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Supplementary Materials

Hypoxia tolerance in fish depends on catabolic preference between lipids and carbohydrates

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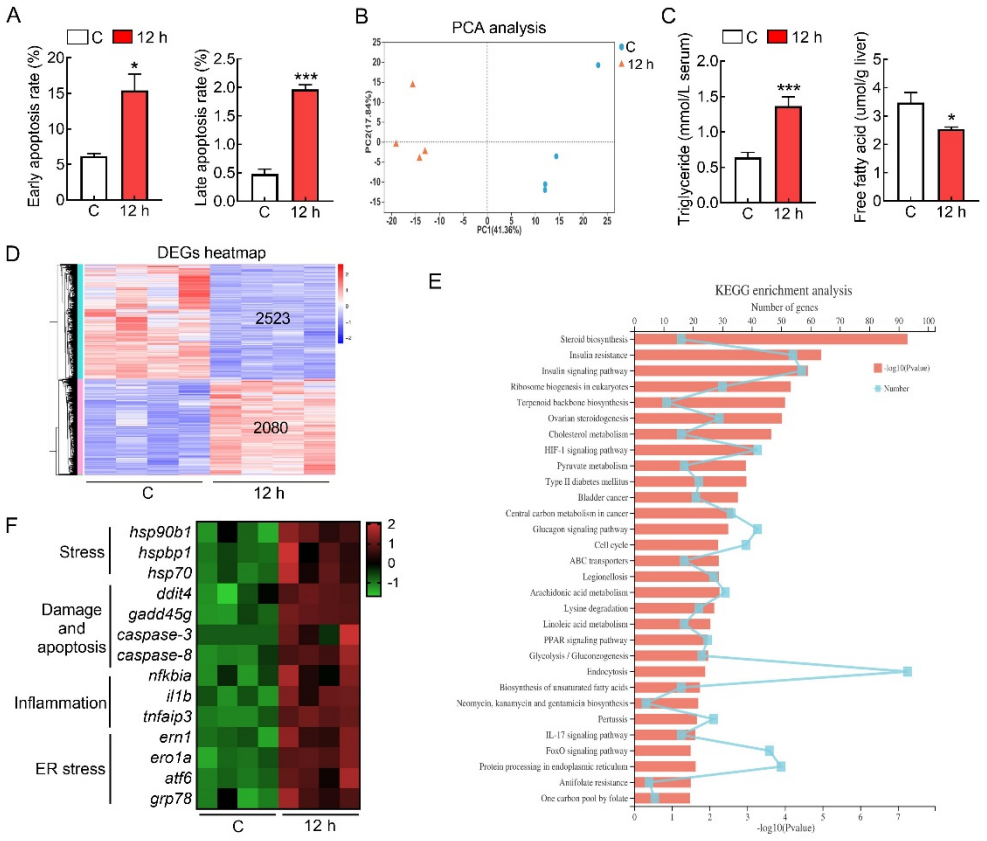
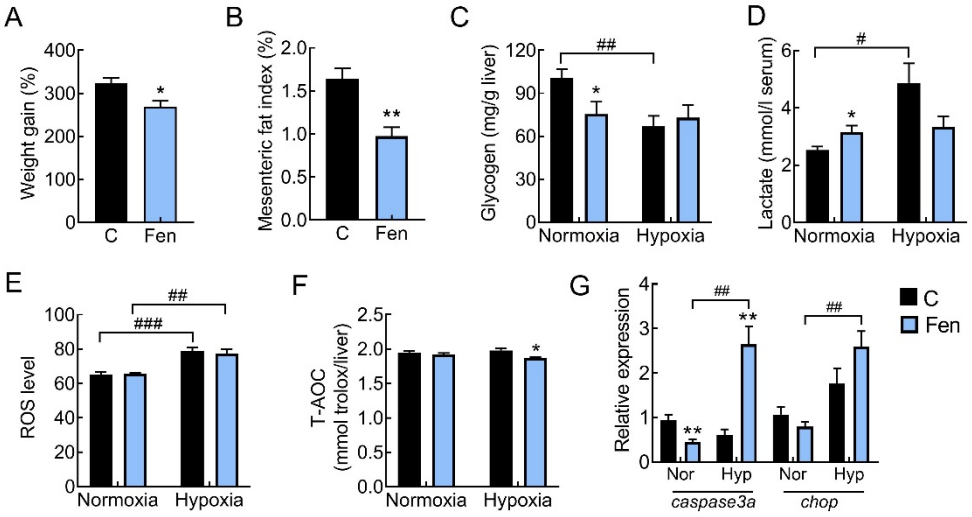


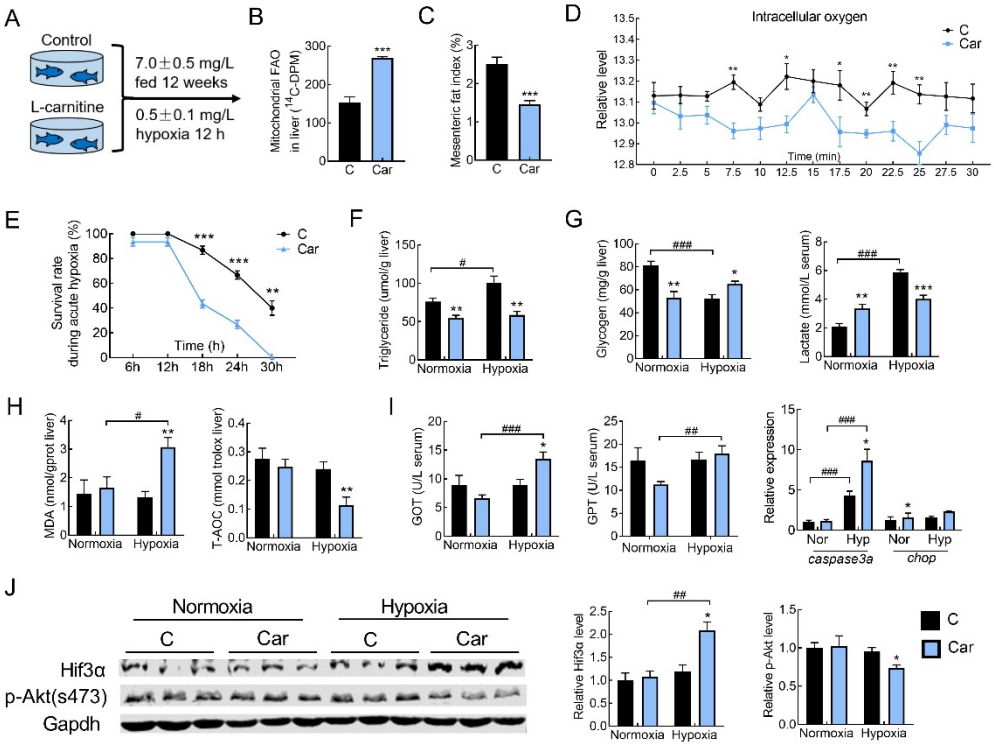
Supplementary Figure S1 Effects of acute hypoxia on apoptosis and metabolism in tilapia

A: Proportion of early and late apoptosis in tilapia primary hepatocytes after exposure to normoxic (C) and hypoxic conditions after 12 h (12 h). B: Principal component analysis (PCA) of RNA-seq. C: Triglyceride (TG) content in serum and free fatty acid (FFA) content in liver. D: All differentially expressed genes (DEGs) from RNA-seq. E: KEGG enrichment analysis of RNA-seq data. F: DEGs of stress and apoptosis. *, **, and *** indicate significant differences (P<0.05), (P<0.01), and (P<0.001), respectively, between C and hypoxia 12 h groups.



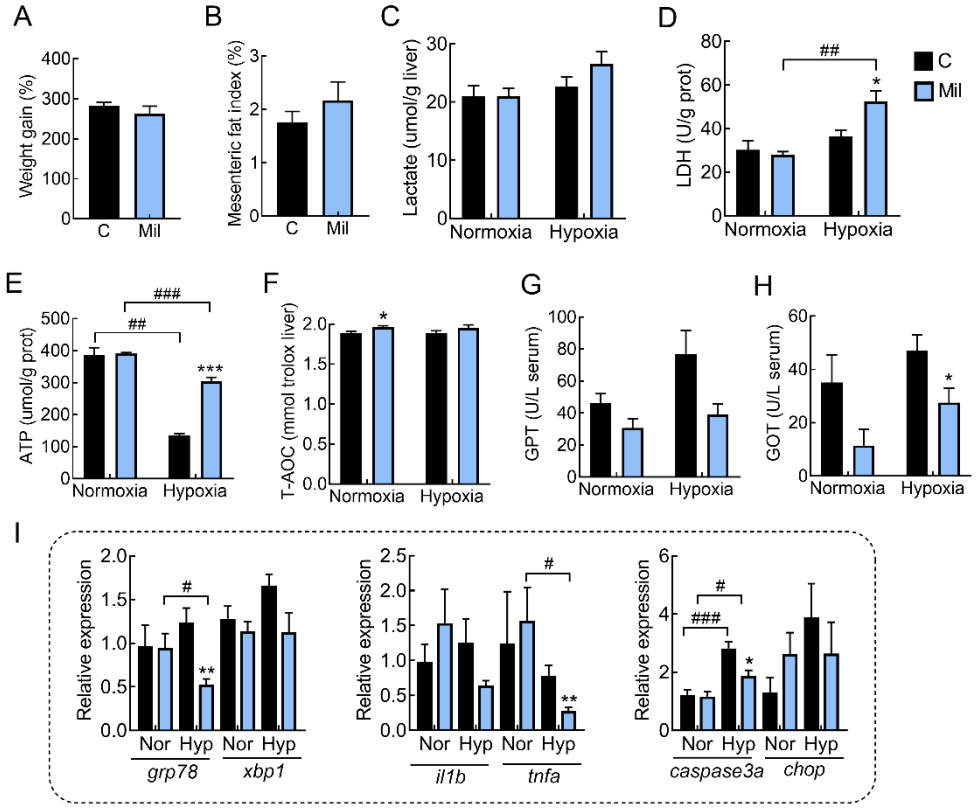
Supplementary Figure S2 Effects of enhanced lipid catabolism (fenofibrate) on tolerance to acute hypoxia in Nile tilapia

A: Weight gain. B: Mesenteric fat index. C: Glycogen content in liver. D: Lactate content in serum. E: Reactive oxygen species (ROS) content in liver. F: Total antioxidant capacity (T-AOC) in liver. G: Gene expression of apoptosis in liver. *, **, and *** indicate significant differences (P<0.05), (P<0.01), and (P<0.001), respectively, between C and Fen diets. $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$ indicate significant differences (P<0.05), (P<0.01), and (P<0.001) between Nile tilapia under normoxic and hypoxic conditions.



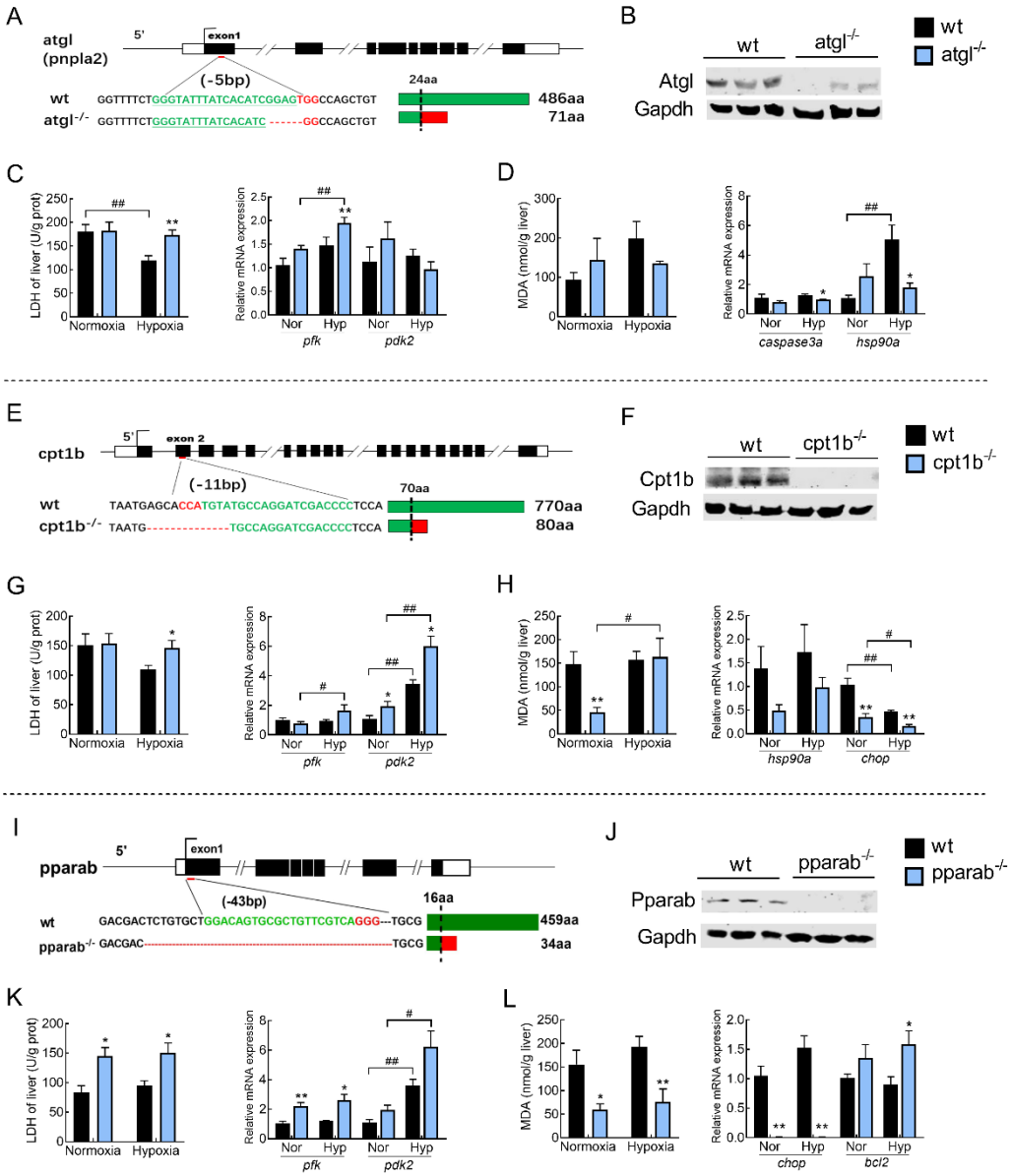
Supplementary Figure S3 Effects of enhanced lipid catabolism (L-carnitine) on tolerance to acute hypoxia in Nile tilapia

A: Nile tilapia were fed control (C) or L-carnitine (Car) diets for 12 weeks and sampled under normoxic and hypoxic conditions after 12 h. B: Mitochondrial fatty acid oxidation (FAO) in liver. C: Mesenteric fat index. D: Intracellular oxygen level in zebrafish hepatocyte line treated with L-carnitine. E: Survival rate of Nile tilapia during acute hypoxia. F: Triglyceride (TG) content in liver. G: Glycogen content in liver, lactate in serum. H: Malondialdehyde (MDA) and total antioxidant capacity (T-AOC) in liver. I: Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in serum, expression of apoptosis genes. J: Protein expression of Hif3α and p-Akt (s473) in liver.



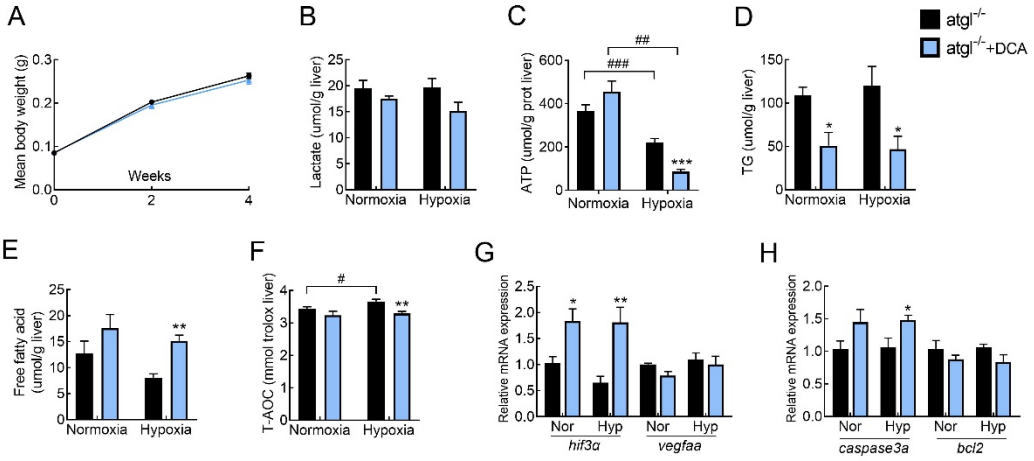
Supplementary Figure S4 Effects of biochemical lipid catabolism inhibition on tolerance to acute hypoxia in Nile tilapia

A: Weight gain. B: Mesenteric fat index. C: Lactate content in liver. D: Lactate dehydrogenase (LDH) activity in liver. E: Adenosine triphosphate (ATP) content in liver. F: Total antioxidant capacity (T-AOC) in liver. G: Glutamic pyruvic transaminase (GPT) in serum. H: Glutamic oxaloacetic transaminase (GOT) in serum. I: mRNA expression of genes related to stress, inflammation, and apoptosis.



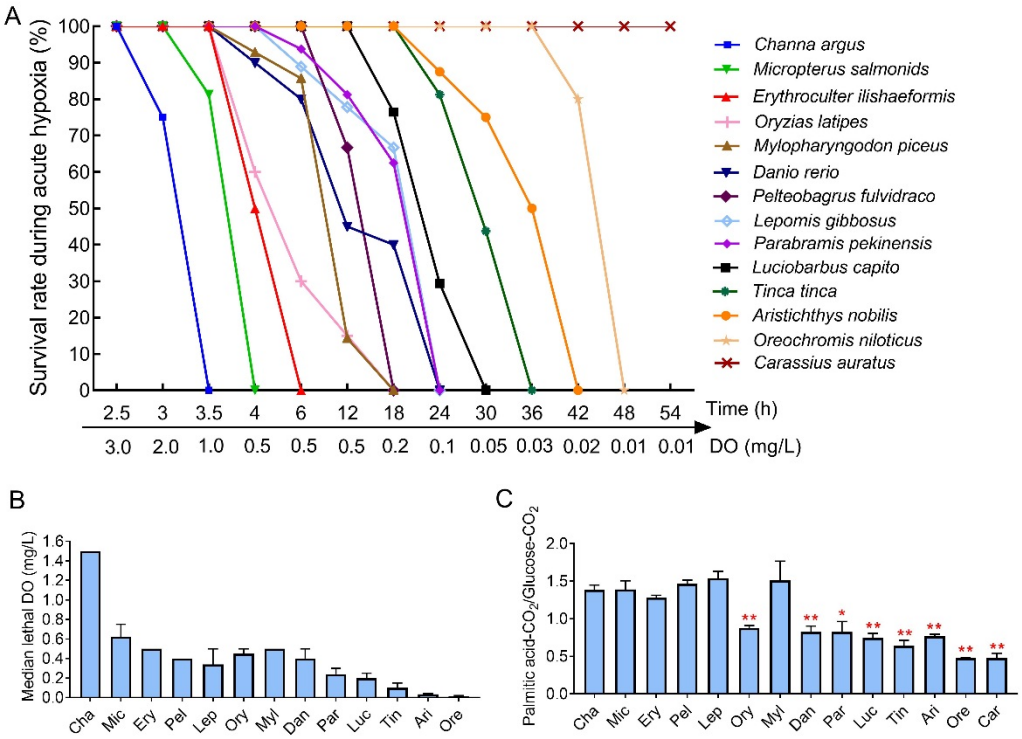
Supplementary Figure S5 Effects of lipid catabolism gene KO on tolerance to acute hypoxia in zebrafish

A, E, I: KO target sites of *atgl*$^{-/-}$, *cpt1b*$^{-/-}$, and *pparab*$^{-/-}$ zebrafish. B, F, J: Liver protein expression of Atgl, Cpt1b, and Pparab in WT and gene KO zebrafish. C, G, K: Lactate dehydrogenase (LDH) activity and gene expression of glycolysis in liver. D, H, L: Malondialdehyde (MDA) content, mRNA expression of genes related to stress and apoptosis in liver.



Supplementary Figure S6 Effects of suppressing glycolysis on tolerance to acute hypoxia in *atgl*$^{-/-}$ zebrafish

A: Mean body weight. B: Lactate content in liver. C: Adenosine triphosphate (ATP) level in liver. D: Triglyceride (TG) content in liver. E: Free fatty acid (FFA) content in liver. F: Total antioxidant capacity (T-AOC) in liver. G: Gene expression of *hif3α* and *vegfaa*. H: mRNA expression of *caspase3a* and *bcl2* genes.



Supplementary Figure S7 Effects of lipid/glucose catabolism ratio on hypoxia tolerance in 14 fish species

A: Repeated survival rate of 14 fish species under acute hypoxia. B: Median lethal DO in 13 fish species (except *Carassius auratus*). C: Ratio of CO_2 from palmitic acid to CO_2 from glucose for 14 fish species. * and ** indicate significant differences (P<0.05) and (P<0.01) between Cha and other fish species.

Supplementary Table S1 Formulation of the experimental diets.

Dietary ingredient (%)	Con	Fen	Car	Mil	DCA
Casein	30	30	30	30	30
Gelatin	5	5	5	5	5
Corn starch	35	35	35	35	35
Soybean oil	7	7	7	7	7
Vitamin premix[a]	0.4	0.4	0.4	0.4	0.4
Mineral premix[b]	0.8	0.8	0.8	0.8	0.8
Choline chloride	0.5	0.5	0.5	0.5	0.5
Butylated hydroxytoluene	0.02	0.02	0.02	0.02	0.02
Dimethyl-beta-propiothetin	0.1	0.1	0.1	0.1	0.1
$Ca(H_2PO_4)_2$	1	1	1	1	1
Carboxymethyl cellulose	4	4	4	4	4
Cellulose	16.18	15.513	16.14	13.68	15.56
Fenofibrate	0	0.667	0	0	0
L-Carnitine	0	0	0.04	0	0
Mildronate	0	0	0	2.5	0
Sodium dichloroacetate	0	0	0	0	0.62
Total	100	100	100	100	100
Nutrients compositions:					
Moisture (%)	8.43	8.58	8.33	8.53	8.37
Total fat (%)	7.12	7.35	7.27	7.55	7.16
Total protein (%)	31.67	31.52	31.88	31.79	32.01

[a] Vitamin premix, (mg or IU/kg): 7000,000 I.U. (international units) Vitamin A, 1500,000 I.U. Vitamin D3, 15,000 mg Vitamin E, 6000 mg Vitamin K3, 8000 mg Vitamin B1, 10,000 mg Vitamin B2, 10,000 mg Vitamin B6, 20 mg Vitamin B12, 60,000 mg Inositol, 20,000 mg calcium pantothenate, 35,000 mg niacinamide, 1000 mg Folic acid, 100 mg Biotin, 50,000 mg Vitamin C, and bran powder etc.

[b] Mineral premix, (g or mg/kg): 40 g Fe, 12 g Zn, 3.6 g Cu, 4 g Mn, 20 g Mg, 25 g K, 400 mg Co, 300 mg I, and zeolite powder ect. Mineral premix and Vitamin premix were bought from Zhejiang Minsheng Biotechnology Co., Ltd.

Supplementary Table S2 The primers for qPCR.

Gene name	Sequences (5' to 3')	GenBank NO.
zebrafish (*Danio rerio*)		
ef1a	F CCCCTGGACACAGAGACTTCATC	NM_131263.1
(eukaryotic translation elongation factor 1 alpha 1)	R ATACCAGCCTCAAACTCACCGAC	
β-actin	F GTCATCACCATCGGCAAT	BC165331.1
(actin, beta 1)	R CGTGGATACCGCAAGATT	
gk	F CCTGGCACTGGATCTTGGAG	NM_001045385.2
(glucokinase)	R TTGGTTTCCACCTTCCAGCC	
pfkl	F TGAACACCGAATGCCCAAGA	XM_693543.8
(phosphofructokinase, liver a)	R CCGGGTCACATGTTCGATCT	
pdk2	F TCAACAGCACCCAGACCTAC	NM_200996.1
(pyruvate dehydrogenase kinase 2b)	R ACGGGAGATGGGAAGACCAT	
hsp90a	F AGGAGAAGAAGAAGCAGGATGA	NM_131328.1
(heat shock protein 90 alpha)	R AGACGGTTGGAGACTGTGAC	
hif3α (hif1al)	F TGGCTGCTAGATGGGACAAG	NM_200405.1
(hypoxia inducible factor 1 subunit alpha, like)	R CCATCAGACAAGCCATCCAGT	
vegfaa	F TGGTAGACATCATCCAGGAGTA	NM_131408.3
(vascular endothelial growth factor Aa)	R CGCCTCATCATTACAGCATCC	
caspase3a	F TTGCTCAGTCACGGCGATG	NM_131877.3
(apoptosis-related cysteine peptidase a)	R CAGCGGTCTCCTCTGAACAG	
chop (ddit3)	F GTTCACCAATCGCCACCAAG	NM_001082825.1
(DNA-damage-inducible transcript 3)	R CCACCTTCGCTGAGGAGTTC	
bcl2	F GCGGAGGGAACAACTCTGAA	NM_001030253.2
(BCL2 apoptosis regulator a)	R ATCCCGTAACACCCGGTAGA	
Nile tilapia (*Oreochromis niloticus*)		
ef1a	F CTACGTGACCATCATTGATGCC	NM_001279647.1
(elongation factor 1a)	R AACACCAGCAGCAACGATCA	
β-actin	F AGCCTTCCTTCCTTGGTATGGAAT	XM_003443127.5
(actin beta)	R TGTTGGCGTACAGGTCCTTACG	
hif1α	F AGGCCAGTACCGAATGTTGG	XM_005477038.4
(hypoxia inducible factor 1 subunit alpha)	R CTGTGGCTGGGAGTTCTTGT	
hif2α (epas1)	F CAGCAAAGAGACGGAGGTGT	XM_025897620.1
endothelial PAS domain protein 1	R ATGGATGCCTTGTCCAGGTG	
hif3α (hif1al)	F TTGATTGGACGCTCAGGCTT	XM_005461141.4
hypoxia-inducible factor 1-alpha like	R GTGCTGACCTGACCTTTGGA	
vegfa	F CCCAGGAGGCAACAAAATGA	XM_005449609.4
(vascular endothelial growth factor Ab)	R TGGCTCACAAACAGCATCGT	
caspase3a	F GGAGTGGACGATACAGACGCAAA	NM_001282894.1
(apoptosis-related cysteine peptidase a)	R TGAAGCTGTGTGACTGGGGCTT	
chop (ddit3)	F TACATGCACCGAGAAGGAGC	XM_003451826.5
(DNA damage inducible transcript 3)	R GACGAGTTGTGATGCAGGGT	
grp78 (bip)	F GCACTTGGGGTGATCCTTC	XM_019361053.2

(endoplasmic reticulum chaperone BiP)	R TGGATATGGGGCTTGCTCTTC	
xbp1	F CACTCAGGCTACGTGTGTCC	XM_013276886.2
(X-box binding protein 1)	R GTCCAGAATGCCCAGTAGCA	
il1b	F GAGCACAGAATTCCAGGATGAAAG	XM_019365841.1
(interleukin-1 beta)	R TGAACTGAGGTGGTCCAGCTGT	
tnfa	F CCTGGCATTCACACTTTGCC	XM_013266976.3
(tumor necrosis factor a)	R TTTTGTCCAGGAGCCTCAGC	

Supplementary Table S3 The information of antibodies used for western blot.

Antibody name	Product source and code	Description
Gapdh	Abways, AB0037	Rabbit, monoclonal
p-Akt (Ser473)	CST, 4060	Rabbit, polyclonal
p-Ampk (Thr172)	CST, 2535	Rabbit, polyclonal
Pdk2	Abways, AY3550	Rabbit, polyclonal
Pdha1	Abways, AB3131	Mouse, Monoclonal
p-Pdha1 (S293)	Abways, CY7247	Mouse, polyclonal
Hif1α	HuaBio, ER1802-41	Rabbit, polyclonal
Hif2α	HuaBio, ET7107-32	Rabbit, monoclonal
Hif3α	HuaBio, ER1910-61	Rabbit, polyclonal
Atgl	HuaBio, Custom	Zebrafish, polyclonal
Cpt1b	HuaBio, Custom	Zebrafish, polyclonal
Pparab	HuaBio, Custom	Zebrafish, polyclonal
800CW IgG	Li-cor, 926-32211	Goat anti-rabbit
680RD IgG	Li-cor, 925-68070	Goat anti-mouse