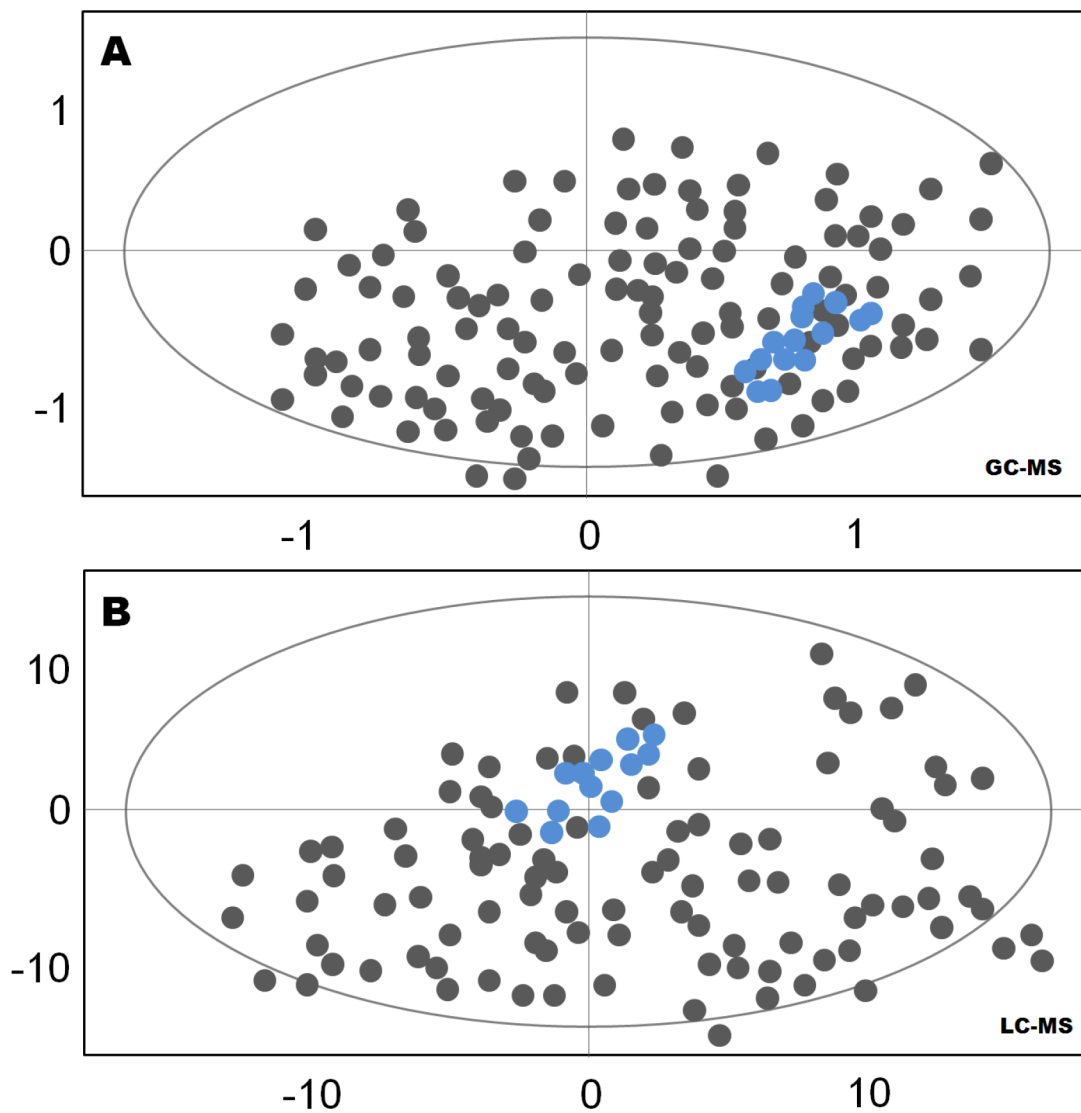
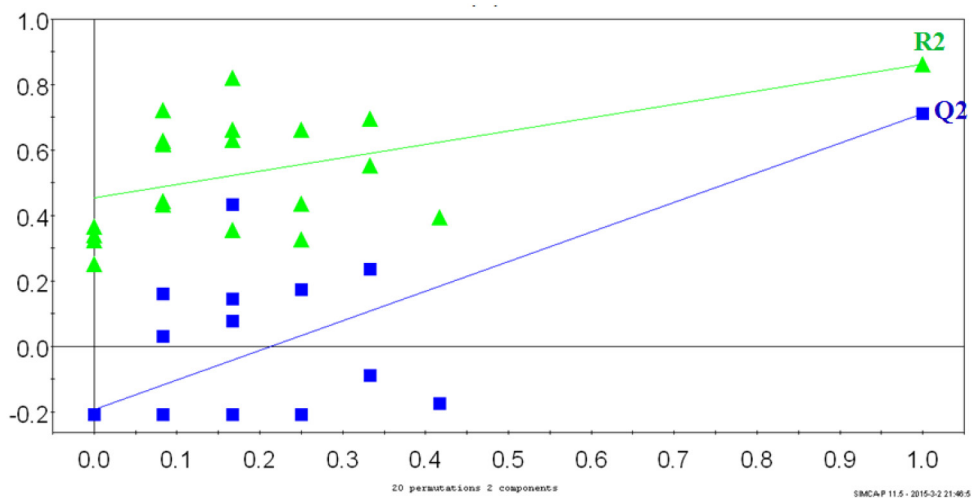


Metabolomics and eicosanoid analysis identified serum biomarkers for distinguishing hepatocellular carcinoma from hepatitis B virus-related cirrhosis

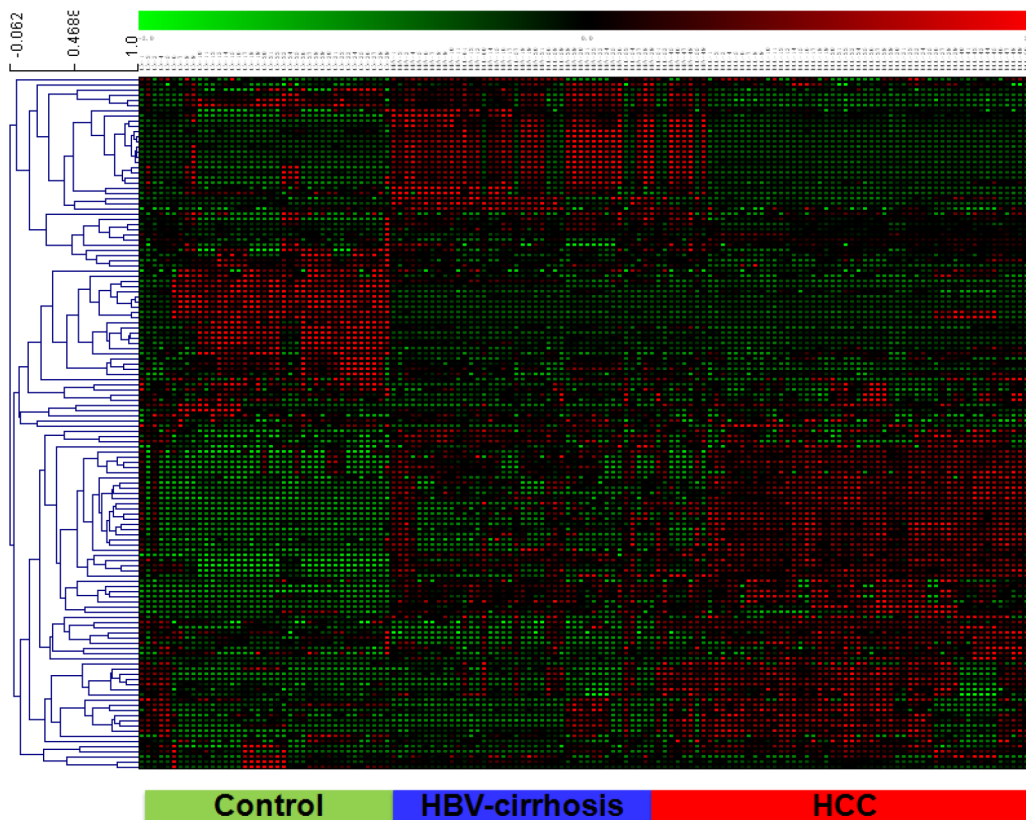
SUPPLEMENTARY MATERIALS



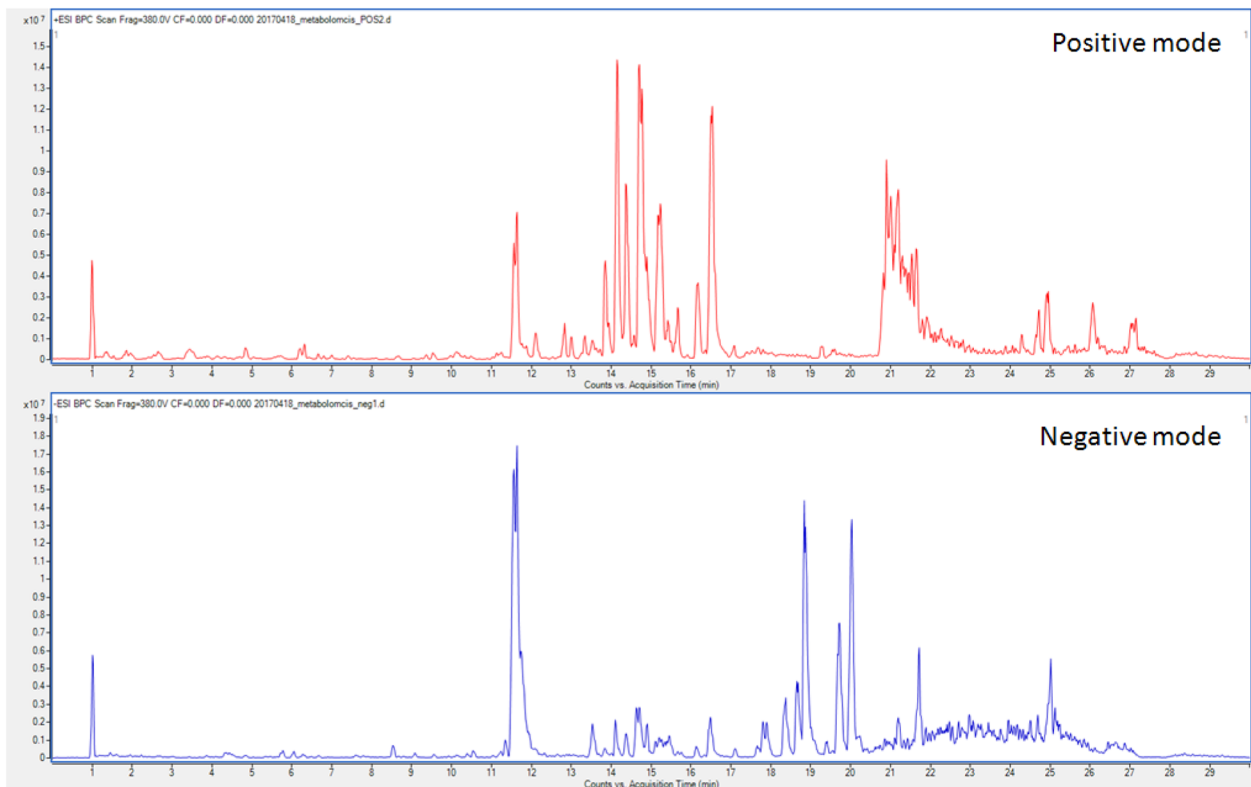
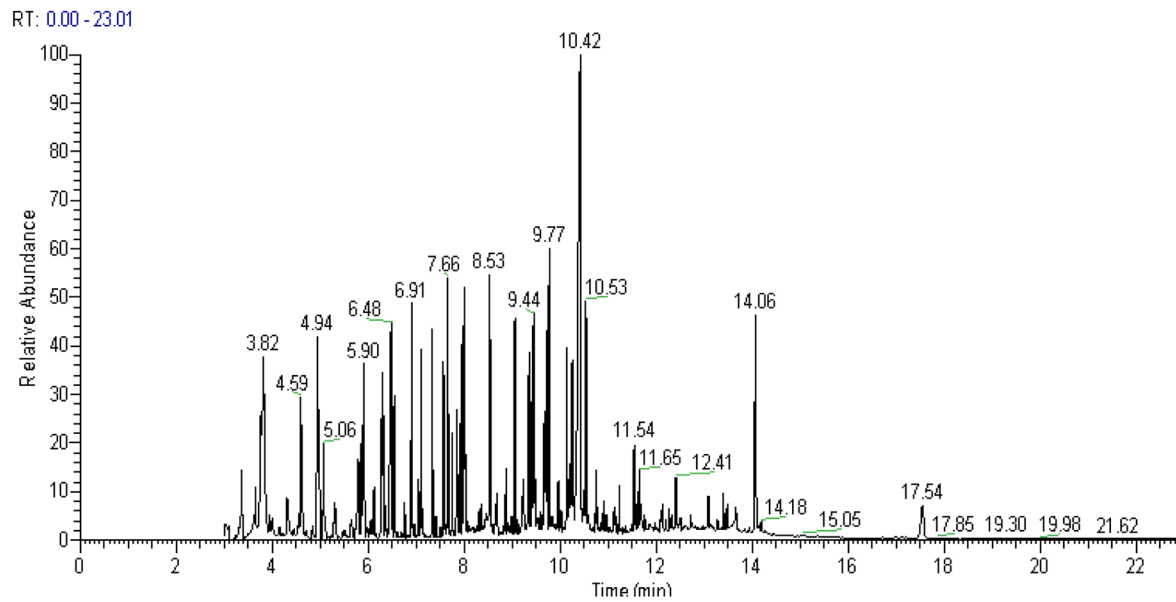
Supplementary Figure 1: The PCA scores plot for QC sample (●) and the other samples (●), showing that the QC samples cluster together. (A) GC-MS; (B) LC-MSI.



Supplementary Figure 2: Validation plot obtained from permutation test (n = 20).



Supplementary Figure 3: Heat map based on the Pearson distance measure and the Ward cluster algorithm for healthy, HBV-cirrhosis and HCC groups. Red color indicates high level of metabolites and green color indicates a low level of metabolites, while black means an equal level groups.



Supplementary Figure 4: The representative TIC chromatography of GC-MS and LC-MS analysis.

Supplementary Table 1: The internal standards used in non-targeted and targeted metabolomics

	Internal standards
Non-targeted metabolomics	Carnitin C16:0-d3, Cholic acid-d4, chenodeoxycholic acid-d4, tryptophan-d5, arachidonic acid-d8, Palmitic acid-d3
Targeted metabolomics	PGF2a-d4; TXB2-d4; 9-HODE-d4; 12,13-EpOME-d4; 15-HETE-d8, 9-HODE-d4, 11,12-EET-d11

Supplementary Table 2: Mass transitions, collision energy, precision and liner range of targeted eicosanoid analysis

See Supplementary File 1

Supplementary Table 3: Validation of LC-MS assay precision, accuracy and autosampler stability of eicosanoids

FAs	Intra-day precision (n = 6)			Inter-day precision (n = 6)			Accuracy (n = 6)		24 hours stability (n =3)
	RSD (%)			RSD (%)			(%)		
	High	Medium	Low	High	Medium	Low	Intra- day	Inter- day	
6-keto-PGF1 α	2.48	1.22	1.55	1.34	7.46	2.06	99.0	113.1	100.0
8-iso-PGF2 α	2.97	2.23	0.70	1.35	1.01	2.06	98.0	110.4	92.1
PGF2 α	1.82	0.96	0.79	1.24	0.91	2.24	97.6	109.1	90.3
PGE2	1.82	0.84	0.48	0.92	1.25	2.44	97.8	107.2	93.0
PGD2	1.94	0.99	0.74	1.20	1.41	2.73	99.2	100.8	94.1
TXB2	1.93	1.28	1.36	1.47	1.31	2.70	98.3	104.0	94.5
LTE4	2.19	1.15	0.52	1.30	1.86	2.74	99.9	96.6	94.2
LTB4	2.08	1.23	0.96	1.86	1.63	2.10	98.8	100.6	94.6
5-HEPE	2.12	1.41	3.91	1.82	2.65	5.58	98.8	100.1	94.0
12-HEPE	2.80	1.09	4.26	2.71	2.06	1.61	100.9	91.5	95.5
15-HEPE	2.59	0.89	3.66	2.15	13.8	2.66	101.1	91.2	93.5
5-HETE	3.61	1.50	2.17	2.69	1.54	1.74	98.7	101.6	97.0
8-HETE	2.02	2.30	3.57	1.81	2.74	2.86	102.0	101.0	93.5
9-HETE	2.81	1.62	5.01	2.35	2.77	1.38	100.5	87.6	97.2
12-HETE	2.36	1.85	3.44	2.67	2.10	1.69	100.6	86.6	93.8
15-HETE	3.04	2.50	3.53	2.10	2.01	1.65	101.7	91.4	97.1
20-HETE	2.58	1.75	6.93	1.99	2.01	3.05	102.0	91.0	93.5
5,6-EET	2.53	2.88	3.78	2.56	1.75	1.79	100.5	90.2	95.9
8,9-EET	1.44	4.09	2.40	1.57	2.01	1.33	100.6	88.4	95.6
11,12-EET	2.53	3.84	8.43	2.19	2.06	1.60	101.7	83.2	97.8
14,15-EET	2.69	2.67	6.84	2.45	2.18	1.62	101.8	83.4	96.3
9-HODE	2.05	1.93	2.14	2.35	2.06	1.73	101.0	89.4	96.8
13-HODE	2.56	1.84	4.00	1.83	1.91	1.60	101.0	90.2	98.5
9,10-DiHOME	2.33	2.18	3.48	1.97	1.46	5.43	101.6	89.7	93.5
12,13-DiHOME	1.87	2.12	6.00	1.21	1.60	2.68	100.9	89.7	97.8
9,10-EpOME	2.72	1.10	6.36	1.80	1.71	1.61	101.8	87.9	96.6
12,13-EpOME	1.93	3.07	7.65	1.90	1.78	3.02	101.6	88.1	94.7
11,12-DiHETrE	3.22	8.17	6.23	1.46	7.76	5.36	101.9	87.3	95.7
14,15-DiHETrE	8.64	1.82	5.23	4.98	1.88	3.74	101.8	94.4	96.8
5-OxoETE	2.52	3.35	7.82	2.13	2.08	2.27	101.1	88.0	93.9

Supplementary Table 4: Recoveries of internal standards

	Recovery (%)		
	High	Medium	Low
PGF2a-d4	71.7	66.5	68.6
TXB2-d4	88.3	74.9	77.1
9-HODE-d4	94.7	69.3	72.2
12,13-EpOME-d4	71.8	61.5	73.2
15-HETE-d8	66.0	70.7	69.0
9-HODE-d4	90.2	68.8	65.4
11,12-EET-d11	82.3	86.1	70.2

Supplementary Table 5: Identification information for LC-MS metabolites

Metabolites	RT (min)	MS1		MS2
		Calculated (Da)	Detected (Da)	
Positive		[M+H] ⁺	[M+H] ⁺	
Glycerol 3-phosphate	1.1	173.0209	173.0212	151.2, 119.3, 78.9
Malate	0.8	135.0287	135.0278	99.0, 71.0, 43.1
Creatinine	1.1	114.1251	114.1255	44.1
Proline	0.9	116.1377	116.1370	70.2
Ornithine	1.2	133.0970	133.0976	70.3
Choline	0.7	105.1147	105.1141	61.2
Carnitine	1.1	162.1124	162.1130	102.4, 84.5, 59.4
Carnitine (10:0)	4.5	317.2553	317.2560	102.4, 84.5
Cholesterol	5.4	387.3621	387.3624	371.3, 369.35
Cortol	5.5	369.2634	369.2637	351.2, 333.2
Cortisol	5.9	363.2166	363.2161	345.2, 121.2
MG (14:0)	10.2	303.2529	303.2525	257.2, 239.1,
DG (30:0)	10.3	541.4826	541.4831	413.2, 352.9, 273.2
DG (32:1)	10.4	567.4982	567.4985	499.2, 413.2, 301.2
DG (40:4)	10.6	678.6074	678.6075	619.1, 380.5, 301.2
TG (47:0)	13.2	793.7279	793.7276	640.2, 542.2
TG (50:2)	14.3	831.7435	831.7433	651.1, 342.2
LPC (14:0)	9.7	468.3083	468.3085	400.3, 184.1, 104.1
LPC (16:0)	10.2	496.3397	496.3395	478.3, 184.1, 104.2
LPC (16:1)	10.1	494.3240	494.3243	476.2, 184.1, 104.3
LPC (18:0)	12.3	524.3710	524.3711	506.2, 184.2, 104.1
PC (32:0)	15.7	734.5693	734.5689	716.2, 184.1, 104.1
PC (40:5)	16.9	836.6163	836.6165	818.2, 184.2, 104.1
PC (42:2)	17.3	870.6945	870.6847	852.2, 184.1
PC (30:5)	17.4	808.5850	808.5852	790.2, 184.1
PC (40:6)	17.4	834.6007	834.6010	816.2, 184.2, 104.1
PC (42:7)	17.6	860.6163	860.6162	842.3, 184.1, 104.1
Negative		[M-H] ⁻	[M-H] ⁻	
Cholic acid	5.7	407.2803	407.2809	391.2, 373.3, 345.2
Deoxycholic acid	5.8	391.2854	391.2857	389.6, 343.3, 327.3
Glycocholic Acid	5.8	464.3018	464.3016	402.4, 74.5
Glycodeoxycholic acid	6.2	448.3069	448.3072	330.2, 74.2
PE (32:0)	16.5	690.5079	690.5082	454.2, 434.2, 255.1
PE (34:1)	16.2	716.5236	716.5240	452.2, 281.3, 255.2

Supplementary Table 6: Calibration curves and figures of selected biomarkers

Metabolites	Regression equation	R2	LLoQ (μM)	RSD (%)
Citrate	$y=417.4x-64.5$	0.995	0.3	99.5
Malate	$y=888.5x-80.4$	0.999	0.3	98.9
Succinate	$y=964.3x-277.5$	0.996	0.2	99.0
Lysine	$y=142.6x-20.1$	0.997	0.1	98.5
Carnitine	$y=297.6x+79.4$	0.996	0.36	99.1
Proline	$y=587x+45.35$	0.996	0.2	97.9
Ornithine	$y=803.7x-217.2$	0.995	0.18	99.6
Serine	$y=197.1x+46.5$	0.999	0.2	98.6
Phenylalanine	$y=694.9x-204.6$	0.995	0.36	97.8
Tyrosine	$y=646,768x+12,653$	0.996	0.4	98.5
Arachidonic acid	$y=124.5x+30.9$	0.996	0.3	98.3
Uric acid	$y=923.3x-11.1$	0.997	0.32	96.9
Arabinose	$y=515.9x-36.7$	0.998	0.3	98.4
Galactose	$y=602.4x-62.4$	0.998	0.2	98.1

Supplementary Table 7: The identified differential metabolites between HCC, HBV-cirrhosis and control

See Supplementary File 2