

Supplementary Materials: Vegfa Impacts Early Myocardium Development in Zebrafish

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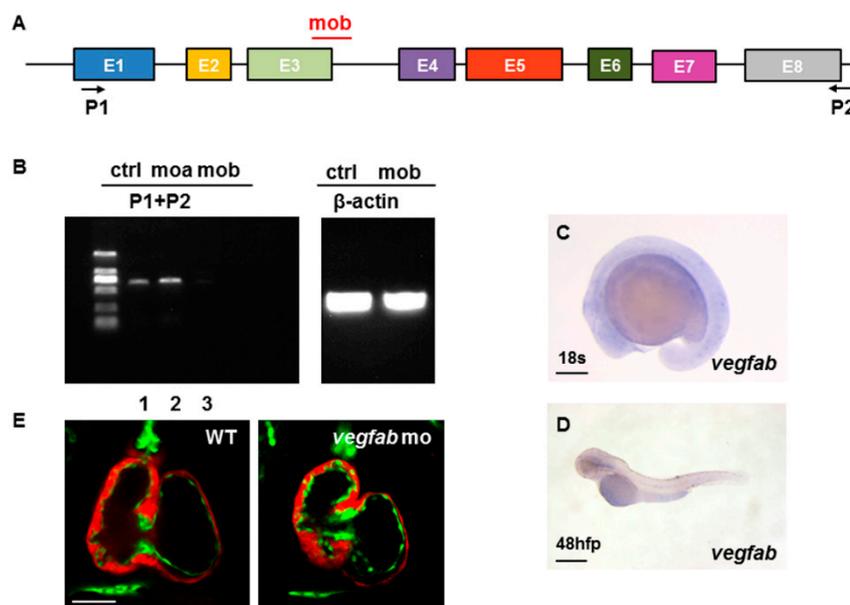


Figure S1. *vegfab* is not involved in early cardiovascular development in zebrafish. (A) Open boxes and black lines denote exons and introns of the early coding and non-coding region of *vegfab*. MO in red mark the target sites of the *vegfab* translation blocking. P1 and P2 indicate the sites of primers; (B) Fragments of *vegfab* sequences were amplified using the indicated primers. In *vegfab* morphant embryos, the normal *vegfab* RT-PCR product was not detected (lane 3). β -actin cDNA was also amplified from each sample as an internal control; (C,D) The expression of *vegfab* during early embryogenesis cannot be detected. Scale bar: 200 μ m (C); 500 μ m (D); (E) The heart development is almost normal with 10ng doses of *vegfab* MO at 96 hpf under the background of *Tg(myl7:mCherry)/Tg(kdrl:EGFP)* transgenic fish. Scale bar: 50 μ m.

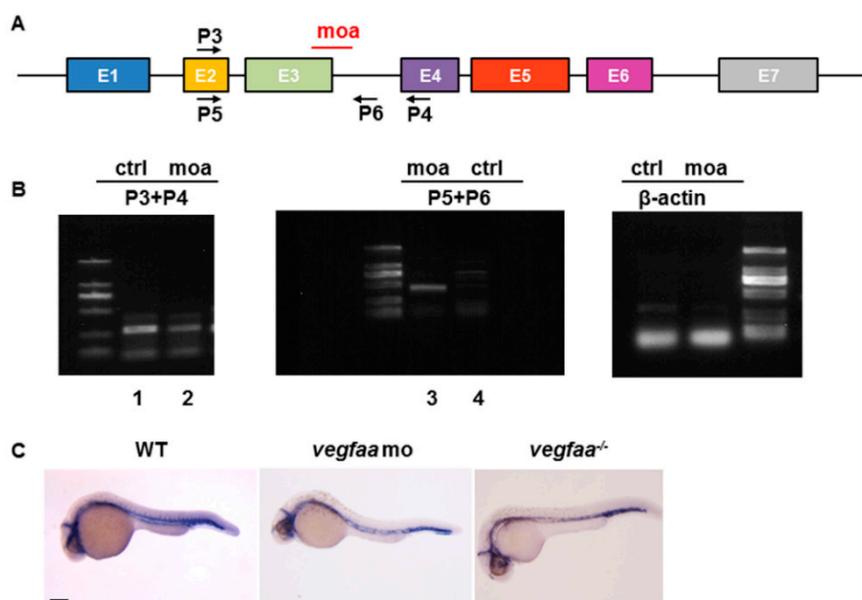


Figure S2. RT-PCR validation of morpholino efficacy for *vegfaa*. (A) Open boxes and black lines denote exons and introns of the first coding and non-coding region of *vegfaa*. MO in red mark the target sites of the *vegfaa* translation blocking. P3, P4, P5, and P6 indicate the sites of primers; (B) One- to two-cell embryos were injected with *vegfaa* MO, and cDNA at 24 hpf was isolated. In wild-type embryos, the normal *vegfaa* spliced P3–P4 RT-PCR product was observed (lane 1) but not the P5–P6 splice donor site PCR product (lane 3). In *vegfaa* morphants, the normal transcription level is decreased (lane 2) and the intron sequences are retained (lane 3). The β -actin cDNA was also amplified from each sample as a reference control; (C) Whole-mount in situ hybridizations (WISH) analysis of *cdh5* in WT, *vegfaa* morphants, and mutants at 30 hpf. The morphants and mutants display identical phenotype in arterial-venous differentiation and intersegmental vessel sprouting. Scale bar: 200 μ m.

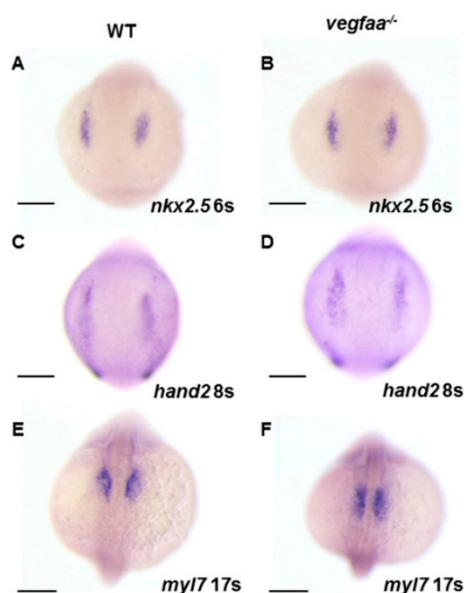


Figure S3. Normal pattern of cardiac progenitors and differentiation in *vegfaa* mutant embryos. (A–D) WISH demonstrates the expression of *nkx2.5* (A,B) and *hand2* (C,D) in wild-type (A,C) and *vegfaa* mutant (B,D) embryos; (E,F) WISH demonstrates the expression of *myl7* in wild-type (E) and *vegfaa* mutant (F) embryos at 17-somite stage. Dorsal views anterior to the top. Gene expression patterns appear normal in *vegfaa* mutants; no expansion or reduction of heart field size is evident. Scale bar: 200 μ m (A–F).

Table S1. Effect of *vegfaa* morpholino dosage on cardiac morphogenesis.

MO Dosage	Die	Severe	Moderate	Noraml	Total
6 ng	23	57	1	1	82
5 ng	17	33	4	2	56
4 ng	4	34	25	3	66
3 ng	1	2	11	42	56

Table S2. List of primers used for RT-PCR.

Primer	5'–3'
P1	GCTTTCTGAGACCTTTACCAATGC
P2	TCTCAAGTCAGTTCGGTTCACCTCC
P3	CCAAAGAAGGGGGAAAGAGCA
P4	ATTATGCTGCGATACGCGTTG
P5	CCCACATACCCAAAGAA
P6	GCTGTCATCATGGCAA