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	Open Source: Micro-manager 1.4. Commercial: LI-COR Odyssey and Amonlite 2.0.
Data analysis	Open Source: Eclipse 4.19 and ImageJ 1.53. Commercial: GraphPad Prism 9 and Imaris 9.2.

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

All data will be made available upon request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	N/A. No statistics were estimated from human clinical samples.
Population characteristics	N/A. No statistics were estimated from human clinical samples.
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	In most cases sample variance was difficult to estimate a priori. Consequently, sample sizes of five or greater were typically chosen. Formal a priori power analysis was not conducted.
Data exclusions	Images and video recording were excluded only if the quality prohibited quantification using our chosen method.
Replication	Individual experiments were always reproduced at least in triplicate. Experimental cohorts were not replicated.
Randomization	Animals were selected based on litter mate pair availability. Genotype groups were assigned after experiments based on PCR identification.
Blinding	Those collecting data were binded to group identity. Data collection and quantification were performed by different individuals. Analysis metadata (typically segmented regions) used for the quantification of acquired data was archived and independently inspected for quality.

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	A comprehensive list is included in methods section of the manuscript (see Tables 1 and 2).
Validation	Western blots were used to validate antibodies against their intended target proteins.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	ATCC CRL-2299 (bEnd3) and HEK293
Authentication	Both cell lines used were ordered directly from a trusted provider. bEnd3 validated only by resistance in TEER assay.
Mycoplasma contamination	Yes, tested with PCR.
Commonly misidentified lines (See ICLAC register)	HEK293 were used only for protein production. Protein concentration and activity were independently determined.

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	C57BL6 wild-type mice or transgenic mice on a C57BL6 background were used for all experiments.
Wild animals	N/A. Only laboratory derived animals were used in this study.
Reporting on sex	Both male and female were used in all experimental groups with the exception of our experimental autoimmune encephalomyelitis (EAE) mice which were all female. We did not analyze the effect of biological sex as it was not expected to be a significant factor with respect to BBB in our animal models.
Field-collected samples	N/A. No samples were collected outside of the laboratory or outside of a pathologists clinic.
Ethics oversight	Animal Care Committee (University Health Network)

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration	N/A. This study is not directly affiliated with any clinical trial.
Study protocol	N/A. This study is not directly affiliated with any clinical trial.
Data collection	Postmortem tissue collected by pathologists.
Outcomes	N/A. No outcomes were tracked for the purposes of this study.

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	Described in first paragraph of methods section "Flow Cytometry and Intracellular Cytokine Staining".
Instrument	BD LSRII or FACS Cantoll cytometer (BD Biosciences).
Software	Flowjo software (Tree Star Inc.). No custom code was used.
Cell population abundance	Analysis of naive cells, 5x10^5 RBC depleted splenocytes and lymph node cells. For each experiment, at least 100,000 live events were acquired and analyzed.

Gating strategy FSC-A/SSC-A : 25-70K/0-45K; SSC-W/SSC-A : 50-90K/10-110K; PI/FSC-A : 0-11K/0-100K

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