**Mouse strains, tissue harvesting and sectioning**

All experiments involving mice were conducted in accordance with policies and procedures described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and were approved by the Institutional Animal Care and Use Committee (IACUC) at The Jackson Laboratory. All mice were bred and housed in a 12/12 hours light/dark cycle. Four months old male C57BL/6J mice were injected intraperitoneally with a lethal quantity of ketamine/xylazine according to IACUC approved procedures. They were perfused with 1X PBS (phosphate buffered saline) and whole brains were removed and fixed in 4% paraformaldehyde for two hours at 4˚C. Following fixation, the tissue was rinsed in 1X PBS, incubated in 10% sucrose for eight hours at 4˚C, then incubated in 30% sucrose overnight at 4˚C. Brains were then frozen in optimal cutting temperature (OCT) compound and stored at -80˚C until sectioning. Frozen brains were sectioned at 25µm and mounted on glass slides, which were stored at -80˚C until required for immunofluorescence staining.

**Immunofluorescence**

Brain sections were incubated overnight at 4˚C in the following primary antibodies: rabbit polyclonal anti-PDGFA (1:50, Santa Cruz Biotechnology), rabbit polyclonal anti-PRKAR1B (1:50, Santa Cruz Biotechnology), goat anti-COL-IV (1:50, EMD Millipore), goat anti-CD31 (1:50, R&D Systems), goat anti-VE-CAD (1:50, R&D Systems), and chicken anti-GFAP (1:300, OriGene). Sections to be stained with anti-COL-IV and anti-CD31 antibodies were immersed in deionized water for 3 minutes at 37˚C and then treated with 0.5mg/ml pepsin in 0.2N HCL for 15 minutes at 37˚C. Slides were then washed in 1X PBS twice for 10 minutes at room temperature. Sections stained with other antibodies were incubated in liberate antibody binding (L.A.B.) antigen retrieval solution (Polysciences, Inc) for 20 minutes at room temperature, before and after which they were washed in 0.5% PBT for 10 minutes at room temperature. With the exception of anti-Col-IV, antibodies were diluted in 0.5% PBTB (1X PBS, .0.5% TritonX-100, 0.5% BSA (bovine serum albumin)) containing 10% normal donkey serum. Anti-Col-IV was diluted in 0.5% PBT. Sections were washed three times in 0.5% PBT then incubated for two hours at room temperature with their respective secondary antibodies (donkey anti-rabbit Alexa Fluor 488, donkey anti-goat Alexa Fluor 568, and donkey anti-chicken Alexa Fluor 647, 1:1000 dilution, Life Technologies). All sections were then counterstained with DAPI (1:1000 in 1X PBS) and then washed with 1X PBS prior to mounting with Aqua PolyMount. Mounted slides were stored at -20˚C until the time of imaging. Images were taken using a Leica SP5 confocal microscope located within the Imaging facility at The Jackson Laboratory.