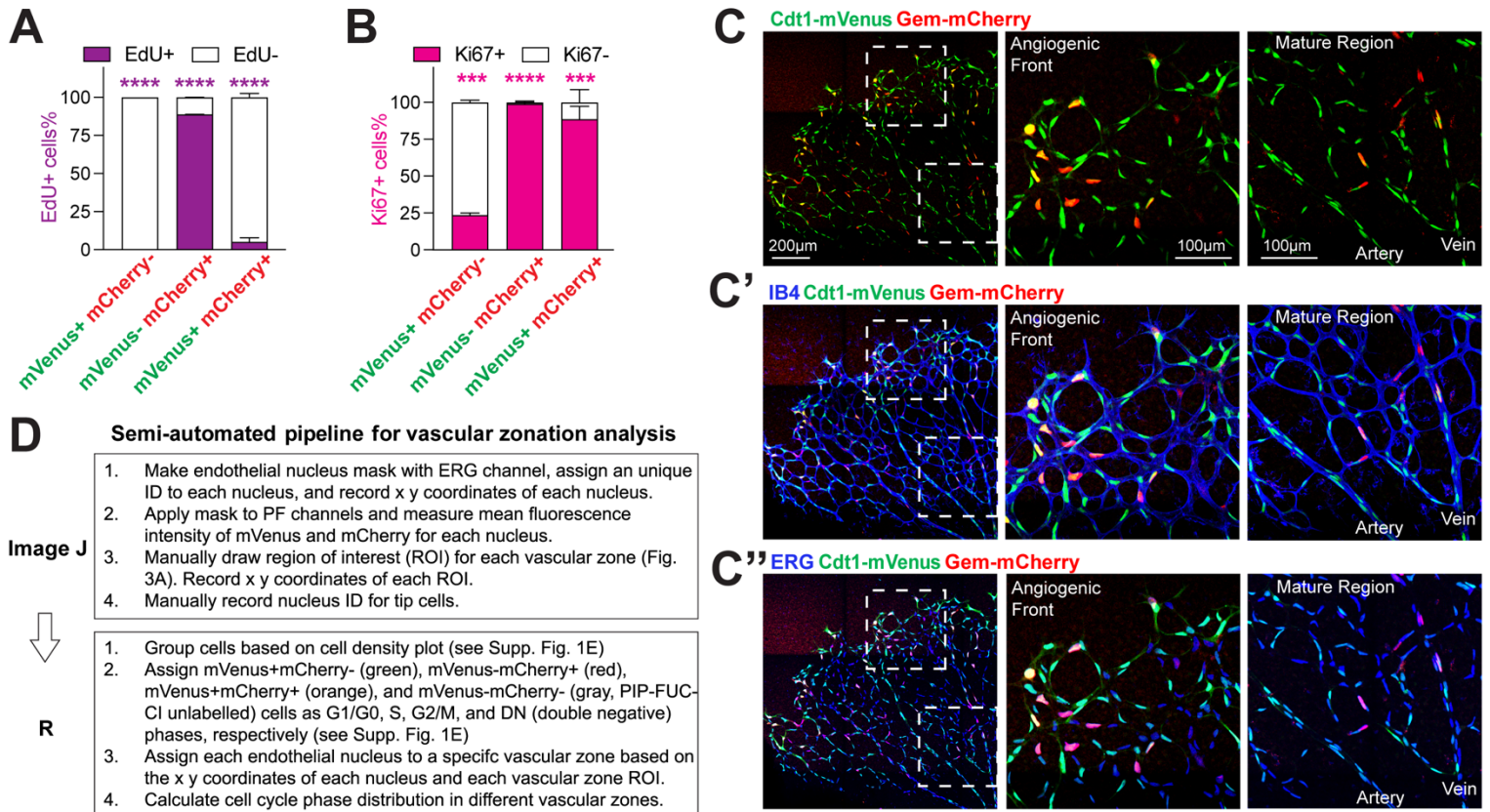


Supplementary Figure 1. PIP-FUCCI reporter mice and workflow for retina analysis. (A) Construct used to generate the PIP-FUCCI reporter mouse line. **(B)** Location of genotyping primers. **(C)** Example of genotyping results on DNA agarose gel. **(D)** Example of ERG mask. **(E)** Workflow for whole retina analysis with ERG mask. **(F)** Quantification of % PIP-FUCCI labeled cells in ERG+ endothelial cells from two *PF/PF;Cdh5-Cre^{ERT2}/+* retinas. **(G)** Accuracy of manual vs. algorithm-assigned cell cycle phases from the same subset of endothelial cells of the same retinal images (PIP-FUCCI labeled and stained for IB4 and ERG). N = 4 pups.



Supplementary Figure 2. Workflow for vascular zonation analysis of the retina.

(A) Quantification of retinal endothelial cells EdU labeled relative to PIP-FUCCI status (related to **Fig. 2D-F**). $n = 3$ pups. **** $p < 0.0001$ by Two-way ANOVA & Sidak's multiple comparisons test. **(B)** Quantification Ki67+ retinal endothelial cells relative to PIP-FUCCI status (related to **Fig. 2G-I**). $n = 2$ pups. **** $p < 0.0001$, *** $p < 0.001$ by Two-way ANOVA & Sidak's multiple comparisons test. **(C-C'')** High resolution view of one representative leaflet of a *PF/PF;Cdh5-Cre^{ERT2/+}* retina stained for IB4 and ERG. Boxed areas on far left (scale bar, 200 μ m) magnified (scale bar 100 μ m on middle (AFC) and far right (MC) regions). **(D)** Workflow for semi-automated vascular zonation analysis of PIP-FUCCI retinal images with ERG mask.

SUPPLEMENTARY TABLES**Supplementary Table 1. PCR Primers**

Primer Name	Primer Sequence	Expected Products
Rosa26 WT-F	TGGAGTTGCAGATCACGAGG	WT = 225 bp
Rosa26 WT-R	TGTTTTGGAGGCAGGAAGCA	PF = no band
Rosa26 PF-F	GCTAACCATGTTTCATGCCTTC	WT = no band
Rosa26 PF-R	CGCCCTCGCCGGACACGCTGAAC	PF = 357 bp
GenCre F	GACCAGGTTTCGTTCACTCA	WT = no band
GenCre R	TAGCGCCGTAAATCAAT	Cre = 400 bp

Supplementary Table 2. Antibodies

Antibody	Species	Company	Catalog No.	Dilution	Final Conc
IsolectinB4-biotin	NA	ThermoFisher	I21414	1:100	10 µg/ml
Streptavidin-Alexa	NA	ThermoFisher	S32351	1:50	20 µg/ml
Ki67-Alexa647	Rat	Biolegend	652407	1:20	25 µg/ml
ERG-Alexa647	Rabbit	Abcam	ab196149	1:100	5 µg/ml
DAPI	NA	Sigma	10236276001	1:1,000	10 ng/ml