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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 Authors & Referees 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

Image Acquisition: Zen Black 2010 SP1 (v.6.0.0.485)
Flow Cytometry: FACSDiva (v.8.01)
Behavior: Behavior testing was performed in a controlled environment as described in methods. Tests were performed in groups of 4 mice at a time with even lighting conditions (30 ± 5 lumens). All trials were recorded using a ceiling mounted camera and Topscan Version 2.00 Software. Data was analyzed using Prism.

Data analysis

Image analysis: Prism (6.0h and 8.0.1), ImageJ (1.51q Build)
3D Image Rendering: Imaris8 (v.8.0.2)
Flow Cytometry Analysis: FlowJo (v.10.0) and FACSDiva (v.8.0.1)
Bulk RNAseq Analysis: FastQC v0.11.2 was used to provide quality control checks on the raw RNAseq sequence data. STAR v2.4.2a was used to align the RNAseq reads to the mouse reference genome (mm9). Cuffdiff v2.2.1 statistical package was used to perform differential expression analysis for RNAseq based on gene and transcript abundance measurements in terms of Fragments Per Kilobase of transcript per Million mapped reads (FPKM), as previously described61. R v3.2.2 statistical package and CummeRbund v2.12.1 R/Bioconductor package were used for visualization of the various output files of the Cuffdiff differential expression analysis including visualization of the changes in gene transcripts with age. FPKM values for genes and transcripts were tabulated and Cluster v3.0 was used to perform hierarchical clustering and cluster analysis. Java TreeView v1.1.6 was used to visualize the output files from hierarchical clustering in the form of heat maps displaying up- or down- differentially regulated genes in aged versus young BECs. Gene Set Enrichment Analysis (GSEA v2.2.0) tool was used to determine whether GO and Pathway gene sets showed statistically significant, concordant differences between young and aged BECs.
Proteomic Analysis: R Segmented package (v3.2.2)
scRNAseq: Sequences from the Nextseq were demultiplexed using bcl2fastq, and reads were aligned to the mm10 genome augmented 2

with ERCC sequences, using STAR version 2.5.2b. Gene counts were made using HTSEQ version 0.6.1p1. Genes were projected into low dimensional principal component space using the robust principal component analysis (rPCA). Single cell PC scores and genes loads for the first 20 PCs were analyzed using the Seurat package in R. All graphs and analyses were generated and performed in R. GeneAnalytics and GeneCards- packages offered by Gene Set Enrichment Analysis (GSEA v2.2.0) tool was used for GO pathway analysis and classification of enriched genes in each subpopulation.

Behavior: Behavior testing was performed in a controlled environment as described in methods. Tests were performed in groups of 4 mice at a time with even lighting conditions (30 ± 5 lumens). All trials were recorded using a ceiling mounted camera and Topscan Version 2.00 Software. Data was analyzed using Prism (6.0h and 8.0.1).

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/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

RNA-seq gene lists with statistics (Fig. 1, Fig. 2, and Extended Data Fig. 1 and 2), full blots (Extended Data Fig.6), and individual data points graphed for Extended Fig. 9n are available as source data files and as Supplementary Tables 1-3 accompanying this article. Requests of datasets obtained from human research will be subject to additional review steps by the IRB that has granted permit for a particular research. Bulk and single cell RNA sequencing datasets that support the findings of this study have been deposited in NCBI GEO with the series accession record GSE127758 [with embedded accession codes GSM3638211 to GSM3638222]. Please contact the corresponding author for additional information.

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 predetermine sample size. For parabiosis and plasma injection studies, sample size was determined based on the number of animals used in prior experiments conducted in the Wyss-Coray lab (Villeda et al., Nature 2011; Villeda et al., Nature Medicine 2014.) Sample sizes are listed in Supplementary Table 4. For in vitro studies, n of 3-6 biologically independent replicates derived from different cell flasks per group were used and experiments were repeated at least 3 times.
Data exclusions	No data was excluded.
Replication	All attempts for replication were successful.
Randomization	All mice were weighed before the start of experiments and those with significant weight differences or visible signs of distress (for example, skin lesions) were not used. Healthy mice of similar weight were assigned randomly into control and treated groups.
Blinding	All researchers were blinded during data collection and image quantification and analysis. Mice were assigned numbers and the numbers corresponding to specific group categories were not revealed until after 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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Immunofluorescence staining:

Primary Antibodies:
Rat monoclonal anti-BrdU (1:500, Abcam, ab6326, clone BU1/75[ICR1])
Rat monoclonal anti-VCAM1 (1:125, Abcam, ab19569, clone M/K-2)
Goat monoclonal anti-Sox2 (1:100, Santa Cruz, sc-17320, clone Y-17)
Goat polyclonal anti-doublecortin (DCX) (1:100, Santa Cruz, sc-8066, clone C-18)
Goat polyclonal VE-Cadherin (Santa Cruz Biotechnology, sc-6458, clone C-19)
Click-iT® Plus EdU Alexa Fluor® 488 Imaging Kit (Thermo/Life Technologies, C-10637)
Mouse monoclonal anti-GFAP (1:1000, Chemicon/Fisher, MAB360MI, clone GA5)
DyLight 488 Lectin (1:200, Vector, DL-1174)
Rabbit monoclonal anti-Aquaporin 4 (1:500, Millipore, AB2218, clone)
Rat monoclonal anti-CD68 (1:600, Serotec, MCA1957, clone FA-11)
Rabbit polyclonal anti-Iba1 (1:250, ProteinTech, 10904-1-AP)
Mouse anti-human-VCAM1 antibody (Novus Biologicals, BBA5, clone BBIG-V1)
Mouse monoclonal anti-human IgG antibody (R&D Systems, MAB002 clone 11711)
Rat monoclonal anti-VCAM-1 (BioxCell, BE0027, clone M/K-2.7)
Rat IgG1 Isotype antibody (BioxCell, BE0088, clone HRPN)

Secondary Antibodies:
Alexa Fluor® 488 donkey anti-goat IgG (1:250, Invitrogen, A-11055)
Alexa Fluor® 488 donkey anti-rat IgG (1:250, Invitrogen, A-21208)
Alexa Fluor® 555 donkey anti-mouse IgG (1:250, Invitrogen, A-31570)
Alexa Fluor® 555 donkey anti-goat IgG (1:250, Invitrogen, A-21432)
Alexa Fluor® 647 donkey anti-mouse IgG (1:250, Invitrogen, A-31571)
Alexa Fluor® 647 donkey anti-rabbit IgG (1:250, Invitrogen, A-31573)
Alexa Fluor® 647 donkey anti-goat IgG (1:250, Invitrogen, A-21447)
Alexa Fluor® 647 donkey anti-rabbit IgG (1:250, Invitrogen, A-31573)
Alexa Fluor® 647 donkey anti-goat IgG (1:250, Invitrogen, A-21447)
Alexa Fluor 488 Azide (Invitrogen, A-10266)
Alexa Fluor 647 Azide (Invitrogen, A-10277)
Cy3 AffiniPure donkey anti-rat IgG (1:250, Jackson Immunoresearch, 712-165-153)
Hoechst 33342 (1:2000, Sigma, 14533-100MG)

Flow Cytometry:

Bran Endothelial Cell Profiling:
Dylight 488 Conjugation Kit (Thermo Scientific, 53024)
Anti-Mouse CD45 PerCP-Cyanine5.5 (1:1000, eBioscience, 45-0451-80, clone 30-F11)
Anti-Mouse CD31 (PECAM-1) PE-Cyanine7 (1:100, eBioscience, 25-0311-81, clone 390)
Anti-Mouse CD11b PerCP-Cyanine5.5 (1:100, eBioscience, 45-0112-80, clone M1/70)
Anti-Mouse TER-119 PerCP-Cyanine5.5 (1:100, eBioscience, 45-5921-80, clone TER119)
Anti- Mouse CD13 Antibody (CD13-APC) (1:50, NOVUS Biologicals, NB100-64843, clone ER-BMDM1)
Anti-Mouse ACSA-2 PE (1:100, Miltenyi Biotec Inc., 130-102-365, clone IH3-18A3)
Anti-Mouse CD16/CD32 (Mouse BD FC Block) (BD Pharmigen, 553142, clone 2.4G2)
Anti-Mouse CD31 (CD31-APC) (1:100, BD Pharmigen, 551262, clone MEC13.3)
Anti-Mouse CD45 (CD45-FITC) (1:100, BD Pharmingen, 553080, clone 30-F11)

Brain-Resident Immune Cell Profiling
Anti-Mouse Ly6C-FITC (1:200, Biolegend 128021
Rabbit monoclonal Anti-Mouse Tmem119 conjugated to AlexaFluor647 (1:400, Abcam ab209064
Anti-Mouse Ly6G APC-Cy7 (1:200, Biolegend 127623
Anti-Mouse Alpha4 PE (1:100, GeneTex GTX74788
Anti-Mouse CD45 Pac Blue (1:100, Biolegend 103126
Anti-Mouse Beta1 PECy7 (1:100, eBioscience 25-0291-80
Anti-Mouse CD11c BV711 (1:200, Biolegend 117349
Anti-Mouse IA/IE AF700 (1:100, Biolegend 107621

Anti-Mouse CD14 PerCP/Cy5.5 (1:100), Biolegend 123313
Anti-Mouse CD19 BV605 (1:100), Biolegend 115539
Anti-Mouse CD3 BV786 (1:100), BD Biosciences 564010
Anti-Mouse Cd11b BV650 (1:100), Biolegend 740551
Anti-Mouse CD206 biotin antibody; streptavidin BUV395. (1:100 BD 564176), Biolegend 141713
Markers for Setting Channel Compensations (for brain immune cell profiling, all 1:100):
Anti-Mouse CD45 APC, Biolegend 103112
Anti-Mouse CD11b ApcCy7, BD Biosciences 557657
Anti-Mouse CD11b PE eBioscience 12-0112-82
Anti-Mouse CD45 PacBlue, Biolegend 103126
Anti-Mouse CD11b PeCy7, Biolegend 101215
Anti-Mouse CDllb-BV7ll, Biolegend 101241
Anti-Mouse CD11b-AF700, Biolegend 101222
Anti-Mouse CD11b-PerCP-Cy5.5, Biolegend 101227
Anti-Mouse CD11b-BV605 Biolegend 101237
Anti-Mouse CD11b-BV785, Biolegend 101243
Anti-Mouse CD11b -BV650 Biolegend 740551
Anti-Mouse CD11b-Biotin Biolegend 101203

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Anti-Mouse CDllb-BV7ll, Biolegend 101241
Anti-Mouse CD11b-AF700, Biolegend 101222
Anti-Mouse CD11b-PerCP-Cy5.5, Biolegend 101227
Anti-Mouse CD11b-BV605 Biolegend 101237
Anti-Mouse CD11b-BV785, Biolegend 101243
Anti-Mouse CD11b -BV650 Biolegend 740551
Anti-Mouse CD11b-Biotin Biolegend 101203

Validation

All antibodies used were validated by manufacturers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Mouse. b.End3 cells; ATCC; CRL-2299

Authentication

Immunofluorescence staining of Bend.3 cells for BBB specific markers of adherens junctions (AJ) and tight junctions (TJ), specifically ß-catenin, Claudin-5, and VECadherin was used to validate the cell line.

Mycoplasma contamination

The b.End3 cells were tested negative for mycoplasma contamination. All cells tested routinely using a PCR kit (PanReac AppliChem PCR Mycoplasma Test Kit, A3744).

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals

NOD-scid IL2Rnull (NSG) immunodeficient mice were purchased from Jackson Laboratory (Bar Harbor, Maine). NSG mice were bred and only young males (3-4 months of age) were used for plasma treatment studies. Aged (13 months of age) males were used for behavior studies.

Heterozygous Slco1c1-CreERT2 breeding males were provided by Professor Markus Schwaninger. Mice were bred and crossed with Vcam1fl/fl mice (B6.129(C3)-Vcam1tm2Flv/J mice) purchased from Jackson Laboratory (Bar Harbor, Maine). Aged (greater than 12 months of age) and young (2-4 months of age) Male mice were used for plasma treatmentstudies following treatment with tamoxifen (an estrogen modulator).

Aged (greater than 12 months of age) C57BL6J males and females were obtained from the National Institute on Aging (NIA), and young C57BL6J males (2-4 months of age) were purchased from Jackson Laboratory and Charles River.
BALB/cNctr-Npc1m1N/J 9-week-old homozygous males and females were generated by breeding heterozygous mice acquired from Jackson.

Aged (17-18 months of age) male and female wildtype and Grn-/- deficient mice (B6.129S4(FVB)-Grntm1.1Far/Mmja) were bred and aged in-house but originally acquired from Jackson.

These transgenic strains were bred and aged in-house. Mice lived under a 12-hour light/dark cycle in pathogenic-free conditions with open access to dry feed and water, in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. In-house aged mice health status was monitored every 2-3 months via weight and physical checks, young (2-4 months of age) mice weighed 20-30 grams and aged (greater than 12 months of age) mice weighed 40-50 grams. Mice found to have health issues were excluded from studies and assessed by the in-house Veterinary Medical Officer.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animals were used under approved protocols from the Institutional Animal Care and Use Committee at the Palo Alto VA or the Animal oversight committee at Stanford University

Human research participants

Policy information about studies involving human research participants

Population characteristics	A previous proteomic data set of human plasma of various aged published in the Wyss-Coray lab was reanalyzed (Britschgi, M. et al. Modeling of pathological traits in Alzheimer's disease based on systemic extracellular signaling proteome. Mol. Cell. Proteomics 10, M111.008862 (2011).) Proteomic Data of plasma sample anonymous donations was received blinded to the participant's identities. For ELISAs and plasma treatment studies in NSG mice: Human blood samples from healthy males in the age range of 18-25 and 65-74 were anonymously donated to the Stanford Blood Center, Palo Alto.
Recruitment	Human blood samples from healthy males in the age range of 18-25 and 65-74 were anonymously donated to the Stanford Blood Center, Palo Alto.
Ethics oversight	Human IRB was approved by Stanford University

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	In vitro: Bend.3 cells were washed once with PBS Cells were detached with 700 µl of accutase (A1110501, Life Technologies) for 5 min and the reaction was stopped by resuspending cells in 2 mL PBS. Cells were centrifuged for 5 min at 1100 rpm, medium was aspirated, and cells were resuspended in 1 mL/well of PBS with 4% PFA (diluted with 8 mL of PBS) and fixed on ice for 10 min. After centrifugation at 1100 rpm for 5 min, cells were resuspended in PBS for one wash followed by 30 min blocking in FACS buffer (PBS + 2% BSA with 2mM EDTA). Following centrifugation, cells were resuspended in 100 µl/sample of FACS buffer. FC blocking antibody (553142, BD Pharmigen) was added for 10 mins followed by addition of each antibody. Samples were incubated in antibodies for 30 min—1 h on ice. After two washes with FACS buffer, cells were resuspended in 500 µl FACS buffer and transferred to flow tubes for analysis. Brain endothelial cells are stained using a antibodies listed in the methods portion of the manuscript. Primary Cells for RNAseq: BEC isolation was based on a previously described procedure. Briefly, mice were anesthetized with avertin and perfused following b lood collection. After thoroughly dissecting the meninges, cortices and hippocampi were collected, minced and digested using the neural dissociation kit according to kit instructions (Miltenyi, 130-092-628). Brain homogenates were filtered through 35 µm in HBSS and centrifuged pellets were resuspended in 0.9 M sucrose in HBSS followed by centrifugation for 15 min at 850xg at 4ºC in order to separate the myelin. This step was repeated for better myelin removal. Cell pellets were eluted in FACs buffer (0.5% BSA in PBS with 2mM EDTA) and blocked for ten min with Fc preblock (CD16/CD32, BD 553141), followed by 20 minute staining with anti-CD31-APC (1:100, BD 551262), anti-CD45-FITC or anti-CD45-APC/Cy7 (1:100, BD Pharmingen Clone 30-F11 553080; Biolegend, 103116), and anti-Cd11b-BV421 (1:100, Biolegend Clone M1/70 101236). Dead cells were excluded by staining with propidium iodide solution (1:3000, Sigma, P4864). Flow cytometry data and cell sorting were acquired on an ARIA II (BD Biosciences) with FACSDiva software (BD Biosciences). FlowJo software was used for further analysis and depiction of the gating strategy. For LPS-treated Slco1c1CreERT2+/-; Vcam1fl/fl mice: BEC isolation was based on a previously described procedure 64. Briefly, mice were sacrificed using carbon dioxide asphyxiation followed by cervical dislocation. Mouse brains were carefully removed from the scull and stored on ice in Buffer A (153mM NaCl, 5.6mM KCl, 1.7mM CaCl2, 1.2mM MgCl2, 15mM HEPES; 10mg/ml bovine serum albumin (BSA) fraction V). After thoroughly dissecting the meninges, cortices and hippocampi were collected and washed several times in Buffer A before the tissues was minced and centrifuged at 300g for 7min at 4°C. The pellet was digested in a 1:1:1 volume mix of tissue, Buffer A, and 0.75% collagenase II (Millipore, C2-22) at 37°C for 50min. The tissue was homogenized by thorough shaking after 25 and 50min of digestion and repetitive up and down pipetting of the cell solution at the end of digestion. The enzymatic reaction was stopped by adding Buffer A. After centrifugation (300g, 7 min, 4°C) the pellet was carefully resuspended in PBS containing 25% BSA (Fisher Scientific, BP1600-1) and centrifuged at 1000g, 30min at 4°C in order to separate the myelin and to enrich for capillary fragments. To deplete for red blood cells the pellet was incubated in Red Blood Cell Lysis Buffer (Sigma, R7757) for 1.5min at room temperature with occasional shaking, followed by a wash step in buffer

A and centrifugation (300g, 7 min, 4°C). For the second digestion, the pellet was resuspended in buffer A containing 1mg/ml Collagenase/Dispase (Roche, 11097113001) and the mixture was incubated at 37°C for 13min. 1μg/ml DNase I (Sigma,10104159001) was added for 2 additional minutes. To quench the reaction buffer A was added and cells were centrifuged at 300g for 7min at 4°C.

For flow cytometry, the enriched BECs were labeled by standard protocols with fluorochrome-conjugated antibodies (identified in antibodies section) in HBSS (Thermo Fisher) containing 10% FBS for 30min on ice. Dead cells were excluded by staining with propidium iodide solution (1:3000, Sigma, P4864). Background fluorescence was determined by the 'fluorescence minusone' method and for VCAM1 a specific IgG1 Isotype control antibody was used. Flow cytometry data were acquired on an ARIA II(BD Biosciences) with FACSDiva software (BD Biosciences). FlowJo software (TreeStar) was used for further analysis.
Flow Cytometry analysis of Brain-resident immune cells: Mice analyzed were: Young (3-month-old) C57BL6J mice (n=5), aged Cre-(n=9) and Cre+ (n=4) 19-month-old mice administered tamoxifen at 17 months as described above in BBB Permeability section, and aged Cre+(22-month-old) mice administered tamoxifen at 2 months of age as described in section: Basal Neurogenesis (n=4). Gates are based on positive LPS-stimulated, Cre- aged mice injected with a fluorescently labeled (DL488) anti-VCAM1 mAb or IgG-DL488 control antibody 2 hours prior to perfusion and analysis by flow cytometry. An aged (19-monthold) Cre + littermate administered tamoxifen at 17 months as described above in BBB Permeability section was also injected with a fluorescently labeled (DL488) anti-VCAM1 mAb to confirm successful deletion of Vcam1 in the hippocampus and cortex.

Mice analyzed were anesthetized by avertin and perfused with 20 mL of cold Medium A (500 ml HBSS, 7.5 mL 1M HEPES, 5.56 mL 45% Glucose. Sterile filtered) for mechanical dissociation of cortex and hippocampus. Tissue was collected and chopped in 2 mL cold Medium A with 80 uL DNase1 then homogenized with a glass douncer. Homogenate was passed through a 100 um cell strainer, washed 3 times with medium A to remove clumps, and centrifuged at 340g for 7 minutes at 4C. The supernatant was removed and the pellet was resuspended in 12 mL of 25% Percoll Plus diluted in Medium A (GE Healthcare Cat #: 17-5445-01) then centrifuged at 950g for 20 minutes. The supernatant was discarded and the remaining cell pellet was washed with 5 mL of Medium A and centrifuged at 340g for 7 minutes at 4C to remove myelin.

The myelin free cell samples were then resuspended in 1 mL FACs buffer (PBS + 1% BSA with 2mM EDTA), transferred to new 2mL eppendorf tubes and centrifuged at 300g for 5 min at 4C. Cells were resuspended in 300 uL of FC block solution (1: 100 CD16/CD32, BD Cat #: 553141) and allowed to incubate on a shaker for 5 minutes at room temperature. After blocking, primary antibodies against Ly6C, Ly6G, Tmem119, Alpha4, Beta 1 (VLA-4), CD45, CD11c, IA/IE, CD14, CD19, CD3, CD11b, CD206 were added to the samples and allowed to incubate 15 minutes. FACs buffer was added to the samples to a final volume of 1 mL and centrifuged at 400g for 5 minutes. Samples were then incubated in 300 uL solution of secondary antibodies, Streptavidin and Alexa Fluor for 15 minutes. FACs buffer was added to the samples to a final volume of 1 mL and centrifuged at 400g for 5 minutes. Samples were washed with 1 mL FACs buffer and stained with a viability dye (Bioscience Fixable Viability Dye eFluor 506 from thermofisher, 65-0866-14). Samples were then washed in 1mL FACs buffer, centrifuged then resuspended in 500 uL of FACs Buffer and filtered through a 40 um cell strainer cap into round-bottom tubes for flow cytometry analysis (Falcon Cat #:32235).

Instrument	Aria II and III, LSRFortessa
Software	FlowJo (v.10.0) and FACSDiva (v.8.0.1)
Cell population abundance	Post-sort fractions for brain endothelial cells were >95%
Gating strategy	In vitro: Cells were gated on forward (FSC = size) and sideward scatter (SSC = internal structure). FSC-A and FSC-W blotting was used to discriminate single cells from cell doublets/aggregates. PI+ dead cells were excluded. CD31+ cells were defined as BECs. For RNAseq: Cells were gated on forward (FSC = size) and sideward scatter (SSC = internal structure). FSC-A and FSC-W blotting was used to discriminate single cells from cell doublets/aggregates. PI+ dead cells were excluded. CD11b+ and CD45+ cells were gated to exclude monocytes/macrophages and microglia. CD31+Cd11b-CD45- cells were defined as the BEC population and were sorted directly into RNAlater (Life Technologies, AM7020) and stored at -80ºC until further processing. If mice were injected with fluorescently labeled FACs anti-mouse VCAM1- DyLight™488 as described above, CD45 was stained in the APC/Cy7 channel, and CD31 +VCAM1+ cells were also gated in the APC and FITC channels. For LPS-treated Slco1c1CreERT2+/-; Vcam1fl/fl mice: Cells were gated on forward (FSC = size) and sideward scatter (SSC = internal structure). FSC-A and FSC-W blotting was used to discriminate single cells from cell doublets/aggregates. PI uptake indicated dead cells, which were excluded. CD11a/b, CD45, and, Ter-119 negative cells were gated to exclude erythrocytes, monocytes/macrophages and microglia. CD13 and ACSA-2 staining was applied to exclude pericytes and astrocytes, respectively. CD31+MECA99+ cells were defined as the BEC population.

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