

**Supplemental information**

**C24:0 avoids cold exposure-induced oxidative  
stress and fatty acid  $\beta$ -oxidation damage**

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## **C24:0 avoids cold exposure-induced oxidative stress and fatty acid $\beta$ -oxidation damage**

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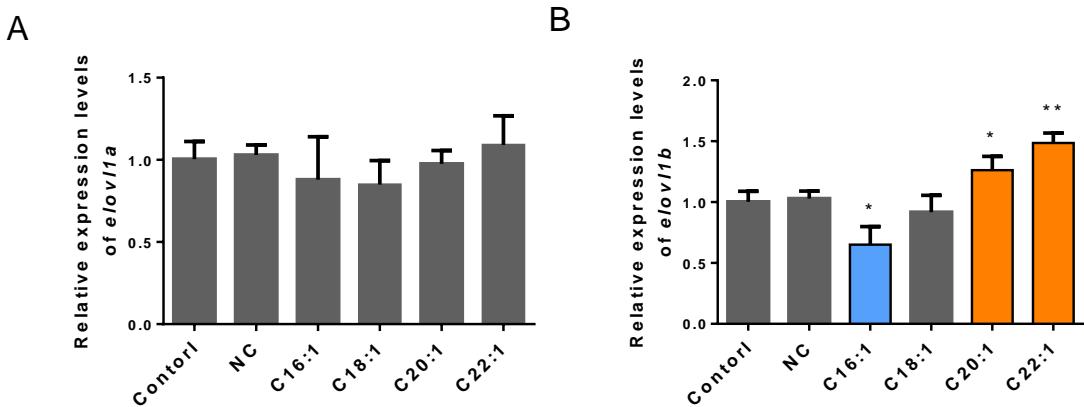
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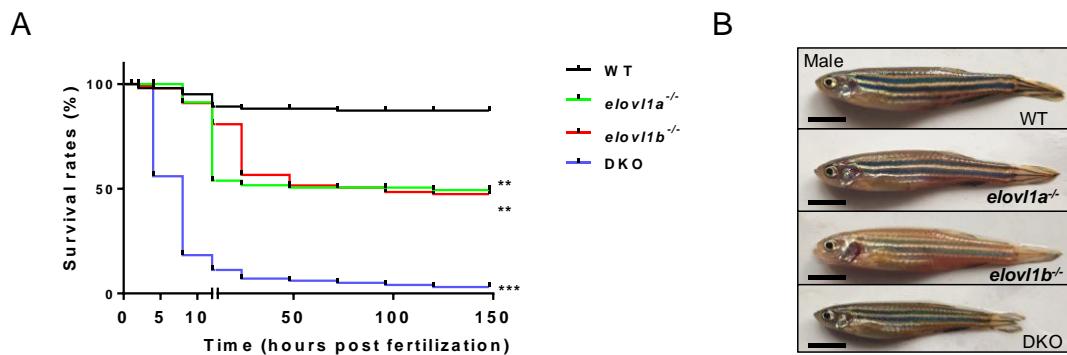
Postal address: No.1 Shizishan Stress, Hongshan District, Wuhan 430070, Hubei Province, China

**Figure S1-S7**

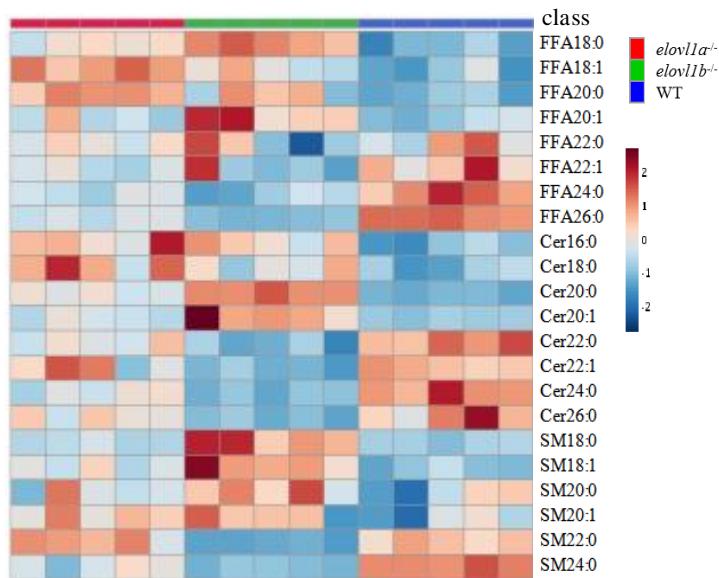
**Table S1**



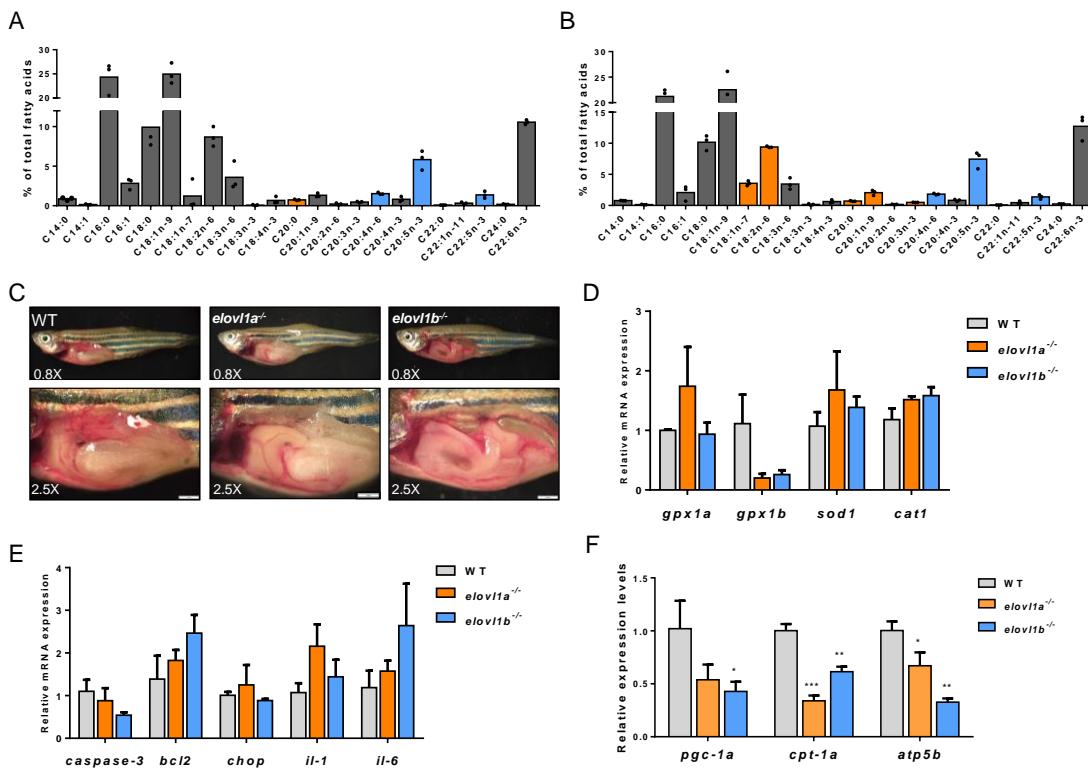
**Figure S1.** Expression levels of *elo11a* (A) and *elo11b* (B) in control and MUFA-treatment zebrafish liver (ZFL) cells at 28°C, related to Figure 1. The blue and orange color respectively meant significant decrease and increase in *elo11b* expression level of the treated groups, compared with the control group. Data were given as means  $\pm$  SD of 3 independent experiments. The statistical analyses were conducted by *T*-test. The asterisks labeled above the error bars indicated significant differences (\* $p < 0.05$ , \*\* $p < 0.01$ ). MUFA, monounsaturated fatty acids; NC, negative control; elo11, fatty acyl elongase 1.



**Figure S2.** The early survival rates of WT, *elo11a*<sup>-/-</sup>, *elo11b*<sup>-/-</sup> and DKO zebrafish (A) and pictures of these four kinds of zebrafish of two-month-old (B), related to Figure 2. Scale bar=5mm. WT, wild-type zebrafish; elo11, fatty acyl elongase 1; *elo11a*<sup>-/-</sup>, *elo11a* knockout zebrafish; *elo11b*<sup>-/-</sup>, *elo11b* knockout zebrafish; DKO, double gene knockout zebrafish.

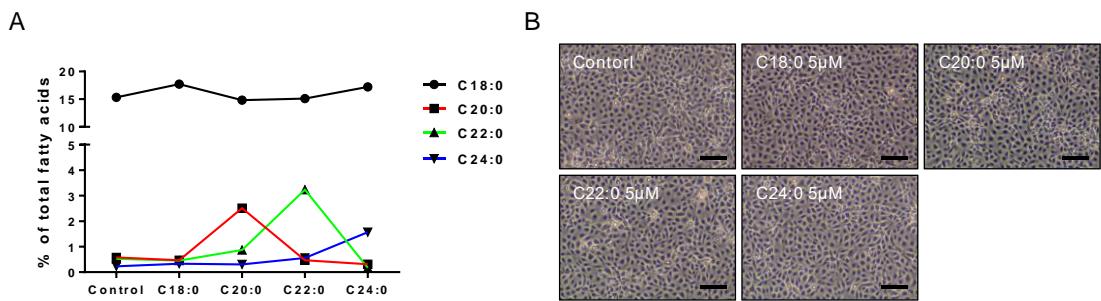


**Figure S3.** Heatmap of saturated and monounsaturated FFA, Cer and SM levels in livers of WT, *elovl1a*<sup>-/-</sup> and *elovl1b*<sup>-/-</sup> zebrafish, related to Figure 2. n=5.  
 WT, wild-type zebrafish; *elovl1*, fatty acyl elongase 1; *elovl1a*<sup>-/-</sup>, *elovl1a* knockout zebrafish; *elovl1b*<sup>-/-</sup>, *elovl1b* knockout zebrafish; FFA, free fatty acids; Cer, ceramide; SM, sphingomyelin.

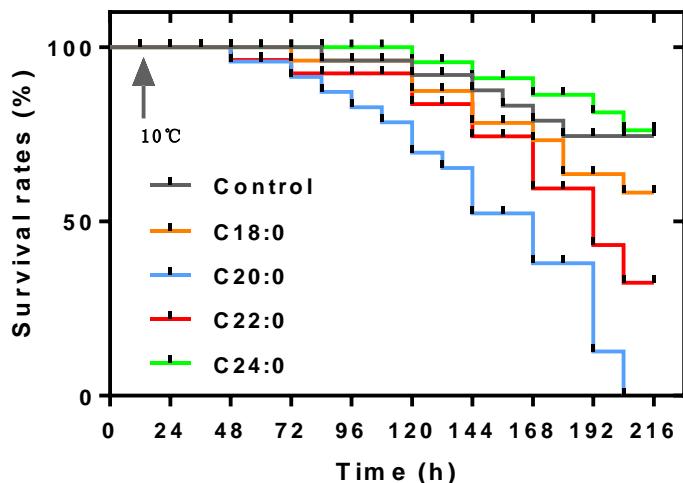


**Figure S4.** *elovl1* deletion impairs the cold resistance of zebrafish. (A and B) Fatty acid compositions in livers of *elovl1a*<sup>-/-</sup> (A) and *elovl1b*<sup>-/-</sup> (B) zebrafish under cold stress, related to Figure 3. The blue and orange color in (A) and (B) respectively meant significant decrease and increase in parameters of *elovl1a*<sup>-/-</sup>/*elovl1b*<sup>-/-</sup> zebrafish, compared with WT zebrafish ( $p < 0.05$ ). (C) The liver lesions of WT, *elovl1a*<sup>-/-</sup> and *elovl1b*<sup>-/-</sup> zebrafish under cold stress, observed under light microscope. (D and E) The expression levels of antioxidant stress-related genes (D) and apoptosis-related genes and inflammation-related genes (E) in livers of WT, *elovl1a*<sup>-/-</sup> and *elovl1b*<sup>-/-</sup> zebrafish at 28°C. (F) The expression levels of  $\beta$ -oxidation-related genes in livers of WT, *elovl1a*<sup>-/-</sup> and *elovl1b*<sup>-/-</sup> zebrafish under cold stress.

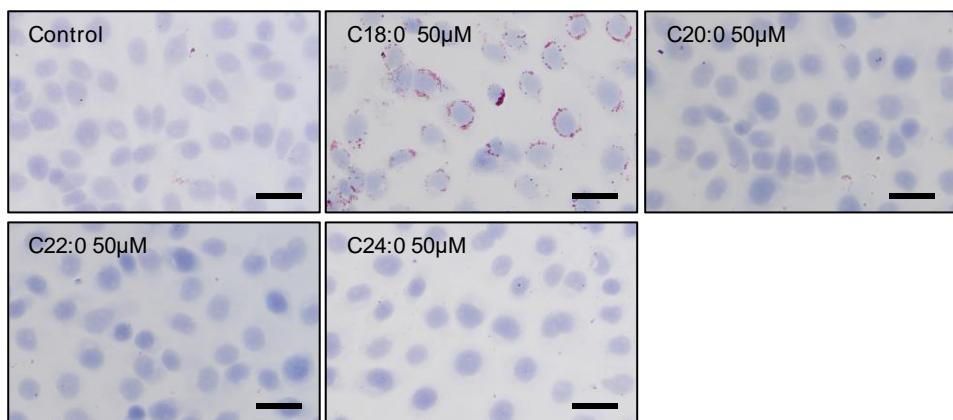
Data were given as means  $\pm$  SD of 3 independent experiments. The statistical analyses were conducted by *T*-test. The asterisks labeled above the error bars indicated significant differences (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). WT, wild-type zebrafish; *elovl1*, fatty acyl elongase 1; *elovl1a*<sup>-/-</sup>, *elovl1a* knockout zebrafish; *elovl1b*<sup>-/-</sup>, *elovl1b* knockout zebrafish; *gpx1*, glutathione peroxidase 1; *sod1*, superoxide dismutase 1, soluble; *cat1*, catalase 1; *chop*, DNA-damage-inducible transcript 3; *bcl2*, BCL2 apoptosis regulator a; *il-1*, interleukin-1; *pgc-1a*, peroxisome proliferator-activated receptor- $\gamma$  coactivator-1a; *cpt-1a*, carnitine palmitoyl transferase 1a; *atp5b*, ATP synthase 5b.



**Figure S5.** The effect of SFA treatment on zebrafish liver (ZFL) cells at 28°C, related to Figure 4. (A) The SFA compositions of ZFL cells incubated with C18-C24 SFA. (B) The proliferate status of ZFL cells with SFA treatment in 28°C, observed under light microscope. Scale bar=10μm. SFA, saturated fatty acids.



**Figure S6.** The survival rates of zebrafish during cold stress, after fed with different saturated fatty acid diets for 2 weeks, related to Figure 5.



**Figure S7.** Oil red O staining of zebrafish liver (ZFL) cells incubated with C18-C24 saturated fatty acids, related to Figure 4. Scale bar=5μm.

**Table S1.** Primers used in this study, related to STAR Methods.

Symbol	Forward	Reverse	accession
Construction of knockout models			
<i>elovl1a</i>	AGCATCAATCCGACTTAT	TGGTTGAACATATCCCTT	ID: 449816
<i>elovl1b</i>	ACCGTAAACCCCTTCAGC	AGATGGCAAGAGTTGCTT	ID: 406725
Real-time quantitative PCR			
<i>β-actin</i>	CACCACCACAGCCGAAAGAG	ACCGCAAGATTCCATACCCA	AF057040.1
<i>18s rRNA</i>	CGGCTACCACATCCAAGGAAGG	GCCCACCTCCGAGATCCAACTA	NR_145818.1
<i>elovl1a</i>	GAGACGTACGTTGCGTCCA	ATGGGACTCTGCATCAACGG	NM_001005989.3
<i>elovl1b</i>	TCAGCTGAAAGAAGCCATGA	AAGGAATGATGGAAGATGTG	NM_213416.2
<i>gpx1a</i>	AGGCACAACAGTCAGGGATT	CAGGAACGCAAACAGAGGG	NM_001007281.2
<i>gpx1b</i>	GCGATGAGCCAATGCCGTCA	GATGCCGCTGGTCAGGAATCTC	NM_001004634.2
<i>sod1</i>	GTCCGCACCTCAACCCCTCA	TCCTCATTGCCACCCCTCC	NM_131294.1
<i>cat1</i>	CAAGGTCTGGTCCCATAAA	TGACTGGTAGTTGGAGGTAA	NM_130912.2
<i>caspase-3</i>	GATCGCAGGACAGGCATGAACC	CATGCCGTGACTGAGCAACA	AB047003.1
<i>chop</i>	AGCGACTGATTGGTGCATGAC	GGTGTTCTCCGTGGTTCGTTCT	NM_001082825.1
<i>bcl2</i>	TGTGCGTGGAAAGCGTCAACC	TCCGATGGTCACTCCTGCCAAG	AY695820.1
<i>il-1</i>	GGGCTCTACCTGAACCACACA	TTGATGCCCTCCAGCTCCTCT	NM_001290418.1
<i>il-6</i>	CCTCAGTCCTGGTGAACGAC	GAACAGGATCGAGTGGACCG	NM_001261449.1
<i>pgc-1a</i>	ATAGAGGAGAGGCGAGTG	GTGTAGCGGTAGGTGATG	AY998087.2
<i>cpt-1a</i>	ATGAGGAGCACCAAAAGAA	GTGGGAAAAGCGTAAAGA	NM_001044854.1
<i>atp-5b</i>	TCACAACCACCAAGAAGG	GGCTACATCATAATGCTCAG	NM_001024429.2
<i>cers2a</i>	GACGGTGTACCTATGCCAA	TGGTCGCTCTGGTTCCCTC	NM_153671.1
<i>cers2b</i>	GAGACGGAGAGTTGCACACA	GGCCAGTTCCAGCATGTAGT	XM_688576.7
<i>cers4b</i>	ACAGATTGAGATCAGCGGGC	CCCAGCAGGAAAATTGTGGC	XM_005163643.4
<i>cers6</i>	TCACACTAACGGGACCAACG	GCCGCGCTCAATCTAAAAGG	XM_688191.8

*elovl1*, fatty acyl elongase 1; *β-actin*, beta-actin; *18s rRNA*, 18S ribosomal RNA; *gpx1*, glutathione peroxidase 1; *sod1*, superoxide dismutase 1, soluble; *cat1*, catalase 1; *chop*, DNA-damage-inducible transcript 3; *bcl2*, BCL2 apoptosis regulator a; *il-1*, interleukin-1; *pgc-1a*, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; *cpt-1a*, carnitine palmitoyltransferase 1a; *atp-5b*, ATP synthase F1 subunit beta; *cers*, ceramide synthase.