# nature research

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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 our Editorial Policies and the Editorial Policy Checklist.

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St	at	ict	100

1 01	an statistical analyses, commit that the following items are present in the figure regend, that legend, main text, or interious 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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	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 <i>d</i> , Pearson's <i>r</i> ), 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 <u>availability of computer code</u>

Data collection

BD FACS Diva v8.0.2 has been used to collect flow cytometry data. ZEN 2011 v14.0.10.201 has been used to collect confocal images.

Data analysis

GraphPad Prism v7.04 has been used to analyze and visualize the data.

For RNAseq FastQC v0.11.5 was used to ensure high per-base sequence quality of reads.

STAR v2.7.4a was used to align and acquire raw count values then RSEM v1.2.25 was used to quantify FPKM expression values.

R v3.6.2 with the following packages was used to perform downstream analysis and visualization of RNAseq data (DEseq2 v1.26.0, pheatmap v1.0.12, rgl v0.100.30).

Expression scatter plots were produced with Excel(version2105 from Microsoft 365).

Kegg pathway and GO analysis were analyzed with online Kegg and GO website at GSEA (MSigDB v6.0, http://software.broadinstitute.org/gsea/index.jsp).

FlowJo v10.2 has been used to analyze flow cytometry data.

For Metabolite data MZmine2 (freeware, v2.53) was used for alignment, gap filling and metabolite identification then PCA was performed using Metaboanalyst 3.0.

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 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

All data supporting the findings of this study are available within the article and its supplementary materials and from the corresponding author upon reasonable request. The RNA-sequencing data of splenic, small intestinal and large intestinal control and Tfam-deficient gdT17 cells, and small intestine tissues of control and Tfamfl/flRorc-cre mice generated in this study have been deposited in the NCBI database under accession code GSE152535 Source data are provided with this paper.

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# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

No sample size calculation was performed. The exact n values used to calculate the statistics are provided and a reasonable sample size was Sample size chosen to ensure adequate reproducibility of results. The sample size was chosen equal or more than 3 to run a student's t-test or other statistics analysis.

Data exclusions Data were excluded when sorted gdT17 cells were of poor quality, for example decreased viability (<50%) due to the long time kept on ice and measurement were failed. Cytokine data were excluded when the PMA and lonomycin stimulation failed to induced cytokine in any samples.

> RNA-seq and metabolome included 3 duplicates for each group. All attempts at replication for RNA-seq and metabolome were successful. Other experiments were replicated several times (2-4) with reproducible results, as indicated in each figure legend.

For experiments comparing knockout vs control mice, or different gdT cell subsets from control mice, or gdT17 cells from different tissues, Randomization aged matched male and female littermate mice were randomized.

> Figure 3c, d: the pathologist who evaluated samples for histology scores was blinded of genotypes during evaluation. Other figures were not blinded since the experiment performing, data collection and analysis were completed by the same author, although the results were confirmed by the rest authors.

# Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

> State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.

Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.

Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.

If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Replication

Blinding

**Timing** 

Data exclusions

Data collection

Research sample

Sampling strategy

Non-participation

State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.

Randomization

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

Research sample

Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

Sampling strategy

Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.

Data collection

Describe the data collection procedure, including who recorded the data and how.

Timing and spatial scale

Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken

Data exclusions

If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Reproducibility

Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.

Randomization

Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.

Blinding

Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Did the study involve field work?

Yes

X No

## Field work, collection and transport

Field conditions

Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

Location

State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

Access & import/export

Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

Disturbance

Describe any disturbance caused by the study and how it was minimized.

# 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.

manisms  ticipants  concern
Anti-CD3-eFluor710 eBioscience 46-0032 Anti-CD4-APC-eFluor® 780 eBioscience 47-0041 Anti-CD8a-APC-Cy7 Biolegend 100714 Anti-IL-22-APC Invitrogen 17-7222-82 Anti-Brdu-APC Biolegend 339807 Anti-CD44-APC Tonbo 20-0441-U100 Anti-TCRgd-BV510 Biolegend 118131 Anti-CD45Rb-PE eBioscience 12-0455-83 Anti-RORgt-PE eBioscience 12-6988-82 Anti-RORgt-APC eBioscience 17-6988-82 Anti-IFN-g-APC eBioscience 17-6988-82 Anti-IFN-g-APC eBioscience 17-7311
Anti-IL-17A-PerCP Cy5.5 eBioscience 45-7177 Anti-IL-17A-PE eBioscience 12-7177 Anti-IL-5-APC Biolegend 504306 Anti-IL-13-Alexa Fluor488 Invitrogen 53-7133 Anti-IL-4-Percp-Cy5.5 Biolegend 504124 Anti-CD45.1-PE eBioscience 12-0453 Anti-CD45.1-BV450 BD 560520
Anti-CD45.2-Percp-Cy5.5 eBioscience 45-0454 Anti-Ki67-PE-Cy7 BD 561283 Anti-Annexin V-APC Invitrogen 17-8007 Anti-DCLK1 Abcam ab31704 Anti-EpCAM-APC BD 563478 Anti-PLZF-PE Biolegend 145803 Anti-IL-4 BIO X CELL BE0045 Anti-IL-13 InvivoGen mabg-mil13-5 Anti-IPh1.2 BioXcell BE0066 Anti-CD16/CD32 Invitrogen 14-0161-86 Anti-Vr4(A gift from Dr. Robert Tigelaar, Clone 17D1) Anti-CD3-PE, BD, Cat#553063 Anti-KLRG1-Alexa488, Invitrogen, Cat#53-5893-82 Goat anti-Rabbit secondary Antibody, Alexa Fluor 488, Thermal Fisher, Cat# A11008
Anti-Vr4 antibody is a gift from Dr. Robert Tigelaar and validated in his publication (Christina L, et al,. Journal of Leukocyte Biology. 2004). All the rest antibodies used are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer's website.

Cell line source(s) State the source of each cell line used. Authentication Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated. Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for Mycoplasma contamination mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines (See <u>ICLAC</u> register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

### Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

## Animals and other organisms

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

Laboratory animals

Mice used in this study were maintained in specific-pathogen-free (SPF) conditions at the University of Florida. All mouse studies were approved by the Animal Care and Use Committees of the University of Florida. Mice were littermate controlled and both male and female mice were used for experiments. Mice were used at 6 to 8-week-old age unless otherwise noted. Tfamfl/fl mice were kindly provided by Navdeep Chandel (Northwestern University). C57BL/6, TcrdCreER mice and Rorc-cre mice were purchased from Jackson Laboratory. Tfamfl/flTcrdCreER mice were generated by crossing Tfamfl/fl mice to TcrdCreER mice.

Wild animals

No wild animals were used in the study

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

All mouse studies were approved by the Animal Care and Use Committees of the University of Florida.

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

# Human research participants

Policy information about studies involving human research participants

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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

### Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration | Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection 
Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

#### Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deli	berate or reckless misuse of agents or technologies generated in the work, or the application of information presented threat to:			
No Yes Public health National security Crops and/or livest				
Any other significa	nt area			
Experiments of concer	n			
Does the work involve an	y of these experiments of concern:			
Confer resistance to Enhance the virule Increase transmiss Alter the host range Enable evasion of Enable the weapor Any other potential ChIP-seq  Data deposition	to render a vaccine ineffective o therapeutically useful antibiotics or antiviral agents note of a pathogen or render a nonpathogen virulent ibility of a pathogen e of a pathogen diagnostic/detection modalities nization of a biological agent or toxin lly harmful combination of experiments and agents  of and final processed data have been deposited in a public database such as GEO.			
Data access links  May remain private before publi	e deposited or provided access to graph files (e.g. BED files) for the called peaks.  For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.			
Files in database submiss	ion Provide a list of all files available in the database submission.			
Genome browser session (e.g. <u>UCSC</u> )				
Methodology				
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.			
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.			
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.			
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.			
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.			
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community			

### Flow Cytometry

#### **Plots**

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- x 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).
- **X** 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 isolation of lymphocytes from intestinal lamina propria was conducted by digesting minced lung tissues with collagenase VIII (Sigma) and DNase I (Sigma) at 37°C for 90 min. Lung lymphocyte isolation was conducted by digesting minced lung tissues with collagenase IV (Sigma) and DNase I (Sigma) at 37°C for 90 min. Fat lymphocyte isolation was conducted by digesting minced peritoneal adipose tissues with collagenase II (Sigma) with DNase I at 37°C for 90 min. Skin lymphocyte isolation was conducted by digesting minced ear skin tissues with collagenase IA (Sigma) with DNase I at 37°C for 60 min. After digestion, cells were further purified by 37.5% and 75% Percoll gradient for 20 min spin at 2,500 rpm. For flow cytometry analysis, the live and dead cells were stained by Live and Dead violet viability kit (Invitrogen) or Zombie Aqua fixable viability kit (BioLegend). anti-CD16/CD32 antibody (Thermo Fisher) was used to block the nonspecific binding followed by surface molecule staining at 4°C for 30 min. Cells were fixed and permeabilized with Foxp3 staining buffer Kit (eBioscience) for transcription factor staining. For cytokine staining, cells were stimulated with 50 ng/ml PMA and 500 ng/ml ionomycin for 4 hours and Brefeldin A (2 μg/ml) was added 2 hours before cells were harvested.

Instrument

BD FACSCantoll and LSRFortessa flow cytometer.

Software

BD FACSDIVA and FlowJo software (version 10) softwares were used for data collection and data analysis respectively.

Cell population abundance

Post-sort gdT17 cells were analyzed on BD LSRII and the purity of gdT17 cells was at least 95%.

Gating strategy

Lymphocytes were gated on FSC-A/SSC-A. Single cells were gated on FSC-A/FSC-H. Live cells were gated on Zombie Aqua negative population. gdT17 cells were gated as CD3+TCRd+RORgt+ or CD3+TCRd+CD44HighCD45Rb-.

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

# Magnetic resonance imaging

#### Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

#### Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Used

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

Not used

### Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.				
Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.				
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).				
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.				
Statistical modeling & infer	nce				
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).				
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.				
Specify type of analysis:	nole brain ROI-based Both				
Statistic type for inference (See <u>Eklund et al. 2016</u> )	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.				
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo)				
Models & analysis  n/a   Involved in the study					
Functional and/or effective cor	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation mutual information).				
Graph analysis  Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph g					

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

etc.).

Multivariate modeling and predictive analysis