

Polysaccharide from *Flammulina velutipes* Attenuates
Markers of Metabolic Syndrome by modulating the Gut
Microbiota and Lipid Metabolism in High fat Diet-fed
Mice

Supplementary Table 1-3

Supplementary Figure 1-5

Supplemental Table 1 Compositions of experimental diets

Ingredients (g / kg)	LAD3001M	TP23100
Sucrose	100	202
Corn Starch	397	132
Casein (protein content>85%)	200	175
Cellulose	50	62
Mineral Mixture	35	61
Maltodextrin	132	125
Soybean Oil	70	30
Lard	0	196
Vitamin Blend	10	12
L-cystine	3	2
Choline Bitartrate	2.5	3
Essential Nutrients (g / 100g, unless noted otherwise)		
Moisture	8.5	8.2
Protein	15.75	18.64
Fat	4.44	19.88
Fiber	3.44	3.86
Ash	1.04	1.43
Calcium	0.014	0.013
Phosphorus	0.16	0.14
Calories (kcal/g)	3.85	4.73
Carbohydrate energy supply ratio (%)	67.4	40.9
Fat energy supply ratio (%)	12.0	45.0
Protein energy supply ratio (%)	20.6	14.1

Supplemental Table 2 Primer sequences used in RT-qPCR assays

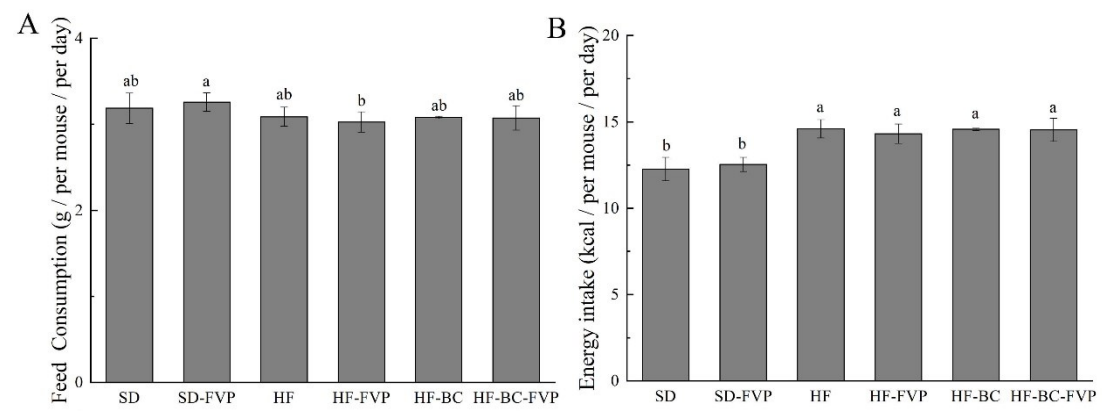
Gene ID	Target gene	Primer Sequence (5'-3')
19017	<i>Ppargc1a</i>	F-GGATATACTTTACGCAGGTCGA R-CGTCTGAGTTGGTATCTAGGTC
70673	<i>Prdm16</i>	F-CAACAAAGAGAAGCCGTTCAAG R-TTTCGGATCTCGGAGAAGTAAG
22227	<i>Ucp1</i>	F-ATTCAGAGGCAAATCAGCTTTG R-GTGTTTCTCTCCCTGAAGAGAA
19013	<i>Ppara</i>	F-CCAAGAATACCAAAGTGCGATC R-TCACAAGCATGAACTCCATAG
19016	<i>Pparg</i>	F-GAGCTGCAAGATTCAGAAGAAG R-GAATCTTTCAGGTCGTGTTTAC
105787	<i>Ampka1</i>	F-GCCCTTCCTTCTCTTGCCCTTTC R-ACCACACCTACTCCTCCTTCACAC
12894	<i>Cpt1</i>	F-CTACATCACCCACCCCATATT R-GATCCCAGAAGACGAATAGGTT
15357	<i>Hmgcr</i>	F-GGTGCAAAGTTCCTTAGTGATG R-GAATAGACACACCACGTTTCATG
20787	<i>Srebp1</i>	F-CAAACAGTTGCGTAAAGAGTCC R-TCTTCTTCCAACCTCGCCTTATT
14102	<i>Fas</i>	F-CAAGTGCAAACCAGACTTCTAC R-GCACTTTCTTTTCCGGTACTTT
107476	<i>Acac1</i>	F-AGGAGGAGGATGGCAGAAGTTGAG R-AAGCAGAGTCGCAGCATGAATGG
2597	<i>Gapdh</i>	F- AGGTCGGTGTGAACGGATTTG R- GGGGTCGTTGATGGCAACA

Supplemental Table 3 Sequence statistics

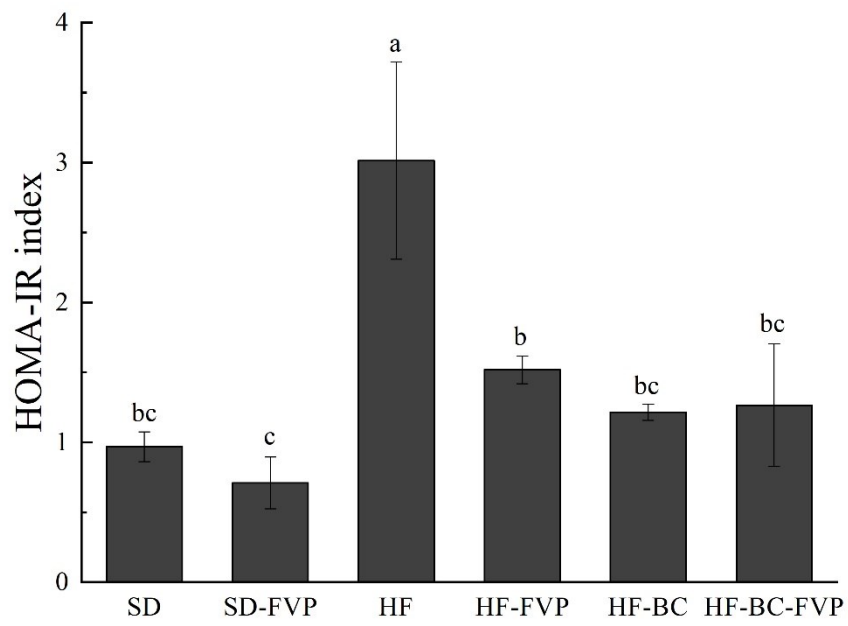
Samples	Raw reads	Q20(%)	Q30(%)	Merge	Merge(%)	Primer	Clean reads	Clean reads(%)	Q20(%)	Q30(%)
SD-1	318230	98.43	95.16	313257	98.44	287224	270296	84.94	99.26	96.76
SD-2	368934	98.25	94.43	363694	98.58	334711	314461	85.24	99.16	96.31
SD-3	358542	98.58	95.57	353356	98.55	328351	309448	86.31	99.3	96.9
SD-4	419796	98.45	95.11	413295	98.45	380546	358900	85.49	99.26	96.68
SD-5	388233	98.52	95.41	382906	98.63	352136	331813	85.47	99.31	96.92
SD-6	391024	98.51	95.4	385003	98.46	358048	337208	86.24	99.3	96.87
SD-7	355813	98.46	95.2	351009	98.65	326255	306225	86.06	99.27	96.74
SD-8	561173	98.15	94.23	553900	98.7	513958	479498	85.45	99.15	96.25
SD-9	457379	98.1	94.04	450669	98.53	420414	394692	86.29	99.18	96.38
SD-10	503039	98.44	95.23	494890	98.38	460758	433523	86.18	99.3	96.87
SD-11	328266	98.27	94.63	323163	98.45	300434	280742	85.52	99.18	96.4
SD-12	447561	98.47	95.25	441116	98.56	410930	386437	86.34	99.29	96.82
SD-FVP-1	306819	98.36	94.73	302527	98.6	276096	260505	84.91	99.22	96.5
SD-FVP-2	491761	98.55	95.5	484545	98.53	445805	419155	85.24	99.28	96.8
SD-FVP-3	418757	98.56	95.51	412479	98.5	382899	361795	86.4	99.35	97.02
SD-FVP-4	399882	98.12	93.98	394071	98.55	362179	338398	84.62	99.11	96.04
SD-FVP-5	420666	98.32	94.79	413863	98.38	381226	356209	84.68	99.08	96.19
SD-FVP-6	444446	98.34	94.93	438070	98.57	407084	381637	85.87	99.24	96.69
SD-FVP-7	423947	98.44	95.13	418287	98.66	388870	365053	86.11	99.23	96.65
SD-FVP-8	440533	98.47	95.25	433989	98.51	403137	379268	86.09	99.26	96.76
SD-FVP-9	438512	98.51	95.35	432352	98.6	402031	377928	86.18	99.26	96.76
SD-FVP-10	437176	98.39	95.07	431009	98.59	400570	375677	85.93	99.25	96.7
SD-FVP-11	374946	98.38	94.94	369132	98.45	343277	323293	86.22	99.22	96.59
SD-FVP-12	463249	98.23	94.47	457089	98.67	425417	396976	85.69	99.17	96.35
HF-1	503074	98.27	94.58	495264	98.45	458734	428636	85.2	99.15	96.3
HF-2	391699	98.55	95.4	386147	98.58	356908	336746	85.97	99.3	96.84
HF-3	442255	98.48	95.34	435777	98.54	405080	381348	86.23	99.31	96.92
HF-4	518157	98.31	94.73	511621	98.74	472531	438057	84.54	99.13	96.28
HF-5	537331	98.19	94.41	529435	98.53	487824	452611	84.23	99.15	96.29
HF-6	372047	98.52	95.47	366147	98.41	340583	320241	86.08	99.26	96.77
HF-7	578167	98.56	95.47	569886	98.57	528722	500225	86.52	99.31	96.94
HF-8	301215	98.5	95.3	297182	98.66	275228	258472	85.81	99.27	96.78
HF-9	553652	98.59	95.64	545974	98.61	508115	479006	86.52	99.32	96.97
HF-10	592537	98.3	94.74	583842	98.53	542607	505658	85.34	99.19	96.44
HF-11	551040	98.33	94.81	543179	98.57	505077	471619	85.59	99.19	96.47
HF-12	481847	98.41	95	475714	98.73	442271	411959	85.5	99.19	96.42
HF-FVP-1	514325	98.35	94.87	507630	98.7	469276	436910	84.95	99.19	96.45
HF-FVP-2	353272	98.54	95.43	348106	98.54	321025	302102	85.52	99.29	96.84
HF-FVP-3	556723	98.57	95.52	549290	98.66	512128	482770	86.72	99.31	96.92
HF-FVP-4	419884	98.46	95.23	413804	98.55	382023	359902	85.71	99.31	96.88
HF-FVP-5	547522	98.2	94.39	539965	98.62	499035	467833	85.45	99.2	96.45

HF-FVP-6	518693	98.54	95.42	511486	98.61	474734	444927	85.78	99.2	96.55
HF-FVP-7	407805	98.48	95.26	401724	98.51	373306	350168	85.87	99.21	96.57
HF-FVP-8	413831	98.14	94.19	408530	98.72	380031	352942	85.29	99.13	96.19
HF-FVP-9	586023	98.42	95.21	576782	98.42	536537	503839	85.98	99.29	96.85
HF-FVP-10	539723	98.4	95.07	531218	98.42	493196	462029	85.6	99.2	96.56
HF-FVP-11	397680	97.7	92.73	391717	98.5	363809	335594	84.39	98.92	95.34
HF-FVP-12	483635	97.52	92.32	477251	98.68	443425	413445	85.49	99.05	95.81
HF-BC-1	531578	98.44	95.15	524808	98.73	485640	456612	85.9	99.33	96.92
HF-BC-2	399040	98.58	95.6	393433	98.59	360997	341102	85.48	99.33	97.01
HF-BC-3	622259	98.44	95.1	613701	98.62	568402	531599	85.43	99.18	96.43
HF-BC-4	413376	98.56	95.5	407924	98.68	375487	352295	85.22	99.26	96.71
HF-BC-5	568420	98.48	95.26	560677	98.64	519261	488706	85.98	99.3	96.86
HF-BC-6	566435	98.53	95.45	558932	98.68	519826	488220	86.19	99.32	96.9
HF-BC-7	582232	98.34	94.7	573989	98.58	533390	497242	85.4	99.11	96.12
HF-BC-8	621223	98.33	94.74	612864	98.65	570220	536301	86.33	99.27	96.68
HF-BC-9	527160	98.26	94.43	519072	98.47	482414	446087	84.62	99	95.76
HF-BC-10	603406	98.53	95.4	595671	98.72	554627	521074	86.36	99.27	96.77
HF-BC-11	375295	98.47	95.19	370915	98.83	345911	323236	86.13	99.2	96.55
HF-BC-12	523864	98.53	95.44	517082	98.71	482031	453983	86.66	99.32	96.96
HF-BC-FVP-1	507196	98.51	95.38	500439	98.67	461951	434532	85.67	99.29	96.87
HF-BC-FVP-2	384745	98.29	94.67	379490	98.63	347919	322374	83.79	99.11	96.21
HF-BC-FVP-3	570216	98.54	95.35	562043	98.57	522714	491056	86.12	99.21	96.58
HF-BC-FVP-4	559373	98.41	95.16	550822	98.47	509248	476689	85.22	99.15	96.56
HF-BC-FVP-5	431172	98.43	95.12	424644	98.49	391857	366639	85.03	99.22	96.66
HF-BC-FVP-6	562629	98.44	95.12	553348	98.35	513223	480798	85.46	99.2	96.51
HF-BC-FVP-7	511247	98.55	95.43	503958	98.57	467872	441050	86.27	99.26	96.74
HF-BC-FVP-8	554015	98.43	95.07	545192	98.41	507429	476627	86.03	99.11	96.28
HF-BC-FVP-9	439086	97.81	93.23	432098	98.41	399990	370765	84.44	99.01	95.78
HF-BC-FVP-10	608864	98.36	94.93	600435	98.62	559329	524798	86.19	99.25	96.67
HF-BC-FVP-11	404944	98.37	94.98	398519	98.41	370779	345506	85.32	99.17	96.44
HF-BC-FVP-12	551845	98.58	95.54	543700	98.52	506594	478110	86.64	99.28	96.83

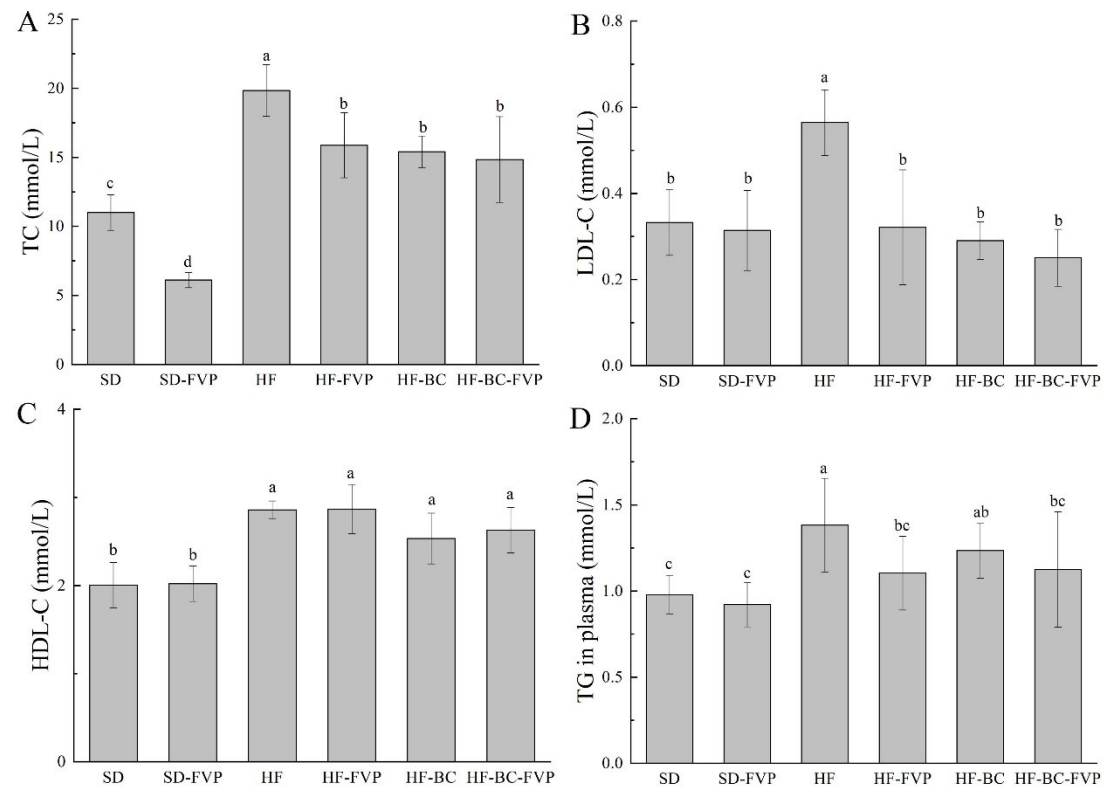
Raw reads : the number of original sequences; RQ20 (%): the proportion of bases with a mass greater than 20% in the original data; RQ30 (%): the proportion of bases with a mass greater than 30% in the original data; Merge : the number of sequences after splicing; Merge (%): The percentage of the spliced sequence to the original number; Primer: the number of sequences after finding and removing the primer; Clean reads: the final optimized sequence Clean reads (%): the percentage of the optimized sequence to the original number; CQ20 (%): the quality of the optimized sequence is greater than 20% in the optimized sequence; CQ30 (%): The proportion of bases with a mass greater than 30% in the optimized sequence.



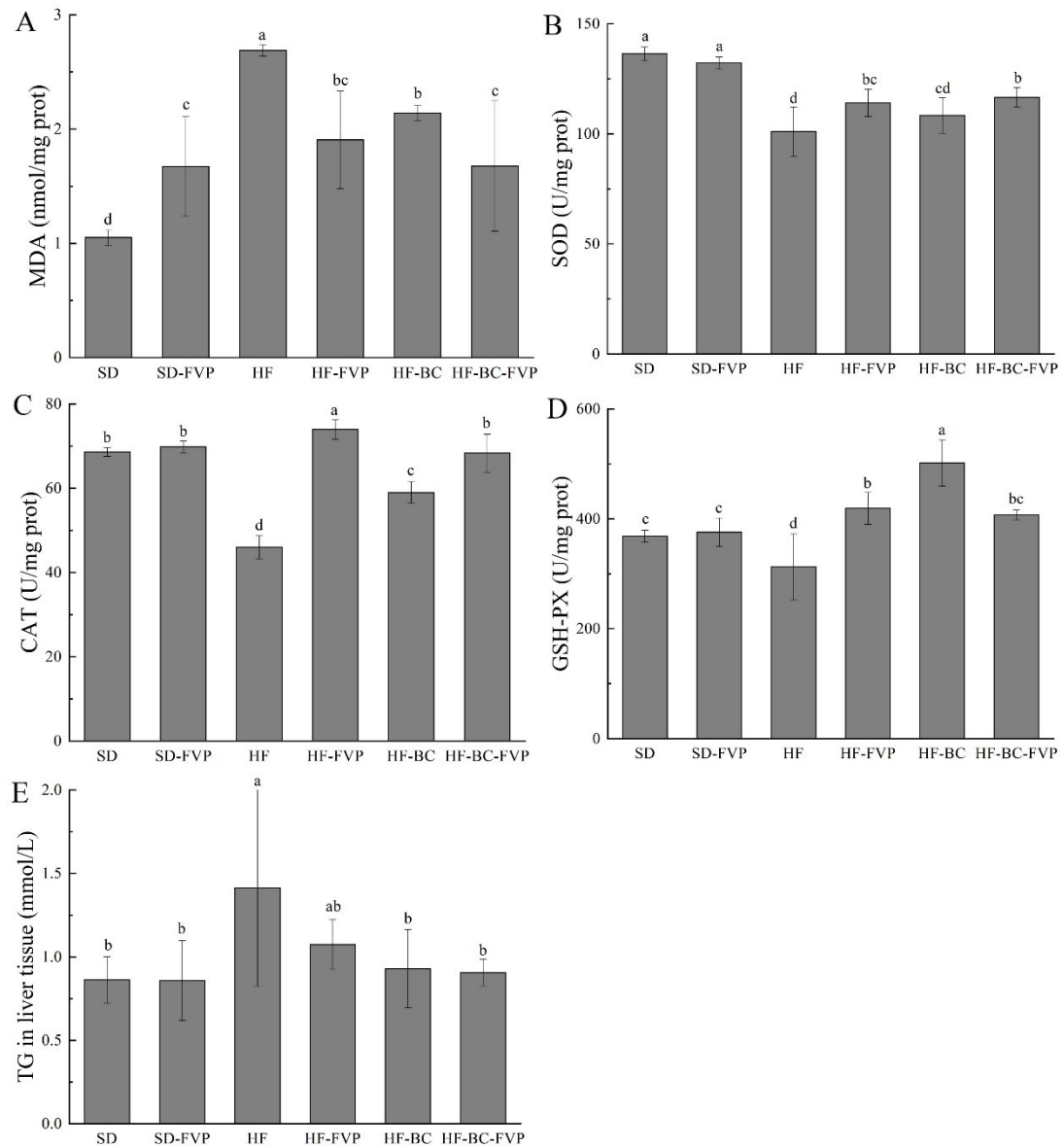
Supplemental Figure 1 (A) Food consumption, (B) Energy intake. Data are expressed as mean $\pm$ SD, n=12. Different lowercase letters (a>b) means significant difference in different treatment groups ($p<0.05$).



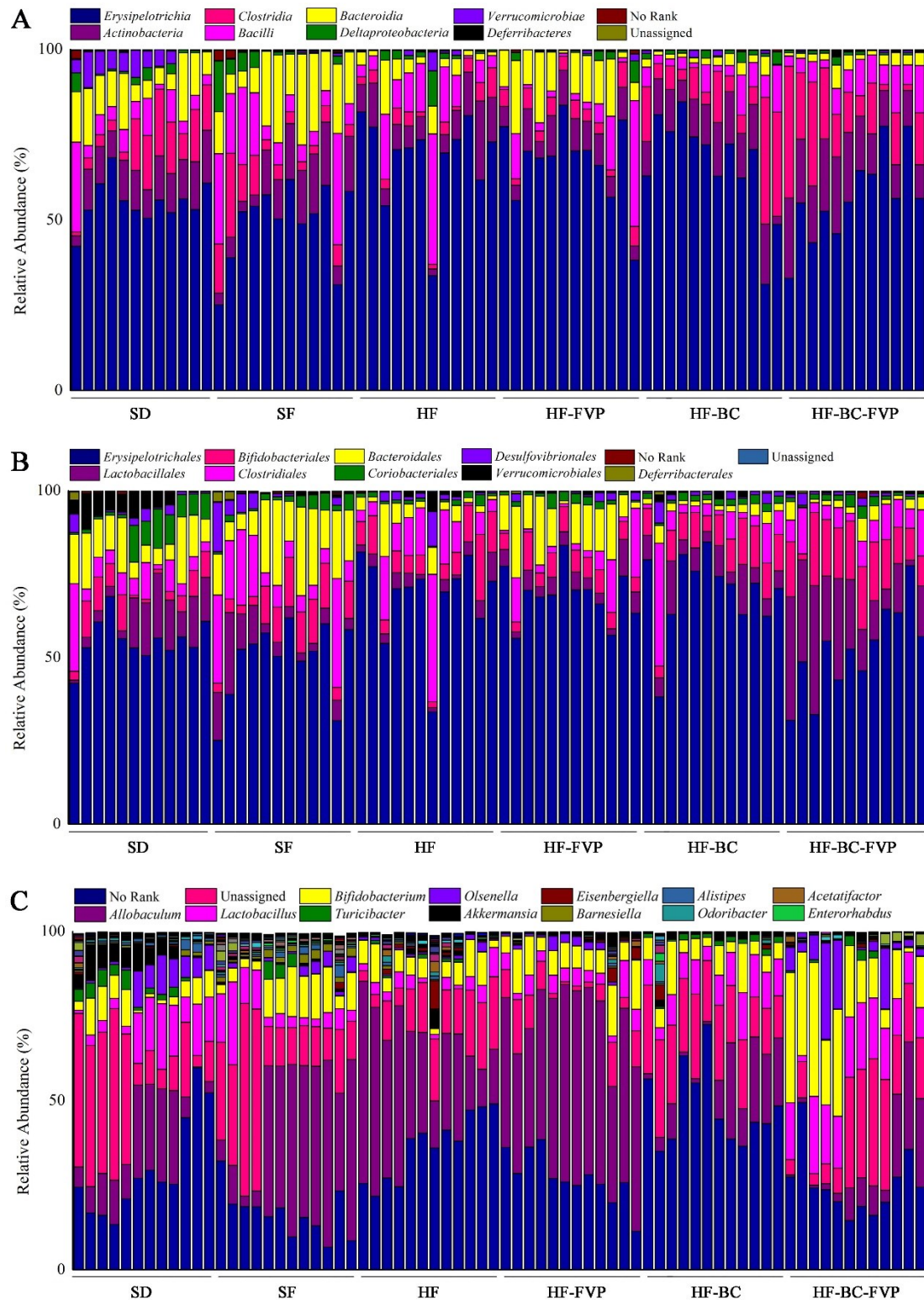
Supplemental Figure 2 HOMA-IR index. Data are expressed as mean $\pm$ SD, n=12. Different lowercase letters (a>b>c) means significant difference in different treatment groups ($p<0.05$).



Supplemental Figure 3 Mice blood lipids profiles: (A) Total cholesterol, (B) LDL cholesterol, (C) HDL cholesterol, (D) Triglyceride. Data are expressed as mean $\pm$ SD, $n=12$. Different lowercase letters (a>b>c>d) means significant difference in different treatment groups ($p<0.05$).



Supplemental Figure 4 Mice liver peroxidation profiles: (A) Malondialdehyde, (B) Superoxide dismutase, (C) Catalase, (D) Glutathione peroxidase, (E) Triglyceride in liver tissue. Data are expressed as mean $\pm$ SD, n=12. Different lowercase letters (a>b>c>d) means significant difference in different treatment groups ($p<0.05$).



Supplemental Figure 5 FVP and FMT induces distinct bacterial composition at class (A), order (B), and genus (C) levels, n=12.