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Reporting Summary

Nature Research 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 Research 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	Sequencing data were collected with the Illumina NovaSeq6000 platform.
Data analysis	Cellranger v3.0.1, Seurat v3.2.0, DoubleFinder v2.0.3, MAST v1.16.0, Enrichr v3.0, ACTIONet v2.1.9, ImageJ; All code will be available at http://compbio.mit.edu/scBBB/ when the manuscript is accepted.

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 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

Raw sequencing reads were aligned to the pre-mRNA annotated Mus musculus reference genome version GRCm38 or Homo sapiens reference genome version GRCh38. Code used in this study is available upon reasonable request from the corresponding authors. Count matrices for all cells analyzed in this study are uploaded with this submission as Supplementary Data and at http://compbio.mit.edu/scBBB/. Interactive website is available at https://nsun.shinyapps.io/scbbb/. Raw sequencing data associated with Figures 1-5 are publicly available in NCBI GEO under accession #GSE173731.

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 calculation was not performed in this study. The number of patient samples used was estimated based on predicted number of cerebrovascular cells profiled per sample (approximately 300-500 cells per patient) to achieve a total of ~3,500 cerebrovascular cells (a benchmark set in previous work of Vanlandewijck, et al. 2018 https://doi.org/10.1038/nature25739). A total of 16 patient samples were used as described in Supplemental Table 1.
Data exclusions	All samples from patients with known genetic mutations were excluded from this study. Furthermore, given the nature of our study in understanding human cerebrovasculature we excluded all patients with known arteriovenous malformations. Finally, to avoid early developmental changes in transcriptional profiles, we restricted patient samples to be within age range of approximately 12-22 years of age. These details are also outlined in Methods section.
Replication	Various findings from the sequencing analyses were confirmed throughout the study at the protein level by indirect immunofluorescent staining using ex vivo tissue from three biologically independent samples. All attempts at replication were successful. Representative images shown in figures
Randomization	Randomization was not necessary in this study given the unbiased experimental approach. In analyzing snRNA-seq data, we projected each cell on a common two-dimensional embedding that corrects for covariates (age, sex, brain regions, sequencing platforms, post-mortem interval)
Blinding	Blinding was not necessary in this study given the unbiased experimental approach. In analyzing snRNA-seq data, cells cluster based on their transcriptional profiles, which does not require blinding given that cell type identities will cluster together regardless of condition

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

Alexa-Fluor488 anti-Chicken, ThermoFisher, A11039, 1:500
Alexa-Fluor488 anti-Mouse, ThermoFisher, A21202, 1:500
Alexa-Fluor488 Plus anti-Rabbit, ThermoFisher, A32790, 1:500
Alexa-Fluor568 anti-Goat, ThermoFisher, A11057, 1:500
Alexa-Fluor546 anti-Rabbit, ThermoFisher, A10040, 1:500
Alexa-Fluor647 anti-Chicken, ThermoFisher, A21449, 1:500
Alexa-Fluor647 anti-Mouse, ThermoFisher, A32787, 1:500
Alexa-Fluor647 anti-Rabbit, ThermoFisher, A31573, 1:500
anti-TMEM16B, Abcam, ab113443, 1:100 dilution,
anti-CLDN5, ThermoFisher, 35-2500, 1:200 dilution, 1:1000 dilution (Western blot)
anti-TJP1, Abcam, ab216880, 1:1000 dilution (Western blot)
anti-FRMD3, Novus, NBP2-33879, 1:100 dilution
anti-CEMIP, LifeSpan Biosciences, LS-C108052-100, 1:100 dilution
anti-KCNMA1, LifeSpan Biosciences, LS-A9575-50, 1:100 dilution

anti-FBLN1, Millipore Sigma, HPA001612-100UL, 1:100 dilution
anti-metallothionein, Abcam, ab192385, 1:100 dilution
anti-VEGFC, Abcam, ab106512, reconstituted to 1 mg/ml in sterile PBS, 1:100 dilution
anti-MT1E, Abcam, ab193618, 1:100 dilution
anti-TSHZ2, LifeSpan Biosciences, LS-B9329-200, 1:100 dilution
anti-PKR, Abcam, ab32052, 1:200 dilution
anti-TRPM3, Abcam, ab56171, 1:100 dilution
anti-GRM8, Lifespan Biosciences, LS-A922-50, 1:100 dilution
anti-SLC20A2, Lifespan Biosciences, LS-C334915, 1:100 dilution
anti-SLC30A10, Abcam, ab229954, 1:300 dilution
anti-GFAP, Abcam, ab4674, 1:400 dilution
anti-AQP4, Millipore Sigma, AB2218, 1:100 dilution
anti-ACTA2, Lifespan Biosciences, LS-B3933, 1:100 dilution
Lycopersicon Esculentum (Tomato) Lectin (LEL, TL), DyLight® 488, Vector Laboratories, DL-1174-1, 1:200 dilution
Lycopersicon Esculentum (Tomato) Lectin (LEL, TL), DyLight® 594, Vector Laboratories, DL-1177-1, 1:200 dilution
Lycopersicon Esculentum (Tomato) Lectin (LEL, TL), DyLight® 649, Vector Laboratories, DL-1178-1, 1:200 dilution
Goat anti-Mouse IgG HRP, ThermoFisher, 31430, 1:10,000 dilution
Goat anti-Rabbit IgG HRP, ThermoFisher, 31460, 1:10,000 dilution

Validation

All primary antibodies were used for indirect immunofluorescence experiments as validation for single nuclear RNA sequencing data analysis. All antibodies used have confirmed reactivity with human targets or human/mouse targets whenever used in cross-species comparisons. To confirm primary staining, we validated antibody signal in three ways: 1) negative controls using only secondary antibodies, 2) expression in expected cell type as predicted by transcriptomic analysis, and 3) in the case of co-staining, imaging at channels with minimal spectral overlap (i.e. 488nm and 647nm). For all antibodies, additional information on key publications and immunogen (if available) can be found on manufacturer's website.

Animals and other organisms

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

Laboratory animals	All animal experiments were approved by the MIT Committee on Animal Care. Mice were grouped housed with food and water provided ab libitum on a standard 12h light/12h dark cycle. 6-week old male C57BL/6J wild-type mice (Jackson Laboratories stock #000664) were used for snRNA-seq and immunofluorescence experiments. 9-week old male B6CBA-Tg(HDexon1)62Gpb/1J mice (CAG repeat length 160 ± 5; Jackson Laboratories stock # 002810) and non-carrier controls were used for R6/2 experiments. No prior procedures were performed on any animal prior to experiments.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	MIT Committee on Animal Care

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