

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

NA

Data analysis

NA

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](#)

Provide your data availability statement here.

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 | NA |
| Reporting on race, ethnicity, or other socially relevant groupings | NA |
| Population characteristics | NA |
| Recruitment | NA |
| Ethics oversight | NA |

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](https://www.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 | For in vitro analyses of T cell function, a minimum of 3 biological replicates are plotted. In each case, a biological replicate represents T cells derived from one mouse. |
| Data exclusions | NA |
| Replication | Independent replicate experiments were performed for all experiments. the number of replicate experiments are described in figure legends |
| Randomization | NA |
| Blinding | Blinding was not undertaken for in vitro T cell experiments. Experiments, data acquisition and analysis were typically undertaken by one individual with analysis checked and verified by a second researcher. |

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 |
|-------------------------------------|---|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Antibodies |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants |

Methods

| n/a | Involved in the study |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Antibodies

| | |
|-----------------|--|
| Antibodies used | All antibodies from BioLegend unless otherwise stated. CD3e, Cat. No 100359, Clone 145-2C11 CD28, Cat. No. 102121, Clone 37.51 |
|-----------------|--|

CD25, Cat. No. 102002, Clone PC61
 CD8b-PE-Cy7, Cat. No. 126616, Clone YTS156.7.7
 CD4-APC, Cat. No. 100412, Clone GK1.5
 CD71-FITC, Cat. No. 113808, Clone R17217
 IFNg-AF488, Cat. No. 505813, Clone XMG1.2
 Tbet-PE, Cat. No. 633810, Clone 4B10
 TNF-PerCP Cy5.5, Cat. No. 506322, Clone MP6-XT22
 ASNS-AF647, Cat. No. 49791, Clone E6C2C, Cell Signaling Technology

Validation

Functional antibodies (CD3 and CD28) are low endotoxin and azide-free formulations. The fluorescently-conjugated antibody clones used in this study are established for use in flow cytometry. CST reports that it uses application-specific validation of their antibodies using positive- and negative-expressing cells, including the use of genetic knockouts, and cross-antibody validation (two or more antibodies that recognise different epitopes of the same protein) <https://www.youtube.com/watch?v=LIRyP34HIG4>. BioLegend states that it tests each antibody lot for specificity and quality using specification criteria based on their intended application. Both facilities operate under ISO 13485:2016 standards.

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

C57BL/6J, AsnsTm1a gene-trap and mCherry-GFP-Map1lc3b autophagy reporter mice were used at 7-12 weeks of age.

Wild animals

NA

Reporting on sex

Both sexes of mice were used.

Field-collected samples

NA

Ethics oversight

Mouse breeding and experiments were reviewed by the host institutions Animal Welfare and Ethical Review Boards, regulated work was approved by and subject to the conditions of UK Home Office Project Licences PP9249234 and P4BD0CE74

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

Plants

Seed stocks

NA

Novel plant genotypes

NA

Authentication

NA

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

Activated mouse T cells were transferred to 5ml flow cytometry tubes and washed and centrifuged in sterile PBS. Cell surface stains were performed by resuspending cell pellets in 100ul of antibody mixes prepared in sterile FACS buffer (PBS + 5% FBS) followed by incubation for 20min at 4oC. For intracellular stains, cells were fixed and permeabilised using FoxP3 fix/permeabilisation buffers (eBioscience) prior to staining. Live-dead discrimination was assessed using LD-Aqua (Life Technologies). Cells were washed and centrifuged in appropriate buffers and resuspended in 200-300ul PBS immediately prior to sample acquisition

Instrument

Cytoflex S (Beckman Coulter) or LSR Fortessa (BD Biosciences)

Software

Flowjo version 10 (Becton Dickinson)

Cell population abundance

For purified CD4 T cells: CD4+ T cells > 90% of live lymphocytes
For mixed LN T cells: CD4+ and CD8+ T cells were 20-50% of live lymphocytes
In all cases, a minimum of 10000 events were collected.

Gating strategy

Sequential gating
1. FSC/SSC (lymphocyte gate)
2. LD-Aqua negative (live-dead discrimination)
3. CD4+ or CD8+ gates
4. Specific markers assessed

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