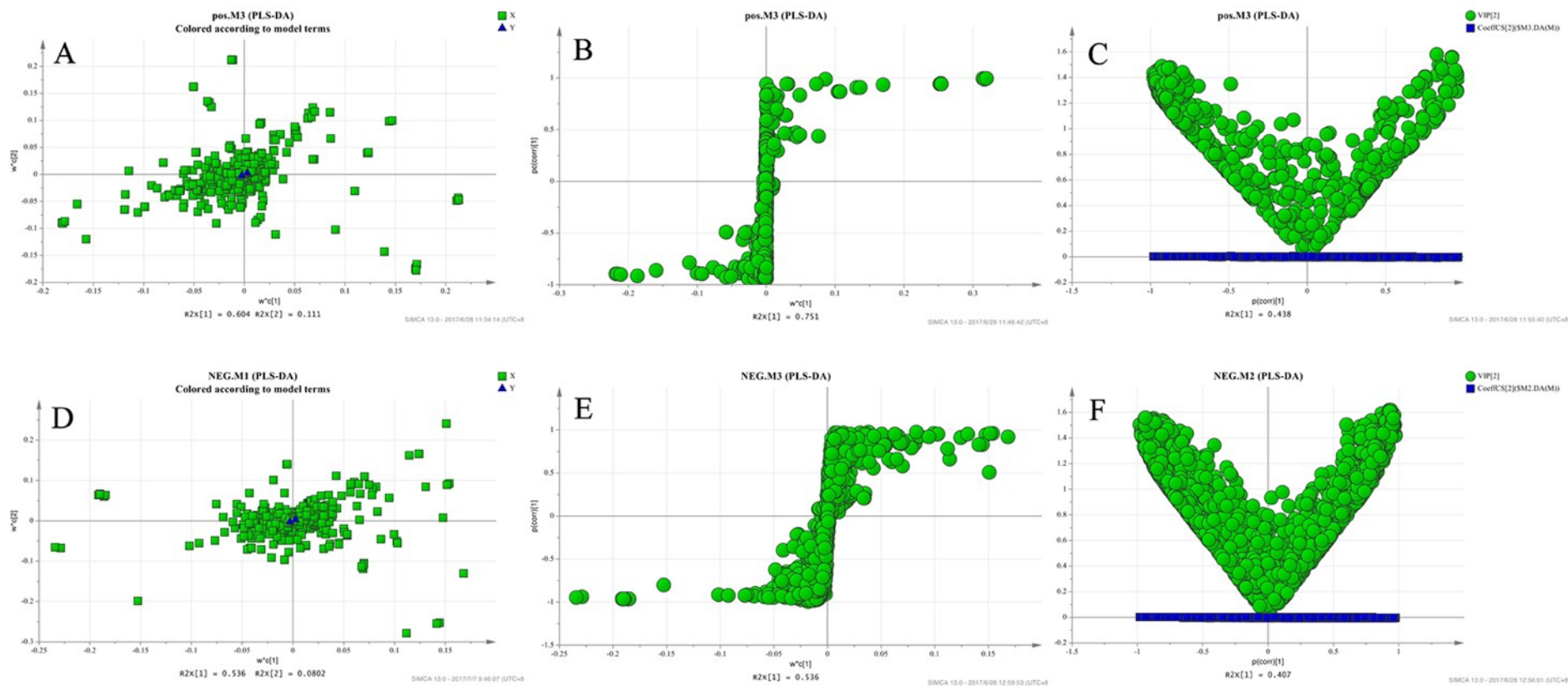


Supplementary data to:

Metabolomics study of the therapeutic mechanism of a Chinese herbal formula on collagen-induced arthritis mice

Zhen Jin, Xin Wu, Jida Zhang, Gang Cao



Fig

ure S1. A, B, and C were represented the loading plot, S score plot, and VIP score plot of PLS-DA model between Control and CIA group in positive mode, respectively. D, E, and F were represented the loading plot, S score plot, and VIP score plot of PLS-DA model between Control and CIA group in negative mode, respectively.

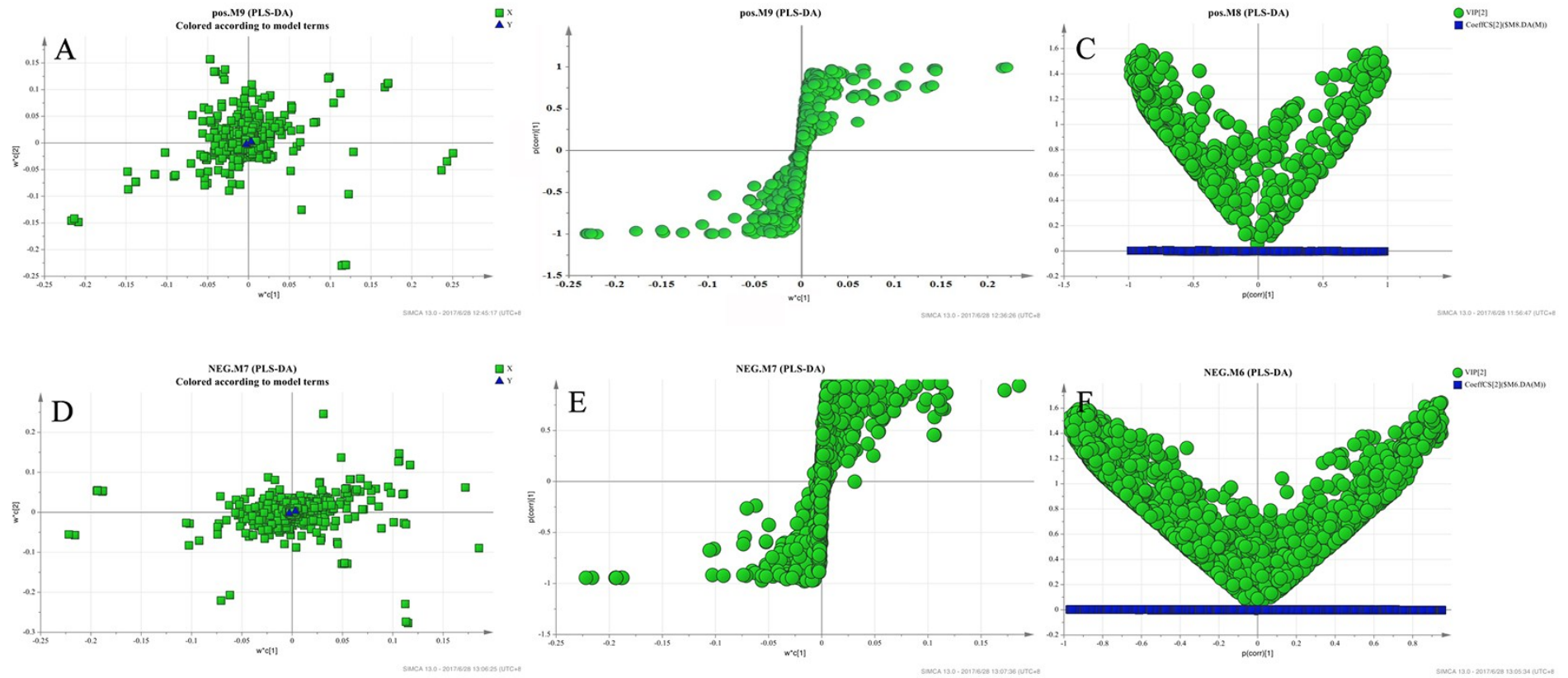


Figure S2. A, B, and C were represented the loading plot, S score plot, and VIP score plot of PLS-DA model between CIA and WJHL group in positive mode, respectively. D, E, and F were represented the loading plot, S score plot, and VIP score plot of PLS-DA model between CIA and WJHL group in negative mode, respectively.

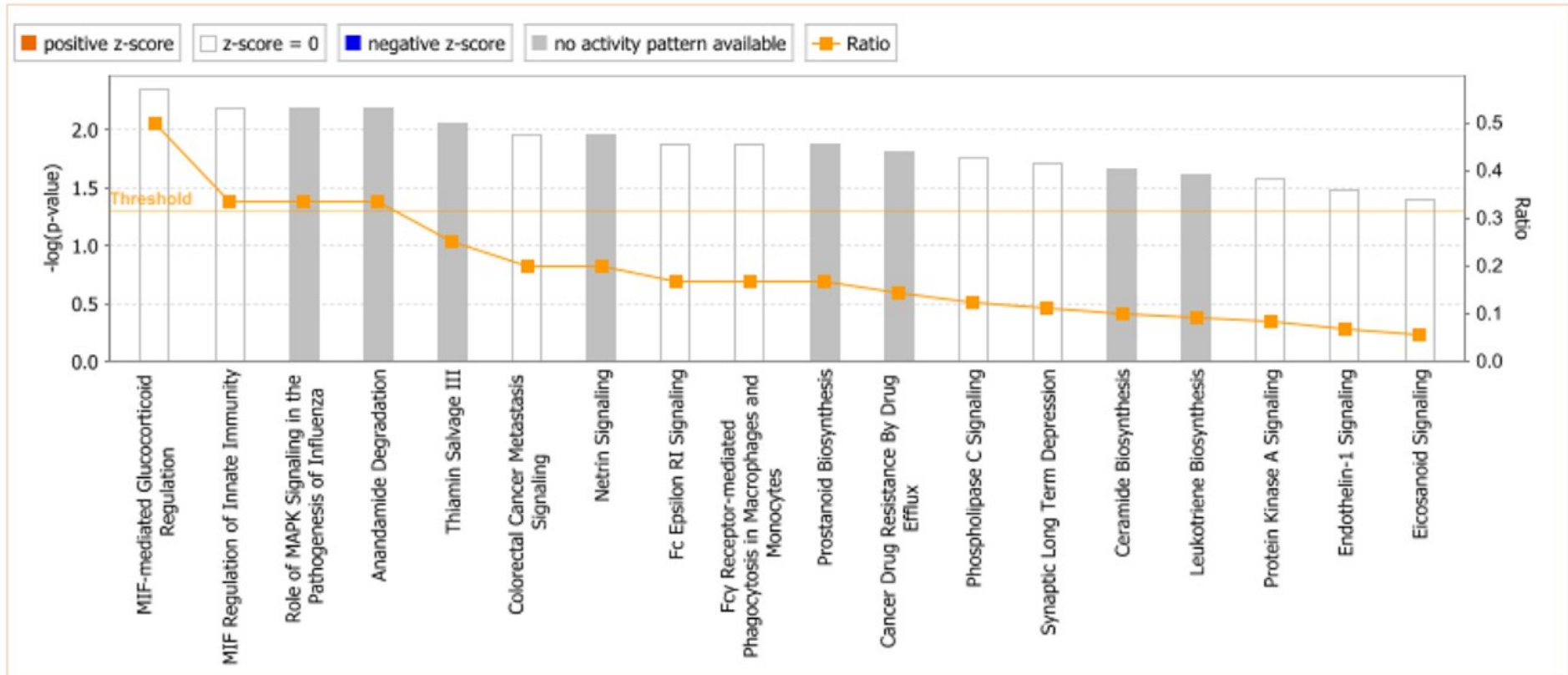


Figure S3. The significant canonical pathways of the treatment of WJHL in CIA model analysed by IPA.

Table S1A. 50 differential metabolites selected from data of positive ion mode in CIA group

Time (min)	Metabolite	Exact mass	Fold change (Z/M)	P-value	Variance
7.76	LysoPC(22:5(4Z,7Z,10Z,13Z,16Z))	569.3484	1.197	0.000	↓
7.52	LysoPC(20:4(5Z,8Z,11Z,14Z))	543.3311	0.473	0.000	↓
4.79	Thiamine	264.1085	1.591	0.000	↓
0.78	L-Lysine	146.1056	-0.641	0.000	↑
8.68	LysoPC(17:0)	509.3479	0.675	0.000	↓
0.88	L-Threonine	119.0582	-0.512	0.000	↑
8.41	DL-Stearoylcarnitine	427.3667	-0.992	0.000	↑
2.03	L-Phenylalanine	165.0790	-0.393	0.000	↑
1.44	L-Leucine	131.0945	-0.344	0.000	↑
7.46	LysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	567.3318	0.580	0.000	↓
8.41	8(S)-HETE	320.2352	-1.795	0.000	↑
12.27	Nervonyl carnitine	101.1204	-0.481	0.001	↑
8.21	Arachidonic Acid	304.2402	-1.790	0.001	↑
0.86	Choline	103.0995	-0.385	0.001	↑
1.21	Uric acid	168.0285	-0.617	0.001	↑
7.42	Retinoic acid	300.2088	-0.961	0.001	↑
8.08	LysoPC(18:1(11Z))	521.3475	0.443	0.001	↓
8.38	LysoPC(22:4(7Z,10Z,13Z,16Z))	571.3634	0.468	0.001	↓
8.22	LysoPC(16:0)	495.3324	0.270	0.001	↓
7.91	Vaccenyl carnitine	425.3505	-0.793	0.001	↑
0.85	L-Arginine	174.1118	-0.763	0.001	↑
3.35	L-Tryptophan	204.0899	0.197	0.001	↓
8.94	LysoPC(15:0)	481.3168	-0.404	0.001	↑
3.32	Indole	117.0580	0.194	0.002	↓
8.14	PC(O-12:0/O-1:0)	439.3066	-1.315	0.002	↑
7.40	Linoelaidyl carnitine	423.3346	-0.743	0.002	↑
7.82	LysoPE(0:0/16:0)	453.2857	-0.432	0.002	↑
7.61	α-Linolenic Acid	278.2243	-1.062	0.002	↑
0.79	L-Histidine	155.0695	-0.376	0.002	↑
11.34	PG(17:1(9Z)/0:0)	496.2820	-0.523	0.002	↑
7.03	Sphinganine	301.2981	-1.237	0.002	↑
8.15	PE(P-16:0/0:0)	437.2908	-0.915	0.003	↑
6.07	Dodecanoylcarnitine	343.2722	-0.564	0.004	↑
8.10	LysoPE(0:0/18:1(11Z))	479.3009	-0.741	0.005	↑
1.11	Phosphoric acid	97.9768	-0.734	0.006	↑
7.39	LysoPE(0:0/20:4(5Z,8Z,11Z,14Z))	501.2852	-0.321	0.007	↑
0.87	β-Alanine	89.0475	-0.322	0.007	↑

7.73	Palmitoyl-L-carnitine	399.3345	-0.495	0.009	↑
11.34	MG(0:0/20:3(11Z,14Z,17Z)/0:0)	380.2908	-1.372	0.009	↑
0.76	4-Hydroxybenzaldehyde	122.0327	0.404	0.011	↓
7.85	Phosphocholine	183.0658	0.217	0.011	↓
8.65	LysoPC(20:2(11Z,14Z))	547.3635	0.700	0.012	↓
9.38	LysoPC(18:0)	523.3620	0.231	0.016	↓
1.06	L-Tyrosine	181.0222	-0.389	0.022	↑
6.96	LysoPC(18:3(6Z,9Z,12Z))	517.3168	0.466	0.022	↓
10.81	PA(15:1(9Z)/0:0)	394.2130	-0.300	0.028	↑
7.24	LysoPC(18:4(6Z,9Z,12Z,15Z))	515.2985	0.400	0.035	↓
7.95	LysoPC(20:3(5Z,8Z,11Z))	545.3477	0.866	0.039	↓
7.40	LysoPE(0:0/18:2(9Z,12Z))	477.2854	-0.479	0.042	↑
7.21	LysoPC(16:1(9Z))	493.3171	0.388	0.042	↓

P value was calculated by one-way ANOVA.

Table S1B. 58 differential metabolites selected from data of negative ion mode in CIA group

Time (min)	Metabolite	Exact mass	Fold change (Z/M)	Significance	Variance
0.89	Hypoxanthine	136.0376	1.100	0.000	↓
7.52	LysoPE(0:0/22:4(7Z,10Z,13Z,16Z))	529.3164	0.530	0.000	↓
8.67	LysoPC(16:0)	495.3321	0.777	0.000	↓
3.39	Tryptamine	160.1003	0.640	0.000	↓
0.91	D-Threose	120.0426	0.451	0.000	↓
0.86	D-Sorbitol	182.0793	-1.263	0.000	↑
7.38	Arachidonic Acid	304.2402	0.532	0.000	↓
7.45	LysoPE(0:0/24:6(6Z,9Z,12Z,15Z,18Z,21Z))	553.3164	0.749	0.000	↓
0.78	L-Lysine	146.1055	-0.590	0.000	↑
7.97	8(S)-HETE	320.2350	-0.900	0.000	↑
1.23	Dehydroascorbic acid	174.0167	0.951	0.000	↓
8.20	9(S)-HODE	296.2349	1.113	0.000	↓
1.21	Oxoglutaric acid	146.0217	0.789	0.000	↓
7.83	Palmitic acid	256.2403	0.320	0.000	↓
1.35	Citric acid	192.0272	0.771	0.000	↓
9.38	α -Linolenic Acid	278.2245	1.386	0.000	↓
2.03	α -ketoisovaleric acid	116.0475	0.709	0.000	↓
6.06	9(S)-HpODE	312.2298	1.567	0.000	↓
0.90	Glyceric acid	106.0270	-0.659	0.000	↑
1.04	Lactic acid	90.0319	-0.464	0.000	↑
8.32	LysoPE(0:0/20:1(11Z))	507.3323	0.565	0.001	↓
1.22	Pyroglutamic acid	129.0429	0.203	0.001	↓
7.21	15(S)-HpETE	336.2298	-1.087	0.001	↑
0.83	L-Histidine	155.0699	-0.265	0.001	↑
8.14	PC(O-12:0/O-1:0)	439.3062	-1.163	0.001	↑

2.39	Pantothenic Acid	219.1105	0.585	0.001	↓
5.65	Octadecanedioic acid	314.2458	-0.424	0.001	↑
2.03	Cinnamic acid	148.0526	-0.347	0.002	↑
0.85	Thiamine	264.1046	-0.755	0.002	↑
5.78	LysoPE(0:0/18:2(9Z,12Z))	477.2853	-1.882	0.002	↑
7.42	Retinoic acid	300.2089	-0.784	0.002	↑
1.38	Levogluconan	162.0530	-0.613	0.002	↑
0.86	L-Threonine	119.0586	-0.341	0.003	↑
3.88	D-Leucic acid	132.0789	-0.829	0.003	↑
8.37	LysoPE(0:0/20:2(11Z,14Z))	505.3159	-0.795	0.003	↑
7.49	LPA(0:0/18:2(9Z,12Z))	434.2435	-0.366	0.005	↑
3.79	Ketoleucine	130.0632	0.642	0.006	↓
9.36	Stearic acid	284.2716	0.315	0.006	↓
10.11	Linoleic acid	280.2401	0.582	0.007	↓
3.37	Phenylpyruvic acid	164.0476	0.744	0.007	↓
7.20	LysoPE(0:0/18:1(11Z))	479.3006	0.544	0.008	↓
8.64	LysoPE(0:0/22:2(13Z,16Z))	533.3473	1.048	0.009	↓
4.87	Phosphoric acid	97.9771	-0.887	0.010	↑
7.46	LysoPC(14:0)	467.3009	0.375	0.011	↓
3.67	Caproic acid	116.0839	0.448	0.011	↓
4.42	Nonanoic acid	158.1308	0.604	0.012	↓
6.14	Bilirubin	584.2631	0.603	0.012	↓
6.96	LysoPE(0:0/22:5(4Z,7Z,10Z,13Z,16Z))	527.3005	0.604	0.013	↓
7.76	LysoPC(16:1(9Z))	493.3168	0.497	0.013	↓
6.96	LysoPE(0:0/20:3(11Z,14Z,17Z))	503.3005	0.678	0.014	↓
4.42	Sebacic acid	202.1205	0.480	0.015	↓
9.36	LysoPC(17:0)	509.3481	0.215	0.018	↓
3.93	Acetylcarnitine	203.1157	-0.561	0.021	↑
3.38	Shikimic acid	174.0528	-0.174	0.021	↑
9.65	MG(0:0/16:1(9Z)/0:0)	328.2615	-0.397	0.024	↑
10.73	MG(0:0/20:4(5Z,8Z,11Z,14Z)/0:0)	378.2803	-0.915	0.024	↑
6.54	Metyrosine	195.0897	-0.179	0.034	↑
3.83	Glycine	75.0323	0.712	0.036	↓

P value was calculated by one-way ANOVA.

Table S2A. 34 differential metabolites selected from data of positive ion mode in WJHL group

Time (min)	Metabolite	Exact mass	Fold change (F/M)	Significance	Variance
1.44	L-Leucine	131.0945	-0.433	0.000	↓
8.68	LysoPC(17:0)	509.3479	0.791	0.000	↑
1.93	L-Phenylalanine	165.0790	-0.482	0.000	↓
7.76	LysoPC(22:5(4Z,7Z,10Z,13Z,16Z))	569.3484	0.793	0.000	↑
7.95	LysoPC(20:3(5Z,8Z,11Z))	545.3477	0.928	0.000	↑
8.41	DL-Stearoylcarnitine	427.3667	-0.916	0.000	↓
4.79	Thiamine	264.1085	1.572	0.000	↑
8.08	LysoPC(18:1(11Z))	521.3475	0.665	0.000	↑
8.22	LysoPC(16:0)	495.3324	0.320	0.000	↑
0.86	Choline	103.0995	-0.314	0.000	↓
0.78	L-Lysine	146.1056	-0.438	0.000	↓
6.07	Dodecanoylcarnitine	343.2722	-0.734	0.001	↓
8.41	8(S)-HETE	320.2352	-1.551	0.001	↓
7.40	Linoelaidyl carnitine	423.3346	-0.806	0.001	↓
7.91	Vaccenyl carnitine	425.3505	-0.806	0.001	↓
8.65	LysoPC(20:2(11Z,14Z))	547.3635	0.947	0.001	↑
3.93	Hexanoylcarnitine	259.1782	-0.678	0.001	↓
8.21	Arachidonic Acid	304.2402	-1.295	0.002	↓
8.15	PE(P-16:0/0:0)	437.2908	-0.820	0.003	↓
7.21	LysoPC(16:1(9Z))	493.3171	0.674	0.004	↑
7.73	Palmitoyl-L-carnitine	399.3345	-0.570	0.005	↓
7.03	Sphinganine	301.2981	-1.015	0.005	↓
0.85	L-Arginine	174.1118	-0.532	0.005	↓
7.42	Retinoic acid	300.2088	-0.723	0.006	↓
7.24	LysoPC(18:4(6Z,9Z,12Z,15Z))	515.2985	0.649	0.007	↑
7.05	LysoPC(18:3(6Z,9Z,12Z))	517.3168	0.903	0.010	↑
8.38	LysoPC(22:4(7Z,10Z,13Z,16Z))	571.3634	0.292	0.017	↑
6.99	Sphinganine-phosphate	381.2644	0.378	0.020	↑
10.00	LysoPE(0:0/22:0)	537.3796	0.386	0.023	↑
6.75	Sphingosine-1-phosphate	379.2488	0.275	0.036	↑
1.21	Uric acid	168.0285	-0.414	0.039	↓
8.10	LysoPE(0:0/18:1(11Z))	479.3009	-0.431	0.043	↓
1.21	Cytosine	111.0432	-0.302	0.046	↓
9.70	LysoPC(18:0)	523.3632	0.175	0.049	↑

P value was calculated by one-way ANOVA.

Table S2B. 41 differential metabolites selected from data of negative ion mode in WJHL group

Time (min)	Metabolite	Exact mass	Fold change (F/M)	Significance	Variance
0.86	3-Methylxanthine	166.0481	1.407	0.000	↑
1.23	Dehydroascorbic acid	174.0167	0.960	0.000	↑

6.14	Bilirubin	584.2631	0.795	0.000	↑
0.89	Hypoxanthine	136.0376	0.877	0.000	↑
7.38	Arachidonic Acid	304.2402	0.392	0.000	↑
8.67	LysoPC(16:0)	495.3321	0.822	0.000	↑
1.38	Levoglucosan	162.0530	-0.970	0.000	↓
7.97	8(S)-HETE	320.2350	-0.726	0.000	↓
7.47	PA(20:4(5Z,8Z,11Z,14Z)/0:0)	458.2428	-0.303	0.000	↓
2.04	L-Phenylalanine	165.0792	-0.464	0.000	↓
1.35	Citric acid	192.0272	0.778	0.000	↑
2.03	Cinnamic acid	148.0526	-0.468	0.000	↓
3.88	D-Leucic acid	132.0789	-1.104	0.000	↓
0.83	L-Histidine	155.0699	-0.367	0.000	↓
8.32	LysoPE(0:0/20:1(11Z))	507.3323	0.722	0.000	↑
3.82	Sebacic acid	202.1205	1.265	0.001	↑
1.04	Lactic acid	90.0319	-0.466	0.001	↓
0.87	Taurine	125.0150	-0.310	0.001	↓
0.90	Glyceric acid	106.0270	-0.522	0.001	↓
0.78	Ornithine	132.0902	-0.342	0.001	↓
7.89	Oleic Acid	282.2558	-0.497	0.001	↓
8.38	LysoPC(14:0)	467.3005	0.315	0.001	↑
6.59	Coprocholic acid	450.3335	3.366	0.001	↑
8.15	PE(P-16:0/0:0)	437.2906	-0.712	0.002	↓
7.52	LysoPE(0:0/22:4(7Z,10Z,13Z,16Z))	529.3164	0.358	0.002	↑
5.78	LysoPE(0:0/18:2(9Z,12Z))	477.2853	-1.559	0.002	↓
8.64	LysoPE(0:0/22:2(13Z,16Z))	533.3473	1.241	0.002	↑
8.14	PC(O-12:0/O-1:0)	439.3062	-0.903	0.002	↓
3.75	LysoPE(0:0/18:3(6Z,9Z,12Z))	475.2635	-1.067	0.004	↓
6.06	9(S)-HpODE	312.2298	1.157	0.008	↑
1.46	L-Leucine	131.0949	-0.379	0.008	↓
3.38	Shikimic acid	174.0528	-0.215	0.009	↓
6.96	LysoPE(0:0/20:3(11Z,14Z,17Z))	503.3005	1.176	0.010	↑
5.07	7-ketodeoxycholic acid	406.2719	2.202	0.011	↑
6.96	LysoPE(0:0/22:5(4Z,7Z,10Z,13Z,16Z))	527.3005	1.034	0.011	↑
0.85	Thiamine	264.1046	-0.483	0.013	↓
6.91	MG(0:0/15:0/0:0)	316.2614	0.169	0.014	↑
0.87	Citrulline	175.0962	0.233	0.016	↑
8.20	9(S)-HODE	296.2349	0.922	0.021	↑
6.58	Octadecanedioic acid	314.2456	0.570	0.022	↑
4.72	Undecanedioic acid	216.1363	-0.341	0.036	↓

P	value	was	calculated	by	one-way	ANOVA.
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Table S3. 39 pathways based on differential metabolites between control group and CIA group through MetPA analysis

Pathway Name	Total	Hits	Raw p	-log(p)	Holm p	FDR	Impact	Details
Phenylalanine, tyrosine and tryptophan biosynthesis	4	3	1.47E-04	8.823	0.011932	0.011577	1	KEGG
Aminoacyl-tRNA biosynthesis	67	9	2.86E-04	8.16	0.022869	0.011577	0	KEGG
Phenylalanine metabolism	9	3	0.0027391	5.9001	0.21639	0.073955	0.64815	KEGG
Glycerophospholipid metabolism	30	7	0.017222	4.0616	1	0.34817	0.30956	KEGG
Glycine, serine and threonine metabolism	32	4	0.021492	3.8401	1	0.34817	0.29197	KEGG
Nitrogen metabolism	9	2	0.035448	3.3397	1	0.47855	0	KEGG
Valine, leucine and isoleucine biosynthesis	11	2	0.051851	2.9594	1	0.52764	0.33333	KEGG
Biosynthesis of unsaturated fatty acids	42	4	0.052112	2.9544	1	0.52764	0	KEGG
Ubiquinone and other terpenoid-quinone biosynthesis	3	1	0.099302	2.3096	1	0.82268	0	KEGG
Glyoxylate and dicarboxylate metabolism	16	2	0.10157	2.2871	1	0.82268	0.2963	KEGG
Glycerolipid metabolism	18	2	0.12408	2.0869	1	0.86438	0.12391	KEGG
Citrate cycle (TCA cycle)	20	2	0.14767	1.9128	1	0.86438	0.12155	KEGG
D-Glutamine and D-glutamate metabolism	5	1	0.16007	1.8321	1	0.86438	0	KEGG
Biotin metabolism	5	1	0.16007	1.8321	1	0.86438	0	KEGG
Linoleic acid metabolism	5	1	0.16007	1.8321	1	0.86438	1	KEGG
Cyanoamino acid metabolism	6	1	0.18893	1.6664	1	0.95645	0	KEGG
Thiamine metabolism	7	1	0.21682	1.5287	1	1	0.4	KEGG
Glutathione metabolism	26	2	0.22266	1.5021	1	1	0.02004	KEGG
Porphyrin and chlorophyll metabolism	27	2	0.23552	1.446	1	1	0.0415	KEGG
Methane metabolism	9	1	0.2698	1.3101	1	1	0	KEGG

Arachidonic acid metabolism	36	2	0.35147	1.0456	1	1	0.42247	KEGG
Valine, leucine and isoleucine degradation	38	2	0.37669	0.97633	1	1	0.0119	KEGG
Fatty acid metabolism	39	2	0.38916	0.94376	1	1	0	KEGG
Histidine metabolism	15	1	0.40858	0.89508	1	1	0.24194	KEGG
Tryptophan metabolism	41	2	0.41379	0.8824	1	1	0.19914	KEGG
Fatty acid biosynthesis	43	2	0.43794	0.82567	1	1	0	KEGG
Retinol metabolism	17	1	0.44882	0.80114	1	1	0.21649	KEGG
Fructose and mannose metabolism	19	1	0.48637	0.72078	1	1	0.03142	KEGG
Butanoate metabolism	20	1	0.5042	0.68478	1	1	0	KEGG
Sphingolipid metabolism	21	1	0.52142	0.6512	1	1	0.14286	KEGG
Pyruvate metabolism	22	1	0.53805	0.6198	1	1	0	KEGG
Alanine, aspartate and glutamate metabolism	24	1	0.56964	0.56275	1	1	0.06329	KEGG
Glycolysis or Gluconeogenesis	26	1	0.59911	0.51231	1	1	0	KEGG
Galactose metabolism	26	1	0.59911	0.51231	1	1	0	KEGG
Fatty acid elongation in mitochondria	27	1	0.6131	0.48923	1	1	0	KEGG
Purine metabolism	68	2	0.68821	0.37367	1	1	0.03141	KEGG
Tyrosine metabolism	42	1	0.77358	0.25673	1	1	0.14045	KEGG
Arginine and proline metabolism	44	1	0.78928	0.23663	1	1	0.08228	KEGG
Primary bile acid biosynthesis	46	1	0.80392	0.21825	1	1	0.02976	KEGG

Raw p value was calculated by one-way ANOVA.

Table S4. 32 pathways based on differential metabolites between CIA group and WJHL group through MetPA analysis

Pathway Name	Total	Hits	Raw p	-log(p)	Holm p	FDR	Impact	Details
Aminoacyl-tRNA biosynthesis	67	5	5.11E-05	9.8825	0.0041359	0.0041359	0	KEGG
Arginine and proline metabolism	44	3	0.031175	3.4681	1	0.95586	0.27364	KEGG
Valine, leucine and isoleucine biosynthesis	11	1	0.035402	3.341	1	0.95586	0.66666	KEGG
Citrate cycle (TCA cycle)	20	2	0.10461	2.2575	1	1	0.08044	KEGG
Phenylalanine, tyrosine and tryptophan biosynthesis	4	1	0.10682	2.2366	1	1	0.5	KEGG
Biosynthesis of unsaturated fatty acids	42	3	0.1084	2.222	1	1	0	KEGG
Biotin metabolism	5	1	0.13174	2.0269	1	1	0	KEGG
Thiamine metabolism	7	1	0.17956	1.7173	1	1	0.4	KEGG
Glycerophospholipid metabolism	30	2	0.202	1.5995	1	1	0.06759	KEGG
Taurine and hypotaurine metabolism	8	1	0.20249	1.597	1	1	0.42857	KEGG
Glycine, serine and threonine metabolism	32	2	0.22271	1.5019	1	1	0	KEGG
Nitrogen metabolism	9	1	0.22481	1.4925	1	1	0	KEGG
Phenylalanine metabolism	9	1	0.22481	1.4925	1	1	0.40741	KEGG
Arachidonic acid metabolism	36	2	0.2646	1.3295	1	1	0.42247	KEGG
Valine, leucine and isoleucine degradation	38	2	0.28563	1.2531	1	1	0	KEGG
Purine metabolism	68	3	0.29258	1.229	1	1	0.03154	KEGG
Fatty acid biosynthesis	43	2	0.33791	1.085	1	1	0	KEGG
Pantothenate and CoA biosynthesis	15	1	0.34645	1.06	1	1	0	KEGG
Histidine metabolism	15	1	0.34645	1.06	1	1	0.24194	KEGG

Glyoxylate and dicarboxylate metabolism	16	1	0.36482	1.0083	1	1	0.2963	KEGG
Retinol metabolism	17	1	0.3827	0.96051	1	1	0.21649	KEGG
Lysine degradation	20	1	0.43343	0.83603	1	1	0	KEGG
Sphingolipid metabolism	21	1	0.44941	0.79981	1	1	0.14286	KEGG
Pyruvate metabolism	22	1	0.46496	0.7658	1	1	0	KEGG
Alanine, aspartate and glutamate metabolism	24	1	0.49479	0.70363	1	1	0.00316	KEGG
Glycolysis or Gluconeogenesis	26	1	0.52299	0.64819	1	1	0	KEGG
Glutathione metabolism	26	1	0.52299	0.64819	1	1	0	KEGG
Fatty acid metabolism	39	1	0.6723	0.39705	1	1	0	KEGG
Pyrimidine metabolism	41	1	0.69079	0.36991	1	1	0.00709	KEGG
Tryptophan metabolism	41	1	0.69079	0.36991	1	1	0.15684	KEGG
Tyrosine metabolism	42	1	0.69965	0.35717	1	1	0	KEGG
Primary bile acid biosynthesis	46	1	0.73269	0.31104	1	1	0.02976	KEGG

Raw p value was calculated by one-way ANOVA.

Table S5. The gradient of mobile phase

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0	0.3	95	5
2	0.3	95	5
12	0.3	5	95
15	0.3	5	95
17	0.3	95	5