

Supplementary Figures:

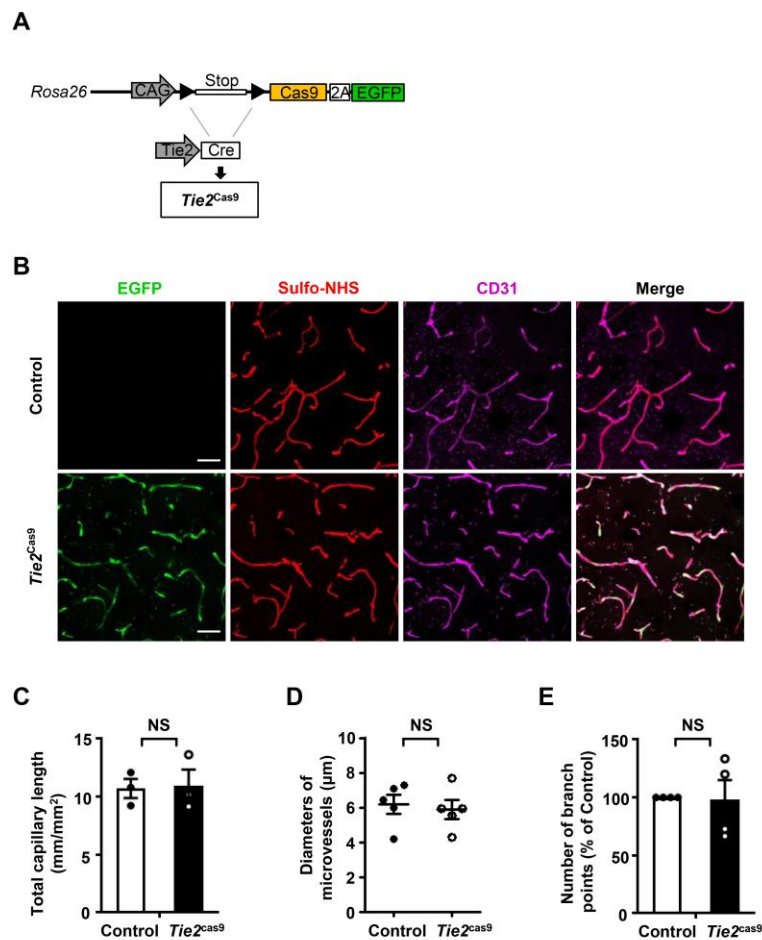


Figure S1. EC-specific Cas9-expressing mouse exhibited a normal brain vasculature.

(A) The strategy used for generating *Tie2*^{Cas9} mice. (B) Sulfo-NHS-LC-Biotin tracer extravasation assay revealed no significant differences in BBB integrity in brain sections of control and *Tie2*^{Cas9} mice. Scale bar, 40 μm . (C-E) Quantification of the cerebral capillary length ($n = 3$ mice), microvessel diameters ($n = 5$ mice), and branch points ($n = 4$ mice), showing a comparable brain vasculature in control and *Tie2*^{Cas9} mice. Data are means \pm SEM.

(NS = not significant, two-tailed unpaired t -test).

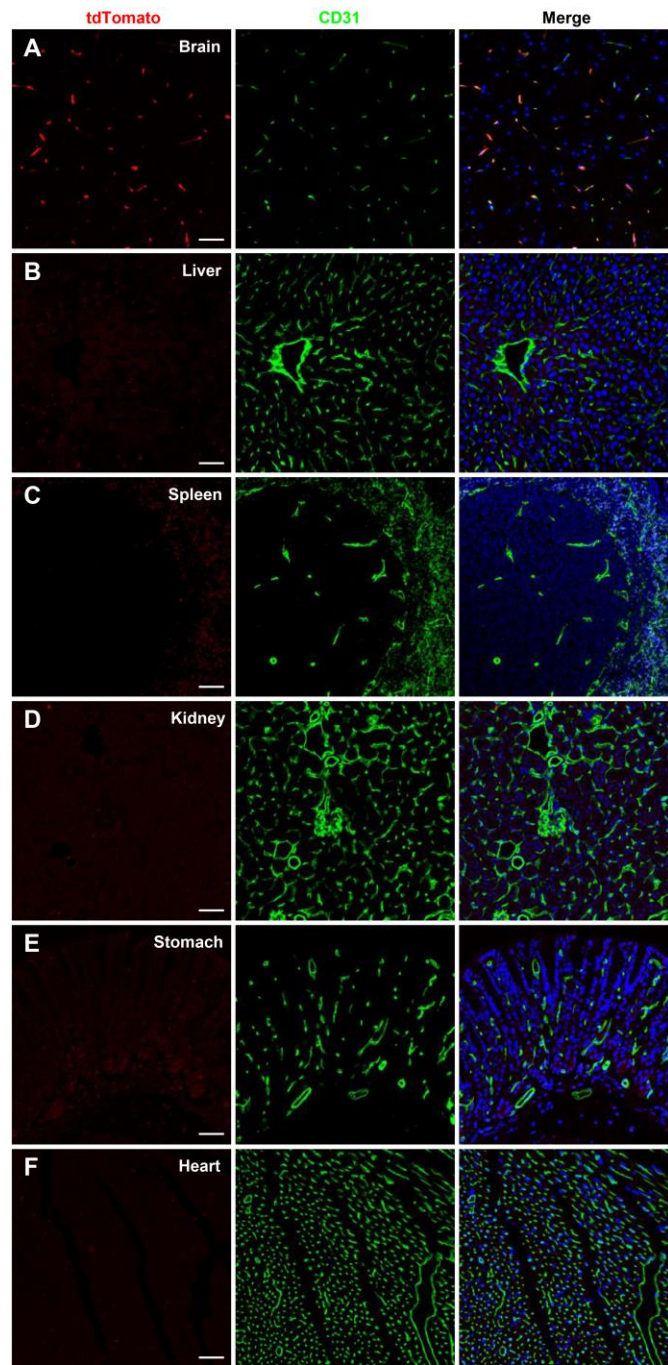


Figure S2. AAV-BR1 specifically transduces ECs in brain.

(A-F) Immunostaining of tdTomato (red), CD31 (green) and DAPI (blue) from brain (A), liver (B), spleen (C), kidney (D), stomach (E) and heart (F) 4 weeks after vector injection, showing that AAV-BR1-tdTomato specifically transduces the brain ECs. Scale bar, 50 μ m.

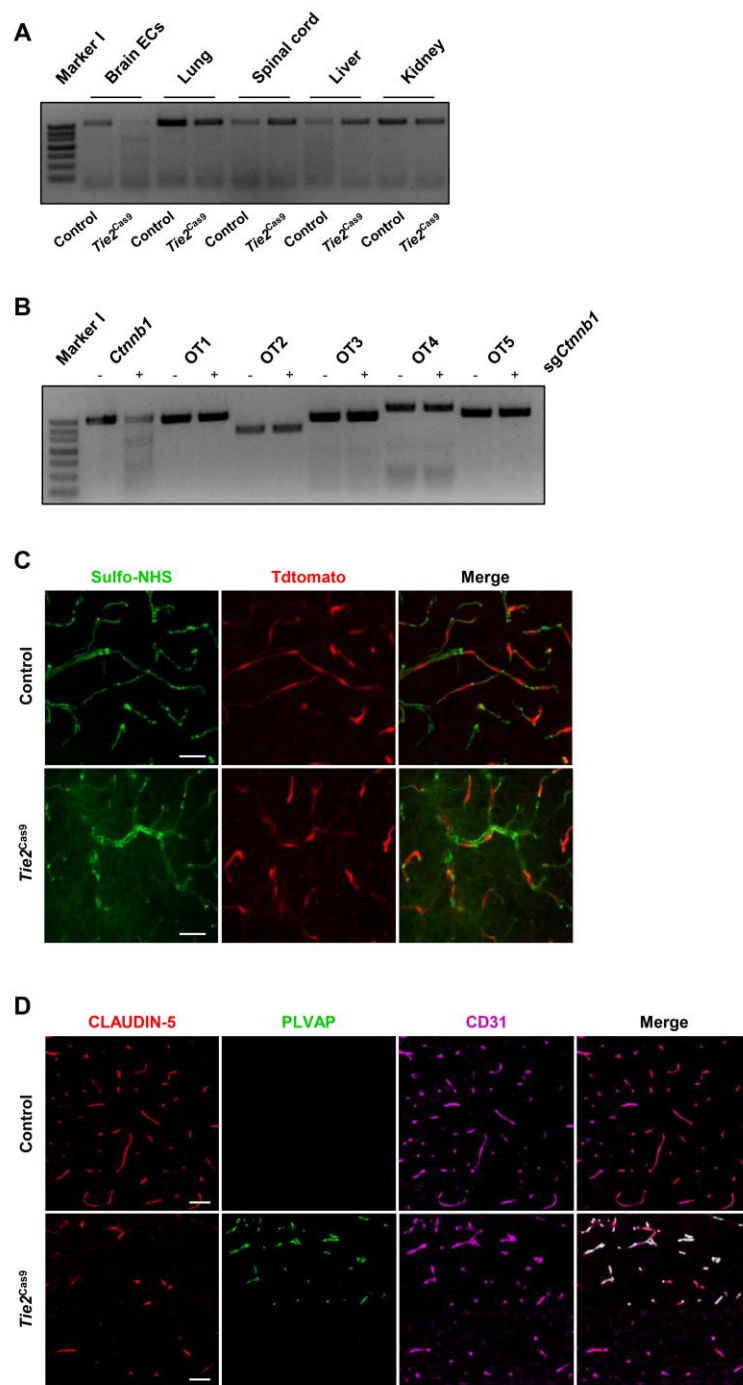


Figure S3. *In vivo* genome editing of brain ECs using AAV-BR1-CRISPR system.

(A) T7E1 analysis on target sites of PCR-amplified genomic DNA in lungs, spinal cord, liver, and kidney from control and *Tie2^{Cas9}* mice 4 weeks after AAV-BR1-*sgCtnnb1*-tdTomato injection. Lane I was loaded with a molecular weight marker (100 bp ladder). (B) T7E1

analysis of the top 5 potential OT sites of PCR-amplified genomic DNA of brain ECs isolated from *Tie2*^{Cas9} mice 4 weeks after AAV-BR1-sg*Ctnnb1*-tdTomato injection. Lane I was loaded with a molecular weight marker (100 bp ladder). (C) Sulfo-NHS-LC-Biotin tracer (green) injection generated BBB defects, mainly in tdTomato positive area. Scale bar, 30 μ m. (D) Immunostaining of CLAUDIN-5 (red), PLVAP (green) and CD31 (purple) showing downregulation of CLAUDIN-5, and upregulation of PLVAP in *Tie2*^{Cas9} mice at P60 (4 weeks after AAV-BR1-sg*Ctnnb1*-tdTomato treatment). Scale bar, 50 μ m.

Supplementary Table:

SgRNA oligos and primers for genotyping, T7E1 analysis, DNA sequencing, and qRT-PCR analysis.

Name	Sequence(5'-3')	Purpose
Tie2 Cre fwd	GAAGGGCAAGATGGATAGGGC	mouse genotyping
Tie2 Cre rev	GCATCGACCGGTAATGCAGGC	mouse genotyping
Cas9 Tg-1	GAGGCAGGAAGCACTTGCTCT	mouse genotyping
Cas9 Tg-2	TGGCGTTACTATGGGAACATACG	mouse genotyping
Cas9 Tg-3	TACACCTGTTCAATTCCCCTGC	mouse genotyping
sgCON fwd	CACCGAGCTCGCCATGTCGGTTCTC	<i>In vitro</i> sgRNA
sgCON rev	CGTGAACCGACATGGCGAGCTCAAA	<i>In vitro</i> sgRNA
<i>Ctnnb1</i> sgRNA1 fwd	CACCGGCGTGGACAATGGCTACTCA	<i>In vitro</i> and <i>in vivo</i> sgRNA
<i>Ctnnb1</i> sgRNA1 rev	CTGAGTAGCCATTGTCCACGCCAAA	<i>In vitro</i> and <i>in vivo</i> sgRNA
<i>Ctnnb1</i> sgRNA2 fwd	CACCGTGACCTGATGGAGTTGGACA	<i>In vitro</i> sgRNA

<i>Ctnnb1</i> sgRNA2 rev	CTGTCCAACCTCCATCAGGTCACAAA	<i>In vitro</i> sgRNA
<i>Ctnnb1</i> sgRNA3 fwd	CACCGGTTGGACATGGCCATGGAGC	<i>In vitro</i> sgRNA
<i>Ctnnb1</i> sgRNA3 rev	CGCTCCATGGCCATGTCCAACCAAAA	<i>In vitro</i> sgRNA
sgRNA1 fwd	AGCACCGTATGCCTACAATCT	T7E1 primer
sgRNA1 rev	CATCTTCTTCCTCAGGGTTGC	T7E1 primer
sgRNA 2 fwd	CTCTTCCCTTCTGCACACTAC	T7E1 primer
sgRNA 2 rev	GGACATTAGTGGGATGAGCA	T7E1 primer
sgRNA 3 fwd	CTCTTCCCTTCTGCACACTAC	T7E1 primer
sgRNA 3 rev	GGACATTAGTGGGATGAGCA	T7E1 primer
<i>Sobp</i> fwd	GTGAGACGACTAGACACGCTTAG	<i>Ctnnb1</i> OT1 T7E1
<i>Sobp</i> rev	GCCTGTCTATCTTATTGCTTTAGC	<i>Ctnnb1</i> OT1 T7E1
<i>Llgl2</i> fwd	CCTACATTGACTTCGGAGAGG	<i>Ctnnb1</i> OT2 T7E1
<i>Llgl2</i> rev	GTCAGGGACAATGTGATTAAGAAC	<i>Ctnnb1</i> OT2 T7E1
<i>Gm10851</i> fwd	CACATACACACATGAGCACATG	<i>Ctnnb1</i> OT3 T7E1
<i>Gm10851</i> rev	CTCAGTGAGGGACTGTCTGAC	<i>Ctnnb1</i> OT3 T7E1
<i>Gtpbp3</i> fwd	CATGAGTCACAGCTGTATTCACTT	<i>Ctnnb1</i> OT4 T7E1
<i>Gtpbp3</i> rev	CTCTAGATCAACTGAGCAAAGCT	<i>Ctnnb1</i> OT4 T7E1
<i>Gm26812</i> fwd	GAGTATTGACCTAGCACCTGATAG	<i>Ctnnb1</i> OT5 T7E1
<i>Gm26812</i> rev	CACGTGGTGAGAGCTGAATC	<i>Ctnnb1</i> OT5 T7E1
<i>Ctnnb1</i> fwd	AGATCGGAAGAGCACACGTCTGAACTCCA GTCACNNNN GTATAGGTAGCAGAATCACGG	DNA sequencing

<i>Ctnnb1</i> rev	TGCTGATTATTTACCAAGCCNNNNAGATC GGAAGAGCGTCGTGTAGGGAAAGAGTG	DNA sequencing
<i>Ctnnb1</i> exon 9 fwd	GGTGCTATTCCACGACT	qRT-PCR
<i>Ctnnb1</i> exon 10 rev	CCCTTCTACTATCTCCTCC	qRT-PCR
GAPDH fwd	TGCCCAGAACATCATCCCT	qRT-PCR
GAPDH rev	GGTCCTCAGTGTAGCCCAAG	qRT-PCR

*NNNN = index from Illumina TruSeq Small RNA Sample Prep Kit