nature portfolio

Corresponding author(s): Zsuzsanna Fabry
zfabry@wisc;edu

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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	The following software were used for data collection: FV10-ASW v4.2 Image Studio Software v5.2.5 BD FACSDiva v6.1.2 Aurora Cytek's Spectroflo
Data analysis	The following software were used for data analysis: FIJI Image Processing Software (NIH) FlowJo Version (v) 10 (TreeStar) Graphpad Prism v6 (GraphPad Software Inc) Excel 16.15 Image Studio Software v5.2.5

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 supporting the findings of this study are 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	Sample size was chosen in accordance with similar published experiments by other independent groups as well as our lab.
Data exclusions	No data were excluded in our experiments.
Replication	All experiments were performed using multiple animals for each condition. Any experiments that were not replicated were validated using an alternative system. For example, in vitro co-cultures of T cell activation were validated using multiple systems and antigens (MOG antigen + 2D2 T cells or brain-derived T cells, OVA antigen + OT-II T cells).
Randomization	All animals were randomly assigned to experimental groups.
Blinding	Experimental groups were blinded for all analysis.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

The following antibodies were used for immunohistochemistry: Podoplanin PE (eBioscience, Catalog #: 12-5381-80), CD31 Alexa647 (BD Biosciences, Catalog #: 563608), Lyve-1 eFluor660 (Thermo Fisher Scientific, Catalog #: 50-0443-80), chicken anti-GFP unconjugated (Novus Biologicals, Catalog #: NB100-1614-0.02ml), CNPase Alexa647 (Biolegend, Catalog #: 836407), MHC II eFluor450 (eBioscience, Catalog #: 48-5321-80), CD11c Alexa488 (Thermo Fisher Scientific, Catalog #: 53-0114-80), CD4 PE (BD Pharmingen, Catalog #: 553049), Goat anti-E-Cadherin unconjugated (R&D Systems, Catalog #: AF748), and rabbit anti-AQP-1 unconjugated (EMD Millipore, Catalog #: AB2219).
The following secondary antibodies were used: Donkey anti-Chicken Alexa488 (Invitrogen, Catalog #: A11039), Donkey anti-Chicken Alexa647 (Invitrogen, Catalog #: A21449), Donkey anti-Goat Alexa568 (Invitrogen, Catalog #: A11057), and/or Donkey anti-rabbit Alexa568 (Invitrogen, Catalog #: A10042).
The following antibodies/dyes were used for flow cytometry:Ghost UV450 (Tonbo Biosciences, Catalog #: 13-0868-T500) or Ghost Violet540 (Tonbo Biosciences, Catalog #: 13-0879-T100) to visualize live/dead cells; CD31 Alexa647 (BD Biosciences, Catalog #:

563608), Podoplanin PE (eBioscience, Catalog #: 12-5381-82), and Lyve-1 Alexa488 (eBioscience, Catalog #: 53-0443-82) to visualize lymphatic endothelial cells; CD45 APC-Cy7 (Biolegend, Catalog #: 103116) or CD45 APC eFluor780 (eBioscience, Catalog #: 47-0451-80) to visualize leukocytes; CD11b PerCP-Cy5.5 (Biolegend, Catalog #: 101227) or CD11b PE-Cy5 (Biolegend, Catalog #: 101210) to visualize myeloid cells; CD11c BV421 (Biolegend, Catalog #: 117329) or CD11c FITC (Biolegend, Catalog #: 117305) to visualize dendritic cells; CD8 PE-Cy7 to visualize cytotoxic T cells (Biolegend, Catalog #: 100721); CD4 BUV496 to visualize T helper cells (BD Bioscience, Catalog #: 564667); B220 BV510 to visualize B cells (Biolegend, Catalog #: 103247); PD-L1 PE-Cy7 to visualize the tolerogenic ligand PD-L1 (Biolegend, Catalog #: 124313).

Validation

All antibodies were validated for use in mice and their applications (immunohistochemistry, flow cytometry) by the corresponding manufacturer.

Animals and other organisms

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

Laboratory animals

Female C57BL/6J wild-type (stock #: 000664), IFN-γ-/- mice (stock #: 002287), 2D2 TCR transgenic mice (stock #: 006912), and OT-II TCR transgenic mice (stock #: 004194) were purchased from Jackson Laboratories. CD11c-eYFP transgenic reporter mice were a generous gift from Dr. Michel C. Nussenzweig at Rockefeller University. CNP-Cre transgenic mice were a generous gift from Brian Popco at the University of Chicago. pZ/EG-OP OVA257-264-OVA323-339 (OVAfl/fl mice) were generated by our lab as previously described in the C57BL/6 background. OVAfl/fl mice were crossed with CNP-Cre mice to generate CNP-OVA transgenic mice that express OVA257-264 and OVA323-339 with GFP under the CNS oligodendrocyte-specific CNPase promoter.
Eight to twelve-week old female mice were used for all EAE experiments along with the appropriate age and sex matched controls.

Wild animals

Not Applicable

Field-collected samples

Not Applicable

Ethics oversight

All experiments were conducted in accordance with guidelines from the National Institutes of Health and the University of Wisconsin Madison Institutional Animal Care and Use Committee.

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

Mice were terminally anesthetized with isoflurane and transcardially perfused with ice cold 0.1M PBS (10 mL). Mice were then decapitated and the skin was cut dorsal to the midline of the skullcap rostrally to expose the brain. The skullcap was then removed along with the brain and dura after separation from the olfactory bulbs. The cribriform plate and its associated tissues which included the olfactory bulbs, the cribriform plate, and dorsal parts of the nasal mucosa were dissected out and placed in a 70-micron strainer submerged in RPMI-1640 in a non-tissue culture treated dish. The tissues were then mechanically dissociated by pushing the tissue through the strainer using a syringe plunger. The mechanically dissociated cells were then spun down at 1258 RCF at 4 degrees celsius for 7 minutes, washed, and resuspended in FACS buffer (1% Bovine Serum Albumin in 0.1M PBS) for FACS staining. Data was collected using an LSRII (BD Biosciences) or Cytek Aurora (Cytek) and analyzed using FlowJo software (TreeStar). Gating was done as shown in all the figures, and data processing was done with excel and GraphPad Prism. Individual numerical values for percentages can be visualized in the quantitation graphs with statistics.

Instrument

LSR II (BD Biosciences) and Cytek Aurora Northern Lights (Cytek)

Software

FlowJo (TreeStar)

Cell population abundance

For sorting experiments, an average of 1,000 - 5,000 cpLECs were sorted per healthy mouse, and 10,000 - 25,000 per EAE mouse. Dead cells were excluded, and LECs identified as CD45negative/low and Podoplanin/CD31+.

Gating strategy

The gating strategies for all experiments can be seen in the figures.

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

Magnetic resonance imaging

Experimental design

Design type	Resting State
Design specifications	Baseline images were taken under anesthesia (scan time was approximately 11 minutes). Mice were then administered 10 uL of Gadolinium into the cisterna magna at a rate of 2 uL/minute under anesthesia, and underwent 5 sequential 3D T1-weighted scans to visualize Gadolinium distribution for a total time of approximately 55 minutes (approximately 11 minutes per scan).
Behavioral performance measures	Not Applicable

Acquisition

Imaging type(s)	Isotropic 3D T1-weighted scans
Field strength	4.7
Sequence & imaging parameters	s TR = 9.3 ms; TE = 4.7 ms; Flip Angle = 20 degrees; Field of View = 40x20x20 mm; Resolution = 256x128x128; Averages = 4; Voxel Size is approximately equal to 156 microns cubed. These resulted in a time scan of approximately 11 minutes.
Area of acquisition	The ROI selected included the whole-head and neck so that the cribriform plate, subarachnoid space, the brain, and the deep cervical lymph nodes could be visualized.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	Not Applicable
Normalization	Not Applicable
Normalization template	Not Applicable
Noise and artifact removal	Not Applicable
Volume censoring	Not Applicable

Statistical modeling & inference

Model type and settings	Not applicable
Effect(s) tested	Not Applicable

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s)	ROI were selected to encompass the whole-head and neck so that the cribriform plate, subarachnoid space, brain, and deep cervical lymph nodes could be visualized.
Statistic type for inference (See Eklund et al. 2016)	Not applicable
Correction	Not applicable

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis