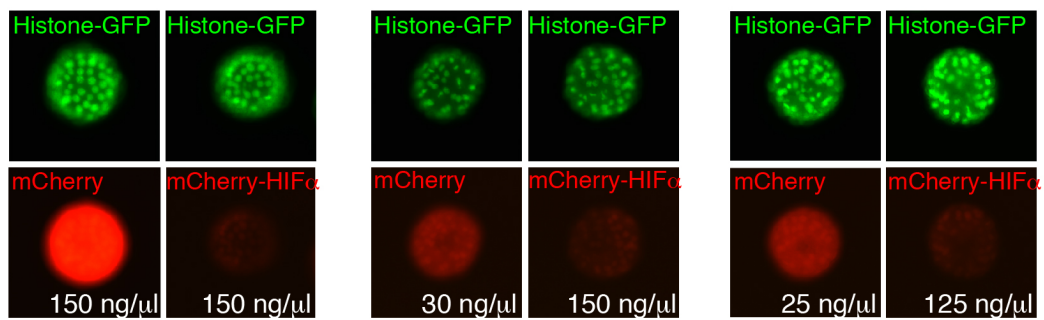


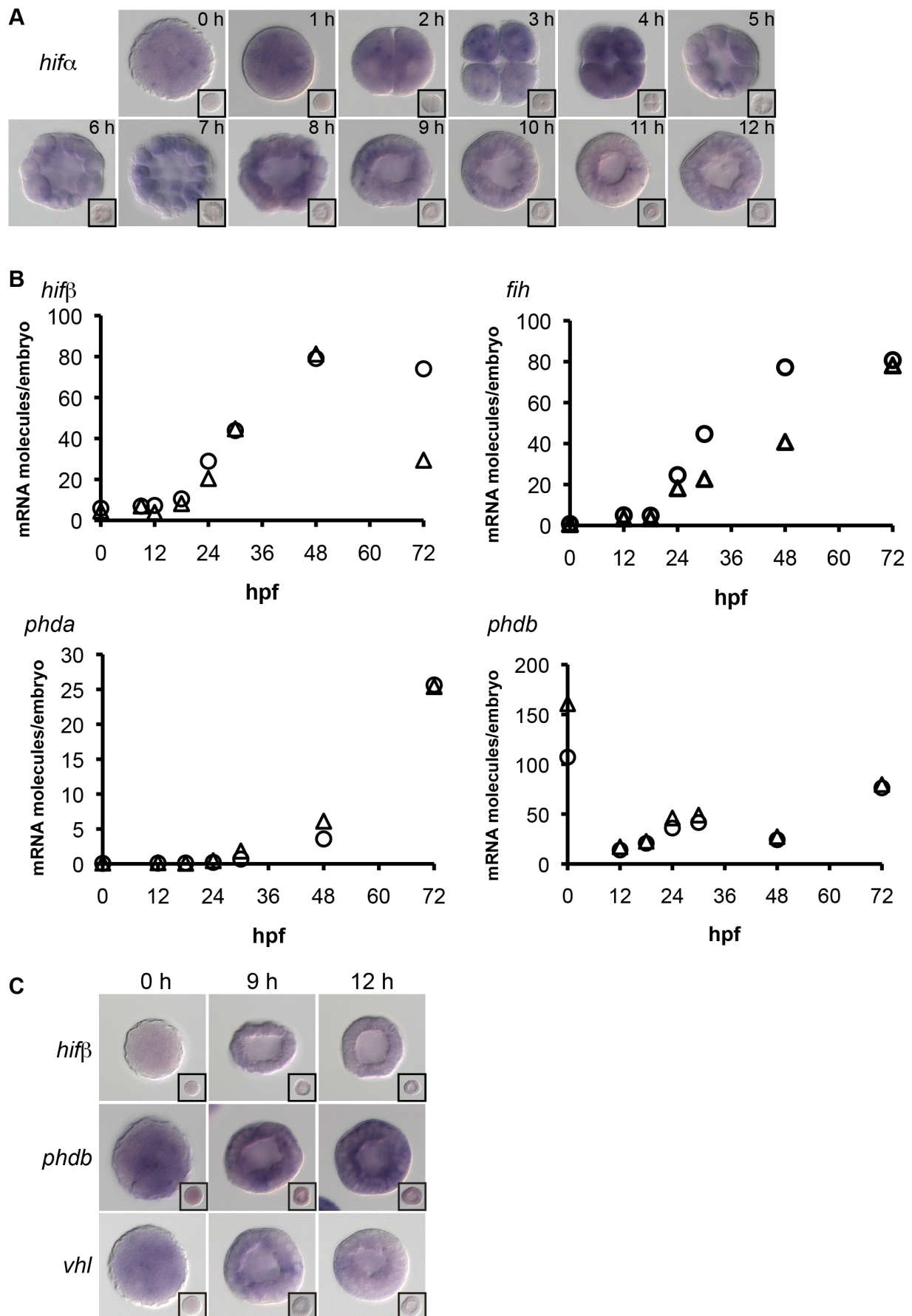
## Supplemental Information

Figure S1



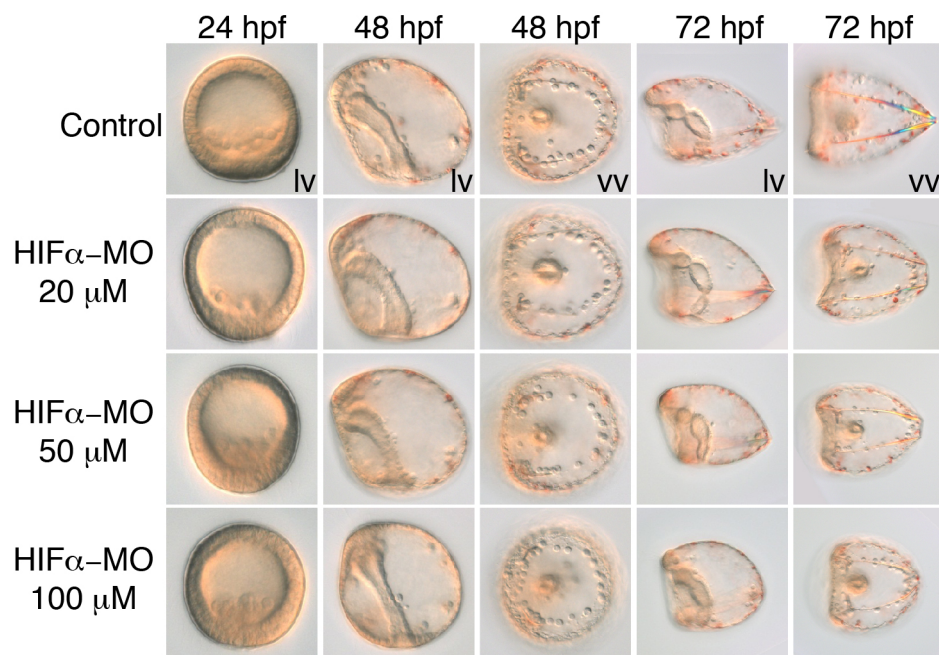
**Figure S1. mCherry-HIF $\alpha$  fusion protein was asymmetrically distributed on one-side of the blastula.** When the same concentration was used, embryos injected with mRNA encoding mCherry only resulted in strong signals in all cells whereas mCherry-HIF $\alpha$  signal is weaker and asymmetric. When the mRNA concentration was adjusted to the same molar ratio (1:5), similar results were observed.

Figure S2



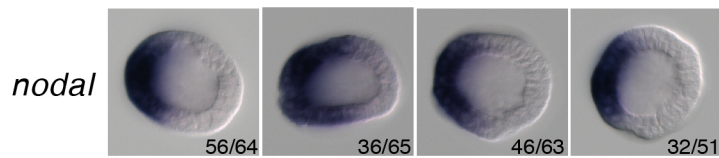
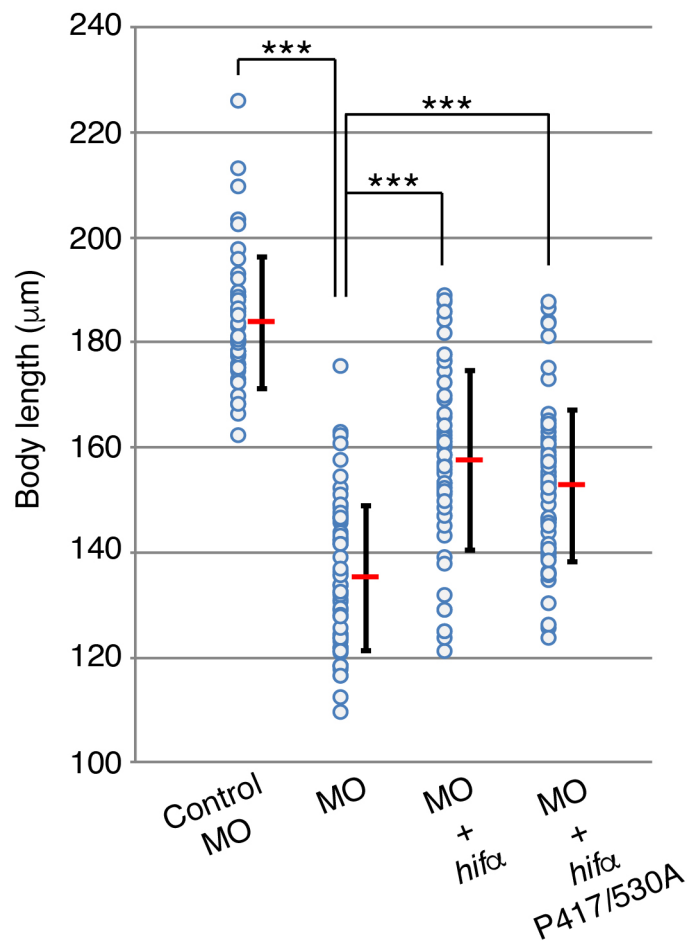
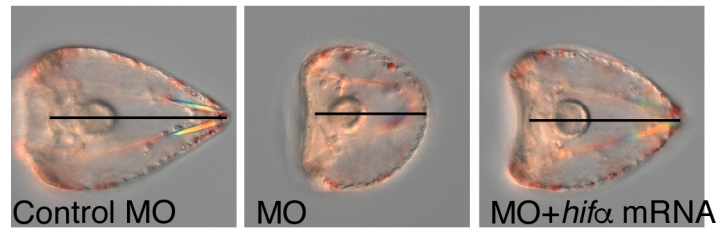
**Figure S2. Expression profiles of components of the hypoxia pathway during sea urchin embryogenesis.** (A) *In situ* hybridization of *hif $\alpha$*  during the first 12 hours (h) of development. (B) QPCR analyses of *hif $\beta$* , *fi $h$* , *phda*, and *phdb* during embryogenesis (hpf, hours post fertilization). The circles and triangles represent data from two sets of non-overlapping primers. (C) *In situ* hybridization of *hif $\beta$* , *phdb*, and *vhl*. The insets in (A) and (C) are embryos hybridized with sense probes.

Figure S3



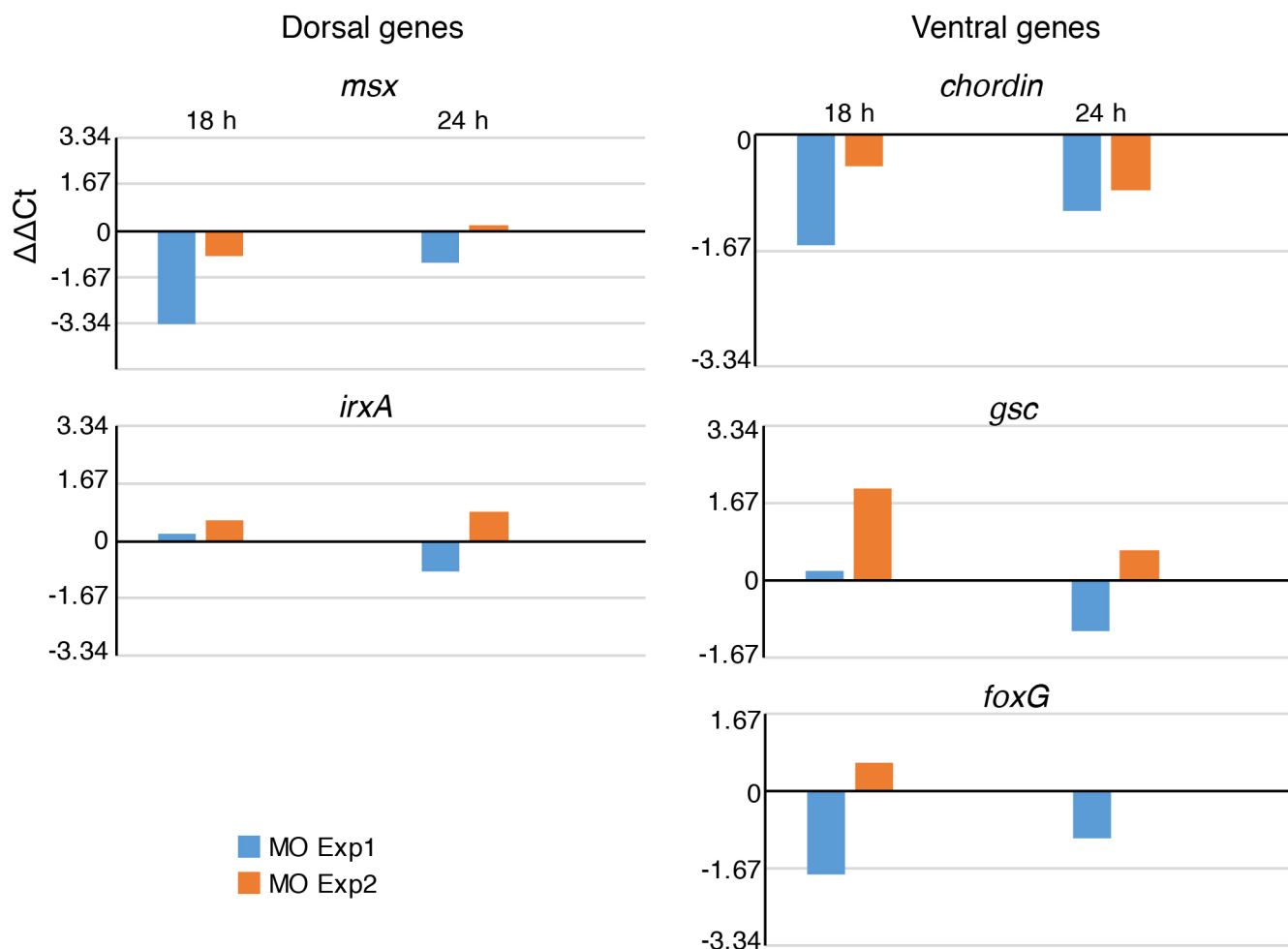
**Figure S3. Phenotypic changes in embryos injected with HIF $\alpha$  MO.** Uninjected embryos (control) and embryos injected with HIF $\alpha$  MO at various concentrations were observed at the mesenchyme blastula (24 hpf), gastrula (48 hpf), and pluteus larva (72 hpf) stages in lateral (lv) or vegetal (vv) view.

Figure S4



**Figure S4. mRNA encoding wild type or mutant HIF $\alpha$  rescued the shortening phenotype and the *nodal* expression pattern of MO morphants.** Embryos injected with control or HIF $\alpha$  MO, or coinjected with HIF $\alpha$  MO and mRNA encoding wild type or mutant HIF $\alpha$  (P417/530A) were raised until 12 hpf for *in situ* hybridization or the pluteus stage (72 hpf) for body length measurements. The body lengths were measured from ventral side to the dorsal apex (black lines in three represented samples). The shortening of the body length caused by HIF $\alpha$  MO could be rescued by mRNA encoding wild type or P417/530A HIF $\alpha$ . The error bars are standard errors and the red lines indicate average body length (T test,  $p < 0.001$ ). Expansion of the *nodal* expression domain was also rescued (bottom panels). The embryos were presented in lateral view with ventral to the left. The numbers in the bottom right-hand corners indicate the ratios of the displayed phenotypes.

Figure S5



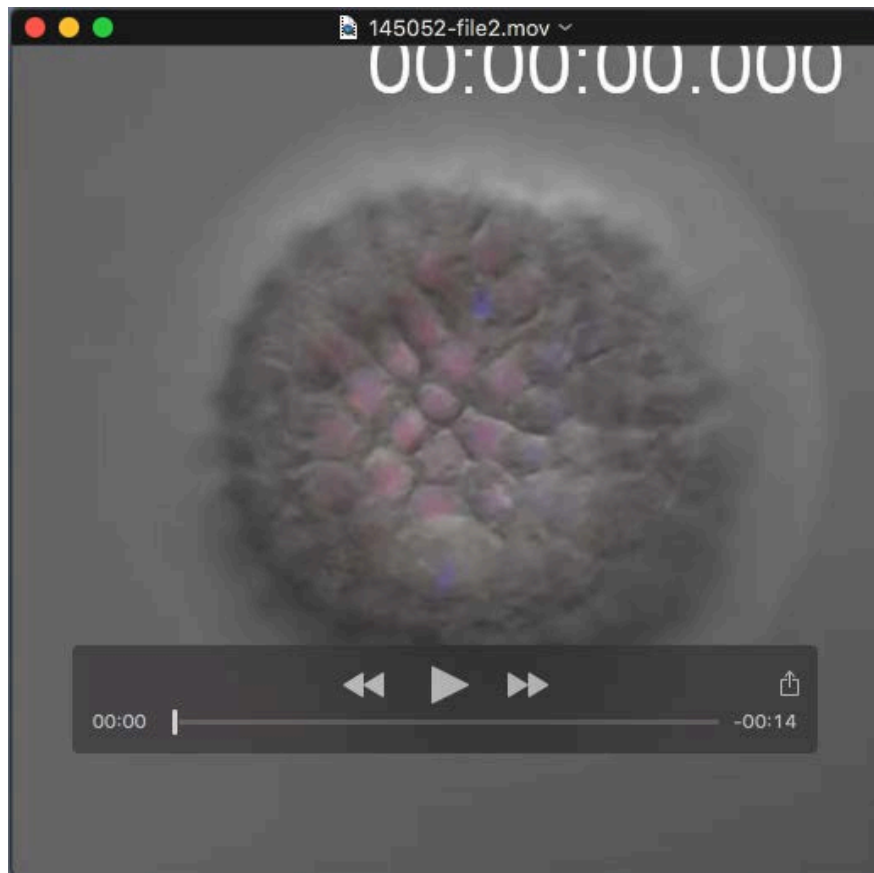
**Figure S5. Effects of HIF $\alpha$  MO on the expression of dorsal and ventral genes.**

QPCR analyses were performed on embryos injected with HIF $\alpha$  MO (80  $\mu$ M) at 18 and 24 hpf. Results from two independent experiments (Exp1 and Exp2) are presented. The Y axis represents  $\Delta\Delta$ Ct, and 1.67  $\Delta\Delta$ Ct roughly equals a 3-fold difference.

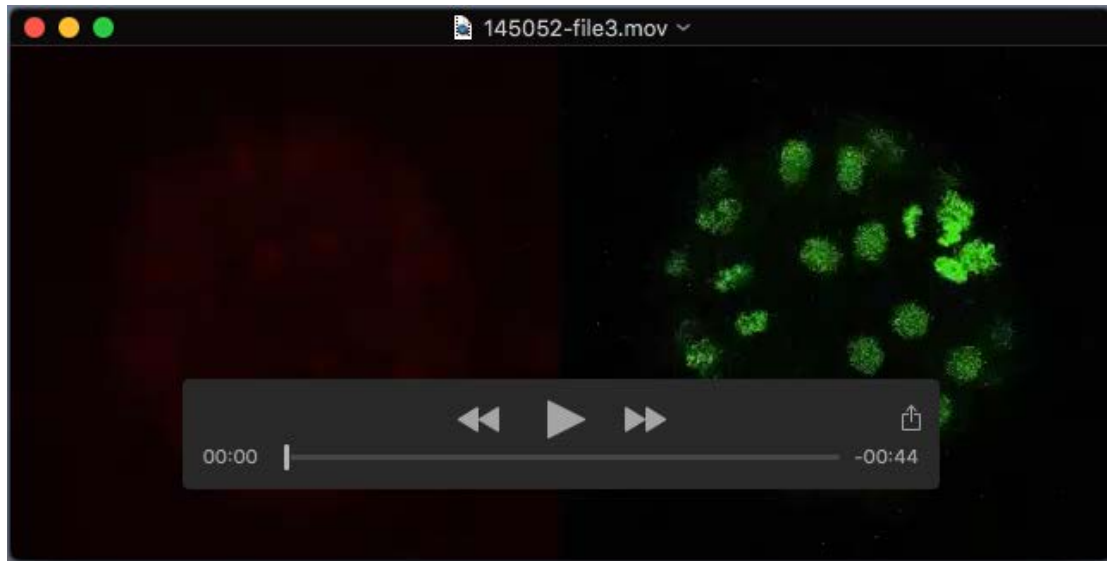
**Table S1.** List of QPCR primers used in this study.

Gene name	Primer set	Forward primer	Reverse primer
<i>chordin</i>	QFR	CAATGAGCTGCGTCAGATGT	GCAGCATTCGCCTTTAGTTC
<i>dlx</i>	QFR	CCAGCTTACAACCTCCAACAGC	TTACCTGAGTTTGAGTGAGTCCA
<i>fih</i>	QFR1	TGGTCGAGACTGCTCTGAAA	CGCCTTCTTGTCGTCAAAGT
<i>fih</i>	QFR4	CAGGCTCATGGAGATGACCA	GCTGGGACCTACTGAATCAT
<i>foxd</i>	QFR	CGCTCGAGTCCAGAGAAAAG	TGTCGAGGGACTTTCACAAA
<i>gsc</i>	QFR	GCGACACGCTCCCTATCTAC	CGATGTCGCCTCTTTCTCTT
<i>hifβ</i>	QFR1	TCTGATGTACCGATTCCAAGC	ATAGCTGTATTGGTGCAGACGA
<i>hifβ</i>	QFR3	CAGTGGGGTGATGGAAGACT	GTGTATGAGGGTGTTCGGTGA
<i>hmx</i>	QFR	TCGTCGTTTGAAGGTTGAAGT	TGATAGACGCATCTTGCTCG
<i>irxa</i>	QFR	TATGGAATGGACCTGAACGG	TATGATCTTTTCGCCCTTGG
<i>msx</i>	QFR	AGCACAAGACAAACCGGAAG	CGTTCGGCTATCGAGAGGTA
<i>nodal</i>	QFR	GACAACCCAAGCAACCACG	CGCACTCCTGTACGATCATG
<i>phda</i>	QFR1	CCGATAAACGCAATCCTCAT	GGGATCCATTCCTCCTGATT
<i>phda</i>	QFR2	TGACAATCCTAACGCAGACG	GAAGACGTCCTCCATGCTTC
<i>phdb</i>	QFR1	AAGGGGAATGTGATGTTGGA	GCAAGCGTAACCTGTTCCAT
<i>phdb</i>	QFR3	CCTAACAGAGATGGGCGCTG	GATGGGTTCTACGTCCACGT
<i>tbx2/3</i>	QFR	ACTGCCGGTACAAGTTCCAC	GACACAGTTCTGCATCCATTG





**Supplementary Movie 1.** Z-stacks (1.01  $\mu\text{m}$ /section, 41 stacks) of the embryo showing in Fig. 1A.



**Supplementary Movie 2.** Time-lapse movie (images acquired every 10 min) of individual embryo injected with mRNAs encoding mCherry-HIF $\alpha$  and Histone-GFP.