Profiling the mouse brain endothelial transcriptome in health and disease models reveals a core blood-brain barrier dysfunction module

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Blood vessels in the CNS form a specialized and critical structure, the blood–brain barrier (BBB). We present a resource to understand the molecular mechanisms that regulate BBB function in health and dysfunction during disease. Using endothelial cell enrichment and RNA sequencing, we analyzed the gene expression of endothelial cells in mice, comparing brain endothelial cells with peripheral endothelial cells. We also assessed the regulation of CNS endothelial gene expression in models of stroke, multiple sclerosis, traumatic brain injury and seizure, each having profound BBB disruption. We found that although each is caused by a distinct trigger, they exhibit strikingly similar endothelial gene expression changes during BBB disruption, comprising a core BBB dysfunction module that shifts the CNS endothelial cells into a peripheral endothelial cell-like state. The identification of a common pathway for BBB dysfunction suggests that targeting therapeutic agents to limit it may be effective across multiple neurological disorders.

The BBB in health.

Results

BBB disruption has been observed in human patients and mouse models of many different neurological diseases including stroke, multiple sclerosis (MS), traumatic brain injury (TBI), epilepsy, cancer, infection and neurodegenerative diseases. The disruption of the BBB can include a loss of tight junction integrity, increase in transcytosis, alterations in transport properties and increases in the expression of leukocyte adhesion molecules. These changes in the BBB result in CNS ion dysregulation, edema and immune infiltration, which can lead to neuronal dysfunction, damage and degeneration. Despite its importance in disease, many questions still remain. What are the molecular mechanisms that lead to BBB dysfunction in each disease? Is disruption of the BBB mediated by the same or different mechanisms in different neurological diseases? How is the BBB repaired? Is BBB dysfunction helpful in wound healing or harmful, initiating neuronal damage?

Here, we have used endothelial cell enrichment followed by RNA sequencing to generate a resource to understand BBB gene expression in health and disease in mice. In health, we enriched for endothelial cells from different organs including the brain, heart, kidney, lung and liver, and sequenced the RNA to generate a BBB-specific gene expression profile. We further used four different disease models including a middle cerebral artery occlusion (MCAO) model of stroke, an experimental autoimmune encephalomyelitis (EAE) model of MS, a cortical impact model of pediatric TBI and a kainic acid model of seizure, each with distinct temporal and spatial patterns of BBB dysfunction and neuroinflammation. For each disease model, we enriched for the endothelial cells and performed RNA sequencing from three timepoints to identify the endothelial gene expression changes following each of the different triggers. This RNA-sequencing database provides a resource for understanding the transcriptional profiles of CNS endothelial cells during health and disease. We found that, although each of the disease models has a unique trigger, they each lead to remarkably similar transcriptional changes to the BBB, suggesting a common mechanism for BBB dysfunction throughout different neurological disorders.

The BBB in health. Transcriptional profiling of different vascular beds. Rosa-tomato; VE-Cadherin-CreERT2 mice were generated to...
enable tamoxifen-inducible expression of tdTomato in endothelial cells. One week following tamoxifen injections in adults, tdTomato fluorescence could be visualized in blood vessels in brain, spinal cord, heart, kidney, lung and liver (Fig. 1). The tdTomato colocalized with CD31+ endothelial cells in each of the tissues, and did not colocalize with immune cells, pericytes or neuronal or glial cell markers (Fig. 1, data not shown). We enriched tdTomato+ endothelial cells from brain, heart, kidney, lung and liver using fluorescence-activated cell sorting (FACS), isolated the RNA and performed RNA sequencing, as well as analyzing whole brain homogenates. Because brain mural cells adhere tightly to the endothelial cells, we added a second set of brain samples with an extra collagenase/disperse digestion step. We termed the first set brain vascular, as it contains endothelial cells with some adherent mural cells, and the second set brain endothelial, as the mural cells are further depleted. Reads were mapped onto the ensembl genome. The brain vascular and brain endothelial cell samples showed high levels of RNA from endothelial cell genes with minimal levels of RNA from neuronal and glial genes. In the brain vascular sample there was a small but present level of mural cell genes estimated to be <2.0% of the RNA, whereas the brain endothelial sample contained <0.05% mural cell RNA (Supplementary Fig. 1). The complete dataset can be found in Supplementary File 1. Brain mural cell genes could thus be identified as genes enriched in the brain vascular compared with the brain endothelial sample (Supplementary File 2).

**BBB-enriched transcriptome.** In Supplementary File 3, we list all of the BBB-enriched genes (>5 counts per million (c.p.m.) in brain endothelial cells, and at least twofold (log2, >1.000) and P <0.05 enriched in brain endothelial cells compared with endothelial cells of each peripheral organ), and in Supplementary Table 1, we list the top 50 BBB-enriched genes (most enriched in the brain endothelial cells compared with the heart, kidney, lung and liver endothelial cell samples and whole brain samples). We used the Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics functional annotation tool to identify signaling pathways, metabolic pathways and protein interactions enriched at the BBB. This identified Wnt–beta-catenin-related pathways, different transport mechanisms and amino acid metabolism as key BBB-enriched pathways. Wnt–beta-catenin signaling has been identified as a key regulator of CNS-specific angiogenesis, BBB induction and maintenance20–30, and this dataset identified BBB-enriched Wnt mediators including Left1, Fzd3, Notum, Apccd1, Axin2, dixdc1 and Tnfrsf19. This dataset identified BBB-enriched components of tight junctions (Supplementary File 4 and Supplementary Table 2), transporters (Supplementary Table 3) and additional BBB-enriched functions including extracellular matrix, metabolic programs and transcription factors (see Supplementary Results & Discussion).

**Peripheral endothelial-enriched transcriptome.** This resource also identified genes enriched in the peripheral endothelial cells compared with brain endothelial cells, as well as genes enriched in each specific vascular bed. In Supplementary File 5, we list all of the peripheral endothelial-enriched genes (c.p.m. >5 in all of the peripheral endothelial samples, with a log2 ratio >1.00 and P <0.05 for at least three of the peripheral endothelial samples compared with the brain endothelial samples). In Supplementary Table 1, we list the 50 most peripheral-enriched genes. Pathways mediating the immune response including leukocyte migration, toll-like receptor signaling, chemokine signaling and antigen presentation are enriched in peripheral endothelial cells compared with brain endothelial cells. Several of these genes are known to mediate the function of peripheral endothelial cells, including Plvap, which regulates transcytosis17–19, and Sele, Selp, Vcam and Icam1, which mediate leukocyte adhesion20–30. Several Hox genes, including Hoxa5 and Hoxb4, are greatly enriched in the peripheral endothelial cells compared with the brain endothelial cells, which may indicate a rostral-caudal axis identity for vasculature. In addition, there are many instances of a peripheral-enriched gene with a corresponding BBB-enriched family member. This includes aquaporins, annexins and semaphorins.

**Cerebrovascular transcriptional response to disease.** **BBB dysfunction in disease models.** Next, we examined the molecular changes to CNS endothelial cells following four different mouse models of neurological diseases and injuries: (1) kainic acid model.
of seizure, (2) EAE model of MS, (3) MCAO model of stroke and (4) a focal cortical impact model of pediatric TBI. Each model is elicited by a different trigger (neuronal overactivity or seizure, auto-inflammation or EAE, ischemia or stroke, and mechanical injury or TBI), but all lead to BBB dysfunction and neuroinflammation.

For each model, we examined BBB dysfunction using a transcardiac perfusion of a molecular tracer (sulfo-NHS-biotin, ~500 Da) at three timepoints: acute, subacute and chronic. These timepoints differed based on the course of the disease (see Methods), but in general the acute timepoint was within 1 d of the onset, the subacute timepoint was 2 d following the acute and the chronic was 1 month post onset.

For the kainic acid model of seizure, there was negligible vascular leakage at the acute timepoint during the active seizures, whereas at the subacute timepoint vascular leakage could be visualized throughout the temporal lobe, and to a lesser extent the hippocampus. At the chronic timepoint there was minimal vascular leakage (Fig. 2a,e and Supplementary Fig. 2). For the EAE model, there was localized BBB leakage at sites of active lesions throughout the spinal cord at the acute stage, and even greater leakage at the subacute timepoint. At the chronic timepoint there were sporadic points of leakage that localized to a subset of the lesions (Fig. 2b,f and Supplementary Fig. 2). For the MCAO model of stroke, there were rare small focal points of vascular leakage on the ipsilateral hemisphere at the acute timepoint, which appeared to result from isolated vessels with a leaky BBB. At the subacute timepoint there was a large discrete region with clear boundaries within the ipsilateral hemisphere that showed vascular leakage. At the chronic timepoint, there was a much smaller region of BBB leakage in a discrete scar (Fig. 2c,g and Supplementary Fig. 2). For TBI, leakage occurred preferentially in the cortex adjacent to the site of impact at the acute timepoint. At the subacute timepoint, the pattern of leakage was more robust, extending into the neighboring cortex. The chronic timepoint was characterized by prominent cavitation with minimal and sporadic BBB leakage. (Fig. 2d,h and Supplementary Fig. 2). We found that the BBB in all four of the disease models exhibited increased permeability to large endogenous molecules, including fibrinogen and IgG, at the subacute timepoint compared with their corresponding controls (Supplementary Figs. 3 and 4).

Although we observed only a few hypertrophic vessels with disorganized Claudin 5 in the seizure model, there were numerous hypertrophic vessels with disorganized Claudin 5 in EAE, stroke and TBI (Supplementary Fig. 5). For each disease we also examined the expression of the inflammatory marker CD45 in the CNS, and found robust inflammation, peaking at the subacute timepoint (Fig. 2, and see the Supplementary Results & Discussion).

We then used endothelial cell enrichment and RNA sequencing to analyze the gene expression for each disease at the three timepoints. In each case the area of CNS corresponding to the inflamed region was dissected for endothelial cell purification. In addition, we used immune-lineage negative selection to limit immune cell contamination. The complete dataset of gene expression is presented in Supplementary File 1. In addition, we performed co-expression analysis to identify modules of genes, named by different colors, that followed statistically similar patterns of expression over all of the health and disease samples. In Supplementary File 1 (tab 4), we present the association of each gene with each module and report the strongest associations after correcting for multiple comparisons (Bonferroni correction (column F) and false discovery rate (FDR) (column G)). This analysis revealed that many of the BBB-enriched genes affiliate with the red or dark-gray modules, while many peripheral-enriched genes affiliate with the black, blue, salmon, pink or tan modules.

Comparisons of gene changes during each disease. To compare the changes in gene expression between each disease we defined the numbers of upregulated genes (log, ratio > 1.000; P < 0.05, minimum mean value of 5 c.p.m. in the disease samples) and downregulated genes (log, ratio < −0.800; P < 0.05, minimum mean value of 5 c.p.m. in the healthy samples) at each timepoint for each disease and determined the overlap of gene changes between timepoints for each disease and between different diseases within a timepoint.

In Fig. 3a, for each disease the overlap of gene changes between timepoints is shown with Venn diagrams. In seizure and TBI models, the most unique changes are observed at the acute timepoint, whereas for EAE and MCAO the most unique changes are observed at the subacute timepoint. For seizure, stroke and TBI models, the most overlap of the changes occurred between the acute and subacute timepoints. For EAE, the most overlap occurred between the subacute and chronic timepoints. These differences likely indicate the distinctions in the severity of the initial insult in the seizure, stroke and TBI models compared with the buildup of inflammation in the EAE model of MS.

In Fig. 3b,c the overlap of gene changes between diseases is highlighted. The largest numbers of overlapping gene changes are observed at the subacute timepoints, with the majority of upregulated genes in seizure, stroke and TBI models also changed in the other disease models. The acute timepoint, on the other hand, showed many disease-specific changes as there were largely nonoverlapping datasets between diseases. Therefore, the triggers for each disease initially elicit different transcriptional changes at the BBB, but then converge on similar changes at the subacute timepoint when the BBB is most leaky. At the chronic timepoint the lowest numbers of changes were observed for seizure, stroke and TBI models.

We determined how the BBB-enriched and peripheral endothelial-enriched datasets responded to each of the diseases (Fig. 3d,e). The BBB-enriched transcriptome was downregulated during each of the diseases, reaching the lowest value at the acute TBI and subacute seizure, EAE and stroke timepoints. The peripheral endothelial-enriched genes were upregulated, peaking at acute TBI and subacute seizure, EAE and stroke timepoints. Therefore, CNS endothelial cells take on a peripheral endothelial cell gene expression profile during BBB dysfunction. Using DAVID Bioinformatics, we identified the signaling and biological pathways altered in the CNS endothelial cells at each timepoint in each disease (Fig. 4, and see the Supplementary Results & Discussion).

Common changes among diseases. The subacute timepoint, when the most severe BBB dysfunction was observed, had the most common gene changes between each disease, with 54 genes upregulated in all four diseases and 136 genes upregulated in at least three of the four diseases (Fig. 5a, Supplementary File 6 and Supplementary Table 4). This is most striking for the TBI and stroke models. For TBI, 61.2% of the upregulated genes at the subacute timepoint are upregulated in at least three of the four diseases, and 85.2% of the genes are upregulated in at least one other disease. For the stroke model, 52.1% of the upregulated genes at the subacute timepoint are upregulated in at least three of the four diseases, and 82.2% of the genes are upregulated in at least one other disease. For the seizure model, 22.9% of the upregulated genes at the subacute timepoint are upregulated in at least three of the four diseases, and 42.1% of the genes are upregulated in at least one other disease. The EAE model showed the most changes, and also the most unique changes. In this model, 16.4% of the upregulated genes at the subacute timepoint are upregulated in at least three of the four diseases, and 54.4% of the genes are upregulated in at least one other disease. The EAE model showed the most changes, and also the most unique changes. In this model, 16.4% of the upregulated genes at the subacute timepoint are upregulated in at least three of the four diseases, and 42.1% of the genes are upregulated in at least one other disease. All four disease models led to remarkably similar gene expression changes at the BBB, and this resource has identified a common BBB dysfunction module that appears to be upregulated in CNS endothelial cells regardless of the trigger. On average, the BBB dysfunction module peaked at the acute timepoint in the TBI model and the subacute timepoint in seizure, EAE and stroke models (Fig. 5b). The genes...
Fig. 2 | BBB leakage and inflammation following different disease models. a–l. Rosa-tdTomato; VE-CadherinCreERT2 mice undergoing a kainic acid seizure model (a,e,i), an EAE model of MS (b,f,j) an MCAO model of stroke (c,g,k) or a pediatric TBI model (d,h,l) were analyzed at three timepoints (acute ‘′), subacute (‘’), chronic (‘’) for BBB leakage using a biotin tracer (green, a–h) or inflammation by staining with an antibody against CD45 (red, i–l).

a–d. Low-magnification images of coronal sections of the brain (a,c,d) and spinal cord (b) of biotin leakage (green) for the subacute timepoint for each disease. In a–d, the ROI for the disease is outlined with a white box. Subsequent images are given at higher magnification of tissue corresponding to the ROIs for controls and acute, subacute and chronic timepoints for each disease for biotin leakage (e–h) and CD45 staining (i–l). The most BBB leakage is observed at the subacute timepoint in each disease. Scale bars, 500 μm. n = number of mice (control, acute, subacute, chronic): seizure: 5, 3, 4, 4; EAE: 4, 4, 5, 5; stroke: 9, 3, 3, 3; TBI: 8, 3, 3, 3.
then returned to baseline expression at the chronic timepoint in the seizure, stroke and TBI models, but remained elevated in the EAE model (Fig. 5b).

We determined the expression of these BBB dysfunction genes during health by determining the expression of each gene in the brain and each peripheral endothelial cell sample. On average, the BBB dysfunction module genes were enriched in each of the peripheral vascular beds when compared with the brain (Fig. 5c), and 68 of the 136 genes were identified as peripheral-enriched (see Supplementary File 6, column FQ). Therefore, in each disease

Fig. 3 | Cerebrovascular transcriptional changes following disease. a. Venn diagrams of the numbers of upregulated (top) and downregulated (bottom) gene changes in the CNS endothelial cells observed in the seizure, EAE, stroke and pediatric TBI models, depicting the overlap of changes found at each of the timepoints. For each timepoint, genes were selected as upregulated if they were increased by log₂(fold change) > 1.00 and had an expression of >5 c.p.m. in the disease condition, with \( P < 0.05 \), and downregulated if they were changed by log₂(fold change) \( \leq -0.800 \) and had an expression of >5 c.p.m. in the control, with \( P < 0.05 \). The timepoint with the largest number of unique changes and the two timepoints with most overlap are highlighted in red. Statistical test: Wald test. \( n = 3 \) mice for each condition as a source of enriched endothelial cells with the exception of TBI control 2 mice for TBI control in red. Statistical test: Wald test.  

b. Bar graphs depicting the number of gene changes at each timepoint for seizure, EAE, stroke and pediatric TBI models, with color coding indicating the number of common changes between diseases.  

c. Bar graphs depicting the number of gene changes at each timepoint for seizure, EAE, stroke and pediatric TBI models, with color coding indicating the number of common changes between diseases.  

d. The average c.p.m. of all of the BBB-enriched genes (d, a list of genes can be found in Supplementary File 3) and peripheral endothelial-enriched genes (e, a list of genes can be found in Supplementary File 5) in the CNS endothelial cells at each timepoint in the seizure, EAE, stroke and pediatric TBI models. On average, there is a decrease in the expression of BBB-enriched genes and an increase in the expression of peripheral-enriched genes following each of the different disease models. Data are presented as mean ± s.e.m. Statistical test: Mann–Whitney \( t \)-test (unpaired, nonparametric, two-tailed): *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) and ****\( P < 0.0001 \); asterisks above error bars represent comparison with control sample; horizontal lines and corresponding asterisks compare samples aligned with each end of the horizontal line. \( n = 518 \) BBB-enriched genes; 1,399 peripheral-enriched genes.
model, CNS endothelial cells take on a 'peripheral' endothelial gene expression pattern. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) term pathways in this BBB dysfunction module include those involved in cell division, blood vessel development, inflammatory response, wound healing, leukocyte migration and focal adhesion, highlighting a role for angiogenesis and inflammation in BBB dysfunction (Fig. 5d). This core BBB dysfunction module of 136 genes includes several genes that have been identified to play a role in BBB dysfunction in different diseases. These include genes that regulate leukocyte trafficking (Sele, Selp) and proteolytic cleavage of ECM (Mmp14). In addition, multiple members of several gene families were upregulated: extracellular proteases of the Serpin family (Serpin1, Serping1), Adams and Adams families (Adam12, Adam19, Adams4, Adams8), collagens (Col1a1, Col1a2, Col3a1, Col5a1, Col5a2, Col12a1), centromere proteins (Cenpe, Cenpf), Igf-binding proteins (Igfbp4, Igfbp5), kinesins (Kif11, Kif15, Kif20b), lysyl oxidases (lox, Loxl2, Lox3), sulfatases (Sulf1, Sulf2), thrombospondins (Thbs1, Thbs2) and pleckstrin-domain-containing genes (Plekho1, Plekho2). Some of the main categories of genes in the BBB dysfunction module were extracellular matrix proteins (Col1a1, Col1a2, Col3a1, Lamb1) and modulators of the extracellular matrix including extracellular proteases (Adam12, Adam19, Adams4, Adams9, Mmp14), extracellular protease inhibitors (Serpin1, Serping1) and matrix crosslinkers (Lox, Loxl2, Lox3). Using immunohistochemistry, we validated that indeed many of these matrix proteins are increased along vessels in each disease (Supplementary Figs. 6 and 7).

The BBB dysfunction module genes could be segregated into three patterns based on the temporal regulation in the diseases (Fig. 5e). Group 1 reached peak upregulation in the acute phase of many of the diseases and consisted of genes that included molecules involved in inflammation (Sele, Timp) and extracellular proteases (Adams4, Adams8). Group 2 peaked at the subacute phase in most of the disease models and included inflammation (Selp, Darc) and cell cycle (Ccn2a, Cenpe, Mki67) genes. Group 3 peaked at early timepoints in seizure, stroke and TBI models, but continued to increase in EAE, and included ECM genes (Col3a1) and inhibitors of angiogenesis (Thbs1, Thbs2).

In the coexpression analysis, the BBB dysfunction module genes were found in different modules including the black, pink, salmon and steel-blue modules (Supplementary File 1, tab 3 column DW and EC, tab 4). Interestingly, while the black, pink and salmon modules were consistent with peripheral endothelial cell genes, the steel-blue module consisted of many genes expressed by fibroblasts. This could suggest that there is an endothelial-to-mesenchymal transition occurring during disease, as has been hypothesized during BBB dysfunction17, or it could occur if endothelial cells engulf fibroblast RNA particles or by fibroblast contamination in the endothelial samples. In addition to the common changes found at the subacute timepoint, there were also common changes found at the acute and chronic timepoints (see Supplementary Results & Discussion).

Changes unique to each disease. The most robust disease-unique changes occurred in the seizure model at the acute timepoint, during seizures and before BBB dysfunction and inflammation. Interestingly, many solute carrier transporters (65 genes) and ABC transporters (11 genes) were downregulated during the seizures corresponding with an upregulation of the glucose transporter Glut1 (Slc2a1), suggesting that the BBB modulates its gene expression in response to neuronal activity, perhaps to focus on glucose transport to meet the heavy energetic demands of the seizures.

The disease with the most unique changes at the subacute timepoint was EAE. Interestingly, many of these unique changes in EAE were family members of genes within the core BBB dysfunction module, including additional members of the following families: Serpin (Serpin1 and Serping1) are upregulated in at least three diseases, while Serpina3f, Serpina3g, Serpina3i, serpinb1a, serpinb9 and serpinb9b are upregulated just in EAE, Mmp (Mmp14 and Mmp23), Adam (Adam12/Adam19 and Adam9), Adams (Adams4/Adams8 and Adamt2/Adamt5), centromere proteins (Cenpe/Cenpf and Cenpa/Cenpi) and kinesins (Kif11/Kif15/Kif20b and Kif18b). This suggests that there is a core BBB dysfunction pathway, and that the more severe the dysfunction, the more additional family members are recruited. There were many additional unique changes in each disease (see Supplementary Results & Discussion).

Regulation of endothelial gene expression by Wnt/beta-catelin signaling. Wnt/beta-catelin signaling has been identified as a key regulator of CNS angiogenesis, BBB formation and BBB maintenance, inducing tight junction, solute transporter and efflux transporter expression, and repressing Phap expression16,17. We used a mouse model (Rosa-Bcat-GOF; VE-Cadherin-CreERT2 mice) to express constitutively active beta-catelin in endothelial cells, and then used endothelial enrichment and RNA sequencing to determine whether activated beta-catelin is sufficient to regulate gene expression in peripheral endothelial cells.

In Supplementary File 7 we present the RNA sequencing data comparing expression in liver and lung endothelial cells from activated beta-catelin (Rosa-Bcat-GOF; VE-Cadherin-CreERT2) and control mice (Rosa-Bcat-GOF). In Supplementary Table 5 we present the top 20 genes that were up- or downregulated in liver and lung endothelial cells by activated beta-catelin. Activated beta-catelin had a greater effect on liver endothelial cells (882 genes altered, P<0.05, c.p.m.>10 in control or Bcat-GOF) than lung endothelial cells (257 genes altered, P<0.05, c.p.m.>10 in control or Bcat-GOF) (Fig. 6a). These findings suggest that the highly permeable liver endothelial cells are more responsive to Wnt/beta-catelin activation than the moderately permeable lung endothelial cells. More BBB-enriched genes were upregulated (34 liver, 12 lung) than downregulated (5 liver, 5 lung) in the peripheral endothelial cells due to activation of beta-catelin, whereas more peripheral-enriched endothelial genes were downregulated (92 liver, 28 lung) than upregulated (50 liver, 23 lung) (Fig. 6a,b). The upregulated genes are more highly expressed in healthy brain endothelial cells than peripheral endothelial cells (Fig. 6c). Therefore, activated beta-catelin caused peripheral endothelial cells to take on more of a brain endothelial gene expression profile.

Lastly, we examined how genes regulated by activated beta-catelin were changed in CNS endothelial cells in different disease models (Fig. 6d). We did not find any correlation between the beta-catelin-activated gene changes and the changes observed in disease. Although the Wnt/beta-catelin pathway has been
implicated in BBB formation and maintenance, our data suggest that loss of Wnt/beta-catenin signaling may not be the driving force behind the transcriptional changes that we observed in the different disease models.

Discussion
We have used endothelial cell sequencing to understand the gene expression changes to the BBB in different neurological disease models that display BBB dysfunction.
Although each of the mouse models has different triggers, we found similar gene expression changes to the BBB in each of the diseases at the subacute timepoint when the BBB was most dysfunctional. These data suggest that although the disparate triggers may initially have different effects on the vasculature, they converge on similar responses in the endothelial cells. This has important implications for the treatment of neurological diseases in which BBB dysfunction is a contributing factor, including epilepsy, MS, stroke and TBI, as it suggests that identifying therapeutics that limit BBB dysfunction in one of the diseases may lead to treatments for others. Increased BBB permeability can be helpful, in allowing the entry of peripheral immune cells to aid in the clearance of debris and wound healing, but can also be detrimental, as it can lead to neuronal dysfunction, intracranial pressure increases and immune-mediated neuronal damage and degeneration. As with all inflammatory processes it is often the scale of the response that is critical, with small amounts being advantageous and large amounts detrimental. Therefore, developing methods to modulate these pathways to control the timing, spatial distribution and amount of BBB dysfunction will be critical.

It is of particular importance to understand the role of each gene of the BBB dysfunction module in regulating alterations in the properties of the BBB. These genes may have negative pathological consequences including leakage of the BBB, driving neuroinflammation and generating a fibrotic scar, or positive consequences including feedback mechanisms that are neuro-protective or for BBB repair. Several of the genes in this BBB dysfunction module have been identified to have key roles in the disease process. For instance, leukocyte adhesion molecules (Sele, Selp) and chemokines (CCL2) and TNF family members (TNFβ) have been shown to drive inflammation and damage4,11-13. On the other hand, upregulation of apelin (Aphl) has been found to be neuroprotective4,14-16.

It is also striking that a subset of this BBB dysfunction module was still elevated at the chronic timepoint after BBB permeability had restored to baseline in many of the diseases. This suggests that a single trigger, such as a 3-h kainic acid-induced seizure, can lead to long-term changes to the vasculature that could potentially have consequences on the function of the brain. For instance, changes in transporters, signaling or the extracellular matrix could alter the CNS microenvironment and have implications for neural circuit function. Persistent changes, such as (mmel176a, scla13, sclb13, scl7a11 or Tlr2), could potentially be used to identify and target therapeutics to a site of previous pathology.

Each disease also displayed unique brain endothelial transcriptional changes at each timepoint, likely reflecting the severity of the vascular insult, different inflammatory changes, amount of neuronal and glial cell death and activation, and differing repair processes in each disease. At the subacute and chronic timepoints, the largest numbers of changes were observed in the EAE model of MS. This is most likely due to the diversity, severity and length of inflammation in this model, which engages both the innate and the adaptive immune systems as the neural tissue is invaded with CD4+ T cells, CD8+ T cells and B cells in addition to innate immune cells. This is reflected in the enhanced immune-interacting gene changes in EAE including leukocyte adhesion molecules (Vcam1), histo-compatibility loci, interferon induced genes, interleukin pathway genes and complement pathway genes.

The most unique changes at the acute timepoint were observed in the kainic acid-induced seizure model, when the seizures were still pervasive but no inflammation or BBB leakage was observed. These unique changes are likely due to the increased metabolic demand in response to high levels of neuronal activity, as it appears that the endothelial cells alter their transport properties to concentrate on delivery of glucose to the neural tissue. This suggests that the BBB is capable of dynamically altering its properties in response to neural activity.

Wnt/beta-catenin signaling is a key regulator of CNS angiogenesis and BBB formation and maintenance, and has been specifically linked to the induction of solute transporters, tight junction proteins, efflux transporters and inhibition of transcytosis12,16. However, it is not known what effect this pathway has on the global gene expression of endothelial cells. We found that constitutively active beta-catenin was sufficient to induce a small subset of the CNS endothelial transcripational program on peripheral endothelial cells, including induction of Pgp and inhibition of Pivap. We only observed a small induction of Cldn5 and no effect on Glut1 expression. One possibility for the discrepancy is that Wnt/beta-catenin signaling has a greater effect on the induction of BBB gene expression during development than on the maintenance. Indeed, both turning on Wnt/beta-catenin signaling and turning down Wnt/beta-catenin signaling are important for the proper formation of CNS vessels17. Another possibility is that although Wnt/beta-catenin signaling is necessary for a wide swathe of BBB-specific gene expression, it is not sufficient to induce many of these genes in peripheral endothelial cells without a second signal. We did not find that the BBB dysfunction module in peripheral endothelial cells was suppressed by activated beta-catenin signaling, suggesting that although this signal is important for development and maintenance of the BBB, loss of this signal is not the only factor in altering brain endothelial gene expression during disease.

It should be noted that although we have achieved high levels of endothelial enrichment, we cannot exclude the fact
that transcripts from other cell populations may be identified through RNA transfer, cell adherence or impurities in the preparations. This is particularly important for the disease models, in which there is extensive cellular damage and many cells are taking up debris from other cell populations. Therefore, it is critical to verify that any observed changes are indeed coming from alterations in CNS endothelial cells, and not interacting cell populations.

In conclusion, we have developed an RNA-sequencing resource to understand the transcriptional program of CNS endothelial cells during health and disease. This resource may provide vital information on the mechanisms by which CNS endothelial cells form their unique properties, on how these properties are disrupted during injury and disease and for investigating novel targets to modulate the BBB as well as deliver drugs across the BBB to treat different neurological diseases.
Fig. 6 | Endothelial transcriptional regulation by activated beta-catenin. a, Total number of gene changes, as identified with \( P < 0.05 \) with a value of >10 c.p.m. in the activated beta-catenin sample in upregulated or control sample in downregulated, in purified liver and lung endothelial cells from control mice and mice expressing activated beta-catenin in endothelial cells. Genes are listed in Supplementary File 7. Genes were stratified based on whether they were identified as BBB-enriched (violet, Supplementary File 3), peripheral-enriched (blue, Supplementary File 5) or neither (green). Statistical test: Wald test. b, BBB-enriched (Supplementary File 3) and peripheral-enriched (Supplementary File 5) endothelial genes were subdivided based on whether they were up- or downregulated in liver or lung endothelial cells (Supplementary File 7) due to activated beta-catenin signaling. Statistical test: Wald test.

c, Average expression levels given in c.p.m. in healthy brain (red), lung (green) or liver (blue) (c.p.m. source, Supplementary File 3) for all genes that were up- or downregulated in liver or lung endothelial cells (Supplementary File 7) due to activated beta-catenin signaling. Statistical test: Wald test. \( n = 518 \) BBB-enriched genes and 1,399 peripheral-enriched genes.

d, GO terms and KEGG pathways as identified by DAVID Bioinformatics that are regulated beta-catenin signaling (identified in Supplementary File 7). Statistical test: EASE score (one-tail). The scale is the log10 of the EASE \( P \) values. \( n \) = number of genes: up in liver: 457, down in liver: 425, up in lung: 140, down in lung: 117.
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Author contributions

R.D. designed and participated in the analysis of all experiments. R.N.M. designed the surgery and Wnt signaling studies, performed cell purification of health and Wnt signaling models, and participated in the analysis of health, disease and Wnt signaling studies and writing of the manuscript. A.L.S. performed the endothelial cell purification and analysis of health and disease models. G.A.W. performed the alignment and normalization of RNA-sequencing data. P.G.S. and M.C.O. designed and performed the gene coexpression analysis. E.S. participated in the design and analysis of the EAE model. L.I.N.-H., R.D.S.-A., T.K. and J.C. participated in the design and analysis of all TBI experiments. T.H., M.Korai and M.Kotoda participated in the design and analysis of all MCAO experiments. S.A. participated in the design and analysis of the Wnt signaling study. A.B. and A.C.C. participated in the design and analysis of disease models.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Mice. Rosa-tdTomato mice were bought from Jackson Laboratories (stock 70999), Rosa-Bcat-GOF mice were generated by Makoto Takato and VE-Cadherin-CreERT2 mice were generated by Ralph Adams. All experiments were performed under Institutional Animal Care and Use Committee (IACUC) approved protocols at University of California San Francisco (UCSF) and University of California Davis (UCD), and mice were housed in a 12 h-light/dark cycle with 2–5 mice per cage. VE-Cadherin-CreERT2 mice were originally in FvB background, and mated to Rosa-tdTomato in C57BL/6. Disease models were performed on the first filial generation, except for EAE which was done after mating VE-Cadherin-CreERT2; six generations into C57BL/6.

Disease models. All disease models were performed on Rosa-tdTomato; VE-Cadherin-CreERT2 mice for endothelial cell purification and wild-type C57BL/6 mice for histological analysis. At 10 d before disease induction, Rosa-tdTomato; VE-Cadherin-CreERT2 mice were injected 100 μl of 20 mg/ml t-mxoxifen solubilized in corn oil for 3 consecutive days to induce expression of the tdTomato transgene. Males were used for MCAO, TBI and seizure, whereas females were used for EAE. Disease models were performed on mice at 2–3 months of age, except for TBI as noted in the TBI section below.

Seizure. Mice were intraperitoneally injected with 20 mg/kg kainic acid to induce seizures. Severity of seizures was scored between 0 and 4 based on observed behaviors: 1, freezing only; 2, freezing and occasional clonus only; 3, repeated and/or prolonged clonus, rearing and falling; 4, jumping and/or death. Mice that scored level 3 were used for brain endothelial cell enrichment or tissue section immunolabeling at different timepoints post kainic acid injection: 3h (acute), 48h (subacute) and 1 month (chronic). For RNA-sequencing experiments, three biological replicates were used for control and for each timepoint, in which each biological replicate examined was from endothelial cells enriched from the temporal lobes of one mouse.

Permanent MCAO. To mimic stroke, we used a permanent focal cerebral ischemia model in mice as previously described by others. Briefly, the permanent focal cerebral ischemia was induced by coagulation of the distal portion of the left middle cerebral artery (MCA). Briefly, mice were anesthetized with 2.5% isoflurane. The left common carotid artery was isolated and temporary ligated using 6–0 surgical nylon monofilament suture. Mice were then placed in the lateral position, and a 2-mm bar hole was made using a dental drill between the left orbit and ear. The distal portion of the left MCA was exposed and coagulated using a small vessel cautzerizer (Fine Science Tools) followed by transection of the artery. The ligation of the common carotid artery was released after 30-min occlusion. Rectal temperature was monitored and maintained at 37 ± 0.5°C using a thermostat-regulated heating pad. Sham-operated mice underwent an identical surgical procedure, including exposure of the common carotid artery and left MCA, except for the coagulation of the distal MCA and the temporary ligation of the common carotid artery. Tissues were harvested at 24h (acute), 72h (subacute) and 1 month (chronic) after MCAO. For RNA-sequencing experiments, three biological replicates were used for control and for each timepoint, in which each biological replicate examined was from endothelial cells enriched from the ipsilateral region of one mouse. For dissections, the infarct region was dissected based on coordinates generated from the leakage analysis on the subacute timepoint (Fig. 2).

EAE. EAE was induced by injecting the MOG35-55 peptide containing emulsion into the footpads. Acute timepoints were taken on the first day post induction and recovery. Follow-up, the disability score levelled off for 1 d. Chronic timepoints were taken 2 weeks after MCAO. For immunostaining, tissue sections were blocked/permeabilized with 0.1% Triton X-100 and 5% goat serum in PBS. Primary antibodies (1/50 dilution): rat anti-mouse CD31 (clone MEC13.3, BD Pharmingen 553370), rabbit anti-mouse CD45 (Abcam ab54269), rabbit anti-Claudin 5 (Life Technologies 341680), rabbit anti-Collagen I (Abcam ab32186), rabbit anti-Fibronogen (Abcam ab54269), rabbit anti-CD31 (BD Pharmingen 553370), rabbit anti-CD45 (Abcam ab54269), and rabbit anti-CD45 (BD Pharmingen 553370). Secondary antibodies (1/1,000 dilution): Life Technologies goat anti-rabbit Alexa Fluor-488 (A11034), goat anti-rabbit Alexa Fluor-594 (A11008), and goat anti-mouse Alexa Fluor-594 (A11001). DAPI was used for nuclear labeling.

Biotin permeability assay. For biotin leakage assays, 0.25 mg/ml sulfo-NHS-biotin dissolved in DPBS was used to transcardially perfuse mice (10 min, 4.5 ml/min) in place of DPBS alone, as described in the Immunostaining section, before fixation with 4% paraformaldehyde (10 min, 4.5 ml/min). Biotin was visualized on cryosections with streptavidin Alexa Fluor-488 (Life Technologies). Three or more mice were used for each analysis.

Quantification of BBB permeability. Biotin permeability: Tissue sections labeled with streptavidin Alexa Fluor-488 for detection of biotin were imaged, and the fluorescence of Alexa Fluor-488 was measured with ImageJ as pixel intensity mean gray value within a 100-pixel-diameter circular region of interest (ROI). For each mouse tissue section, three ROIs were measured within the tissue areas exhibiting leakage to biotin, which correspond to the temporal lobe in the seizure model, spinal cord lesions in EAE, infract in the stroke model and impact in the TBI model. Meningeal labeling was not included in ROIs. The average of the three ROI measurements represents each condition. Fibronogen permeability: The same methods were used as in biotin permeability quantification, except fibronogen was visualized with fibronogen antibody and Alexa Fluor-594-conjugated secondary antibody.

Endothelial cell enrichment. Endothelial cells were enriched from Rosa-tdTomato; VE-Cadherin-CreERT2 mice based on methods previously described. A graphical representation of the protocol, including modifications, is found in Supplementary Figs. 8 and 9. Modifications to the procedure include: Isolated cells suspensions were restandarded in 0.5% BSA and incubated with Myelin Removal Beads II for 15 min at 4°C (Miltenyi Biotec) according to the manufacturer’s protocol before FACs purification. For immunostaining, cell suspensions were blocked in IgG serum from rat (Sigma-Aldrich I8015, 1/100 in 0.5% BSA DPBS) on ice for 20 min, washed in 0.5% BSA DPBS, resuspended in 0.5% BSA DPBS, incubated at 4°C for 20 min, washed twice and resuspended in 0.5% BSA DPBS. For negative selection of pericytes and dead cells, cells were labeled with Alexa Fluor-488-conjugated rat anti-PDGFRβ (clone AB95; R&D Systems) and Alexa Fluor-488-conjugated rat anti-NG2 (Bioss bs-1119G-A488) or FITC-conjugated rat anti-mCD141 (clone 24.22, BDPharmingen 558744) at 1100 and DAPI at 0.5 μg/ml. For negative selection of immune cells, cells were labeled with: FITC-conjugated rat anti-CD11b (clone M1/70, eBioscience 11-0121-81) and anti-mCD45 (clone 30-F11, eBioscience 9481-85) at 1/100. With FACs, cells were sorted for tdTomato, excluding cells positive for FITC/A488, both FITC/A488 and tdTomato, doublets and DAPI+ dead cells. Cells were directly sorted into 750 μl Trizol (Life Technologies). Phenol-chloroform extraction was used to isolate nuclear acids and RNA was purified with Qiagen RNeasy Micro Kit. For the brain endothelial samples, a collagenase and dispase digestion step was added after trituration. Each brain was incubated in 1 mg/ml collagenase, 0.4 mg/ml dispase and 625 units of DNase in 10 mM ethyol saline stock solution at 37°C, 95% v/v CO2 and 5% CO2 for 30 min. Cell sorting was conducted at the UCSF Parnassus Flow Cytometry Core using a Beckman Coulter MoFlo XDP.

RNA sequencing and bioinformatics. RNA sequencing for health and disease samples was done with the Gladstone Genomics core. Quality of purified RNA
was accessed using an Agilent bioanalyzer. Complementary DNA was generated from full-length RNA using the NuGEN Ovation RNA-Seq V2 kit, which uses the single primer isothermal amplification method to deplete ribosomal RNA. After checking cDNA size and quality on the bioanalyzer, the cDNA was quantified by NanoDrop and then sheared by the Covaris S2 Sonicator to yield uniform-size fragments. The NuGEN Ovation V2 kit was used to ligate adapters and for barcoding and amplification. Libraries were purified using Agencourt XP magnetic beads, quality controlled with Agilent Bioanalyzer 2100 and quantified by KAPA quantitative PCR. Libraries were pooled and sequenced on HiSeq2500 using paired-end 100-base pair reads. For mapping, trimming of FASTQ format sequences was performed using FastX_Trimmer, and sequence quality control was assessed using FastQC v.0.10.1. Alignment to the Ensemble reference genome mm9 (v67) was performed using the splice-aware aligner TopHat v.2.0.11 and bowtie2 v.2.2.21 with parameters --no-coverage-search -m 2 -a 5 -p 7. Alignment files were sorted using SAMTools v.0.1.19. Count tables were generated using HTSeq-0.6.1. Differential expression was analyzed using DESeq v1.18.1. Reads were filtered using the built-in independent filtering function of DESeq2, and P values were calculated using a negative binomial distribution as a model for expected gene expression. FDR values were calculated with the built-in function using the Benjamin–Hochberg method.

For health, enrichment in one sample compared with another was calculated for genes with >2-fold enrichment, c.p.m. > 5 in the higher sample and P < 0.05. The BBB-specificity score for each gene was calculated as \[ \log_{2}(\text{BE} + 0.1) - \log_{2}(\text{HE} + 0.1) + \log_{2}(\text{KE} + 0.1) + \log_{2}(\text{LiE} + 0.1) - \log_{2}(\text{LuE} + 0.1) - \log_{2}(\text{KE} + 0.1) - \log_{2}(\text{LiE} + 0.1) - \log_{2}(\text{LuE} + 0.1) \]

Average(KE, LiE, LuE, LiE). For disease, upregulation was scored for each timepoint as \[ \log_{2}(\text{fold change}) < 0.800, \text{ c.p.m.} > 5 \text{ in the higher sample and P < 0.05.} \]

A lower cut-off was selected for downregulated genes (log, (fold change) < -0.800) than upregulated genes (log, (fold change) > 1.00) as we found that there were more robust upregulated changes than downregulated changes. This is likely because although we dissected around the disease region we still will have obtained a mixture of vessels that are leaky and vessels that are unaffected. Thus, it is easier to observe larger amplitudes due to a 0 value in the lower sample, it was scored as a log2(fold change)

\[ \log_{2}(\text{BE} + 0.1) + \log_{2}(\text{BE} + 0.1) + \log_{2}(\text{BE} + 0.1) + \log_{2}(\text{BE} + 0.1) + \log_{2}(\text{BE} + 0.1) + \log_{2}(\text{BE} + 0.1) \]

Peripheral endothelial-enrichment score was calculated as \[ \log_{2}(\text{HE} + 0.1) + \log_{2}(\text{BE} + 0.1) + \log_{2}(\text{KE} + 0.1) + \log_{2}(\text{LiE} + 0.1) - \log_{2}(\text{LuE} + 0.1) - \log_{2}(\text{LuE} + 0.1) \]

Average(KE, LiE, LuE, LiE). Disease model control and experimental tissue identities from EAE, MCAO and TBI models could not be blinded to researchers for measurement of vascular length, demarcation of lesions and observation of tracer permeability and cellular morphology. Since the appearances of control and disease tissues are easily identifiable. One mouse each for TBI control acute and chronic was excluded from RNA sequencing due to signs of hemorrhage at the surface of the brain. No other data points were excluded from analyses. Information on sex, age and number of mice used for each experiment is noted in specific sections in the Methods and figure legends.

**Statistical analysis.** Statistical significance of differences between groups was analyzed using the following software and statistical tests: (1) DESeq v1.18.1 Wald test (P values) and Benjamini–Hochberg method (FDR). (2) GraphPad Prism t-test (unpaired, parametric, equal standard deviation, two-tailed); Mann–Whitney t-test (unpaired, nonparametric, two-tailed) and Friedman test (matched data, nonparametric) with post hoc Dunn’s multiple comparison test. (3) DAVID Bioinformatics EASE score (a modified one-tailed Fisher exact test). (4) Microsoft Excel t-test (unpaired or paired, equal standard deviation, two-tailed). Application of these statistical methods to specific experiments is noted in the figure legends.

**Accession codes.** On the Gene Expression Omnibus, the cerebrovascular response to injury and disease can be found at GSE95401; gene expression profiles of liver and lung endothelial cells during normal and upregulated Wnt/beta-catenin signaling can be found at GSE95201.

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

**Data availability.** The data that support the findings of this study are available from the corresponding author upon request. RNA-sequencing files were deposited in the Gene Expression Omnibus repository and are available to use. Specific experimental details are also organized in the accompanying Nature Research Reporting Summary.

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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 | no software was used |
|-----------------|----------------------|

| Data analysis  | RNA seq analysis: FASTX, Trimmmer, FASTQ, Tophat 2.0.11, bowtie 2 2.2.21, SAMtools v.0.1.19, HTSeq 0.6.1, DESeq2, Microsoft Excel |

Coexpression: R statistical computing environment [http://cran.us.rproject.org], WGCNA R package
Pathway analysis: DAVID BIOINFORMATICS [https://david.ncifcrf.gov/]
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | We determined sample size based on standard practice in the literature for RNA sequencing analysis |
|-------------|--------------------------------------------------------------------------------------------------|
| Data exclusions | We excluded 2 control mice for the TBI disease model for RNA sequencing due to signs of brain hemorrhage. |
| Replication | To verify the reproducibility of the findings, RNA sequencing results from each biological sample of each condition were compared and determined to have similar expression patterns. Tissue permeability, tissue/cellular morphology or protein expression experiments were conducted 2 or more times. All attempts at replication were successful. |
| Randomization | Animals were assigned to various experimental groups at random. |
| Blinding | Disease model control and experimental tissue identity from EAE, MCAO and TBI models could not be blinded to researchers for measurement of vascular length, demarcation of region of interest and observation of tropermability and cellular morphology since the appearance of control and disease tissues are easily identifiable. |

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| n/a | Involved in the study |
|-----|-----------------------|
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| ☒ | 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

- rat anti-mouse CD31 (clone MEC13.3, BD Pharmingen 553370), rat anti-mouse CD45 (clone YW62.3, BioRad MCA1031GA), rat anti-mouse CD140B (clone APR5, eBioscience 14-1402-82), rabbit anti Fibrinogen (Abcam ab34269), rabbit anti Claudin 5 (Life Technologies 341600), rabbit anti Collagen I (Abcam ab21286), rabbit anti Collagen III (Abcam ab7778), rabbit anti Decorin (Biomatik CAC07220), rabbit anti Lumican (Biomatik CAU25816), rabbit anti SPP1 (Abcam ab8448), alexa-488 conjugated rat anti-PDGFRB (clone APR5 Novus NRP1-43349AF488), alexa-488 conjugated rabbit anti NG2 (Bioss bs:1192-A488), FITC conjugated rat anti mCD13 (clone R-3-242 BD Pharmingen 558744), FITC anti-mCD11b (clone M1/70, ebioscience 11-0117-81), FITC anti-mCD45 (clone 30-F11, ebioscience 0451-85), alexa-647-conjugated Rat mAb anti-CDS1 (clone 390 Molecular Probes A31716) |

Validation

Antibody were validated by manufacturers or in published studies:

- rat anti-mouse CD31 (clone MEC 13.3, BD Pharmingen 553370)
- Baldwin, H. S. et al. Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31): alternatively spliced, functionally distinct isoforms expressed during mammalian cardiovascular development. Development 120, 2539-2553 (1994).
- rat anti-mouse CD45 (clone YW62.3, BioRad MCA1031GA)
- Watt, S. M., Gilmore, D. J., Davis, J. M., Clark, M. R. & Waldmann, H. Cell-surface markers on haemopoietic precursors. Reagents for the isolation and analysis of progenitor cell subpopulations. Mol Cell Probes 1, 297-326 (1987).
- rat anti-mouse CD140B (clone APR5, eBioscience 14-1402-82), He, L. et al. Analysis of the brain mural cell transcriptome. Sci Rep 6, 35108, doi:10.1038/srep35108 (2016).
- rabbit anti Fibrinogen (Abcam ab34269),
Animals and other organisms

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| Laboratory animals | Rosa-tdtTomato were bought from Jackson Laboratories (stock 07909), Rosa-mat-GFP were generated by Makoto Takato, and VE-Cadherin-CreERT2 mice were generated by Ralf Adams. Disease models were performed on mice 2-3 months of age, except for TBI which was performed at P21 Rkainic acid, MCAC and TBI were all performed on male mice, EAE was performed on female mice. |
| Wild animals | Wild animals were not used in the study. |
| Field-collected samples | Field collected samples were not used in the study. |
| Ethics oversight | All experiments were performed under IACUC approval at UCSF and UCSD. |

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

Flow Cytometry

Plots

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### Methodology

**Sample preparation**

Endothelial cell purity was validated by the gene expression profiles of isolated endothelial cells. Homogenates of brain, spinal cord, heart, kidney, lung or liver were enzymatically and mechanically dissociated for FACS. Sorting was based on positive selection for tdTomato fluorescent protein or anti-CD31-A647 and negative selection with pericyte, immune cell and dead cell markers. The detailed process is outlined in the methods section and in a graphic is presented in Supplementary Figure 8.

**Instrument**

Beckman Coulter MoFlo XDP/BD biosciences FACS Aria II

**Software**

MoFlo/FACSDiva

**Cell population abundance**

We achieved >98% purity of endothelial cells. Purity was measured post-RNA sequencing by comparing the cell-specific signature of endothelial cells to published RNA sequencing data of brain endothelial cells, pericytes, neurons and astrocytes.

**Gating strategy**

From the starting tissue homogenate, FSC/SSC was adjusted to isolate single cells then followed by live cell DAPI/negative selection-FITC/488 dump. Gating for endothelial cells (Tomato+ or A647) was set conservatively within the tomato+ only or A647 + only cell population. No cells double positive for tomato DAPI, Tomato FITC/488, A647 DAPI, A647 FITC/488 were collected.

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