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	SpectroFlo v3.3.0 QuantStudio™ Design and Analysis Software v1.5.1 BioTek Gen5 v2.07 AxioVision v4.8
Data analysis	Flowjo 10.10.0 Microsoft Escel v2511 GraphPad Prism v10.4.1 Adobe Photoshop v27.3.1

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

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

> All data supporting the findings of this study are available within the paper and its Supplementary Information. QPCR primer sequences are provided in Material and Methods

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	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	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	NA
Data exclusions	NA
Replication	All experiments were performed with two or three biological replicates
Randomization	NA
Blinding	NA

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	rabbit anti-IL-17A polyclonal antibody (Cat# PA5-114455, Invitrogen) donkey anti-rabbit IgG antibody conjugated to Alexa Fluor 488 (Cat# A5-21206, Invitrogen) anti-mCD3 antibodies (clone 17A2, Invitrogen) anti-mIL-4 antibodies (clone 11B11, Invitrogen) anti-mCD28 antibody (clone 37.51, BioLegend) anti-mIFNg antibody (clone XMG1.2, BioLegend) anti-mCD16/CD32 antibodies (BD Pharmingen) anti-mCD4 antibody (clone GK1.5, BioLegend) anti-mRORγT (clone Q31-378, BD Pharmingen) anti-mIL-17A (clone TC11-18H10.1, BioLegend).
Validation	Validation statement can be found in the manufacture's websites: Invitrogen, Thermo Fisher Scientific: https://www.thermofisher.com/us/en/home/life-science/antibodies.html BioLegend, https://www.biolegend.com/nl-be/antibodies-reagents BD Biosciences, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents

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	Mus musculus, C57BL/6J strain
Wild animals	Not applicable
Reporting on sex	Not applicable
Field-collected samples	Not applicable
Ethics oversight	All procedures involving mice were approved by the University of Michigan's Institutional Animal Care and Use Committee, as per the protocol numbers PRO00010437 and PRO00011303.

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

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable

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	Mouse induced Th17 cells were differentiated in 96-well plates (Fisher Brand) at a density of 2×10^5 cells per well and subjected to the designated treatments. After treatment, plates were centrifuged at 1,200 rpm for 2 minutes, and the culture medium was carefully removed. Cells were first blocked with anti-CD16/CD32 antibodies (Fc block; BD Pharmingen), followed by cell surface staining with fluorochrome-conjugated anti-mouse CD4 antibody (clone GK1.5, BioLegend). Subsequently, the cells were fixed and permeabilized using the Foxp3 staining buffer set (eBioscience), and then stained for intracellular RORγT (clone Q31-378, BD Pharmingen) and IL-17A (clone TC11-18H10.1, BioLegend).
Instrument	Flow cytometry was performed using a Cytek Aurora analyzer (Cytek Biosciences).
Software	SpectroFlo v3.3.0 was used to collect flow data, and Flowjo v10.10.0 was used to analyze the flow data.
Cell population abundance	Not applicable
Gating strategy	Please see Supplementary Figure 1. for gating strategy.

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