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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 Authors & Referees 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<br>*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<br>*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 | Flowcytometry data were collected using BD FACSDIVA V8.0.1.<br>Imaging data were collected using FV10-ASW V3.1.<br>Protein purification data were collected using UNICORN V7.0. |
| Data analysis | Flowcytometry data were processed and analyzed using FlowJo V10.<br>Imaging data were processed and analyzed using Imaris V7.6.5 and ImageJ 1.52.<br>Protein purification data were processed and analyzed using UNICORN V7.0 |

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/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

Data generated during this study are included in this published article (and its supplementary information files) or available from the corresponding author upon reasonable request.

# 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 | The sample size for each experiment is indicated in the figure legend. The sample size was chosen empirically to provide a sufficient level of statistical power for detecting indicated biological effects. No statistical methods were used to pre-determine the sample size. |
| Data exclusions | No data were excluded from analyses. |
| Replication | All experimental findings were reproducible across multiple independently repeated experiments, as indicated. |
| Randomization | Where applicable, littermate animals of desired genotypes were randomly assigned into experimental and control groups. |
| Blinding | Imaging analyses were conducted in a blinded manner. |

# 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 |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | Antibodies included BV421-anti-CD4 (GK1.5, 562891), APC-Cy7-anti-CD19 (1D3, 557655), PE-Cy7-anti-CD95 (Jo2, 557653), AlexaFluor-700-anti-B220 (RA3-6B2, 557957), eFlour450-anti-B220 (RA3-6B2, 558108), APC-anti-CD138 (281-2, 558626) from BD Biosciences; eFlour450-anti-GL7 (GL-7, 48-5902-82) and FITC-anti-GL7 (GL-7, 53-5902-82) from eBioscience; PerCP Cy5.5 anti-IgD (HK1.4, 405709), biotinylated anti-CCR7 (4B12, 120103), and streptavidin-APC (405207) from Biolegend. Antibodies were used at 1:200 dilution for staining cells or tissue sections. |
| Validation | Based on information provided by commercial vendors. |

## Eukaryotic cell lines

Policy information about cell lines

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| Cell line source(s) | A20 and 293T cell lines were originally from the American Type Culture Collection (Manassas, VA, USA). |
| Authentication | No specific procedure was taken to authenticate the cell line identity. |
| Mycoplasma contamination | The cell lines have been tested negative for mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No cell lines used are in the database of commonly misidentified cell lines. |

## Animals and other organisms

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

| | |
|---|---|
| Laboratory animals | C57BL/6 (Jax664), µMT (Jax2288), GFP-expressing (Jax4353), CFP-expressing (Jax 4218), dsRed-expressing (Jax 6051), OVA323–339-specific T-cell receptor transgenic OT-II (Jax 4194), and HEL-specific Ig-transgenic MD4 (Jax2595) mice were originally from the Jackson Laboratory. GPR174-deficient mice were generated by standard gene targeting procedures to replace the Gpr174 open reading frame with a lacZ/neo cassette using 129SvEvBrd embryonic stem cell line (Texas A&M Institute for Genomic Medicine, TG0128). These mutant mice were backcrossed to C57BL/6 for 12 generations. Relevant mice on the C57BL/6 background were interbred to obtain GFP-expressing MD4 mice and dsRed-expressing GPR174-sufficient or -deficient dsRed MD4 mice. Age-matched littermates between 6 and 12 weeks of age and of indicated sexes were used for experiments. All mice were maintained under specific-pathogen free conditions and were used in accordance of governmental and Tsinghua guidelines for animal welfare. |
| Wild animals | The study did not involve wild animals. |
| Field-collected samples | The study did not involve samples collected from the field. |
| 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.

## 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 | Spleen and LN single-cell suspension was incubated in MACS buffer (phosphate-buffered saline (PBS) supplemented with 1% fetal bovine serum (FBS) and 5 mM EDTA) containing 20 µg/ml 2.4G2 (BioXcell) for 20 min before being stained with indicated monoclonal antibodies. |
| Instrument | LSR II or FACSAria III cytometer (BD Biosciences) |
| Software | Flowcytometry data were processed and analyzed using FlowJo V10. |
| Cell population abundance | At least 10000 events were acquired for cells in the defined gate. |
| Gating strategy | For all experiments FSC-A/ SSC-A gates of the starting cell population were used to identify viable cells. Singlet cells were identified using FSC-H/ FSC-W gating. Isotype control was used to distinguish between background and marker-positive events. |

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