Periplocymarin alleviates doxorubicin-induced heart failure and excessive accumulation of ceramides

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Supplementary information

Supplementary method

1. Cell viability assay

H9c2 cells were seeded in a 96-well plate with 5000 cells/well, and treated with the following conditions: fresh culture medium alone (control), fresh culture medium with different concentrations (0-100μM) of periplocymarin. Cell viability was assessed by a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Gaithersburg, MD, USA) according to manufacturer's instructions. Briefly, after treatment, the CCK-8 solution was added to the culture medium and incubated at 37°C for 4 h. The absorbance was read at 450 nm with a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability was calculated by (experimental group absorbance value/control group absorbance value) × 100%.

2. Determination of intracellular ROS

The cellular ROS level was determined using ROS detection kits (Beyotime Biotechnology, China). In brief, following DOX and periplocymarin treatment, H9c2 cells were washed third with 1 washing buffer and then incubated with serum free DMEM (10 mM DCFH-DA) at 37 ℃ for 30 min. The fluorescence intensity of DCF was quantified by using the confocal microscope at Ex./Em. = 488/525 nm.

Supplementary data

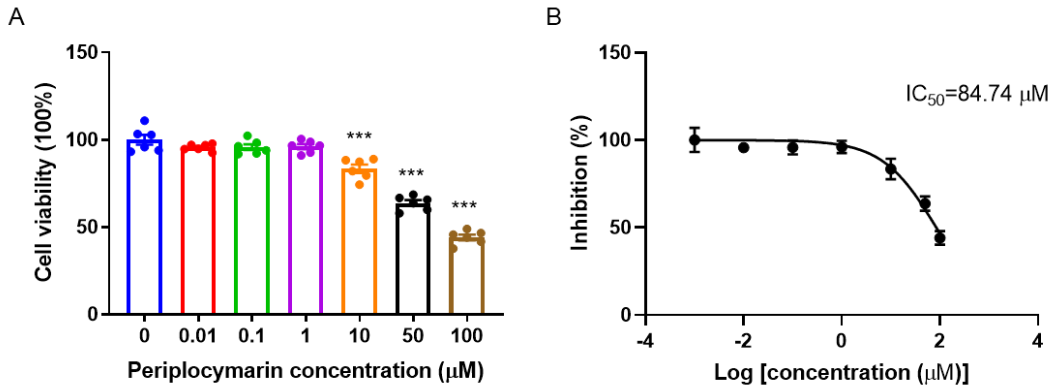

Fig. S1. Effect of periplocymarin on the cell viability of H9c2 cell. H9c2 cells were treated with different doses (0, 0.01, 0.1, 1, 10, 50 and 100 μM) of periplocymarin for 24 h. After treatment, cell viability of H9c2 cells was detected by CCK-8 assay (A). And the IC_{50} was calculated (B). Each experiment was repeated at least three times. Data shown are means ± SEM; n=6, ***p<0.001 vs. control.

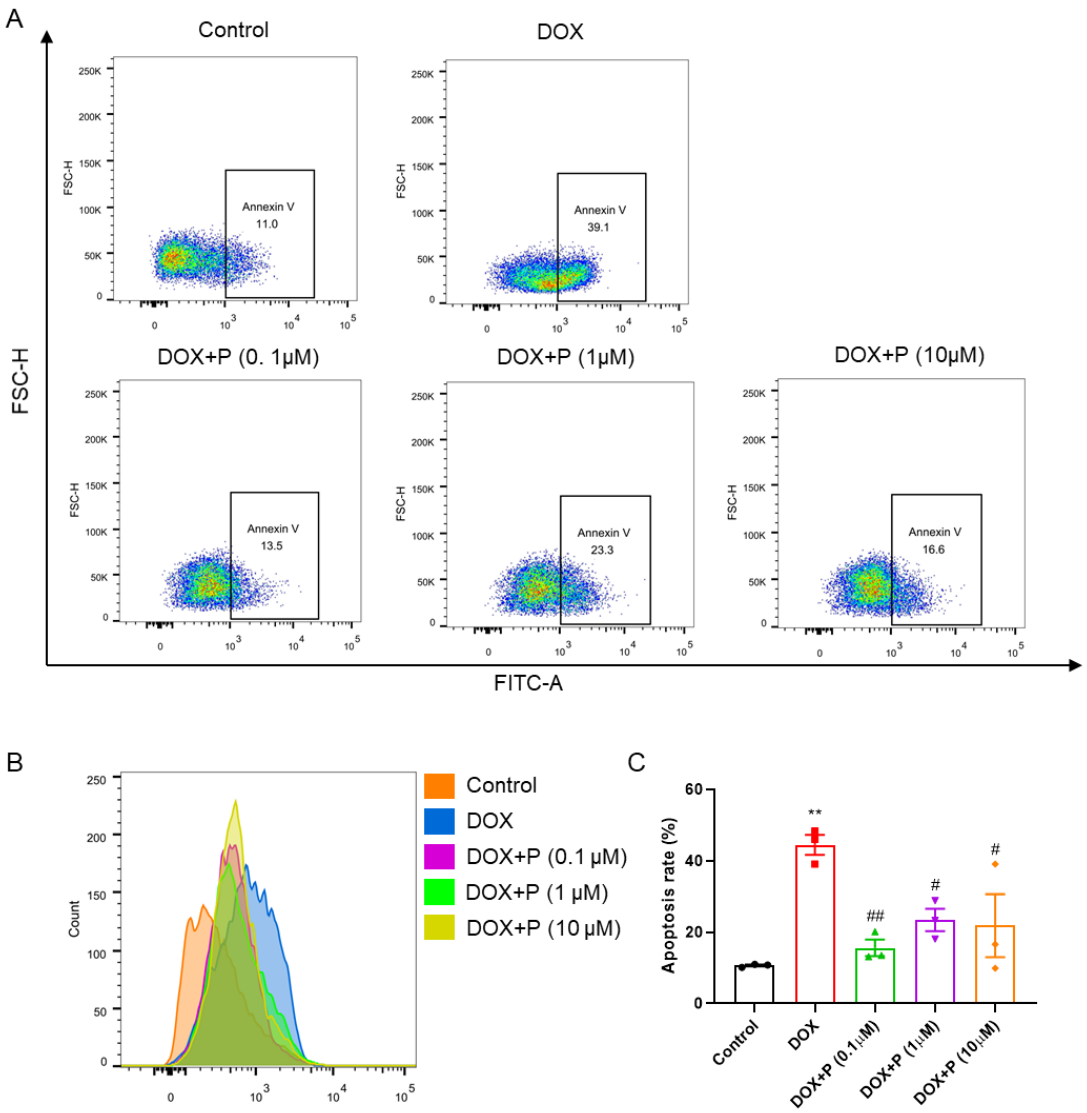

Fig. S2. Periplocymarin inhibits DOX-induced myocardial apoptosis in vitro.

(A). Flow cytometric scatterplot of Annexin-V-FLTC staining of cell apoptosis.

(B). Flow cytometric histogram of Annexin-V-FLTC staining of cell apoptosis.

(C). Quantitative analysis of apoptotic cells in different groups using bar graphs. Data shown are means ± SEM; n=3. **p<0.01 vs. control group, $^{\#}$p<0.01, $^{\#\#}$p<0.01 vs DOX group.

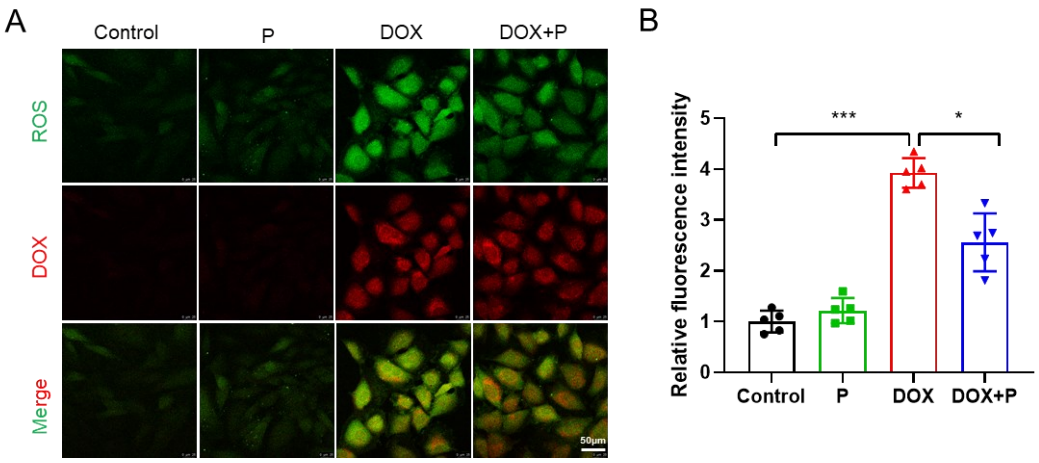

Fig. S3. The effect of DOX and periplocymarin on the production of ROS was measured by DCFH-DA staining via confocal microscope(A). And the quantitative analysis of mean DCF fluorescence intensity in different groups(B). Data shown are means ± SEM; n=5, *p<0.05, ***p < 0.001.

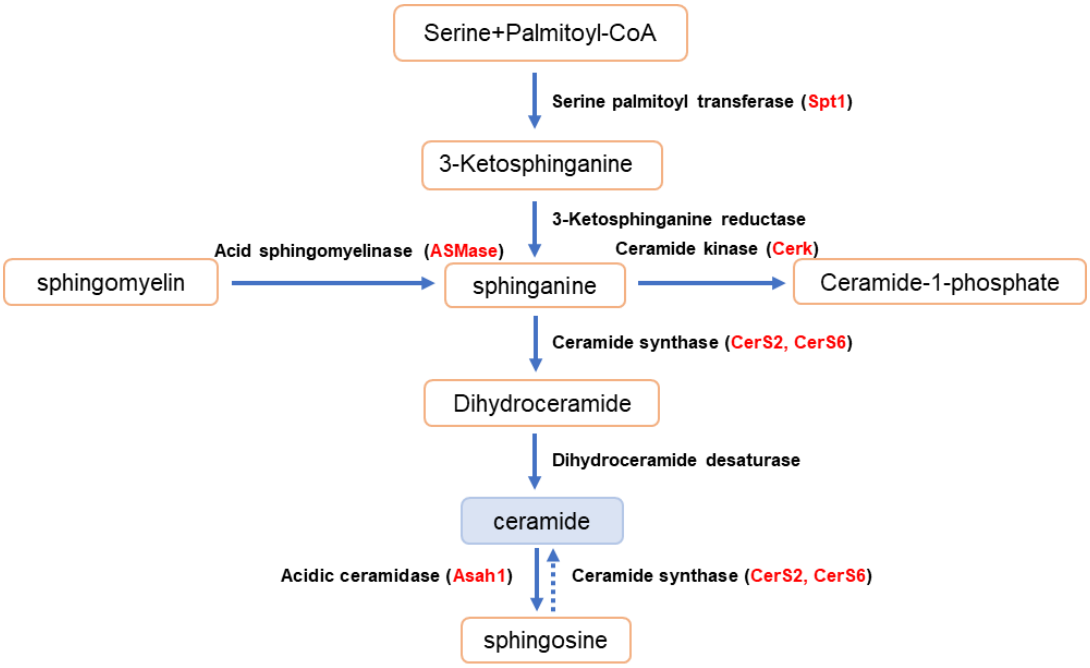

Fig. S4. Schematic representation of ceramide biosynthesis pathways.

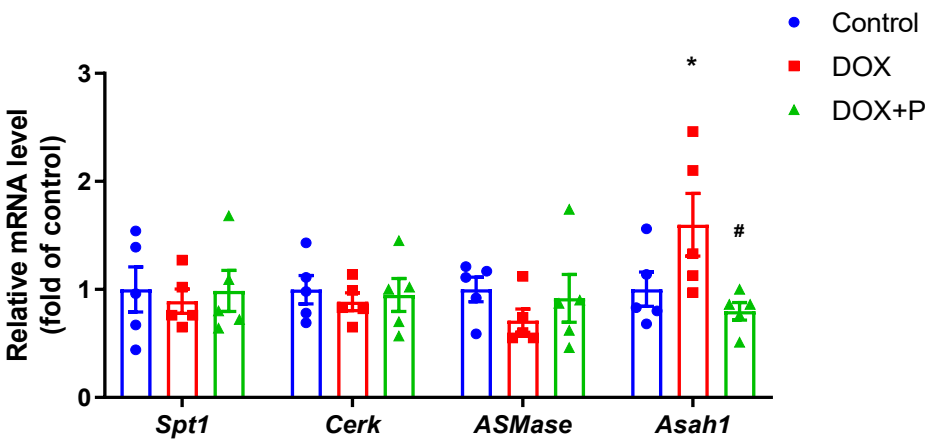

Fig. S5. Real-time PCR analysis of *Spt1, Cerk, ASMase* and *Asah1* mRNA levels in the hearts. Data shown are means ± SEM; n=5, *p<0.05 vs. control, #p < 0.05 vs. model.

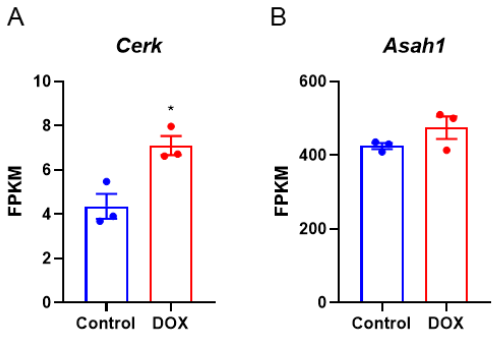

Fig. S6. The expressions of *Cerk* (A) and *Asah1* (B) in the RNA sequencing data (GSE157282).

Table S1. Primers for quantitative RT-PCR.

PCR production	Forward primer	Reverse primer
Rat Cers1	GTCTGTCGCCTATCTCCGTG	GTCCCTGAAAGGGTGTCCTG
Rat Cers2	TCCCTTTTCCCCTCCCTTCA	AGTCTGACACAGTCATGTTTTATTT
Rat Cers3	GCCCCGCAGCTTTTGAAG	CATTGGCTAGAGGTGTGGCA
Rat Cers4	TCTGTGTTCCTTCGTGGGTG	AGGGCCAAATTCAGGGTCTG
Rat Cers5	CAGCAGGTCTCTTCTCCGTG	AGCCAAGGCATCTTAGAGATAGA
Rat Cers6	GTTAGCCTGGAGCTGGTCTC	TCCCCTGCCCTCTGAAAATG
Mouse Cers1	AGAGCCCATGCCGAGTTAT	TCCAGGCGCTCTCAGGTAA
Mouse Cers2	TACGCGGGATGGAAGAACAC	TGGGCCATACGCAGAATGAA
Mouse Cers3	ACTTTCGAGGGTGGGTCTCT	AGCCAGTATCTCTCCGACCA
Mouse Cers4	TTCTGAACCTCCAGCATGTCG	GATCCTGCACACCCATCCAC
Mouse Cers5	CTGGTCGATGCCTTGGTTCT	CTCGCACCATGTTGTTGACG
Mouse Cers6	CGGCTGGGCATATTTCCTCT	AAAGGGTTCCACTTCCCAGC
Mouse ASAH1	AAAGTCTTCTCACCTGGGTCC	GATTTATGGTGTGCCACGGA
Mouse SPT1	GAGAGCCTGTCCTTTGGAGTC	AGCAGTATCCTTGACCGGAG
Mouse Cerk	TCGGTACTGGTGTCGGAGAT	CGGTCTTGAAGTCAGGCTCT
Mouse Asmase	GAGGAAACTCTGAGCCGACC	GTAGACCAGGTTGTGCCAGG
Mouse 18S	GAAACGGCTACCACATCCAAGG	GCCCTCCAATGGATCCTCGTTA

Table S2. Metabolites in positive ion mode by MRM assay.

Sequence	Q1	Q3	MRM Name	DP	EP	CE	CXP	Chemical Formula
1	620.7	264.3	Ceramide(d18:1/22:0 OH)	100	10	40	10	$C_{40}H_{79}NO_4$
2	572.7	264.3	Ceramide(d18:1/20:2)	100	10	40	10	$C_{38}H_{71}NO_3$
3	600.7	264.3	Ceramide(d18:1/22:2)	100	10	40	10	$C_{40}H_{75}NO_3$
4	576.57	264.3	Ceramide(d18:1/20:0)	100	10	40	10	$C_{38}H_{75}NO_3$
5	548.6	264.3	Ceramide(d18:1/18:0)	100	10	40	10	$C_{36}H_{71}NO_3$
6	604.6	264.3	Ceramide(d18:1/22:0)	100	10	40	10	$C_{40}H_{79}NO_3$
7	632.6	264.3	Ceramide(d18:1/24:0)	100	10	40	10	$C_{42}H_{83}NO_3$
8	630.7	264.3	Ceramide(d18:1/24:1)	100	10	40	10	$C_{42}H_{81}NO_3$
9	628.7	264.3	Ceramide(d18:1/24:2)	100	10	40	10	$C_{42}H_{79}NO_3$
10	646.7	264.3	Ceramide(d18:1/25:0)	100	10	40	10	$C_{43}H_{85}NO_3$
11	520.5	264.3	Ceramide(d18:1/16:0)	100	10	40	10	$C_{34}H_{67}NO_3$
12	536.7	264.3	Ceramide(d18:1/16:0 OH)	100	10	40	10	$C_{34}H_{67}NO_4$
13	518.5	264.3	Ceramide(d18:1/16:1)	100	10	40	10	$C_{34}H_{65}NO_3$
14	516.5	264.3	Ceramide(d18:1/16:2)	100	10	40	10	$C_{34}H_{63}NO_3$
15	562.7	264.3	Ceramide(d18:1/18:1 OH)	100	10	40	10	$C_{36}H_{69}NO_4$
16	546.7	264.3	Ceramide(d18:1/18:1)	100	10	40	10	$C_{36}H_{69}NO_3$
17	560.7	264.3	Ceramide(d18:1/18:2 OH)	100	10	40	10	$C_{36}H_{67}NO_4$
18	544.7	264.3	Ceramide(d18:1/18:2)	100	10	40	10	$C_{36}H_{67}NO_3$
19	592.7	264.3	Ceramide(d18:1/20:0 OH)	100	10	40	10	$C_{38}H_{75}NO_4$
20	590.7	264.3	Ceramide(d18:1/20:1 OH)	100	10	40	10	$C_{38}H_{73}NO_4$
21	574.7	264.3	Ceramide(d18:1/20:1)	100	10	40	10	$C_{38}H_{73}NO_3$
22	602.7	264.3	Ceramide(d18:1/22:1)	100	10	40	10	$C_{40}H_{77}NO_3$
23	616.7	264.3	Ceramide(d18:1/22:2 OH)	100	10	40	10	$C_{40}H_{75}NO_4$
			Ceramide(d18:1/23:0) or					
24	618.7	264.3	Ceramide(d18:1/22:1 OH)	100	10	40	10	$C_{41}H_{81}NO_3$
25	644.7	264.3	Ceramide(d18:1/24:2 OH)	100	10	40	10	$C_{42}H_{79}NO_4$
26	676.7	264.3	Ceramide(d18:1/26:0 OH)	100	10	40	10	$C_{44}H_{87}NO_4$
27	660.7	264.3	Ceramide(d18:1/26:0)	100	10	40	10	$C_{44}H_{87}NO_3$
28	658.7	264.3	Ceramide(d18:1/26:1)	100	10	40	10	$C_{44}H_{85}NO_3$
29	656.7	264.3	Ceramide(d18:1/26:2)	100	10	40	10	$C_{44}H_{83}NO_3$
30	534.7	264.3	Ceramide(d18:1/16:1 OH)	100	10	40	10	$C_{34}H_{65}NO_4$
31	648.7	264.3	Ceramide(d18:1/24:0 OH)	100	10	40	10	$C_{42}H_{83}NO_4$

Table S3. extracted ions in six QC samples in serum.

Num.	Name	Peak area								RSD
1	Cer 16:1 OH	40957.33	42072.67	39095.00	47261.67	40753.00	42067.33	46722.67	44366.33	6.82
2	Cer 24:0 OH	17542287.67	20139209.33	18902600.33	22357009.33	25368105.00	23334785.00	22243591.33	22534429.00	11.75
3	Cer 22:0 OH	403225.00	464681.33	434717.33	502075.33	503635.33	565653.67	533035.33	530323.33	11.06
4	Cer 20:2	583076.00	657150.33	501883.67	734766.67	650536.00	610082.00	667626.67	639345.00	10.83
5	Cer 22:2	9095407.00	11675648.00	12032290.33	12569225.00	14091595.00	13294734.00	14048312.67	13984947.00	13.48
6	Cer 20:0	1208971.00	1364833.67	1329733.33	1269860.67	1544478.67	1453638.33	1528147.67	1495194.67	8.91
7	Cer 18:0	1172245.33	1048313.00	1043541.67	1141032.67	1230636.00	1427889.67	1186068.00	1011843.33	11.58
8	Cer 22:0	22291013.00	27458378.67	24482229.67	27089309.33	28500834.67	25956107.33	29594552.67	26172444.33	8.70
9	Cer 24:0	25678963.33	30858387.00	28164602.33	32706120.00	30346357.33	32286372.00	32331243.00	31509295.67	7.97
10	Cer 24:1	20284462.00	23611153.33	20960432.33	24423168.33	28180204.33	25683637.67	26694333.00	24894683.00	11.07
11	Cer 24:2	2040810.00	2188578.67	2050077.00	2066107.33	2798930.00	2118749.67	2303747.67	1979804.33	12.06
12	Cer 25:0	1004244.67	1123759.00	1092889.33	1152795.33	1161579.67	1019248.33	1048123.33	1125398.33	5.54
13	Cer 16:0	2337330.67	2214703.67	2358335.33	2400227.67	2755127.67	2335109.67	2507163.67	1960689.33	9.63
14	Cer 16:0 OH	120066.33	108016.67	114340.00	105309.00	122338.33	80302.00	115727.67	127318.67	13.07
15	Cer 16:1	251157.33	269641.67	280031.67	291481.00	284053.00	298575.33	291177.67	307934.00	6.23
16	Cer 16:2	46027.33	45642.00	43470.33	44446.67	40848.00	51395.00	44671.33	45314.00	6.58
17	Cer 18:1 OH	225475.67	229877.00	204522.67	233264.67	234492.67	235199.67	285298.33	231139.33	9.65
18	Cer 18:1	84510.67	72810.00	86941.00	69619.00	95085.33	66304.33	77806.33	83554.67	12.19
19	Cer 18:2 OH	40208.33	43546.33	35318.67	38673.33	44555.33	41619.67	50531.00	34773.67	12.57
20	Cer 18:2	203590.00	201002.67	206480.33	219748.33	208615.33	209347.67	200768.00	228791.33	4.66
21	Cer 20:0 OH	66459.67	64637.00	81735.33	68488.00	70650.33	79128.00	80926.00	69975.67	9.35
22	Cer 20:1 OH	542820.67	614854.00	598790.33	657460.33	680232.33	688509.67	706223.00	594825.33	8.88
23	Cer 20:1	65859.00	84900.33	75186.67	76121.00	75660.00	81995.67	73488.33	71185.67	7.86
24	Cer 22:1	1112775.33	1355044.00	1188919.67	1322446.00	1500953.67	1433221.33	1412779.00	1569929.33	11.23
25	Cer 22:2 OH	979632.33	1321039.00	1178052.33	1370225.33	1154753.67	1507951.67	1257452.00	1300267.00	12.58
26	Cer 23:0	10617832.67	12818032.33	12142866.67	13162491.00	14029670.00	14437224.67	13963362.67	11648370.67	10.26
27	Cer 24:2 OH	414285.33	337230.67	364557.00	389917.00	439423.67	449131.67	370489.00	383552.67	9.74
28	Cer 26:0 OH	54474.33	76545.33	65599.00	62544.67	63499.00	71544.33	75020.67	64676.67	10.92
29	Cer 26:0	279430.67	240734.67	295269.67	296960.33	230243.33	269717.00	243781.00	263029.33	9.49
30	Cer 26:1	171103.67	176815.33	150106.00	171783.67	208170.33	185492.33	172471.00	173909.67	9.24
31	Cer 26:2	40945.00	40135.67	47318.00	49208.00	55820.33	46351.33	45314.00	49924.00	10.76

Table S4. The model parameters of OPLS-DA in serum.

	R^2X	R^2Y	Q^2(cum)
Control VS DOX	0.898	0.902	0.863
DOXVS DOX+P	0.932	1	0.941

Table S5. VIP value of different metabolites come from control and DOX group in serum.

Name	VIP
Cer 24:1	3.49954
Cer 24:0 OH	3.32994
Cer 23:0	1.40985
Cer 24:2	1.25155
Cer 22:2	1.17972
Cer 16:0	1.07731

Table S6. VIP value of different metabolites come from DOX and DOX+P group in serum.

Name	VIP
Cer 22:0	1.45505
Cer 25:0	1.26429
Cer 24:1	1.2335
Cer 24:0	1.20111
Cer 26:1	1.18804
Cer 22:2 OH	1.12223
Cer 18:0	1.10362
Cer 20:0	1.09678
Cer 16:0	1.09228
Cer 20:1 OH	1.06412
Cer 20:2	1.06328
Cer 22:1	1.0523
Cer 22:0 OH	1.03578
Cer 24:2	1.02023
Cer 18:2 OH	1.00816

Table S7. extracted ions in six QC samples in H9C2 cell.

Num.	Name	Peak area						RSD
1	Cer 16:1 OH	14165.43	14793.71	15796.18	14480.90	14852.80	15844.48	4.60
2	Cer 24:0 OH	256230.67	296928.10	314923.32	345786.32	306094.43	358605.68	11.68
3	Cer 22:0 OH	12681.89	14440.39	13446.25	16155.97	15194.60	15060.63	8.70
4	Cer 20:2	2791.24	2729.74	2650.21	2865.83	2527.82	2649.15	4.42
5	Cer 22:2	26585.56	24496.78	25206.62	27625.12	26041.22	26167.40	4.17
6	Cer 20:0	44872.91	52191.53	50538.39	54513.34	51504.33	56801.25	7.84
7	Cer 18:0	96211.68	116910.22	120795.41	130497.07	118275.75	132562.38	10.89
8	Cer 22:0	111684.12	123218.55	132679.22	153618.32	136894.21	148012.66	11.55
9	Cer 24:0	236366.65	230651.23	257624.13	238819.05	269519.50	270289.00	6.99
10	Cer 24:1	272578.96	263203.57	299541.98	306601.58	283905.48	286669.74	5.67
11	Cer 24:2	15222.52	15752.90	18811.80	19368.57	18712.25	20123.36	11.20
12	Cer 25:0	6923.43	6961.64	8292.65	7886.68	8698.97	8640.55	10.09
13	Cer 16:0	192008.26	244966.79	247650.32	273275.96	257930.42	286927.69	13.07
14	Cer 16:0 OH	1343.58	1137.92	1289.08	1496.57	1353.26	1238.82	9.22
15	Cer 16:1	1562.06	1486.20	1573.48	1764.49	1759.08	1692.59	7.04
16	Cer 16:2	12084.54	13976.63	15276.49	16482.01	14172.08	16930.57	12.08
17	Cer 18:1 OH	2477.66	2761.07	2554.80	3156.52	3325.17	3248.75	12.65
18	Cer 18:1	3983.48	3885.15	3699.33	4554.74	4330.40	4182.10	7.60
19	Cer 18:2 OH	1103.54	1056.05	1005.92	1014.41	1032.44	1038.23	3.37
20	Cer 18:2	1997.23	1797.24	1892.46	2277.37	2249.83	2007.37	9.42
21	Cer 20:0 OH	38351.43	44129.10	39322.80	47845.47	39347.05	49631.56	11.23
22	Cer 20:1 OH	3385.55	4052.13	3482.63	3944.52	3915.19	4707.04	12.06
23	Cer 20:1	2369.33	2173.33	1825.64	2478.89	1944.75	2123.40	11.47
24	Cer 22:1	13169.34	13862.57	13295.58	16457.41	12694.06	13506.47	9.71
25	Cer 22:2 OH	11397.97	11999.50	13382.58	14087.05	13372.82	14935.29	9.91
26	Cer 23:0	29980.34	34558.55	34535.73	36055.62	38382.40	39229.42	9.34
27	Cer 24:2 OH	4714.03	5086.75	4854.01	6012.10	5108.58	5046.09	8.86
28	Cer 26:0 OH	4586.29	5654.14	4940.01	5593.88	5366.88	5038.29	7.98
29	Cer 26:0	7865.74	7651.88	7776.36	7805.65	7507.77	8955.63	6.56
30	Cer 26:1	10135.46	10511.32	9119.11	9500.62	9967.25	10070.82	5.02
31	Cer 26:2	1537.72	1746.38	1741.65	1838.68	1922.78	1798.53	7.35

Table S8. The model parameters of OPLS-DA in H9C2 cell.

	R^2X	R^2Y	Q^2(cum)
Control VS DOX	0.912	0.975	0.904
Control VS DOX+P	0.972	0.984	0.981

Table S9. VIP value of different metabolites come from control and DOX group in H9C2 cell.

Name	VIP
Cer 24:0 OH	2.57948
Cer 24:1	2.49988
Cer 16:0	2.12698
Cer 24:0	2.11194
Cer 22:0	1.57051
Cer 18:0	1.49148

Table S10. VIP value of different metabolites come from DOX and DOX+P group in H9C2 cell.

Name	VIP
Cer 24:0	2.5083
Cer 24:0 OH	2.25757
Cer 24:1	2.12897
Cer 16:0	1.95275
Cer 18:0	1.02906

Table S11. Periplocymarin related targets

Gene	Score
ATP1A1	122.778
GLRA3	80.882
GABRB3	80.882
GLRA1	23
NRXN1	22.373
GLRA2	22.373
GLRB	22.373
GLRA4	22.373
ATP1A3	15.365
GABRB1	15.365
GABRB2	15.365
ATP1A2	15.365
FKBP1A	8.253
TOP2A	8.253

SLC47A1	8.253
ATP1A4	8.253
CYP3A4	8.253
TUBB	8.253
MAP2	8.253
TUBB4A	8.253
TUBA3E	8.253
CYP51A1	8.253
BCL2	8.253
TUBB2B	8.253
HMGCR	8.253
NR1I2	8.253
KCNH2	8.253
MTOR	8.253
TUBB4B	8.253
ITGB2	8.253
CYP3A7-CYP3A51P	8.253
TUBA3C	8.253
MAPT	8.253
TUBA1C	8.253
TUBB2A	8.253
TUBA1B	8.253
PADI4	8.253
MLNR	8.253
CYP3A7	8.253
SLCO1B3	8.253
ITGAL	8.253
TUBB1	8.253
TUBA4A	8.253
ALB	8.253
HDAC2	8.253
MAP4	8.253
ABCB1	8.253
FGF2	8.253
TUBA3D	8.253
SLCO1B1	8.253
TUBA1A	8.253
GPHN	5.859
GABRA2	5.859
CHRNA4	5.859
SHANK3	5.859
ADORA2A	5.859
GABRG3	5.859

IGSF9B	5.859
CX3CL1	5.859
RELN	5.859
APOE	5.859
GSS	5.859
NTSR1	5.859
GRIN3B	5.859
CHRNA7	5.859
UNC13B	5.859
GNMT	5.859
GABRE	5.859
NPAS4	5.859
GABRA3	5.859
GABRG1	5.859
ATP1B1	5.859
NR3C1	5.859
GABRA4	5.859
DRD2	5.859
DLG4	5.859
NLGN1	5.859
AOC1	5.859
HRH1	5.859
GLDC	5.859
GRIN2B	5.859
DRD4	5.859
RIMS2	5.859
NLGN2	5.859
ALAS2	5.859
SLC6A9	5.859
DIAPH1	5.859
DRD3	5.859
GRIN3A	5.859
BDNF	5.859
GABRA5	5.859
FXYD2	5.859
SLC6A5	5.859
GABRA6	5.859
GABRA1	5.859
GABRG2	5.859
RIMS1	5.859
CHRNA9	5.859
GRIK1	5.859
GRIK2	5.859
RAD51	5.859

GRIN1	5.859
SRR	5.859
NLGN3	5.859
CXCR4	5.785
GAA	5.785
MGAM	5.785
KDM5D	5.785
ALKBH3	5.785
OGFOD2	5.785
P3H3	5.785
TMLHE	5.785
ESR1	5.785
P4HTM	5.785
PLOD1	5.785
PTGIS	5.785
EGLN2	5.785
ALKBH2	5.785
BBOX1	5.785
PGR	5.785
AR	5.785
P2RY12	5.785
PTGIR	5.785
PRKCA	5.785
PAM	5.785
AMY2A	5.785
SLC23A1	5.785
OGFOD1	5.785
DHFR	5.785
DBH	5.785
EGLN3	5.785
SMO	5.785
PLOD3	5.785
P4HA1	5.785
SI	5.785
PTGFR	5.785
EGLN1	5.785
P3H2	5.785
LCT	5.785
LRP2	5.785
ANXA1	5.785
CYP19A1	5.785
PLOD2	5.785
P3H1	5.785
PRKCD	5.785

PHYH	5.785
NR3C2	5.785
TUBB3	3.351
HDAC1	3.351
CYP3A5	3.351
EHHADH	3.351
CHRM3	3.351
CHRM1	3.351
TUBA8	3.351
ATP4A	3.351
ATP12A	3.351
TUBB6	3.351
CYP3A43	3.351
TUBB8	3.351
CHRNA2	3.351
FKBP1B	3.351
HTR3A	3.351
ADA	3.351
CHRM2	3.351
TOP2B	3.351
CACNB1	2.608
CACNA2D1	2.608
PLAT	2.608
CACNA1H	2.608
SCN1A	2.608
PDE4A	2.608
NDRG1	2.608
MAPK1	2.608
SNX6	2.608
PRDX5	2.608
CACNA1F	2.608
GABRD	2.608
ESR2	2.608
FGF1	2.608
PRLR	2.608
CACNA1A	2.608
TNFRSF1B	2.608
CFL1	2.608
FXYD1	2.608
HGS	2.608
CACNA1I	2.608
NFKB2	2.608
CACNA2D2	2.608
SERPINC1	2.608

TNF	2.608
HSD3B2	2.608
SHBG	2.608
PTGER1	2.608
MMP9	2.608
CACNA1S	2.608
MAPK3	2.608
FGG	2.608
PTGER4	2.608
PDE4D	2.608
TRADD	2.608
GABRP	2.608
TPT1	2.608
CACNA2D3	2.608
NEU1	2.608
PLA2G4A	2.608
CACNB4	2.608
GTF2F2	2.608
F10	2.608
CACNA1B	2.608
SMAD2	2.608
CACNA1C	2.608
IFNG	2.608
EGF	2.608
CACNA1D	2.608
HSD3B1	2.608
CACNB3	2.608
CA4	2.608
FGF4	2.608
FGB	2.608
GABRQ	2.608
PDE4C	2.608
PTGER2	2.608
FASN	2.608
EZH2	2.608
PTGER3	2.608
CES1	2.608
CYP11B2	2.608
CACNA1G	2.608
FXYD7	2.608
CLCN2	2.608
IKBKB	2.608
NEU2	2.608
FGA	2.608

PDE4B	2.608
ADD1	2.608
CACNB2	2.608
TNFRSF1A	2.608
CA2	2.608
VDR	2.608
EGFR	2.608
CYP27B1	2.608
CACNG1	2.608
CERS1	2.394
CERS2	2.394
CERS3	2.394
CERS5	2.394
CERS4	2.394
CERS6	2.394
ZNF471	2.394
THOC5	2.394
RECK	2.394
CCNO	2.394
ZNF99	2.394
SPSB1	2.394
KRTAP10-11	2.394
CREBBP	2.394
ZC3H15	2.394
CCDC117	2.394
ATP13A3	2.394
DNM2	2.394
GBP2	2.394
BFSP2	2.394
COG3	2.394
RAB33A	2.394
LSR	2.394
PPM1K	2.394
RAB19	2.394
HMBOX1	2.394
NRAS	2.394
RAB41	2.394
ARMC2	2.394
SLC6A3	2.394
TFAP2C	2.394
ABCC2	2.394
DCT	2.394
PNKD	2.394
EPHA2	2.394

TBC1D10C	2.394
C17orf51	2.394
FDX1L	2.394
KRTAP4-3	2.394
FBP1	2.394
MPV17	2.394
SLAMF6	2.394
UAP1L1	2.394
KRTAP8-1	2.394
LOC101060321	2.394
VASH2	2.394
SLC25A33	2.394
NMT1	2.394
LRRC23	2.394
MTCH2	2.394
CEACAM19	2.394
ZNF366	2.394
C1orf137	2.394
C5orf66	2.394
MYL6B	2.394
FBN3	2.394
ANGEL2	2.394
TMEM179B	2.394

Table S13. The top 20 pathways

GO	Description
hsa04080	Neuroactive ligand-receptor interaction
hsa04540	Gap junction
hsa04010	MAPK signaling pathway
has04024	cAMP signaling pathway
hsa05200	Pathways in cancer
hsa04020	Calcium signaling pathway
has04071	Sphingolipid signaling pathway
has04728	Dopaminergic synapse
hsa04721	Synaptic vesicle cycle
hsa00140	Steroid hormone biosynthesis
hsa00310	Lysine degradation
hsa04611	Platelet activation

hsa05014	Amyotrophic lateral sclerosis (ALS)
hsa05169	Epstein-Barr virus infection
hsa00052	Galactose metabolism
hsa04750	inflammatory mediator regulation of trp channels
hsa00260	Glycine, serine and threonine metabolism
hsa04060	Cytokine-cytokine receptor interaction
hsa04514	Cell adhesion molecules (CAMs)
has05204	Chemical carcinogenesis