

1 **Supplementary Material**

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3 **Material and Method**

4 **Western blot analysis**

5 Thirty embryos were pooled as one sample in homogenization buffer (100 mM imidazole, 5  
6 mM EDTA, 200 mM sucrose, and 0.1% sodium deoxycholate; pH 7.6), and then  
7 homogenized. After centrifugation at 4°C and 10,000 rpm for 10 min, the supernatant (a  
8 volume equivalent to 50 µg protein) was supplemented with electrophoresis sample buffer  
9 (250 mM Tris base, 2 mM Na<sub>2</sub>EDTA, 2% SDS, and 5% dithiothreitol), and then incubated at  
10 95°C for 10 min. The denatured samples were subjected to 10% sodium dodecyl  
11 sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride  
12 membranes (Millipore, Billerica, MA). After blocking in blocking buffer, the blots were  
13 incubated overnight at 4°C with monoclonal anti-human estrogen-related alpha antibody  
14 (1:1000, #ab41868, Abcam). After washing in PBST, the membrane was incubated for 2 h in  
15 horseradish peroxidase-conjugated goat anti-mouse IgG (Jackson Laboratory, Bar Harbor,  
16 ME), diluted 1:2000 in PBST. The immunoreactive bands were detected by chemiluminescent  
17 reaction with WesternBright ECL HRP substrate (Advansta, Inc., Menlo Park, CA). The  
18 β-actin detected by a rabbit anti-β-actin antibody (1:1000, #ab8227; Abcam, Cambridge, UK)  
19 was used as an internal control.

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22 **Supplementary Table**

23 **Table S1.** Primers used for Q-PCR.

Gene	Protein Name		Primer sequence	Accession No.
<i>esrra</i>	Estrogen-related receptor $\alpha$	F	5'- GCTACTCCCCACCCCTCTAC-3'	NM_212955.1
		R	5'-TTAACGCATACTTGCAACGC-3'	
<i>atp6v1a</i>	V-H <sup>+</sup> -ATPase subunit A	F	5'-GAGGAACCACTGCCATTCCA-3'	NM_201135.2
		R	5'-CAACCCACATAAATGATGACATC-3'	
<i>slc4a1b</i>	Anion exchanger 1b	F	5'-ATCACCTTCGGAGGTCTGC-3'	NM_001168266.1
		R	5'-ACAGGTTGAGCAGCGATCAG-3'	
<i>slc9a3.2</i>	Sodium-hydrogen exchanger 3b	F	5'-TGCAGACAGCGCCTCTAGC-3'	NM_001113479.1
		R	5'-TGTGGCCTGTCTCTGTTTGC-3'	
<i>cyc1</i>	Cytochrome c-1	F	5'-CACCATGAGCCAGGTTGCTA-3'	NM_001037393.2
		R	5'-TAAGCAGAGCACCAACCAAC-3'	
<i>cycsb</i>	Cytochrome c, somatic b	F	5'-GGCATTGTCTGGGGTGAAGA-3'	NM_001002068.1
		R	5'-GATCTGCTCTCTCGCCCTTC-3'	
<i>atp5b</i>	ATP synthase subunit beta, mitochondrial	F	5'- AGGGATTATGCTGCTCCTGC -3'	NM_001024429.2
		R	5'-AGGGCATTGAGAATGGGTGG -3'	
<i>g6pd</i>	Glucose-6-phosphate dehydrogenase	F	5'-AGCCTTCTGAAATGATGGGCA -3'	XM_694076.6
		R	5'-ATCTGACTGGTGAAATGCGGT -3'	
<i>cs</i>	Citrate synthase	F	5'- TTCAACCTTCACTGCGAGC -3'	NM_199598.1
		R	5'-CTTGGGGCTAGTCTGCTGAT-3'	
<i>rpl13a</i>	Ribosomal protein L13a	F	5'-TCTGGAGGACTGTAAGAGGTATGC-3'	NM_212784.1
		R	5'-CTAGACGCACAATCTTGAGAGCAG-3'	

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25 **Supplementary Figure Legends**

26 **Figure S1.** Effects of ERR $\alpha$  knockdown on expression of energy metabolism genes from

27 glycolysis (A), TCA cycle (B) and oxidative phosphorylation (C). MOs were injected into

28 embryos at the 1~2 cell stage, and the levels of the indicated mRNAs were analyzed by

29 Q-PCR at 3 dpf, with *rpl13a* as an internal control,. Values are the mean  $\pm$  SD (n = 6).

30 \*Significant difference from the respective control (ctrl MO) group ( $p < 0.05$ , Student's t-test).

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32 **Figure S2.** A: Effects of MO dosages on the development of zebrafish embryos. Embryos

33 injected with 4ng  $ERR\alpha$  MO showed abnormal development. B: Western blot analysis of

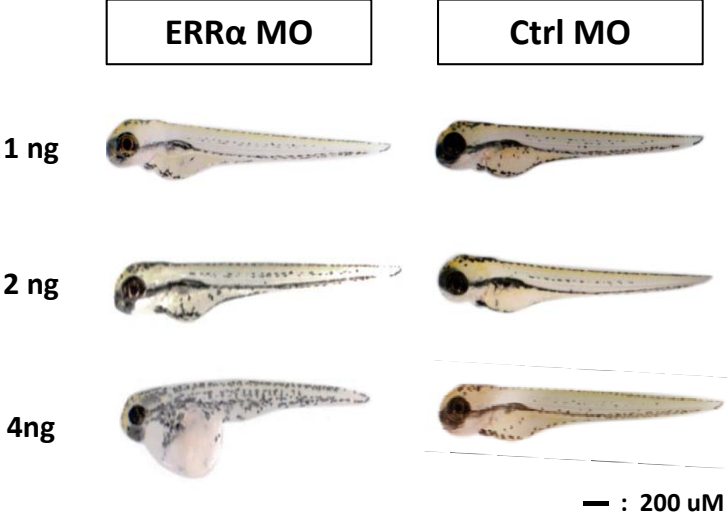
34 effectiveness of  $ERR\alpha$  knockdown (injected with 2ng/embryo) in 3-dpf embryos.  $ERR\alpha$

35 protein detected by anti-mouse  $ERR\alpha$  antibody (Abcam, 1:1000) were observed at ~55 kD.

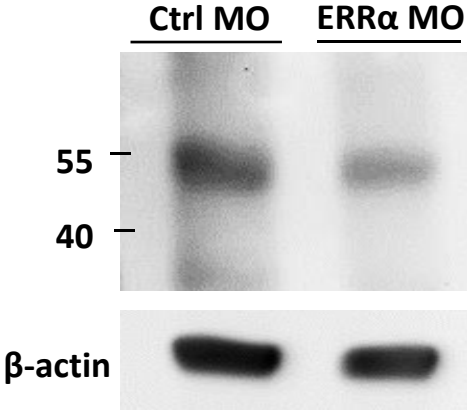
36 The intensity of signal was decreased in  $ERR\alpha$  morphants.

# Supplementary Figure S1

## A



## B



## Supplementary Figure S2

