

Supplementary Materials

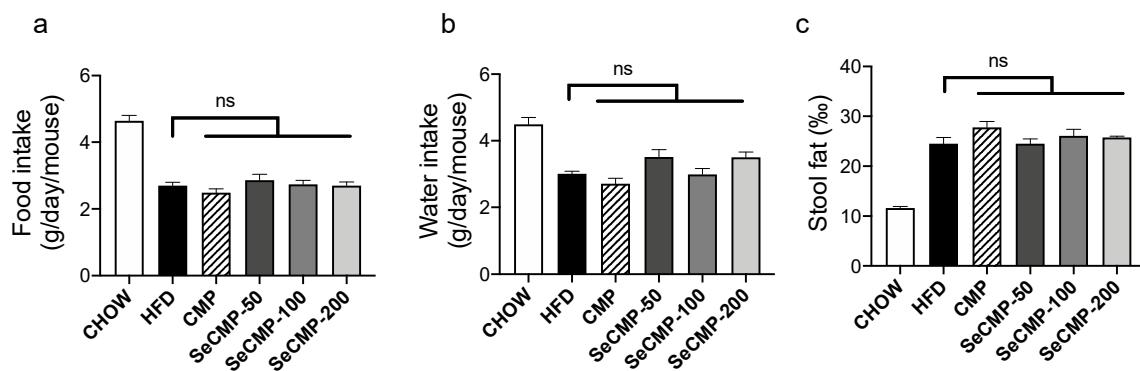


Figure S1. Polysaccharides supplementation do not affect energy intake, water intake and energy extraction. (a) Food intake and (b) water intake were determined once per week of each cage and converted to daily intake per mouse. (c) Stool fat was assessed by gravimetric analysis. Data are expressed as mean \pm SE ($n = 8$ mice/group). Data were analyzed using one-way ANOVA analysis with Tukey's post hoc test or the non-parametric Kruskal-Wallis test with Dunnnett's T3 multiple comparison test; ns represents not significant. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg selenium-deficient *C. militaris* crude polysaccharides; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

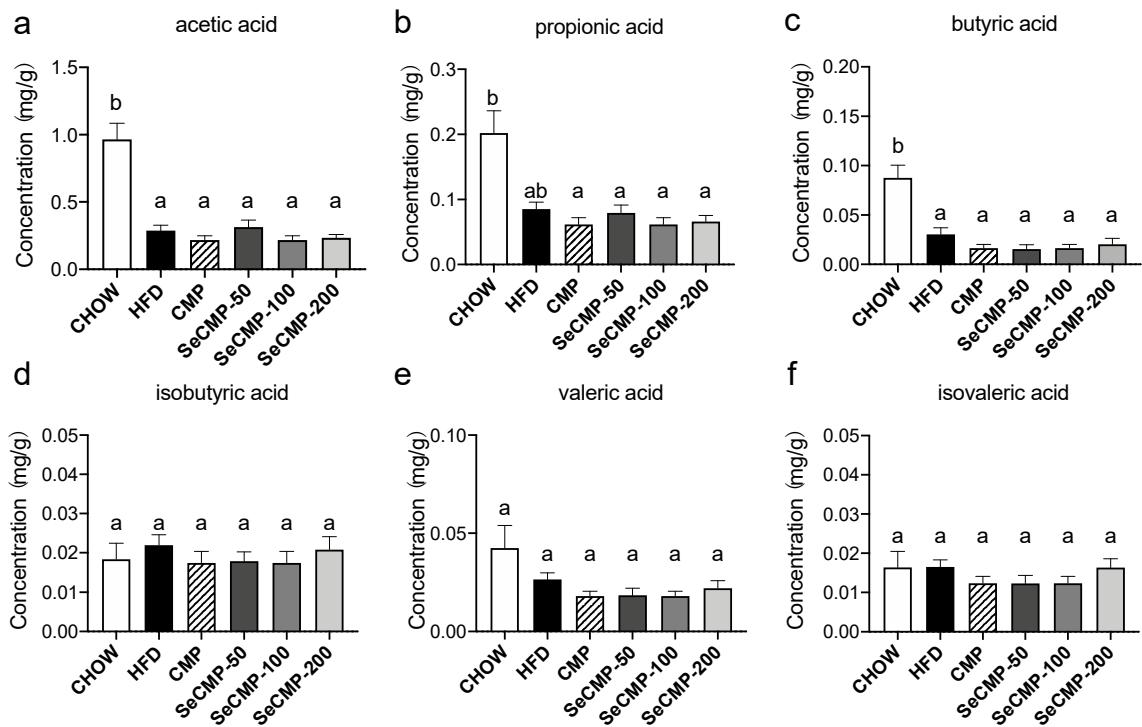


Figure S2. Selenium-deficient *C. militaris* crude polysaccharides (CMP) and selenium-deficient *C. militaris* crude polysaccharides (SeCMP) do not affect production of short chain fatty acids. Linear chain of (a) acetate, (b) propionate, (c) butyrate and (e) valeric acid and branched chain (d) isobutyric acid and (f) isovaleric acid were quantified using GC-MS. Data represent mean \pm SE ($n = 8$ mice/group). Data were analyzed using Tukey's post hoc test or the non-parametric Kruskal-Wallis test with Dunnett's T3 multiple comparison test. Superscript characters indicate significant variation among treatments. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg CMP; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

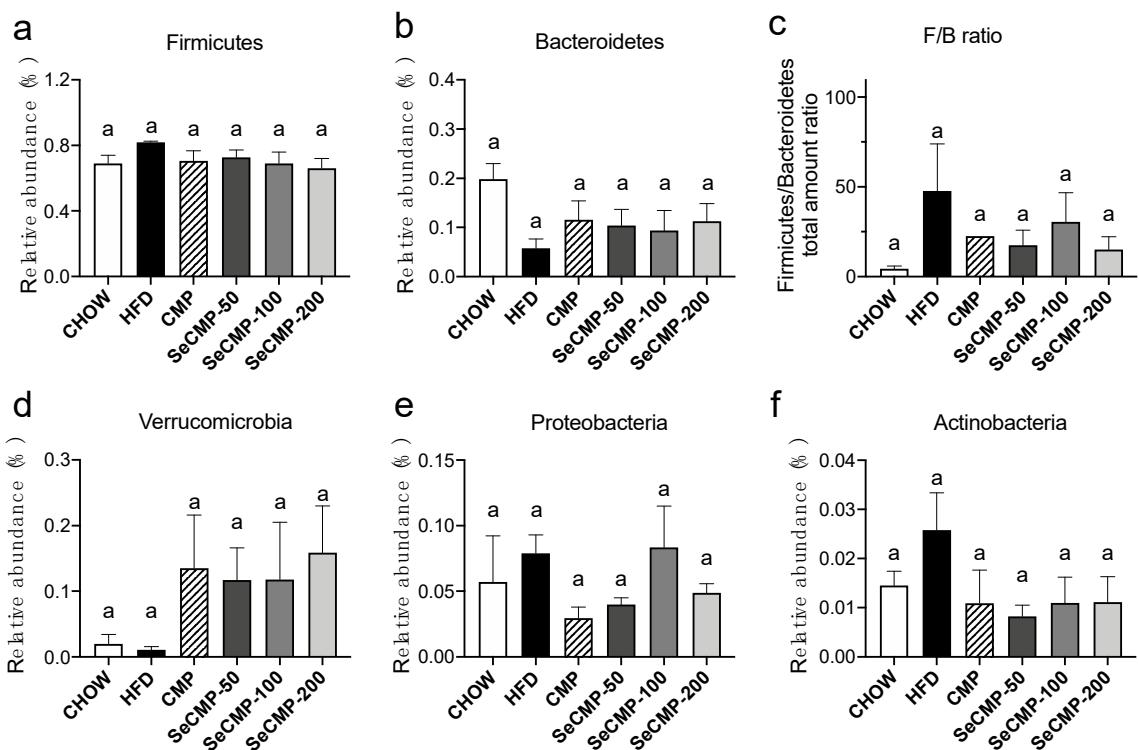


Figure S3. The relative abundance of top 5 phyla and F/B ratio, including (a) Firmicutes, (b) Bacteroidetes, (c) Firmicutes/Bacteroidetes ratio, (d) Verrucomicrobia, (e) Proteobacteria and (f) Actinobacteria after polysaccharides administration. Data represent mean \pm SE ($n = 5\text{-}6$ mice/group). Data were analyzed using Tukey's post hoc test or the non-parametric Kruskal-Wallis test with Dunnett's T3 multiple comparison test. Superscript characters indicate significant variation among treatments. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg CMP; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

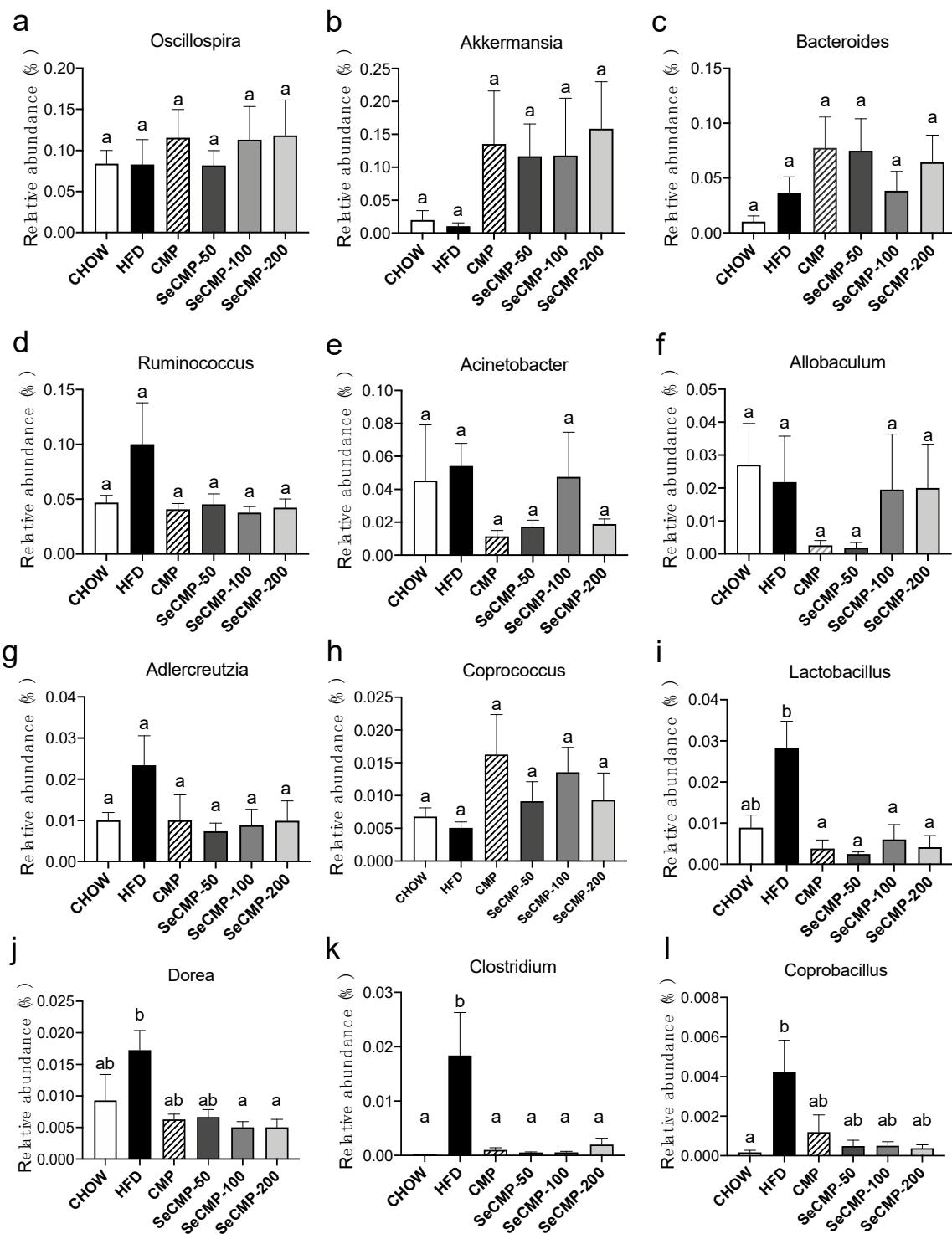


Figure S4. The relative abundance of top 10 genera and key genera identified in LEfSe comparsion test. Data represent mean \pm SE ($n = 5-6$ mice/group). Data were analyzed using Tukey's post hoc test or the non-parametric Kruskal-Wallis test with Dunnett's T3 multiple comparison test. Superscript characters indicate significant variation among treatments. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg CMP; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

Table S1. Weight of organs of mice under different feeding treatment.

| | Pancreas (g) | Heart (g) | Spleen (g) | Lung (g) | Kidney (g) |
|-----------|--------------------------|--------------------------|---------------------------|---------------------------|--------------------------|
| CHOW | 0.108±0.005 ^a | 0.150±0.007 ^a | 0.091±0.007 ^b | 0.166±0.004 ^b | 0.352±0.018 ^b |
| HFD | 0.114±0.004 ^a | 0.129±0.006 ^a | 0.064±0.004 ^a | 0.142±0.006 ^{ab} | 0.274±0.008 ^a |
| CMP | 0.129±0.008 ^a | 0.122±0.007 ^a | 0.076±0.008 ^{ab} | 0.139±0.006 ^a | 0.262±0.009 ^a |
| SeCMP-50 | 0.116±0.009 ^a | 0.128±0.005 ^a | 0.068±0.004 ^{ab} | 0.140±0.005 ^a | 0.264±0.006 ^a |
| SeCMP-100 | 0.108±0.005 ^a | 0.124±0.008 ^a | 0.068±0.007 ^{ab} | 0.142±0.009 ^a | 0.258±0.008 ^a |
| SeCMP-200 | 0.118±0.008 ^a | 0.130±0.007 ^a | 0.075±0.007 ^{ab} | 0.143±0.006 ^{ab} | 0.273±0.008 ^a |

Data are expressed as mean ± SE ($n = 8$ mice/group). Data were analyzed using one-way ANOVA analysis with Tukey's post hoc test or the non-parametric Kruskal-Wallis test with Dunnett's T3 multiple comparison test. Superscript characters indicate significant variation between different values within a column. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg selenium-deficient *C. militaris* crude polysaccharides; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

Table S2. Biochemistry analysis of serum of mice.

| | CHOW | HFD | CMP | SeCMP-50 | SeCMP-100 | SeCMP-200 |
|----------------|---------------------------|---------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| ALT (IU/L) | 29.05±1.45 ^a | 28.64±1.42 ^a | 33.07±5.54 ^a | 26.17±1.04 ^a | 28.50±1.98 ^a | 25.88±1.11 ^a |
| AST (IU/L) | 47.54±1.53 ^b | 46.98±1.98 ^b | 41.29±3.15 ^{ab} | 41.13±2.20 ^{ab} | 42.91±2.85 ^{ab} | 34.73±1.51 ^a |
| UA (μmol/L) | 243.06±10.02 ^b | 223.07±5.50 ^b | 189.15±2.07 ^a | 213.13±6.04 ^{ab} | 227.49±6.24 ^b | 211.20±4.98 ^a |
| CRE (μmol/L) | 167.37±9.75 ^b | 146.00±2.40 ^{ab} | 130.65±1.84 ^a | 123.85±1.31 ^a | 153.12±3.81 ^b | 138.08±2.34 ^a |
| ALB (g/L) | 23.92±2.33 ^a | 22.70±2.28 ^a | 22.06±1.25 ^a | 22.97±1.95 ^a | 21.45±1.06 ^a | 15.47±1.28 ^a |
| T-BIL (μmol/L) | 2.67±0.77 ^a | 4.50±1.30 ^a | 1.56±0.45 ^a | 1.92±0.55 ^a | 1.63±0.47 ^a | 2.10±0.560 ^a |

AST, aspartate aminotransferase; ALT, alanine aminotransferase; UA, uric acid; CREA, creatinine; ALB, albumin; T-BIL, total bilirubin. Data represent mean ± SE ($n = 6$ to 8 mice/group). Data were analyzed using one-way ANOVA analysis with Tukey's post hoc test or the non-parametric Kruskal-Wallis test with Dunnett's T3 multiple comparison test. Superscript characters indicate significant variation between different values within a row. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg CMP; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

Table S3. Group significance using ANOSIM analysis based on Bray-Curtis distance.

| Group 1 | Group 2 | Sample size | Permutations | R* | p-value | q-value [#] |
|----------|----------|-------------|--------------|----------|---------|----------------------|
| CHOW | HFD | 11 | 999 | 0.970667 | 0.002 | 0.006 |
| CHOW | CMP | 11 | 999 | 0.989333 | 0.002 | 0.006 |
| CHOW | SeCM-50 | 11 | 999 | 1 | 0.006 | 0.015 |
| CHOW | SeCM-100 | 11 | 999 | 0.973333 | 0.002 | 0.006 |
| CHOW | SeCM-200 | 11 | 999 | 1 | 0.002 | 0.006 |
| HFD | CMP | 12 | 999 | 0.32963 | 0.015 | 0.028125 |
| HFD | SeCM-50 | 12 | 999 | 0.582407 | 0.002 | 0.006 |
| HFD | SeCM-100 | 12 | 999 | 0.199074 | 0.055 | 0.091667 |
| HFD | SeCM-200 | 12 | 999 | 0.298148 | 0.015 | 0.028125 |
| SeCM-50 | CMP | 12 | 999 | 0.051852 | 0.277 | 0.377727 |
| SeCM-50 | SeCM-100 | 12 | 999 | -0.01019 | 0.563 | 0.603214 |
| SeCM-50 | SeCM-200 | 12 | 999 | 0.096296 | 0.194 | 0.291 |
| SeCM-100 | CMP | 12 | 999 | -0.02593 | 0.465 | 0.536538 |
| SeCM-100 | SeCM-200 | 12 | 999 | -0.11111 | 0.803 | 0.803 |
| SeCM-200 | CMP | 12 | 999 | 0.031481 | 0.307 | 0.38375 |

* R ranges $-1 \leq R \leq 1$. The more R approaches -1, the worse grouping; the more R approaches 1, the better grouping. [#] Q-value represents the adjust p value. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg selenium-deficient *C. militaris* crude polysaccharides; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

Table S4. Group significance by PERMANOVA analysis based on Bray-Curtis distance.

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F* | p-value | q-value [#] |
|----------|----------|-------------|--------------|-----------|---------|----------------------|
| CHOW | HFD | 11 | 999 | 4.419134 | 0.004 | 0.01 |
| CHOW | CMP | 11 | 999 | 3.864 | 0.003 | 0.01 |
| CHOW | SeCM-50 | 11 | 999 | 5.791691 | 0.004 | 0.01 |
| CHOW | SeCM-100 | 11 | 999 | 3.896248 | 0.004 | 0.01 |
| CHOW | SeCM-200 | 11 | 999 | 3.992201 | 0.002 | 0.01 |
| HFD | CMP | 12 | 999 | 2.22121 | 0.013 | 0.027857 |
| HFD | SeCM-50 | 12 | 999 | 3.503364 | 0.002 | 0.01 |
| HFD | SeCM-100 | 12 | 999 | 1.674476 | 0.067 | 0.111667 |
| HFD | SeCM-200 | 12 | 999 | 2.210875 | 0.018 | 0.03375 |
| SeCM-50 | CMP | 12 | 999 | 1.232954 | 0.252 | 0.343636 |
| SeCM-50 | SeCM-100 | 12 | 999 | 0.791944 | 0.742 | 0.795 |
| SeCM-50 | SeCM-200 | 12 | 999 | 1.321897 | 0.174 | 0.261 |
| SeCM-100 | CMP | 12 | 999 | 0.851569 | 0.553 | 0.638077 |
| SeCM-100 | SeCM-200 | 12 | 999 | 0.631252 | 0.816 | 0.816 |
| SeCM-200 | CMP | 12 | 999 | 0.988005 | 0.42 | 0.525 |

* pseudo-F the higher, the better grouping. [#]Q-value represents the adjust p value. CHOW: normal diet + sterile water; HFD: high-fat diet + sterile water; CMP: high-fat diet + 100 mg/kg selenium-deficient *C. militaris* crude polysaccharides; SeCMP-50: high-fat diet + 50 mg/kg SeCMP; SeCMP-100: high-fat diet + 100 mg/kg SeCMP; SeCMP-200: high-fat diet + 200 mg/kg SeCMP.

Table S5. Q-RCP primer sequences.

| Target | Direction | Primer sequence (5'-3') |
|---------------|-----------|--------------------------|
| GAPDH | Forward | GCATCCACTGGTGTGCC |
| | Reverse | TCATCATACTGGCAGGTTTC |
| FAS | Forward | CAAGTGCAAACCAGACTTCTAC |
| | Reverse | GCACTTCTTTCCGGTACTTT |
| ACC-1 | Forward | CCCAGAGATGTTCGGCAGTCAC |
| | Reverse | GTCACGGATGTCGGAAGGCAAAGG |
| SREBP1c | Forward | TCCACCATCGGCACCCACTG |
| | Reverse | GGCACTGGCTCCTCTTGATTCC |
| MTP | Forward | TTGTGTGTGATCTCCGTATTCA |
| | Reverse | GTTGTAAAGACGGTCTCAGGTA |
| CYP7A1 | Forward | GTGATGTTGAAGCCGGATATC |
| | Reverse | TTTATGTGCGGTCTTGAACAAG |
| CPT1 | Forward | CTACATCACCCCAACCCATATT |
| | Reverse | GATCCCAGAACGACGAATAGGTT |
| HSL | Forward | GCTGGGCTGTCAAGCACTGT |
| | Reverse | GTAACTGGTAGGCTGCCAT |
| ATGL | Forward | CAGAGATGGACTTCGATTCCCTT |
| | Reverse | CAGGTGCTCTAGAATTGATCT |
| FATP | Forward | CCTCTCTGTTCTGATTGTT |
| | Reverse | GTCCAGCATATACCACTACTGG |
| TNF- α | Forward | TAGCCAGGAGGGAGAACAGA |
| | Reverse | TTTCTGGAGGGAGATGTGG |
| IL-10 | Forward | TTCTTCAAACAAAGGACCAGC |
| | Reverse | GCAACCCAAGTAACCCCTAAAG |
| IL-6 | Forward | CTCCCAACAGACCTGTCTATAC |
| | Reverse | CCATTGCACAACCTTTCTCA |
| ZO-1 | Forward | CTGGTGAAGTCTCGGAAAAATG |
| | Reverse | CATCTCTGCTGCCAAACTATC |
| Ocln | Forward | TGCTTCATCGCTTCCTTAGTAA |
| | Reverse | GGGTTCACTCCCATTATGTACA |