Supplementary Information

Nano-infrared imaging of primary neurons

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Keywords: O-PTIR; s-SNOM; neuron; Amyloid-beta; Alzheimer's disease

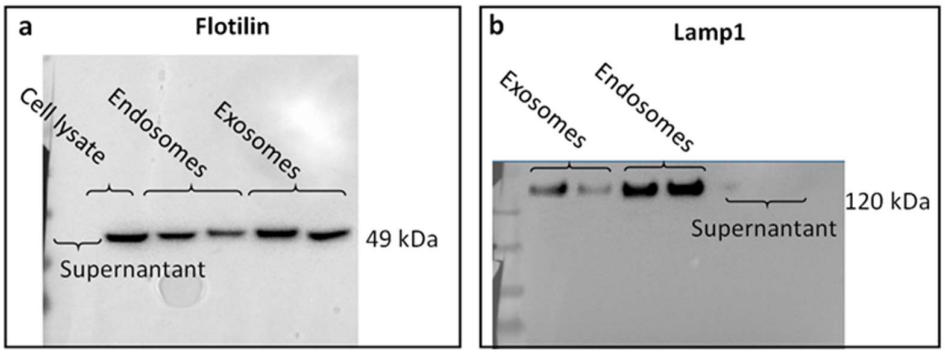

Figure S1. Characterization of exosomal preparation. Representative Western blots of cell lysate, supernatant, and fractions containing exosomes and endosomes are shown. (**a**) Representative Western blot with flotillin, a marker of exosomes. (**b**) Representative Western blot with Lamp1, a marker of endosomes.

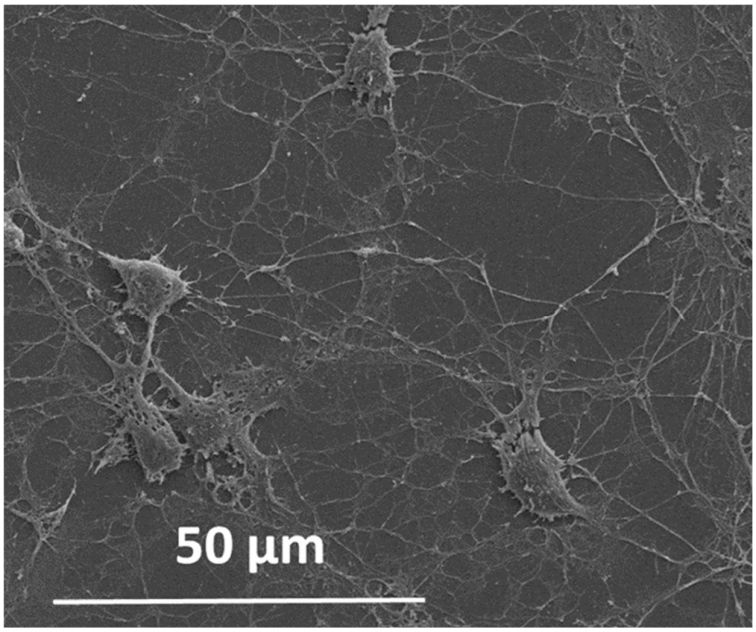

Figure S2. Sample characterization**.** Primary neurons were seeded on 100 nm gold-coated chips. After 19 days of growth and maturation, neurons were fixed with 4% paraformaldehyde in phosphate buffer saline for 15 min at room temperature, washed, and air-dried. Examining the neurons using surface scanning electron microscopy confirmed that air-drying did not affect the quality of the sample.

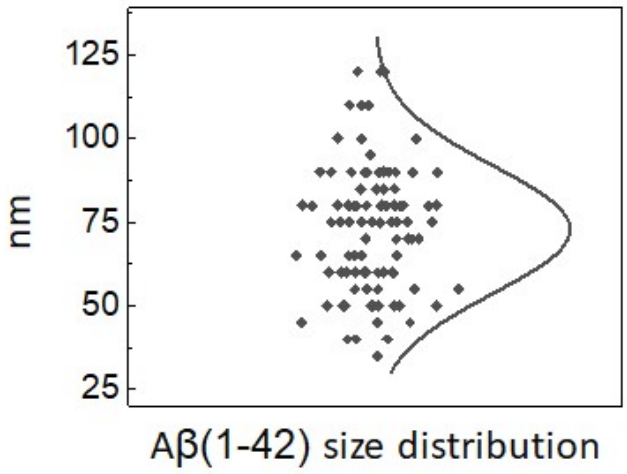

Figure S3. Aβ-size distribution. To calibrate STED micro-scope we used 20 nm fluorescent beads, Ø = 25 nm. The plot represents size distribution of Aβ(1–42) particles measured by S TED.

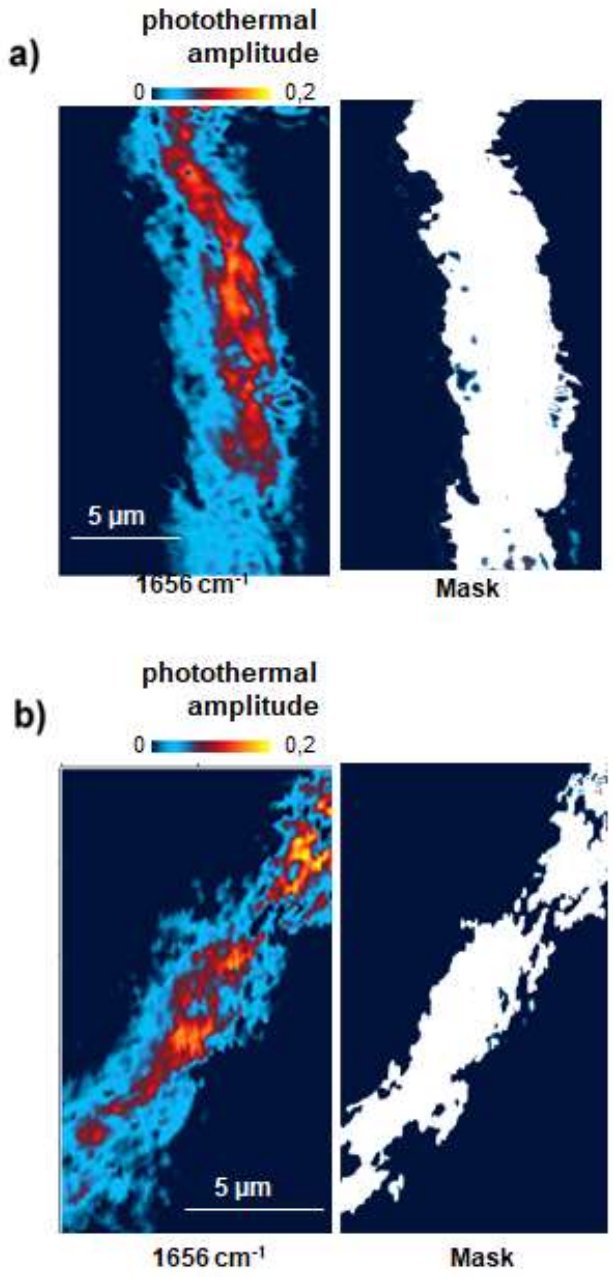

Figure S4. Mask rendering. O-PTIR maps were acquired from wild-type (**a**) and AD transgenic neurons (**b**). Blue-yellow gradient indicates elevations of the intensities at 1650 cm^{-1}. Right panels: asks which were rendered using threshold set to 0.03 a.u. of photothermal amplitude of 1650 cm^{-1} map.

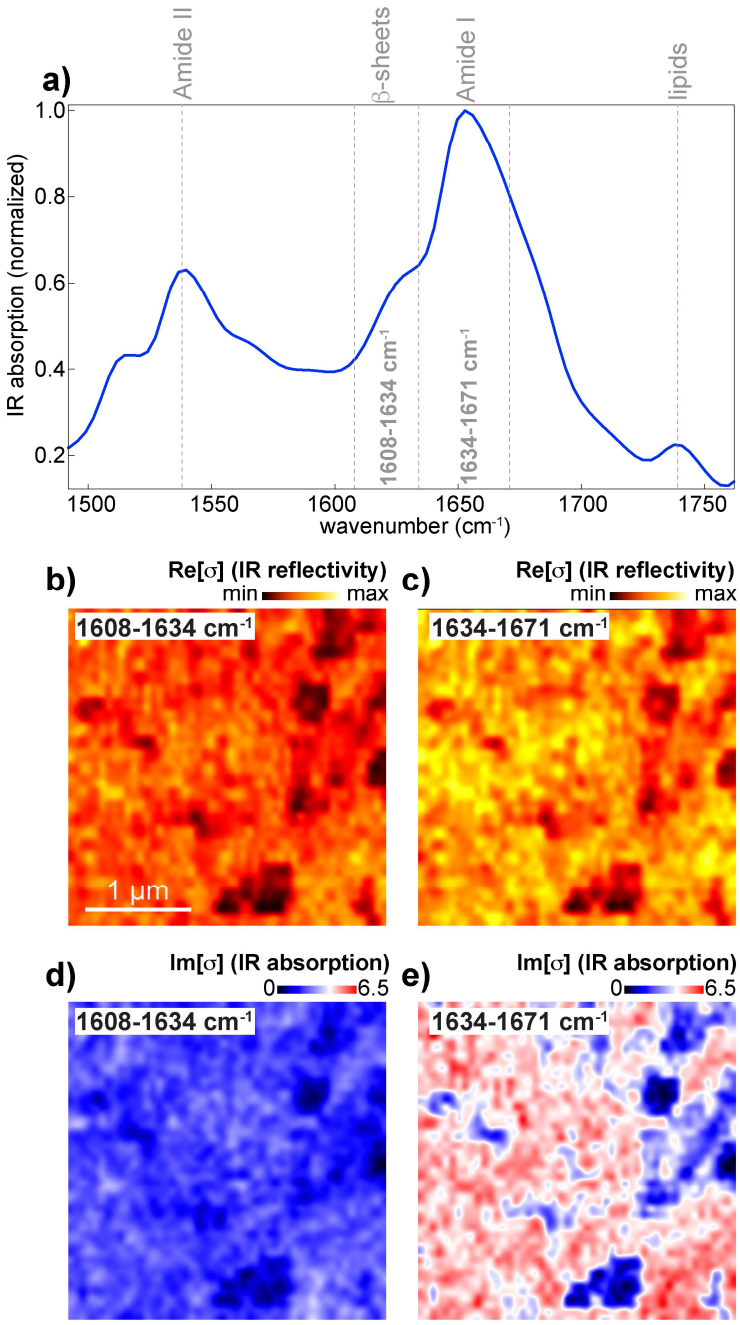

Figure S5. Example of s-SNOM hyperspectral nanoimaging. (**a**) Point spectrum from an arbitrary pixel of the HS map indicating the spectral ranges 1608–1634 cm^{-1} and 1634–1671 cm^{-1} used for nanoimaging analysis. (**b–c**) Nanoscale IR reflectivity integrated across the spectral ranges 1608-1634 cm^{-1} and 1634-1671 cm^{-1}, respectively, reconstructed from a 3 × 3 μm^2 HS map of wild-type neurons. (**d–e**) Nanoscale IR absorption images integrated within the spectral ranges 1608–1634 cm^{-1} and 1634–1671 cm^{-1}, respectively.