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SUPPLEMENTARY INFORMATION

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Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex

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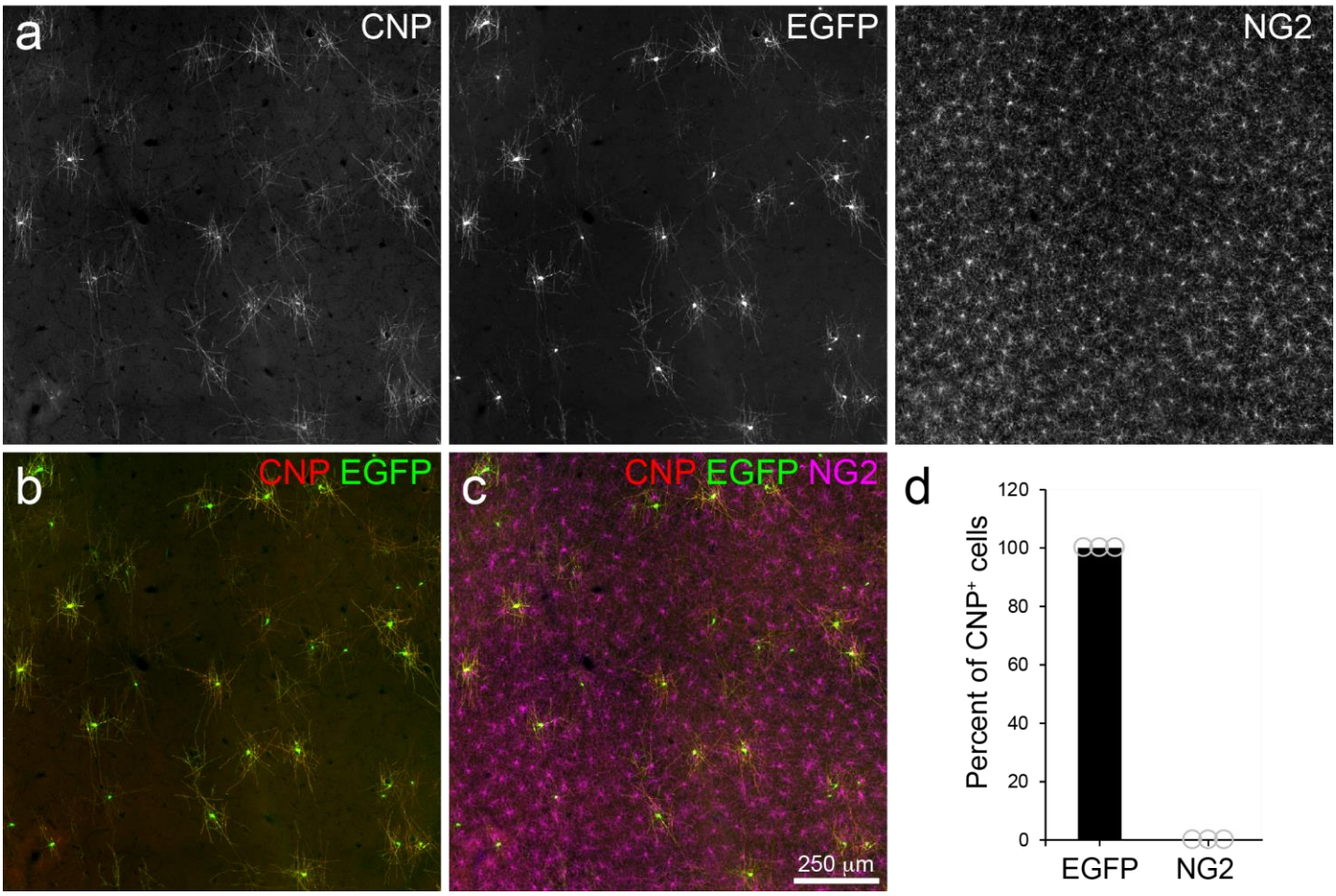
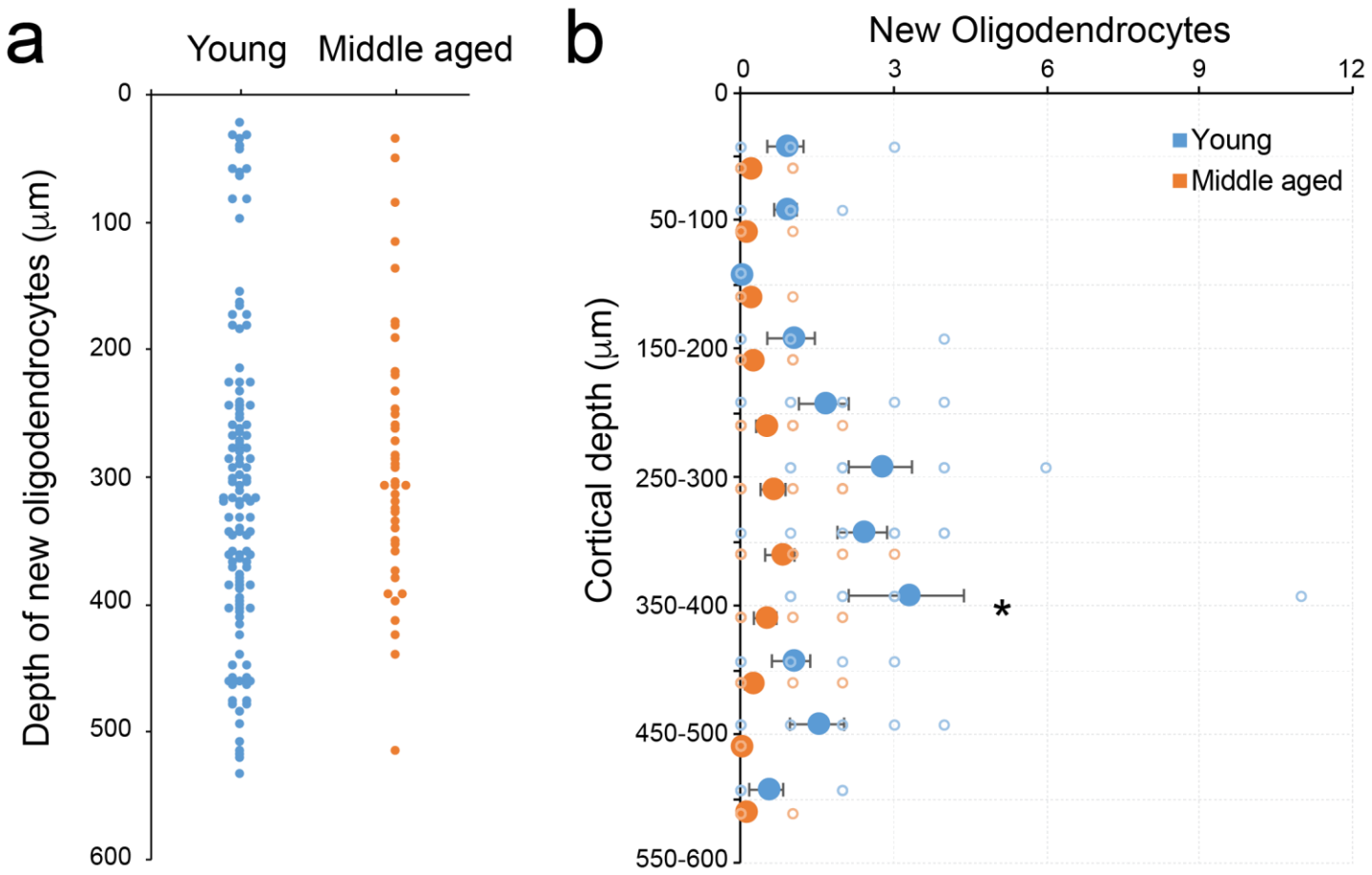


Supplementary Figure 1

Specific expression of EGFP in all cortical oligodendrocytes

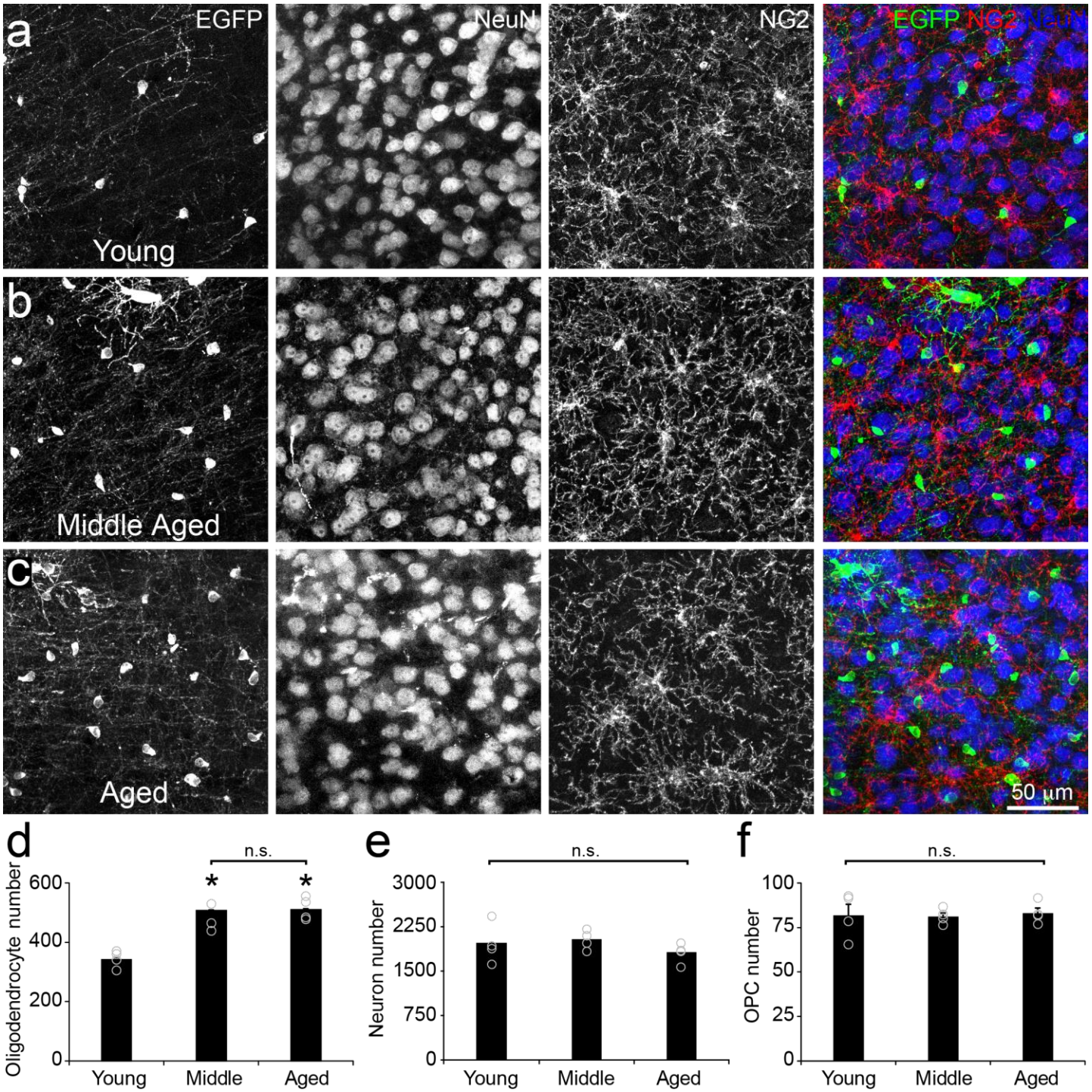
a, Horizontal section from layer I cortex of a *Mobp-EGFP* mouse immunostained with CNP (left), EGFP (middle) and NG2 (right) (P30). **b**, Overlay of CNP and EGFP immunostaining shown in **a**. **c**, Overlay of CNP, EGFP, and NG2 immunostaining shown in a. Note lack of cells co-labeled with EGFP and NG2. All EGFP+ cells were labeled with CNP and are oligodendrocytes. **d**, Quantification of proportion of CNP+ colabeled with EGFP or NG2 (n = 3 mice; CNP+ cells = 356; mean ± SEM).



Supplementary Figure 2

Oligodendrogenesis is uniform across cortical layers

a, Depth of newly formed oligodendrocytes in Young and Middle-aged mice (Young, 2-4 months, n = 8 mice; Middle-aged, 11-14 months, n = 13 mice). **b**, Quantification of total oligodendrocyte number in layers I-IV of somatosensory cortex (2-4 months, n= 8 mice, p=0.0233, one-way ANOVA with Tukey post hoc test, 300-350µm vs. 0-50µm, * = p = 0.0436, q(7) = 6.48; 300-350µm vs. 100-150µm, * = p = 0.0357, q(7) = 6.75 ; 11-14 months, n =13 mice, one-way ANOVA, p = 0.06, F(4, 51) = 2.40; mean ± SEM).

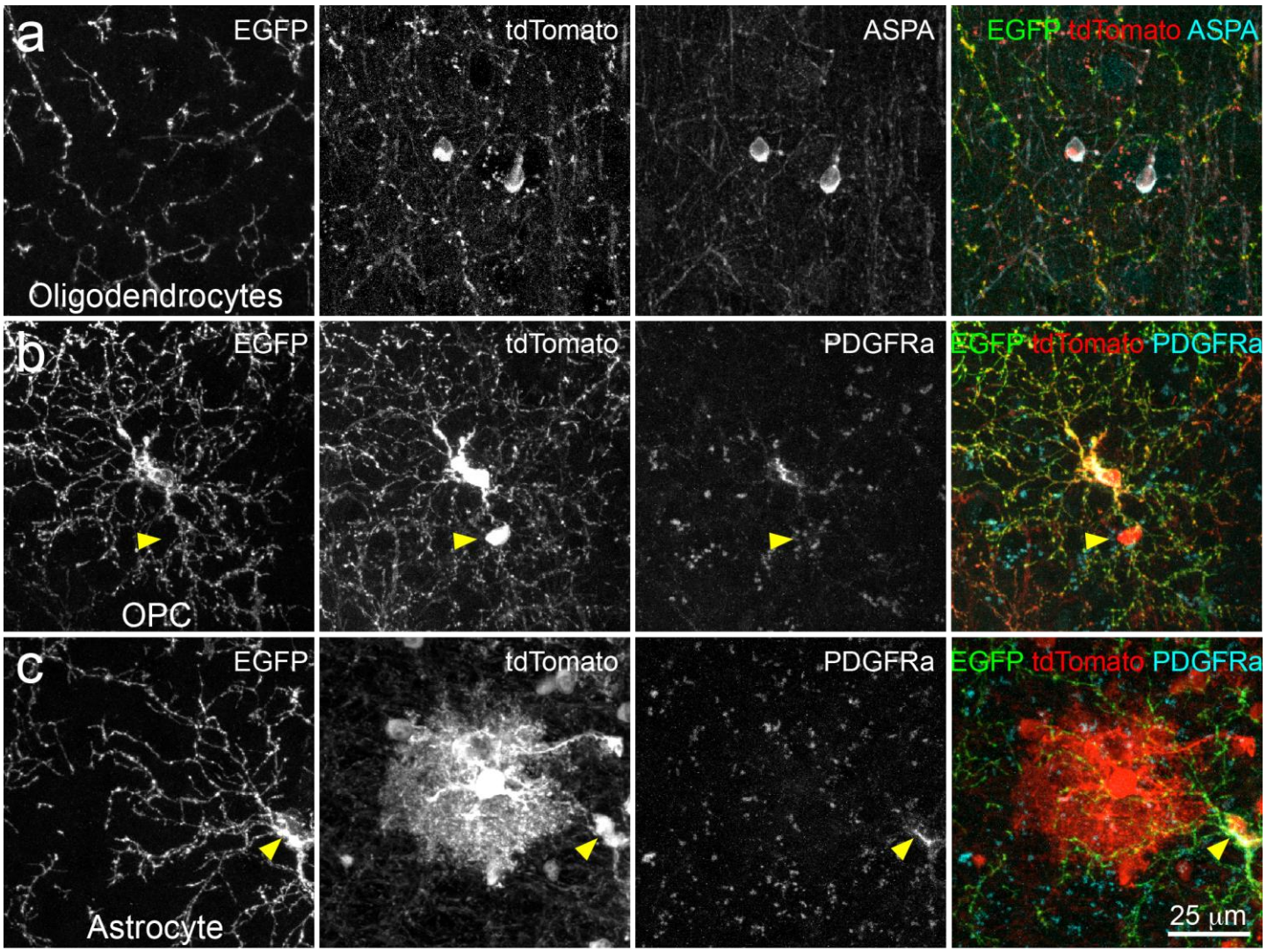


Supplementary Figure 3

Neuron and OPC numbers remain constant over the adult lifespan

a-c, Immunostaining of coronal sections somatosensory cortex from Young (P60; **a**), Middle-aged (P365; **b**), and Aged (P665; **c**) *Mobp-EGFP* mice. Antibodies against EGFP, NeuN, and NG2 were used to label mature oligodendrocytes, neurons, and oligodendrocyte precursor cells (OPCs). **d**, Quantification of number of EGFP+ oligodendrocytes in a 0.6 mm^2 area over age (n = 4 mice per group; One-way ANOVA with Tukey's posthoc test, Young vs. Middle, p = 0.004, q(11) = 6.44; Young vs. Aged, p = 0.003, q(11) = 6.56; Middle vs. Aged, p = 0.90, q(11) = 0.12; n.s. = not significant; * = p < 0.005). **e**, Quantification of number of NeuN+ neurons in a 0.6 mm^2 area over age (n = 4 mice each group; One-way ANOVA p = 0.44, F(2, 9) = 0.90; n.s. = not significant). **f**, Quantification of

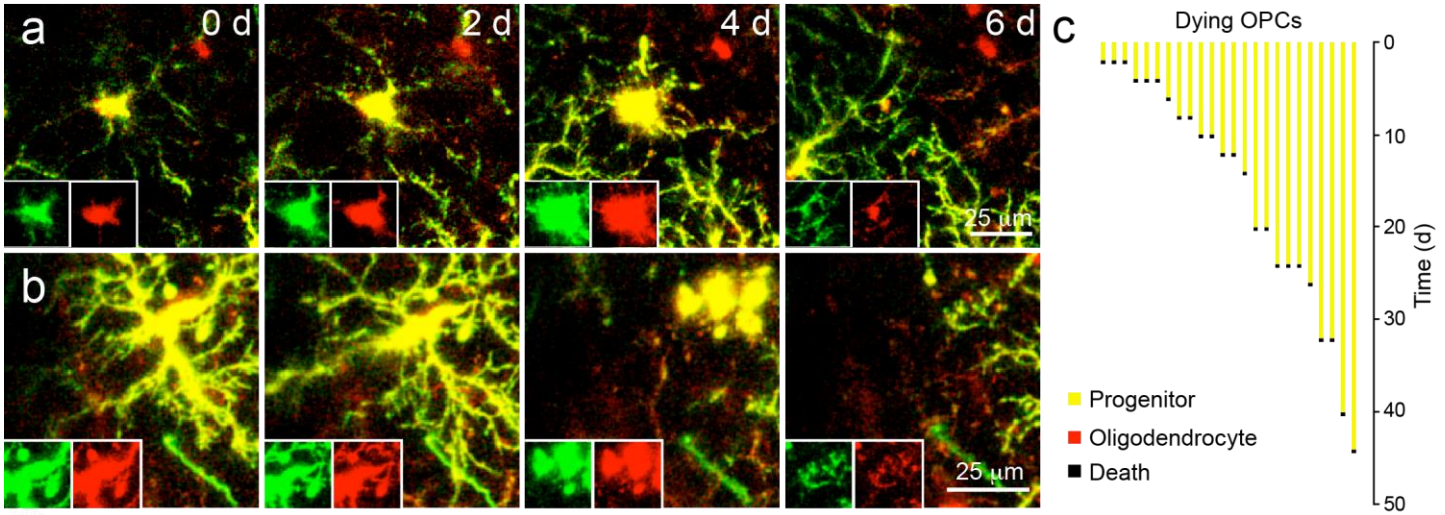
oligodendrocyte precursor cell number in a 0.6 mm^2 area in young, middle-aged and aged mice (n = 4 mice each group; p = 0.95, F(2, 9) = 0.05, One-way ANOVA; n.s. = not significant). **d-f**, Data is presented as mean ± SEM.



Supplementary Figure 4

Specificity of expression in fate-mapped mouse OPCs

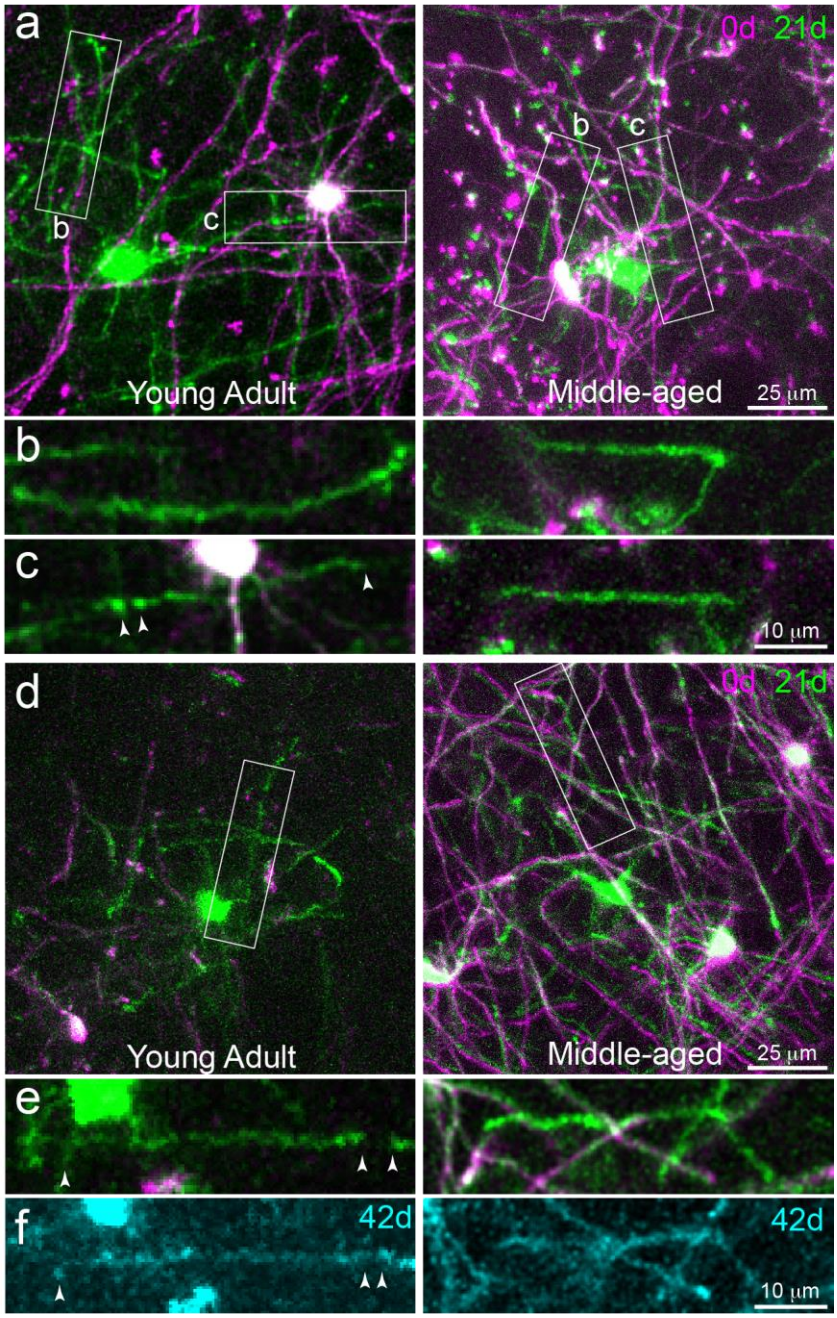
Maximal projections from triple transgenic mice (*Olig2-CreER; R26-lsl-tdTomato;NG2-mEGFP*) at P150 (100 mg/kg tamoxifen injected for five days at P30, n = 3 mice) immunostained for EGFP, tdTomato and a marker of mature oligodendrocytes or OPCs. **a**, A subset of mature oligodendrocytes (ASPA+) express tdTomato. **b**, OPCs that express membrane-anchored EGFP and tdTomato were immunoreactive for PDGF receptor alpha (PDGFRa). Note mature oligodendrocyte that does not express EGFP and is not labeled with PDGF receptor alpha (yellow arrowhead). **c**, A small population of astrocytes are recombined and express tdTomato only. Astrocytes are not immunolabeled with PDGF receptor alpha and are distinguished by their distinct bushy morphology. Note the OPC labeled with PDGF receptor alpha that expresses both EGFP and tdTomato (yellow arrowhead).



Supplementary Figure 5

In vivo imaging of OPC death

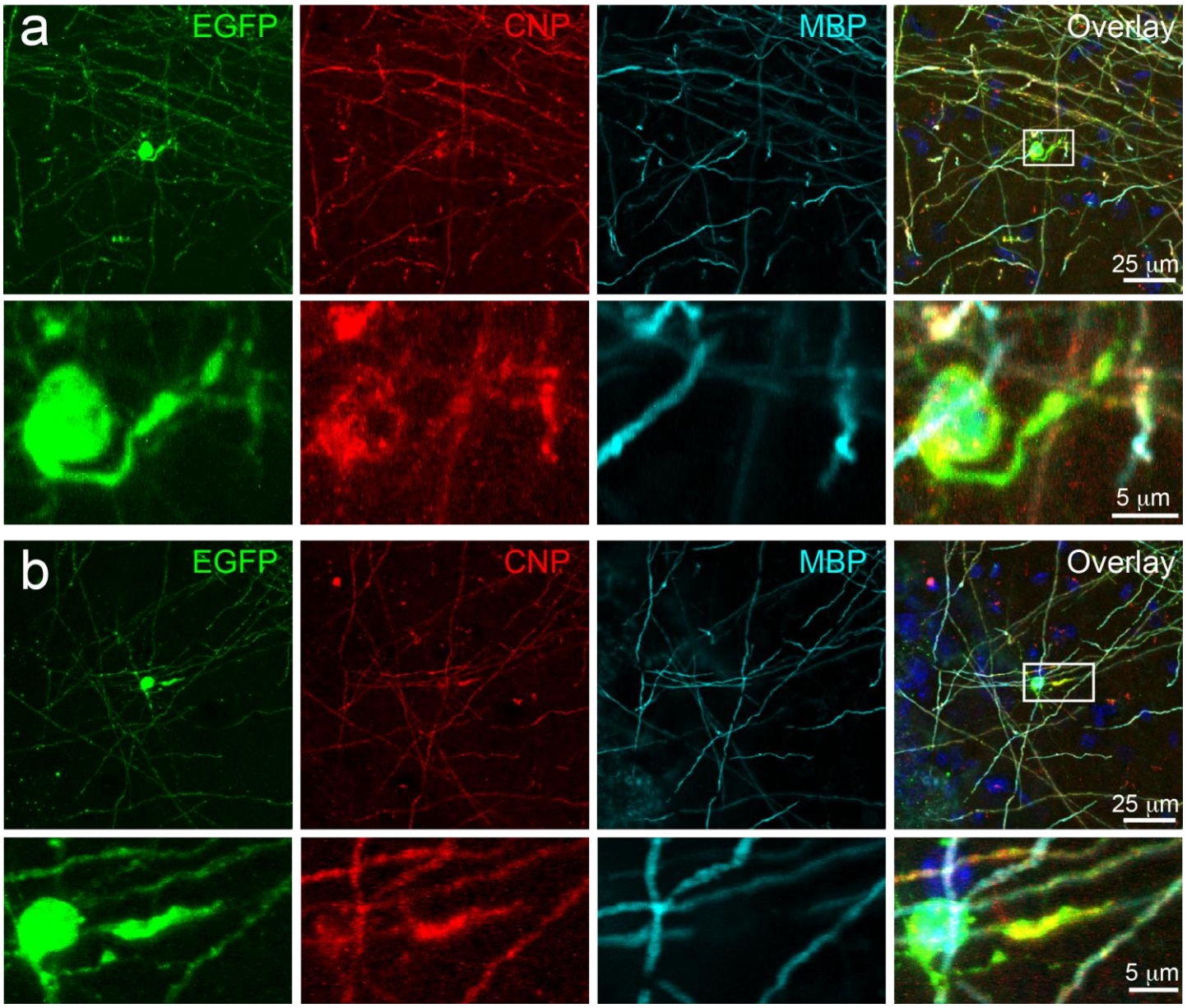
a-**b**, Maximal intensity projection of dying OPCs from triple transgenic mice (*Olig2-CreER; R26-lsl-tdTomato;NG2-mEGFP)*. **a**, P219; depth= 90-132 μm; **b**, P203; depth= 102-159 μm). Images were acquired every two days for six days. Inset panels show the cell soma at higher resolution and each individual fluorescent channel. **c**, Quantification of time-course of OPC death over 1.5 months in >P190 mice. (n = 3 mice; dying OPCs = 24).



Supplementary Figure 6

Adult-born oligodendrocytes ensheath unmyelinated regions of axons

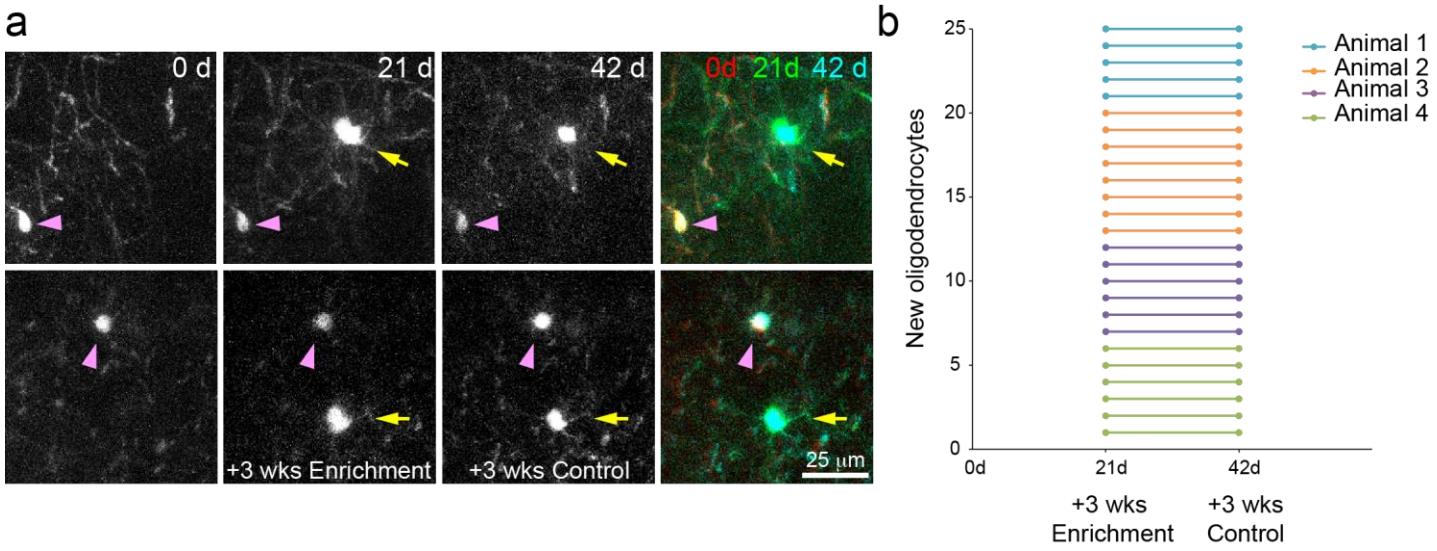
a-c, Maximal intensity projection of newly generated oligodendrocytes (P96; Left- depth= 129-159 μm; Right- P426, depth= 78-108 μm). Images were acquired at baseline (0d) and after 3 weeks of sensory enrichment (21d). These new oligodendrocytes generate Isolated and Interrupted sheaths shown in **b** and **c** indicating they ensheath unmyelinated stretches of axons. (Young Adult, n = 8 mice; Middle-aged, n = 13 mice) **d-f**, Maximal intensity projection of newly generated oligodendrocytes (P135; Left- depth= 15-33 μm; Right- P426, depth= 68-98 μm). Images were acquired at baseline (0d), after 3 weeks of sensory enrichment (21d), and following an additional 3 weeks in standard housing (42d). These newly generated sheaths are stable over the next 3 weeks shown in **e** (green) and **f** (pseudo-colored to cyan). (Young Adult, n = 8 mice; Middle-aged, n = 13 mice)



Supplementary Figure 7

Internodes with abnormal morphology in layer I of cortex

a-**b**, Individual oligodendrocytes in a fixed horizontal section from layer I of somatosensory cortex immunostained with EGFP, CNPase (CNP), and MBP (P365; *Mobp-EGFP* mouse, n = 3 mice). Note the presence of myelin internodes with abnormal morphology near the cell soma (boxes). Areas from **a**, **b** are shown below at higher magnification. Note the internodes with abnormal morphologies are not MBP positive.



Supplementary Figure 8

Sensory enrichment results in the stable integration of new oligodendrocytes

a, Maximal intensity projection of a newly generated oligodendrocytes (P365; to- depth= 105-114 μm; bottom- depth= 303-318 μm). Images were acquired at baseline (0d), after 3 weeks of sensory enrichment (21d), and following an additional 3 weeks in standard housing (42d). Pink arrowheads designate existing oligodendrocytes. Yellow arrows designate new oligodendrocytes.
b, Quantification of oligodendrocyte addition after sensory enrichment (21d) and stability following 3 additional weeks in standard housing (42d; n= 4 mice, persistent new oligodendrocytes= 25/25).