Supplementary Information for

Asiatic acid alleviates metabolism disorders in ob/ob mice: mechanistic insights

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1. Chromatographic and mass spectrometry conditions for untargeted metabolomics analysis

Sample analysis was performed using UHPLC-Q Exactive (Thermo Scientific, USA), equipped with an electrospray ion source, and mass spectrometry detection was performed in positive and negative ion modes, respectively. The column was an ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.8 μm; Waters, Milford, USA) with a flow rate of 0.4 mL/min and a column temperature of 40°C. Mobile phase A is composed of 95% water + 5% acetonitrile (containing 0.1% formic acid); mobile phase B is composed of 47.5% acetonitrile + 47.5% isopropanol + 5% water (containing 0.1% formic acid). The gradient elution program is: 0~0.1 min, 0~5% B; 0.1~2 min, 5~25% B; 2~9 min, 25~100% B; 9~13 min, 100~100% B; 13~13.1 min, 100~0% B; 13.1~16 min, 0~0% B. Scanning range is *m/z* 70~1050, positive ion mode spray voltage is 3500 V, negative ion mode spray voltage is -2800 V, the heating temperature was 400°C, the capillary temperature was 320°C, the sheath gas flow rate was 40 arb, and the auxiliary gas flow rate was 10 arb.

2. Metabolomics data processing

The collected raw data were imported into the metabolomics processing software Progenesis QI (Waters Corporation, Milford, USA) for baseline filtering, peak identification, integration, retention time correction, and peak alignment. A data matrix of information including intensities was then produced. Characteristic peaks were then searched and identified, with primary and secondary mass spectral information matched with the metabolite database. The mass deviation was set to less than 10 ppm and according to the 80% filtering rule the relative standard deviation (RSD) in the QC samples was less than 30%. To preprocess the data, they were logarithmically transformed to reduce errors caused in the experiment and analysis process, standardize the data structure. Metabolites that meet the criteria were then identified as the final identification result. The main

databases used were public databases including HMDB (http://www.hmdb.ca/), Metlin (https://metlin.scripps.edu/), and Lipidmaps (https://www.lipidmaps.org/).

To reveal the differences between groups, metabolites were then subjected to multivariate statistical analysis, including Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). To ensure the reliability of the model, cross-validation was performed on the OPLS-DA model. The cumulative interpretation rate of the model was evaluated by R^2 (cum); the predictive ability of the model was evaluated by Q^2 (cum). When Q^2 (cum) > 0.5, the model was determined to have a good predictive ability. For the screening of differential metabolites, Student's T (Unpaired) test was used to screen metabolites with p-value < 0.05, VIP > 1. Finally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) was used to perform pathway enrichment analysis on the metabolites.

3. Molecular docking

Mock docking between the ligand-binding domains (LBD) of PPAR-γ (PDB ID: 5y2o) and AA (Pubchem ID: 119034) was performed using AutoDock. The PPAR-γ agonist pioglitazone (Pubchem ID: 4829) was used as a positive control. Predefined binding sites for the LBD of PPAR-γ were used as docking pockets. Docking simulations were run to predict binding modes within the active site of the target receptor. Use the Discovery Studio Visualizer to analyze optimal conformations for possible hydrophobic interactions and H-bonding. The active sites were defined suing Site Finder module. Docking was performed with the setting of Triangle Matcher Placement and Rigid Receptor Refinement. Other parameters were established by default in software.

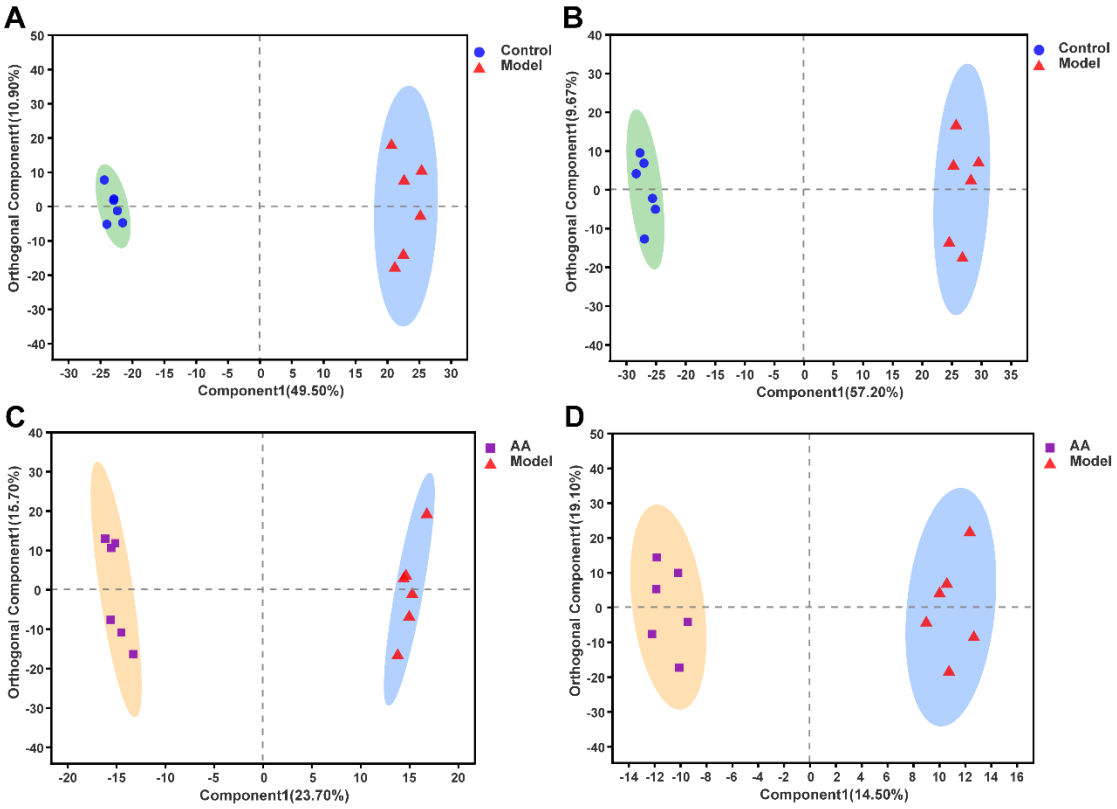

Figure S1. OPLS-DA score plot of between groups. (A) OPLS-DA score plot of Model group and Control group in positive mode, (B) and negative mode; (C) AA group and Model group in positive mode, (D) and negative mode. (n = 6).

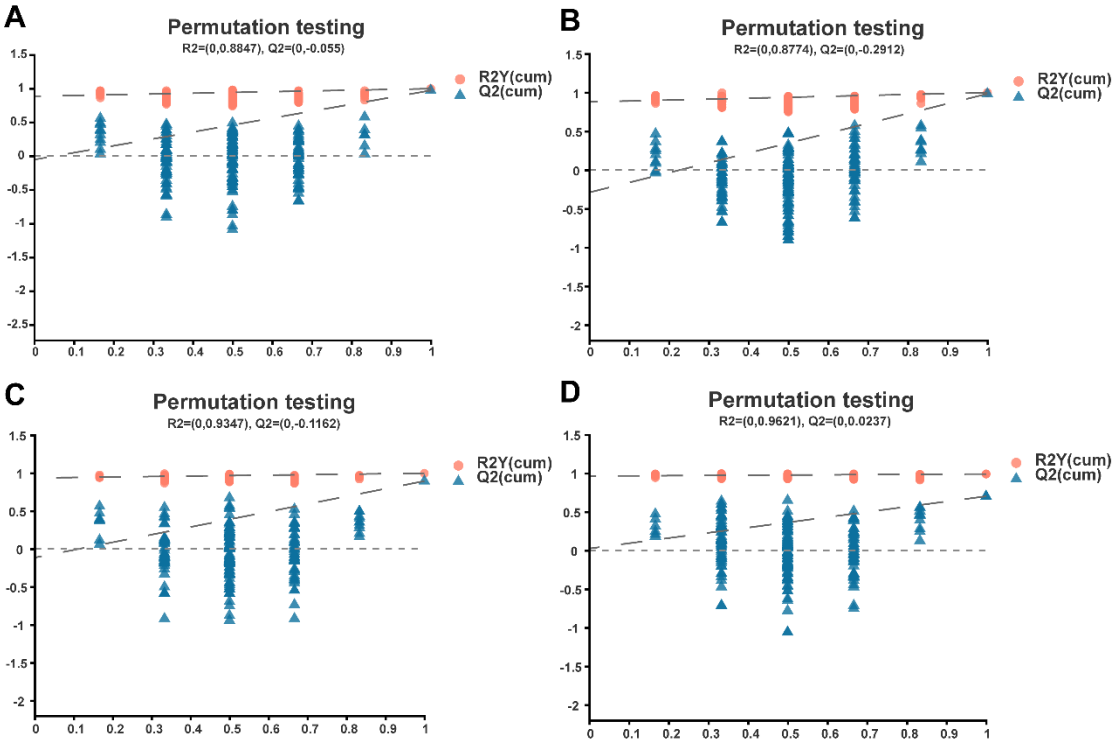

Figure S2. Permutation testing. (A) OPLS-DA model validation of Model group vs Control group in positive mode, $R^2X(cum) = 0.604$, $R^2Y(cum) = 0.996$, $Q^2(cum) = 0.97$; (B) and negative mode,

$R^2X(cum) = 0.669$, R^2Y (cum) = 0.997, Q^2 (cum) = 0.982; (C) AA group vs Model group in positive mode, R^2X (cum) = 0.394, R^2Y (cum) = 0.996, Q^2 (cum) = 0.893; (D) and negative mode, R^2X (cum) = 0.336, R^2Y (cum) = 0.989, and Q^2 (cum) = 0.7. (n = 6).

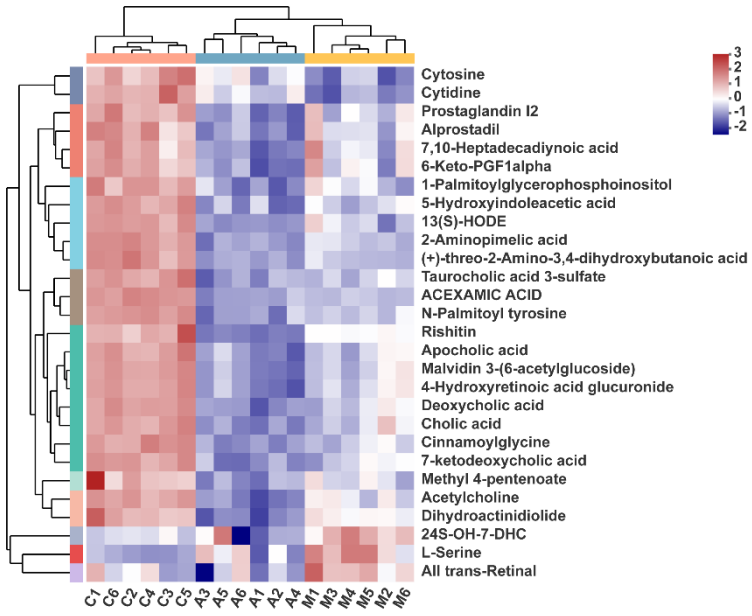

Figure S3. Metabolite expression cluster analysis.

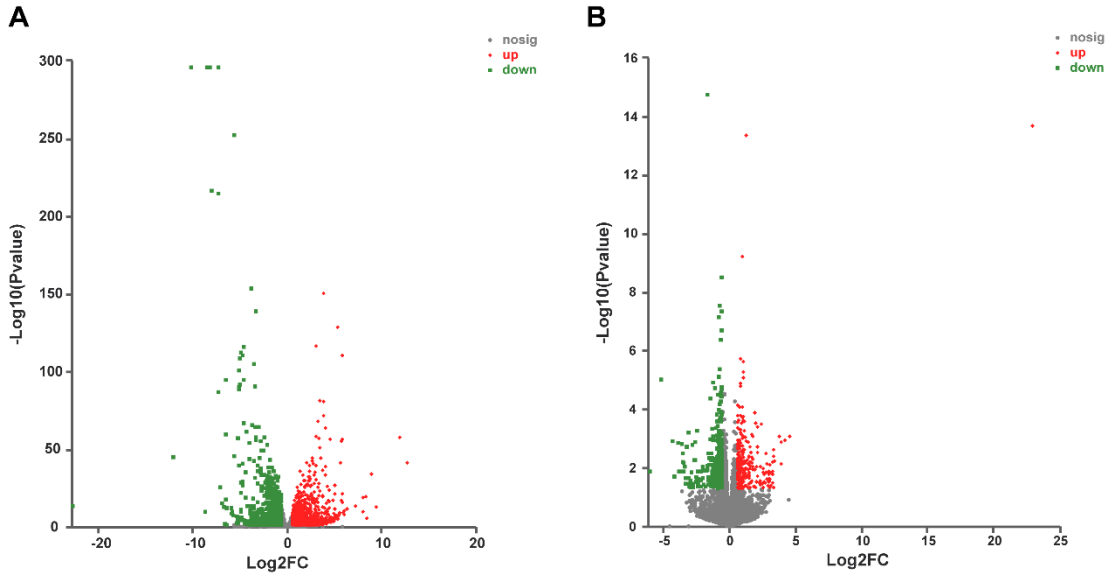

Figure S4. Differential gene volcano map. (A) Model group vs Control group, (B) and AA group vs Model group.

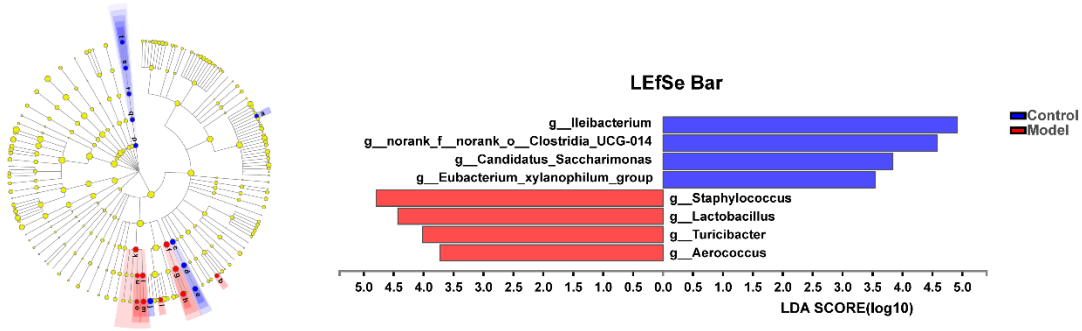

Figure S5. Microbial communities were compared by LEfSe analysis between Model group and Control group.

Table S1. Differential Metabolite Summary Table.

NO.	Mode	Metabolite	Formula	m/z	Rt(min)	VIP		FC		Trend		Pathway
						Model vs. Control	AA vs. Model	Model vs. Control	AA vs. Model	Model vs. Control	AA vs. Model	
1	pos	Cytosine	$C_4H_5N_3O$	112.0513	0.73	1.36	1.06	0.93	1.03	↓***	↑#	c
2	pos	Acetylcholine	$C_7H_{15}NO_2$	146.1179	0.68	1.32	1.77	0.93	0.94	↓***	↓###	a;b;g
3	pos	L-Serine	$C_3H_7NO_3$	147.0768	0.59	1.38	1.46	1.07	0.96	↑**	↓#	-
4	pos	Dihydroactinidiolide	$C_{11}H_{16}O_2$	181.1228	5.03	1.23	2.33	0.93	0.9	↓**	↓###	-
5	pos	5-Hydroxyindoleacetic acid	$C_{10}H_9NO_3$	192.0660	1.72	1.69	1.22	0.89	0.96	↓***	↓#	-
6	pos	Rishitin	$C_{14}H_{22}O_2$	223.1698	4.71	1.53	2.54	0.9	0.89	↓***	↓###	-
7	pos	Cytidine	$C_9H_{13}N_3O_5$	244.0935	1.11	2.13	1.74	0.81	1.09	↓***	↑##	c
8	pos	Methyl 4-pentenoate	$C_6H_{10}O_2$	251.1259	5.09	1.35	1.46	0.89	0.94	↓**	↓	-
9	pos	7,10-Heptadecadiynoic acid	$C_{17}H_{26}O_2$	263.2012	6.94	1.04	1.75	0.93	0.92	↓*	↓#	-
10	pos	All trans-Retinal	$C_{20}H_{28}O$	317.2482	8.16	1.01	2.27	1.07	0.9	↑	↓#	-
11	pos	Prostaglandin I2	$C_{20}H_{32}O_5$	353.2331	4.71	1.75	2.09	0.86	0.89	↓**	↓#	b;f
12	pos	Apocholic acid	$C_{24}H_{38}O_4$	355.2639	5.9	2.18	1.69	0.83	0.92	↓***	↓#	-
13	pos	24S-OH-7-DHC	$C_{27}H_{44}O_2$	401.3423	9.41	1.15	1.59	1.06	0.93	↑	↓#	-
14	pos	1-Palmitoylglycerophosphoinositol	$C_{25}H_{49}O_{12}P$	555.2941	9.02	1.18	1.08	0.95	0.98	↓***	↓	-
15	neg	(+)-threo-2-Amino-3,4-dihydroxybutanoic acid	$C_4H_9NO_4$	116.0342	1.48	1.44	1.15	0.87	0.98	↓***	↓	-
16	neg	2-Aminopimelic acid	$C_7H_{13}NO_4$	156.0658	1.48	1.91	1.95	0.77	0.93	↓***	↓##	-
17	neg	ACEXAMIC ACID	$C_8H_{15}NO_3$	172.0973	3.41	1.68	1.15	0.84	0.98	↓***	↓#	-
18	neg	Cinnamoylglycine	$C_{11}H_{11}NO_3$	204.0662	3.59	2.09	2.58	0.67	0.85	↓***	↓##	-
19	neg	13(S)-HODE	$C_{18}H_{32}O_3$	295.2282	7.53	1.31	1.55	0.9	0.96	↓***	↓	d
20	neg	Alprostadil	$C_{20}H_{34}O_5$	353.2339	6.29	1.36	2.51	0.86	0.89	↓**	↓#	-

21	neg	6-Keto-PGF1alpha	$C_{20}H_{34}O_6$	369.2286	4.7	1.09	2.68	0.9	0.89	↓*	↓#	e
22	neg	Deoxycholic acid	$C_{24}H_{40}O_4$	391.2860	6.36	1.9	2.78	0.8	0.89	↓***	↓##	a
23	neg	Cholic acid	$C_{24}H_{40}O_5$	407.2807	6.51	1.54	2.53	0.88	0.93	↓***	↓#	a
24	neg	N-Palmitoyl tyrosine	$C_{25}H_{41}NO_4$	418.2968	8.55	1.6	1.55	0.85	0.95	↓***	↓#	-
25	neg	7-ketodeoxycholic acid	$C_{24}H_{38}O_5$	451.2707	6.02	2.05	2.65	0.79	0.9	↓***	↓#	-
26	neg	4-Hydroxyretinoic acid glucuronide	$C_{26}H_{36}O_9$	537.2388	5.91	1.69	2.72	0.85	0.9	↓***	↓##	-
27	neg	Malvidin 3-(6-acetylglucoside)	$C_{25}H_{27}O_{13}^+$	570.1194	5.91	1.98	2.9	0.8	0.88	↓***	↓##	-
28	neg	Taurocholic acid 3-sulfate	$C_{26}H_{45}NO_{10}S_2$	594.2420	4.37	1.8	1.83	0.84	0.95	↓***	↓	-

The direction of the arrow indicates the changing trend of the relative content of the metabolites. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus the Control group，# $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus the Model group. (n = 6). a: Bile secretion; b: cAMP signaling pathway; c: Pyrimidine metabolism; d: PPAR signaling pathway; e: Arachidonic acid metabolism; f: VEGF signaling pathway; g: Insulin secretion.