1 Multi-omics analysis reveals the anti-fatigue mechanism of *Acanthopanax senticosus*

2 leaf extract

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Methods

Gastrointestine simulation

We built a 4-compartment gastrointestinal tract simulator following the work of K. Molly et al (1). The composition schematic and real images are shown in Figure S4. The first three chambers simulated the stomach, duodenum, and small intestine to stimulate *in vivo* environment, and the fourth chamber served as an intestinal microbial fermenter. 35 g of healthy human feces were collected and mixed with sodium phosphate buffer (w/V=1:4). After centrifuging, the supernatant was collected as inoculum. During the 7-day acclimation period, 200 ml of nutrient medium (g/L: arabinogalactan 1, pectin 2, xylan 1, starch 4.2, glucose 0.4, annual extract 3, peptone 1, mucin 4, cystine 0.5, $NaHCO_3$ 0.4, NaCl 0.08, K_2HPO_4 0.04, KH_2PO_4 0.04, $CaCl_2$ 0.008, $MgSO_4$-$7H_2O$ 0.008, $FeCl_2$ 0.008, vitamin C 0.008, $MnSO_4$ 0.008, Tween-80 1, hemoglobin 0.005) was pumped into stomach chamber at 10:00 and 16:00. Every time after simulated feeding, 100 mL of gastric juice (NaCl 2.0 g/L, protease (10000 activity units) 0.8 g/L, 7 ml of concentrated hydrochloric acid, pH 1.5) was added to react for 1 h (2). All fluids were then pumped into the duodenal chamber where pH was then adjusted to 7.0 with 150 mM $NaHCO_3$. After the contents were transferred to the small intestine, 150 ml of pancreatic juice (g/L: pig's bile salt 4, pancreatic enzyme 0.9) was pumped into it. When the pH was stabilized at 6.4, all contents were pumped into the reactor chamber. During the 14-day intervention, the steps remained the same except for the addition of 1 g ASLE to the food solution at each meal. The pump rotation time and speed were set to automatically discharge a portion of the solution from the reactor tank. 10% CO_2 + 90% N_2 was used as the headspace gas for the anaerobic environment. During the intervention, the effluent was collected and stored every 2 days and stored at -80°C. Schematic diagram and actual picture of gastrointestine simulator were shown in Fig S4 A&B.

49   **Table S1**. Primers used in qPCR.

Gene name	Primer
IL1b	F:5'-TCCATGAGCTTTGTACAAGGA-3'
	R:5'-AGCCCATACTTTAGGAAGACA-3'
IL6	F:5'-GTTCTCTGGGAAATCGTGGA-3'
	R:5'-TGTACTCCAGGTAGCTA-3'
COX2	F:5'-GTTCTCTGGGAAATCGTGGA-3'
	R:5'-TGTACTCCAGGTAGCTA-3'
iNOS	F:5'-TGTCTGCAGCACTTGGATCA-3'
	R:5'-AACTTCGGAAGGGAGCAATG-3'
Occludin	F:5'-ATGTCCGGCCGATGCTCTC-3'
	R:5'-TTTGGCTGCTCTTGGGTCTGTAT-3'
ZO1	F:5'-TTTTTGACAGGGGGAGTGG-3'
	R:5'-TGCTGCAGAGGTCAAAGTTCAAG-3'
Claudin1	F:5'-CCCGAGCCTTGATGGTAA-3'
	R:5'-CACTAATGTCGCCAGACCTGA-3'
Muc2	F:5'-ACGTGTCATATTTGCACCTCT-3'
	R:5'-TCAACATTGAGAGTGCCAACT-3'
Nrf2	F:5'-GTCTTCACTGCCCCTCATC-3'
	R:5'-TCGGGAATGGAAAATAGCTCC-3'
HO1	F:5'-TCAGTCCCAAACGTCGCGGT-3'
	R:5'-GCTGTGCAGGTGTTGAGCC-3'
Gclc	F:5'-ACATCTACCACGCAGTCAAGGACC-3'
	R:5'-CTCAAGAACATCGCCTCCATTCAG-3'
Foxo3a	F:5'-AGTGGATGGTGCGCTGTGT-3'
	R:5'-CTGTGCAGGGACAGGTTGT-3′
TNFα	F:5'-AGACCCTCACACTCAGATCA-3'
	R:5'-TCTTTGAGATCCATGCCGTTG-3'

Nfkb1	F:5'-ATGGCAGACGATGATCCCTAC-3'
	R:5'-TGTTGACAGTGGTATTTCTGGTG-3'
Gys2	F:5'-ACCAAGGCCAAAACGACAG-3'
	R:5'-GGGCTCACATTGTTCTACTTGA-3'
Pygl	F:5'-GAGAAGCGACGGCAGATCAG-3'
	R:5'-CTTGACCAGAGTGAAGTGCAG-3'
Gsk3b	F:5'-TGGCAGCAAGGTAACCACAG-3'
	R:5'-CGGTTCTTAAATCGCTTGTCCTG-3'
Fbp1	F:5'-TATGGTGGAAAGGGACGGGAA -3'
	R:5'-CCTCTGGTGATACTCAAGGATGG-3'
Idh2	F:5'-GGAGAAGCCGGTAGTGGAGAT-3'
	R:5'-GGTCTGGTCACGGTTTGGAA-3'
Pck1	F:5'-TGCATAACGGTCTGGACTTC-3'
	R:5'-CAGCAACTGCCCGTACTCC-3'
Pcx	F:5'-CTGAAGTTCCAAACAGTTCGAGG-3'
	R:5'-CGCACGAAACACTCGGATG-3'
Pfkl	F:5'-GGAGGCGAGAACATCAAGCC-3'
	R:5'-CGGCCTTCCCTCGTAGTGA-3'
18S	F:5'-AAACGGCTACCACATCCAAG-3'
	R:5'-CCTCCAATGGATCCTCGTTA-3'

51 **Table S2** The exhaustion distance of mice during each exercise session on the treadmill

	EC	ASLEL	ASLEM	ASLEH
1	801.45±167.93	785.39±183.79	751.50±82.37	737.78±237.38
2	715.67±108.46	720.10±268.35	736.37±62.20	725.18±282.11
3	617.50±267.16 *	655.83±314.42	793.30±173.36	762.33±268.87
4	505.75±181.50 *	728.42±229.14	646.00±168.14	762.50±211.48
5	574.83±124.92 *	649.75±263.54	673.75±176.30	730.36±189.09
6	529.83±112.38 *	775.54±92.58	627.58±149.19	610.00±195.93
7	598.92±149.08 *	693.17±225.20	717.91±108.82	723.18±256.01
8	589.00±193.63	667.83±276.03	794.82±130.91	781.70±215.07
9	604.08±259.27 *	629.17±260.61	683.08±123.37	694.00±279.35
10	657.50±175.20	668.50±244.64	721.08±136.59	755.34±183.58
11	618.43±33.49 *	750.60±74.05	784.25±46.56	838.25±176.43

52 All data are expressed as mean ± SD (n=8). The asterisk (*) represents significant differences
53 compared to the exhaustion distance in the first trial ($p < 0.05$).

54   **Table S3** Metabolites regulated by exercise and ASLE in ileum contents

Compound name	Chemical shift	EC vs. NC		ASLEH vs. EC	
		Fold change	p value	Fold change	p value
L-isoleucine	0.93t), 1.00(d), 1.46(m)	1.404	0.014		▢
L-leucine	0.95(t),1.70(m)	1.404	0.013		
L-Valine	0.98(d),1.03(d)	1.319	0.012		
L-alanine	1.47(d)	1.278	0.041		
alpha-Hydroxyisobutyric acid	1.34(s)	1.260	0.001		
L-Glutamic acid	2,04(s),2.12(s),2.34(s)	1.281	0.002		
L-Methionine	2,04(s),2.12(s),2.34(s)	0.902	0.124		
Choline	3.19(s),3.51(dd)	1.222	0.045		
phenylacetic acid	3.52(s)	0.588	0.012		
Glycine	3.54(s)	1.005	0.956		
L-carnitine	4.60(m)	1.147	0.033		
L-Tyrosine	6.90(ddd),6.93(ddd),7.19(ddd)	1.713	0.016		
L-Phenylalanine	7.37(m)	1.404	0.012		
Oxoglutaric acid	3.00(m)	1.547	0.013	1.113	0.023
creatinine	3.03(s)	1.762	0.023	1.022	0.295
D-Glucose	3.46(dt),3.73(dd),3.89(dd)	0.592	0.018	0.716	0.267
Taurine	3.25(t),3.42(t)	0.683	0.065	0.600	0.020
Trimethylamine N-oxide	3.25(s)	0.472	0.065	0.519	0.018
Ethanol	1.17(t),3.65(q)	0.743	0.378	0.796	0.757
Acetic acid	1.91(m)	1.021	0.767	0.985	0.179
Indoleacetylglycine	3.80(s)	0.767	0.066	0.814	0.050
L-Tryptophan	3.29(dd),4.05(dd)			0.750	0.036
Sarcosine	2.73(s)			1.278	0.017
Trimethylamine	2.89(s)			0.900	0.094
cis-Aconitic acid	3.12(s)			1.458	0.003
Creatine	3.92(s)			1.116	0.021

| Allantoin | 5.38(s) | | 1.134 | 0.704 |

The raw data associated with this table is in Supplementary information 3. "-" represents VIP<2.

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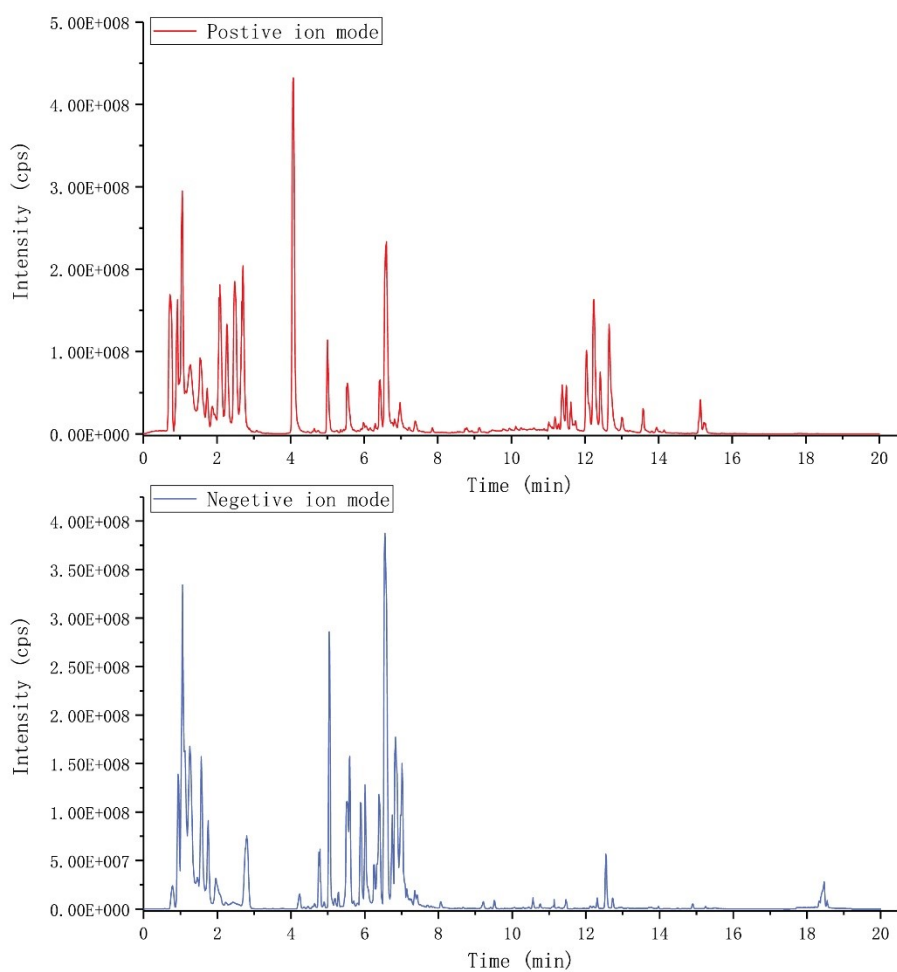
Figure S1. Ultra performance liquid chromatography mass spectrometry chromatograms of *Acanthopanax senticosus* leaves extracts. The molecular information corresponding to the retention time is displayed in the supplementary material 2.

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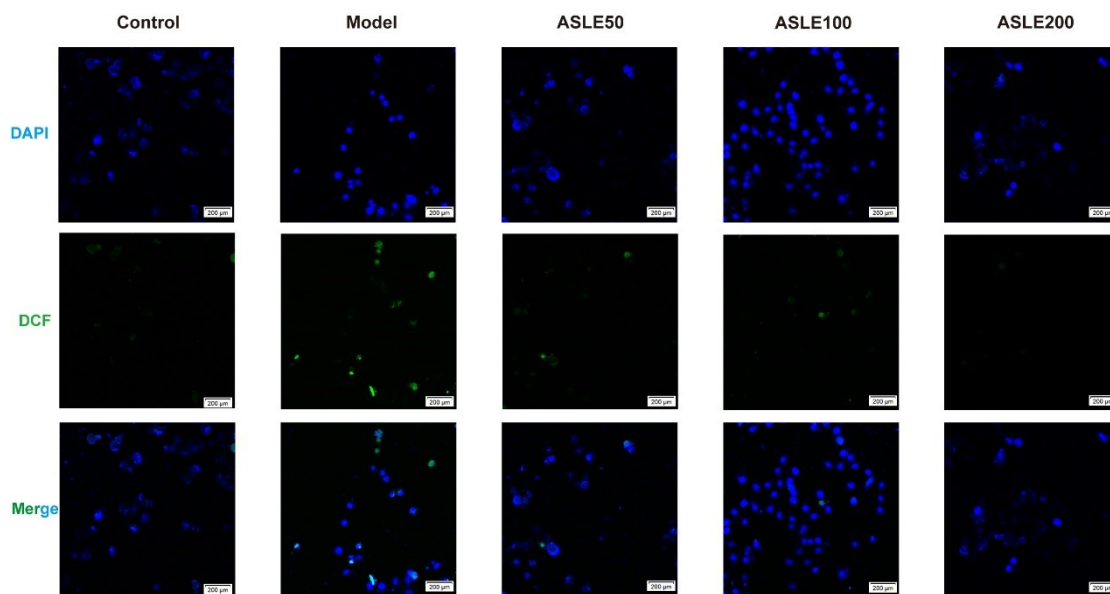
Figure S2. Effects of H2O2 on reactive oxygen species (ROS). DCFH-DA (2',7'-Dichlorodihydrofluorescein diacetate) can freely cross the cell membrane and hydrolyzed by intracellular enzymes and can be oxidized by ROS to generate green fluorescence.

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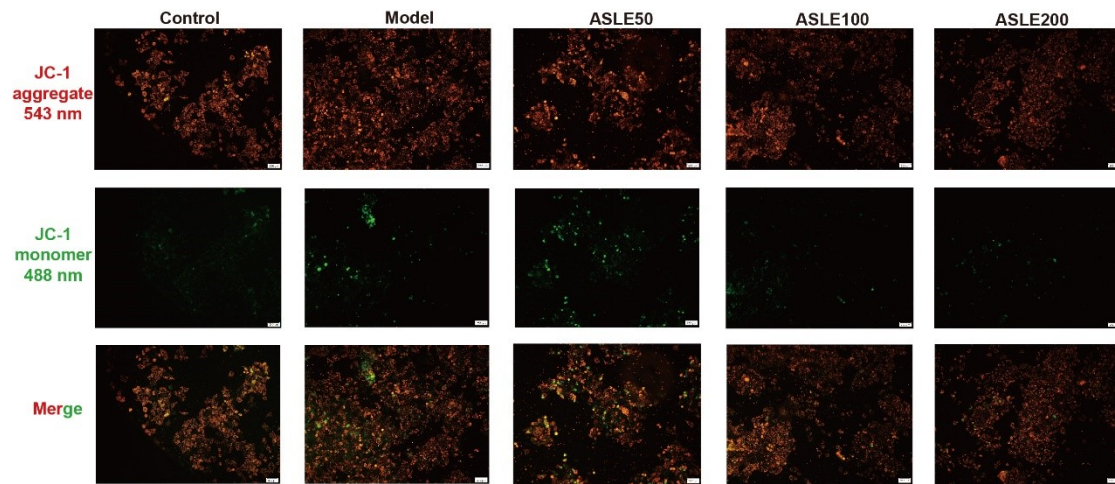
Figure S3. Effects of H2O2 and ASLE on mitochondrial membrane potential. When the mitochondrial membrane potential is high, JC-1 aggregates in the matrix of mitochondria and generates red fluorescence; When the mitochondrial membrane potential is abnormal, JC-1 is a monomer and can generate green fluorescence.

Gastrocnemius muscle
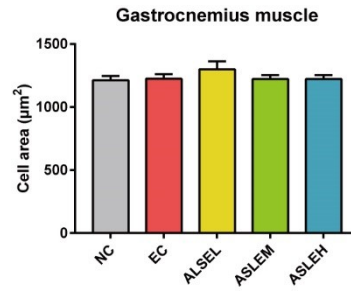
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70 **Figure S4**. Cell area of gastrocnemius cell. The cross-sectional area of all muscle fibers
71 in one field of view was counted and the results were expressed as mean ± SEM
72 (average area of all myocytes in one random microscopic field of view).

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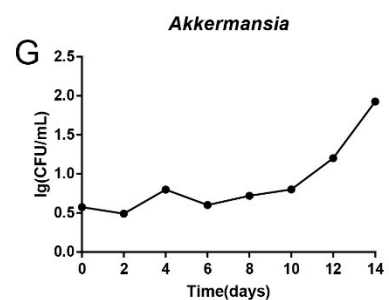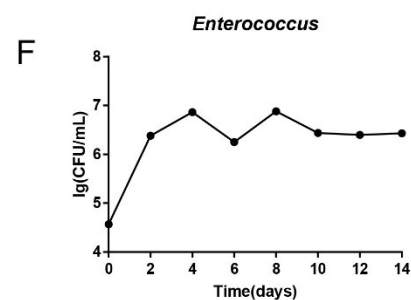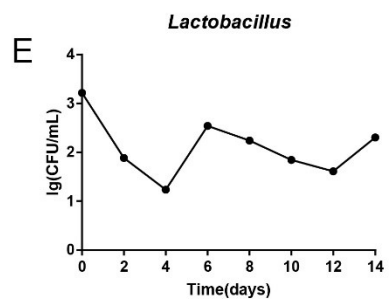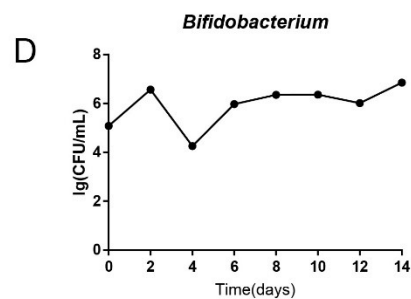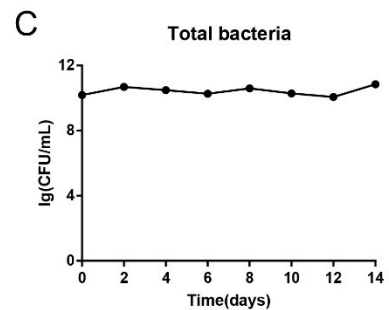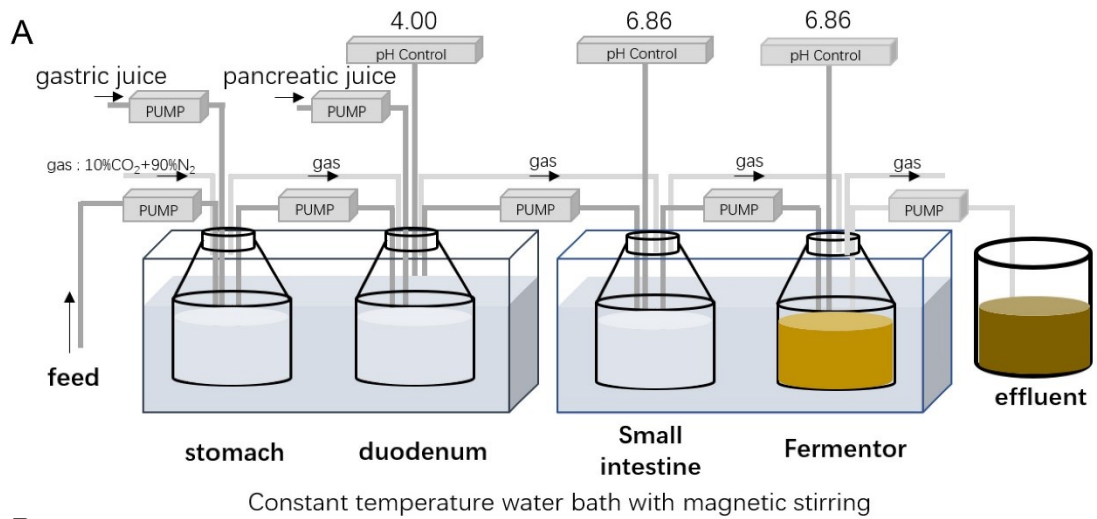
Figure S5. Effect of ASLE on bacteria in gastrointestine stimulator. (A) Schematic diagram of gastrointestine stimulator; (B) Actual picture of gastrointestine stimulator; (C-G) Effect of ASLE on bacterial counts at genus level.
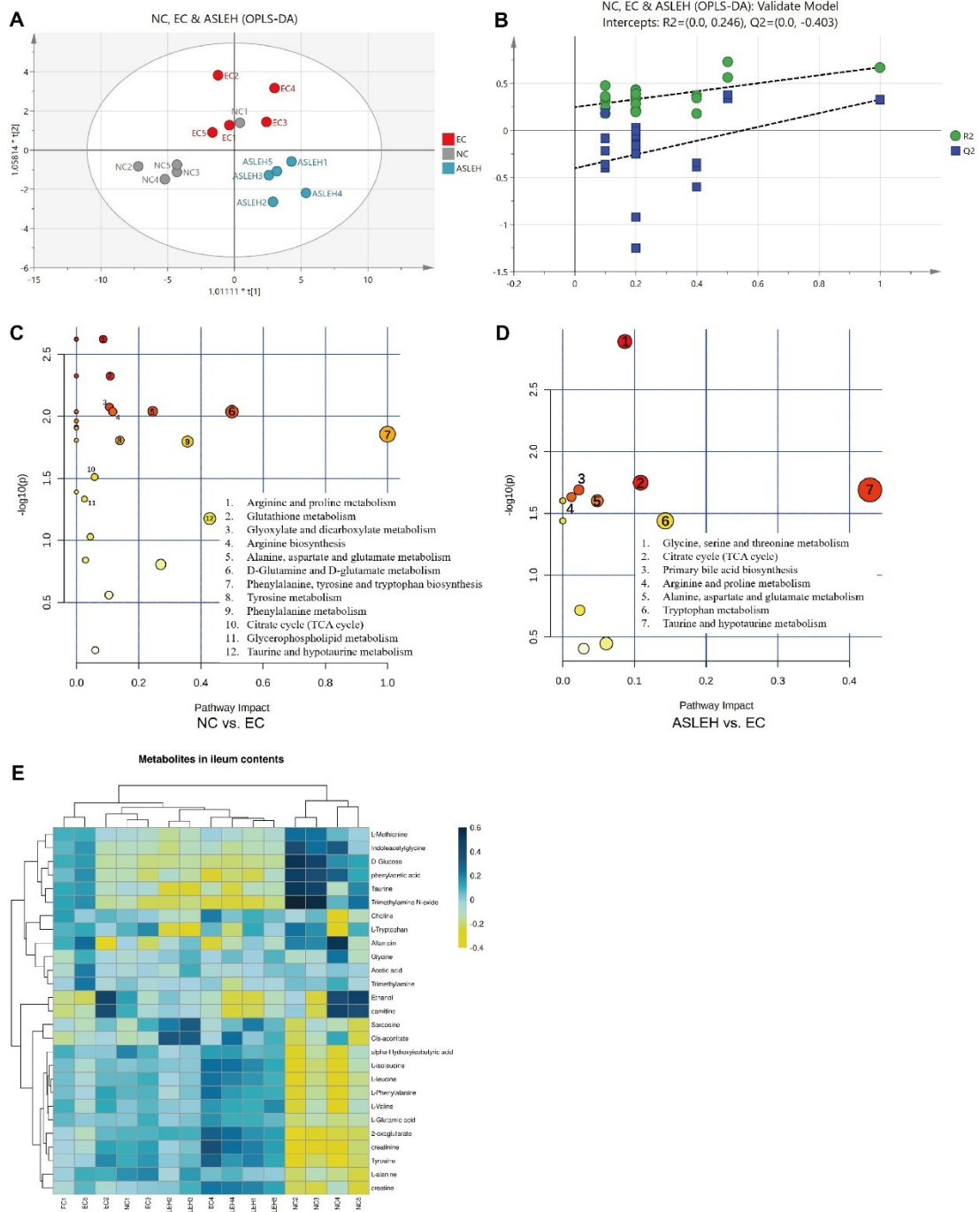
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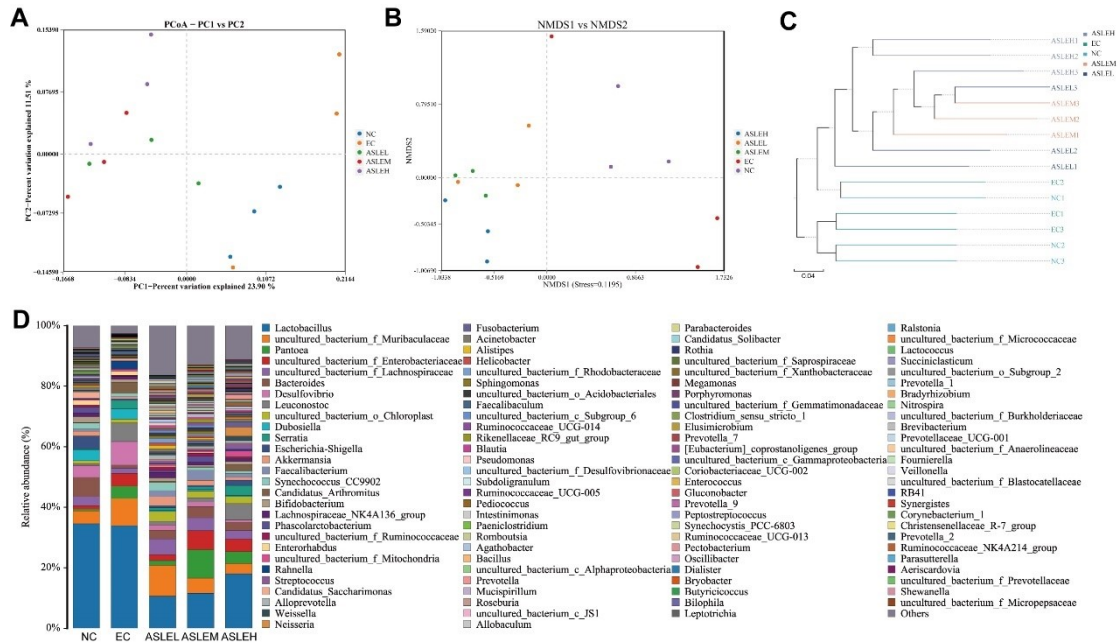
Figure S6. β-diversity of microbiota in cecum contents. (A)PCoA, (B)NMDS, (C)UPGMA, and (D) microbiota composition at genus level of cecum microbiota (n=3).

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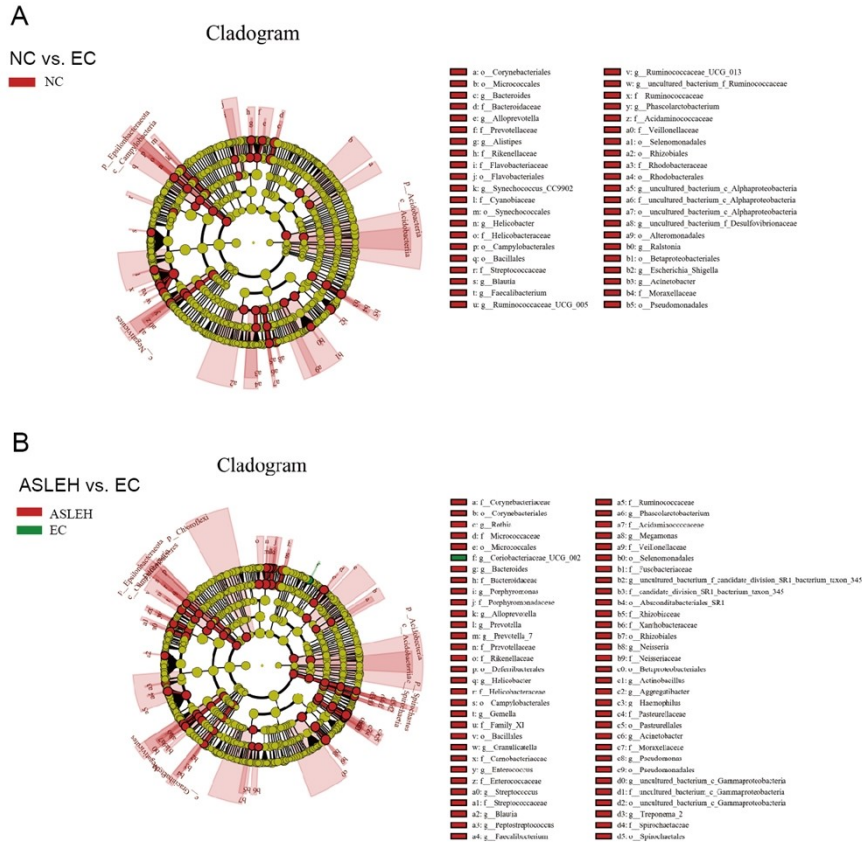
Figure S7. Cladogram of microbiota based on a LDA score above 3.0 (A)NC vs. EC;
(B) ASLEH vs. EC. LDA: linear discriminant analysis.

Figure S7. Metabolites in ileum contents altered by excessive exercise and ASLE supplementation. (A)OPLS-DA diagram;（B）OPLS-DA model permutation；regulated pathways of (C) EC vs. NC group and (D)ASLEH vs. EC group (n=5); (E) Heatmap of metabolites based on euclidean distance.

87 **Reference**
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