Supplementary Material for *In vivo* imaging of myelin in the vertebrate central nervous system using third harmonic generation microscopy

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Supplementary Text

The appearance of "holes" in THG images of white matter tracts in the spinal cord was due to the presence of axons

Transgenic mice expressing YFP in a subset of pyramidal neurons and DRGs were perfused and the spinal cord was sectioned. THG and 2PEF imaging were performed simultaneously. Some of the "holes" in the THG image of myelin are seen to correspond to fluorescently labeled axon tips (Fig. S1), suggesting that the "holes" in the myelin seen in Fig. 1b and 1c are axons viewed on axis.

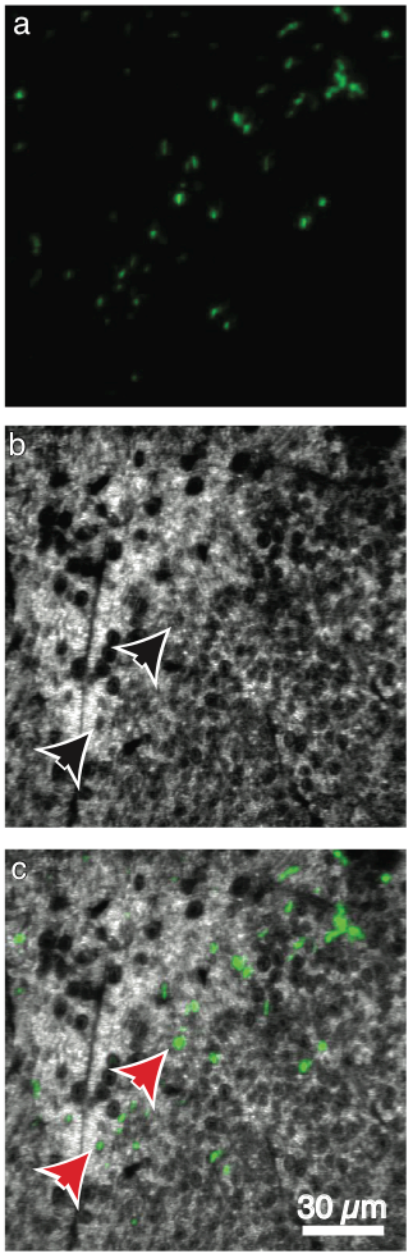

Figure S1: Apparent holes in THG images of the dorsal corticospinal tract were axons seen on-axis. Fixed sections of spinal cord from mice expressing YFP in a subset of pyramidal neurons and DRG neurons were imaged using 2PEF and THG simultaneously. The axons (a) were seen on-axis perpendicular to the cut, and appear as dark holes (*black arrows*) in the THG image (b). Overlaying the images revealed some axons (*red arrows*) in the myelin relief (c), indicating that the holes were axons or axon bundles. Because this is a 2D-projection, not all axons have a corresponding visible hole.