Supporting Information

DESI then MALDI mass spectrometry imaging of lipid and protein distributions in single tissue sections

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H&E staining protocol:

All chemicals used for the H&E staining were purchased from Sigma-Aldrich (St. Louis, MO, USA). H&E staining was preformed after MALDI matrix was washed. It was noted that the matrix washing step is critical for assuring high quality H&E stained sections, since sliding and cracking of the tissue can readily occur at this stage. H&E staining was performed according to the following protocol: 1) fix in MeOH(30 seconds), 2) rinse in water (10 dips), 3) stain in Harris modified hematoxylinsolution(1.5 minutes), 4) rinse in water (10 dips), 5) blue in 0.1% ammonia (quick dip), 6) rinse in water (10 dips), 7) counterstain in Eosin Y (8 seconds), 8) rinse and dehydrate in 100% EtOH (10 dips), 9) rinse and dehydrate again in 100% EtOH (10 dips), 10) dip in xylene (6 dips) and 11) dip again in xylene (6 dips). Sections were allowed to dry at room temperature and covered with histological mounting medium (Permount®, Fisher Chemicals, Fair Lawn, NJ) and a glass cover slide.

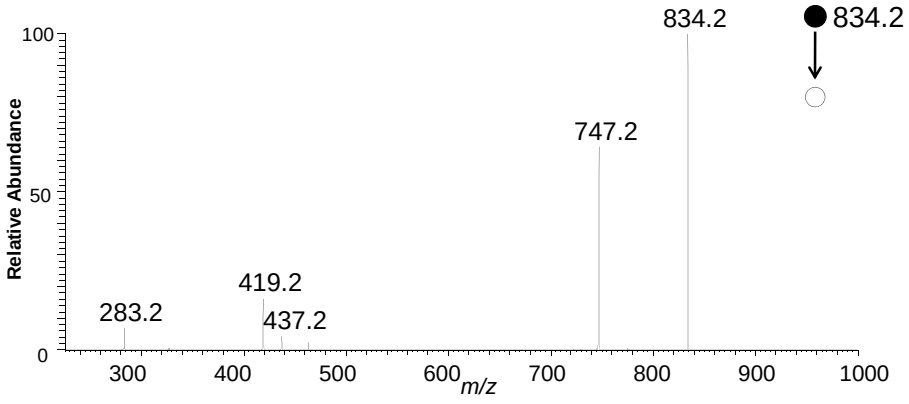

Figure S1. Negative ion mode MS/MS spectrum of *m/z* 834.2, identified as PS(18:0/22:6). The most abundant fragment ion in the CID MS/MS spectrum of this molecule is $[M-H-87]^-$ corresponding to the loss of the serine portion of the head group by cleavage of the oxygen-carbon bond. The next most abundant peak, at *m/z* 419.2, result from the loss of 87 followed by the loss of one of the two fatty acid chains. The other peak at *m/z* 283.2 is related to the fatty acid anions.

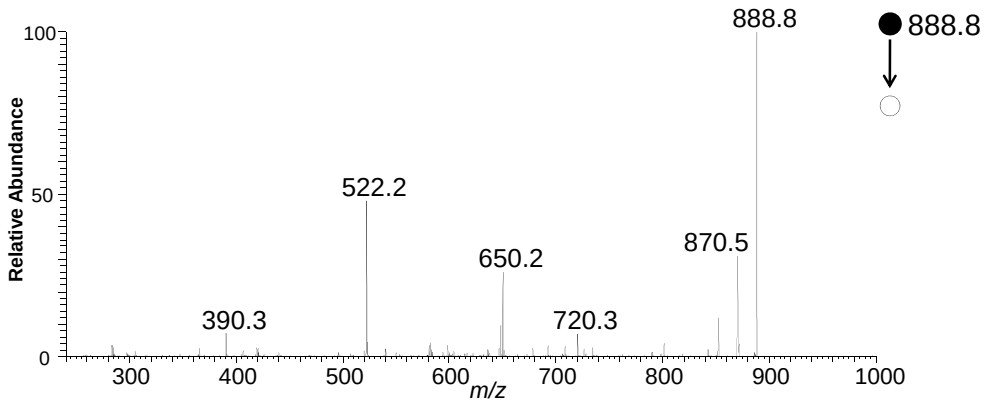

Figure S2. Negative ion mode MS/MS spectrum of *m/z* 888.8, identified as ST(24:1). CID gives rise to an abundant peak at *m/z* 522.2, corresponding to the loss of water and the amide linked ketene by cleavage of the amide bond. The fragment of *m/z* 390.3 arises from cleavage of the C2 to C3 bond of the sphingosine backbone along with loss of the head group via cleavage of the C1–O bond. The fragment ion of *m/z* 650.2 comes from loss of the long-chain base after the second carbon.

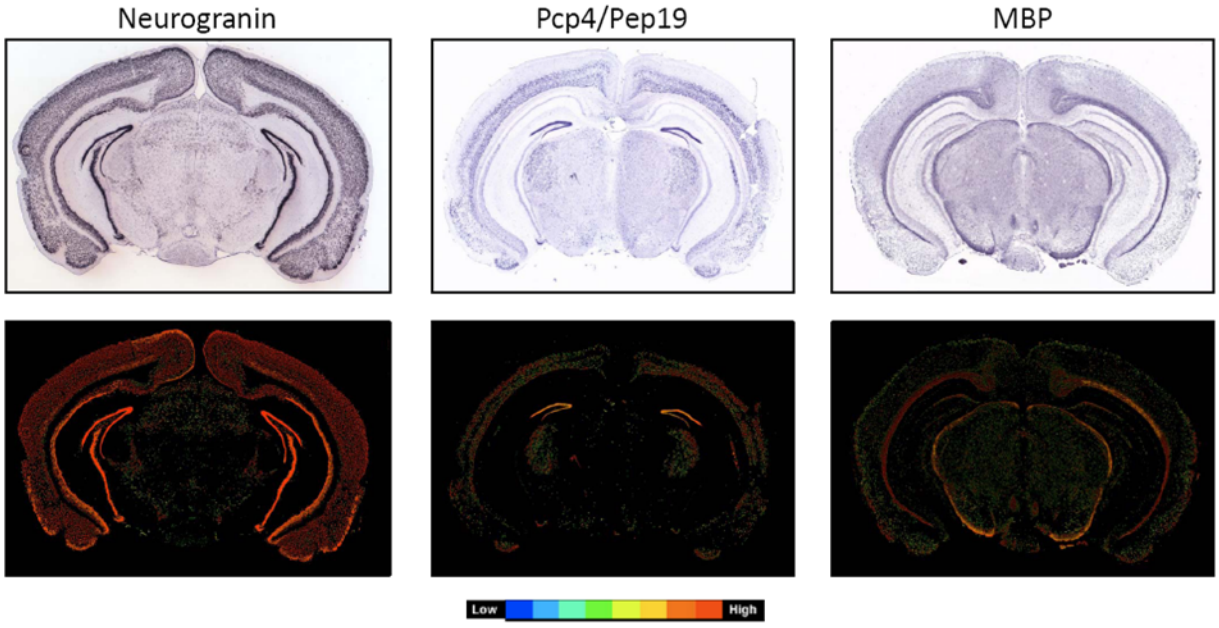

Figure S3. mRNA in situ hybridization images from the Allen Brain Atlas (http://mouse.brain-map.org/welcome.do) for correlation of mass spectrometry imaging of lipid and protein distribution. The upper panels show the mRNA distribution results as detected by in situ hybridization with a colorimetric reaction based on a horseradish peroxidase (HRP)-conjugated anti-digoxigenin antibody, and the lower panels present quantitation of the mRNA expression levels from low to high according to the color scale

bar. Hybridization is from coronal brain sections of male C57BL/6J mice. Neurogranin: Neurog1-Coronal-05-0560: Neurog1_198 (position: 4950); age 55 days. PCP4/PEP-19: Pcp4_178_1850 (position 4450); age 56 days. MBP: Mbp-Coronal-2337:Mbp_200_2337 (position 5000); age 56 days.

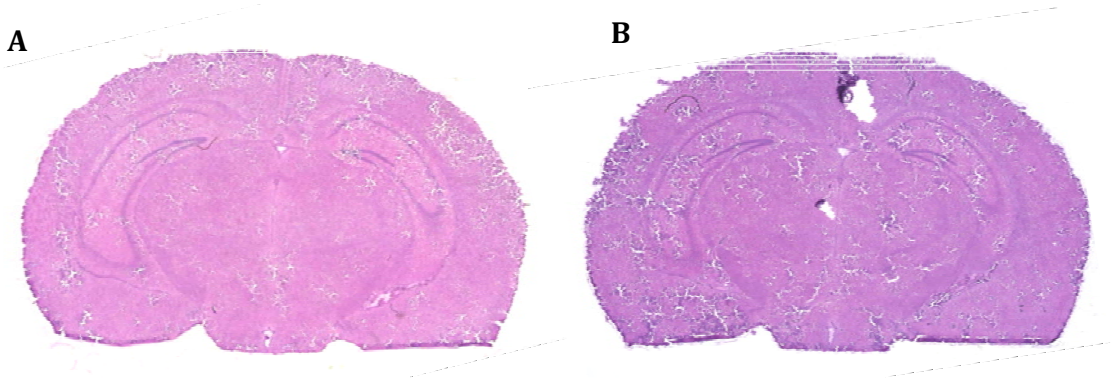

Figure S4. Optical image of the H&E stained tissue section which (A) was first imaged by DESI-MS and then imaged by MALDI-MS and (B) control section which was solely imaged by MALDI-MS.

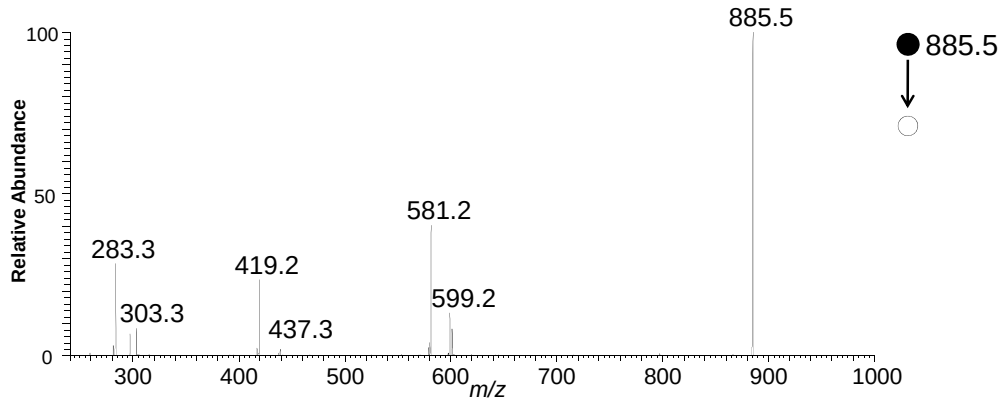

Figure S5. Negative ion mode MS/MS spectrum of *m/z* 885.5, identified as PI (18:0/20:4). The most abundant ion recorded at *m/z* 581.2 corresponds to the loss of the neutral carboxylic acid (20:4). Meanwhile, the ion of *m/z* 599.2 corresponds to the loss of C18:0 ketene. The peaks at *m/z* 419 were generated by the loss of the inositol portion and ketene from C20:4. Other peaks at *m/z* 283.3 and *m/z* 303.3 are related to the fatty acid anions.

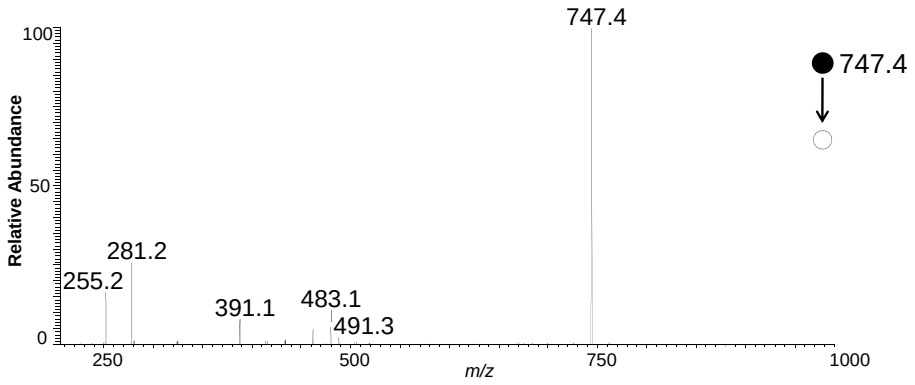

Figure S6. Negative ion mode MS/MS spectrum of m/z 747.4, identified as PG(16:0/18:1). The ion at *m/z* 491, is due to the neutral loss of 16:0 fatty acid. The spectrum also contain the ion at *m/z* 483.1, reflecting neutral loss of the fatty acyl substituent at sn-2 as a ketene. The other peaks at *m/z* 281.2 and *m/z* 255.2 are related to the fatty acid anions.

References:

1. N. E. Manicke, A. L. Dill, D. R. Ifa, R. G. Cooks, High-resolution tissue imaging on an orbitrap mass spectrometer by desorption electrospray ionization mass spectrometry. *Journal of Mass Spectrometry* 2010, *45*. 223-226, DOI: 10.1002/jms.1707.