

Supplementary Material

Experimental

In Vivo PLA Assay

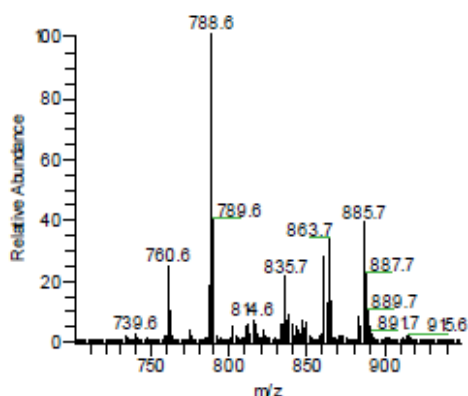
EA. hy926 cells were seeded onto 100 mm culture plates at a density of 2×10^6 cells per plate and grown into confluence before use. Cells were preincubated for 2 h with 1 ml liposomes suspension and induced by 0.2 mM H_2O_2 for 0 h and 1 h, respectively. The liposomes suspension was prepared as published protocols¹. Briefly, the bis-BODIPY-PC, a molecular probe providing a continuous fluorescence response to PLA action, and PS16:0/16:0 at a molar ratio of 1:9 were dissolved in chloroform and dried under gentle stream of nitrogen, then desiccated overnight. The dried film of lipids was rehydrated in PBS at a concentration of 1 μ M of bis-BODIPY-PC and sonicated for 30 min on ice. Liposomes were used immediately after preparation. Treated cells were added with a volume of 2 ml chloroform/methanol (v/v=2/1, 0.01% BHT) and a volume of 1 ml 1 M KCl, mixed well and centrifuged ($2,000 \times g$, 4 °C, 5 min). The organic layer was then recovered and dried under gentle nitrogen stream and redissolved in 100 μ l chloroform (0.01% BHT) for thin layer chromatography (TLC) analysis as previously described². The resulting samples were spotted onto a TLC plate (Merck silica gel 60) in a solvent system of chloroform/methanol/water (v/v/v=65/25/4). Intensities of fluorescent BODIPY-LPC were semi-quantified by a UVP imaging system with the LabWorks 4.6 software (UVP Products, Upland, CA, USA). PLA activity was assayed by the formation of BODIPY-LPC and expressed as the fold of control.

References

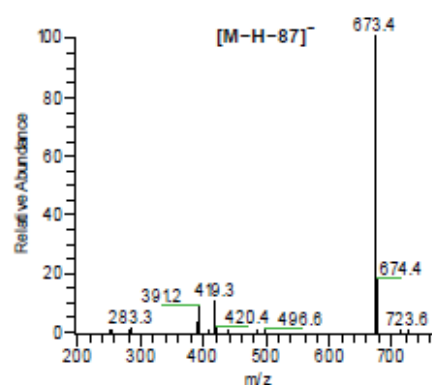
1. T. Meshulam, H. Herscovitz, D. Casavant, J. Bernardo, R. Roman, R. P. Haugland, G. S. Strohmeier, R. D. Diamond and E. R. Simons, *The Journal of biological chemistry*, 1992, **267**, 21465-21470.
2. G. F. Scherer, R. U. Paul, A. Holk and J. Martinec, *Biochemical and Biophysical Research Communications*, 2002, **293**, 766-770.

Figures

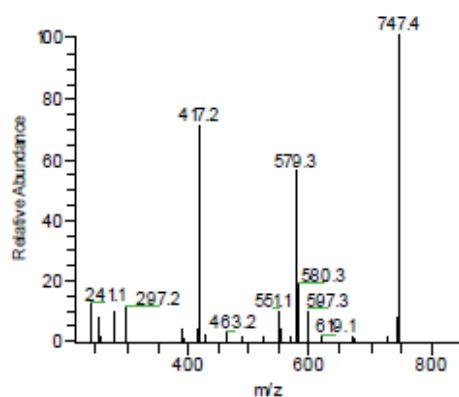
(A) MS¹ of PI/PS



(C) MS² of PS34:2, m/z 760



(B) MS² of PI34:2, m/z 833



| | | |
|--------------|---------|--|
| PS 16:0/18:1 | m/z 391 | [M-88-C ₁₇ H ₃₃ COOH] ⁻ |
| PS 18:0/16:1 | m/z 419 | [M-88-C ₁₆ H ₂₉ COOH] ⁻ |
| PI 16:0/18:2 | m/z 255 | [C ₁₆ H ₃₁ COO] ⁻ |
| | m/z 577 | [M-H ₂ O-C ₁₆ H ₃₁ CO] ⁻ |
| | m/z 279 | [C ₁₇ H ₃₁ COO] ⁻ |
| | m/z 553 | [M-H ₂ O-C ₁₇ H ₃₁ CO] ⁻ |
| PI 18:1/16:1 | m/z 281 | [C ₁₇ H ₃₃ COO] ⁻ |
| | m/z 551 | [M-H ₂ O-C ₁₇ H ₃₃ CO] ⁻ |
| | m/z 253 | [C ₁₆ H ₂₉ COO] ⁻ |
| | m/z 579 | [M-H ₂ O-C ₁₆ H ₂₉ CO] ⁻ |

Fig. S1. Negative-ion MSⁿ analysis of PI and PS molecular species from EA.hy926 cells. (A) Averaged MS¹ spectrum of PI/PS. (B) MS² spectrum of PI34:2. (C) MS² spectrum of PS34:2. Featured fragment ions of PI34:2 and PS34:2 are listed.

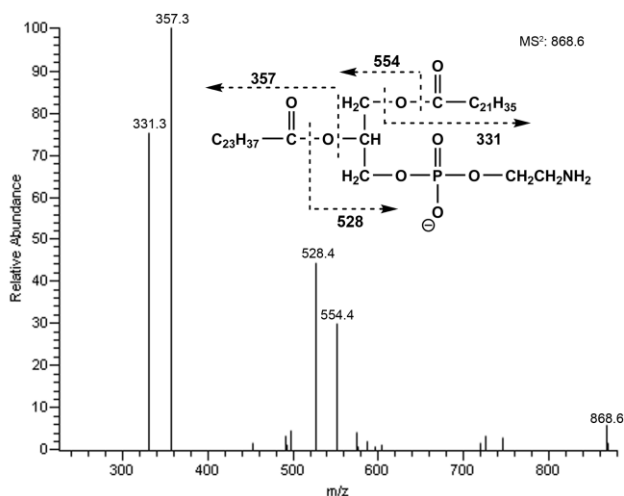


Fig S2. The MS² analysis of PE46:9 (m/z 868.6) as illustration of qualitative identification of long chain polyunsaturated fatty acid containing phospholipids. The MS² fragment ions m/z 331.3, 357.3, 528.4 and 554.4 were corresponding to [C₂₁H₃₅COO]⁻, [C₂₃H₃₇COO]⁻, [M-C₂₃H₃₇CO]⁻ and [M-C₂₁H₃₅CO]⁻, respectively.

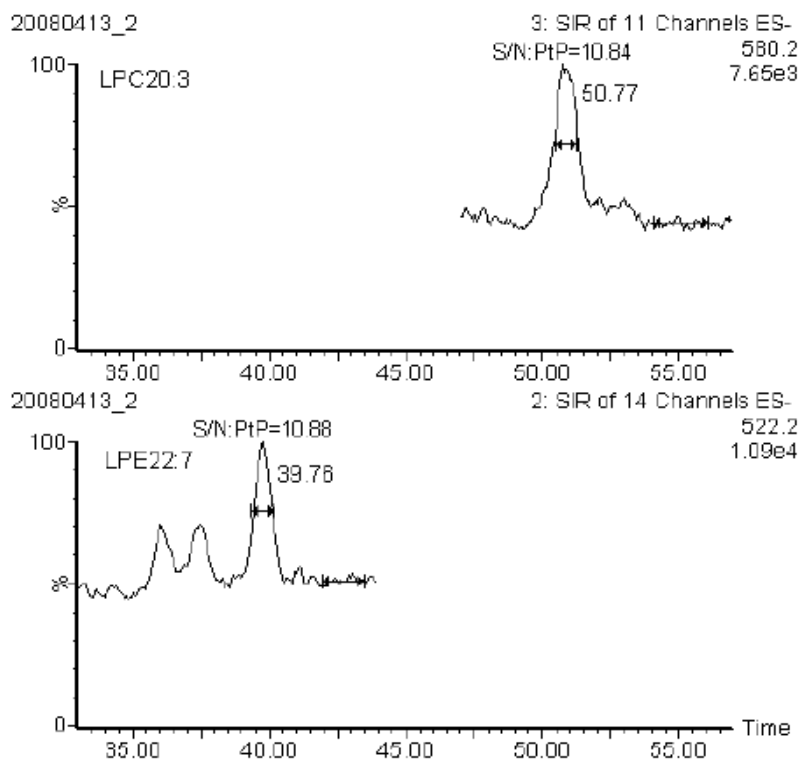


Fig. S3. Extracted ions chromatographs of LPC 20:3 and LPE 22:7 in EA.hy926 cells. The S/N:PtP (signal to noise ratio: peak to peak) were 10.84 and 10.88, respectively.

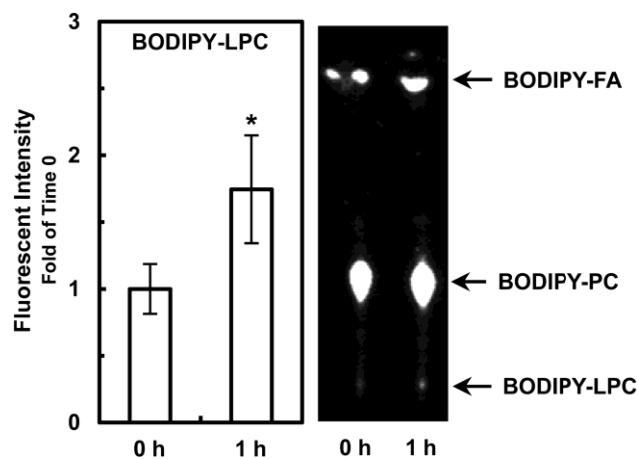


Fig. S4 TLC assay of *in vivo* PLA activity. Cells were treated by 0.2 mM H₂O₂ for 0 h and 1 h. The bis-BODIPY-PC was attached by fluorescent substituent to each of its fatty acid chains and provided a continuous fluorescence response to PLA. The PLA activity was expressed by normalizing the formation of fluorescent BODIPY-LPC of time 1 h to that of time 0. **P* < 0.05, compared with time 0.

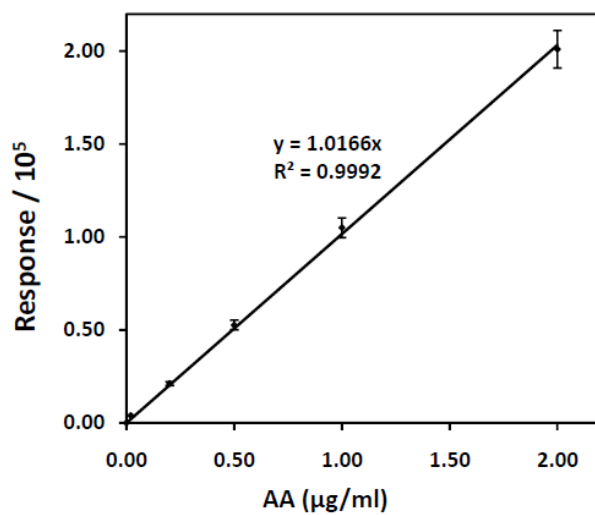


Fig. S5. The external standard calibration curve of AA ranging from 0.02 to 2 µg. The equation of standard curve was $y=1.0166x$, $R^2=0.9992$.

Table S1 The carboxylates assignment of phospholipids in EA.hy926 cells.

| <i>a</i> No. | <i>b</i> <i>sn-1/sn-2</i> | No. | <i>sn-1/sn-2</i> | No. | <i>sn-1/sn-2</i> | No. | <i>sn-1/sn-2</i> |
|--------------|---------------------------|-----|------------------------|-----|-----------------------|-----|-----------------------|
| | PG | | 18:0e/24:6, | 127 | 18:0e/18:0 | 191 | 20:0e/20:4 |
| 001 | 16:0/16:0,14:0/18:0 | 066 | 20:1e/22:5,22:2e/20:4 | 128 | 16:1/20:4,18:2/18:3 | 192 | 18:0e/22:3,18:1e/22:2 |
| 002 | 16:0/18:2,16:1/18:1 | | 18:1e/24:4, | 129 | 16:0/20:4, | 193 | 18:0e/22:2,18:1e/22:1 |
| 003 | 16:0/18:1,16:1/18:0 | 067 | 20:1e/22:4,22:1e/20:4 | 130 | 18:1/18:3,18:2/18:2 | 194 | 18:2/22:6,18:1/22:7 |
| 004 | 16:0/18:0 | | 18:0e/24:4, | 131 | 16:1/20:2,18:1/18:2 | 195 | 18:1/22:6 |
| 005 | 16:1/20:3,18:2/18:2 | 068 | 20:1e/22:3,22:0e/20:4 | 132 | 18:1/18:1,18:0/18:2 | 196 | 18:0/22:6,20:2/20:4 |
| 006 | 16:0/20:3,18:1/18:2 | 069 | 20:4/22:6,20:1e/22:2 | 133 | 18:0/18:1 | 197 | 18:1/22:4,20:1/20:4 |
| 007 | 18:0/18:2,18:1/18:1 | | 20:3/22:6,22:4/20:5, | 134 | 16:0e/22:6,18:1e/20:5 | 198 | 18:0/22:4, |
| 008 | 18:0/18:1 | 070 | 20:0e/22:2,20:1e/22:1 | 135 | 16:1e/22:4, | 199 | 18:1/22:3,20:0/20:4 |
| 009 | 18:0/18:0 | 071 | 20:2/22:6,22:4/20:4, | | 18:0e/20:5,18:1e/20:4 | 200 | 18:0/22:3,20:1/20:2 |
| | PE | | 20:0e/22:1,20:1e/22:0 | 136 | 16:0e/22:4, | 201 | 18:0/22:2, |
| 010 | 16:1e/16:1 | 072 | 20:1/22:6,22:4/20:3, | 137 | 18:0e/20:4,18:1e/20:3 | 202 | 20:1/20:1,20:0/20:2 |
| 011 | 16:0e/16:1,16:1e/16:0 | | 22:2/20:5,20:0e/22:0 | 138 | 16:1e/22:2, | 203 | 18:0/22:1, |
| 012 | 16:0e/16:0 | 073 | 20:0/22:6,20:2/22:4, | 139 | 18:0e/20:3,18:1e/20:2 | 204 | 18:1/22:0,20:0/20:1 |
| 013 | 14:0/18:2,16:1/16:1 | | 20:4/22:2,20:5/22:1 | 140 | 16:1e/22:1,18:1e/20:1 | 205 | 18:0/22:0 |
| 014 | 14:1/18:0,16:0/18:1 | 074 | 20:1/22:4,20:3/22:2, | 141 | 16:1/22:4,18:1/20:4 | 206 | 20:1/22:3 |
| 015 | 16:0/16:0,14:0/18:0 | | 20:4/22:1,20:5/22:0 | 142 | 16:1/22:3,18:1/20:3 | 207 | 20:1/22:2 |
| | 16:2e/18:1, | 075 | 20:0/22:4,20:2/22:2, | 143 | 16:0/22:3,18:1/20:2 | 208 | 20:1/22:1,18:0/24:2 |
| 016 | 16:1e/18:2,18:2e/16:1 | | 20:3/22:1,20:4/22:0 | 144 | 16:0/22:2,18:1/20:1 | 209 | 20:0/22:1,20:1/22:0 |
| 017 | 16:1e/18:1,18:1e/16:1 | 076 | 20:1/22:2, | 145 | 16:0/22:1,18:1/20:0 | 210 | 20:0/22:0 |
| 018 | 16:0e/18:1,18:1e/16:0 | | 20:2/22:1,20:3/22:0 | 146 | 18:0/20:0,18:1e/22:6 | 211 | PS |
| 019 | 16:0e/18:0,18:0e/16:0 | 077 | 20:0/22:2, | 147 | 18:0/20:0,18:1e/22:6 | 212 | 16:1/18:1 |
| 020 | 16:1/18:2 | | 20:1/22:1,20:2/22:0 | 148 | 18:0e/22:6 | 213 | 16:0/18:1,18:0/16:1 |
| 021 | 16:0/18:2,18:1/16:1 | 078 | 20:0/22:1,20:1/22:0 | 149 | 20:1e/20:4 | 214 | 16:0/18:0 |
| 022 | 16:0/18:1,18:0/16:1 | 079 | 20:0/22:0 | 150 | 20:5/20:6, | 215 | 18:0/18:2,18:1/18:1 |
| 023 | 16:0/18:0 | | 18:1e/26:5, | 151 | 18:0e/22:4,20:0e/20:4 | 216 | 18:0/18:1 |
| 024 | 16:1e/20:5 | 080 | 22:1e/22:5,24:2e/20:4 | 152 | 18:1e/22:2,20:1e/20:2 | 217 | 18:0/18:0 |
| 025 | 16:1e/20:4 | | 18:0e/26:5,22:0e/24:5, | 153 | 20:5/20:4,18:1e/22:1 | 218 | 18:0/20:4,18:1/20:3 |
| 026 | 16:0e/20:4,16:1e/20:3 | 081 | 22:1e/22:4,24:1e/20:4 | 154 | 20:4/20:4,20:1e/20:0 | 219 | 18:0/20:3,18:1/20:2 |
| 027 | 16:0e/20:3,16:1e/20:2 | 082 | 22:5/22:6 | 155 | 20:3/20:4,20:0e/20:0 | 220 | 18:0/20:2,18:1/20:1 |
| 028 | 18:1e/18:1,16:1e/20:1 | 083 | 22:4/22:6 | 156 | 18:0/22:6 | 221 | 16:1/22:0, |
| 029 | 16:0e/20:1,18:0e/18:1 | 084 | 22:3/22:6 | 157 | 18:1/22:4,20:1/20:4 | 222 | 18:0/20:1,18:1/20:0 |
| 030 | 16:0e/20:0,18:0e/18:0 | 085 | 22:2/22:6,22:4/22:4 | 158 | 18:1/22:3,20:0/20:4 | 223 | 18:0/22:4 |
| 031 | 16:1/20:5,16:2/20:4 | 086 | 22:1/22:6 | 159 | 18:1/22:2,20:1/20:2 | 224 | 18:0/22:3 |
| 032 | 16:0/20:5,16:1/20:4 | 087 | 22:0/22:6,22:2/22:4 | 160 | 18:0/22:2, | 225 | 18:0/22:2,18:1/22:1 |
| 033 | 16:0/20:4,16:1/20:3 | 088 | 22:1/22:4 | 161 | 20:0/20:2,20:1/20:1 | 226 | 16:0/24:1,20:0/20:1 |
| 034 | 16:0/20:3,18:1/18:2 | 089 | 22:0/22:4,22:2/22:2 | 162 | 18:0/22:1, | 227 | 18:0/22:0 |
| 035 | 18:1/18:1,18:0/18:2 | 090 | 22:1/22:2 | 163 | 18:1/22:0,20:0/20:1 | 228 | 18:1/24:2 |
| 036 | 18:0/18:1 | 091 | 22:0/22:2,22:1/22:1 | 164 | 18:0/22:0, | 229 | 18:1/24:1 |
| 037 | 16:0/20:0,18:0/18:0 | 092 | 22:0/22:1 | 165 | 20:0/20:0,20:1e/22:6 | 230 | 18:0/24:1 |
| 038 | 16:0e/22:6,18:1e/20:5 | 093 | 22:0/22:0 | 166 | 20:0e/22:6 | 231 | LPE |
| 039 | 18:1e/20:4 | 094 | 22:0/24:6 | 167 | 22:1e/20:4 | 232 | ^c 16:1e/H |
| | 16:0e/22:4,16:1e/22:3, | 095 | 24:2/22:3,24:1/22:4 | 168 | 20:5/22:6,20:0e/22:4 | 233 | 18:2e/H |
| 040 | 18:0e/20:4,18:1e/20:3 | 096 | 22:5/24:6 | 169 | 22:0e/20:3 | 234 | 18:1e/H |
| 041 | 16:0e/22:3,18:0e/20:3 | 097 | 22:4/24:6 | 170 | 18:1/24:8 | 235 | 18:1/H |
| | 20:1e/18:1,18:1e/20:1, | 098 | 22:4/24:5 | 171 | 20:1/22:4 | 236 | 18:0/H |
| 042 | 16:0e/22:2,16:1e/22:1 | 099 | 22:4/24:4 | 172 | 20:0/22:4 | 237 | 20:4/H |
| 043 | 16:0e/22:1,18:1e/20:0 | 100 | 22:3/24:4 | 173 | 20:1/22:2 | 238 | 20:3/H |
| 044 | 18:1/20:6,20:0e/20:0 | 101 | 22:2/24:4 | 174 | PI | 239 | 20:2/H |
| 045 | 18:1/20:5 | 102 | 22:1/24:4 | 175 | 16:0/16:1 | 240 | 22:7/H |
| 046 | 18:1/20:4 | 103 | 22:0/24:4 | 176 | 16:0/16:0 | 241 | 22:6/H |
| 047 | 18:0/20:4,18:1/20:3 | 104 | 22:1/24:2 | 177 | 16:0/18:2,18:1/16:1 | 242 | 22:1/H |
| 048 | 18:0/20:3,18:1/20:2 | 105 | 24:3/24:6 | 178 | 16:0/18:1,16:1/18:0 | 243 | 22:0/H |
| 049 | 18:0/20:2,18:1/20:1 | | PC | 179 | 16:0/18:0 | 244 | 24:2/H |
| 050 | 18:0/20:1,18:2e/22:6 | 106 | 14:0/16:1 | 180 | 16:0e/20:3,18:1e/18:2 | 245 | PA |
| 051 | 18:0/20:0,18:1e/22:6 | 107 | 14:0/16:0 | 181 | 16:0e/20:2,18:0e/18:2 | 246 | 14:0/16:0 |
| 052 | 18:1e/22:5 | 108 | 14:1e/18:0 | 182 | 16:0e/20:1,18:0e/18:1 | 247 | 16:1/16:1 |
| 053 | 18:1e/22:4 | 109 | 14:0e/18:0 | 183 | 16:0e/20:0,18:0e/18:0 | 248 | 16:0/16:1 |
| 054 | 18:1e/22:3 | 110 | 16:1/16:1,14:1/18:1 | 184 | 16:0/20:4, | 249 | 16:0/18:1 |
| | 16:0e/24:3,18:1e/22:2, | 111 | 16:0/16:1,14:1/18:0 | 185 | 16:1/20:3,18:2/18:2 | 250 | 18:1/18:1 |
| 055 | 18:0e/22:3,20:1e/20:2 | 112 | 16:0/16:0 | 186 | 16:0/20:3, | 251 | 18:0/18:1 |
| 056 | 18:0e/22:2,20:1e/20:1 | 113 | 16:1e/18:3 | 187 | 16:1/20:2,18:1/18:2 | 252 | 18:0/20:4 |
| 057 | 18:0e/22:1,20:0e/20:1 | 114 | 16:0e/18:2,16:1e/18:1 | 188 | 18:1/18:1 | 253 | 18:0/20:3 |
| | 18:1/22:6, | 115 | 16:0e/18:1 | 189 | 18:0/18:1 | 254 | LPC |
| 058 | 20:3/20:4,18:0e/22:0 | 116 | 16:0e/18:0 | 190 | 18:0/18:0 | 255 | 16:1/H |
| | 18:0/22:6, | 117 | 16:0/18:3,16:1/18:2 | 191 | 18:0e/20:4 | 256 | 16:0/H |
| 059 | 18:2/22:4,20:2/20:4 | 118 | 16:0/18:2,16:1/18:1 | 192 | 16:0e/22:3,18:0e/20:3 | 257 | 18:1/H |
| | 18:1/22:4, | 119 | 16:0/18:1,18:0/16:1 | 193 | 18:1e/20:1 | 258 | 18:0/H |
| 060 | 18:3/22:2,20:1/20:4 | 120 | 16:0/18:0 | 194 | 16:0e/22:1,18:0e/20:1 | 259 | 20:3/H |
| | 18:0/22:4,18:2/22:2, | 121 | 16:1e/20:5 | 195 | 18:1/20:4 | 260 | 20:2/H |
| 061 | 20:0/20:4,20:1/20:3 | 122 | 16:0e/20:5,16:1e/20:4 | 196 | 18:0/20:4,18:1/20:3 | 261 | 20:1/H |
| 062 | 18:1/22:2,20:1/20:2 | 123 | 16:0e/20:4,18:1e/18:3 | 197 | 18:0/20:3 | 262 | 20:0/H |

| | | | | | | | |
|-----|---------------------|-----|-----------------------|-----|----------------------|-----|--------|
| 063 | 18:0/22:2,20:0/20:2 | 124 | 16:1e/20:2,18:1e/18:2 | 188 | 18:0/20:2 | 255 | 22:6/H |
| 064 | 18:0/22:1,20:0/20:1 | 125 | 16:0e/20:2,18:1e/18:1 | 189 | 18:0/20:1,18:1/20:0 | | |
| 065 | 18:0/22:0,20:0/20:0 | 126 | 16:0e/20:1,18:0e/18:1 | 190 | 18:0/20:0,20:3e/20:4 | | |

^a The numbering is in the same order as that of Table 1. ^b All detected isobaric species are listed in the form of *sn-1/sn-2*. The *sn*-position determination is not unequivocal. ^c The letter "H" represents for the hydrogen atom.

Table S2 The PC1 and PC2 values of the "outliers" in PCA-loading plot.

| Species | PC1 | PC2 |
|----------------|---------|---------|
| PE36:6 | -0.1032 | 0.1234 |
| PE44:7 | -0.0608 | 0.1335 |
| PE44:5 | -0.0511 | 0.1186 |
| PE44:2 | -0.0731 | 0.1077 |
| PE44:0 | -0.0649 | 0.1303 |
| ePC34:3 | -0.1152 | 0.0526 |
| PC38:5 | -0.1223 | -0.0638 |
| PC40:11/e40:4 | 0.0300 | -0.0246 |
| ePC42:5 | -0.0841 | -0.1050 |
| PC42:5 | -0.0558 | -0.1206 |
| PI38:0/ePI40:7 | -0.1069 | 0.1133 |
| ePI40:4 | -0.1064 | 0.0507 |
| PI40:2 | -0.0817 | 0.0822 |
| eLPE18:2 | 0.1014 | 0.0129 |
| eLPE18:1 | 0.1396 | 0.0371 |
| LPE18:1 | 0.1797 | -0.0126 |
| LPE18:0 | 0.1514 | 0.0420 |
| LPE20:4 | 0.1968 | 0.0136 |
| LPE20:3 | 0.1834 | 0.0021 |
| LPE22:1 | 0.1857 | 0.0250 |
| LPE22:0 | 0.1980 | -0.0302 |
| LPC16:1 | 0.1982 | 0.1341 |
| LPC16:0 | 0.1924 | 0.1959 |
| LPC18:1 | 0.1759 | 0.1669 |
| LPC18:0 | 0.1952 | 0.2365 |
| LPC20:2 | 0.1906 | 0.2495 |
| LPC20:1 | 0.1722 | 0.1037 |
| LPC20:0 | 0.1533 | 0.0352 |