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	Confocal imaging: Zen Black (v2.3). qPCR analyses: Roche LightCycler 480 SEM: 3View2 (v3.3.1, Gatan) CLEM: Bigwarp plugin of ICY RNA ISH data: Allen Brain Atlas (developingmouse.brain-map.org/) L/D sort: BD FACSDiva v8.0.1
Data analysis	Data representation: Prism 9.3.1 (GraphPad Software, Inc) imaging analysis: Fiji (1.52p) and Zen Blue (v2.5). Co-loc analyses: QuPath 0.4.0. EM 3D reconstructions: MIB (v2.5) and Imaris (v9.3). qPCR data analyses: qbase+ (v3.2) scRNA-Seq analyses: scater R package (v1.14.6) ; Seurat (v3.1.4) ; DoubletFinder (v2.0.2); clusterProfiler R package (v3.14.3); R environment (v4.0.5); python environment (v3.9.9); BBKNN (v.1.5.1); biomaRt package (v2.46.3); Harmony (v1.0); 10x Cell ranger software (v2.0.1). Custom code and information can be found on our GitHub: https://github.com/VandenbrouckeLab/Base_barrier_cells. An archive of the Github repository can be downloaded from Zenodo: https://doi.org/10.5281/zenodo.10043815.

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 raw sequencing data produced in this publication has been deposited in NCBI's Gene Expression omnibus and is accessible through GEO Series accession number GSE227969 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227969). Previously published single cell datasets were also used, and these are accessible via various repositories or websites: Shah et al. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE100320A), Vanlandewijck et al. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE98816), Desisto et al. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150219), Zeisel et al. (http://mousebrain.org/), Pietilä et al. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227713 , https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE233270 , https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE228882), Dani et al. (https://singlecell.broadinstitute.org/single_cell/study/SCP1365/choroid-plexus-cell-atlas) and Yang et al. (https://twc-stanford.shinyapps.io/scrna_brain_covid19/). All single cell datasets provided in this manuscript can be accessed in an online tool via the following link: https://www.single-cell.be/ChP_base_barrier_cells. Additional supplemental information has been made available at https://doi.org/10.5281/zenodo.14703050.

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	Human samples included are from a 5-week-old female.
Reporting on race, ethnicity, or other socially relevant groupings	A neuropathologist (H.G.W.L.) reviewed the human tissue specimens and recorded non-identifying information.
Population characteristics	A neuropathologist (H.G.W.L.) reviewed the human tissue specimens and recorded non-identifying information. Human brain tissue samples were obtained from autopsies performed at BCH as part of the requested clinical service. The example shown (5-week-old female, congenital diaphragmatic defect hepatomegaly) did not have identified neurological disease although the gyral pattern was described as non-specifically abnormal.
Recruitment	We selected samples on anatomical description and H&E check to identify choroid plexus and it's attachment to brain tissue in human FFPE samples.
Ethics oversight	Human samples were obtained under an IRB approved protocol at Boston Children's Hospital. A neuropathologist (H.G.W.L.) reviewed the human tissue specimens and recorded non-identifying information. This study was approved by Institutional Postmortem Research Committee of Boston Children's Hospital (IRB-P00003747). Use is compliant with HIPAA informed consent procedures. Parents provided signed informed consent for research based on a written explanation of the anticipated use of samples not required for clinical purposes.

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	No statistical methods were used to pre-determine sample sizes but are based on previous experience with every readout experiment. As most of our analyses were microscopy read-outs aimed at showing the presence/arrival of cells across time, expression of markers and anatomical investigations, we were able to keep number of mice low. For analyses with statistical comparisons between ventricles/treatments, we used an N=4 per group for microscopy (based on experience with read-outs) and N=6 for qPCR read-outs (based on previous experience). For scRNA-Seq analyses, we used pooled samples from 8 mice per group condition. For human post mortem IHC analyses, we had access to only a single sample. For human snRNA-Seq data, we used published dataset from Yang et al, 2021.
Data exclusions	No animals or data points were excluded from analyses for any biological reasons. However, due to difficulty in getting good quality sections/samples of the ChP base, many mice/samples did not end up being useful. For microscopy read-out of CLDN11 co-loc analyses, we used a selection criteria to only include sections with clear CLDN11 signal to ensure correct anatomical location.

Replication	Most of the findings in this study are based on single-cell RNA sequencing analyses and anatomical validation of cell subtype marker expression. Given the stable anatomical and transcriptional identity of these cells across animals, replication of these analyses was not deemed necessary. A subset of experiments were repeated at least two times and showed consistent results: multiplex RNA FISH for Dpep1 and Igfbp6 mRNA; immunostaining for CLDN11/ZO1/CDH1.
Randomization	Treatment/conditions were randomized over cages to avoid cage effects. For experiments other than those involving animals, we did not perform treatments and hence no randomization was needed.
Blinding	The majority of our experiments comprised transcriptional and marker/anatomical analyses in naive adult mice without a treatment/ comparing groups, hence no blinding was needed. Nevertheless, investigators were blinded for group allocation during sampling and analyses when different groups where compared. Exceptions were: blinding aging microscopy samples had no value since size of brains give away age group. For embryonal lineage tracing all mice had identical genotypes, separate ventricles (sections) can be recognized anatomically.

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	Primary antibodies used in this study: Rabbit anti CLDN11 (1/800, ThermoFisher Scientific, 36-4500); Rat anti CD31 (1/100, BD Pharmigen, 553370); Mouse anti ACTA2-Cy3 (1/400, Sigma Aldrich, C6198); Rabbit anti ZO1 (1/500, Invitrogen, 617300); Mouse anti CDH1 (1/200, Becton Dickinson, 610181); Chicken anti GFP (1/750, Abcam, ab13970); Rabbit anti LAMININ (1/500, ThermoFisher Scientific, PA1-16730). Secondary antibodies used in this study: Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight™ 488 (1/500, Thermofisher 35503), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight™ 488 (1/500, Thermofisher 35553), Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568 (1/500, Thermofisher A11036), Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 633 (1/500, Thermofisher A21094), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight™ 633 (1/500, Thermofisher 35563), Streptavidin Protein, DyLight™ 633 (1/500, Thermofisher 21844), Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor™ 488 (1/500, Thermofisher A11039) and Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (1/500, Thermofisher A11012).
Validation	All antibodies were used exclusively for immunohistochemistry (IHC). Dilutions were based on manufacturer recommendations (typically 1/500) and adjusted empirically based on staining quality. To assess background and nonspecific binding, a secondary antibody–only control condition was included in all experiments.

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	C57BL/6J mice (Mus musculus), B6.129S4-Pdgfratm11(EGFP)Sor/J 58 and B6(C)-Ccr2tm1.1Cln/J (strain 027619)59 were maintained in the VIB Center for Inflammation Research animal facility. Mice were housed in individually ventilated cages in specific pathogen-free conditions, under a 14-10 h light/dark cycle, at 20-22 degrees Celsius and 40-60% relative humidity, with food and water ad libitum. We used a mix of male and female mice of 7 to 12 weeks old, unless specified elsewhere. Embronic mouse experiments were performed in Colorado and samples shipped to Belgium. Embryonic Tbx18tm3.1(cre/ERT2)Sev/J 31x Ai14fl/fl (Gt(ROSA)26Sortm14(CAG-tdTomato)Hze mice and Mesp1tm(cre)Ysa 29x Ai14fl/fl (Gt(ROSA)26Sortm14(CAG-tdTomato)Hze were used at ages E10-18.
Wild animals	This study did not involve the use of wild animals.

Reporting on sex	Throughout this study, we used both male and female mice unless stated differently. For embryonic mouse samples, sex was unknown.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	Animal studies (EC2020-005, EC2021-048, EC2022-004 and EC2023-039) were approved by the ethical committee of Ghent University Center for Inflammation Research, Belgium. Embryonic C57BL/6J mouse work was performed in accordance with the IACUC and relevant guidelines of Boston Children's Hospital and Masaryk University.

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

Plants

Seed stocks	N/A.
Novel plant genotypes	N/A.
Authentication	N/A.

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	Flow cytometry was used to isolate single live cells for scRNA-Seq analyses only. Mice for all groups were first sedated as described above, transcardially perfused with PBS with 1% heparin after which brain tissue was excised and lateral and fourth ventricle choroid plexus tissue were carefully microdissected and collected in serum-free RPMI at 4 °C. As choroid plexus tissue is small and does not allow for single mouse scRNA-Seq analyses, we pooled choroid plexus tissues from 8 mice to generate single pooled samples for naive 7-week-old (female), 22 week old (male) and 82 week old (male) mouse groups, for the lateral and fourth ventricle ChP tissue separately. After tissue collection, samples were pre-cut with small scissors and diluted and digested in 2:3 stock solution of CollagenaseI (10 U/ml, Worthington), Collagenase IV (400 U/ml, Worthington) and DNaseI (30 U/ml, Worthington). Incubation was at 37 °C for 30 min with up/down pipetting every 10 min. Samples were strained through a 70 µm filter (Falcon) and washed with a total of 700 µl RPMI. Samples were centrifuged for 7 min 300 g at 4 °C, supernatant discarded and pellet resuspended in 250 µl FACS buffer (1x HBSS without Ca2+/Mg2+, 2 mM EDTA, 2% BSA).
Instrument	BD FACS ARIA II
Software	BD FACSDiva v8.0.1
Cell population abundance	Sorting data were not recorded nor included in the manuscript.
Gating strategy	Simple L/D sort based on eFLuor V525 for following scRNA-Seq. Sorting data were not recorded nor included in the manuscript.

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