Supplemental Material

Immunotoxic potentials of bisphenol F mediated through lipid signaling pathways on macrophages

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Table S1. Differential lipid metabolites induced by BPF exposure in macrophages

	Lipid	Composition	m/z	Formula	Errors (ppm)
SM	SM d40:1	SM(d22:0/18:1)	786.6615	C45 H91 O6 N2 P1	1
	SM d40:2	SM(d22:1/18:1)	784.6458	C45 H89 O6 N2 P1	1
	SM d42:1	SM(d14:0/28:1)	814.6928	C47 H95 O6 N2 P1	1
	SM d42:1	SM(d24:0/18:1)	814.6928	C47 H95 O6 N2 P1	1
	SM d42:2	SM(d18:1/24:1)	812.6771	C47 H93 O6 N2 P1	1
	SM d42:4	SM(d22:0/20:4)	808.6512	C47 H89 O6 N2 P1	1
	SM d43:1	SM(d25:0/18:1)	828.7084	C48 H97 O6 N2 P1	1
	SM d43:2	SM(d18:1/25:1)	826.6928	C48 H95 O6 N2 P1	1
Cer	Cer d34:2	Cer(d18:2/16:0)	535.4964	C34 H65 O3 N1	1
	Cer d40:2	Cer(d18:1/22:1)	619.5903	C40 H77 O3 N1	1
	Cer d41:1	Cer(d17:1/24:0)	635.6216	C41 H81 O3 N1	1
	Cer d42:1	Cer(d18:1/24:0)	649.6373	C42 H83 O3 N1	1
	Cer d42:2	Cer(d18:1/24:1)	647.6216	C42 H81 O3 N1	1
	Cer d42:3	Cer(d18:2/24:1)	645.6063	C42 H79 O3 N1	0
	Cer d44:2	Cer(d18:1/26:1)	675.6529	C44 H85 O3 N1	0
So	So 18:1	So (d18:1)	299.2824	C18 H38 O2 N1	1
	So 18:0	So (d18:0)	301.2981	C18 H40 O2 N1	1
PC	PC 26:0	PC(12:0/14:0)	649.4683	C34 H68 O8 N1 P1	0
	PC 31:1	PC(17:1/14:0)	717.5309	C39 H76 O8 N1 P1	0
	PC 32:1	PC(16:0/16:1)	731.5465	C40 H78 O8 N1 P1	0
	PC 35:1	PC(19:1/16:0)	773.5935	C43 H84 O8 N1 P1	0
	PC 35:1	PC(17:0/18:1)	773.5935	C43 H84 O8 N1 P1	0
	PC 35:3	PC(18:0/17:3)	769.5622	C43 H80 O8 N1 P1	1
	PC 36:2	PC(18:1/18:1)	785.5935	C44 H84 O8 N1 P1	1
	PC 42:10	PC(20:4/22:6)	853.5622	C50 H80 O8 N1 P1	1
	PC 44:2	PC(26:1/18:1)	897.7187	C52 H100 O8 N1 P1	1
	PC O-32:1	PC(16:0e/16:1)	717.5672	C40 H80 O7 N1 P1	1
	PC O-33:1	PC(16:0e/17:1)	731.5829	C41 H82 O7 N1 P1	1
	PC O-34:2	PC(16:0e/18:2)	743.5829	C42 H82 O7 N1 P1	1
	PC O-34:4	PC(14:0e/20:4)	739.5516	C42 H78 O7 N1 P1	0
	PC O-38:5	PC(16:0e/22:5)	793.5985	C46 H84 O7 N1 P1	0
	PC O-42:6	PC(20:0e/22:6)	847.6455	C50 H90 O7 N1 P1	0
	PC P-32:1	PC(16:0p/16:1)	715.5516	C40 H78 O7 N1 P1	0
	PC P-34:0	PC(16:0p/18:0)	745.5985	C42 H84 O7 N1 P1	0
	PC P-38:3	PC(18:0p/20:3)	795.6142	C46 H86 O7 N1 P1	1
	PC P-40:4	PC(18:0p/22:4)	821.6298	C48 H88 O7 N1 P1	0
	PC P-40:5	PC(18:0p/22:5)	819.6142	C48 H86 O7 N1 P1	0
PE	PE 36:2	PE(18:1/18:1)	743.5465	C41 H78 O8 N1 P1	1
	PE 36:3	PE(18:1/18:2)	741.5309	C41 H76 O8 N1 P1	1
	PE 36:4	PE(16:0/20:4)	739.5152	C41 H74 O8 N1 P1	1
	PE 36:5	PE(16:0/20:5)	737.4996	C41 H72 O8 N1 P1	0
	PE 38:1	PE(18:0/20:1)	773.5935	C43 H84 O8 N1 P1	0
	PE 38:3	PE(18:0/20:3)	769.5622	C43 H80 O8 N1 P1	0
	PE 40:5	PE(20:1/20:4)	793.5689	C45 H80 O8 N1 P1	0

	PE 40:6	PE(18:1/22:5)	791.5465	C45 H78 O8 N1 P1	0
	PE P-40:2	PE(18:1p/22:1)	783.6142	C45 H86 O7 N1 P1	0
PS	PS 36:1	PS (18:0/18:1)	789.5520	C42 H80 O10 N1 P1	0
	PS 36:2	PS (18:1/18:1)	787.5363	C42 H78 O10 N1 P1	0
	PS 40:6	PS(18:0/22:6)	835.5343	C46 H78 O10 N1 P1	0

Table S2. Effects of BPF on lipidomics-related gene expression in 10^{-10} M and 10^{-8} M BPF-treated macrophages

Lipid Metabolic Pathways	Gene	Gene Expression Fold			
		10^{-10} M of BPF/ Control		10^{-8} M of BPF/ Control	
		P	Fold	P	Fold
(1). Cer De novo synthetic pathway	Sptlc	0.05	1.6	0.03	2.3
	CerS	0.04	1.6	0.03	3.0
	SphK2	0.10	1.2	0.10	4.3
(2). SM hydrolysis	SMase	0.03	2.3	0.11	2.9
(3). Cer degradation pathway	CDase	0.02	1.3	0.12	2.7
	S1P lyase	0.32	2.6	0.05	3.9
(4).Kennedy pathway	Ccpt	0.11	2.2	0.03	2.0
	Pcyt2	0.02	3.4	0.11	3.5
	Cept	0.20	-0.8	0.04	-0.5
(5). PS Decarboxylation Pathway	Psd	0.05	-0.4	0.03	-0.6
(6). PEMT methylation pathway	Pemt	0.04	1.3	0.02	2.0
(7). PS synthesis	Pss1	0.03	-0.5	0.02	0.9
	Pss2	0.04	-0.4	0.24	-0.6

Figure S1. Apoptosis-related gene expression in macrophages following BPF and BPA exposure. Data were analyzed as means ± SD of eight replicate experiments (the symbol represents statistical significance between treated group and control group: * $P < 0.05$, ** $P < 0.01$; the same as below).

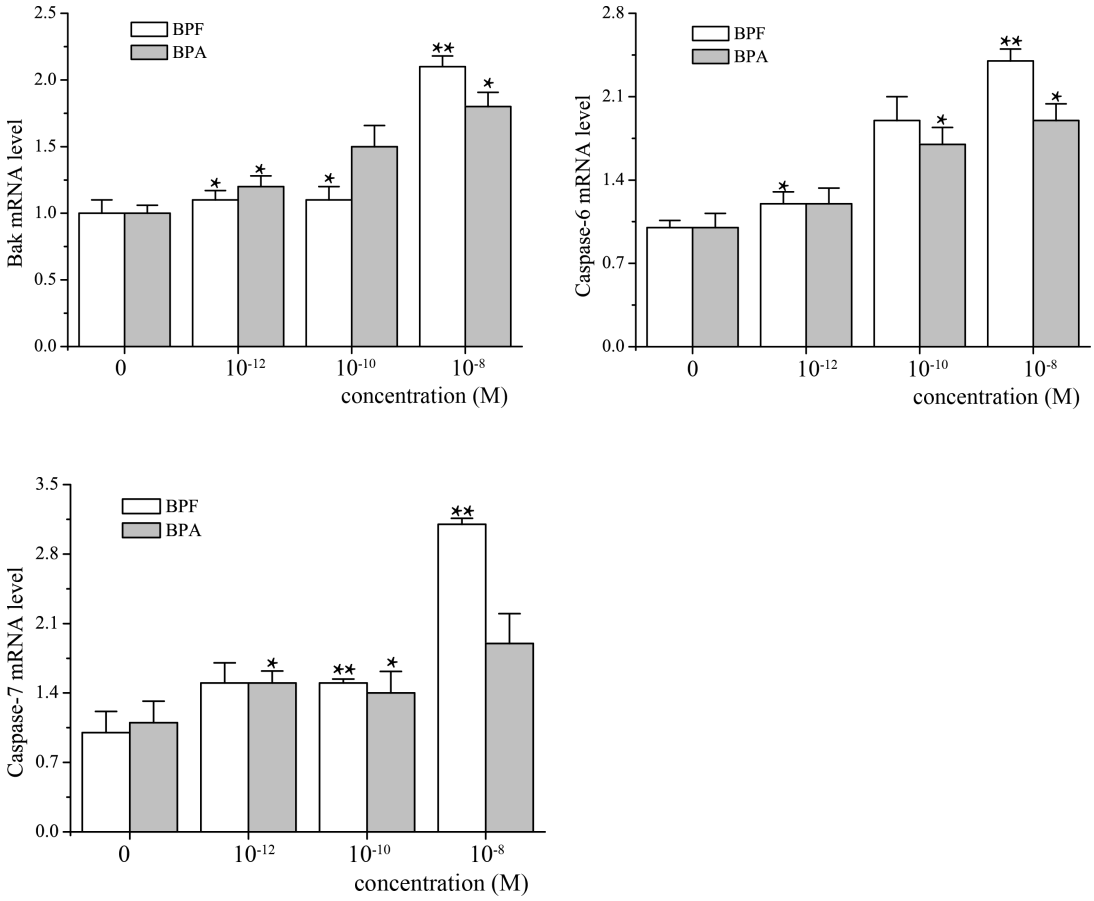
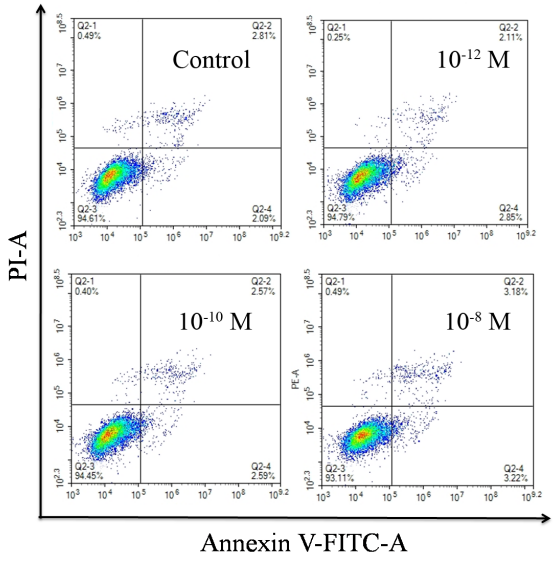


Figure S2. (A) Effects of BPA on macrophage apoptosis. Macrophages were administrated with BPF for 24 h, and Annexin V-FITC/PI was used to determine the cell apoptosis via flow cytometry. (B) The percentages of apoptotic cells were calculated from the ratio of apoptotic cells to total cells counted.

(A)



(B)

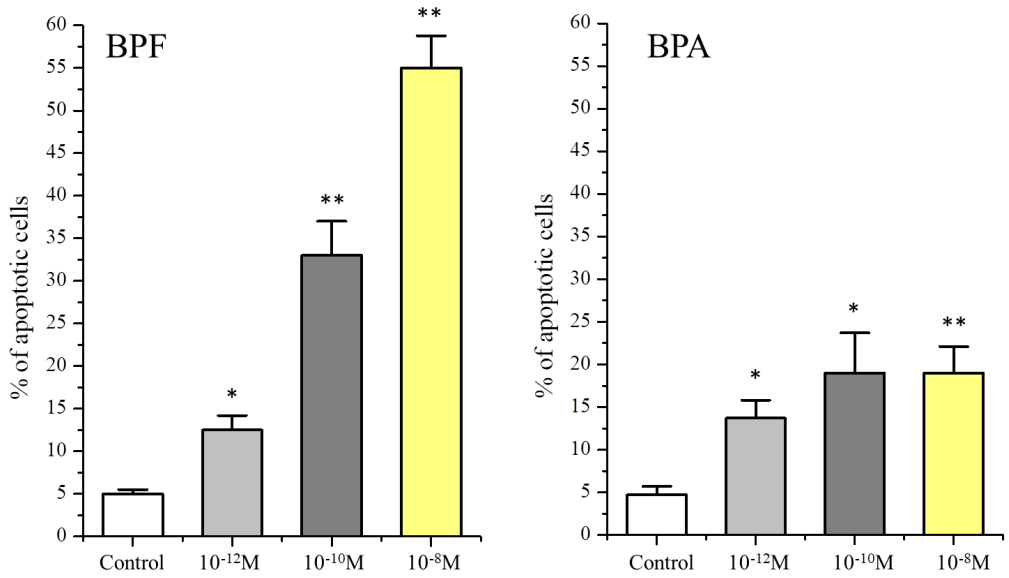


Figure S3. ROS formation in macrophages following BPF and BPA exposure. (A) MDA level; (B) GSH-Px activities; (C) SOD activities; (D) Effect of NAC on ROS generation. Macrophages were treated to NAC for 1 h firstly, and then co-incubated with 10^{-8} M of BPF or BPA.

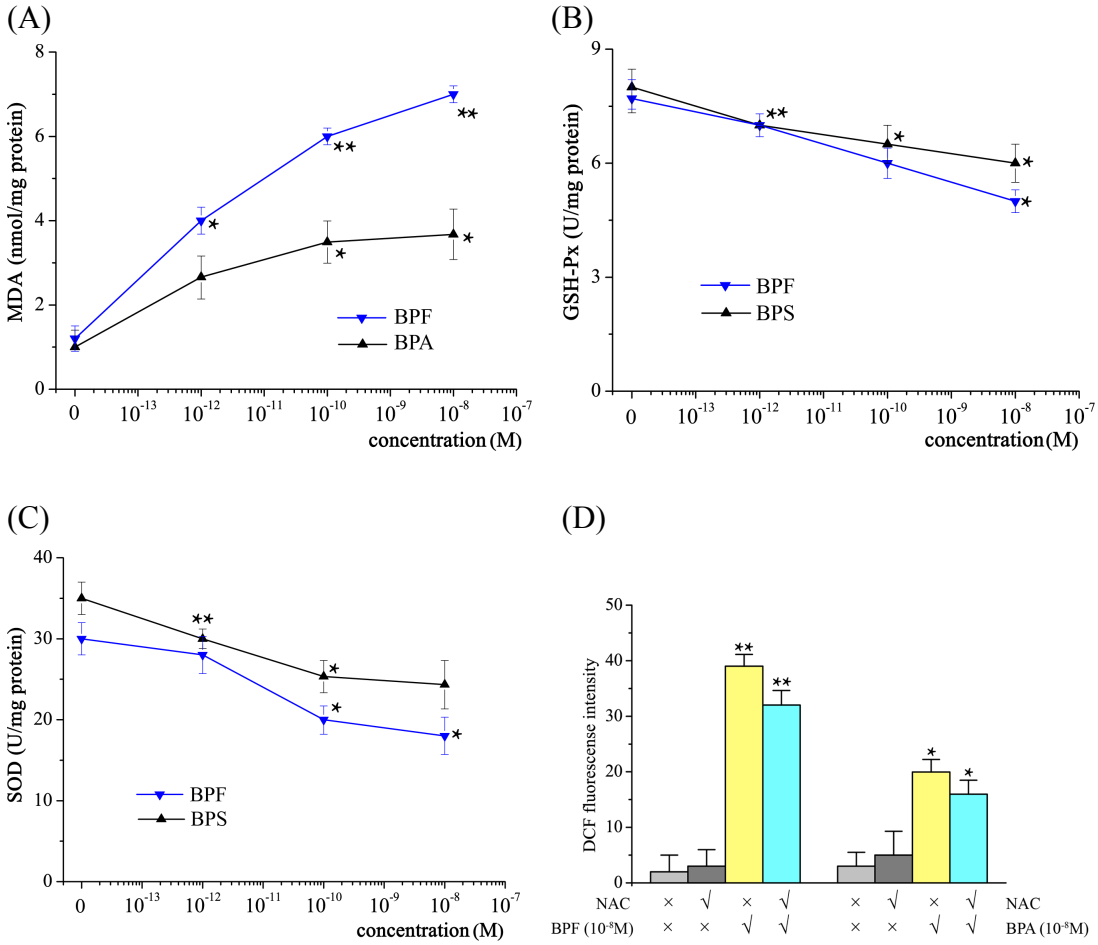


Figure S4. Expression and secretion of immune-related cytokines in macrophages (TGF-β and IL-10) following BPF (A and B) and BPA exposure (C and D).

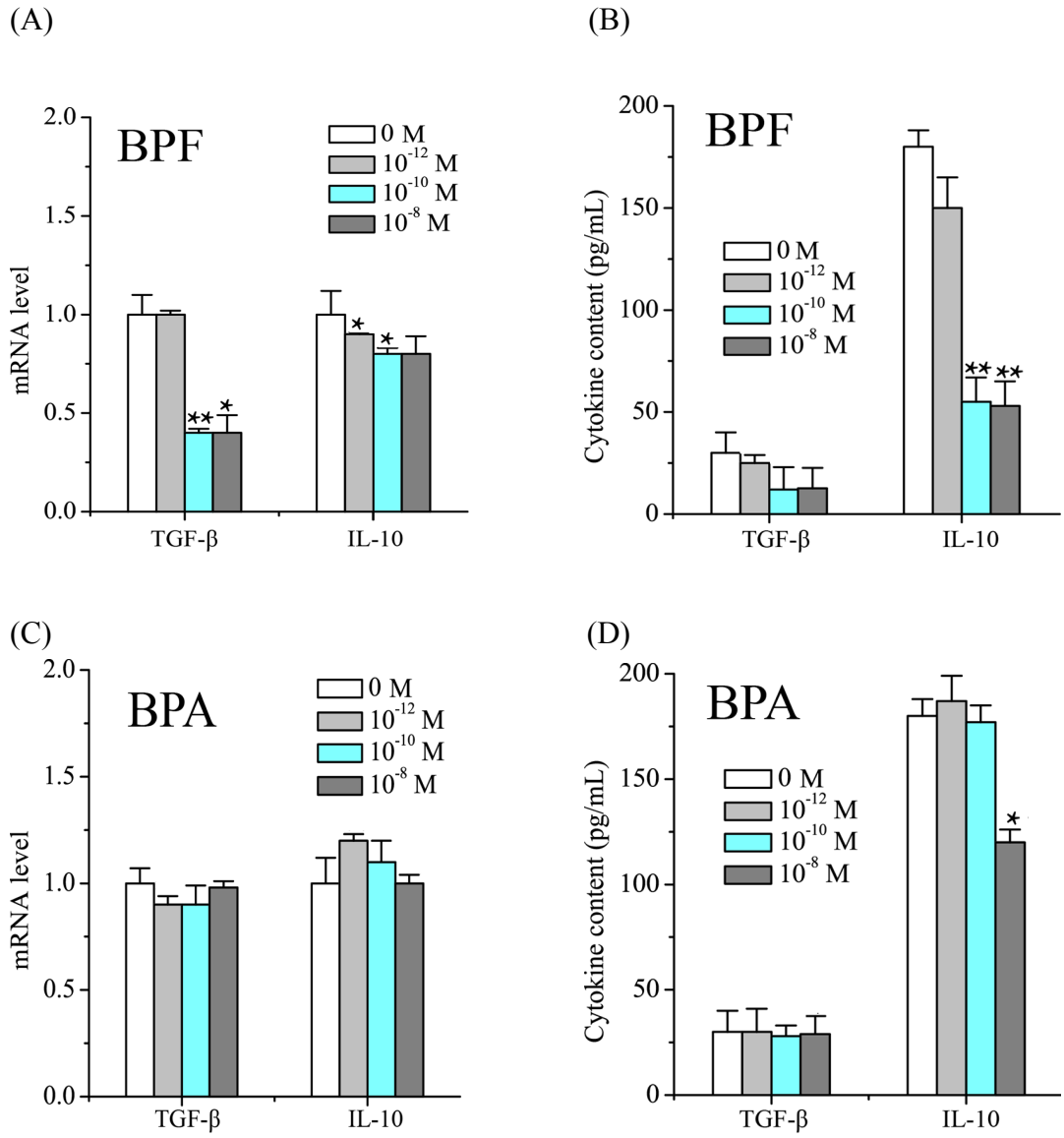


Figure S5. Lipidomics analysis in macrophages following BPF exposure. The PLS-DA score plots in positive (A) and negative ionization modes (B). The doses of 10^{-8}, 10^{-10} and 10^{-12} M BPF, negative control (0.002% DMSO in culture medium) and positive control (M1 phenotype) were used to multivariate statistical analysis (n=8).

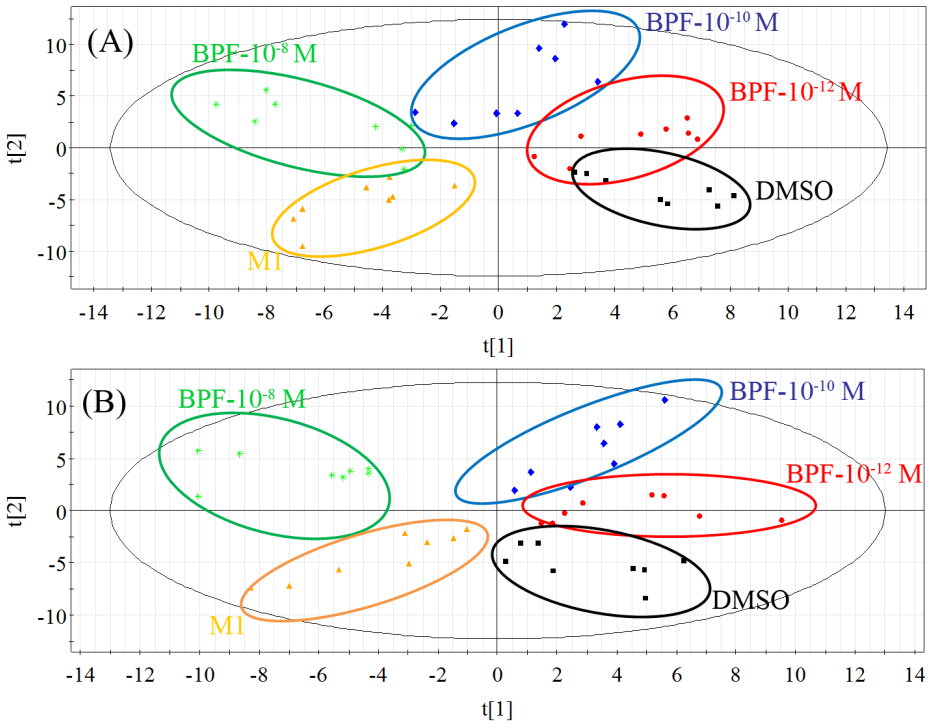
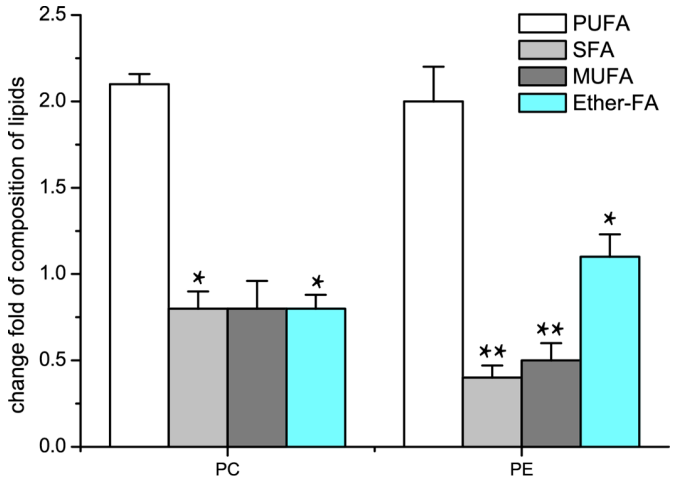
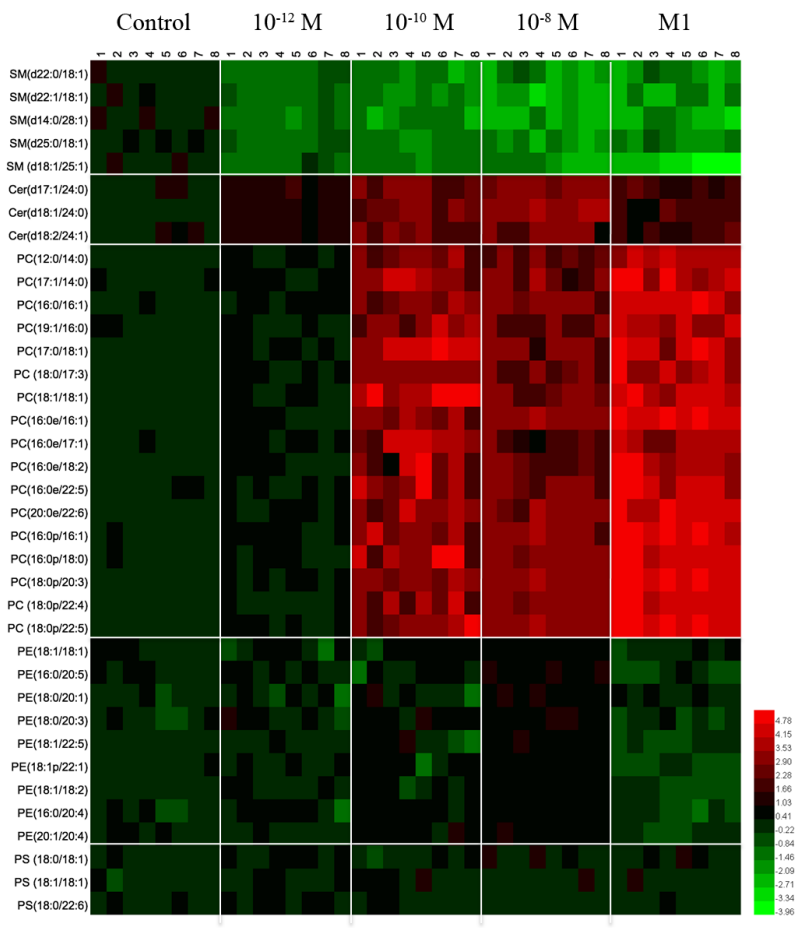


Figure S6. (A) Composition of acyl chains in four GPs categories including PC, PE, PI and PG. The identified chain composition of lipids by software LipidSearch were used the calculation of percentage of ether-linked FA, PUFA, MUFA- and SFA. (B) Heat map analysis for identified subclass lipids. The individual samples were represented in the vertical axis, and the identified lipids were represented in the horizontal axis. Up-, un- and down-regulated lipids were represented in red, black and green, respectively.

(A)



(B)


Control
10-12 M
10-10 M
10-8 M
M1
1 2 3 4 5 6 7 8
SM(d22:0/18:1)
SM(d22:1/18:1)
SM(d14:0/28:1)
SM(d25:0/18:1)
SM (d18:1/25:1)
Cer(d17:1/24:0)
Cer(d18:1/24:0)
Cer(d18:2/24:1)
PC(12:0/14:0)
PC(17:1/14:0)
PC(16:0/16:1)
PC(19:1/16:0)
PC(17:0/18:1)
PC (18:0/17:3)
PC(18:1/18:1)
PC(16:0e/16:1)
PC(16:0e/17:1)
PC(16:0e/18:2)
PC(16:0e/22:5)
PC(20:0e/22:6)
PC(16:0p/16:1)
PC(16:0p/18:0)
PC(18:0p/20:3)
PC (18:0p/22:4)
PC (18:0p/22:5)
PE(18:1/18:1)
PE(16:0/20:5)
PE(18:0/20:1)
PE(18:0/20:3)
PE(18:1/22:5)
PE(18:1p/22:1)
PE(18:1/18:2)
PE(16:0/20:4)
PE(20:1/20:4)
PS (18:0/18:1)
PS (18:1/18:1)
PS(18:0/22:6)
4.78
4.15
3.53
2.90
2.28
1.66
1.03
0.41
-0.22
-0.84
-1.46
-2.09
-2.71
-3.34
-3.96