

Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

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 | LSRli, LSRFortessa, FACSaria Fusion(Becton Dickinson), Zeiss LSM 410 confocal microscope, Zeiss LSM880 confocal microscopy, Zeiss AxioObserver Z1 equipped with Axiocam MRC5 camera, QuantStudio 6 Flex Real-time PCR System (Applied Biosystem) and SpectralMax iD3. |
| Data analysis | FlowJo 10.6.0, Prism 8 (Graph pad software), Adobe Photoshop, Adobe Illustrator, Canvas 12 and Adobe Illustrator |

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](#)

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, 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.

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

The sample size for each experiment is indicated in the figure legends. The minimum sample size was chosen to reach statistical significance compared to control mice.

Data exclusions

No data were excluded

Replication

For all experiments, at least three replicates were analyzed in at least two independent experiments. The experimental findings were reliably reproduced.

Randomization

Animals were allocated to groups based on genotype.

Blinding

No blinding was used as no subjective scoring methods were used.

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-CD45.1 FITC (clone A20), BD Bioscience, Cat#553775, RRID: AB_395043
 anti-CD45.1 Biotin (clone A20), BD Bioscience, Cat#553774, RRID: AB_395042
 anti-CD45.1 PE/Cy7 (clone A20) BioLegend Cat# 110730, RRID: AB_1134168
 anti-CD45.2 Alex594 (clone 104) BioLegend Cat#109850, RRID: AB_2629589
 anti-CD45.2 PE (clone 104) BioLegend Cat#109808, RRID: AB_313445
 anti-CD45.2 FITC (clone 104) BD Bioscience Cat#553772, RRID: AB_395041
 anti-TCRab BV510 (clone H57-597) BD Bioscience Cat#563221, RRID: AB_2738078
 anti-TCRab Alex594 (clone H57-597) BioLegend Cat#109238, RRID: AB_2563324
 anti-TCRab eFluor 450 (clone H57-597) eBioscience Cat#48-5961-82, RRID: AB_11039532
 anti-TCRab PE Cy7 (clone H57-597) BD Bioscience Cat#560729, RRID: AB_1937310
 anti-TCRab NALE (clone H57-597) BD Bioscience Cat#553166, RRID: AB_394678
 anti-CD28 NALE (clone 37.51) BD Bioscience Cat#553294, RRID: AB_394763
 anti-CD4 BV786 (clone GK1.5) BD Bioscience Cat#563331, RRID: AB_2738140
 anti-CD4 APC-eFluor780 (clone GK1.5) eBioscience Cat#47-0042-82, RRID: AB_1272183
 anti-CD5 APC (53-7.3) eBioscience Cat#17-0051-82, RRID: AB_469331
 anti-CD8a Pacific Blue (clone 53-6.7) BD Bioscience Cat#558106, RRID: AB_397029
 anti-CCR7 APC (clone 4B12) eBioscience Cat#17-1971-82, RRID: AB_469444
 anti-CCR7 Biotin (clone 4B12) eBioscience Cat#13-1971-82, RRID: AB_466642
 anti-CD69 PE (clone H1.2F3) BioLegend Cat#104508, RRID: AB_313111
 anti-CD69 Pacific Blue (clone H1.2F3) BioLegend Cat#104524, RRID: AB_2074979
 anti-CD69 PE/Cy7 (clone H1.2F3) BioLegend Cat#104512, RRID: AB_493564
 anti-CD69 APC (clone H1.2F3) BioLegend Cat#104514, RRID: AB_492843
 anti-Vα 11.1, 11.2 Biotin (clone RR8-1) BD Bioscience Cat#553221, RRID: AB_394716
 anti-Vα 11.1, 11.2 FITC (clone RR8-1) BD Bioscience Cat#553222, RRID: AB_394717
 anti-Vβ3 PE (clone KJ25) BD Bioscience Cat#553209, RRID: AB_394709
 anti-Vβ5 FITC (clone MR9-4) BD Bioscience Cat#553189, RRID: AB_394697
 anti-Vβ5 Biotin (clone MR9-4) BD Bioscience Cat#553188, RRID: AB_394696
 anti-Vβ5 PE (clone MR9-4) BD Bioscience Cat#553190, RRID: AB_394698
 anti-Vβ6 Biotin (clone RR4-7) BD Bioscience Cat#553192, RRID: AB_394699
 anti-Vβ6 FITC (clone RR4-7) BD Bioscience Cat#553193, RRID: AB_394700
 anti-Vβ6 PE (clone RR4-7) BD Bioscience Cat#553194, RRID: AB_394701
 anti-Vβ8 PE (clone F23.1) BD Bioscience Cat#553862, RRID: AB_395098
 anti-Vβ11 PE (clone RR3-15) BD Bioscience Cat#553198, RRID: AB_394704
 anti-CD25 Alex 594 (clone PC61) BioLegend Cat#102045, RRID: AB_2563963
 anti-CD25 BV510 (clone PC61) BD Bioscience Cat#563037, RRID: AB_2737969
 anti-CD25 PE (clone PC61) BD Bioscience Cat#553866, RRID: AB_395101
 anti-CD25 Biotin (clone 7D4) BD Bioscience Cat#553070, RRID: AB_394602
 anti-CD25 PE/Cy7 (clone PC61) eBioscience Cat#25-0251-82, RRID: AB_469608
 anti-CD132 PE (clone 4G3) BD Bioscience Cat#554457, RRID: AB_395404
 anti-Foxp3 PE (clone FJK-16s) eBioscience Cat#12-5773-82, RRID: AB_467576
 anti-Foxp3 eFluo660 (clone FJK-16s) eBioscience Cat#50-5773-82, RRID: AB_11218868
 anti-ZAP-70 PE (clone 1E7.2) BioLegend Cat#313404, RRID: AB_2304732
 anti-Thy1.1 Alex 647 (T43-94) BD Bioscience Cat#565500, RRID: AB_2739268
 anti-Bim BD Bioscience Cat#559685, RRID: AB_397305
 anti-Bcl-2 PE (clone 100) BioLegend Cat#658707, RRID: AB_2563281
 anti-Foxo1 (clone C29H4) Cell signaling Cat#2880P, RRID: AB_2106495
 anti-cRel PE (clone REA397) Miltenyi Biotec Cat#130-124-715, RRID: AB_2651454
 Normal Goat Serum Jackson ImmunoResearch Cat#005-000-121, RRID: AB_2336990
 anti-GFP abcam Cat#ab13970, RRID: AB_300798
 Goat anti-chicken IgY Alex 488 abcam Cat#ab150173, RRID: AB_2827653
 anti-RFP ROCKLAND Cat#600-401-379, RRID: AB_2209751
 Goat anti-rabbit IgG(H+L) Alex 488 Thermo Fisher Cat#A-11070, RRID: AB_142134
 Goat anti-rabbit IgG(H+L) Alex 568 Thermo Fisher Cat#A-11011, RRID: AB_143157
 Goat anti-rabbit IgG(H+L) Alex 546 Thermo Fisher Cat#A-11071, RRID: AB_2534115
 anti-IL-2 Alex 647 (clone JES6-5H4) BioLegend Cat#503814, RRID: AB_493517
 anti-TGFβ (clone 1D11) Thermo Fisher Cat#MA5-23795, RRID: AB_2609812
 anti-IL-2 (clone JES6-5H4) BioXcell Cat#BE0042, RRID: AB_1107703
 anti-IL-2 (clone JES6-1A12) BioXcell Cat#BE0043, RRID: AB_1107702
 anti-IL-2 (clone S4B6-1) BioXcell Cat#BE0043-1, RRID: AB_1107705

Validation

All antibodies are commercially available and have been validated by the manufacture or in previous reports.

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

Mouse: CD45.1+/B6, Charles River #564
 Mouse: CD45.2+/B6, Charles River #027

Mouse: CBA/J, Jackson Laboratory #000656
 Mouse: B6/CBA/J, this paper
 Mouse: Rag-GFP, Yu et al., 1999
 Mouse: hBcl-2Tg, Sentman et al., 1991
 Mouse: Rosa26tdTomato (Loxp-STOP-Loxp), Jackson Laboratory #007908
 Mouse: IL2-/-, Jackson Laboratory #002229
 Mouse: AND TCRTg, Kaye et al., 1989
 Mouse: POCtg, Oehen et al., 1996
 Mouse: OTII TCRTg, Jackson Laboratory #004194
 Mouse: RIP-mOVA, Jackson Laboratory #005431
 Mouse: ZAP70-/-, Jackson Laboratory #004225
 Mouse: ZAP70Tg, this paper
 Mouse: ZAP70-/-ZAP70Tg (ZAP70TgKO), this paper
 Mouse: ZAP70+/-ZAP70Tg (ZAP70TgWT), this paper
 Mouse: E8III-Cre, Park et al., 2010
 Mouse: Nur77-GFP, Jackson Laboratory #016617
 Mouse: IL2rgfl/fl, McCaughy et al., 2012
 Mouse: IL2rgfl/flE8III-Cre (gockO), this paper
 Mouse: IL2rgfl/flhBcl-2TgE8III-Cre (gockOhBcl-2Tg), this paper
 Mouse: Tgfb1fl/fl, Jackson Laboratory #028701
 Mouse: Tgfb1fl/flE8III-Cre (TGFB1dKO), this paper
 Mouse: IL2-/-Tgfb1fl/flE8III-Cre (IL-2KOTGFB1dKO), this paper
 Mouse: IL2rgfl/flTgfb1fl/flE8III-Cre (gockOTGFB1dKO), this paper
 Mouse: hBcl-2TgTgfb1fl/flE8III-Cre (hBcl-2TgTGFB1dKO), this paper
 Mouse: IL2rgfl/flhBcl-2TgTgfb1fl/flE8III-Cre (gockOhBcl-2TgTGFB1dKO), this paper
 Mouse: Rag-/-, Jackson Laboratory #008449
 Mouse: Cd25-/-, Jackson Laboratory #002462
 Mouse: Cd28-/-, Shahinian et al., 1993
 Mouse: B7DKO, Borriello et al., 1997
 Mouse: Socs1-/-Ifng-/-, Marine et al., 1999
 Mouse: Foxo1fl/fl, Jackson Laboratory #024756
 Mouse: Foxo3fl/fl, Jackson Laboratory #024668
 Mouse: Foxo1fl/flFoxo3fl/flE8III-Cre (FoxodKO), this paper
 Mouse: Foxo1fl/flFoxo3fl/flhBcl-2TgE8III-Cre (hBcl-2TgFoxodKO), this paper
 Mouse: Foxp3-RFP backcrossed to B6, Jackson Laboratory #008374
 Mouse: Foxp3-RFP backcrossed to Rag-GFP, this paper
 Mouse: Foxp3-RFP backcrossed to Nur77-GFP, this paper
 Mouse: Foxp3-GFP backcrossed to B6, Bettelli et al., 2006
 Mouse: Foxp3-GFP backcrossed to B7DKO, this paper
 Mouse: Foxp3-GFP backcrossed to hBcl-2Tg, this paper
 Mouse: Foxp3-GFP backcrossed to FoxodKO, this paper
 Mouse: Foxp3-GFP backcrossed to hBcl-2TgFoxodKO, this paper
 Mouse: Foxp3-GFP backcrossed to TGFB1dKO, this paper
 Mouse: Foxp3-GFP backcrossed to hBcl-2TgTGFB1dKO, this paper
 Mouse: IL-2-Cre, this paper
 Mouse: IL-2-CreRosatdTomato, This paper
 Mouse: IL-2-CreRosatdTomato backcrossed to Rag-GFP, this paper
 Mouse: IL-2-CreRosatdTomato backcrossed to Foxp3-GFP, this paper
 Mouse: IL-2-CreRosatdTomato backcrossed to ZAP70TgKO, this paper
 Mouse: IL-2-CreRosatdTomato backcrossed to Cd28-/-, this paper
 Mouse: IL-2-CreRosatdTomato backcrossed to TGFB1dKO, this paper
 Mouse: B6/CBA/J + ZAP70TgKO to B6/CBA/J F1 mixed chimera, this paper
 Mouse: AND.H-2bkPCC-Rag+/-, this paper
 Mouse: AND.H-2bkPCC+Rag+/-, this paper
 Mouse: OTII.H-2bkOVA-Rag+/-, this paper
 Mouse: OTII.H-2bkOVA+Rag+/-, this paper

Wild animals

No wild animals were used in this study.

Reporting on sex

Both male and female mice were used and analyzed at age 6-10 weeks old. Animals that develop autoimmune disease were used at 3-4 weeks before the onset of disease.

Field-collected samples

No field collected samples were used in this study.

Ethics oversight

All animal experiments were approved by the National Cancer Institute Animal Care and Use Committee and were maintained in accordance with US National Institutes of Health guidelines.

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	Single cell suspension were prepared in cold HBSS supplemented with 0.5% BSA and 0.5% NaN ₃ .
Instrument	LSRII, LSRFortessa, FACSAria Fusion(Becton Dickinson).
Software	FlowJo 10.6.0
Cell population abundance	>95% on sorted cells, which was determined by flow cytometry analysis on post sorted cells.
Gating strategy	Live cells were defined by FSC gating and staining with propidium iodide or LIVE/DEAD Fixable Aqua Dead Cell Stain Kit. All gating strategies are stated in the manuscript.

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