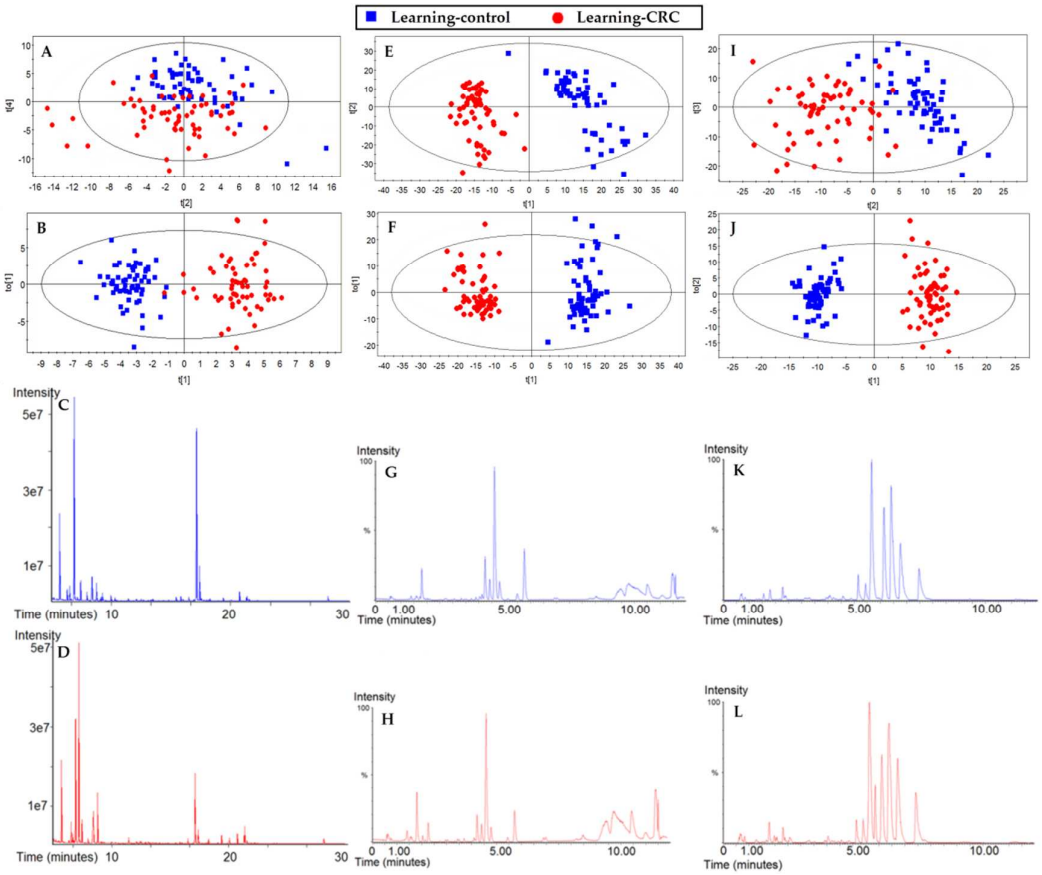
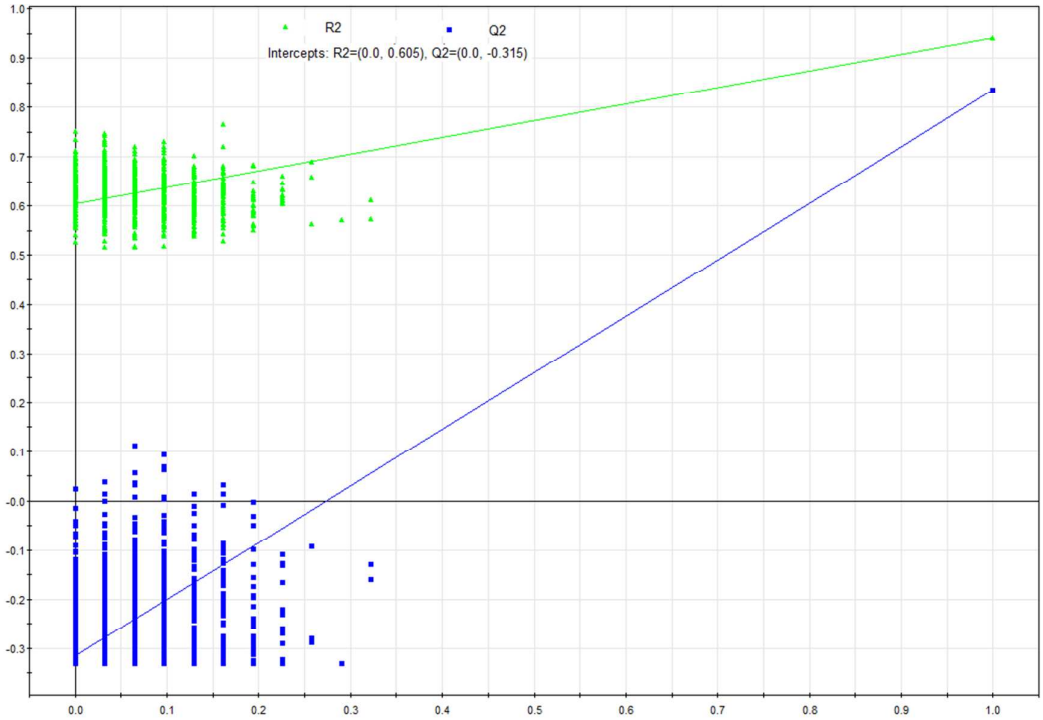
Supplementary Materials

1. Supplementary figures



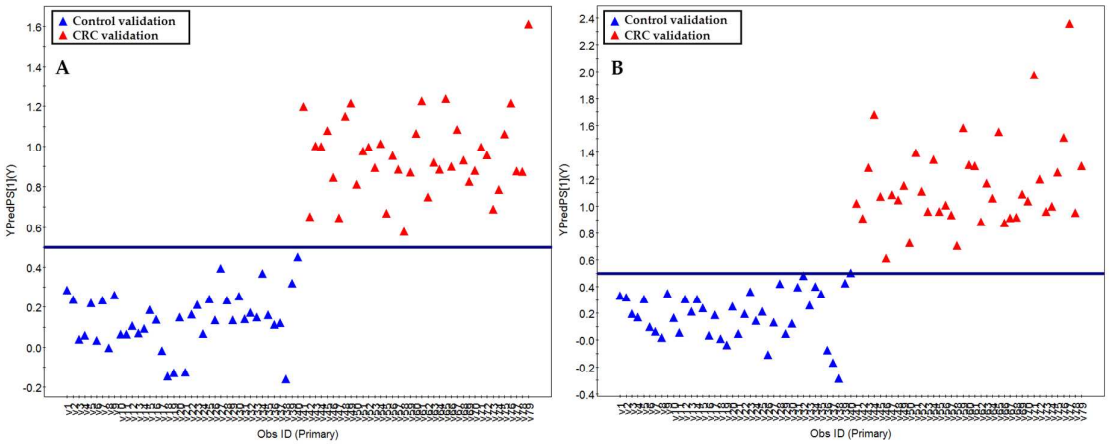
Supplementary Fig. 1

The results from GC-TOFMS (A-D), UPLC-QTOFMS ES+ (E-H), and UPLC-QTOFMS ES- (I-L) analysis. PCA and OPLS-DA model for 62 CRC patients (red dots) and 62 healthy controls (blue squares) based on total spectral data of GC-TOFMS (A and B); UPLC-QTOFMS ES+ (E and F); and UPLC-QTOFMS ES- (I and J). At the bottom of OPLS-DA model, three representative chromatograms of CRC (red) and healthy control sample (blue) derived from GC-TOFMS, UPLC-QTOFMS ES+ and ES-, respectively (C-D, G-H and K-I).



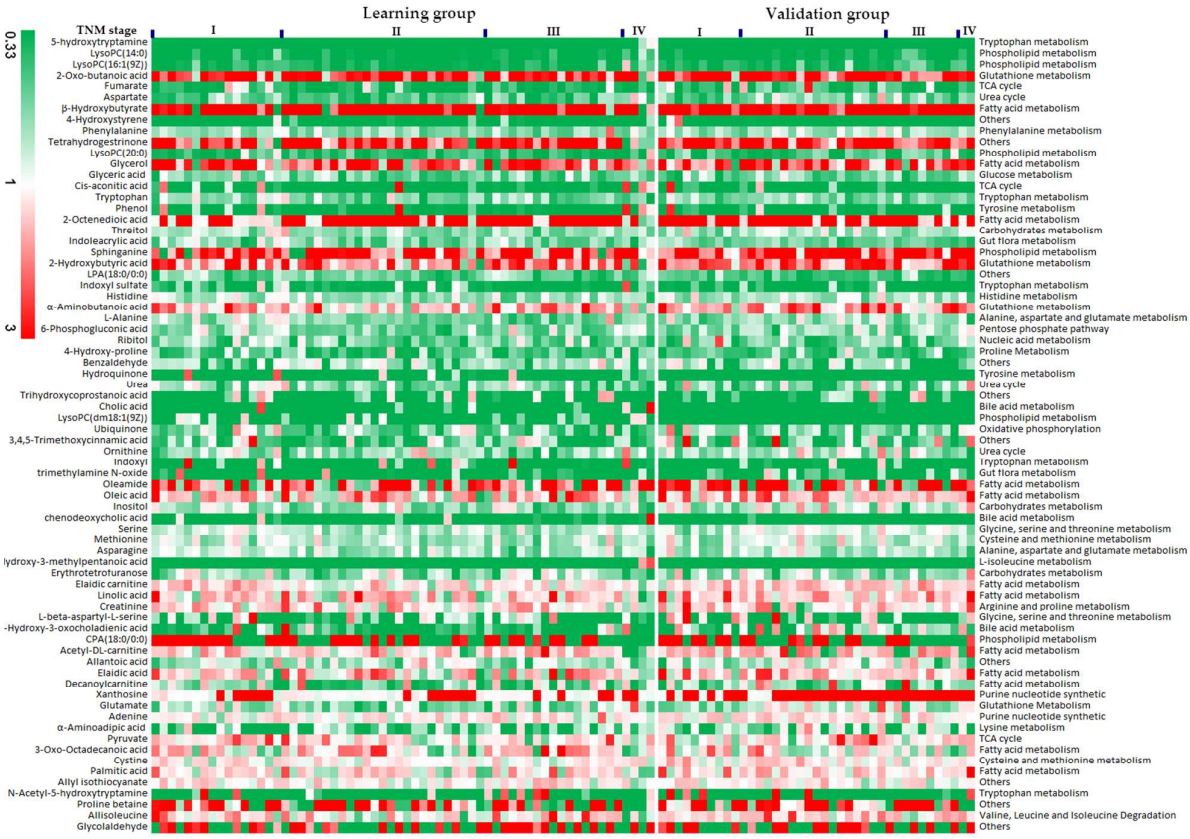
Supplementary Fig. 2

A 999-time permutation test validated the OPLS-DA model with 249 annotated metabolites between healthy controls and CRC patients in the learning data set. Y-axis intercepts: R2= (0.0, 0.605), Q2= (0.0, -0.309).



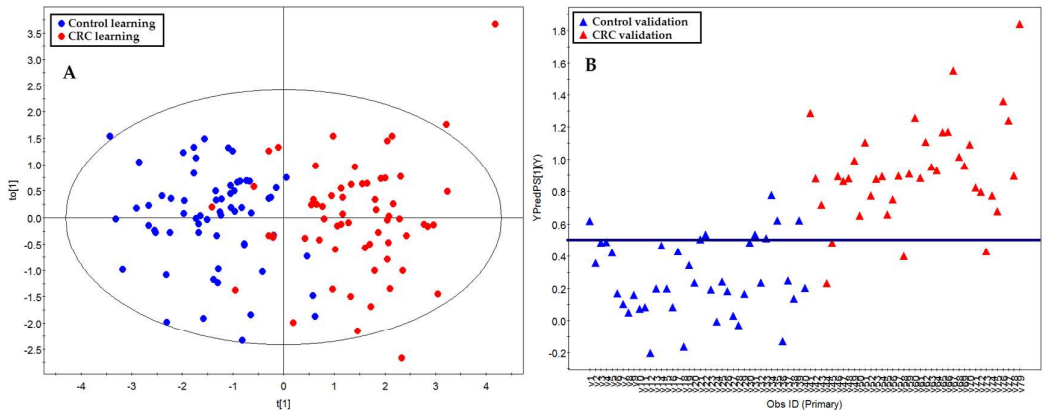
Supplementary Fig. 3

Y-predicted scatter plots of samples in validation groups. A, the model was built with males only in the training dataset. B, the model was built with females only in the training dataset. A number of 40 healthy controls (blue triangle) and 39 CRC patients (red triangle) as a validation group were used to test the prediction ability for the OPLS-DA model using a cut-off of 0.5.



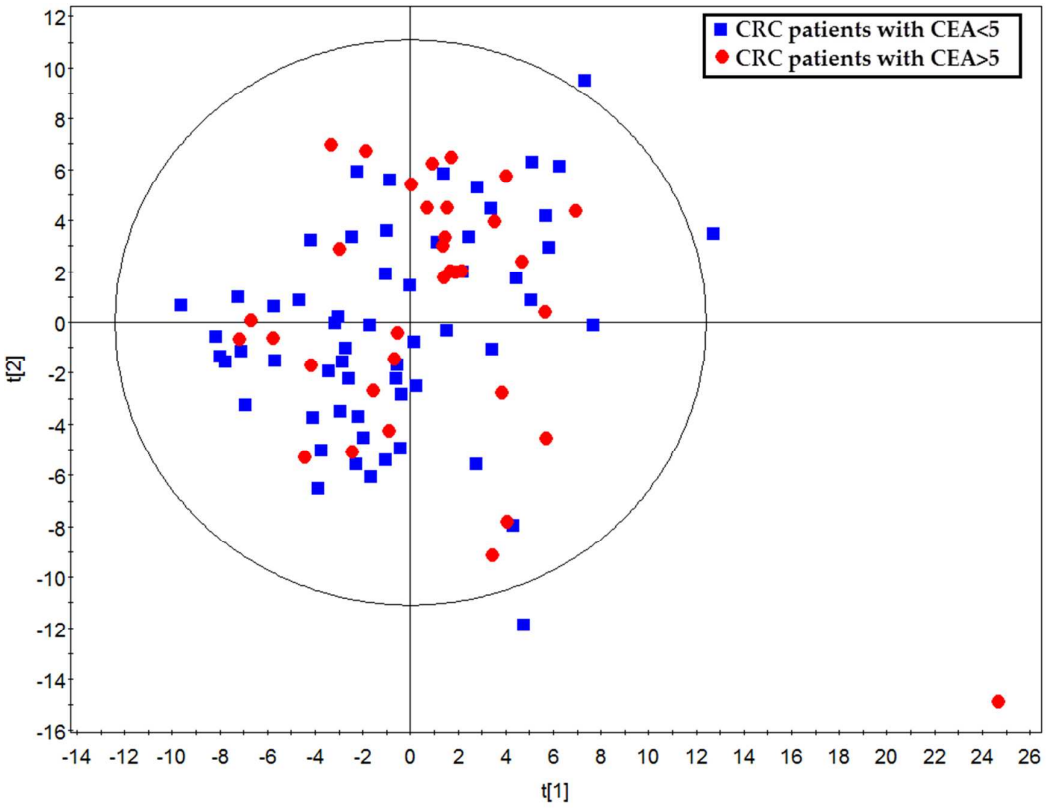
Supplementary Fig. 4

Fold changes (FC) heat-map of differentially expressed metabolites between CRC patients (both in training set and testing set). Each row represents one metabolite and each column is one patient. Fold changes (FC) are the intensity ratios of every patient to the mean value of learning healthy controls.



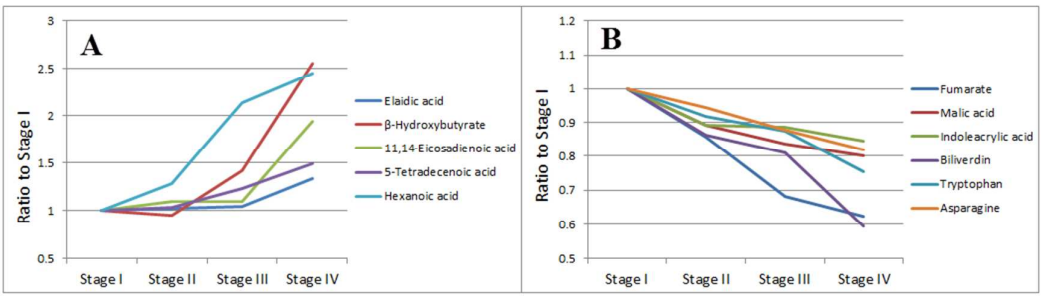
Supplementary Fig. 5

Diagnostic potential of 10 shared differential metabolites identified in two CRC metabonomics studies with two independent cohorts. A, OPLS-DA scores plot of in the training dataset. The OPLS-DA model was constructed using data from 62 CRC patients (red dots) and 62 healthy controls (blue dots). B, Y-predicted scatter plot of samples in validation groups. A number of 40 healthy controls (blue triangle) and 39 CRC patients (red triangle) were used to test the prediction ability for the erected OPLS-DA model. The prediction ability of the OPLS-DA model was made with a cut-off of 0.5.



Supplementary Fig. 6

PCA scores plot for all the CRC samples with high (CEA>5) or low CEA (CEA<5) values, suggesting that there is no statistical correlation between the metabonomics data and the CEA values in CRC subjects.



Supplementary Fig. 7

Metabolites with expression levels changed in a consistent trend (A, increase, B, decrease) from stage I to IV of CRC (values normalized to stage I). The data with the CRC patients in the learning group and validation group were combined, to increase the sample size, especially for stage IV patients,

2. Supplementary Table

Supplementary Table 1. Identified serum metabolites derived from GC-TOFMS and UPLC-QTOFMS

No.	Compounds	Platform [b]	Library [c]	Retention time (min)	M [d]	MR [e]	Missing number [f]
1	11,14-Eicosadienoic acid [a]	G	std	22.5	67		
2	12a-Hydroxy-3-oxocholadienic acid	P	HMDB	3.18	386.2453	386.2457	58
3	12-Ketodeoxycholic acid	N	HMDB	3.18	390.2756	390.277	111
4	12-keto-tetrahydro-Leukotriene B4	P	HMDB	6.69	338.2425	338.2457	0
5	13-cis-Retinoic acid	P	HMDB	5.14	300.207	300.2089	133
6	1b-Hydroxycholic acid	N	HMDB	9.40	424.2797	424.2825	0
7	1-Methylhistamine	P	HMDB	9.41	125.0942	125.0953	0
8	1-Monopalmitin	G	std	23.35	57		
9	2,3-Dihydroxybutanoic acid [a]	G	NIST	9.74	117		
10	24-Hydroxycalcitriol	N	HMDB	3.82	432.3222	432.324	0
11	2-Arachidonylglycerol	N	HMDB	3.38	378.2754	378.277	97
12	2-Ethyl-3-hydroxypropionic acid	G	NIST	8.18	73		
13	2-Hydroxy-3-methylbutyric acid [a]	P	std	1.82	118.0643		0
14	2-Hydroxy-3-methylpentanoic acid	P	HMDB	2.65	132.0772	132.0786	0
15	2-Hydroxybutyric acid [a]	G	std	6.66	131		
16	2-Methylacetoacetic acid	P	HMDB	0.82	116.0472	116.0473	0
17	2-Methylbutyroyl carnitine	P	HMDB	1.85	245.161	245.1627	0
18	2-Oxo-3-methylbutanoic acid	G	NIST	6.41	89		
19	2-Oxo-4-methylthiobutanoic acid	P	HMDB	3.53	148.0152	148.0194	0
20	2-Oxo-4-methylvaleric acid	G	std	7.74	89		
21	2-Oxo-butanoic acid	G	NIST	6.79	89		
22	2-Oxo-pentanedioic acid	G	std	12.81	73		
23	2-Oxopentanoic acid	G	NIST	7.94	89		
24	2-Phenylethanol glucuronide	P	HMDB	3.01	298.1029	298.1053	0
25	2-Piperidinecarboxylic acid	G	std	10.13	156		
26	3,4,5-Trimethoxycinnamic acid	P	HMDB	2.25	238.0834	238.0841	0
27	3,4-Dihydroxybutanoic acid	G	NIST	10.95	73		
28	3-Furoic acid	P	HMDB	1.81	112.0111	112.016	0
29	3-Hydroxybutyric acid	G	std	7.19	117		
30	3-Hydroxy-hexadecanoic acid	N	HMDB	3.97	272.2345	272.2351	1
31	3-Hydroxy-octadecanoic acid	N	HMDB	4.52	300.2657	300.2664	7
32	3-Oxo-Octadecanoic acid	P	HMDB	4.24	298.25	298.2508	0
33	4,8-Dimethylnonanoyl carnitine	P	HMDB	3.58	329.2556	329.2566	0
34	4-Aminobutanoic acid	G	std	14	326		
35	4-Aminophenol	N	HMDB	0.76	109.0532	109.0528	133
36	4-Hydroxy-3-methoxymandelic acid	N	std	0.73	198.0379		14

37	4-Hydroxyisovaleric acid	P	HMDB	0.76	118.0625	118.063	11
38	4-Hydroxy-proline	G	std	12.16	140		
39	4-Hydroxystyrene	N	HMDB	1.81	120.0573	120.0575	85
40	4-Oxo-Retinoic acid	N	HMDB	6.11	314.1896	314.1882	0
41	5,8-Tetradecadienoic acid	N	HMDB	4.28	224.1765	224.1776	3
42	5-Aminoimidazole	P	HMDB	0.69	83.0439	83.0483	0
43	5-Dodecenoic acid	N	HMDB	4.06	198.161	198.162	55
44	5Hydroxytryptamine	P	std	1.63	176.1035		85
45	5-Oxoproline [a]	G	std	12.14	156		
46	5-Tetradecenoic acid	P	HMDB	4.66	226.1922	226.1933	0
47	6-Phosphogluconic acid [a]	G	std	21.28	73		
48	7,10-Hexadecadienoic acid	P	HMDB	4.88	252.2081	252.2089	2
49	8,11,14-eicosatrienoic acid	G	NIST	22.37	67		
50	9-HODE	P	HMDB	4.46	296.2343	296.2351	8
51	Acetyl carnitine	P	std	0.77	203.1146		10
52	Acetyl-N-formyl-5-methoxykynurenamine	N	HMDB	1.90	264.1116	264.111	0
53	Adenine	P	std	1.15	135.0666		0
54	Adenylylselenate	N	HMDB	0.77	474.963	474.9644	13
55	Alanine [a]	G	std	6.33	116		
56	Allantoic acid	N	HMDB	0.59	176.054	176.0546	0
57	Allisoleucine	G	NIST	9.03	158		
58	Allyl isothiocyanate	P	HMDB	0.77	99.0139	99.0143	0
59	Aminomalonic acid	G	std	11.46	73		
60	Androsterone sulfate	P	HMDB	2.44	370.1804	370.1814	3
61	Arabinofuranose	G	NIST	15.5	217		
62	Arabinose	G	std	14.05	103		
63	Arabitol	G	std	14.62	73		
64	Arachidonic acid [a]	G	std	22.26	91		
65	Arginine	P	std	0.64	174.1105		1
66	Asparagine	G	std	14.02	116		
67	Aspartate	G	std	12.08	232		
68	Aspartylysine	N	HMDB	3.52	261.1359	261.1325	0
69	Barium	N	HMDB	0.63	137.9012	137.9052	0
70	Benzaldehyde	P	HMDB	1.41	106.0415	106.0419	0
71	Benzamide	N	HMDB	0.83	121.0517	121.0528	10
72	Benzoic acid [a]	G	std	8.52	105		
73	Betaine	P	std	0.65	117.0781		0
74	Biliverdin	P	HMDB	9.23	582.2471	582.2479	0
75	Butyrylcarnitine	N	HMDB	1.54	231.1454	231.1471	0
76	Carnitine	P	std	0.64	161.1035		0
77	Carnitine (18:1)	P	HMDB	4.55	425.3494	425.3505	0
78	Carnosic acid	P	HMDB	2.72	332.1981	332.1988	1
79	Chenodeoxycholic acid	N	std	3.71	392.2918		97

80	Chenodeoxyglycocholic acid	N	HMDB	3.42	449.314	449.3141	0
81	Cholesterol	G	std	28.17	129		
82	Cholesterol sulfate	N	HMDB	4.38	466.3116	466.3117	11
83	Cholic acid	N	std	3.21	408.2869		98
84	Cis-5-Tetradecenoyl carnitine	P	HMDB	3.69	369.287	369.2879	0
85	Cis-Aconitate	N	std	1.43	173.9978		21
86	Citrate	G	std	16.16	157		
87	Citrulline	G	std	13.31	142		
88	Corticosterone	P	HMDB	5.10	346.2113	346.2144	2
89	CPA(18:0/0:0)	N	HMDB	4.05	420.2629	420.2641	130
90	Creatinine [a]	G	std	12.54	115		
91	Cysteine	G	std	12.52	220		
92	Cystine	G	std	21.83	218		
93	Decanoic acid	N	std	3.82	172.141		4
94	Decanoyl carnitine	P	HMDB	3.15	315.2398	315.241	0
95	DHEA sulfate	N	HMDB	2.39	368.1649	368.1657	0
96	Docosahexaenoic acid [a]	G	std	23.24	91		
97	Dodecanoic acid [a]	G	std	13.86	117		
98	Dodecanoyl carnitine	P	HMDB	3.53	343.271	343.2722	0
99	Dopamine	P	std	0.76	153.075		66
100	dUMP	P	HMDB	0.78	308.0377	308.0409	0
101	Eicosenoic acid	N	HMDB	7.76	310.2859	310.2871	0
102	Elaidic acid	G	std	21.42	117		
103	Erythrotetrofuranose	G	NIST	16.51	73		
104	Estrone glucuronide	N	HMDB	2.04	446.1954	446.1941	118
105	Ethanolamine	G	NIST	5.59	102		
106	Fructose	G	std	16.77	103/103		
107	Fumarate	G	std	9.91	245		
108	Galactose	G	NIST	17.07	191		
109	Gluconic acid	G	NIST	18.01	73/73		
110	Gluconolactone	G	NIST	16.49	140/140		
111	Glucopyranose	G	NIST	18.29	204		
112	Glucose	G	std	17.15	205/319		
113	Glucose-6-phosphate	G	NIST	22.25	204		
114	Glucuronic acid-1-phosphate	N	HMDB	0.72	274.0044	274.009	75
115	Glutamate [a]	G	std	13.38	246		
116	Glutamine [a]	G	std	15.45	156		
117	Glutamylalanine	P	HMDB	2.09	218.0909	218.0903	131
118	Glyceraldehyde [a]	G	std	7.81	73		
119	Glyceric acid	G	std	9.57	102		
120	Glycerol	G	std	8.78	205		
121	Glycerolphosphate	G	std	15.23	73		
122	Glycine	G	std	9.28	174/102		
123	Glycochenodeoxycholic acid	P	std	3.41	471.2952		52

124	Glycochenodeoxycholic acid 3-glucuronide	N	HMDB	2.97	625.3436	625.3462	0
125	Glycocholic acid	N	std	4.17	465.3077		107
126	Glycolaldehyde	N	HMDB	0.72	60.0216	60.0211	82
127	Guanidinosuccinic acid	P	std	0.75	175.0581		74
128	Hexanoic acid	G	std	5.92	173		
129	Hexanoyl carnitine	P	HMDB	2.23	259.1764	259.1783	64
130	Hippuric acid	N	HMDB	1.36	179.0594	179.0582	161
131	Histidine [a]	G	std	17.51	154		
132	Histidinol	N	std	0.83	141.0776		152
133	Homovanillic acid	P	std	1.79	182.0124		0
134	Hydroquinone	N	std	1.39	110.0345		113
135	Hydroxyacetic acid	G	std	5.95	147		
136	Hypotaurine	G	std	13.42	188		
137	Hypoxanthine [a]	G	std	15.93	265		
138	Indole	P	HMDB	1.81	117.0575	117.0578	0
139	Indoleacrylic acid	P	HMDB	1.6	187.0619	187.0633	0
140	Indoxyl	N	HMDB	1.49	133.0513	133.0528	103
141	Indoxyl sulfate	N	HMDB	1.48	213.0087	213.0096	26
142	Inosine	N	std	0.83	268.0807		79
143	Inositol	G	std	19.92	73		
144	Isoleucine [a]	G	std	9.12	158		
145	Isovalerylcarnitine	N	HMDB	1.85	245.161	245.1627	0
146	Kynurenic acid	N	HMDB	0.83	189.0462	189.0426	0
147	Lactate	G	std	5.7	117		
148	Leucine	G	std	8.79	158		
149	Leucyl-proline	P	HMDB	0.77	228.1455	228.1474	0
150	Linoelaidyl carnitine	P	HMDB	4.12	423.3331	423.3349	0
151	Linolic acid	G	std	21.33	81		
152	LPA(16:0/0:0)	P	HMDB	4.043	410.2429	410.2434	0
153	LPA(18:0/0:0)	P	HMDB	4.53	438.2761	438.2746	3
154	LPA(18:1/0:0)	P	HMDB	4.1062	436.2588	436.259	0
155	Lysine	G	std	17.57	156		
156	LysoPC(14:0)	P	HMDB	3.91	467.3003	467.3012	0
157	LysoPC(16:0)	N	HMDB	4.02	495.3327	495.3325	1
158	LysoPC(16:1)	P	HMDB	4.05	493.3161	493.3168	0
159	LysoPC(20:0)	P	HMDB	7.43	551.3947	551.3951	0
160	LysoPC(22:1)	P	HMDB	7.61	577.4075	577.4108	0
161	LysoPC(P-18:1)	P	HMDB	5.09	505.3511	505.3532	0
162	LysoPE(18:0/0:0)	P	HMDB	4.05	481.3163	481.3168	0
163	Malic acid	G	std	11.69	73		
164	Mannose [a]	N	std	0.73	180.0625		0
165	Mercaptoacetic acid	G	NIST	10	160		
166	Mesobilirubinogen	P	HMDB	3.4	592.3246	592.3261	0

167	Methionine [a]	G	std	12.09	176		
168	Methylcysteine	G	std	10.85	162		
169	Methylmaleic acid	G	NIST	9.87	117		
170	Myristic acid [a]	G	std	16.58	285		
171	N-Acetyl-5-hydroxytryptamine	N	std	2.25	218.0909		128
172	N-Acetyl-aspartic acid	N	std	0.76	175.0363		0
173	N-Acetylaspartylglutamic acid	N	HMDB	0.83	304.0867	304.0907	0
174	N-Acetylglutamine	G	NIST	13.21	274		
175	N-Formyl-methionine	N	HMDB	0.75	177.043	177.046	98
176	Nicotinamide	P	std	1.15	122.0359		4
177	Nonanoic acid	G	std	10.06	117		
178	Octadecanoic acid [a]	G	std	21.56	117		
179	Octanoic acid [a]	G	std	7.27	151		
180	Octanoyl carnitine	P	HMDB	2.73	287.2086	287.2097	0
181	Octenedioate	P	HMDB	1.43	172.0719	172.0735	0
182	Oleamide [a]	G	std	22.5	144		
183	Oleic acid [a]	G	std	21.38	129/117		
184	Ornithine	G	std	16.07	142		
185	Oxalic acid	G	std	6.76	147		
186	Oxaloacetic acid	G	NIST	12.44	98		
187	Palmitic acid	G	std	19.41	117		
188	Palmitoleic acid	G	std	19.11	117		
189	Palmitoyl carnitine	P	HMDB	4.4	399.3324	399.3349	0
190	p-Cresol [a]	G	std	7.13	152		
191	Pentaglutamyl folate	P	HMDB	0.64	957.3135	957.3101	0
192	Perillic acid	N	HMDB	2.71	166.0996	166.0994	145
193	Phenol	N	HMDB	0.83	94.0413	94.0419	39
194	Phenylalanine [a]	G	std	13.5	218		
195	Phosphate	G	std	8.78	299		
196	Phosphohydroxypyruvic acid	N	HMDB	9.42	183.9775	183.9773	0
197	Phytosphingosine	P	HMDB	3.36	317.2917	317.293	0
198	Pregnenolone sulfate	N	HMDB	2.6	396.1965	396.197	7
199	Proline [a]	G	std	9.19	142		
200	Proline betaine	P	HMDB	0.69	143.0937	143.0946	0
201	Propionyl carnitine	P	HMDB	1.27	217.1302	217.1314	0
202	Pyrrole-2-carboxylic acid	G	NIST	10.34	240		
203	Pyruvate	G	std	5.56	174		
204	Quinolinic acid	P	HMDB	0.71	167.0197	167.0219	21
205	Retinyl ester	N	HMDB	5.04	302.2239	302.2246	1
206	Rhamnose [a]	G	std	14.71	73		
207	Ribitol	G	NIST	14.44	171		
208	Sagittariol	P	HMDB	6.03	306.2549	306.2559	0
209	Serine	G	std	10	204/116		
210	Sorbitol [a]	G	std	14.08	73		

211	Sphinganine	P	HMDB	3.72	301.297	301.2981	0
212	Sphingosine-1-phosphate	P	HMDB	3.86	379.2493	379.2487	0
213	Stearoylcarnitine	N	HMDB	5.25	427.3664	427.3662	0
214	Succinic acid	G	std	9.43	147		
215	Sulfolithocholylglycine	P	HMDB	2.52	513.2764	513.276	0
216	Taurine	N	std	0.71	125.0183		0
217	Taurochenodesoxycholic acid	P	HMDB	2.56	499.2964	499.2968	71
218	Tetradecanedioic acid	N	HMDB	3.15	258.1823	258.1831	138
219	Tetrahydrodeoxycortisol	P	HMDB	6.36	350.2431	350.2457	0
220	Tetrahydrogestrinone	P	HMDB	0.72	284.1093	284.1049	3
221	Tetrahydropteridine	P	HMDB	9.48	136.0758	136.0749	0
222	Threitol	G	std	11.9	73		
223	Threonic acid	G	std	12.32	73/292		
224	Threonine [a]	G	std	10.34	73		
225	Thyroxine	P	std	2.93	776.6879		4
226	trans-Hexadec-2-enoyl carnitine	P	HMDB	4	397.3178	397.3192	0
227	Tridecanoic acid	N	HMDB	4.64	214.1923	214.1933	116
228	Trihydroxycoprostanoic acid	N	HMDB	4.73	464.3505	464.3502	102
229	Trimethylamine N-oxide	P	std	0.66	75.0607		119
230	Tryptophan [a]	G	std	21.32	202		
231	Tyrosine [a]	G	std	17.8	218		
232	Ubiquinone	P	HMDB	3.56	250.1199	250.1205	0
233	Ubiquinone Q2	P	HMDB	7.21	318.1869	318.1831	0
234	Urea	G	std	8.49	171/171		
235	Uric acid [a]	G	std	20.07	441		
236	Uridine	N	std	0.77	244.0688		5
237	Urobilin	P	HMDB	3.37	594.3371	594.3417	0
238	Urobilinogen	P	HMDB	3.41	596.3556	596.3574	0
239	Valine [a]	G	std	8	144		
240	Xanthine	N	std	0.77	152.033		5
241	Xanthosine	N	HMDB	5	284.0779	284.0757	152
242	Xylose	G	std	13.86	73		
243	α-Aminoadipic acid	G	NIST	12.43	218		
244	α-Aminobutanoic acid	G	std	7.36	130		
245	α-CEHC	N	HMDB	1.91	278.1522	278.1518	0
246	α-Tocopherol	G	std	27.93	237		
247	β-Alanine	G	std	10.93	174		
248	β-aspartyl-glutamate	P	HMDB	3.09	262.0825	262.0801	0
249	β-aspartyl-serine	P	HMDB	2.24	220.0743	220.0695	0

[a] Metabolites that can be identified by GC-TOFMS and UPLC-QTOFMS; [b] platform, G: data obtained from GC-TOFMS, P: data obtained from positive ion mode of UPLC-QTOFMS, N: data obtained from negative ion mode of UPLC-QTOFMS. [c] std, metabolites validated by reference standards by comparing the mass spectrum or accurate mass and the retention time. For GC-TOFMS, the mass spectrum similarity higher than 70% and retention time difference less than 0.5 min, for

UPLC-QTOFMS, the accurate mass difference less than 0.05Da and retention time difference less than 0.5 min, compare with our in-house library; NIST, annotated with National Institute of Standards and Technology (NIST) database with mass spectrum similarity more than 70%; HMDB, annotated with Human Metabolome Database (HMDB) by comparing the accurate mass difference less than 0.005; [d] in GC-TOFMS platform, M stands for quantity mass, while in UPLC-QTOFMS platform, M stands for the detected monoisotopic molecular weight. [e] MR, stands for the monoisotopic molecular weight recorded in HMDB database. [f] The number of missing values in each metabolite detected with UPLC-QTOFMS.

Supplementary Table 2. Most significant metabolites markers identified in our two CRC metabonomics studies.

No.	Differential metabolite	Identified in our previous study [b]		Identified in the current study		20 most significant markers identified in our HCC study [e]
		FC [c] R/C	P [d]	FC [c] R/C	P [d]	
1	Pyruvate [a]	2.1	9.57E-13	1.30	1.10E-03	Oleamide
2	2-hydroxybutanoic acid [a]	1.4	2.84E-04	2.09	7.43E-11	Arabinose
3	3-hydroxybutanoic acid [a]	1.4	1.70E-02	8.59	9.87E-15	2,3-dihydroxyl-propanoic acid
4	Urea [a]	-1.4	1.15E-03	-1.37	1.65E-06	Cystine
5	4-hydroxyproline [a]	-1.5	1.71E-04	-1.80	3.18E-07	4-ketoglucose
6	Ornithine [a]	-1.4	3.93E-04	-1.27	5.43E-06	Inosine
7	Tryptophan [a]	-1.6	2.04E-05	-1.45	7.31E-12	2,3-dihydroxy-2(3H)-furanone
8	Oleic acid [a]	1.1	4.23E-02	1.55	9.31E-06	Creatinine
9	Phenylalanine [a]	-1.2	3.34E-02	-1.35	1.30E-13	3-amino-2-piperidone
10	Glutamic acid [a]	-1.7	1.21E-06	-1.29	1.78E-03	Ornithine
11	Oleamide	-3	2.04E-15	2.55	7.91E-06	Glucosamine
12	Palmitic acid	-1.4	9.00E-04	1.24	2.46E-03	Citrulline
13	Valine [a]	-1.5	3.09E-05	-1.14	5.32E-03	Phosphoric acid
14	Threonic acid [a]	-1.6	5.51E-05	-1.09	2.34E-02	Arachidonic acid
15	Malic acid [a]	1.3	3.82E-03	1.17	5.73E-03	Fumaric acid
16	2-Piperidinecarboxylic acid [a]	-1.3	7.90E-03	-1.53	4.53E-03	α-tocopherol
17	Hippurate [a]	-1.5	3.77E-02	-8.14	2.49E-03	Lysine
18	Lysine [a]	-1.4	2.91E-03	-1.14	4.34E-03	Cysteine
19	Myristic acid	-1.3	3.40E-03	1.25	1.95E-02	Docosahexaenoic acid
20	Carnitine [a]	1.3	7.30E-03	1.18	3.14E-03	Aspartic acid
21	Nicotinamide	-1.2	3.20E-03	1.11	2.00E-02	
22	Arginine [a]	-1.3	1.29E-02	-1.16	2.42E-02	
23	Lactate	1.3	8.99E-03	1.02	7.57E-01	
24	Leucine	-1.5	1.20E-04	-1.09	6.33E-02	
25	Proline	-1.3	1.01E-02	-1.10	1.13E-01	
26	Threonine	-1.4	5.80E-03	-1.08	1.03E-01	
27	Citrulline	-1.4	3.94E-04	-1.09	5.68E-02	
28	Tyrosine	-1.5	9.89E-05	1.15	1. 83E-01	
29	Glycerol phosphate	1.5	3.89E-05	-1.09	5.90E-02	
30	Dopamine	-1.3	9.80E-03	-1.23	5.62E-01	
31	Uridine	1.1	1.14E-06	1.01	7.68E-01	

[a] The differential metabolites identified in both studies with the same up and down direction. [b] Our previous publication refers to: Qiu Y., Cai G., Su M., et al. Serum metabolite profiling of human colorectal cancer using GC-TOFMS and UPLC-QTOFMS. J Proteome Res 2009; 8, 4844-50. [c] The fold change (FC) was calculated by the average value of CRC group to that of control group. FC with a value larger than 1.0 indicates a higher level of the serum metabolite in patients while a FC value lower than 1.0 indicates a lower level, compared to healthy controls. [d] P values were calculated from nonparametric Wilcoxon-Mann-Whitney test. [e] Our HCC study refers to: Chen T., Xie G., Wang X. et al.

Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. Mol Cell Proteomics 2011; 10 (7): M110 004945. HCC, hepatocellular carcinoma.

3. Quality control

The relative standard derivation (RSD) of the internal standard(s) was used to control the quality of the whole data set. For GC-TOFMS, the overall RSD was set to below 20%, and for UPLC-QTOFMS, that was set to below 15%.

The relative standard derivations (RSDs) for the internal standards were 18.81% for chlorophenylalanine and 18.95 % for heptadecanoic acid in GC-TOFMS analysis over all samples. In the UPLC-QTOFMS analysis, the RSD was 12.06 % for negative mode and 14.79 % for positive mode.

In additional to internal standards, a QC sample with mixed (external) standard compounds was also used to control the quality of the sample analysis. Each 10 samples run was followed by a QC sample. Using the same parameters, data analysis of these QC samples was performed. The relative standard derivations (RSD) were calculated in the supplementary Table 3. The results showed acceptable data quality in the current study with all the RSDs less than 20% in GCTOFMS and RSDs less than 15% in UPLC-QTOFMS (except for uridine).

Supplementary Table 3. Reference standards used in the quality control samples in GC-TOFMS, UPLC-QTOFMS (ES-), and UPLC-QTOFMS (ES+).

GC-TOFMS		UPLC-QTOFMS (ES-)		UPLC-QTOFMS (ES+)	
Reference standards	RSD (%)	Reference standards	RSD (%)	Reference standards	RSD (%)
L-Lactate	16.42	3-indolepropionic acid	2.43	2-Aminoisobutyric acid	10.48
L-Alanine	13.72	D-Panthenol	2.15	L-Serine	12.56
3-Hydroxybutyric acid	15.71	Palmitoleic acid	3.77	Betaine	10.73
2-hydroxybutanoic acid	11.32	Eicosapentaenoic acid	3.83	Nicotinic acid	12.59
á-Amino isobutyric acid	14.70	Nimodipine	3.08	L-carnitine	10.26
L-Valine	10.75			L-arginine	9.83
L-Leucine	9.07			Uridine	19.59
L-Isoleucine	9.14				
L-Proline	18.33				
L-Serine	11.84				
L-Threonine	11.15				
L-Aspartic acid	14.56				
L-Methionine	9.69				
L-Phenylalanine	7.92				
L-Glutamine	19.75				
d-Galactose	12.56				
Palmitoleic acid	14.72				
Palmitic acid	18.20				
Myo-Inositol	13.58				
Linolic acid	16.48				
Oleic acid	17.86				
Cholesterol	17.71				
Average	13.87		3.05		12.29

RSD, relatively standard derivation.