Electronic Supplementary Material (ESI) for RSC Advances.

Figure legends

Fig S1 Representational base peak intensity (BPI) chromatograms from HT-29 cells derived from UPLC/MS under negative ion modes.

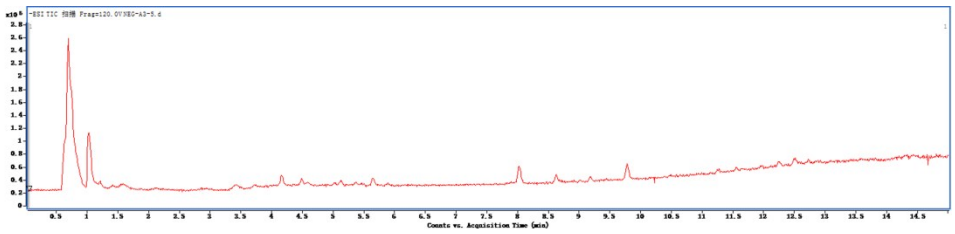


Fig S2 Representational base peak intensity (BPI) chromatograms from HT-29 cells derived from UPLC/MS under positive ion modes.

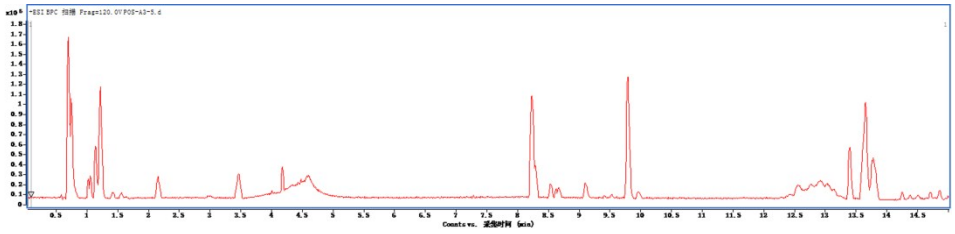


Fig S3 Cross-validation of the PLS-DA model under negative ion modes.

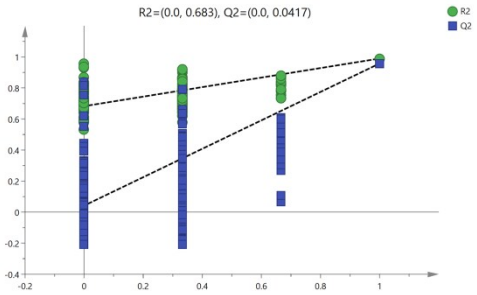
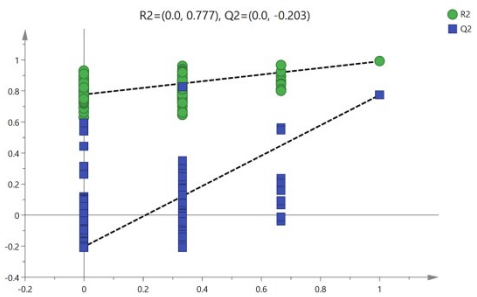


Fig S4 Cross-validation of the PLS-DA model under positive ion modes.



Tables

Table S1 Changed metabolites of HT-29 cells after Cu exposure in negative mode

M/Z[a]	R.T. (min) [b]	Metabolites	Adduct[c]	△m[d]	VIP	p value[e]	Fold change[f]
404.047	1.020	Gluconasturtiin	M-H_2O-H	0.00070	1.89	5.877E-08	0.02
218.103	2.917	Pantothenic acid	M-H	0.00010	2.24	4.209E-07	0.41
306.077	1.016	Glutathione	M-H	0.00078	7.40	4.873E-07	0.01
132.030	0.703	L-Aspartic acid	M-H	0.00008	1.42	3.651E-05	0.37
579.028	0.881	UDP-D-galacturonate	M-H	0.00051	2.65	5.698E-05	0.32
146.046	0.710	L-Glutamic acid	M-H	0.00009	1.85	9.575E-05	0.44
124.007	0.707	Taurine	M-H	0.00008	1.47	0.00014	0.33
578.258	9.998	Vicriviroc carboxylic acid	M+FA-H	0.00204	1.50	0.00282	1.90
221.155	11.278	Isokobusone	M-H	0.00003	1.22	0.00350	0.84
275.054	0.713	2',4'-Dihydroxy-2-biphenylcarboxylic acid	M+FA-H	0.00208	1.61	0.00556	0.53
653.139	1.026	3-Methylellagic acid 2-(4-galactosylglucoside)	M-H	0.00334	1.43	0.00778	0.06
347.172	11.965	2-Propylglutaric acid	2M-H	0.00053	1.38	0.00794	0.48
426.025	0.945	ADP	M-H	0.00328	1.39	0.01129	0.53
164.072	2.179	L-Phenylalanine	M-H	0.00004	1.19	0.01170	0.62
935.139	0.782	Cromoglicic acid	2M-H	0.00820	1.54	0.01280	0.03
180.067	1.174	2-Phenylacetamide	M+FA-H	0.00008	1.30	0.01624	0.65
533.053	12.283	Nitroso-sulfamethoxazole	2M-H	0.00252	2.26	0.01945	2.79
223.029	8.651	3,3'-Thiobispropanoic acid	M+FA-H	0.00061	1.31	0.02136	1.30
133.014	0.791	Pyruvic acid	M+FA-H	0.00000	4.57	0.02470	0.67
520.268	10.917	1-Oleoylglycerophosphoserine	M-H	0.00004	1.12	0.02775	0.49
635.142	1.021	Proanthocyanidin A2	M+FA-H	0.00106	1.38	0.03341	0.01
239.060	13.208	Cysteinyl-Histidine	M- H_2O -H	0.00046	1.27	0.03520	0.75
239.060	8.643	Histidinyl-Cysteine	M- H_2O -H	0.00054	2.25	0.04233	1.25
129.056	4.327	Tiglic aldehyde	M+FA-H	0.00004	1.41	0.02701	0.58
497.077	12.284	Pyridoxine 5'-phosphate	2M-H	0.00342	3.59	0.01966	2.03
333.092	6.136	(S)-a-Amino-2,5-dihydro-5-oxo-4-isoxazolepropanoic acid N2-glucoside	M-H	0.00185	1.85	0.01891	0.62
191.020	1.039	Citric acid	M-H	0.00004	3.81	0.02527	0.78
546.286	9.595	LysoPE(20:4(5Z,8Z,11Z,14Z)/0:0)	M+FA-H	0.00215	4.54	0.01691	0.31
179.056	0.678	Alpha-D-Glucopyranoside	M-H	0.00011	1.01	0.01770	0.74

[a]M/Z, mass/charge number of peaks in mass spectra. [b]R.T.(min), retention time of metabolites in chromatography. [c]The formation of peaks in mass spectra, in which FA represents formic acid. [d]△m, the mass discrepancies between measured values and theoretical values during metabolite identification in database HMDB and METLIN. [e] Differences between the control and the treated are analyzed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. $p < 0.05$ is considered as significant. [f]The change of metabolite abundance is expressed as the ratio of the average content in treatment and in control (n=6). A value >1 represents upregulation, while a value <1 indicates downregulation.

Table S2 Changed metabolites of HT-29 cells after Cu exposure in positive mode

M/Z	R.T. (min)	Metabolites	adduct	△m	VIP	p value	Fold change
308.091	1.009	Glutathione	M+H	0.00030	11.63	8.347E-09	0.04
330.073	0.896	Glutathione	M+Na	0.00008	2.85	1.03E-09	0.06
346.045	1.023	Glutathione	M+K	0.00227	1.28	1.819E-06	0.13
220.118	2.919	Pantothenic acid	M+H	0.00016	2.60	1.932E-07	0.40
300.290	9.446	Sphingosine	M+H	0.00008	1.90	3.086E-05	0.44
146.165	0.584	Spermidine	M+H	0.00018	2.65	0.00130	0.48
148.060	0.725	L-Glutamic acid	M+H	0.00082	1.43	0.00105	0.49
132.077	0.723	Creatine	M+H	0.00006	4.75	0.00071	0.52
205.097	4.090	L-Tryptophan	M+H	0.00014	1.69	0.00521	0.60
166.086	2.182	L-Phenylalanine	M+H	0.00005	3.50	0.01457	0.63
298.097	4.146	5'-Methylthioadenosine	M+H	0.00012	2.33	0.00374	0.67
613.160	1.023	Oxidized glutathione	M+H	0.00047	2.53	0.01736	1.87
323.070	1.869	4-Hydroxy-L-glutamic acid	2M+H	0.00180	1.23	3.957E-07	68.29
756.558	12.559	PC(14:1(9Z)/20:2(11Z,14Z))	M+H	0.00410	3.36	0.00086	0.09
778.570	11.662	PE(22:4(7Z,10Z,13Z,16Z)/P-18:1(11Z))	M+H	0.00446	1.01	0.00714	0.09
162.058	1.494	S-Allylcysteine	M+H	0.00020	1.37	9.684E-05	0.22
760.581	13.547	PC(16:0/18:1(11Z))	M+H	0.00452	3.64	2.841E-06	0.27
784.585	13.309	PC(22:2(13Z,16Z)/14:1(9Z))	M+H	0.00047	2.00	0.01480	0.38
703.575	13.621	SM(d18:0/16:1(9Z))	M+H	0.00055	2.62	0.00528	0.41
563.552	13.628	Oleamide	2M+H	0.00103	4.00	0.04920	0.46
725.557	13.561	SM(d18:0/16:1(9Z)) ;Sphingomyelin	M+Na	0.00052	1.15	0.02866	0.49
184.073	0.699	Tryptophanol;Indole-3-ethanol	M+Na	0.00007	2.33	0.02410	0.57
732.552	12.078	PC(14:0/18:1(9Z))	M+H	0.00202	1.66	0.01265	2.03
372.311	9.670	Tetradecanoylcarnitine	M+H	0.00024	2.21	0.00079	0.05
426.358	10.695	Vaccenyl carnitine	M+H	0.00039	2.38	1.377E-05	0.08
398.327	9.918	9-Hexadecenoylcarnitine	M+H	0.00081	1.86	0.00047	0.12
246.170	4.577	2-Methylbutyroylcarnitine	M+H	0.00009	2.06	0.00048	0.12
232.154	3.513	Isobutyryl-L-carnitine	M+H	0.00038	1.40	0.00169	0.19
714.540	14.882	PC(P-18:1(9Z)/14:1(9Z))	M+H	0.00312	1.85	0.00069	0.20
692.560	14.951	PC(o-14:0/16:0)	M+H	0.00072	1.26	0.04381	0.23
428.374	11.290	Stearoylcarnitine	M+H	0.00042	1.63	0.00011	0.30
392.149	3.868	7-Hydroxydehydroglaucine	M+Na	0.00171	1.64	0.00080	0.38
252.232	11.689	Herculin	M+H	0.00000	1.14	0.03766	0.42
137.071	0.702	N-Methylnicotinamide	M+H	0.00017	2.18	0.00525	0.49
722.507	11.899	PE(P-18:1(11Z)/18:4(6Z,9Z,12Z,15Z))	M+H	0.00511	1.08	0.00118	0.50
172.169	9.096	N,2,3-Trimethyl-2-(1-methylethyl)butanamide	M+H	0.00038	1.29	0.01654	0.54
478.293	10.218	LysoPE(0:0/18:2(9Z,12Z))	M+H	0.00043	1.14	0.03316	0.55
278.248	10.045	Palmitic amide	M+Na	0.00239	1.34	0.00728	0.56
309.206	11.354	Methylgingerol	M+H	0.00007	1.41	0.02574	0.58
203.058	0.686	Kynuramine	M+K	0.00033	2.26	0.02424	0.59
188.070	4.090	Indoleacrylic acid	M+H	0.00032	1.13	0.00435	0.61
371.101	14.240	meta-O-Dealkylated flecainide	M+K	0.00352	1.83	0.00046	1.67
413.267	12.110	12-Ketodeoxycholic acid	M+Na	0.00073	6.23	0.04946	1.75
265.180	14.862	12-Oxo-2,3-dinor-10,15-phytodienoic acid	M+H	0.00021	1.27	0.00753	3.65
345.337	12.377	MG(18:0e/0:0/0:0)	M+H	0.00019	1.35	0.03027	10.77
339.287	14.543	1,2,4-Nonadecanetriol	M+Na	0.00016	1.20	0.02724	12.99
367.319	12.393	MG(18:0e/0:0/0:0)	M+Na	0.00030	1.85	0.02767	14.80
401.342	12.042	7-Ketocholesterol	M+H	0.00032	4.17	0.001991	47.22

[a]M/Z, mass/charge number of peaks in mass spectra. [b]R.T.(min), retention time of metabolites in chromatography. [c]The formation of peaks in mass spectra, in which FA represents formic acid. [d]△m, the mass discrepancies between measured values and theoretical values during metabolite identification in database HMDB and METLIN. [e] Differences between the control and the treated are analyzed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. $p < 0.05$ is considered as significant. [f]The change of metabolite abundance is expressed as the ratio of the average content in treatment and in control (n=6). A value >1 represents upregulation, while a value <1 indicates downregulation.