Supplementary Materials and Methods:

Zebrafish Experiments:

Imaging inhibitor-treated embryos − 28-30 hpf embryos were fixed overnight, then mounted in low melt agarose the following day, and somites 7-12 were imaged (20X objective, NA=0.8, laser power=20%, pinhole = 182 µm, 32 µm step size, 16 total slices) using a LSM780 microscope and ZenPro imaging software. Intersomitic vessel length was measured using the Distance Tracking function in ZenPro.

pERK immunostaining − Briefly, embryos were fixed in freshly made 4% PFA / PBS / 0.1% Tween-20 overnight at 4°C, then dehydrated to absolute methanol at 4°C. Endogenous peroxidase activity was quenched by incubation in 3% H_2O_2 / 97% methanol at 4°C for 1 h, then embryos were washed extensively in methanol and stored for at least 2 days at -20°C. Embryos were then rehydrated to PBS / 0.1% Tween-20 (PBST), and cryoprotected in PBST / 30% sucrose overnight at 4°C. The following day, embryos were equilibrated for 5 min in 150 mM Tris-HCl (pH 9.0) at room temperature (RT), then this was replaced with 70°C 150 mM Tris-HCl (pH 9.0) and the embryos were maintained at this temperature for 15 min for antigen retrieval. After cooling to RT, embryos were washed in PBST, rinsed multiple times in ddH_2O, then incubated in acetone at -20°C for 20 min. Embryos were then washed in PBST at RT, transferred to TBS / 0.1% Tween-20 / 0.1% Triton X-100 (TBSTx), then blocked (TBST / 0.8% Triton X-100 / 1% BSA / 10% goat serum) overnight at 4°C. The next day embryos were incubated with anti-phospho ERK antibody (phospho-p44/42 MAPK (Thr202/Tyr204) XP rabbit monoclonal antibody, Cell Signaling Technologies, #4370) (1:250) in TBST / 0.8% Triton X-100 / 1% BSA / 1% goat serum overnight at 4°C for 3 days. Embryos were then washed extensively in TBSTx, then washed in maleic acid buffer (150 mM maleic acid (pH 7.4) / 100 mM NaCl / 0.001% Tween-20) (MABT), then incubated in HRP-conjugated goat anti-rabbit IgG secondary antibody (Jackson ImmunoResearch, 111-035-144) (1:1,000) in 2% Blocking Reagent (Sigma,

11096176001) / MABT overnight at 4°C for 2 days. Embryos were washed in 2% blocking reagent / MABT at RT, washed in MABT, transferred to PBS, then incubated (upright, rocking) in 150 µL of Alexa-568 TSA reagent (Life Technologies, T20950) (1:50) for 3 h in the dark at RT. Embryos were washed extensively in TBSTx, then blocked in TBSTx / 10% goat serum / 1% BSA for 1 h at RT, and incubated with rat anti-GFP (Chromotek, 3h9) (1:500) overnight 4°C in TBST / 0.5% Triton X-100 / 1% goat serum / 1% BSA, washed in TBST / 0.5% Triton X-100, washed in TBSTx, then incubated with Alexa-488 conjugated goat anti-Rat IgG (Life Technologies, A11006) (1:250) overnight at 4°C. Embryos were then washed extensively, briefly post-fixed in 4% PFA, washed in PBS, then mounted in low-melt agarose for confocal analysis. Embryos without primary pERK antibody were always included as a negative control for tyramide amplification. Images were collected at a step size of 1.5 µm, for 43 total slices, using a Plan Apochromat 20X/0.8 objective, laser power=2.20% (488 nm), 5.50% (561 nm), pinhole=57 µm, on a LSM800 confocal laser scanning microscope using ZenBlack imaging software. Stacks were exported to ImageJ where individual cells were counted and the stack was compressed to a Maximum Intensity Projection (MIP). Vessels were imaged as above (for sprout length) and GFP^+ vessels with pERK staining were scored as positive.

Time-lapse confocal microscopy − 2 embryos were analyzed per experiment, and the movies shown are representative of these results (DMSO=6 embryos, SL327=6 embryos). Images were collected every 10 minutes, at a 5 µm step size, with a total stack size of 205 µm, using a Plan Apochromat 20X objective, laser power=20.8% (488), 56.4% (546), NA=0.8, WD=0.55 mm, pinhole=57 µm, on a LSM800 confocal laser scanning microscope using ZenBlack imaging software. Images were compressed to a maximum intensity projection (MIP), then stitched together as a movie in Zen and exported as an AVI (with no compression).

Imaging transplantation experiments: Embryos were embedded in low melt agarose, then imaged using a LSM 780 confocal microscope (Zeiss) with the following conditions: Plan-Apochromat 20x/0.8 M27 objective, 488 laser power at 4.5%, 594 laser power at 4.7%, 647 laser power at 5%. A total tack of 96 µm was collected, scanning every 2 µm (averaging set to 8). The pinhole was set to 126 µm (488), 55 µm (561), 113 µm (640). Stacks were exported to ImageJ where individual cells were counted and the stack was compressed to a Maximum Intensity Projection (MIP).

In situ hybridization primers −
JDW 436 (T7 *drhlx* FWD): 5'-
GCAAATTAATACGACTCACTATAGGGAGAGCGGTGGATAGCATGAAGAAAC

JDW 437 (SP6 *drhlx* REV): 5'-
TATGCGATTTAGGTGACACTATAGAACAGGTTGGAAAACACAGCTCTG
JDW 295 (SP6 *drdusp5* FWD): 5'-
TATGCGATTTAGGTGACACTATAGAACTTTGTCGCACTTGACGAGTA
JDW 296 (T7 *drdusp5* REV): 5'-
CGAAATTAATACGACTCACTATAGGGTTGGACTCAGTTTGAATGATGG
JDW 297 (SP6 *drdll4* FWD): 5'-
TATGCGATTTAGGTGACACTATAGAAGGACATCGTGTCCCAAGAGGA
JDW 298 (T7 *drdll4* REV): 5'-
CGAAATtaatacgactcactatagggCAATCCAAGAAGACCCGGGCA

Mouse experiments:

Immunohistochemistry on sections − For frozen sections, after equilibration at -20°C, 10 µm sections were collected on Superfrost Plus slides (Fisher, #12-550-15) using a Leica CM1850 cryotome. Sections were stored at -80°C until ready for processing. For IHC, slides were dried for 10 minutes at 42°C, washed 3X in PBS, permeabilized in 0.3% Triton-X100 / 1X PBS, washed in PBS, blocked in TNB (0.1 M Tris-HCl (pH 7.5) / 0.15 M NaCl / 0.5% Blocking Reagent (Roche, #10447200)) for 1 h at room temperature, then incubated with anti-ERG1/2/3 (Santa Cruz Biotechnology, SC-353) (1:200) overnight at 4°C. Tissue was washed in PBS, incubated with Alex 594 conjugated goat anti-rabbit (Life Technologies, A11034) (1:150) for 1.5 h at room temperature, washed in PBS, then stained for CD31 (BD Biosciences, #550274) for 2 h at room temperature, washed in PBS, incubated in Alexa 488 conjugated goat anti-rat (Life Technologies, A11006) (1:150) for 1.5 at room temperature, washed in PBS, counterstained in DAPI (300 nM) (Life Technologies, #D3571) for 10 min at room temperature, washed in PBS, coverslipped in aqueous mounting media (Vector Labs, #H-5501), cured, then sealed with nail polish. Images were acquired using a EC Plan-Neofluar 10x/0.30 M27 objective with laser power 6.5% (405 nm); 11% (488 nm); 6% (561 nm); or a 40x/1.4 oil DIC M27 objective with laser power 0.8% (405 nm); 4% (488 nm); 4% (561 nm) using a Zeiss LSM 780 confocal microscope. Confocal stacks of 12.25 µm were collected with a step size of 1.75 µm. Stacks were compressed to a maximum intensity projection using ZenBlue (Zeiss), and images were exported to Adobe Photoshop and Illustrator.

X-gal staining on sections − 10 µm cryosections (processed as detailed above) were dried for 10 min at room temperature, washed in PBS, post-fixed (2% PFA / 0.2% glutaraldehyde / 1X PBS / 0.02% sodium deoxycholate / 0.01% IGEPAL CA-630) for 5 min at room temperature, washed in

Permeabilization Buffer (1X PBS / 0.02% sodium deoxycholate / 0.01% IGEPAL CA-630) at room temperature, then incubated in Staining Buffer (1 mg/mL X-gal / 5 mM postassium ferricyanide / 5 potassium ferrocyanide / 2 mM $MgCl_2$ in Permeabilization Buffer) overnight at 37°C, washed in PBS, fixed, washed, then counter-stained with nuclear fast red (Vector Labs, #H-3404) for 10 min, washed in dH_2O, dehydrated to absolute ethanol, washed in xylene, and mounted in Entellan New (Electron Microscopy Sciences, #14800). Slides were imaged on a Zeiss Axio Zoom.V16 stereoscope using Zen Blue software. Images were exported in Adobe Photoshop.

Collecting ECs from mouse embryos by FACS − Following collagenase digestion, cells were washed and blocked in HBSS++ (HBSS without calcium, magnesium, or phenol red (Gibco, Cat#14175095) / 2% FBS (HyClone, Cat #SH30910.03) / 1% penicillin-streptomycin (Lonza, Cat #12001-324) / 1% 1M HEPES (Gibco, Cat #15630080). Cells were then incubated in HBSS++ with BV421 Rat anti-mouse CD31 (BD Biosciences, Cat #562939) (1:100) for 90 min at 4°C, washed in HBSS++, then stained in TO-PRO-3 Iodide (Life Technologies, Cat # T3605) (1:1,000) for 15 min at 4°C, all while rocking gently. Cells were then washed and filtered through a 35 μm cell strainer (Falcon, Cat#352235). The single cell suspension was sorted at the Cytometry and Cell Sorting Core (CCSC) (BCM) on a FACSAria II (BD Biosciences) using the following gating parameters: Sort set up to 85 μm, precision set to purity. Events were recorded until 25,000 live endothelial cells per embryo were collected into 350 μL of RLT Buffer (Qiagen) for downstream processing. Embryos with EC viability lower than 70% were not used for downstream analysis.

Analysis of retinal vasculature – Following administration of tamoxifen at P1 and P3, pups were euthanized at P8 by CO_2 asphyxiation, tail tissue was acquired for DNA genotyping, and eyes were enucleated and placed in 4% PFA at 4°C overnight. The following morning, retinas were isolated, then partially cut into 4 quadrants (i.e. leaflets) to allow for flat mounting. Isolated retinas were first blocked and permeabilized overnight with gentle shaking at 4°C in retina blocking buffer (1% BSA / 0.5% Triton-X100 / 1X PBS / pH 7.2). The following morning, retinas were washed 3 times for 5 minutes each wash in 1X PBS. Retinas were then equilibrated to room temperature in Pblec solution (1X PBS / 1 mM $CaCl_2$ / 1 mM $MgCl_2$ / 0.1 mM $MnCl_2$ / 1% Triton-X100 / pH 6.8) 3 times, for 20 minutes each time. Retinas were then incubated overnight with gentle shaking at 4°C with biotinylated *Griffonia simplicifolia* Isolectin B4 (Vector Labs, B-1205) at a concentration of 1:50 prepared in Pblec solution. Retinas were rinsed twice with 1X PBS, followed by 3X 10-minute washes in retina wash buffer (retina blocking buffer diluted 1:1 in

1X PBS), then incubated in DyLight 594 Streptavidin (Vector Labs, SA-5594) prepared in retina blocking buffer for 2 h at room temperature with gentle agitation. After incubation, retinas were rinsed twice with 1X PBS, followed by 3X 10-minute washes in retina wash buffer. Retinas were mounted on glass slides and coverslipped using Fluoromount-G mounting medium (SouthernBiotech, 0100-01). For P6 retinal harvests, pups were given a single, 50 µL dose (10 mg/mL) of tamoxifen (equivalent to 50 µg per mouse) by subcutaneous injection at P1, and harvested eyes were fixed in 4% PFA and then retinas were collected after 2 hours and processed identically as P8 retinas.

To quantify vascular branching in the retina, a single 20X image was taken from a middle point of the central plexus for each of the 4 quadrants per retina (i.e. 4 images per retina examined). All branch points were tallied using ImageJ software within each full 20X field of view and averaged across the 4 quadrants for a single averaged measurement of vascular branching per biological sample. This was performed from 1 retina per animal, from 5 different animals for both control ($ERG^{fl/+}$) and experimental (ERG^{iECKO}) groups at P8, and 3 animals for control ($ERG^{fl/+}$) and 2 animals for the experimental (ERG^{iECKO}) groups at P6. Data was graphed using GraphPad Prism software and shown as an average ± SEM. Comparisons were made using a two-way, unpaired Student's T-test for the P8 retinas. Images for both ages were obtained on a Leica TCS SPE confocal microscope with a 20X objective lens (1,024 x 1,024 pixels) at 15% power (561 nm).

Radial expansion of the retinal vasculature was quantified using the integrated length calculator function in the Zen Pro software suite (Zeiss). Images were taken on a Zeiss Axio Zoom.V16 fluorescent stereoscope at a 65X magnification. At this magnification, the entirety of a retinal quadrant to the optic nerve could be collected in one image. Images were taken for each quadrant (4 per retina), and a single line was drawn from the center of the optic nerve, through the center of the quadrant, to the vascular front. Total length was recorded for each of the 4 quadrants for a single retina and the average determined. This was performed for all retinas from both control and experimental groups. Radial expansion was graphed using GraphPad Prism software and shown as an average ± SEM. Comparisons were made using a two-way, unpaired Student's T-test for the P8 retina data.

To quantify sprouting within the P6 retina, a single 40X image (1,024 x 1,024 pixels) was acquired from each leaflet (i.e. 4 images per retina). Major sprouting vessels at the vascular front were tallied within each full 40X field of view and averaged across the four leaflets to yield a single measurement per animal. One retina per animal was analyzed, and for P6 3 control ($ERG^{fl/+}$) and 2 experimental (ERG^{iECKO}) animals were quantified. Data was graphed using

GraphPad Prism software and shown as an average ± SEM. Images were taken on a Leica TCS SPE confocal microscope using a 20X objective with a 2X zoom magnification at 22% laser power (561 nm).

Cloning:

ETS concatemer − Oligos containing the previously EMSA-validated ETS site B (site #2, GCGTTTCCTGCGGG) of the minimal 30 bp murine *Dll4* intron 3 arterial enhancer (F2-6) (Wythe et al., 2013) were synthesized as a multimer (8X), and the duplex was annealed and cloned into pDONR221 to generate JDW 295 (8X WT) and JDW 242 (8X MT, GCGTTTttTGCGGG) constructs. Subsequently, this was recombined into pGL3-Pro-DV (JDW 250/ETS WT; JDW 241/ETS MT).

HLX enhancers − Nucleotides of the gene regulatory element *HLX-3a*, spanning 1,565 bp on human chromosome 1, beginning approximately 3 kb upstream of the 5' UTR of *HLX*, were directly synthesized as a gene block (IDT) with all ETS sites intact, or all ETS sites mutated as follows: ETS A: TTCC>TTaa*;* ETS B: GGAA>aaAA*;* ETS C: TTCC>TTtt; ETS D: TTCC>TTtt; ETS E: GGAA>aaAA; ETS F: GGAA>ttAA; ETS G: GGAA>aaAA; ETS H: TTAA>TTtt; ETS I: GGAA>GGgg; ETS J: GGAA>aaAA; ELK1: TCCG>TttG; ETS K: TTCC>TTtt; ETS L: GGAA>ctct; ETS M: GGAA>aaAA; ETS N: GGAA>aaAA; ETS O: TTCC>ccCC; ETS P: GGAA>aaAA; ETS Q: GGAA>aaAA
Subsequently, primers containing flanking attB1/B2 sites and BamHI and NotI restriction sites (5' and 3', respectively) were designed to amplify the entire region (*HLX-3a*) or the smaller fragment (*HLX-3b*), from both wild-type and mutant templates, and were directly cloned into pDONR221 via a BP clonase reaction.

HLX Enhancer Activity Assays − Following sequence verification, *HLX-3a/3b* donor clones were recombined with a destination vector, pGL4.23[luc2 minP]-DV, for *in vitro* luciferase analysis. pGL4.23[luc2 minP]-DV was created by digesting the parental vector (pGL4.23[luc2 minP]) with NheI and inserting a PCR-amplified gateway RFA cassette via Cold Fusion (SBI). The RFA insert was amplified using the following primers, JDW 356 (FWD): 5'-ACTGGCCGGTACCTGAGCTCACAAGTTT GTACAAAAAAGC; JDW 357 (REV): 5'-CAGATCTTGATATCCTCGAGCCACTTTG TACAAGAAAGCT. For *in vivo* analyses, the *HLX* pDONR clones were recombined with a modified pTol2-E1b-EGFP destination vector. Briefly, to stabilize reporter activity, pT2KXIGQ-DV (Smith et al., 2013) (a kind gift of Nadav Ahituv, UCSF), which has a gateway RFA cassette inserted between XhoI and BglII, and is a variant of

pT2KXIGQ (Li et al., 2010), originally derived from pT2KXIG (Kawakami et al., 2004), was digested with ClaI and a WPRE element was amplified by PCR and placed downstream of EGFP, upstream of the poly(A), using Cold Fusion (SBI).
JDW 348 (FWD): 5'-GGTGGAGCTCGAATTAATTCATCGATTCAACCTCTGGATTACAA AATTTGTG; JDW 360 (REV): 5'-CTTATCATGTCTGGATCATCATCGATTCGAG GTCGAGGTCGACGGTAT. This construct was validated using a *myh6* (aka *cmlc2*) promoter (data not shown).

Wild-type and mutant ERG constructs − The ORF of human ERG was amplified by PCR, adding a 3X-FLAG sequence and a Gly-Gly-Ala-Gly-Gly flexible linker (Sabourin et al., 2007) at the amino terminus, with two stop sequences and an XbaI site at the carboxy terminus.
JDW 497 (FWD) (ERG homology is underlined, linker is in bold): GCCACCATGGACTAC AAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGAT GACAAG**GGTGGTGCTGGTGGT**ATGGCCAGCACTATTAAGGAAGCC
JDW 498 (with an attB2 site):
CCCCACCACTTTGTACAAGAAAGCTGGGTCTAGATTATTAGTAGTAAGTGCCCAGATGAGA AGGC
Then an attB1 site was added at the 5' end by PCR, using the same REV primer as above:
JDW 499 (FWD):
GGGGACAAGTTTGTACAAAAAAGCAGGCTGCCACCATGGACTACAAAGACCATGAC GG
This PCR product was cloned via a BP reaction directly into pDONR221, and then used as the template for site directed mutagenesis. The primers are listed below:
JDW 500 (S215A FWD; TCT>GcC): gatgttgataaagccttacaaaacgccccacggttaatgcatgcta,
JDW 501 (S215A REV; TCT>GcC): tagcatgcattaaccgtggggcgttttgtaaggctttatcaacatc.
JDW 448 (S96A FWD; AGC>gGC): 5'-ggaagatggtgggcgccccagacaccgttg
JDW 449 (S96A REV; AGC>gGC): 5'- caacggtgtctggggcgcccaccatcttcc
JDW 450 (S276A FWD; TCT>gCT) 5'-gaaagctgctcaaccagctccttccacagtgcc
JDW 451 (S276A REV; TCT>gCT) 5'-ggcactgtggaaggagctggttgagcagctttc

All constructs were then recombined by a LR clonase reaction into pCS2-DEST (Addgene # 22423) (Villefranc et al., 2007) for *in vitro* validation by immunofluorescence and western blot (using by anti-Myc and anti-Flag antibodies).

pERG western blots and co-immunoprecipitation experiments:
For pERG western blots, ERG was immunoprecipitated from cell lysates (in RIPA buffer). 25 µL of Dynabeads M-280 sheep anti-mouse IgG was mixed overnight at 4°C with 5 µL of anti-ERG

antibody (BioCare) in 500 µl ChIP dilution buffer. 1 mg of cell lysate in RIPA was added to the mixture and rotated overnight at 4°C. The protein complexes bound to the beads were collected using a magnetic separator, and Western blotting was performed on SDS-PAGE gels. Blots were probed with anti-pERG^{S215} [provided by Dr. Peter Hollenhost, (Selvaraj et al., 2015)] and then stripped and reprobed with anti-ERG (BioCare). The specificity of this antibody was determined by performing western blots on wild-type and S215A mutant ERG. No signal was observed for S215A ERG. Similar procedures to those described above were used for co-immunoprecipitation experiments in HUVEC, except non-denaturing buffer was used (20 mM Tris-HCl (pH 8.0) / 137 mM NaCl / 1% NP-40 / 2 mM EDTA). Anti-V5 antibody (mouse monoclonal, Thermo Fisher Scientific) was used as a negative control for co-immunoprecipitation experiments. Blots were probed with anti-p300 (Santa Cruz Biotechnology) and anti-ERG (BioCare). Co-immunoprecipitation experiments were performed in BAECs to assess the interaction between Myc-p300 (a gift from Dr. Tso-Pang Yao, Addgene plasmid #30489) and either wild-type or mutant Flag-ERG (cloning described below). Briefly, BAECs on a 10 cm plate were transfected with 6 µg of Myc-p300 and 6 µg of Flag-ERG constructs using Lipofectamine 2000. After 48 h, cells were treated with VEGF (50 ng/mL) for 1 h. Flag-ERG was precipitated from two 10 cm plates of BAEC (~ 1 mg of total input protein per IP) overnight at 4°C using 5 µL of Flag antibody (Cat. #F1804, Sigma) and Dynabeads (Sacilotto et al.). V5 antibody was used as a negative control (as above). The samples were lysed in Non-denaturing lysis buffer (20 mM Tris HCl (pH 8) /137 mM NaCl / 1% Nonidet P-40 / 2 mM EDTA) and run on a 4% SDS-PAGE gel, transferred using a semi-dry blotter (15V, 1 h) and then blotted with anti-Flag (1:1,000) or anti-Myc antibody (Cat. #sc-4084) (1:500). The signals were detected using standard chemiluminescence.

Microarray processing and analysis:

Processing − Low Input Quick Amp Labeling Kit from Agilent (Cat#0006177230) was used to generate fluorescent cyanine 3-labeled complimentary RNA (cRNA) from total RNA (150 ng) for one-color processing. Labeled cRNA was purified using RNeasy Mini Kit (Qiagen, Cat# 74104). For microarray hybridization, 600 ng of cyanine 3-labeled cRNA was fragmented and hybridized on Agilent G3 Human 8x60K microarrays at 65°C for 17 hours at 20 rpm using the Agilent gene expression hybridization kit (part number 5188-5281). The hybridized microarrays were dissembled at room temperature in gene expression wash buffer 1 (part number 5188-5325), then washed in gene expression wash buffer 1 at room temperature for 1 minute. This was followed by a wash for 1 minute in gene expression wash buffer 2 (part number 5188-5326) at

37°C. The processed microarrays were scanned with an Agilent DNA microarray scanner (Scanner Model G2505B-C), and extracted with Agilent feature extraction software (version 10.7.3.1).

Analysis − Data was checked for overall quality using R (v2.15.3) with the Bioconductor framework and the Array Quality Metrics package installed. All samples passed quality control, but two outliers (1 sample from control siRNA, non-stimulated and 1 sample from ERG siRNA, VEGF stimulated) were identified from visualizing correlation coefficient data and were removed from subsequent analysis. Data was imported into GeneSpring v12.6.1 for analysis. During import, the data was normalized using the recommended Agilent spatial detrending method with a "per probe" median centered normalization. All data analysis and visualization were performed on log2 transformed data. Data was first filtered to remove probes that showed low signal and positive control probes were also eliminated from further analysis. Only probes that were above the 20th percentile of the distribution of intensities in 80% of any of the 4 groups were allowed to pass through this filtering. One-way ANOVA with a Benjamini-Hochberg FDR corrected $p<0.05$ was performed, revealing 498 significantly varying probes. A post-hoc Tukey's HSD test was used following the ANOVA to identify significant differences between groups. The probes that were significantly different between control siRNA and ERG siRNA in the presence of VEGF stimulation (n=357 probes) were cross-referenced with the list of VEGF regulated genes (control siRNA, no stimulation vs. control siRNA, 1 h VEGF stimulation, n=196 probes) to identify a group of 44 unique genes that were both ERG regulated and VEGF-dependent. Microarray data was submitted to ArrayExpress (accession number: E-MTAB-5207).

ChIP-seq experiments:

Processing − ChIP DNA was prepared for Illumina sequencing by blunt-end repair, dA-tailing, and ligation of Illumina adaptors using a NEBNext DNA library preparation kit (New England Biolabs, catalogue #E6040L). Total ChIP DNA (approximately 200-500 ng) and 220 ng of DNA input (WCE) was end repaired for 30 minutes at room temperature, and then purified using column purification with either DNA Clean and Concentrator (Zymogen, catalogue #D4014) or PCR purification columns (Qiagen, catalogue #28106) as recommended by the manufacturers' protocols. Blunt-end repaired DNA was dA-tailed for 40 minutes at 37°C, then column purified. dA-tailed DNA was ligated to Illumina adaptors (final concentration 6.67 nM) that have a T-overhang. USER enzyme was used to cleave the uracil hairpin of the Illumina adaptor, and adaptor-ligated DNA was column purified. The library was PCR amplified for 16-18 cycles using a universal primer and a barcoded primer (New England Biolabs, catalogue #E7335L). PCR-

amplified DNA was purified using PCR column purification and eluted in 20 µL of elution buffer for preparation of gel extraction, or 30 µL of TE for preparation of Pippin Prep size selection. Library samples were size selected from 200-350 bp using a 2% agarose dye-free automated size selection cassette from Pippin Prep (Sage Sciences, catalogue #CDF2010).

Analysis − Samples were submitted for quality control analysis to the Donnelley Sequencing Center (University of Toronto) or The Center for Applied Genomics (Hospital for Sick Children) for Bioanalyzer analysis and library quantification using KAPA Biosystems. Libraries were sequenced using Illumina HiSeq2500. The flowcells were prepared and processed by the sequencing facility according to the manufacturer's protocol, with 100-bp single-end sequencing for 75 cycles. ChIP-seq and input reads were aligned to hg19 [GRCh37] genome assembly with Burrow-Wheeler Aligner (BWA), using default parameters (Li and Durbin, 2009), and quality control indicators were measured according to the ENCODE Consortium guidelines (Landt et al., 2012). Peaks were called for each sample relative to the WCE input using MACS2 with a cutoff of false-discovery rate ($q \leq 0.05$) (Zhang et al., 2008). ChIP-seq data was submitted to ArrayExpress (accession number: E-MTAB-5148). We also utilized HUVEC H3K4me3 (pooled signal from biological replicates, ENCODE accession numbers: ENCFF000BTS, ENCFF000BTL) and HUVEC H3K4me1 (pooled signal from biological replicates, ENCODE accession numbers: ENCFF000BTD, ENCFF000BSY, ENCFF000BSX) from the Encyclopedia of DNA Elements Consortium (Consortium, 2012). Vertebrate conservation across 100 genomes was extracted from UCSC Genome Browser. Further details regarding quality control of ChIP-seq experiments can be found in the supplementary Materials and Methods (Table S4).

Motif analysis − Motif enrichment analysis was performed using the tool peak-motifs (Thomas-Chollier et al., 2012) from the Regulatory Sequence Analysis tools (Medina-Rivera et al., 2015) with parameters: -markov auto -disco oligos,positions -nmotifs 5 -minol 6 -maxol 7 -no_merge_lengths -2str -origin center . peak-motifs builds motifs using over-represented oligos present in the sequence set given as input, the tool is also able to compare the discovered motifs with any given set of known motifs in order to identify the putative transcription factors that could be binding the sequences, for this task we used the none redundant JASPAR motif collection for vertebrates (Castro-Mondragon et al., 2016; Mathelier et al., 2016). Secondary motif sets were discovered by using a control sequence set as background to eliminate motifs enriched in such control set and increase the signal of other motifs with less representation but that still can have biological and statistical significance. Further details regarding motif analysis of ERG target genes can be found in the supplementary Materials and Methods (Table S3).

CRISPR-mediated *HLX* enhancer deletion:

The gRNAs were ordered as standard DNA oligomers from Integrated DNA Technologies (IDT) (see below for sequences). The oligomers were then annealed, phosphorylated, and cloned into pSpCas9(BB)-2A-GFP (PX458) under control of the U6 promoter (Addgene Plasmid ID 48138). TeloHAECs were transfected with 6 µg of PX458 plasmid containing each 5' and 3' gDNA or two scrambled control gRNAs using Lipofectamine 2000 (2 µL/1 µg DNA, Invitrogen) in 100 mm dishes. After 48 h, TeloHAECs were FACS sorted to isolate GFP^+ cell populations. Following expansion of the GFP^+ cells, they were then seeded as single cell colonies in 96 well plates. Subsequently, the cells were cultured, expanded and genotyped. Genomic DNA was isolated from cell pellets after overnight incubation at 56°C in lysis buffer (100 mM NaCl / 10 mM Tris-HCl (pH 8) / 25 mM EDTA (pH 8) / 0.5% SDS / 0.2 mg/mL ProK) and phenol-chloroform extraction. For genotyping, PCR reactions were performed using Taq Polymerase (Invitrogen) as per manufacturer's instructions. A forward anchor primer was used along with a reverse primer nested either within the deletion region (wild type allele) or 3' of the deletion region (deletion allele). The PCR products were separated by gel electrophoresis and visualized using MiniBIS Pro (DNR Bio-Imaging Systems). Furthermore, the PCR products were gel extracted (QIAquick Gel Extraction Kit) and the deletion was confirmed by DNA sequencing.

Guide sequences for CRISPR experiments:

*HLX*_3'_gRNA_F	5'-CACCGTCCAAGGTTTGCGACGCTCC
*HLX*_3'_gRNA_R	5'-AAACGGAGCGTCGCAAACCTTGGAC
*HLX*_5'_gRNA_F	5'-CACCGATTGCATAAGCCCCTGATTC
*HLX*_5'_gRNA_R	5'-AAACGAATCAGGGGCTTATGCAATC
SCR_1_gRNA_F	5'-CACCGGCACTACCAGAGCTAACTCA
SCR_1_gRNA_R	5'-AAACTGAGTTAGCTCTGGTAGTGCC
SCR_2_gRNA_F	5'-CACCGACAACTTTACCGACCGCGCC
SCR_2_gRNA_R	5'-AAACGGCGCGGTCGGTAAAGTTGTC

Genotyping primers for HLX CRISPR experiments:

*HLX*_Enh_Anchor:	5'-TTGACCTGTGCTCAGTGTGG
*HLX*_Enh_WT:	5'-TATCGCATTGGCTGGGGTTT
*HLX*_Enh_Del:	5'-GAGGGTTCTGGTGAGCCTTC

Table S1: Primers used for murine genotyping

MGI #	Allele	Forward 5'-3'	Reverse 5'-3'	Band Size
NA	*Erg*$^{KO/+}$ WT allele	AATGCTCTGGTAAGGCACACAAGG	AGAGTCTCTGCACACAGAACTTCC	312 bp
NA	*Erg*$^{KO/+}$ lacZ allele	GCTACCATTACCAGTTGGTCTGGTGTC	AGAGTCTCTGCACACAGAACTTCC	644 bp
NA	*Erg*$^{fl/+}$ WT allele	AATGCTCTGGTAAGGCACACAAGG	AGAGTCTCTGCACACAGAACTTCC	312 bp
NA	*Erg*$^{fl/+}$ Flox allele	GAGATGGCGCAACGCAATTAATG	AGAGTCTCTGCACACAGAACTTCC	346 bp
3848982	Cdh5-PAC-CreERT2	TCCTGATGGTGCCTATCCTC	CCTGTTTTGCACGTTCACCG	548 bp

Table S2: Primers used for quantitative PCR: qPCR primers were designed using Primer3 (http://bioinfo.ut.ee/primer3/), selecting for an amplicon length of 80-140 bp, spanning at least one exon-exon boundary with an intervening intron greater than 400 bp to favor mRNA amplification, with an efficiency of greater than 80% as determined by qPCR dissociation (melting curve), with a single amplicon (confirmed by melt curve dissociation).

Gene	Forward Primer	Reverse Primer
Human Genes:		
ADRB2 mRNA	TCA CAC GGG GTA TTT TAG GC	CAA GGG GTT TTG GAG AAA CA
APOLD1 pre-mRNA	GCT GAC CGC GTG TCT ATG T	AGC AGC AGT CCC TGG AAG
CCRL2 mRNA	TGC CAG CTG ATG AGA CAT TC	ACC TCT GCT CCC TGA ACC TT
CHAC1 mRNA	GCA GCG ACA AGA TGC CTG	CTT CAG GGC CTT GCT TAC CT
CHAC1 pre-mRNA	GAC TTC GCC TAC AGC GAC A	AAG ATG GTG AGC ATC CAA CC
DLL4 mRNA	TGC GAG AAG AAA GTG GAC AG	ACA GTA GGT GCC CGT GAA TC
DLL4 pre-mRNA	TGC GAG AAG AAA GTG GAC AG	ACC CTC CCT CAC CAG AAG T
DUSP5 mRNA	ATG GAT CCC TGT GGA AGA CA	TCA CAG TGG ACC AGG ACC TT
DUSP5 pre-mRNA	CCA ACT CAC TCC CAC CAT CT	CGG AAC TGC TTG GTC TTC AT
EGR3 mRNA	AAG AGC AGG GGG TTG TGA AT	CCT CCT GGC AAA CTT CAT CT
EGR3 pre-mRNA	ACT CAC CCC TCC CCT CTC TC	GGA AGG AGC CGG AGT AAG AG
FJX mRNA (single exon)	CTT TCT GGA CAA TGA GGC GG	GCG GCT CGT TAT ACT TGT CC
FUT1 mRNA	TTG GTC TTT GTC TGC AGG TG	ACG GTA CCT GCC AGT TTG TC
HLX mRNA	GCC TGG AGA AAA GGT TTG AG	CTG GAA CCA CAC CTT CAC CT
HLX pre-mRNA	GGC CCT CTT GTC TTT CTT CC	CTT TCC TCT GCA GGT TGG AG
KCNJ2AS1 mRNA	AGC GGG AGG AAG GTC ATA AT	TTG CTG CAC ATC CTT CAG TC
NDRG1 mRNA	CTC GCT GAG GCC TTC AAG TA	GGG TGC CAT CCA GAG AAG T
NDRG1 pre-mRNA	TTG GTC TTG GAT TTG CTT CC	GAG ACA TGT CCC TGC TGT CA
NRARP mRNA (single exon)	CAT TGA AAT GGA GGC ACA GA	ACC CAC ACA CAG CTT CGA TA
PIK3R1 mRNA	TGT CCG GGA GAG CAG TAA AC	AGC CAT AGC CAG TTG CTG TT
PIK3R1 pre-mRNA	CAG ATG GCA AAG TGT GCT GT	GCC CAA TCC TTT CTG ACA CT
SLC25A25 mRNA	CTG TGG CAC CAT GTC CAG TA	GGT CCG CAG GAT ATG TTT GA
SLC25A25 pre-mRNA	AGG GCG AGC TCC AGG TAG	GCA CCC TTC CTC TGG ACA GT
SDF2L1 mRNA	AGG CTC ACG CAT GTG CTT AC	TGT CCA TAG GTC CAG GTC GT
SDF2L1 pre-mRNA	CAT GGT GTC ACC TTT TGC AG	CCA AAG GCA CTC ACC TCC TA
TBP mRNA	TCG GAG AGT TCT GGG ATT GT	CAC GAA GTG CAA TGG TCT TT
ZNF555 mRNA	GCA TCG AAG ATC AAA CCA CA	GAT GTG GAA TCT GGC TGA GG
Zebrafish genes:		
dll4 mRNA	GGA CAA ATG CAC CAG TAT GC	GTT TGC GCA GTC GTT AAT GT
tbp mRNA	TGG GTT TCC CTG CCA AAT TCT T	GGA AAT AAC TCC GGT TCA TAG CTG C
Murine genes:		
Cdh5 mRNA	TCAACGCATCTGTGCCAGAGA	CACGATTTGGTACAAGACAGTG
Erg mRNA	GGAGCTGTGCAAGATGACAA	TCAGATGTGGAAGGGGAGTC
Dll4 mRNA	ACAAGAATAGCGGCAGTGGT	GGATGTTGAGTGAGAAGGTTCC
Hlx mRNA	TTCAGCATCAATTCCAAGACACA	ACCTCTTCTCCAGGCCTTTTCT
Dusp5 mRNA	TGCACCACCCACCTACACTA	AGGACCTTGCCTCCTTCTTC
Ccdn1 mRNA	CCAACAACTTCCTCTCCTGCT	GACTCCAGAAGGGCTTCAATC

Pik3r1 mRNA	CAAAGCGGAGAACCTATTGC	CCGGTGGCAGTCTTGTTAAT
Fjx1 mRNA	CTCTACAGTCGCCACGAACC	AAATGTGTTTGGCGAGGAAG
Egr3 mRNA	GACTCGGTAGCCCATTACAATCAG	GTAGGTCACGGTCTTGTTGCC
Nrarp mRNA	TGGTGAAGCTGTTGGTCAAG	CTTGGCCTTGGTGATGAGAT
Sdf2l1 mRNA	TCATCACCCTCACCGTCTTCCC	AACCTGCACACGCACCACTTC
Rasip1 mRNA	CCGTCCCTACTTCCTTCTGCTC	ACGTGCTGCTCTCGTGTCAT
Cldn5 mRNA	GCAAGGTGTATGAATCTGTGCTGG	GCGCCGGTCAAGGTAACAAAG
Sox18 mRNA	AGGACGAGCGCAAGCGACTG	CGTGTTCAGCTCCTTCCACGCT
Gapdh mRNA	GATGGCAACAATCTCCACTTTGC	GCCGCATCTTCTTGTGCAGT
ChIP primers:		
DLL4 intron 3	GTT TCC TGC GGG TTA TTT TT	CTT TCC AAA GGA GCG GAA T
HLX enhancer	TTG AAA GGG GAA GTG CTA GG	GGC TCA AAC TCG GGA CTA AA

Table S3: Motif analysis of ERG target genes.

Primary motifs were discovered using peak-motifs in ERG peaks overlapping extended (10 kb) transcriptional start sites (TSSs) of the corresponding gene data sets. Motifs were annotated as belonging to a transcription factor (TF) by similarity to Jaspar vertebrates non-redundant annotate motifs.

Secondary motifs were discovered using peak-motifs in the ERG peaks overlapping extended TSSs for the specified data set using a control background corresponding to ERG peak regions overlapping extended TSS for genes with opposite expression pattern. Motifs were annotated as belonging to a TF by similarity to Jaspar vertebrates non-redundant motifs.
** Indicates data sets where the Secondary Motifs cotained one or more TFs not contained in the Primary Motifs set.*

Data Set	Num. Peaks	Primary Motifs
ERG target genes	221	Gabpa, ETV6, ELF5, ETS1, ERG, Gabpa, ELK4, ETS1, NFIC, SPIB, ETV6, NKX6-1, NKX6-2, BSX, Tcfl5, ZBTB33, TEAD4, EBF1, MZF1, ERF, SP1, SP2, KLF5, SPIC, FOSL1, JUNB, FOSL2, OTX2, Pitx1, Arid3b, FOXD2, ELK3
ERG-sensitive VEGF regulated genes	62	ELF5, ETS1, ERG, REL, RELA, Stat4, SP1, KLF5, SP2, EWSR1-FLI1, SPIB, BATF::JUN, MAFG::NFE2L1, Gfi1, Gfi1b, ETV2, POU5F1B, POU3F4, POU2F1, NKX2-8, NKX2-3, FOSL1, FOSL2, JUNB, JDP2, NFE2, HIC2, Hic1, MXF1

Data Set	Num. Peaks	Primary Motifs	Secondary Motifs	
ERG target genes (down-regulated following *ERG* knock-down)	187	Gabpa, ETV6, ELF5, ETS1, ERG, ELK4, NFIC, SPIB, TEAD4, EBF1, MZF1, ERF, SP1, SP2, KLF5	HOXD11 , NFATC2, REST, NHLH1, Ascl2, Myog, ZNF263, Zfx	*
ERG target genes (upregulated following *ERG* knock-down)	34	REL, RELA, SPIB, EWSR1-FLI1, ETV2; SP1, KLF5, SP2, TEAD4, POU3F4, TEAD3, Bcl6; Ahr::Arnt; ETS1, ELF5, ERF, FOSL2, FOSL1, JUNB, EWSR1-FLI1	SP2, LKF5, SP1 ; CREB, ATF7; NRF1 ; NKX6-1, mix-a, Alx4 ; USF2, PKNOX2, FOS::JUN ; Hic1, NFIX, NFIC ; JDP2(var.2), CREB1, ATF7	*
ERG-sensitive VEGF regulated genes (down-regulated following *ERG* knock-down)	50	ELF5, ETS1, ERG, RELA, REL, SPIB, KLF5, SP1, SP2, HOXC10, CDX1, HOXD11, ETV2, Nkx3-1, NKX2-3, NKX3-2, MZF1, JDP2, NFE2	ZBTB7C, ZNF354C, ZBTB7A, Nkx3-1, NKX3-2, NKX2-3, Nr2e1, Nkx3-1, NKX3-2, NKX2-3	*
ERG-sensitive VEGF regulated genes (upregulated following *ERG* knock-down)	12	EWSR1-FLI1, SP2, KLF5, SP1, ELF5, REL, SPIB, HOXA5,NFIC, SP1, Nr2e1	Nr2e1, FOXP3, FOXI1, FOXD, Nr2e1. Atf3	*

Table S4: Summary and quality control analysis of ChIP-seq experiments. A) Sequencing statistics and QC measurements for individual libraries. ENCODE practice (see *Landt et al.,* 2012 and https://genome.ucsc.edu/ENCODE/protocols/dataStandards/ChIP_DNase_FAIRE_DNAme_v2_2011.pdf for a detailed explanation of QC). Normalized strand-cross correlation (NSC) coefficient and the relative strand cross correlation (RSC) are a peak-calling independent means to assess data quality and NSC values above 1.05 and RSC above 0.8 indicate high quality transcription factor ChIP-seq data. PCR bottleneck coefficient (PBC) indicates PCR based artifacts. We observe a low PBC score in the WL375 library however this library is comparable to the better replicate WL379 and so we retained both experiments for analysis. B) Irreproducible discovery rate (IDR) analysis of ChIP-seq replicates. As a rule of thumb, ENCODE recommends that reproducible replicates possess Nt/Np and N1/N2 ratios within a factor of 2 ($0.5 \leq N \leq 2$). C) Summary of number peaks and conserved peaks in HUVEC and BAEC experiments.

A)

	Species	Factor	# Raw Reads	# Mapped Reads	# Unique Mapped Reads	% Unique Mapped	PBC	NRF	NSC	RSC	# Peaks
WL375	hsap	Erg	17155982	14662609	13425327	78	0.3	0.37	2.13	5.16	15104
WL379	hsap	Erg	21892688	19915133	18082059	83	0.93	0.92	1.14	1.11	22194
WL373	hsap	H3K27Ac	22680375	18749798	17255873	76	0.83	0.83	1.22	1.22	39183
WL377	hsap	H3K27Ac	29876586	28064462	26908616	90	0.82	0.8	2.25	1.15	54830
WL376	hsap	Input	27173361	20910309	19096572	70	0.93	0.93	1.03	0.74	NA
WL380	hsap	Input	22088949	20466251	18560981	84	0.97	0.96	1.03	0.58	NA
WL383	btau	Erg	27258172	25175440	17419369	64	0.95	0.94	1.05	0.21	24531
WL387	btau	Erg	26755810	25276242	18369459	69	0.92	0.92	1.16	0.80	23327
WL384	btau	Input	26864153	25540351	18730958	70	0.95	0.94	1.02	0.23	NA
WL388	btau	Input	21820116	20694192	16120267	74	0.81	0.81	1.02	0.17	NA

B)

Species	ChIP	Rep 1	Rep 2	Nt	Np	Nt / Np	N1	N2	N1 / N2	# Peaks from Merged Reps
btau	Erg	WL383	WL387	22169	17550	1.26	20965	25724	0.82	35183
hsap	Erg	WL375	WL379	7773	13275	0.59	27176	13711	1.98	31175
hsap	H3K27Ac	WL373	WL377	26102	37690	0.69	22333	44855	0.50	60555

C)

Species	Factor	Rep 1	Rep2	# Peaks	Conserved Peaks
btau	Erg	WL383	WL387	34773	8464
hsap	Erg	WL375	WL379	31175	8337

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Supplemental Figures

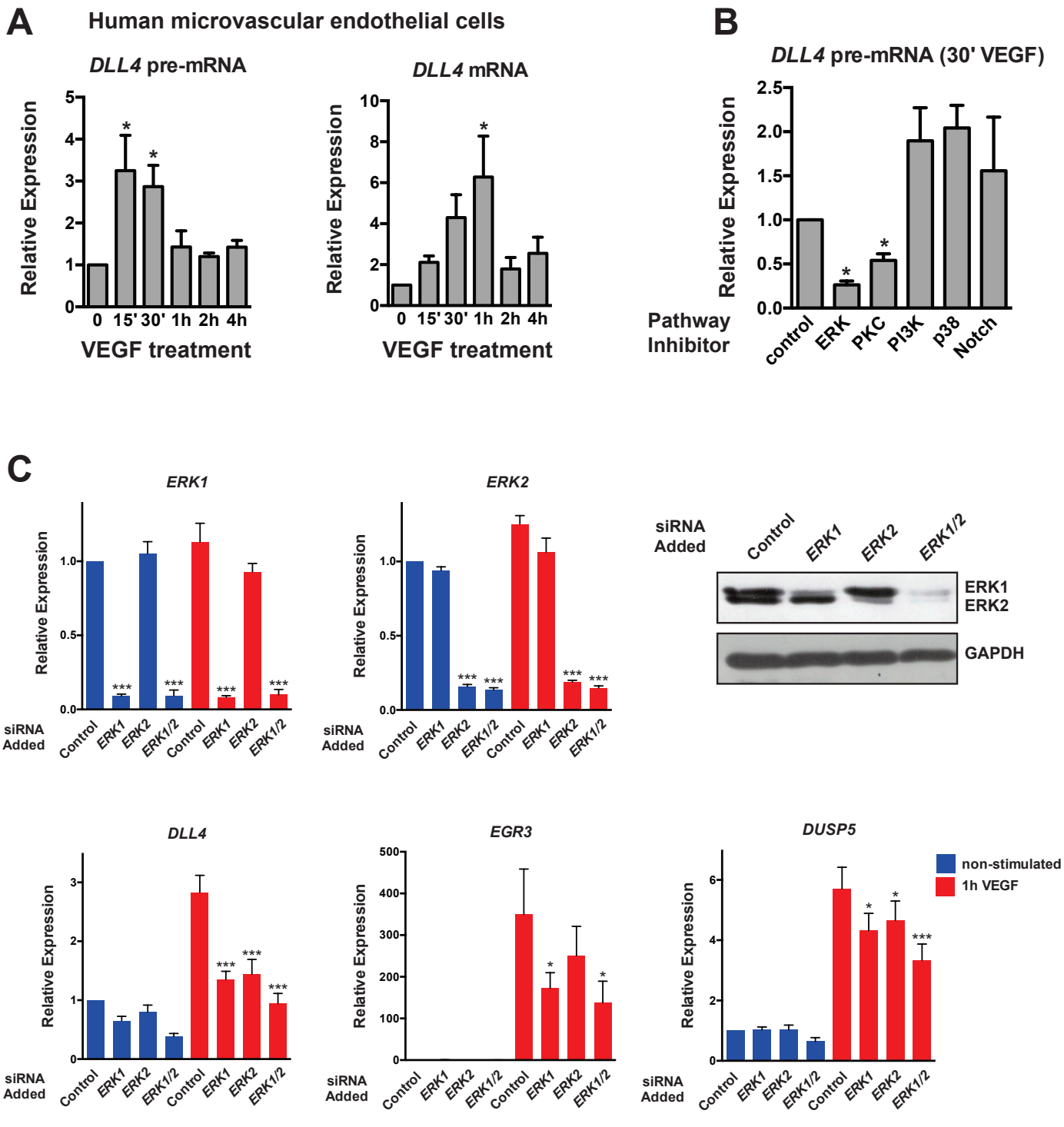
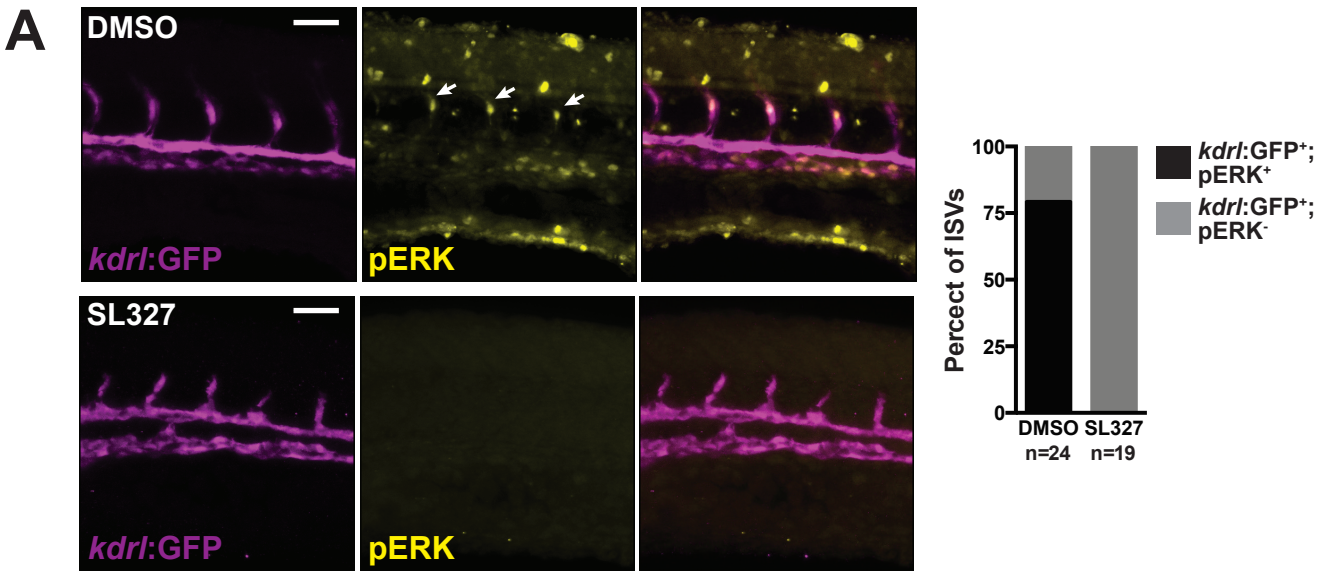


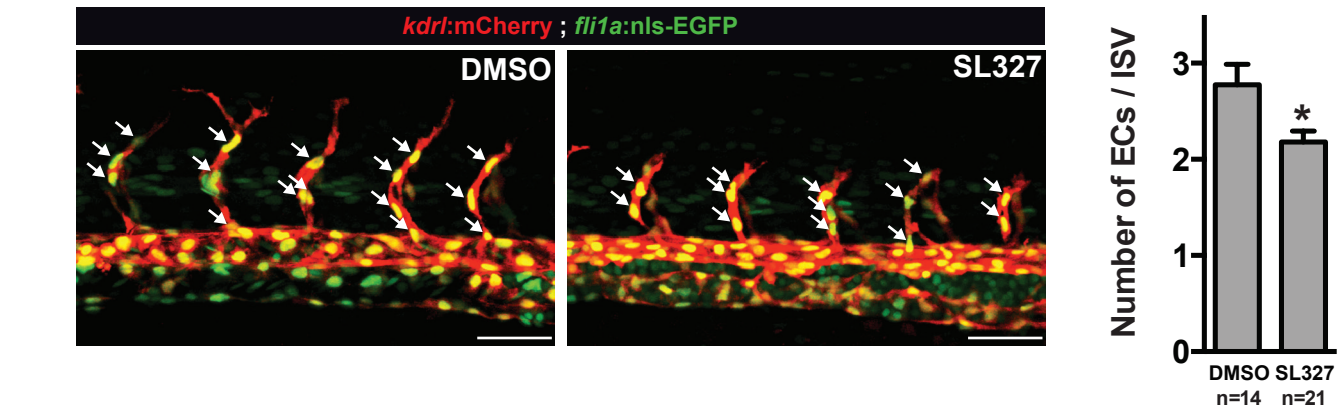
Figure S1: Transcriptional induction of *DLL4* is dynamic and requires MAPK/ERK signaling

A) Induction kinetics of *DLL4* pre-mRNA and mRNA in dermal microvascular ECs as assessed by qRT-PCR (n=3).

B) MAPK-dependence (MEK and PKC inhibitors) of *DLL4* induction in dermal microvascular ECs assessed through the use of small molecule inhibitors of these pathways (n=3).

C) *ERK1* and *ERK2* were knocked down in HUVEC using siRNA. qRT-PCR reveals that the induction of *DLL4, EGR3* and *DUSP5* is ERK1/2-dependent. N = 4-5. A representative western blot (of 3) reveals the extent of ERK1/2 knockdown. GAPDH is shown as a loading control.





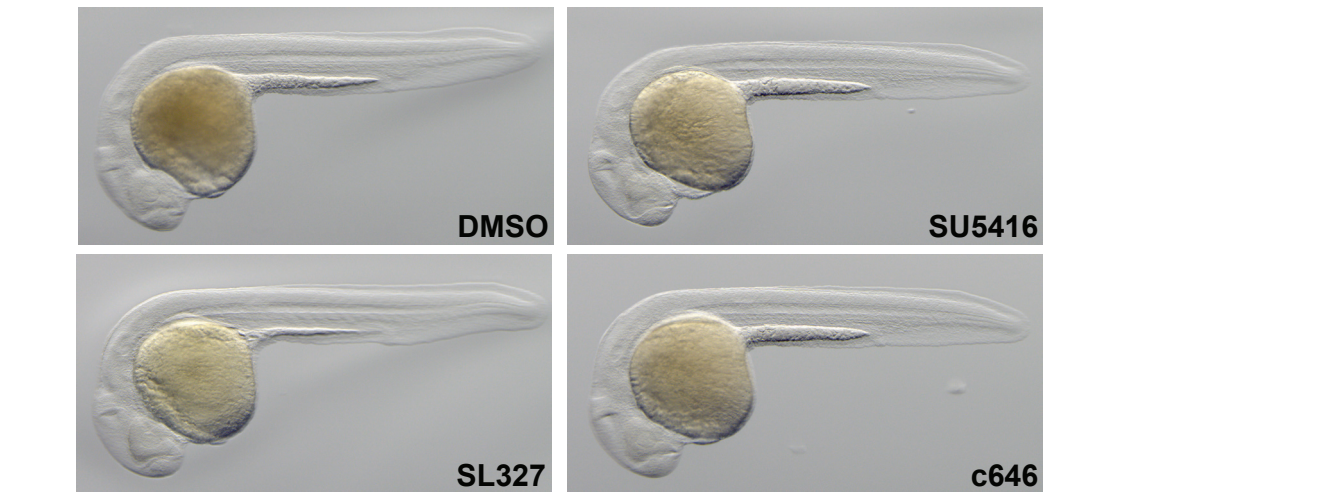


Figure S2: Characterization of sprouting defects in embryos treated with MEK inhibitor (SL327)

A) pERK is enriched in tip cells during ISV formation in zebrafish and this is dependent on MEK signaling as it is negated in MEK-inhibitor (SL327) treated embryos (treated from 18-20 hpf to ~24 hpf). Quantification of the number of ISVs that stained positive for pERK is indicated. 24 hpf. Scale bar = 50 μm.

B) The number of endothelial cells per intersomitic vessel was quantified in *Tg(kdrl*:mCherry;*fli1a*:nls-EGFP) embryos exposed to DMSO or SL327 (30 μM). The number of mCherry/EGFP double-positive cells was counted per intersomitic vessel and averaged per embryo. The number of embryos quantified is indicated.

C) Transmitted light images of DMSO, SL327 (30 μM), SU5416 (5 μM) and c646 (3 μM) treated embryos revealing normal overall development and lack of tissue necrosis or developmental delay.

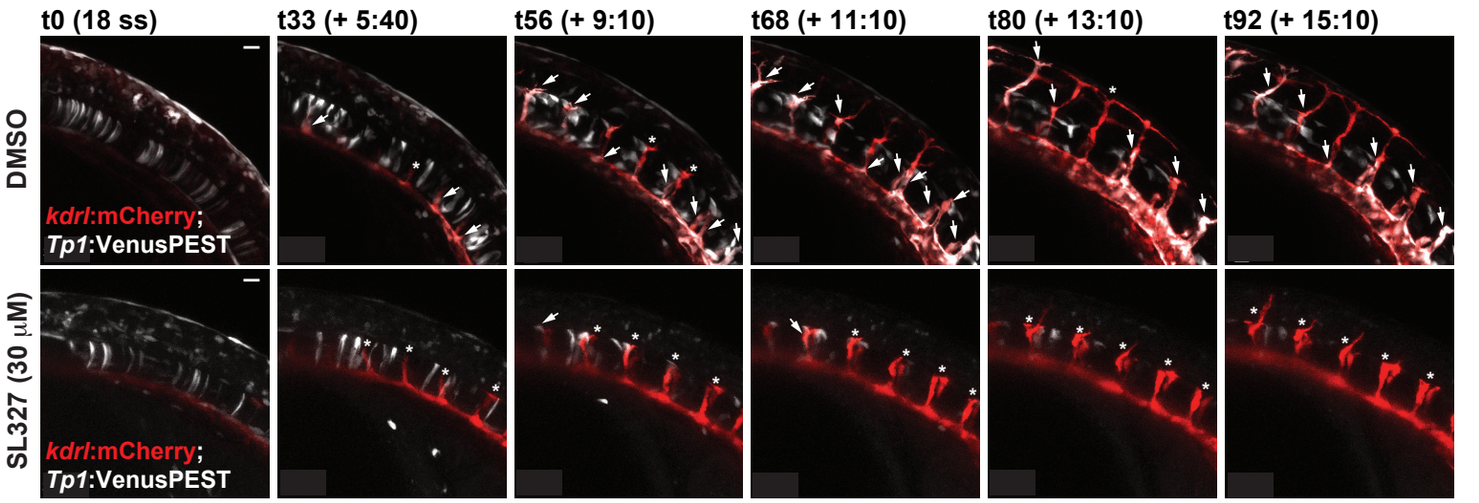
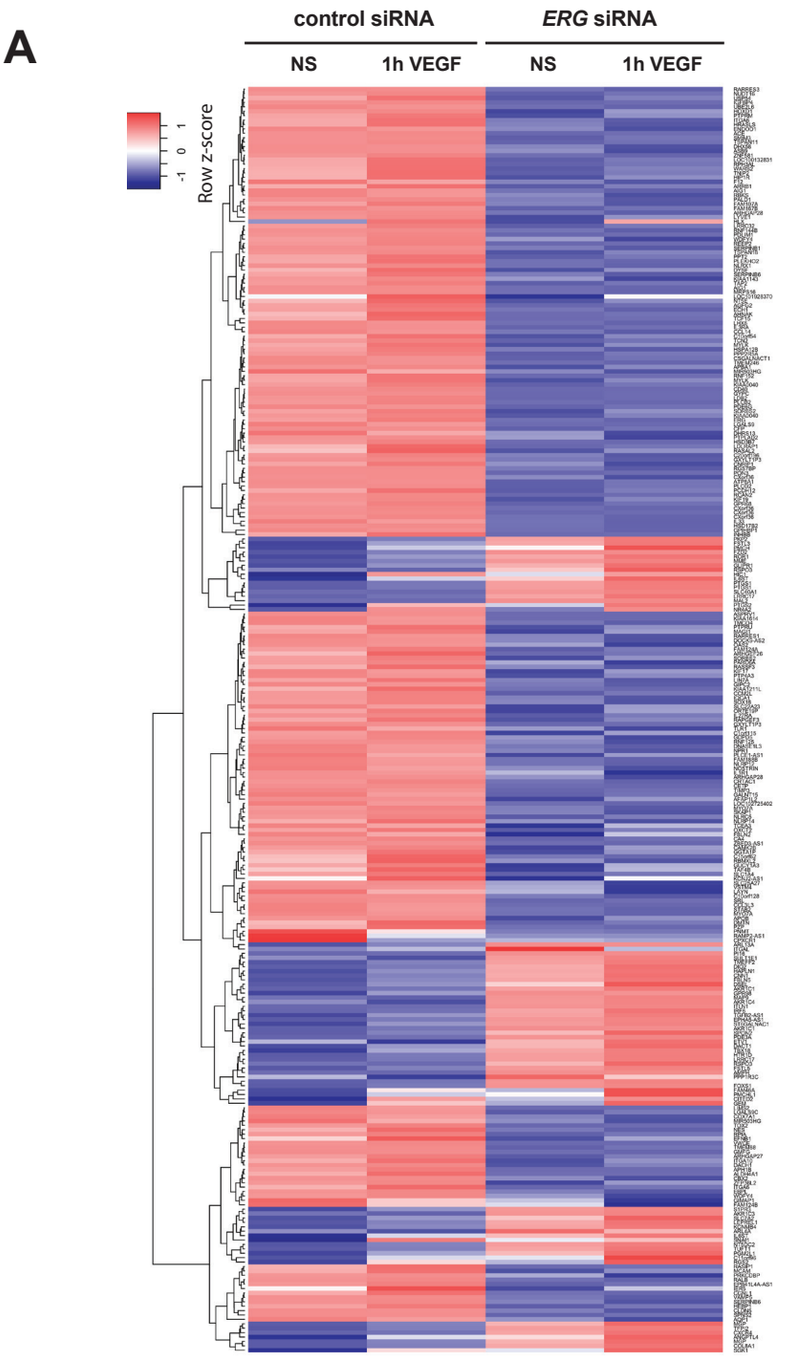


Figure S3: Notch induction in the vasculature is compromised in embryos treated with MEK inhibitor (SL327)

Notch activity is reduced in the vasculature of SL327-treated *Tg*(*kdrl*:mCherry); *Tg*(*Tp1bglob*:Venus-PEST) embryos. Still images from time-lapse microscopy of a representative experiment are shown. Arrows indicate Notch signaling-positive ISVs, while asterisks indicate Notch-negative ISVs. Scale bar = 20 µm. See Videos S1 and S2.



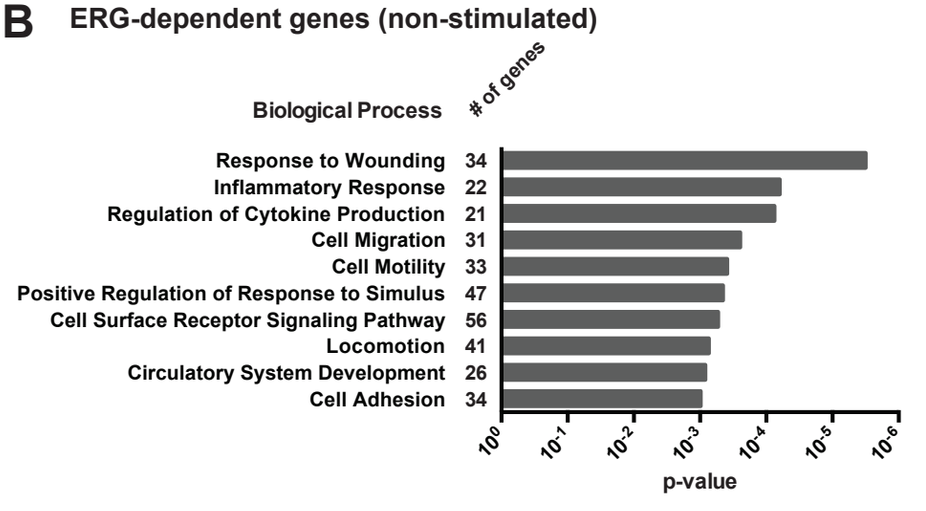
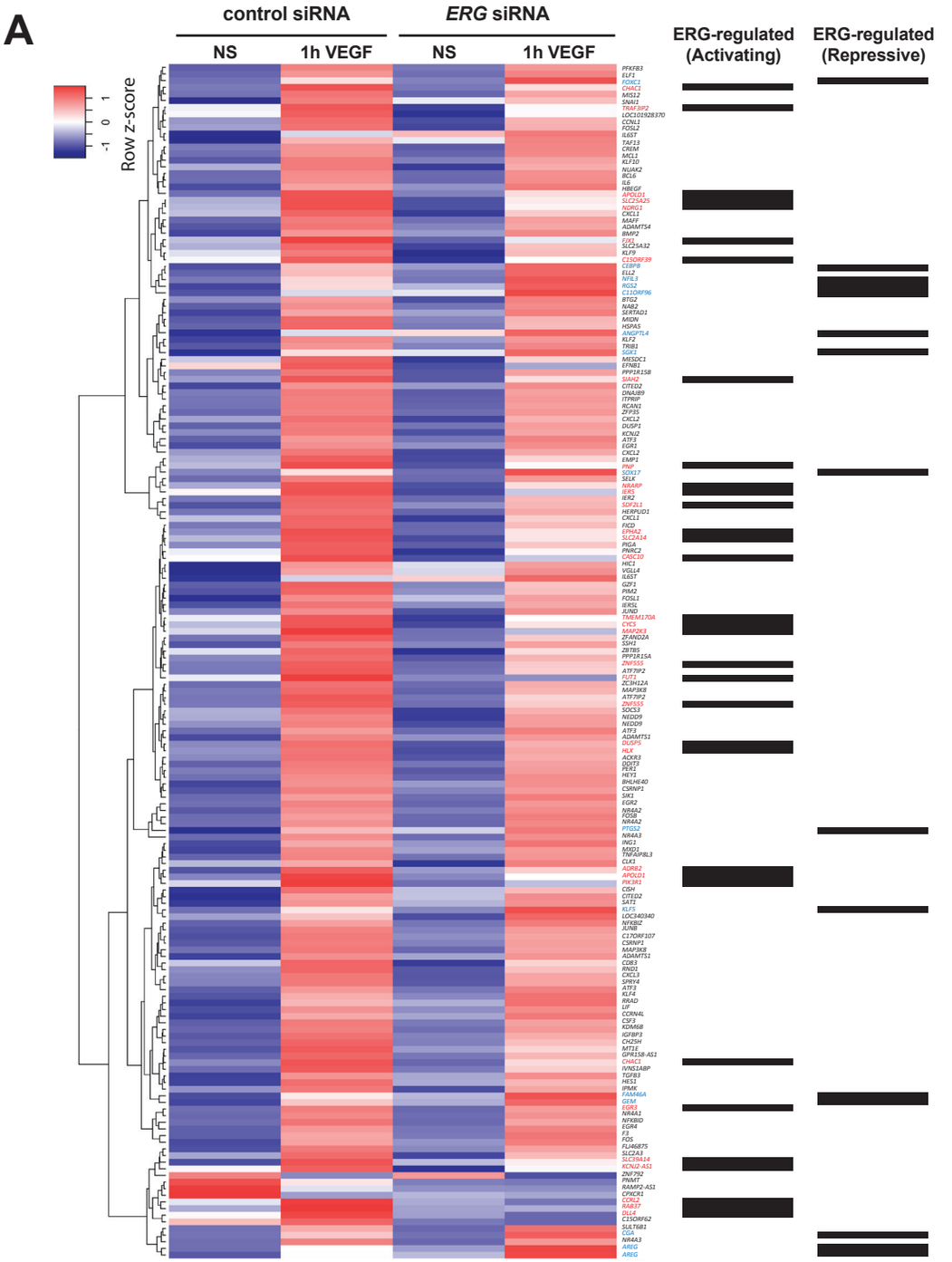


Figure S4: A network of genes are regulated by ERG in endothelial cells
A) Heat map depicting hierarchical clustering of significantly regulated genes in control- and *ERG* siRNA-transfected cells +/- 1h VEGF treatment. NS = not stimulated. Shown are genes that are differentially expressed between 'Control siRNA-NS' and '*ERG* siRNA-NS'.
B) Gene Ontology analysis of dysregulated genes ('Control siRNA-NS' vs. '*ERG* siRNA-NS'). Selected, representative GO terms are displayed with their associated p-value. The number of genes in each GO category is indicated. The GO terms depicted are: Response to wounding (GO:0009611), Inflammatory response (GO:0006954), Regulation of cytokine production (GO:0001817), Cell migration (GO:0016477), Cell motility (GO:0048870), Positive regulation of response to stimulus (GO:0048584), Cell surface receptor signaling pathway (GO:0007166), Locomotion (GO:0040011), Circulatory system development (GO:0072359), Cell adhesion (GO:0007155).



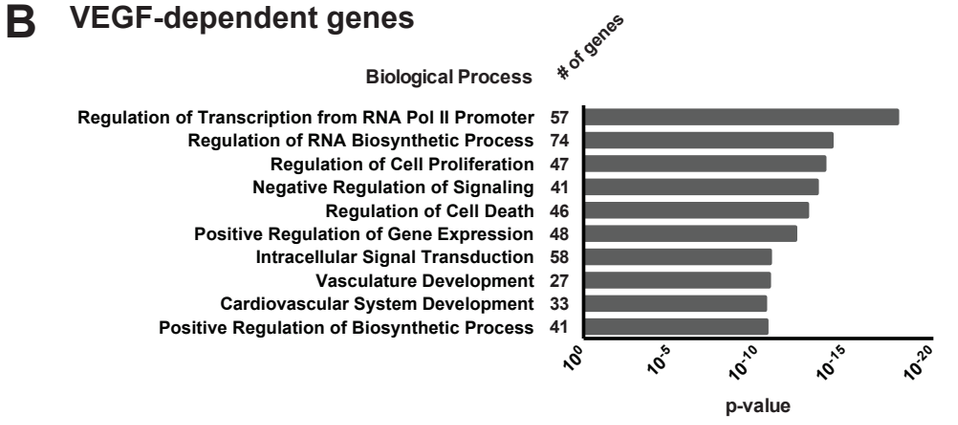


Figure S5: A network of genes are regulated by VEGF and ERG in endothelial cells
A) Heat map of genes that are differentially expressed between 'Control siRNA-NS' and 'Control siRNA-1h VEGF'. The VEGF-inducible genes that are dysregulated upon *ERG* knock-down are highlighted in red (activated) or blue (repressed), and indicated to the right.

B) Gene Ontology analysis of VEGF inducible genes ('Control siRNA-NS' vs. 'Control siRNA-1h VEGF'). Representative GO terms are indicated: Regulation of transcription from RNA Polymerase II promoter (GO:0006357), Regulation of RNA biosynthetic processes (GO:2001141), Regulation of cell proliferation (GO:0042127), Negative regulation of signaling (GO:0023057), Regulation of cell death (GO:0010941), Positive regulation of gene expression (GO:0010628), Intracellular signal transduction (GO:0035556), Vasculature development (GO:0001944), Cardiovascular system development (GO:0072358), Positive regulation of biosynthetic process (GO:0009891).

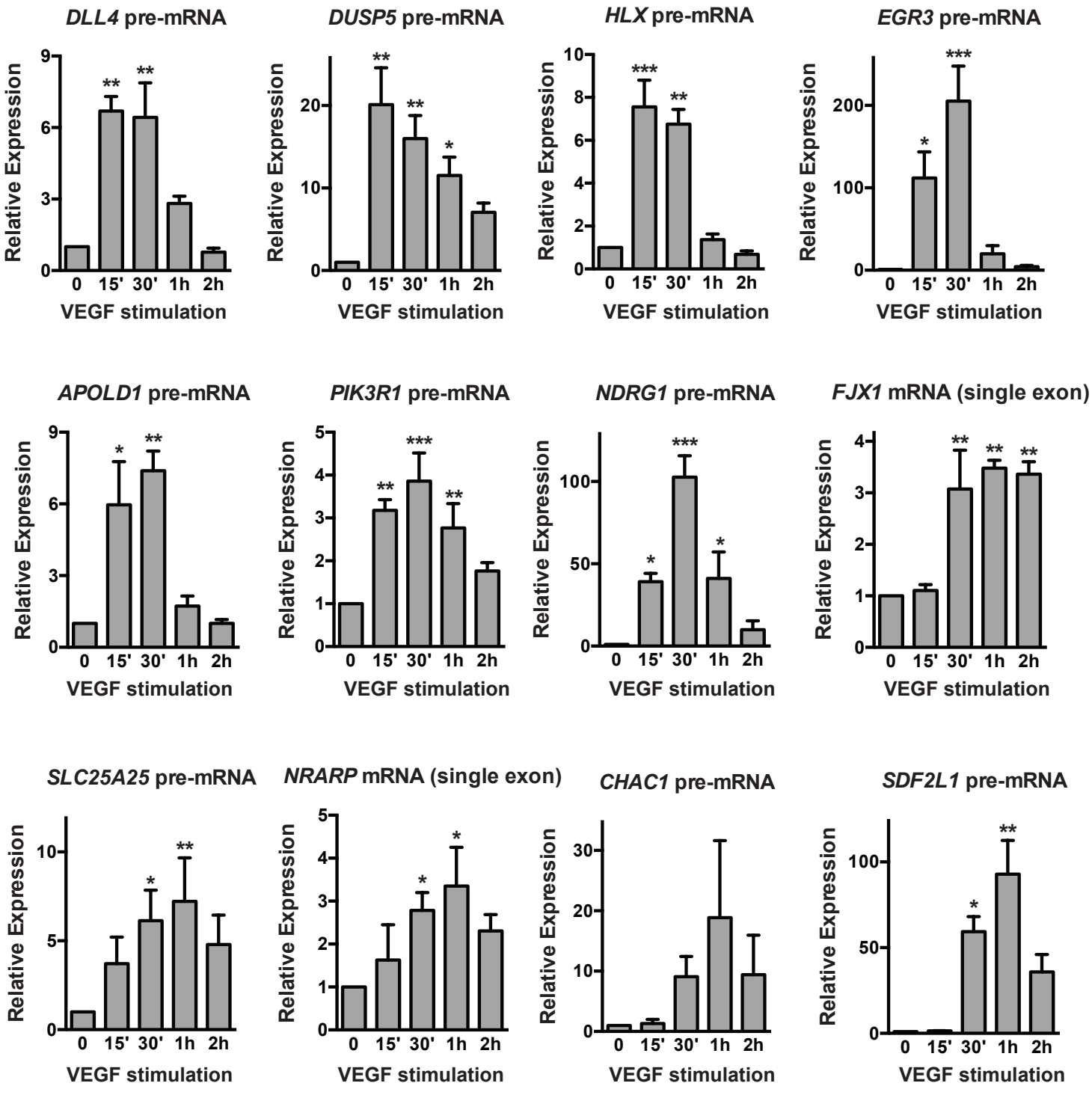
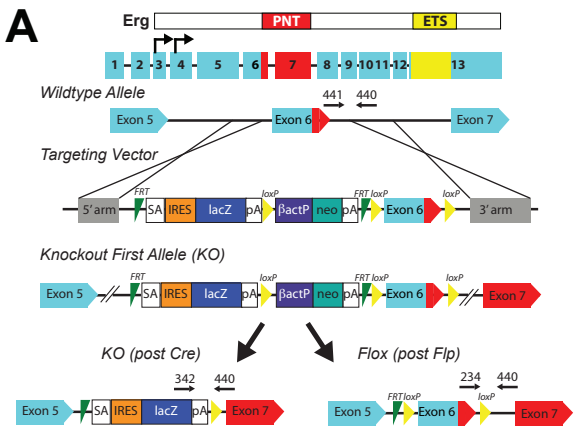
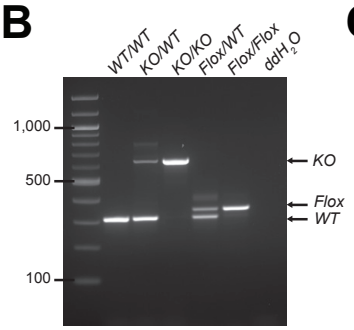


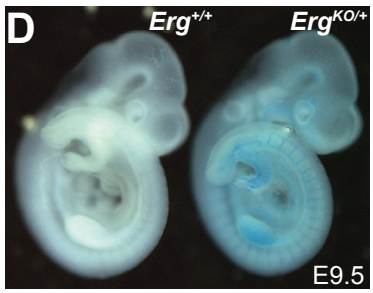
Figure S6: ERG-/VEGF-regulated genes are dynamically expressed
Kinetics of a subset of ERG-/VEGF-regulated genes, as measured by qRT-PCR of pre-mRNA in HUVEC (n=3). Transcriptional induction of these genes is dynamic, with peak transcription between 15'-1h. Note that some of this data is also found in Fig. 1A,E, 7A, but is included here to facilitate direct comparison.

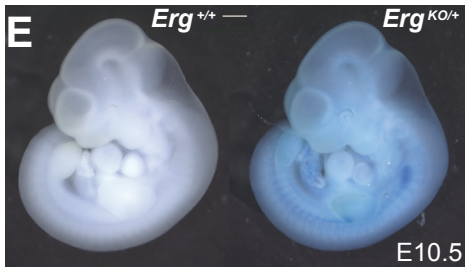


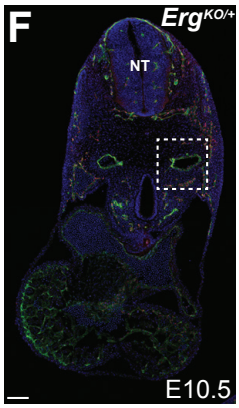


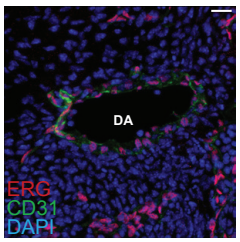
C

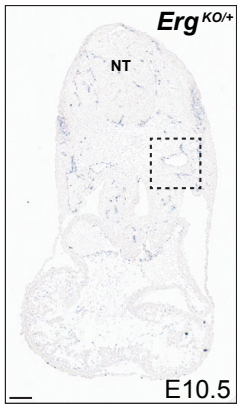
Adult (male and female)				
genotype	$Erg^{+/+}$	$Erg^{KO/+}$	$Erg^{KO/KO}$	total
predicted	25%	50%	25%	100%
observed	40%	60%	0%	100%
n	50	74	0	124
E11.5				
predicted	25%	50%	25%	100%
observed	31%	44%	14%	100%
n	6	12	3	21
E10.5				
predicted	25%	50%	25%	100%
observed	26%	44.5%	29.5%	100%
n	57	98	65	220
E10.5				
phenotype	$Erg^{+/+}$	$Erg^{KO/+}$	$Erg^{KO/KO}$	
hemorrhage	0% (0)	3% (3)	43% (28)	
small size	7% (4)	7% (7)	86% (56)	
total	57	98	65	220

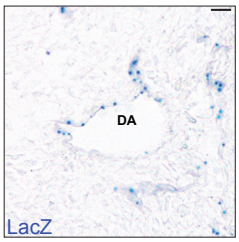


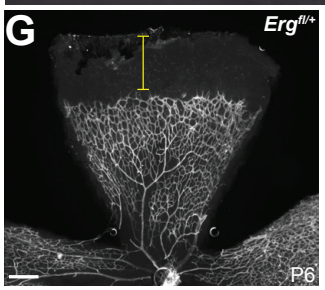


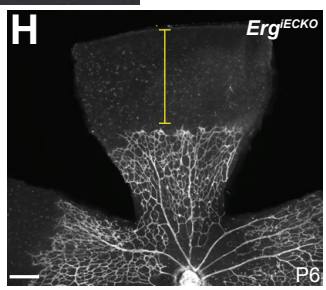


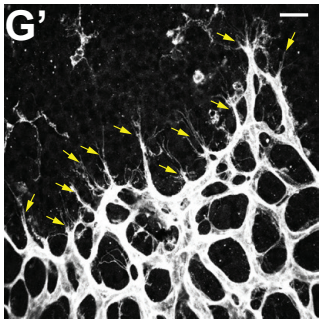


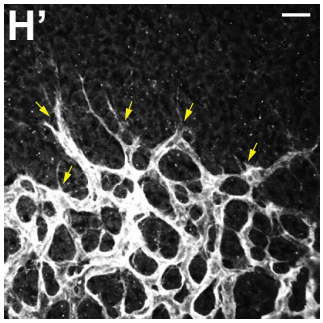


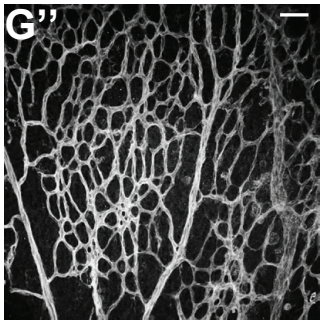


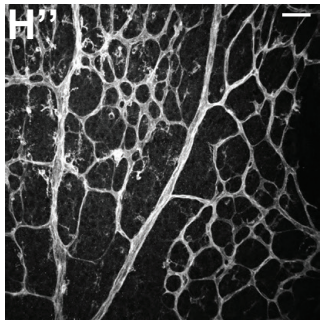


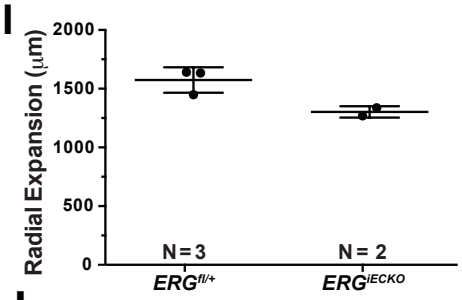


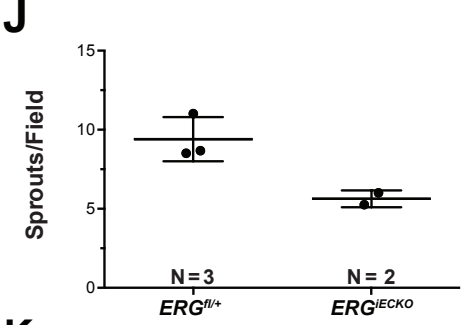












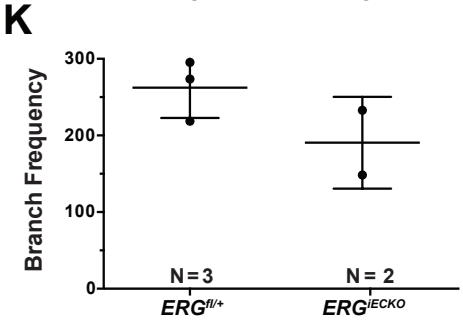


Figure S7: Deletion of *Erg* in mouse embryos results in embryonic lethality, accompanied by defects in angiogenesis, vascular remodeling and vascular integrity

A) Strategy for the generation of an *Erg* knock-out/LacZ knock-in first, conditional ready allele (also known as Erg^{tm1a}). Homologous recombination was used to insert an IRES-LacZ / β-Actin::Neo cassette into intron 5 of *Erg.* Following Cre-mediated recombination, the FRT-flanked β-actin::NEO selection cassette is deleted, leaving an ERG-ires-LacZ gene-trap (also known as Erg^{KO} or Erg^{tm1c}). Following Flp-mediated recombination of Erg^{tm1a}, loxP sites are left flanking exon 6, generating a conditional loss of function allele (also known as Erg^{flox} or Erg^{tm1b}). Genotyping primers are indicated. PNT=pointed domain; ETS=ETS DNA binding domain.

B) Agarose gel electrophoresis of PCR genotyping products to identify *Erg* mutants. 100 bp ladder on the far left lane.

C) Expected and observed ratios of genotypes from the progeny of $Erg^{+/-}$ crosses. Deletion of *Erg* results in embryonic lethality ~E11.5, and increased presence of hemorrhage and smaller embryo size. No defects were observed in heterozygous mutant embryos.

D) Phase images of wholemount, E9.5 $Erg^{+/+}$ and $Erg^{KO/+}$ embryos following lacZ staining.

E) Phase images of wholemount, E10.5 $Erg^{+/+}$ and $Erg^{KO/+}$ embryos following lacZ staining. Scale bar = 500 μm.

F) Immunohistochemistry for ERG (red), CD31 (green), and DAPI (blue) in the left panel, and LacZ staining in the right panel, from serial sections of an $Erg^{KO/+}$ E10.5 embryo, show overlap of Erg and gene-trap *Erg-ires-LacZ* activity. Scale bar = 100 μm. Lower panels are magnified views of the boxed area in the upper panels, scale bar = 20 μm. NT, neural tube; DA, dorsal aorta.

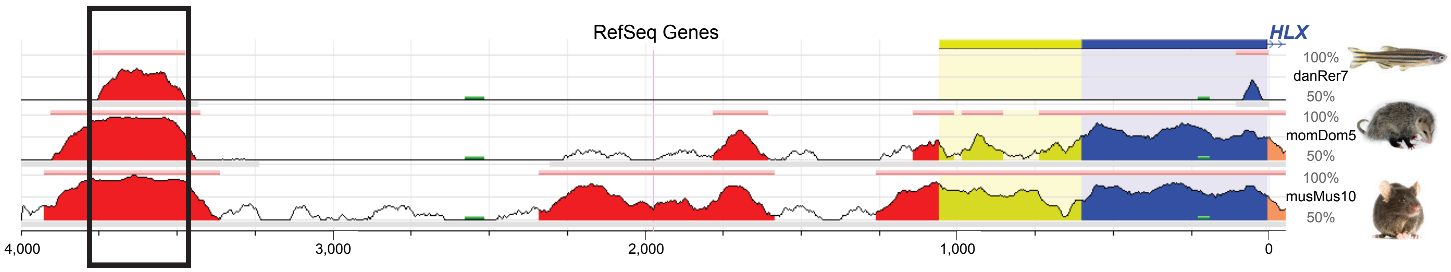
G, H) Representative images of the total retinal vasculature (G, H) and magnified view of the distal, vascular front (G', H') and proximal region (G'', H'') stained with IB4 in $Erg^{fl/+}$ (G, G', G'') and Erg^{iECKO} (H, H', H'') retinas at P6 following tamoxifen administration at P1. Scale bar = 100 μm. The bar in (G, H) indicates avascular area in each retina. Arrows in (G', H') indicate sprouting vessels at the vascular front. Scale bar = 100 μm.

I) Quantification of radial expansion of the $IB4^+$ vasculature within the P6 retina.

J) Vascular sprouting as determined by quantification of $IB4^+$ sprouts in the distal retinal vascular plexus at P6.

K) Vascular density as determined by quantification of $IB4^+$ branches in the proximal retinal vascular plexus at P6.

A



B

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                                                                         ETS-D
hg19     TAGGGTCTCC-GC----TGCGTAGACC-AAGC-----TCAAGTTAATTCGGT---TTAGCCAGGCCCTTTTCTTTCCTGTTAA-------ATTCTCA-AC
mm10     TAGGCACAGCAGCAACTTGCAAAGTTTTAGCCACTTTTAAGG-ATGCCAGTGTGTGAACGAAGTTCAAGTAATTTGATTTAATCGGGCCCTTTTCTTTC
danRer7  T--G-TTTTC--------------------GT--CTTT---------CCAG---------CACGCTCTA---TTT-------------------T-----
         *  *     *                    *      *          * *          * *  *      **                   *

                                                                                   HLX-3b FWD
         CCCCATGGCTACCAG-CCCCTGGGGAGCCCTCGCCTCTACCCCCAGGACCTCCCTGCCCG----------GC-TCAGTAGCTCTGGGCCAGTTCATTTCG
         CCTGGTGGTTACAAGGTCCCTTGGGAGTCCACGCCTCTGCCCCCAGGGCCTCCCTGACTAGGTGCACAACGCAACAACAACTCTGGGCTAGTTCATTTCA
         -----TGGC-ACA-GC--AATGTAAAGTT--------------------------GTC-ATGTGCATATGGA-TGA-------------AG----TATC-
              ***  **  *     *    **                            * *            *    *             **    * **

                      ETS-E                                     mef2-a                ETS-G   ETS-H
         CCACAGCCCGGAGCCTCTCGGAAAGCAAAGATTAAAGGGGAAAGTCGCAGCTGTATATTTATATTTTCATTGCTAGAAGGGAATTGATTTCCGTGCATTT
         CCACAGCCCGGGGACGCTCGGAAAGCAAAGATTAAAGGCGAAAGTCGCAGCTGTGTATTTATATTTTCATTGCTAGAAGGGAATTGATTTCCGTGCATTT
         -----------------------------------------------GCCTCAGTAGT-TAATTGTGTTTTCGTTTC-AGCGGAGAATAGATTTCC-TCTGTTT
                                                    * * ***  ** ** ** * ***** ** * **  * **** ******* *   ***

                                     ets                                 gata          srf       ETS-I
         ATTTTGGTAGTTGTAATACATAAGGGCGGATTTGCGTCACCCGAGCAACTTGCCGGTGGAGATAAAGTTGCACAAATATTGAAAGG-GGAAGTGCTAGGA
         ATTTTGGAAGTTGTAATACAGAAGAGCGGATTTGCGTCACCACAGCAACTTGCCGGTGGAGATAAAGTTGCACAAATATTGAAAGG-GGAAGTGCTAGGA
         ATT---------ATAA-GCATAAGGACGGATTTGCGTCACC-GTGCGACTTGCGAGTTGAGATAAAGTTGCACAAATATTGAAAAAGGAAGTGCTAACA
         ***          ***  ** ***  ***************   ** ******  ** **************************   **********  *

                                  ETS-J     ets                    mef2-b               mef2-c
         GTCATTATAGAGTTTTT---CT-----CCGGAAGAAATAAGGAT-TTCTGCAGTATCCTAAAATACTAAGGCCGCTTCTATTTGAGACC-AATCTCGCA
         GTCATTATAGAGTTTTT---CT-----CCGGAAGAAATAAGGAT-TTCTGCAGTATCCTAAAATACTAGGACCGCTTCTATTTGAGACC-AATCTCGCA
         GTCATTATAAAGTTCCACCTCTGTGAGTCGGAGGAAATAAGGGTGCTCTGCTGTATCCTAAAATACTAAAGCATCTTCTATTTTGGCCTAAATCTTGCA
         ********* ****      **      **** ********* *  ***** ****************   *  **********  * *  ***** ***

              ELK1                                                                    ETS-k         sox
         GGCACATCCGCTCATTTAGTCCCGAGTTTGAGCCCATCAAAAAA-CAGGAGATGACCTGAACTCCG-GCGAGCCCAGGGTTTCCTGCTGCTTTCTTGGTT
         GGCATATCCGCTCATTTAG-CCCAAGTTTGAGCCCATCAAAAAAACAGGAGATGACCTGAACTCCGGGCGAGCCCCGGGTTTCCTGCTGCTTTCTTGGTT
         GGAATATCCGCTCATTTAG-CCTGAATCAGAGCTCATCAAAAA--CAGG--ATGACCTG--CATCGA--GAAACTCCTGTTTCCTAATT-----------
         ** * ************** **  * *  **** *********  ****  ********  *  **   **       *******  **

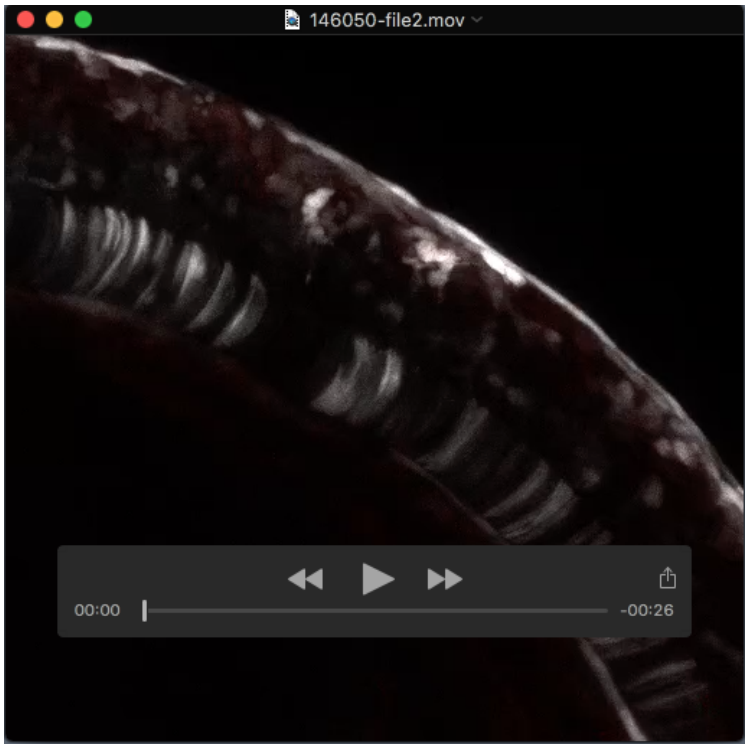
                             HLX-3b REV                                                       ETS-L
         CTGAAGAGTGGGGAGTAAGGAGGGCGGGAGTCT-GCGGGCTCAGAACTCGGCGAGGGGCCTGCAGGGGCCAGGCTTGGGCCTGGGG-AAGGGGTAGAGGG
         CTGAAGGGTAGGG-GTAAGGAGGAGGGCAGTGTTCCAAGCTCCCGCCAAAGCGACTGGTCTACAGGGCTTGGGCCTGGAGCAAGAGAGAGTGACCCAAAG
         CCACAGAGCAGAA----AACAC------A--CACACATACACAC---------------ACACACGAGTCG----------------GAGTGTTAGC---
         *   ** *  *      *  *       *      *   * *                   ** *                      ** *

         GGC--------------------G---G--CG----GG--G----G-TC--GCTC-CAA---------------A--GACTT
         TCCTTCCCCTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCCTCTCCTCTCCTCTCCTCTCC
         ----------------ATTTG--A---T--TG----TG--G----A-TC--ATTT-TTT------------------AATCA
                                                        *     *
```

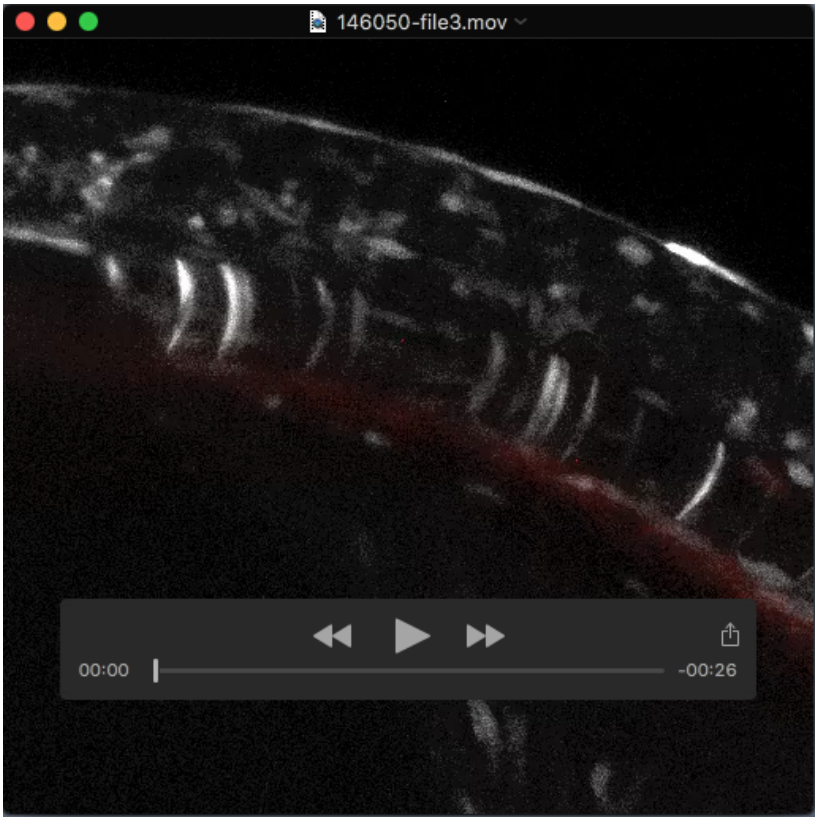
Figure S8: Comparison of conservation between an upstream human and zebrafish *HLX* enhancer

A) Shown are browser screenshots comparing evolutionary conserved regions (ECR) across multiple, distantly-related vertebrates, with the zebrafish, possum, and mouse genomes aligned to the human genome (chr1:221049800-221053860). Peaks indicate sequence conservation. Red denotes intergenic sequence, yellow indicates the 5' UTR, blue denotes exons, and salmon shows intronic regions. The x-axis denotes position in the base genome and the y-axis represents the percent identity between the base and aligned genomes. The region analyzed in (B) is underlined.

B) ClustalW alignment of a portion of the *HLX-3a* (and *HLX-3b*) enhancer regions from human (hg19), mouse (mm10), and zebrafish (danRer7). Asterisks below the nucleotides indicate sequence conservation across all three species. The human *HLX-3a* region contains 26 ETS consensus sequences (GGA(A/T)) (not shown). The sites are numbered relative to their positive within *HLX-3a*. As H3K27ac flanked *HLX-3a* region did not direct expression in the endothelium (see main text and methods), we focused on the smaller, conserved, region identified by ERG ChIP-seq, *HLX-3b* (denoted by primers). *HLX-3b* contained 6 deeply conserved ETS sites (ETS-H, ets, ETS-I, ETS-J, ELK1, and ETS-K), as well as 3 deeply conserved MEF2 sites, an SRF site, and a GATA site. All ETS sites tested by mutation analysis are indicated in blue and capital letters, while lowercase and gray coloring indicates sites that were not experimentally validated. The human *HLX-3b* enhancer contained 9 total ETS sites.



Supplementary Movie 1. Notch signaling is active in the developing vasculature. Representative time-lapse confocal microscopy of a *Tg(kdrl:mCherry)*; *Tg(Tp1:VenusPEST)* embryo treated with DMSO demonstrates Notch activity within the axial and sprouting vasculature (n=6). Embryos were mounted in E3 with tricaine. Treatment began at 18 ss, and continued, at RT, for 15 hours and 10 minutes. See Fig. S3 for still images.



Supplementary Movie 2. Notch signaling is lost in the vasculature upon MAPK inhibition. Representative time-lapse confocal microscopy of a *Tg(kdrl:mCherry)*; *Tg(Tp1:VenusPEST)* embryo treated with SL327 (30 uM) demonstrates reduced Notch activity within the vasculature, and decreased sprout elongation (n=6). Treatment began at 18 ss, and continued, at RT, for 15 hours and 10 minutes. See Fig. S3 for still images.