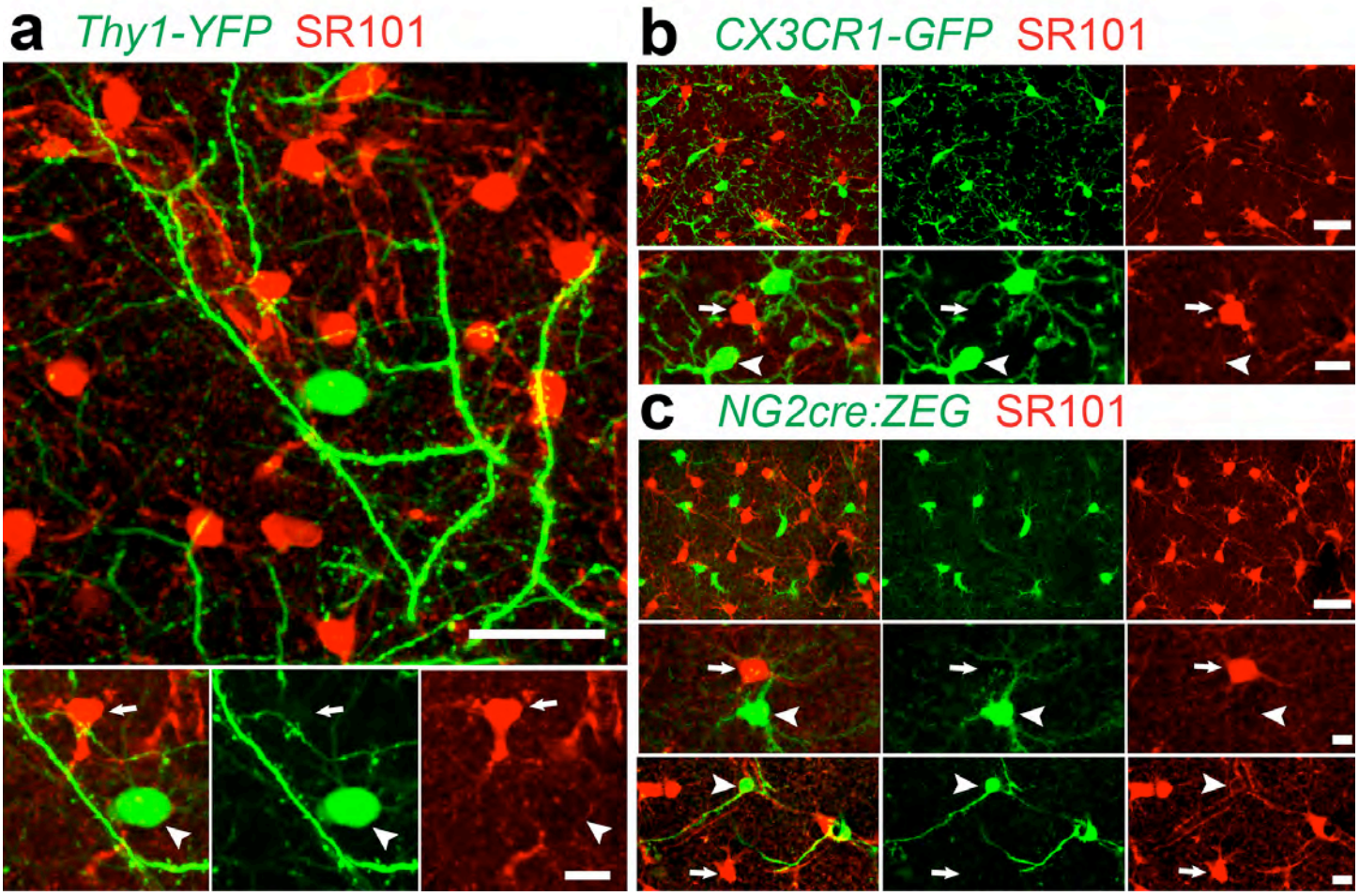

Supplementary Figure 1

SR101 does not label neurons, microglia, NG2 cells, or vascular pericytes.

a) In vivo two photon fluorescence image captured from the cortex of a *Thy1-YFP* transgenic mouse demonstrating that YFP (green) labeled neuronal cell bodies (arrowheads) and dendrites are not labeled with SR101 (red, arrows). scale bars 25µm, 10µm. **b)** In vivo image captured from the cortex of a *CX3CR1-GFP* transgenic mouse demonstrating that GFP labeled microglia (green, arrowheads) are not labeled with SR101 (red, arrows), scale bars 25µm, 10µm. **c)** In vivo image captured from the cortex of an *NG2cre:ZEG* transgenic mouse demonstrating that GFP labeled NG2 cells and vascular pericytes (green, arrowheads) are not labeled with SR101 (red, arrows), scale bars 25µm, 10µm. Images representative of data acquired from 3 mice for each genotype.

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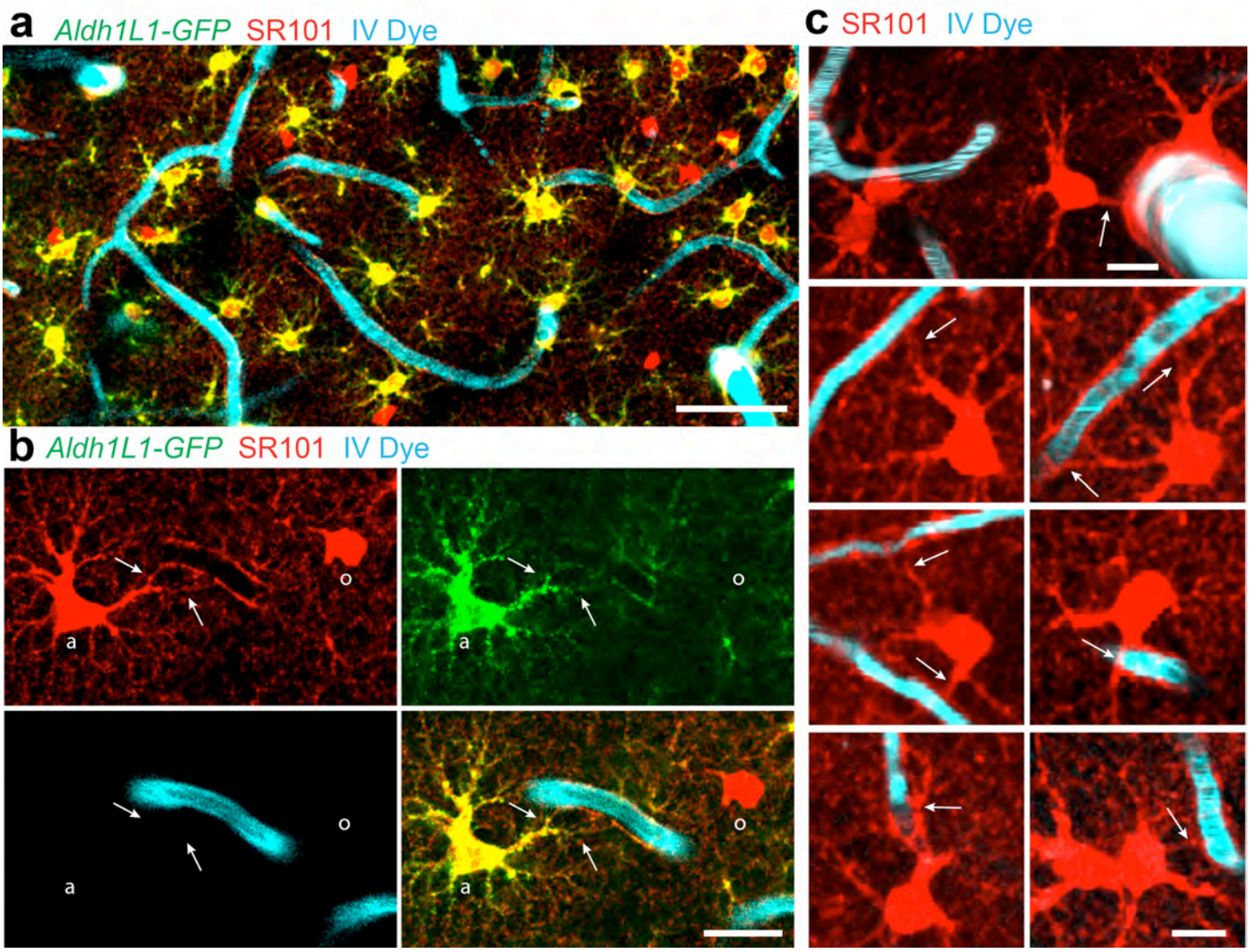

Supplementary Figure 2

Morphological properties of single SR101 -labeled cells.

a) Low magnification in vivo image showing SR101 (red) labeling in an Aldh1L1-GFP (green) transgenic mouse with blood vessels imaged via intravascular injection of a high molecular weight dextran (cyan), scale bar 50µm. **b)** In vivo image showing a single astrocyte (a) and a single oligodendrocyte (o) both labeled with SR101 (red) but only the astrocyte showing the typical morphology with perivascular endfeet (arrows), scale bar 20µm. **c)** Multiple examples of single astrocytes labeled with SR101 with perivascular endfeet (arrows), scale bars 10µm. Images representative of data acquired from 3 mice and quantification can be found in the main text.

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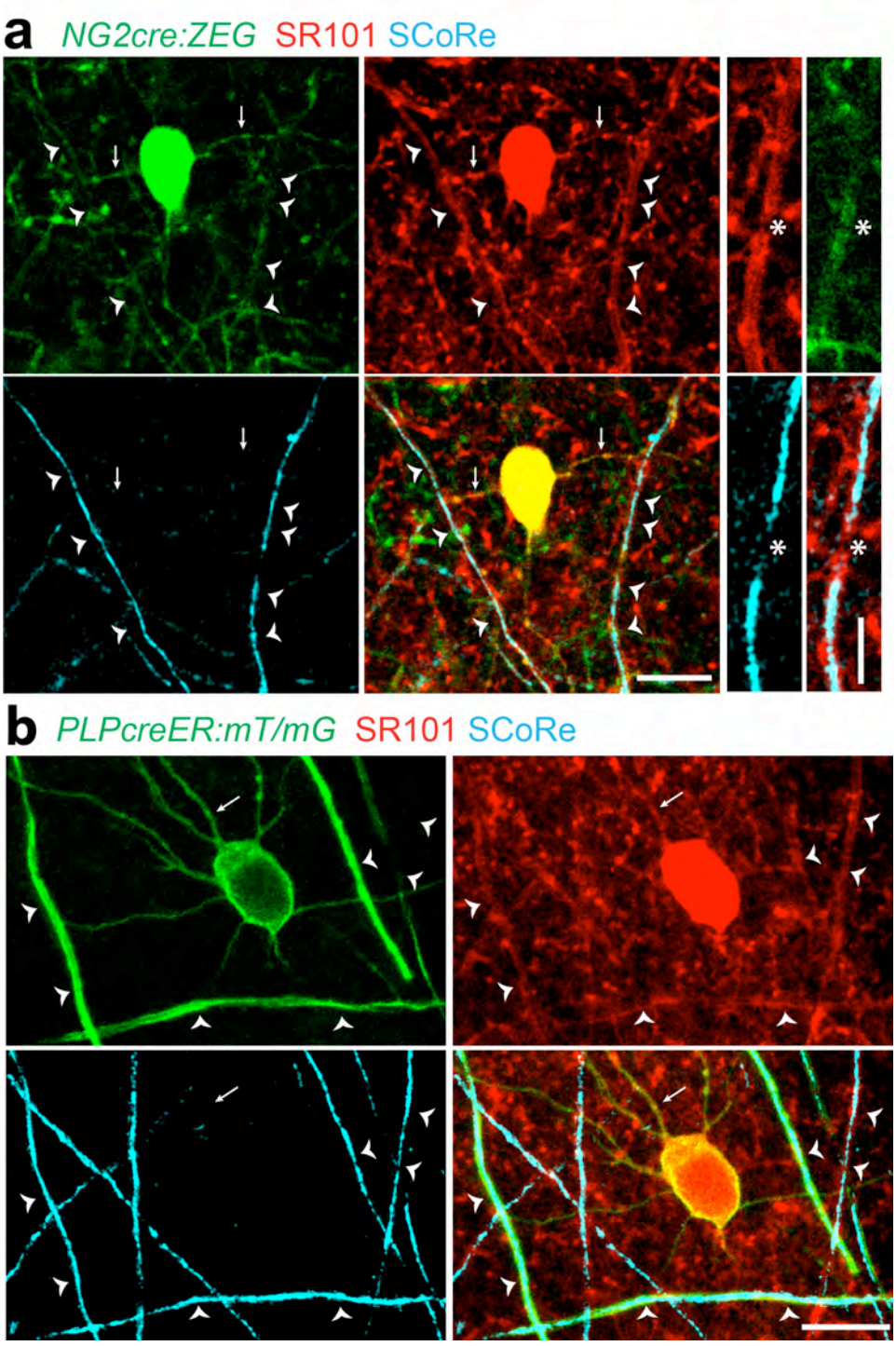

Supplementary Figure 3

SR101 labels oligodendrocyte cell bodies and myelinating processes.

a) In vivo image showing a single GFP (green) labeled oligodendrocyte from an *NG2cre:ZEG* transgenic mouse showing SR101 (red) labeling of proximal non-myelinating processes (arrows) and SCoRe (cyan) labeled myelinating processes (arrowheads). * depicts a node of Ranvier, scale bars 10μm, 5μm. **b)** In vivo image from a *PLPcreER:mT/mG* transgenic mouse showing SR101 (red) labeling in proximal non-myelinating processes (arrow) and in membrane tethered GFP-labeled (green), SCoRe-labeled (cyan), myelinating processes (arrowheads) scale bar 10μm.

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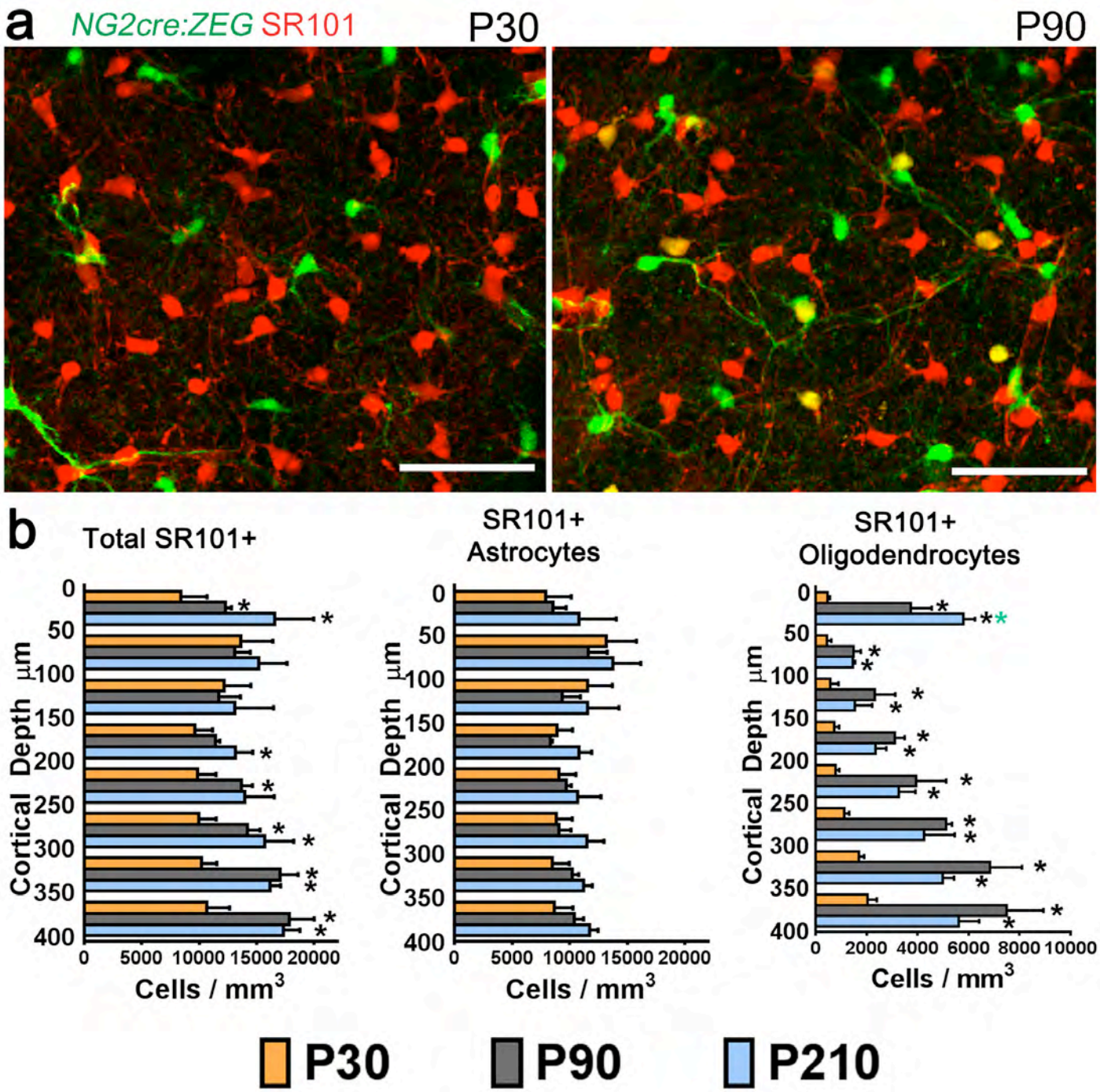

Supplementary Figure 4

Developmental density and identity of SR101 -labeled cells.

a) Representative in vivo two photon fluorescence images captured from cortex of *NG2cre:ZEG* mice labeled with SR101 at P30 and P90 demonstrating the differences in the density of GFP (green) SR101 (red) labeled oligodendrocytes, scale bars 50µm. **b)** Quantification showing the total density of SR101 labeled cells (left), cells identified as astrocytes (middle) and the cells identified as oligodendrocytes (right) at postnatal (P) days 30, 90 and 210 by cortical depth, error bars = s.d. * $P < 0.05$ compared to P30, * $P < 0.05$ compared to P90, student's t-test, data acquired from 3 mice at each age.

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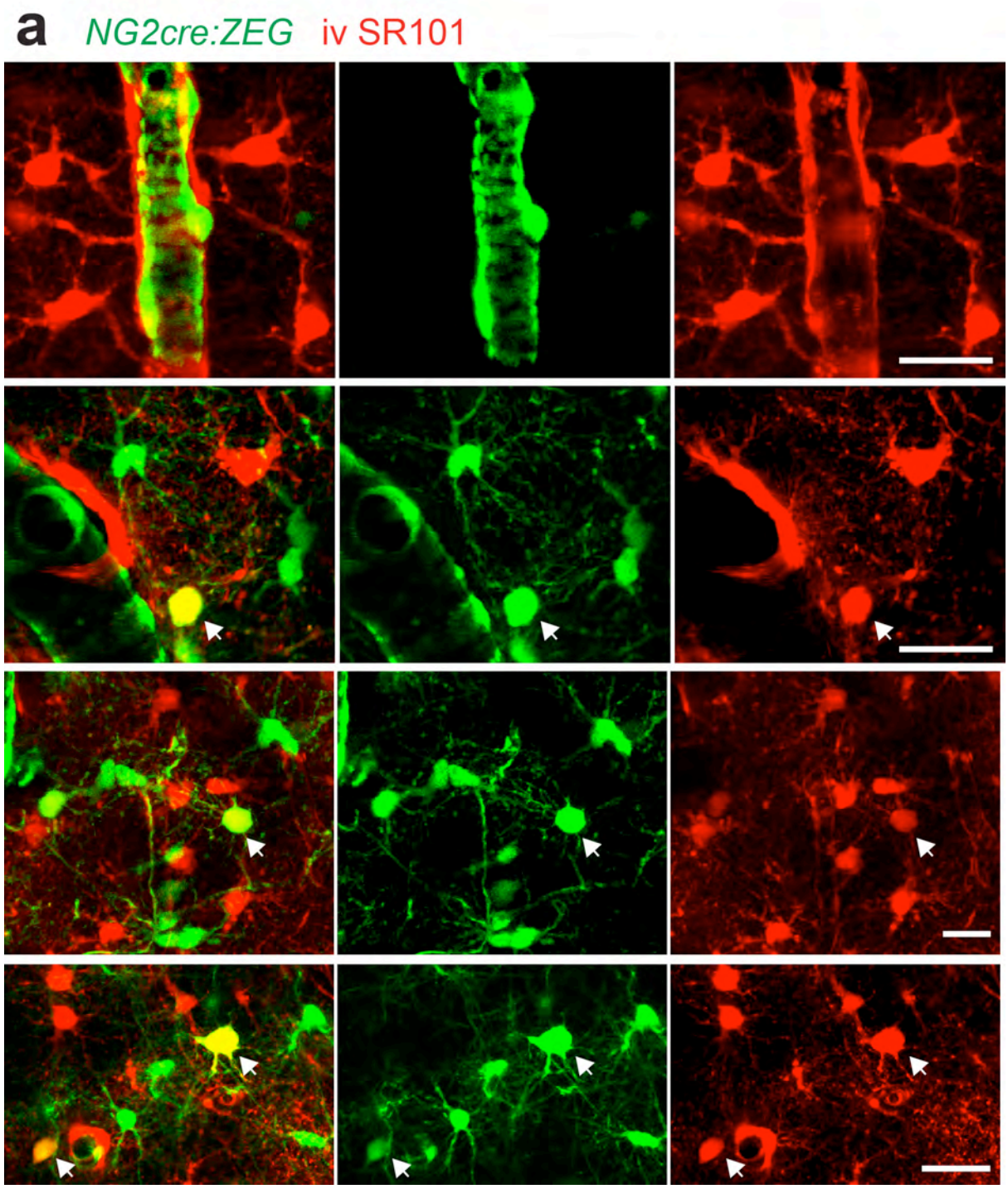

Supplementary Figure 5

Intravenous injection of SR101 labels oligodendrocytes and astrocytes *in vivo*.

a) In vivo images captured through a thinned skull 24 hours after intravenous injection of SR101 in *NG2cre:ZEG* transgenic mice. Arrows indicate labeling of mature oligodendrocytes with SR101 (red) and GFP (green) while other GFP labeled cells (NG2 cells and vascular pericytes) are not labeled, consistent with topical application of SR101 to an open skull preparation. Scale bars 20μm. Images representative of data acquired from 3 mice.

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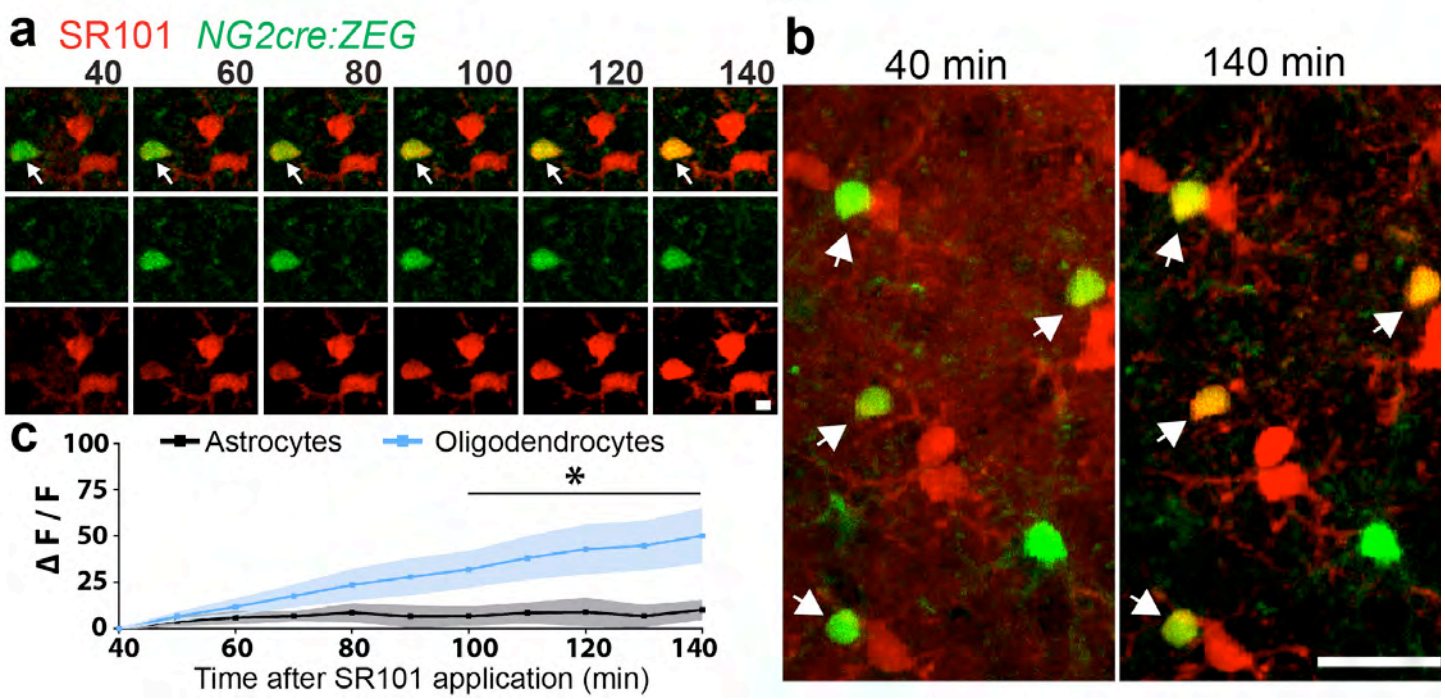
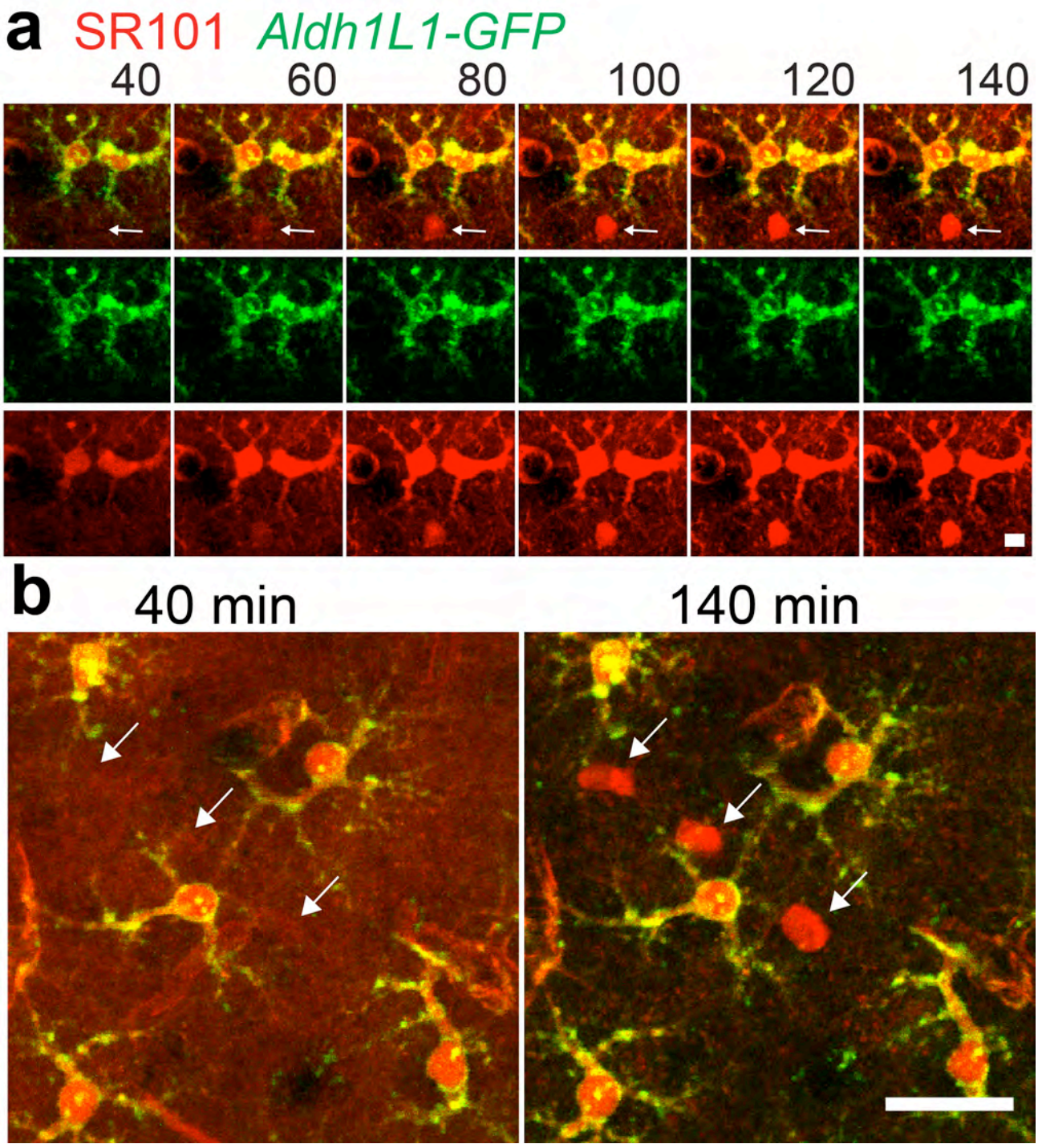


Supplementary Figure 6

Temporal dynamics of cell-specific labeling with SR101 in *NG2cre:ZEG* mice.

a) In vivo time lapse images of a GFP+ (green, arrow) oligodendrocyte in an *NG2cre:ZEG* mouse showing SR101 (red) labeling over time (indicated in minutes), scale bars 5µm. **b)** Images taken at 40 and 140 minutes after SR101 labeling indicating dye spread from astrocytes to oligodendrocytes (arrows) in *NG2cre:ZEG* mice , scale bar 50µm. **c)** Quantification showing significant differences in the change in fluorescent intensity starting at 40 min after SR101 labeling , error bars = s.e.m. **P* < 0.05, t-test, data acquired from 3 mice.

Nature Methods: doi:10.1038/nmeth.3140

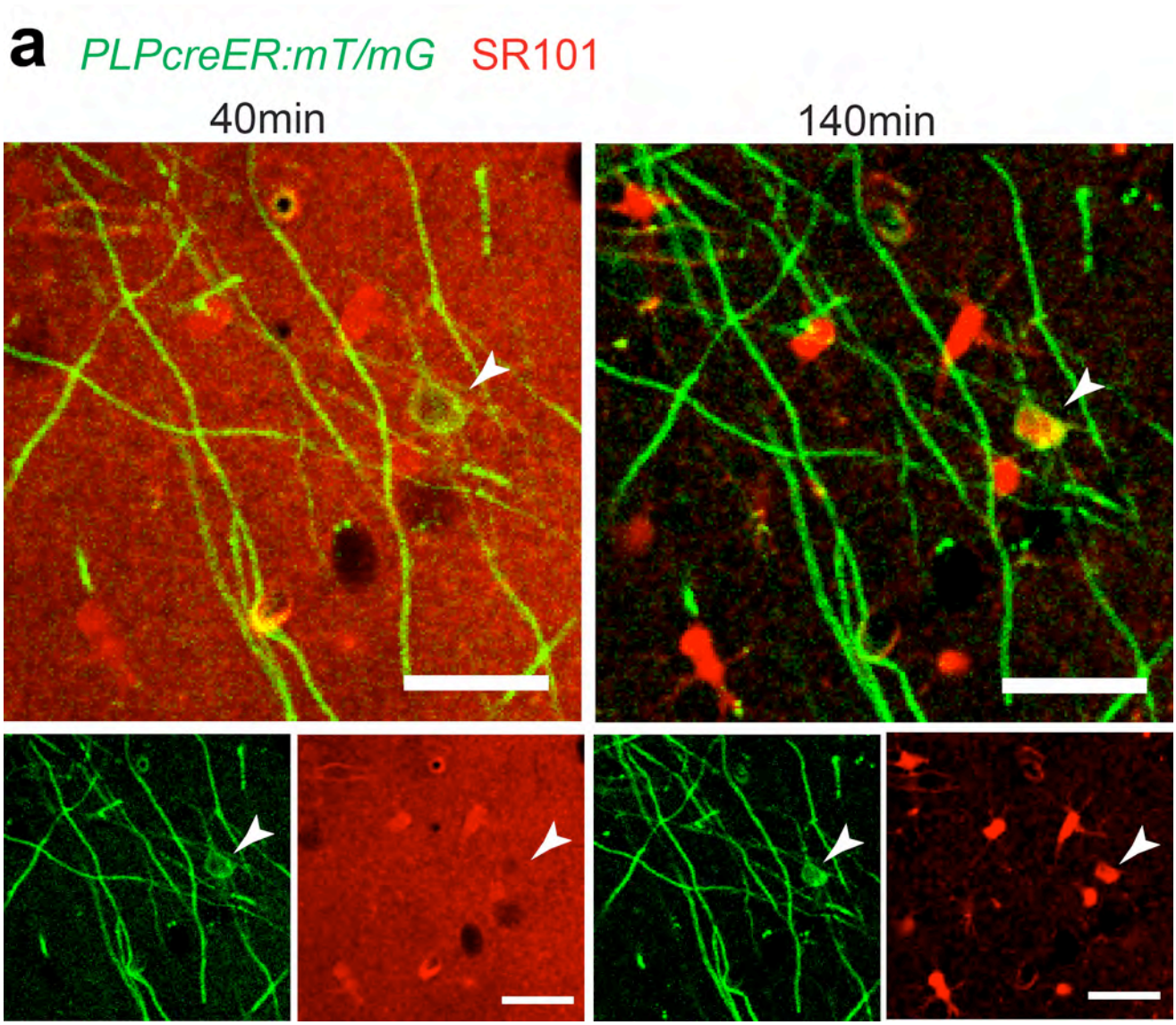


Supplementary Figure 7

Temporal dynamics of cell-specific labeling with SR101 in *Aldh1L1-GFP* mice.

a) In vivo time lapse images showing SR101(red) labeling over time of a GFP- (arrow) cell in an *Aldh1L1-GFP* mouse, scale bar 5µm. **b)** Representative images taken at 40 and 140 minutes after SR101 labeling indicating spread of the dye from astrocytes to oligodendrocytes (arrows), scale bar 50µm. Images representative of data acquired from 3 mice.

Nature Methods: doi:10.1038/nmeth.3140

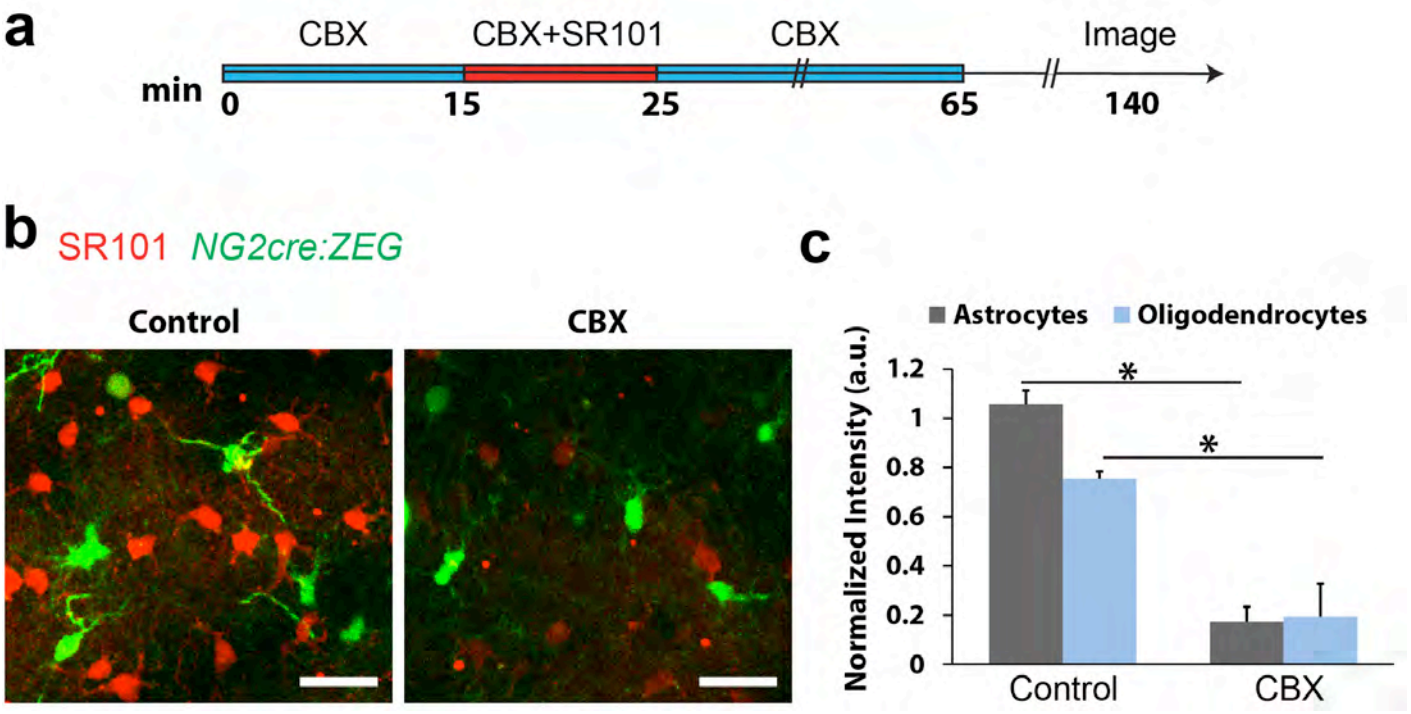


Supplementary Figure 8

Temporal dynamics of cell-specific labeling with SR101 in *PLPcreER:mT/mG* mice.

a) In vivo image captured from the cortex of a *PLPcreER:mT/mG* transgenic mouse demonstrating that membrane bound GFP (green) labeled myelinating oligodendrocytes (arrowheads) are not initially labeled with SR101 (red) but become labeled between 30 and 120 minutes after SR101 application, scale bars 25μm. Images representative of data acquired from 3 mice.

Nature Methods: doi:10.1038/nmeth.3140



Supplementary Figure 9

Carbenoxolone inhibits SR101 labeling of both oligodendrocytes and astrocytes.

a) Timeline of the experimental procedure for blocking SR101 labeling in vivo with the gap junction blocker carbenoxolone (CBX, 100μM). **b)** In vivo images from *NG2cre:ZEG* (green) mice showing SR101 (red) labeling in control conditions and after CBX treatment, scale bar 25μm. **c)** Fluorescence intensity quantification of SR101 labeling normalized to GFP intensity showing significant inhibition of SR101 labeling under CBX treatment in both astrocytes and oligodendrocytes, error bars = s.d. *P < 0.002, student's t-test, data acquired from 3 mice for each treatment.

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Supplementary Methods

Animals

All animal procedures were approved by the institutional animal care and use committee (IACUC) at Yale University. Male and female mice aged P21-P220 housed in a 12 hr light/dark cycle housed with 3-5 animals per cage of the following transgenic lines were used: NG2cre[8] (Jax #008533) PLPcreER[9] (Jax #005975), mT/mG[10] (Jax #007576), Z/EG[11] (Jax #003920), CX3CR1-GFP[12] (Jax #005582), Thy1-YFP-H[13] (Jax #003782), and Aldh1L1-GFP[14]. To induce cre recombination in PLPcreER:mT/mG a single intraperitoneal injection of 0.5mg tamoxifen dissolved in ethanol:sunflower oil (1:4 ratio) was administered to P30 mice in order to label a subset of all oligodendrocytes to visualize single cells. Animals were assigned to experimental groups randomly and no animals were excluded from analysis. No investigator blinding was necessary for the reported experiments and a sample size of 3 animals was used for each experiment.

In vivo imaging

The cranial window procedure was used for topical application of fluorescent dyes. The thin skull procedure was used for imaging after intravenous injection of fluorescent dyes[15]. Briefly, animals were anesthetized with intraperitoneal injection of ketamine and xyalazine. The skin covering the skull was thoroughly shaved and sterilized and a midline scalp incision was performed to expose the underlying skull. A custom made plate was fixed to the skull with cyanoacrylate glue. An area no larger than 1mm was thinned to a thickness of 20-30µm with a high speed drill and a microsurgical blade for thin skull imaging or removed for acute craniotomy imaging with dye labeling. Dye was applied to the cortical surface as described below. After dye labeling a #0 glass coverslip was cut to size, placed over the craniotomy and glued in place. Images were captured using a mode-locked MaiTai tunable laser (Spectra Physics) with a two-photon microscope (Prairie Technologies) using at 20x water immersion objective (1.0 NA, Zeiss). The near infrared laser was tuned to the following wavelengths for two-photon excitation: SR101: 800-900nm, Cascade Blue dextran: 820nm, GFP/YFP: 800-900nm. Spectral confocal reflectance (SCoRe) in vivo imaging was performed as described previously[7]. A Leica SP5 confocal microscope with a 20x water immersion objective (1.0 NA, Leica) was used. Reflected light from 488nm, 561nm, and 633nm lasers was collected using photo-detectors centered on wavelengths of 486-491nm, 559-564nm, and 631-636nm respectively. Reflected signals were combined into a single channel and displayed as a color composite to detect the entire myelinated axon. All images were analyzed using ImageJ software.

Fluorescent dye labeling and pharmacology

Sulforhodamine 101 (Sigma-Aldrich cat# S7635) was applied to the cortical surface after craniotomy for 5-10 minutes at a concentration of 50µM and then washed thoroughly for 10 minutes or 100µL was injected intravenously at a concentration of 5mM dissolved in phosphate buffered saline (PBS). Cascade Blue dextran (Life Technologies, cat# D1976) was injected intravenously to visualize the cortical vasculature. To block gap junctions carbenoxolone (CBX, Sigma-Aldrich cat# C4790) dissolved in PBS at 100µM was applied topically to the surface of the craniotomy.

Quantification and statistics

SR101 Cell Density – the density and percentage of GFP labeled SR101 labeled cells in Aldh1L1-GFP and NG2cre:ZEG at P30, 90 and P210 was determined 120-140 minutes after topical SR101 application (Figure 1 and Supplementary Fig. 4). 400 micron Z stacks were captured from 3 mice at each age and each genotype and the numbers of double and single labeled cells were quantified in 50 micron Z projections. The proportion of GFP+SR101+ cells in NG2cre:ZEG mice was corrected due to the reported ~85% cre recombination rate in these mice[8] for quantification in Fig. 1e and Supplementary Fig. 4. Statistical differences were determined using unpaired Student's t-tests.

Nature Methods: doi:10.1038/nmeth.3140

SR101 Cell Morphology – Z stacks were acquired from Aldh1L1-GFP transgenic mice labeled with SR101 after IV administration of Cascade Blue dextran. Single SR101-labeled GFP positive and negative cells were analyzed and the presence or absence of a primary process terminating on a blood vessel (perivascular end-foot) was noted and quantified from 3 mice with total numbers indicated in the main text.

Fluorescence Intensity Time Lapse – Single regions of interest (ROIs) of the same size were manually selected over SR101-labeled GFP-negative and positive cells in NG2cre:ZEG mice from time lapse sequences acquired every 10 minutes from 40 to 140 minutes after dye application with identical microscope and laser settings at each time point. Mean fluorescence intensity values were measured within these ROIs and averaged for each cell type and mouse (F_2). These values were averaged and graphed for each time point as an average change in fluorescence intensity relative to average fluorescence intensity at 40 min (F_1) ((F_2-F_1) / F_1 = $\Delta F/F$) (Supplementary Fig. 6). Statistical differences were determined using unpaired Student's t-tests between astrocytes and oligodendrocytes at each time point. Values represent averages from 221 astrocytes and 123 oligodendrocytes from 3 mice.

Pharmacology – Under control and CBX conditions, ROIs of the same size were manually selected over SR101 labeled GFP negative and positive cells in NG2cre:ZEG mice from images acquired at 140 minutes after dye application with identical microscope and laser settings for each condition. Mean fluorescence intensity values were measured within these ROIs and averaged for each cell type and mouse. SR101 intensity values were averaged and graphed for each condition and normalized to average fluorescence intensity of all GFP labeled cells in the image (Supplementary Fig. 9). Statistical differences were determined using unpaired Student's t-tests between control and CBX treatments. Values represent averages from 3 mice for each treatment.

Supplementary References

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