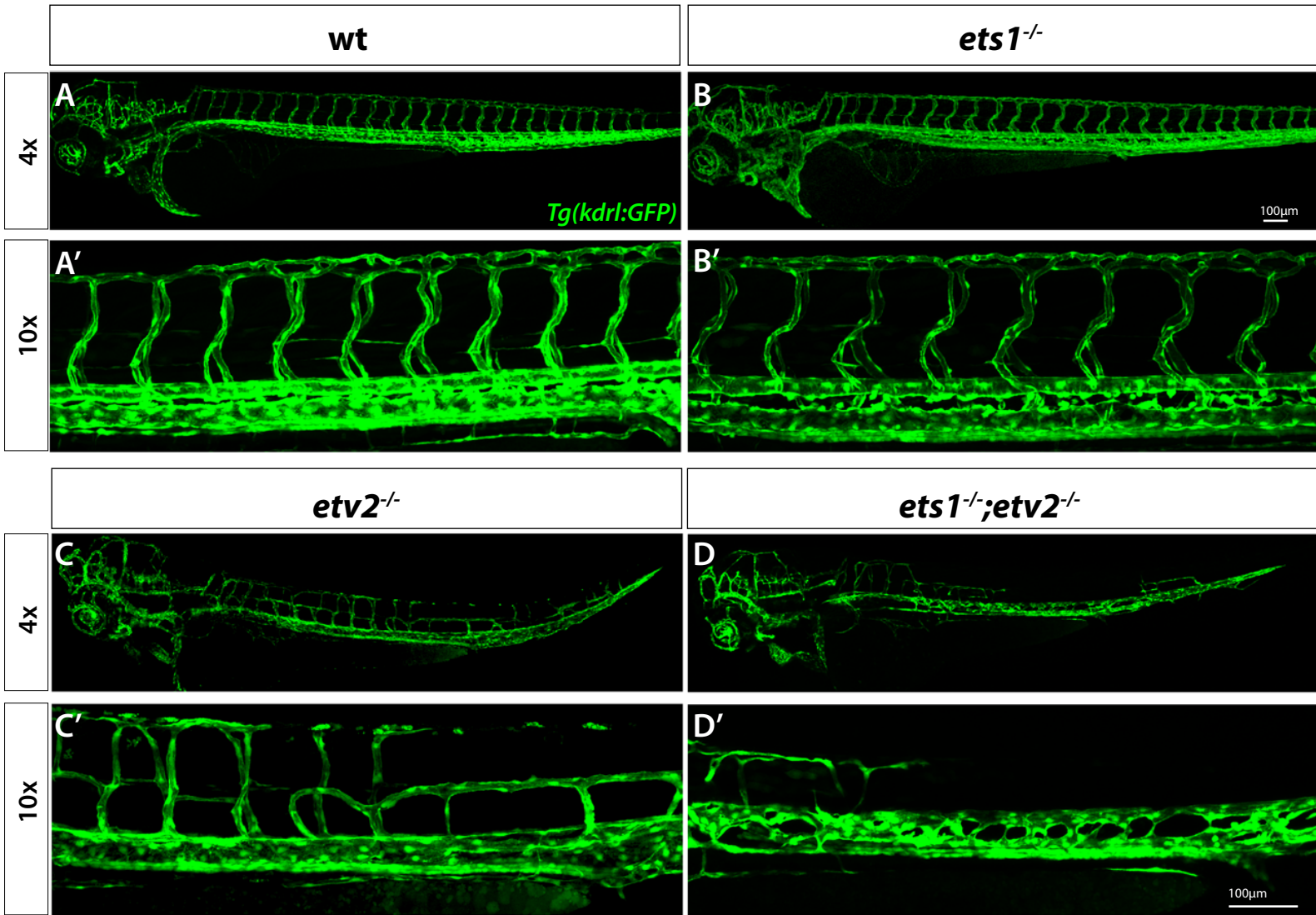


**Supplemental Figure S1. Generation of *ets1<sup>ci14</sup>* mutants and analysis of *ets1* expression.** (A) DNA sequencing chromatogram shows the 5 bp TCTGG deletion in *ets1<sup>ci14</sup>* mutants. (B) Alignment between predicted mutant and wild-type Ets1 protein sequences. Note the severely truncated Ets1 mutant protein (underlined in red). (C) Schematic of wild-type Ets1 protein domain structure and predicted phosphorylation sites. Note the Pointed (PNT) and DNA binding ETS domains. The truncated protein in the *ets1<sup>ci14</sup>* mutant lacks both the PNT and ETS domains. (D) qPCR analysis of *ets1* expression in *ets1<sup>ci14</sup>* mutants at 15-somite stage (15 ss) and 24 hpf. Note the persistent expression of *ets1* in the mutants. (E,F) In situ hybridization for *ets1* in wildtype and *ets1<sup>ci14</sup>* embryos at 24 hpf. Note the presence of the *ets1* transcript in *ets1<sup>ci14</sup>* embryos.



**Supplemental Figure S2. *ets1*<sup>-/-</sup>; *etv2*<sup>-/-</sup> embryos display severe vascular defects compared to *etv2*<sup>-/-</sup> embryos.** (A-D') Confocal micrographs of 80 hpf *Tg(kdrl:GFP)* wild-type, *ets1*<sup>-/-</sup>, *etv2*<sup>-/-</sup> and *ets1*<sup>-/-</sup>; *etv2*<sup>-/-</sup> embryos. Note the severe vascular defects in *ets1*<sup>-/-</sup>; *etv2*<sup>-/-</sup> embryos compared to the *etv2*<sup>-/-</sup> embryos

# MSA

The multiple sequence alignment result as produced by T-coffee.

T-COFFEE, Version\_11.00.d625267 (2016-01-11 15:25:41 - Revision d625267 - Build 507)

Cedric Notredame

SCORE=99

\*

AD AVG GOOD

\*

Human : 99  
Mouse : 99  
Chicken : 99  
Zebrafish : 99  
cons : 9

```
Human MKAAVDLKPTLTIIKTEKVDLELFPSPDMECADVPLLTPSSKEMMSQALKATFSGFTKEQQLGIPKDP
Mouse MKAAVDLKPTLTIIKTEKVDLELFPSPDMECADVPLLTPSSKEMMSQALKATFSGFTKEQQLGIPKDP
Chicken MKAAVDLKPTLTIIKTEKVDIDLFPSPDMECADVPLLTPSSKEMMSQALKATFSGFAKEQQLGIPKDP
Zebrafish MTAAVDIKP-LTIIKSEKVD-----DLECADVPLLTPGSKEMMSQALLATFSGFTREQQRLSIPKDP
```

cons \*.\*\*\*\*:\* \*\*\*:\*\*\*\* \*:\*:\*\*\*\*\*.\*:\*\*\*\*\* \*\*\*:\*\*:\*:\*\*\*\*.\*\*\*\*\*

```
Human RQWTETHVRDWMWAVNEFSLKGVDFQKFCMNGAALCALGKDCFLELAPDFVGDILWEHLEILQKEDVK
Mouse RQWTETHVRDWMWAVNEFSLKGVDFQKFCMNGAALCALGKCFLELAPDFVGDILWEHLEILQKEDVK
Chicken RQWTETHVRDWMWAVNEFSLKGVDFQKFCMNGAALCALGKCFLELRPFDVGDILWEHLEILQKEEAK
Zebrafish RQWTEGHVREWL TWTVNEFSLKNVDFHKFMSMGASL CALGKERFLDLAPDFVGDILWGHLEMLQKEDPK
```

cons :.\*\*\* \*\*\*:.\*: \*:\*:\*\*\*\*\*.\*\*\*:\*.\*:\*:\*\*\*\*\*: \*\*:\* \*\*\*\*\* \*\*\*:\*\*\*\*: \*

```
Human PYQVNGVNPAYPESRYTSDYFISYGIEHAQCVPVSEFSEPSFITESYQTLHPISSEELLSLKYENDYPS
Mouse PYQVNGANPTYPECYTSDYFISYGIEHAQCVPVSEFSEPSFITESYQTLHPISSEELLSLKYENDYPS
Chicken PYPANGVNAAYPESRYTSDYFISYGIEHAQCVPVSEFSEPSFITESYQTLHPISSEELLSLKYENDYPS
Zebrafish HFPVSSLSSSFQESRYPSEYFFNYGIEHPQCVPVSEYSEPSFITESYQTLHPISSEDL SLKYESEYPN
```

cons : ... ..: \*\* \*:\*:\*\*\*.\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*.

```
Human VILRDPLQDRTLQNDYFAIKQEVVTPDNMCMGRSTRGKLGQDSFESIESYDSCDRLTQSWSSQSSFNS
Mouse VILQDPLQDRTLQTDYFAIKQEVLPDNMCLGRASRGKLGQDSFESVESYDSCDRLTQSWSSQSSFNS
Chicken VILRDPVQDSDLQTDYFTIKQEVVTPDNMCMGRASRGKLGQDSFESIESYDSCDRLTQSWSSQSSFNS
Zebrafish VILRDA-PLNPLQGDYFVSVKQEVVSPDNMCMVGRISRGKLGQDSFESIDSFESCDRLTQSWSSQSSFNS
```

cons \*\*\*:\*. :. \*\* \*\*\*:\*\*:\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\* \*\*\*\*\*:\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*

```
Human LQRVPSYDSFDSEDPYAAPLNHKPKGTFKDYVRDRADLNKDKPVIPAAALAGYTGSGPIQLRQFLLELL
Mouse LQRVPSYDSFDYEDYAAPLNHKPKGTFKDYVRDRADLNKDKPVIPAAALAGYTGSGPIQLWQFLLELL
Chicken LQRVPSYDSFDSEDPYAAPLNHKPKGTFKDYVRDRADNMNPKPVIPAAALAGYTGSGPIQLWQFLLELL
Zebrafish LQRVPSYDSFDSEDPYALHAHKPKGTFKDYVRERSDL SKDKPVIPAAALAGYTGSGPIQLWQFLLELL
```

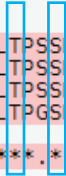
cons \*\*\*\*\* \*\*\*:\*\* \*\*\*\*\*:\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

```
Human TDKSCQSFISWTGDGWEFKLSDPDEVARRWGKRKNPKPMNYEKL SRGLRYYYDKNIIHKTAGKRYVYRF
Mouse TDKSCQSFISWTGDGWEFKLSDPDEVARRWGKRKNPKPMNYEKL SRGLRYYYDKNIIHKTAGKRYVYRF
Chicken TDKSCQSFISWTGDGWEFKLSDPDEVARRWGKRKNPKPMNYEKL SRGLRYYYDKNIIHKTAGKRYVYRF
Zebrafish TDKSCQSFISWTGDGWEFKLSDPDEVARRWGKRKNPKPMNYEKL SRGLRYYYDKNIIHKTSKGKRYVYRF
```

cons \*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*

```
Human VCDLQSLGTYTPEELHAML DVKPDAD
Mouse VCDLQSLGTYTPEELHAML DVKPDAD
Chicken VCDLQSLGTYTPEELHAML DVKPDAD
Zebrafish VCDLKSLLGTYTPEELHTML DVKPDTE
```

cons \*\*\*\*:\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*



**Supplemental Figure S3. Ets1 protein sequence is highly conserved across multiple vertebrate species.** Alignment output of human, mouse, chicken and zebrafish Ets1 protein sequences using the T-COFFEE structural alignment tool (<http://tcoffee.org.cat/apps/tcoffee/do:expresso>). The light blue boxes indicate the conserved Threonine and Serine phosphorylation sites present in all four species.