

SUPPLEMENTAL MATERIAL

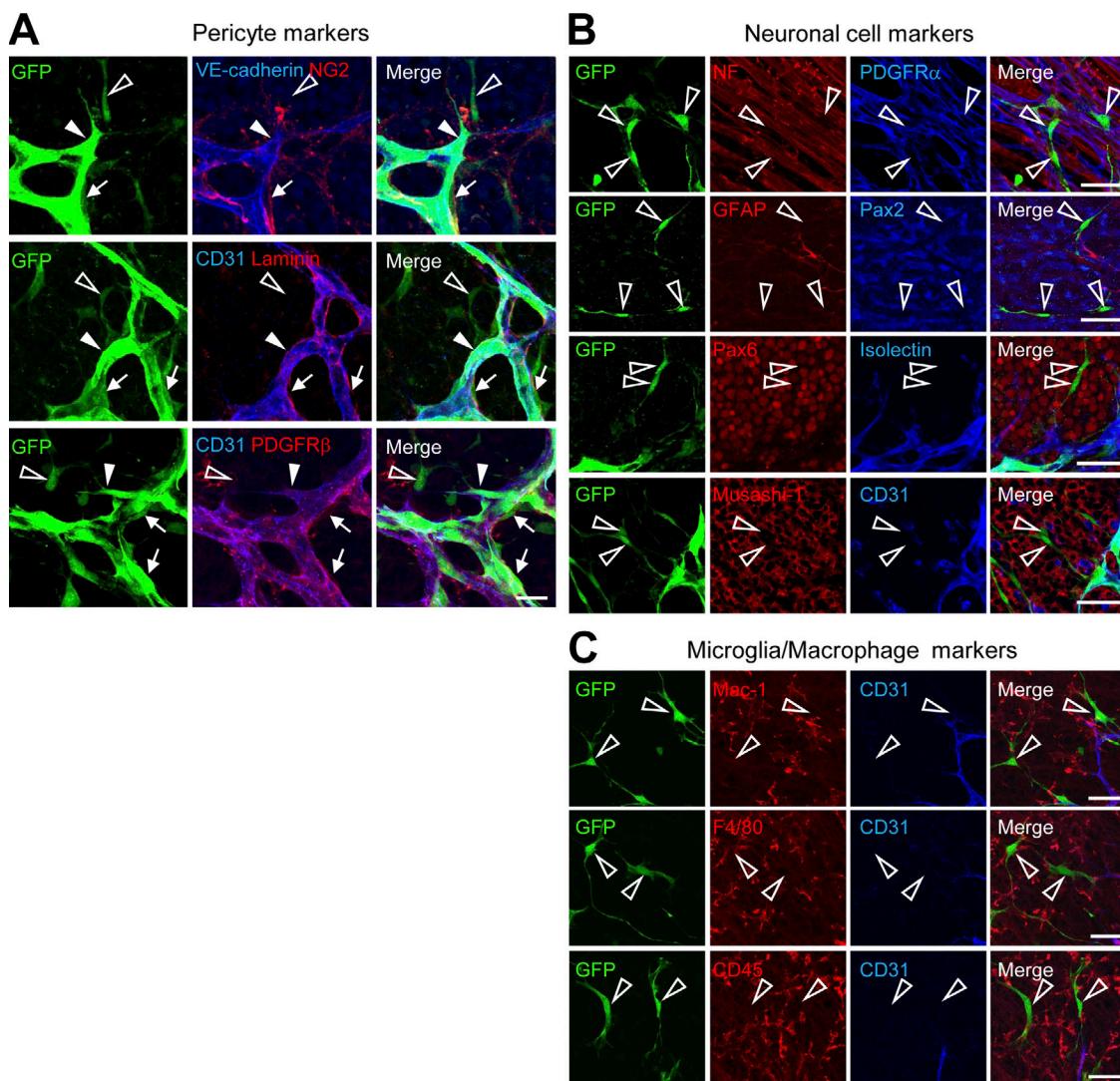
Kubota et al., <http://www.jem.org/cgi/content/full/jem.20102187/DC1>

Figure S1. Characterization of *Po-Cre*⁺ spindle-shaped cells in the avascular area. IHC of retinas from *Po-Cre*⁺/*FloxCAT-EGFP* mice at P4 stained with antibodies of markers for pericytes (A), astrocytes/neurons/retinal progenitors/photoreceptors (B), and microglia/macrophages (C). Representative images from at least three independent experiments. Pericytes (arrows). GFP⁺ spindle-shaped cells (open arrowheads). GFP⁺ endothelial cells (closed arrowheads). Bars: (B and C) 50 μ m; (A) 20 μ m.

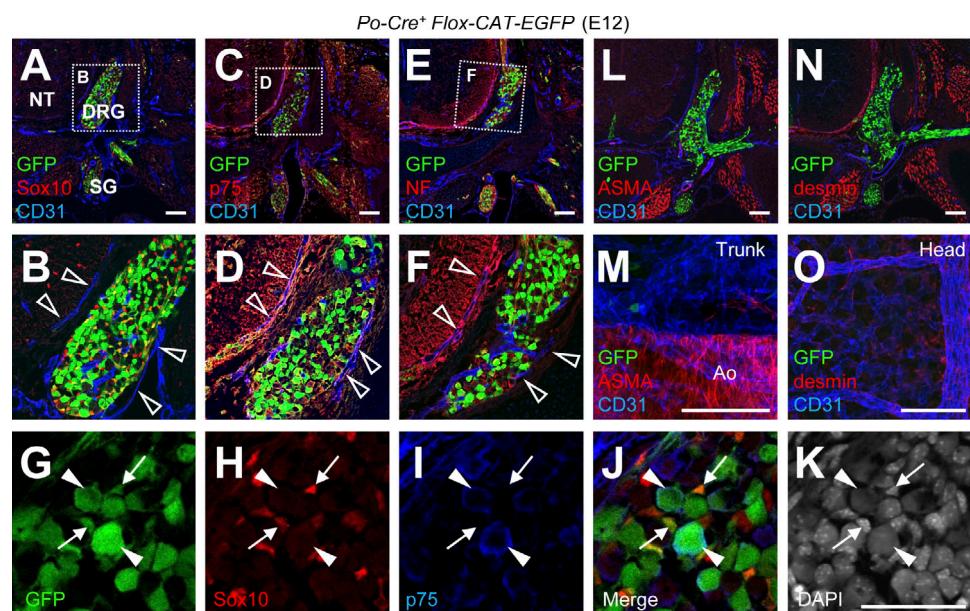


Figure S2. **Po-Cre does not mark embryonic endothelial cells.** Trunk sections (A–L and N) or whole-mount samples (M and O) of *Po-Cre⁺ Flox-CAT-EGFP* embryos (E12) immunostained with indicated antibodies. Representative images from at least three independent experiments. Ao, dorsal aorta. DRG, dorsal root ganglia; SG, sympathetic ganglia; NT, neural tube. Open arrowheads, endothelial cells. GFP⁺sox10⁺ cells (arrows in G–K). GFP⁺p75⁺ cells (closed arrowheads in G–K). Bars, 50 μ m.

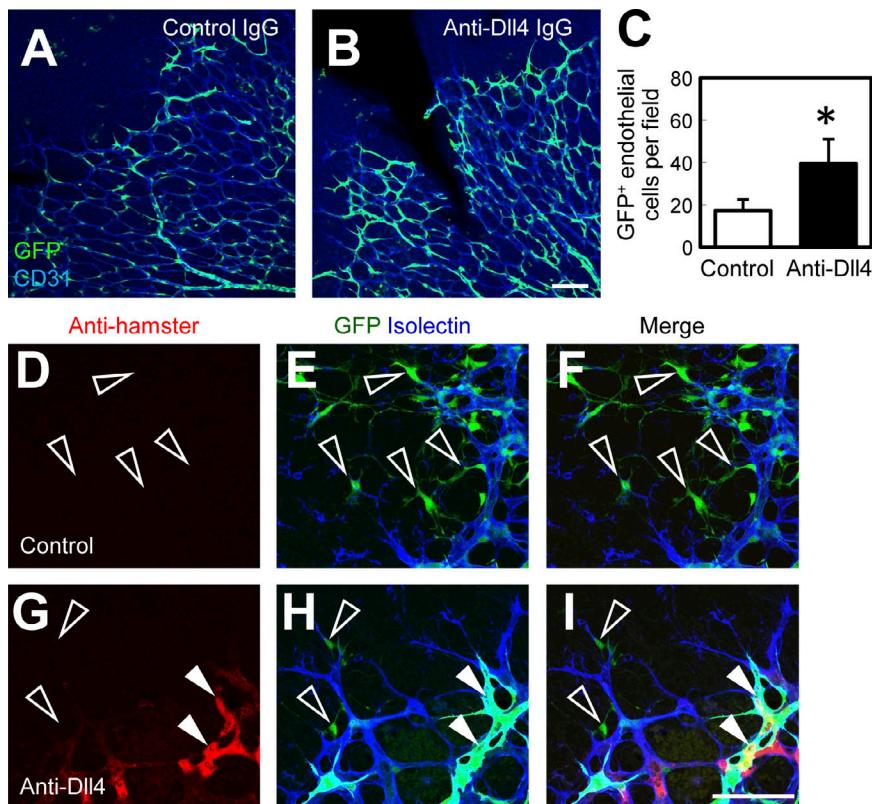


Figure S3. Recruitment of PVPs is enhanced by Dll4 neutralization. (A and B) IHC for GFP (green) and CD31 (blue) in P5 retinas of *Po-Cre⁺/FloxCAT-EGFP* mice after pulse treatment from P1 to P5 with hamster IgGs (A) or Dll4 neutralizing antibodies (B). Representative images from eight independent experiments. (C) Quantification of GFP⁺ endothelial cells ($n = 8$). (D–I) Immunostaining with anti-hamster IgG combined with IHC for GFP (green) and CD31 (blue) in retinas of P5 *Po-Cre⁺/FloxCAT-EGFP* mice 12 h after treatment with hamster IgGs or Dll4 neutralizing antibodies. Representative images from three independent experiments. Closed arrowheads, GFP⁺ endothelial cells. Open arrowheads, PVPs. Bars, 100 μ m. *, P < 0.05.

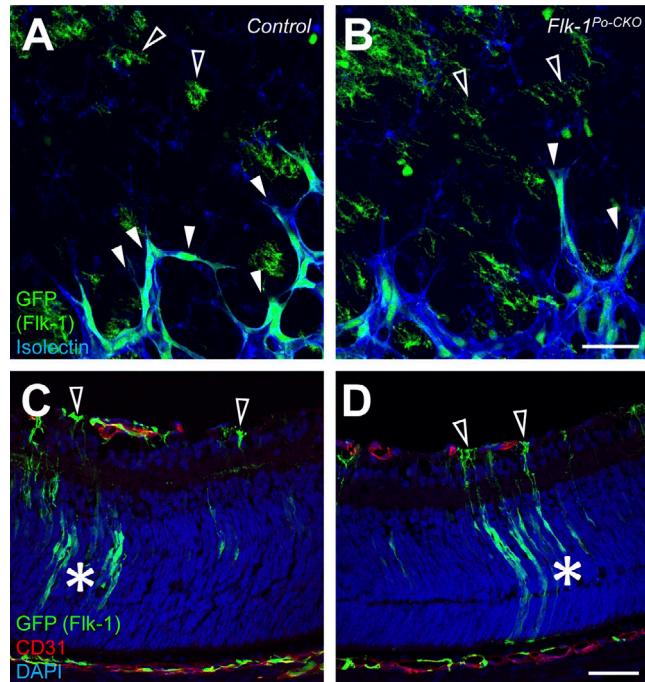


Figure S4. Flik-1 expression in $\text{Flk-1}^{\text{Po-CKO}}$ mice shows reduction in endothelial cells, but not in Muller cells and photoreceptors.

(A and B) Whole-mount IHC for GFP (green) and Isolectin (blue) in P5 retinas of $\text{Flk-1}^{\text{Flox/EGFP}}$ mice (control) or $\text{Po-Cre}^+/\text{Flk-1}^{\text{Flox/EGFP}}$ ($\text{Flk-1}^{\text{Po-CKO}}$) mice. Representative images from three independent experiments. Closed arrowheads, endothelial cells. Open arrowheads, Muller cells. (C and D) Section IHC in P5 retinas of $\text{Flk-1}^{\text{Flox/EGFP}}$ mice (control) or $\text{Po-Cre}/\text{Flk-1}^{\text{Flox/EGFP}}$ ($\text{Flk-1}^{\text{Po-CKO}}$) mice. Representative images from three independent experiments. Asterisks represent hotoreceptors in the outer nuclear layer. Open arrowheads, Muller cells. Bars, 50 μm .

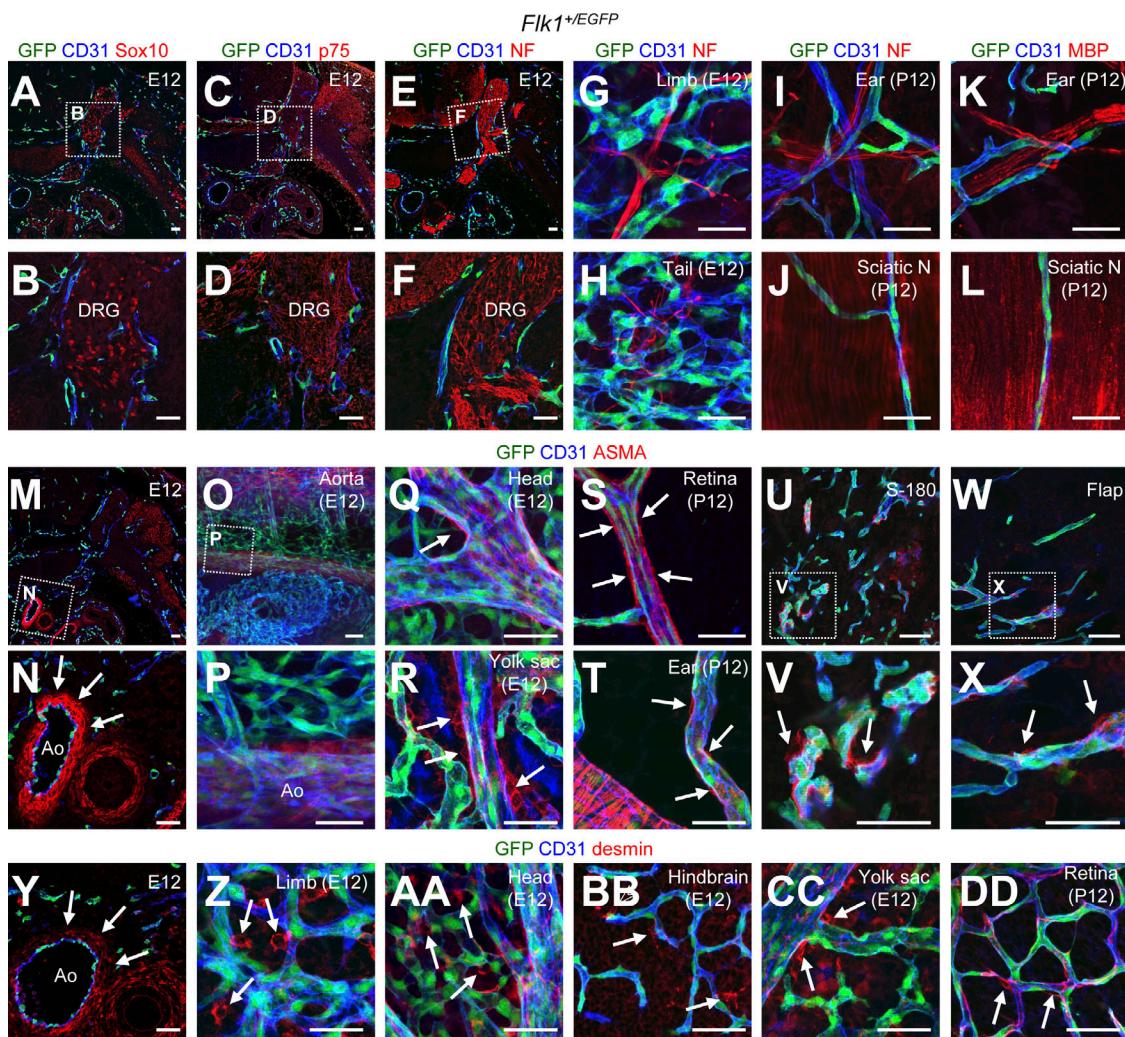


Figure S5. *Flk-1* is expressed in endothelial cells, but not neural crest cells, neurons, Schwann cells, or pericytes/vSMCs. IHC with the indicated antibodies in various tissues harvested from *Flk-1^{+/EGFP}* mice at E12, P12, 10 d after S-180 transplantation (3 mo old), or 7 d after ischemic flap elevation (3 mo old). Representative images from at least three independent experiments. Dorsal root ganglia (DRG). Pericytes/vSMCs (arrows). Dorsal Aorta (Ao). Bars, 50 μ m.

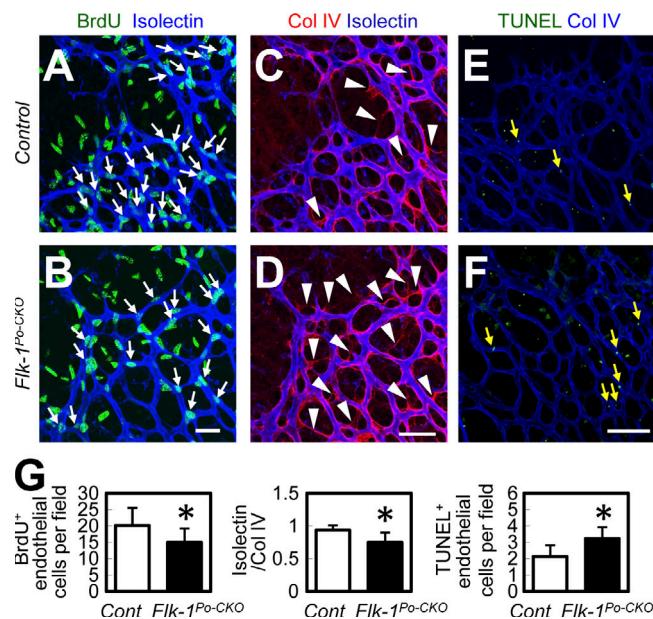


Figure S6. *Flk-1*^{Po-CKO} mice show decreased endothelial proliferation, increased endothelial apoptosis, and increased vessel regression. (A–F) IHC with indicated antibodies. Arrows, endothelial proliferation. Arrowheads, empty sleeves. Yellow arrows, apoptosis. Representative images from eight independent experiments. (G) Quantification of BrdU⁺ endothelial cells, ratio of IsolectinB4⁺ vessels to collagen IV⁺ vessels, and TUNEL⁺ endothelial cells ($n = 8$, respectively). Bars, 100 μ m.