Supplementary Material

Isolation of microvascular pericytes from mouse brain

For pericyte isolation from brain microvessels, we followed the method described by Sawada et al., 2012 [8] with a slight modification. Neonatal brains from control (n=5) and PC-cKO (n=3) mice were pooled and homogenized in 30% BSA-PBS (Ca^{2+} and Mg^{2+} free) filtered solution by using a Wheaton dounce homogenizer on ice, followed by consecutive centrifugations at 4°C for 10 minutes each, with pellet suspension in 30% BSA-PBS every time and increasing speed to remove the myelin: 500 *g*, 800 *g* and 1000 *g*, respectively. After the last centrifugation, the supernatants were removed and pellets were suspended with 0.5% BSA-PBS. The resulting solution was first filtrated through a 100µm cell strainer to remove big vessels and debris, and then through a 40µm cell strainer to remove single cells. The microvessels on the membrane were washed and collected with 0.5% BSA-PBS, and centrifuged at 800 *g* for 5 minutes. Then, microvessels were incubated with 1.5mL of 1mg/mL collagenase/dispase (SCR139, Millipore, CA) in PBS containing Ca^{2+} and Mag^{2+} at 37°C for 2h. Next, cells were washed and centrifuged once with 2mM EDTA/BSA-PBS, and once with pericyte culture medium (PC medium, #1201, ScienceCell™, CA) at 800 *g* for 5 minutes. Finally, cells were suspended in PC medium and seeded in fibronectin-coated 60mm dish. After 5 passages only pericytes survived in the PC medium.

MTT assay

Proliferation of brain-isolated pericytes from control and PC-cKO mice was measured with the MTT kit (ab211091, Abcam, Cambridge, MA, USA) following

the manufacturer's instructions. Pericytes were seeded in 96-well plate in triplicate for each genotype. Culture medium was replaced with 50µL of serum-free pericyte basal medium plus 50µL of MTT working solution. The culture multi-well plate was incubated at 37°C for 3 h and then supplemented with 150µL of MTT solvent. After incubation on a shaker at room temperature for 15min, the absorption at 590nm was recorded using the EnSpire Multimode Plate Reader (Perkin Elmer, MA, USA).

Blood glucose measurements

Blood glucose (mg/dL) in the pups was measured using the AlphaTRAK blood glucose monitoring system (71676-01, Zoetis, NJ). Blood was applied from the tail directly on the strips, and a control solution containing a fixed amount of glucose was used to confirm that both the meter and test strips worked together properly every time the device was used.

Real-Time Quantitative PCR

Total RNA was isolated and purified from P5 pooled-retinas (*n*=12 control retinas and *n*=12 PC-cKO retinas) using NucleoSpin® RNA Plus (#740984.50, Takara Bio USA, CA). c-DNA was synthetized by using the Superscript IV Reverse Transcriptase™ (18090010, ThermoFisher Scientific, MA). Primers sequence for mouse VE-Cadherin (Cdh5): Forward (5' to 3') CCTGAGGCAATCAACTGTGC; Reverse (3' to 5') GAGGAGCTGATCTTGTCCGT. Primers sequence for mouse 18S: Forward (5' to 3') CAGATGGGCACCAACAAAGT; Reverse (3' to 5') CATATACTTGCCGCCCAAGC. Amplification and detection was performed

using the Power SYBR™ Green PCR Master Mix (#4367659, ThermoFisher Scientific). VE-Cadherin mRNA expression from the retinal vasculature was calculated using the comparative *Ct* method for triplicate reactions, normalizing the values with those of the 18S mRNA expression.

Protein extraction and western blot analyses

For brain-isolated pericytes, proteins were extracted using a RIPA buffer (R3792, Teknova, CA) and Laemmli's buffer (BP-111R, Boston BioProducts, MA). For retina studies, proteins were extracted from pooled retinas (n=5 control mice and n=6 PC-cKO mice), and homogenized in lysis buffer (10mmol/L Tris-HCl, pH7.5, 1mmol/L EDTA, 1mmol/L EGTA, 150mmol/L NaCl, 0.5% Nonidet P40, 1% Triton X-100, and β-mercaptoethanol) containing a protease inhibitor cocktail (Complete 50X, Roche, France) and a phosphatase inhibitor cocktail (PhosII and PhosIII, Sigma-Aldrich). Protein concentration was determined using a bicinconinic acid assay (23227, Pierce ™, MA). Proteins (20-30µg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 4-15% acrylamide gel and electroblotted onto PVDF membrane (162-0177, Bio-Rad,). Membranes were blocked with 5% bovine serum albumin (BSA, BP1600-100, Fisher Bioreagents™, PA) in Tris-buffered saline (TBS) for 1 hour at room temperature and incubated overnight at 4°C with the following antibodies: rabbit anti-NG2 Chondroitin Sulfate Proteoglycan antibody (AB5320, Millipore); mouse anti-N-Cadherin antibody (BD Pharmingen™); rabbit anti-VE-Cadherin antibody (CP2231 ECM Biosciences, KY); rabbit anti-Rras antibody (A4862, Annaspec); mouse anti-β-actin antibody (A2228, Sigma Aldrich); mouse anti-VCAM-1 antibody 6G9

antibody (NBP1-47491, Novus biological, CO); mouse anti-Cre recombinase antibody (ab24607, abcam); mouse anti-GAPDH antibody (SC-32233, Santa Cruz Biotechnology); mouse anti-PDGFRβ antibody (AF1042 R&D Systems, MN); rabbit anti-integrin α1 antibody (#71747, Cell Signaling, MA); Anti-rabbit IgG HRP-conjugated (W401B, Promega, WI, USA) or anti-mouse IgG HRP-conjugated (W402B) were used as secondary antibodies and the Western lightning Plus-ECL kit (NEL103001EA, Perkin Elmer, MA, USA) was used for chemiluminescence detection.

Supplementary Figures

Supplementary fig. 1. Offspring genotyping.

(**A**) The floxed and wild-type *Rras* allele structures (**B**) The image shows sybr®-stained PCR products after gel electrophoresis. Two sets of primers were used to amplify the target sequences from mouse tissue. The top DNA band (680-700bp) framed in black corresponds to the amplification product obtained with the Cre primers. The lower bands (384bp and 513bp) framed in red correspond to the amplification obtained with the *Rras*$^{fl/fl}$ primers. DNA bands in sample #3 indicate amplification of the target sequences indicative of a PC-cKO mouse. The gel also shows all potential offspring genotypes resulting from the *pdgfrb*-Cre × *Rras*$^{fl/fl}$ crossing.

Supplementary fig. 2. Pericyte-targeted ablation of *Rras*

Immunostaining of capillary-size vessels from different tissues showed lack of R-Ras in pericytes but not in ECs of PC-cKO mice. CD31 and NG2 were used as markers for ECs and pericytes, respectively.

Supplementary fig. 3. Histology of *Rras* PC-cKO organs.

H&E staining in several major organs collected from 6-week old control and *Rras* PC-cKO mice. No obvious sign of abnormality was found by histological examinations in the *Rras* PC-cKO tissues.

Supplementary fig. 4. Immunostaining of P5 retina.

(**A**) Whole-mounted P5 retinas were immunostained for 2 pericytes markers, NG2 and PDGFR, co-stained with IB4, and analyzed by confocal 3-D imaging. An aqueous mounting medium Fluoromount-G was used to mount the stained retinas. (**B**) P5 retinas were immunostained for desmin, a pericyte marker, co-stained with IB4, and analyzed by confocal 3D imaging. (**C**) Immunostaining of VE-cadherin in P5 retina mounted using Fluoromount-G.

Supplementary fig. 5. Microvessels diameter.

(**A**) Vein width was measured in maximum intensity projection confocal images from P5 retinas. Each dot represents the average vein width for each vein measured at its distal, medial and proximal position in each picture analyzed. (**B**) Capillary diameter between arteries and veins close to the central disc was measured in 3-D reconstructed confocal images. Each dot represents the average capillary diameter in each section analyzed. The following equation

was used: Diameter3D= [6 x Volume / π]$^{1/3}$. NIS-Elements software (Nikon) was used to perform all analyses.