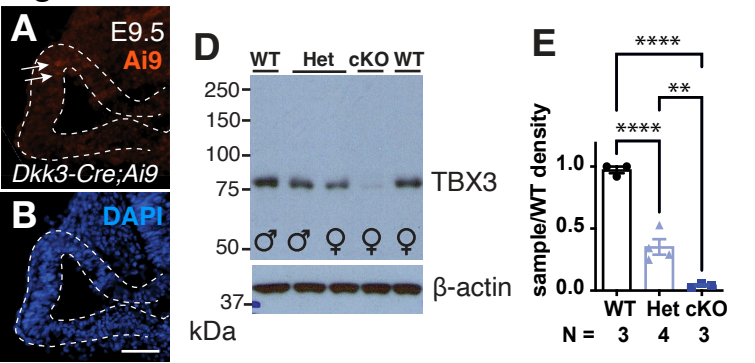
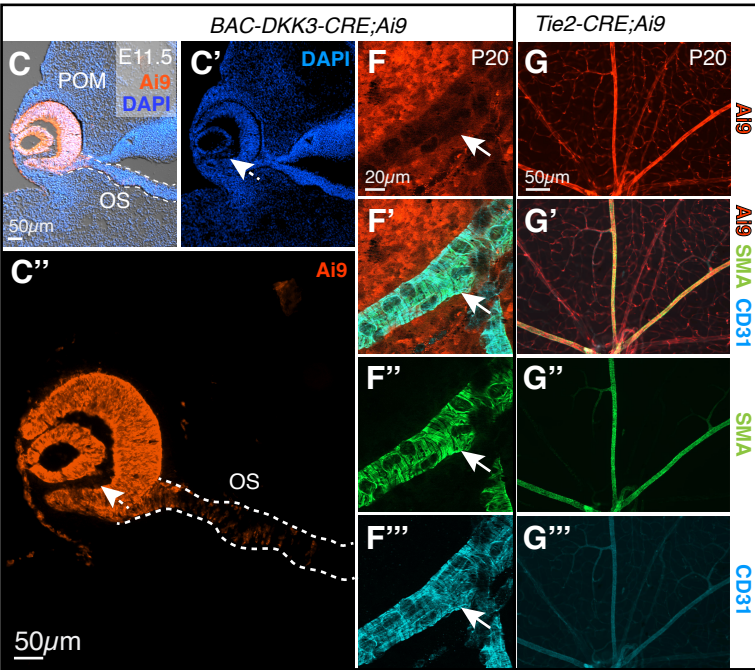


**Figure S1**

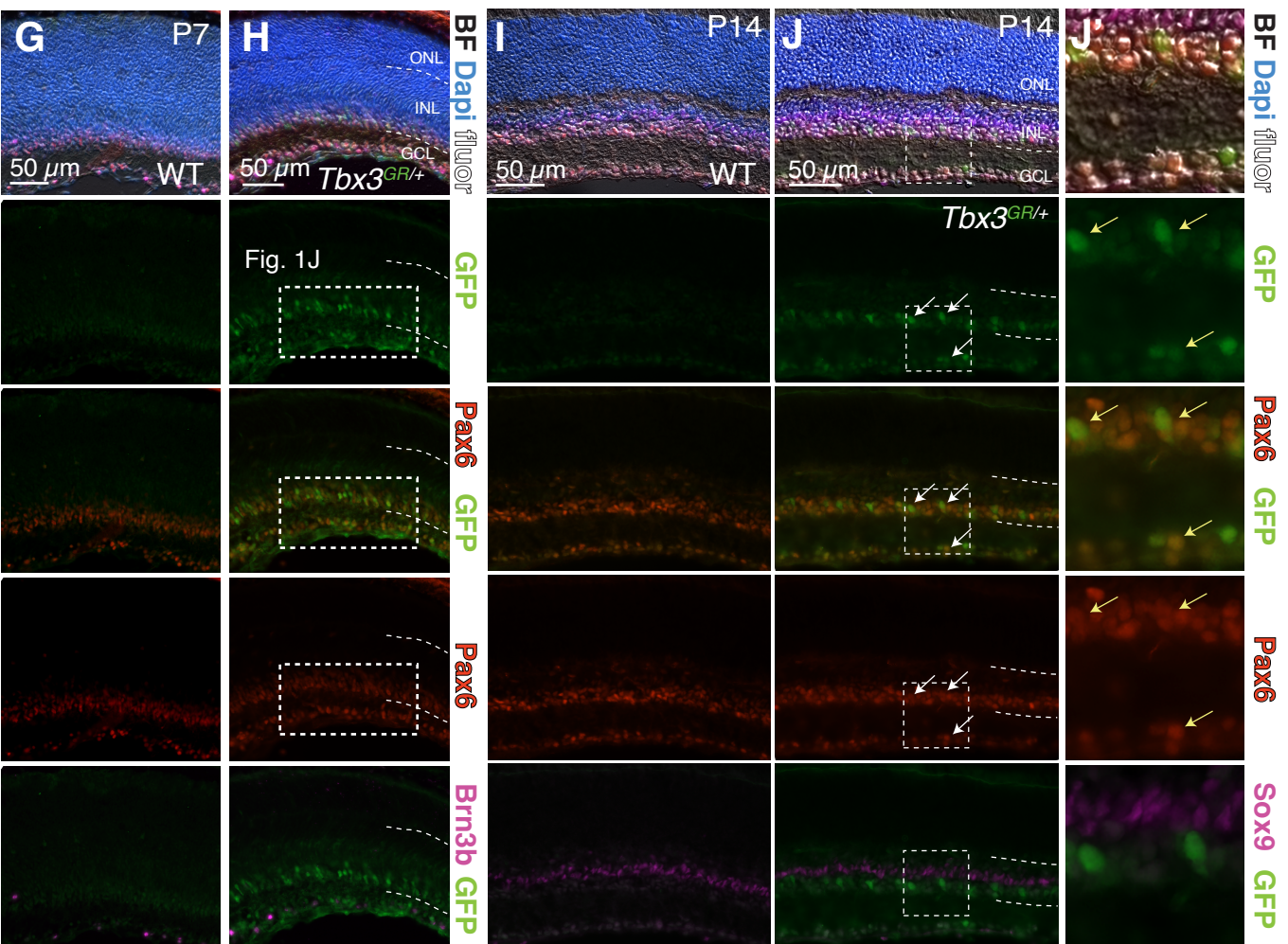
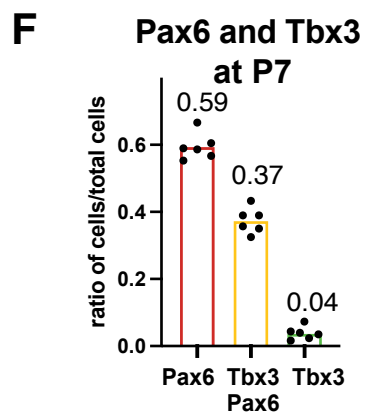
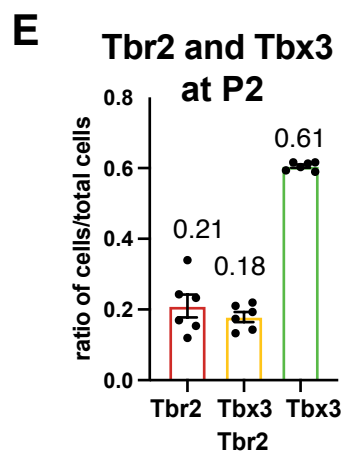
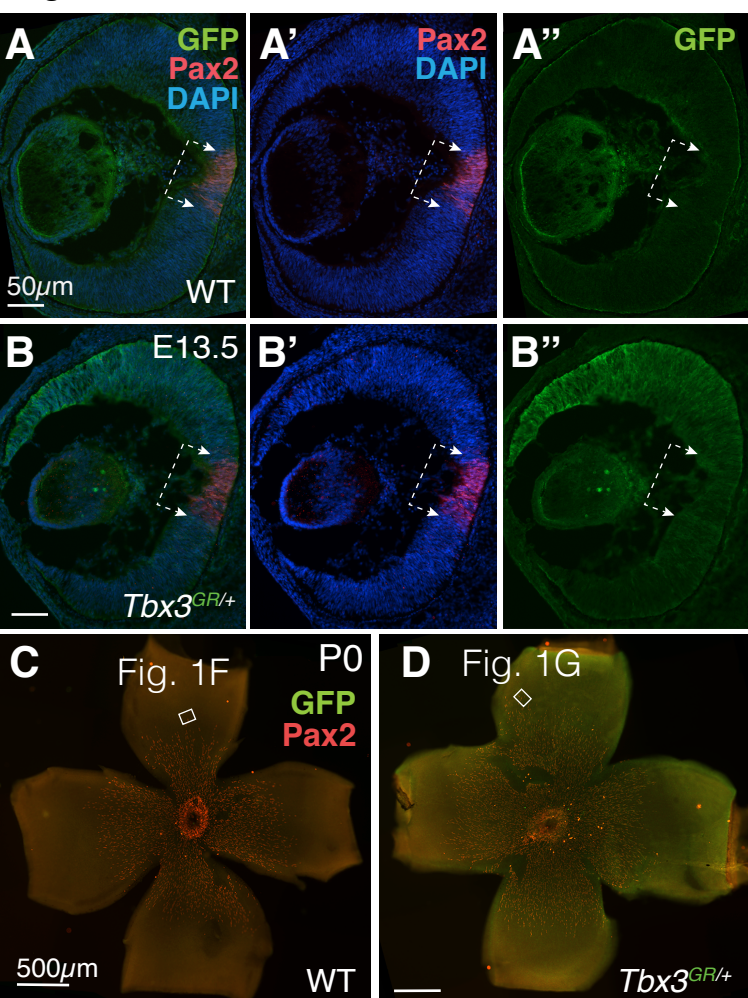
**Supplemental Figure S1. The optic cup-expressing Cre driver, *BAC-Dkk3-CRE*, removes *Tbx3* from the retina and optic stalk.**

(A,B) *BAC-Dkk3-CRE* expression pattern is detected using Ai9 reporter line expressing tdTomato. Cre activity is first detectable in the optic vesicle (dashed lines) at E9.5 (arrows). (C-C'') At E13.5, the only structures expressing tdTomato (red) are the neural retina, overlying ectoderm and optic stalk (OS). The red channel was enhanced to show that cells between the neural retina and lens were not stained (arrow). (D,E) Western blot of P0 retinas collected from wild-type (WT, *Tbx3*<sup>Fl/+</sup>), heterozygous (Het, *BAC-Dkk3-CRE;Tbx3*<sup>ΔFl/+</sup>), and homozygous knockout (cKO, *BAC-Dkk3-CRE;Tbx3*<sup>ΔFl/ΔFl</sup>) siblings. Graph shows mean  $\pm$  s.e.m. of 2 litters. No statistical differences in *Tbx3* expression were observed in males and females. Ordinary one-way ANOVA test with multiple comparisons was used to determine statistical significance. \*\*\*\*,  $p=0.00005$ ; \*\*,  $p\leq 0.01$ .

(F-F'') At P20, a close-up of a blood vessel (arrow) stained with an antibody against alpha-smooth muscle actin (SMA) and blood vessel marker, CD31, shows a lack of tdTomato. Surrounding cells are expressing the Cre reporter. (G-G'') Another transgenic line expressing Cre under the control of *Tie2* has overlapping expression with blood vessel markers, SMA and CD31.



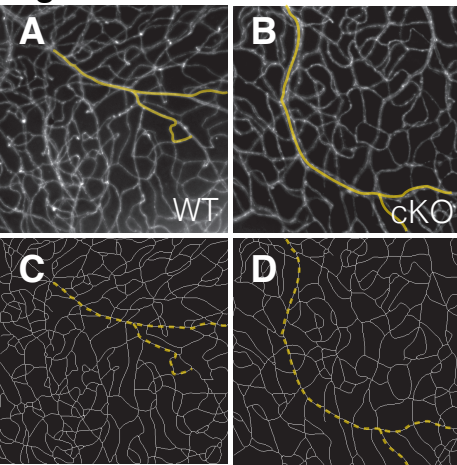
**Figure S2**



**Supplemental Figure S2. Tbx3 is expressed in embryonic neural retinal progenitors, Pax2-positive embryonic and perinatal astrocyte precursors, retinal ganglion and amacrine cells.**

(A-B'') Embryonic retinal sections from sibling WT and Tbx3-GFP knock-in mice (Tbx3<sup>GR/+</sup>) immunostained with anti-GFP (green) and Pax2 (red) antibodies (bracket marks Pax2-positive cells). DAPI labels nuclei. Scale bars indicate 50  $\mu$ m. (C,D) Flat mounts from sibling P0 WT and Tbx3<sup>GR/+</sup> immunostained with anti-GFP (green) and Pax2 (red) antibodies. Images were taken and enhanced using the same conditions. Box marks region imaged by the confocal microscope in Figs. 1I and 1J. Dorsal (D) is up in panels A-D. (E,F) Quantitation of cells counted in six regions of central retinal sections at P2 (Tbr2/GFP) and P7 (Pax6/GFP) in three Tbx3-GFP mice at each age. The average ratio of the indicated marked cells labels each bar. (G-H) Representative image of a P7 WT (G) and Tbx3<sup>GR/+</sup> (H) retina immunostained with anti-Pax6 and Brn3b antibodies. Boxed area indicates the region amplified in Fig. 1J. (I-J') Representative image of a P4 WT (I) and Tbx3<sup>GR/+</sup> (J) retina immunostained with anti-Pax6 and Sox9 antibodies. Boxed area in J indicates the region amplified for panels in J'. Arrows point to double labeled cells (Pax6/GFP).

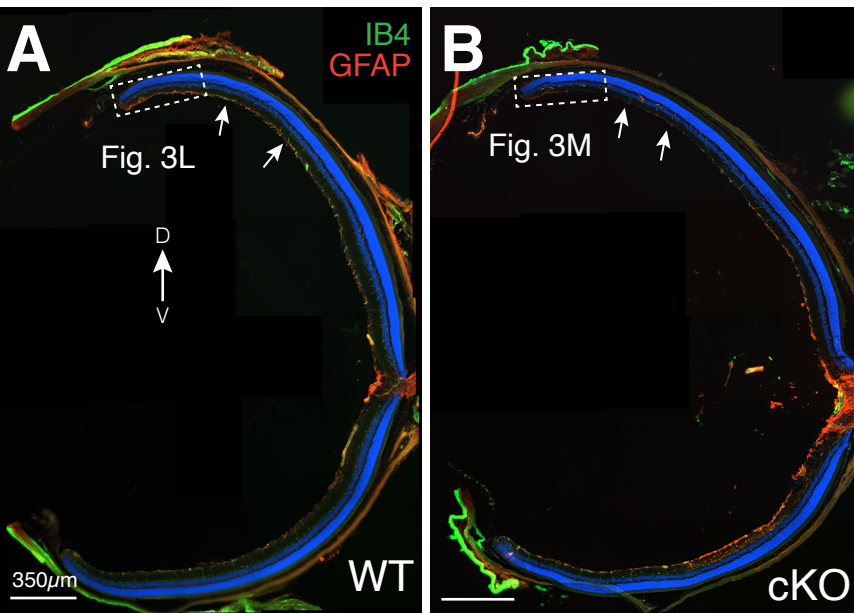
## Figure S3



**Supplemental Figure S3. Example images of how branch points and density were calculated.**

(A,B) Flat mount retinas of CD31-positive dorsal retinal vasculature in WT and Tbx3 cKO mice; images were processed using ImageJ and (C,D) transformed into skeletonized versions of the corresponding images. The skeletonized images from WT ( $n = 3$ ) and cKO ( $n = 3$ ) were used for analysis. Yellow and dashed lines trace the same vessels in A and C, B and D.

**Figure S4**



**Supplemental Figure S4. Blood vessel and astrocyte lattice formation are perturbed in P26 conditional knockout mice.**

A,B) Transverse sections (20 $\mu$ m) of P26 eyes through the optic nerve shows the regions taken to make Fig. 3 (dashed box). Blood vessels were stained with IB4 (green); astrocytes and Müller glia were stained with anti-GFAP antibody (red). Dorsal is up. Note the astrocytes in the dorsal inner limiting membrane in the wild-type (A, arrows), but much fewer in the cKO (B).

## Supplemental Tables

**Supplemental Table S1. PCR primers and conditions for PCR reactions**

Target	Primer name	5'→3' Sequence	PCR conditions					
			Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
<b>Tbx3-flox</b>	Tbx3 Geno01	AAGTCATGGAGCTCGTATCGCG	94° C 2 min	94° C 20 sec	Repeat Step 2 35X	72° C 5 min		
	Tbx3 Geno02	GTGTGAGACAGAGAAATCAGTGG		55° C 20 sec				
	Tbx3 Geno03	CCAAC TGGTATCTTGATAAACCTC		72° C 20 sec				
<b>CRE</b>	5' CreR 368	AAAACGTTGATGCCGGTGAA	94° C 2 min	94° C 15 sec	Repeat Step 2 37X	72° C 5 min		
	3' CreR 922	CCGGTATTGAAACTCCAGCG		60° C 15 sec				
				72° C 30 sec				
<b>Sex</b>	SX_F	GATGATTTGAGTGGAAATGTGAGGTA	94° C 3 min	94° C 20 sec	Repeat Step 2 35X	72° C 5 min		
	SX_R	CTTATGTTTATAGGCATGCACCATGTA		57° C 20 sec				
				72° C 20 sec				
<b>Tbx3<sup>GR</sup></b>	SH65A Tbx3-G	CATACGTGTATATGATGGGAGGTTG	94° C 2 min	94° C 20 sec	Repeat Step 2 10X	94° C 15 sec	Repeat Step 4 28X	72° C 2 min
	SH57A Tbx3-G	GCAATCTATACATGTCTCGGAG		65-0.5° C 15 sec		60° C 15 sec		
				68° C 10 sec		72° C 10 sec		
<b>Ai9</b>	5 Ai9WT oIMR9020	AAGGGAGCTGCAGTGGAGTA	94° C 2 min	94° C 20 sec	Repeat Step 2 10X	94° C 15 sec	Repeat Step 4 28X	72° C 2 min
	3 Ai9 WT oIMR9021	CCGAAAATCTGTGGGAAGTC		65-0.5° C 15 sec		60° C 15 sec		
	5 Ai9WPRE oIMR9103	GGCATTAAAGCAGCGTATCC		68° C 10 sec		72° C 10 sec		
	3 Ai9 oIMR9105	CTGTTCTGTACGGCATGG						

**Supplemental Table S2. Antibodies and conditions used for immunostaining.**

	Species raised	Company	Catalog #	Lot #	Block conditions	1° Antibody concentration	2° Ab Co. & cat#	Antibody ID	(PMID)
<b>GFP</b>	Chicken	AbCam	AB13970	GR89472-2 2	1X PBS, 10% HIGS, 0.3% Triton	1:1500	AbCam AB150169	AB_300798	33774011
<b>Pax2</b>	Rabbit	Biologend	901002 (Covance# PRB-276P)	B287354	1X PBS, 10% HIGS, 0.3% Triton	1:1000	Invitrogen A21428	AB_2565001 AB_291611	19505455 33428890
<b>Pax6</b>	Mouse	DSHB	Pax6	n/a	1X PBS, 10% HIGS, 0.5% Triton	1:50	Invitrogen A31570	AB_528427	12648492 18626943
<b>Tbr2</b>	Rabbit	AbCam	AB23345	GR3378360 -1	1X PBS, 10% HIGS, 0.5% Triton	1:1000	Invitrogen A31572	AB_778267	28078709 27565344
<b>Sox9</b>	Rabbit	Millipore	AB5535	3892468	1X PBS, 5% HIGS, 0.3% Triton	1:4000	AbCam AB150079	AB_2239761	18626943 33960384
<b>Brn3b</b>	Goat	Santa Cruz	SC-6026	D1012	1X PBS, 10% HIGS, 0.5% Triton	1:250	Invitrogen A34849	AB_673441	21935940 27957530
<b>GFAP</b>	Mouse	Sigma	G3893	122914	1X PBS, 5% HIGS, 0.3% Triton	1:500	Invitrogen A21424	AB_477010	6198232 26996101
<b>PDGFR<math>\alpha</math></b> <b>(CD140a)</b>	Rat	BD Biosciences	558774	8109820	1X PBS, 5% HIGS, 5% HIDS, 0.3% Triton	1:500	Jackson 112-605-167	AB_397117	28943241 8982160 8875964
<b>OPN4</b>	Rabbit	Advanced Targeting Systems Bio	AB-N38 (clone UF006)	135-4	1X PBS, 5% HIGS, 5% HIDS, 0.3% Triton	1:2500	Invitrogen A31572	AB_1608077	20503419 11823848 11834834
<b>Islet1/2</b>	Mouse	DSHB	39.4D5	n/a	1X PBS, 10% HIDS, 0.3% Triton	1:100	Invitrogen A31570	AB_2314683	1350865 31313880
<b>CD31</b>	Rat	BD Biosciences	550274 (clone MEC13.3)	8079850	1X PBS, 5% HIGS, 0.1% Triton	1:250	Invitrogen A11077	AB_393571	9284815 7956830
<b>IB4-FITC</b>	Griffonia simplicifolia	Sigma	L2895	125M4177V	1X PBS, 5% HIGS, 0.3% Triton	1:50	-	-	7107706 29511172
<b><math>\alpha</math>SMA-FITC</b>	Mouse	Sigma	F3777 (Clone 1A4)	n/a	1X PBS, 5% HIGS, 0.3% Triton	1:500	-	-	29874128