Supplementary Figure S1

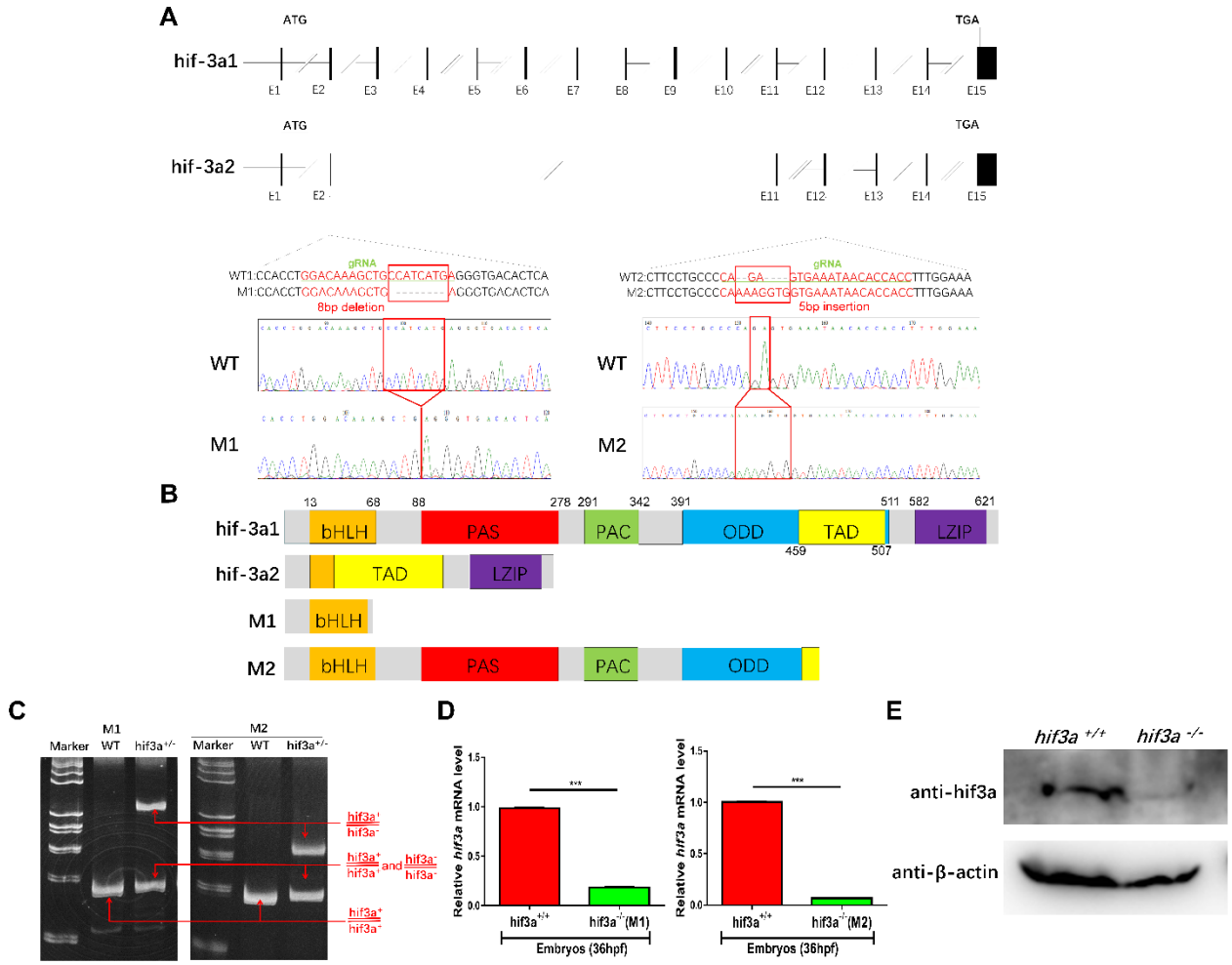


Figure S1. Generation of *hif-3a*$^{-/-}$ zebrafish using CRISPR/Cas9 technology. (A) Schematic of the targeting site in *hif-3a* and the resulting nucleotide sequence in mutant 1 (M1: *Hif-3a*$^{ihb20180620/ihb20180620}$) and mutant 2 (M2: *hif-3a*$^{ihb20180621/ihb20180621}$). (B) The predicted protein products of *hif-3a* in the mutants and their wild-type siblings. (C) Verification of the efficiency of CRISPR/Cas9-mediated disruption of zebrafish *hif-3a* disruption by heteroduplex mobility assay (HMA). (D) The relative mRNA expression levels of *hif-3a* in the wild-type zebrafish and the homozygous mutants (10 embryos for each, 3 replicates; 36 hpf) were quantified by qRT-PCR. (E) *hif-3a* protein level in the wild-type and homozygous mutant embryos (200 embryos) under normoxic conditions detected by anti-hif-3a antibody. *** $p < 0.001$.

Supplementary Figure S2

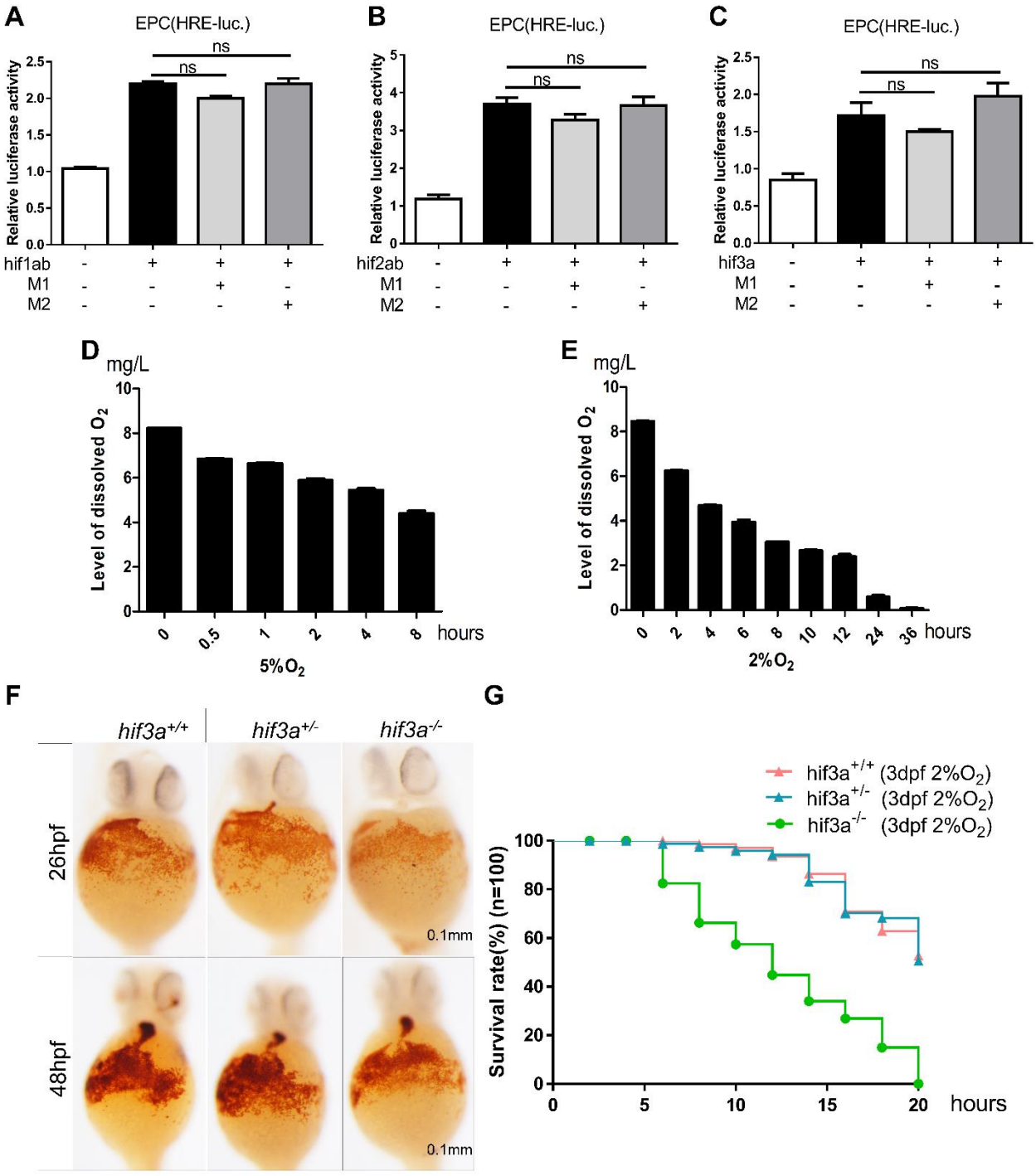


Figure S2. (A, B, C) The predicted truncated proteins in M1 and M2 mutants had no effect on the transcriptional activity of *hif-1ab, hif-2ab* and *hif-3a.* (D, E) The actual levels of dissolved O_2 in water were measured with an LDO101 probe at different time points when the flasks were put into Invivo2 Hypoxia workstation set at 5% O_2 and 2% O_2 respectively (3 replicates). (F) O-dianisidine staining of functional hemoglobin in the mature primitive erythrocytes in *hif-3a*$^{+/+}$ (left), *hif-3a*$^{+/-}$ (middle) and *hif-3a*$^{-/-}$ zebrafish (right) at 26 hpf and 48hpf. (G) The survival rate curve of *hif-3a*$^{-/-}$ zebrafish larvae, *hif-3a*$^{+/-}$ zebrafish larvae and their WT siblings (100 larvae). The oxygen concentration of the hypoxia workstation (Ruskinn INVIVO2 400) was adjusted to 2% prior to experimentation. The dead larvae were counted once every two hours. M1, mutant1; M2, mutant 2; hpf, hours post-fertilization; dpf, days post-fertilization.

Supplementary Figure S3

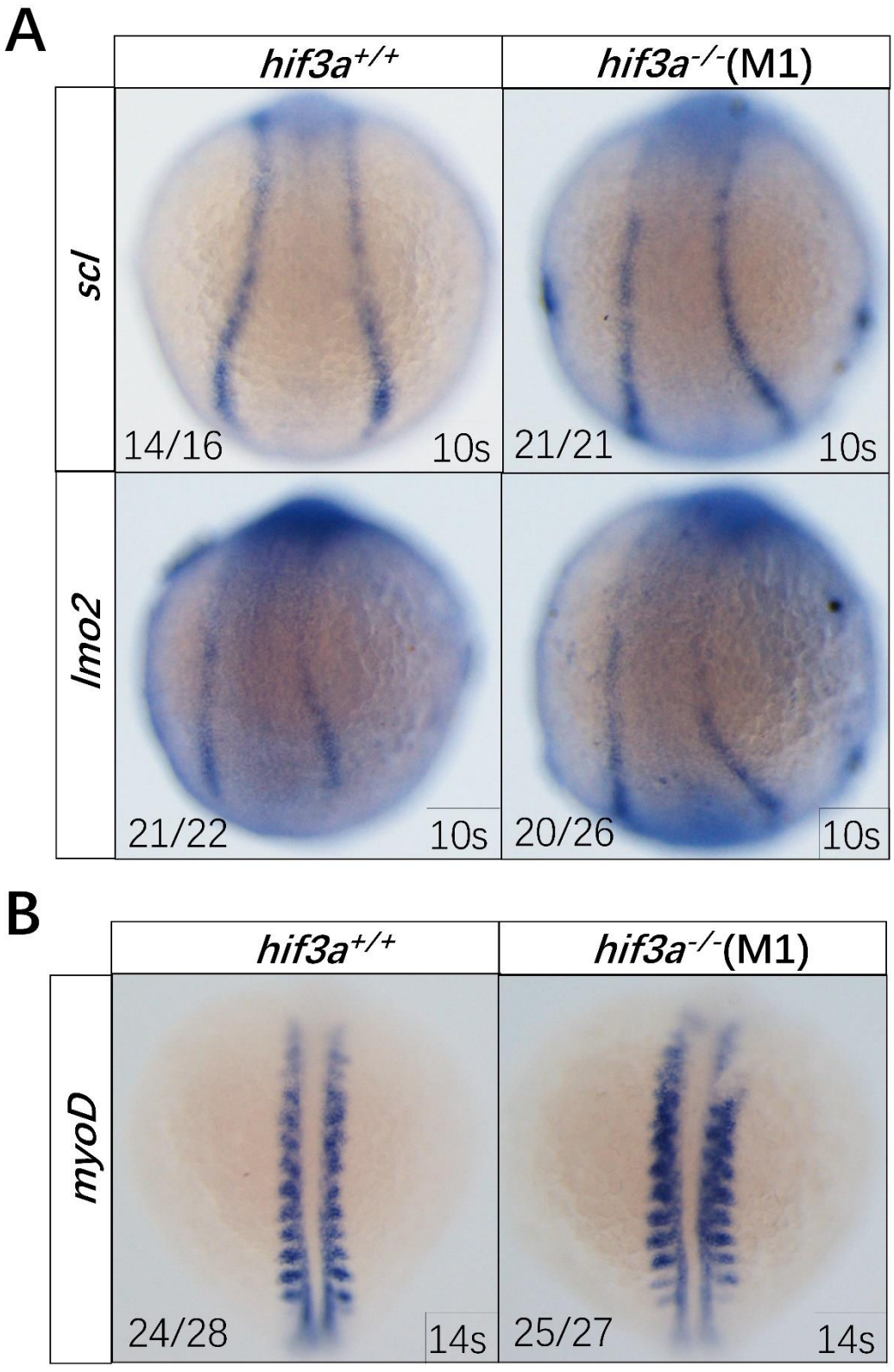


Figure S3. (A) Disruption of *hif-3a* did not alter the expression of *scl* and *lmo2* at the 10 s stage in the posterior lateral mesoderm. (B) Disruption of *hif-3a* did not alter the expression of *myoD* (the somatic mesoderm marker) at 14 s. The number of stained embryos was indicated in the left lower corner of each representative picture. M1, mutant 1; s, somite.

Supplementary Figure S4

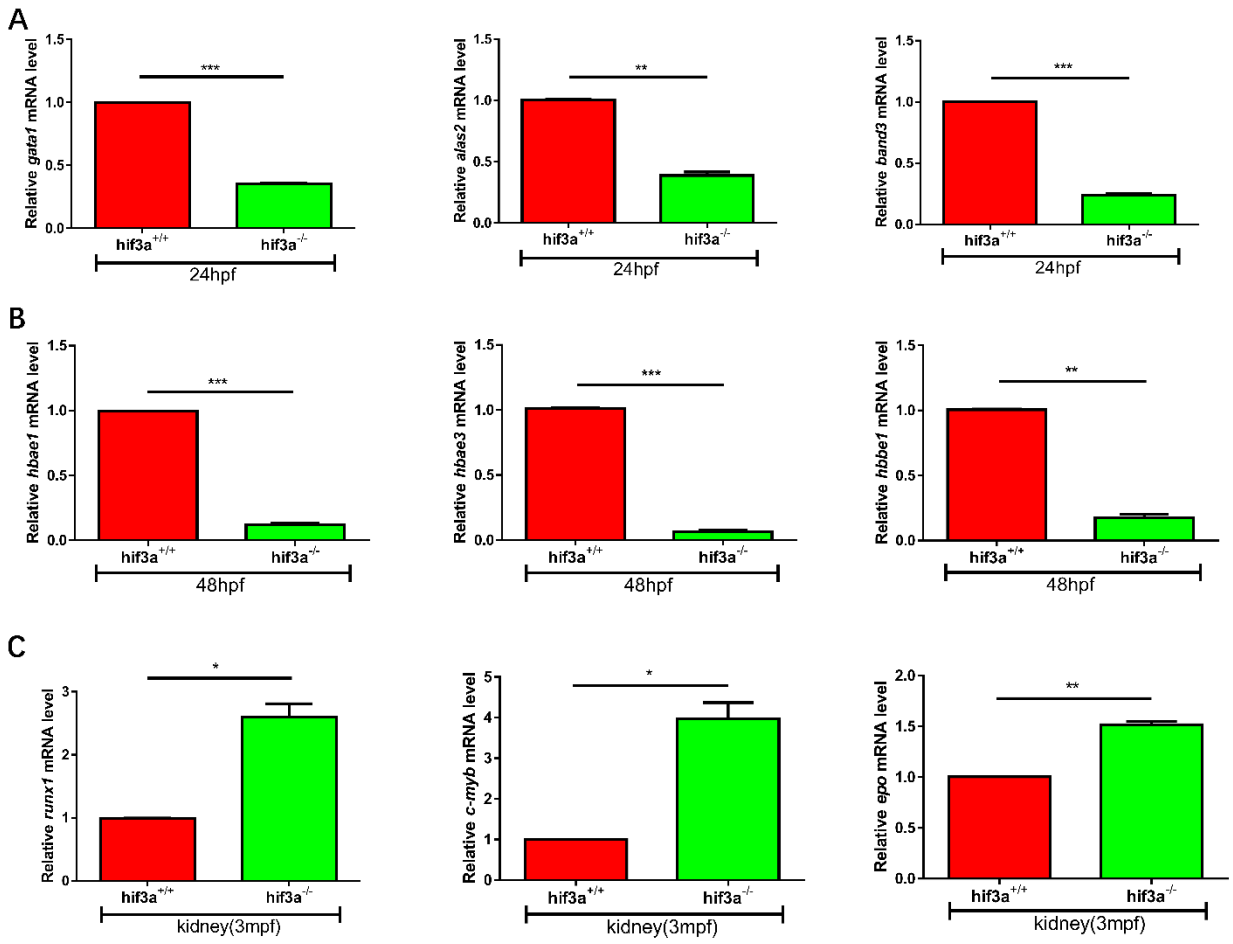


Figure S4. (A) qRT-PCR assays confirmed that the expression levels of *gata1*, *alas2*, and *band3* were reduced in *hif-3a*-null larvae at 24 hpf (30 embryos for each, 3 replicates). (B) Quantitative RT-PCR assays confirmed that the expression levels of *hbae1*, *hbae3* and *hbbe1* were reduced in *hif-3a*-null larvae at 48 hpf (30 embryos for each, 3 replicates). (C) qRT-PCR assays showing that the expression levels of *runx1, c-myb* and *epo* were increased in *hif-3a*-null kidneys at 3 mpf (3 replicates). hpf, hours post-fertilization; mpf, months post-fertilization. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Figure S5

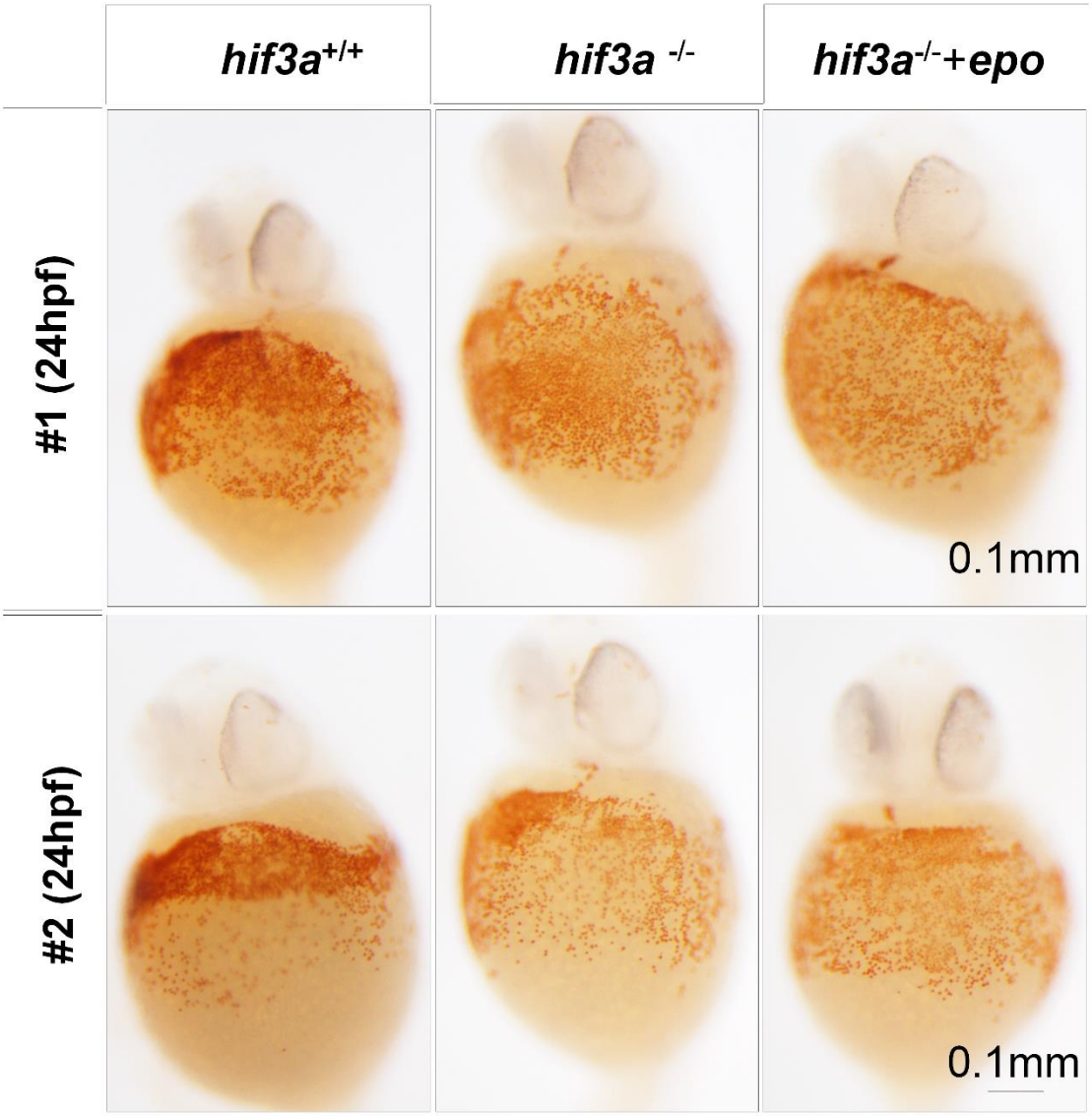


Figure S5. O-dianisidine staining indicated that co-injection of *epo* mRNA (500 pg/embryo) could not restore hemoglobin levels in *hif-3a*$^{-/-}$ larvae at 24 hpf. hpf, hours post-fertilization.

Supplementary Figure S6

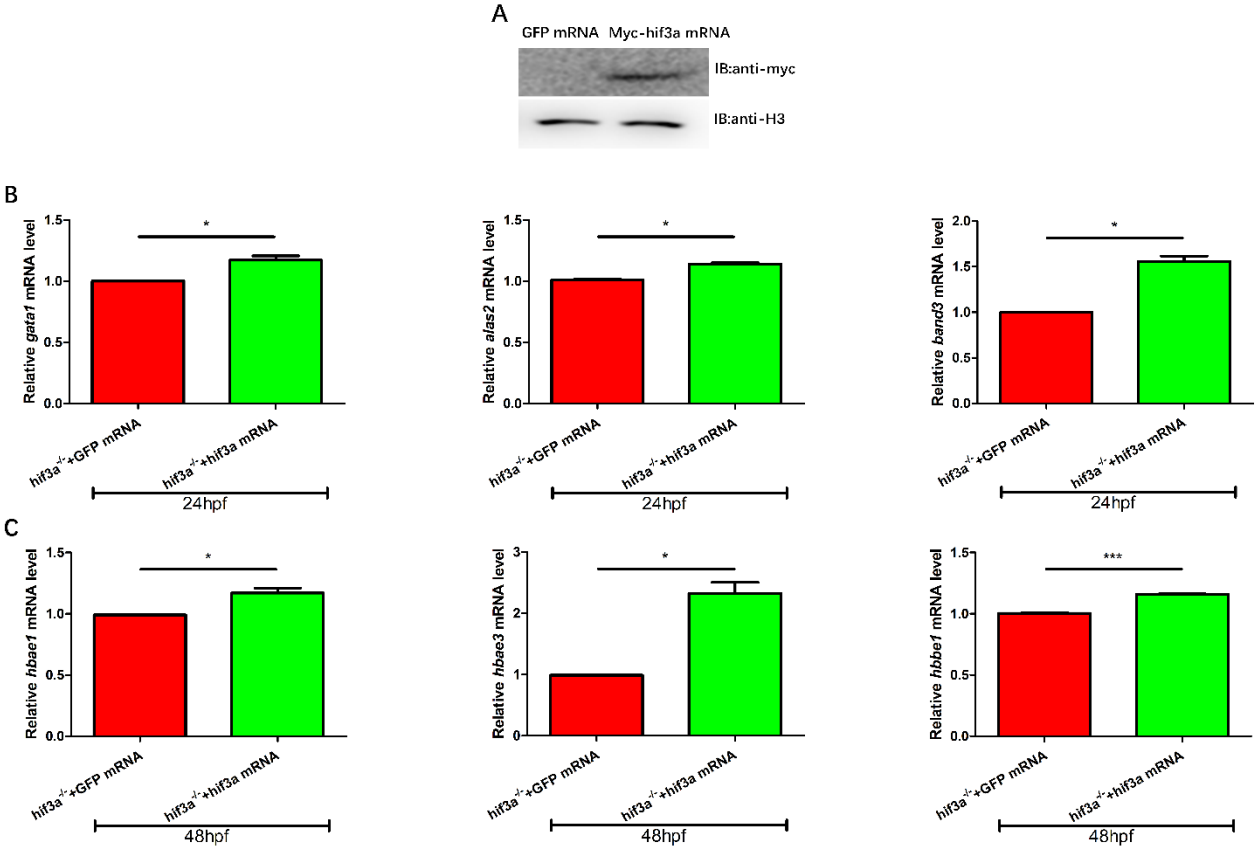


Figure S6. (A) Expression of myc-hif-3a in the injected embryos was confirmed by western blot assays (200 embryos). (B) qRT-PCR confirmed the restoration of *gata1*, *alas2* and *band3* by injection of *hif-3a* mRNA in *hif-3a*-null embryos at 24 hpf as compared with by the injection of the GFP mRNA control. (C) qRT-PCR confirmed the restoration of *hbae1*, *hbea3* and *hbbe1* by the injection of *hif-3a* mRNA in *hif-3a*-null embryos at 48 hpf as compared to the injection of the GFP mRNA control. IB, immunoblotting; hpf, hours post-fertilization. 30 embryos for each, 3 replicates; * $p < 0.05$;*** $p < 0.001$.

Supplementary Figure S7

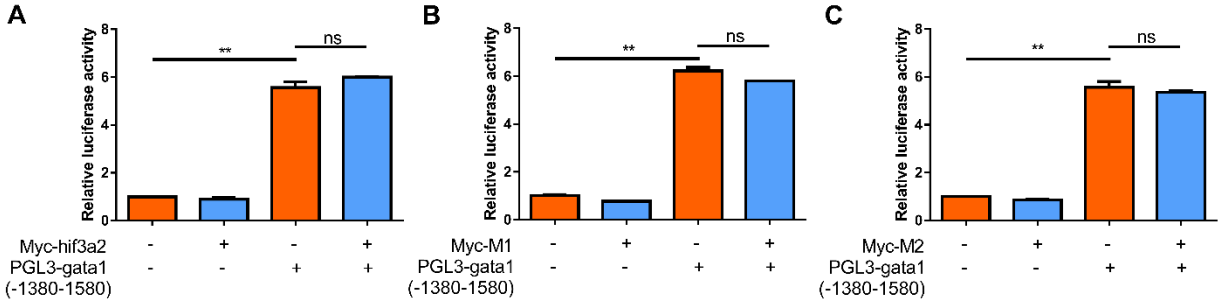


Figure S7. Luciferase reporter assays indicate that *hif-3a2*(A), *hif-3a* mutant M1(B) and M2(C) could not active *gata1* promoter in EPC cells. ** $p < 0.01$; ns, no significance.

Supplementary Figure S8

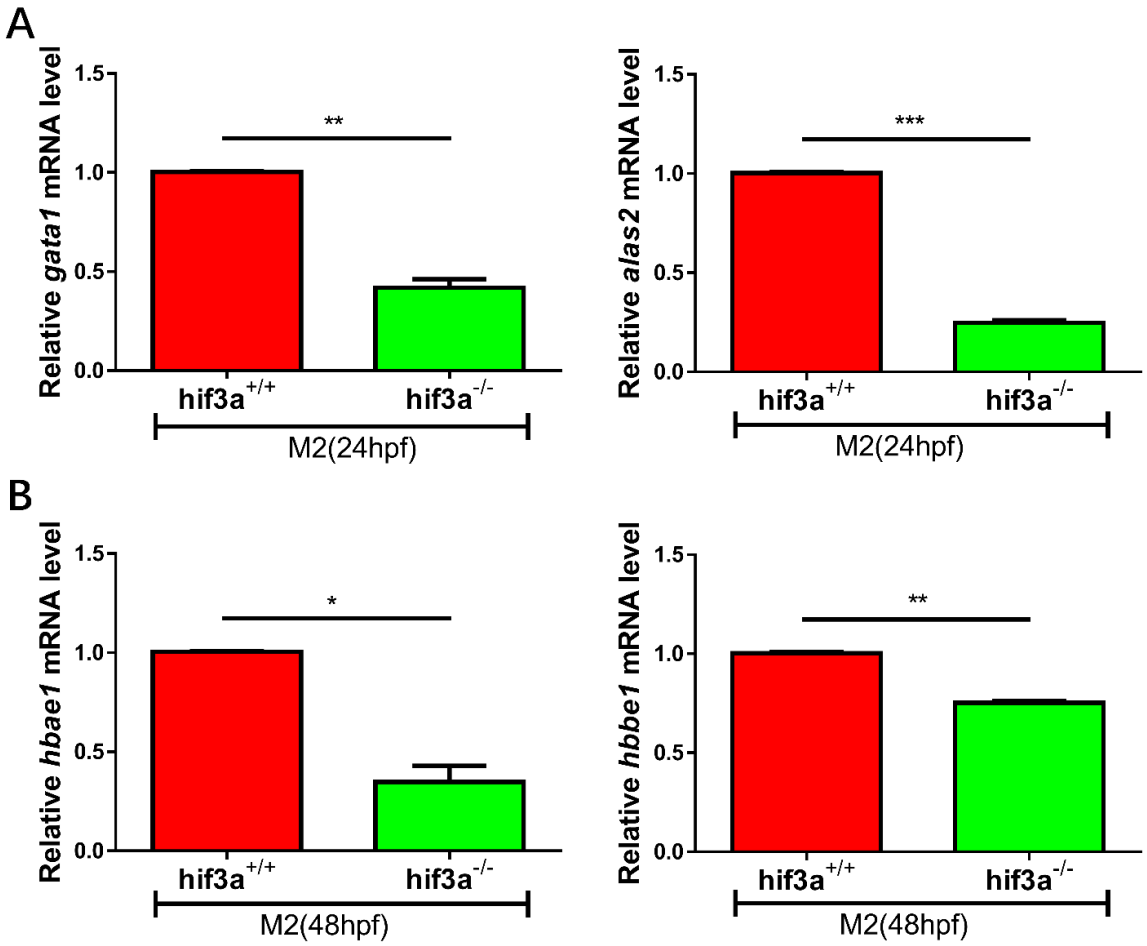


Figure S8. (A) Expression levels of erythrocytic markers *gata1* and *alas2* in wild-type and *hif3a*$^{-/-}$ (M2: *hif1al*$^{ihb20180621/ihb20180621}$) zebrafish larvae at 24 hpf. (B) Expression levels of *hbae1* and *hbbe1* were quantified by qRT-PCR at 48hpf. Values graphed are the means of three independent experiments performed in triplicates; error bars indicate the standard error of the mean (S.E.M.). hpf, hours post-fertilization. n=30; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Figure S9

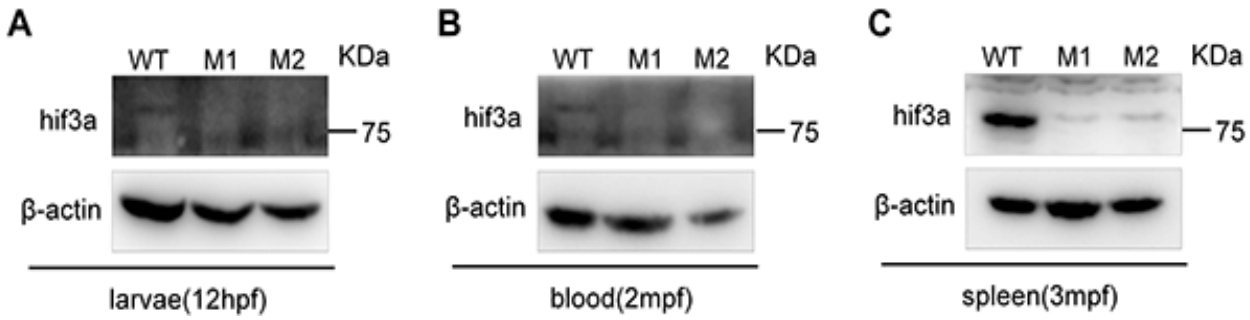


Figure S9. (A, B, C) Western blot analysis of hif-3a protein level in larvae (12hpf; 200 embryos), blood (2mpf; 3 zebrafish for each, 3 replicates) and spleens (3mpf; 3 zebrafish for each; 3 replicates) from wild-type and M1, M2 mutants. WT, wildtype; M1, mutant 1; M2, mutant 2; mpf, months post-fertilization.

Supplementary Figure S10

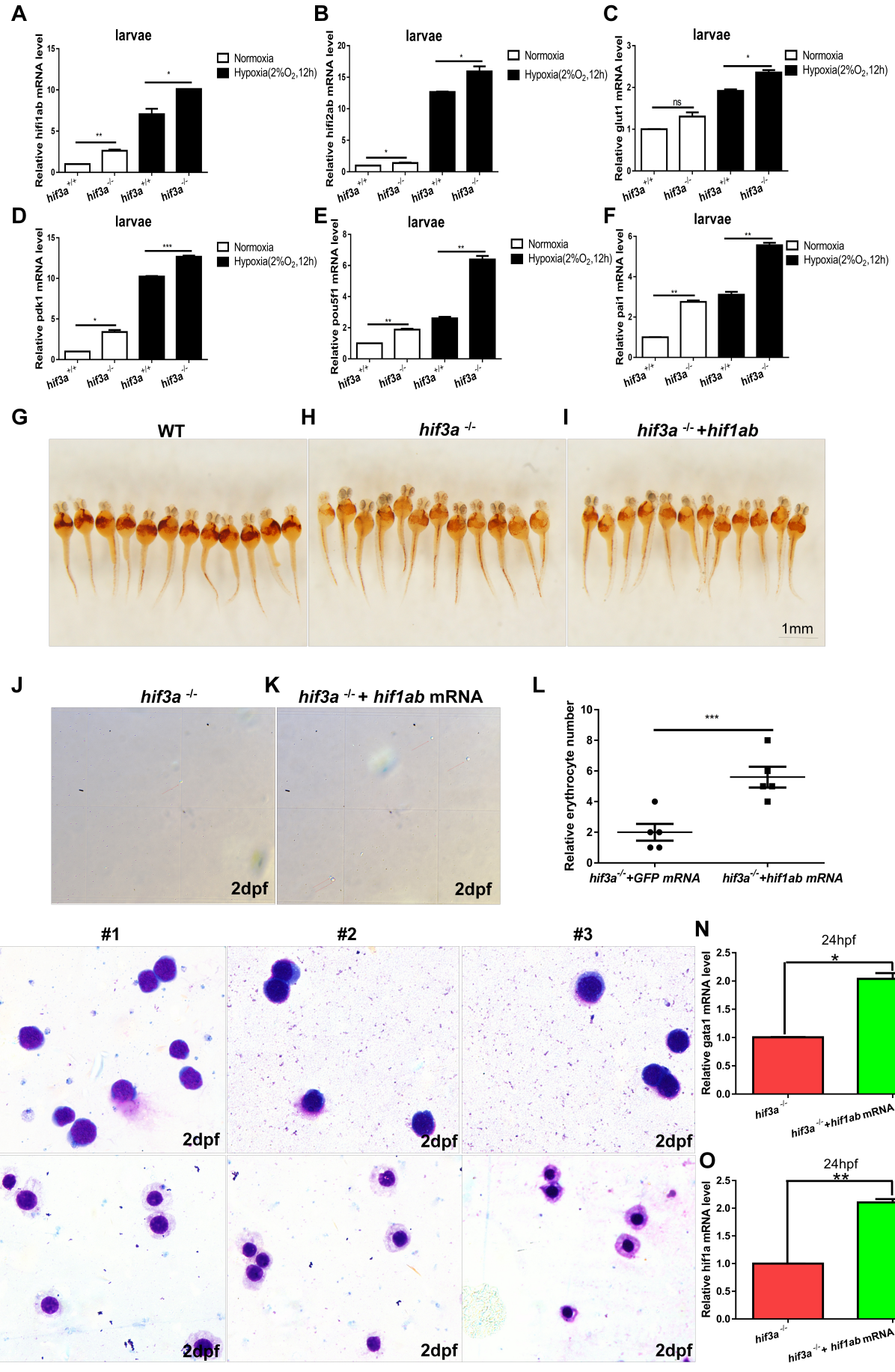


Figure S10. (A, B) qRT-PCR analysis of *hif1a*(A) and *hif2a*(B) expression in WT(*hif-3a*$^{+/+}$) and *hif- 3a*-null(*hif-3a*$^{-/-}$) zebrafish embryo (10 embryos for each, 3 replicates; 3dpf) under normoxia and hypoxia (2% O_2 for 12 hours). (C-F) Expression levels of the *hif-1a* down-stream targets *glut1*(C), *pdk1*(D) and *hif-2a* down-stream targets *pou5f1*(E), *pai1*(F) were increased in *hif-3a*$^{-/-}$ larvae (10 embryos for each, 3 replicates; 3 dpf) under normoxia and hypoxia (2% O_2 for 12 hours). (G-I) O-dianisidine staining indicated that co-injection of *hif-1ab* mRNA (500 pg/embryo) partially restored hemoglobin levels in *hif-3a*$^{-/-}$ larvae at 2 dpf. (J-K) Erythrocyte number counting indicated that co-injection of *hif-1ab* mRNA (500 pg/embryo) increased erythrocyte in *hif-3a*$^{-/-}$ larvae at 2 dpf. (M) May-Grunwald-Giemsa staining indicated that co-injection of *hif-1ab* mRNA (500 pg/embryo) restored erythrocytic maturation in *hif-3a*$^{-/-}$ larvae at 2 dpf. (N) Co-injection of *hif-1ab* mRNA (500 pg/embryo) restored gata1 expression in *hif-3a*$^{-/-}$ embryos at 24 hpf. (O) Expression of micro-injected *hif-1ab* mRNA in *hif-3a* $^{-/-}$ embryos at 24 hpf was confirmed by qRT-PCR. Hpf, hours post fertilization; dpf, days post-fertilization. *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

Supplementary Figure S11

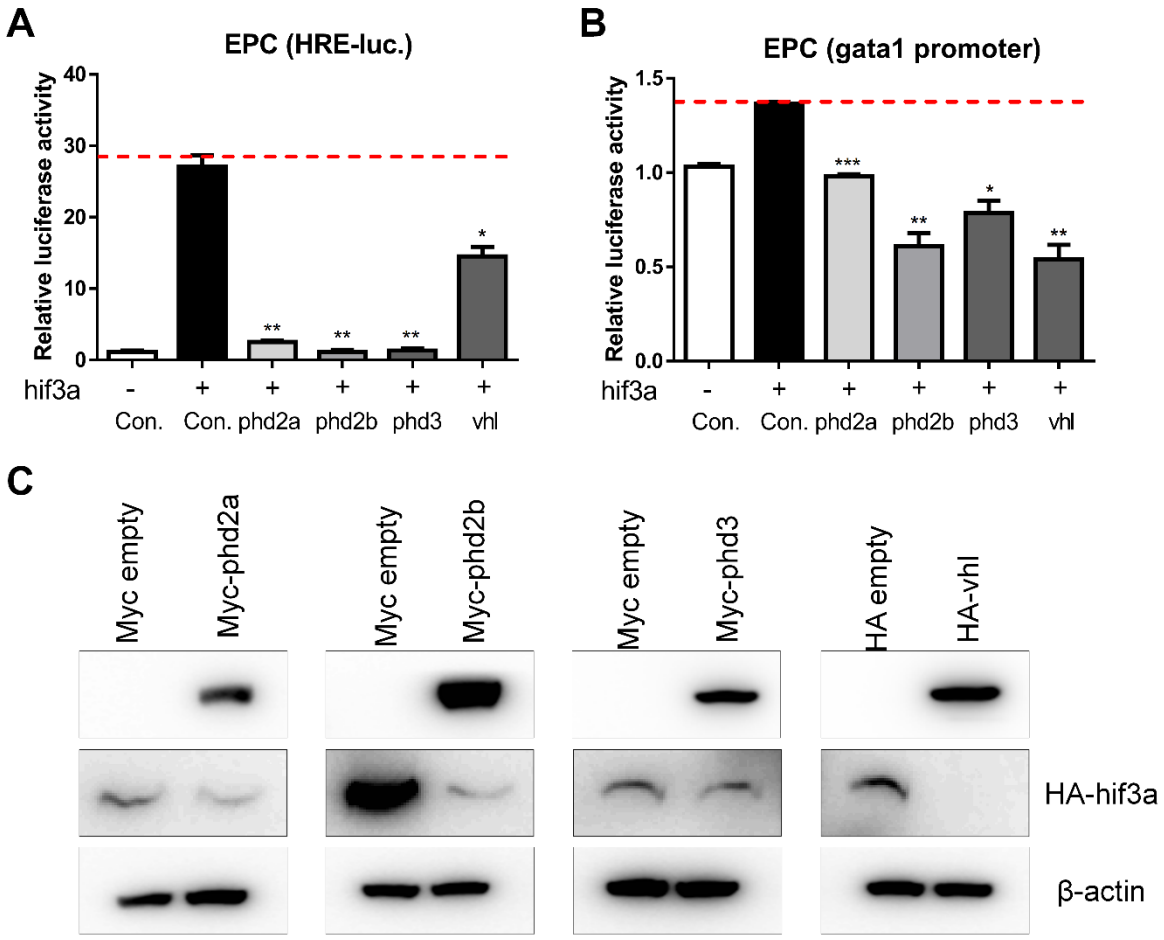
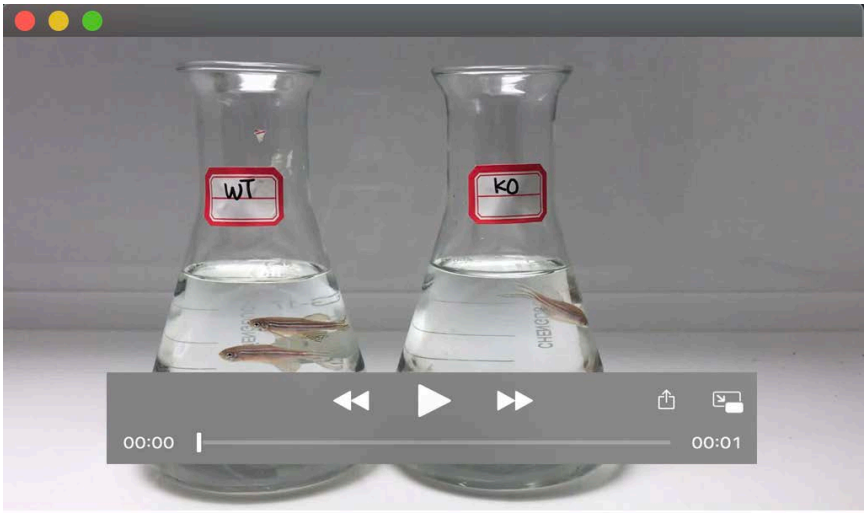


Figure S11. (A) Co-expression of *phd2a, phd2b, phd3* and *vhl* together with *hif-3a* suppressed the activity of HRE luciferase reporter induced by hif-3a in EPC cells. (B) Co-expression of *phd2a, phd2b, phd3* and *vhl* together with *hif-3a* suppressed the activity of gata1 promoter luciferase reporter induced by hif-3a in EPC cells. *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$. (C) Western blot analysis indicated that hif-3a protein level was decreased when *phd2a, phd2b, phd3* or *vhl* were co-expressed in EPC cells. Con, control. Myc empty, pCMV-Myc empty vector.



Movie 1. Wildtype (left, WT) and *hif3-a* null (right, KO) zebrafish (3 mpf, body weight = 0.32 ± 0.02 g) placed in a hypoxia workstation at the beginning (5% O_2) (before 30 min).

Movie 2. Wildtype (left, WT) and *hif3-a* null (right, KO) zebrafish (3 mpf, body weight = 0.32 ± 0.02 g) placed in a hypoxia workstation for a while (5% O_2).