

Supporting Information

Integrative Proteomics and Metabolomics Analysis Reveals the Toxicity of Cationic Liposomes to Human Normal Hepatocyte Cell Line L02

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Figure S1. Representative total ion current chromatograms of significantly changed metabolites in positive and negative ion mode using the two kinds of columns. The labels of A, B were obtained from the T3 column in positive and negative ion mode, respectively; with the labels of C, D from the Amide column in positive and negative mode, respectively.

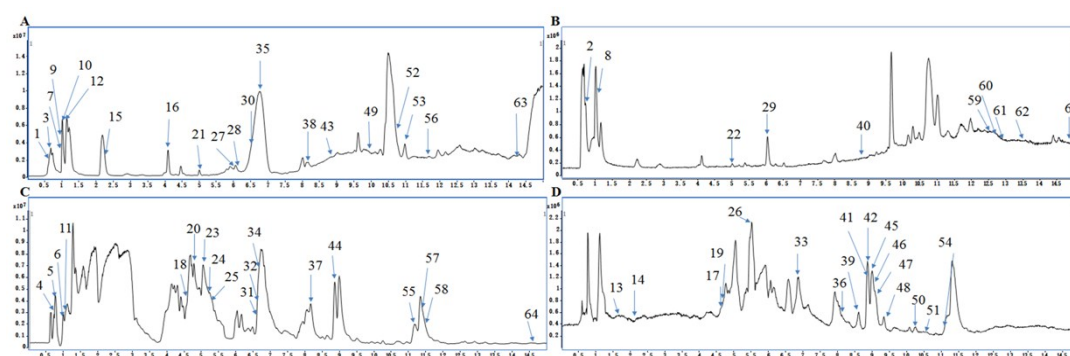


Figure S2. Hierarchical clustering and the heatmap based on the 65 metabolites showed significant differences between CLs and control groups from metabolomics data.

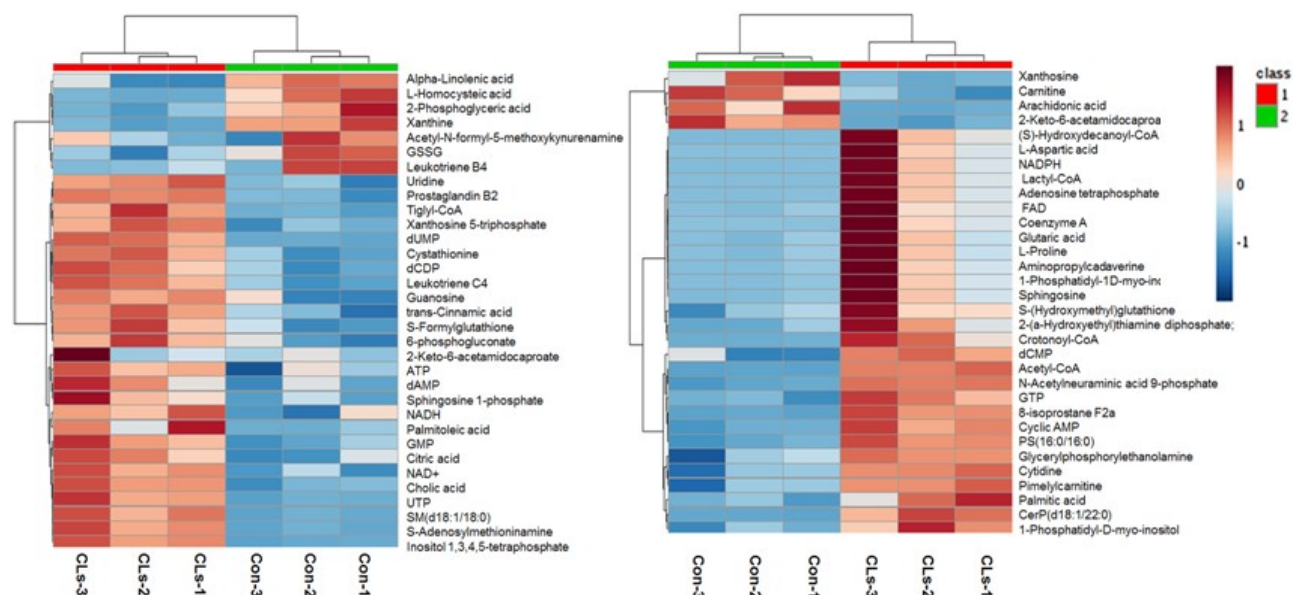


Table S1. Identification details of the metabolites that displayed significant changes in the control and the CLs groups by UHPLC-Q-TOF/MS

No.	RT (min)	VIP	p-val	m/z	Column	Ion	Metabolites	FC(CLs/C)
1	0.6892	1.503	0.046	337.9483	T3	M+H	Leukotriene B4	-0.027
2	0.7036	0.876	0.015	160.11	T3	M-H	Carnitine	-0.37
3	0.736	1.552	0.003	309.0783	T3	M+H	dUMP	1.761
4	0.7561	1.419	0.011	330.0575	AMIDE	M+H	Cyclic AMP	3.016
5	0.8046	2.61	0.026	786.6012	AMIDE	M+H	FAD	24.62
6	1.0143	1.261	0.035	470.1286	AMIDE	M+H	2-(α -Hydroxyethyl) thiamine diphosphate	1.652
7	1.0219	1.457	0.015	613.1449	T3	M+H	GSSG	-8.279
8	1.024	4.529	0.012	306.0908	T3	M-H	dCMP	10.143
9	1.033	1.252	0.043	666.1099	T3	M+H	NADH	0.696
10	1.063	1.373	0.017	193.0187	T3	M+H	Citric acid	2.091
11	1.087	1.402	0.031	153.0251	AMIDE	M+H	Xanthine	-0.13
12	1.159	1.336	0.021	364.1382	T3	M+H	GMP	0.068
13	1.621	2.441	0	61.9887	AMIDE	M-H	Carbonic acid	2.923
14	2.085	3.022	0	388.2526	AMIDE	M-H	N-Acetylneuraminic acid 9-phosphate	4.106
15	2.481	2.824	0.004	524.3719	T3	M+H	GTP	4.445

16	4.116	1.213	0.047	409.1717	T3	M+H	Cholic acid	1.274
17	4.649	1.179	0	848.6473	AMIDE	M-H	Tiglyl-CoA	0.708
18	4.685	13.21	0.045	134.1176	AMIDE	M+H	L-Aspartate	638.242
19	4.725	2.155	0	303.0834	AMIDE	M-H	Arachidonic acid	-2.35
20	4.897	1.104	0.006	836.1088	AMIDE	M+H	Crotonoyl-CoA	2.169
21	4.991	1.519	0.036	265.0152	T3	M+H	Acetyl-N-formyl-5-methoxykynurenamine	-0.065
22	4.992	1.658	0.012	185.0378	T3	M-H	2-Phosphoglyceric acid	-0.05
23	5.05	1.272	0.002	938.1697	AMIDE	M+H	(S)-Hydroxydecanoyl-CoA	4.07
24	5.193	1.39	0.036	768.4021	AMIDE	M+H	Coenzyme A	6.502
25	5.207	1.457	0.049	188.1123	AMIDE	M+H	2-Keto-6-acetamidocaproate	0.045
26	5.577	2.245	0.021	283.0689	AMIDE	M-H	Xanthosine	-2.462
27	6.038	1.722	0.001	356.0513	T3	M+H	S-Adenosylmethioninamine	5.967
28	6.041	1.709	0.001	732.0897	T3	M+H	SM(d18:1/18:0)	0.547
29	6.056	2.398	0	353.0642	T3	M-H	8-isoprostane F2a	2.379
30	6.516	1.634	0.003	332.1923	T3	M+H	dAMP	0.196
31	6.692	1.051	0.025	745.7027	AMIDE	M+H	NADPH	3.593
32	6.719	1.245	0.022	587.0449	AMIDE	M+H	Adenosine tetraphosphate	4.793
33	6.787	1.942	0	182.0558	AMIDE	M-H	L-Homocysteic acid	-2.033
34	6.788	1.25	0.025	840.7702	AMIDE	M+H	Lactyl-CoA	5.032

35	6.803	1.707	0.001	485.1685	T3	M+H	UTP	0.311
36	8.152	1.207	0.006	386.0432	AMIDE	M-H	dCDP	0.811
37	8.232	1.861	0	500.2886	AMIDE	M+H	Inositol 1,3,4,5-tetrphosphate	2.766
38	8.315	1.758	0	380.2848	T3	M+H	Sphingosine 1-phosphate	0.045
39	8.584	1.229	0.006	275.0888	AMIDE	M-H	6-phosphogluconate	0.905
40	8.857	6.074	0.01	302.1015	T3	M-H	Pimelylcarnitine	23.452
41	8.857	3.472	0.017	242.0805	AMIDE	M-H	Cytidine	8.117
42	8.857	1.013	0.014	243.0835	AMIDE	M-H	Uridine	0.675
43	8.88	1.827	0.006	255.1429	T3	M+H	Palmitoleic acid	0.043
44	8.897	2.028	0.024	471.1592	AMIDE	M+H	1-Phosphatidyl-1D-myo-inositol 3-phosphate	15.913
45	9.022	2.116	0	808.273	AMIDE	M-H	Acetyl-CoA	1.986
46	9.101	4.742	0.005	214.0489	AMIDE	M-H	Glycerolphosphorylethanolamine	12.328
47	9.225	1.376	0.004	282.0651	AMIDE	M-H	Guanosine	1.049
48	9.346	1.63	0.002	624.1823	AMIDE	M-H	Leukotriene C4	1.538
49	9.992	1.614	0.002	665.3125	T3	M+H	NAD ⁺	0.258
50	10.33	1.757	0.033	333.0594	AMIDE	M-H	Prostaglandin B2	2.233
51	10.56	1.133	0.008	334.1258	AMIDE	M-H	S-Formylglutathione	0.79
52	10.88	1.595	0.005	279.1436	T3	M+H	Alpha-Linolenic acid	-0.121

53	11.02	1.28	0.032	508.3249	T3	M+H	ATP	0.278
54	11.03	1.497	0.007	221.0602	AMIDE	M-H	Cystathionine	1.359
55	11.32	1.18	0.019	300.3377	AMIDE	M+H	Sphingosine	5.131
56	11.51	1.915	0	524.2831	T3	M+H	Xanthosine 5-triphosphate	4.546
57	11.52	1.281	0.045	116.0708	AMIDE	M+H	L-Proline	7.775
58	11.53	1.738	0.043	133.0974	AMIDE	M+H	Glutaric acid	14.122
59	12.53	3.599	0	389.2858	T3	M-H	1-Phosphatidyl-D-myo-inositol	5.298
60	12.54	1.733	0	147.0238	T3	M-H	Trans-Cinnamic acid	1.212
61	12.89	2.023	0.006	700.5618	T3	M-H	CerP(d18:1/22:0)	1.809
62	13.48	1.824	0.012	336.3422	T3	M-H	S-(Hydroxymethyl)glutathione	1.644
63	14.39	1.949	0	257.2312	T3	M+H	Palmitic acid	1.001
64	14.45	1.002	0.031	160.1811	AMIDE	M+H	Aminopropylcadaverine	4.247
65	14.94	1.164	0.001	734.5755	T3	M-H	PS(16:0/16:0)	0.59

CLs, Cationic liposomes group; C, Control group.

Fold change was calculated as the logarithm of the average mass response (area) ratio between the two groups (fold change = \log_2 [CLs/C]).

Table S2. Differentially expressed proteins in the CLs group identified by the iTRAQ based quantitative proteomics approach.

Accession	Protein description	FC	p-val	Peptides	Unused	Gene Name
sp P23528	Cofilin-1	0.099	0.0408	233	55.98	CFL1
sp P13639	Elongation factor 2	0.103	0.0002	19	173.91	EEF2
sp P08238	Heat shock protein HSP 90-beta	0.184	0.0017	23	167.69	HSP90AB1
sp P30085	UMP-CMP kinase	0.192	0.0002	836	21.99	CMPK1
sp P68104	Elongation factor 1-alpha 1	0.198	0.0029	34	136.18	EEF1A1
sp P17987	T-complex protein 1 subunit alpha	0.211	0.0003	117	81.57	TCP1
sp P60842	Eukaryotic initiation factor 4A-I	0.265	0.0066	141	74.18	EIF4A1
sp P23588	Eukaryotic translation initiation factor 4B	0.305	0.0001	242	55.13	EIF4B
sp P50991	T-complex protein 1 subunit delta	0.305	0.0003	142	74.03	CCT4
sp P78371	T-complex protein 1 subunit beta	0.305	0.0002	83	96.04	CCT2
sp P31153	S-adenosylmethionine synthase isoform type-2	0.308	0.0023	933	19.8	MAT2A
sp P61081	NEDD8-conjugating enzyme Ubc12	0.347	0.0023	1279	13.69	UBE2M
sp P30520	Adenylosuccinate synthetase isozyme 2	0.356	0.0037	468	34.88	ADSS
sp P06493	Cyclin-dependent kinase 1	0.402	0.0095	533	31.7	CDK1
sp Q14376	UDP-glucose 4-epimerase	0.406	0.0119	1409	12.14	GALE
sp Q13347	Eukaryotic translation initiation factor 3 subunit I	0.409	0.0228	484	34.03	EIF3I

sp Q15181	Inorganic pyrophosphatase	0.413	0.0006	410	38.65	PPA1
sp Q04760	Lactoylglutathione lyase	0.421	0.0045	1590	10.17	GLO1
sp Q04637	Eukaryotic translation initiation factor 4 gamma 1	0.425	0.0032	115	82.5	EIF4G1
sp P15170	CBR1	0.437	0.0282	322	44.84	GSPT1
sp P46779	60S ribosomal protein L28	0.441	0.0211	901	20.43	RPL28
sp P15531	Nucleoside diphosphate kinase A	0.461	0.004	328	44.61	NME1
sp P30044	Peroxiredoxin-5, mitochondrial	0.483	0.0038	192	62.96	PRDX5
sp P19623	Spermidine synthase	0.488	0.0113	849	21.64	SRM
sp O95340	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2	0.53	0.0382	330	44.46	PAPSS2
sp Q01581	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	0.545	0.0261	273	50.56	HMGCS1
sp P13929	Beta-enolase	0.555	0.0136	446	36.46	ENO3
sp P10809	60 kDa heat shock protein, mitochondrial	0.565	0.0051	29	148.94	HSPD1
sp P11586	C-1-tetrahydrofolate synthase, cytoplasmic	0.586	0.0398	97	89.56	MTHFD1
sp P60174	Triosephosphate isomerase	0.619	0.0332	87	95.3	TPI1
sp Q06210	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 1	0.619	0.0139	276	50.03	GFPT1
sp P16152	Carbonyl reductase [NADPH] 1	0.637	0.0136	477	34.47	CBR1
sp Q16851	UTP--glucose-1-phosphate uridylyltransferase	0.643	0.021	367	40.99	UGP2
sp P37268	Squalene synthase	0.649	0.0086	748	24.55	FDFT1
sp Q99613	Eukaryotic translation initiation factor 3 subunit C	0.661	0.0095	417	38.33	EIF3C

sp P55884	Eukaryotic translation initiation factor 3 subunit B	1.541	0.0494	275	50.17	EIF3B
sp Q96RP9	Elongation factor G, mitochondrial	1.584	0.0183	388	39.94	GFM1
sp P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	1.691	0.0152	288	47.87	SDHA
sp P47985	Cytochrome b-c1 complex subunit Rieske, mitochondrial	1.753	0.0342	1584	10.19	UQCRFS1
sp P22695	Cytochrome b-c1 complex subunit 2, mitochondrial	1.836	0.0059	235	55.72	UQCRC2
sp P31930	Cytochrome b-c1 complex subunit 1, mitochondrial	1.871	0.0154	333	44.06	UQCRC1
sp Q99714	3-hydroxyacyl-CoA dehydrogenase type-2	1.905	0.0021	156	71.22	HSD17B10
sp O00116	Alkyldihydroxyacetonephosphate synthase, peroxisomal	1.923	0.0411	462	35.11	AGPS
sp P11021	78 kDa glucose-regulated protein	1.976	0.0008	37	132.39	HSPA5
sp P55809	Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	2.014	0.0132	247	54.77	OXCT1
sp O60568	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	2.032	0.0256	244	54.96	PLOD3
sp P51659	Peroxisomal multifunctional enzyme type 2	2.051	0.0382	213	59.35	HSD17B4
sp P48506	Glutamate--cysteine ligase catalytic subunit	2.051	0.0319	979	18.63	GCLC
sp Q9Y6K5	2'-5'-oligoadenylate synthase 3	2.187	0.0167	659	26.94	OAS3
sp P04062	Glucosylceramidase	2.187	0.0168	1363	12.59	GBA
sp P27824	Calnexin	2.187	0.0002	125	78.64	CANX
sp P39023	60S ribosomal protein L3	2.249	0.0052	291	47.72	RPL3
sp P27635	60S ribosomal protein L10	2.249	0.0117	630	28.22	RPL10
sp Q8NBJ5	Procollagen galactosyltransferase 1	2.249	0.0009	302	46.62	COLGALT1

sp Q96C36	Pyrroline-5-carboxylate reductase 2	2.269	0.0194	566	30.36	PYCR2
sp P60228	Eukaryotic translation initiation factor 3 subunit E	2.312	0.0489	438	36.81	EIF3E
sp P49189	4-trimethylaminobutyraldehyde dehydrogenase	2.312	0.0004	646	27.39	ALDH9A1
sp Q13423	NAD(P) transhydrogenase, mitochondrial	2.355	0.0061	200	61.85	NNT
sp P55084	Trifunctional enzyme subunit beta, mitochondrial	2.355	0.0009	251	54.06	HADHB
sp P53597	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	2.398	0.0121	980	18.62	SUCLG1
sp P31939	Bifunctional purine biosynthesis protein PURH	2.398	0.0009	182	65.13	ATIC
sp P31939	Bifunctional purine biosynthesis protein PURH	2.398	0.0009	182	65.13	ATIC
sp O00410	Importin-5	2.421	0.0121	179	65.27	IPO5
sp P48047	ATP synthase subunit O, mitochondrial	2.443	0.0206	771	23.7	ATP5O
sp P22392	Nucleoside diphosphate kinase B	2.488	0.0005	1171	15.72	NME2
sp P49411	Elongation factor Tu, mitochondrial	2.511	0.0013	134	76.56	TUFM
sp O75390	Citrate synthase, mitochondrial	2.511	0.0125	203	60.88	CS
sp P43490	Nicotinamide phosphoribosyltransferase	2.558	0.0138	937	19.75	NAMPT
sp P06576	ATP synthase subunit beta, mitochondrial	2.558	0.0007	50	119.6	ATP5B
sp P40939	Trifunctional enzyme subunit alpha, mitochondrial	2.63	0.0001	91	93.27	HADHA
sp P14625	Endoplasmin	2.631	0.0003	42	126.88	HSP90B1
sp P43034	Platelet-activating factor acetylhydrolase IB subunit alpha	2.654	0.0057	1215	14.8	PAFAH1B1
sp P38646	Stress-70 protein, mitochondrial	2.857	0.0001	4669	129.31	HSPA9

sp P08243	Asparagine synthetase [glutamine-hydrolyzing]	2.937	0.0028	1304	13.31	ASNS
sp P11172	Uridine 5'-monophosphate synthase	3.311	0.0461	1202	15.03	UMPS
sp P54886	Delta-1-pyrroline-5-carboxylate synthase	3.311	0.0046	217	58.45	ALDH18A1
sp P19367	Hexokinase-1	3.341	0.0011	90	93.61	HK1
sp P00390	Glutathione reductase, mitochondrial	4.166	0.0215	524	32.12	GSR
sp Q8IV08	Phospholipase D3	4.365	0.0059	2834	3.75	PLD3
sp O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	4.528	0.0097	1240	14.43	IDH3B
sp P09651	Heterogeneous nuclear ribonucleoprotein A1	4.528	0.0218	71	98.95	HNRNPA1
sp P99999	Cytochrome c	4.613	0.0283	967	18.94	CYCS
sp P61247	40S ribosomal protein S3a	4.786	0.0077	298	46.93	RPS3A
sp P22102	Trifunctional purine biosynthetic protein adenosine-3	4.786	0.0007	208	60.11	GART
sp P08670	Vimentin	4.875	0.0004	35	135.88	VIM
sp P47712	Cytosolic phospholipase A2	4.875	0.0013	672	26.69	PLA2G4A
sp O14561	Acyl carrier protein, mitochondrial	5.152	0.0054	1317	13.09	NDUFAB1
sp P37837	Transaldolase	5.296	0.0001	216	58.58	TALDO1
sp P62899	60S ribosomal protein L31	8.953	0.0151	853	21.54	RPL31

Proteins discovered in at least two out of three experiments with unused prot score >2, fold change of >1.5 or ≤ 0.667 , and P-value ≤ 0.05 were presented in the table. Proteins shaded in gray indicate up-regulated, and unshaded are down-regulate.