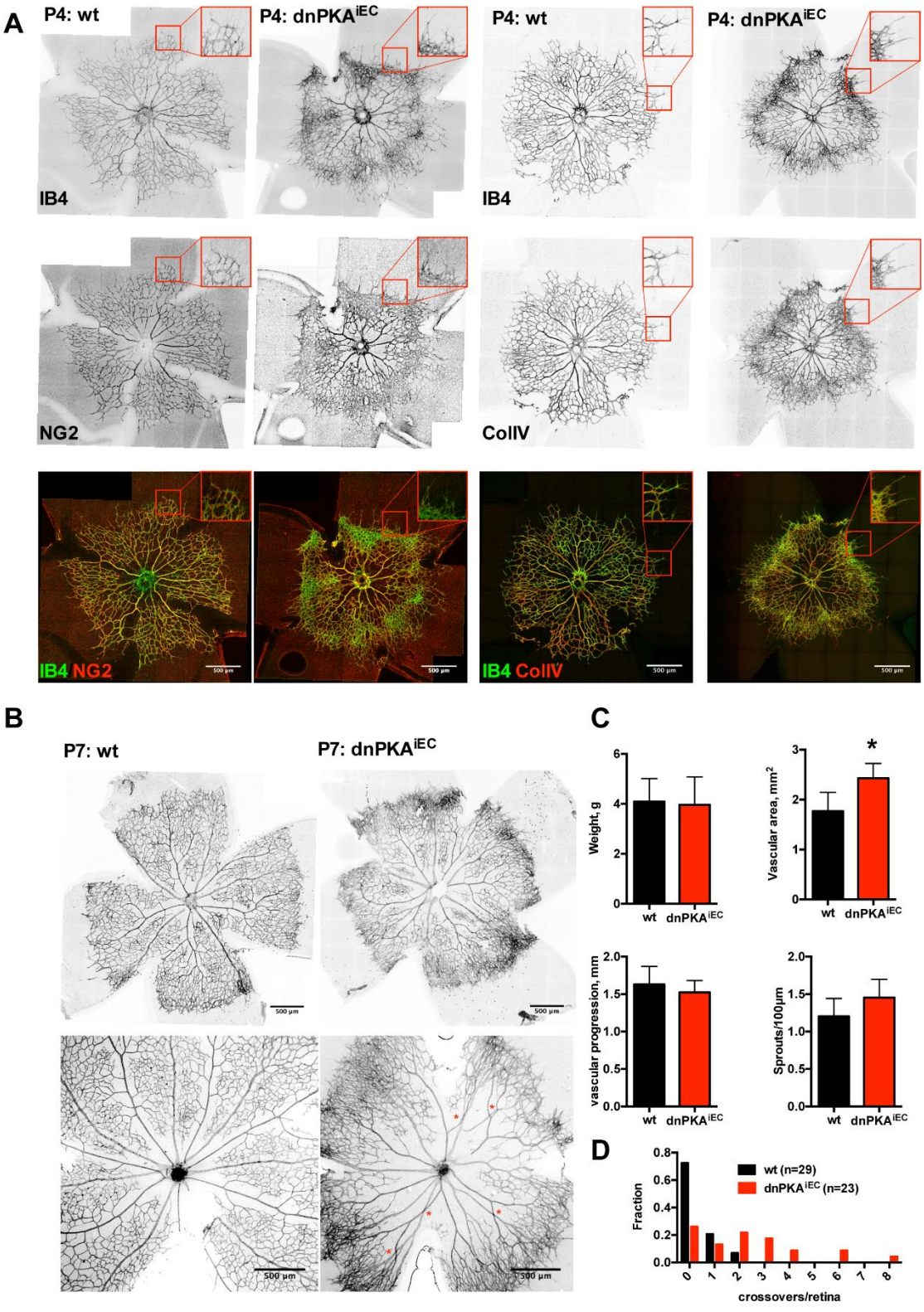
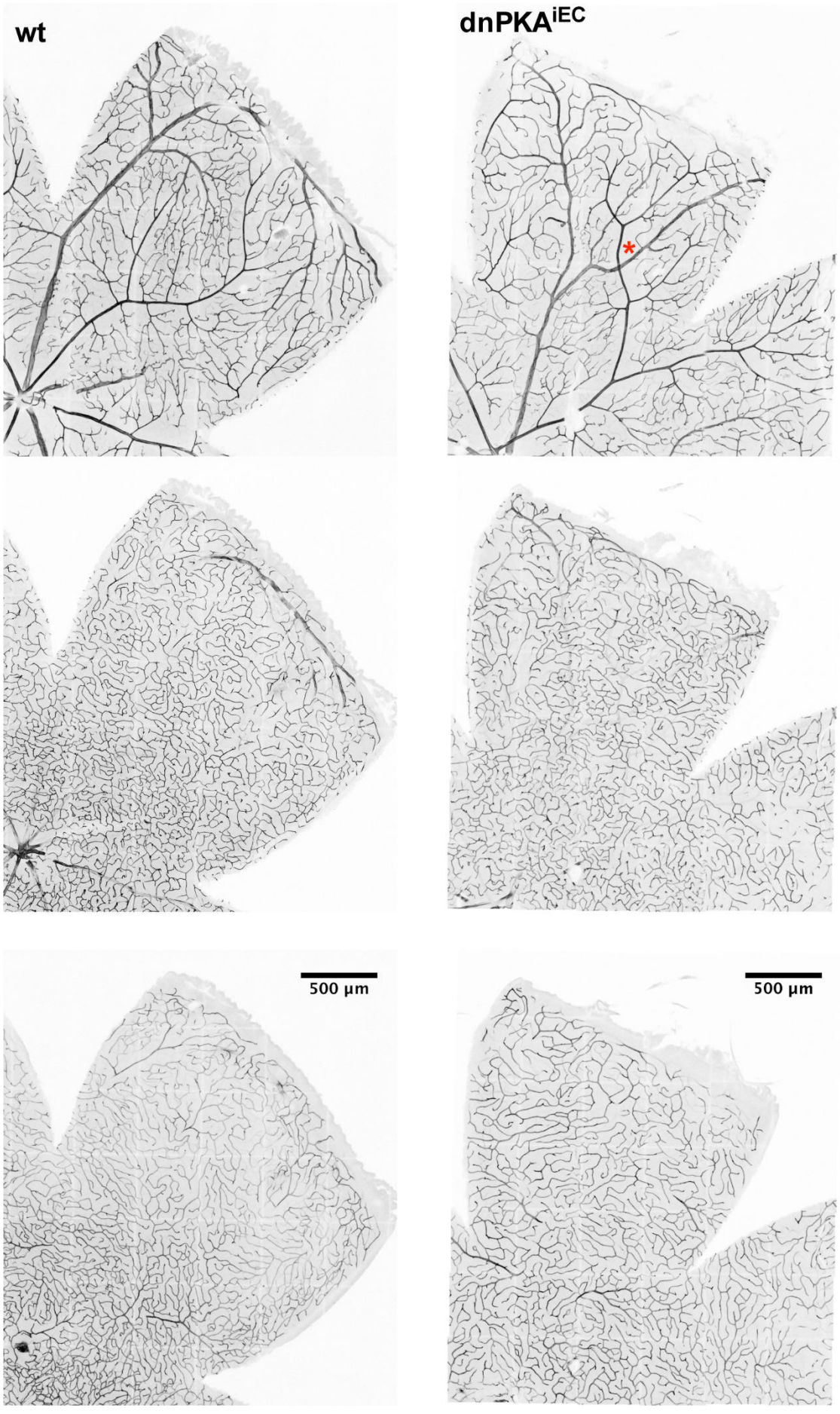
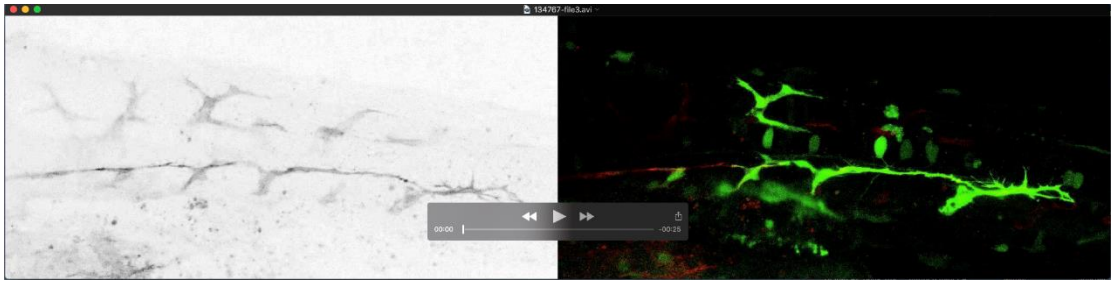


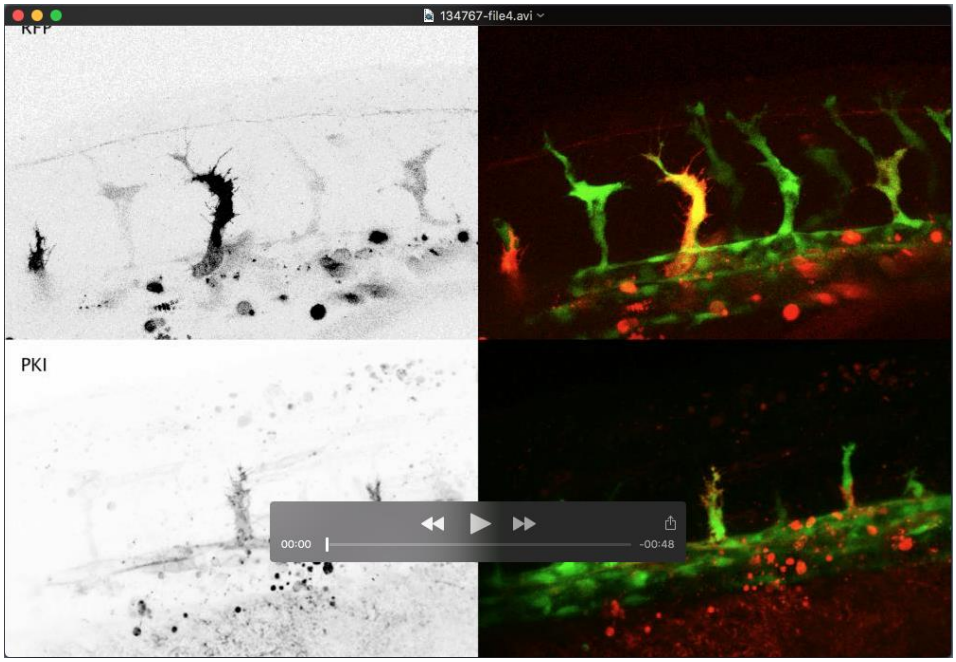
Suppl. Fig. 1. Characterization of retinal vasculature in dnPKA^{iEC} mice. (**A**) Retinas from Cdh5-CreERT2 (wt) and dnPKA^{iEC} littermates were isolated at P4 and co-stained for endothelial cells with isolectin B4-Alexa F568 and either a pericyte marker NG2 or basement membrane protein collagen IV (ColIV). For NG2 staining, 7 wt and 6 dnPKA^{iEC} pups from three different litters were analyzed. For collagen IV, 8 wt and 10 dnPKA^{iEC} from three different litters were analyzed. Shown are representative images. (**B**) Retinas from Cdh5-CreERT2 (wt) and dnPKA^{iEC} littermates were isolated at P7 and the vasculature was visualized with isolectin B4-Alexa F568. Upper panels show overview over the whole retinal vasculature; lower panels are zoomed into the central part of retinas (different from the ones shown in the upper panels) to demonstrate arteriovenous crossovers (indicated by red asterisks). (**C**) Quantification of weight of the pups, retinal vascular area, vascular progression and number of sprouts in Cdh5-CreERT2 (wt) and dnPKA^{iEC} littermates at P7. Shown are means ± SD of at least 8 mice from at least three different litters. (**D**) Quantification of arteriovenous crossovers in P7 retinas of Cdh5-CreERT2 (wt) and dnPKA^{iEC} littermates. Number of analyzed retinas is indicated on the figure.


wt
dnPKA^iEC
*
500 µm
500 µm

Suppl. Fig. 2. Characterization of retinal vasculature in dnPKA^{iEC} mice at P30. Retinas from Cdh5-CreERT2 (wt) and dnPKA^{iEC} littermates were isolated at P30 and the vasculature was visualized with isolectin B4-Alexa F568. Shown are superficial vascular plexus (upper panels), inner deeper plexus (middle panels) and outer deeper plexus (lower panels). Arteriovenous crossover in the superficial plexus is indicated by red asterisk (right upper panel).



Movie 1. Tg(*Kdrl:Ras-mCherry*)^{s916} embryos were injected with plasmid expressing PKI-GFP fusion under control of endothelial *fli1ep* promoter and vascular development was monitored from 29 till 42hpf. Membrane-bound mCherry fluorescent protein (black in left panel and red in the right) depicts higher mobility and excessive filopodia formation in PKI-GPF-expressing cells (green in the right panel) compared to the wild type GFP-negative cells. Time frame: 25min.



Movie 2. Tg(*fli1a:eGFP*)^{y1} embryos were injected with plasmid expressing RFP or PKI-GFP fusion under control of endothelial *fli1ep* promoter and and growth and lumeniyation of ISVs were monitored from 29 till 45hpf. Time frame: 10min.

Supplementary Methods to Nedvetsky et al.

Genotyping

All genotyping PCR were performed using MyTaq Red DNA Polymerase (Bioline) using following PCR conditions:

Step 1:	95°C x 1min
Step2 (36 cycles):	95°C x 15sec
	62°C x 15sec
	72°C x 30sec

Primers used for genotyping are listed below. To genotype Prkar2a^{tm1Gsm} mice, PCR product (328bp) was digested with ScrFI. ScrI digest of the wild type allele results in bands of 174, 101 and 52bp; digest of transgenic allele gives 226 and 101 bp bands.

Genotyping primers:

1. Prkar2a^{tm1Gsm}

Forward: GTAGGCATGACTGTAGGGGC

Reverse: CTGTTGTACTGCTGGATGTTCCG

PCR product (both wt and transgene): 328bp. Further digest with ScrFI is required to discriminate between wild type and transgene (see above).

2. Cre (used to genotype Tg(Cdh5-cre/ERT2)^{1Rha} and Tg(Tek-cre)$^{1Ywa/J}$

Forward 1: ACTGGGATCTTCGAACTCTTTGGAC

Reverse 1: GATGTTGGGGCACTGCTCATTCACC

Forward 2: CCATCTGCCACCAGCCAG

Reverse 2: TCGCCATCTTCCAGCAGG

These primer were described by others in http://www.ics-mci.fr/mousecre/pdf/standard_cre_genotyping.pdf. PCR results in 420bp control band (present both in wild type and Cre-positive mice) and 281bp band present in Cre-positive samples only.

3. Tg(Pdgfb-icre/ERT2)^{1Frut}

Forward: GTGCCTGGCTAGAGATCCTG

Reverse: GATGTGGGAGAGGATGAGGA

No band present in wild type samples; 410bp band in transgenes.

4. Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$

Forward 1: TTCCCCTGCAGGACAACGCC

Reverse 1: GGCTTCTGAGGACCGCCCTG

Forward 2: GTGGGGCTCACCTCGACCATGG

Reverse 2: GAGGGCGATGCCACCTACGG

Wild type: 148bp band; heterozygotes: 148 and 268pb; homozygote transgene: 268bp.

QPCR primers

Following primers were used to perform QPCR analysis:

Target	**Forward primer**	**Reverse primer**
Dll4 (NM_019454.3)	GGCAAACTGCAGAACCACAC	TGGCTTCTCACTGTGTAACCG
Jag1 (NM_013822.5)	CAGGGTCTACGCCTGTCATC	AAAGTGTAGGACCTCGGCCA
Notch1 (NM_008714.3)	ACAGTGCAACCCCCTGTATG	CCGCAGAAAGTGGAAGGAGT
Hes1 (NM_008235.2)	CAACACGACACCGGACAAAC	GGAATGCCGGGAGCTATCTT
Hey1 (NM_010423.2)	TGCAGTTAACTCCTCCTTGCC	CGCCGAACTCAAGTTTCCATT
Hey2 (NM_013904.1)	GAACAATTACCCTGGGCACG	TTCGATCCCGACGCCTTTTT

VEGFR1 (NM_010228.3)	TACCTCACCGTGCAAGGAAC	AAGGAGCCAAAAGAGGGTCG
VEGFR2 (NM_010612.2)	GTGCAGGATGGAGAGCAAGG	GCTGTCCCCTGCAAGTAATCT
ESM1 (NM_023612.3)	GCAAGAGGACAGTGCTGGAT	GGTGCCATAGGGACAGTCTTT
Cdh5 (NM_009868.4)	CCTGAGGCAATCAACTGTGC	GGAGGAGCTGATCTTGTCCG
Cxcl2 (NM_009140.2)	TGAACAAAGGCAAGGCTAACTG	CAGGTACGATCCAGGCTTCC
Nrp1 (NM_008737.2)	GCTGTGAAGTGGAAGCACCT	GGAAGTCATCACCTGTGCCA
PECAM (NM_001032378.1)	AGCCAACAGCCATTACGGTTA	GAGCCTTCCGTTCTCTTGGT
GAPDH (NM_008084.3)	GGGTTCCTATAAATACGGACTGC	ACGGCCAAATCCGTTCACA

To validate the specificity, each primer pair was tested to give a single band of expected size in PCR prior to use in QPCR.