Supplementary methods

Vascular labelling with Lycopersicon esculentum lectin

Dylight 649-labeled *Lycopersicon esculentum* (DL-1178-1, Vector Laboratories, Burlingame, CA) was delivered retroorbitally 10 min before euthanasia with a ketamine (100 mg/kg) / xylazine (20 mg/kg) combination. Mice were anesthetized with sodium pentobarbital (120 mg/kg, i.p.) and perfused transcardially with PBS followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed and post-fixed overnight at 4°C in 4% PFA in PBS. Samples were washed in PBS for 1 h (PBS exchanged at least twice) and stored in PBS + at 4 °C until further processing.

Brains were then processed with the iDISCO immunolabeling protocol, as found at http://www.idisco.info. Briefly, after dehydratation, the brains were incubated in Methanol / 2 Volumes of Dichloromethane (DCM, Sigma 270997) for 3 hours, in 100 % DCM 15 minutes twice and left in Dibenzyl Ether (DBE, Sigma 108014) until observation.

Light-sheet microscopy imaging

Image acquisition were realized on a light-sheet microscope (Ultramicroscope II, LaVision Biotec) equipped with a sCMOS camera and a 2x NA 0.5 Olympus MVPLAPO objective. Dylight 649 was Illuminated by a 640 nm laser and detected using a 680/30 nm emission filter. The system was controlled by the Imspector software (LaVision Biotec). Images were made with a zoom of 6.3 resulting in a 12.6x magnification. The light sheet numerical aperture was set to 0.135, which corresponds to a 4 µm thick light sheet. We used illumination from both left and right sides to ensure a uniform illumination of the field of view. 200 µm thick Z-stack were acquired with a step size of 2 µm resulting in 200 images stacks. We acquired the same 4 regions from one brain to the other. To do so, brains were cut on the midsagittal plane. The hemi brain was oriented with the midsagittal on the bottom of the microscope observation chamber. The 4 regions were selected at a depth of 3 mm from the surface of the cortex.

Image analysis

Images were cropped to keep a center square of 500 x 500 µm where the light sheet is the thinnest, allowing to get the best resolution in Z. Z-stacks were analyzed using the filament module of Imaris 9.5 (Oxford instruments). First, threshold value for segmentation was determined using Otsu algorithm in FIJI [1]. This value was then reported in the Imaris Filament module, using the threshold (loops) algorithm setting (Bitplane, http://www.bitplane.com/imaris/imaris). After the analysis was done, vascular volume was obtained.

Preparation of Subcellular Fractions from brain Microvessels

For subcellular fraction analysis, freshly isolated brain microvessels were snap frozen and then cytosolic, membrane, nuclear, and cytoskeletal fractions were extracted by using the ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem) according to the manufacturer's instructions. For each point, three brains from the same genotype were pooled. A total of 200 µL of extraction Buffer I/II or 100 µL of Extraction Buffer III/IV was used for the fractionation. Fractionated protein sample lysates were separated into 7% or 12% (wt/vol) polyacrylamide gel and transferred on poly(vinylidene difluoride) membrane. Membranes were blocked for 1 h in 5% nonfat milk that was dissolved in Tris-buffered saline solution and were incubated in primary antibody at 4°C overnight against PDZRN3 (1:500, gift of Henry H. Ho, CA, USA), Ve cadherin (1:500, Invitrogen, 35-2500), Claudin5 (1:1000, Invitrogen, 35-2500), beta catenin (1:2000, Sigma, C2206), Occludin (1:500, Thermofisher, 33-1500), alpha tubulin (1:20,000, Sigma, T5168).

Behavior testing

Visual *behavior testing*. Ameroid Constrictor treated mice underwent visual behavior testing one day before the y-maze test using a visual cliff. Briefly, a transparent Plexiglass square arena was divided into two equal parts by aligning the middle of the arena with the edge of a table. The side sitting on the tabletop was considered a "shallow" side and the other one that is positioned over the floor area (50 cm high) a "deep" side. Each animal was placed on the shallow side and the total time the animal spent exploring each side of the arena was recorded within a 5 min trial, and the percentage time spent in the shallow side was analyzed.

Nesting test.

Age-matched animals of each genotype and gender were housed to individual testing cages approximately 1 h before the dark. One nestlet (square mats 5x5 cm, Serlab, UK) was placed in each cage. The day after, the capacity of each mouse to build a nest was evaluated on a rating scale of 1–5 based on the shape of the nest as described [27]. Briefly, rating scale #1 correspond to nestlet not touched, #2 partially torn, #3 nestlet partially torn with cotton spread around the cage, #4 build nest but still flat and #5 nest is nearly perfect with mouse inside.

RNA preparation and quantitative PCR

Total RNA was extracted from brain microvessels that had been snap-frozen in liquid nitrogen by using Tri Reagent® (Molecular Research Center Inc) according to the manufacturer's instructions. Reverse transcription was carried out by using M-MLV reverse transcriptase (Promega) with a mix of random and oligodt primers. Real-time PCR was performed on an AriaMx Real Time PCR system (Agilent Technologies) using B-R SYBER® Green SuperMix (Quanta Biosciences). Primers used for gene expression are listed below (Forward; Reverse). Cycle time values were measured as a function of P0 mRNA levels in the same tissue. The relative expression of each mRNA was calculated by the comparative threshold cycle method.

The following primers sets used were listed below:

Gene	Forward, 5'- -3'	Reverse, 5'- -3'
Pecam	AGGGGACCAGCTGCACATTAGG	AGGCCGCTTCTCTTGACCACTT
Cdh5	GTTCAAGTTTGCCCTGAAGAA	GTGATGTTGGCGGTGTTGT
Cldn5	ACGGGAGGAGCGCTTTAC	GTTGGCGAACCAGCAGAG
Ocln	GTCCGTGAGGCCTTTTGA	GGTGCATAATGATTGGGTTTG
Gpr124	CCCCTCATAATCTGCGGCAT	ACTACTCCTGAGCCTGGTGG
Lef1	ACCCGTGATGGGATAAACAG	TCCTGAAATCCCCACCTTC
Axin2	AACCTATGCCCGTTTCCTCT	CTGGTCACCCAACAAGGAGT
Ndp	AAAGCAAGCCATGTGACAGA	CAGTCCTTTCAAGGCCAGAG
Gpr124	CCCCTCATAATCTGCGGCAT	ACTACTCCTGAGCCTGGTGG
Nkd1	GTCTCCCCAAACCACAGGAG	GCCAAGGGCTAGTTCCCTTT
P0	GCGACCTGGAAGTCCAACT	CCATCAGCACCACAGCCTTC