

Supplementary information to:

**Identification of key metabolic changes during liver fibrosis progression in rats
using a urine and serum metabolomics approach**

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UHPLC conditions

Waters AcquityTMUHPLC (consisting of a vacuum degasser, autosampler, binary pump, photodiode array detector, and oven) was equipped with an ACQUITY UPLC[®] BEH C18 column (2.1mm×50mm, i. d. 1.7 μ m, Waters Corp). The analytical column was maintained at a temperature of 40°C and the mobile phase was composed of acetonitrile (A) and water (B), each containing 0.1% formic acid. The gradient for the urine sample was as follows: 0-8min, 1-40%A; 8-10min, 40-98%A; 10-13min, 98-100%A. The gradient for serum sample was used: 2–100% A for 15 min. The injection volume was 2 μ L with a flow rate of 0.4 mL/min. The eluent was introduced to the mass spectrometer directly.

MS conditions

MS analysis was performed on a Q-TOF analyzer in the SYNAPTHDMS system (Waters Corporation) in the positive-ion (ESI⁺) and negative-ion (ESI⁻) mode, using the following parameters: capillary voltage, 1300V(ESI⁺), 1500V(ESI⁻); sample cone voltage, 60 V(ESI⁺), 770V(ESI⁻); source temperature, 110°C; desolvation temperature, 350°C; desolvation gas flow, 750 L/h; cone gas flow, 20 L/h. MS data were collected in the full scan mode from m/z 100–1500. All the data were acquired using an independent reference lock mass via the LockSprayTM interface to ensure accuracy and reproducibility during the MS analysis. Leucine enkephalin was used as the reference ion ($[M+H]^+ = 556.2771$) and $[M-H]^- = 554.2615$) at a concentration of 1 ng/mL under a flow rate of 30 μ L/min. The data were collected in the centroid mode, and the LockSpray frequency was set at 15 s and was averaged over five scans for correction.

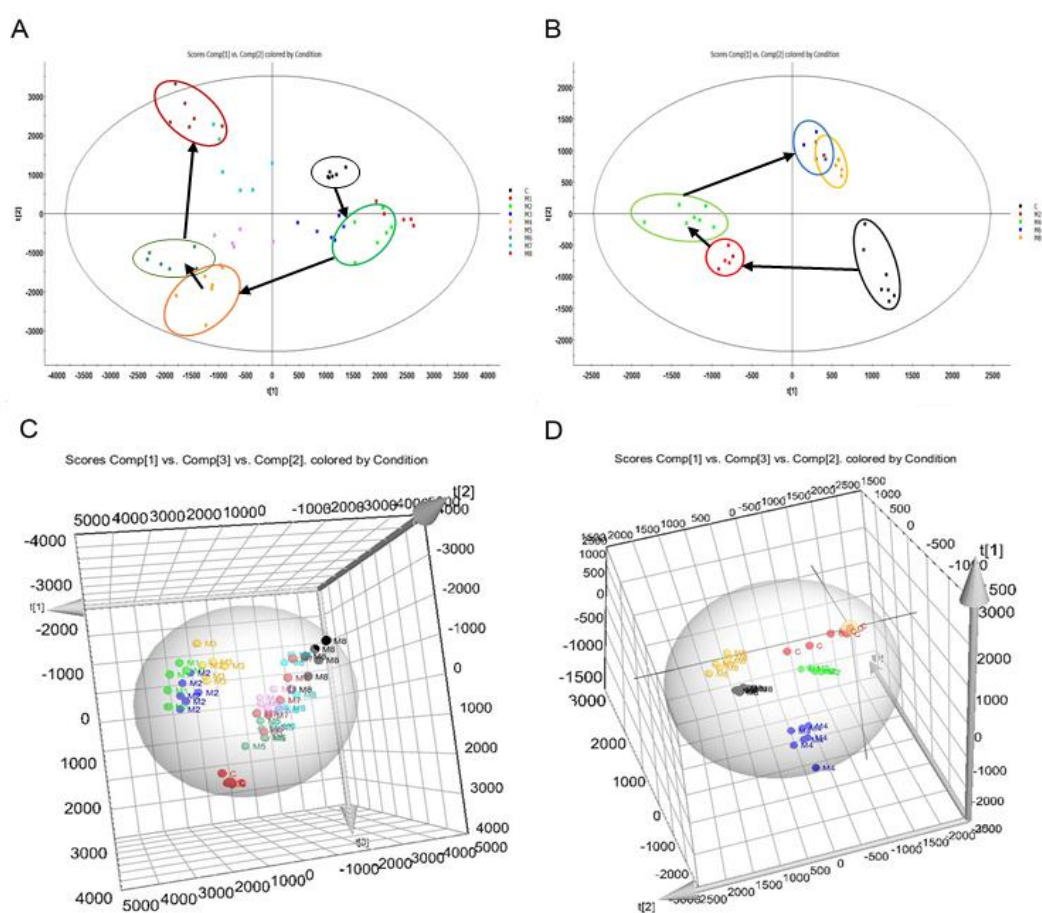


Figure S1. Trajectory analysis of PLS-DA and 3D PLS-DA Score plots for the liver fibrosis. (A, C) the urine samples in negative mode ($R^2Y=53\%$, $Q^2=44\%$). (B, D) The serum samples in negative mode ($R^2Y=97\%$, $Q^2=95\%$).

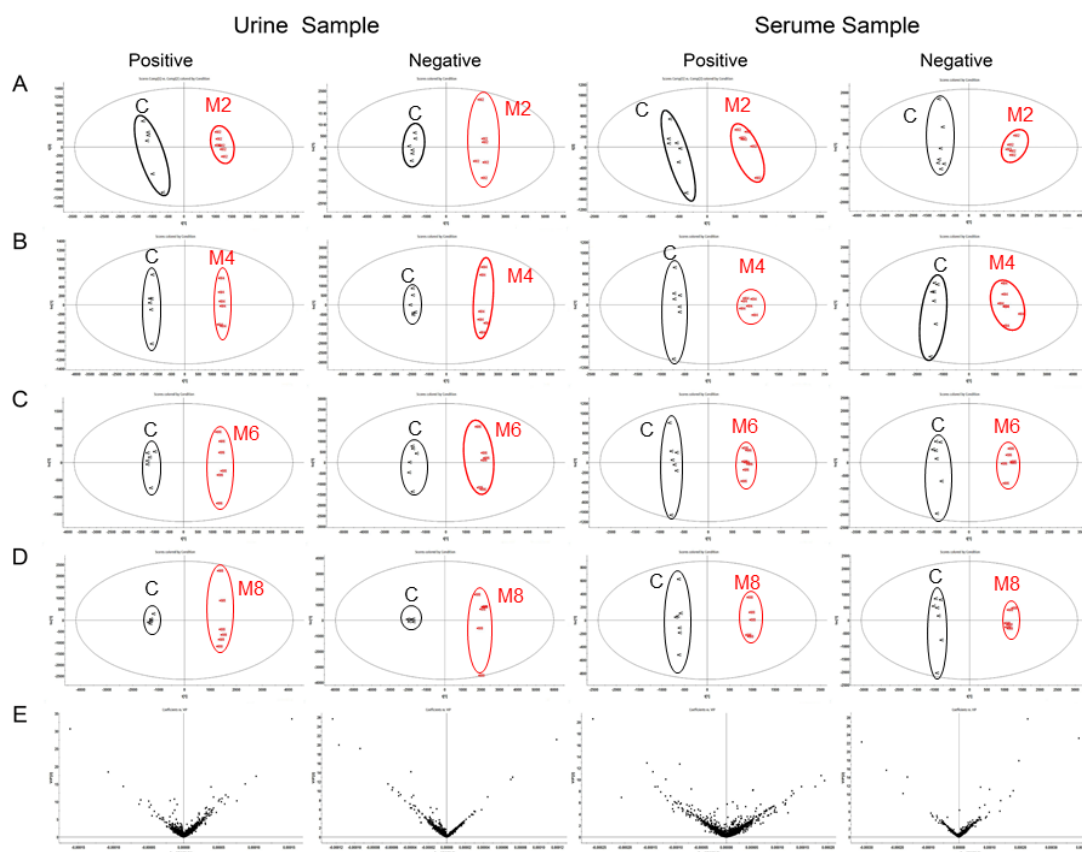


Figure S2. The screening of the metabolite markers. OPLS-DA score plots of urine and serum samples analysed in positive and negative modes to compare liver metabolite profiles between the control and model groups at weeks 2(A), 4(B), 6(C), and 8(D). (E) VIP score plots constructed from OPLS-DA models of urine and serum samples at week 8.

Table S1 Cross-validated data of OPLS-DA models in liver fibrosis model groups compared to control group.

Model	Positive ion mode			Negative ion mode		
	R ² X	R ² Y	Q ²	R ² X	R ² Y	Q ²
Urine						
C vs M2 (week 2)	0.49	0.99	0.96	0.64	0.99	0.97
C vs M4 (week 4)	0.58	0.99	0.98	0.71	0.99	0.97
C vs M6 (week 6)	0.59	0.99	0.98	0.68	0.99	0.96
C vs M8 (week 8)	0.74	0.99	0.97	0.66	0.99	0.97
Serum						
C vs M2 (week 2)	0.69	0.99	0.96	0.85	0.98	0.97
C vs M4 (week 4)	0.70	0.98	0.97	0.85	0.98	0.97
C vs M6 (week 6)	0.69	0.99	0.96	0.80	0.99	0.98
C vs M8 (week 8)	0.69	0.99	0.99	0.82	0.98	0.99

Table S2. Differential metabolites from different groups at weeks 2, 4, 6 and 8.

No.	Metabolite	m/z	RT (min)	HMDB	2weeks model vs control			4weeks model vs control			6weeks model vs control			8weeks model vs control			
					FC ^a	P ^b	P ^c	FC ^a	P ^b	P ^c	FC ^a	P ^b	P ^c	FC ^a	P ^b	P ^c	VIP ^d
urine																	
1	Valyl-Tyrosine	162.06	3.69	HMDB29139	-0.54	4.42E-02	2.29E-02	-1.11	2.82E-04	5.11E-04	-1.21	2.34E-04	4.88E-04	-3.02	3.70E-10	4.85E-06	9.05
2	Ursocholic acid	385.14	3.70	HMDB00917	2.67	P ^b >0.05	P ^c >0.05	3.64	1.35E-02	1.89E-02	2.62	2.20E-01	P ^c >0.05	2.94	P ^b >0.05	8.99E-03	3.50
3	Uric acid	160.04	3.69	HMDB00289	0.43	P ^b >0.05	P ^c >0.05	1.18	2.65E-02	4.18E-02	1.15	1.19E-02	6.75E-03	3.01	P ^b >0.05	1.34E-05	3.91
4	Tyrosyl-Cysteine	220.12	2.02	HMDB29102	-0.01	P ^b >0.05	P ^c >0.05	0.02	P ^b >0.05	P ^c >0.05	1.5	1.72E-05	3.02E-04	2.26	9.18E-08	3.63E-05	13.69
5	Thymidine glycol	162.08	1.00	HMDB42036	-0.3	P ^b >0.05	3.15E-02	-0.65	P ^b >0.05	1.56E-04	-0.65	4.92E-01	4.81E-04	-0.78	1.78E-03	4.74E-06	3.13
6	Prolyl-Threonine	174.11	3.79	HMDB29027	-1.21	2.69E-05	1.32E-03	-1.08	1.00E-03	1.91E-03	-1.51	3.28E-05	3.45E-04	-1.58	8.25E-07	3.19E-04	10.13
7	Phosphoguanidinoacetate	453.28	7.96	HMDB03705	0.33	2.84E-02	P ^c >0.05	0.09	4.57E-02	P ^c >0.05	0.3	1.07E-03	P ^c >0.05	0.78	1.86E-04	2.10E-04	4.84
8	Phenylalanyl-Threonine	328.10	2.82	HMDB29005	-0.4	P ^b >0.05	2.66E-02	-0.8	2.65E-03	6.87E-05	-1.1	3.69E-05	1.97E-05	-1.6	P ^b >0.05	1.10E-06	4.91
9	p-Cresol sulfate	173.01	1.01	HMDB11635	-1.85	P ^b >0.05	2.38E-06	0.59	P ^b >0.05	2.17E-03	1.3	2.29E-03	4.94E-02	0.89	P ^b >0.05	2.67E-02	6.78
10	Normetanephrine	373.27	7.95	HMDB00819	-0.52	P ^b >0.05	1.22E-02	-1.28	2.73E-04	3.98E-05	-1.54	4.40E-05	1.45E-04	-2.42	4.32E-08	2.07E-06	8.96
11	Nonate	187.10	5.37	HMDB11717	1.06	7.16E-05	2.31E-06	0.83	9.41E-05	7.57E-04	0.78	1.97E-05	1.27E-04	1.87	1.18E-10	5.25E-07	7.14
12	Methylmalonic acid	233.01	2.20	HMDB00202	1.17	P ^b >0.05	1.04E-04	-0.48	P ^b >0.05	P ^c >0.05	0.63	1.68E-01	P ^c >0.05	2.28	9.10E-06	1.75E-03	3.71
13	Mesaconic acid	189.04	1.10	HMDB00749	0.48	1.80E-03	3.80E-03	-0.21	P ^b >0.05	P ^c >0.05	0.08	6.89E-03	P ^c >0.05	0.89	1.50E-06	1.15E-04	3.65
14	L-Urobilin	303.07	1.78	HMDB04159	-1.32	P ^b >0.05	1.97E-02	0.81	P ^b >0.05	P ^c >0.05	-0.79	8.40E-01	P ^c >0.05	1.51	5.57E-03	2.42E-02	4.73
15	L-Tryptophan	221.09	3.20	HMDB00929	-0.35	P ^b >0.05	P ^c >0.05	0.08	P ^b >0.05	P ^c >0.05	1.95	5.63E-05	1.40E-02	4.11	1.44E-16	3.21E-10	8.22
16	L-Leucine	389.27	8.81	HMDB00687	-0.12	P ^b >0.05	P ^c >0.05	-0.76	P ^b >0.05	1.82E-03	-1.37	4.30E-04	1.58E-04	-2.57	1.34E-07	1.67E-06	5.58
17	Lipoamide	295.23	8.23	HMDB00962	-0.91	9.20E-11	1.47E-05	-2.37	3.42E-17	1.78E-10	-2.61	1.72E-17	8.94E-11	-2.39	7.89E-18	1.68E-10	4.81
18	Kynurenic acid	268.11	1.40	HMDB00715	-1.09	5.04E-06	4.23E-06	-1.18	2.28E-05	7.86E-07	-1.47	3.95E-06	1.99E-08	-0.78	1.78E-04	5.76E-05	6.07
19	Isoleucyl-Proline	334.11	1.51	HMDB28915	-0.04	P ^b >0.05	P ^c >0.05	-0.12	1.73E-04	P ^c >0.05	-0.36	7.60E-02	2.38E-02	0.22	4.95E-04	6.08E-06	4.61
20	Isocitricacid	259.09	0.72	HMDB00193	-0.49	5.12E-02	2.74E-03	-1.48	7.60E-07	6.75E-07	-1.58	6.90E-07	3.65E-07	-1.51	4.93E-08	1.47E-06	7.34

21	Indoxyl sulfate	313.24	9.72	HMDB00682	0.31	$P^b > 0.05$	$P^c > 0.05$	0.95	2.60E-06	1.06E-04	0.88	9.75E-07	2.40E-05	0.89	3.52E-04	3.82E-07	19.40
22	Hexanoylglycine	661.49	8.24	HMDB00701	-0.19	$P^b > 0.05$	$P^c > 0.05$	-0.16	$P^b > 0.05$	$P^c > 0.05$	-1.65	1.63E-03	7.35E-05	-3.66	2.56E-06	1.60E-06	3.37
23	Ethyl methylsuccinate	159.07	3.24	HMDB59893	0.85	2.11E-04	3.68E-05	0.47	1.95E-03	5.33E-03	0.73	3.87E-06	3.57E-04	1.92	1.00E-12	4.84E-07	4.25
24	DG(18:4(6Z,9Z,12Z,15Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	240.10	3.20	HMDB07353	1.39	5.96E-08	9.31E-04	-3.65	3.76E-03	2.68E-04	-5.45	1.99E-03	1.40E-04	-5.87	1.66E-03	1.32E-04	6.24
25	Dehydrospermidine	188.05	0.53	HMDB12925	-2.04	1.90E-19	1.36E-09	-2.7	1.58E-20	6.80E-11	-2.45	1.10E-19	1.61E-10	-1.96	2.52E-19	8.86E-10	3.81
26	cis-Aconitic acid	451.27	8.86	HMDB00072	0.8	4.83E-04	3.45E-03	-0.34	$P^b > 0.05$	$P^c > 0.05$	-0.18	2.95E-01	$P^c > 0.05$	0.69	5.07E-03	8.12E-05	6.81
27	Adrenochrome o-semiquinone	205.10	2.49	HMDB12883	-1.38	$P^b > 0.05$	7.65E-03	-0.37	$P^b > 0.05$	$P^c > 0.05$	1.37	6.28E-04	1.49E-02	3.53	1.97E-15	1.58E-08	10.41
28	Adrenochrome	147.03	0.88	HMDB12884	0.07	2.18E-02	$P^c > 0.05$	-0.86	1.23E-02	8.77E-06	-1.28	2.22E-04	1.93E-07	-4.73	1.34E-10	3.48E-15	21.14
29	Acetaminophen glucuronide	267.13	4.89	HMDB10316	-0.96	4.04E-03	4.17E-03	-0.49	$P^b > 0.05$	$P^c > 0.05$	-1.06	1.30E-02	7.20E-03	-4.44	4.29E-08	1.31E-05	7.51
30	9-HOTE	255.07	6.03	HMDB10224	-0.97	5.11E-04	1.16E-02	-2.48	9.39E-08	2.85E-04	-2.54	1.28E-07	2.28E-04	-2.64	2.21E-08	2.09E-04	3.00
31	6-Keto-prostaglandin F1a	164.07	4.14	HMDB02886	-0.65	$P^b > 0.05$	$P^c > 0.05$	-1.85	2.88E-05	8.65E-04	-2.39	4.40E-06	2.34E-04	-2.62	4.28E-07	1.82E-04	3.71
32	5-L-Glutamyl-aurine	263.14	3.48	HMDB04195	-1.21	$P^b > 0.05$	$P^c > 0.05$	0.43	$P^b > 0.05$	$P^c > 0.05$	2.54	9.14E-07	1.49E-04	3.16	2.46E-08	6.43E-05	17.63
33	5-Hydroxyindoleacetyl glycine	166.09	1.81	HMDB04185	-1.64	5.31E-06	2.87E-07	-1.03	5.95E-03	3.28E-04	-1.44	3.55E-04	7.18E-07	-2.17	3.33E-07	1.67E-06	3.52
34	5-Hydroxy-6-methoxyindole glucuronide	183.19	10.57	HMDB10363	0.22	$P^b > 0.05$	$P^c > 0.05$	1.05	1.72E-03	3.39E-02	0.77	1.01E-02	$P^c > 0.05$	-2.64	$P^b > 0.05$	7.37E-03	8.47
35	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	206.08	5.14	HMDB04083	-1.63	1.86E-06	4.06E-04	-1.38	7.32E-05	5.52E-04	-1.41	1.99E-04	3.75E-04	-1.16	5.31E-05	1.00E-03	3.99
36	3-Methyldioxyindole	381.08	0.68	HMDB04186	-1.12	9.30E-08	2.06E-07	-0.38	$P^b > 0.05$	1.55E-03	-0.81	1.60E-02	1.41E-05	-4.04	4.26E-14	5.87E-11	7.57
37	3-Indolecarboxylic acid glucuronide	171.07	3.94	HMDB13189	0.16	$P^b > 0.05$	$P^c > 0.05$	-0.59	$P^b > 0.05$	$P^c > 0.05$	-0.97	2.92E-01	4.96E-02	-5.08	2.08E-04	3.82E-04	4.69
38	3-Dehydrocarnitine	173.08	4.42	HMDB12154	0.45	4.55E-02	$P^c > 0.05$	0.47	2.24E-03	1.97E-02	0.59	1.26E-04	1.42E-02	0.87	5.44E-04	2.82E-03	4.94
39	2-Hydroxy-2-(2-oxopropyl)butanedioic acid	359.05	0.85	HMDB59927	1.27	4.79E-04	3.15E-03	0.32	$P^b > 0.05$	$P^c > 0.05$	-0.03	3.30E-01	$P^c > 0.05$	1.83	4.48E-07	4.79E-04	3.11
40	13-HOTE	217.12	1.37	HMDB10203	0.09	$P^b > 0.05$	$P^c > 0.05$	-1.4	5.87E-03	1.23E-03	-1.97	8.42E-04	8.06E-05	-1.8	2.66E-04	1.59E-04	3.30
41	13E-Tetranor-16-carboxy-LTE4	215.02	0.92	HMDB12575	-1.13	$P^b > 0.05$	4.02E-02	0.52	$P^b > 0.05$	$P^c > 0.05$	2.22	2.47E-02	2.82E-03	4.25	1.01E-08	1.08E-04	4.74
serum																	
1	Uric acid	167.02	0.91	HMDB00289	1.79	5.94E-03	2.45E-06	1.81	5.58E-03	9.00E-06	1.84	2.90E-04	6.23E-05	2.10	1.10E-05	7.43E-05	3.33

2	Ubiquinone-2	301.19	10.79	HMDB06709	-3.07	1.46E-20	5.16E-06	-3.07	4.37E-20	5.16E-06	-3.75	2.26E-20	5.20E-06	-3.69	2.46E-20	5.12E-06	3.03
3	Tryptophyl-Lysine	315.2	12.22	HMDB29088	-6.21	$P^b > 0.05$	8.56E-06	-6.24	2.10E-19	8.56E-06	-5.13	3.07E-19	1.04E-05	-5.53	2.65E-19	2.37E-07	2.72
4	Tetrahydrodipicolinate	154.04	11.35	HMDB12289	0.35	$P^b > 0.05$	1.64E-03	0.2	$P^b > 0.05$	1.54E-03	1.16	8.58E-12	7.31E-06	1.13	1.50E-11	2.41E-06	3.91
5	Taurocholic acid 3-sulfate	640.26	10.77	HMDB02581	0.05	2.03E-10	9.85E-04	0	2.30E-10	9.65E-04	0.22	$P^b > 0.05$	4.32E-05	-0.21	3.13E-09	7.17E-05	3.39
6	Sulfolithocholylglycine	512.27	6.08	HMDB02639	3.78	$P^b > 0.05$	3.06E-04	3.55	$P^b > 0.05$	8.84E-04	3.66	$P^b > 0.05$	1.22E-04	5.40	7.65E-03	3.64E-04	13.55
7	Stearoylcarnitine	410.38	11.7	HMDB00848	5.04	2.44E-12	8.79E-06	5.09	1.93E-12	8.77E-06	4.71	1.84E-11	8.53E-06	4.49	$P^b > 0.05$	8.64E-06	3.20
8	Sphinganine	302.31	8.96	HMDB00269	-0.7	2.95E-11	$P^c > 0.05$	-0.74	6.15E-11	$P^c > 0.05$	-0.68	1.02E-08	$P^c > 0.05$	-0.64	1.71E-08	2.25E-02	3.91
9	SM(d18:1/24:1(15Z))	857.67	12.94	HMDB12107	-0.31	3.38E-12	6.03E-03	-0.36	6.24E-12	6.90E-03	-0.42	6.95E-13	3.79E-03	-0.33	1.07E-12	1.67E-02	5.42
10	S-(PGJ2)-glutathione	640.29	12.23	HMDB13063	-0.95	1.89E-13	3.53E-06	-1.01	3.18E-13	3.52E-06	-1.52	3.64E-11	3.84E-06	-1.49	$P^b > 0.05$	3.52E-06	9.62
11	PG(18:3(6Z,9Z,12Z)/18:3(6Z,9Z,12Z))	789.45	4.33	HMDB10666	1.27	9.81E-05	$P^c > 0.05$	1.29	6.67E-05	$P^c > 0.05$	-3.32	2.70E-06	4.19E-08	-1.75	4.96E-05	3.57E-08	2.24
12	PC(18:1(11Z)/20:5(5Z,8Z,11Z,14Z,17Z))	850.56	12.96	HMDB08083	0.7	$P^b > 0.05$	8.90E-06	0.72	$P^b > 0.05$	1.12E-05	-0.45	$P^b > 0.05$	2.51E-05	-2.05	3.61E-03	1.64E-04	5.45
13	Nonate	187.1	4.46	HMDB11717	-1.28	8.20E-15	1.54E-03	-1.31	2.15E-14	1.08E-10	-0.48	3.65E-08	5.95E-05	-0.85	5.43E-11	8.09E-05	3.99
14	N-Acetyl-L-methionine	192.07	6	HMDB11745	-4.92	3.86E-19	$P^c > 0.05$	-4.92	1.09E-18	$P^c > 0.05$	-5.07	1.14E-18	1.03E-07	-5.27	1.05E-18	2.01E-07	2.33
15	N-Acetylgalactosamine	244.08	0.63	HMDB00212	-1.45	1.17E-10	9.99E-07	-1.56	1.97E-10	8.23E-07	-1.46	1.52E-09	1.53E-06	-1.81	2.27E-10	1.31E-06	4.55
16	Myo-inositol hexakisphosphate	682.87	4.75	HMDB03502	-7.55	6.03E-18	3.73E-02	-7.55	1.66E-17	4.58E-02	-3.62	1.05E-16	1.08E-02	-2.97	3.25E-16	4.22E-03	2.24
17	Muricholic acid	453.28	7	HMDB00865	1.66	$P^b > 0.05$	$P^c > 0.05$	1.62	$P^b > 0.05$	$P^c > 0.05$	2.57	3.53E-03	$P^c > 0.05$	2.49	5.34E-03	7.30E-02	7.10
18	Methylglutaric acid	169.04	0.92	HMDB00422	-3.79	9.18E-21	$P^c > 0.05$	-3.82	2.60E-20	$P^c > 0.05$	-3.96	2.88E-20	$P^c > 0.05$	-4.25	2.24E-20	1.73E-02	8.34
19	LysoPC(20:4(5Z,8Z,11Z,14Z))	544.34	10.2	HMDB10395	-0.17	1.70E-03	5.93E-03	-0.15	3.65E-03	4.75E-03	-0.82	1.28E-04	9.13E-05	-1.29	6.45E-06	2.82E-03	16.88
20	LysoPC(18:2(9Z,12Z))	520.34	10.2	HMDB10386	0.17	$P^b > 0.05$	1.39E-04	0.13	8.34E-02	2.74E-04	0.31	$P^b > 0.05$	5.89E-04	-0.178	4.27E-02	1.68E-04	9.48
21	LysoPC(18:0)	524.37	12.22	HMDB10384	-0.54	1.46E-05	3.20E-04	-0.52	3.68E-05	9.11E-04	-0.98	6.75E-06	1.28E-06	-0.50	3.37E-04	4.65E-05	27.20
22	LysoPC(17:0)	508.34	12.23	HMDB12108	-0.79	1.11E-12	6.17E-10	-0.82	2.86E-12	5.10E-09	-1.63	2.47E-14	5.37E-09	-1.66	2.09E-14	4.95E-09	5.77
23	LysoPC(15:0)	482.32	10.07	HMDB10381	0.58	$P^b > 0.05$	1.74E-02	0.56	$P^b > 0.05$	1.73E-02	0.56	5.45E-01	1.97E-02	1.83	1.46E-05	2.02E-02	4.85
24	L-Valine	118.09	0.65	HMDB00883	-0.25	1.24E-02	2.39E-03	-0.41	8.18E-03	1.25E-03	0.88	1.85E-03	2.47E-04	1.31	1.46E-07	4.08E-04	3.05
25	L-Tryptophan	205.1	2.27	HMDB00929	1.21	8.21E-04	3.40E-04	1.16	1.79E-03	3.16E-04	1.75	9.35E-12	5.60E-04	0.96	5.66E-04	3.78E-04	5.21

26	L-Leucine	132.1	1.3	HMDB00687	-0.1	1.04E-03	3.35E-04	-0.22	6.46E-04	2.87E-04	1.09	2.94E-07	6.89E-04	0.93	1.41E-05	4.05E-04	4.62
27	Lithocholyltaurine	528.31	10.2	HMDB00722	-0.59	1.13E-06	$P^c > 0.05$	-0.58	3.10E-06	$P^c > 0.05$	-2.31	9.79E-10	4.03E-02	-2.76	$P^b > 0.05$	9.65E-04	3.26
28	Leukotriene C5	668.29	12.23	HMDB12993	-0.46	6.04E-15	$P^c > 0.05$	-0.51	1.21E-14	$P^c > 0.05$	-0.25	5.53E-09	1.73E-03	-0.56	7.42E-13	1.89E-02	6.19
29	L-Carnitine	162.11	0.63	HMDB00062	-0.98	2.57E-09	1.42E-07	-1.05	4.14E-09	7.22E-07	-0.92	1.31E-07	3.96E-05	-1.16	1.15E-08	3.47E-06	2.99
30	Lactosylceramide (d18:1/12:0)	806.57	12.79	HMDB04866	1.17	$P^b > 0.05$	1.36E-02	1.39	4.03E-02	3.70E-02	-0.35	$P^b > 0.05$	2.34E-02	-1.18	$P^b > 0.05$	1.42E-01	3.87
31	L-Acetylcarnitine	204.12	0.9	HMDB00201	-0.81	2.61E-04	2.96E-02	-0.87	3.66E-04	$P^c > 0.05$	-1	5.27E-04	1.93E-03	-0.99	5.65E-04	1.50E-02	2.64
32	Kinetensin 4-7	616.29	10.2	HMDB12986	-3.42	1.47E-20	3.65E-09	-3.58	3.63E-20	2.58E-08	-3.61	4.50E-20	4.32E-04	-0.89	7.18E-14	8.19E-07	3.12
33	Indoxyl sulfate	212	4.34	HMDB00682	2.24	1.00E-05	8.20E-04	2.14	3.74E-05	5.74E-04	1.02	$P^b > 0.05$	1.86E-02	1.45	5.37E-03	3.13E-04	2.08
34	Hippuric acid	178.05	2.9	HMDB00714	2	5.13E-02	1.29E-03	1.96	5.96E-02	5.36E-03	1.82	4.94E-02	2.63E-04	1.95	2.54E-02	8.09E-04	2.52
35	Glycyl-Phenylalanine	223.11	1.15	HMDB28848	1.7	8.20E-03	$P^c > 0.05$	1.59	1.87E-02	$P^c > 0.05$	2.26	2.56E-07	6.72E-05	2.83	1.64E-11	1.07E-05	2.33
36	Glycocholic acid	464.3	6.76	HMDB00138	1.74	$P^b > 0.05$	$P^c > 0.05$	1.52	$P^b > 0.05$	$P^c > 0.05$	2.18	4.54E-03	$P^c > 0.05$	2.25	1.59E-03	4.72E-02	5.35
37	D-Urobilinogen	1179.6	10.2	HMDB04158	0.33	$P^b > 0.05$	5.15E-07	0.3	$P^b > 0.05$	1.91E-06	-0.31	2.37E-02	9.71E-07	-1.47	2.64E-05	1.05E-06	4.73
38	D-Mannose	225.06	0.63	HMDB00169	0.38	$P^b > 0.05$	1.45E-10	0.38	$P^b > 0.05$	9.04E-10	-0.27	1.64E-04	7.00E-06	-0.33	5.18E-05	8.13E-09	2.97
39	Cholesterol	369.35	11.67	HMDB00067	3.3	2.33E-07	5.84E-09	3.5	2.41E-08	2.27E-08	3.27	2.05E-08	1.26E-06	2.81	9.55E-06	9.58E-07	15.38
40	Adrenochrome o-semiquinone	182.08	1.14	HMDB12883	1.11	8.26E-04	4.94E-07	1.06	1.61E-03	2.30E-06	1.17	9.10E-07	1.20E-06	1.31	2.62E-08	6.54E-06	3.82
41	9,12,13-TriHOME	329.23	6.52	HMDB04708	-2.3	9.61E-15	2.53E-07	-2.33	2.46E-14	4.44E-06	-1.52	3.80E-12	$P^c > 0.05$	-1.77	8.26E-13	1.39E-05	3.69
42	6-Hydroxymelatonin	497.24	3.39	HMDB04081	-0.67	1.29E-08	3.69E-07	-0.68	3.14E-08	3.26E-07	-3.63	7.16E-13	6.44E-07	-4.03	4.88E-13	1.51E-06	2.68
43	3-Oxohexanoic acid	129.05	3.22	HMDB10717	2.17	3.95E-05	2.83E-03	2.06	1.49E-04	3.15E-03	1.39	1.84E-02	2.49E-04	1.61	1.64E-03	2.36E-04	2.06
44	3-Methylhistidine	192.07	2.2	HMDB00479	-4.55	4.03E-18	$P^c > 0.05$	-4.56	1.12E-17	$P^c > 0.05$	-4.99	1.04E-17	3.04E-02	-5.02	1.02E-17	3.44E-04	2.68
45	3-Methylene-indolenine	152.05	12.13	HMDB11664	0.38	$P^b > 0.05$	1.35E-03	0.23	$P^b > 0.05$	1.78E-03	1.17	3.46E-12	7.28E-05	1.16	3.03E-12	5.77E-03	21.89
46	2-Phenylethanol glucuronide	297.1	4.54	HMDB10350	1.59	$P^b > 0.05$	$P^c > 0.05$	1.45	$P^b > 0.05$	$P^c > 0.05$	1.51	2.40E-02	8.77E-04	1.74	3.24E-03	9.28E-04	2.82

^a Fold change was calculated as a binary logarithm of the average mass response (normalized peak area) ratio between the model group vs the control group, where a positive value means that the average mass response of the metabolite in each group is larger than that in the control group. ^b The P-value was calculated from ANOVA. ^c The P-value was calculated from Student's t-test. ^d VIP was obtained from the PLS-DA model. RT represented Retention Time.

Table S3 Location-based metabolite sets for liver fibrosis from urine and serum Metabolomics Pathway Analysis

NO.	Pathway	Total	Hits	Metabolites	Raw p	-Log(p)	Impact
urine							
1	Tryptophan metabolism	79	5	L-Tryptophan; Kynurenic acid; 5-Hydroxyindoleacetyl glycine; 3-Methyldioxyindole; 4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	0.00	7.70	0.11
2	Citrate cycle (TCA cycle)	20	2	cis-Aconitic acid; Isocitric acid	0.01	4.38	0.11
3	Valine, leucine and isoleucine degradation	40	2	L-Leucine; Methylmalonic acid	0.05	3.07	0.02
4	Glyoxylate and dicarboxylate metabolism	50	2	cis-Aconitic acid; Isocitric acid	0.07	2.67	0.03
serum							
1	Sphingolipid metabolism	25	3	Sphinganine; Lactosylceramide; Sphingomyelin(SM)	0.00	5.90	0.15
2	Valine, leucine and isoleucine biosynthesis	27	2	L-Valine; L-Leucine	0.04	3.26	0.03
3	Glycerophospholipid metabolism	39	2	LysoPC(17:0); PC(18:1(11Z)/20:5(5Z,8Z,11Z,14Z,17Z	0.07	2.60	0.10
4	Valine, leucine and isoleucine degradation	40	2	L-Leucine; L-Valine	0.08	2.56	0.02
5	Primary bile acid biosynthesis	47	2	Cholesterol; Glycocholic acid	0.10	2.28	0.06
6	Tryptophan metabolism	79	2	L-Tryptophan; 6-Hydroxymelatonin	0.23	1.45	0.11
7	Aminoacyl-tRNA biosynthesis	75	3	L-Valine; L-Leucine; L-Tryptophan	0.05	2.91	0.00

Table S4. ROC curves for the diagnosis of liver fibrosis based on the potential biomarkers of the urine and serum samples at week 8.

No.	Metabolites	AUC	Sensitivity	1-Specificity	95% CI
urine					
1	L-Tryptophan	1.00	1.00	0.00	1.00-1.00
2	Kynurenic acid	1.00	1.00	0.00	1.00-1.00
3	5-Hydroxyindoleacetyl glycine	1.00	1.00	0.00	1.00-1.00
4	3-Methyldioxyindole	1.00	1.00	0.00	1.00-1.00
5	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	0.88	1.00	0.14	0.657-1.105
6	cis-Aconitic acid	1.00	1.00	0.00	1.00-1.00
7	Isocitric acid	1.00	1.00	0.00	1.00-1.00
8	L-Leucine	1.00	1.00	0.00	1.00-1.00
9	Methylmalonic acid	1.00	1.00	0.00	1.00-1.00
serum					
1	Sphinganine	1.00	1.00	0.00	1.00-1.00
2	Lactosylceramide	0.98	1.00	0.14	0.904-1.049
3	L-Valine	1.00	1.00	0.00	1.00-1.00
4	L-Leucine	1.00	1.00	0.00	1.00-1.00
5	L-Tryptophan	1.00	1.00	0.00	1.00-1.00
6	LysoPC(17:0)	1.00	1.00	0.00	1.00-1.00
7	PC(18:1(11Z)/20:5(5Z,8Z,11Z,14Z,17Z))	0.88	1.00	0.14	0.657-1.105
8	Cholesterol	1.00	1.00	0.00	1.00-1.00
9	Glycocholic acid	0.90	0.83	0.14	0.737-1.073
10	Sphingomyelin (d18:1/24:1(15Z))	1.00	1.00	0.00	1.00-1.00

Table S5. ROC curves for the diagnosis of liver fibrosis based on the potential biomarkers of the urine and serum samples at week 2.

No.	Metabolites	AUC	Sensitivity	1-Specificity	95% CI
urine					
1	Kynurenic acid	1.00	1.00	0.00	1.00-1.00
2	5-Hydroxyindoleacetyl glycine	1.00	1.00	0.00	1.00-1.00
3	3-Methyldioxyindole	1.00	1.00	0.00	1.00-1.00
4	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	0.95	1.00	0.14	0.839-1.066
5	cis-Aconitic acid	0.90	0.83	0.14	0.715-1.094
serum					
1	Sphinganine	1.00	1.00	0.00	1.00-1.00
2	Sphingomyelin	1.00	1.00	0.00	1.00-1.00
3	L-Leucine	0.86	1.00	0.14	0.579-1.116
4	L-Tryptophan	0.97	1.00	0.14	0.886-1.057
5	LysoPC(17:0)	1.00	1.00	0.00	1.00-1.00

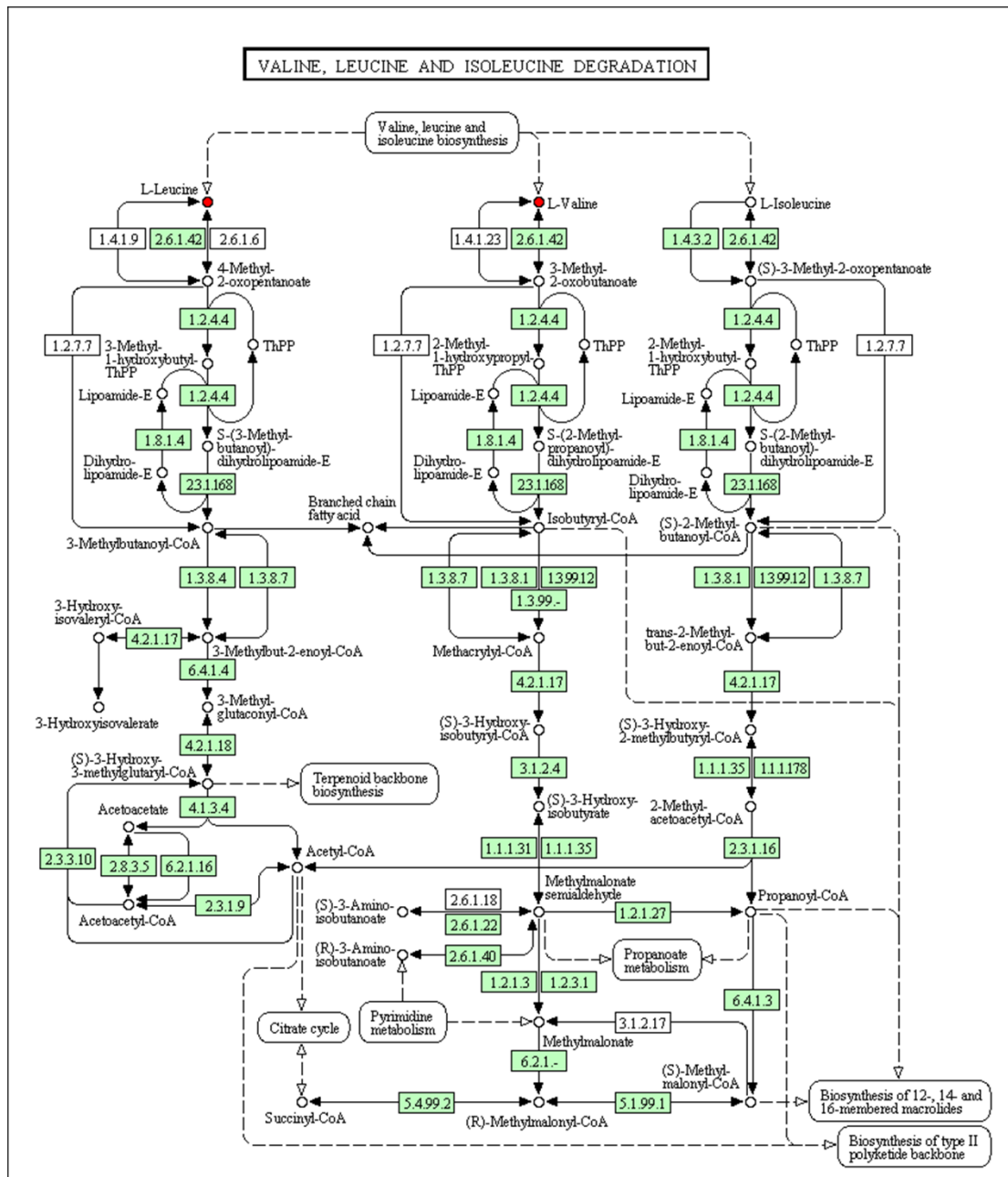


Figure S4. Valine, leucine and isoleucine degradation pathways based on the KEGG database. (Metabolites in red represent the identified potential biomarker in this study)

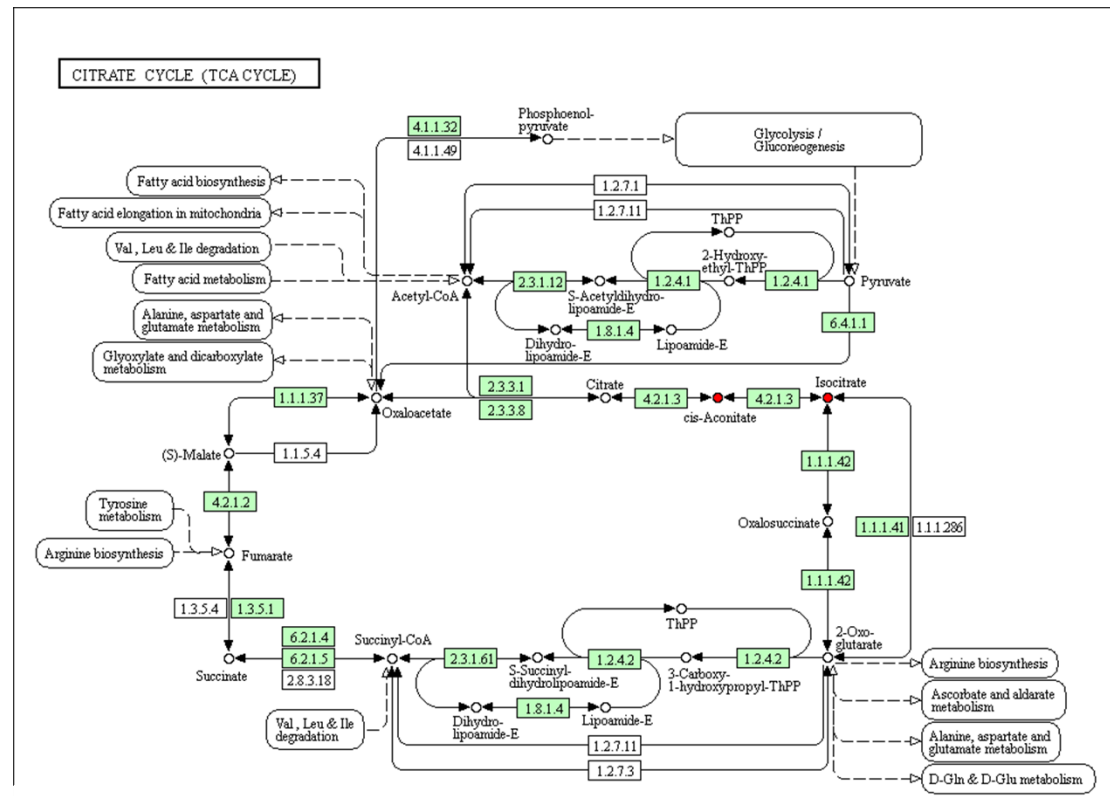


Figure S5. Citrate cycle (TCA cycle) pathways based on the KEGG database. (Metabolites in red represent the identified potential biomarker in this study)

