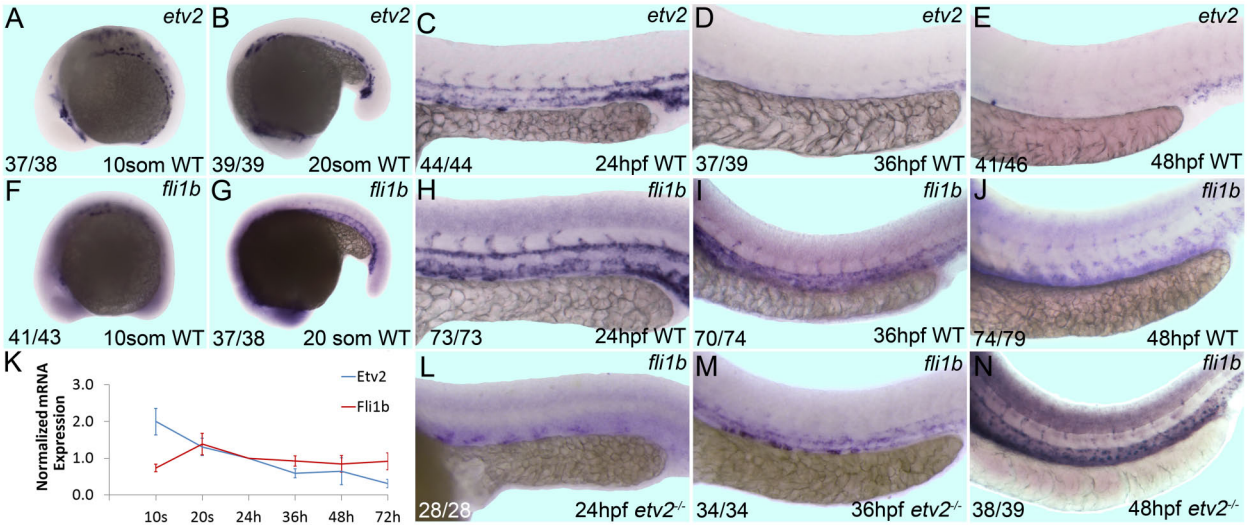
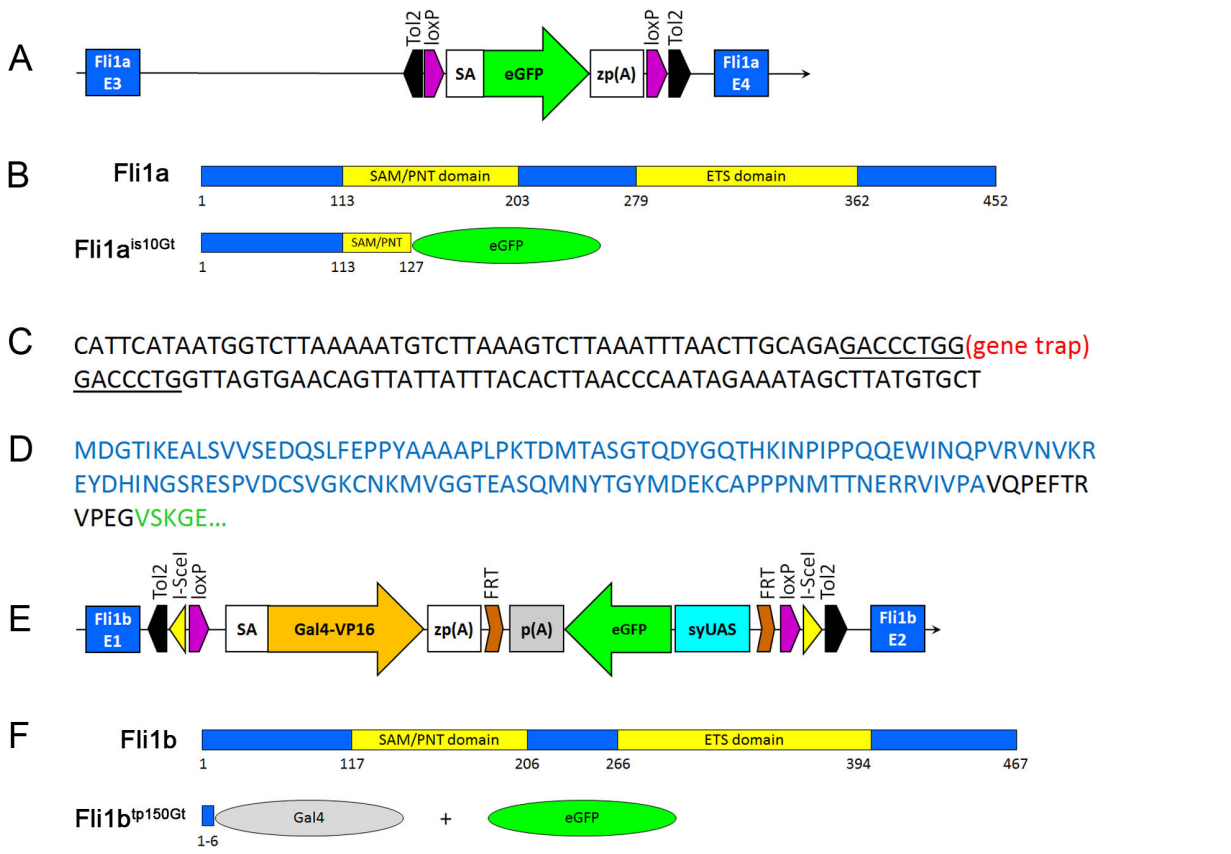
SUPPLEMENTAL MATERIAL

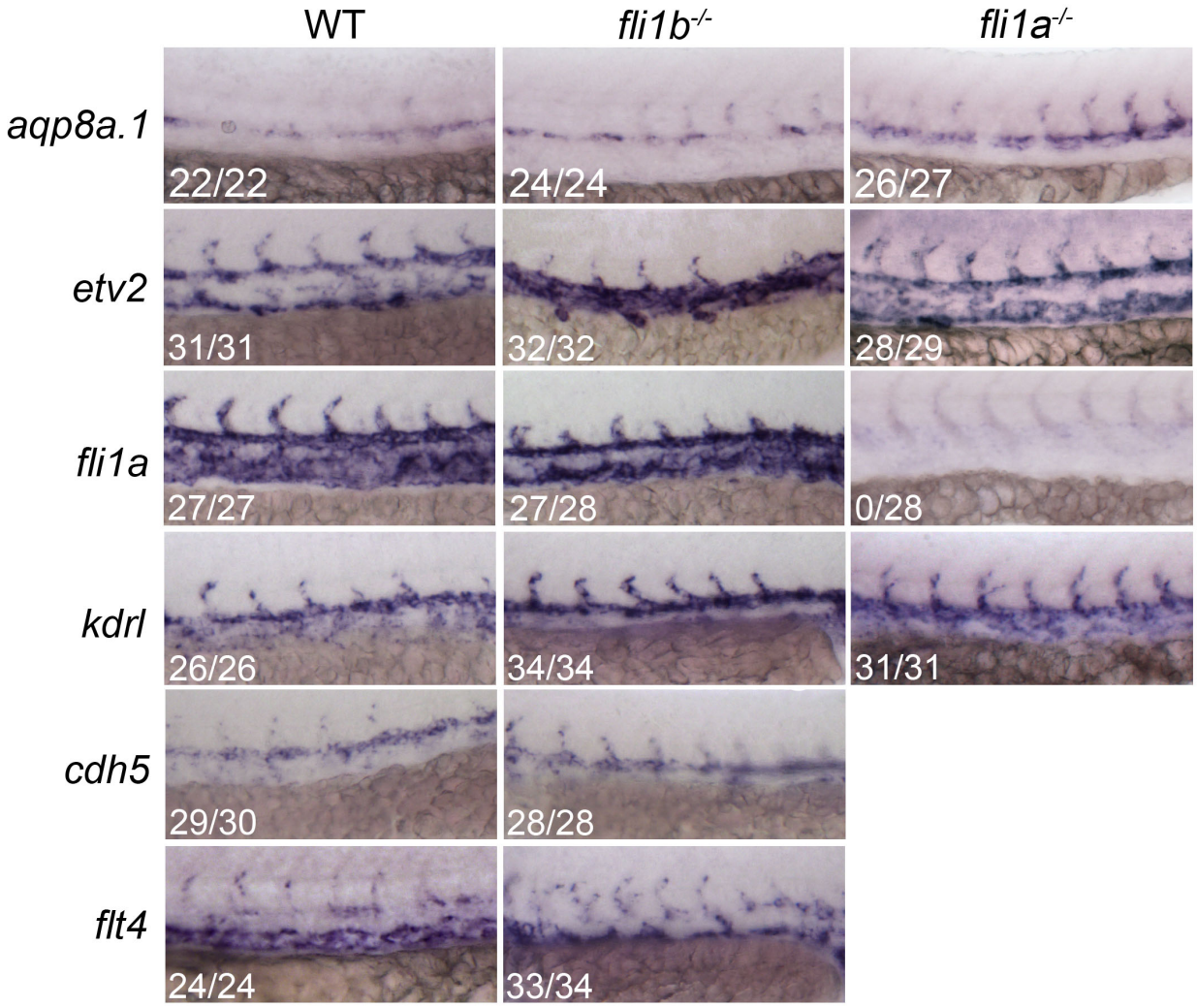


Supplemental Figure I: Comparison of *etv2* and *fli1b* expression. Expression of *etv2* (**A-E**) and *fli1b* (**F-J**) in the trunk vasculature from the 10-somite stage through 48 hpf (lateral views, anterior to the left). *Etv2* expression was restricted to endothelial progenitor cell (EPC) populations at the 10-somite (**A**) and 20-somite stages (**B**), and was strongly expressed in the vascular endothelium by 24 hpf (**C**). Expression of *etv2* was reduced, but detectable at 36 hpf (**D**) and at 48 hpf (**E**). *Fli1b* expression was first observed at 10 somites (**F**) and strongly expressed beginning at 20 somites (**G**). Note that *fli1b* expression appeared more intense than *etv2* at 36 hpf and 48 hpf (**I,J**). **K,** Relative expression of *etv2* and *fli1b* as analyzed by RT-PCR from the 10 somite stage to 72 hpf. Data points represent an average of two independent samples of pooled embryos, each run in duplicate, and normalized to the 24 hpf expression level. Error bars represent ± 1 s.d. **L-N**, *Fli1b* expression in the trunk vasculature of *etv2*$^{-/-}$ embryos from 24-48 hpf. *Fli1b* expression in *etv2*$^{-/-}$ mutant embryos was low at 24 hpf (**L**), but increased during the 36 to 48 hpf period corresponding to the angiogenic recovery of *etv2*$^{-/-}$ embryos (**M**,**N**).Fractions indicate the number of embryos with the observed staining pattern (numerator) and the total evaluated (denominator).

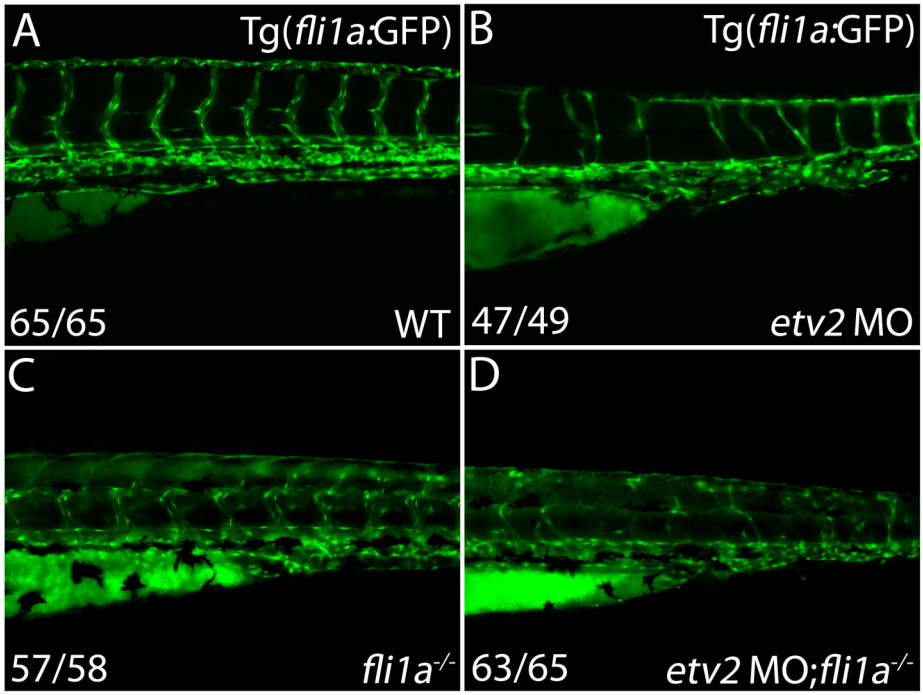


Supplemental Figure II: The *fli1a*^{is10Gt} and *fli1b*^{tpl50Gt} gene-trap lines have lost *fli1a* or *fli1b* expression, respectively, but develop a normally appearing vasculature. Schematic and functional confirmation of the *fli1a* gene trap (**A-D;I-L**) and *fli1b* gene trap lines (**E-H, M-P**). (**A**) The *fli1a*^{is10Gt} gene trap allele contains *Tol2* inverted repeat sequences (black arrows), carp β-

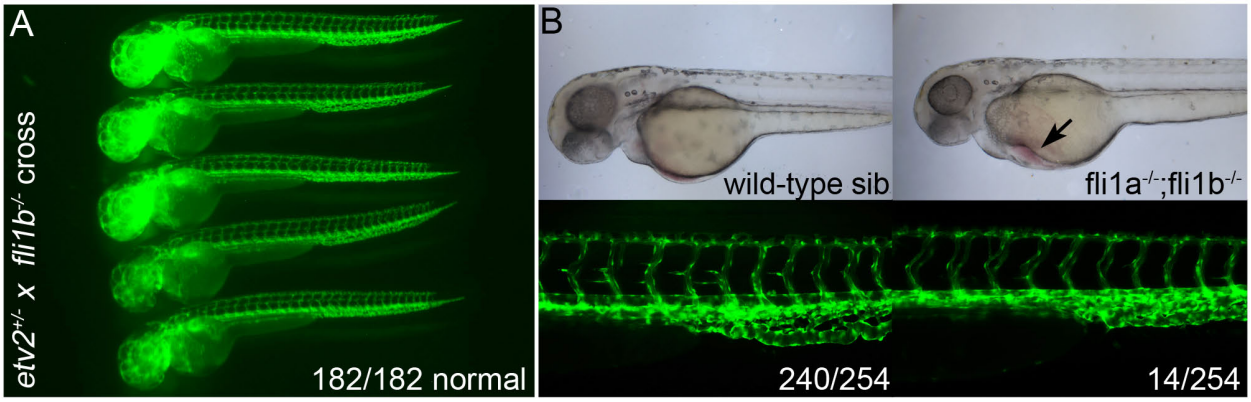
actin splice acceptor (SA) from GBT-R15,[1] enhanced green fluorescent protein (eGFP, green), zebrafish β-actin polyadenylation and transcriptional termination signals (zp(A), white) and *loxP* sites (purple). Flanking exons (blue boxes) of the zebrafish *fli1a* gene are shown. (**B**) The *fli1a* gene trap is inserted between exon 3 and 4 resulting in a 127 AA truncated GFP-fusion protein lacking a functional ETS domain. (**C**) The DNA sequence flanking the *fli1a*^{is10Gt} gene-trap is shown. Note the 8 base pair duplication indicative of Tol2-mediated transposition events (underlined). (**D**) Sequence of the expected Fli1a-GFP fusion protein. Fli1a is in blue, eGFP is in green. (**E**) The *fli1b*^{tpl50Gt} gene trap allele contains the same carp β-actin splice acceptor (SA), an AUG-less Gal4-VP16 (orange), a zebrafish β-actin 3′ untranslated region and transcriptional termination / polyadenylation cassette [zp(A)], an enhanced green fluorescent protein (eGFP, green) zebrafish β-actin 3'UTR and polyadenylation site (pA, gray) from the GBT-R15 vector,[1] and the hybrid yeast-based UAS (syUAS, light blue) (described in Methods). Tol2 5′ and Tol2 3′ are miniTol2 transposon arms as previously described.[2] (**F**) The *fli1b* gene trap is inserted between exon 1 and 2 resulting in 6 AA of Fli1b protein fused with Gal4 domain which transactivates the UAS-GFP reporter. (**G**) The DNA sequence flanking the *fli1b*^{tpl50Gt} gene-trap is shown. Note the 8 base pair duplication indicative of Tol2-mediated transposition events (underlined). (**H**) The expected Fli1b-Gal4 fusion protein. Fli1b sequence is in blue, AUG-less Gal4 is in orange. (**I,J**) *Fli1a* expression is greatly diminished in *fli1a*$^{-/-}$ mutants relative to wild-type embryos as confirmed by WISH at 24 hpf. (**K**) Vascular patterning is normal in *fli1a*$^{-/-}$ embryos, as evident by the endothelial GFP expression at 48 hpf. (**L**) qRT-PCR quantitation of full-length *fli1a* mRNA levels in *fli1a*$^{-/-}$ embryos. (**M,N**) *Fli1b* expression was disrupted in 24 hpf *fli1b*$^{-/-}$ embryos as detected by WISH. (**O**) Vascular patterning was normal in 48 hpf *fli1b*$^{-/-}$ embryos as observed by endothelial GFP expression. Fractions indicate the number of embryos with the normal pattern of expression (numerator) and the total evaluated (denominator). (**P**) RT-PCR quantitation of full-length *fli1b* mRNA levels in *fli1b*$^{-/-}$ embryos.



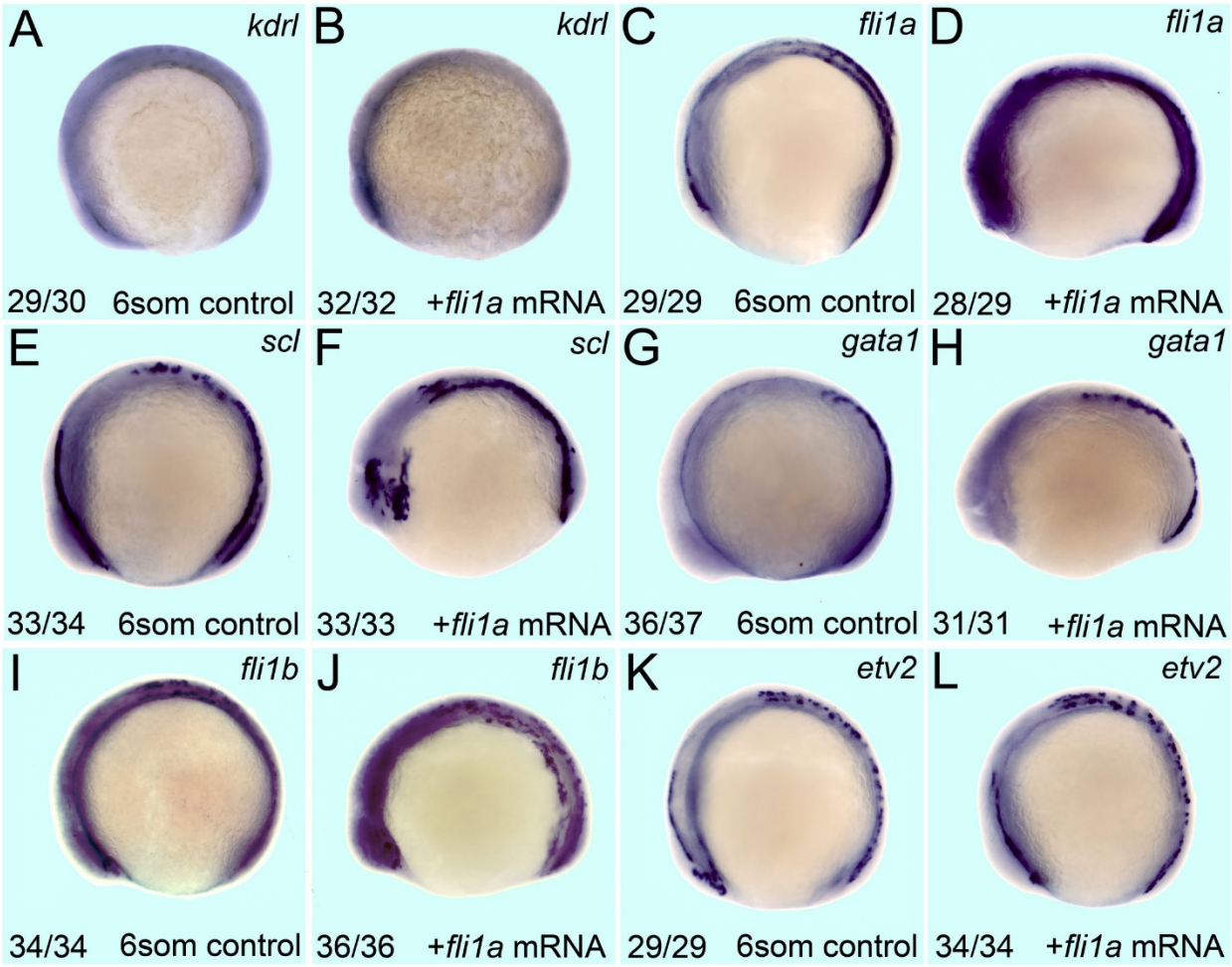
Supplemental Figure III: Vascular endothelial marker expression is normal in 24 hpf *fli1a*$^{-/-}$ and *fli1b*$^{-/-}$ embryos as determined by whole-mount ISH. Lateral views with the anterior oriented to the left are shown in panels. Fractions indicate the number of embryos staining positive for the indicated vascular marker (numerator) and the total evaluated (denominator). Note the reduction of *fli1a* marker expression in *fli1a*$^{-/-}$ mutants.



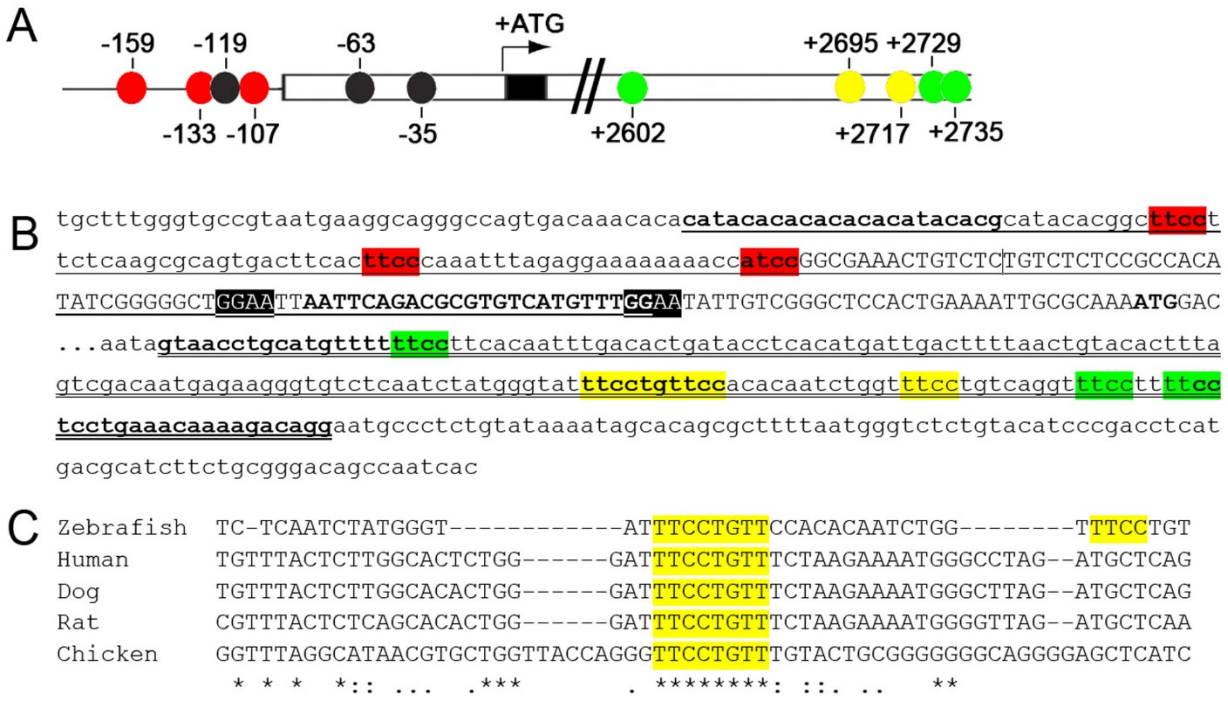
Supplemental Figure IV: Loss of Fli1a does not significantly alter the *etv2* morphant phenotype. Intersegmental vessel patterning in control 65 hpf wild-type Tg(*fli1a*:GFP) embryos (**A**) was disrupted by *etv2* MO knockdown, yielding a number of mispatterned but full-length ISVs (**B**). Fluorescent ISVs labeled with the *fli1a*^{is10Gt} transgene observed in age-matched *fli1a*$^{-/-}$ embryos (**C**) were also impaired by *etv2* MO knockdown (**D**), but the vascular phenotype in these double-knockdown embryos was indistinguishable from the *etv2* morphant phenotype. Fractions indicate the number of embryos appearing as shown (panels A,C) or the number of embryos with greater than 15 partial or full ISVs at 65 hpf (panels C,D) (numerator) and the total evaluated (denominator).



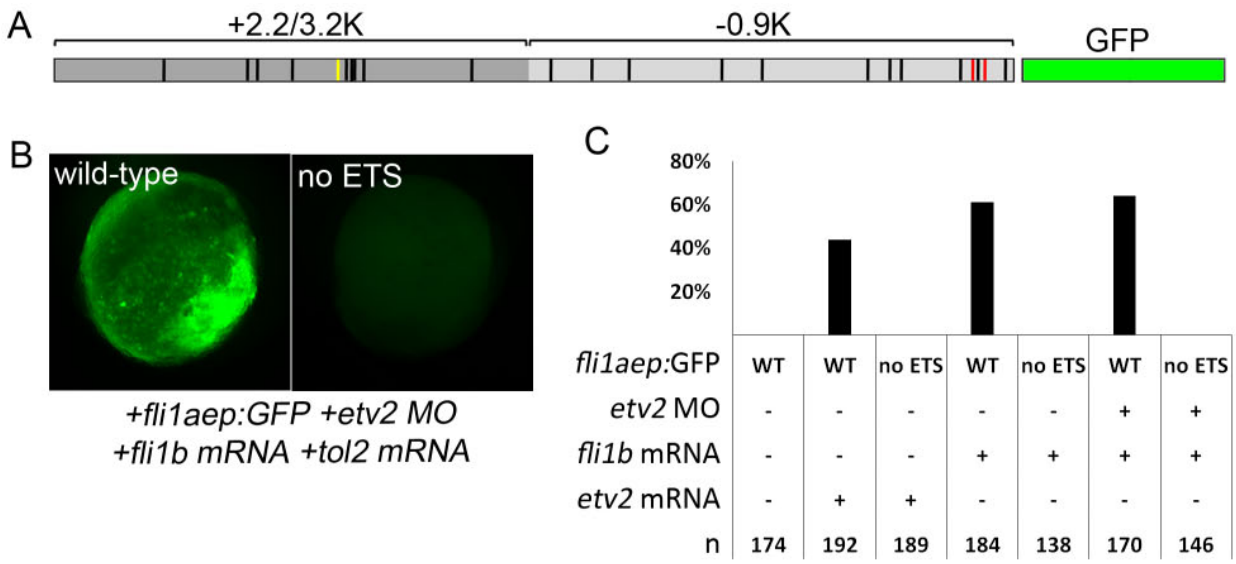
Supplemental Figure V: Combinatorial mutant phenotypes in 48 hpf embryos. (**A**) Representative embryos from crosses of *etv2*$^{+/-}$ and *fli1b*$^{-/-}$ zebrafish which yielded 182 embryos with no observable vascular phenotype. *Etv2*$^{+/-}$*;fli1b*$^{+/-}$ embryos (expected to comprise 50% of the embryos evaluated) were indistinguishable from age-matched wild-type embryos or the remaining *etv2*$^{+/+}$*;fli1b*$^{+/-}$ clutch-mates. (**B**) In-crosses of *fli1a*$^{+/-}$*;fli1b*$^{+/-}$ double carriers yielded a total of 254 embryos, 14 of which (approximately 1/16th) had mild edema (arrow) and lacked circulation but retained normal vascular patterning, corresponding to the expected ratio of double *fli1a*$^{-/-}$*;fli1b*$^{-/-}$ mutants, while the remaining 240 where phenotypically normal). Fractions indicate the number of embryos appearing as shown (numerator) and the total evaluated (denominator).



Supplemental Figure VI: *Fli1a* overexpression does not induce expression of other vascular endothelial markers. Uninjected controls are shown in panels A, C, E, G, I and K. Whole-mount *in situ* analysis of embryos is shown in lateral view with anterior of embryo oriented left. (**A-H**) Effect of *fli1a* overexpression on vascular markers during the indicated stages. Fli1a mRNA does not induce ectopic marker expression of *kdrl* (**B**)*, scl* (**F**), *gata 1* (**H**), *fli1b* (**J**) or *etv2* (**L**). Note the intense Fli1a staining in *fli1a* mRNA injected embryos (**D**) relative to uninjected controls (**C**) indicating successful mRNA injection. The fraction of embryos with ectopic expression is shown in the lower left corner of each panel, the age and injected mRNA in the bottom left, and gene probed by *in situ* in the upper right.



Supplemental Figure VII: The zebrafish *fli1a* gene contains multiple ETS-binding sites within the proximal promoter and first intron. (**A**) Putative ETS-binding sites (colored dots) in the proximal promoter and first intron of the zebrafish *fli1a* gene. Sites previously identified as functional binding targets for *etv2* in the mouse Fli1 gene[3] are shown in red, specifically -159, -133, and -107 correspond to murine -200, -228 and -256, respectively. The consensus intronic FOX-ETS site is shown in yellow and adjacent putative ETS sites are shown in green. (**B**) Regional sequence of the zebrafish *fli1a* (NM_131348) showing exonic and intronic regions in caps and lowercase, respectively. Primer sequences used for ChIP are shown in bold. The promoter fragment enriched by ChIP qPCR is underlined once, and the enriched intronic FOX-ETS fragment is underlined twice. (**C**) Conservation of the FOX-ETS motif.



Supplemental Figure VIII: *Fli1b* induces expression of *fli1a* in an ETS-binding site dependent manner. (**A**) Schematic *fli1a[ep]*:GFP reporter construct showing the 23 putative ETS-binding sites identified within the *fli1a* minimal enhancer-promoter sequence. ETS sites previously identified as sufficient for vascular-specific expression of *fli1a* by Abedin *et al*.[4] (red highlight) and the putative intronic FOX-ETS site identified herein (yellow highlight). (**B**) Strong ectopic expression in 8-somite staged wild-type embryos injected with *fli1b* mRNA, *tol2* mRNA, *etv2* MO, and the wild-type *fil1aep*:GFP construct (left embryo) and the corresponding lack of GFP readout in embryos similarly injected but using the mutated (noETS) *fli1a[ep]:*GFP construct (right embryo). (**C**) Percentage of fluorescent embryos injected with different combinations of wild-type (WT) or mutant ETS sites (no ETS) *fli1a[ep]:GFP* construct, *etv2* MO, *fli1b* or *etv2* mRNA.

```
   1>GATCTCATCTTGACCCATAAACATACACTAAAACCCAACAGTCAACTTTATCAAATGAAATGAGTGTAATTAACTCAAAATTTACTGAATGTTGATTCTA>100
   1>GATCTCATCTTGACCCATAAACATACACTAAAACCCAACAGTCAACTTTATCAAATGAAATGAGTGTAATTAACTCAAAATTTACTGAATGTTGATTCTA>100

 101>CTCATTCGAAAAGAGTTTTAAACTCAGTGTTGAAGGTAGTGAGTTCATTAAATACCTCATTACTTCAACTTAACTGGAGTAAGTTCATGGTACTCACATA>200
 101>CTCATTCGAAAAGAGTTTTAAACTCAGTGTTGAAGGTAGTGAGTTCATTAAATACCTCATTACTTCAACTTAACTGGAGTAAGTTCATGGTACTCACATA>200

 201>GATTAGTTTAGCTCAAATGGTTTGTAGCAGTCGGTTCCCTCAAGCAGTTTGAGTTGCCTTAACTTTTTGGGTTTTACAGTACTCAGTTGGTTTGAGTTCA>300
 201>GATTAGTTTAGCTCAAATGGTTTGTAGCAGTCGGTTAACTCAAGCAGTTTGAGTTGCCTTAACTTTTTGGGTTTTACAGTACTCAGTTGGTTTGAGTTCA>300

 301>CTTCACTTATTGGGTTCAACATGTGCTCAAATTGCATCGTTTAATCAAATAGATTAAGTTCACAGTACTCATTAGGATTAGTTTTTGAACTTAAATGGTT>400
 301>CTTCACTTATTGGGTTCAACATGTGCTCAAATTGCATCGTTTAATCAAATAGATTAAGTTCACAGTACTCATTAGGATTAGTTTTTGAACTTAAATGGTT>400

 401>TGTTGCAGTCGGTTTCCTCAAACGGTTTGAGTTTCCATAACTTTATGGGTTTTTCAGTGTAGTAAACATATGCATCCACAGATTATAATAGTAACCTGCA>500
 401>TGTTGCAGTCGGTTTAATCAAACGGTTTGAGTTTAAATAACTTTATGGGTTTTTCAGTGTAGTAAACATATGCATCCACAGATTATAATAGTAACCTGCA>500

 501>TGTTTTTTCCTTCACAATTTGACACAGATACCTCACATGATTGACTTTTAACTGTACACTTTAGTCGACAATGAGAAGGGTGTCTCAATCTATGGGTATT>600
 501>TGTTTTTTAATTCACAATTTGACACAGATACCTCACATGATTGACTTTTAACTGTACACTTTAGTCGACAATGAGAAGGGTGTCTCAATCTATGGGTATT>600

 601>TCCTGTTCCACACAATCTGGTTTCCTGTCAGGTTTCCTTTTCCTCCTGAAACAAAAGACAGGAATGCCCTCTGTATAAAATAGCACAGCGCTTTTAATGG>700
 601>TAATGTTAAACACAATCTGGTTTAATGTCAGGTTTAATTTTAATCCTGAAACAAAAGACATTAATGCCCTCTGTATAAAATAGCACAGCGCTTTTAATGG>700

 701>GTCTCTGTACATCCCGACCTCATGACGCATCTTCTGCGGGACAGCCAATCACGATCAAGTTGGCTTATGAAAACAGTTTTTTGTCCCCCTGTTAAGTTAA>800
 701>GTCTCTGTACATCCCGACCTCATGACGCATCTTCTGCGGGACAGCCAATCACGATCAAGTTGGCTTATGAAAACAGTTTTTTGTCCCCCTGTTAAGTTAA>800

 801>AAGCACGGTAGCATACATGCATTATTCATCATGCAGCATATTCAGCAGTACAAAGATGAGCTCTTGACACACCTCAGCGTAAAATCTCTATTCCAGCGTA>900
 801>AAGCACGGTAGCATACATGCATTATTCATCATGCAGCATATTCAGCAGTACAAAGATGAGCTCTTGACACACCTCAGCGTAAAATCTCTATTAAAGCGTA>900

 901>ATCCTGCGGTTCTGGATGGTTTAAGCGAGCCTTGCAGACACGGCCAATTTATAGCCATGTTGAAAATAGCAGCTTTGTGAGCATTTCGACCTCAATGTAC>1000
 901>ATCCTGCGGTTCTGGATGGTTTAAGCGAGCCTTGCAGACACGGCCAATTTATAGCCATGTTGAAAATAGCAGCTTTGTGAGCATTTCGACCTCAATGTAC>1000

1001>CAAGAATgcgtgctcggtaactgaaaaagaggggtggagattttggatttagagatGGAAtcacatgcacgctgtatatgctatataaaaatagcaggc>1100
1001>CAAGAATgcgtgctcggtaactgaaaaagaggggtggagattttggatttagagatttaatcacatgcacgctgtatatgctatataaaaatagcaggc>1100

1101>atttaatgcatgcaaaatatgtttcgacactaaacactacaagtcGGAAatatatctatttttctttttttaacgaaatgagacatttaatgcacagtaa>1200
1101>atttaatgcatgcaaaatatgtttcgacactaaacactacaagtcttaaatatatctatttttctttttttaacgaaatgagacatttaatgcacagtaa>1200

1201>atgtagtctaacacgcacaataTTCCtattattgtccatgaaaaatgtgaggtcatatgctactgatttaaagaaatgttgaacagcacagcacacaaga>1300
1201>atgtagtctaacacgcacaatattaatattattgtccatgaaaaatgtgaggtcAtatgctactgatttaaagaaatgttgaacagcacagcacacaaga>1300

1301>ggtatcagcctctctatatggtccatacagcaccaggctcgctgcgttcatgtgcattcagaggcgcgcacacgtgtggagcagacagactcgacgcagc>1400
1301>ggtatcagcctctctatatggtccatacagcaccaggctcgctgcgttcatgtgcattcagaggcgcgcacacgtgtggagcagacagactcgacgcagc>1400

1401>gggctttgctttttattcatGGAAgcccattttagaagaagctcgggcagactcgtcacattcttcacttcaccataggtagatcaaatcaaagttattg>1500
1401>gggctttgctttttattcatttaagcccattttagaagaagctcgggcagactcgtcacattcttcacttcaccataggtagatcaaatcaaagttattg>1500
```

1501>gatataac**GGAA**caatattcatcatattatgagattattattagaccgagtgcattagacatatttgtattcgatcgttgaaaaagtatatattttattt>1600
1501>gatataacttaacaatattcatcatattatgagattattattagaccgagtgcattagacatatttgtattcgatcgttgaaaaagtatatattttattt>1600

1601>tagccttaaactatggttagcctatattaatcagcataatctgttatttcagacgttgtattaatgattatttctagcacagctttgatgcatcaatgca>1700
1601>tagccttaaactatggttagcctatattaatcagcataatctgttatttcagacgttgtattaatgattatttctagcacagctttgatgcatcaatgca>1700

1701>atgtttctacaaacccgccttgtttaatgtc**GGAA**aaaaagcgcaacaaatgtaaagagttggcaggcattctgtaaatat**TTCC**aggcaaaaacatgca>1800
1701>atgtttctacaaacccgccttgtttaatgtcttaaaaaaagcgcaacaaatgtaaagagttggcaggcattctgtaaatatttaaaggcaaaaacatgca>1800

1801>caaa**GGAA**agaaacaccattgttttggtgtcttatcttcgctggacaaatatcctgctttgggtgccgtaatgaaggcagggccagtgacacacacacat>1900
1801>caaattaaagaaacaccattgttttggtgtcttatcttcgctggacaaatatcctgctttgggtgccgtaatgaaggcagggccagtgacacacacacat>1900

1901>acacacacacacatacacgcatacacggc**TTCC**ttctcaagcgcagtgacttcac**TTCC**caaatttaga**GGAA**aaaaaaccatccggcgaaactgtctct>2000
1901>acacacacacacatacacgcatacacggcttaattctcaagcgcagtgacttcacttaacaaatttagattaaaaaaaaccataaggcgaaactgtctct>2000

2001>gtctctccgccacatatcgggggct**GGAA**ttaattcagacgcg>2043
2001>gtctctccgccacatatcgggggctttaattaattcagacgcg>2043

Supplemental Figure IX: Sequence comparison of the wild-type *fli1a[ep]* sequence (top rows) containing intact ETS sites (highlighted in yellow and underlined) versus the mutated version with disruption of all 23 putative ETS sites. The intronic region (+2095 through +3100 nt from the transcription start site) is positioned upstream and shown in capital letters, while the core promoter-enhancer region (-1079 to ATG start) is downstream and shown in lowercase letters.

Supplemental Material References

1. Petzold AM, Balciunas D, Sivasubbu S, Clark KJ, Bedell VM, Westcot SE, Myers SR, Moulder GL, Thomas MJ, Ekker SC. Nicotine response genetics in the zebrafish. Proceedings of the National Academy of Sciences of the United States of America. Nov 3 2009:10644:18662-18667.
2. Balciunas D, Wangensteen KJ, Wilber A, Bell J, Geurts A, Sivasubbu S, Wang X, Hackett PB, Largaespada DA, McIvor RS, Ekker SC. Harnessing a high cargo-capacity transposon for genetic applications in vertebrates. PLoS genetics. Nov 10 2006:211:e169.
3. Abedin MJ, Nguyen A, Jiang N, Perry CE, Shelton JM, Watson DK, Ferdous A. Fli1 acts downstream of Etv2 to govern cell survival and vascular homeostasis via positive autoregulation. Circulation research. May 23 2014:11411:1690-1699.
4. Villefranc JA, Amigo J, Lawson ND. Gateway compatible vectors for analysis of gene function in the zebrafish. Developmental dynamics : an official publication of the American Association of Anatomists. Nov 2007:23611:3077-3087.