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Supplemental information

Propionate induces intestinal oxidative stress via Sod2 propionylation in zebrafish

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Supplemental Figures

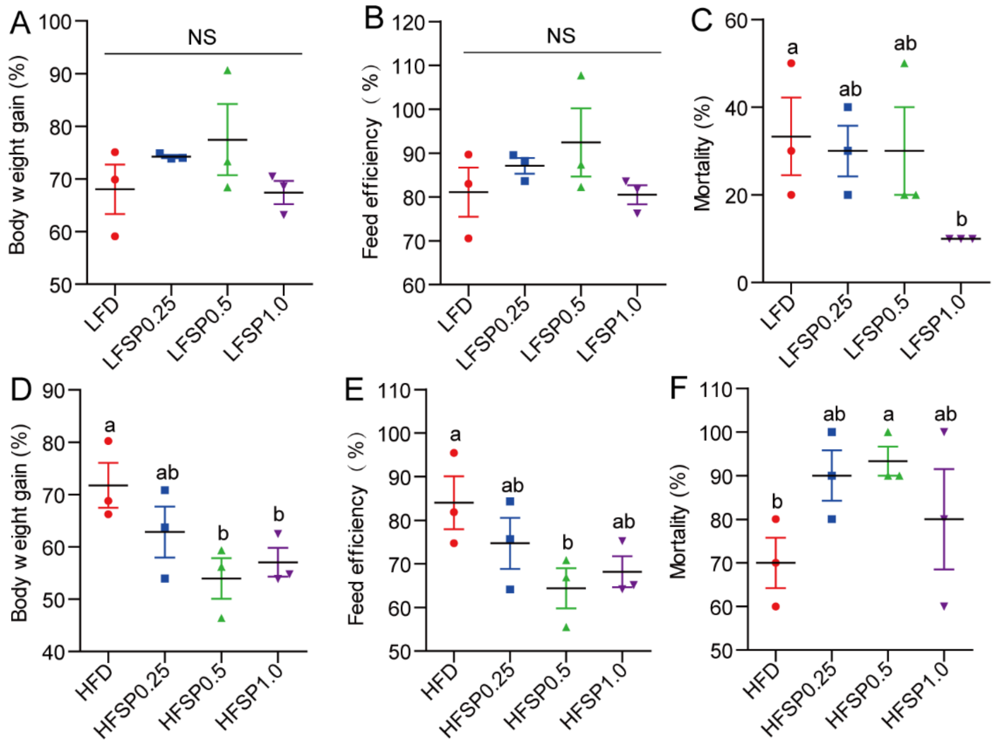

Figure S1. Growth, feed efficiency and mortality challenged by *Aeromonas veronii* of zebrafish, related to Figure 1.

(A) Body weight gain of LFD, LFSP0.25, LFSP0.5 and LFSP1.0-fed zebrafish at the end of 2-wk feeding trial (n = 3 tanks, 20 fish/tank).

(B) Feed efficiency of LFD, LFSP0.25, LFSP0.5 and LFSP1.0-fed zebrafish at the end of 2-wk feeding trial (n = 3 tanks, 20 fish/tank).

(C) Mortality of LFD, LFSP0.25, LFSP0.5 and LFSP1.0-fed zebrafish challenged by *Aeromonas veronii* at the end of 2-wk feeding trial (n = 3 tanks, 10 fish/tank).

(D) Body weight gain of HFD, HFSP0.25, HFSP0.5 and HFSP1.0-fed zebrafish at the end of 2-wk feeding trial (n = 3 tanks, 20 fish/tank).

(E) Feed efficiency of HFD, HFSP0.25, HFSP0.5 and HFSP1.0-fed zebrafish at the end of 2-wk feeding trial (n = 3 tanks, 20 fish/tank).

(F) Mortality of HFD, HFSP0.25, HFSP0.5 and HFSP1.0-fed zebrafish challenged by *Aeromonas veronii* at the end of 2-wk feeding trial (n = 3, 10 fish/replicate).

Values are means ± SEM. Means without a common letter are significantly different ($p < 0.05$). NS, not significant. Duncan’s test. LFD, low fat diet; LFSP, propionate-supplemented LFD; HFD, high fat diet; HFSP, propionate-supplemented HFD.

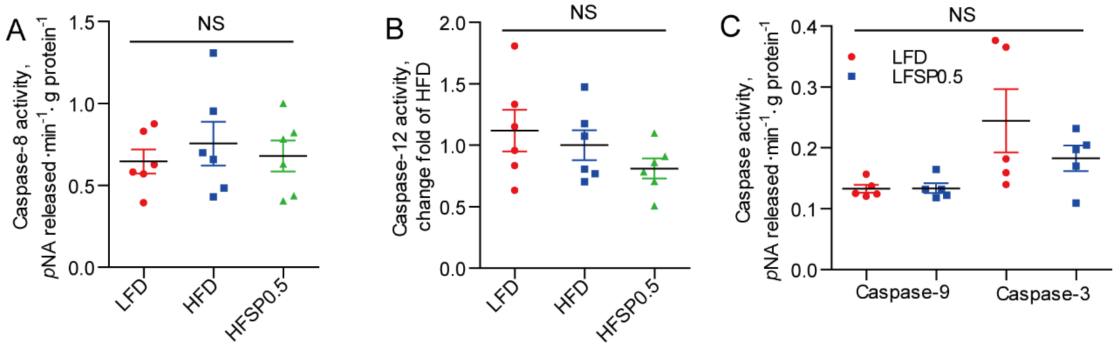

Figure S2. Caspase activity in the intestinal of zebrafish, related to Figure 1. (A) Caspase-8 and (B) caspase-12 activity in one-month-old zebrafish fed LFD, HFD or HFSP0.5 for 2 wks (n = 6). (C) Caspase-9 and caspase-3 activity in the intestine of one-month-old zebrafish fed LFD or LFSP0.5 (n = 5). Values are means ± SEM. NS, not significant. Duncan's test.

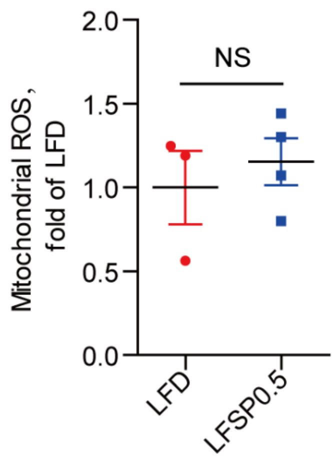

Figure S3. Mitochondrial ROS in the intestine of one-month-old zebrafish, related to Figure 2. Mitochondrial ROS in the intestine of zebrafish fed LFD and LFSP0.5 (n = 3 - 4). Values are means ± SEM. NS, not significant. Student's *t*-test.

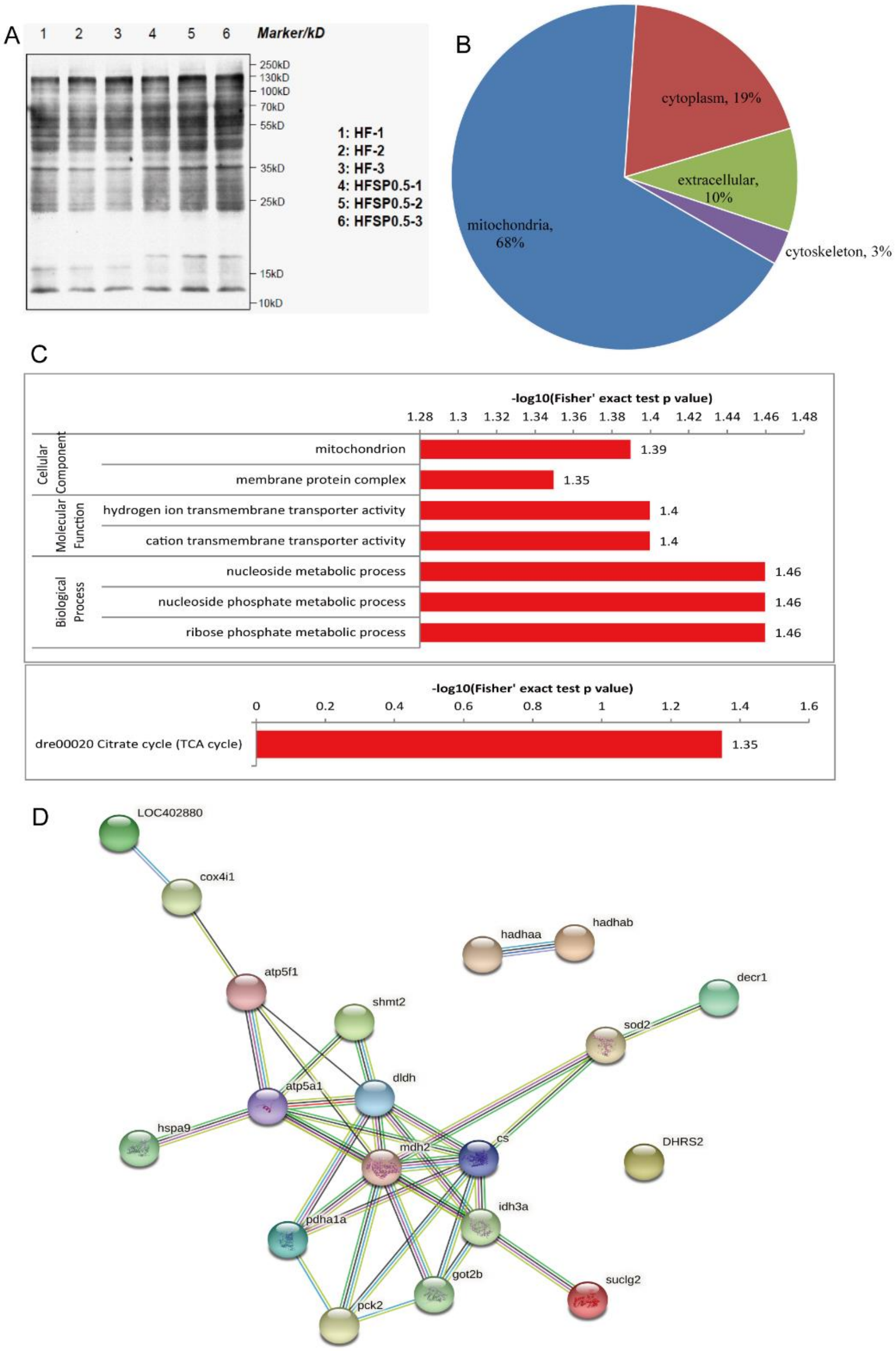

Figure S4. Propionate induces intestinal mitochondrial protein hyper-propionylation in high fat diet, related to Figure 4. (A) Immunoblotting validation of Kpro from zebrafish intestine. Lanes 1-3: biological sample of HFD group, lanes 4-6: biological sample of HFSP0.5 group (n = 3). (B) Subcellular distribution of increased lysine-propionylated proteins in zebrafish intestine of HFSP0.5 group compared to HFD group. (C) GO enrichment and KEEG pathway analysis of differentially represented Kpro proteins. (D) STRING analysis revealed mitochondrial Kpro protein interaction networks. Interactions of the identified Kpro proteins were mapped by searching the STRING database with a high confidence value of 0.7.

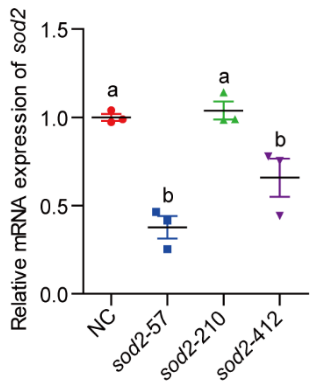

Figure S5. Efficiency of *sod2 si*RNA, related to Figure 5. Values are means ± SEM (n = 3). Means without a common letter are significantly different ($p < 0.05$). Duncan's test. NC, negative control.

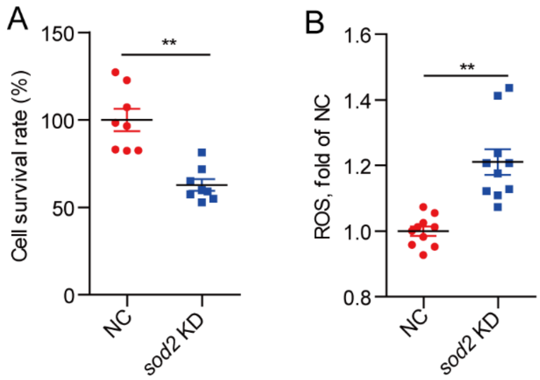

Figure S6. Cell viability and ROS in ZF4 cells with *sod2* knockdown, related to Figure 5. Values are means ± SEM (n = 8). **, $p < 0.01$. Student's *t*-test.

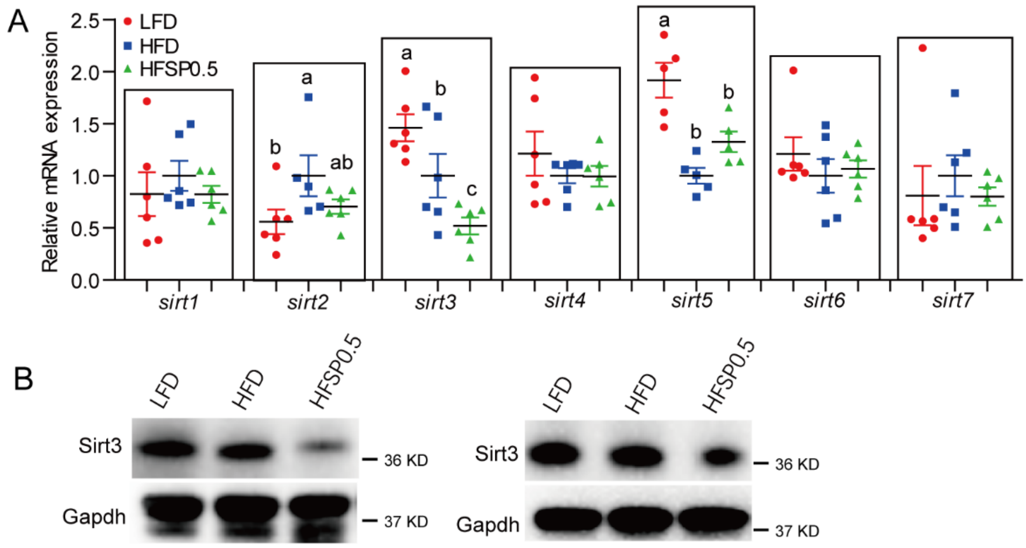

Figure S7. (A) Intestinal mRNA expression of genes encoding sirtuins and (B) protein expression of Sirt3 in zebrafish fed LFD, HFD or HFSP0.5 diet for 2 wks, related to Figure 6. Values are means ± SEM (n = 5 - 6). Means without a common letter are significantly different ($p < 0.05$). Duncan's test.

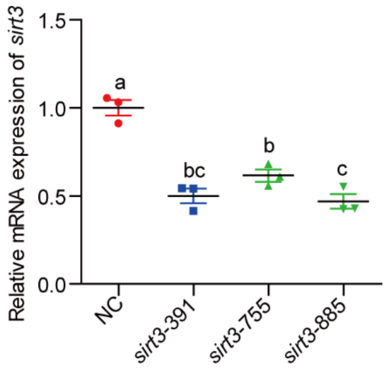

Figure S8. Efficiency of *sirt3 si*RNAs, related to Figure 6. Values are means ± SEM (n = 3). Means without a common letter are significantly different (p < 0.05). Duncan's test. NC, negative control.

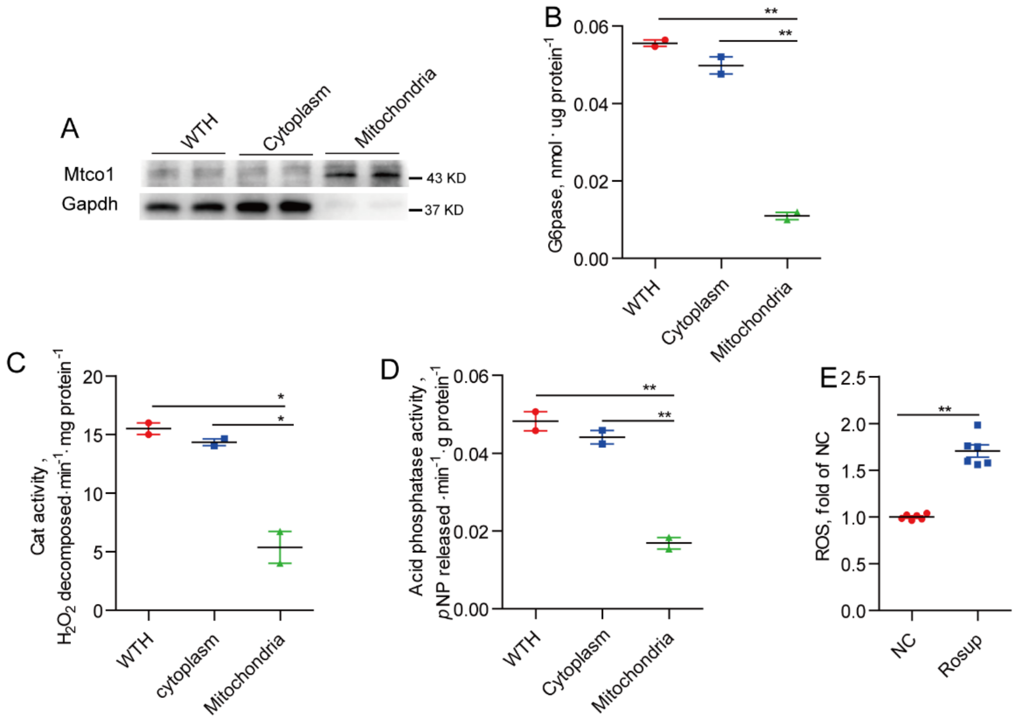

Figure S9. Positive control and purity assessment of isolated mitochondria, related to STAR Methods. (A) Mtco1 and Gapdh level in in WTH, cytoplasm and mitochondria fractions. (B) G6pase level in WTH, cytoplasm and mitochondria fractions. (C) Cat activity in WTH, cytoplasm and mitochondria fractions. (D) Acid phosphatase activity in WTH, cytoplasm and mitochondria fractions. (E) ROS generated by isolated mitochondria when stimulated with positive control Rosup. Values are means ± SEM (n = 2).*, $p < 0.05$, **, $p < 0.01$. Student's *t*-test. WTH, whole tissue homogenate.

Supplemental Tables

Table S1. Ingredient and nutrient composition of low fat experimental diets used for one-month-old zebrafish (dry matter, g/kg), related to STAR Methods.

	One-month-old zebrafish			
Ingredients (g/kg dry diet)	LFD	LFSP0.25	LFSP0.5	LFSP1.0
Casein	400	400	400	400
Geltin	100	100	100	100
Wheat flour	350	350	350	350
Sodium propionate[1]	0	2.5	5	10
Soybean oil	60	60	60	60
Lard oil	0	0	0	0
Lysine	3.3	3.3	3.3	3.3
Ascorbyl phosphater	1	1	1	1
Vitamin premix[2]	2	2	2	2
Mineral premix[3]	2	2	2	2
Dicalcium phosphate	20	20	20	20
Choline chloride	2	2	2	2
Sodium alginate	20	20	20	20
Microcrystalline cellulose	39.7	37.2	34.7	29.7
Total	1000	1000	1000	1000

[1]Sigma;

[2]Vitamin premix (g/kg): thiamine, 0.438; riboflavin, 0.632; pyridoxine·HCl, 0.908; *d*-pantothenic acid, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; *dl*-α-tocopherol-acetate, 12.632;

[3]Mineral premix (g/kg): $CoCl_2 \cdot 6H_2O$, 0.074; $CuSO_4 \cdot 5H_2O$, 2.5; $FeSO_4 \cdot 7H_2O$, 73.2; NaCl, 40.0; $MgSO_4 \cdot 7H_2O$, 284.0; $MnSO_4 \cdot H_2O$, 6.50; KI, 0.68; Na_2SeO_3, 0.10; $ZnSO_4 \cdot 7H_2O$, 131.93; cellulose, 501.09;

LFD, low-fat diet; LFSP0.5, low-fat diet supplemented with 0.5% sodium propionate; HFD, high-fat diet; HFSP0.5, high-fat diet supplemented with 0.5% sodium propionate.

Table S2. Ingredient and nutrient composition of the high fat experimental diets used for one-month-old zebrafish (dry matter, g/kg), related to STAR Methods.

	One-month-old zebrafish			
Ingredients (g/kg dry diet)	LFD	LFSP0.25	LFSP0.5	LFSP1.0
Casein	400	400	400	400
Geltin	100	100	100	100
Wheat flour	250	250	250	250
Sodium propionate[1]	0	2.5	5	10
Soybean oil	80	80	80	80
Lard oil	80	80	80	80
Lysine	3.3	3.3	3.3	3.3
Ascorbyl phosphater	1	1	1	1
Vitamin premix[2]	2	2	2	2
Mineral premix[3]	2	2	2	2
Dicalcium phosphate	20	20	20	20
Choline chloride	2	2	2	2
Sodium alginate	20	20	20	20
Microcrystalline cellulose	39.7	37.2	34.7	29.7
Total	1000	1000	1000	1000

[1]Sigma;

[2]Vitamin premix (g/kg): thiamine, 0.438; riboflavin, 0.632; pyridoxine·HCl, 0.908; *d*-pantothenic acid, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; *dl*-α-tocopherol-acetate, 12.632;

[3]Mineral premix (g/kg): $CoCl_2·6H_2O$, 0.074; $CuSO_4·5H_2O$, 2.5; $FeSO_4·7H_2O$, 73.2; NaCl, 40.0; $MgSO_4·7H_2O$, 284.0; $MnSO_4·H_2O$, 6.50; KI, 0.68; Na_2SeO_3, 0.10; $ZnSO_4·7H_2O$, 131.93; cellulose, 501.09;

LFD, low-fat diet; LFSP0.5, low-fat diet supplemented with 0.5% sodium propionate; HFD, high-fat diet; HFSP0.5, high-fat diet supplemented with 0.5% sodium propionate.

Table S5. *Si*RNA sequences, related to STAR Methods.

	Sense (5'-3')	Antisense (5'-3')
sod2-57	GCAGAGUCGGAUAUGUUCGTT	CGAACAUAUCCGACUCUGCTT
sod2-210	GCAAGCACCAUGCAACAUATT	UAUGUUGCAUGGUGCUUGCTT
sod2-412	GGCCAUAAAGCGUGACUUUTT	AAAGUCACGCUUUAUGGCCTT
sirt3-391	GCAGGCAUCAGCACACCAATT	UUGGUGUGCUGAUGCCUGCTT
sirt3-755	GCAGGAACAGUACCCAAAUTT	AUUUGGGUACUGUUCCUGCTT
sirt3-885	GCACUUCUUCACCUACCUUTT	AAGGUAGGUGAAGAAGUGCTT
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Table S6. Quantitative PCR primers, related to STAR Methods.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
rps11	ACAGAAATGCCCCTTCACTG	GCCTCTTCTCAAAACGGTTG
sirt3	CATTAAATGTGGTGGAACAAGAGGCCTG	AGTTCCTCTCCTTTGTAATCCCTCCGAC
sod2	ATGCTGTGCAGAGTCGGATATG	GCTGAAGGGAGACTTGGGTT
claudin-15	CTCTGCTCGCTCTATCTGGTTA	TTTCACGGGTGGGATGGTAT
occludin	GGCTCCTATGCTGGTTCCTA	AACTTCCTGCCCTGAGACACT
sirt1	CCAAACGAAAGAAACGCAAAGA	CACAGGAAACAGACACCCCAG
sirt2	AGAAGTACAACCTGCCTTACC	CTTTCCCCAAAAAACACAATA
sirt4	TTTCTTCGCACCAGCCTAAC	CACAGTCCAAACACACAACCC
sitrt5	CCACGGTAGTCTGTTTAAAACCCGCTG	AGTGATATTTGAAGCGTTGGGTAGCAGG
sirt6	CTACAGGCATACCCGACTTCA	CCATCACTCCGACCACCG
sirt7	GCTGTGATGGCTCTGTTGAT	GCGGACTGAGGGGTTTAG
Universal bacteria	CCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG
Fusobacteria (*phylum*)	KGGGCTCAACMCMGTATTGCGT	TCGCGTTAGCTTGGGCGCTG
Proteobacteria (*phylum*)	TCGTCAGCTCGTGTYGTGA	CGTAAGGGCCATGATG
Firmicutes (*phylum*)	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC
Cetobacterium (*genus*)	AGTTTGATCCTGGCTCAGGATG	GAGGCAAGTTCCTTACGCGTT
Plesiomonas (*genus*)	CTCCGAATACCGTAGAGTGCTATCC	CTCCCCTAGCCCAATAACACCTAAA
Aeromonas (*genus*)	GAAGGCCAAGTCGGCCGCCAG	ATCTTGGCACGCCCGGGTTTTC