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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	*Provide a description of all commercial, open source and custom code used to analyse the data in this study, specifying the version used OR state that no software was used.*

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 upon request. 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 RNA-seq and DNA methylation data have been deposited Gene Expression Omnibus (GEO) under accession code GSE120452. All other data from the manuscript are available from the corresponding author on request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 predetermined for any experiments in the manuscript. We utilized sample sizes that are consistent with recent studies on a similar topic, and made an effort to avoid needless use of animals. In addition, we used statistical analysis consistent with the sample size of each experiment.
Data exclusions	We performed outlier tests on each data set using the ROUT method (can detect any number of outliers) with a Q value=1% using graphpad prism. Only samples flagged as outliers using this criteria was removed from the analysis.
Replication	All attempts at replication were successful.
Randomization	Experimental animals were always allocated along with a litter-mate control. If no litter-mate control was available animals were not used.
Blinding	Blinding was not relevant in this study, as groups consisted of previously genotyped mice in order to have correct experimental and control groups.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	For flow cytometry. The antibody target and clone number are provided in the methods, and specific fluorochrome color are labeled in the figures. We did not provide the company and catalog number for antibodies in the methods because antibodies were purchased from multiple suppliers (Tonbo, BD Biosciences, eBioscience, and Biolegend) if the clone was available from different companies in order to minimize costs. All antibodies from each supplier were tested prior to usage for analysis. Antibodies were titrated for proper amount based on manufactures instructions.
Validation	For flow cytometry. The antibody target and clone number are provided in the methods, and specific fluorochrome color are labeled in the figures. We did not provide the company and catalog number for antibodies in the methods because antibodies were purchased from multiple suppliers (Tonbo, BD Biosciences, eBioscience, and Biolegend) if the clone was available from

different companies in order to minimize costs. All antibodies from each supplier were tested prior to usage for analysis. Antibodies were titrated for proper amount based on manufactures instructions.

Animals and other organisms

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

Laboratory animals	C57BL/6 Uqcrfsfl/fl (RISPfl/fl) are previously described1. C57BL/6 mice harboring a loxp flanked exon 1 of the Uqcrq gene (encodes QPC, QPCfl/fl mice) were generated by Ozgene (Locus map in Extended Data Figure 2a). The frt-flanked neomycin resistance cassette was removed by crossing QPCfl/fl mice to mice expression FLP-recombinase (FLPo Mice Jackson Lab, Stock no. 011065). Loss of Neo-cassette was confirmed using PCR. QPCfl/fl mice were genotyped using the following primers: 1. 5'-CTTCCGCTCCTCCCGGAAGT-3', 2. 5'-TTCCCAAACTCGCGGCCCATG-3' and 3. 5'-CAATTCCAGCCAACAGTCCC-3' which allow identification of the Uqcrq wild-type, loxp-flanked and excised alleles. RISPfl/fl and QPCfl/fl mice were checked for C57BL/6 status and both are >99% C57BL/J using the genome scanning service from Jackson Laboratories. Foxp3YFP-Cre (stock no. 016959), Foxp3eGFP-Cr-eERT2 (stock no. 016961), and ROSA26SorCAG-tdTomato (Ai14, stock no. 007908) mice were obtained from Jackson Laboratory. Rag-1-deficient animals where acquired from Jackson lab (Cat# 002216) and colitis was induced based on the protocol described by Workman and collegues. Age of mice that were used in study is described in figure legends for each experiment. In brief, male and female experimental and control matched littermate animals were used at 10 days postnatal, 21 days postnatal, and as adults 8-12 weeks of age.
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*

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, lymph nodes (always included all superficial and periaortic lymph nodes) and thymus were harvested from 10-day post-natal, 3-week-old and adult (8-12-week-old) mice. To obtain a single-cell suspension, tissues were disrupted using scored 60mm petri dishes in PBS containing 2% FBS and filtered through a 70µM nylon mesh filter. Total spleen, lymph node and thymus counts were obtained using the Cellometer K2 Counter using AOPI stain (Nexcelom). Single cell suspensions were stained with antibodies against CD45.2 (Clone: 104), CD4 (Clone: RM4-5), CD8 (Clone: 53-6.7), CD25 (Clone: pc61.5), CD44 (Clone: IM7), CD62L (Clone: MEL-14) to obtain absolute cell numbers. For intracellular protein expression, samples were fixed using the Foxp3/Transcription Factor Staining Buffer Set (Invitrogen Catalog no. 00-5523-00) according to the manufactures instructions and stained with FOXP3 (Clone: NRRF-30), HELIOS (Clone: 22F6), EOS (Clone: ESB7C2) and Ki-67 (Clone: SalA15). CD4+ T cells were purified from single cell suspension using the EasySepTM mouse CD4+ T cell isolation Kit (Stemcell, Catalog no. 19852) according to the manufactures instructions. CD4+ T cells were further stained with antibodies against CTLA-4 (Clone: UC10-4B9), GITR (Clone: DTA-1), ICOS (Clone: C398.4A), OX40 (Clone: OX-86), CD69 (Clone: H1.2F3), CD103 (Clone: 2E7), NRP1 (Clone: 3DS304M), CD73 (Clone: TY/11.8), PD-1 (Clone: 29F.1A12), and TIGIT (Clone: GIGD7).
Instrument	BD LSR Fortessa or BD FACSymphony
Software	BD FASC Diva was used for collection of the data. All data was analyzed using FlowJo software.
Cell population abundance	An aliquot of the sorted cells were always collected and run on a cytometer to verify purity of the samples collected. In addition, cell counts were performed on samples post sort to verify correct cell numbers.
Gating strategy	CD4+ cells were identified from whole spleen and lymph nodes first by discrimination of cells by size (FSC-A by SSC-A). Singlets were then distinguished using FSC-A by FSH-H. CD45+ Live cells were further gated for using a Ghost Dye (Tonbo) versus CD45. Following this CD4 cells were discriminated by CD8 versus CD4. Once at this point CD4+ Foxp3+ (or Foxp3-YFP+) CD25+ cells were gated as shown in Figure 1. The same strategy was used for samples that had previously undergone depletion of non-CD4+ cells except the live dead discrimination was used versus CD4 staining. Representative Tdtomato-RFP gating verses Foxp3-GFP is shown in extended data figure 4.

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