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⁻/⁻; hif-3aihb20180621/ihb20180621) and mutant 2 (M2: hif-3a⁻/⁻; hif-3aihb20180621/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

A

EPC(HRE-luc.)

Relative luciferase activity

ns

- + + +
hi3ab
M1
M2

B

EPC(HRE-luc.)

Relative luciferase activity

ns

- + + +
hi3ab
M1
M2

C

EPC(HRE-luc.)

Relative luciferase activity

ns

- + + +
hi3ab
M1
M2

D

mg/L

Level of dissolved O₂

5% O₂

0 0.5 1 1.5 2 6 6 hours

E

mg/L

Level of dissolved O₂

2% O₂

0 0.5 1 1.5 2 6 6 hours

F

hi3a+/+  hi3a−/−  hi3a−−

28hpf

0.1mm

48hpf

0.1mm

G

Survival rate (%)(n=10)

hi3a+/+(3dpf 2%O₂)

hi3a−/+ (3dpf 2%O₂)

hi3a−− (3dpf 2%O₂)

hours

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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.
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.
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.
**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.

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<th>hif3a⁺⁺⁺</th>
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**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 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-3a*2(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<sup>−/−</sup> (M2: hif1al<sup>hhb20180621/ihb20180621</sup>) 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

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

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**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% O₂ 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% O₂ 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.
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% O₂) (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% O₂).