclusion of dead cells by gating on dye-negative cells.

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