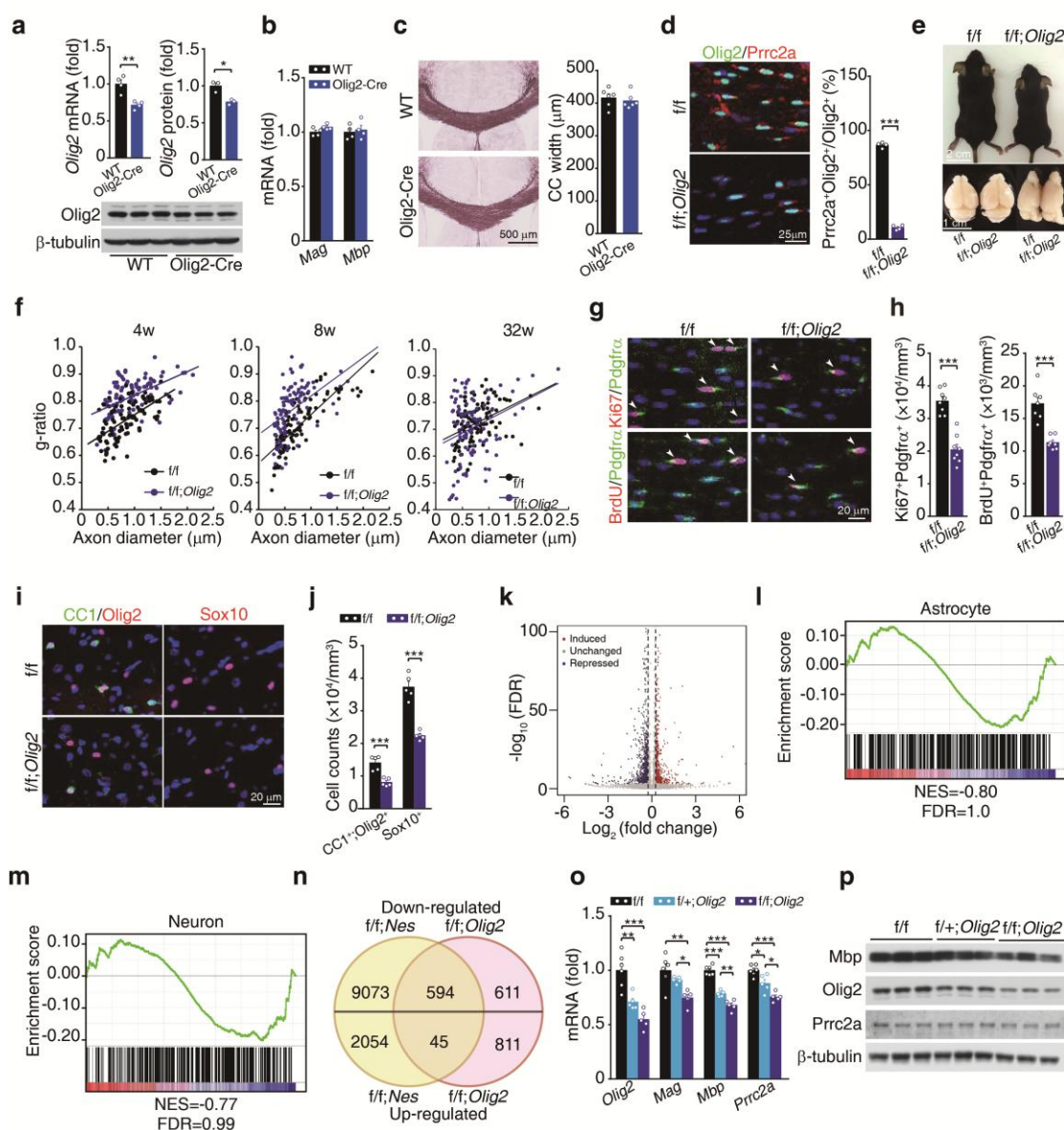


Figure S5



Supplementary Figure 5, related to Figure 6. *Prrc2a* deletion in the oligodendroglial lineage leads to hypomyelination.

(a) The gene (left upper panel) and protein (right upper panel and bottom panel) expression of *Olig2* in brain tissues from WT and Olig2-cre mice at 8 weeks old (two-tailed unpaired Student's *t*-test, * $P < 0.05$, ** $P < 0.01$; RNA level, $n = 4$ per group; protein level, $n = 3$ per group).

(b) The *Mag* and *Mbp* expressions of brain tissues from wild-type and Olig2-cre mice at 8 weeks old ($n = 4$ per group).

(c) Black gold staining of corpus callosum in brain slices from WT and Olig2-Cre mice at 4 weeks old. The quantification of corpus callosum width at the midline was shown in right bar (n=6 per group).

(d) Prrc2a co-immunostained with Olig2 in corpus callosum from control and *Prrc2a^{ff};Olig2-cre* mice at P9. The quantification of the percentage of Prrc2a expression in Olig2 positive cells (two-tailed unpaired Student's *t*-test, ****P*<0.001, n = 4 each group) was shown in the right panel.

(e) Representative picture of *Prrc2a^{ff};Olig2^{cre+/-}* and control mice at P28.

(f) Scatterplots of the myelin g ratios of the corpus callosum at 4, 8 and 32 week-old *Prrc2a^{ff};Olig2^{cre+/-}* and control mice (general linear model and ANCOVA analysis, 4w: *P*<0.001; 8w: *P*<0.001, 32w: *P*=0.124; More than 100 axons from each genotype and time points were analyzed).

(g) *Prrc2a^{ff};Olig2-cre* and control mice at P9 were sacrificed and the brain sections of corpus callosum were immunostained with Pdgfr α and Ki67 (upper panel) or mice at P6 were intraperitoneally injected with 50mg/kg BrdU, and 2 hours later, the mice were sacrificed and the brain sections of corpus callosum were immunostained for Pdgfr α and BrdU (bottom panel). Arrowheads indicate the proliferating OPCs (Pdgfr α and Ki67/BrdU double-positive cells).

(h) The quantification of Pdgfr α ⁺Ki67⁺ or Pdgfr α ⁺BrdU⁺ in corpus callosum from the indicated genotype mice (two-tailed unpaired Student's *t*-test, ****P*<0.001, Pdgfr α ⁺Ki67⁺: n=8 each group; Pdgfr α ⁺BrdU⁺: n=7 each group).

(i) Immunostaining of CC1/Olig2 or Sox10 in hippocampus from 4-week-old mice with

143 indicated genotypes.

144 **(j)** The quantification of CC1⁺ Olig2⁺ or Sox10⁺ cells (two-tailed unpaired Student's *t*-test,
145 ****P*<0.001, n=5 per group).

146 **(k)** Volcano plot of RNA-seq data showing *Prrc2a*-regulated genes from brain tissue samples
147 of 4-week-old *Prrc2a*^{f/f}; *Olig2*^{Cre+/-} versus control mice.

148 **(l)** GSEA plots evaluating the changes in astrocyte related genes from brain tissue samples of
149 *Prrc2a*^{f/f}; *Olig2*^{Cre+/-} versus control mice.

150 **(m)** GSEA plots evaluating the changes in neuron related genes from brain tissue samples of
151 *Prrc2a*^{f/f}; *Olig2*^{Cre+/-} versus control mice.

152 **(n)** Overlapped DEGs from *Prrc2a*^{f/f}; *Nestin*^{Cre+/-} and *Prrc2a*^{f/f}; *Olig2*^{Cre+/-} versus their controls.

153 **(o)** RT-qPCR analysis of the myelin related gene expression in *Prrc2a* deficiency and control
154 mice at 6 weeks old (one-way ANOVA followed Tukey test, **P*<0.05, ***P*<0.01,
155 ****P*<0.001, f/f, n=6; f/+; *Olig2*, n=5; f/f; *Olig2*, n=5).

156 **(p)** Western blot analysis of protein expression using the indicated antibodies in isolated
157 hippocampus from *Prrc2a* deficiency and control mice at 6 weeks old.