Supplementary Information

Michler's ethylketone as a novel negative-ion matrix for the enhancement of lipid MALDI tissue imaging

Yiyang Shi,‡[ab] Hao Hu,‡[ab] Qichen Hao,‡[ab] Ran Wu,[ab] Lei Wang,[ab] Liang Qin,[ab] Wei Gu,[b] Haiqiang Liu,[ab] Dongxu Jiang,[ab] Liya Hong,[c] Yijun Zhou,[b] Xiangyi Liu,[d] Jinchao Feng,[b] Kun Xue*[b] and Xiaodong Wang*[ab]

[a]Key Laboratory of Mass Spectrometry Imaging and Metabolomics (Minzu University of China), State Ethnic Affairs Commission, Beijing 100081, China

[b]College of Life and Environmental Sciences, Centre for Imaging & Systems Biology, Minzu University of China, Beijing 100081, China

[c]College of Agronomy, Henan Agricultural University, Zhengzhou 450046, China

[d]Department of Laboratory Medicine, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China

‡ Y. S., H. H., and Q. H. contributed equally to this manuscript.

***Corresponding author:**

Prof. Xiaodong Wang, Ph.D, Prof. Kun Xue, Ph.D

Key Laboratory of Mass Spectrometry Imaging and Metabolomics (Minzu University of China), State Ethnic Affairs Commission

Centre for Imaging & Systems Biology, College of Life and Environmental Sciences, Minzu University of China

#27 Zhongguancun South Avenue, Beijing, 100081, China

Email: Xiaodong@muc.edu.cn (X. W.) and xuekun@muc.edu.cn (K. X.)

Tel.: +86-10-68932922; **Fax**: +86-10-68936927

Supplementary Information--Experimental Section

Chemicals and Materials. Four compounds of 4,4'-Bis(diethylamino)benzophenone (MEK), 2,5-dihydroxybenzoic acid (DHB), 2-mercaptobenzothiazole (2-MBT), and 2,6-dihydroxyacetophenone (DHA) were purchased from Sigma-Aldrich (St. Louis, MO). PE (15:0/15:0), PE (18:0/18:0), PG (14:0/14:0), PG (17:0/17:0), PS (16:0/16:0), PI (16:0/16:0), and PA (16:0/18:1) were purchased from Avanti Polar Lipids (Alabaster, AL). LC/MS-grade methanol (MeOH), chloroform ($CHCl_3$), ammonium hydroxide solution ($NH_3 \cdot H_2O$), trifluoroacetic acid (TFA), formic acid (FA), hematoxylin and eosin (H&E) stain solution, were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water used throughout the experiments was obtained from a Milli-Q system (Millipore, USA). Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seeds were collected from Jiujiang city (Jiangxi Province, China) in December 2019. The rat brain and liver were harvested from a 6-week-old male Sprague-Dawley rat (Shanghai Super-B&K Laboratory Animal Corp. Ltd, Shanghai, China). All the harvested animal organ tissues were slowly immersed into liquid nitrogen to avoid shattering, and then stored at -80ºC until use. All the animal experiments were approved by the Ethics Committee of College of Life and Environmental Sciences, Minzu University of China.

UV-vis Absorption Spectroscopy. Ultraviolet-visible (UV-vis) absorption spectra of MEK, DHB, 2-MBT, and DHA solutions at the concentration of 2×10^{-5} mol/L were acquired on a UV-vis spectrometer (V-750, Jasco, Japan). For MEK matrix solution preparation, 64.8 mg of MEK was dissolved in 1 mL solvent of MeOH/H_2O/$NH_3 \cdot H_2O$ (80:20:0.1, v/v/v). For the other three matrix solution preparations, 30.8 mg of DHB, 33.4 mg of 2-MBT, and 30.4 mg of DHA were dissolved in 1.0 mL solvent of MeOH/H_2O/TFA (80:20:0.1, v/v/v), respectively. These matrices solutions were then diluted to the final concentration of 2×10^{-5} mol/L with the corresponding solvents. The wavelength scanning range from 200 to 800 nm was used for UV-

vis absorption spectrum acquirement.

Tissue Sectioning and Matrix Coating. All the selected fresh frozen tissues samples were sectioned into 12 μm (rat liver and rat brain) and 25 μm (germinating Chinese-yew seed) thickness slices with a Leica CM1860 cryostat (Leica Microsystems Inc., Wetzlar, Germany) at the chamber temperature of -20 °C to meet the following requirements. After sectioning, all of these serial cryo-sectioned tissue slices were immediately thaw-mounted on the indium tin oxide (ITO)-coated microscope glass slides purchased from Bruker Daltonics (Bremen, Germany). After air-drying, these tissue sections were coated with matrix solutions (i.e., MEK, 2-MBT, and DHA) by a GET-Sprayer (I) (HIT Co., Ltd, Beijing, China). The matrix solutions were sprayed ten cycles (5 s spray, and 45 s drying time) on the surfaces of the rat liver, rat brain, and germinating Chinese-yew seed tissue sections to pre-seed a thin layer of matrix. After air-drying in a vented fume hood, the matrix solution was evenly sprayed with fifty more cycles on the same tissue sections.

Optimization of Matrix Solution Composition. The L9 (3^3) orthogonal array testing were carried out for the optimization of MEK, 2-MBT, and DHA matrix solution compositions. The concentration of MEK, the % MeOH, and the % $NH_3 \cdot H_2O$ (MEK) or the % FA (2-MBT) or the % TFA (DHA) were selected as three variables for these optimizations. For each variable, three levels were chosen for the matrix optimization. For MEK solution optimization, the concentrations of MEK were prepared at 0.028 mol/L, 0.037 mol/L, and 0.046 mol/L; the % MeOH was prepared at 70%, 80%, and 90% in H_2O; the % $NH_3 \cdot H_2O$ was prepared at 0.8%, 0.9%, and 1.0%. For 2-MBT solution optimization, the concentrations of 2-MBT were prepared at 0.060 mol/L, 0.090 mol/L, and 0.120 mol/L; the % MeOH was prepared at 70%, 80%, and

90% in H_2O; the % FA was prepared at 1.0%, 2.0%, and 3.0%. For DHA solution optimization, the concentrations of DHA were prepared at 0.066 mol/L, 0.112 mol/L, and 0.158 mol/L; the % MeOH was prepared at 70%, 80%, and 90% in H_2O; the % TFA was prepared at 0.1%, 0.2%, and 0.3%. Serial parallel-sectioned tissue slices (12-μm thickness) of rat liver were used for the optimization of MEK, 2-MBT, and DHA matrix solutions.

Microscopy Visualization. For the evaluation of matrix crystal morphology, microscopy visualization was performed. The concentration of MEK in the matrix solution was prepared at 0.028 mol/L, 0.037 mol/L, and 0.046 mol/L, dissolved in 90% MeOH containing 1% $NH_3 \cdot H_2O$. The GET-Sprayer (I) (HIT Co., Ltd, Beijing, China) sprayed these matrix solutions onto series 5.0×5.0 cm glass slides. After air-drying, the matrix crystals were observed by an Olympus research grade upright microscope (BX35, Olympus Life Science, Tokyo, Japan) at three multiples of 10×, 20× and 40× magnifications.

Chemical Stability Evaluation. MEK was prepared at the optimal concentration and sprayed onto the surfaces of a clean glass slide (2.5×2.5 cm) and a rat liver tissue section using GET-Sprayer (I) (HIT Co., Ltd, Beijing, China). After air-drying in a vented fume hood, the glass slides were loaded and stored in the high-vacuum MALDI ion source (~10^{-7} mbar) with a modified standby mode (the high voltage on target plate is switched off, and the UV laser in MALDI source is in a non-excited state) for chemical stability evaluation. Optical images of matrix-coated clean glass slide were captured under the Olympus BX35 microscope at different time points during vacuum-storage (*i.e.*, 0, 12, 24, and 48 h), and mass spectra of lipids detected in MEK-coated rat liver tissue section were obtained by (-)MALDI-TOF/TOF MS at each storage time points, *i.e.*, 0, 12, 24, and 48 h.

Lipid Standard Preparation for MALDI-MS Analysis. A series of lipid standard solutions of PE (15:0/15:0), PE (18:0/18:0), PG (14:0/14:0), PG (17:0/17:0), PS (16:0/16:0), PI (16:0/16:0), and PA (16:0/18:1) were dissolved in chloroform/methanol (50:50, v/v) at the concentration of 10 μg/μL as the storage solution and finally diluted to 1.0 μg/μL for the following MALDI-MS detection. MEK, 2-MBT, and DHA were prepared at its optimal concentrations. Before MS analysis, 1 μL of each of 7 standard solutions was spotted onto a clean Bruker's Anchorchip target plate, respectively. After air-drying in a vented fume hood, 1 μL of each MEK, 2-MBT, and DHA matrix solutions was added to cover the corresponding dried lipid standard. Air-drying again, the dried lipid standard-matrix mixture spots were detected by the MALDI-TOF MS analysis. Three different UV laser excitation energies, including 20%, 60%, and 100% of full UV laser energies (355 nm), were chosen as the three variables for lipid standard detections.

MALDI-MS. The Bruker Autoflex Speed MALDI time-of-fight (TOF)/TOF mass spectrometer (Bruker Daltonics, Billerica, MA) was used for *in situ* detection and imaging of lipids in tissues. In this type TOF mass spectrometer, the MALDI source is equipped with a 2000 Hz solid-state Smartbeam Nd:YAG UV laser (355 nm) (Azura Laser AG, Berlin, Germany). For MALDI-MS profiling, all mass spectra were acquired over the mass range from 100 to 1500 Da, and recorded by accumulating 100 scans at 500 laser shots per scan. Each spectrum was acquired by applying the optimized laser power for each matrix, in the range of 75% to 85% of the full scale of the Nd:YAG UV laser power with a global attenuator offset of 20%, which is ca. 1.6 mJ per pulse according to the manufacturer’s product specification. Thus, 45% to 55% of the full scale of the Nd:YAG UV laser power with a global attenuator offset of 20% is equivalent to 1.2 to 1.4 mJ per laser shot. For MALDI-MSI, MS images of endogenous lipids were obtained at a spatial resolution of 150 μm (rat brain) and 100 μm (germinating

Chinese-yew seed) with 500 laser shots per position. For MS data acquisition, a mixed matrix and standard solution, including DHB ($[M-H]^-$, *m/z* 153.02), CHCA ($[M-H]^-$, *m/z* 188.04), SA ($[M-H]^-$, *m/z* 223.06), MEK($[M-H]^-$, *m/z* 323.21), SA ($[2M-H]^-$, *m/z* 447.13), Angiotensin II ($[M-H]^-$, *m/z* 1044.53), Angiotensin I ($[M-H]^-$, *m/z* 1294.67), Substance and P ($[M-H]^-$, *m/z* 1345.72), was used for external mass calibration. The matrix ions of MEK ($[M-H]^-$, *m/z* 323.21), 2-MBT ($[M-H]^-$, *m/z* 165.98), and DHA ($[M-H]^-$, *m/z* 151.04), were chosen in combination with one standard compound ion of Angiotensin II ($[M-H]^-$, *m/z* 1044.53) for internal mass calibration. The cubic enhanced mode was chosen for both external and internal mass calibration processing.

Data Analysis. Bruker *FlexAnalysis* 3.4 software was used to view and process the profiling data. The peak lists generation were derived by setting the mass window as 0.05% and signal-to-noise (S/N) ratio of 3. With the aid of two databases, LIPID MAPS (https://www.lipidmaps.org/) and HMBD (https://hmdb.ca/), lipids matching and statistics were performed on the inverted peak table within the allowable mass error range of ±10 ppm. In the process of searching the lipid database in negative-ion mode, we consider the results in the ion adduct forms of $[M-H]^-$. The software of Bruker *FlexImaging* 4.1 was used to complete the ion maps reconstruction of the detectable lipids.

Histological Staining. After MALDI-MSI experiments, the same tissue sections were sequentially washed by 70%, 90%, and 100% methanol solutions to remove the matrix, and then hematoxylin and eosin (H&E) staining was performed to obtain standard histological optical images. An Epson Perfection V550 Photo Scanner was used to take optical images of the tissue sections.

LC-MS/MS Data Acquisition and Analysis. Most of the detected mass-matched lipid

compounds were structurally confirmed by LC/MS/MS analysis. A Waters ACQUITY UPLC system coupled online to an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), equipped with an ESI source was used. The mobile phase consisted of 0.01% formic acid aqueous solution (solvent A) and 0.01% formic acid ACN/isopropanol (1:1) (solvent B) for binary gradient elution. The elution gradient within 5 minutes was 5% to 45% solvent B; Under the conditions of Waters BEH C8 column (2.1×50 mm, 1.7 μm), flow rate of 0.35 mL/min, column temperature of 45°C, lipids were separated using 45% to 100% solvent B for 15 min and 100% solvent B for 2 min before the next injection. For LC-MS measurement scans in the mass range *m/z* 30 to 2000, FTMS detection mode was set to 15000 FWHM (M/Z 400) mass resolution. MS/MS experiments were performed using collision-induced dissociation (CID) for the top five most abundant ions in the measurement scan at 30% standardized collision energy. Automatic gain control and a time limit of 2×10^5 ion counts per ion implantation is 100 ms in an ion trap. During MS/MS data acquisition, dynamic exclusion was used, with an ion exclusion time of 15 seconds. Lipid allocation was obtained by comparing the obtained MS/MS spectra with those in the standard MS/MS library of METLIN, HMDB, or lipid Atlas database, with some manual spectral interpretation.

Supplementary Information--Figures

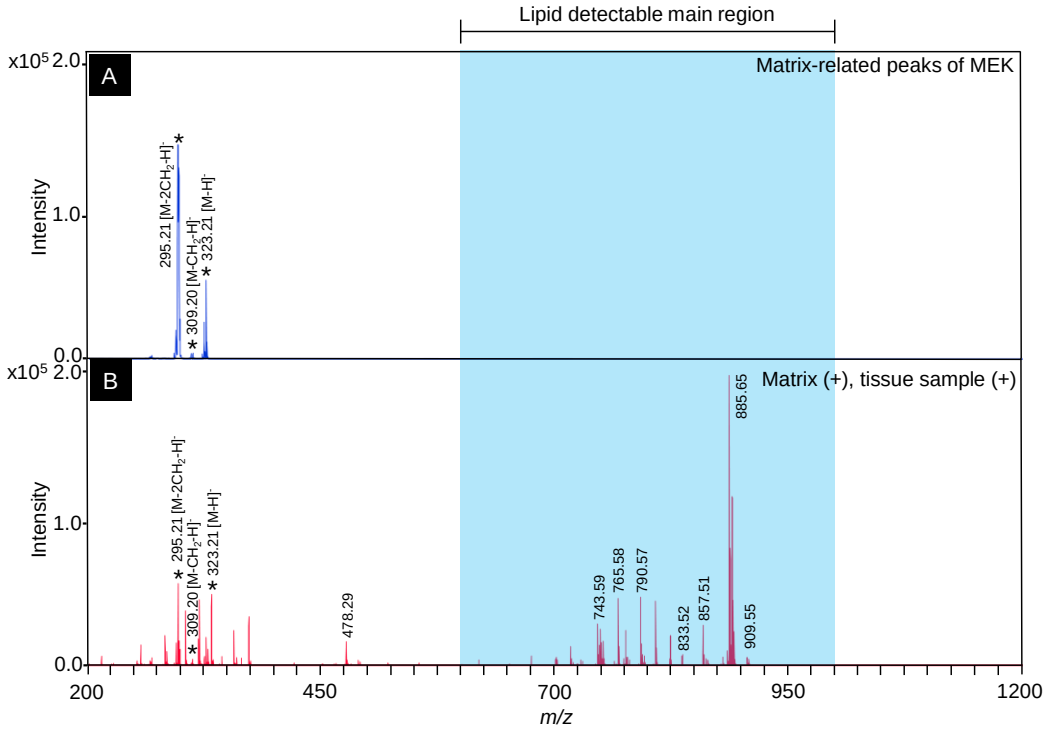

Fig. S1 Comparison of mass spectra acquired using MEK as the matrix from the laser irradiation of glass slides coated without (A, blue) and with (B, red) rat liver tissue section by MALDI-TOF/TOF MS in the negative ion mode. The matrix solution containing 0.028 mol/L MEK, was prepared in 80% MeOH solution containing 1.0% $NH_3 \cdot H_2O$ (without optimization). The matrix-related signals are labelled with asterisks.

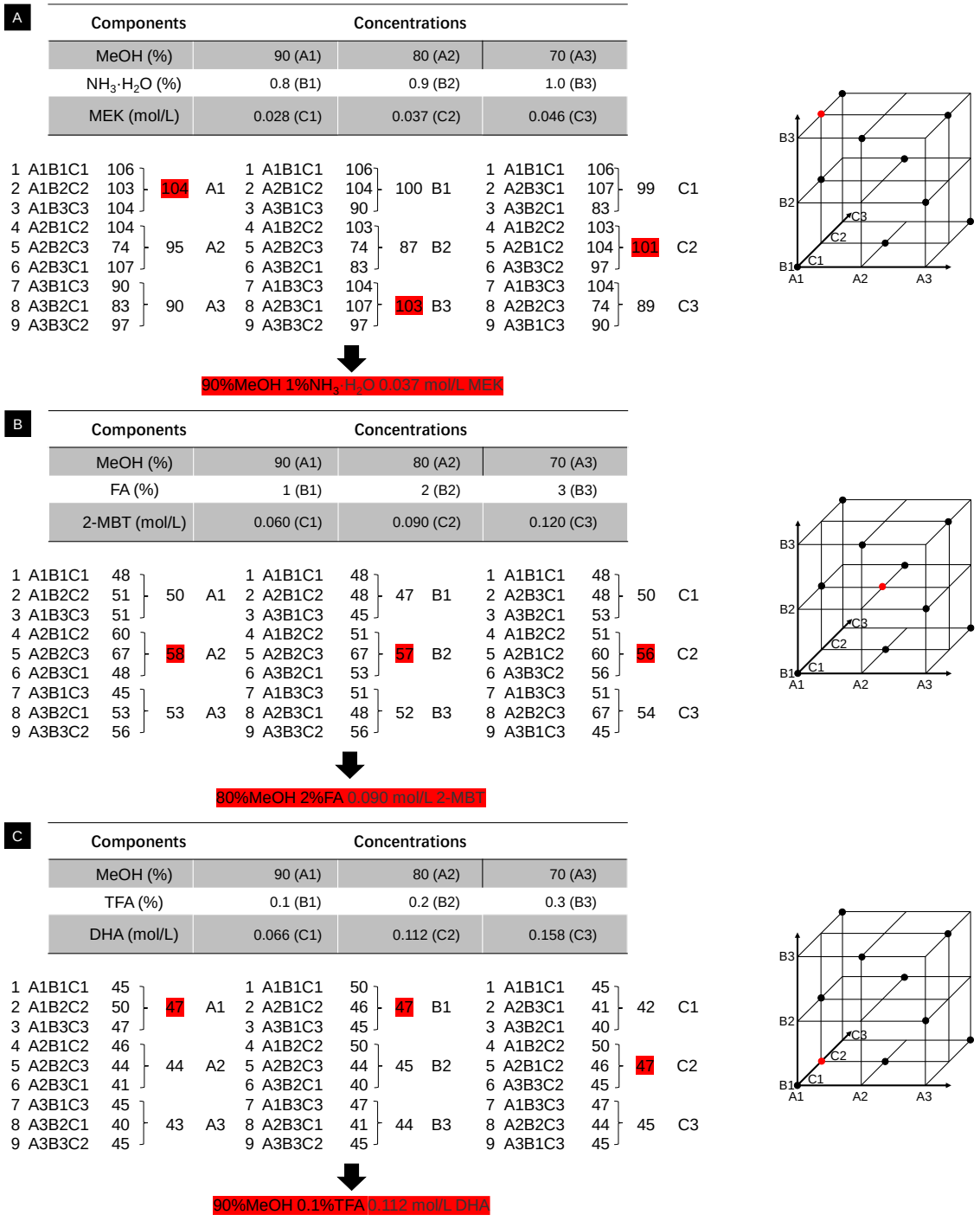

Fig. S2 Orthogonal array testing for the optimization of MEK (A), 2-MBT (B), and DHA (C) matrix solution compositions. Rat-liver tissue sections (n=3) were used for lipid *in situ* detection by MALDI-TOF/TOF MS using MEK, 2-MBT, and DHA as the matrices, respectively. The numbers of detected ion signals were the average numbers from nine independent detection experiments (n=3*3).

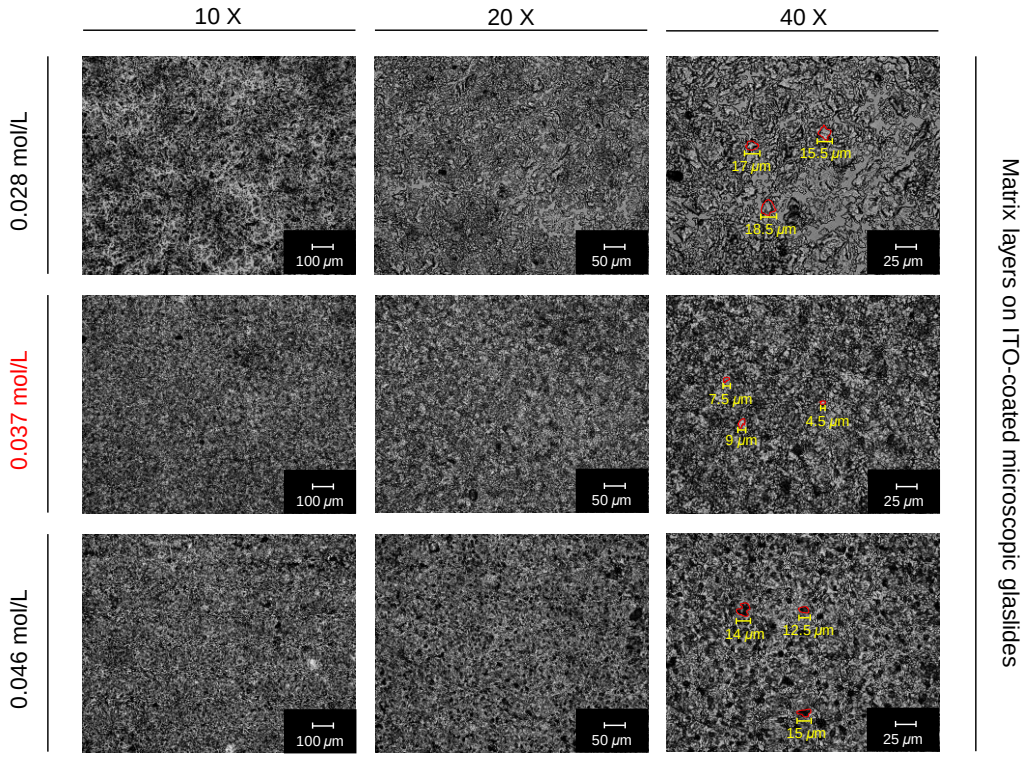

Fig. S3 Microscope images of the MEK-coated ITO glass slides under 10×, 20×, and 40× magnification microscope, respectively. The used MEK solutions were prepared at a concentration of 0.028, 0.037, or 0.046 mol/L, dissolved in 90% MeOH containing 1.0% $NH_3 \cdot H_2O$.

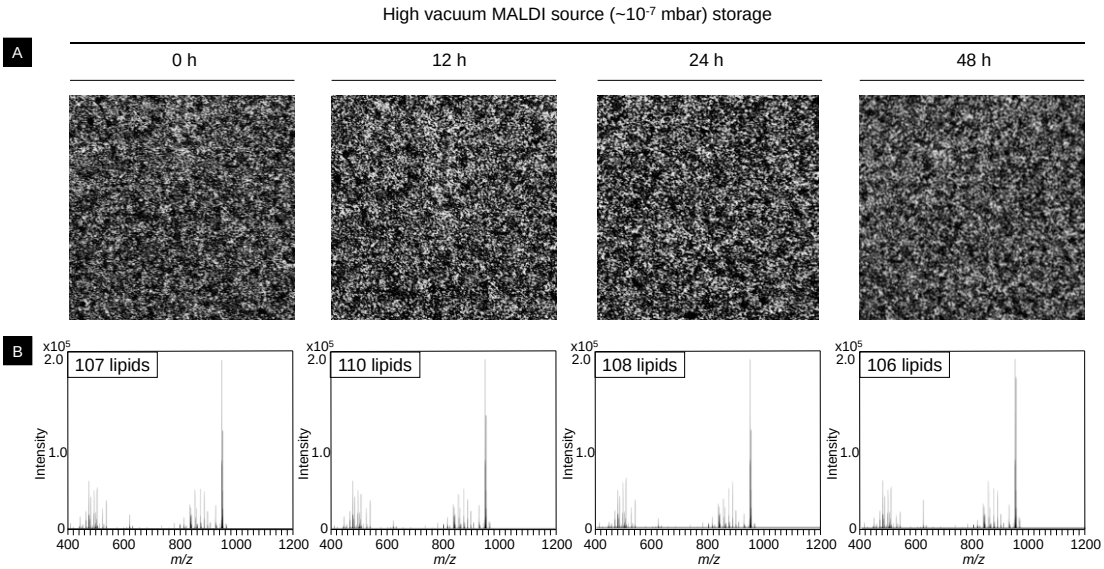

Fig. S4 Comparison of the morphologies of MEK matrix crystals and the number of detectable lipids in rat liver tissue sections stored in a high-vacuum MALDI ion source (~10^{-7} mbar) with different storage times (0, 12, 24, and 48 h). The used MEK matrix solution containing 0.037 mol/L MEK, was prepared in 80% MeOH solution containing 1.0% $NH_3 \cdot H_2O$.

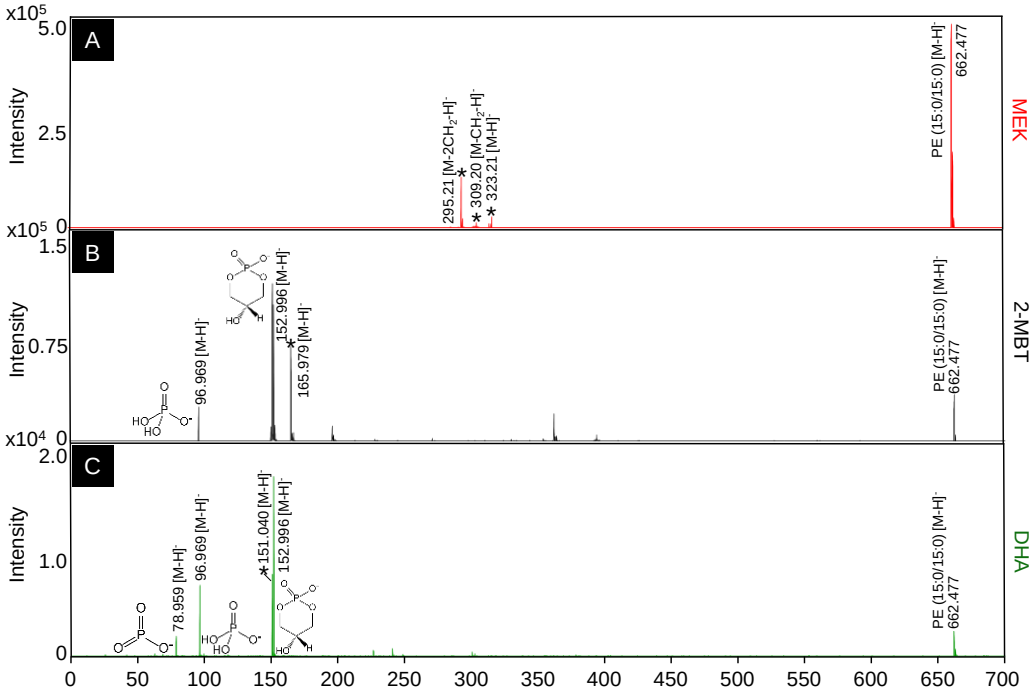

Fig. S5 Comparison of mass spectra of the lipid standard of PE (15:0/15:0) detected by (-) MALDI-TOF MS using MEK, 2-MBT, and DHA as the matrices, respectively. The matrix-related signals are labelled with asterisks.

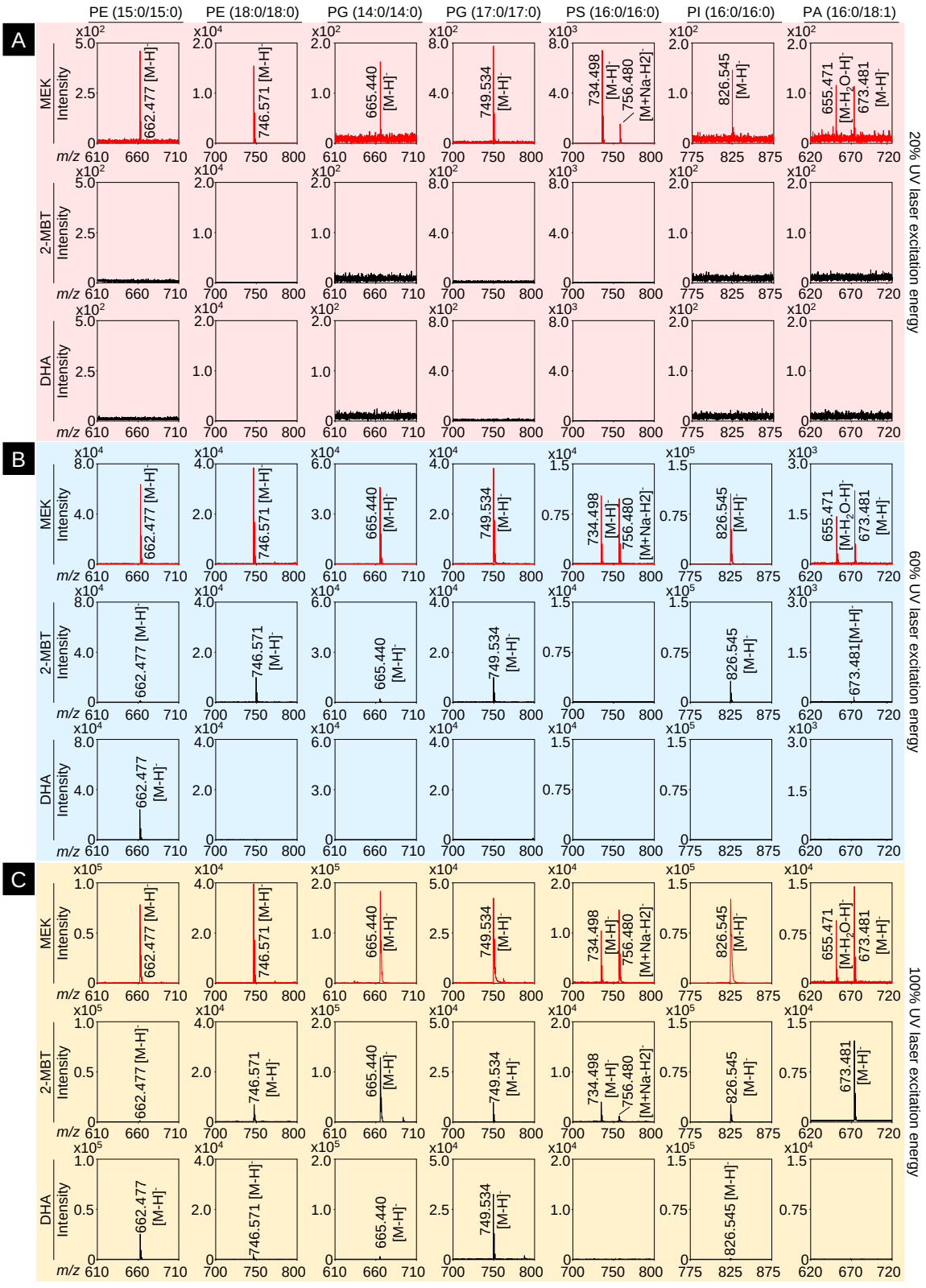

Fig. S6 Comparison of mass spectra of 7 lipid standards detected by (-)MALDI-TOF MS at 20% (A), 60% (B), and 100% (C) UV_{355} laser excitation energies using MEK, 2-MBT, and DHA as the matrices, respectively.

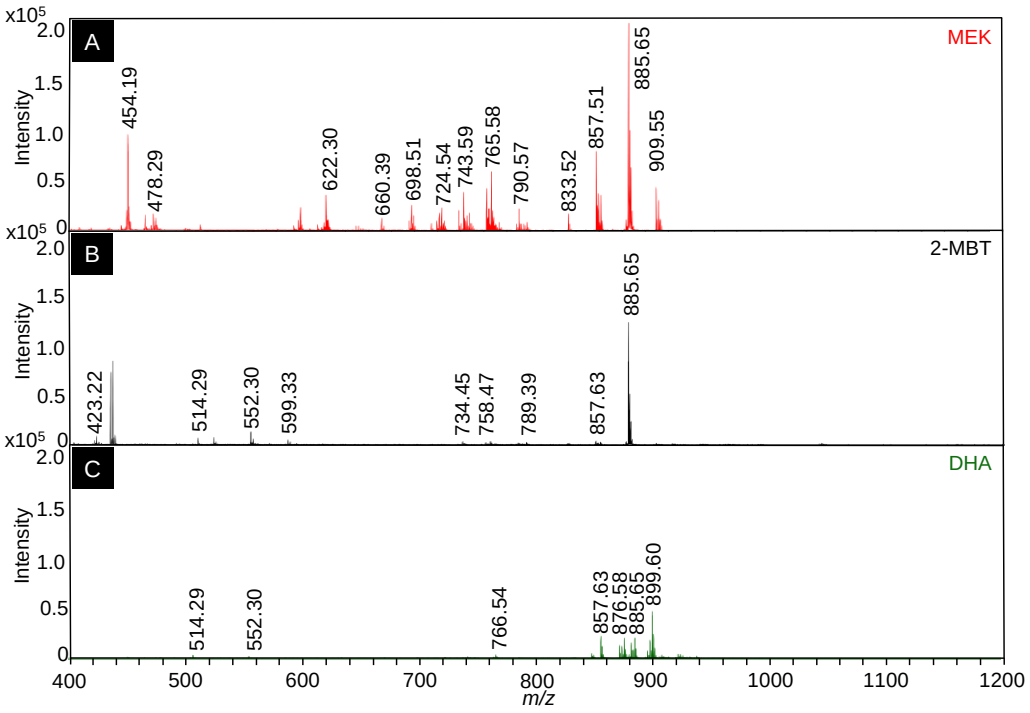

Fig. S7 Comparison of mass spectra acquired using MEK (A), 2-MBT (B) and DHA (C) as the matrices from UV laser irradiation of serial 12-μm tissue sections of the same rat liver by (-)MALDI-TOF/TOF MS.

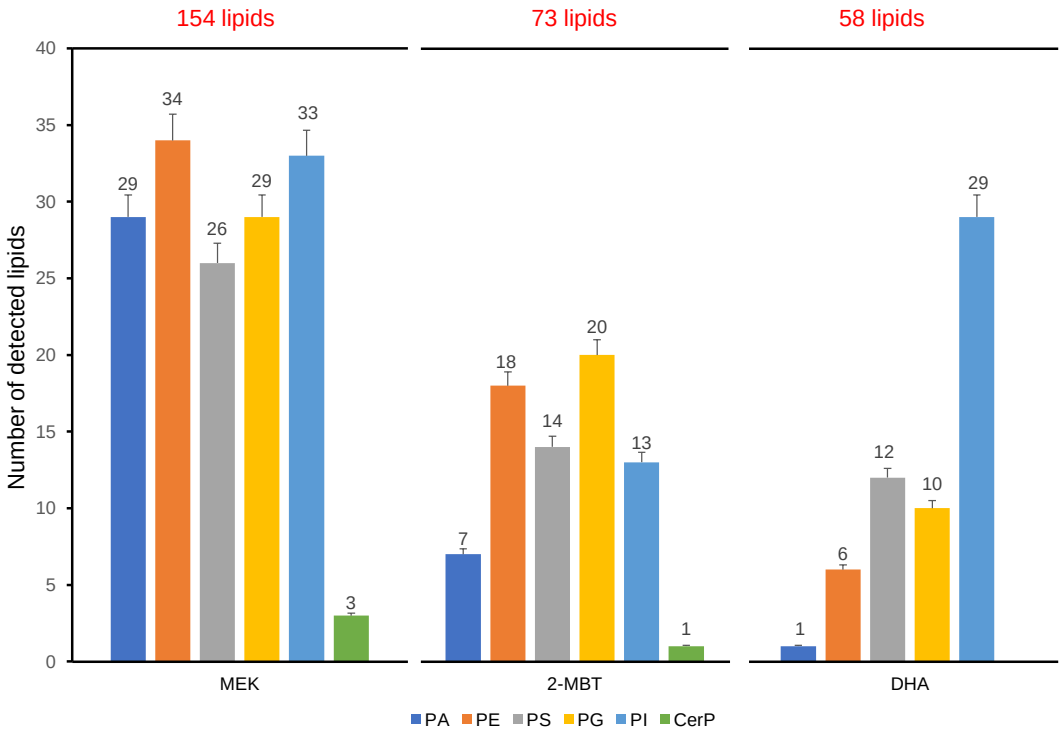

Fig. S8 Comparison of the number of lipid entities detected in the parallel rat liver tissue sections by MALDI-TOF/TOF MS in negative-ion mode using MEK, 2-MBT, and DHA as the matrices.

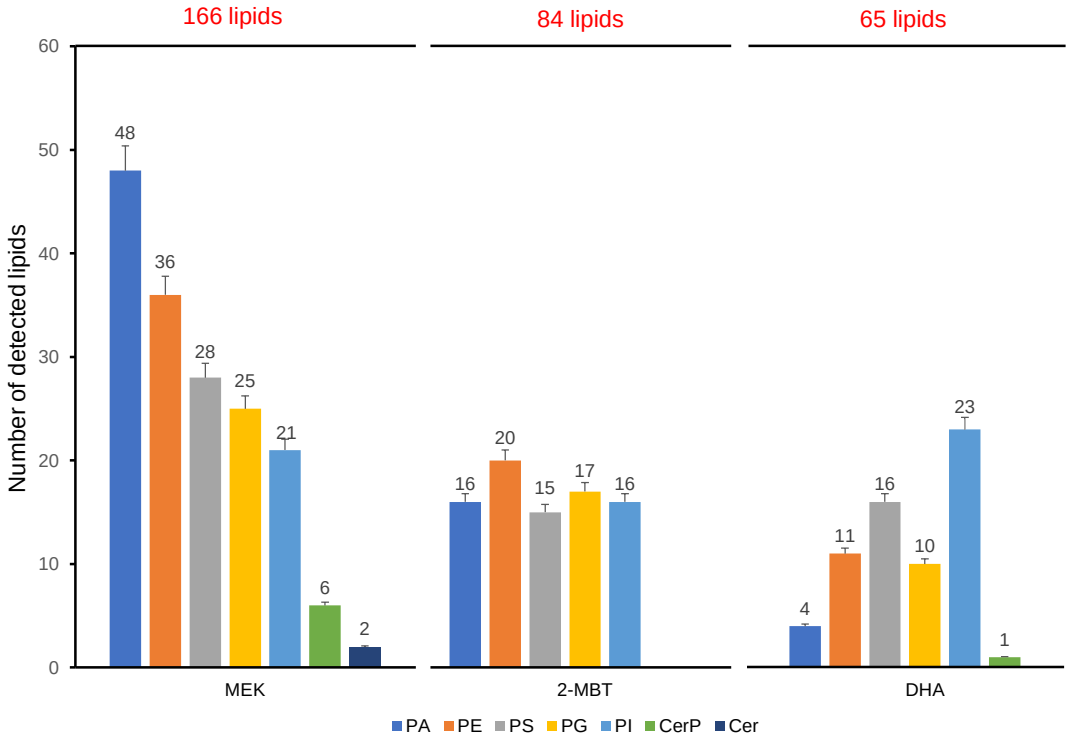

Fig. S9 Comparison of the number of lipid entities detected on the parallel rat brain tissue sections by MALDI-TOF/TOF MS in negative-ion mode using MEK, 2-MBT, and DHA as the matrices.

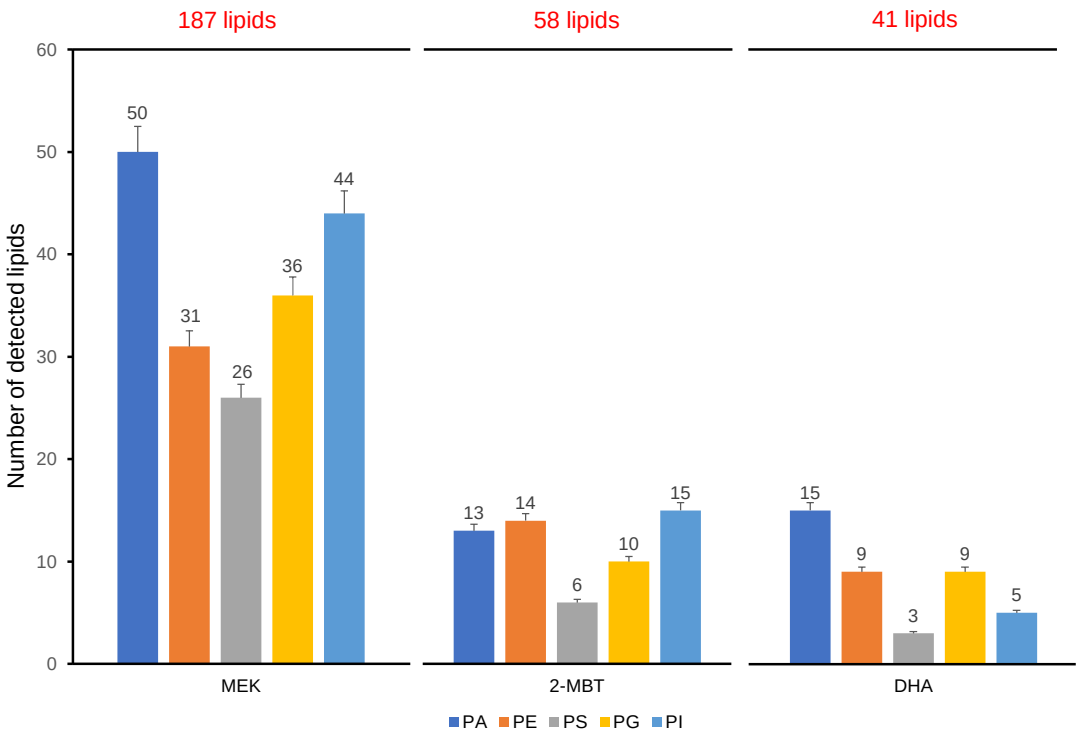

Fig. S10 Comparison of the number of lipid entities detected on the parallel germinating Chinese-yew seed tissue sections by MALDI-TOF/TOF MS in negative-ion mode using MEK, 2-MBT, and DHA as the matrices.

Supplementary Information--Tables

Supplementary Information Table S1. Lipids detected and assigned in a rat liver tissue section by (-)MALDI-TOF/TOF MS using MEK as the matrix.

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
1	407.222	407.2204	4	$[M-H]^-$	LysoPA(16:1)	$C_{19}H_{37}O_7P$	
2	410.194	410.1949	2	$[M-H]^-$	PE(12:0)	$C_{17}H_{34}NO_8P$	
3	429.202	429.2048	7	$[M-H]^-$	LysoPA(18:4)	$C_{21}H_{35}O_7P$	
4	433.236	433.2361	0	$[M-H]^-$	LysoPA(18:2)	$C_{21}H_{39}O_7P$	
5	437.268	437.2674	1	$[M-H]^-$	LysoPA(18:0)	$C_{21}H_{43}O_7P$	419, 285, 150, 78
6	440.206	440.2055	1	$[M-H]^-$	LysoPS(12:0)	$C_{18}H_{36}NO_9P$	
7	454.188	454.1848	7	$[M-H]^-$	PS(12:0)	$C_{18}H_{34}NO_{10}P$	
8	461.264	461.2674	7	$[M-H]^-$	LysoPA(20:2)	$C_{23}H_{43}O_7P$	
9	463.285	463.2830	4	$[M-H]^-$	LysoPA(20:1)	$C_{23}H_{45}O_7P$	
10	464.277	464.2783	3	$[M-H]^-$	LysoPE(O-18:1)	$C_{23}H_{48}NO_6P$	
11	478.293	478.2939	2	$[M-H]^-$	LysoPE(18:1)	$C_{23}H_{46}NO_7P$	
12	480.308	480.3096	3	$[M-H]^-$	LysoPE(18:0)	$C_{23}H_{48}NO_7P$	
13	497.323	497.3249	4	$[M-H]^-$	LysoPG(O-18:0)	$C_{24}H_{51}O_8P$	
14	502.297	502.2939	6	$[M-H]^-$	LysoPE(20:3)	$C_{25}H_{46}NO_7P$	502, 459, 441 305, 287, 122
15	503.241	503.2416	1	$[M-H]^-$	LysoPG(18:4)	$C_{24}H_{41}O_9P$	
16	504.345	504.3460	2	$[M-H]^-$	CerP(26:1)	$C_{26}H_{52}NO_6P$	
17	505.257	505.2572	0	$[M-H]^-$	LysoPG(18:3)	$C_{23}H_{43}O_9P$	
18	506.321	506.3252	8	$[M-H]^-$	LysoPE(20:1)	$C_{25}H_{50}NO_7P$	506, 463, 445 309, 291, 122
19	507.272	507.2729	2	$[M-H]^-$	LysoPG(18:2)	$C_{24}H_{45}O_9P$	507, 279
20	515.228	515.2263	3	$[M-H]^-$	LysoPI(12:0)	$C_{21}H_{41}O_{12}P$	
21	516.314	516.3096	9	$[M-H]^-$	LysoPS(18:3)	$C_{26}H_{48}NO_7P$	
22	533.288	533.2885	9	$[M-H]^-$	LysoPG(20:3)	$C_{26}H_{47}O_9P$	
23	536.377	536.3722	9	$[M-H]^-$	LysoPE(22:0)	$C_{27}H_{56}NO_7P$	339, 140, 122, 96, 78
24	537.319	537.3198	2	$[M-H]^-$	LysoPG(20:1)	$C_{26}H_{51}O_9P$	
25	539.332	539.3355	6	$[M-H]^-$	LysoPG(20:0)	$C_{26}H_{53}O_9P$	
26	540.335	540.3307	8	$[M-H]^-$	LysoPS(O-19:0)	$C_{25}H_{52}NO_9P$	
27	550.318	550.3151	5	$[M-H]^-$	LysoPS(20:1)	$C_{26}H_{50}NO_9P$	
28	552.299	552.2943	9	$[M-H]^-$	PE(20:1)	$C_{25}H_{48}NO_{10}P$	
29	557.309	557.3096	1	$[M-H]^-$	LysoPI(O-16:0)	$C_{25}H_{51}O_{11}P$	
30	559.320	599.3202	0	$[M-H]^-$	LysoPI(18:0)	$C_{27}H_{53}O_{12}P$	599, 283, 265
31	565.355	565.3511	7	$[M-H]^-$	LysoPG(22:1)	$C_{28}H_{55}O_9P$	
32	566.315	566.3100	3	$[M-H]^-$	PE(21:1)	$C_{26}H_{50}NO_{10}P$	

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
33	567.293	567.2940	2	$[M-H]^-$	PG(20:1)	$C_{26}H_{49}O_{11}P$	
34	571.289	571.2899	2	$[M-H]^-$	LysoPI(16:0)	$C_{25}H_{49}O_{12}P$	553, 259, 237
35	572.294	572.2994	9	$[M-H]^-$	LysoPS(22:4)	$C_{28}H_{48}NO_9P$	
36	579.294	579.2940	0	$[M-H]^-$	PA(24:2)	$C_{27}H_{49}O_{11}P$	
37	580.293	580.2892	7	$[M-H]^-$	PS(20:1)	$C_{26}H_{48}NO_{11}P$	
38	583.321	583.3253	7	$[M-H]^-$	LysoPI(O-18:1)	$C_{27}H_{53}O_{11}P$	
39	595.285	595.2889	6	$[M-H]^-$	LysoPI(18:2)	$C_{27}H_{49}O_{12}P$	
40	596.286	596.2841	3	$[M-H]^-$	PS(20:1)	$C_{26}H_{48}NO_{12}P$	
41	597.305	597.3045	8	$[M-H]^-$	LysoPI(18:1)	$C_{27}H_{51}O_{12}P$	597, 316, 281, 241, 152
42	606.304	606.3049	2	$[M-H]^-$	PS(22:2)	$C_{28}H_{50}NO_{11}P$	
43	609.309	609.3045	7	$[M-H]^-$	PG(22:2)	$C_{28}H_{51}O_{12}P$	
44	613.374	613.3722	3	$[M-H]^-$	LysoPI(O-20:2)	$C_{29}H_{59}O_{11}P$	
45	617.418	617.4188	1	$[M-H]^-$	PA(30:1)	$C_{33}H_{63}O_8P$	617, 253, 235, 78
46	618.450	618.4504	6	$[M-H]^-$	PE(O-28:1)	$C_{33}H_{66}NO_7P$	
47	619.287	619.2889	3	$[M-H]^-$	LysoPI(20:4)	$C_{29}H_{49}O_{12}P$	619, 301, 285
48	620.325	620.3205	7	$[M-H]^-$	PE(24:3)	$C_{29}H_{52}NO_{11}P$	
49	621.308	621.3045	6	$[M-H]^-$	LysoPI(20:3)	$C_{29}H_{51}O_{12}P$	
50	622.295	622.2998	8	$[M-H]^-$	PS(22:2)	$C_{28}H_{50}NO_{12}P$	
51	625.338	625.3358	4	$[M-H]^-$	LysoPI(20:1)	$C_{29}H_{55}O_{12}P$	
52	630.340	630.3413	2	$[M-H]^-$	PE(26:4)	$C_{31}H_{54}NO_{10}P$	
53	635.315	635.3202	8	$[M-H]^-$	PG(24:3)	$C_{30}H_{55}O_{12}P$	
54	641.450	641.4552	8	$[M-H]^-$	PA(O-33:3)	$C_{36}H_{67}O_7P$	
55	648.347	648.3518	7	$[M-H]^-$	PE(26:3)	$C_{31}H_{56}NO_{11}P$	
56	650.335	650.3311	6	$[M-H]^-$	PS(24:2)	$C_{30}H_{54}NO_{12}P$	
57	651.313	651.3151	3	$[M-H]^-$	PG(24:3)	$C_{30}H_{53}O_{13}P$	
58	653.330	653.3308	1	$[M-H]^-$	PG(24:2)	$C_{30}H_{55}O_{13}P$	
59	660.387	660.3882	2	$[M-H]^-$	PE(28:3)	$C_{33}H_{60}NO_{10}P$	
60	669.456	669.4501	9	$[M-H]^-$	PA(34:3)	$C_{37}H_{67}O_8P$	669, 441, 363, 305, 227
61	671.460	671.4657	8	$[M-H]^-$	PA(34:2)	$C_{37}H_{69}O_8P$	671, 415, 391, 279, 255
62	672.498	672.4974	1	$[M-H]^-$	PE(O-32:2)	$C_{37}H_{72}NO_7P$	
63	673.481	673.4814	1	$[M-H]^-$	PA(34:1)	$C_{37}H_{71}O_8P$	673, 279, 255, 237, 78
64	674.514	674.5130	1	$[M-H]^-$	PE(O-32:1)	$C_{37}H_{74}NO_7P$	
65	679.495	679.4920	4	$[M-H]^-$	PG(O-30:0)	$C_{36}H_{73}O_9P$	
66	685.406	685.4086	4	$[M-H]^-$	PG(30:4)	$C_{36}H_{63}O_{10}P$	
67	687.532	687.5334	2	$[M-H]^-$	PA(O-36:1)	$C_{39}H_{77}O_7P$	
68	696.496	696.4974	2	$[M-H]^-$	PE(O-34:4)	$C_{39}H_{72}NO_7P$	

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
69	698.507	698.5130	9	$[M-H]^-$	PE(O-34:3)	$C_{39}H_{74}NO_7P$	
70	699.494	699.4970	4	$[M-H]^-$	PA(36:2)	$C_{39}H_{73}O_8P$	281, 263, 96, 78
71	700.532	700.5287	5	$[M-H]^-$	PE(O-34:2)	$C_{39}H_{76}NO_7P$	
72	701.517	701.5127	6	$[M-H]^-$	PA(36:1)	$C_{39}H_{75}O_8P$	
73	714.514	714.5079	9	$[M-H]^-$	PE(34:2)	$C_{39}H_{74}NO_8P$	698, 575
74	715.562	715.5647	3	$[M-H]^-$	PA(O-38:1)	$C_{41}H_{81}O_7P$	
75	717.582	717.5804	3	$[M-H]^-$	PA(O-38:0)	$C_{41}H_{83}O_7P$	
76	718.536	718.5392	4	$[M-H]^-$	PE(34:0)	$C_{39}H_{78}NO_8P$	718, 508, 490 424, 311, 227
77	719.485	719.4869	2	$[M-H]^-$	PG(32:1)	$C_{38}H_{73}O_{10}P$	719, 463, 253, 235
78	720.502	720.4974	6	$[M-H]^-$	PE(O-36:6)	$C_{41}H_{72}NO_7P$	
79	721.500	721.5025	3	$[M-H]^-$	PG(32:0)	$C_{38}H_{75}O_{10}P$	721, 465, 255, 237
80	722.512	722.5130	1	$[M-H]^-$	PE(O-36:5)	$C_{41}H_{74}NO_7P$	
81	724.535	724.5287	9	$[M-H]^-$	PE(O-36:4)	$C_{41}H_{76}NO_7P$	
82	725.518	725.5127	7	$[M-H]^-$	PA(38:3)	$C_{41}H_{75}O_8P$	725, 305, 283, 265, 78
83	726.575	726.5807	8	$[M-H]^-$	CerP(42:2)	$C_{42}H_{82}NO_6P$	
84	727.524	727.5283	6	$[M-H]^-$	PA(38:2)	$C_{41}H_{77}O_8P$	
85	728.596	728.5964	5	$[M-H]^-$	CerP(42:1)	$C_{42}H_{84}NO_6P$	
86	730.579	730.5756	5	$[M-H]^-$	PE(O-36:1)	$C_{41}H_{82}NO_7P$	730, 673, 448 281, 263, 225
87	738.513	738.5079	7	$[M-H]^-$	PE(36:4)	$C_{41}H_{74}NO_8P$	740, 599
88	739.561	739.5647	5	$[M-H]^-$	PA(O-40:3)	$C_{43}H_{81}O_7P$	
89	740.528	740.5236	6	$[M-H]^-$	PE(36:3)	$C_{41}H_{76}NO_8P$	742, 601
90	741.580	741.5804	5	$[M-H]^-$	PA(O-40:2)	$C_{43}H_{83}O_7P$	
91	743.593	743.5960	4	$[M-H]^-$	PA(O-40:1)	$C_{43}H_{85}O_7P$	743, 365, 347, 241, 78
92	745.505	745.5025	3	$[M-H]^-$	PG(34:2)	$C_{40}H_{75}O_{10}P$	
93	746.511	746.5130	3	$[M-H]^-$	PE(O-38:7)	$C_{43}H_{74}NO_7P$	
94	747.513	747.5182	7	$[M-H]^-$	PG(34:1)	$C_{44}H_{77}O_{10}P$	747, 491, 281, 263, 255
95	748.527	748.5287	2	$[M-H]^-$	PE(O-38:6)	$C_{43}H_{76}NO_7P$	
96	749.538	749.5338	6	$[M-H]^-$	PG(34:0)	$C_{40}H_{79}O_{10}P$	749, 493, 283, 265, 255
97	750.545	750.5443	4	$[M-H]^-$	PE(O-38:5)	$C_{43}H_{78}NO_7P$	750, 329, 241
98	753.541	753.5440	4	$[M-H]^-$	PA(40:3)	$C_{43}H_{79}O_8P$	
99	754.578	753.5756	3	$[M-H]^-$	PE(O-38:3)	$C_{42}H_{82}NO_7P$	
100	760.512	760.5134	2	$[M-H]^-$	PS(34:1)	$C_{40}H_{76}NO_{10}P$	760, 673, 506

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
101	762.525	762.5291	5	$[M-H]^-$	PS(34:0)	$C_{40}H_{78}NO_{10}P$	762, 283, 265, 255
102	763.568	763.5647	4	$[M-H]^-$	PA(O-42:5)	$C_{45}H_{81}O_7P$	
103	765.583	765.5804	3	$[M-H]^-$	PA(O-42:4)	$C_{45}H_{83}O_7P$	
104	767.502	767.5080	8	$[M-H]^-$	PI(O-30:0)	$C_{39}H_{77}O_{12}P$	767, 337, 319, 293, 78
105	769.615	769.6117	4	$[M-H]^-$	PA(O-42:2)	$C_{45}H_{87}O_7P$	
106	770.575	770.5705	6	$[M-H]^-$	PE(38:2)	$C_{43}H_{82}NO_8P$	
107	771.622	771.6273	7	$[M-H]^-$	PA(O-42:1)	$C_{45}H_{89}O_7P$	
108	772.587	772.5862	2	$[M-H]^-$	PE(38:1)	$C_{43}H_{84}NO_8P$	772, 367, 225
109	773.537	773.5338	9	$[M-H]^-$	PG(36:2)	$C_{42}H_{79}O_{10}P$	
110	776.554	776.5600	8	$[M-H]^-$	PE(O-40:6)	$C_{45}H_{80}NO_7P$	
111	781.575	781.5753	0	$[M-H]^-$	PA(42:3)	$C_{45}H_{83}O_8$	781, 573, 555 275, 257, 241
112	788.544	788.5447	1	$[M-H]^-$	PS(36:1)	$C_{42}H_{80}NO_{10}P$	391, 309, 255, 184, 165, 78
113	789.605	789.6015	4	$[M-H]^-$	PG(O-38:1)	$C_{44}H_{87}O_9P$	
114	790.573	790.5704	3	$[M-H]^-$	PS(36:0)	$C_{42}H_{82}NO_{10}P$	790, 703, 255, 96, 78
115	791.619	791.6172	2	$[M-H]^-$	PG(O-38:0)	$C_{44}H_{89}O_9P$	
116	793.528	793.5236	6	$[M-H]^-$	PI(O-32:1)	$C_{41}H_{79}O_{12}P$	
117	798.565	798.5655	6	$[M-H]^-$	PS(O-38:3)	$C_{44}H_{82}NO_9P$	
118	799.528	799.5283	0	$[M-H]^-$	PA(44:8)	$C_{47}H_{77}O_8P$	799, 331, 313, 78
119	801.454	801.4560	2	$[M-H]^-$	PI(32:4)	$C_{41}H_{71}O_{13}P$	
120	805.480	805.4873	9	$[M-H]^-$	PI(32:2)	$C_{41}H_{75}O_{13}P$	
121	807.556	807.5546	2	$[M-H]^-$	PG(O-40:6)	$C_{46}H_{81}O_9P$	
122	816.577	816.5760	1	$[M-H]^-$	PS(38:1)	$C_{44}H_{84}NO_{10}P$	816, 729, 391, 255, 96, 78
123	818.594	818.5917	3	$[M-H]^-$	PS(38:0)	$C_{44}H_{86}NO_{10}P$	818, 311, 283, 265
124	819.641	819.6485	9	$[M-H]^-$	PG(O-40:0)	$C_{46}H_{93}O_9P$	
125	833.516	833.5186	3	$[M-H]^-$	PI(34:2)	$C_{43}H_{79}O_{13}P$	
126	834.525	834.5291	5	$[M-H]^-$	PS(40:6)	$C_{46}H_{78}NO_{10}P$	834, 327, 309, 281
127	835.585	835.5859	1	$[M-H]^-$	PG(O-42:6)	$C_{48}H_{85}O_9P$	
128	838.556	838.5604	5	$[M-H]^-$	PS(40:4)	$C_{46}H_{82}NO_{10}P$	
129	857.512	857.5186	8	$[M-H]^-$	PI(36:4)	$C_{45}H_{79}O_{13}P$	857, 601, 303, 285, 255
130	858.534	858.5291	6	$[M-H]^-$	PS(42:8)	$C_{48}H_{78}NO_{10}P$	

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
131	859.538	859.5342	6	$[M-H]^-$	PI(36:3)	$C_{45}H_{81}O_{13}P$	859, 595, 577, 281
132	860.543	860.5447	2	$[M-H]^-$	PS(42:7)	$C_{48}H_{80}NO_{10}P$	
133	861.556	861.5499	7	$[M-H]^-$	PI(36:2)	$C_{45}H_{83}O_{13}P$	861, 307, 289, 255
134	862.565	862.5604	5	$[M-H]^-$	PS(42:6)	$C_{48}H_{82}NO_{10}P$	
135	863.561	863.5655	5	$[M-H]^-$	PI(36:1)	$C_{45}H_{85}O_{13}P$	
136	865.504	865.5025	2	$[M-H]^-$	PG(44:12)	$C_{50}H_{75}O_{10}P$	
137	871.563	871.5706	9	$[M-H]^-$	PI(O-38:4)	$C_{47}H_{85}O_{12}P$	
138	881.518	881.5186	0	$[M-H]^-$	PI(38:6)	$C_{47}H_{79}O_{13}P$	
139	882.523	882.5291	7	$[M-H]^-$	PS(44:10)	$C_{50}H_{78}NO_{10}P$	
140	883.534	883.5342	0	$[M-H]^-$	PI(38:5)	$C_{47}H_{81}O_{13}P$	883, 329, 311, 255
141	885.653	885.6590	7	$[M-H]^-$	PG(44:2)	$C_{50}H_{95}O_{10}P$	
142	886.565	886.5604	5	$[M-H]^-$	PS(44:8)	$C_{50}H_{82}NO_{10}P$	
143	888.572	888.5760	4	$[M-H]^-$	PS(44:7)	$C_{50}H_{84}NO_{10}P$	
144	889.697	889.6903	8	$[M-H]^-$	PG(44:0)	$C_{50}H_{99}O_{10}$	889, 331, 313, 277, 259
145	891.599	891.5968	3	$[M-H]^-$	PI(38:1)	$C_{47}H_{89}O_{13}P$	
146	893.555	893.5549	0	$[M-H]^-$	PI(O-40:7)	$C_{49}H_{83}O_{12}P$	
147	897.590	897.5862	4	$[M-H]^-$	PI(O-40:5)	$C_{49}H_{87}O_{12}P$	
148	899.604	899.6019	2	$[M-H]^-$	PI(O-40:4)	$C_{49}H_{89}O_{12}P$	
149	909.547	909.5499	3	$[M-H]^-$	PI(40:6)	$C_{49}H_{83}O_{13}P$	909, 327, 309, 283
150	911.565	911.5655	1	$[M-H]^-$	PI(40:5)	$C_{49}H_{85}O_{13}P$	911, 329, 283, 265
151	913.586	913.5812	5	$[M-H]^-$	PI(40:4)	$C_{49}H_{87}O_{13}P$	913, 581, 331, 283, 265
152	933.683	933.6801	3	$[M-H]^-$	PI(O-42:1)	$C_{51}H_{99}O_{12}P$	
153	937.584	937.5812	3	$[M-H]^-$	PI(42:6)	$C_{51}H_{87}O_{13}P$	
154	957.543	957.5499	7	$[M-H]^-$	PI(44:10)	$C_{53}H_{83}O_{13}P$	

[a] Structurally specific CID ions of lipids were detected by LC-MS/MS using CID. Fragment ions shown in blue were detected by *in situ* MALDI-TOF/TOF MS/MS. Abbreviations: PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; CerP, ceramide 1-phosphate; Cer, ceramides. Highlighted lipids were also detected using MEK matrix without optimization (*i.e.*, 0.028 mol/L MEK, prepared in 80% aqueous MeOH solution containing 1.0% $NH_3 \cdot H_2O$).

Supplementary Information Table S2. Orthogonal-array testing for lipid ion signal detection on rat liver tissue sections by (-)MALDI-TOF/TOF MS for MEK matrix solution composition optimization.

Lipid ion signals (*m/z*)	Optimization of MEK matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
410.194	√	√	√	√	√	√	√	√	√
437.268	√	√	√	√	√	√	√	√	√
454.188	√	√	√	√		√	√	√	√
464.277	√	√	√	√		√	√	√	√
478.293	√	√	√	√	√	√	√		√
480.308	√	√	√	√	√	√		√	
515.228	√	√	√	√	√	√	√	√	√
516.314	√	√	√	√	√	√	√	√	√
597.305	√	√	√	√		√	√		√
606.304	√	√	√	√	√	√	√	√	√
609.309	√		√	√	√	√	√	√	√
613.374	√	√	√	√	√	√	√		√
617.418	√	√	√	√		√	√	√	√
618.450	√	√	√	√	√	√	√	√	√
619.287	√	√	√	√		√	√	√	
620.325	√	√	√	√		√	√		√
671.460	√	√	√	√	√	√	√	√	√
672.498	√	√	√	√	√	√	√	√	√
673.481	√	√	√	√	√	√	√	√	√
674.514	√	√	√	√	√	√	√	√	√
679.495	√	√	√	√		√	√	√	√
685.406	√	√	√	√	√	√		√	√
687.532	√	√	√	√	√	√	√	√	√
696.496	√	√	√	√	√	√	√		√
698.507	√	√	√	√	√	√	√	√	√
699.494	√	√	√	√		√	√	√	√
700.532	√	√	√	√	√	√	√	√	√
701.517	√	√	√	√		√	√	√	√
714.514	√	√	√	√	√	√	√	√	√
715.562	√			√	√	√	√	√	√
717.582	√	√	√	√	√	√	√	√	√
718.536	√	√	√	√	√	√	√		√
719.485	√	√	√	√	√	√	√	√	√
720.502	√	√	√	√	√	√	√		√
721.500	√	√	√	√	√	√	√	√	√

Lipid ion signals (*m/z*)	Optimization of MEK matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
722.512	√	√	√	√	√	√	√	√	√
724.535	√	√	√	√	√	√	√	√	√
725.518	√	√	√	√	√	√		√	√
726.575	√	√	√	√	√	√	√	√	√
727.524	√	√	√	√		√	√	√	√
728.596	√	√	√	√	√	√	√		√
730.579	√	√	√	√		√	√	√	√
738.513	√	√	√	√	√	√	√	√	√
739.561	√	√	√	√		√	√	√	√
740.528	√	√	√	√		√	√		√
741.580	√	√	√	√	√	√	√	√	√
743.593	√	√	√	√	√	√	√	√	√
745.505	√	√	√	√	√	√	√		√
746.511	√	√	√	√		√		√	√
747.513	√	√	√	√		√	√	√	√
748.527	√	√	√	√	√	√		√	√
749.538	√	√		√	√	√		√	√
750.545	√	√	√	√	√	√	√	√	√
753.541	√	√	√	√		√	√	√	√
754.578	√	√	√	√	√	√		√	√
760.512	√	√	√	√	√	√	√		√
762.525	√	√	√	√		√	√	√	√
763.568	√	√	√	√	√	√	√		√
765.583	√	√	√	√	√	√	√	√	√
767.502	√	√	√	√		√			√
769.615	√	√	√	√	√	√	√		√
770.575	√	√	√	√	√	√	√	√	√
771.622	√	√	√	√		√	√		√
772.587	√	√	√	√	√	√			√
773.537	√	√	√	√	√	√	√		√
776.554	√	√	√	√	√	√	√		
781.575	√	√	√	√		√			
788.544	√	√	√	√	√	√	√	√	√
789.605	√	√	√	√	√	√	√	√	√
790.573	√	√	√	√		√	√	√	√
791.619	√	√	√	√	√	√	√	√	√
793.528	√	√	√	√		√	√		√
798.565	√	√	√	√	√	√		√	
799.528	√	√	√	√	√	√	√	√	√
801.454	√	√	√	√	√	√	√	√	√

Lipid ion signals (*m/z*)	Optimization of MEK matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
805.480	√	√	√	√	√	√	√	√	√
807.556	√	√	√	√	√	√	√	√	√
816.577	√	√	√	√	√	√		√	√
818.594	√	√	√	√	√	√	√	√	√
819.641	√	√	√	√		√	√	√	√
833.516	√	√	√	√		√	√	√	√
834.525	√	√	√	√	√	√	√	√	
835.585	√	√	√	√	√	√			√
838.556	√	√	√		√	√	√	√	√
857.512	√	√	√	√	√	√	√	√	√
858.534	√			√	√	√	√	√	√
859.538	√	√	√	√		√	√	√	√
860.543		√	√	√	√	√	√	√	√
861.556	√	√	√	√	√	√		√	√
862.565	√	√	√	√		√	√	√	√
863.561	√	√	√	√	√	√	√		√
865.504	√	√	√	√	√	√	√	√	√
871.563	√	√	√	√		√	√	√	√
881.518	√	√	√	√	√	√	√	√	√
882.523	√	√	√	√	√	√	√	√	√
883.534	√	√	√	√	√	√	√	√	
885.653	√	√	√	√	√	√	√	√	√
886.565	√	√	√	√		√	√	√	√
888.572	√	√	√	√	√	√		√	√
889.697	√	√	√	√		√	√	√	
891.599	√	√	√	√	√	√			√
893.555	√	√	√	√	√	√	√	√	√
897.590	√	√	√	√		√	√	√	√
899.604	√		√			√	√	√	√
909.547	√	√	√	√		√		√	√
911.565	√	√	√	√		√	√	√	
913.586	√	√	√		√	√	√		
Total number of detected lipids	**106**	**103**	**104**	**104**	**74**	**107**	**90**	**83**	**97**

Supplementary Information Table S3. Orthogonal-array testing for lipid ion signal detection on rat liver tissue sections by (-)MALDI-TOF/TOF MS for 2-MBT matrix solution composition optimization.

Lipid ion signals (*m/z*)	Optimization of 2-MBT matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
417.306	√	√	√	√	√	√	√	√	√
419.228	√	√	√	√	√	√	√	√	√
422.230	√	√	√	√	√	√	√	√	√
423.217	√	√	√	√	√	√	√	√	√
433.236	√	√	√	√	√	√	√		√
459.257		√	√		√			√	
464.277	√	√	√	√	√	√	√	√	√
507.272	√	√	√	√	√	√	√	√	√
514.292	√				√	√	√	√	√
521.421	√	√	√	√	√	√	√	√	√
522.393	√	√	√	√	√	√		√	√
523.304	√	√	√	√	√	√	√	√	√
552.299		√	√	√	√	√	√	√	√
557.309	√	√	√	√	√	√	√	√	√
559.320	√	√	√	√	√		√	√	
567.293	√	√	√	√	√	√			√
599.328	√		√	√	√	√	√	√	√
616.476	√	√	√	√	√	√	√	√	√
679.495	√	√		√	√	√	√	√	√
687.532	√	√	√	√	√	√	√	√	√
734.453			√	√	√	√	√	√	√
735.493	√	√	√	√	√	√		√	√
736.494	√	√	√	√	√	√	√	√	√
742.538	√	√	√	√	√		√		√
749.538	√	√		√	√	√	√	√	√
750.545	√	√	√	√	√	√	√	√	√
751.630	√	√	√	√	√	√	√	√	√
754.578	√	√	√	√	√	√	√	√	√
758.470	√	√	√	√	√	√	√	√	√
759.430	√	√	√	√	√	√	√	√	√
760.512	√	√	√	√	√	√	√	√	√
766.539				√	√				√
772.587	√	√	√	√	√	√	√	√	√
773.537				√	√				√
777.699	√	√	√	√	√	√	√	√	√

Lipid ion signals (*m/z*)	Optimization of 2-MBT matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
780.420	√	√	√	√	√	√	√	√	√
782.480		√	√	√	√	√	√	√	√
783.430	√	√	√	√	√	√		√	√
785.470				√	√	√	√	√	√
786.565	√	√	√	√	√	√	√	√	√
787.470				√	√	√	√	√	√
789.605			√	√	√	√	√	√	√
790.573	√	√	√	√	√	√	√	√	√
796.480		√	√	√	√	√	√	√	√
801.454	√	√	√	√	√	√	√	√	√
802.590	√	√	√	√	√	√	√	√	√
803.660	√	√	√	√	√	√	√	√	√
804.410	√	√	√	√	√			√	√
806.470			√	√	√			√	√
807.556	√	√	√	√	√	√	√	√	√
808.513				√	√			√	√
848.546	√	√	√	√	√	√		√	√
849.550		√		√	√		√	√	√
850.563	√	√	√	√	√	√	√	√	√
857.512		√		√	√			√	√
875.572	√	√	√	√	√		√		√
876.583		√	√	√	√		√	√	
877.594	√	√	√	√	√	√			√
879.601	√	√	√	√	√			√	
878.498	√				√	√			
883.534	√	√	√	√	√				
885.653	√	√		√	√			√	
887.673	√				√	√			√
888.572			√		√				
913.586				√	√				
933.683					√				
957.543					√				
Total number of detected lipids	**48**	**51**	**51**	**60**	**67**	**48**	**45**	**53**	**56**

Supplementary Information Table S4. Orthogonal-array testing for lipid ion signal detection on rat liver tissue sections by (-)MALDI-TOF/TOF MS for DHA matrix solution composition optimization.

Lipid ion signals (*m/z*)	Optimization of DHA matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
507.272	√	√	√	√	√	√	√	√	√
514.292	√	√	√	√	√	√	√	√	√
552.299	√	√	√	√	√	√	√	√	√
419.228	√	√	√	√	√	√	√	√	√
699.494	√	√	√	√	√	√	√	√	√
722.512	√	√	√	√	√	√	√	√	√
738.513	√	√	√	√	√		√	√	√
740.528	√	√	√	√	√	√	√	√	√
742.538	√	√	√	√	√	√	√	√	√
748.527	√	√		√	√	√	√	√	√
765.583	√	√	√	√	√	√	√	√	√
766.539	√	√	√	√	√	√	√	√	√
767.502	√	√	√	√	√		√	√	√
785.470	√	√	√	√	√	√	√	√	√
791.619	√	√	√		√	√	√	√	√
834.525	√	√	√	√	√	√	√	√	√
835.585	√	√	√	√	√	√	√	√	√
848.546	√	√	√	√	√	√	√	√	√
849.550		√	√	√	√	√	√	√	√
850.563	√	√	√	√		√	√	√	√
852.616	√	√	√		√	√	√	√	√
857.512		√	√	√	√	√	√	√	√
859.538	√	√		√	√		√	√	√
860.543	√	√	√	√	√	√	√	√	√
862.565	√	√	√	√	√	√	√	√	√
865.504	√	√	√		√	√	√	√	√
871.563	√	√	√	√	√	√	√	√	√
873.556		√	√	√	√	√	√	√	√
875.572	√	√	√	√	√	√	√	√	√
876.583	√	√	√	√	√		√		√
877.594	√	√	√	√		√	√	√	√
879.601	√	√		√	√		√		√
878.498	√	√	√	√	√	√	√	√	√
881.518	√	√	√	√	√	√	√	√	√
882.523	√	√	√	√	√	√	√	√	√

Lipid ion signals (*m/z*)	Optimization of DHA matrix solution composition								
	Concentration factor combinations of three selected variables*								
	1 (A1B1C1)	2 (A1B2C2)	3 (A1B3C3)	4 (A2B1C2)	5 (A2B2C3)	6 (A2B3C1)	7 (A3B1C3)	8 (A3B2C1)	9 (A3B3C2)
885.653	√	√	√	√	√	√	√		√
886.565	√	√	√	√	√	√	√	√	√
888.572	√	√	√	√	√	√			√
889.697	√	√	√	√	√	√	√	√	√
891.639	√	√	√	√	√	√	√	√	√
895.539	√	√	√	√		√	√	√	√
899.604	√	√	√	√	√	√	√	√	√
909.547	√	√	√	√	√	√		√	√
911.565	√	√	√	√	√	√	√	√	√
913.586	√	√	√	√	√	√	√		√
915.590	√	√	√	√	√		√		
923.609		√	√						
927.592		√	√	√	√		√		
933.683	√	√	√	√					
937.584	√	√	√	√		√			
Total number of detected lipids	**45**	**50**	**47**	**46**	**44**	**41**	**45**	**40**	**45**

Supplementary Information Table S5. Comparison of lipids ion signals detected in three parallel rat liver tissue sections by (-)MALDI-TOF/TOF MS using optimized MEK, 2-MBT, and DHA as the matrices, respectively.

Detected lipid ion signals (*m/z*)	Rat liver tissue sections		
	MEK	2-MBT	DHA
407.222	√		
410.194	√		
417.306		√	
419.228		√	
422.230		√	
423.217		√	
429.202	√		
433.236	√	√	
437.268	√		
440.206	√		
454.188	√		
459.257		√	
461.264	√		
463.285	√		
464.277	√	√	
478.293	√		
480.308	√		
497.323	√		
502.297	√		
503.241	√		
504.345	√		
505.257	√		
506.321	√		
507.272	√	√	√
514.292		√	√
515.228	√		
516.314	√		
521.421		√	
522.393		√	
523.304		√	
533.288	√		
536.377	√		
537.319	√		
539.332	√		

Detected lipid ion signals (*m/z*)	Rat liver tissue sections		
	MEK	2-MBT	DHA
540.335	√		
550.318	√		
552.299	√	√	√
557.309	√	√	
559.320	√	√	
565.355	√		
566.315	√		
567.293	√	√	
571.289	√		
572.294	√		
579.294	√		
580.293	√		
583.321	√		
595.285	√		
596.286	√		
597.305	√		
599.328		√	
606.304	√		
609.309	√		
613.374	√		
616.476		√	
617.418	√		
618.450	√		
619.287	√		
620.325	√		
621.308	√		
622.295	√		
625.338	√		
630.340	√		
635.315	√		
641.450	√		
648.347	√		
650.335	√		
651.313	√		
653.330	√		
660.387	√		
669.456	√		
671.460	√		
672.498	√		
673.481	√		
674.514	√		

Detected lipid ion signals (*m/z*)	Rat liver tissue sections		
	MEK	2-MBT	DHA
679.495	√	√	
685.406	√		
687.532	√	√	
696.496	√		
698.507	√		
699.494	√		√
700.532	√		
701.517	√		
714.514	√		
715.562	√		
717.582	√		
718.536	√		
719.485	√		
720.502	√		
721.500	√		
722.512	√		√
724.535	√		
725.518	√		
726.575	√		
727.524	√		
728.596	√		
730.579	√		
734.453		√	
735.493		√	
736.494		√	
738.513	√		√
739.561	√		
740.528	√		√
741.580	√		
742.538		√	√
743.593	√		
745.505	√		
746.511	√		
747.513	√		
748.527	√		√
749.538	√	√	
750.545	√	√	
751.630		√	
753.541	√		
754.578	√	√	
758.470		√	

Detected lipid ion signals (*m/z*)	Rat liver tissue sections		
	MEK	2-MBT	DHA
759.430		√	
760.512	√	√	
762.525	√		
763.568	√		
765.583	√		√
766.539		√	√
767.502	√		√
769.615	√		
770.575	√		
771.622	√		
772.587	√	√	
773.537	√	√	
776.554	√		
777.699		√	
780.420		√	
781.575	√		
782.480		√	
783.430		√	
785.470		√	√
786.565		√	
787.470		√	
788.544	√		
789.605	√	√	
790.573	√	√	
791.619	√		√
793.528	√		
796.480		√	
797.410		√	
798.565	√	√	
799.528	√	√	
800.658		√	
801.454	√	√	
802.590		√	
803.660		√	
804.410		√	
805.480	√		
806.470		√	
807.556	√	√	
808.513		√	
816.577	√		
818.594	√		

Detected lipid ion signals (*m/z*)	Rat liver tissue sections		
	MEK	2-MBT	DHA
819.641	√		
833.516	√		
834.525	√		√
835.585	√		√
838.556	√		
848.546		√	√
849.550		√	√
850.563		√	√
851.565			√
852.616		√	√
853.727		√	
857.512	√	√	√
858.534	√		
859.538	√	√	√
860.543	√		√
861.556	√	√	√
862.565	√		√
863.561	√		
865.504	√		√
871.563	√		√
873.556			√
875.572		√	√
876.583		√	√
877.594		√	√
879.601		√	√
878.498		√	√
881.518	√		√
882.523	√		√
883.534	√	√	
885.653	√	√	√
886.565	√		√
887.673		√	√
888.572	√	√	√
889.697	√		√
891.599	√		√
893.555	√		√
895.539			√
897.590	√		√
899.604	√		√
909.547	√		√
911.565	√		√

Detected lipid ion signals (*m/z*)	Rat liver tissue sections		
	MEK	**2-MBT**	**DHA**
913.586	√	√	√
915.590			√
921.545			√
923.609			√
925.612			√
927.592			√
933.683	√		√
937.584	√		√
939.595			√
957.543	√		√
Total number of detected lipids	**154**	**73**	**58**

Supplementary Information Table S6. Lipids detected and assigned in a rat brain tissue section by (-)MALDI-TOF/TOF MS using MEK as the matrix.

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
1	410.194	410.1949	2	$[M-H]^-$	PE(12:0)	$C_{17}H_{34}NO_8P$	
2	420.253	420.2521	3	$[M-H]^-$	CerP(20:1)	$C_{20}H_{40}NO_6P$	
3	436.283	436.2834	1	$[M-H]^-$	LysoPE(O-16:1)	$C_{21}H_{44}NO_6P$	
4	449.307	449.3038	7	$[M-H]^-$	LysoPA(O-20:1)	$C_{23}H_{47}O_6P$	
5	450.265	450.2626	5	$[M-H]^-$	LysoPE(16:1)	$C_{21}H_{42}NO_7P$	
6	451.316	451.3194	8	$[M-H]^-$	LysoPA(O-20:0)	$C_{23}H_{49}O_6P$	
7	454.186	454.1848	3	$[M-H]^-$	PS(12:0)	$C_{18}H_{34}NO_{10}P$	
8	455.245	455.2416	7	$[M-H]^-$	LysoPG(14:0)	$C_{20}H_{41}O_9P$	
9	461.264	461.2674	7	$[M-H]^-$	LysoPA(20:2)	$C_{23}H_{43}O_7P$	
10	464.314	464.3147	2	$[M-H]^-$	LysoPE(O-18:1)	$C_{23}H_{48}NO_6P$	464, 403, 267, 140
11	469.298	469.2936	9	$[M-H]^-$	LysoPG(O-16:0)	$C_{22}H_{47}O_8P$	
12	474.265	474.2626	5	$[M-H]^-$	LysoPE(18:3)	$C_{23}H_{42}NO_7P$	474, 277, 259
13	491.318	491.3143	7	$[M-H]^-$	LysoPA(22:1)	$C_{25}H_{49}O_7P$	
14	506.324	506.3252	2	$[M-H]^-$	LysoPE(20:1)	$C_{25}H_{50}NO_7P$	506, 309, 291, 140, 79
15	518.251	518.2525	3	$[M-H]^-$	LysoPS(20:2)	$C_{24}H_{42}NO_9P$	
16	519.275	519.2729	4	$[M-H]^-$	PA(22:2)	$C_{25}H_{45}O_9P$	
17	520.263	520.2681	9	$[M-H]^-$	LysoPS(18:2)	$C_{24}H_{44}NO_9P$	
18	522.287	522.2838	6	$[M-H]^-$	LysoPS(18:1)	$C_{24}H_{46}NO_9P$	
19	523.266	523.2678	3	$[M-H]^-$	PA(22:1)	$C_{24}H_{45}O_{10}P$	
20	524.295	524.2994	8	$[M-H]^-$	LysoPS(18:0)	$C_{24}H_{48}NO_9P$	524, 437, 283, 265
21	532.342	532.3409	2	$[M-H]^-$	LysoPE(22:2)	$C_{27}H_{52}NO_7P$	532, 335, 214, 196
22	540.334	540.3307	6	$[M-H]^-$	LysoPS(O-18:0)	$C_{25}H_{52}NO_9P$	
23	546.288	546.2838	8	$[M-H]^-$	LysoPS(20:3)	$C_{26}H_{46}NO_9P$	
24	548.464	548.4684	8	$[M-H]^-$	CerP(34:3)	$C_{34}H_{63}NO_4$	
25	549.355	549.3562	2	$[M-H]^-$	PA(26:0)	$C_{28}H_{55}O_8P$	
26	550.314	550.3151	2	$[M-H]^-$	PE(20:1;O)	$C_{26}H_{50}NO_9P$	
27	552.298	552.2943	7	$[M-H]^-$	PE(20:1)	$C_{25}H_{48}NO_{10}P$	
28	553.314	553.3147	1	$[M-H]^-$	PG(20:0)	$C_{26}H_{51}O_{10}P$	
29	557.301	557.3096	9	$[M-H]^-$	LysoPI(O-16:0)	$C_{25}H_{51}O_{11}P$	
30	564.406	564.4035	4	$[M-H]^-$	LysoPE(24:0)	$C_{29}H_{60}NO_7P$	
31	566.414	566.4191	9	$[M-H]^-$	LysoPE(O-24:0)	$C_{29}H_{62}NO_7P$	
32	572.297	572.2994	4	$[M-H]^-$	LysoPS(22:4)	$C_{28}H_{48}NO_9P$	
33	575.402	575.4082	9	$[M-H]^-$	PA(O-28:1)	$C_{31}H_{61}O_7P$	
34	576.335	576.3307	7	$[M-H]^-$	LysoPS(22:2)	$C_{28}H_{52}NO_9P$	

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
35	580.363	580.3620	2	$[M-H]^-$	LysoPS(22:0)	$C_{28}H_{56}NO_9P$	
36	588.443	588.4399	7	$[M-H]^-$	CerP(32:1)	$C_{32}H_{64}NO_6P$	
37	589.387	589.3875	8	$[M-H]^-$	PA(28:1)	$C_{31}H_{59}O_8P$	
38	599.545	599.5416	8	$[M-H]^-$	Cer(36:0)	$C_{36}H_{73}NO_5$	599, 301, 283, 241, 223
39	603.437	603.4395	4	$[M-H]^-$	PA(O-30:1)	$C_{33}H_{65}O_7P$	
40	605.455	605.4552	3	$[M-H]^-$	PA(O-30:0)	$C_{33}H_{67}O_7P$	
41	615.409	615.4031	9	$[M-H]^-$	PA(30:2)	$C_{33}H_{61}O_8P$	
42	616.472	616.4712	1	$[M-H]^-$	CerP(34:1)	$C_{34}H_{68}NO_6P$	
43	617.418	617.4188	1	$[M-H]^-$	PA(30:1)	$C_{33}H_{63}O_8P$	
44	618.450	618.4504	1	$[M-H]^-$	PE(O-28:1)	$C_{32}H_{62}NO_8P$	
45	619.431	619.4344	5	$[M-H]^-$	PA(30:0)	$C_{33}H_{65}O_8P$	
46	620.466	620.4661	0	$[M-H]^-$	PE(O-28:0)	$C_{33}H_{68}NO_7P$	
47	631.470	631.4708	1	$[M-H]^-$	PA(O-32:1)	$C_{35}H_{69}O_7P$	
48	643.435	643.4344	0	$[M-H]^-$	PA(32:2)	$C_{35}H_{65}O_8P$	
49	645.456	645.4501	9	$[M-H]^-$	PA(32:1)	$C_{35}H_{67}O_8P$	
50	646.613	646.6144	2	$[M-H]^-$	Cer(42:2)	$C_{42}H_{81}NO_3$	
51	647.467	647.4657	2	$[M-H]^-$	PA(32:0)	$C_{35}H_{69}O_8P$	647, 339, 321, 171, 78
52	648.491	648.4974	10	$[M-H]^-$	PE(O-30:0)	$C_{35}H_{72}NO_7P$	
53	650.408	650.4039	6	$[M-H]^-$	PS(26:0)	$C_{32}H_{62}NO_{10}P$	
54	651.439	651.4395	1	$[M-H]^-$	PA(O-34:5)	$C_{37}H_{65}O_7P$	
55	655.378	655.3828	1	$[M-H]^-$	LysoPI(22:0)	$C_{31}H_{61}O_{12}P$	
56	664.458	664.4559	3	$[M-H]^-$	PS(O-28:0)	$C_{34}H_{68}NO_9P$	
57	671.462	671.4657	5	$[M-H]^-$	PA(34:2)	$C_{37}H_{69}O_8P$	
58	673.485	673.4814	5	$[M-H]^-$	PA(34:1)	$C_{37}H_{71}O_8P$	673, 417, 391, 281, 255
59	675.493	675.4970	5	$[M-H]^-$	PA(34:0)	$C_{37}H_{73}O_8P$	675, 447, 363, 311, 227
60	677.479	677.4763	4	$[M-H]^-$	PG(O-30:1)	$C_{36}H_{71}O_9P$	
61	679.345	679.3464	2	$[M-H]^-$	PG(26:3)	$C_{32}H_{57}O_{13}P$	
62	695.466	695.4657	0	$[M-H]^-$	PA(36:4)	$C_{39}H_{69}O_8P$	
63	699.496	699.4970	1	$[M-H]^-$	PA(36:2)	$C_{39}H_{73}O_8P$	
64	701.515	701.5127	3	$[M-H]^-$	PA(36:1)	$C_{39}H_{75}O_8P$	
65	702.548	702.5443	5	$[M-H]^-$	PE(O-34:1)	$C_{39}H_{78}NO_7P$	
66	703.525	703.5283	5	$[M-H]^-$	PA(36:0)	$C_{39}H_{77}O_8P$	
67	713.544	713.5491	7	$[M-H]^-$	PA(O-38:2)	$C_{41}H_{79}O_7P$	
68	715.566	715.5647	2	$[M-H]^-$	PA(O-38:1)	$C_{41}H_{81}O_7P$	
69	717.451	717.4501	1	$[M-H]^-$	PA(38:7)	$C_{41}H_{67}O_8P$	
70	718.535	718.5392	6	$[M-H]^-$	PE(34:0)	$C_{39}H_{78}NO_8P$	700, 227, 168, 96, 78

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
71	719.482	719.4869	7	$[M-H]^-$	PG(32:1)	$C_{38}H_{73}O_{10}P$	
72	721.507	721.5025	6	$[M-H]^-$	PG(32:0)	$C_{38}H_{75}O_{10}P$	
73	722.516	722.5130	4	$[M-H]^-$	PE(O-36:5)	$C_{41}H_{74}NO_7P$	
74	723.493	723.4970	6	$[M-H]^-$	PA(38:4)	$C_{41}H_{73}O_8P$	
75	725.515	725.5127	3	[M-H]	PA(38:3)	$C_{41}H_{75}O_8P$	
76	726.583	726.5807	3	$[M-H]^-$	CerP(42:2)	$C_{42}H_{82}NO_6P$	
77	727.525	727.5283	5	$[M-H]^-$	PA(38:2)	$C_{41}H_{77}O_8P$	
78	729.543	729.5440	1	$[M-H]^-$	PA(38:1)	$C_{41}H_{79}O_8P$	
79	731.554	731.5596	8	$[M-H]^-$	PA(38:0)	$C_{41}H_{81}O_8P$	731, 503, 367, 363, 227
80	732.593	732.5913	2	$[M-H]^-$	PE(O-36:0)	$C_{41}H_{84}NO_7P$	
81	739.566	739.5647	2	$[M-H]^-$	PA(O-40:3)	$C_{43}H_{81}O_7P$	
82	740.485	740.4872	3	$[M-H]^-$	PS(O-34:4)	$C_{40}H_{72}NO_9P$	
83	742.544	742.5392	6	$[M-H]^-$	PE(36:2)	$C_{41}H_{78}NO_8P$	742, 196, 152
84	743.465	743.4657	0	$[M-H]^-$	PA(40:8)	$C_{43}H_{69}O_8P$	483, 329, 311, 277, 78
85	744.552	744.5549	4	$[M-H]^-$	PE(36:1)	$C_{41}H_{80}NO_8P$	744, 311, 253
86	745.615	745.6117	4	$[M-H]^-$	PA(O-40:0)	$C_{43}H_{87}O_7P$	745, 491, 253, 227, 209
87	746.570	746.5705	1	$[M-H]^-$	PE(36:0)	$C_{41}H_{82}NO_8P$	746, 311, 255
88	749.536	749.5338	3	$[M-H]^-$	PG(34:0)	$C_{40}H_{79}O_{10}P$	749, 283, 255
89	754.617	754.6120	7	$[M-H]^-$	CerP(44:2)	$C_{44}H_{86}NO_6P$	
90	757.573	757.5753	3	$[M-H]^-$	PA(40:1)	$C_{43}H_{83}O_8P$	757, 447, 445, 311, 309
91	762.527	762.5291	3	$[M-H]^-$	PS(34:0)	$C_{40}H_{78}NO_{10}P$	
92	763.586	763.5859	0	$[M-H]^-$	PG(O-36:0)	$C_{42}H_{85}O_9P$	
93	764.528	764.5236	6	$[M-H]^-$	PE(38:5)	$C_{43}H_{76}NO_8P$	764, 303, 281, 263
94	765.587	765.5804	9	$[M-H]^-$	PA(O-42:4)	$C_{45}H_{83}O_7P$	
95	767.468	767.4657	3	$[M-H]^-$	PA(42:10)	$C_{45}H_{69}O_8P$	
96	769.610	769.6117	2	$[M-H]^-$	PA(O-42:2)	$C_{45}H_{87}O_7P$	
97	772.587	772.5862	1	$[M-H]^-$	PE(38:1)	$C_{43}H_{84}NO_8P$	772, 339, 253
98	773.650	773.6430	9	$[M-H]^-$	PA(O-42:0)	$C_{45}H_{91}O_7P$	
99	774.604	774.6018	3	$[M-H]^-$	PE(38:0)	$C_{43}H_{86}NO_8P$	774, 311, 283
100	776.565	776.5600	6	$[M-H]^-$	PE(O-40:6)	$C_{45}H_{80}NO_7P$	
101	777.562	777.5651	4	$[M-H]^-$	PG(36:0)	$C_{42}H_{83}O_{10}P$	777, 283
102	779.557	779.5596	3	$[M-H]^-$	PA(42:4)	$C_{45}H_{81}O_8P$	779, 501, 413, 365, 277
103	788.650	788.6539	5	$[M-H]^-$	PE(O-40:0)	$C_{45}H_{92}NO_7P$	788, 367, 241
104	789.607	789.6015	7	$[M-H]^-$	PG(O-38:1)	$C_{44}H_{87}O_9P$	
105	790.565	790.5604	2	$[M-H]^-$	PS(36:0)	$C_{42}H_{82}NO_{10}P$	283, 265, 184, 78

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
106	791.617	791.6172	0	$[M-H]^-$	PG(O-38:0)	$C_{44}H_{89}O_9P$	
107	792.555	792.5549	0	$[M-H]^-$	PE(40:5)	$C_{45}H_{80}NO_8P$	792, 329, 283
108	793.572	793.5753	4	$[M-H]^-$	PA(44:1)	$C_{46}H_{83}O_8P$	793, 253, 235, 225, 207
109	795.533	795.5393	8	$[M-H]^-$	PI(O-32:0)	$C_{41}H_{81}O_{12}P$	795, 277, 259, 225, 207
110	797.606	797.6066	1	$[M-H]^-$	PA(44:2)	$C_{46}H_{87}O_8P$	
111	799.623	799.6222	1	$[M-H]^-$	PA(44:5)	$C_{46}H_{89}O_8P$	
112	802.633	802.6331	0	$[M-H]^-$	PE(40:0)	$C_{45}H_{90}NO_8P$	802, 367, 255
113	804.613	804.6124	0	$[M-H]^-$	PS(O-38:0)	$C_{44}H_{88}NO_9P$	
114	805.601	805.5964	5	$[M-H]^-$	PG(38:0)	$C_{44}H_{87}O_{10}P$	
115	806.608	806.6069	1	$[M-H]^-$	PE(O-42:5)	$C_{47}H_{86}NO_7P$	
116	807.598	807.5909	9	$[M-H]^-$	PA(44:4)	$C_{47}H_{85}O_8P$	807, 367, 349, 303, 78
117	809.606	809.6066	1	$[M-H]^-$	PA(44:3)	$C_{47}H_{87}O_8P$	
118	810.637	810.6382	6	$[M-H]^-$	PE(O-42:3)	$C_{47}H_{90}NO_7P$	
119	811.580	811.5859	7	$[M-H]^-$	PG(O-40:4)	$C_{46}H_{85}O_9P$	
120	816.684	816.6852	1	$[M-H]^-$	PE(O-42:0)	$C_{47}H_{96}NO_7P$	
121	821.553	821.5549	2	$[M-H]^-$	PI(O-34:1)	$C_{43}H_{83}O_{12}P$	
122	822.602	822.6018	0	$[M-H]^-$	PE(42:4)	$C_{47}H_{86}NO_8P$	822, 339, 303
123	823.548	823.5495	2	$[M-H]^-$	PI(40:5)	$C_{46}H_{81}O_{10}P$	823, 493,329, 283, 265
124	824.617	824.6175	0	$[M-H]^-$	PE(42:3)	$C_{47}H_{88}NO_8P$	824, 367, 277
125	826.590	826.5968	8	$[M-H]^-$	PS(O-40:3)	$C_{46}H_{86}NO_9P$	
126	830.625	830.6281	4	$[M-H]^-$	PS(O-40:1)	$C_{46}H_{90}NO_9P$	
127	832.639	832.6437	6	$[M-H]^-$	PS(O-40:0)	$C_{46}H_{92}NO_9P$	
128	833.627	833.6277	1	$[M-H]^-$	PG(40:0)	$C_{46}H_{91}O_{10}P$	
129	835.588	835.5859	3	$[M-H]^-$	PG(O-42:6)	$C_{48}H_{85}O_9P$	
130	836.549	836.5447	5	$[M-H]^-$	PS(40:5)	$C_{46}H_{80}NO_{10}P$	836, 749, 471, 413, 335, 277
131	837.605	837.6015	4	$[M-H]^-$	PG(O-42:5)	$C_{48}H_{87}O_9P$	
132	850.633	850.6331	0	$[M-H]^-$	PE(44:4)	$C_{40}H_{90}NO_8P$	850, 365, 305
133	851.605	851.6019	4	$[M-H]^-$	PI(O-36:0)	$C_{45}H_{89}O_{12}P$	
134	852.650	852.6488	1	$[M-H]^-$	PE(44:3)	$C_{49}H_{92}NO_8P$	
135	855.504	855.5029	1	$[M-H]^-$	PI(36:5)	$C_{45}H_{77}O_{13}P$	
136	856.640	856.6437	4	$[M-H]^-$	PS(O-42:2)	$C_{48}H_{92}NO_9P$	
137	857.625	857.6277	3	$[M-H]^-$	PG(42:2)	$C_{48}H_{91}O_{10}P$	
138	858.652	858.6594	9	$[M-H]^-$	PS(O-42:1)	$C_{48}H_{94}NO_9P$	
139	859.640	859.6434	4	$[M-H]^-$	PG(42:1)	$C_{48}H_{93}O_{10}P$	
140	860.675	860.6750	0	$[M-H]^-$	PS(O-42:0)	$C_{48}H_{96}NO_9P$	
141	861.655	861.6590	5	$[M-H]^-$	PG(42:0)	$C_{48}H_{95}O_{10}P$	

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
142	865.585	865.5812	4	$[M-H]^-$	PI(36:0)	$C_{45}H_{87}O_{13}P$	571, 447, 391, 311
143	867.614	867.6121	2	$[M-H]^-$	PG(44:5)	$C_{49}H_{89}O_{10}P$	867, 327, 309, 275, 257, 225
144	872.634	872.6386	5	$[M-H]^-$	PS(42:1)	$C_{48}H_{92}NO_{10}P$	
145	873.586	873.5862	0	$[M-H]^-$	PI(O-38:3)	$C_{47}H_{87}O_{12}P$	
146	874.655	874.6543	1	$[M-H]^-$	PS(42:0)	$C_{48}H_{94}NO_{10}P$	874, 787, 311, 96, 78
147	877.591	877.5964	6	$[M-H]^-$	PG(44:6)	$C_{50}H_{87}O_{10}P$	
148	879.637	879.6332	5	$[M-H]^-$	PI(O-38:0)	$C_{47}H_{93}O_{12}P$	
149	881.626	881.6277	2	$[M-H]^-$	PG(44:4)	$C_{50}H_{91}O_{10}P$	
150	883.535	883.5342	1	$[M-H]^-$	PI(38:5)	$C_{47}H_{81}O_{13}P$	883, 303, 285, 281
151	884.718	884.7114	7	$[M-H]^-$	PE(46:1)	$C_{51}H_{100}NO_8P$	884, 365, 339
152	886.564	886.5604	4	$[M-H]^-$	PS(44:8)	$C_{50}H_{82}NO_{10}P$	886, 799, 471, 463, 335, 327
153	887.669	887.6747	6	$[M-H]^-$	PG(44:1)	$C_{50}H_{97}O_{10}P$	
154	889.696	889.6903	6	$[M-H]^-$	PG(44:0)	$C_{50}H_{99}O_{10}P$	331, 313, 277, 259, 241
155	891.598	891.5968	1	$[M-H]^-$	PI(38:1)	$C_{47}H_{89}O_{13}P$	
156	893.610	893.6125	3	$[M-H]^-$	PI(38:0)	$C_{47}H_{91}O_{13}P$	893, 303, 285, 241, 223
157	894.629	894.6230	7	$[M-H]^-$	PS(44:4)	$C_{50}H_{99}NO_{10}P$	
158	899.601	899.6019	1	$[M-H]^-$	PI(O-40:4)	$C_{49}H_{89}O_{12}P$	
159	902.686	902.6856	1	$[M-H]^-$	PS(44:0)	$C_{50}H_{98}NO_{10}P$	902, 815, 96, 78
160	905.646	905.6488	3	$[M-H]^-$	PI(O-40:1)	$C_{49}H_{95}O_{12}P$	
161	907.666	907.6645	2	$[M-H]^-$	PI(O-40:0)	$C_{49}H_{97}O_{12}P$	
162	909.548	909.5499	2	$[M-H]^-$	PI(40:6)	$C_{49}H_{83}O_{13}P$	909, 335, 281, 263, 253, 235
163	935.693	935.6958	3	$[M-H]^-$	PI(O-42:0)	$C_{51}H_{101}O_{12}P$	
164	943.628	943.6281	0	$[M-H]^-$	PI(42:3)	$C_{51}H_{93}O_{13}P$	
165	961.581	961.5812	0	$[M-H]^-$	PI(44:8)	$C_{53}H_{87}O_{13}P$	
166	967.621	967.6281	9	$[M-H]^-$	PI(44:5)	$C_{53}H_{93}O_{13}P$	967, 337, 319, 309, 281, 263

[a)] Structurally specific CID ions of lipids were detected by LC-MS/MS using CID. Fragment ions shown in blue were detected by *in situ* MALDI-TOF/TOF MS/MS. Abbreviations: PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; Cer, ceramide; CerP, ceramide 1-phosphate.

Supplementary Information Table S7. Comparison of lipid ion signals detected in three parallel rat brain tissue sections by (-)MALDI-TOF/TOF MS using optimized MEK, 2-MBT, and DHA as the matrices, respectively.

Detected lipid ion signals (*m/z*)	Rat brain tissue sections		
	MEK	2-MBT	DHA
410.194	√	√	
420.253	√		
436.283	√		
449.307	√		
450.265	√	√	
451.316	√		√
454.186	√		
455.245	√		
461.264	√		√
464.314	√		
469.298	√		
474.265	√		√
491.318	√		
506.324	√		√
514.293		√	
518.251	√		
519.275	√		√
520.263	√		
522.287	√		
523.266	√		
524.295	√		
532.342	√		
540.334	√		√
546.288	√		
548.464	√		
549.355	√		
550.314	√		
552.298	√		
553.314	√		√
557.301	√		
564.406	√		√
566.414	√		
572.297	√		
575.402	√	√	
576.335	√		
580.363	√		

Detected lipid ion signals (*m/z*)	Rat brain tissue sections		
	MEK	2-MBT	DHA
588.443	√		√
589.387	√		
599.545	√	√	
603.437	√		
605.455	√		√
615.409	√		
616.472	√		
617.418	√		
618.450	√		
619.431	√		
620.466	√		
631.470	√		
643.435	√		
645.456	√		
646.613	√		
647.467	√		
648.491	√		
650.408	√		
651.439	√		
655.378	√		
664.458	√		
671.462	√		
673.485	√		
675.493	√		
677.479	√		
679.345	√	√	
695.466	√		
699.496	√		√
701.515	√	√	
702.548	√		
703.525	√		
713.544	√		
715.566	√	√	
717.451	√		
718.535	√	√	
719.482	√		
721.507	√		
722.516	√	√	√
723.493	√	√	
725.515	√	√	
726.583	√	√	

Detected lipid ion signals (*m/z*)	Rat brain tissue sections		
	MEK	2-MBT	DHA
727.525	√		
729.543	√		√
731.554	√		
732.593	√	√	
739.566	√	√	
740.485	√	√	√
742.544	√	√	√
743.465	√		√
744.552	√	√	√
745.615	√	√	
746.570	√		
749.536	√	√	
754.617	√		
757.573	√	√	
762.527	√		√
763.586	√	√	
764.528	√		
765.587	√	√	
766.536		√	
767.468	√	√	√
769.610	√	√	
772.587	√	√	
773.650	√	√	
774.604	√		√
776.565	√	√	√
777.562	√		
779.557	√	√	
788.650	√	√	
789.607	√	√	√
790.565	√		
791.617	√		√
792.555	√		
793.572	√	√	
795.533	√	√	
797.606	√	√	
799.623	√	√	√
802.633	√	√	√
804.613	√	√	
805.601	√		√
806.608	√	√	
807.598	√		

Detected lipid ion signals (*m/z*)	Rat brain tissue sections		
	MEK	2-MBT	DHA
809.606	√		
810.637	√	√	√
811.580	√		
816.684	√	√	
821.553	√	√	√
822.602	√	√	
823.548	√	√	
824.617	√	√	
826.590	√		
830.625	√	√	
832.639	√	√	
833.627	√		
834.521		√	√
835.588	√	√	√
836.549	√	√	√
837.605	√	√	
850.633	√	√	
851.605	√	√	√
852.650	√		√
855.504	√	√	
856.640	√		√
857.625	√	√	√
858.652	√	√	
859.640	√		√
860.675	√	√	√
861.655	√	√	√
862.569		√	√
863.718		√	
865.585	√	√	√
867.614	√	√	
872.634	√	√	√
873.586	√	√	
874.655	√	√	√
877.591	√	√	√
879.637	√	√	√
881.626	√	√	√
883.535	√		
884.718	√	√	√
885.657		√	
886.564	√	√	√
887.669	√	√	√

Detected lipid ion signals (*m/z*)	Rat brain tissue sections		
	MEK	**2-MBT**	**DHA**
889.696	√	√	√
891.638	√	√	√
893.610	√	√	√
894.629	√	√	√
896.631			√
899.601	√	√	√
900.669			√
901.581		√	√
902.686	√	√	√
903.596		√	
905.646	√	√	√
907.666	√	√	√
909.548	√	√	√
923.713			√
925.617		√	√
935.693	√		√
943.628	√		√
961.581	√		√
967.621	√		
Total number of detected lipids	**166**	**84**	**65**

Supplementary Information Table S8. Lipids detected and assigned in a germinating Chinese-yew seed tissue section by (-)MALDI-TOF/TOF MS using MEK as the matrix.

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
1	409.235	409.2361	3	$[M-H]^-$	LysoPA(16:0)	$C_{19}H_{39}O_7P$	
2	419.220	419.2204	1	$[M-H]^-$	LysoPA(17:2)	$C_{20}H_{37}O_7P$	
3	421.272	421.2725	0	$[M-H]^-$	LysoPA(O-18:1)	$C_{21}H_{43}O6P$	
4	423.285	423.2881	8	$[M-H]^-$	LysoPA(O-18:0)	$C_{21}H_{45}O6P$	
5	424.248	424.2470	2	$[M-H]^-$	LysoPE(14:0)	$C_{19}H_{40}NO_7P$	
6	429.203	429.2048	4	$[M-H]^-$	LysoPA(18:4)	$C_{21}H_{35}O_7P$	
7	445.238	445.2361	4	$[M-H]^-$	LysoPA(18:3)	$C_{22}H_{39}O_7P$	
8	449.306	449.3038	5	$[M-H]^-$	LysoPA(O-20:1)	$C_{23}H_{47}O_6P$	
9	459.251	459.2517	2	$[M-H]^-$	LysoPA(20:3)	$C_{23}H_{41}O_7P$	
10	467.277	467.2779	1	$[M-H]^-$	LysoPG(O-16:1)	$C_{22}H_{45}O_8P$	
11	471.251	471.2517	2	$[M-H]^-$	LysoPA(20:4)	$C_{24}H_{41}O_7P$	
12	474.265	474.2626	5	$[M-H]^-$	LysoPE(18:3)	$C_{23}H_{42}NO_7P$	474, 277, 259
13	482.288	482.2888	1	$[M-H]^-$	LysoPS(O-16:0)	$C_{22}H_{46}NO_8P$	
14	485.263	485.2674	9	$[M-H]^-$	LysoPA(22:4)	$C_{25}H_{43}O_7P$	
15	489.296	489.2987	6	$[M-H]^-$	LysoPA(22:2)	$C_{25}H_{47}O_7P$	
16	491.315	491.3143	1	$[M-H]^-$	LysoPA(22:1)	$C_{25}H_{49}O_7P$	491, 152, 78
17	493.259	493.2572	4	$[M-H]^-$	LysoPG(18:1)	$C_{23}H_{43}O_9P$	
18	502.290	502.2939	8	$[M-H]^-$	LysoPE(20:3)	$C_{25}H_{46}NO_7P$	502, 459, 305 214, 194
19	505.256	505.2572	2	$[M-H]^-$	LysoPG(18:3)	$C_{24}H_{43}O_9P$	
20	506.249	506.2525	7	$[M-H]^-$	LysoPS(18:3)	$C_{23}H_{42}NO_9P$	
21	507.276	507.2729	6	$[M-H]^-$	PA(21:1)	$C_{24}H_{45}O_9P$	
22	509.250	509.2521	4	$[M-H]^-$	PA(20:1)	$C_{23}H_{43}O_{10}P$	
23	510.251	510.2474	7	$[M-H]^-$	PS(16:0)	$C_{22}H_{42}NO_{10}P$	
24	516.239	516.2368	4	$[M-H]^-$	LysoPS(18:4)	$C_{24}H_{40}NO_9P$	
25	519.270	519.2729	6	$[M-H]^-$	PA(22:2)	$C_{25}H_{45}O_9P$	
26	522.320	522.3201	2	$[M-H]^-$	PE(20:0)	$C_{25}H_{50}NO_8P$	
27	533.288	533.2885	1	$[M-H]^-$	LysoPG(20:3)	$C_{26}H_{47}O_9P$	
28	535.306	535.3042	4	$[M-H]^-$	LysoPG(20:2)	$C_{26}H_{49}O_9P$	
29	536.293	536.2994	1	$[M-H]^-$	PE(20:1)	$C_{25}H_{48}NO_9P$	
30	543.255	543.2576	5	$[M-H]^-$	LysoPI(14:0)	$C_{23}H_{45}O_{12}P$	
31	546.288	546.2838	8	$[M-H]^-$	LysoPS(20:3)	$C_{26}H_{46}NO_9P$	
32	547.265	547.2678	5	$[M-H]^-$	PA(23:3)	$C_{26}H_{45}O_{10}P$	
33	550.313	550.3151	4	$[M-H]^-$	PE(21:1)	$C_{26}H_{50}NO_9P$	
34	553.317	553.3147	4	$[M-H]^-$	PG(20:0)	$C_{26}H_{51}O_{10}P$	
35	555.259	555.2576	3	$[M-H]^-$	LysoPI(15:1)	$C_{24}H_{45}O_{12}P$	
36	557.305	557.3096	8	$[M-H]^-$	LysoPI(O-16:0)	$C_{25}H_{51}O_{11}P$	
37	559.305	559.3042	1	$[M-H]^-$	LysoPG(22:4)	$C_{28}H_{49}O_9P$	

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
38	561.356	561.3562	0	$[M-H]^-$	PA(26:1)	$C_{29}H_{55}O_8P$	
39	563.295	563.2991	7	$[M-H]^-$	PA(24:2)	$C_{27}H_{49}O_{10}P$	
40	569.273	569.2732	4	$[M-H]^-$	LysoPI(16:1)	$C_{25}H_{47}O_{12}P$	
41	575.404	575.4082	7	$[M-H]^-$	PA(O-28:1)	$C_{31}H_{61}O_7P$	
42	576.330	576.3307	1	$[M-H]^-$	PE(23:2)	$C_{28}H_{52}NO_9P$	
43	577.386	577.3875	3	$[M-H]^-$	PA(27:0)	$C_{30}H_{59}O_8P$	577, 227, 213 195, 78
44	578.380	578.3827	5	$[M-H]^-$	PE(24:0)	$C_{29}H_{58}NO_8P$	
45	583.325	583.3253	1	$[M-H]^-$	LysoPI(O-18:1)	$C_{27}H_{53}O_{11}P$	583, 301, 28, 225, 207
46	587.370	587.3718	3	$[M-H]^-$	PA(28:2)	$C_{31}H_{57}O_8P$	
47	589.355	589.3511	7	$[M-H]^-$	PA(27:2)	$C_{30}H_{55}O_9P$	
48	597.306	597.3045	3	$[M-H]^-$	PG(21:1)	$C_{27}H_{51}O_{12}P$	
49	599.324	599.3202	7	$[M-H]^-$	LysoPI(18:0)	$C_{27}H_{53}O_{12}P$	599, 301, 283 241, 223
50	601.425	601.4239	2	$[M-H]^-$	PA(O-30:2)	$C_{33}H_{63}O_7P$	
51	603.440	603.4395	1	$[M-H]^-$	PA(O-30:1)	$C_{33}H_{65}O_7P$	
52	604.432	604.4348	5	$[M-H]^-$	LysoPE(O-27:2)	$C_{32}H_{64}NO_7P$	604, 517, 501 365, 347
53	605.458	605.4552	5	$[M-H]^-$	PA(O-30:0)	$C_{33}H_{67}O_7P$	
54	606.445	606.4504	9	$[M-H]^-$	LysoPE(O-27:1)	$C_{32}H_{66}NO_7P$	606, 367, 349
55	608.324	608.3205	6	$[M-H]^-$	PE(23:2)	$C_{28}H_{52}NO_{11}P$	
56	611.374	611.3718	4	$[M-H]^-$	PA(30:4)	$C_{33}H_{57}O_8P$	
57	613.338	613.3358	4	$[M-H]^-$	LysoPI(19:0)	$C_{28}H_{55}O_{12}P$	
58	615.405	615.4031	4	$[M-H]^-$	PA(30:2)	$C_{33}H_{61}O_8P$	615, 253, 235 225, 78
59	617.415	617.4188	6	$[M-H]^-$	PA(30:1)	$C_{33}H_{63}O_8P$	617, 255, 237 225, 78
60	618.415	618.4140	2	$[M-H]^-$	PE(27:1)	$C_{32}H_{62}NO_8P$	
61	620.424	620.4297	9	$[M-H]^-$	PE(27:0)	$C_{32}H_{64}NO_8P$	
62	622.368	622.3726	7	$[M-H]^-$	PS(24:0)	$C_{30}H_{58}NO_{10}P$	
63	624.427	624.4246	4	$[M-H]^-$	LysoPS(O-25:0)	$C_{31}H_{64}NO_9P$	
64	625.389	625.3875	2	$[M-H]^-$	PA(31:4)	$C_{34}H_{59}O_8P$	
65	629.459	629.4552	6	$[M-H]^-$	PA(O-32:2)	$C_{35}H_{67}O_7P$	
66	630.415	630.4140	2	$[M-H]^-$	PE(28:2)	$C_{33}H_{62}NO_8P$	630, 196, 152
67	636.392	636.3882	6	$[M-H]^-$	PS(25:0)	$C_{31}H_{60}NO_{10}P$	
68	641.369	641.3671	3	$[M-H]^-$	LysoPI(21:0)	$C_{30}H_{59}O_{12}P$	
69	645.456	645.4501	9	$[M-H]^-$	PA(32:1)	$C_{35}H_{67}O_8P$	645, 281, 263 227,78
70	647.465	647.4657	1	$[M-H]^-$	PA(32:0)	$C_{35}H_{69}O_8P$	
71	648.462	648.4610	2	$[M-H]^-$	PE(29:0)	$C_{34}H_{68}NO_8P$	648, 438, 241

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
72	651.435	651.4395	7	$[M-H]^-$	PA(O-34:5)	$C_{37}H_{65}O_7P$	
73	655.312	655.3100	3	$[M-H]^-$	PI(21:1)	$C_{29}H_{53}O_{14}P$	
74	656.425	656.4297	7	$[M-H]^-$	PE(30:3)	$C_{35}H_{64}NO_8P$	
75	659.464	659.4657	3	$[M-H]^-$	PA(33:1)	$C_{36}H_{69}O_8P$	659, 417, 281, 263, 241
76	661.405	661.4086	5	$[M-H]^-$	PG(28:2)	$C_{34}H_{63}O_{10}P$	
77	662.475	662.4766	2	$[M-H]^-$	PE(30:0)	$C_{35}H_{70}NO_8P$	662, 452, 406, 255, 227
78	671.496	671.5021	9	$[M-H]^-$	PA(O-35:2)	$C_{38}H_{73}O_7P$	
79	673.480	673.4814	2	$[M-H]^-$	PA(34:1)	$C_{37}H_{71}O_8P$	673, 391, 281 255, 237
80	674.476	674.4766	1	$[M-H]^-$	PE(31:1)	$C_{36}H_{70}NO_8P$	674, 450, 420, 253, 241
81	675.458	675.4607	4	$[M-H]^-$	PG(O-30:2)	$C_{36}H_{69}O_9P$	
82	679.381	679.3828	3	$[M-H]^-$	PG(27:2)	$C_{33}H_{61}O_{12}P$	
83	683.464	683.4657	2	$[M-H]^-$	PA(35:3)	$C_{38}H_{69}O_8P$	683, 441, 305 287, 241
84	685.481	685.4814	1	$[M-H]^-$	PA(35:2)	$C_{38}H_{71}O_8P$	685, 307, 289 241, 78
85	686.472	686.4766	7	$[M-H]^-$	PE(32:2)	$C_{37}H_{70}NO_8P$	
86	687.499	687.4970	3	$[M-H]^-$	PA(35:1)	$C_{38}H_{73}O_8P$	
87	688.486	688.4932	9	$[M-H]^-$	PE(32:1)	$C_{37}H_{72}NO_8P$	
88	689.478	689.4763	2	$[M-H]^-$	PG(O-31:2)	$C_{37}H_{71}O_9P$	
89	693.450	693.4501	1	$[M-H]^-$	PA(36:5)	$C_{39}H_{67}O_8P$	693, 439, 303, 285, 253
90	694.452	694.4453	10	$[M-H]^-$	PE(33:5)	$C_{38}H_{66}NO_8P$	
91	696.465	696.4610	6	$[M-H]^-$	PE(33:4)	$C_{38}H_{68}NO_8P$	696, 454, 420, 275, 241
92	697.485	697.4814	5	$[M-H]^-$	PA(36:3)	$C_{39}H_{71}O_8P$	697, 307, 289 253, 78
93	698.481	698.4766	6	$[M-H]^-$	PE(33:3)	$C_{38}H_{70}NO_8P$	
94	699.500	699.4970	4	$[M-H]^-$	PA(36:2)	$C_{39}H_{73}O_8P$	699, 363, 335, 227, 209
95	700.496	700.4923	5	$[M-H]^-$	PE(33:2)	$C_{38}H_{72}NO_8P$	700, 476, 420, 279, 241
96	704.454	704.4508	5	$[M-H]^-$	PS(30:1)	$C_{36}H_{68}NO_{10}P$	704, 476, 253 235, 227
97	708.467	708.4610	8	$[M-H]^-$	PE(34:5)	$C_{39}H_{68}NO_8P$	708, 498, 406 301, 227
98	710.473	710.4766	5	$[M-H]^-$	PE(34:4)	$C_{39}H_{70}NO_8P$	710, 502, 422 404, 305, 225

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a)]
				Ion form	Compound	Molecular formula	
99	711.498	711.4970	1	$[M-H]^-$	PA(37:3)	$C_{40}H_{73}O_8P$	
100	712.529	712.5287	0	$[M-H]^-$	PE(O-35:3)	$C_{40}H_{76}NO_7P$	
101	713.511	713.5127	2	$[M-H]^-$	PA(37:2)	$C_{40}H_{75}O_8P$	
102	715.491	715.4920	1	$[M-H]^-$	PG(O-33:3)	$C_{39}H_{73}O_9P$	
103	716.525	716.5236	2	$[M-H]^-$	PE(34:1)	$C_{39}H_{76}NO_8P$	716, 478, 434 281, 255
104	719.480	719.4869	10	$[M-H]^-$	PG(32:1)	$C_{38}H_{73}O_{10}P$	719, 483, 465 255, 237
105	725.440	725.4399	1	$[M-H]^-$	PG(33:5)	$C_{39}H_{67}O_{10}P$	
106	727.452	727.4556	5	$[M-H]^-$	PG(33:4)	$C_{39}H_{69}O_{10}P$	
107	741.469	741.4712	3	$[M-H]^-$	PG(34:4)	$C_{40}H_{71}O_{10}P$	741, 463, 275 253, 235
108	742.470	742.4665	5	$[M-H]^-$	PS(33:3)	$C_{39}H_{70}NO_{10}P$	
109	745.481	745.4814	1	$[M-H]^-$	PA(40:7)	$C_{43}H_{71}O_8P$	745, 417, 327 281, 263
110	747.495	747.4970	3	$[M-H]^-$	PA(40:6)	$C_{43}H_{73}O_8P$	747, 485, 467 331, 313, 279
111	749.533	749.5338	1	$[M-H]^-$	PG(34:0)	$C_{40}H_{79}O_{10}P$	749, 511, 493 283, 265, 255
112	754.466	754.4665	1	$[M-H]^-$	PS(34:4)	$C_{40}H_{70}NO_{10}P$	
113	755.565	755.5596	7	$[M-H]^-$	PA(40:2)	$C_{43}H_{81}O_8P$	755, 489, 471 335, 317, 283
114	756.588	756.5913	4	$[M-H]^-$	PE(O-38:2)	$C_{43}H_{84}NO_7P$	
115	765.490	765.4923	3	$[M-H]^-$	PI(O-30:1)	$C_{39}H_{75}O_{12}P$	
116	769.502	769.5025	1	$[M-H]^-$	PG(36:4)	$C_{42}H_{75}O_{10}P$	769, 483, 465 303, 255, 237
117	772.549	772.5498	1	$[M-H]^-$	PS(O-36:2)	$C_{42}H_{80}NO_9P$	
118	773.509	773.5127	5	$[M-H]^-$	PA(42:7)	$C_{45}H_{75}O_8P$	773, 485, 467 331, 313, 305
119	781.578	781.5753	3	$[M-H]^-$	PA(42:3)	$C_{45}H_{83}O_8P$	781, 433, 415 365, 279, 261
120	784.519	784.5134	7	$[M-H]^-$	PA(36:3)	$C_{42}H_{76}O_{10}P$	
121	795.514	795.5182	5	$[M-H]^-$	PG(38:5)	$C_{40}H_{77}O_{10}P$	795, 483, 465 329, 255, 237
122	809.528	809.5338	7	$[M-H]^-$	PG(39:5)	$C_{45}H_{79}O_{10}P$	
123	815.505	815.5080	4	$[M-H]^-$	PI(O-34:4)	$C_{43}H_{77}O_{12}P$	
124	817.524	817.5236	0	$[M-H]^-$	PI(O-34:3)	$C_{43}H_{79}O_{12}P$	
125	818.535	818.5342	1	$[M-H]^-$	PS(O-40:7)	$C_{46}H_{78}NO_9P$	
126	819.540	819.5393	1	$[M-H]^-$	PI(O-34:2)	$C_{43}H_{81}O_{12}P$	
127	823.548	823.5495	2	$[M-H]^-$	PG(40:5)	$C_{46}H_{81}O_{10}P$	823, 493, 329 283, 265

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
128	827.472	827.4716	1	$[M-H]^-$	PI(34:5)	$C_{43}H_{73}O_{13}P$	
129	831.538	831.5393	2	$[M-H]^-$	PI(O-35:3)	$C_{44}H_{81}O_{12}P$	
130	832.521	832.5134	9	$[M-H]^-$	PS(40:7)	$C_{46}H_{76}NO_{10}P$	
131	833.519	833.5186	1	$[M-H]^-$	PI(34:2)	$C_{43}H_{79}O_{13}P$	
132	834.523	834.5291	7	$[M-H]^-$	PS(40:6)	$C_{46}H_{78}NO_{10}P$	834, 747, 528 305, 287
133	835.547	835.5495	3	$[M-H]^-$	PG(41:6)	$C_{47}H_{81}O_{10}P$	
134	836.544	836.5447	8	$[M-H]^-$	PS(40:5)	$C_{46}H_{80}NO_{10}P$	836, 506, 329 283, 265
135	837.567	837.5651	2	$[M-H]^-$	PG(41:5)	$C_{47}H_{83}O_{10}P$	
136	839.619	839.6172	2	$[M-H]^-$	PG(O-42:4)	$C_{48}H_{89}O_9P$	839, 277, 259 241, 223
137	841.601	841.5964	6	$[M-H]^-$	PG(41:3)	$C_{47}H_{87}O_{10}P$	
138	842.575	842.5705	5	$[M-H]^-$	PE(44:8)	$C_{49}H_{82}NO_8P$	
139	843.535	843.5393	5	$[M-H]^-$	PI(O-36:4)	$C_{45}H_{81}O_{12}P$	
140	845.552	845.5549	3	$[M-H]^-$	PI(O-36:3)	$C_{45}H_{83}O_{12}P$	
141	846.566	846.5655	1	$[M-H]^-$	PS(O-42:7)	$C_{48}H_{82}NO_9P$	
142	847.558	847.5495	1	$[M-H]^-$	PG(42:7)	$C_{48}H_{81}O_{10}P$	
143	855.615	855.6121	3	$[M-H]^-$	PG(42:3)	$C_{48}H_{89}O_{10}P$	
144	857.622	857.6277	7	$[M-H]^-$	PG(42:2)	$C_{48}H_{91}O_{10}P$	
145	858.658	858.6594	2	$[M-H]^-$	PS(O-42:1)	$C_{48}H_{94}NO_9P$	
146	859.575	859.5706	5	$[M-H]^-$	PI(O-37:3)	$C_{46}H_{85}O_{12}P$	
147	861.652	861.6590	8	$[M-H]^-$	PG(42:0)	$C_{48}H_{95}O_{10}P$	
148	862.567	862.5604	7	$[M-H]^-$	PS(42:6)	$C_{48}H_{82}NO_{10}P$	
149	863.606	863.6019	6	$[M-H]^-$	PI(O-37:1)	$C_{46}H_{89}O_{12}P$	
150	864.579	864.5760	3	$[M-H]^-$	PS(42:5)	$C_{48}H_{84}NO_{10}P$	
151	867.619	867.6121	8	$[M-H]^-$	PG(43:4)	$C_{49}H_{89}O_{10}P$	
152	868.609	868.6073	2	$[M-H]^-$	PS(42:3)	$C_{48}H_{88}NO_{10}P$	
153	869.557	869.5549	2	$[M-H]^-$	PI(O-38:5)	$C_{47}H_{83}O_{12}P$	869, 305, 287 241, 223
154	871.577	871.5706	8	$[M-H]^-$	PI(O-38:4)	$C_{47}H_{85}O_{12}P$	
155	872.638	872.6386	1	$[M-H]^-$	PS(42:1)	$C_{48}H_{92}NO_{10}P$	
156	875.594	875.6019	9	$[M-H]^-$	PI(O-38:2)		
157	877.622	877.6175	5	$[M-H]^-$	PI(O-38:1)	$C_{47}H_{91}O_{12}P$	
158	880.605	880.6073	3	$[M-H]^-$	PS(43:4)	$C_{49}H_{88}NO_{10}P$	
159	881.624	881.6277	4	$[M-H]^-$	PG(44:4)	$C_{50}H_{91}O_{10}P$	
160	883.642	883.6434	2	$[M-H]^-$	PG(44:3)	$C_{50}H_{93}O_{10}P$	883, 327, 309 275, 257
161	884.710	884.7114	2	$[M-H]^-$	PE(46:1)	$C_{51}H_{100}NO_8P$	884, 367, 337
162	885.654	885.6590	6	$[M-H]^-$	PG(44:2)	$C_{50}H_{95}O_{10}P$	885, 327, 309 241, 223

No.	Measured *m/z*	Calculated *m/z*	Error |ppm|	Assignment			Structurally specific CID ions (*m/z*)[a]
				Ion form	Compound	Molecular formula	
163	887.681	887.6747	7	$[M-H]^-$	PG(44:1)	$C_{50}H_{97}O_{10}P$	
164	888.676	888.6699	7	$[M-H]^-$	PS(43:0)	$C_{49}H_{96}NO_{10}P$	
165	889.688	889.6903	3	$[M-H]^-$	PG(44:0)	$C_{50}H_{99}O_{10}P$	
166	891.637	891.6332	4	$[M-H]^-$	PI(O-39:1)	$C_{48}H_{93}O_{12}P$	891, 327, 309 241, 223
167	893.649	893.6488	0	$[M-H]^-$	PI(O-39:0)	$C_{48}H_{95}O_{12}P$	
168	895.574	895.5706	6	$[M-H]^-$	PI(O-40:6)	$C_{49}H_{85}O_{12}P$	895, 327, 309 225,207
169	896.638	896.6386	1	$[M-H]^-$	PS(44:3)	$C_{50}H_{92}NO_{10}P$	
170	898.655	898.6543	1	$[M-H]^-$	PS(44:2)	$C_{50}H_{94}NO_{10}P$	811, 562, 473, 337, 96, 78
171	899.601	899.6019	1	$[M-H]^-$	PI(O-40:4)	$C_{49}H_{89}O_{12}P$	
172	903.599	903.5968	2	$[M-H]^-$	PI(39:2)	$C_{48}H_{89}O_{13}P$	
173	905.650	905.6488	1	$[M-H]^-$	PI(O-40:1)	$C_{49}H_{95}O_{12}P$	
174	907.665	907.6645	6	$[M-H]^-$	PI(O-40:0)	$C_{49}H_{97}O_{12}P$	
175	909.546	909.5499	4	$[M-H]^-$	PI(40:6)	$C_{49}H_{83}O_{13}P$	909, 301, 283 241, 223
176	911.561	911.5655	5	$[M-H]^-$	PI(40:5)	$C_{49}H_{85}O_{13}P$	911, 303, 285 241, 223
177	923.603	923.6019	1	$[M-H]^-$	PI(O-42:6)	$C_{51}H_{89}O_{12}P$	
178	925.580	925.5812	1	$[M-H]^-$	PI(41:5)	$C_{50}H_{87}O_{13}P$	
179	927.597	927.5968	0	$[M-H]^-$	PI(41:4)	$C_{50}H_{89}O_{13}P$	
180	929.657	929.6488	9	$[M-H]^-$	PI(O-42:3)	$C_{51}H_{95}O_{12}P$	
181	931.634	931.6281	6	$[M-H]^-$	PI(41:2)	$C_{50}H_{93}O_{13}P$	
182	933.551	933.5499	1	$[M-H]^-$	PI(41:8)	$C_{51}H_{83}O_{13}P$	
183	935.699	935.6958	3	$[M-H]^-$	PI(O-42:0)	$C_{51}H_{101}O_{12}P$	
184	953.518	953.5186	1	$[M-H]^-$	PI(44:12)	$C_{53}H_{79}O_{13}P$	953, 667, 649, 307, 289
185	957.553	957.5499	3	$[M-H]^-$	PI(44:10)	$C_{53}H_{83}O_{13}P$	
186	961.669	961.6751	6	$[M-H]^-$	PI(43:1)	$C_{52}H_{99}O_{13}P$	
187	975.693	975.6907	2	$[M-H]^-$	PI(44:1)	$C_{53}H_{101}O_{13}P$	

[a] Structurally specific CID ions of lipids were detected by LC-MS/MS using CID. Fragment ions shown in blue were detected by *in situ* MALDI-TOF/TOF MS/MS. Abbreviations: PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol.

Supplementary Information Table S9. Comparison of lipid ion signals detected in three parallel germinating Chinese-yew seed tissue sections by (-)MALDI-TOF/TOF MS using optimized MEK, 2-MBT, and DHA as the matrices, respectively.

Detected lipid ion signals (*m/z*)	Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seed tissue sections		
	MEK	2-MBT	DHA
409.235	√	√	
419.220	√		
421.272	√		
423.255	√		√
424.248	√	√	
429.203	√		
445.238	√		
449.306	√	√	√
451.286			√
459.251	√	√	
464.317			√
467.277	√		√
471.251	√		
474.265	√	√	
478.293			√
482.288	√	√	
485.263	√		
489.296	√		
491.315	√	√	√
493.259	√		√
502.290	√	√	
504.308		√	√
505.256	√		√
506.249	√		√
507.276	√		
509.250	√	√	
510.251	√		√
516.239	√		
519.270	√		
522.320	√		
533.288	√		√
535.306	√		√
536.293	√		
543.255	√	√	
546.288	√		
547.265	√		

Detected lipid ion signals (*m/z*)	Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seed tissue sections		
	MEK	2-MBT	DHA
550.313	√		√
553.317	√		√
555.259	√		
557.305	√		
559.305	√	√	√
561.356	√		
563.295	√		
569.273	√		√
575.404	√		
576.330	√		
577.386	√		
578.380	√		√
583.325	√	√	
587.370	√		
589.355	√		
597.306	√	√	√
599.324	√	√	
601.425	√		√
603.440	√		
604.432	√		
605.458	√	√	
606.445	√		
608.324	√		
611.374	√		√
613.338	√		
615.405	√	√	
617.415	√		
618.415	√		
620.424	√		
622.368	√		
624.427	√		
625.389	√		√
629.459	√	√	
630.415	√	√	
636.392	√		
639.409		√	√
641.369	√		
645.456	√	√	
647.465	√		
648.462	√		√
651.435	√		

Detected lipid ion signals (*m/z*)	Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seed tissue sections		
	MEK	2-MBT	DHA
655.312	√		
656.425	√	√	
658.484		√	
659.464	√	√	
661.405	√		
662.475	√		
671.496	√	√	
673.480	√		
674.476	√	√	√
675.458	√		
676.350		√	
679.381	√		
683.464	√		
685.481	√		√
686.472	√		
687.499	√		√
688.486	√		
689.478	√		
693.450	√		√
694.452	√		√
696.465	√		
697.485	√	√	√
698.481	√		
699.500	√	√	√
700.496	√	√	√
702.398		√	
704.454	√		
708.467	√		
710.473	√		
711.498	√	√	
712.529	√		√
713.511	√		
715.491	√		
716.525	√		
719.480	√		√
725.440	√	√	
727.452	√	√	
741.469	√		
742.470	√		
745.481	√	√	
747.495	√		

Detected lipid ion signals (*m/z*)	Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seed tissue sections		
	MEK	2-MBT	DHA
749.533	√		√
754.466	√		
755.565	√		
756.588	√	√	
765.490	√		
769.502	√		
772.549	√		
773.509	√		√
774.510		√	
775.585		√	
777.565		√	
781.578	√		
782.477		√	
784.519	√		
795.514	√		
809.528	√	√	
815.505	√	√	√
817.524	√	√	
818.535	√		
819.540	√	√	
823.548	√		
827.472	√		
831.538	√	√	
832.521	√		
833.519	√		
834.523	√	√	
835.547	√	√	
836.544	√	√	
837.567	√		
839.619	√		
841.601	√	√	
842.575	√		
843.535	√	√	
845.552	√		
846.566	√		
847.558	√		
855.615	√		
857.622	√	√	
858.658	√		
859.575	√	√	
861.652	√	√	

Detected lipid ion signals (*m/z*)	Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seed tissue sections		
	MEK	2-MBT	DHA
862.567	√		
863.606	√		
864.579	√	√	
867.619	√		
868.609	√		
869.557	√		
871.577	√		
872.638	√		
873.532		√	
875.594	√	√	
877.622	√		
880.605	√		
881.624	√		
883.642	√		
884.710	√		
885.654	√		
887.681	√		
888.676	√		
889.688	√		
891.637	√		
893.649	√		
895.574	√		
896.638	√		
898.655	√		
899.601	√		
901.486		√	
903.599	√		
905.650	√		√
907.665	√		
909.546	√		
911.561	√		
923.603	√		
925.580	√		√
927.597	√		
929.657	√		
931.634	√		
933.551	√		
935.699	√		
953.518	√		
957.553	√		
961.669	√		

Detected lipid ion signals (*m/z*)	Germinating Chinese-yew (*Taxus chinensis* var. *mairei*) seed tissue sections		
	MEK	**2-MBT**	**DHA**
975.693	√	√	
Total number of detected lipids	**187**	**58**	**41**