Supplementary Information

Table S1. PCR primers for genotyping.

Target	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product length (bp)
Phd2	CAAATGGAGATGGAAGATGC	TCAACTCGAGCTGGAAACC	floxed, 840 wt, 389
Phd2	AACTCCGCCAAGCAGGTCAGAA	CCCGAAGAACGATACCGTCGAG	deleted, 140
GFAPCre	ACTCCTTCATAAAGCCCTCG	ATCACTCGTTGCATCGACCG	GFAPCre, 230

Table S2. qPCR primers.

Target	Forward primer (5' to 3')	Reverse primer (5' to 3')
Phd2	TAAACGGCCGAACGAAAGC	GGGTTATCAACGTGACGGACA
VEGF-A	GCACATAGGAGAGATGAGCTTCC	CTCCGCTCTGAACAAGGCT
PDGF-A	CCTGTGCCCATTCGCAGGAAGAGA	TTGGCCACCTTGACACTGCGGTG
EPO	TAGCCTCACTTCACTGCTTCG	GCTTGCAGAAAGTATCCACTGT
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

Table S3. Antibodies and other staining reagents

Antibodies/staining reagents	Application	Target Cells/Tissues	Supplier	Catalog #	Final Conc. /dilution
Rat anti-GFAP	Whole mount IF	astrocytes	Life Technologies (Thermo Fisher)	I-30300	1:500
Rabbit anti-Pax2	Whole mount IF	astrocytes	Abcam	Ab79389	1 µg/ml
Rabbit anti-Collagen IV	Whole mount IF	basement membrane	Millipore (Thermo Fisher)	AB755P	2 µg/ml
Goat anti- PDGFRα	Whole mount IF	astrocytes	R&D Systems (Thermo Fisher)	AF1062	1:100
Rat anti-BrdU -Biotin	Whole mount IF	proliferative cells	Abcam	Ab171059	1:1000
Anti- Caspase 3 (against active fragment)	Whole mount IF	Apoptotic cells	Cell Signaling	9661S	1:200
Mouse anti-actin α-Smooth Muscle -FITC	Whole mount IF	VSMCs	Sigma-Aldrich	F3777	1:100
Anti-VEGF	Intravitreal injection	Ocular VEGF-A	Cedarlane	CL9188AP	1.8 µg/µl; 1 µl per eye
Rat anti-PHD2	Blotting	N/A	Maine Biotechnologies	Custom-made	1:100
Anti-HIF-1α	Blotting	N/A	Novus Biologicals	NB100-449	1:200
Anti-HIF-2α	Blotting	N/A	Novus Biologicals	NB100-132	1:100
Anti-ß-actin	Blotting	N/A	Santa Cruz Biotechnology	sc-1616	1:200
Goat anti-rabbit Alexa Fluor®-488	Whole mount IF	N/A	Life Technologies (Thermo Fisher)	A-11034	1:200
Goat anti-rat IgG-Alexa Fluor®-488	Whole mount IF	N/A	Life Technologies (Thermo Fisher)	A-11006	1:200
Donkey anti-rabbit IgG-Cy3	Whole mount IF	N/A	Jackson ImmunoResearch; West Grove, PA	11473299	2 µg/ml
Donkey anti-goat Alexa Fluor®-488	Whole mount IF	N/A	Life Technologies (Thermo Fisher)	A-11055	1:200
Goat anti-mouse IgG-Alexa Fluor®-488	Whole mount IF	N/A	Life Technologies (Thermo Fisher)	A-11001	1:200
IB_4-Alexa Fluor®-594	Whole mount	Endothelial cells	Life Technologies (Thermo Fisher)	I-21413	1:100
IB_4-Alexa Fluor®647	Whole mount	Endothelial cells	Life Technologies (Thermo Fisher)	I-32450	1:100
Streptavidin-DyLight®-549	Whole mount IF	Proliferative cells	Jackson ImmunoResearch; West Grove, PA	016-500-084	1:500

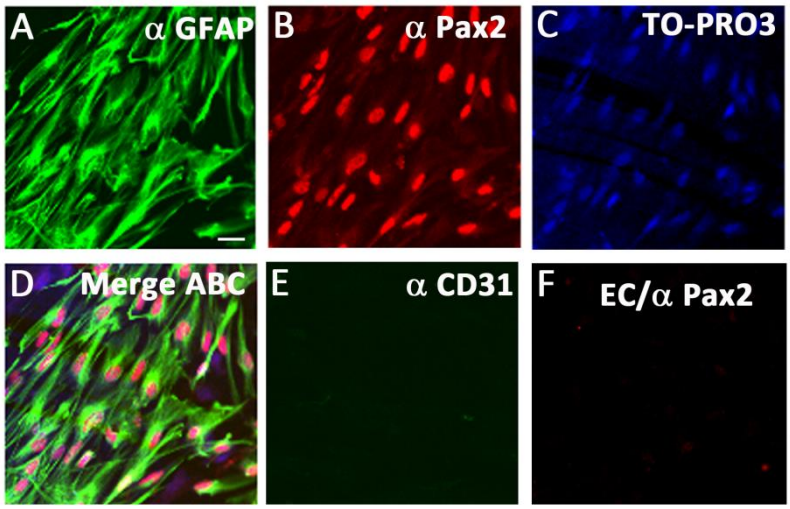

Figure S1. Primary retinal astrocyte cultures. A-E. Examples from wild-type mice, stained with anti-GFAP and anti-Pax2 to show astrocytic identity. Anti-CD31 staining indicated lack of contaminating ECs. F. Endothelial cell culture, showing that ECs do not express Pax2, thus further confirming lack of contaminating ECs in astrocyte cultures as shown in A-E. Scale bar, 50 μm.

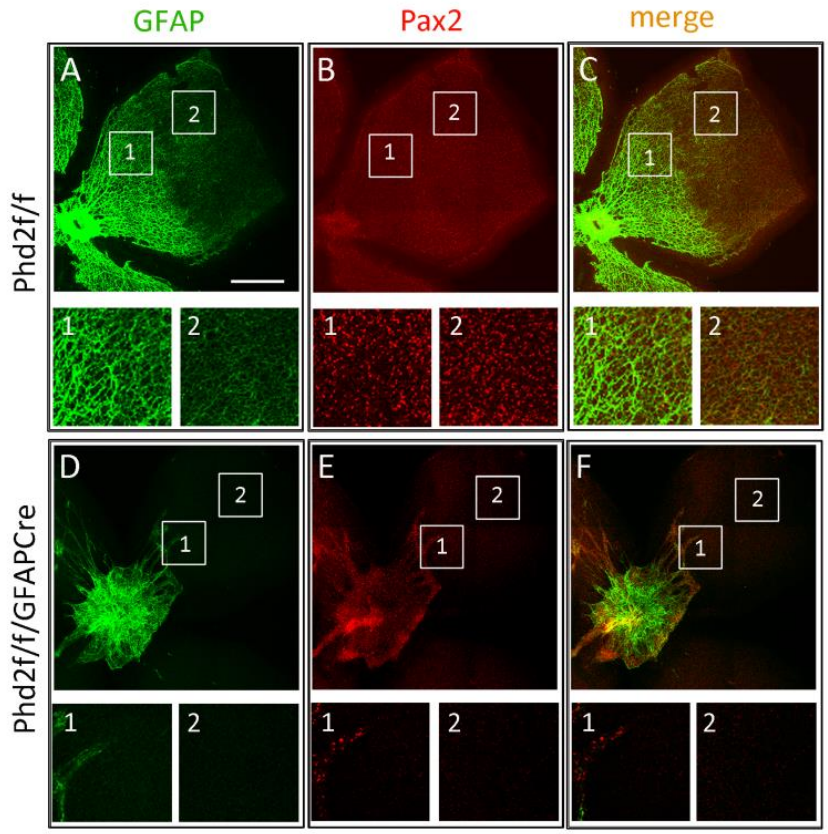

Figure S2. Retinal astrocyte migration and differentiation was severely blocked in Phenotype B mice (representing 9% of all *Phd2*$^{f/f}$*/GFAP*^{Cre} mice). Retinas were dissected at P5, and stained with anti-GFAP and anti-Pax2. Panels 1 and 2 match white boxes in main images. Note that D-F, GFAP^{+} and Pax2^{+} cells are virtually absent beyond box 1. Images shown are representative of 4 mice per group. Scale bar, 500 μm.

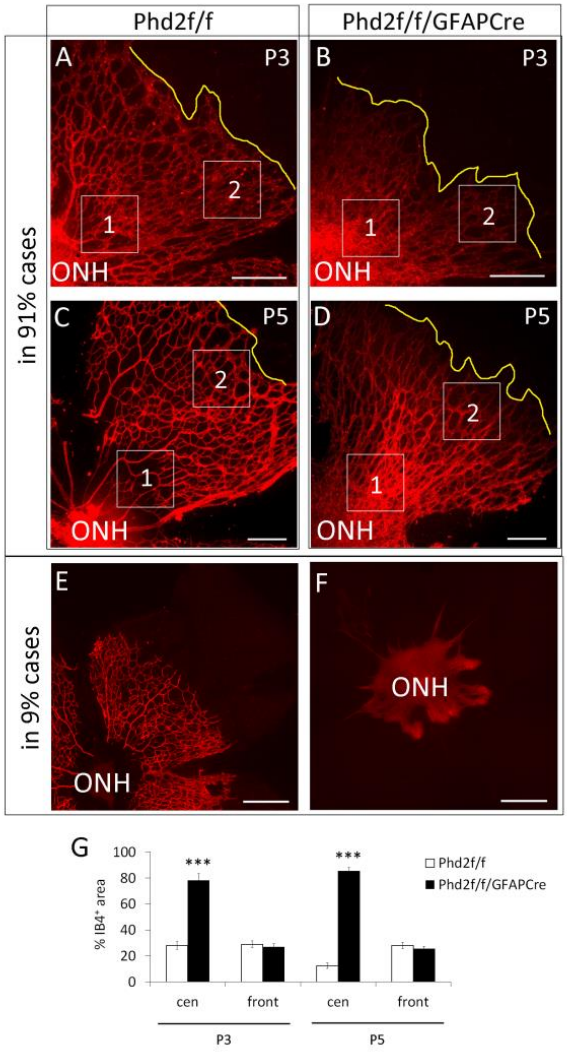

Figure S3. Presence of two distinct retinal vascular phenotypes in *Phd2$^{f/f}$/GFAP^{Cre}* mice. A-D, Phenotype A (representing 91% cases). Wavy yellow lines mark vascular front. Note dense IB4^{+} signals near ONH in *Phd2$^{f/f}$/GFAP^{Cre}* mice. E and F. Phenotype B (found in 9% of *Phd2$^{f/f}$/GFAP^{Cre}* mice). In this subtype, IB4^{+} cells failed to migrate towards the retinal periphery, but were instead clumped near the ONH. G. Quantification of retinal vascular development in Phenotype A mice. Box 1 is near the central area of the retina ("cen") and Box 2 just behind the vascular front. Scale bars: 200 μm for A-D; E and F, 500 μm. n = 6 mice/group. ***, $p < 0.001$.

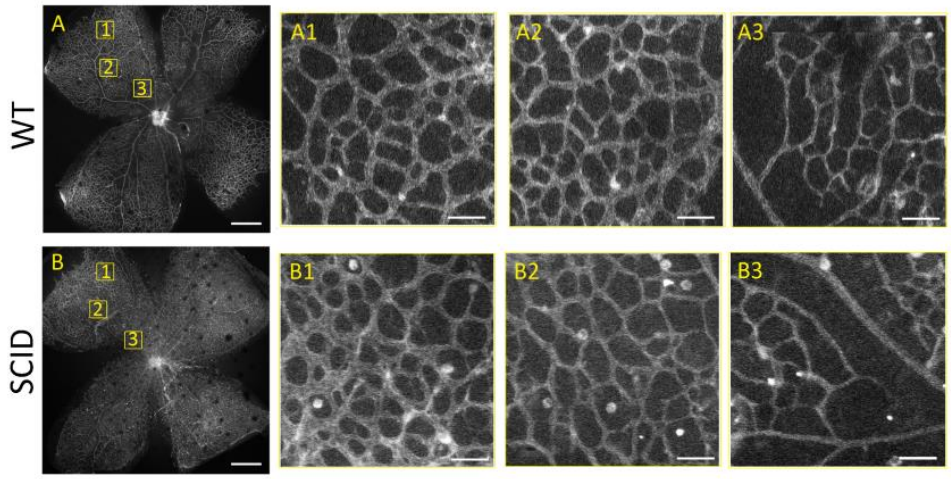

Figure S4. Developmental vascular pruning in mouse retinas is independent of T cells. Retinas were dissected from wilt-type (WT) and SCID mice at P8, and subject to IB_4 staining. Stained retinas were imaged by confocal microscopy as flat-mounts. Yellow boxes 1, 2, and 3 are expanded and shown to the right of the main panels. Images are representative of 4 mice per group. Scale bars: A and B, 500 μm. A1-A3 and B1-B3, 50 μm.