nature portfolio

Corresponding author(s): Jonathan Kipnis

Last updated by author(s): Jan 20, 2023

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 softwares were used to collect the data in this study:
- SpectroFlo v 2.2.0.3 (Cytek)
- VS200 AWS (Olympus)
- Leica Application Suite v 4.2.1.23810 (Leica)
- Nikon Elements v. 5.20 (Nikon)
- Image Lab v 3.0.1 (Bio-Rad)
- Imspector Pro v 7.0.0 (LaVision)
- Siemens syngo MR E11

Data analysis	The following softwares were used to analyze the data in this study: - FIJI image processing software (NIH) - v 2.14.0/1.54f - Prism v 8.3.0 (GraphPad Software, Inc) - FastQC v 0.11.5 - R v 3.5.0 - RStudio v 1.4.1717 - Bioconductor DESeq2 v3.5 - FlowJo software v 10 (BD Biosciences) - Gen5 software v 3.11 (BioTek) - Napari (v 0.4.17rc8) - ITK-SNAP 4.0.0 beta - Custom code used for single-cell RNA-seq analysis is available from the corresponding authors upon request.

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

snRNA-seq data are available from Gene Expression Omnibus under accession number GSE213895. All data are available in the main text or the supplementary information files.

ISH imaging of Sema3a and Sema3d was accessed through the Allen Brain Atlas ISH portal. https://mouse.brain-map.org/

Human research participants

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

Reporting on sex and gender	Biological sex was used for demographic data in the human healthy participants part of this study. Findings may apply to both sexes since both male and female participants are recruited. Self-reporting method was used to determine sex during participants' recruitment to the study. Sex was not considered in the study design. Sex-based analyses are not performed since sample size of human participants is small (n=10).
Population characteristics	All healthy participants undergo a clinical evaluation during their research participation visits evaluating their current health status, past medical history, and medical exam. None of the participants has chronic disease or is under any treatment regimen, except for one participant who has hyperlipidemia that is controlled with low-dose statins. All demographic data including the age and sex of the patients is included in the supplementary data file.
Recruitment	Participants are recruited from an NIH Clinical Center database containing contact information of individuals applying to NIH for volunteering in NIH research as healthy subjects. Contact information in the database was used to reach out to volunteers and describe the details of the study protocol. Volunteers accepted to participate in the study protocol were enrolled after confirming that they are eligible for 3T MRI scan (no metal implants in the body) and intravenous gadolinium-based contrast agent injection (normal renal function, no history of allergic reaction after MRI-contrast injection). Participant selection was solely based on voluntary participation, and we are unaware of any self-selection bias or other biases present in this cohort that may impact results.
Ethics oversight	National Institutes of Health Institutional Review Board; Imaging and demographic data were collected from 10 healthy volunteers with institutional review board approval and after written, informed consent, as part of the National Institute of Neurological Disorders and Stroke's "Evaluation of Progression in Multiple Sclerosis by Magnetic Resonance Imaging" protocol (NCT00001248).

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	Statistical methods were not used to recalculate or predetermine study sizes but were based on similar experiments previously published (Cugurra et al., Science (2021); Rustenhoven et al., Cell (2021); Mazzitelli et al. (2022)).
Data exclusions	No data were excluded for analysis.
Replication	All experiments were replicated in at least two independent experiments for a total of at least 3 mice per group, and all replication was successful. All representative images are representative of the same experiment performed in at least 3 animals. For in vitro experiments, cells from the same animal were resuspended and plated with treatments and controls in adjacent wells.
Randomization	For all experiments, animals from different cages were randomly assigned to different experimental groups. Because all variables were controlled for, no covariates were present. For in vitro studies, cells from the same animal were harvested and resuspended. Treatments and controls were plated adjacent to one another.
Blinding	For all experiments, the researchers were blinded, where possible, for at least one of the independent experiments. These experiments included tracer studies and experiments using anti-Itga6 treatment. Because EAE causes ascending paralysis it was not possible for researchers to be blinded to disease state in these studies.

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

Flow cytometry
CD45 Rat BV750 30-F11 746947 BD Bioscience 1/200
Ly6G Rat BUV661 N418 741587 BD Bioscience 1/200
Ly6G Rat BV421 N418 Biolegend 1/200
Ly6C Rat PerCP Cy5.5 HK1.4 128011 Biolegend 1/200
Ly6C Rat Alexa Fluor 700 HK1.4 Biolegend 1/200
CD11b Rat BUV563 M1/70 741242 BD Bioscience 1/200
TCR-β A Hamster BUV805 H57-597 748405 BD Bioscience 1/200
CD4 Rat Alexa Fluor 647 RM4-5 100533 Biolegend 1/200
CD8a Rat Alexa Fluor 532 53-6.7 58-0081-80 Invitrogen 1/200
F4/80 Rat BV605 BM8 123133 Biolegend 1/200
F4/80 Rat Alexa Fluor 700 BM8 123130 Biolegend 1/200
CD64 Mouse APC X54-5/7.1 139306 Biolegend 1/200
CD206 Rat Alexa Fluor 488 C068C2 141710 Biolegend 1/200
CD11c A Hamster BUV737 N418 749039 BD Bioscience 1/200
MHC-II Rat APC-Cy7 aSMA 107628 Biolegend 1/1000
Foxp3 Rat PE-eFluor 610 FJK-16s 61-5773-82 Invitrogen 1/200
Ror-gt Mouse BV650 Q31-378 564722 BD Bioscience 1/200
CD38 Rat Alexa Fluor 647 90 102716 Biolegend 1/200
CD19 Rat BV480 1D3 566107 BD Bioscience 1/200

Immunostaining
Aldh1a2 Rabbit - Poly HPA010022 Sigma 1/1000 FFPE, cryosections
Aquaporin-4 Rabbit - Poly A5971 Sigma 1/1000 FFPE, cryosections
CD3 Rat Alexa Fluor 594 17A2 100240 Biolegend 1/200 Cryosections
CD13 Goat - Poly AF2335 R&D Systems 1/2500 FFPE, cryosections
CD45 Rat Alexa Fluor 647 30-F11 103124 Biolegend 1/500 Cryosections
CD206 Goat - Poly AF2535 R&D Systems 1/500 FFPE, cryosections

Claudin-5 Mouse Alexa Fluor 488 4C3C2 352588 Invitrogen 1/100 Methanol fixed, FFPE
Claudin-11 Rabbit - Poly 36-4500 Invitrogen 1/100 Methanol fixed
Collagen I Rabbit - Poly EPR24331-53 Abcam 1/200 FFPE
Crabp2 Rabbit - Poly 10225-1 Thermo Fisher Scientific 1/500 Cryosections
Dpp4 Goat - Poly AF954 R&D Systems 1/1000 FFPE, cryosections
Dpp4 (human) Rabbit - Poly HPA071236 Sigma 1/500 FFPE
E-Cadherin Rat Alexa Fluor 647 DECMA-1 147308 Biolegend 1/200 Cryosections
E-Cadherin Rabbit - Poly 20874-1-AP Proteintech 1/100 FFPE
EMA (human) Mouse - E29 M061329-2 Dako 1/500 FFPE
Fibronectin Rabbit - Poly ab2413 Abcam 1/500 FFPE
GFAP Rabbit - Poly Z0334 Dako 1/2500 FFPE
GFP Rabbit Alexa Fluor 488 Poly A21311 Thermo Fisher Scientific 1/500 Cryosections
GLUT1 Rabbit - Poly RB9052P1 Thermo Fisher Scientific 1/1000 Cryosections
GR-1 Rabbit - Rat RB6-8C5 Thermo Fisher Scientific 1/500 Cryosections
Iba1 Goat - Poly ab7076 Abcam 1/100 FFPE
Laminin Rabbit - Poly ab11575 Abcam 1/500 FFPE, cryosections
Lyve-1 Rat eFluor 660 ALY7 50-0443-82 Invitrogen 1/200 Cryosections
Lyve-1 Goat - Poly AF2125 R&D Systems 1/500 Cryosections, FFPE
Mesothelin Rabbit - Poly PA5-79698 Invitrogen 1/500 FFPE
MHC-II Rat FITC M5/114.15.2 11-5321-82 Invitrogen 1/1000 Methanol fixed
Occludin Mouse Alexa Fluor 594 OC-3F10 331594 Invitrogen 1/200 Methanol fixed
Pecam-1 A hamster - 2H8 MAB1398Z Millipore 1/200 Cryosections
Plvap Rat - MECA-32 553849 BD Bioscience 1/200 Cryosections
Podocalyxin Goat - Poly AF1556 R&D Systems 1/1000 FFPE, cryosections
Podoplanin Rat Alexa Fluor 488 8.1.1 35-5381-82 Invitrogen 1/200 FFPE
S100A8 Goat - Poly AF3059 R&D Systems 1/2000 Cryosections
Slc6a13 Rabbit - Poly AB1572 Millipore 1/200 FFPE
Slc38a2 Rabbit - Poly HPA035180 Sigma 1/200 FFPE
αSMA Mouse FITC 1A4 F3777 Sigma 1/2500 FFPE, cryosections
Streptavidin - Alexa Fluor 647 - S32357 Thermo Fisher Scientific 1/1000 Cryosections
VE-Cadherin Goat - Poly AF1002 R&D Systems 1/100 FFPE
vWF Rabbit - Poly A008229-5 Dako 1/500 FFPE, cryosections

Validation

Each antibody was validated for the species (mouse or human) and application (immunohistochemistry, flow cytometry) by the correspondent manufacturer. The usage was described in full detail the methods section of the manuscript.

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	All mice were housed and bred in a temperature (22 ºC) and humidity-controlled (33 - 39 %) environment with a 12-hour light dark/ cycle and provided with food and water ad libitum. No more than 5 mice were housed together per cage. Mice were bred in house, purchased from Jackson Laboratories, or Taconic Biosciences. Aged mice were provided by the National Institutes of Aging and used at >20 months of age. All mice were habituated for at least 1 week before experimentation, and used between 2 – 4 months, unless specified otherwise. The following strains were used: C57Bl/6J (JAX000664), Ms4a3-Cre (C57BL/6J-Ms4a3em2(cre)Fgnx/J, JAX:036382), Ai6/LSL-zsGreen (B6.Cg-Gt(ROSA)26Sortm6(CAG-ZsGreen1)Hze/J, JAX007906), Ai9/LSL-tdTomato (B6.Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J, JAX007909), Cdh5-CreERT2 (C57BL/6-Tg(Cdh5-cre/ERT2)1Rha, Taconic 13073 72), Col1a2-CreERT (Tg(Col1a2-cre/ERT,-ALPP)7Cpd/J, JAX029235), Prox1-EGFP (gifted by Y.K. Hong, Division of Plastic and Reconstructive Surgery, Department of Surgery, Keck School of Medicine, University of South California, Los Angeles, CA, USA), Cx3cr1-EGFP (B6.129P2(Cg)-Cx3cr1tm1Litt/J, JAX:005582), Dpp4-CreERT2 and Slc47a1-CreERT2 were generated in house.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex was not considered in study design, however experiments were performed on both sexes and we did not observe sex-specific effects.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All experiments were performed under the approval of the Institutional Animal Care and Use Committee at Washington University in St. Louis (#200-043, #23-0145).

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 ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT00001248
Study protocol	The full protocol is not available, as it covers additional studies beyond the data and analysis presented here. Some of these studies involve confidential disclosure agreements.

Data collection	NIH Clinical Center, data collected between June 2021 and October 2022, 7T images were acquired in February 2022 and March 2023 from the same healthy volunteer.
Outcomes	In this exploratory analysis, our primary focus was on identifying and quantifying bridging veins with "spreading paravascular enhancement" (SPE) following the administration of a gadolinium-based contrast agent (GBCA) over a 4-hour period. We hypothesized that SPE indicates a paravascular gradient of cerebrospinal fluid (CSF) transportation from bridging veins to parasagittal dura. The quantification process involved assessing the contrast enhancement in identified bridging veins, detailed further in the manuscript. Additionally, we explored a secondary outcome to understand relationships between demographic factors and signal changes. Our hypothesis, supported by the absence of GBCA signal changes similar to SPE in lateral ventricles, suggested independence from CSF gradient. Using signal-intensity ratio values, we quantified these changes, enabling a comprehensive analysis of the SPE phenomenon. Comparisons between signal changes in bridging veins with paravascular enhancement and lateral ventricles provided insights into the distribution and characteristics of the observed contrast enhancement. This analysis is exploratory, emphasizing preliminary findings and hinting at potential directions for future hypothesis-driven studies to unravel the implications of these phenomena in health and disease.

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 euthanized by lethal injection of Euthasol (10 % v/v, i.p.) followed by transcardiac perfusion of PBS with heparin (5 U/mL). Tissues were microdissected as described above in ice-cold RPMI and placed into a solution of pre-warmed RPMI supplemented with collagenase VIII (1 mg/mL, Sigma-Aldrich) and DNase I (0.5 mg/mL, Sigma-Aldrich), and FBS (2 %, Gibco) for dissociation. Prior to enzymatic dissociation, brains were chopped with scissors and triturated ten times with a P1000 pipette. Tissues were dissociated for 20 minutes (leptomeninges, dura) and 40 minutes (brains). Tissues were passed through a 70 ?m cell strainer and washed through with one volume of 10 % FBS in RPMI and 1 volume of fluorescence-activated cell sorting (FACS) buffer (2 % BSA, 1 mM ethylenediamine acetic acid (EDTA)) to quench enzyme activity. Brain samples were resuspended in 3 volumes of 22 % (w/v) BSA in PBS and centrifuged at 1000 x g for 10 minutes. Suspensions were transferred to a V-bottom plate and pelleted (450 x g, 5 min). Viability staining was performed using Zombie NIR (1:500 in PBS, BioLegend) for 20 minutes at room temperature. Suspensions were then pelleted and resuspended in anti-CD16/32 antibody (1:100, BioLegend) diluted in FACS buffer to block Fc receptors. Cell surface stains, diluted appropriately in FACS buffer, were then added for 30 minutes on ice. For intracellular staining, suspensions were fixed and permeabilized using the Transcription Factor Fixation/Permeabilization Kit (eBioscience) per the manufacturer's instructions. Antibodies against intracellular proteins, diluted in permeabilization buffer, were added for 30 minutes on ice.
Instrument	Flow cytometry was performed using an Aurora spectral flow cytometer (Cytek Biosciences, CA, USA).
Software	Data were collected on SpectroFlo (v2.2.0.3; Cytek) and analyzed with FlowJo (v10; BD Biosciences, NJ, USA).
Cell population abundance	For each individual experiment, single-cell suspensions were incubated with viability dyes. Positive populations were gated based on negative control staining. In general, populations are given as a percentage of live, CD45+ cells.
Gating strategy	Gating strategies are described in figures. Briefly: Ly6Chi monocytes: Live/CD45+/CD11b+/Ly6G-/F4-80-/CD64-/Ly6C+ OR CD45hi/Ly-6Gint/Ly-6C+ Neutrophils:Live/CD45+/CD11b+/Ly6G+ DCs: Live/CD45+/CD11c+/MHC-II+ B cells: Live/CD45+/CD11b-/Ly6G-/CD19+/TCR-b- T cells: Live/CD45+/CD11b-/Ly6G-/CD19-/TCR-b+ CD4 T cells: Live/CD45+/CD11b-/Ly6G-/CD19-/TCR-b+/CD4+ Th17: Live/CD45+/CD11b-/Ly6G-/CD19-/TCR-b+/CD4+/Ror-gt+/Foxp3- CD8 T cells: Live/CD45+/CD11b-/Ly6G-/CD19-/TCR-b+/CD8+ Macrophages: Live/CD45+/CD11b+/Ly6G-/F4-80+/CD64+/CD206+ CD38+/MHC-II- macrophages: Live/CD45+/CD11b+/Ly6G-/F4-80+/CD64+/CD206+/CD38+/MHC-II- CD38+/MHC-II+ macrophages: Live/CD45+/CD11b+/Ly6G-/F4-80+/CD64+/CD206+/CD38+/MHC-II+ CD38-/MHC-II+ macrophages: Live/CD45+/CD11b+/Ly6G-/F4-80+/CD64+/CD206+/CD38-/MHC-II+ Endothelial cells: Live/CD45-/CD31+ Fibroblasts: Live/CD45-/CD31-/Pdpn+

☒ 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	Contrast-enhanced MRI with gadolinium-based contrast agent (GBCA, gadobutrol)
Design specifications	Each participant underwent 2 sessions of scans. In the first session, pre-GBCA and early time point post-GBCA images were acquired. The second imaging session was done 4 hours after the GBCA administration. 7 participants underwent both sessions in the same day with one time GBCA administration. 3 participants underwent 2 sessions in different days with GBCA administration in each session (48, 42, and 83 days between the 2 sessions).
Behavioral performance measures	Not applicable

Acquisition

Imaging type(s)	Structural MRI
Field strength	3 tesla
Sequence & imaging parameters	See Supplemental Table 4
Area of acquisition	Whole brain field-of-view

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	ITK-SNAP for image coregistration
Normalization	None
Normalization template	Not applicable
Noise and artifact removal	None
Volume censoring	None

Statistical modeling & inference

Model type and settings	None
Effect(s) tested	Paired t-test

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

Anatomical location(s)	Anatomical locations were determined by visual assessments. Vessels on the cortical surface of the brain conjoining to the major venous sinuses were identified as bridging veins. Bridging veins were assessed on pre-GBCA, early time point post-GBCA and late time point post-GBCA. Vessels showing a spreading pattern of contrast enhancement around them over time were identified. Contrast enhancing areas around the vessel that is visible to raters in both time points are identified as region-of-interest (ROI). Hand-traced ROI masks were generating to document ROI size.
Statistic type for inference (See Eklund et al. 2016)	None
Correction	None

Models & analysis

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