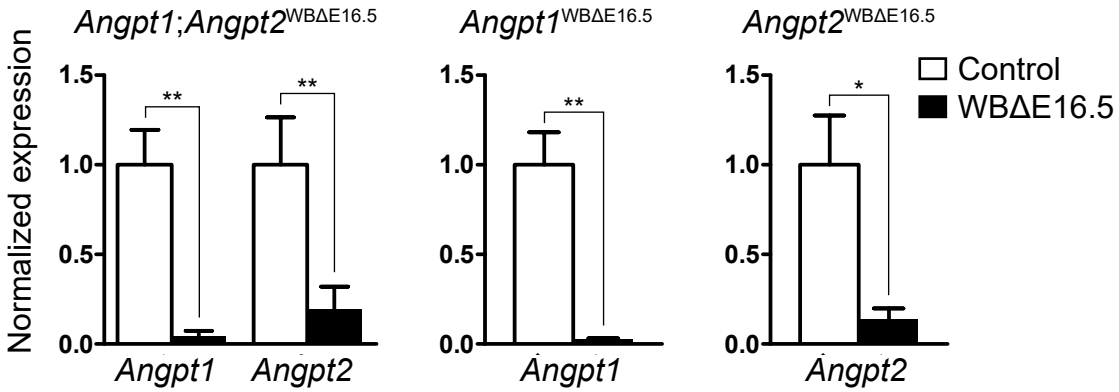
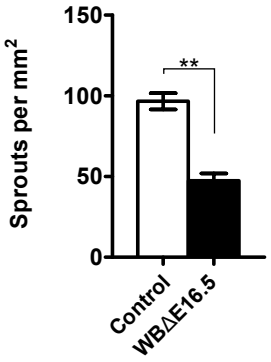


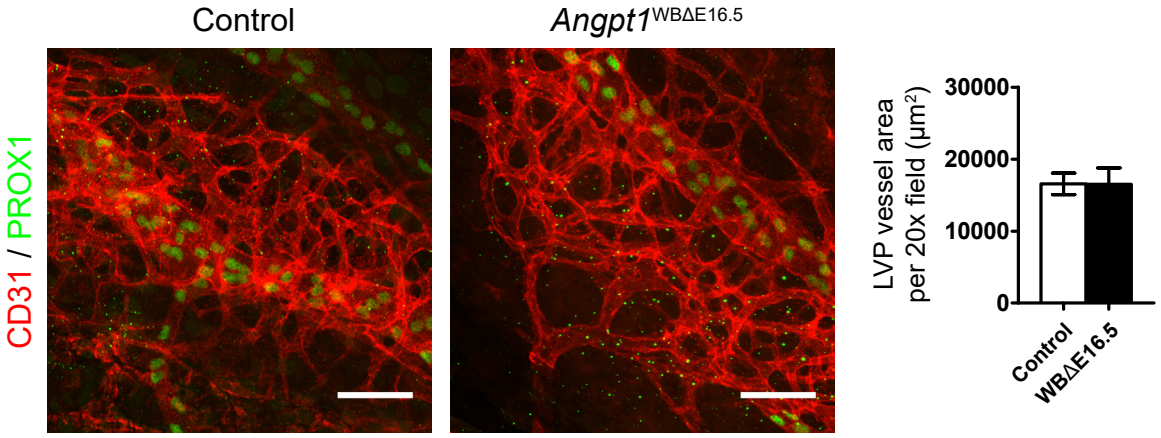
Supplemental figure 1. Mouse model knockout efficiency was confirmed by real-time PCR

RT qPCR was used to validate all knockout mouse models used in this study. Normalized *Angpt1* and *Angpt2* mRNA expression is shown in lung tissue from adult *Angpt1*;*Angpt2*$^{WB\Delta E16.5}$, *Angpt1*$^{WB\Delta E16.5}$, *Angpt1*$^{WB\Delta E16.5}$ and littermate control animals.n = 4-6 animals per group.
* $p \leq 0.05$, ** $p \leq 0.01$ as determined by 2-way ANOVA followed by Bonferonni's correction (*Angpt1*;*Angpt2*$^{WB\Delta E16.5}$) or Student's t-test (*Angpt1*$^{WB\Delta E16.5}$, *Angpt1*$^{WB\Delta E16.5}$).



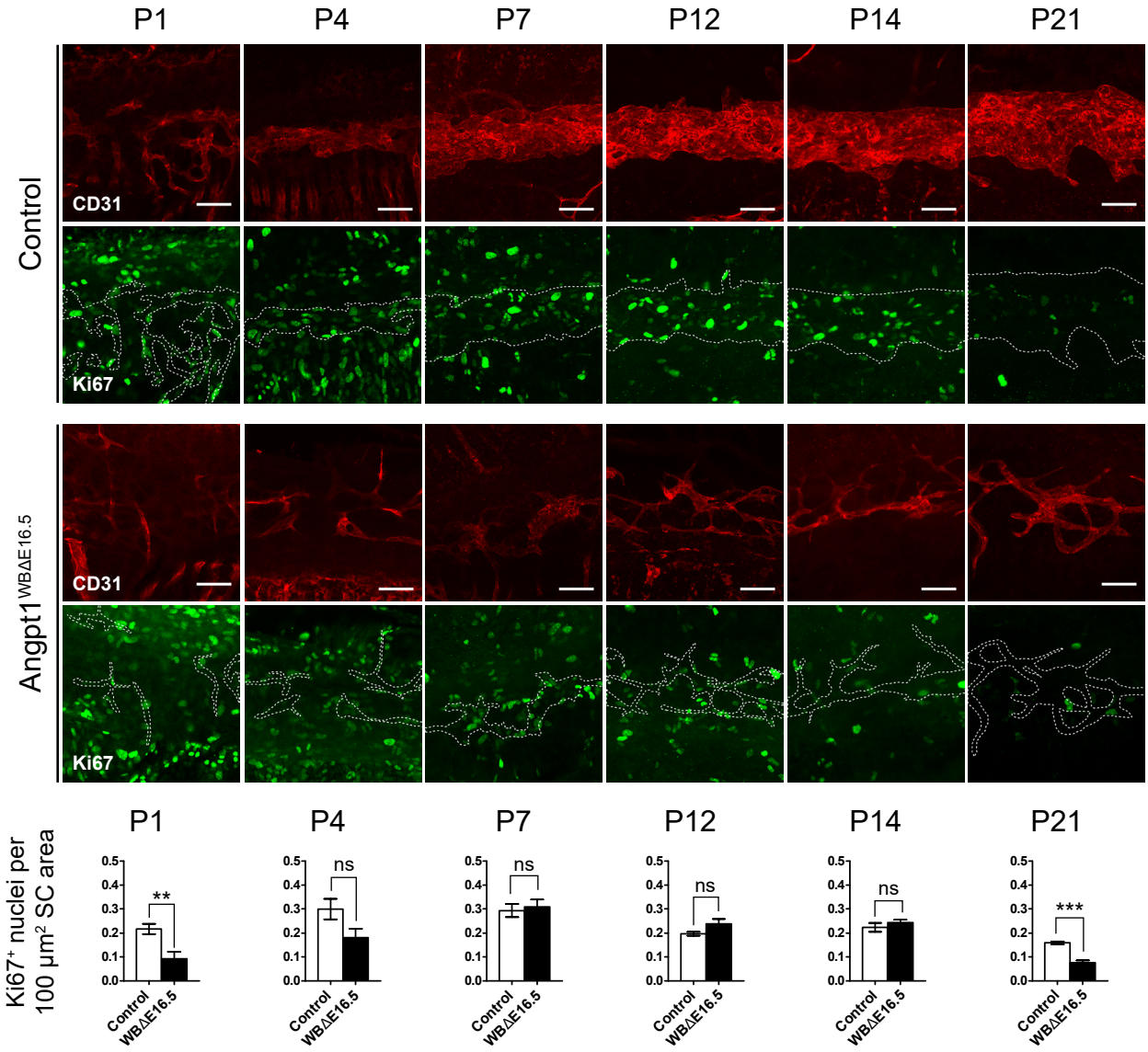
Supplemental figure 2. Fewer CD31-positive sprouts leave the limbal vascular plexus of Angpt1$^{WB\Delta E16.5}$ mice to form Schlemm's canal.

Compared to littermate controls, fewer CD31-positive endothelial sprouts were observed leaving the limbal vascular plexus (LVP) of *Angpt1*$^{WB\Delta E16.5}$ mice at postnatal day 1 (P1). n = 3 control and 3 Angpt1 KO mice. ** $p \leq 0.01$ as determined by Student's t-test.



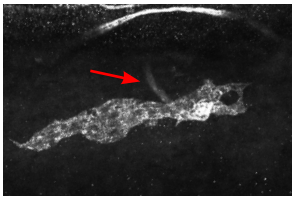
Supplemental figure 3. Sprouting angiogenesis outside of Schlemm's canal is uneffected in ANGPT1 knockout mice.

Vascular area of the limbal vascular plexus (LVP) was normal in *Angpt1*$^{WB\Delta E16.5}$ *(*Angpt1 KO) mice at postnatal day 1. Scale bars represent 50 μm. n = 3 control and 3 Angpt1 KO mice.



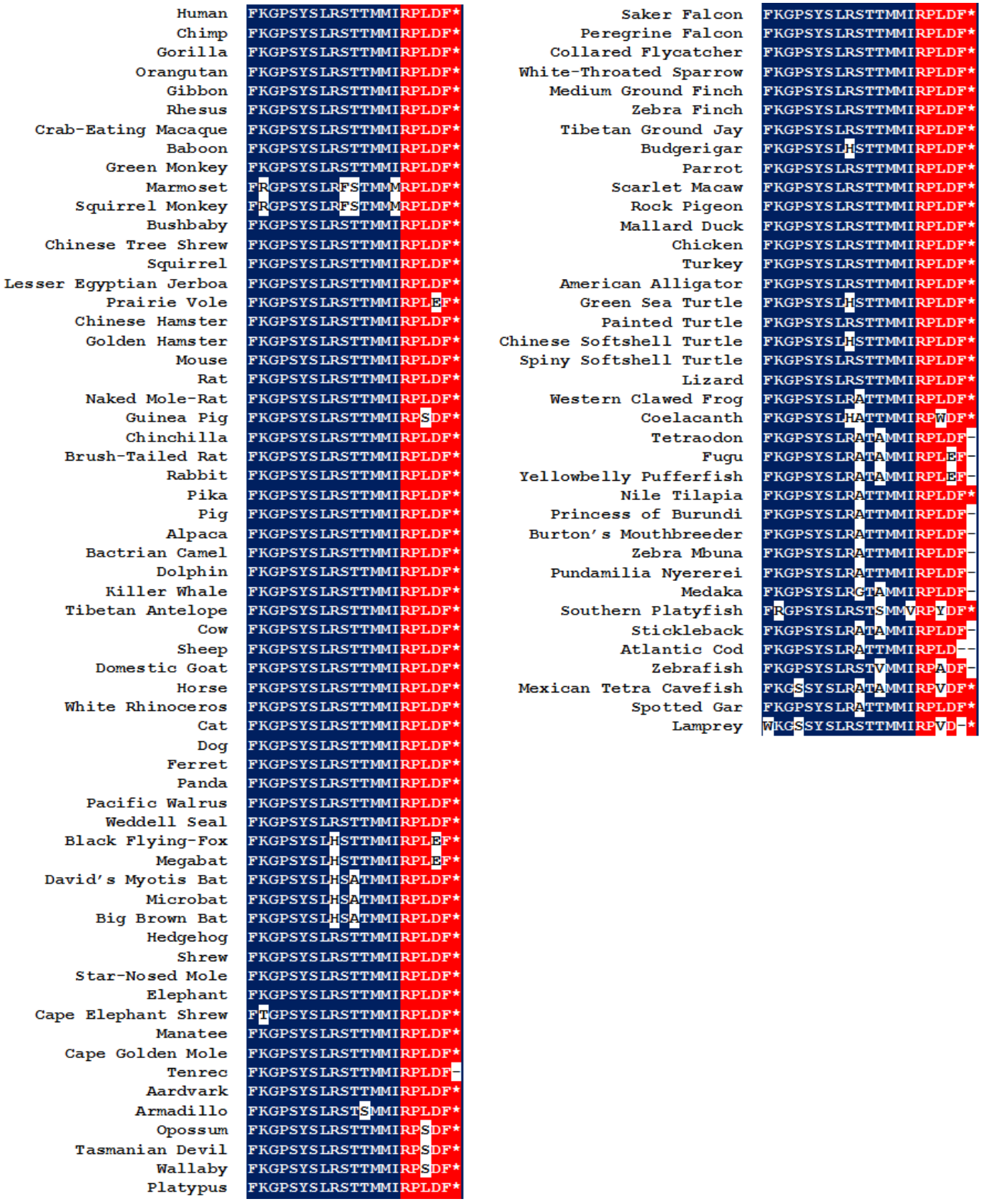
Supplemental figure 4. ANGPT1 knockout mice have reduced endothelial proliferation in Schlemm's canal.

Ki67 staining of Schlemm's canal throughout development showed that *Angpt1*$^{WB\Delta E16.5}$ mice had reduced endothelial cell proliferation in sprouts emerging from the limbal vascular plexus and Schlemm's canal. Starting with fewer sprouts and lacking increased proliferation compared to controls, knockout mice were unable to catch up and form a mature Schlemm's canal. Dashed lines in Ki67 panels highlight CD31-positive sprout and Schlemm's canal area from the matching CD31 panels, which are reproduced here from Figure 2 in the main text. Scale bars indicate 50 µm. Littermate controls were used for all timepoints, and eyes from 3-6 mice were analyzed per group.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ as determined by Student's t-test.



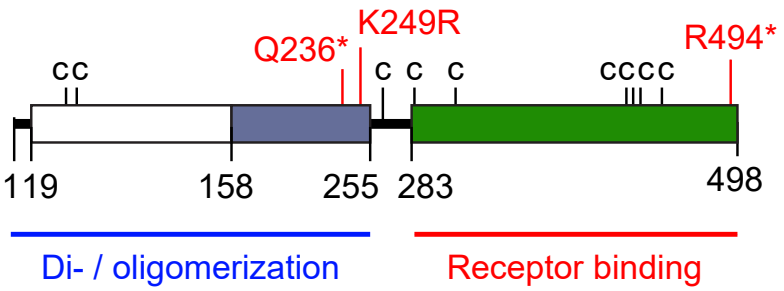
Supplemental figure 5. Isolated SC fragments observed in Angpt1 knockout mice are connected to the episclaral veins by at least one drainage vessel.

When observed by confocal microscopy, isolated, CD31-positive Schlemm's canal fragments present in Angpt1 knockout mice were found to be connected to the limbal capillaries and episclaral vasculature by drainage channels--suggesting that they may retain some drainage function.



Supplemental figure 6. The ANGPT1 C-terminus is highly conserved throughout vertebrate evolution
Alignment of the C-terminal region of the ANGPT1 protein. Red labeled residues indicate the region deleted in the p.R494* PCG subject.

A

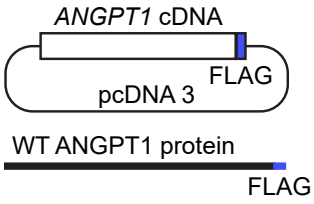


B

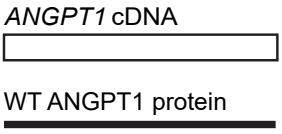
Wild-type

ANGPT1 constructs

ANGPT1-FLAG



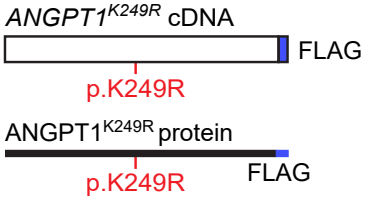
ANGPT1



Missense variant

ANGPT1^{K249R} constructs

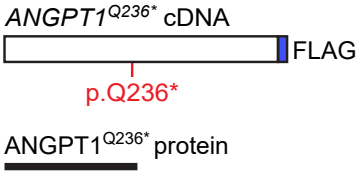
K249R-FLAG



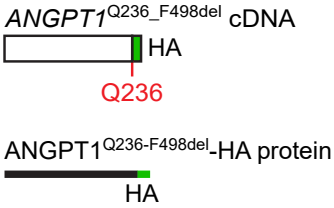
Nonsense variants

ANGPT1^{Q236*} constructs

Q236*-FLAG

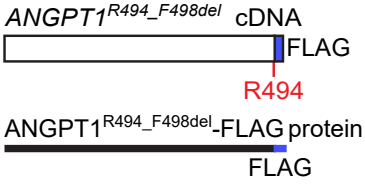


Q236_F498del-HA

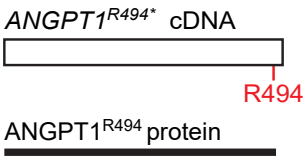


ANGPT1^{R494*} constructs

R494_F498del-FLAG

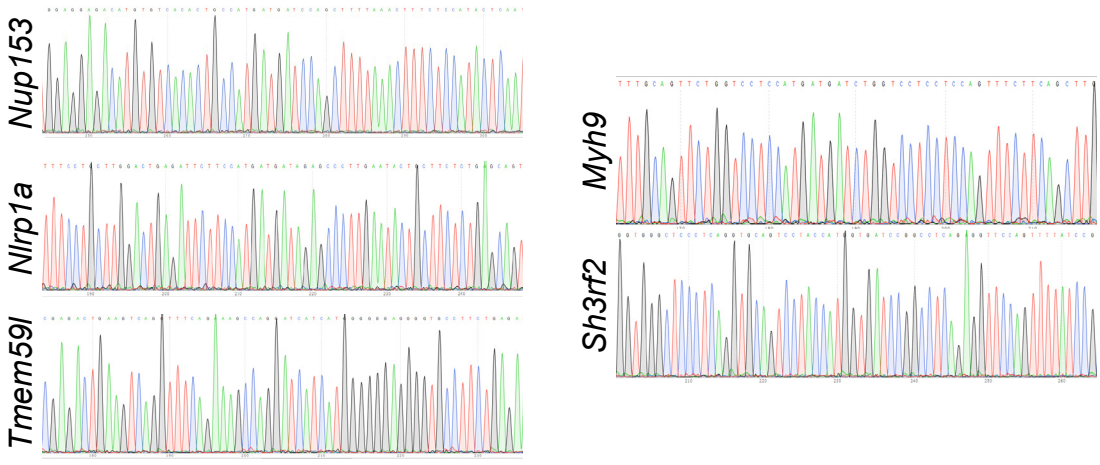


R494*



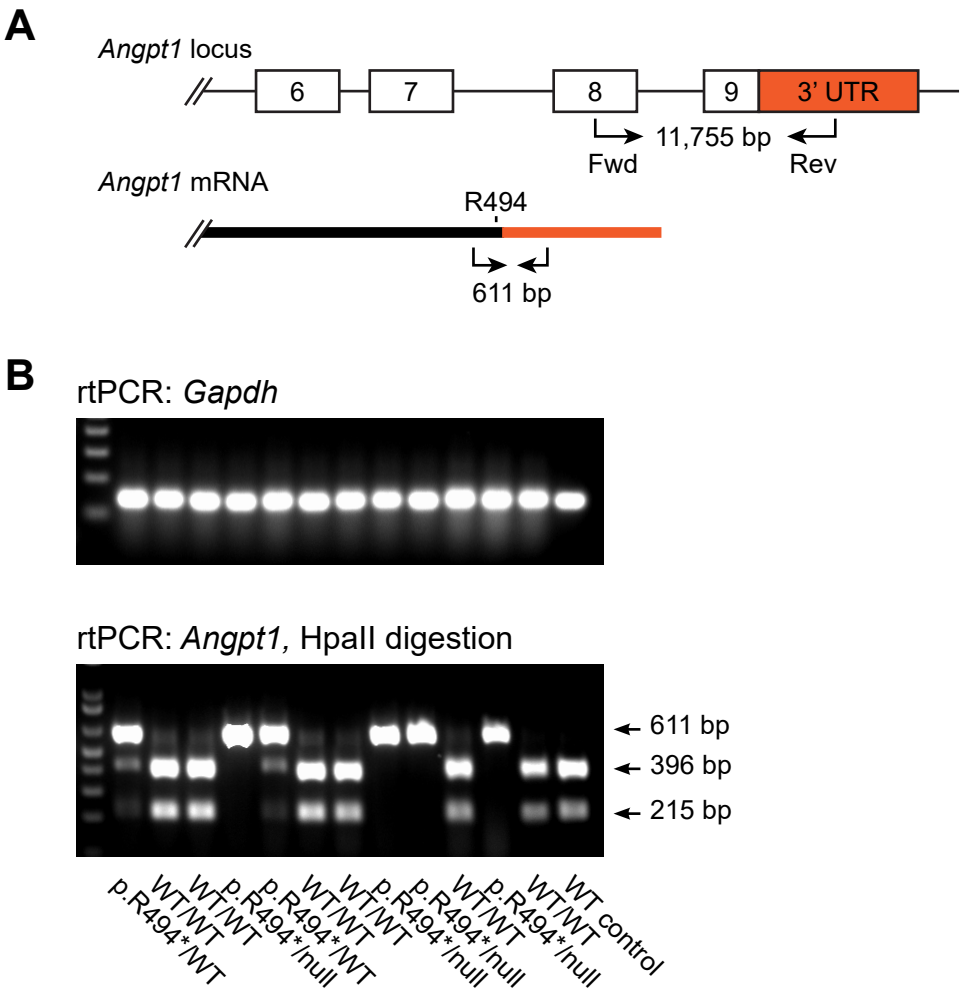
Supplemental figure 7. Schematic representation of the ANGPT1-expressing plasmid vectors designed for in vitro experiments

(A) Simplified drawing of an ANGPT1 protein monomer, with the locations of PCG protein mutations indicated. Note location of the final amino acid, F498. **(B)** Schematic of plasmid constructs used in this study. Within each category, constructs are described in the order of appearance in the manuscript. Site directed mutagenesis was used to insert the p.Q236* mutation into a WT ANGPT1-FLAG expressing plasmid. Therefore, this premature stop codon is upstream of the plasmid FLAG tag and truncated protein expressed does not contain the C-terminal FLAG. Missense variant proteins with functional C-terminal tags (ANGPT1$^{Q236_F498del)}$-HA and ANGPT1^{R494_F498del}-FLAG) were generated by truncating the ANGPT1 cDNA at the site of the mutation and inserting the epitope tag sequence in-frame with no intervening stop codon.

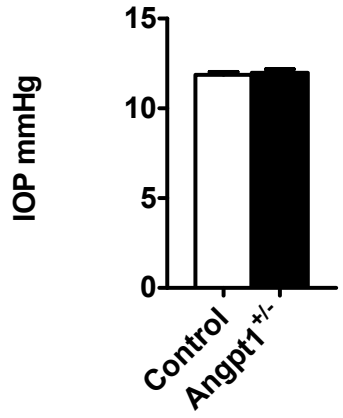


Supplemental figure 8. Analysis of off-target cleavage at predicted sites in the mouse genome

The MIT Crispr design tool (crispr.mit.edu) was used to obtain a list of likely off-target mutation sites for our selected sgRNA sequence (**Table S2**, 5.1_gRNA, 5'-CAAGGGCCGGATCATCATGG). The top five hits were selected for Sanger sequencing to confirm a lack of off target mutations in the selected N1 founders.

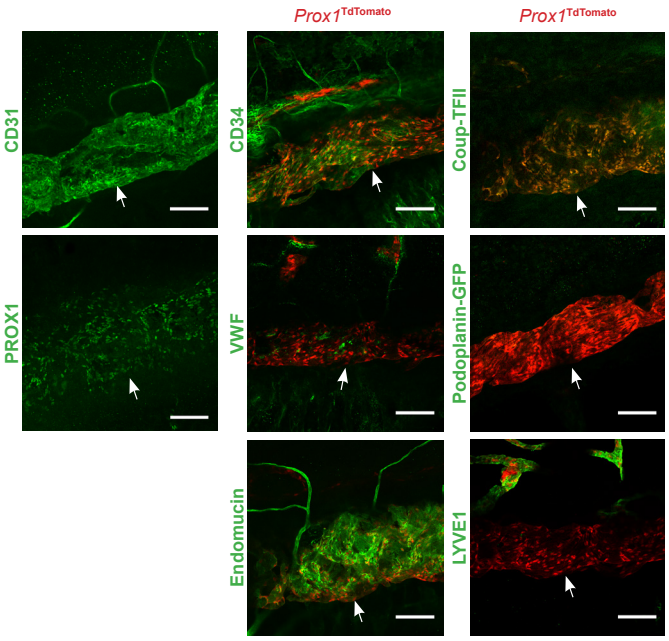


Supplemental figure 9. Angpt1$^{p.R494*}$ mRNA is transcribed and escapes nonsense mediated decay (**A**) cDNA was generated from whole E10.5 *Angpt1*$^{p.R494/null}$ embryos and rtPCR was used to amplify a 611 bp PCR product incorperating the region modified in *Angpt1*$^{p.R494*}$. (**B**) Digestion with HpaII cleaves the WT amplicon into 396 and 215 bp fragments. This HpaII site is disrupted by the p.R494* mutation, and the presence of undigested 611 bp amplicons in *Angpt1*$^{p.R494*}$ embryos confirms that mutant mRNA is produced and escapes nonsense mediated decay.



Supplemental figure 10. Angpt1$^{null\,/\,WT}$ heterozygous mice have normal IOP
When measured at 14 weeks, ANGPT1 heterozygous mice have normal IOP. n = 8 (Angpt1 heterozygote) and 10 (control littermates).

A



B

Protein	Expression	Present in SC	Reference
Prox1	LEC	Yes	(1-3)
Podoplanin	LEC	**No**	(1)
LYVE1	LEC	**No**	(1-3)
VEGFR3	LEC	Yes	(1-3)
CCL21	LEC	Yes	(2)
Coup TFII	BEC/LEC	Yes	Preliminary data
CD31	BEC/LEC	Yes	(1-3)
VE-Cad	BEC/LEC	Yes	(1,3)
Col IV	BEC/LEC	Yes	(1,2)
CD34	BEC	Yes	(3)
VWF	BEC	Yes	(3)
VEGFR2	BEC	Yes	(1-3)
Endomucin	BEC	Yes	(3)

(1) Park et al. *Lymphatic regulator PROX1 determines Schlemm's canal integrity and identity.* JCI. 2014
(2) Aspelund et al. *The Schlemm's canal is a VEGF-C/VEGFR-3–responsive lymphatic-like vessel.* JCI. 2014
(3) Kizhatil et al. *Schlemm's Canal Is a Unique Vessel with a Combination of Blood Vascular and Lymphatic Phenotypes that Forms by a Novel Developmental Process*. PLoS Biol 2014

Supplemental figure 11. At the molecular level, Schlemm's canal resembles a large, PROX1-expressing vein.

(**A**) Schlemm's canal was stained with antibodies for a selection of characteristic markers of blood and lymphatic endothelium in either WT (CD31, PROX1) or *Prox1*^{TdTomato} (CD34, Van-Willibrant Factor, Endomucin, CoupTFII, Podoplanin-GFP, LYVE1) mice. As previously described, Schlemm's canal phenotypically resembles a vein which expresses PROX1, but few other markers of the lymphatic endothelium. (**B**) A literature survey highlights the unique molecular character of Schlemm's canal. Scale bars in (A) indicate 100 μm, white arrowheads indicate Schlemm's canal.

Table S1. Selected sgRNA sequences tested for generation of an ANGPT1^{R494*} mouse line

Name	Sequence	PAM site	
2.1_gRNA	CACCATGATGATCCGGCCCT	TGG	
3.1_gRNA	GTCCAAGGGCCGGATCATCA	TGG	
4.1_gRNA	CATCATGGTGGTGGAACGTA	AGG	
5.1_gRNA	CAAGGGCCGGATCATCATGG	TGG	*-Selected for founder generation*
6.1_gRNA	GGGCCGGATCATCATGGTGG	TGG	

Table S2. Predicted off-target cleavage sites for sgRNA 5.1_sgRNA

Off-target sequence	Score	Mismatches	Gene	UCSC	Locus
TAAAAGCTGGATCATCATGGCAG	1.4	4MMs [1:4:5:8]	*Nup153*	NM_175749	chr13:-46789339
CAAGGGCTCTATCATCATGGAAG	1.3	3MMs [8:9:10]	*Nlrp1a*	NM_001004142	chr11:-70955857
GAAAGCCAGGATCATCATGGGGG	0.8	4MMs [1:4:6:8]	*Tmem59l*	NM_182991	chr8:+73010361
GGAGGACCAGATCATCATGGAGG	0.5	4MMs [1:2:6:9]	*Myh9*	NM_022410	chr15:-77605005
CTGAGGCCGGATCACCATGGTAG	0.4	4MMs [2:3:4:15]	*Sh3rf2*	NM_001146299	chr18:-42312735

The MIT Crispr design tool (crispr.mit.edu) was used to obtain a list of likely off-target mutation sites for our selected sgRNA sequence (**Table S2**, 5.1_gRNA, 5'-CAAGGGCCGGAT-CATCATGG).

Table S3. Primers for gDNA PCR amplification and direct Sanger sequencing of the human ANGPT1 gene

Exon	Forward Primer	Reverse Primer
1	AAGGAGCAAGTTTTGCGAGA	AAAGGAAAAAGGTCCGTGCT
2	AACTGGGAGGCCTTGCTTAT	TGTTGAGTCTGTGGACTCTGG
3	TTTGATTCGTGACTGAAGTTTGA	TTGGCAGAGAGGTGAAGGAT
4	TTCAGGAACCAATTGAATTATAAGG	AACAATACCAAAGTGAGGAAGACA
5	GCTATTATTGGAGTCAGTTTGGCTA	TGGGATCTGGCTTACATCTTG
6	GCAGACCTGTTCGCCTTATT	AAAACACCAAAAAGCACCAT
7	CCTCTCTAAAGTAACAACTGCATCTC	TGGCTAGGTAAAAGGTAGTCGAA
8	AGCTGGTCTTCTGGGTTCTG	AGAATGGCCCCATAGGACTT
9	TTGCCTCTCCTTCTCTCTTCTA	TCTCCGGATTTCTTTGTTGC