

## Supporting Information for

### More From Less: High-Throughput Dual Polarity Lipid Imaging of Biological Tissues

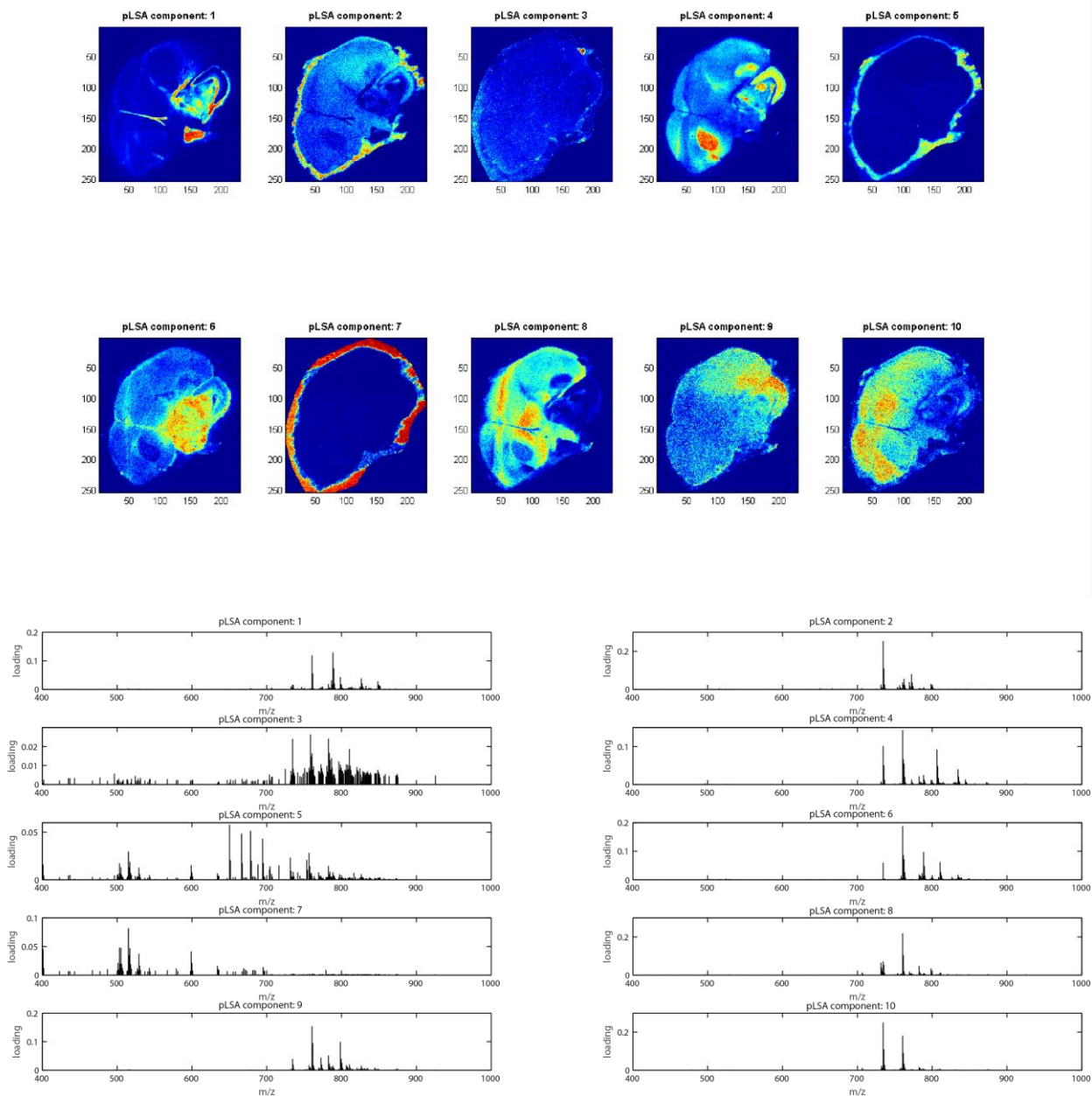
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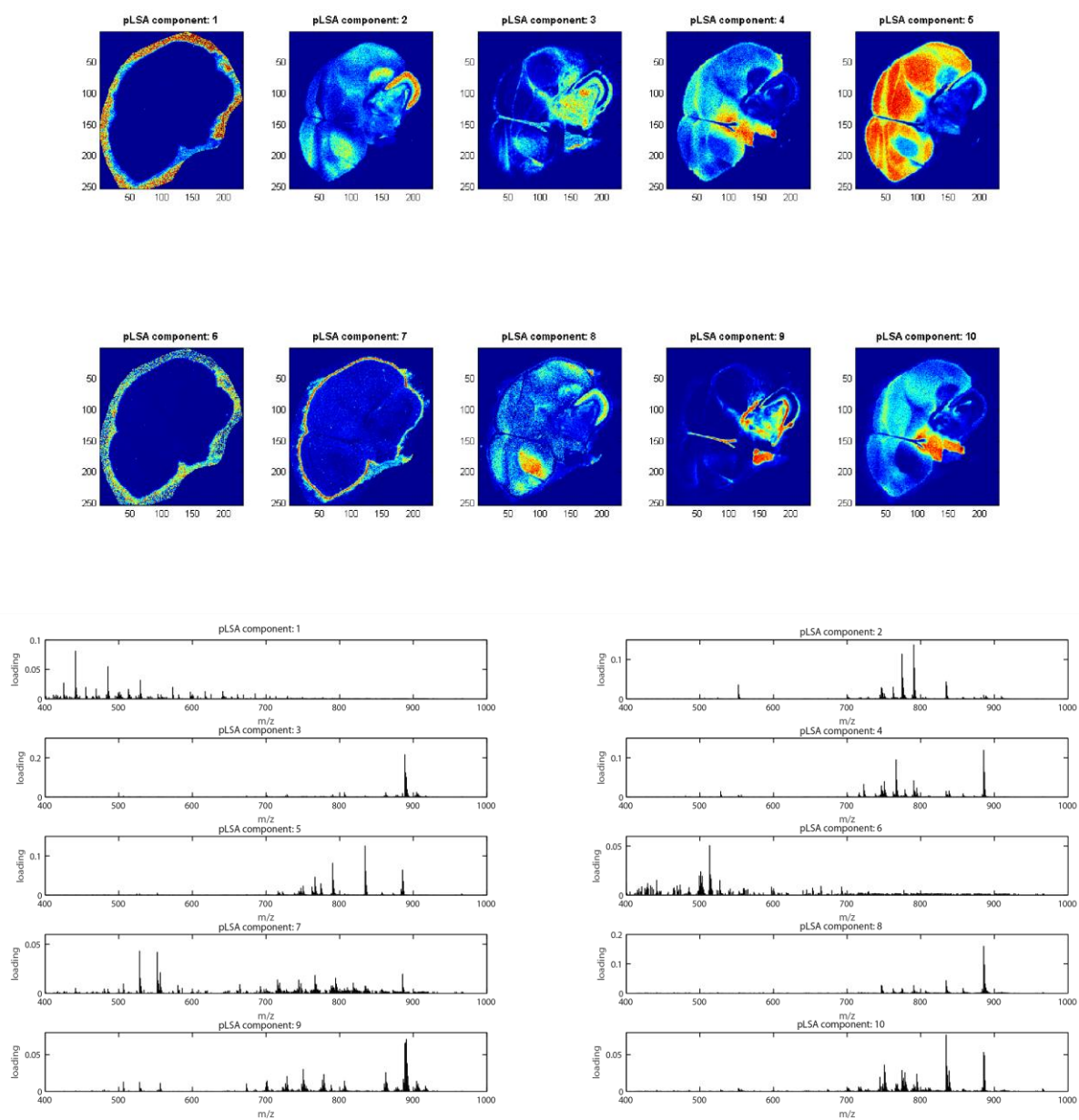
<sup>2</sup> Bio-Imaging Lab, University of Antwerp, 2610 Wilrijk, Belgium

\* Corresponding Author

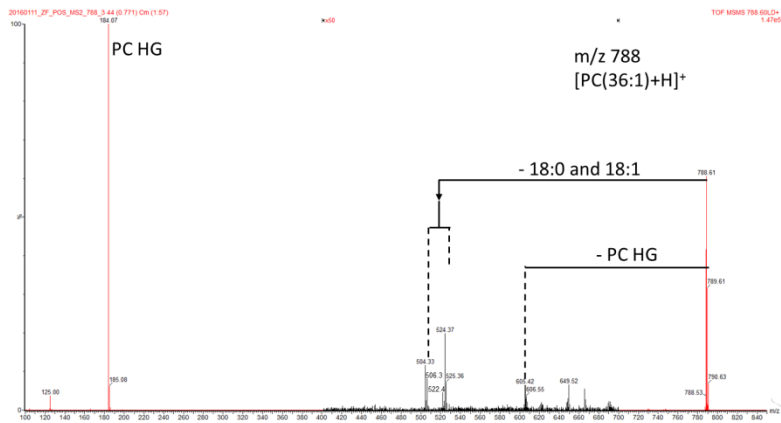
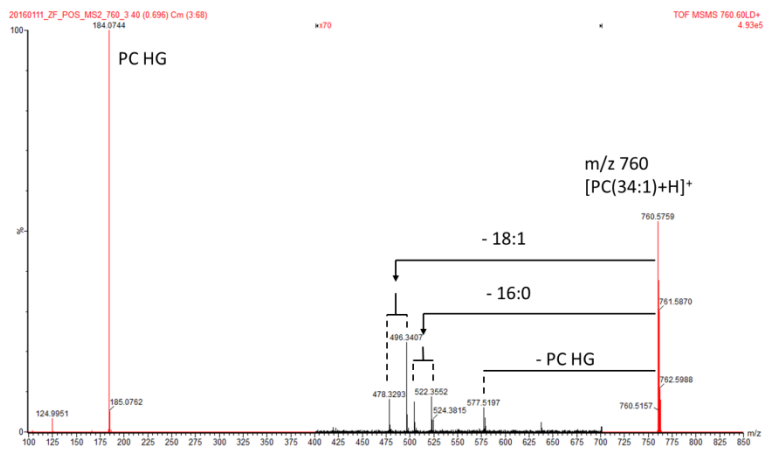
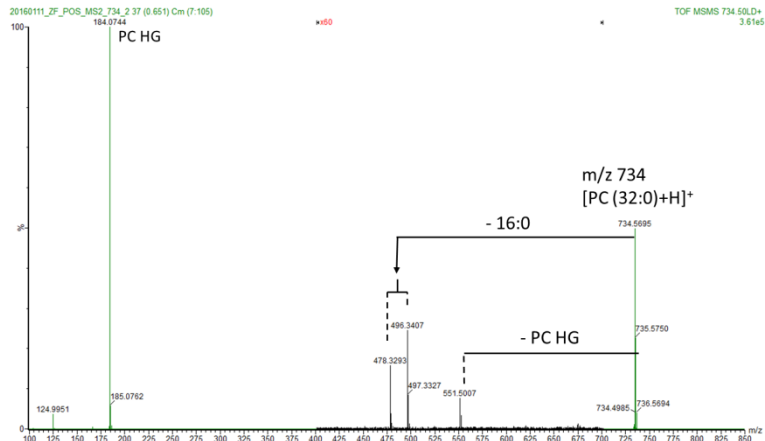
[s.ellis@maastrichtuniversity.nl](mailto:s.ellis@maastrichtuniversity.nl)

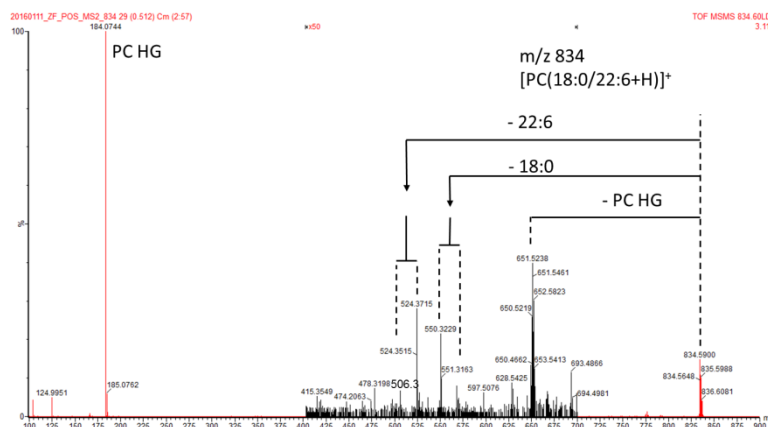
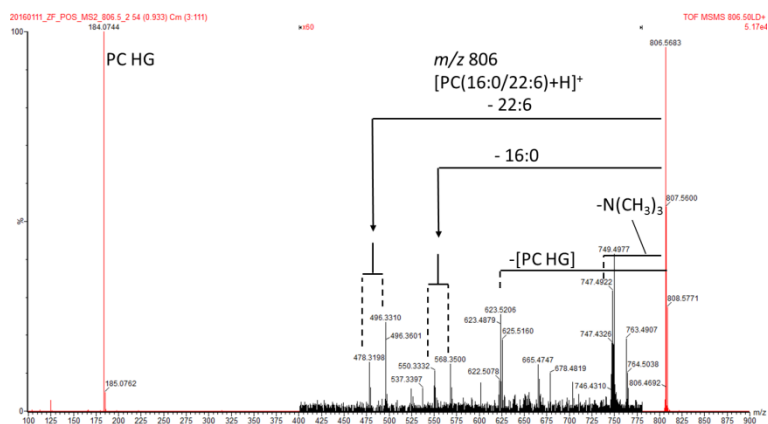
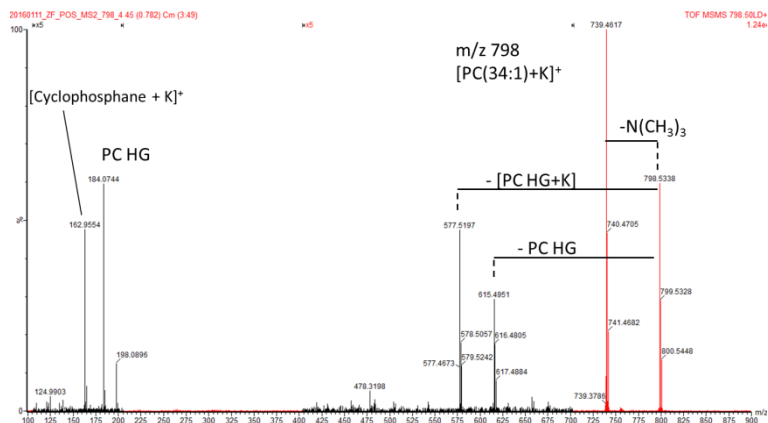


**Figure S1.** Positive ion pLSA component images from zebra finch brain tissue (top) and the corresponding loadings spectra (bottom). Note the signal observed in components 2, 5 and 7 arise from matrix-related background adjacent to the tissue.

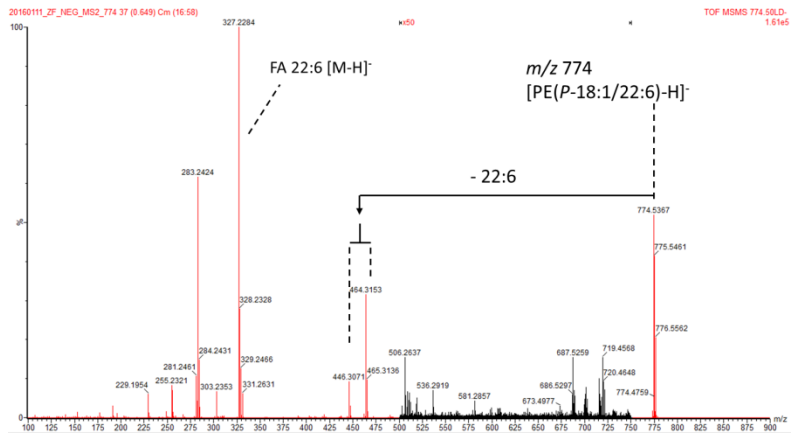
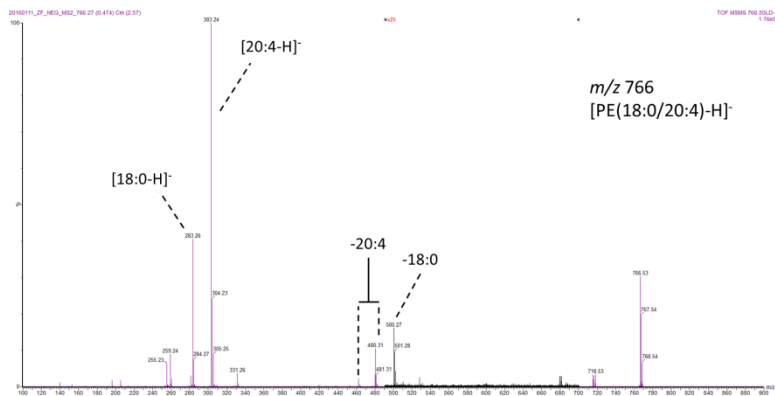
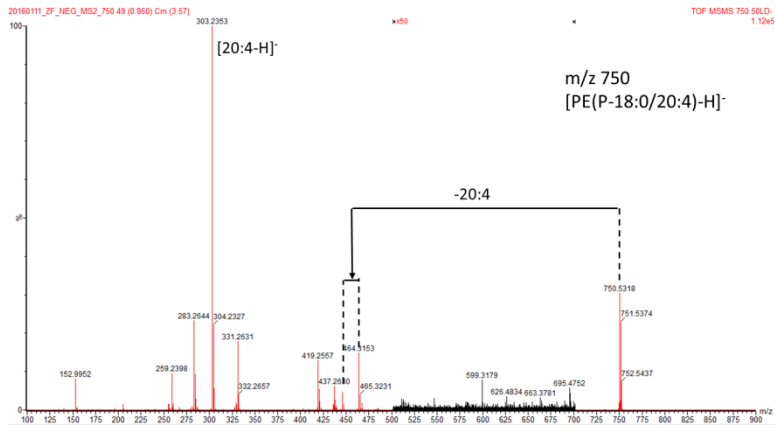


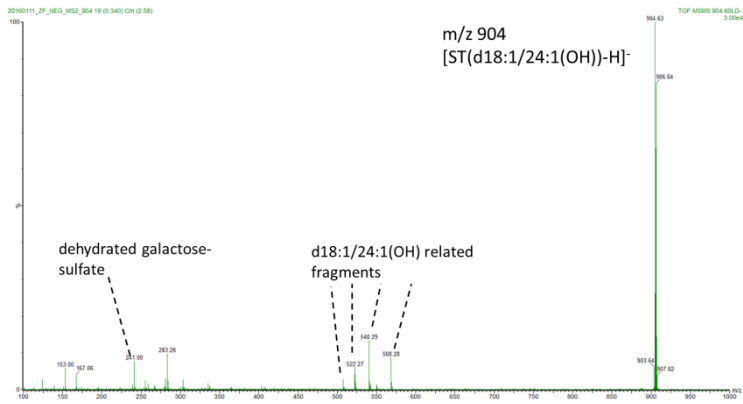
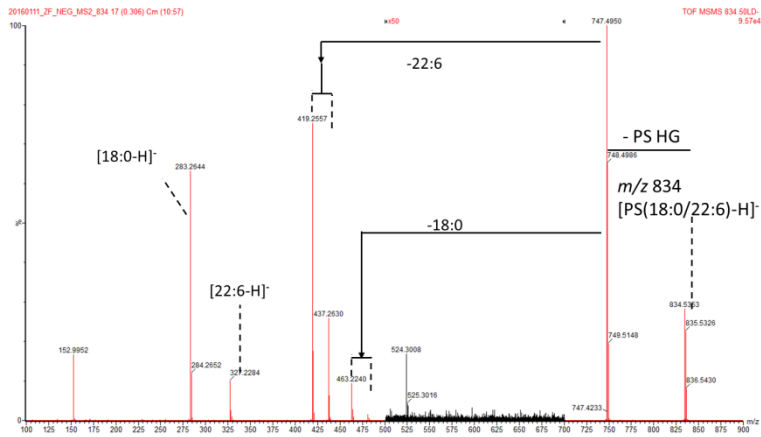
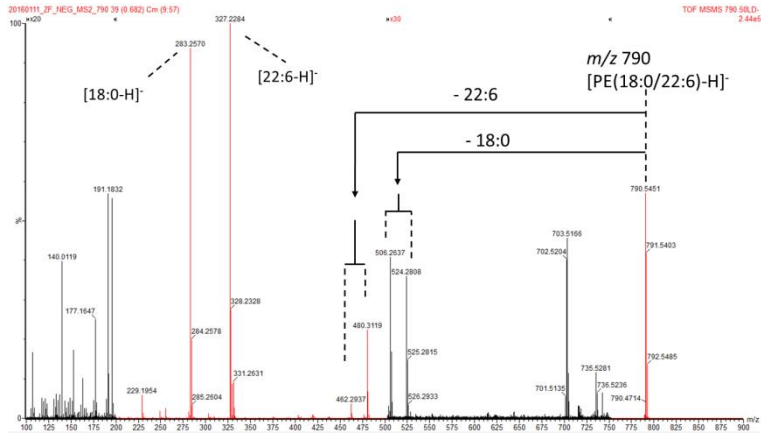
**Figure S2.** Negative ion pLSA component images from zebra finch brain tissue (top) and the corresponding loadings spectra (bottom). Note the signal observed in components 1, 6 and 7 arise from matrix-related background adjacent to the tissue.

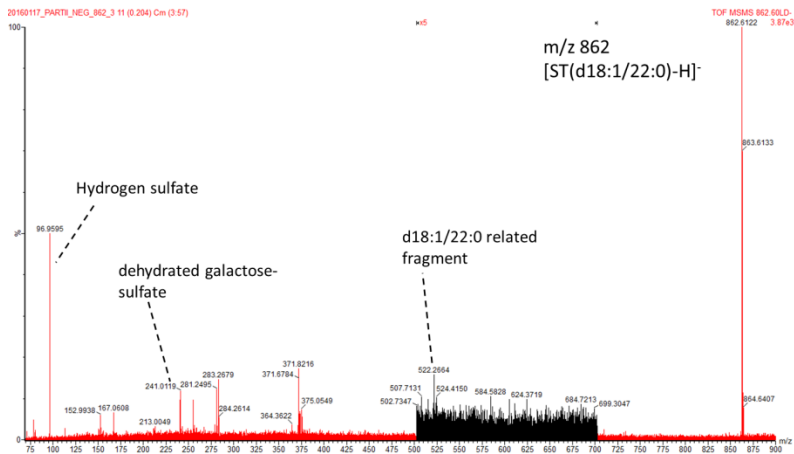
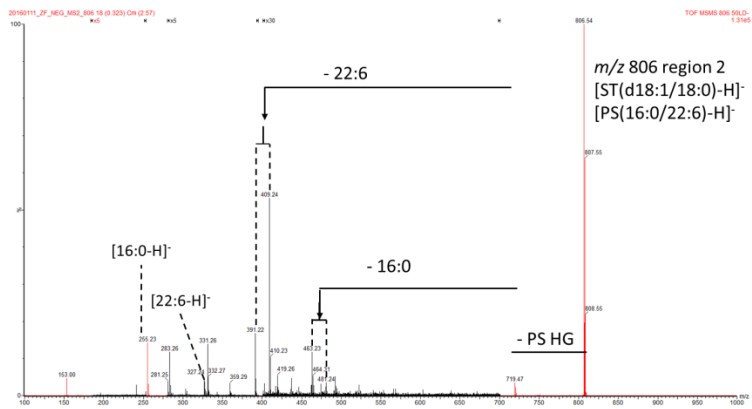
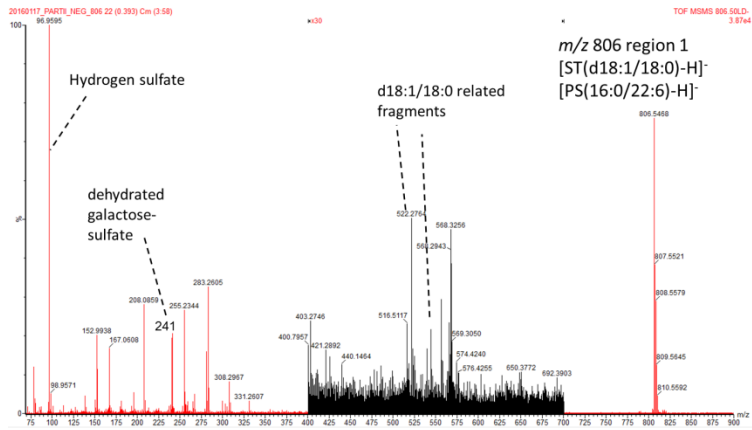




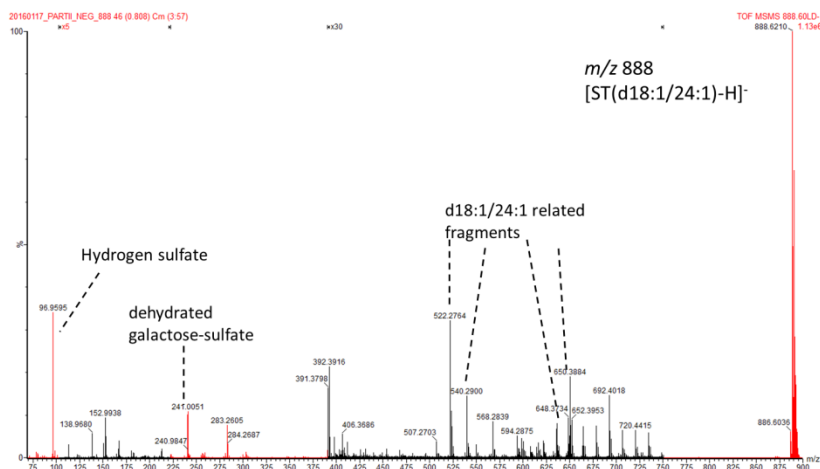
**Figure S3.** Positive ion MS/MS spectra acquired from zebra finch brain tissue. Only the most dominant lipid assignment is provided. In many cases, due to both the complexity of the lipidome and the relatively wide (several Da) isolation window that was required to produce sufficient signal, the isolation of more than one lipid structure is observed. Polyunsaturated fatty acids are assumed to be at the sn2 position. HG=headgroup



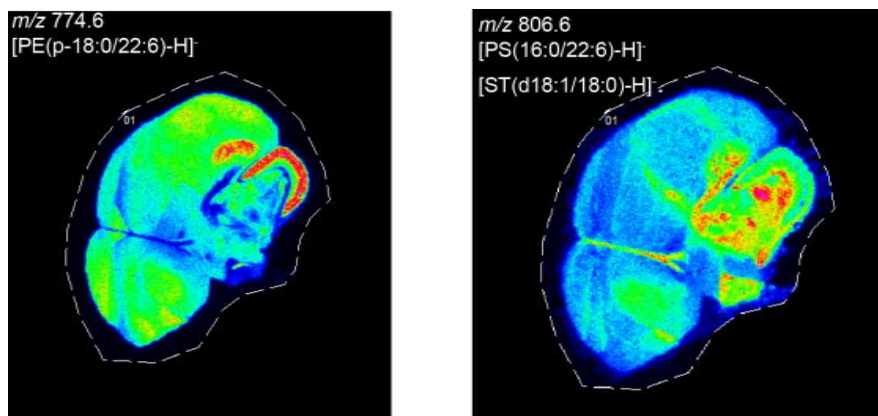




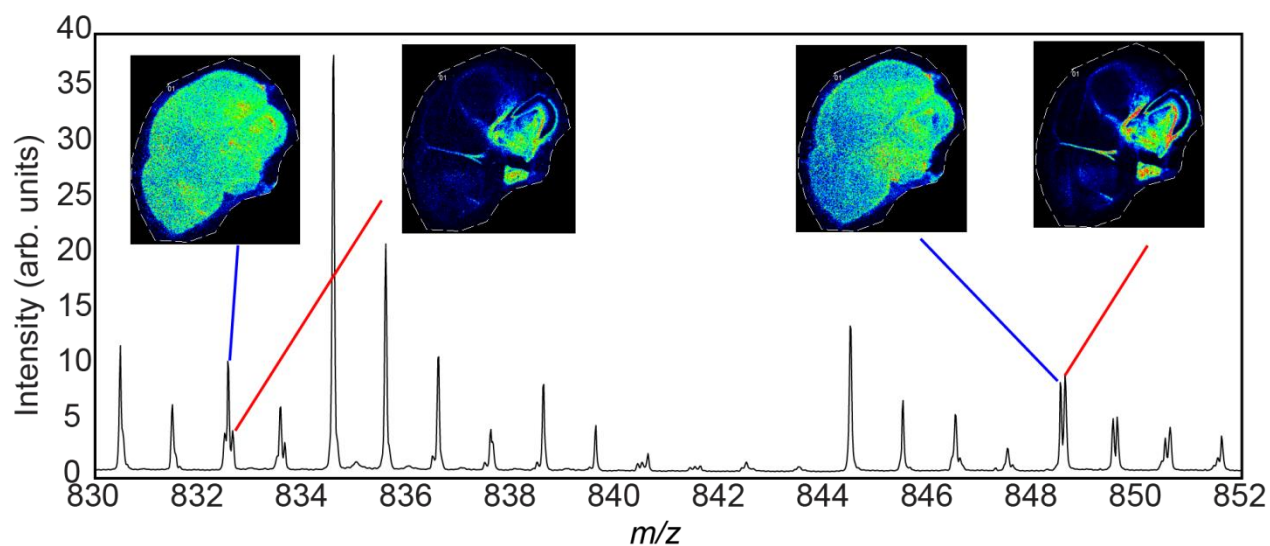




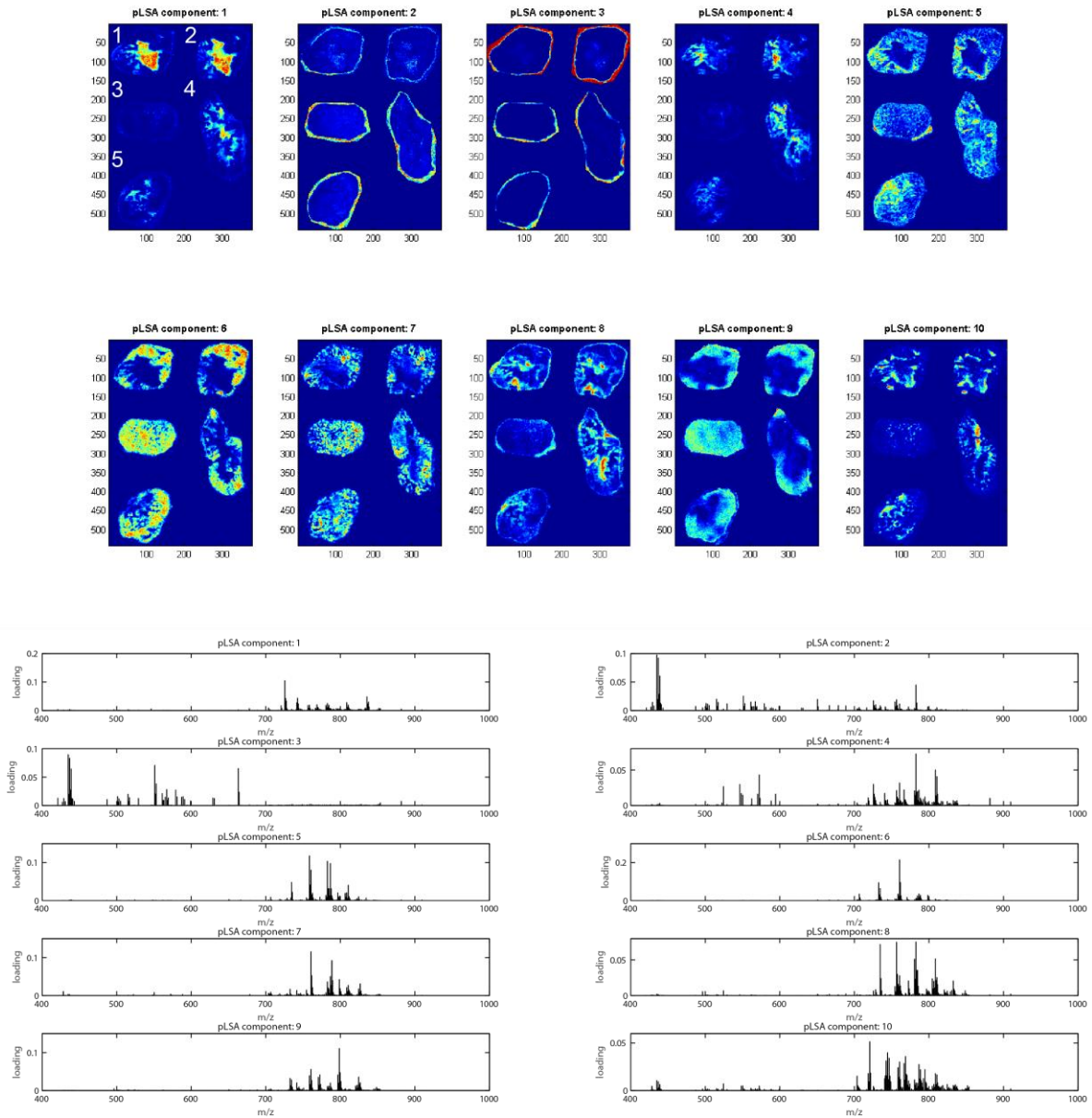
**Figure S4.** Negative ion MS/MS spectra acquired from zebra finch brain tissue. Only the most dominant lipid assignment is provided. In many cases, due to both the complexity of the lipidome and the relatively wide (several Da) isolation window that was required to produce sufficient signal the isolation of more than one lipid structure is observed. Polyunsaturated fatty acids are assumed to be at the sn2 position. HG=headgroup



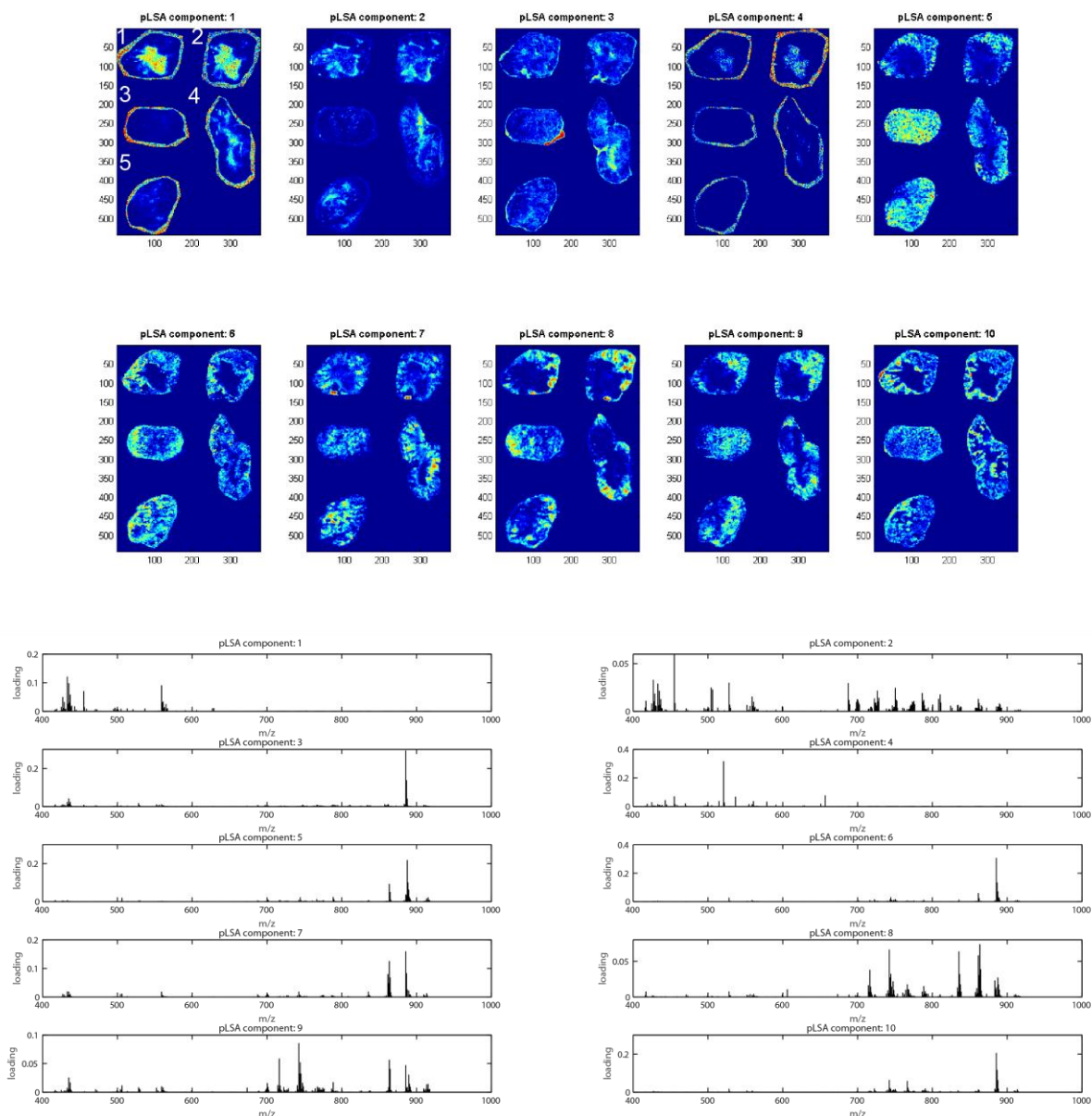
**Figure S5.** Negative ion images of  $m/z$  774.6 ( $[PE(p-18:0/22:6)-H]^-$ ) and  $m/z$  806.6 ( $[mixture\ of\ [PS(p-16:0/22:6)-H]^-$  and  $[ST(d18:1/18:0)-H]^-]$ ) acquired from zebra finch brain tissue.



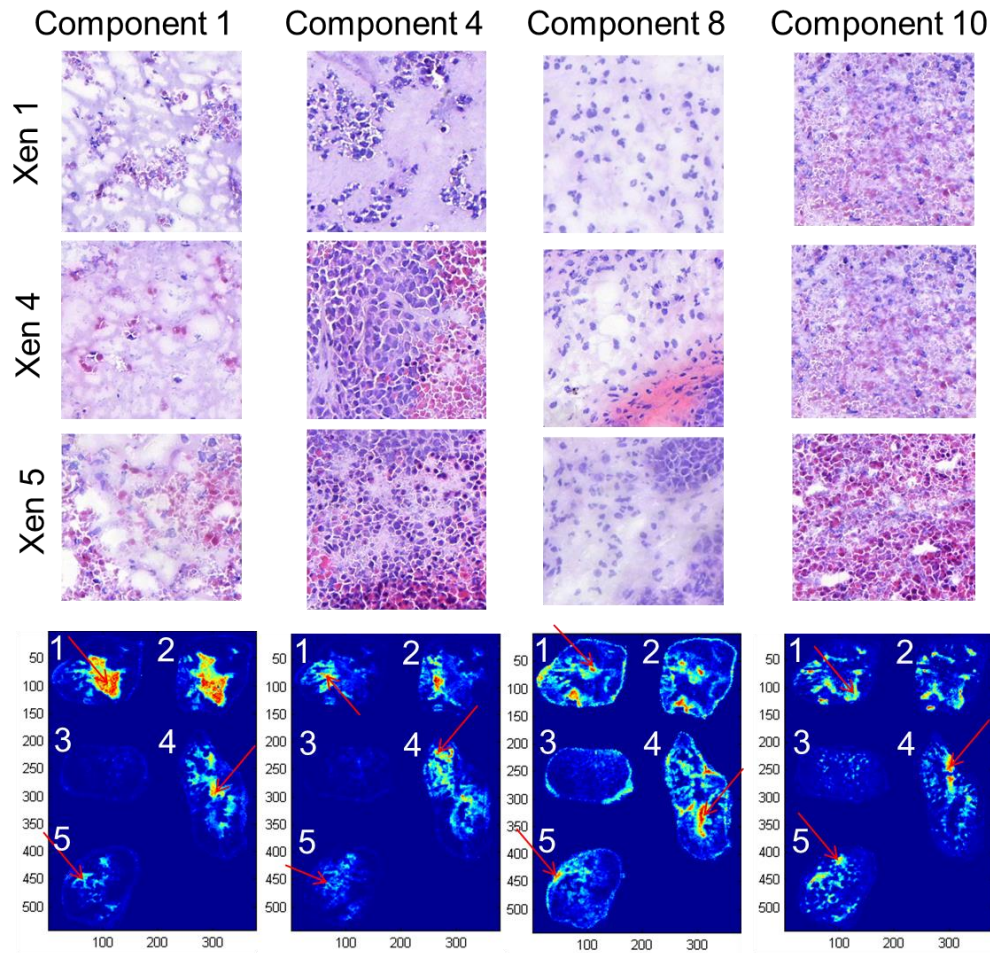
**Figure S6.** Positive ion images of near-isobaric ions observed at nominal mass  $m/z$  832 and 848. The ions indicated in red with the higher mass defect are tentatively assigned as the sodium and potassium adducts of GalCer(d18:1/24:1) while the ions in blue are tentatively assigned as sodium and potassium adducts of PC (38:4).



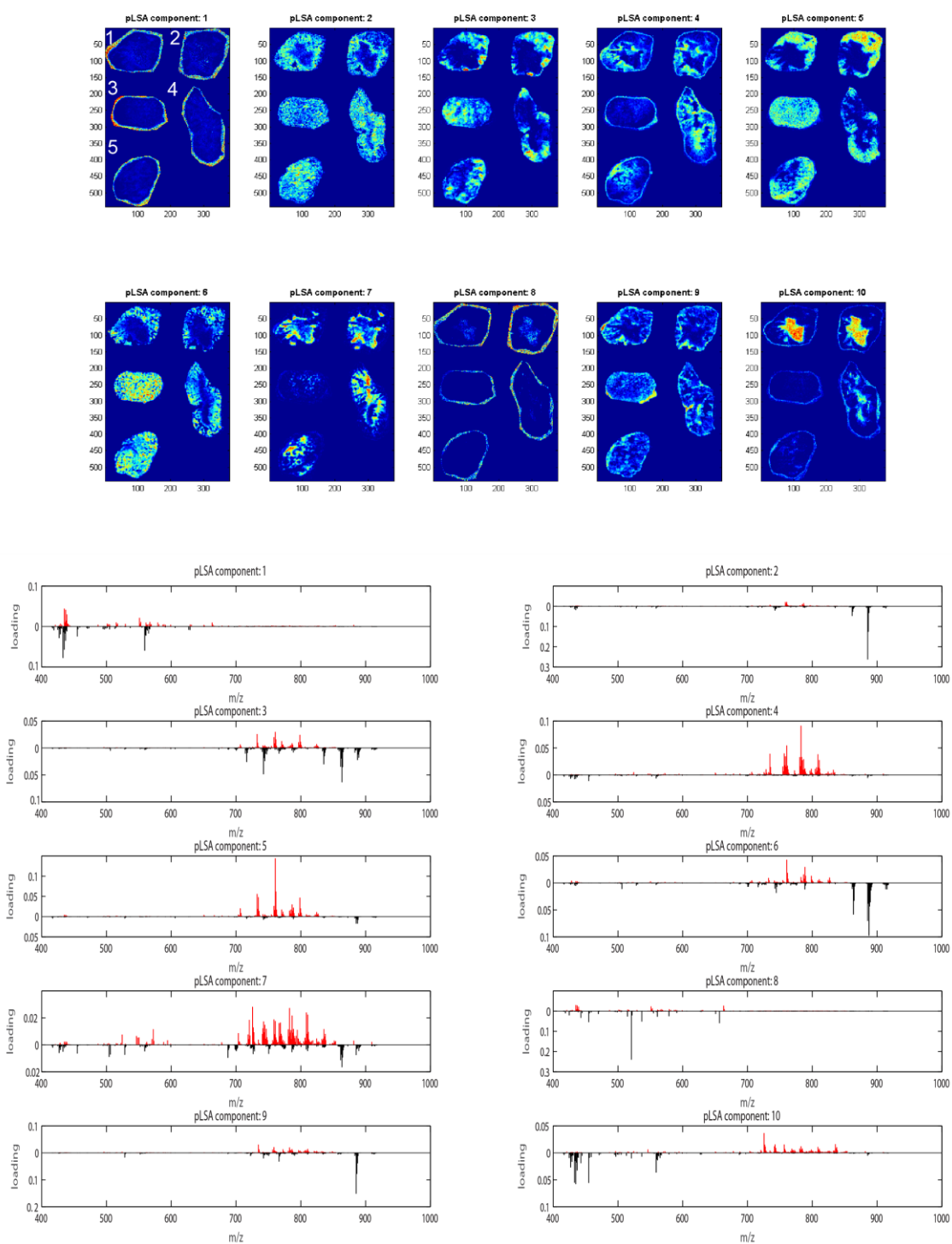
**Figure S7.** Positive ion pLSA component images from the xenografts (top) and the corresponding loadings spectra (bottom). Sections one and two are adjacent sections from the same xenograft. Note the signal observed in components 2 and 3 arise from matrix-related background adjacent to the tissue.



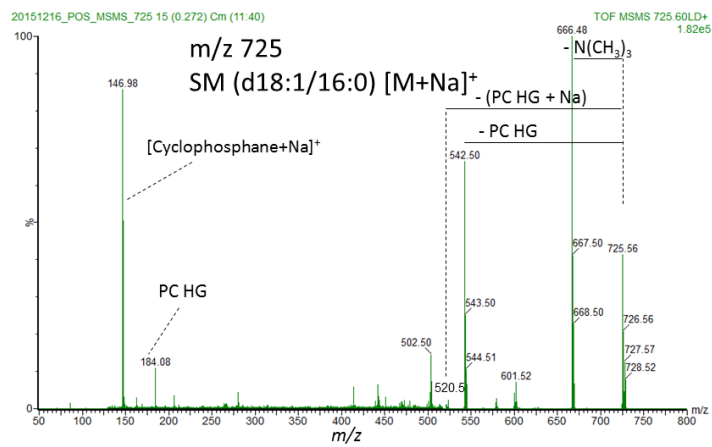
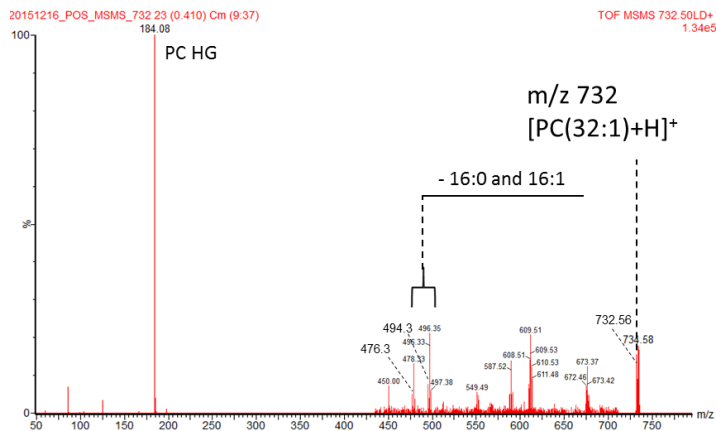
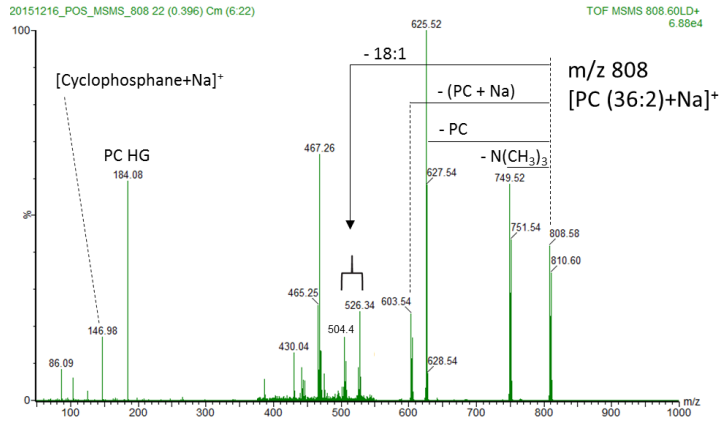
**Figure S8.** Negative ion pLSA component images from the xenografts (top) and the corresponding loadings spectra (bottom). Sections one and two are adjacent sections from the same xenograft. Note the signal observed in components 1 and 4 arise from matrix-related background adjacent to the tissue.

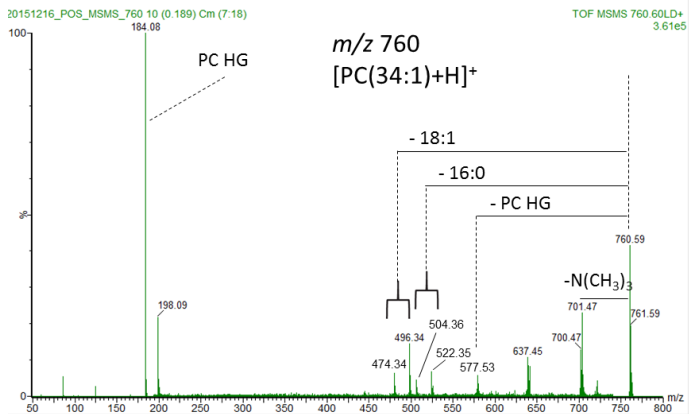
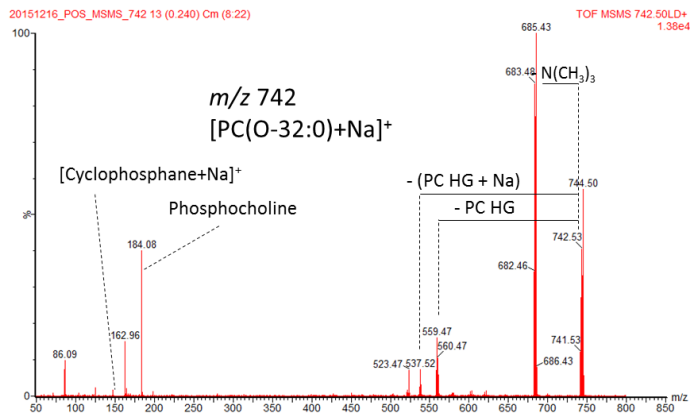
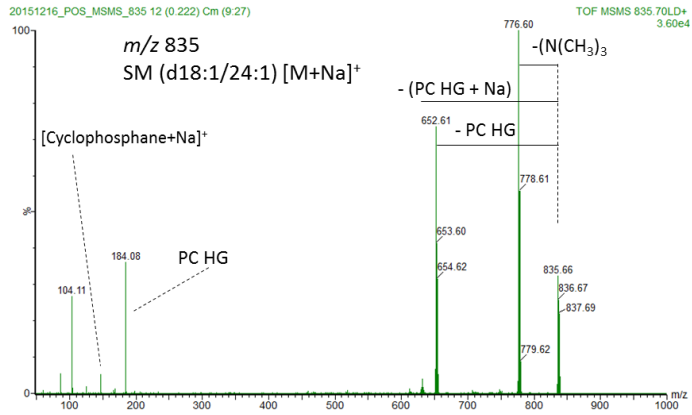


**Figure S9.** 5 selected pLSA component images generated from the positive ion dataset of the xenografts. Components correlate with histologically different tissue regions. Above each pLSA image are enlarged images of the H&E stained section from different xenografts that correspond in location to the regions indicated by the red arrows. Sections one and two are adjacent sections from the same xenograft.

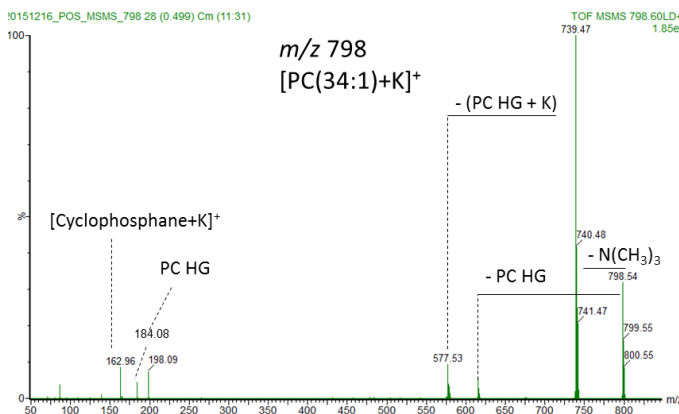
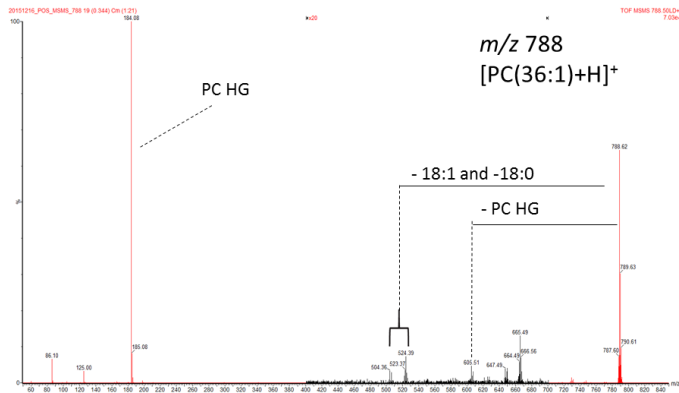


**Figure S10.** Combined positive and negative ion pLSA component images from the xenografts (top) and the corresponding loadings spectra (bottom). Positive ion contributions are indicated as red peaks and negative ions as black peaks. Sections one and two are adjacent sections from the same xenograft.

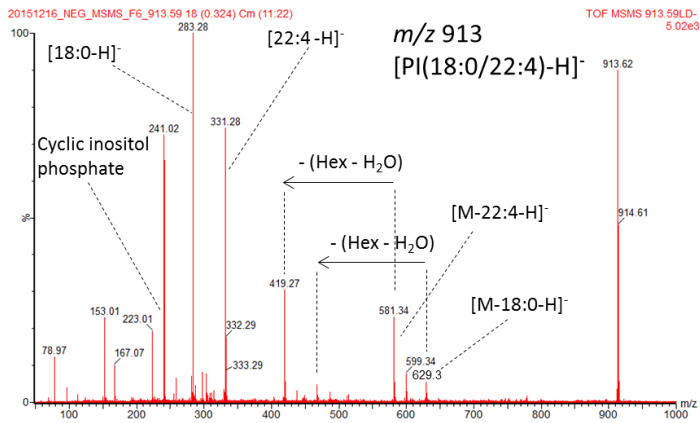
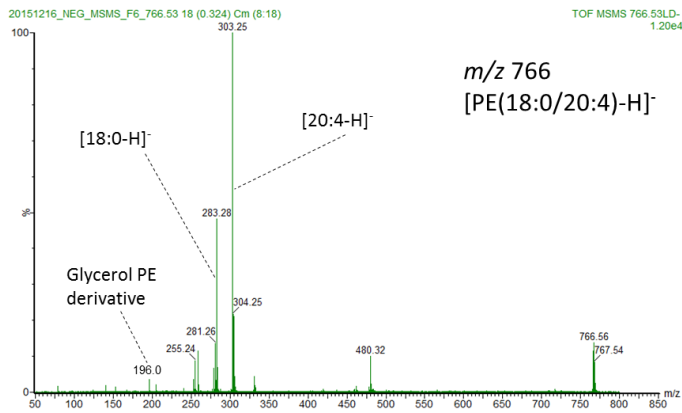
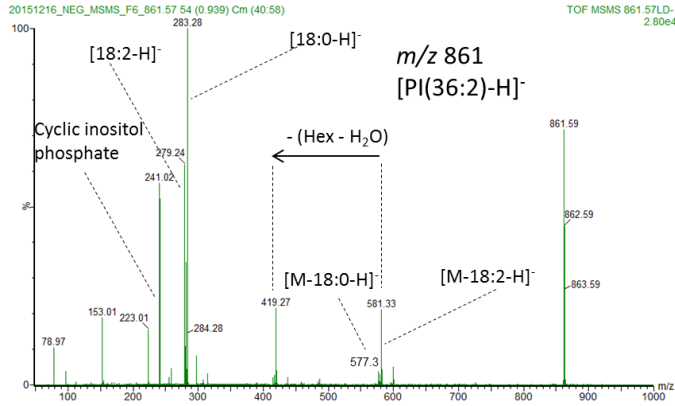


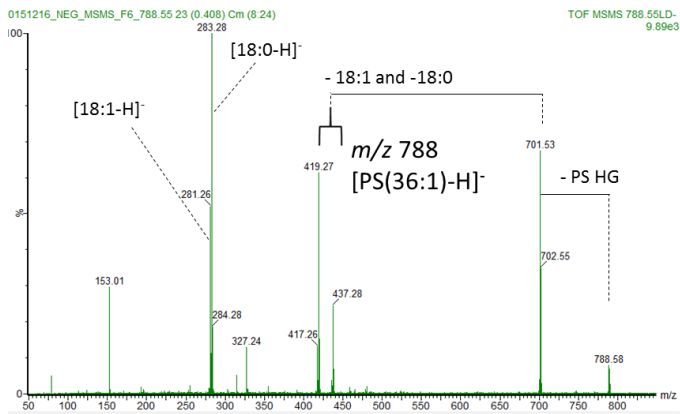
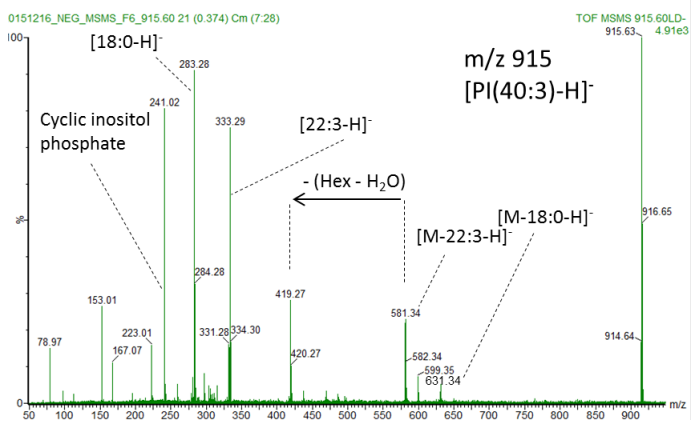
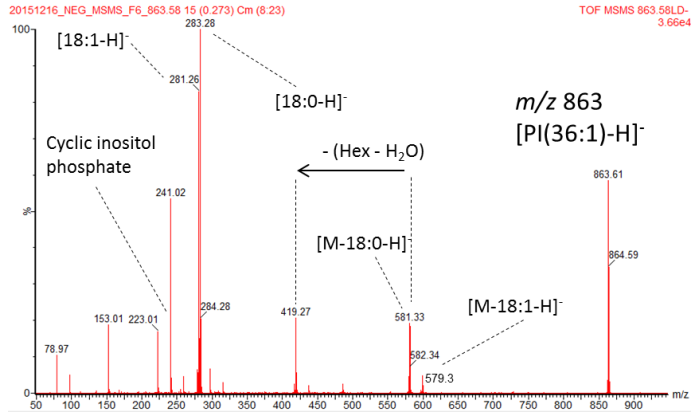


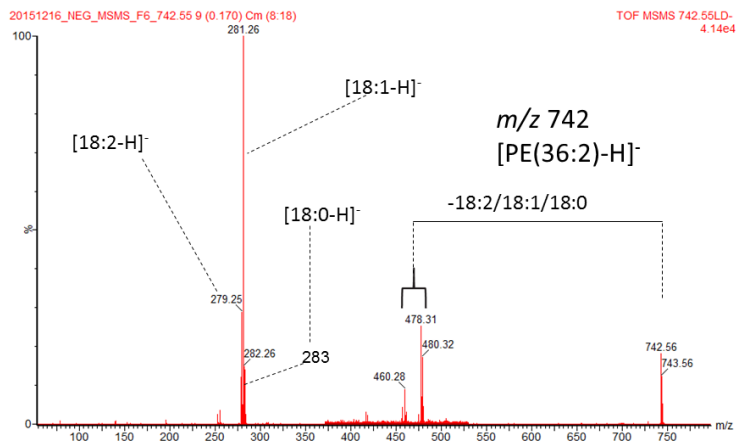




**Figure S11.** Positive ion MS/MS spectra acquired from the xenografts. Only the most dominant lipid assignment is provided. In many cases, due to both the complexity of the lipidome and the relatively wide (several Da) isolation window that was required to produce sufficient signal the isolation of more than one lipid structure is observed. Polyunsaturated fatty acids are assumed to be at the sn2 position. HG=headgroup







**Figure S12.** Positive ion MS/MS spectra acquired from the xenografts. Only the most dominant lipid assignment is provided. In many cases, due to both the complexity of the lipidome and the relatively wide (several Da) isolation window that was required to produce sufficient signal the isolation of more than one lipid structure is observed. Polyunsaturated fatty acids are assumed to be at the sn2 position. HG=headgroup

