

Supplemental information to:

Resveratrol Induces Expression of Metabolic and Antioxidant Machinery and Protects Tilapia under Cold Stress

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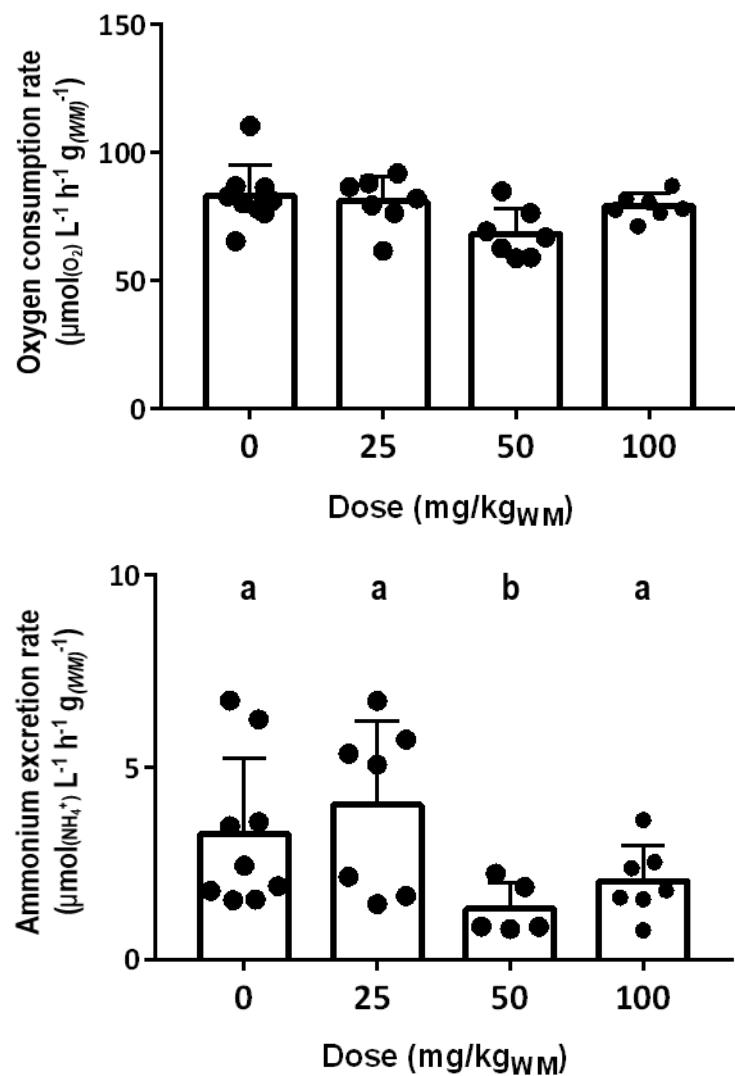


Figure S1. The effect of different RSV dosages on juvenile tilapia oxygen consumption (A) and ammonium excretion (B) rate. Values that are significantly different ($p < 0.05$) among groups are indicated by different letters. Data are presented as mean \pm SD ($n = 5-9$).

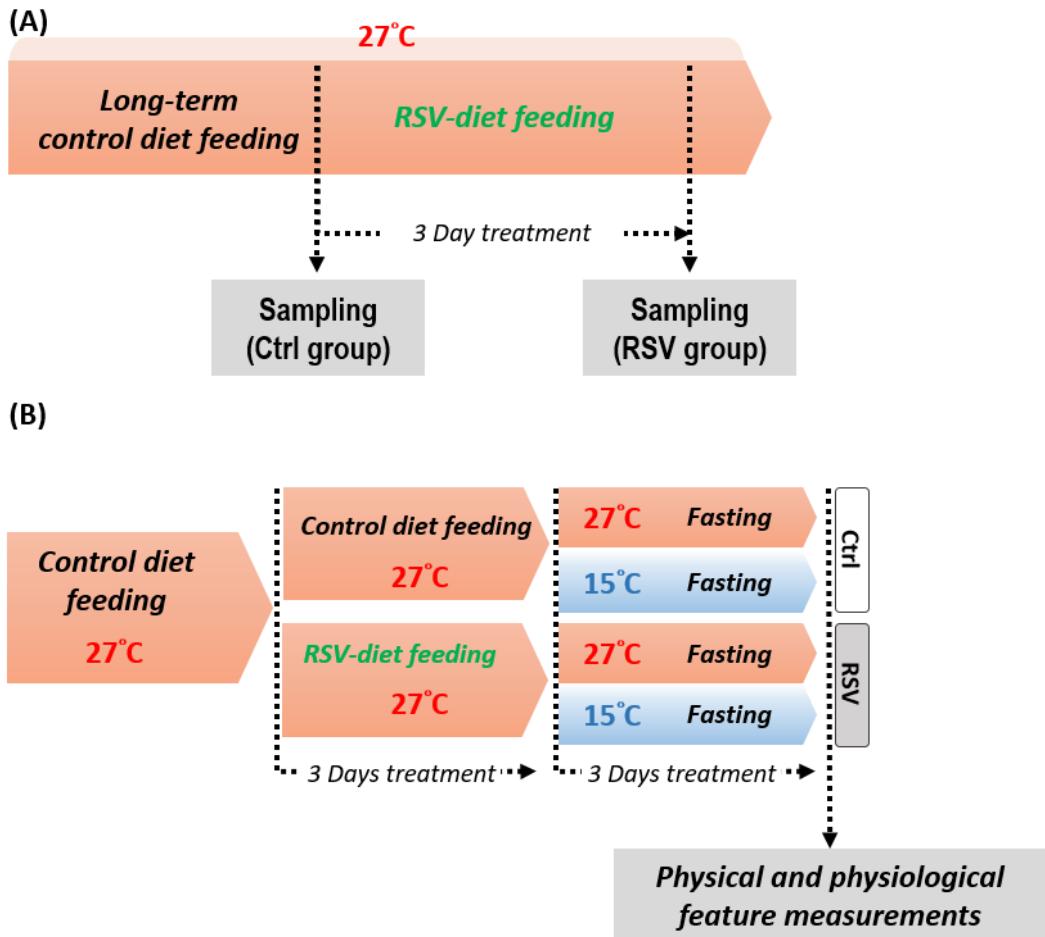


Figure S2. Experimental design. Tilapia were reared at 27 °C and fed with Ctrl-diet before experiments. (A) The effects of RSV-diet on gene expression of SIRT homologues and SIRT-related genes in tilapia liver were estimated after three days of RSV-diet feeding and compared with the fish fed on control diet. (B) After feeding with the Ctrl and RSV-diets for three days, two treatment groups were subdivided into 27 °C and 15 °C conditions and fasted for three days. Physical and physiological features were assessed after the temperature treatments.

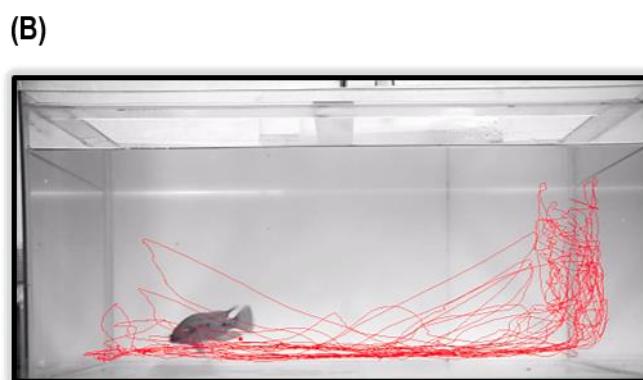
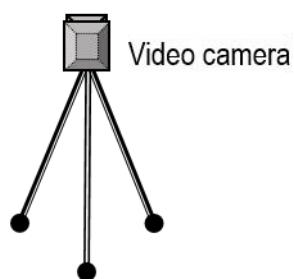
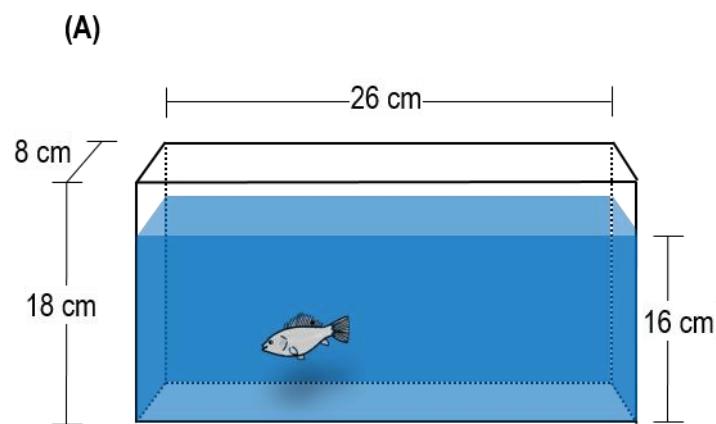


Figure S3. Behavioral experiment set-up. (A) An individual tilapia was placed in a novel tank (26 cm long, 8 cm wide and 18 cm deep; water level: 16 cm) and swimming performance was recorded. (B) Trajectory tracking was performed for 5 min, and tracks were used to analyze the prolonged swim speed and spatial preference of fish.

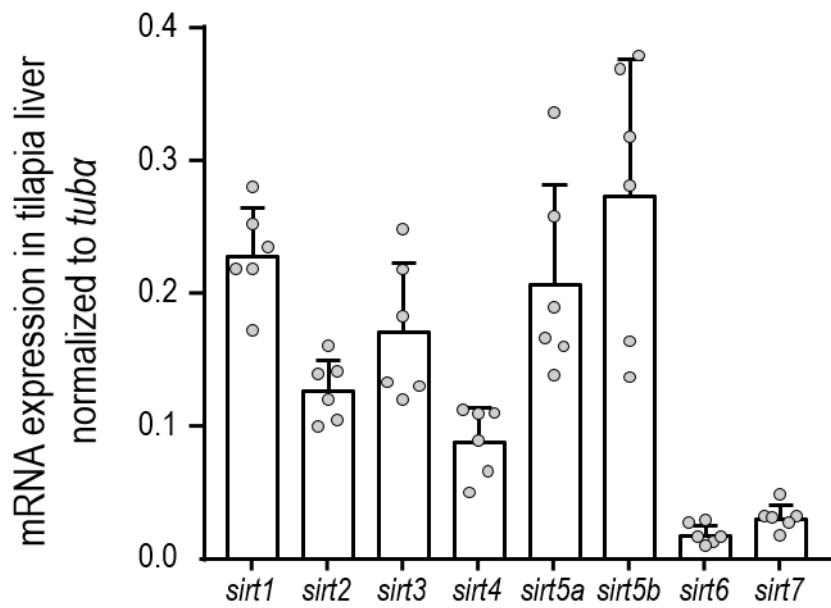


Figure S4. Sirtuin homologue expression in tilapia liver. qPCR analysis of relative mRNA expression levels of sirtuin homologues in liver of tilapia juveniles. Data are expressed as mean \pm SD ($n = 6$)

Table S1. qRT-PCR primer sequences.

| Gene Name | Abbreviation | | Primer Sequence (5'→3') | Primer Efficiency (%) | Amplicon Size (bp) | Accession Numbers |
|--|--------------|---|----------------------------|-----------------------|--------------------|-------------------|
| Sirtuin-1 | SIRT1 | F | AACTTGACGACACCGCTGTCTTG | 99 | 137 | XM_005473846 |
| | | R | GCTTGCATGTGAGGGACCTGTCATC | | | |
| Sirtuin-2 | SIRT2 | F | CATGGAGAAAAGCAGGGCAGGTTAAC | 93 | 161 | XM_003449264 |
| | | R | GCTCTTCCTTCCATCCCAGAAGATCA | | | |
| Sirtuin-3 | SIRT3 | F | AGTTCTGTCCAGACATGCACGATCT | 98 | 124 | XM_005457371 |
| | | R | CCTGTTGAACTGTTGCTCCCGTATG | | | |
| Sirtuin-4 | SIRT4 | F | TTCAGAGTCCCTCCTGTGATGACT | 96 | 154 | XM_025907266 |
| | | R | ACACCTGTAAAGATGACCCCCACAAC | | | |
| Sirtuin-5a | SIRT5a | F | CCACCGAGCTGGATCCAAAAAC | 94 | 179 | XM_003457306 |
| | | R | TGGGCAAATTCTGGACTGGGAT | | | |
| Sirtuin-5b | SIRT5b | F | CGGCTCTGTGCTCTGATAGAAGGT | 95 | 190 | XM_003438119 |
| | | R | GACTCATGTTGCTGCCAAAGCTTGAG | | | |
| Sirtuin-6 | SIRT6 | F | CGCAAGGGTGGCAAACACTGGTTAT | 97 | 197 | XM_003437978 |
| | | R | TTGACATCAGCGGTGGTCTCAGT | | | |
| Sirtuin-7 | SIRT7 | F | GCATGAACAGACCAGCAAGCAAAA | 93 | 100 | XM_003455810 |
| | | R | CACTTGCCGTGAATTTCAGCACA | | | |
| Glyceraldehyde 3-phosphate dehydrogenase 1 | GAPDH1 | F | TCATCCCTGAGCTCAATGGCAA | 98 | 187 | XM_005455438 |
| | | R | AGACCTGGTGTCTGTGTATCC | | | |
| Glyceraldehyde 3-phosphate dehydrogenase 2 | GAPDH2 | F | TCTGGGATAACACAGAGGACCAAGGT | 98 | 102 | XM_003452690 |
| | | R | AAGTTGTCGGTGAGTGCAATGCCA | | | |
| Protein kinase AMP-activated catalytic subunit alpha 1 | PRKAA1 | F | GTGCAGCGATAGTCAAGCCTCAC | 96 | 127 | NM_001319868 |
| | | R | CTCCTAACACCCTGGTGTGCTTGA | | | |
| Peroxisome proliferator-activated receptor alpha | PPARA | F | CATGATGGAGCCCAAATTCCAGTTG | 91 | 153 | NM_001290066 |
| | | R | ATGCTTCCTGCAACTGCTCTACTAG | | | |
| Peroxisome proliferator activated receptor alpha | PPARAB | F | TCCCCATCACCAACATGGTCGACA | 94 | 175 | XM_003443920 |
| | | R | TGGGACACCAAAGGAGCTGAGAG | | | |

| | | | | | | |
|------------------------------------|--------------|---|----------------------------|----|-----|--------------|
| PPARG related coactivator 1 | PPARGC1a | F | TACCTAACGCCACCGATGACATT | 92 | 96 | XM_005468189 |
| | | R | CATGCCACTCTTCTCCCTGCTACT | | | |
| Forkhead box protein 1 | FOXO1 | F | CAAACGCCAGTTCATCAGAAACCT | 92 | 121 | XM_025910765 |
| | | R | GGTAATGATGCACACAGTTGCTGT | | | |
| Forkhead box protein 3 | FOXO3 | F | ACAACCACAATCACAGCTCTGAG | 96 | 196 | XM_005454618 |
| | | R | CGATCCTGATAGTTCCCTCCATTGC | | | |
| Insulin receptor substrate 2a | IRS2A | F | TGGGAGGTCATCTCTAGCAGACTACA | 90 | 168 | XM_003440900 |
| | | R | CCCTGGGAGTCTTCTCACTATCCAC | | | |
| Catalase | CAT | F | GCGACAGAGACTTGCCAGAAC | 90 | 181 | XM_019361816 |
| | | R | ACGGCTGTAAACATGCAAGGTG | | | |
| Mitochondrial uncoupling protein 2 | UCP2 | F | TTCGTTACCACAGTGATCGCCT | 91 | 154 | XM_003452255 |
| | | R | ACGAGGGCACGAATCCTTGTA | | | |
| Superoxide dismutase 1 | SOD1 | F | CACCCCTCACAGGTCTGACTCC | 92 | 136 | XM_003446807 |
| | | R | AATGACTCCACAGGCCAGACGT | | | |
| Superoxide dismutase 2 | SOD2 | F | GAACATGCTTGAGAGCTGGACA | 95 | 160 | XM_003449940 |
| | | R | CAGCTGCATGATCTCTGCACTGAC | | | |
| Superoxide dismutase 3 | SOD3 | F | GAAAGTCAAGGTCTCCTCCGGTT | 98 | 194 | XM_003454189 |
| | | R | TCTTCCCTTGCTGAGGCTCAAAGT | | | |
| Tubulin alpha chain | TUB α | F | GCCTTCAGCAACCGATTCTT | 98 | 115 | XM_019352023 |
| | | R | CAGCATGCATTGCCATTG | | | |

F, forward primer; R, reverse primer

Table S2. Swimming phenotypes of Ctrl- and RSV-fed tilapia at 15 °C for three days.

| Phenotypes | Ctrl Diet Feeding | RSV-Containing Diet Feeding |
|------------------|-------------------|-----------------------------|
| Erratic Swimming | (6/9) | (0/9) |

Numbers in parentheses indicate (number of fish with phenotype/total experimental fish)