SUPPLEMENTARY APPENDIX

ETV6 (TEL1) regulates embryonic hematopoiesis in zebrafish

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Supplementary Methods

Bioinformatics

Human ETV6 was used in a TBLASTN search of zebrafish (*Danio rerio*) and Japanese pufferfish (*Takifugu rubripes*) EST databases that identified putative *etv6* sequences (GenBank Acc. No. AY693994 and AF340230, respectively). Nucleotide sequences were assembled using Sequencher 4.10.1 (Gene Codes Corporation), and intron/exon boundaries determined by alignment of cDNA and genomic sequences, applying the GT-AG splice rule where possible.[1] Multiple protein sequences were aligned using ClustalX 1.83,[2] which was used to create bootstrapped phylogenetic trees of 1000 replicates utilizing the Neighbor-Joining algorithm NJplot (http://pbil.univ-lyon1.fr/software/njplot.html) that were viewed in Treeview 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). The sequences analyzed were ETV6: human (NP_001978), mouse (NP_030987), zebrafish (AAH65661) and Japanese pufferfish (AAK54061) and ETV7: human (NP_057219), zebrafish (NP_001076493) and Japanese pufferfish (XP_003972996). Ensembl (http://www.ensembl.org) was employed to perform synteny analysis of the Japanese pufferfish (FUGU4) and human (GRCh37) genome assemblies.

Histochemical analysis

Staining of whole embryos for haemoglobin with *O*-dianisidine used a previously described method.[3] Sudan black B and myeloperoxidase staining were both performed according to the manufacturer's protocol (Sigma-Aldrich), except that the incubation time for the latter was decreased to 7 min. For differential cell counts, blood was pooled from embryos by nicking the ventral part of the trunk in PBS containing 1 mM EDTA and 2% (v/v) FCS, and smears prepared by centrifugation at 7000 rpm for 5 min using a Cytospin 4 (Thermo Scientific). Slides

were fixed and stained with Wright-Geimsa according to the manufacturer's protocol (Sigma-Aldrich). Sectioning and counterstaining of WISH embryos was performed as described.[4]

Imaging and quantification

Images were taken on an MVX10 microscope (Olympus) using a DP72 camera (Olympus). Following whole mount *in situ* hybridization, individual cells were manually counted or an area of staining quantified using CellSens Dimensions 1.6 software (Olympus).

Statistical analyses

All statistical analyses were performed using GraphPad Prism software version 4. Statistical significances between wild type and morphant embryos were determined using an unpaired independent Student *t* test. Probability (*p*) values less than 0.05 were considered significant. Sample populations in each group were at least 30.

Supplementary Table 1: Oligonucleotide primers used for Q-RT-PCR.

Gene	**Forward primer**	**Reverse primer**
c-myb	5'-GGTCCTCATGCCAAGTCAG	5'-CGGAGTTGGGCTGACTTTAG
gata1	5'-CTCCTCTGAGCCTTCTCGTTGG	5'-GTCTGATGAGGGGTCTGTTCTGGC
β-e-g	5'-ATCTTCGCCAAGGCTGACTA	5'-GCATAGGTGGCCTTGATGTT
epo	5'-TACTGCTGATGGTGCTGGAG	5'-GACTGGACCTCCTGAGCTTG
epor	5'-GCCCTGTTCTTCACCTCTCTGG	5'-CTTCTGCTCTGGTGTTGGTATGTC
lyz	5'-ACTGGGACGCTGTGATGTTTAC	5'-GTAAGAATCCCAGGTTTCCCAT
mmp9	5'-GCTGCTCATGAGTTTGGACA	5'-GGTGGAAGCAGTGGTTGTTT
ikaros	5'-AAGCGAAGTCACACTGAAGAAAG	5'-CAGATGTCCAGTGAGAGCGTC
rag1	5'-ACACTGCCTTAACCATTACCG	5'-GTCAAACACACAGACTTCACATC

References

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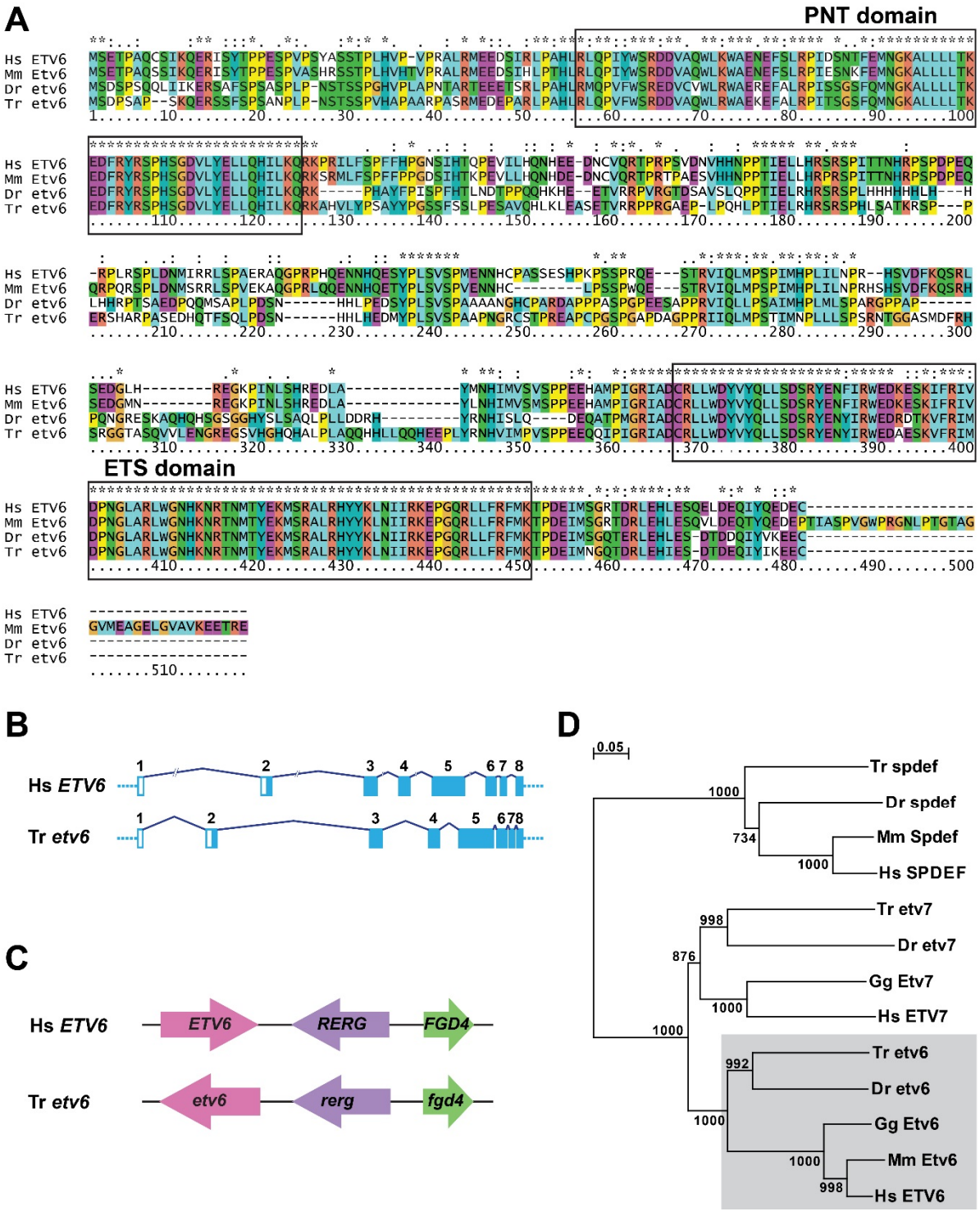
Supplementary Figures



Supplementary Figure 1. Characterization of teleost *etv6* genes.

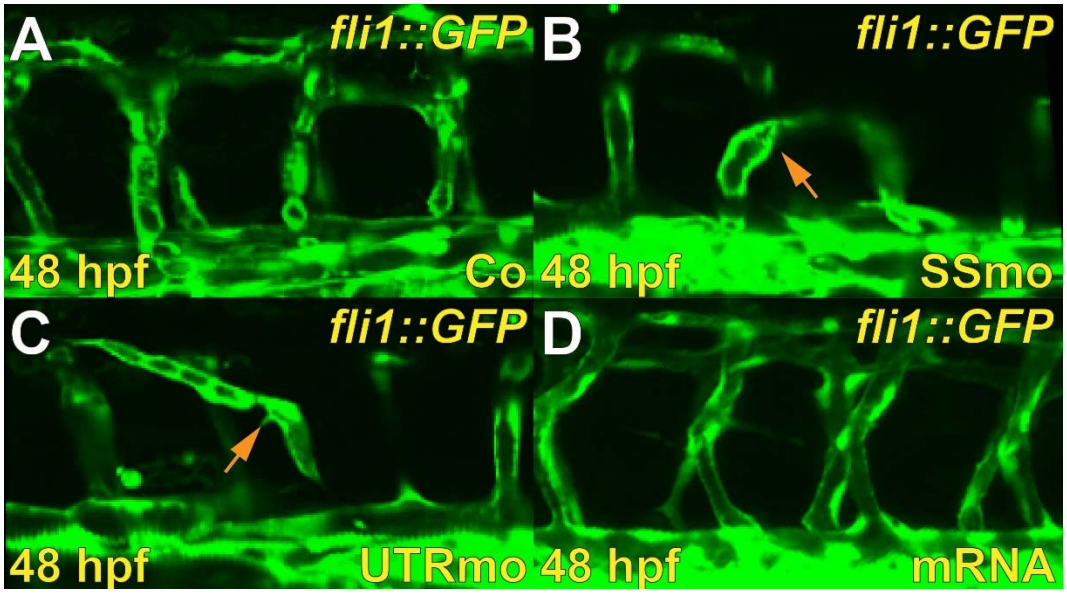
A. Alignment of ETV6 proteins from human (*Homo sapiens*, Hs ETV6), mouse (*Mus musculus*, Mm Etv6), zebrafish (*Danio rerio*, Dr etv6) and pufferfish (*Takifugu rubripes*, Tr etv6). The identical, conserved, and semi-conserved residues are denoted by asterices, colons,

and periods, respectively. The boxes demarcate the highly conserved PNT and ETS domains.

B. Splicing structure of human (Hs) *ETV6* and pufferfish (Tr) *etv6* genes, with exons represented by numbered rectangles and coding regions shaded.

C. Synteny analysis of human (Hs) *ETV6* and pufferfish (Tr) *etv6* gene loci, with adjacent conserved genes color-matched.

D. Phylogenic analysis of ETV6 proteins and related ETS family members, ETV7 and SPDEF, from human (Hs), mouse (Mm), chicken (*Gallus gallus*, Gg), zebrafish (Dr) and pufferfish (Tr). Boostrap values (n=1000) for each branch are indicated with the ETV6 clade shaded.



Supplementary Figure 2. Knockdown of *etv6* affects early vessel development.

A-D. Confocal analysis of *fli1::GFP* embryos injected with control (Co) or *etv6*-specific morpholinos (SSmo, UTRmo) or *etv6* mRNA (mRNA) and visualized at 48 hpf. Arrows indicate disrupted vessels in morphant embryos.