nature portfolio

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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	Seahorse Wave Analyzer Software v2.6 - Seahorse. XFe96 Analyzer BD FACS Diva v8.01 - BD LSR II and Fortessa X20 (4-laser) flow cytometer Attune NxT software - Invitrogen Attune NxT Flow Cytometer MESO QuickPlex SQ 120 reader - p-S65-Ub ELISA JEM1400 plus transmission electron microscope - TEM analysis SpectraMax Plus Microplate Reader - ELISA Olympus DP80 digital microscope - Immunofluorescence Appliedbiosystems QuantStudio 3 -qRT-PCR Azure biosystems 300Q - Immunoblot

Data analysis	Zeiss ZEN (blue edition) v3.4 - Image analysis Flow Jo v10 - Flow cytometry Seahorse Wave Desktop Software v2.6 - Seahorse analysis ImageJ v1.53t - Image analysis Prism v10.0 for statistical analysis Bulk RNA sequencing data was processed using R v4.2.1, GSEA 4.3.2. Public single cell RNA sequencing data were annotated and aligned using CellRanger (v3.0.0), and analyzed using R 4.1.0, with the following packages: Seurat (v4).

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

For the Single cell RNA sequencing data, we obtained the data from a public database ArrayExpress (E-MTAB-9478), and further analyzed the data.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

Life sciences study design

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

Sample size	Sample size was not pre-determined by any statistical methods. In each experiment, at least two biological replicates were analyzed, and similar to those reported in previous publications (PMID: 33406440, PMID: 27637146). There were some exceptions. In Fig.3F, the sample size n = 2 for CreERRptorfl/fl due to the limited mice available for this experiment.
Data exclusions	Mice with complete absence of germinal centers and lack of Alum spots after immunization were considered as failed intraperitoneal immunization, and the mice with assay values fell below the detection limit, were excluded from analysis.
Replication	All experiments included biological replicates. Each experiment reflects at least two independent replicates.
Randomization	For in vivo experiments we matched the sex and age of the mice in experimental batches. To minimize the cage effect and litter effect, we co-house control and experimental mice and use littermate control when it was possible.
Blinding	In all experiments, data collection and analysis were not conducted in a blinded manner, in accordance with standard practices in the field.

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
☒	☐	Plants

Methods

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

Antibodies

Antibodies used

Flow cytometry antibodies
Antibody Clone Manufacturer Cat no: Dilution
Alexa Fluor488 anti-mouse IgG1 RMG1-1 Biolegend 406625 1:200
PE/Cy7 anti-mouse CD19 ID3 Biolegend 115520 1:300
PerCP/Cy5.5 anti-mouse CD19 6D5 Biolegend 115534 1:200
BV605 Anti-mouse B220 RA3-6B2 Biolegend 103244 1:300
FITC anti-mouse CD4 GK1.5 Biolegend 100510 1:300
BV711 anti-mouse CD138 281-2 Biolegend 142519 1:300
APC/Cy7 anti-mouse IgD 11-26c.2a Biolegend 405715 1:400
APC anti-mouse IgG1 RMG1-1 Biolegend 406609 1:200
PE/Cy7 anti-mouse CD86 GL-1 Biolegend 105014 1:300
PE/Cy7 anti-mouse CD21/CD35 7E9 Biolegend 123420 1:500
PE anti-mouse CD98 RL388 Biolegend 128208 1:500
APC anti-mouse CD45.1 A20 Biolegend 110714 1:400
Pacific Blue anti-mouse GL-7 GL7 Biolegend 144614 1:300
BV605 Anti-mouse CD25 PC61 Biolegend 102036 1:300
FITC anti-mouse Ly-51 6C3 Biolegend 108305 1:200
FITC anti-mouse CD23 B3B4 Biolegend 101606 1:200
PE anti-mouse CD45.2 104 Biolegend 109808 1:300
PE/Cy7 anti-mouse CD24 M1/69 Biolegend 101822 1:1000
Alexa Fluor488 anti-mouse CD278 C398.4A Biolegend 313514 1:200
PE anti-mouse CD93 AA4.1 Biolegend 136503 1:300
APC-eFluor 780 anti-mouse TCR beta H57-597 ThermoFisher 47-5961-82 1:300
PE-Cy7 anti-mouse PD1 J43 ThermoFisher 25-9985-82 1:400
FITC Anti-mouse IgA mA-6E1 ThermoFisher 11-4204-82 1:200
APC anti-mouse CXCR4 2B11 ThermoFisher 17-9991-82 1:100
eFluor 450 anti-mouse IgM II/41 ThermoFisher 48-5790-82 1:200
PE-Cyanine7 Ki-67 SolA15 Thermo Fisher 25-5698-82 1:500
PE Anti-mouse CD43 S7 BD Biosciences 553271 1:300
Biotinylated anti-mouse CXCR5 2G8 BD Biosciences 551960 1:100
BV421 Anti-mouse/human Bcl6 K112-91 BD Biosciences 563363 1:50
AF647 anti-mouse CD95 Jo2 BD Biosciences 563647 1:300
APC anti-mouse IL-7RA A7R34 Tonbo Bioscience 20-1271-U100 1:200
Brilliant Violet 421™ anti-mouse CD107a 1D4B Biolegend 121618 1:100
Alexa Fluor® 700 anti-mouse/human CD45R/B220 RA3-6B2 Biolegend 103232 1:300
Alexa Fluor® 488 anti-mouse/human GL7 GL7 Biolegend 144611 1:500
Alexa Fluor® 647 anti-mouse CD98 RL388 Biolegend 128210 1:500
APC anti-mouse CD71 R17217 (RI7 217.1.4) ThermoFisher, 17-0711-82 1:500
FITC Anti-Mouse CD45.2 104 Cytek 35-0454-U100 1:300
PE Phospho-S6 Ribosomal Protein (Ser235/236) D57.2.2E Cell Signaling 5316S 1:400
Alexa Fluor® 647 Phospho-4E-BP1 (Thr37/46) 236B4 Cell Signaling 5123s 1:200
PGC1 alpha Antibody N/A Novusbio NBP1-04676 1:200
Recombinant Anti-mtTFA antibody EPR23548-120 Abcam ab252432 1:500
Alexa Fluor® 647 Anti-MTCO1 antibody 1D6E1A8 Abcam ab198600 1:200
Alexa Fluor® 488 Donkey anti-rabbit IgG (min.x-reactivity) N/A Biolegend 406416 1:500
Alexa Fluor® 647 Donkey anti-rabbit IgG (min.x-reactivity) N/A Biolegend 406414 1:500
Ghost Dye™ Violet 510 N/A Cytek 13-0870-T500 1:1000
NP-PE N/A Biosearch N-5070-1 1:200

Antibody for immunofluorescence

Antibody	Clone	Manufacturer	Cat no:	Dilution
Alexa Fluor® 647 anti-mouse IgD	11-26c.2a	Biolegend	405708	1:100
Alexa Fluor® 594 anti-mouse CD21/CD35	7E9	Biolegend	123426	1:200
Streptavidin, Alexa Fluor™ 488 conjugate	N/A	ThermoFisher	S32354	1:1000
Biotinylated Peanut Agglutinin (PNA)	N/A	Vector laboratories	B-1075	1:1000

Immunoblot antibody (1:1000 dilution except Alpha Tubulin and Lamin B1 were diluted at 1:5000)

Antibody	Clone	Manufacturer	Cat no:
Phospho-4E-BP1 (Thr37/46) () Rabbit mAb	236B4	Cell signaling	2855s
Phospho-S6 Ribosomal Protein (Ser235/236)	D57.2.2E)	Cell signaling	4858s
Phospho-p70 S6 Kinase (Thr389) Antibody	N/A	Cell signaling	9205s
LAMP1 (C54H11) Rabbit mAb	C54H11	Cell signaling	3243S
RagA (D8B5) Rabbit mAb	D8B5	Cell signaling	4357S
Raptor (24C12) Rabbit mAb	24C12	Cell signaling	2280S
AID Monoclonal Antibody	mAID-2	ThermoFisher	14-5959-80
Anti-TFE3 antibody produced in rabbit	N/A	Sigma	HPA023881
TFEB Antibody	N/A	Bethyl Laboratories	A303-673A-T
Alpha Tubulin Polyclonal antibody	N/A	Proteintech	11224-1-AP
Lamin B1 Monoclonal antibody	3C10G12	Proteintech	66095-1-Ig

Validation

All antibodies employed in this study have been validated by commercial vendors. Specification sheet containing technical usage information and QC data for each antibody clone can be obtained from the manufacturer's website by searching for the associated catalog number. Further information can be obtained from the vendor's websites:
https://www.bdbiosciences.com/en-gb/products/reagents/flow-cytometry-reagents/research-reagents/quality-and-reproducibility
https://www.biolegend.com/en-us/quality/quality-control
https://www.abcam.com/primary-antibodies/a-guide-to-antibody-validation
https://www.ptglab.com/
https://www.cellsignal.com/learn-and-support/videos-and-webinars/cst-antibody-validation-documentary?gclid=CjwKCAiAmJGgBhAZEiwA1JZolqXP7c01OXJ8shE-lMEmfmQIzVesF22qol99dmdWL4DEEgZACFKj5hoCyLsQAvD_BwE&gclsrc=aw.ds

The specificity of the antibody staining was experimentally assessed and validated using single-stain or unstained controls.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The retroviral packaging Plat-E cells were a gift from Dr. Hongbo Chi (St. Jude Children’s Research Hospital) and from female origin

Authentication

Plat-E cell line was not authenticated.

Mycoplasma contamination

The cell lines were not tested for mycoplasma in our lab.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines used in this study

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

Mice were maintained in Mayo Clinic animal facility under specific pathogen-free conditions with food and water in 12-hour dark-night cycles, in compliance with Mayo Clinic Institutional Animal Care and Use Committee guidelines and the protocol number is A00003354-18-R23. ROSA26-Cre-ERT2, Rragafl/flRragbfl/fl, Rptorfl/fl, Tfebfl/fl, Tfe3–/– , CD45.1+ (RRID: IMSR_JAX:002014), C57BL/6J (RRID: IMSR_JAX:000664), and Rag1–/– (RRID: IMSR_JAX:002216), B6.129S2-Ighmtm1Cgn/J (RRID:IMSR_JAX:002288) and B6.129P2-Aicdatm1(cre)Mnz/J (RRID:IMSR_JAX:007770) were used in this study. And the mice were analyzed at the ages between 8 and 12 weeks as described in the manuscript.

Wild animals

No wild animals were used in this study.

Reporting on sex

Mice of both sexes were used.

Field-collected samples

No field collected samples were used this study.

Ethics oversight

All experiments were performed as outlined in protocol A00003354-18 approved by Mayo Clinic Institutional Animal Care and Use Committee.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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

Briefly, cells from spleens, mesenteric lymph nodes, and Peyer's patches were harvested by mashing with the syringe plunger on 70 μm cell strainers in cold preparing buffer (2% FBS in PBS). RBCs were depleted by incubating splenocytes with ACK Lysis Buffer (Gibco) for 1-2 mins at room temperature (20°C). Single cell suspensions were incubated with Fixable Viability Dye eFluor™ 510 (eBioscience) in PBS, followed by surface antibodies (30 mins on ice) in FACS buffer (PBS supplemented with 1% BSA). For intracellular staining, cells were fixed at RT with fresh True-Nuclear™ Transcription Factor Fix working solution. (Biolegend, Cat # 424401) for 30 min, and washed twice with True-Nuclear™ 1X Perm Buffer (Biolegend, Cat # 424401). Antibodies were prepared in the 1X Perm Buffer, and the cells were incubated with the antibodies at room temperature for at least 30 minutes in the dark. For LAMP1 staining, cells were fixed using Fixation/Permeabilization solution (BD, Cat # 554714) for 20 minutes at 4°C, followed by washing with 1× BD Perm/Wash™ buffer (BD, Cat # 554714)for twice. LAMP1 antibody was prepared using 1× BD Perm/Wash™ buffer, and cells were incubated with the antibody at 4°C for 30 minutes in the dark. For the phosflow staining, cells were fixed with 1X BD Phosflow™ Lyse/Fix Buffer (BD, Cat # 558049) in the water bath at 37°C for 10 minutes in the dark, followed by personalizing with cold BD Phosflow™ Perm Buffer III (BD, Cat # 558050)at 4°C for 30 minutes in the dark. Cells were incubated with the antibodies prepared in the FACS buffer after two washes with FACS buffer. For the indicators for mitochondrial membrane potential (TMRM, MTDR), mitochondrial ROS (MitoSox), and reactive oxygen species (CellROX) staining, cells were stained with viability dye and indicators (5 μM) at 37°C for 20 minutes in the dark, followed by surface staining at 4°C for 30 minutes in the dark. After antibody staining, cells were washed twice and resuspended in FACS buffer. Flow cytometry was performed on Invitrogen Attune NxT Flow Cytometer, BD Fortessa X-20 or LSR II instruments (both BD).

Instrument

Samples were collected using Attune NxT Flow Cytometer or BD LSR II and Fortessa X20 (4-laser) flow cytometer

Software

Data was collected using BD FACS Diva v8.01, Attune NxT software and analyzed with FlowJo v10 (Tree Star)

Cell population abundance

Cell Sorting

Total B cells were isolated using the EasySep Mouse B Cell Isolation Kit (Stemcell Technologies, Cat # 19854) from the spleens (B cell frequency: 10%-30%), and Purity validated by flow cytometry was >95%.

For bulk RNA sequencing analysis of the GC B cells, B cells from the spleens of immunized mice were enriched with EasySep Mouse B Cell Isolation Kit (Stemcell Technologies, Cat # 19854), then the GC B cells (Fas+GL7+, Post-sort frequency: >95%) cells were sorted from the enriched B cells (GC B cells, Pre-sort frequency: ~10%) using BD FACSMelody. For Vector, WT TFEB or Ca TFEB transduced B cells sorting, the pre-sort GFP frequency was 50%, 50%, 30% respectively, and the GFP+ cells were sorted out using BD FACSMelody, the post-sort GFP frequencies were all >95%.

Gating strategy

Debris were excluded based on SSC-A vs. FSC-A gating. Doublets were then excluded by plotting FSC-H vs. FSC-A. Dead cells were excluded from analysis by gating on cells negative for the live/dead dye staining.

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