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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Animal sample size estimates were determined using power analysis (power=90% and alpha=0.05) based on the mean and standard deviation from our previous studies and/or pilot studies using 4-5 animals per group.

2. Data exclusions

Describe any data exclusions.

No samples were excluded from analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For T cell adoptive transfer colitis co-housed littermate recipients were randomly assigned to different treatment groups such that each cage contained all treatment conditions.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Pathology analysis was single-blind. All other animal studies were not blinded

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Flow jo (v9.9.6,Tree Star) was used for all flow cytometry analysis. Prism7 (v7.0) was used to generate all graphs and statistics with the exception of RNA-seq. RNA-seq analysis was performed using STAR (v2.5.2b), featureCounts is part of the Subread package(v1.5.1), DESeq2 (v1.14.1), R (v3.3.1), ComBat is part of the sva R package (v3.22.0), Ingenuity Pathway Analysis (www.Ingenuity.com). Gene set enrichment analysis (GSEA, http://www.broad.mit.edu/gsea/). Nucleotide sequences of the TCRα and TCRβ families were retrieved from the IMGT database (http://www.imgt.org). The potential MHCII epitopes were predicted with online software RANKPEP (http://imed.med.ucm.es/Tools/rankpep.html).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No restricted materials were used. (Tetramers are available through the NIH tetramer core facility. TCRtg mouse lines have been deposited and will be available at Jackson laboratory)

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The antibodies are described below. All antibodies were purchased from BD, eBioscience, Biolegend or BioXcell. All antibodies were validated by manufacturers and in previous publications.

Antibodies for flow cytometry:
antibody/clone/color/company
CD3 145-2C11 AF700/PerCP-Cy5.5 BD
CD4 RM4-5 BUV395/PE-Cy7 BD
CD25 PC61 APC ebioscience
CD44 IM7 AF700 ebioscience
CD45.1 A20 BV421 BD
CD45.1 A20 APC-eF780/PerCP-Cy5.5 ebioscience
CD45.2 104 PerCP-Cy5.5/APC ebioscience
CD62L MEL-14 PE ebioscience
CXCR5 L138D7 PE Biolegend
NPR-1(CD304) 3E12 BV421 Biolegend
ST2 RMST2-2 PE ebio
TCRβ H57-597 APC-eF780/BV711 BD
TCR Vβ6 RR4-7 APC ebioscience
TCR Vβ6 RR4-7 FITC BD
TCR Vβ8.1/8.2 MR5-2 FITC BD
TCR Vβ14 14-2 FITC BD
Bcl-6 K112-91 BV421 BD
c-Maf T54-853 PE BD
Foxp3 FJK-16s FITC/PE-Cy7/APC ebioscience
GATA3 TWAJ FITC ebioscience
Helios 22F6 PE ebioscience
RORγt B2D APC/PE ebioscience
RORγt Q31-378 BV421 BD
T-bet eBio4B10 PE/APC ebioscience
IL-10 JES5-16E3 BV421 BD
IL-17A eBio17B7 APC ebioscience
IFN-γ XM61.2 FITC Biolegend

Antibodies for cell culture and mouse treatment
antibody/clone/company
anti-CD3e 145-2C11 Bioxcell
anti-CD28 37.51 Bioxcell
anti-IL4 11B11 Bioxcell
anti-IFNg XMG1.2 Bioxcell
anti-TGFb 1D11.16.8 Bioxcell
anti-IL10Ra 1B1.3A Bioxcell

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The NFAT-GFP 58α−β− hybridoma cell line was kindly provided by Dr. K. Murphy.

b. Describe the method of cell line authentication used.

The NFAT-GFP 58α−β− hybridoma cell line can faithfully report TCR stimulation as shown in previous publications:
Yang et. al., Nature, 2014, 510(7503):152-6.
Ise et. al., Nat Immunol, 2010, 11(2): 129–135.

c. Report whether the cell lines were tested for mycoplasma contamination.

mycoplasma contamination was not tested

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

WT C57Bl/6 mice were obtained from Jackson Laboratories or Taconic Farm. Il10-/-, CD4-dnTGFbRII, CD4cre, CD45.1, Foxp3creYFP, Il23rgfp, Maf fl/fl; Stat3 fl/fl and Gata3 fl/fl mice were on C57Bl/6 background. Littermates with matched sex (both males and females) were used. Except the aged mice (6-12 month old) analyzed in the experiments of Fig. 4e, mice in all the experiments were 6-12 week old at the starting point of treatments.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	The detailed information is provided in the manuscript methods section 'Isolation of lymphocytes' and 'T cell culture', 'MHCII tetramer production and staining' Intestinal tissues were sequentially treated with PBS containing 1 mM DTT at room temperature for 10 min, and 5 mM EDTA at 37°C for 20 min to remove epithelial cells, and then minced and dissociated in RPMI containing collagenase (1 mg/ml collagenase II; Roche), DNase I (100 µg/ml; Sigma), dispase (0.05 U/ml; Worthington) and 10% FBS with constant stirring at 37°C for 45 min (SI) or 60 min (LI). Leukocytes were collected at the interface of a 40%/80% Percoll gradient (GE Healthcare). The Peyer's patches and cecal patch were treated in a similar fashion except for the first step of removal of epithelial cells. Lymph nodes and spleens were mechanically disrupted. Fluorescence-activated cell sorting and magnetic-activated cell sorting were used to further isolate indicated cells populations.
6. Identify the instrument used for data collection.	LSRII or AriaII (BD Biosciences)
7. Describe the software used to collect and analyze the flow cytometry data.	Diva (BD Biosciences) and Flowjo 9.9.6 (Tree Star)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	The purities of sorted T cells were more than 98%
9. Describe the gating strategy used.	Based on the pattern of FSC-A/SSC-A, cells in the lymphocyte gate were used for analysis of T cell subsets. Singlets were gated according to the pattern of SSC-H vs. SSC-W. Positive populations were determined by the specific antibodies, which were distinct from negative populations.

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