Supplementary material as noted in the text

HILIC-ESI-FTMS with all ion fragmentation (AIF) scans as a tool for fast lipidome investigation

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Lipid identification by HILIC-ESI-FTMS in positive and negative ion modes

Although high resolution/accuracy FTMS instruments provide useful information on plasma lipids, accurate *m/z* values may introduce misleading results because of the presence of isobaric PL species. Elution times of lipid classes during HILIC separation, along with a comparison between ionization in positive and negative ion mode, can be exploited for a more confident assignment. First, this approach was employed to process data generated from plasma samples which were extracted in triplicate and analyzed in both ESI modes. Peak areas were normalized with respect to the specific PL class and only values with a RSD<20% on replicate analyses were examined further [1]. PI, PE, PE-O, LPE and LPE-O attributions were first obtained in negative ion mode and then a targeted quest was performed in positive ion mode, searching for protonated adducts of PE and ammonia adducts of PI. Choline-head containing lipids (i.e., PC, SM and LPC) were investigated as demethylated species in negative ion mode and then a focused analysis was performed also in ESI(+) for their positive ions (note that in this case the neat positive charge is related to the choline head, due to protonation of the phosphate group). Only species assigned in both polarities were considered for data processing. In ESI(-), Hex_1Cer ionizes mainly as deprotonated species while Hex_2Cer and Hex_3Cer ionize as chlorinated adducts. In positive ion mode, sodiated adducts contributed up to 25-30% to the peak signal (spectra not shown), while protonated adducts were the most abundant. Quehenberger *et al.* [2] reported the occurrence of PG, PS and PA in plasma samples; unfortunately, PS and PA did not provide a well definite chromatographic band and their intensity was relatively low, while only four PG passed the RSD criteria among replicates, i.e. PG 34:1, PG 36:1, PG 36:2, and PG 36:3. Since ions of these species were not retrieved in positive ion mode, they were not longer considered in the present work. We have recently reported the identification of neutral and acidic glycosphingolipids in human dermal

fibroblasts [3], evaluating the globosides (Gbl) levels. As already mentioned, extraction of ion current for *m/z* 264.270 allowed the identification of ceramide-based classes (see **Figure 4**, plot B); peak signals with *m/z* values compatible with (Gbl) were retrieved at around 10 minutes. Interestingly, as noticed for human dermal fibroblasts [3], the main (Gbl) detected in plasma were globotetraosyl Gb4b 34:1;2 and Gb4b 42:2;2, detected at *m/z* 1225.743 and *m/z* 1335.852 as deprotonated species, and at *m/z* 1261.719 and *m/z* 1371.882 as chlorinated adducts, respectively. Nevertheless, signals in positive ion mode did not fit RSD criteria and were not further considered; **Table S1** summarizes all lipid species identified in plasma sample by the adopted approach.

[1] W. B. Dunn *et al.*, "Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry," *Nat. Protoc.*, vol. 6, no. 7, pp. 1060–1083, Jul. 2011, doi: 10.1038/nprot.2011.335.

[2] O. Quehenberger *et al.*, "Lipidomics reveals a remarkable diversity of lipids in human plasma1," *J. Lipid Res.*, vol. 51, no. 11, pp. 3299–3305, 2010, doi: 10.1194/jlr.M009449.

[3] C. D. Calvano, G. Ventura, A. M. Sardanelli, I. Losito, F. Palmisano, and T. R. I. Cataldi, "Identification of neutral and acidic glycosphingolipids in the human dermal fibroblasts," *Anal. Biochem.*, vol. 581, no. April, p. 113348, 2019, doi: 10.1016/j.ab.2019.113348.

Table S1. Summary of glycosphingolipids and phospholipids identified in plasma sample of a healthy volunteer. Data are reported as class normalized intensities.

Specie #	Sum composition	Formula	Positive HRMS		Negative HRMS		
			adduct	*m/z*	adduct	*m/z*	Class-normalized relative abundance
HexCer (1.8-2.5 min)							
1	32:1;2	$C_{38}H_{73}NO_8$	$[HexCer+H]^+$ $[HexCer+Na]^+$ Intensity ratio: between 1 and 0.4	672.541, 694.523	$[HexCer-H]^-$ $[HexCer+Cl]^-$ Intensity ratio: between 1 and 0.5	670.526, 706.503	0.54 ± 0.05
2	33:1;2	$C_{39}H_{75}NO_8$		686.557, 708.538		684.542, 720.519	0.44 ± 0.02
3	34:1;2	$C_{40}H_{77}NO_8$		700.572, 722.554		698.558, 734.534	18.7 ± 1.2
4	34:2;2	$C_{40}H_{75}NO_8$		698.557, 720.538		696.542, 732.519	0.63 ± 0.03
5	36:1;2	$C_{42}H_{88}NO_8$		728.603, 750.585		726.589, 762.566	1.65 ± 0.04
6	36:2;2	$C_{42}H_{79}NO_8$		726.588, 748.570		724.573, 760.550	0.28 ± 0.02
7	38:1;2	$C_{44}H_{85}NO_8$		756.635, 778.617		754.620, 790.597	2.3 ± 0.1
8	38:2;2	$C_{44}H_{83}NO_8$		754.619, 770.650		752.605, 788.581	0.27 ± 0.02
9	39:1;2	$C_{45}H_{87}NO_8$		770.650, 792.632		768.636, 804.613	1.05 ± 0.03
10	40:1;2	$C_{46}H_{89}NO_8$		784.666, 806.648		782.652, 818.628	17.1 ± 0.4
11	40:2;2	$C_{46}H_{87}NO_8$		782.650, 804.632		780.636, 816.613	2.8 ± 0.1
12	41:1;2	$C_{47}H_{91}NO_8$		798.682, 820.664		796.667, 832.644	9.0 ± 0.4
13	41:2;2	$C_{47}H_{89}NO_8$		796.666, 818.648		794.652, 830.628	1.85 ± 0.05
14	42:1;2	$C_{48}H_{93}NO_8$		812.697, 834.679		810.683, 846.660	21.3 ± 0.4

15	42:2;2	$C_{48}H_{91}NO_8$		810.682, 832.664		808.667, 844.644	20.7 ± 0.3
16	43:1;2	$C_{49}H_{95}NO_8$		826.713, 848.695		824.698, 860.675	0.8 ± 0.04
17	43:2;2	$C_{49}H_{93}NO_8$		824.697, 846.679		822.683, 858.660	0.47 ± 0.02
Hex2Cer (4.5-6.5 min)							
Specie #	**Sum composition**	**Formula**	**Positive HRMS**		**Negative HRMS**		
			adduct	***m/z***	**adduct**	***m/z***	
18	32:1;2	$C_{44}H_{84}NO_{13}$		834.594, 856.576		868.556, 832.579	4.4 ± 0.5
19	33:1;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		882.571, 846.595	1.22 ± 0.05
20	34:1;2	$C_{45}H_{85}NO_{13}$		862.625, 884.607		896.587, 860.610	62.5 ± 0.2
21	34:2;2	$C_{46}H_{85}NO_{13}$		860.609, 882.591		894.571, 858.595	5.21 ± 0.12
22	36:1;2	$C_{48}H_{91}NO_{13}$		890.656, 912.638		924.618, 888.642	2.4 ± 0.3
23	38:1;2	$C_{50}H_{95}NO_{13}$		918.688, 940.67		952.65, 916.673	0.66 ± 0.04
24	40:1;2	$C_{52}H_{99}NO_{13}$	$Hex2Cer+H]^+$ $[Hex2Cer+Na]^+$	946.719, 968.701	$[Hex2Cer + Cl]^-$ $[Hex2Cer-H]^-$	980.681, 944.704	2.51 ± 0.02
25	40:2;2	$C_{52}H_{97}NO_{13}$		944.703, 966.685		978.665, 942.689	1.82 ± 0.1
26	41:1;2	$C_{53}H_{101}NO_{13}$	Intensity ratio: between 1 and 0.3	960.735, 982.717	Intensity ratio: between 1 and 0.3	994.697, 958.72	0.78 ± 0.04
27	41:2;2	$C_{43}H_{99}NO_{13}$		958.719, 980.701		992.681, 956.704	0.48 ± 0.01
28	42:1;2	$C_{54}H_{103}NO_{13}$		974.75, 996.732		1008.712, 972.736	3.02 ± 0.12
29	42:2;2	$C_{54}H_{101}NO_{13}$		972.735, 994.717		1006.697, 970.72	15.1 ± 0.4
Hex3Cer (8.5-9.5 min)							
30	32:1;2	$C_{44}H_{84}NO_{13}$	$Hex3Cer+H]^+$ $[Hex3Cer+Na]^+$	848.609, 870.591	$Hex3Cer + Cl]^-$ $[Hex3Cer-H]^-$	1030.609, 994.632	1.90 ± 0.11

31	34:0;2	$C_{44}H_{84}NO_{13}$	Intensity ratio: between 1 and 0.3	848.609, 870.591	Intensity ratio: between 1 and 0.3	1060.656, 1024.679	6.3 ± 0.2
32	34:1;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1058.64, 1022.663	37.1 ± 0.2
33	34:2;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1056.624, 1020.648	3.7 ± 0.2
34	36:1;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1086.671, 1050.695	5.58 ± 0.17
35	36:2;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1084.656, 1048.679	1.86 ± 0.10
36	38:1;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1114.703, 1078.726	2.67 ± 0.13
37	38:2;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1112.687, 1076.71	1.13 ± 0.07
38	40:1;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1144.75, 1108.773	8.0 ± 0.4
39	40:2;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1142.734, 1106.757	6.3 ± 0.4
40	42:0;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1140.718, 1104.742	1.17 ± 0.05
41	42:1;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1172.781, 1136.804	7.9 ± 0.3
42	42:2;2	$C_{44}H_{84}NO_{13}$		848.609, 870.591		1170.765, 1134.788	16.6 ± 0.7
			PI (7.5 – 9.0 min)				
43	32:1	$C_{41}H_{77}O_{13}P$	$[PI+NH_4]^+$	826.544	$[PI-H]^-$	807.503	2.2 ± 0.2
44	33:1	$C_{42}H_{79}O_{13}P$	$[PI+H]^+$	840.56		821.519	0.1 ± 0.01
45	34:1	$C_{43}H_{81}O_{13}P$	$[PI+Na]^+$	854.575		835.534	9.9 ± 0.9
46	34:2	$C_{43}H_{77}O_{13}P$		852.56		833.519	3.2 ± 0.2
47	36:0	$C_{45}H_{87}O_{13}P$	Intensity ratio: between 1, 0.3 and 0.2	884.622		865.581	0.60 ± 0.11
48	36:1	$C_{45}H_{85}O_{13}P$		882.607		863.566	9.0 ± 0.3
49	36:2	$C_{45}H_{83}O_{13}P$		880.591		861.550	15.0 ± 0.4
50	36:3	$C_{45}H_{81}O_{13}P$		878.575		859.534	2.9 ± 0.3
51	36:4	$C_{45}H_{79}O_{13}P$	Sodiated and protonated adducts give rise to quasi-isobaric species (i.e. 887.562 ± 0.003 may be $[PI\ 36:1\ +Na]^+$ or $[PI\ 38:4\ +H]^+$;	876.56		857.519	4.4 ± 0.2
52	38:3	$C_{47}H_{85}O_{13}P$		906.607		887.566	8.6 ± 0.3
53	38:4	$C_{47}H_{83}O_{13}P$		904.591		885.550	39.3 ± 0.4
54	38:5	$C_{47}H_{81}O_{13}P$		902.575		883.534	1.7 ± 0.2
55	38:6	$C_{47}H_{79}O_{13}P$		900.56		881.519	0.49 ± 0.01
56	40:5	$C_{49}H_{85}O_{13}P$		930.607		911.566	0.58 ± 0.04

57	40:6	$C_{49}H_{83}O_{13}P$	So, those adducts were not considered.	928.591		909.550	2.05 ± 0.07
			PE (9.5 – 10.5 min)				
58	34:0	$C_{39}H_{77}NO_8P$	$[PE+H]^+$ $[PE+Na]^+$ Intensity ratio: between 1 and 0.2 Sodiated and protonated adducts give rise to quasi-isobaric species.	720.554	$[PE-H]^-$	718.539	0.19 ± 0.01
59	34:1	$C_{39}H_{76}NO_8P$		718.538		716.524	3.15 ± 0.10
60	34:2	$C_{39}H_{74}NO_8P$		716.522		714.508	3.9 ± 0.2
61	34:3	$C_{39}H_{72}NO_8P$		714.507		712.492	0.11 ± 0.02
62	35:1	$C_{40}H_{80}NO_8P$		732.554		730.539	0.18 ± 0.01
63	36:0	$C_{41}H_{82}NO_8P$		748.585		746.571	0.22 ± 0.01
64	36:1	$C_{41}H_{80}NO_8P$		746.569		744.555	3.23 ± 0.03
65	36:2	$C_{41}H_{78}NO_8P$		744.554		742.539	17.2 ± 0.2
66	36:3	$C_{41}H_{76}NO_8P$		742.538		740.524	4.32 ± 0.08
67	36:4	$C_{41}H_{74}NO_8P$		740.522		738.508	8.6 ± 0.2
68	36:5	$C_{41}H_{72}NO_8P$		738.507		736.492	0.34 ± 0.02
69	37:2	$C_{42}H_{80}NO_8P$		758.569		756.555	0.12 ± 0.01
70	37:4	$C_{42}H_{76}NO_8P$		754.538		752.524	0.47 ± 0.01
71	38:1	$C_{42}H_{77}NO_8P$		774.601		772.586	0.14 ± 0
72	38:2	$C_{43}H_{82}NO_8P$		772.585		770.571	0.13 ± 0.01
73	38:3	$C_{43}H_{80}NO_8P$		770.569		768.555	2.29 ± 0.03
74	38:4	$C_{43}H_{78}NO_8P$		768.554		766.539	24.8 ± 0.2
75	38:5	$C_{43}H_{76}NO_8P$		766.538		764.524	6.00 ± 0.08
76	38:6	$C_{43}H_{74}NO_8P$		764.522		762.508	12.9 ± 0.5
77	38:7	$C_{43}H_{72}NO_8P$		762.507		760.492	0.18 ± 0.02
78	39:4	$C_{44}H_{80}NO_8P$		782.569		780.555	0.14 ± 0.01
79	39:6	$C_{44}H_{76}NO_8P$		778.538		776.524	0.30 ± 0.01
80	40:3	$C_{45}H_{84}NO_8P$		798.601		796.586	0.18 ± 0.03
81	40:4	$C_{45}H_{82}NO_8P$		796.585		794.571	0.52 ± 0.03
82	40:5	$C_{45}H_{80}NO_8P$		794.569		792.555	1.03 ± 0.04
83	40:6	$C_{45}H_{78}NO_8P$		792.554		790.539	6.19 ± 0.12
84	40:7	$C_{45}H_{76}NO_8P$		790.538		788.524	1.16 ± 0.02
85	40:9	$C_{45}H_{72}NO_8P$		786.507		784.492	0.37 ± 0.03
86	42:10	$C_{47}H_{74}NO_8P$		812.522		810.508	0.31 ± 0.03
87	42:11	$C_{47}H_{72}NO_8P$		810.507		808.492	0.74 ± 0.03
88	42:12	$C_{47}H_{70}NO_8P$		808.491		806.477	0.46 ± 0.02
			PE-O (9.5 – 10.5 min)				
89	34:1	$C_{39}H_{77}NO_7P$	$[PE\text{-}O +H]^+$	704.559	$[PE\text{-}O - H]^-$	702.544	0.36 ± 0.02
90	34:2	$C_{39}H_{76}NO_7P$		702.543		700.529	1.84 ± 0.08

91	34:3	$C_{39}H_{74}NO_7P$	$[PE\text{-}O +Na]^+$	700.528		698.513	3.54 ± 0.07
92	35:3	$C_{40}H_{76}NO_7P$		714.543		712.529	0.28 ± 0.01
93	36:1	$C_{41}H_{82}NO_7P$	Intensity ratio: between 1 and 0.2	732.59		730.576	0.12 ± 0.02
94	36:2	$C_{41}H_{80}NO_7P$		730.575		728.56	1.69 ± 0.04
95	36:3	$C_{41}H_{78}NO_7P$		728.559		726.544	7.95 ± 0.16
96	36:4	$C_{41}H_{76}NO_7P$		726.543		724.529	3.7 ± 0.2
97	36:5	$C_{41}H_{74}NO_7P$	Sodiated and protonated adducts give rise to quasi-isobaric species.	724.528		722.513	9.2 ± 0.5
98	36:6	$C_{41}H_{72}NO_7P$		722.512		720.497	0.39 ± 0.05
99	37:5	$C_{42}H_{76}NO_7P$		738.543		736.529	0.79 ± 0.01
100	37:6	$C_{42}H_{74}NO_7P$		736.528		734.513	0.14 ± 0.03
101	38:2	$C_{43}H_{84}NO_7P$		758.606		756.591	0.21 ± 0.01
102	38:3	$C_{43}H_{82}NO_7P$		756.59		754.576	0.61 ± 0.01
103	38:4	$C_{43}H_{80}NO_7P$		754.575		752.56	2.48 ± 0.07
104	38:5	$C_{43}H_{78}NO_7P$		752.559		750.544	23.1 ± 0.7
105	38:6	$C_{43}H_{76}NO_7P$		750.543		748.529	14.9 ± 0.4
106	38:7	$C_{43}H_{74}NO_7P$		748.528		746.513	6.25 ± 0.16
107	39:5	$C_{44}H_{80}NO_7P$		766.575		764.56	0.20 ± 0.01
108	39:6	$C_{44}H_{78}NO_7P$		764.559		762.544	0.28 ± 0.01
109	39:7	$C_{44}H_{76}NO_7P$		762.543		760.529	0.51 ± 0.03
110	40:3	$C_{45}H_{86}NO_7P$		784.621		782.607	0.17 ± 0.02
111	40:4	$C_{45}H_{84}NO_7P$		782.606		780.591	0.32 ± 0.01
112	40:5	$C_{45}H_{82}NO_7P$		780.59		778.576	2.06 ± 0.04
113	40:6	$C_{45}H_{80}NO_7P$		778.575		776.56	2.93 ± 0.08
114	40:7	$C_{45}H_{78}NO_7P$		776.559		774.544	10.1 ± 0.2
115	40:8	$C_{45}H_{76}NO_7P$		774.543		772.529	4.41 ± 0.08
116	42:5	$C_{47}H_{86}NO_7P$		808.621		806.607	0.29 ± 0.01
117	42:6	$C_{47}H_{84}NO_7P$		806.606		804.591	0.37 ± 0
118	42:7	$C_{47}H_{82}NO_7P$		804.59		802.576	0.66 ± 0.04
119	42:8	$C_{47}H_{80}NO_7P$		802.575		800.560	0.25 ± 0.01
			LPE (11.0 – 12.5 min)				
120	16:0	$C_{21}H_{43}NO_7P$	$[LPE+H]^+$	454.293	$[LPE\text{-}H]^-$	452.278	5.59 ± 0.18
121	16:1	$C_{21}H_{42}NO_7P$		452.277		450.263	0.23 ± 0.01
122	17:0	$C_{22}H_{46}NO_7P$		468.308		466.294	0.32 ± 0.02
123	18:0	$C_{23}H_{48}NO_7P$		482.324		480.31	16.3 ± 0.9
124	18:1	$C_{23}H_{46}NO_7P$		480.308		478.294	14.7 ± 0.3
125	18:2	$C_{23}H_{44}NO_7P$		478.293		476.278	15.3 ± 0.3
126	18:3	$C_{23}H_{42}NO_7P$		476.277		474.263	0.23 ± 0.02
127	20:2	$C_{25}H_{48}NO_7P$		506.324		504.31	0.54 ± 0.02

128	20:3	$C_{25}H_{46}NO_7P$		504.308		502.294	3.16 ± 0.07
129	20:4	$C_{25}H_{44}NO_7P$		502.293		500.278	21.8 ± 0.9
130	20:5	$C_{25}H_{42}NO_7P$		500.277		498.263	0.76 ± 0.05
131	22:0	$C_{27}H_{56}NO_7P$		538.387		536.372	0.24 ± 0.01
132	22:2	$C_{27}H_{52}NO_7P$		534.355		532.341	0.35 ± 0.01
133	22:4	$C_{27}H_{48}NO_7P$		530.324		528.31	0.75 ± 0.06
134	22:5	$C_{27}H_{46}NO_7P$		528.308		526.294	2.66 ± 0.06
135	22:6	$C_{27}H_{44}NO_7P$		526.293		524.278	16.6 ± 0.2
136	24:0	$C_{29}H_{60}NO_7P$		566.418		564.403	0.45 ± 0.03
LPE-O (11.0 – 12.5 min)							
137	16:1	$C_{21}H_{44}NO_6P$		438.298		436.283	22.3 ± 0.7
138	17:1	$C_{22}H_{46}NO_6P$		452.314		450.299	2.82 ± 0.15
139	18:1	$C_{23}H_{48}NO_6P$		466.329		464.315	38.9 ± 0.9
140	18:2	$C_{23}H_{46}NO_6P$		464.314		462.299	13.3 ± 0.4
141	18:3	$C_{23}H_{44}NO_6P$		462.298		460.283	0.38 ± 0.03
142	19:1	$C_{24}H_{50}NO_6P$		480.345		478.330	0.37 ± 0.01
143	20:0	$C_{25}H_{54}NO_6P$		496.376		494.362	0.36 ± 0.01
144	20:1	$C_{25}H_{52}NO_6P$	$[LPE\text{-}O + H]^+$	494.361	$[LPE\text{-}O - H]^-$	492.346	4.4 ± 0.2
145	20:2	$C_{25}H_{50}NO_6P$		492.345		490.330	0.77 ± 0.06
146	22:0	$C_{27}H_{58}NO_6P$		524.407		522.393	0.90 ± 0.04
147	22:1	$C_{27}H_{56}NO_6P$		522.392		520.377	2.91 ± 0.14
148	22:2	$C_{27}H_{54}NO_6P$		520.376		518.362	0.75 ± 0.09
149	24:0	$C_{29}H_{62}NO_6P$		552.439		550.424	2.82 ± 0.14
150	24:1	$C_{29}H_{60}NO_6P$		550.423		548.409	4.07 ± 0.03
151	24:2	$C_{21}H_{44}NO_6P$		548.407		546.393	4.35 ± 0.06
152	26:2	$C_{21}H_{44}NO_6P$		576.439		574.424	0.59 ± 0.03
PC (14.5 – 16.0 min)							
153	30:0	$C_{38}H_{76}NO_8P$		706.538	$[PC\text{-}Me]^-$ $[PC + HCOO]^-$ Intensity ratio: between 1 and 0.9	690.508, 750.529	0.56 ± 0.02
154	32:0	$C_{40}H_{80}NO_8P$		734.569		718.539, 778.56	1.86 ± 0.05
155	32:1	$C_{40}H_{78}NO_8P$	$[PC + H]^+$	732.554		716.524, 776.545	1.29 ± 0.03
156	32:2	$C_{40}H_{76}NO_8P$		730.538		714.508, 774.529	0.31 ± 0.02
157	33:1	$C_{41}H_{80}NO_8P$		746.569		730.539, 790.56	0.25 ± 0.01

158	33:2	$C_{41}H_{78}NO_8P$	744.554		728.524, 788.545	0.19 ± 0.01
159	34:1	$C_{42}H_{82}NO_8P$	760.585		744.555, 804.576	14.6 ± 0.2
160	34:2	$C_{42}H_{80}NO_8P$	758.569		742.539, 802.56	25.2 ± 0.5
161	34:3	$C_{42}H_{78}NO_8P$	756.554		740.524, 800.545	0.53 ± 0.01
162	35:1	$C_{43}H_{84}NO_8P$	774.601		758.571, 818.592	0.16 ± 0.01
163	35:2	$C_{43}H_{82}NO_8P$	772.585		756.555, 816.576	0.37 ± 0.02
164	36:1	$C_{44}H_{86}NO_8P$	788.616		772.586, 832.607	1.46 ± 0.03
165	36:2	$C_{44}H_{84}NO_8P$	786.601		770.571, 830.592	10.3 ± 0.2
166	36:3	$C_{44}H_{82}NO_8P$	784.585		768.555, 828.576	6.50 ± 0.09
167	36:4	$C_{44}H_{80}NO_8P$	782.569		766.539, 826.56	11.1 ± 0.2
168	36:5	$C_{44}H_{78}NO_8P$	780.554		764.524, 824.545	0.50 ± 0.01
169	37:4	$C_{45}H_{82}NO_8P$	796.585		780.555, 840.576	0.21 ± 0.02
170	38:3	$C_{46}H_{86}NO_8P$	812.616		796.586, 856.607	2.37 ± 0.06
171	38:4	$C_{48}H_{88}NO_8P$	810.601		794.571, 854.592	8.9 ± 0.2
172	38:5	$C_{46}H_{82}NO_8P$	808.585		792.555, 852.576	3.0 ± 0.2
173	38:6	$C_{46}H_{80}NO_8P$	806.569		790.539, 850.56	4.75 ± 0.14
174	40:4	$C_{48}H_{88}NO_8P$	838.632		822.602, 882.623	0.24 ± 0.02
175	40:5	$C_{48}H_{86}NO_8P$	836.616		820.586, 880.607	0.73 ± 0.01
176	40:6	$C_{48}H_{84}NO_8P$	834.601		818.571, 878.592	3.92 ± 0.11
177	40:7	$C_{48}H_{82}NO_8P$	832.585		816.555, 876.576	0.68 ± 0.03

178	40:8	$C_{48}H_{80}NO_8P$	830.569		814.539, 874.56	0.14 ± 0.02
			PC-O (14.5 – 16.0 min)			
179	30:0	$C_{38}H_{78}NO_7P$			676.529	1.12 ± 0.01
180	30:1	$C_{38}H_{76}NO_7P$			674.513	0.41 ± 0.01
181	32:0	$C_{40}H_{82}NO_7P$			704.560	9.3 ± 0.2
182	32:1	$C_{40}H_{80}NO_7P$			702.544	2.95 ± 0.07
183	32:2	$C_{40}H_{78}NO_7P$			700.529	0.39 ± 0.02
184	34:0	$C_{42}H_{86}NO_7P$			732.591	0.39 ± 0.03
185	34:1	$C_{42}H_{84}NO_7P$			730.576	8.0 ± 0. 2
186	34:2	$C_{42}H_{82}NO_7P$			728.560	8.8 ± 0.4
187	34:3	$C_{42}H_{80}NO_7P$			726.544	4.8 ± 0.2
188	36:1	$C_{44}H_{88}NO_7P$			758.607	0.34 ± 0.03
189	36:2	$C_{44}H_{86}NO_7P$			756.591	1.44 ± 0.06
190	36:3	$C_{44}H_{84}NO_7P$			754.576	3.09 ± 0.11
191	36:4	$C_{44}H_{82}NO_7P$	$[PC\text{-}O + H]^+$		$[PC\text{-}O - Me]^-$	752.560	7.0 ± 0.2
192	36:5	$C_{44}H_{80}NO_7P$			750.544	14.8 ± 0.7
193	36:6	$C_{44}H_{78}NO_7P$			748.529	0.56 ± 0.01
194	38:3	$C_{46}H_{88}NO_7P$			782.607	0.25 ± 0.02
195	38:4	$C_{46}H_{86}NO_7P$			780.591	3.5 ± 0.2
196	38:5	$C_{46}H_{84}NO_7P$			778.576	15.2 ± 0.4
197	38:6	$C_{46}H_{82}NO_7P$			776.560	9.8 ± 0.3
198	38:7	$C_{46}H_{80}NO_7P$			774.544	4.6 ± 0.2
199	38:8	$C_{46}H_{78}NO_7P$			772.529	0.64 ± 0.06
200	40:4	$C_{48}H_{90}NO_7P$			808.623	0.30 ± 0.02
201	40:5	$C_{48}H_{88}NO_7P$			806.607	1.13 ± 0.04
202	40:10	$C_{48}H_{78}NO_7P$			796.529	0.49 ± 0.02
203	42:5	$C_{50}H_{92}NO_7P$			834.638	0.70 ± 0.03
			SM (16.0 – 17.0 min)			
204	32:1;2	$C_{37}H_{75}N_2O_6P$	675.544	$[SM - Me]^-$ $[SM + HCOO]^-$ Intensity ratio: between 1 and 0.3	659.513, 719.534	5.66 ± 0.19
205	32:2;2	$C_{37}H_{73}N_2O_6P$	673.528		657.498, 717.519	0.39 ± 0.01
206	33:1;2	$C_{38}H_{77}N_2O_6P$	689.559		673.529, 733.550	1.95 ± 0.03
207	34:0;2	$C_{39}H_{81}N_2O_6P$	705.591		689.56, 749.581	1.7 ± 0.3

208	34:1;2	$C_{39}H_{79}N_2O_6P$	703.575		687.545, 747.566	46.2 ± 0.3
209	34:2;2	$C_{39}H_{77}N_2O_6P$	701.559		685.529, 745.550	6.95 ± 0.07
210	35:1;2	$C_{40}H_{81}N_2O_6P$	717.591		701.560, 761.581	1.00 ± 0.01
211	36:0;2	$C_{41}H_{85}N_2O_6P$	733.622		717.592, 777.613	0.36 ± 0.05
212	36:1;2	$C_{41}H_{83}N_2O_6P$	731.606		715.576, 775.597	7.82 ± 0.08
213	36:2;2	$C_{41}H_{81}N_2O_6P$	729.591		713.560, 773.581	4.22 ± 0.08
214	37:1;2	$C_{42}H_{85}N_2O_6P$	745.622		729.592, 789.613	0.4 ± 0.01
215	38:0;2	$C_{43}H_{89}N_2O_6P$	761.653		745.623, 805.644	0.19 ± 0.01
216	38:1;2	$C_{43}H_{87}N_2O_6P$	759.637		743.607, 803.628	3.05 ± 0.04
217	38:2;2	$C_{43}H_{85}N_2O_6P$	757.622		741.592, 801.613	1.33 ± 0.04
218	39:1;2	$C_{44}H_{89}N_2O_6P$	773.653		757.623, 817.644	0.65 ± 0.01
219	40:1;2	$C_{45}H_{91}N_2O_6P$	787.669		771.639, 831.660	3.46 ± 0.10
220	40:2;2	$C_{45}H_{89}N_2O_6P$	785.653		769.623, 829.644	2.55 ± 0.07
221	41:1;2	$C_{46}H_{93}N_2O_6P$	801.684		785.654, 845.675	1.08 ± 0.02
222	41:2;2	$C_{46}H_{91}N_2O_6P$	799.669		783.639, 843.660	0.97 ± 0.01
223	42:1;2	$C_{47}H_{95}N_2O_6P$	815.700		799.670, 859.691	2.87 ± 0.01
224	42:2;2	$C_{47}H_{93}N_2O_6P$	813.684		797.654, 857.675	7.24 ± 0.15
			LPC (17 – 18.0 min)			
225	15:0	$C_{23}H_{48}NO_7P$	$[LPC+H]^+$ 482.324	$[LPC-Me]^-$ $[LPC+HCOO]^-$	466.294, 526.315	0.24 ± 0.01
226	16:0	$C_{24}H_{50}NO_7P$	496.340		480.31, 540.331	41.3 ± 0.5

227	16:1	$C_{24}H_{48}NO_7P$	494.324	Intensity ratio: between 1 and 0.4	478.294, 538.315	0.79 ± 0.03
228	17:0	$C_{25}H_{52}NO_7P$	510.355		494.325, 554.346	1.07 ± 0.01
229	18:0	$C_{26}H_{54}NO_7P$	524.371		508.341, 568.362	21.6 ± 0.4
230	18:1	$C_{26}H_{52}NO_7P$	522.355		506.325, 566.346	13.3 ± 0.2
231	18:2	$C_{26}H_{50}NO_7P$	520.340		504.31, 564.331	13.1 ± 0. 2
232	18:3	$C_{26}H_{48}NO_7P$	518.324		502.294, 562.315	0.18 ± 0.01
233	20:1	$C_{28}H_{56}NO_7P$	550.387		534.357, 594.378	0.15 ± 0.01
234	20:2	$C_{28}H_{54}NO_7P$	548.371		532.341, 592.362	0.14 ± 0.01
235	20:3	$C_{28}H_{52}NO_7P$	546.355		530.325, 590.346	2.04 ± 0.04
236	20:4	$C_{28}H_{50}NO_7P$	544.340		528.31, 588.331	4.43 ± 0.05
237	20:5	$C_{28}H_{48}NO_7P$	542.324		526.294, 586.315	0.25 ± 0.01
238	22:5	$C_{30}H_{52}NO_7P$	570.355		554.325, 614.346	0.23 ± 0.01
239	22:6	$C_{30}H_{50}NO_7P$	568.340		552.310, 612.331	1.16 ± 0.04
			LPC-O (17 – 18.0 min)			
240	16:1	$C_{24}H_{50}NO_6P$			464.315	35.8 ± 0.3
241	18:0	$C_{26}H_{56}NO_6P$			494.362	11.3 ± 0.2
242	18:1	$C_{26}H_{54}NO_6P$			492.346	25.9 ± 0. 2
243	18:2	$C_{26}H_{52}NO_6P$			490.330	3.2 ± 0.2
244	20:0	$C_{28}H_{60}NO_6P$			522.393	2.5 ± 0.2
245	20:1	$C_{28}H_{58}NO_6P$	$[LPC\text{-}O + H]^+$		$[LPC\text{-}O - Me]^-$ 520.377	2.0 ± 0.2
246	20:2	$C_{28}H_{56}NO_6P$			518.362	0.31 ± 0.05
247	22:0	$C_{60}H_{64}NO_6P$			550.424	4.0 ± 0.2
248	22:1	$C_{60}H_{62}NO_6P$			548.409	3.38 ± 0.02
249	22:2	$C_{30}H_{60}NO_6P$			546.393	0.43 ± 0.02
250	23:0	$C_{31}H_{66}NO_6P$			564.440	0.40 ± 0.03
251	24:1	$C_{32}H_{66}NO_6P$			576.440	9.0 ± 0.3

252	24:2	$C_{32}H_{64}NO_6P$			574.424	1.53 ± 0
253	26:2	$C_{34}H_{68}NO_6P$			602.455	0.47 ± 0.02

Table S2. Summary of sphingoid base signals found for SM and Hex_2Cer of plasma samples. Data are reported in relative terms (%). Mean values and standard deviations obtained from three replicates are reported.

C18 sphingosine (*m/z* 264)		Sphingadiene (*m/z* 262)		C16 sphingosine (*m/z* 236)	
Hex2Cer	**SM**	**Hex2Cer**	**SM**	**Hex2Cer**	**SM**
47 ± 1	30 ± 1	5.5 ± 0.7	10.0 ± 0.4	2.2 ± 0.2	4.7 ± 0.3

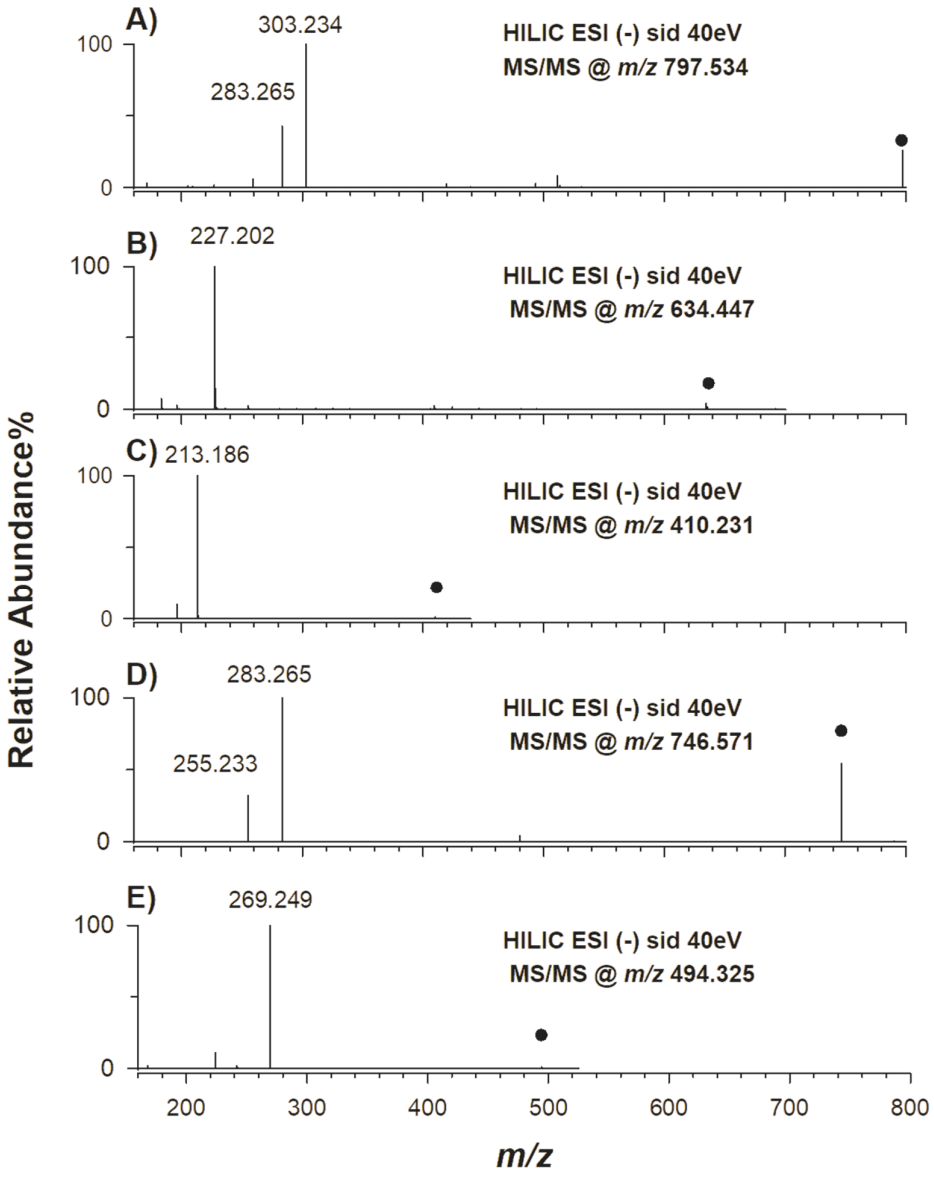

Figure S1. ESI-MS/MS (normalized collisional energy used: 30%) spectra related to standard phospholipids referred to A) PG 18:0/20:4 at *m/z* 797.534, B) PE 14:0/14:0 at *m/z* 634.447, C) LPE 13:0/0:0 at *m/z* 410.231, D) PC 16:0/18:0 at *m/z* 746.571 and E) LPC 17:0/0:0 at *m/z* 494.325. Precursor ions in A-C were isolated as deprotonated molecules $[M-H]^-$, while precursors in D and E were isolated as demethylated ion $[M-CH_3]^-$. Molecular structures for standard PL are reported in Figure 1.

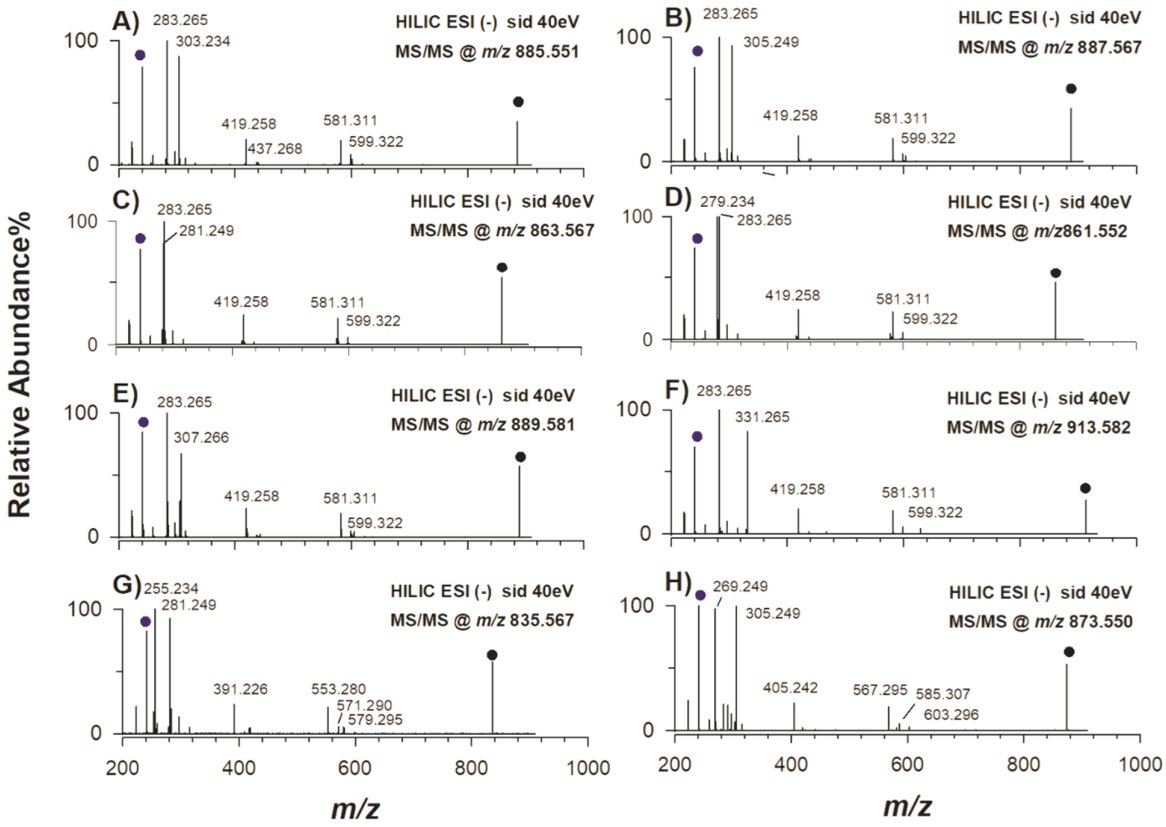

Figure S2. FT MS/MS spectra obtained in negative ion mode on deprotonated species of the main PI found in bovine liver standard extract : A) PI 18:0/20:4 at *m/z* 885.551, B) PI 18:0/20:3 at *m/z* 887.567, C) PI 18:0/18:1 at *m/z* 863.567, D) PI 18:0/18:2 at *m/z* 861.552, E) PI 18:0/20:2 at *m/z* 889.581, F) PI 18:0/22:4 at *m/z* 913.582, G) PI 16:0/18:1 at *m/z* 835.567, H) PI 17:0/20:3 at *m/z* 873.550.

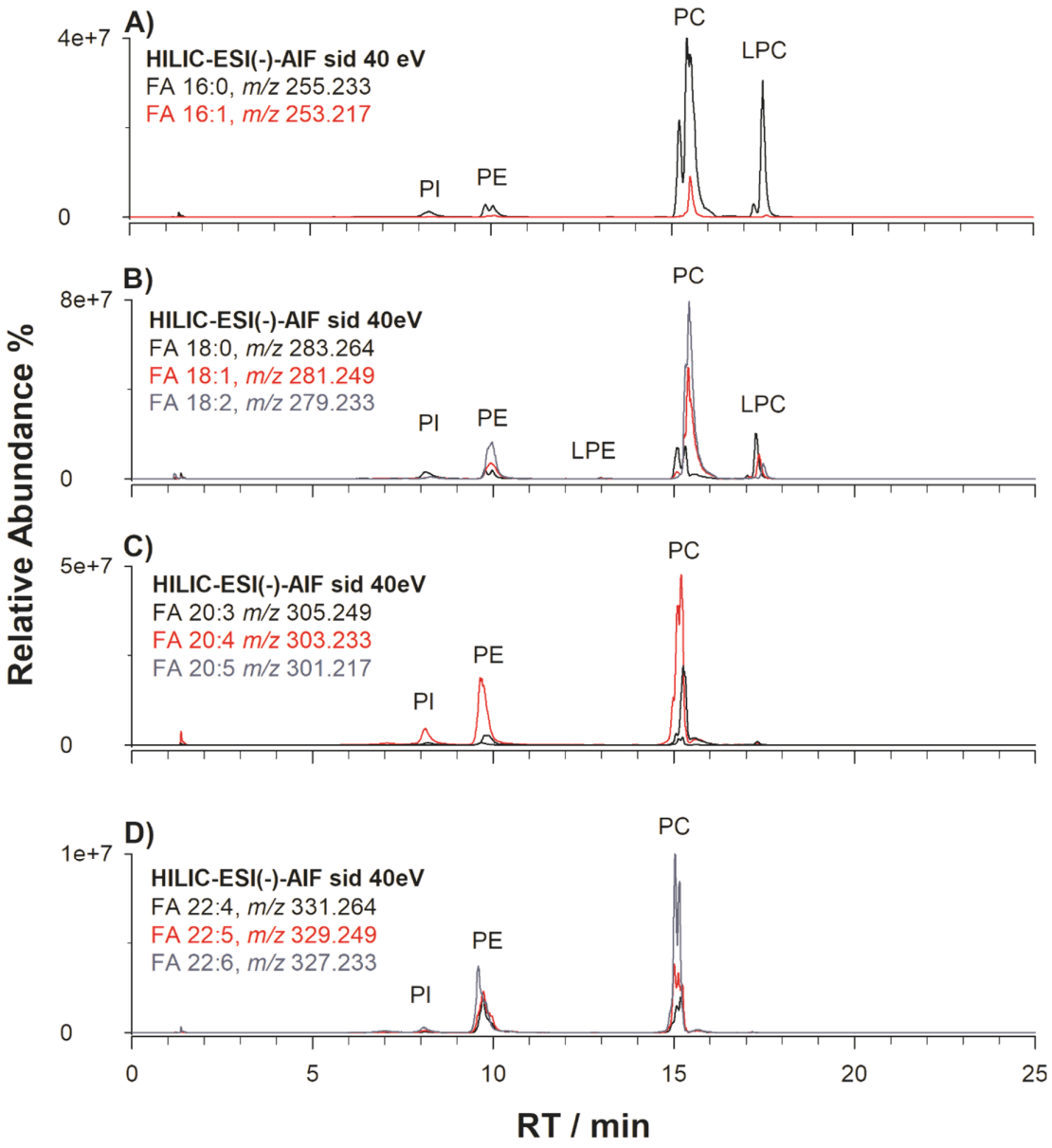

Figure S3. Extracted ion current chromatograms (XIC) obtained upon HILIC-ESI-FTMS analysis of a plasma sample using all ion fragmentation (AIF). XIC chromatograms were obtained using narrow windows centred on selected *m/z* values corresponding to those of the most interesting carboxylate ions in the different lipid classes (see Table 2).

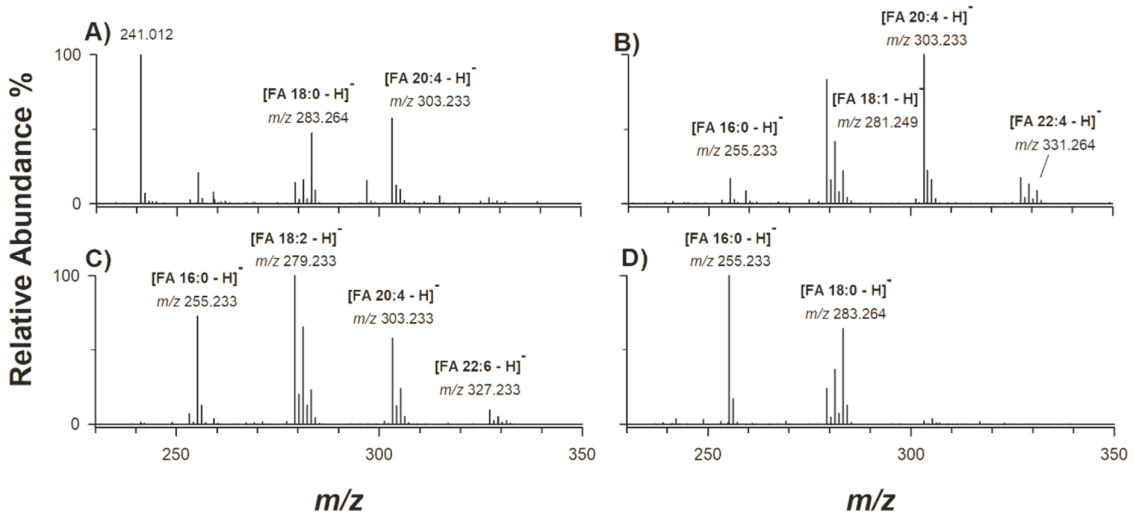

Figure S4. AIF MS spectra averaged under the HILIC bands related to the PL classes of PI (A), PE (B), PC (C) and LPC (D), as obtained upon HILIC-ESI(-)-FTMS analysis of a plasma lipid extract.

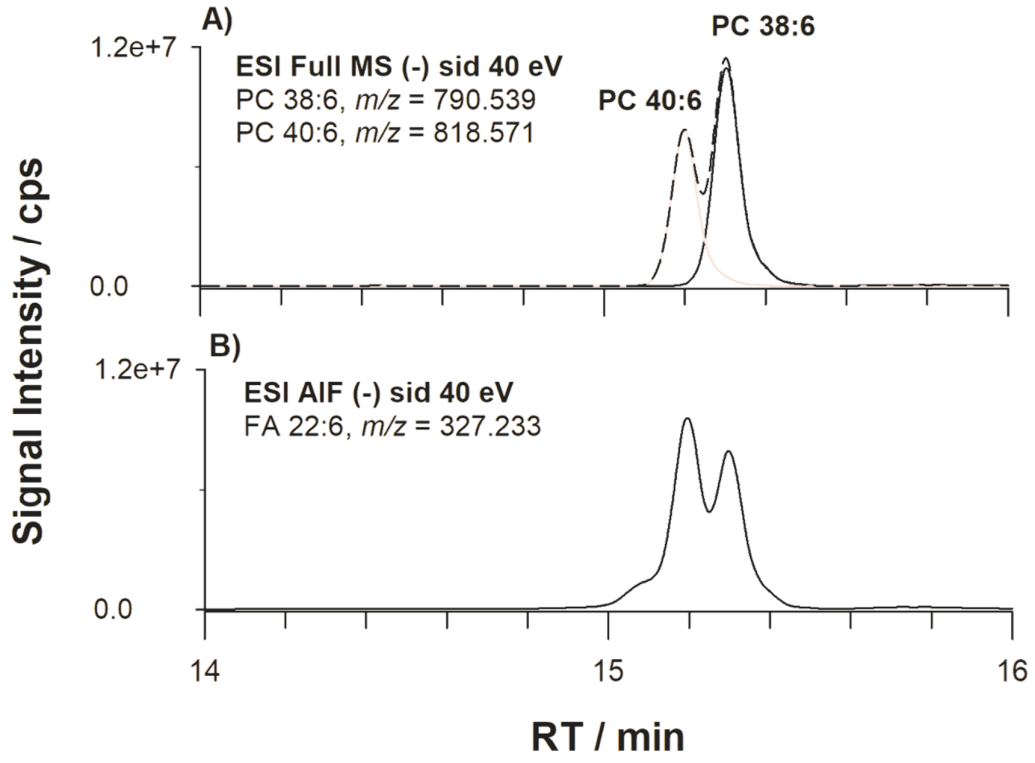

Figure S5. Extracted ion current chromatograms referred to: (A) the carboxylate ion of FA 22:6, and (B) the negative ions related to PC 40:6 and PC 38:6, obtained upon HILIC-ESI(-)FTMS analysis of a plasma extract with AIF MS and Full MS acquisition, respectively. Note that Source Induced Fragmentation (sid) at an energy of 40 eV was applied in both cases.

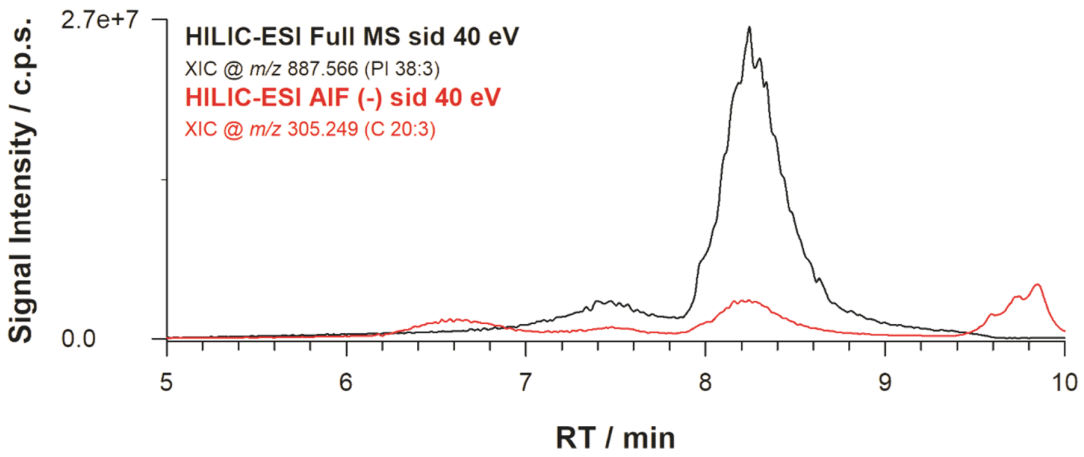

Figure S6: Superimposition of XIC traces referred to PI 38:3 and to the carboxylate of FA 20:3, obtained using Full MS and AIF MS acquisition modes, respectively, during the HPLC-ESI(-)-FTMS analysis of the lipid extract of human dermal fibroblasts.