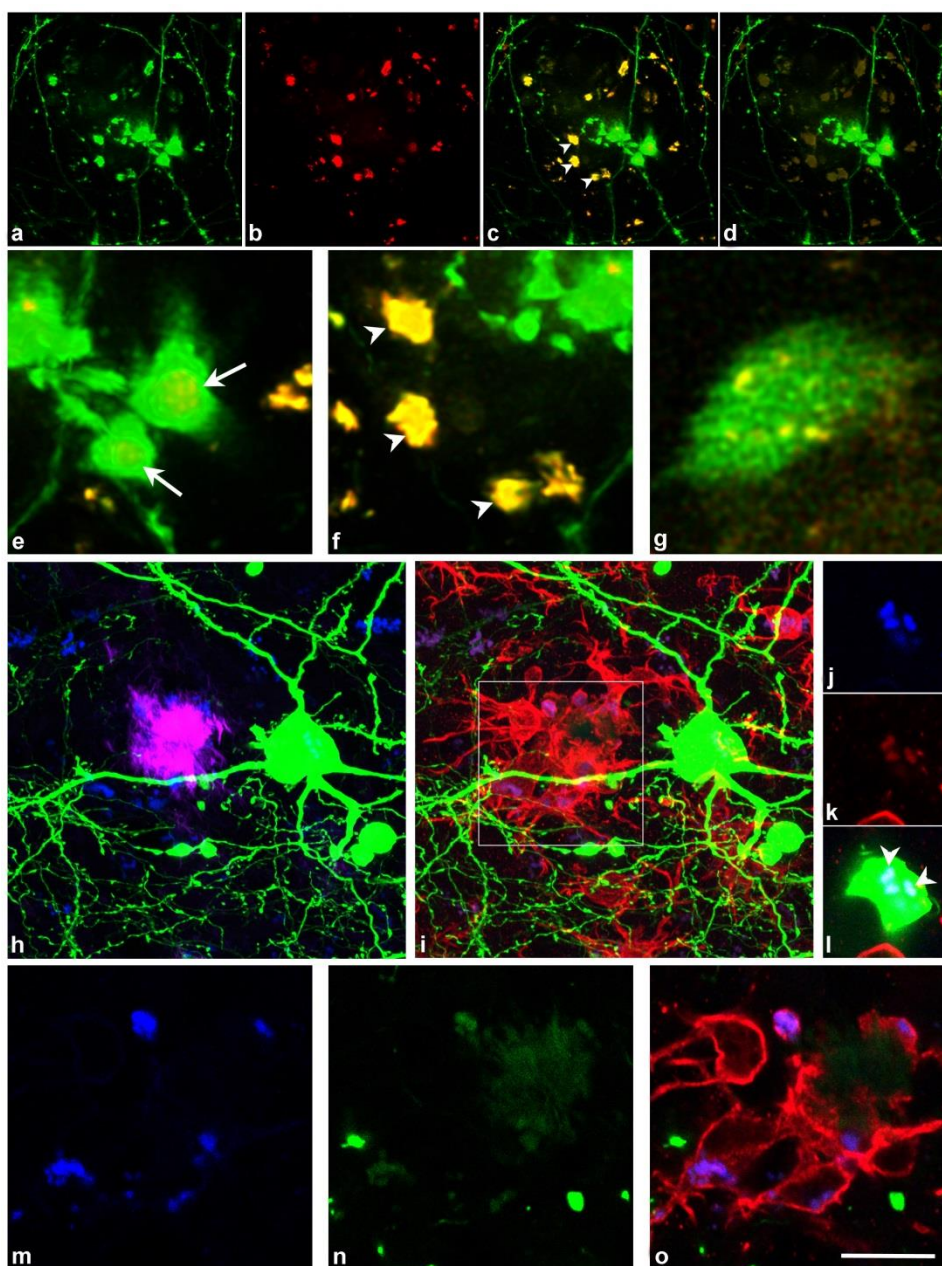


SUPPLEMENTARY FIGURE 1

High plasticity of axonal pathology in Alzheimer's disease mouse models

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Supplementary Fig. 1 Location of auto-fluorescent spots. (a–d), Two-photon *in vivo* images of the same GFP-expressing AxD (dys 1) around an A β plaque stained with Methoxy-X04 (blue) in the somatosensory cortex of the dE9 mouse (the same AxD is shown in Fig. 2). Two-photon excitation of GFP-expressing neuronal structures was performed at 880 nm and the signal was detected using a bandpass (BP) 500–550 nm filter (a). To exclude false positive fluorescent spots from the analysis, we also recorded emissions at 590–650 nm (b). By combining both images, the auto-fluorescent spots can be visualized as yellow structures (arrowheads, the same as in f) (c). When necessary, Photoshop was used to facilitate the visualization of GFP-expressing structures by manually darkening those auto-fluorescent structures that were alone in the neuropil and not inside any GFP-expressing structure (d). (e–g), Higher magnification of two-photon *in vivo* images showing that the auto-fluorescent spots can be inside GFP-expressing AxDs (arrow, E), in the neuropil (arrowheads, f) and inside neuronal cell bodies (lipofuscin, g). (h–o), Immunohistochemistry for Iba-1 (red) was performed to better define the presence of the auto-fluorescence. (h), Maximum projection of images (44 images, $z = 0.4 \mu\text{m}$) showing a GFP-expressing pyramidal neuron and numerous neuronal processes (green) near an A β plaque stained with Methoxy-X04 (magenta). The far-red fluorescence was also recorded to facilitate the visualization of auto-fluorescent spots (blue). (i), Same stack of images and field of view as in h adding the fluorescence recorded for Alexa 594 (immunofluorescence of Iba-1, red) and excluding the fluorescence recorded for Methoxy-X04 (A β plaque, magenta). (j–l), Single optical section showing a higher magnification of the neuronal body of the pyramidal neuron in (h, i). Auto-fluorescent spots (recorded with far-red fluorescence (j), Alexa 594 (k) and GFP channel (l)) are inside the neuronal cell body (lipofuscin (white), arrowheads) (l). (m–o), Single optical section showing a higher magnification of the region in i that was delimited by a square. Auto-fluorescent spots (arrowheads, recorded with far-red (m), GFP (n) and Alexa 594 channel (o)) are enclosed within microglial processes (red) (o). Scale bar (in o): 25.5 μm in a–d; 5.2 μm in e–g; 12.5 μm in h, i; 6 μm in j–l; 6.3 μm in m–o