Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1

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An aged circulatory environment can activate microglia, reduce neural precursor cell activity and impair cognition in mice. We hypothesized that brain endothelial cells (BECs) mediate at least some of these effects. We observe that BECs in the aged mouse hippocampus express an inflammatory transcriptional profile with focal upregulation of vascular cell adhesion molecule 1 (VCAM1), a protein that facilitates vascular–immune cell interactions. Concomitantly, levels of the shed, soluble form of VCAM1 are prominently increased in the plasma of aged humans and mice, and their plasma is sufficient to increase VCAM1 expression in cultured BECs and the hippocampi of young mice. Systemic administration of anti-VCAM1 antibody or genetic ablation of Vcam1 in BECs counteracts the detrimental effects of plasma from aged individuals on young brains and reverses aging aspects, including microglial reactivity and cognitive deficits, in the brains of aged mice. Together, these findings establish brain endothelial VCAM1 at the blood–brain barrier as a possible target to treat age-related neurodegeneration.

Brain structure and function deteriorate with age, steadily driving cognitive impairments and susceptibility to neurodegenerative disorders in humans. How aging leads to these manifestations is poorly understood, but an increase in the activation of microglia, frequently referred to as ‘neuroinflammation’ (refs. 1–4), and a precipitous loss of stem cell numbers and activity in the dentate gyrus (DG) of the hippocampus, one of two neurogenic regions of the adult mammalian brain, are commonly noted. The hippocampus is crucial for learning and memory and is particularly vulnerable to age-related neurodegeneration and diseases such as Alzheimer’s disease (AD)5.

Although many of these age-related changes in the brain may be the consequences of cell-intrinsic and brain-localized mechanisms of aging, we asked whether changes in secreted signaling proteins, dubbed the communicome, could be used to understand, characterize and quantify aspects of brain aging and cognitive impairment. Indeed, not only are such changes in plasma or cerebrospinal fluid manifestations of aging, we asked whether changes in secreted signaling proteins, dubbed the communicome, could be used to understand, characterize and quantify aspects of brain aging and cognitive impairment. Indeed, not only are such changes in plasma or cerebrospinal fluid proteomes abundant with aging and disease15, but factors in blood or plasma from young mice or humans are also sufficient to increase brain function in the hippocampus4,6,10 and the subventricular zone (SVZ)12. Conversely, young mice exposed to blood from aged individuals showed reduced neurogenesis and cognitive function in the hippocampus4,13. Considering the tight regulation of transport of molecules across the blood–brain barrier (BBB) and its role as a protective barrier with limited permeability to macromolecules14, it is currently unclear how factors promoting youth or aging may modulate brain function1. Here we investigated the interaction between the circulating communicome and BECs in the context of brain aging.

Results
Aged BECs are transcriptionally activated. To determine the transcriptional changes associated with aging in BECs, we acutely isolated and pooled primary CD31+ BECs from the cortices and hippocampi of young (3-month-old) and aged (19-month-old) mice and analyzed their transcriptome using RNA-seq (Extended Data Fig. 1a,b). Unsupervised clustering analysis revealed prominent age-dependent differences in the transcriptome, with over 1,000 differentially expressed genes (Fig. 1a). Cell purity was confirmed on the basis of high gene expression values for genes with BEC-specific expression and very low or undetectable expression for other cell-type-specific markers of the central nervous system (CNS) (Fig. 1b and Extended Data Fig. 1c). GeneAnalytics pathway analysis of differentially expressed genes identified numerous pathways associated with aging (Supplementary Table 1), including cell adhesion, immune cell activation, stress response and vascular remodeling15. Analysis of the highly expressed and differentially expressed transcripts revealed an inflammatory and activated profile with age,

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as illustrated by the increase in mRNA levels for the major histocompatibility complex (MHC) class I molecules B2m (β2-microglobulin) and H2-K1, two of the most highly expressed transcripts in BECs (Fig. 1c). Tspo, a marker of neuroinflammation commonly used in human positron emission tomography (PET) scans to assess the level of microglial activation in neurodegenerative diseases,16, was highly expressed in BECs and also had significantly increased expression with age, as was the case for Vwf (von Willebrand factor), which encodes a blood glycoprotein involved in hemostasis that has elevated levels under acute and chronic inflammation and is known to promote vascular inflammation17 (Fig. 1c).

VCAM1 levels increase with age and exposure to systemic inflammatory mediators. To identify proteins whose abundance changes with age in humans and are possibly associated with the BBB and cerebrovascular dysfunction, we searched for proteins involved in vascular function in the healthy aging control group in a previously published plasma proteomics dataset from our laboratory.7 Concentrations of 31 factors correlated significantly with age (Fig. 1d and Supplementary Table 2; P < 0.05). Of these, eight were expressed in mouse BECs at the transcript level (Supplementary Table 2 and Extended Data Fig. 1d,f) and five have vascular-, endothelial- or angiogenesis-related functions (Supplementary Table 2; GeneCards). Among the proteins expressed in or related to the vasculature, soluble VCAM1 (sVCAM1) correlated most strongly with age (Fig. 1e) and its levels were increased in an independent cohort of healthy individuals (Fig. 1f). VCAM1, a member of the immunoglobulin superfamily, is upregulated on endothelium in response to inflammation throughout the body, where it facilitates leukocyte tethering through the integrin receptor α4β1 (also known as VLA-4) and transmigration into tissues18,19. VCAM1 is shed constitutively through proteolytic cleavage by the membrane-bound metalloprotease ADAM17, resulting in high quantities of plasma sVCAM1 (ref. 20). Similarly to humans, mice showed a significant increase in plasma sVCAM1 abundance with more advanced age (19 months) that was not seen in middle age (10–12 months; Fig. 1g).

This increase in sVCAM1 levels in plasma was associated with a significant increase in Vcam1 mRNA expression in cells immunoreactive for lectin and MECA-99, markers of cerebral blood vessels, in the DG of aged mice (Fig. 1h,i,21,22). This increase was confirmed by quantifying CD31+VCAM1+ BECs in the cortices and hippocampi of young and aged mice (Extended Data Fig. 1g–i). Interestingly, exposure to blood from aged mice through heterochronic parabiosis induced a similar increase in VCAM1 immunoreactivity in young mice (Fig. 1j and Extended Data Fig. 1k) and a concomitant increase in sVCAM1 levels in plasma (Extended Data Fig. 1j). In a bulk population of BECs, mRNA levels of molecules involved in leukocyte adhesion were low or undetectable (Extended Data Fig. 1d). At the protein level, VCAM1 expression was visible in a small percentage of BECs (Fig. 1h–j, 2a–j and 3c,d) and, in Extended Data Fig. 1g–i, we performed single-cell RNA-seq (scRNA-seq) on Vcam1+enriched BECs to characterize the unique molecular and phenotypic nature of rare VCAM1+ BECs. Full-length scRNA-seq was performed on 160 and 112 BECs isolated from pooled hippocampi from young and aged mice, respectively. We infused a fluorescently labeled anti-VCAM1 monoclonal antibody retro-orbitally before mouse perfusion and tissue dissection, which allowed us to enrich VCAM1+ BECs by up to 50% via cell sorting (Methods and Extended Data Fig. 1g–i). All isolated cells expressed both pan-endothelial (Pecam1, Cldn5) and BBB-specific (Sko1c1, Slc2a1, Abb1a, Ocn) markers (Extended Data Fig. 1l; refs. 16–20). Furthermore, we verified that VCAM1 protein levels correlated with Vcam1 mRNA levels (Extended Data Fig. 1m,n). Unsupervised clustering in principal-component space using the top 2,500 correlated and anticorrelated genes identified three molecularly distinct populations (Fig. 2b). The isolated VCAM1+ cells were confined to two subpopulations (Fig. 2c and Supplementary Table 3). Interestingly, none of the clusters was significantly enriched for cells from young or aged mice, indicating that strong sources of variation exist besides age that result in transcriptional heterogeneity between the BEC subpopulations (Extended Data Fig. 1o). In spite of this, a direct comparison of Vcam1 expression levels within isolated CD31+VCAM1+ BECs showed significantly higher Vcam1 mRNA levels in BECs from aged as compared to young mice (Extended Data Fig. 1p). Transcripts for other adhesion molecules, namely Sele (E-selectin) and Selp (P-selectin), were undetectable in the isolated BECs with scRNA-seq, in agreement with the absence of their detection in bulk RNA-seq (Extended Data Fig. 1d). Vcam1 transcript, primarily enriched in the venous Vcam1+ cluster (Fig. 2h), was coexpressed in few VCAM1+ BECs from young or aged mice as well as all BECs analyzed and did not show increased expression with age (Extended Data Fig. 1q).

Fig. 1 | BECs are activated with age, and systemic and cerebrovascular VCAM1 levels increase with age and heterochronic parabiosis. a, Heat map displaying up- and downregulated genes in young versus aged BECs based on bulk RNA-seq (n = 6 young and 6 aged biologically independent samples; each sample corresponds to biologically independent cortices or hippocampi pooled from two mice). There were 1,006 genes with significant differential expression (q < 0.05, Cuffdiff statistical package). b, Fragments per kilobase of transcript per million mapped reads (FPKM) of cell-type-specific markers for BECs. n = 6 young and 6 aged biologically independent samples. Data are shown as the mean ± s.e.m. c, FPKM values of inflammation- and activation-related genes. n = 6 young and 6 aged biologically independent samples. Data are shown as the mean ± s.e.m. Specific q values shown were derived from the Cuffdiff statistical package. See the Methods for details; source data are available online. d, Heat map showing changes in gene expression for 31 of 74 human plasma factors with aging (P < 0.05, Spearman’s correlation coefficient). A multiplex assay was used (n = 118 healthy humans). e, Spearman correlation of sVCAM1 protein levels and age (Spearman’s correlation coefficient = 0.47, P = 7.7 × 10−6, q = 5.72 × 10−5). f, Human sVCAM1 concentration in plasma from 11 young (<25 years old) and 11 aged (>65 years old) individual healthy donors, as determined by ELISA. **P = 0.0033, Student’s t test, two tailed. Data are shown as the mean ± s.e.m. g, Mouse sVCAM1 concentration in plasma from young (3-month-old; n = 8), middle-aged (8- to 10-month-old; n = 10) and aged (19-month-old; n = 8) mice as determined by ELISA. Data are shown as the mean ± s.e.m. ***P = 0.0001, ****P < 0.0001, one-way analysis of variance (ANOVA) with Tukey’s multiple-comparisons test. mo, months. h, Representative confocal images in the DG of young (3-month-old) or aged (18-month-old) mice given retro-orbital (ro.) injections of fluorescently conjugated anti-VCAM1 and anti-MEA-99 antibodies 2 h before perfusion. Hoechst labels cell nucleus. Arrowheads indicate VCAM1+ vessels. Three-dimensional renderings (3D) of the two-dimensional images (2D) are displayed. Scale bars, 50 μm. i, Quantification of VCAM1+lectin+ brain vasculature in hippocampi from young, middle-aged and aged mice. n = 12 young (3- to 4-month-old), 5 middle-aged (12-month-old), 11 aged (18-month-old) and 6 very aged (24-month-old) mice. VCAM1 was quantified in four separate cohorts of mice spaced 6 months or more apart. Data are shown as the mean ± s.e.m. ***P = 0.0002, ****P < 0.0001, one-way ANOVA with Tukey’s multiple-comparisons test. j, Quantification in the DG of VCAM1+lectin+ brain vasculature of young isochronic or heterochronic parabionts 5 weeks after surgery. Representative images are shown in Extended Data Fig. 1k. **P = 0.0071, Student’s t test, two tailed. Data are shown as the mean ± s.e.m. n = 8 mice in the young isochronic (Iso) group and 13 mice in the young heterochronic (Het) group from two independent experiments.
Among the three unique clusters, we identified one population largely negative for Vcam1 mRNA characterized by expression of genes relating to BBB metabolism, transport and the capillary phenotype (Fig. 2c–f). Interestingly, the remaining two clusters were both positive for Vcam1 expression but were molecularly distinct, with one expressing slightly higher levels of Vcam1 (C1) than the other (C2) (Fig. 2c). By using a biased classification method with known markers of the three main vessel types found in the BBB (Extended Data Fig. 2a; refs. 23–26), we found the Vcam1+ C2 cluster to express significantly higher levels of proinflammatory genes (Vwf among others) and postcapillary venous (venule and vein) markers (Nr2f2, Ephb4), whereas the Vcam1+ C1 cluster expressed genes involved in vascular remodeling and Notch signaling (Vegf, Notch1 and Edn1, among others) and arterial (artery and arteriole) classification markers (Bmx, Efnb2, Jag1) (Fig. 2d and Extended Data Fig. 2a,b; GeneCards; refs. 23–26).

We further applied the Mann–Whitney test to find differentially expressed genes between the three BEC subpopulations in an unbiased manner (Fig. 2e). Indeed, the Vcam1+ venous cluster was enriched for inflammatory and cytokine signaling genes.
(Tspo, Lrg1, Hif1a and B2m, among others) and pathways including tumor necrosis factor (TNF-α), NF-kB and cytokine signaling, among others (Fig. 2e–i; GeneCards). The Vcam1+ arterial cluster was differentially characterized by genes involved in matrix remodeling, migration and proliferation (Fbn5, Mgp, Bgn) and pathways including innate immunity, integrin and vasoendothelial growth factor (VEGF) signaling (Fig. 2e–g), Notch signaling genes and factors related to neurogenesis (Extended Data Fig. 2a,b; GeneCards; refs. 25–28). Venous and arterial Vcam1+ BECs expressed Tnfrsf1a, Il1r1, Il6ra and Il6st (Fig. 2i), which encode receptors for the inflammatory cytokines TNF-α, IL-1β and IL-6, respectively, known to induce Vcam1 expression through NF-kB signaling9,20,21 and increased in the circulation in neurodegeneration and aging22,23. Indeed, recombinant TNF-α and IL-1β, but not IL-6, were sufficient to induce Vcam1 expression in the hippocampus following retro-orbital injection in mice (Fig. 2i) or in cultured BECs (Extended Data Fig. 2c–e).

Plasma from aged mice increases Vcam1 expression, reduces NPC activity and increases microglial reactivity. To determine whether soluble factors in blood can increase cerebrovascular Vcam1 levels, we added plasma from aged mice to cultured BECs or infused it into young mice. Plasma from aged mice added to acutely isolated primary mouse BECs or a transformed mouse brain endothelial cell line, Bend.3 cells, significantly increased Vcam1 protein amount in comparison to plasma from young mice, whereas other adhesion molecules, namely ICAM1, E-selectin and P-selectin, were not significantly upregulated at the protein (Extended Data Fig. 2f–i) or mRNA (Extended Data Fig. 1d,q) level. Likewise, plasma from aged but not young mice infused into young mice (retro-orbitally) caused a significant increase in Vcam1 expression in lectin-positive blood vessels and acutely isolated BECs (Fig. 3a–d), whereas ICAM1 levels were not changed (Extended Data Fig. 2j,k).

In line with previous findings24–26, infusions with plasma from aged mice reduced the numbers of BrdU+ proliferating cells overall, as well as the numbers of BrdU+SOX2+ neural progenitor cells (NPCs; Fig. 3e,f) and doublecortin (DCX)+ immature neurons (Fig. 3g,h), in the granule cell layer (GCL) of the DG of young mice. Administration of recombinant TNF-α or lipopolysaccharide (LPS) in young mice also reduced NPC proliferation (Extended Data Fig. 2l,m). There was no change in the number of quiescent BrdU+SOX2+GFAP+ neural stem cells in the SGZ in mice treated with plasma from aged mice (Fig. 3f). Acute injections with plasma from aged mice also induced a prominent response in microglia, manifested as increased IBA1 immunoreactivity overall, expression of CD68 in IBA1+ cells and increased numbers of CD68+IBA1+ microglia (Fig. 3i,j). The total number of microglia did not change with this short-term plasma treatment (Fig. 3j).

Similarly to plasma from aged mice, repeated injections with plasma from aged humans over 3 weeks induced a prominent increase in BEC-specific Vcam1 expression in young immunodeficient NOD-scid IL-2Rγnull (NSG) mice (Extended Data Fig. 3a,b), reduced NPC activity and increased microglial reactivity (Extended Data Fig. 3c,d). NSG mice lack T and B lymphocytes and have defective natural killer cells, but VLA-4-expressing innate immune cells of the myeloid lineage, including neutrophils and monocyes, are intact27. Injection of human cord plasma (retro-orbitally) into aged NSG mice rejuvenates their brains28.

Genetic deletion of Vcam1 in BECs prevents effects of plasma from aged mice. To test whether Vcam1 abundance is simply a correlate of vascular inflammation or this protein is a possible mediator of the detrimental effects of plasma from aged individuals on the hippocampus, we deleted Vcam1 in BECs by using an Scler1-CreERT2 mouse, which encodes tamoxifen-inducible Cre recombinase under the control of the brain endothelial- and epithelial-specific Scler1 promoter29. Although unspecific recombination in tamoxifen-treated Scler1-CreERT2 mice can occur in granule neurons and possibly other cell types, when these mice were crossed with a tdTomato reporter line30, we did not detect any expression of Vcam1 protein in SOX2+, GFAP+, NeuN+ or DCX+ cells in the DG of the hippocampus (Extended Data Fig. 4a,c), even in aged mice, although as expected aquaporin-4 (AQP4)+ astrocytic end-feet outlined Vcam1+ vessels (Extended Data Fig. 3j,k). We confirmed that Scler1-CreERT2 (Cre+) mice underwent BEC-specific

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**Fig. 2 | Single-cell RNA-seq of Vcam1+–enriched BECs from young and aged mice.** a, Schematic of the BBB. Nutrient-rich, oxygenated blood is pumped into the brain through cerebral arterial BECs (arteries and arterioles), which are protected and supported by smooth muscle cells (SMCs) that cover the endothelium and form a basement membrane layered by astrocytic end-feet of the brain parenchyma. The blood is transferred to highly specialized capillaries, which comprise BECs that form unique tight junctions and are wrapped by pericytes (Peric.) within the endothelial basement membrane; these are in turn covered by astrocytic end-feet. BBB capillaries are the site of controlled transport of fluids and solutes into the CNS. Immune surveillance and occasional extravasation of leukocytes (Leuk.) into the CNS parenchyma occur at the level of postcapillary venous cells (venules and veins), the vascular segments into which blood flows after passing through the capillaries. Postcapillary venules have enlarged perivascular space between the endothelial and astrocytic basement membranes where immune cells can occasionally reside24,25. b, Unbiased clustering of 112 aged and 160 young hippocampal BECs using whole-transcriptome data and visualization with tSNE reveals three molecularly distinct BEC populations. c, Violin plots of Vcam1 show differing levels of the transcript in each of the cell clusters. Minima, maxima, medians and percentiles are listed in Supplementary Table 4; n=146 capillary BECs, n=59 venous BECs and n=67 arterial BECs pooled from eight mouse hippocampi. c.p.m., counts per million. d, Dot plot comparing the expression (scaled transcript counts and percent of population expressing) of various classical inflammatory, Notch signaling, arteriolar, venular and capillary markers between the three clusters (cluster 0, Vcam1 negative; cluster 1, Vcam1 positive; cluster 2, Vcam1 positive). e, Heat map of scaled expression of the top ten most enriched genes (differentially expressed with P<0.05, Mann-Whitney test, two sided) in each cluster. Genes are ranked by highest log-transformed fold change when compared to all other cells. f, tSNE visualization colored by Vcam1 expression level. Clusters are further annotated by their putative functional phenotype and vessel segmental identity; n=146 capillary BECs, n=59 venous BECs and n=67 arterial BECs pooled from the hippocampi of eight mice. g, GeneAnalytics (GEA package) BEC pathway analysis of the Vcam1+ venous and arterial hippocampal BEC clusters. The top ten pathways containing Vcam1 are highlighted here, along with the number of genes in each pathway enriched and the score assigned to each pathway. h, Violin plots of expression of various inflammation-related genes in each of the three distinct clusters. The age-related chemokine Ccl11 and its receptor, Ccr3, were not found to be expressed in isolated CD31+ BECs. Minima, maxima, medians and percentiles are listed in Supplementary Table 4; n=146 capillary BECs, n=59 venous BECs and n=67 arterial BECs pooled from the hippocampi of eight mice. i, Violin plots of expression of cytokine receptors enriched in the Vcam1+ venous cluster. Minima, maxima, medians and percentiles are listed in Supplementary Table 4; n=146 capillary BECs, n=59 venous BECs and n=67 arterial BECs pooled from the hippocampi of eight mice. j, Young (2.5-month-old) mice were injected with PBS control (n=5 mice at high dose and 3 mice at low dose), TNF-α (n=3 mice at high dose and 4 mice at low dose), IL-1β (n=4 mice at high dose and 4 mice at low dose) or IL-6 (n=4 mice at low dose) retro-orbitally (i.o.) daily over 5 d (2 µg per injection; low dose) or acutely (10 µg; high dose). Representative confocal images (bottom) and quantification (top) are shown for Vcam1 staining in the DG. Scale bar, 100 µm. Data are shown as the mean±s.e.m. *P=0.027, **P=0.041, ***P=0.028, ****P=0.006, one-way ANOVA with Dunnett’s multiple-comparisons test.
Vcam1 deletion following tamoxifen injections while using a systemic LPS inflammation model (Extended Data Fig. 4d–g). Systemic LPS administration significantly upregulated BEC-specific VCAM1 levels in tamoxifen-treated Slco1c1-CreERT2–/–; Vcam1flox (Cre+) control mice, but not in tamoxifen-treated Cre+ littermates (Extended Data Fig. 4d–g), and reduced VCAM1 protein amount in the blood vessels of Cre+ mice (Extended Data Fig. 3j,k).

We next investigated how short-term administration of plasma from aged mice affects young mice in the absence of brain endothelial- and epithelial-specific Vcam1 (Fig. 4a). While VCAM1 expression was absent in Cre+ brains, sVCAM1 levels remained high in the plasma of all tamoxifen-treated mice, indicating unperturbed peripheral expression (Fig. 4b–d). Notably, Vcam1 deletion in Cre+ brain endothelium abrogated the detrimental effects of plasma from
aged mice on hippocampal NPC activity and microglial reactivity (Fig. 4e–k). Thus, Cre+ mice treated with plasma from young or aged mice had equal levels of NPCs, as shown by equal numbers of cells in the BrdU+, BrdU+SOX2+ and DCX+ NPC populations (Fig. 4e–i). Moreover, plasma from aged mice also failed to induce microglial reactivity, as indicated by the levels of CD68 in IBA1+ cells (Fig. 4j,k). Similarly to wild-type mice (Fig. 3), Cre– control mice showed reduced NPC activity and increased microglial activation when exposed to plasma from aged mice (Fig. 4e–k). Likewise, long-term administration of plasma from aged mice did not induce microglial reactivity or inhibit NPC activity in the DG of young Cre+ mice lacking VCAM1 (Extended Data Fig. 5a–j). Interestingly, young mice lacking brain endothelial and epithelial Vcam1 for 3 weeks of young adulthood showed a lower baseline number of BrdU+SOX2+ NPCs in the hippocampus, whereas microglial number and activation were not affected (Extended Data Fig. 3e–i). It is possible that VCAM1 has additional functions in the maintenance of adult NPCs in young mice, as reported for the SVZ+ and discussed in more detail below. Notably, depletion of sVCAM1 from the plasma of aged humans before in vivo administration (Extended Data Fig. 6a–d) did not significantly change its adverse effects on NPC activity and microglial reactivity in young mice (Extended Data Fig. 6e–j), indicating that high levels of circulating sVCAM1 do not drive aging phenotypes in the young brain.

Monoclonal anti-VCAM1 antibody prevents detrimental effects of plasma from aged humans and mice. Leukocytes bind to VCAM1 primarily through α4β1 integrin, also known as VLA-4 (ref. 38), and an antibody against VLA-4 is used to treat multiple sclerosis and Crohn’s disease39. Blockade of the VCAM1–VLA-4 interaction also reduced seizures in a mouse model39, prompting us to determine whether anti-VCAM1 antibody treatment would mimic the effects of genetic Vcam1 deletion and antagonize the effects of plasma from aged individuals (Fig. 5a). Treatment with a well-characterized anti-VCAM1 antibody that binds to immunoglobulin domains 1 and 4 of the extracellular domain of the protein and prevents leukocyte tethering38 did not affect the increase in VCAM1 expression in brain endothelium following infusion with plasma from aged humans (Fig. 5b,c), whereas it completely prevented the inhibitory effects of plasma from aged humans on NPC activity (Fig. 5c,f) and microglial reactivity (Fig. 5d,g). In contrast, in PBS-treated control mice, anti-VCAM1 treatment had no effects on these parameters. Anti-VCAM1 antibody treatment similarly prevented the detrimental effects of plasma from aged humans in young NSG mice (Extended Data Fig. 6k–p).

To determine whether plasma from aged mice reduced the survival of newborn cells in the DG of the hippocampus and whether anti-VCAM1 treatment antagonized this effect, we pulsed young mice with 5-ethyl-2‘-deoxyuridine (EdU) before treatment with plasma from aged mice and antibodies (Fig. 5h). As shown earlier, young mice treated with plasma from aged mice and IgG isotype control antibody had reduced cell proliferation as assessed by numbers of BrdU+ cells and BrdU+SOX2+ NPCs, and this was reversed with anti-VCAM1 treatment (Fig. 5i,j). Interestingly, plasma from aged mice also reduced survival of EdU-labeled cells overall, EdU+SOX2+GFAP+ neural stem cells and newly formed Edu+NeuN+ neurons whereas it increased the number of EdU+GFAP+ astrocytes (Fig. 5k–n). These effects of plasma from aged mice were prevented by anti-VCAM1 treatment. Survival of immature Edu+DCX+ cells was not affected (Fig. 5l–n).

To investigate whether these overall beneficial effects of VCAM1 inhibition and depletion were linked to overt changes in BBB integrity, we injected young and aged Vcam1-deficient mice and mice exposed to traumatic brain injury (TBI) with a non-fixable 70-kDa tracer and perfused them 3 h later with a fixable 2-MDa tracer. We observed no differences in dextran diffusion in the hippocampus as a result of BEC VCAM1 expression, with the caveat that non-fixable tracers may underestimate BBB permeability40 (Extended Data Fig. 7a–d). Additionally, we did not observe an age-related change in the mRNA levels of the major tight junction or BBB permeability genes Cldn5 (claudin-5), Ocn (occludin), Tjp1 (ZO-1) and Pvel (plasmalemma vesicle-associated protein) at the single-cell level, although in bulk populations of BECs Tjp1 or Pvel levels were reduced with age (Fig. 1b and Extended Data Fig. 1e,r). Likewise, we observed no significant changes in the number of microglia, T cells, B cells, neutrophils, monocytes, macrophages or natural killer cells between young and aged mice with and without short- or long-term deletion of Vcam1 in BECs when using flow cytometry (Extended Data Fig. 7e,f and 8). Expression of VCAM-4 did not affect this result with this antibody to VCAM-4, which had increased numbers in 22-month-old Cre+ mice lacking Vcam1 in BECs (Extended Data Fig. 8c); in addition, we observed an increase in the number of CD45+CD11b+CD11c+IAIE+ dendritic cells in the brains of aged mice regardless of VCAM1 expression (Extended Data Fig. 8).

To determine whether the VCAM1 ligand VLA-4 is similarly involved in regulating age-related NPC activity and microglial reactivity, we treated aged wild-type mice with anti-VLA-4 antibody, which did not affect VCAM1 levels in the hippocampus.
(Extended Data Fig. 9a,b,d). While we observed a reduction in the numbers of IBA1^{+}CD68^{+} activated microglia, neural progenitor proliferation was unaffected (Extended Data Fig. 9c,e,f).

To determine whether the increase in VCAM1 as a result of normal aging (Fig. 1) might mediate similar negative effects on the aged brain as plasma from aged humans and mice does on a young brain, we treated aged male wild-type mice with anti-VCAM1 antibody (Fig. 6a). Strikingly, mice treated with anti-VCAM1 antibody for 3 weeks exhibited a general increase in the number of BrdU^{+} cells as well as BrdU^{+}SOX2^{+} NPCs (Fig. 6b,d) and a significant reduction in the number of IBA1^{+}CD68^{+} activated microglia (Fig. 6c,e). In agreement with these results, Cre^{+} mice lacking Vcam1 long term from 2 to 18 months showed reduced microglial activation and increased NPC proliferation (Fig. 6f-h and Extended Data Fig. 9g,h). Anti-VCAM1 antibody treatment similarly promoted NPC proliferation and reduced microglial activation in aged female mice, which, like male mice, showed age-related increases in the levels of soluble and BEC-specific VCAM1 (Extended Data Fig. 5k–o).
Fig. 4 | Brain endothelial- and epithelial-specific Vcam1 deletion in young mice mitigates the effects of administration of plasma from aged mice.

a, Experimental design. n = 7 Cre− mice and 8 Cre+ mice administered young mouse plasma and 8 Cre+ mice administered aged mouse plasma. Plasma administration in these transgenic mice was performed one additional time in a long-term paradigm with similar results (Extended Data Fig. 5). Plasma administration was performed in eight independent experiments with similar results (Supplementary Table 3). b, Representative confocal images in the DG of staining for Vcam1, MECA-99 and AQP4. Hoechst labels cell nuclei. Scale bars, 200 µm (merged images, left) and 50 µm (magnified Vcam1 and MECA-99 merged images, right). Tissue was stained and Vcam1 was measured in all 31 mice in this study.

c, Quantification of Vcam1+lectin+ vasculature. ***P = 0.0031, two-way ANOVA with Tukey’s multiple-comparisons test. Data are shown as the mean ± s.e.m. n = 7 Cre− mice and 8 Cre+ mice administered young mouse plasma and 8 Cre+ mice administered aged mouse plasma.

d, Mouse sVcam1 concentration in plasma samples, as determined by ELISA. *P = 0.022, two-way ANOVA with Tukey’s multiple-comparisons test. Data are shown as the mean ± s.e.m. n = 7 Cre− mice and 8 Cre+ mice administered young mouse plasma and 8 Cre+ mice administered aged mouse plasma.

e–g, BrdU quantification (e) and representative confocal images (f) and BrdU+SOX2+ quantification (g) in the DG of brain sections immunostained for BrdU and SOX2. White dashed lines outline the SGZ. Scale bars, 100 µm. *P = 0.02, **P = 0.017, two-way ANOVA with Tukey’s multiple-comparisons test. Data are shown as the mean ± s.e.m. n = 7 Cre− mice and 8 Cre+ mice administered young mouse plasma and 8 Cre+ mice administered aged mouse plasma.

h, DCX quantification (h) and representative confocal images (i) in the GCL. Hoechst labels cell nuclei. Scale bar, 100 µm. **P = 0.0015, two-way ANOVA with Tukey’s multiple-comparisons test. Data are shown as the mean ± s.e.m. n = 7 Cre− mice and 8 Cre+ mice administered young mouse plasma and 8 Cre+ mice administered aged mouse plasma.

i–k, Representative confocal images (j) and quantification (k) in the DG of CD68 and IBA1. Hoechst labels cell nuclei. Scale bars, 100 µm. ***P = 0.0008, ****P = 0.0061, two-way ANOVA with Tukey’s multiple-comparisons test. Data are shown as the mean ± s.e.m. n = 7 Cre− mice and 8 Cre+ mice administered young mouse plasma and 8 Cre+ mice administered aged mouse plasma.
To test whether anti-VCAM1 therapy exerts cognitive benefits, we treated young and aged NSG and C57BL/6 mice with anti-VCAM1 antibody or IgG as a control and tested them in contextual fear conditioning, novel object recognition and Barnes maze tasks, established paradigms to assess age-related impairments in hippocampal-dependent learning and memory.16,17 Remarkably, aged C57BL/6 mice treated with anti-VCAM1 antibody, but not with IgG, reached the escape hole in the Barnes maze with similar efficiency as young mice on the final two testing days, and they showed significantly increased interaction with a novel object in comparison to IgG-treated mice (Fig. S1j). Thirteen-month-old NSG mice, which exhibit accelerated age-related cognitive decline18, showed increased interaction with a novel object (Extended Data Fig. S1i) but no significant difference in contextual freezing (Extended Data Fig. S1g,k) upon treatment with anti-VCAM1 antibody for 1 month. Likewise, a cohort of 23-month-old C57BL/6 wild-type mice, in which all mice exhibited similar baseline freezing (Extended Data Fig. S1l), demonstrated a significant increase in contextual freezing (Fig. S1k and Extended Data Fig. S1n) but not cued (Extended Data Fig. S1m) memory following treatment with anti-VCAM1 antibody. All in vivo experiments are summarized in Supplementary Table 4.

Discussion
Our studies uncovered a new role for the endothelial cell adhesion molecule VCAM1 in regulating brain function with aging. Maybe most surprisingly and of potential therapeutic relevance, administration of anti-VCAM1 antibody in aged mice led to increased NPC activity, reduced microglial reactivity and improved hippocampal-dependent learning and memory (Fig. 6 and Extended Data Figs. 5 and 9). Moreover, long-term genetic deletion of Vcam1 in BECs during adulthood also reduced microglial activation and resulted in increased NPC numbers in the hippocampus of aged mice (Fig. 6d–f). On the basis of our experimental evidence, we propose the following model: (1) factors in plasma from aged individuals, including TNF-α and IL-1β, induce BEC expression of VCAM1; (2) VCAM1 facilitates tethering, but not transmigration, of leukocytes, which sustains BEC inflammation; and (3) inflamed and activated venous and arterial VCAM1+ BECs relay signals to the parenchyma to activate microglia, inhibit NPC activity and impair cognition (Extended Data Fig. 10). Naturally, future work will have to refine, revise and test other aspects of this model.

We report that VCAM1 expression on BECs, the major cell type of the BBB, is upregulated during normal aging or by exposure to dialyzed plasma from aged individuals. As VCAM1 is shed constitutively by ADAM17 (refs. 20,41), we observe a concomitant increase in sVCAM1 levels with aging in blood (Fig. 1). Interestingly, sVCAM1 levels correlated negatively with cognitive impairment and cerebrovascular dysfunction in 680 elderly participants.42 Indeed, VCAM1 is increased not only with normal aging in mice43 and humans44, but also in peripheral endothelium in atherosclerosis,45 cancer and inflammatory diseases46 and in BECs in AD,46 multiple sclerosis49 and epilepsy.50 While Elahy et al. reported no increase in VCAM1 expression in aged mice,51 we detected the increase with age by immunofluorescence and flow cytometry only after retro-orbital injection of fluorescently conjugated anti-VCAM1 antibody before mouse perfusion and cell processing or by using scRNA-seq (Figs. 1 and 3, and Extended Data Fig. 1).

The circulating factors mediating the observed pro-aging effects on the brain are unknown at this point, but because we dialyzed the plasma, removing most metabolites and small molecules, we think proteins are responsible for communicating many of the circulatory signals to the brain. Indeed, circulating cytokines and chemokines with detrimental effects on the brain have increased levels in blood with advanced age,52 and TNF-α, IL-1β and IL-4 induce expression of endothelial VCAM1 through NF-κB signaling53,54, in line with our findings (Fig. 2). In contrast, the shed form of VCAM1 is unlikely to be a culprit, because depletion of sVCAM1 from the plasma of aged individuals did not affect its capacity to increase VCAM1 levels, activate microglia or inhibit NPC activity (Extended Data Fig. 6).

How then do increased levels of VCAM1 in BECs result in brain dysfunction with age, as proposed in our model? It is possible that leukocytes expressing VLA-4 bind to VCAM1 expressed on venule BECs, releasing detrimental factors toward the endothelium (as seen in atherosclerosis) and/or activating BECs via VCAM1 (refs. 51–53). Indeed, ligand binding to VCAM1 can induce Ca2+ mobilization, hydrogen peroxide production, and p38 MAPK and protein kinase C (PKC)-α activation, leading to concerted activation of endothelial cells.55 While these events are necessary for transcytosis of leukocytes, aging or plasma from aged individuals, in our hands, did not result in upregulation of Icam1, E-selectin or P-selectin, proteins required for transcytosis. Indeed, during normal aging, leukocyte recruitment into the brain parenchyma is minimal or absent (Extended Data Figs. 7 and 8; refs. 43,52). Likewise, hetero...
ochronic parabiosis studies using a GFP-expressing transgenic mouse showed no evidence of infiltrating GFP+ leukocytes in the brains of GFP+ parabionts. Nevertheless, our studies with systemically administered anti-VCAM1 or anti-VLA-4 antibodies (Figs. 5 and 6, Extended Data Figs. 5, 6 and 9, and Supplementary Table 4) make it reasonable to conclude that leukocytes—but not T, B or natural killer cells, which are absent in NSG mice—are involved in the adverse effects of plasma from aged individuals on the brain. Interestingly, anti-VLA-4 antibody did not affect NPC activity in aged mice, possibly because leukocytes could still tether to BECs through other pathways. Alternatively, progenitor proliferation in aged mice, already reduced to less than 10% of that in young mice, may no longer be susceptible to rescue by peripheral modulation of leukocyte binding to brain endothelium.
Fig. 6 | VCAM1 perturbation reverses age-related impairments and improves hippocampal-dependent learning and memory. a. Experimental design for anti-VCAM1 treatment. n = 7 mice per group. b, Representative confocal images of BrdU and SOX2 from the experiment described in a. Arrowheads indicate proliferating NPCs. The white lines outline the SGZ. Scale bar, 100 µm. n = 7 mice per group. c, Representative confocal images of CD68, IBA1 and Hoechst from the experiment described in a. Scale bar, 100 µm. n = 7 mice per group. d, Quantification in the DG of BrdU and SOX2. n = 7 mice per group. Data are shown as the mean ± s.e.m. **P = 0.0341, ***P = 0.0027, two-tailed Student’s t test. e, Quantification in the DG of CD68 and IBA1 from confocal images. n = 7 mice per group. Data are shown as the mean ± s.e.m. **P = 0.0075, *P < 0.001, two-tailed Student’s t test. f, Experimental design for conditional deletion of Vcam1 in young (2-month-old) mice followed by aging to 18 months. n = 8 mice per group. g, Quantification of total BrdU+ proliferating cells and BrdU+SOX2+ NPCs in immunostained sections of DG. n = 8 mice per group. Data are shown as the mean ± s.e.m. **P = 0.0075, ***P = 0.0263, two-tailed Student’s t test. h, Quantification in the DG of CD68 and IBA1. Hoechst labels cell nuclei. n = 8 mice per group. Data are shown as the mean ± s.e.m. **P = 0.0068, **P = 0.0169, two-tailed Student’s t test. i, Escape latency on days 1–5 from the Barnes maze (a) and percentage of time spent exploring objects in the novel object placement task (j) for IgG-treated young adult C57BL/6 mice (5 months old; n = 15) and IgG-treated aged mice (17 months old; n = 15) or aged mice treated with anti-VCAM1 monoclonal antibody (17 months old; n = 15). All mice received intraperitoneal injections every 3 d for 3 weeks before initiation of the behavioral studies and throughout the duration of the studies. **P = 0.0217, ***P < 0.001, ****P < 0.0001, two-way repeated-measures ANOVA with Bonferroni’s post hoc test for time x group comparisons and one-way ANOVA with Tukey’s post hoc test for group comparisons. Data are shown as the mean ± s.e.m. k, Quantification of freezing behavior in fear conditioning contextual trials with 23-month-old C57BL/6 mice injected with anti-VCAM1 monoclonal antibody or IgG every 3 d for 1 month. Averages from trials 3–5 are shown. n = 7 PBS-, n = 12 IgG- and n = 13 anti-VCAM1-treated mice. **P = 0.0075, ***P = 0.0265, one-way ANOVA with Tukey’s post hoc test for group comparisons. Data are shown as the mean ± s.e.m.
Finally, in the third step of our model, we propose that activated VCAM1+ BECs function as relay stations of circulatory signals (or cells) communicating information as part of the neurovascular unit to surrounding glia and neurons. Intriguingly, we observe VCAM1 expression in only a small subset of BECs, even after LPS stimulation (Figs. 1 and 3, and Extended Data Figs. 1, 3 and 8). scRNA-seq of isolated hippocampal BECs identified three unique subpopulations, in line with a recent scRNA-seq study of mouse brain endothelium:\(^1\) (1) Vcam1-negative capillaries expressing characteristic BBB genes related to transport and metabolism, (2) Vcam1-positive arterial BECs enriched in Notch signaling markers and (3) Vcam1-positive venous BECs expressing inflammatory gene transcripts (Fig. 2 and Extended Data Figs. 1 and 2). Considering that even young, healthy mice contain VCAM1-expressing subpopulations of BECs, we hypothesize that these cells function as the above-mentioned relay stations and environmental sensors. In addition to the canonical roles of VCAM1 in leukocyte extravasation and inflammation in venous BECs, Vcam1-positive arterial BECs are enriched for transcripts related to Notch signaling and vascular remodeling. We speculate that the regenerative effect of Vcam1 deletion in young mice (Fig. 4) and, possibly, the perturbation of homeostasis in the SVC neural stem cell niche\(^2\) (see discussion below) may involve these specialized Vcam1-positive arterial BECs.

Given that the transcriptional profile of BECs changes drastically with age, exhibiting an overall activated, proinflammatory signature (Fig. 1), it could be expected that BBB function is different between young and aged mice. Indeed, it is generally agreed that the ultrastructural composition, the activity of the neurovascular unit and the transport of various types of molecules change with age\(^3\). Montagne et al. used advanced dynamic contrast-enhanced magnetic resonance imaging in brains in living humans and reported that the BBB deteriorates and becomes more permeable in the aged hippocampus\(^4\). Although studies focused on normal aging remain limited, Bien-Ly et al. showed that multiple mouse models of AD have limited uptake of therapeutic antibodies owing to intact BBB and limited permeability\(^5\). Although fixable large and non-fixable small dextran tracers indicate no overt leakage of the BBB with aging or following genetic Vcam1 deletion in our studies (Extended Data Fig. 7a–d), we observed some age-related differences in tight junction gene expression with aging (Fig. 1b and Extended Data Fig. 1e,r). In conclusion, it is possible that VCAM1 contributes to the overall regulation of BBB function with aging, but additional studies involving more refined methods will be required to test this possibility.

Apart from the new role in hippocampal aging and function we describe here, VCAM1 is required under non-pathological conditions for type B neural stem cell anchoring to the neurogenic niche of the SVZ\(^6\), where it is highly expressed in endothelial cells of the lateral ventricles and epithelial cells of the choroid plexus\(^7\). Kokovay et al. showed that VCAM1 expression increases in the lateral ventricles as a result of increased inflammatory cytokine signaling, leading to production of reactive oxygen species and restriction of neural stem cell proliferation and lineage progression in the SVZ\(^8\). Intraventricular infusion of anti-VCAM1 antibody activated type B neural stem cells to a proliferative state\(^9\). Additionally, it was recently shown that VCAM1 expression in radial glial cells is necessary for embryonic neurogenesis and development of the SVZ neurogenic niche\(^10\). Although this seems to contradict our findings, we indeed observed that genetic deletion of Vcam1 in young Cre+ mice in which Vcam1 was flanked by loxp sites resulted in a reduction in baseline NPC activity. Thus, VCAM1 seems to have dual roles in regulating adult NPC activity, supporting a homeostatic role related to anchoring of stem cells in their niche, as well as a role in aging-related inflammation, which inhibits NPC activity. In other words, genetic ablation of brain endothelial and epithelial Vcam1 in young animals may reduce NPC activity owing to depletion of the quiescent neural stem cell population. Increased VCAM1 with aging and inflammation, on the other hand, may reduce NPC activity by restricting neural stem cell activation and lineage progression. Additionally, activated microglia might also be directly inhibiting NPC activity via secretion of inflammatory soluble factors\(^11\).

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41591-019-0440-4.

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**Methods**

Mice. NSG immunodeficient mice were purchased from Jackson Laboratory. NSG mice were bred and only males were used for plasma treatment studies. Heterozygous SklO1c1-CreERT2 breeding mice were provided by M. Schwaninger. Mice were bred and crossed with Vcam1fl/fl (B6.129-C3Vcam1tm2Flv/J) mice purchased from Jackson Laboratory. Male mice were used for plasma treatment studies following treatment with tamoxifen (an estrogen modulator). Aged (greater than 12 months of age) C57BL/6J males and females were obtained from the National Institute on Aging (NIA), and young C57BL/6J males (2–4 months of age) were purchased from Jackson Laboratory and Charles River. BALB/c Nctr- were purchased from Jackson Laboratory and Charles River. BALB/c Nctr- younger than 12 months of age) C57BL/6J males and females were obtained from the Animal Welfare Act and were in accordance with institutional guidelines and approved by the VA Palo Alto Committee on Animal Research and the institutional administrative panel of laboratory animal care at Stanford University.

Human blood samples. Human blood samples from healthy males in the age range of 18–25 and 65–74 years were anonymously donated to the Stanford Blood Center, Palo Alto. Blood was centrifuged at 1,600 × g for 10 min at 4°C, and plasma was collected and centrifuged again at 1,600 × g for 10 min at 4°C. Plasma was dialyzed by using cassettes (Slide-A-Lyzer Dialysis Cassettes, 3.5k MWCO, 12 ml) before each use. Plasma was transferred into 1,000 µl aliquots and stored at −80°C until further processing. If mice were injected with fluorescently labeled anti-mouse VCAM1-DL488 as described above, CD45 was stained in the APC/Cy7 channel and CD31+VCAM1+ cells were also gated in the APC and FITC channels.

**Cell culture and plasma treatment.** Cells were cultured in DMEM supplemented with 10% FBS and 1% Pen-Strep. Cells were plated in 24-well plates at 5 × 10^4 cells/well and cultured overnight in a 5% CO2 incubator. After overnight incubation, medium was discarded and replaced with fresh medium containing plasma and treated with tamoxifen (an estrogen modulator). Aged (greater than 12 months of age) C57BL/6J males were injected with either LPS (100 µg/kg of body weight) or PBS. Experimental mice received intraperitoneal and subcutaneous injections of sterile 0.9% saline with 5% glucose levels during the procedure. Mice received a third LPS injection followed by retro-orbital injections of either 100 µg fluorescently labeled (DyLight 488, Thermo Scientific, 53025) In VivoMab anti-mouse CD106 (VCAM1, clone M/K-2.7, BioXcell, BE0027) or fluorescently labeled rat IgG1 isotype antibody (clone 1A11, BioXcell, BE0009). Two hours after the last LPS injection, mouse brains were collected for BEC isolation and flow cytometry analysis.

Human blood samples. Human blood samples from healthy males in the age range of 18–25 and 65–74 years were anonymously donated to the Stanford Blood Center, Palo Alto. Blood was centrifuged at 1,600 × g for 10 min at 4°C, and plasma was collected and centrifuged again at 1,600 × g for 10 min at 4°C. Plasma was dialyzed by using cassettes (Slide-A-Lyzer Dialysis Cassettes, 3.5k MWCO, 12 ml) before each use. Plasma was transferred into 1,000 µl aliquots and stored at −80°C until further processing. Plasma was dialyzed and immediately snap-frozen on dry ice and stored at −80°C until further processing. Plasma was dialyzed in 4 liters of fresh PBS with a stir bar for 45 min at room temperature, with fresh PBS added every 20 min. Cassettes were transferred to 4 liters of fresh PBS with a stir bar for overnight dialysis at 4°C. Plasma samples from five aged individuals >65 years old were pooled together for aged human plasma injections in vitro studies. Plasma samples anonymously donated by five young adults <25 years old were pooled together for in vitro use. Plasma was aliquoted to prevent more than one freeze–thaw cycle and stored at −80°C until further use.

**Plasma collection, dialysis and processing.** Mouse. Approximately 500 µl of blood was drawn from the heart in 250 µM EDTA (Sigma-Aldrich, CAS no. 60-00-4) and immediately transferred to ice. Blood was centrifugated at 1,000 × g at 0 min at 10°C for 4°C. Plasma was dialyzed and immediately snap-frozen on dry ice and stored at −80°C until further processing. Plasma was dialyzed in 4 liters of fresh PBS with a stir bar for overnight dialysis at 4°C. Plasma samples from five aged individuals >65 years old were pooled together for aged human plasma injections in vitro studies. Plasma samples anonymously donated by five young adults <25 years old were pooled together for in vitro use. Plasma was aliquoted to prevent more than one freeze–thaw cycle and stored at −80°C until further use.

**Proteomics (human plasma, VCAM1 analysis).** Britschgi et al. measured plasma factors in cognitively normal individuals and patients with AD by multiplex assay, which measured 74 cytokines, chemokines, growth factors and related proteins in plasma using various multiplex immunoassays as described. We used the raw plasma data generated in which low values were replaced with the lowest detectable value measured in patients with AD or controls (Data 4 of the supplementary data) and focused our analysis on subjects who were cognitively normal (n = 118 subjects; 59 males and 59 females). The age range was between 50 and 90 years with an average age of 68 years. We replaced QNS (quantity not sufficient) values in the dataset by NA and log-transformed the data to measure the strength of the relationships between age and levels of plasma factors, we used the R Segmented package to calculate Spearman’s correlation coefficients. To visualize the changes in levels of plasma factors with aging, mean value per decade were calculated for each plasma factor and hierarchical clustering was applied.

**RNA sequencing.** Bulk RNA-seq. Mouse hippocampi and cortices (two pooled brains per sample; n = 6 young (3-month-old C57BL/6J male) samples or n = 6 aged (19-month-old C57BL/6J male) samples were dissected using the neural dissociation kit (Millenyi, 130-902-628) following perfusion. BECs (average of 81,000 C57BL/6J CD45 +CD11b+ cells per pooled sample) were isolated using multiple-lane flow cytometry and sorted directly into RNA-later as described above. Frozen cells were thawed to room temperature before centrifugation for 10 min at 1,000g. Total RNA was isolated from the cells with the RNeasy Plus Micro kit (Qiagen, 74204). RNA quantity and quality were assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies). All samples passed a quality-control threshold (RIN ≥ 8.5) to proceed to library preparation and RNA-seq.

Total mRNA was transcribed into full-length cDNA by using the Smart-Seq v4 Ultra-Low-Input RNA kit from Clontech according to the manufacturer’s instructions. Samples were validated with an Agilent GeneChip Human G4128A Agilent High-Sensitivity DNA kit. Full-length cDNA (150 pg) was processed with the Nextera XT kit from Illumina for library preparation according to the manufacturer’s protocol. Library quality was verified with an Agilent 2100 Bioanalyzer and Agilent High-Sensitivity DNA kit. Sequencing was carried out with an Illumina HiSeq 2000 (paired-end, 2 × 100 bp) sequence.

FastQC v0.11.2 was used to provide quality-control checks on the raw RNA-seq data. STAR v2.4.2a was used to align the RNA-seq reads to the mouse reference genome (mm9). The Cuffdiff v2.2.1 statistical package was used to perform differential expression analysis for RNA-seq on the basis of gene and transcript abundance measurements in FPKM, as previously described. The R v3.3.2 statistical package and v2.12.1 R package were used for visualization of the various output files of the Cuffdiff differential expression analysis including visualization of 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 TreeMap v1.1.6 was used to visualize the output files from hierarchical clustering in the form of heat maps displaying genes that were differentially up- or downregulated in BECs from aged versus young mice. The 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 BECs from young and aged mice.

**Single-cell RNA-seq of VCAM1−enriched BECs.** Four young (3-month-old) and four aged (19-month-old) C57BL/6J male mice were injected (retro-orbitally) with fluorescently labeled anti-VCAM1 monoclonal antibody 2h before they were killed and cells were gated for single-cell isolation of CD31+VCAM1+ cells from pooled hippocampi following perfusion. Gates were based on positive-control

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LPS-stimulated mice injected with fluorescently labeled anti-VCAM1-DL488 monoclonal antibody or IgG-DL488 control antibody.

Hippocampi (from both hemispheres) were pooled from four young (3-month-old) or aged (19-month-old) C57BL/6J males, sorted into lysate buffer in 96-well plates and then snap frozen and stored at −80°C until RNA extraction and library preparation. Two 96-well plates per group contained BECs that were 50% enriched for high expression of VCAM1 on the basis of flow cytometry gating; unbiased Cd31+ cells were also collected into two 96-well plates per group.

cDNA synthesis, library preparation and sequencing. Cell lysis and cDNA synthesis were performed with the Smart-Seq2 protocol as described previously13, with some modifications. After cDNA amplification (23 cycles), cDNA concentrations were determined via capillary electrophoresis and cells were cherry-picked to improve quality and decrease cost of sequencing. Cell selection was done using custom custom scripts and simultaneously normalized cDNA concentrations to ~0.2 ng μl−1 per sample, by using the TTP Labtech Mosquito HTS and Manitas (Formulaatrix) robotic platforms. Libraries were prepared with Illumina Nextera XT kits following the manufacturer’s instructions. Libraries were then sequenced on a NextSeq instrument (Illumina) by using 2 × 75 bp paired-end reads and 2 × 8 bp index reads with a 200-cycle kit (Illumina, 2012861) and pooled by the Mosquito liquid handler. Library quality was assessed via capillary electrophoresis on a Fragment Analyzer (AATI) and quantified by qPCR. Samples were sequenced at an average of 700,000 reads per cell.

Bioinformatics pipeline. Sequences from the NextSeq were demultiplexed with bcftools and reads were aligned to the mm10 genome augmented with ERCC sequences, by using STAR version 2.5.2b. Gene counts were made with HTSeq version 0.6.1p1. We applied standard algorithms for cell filtration, feature selection and dimension reduction. First, genes appearing in fewer than three cells, cells with fewer than 500 genes and cells with fewer than 50,000 reads were excluded from the analysis. Of the remaining reads, more than 30% of reads as ERCC sequences or more than 5% mitochondrial or 3% ribosomal sequences were also excluded. Counts were log normalized (log(1 + counts per million)) and then scaled via linear regression against the number of reads, the percentage mapping to ribosomal genes and the percentage mapping to mitochondrial genes. To select for relevant features, genes were first filtered to a set of 3,000 with the highest positive and negative pairwise correlations. Genes were then projected into low-dimensional principal-component space with robust principal-component analysis (rPCA). Single-cell principal-component scores and gene loads for the first 20 principal components were analyzed with the Seurat package in R. Briefly, a shared nearest-neighbor graph was constructed on the basis of the Euclidean distance metric in principal-component space and cells were clustered via the Louvain method. Cells and clusters were then visualized with 3D t-distributed stochastic neighbor embedding on the same distance metric. Differential gene expression analysis was done by applying the Mann–Whitney U test to the BEC clusters obtained by unsupervised clustering. P values were adjusted via the false–discovery rate (FDR) or Bonferroni method. All graphs were generated and analyses performed in R.

GeneAnalytics and GeneCards packages offered by the GSEA tool were used for GO pathway analysis and classification of enriched genes in each subpopulation.

Cell culture. For all studies, Bend.3 cells (a gift of the Butcher laboratory; purchased from the ATCC) were used. Bend.3 cells are immortalized brain endothelial cells isolated from BALB/c mice (CRL-2299, ATCC). These cells were seeded at 40,000 cells cm−2 in MCDB-131 HUVEC medium (10372019, Gibco), followed by treatment in DMEM with 10% pooled and dialyzed serum derived from plasma from young (25 years or younger) or aged (65 years or older) humans (Bend.3 cells) or plasma from mice (3-month-old) or aged (19-month-old) mice (primary BECs and Bend.3 cells) for 16 h. Plasma was warmed to 37 °C and filtered through a 22-μm filter before being added to cells. The following day, cells were fixed in cold 4% paraformaldehyde for 10 min, followed by three 5-min washes with 1% 0.25% Triton X-100 (TBS++) and primary antibody (1:250) overnight: anti-VCAM1 (ab19569, Abcam) and anti-VE-cadherin (sc-6458, Santa Cruz Biotechnology). Cells were blocked in TBS++ and secondary antibody (1:250) for 45 min the next day (Alexa Fluor 488: A10266, Life Technologies; Alexa Fluor 647: A10277, Life Technologies).

TNF-α dosage response. Bend.3 cells were seeded overnight as described above and serum starved for 1 h before being cultured for 24 h in varying concentrations of TNF-α (5 ng ml−1 to 156.25 pg ml−1) in DMEM, followed by staining with antibodies to CD31 and VCAM1 and flow cytometry analysis.

In vitro cytokine treatment and flow cytometry analysis. Bend.3 cells were seeded at 10,000 cells per well in six-well plates (seeded at 400,000 cells per well) were serum starved for 1 h followed by overnight treatment for 16 h with recombinant TNF-α (10 ng ml−1). IL-1β (10 ng ml−1) or IL-6 (30 ng ml−1). After overnight treatment, medium was aspirated and 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 of PBS. Cells were centrifuged for 5 min at 300 t.c.f. and medium was aspirated. After centrifugation, cells were resuspended in PBS for one wash followed by blocking for 30 min in FACS buffer (PBS + 1 g/μl glucose + 30 mM sodium pyruvate + 2% BSA with 2 mM EDTA). Following centrifugation, cells were resuspended in 100 μl per sample of FACS buffer. Fc blocking antibody (553142, BD Pharmingen) was added for 10 min followed by addition of each antibody (1:50). Samples were incubated in antibodies for 30 min to 1 h with shaking at room temperature and covered from light. After one wash, with FACS buffer, cells were resuspended in 500 μl of FACS buffer and transferred to flow tubes for analysis. Bend.3 cells were stained with a conjugated anti-CD31 antibody (CD31-APC; 551262, BD Pharmingen) and an anti-VCAM1 antibody (BE0027, BioXcell) fluorescently conjugated with the DyLight 488 Conjugation kit according to the manufacturer’s instructions (53024, Thermo Scientific). FSC-A and FSC-W blotting was used to identify single cells and cell aggregates. PI+ dead cells were excluded. Cells were gated on the basis of positive staining for CD31 and VCAM1 in comparison to n-1 control staining and IgG isotype control staining.

In vitro VCAM1 analysis following plasma administration. Immunofluorescence analysis. Bend.3 cells or primary BECs were seeded in eight-well chamber slides (154534, Thermo Scientific) overnight with 40,000 cells cm−2. Cells were serum starved for 1 h via incubation in DMEM with no added supplements (11965-092, Gibco), followed by treatment in DMEM with 10% pooled and dialyzed serum derived from plasma from young (25 years or younger) or aged (65 years or older) humans (Bend.3 cells) or plasma from young (3-month-old) or aged (19-month-old) mice (primary BECs and Bend.3 cells) for 16 h. Plasma was warmed to 37 °C and filtered through a 22-μm filter before being added to cells. The following day, cells were fixed in cold 4% paraformaldehyde for 10 min, followed by three 5-min PBS washes and blocking for 1 h in TBS with 3% donkey serum and 0.25% Triton X-100 (TBS++). Cells were blocked in TBS++ and primary antibody (1:250) overnight: anti-VCAM1 (ab19569, Abcam) and anti-VE-cadherin (sc-6458, Santa Cruz Biotechnology). Cells were blocked in TBS++ and secondary antibody (1:250) for 45 min the next day (Alexa Fluor 488: A10266, Life Technologies; Alexa Fluor 647: A10277, Life Technologies).

In vitro cytokine treatment and flow cytometry analysis. Bend.3 cells were seeded for 1 h followed by overnight treatment for 16 h with 10% plasma-derived serum from young or aged mice or humans, as described above for in vitro VCAM1 analysis. After overnight treatment with plasma, medium was aspirated and 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 of PBS. Cells were centrifuged for 5 min at 1,100 r.p.m., medium was aspirated and cells were resuspended in 1 ml per well of PBS with 4% paraformaldehyde (diluted with 8 ml of PBS) and fixed on ice for 10 min. After centrifugation at 1,100 r.p.m. for 5 min, cells were resuspended in PBS for one wash followed by blocking for 30 min in FACS buffer (PBS + 2% BSA with 2 mM EDTA). Following centrifugation, cells were resuspended in 100 μl per sample of FACS buffer. Fc blocking antibody (553142, BD Pharmingen) was added for 10 min followed by addition of each antibody. Samples were incubated in antibodies for 30 min to 1 h on ice. After two washes with FACS buffer, cells were resuspended in 500 μl of FACS buffer and transferred to flow tubes for analysis. Bend.3 cells were stained with a conjugated anti-CD31 antibody (CD31-APC; 551262, BD Pharmingen), an anti-VCAM1 antibody (BE0027, BioXcell) conjugated with the DyLight 488 Conjugation kit according to the manufacturer’s instructions (53024, Thermo Scientific) and rat anti-mouse E-selectin-DL405 (clone RB40.34, a gift of the Butcher laboratory). Plasma-treated Bend.3 cells were also stained with anti-CD31-PE/Cy7 (102418, BioLegend), anti-ICAM1-DL394 (clone TN1/1.7.4, a gift of the Butcher laboratory) and anti-P-selectin-DL405 (clone 10E9.6, a gift of the Butcher laboratory). Cells were gated on forward (FSC, size) and side (SSC, internal structure) scatter. FSC-A and FSC-W blotting was used to discriminate single cells from cell doublets and aggregates. PI− dead cells were excluded. CD31+ cells were defined as BECs.

In vivo mouse studies. Parabiosis. Isochronic and heterochronic parabiosis was performed as described16. In brief, mirror-image incisions through the skin were made on the left and right flanks of mice and shorter incisions were made through the abdominal wall. Parabionts were sutured together at their adjacent peritoneal openings. The parabionts’ elbow and knee joints were also sutured together and the skin of each mouse was stapled together. For 1 week during recovery after surgery,
each parabiont mouse received daily subcutaneous injections of Baytril antibiotic solution (5 µg per gram of body weight in saline to give a volume of approximately 1% of the weight of each mouse) and buprenorphine (0.1 mg kg⁻¹, 0.05 mg per kg) as stress relief, prevention of infection and hydration and were monitored regularly.

In vivo cytokine injections. For low-dose cytokine injections, young (2.5-month-old) C57BL/6 littersmates were injected every other day (0.08 mg per kg retro-orbitally) with recombinant proinflammatory cytokines (TNF-α, IL-1β or IL-6) for 5 d (n = 4 mice per group for four groups). Mice were injected retro-orbitally with 0.05 mg of fluorescently labeled (DyLight 488, Thermo Scientific, 53025) rat anti-mouse-VCAM1 antibody (clone M-K-2.7, BioXcell, BE0027) before perfusion. Two hours after the final injections, mice were anesthetized with Avertin followed by PBS perfusion.

For high-dose acute cytokine treatment, young (2.5-month-old) C57BL/6 littersmates were injected (0.4 mg per kg retro-orbitally) with TNF-α, IL-1β or PBS control (n = 3–4 mice per group for four groups). Mice were injected retro-orbitally with 0.05 mg of fluorescently labeled (DyLight 488, Thermo Scientific, 53025) rat anti-mouse-VCAM1 antibody (clone M-K-2.7, BioXcell, BE0027) approximately 16 h later. Two hours after antibody injections, mice were anesthetized with Avertin followed by PBS perfusion. Full information on the cytokines used can be found in Supplementary Table 5.

C57BL/6 mice: plasma injections in young mice. Young (3-month-old) C57BL/6 male mice were treated with seven injections of dialyzed and pooled plasma from young (3-month-old) or aged (18-month-old) mice (150 µl retro-orbitally), derived from 8–10 mice per pooled plasma sample. Mice were treated acutely over 4 d, with two injections per day separated by 10–12 h. During both the morning and evening injections on day 3 and the morning injection on day 4, mice were pulse with BrdU to label proliferating cells (100 mg per kg intraperitoneally; B5002-5G, Sigma-Aldrich). Mice received a seventh plasma injection along with BrdU on day 4 followed by perfusion 3 h after the last injection.

C57BL/6 mice: aged mice treated with anti-VCAM1 or anti-VALA-4 monochlonal antibody. Aged (16-month-old) C57BL/6 mice received intraperitoneal injections of anti-VCAM1 monoclonal antibody (BE0027, BioXcell) or IgG isotype control (9 mg per kg) every 3 d for a total of seven injections. Mice also received BrdU daily (100 µg per kg intraperitoneally) for the last 6 d before perfusion 24 h after the last BrdU injection and 48 h after the last antibody injection. Similarly, aged (16-month-old) C57BL/6 mice received intraperitoneal injections of anti-VALA-4 monoclonal antibody (BE0071, BioXcell) or IgG isotype control (9 mg per kg) every 3 d for a total of seven injections. Mice also received BrdU daily (100 µg per kg intraperitoneally) for the last 6 d before perfusion 24 h after the last BrdU injection and 48 h after the last antibody injection.

C57BL/6 mice: young mice treated with anti-VCAM1 monoclonal antibody and aged plasma injections. Young (3-month-old) C57BL/6 male mice were treated with nine injections of dialyzed and pooled plasma (150 µl retro-orbitally) from aged (18-month-old) mice, derived from 8–10 mice per pooled plasma sample. Mice also received intraperitoneal injections of anti-VCAM1 monoclonal antibody or IgG isotype control (9 mg per kg) every 3 d for a total of nine injections. Mice were injected with PBS as a baseline control. Before the start of the experiment, mice received daily EdU (Invitrogen, E10415) injections (100 mg per kg intraperitoneally) for 4 d to label newly born surviving cells. Mice were treated over 4 weeks, with one injection every third day for a total of nine injections. Starting on day 23 after the first injection of plasma and antibody, mice received daily injections of BrdU (100 µg per kg intraperitoneally; B5002-5G, Sigma-Aldrich) to label proliferating cells for 4 d followed by perfusion 24 h after the last BrdU injection.

C57BL/6 mice: aged mice treated with anti-VCAM1 monoclonal antibody and aged plasma injections. Mice were treated with seven injections of dialyzed and pooled plasma from aged humans (AHP; >65 years, dialyzed plasma from five individuals pooled, 150 µl per injection retro-orbitally) every 3 d for 3 weeks, totaling seven injections. They also received daily EdU injections (150 µg per kg intraperitoneally) for 3 d, beginning 2 weeks after plasma treatment, followed by daily BrdU injections (150 µg per kg intraperitoneally) beginning on day 18 for 3 d followed by perfusion. n = 6–7 mice per group.

C57BL/6 mice: acute plasma paradigm with anti-VCAM1. Three-month-old NSG mice received rat anti-mouse-VCAM1 monoclonal antibody or rat IgG isotype control (9 mg per kg intraperitoneally) on day 0 and day 3. Mice were given retro-orbital injections (50 µl) of plasma from aged humans (AHP; >65 years, pooled from five individuals) or PBS as control twice daily for seven total injections. Mice were pulse with EdU (100 mg per kg intraperitoneally) 16 h and 2 h before perfusion to label proliferating cells.

NSG mice: long-term plasma paradigm with anti-VCAM1. NSG mice received injections with pooled plasma from aged humans (AHP; >65 years, 150 µg, retro-orbitally) every 3 d for seven total injections. In addition, mice received intraperitoneal injections of an anti-VCAM1 blocking monoclonal antibody or IgG isotype control (9 mg per kg) every 3 d for a total of seven injections. They also received daily BrdU injections (150 µg per kg intraperitoneally) for 4 d beginning on day 16.

NSG mice: treatment with sVCAM1-depleted plasma. sVCAM1 was immunoprecipitated from the pooled plasma of aged humans (65–74 years of age, n = 5) by using superparamagnetic microbeads conjugated to a mouse anti-human-VCAM1 antibody (BBA5, Novus Biologicals) or monoclonal mouse anti-human-IgG antibody (MAb002, R&D Systems) as a control. To first conjugate Dynabeads to the anti-IgG or anti-VCAM1 antibody, 500 µl (0.5 µg ml⁻¹) of antibody was added to 25 mg of Dynabeads and beads were incubated on a rotator overnight at 37 °C and prepared according to the instructions in the manual (14311D, Thermo Scientific). The following day, 8 mg of conjugated anti-VCAM1 monoclonal antibody was added to Dynabeads; a similar amount of anti-IgG monoclonal antibody bound to Dynabeads was added to aliquots of 0.5 ml of dialyzed pooled human plasma and samples were incubated at 4 °C with rotation overnight. Depleted plasma was collected the next day and magnetic VCAM1–saturated Dynabeads were removed with a magnetic bar through serial transfers of the plasma to fresh tubes. Plasma was stored at −80 °C until mouse treatment.

Following pulldown of sVCAM1, pooled depleted plasma from aged humans (IgG versus sVCAM1 depleted) or saline (200 µl per mouse) was injected retro-orbitally into young (4-month-old) NSG mice (n = 7–8 mice per group) twice daily for 4 d for seven total injections. Mice were also injected with BrdU (100 mg per kg intraperitoneally) starting on the third day. Mice were anesthetized with Avertin followed by saline perfusion on the fourth day of treatment, 4 h after the third BrdU and seventh plasma injections. One mouse per group received intra-orbital injections of 100 µg of fluorescently labeled (DyLight 488, Thermo Scientific, 53025) in VivoMAB anti-mouse-CD106 antibody (VCAM1, clone M-K-2.7, BioXcell, BE0027) and fluorescently labeled (DyLight 550, Thermo Scientific, 84530) rat anti-mouse-MECA-99 antibody (a gift of the Butcher laboratory) 3 h before perfusion.

Conditional Vcam1-knockout mice: LPS treatment. Young (4-month-old) Slo1-CreERT2+/−; Vcam1tm2(Cre)−/+ (Cre+ ) mice received tamoxifen (100 mg per kg intraperitoneally) once daily for 5 d. After 3–5 resting period, mice were treated with LPS at time points of 0, 2 and 4 h (0.5 mg per kg intraperitoneally) and fluorescently labeled anti-VCAM1 monoclonal antibody (100 µg retro-orbitally) 2 h before cell isolation and flow cytometry analysis.

BEC isolation was based on a previously described procedure. Briefly, mice were transcardially perfused with ice-cold heparinized phosphate-buffered saline (PBS) containing 25% BSA (Fisher Scientific, BP1600-1) and 10 µg ml⁻¹ of antibiotic and collected at 1,000 g for 30 min at 4 °C to separate the meninges and to enrich for capillary fragments. To deplete red blood cells, the pellet was incubated in Red Blood Cell Lysis Buffer (Sigma, R7757) for 1.5 min 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 1 mg ml⁻¹ collagenase/diisopropyl fluorophosphate (Roche, 1107113001) and the digestion was incubated at 37 °C for 15 min. DNase I (1 µg ml⁻¹; Sigma, 10104159001) was added for two additional minutes. To quench the reaction, buffer A was added and cells were centrifuged at 300g for 7 min at 4 °C.

For flow cytometry, enriched BECs were labeled by standard protocols with fluorochrome-conjugated antibodies (listed in the antibodies section) in HBSS (Thermo Fisher) containing 10% FBS for 30 min on ice. Dead cells were excluded by staining with PI solution (1,300; Sigma, P4864). Background fluorescence was determined by the fluorescence-minus-one method, and for VCAM1 a specific IgG1 isotype control antibody was used. Flow cytometry data were acquired on
The left hemisphere was thawed and suspended in 300 µl of a custom lysis buffer (200 mM Tris, 4% CHAPS, 1 M NaCl, 8 M urea, pH 8.0). Tissues were then homogenized with a Branson Digital Sonifier sonicator set to 20% amplitude for 3 s and then washed for 30 s on ice; sonication was repeated three times. Samples were centrifuged at 14,000 g for 20 min at 4°C, and supernatant was extracted for analysis. Fluorescence for FITC and Texas Red was measured with a Theramo Fisher Varioskan Flash microplate reader. Fluorescent signal was standardized to the quantity of protein per sample determined by Pierce BCA Protein Assay (Thermo Fisher, 32227).

For a control, aged (19-month-old) Cre– littermate administered tamoxifen at 17 months and aged (22-month-old) mice administered tamoxifen at 2 months of age as described in the section on basal NPC activity (n = 4).

Mice were analyzed and perfused with 20 ml of medium A (500 ml of HBSS, 7.5 ml of 1 M HEPEs, 5.56 ml of 45% glucose, sterile filtered) for mechanical dissociation of the cortex and hippocampus. Tissue was collected and chopped in 2 ml of cold medium A with 20 µl of DNase I and then homogenized with a glass douncer. Homogenate was passed through a 100-µm strainer, washed three times with medium A to remove clumps and centrifuged at 340 x g for 7 min at 4°C. The supernatant was removed and the pellet was resuspended in 12 ml of 25% Percoll Plus diluted in medium A (GE Healthcare, 17-5445-01) and then centrifuged at 950 x g for 20 min. The supernatant was discarded and the remaining cell pellet was washed with 5 ml of medium A and centrifuged at 340 x g for 7 min at 4°C to remove myelin.

The myelin-free cell samples were then resuspended in 1 ml of FACS buffer (PBS + 1% BSA with 2 mM EDTA), transferred to new 2-ml Eppendorf tubes and centrifuged at 300 g for 5 min at 4°C. Cells were resuspended in 300 µl of Fc blocking solution (1:100 CD16/CD32; BD, 553141) and allowed to incubate on a shaker for 5 min at room temperature. After blocking, primary antibodies against Ly6G, Ly6C, TMEM119, αβ2 (VLA-4), CD45, CD11c, IA/IE, CD14, CD19, CD3, CD11b and CD206 were added to the samples and allowed to incubate for 15 min.

In VivoMAb anti-mouse-CD106 antibody (VCAM1, clone M/K-2.7, BioXcell, BE0027) and fluorescently labeled (DyLight 550, Thermo Scientific, 84530) rat anti-mouse-MECA-99 antibody (a gift of the Butcher laboratory) 3 h before perfusion. Mice were anesthetized with Avertin followed by saline perfusion 16 h after the final BrdU injections.

Blood–brain barrier permeability. Aged (17-month-old) Cre+ or Cre– littermates were injected once daily with tamoxifen (Sigma, T5648, prepared in sunflower seed oil; 20 mg/ml solution; 100 µg per kg intraperitoneally) for four consecutive days, followed by 2 weeks of rest. Mice received BrdU (100 µg per kg intraperitoneally) for 4 days after the last BrdU injection along with a third BrdU injection on day 4 followed by perfusion 3 h before perfusion.

Fluorescence of FITC and Texas Red was measured with a Theramo Fisher Varioskan Flash microplate reader. Fluorescent signal was standardized to the quantity of protein per sample determined by Pierce BCA Protein Assay (Thermo Fisher, 32227).

The right hemisphere was fixed in 4% paraformaldehyde for immunostaining and the left hemisphere was frozen at −80°C for tissue homogenate analysis until further processing.

**Flow cytometry analysis of brain-resident immune cells.** Mice were analyzed as follows: young (3-month-old) C57BL/6j mice (n = 5), aged Cre+ (n = 9) and Cre– (n = 4) 19-month-old mice administered tamoxifen at 17 months as described above in the section on BBB permeability, and aged Cre+ (22-month-old) mice administered tamoxifen at 2 months of age as described in the section on basal NPC activity (n = 4).

Gates were based on positive-control LPs-stimulated Cre+ aged mice injected with fluorescently labeled anti-VCAM1-ΔL488 monoclonal antibody or IgG-ΔL488 control antibody 2 h before perfusion and analysis by flow cytometry. An aged (19-month-old) Cre– littermate administered tamoxifen at 17 months and aged (22-month-old) mice were injected with fluorescently labeled anti-VCAM1-ΔL488 monoclonal antibody to confirm successful deletion of Vcan1 in the hippocampus and cortex.
Novel object recognition. The novel object recognition test was performed in a large acrylic cube measuring 45 cm (17.5 in) tall by 44.5 cm (17.5 in) wide with a light ceiling. On day 1 of testing, the mice were allowed to habituate to the testing environment for 5 min. Two objects, a black cat/tabby cat or gray cat/orange dog (silicone toys measuring 5.1 cm (2 in) tall by 3.2 cm (1.25 in) wide), were placed along one wall of the cubic field 5.1 cm (2 in) away from the corners. The mice were placed inside the cubic field facing away from the objects and allowed 15 min to explore and interact with the objects. Object interaction was defined as close investigation within 1 cm of the object. Novel object interaction percentage was calculated with the formula (time interaction with novel object × 100)/(time interaction with sample object + time interaction with novel object) from day 2. Sample object interaction percentage was calculated using the formula (time interaction with sample object × 100)/(time interaction with sample object + time interaction with novel object) from day 2. Interaction times were manually scored by the observer. Corresponding data are shown in Fig. 6.

Fear conditioning. The fear conditioning test was performed as previously described6. Briefly, mice were first habituated to the open-field arena (40 cm × 40 cm × 35 cm), which contained wall-mounted visual cues, for a period of 5 min. Mice were given one 5-min trial during which they explored two identical objects. Mice were then habituated to the maze by being randomly assigned to novel starting objects of either 25-ml cell culture flasks filled with sand or Lego towers (3 cm × 3 cm × 7.5 cm). Subsequently, mice explored the same arena for 5 min but with one object replaced with the novel, distinct object. Interactions with objects (sniffing or exploring within 2 cm of the object; excluding time spent sitting on top of the object) were manually timed in a blinded fashion to assess the percentage of time spent exploring each object. Single object interaction time/total interaction time with both objects) × 100. Corresponding data are shown in Fig. 6.

Barnes maze. A modified Barnes maze test was performed similarly to the test previously described and tailored minimally to accommodate the mild deficiencies of aged mice. Briefly, a large circular maze containing 16 holes on the outer edge was placed over a pedestal approximating the size of the mice and raised above the floor. The escape hole consisted of a PVC elbow joint connector that was similar in texture to the maze, while the other holes left were open to the floor. Distinct visual cues were placed at four equally spaced points around the maze. An overhead light, two additional standing lights and a fan blowing on the maze provided motivation to find the escape hole. The escape hole position was fixed for all days of the task. Mice performed four trials per day for 5 d as follows. For each trial conducted on the same day, the starting location for the mouse was altered to a random field, the tabby cat and orange dog, was replaced by a novel object, an orange tiger and yellow lion, respectively, while the second object (black cat and gray cat) was kept constant from day 1. The mice were placed inside the field facing away from the objects and allowed 15 min to explore and interact with the objects. Object interaction was defined as close investigation within 1 cm of the object. Novel object interaction percentage was calculated with the formula (time interaction with novel object × 100)/(time interaction with sample object + time interaction with novel object) from day 2. Sample object interaction percentage was calculated using the formula (time interaction with sample object × 100)/(time interaction with sample object + time interaction with novel object) from day 2. Interaction times were manually scored by the observer. Corresponding data are shown in Fig. 9.

Immunostaining. Staining without BrDU or EdU. Brain sections were washed three times for 10 min in TBST and then blocked in TBSS++ (TBSS + 3% donkey serum) (1:30787, Jackson ImmunoResearch) + 0.25% Triton X-100 (T8787, Sigma-Aldrich)) for 1.5 h, followed by incubation for 72 h on a rocking platform at 4 °C in primary antibodies (see antibodies listed below). For secondary staining, brain sections were washed three times for 10 min in TBST, followed by incubation for 1.5 h in secondary antibody mix. Following incubation with secondary antibody, there were four 10-min washes in TBST; the second wash contained Hoescht (1:2,000) with TBST. Brain sections were mounted on Superfrost microscope slides (12-550-15, Fisher Scientific) with Fluoromount Aqueous Mounting Medium (Sigma-Aldrich, F4680). Slides were stored at 4 °C.

EdU staining. Brain sections were washed three times for 10 min in TBST and then transferred to 95 °C for 10 min in 10 mM sodium citrate (pH 6), followed by three 10-min washes in TBST. Sections were incubated in 3 M HCl for 30 min at 37 °C and then washed three times for 10 min in TBST. Sections were blocked for 1.5 h in TBSS++ and then transferred to primary antibody mix for incubation for 72 h on a rocking platform at 4 °C. Secondary staining started with three washes for 5 min each in TBST, followed by incubation with secondary antibody mix for 1.5 h (all antibodies listed below). After three 10-min washes in TBST, sections were mounted with Fluoromount. Slides were stored at 4 °C.

ELISA. Mouse plasma samples were used to measure sVCAM1 with the VCAM1 ELISA kit (Raybiotech, ELM-VCAM-1) according to the instructions in the manual. sVCAM1 was measured in human plasma samples with the human sVCAM-1/C1016 Quantikine ELISA kit (R&D Systems, DVC00), following the instructions in the manual. Optical density was measured at 450 nm and 540 nm on a Varioskan Flash Multimode Reader (S2500400, Thermo Scientific).

Antibodies. Immunofluorescent staining or western blot analysis. The primary antibodies used included rat monoclonal anti-BrDU (1:500; Abcam, ab6326, clone BU1/75[IC1]); rat monoclonal anti-VCAM1 (1:125; Abcam, ab19568, clone M/K-2); goat monovalent anti-Sox2 (1:100; Santa Cruz Biotechnology, sc-17320, clone Y-17); goat polyclonal anti-doublecortin (DCX) (1:100; Santa Cruz Biotechnology, sc-8066, clone C-18); goat polyclonal anti-VE-cadherin (Santa Cruz Biotechnology, sc-6458, clone C-19); the Click-it Plus Edu Alexa Fluor 488 Imaging Kit (Thermo/Life Technologies, C-10637); mouse monoclonal anti-GFAP (1:100; Chemicon/Fisher, MAB360M, clone GA5); DiLight 488 lectin (clone 1:200; Vector, DL-1174); rabbit monoclonal anti-aquaporin 4 (1:500; Millipore, AB-510); goat polyclonal anti-CD68 (1:1000; BD Biosciences, 550201, clone EA-11); rabbit polyclonal anti-Iba1 (1:250; ProteinTech, 10904-1-AF); mouse anti-human VCAM1 (Novus Biologicals, BBA5, clone BBIG-V1); mouse monoclonal anti-human IgG (R&D Systems, MAB002, clone 11711); rat monoclonal anti-VCAM1 (BioXcell, BE0072, clone M/K-2.7) and rat IgG1 isotype control antibody (BioXcell, BE0088, clone HRPN).

Secondary antibodies used included 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-rabbit-IgG (1:250; Invitrogen, A-31571); Alexa Fluor 647 donkey anti-rat-IgG (1:250; Invitrogen, A-31573); Alexa Fluor 647 donkey anti- 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 anti-rat-IgG (Invitrogen, A-10277); Cy3 AffiniPure donkey anti-rat-IgG (1:250; Jackson ImmunoResearch, 712-165-153); and Hoechst 33342 (1:2,000; Sigma, 14533-100M).

Flow cytometry antibodies. The antibodies used for BEC profiling included the DiLight 488 Conjugation Kit (Thermo Scientific, 53024); anti-mouse-CD45 PerCP-Cy5.5 (5.1:1000; e Bioscience, 45-0451-80, clone 30-F11); anti-mouse-CD31 (PECAM-1) PE-Cy5.5 (1:100; e Bioscience, 25-0311-81, clone 390); anti-mouse-CD11b PerCP-Cy5.5 (5:100; e Bioscience, 45-0112-80, clone M1/70); anti-mouse-Ter-119 PerCP-Cy5.5 (1:100; e Bioscience, 45-5921-80, clone TER119); anti-mouse-CD13 (CD13-APC) (1:50; Novus Biologicals, NB016-64834, clone ER-BMDM1); anti-mouse AC1A 2 PE (1:100; Millenyi Biotech, 130-102-365, clone I3H-18A3); anti-mouse-CD16/32 (Mouse BD FC Block).
The antibodies used for profiling of brain-resident immune cells included anti-mouse-Ly6C-FITC (1:200; BioLegend, 128021); rabbit monoclonal anti-mouse-Tim-1 conjugated to Alexa Fluor 647 (1:400; Abcam, ab209064); anti-mouse-Ly6G APC-Cy7 (1:200; BioLegend, 127623); anti-mouse-α, PE (1:100; GeneTex, GTX74788); anti-mouse-CD45 Pacific Blue (1:100; BioLegend, 103126); anti-mouse-β2, PE-Cy7 (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; BioLegend, 364010); anti-mouse-CD11b BV605 (1:100; BioLegend, 740551); and anti-mouse-CD206 biotin and streptavidin BUV395 (1:100; BD, 564176) and Biocytin, 14,17,131.

The antibodies used to detect markers for setting channel compensations (in profiling of brain-resident immune cells; all used at 1:100) included anti-mouse-CD45 APC (BioLegend, 103312); anti-mouse-CD11b APC-Cy7 (BD Biosciences, 557657); anti-mouse-CD11b PE (eBioscience, 12-0112-82); anti-mouse-CD45 PerCP-Cy7 (BioLegend, 101215); anti-mouse-CD11b BV711 (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 BV605 (BioLegend, 740551); and anti-mouse-CD11b biotin (BioLegend, 101203).

Statistics and reproducibility. NPC activity. For quantification of the number of BrdU+, EdU+, Sox2+ , Dcx+, NeuN+ and Gap43+ cells in mice, confocal z stacks of six coronal brain sections spanning the hippocampus (40 µm thick, 200 µm apart) were captured on a Zeiss confocal microscope for each brain sample. VCAM1 and lectin signals were thresholded and analyzed through ‘% Area’ and manual cell counts; IBA1 and CD68 signals were counted the numbers of IBA1+ and IBA1+ cells in the DG were calculated by normalizing the total counts to the volume of the DG determined by the dimensions of the images captured with the Zeiss confocal microscope. Statistical tests. Data were analyzed with an unpaired Student’s t test and one- or two-way ANOVA with Tukey’s multiple-comparisons test or Sidak’s multiple-comparisons test, respectively. Proteomics data were analyzed by Spearman’s correlation coefficient. Bulk RNA-seq data were analyzed by using the Cuffdiff v2.2.1 statistical package. scRNA-seq data were analyzed by applying the Mann–Whitney U test to the BEC clusters obtained from unsupervised clustering. P values were adjusted via FDR or Bonferroni correction. P values equal to or lower than 0.05 were considered statistically significant. Significance was assessed on the basis of P values and heteroscedastic variance between groups that were statistically compared. For the violin plots, the data corresponding to the minima, maxima, center and percentile values in Fig. 1 and Extended Data Figs. 1 and 2 are shown in Supplementary Table 3. Unless otherwise noted, experiments were repeated three or more times independently with similar results. A summary of all in vivo studies and n groups can be found in Supplementary Table 4.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
RNA-seq gene lists with statistics (Figs. 1 and 2, and Extended Data Figs. 1 and 2), full blot (Extended Data Fig. 6) and individual data points graphed for Extended Data Fig. 9n are available as source data files and as Supplementary Tables 1–6 accompanying this article. Requests for datasets obtained from human research will be subject to additional review steps by the IRB that granted the permit for particular research. Bulk and scRNA-seq datasets that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) with the series accession numbers GSE127963 (extended accession GSM3638211 to GSM3638222) and GSE127963. Please contact the corresponding author for additional information.

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Extended Data Fig. 1 | see figure caption on next page.
Extended Data Fig. 1 | Bulk and single-cell transcriptome and proteome profiling of young and aged BECs reveals an increased inflammatory signature with aging. a, Schematic of flow sorting of CD31⁺CD45⁻ BECs from mouse cortex and hippocampus. n = 6 young and 6 aged biologically independent samples; each sample pools the cortices and hippocampi from two biologically independent mice. There were 1,006 significantly differentially expressed genes (*q < 0.05, Cuffdiff statistical package*). b, FACS gating strategy to isolate single BECs. 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. c, FPKM values of CNS cell-type-specific markers. n = 6 young and 6 aged biologically independent samples. Data are shown as the mean ± s.e.m. d, FPKM values of leukocyte binding adhesion molecules, including Vcam1. n = 6 young and 6 aged biologically independent samples. Bars show the mean; error bars are derived from s.e.m. Specific q values shown were derived from the Cuffdiff statistical package (*q = 0.0015). See the Methods for details; source data are available online.

e, FPKM values of tight junction genes. n = 6 young and 6 aged biologically independent samples. Data are shown as the mean ± s.e.m. *q = 0.16, **q = 0.0013, ***q = 0.0015, Cuffdiff statistical package. See the Methods for details; source data are available online.

f, FPKM values of transcripts in s.e.m. ± n, FPKM values of tight junction genes. n = 6 young and 6 aged biologically independent samples. Data are shown as the mean ± s.e.m. *q = 0.16, **q = 0.0013, ***q = 0.0015, Cuffdiff statistical package. See the Methods for details; source data are available online.

g, CD31⁺CD45⁻ cells were defined as the BEC population. Vcam1⁺ and CD45⁺ gates. The scatterplot shows VCAM1 fluorescence intensity as measured by FACS and corresponding transcript counts (per million).

h, Flow gating and histogram plots of pooled (n = 4 mice per age group) young or aged hippocampi isolated from healthy mice injected with anti-VCAM1-DL488 or lgG-DL488 isotype control (r.o.) 2 h before perfusion to label BECs in vivo followed by brain dissociation, staining and FACS. i, Validation of the correlation (Spearman's rho = 0.704) between protein and mRNA expression in all isolated BECs. j, Confocal images in the DG of VCAM1, lectin and AQP4 of young isochronic or heterochronic parabionts 5 weeks after surgery. Quantification is shown in Fig. 1j. Hoechst labels cell nuclei. Scale bar, 100 µm. n = 8 mice in the young isochronic group and 13 mice in the young heterochronic group from two independent experiments; representative images are shown. k, Box plot of expression levels of classical pan-endothelial and BBB-specific transcripts (n = 272 BECs in total). Minima, maxima, medians and percentiles are listed in Supplementary Table 3. l, Box plot of expression levels of classical pan-endothelial and BBB-specific transcripts (n = 146 capillary BECs, n = 59 venous BECs, n = 67 arterial BECs pooled from the hippocampi of eight mice). m, Overlay of Vcam1 mRNA levels on corresponding coordinates on the CD31 versus VCAM1 fluorescence intensity plots obtained during FACS sorting.

n, Validation of the correlation (Spearman's rho = 0.704) between protein and mRNA levels for 77 single BECs sorted from both the VCAM1⁺ and VCAM1⁻ gates. The scatterplot shows VCAM1 fluorescence intensity as measured by FACS and corresponding transcript counts (per million).

o, tSNE visualization colored by cell identity (aged versus young) (n = 160 young BECs and 112 aged BECs pooled from the hippocampi of eight mice). p, Comparison of VCAM1 expression levels in young and aged hippocampal CD31⁺ BECs collected from the VCAM1⁺ gate during FACS sorting (bars, mean; error bars, s.d.; n = 160 young BECs and 112 aged BECs pooled from the hippocampi of four mice per age group). *P = 0.017, two-tailed Mann-Whitney test.

q, Violin plots of mRNA levels of Vcam1 in all isolated BECs (bottom) and specifically in VCAM1⁺-enriched BECs (top). Other adhesion molecules, namely Pshele and Sele, were not found to be expressed in isolated CD31⁺ BECs. All BECs: n = 160 young and 112 aged BECs pooled from the hippocampi of four mice per age group; VCAM1⁺-enriched BECs: n = 56 VCAM1⁺ young BECs and 44 VCAM1⁺ aged BECs pooled from the hippocampi of four mice per age group. Minima, maxima, medians and percentiles are listed in Supplementary Table 3. r, Violin plots of tight junction markers in all isolated young and aged BECs: n = 160 young BECs and 112 aged BECs pooled from the hippocampi of four mice per age group. Minima, maxima, medians and percentiles are listed in Supplementary Table 3.
Extended Data Fig. 2 | see figure caption on next page.
Extended Data Fig. 2 | Single-cell transcriptome profiling of VCAM1<sup>+</sup>-enriched BECs reveals specialized subclusters and plasma from aged individuals upregulates VCAM1 on cultured BECs. a, Violin plots of classical arterial (top) and venous (bottom) markers in each cluster. Putative neurogenic secreted factors include Jag1 and Ephb2. Minima, maxima, medians and percentiles are listed in Supplementary Table 3. n = 146 capillary BECs, n = 59 venous BECs, n = 67 arterial BECs pooled from the hippocampi of eight mice. b, Violin plots of various genes related to angiogenesis and Notch signaling in each of the three distinct clusters. Putative neurogenic secreted factors include Vegfc. Minima, maxima, medians and percentiles are listed in Supplementary Table 3. n = 146 capillary BECs, n = 59 venous BECs, n = 67 arterial BECs pooled from the hippocampi of eight mice. c, Representative images of Bend.3 cells immunostained for BBB-specific markers of adherens junctions (AJ) and tight junctions (TJ), specifically β-catenin, claudin-5 and VE-cadherin. All Bend.3 cells and primary BECs were validated with these markers before experimentation; confirmed independently in >10 experiments. Hoechst labels cell nuclei. Scale bar, 100 µm. d, Dose-response graph depicting cultured Bend.3 cells stimulated overnight with increasing concentrations of recombinant mouse TNF-α followed by flow cytometry to quantify the percentage of CD31<sup>+</sup>-VCAM1<sup>+</sup> cells. n = 2 pooled samples per condition. e, CD31 and VCAM1 quantification (left) and histogram (right) of Bend.3 cells stimulated overnight with recombinant mouse TNF-α, IL-1β or IL-6 followed by flow cytometry to measure VCAM1. n = 3 biologically independent samples per condition. ****P < 0.0001, one-way ANOVA with Tukey’s post hoc test for group comparisons. Data are shown as the mean ± s.e.m; experiment repeated four times independently with similar results. f, Primary BECs and Bend.3 cells cultured in 10% plasma from young or aged mice (YMP, 3 months old; AMP, 18 months old) or young or aged humans (<25 years or >65 years, YHP/ AHP) for 16 h and then stained for VCAM1. Representative images are shown. Scale bar, 100 µm. Each plasma treatment experiment in primary BECs or Bend.3 cells with mouse or human plasma was repeated at least three times independently with similar results. g, Quantification of the percentage of area with VCAM1 staining. Primary BECs treated with plasma from young or aged mice: n = 7 YMP and 9 AMP biologically independent replicates pooled from two experiments. *P = 0.0343. Bend.3 cells with YMP or AMP: n = 4 biologically independent replicates per group derived from different cell flasks. ***P = 0.0003. Bend.3 cells with YHP or AHP: n = 6 biologically independent replicates derived from different cell flasks per group. ****P < 0.0001. P values were determined by two-tailed Student’s t test. Data are shown as the mean ± s.e.m. h, Bend.3 cells cultured in 10% plasma from young or aged mice for 16 h followed by flow cytometry for CD31 and VCAM1, n = 5 biologically independent replicates per group. A graph of CD31 and VCAM1 quantification is shown with a histogram of Bend.3 cells. **P = 0.0082, two-tailed Student’s t test. Data are shown as the mean ± s.e.m. I, Quantification of the percentage of CD31<sup>+</sup> Bend.3 cells treated with plasma from young or aged mice and co-stained for CD31 and ICAM1. E-selectin or P-selectin, n = 5 biologically independent replicates per group for ICAM1; n = 6 biologically independent replicates per group for E-selectin and P-selectin. Data are shown as the mean ± s.e.m. Histogram plots are shown to the right of quantification. Not significant; P = 0.2355 (ICAM1), P = 0.1959 (E-selectin), P = 0.0825 (P-selectin), two-tailed Student’s t test. j, Representative images of ICAM1, MECA-99, lectin and Hoechst (to label cell nuclei) of young (3-month-old) mice that received seven retro-orbital injections of pooled plasma from young (3-month-old) or aged (18-month-old) mice over 4 d as described in the schematic in Fig. 3a. n = 10 mice treated with YMP and 11 mice treated with AMP. Scale bar, 100 µm. k, Quantification on the right using n = 4 mice per group. Data are shown as the mean ± s.e.m. Not significant; P = 0.5222, two-tailed Student’s t test. l, Quantification in the DG of total BrdU<sup>+</sup>SOX2<sup>+</sup> NPCs in young (3-month-old) mice injected retro-orbitally daily over 5 d (2 µg per injection) with TNF-α (n = 4 mice per group) or with three LPS injections (0.5 mg per kg intraperitoneally) at 28 h, 22 h and 2 h before perfusion (n = 8 mice per group). In each experiment, mice were pulsed with BrdU every 8 h for three injections before perfusion. *P = 0.0194 (TNF-α), *P = 0.0122 (LPS), two-tailed Student’s t test. Data are shown as the mean ± s.e.m.
Extended Data Fig. 3 | see figure caption on next page.
Extended Data Fig. 3 | Assessment of Slco1c1-Cre\textsuperscript{ERT2/+}; Vcam1\textsuperscript{fl/fl} young mice, Sudan Black B treatment quenches autofluorescence caused by lipofuscin revealing VCAM1 cerebrovascular specificity and immunodeficient mice exposed to plasma from aged humans over 3 weeks have increased hallmarks of brain aging. 

**a**, Schematic. \(n=5\) mice per group. **b**, Quantification in the DG of VCAM1 from immunostained confocal images. Data are shown as the mean \(\pm\) s.e.m. *\(P=0.0451\), unpaired two-tailed Student’s \(t\) test. 

**c**, Quantification in the DG of BrdU\(^+\) and SOX2\(^+\) NPCs and triple-labeled GFAP\(^+\) neural stem cells from confocal images of immunostained sections. Scale bar, 100\(\mu\)m. \(n=5\) mice per group. Data are shown as the mean \(\pm\) s.e.m. ***\(P=0.007\), **\(P=0.0227\), *\(P=0.0038\), unpaired two-tailed Student’s \(t\) test. 

**d**, Quantification in the DG of IBA1 and CD68 from confocal images of immunostained sections. \(n=5\) mice per group. Data are shown as the mean \(\pm\) s.e.m. *\(P=0.0454\), unpaired two-tailed Student’s \(t\) test. 

**e**, Experimental design. \(n=6\) Cre\(^–\) and 7 Cre\(^+\) mice per group. 

**f**, Quantification of the percentage of VCAM1\(^+\) area in the lectin\(^+\) vasculature of immunostained sections from six Cre\(^–\) and five Cre\(^+\) mice per group. ****\(P<0.0001\), unpaired two-tailed Student’s \(t\) test. Data are shown as the mean \(\pm\) s.e.m.

**g**, **h**, Quantification of the total number of BrdU\(^+\) cells and BrdU\(^+\)SOX2\(^+\) NPCs (g) and average number of DCX\(^+\) immature neurons (h) per section in the DG of immunostained sections. \(n=6\) Cre\(^–\) and 7 Cre\(^+\) mice per group. Bars, mean; error bars, s.e.m. Staining experiments were repeated twice with similar results; similar mouse experiments using these validated transgenic mice were repeated four times with similar results (Supplementary Table 4). 

**i**, Quantification of IBA1 and CD68 in the DG of immunostained sections. \(n=6\) Cre\(^–\) and 7 Cre\(^+\) mice per group. \(P=0.0012\), **\(P=0.0021\), ***\(P=0.0028\), unpaired two-tailed Student’s \(t\) test. Data are shown as the mean \(\pm\) s.e.m. 

**j**, Confocal images of brain sections of aged Cre\(^+\) or Cre\(^–\) mice treated with tamoxifen in young adulthood (aged 2 months) and aged to 18 months stained for VCAM1 (or IgG isotype control), AQP4 and GFAP. Hoechst labels cell nuclei. Brain sections from aged (18-month-old) mice were treated with Sudan Black B to remove lipofuscin background in the granular and hilus layers of the DG. Sudan Black B treatment removes the majority of lipid-based artifacts typically seen in aged tissues without suppressing immunofluorescent labeling. Scale bar, 100\(\mu\)m. Experiment repeated three times with similar results. 

**k**, Brain sections from aged (18-month-old) Cre\(^+\) and Cre\(^–\) mice were immunostained with the regular protocol, without Sudan Black B treatment. Heavy lipofuscin background is present in the Cy3 fluorescence channel. Experiment repeated three times with similar results.
Extended Data Fig. 4 | see figure caption on next page.
Extended Data Fig. 4 | VCAM1 is not expressed in CNS cell types other than BECs in the hippocampus, is increased during neurodegeneration and is deleted in brain endothelium for Slco1c1-Cre<sup>ERT2</sup>; Vcam1<sup>fl/fl</sup> transgenic mice. a, Representative 2D and 3D z-stacked high-magnification confocal images (51 slices with an interval of 0.4 µm) of VCAM1 in the granular layer of the DG of the hippocampus of a young (3-month-old) NSG mouse acutely treated with plasma from aged humans. Brain sections were co-stained for DCX and NeuN to label immature and mature granule neurons, respectively. VCAM1 is not expressed in these cell types. Light blue lines outline the granule layer. Experiment repeated three times independently with similar results. 2D scale bar, 50 µm. Two 3D renderings of the 2D images are displayed. 3D scale bar, 20 µm. b, Quantification of VCAM1, AQP4 and lectin, with Hoechst labeling of cell nuclei, in the hippocampus and cerebellum of EAE (multiple sclerosis), Npc1<sup>−/−</sup> (Niemann Pick disease type C) and Grn<sup>−/−</sup> (frontotemporal dementia) disease models. EAE: n = 4 naive and 8 EAE induced mice, *P = 0.006, **P = 0.0125; Npc1<sup>−/−</sup>: n = 6 mice per group, ***P = 0.0274, ****P < 0.0001; Grn<sup>−/−</sup>: n = 4 mice per group, *****P = 0.0004. P values were determined by unpaired two-tailed Student’s t test. Data are shown as the mean ± s.e.m. c, Representative 2D and 3D z-stacked high-magnification confocal images (51 slices with an interval of 0.4 µm) of VCAM1 in the granular layer of the DG of the hippocampus co-stained with SOX2 and GFAP to label neural stem and progenitor cells (SOX2<sup>+</sup>GFAP<sup>+</sup>) and hilus GFAP<sup>+</sup> astrocytes. VCAM1 is not expressed in these cell types in the DG. Light blue lines outline the granule layer. Experiment repeated three times independently with similar results. 2D scale bar, 50 µm. Two 3D renderings of the 2D images are displayed. 3D scale bar, 20 µm. d, Cre<sup>+</sup> or Cre<sup>−</sup> littermates (3 months old) were treated daily with tamoxifen (i.p., 150 mg per kg) for 5 d followed by 4 d of rest. Mice received three LPS injections (0.5 mg per kg i.p.) at 28, 22 and 2 h before perfusion. Mice also received a retro-orbital injection of fluorescently conjugated mouse anti-VCAM1 monoclonal antibody (100 µg) 2 h before perfusion. FACS gating strategy to analyze single BECs. PI<sup>+</sup> dead cells were excluded. Cells negative for CD11a, CD11b, CD45 and Ter-119 were gated to exclude erythrocytes, monocytes/macrophages and microglia. CD13 and ACSA-2 staining were applied to exclude pericytes and astrocytes, respectively. CD31<sup>+</sup>MECA-99<sup>+</sup> cells were defined as the BEC population. e, f, Quantification (e) of flow cytometry (f) that was performed on primary BECs isolated from Cre<sup>+</sup> or Cre<sup>−</sup> mice treated as described in d. n = 3 Cre<sup>+</sup> or Cre<sup>−</sup> mice received LPS, while one Cre<sup>−</sup> mouse was given PBS vehicle control instead. The VCAM1 gate was set on the basis of a Cre<sup>−</sup> mouse injected with fluorescently conjugated IgG. **P = 0.0011, unpaired two-tailed Student’s t test. Data are shown as the mean ± s.e.m. g, Representative confocal images of cortex and DG for VCAM1 and Hoechst (to label cell nuclei) in LPS-stimulated mice as described in d. Loss of Vcam1 in Cre<sup>+</sup> mice, but not Cre<sup>−</sup> mice, in BBB endothelium but not meninges is shown. Experiment repeated three times independently with similar results. Scale bar, 100 µm.
Extended Data Fig. 5 | see figure caption on next page.
Extended Data Fig. 5 | Brain endothelial- and epithelial-specific Vcam1 deletion in young mice mitigates the negative effects of administration of plasma from aged individuals and anti-VCAM1 antibody reduces hallmarks of brain aging in female mice. a, Experimental design. n = 8 mice per group. b, c, Representative confocal images (b) and quantification (c) in the DG of VCAM1, MECA-99 and AQP4. Hoechst labels cell nuclei. Scale bar, 100 µm. Arrows indicate VCAM1+ vessels. n = 4 mice per group analyzed. Data are shown as the mean ± s.e.m. ***P = 0.0002, two-way ANOVA with Tukey’s multiple-comparisons test. d–f, Quantification of the total number of BrdU+ cells, BrdU+SOX2+ NPCs and DCX+ immature neurons in the DG of immunostained sections. n = 8 mice per group. Data are shown as the mean ± s.e.m. *P = 0.0193, **P = 0.0283, ***P = 0.0015, two-way ANOVA with Tukey’s multiple-comparisons test. g, h, Quantification of the total number of surviving EdU/DCX+ immature neurons and EdU/NeuN+ neurons in the DG of immunostained sections. n = 8 mice per group. Data are shown as the mean ± s.e.m. *P = 0.0181, two-way ANOVA with Tukey’s multiple-comparisons test. i, j, Quantification of IBA1 and CD68 in the DG of immunostained sections. n = 8 mice per group. Data are shown as the mean ± s.e.m. ****P < 0.0001 for both, two-way ANOVA with Tukey’s post hoc test. k, Schematic. Aged (18-month-old) C57BL/6J female mice received intraperitoneal injections of a mouse-specific anti-VCAM1 monoclonal antibody or IgG isotype control (9 mg per kg) every 3 d for a total of seven injections. Mice also received BrdU daily (100 mg per kg i.p.) for six consecutive days followed by perfusion 2 d after the last injection. n = 9 IgG-treated and 10 anti-VCAM1 monoclonal-antibody-treated mice per group. l, Quantification of VCAM1 and lectin staining from confocal images in the DG. n = 3 mouse brain sections stained and quantified per group. Data are shown as the mean ± s.e.m. *P = 0.0128, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. m, Quantification of BrdU+ and BrdU+SOX2+ staining from confocal images in the DG. n = 9 IgG-treated and 10 anti-VCAM1 monoclonal-antibody-treated mice per group. Data are shown as the mean ± s.e.m. *P = 0.0325, ***P = 0.0003, unpaired two-tailed Student’s t test. n, Quantification of IBA1 and CD68 staining from confocal images in the DG. n = 9 IgG-treated and 10 anti-VCAM1 monoclonal-antibody-treated mice per group. **P = 0.0008, *P = 0.0427, unpaired two-tailed Student’s t test. Data are shown as the mean ± s.e.m. o, sVCAM1 ELISA of the plasma of young (4-month-old) and aged (18-month-old) female mice. n = 6 mice per group. ****P < 0.0001, unpaired two-tailed Student’s t test. Data are shown as the mean ± s.e.m.
Extended Data Fig. 6 | see figure caption on next page.
Extended Data Fig. 6 | Circulating sVCAM1 does not contribute to inhibitory effects of administration of plasma from aged individuals while anti-VCAM1 antibody prevents the inhibitory effects of plasma from aged humans. a, Experimental design. n = 7 mice per group. b, Ponceau S staining showing total protein pulldown from plasma by both IgG and anti-VCAM1 monoclonal antibody conjugated to beads. Experiment repeated three times with similar results. c, Western blot showing human sVCAM1 (93 kDa) pulled down during immunodepletion by anti-human-VCAM1 antibody but not IgG. Experiment repeated three times with similar results. Source data are available online. d, Human sVCAM1 ELISA of depleted plasma. n = 4 mice per group. Data are shown as the mean ± s.e.m. e, f, Representative confocal images (e) and quantification (f) in the DG of VCAM1, MECA-99 and AQP4. Hoechst labels cell nuclei. Scale bar, 50 µm for merged images and 20 µm for the ×4 single-channel VCAM1 images outlined with white boxes. Arrows indicate VCAM1+ vessels. n = 5 mice per group analyzed. Data are shown as the mean ± s.e.m. **P = 0.0004, ***P = 0.0025, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. g, Quantification of the total number of BrdU+ and BrdU+SOX2+ NPCs in the DG of immunostained sections. n = 7 mice per group. Data are shown as the mean ± s.e.m. *P = 0.0237, **P = 0.0123, ***P = 0.0320, ****P = 0.0094, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. h, i, Quantification (h) and representative confocal images (i) of the DG for DCX and Hoechst (to label cell nuclei). Scale bar, 100 µm. n = 5 mice per group analyzed. Data are shown as the mean ± s.e.m. **P = 0.0017, *P = 0.0385, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. j, Quantification of the IBA1 and CD68 staining from confocal images in the DG. n = 7 mice per group. *P = 0.0156, **P = 0.0242, ***P = 0.0034, ****P = 0.0237, P = 0.0546 (PBS as compared to anti-VCAM1 activated microglia counts), one-way ANOVA with Tukey’s multiple-comparisons post hoc test. k, Experiment schematic. n = 9 PBS-treated, 8 AHP+IgG-treated and 8 AHP+anti-VCAM1 monoclonal-antibody-treated mice. l, Quantification in the DG of VCAM1 in lectin+ blood vessels from immunostained confocal images. n = 9 PBS-treated, 8 AHP+IgG-treated and 7 AHP+anti-VCAM1 monoclonal-antibody-treated mice analyzed. Data are shown as the mean ± s.e.m. **P = 0.006, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. m, Quantification in the DG of CD68 in IBA1+ stained microglia from immunostained confocal images. n = 9 PBS-treated, 8 AHP+IgG-treated and 8 AHP+anti-VCAM1 monoclonal-antibody-treated mice. Data are shown as the mean ± s.e.m. **P = 0.0067, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. n, o, Quantification of BrdU+SOX2+ progenitor cells (n) and DCX+ immature neurons (o) from confocal images. n = 9 PBS-treated, 8 AHP+IgG-treated and 8 AHP+anti-VCAM1 monoclonal-antibody-treated mice. Data are shown as the mean ± s.e.m. *P = 0.018, **P = 0.0386, ***P = 0.0344, ****P = 0.0167, one-way ANOVA with Tukey’s multiple-comparisons post hoc test. p, Quantification of the total numbers of EdU+ surviving cells in the DG of immunostained sections. n = 9 PBS-treated, 8 AHP+IgG-treated and 8 AHP+anti-VCAM1 monoclonal-antibody-treated mice. Data are shown as the mean ± s.e.m. ***P = 0.0009, ****P = 0.0002, one-way ANOVA with Tukey’s multiple-comparisons post hoc test.
Extended Data Fig. 7 | see figure caption on next page.
Extended Data Fig. 7 | BBB integrity is not compromised with aging or conditional deletion of Vcam1. a, Quantification of fluorescent signal measured with a microplate reader from homogenized brain tissue samples from mice that were injected with Texas Red-labeled 70-kDa dextran retro-orbitally and perfused with FITC-labeled 2-MDa dextran 3 h after injection. Cre– and Cre+ mice were used. n = 3 young (5-month-old) Cre– mice, 5 aged (19-month-old) Cre– mice, 2 young Cre– mice that underwent TBI as a positive control, 3 young Cre– control mice not injected with dextran and 3 aged Cre– control mice not injected with dextran. Data are shown as the mean ± s.e.m. b, Quantification of the fluorescent signal from homogenized brain tissue samples measured with a microplate reader. Cre– and Cre+ mice were used as described in a. n = 3 young Cre– mice, 5 aged Cre– mice and 5 ‘aged Vcam1-ST’ (19-month-old) mice, which are Cre+ mice that were treated with tamoxifen for 4 d, 2 months before they were killed, and that were infused with dextran before being killed as described in a. Data are shown as the mean ± s.e.m. c,d, Quantification of mean fluorescence intensity from confocal images of tissue sections from mice injected as in a and b. Cre– and Cre+ mice were used as described in a. n = 4 young Cre– mice, 5 aged Cre– mice, 5 aged Vcam1-ST Cre+ mice that were treated with tamoxifen and that were infused with dextran as described in a and b, and 1 young and 1 aged Cre– control mice not infused with dextran. Data are shown as the mean ± s.e.m. P = 0.895 (young versus aged), 0.9097 (aged versus aged Vcam1-ST), one-way ANOVA with Tukey’s multiple-comparisons post hoc test. e, Schematic of flow cytometry analysis of various immune cell populations from mouse cortex and hippocampus. f, Flow cytometry gating strategy for individual hippocampal immune cell populations labeled for various immune cell markers, including α4β1 integrins (VLA-4). n-1 was used to gate for VLA-4+ cell populations.
Extended Data Fig. 8 | Brain-resident leukocyte composition does not change with aging or conditional deletion of Vcam1. a, Mouse model and experimental groups. n = 5 young Cre− mice, 9 aged Cre− mice, 4 Vcam1-LT mice and 4 Vcam1-ST mice. Cre− and Cre+ mice were used. The tamoxifen treatment paradigm is described in the schematic. 

b, Gating plots of CD31+VCAM1+ hippocampal and cortex cells isolated from one LPS-stimulated aged (19-month-old) Cre+ (Vcam1 deletion short term) mouse and one Cre− mouse injected with anti-VCAM1-DL488 (r.o.) 2 h before killing to confirm VCAM1 levels on BECs were reduced. One additional Cre− mouse was treated with LPS and injected with IgG-DL488 isotype control before killing to serve as a control for VCAM1 gating.

c–j, Quantification of various cell populations present in young Cre− (n = 5), aged Cre− (n = 9), aged Vcam1-LT (n = 4) and aged Vcam1-ST (n = 4) mice per group. Data are shown as the mean ± s.e.m. *P = 0.0413, **P = 0.0245, ***P = 0.0429, ****P = 0.0023, one-way ANOVA with Tukey’s multiple-comparisons post hoc test.
Extended Data Fig. 9 | VCAM1 and VLA-4 perturbations reduce hallmarks of brain aging. a, Experimental design for anti-VLA-4. n = 7 mice per group. b,d, Representative confocal images (b) and quantification (d) of VCAM1, lectin and Hoechst (to label cell nuclei). Scale bar, 100 µm. n = 3 mice per group analyzed. Data are shown as the mean ± s.e.m. c, f, Representative confocal images (c) and quantification (f) in the DG of CD68, IBA1 and Hoechst. Scale bar, 100 µm. n = 7 mice per group. Data are shown as the mean ± s.e.m. *P = 0.0436, **P = 0.0175, two-tailed Student’s t test. e, Quantification of confocal images of the DG of NPCs co-labeled with BrdU and SOX2. n = 7 mice per group. Data are shown as the mean ± s.e.m. g, h, Quantification of IBA1+ and IBA1+CD68+ microglia in DG from the experiment described in Fig. 6f. n = 8 mice per group. Data are shown as the mean ± s.e.m. i–k, Thirteen-month-old NSG mice were injected with anti-VCAM1 monoclonal antibody or IgG every 3 d for 1 month and underwent novel object recognition or fear conditioning tasks during the last week (n = 11 mice per group). i, Quantification of the percentage of time spent exploring objects in the novel object placement task. j, Percentage freezing observed during the training phase. k, The average of trials 3–5 for contextual freezing. Data are shown as the mean ± s.e.m. *P = 0.0493, two-way ANOVA with Tukey’s multiple-comparisons test. There were no significant differences between groups for contextual freezing (P = 0.2722, two-tailed Student’s t test). l–n, Twenty-three-month-old C57BL/6 mice were injected with anti-VCAM1 or IgG every 3 d for 1 month and underwent fear conditioning during the last week (n = 7 PBS-, 12 IgG- and 13 anti-VCAM1-treated mice per group). l, Percentage freezing observed during training. Cued (m) and contextual (n) tests. Data are shown as the mean ± s.e.m. *P = 0.0493, two-way ANOVA with Tukey’s multiple-comparisons test between groups at each time point. Source data are available online.
Extended Data Fig. 10 | Blood from aged individuals inhibits hippocampal NPC activity and activates microglia through VCAM1 at the BBB. In young, healthy mice, neurovascular homeostasis is maintained with low expression levels of systemic sVCAM1 and BBB-specific VCAM1, active neurogenesis with neural stem cells (NSCs) differentiating into NPCs, immature neurons and mature neurons, and nonreactive microglia in a low-inflammation environment. During aging or exposure to plasma from aged individuals, we propose that (1) inflammatory factors in plasma from aged individuals (IL-1β and TNF-α, among others) induce arterial and venous BEC activation and upregulation of VCAM1 through their cytokine receptors TNFRSF1A and IL1R1. (2) Venous VCAM1 facilitates tethering, but not transmigration, of leukocytes, which sustain BEC inflammation. (3) Inflamed and activated venous and arterial VCAM1+ brain endothelium relays (unknown) signals to the parenchyma, leading to loss of homeostasis, decline in NPC activity and chronic activation of microglia. (4) Anti-VCAM1 monoclonal antibody protects young brains from the detrimental effects of plasma from aged individuals by reducing BEC-mediated inflammation. (5) Anti-VCAM1 monoclonal antibody rejuvenates aged brains by reducing BEC-mediated inflammation and the reduction mediated by VCAM1+ BECs in NPC proliferation.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- 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
- 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: FACSDivia (v.8.0.1)
- 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 FACSDivia (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 described. 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 bc12fastq, 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).

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).

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.

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.

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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])
- Goat 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, MAB360M1, 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, BBAS, 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® 647 donkey anti-rabbit IgG (1:250, Invitrogen, A-31573)
- Alexa Fluor® 647 donkey anti-goat IgG (1:250, Invitrogen, A-31573)
- Alexa Fluor® 647 donkey anti-rabbit IgG (1:250, Invitrogen, A-31573)
- Alexa Fluor® 647 donkey anti-mouse IgG (1:250, Invitrogen, A-31571)
- Alexa Fluor® 647 donkey anti-rabbit IgG (1:250, Invitrogen, A-31573)

**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 CD3 (PECAM-1) PE-Cyanine7 (1:100, eBioscience, 25-0311-81, clone 300)
- 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 Pharmingen, 551262, clone MECA13.3)
- 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 APC, Biolegend 103126
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 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

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 polyclonal 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, BA5, 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:
Brain 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 CD3 (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 MEIC3.3)
- 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 AlexaFluoer647 (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 141713
Anti-Mouse CD206 biotin antibody; streptavidin BV395. (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 Cd11b-BV711, 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 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 Bend3 cells for BBB specific markers of adherens junctions (AJ) and tight junctions (TJ), specifically β-catenin, Claudin-5, and VECAderhin 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 treatment studies 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) 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 Gmr-/- deficient mice (B6.129(FVB)-Gnmtm1.1Far/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.
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 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 blood 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), 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 skull 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 4ºC. The pellet was digested in a 1:1:1 volume mix of tissue, Buffer A, and 0.75% collagenase II (Millipore, C2-22) at 37ºC 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, 4ºC) the pellet was carefully resuspended in PBS containing 25% BSA (Fisher Scientific, BP1600-1) and centrifuged at 1000g, 30min at 4ºC 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...
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. CD51+ 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. CD51+ cells were defined as BECs.

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. Background fluorescence was determined by the ‘fluorescence minus one’ 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+ intermative 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 mL DNase I 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, CD6, 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 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+ dead cells were excluded. CD31+ cells were defined as BECs.

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