Supplementary Information For

Streamlined Analysis of Cardiolipins in Prokaryotic and Eukaryotic Samples Using Norharmane Matrix by MALDI-MSI

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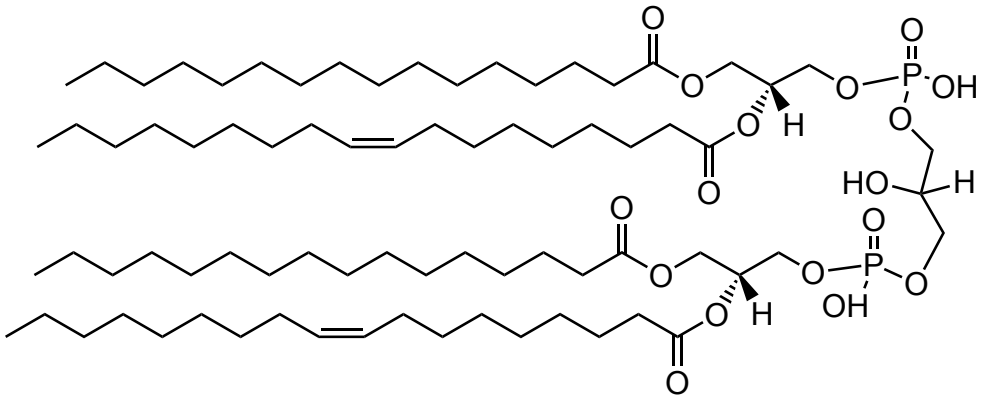


Figure S1. Cardiolipin generic structure based on synthetic standard used in these studies. Reference from Avanti Polar Lipids: 1',3'-bis[1-palmitoyl-2-oleoyl-sn-glycero-3-phospho]-glycerol (sodium salt, not shown here), referred to in this work as CL (68:2). Observed as a quasi-molecular ion $[M-H]^-$ at *m/z* 1404 in MALDI negative ion mode.

Supplemental Table 1. The physical properties of matrices used in these experiments.

Property	9AA	NRM	DAN	DHB	CHCA
n-octanol/water partition coefficient, (XLOGP3)	2.74, (2.8)	3.17, (3.2)	0.89, (2.1)	1.74, (1.6)	N/A*, (1.4)
pKa	10.0	13.2	4.5	3.0 ⌘	1.2 ⌘
Chemical Formula	$C_{13}H_{10}N_2$	$C_{11}H_8N_2$	$C_{10}H_{10}N_2$	$C_7H_6O_4$	$C_{10}H_7NO_3$
Molar Mass	194.084	168.069	158.084	154.027	189.043

*N/A, not available. ⌘Values from Remily-Wood, E.; Dirscherl, H; Koomen, J. M. Acid hydrolysis of proteins in matrix assisted laser desorption ionization matrices; *J Am Soc Mass Spectrom*, 2009, *20*, *2106-2115*.

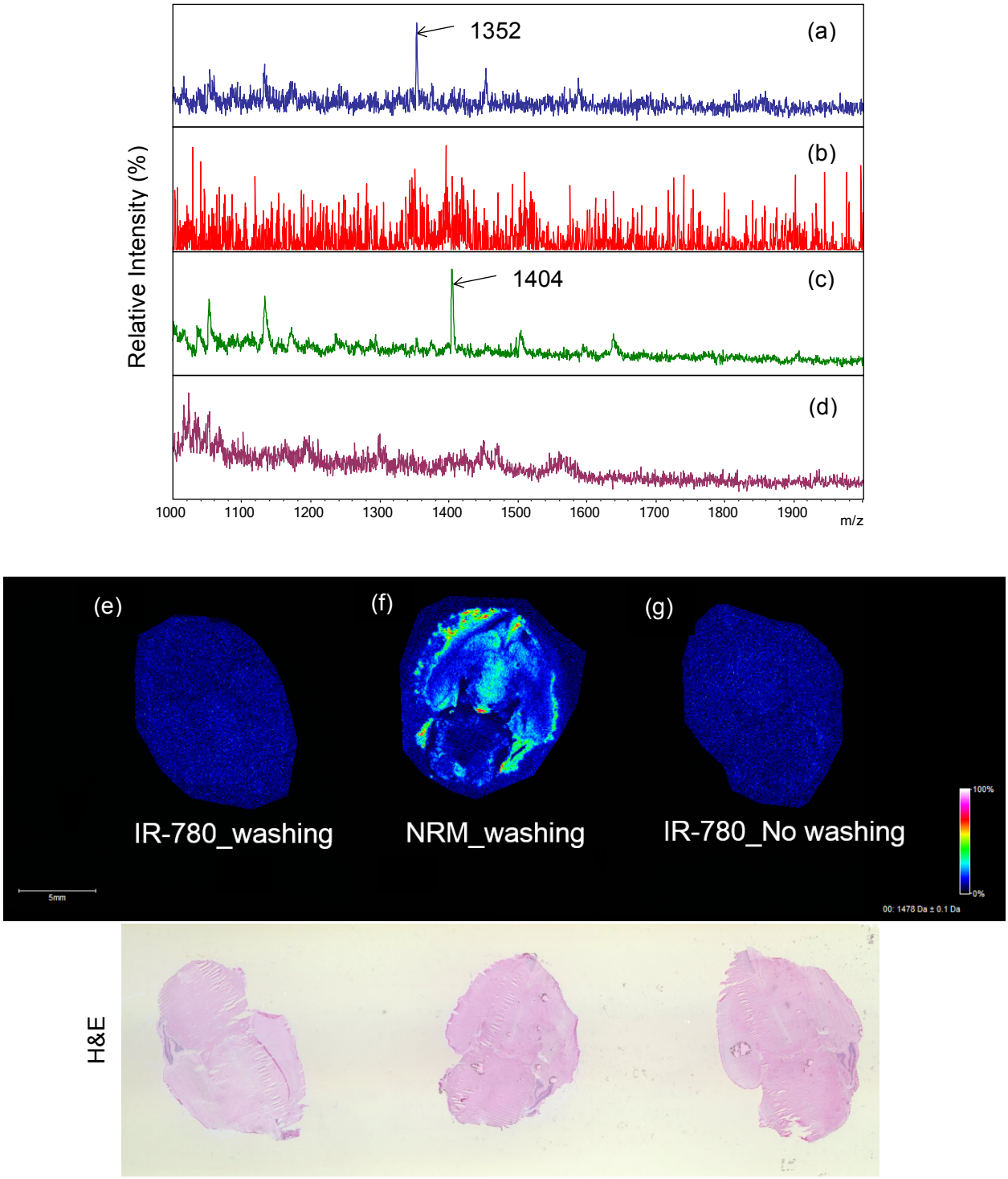


Figure S2. Comparison of CL detection by IR-780 matrix versus NRM. (top panel) MALDI mass spectra of 250 ng/mL of CL(64:0) and (68:2) with NRM (a) and (c) and IR-780 (b) and (d), respectively. Autoflex MALDI-TOF linear negative ion mode. (bottom panel) Ion map of *m/z* 1478 (CL (74:7)) in brain tissue with or without washing. (e) IR-780 and (f) NRM used as matrix with tissue washing. (g) IR-780 used as matrix without tissue washing. The spatial resolution was set to 50 μm and normalized by TIC. Negative ion mode, rapifleX MALDI-TOF.

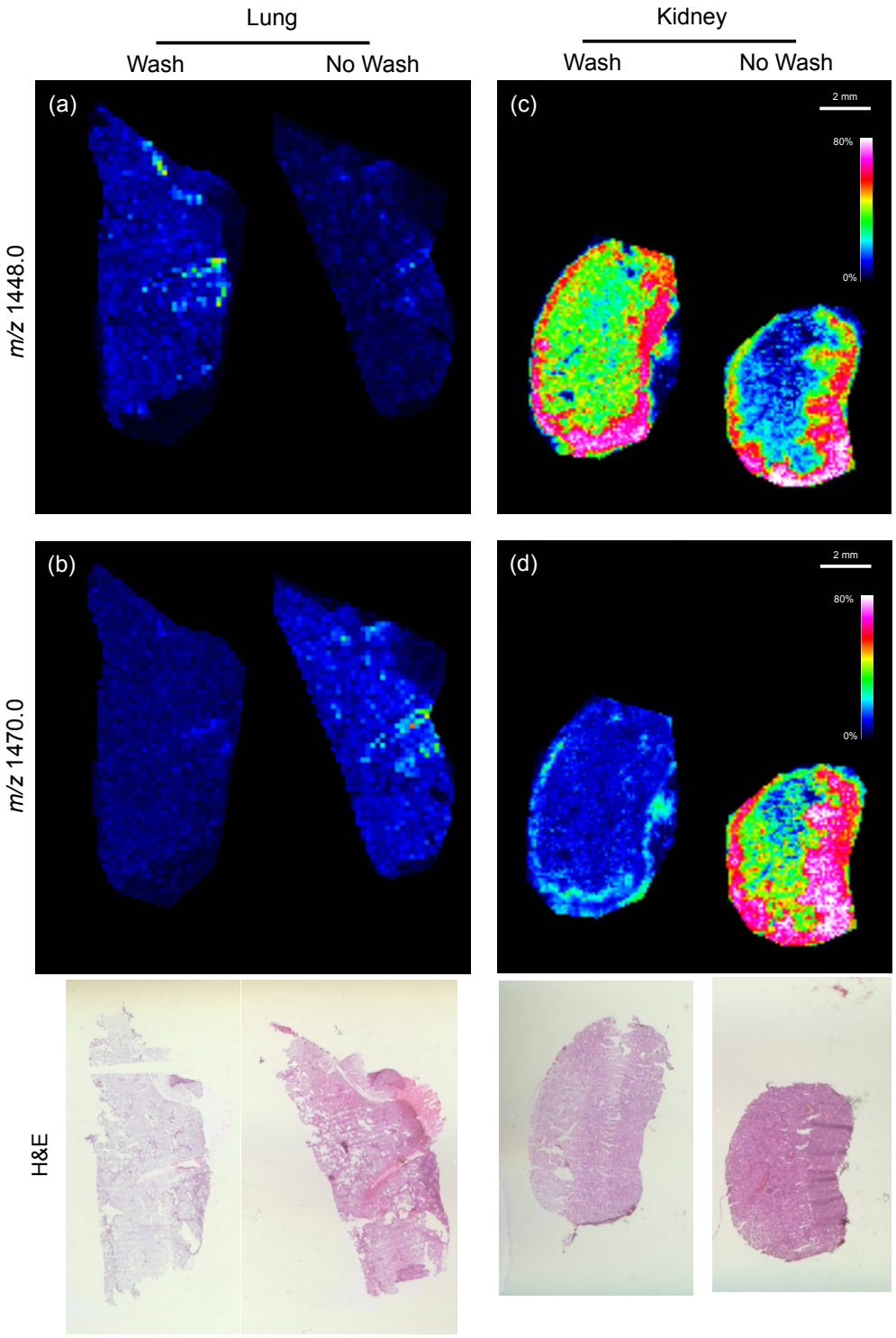


Figure S3. MALDI Ion maps of CL ions on various tissue (a) and (b) on mouse lung, (c) to (d) kidney. The spatial resolution was set to 100 μm and 200 μm for kidney and lung, respectively. The data was normalized by TIC. All results were obtained from reflectron negative ion mode using a rapifleX MALDI-TOF.

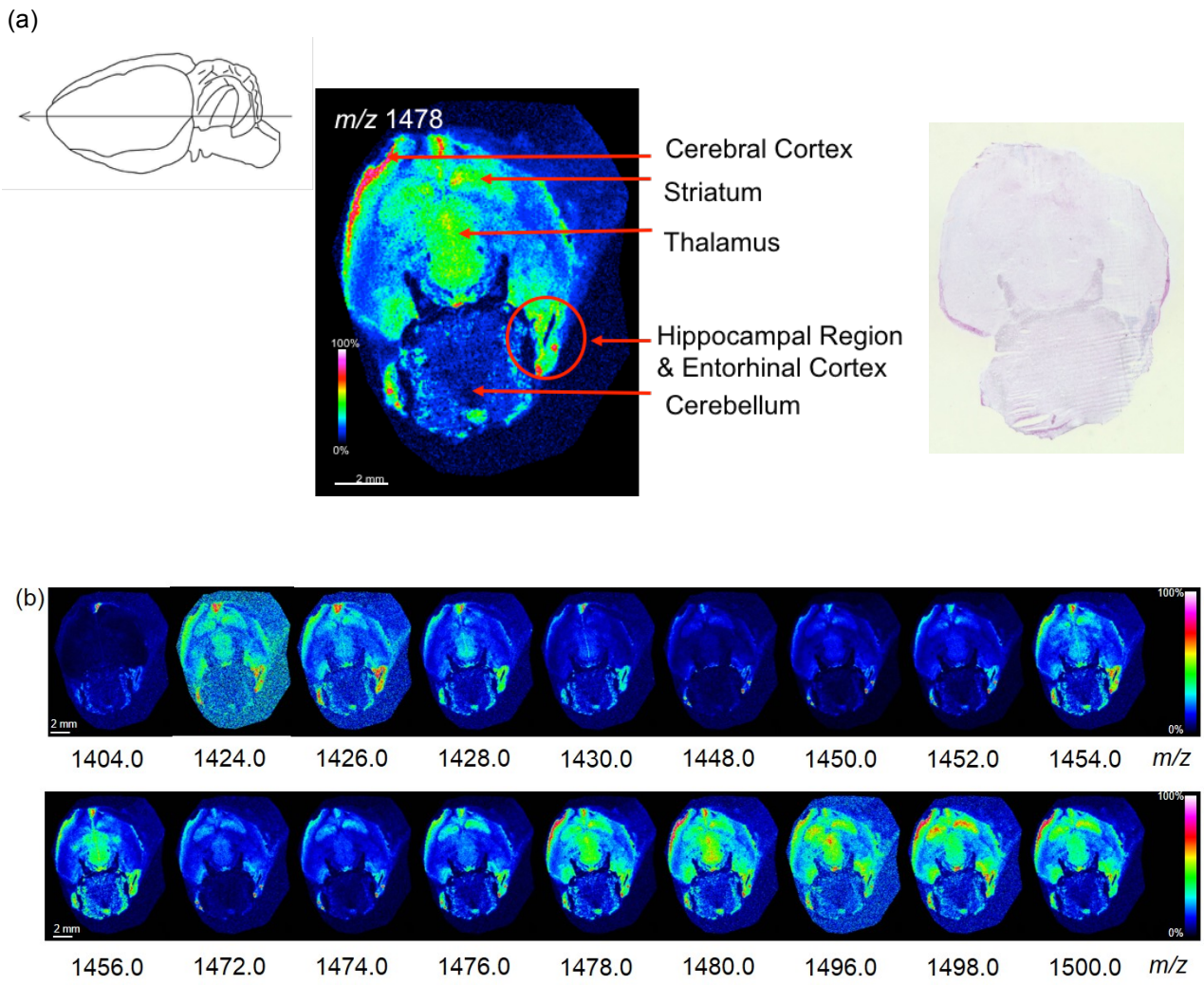


Figure S4. MALDI image of CL distributions in mouse brain. (a, left) Horizontal sectioning plane. (a, middle) MALDI-MSI map of the ion *m/z* 1478 corresponding to CL(74:7) in mouse brain with regional annotation, (a, right) H&E post-acquisition of section in (a) and (b). The spatial resolution was set to 50 μm, normalized by TIC. Reflectron negative ion mode using a rapifleX MALDI-TOF.

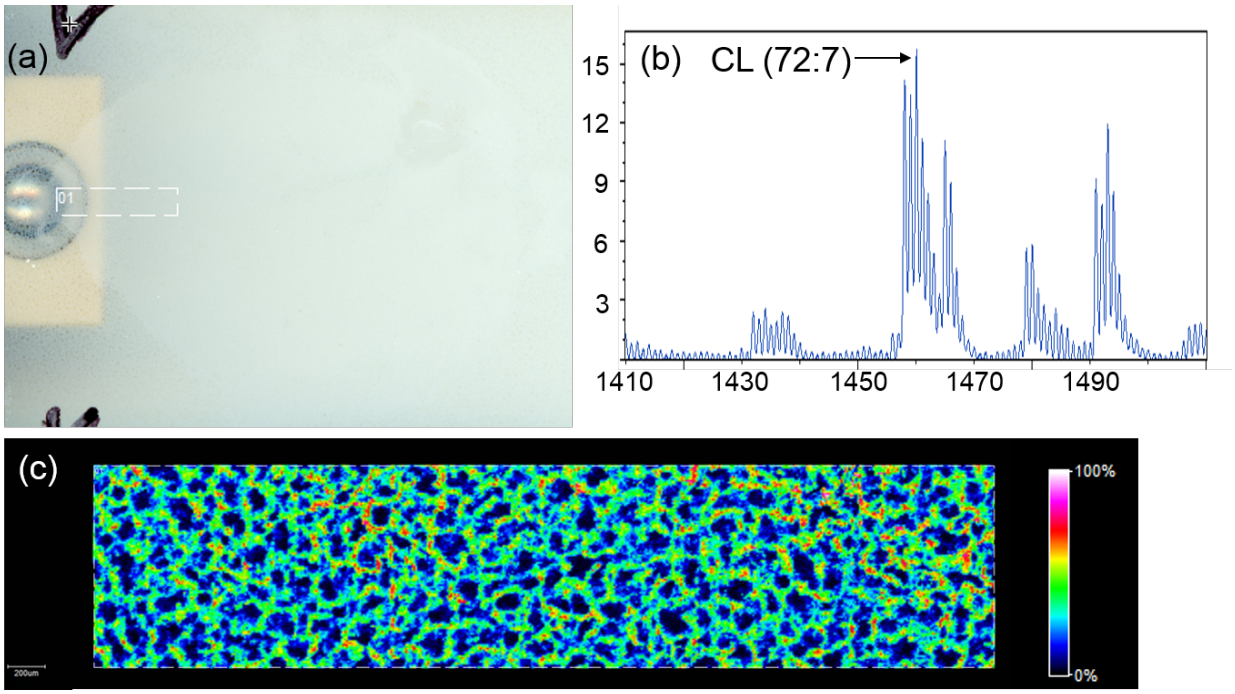
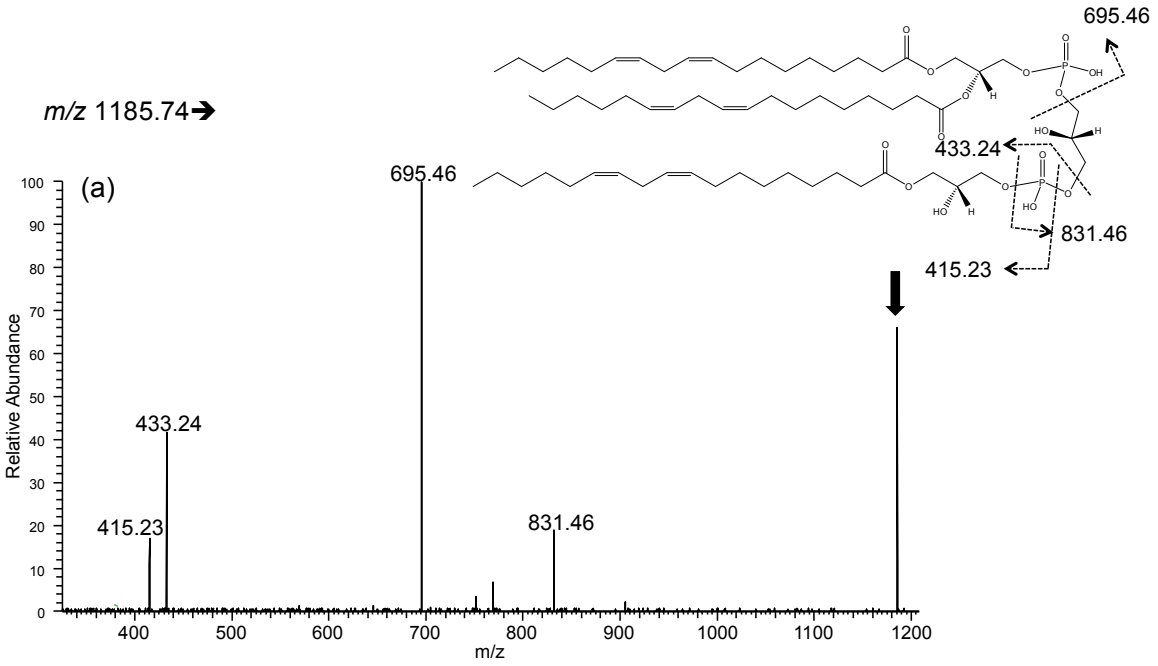


Figure S5. Image of mouse liver at high spatial resolution. (a) capture area, (b) averaged mass spectrum after scanning selected area in (a), and (c) ion map of *m/z* at 1450 (CL (72:7)) in the selected area in the liver sample. The spatial resolution was set to 10 μm and normalized by TIC. Reflectron negative ion mode on a rapifleX MALDI-TOF.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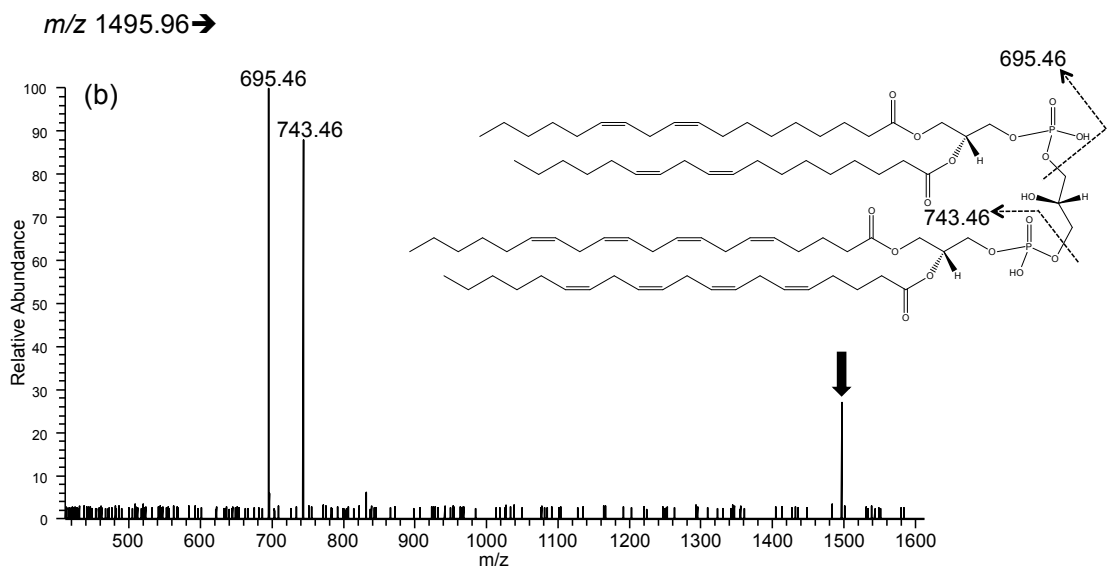
(a)
695.46
433.24
831.46
415.23
Relative Abundance
m/z


m/z 1495.96➔
(b)
695.46
743.46
Relative Abundance
m/z

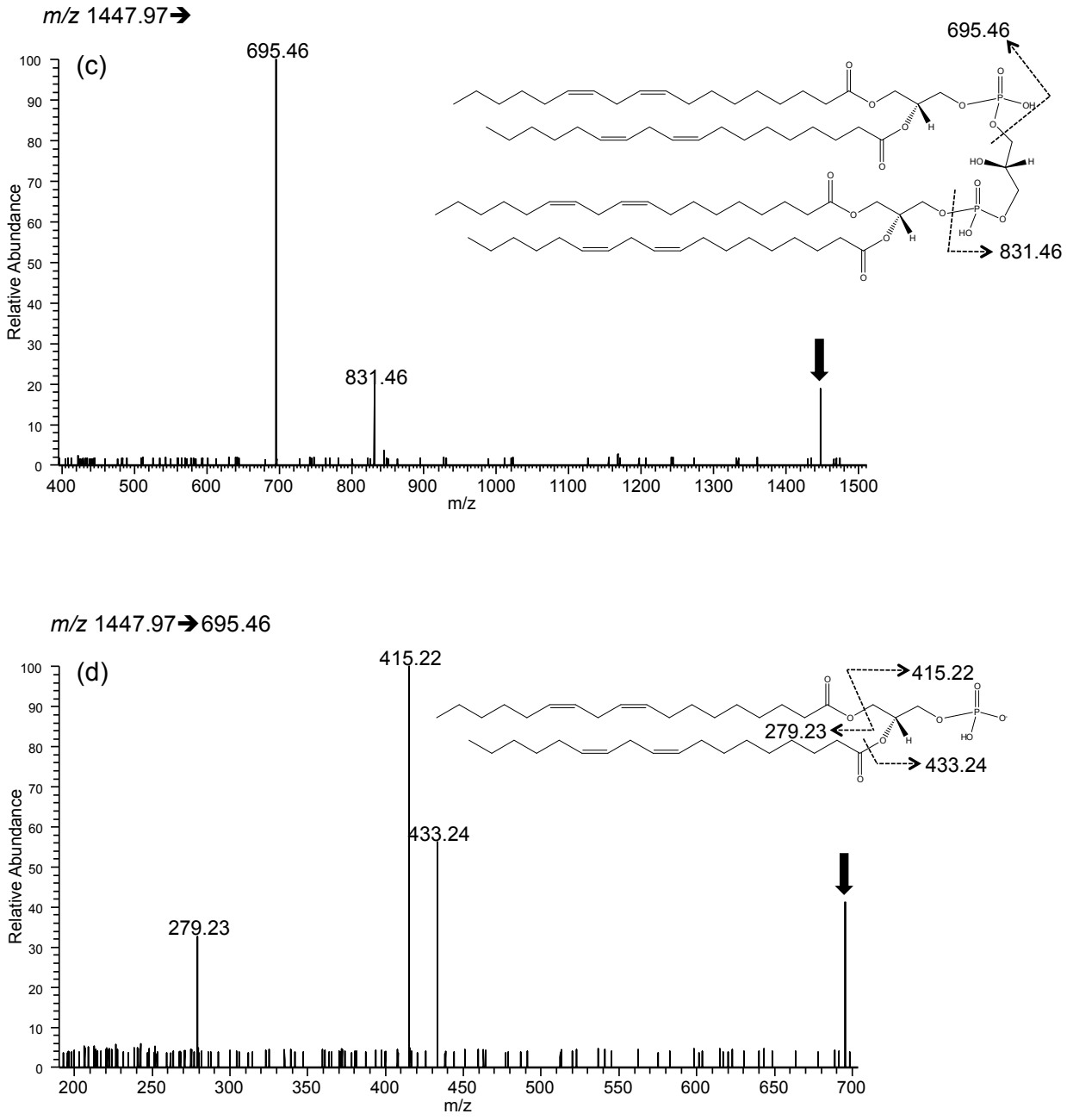


Figure S6. MALDI MS/MS spectrum of CL(54:6, m/z 1185.74), CL(76:12, 1495.96), CL(72:8, m/z 1447.97), and MS^3 spectrum of CL(72:8, 1447.97-> 695.46) using MALDI Ion Trap/Orbitrap Mass Spectrometry. The collision energy was set from 28 to 30. The dashed line on the CLs indicated fragments of the lipids. The bold arrow mark represented the precursor ion of MS/MS and MS^3.

Supplementary Table 2. The identified CL ions in MALDI Imaging experiments with kidney tissue.

Number	CL List	*m/z* (Observed)	*m/z* (Theoretical)	Molecular Formula	Error (ppm)
1	CL (54:6)	1185.7374	1185.7353	$C_{63}H_{112}O_{16}P_2$	1.77
2	CL (68:3)	1401.9839	1401.9806	$C_{77}H^{144}O_{17}P_2$	2.35
3	CL (68:2)	1403.9998	1403.9963	$C^{77}H_{146}O_{17}P_2$	2.49
4	CL (70:6)	1423.9682	1423.9649	$C_{79}H_{142}O_{17}P_2$	2.32
5	CL (70:5)	1425.9833	1425.9806	$C_{79}H_{144}O_{17}P_2$	1.89
6	CL (70:4)	1427.9989	1427.9962	$C_{79}H_{146}O_{17}P_2$	1.89
7	CL (70:3)	1430.0109	1430.0119	$C_{79}H_{148}O_{17}P_2$	-0.70
8	CL (70:2)	1432.0213	1432.0275	$C_{79}H_{150}O_{17}P_2$	-4.33
9	CL (72:8)	1447.9674	1447.9650	$C_{81}H_{142}O_{17}P_2$	1.66
10	CL (72:7)	1449.9805	1449.9806	$C_{81}H_{144}O_{17}P_2$	-0.07
11	CL (72:6)	1451.9944	1451.9962	$C_{81}H_{146}O_{17}P_2$	-1.24
12	CL (72:5)	1454.0065	1454.0119	$C_{81}H_{148}O_{17}P_2$	-3.71
13	CL (72:4)	1456.0210	1456.0275	$C_{81}H_{150}O_{17}P_2$	-4.46
14	CL (74:10)	1471.9653	1471.9649	$C_{83}H_{142}O_{17}P_2$	0.27
15	CL (74:9)	1473.9808	1473.9806	$C_{83}H_{144}O_{17}P_2$	0.14
16	CL (74:8)	1475.9959	1475.9962	$C_{83}H_{146}O_{17}P_2$	-0.20
17	CL (74:7)	1478.0096	1478.0119	$C_{83}H_{148}O_{17}P_2$	-1.56
18	CL (74:6)	1480.0208	1480.0275	$C_{83}H_{150}O_{17}P_2$	-4.53
19	CL (76:12)	1495.9664	1495.9649	$C_{85}H_{142}O_{17}P_2$	1.00
20	CL (76:11)	1497.9799	1497.9806	$C_{85}H_{144}O_{17}P_2$	-0.47
21	CL (76:10)	1499.9933	1499.9962	$C_{85}H_{146}O_{17}P_2$	-1.93
22	CL (76:9)	1502.0095	1502.0119	$C_{85}H_{148}O_{17}P_2$	-1.60
23	CL (76:8)	1504.0208	1504.0275	$C_{85}H_{150}O_{17}P_2$	-4.45
24	CL (76:7)	1506.0361	1506.0432	$C_{85}H_{152}O_{17}P_2$	-4.71
25	CL (78:14)	1519.9643	1519.9649	$C_{87}H_{142}O_{17}P_2$	-0.39
26	CL (78:13)	1521.9802	1521.9806	$C_{87}H_{144}O_{17}P_2$	-0.26
27	CL (78:12)	1523.9974	1523.9962	$C_{87}H_{146}O_{17}P_2$	0.79
28	CL (80:16)	1543.9639	1543.9649	$C_{89}H_{142}O_{17}P_2$	-0.65

*N.D. means Not Detected. **N.A. means Not Applicable.

Supplementary Table 3. The identified CL ions in MALDI Imaging experiments with heart tissue.

Number	CL List	*m/z* (Observed)	*m/z* (Theoretical)	Molecular Formula	Error (ppm)
1	CL (54:6)	1185.7388	1185.7353	$C_{63}H_{112}O_{16}P_2$	2.95
2	CL (68:3)	1401.9842	1401.9806	$C_{77}H^{144}O_{17}P_2$	2.57
3	CL (68:2)	N.D. *	1403.9963	$C^{77}H_{146}O_{17}P_2$	N.A.**
4	CL (70:6)	1423.9706	1423.9649	$C_{79}H_{142}O_{17}P_2$	4.00
5	CL (70:5)	1425.9831	1425.9806	$C_{79}H_{144}O_{17}P_2$	1.75
6	CL (70:4)	1427.9982	1427.9962	$C_{79}H_{146}O_{17}P_2$	1.40
7	CL (70:3)	N.D. *	1430.0119	$C_{79}H_{148}O_{17}P_2$	N.A. **
8	CL (70:2)	N.D. *	1432.0275	$C_{79}H_{150}O_{17}P_2$	N.A. **
9	CL (72:8)	1447.9704	1447.965	$C_{81}H_{142}O_{17}P_2$	3.73
10	CL (72:7)	1449.9807	1449.9806	$C_{81}H_{144}O_{17}P_2$	0.07
11	CL (72:6)	1451.9957	1451.9962	$C_{81}H_{146}O_{17}P_2$	-0.34
12	CL (72:5)	1454.0084	1454.0119	$C_{81}H_{148}O_{17}P_2$	-2.41
13	CL (72:4)	1456.0202	1456.0275	$C_{81}H_{150}O_{17}P_2$	-5.01
14	CL (74:10)	1471.9665	1471.9649	$C_{83}H_{142}O_{17}P_2$	1.09
15	CL (74:9)	1473.9853	1473.9806	$C_{83}H_{144}O_{17}P_2$	3.19
16	CL (74:8)	1475.9989	1475.9962	$C_{83}H_{146}O_{17}P_2$	1.83
17	CL (74:7)	1478.0126	1478.0119	$C_{83}H_{148}O_{17}P_2$	0.47
18	CL (74:6)	1480.0236	1480.0275	$C_{83}H_{150}O_{17}P_2$	-2.64
19	CL (76:12)	1495.9704	1495.9649	$C_{85}H_{142}O_{17}P_2$	3.68
20	CL (76:11)	1497.9808	1497.9806	$C_{85}H_{144}O_{17}P_2$	0.13
21	CL (76:10)	1499.9946	1499.9962	$C_{85}H_{146}O_{17}P_2$	-1.07
22	CL (76:9)	1502.0087	1502.0119	$C_{85}H_{148}O_{17}P_2$	-2.13
23	CL (76:8)	N.D. *	1504.0275	$C_{85}H_{150}O_{17}P_2$	N.A. **
24	CL (76:7)	N.D. *	1506.0432	$C_{85}H_{152}O_{17}P_2$	N.A. **
25	CL (78:14)	1519.9604	1519.9649	$C_{87}H_{142}O_{17}P_2$	-2.96
26	CL (78:13)	1521.9803	1521.9806	$C_{87}H_{144}O_{17}P_2$	-0.20
27	CL (78:12)	1523.9964	1523.9962	$C_{87}H_{146}O_{17}P_2$	0.13
28	CL (80:16)	1543.9687	1543.9649	$C_{89}H_{142}O_{17}P_2$	2.46

*N.D. means Not Detected. **N.A. means Not Applicable.

Supplementary Table 4. The identified CL ions in MALDI Imaging experiments with spleen tissue.

Number	CL List	*m/z* (Observed)	*m/z* (Theoretical)	Molecular Formula	Error (ppm)
1	CL (54:6)	1185.7387	1185.7353	$C_{63}H_{112}O_{16}P_2$	2.87
2	CL (68:3)	1401.9836	1401.9806	$C_{77}H^{144}O_{17}P_2$	2.14
3	CL (68:2)	1404.0004	1403.9963	$C^{77}H_{146}O_{17}P_2$	2.92
4	CL (70:6)	1423.9689	1423.9649	$C_{79}H_{142}O_{17}P_2$	2.81
5	CL (70:5)	1425.9841	1425.9806	$C_{79}H_{144}O_{17}P_2$	2.45
6	CL (70:4)	1427.9988	1427.9962	$C_{79}H_{146}O_{17}P_2$	2.66
7	CL (70:3)	1430.0122	1430.0119	$C_{79}H_{148}O_{17}P_2$	0.21
8	CL (70:2)	N.D. *	1432.0275	$C_{79}H_{150}O_{17}P_2$	N.A. **
9	CL (72:8)	1447.97	1447.965	$C_{81}H_{142}O_{17}P_2$	3.45
10	CL (72:7)	1449.9836	1449.9806	$C_{81}H_{144}O_{17}P_2$	2.07
11	CL (72:6)	1451.9989	1451.9962	$C_{81}H_{146}O_{17}P_2$	1.86
12	CL (72:5)	1454.0113	1454.0119	$C_{81}H_{148}O_{17}P_2$	-0.41
13	CL (72:4)	1456.025	1456.0275	$C_{81}H_{150}O_{17}P_2$	-1.72
14	CL (74:10)	1471.9688	1471.9649	$C_{83}H_{142}O_{17}P_2$	2.65
15	CL (74:9)	1473.9846	1473.9806	$C_{83}H_{144}O_{17}P_2$	2.71
16	CL (74:8)	1476.0006	1475.9962	$C_{83}H_{146}O_{17}P_2$	2.98
17	CL (74:7)	1478.0145	1478.0119	$C_{83}H_{148}O_{17}P_2$	1.76
18	CL (74:6)	1480.0248	1480.0275	$C_{83}H_{150}O_{17}P_2$	-1.82
19	CL (76:12)	1495.9662	1495.9649	$C_{85}H_{142}O_{17}P_2$	0.87
20	CL (76:11)	1497.9825	1497.9806	$C_{85}H_{144}O_{17}P_2$	1.27
21	CL (76:10)	1499.9979	1499.9962	$C_{85}H_{146}O_{17}P_2$	1.13
22	CL (76:9)	1502.0111	1502.0119	$C_{85}H_{148}O_{17}P_2$	-0.53
23	CL (76:8)	1504.0285	1504.0275	$C_{85}H_{150}O_{17}P_2$	0.66
24	CL (76:7)	N.D. *	1506.0432	$C_{85}H_{152}O_{17}P_2$	N.A. **
25	CL (78:14)	N.D. *	1519.9649	$C_{87}H_{142}O_{17}P_2$	N.A. **
26	CL (78:13)	N.D. *	1521.9806	$C_{87}H_{144}O_{17}P_2$	N.A. **
27	CL (78:12)	N.D. *	1523.9962	$C_{87}H_{146}O_{17}P_2$	N.A. **
28	CL (80:16)	N.D. *	1543.9649	$C_{89}H_{142}O_{17}P_2$	N.A. **

*N.D. means Not Detected. **N.A. means Not Applicable.

Supplementary Table 5. The identified CL ions in MALDI Imaging experiments with brain tissue.

Number	CL List	*m/z* (Observed)	*m/z* (Theoretical)	Molecular Formula	Error (ppm)
1	CL (54:6)	N.D. *	1185.7353	$C_{63}H_{112}O_{16}P_2$	N.A.**
2	CL (68:3)	1401.9825	1401.9806	$C_{77}H^{144}O_{17}P_2$	1.36
3	CL (68:2)	1403.9965	1403.9963	$C^{77}H_{146}O_{17}P_2$	0.14
4	CL (70:6)	1423.9667	1423.9649	$C_{79}H_{142}O_{17}P_2$	1.26
5	CL (70:5)	1425.9829	1425.9806	$C_{79}H_{144}O_{17}P_2$	1.61
6	CL (70:4)	1427.9988	1427.9962	$C_{79}H_{146}O_{17}P_2$	1.82
7	CL (70:3)	1430.0106	1430.0119	$C_{79}H_{148}O_{17}P_2$	-0.91
8	CL (70:2)	N.D. *	1432.0275	$C_{79}H_{150}O_{17}P_2$	N.A.**
9	CL (72:8)	1447.9678	1447.965	$C_{81}H_{142}O_{17}P_2$	1.93
10	CL (72:7)	1449.9833	1449.9806	$C_{81}H_{144}O_{17}P_2$	1.86
11	CL (72:6)	1451.997	1451.9962	$C_{81}H_{146}O_{17}P_2$	0.55
12	CL (72:5)	1454.0143	1454.0119	$C_{81}H_{148}O_{17}P_2$	1.65
13	CL (72:4)	1456.0294	1456.0275	$C_{81}H_{150}O_{17}P_2$	1.30
14	CL (74:10)	1471.9677	1471.9649	$C_{83}H_{142}O_{17}P_2$	1.90
15	CL (74:9)	1473.9826	1473.9806	$C_{83}H_{144}O_{17}P_2$	1.36
16	CL (74:8)	1475.9983	1475.9962	$C_{83}H_{146}O_{17}P_2$	1.42
17	CL (74:7)	1478.0142	1478.0119	$C_{83}H_{148}O_{17}P_2$	1.56
18	CL (74:6)	1480.0232	1480.0275	$C_{83}H_{150}O_{17}P_2$	-2.91
19	CL (76:12)	1495.9681	1495.9649	$C_{85}H_{142}O_{17}P_2$	2.14
20	CL (76:11)	1497.983	1497.9806	$C_{85}H_{144}O_{17}P_2$	1.60
21	CL (76:10)	1499.9992	1499.9962	$C_{85}H_{146}O_{17}P_2$	2.00
22	CL (76:9)	1502.013	1502.0119	$C_{85}H_{148}O_{17}P_2$	0.73
23	CL (76:8)	1504.0225	1504.0275	$C_{85}H_{150}O_{17}P_2$	-3.32
24	CL (76:7)	N.D. *	1506.0432	$C_{85}H_{152}O_{17}P_2$	N.A.**
25	CL (78:14)	1519.9665	1519.9649	$C_{87}H_{142}O_{17}P_2$	1.05
26	CL (78:13)	1521.9837	1521.9806	$C_{87}H_{144}O_{17}P_2$	2.04
27	CL (78:12)	1523.9994	1523.9962	$C_{87}H_{146}O_{17}P_2$	2.10
28	CL (80:16)	N.D.*	1543.9649	$C_{89}H_{142}O_{17}P_2$	N.A.**

*N.D. means Not Detected. **N.A. means Not Applicable.

Mouse Lung Inflation Method

Uninfected solid organs (kidney, lung) were collected from female C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA), 6–8 weeks of age. Briefly, mice were housed in biosafety level 2 microisolator cages and provided food and water ad libitum. Mice were euthanized by carbon dioxide narcosis prior to tissue collection. Lung inflation was followed a previous report. Briefly, mice were pinned to a dissection board, the fur wetted with 70% ethanol, and the skin was opened along the midline from the mid-abdomen to the jawline to cleanly expose the thorax. Next, the muscle layer was opened exposing the liver and diaphragm, the liver was pulled toward the stomach and the diaphragm was cut, deflating the lungs. The thoracic cavity was prepared by removing the entire ventral rib cage, cutting near the line formed by the intersection of the ventral/dorsal rib segments, taking care not to disturb the heart, lungs, or trachea. The salivary glands were removed, fully exposing the trachea. For these studies, the thymus was removed prior to lung inflation, although optional. A sterile, 5 mL Leur-lock syringe was filled with 2 mL of a solution of 2% porcine gelatin in molecular-grade water cooled before use. Two lengths of suture (4-0 Vicryl Braided, Ethicon, Sommerville, NJ) were threaded under the trachea approximately 2-3 mm apart and approximately 2 mm from the larynx. Both sutures were loosely tied with a double-twisted single knot. A catheter needle (Jelco 20G1-1/4 IV catheter, Smiths Medical, Minneapolis, MN) with a Leur-lock fitting was inserted into the trachea between the larynx and the first suture. When the catheter tip reached the tracheal interior, the needle was retracted to prevent perforation and the catheter was gently guided into the trachea below the second suture. The suture knots were subsequently tightened to secure the catheter. The syringe containing the gelatin solution was affixed to the Luer end of the catheter and the lungs inflated with ~1-1.5 mL of gelatin solution. The inflation was complete when the distal fringes of the lungs inflate, especially the accessory lobe. Subsequently, the trachea and catheter tube were secured, the catheter tube was pinched by twisting to prevent backflow, and the trachea cut using forceps. Any remaining connections to the spine were gently cut away and the inflated lungs and heart were lifted out of the pleural cavity. The descending aorta and inferior vena cava were cut and the lungs (dorsal side down) were placed onto a small aluminum foil square (approximately 2.5 cm x 2.5 cm), floated on a pool of liquid nitrogen and allowed to freeze for at least 2 minutes. Frozen, inflated lungs were stored in a foil envelope inside sealed plastic storage bags at -80°C until analysis could be completed. Kidneys were also collected for on-tissue derivatization of LPS model tissue. Tissues were excised then snap-frozen by floating on foil in a pool of liquid nitrogen and stored at −80°C for sectioning.