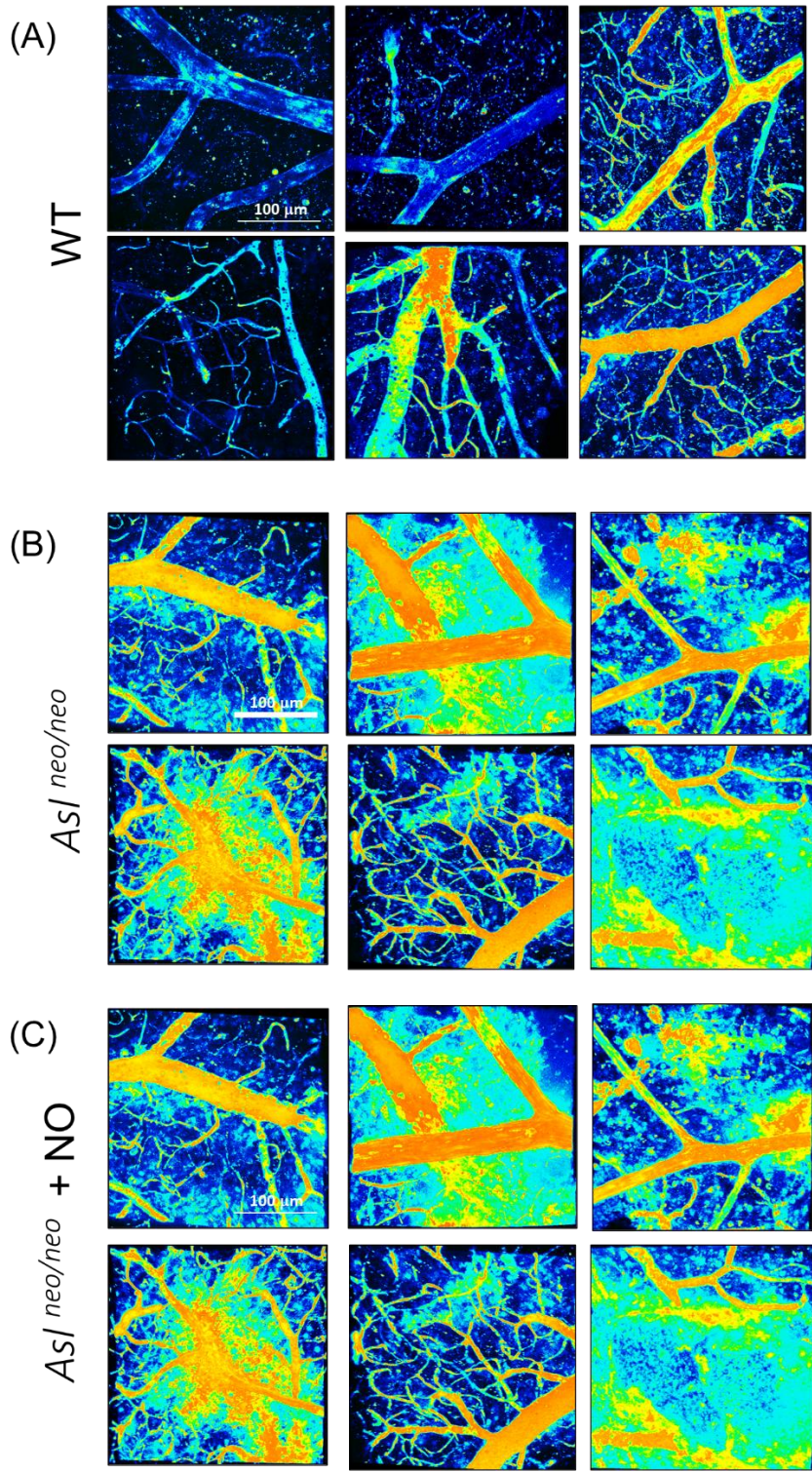


Supplementary Figure 1. Effects of NO Donor on mRNA expression of BBB genes

mRNA expression of *CLDN5*, *CLDN1*, and *CDH5* in HBMECs treated with 0, 10, or 25 μM of S-nitroso-N-acetylpenicillamine (SNAP), an NO donor (n = 3). Bar graphs represent mean values while error bars represent the standard deviation. One-way ANOVA, multiple comparisons.



Supplementary Figure 2. Additional images to supplement Figure 4. Two-photon images of cerebral vasculature in (A) WT, (B) mutant ($As1^{Neo/Neo}$), and (C) sodium nitrite-treated mutant mice ($As1^{Neo/Neo} + NO$) after Evans blue-fluorescent tracer injection.

Gene		FPKM		log2(fold change)
		siControl	siASL	
<i>ASL</i>	Argininosuccinate lyase	15.06	0.52	-4.86
<i>CLDN1</i>	Claudin-1	0.22	5.10	4.52
<i>CLDN5</i>	Claudin-5	148.30	45.59	-1.70
<i>CDH5</i>	Cadherin-5	668.29	177.28	-1.91
<i>ABCB1</i>	Multidrug resistance protein 1 (P-glycoprotein 1)	1.47	1.07	-0.45
<i>HSP90AA1</i>	Heat shock protein HSP 90-alpha	369.38	437.26	0.24

Supplementary Table 1. Effects of ASL deficiency on expression of BBB-associated genes in human aortic endothelial cells (HAECs).

Patient	1	2	3	4
Sex	Female	Male	Female	Male
Age of diagnosis	Birth	Birth	-	3.5 years
Diagnosis	Newborn screen, biochemical testing	Clinical symptoms, biochemical testing	-	Clinical symptoms, biochemical testing
Genotype (NM_000048.3)	c.1060C>T (p.Gln354Ter), homozygous	c.765dupG (p.Met256Aspfs*79), c.1193C>A (p.Ala398Asp)	-	c.557G>A (p.Arg186Gln), c.857A>G (p.Gln286Arg)
Patient demographics at the time of MRI				
Age	3 y 9 mo	10 y	33 y	21 y
Weight in kg (Z-score)	16.5 (0.53)	30.3 (-0.59)	55.7	88.1
Height in cm (Z-score)	102.4 (0.72)	122 (-2.72)	155.2	185.1
Treatment at the time of MRI				
Liver transplant (age)	Yes (4 years old)	No	No	No
Arginine dose (g/m ²)	2.2	1.8	10	1.87
Nitrogen scavengers	Yes	Yes	No	No
Protein restriction (% DRI)	No (100%)	No (100%)	No (Not counting protein)	Yes (50%)
Phenotype				
Highest recorded ammonia in medical record (μmol/L)	350	216	<9	27
Number of hyperammonemic episodes	6	5	0	0
Intellectual Disability	No	Yes	Yes	Yes
Seizures	No	No	Yes	No
Spasticity	No	Yes	Yes	No
Hypertension	No	No	No	Yes

"-" datum not available

Supplementary Table 2. Demographic data of the patients with ASLD evaluated in the study.