nature neuroscience

SUPPLEMENTARY INFORMATION

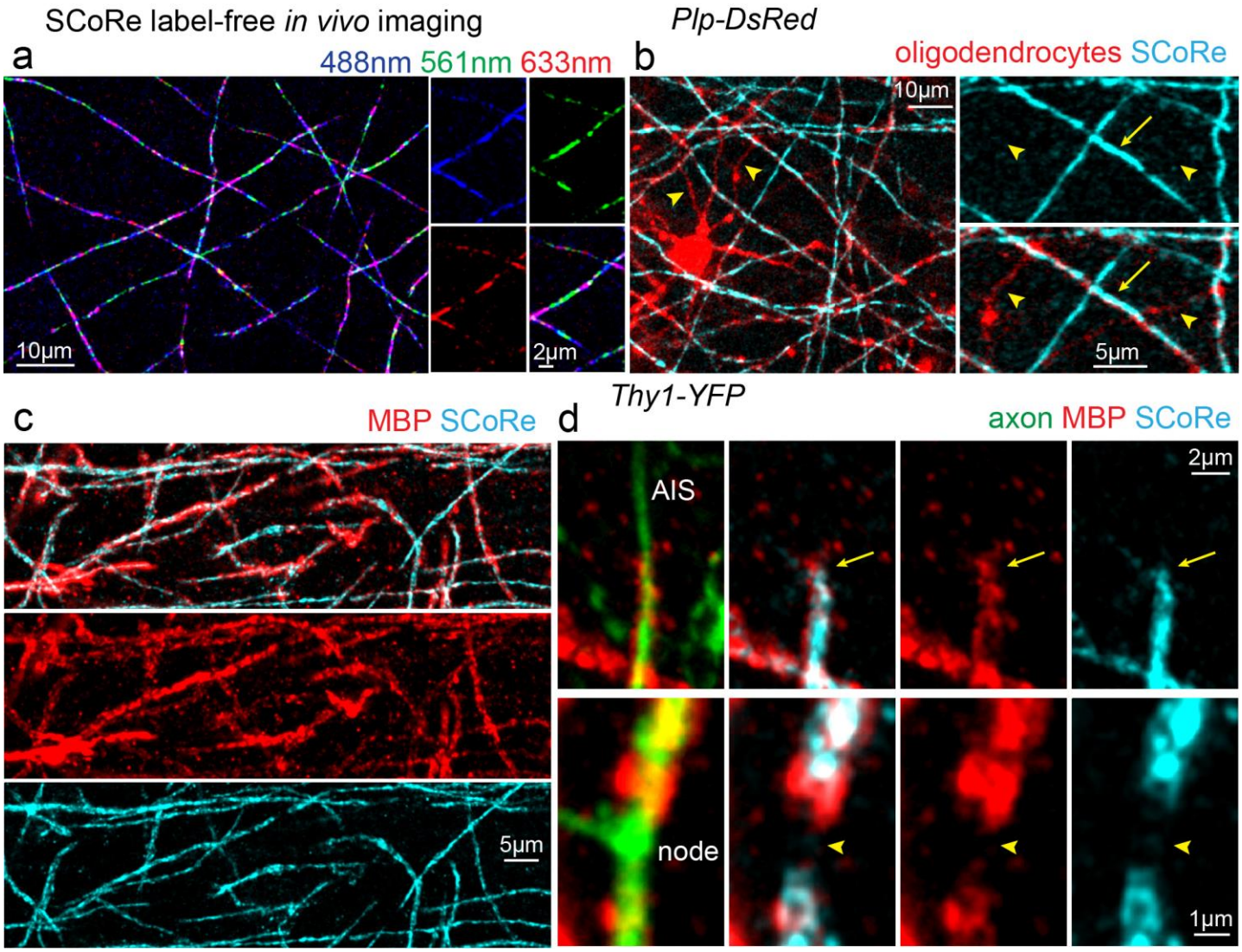
https://doi.org/10.1038/s41593-018-0120-6

In the format provided by the authors and unedited.

Lifelong cortical myelin plasticity and age-related degeneration in the live mammalian brain

Robert A. Hill[1]*, Alice M. Li[1] and Jaime Grutzendler[1,2]*

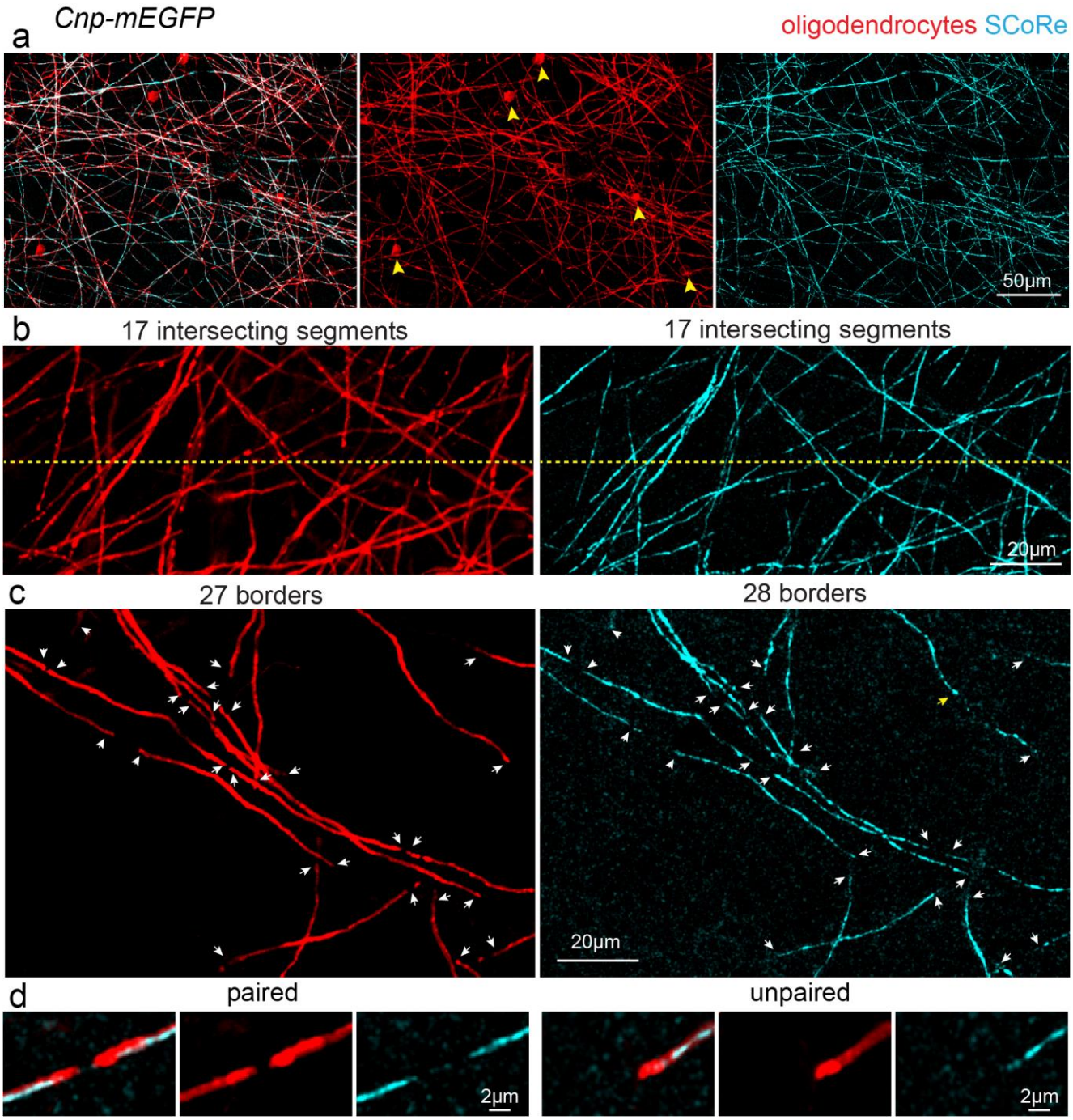
[1]Department of Neurology, Yale School of Medicine, New Haven, CT, USA. [2]Department of Neuroscience, Yale School of Medicine, New Haven, CT, USA.
*e-mail: robert.hill@yale.edu; jaime.grutzendler@yale.edu



Supplementary Figure 1

Spectral confocal reflection (SCoRe) microscopy allows label-free myelin imaging in vivo and in fixed tissues.

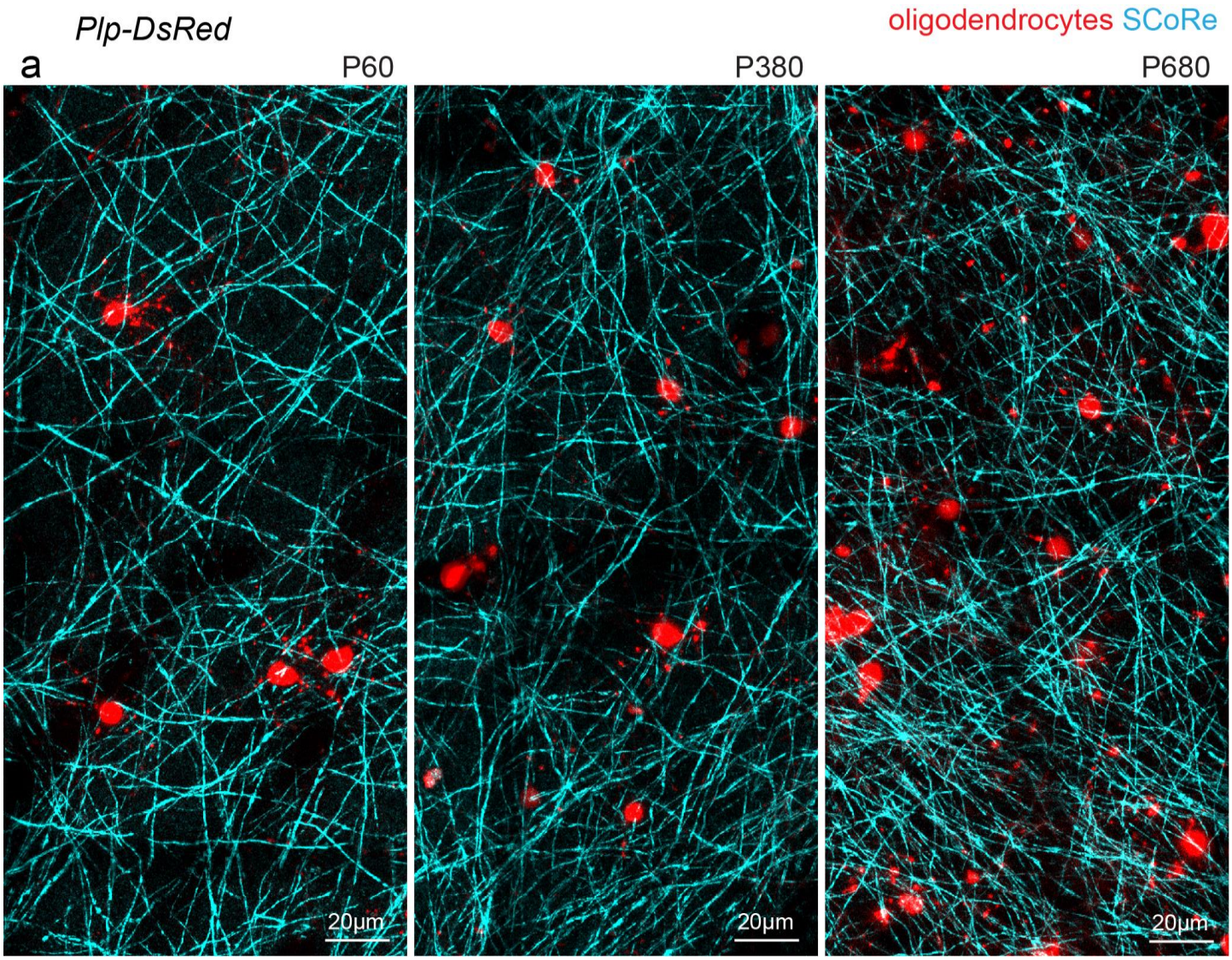
(**a**) In vivo image captured from layer I of the mouse somatosensory cortex showing the separated reflective wavelengths of single myelin fibers using SCoRe microscopy. All other SCoRe images were combined into a single color. (**b**) In vivo image of an oligodendrocyte in the cortex of a transgenic mouse with DsRed fluorescent protein expressed exclusively in mature oligodendrocytes (*Plp-DsRed*) showing the specificity of the SCoRe signal for portions of the oligodendrocyte forming compact myelin (arrows) and not the proximal processes extending from the oligodendrocyte cell body (arrowheads). (**c**) Confocal images in fixed brain slices showing the overlap of SCoRe signal with that of immunofluorescence for myelin basic protein (MBP). (**d**) Confocal images showing the beginning portion of myelination at the axon initial segment (AIS) visualized with SCoRe microscopy and with MBP staining in the top image (arrow) as well as a break in myelination at a node of Ranvier in the bottom image (arrowhead). Each image is representative of at least three locations in at least three animals.



Supplementary Figure 2

Overlap between fluorescent and SCoRe signals for in vivo detection of myelin.

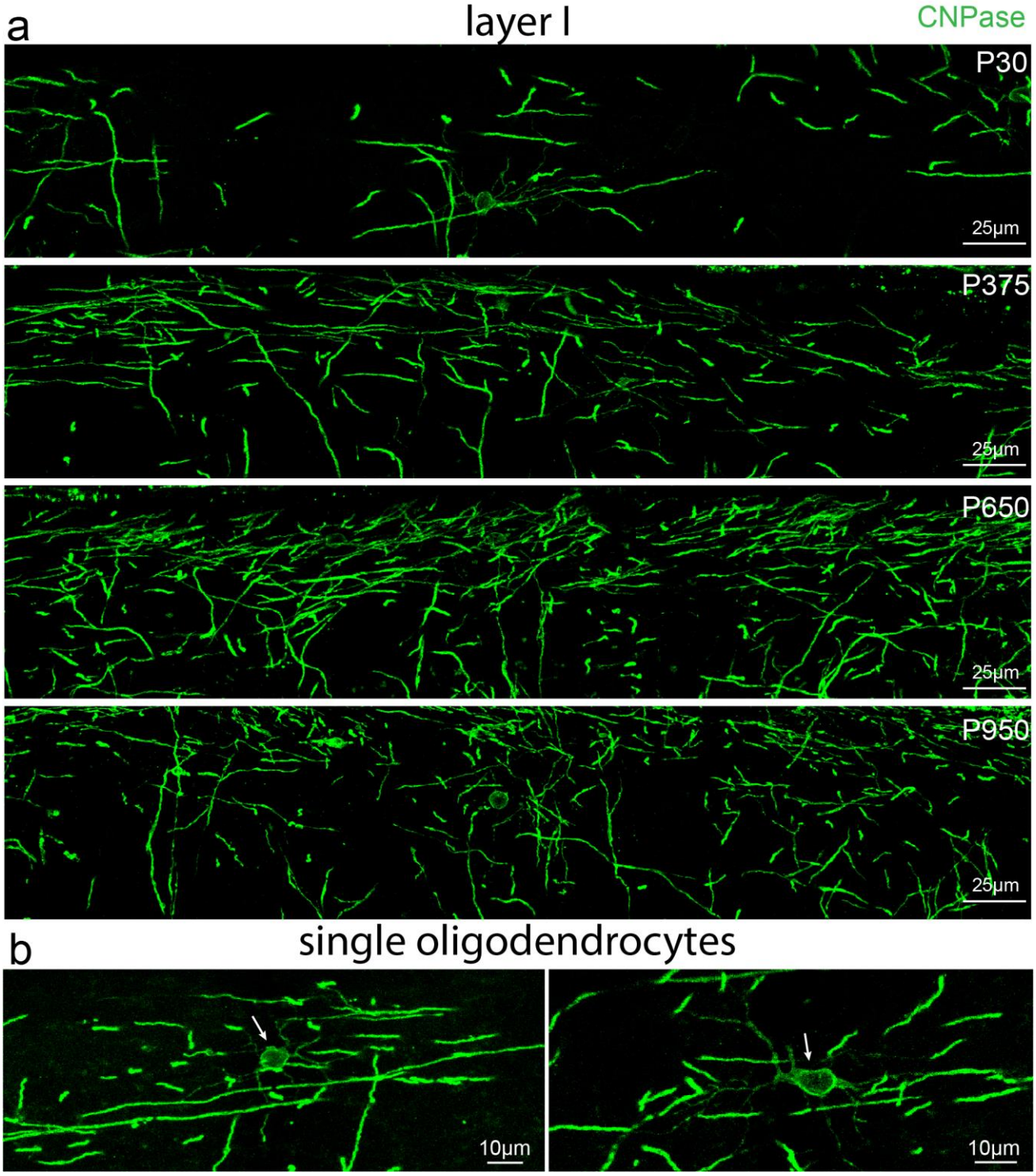
(**a**) In vivo image captured from layer I of the somatosensory cortex in a transgenic mouse with membrane tethered EGFP expressed exclusively in mature oligodendrocytes (*Cnp-mEGFP*) showing the overlap between fluorescence and SCoRe. Single oligodendrocyte cell soma can be seen in the fluorescence (yellow arrowheads) but not the SCoRe image due to the specificity of SCoRe for myelin. (**b**) The number of myelin segments intersecting the yellow line can be quantified as a proxy for equivalent myelin detection between mEGFP and SCoRe as shown in Figure 1g. (**c**) Reliable detection of myelin segment borders (arrowheads) using both fluorescence and SCoRe as shown in Figure 1g. (**d**) Classification of myelin segments as paired or unpaired for quantification of internode plasticity as shown in Figure 3h-i. Each image is representative of at least three locations in at least three animals.



Supplementary Figure 3

Lifelong changes in layer I oligodendrocyte and myelin density.

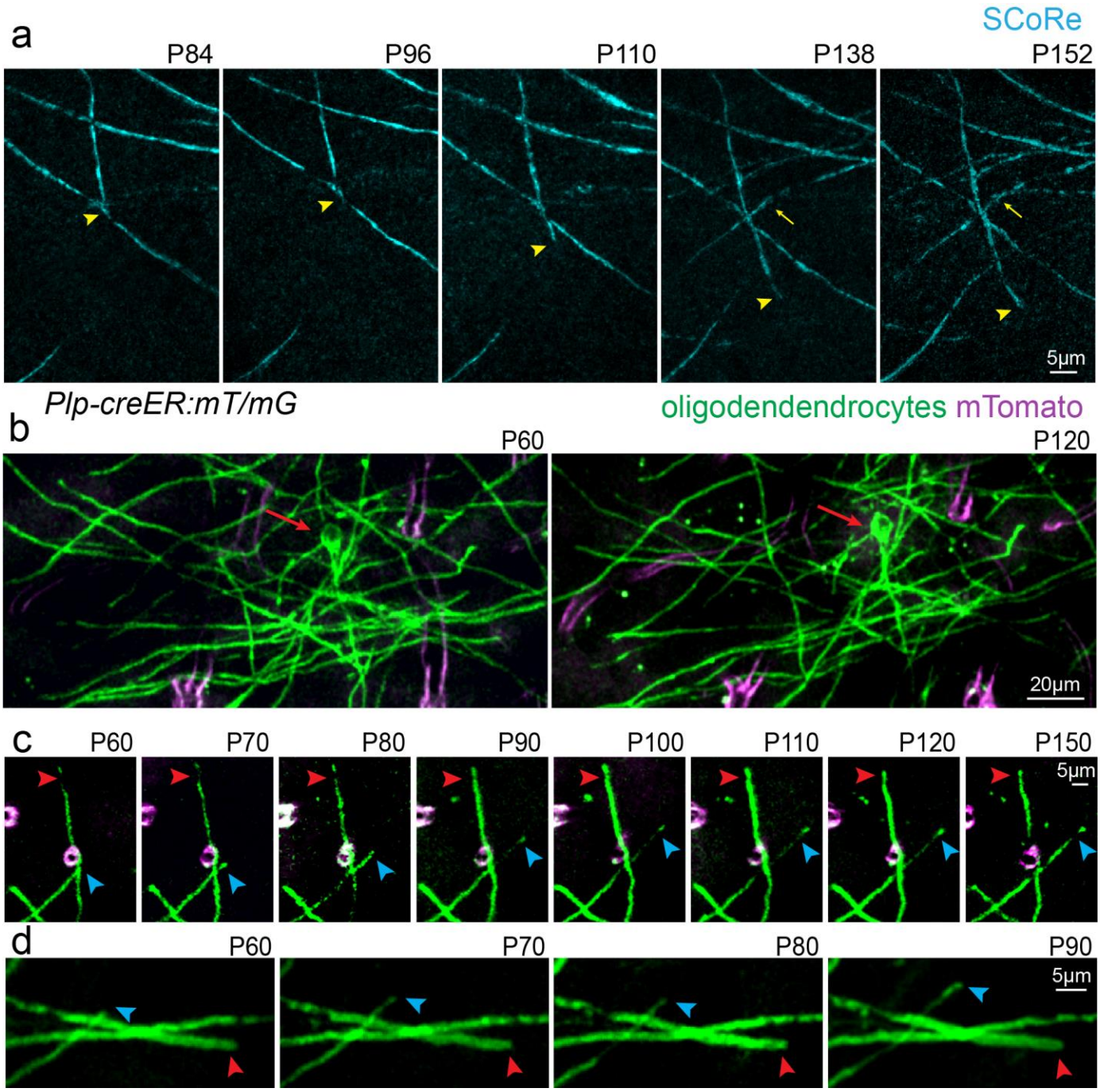
(**a**) Oligodendrocyte (*Plp-DsRed*) and SCoRe imaging captured in vivo from the somatosensory cortex at the ages indicated showing significant changes in both oligodendrocyte cell soma and myelin fiber density. Each image is representative of at least three locations in at least three animals.



Supplementary Figure 4

Lifelong changes in layer I myelination.

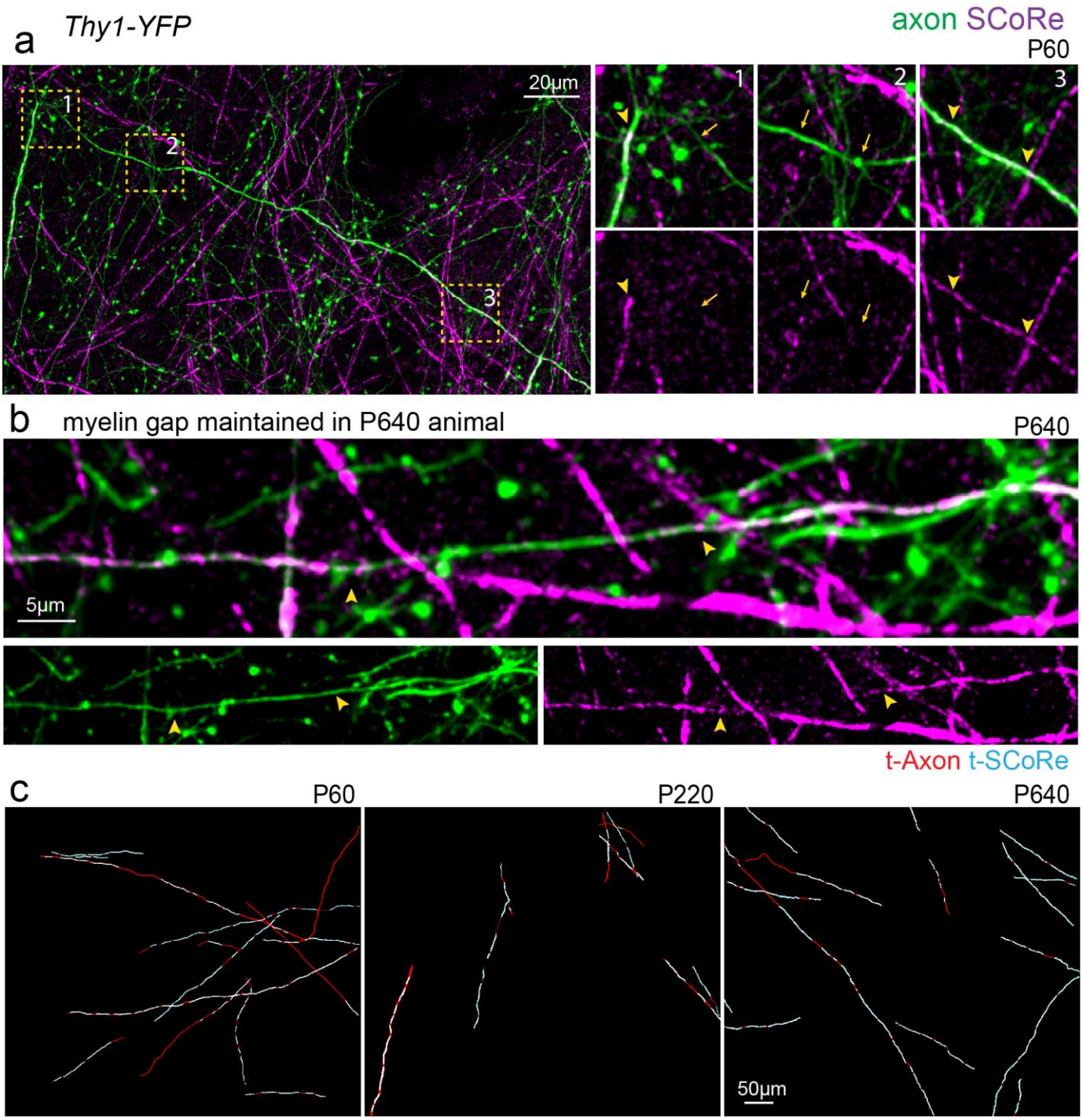
(**a**) Images of oligodendrocyte specific CNPase staining captured from the somatosensory cortex showing age-dependent changes in the upper layers of the cortex. (**b**) Examples of single oligodendrocyte cell soma (arrows) revealed by CNPase staining in layer I of the cortex. Each image is representative of at least three locations in at least three animals.



Supplementary Figure 5

Evidence of myelin plasticity through internode remodeling.

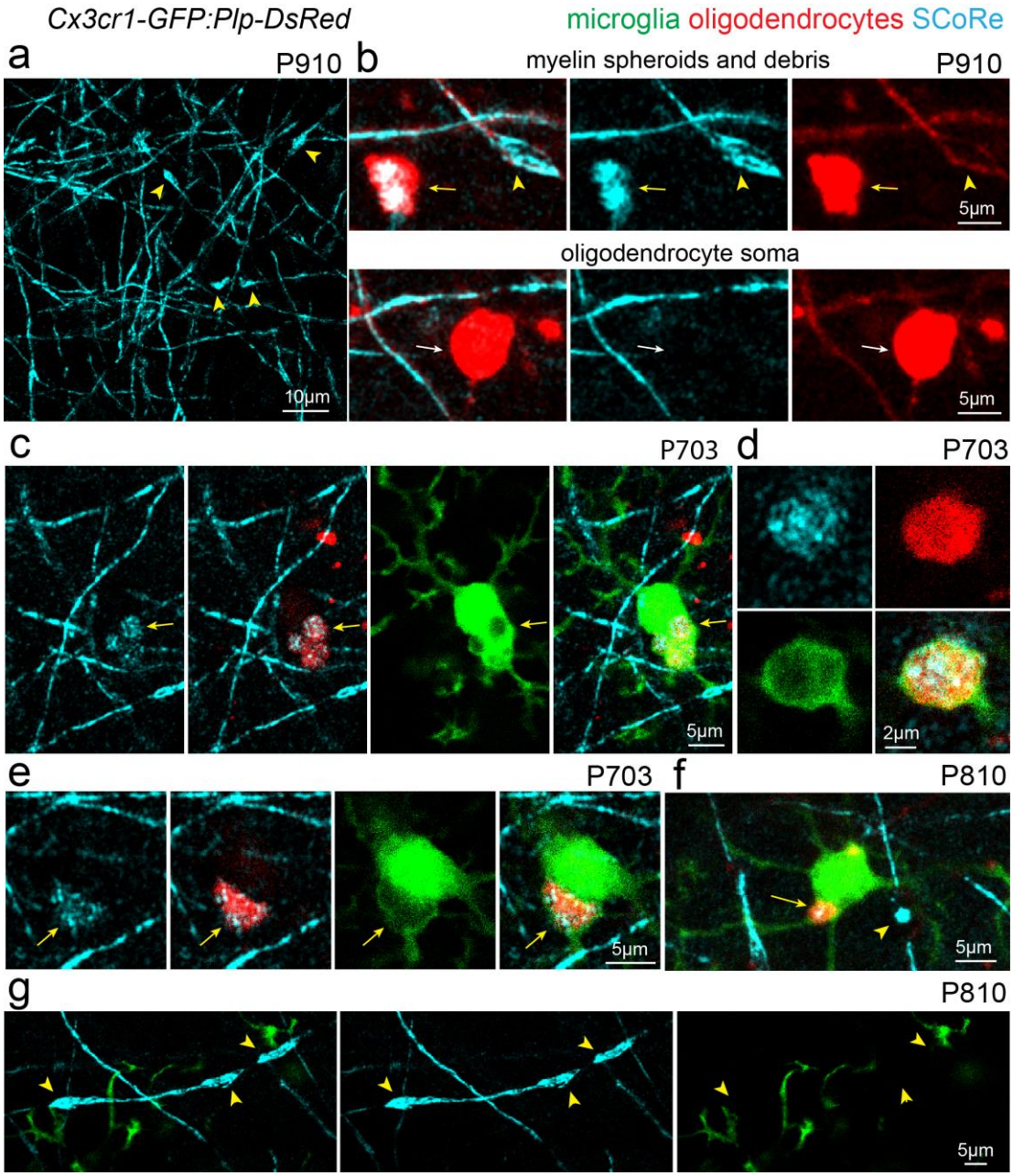
(**a**) In vivo time-lapse SCoRe images showing addition of single myelin internodes (yellow arrows) and extension of a single myelin internode (yellow arrowhead) over 68 days (**b**) In vivo two-photon fluorescence images of a single oligodendrocyte (red arrow) imaged over 60 days in a transgenic mouse (*Plp-creER:mT/mG*) with membrane tethered GFP expressed specifically in mature oligodendrocytes and membrane tethered Tomato (mTomato) expressed predominantly in cerebral blood vessels. (**c-d**) In vivo time-lapse images showing extension of single internodes (blue arrowheads) and stability of other internodes from the same oligodendrocyte (red arrowheads). Each image is representative of at least three locations in at least three animals.



Supplementary Figure 6

In vivo imaging of myelin distribution along single cortical axons.

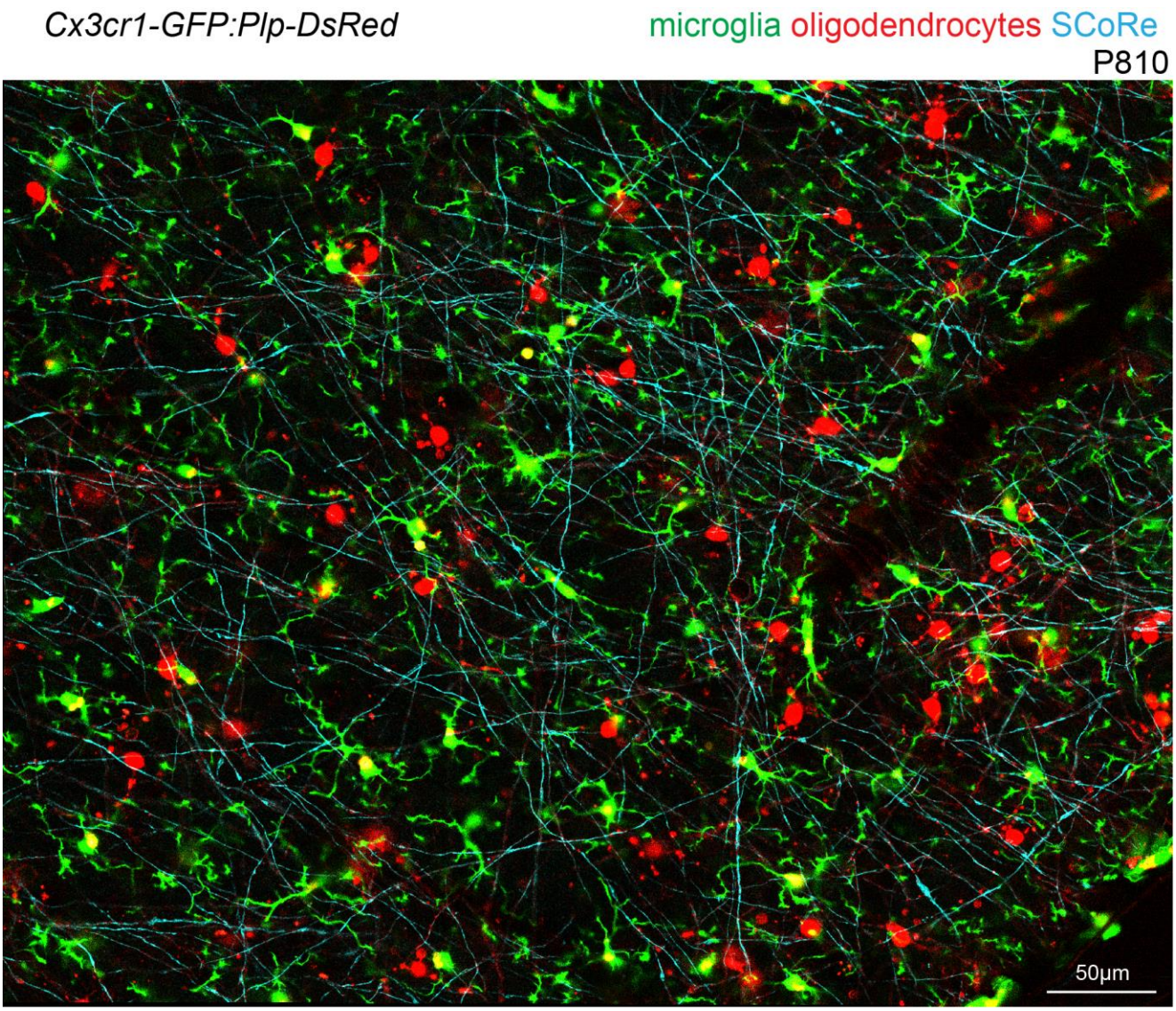
(**a**) In vivo images captured from the cortex of a P60 *Thy1-YFP* transgenic mouse showing a partially myelinated axon with arrowheads designating myelin segments and arrows pointing to unmyelinated regions. (**b**) Image captured from the cortex of a P640 mouse showing an unmyelinated region along a single axon in late adulthood. (**c**) Representative traced axons from mice at the ages indicated showing age-dependent increase in myelin coverage along single axons. Each image is representative of at least three locations in at least three animals.



Supplementary Figure 7

Myelin degeneration and debris accumulation in microglia in advanced aging.

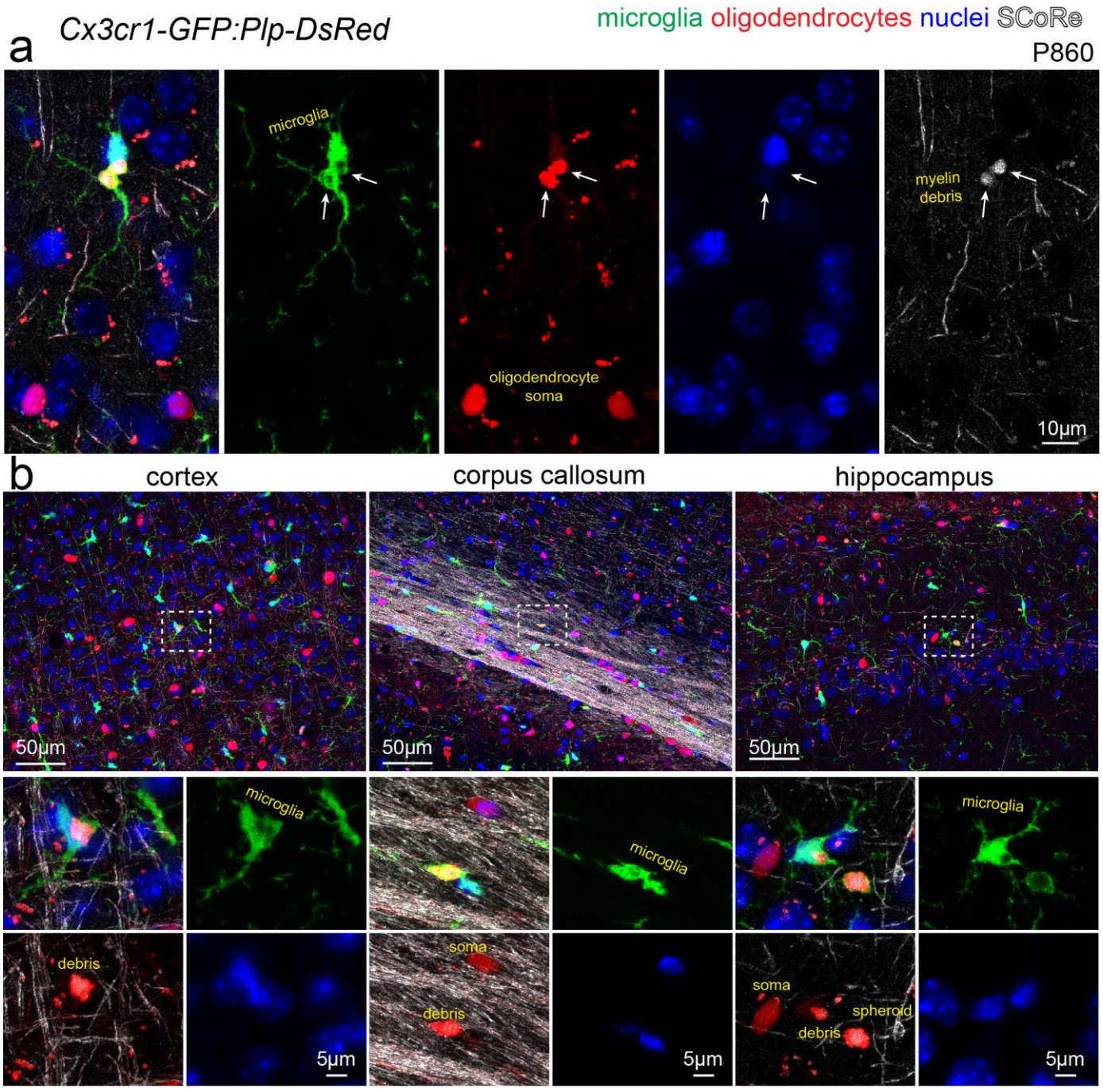
(**a-b**) In vivo images captured from the cortex of a 910-day old mouse with mature oligodendrocytes labeled with DsRed (*Plp-DsRed*) showing examples of myelin pathology detected in aged mice revealed by SCoRe and DsRed fluorescence. Myelin spheroids (yellow arrowheads) can be detected using SCoRe and are only found in aged mice. Myelin debris (yellow arrows) can also be detected using SCoRe and the vast majority were found to have accumulation of DsRed fluorescent protein in the *Plp-DsRed* transgenic mice. Myelin debris and oligodendrocyte cell bodies (white arrows) can be distinguished due to the lack of SCoRe signals in addition to the proximal processes extending from the cell body. (**c**) In vivo image captured from the cortex of a *Cx3cr1-GFP:Plp-DsRed* transgenic mouse showing accumulation of reflective and DsRed labeled myelin debris within microglia (yellow arrows). (**d**) High resolution in vivo image of a single myelin debris accumulation engulfed within a microglia process (**e-g**) In vivo images showing examples of myelin debris engulfed by microglia (yellow arrows) with no preferential microglial association with myelin spheroids (yellow arrowheads). Each image is representative of at least three locations in at least three animals.



Supplementary Figure 8

In vivo imaging of myelin, oligodendrocytes and microglia.

Low magnification in vivo image captured from the cortex of a Cx3cr1-GFP:Plp-DsRed transgenic mouse showing the capabilities of imaging myelination (SCoRe), oligodendrocytes (Plp-DsRed) and microglia (Cx3cr1-GFP) in an 810 day old mouse. This image is representative of at least three locations in at least three animals.



Supplementary Figure 9

Myelin debris accumulation in microglia in advanced aging.

(a) Image captured of a tissue section stained with nuclear dye from an 860-day old *Cx3cr1-GFP:Plp-DsRed* transgenic mouse showing myelin debris accumulation (white arrows) within a single microglia. These debris are characterized by bright DsRed and SCoRe labeling and no nuclear dye labeling. Two oligodendrocyte cell soma are shown in the lower portion of the image characterized by DsRed expression with nuclear dye labeling. (**b**) Images from 860-day old *Cx3cr1-GFP:Plp-DsRed* mice showing the presence of myelin debris within microglia in the cerebral cortex, corpus callosum, and the hippocampus suggesting myelin degeneration is a wide spread phenomenon in the aging brain. Each image is representative of at least three locations in at least three animals.