**Supplemental figure captions**

S1. Scheme of the experimental setup for matrix sublimation on thin tissue sections. See Methods for details.

S2. Photomicrographs and scheme of the experimental setup for dry matrix deposition on thin tissue sections. See Methods for details.

S3. Imaging MS of phospholipids in the negative ionization mode from a coronal mouse brain section. A) Photomicrograph of the section after 2,5-DHB matrix sublimation and positive and negative mode imaging MS data acquisitions with a lateral resolution of 100 µm. The imaging array was shifted by 50x50 µm after acquisition in the positive ion mode. The outlines of the major brain substructures are clearly visible. B) Phospholipid sum spectrum obtained after imaging MS data acquisition. C) Overlay of 3 different phospholipid ion images expressed in different regions of the section.

S4. Effects of TIC normalization on observed phospholipid distributions. The shown examples are from the imaging MS dataset presented in Figure 2. Whereas in some cases normalization seems to have improved the quality of the image (m/z 660.07 and 776.16) in other cases, obvious distortions were observed such as edge effects (m/z 746.66 and
820.66), oversaturation ($m/z$ 772.67), loss of information ($m/z$ 746.66 and 797.67), and of greater concern ‘fabrication’ of information ($m/z$ 820.66).

S5. High spatial resolution imaging MS. A) Scheme of the MALDI-TOF coaxial laser illumination ion source installed in a linear TOF instrument. Precise control of the sample plate position (mounted on a Piezo stage), the laser energy and the number of laser shots allow to image tissue sections with lateral resolutions as low as 5 µm (with permission from reference (48)). B) Phospholipid profile acquired summing signals from 200 shots on tissue (10 series of 20 shots at different positions) with an estimated laser focus on target of 5 µm in diameter.
Supplemental Figure 1
Supplemental Figure 2

20 µm sieve
Supplemental Figure 3
Supplemental Figure 5

A) Diagram of experimental setup: Sample illumination, UV laser (337 nm), Attenuator, 20 Hz, 15 kV Target plate, Piezo Stage, TOF, MCP detector, CCD camera, Transient recorder.

B) Graph showing Mass-to-charge (m/z) distribution with % intensity along the x-axis from 600 to 900.