

Supplementary Methods:

Production of Transgene Vector:

pKTol2H70-mC-hVEGF-gcG was made by cloning a floxed mCherry cassette into pKTol2H70-hVEGF-gcG that was linearized with PstI digestion. The floxed mCherry cassette was amplified from pKT2C-LP2-mC-CTLA4Ig using MISC-LP2-mC-F1 [ATTGTTTTTTTCCTGCAGTTCTCATCATTTTTGGCAAAGAATTGATAAC] and MISC-LP2-mC-R1 [TTGATTTCAAGAAACGGCAAGCCATGGTCTCGAGGAATTCATAAC] primers. The primers introduced overlapping sequences allowing the cloning to be done using In-Fusion PCR cloning kit (Clontech). pKT2C-LP2-mC-CTLA4Ig was a kind gift of Dr. Dan Carlson from the University of Minnesota.

pKTol2H70-hVEGF-gvG was made by cloning a 1.6kb XmaI to NheI fragment of pKTol2gC-GFP that contained the *Xenopus* gamma-crystallin driven GFP expression into pKTol2H70-hVEGF opened with NheI and AgeI.

pKTol2H70-hVEGF was made by amplifying human cloning VEGF 165 cDNA into pKTol2H70-GFP. The human VEGF165 was amplified from cDNA with CDS-hVEGF165-F1 [TTCTCGAGACCATGAACTTTCTGCTGTCTTGGGT] and CDS-hVEGF165-R1 [AAAGATCTCACCGCCTCGGCTTGTGACA] primers and TOPO cloned into pCR4 (Invitrogen). The .58 kb XhoI to BglII hVEGF165 fragment was digested from the pCR4 vector and cloned into pKTol2H70-GFP in place of the GFP cassette, which was removed as an XhoI to BglII fragment.

pKTol2H70-GFP was made by replacing the mini-CAGs promoter of pKTol2C-GFP with the hsp70 promoter from zebrafish³⁴. The original 1.5kb promoter described by Halloran et al. contains upstream regulatory elements, a non-coding exon, and the majority of intron 1. However it lacks the complete splice-acceptor of intron 1. In order to capture the full splice acceptor sequence following intron 1 of the hsp70 promoter, we re-isolated the promoter by amplifying it from zebrafish genomic DNA using MISC-HSP-F1 [TTCCCGGGTGTGCGCTTGGTGATTTCC] and MISC-HSP-R1 [AACTCGAGATTGATTCAAGAACTGCA] primers. The initial PCR product was TOPO cloned into pCR4. The hsp70 promoter was cut from the pCR4 vector using XmaI and XhoI restriction endonucleases. The mini-CAGs promoter of pKTol2C-GFP was removed with XmaI and XhoI and replaced by the zebrafish hsp70 promoter.

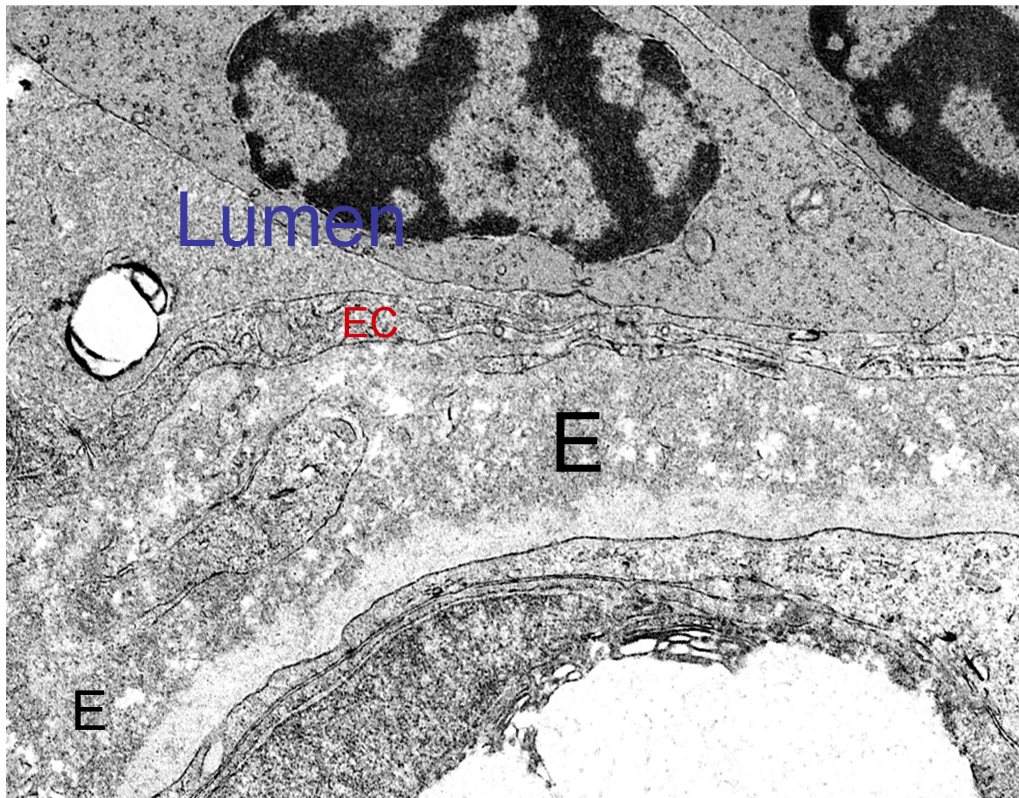
pKTol2C-GFP was made by cloning the 2.2 kb SpeI to AgeI mini-CAGs promoter and EGFP cassette from pKT2C-GFP³⁵ into pKTol2-SE³⁶ opened with AgeI and SpeI.

pKTol2gC-GFP was made by cloning the *Xenopus* gamma crystallin promoter into pKTol2C-GFP. The gamma-crystallin promoter was amplified from T2/ γ -Cry-GM2³⁷ with MISC-gCrystallin-F1 [TACTAGTTCTTTAAACTGTTACAAAGTGTGGACC] and MISC-gCrystallin-R2 [AACTCGAGTTAGTCAGGCTGTGGATCCCCGCAAGTC]. The PCR product was digested with SpeI and XhoI and cloned in place of the SpeI to XhoI mini-CAGs promoter of pKTol2C-GFP.

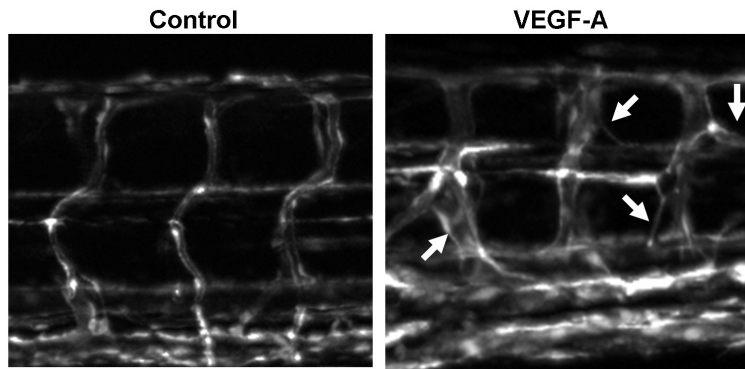
PCR: Extracted zebrafish RNA was purified with RNeasy columns (Qiagen). Quantitative RT-PCR was performed using the QuantiTech SYBR Green RT-PCR kit (Qiagen) according to the manufacturer's instructions. RNA (10 ng) was added to 20 μ l reactions with QuantiTech SYBR Green RT master mix, QuantiTech RT mix, and 0.5 pmol/ μ l of each of the oligonucleotide primers. The primer pairs used were: VEGF-A [forward, CTACTGCCATCCAATCGAGA; reverse, TGGTGATGTTGGACTCCTCA], mCherry [forward, CCCCGTAATGCAGAAGAAGA; reverse, TCTTGGCCTTGTAGGTGGTC], and GAPDH [forward, GATACACGGAGCACCAGGTT; reverse,

GCCATCAGGTCACATACACG]. The reactions were run on a 7500 Real Time PCR System (Applied Biosystems) using the following conditions: reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 15 min, and 40 cycles of two-step PCR: 95°C denaturation for 15 s and 60°C annealing and elongation for 1 min. Data were normalized to zebrafish GAPDH levels. For standard PCR, purified zebrafish RNA (500 ng) was converted to cDNA using the Superscript II First-Strand Synthesis System (Invitrogen) and added to 20 µl reactions with Choice Taq Blue Mastermix (Denville Scientific) and 0.5 pmol/µl of each of the PLCβ3 oligonucleotide primers [forward, CCGTTGTTACACTGAAGGT; reverse, GCTTTGAGTAAGAAGGTGTTG]. The following PCR conditions were used: initial denaturation at 95 °C for 10 min, 40 cycles of 95°C denaturation for 30 s, 54°C annealing for 30 s, and 72°C elongation for 1 min, and lastly 72°C elongation for 10 min.

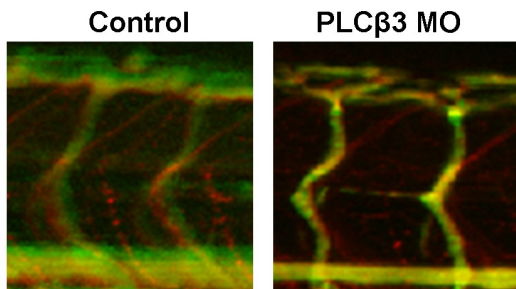
Supplementary Figures:



Supplementary Figure 1: VEGF-induction in zebrafish causes edema. Heat shock induction of VEGF was performed in transgenic zebrafish. VEGF-induced zebrafish were fixed in KII fixative for 2 hours at room temperature, transferred to 0.1 M Na Cacodylate at 4°C, and electron microscopy was performed. E: edema, EC: endothelial cell.



Supplementary Figure 2: Chronic VEGF-induction causes increased angiogenesis. Fli-GFP zebrafish were crossed to the VEGF-inducible zebrafish and Fli-GFP:pkTol2-VEGF offspring were heat shocked three times at 3 and 4 dpf to cause VEGF-induced CVH. Corresponding Fli-GFP controls were also heat shocked at these time points. Vascular structure was imaged in control (left panel) and VEGF-induced zebrafish (right panel) at 4 dpf. White arrows indicate areas of increased vascular structure and angiogenesis.



Supplementary Figure 3: Knockdown of PLC β 3 in zebrafish does not affect basal vascular permeability in absence of VEGF-induction. Microangiography was performed on 3 d.p.f. zebrafish with red permeabilizing tracer and green ISV marker. Control (left panel) and PLC β 3 MO-injected (right panel) wildtype zebrafish were imaged in real-time and representative images are depicted.

Video 1: Three dimensional imaging of VEGF-induced VP. Microangiography was performed on 3 dpf control zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Approximately 30 minutes following heat shock, three dimensional rotating confocal imaging was performed to visualize extravasation of Texas Red-dextran.

Video 2: Three dimensional imaging of VEGF-induced VP. Microangiography was performed on 3 dpf VEGF-induced zebrafish with 70 KDa Texas Red-dextran (permeabilizing

agent) and 2000 KDa FITC-dextran (marker of the veins). Approximately 30 minutes following VEGF-induction by heat shock, three dimensional rotating confocal imaging was performed to visualize extravasation of Texas Red-dextran.

Video 3: Live fluorescence microscopy of VEGF-induced VP. Microangiography was performed on 3 dpf control zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Live imaging was performed after heat shock for 52 minutes using merged z-stacks at each time point throughout the series.

Video 4: Live fluorescence microscopy of VEGF-induced VP. Microangiography was performed on 3 dpf VEGF-induced, control MO-injected zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Live imaging was performed immediately following heat shock induction of VEGF for 52 minutes using merged z-stacks at each time point throughout the series.

Video 5: Live fluorescence microscopy of VEGF-induced VP. Microangiography was performed on 3 dpf VEGF-induced, PLC β 3 MO-injected (c) zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Live imaging was performed immediately following heat shock induction of VEGF for 52 minutes using merged z-stacks at each time point throughout the series.

Video 6: Real-time surface projection imaging of VEGF-induced VP. Microangiography was performed on 3 dpf control zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Confocal surface projection time lapse imaging was performed after heat shock for 52 minutes using merged z-stacks at each time point throughout the real-time imaging series.

Video 7: Real-time surface projection imaging of VEGF-induced VP. Microangiography was performed on 3 dpf VEGF-induced, control MO-injected zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Confocal surface projection time lapse imaging was performed immediately following heat shock induction of VEGF for 52 minutes using merged z-stacks at each time point throughout the real-time imaging series.

Video 8: Real-time surface projection imaging of VEGF-induced VP. Microangiography was performed on 3 dpf VEGF-induced, PLC β 3 MO-injected zebrafish with 70 KDa Texas Red-dextran (permeabilizing agent) and 2000 KDa FITC-dextran (marker of the veins). Confocal surface projection time lapse imaging was performed immediately following heat shock induction of VEGF for 52 minutes using merged z-stacks at each time point throughout the real-time imaging series.