



Figure 1 - figure supplement 1. Antibodies used in this study labeled the expected structures as assessed by IF volume reconstruction and SEM imaging. A. MBP immunofluorescence on a single ultrathin section (70 nm) with a 10 μm scale bar. B. High-magnification view of a single axon with a 1 μm scale bar. C. High-magnification view of a single axon with a 1 μm scale bar. D. 3D reconstruction of GABA (red), MBP (grey), and DAPI (blue) with a 10 μm scale bar. E. 3D reconstruction of GABA (red), MBP (blue), Tubulin (green), GS (orange), and DAPI (purple) with a 10 μm scale bar.

nm) from layer 5 of the adult mouse cortex. Interruption in the MBP staining indicates a node of Ranvier (magenta box) B. MBP staining corresponds exactly to the myelin sheath as seen in the SEM of the axon boxed in green in A. C. Nodes of Ranvier (magenta box in A) do not stain for MBP, but the axonal path can be traced using cytoskeletal markers which persist through the nodes (alpha tubulin in red). D. Volume reconstruction of myelinated axons immunolabeled with MBP (white) and GABA (red) in cortical layer 5 (43 sections, 70 nm each). Nuclei are stained with DAPI (blue). SEM of layer 5 of mouse cortex overlaid with immunofluorescence for GABA (red), MBP (cyan), α tubulin (green), glutamine synthetase (orange).