Supporting Information

For

Carboxylic Submetabolome-Driven Signature Characterization of COVID-19 Asymptomatic Infection

Jing Xu,[a,†] Yu Yuan,[b,†] Yao-Yu Chen,[a] Cai-Feng Xiong,[a] Zheng Zhang,*[c] Yu-Qi Feng *[a, d]

a. Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China.

b. Hubei Key Lab of Environment and Health Incubating, Department of Occupation and Environmental Health, Huazhong University of Science & Technology, Wuhan 430030, P.R. China.

c. School of Life Sciences, Central China Normal University, 152 Luoyu Rd, Wuhan 430079, P.R. China.

d. School of Health Sciences, Wuhan University, Wuhan 430071, P.R. China.

† These authors contributed equally to this work.

*To whom correspondence should be addressed. Tel.: +86-27-68755595; fax: +86-27-68755595. E-mail address: yqfeng@whu.edu.cn, zhangzheng_whu@126.com.

Table of contents

Chemicals and reagents

9-decenoic acid, undecenoic acid, 3-hydroxydecanoic acid, 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), cholic acid, glycoursodeoxycholic acid (GUDCA) was purchased from J&K Chemical (Beijing, China). Analytical grade ethyl acetate (EA), hydrochloric acid (HCl), formic acid (FA), 2-chloro-1-methylpyridinium iodide (CMPI), triethylamine (TEA), HPLC-grade acetonitrile (ACN), were obtained from Tedia Co. (Fairfield, OH, USA). 2-dimethylaminoethylamine (DMED) was supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The isotope labeling reagent of d_4-DMED was synthesized according to our previous work[28]. Ultrapure water was purified by a Milli-Q apparatus (Millipore, Bedford, MA).

Carboxylic metabolite identification

Thermo Sieve 2.2.0 was used to extract MS spectral peaks of raw data for information about detected carboxylic features, such as accurate *m/z*, peak intensity, and RT. RTs were calibrated by retention index (RI) according to our previously reported method (Zheng SJ et al., *Anal. Chem.* 2018, 90, 8412-8420). An in house Matlab-based software was used to extract the peak pairs with 4.025 Da mass difference, identical RI (Deviation is less than 1%), and 0.5-2 peak intensity ratio. Based on the accurate *m/z*, prospective molecular formulas of DMED-labeled carboxylic features were generated by Thermo Xcalibur 4.2.47 software. A mass tolerance of 10.0 ppm was set, and elements of C, H, N and O were used. Then, an in-house chemically labeled metabolite database (CLMD) (http://www.clmdb.com) and several standards were employed for the identification of detected carboxylic features. The acquired formulas not included in CLMD were further searched in HMDB (https://hmdb.ca) or METLIN (https://metlin.scripps.edu/) for identification.

Table S1. Baseline characteristics of participants.

Variables	Healthy Control (N=122)	COVID-19 (N=62)
Sex-no. (%)		
Male	62 (50.8)	30 (48.4)
Female	60 (49.2)	32 (52.6)
Age (years)		
Mean±SD.	44.0 ± 13.5	45.0 ± 13.9
Median（IQR）	43.0 (35.0-52.0)	42.0 ± (36.0 -53.8)
Range	19.0-84.0	19.0-88.0
IgM (g/L)		
Mean±SD.	0.32 ± 0.10	0.68 ± 1.11
Median（IQR）	0.28 (0.26-0.33)	0.38 (0.33-0.52)
Range	0.25-0.66	0.28-8.7
Seropositive ratio (%)	0 (0/122)	11.3 (7/62)
IgG (g/L)		
Mean±SD.	0.15 ± 0.04	12.43 ± 12.49
Median（IQR）	0.14 (0.12-0.18)	7.95 (2.95-18.40)
Range	0.1-0.27	0.13-54.0
Seropositive ratio (%)	0 (0/122)	90.3 (56/62)

*no. (%), number (percentage); SD, standard deviation; IQR, interquartile range.

Table S2. Clinical data of the COVID-19 asymptomatic patients and healthy control.

Test ID	Sex	Age	IgG (g/L)	IgM (g/L)	Nucleic Acid Dection
1	Male	74			Negative
2	Male	73	13.95	1.04	Positive
3	Male	74			Negative
4	Male	34	0.13	0.27	Negative
5	Male	34			Negative
6	Male	34	2.80	0.33	Positive
7	Female	54			Negative
8	Female	54	1.89	0.31	Positive
9	Female	52			Negative
10	Female	57			Negative
11	Female	62			Negative
12	Female	62	22.19	0.77	Positive
13	Male	31			Negative
14	Male	31	0.22	0.34	Negative
15	Male	31	32.32	0.41	Positive
16	Female	36	7.34	0.29	Positive
17	Female	35			Negative
18	Female	35			Negative
19	Male	35			Negative
20	Male	37	0.10	0.26	Negative
21	Male	37	5.15	1.14	Positive
22	Female	46			Negative
23	Female	46			Negative
24	Female	46	53.98	0.36	Positive
25	Female	88	5.12	0.36	Positive
26	Female	91			Negative
27	Female	84			Negative
28	Female	54			Negative
29	Female	52			Negative
30	Female	54	34.04	0.86	Positive
31	Male	52			Negative
32	Male	53	19.42	0.39	Positive
33	Male	54	0.13	0.27	Negative
34	Male	60			Negative
35	Male	60	26.37	0.43	Positive
36	Male	61			Negative
37	Female	52			Negative
38	Female	52	6.17	0.30	Positive
39	Female	52			Negative
40	Female	35			Negative

Table S2. Clinical data of the COVID-19 asymptomatic patients and healthy control. (continued)

Test ID	Sex	Age	IgG(g/L)	IgM(g/L)	Nucleic Acid Dection
41	Female	35	8.95	0.30	Positive
42	Female	35	0.18	0.28	Negative
43	Female	41			Negative
44	Female	40	48.84	0.34	Positive
45	Female	38			Negative
46	Male	19	0.13	0.27	Negative
47	Male	19	0.14	0.66	Negative
48	Male	19	0.56	0.33	Positive
49	Female	56	0.17	0.30	Negative
50	Female	56			Negative
51	Female	58	2.65	0.38	Positive
52	Male	38	6.71	0.86	Positive
53	Male	39			Negative
54	Male	38	0.19	0.26	Negative
55	Female	32	17.71	0.33	Positive
56	Female	32			Negative
57	Female	32	0.16	0.28	Negative
58	Male	62	0.14	0.26	Negative
59	Male	63			Negative
60	Male	64	8.43	8.70	Positive
61	Male	43	0.13	0.28	Negative
62	Male	41			Negative
63	Male	42	0.13	0.33	Positive
64	Female	43			Negative
65	Female	43			Negative
66	Female	42	14.43	0.34	Positive
67	Female	25	4.55	0.33	Positive
68	Female	25	0.13	0.28	Negative
69	Female	25			Negative
72	Male	52			Negative
73	Male	40	0.12	0.44	Negative
74	Male	36			Negative
75	Male	39	18.49	1.37	Positive
76	Female	49			Negative
77	Female	49	8.50	0.46	Positive
78	Female	49			Negative
79	Female	63			Negative
80	Female	56			Negative
81	Female	59	39.72	0.33	Positive
82	Female	50			Negative
83	Female	50			Negative

Table S2. Clinical data of the COVID-19 asymptomatic patients and healthy control (continued).

Test ID	Sex	Age	IgG(g/L)	IgM(g/L)	Nucleic Acid Dection
84	Female	50	1.28	0.32	Positive
85	Female	23			Negative
86	Female	21	0.10	0.38	Negative
87	Female	21	10.01	0.38	Positive
88	Female	35			Negative
89	Female	36	3.67	0.42	Positive
90	Female	35			Negative
91	Female	45			Negative
92	Female	45			Negative
93	Female	45	9.87	0.34	Positive
94	Male	40			Negative
95	Male	42	0.13	0.28	Positive
96	Male	43	0.25	0.26	Negative
97	Female	43			Negative
98	Female	42	2.11	0.34	Positive
99	Female	42			Negative
100	Male	47			Negative
101	Male	51			Negative
103	Female	69			Negative
105	Female	68	7.93	0.35	Positive
106	Male	49	2.31	0.47	Positive
107	Male	46	0.10	0.26	Negative
108	Male	48			Negative
109	Female	43	0.10	0.31	Negative
111	Female	41	13.89	0.35	Positive
112	Male	38	28.15	0.42	Positive
113	Male	38			Negative
114	Male	37	0.16	0.26	Negative
115	Female	35			Negative
116	Female	35			Negative
117	Female	36	3.33	0.31	Positive
118	Female	38	0.17	0.30	Positive
119	Female	34			Negative
120	Female	38			Negative
121	Male	35	0.18	0.28	Negative
122	Male	35			Negative
124	Male	42	0.20	0.26	Negative
125	Male	42	12.04	0.53	Positive
126	Male	41			Negative
127	Male	57	0.16	0.28	Negative
128	Male	55	3.89	0.45	Positive
129	Male	52			Negative
130	Male	30			Negative

Table S2. Clinical data of the COVID-19 asymptomatic patients and healthy control (continued).

Test ID	Sex	Age	IgG(g/L)	IgM(g/L)	Nucleic Acid Dection
131	Male	30	0.11	0.26	Negative
132	Male	30	18.13	1.98	Positive
133	Male	34	0.10	0.38	Negative
134	Male	34	22.75	0.37	Positive
135	Male	34			Negative
136	Male	23	7.97	0.34	Positive
137	Male	28			Negative
138	Male	23	0.11	0.66	Negative
139	Male	51			Negative
141	Male	51	0.21	0.50	Negative
142	Male	44	0.19	0.37	Negative
143	Male	45	25.13	0.46	Positive
144	Male	45			Negative
145	Male	39			Negative
147	Male	39	10.90	1.04	Positive
148	Male	63			Negative
149	Male	64	34.68	1.11	Positive
150	Male	66			Negative
151	Female	32	4.29	0.30	Positive
152	Female	32			Negative
154	Female	38	4.81	0.32	Positive
155	Female	38			Negative
158	Male	56	0.21	0.28	Negative
161	Female	37			Negative
162	Female	37	11.66	0.32	Positive
163	Male	28			Negative
164	Male	28	0.27	0.28	Negative
165	Male	28	1.63	0.38	Positive
167	Male	21	0.18	0.33	Negative
168	Male	21	0.12	0.25	Negative
169	Female	48	30.16	2.75	Positive
170	Female	48			Negative
173	Female	52			Negative
175	Female	48			Negative
177	Female	48			Negative
178	Male	35			Negative
183	Male	42	0.14	0.25	Negative
184	Male	49	0.13	0.30	Positive
185	Male	49			Negative
186	Male	46			Negative
195	Female	40	18.08	0.43	Positive
197	Female	47			Negative

Table S2. Clinical data of the COVID-19 asymptomatic patients and healthy control (continued).

Test ID	Sex	Age	IgG(g/L)	IgM(g/L)	Nucleic Acid Dection
203	Male	22	6.26	0.77	Positive
216	Male	66	27.58	0.36	Positive
233	Female	27			Negative
239	Male	61	1.19	0.30	Positive
246	Female	43	2.86	0.30	Positive
249	Female	30			Negative
250	Male	72			Negative
252	Male	71	23.17	1.01	Positive
253	Male	40	4.13	1.25	Positive
259	Male	40			Negative
264	Male	51			Negative
265	Female	61			Negative
267	Female	62	0.17	0.67	Positive
269	Male	37	2.60	0.38	Positive
272	Female	33	3.23	0.49	Positive
273	Female	33	0.15	0.27	Negative
274	Female	55			Negative
275	Female	64			Negative

Table S3. ID of the carboxylic features screened by CIL-LC-MS strategy

ID	*m/z*	RI	Primary ID	*m/z*	RI
1	221.1652	695	49	315.2644	1172
2	227.2120	765	50	318.1817	755
3	231.2070	678	51	321.2539	977
4	235.1807	792	52	328.2961	1178
5	235.1809	869	53	328.2962	875
6	236.1252	678	54	330.2390	700
7	237.1963	817	55	336.2285	667
8	237.1963	831	56	336.3013	988
9	237.1963	845	57	336.3013	1005
10	239.2122	814	58	337.2486	1085
11	241.2276	900	59	341.3529	1700
12	241.2276	923	60	343.2956	1372
13	243.2434	1000	61	351.3009	1082
14	246.1605	601	62	355.3681	1800
15	249.2326	1121	63	356.3271	1367
16	255.2070	716	64	358.2703	804
17	255.2432	997	65	366.2752	979
18	257.2589	1100	66	367.2593	1145
19	259.2017	779	67	371.3057	1249
20	259.2383	926	68	372.2859	862
21	259.2384	864	69	382.2702	779
22	260.1760	657	70	382.3068	1308
23	265.2277	934	71	383.3269	1118
24	269.2590	1068	72	383.3272	982
25	269.2590	1094	73	385.2233	784
26	271.2380	984	74	385.285	1091
27	271.2744	1200	75	386.3014	919
28	273.2176	746	76	389.2187	718
29	276.2106	968	77	389.2187	748
30	285.2904	1300	78	391.3323	1276
31	286.2493	704	79	394.3065	1153
32	289.2639	1327	80	409.4156	2022
33	295.2744	1199	81	423.3582	1683
34	296.2696	946	82	440.1773	865
35	297.2901	1274	83	441.4054	2028
36	297.2901	1291	84	463.3896	1079
37	299.2330	998	85	463.3898	910
38	299.3058	1400	86	463.3898	964
39	300.2652	763	87	463.3899	1106
40	301.2489	1069	88	463.3901	980
41	307.2746	822	89	471.3585	1674
42	309.2174	939	90	479.3481	922
43	309.2174	954	91	479.3849	987
44	309.2174	970	92	487.4261	1310
45	311.2334	847	93	503.4208	1122
46	311.3059	1392	94	520.4110	1091
47	313.2488	1082	95	520.4111	958
48	313.3215	1500	96	541.4689	1066

Table S4. The qualitative results of features in asymptomatic and healthy serum with CIL-LC-MS strategy（Please see the .xlsx file named “Table S4-Quantitative analysis”）

Table S5. The identification and statistical analysis result of the discriminative features

ID	DMED labeled *m/z*	d_4-DMED labeled *m/z*	DMED labeled RT (min)	RI	Unlabeled molecular	Name	CAS	Database	MSI	*p* value	FC (AS/HC)	VIP	Difference rank
12	241.2276	245.2526	15.14	923	C10H18O2	9-Decenoic acid	14436-32-9	CLMD	1	3.13E-06	0.75	1.58	1
17	255.2432	259.2682	16.63	997	C11H20O2	Undecylenic acid	112-38-9	CLMD	1	1.05E-11	0.43	2.16	1
21	259.2384	263.2634	13.84	864	C10H20O3	3-Hydroxydecanoic acid	14292-26-3	CLMD	1	2.53E-06	0.60	1.63	1
78	391.3323	395.3573	21.23	1276	C20H32O3	12(R)-HETE	82337-46-0	CLMD	1	7.10E-11	0.43	2.24	1
91	479.3849	483.4099	16.43	987	C24H40O5	Cholic acid	81-25-4	CLMD	1	3.71E-03	1.80	0.96	2
95	520.4111	524.4361	15.85	958	C26H43O5N	GUDCA	64480-66-6	HMDB	1	8.29E-05	1.97	1.27	1
19	259.2017	263.2267	11.62	779	C9H16O4	3-(Hexanoyloxy)propanoic acid			2	7.00E-03	1.47	1.18	1
37	299.2330	303.2580	16.65	998	C12H20O4	3-(2-Enoyloxy)propanoic acid			2	1.39E-02	1.33	1.16	1
72	383.3272	387.3522	16.34	982	C18H32O4	15,16-DiHODE		HMDB	2	6.71E-07	2.41	1.86	1
3	231.2070	235.2320	7.80	678	C8H16O3	3-Hydroxyvalproic acid	58888-84-9	Metlin	3	5.32E-04	0.83	1.10	1
						4-Hydroxyvalproic acid	60113-82-8	Metlin					
						5-Hydroxyvalproic acid	53660-23-4	Metlin					
						2-hydroxy caprylic acid	617-73-2	Metlin					
						3-hydroxy caprylic acid	33796-86-0	Metlin					
						6-hydroxy caprylic acid	64165-18-0	Metlin					
						8-hydroxy caprylic acid	764-89-6	Metlin					
						3-hydroxy-isoheptanoic acid		Metlin					
						2-hydroxy-octanoic acid	617-73-2	Metlin					
						3-hydroxy-octanoic acid	33796-86-0	Metlin					
						4-hydroxy-octanoic acid	7779-55-7	Metlin					
						5-hydroxy-octanoic acid	17369-50-5	Metlin					
						7-hydroxy-octanoic acid	17173-14-7	Metlin					
						4-Butoxybutanoic acid	55724-73-7	Metlin					
4	235.1807	239.2057	12.05	792	C10H12O2	Benzenebutanoic acid	1821-12-1	CLMD	3	1.47E-02	1.12	1.02	3
						2,4-Decadiynoic acid		Metlin					
						4-Isopropylbenzoic acid	536-66-3	Metlin					
						3-Phenylbutyric acid	4593-90-2	Metlin					
						2-Phenylbutyric acid	90-27-7	Metlin					
						4-Phenylbutyric acid	1821-12-1	Metlin					
5	235.1809	239.2059	13.93	869	C10H12O2	2-(4-Ethylphenyl)acetic acid	14387-10-1	Metlin	3	1.30E-03	0.78	1.15	1
7	237.1963	241.2213	12.70	817	C10H14O2	Perillic acid	7694-45-3	Metlin	3	5.01E-02	1.23	1.14	3
						2,6,8-Decatrienoic acid	94450-21-2	Metlin					
						Myrtenic acid	19250-17-0	Metlin					
8	237.1963	241.2213	13.03	831	C10H14O2	Perillic acid	7694-45-3	Metlin	3	1.24E-03	1.36	1.30	1
						2,6,8-Decatrienoic acid	94450-21-2	Metlin					
						Myrtenic acid	19250-17-0	Metlin					
10	239.2122	243.2372	12.62	814	C10H16O2	cis, cis-stillingic acid		Metlin	3	3.60E-02	1.03	1.05	3
						2,4-decadienoic acid	63889-75-8	Metlin					
						trans, cis-stillingic acid		Metlin					
						2,6-decadienoic acid		Metlin					
						4E,6E-decadienoic acid	60388-65-0	Metlin					

Table S5. The identification and statistical analysis result of the discriminative features (continued)

ID	DMED labeled *m/z*	d_4-DMED labeled *m/z*	DMED labeled RT (min)	RI	Unlabeled molecular	Name	CAS	Database	MSI	*p* value	FC (AS/HC)	VIP	Difference rank
10	239.2122	243.2372	12.62	814	C10H16O2	Aleprestic acid		Metlin	3	3.60E-02	1.03	1.05	3
						2,5-decadienoic acid		Metlin					
						2E,7Z-decadienoic acid		Metlin					
						3,5-decadienoic acid		Metlin					
						4,8-decadienoic acid		Metlin					
						4E,9-decadienoic acid		Metlin					
						5E,9-decadienoic acid		Metlin					
						5E,8E-decadienoic acid		Metlin					
						6E,8E-decadienoic acid		Metlin					
						7,9-decadienoic acid		Metlin					
						Chrysanthemic acid		Metlin					
13	243.2434	247.2684	16.70	1000	C10H20O2	Capric acid	334-48-5	Metlin	3	1.56E-02	1.19	1.14	3
						2-methyl nonaoic acid		Metlin					
						8-methyl Nonanoic acid		Metlin					
						3-methyl-nonanoic acid		Metlin					
						7-methyl-nonanoic acid		Metlin					
						8-methyl-nonanoic acid		Metlin					
						4-Methylnonanoic acid	45019-28-1	Metlin					
						4-Ethyloctanoic acid	16493-80-4	Metlin					
16	255.2070	259.2320	9.60	716	C10H16O3	(3R)-3-isopropenyl-6-oxoheptanoic acid		Metlin	3	6.77E-02	0.77	1.03	3
						(3S)-3-isopropenyl-6-oxoheptanoic acid		Metlin					
						9-oxo-2E-decenoic acid		Metlin					
						9-oxo-2Z-decenoic acid		Metlin					
						5-oxo-7E-decenoic acid		Metlin					
						(1'R)-Nepetalic acid	21651-54-7	Metlin					
						(S)-Oleuropeic acid	5027-76-9	Metlin					
						(E)-10-Oxo-8-decenoic acid	69152-89-2	Metlin					
						Nepetalic acid		Metlin					
24	269.2590	273.2840	17.89	1068	C12H22O2	5-methyl-2-undecenoic acid		Metlin	3	7.94E-07	0.39	1.55	1
						2-lauroleic acid		Metlin					
						Linderic acid		Metlin					
						6-lauroleic acid		Metlin					
						7-lauroleic acid		Metlin					
						9-lauroleic acid		Metlin					
						10-Lauroleic acid		Metlin					
						11-lauroleic acid		Metlin					
						5-dodecenoic acid	2430-94-6	Metlin					
33	295.2744	299.2994	19.99	1199	C14H24O2	13-Tetradecynoic acid	82909-47-5	Metlin	3	2.30E-11	0.31	2.18	1
						9-Tetradecynoic acid	55182-92-8	Metlin					
						8-Tetradecynoic acid	55182-90-6	Metlin					
						7-Tetradecynoic acid	55182-86-0	Metlin					

Table S5. The identification and statistical analysis result of the discriminative features (continued)

ID	DMED labeled *m/z*	d_4-DMED labeled *m/z*	DMED labeled RT (min)	RI	Unlabeled molecular	Name	CAS	Database	MSI	*p* value	FC (AS/HC)	VIP	Difference rank
33	295.2744	299.2994	19.99	1199	C14H24O2	3-Tetradecynoic acid	55182-76-8	Metlin	3	2.30E-11	0.31	2.18	1
						5Z,8Z-tetradecadienoic acid	39039-37-7	Metlin					
						2E,4E-tetradecadienoic acid	29826-00-4;	Metlin					
						Alepric acid	2519-24-6	Metlin					
						3Z,5Z-tetradecadienoic acid	25091-12-7	Metlin					
						3Z,5E-tetradecadienoic acid	23400-52-4	Metlin					
						3,4-tetradecadienoic acid	112146-20-0	Metlin					
						5,8-tetradecadienoic acid	10390-16-6	Metlin					
						10Z,12E-tetradecadienoic acid		Metlin					
						4-Tetradecynoic acid		Metlin					
						5-Tetradecynoic acid		Metlin					
						6-Tetradecynoic acid		Metlin					
						10-Tetradecynoic acid		Metlin					
						11-Tetradecynoic acid		Metlin					
						12-Tetradecynoic acid		Metlin					
						14:2(6,9)		Metlin					
40	301.2489	305.2739	17.92	1069	C12H22O4	Dodecanedioic acid	693-23-2	Metlin	3	8.78E-02	1.26	1.12	3
						Octanedioic acid, 2,2,7,7-tetramethyl-		Metlin					
42	309.2174	313.2424	15.48	939	C13H18O4	3-(1-hydroxy-4-phenylbutoxy)propanoic acid			3	7.71E-02	1.25	1.14	3
43	309.2174	313.2424	15.78	954	C13H18O4	3-(1-hydroxy-4-phenylbutoxy)propanoic acid			3	1.34E-01	1.18	1.11	3
44	309.2174	313.2424	16.09	970	C13H18O4	3-(1-hydroxy-4-phenylbutoxy)propanoic acid			3	1.45E-02	1.36	1.22	1
47	313.2488	317.2738	18.16	1082	C13H22O4	7-(2-Formyl-5-hydroxycyclopentyl)heptanoic acid	55502-82-4	Metlin	3	2.37E-02	0.36	1.15	1
						2,7-Diethyl-4-methyloct-4-enedioic acid	55005-88-4	Metlin					
						2-(Non-1-en-1-yl)butanedioic acid	28299-28-7	Metlin					
						2-Carboxy-4-dodecanolide		Metlin					
49	315.2644	319.2894	19.58	1172	C13H24O4	Isooctyl hydrogen glutarate	94248-70-1	Metlin	3	3.94E-02	1.32	1.07	1
						2,4-Dibutylpentanedioic acid	92155-85-6	Metlin					
						6,6-Dimethylundecanedioic acid	89131-65-7	Metlin					
						3-(2,4-Dimethylpentan-2-yl)hexanedioic acid	88438-11-3	Metlin					
						2-Octylpentanedioic acid	59039-06-4	Metlin					
						8-(Tetrahydro-2H-pyran-2-yloxy)octanoic acid	54699-43-3	Metlin					
						2-(Decanoyloxy)propanoic acid	51119-25-6	Metlin					
						Decylmalonic acid	4372-29-6	Metlin					
						Dodecanedioic acid monomethyl ester	3903-40-0	Metlin					
						Nonanedioic acid, 2,2,8,8-tetramethyl-	30313-67-8	Metlin					
						2-methyl-dodecanedioic acid		Metlin					

Table S5. The identification and statistical analysis result of the discriminative features (continued)

ID	DMED labeled *m/z*	d_4-DMED labeled *m/z*	DMED labeled RT (min)	RI	Unlabeled molecular	Name	CAS	Database	MSI	*p* value	FC (AS/HC)	VIP	Difference rank
						3-methyl-dodecanedioic acid		Metlin					
						4-methyl-dodecanedioic acid		Metlin					
						6-methyl-dodecanedioic acid		Metlin					
58	337.2486	341.2736	18.20	1085	C15H22O4	Xanthoxic acid		Metlin	3	8.15E-01	0.88	1.00	3
						4'-Dihydroabscisic acid	84026-26-6	Metlin					
61	351.3009	355.3259	18.16	1082	C17H28O3	12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid	54397-84-1	Metlin	3	6.22E-07	0.54	1.90	1
						12-hydroxy-5,8,10-heptadecatrienoic acid	50683-78-8	Metlin					
						(13S)-13-Hydroxyheptadeca-9,11,15-trienoic acid	168781-98-4	Metlin					
90	479.3481	483.3731	15.13	922	C23H36O6	(3R,5R)-3,5-Dihydroxy-7-{(1S,2S,8S,8aR)-2-methyl-8-[(2-methylbutanoyl)oxy]-1,2,6,7,8,8a-hexahydro-1-naphthalenyl}heptanoic acid		Metlin	3	1.95E-04	0.40	1.31	1
39	300.2652	304.2902	11.12	763	C12H23O3N				4	2.32E-02	1.08	1.04	3
50	318.1817	322.2067	10.85	755	C13H13O4N				4	1.43E-01	1.29	1.00	3
53	328.2962	332.3212	14.09	875	C14H27O3N				4	1.98E-02	1.11	1.19	3
54	330.2390	334.2640	9.07	700	C12H21O5N				4	4.27E-02	1.28	1.15	1
55	336.2285	340.2535	7.13	667	C14H19O4N				4	7.56E-03	0.69	0.81	2
64	358.2703	362.2953	12.38	804	C14H25O5N				4	7.12E-02	1.24	1.14	3
65	366.2752	370.3002	16.28	979	C16H25O4N				4	1.36E-02	0.64	0.88	2
68	372.2859	376.3109	13.77	862	C15H27O5N				4	5.82E-02	1.30	1.12	3
69	382.2702	386.2952	11.61	779	C16H25O5N				4	4.26E-02	0.81	0.75	2
73	385.2233	389.2483	11.78	784	C17H18O4N2				4	1.36E-04	0.76	1.13	1
75	386.3014	390.3264	15.07	919	C16H29O5N				4	4.12E-02	1.29	1.18	1
76	389.2187	393.2437	9.66	718	C16H18O5N2				4	1.73E-02	0.79	0.82	2
77	389.2187	393.2437	10.62	748	C16H18O5N2				4	1.31E-02	0.80	0.88	2
92	487.4261	491.4511	21.77	1310	C22H44O5N2				4	3.78E-05	0.75	1.15	1
93	503.4208	507.4458	18.80	1122	C22H44O6N2				4	4.19E-05	0.80	1.30	1
96	541.4689	545.4939	17.87	1066	C26H50O5N2				4	1.36E-01	1.12	1.03	3

* The 21 significant variables irrelevant to age and gender were marked as red.

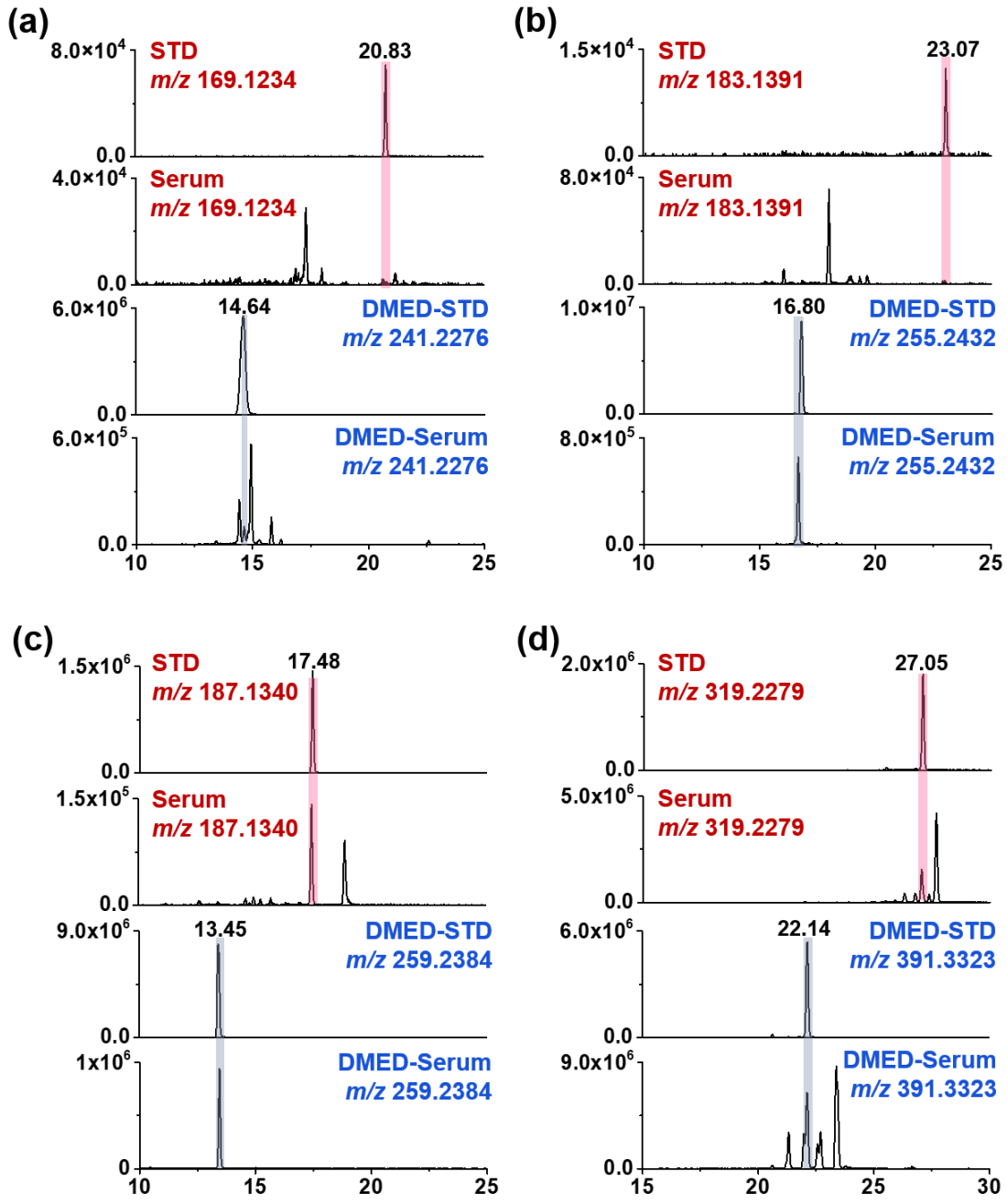

Figure S1. Comparation on the sensitivity of regular untargeted method and CIL-LC-MS method. Extracted ion chromatograms of unlabeled standard (STD), unlabeled serum (Serum), DMED labeled standard (DMED-STD) and DMED labeled standard (DMED-Serum) for (a) 9-decenoic acid; (b) undecylenic acid; (c) 3-hydroxydecanoic acid; (d) 12-HETE.

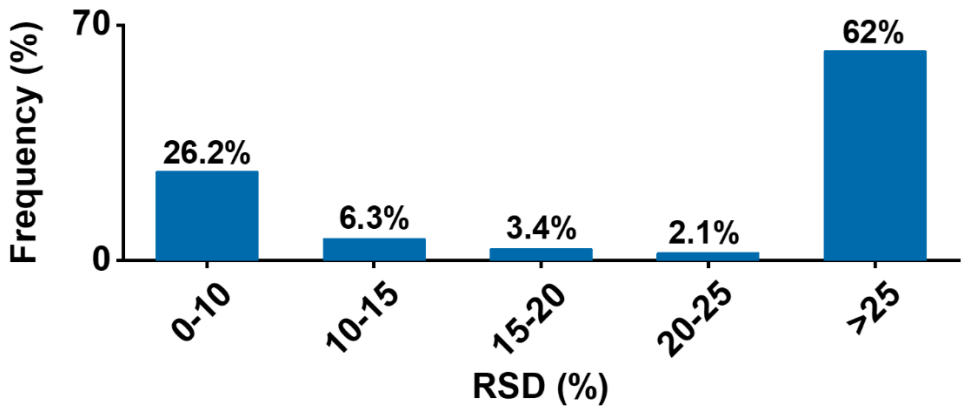

Figure S2. Frequency histogram of RSDs for metabolites in serum that detected by regular untargeted method.

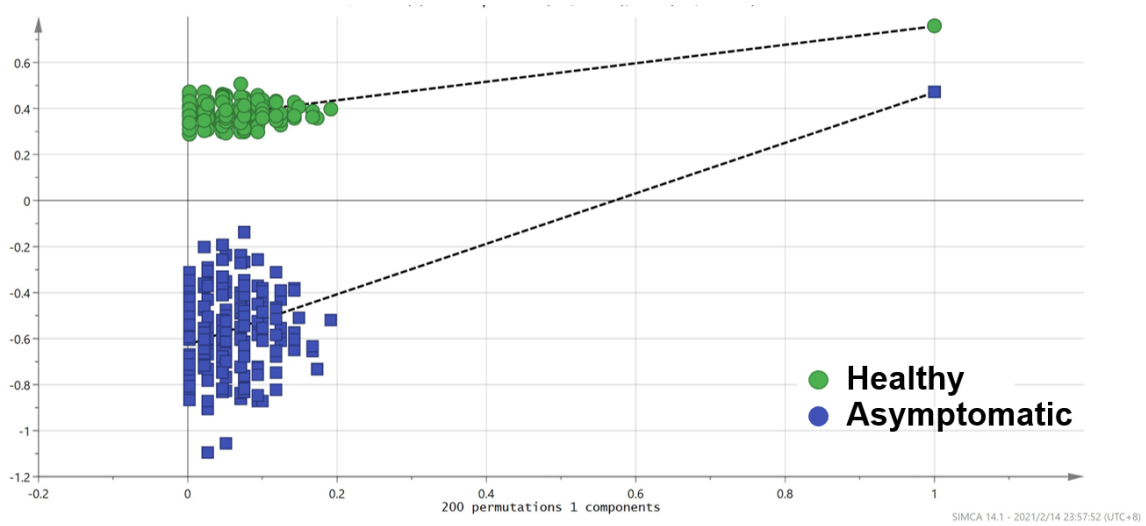

Figure S3. The validation plot for the OPLS-DA models.

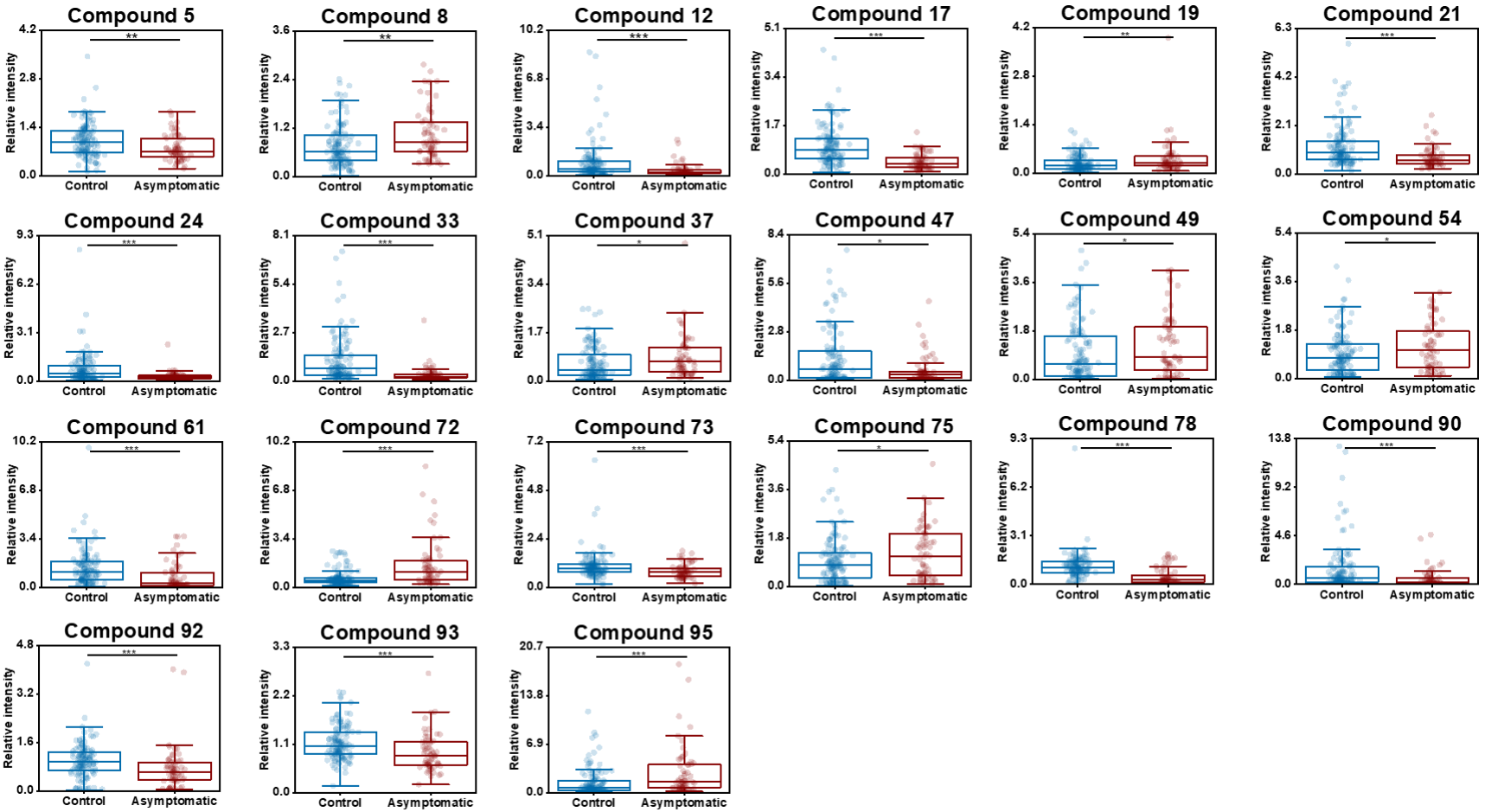

Figure S4. Boxplot of 21 discriminative features between healthy control and asymptomatic patient group. *P* value was calculated by Kruskal-Wallis test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

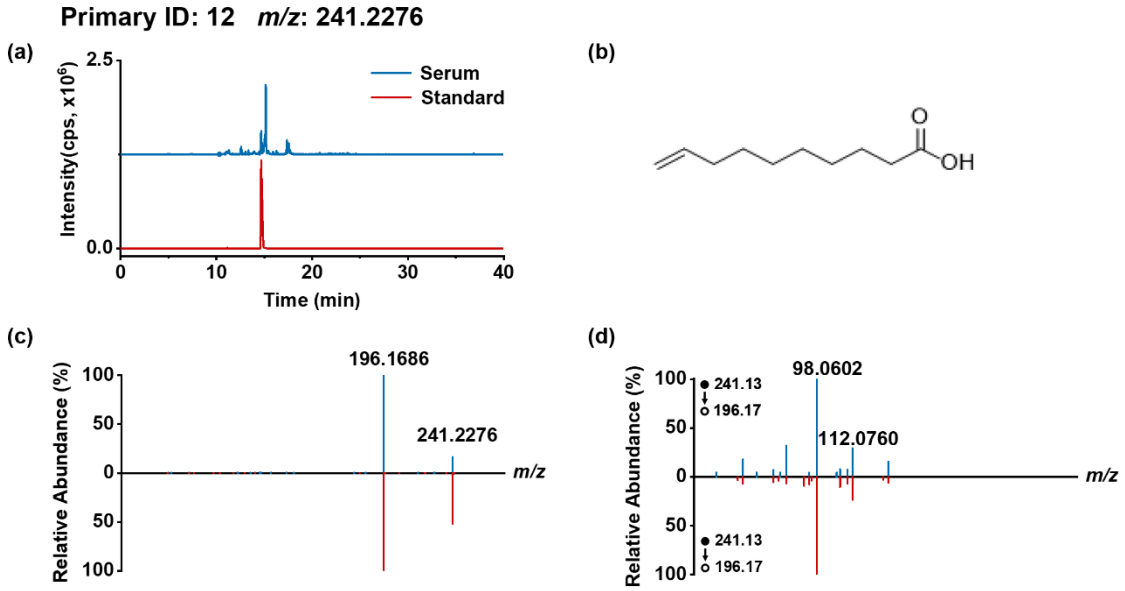

Figure S5. Chromatogram and MS^n spectra of 9-decenoic acid. (a) The XIC of 9-decenoic acid in serum sample and in standard solution; (b)The chemical structure of 9-decenoic acid; (c) The MS^2 spectrum of 9-decenoic acid in serum sample and in standard solution; (d) The MS^3 spectrum of fragment ion *m/z* 196.17 (241.13-196.17) in serum sample and in standard solution.

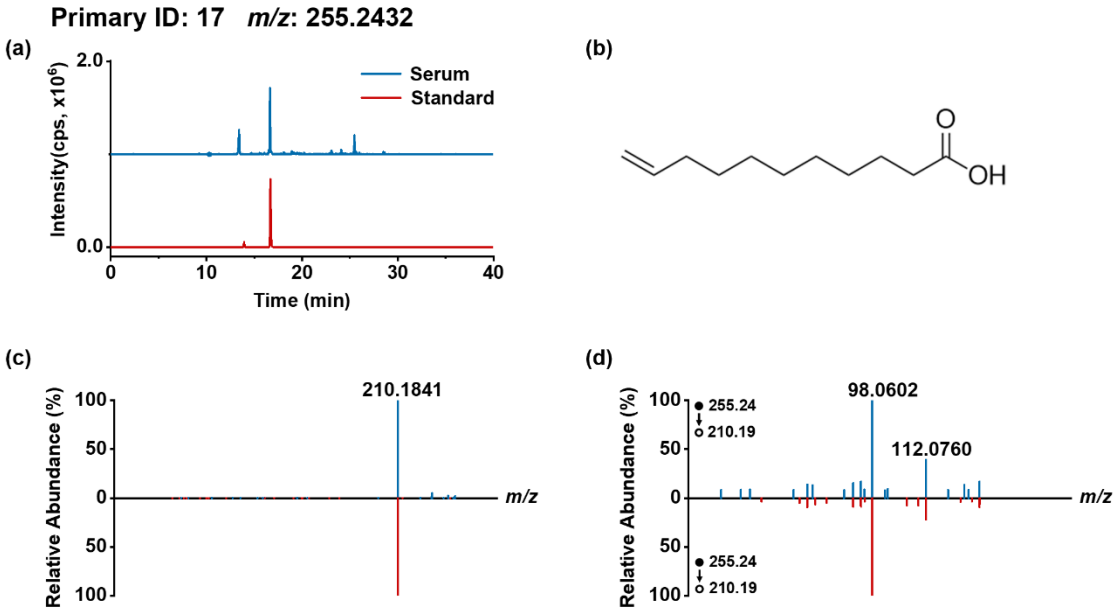

Figure S6. Chromatogram and MS^n spectra of undecylenic acid. (a) The XIC of undecylenic acid in serum sample and in standard solution; (b)The chemical structure of undecylenic acid; (c) The MS^2 spectrum of undecylenic acid in serum sample and in standard solution; (d) The MS^3 spectrum of fragment ion *m/z* 210.19 (255.24-210.19) in serum sample and in standard solution.

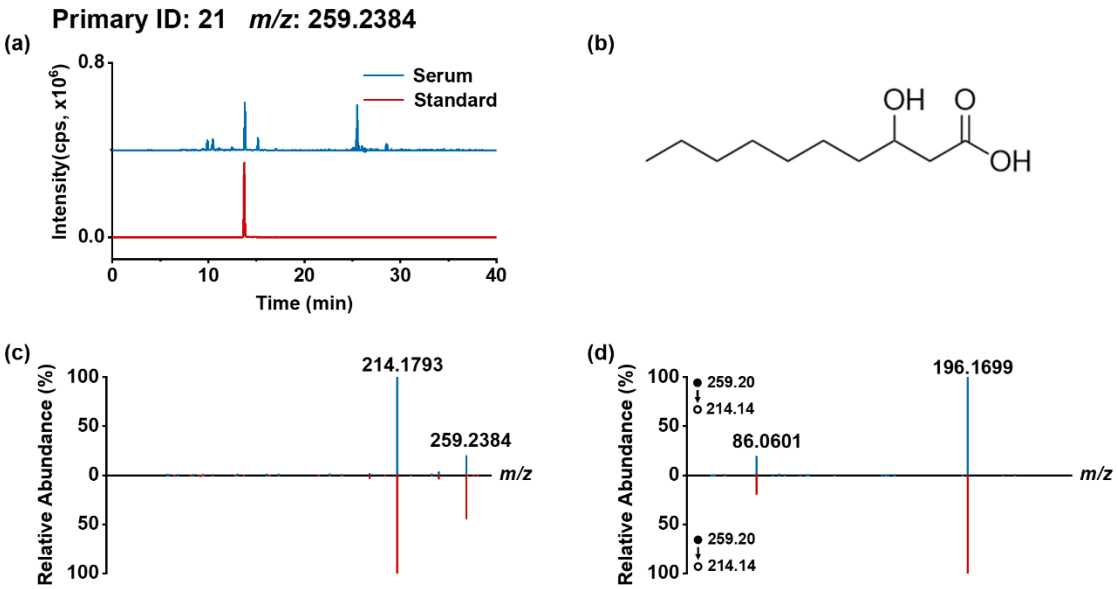

Figure S7. Chromatogram and MS^n spectra of 3-hydroxydecanoic acid. (a) The XIC of 3-hydroxydecanoic acid in serum sample and in standard solution; (b)The chemical structure of 3-hydroxydecanoic acid; (c) The MS^2 spectrum of 3-hydroxydecanoic acid in serum sample and in standard solution; (d) The MS^3 spectrum of fragment ion *m/z* 214.14 (259.20-214.14) in serum sample and in standard solution.

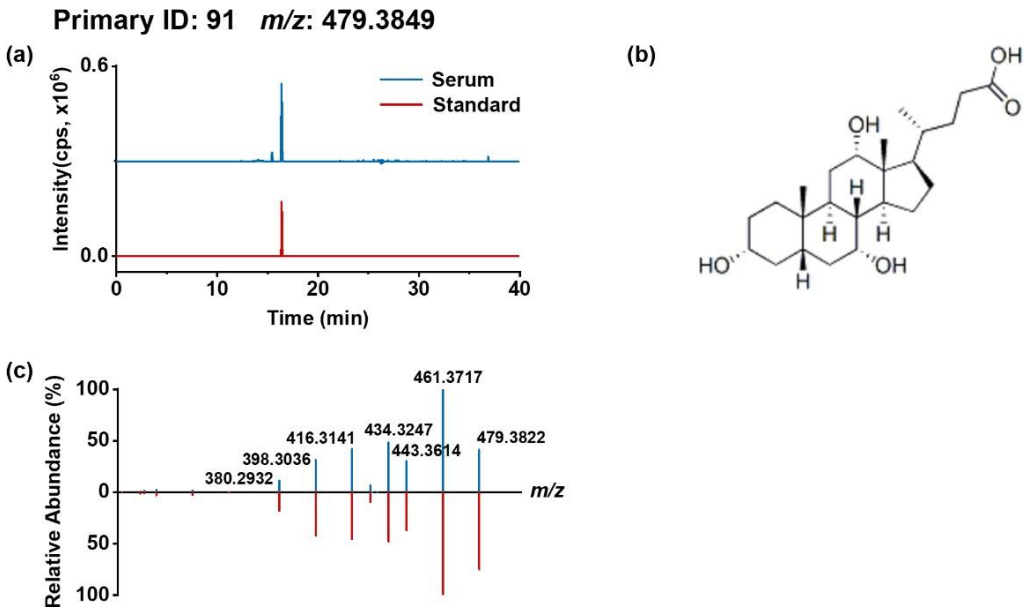

Figure S8. Chromatogram and MS^n spectra of cholic acid. (a) The XIC of cholic acid in serum sample and in standard solution; (b)The chemical structure of cholic acid; (c) The MS^2 spectrum of cholic acid in serum sample and in standard solution.

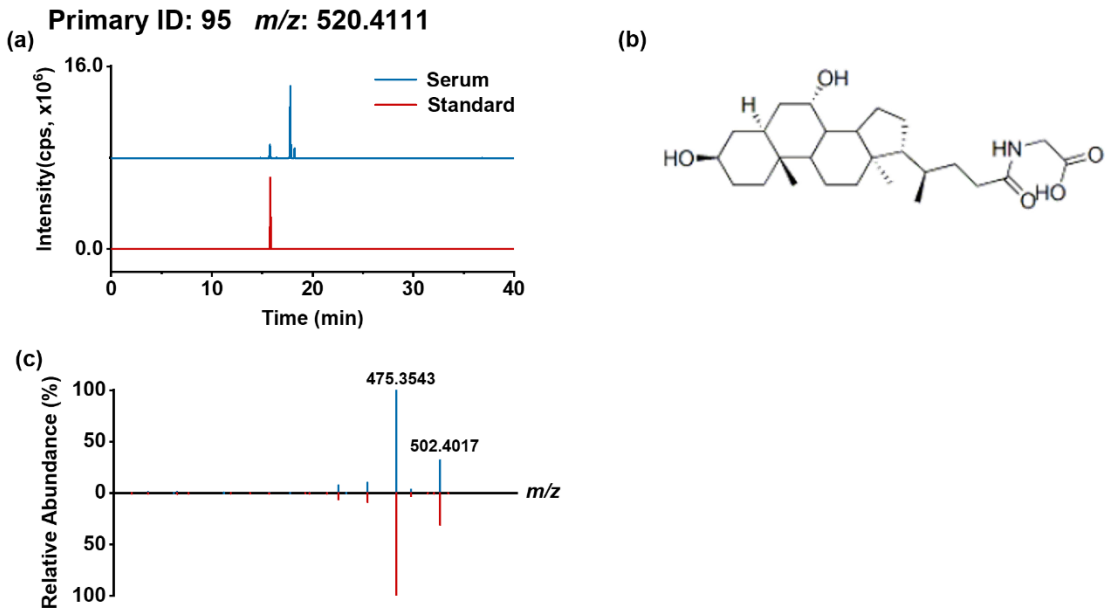

Figure S9. Chromatogram and MS^n spectra of GUDCA. (a) The XIC of GUDCA in serum sample and in standard solution; (b)The chemical structure of GUDCA; (c) The MS^2 spectrum of GUDCA in serum sample and in standard solution.

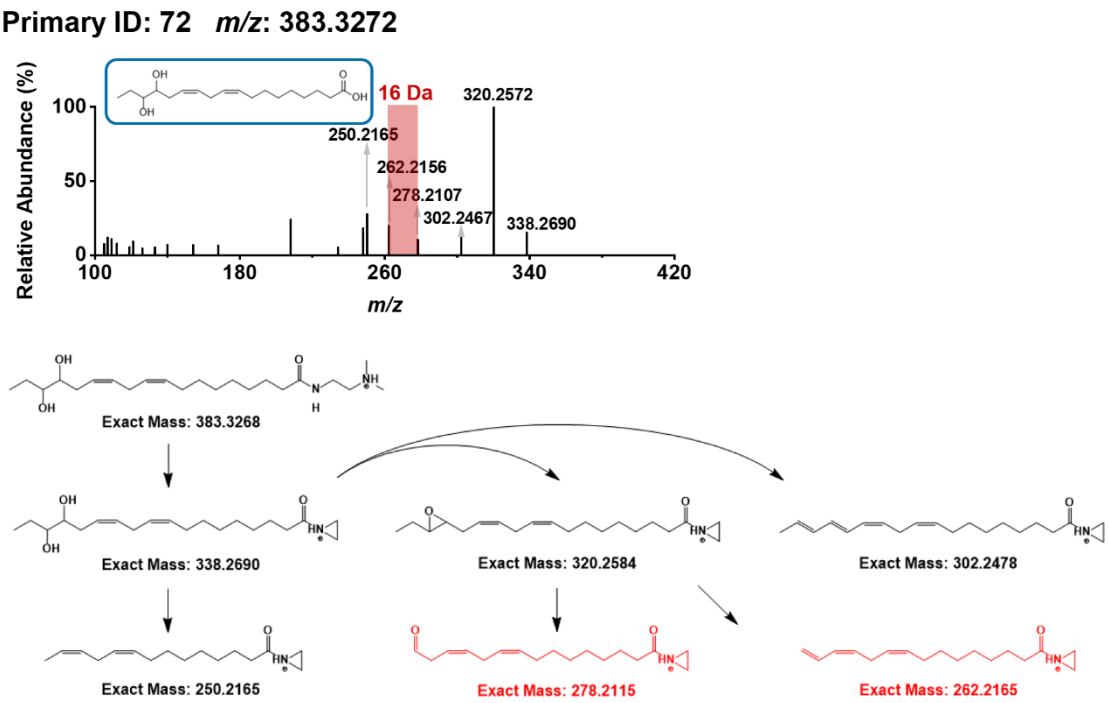

Figure S10. MS^n spectra and fragmentation pattern of 15,16-DiHODE in serum. (a) The spectrum of *m/z* 383.3268; (b) The possible fragmentation pathways.

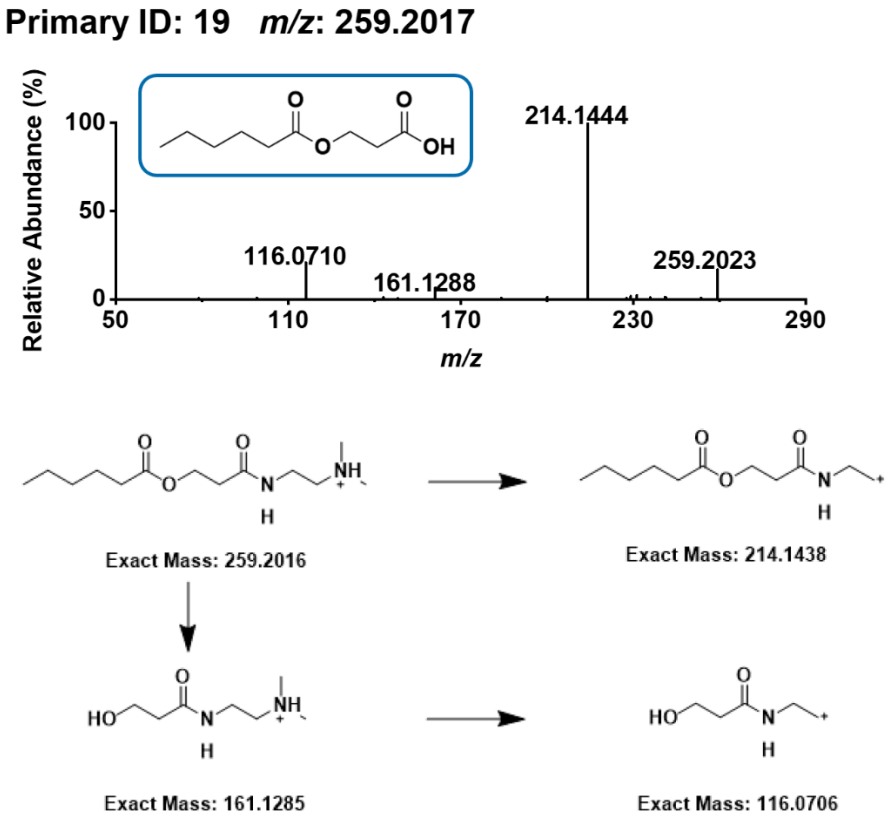

Figure S11. MS^n spectra and fragmentation pattern of 3-(hexanoyloxy)propanoic acid in serum. (a) The MS^2 spectrum of *m/z* 259.2017; (b) The possible fragmentation pathways.

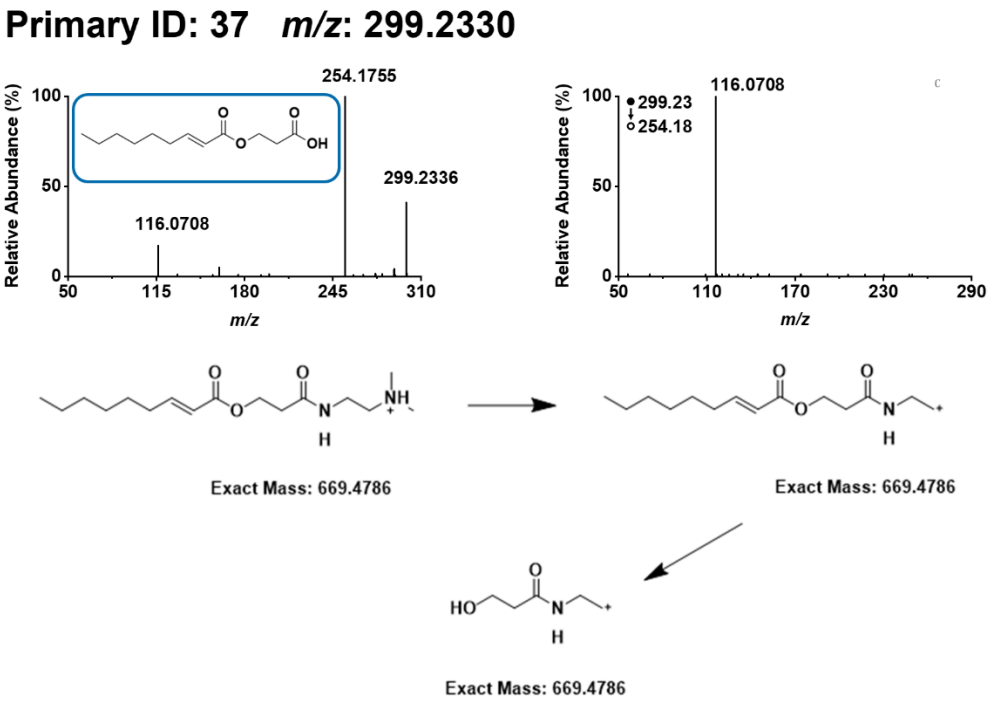

Figure S12. MS^n spectra and fragmentation pattern of 3-(2-enoyloxy) propanoic acid in serum. (a) The MS^2 spectrum of *m/z* 299.2330; (b) The MS^3 spectrum of fragment ion *m/z* 254.18 (299.23-254.18); (c) The possible fragmentation pathways.