SUPPLEMENTARY INFORMATION

The oligodendrocyte-specific G-protein coupled receptor GPR17 is a cell intrinsic timer of myelination

Ying Chen, Heng Wu, Hisami Koito, Jianrong Li, Jenny Hoang, Feng Ye, Shuzong Wang, Sabine S. Escobar, Alex Gow, Heather A. Arnett, Bruce D. Trapp, Nitin J. Karandikar, Jenny Hsieh and Q. Richard Lu

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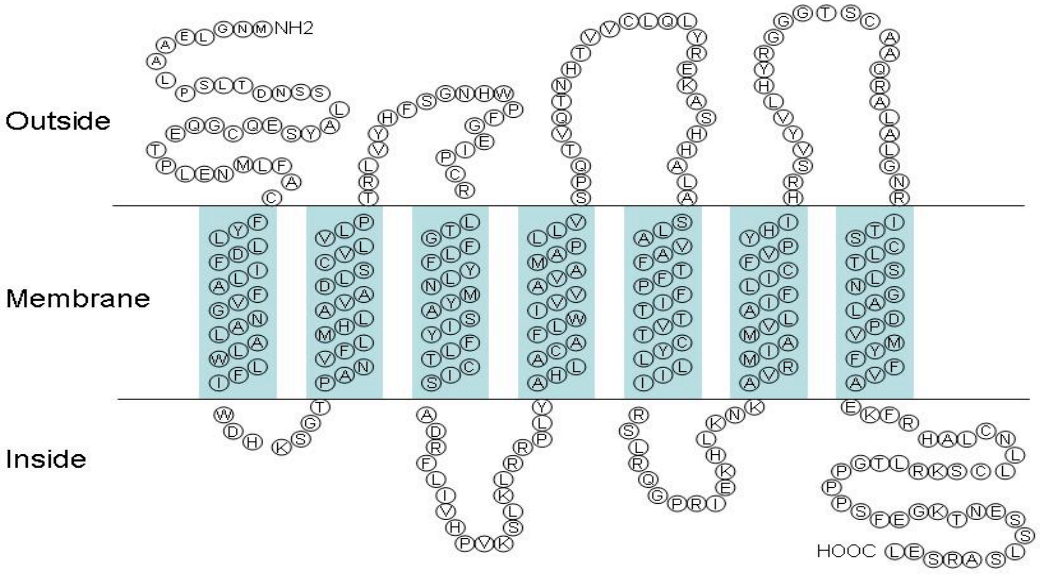

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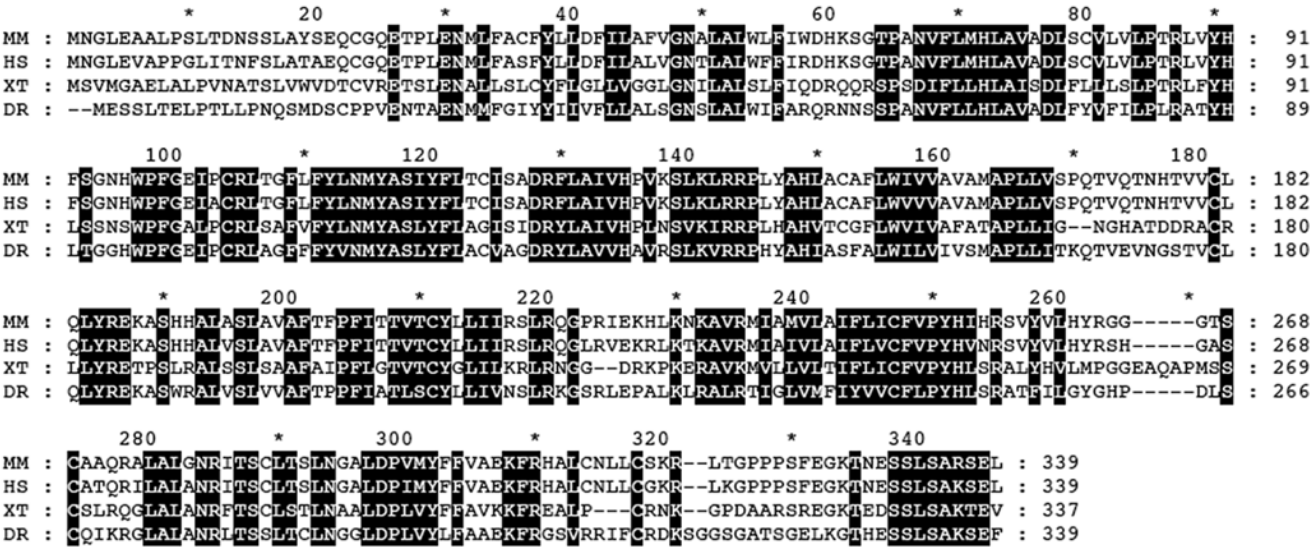
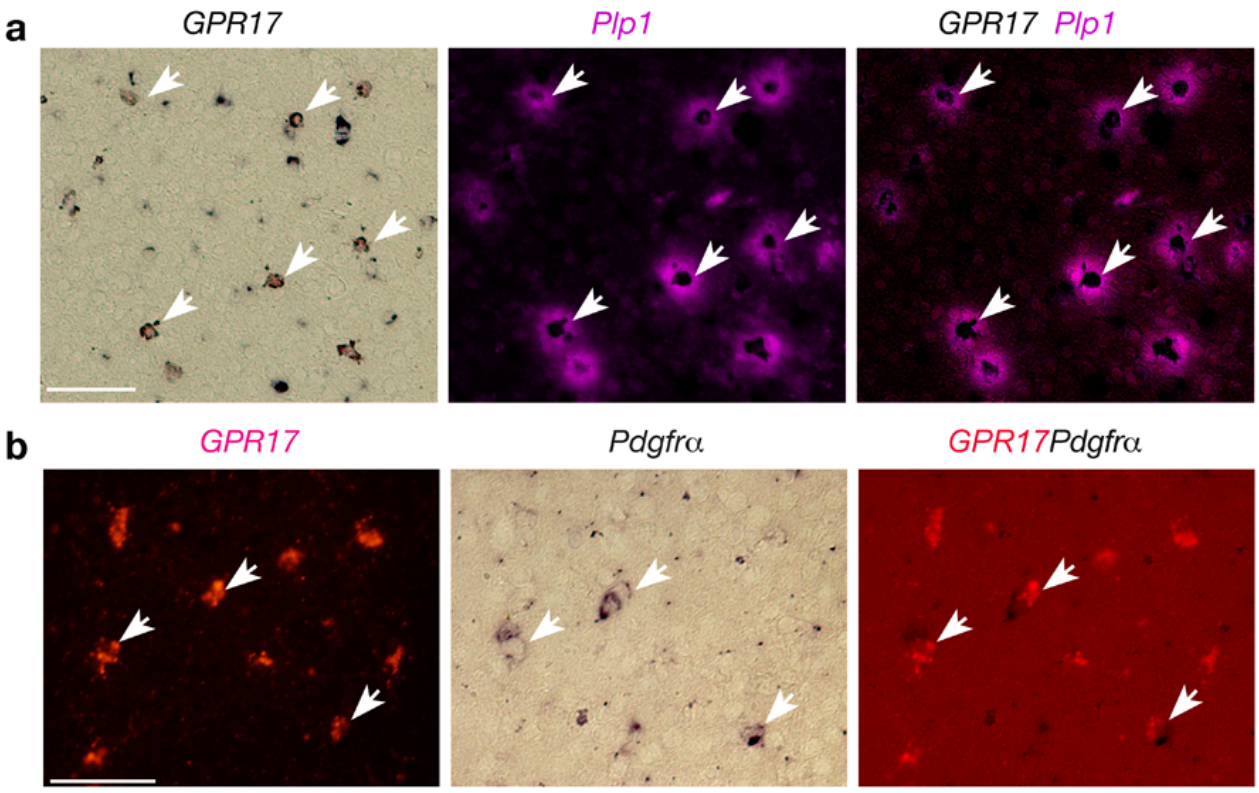


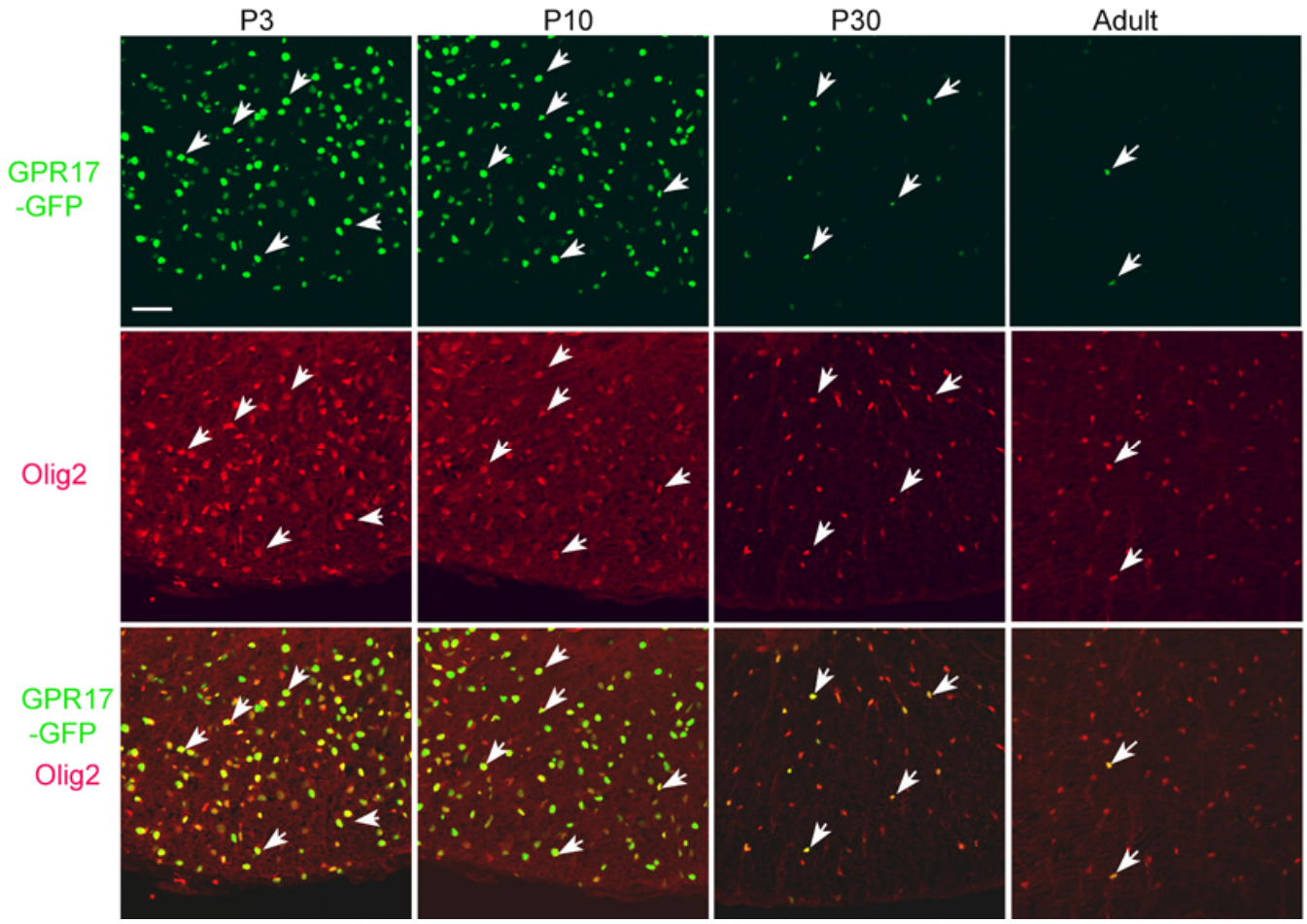
Supplementary Figure 1. Primary sequence and structure of GPR17 protein

a) The predicted amino acid sequence of mouse *GPR17* encodes a 339 amino acid residue with typical seven transmembrane motifs as a rhodopsin P2Y type. **b**) Alignment of evolutionarily conserved GPR17 among vertebrate species including human, rat, *Xenopus* and Zebrafish. Conserved residues are boxed.



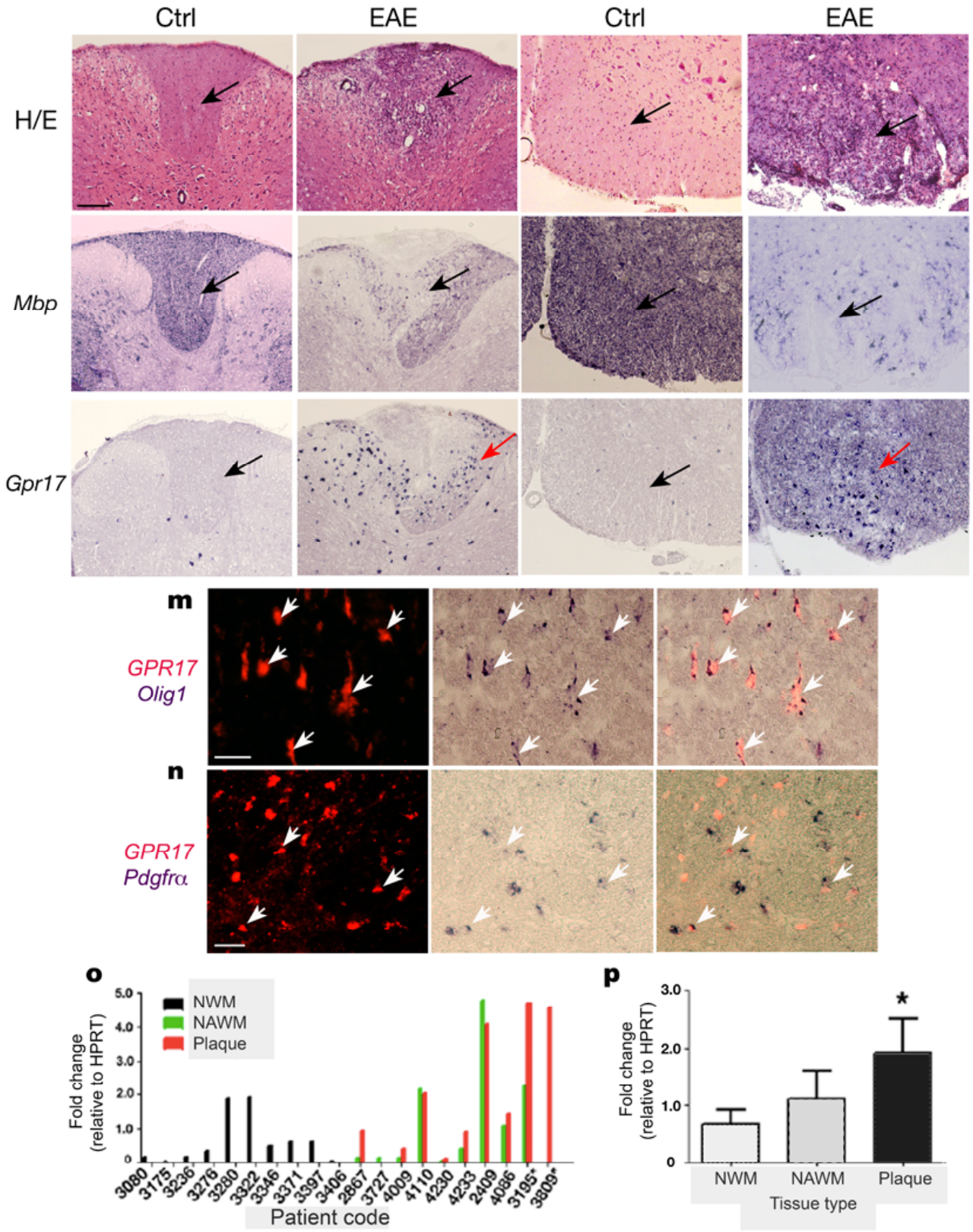
Supplementary Figure 2. *GPR17* expression in oligodendrocyte lineage cells

Double *in situ* hybridization on the cortical sections from wildtype mice at P14 with colorimetric or tyramide fluorescent amplification of antisense probes to murine *GPR17*, *Plp1***(a)** or *Pdgfrα* **(b)** as indicated. Arrows indicate co-labeling cells. Scale bars in **a** and **b**: 100 μm.



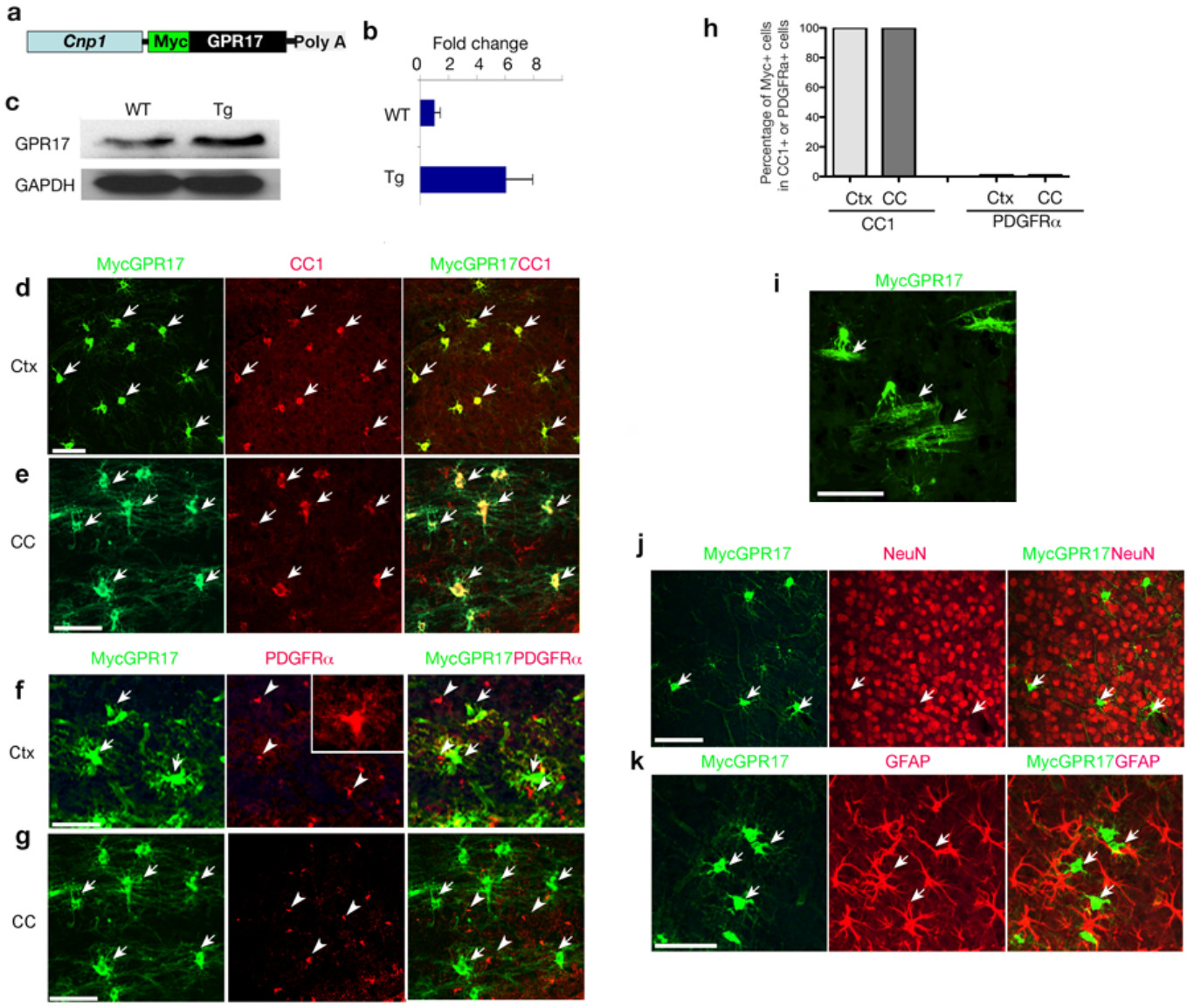
Supplementary Figure 3. Transient expression of *GPR17* in the spinal cord during development

Spinal cord sections from *GPR17-GFP+/-* knockin mice at P3, P10, P30 and adulthood were immunostained with an antibody against Olig2. Arrows indicate GFP+ cells (upper), Olig2+ cells (middle) and double GFP+/Olig2+ cells (lower panel), respectively. Notably, the number of GFP+/Olig2+ cells decreases at P30 and adulthood compared to early postnatal stages P3 and P10. Scale bars in **a**: 100 μm.



Supplementary Figure 4. *GPR17* upregulation in demyelinating lesions of EAE mice and human MS

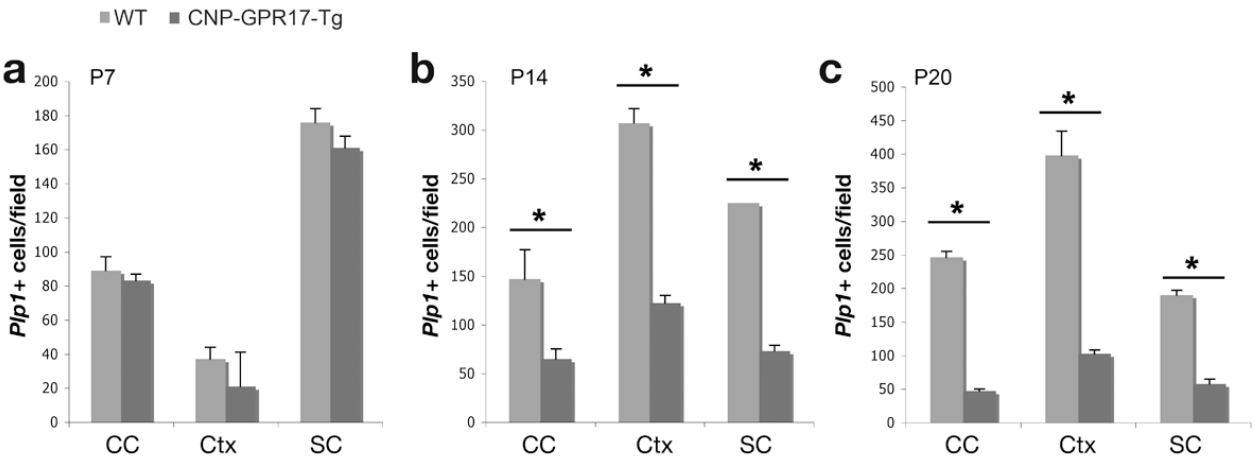
a-l) Spinal cord sections from control and EAE mice (clinical score 3 and 4) were subjected to H/E histology analysis and in situ hybridization for *GPR17* and *Mbp*. Adjacent dorsal funiculus regions (**a,b; e,f; i,j**) and ventral white matter regions (**c,d; g,h; k,l**) are shown. Arrows indicate the control and inflammatory demyelinating lesions with enormous immune cell infiltration (**b,d**) and corresponding *Mbp* and *GPR17* expression in the adjacent sections. **m-n)** Double fluorescent in situ hybridization was performed with antisense riboprobes directed against *Olig1/GPR17* (**m)** and *Pdgfrα*/*GPR17* (**n**) on white matter lesions of EAE mice. Arrows indicate the double labeling cells. **o**) A bar chart shows the relative values of *GPR17* expression vs. *HPRT* (hypoxanthine-guanine phosphoribosyltransferase) derived from a cohort of 20 MS tissues by quantitative real time PCR analysis. **p**) A histograph shows the level of *GPR17* expression at indicated regions of MS. NWM, normal white matter samples; NAWM, MS normal appearing white matter samples; and Plaque, MS Plaque. Data are present as means ± SDs. *P<0.01. One-way ANOVA with Newman-Keuls Multiple comparison test. Scale bars: **a-l**, 200 μm; and **m-n**, 100 μm.



Supplementary Figure 5. Generation of transgenic mice with *GPR17* expression in oligodendrocytes

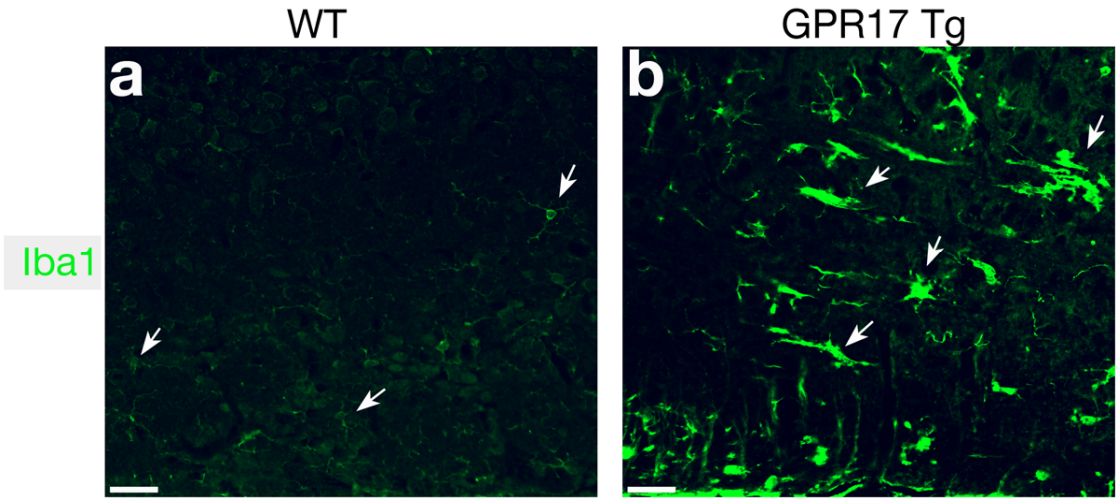
a) Schematic representation of the *CNP-GPR17* transgene consisting of mouse CNP1 promoter myc-tagged GPR17 followed by bovine beta-globin polyA signal**. b-c**) Amounts of *GPR17* mRNA and protein from the brain of transgenic and wild-type progeny of a transgenic founder at P12 were subject to semi-quantitative RT-PCR (**b**) and Western blot (**c**) analysis. Values were normalized to GAPDH for each sample. Transcript amounts in transgenic samples were expressed as folds over the wild-type value (n=3). **d-g**) Postnatal CNS expression of the *GPR17*

transgene at P12. Immunohistochemistry against Myc tag, CC1 (**d,e)** and PDGFRα (**f,g)** was performed in the cerebral cortex and the corpus callosum (CC). Arrows and arrowheads indicate Myc+, CC1+ or PDGFRα+ cells, respectively. Inset in **f** indicates a PDGFRα+ cell. **h**) Quantification of Myc+ cells expressing CC1 or PDGFRα in the cortex (Ctx) and the corpus callosum (CC) (n=3). **i**) Expression of the *GPR17* transgene product (Myc+) is detected in multiple parallel processes (arrows) of oligodendrocytes wrapping around the axons in the brain at P12. **j-k**) The cortex of *CNP-GPR17* transgenic mice was immunostained with Myc, neuronal marker (NeuN, **j)** or astrocyte marker GFAP (**k**). Arrows indicate the location of Myc-GPR17+ cells. Scale bars in **d-g** and **i-k**: 50 μm.



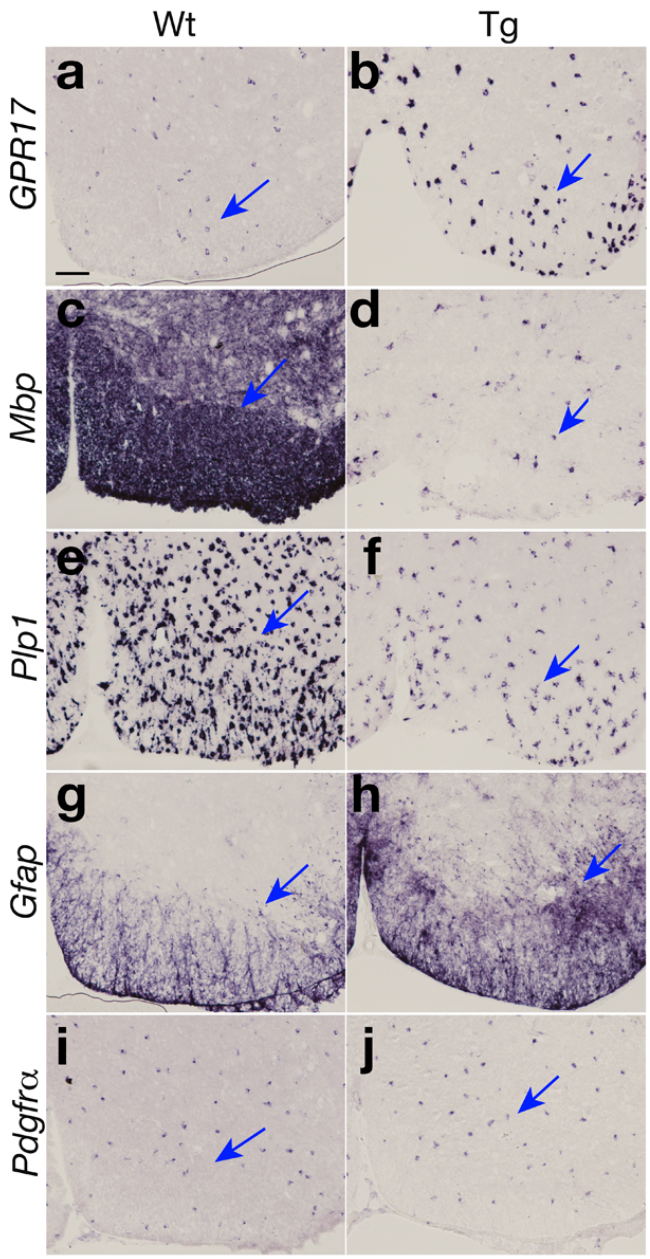
Supplementary Figure 6. Oligodendrocyte formation in *GPR17* transgenic mice during CNS development

In situ hybridization was performed on transverse spinal cord and brain sections of *WT* and *CNP1-GPR17* transgenic mouse from P7 (**a**), P14 (**b**) and P20 (**c**) with a probe to murine *Plp1*. The number of *Plp*+ oligodendrocytes per field (0.08 mm^2) at corresponding regions of control and transgenic mice was quantified in the corpus callosum (CC), cortex (Ctx), and spinal cord (SC). Error bars represent SDs. *P<0.01, Student *t*-test, n=3.



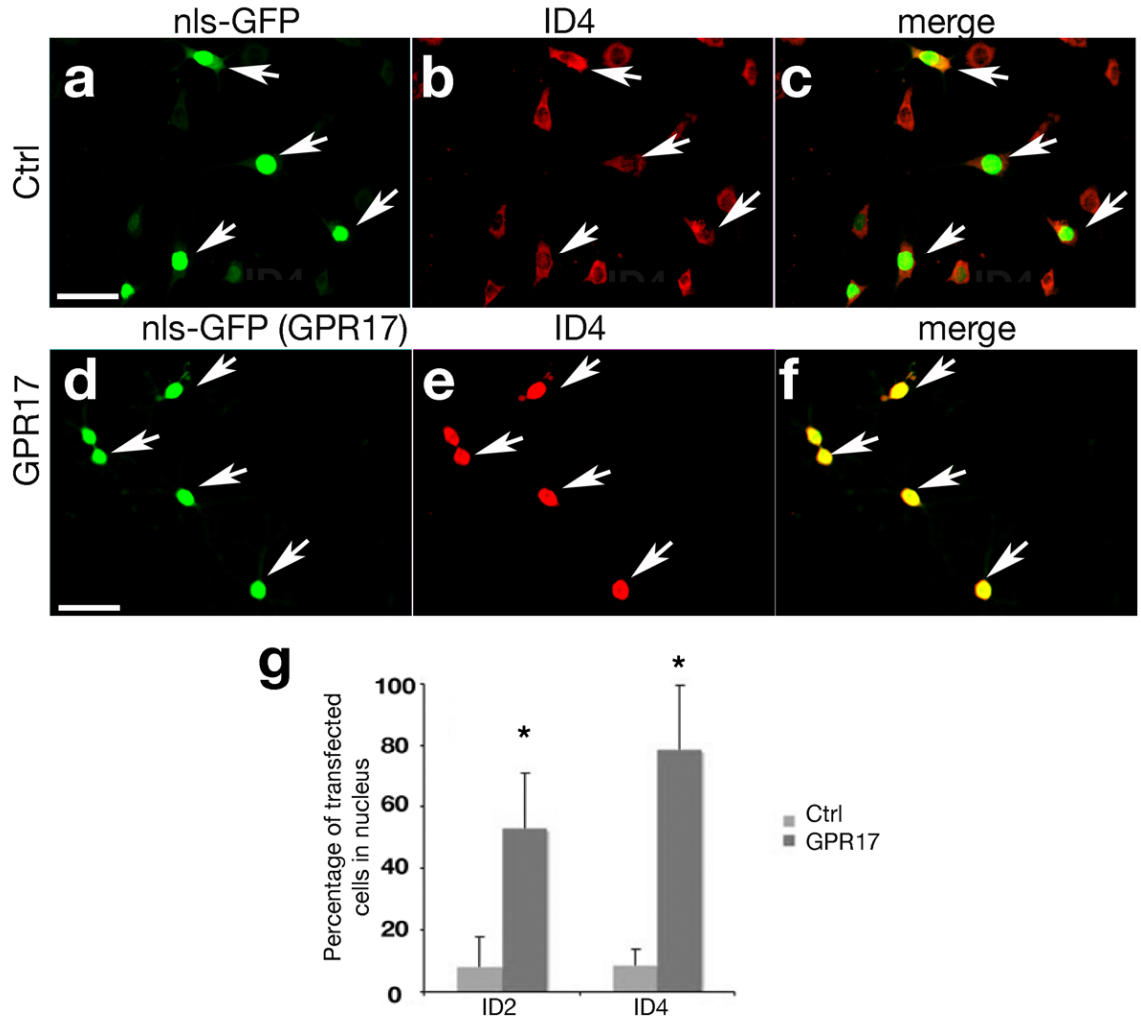
Supplementary Figure 7. Microglia activation in the brain of *GPR17* transgenic mice

Brain sections of wildtype (WT) and *GPR17* transgenic mice at P20 were immunostained with a microglia marker Iba1. Arrows in **b** indicate hyper-ramified activated microglia in the cerebral white matter compared to resting microglia in WT mice (**a**). Scale bars in **a** and **b**: 50 µm.



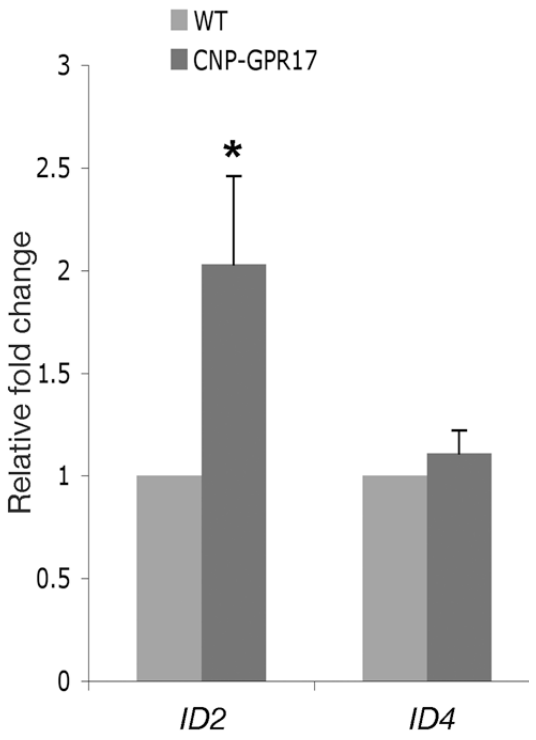
Supplementary Figure 8. Myelination defects and gliosis in the spinal cord of *GPR17* transgenic mice

a) *In situ* hybridization was performed with antisense riboprobes against *GPR17* **(a-b)**, *Mbp* (**c-d**), *Plp1* (**e-f**), *Gfap* **(g-h)** and *Pdgfrα* **(i-j)** on spinal cross-sections of wild-type (Wt) and *CNP-GPR17* transgenic (Tg) littermates at P20. Arrows indicate in situ labeling cells. Note: intense GPR17 expression in Tg mice (**b**) with reduced myelin gene expression (**d,f**) and *Gfap* upregulation (**h**). Expression of *Pdgfrα* is comparable between wildtype and transgenic mice (**i-j**). Scale bar in **a-j**: 100 µm.



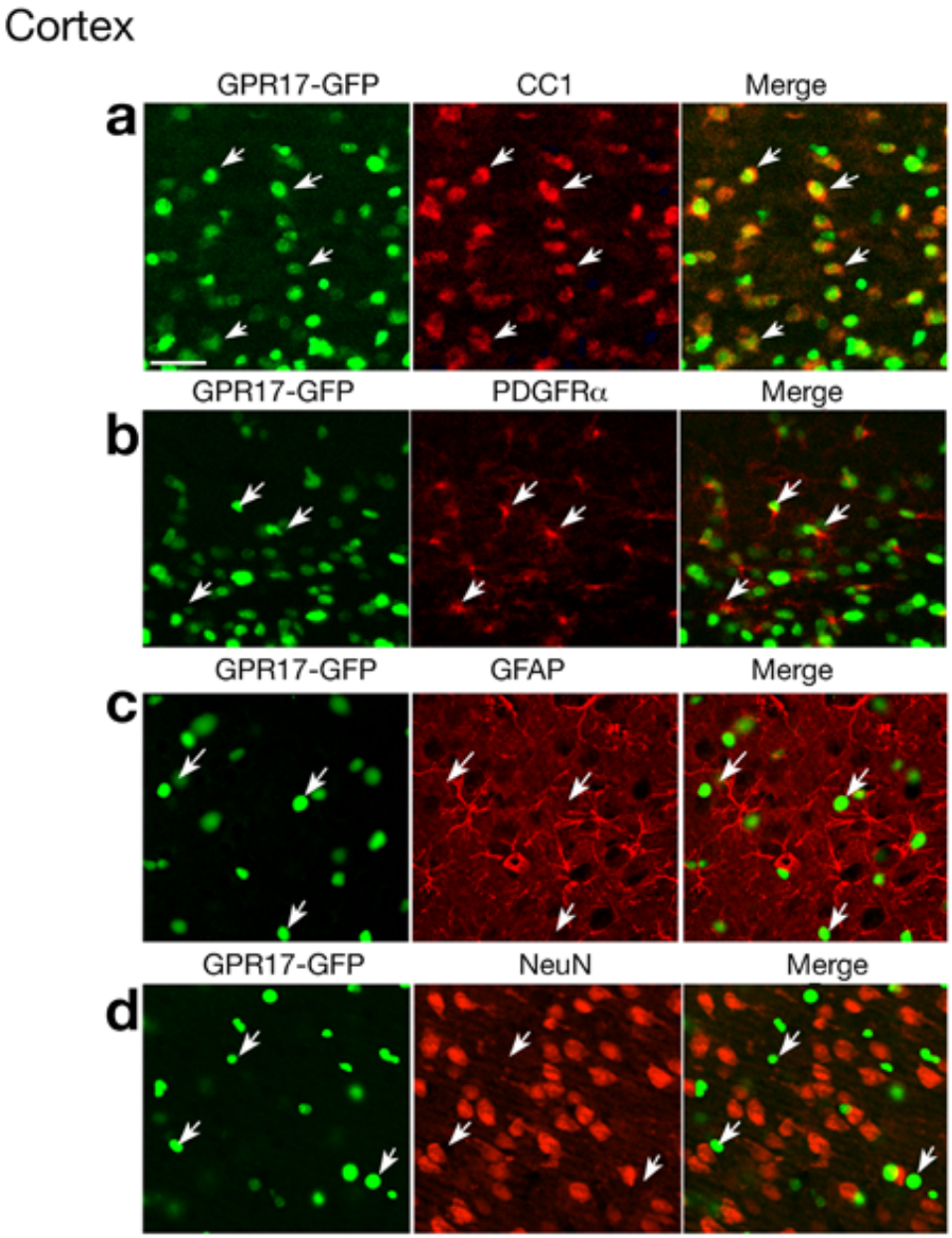
Supplementary Figure 9. Nuclear translocation of ID4 in *GPR17* overexpressing neural precursor cells

HCN precursor cells were transfected with control pCIG (**a-c**) and *GPR17* (**d-f**) expression vectors were immunostained with an antibody to ID4. **c** and **f** are the overlay of **a,b** and **d,e**, respectively. Note that ID4 staining was detected in the nucleus of *GPR17*-transfected cells indicated by nuclear-localized GFP (nls-GFP) (**e,f**, arrows) while in the cytoplasm of control GFP transfected cells (**b, c**). **g**) Quantification of cells with nuclear localized ID2 and ID4 expression after transfected with control and *GPR17* expressing vectors. All data shown are from at least three experiments (>1500 cell count) in parallel cultures with error bars representing SDs, *P<0.01. Student *t*-test. Scale bar in **a-f**: 50 μm.



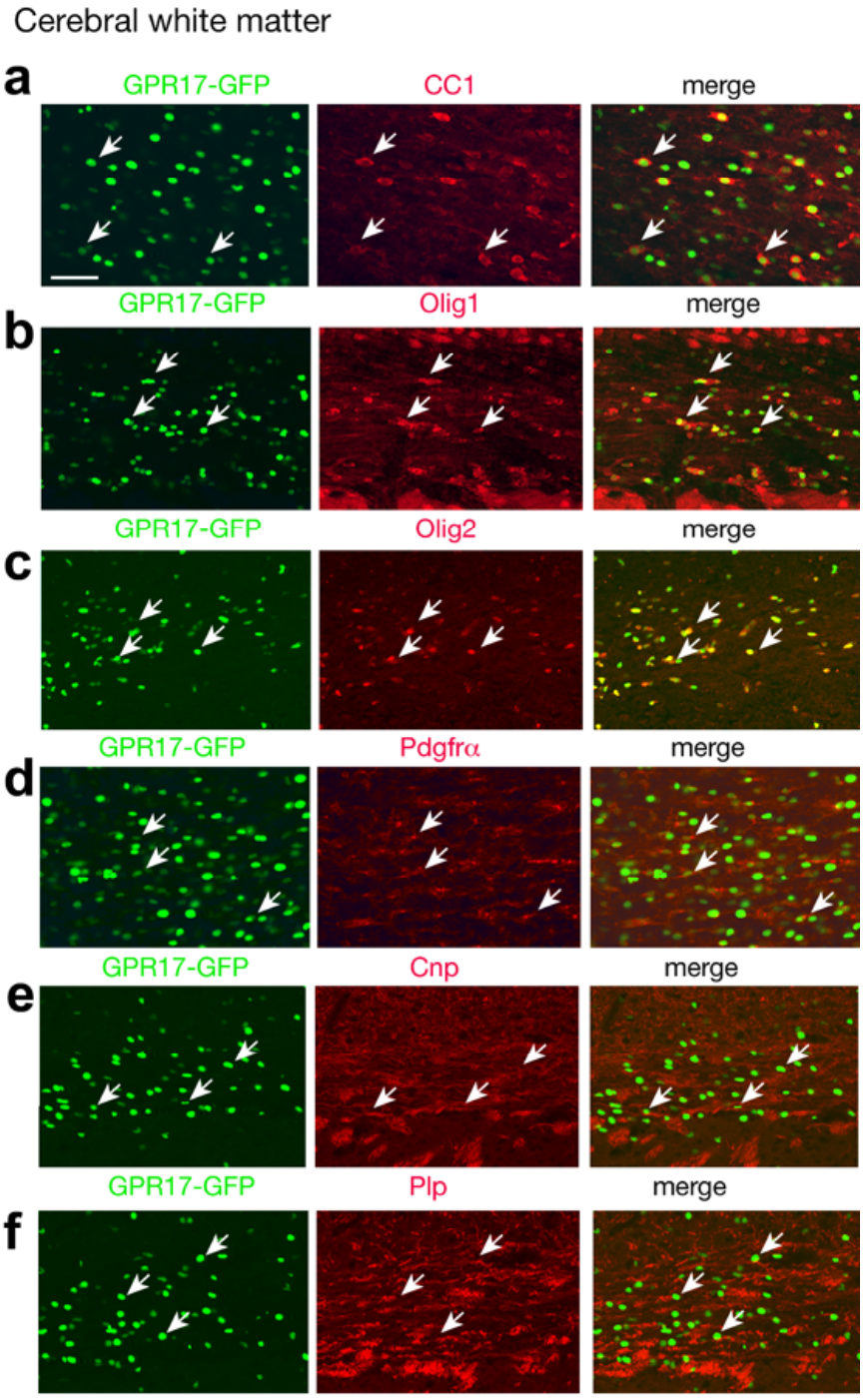
Supplementary Figure 10. ID2 and ID4 expression in *GPR17* transgenic mice

Total RNAs extracted from the corpus callosum of wildtype and *GPR17* transgenic mice at P20 were subjected to qRT-PCR analysis for *ID2* and *ID4*. Note: there is a significant increase of the *ID2* level in the CNP-GPR17 Tg versus control (*P<0.01, Student *t*-test).



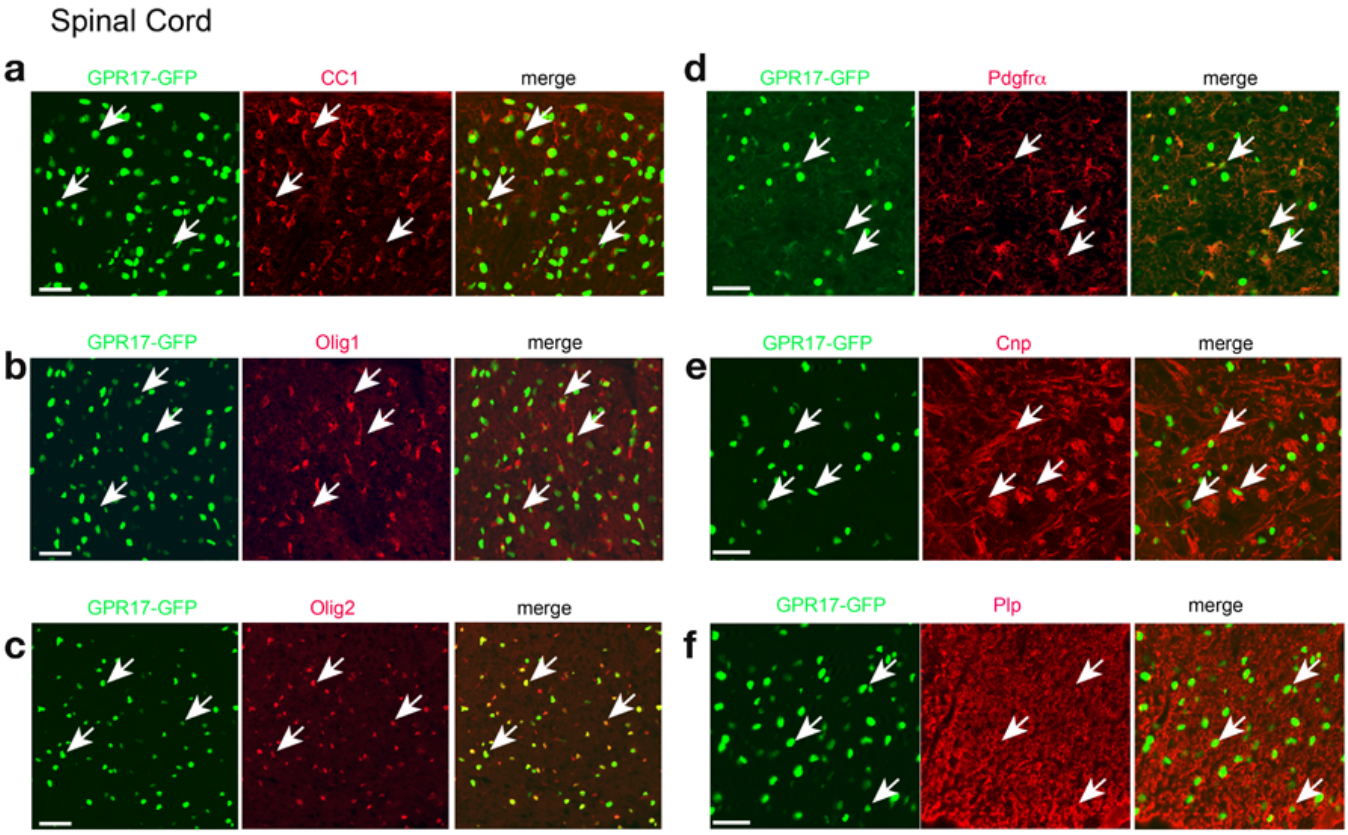
Supplementary Figure 11. GPR17 expression is restricted to the oligodendrocyte lineage in the cortex

Cortical sections of *GPR17-GFP*+/- mice at P14 were immunostained with GFP and markers for oligodendrocytes (CC1, PDGFRα), neurons (NeuN) and astrocytes (GFAP) as indicated. The images were taken from cortical layers 4-6, which are known to possess numerous oligodendrocytes and myelinated tracts. Arrows in **a** and **b** indicate CC1+/GFP+ or PDGFRα+/GFP+ co-labeling cells, respectively. **c-d**) GFP expressing cells (green) were not detected in NeuN+ neurons (red) and GFAP+ astrocytes (red) in the brain. Arrows indicate the location of GPR17-GFP+ cells. Scale bar: 50 μm.



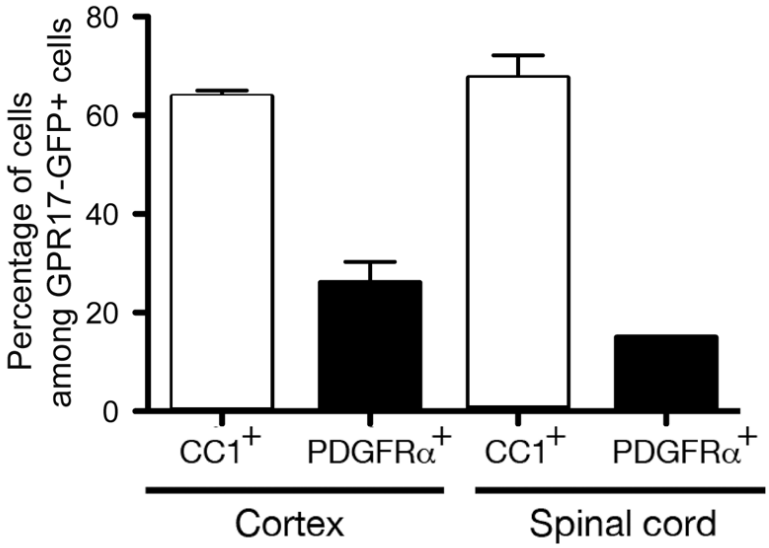
Supplementary Figure 12. GPR17 is expressed in the oligodendrocyte lineage in the cerebral white matter

The sections from the brain of *GPR17*+/- mice at P14 were immunostained with GFP and markers for oligodendrocytes CC1 **(a)**, Olig1 **(b)**, Olig2 **(c),** PDGFRα **(d)**, CNP **(e)** and Plp **(f)** as indicated. Arrows in indicate co-labeling cells of GFP with corresponding markers, respectively. Scale bar: 50 μm.



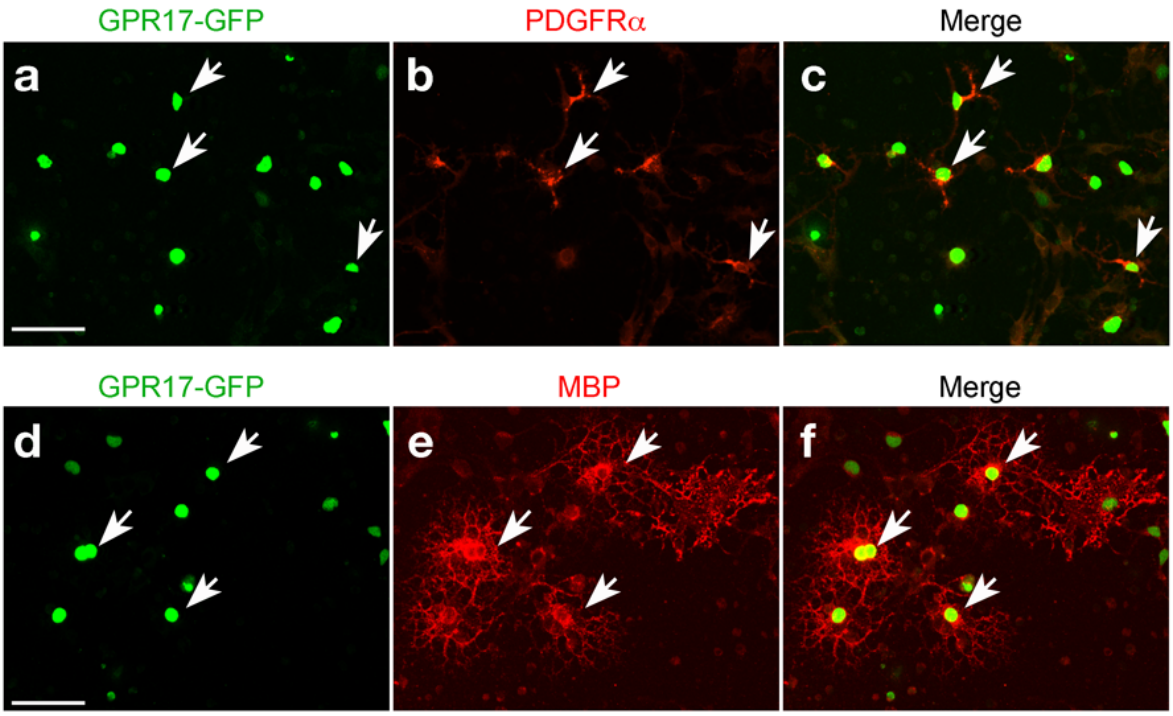
Supplementary Figure 13. GPR17 is expressed in the oligodendrocyte lineage in the spinal cord

The sections from the spinal cord of *GPR17*+/- mice at P14 were immunostained with GFP and markers for oligodendrocytes CC1 **(a)**, Olig1 **(b)**, Olig2 **(c),** PDGFRα **(d)**, CNP **(e)** and Plp **(f)** as indicated. Arrows in indicate co-labeling cells of GFP with corresponding markers, respectively. Scale bar: 50 μm.



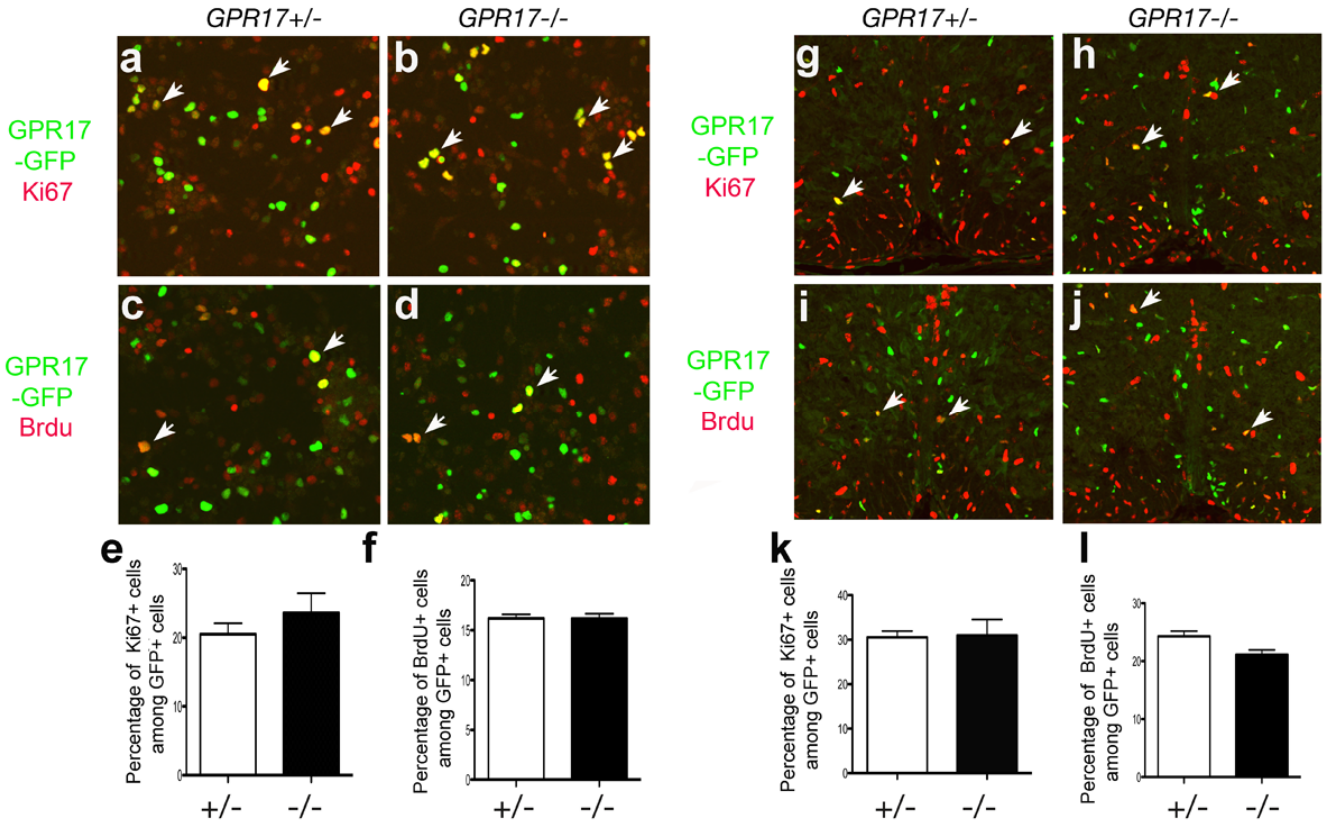
Supplementary Figure 14. The percentage of oligodendrocyte precursors and differentiated oligodendrocytes among GPR17-GFP+ cells in the CNS.

Cortical and spinal cord sections of *GPR17-GFP*+/- mice at P10 were immunostained with GFP and markers for differentiated oligodendrocytes (CC1) and their precursors (PDGFRα). The percentage of CC1+ or PDGFRα+ cells among GPR17+ cells was quantified as indicated (>1,000 *GPR17*+ cells were analyzed).



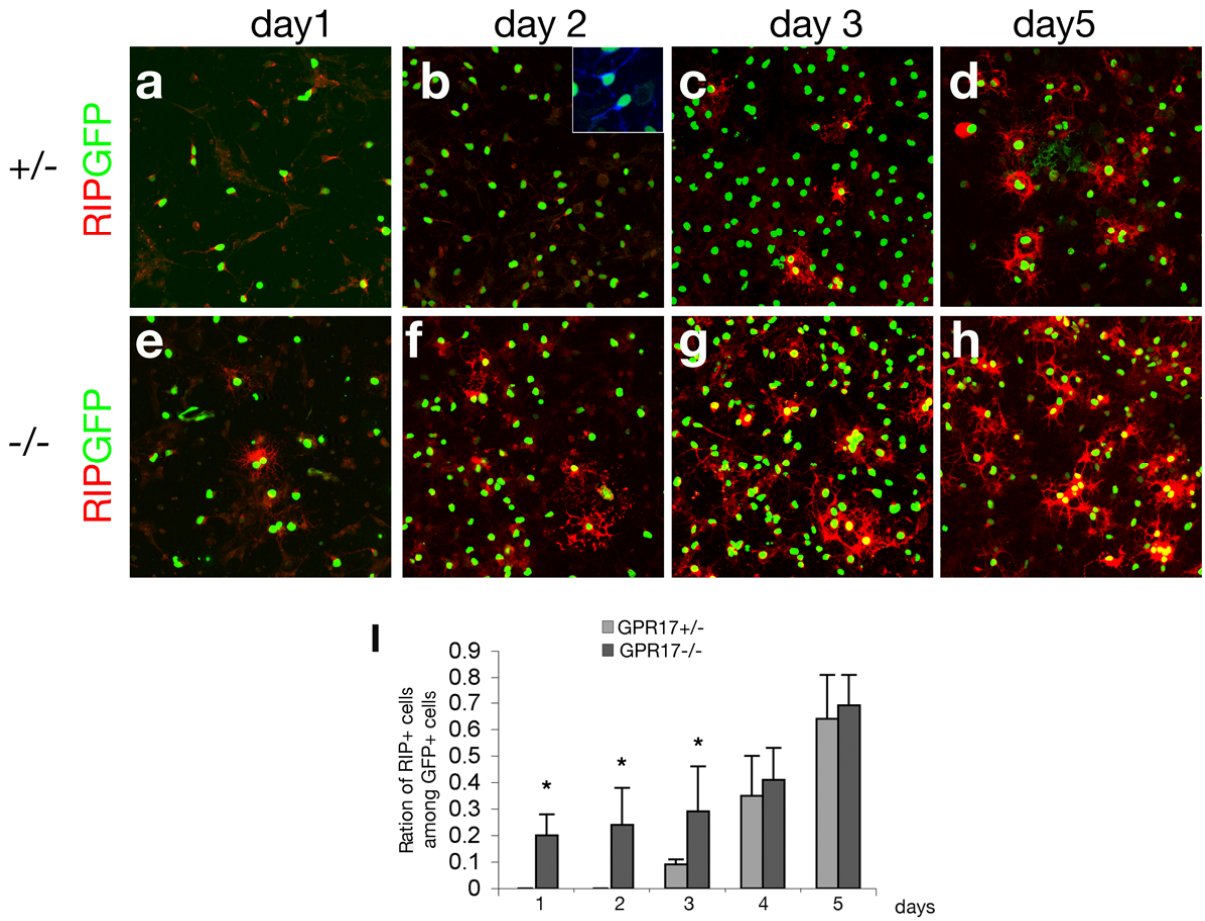
Supplementary Figure 15. Expression of GPR17 in oligodendrocytes and their precursors in vitro

Cortical progenitor cells isolated from *GPR17-GFP+/-* embryos at E15.5 were cultured in the oligodendrocyte growth medium to enrich oligodendroglial precursor cells for 5 days (**a-c**). These precursor cells were then plated in the differentiation medium for 3 days (**d-f**). Cells were immunostained with antibodies to PDGFRα **(a-c)** and MBP **(d-f).** Arrows in indicate co-labeling cells. Scale bars: 50 μm.



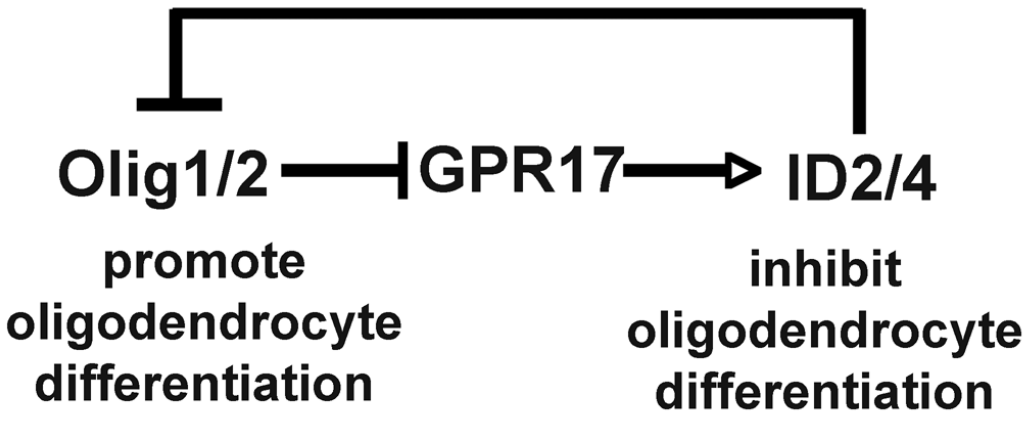
Supplementary Figure 16. Normal OPC proliferation in the absence of *GPR17* in vitro and in vivo

a-d) Cortical progenitor cells isolated from *GPR17*+/- and -/- embryos at E15.5 were cultured in oligodendrocyte growth medium for 5 days. After 1 hr BrdU pulse, cells were immunostained antibodies to Ki67 **(a, b)** or Brdu **(c, d)**. Arrows in indicate co-labeling cells for GPR17-GFP/Ki67 or GPR17-GFP/BrdU, respectively. **e-f)** Quantification of GFP+ cells expressing Ki67 **(e)** or Brdu **(f)** in oligodendrocyte progenitor cell culture**.** The mean values are derived from three independent experiments. Error bars present SDs. **g-j)** Sections from the ventral spinal cord of *GPR17*+/- and -/- mice at e18.5 were immunostained with Ki67 **(g, h)** and Brdu **(i, j)** 2 hrs after BrdU injection to the pregnant mother (100 mg BrdU/kg body weight). Arrows indicate co-labeling cells for GPR17-GFP/Ki67 or GPR17-GFP/BrdU in the ventral spinal cord, respectively. **k-l)** Quantification of GFP+ cells expressing Ki67 **(k)** or Brdu **(l).** The mean values are derived from three +/- and -/- littermates. Scale bars: 50 μm. Error bars present SDs.



Supplementary Figure 17. Accelerated oligodendrocyte maturation in *GPR17* null progenitor cells in vitro

Cortical progenitor cells from *GPR17+/-* **(a-d)** and -/- **(e-h)** embryos at E15.5 were cultured in the oligodendrocyte growth media and immunostained at defined days as indicated. Cells were immunostained antibodies to a differentiated oligodendrocyte marker RIP and GFP. GFP+ cells represent the *GPR17* expressing cells. In control *GPR17+/-* culture, RIP+ cells were not detected 2 days after plating (**a-d**) while appearance of RIP+ cells began on day 1 after plating (**e-h**)**.** At day 1 and 2, those neural progenitor cells were maintained as OPCs expressing PDGFRα **(b,** blue labeling; insert**),** while RIP+ cells from *GPR17-/-* culture were readily detected at one day after plating. **i**) Quantification of cells expressing RIP at indicated days in culture. All data shown are derived from three experiments in parallel cultures of at least three age-matching littermates with error bars representing SDs. (> 1500 cell counts, *P<0.01, Student *t*-test).



Supplementary Figure 18. A schematic model depicts Olig1-GPR17-ID2/4 negative regulatory loop in modulating oligodendrocyte differentiation.

Olig1 promotes myelin gene expression while repressing *GPR17* expression. Conversely, GPR17 signaling inhibits oligodendrocyte differentiation through activation and nucleus translocation of ID2/4, which block Olig1/2 function by forming non-functional heterodimers