Multimodal Chemical Imaging of a Single Brain Tissue Section using

ToF-SIMS, MALDI-ToF and Immuno/Histochemical Staining

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Supporting Information Figure S-1: A flow chart of multimodal imaging workflows of a single brain tissue section. A) Brightfield image of analyzed cerebellum region from a coronal rat brain tissue section. Multimodal imaging of coronal rat brain tissue section starts with B) dual polarity ToF-SIMS imaging of lipids followed by C) dual polarity MALDI-ToF imaging mass spectrometry of lipids using 1,5-DAN matrix applied via sublimation. Multimodal ToF-SIMS and MALDI-ToF Imaging mass spectrometry of a single brain tissue section can be followed by D) H&E staining, or E) specific histological staining (e.g. luxol fast blue and cresyl violet staining), or F) chromogenic immunohistochemical staining counter-stained with hematoxylin, or G) immunofluorescence and/or fluorescence staining.



Supporting Information Figure S-2: Representative ToF-SIMS spectra in negative ion mode A) in a mass range of m/z 0-900, B) m/z 200-450, and C) m/z 600-900 obtained from cerebellum of coronal rat brain tissue section.



Supporting Information Figure S-3: Representative ToF-SIMS spectra in positive ion mode A) in a mass range of m/z 0-900, B) m/z 200-450, and C) m/z 600-8500 obtained from cerebellum of coronal rat brain tissue section.



Supporting Information Figure S-4: Representative MALDI-ToF-MS spectra in negative ion mode A) in a mass range of *m/z* 0-2000, B) *m/z* 600-1000, and C) *m/z* 200-450 obtained from cerebellum of coronal rat brain tissue section.



Supporting Information Figure S-5: Representative MALDI-ToF-MS spectra in positive ion mode A) in a mass range of m/z 0-2000, B) m/z 600-1000 and, C) m/z 200-450 obtained from cerebellum of coronal rat brain tissue section.