Electronic Supplementary Material (ESI) for Analyst.

Thermal evaporation as sample preparation for silver-assisted laser desorption/ionization mass spectrometry imaging of cholesterol in amyloid tissues

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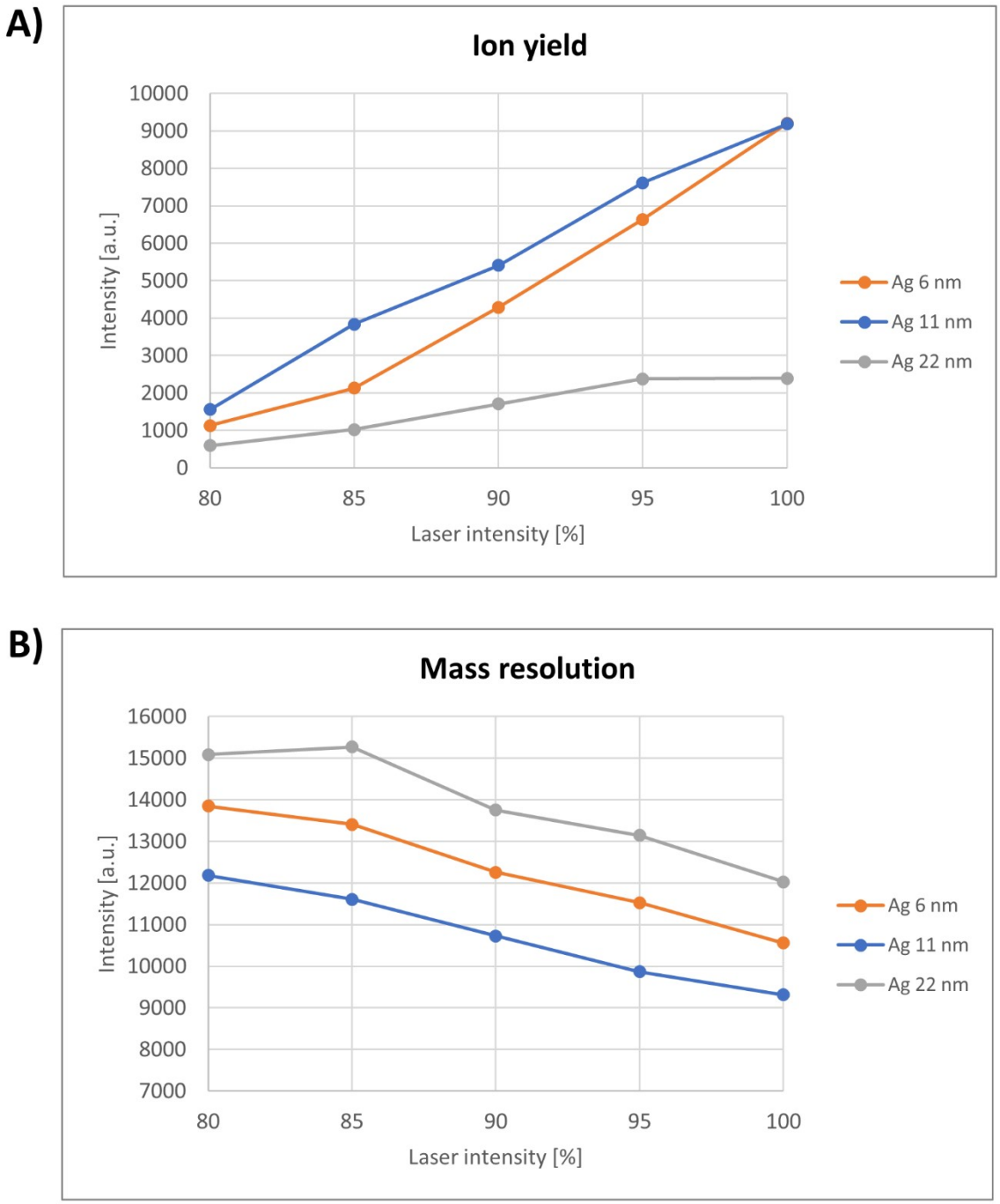
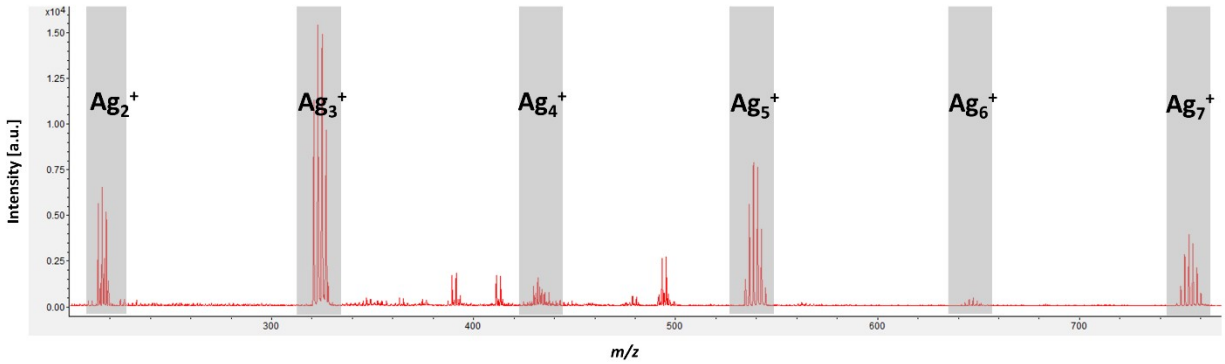
Supplementary Tab. 1. Thermal evaporation settings.

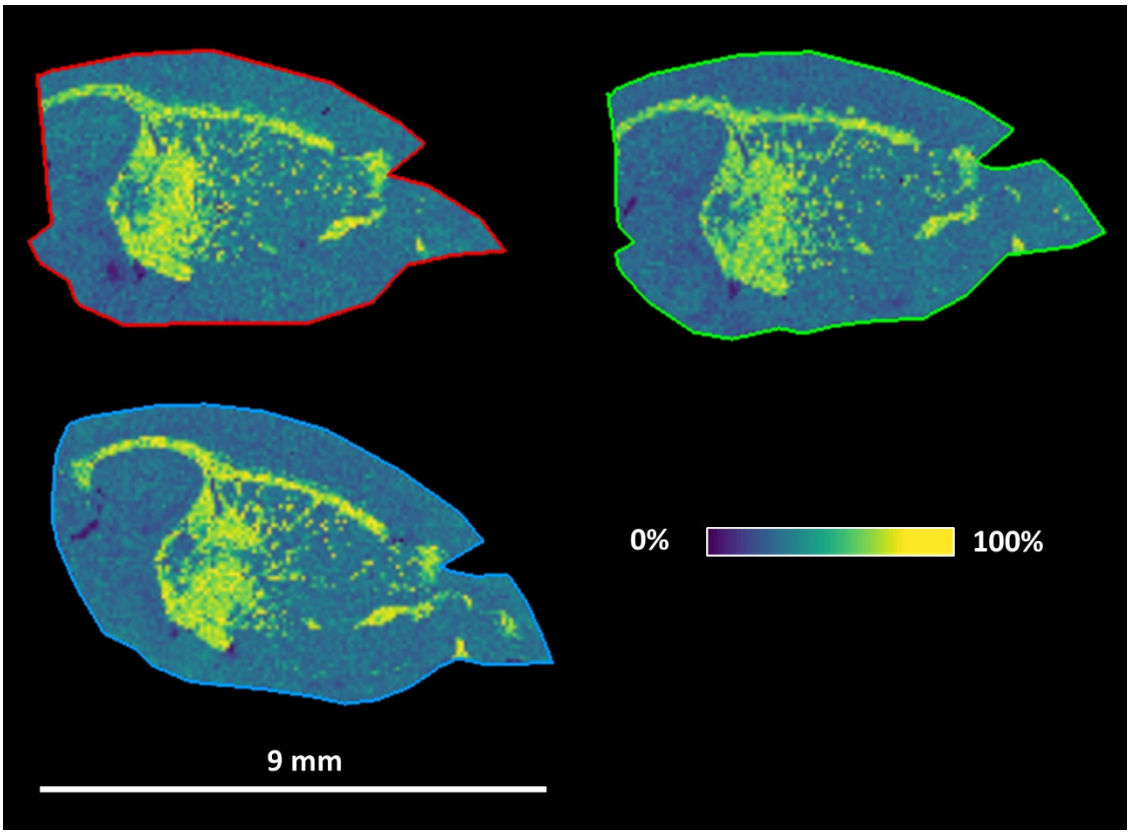
Name	Value	Units
Rise Time 1	00:30	mm:ss
Soak Power 1	10.0	%
Soak Time 1	00:30	mm:ss
Rise Time 2	00:30	mm:ss
Soak Power 2	15.0	%
Soak Time 2	00.20	mm:ss
Idle Ramp Time	00.30	mm:ss
Rate	5.000	Å/s
Final Thickess	0.060 or 0.11 or 0.22	kÅ
Thickess Set Point	0.060 or 0.11 or 0.22	kÅ
Control Gain	10	Å/s/%



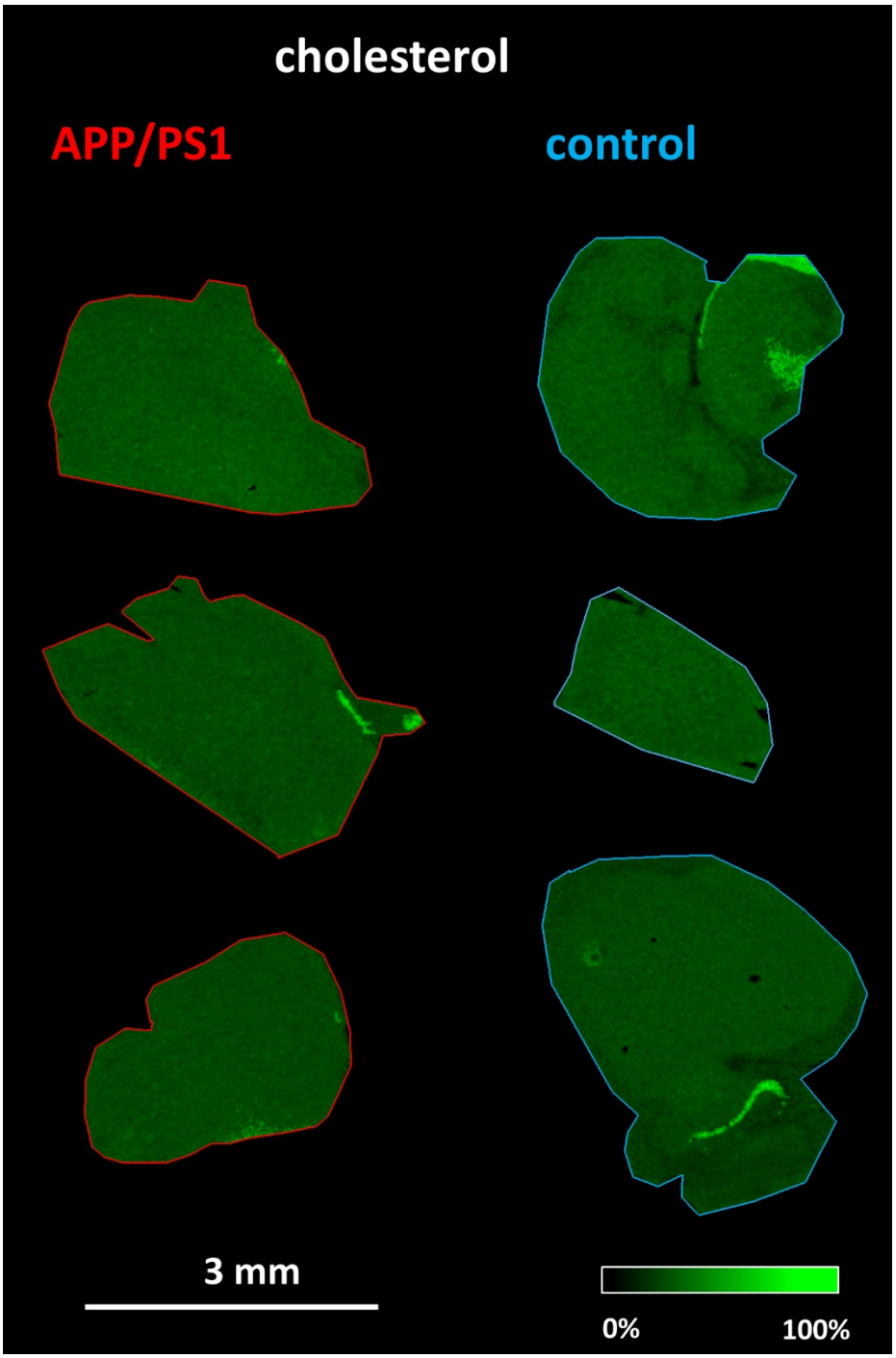
Supplementary Fig. 1. A) Ion yield and B) mass resolution plots as a function of laser intensity for different thicknesses of Ag. Cholesterol at m/z 493.2 $[M + {}^{107}Ag]^+$. Average of 5 spectra from AgLDI of a mouse brain cortex region.



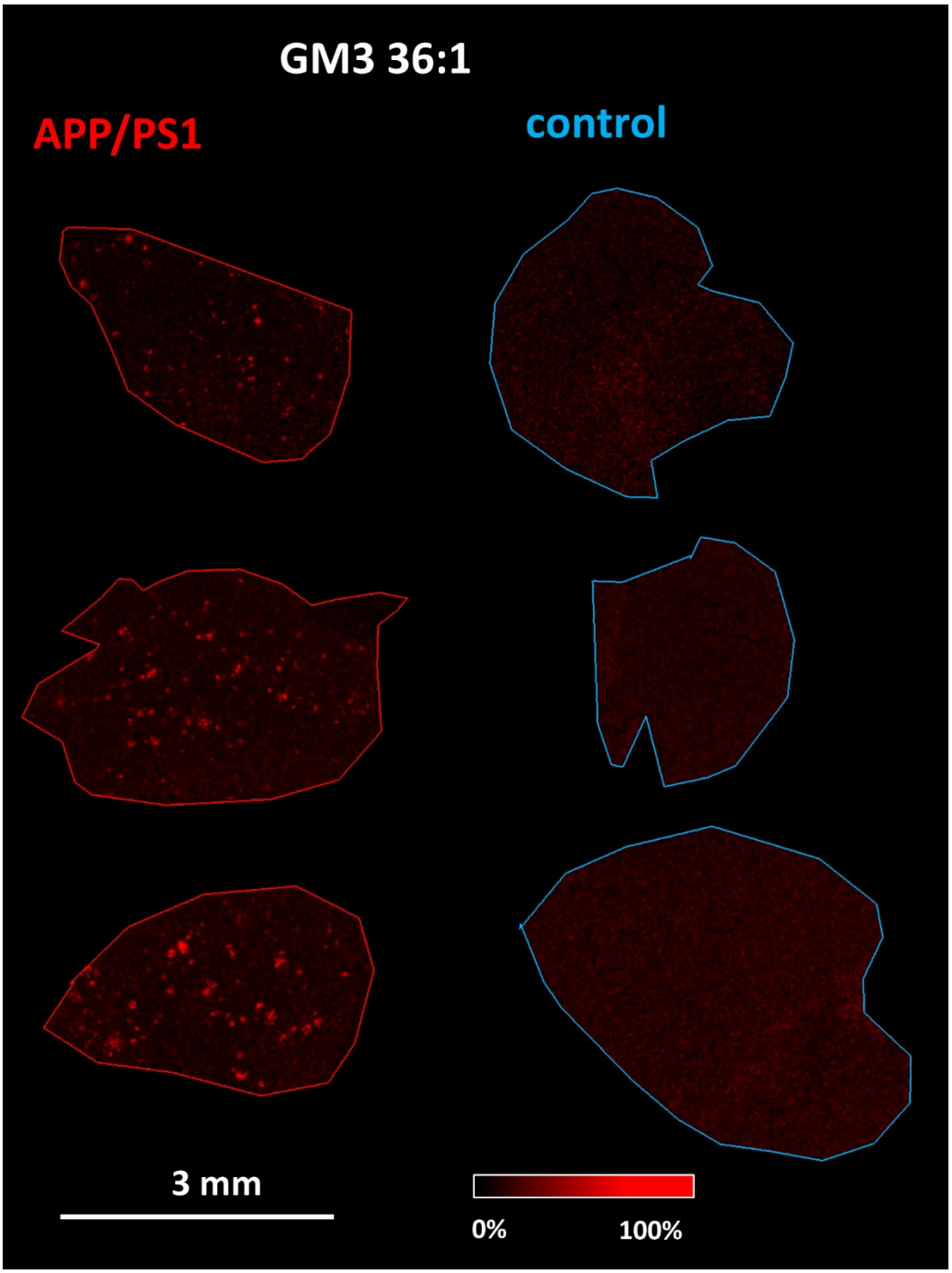
Supplementary Fig. 2. Representative spectra from AgLDI of a mouse brain section in the cortex region in positive ion mode. Section with Ag film with a thickness of 22 nm deposited using thermal evaporation showing various silver clusters.



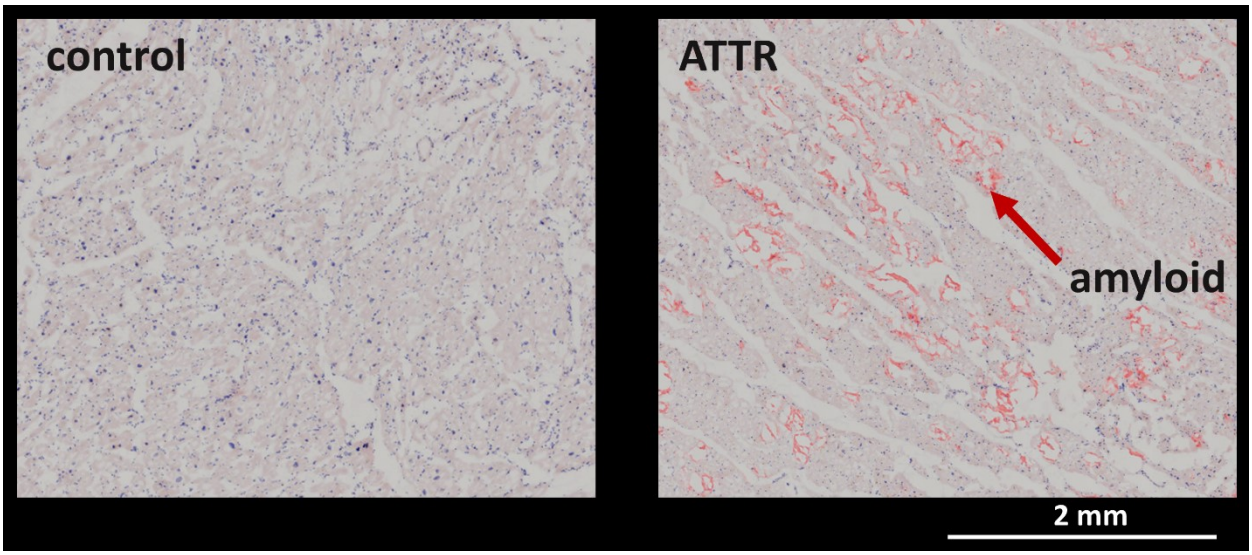
Supplementary Fig. 3. Reproducibility of the sample preparation process and analysis. AgLDI MSI (Ag 6 nm) of three consecutive sections of a single brain. Distribution of cholesterol at *m/z* 493.2 $[M + {}^{107}Ag]^+$ using a spatial resolution of 70 µm.



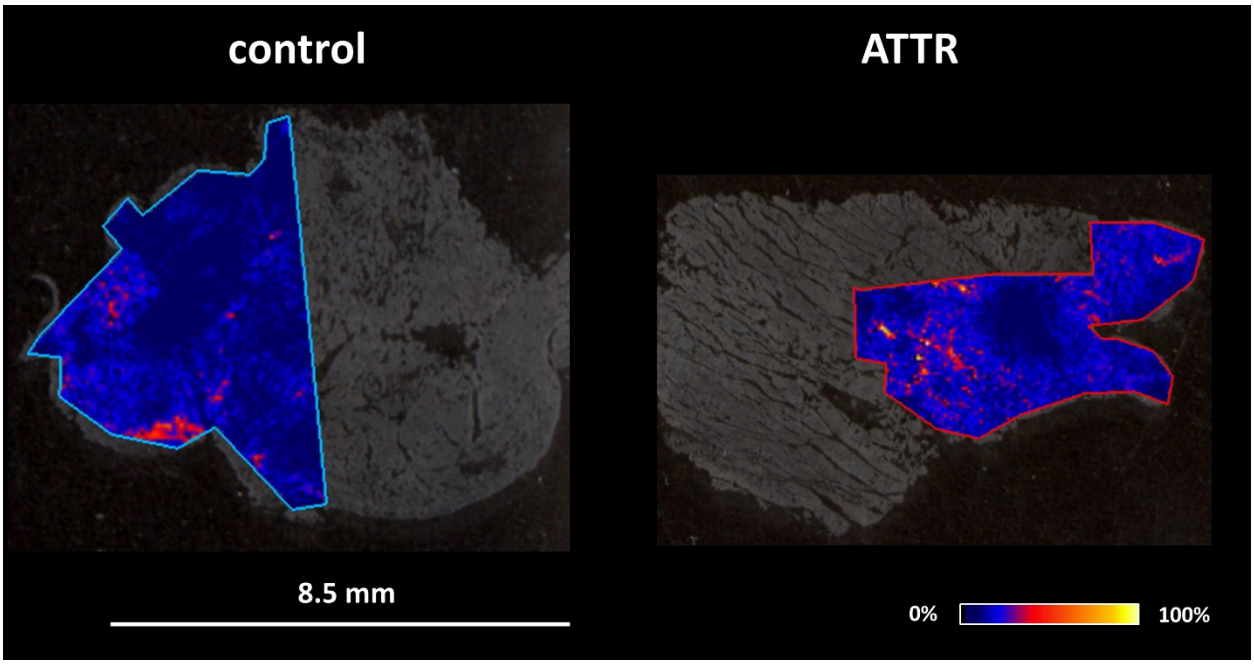
Supplementary Fig. 4. AgLDI MSI (Ag 6 nm) analysis of a frontal cortex section (APP/PS1 mice and age-matched WT controls). Ion images of cholesterol (green, *m/z* 493.2, $[M + {}^{107}Ag]^+$) at a spatial resolution of 20 µm.



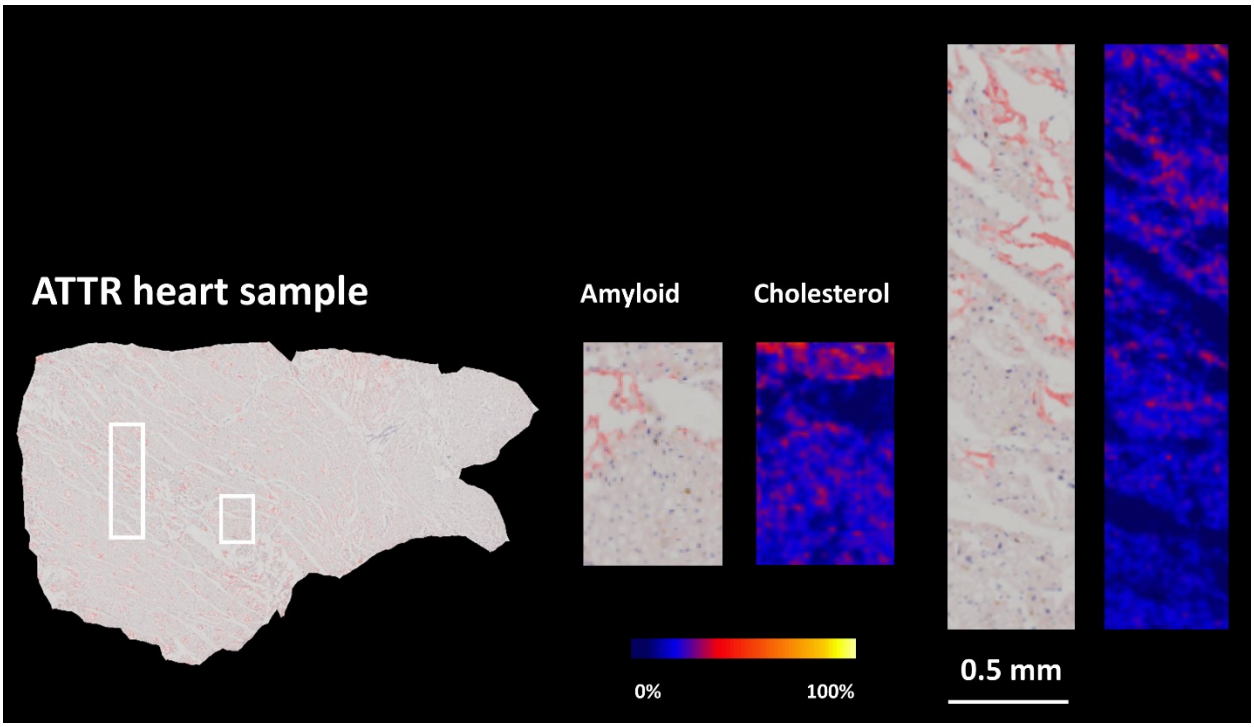
Supplementary Fig. 5. MALDI MSI (Ag 6 nm) analysis of a frontal cortex section (APP/PS1 mice and age-matched WT controls). Ion images of ganglioside (red, *m/z* 1179.7 $[M - H]^-$) at a spatial resolution of 20 μm.



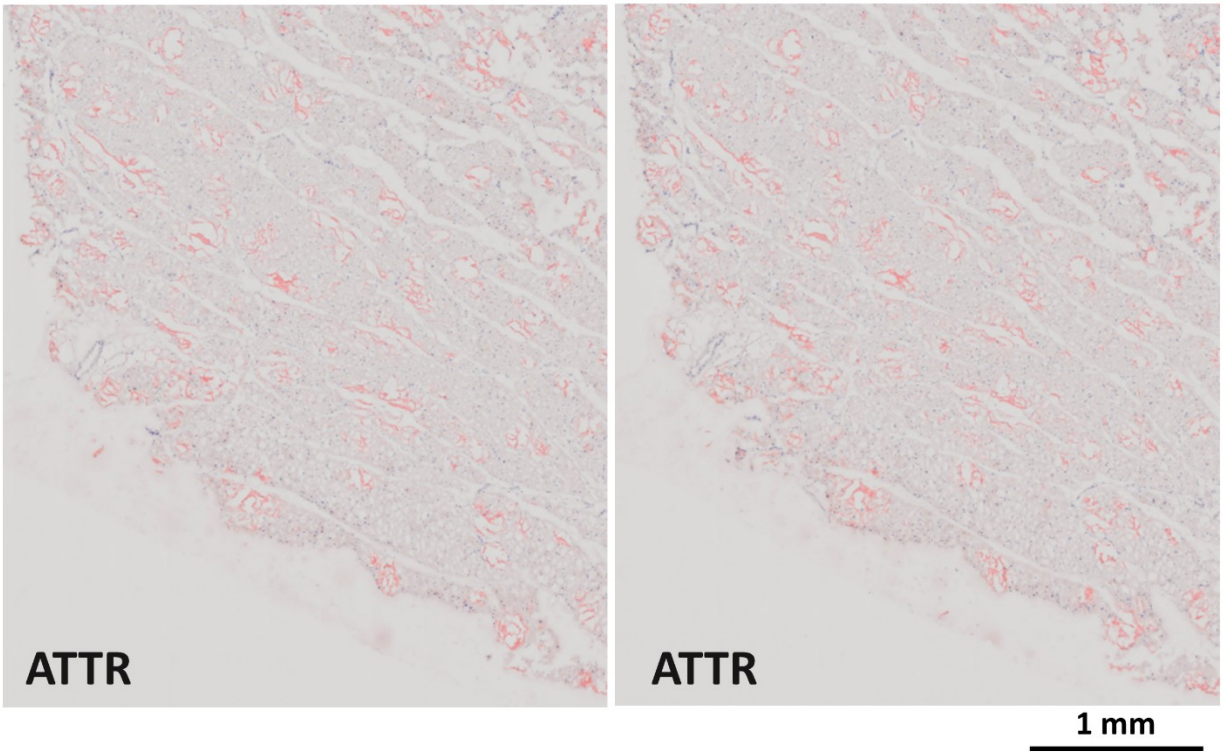
Supplementary Fig. 6. Congo red amyloid staining. Images of a control myocardial section (left) and an ATTR-affected myocardial section (right) under a light microscope.



Supplementary Fig. 7. AgLDI MSI (Ag 6 nm) analysis of control (left) and ATTR-affected (right) myocardial section. Ion image cholesterol (m/z 493.2, $[M + {}^{107}Ag]^{+}$) at a spatial resolution of 20 μm.



Supplementary Fig. 8. Congo red amyloid staining and AgLDI MSI (Ag 6 nm) analysis of ATTR-affected myocardial section. Ion image cholesterol (m/z 493.2, $[M + {}^{107}Ag]^{+}$) at a spatial resolution of 10 μm.



Supplementary Fig. 9. Congo red amyloid staining. Images showing non-serial sections of an ATTR-affected myocardial section under a light microscope. The serial sections in between were analyzed by AgLDI.