# Figure S1

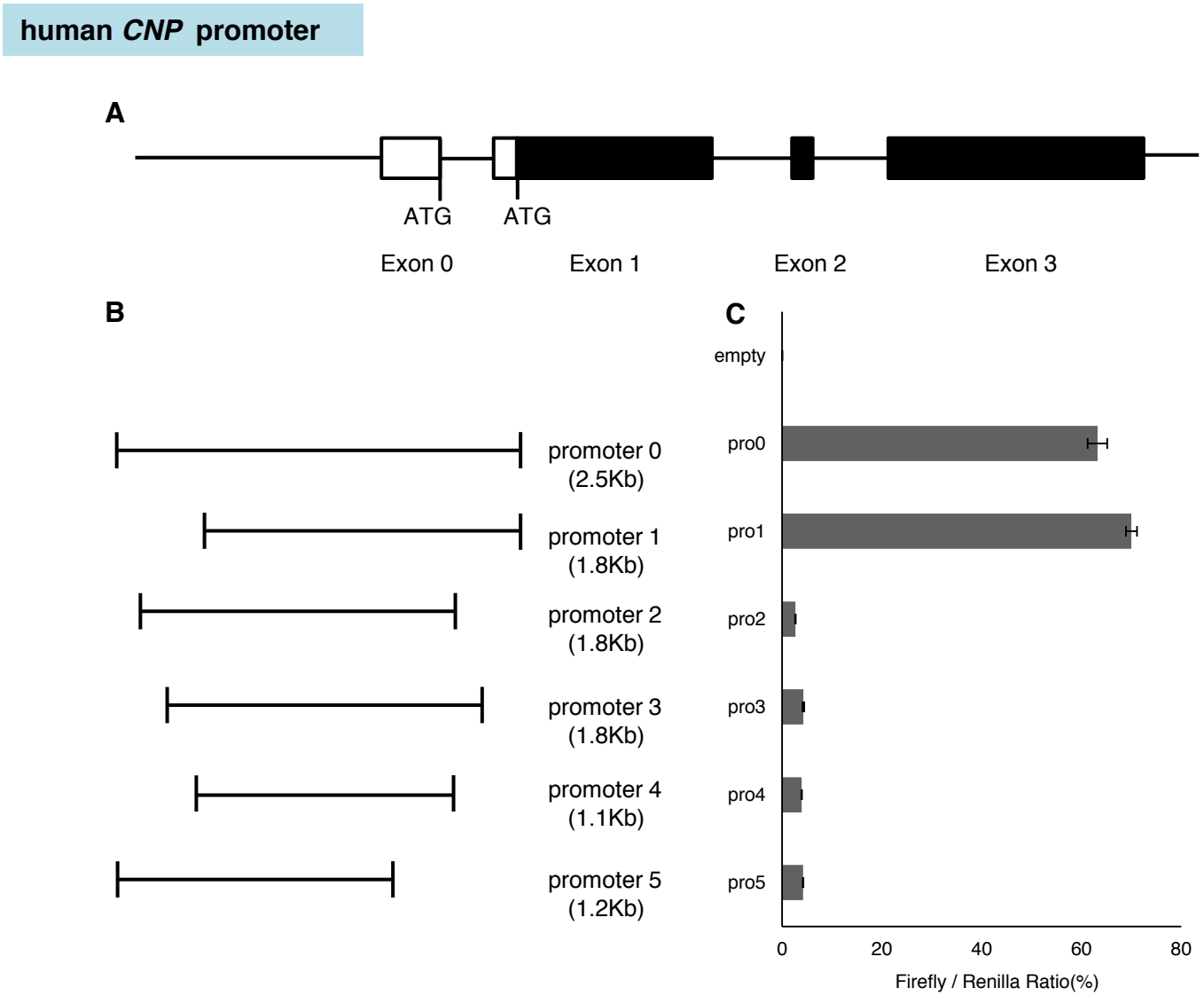


Figure S1 A schematic diagram of the human C*NP* gene (A) and six CNP promoter constructs (B). (C) The transcriptional activities of each promoter construct was evaluated using dual-luciferase reporter assay system (Promega, USA) in a oligodendroglial precursor cell line, Oli-neu cells (1). In this system, the expression of the firefly luciferase driven by each cloned human CNP promoter DNA fragment was correlated with a co-transfected control reporter expressing Renilla luciferase (2) in Oli-neu cells. The data are represented as the ratio of firefly to Renilla luciferase activity (Fluc/ Rluc%). Based on the efficient of transcriptional activity and the size of DNA fragment to fit in the scAAV vector, promoter 1 was selected to use in our study.

# Figure S2

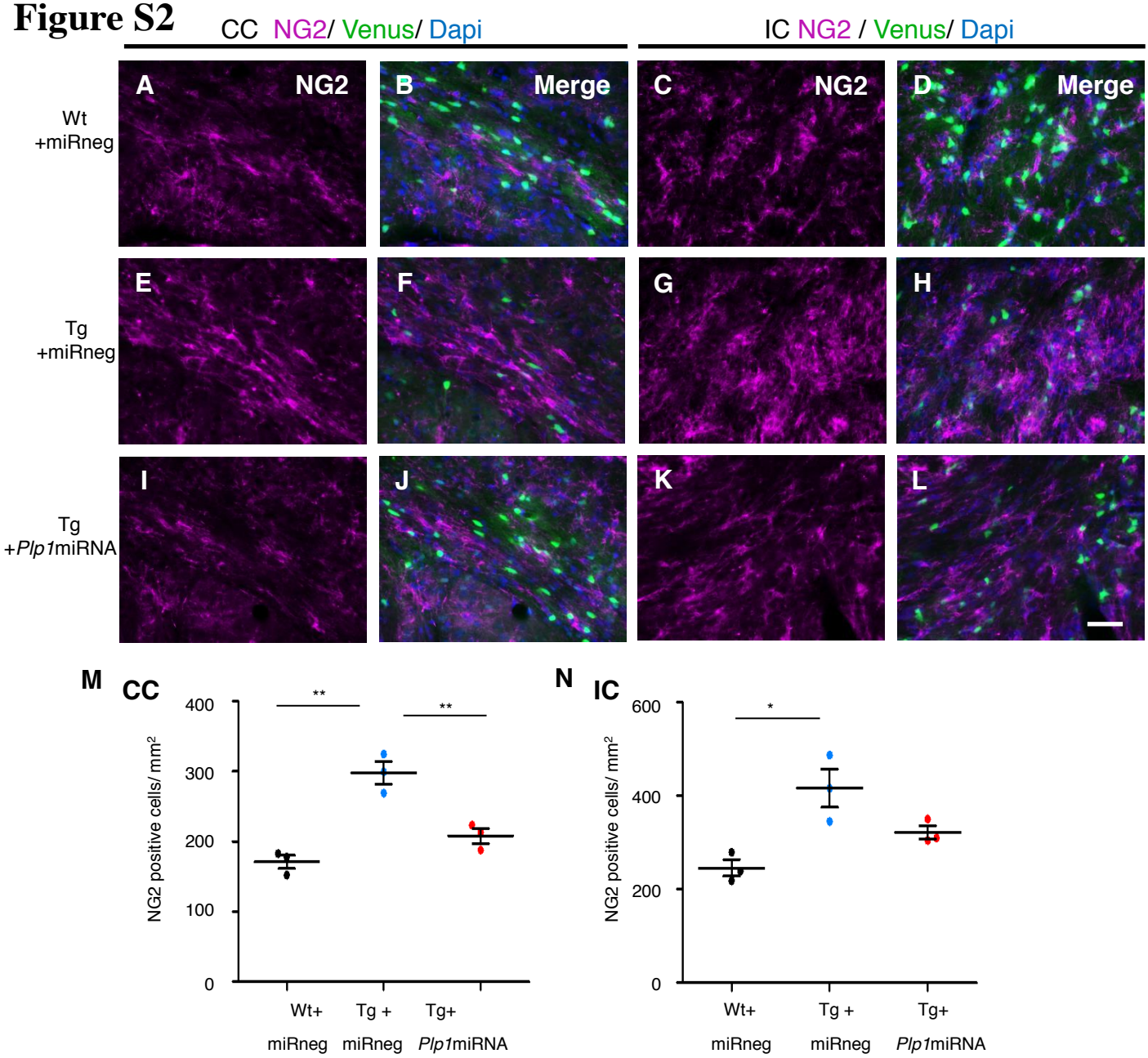





Figure S2. scAAV.CNP.Venus.Plp1miRNA treatment prevented the enhancement of proliferation of oligodendrocyte progenitor cells (OPCs) in *Plp1*-Tg mouse. Coronal sections of P25 mouse brains with an OPC marker NG2 immunostaining in CC (A-B, E-F, I-J) and IC (C-D, G-H, K-L) of Wt+miRneg mice (A-D), Tg+miRneg mice (E-H), and Tg+*Plp1*miRNA mice (I-L). Scale bar was 50 μm. Quantification of the densities of NG2 positive OPCs (n = 3 mice per group. Each value represents the average of cell densities obtained from 3 images.) in CC (M) and IC (N). Statistical significance was determined using One-way ANOVA with Bonferroni's post-test. *P<0.05, **P<0.01.

# Figure S3

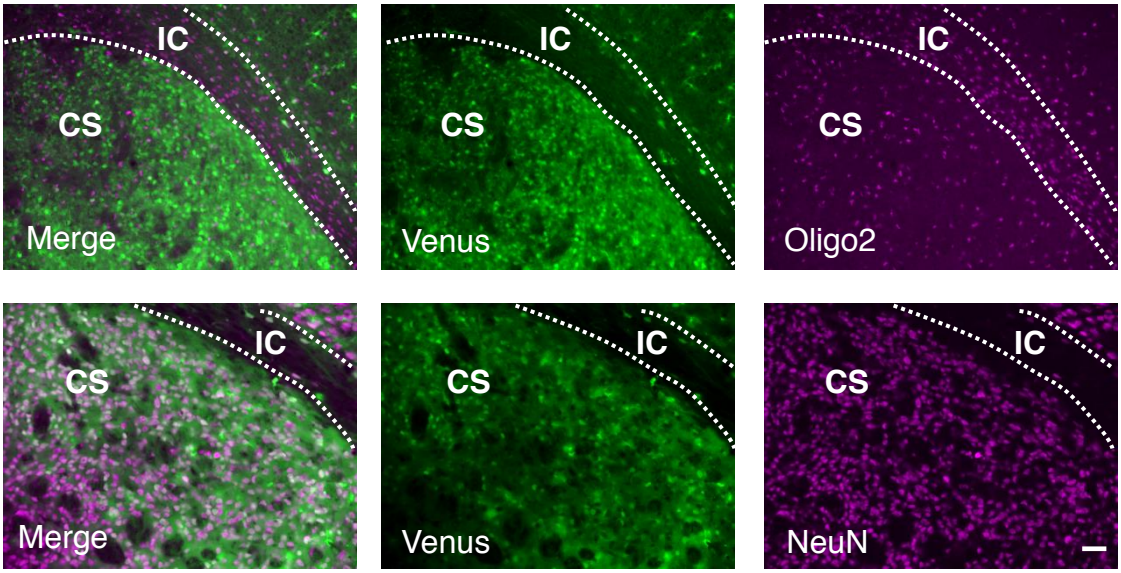


Figure S3 CAG promoter-driven scAAV barely express transgene in oligodendrocyte cells. One week prior to analysis, $1.2\times10^9$ genome copies scAAV.CAG.Venus.miRneg was injected respectively into right CS and IC of P10 wild-type mice (n=5). Immunostaining of the coronal brain sections indicated that the AAV-derived Venus expression was rarely present in olig2-positive oligodendrocyte cells (upper row) , but mainly present in NeuN-positive neurons (lower row). Scale bar=50μm.

# Figure S4

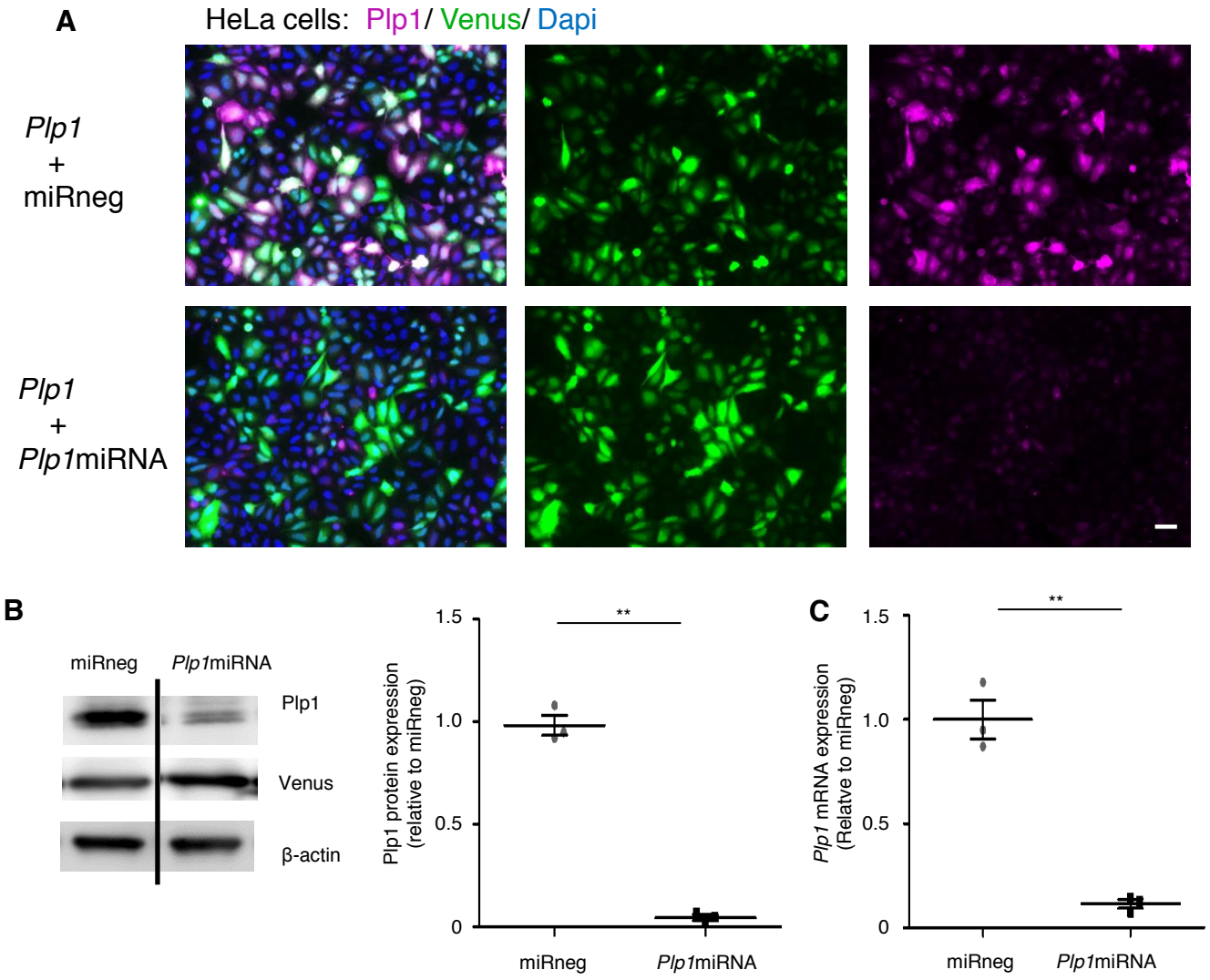


Figure S4 Artificial *Plp1* miRNA suppresses *Plp1* mRNA and protein expression *in vitro*. A mouse *Plp1* cDNA expression plasmid vector (pCAG-*Plp1*-Flag) (3) was co-transfecting with AAV backbone plasmid pscw.CAG.Venus.miRneg or pscw.CAG.Venus.*Plp1*miRNA in HeLa cells. 24 hours later, immunostaining was performed to detected Plp1 expression in Venus positive HeLa cells (A, Scale bar = 50μm). (B) The expression of Plp1 was detected by western blotting. β-actin was used as a control. The lanes were run on the same gel but were noncontiguous. Relative quantity of the Plp1 bands was shown in the right graph (data were obtained from 3 repeated experiments). (C) The expressions of *Plp1* mRNA were detected by quantitative RT-PCR (data were obtained from 3 repeated experiments). Statistical significant were determined using two tailed Student's t-test. **P<0.01.

# Figure S5

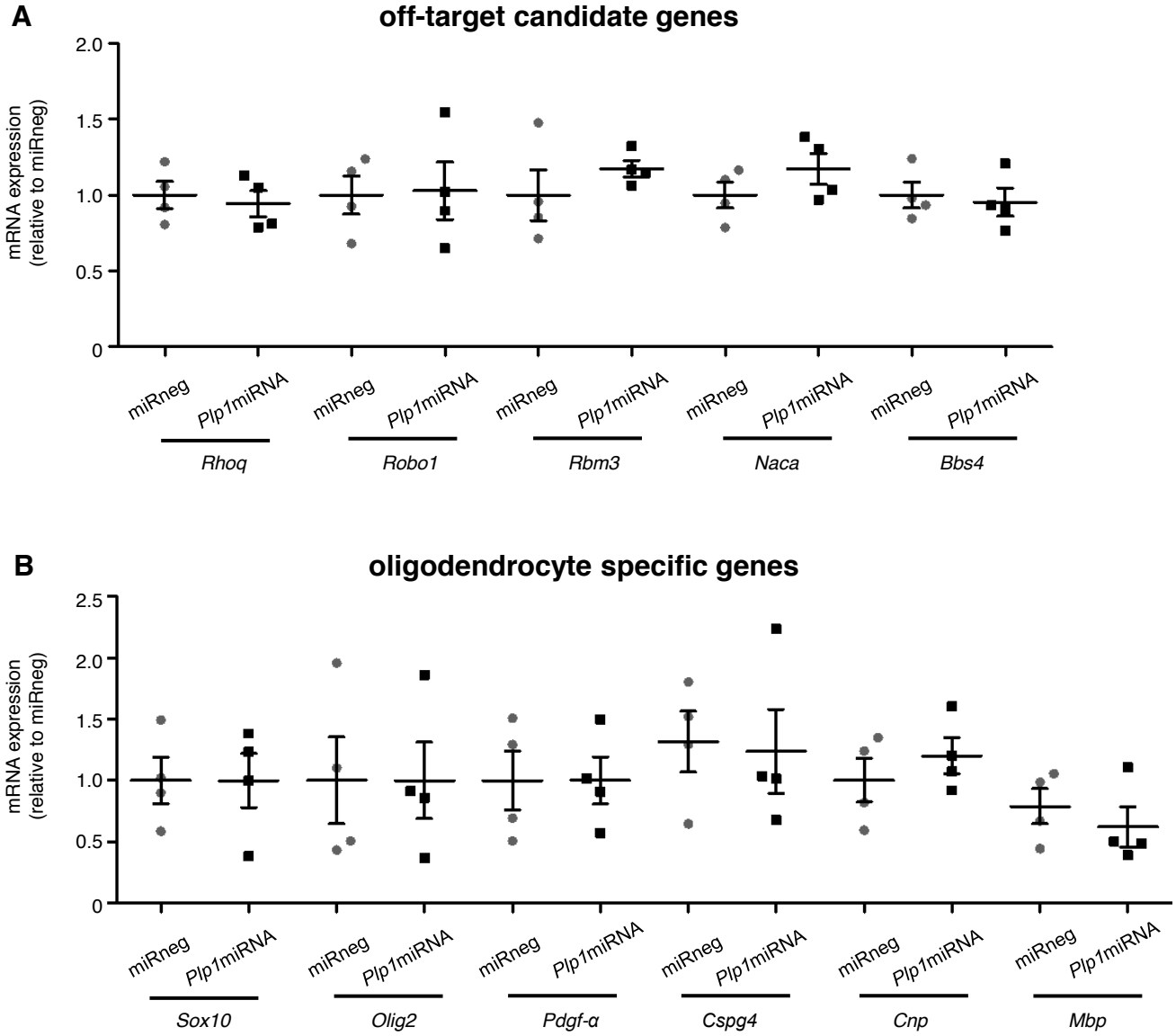


Figure S5 No significant changes in the mRNA level of off-target candidate genes (A) and oligodendrocyte specific genes (B) in response to the artificial *Plp1*miRNA. Venus-positive oligodendrocyte cells from wild-type mice treated with scAAV.CNP.Venus.miRneg (miRneg group) or scAAV.CNP.Venus.*Plp1*miRNA (*Plp1*miRNA group) were isolated by fluorescence-activated cell sorter (FACS). The values of black bars showed the mRNA expression of each gene in *Plp1*miRNA group relative to miRneg group (n=4 samples per group, in each sample, Venus-positive cells from 3 mice were pooled). Statistical significant were determined using two taied Student's t-test.

# Figure S6

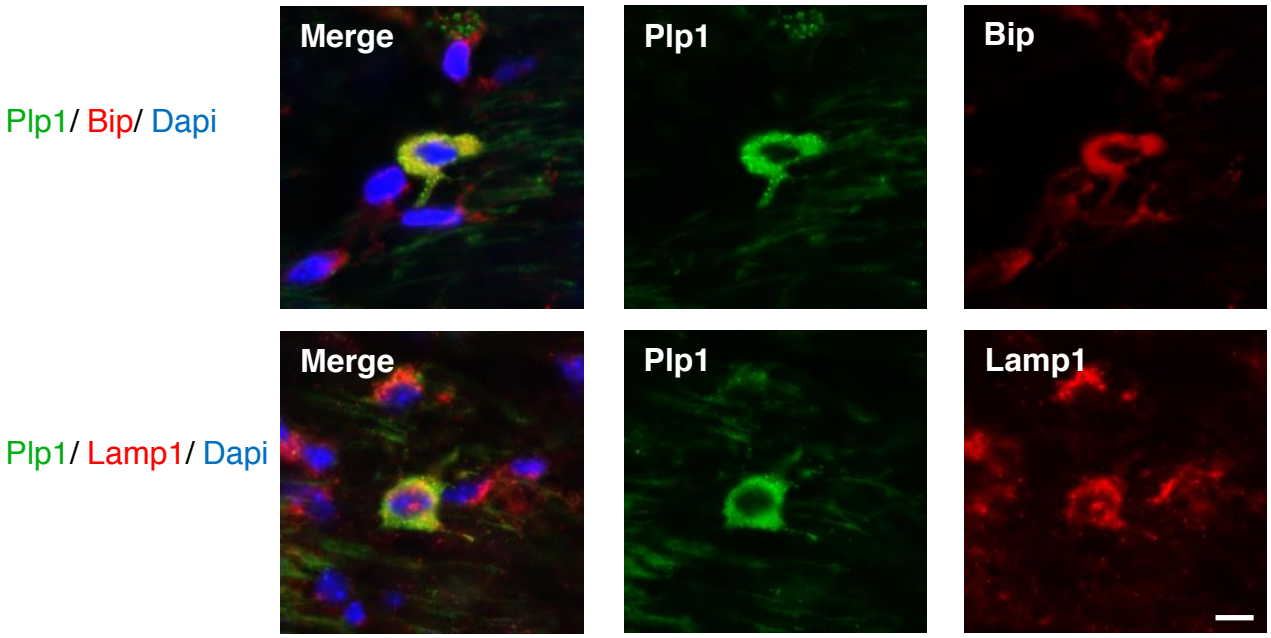


Figure S6 The intracellular localization of cytoplasmic accumulating Plp1 in *Plp1*-Tg mice (P25, corpus callosum) is detected by co-immunostaining with endoplasmic reticulum marker, Bip (upper row), and late endosomes/ lysosomes marker, Lamp1(lower row). Scale bar is 10μm.

# Figure S7

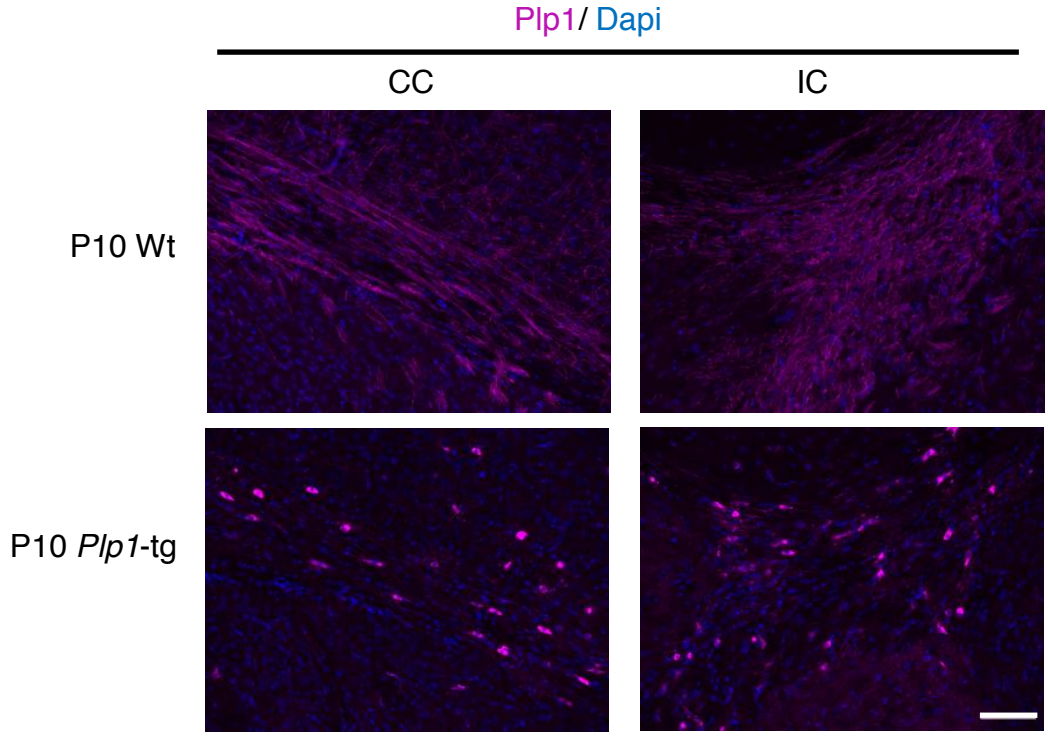


Figure S7 Cytoplasmic accumulation of Plp1 was already present in the oligodendrocytes of *Plp1*-Tg mice at postnatal day 10 (P10). Plp1 immunostaining was performed with the coronal brain sections at CC (left) and IC (right) of wild-type mice (upper row) and *Plp1*-tg mice (lower row). Scale bar is100μm.

# Figure S8

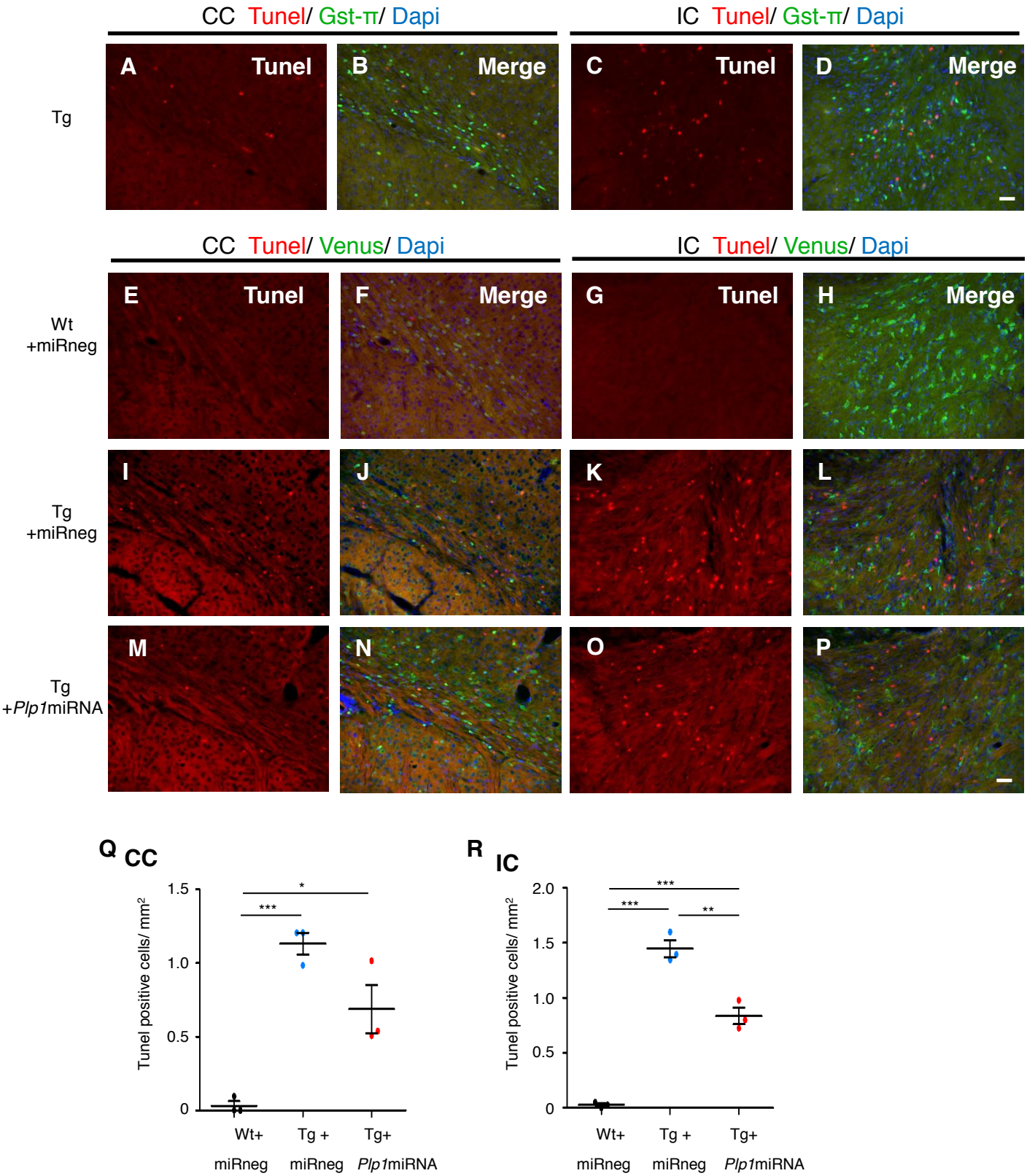





Figure S8. scAAV.CNP.Venus.Plp1miRNA treatment prevented the apoptosis of mature oligodendrocytes in *Plp1*-Tg mouse. Coronal sections of P25 *Plp1*-Tg mouse brains with a mature oligodendrocyte marker Gst-π immunostaining and TUNEL assay in CC (A-B) and IC (C-D). TUNEL positive cells were mostly positive for Gst-π. TUNEL assays in CC (E-F, I-J, M-N) and IC (G-H, K-L, O-P) of Wt+miRneg mice (E-H), Tg+miRneg mice (I-L), and Tg+*Plp1*miRNA mice (M-P). Scale bar was 50 μm. Quantification of the densities of TUNEL positive cells (n = 3 mice per group. Each value represents the average of cell densities obtained from 3 images) in CC (Q) and IC (R). Statistical significance was determined using One-way ANOVA with Bonferroni's post-test. *P<0.05, **P<0.01, ***P<0.001.

**Table S1 Primers used in RT-qPCR and *CNP* promoter cloning**

| Gene | Primers (forward) | Primers (reverse) |
|---|---|---|
| *Plp1* | GTTCCAGAGGCCAACATCAAGCTC | AGCCATACAACAGTCAGGGCATAG |
| *Sox10* | AACCTCATCCCTTGCCTAACT | TTGCCTCTGACTCTTTCCTG |
| *Olig2* | GGGAGGTCATGCCTTACGC | CTCCAGCGAGTTGGTGAGC |
| *Pdgfr-α* | CGTCTGGTCCTATGGCATTCTG | TCTCTTCTCGGGCTCACTGTTC |
| *Cspg4* | CTCTTGCTCCAGCTCCACTC | AGTGAGCACTACAGTGTGGG |
| *Cnpase* | GGAGATGAACCCAAGGAGAAGC | GGTGTCACAAAGAGAGCAGAGATGG |
| *Mbp* | TCACACACGAGAACTACCCATT | TGGTGTTCGAGGTGTCACAA |
| *Mog* | CCTGCTTCTTCAGAGACCACT | CGATGAGAGTCAGCACACCG |
| *Rhoq* | AGCAGTACCTCTTGGGACTC | AACGAGGCTGGATTTACCACG |
| *Robo1* | TGGCGACATGGGATCATACA | TTACAACGAAATGTGGCGGC |
| *Rbm3* | CCCAGCTACATGGGCAAAC | GGAGCTGGTGCATAGGACT |
| *Naca* | TCTTTCTGCCGCCATCTTGG | GGCTGAAGACATAGGAAGCACA |
| *Bbs4* | CGGGAAACGGTCGGCTT | ACAGATGCAGGAACCTGAGTT |
| *Actb* | CACAGCTTCTTTGCAGCTCCTT | GACGACCAGCGCAGCGATA |
| *CNP* prompter | GGCGGTACCACTAGTTAATGCAAA AGTCCATGATG | GCTACGCGTGAATTCGGGAGAAGCCT CTGTTCTGT |

References

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2. McNabb DS, Reed R, and Marciniak RA. Dual luciferase assay system for rapid assessment of gene expression in Saccharomyces cerevisiae. Eukaryot Cell. 2005;4(9):1539-49.

3. Yu LH, et al. Effect of curcumin in a mouse model of Pelizaeus-Merzbacher disease. *Mol Genet Metab*. 2012;106(1):108-14.