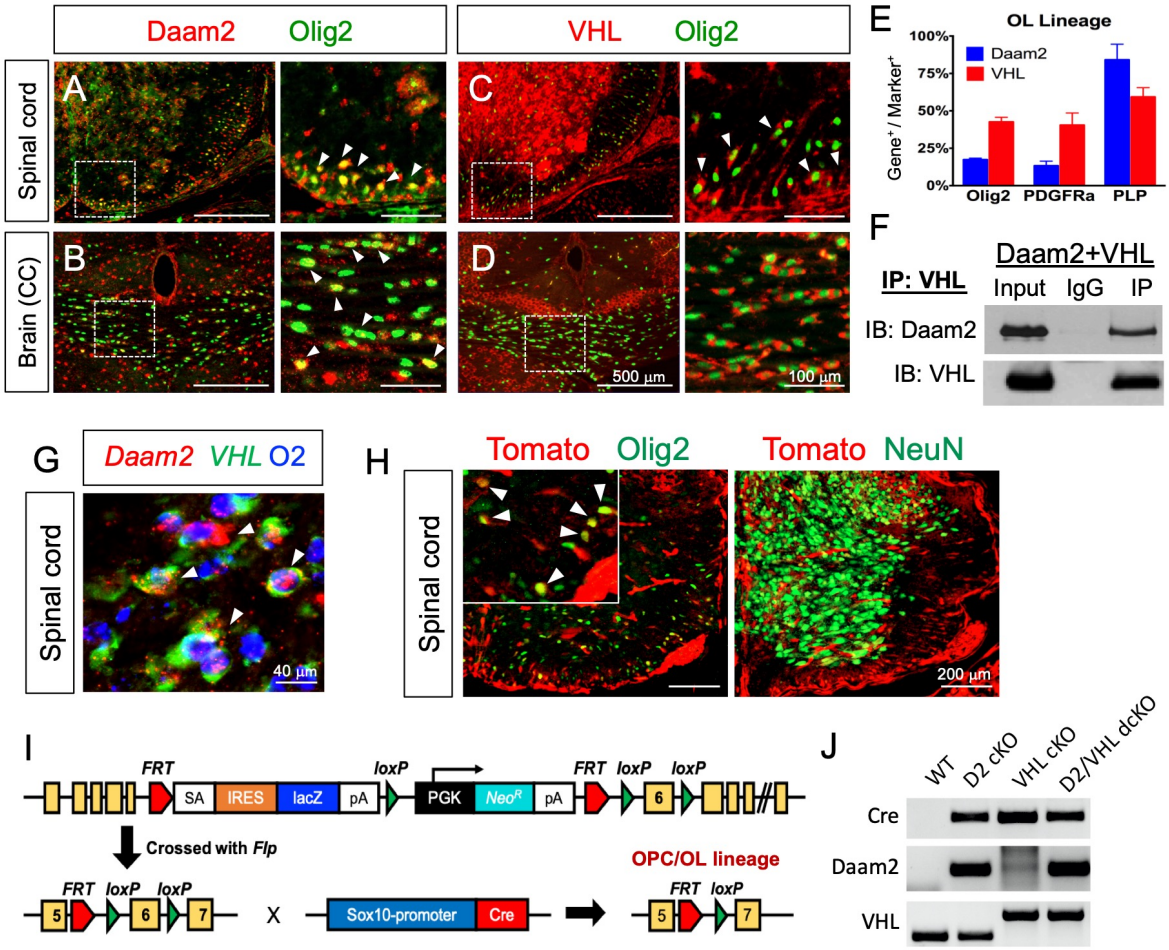
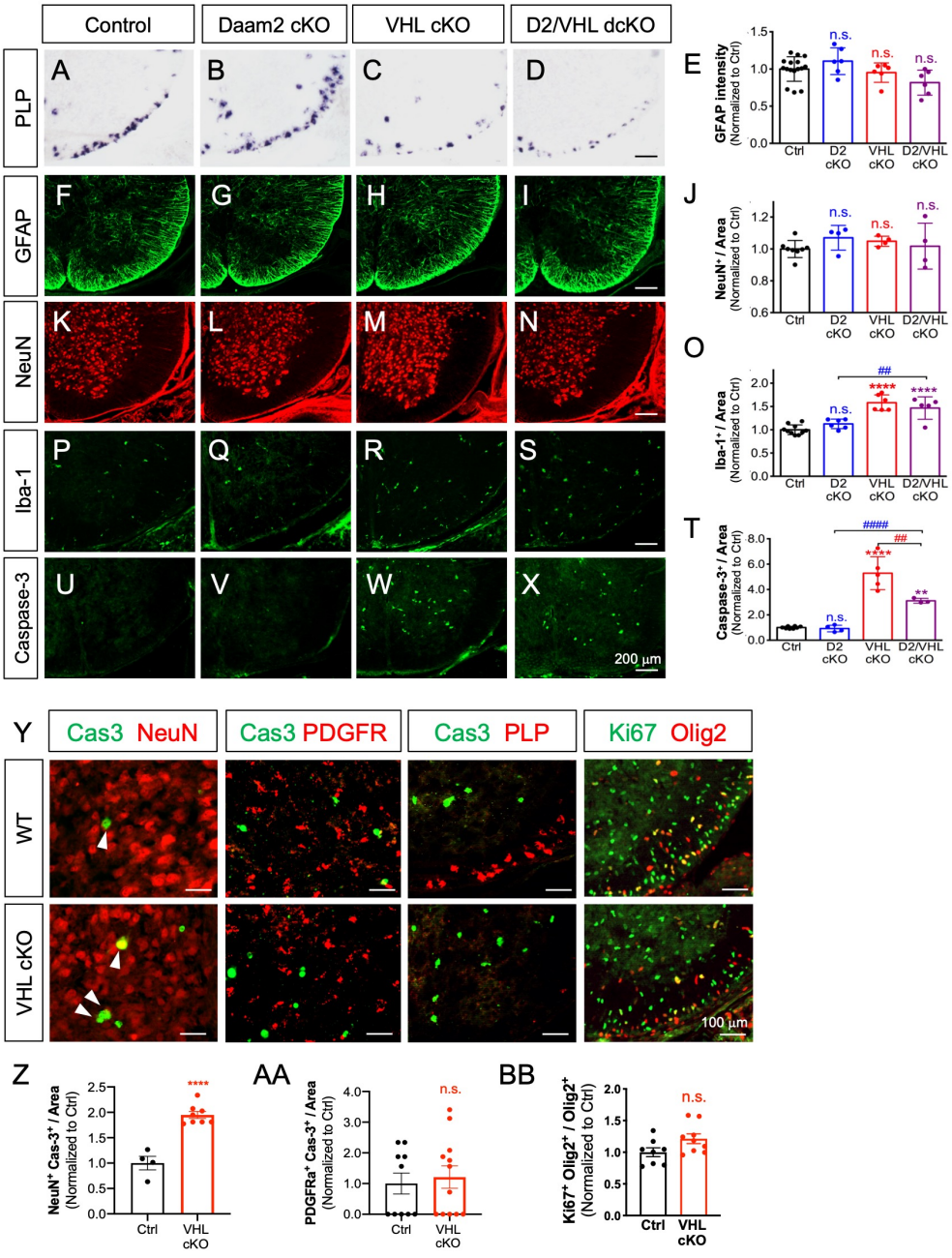
**Supplemental Figure S1.** Related to Figure 1




**Supplemental Figure S1. Expressional analysis of Daam2 and VHL in OL lineage during development and generation of *Sox10-Cre* derived conditional alleles.** (A-E) Daam2 and VHL are expressed in OL lineage in mouse spinal cord at postnatal day 3 (P3) and corpus callosum of the brain at P14. (A-B) Fluorescence *in situ* hybridization of Daam2 and immunofluorescence staining of Olig2. (C-D) Immunofluorescence staining of VHL and Olig2. Zoomed-in images are shown in the adjacent panels on the right which are indicated in the dashed box. Arrowheads indicate colocalization. (E) Quantification of Daam2 and VHL in the OL lineage. Data are shown as mean ± SD, from at least 3 different sections from 3 different animals. (F) Co-immunoprecipitation of Daam2 and VHL in the oligodendroglia precursor cell line Oli-neu confirms that Daam2 and VHL physically associate in OL lineage. (G) Co-expression of *Daam2* and *VHL* transcripts in OL-lineage cells using fluorescence *in situ* hybridization of Daam2 and VHL with immunofluorescence staining of Olig2 in the corpus callosum at P14. (H) Confirmation of *Sox10-Cre* derived recombination efficacy by intercrossing with a *ROSA-LoxP-STOP-LoxP-tdTomato* reporter. We have confirmed that 88% of all Olig2+ cells are colocalized with tdTomato (n=7, SD 4%), but no overlap with NeuN+ neurons. (I) Schematic illustrating the OL lineage-specific *Daam2* conditional knockout allele. The LacZ and Neomycin cassettes in *Daam2*$^{Tm1a}$ animals were deleted by crossing with FLP

mice to generate *Daam2*$^{F/F}$ mice. LoxP sites flank exon 6 of the *Daam2* gene in *Daam2*$^{F/F}$ mice, yielding a frameshift mutation upon Cre-mediated recombination. OL lineage-specific deletion of this exon was achieved by crossing with the *Sox10-Cre* line. (J) PCR confirmation of the genotypes of the four different groups of animals.

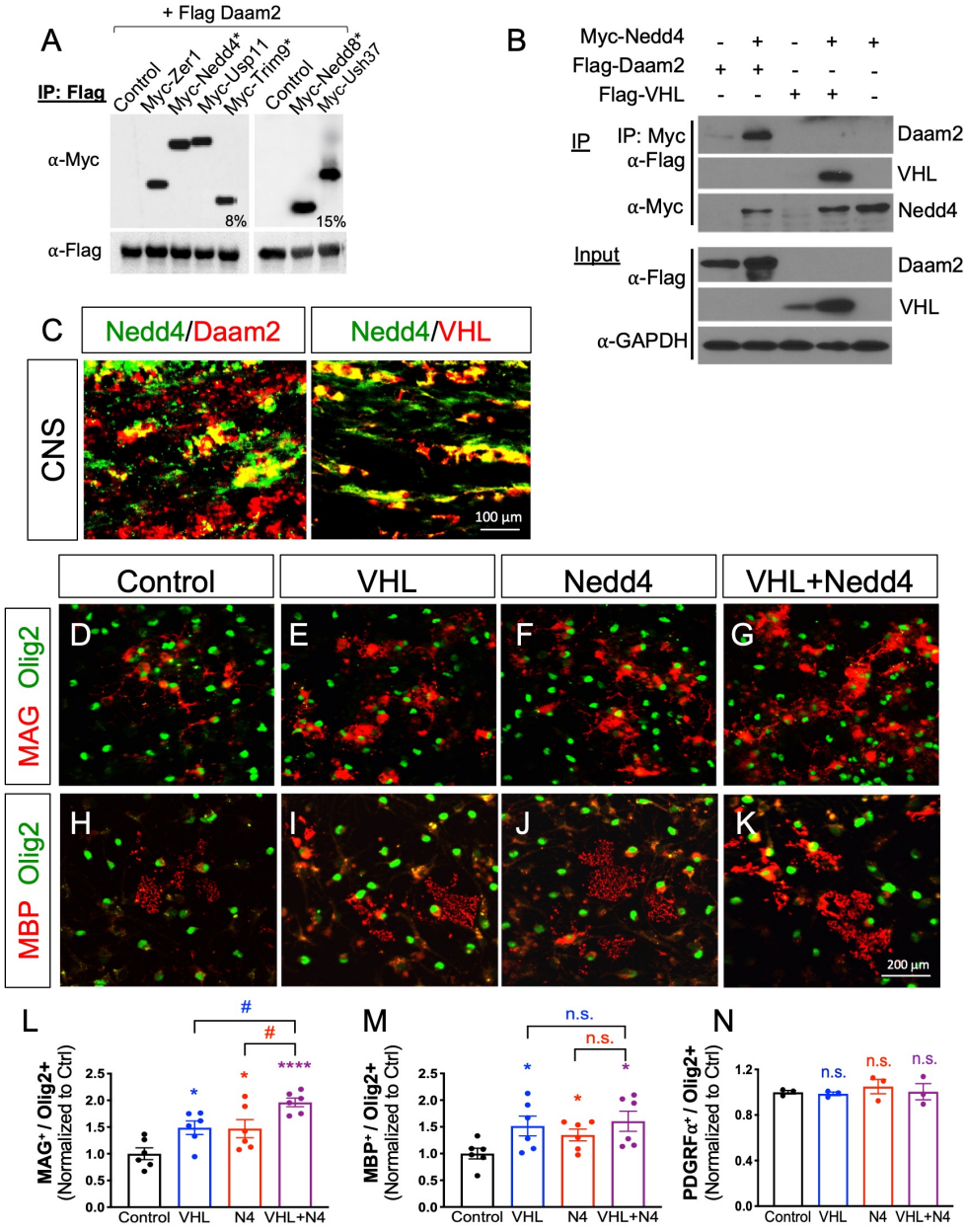
**Supplemental Figure S2.** Related to Figure 1




**Supplemental Figure S2. Expression analysis of various cell type markers in the OPC-Specific conditional knockout mice.** (A-D) *In situ* hybridization of PLP in the P0 spinal cord. (E-X) Immunofluorescence staining of astrocytic marker GFAP (E-I), neuronal marker NeuN (J-N), microglial marker Iba-1 (O-S), and apoptosis marker active Caspase-3 (T-X) in the spinal cord of *WT*, *Daam2* cKO, *VHL* cKO and *Daam2-VHL* dcKO mice at P0. No significant differences were found between all 4 groups in terms of GFAP intensity (E) and number of NeuN+ cells (J). Increases in Iba-1+ microglia (O) and Caspase-3+ apoptotic cells (T) were observed in *VHL* cKO and *Daam2-VHL* dcKO animals compared to *Daam2* cKO and control. (E-T) Each data point

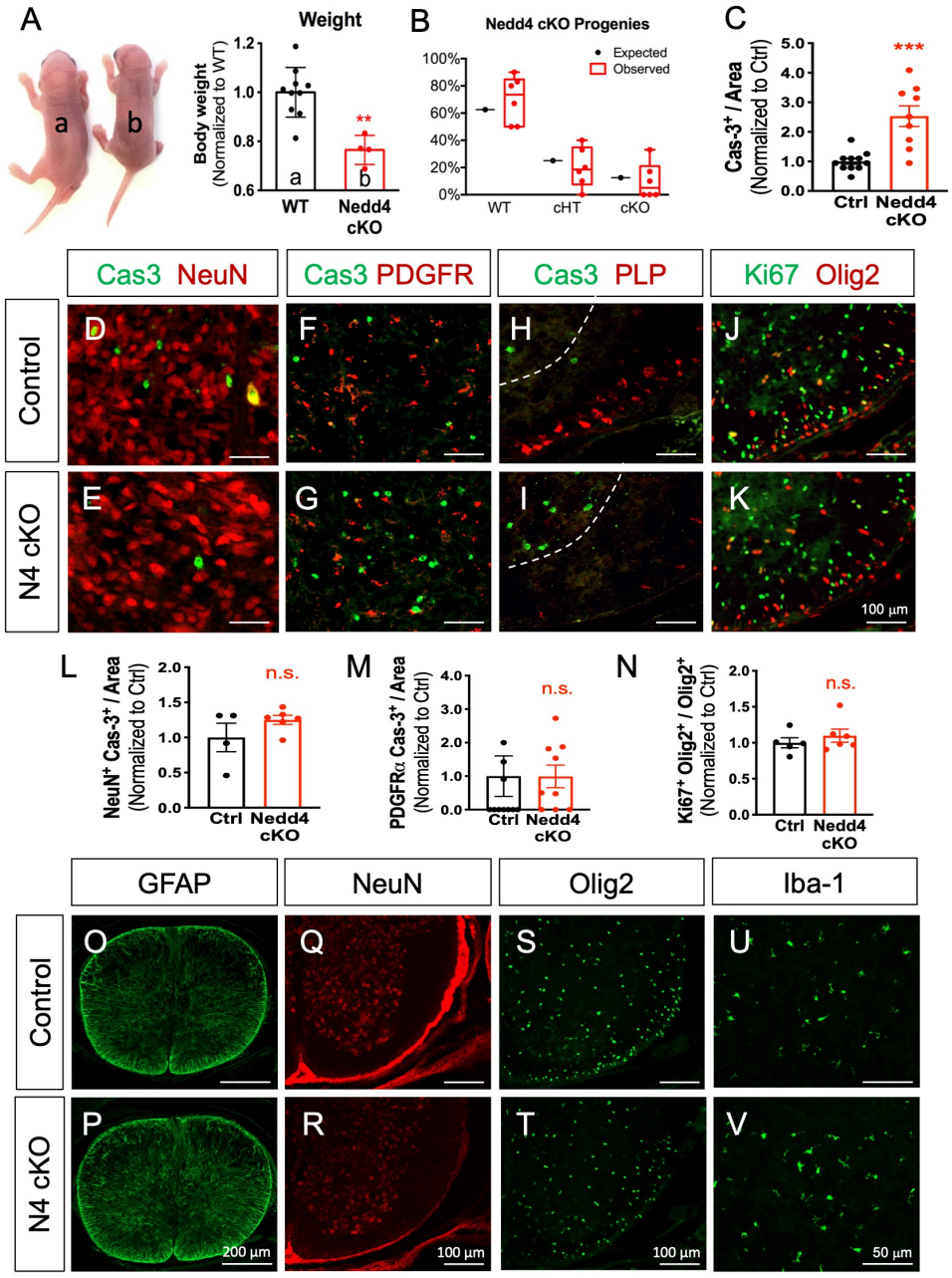
represents one animal. Data were acquired from at least three independent litters. Values were normalized to control (One-way ANOVA with multiple comparisons, * for comparison with control, # for comparison with *Daam2-VHL* cKO, p**<0.01, p****<0.0001, $p^{\#\#}$<0.01, $p^{\#\#\#\#}$<0.0001) (Y-BB) Confirmation of cell death and proliferation in *VHL* cKO and controls with various lineage markers. An increase in Caspase-3+ NeuN+ cells was found in *VHL* cKO mice. Each data point represents individual images from n=3-4 mice (Student's t-test, * for comparison with control, p****<0.0001).

## **Supplemental Figure S3.** Related to Figure 3




**Supplemental Figure S3. Dam2-VHL-Nedd4 are associated and *Nedd4* increases OPC differentiation *in vitro*.** (A) Co-immunoprecipitation of Daam2 and candidates in 293T cells (* indicate E3 ligases). (B) Co-immunoprecipitation (Co-IP) of Nedd4 with Daam2 and VHL in 293T cells. (C) Fluorescence *in situ* hybridization and immunofluorescence staining of Nedd4, Daam2 and VHL showed the colocalization of Nedd4/Daam2 and Nedd4/VHL in the corpus callosum of P14 mice. (D-N) *In vitro* functional analysis of Nedd4, VHL, and Nedd4-VHL in cultured OPC differentiation (Student's t-test* for comparison with control, p*<0.05, p****<0.0001, p$^{\#}$<0.05)

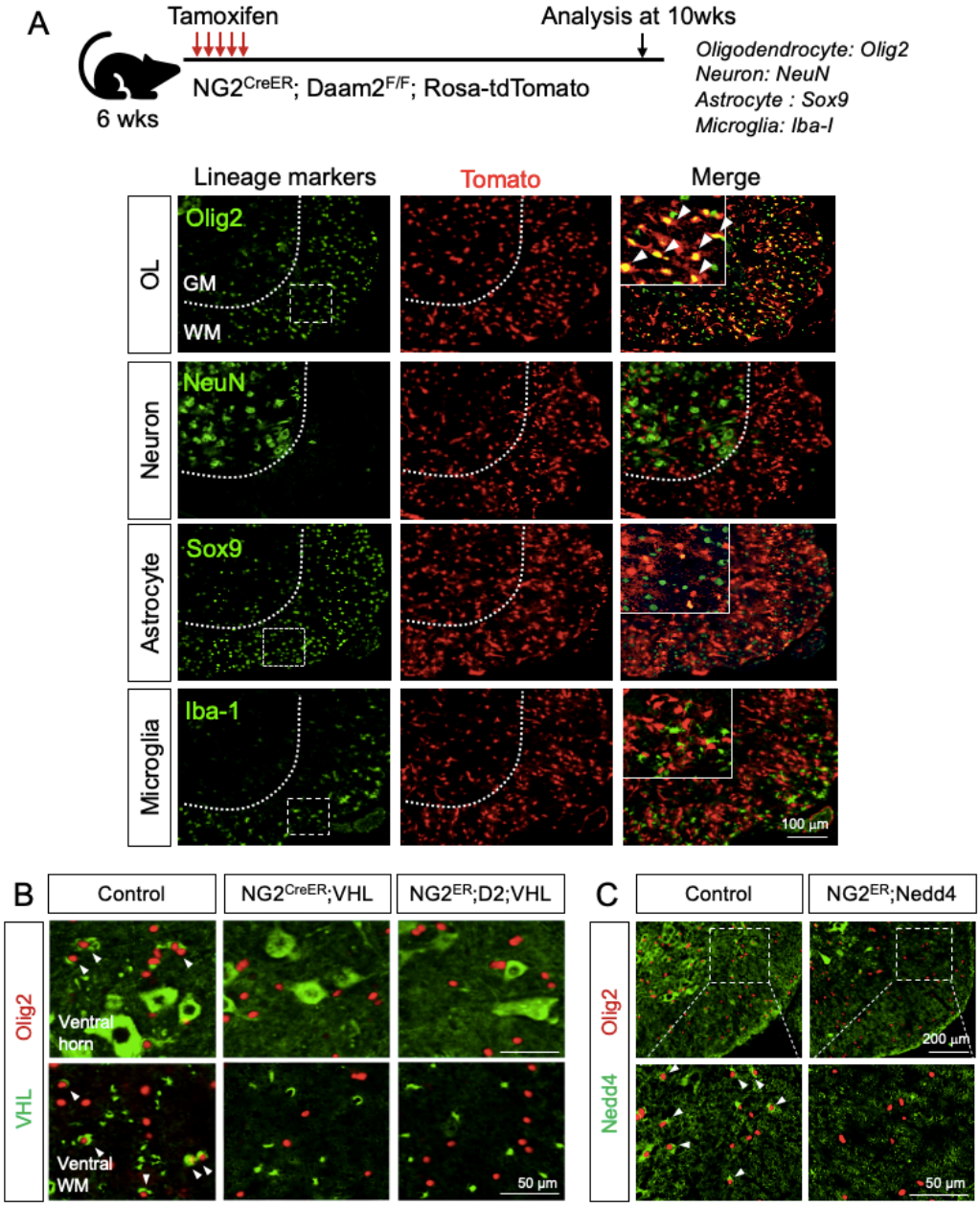
**Supplemental Figure S4.** Related to Figure 4




**Supplemental Figure S4. Characterization of OPC-specific *Nedd4* conditional knockout mice.** (A) Size and bodyweight of mice harboring OL lineage-specific deletion of *Nedd4* (*Nedd4* cKO) compared to control (Student's t-test, p**<0.001). Each data point represents an individual animal from 3 litters. Plotted values are normalized to control. (B) Genotypes of offspring from crosses of *Sox10-Cre; Nedd4*$^{F/+}$ and *Nedd4*$^{F/+}$ at birth. Black line indicates expected yield based on the mendelian ratio; red box plot (min to max) indicates the observed values from each litter. Each data point represents one litter. Data were acquired from six independent litters. There are no significant

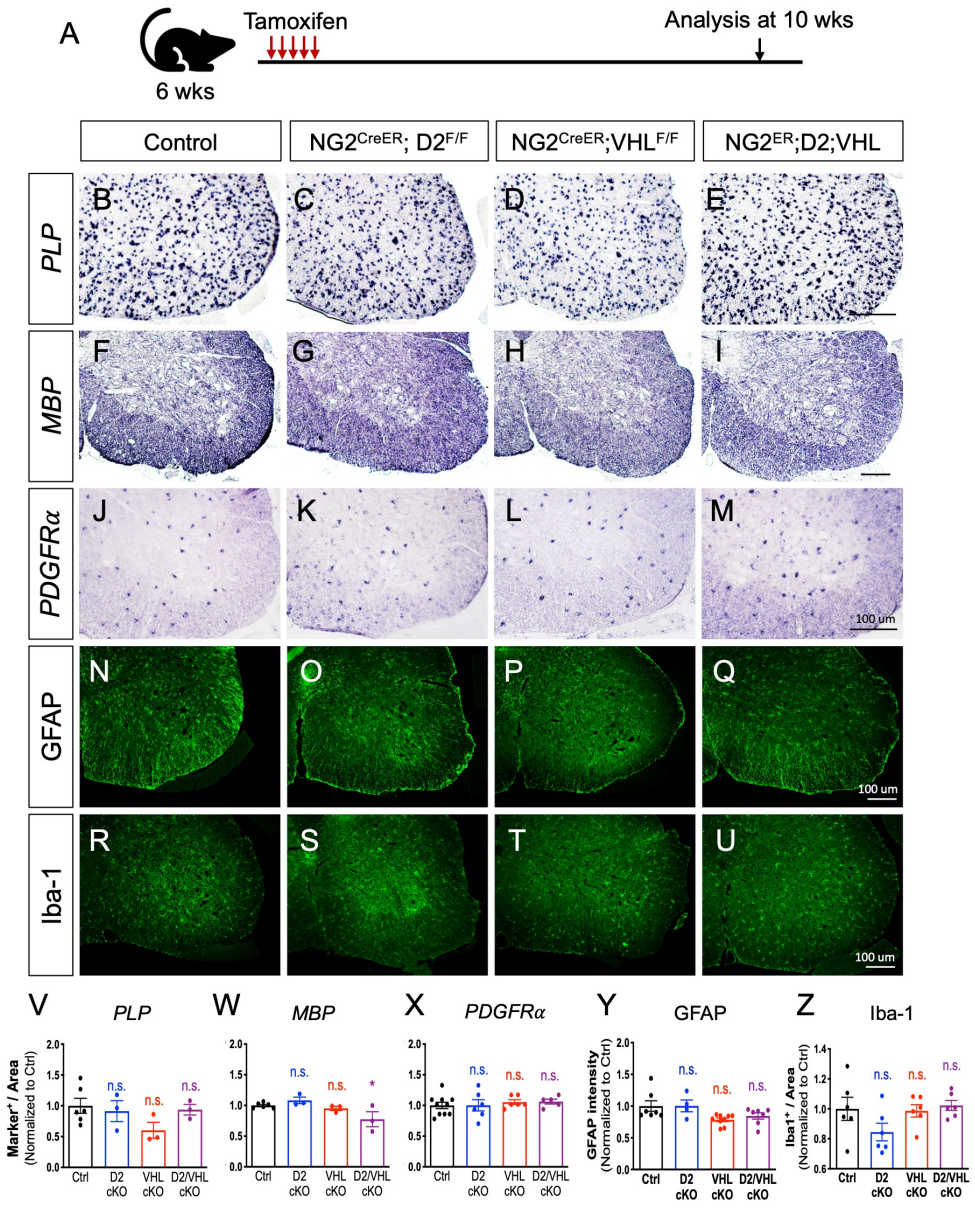
differences between the expected and observed values in newborn mice, suggesting no embryonic lethality associated with OL lineage-specific *Nedd4* deletion (Chi-squared test, p=0.1351). However, given the perinatal lethality, all mice were harvested and analyzed at P0. (C-I) Immunofluorescence staining of cell apoptotic marker Caspase-3 in the spinal cord of *Nedd4* cKO vs control mice. A significant increase in Caspase-3+ apoptotic cells per unit area was observed in *Nedd4* cKO compared to control mice. Data were acquired from 4 animals per group (student's t-test, p***<0.001). We did not observe differences in Caspase-3 staining in both NeuN+ neurons (D-E, L) and OPCs/OLs (F-I, M). (J-K, N) Immunofluorescence staining of cell proliferative marker Ki67 in OLs. (student's t-test). (H-O) Immunofluorescence staining of other cell type markers, including astrocytic marker GFAP (O-P), neuronal marker NeuN (Q-R), OL-lineage marker Olig2 (S-T), and microglial marker Iba1 (U-V) in the spinal cords of control vs *Nedd4* cKO mice at P0.

**Supplemental Figure S5.** Related to Figure 5



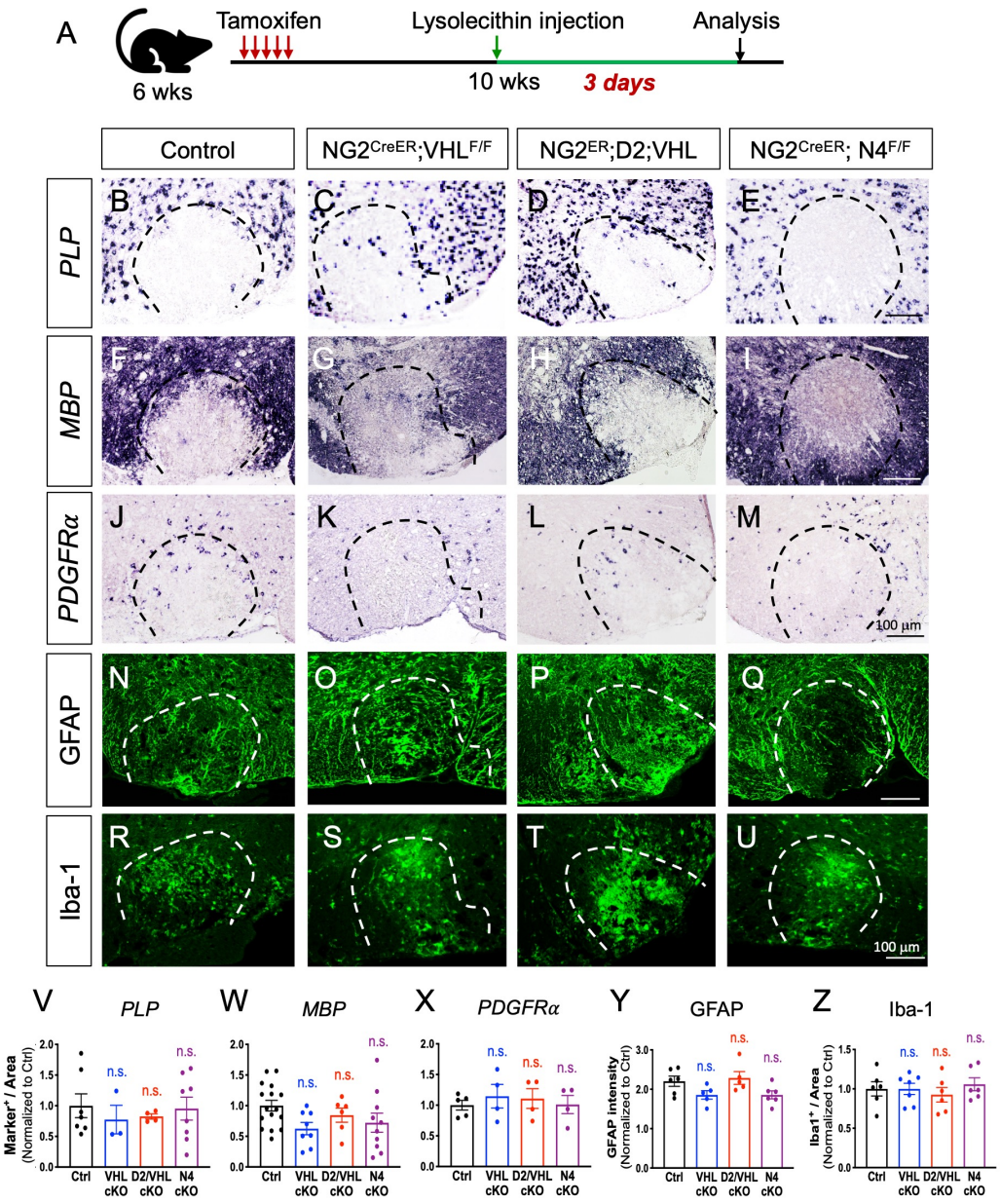

**Supplemental Figure S5. Generation of *NG2-CreER* derived conditional knockout mice and gene deletion.** (A) Experimental scheme of generation of *NG2-CreER* derived *Daam2* mutants (*NG2-CreER*$^{+/-}$*; Daam2*$^{F/F}$) intercrossing with a *ROSA-LoxP-STOP-LoxP-tdTomato* reporter to confirm the Cre recombination efficacy. Tamoxifen was administered at 6 weeks and spinal cords were harvested and analyzed at 10 weeks. We have confirmed that 63% of Olig2+ cells were positive for tdTomato after injection with tamoxifen (n=4, SD 8%), mild overlap with Sox9+ astrocytes, but no overlap with NeuN+ neurons or Iba-1+ microglia. (B-C) Immunostaining of VHL and Nedd4 in Olig2+ cells in *NG2-CreER*-derived conditional knockouts.

**Supplemental Figure S6.** Related to Figure 5



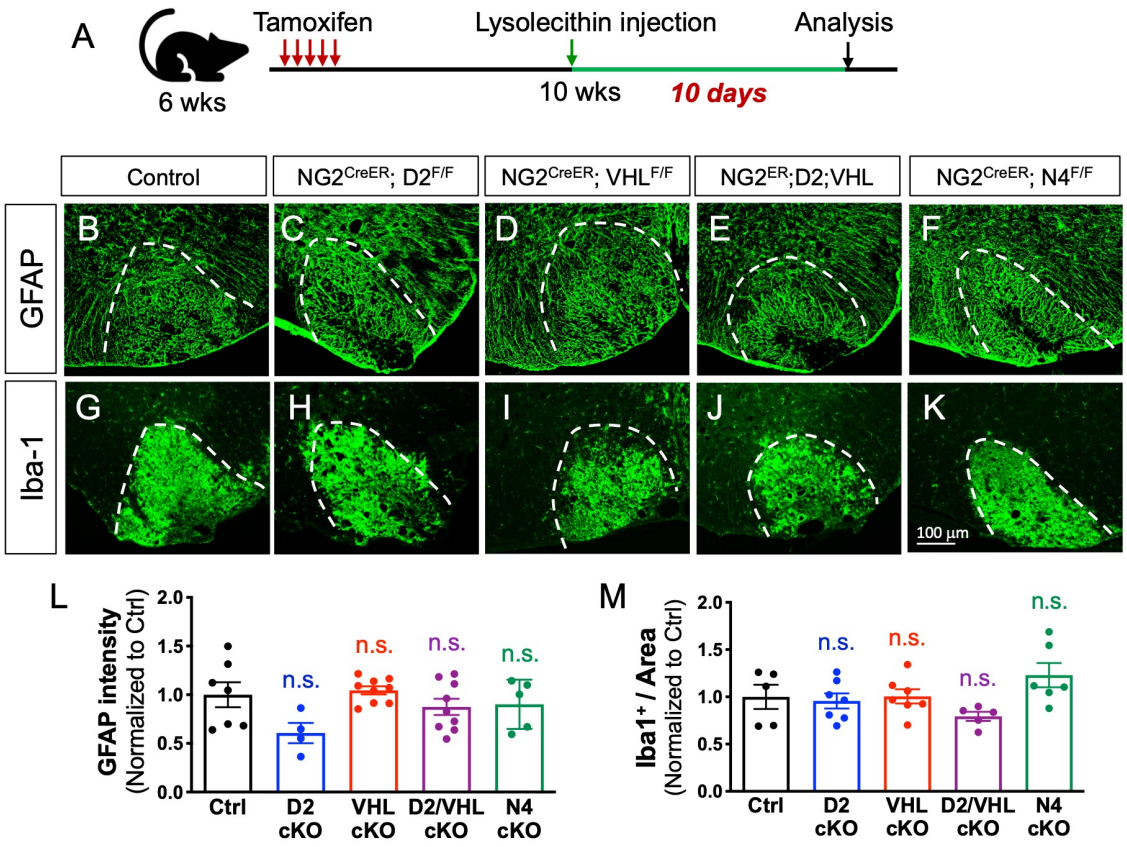

**Supplemental Figure S6. Various cellular phenotyping in *NG2-CreER* derived conditional knockout mice.** (A) Experimental scheme of the deletion of *Daam2*, *VHL*, *Daam2-VHL* in *NG2-CreER* derived mutants (*NG2-CreER*$^{+/-}$*; Daam2*$^{F/F}$*, NG2-CreER*$^{+/-}$*; VHL*$^{F/F}$*, NG2-CreER*$^{+/-}$*; Daam2*$^{F/F}$*; VHL*$^{F/F}$). Tamoxifen was administered at 6 weeks and spinal cords were harvested and analyzed at 10 weeks. (B-M) *In situ* hybridization for mature OLs (PLP and MBP) and OPCs (PDGFRα) in *NG2-CreER* derived conditional knockouts. (N-U) Immunofluorescence staining of astrocyte (GFAP) and microglia (Iba-1) in *NG2-CreER* derived conditional knockouts. (V-Z) Quantification of PLP+, MBP+, PDGFRα+, GFAP+, Iba-1+. Data points represent individual images from multiple animals. Values were normalized to control (One-way ANOVA with multiple comparisons, p*<0.05).

**Supplemental Figure S7.** Related to Figures 5 and 6



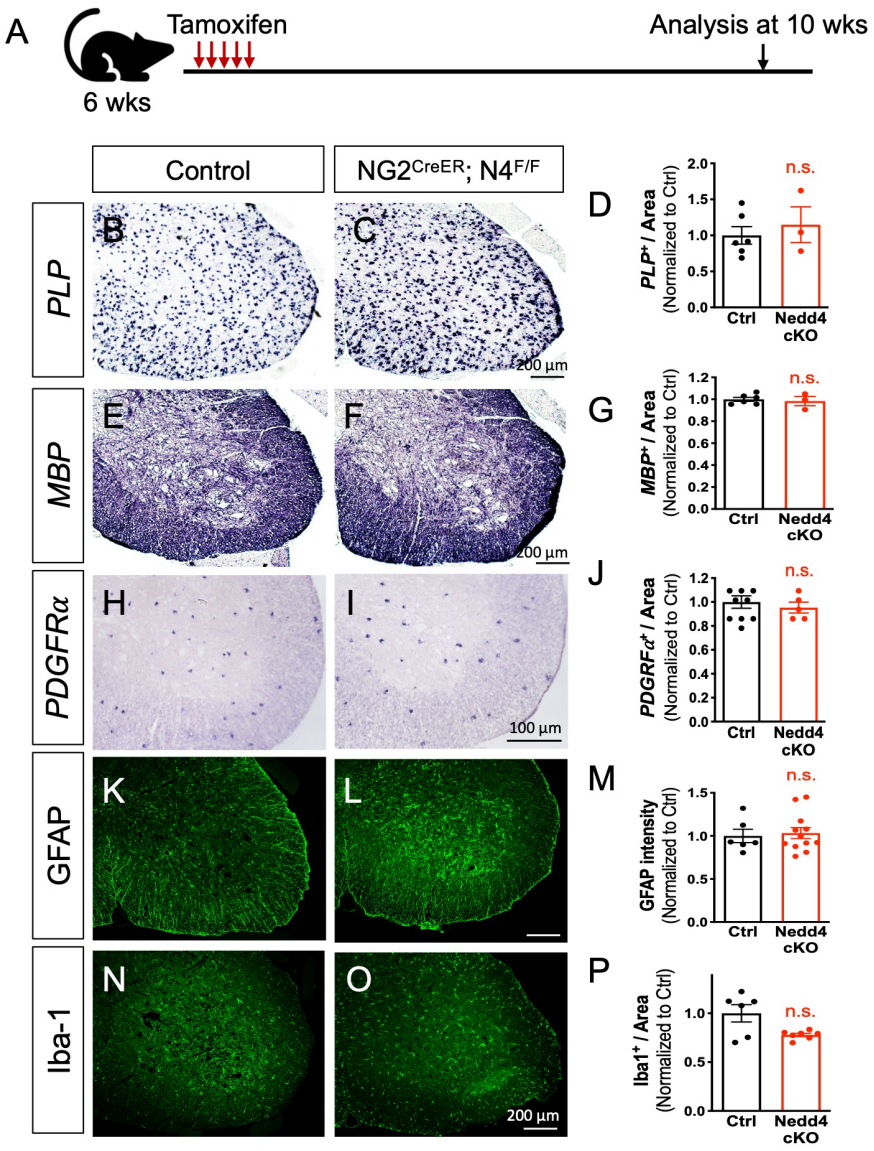

**Supplemental Figure S7. *NG2-CreER* derived conditional knockout do not affect OPC recruitment and demyelination at 3 days post lesion.** (A) Experimental scheme of the lysolecithin (LPC)-induced demyelination in *NG2-CreER* derived knockouts. Tamoxifen was administered 4 weeks prior to LPC injection to induce demyelination. Spinal cords were then harvested and analyzed 3 days after LPC injection. (B-M) *In situ* hybridization was performed for mature markers MBP+, PLP+, and OPC marker PDGFRα+ in the lesion. (N-U) Immunofluorescence staining of astrocytic marker GFAP and microglial marker Iba-1 inside the lesions. (V-Z) Quantification of PLP+, MBP+, PDGFRα+, GFAP+, Iba-1+. Data points represent individual images from multiple animals. Values were normalized to control (One-way ANOVA with multiple comparisons).

**Supplemental Figure S8.** Related to Figures 5 and 6



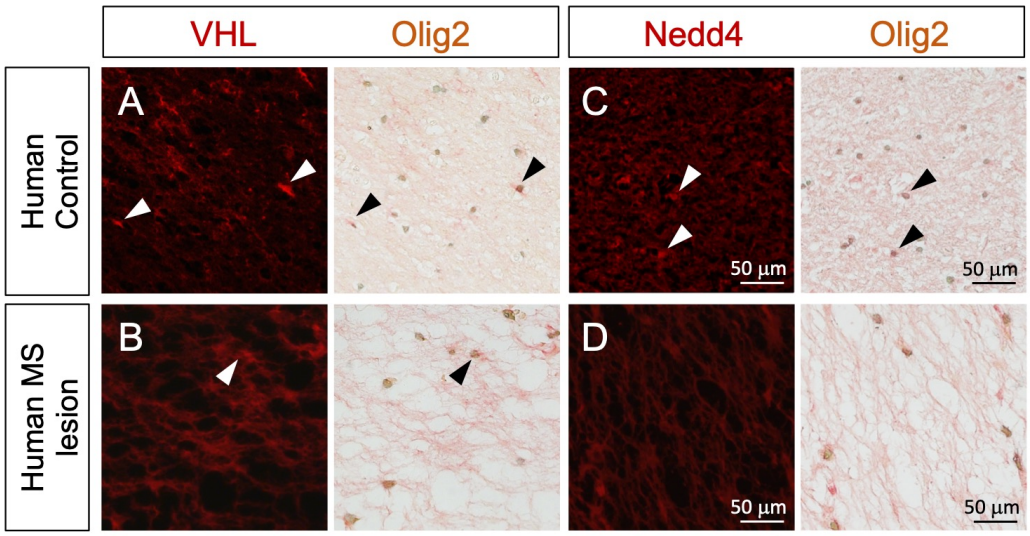

**Supplemental Figure S8. Reactive astrocytes and inflammatory response markers are unaffected in LPC-induced demyelination.** (A) Experimental scheme of the lysolecithin (LPC)-induced demyelination in *NG2-CreER* derived knockouts. The mice were treated with tamoxifen at 4 weeks prior to LPC injection to induce demyelination. Spinal cords were then harvested and analyzed 10 days after LPC injection. (B-K) Immunofluorescence staining of astrocytic marker GFAP and microglial marker Iba-1 inside the lesions. (L-M) Quantification of GFAP+ and Iba-1+ cells. Data points represent individual images from multiple animals. Values were normalized to control (One-way ANOVA with multiple comparisons).

**Supplemental Figure S9.** Related to Figure 6




**Supplemental Figure S9. Various cellular phenotyping in *NG2-CreER* derived *Nedd4* conditional knockout mice.** (A) Experimental scheme of the deletion of *NG2-CreER* derived *Nedd4* mutants (*NG2-CreER*$^{+/-}$*; Nedd4*$^{F/F}$). The mice were treated with tamoxifen at 6 weeks and spinal cords were harvested and analyzed at 10 weeks. (B-J) *In situ* hybridization for mature OL (PLP and MBP) and OPC marker (PDGFRα) in *NG2-CreER*$^{+/-}$*; Nedd4*$^{F/F}$. (K-P) Immunofluorescence staining of astrocyte (GFAP) and microglia (Iba-1) in *NG2-CreER*$^{+/-}$*; Nedd4*$^{F/F}$. Data points represent individual images from multiple animals. Values were normalized to control (Student's t-test).

**Supplemental Figure S10.** Related to Figure 6




**Supplemental Figure S10. VHL and Nedd4 expression in human MS lesions.** Immunohistochemical staining (IHC) of VHL (A-B) and Nedd4 (C-D) with Olig2. Solid arrowheads indicate colocalization with Olig2 in healthy brains and MS lesions.