Table S1 The primer sequences for real-time PCR.

Genes	**Sequence**
18S	F:5’- GCAAGACGGACCAGAGCG-3’
	R:5’- GTGGTGCCCTTCCGTCAA-3’
Tet2	F:5’- AGAGAAGACAATCGAGAAGTCGG-3’
	R:5’- CCTTCCGTACTCCCAAACTCAT-3’
Malat1	F:5’- CAGTGCTGGGTGGGAATGTA-3’
	R:5’- CAATCCCACCCCAACAACTT-3’
Sox2ot	F:5’- TGGGATGAGTGAAAGCCTCT-3’
	R:5’- TCTCTCCCATCAGCGTCCT-3’
Gm15477	F:5’- ACAAAACCAGAAAGAGGATTGAAGAT-3’
	R:5’- GCGTGCTGCCATGCTTTA-3’
Snhg1	F:5’- TTGTGGGTTTACAGAGATGGTG-3’
	R:5’- CGCAGCTCATTCTTTTCCTC-3’

Table S2 The relative abundance of the top 10 gut bacterial genera at the phylum, class, order, family and genus level (%) (Mean ± SEM)

level	CON	HFD	AGE	EX
Young				
phylum				
Actinobacteria	0.0098 ± 0.0022	0.0223 ± 0.0138	0.0258 ± 0.0109	0.0324 ± 0.0101*
Bacteroidetes	0.5379 ± 0.0316	0.4053 ± 0.0625	0.3968 ± 0.0421*	0.4592 ± 0.0361
Deferribacteres	0.0173 ± 0.0036	0.0358 ± 0.0044*	0.0147 ± 0.0023$^{\#}$	0.0163 ± 0.0053$^{\#}$
Firmicutes	0.3346 ± 0.0221	0.4302 ± 0.0550	0.4338 ± 0.0427*	0.3569 ± 0.0354
Proteobacteria	0.0938 ± 0.0123	0.1019 ± 0.0121	0.1270 ± 0.0162	0.1332 ± 0.0121$^{\#}$
TM7	UD	UD	UD	0.0003 ± 0.0002
Tenericutes	0.0001 ± 0.0001	0.00 ± 0.00	0.0003 ± 0.0002	0.0007 ± 0.0003$^{*\#}$
Verrucomicrobia	0.0035 ± 0.0021	0.0036 ± 0.0022	0.0006 ± 0.0004$^{*\#}$	UD
class				
Coriobacteriia	0.0086 ± 0.0019	0.0222 ± 0.0138	0.0248 ± 0.0106	0.0305 ± 0.0099*
Bacteroidia	0.5379 ± 0.0316	0.4053 ± 0.0625	0.3968 ± 0.0421*	0.4592 ± 0.0361
Deferribacteres	0.0173 ± 0.0036	0.0358 ± 0.0044*	0.0147 ± 0.0023$^{\#}$	0.0163 ± 0.0053$^{\#}$
Bacilli	0.0067 ± 0.0023	0.0011 ± 0.0005*	0.0012 ± 0.0004*	0.0049 ± 0.0026

Clostridia	0.3031 ± 0.0239	0.3857 ± 0.0582	0.4178 ± 0.0448*	0.3338 ± 0.0379
Erysipelotrichi	0.0249 ± 0.0117	0.0434 ± 0.0164	0.0148 ± 0.0038$^{\#}$	0.0181 ± 0.0052
Betaproteobacteria	0.0001 ± 0.0001	0.0028 ± 0.0026	0.0006 ± 0.0004	0.0074 ± 0.0063
Deltaproteobacteria	0.0907 ± 0.0121	0.0882 ± 0.0128	0.0932 ± 0.0138	0.1064 ± 0.0165
Epsilonproteobacteria	0.0022 ± 0.0007	0.0095 ± 0.0023*	0.0235 ± 0.0100*	0.0021 ± 0.0008$^{\#\$}$
Gammaproteobacteria	0.0007 ± 0.0004	0.0012 ± 0.0003	0.0095 ± 0.0069	0.0169 ± 0.0140
order				
Coriobacteriales	0.0086 ± 0.0019	0.0222 ± 0.0138	0.0248 ± 0.0106	0.0305 ± 0.0099*
Bacteroidales	0.5379 ± 0.0316	0.4053 ± 0.0625	0.3968 ± 0.0421*	0.4592 ± 0.0361
Deferribacterales	0.0173 ± 0.0036	0.0358 ± 0.0044*	0.0147 ± 0.0023$^{\#}$	0.0163 ± 0.0053$^{\#}$
Clostridiales	0.3031 ± 0.0239	0.3857 ± 0.0582	0.4178 ± 0.0448*	0.3338 ± 0.0379
Erysipelotrichales	0.0249 ± 0.0117	0.0434 ± 0.0164	0.0148 ± 0.0038$^{\#}$	0.0181 ± 0.0052
Burkholderiales	0.0001 ± 0.0001	0.0028 ± 0.0026	0.0006 ± 0.0004	0.0074 ± 0.0063
Desulfovibrionales	0.0907 ± 0.0121	0.0882 ± 0.0128	0.0932 ± 0.0138	0.1064 ± 0.0165
Campylobacterales	0.0022 ± 0.0007	0.0095 ± 0.0023*	0.0235 ± 0.0100*	0.0021 ± 0.0008$^{\#\$}$
Enterobacteriales	0.0007 ± 0.0004	0.0012 ± 0.0003	0.0095 ± 0.0069	0.0169 ± 0.0140
Verrucomicrobiales	0.0035 ± 0.0021	0.0036 ± 0.0022	0.0006 ± 0.0004$^{*\#}$	UD

family				
Coriobacteriaceae	0.0086 ± 0.0019	0.0222 ± 0.0138	0.0248 ± 0.0106	0.0305 ± 0.0099*
Bacteroidales	0.0716 ± 0.0075	0.1447 ± 0.0228*	0.1234 ± 0.0258	0.1108 ± 0.0127*
Bacteroidaceae	0.0269 ± 0.0116	0.1123 ± 0.0390	0.0545 ± 0.0208	0.0815 ± 0.0145*
S24-7	0.4131 ± 0.0359	0.1085 ± 0.0220*	0.1646 ± 0.0288*	0.1513 ± 0.0354*
Deferribacteraceae	0.0173 ± 0.0036	0.0358 ± 0.0044*	0.0147 ± 0.0023$^{\#}$	0.0163 ± 0.0053$^{\#}$
Clostridiales	0.1634 ± 0.0159	0.2214 ± 0.0326	0.2337 ± 0.0261*	0.1855 ± 0.0263
Lachnospiraceae	0.0303 ± 0.0030	0.0500 ± 0.0117	0.0552 ± 0.0082*	0.0424 ± 0.0074
Ruminococcaceae	0.0965 ± 0.0099	0.0984 ± 0.0185	0.1182 ± 0.0157	0.0982 ± 0.0132
Erysipelotrichaceae	0.0249 ± 0.0117	0.0434 ± 0.0164	0.0148 ± 0.0038$^{\#}$	0.0181 ± 0.0052
Desulfovibrionaceae	0.0907 ± 0.0121	0.0882 ± 0.0128	0.0932 ± 0.0138	0.1064 ± 0.0165
genus				
Coprococcus	0.0029 ± 0.0004	0.0054 ± 0.0025	0.0066 ± 0.0009*	0.0084 ± 0.0034*
Ruminococcus	0.0093 ± 0.0014	0.0051 ± 0.0012*	0.0056 ± 0.0009	0.0086 ± 0.0017
Helicobacter	0.0022 ± 0.0007	0.0095 ± 0.0023*	0.0234 ± 0.0100*	0.0020 ± 0.0008$^{\#\$}$
[Ruminococcus]	0.0100 ± 0.0013	0.0171 ± 0.0038	0.0131 ± 0.0019	0.0118 ± 0.0027
Odoribacter	0.0120 ± 0.0017	0.0143 ± 0.0026	0.0245 ± 0.0090	0.0218 ± 0.0072

Parabacteroides	0.0047 ± 0.0012	0.0061 ± 0.0028	0.0073 ± 0.0024	0.0609 ± 0.0089$^{*\#\$}$
Allobaculum	0.0226 ± 0.0119	0.0287 ± 0.0150	0.0119 ± 0.0039	0.0114 ± 0.0038
Mucispirillum	0.0173 ± 0.0036	0.0358 ± 0.0044*	0.0147 ± 0.0023$^{\#}$	0.0163 ± 0.053$^{\#}$
Oscillospira	0.0333 ± 0.0030	0.0391 ± 0.0079	0.0423 ± 0.0074	0.0362 ± 0.0071
Bacteroides	0.0269 ± 0.0116	0.1123 ± 0.0390	0.0545 ± 0.0208	0.0815 ± 0.0145*
Middle-aged				
phylum				
Actinobacteria	0.0180 ± 0.0061	0.0093 ± 0.0035	0.0036 ± 0.0011*	0.0158 ± 0.0053
Bacteroidetes	0.5563 ± 0.0437	0.4266 ± 0.0352*	0.5026 ± 0.0668	0.5235 ± 0.0283
Cyanobacteria	0.0013 ± 0.0009	UD	UD	UD
Deferribacteres	0.0477 ± 0.0120	0.0447 ± 0.0178	0.0310 ± 0.0082	0.0130 ± 0.0020$^{\#}$
Firmicutes	0.3438 ± 0.0363	0.4408 ± 0.0440	0.4341 ± 0.0573	0.4192 ± 0.0276
Fusobacteria	UD	UD	UD	UD
Proteobacteria	0.0298 ± 0.0063	0.0739 ± 0.0372	0.0249 ± 0.0065	0.0249 ± 0.0075
TM7	0.0008 ± 0.0002	0.0010 ± 0.0005	0.0003 ± 0.0002*	0.0001 ± 0.0001*
Tenericutes	0.0011 ± 0.0006	0.0010 ± 0.0006	0.0012 ± 0.0007	0.0016 ± 0.0006
Verrucomicrobia	UD	0.0018 ± 0.0013	0.0012 ± 0.0012$^{\#}$	0.0001 ± 0.0001$^{\#}$

class				
Actinobacteria	0.0072 ± 0.0043	0.0005 ± 0.0001*	0.0002 ± 0.0001$^{*\#}$	0.0006 ± 0.0002$^{*\$}$
Coriobacteriia	0.0109 ± 0.0028	0.0088 ± 0.0035	0.0035 ± 0.0010*	0.0152 ± 0.0053
Bacteroidia	0.5563 ± 0.0437	0.4266 ± 0.0352*	0.5026 ± 0.0668	0.5235 ± 0.0283
Deferribacteres	0.0477 ± 0.0120	0.0447 ± 0.0178	0.0310 ± 0.0082	0.0130 ± 0.0020$^{\#}$
Bacilli	0.0043 ± 0.0011	0.0011 ± 0.0004*	0.0022 ± 0.0003$^{\#}$	0.0020 ± 0.0003
Clostridia	0.2989 ± 0.0408	0.4149 ± 0.0480	0.4132 ± 0.0574	0.3532 ± 0.0335
Erysipelotrichi	0.0406 ± 0.0105	0.0248 ± 0.0070	0.0186 ± 0.0085*	0.0640 ± 0.0254$^{\$}$
Deltaproteobacteria	0.0222 ± 0.0054	0.0280 ± 0.0077	0.0182 ± 0.0041	0.0129 ± 0.0036
Epsilonproteobacteria	0.0044 ± 0.0029	0.0441 ± 0.0396	0.0036 ± 0.0031	0.0031 ± 0.0020
Gammaproteobacteria	0.0008 ± 0.0004	0.0015 ± 0.0007	0.0030 ± 0.0018	0.0078 ± 0.0064
order				
Bifidobacteriales	0.0071 ± 0.0043	0.0005 ± 0.0001*	0.0002 ± 0.0001$^{*\#}$	0.0005 ± 0.0002$^{*\$}$
Coriobacteriales	0.0109 ± 0.0028	0.0088 ± 0.0035	0.0035 ± 0.0010*	0.0152 ± 0.0053
Bacteroidales	0.5563 ± 0.0437	0.4266 ± 0.0352*	0.5026 ± 0.0668	0.5235 ± 0.0283
Deferribacterales	0.0477 ± 0.0120	0.0447 ± 0.0178	0.0310 ± 0.0082	0.0130 ± 0.0020$^{\#}$
Lactobacillales	0.0017 ± 0.0006	0.0011 ± 0.0004	0.0021 ± 0.0003	0.0017 ± 0.0003

Clostridiales	0.2989 ± 0.0408	0.4149 ± 0.0480	0.4132 ± 0.0574	0.3532 ± 0.0335
Erysipelotrichales	0.0406 ± 0.0105	0.0248 ± 0.0070	0.0186 ± 0.0085*	0.0640 ± 0.0254$^{\$}$
Desulfovibrionales	0.0222 ± 0.0054	0.0280 ± 0.0077	0.0182 ± 0.0041	0.0129 ± 0.0036
Campylobacterales	0.0044 ± 0.0029	0.0441 ± 0.0396	0.0036 ± 0.0031	0.0031 ± 0.0020
Enterobacteriales	0.0005 ± 0.0001	0.0015 ± 0.0007	0.0029 ± 0.0018	0.0076 ± 0.0064
family				
Bacteroidaceae	0.0855 ± 0.0172	0.0824 ± 0.0185	0.0765 ± 0.0202	0.0556 ± 0.0087
Porphyromonadaceae	0.0130 ± 0.0054	0.0295 ± 0.0108	0.0533 ± 0.0103*	0.0385 ± 0.0102
Rikenellaceae	0.0525 ± 0.0076	0.0320 ± 0.0093	0.0427 ± 0.0119	0.0559 ± 0.0075
S24-7	0.3820 ± 0.0329	0.1662 ± 0.0186*	0.2457 ± 0.0382*	0.2261± 0.0291*
Deferribacteraceae	0.0477 ± 0.0120	0.0447 ± 0.0178	0.0310 ± 0.0082	0.0130 ± 0.0020$^{\#}$
Lachnospiraceae	0.0286 ± 0.0041	0.0811 ± 0.0162*	0.0578 ± 0.0088*	0.0651 ± 0.0025*
Ruminococcaceae	0.0767 ± 0.0142	0.1224 ± 0.0179	0.1253 ± 0.0205	0.1216 ± 0.0155
Erysipelotrichaceae	0.0406 ± 0.0105	0.0248 ± 0.0070	0.0186 ± 0.0085*	0.0640 ± 0.0254$^{\$}$
Desulfovibrionaceae	0.0221 ± 0.0054	0.0278 ± 0.0077	0.0181 ± 0.0041	0.0129 ± 0.0036
Helicobacteraceae	0.0044 ± 0.0029	0.0441 ± 0.0396	0.0036 ± 0.0031	0.0031 ± 0.0020
genus				

Oscillospira	0.0272 ± 0.0053	0.0422 ± 0.0072	0.0572 ± 0.0116*	0.0613 ± 0.0079*
Parabacteroides	0.0130 ± 0.0054	0.0294 ± 0.0109	0.0533 ± 0.0103*	0.0385 ± 0.0102
Bacteroides	0.0855 ± 0.0172	0.0824 ± 0.0185	0.0765 ± 0.0202	0.0556 ± 0.0087
Mucispirillum	0.0477 ± 0.0120	0.0447 ± 0.0178	0.0310 ± 0.0082	0.0130 ± 0.0020$^{\#}$
Bilophila	0.0056 ± 0.0016	0.0216 ± 0.0073*	0.0119 ± 0.0024*	0.0094 ± 0.0033
[Ruminococcus]	0.0050 ± 0.0010	0.0160 ± 0.0042*	0.0123 ± 0.0025*	0.0097 ± 0.0008*
Coprococcus	0.0021 ± 0.0007	0.0213 ± 0.0087*	0.0122 ± 0.0024*	0.0143 ± 0.0018*
Allobaculum	0.0350 ± 0.0075	0.0117 ± 0.0057*	0.0116 ± 0.0053*	0.0570 ± 0.0231$^{\#\$}$
Odoribacter	0.0033 ± 0.0033	0.0187 ± 0.0079	0.0055 ± 0.0040	0.0001 ± 0.0001
Helicobacter	0.0044 ± 0.0029	0.0441 ± 0.0396	0.0036 ± 0.0031	0.0031 ± 0.0020

* $p<0.05$ versus CON group by Mann-Whitney U test

$^{\#}$ $p<0.05$ versus HFD group by Mann-Whitney U test

$^{\$}$ $p<0.05$ versus AGE group by Mann-Whitney U test

UD: under the detection limit, means the values is 0 when detecting.