Supporting Information

Imaging Mass Spectrometry of Diversified Cardiolipin Molecular Species in the Brain

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Figure S-1a: Negative mode MALDI Imaging mass spectrometry of untreated and EDC/PLC treated rat brain tissue.

Figure S-1b: Mapping the spatial distribution of CL species in rat brain.

Figure S-2: Representative CL spectra (m/z 1420-1540) from a single imaged location (one pixel) at lateral spatial resolutions of 200, 100, and 50 microns.

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Table S-1a: Effect of EDC/PLC Treatment on IMS Positive Ion Mode Intensities of Rat Brain Tissue Lipids.

Table S-1b: Effect of EDC/PLC Treatment on IMS Negative Ion Mode Intensities of Rat Brain Tissue Lipids.

Table S-2: Brain Lipid Accurate Masses.

Table S-3: CL/Mitochondrial Intensity Ratios for Individual CL Species in Various Anatomical Regions of the Brain.
Figure S-1a: Negative mode MALDI Imaging mass spectrometry of untreated and EDC/PLC treated rat brain tissue. Left hemisphere MALDI images of various sulfatide, phospholipid and ganglioside species of either untreated (a, top row) or EDC-PLC treated (a, bottom row) rat brain coronal sections. The corresponding optical images are of serial tissue sections that were also either untreated or EDC-PLC treated. The negative ion mass spectra of the PL regions of untreated rat brain coronal sections (b, full m/z range; d, m/z range 760-940), and EDC-PLC treated rat brain coronal sections (c, full m/z range; e, m/z range 760-940). MALDI-IMS was performed at a spatial resolution of 200 microns. * indicates an isobaric species for ST (d18:1/20:0) and PS (40:6); ** indicates sodium adduct.
Figure S-1b: Mapping the spatial distribution of CL species in rat brain. (a) Relative-intensity heat maps of CL species (m/z 1424, 1426, 1428, 1446, 1448, 1450, 1452, 1454, 1456, 1470, 1472, 1474, 1476, 1478, 1494, 1496, 1498, 1500, 1502, 1522 and 1524 from EDC-PLC treated rat brain coronal sections (right hemisphere). Parentheses indicate CL fatty acyl chain carbon number and the number of double bonds, respectively. Scale bar = 1 mm. Spatial resolution = 200 microns.

Figure S-2: Representative CL spectra (m/z 1420-1540) from a single imaged location (one pixel) at lateral spatial resolutions of 200, 100 and 50 μm. The y axis represents the absolute intensity from one pixel at three different spatial resolutions.
Figure S-3: Positive mode MALDI Imaging mass spectrometry of untreated and EDC/PLC treated rat brain tissue. (a) Heat maps of untreated rat brain coronal sections and EDC-PLC treated rat brain coronal sections (left hemispheres) for various PC species. MALDI imaging was performed at a spatial resolution of 200 microns; (b) averaged positive ion spectrum for the imaged area of the untreated tissue (m/z 400-2000; zoomed spectrum m/z 700-860); (c) averaged positive ion spectrum for the EDC-PLC treated tissue (m/z 400-2000; zoomed spectrum m/z 700-860).
Figure S-4: Lipid spectra from various anatomical regions of rat brain. Spectra from selected regions from the right hemisphere of rat brain and corresponding individual pixels (circles, heat map) were generated for the phospholipid region (m/z 760-940). Regions include: the medial dorsal thalamic nuclear (A), the habenular nuclear (Hb) and dorsal 3\textsuperscript{rd} ventricle (D3V) (B), the cortex (C), the dentate gyrus (DG) (D), the external capsule white matter (E) and the CA2 regions (F). The heat maps shown were that for ST (m/z 806.5) and PI (m/z 885.5). Scale bar = 1 mm for the heat map image. Spatial resolution = 200 microns.
Figure S-5: Negative mode FT-ICR MALDI-IMS of EDC/PLC treated rat brain tissue. Heat maps for GM1 (d18:1/20:0), ST (d18:1/18:0) and PI (18:0/20:4) with corresponding H&E imaged area and overall negative ion spectrum (m/z range 600-2000) of an EDC-PLC treated rat brain coronal section (right hemisphere). Imaging was performed at a spatial resolution of 100 microns. Scale bar = 1 mm.
Figure S-6: UltraFlextreme MALDI-IMS analysis of intermediate fragments from the CL m/z 1448, 1476, and 1496 clusters. MS/MS analysis was performed on the CL clusters centered around m/z 1448 (72:X), 1476 (74:X), and 1498 (76:X). Heat maps (bar = 1 mm) for fragments of m/z 415.3, 417.3, 419.3, 695.5, 697.5 and 699.5 are shown along with a matching optical image. Spatial resolution = 200 microns.
Figure S-7: Ultraflex II MS/MS imaging analysis of the CL cluster centered around m/z 1474. MS/MS analysis was performed on the CL cluster centered around m/z 1474. The top panel indicates the averaged MS/MS mass spectrum for the entire area outlined in the optical image. Panels below are individual heat maps (bar = 1 mm) for the various CL daughter ions. Imaging was performed at a spatial resolution of 750 microns in order to generate enough parent signal for subsequent MS/MS analysis.
Figure S-8: Ultraflex II MS/MS imaging analysis of CL fatty acyl fragments in various brain regions. MS/MS analysis was performed on the CL cluster centered around \( m/z \) 1474. An optical image (bar = 1 mm) and a heat map of \( m/z \) 279 species (C18:2) is shown. Circled areas in the optical and heat map images correspond to the Hb/D3V (A), dentate gyrus (B), white matter (C) and cortical (D) regions of the brain. Spectra (\( m/z \) 250-350) from individual pixels from the various regions are shown. The spatial resolution was 750 microns.
**Supplementary Table S-1a.** Effect of EDC/PLC Treatment on IMS Positive Ion Mode Intensities of Rat Brain Tissue Lipids

| Positive mode m/z | Lipids** | EDC/PLC Treated Intensities (a.u.)* | Untreated Intensities (a.u.)* |
|-------------------|----------|-----------------------------------|-------------------------------|
| 730.6             | PC(16:1/16:1) | 1.91                              | 54.38                         |
| 734.6             | PC(16:0/16:0) | 22.02                             | 122.10                        |
| 760.6             | PC(16:0/18:1)| 13.98                             | 144.00                        |
| 788.6             | PC(18:0/18:1)| 6.24                              | 59.51                         |
| 810.6             | PC(18:0/20:4)| 5.17                              | 78.75                         |
| 731.6             | SM(d18:1/18:0)| 18.38                            | 42.28                         |
| 813.6             | SM(d18:1/24:1)| 1.11                              | 12.14                         |
| 744.5             | PE(18:1/18:1)| 0.26                              | 1.71                          |
| 746.5             | PE(18:0/18:1)| 0.69                              | 12.78                         |

*a.u.= arbitrary units. Values were derived from heat maps on a scale from 0-255 (detector maximum) as determined by Bruker FlexImaging Software. PC= phosphatidylcholine; SM= sphingomyelin; PE=phosphatidylethanolamine. ** see reference 9.

**Supplementary Table S-1b.** Effect of EDC/PLC Treatment on IMS Negative Ion Mode Intensities of Rat Brain Tissue Lipids

| Negative mode m/z | Lipids** | EDC/PLC Treated Intensities (a.u.)* | Untreated Intensities (a.u.)* |
|-------------------|----------|-----------------------------------|-------------------------------|
| 806.5             | ST(d18:1/18:0) | 43.95                             | 31.42                         |
| 834.5             | PS(18:0/22:6)*** | 5.69                             | 7.36                          |
|                   | ST(d18:1/20:4)*** | 1.11                             | 12.14                         |
| 906.6             | ST(d18:1/h24:0) | 40.93                             | 23.38                         |
| 1544.9            | GM1(d18:1/18:0) | 7.34                              | 2.91                          |
| 1476.0            | CL(18:1)_{(18:2)/(20:4)} | 0.27                             | ND                           |
| 1450.0            | CL(18:1)_{(18:2)} | 0.16                             | ND                           |

*a.u.= arbitrary units. Values were derived from heat maps on a scale from 0-255 (detector maximum) as determined by Bruker FlexImaging Software. ND= not detected. ST= sulfatide; PS= phosphatidylserine; GM1= ganglioside GM1; CL=cardiolipin. ** see table S-3 supplementary data. *** isobaric species.
**Supplementary Table S-2. Brain Lipid Accurate Masses**

| m/z   | Species                  | Ion          | Reference* |
|-------|--------------------------|--------------|------------|
| 747.516 | PG(34:1)                 | [M-H]-       |            |
| 762.507 | PE(38:6)                 | [M-H]-       |            |
| 766.538 | PE(38:4)                 | [M-H]-       |            |
| 778.514 | ST(d18:1/16:0)            | [M-H]-       | Angel, Hsu |
| 804.530 | ST(d18:1/18:1)            | [M-H]-       | Angel      |
| 806.545 | ST(d18:1/18:0)            | [M-H]-       | Angel, Hsu |
| 820.524 | ST(d18:1/h18:1)           | [M-H]-       |            |
| 821.545 | PI(e16:0/18:1)            | [M-H]-       | Angel      |
| 822.540 | ST(d18:1/h18:0)           | [M-H]-       | Angel      |
| 834.576 | ST(d18:1/20:0)            | [M-H]-       | Hsu        |
| 835.523 | PI(34:1)                 | [M-H]-       |            |
| 850.571 | ST(d18:1/h20:0)           | [M-H]-       | Angel      |
| 857.518 | PI(16:0/20:4)             | [M-H]-       | Angel      |
| 862.608 | ST(d18:1/22:0)            | [M-H]-       | Angel, Hsu |
| 878.602 | ST(d18:1/h22:0)           | [M-H]-       | Angel, Hsu |
| 881.518 | PI(16:0/22:6)             | [M-H]-       | Angel      |
| 883.534 | PI(18:1/20:4)             | [M-H]-       | Angel      |
| 885.549 | PI(18:0/20:4)             | [M-H]-       | Angel      |
| 888.622 | ST(d18:1/24:1)            | [M-H]-       | Hsu, Angel |
| 890.639 | ST(d18:1/24:0)            | [M-H]-       | Hsu, Angel |
| 902.602 | ST(d18:1/h24:2)           | [M-H]-       |            |
| 904.618 | ST(d18:1/h24:1)           | [M-H]-       | Hsu, Angel |
| 906.634 | ST(d18:1/h24:0)           | [M-H]-       | Hsu, Angel |
| 916.654 | ST(d18:1/26:1)            | [M-H]-       | Angel, Hsu |
| 918.670 | ST(d18:1/26:0)            | [M-H]-       |            |
| 932.649 | ST(d18:1/h26:1)           | [M-H]-       | Hsu        |
| 1382.818 | GM2(d18:1/18:0)          | [M-H]-       | Woods      |
| 1425.980 | CL(16:1)/1/(18:2)/1/(18:1) | [M-H]-       | Tyurin     |
| 1427.996 | CL(16:1)/1/(18:1)         | [M-H]-       | Tyurin     |
| 1445.949 | CL(16:1)/1/(16:0)/1/(18:3)/1/(22:5) | [M-H]-       | Tyurin     |
| 1447.964 | CL(16:1)/1/(18:1)/1/(18:3)/1/(20:4) | [M-H]-       | Tyurin     |
| 1449.980 | CL(18:1)/1/(18:2)/1/(18:3)/1/(18:2)/1/(20:3) | [M-H]-       | Tyurin     |
| 1451.995 | CL(18:1)/1/(18:2)/1/(18:3)/1/(18:2)/1/(20:3) | [M-H]-       | Bayir      |
| 1454.011 | CL(16:0)/1/(18:2)/1/(20:3)/1/(18:2)/1 | [M-H]-       | Bayir      |
Accurate masses for brain lipids. Abbreviations: PI = phosphatidylinositol, PG = phosphatidylglycerol, ST = sulfatide, CL = cardiolipin, G = ganglioside, h = hydroxy fatty acid, d = dihydrosphingosine, e = ether linkage. Ganglioside nomenclature follows Woods et al 2011.

Chain assignments are given according to literature reports. Ion m/z values are given as the apex of the monoisotopic peak. ** = minor species. CL(XX:XX) indicates CL(total number of fatty acyl carbons: total number of double bonds). ^a= See references 9,10,11,12, 22, 24, 26

| Mass          | Molecule Description                                  | Formula      | Charge | Reference |
|---------------|-------------------------------------------------------|--------------|--------|-----------|
| 1456.027      | CL(18:1)4; CL(16:0)1/(18:1)2/(20:2)1                   | [M-H]-       | Bayir  |
| 1469.949      | CL(74:11)                                              | [M-H]-       |        |
| 1471.964      | CL(74:10)                                              | [M-H]-       |        |
| 1473.980      | CL(18:1)1/(18:2)2/(20:4)1; CL(16:0)1/(18:1)1/(18:2)1/(22:6)1 | [M-H]-       | Bayir  |
| 1475.996      | CL(16:1)1/(18:1)2/(22:5)1; CL(16:1)1/(18:2)1/(20:3)1/(20:2)1; CL(16:0)1/(18:1)2/(22:6)1; CL(16:0)1/(18:1)1/(20:4)1/(20:3)1; CL(18:1)2/(18:2)1/(20:4)1 | [M-H]-       | Bayir, Tyurin |
| 1478.011      | CL(18:1)9/(20:4)1; CL(16:1)1/(18:2)1/(20:3)1/(20:1)1,** | [M-H]-       | Bayir  |
| 1480.027      | CL(18:1)2/(18:0)1/(20:4)1                             | [M-H]-       | Bayir  |
| 1495.964      | CL(16:1)1/(18:2)1/(20:3)1/(22:6)1                      | [M-H]-       | Bayir  |
| 1497.980      | CL(18:1)1/(18:2)1/(20:4)2                             | [M-H]-       | Tyurin, Bayir |
| 1499.996      | CL(18:1)2/(20:4)2; CL(18:0)1/(18:2)2/(22:6)1          | [M-H]-       | Tyurin, Bayir |
| 1502.011      | CL(18:1)2/(20:4)1/(20:3)1; CL(16:0)1/(18:1)1/(20:4)1/(22:4)1 | [M-H]-       | Tyurin, Bayir |
| 1516.839      | GM1(d18:1/16:0)                                         | [M-H]-       |        |
| 1542.855      | GM1(d18:1/18:1)                                         | [M-H]-       | Angel, Woods |
| 1544.869      | GM1(d18:1/18:0)                                         | [M-H]-       | Angel, Woods |
| 1572.896      | GM1(d20:1/18:0)                                         | [M-H]-       | Woods  |
| 1817.953      | GD1(d18:1/18:0)                                         | [M-H2O-H]-   | Woods  |
| 1842.969      | GD1(d20:1/18:1)                                         | [M-H2O-H]-   | Woods  |
| 1845.981      | GD1(d20:1/18:0)                                         | [M-H2O-H]-   | Woods  |
| 1857.940      | GD1(d18:1/18:0)                                         | [M-2H+Na]-   | Woods  |
| 1882.892      | GD1(d20:1/18:0)                                         | [M-2H+Na]-   | Woods  |
| 1953.964      | GT2(d18:1/18:0)                                         | [M-H2O-H]-   | Woods  |
**Supplementary Table S-3:** CL/Mitochondria Intensity Ratios for Individual CL Species in Various Anatomical Regions of the Brain

| Species          | Cortex | Hippo-campus | Thalamus | D3V and Hb | LV | Hippo/Cortex | Hippo/Thalamus | Hippo/average(Cortex+Thalamus) |
|------------------|--------|--------------|----------|------------|----|--------------|----------------|--------------------------------|
| CL(70:5), m/z 1425.980 | 0.11   | 0.26         | 0.11     | 0.61       | 0.00 | 2.30         | 2.42           | 2.36                  |
| CL(70:4), m/z 1427.996 | 0.10   | 0.46         | 0.17     | 0.84       | 0.50 | 4.72         | 2.71           | 3.44                  |
| CL(72:8), m/z 1447.964 | 0.41   | 0.51         | 0.37     | 1.29       | 0.72 | 1.24         | 1.37           | 1.30                  |
| CL(72:7), m/z 1449.980 | 0.45   | 0.88         | 0.45     | 1.32       | 1.81 | 1.94         | 1.93           | 1.93                  |
| CL(72:6), m/z 1451.996 | 0.24   | 0.43         | 0.20     | 1.26       | 3.02 | 1.84         | 2.19           | 2.00                  |
| CL(72:5), m/z 1454.011 | 0.27   | 0.48         | 0.33     | 1.39       | 2.31 | 1.77         | 1.43           | 1.58                  |
| CL(72:4), m/z 1456.027 | 0.07   | 0.23         | 0.08     | 0.99       | 1.89 | 3.53         | 2.95           | 3.22                  |
| CL(74:11), m/z 1497.980 | 0.11   | 0.04         | 0.10     | 0.26       | 0.00 | 0.38         | 0.43           | 0.40                  |
| CL(74:10), m/z 1499.996 | 0.24   | 0.28         | 0.24     | 1.21       | 0.17 | 1.19         | 1.20           | 1.19                  |
| CL(74:9), m/z 1473.980 | 0.37   | 0.43         | 0.37     | 1.28       | 1.92 | 1.16         | 1.17           | 1.17                  |
| CL(74:8), m/z 1475.996 | 0.53   | 0.76         | 0.50     | 1.20       | 2.00 | 1.43         | 1.51           | 1.47                  |
| CL(74:7), m/z 1478.011 | 0.20   | 0.61         | 0.22     | 1.19       | 1.87 | 3.09         | 2.86           | 2.97                  |
CL/mitochondria ratios for individual CL species are given for various anatomical regions of the brain. D3V and Hb = dorsal 3rd ventricle and Habenular region; LV = lateral ventricle. Intensity ratios of CL/mitochondria in the hippocampus relative to the thalamus and cortex are also given. Ratios are displayed as a heat map from low CL/mitochondrial intensities (purple) to high CL/mitochondrial intensities (red). CL (XX:XX) indicates the total number of carbons:double bonds within the CL species.