# Supplementary Materials

**Table S1.** Primer sequences for methylation-specific PCR (MS-PCR), quantitative real-time PCR (q-PCR), double in situ hybridization (D-ISH) and dual-luciferase reporter assay.

| Primer name | Sequence (5′ to 3′) | Product size (bp) | Annealing temperature (℃) | Accession number |
|---|---|---|---|---|
| HIF-1$\alpha$ (MS-PCR) | F: TAGTAGTATTGTTTTAGAAAGTGAA<br>R: AACAAACCAAAATTAAAACTCTCTA | 234 | 50.7 | 109628061 |
| LDH-A (MS-PCR) | F: GGGATATAAGGTATGATTGTTTTATT<br>R: TCACACACAACTATTTATATTTCCTA | 243 | 57.0 | 109638975 |
| HIF-1$\alpha$ (q-PCR) | F: CATGGACACAGGAATTGTACCC<br>R: ATAGCTGATGGTGAGCCTCATG | 196 | 60.0 | XM_020084937.1 |
| LDH-A (q-PCR) | F: TGTCAAGTACAGCCCCAACTGC<br>R: TGGAGCTTCTCTCCCATGAGGT | 165 | 60.0 | XM_020102195.1 |
| 18S (q-PCR) | F: ATTGACGGAAGGGCACCAC<br>R: ATGCACCACCACCCACAGA | 134 | 60.0 | EF126037.1 |
| HIF-1$\alpha$ (D-ISH) | F: cgc-atttaggtgacactatagaagcgGATGTAATGCTTCCCTCCTCG<br>R: ccg-taatacgactcactatagggagacaTGCTACTTCCTACTTCGCTGA | 535 | 57.0 | XM_020084937.1 |
| LDH-A (D-ISH) | F: cgc-atttaggtgacactatagaagcgGGCAGCAAGAACAAGGTCACA<br>R: ccg-taatacgactcactatagggagacaGCTGGAAGGGTGGATGTGG | 555 | 57.0 | XM_020102195.1 |

| | | | | |
|---|---|---|---|---|
| pc3.1~HIF-1$\alpha$ | F: cttggtaccgagctcggatccATGGACACAGGAATTGTACCCG<br>R: ccacactggactagtggatccTCAGTTTACGTGGTCCAGTGCG | 2388 | 60.0 | XM_020084937.1 |
| pc3.1~HIF-1$\beta$ | F: cttggtaccgagctcggatccATGTTCTTCCACTCGGACATGTC<br>R: ccacactggactagtggatccTCACTCATTAAACGAAGGGTAGATTG | 2259 | 60.0 | XM_020092959.1 |
| pGL~LDH-A | F: ctatcgataggtaccgagctcTAAGAGGCTGACACCCCAAC<br>R: cagtaccggaatgccaagcttTAATTCCCGGATGAGCACGG | 805 | 62.0 | 109638975 |
| pGL~Lf2 | F: ctatcgataggtaccgagctcCTGCTCCACCAGGGACCATT<br>R: cagtaccggaatgccaagcttTAATTCCCGGATGAGCACGG | 415 | 62.0 | 109638975 |
| pGL~Lf1 | F: ctatcgataggtaccgagctcCCCAGCTCATTGCGTATGC<br>R: cagtaccggaatgccaagcttTAATTCCCGGATGAGCACGG | 275 | 62.0 | 109638975 |
| pGL~Lf0 | F: ctatcgataggtaccgagctcCGTTCACGGAGCAGCCTT<br>R: cagtaccggaatgccaagcttTAATTCCCGGATGAGCACGG | 246 | 62.0 | 109638975 |
| pGL~Lf2m | F: ctatcgataggtaccgagctcCTGCTCCACCAGGGACCATT<br>R: ATGTTGCCGGTAGGCAGGAT<br>F: ATCCTGCCTACCGGCAACAT<br>R: cagtaccggaatgccaagcttTAATTCCCGGATGAGCACGG | 407 | 62.0 | 109638975 |
| pGL~LDH-Am | F: ctatcgataggtaccgagctcTAAGAGGCTGACACCCCAAC<br>R: ATGTTGCCGGTAGGCAGGAT<br>F: ATCCTGCCTACCGGCAACAT<br>R: cagtaccggaatgccaagcttTAATTCCCGGATGAGCACGG | 797 | 62.0 | 109638975 |

**A**
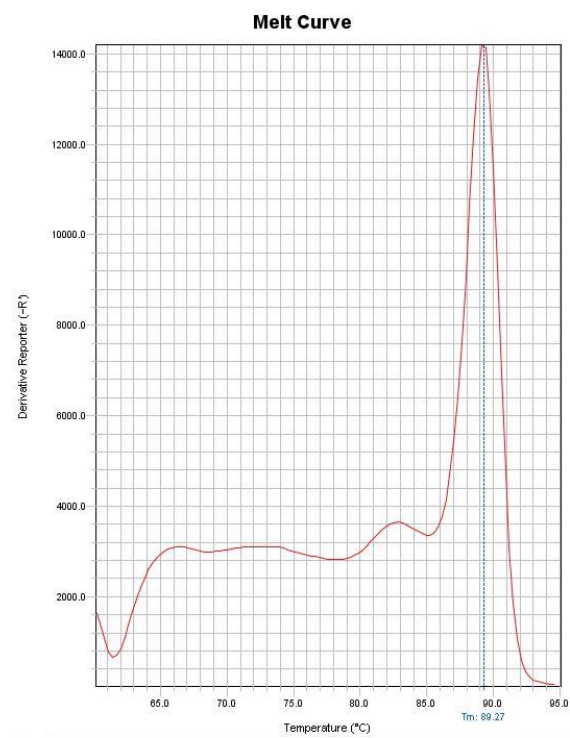


**B**
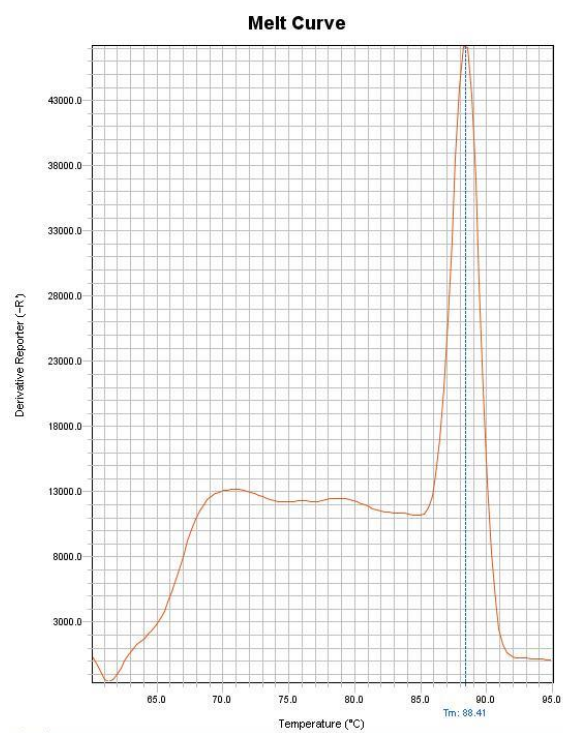


**C**
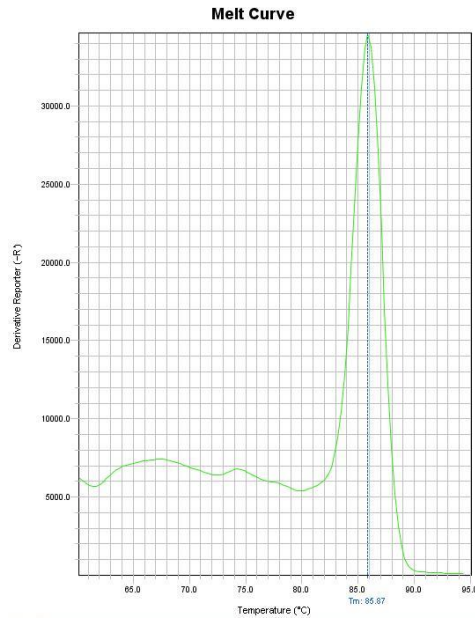
**Figure S1.** The melt curves of primers for three genes *HIF-1α* (**A**), *LDH-A* (**B**), *18S* (**C**) in

q-PCR.

**Table S2.** The gene sequence analysis results of *HIF-1α* and *LDH-A*.

| Gene | Length (bp) | 5′ UTR (bp) | Intron (number, bp) | Exon (number, bp) | 3′ UTR (bp) | mRNA (bp) | Gene ID |
|------|------|------|------|------|------|------|------|
| *HIF-1α* | 12737 | 262 | 14, 9069 | 15, 2346 | 1060 | 3668 | 109628061 |
| *LDH-A* | 5999 | 134 | 7, 3815 | 7, 999 | 1051 | 2184 | 109638975 |
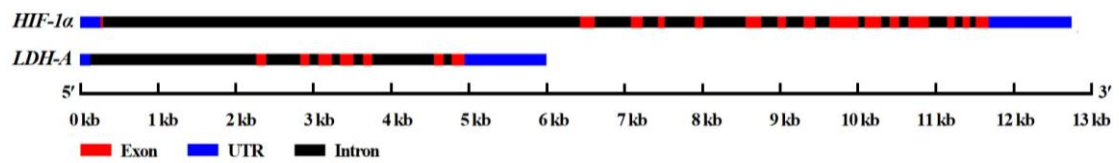


**Figure S2.** The gene sequence analysis results of *HIF-1α* and *LDH-A*. The red rectangles and

black rectangles represent exon and intron sequences, respectively. 5′ UTR and 3′ UTR are

expressed by blue rectangles on the left and right, respectively.

**Table S3.** The protein sequence information of HIF-1α and LDH-A.

| Protein | amino acid (aa) | Molecular weight (kDa) | Isoelectric point (pI) | Domain (number) | Transmembrane helices | Accession number |
|---------|-----------------|------------------------|------------------------|-----------------|------------------------|------------------|
| HIF-1α | 781 | 87463.97 | 4.90 | 7 | 0 | XP_019940496.1 |
| LDH-A | 332 | 36368.38 | 7.75 | 1 | 0 | XP_019957754.1 |

All "0" in the table indicates none. Values in the column of "Domain" represent the number of domains. Their names and amino acid sites (start - end) were as follows. The 7 domains in HIF-1α protein were Helix loop helix domain (HLH): 22 - 77; PAS domain (PAS): 88 - 154, 230 - 296; Motif C-terminal to PAS motifs (PAC): 302 - 345; Coiled coil region (coiled coil): 355 - 382; Low complexity region (low complexity): 394 - 414, 486-506. The 1 domain in LDH-A protein was low complexity: 25 - 39.
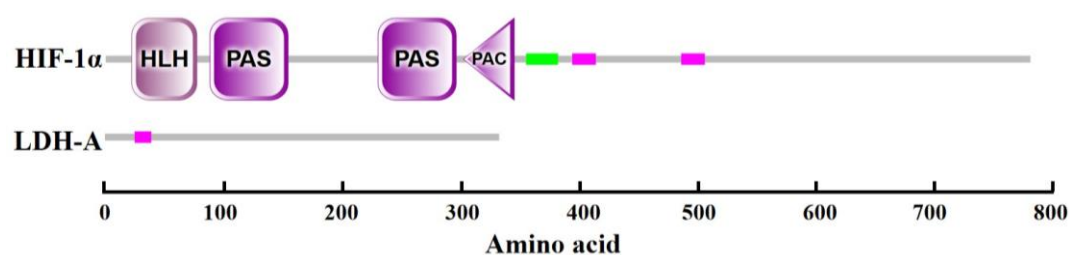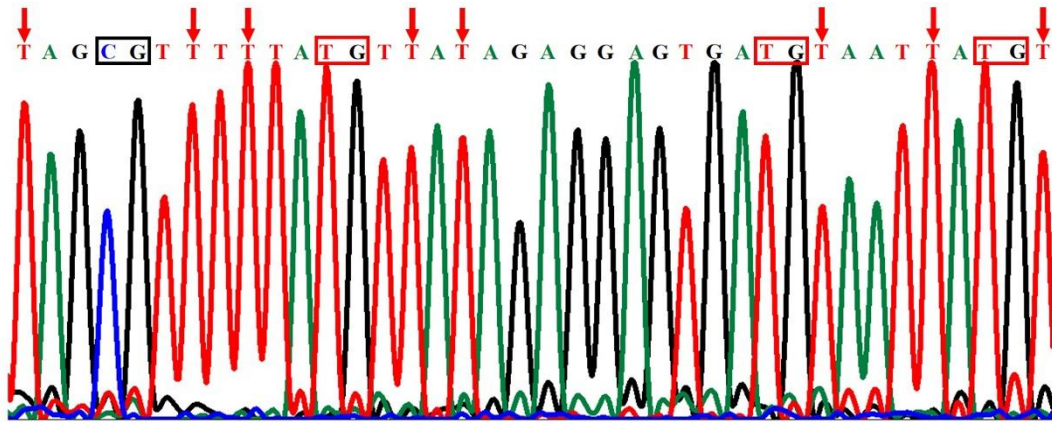


**Figure S3.** Domain analyses of HIF-1α and LDH-A protein. Domains are same as those in Table S3. The green and magenta boxes represented coiled coil and low complexity, respectively.

**A**
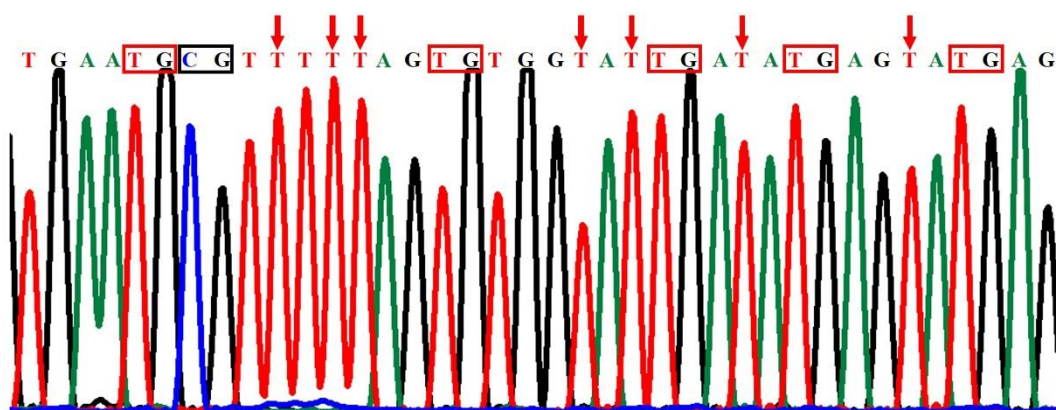


**B**

**Figure S4.** The part DNA sequences of *HIF-1α* gene (**A**) and *LDH-A* gene (**B**) modified by bisulfite. Red arrows denote the bases which are successfully modified from cytosines to thymines by bisulfite. Similarly, the red boxes indicate successfully modified bases which are unmethylated cytosines (changed from cytosines to thymines) in CpG dinucleotides, and the black boxes mean unmethylated cytosines (not changed from cytosines to thymines) in CpG dinucleotides.
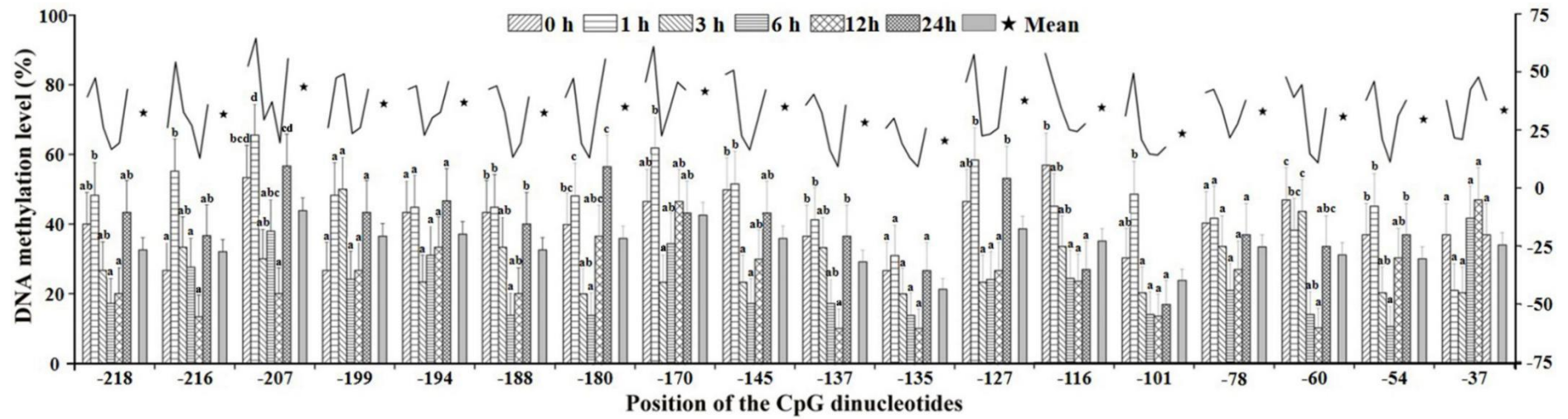
**Figure S5.** The single CpG dinucleotides methylation level of *HIF-1α* gene in the six treatment groups of Japanese flounder (*Paralichthys olivaceus*). The columns which are following the left ordinate represent the methylation level values of 18 CpG dinucleotides in six hypoxia treatment groups, and the lines following the right ordinate show the methylation level changing trends of them. Furthermore, the five-pointed stars (★) denote the methylation level average values, which follow the right ordinate, of corresponding CpG dinucleotides. In addition, different letters above the columns indicate significant statistical differences ($P < 0.05$).
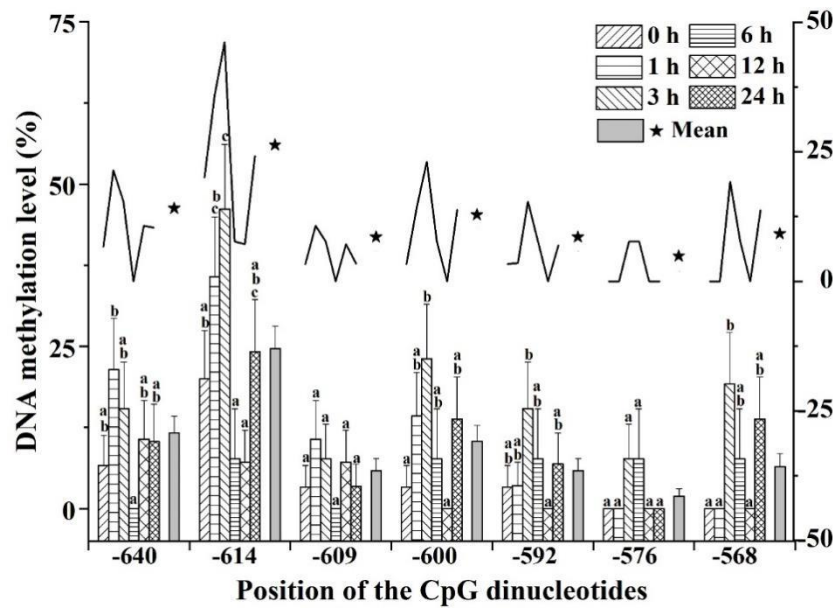
**Figure S6.** The single CpG dinucleotides methylation level of *LDH-A* gene in the six hypoxia treatment groups of Japanese flounder. The columns which are following the left ordinate represent the methylation level values of 7 CpG dinucleotides in six hypoxia treatment groups, and the lines following the right ordinate show the methylation level changing trends of them. Furthermore, the five-pointed stars (★) denote the methylation level average values, which follow the right ordinate, of corresponding CpG dinucleotides. Different letters above the columns indicate significant statistical differences ($P < 0.05$).

**Table S4.** Linear regression analysis results of expression and 18 single CpG dinucleotides methylation level in *HIF-1α* gene.

| CpG site | $R^2$ | $P$ | Significance level | Function |
|---|---|---|---|---|
| **-218** | **0.700** | **0.038** | * | **Y = 5.84 - 0.10X** |
| -216 | 0.270 | 0.291 | n.s. | --- |
| -207 | 0.369 | 0.201 | n.s. | --- |
| -199 | 0.252 | 0.310 | n.s. | --- |
| -194 | 0.254 | 0.308 | n.s. | --- |
| **-188** | **0.906** | **0.003** | ** | **Y = 6.33 - 0.12X** |
| -180 | 0.344 | 0.221 | n.s. | --- |
| -170 | 0.195 | 0.381 | n.s. | --- |

| | | | | |
|---|---|---|---|---|
| **-145** | **0.709** | **0.035** | ***** | **Y = 5.83 - 0.09X** |
| **-137** | **0.688** | **0.041** | ***** | **Y = 5.57 - 0.11X** |
| **-135** | **0.687** | **0.041** | ***** | **Y = 5.93 - 0.16X** |
| -127 | 0.486 | 0.124 | n.s. | --- |
| **-116** | **0.710** | **0.035** | ***** | **Y = 6.00 - 0.10X** |
| -101 | 0.576 | 0.080 | n.s. | --- |
| **-78** | **0.948** | **0.001** | ****** | **Y = 8.91 - 0.19X** |
| **-60** | **0.753** | **0.025** | ***** | **Y = 5.26 - 0.09X** |
| -54 | 0.638 | 0.057 | n.s. | --- |
| -37 | 0.354 | 0.213 | n.s. | --- |

The word n.s. indicates $P > 0.05$, and the signs *, ** represent $P < 0.05$, $P < 0.01$ respectively.

**Table S5.** Linear regression analysis results of expression and 7 single CpG dinucleotides methylation level in *LDH-A* gene.

| CpG site | $R^2$ | $P$ | Significance level |
|---|---|---|---|
| -640 | 0.068 | 0.618 | n.s. |
| -614 | 0.012 | 0.835 | n.s. |
| -609 | 0.105 | 0.530 | n.s. |
| -600 | 0.121 | 0.498 | n.s. |
| -592 | 0.188 | 0.391 | n.s. |
| -576 | 0.373 | 0.198 | n.s. |
| -568 | 0.296 | 0.265 | n.s. |

The word n.s. indicates $P > 0.05$.