

Supplementary Information:

***In situ* characterizing membrane lipid phenotype of Human Lung Cancer Cell Lines using mass spectrometry profiling**

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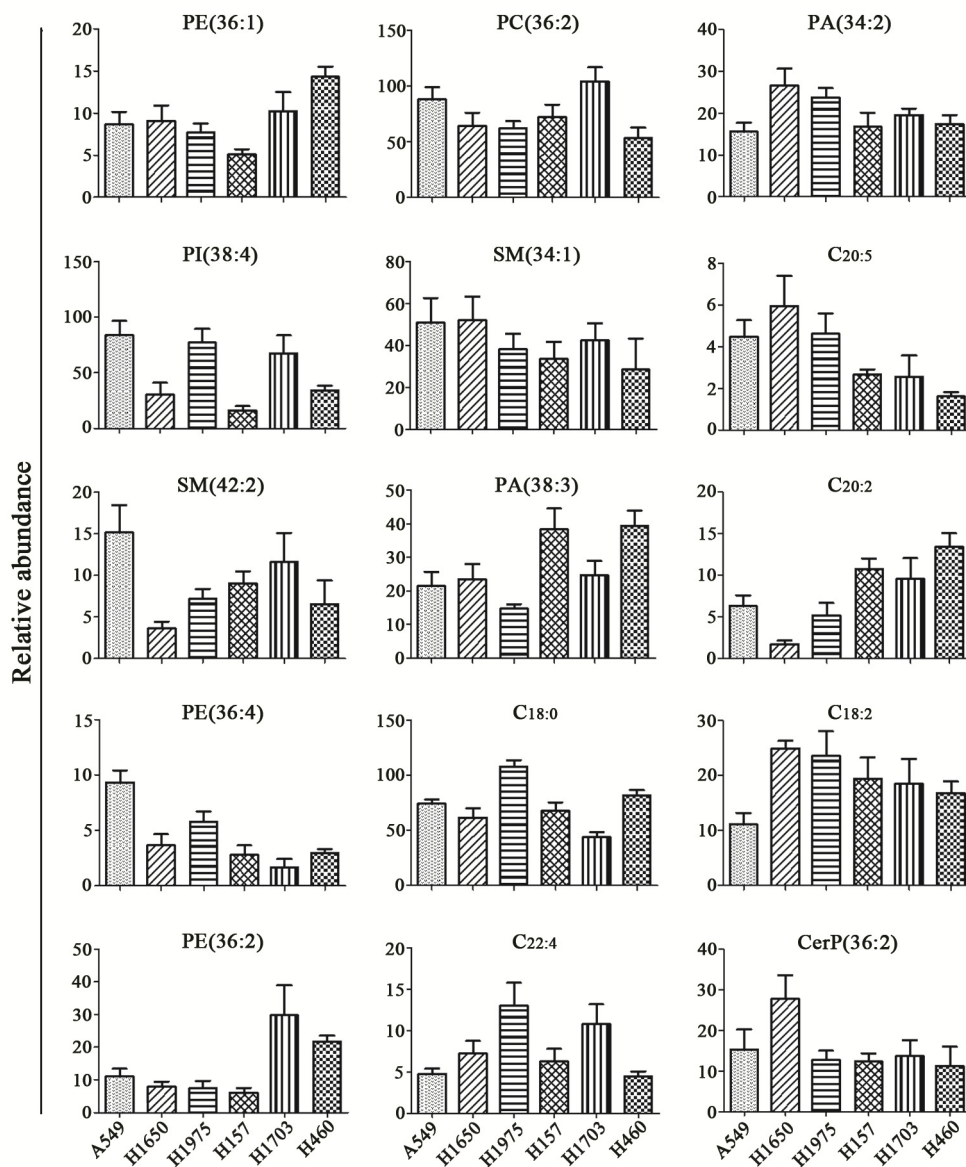


Figure S1. Fifteen important lipids (PE 18:0_18:1, PI 18:0_20:4, SM 42:2, PE 16:0_20:4, PE 36:2, PC 36:2, SM 34:1, PA 38:3, C_{18:0}, C_{22:4}, PA 34:2, C_{20:5}, C_{20:2}, C_{18:2}, and CerP 36:2) used to discriminate six NSCLC cell lines have significant differences in their levels. All data are expressed as mean ± SD and statistically significant differences (*p* values) of lipids between every two cell lines were listed in Supplementary Table S2.

Supplementary Table S1 Identification of significantly changed lipids using tandem mass spectrometry.

Lipids	Molecular Formula	Ion Form	Observed m/z	Theoretical m/z	Error /Da	Isotope distribution M : (M+1)		fragments
						Observed / Theoretical	Observed m/z	
CerP 36:2	C ₃₆ H ₇₀ NO ₆ P	[M+Na] ⁺	666.4832	666.4833	-0.0001	100.00:36.89/100.00:40.34		
		[M+K] ⁺	682.4575	682.4572	0.0003	100.00:43.01/100.00:40.35		
PA 34:2	C ₃₇ H ₆₉ O ₈ P	[M+Na] ⁺	695.4620	695.4622	-0.0002	100.00:37.02/100.00:41.12		
		[M+K] ⁺	711.4356	711.4362	-0.0006	100.00:38.54/100.00:41.13		
SM 34:1	C ₃₉ H ₇₉ N ₂ O ₆ P	[M+Na] ⁺	725.5569	725.5568	0.0001	100.00:49.01/100.00:44.06	725.5572/666.4828/542.4909	725.5568/666.4833/542.4908
		[M+K] ⁺	741.5300	741.5307	-0.0007	100.00:47.60/100.00:44.07	741.5307/682.4572/558.4647	741.5307/682.4572/558.4647
PC 30:0	C ₃₈ H ₇₆ NO ₈ P	[M+H] ⁺	706.5390	706.5381	0.0009	100.00:46.13/100.00:42.66		706.5382/184.0733
		[M+Na] ⁺	728.5195	728.5201	-0.0006	100.00:37.80/100.00:42.65	728.5201/669.4466/545.4540/523.4721	728.5201/669.4466/545.4540/523.4721
		[M+K] ⁺	744.4940	744.4940	0.0000	100.00:44.21/100.00:42.66	744.4945/685.4200/561.4257	744.4940/685.4205/561.4256
PA 36:2	C ₃₉ H ₇₃ O ₈ P	[M+Na] ⁺	723.4935	723.4935	0.0000	100.00:38.50/100.00:43.33		
		[M+K] ⁺	739.4669	739.4675	-0.0006	100.00:47.48/100.00:43.34		
PC 32:1	C ₄₀ H ₇₈ NO ₈ P	[M+H] ⁺	732.5537	732.5538	-0.0001	100.00:39.94/100.00:44.85		732.5538/184.0740
		[M+Na] ⁺	754.5350	754.5357	-0.0007	100.00:42.35/100.00:44.83	754.5356/695.4624/571.4695/549.4878	754.5357/695.4622/571.4697/549.4877
		[M+K] ⁺	770.5094	770.5097	-0.0003	100.00:40.70/100.00:44.85	770.5096/711.4363/587.4436	770.5097/711.4362/587.4436
PC 32:0	C ₄₀ H ₈₀ NO ₈ P	[M+Na] ⁺	756.5517	756.5514	0.0003	100.00:40.49/100.00:44.86		756.5511/697.4782/573.4852
		[M+K] ⁺	772.5261	772.5253	0.0008	100.00:38.88/100.00:44.87		772.5251/713.4519
PA 38:3	C ₄₁ H ₇₅ O ₈ P	[M+Na] ⁺	749.5092	749.5092	0.0000	100.00:50.02/100.00:45.51		
		[M+K] ⁺	765.4824	765.4831	-0.0007	100.00:45.13/100.00:45.52		
PC 34:1	C ₄₂ H ₈₂ NO ₈ P	[M+H] ⁺	760.5856	760.5851	0.0005	100.00:50.50/100.00:47.06		760.5850/184.0734
		[M+Na] ⁺	782.5662	782.5670	-0.0008	100.00:53.30/100.00:47.04	782.5669/723.4936/599.5010	782.5670/723.4935/599.5010
		[M+K] ⁺	798.5401	798.5409	-0.0008	100.00:51.03/100.00:47.06	798.5408/739.4676/615.4749	798.5409/739.4675/615.4749
PC 36:1	C ₄₄ H ₈₆ NO ₈ P	[M+Na] ⁺	810.5995	810.5983	0.0012	100.00:51.64/100.00:49.25		810.5987/751.5243/627.5324
PC 36:2	C ₄₄ H ₈₄ NO ₈ P	[M+Na] ⁺	808.5832	808.5827	0.0005	100.00:51.86/100.00:49.23		808.5829/749.5089/625.5166
		[M+K] ⁺	824.5563	824.5566	-0.0003	100.00:48.62/100.00:49.24		824.5566/765.4831/641.4906
SM 42:2	C ₄₇ H ₉₃ N ₂ O ₆ P	[M+Na] ⁺	835.6677	835.6663	0.0014	100.00:57.31/100.00:52.87		
		[M+K] ⁺	851.6407	851.6403	0.0004	100.00:56.37/100.00:52.88		
C _{16:0}	C ₁₆ H ₃₂ O ₂	[M-H] ⁻	255.2330	255.2330	0.0000	100.00:18.44/100.00:17.74		
C _{16:1}	C ₁₆ H ₃₀ O ₂	[M-H] ⁻	253.2173	253.2173	0.0000	100.00:18.71/100.00:17.72		
C _{18:0}	C ₁₈ H ₃₆ O ₂	[M-H] ⁻	283.2642	283.2643	-0.0001	100.00:16.13/100.00:19.95		
C _{18:1}	C ₁₈ H ₃₄ O ₂	[M-H] ⁻	281.2478	281.2486	-0.0008	100.00:18.63/100.00:19.92		
C _{18:2}	C ₁₈ H ₃₂ O ₂	[M-H] ⁻	279.2329	279.2330	-0.0001	100.00:17.62/100.00:19.90		
C _{20:1}	C ₂₀ H ₃₈ O ₂	[M-H] ⁻	309.2800	309.2799	0.0001	100.00:18.07/100.00:22.13		
C _{20:2}	C ₂₀ H ₃₆ O ₂	[M-H] ⁻	307.2646	307.2643	0.0003	100.00:17.12/100.00:22.11		
C _{20:3}	C ₂₀ H ₃₄ O ₂	[M-H] ⁻	305.2488	305.2486	0.0002	100.00:17.84/100.00:22.09		
C _{20:4}	C ₂₀ H ₃₂ O ₂	[M-H] ⁻	303.2329	303.2330	-0.0001	100.00:20.36/100.00:22.06		
C _{20:5}	C ₂₀ H ₃₀ O ₂	[M-H] ⁻	301.2174	301.2173	0.0001	100.00:22.92/100.00:22.04		
C _{22:4}	C ₂₂ H ₃₆ O ₂	[M-H] ⁻	331.2642	331.2643	-0.0001	100.00:20.08/100.00:24.27		
C _{22:5}	C ₂₂ H ₃₄ O ₂	[M-H] ⁻	329.2486	329.2486	0.0000	100.00:22.56/100.00:24.25		
C _{22:6}	C ₂₂ H ₃₂ O ₂	[M-H] ⁻	327.2329	327.2330	-0.0001	100.00:21.12/100.00:24.23		
PE18:0_18:1	C ₄₁ H ₈₀ NO ₈ P	[M-H] ⁻	744.5545	744.5549	-0.0004	100.00:39.98/100.00:45.93		744.5549/283.2643/281.2486
PE 36:2	C ₄₁ H ₇₈ NO ₈ P	[M-H] ⁻	742.5408	742.5392	0.0016	100.00:46.18 /100.00:45.90		742.5392/283.2643/281.2487/279.2330
PE 16:0_20:4	C ₄₁ H ₇₄ NO ₇ P	[M-H] ⁻	722.5121	722.5130	-0.0009	100.00:49.31/100.00:45.82		722.5130/303.2330/255.2329
PE 38:4	C ₄₃ H ₇₈ NO ₇ P	[M-H] ⁻	750.5450	750.5443	0.0007	100.00:47.83/100.00:48.03		750.5443/464.3147/331.2643/303.2329/
						283.2643/255.2328		/ 283.2643/255.2326
PI 18:0_20:4	C ₄₇ H ₈₃ O ₁₃ P	[M-H] ⁻	885.5494	885.5499	-0.0005	100.00:46.97/100.00:52.27		885.5500/581.3095/303.2330/283.2643

Supplementary Table S2 Lipids with significant differences among six NSCLC cell lines.

	PE(36:1)	PI(38:4)	SM(42:2)	PE(36:4)	PE(36:2)	PC(36:2)	SM(34:1)	PA(38:3)	C18:0	C22:4	PA(34:2)	C20:5	C20:2	C18:2	CerP(36:2)
A549 vs. H157	1.27E-10	1.50E-56	2.75E-18	2.87E-11	1.04E-10	3.63E-08	2.14E-09	1.43E-33	6.62E-05	1.42E-07	0.102428	1.00E-12	3.77E-22	6.13E-18	1.61E-02
A549 vs. H1650	0.2813946	5.75E-44	2.87E-11	2.14E-61	3.79E-03	5.71E-09	0.604839	1.35E-02	7.04E-10	2.39E-07	5.32E-10	7.32E-07	3.67E-23	2.87E-11	1.28E-15
A549 vs. H1703	6.07E-05	2.18E-08	3.81E-08	7.13E-81	3.68E-40	1.64E-08	3.22E-03	4.31E-03	2.87E-11	2.23E-28	6.33E-08	9.67E-09	2.71E-08	5.95E-15	0.224904
A549 vs. H1975	1.36E-02	4.13E-02	8.26E-27	1.41E-36	3.21E-06	3.02E-17	9.83E-06	1.23E-08	8.52E-52	5.35E-42	1.16E-23	0.964623	2.44E-03	1.14E-31	4.68E-02
A549 vs. H460	4.01E-32	2.32E-40	1.54E-10	7.32E-69	9.47E-19	6.75E-26	5.99E-14	9.87E-36	1.30E-06	0.541492	1.95E-04	2.87E-11	3.68E-41	5.84E-10	1.72E-03
H157 vs. H1650	8.49E-10	2.17E-06	2.87E-11	2.92E-04	1.21E-05	7.91E-04	1.80E-07	1.86E-10	3.09E-04	2.00E-03	1.48E-09	2.87E-11	8.04E-54	9.67E-09	2.26E-22
H157 vs. H1703	3.88E-11	2.46E-41	8.41E-05	9.85E-06	2.87E-11	1.14E-23	1.05E-03	2.90E-25	5.23E-11	4.78E-09	8.94E-05	0.351637	2.10E-03	0.271503	0.227006
H157 vs. H1975	1.69E-10	2.87E-11	3.11E-03	3.18E-11	9.27E-03	3.59E-04	0.07946	6.96E-50	1.01E-61	1.04E-10	5.15E-19	2.87E-11	2.87E-11	3.20E-06	0.669015
H157 vs. H460	2.87E-11	3.54E-09	1.72E-03	0.813005	2.87E-11	2.79E-10	0.065417	0.447773	2.01E-16	5.84E-10	0.064595	4.29E-11	2.97E-10	3.76E-03	0.459773
H1650 vs. H1703	2.82E-03	1.84E-27	2.87E-11	6.22E-17	4.62E-48	2.74E-10	1.34E-03	0.468798	9.44E-11	2.28E-13	5.84E-10	2.87E-11	2.87E-11	2.11E-07	5.94E-19
H1650 vs. H1975	4.54E-04	2.87E-11	3.18E-11	8.03E-18	0.859184	0.929315	1.58E-05	5.32E-10	3.18E-11	8.79E-27	8.58E-06	9.85E-06	2.87E-11	0.120575	3.72E-21
H1650 vs. H460	5.08E-29	0.188065	1.29E-04	1.04E-03	4.17E-27	4.85E-04	1.42E-07	3.88E-11	5.32E-10	1.17E-08	5.32E-10	2.87E-11	2.61E-69	2.87E-11	7.44E-09
H1703 vs. H1975	4.65E-10	1.66E-02	4.62E-11	4.76E-44	2.87E-11	4.04E-34	0.118737	8.43E-16	2.87E-11	3.05E-06	9.37E-09	3.66E-09	2.49E-10	1.70E-08	0.43401
H1703 vs. H460	2.42E-20	1.16E-23	5.66E-06	1.40E-08	2.61E-12	1.20E-42	5.92E-07	1.87E-27	2.87E-11	3.87E-30	1.81E-05	1.54E-04	2.28E-07	0.261176	4.28E-02
H1975 vs. H460	3.61E-39	2.87E-11	0.544408	2.69E-27	2.87E-11	2.57E-03	3.89E-04	7.86E-52	2.10E-38	1.16E-43	4.29E-11	2.87E-11	2.87E-11	7.39E-08	0.15581

P values labelled in red represent no statistical differences. The p values were obtained from One-Way ANOVA or Wilcoxon-Mann-Whitney test.

Supplementary Table S3. Correlation among saturated, monounsaturated, and polyunsaturated lipids in A549 cells.

	C_{16:0}	C_{18:0}	PC(30:0)	PC(32:0)	C_{16:1}	C_{18:1}	C_{20:1}	PC(32:1)	PC(34:1)	PC(36:1)	SM(34:1)	C_{20:3}	C_{20:4}	C_{22:4}	C_{22:5}	C_{22:6}	PE(36:4)	PE(38:4)	PI(38:4)
C_{16:0}	1	-0.5644	0.05539	-0.00111	0.80556	0.10256	0.14794	-0.1337	-0.02959	-0.02603	-0.20578	-0.18265	-0.56796	-0.13504	0.00512	0.05273	-0.49677	-0.44516	-0.65117
C_{18:0}		1	-0.18799	-0.07141	-0.51902	0.13103	0.23515	0.04205	0.22358	0.41802	0.103	0.604	0.58932	0.24004	0.24138	0.2921	0.19244	0.24627	0.4594
PC(30:0)			1	0.90122	0.35573	0.16307	0.0941	-0.4723	-0.79444	-0.503	0.62625	0.01268	-0.05584	-0.1515	0.15729	0.14972	-0.21735	-0.26897	-0.28187
PC(32:0)				1	0.18754	0.21513	0.14572	-0.72325	-0.73838	-0.29967	0.72147	0.10389	0.07453	-0.17686	0.14038	0.15462	-0.297	-0.25384	-0.26674
C_{16:1}					1	0.07631	0.08743	-0.12125	-0.2832	-0.27119	0.00957	-0.15862	-0.50923	-0.07809	0.06118	0.09455	-0.4069	-0.4941	-0.64983
C_{18:1}						1	0.74594	-0.11724	-0.02914	0.4505	-0.12659	0.60178	-0.07809	-0.24449	0.44516	0.68498	-0.30456	-0.44605	-0.33437
C_{20:1}							1	-0.0683	0.17553	0.45184	-0.03715	0.53237	-0.03226	0.06073	0.66808	0.71257	-0.28053	-0.26318	-0.21424
PC(32:1)								1	0.46652	0.12747	-0.47809	-0.20222	-0.13504	0.12214	-0.0198	0.00156	0.42736	0.21735	0.38509
PC(34:1)									1	0.52881	-0.44383	0.05762	0.02736	0.30634	0.12347	0.0416	0.17286	0.21201	0.39889
PC(36:1)										1	-0.29166	0.31479	0.14705	-0.00423	0.27564	0.39978	0.01179	0.0861	0.1119
SM(34:1)											1	-0.10478	0.3139	0.17686	0.05584	-0.04205	-0.07809	-0.05095	0.02469
C_{20:3}												1	0.1733	-0.19689	0.24182	0.46696	-0.20979	-0.25784	-0.11368
C_{20:4}													1	0.49455	0.27786	0.10434	0.44383	0.3931	0.5119
C_{22:4}														1	0.29655	0.02781	0.40912	0.47853	0.43582
C_{22:5}															1	0.7495	0.02425	-0.00289	0.15551
C_{22:6}																1	-0.07987	-0.08699	-0.04472
PE(36:4)																	1	0.7277	0.77264
PE(38:4)																		1	0.71969
PI(38:4)																			1

Correlation coefficient and p values were listed between every two lipids.

Supplementary Table S5. Correlation among saturated, monounsaturated, and polyunsaturated lipids in H1975 cells.

	C _{16:0}	C _{18:0}	PC(30:0)	PC(32:0)	C _{16:1}	C _{18:1}	C _{20:1}	PC(32:1)	PC(34:1)	PC(36:1)	SM(34:1)	C _{20:3}	C _{20:4}	C _{22:4}	C _{22:5}	C _{22:6}	PE(36:4)	PE(38:4)	PI(38:4)
C _{16:0}	1	0.43226	-0.38776	-0.43671	0.88476	-0.47898	-0.66007	0.41268	0.4812	-0.71657	-0.65784	-0.85495	-0.57375	-0.50033	-0.72859	-0.81446	-0.41179	-0.66274	-0.52303
C _{18:0}		1	-0.23026	-0.14483	0.52392	-0.66452	-0.49588	0.33437	0.40245	-0.36196	-0.34372	-0.31346	-0.14616	-0.04828	-0.13148	-0.28231	-0.10701	-0.28587	-0.05006
PC(30:0)			1	0.8376	-0.44872	0.5079	0.50968	0.10345	-0.22403	-0.07809	0.67297	0.42781	0.22447	0.25606	0.53771	0.44294	0.39711	0.38109	0.07675
PC(32:0)				1	-0.40156	0.4683	0.54572	-0.13103	-0.26541	0.14794	0.74683	0.49544	0.26496	0.22403	0.53726	0.4772	0.25517	0.32592	0.10167
C _{16:1}					1	-0.46919	-0.56574	0.27164	0.4198	-0.56529	-0.58754	-0.7366	-0.64494	-0.58487	-0.70412	-0.69967	-0.47408	-0.70723	-0.61068
C _{18:1}						1	0.87052	-0.2881	-0.43048	0.2921	0.48254	0.52659	0.01357	-0.11591	0.24627	0.45228	-0.09277	0.10879	-0.21958
C _{20:1}							1	-0.40423	-0.56618	0.51368	0.62892	0.78509	0.18621	0.04383	0.51146	0.6743	-0.04516	0.20133	-0.08877
PC(32:1)								1	0.62447	-0.67653	-0.4376	-0.38598	-0.204	-0.13949	-0.15818	-0.29433	-0.07853	-0.23204	-0.12925
PC(34:1)									1	-0.61691	-0.47275	-0.60979	-0.50122	-0.25695	-0.38643	-0.45717	-0.06251	-0.28142	-0.10345
PC(36:1)										1	0.45406	0.68098	0.52481	0.38465	0.47987	0.61735	0.22314	0.44828	0.43226
SM(34:1)											1	0.67119	0.32636	0.32592	0.65428	0.63337	0.34683	0.56752	0.196
C _{20:3}												1	0.59066	0.37931	0.72859	0.83092	0.2178	0.46518	0.30679
C _{20:4}													1	0.74994	0.68498	0.60311	0.53815	0.62581	0.65606
C _{22:4}														1	0.77798	0.61157	0.78687	0.80067	0.83893
C _{22:5}															1	0.89055	0.5733	0.72592	0.55773
C _{22:6}																1	0.45406	0.70189	0.47275
PE(36:4)																	1	0.83048	0.75172
PE(38:4)																		1	0.82603
PI(38:4)																			1

Correlation coefficient and p values were listed between every two lipids.

Supplementary Table S7. Correlation among saturated, monounsaturated, and polyunsaturated lipids in H1703 cells.

	C_{16:0}	C_{18:0}	PC(30:0)	PC(32:0)	C_{16:1}	C_{18:1}	C_{20:1}	PC(32:1)	PC(34:1)	PC(36:1)	SM(34:1)	C_{20:3}	C_{20:4}	C_{22:4}	C_{22:5}	C_{22:6}	PE(36:4)	PE(38:4)	PI(38:4)
C_{16:0}	1	0.29477	0.25918	-0.42959	0.15907	-0.29477	-0.32992	0.36463	0.30501	0.36151	-0.40868	-0.00289	0.33304	0.08254	0.17553	0.36774	0.48743	0.38776	0.23204
C_{16:1}		0.1138	0.1667	0.0178	0.4011	0.1138	0.075	0.0476	0.1012	0.0497	0.0249	0.9879	0.0721	0.6646	0.3535	0.0456	0.0063	0.0342	0.2173
C_{18:0}		1	0.10656	0.1297	-0.1564	-0.51012	-0.40378	-0.00289	-0.05317	0.15106	0.14394	0.01313	0.47364	0.14171	0.18665	0.10923	0.103	0.14928	0.38776
C_{18:1}			0.5752	0.4945	0.4092	0.004	0.0269	0.9879	0.7802	0.4256	0.4479	0.9451	0.0082	0.4551	0.3233	0.5656	0.5881	0.4311	0.0342
PC(30:0)			1	-0.54572	-0.21246	-0.44917	-0.4287	0.53281	0.62759	0.71613	-0.31746	0.04605	0.47008	0.36151	0.47052	0.51457	0.44783	0.5119	0.39711
PC(32:0)				1	-0.01491	0.14572	0.18487	-0.81268	-0.80556	-0.77219	0.81935	-0.15462	-0.22981	-0.24983	-0.38999	-0.4772	-0.48209	-0.45139	-0.06919
PC(32:1)					1	0.57953	0.46563	0.06118	0.1168	-0.15462	-0.13682	0.26318	-0.18176	-0.05006	0.07542	0.04828	-0.10478	-0.29833	-0.58977
PC(34:1)						1	0.91413	-0.27697	-0.19956	-0.38732	0.11235	0.32414	-0.38287	-0.08788	-0.22848	-0.3891	-0.54572	-0.68632	-0.83092
PC(36:1)							1	-0.31168	-0.31568	-0.34549	0.20667	0.30857	-0.41357	-0.09055	-0.29077	-0.503	-0.65829	-0.75039	-0.90211
SM(34:1)								1	0.89188	0.84071	-0.88343	0.03537	0.22314	0.16663	0.35751	0.53415	0.53415	0.42603	0.17775
C_{20:3}									1	0.82247	-0.79755	0.14082	0.31079	0.2356	0.45628	0.64494	0.58576	0.47275	0.21958
C_{20:4}										1	-0.67475	0.04828	0.30323	0.25339	0.30011	0.45673	0.44071	0.45228	0.29344
C_{22:4}											1	-0.07497	-0.14171	-0.05762	-0.28187	-0.4416	-0.46474	-0.33793	-0.11279
C_{22:5}												1	0.52169	0.64716	0.5911	0.35884	0.10033	0.01268	-0.21246
C_{22:6}													1	0.79355	0.79889	0.67653	0.5644	0.51324	0.51235
PE(36:4)														1	0.84516	0.62225	0.39488	0.45584	0.23693
PE(38:4)															1	0.86073	0.63737	0.61023	0.33348
PI(38:4)																1	0.8594	0.7673	0.47853

Correlation coefficient and p values were listed between every two lipids.

