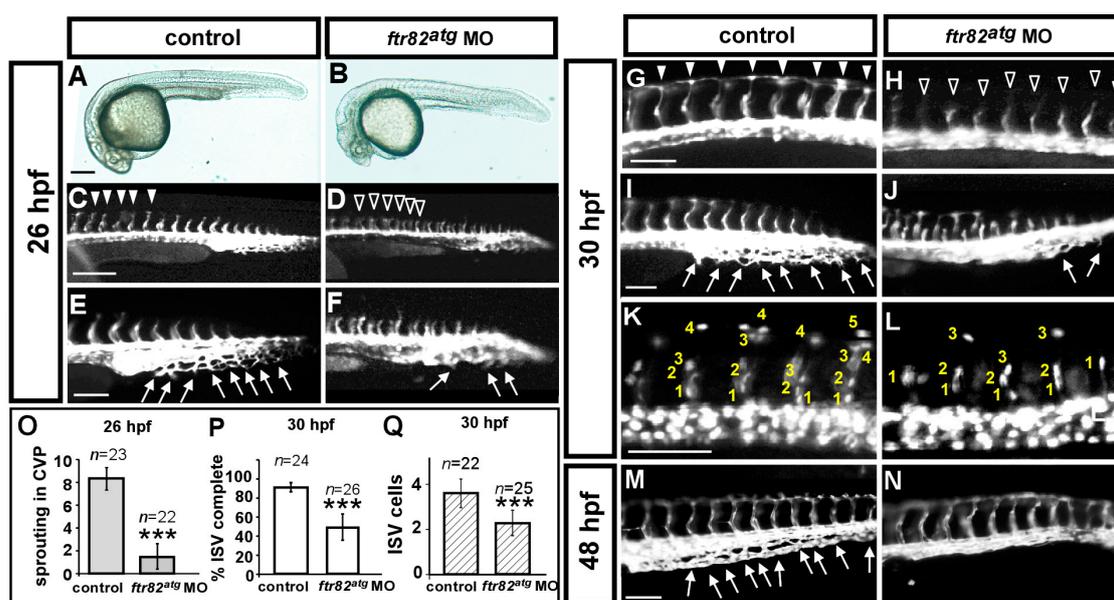


## Supplementary Materials: Ftr82 Is Critical for Vascular Patterning during Zebrafish Development

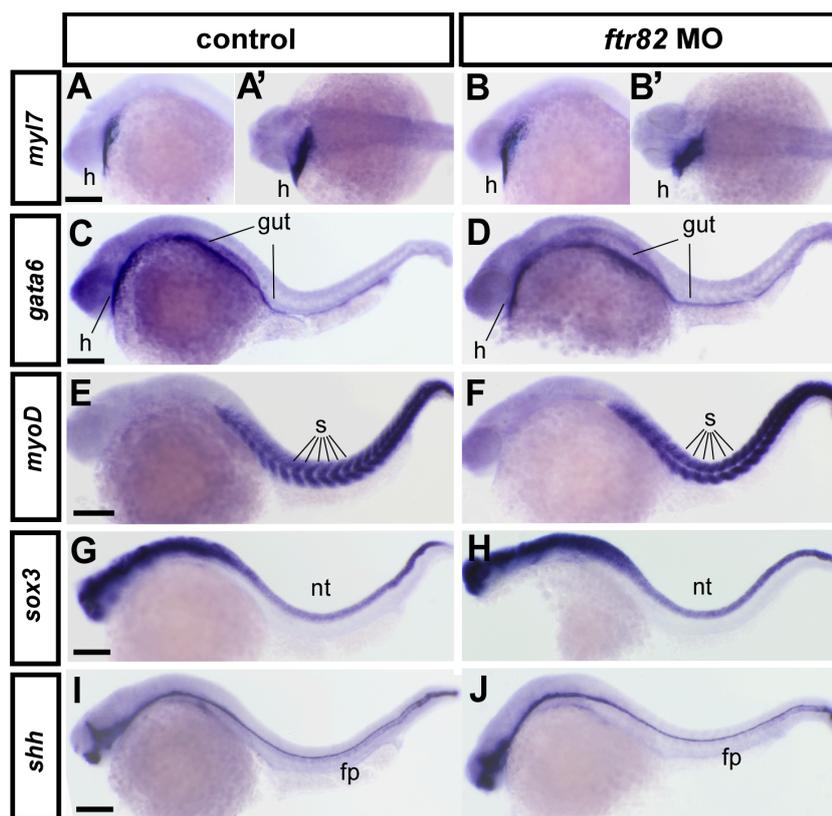
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Table S1. Quantitative PCR (qPCR) primer sequences used in this study.

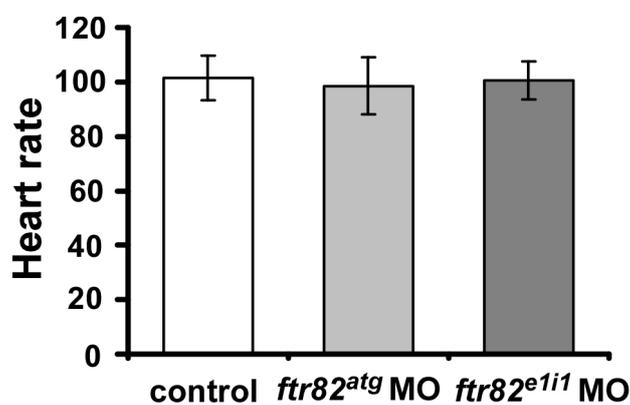
qPCR Primers	Sequence
<i>β-actin</i> _qf	5'-CTCTTCCAGCCTTCCTTCCT-3'
<i>β-actin</i> _qr	5'-CTTCTGCATACGGTCAGCAA-3'
<i>flt4</i> _f1	5'-ACTCGGGTTATTACCGCTGCTTCT-3'
<i>flt4</i> _r1	5'-TGGATGCTCTGGGTCTCGAACAAA-3'
<i>flk1</i> _qf	5'-ACTTTGAGTGGGAGTTTCATAAGGA-3'
<i>flk1</i> _qr	5'-TTGGACCGGTGTGGTGCTA-3'
<i>mrc1</i> _qf	5'-CTAGCAAGCCTGAAGGTGCC-3'
<i>mrc1</i> _qr	5'-TGAGAGGCTGGGTAGTTGGG-3'
<i>ephrinb2</i> _qf	5'-CTGGAACACCACGAACACC-3'
<i>ephrinb2</i> _qr	5'-CACACGTGGGCAAACACTATGT-3'
<i>ftr82</i> _qf	5'-TCCCTGTGACTTCTGCACTG-3'
<i>ftr82</i> _qr	5'-CCTCTTGAAGGTGGCAGACT-3'



**Figure S1.** Knockdown of *ftr82* causes defects in vascular development in zebrafish by using translational block ATG morpholino. (A–F) Bright-field images show the morphology of uninjected control *Tg(flk:gfp)* embryos (control, A) and *ftr82* translational block ATG morpholino injection (*ftr82<sup>atg</sup>* MO) at 26 hpf (B); (C–F) Loss of *ftr82* shows intersegmental vessel (ISV) growth defect (hollow arrowheads in (D)) compared to control (arrowheads in (C)), and less angiogenic sprouting from the tail caudal vein in *ftr82* morphants (arrows in E,F) at 26 hours post-fertilization (hpf). (E) and (F) are enlarged figures from (C) and (D), respectively; (G–L) At 30 hpf, in uninjected control embryos, ISV have reached the dorsal longitudinal anastomotic vessel (DLAV) at the dorsal part of the embryo (G, arrowheads) and the caudal vein plexus (CVP) formed loop structures at the tail (I, arrows). Knockdown of *ftr82* at the same stage showed ISVs growth stall in the middle line of somite (H, hollow arrowheads) and less honeycomb structure in the CVP (J, arrows); Reduced number of cells per ISV was photographed and counted in *ftr82* morphant embryos ( $2.0 \pm 0.5$ ,  $n = 25$ ) versus in control *Tg(fli1a:negfp)<sup>y7</sup>* ( $3.8 \pm 0.4$ ,  $n = 22$ ) (K,L,Q). At 48 hpf, less or no capillaries loop in the CVP was observed (N) compared to control (arrows in M); (O) Quantification of angiogenic sprouting from the caudal vein shows a 4.5-fold decreased in *ftr82* morphants at 26 hpf ( $n = 23$  in control and in  $n = 22$  in *ftr82* MO); (P) Quantification of percentage of completed ISV in *ftr82* morphants shows a ~40% decrease ( $n = 24$  in control and  $n = 26$  in *ftr82<sup>atg</sup>* MO) at 30 hpf. \*\*\* refers to  $p < 0.0001$  by an unpaired Student's *t*-test. Scale bars are 200  $\mu\text{m}$  for A–D, 100  $\mu\text{m}$  for E–N.



**Figure S2.** The gross developmental process is unaffected in *ftr82* morphants. Knockdown of *ftr82* at 24 hpf did not alter the expression pattern of heart, gut, somite, or neural systems by examining the expression of *myl7* (heart marker, h, **A** and **B** are lateral view and **A'** and **B'** are dorsal view), *gata6* (heart and gut marker, **C,D**), *myoD* (somite marker, s, **E,F**), and *sox3* (neural tube marker, nt, **G,H**) and *shh* (floor plate marker, fp, **I,J**). Scale bars in all figures are 250  $\mu$ m.



**Figure S3.** Knockdown of *ftr82* did not cause developmental delay by measuring heart rate. Quantification of zebrafish heart beats per minute in uninjected control and *ftr82* morphants shows no difference ( $n = 12$  in control,  $n = 12$  in *ftr82<sup>atg</sup>* MO and  $n = 9$  in *ftr82<sup>e1i1</sup>* MO) at 24–25 hpf. The mean heart rate determined by direct visual examination of ventricle beating in control was  $101.5 \pm 8.2$  beats per minute,  $98.5 \pm 10.5$  beats per minute in *ftr82<sup>atg</sup>* MO, and  $100.6 \pm 6.9$  beats per minute in *ftr82<sup>e1i1</sup>* MO.