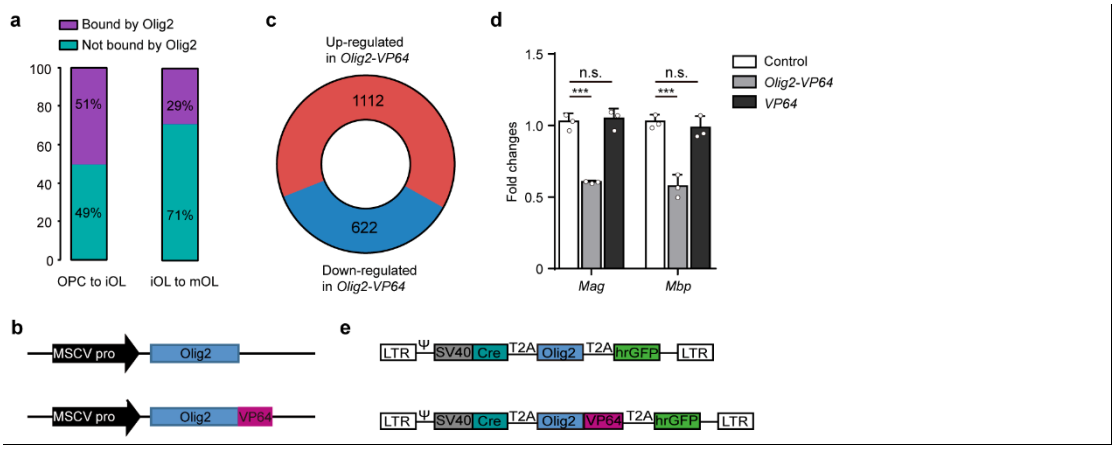
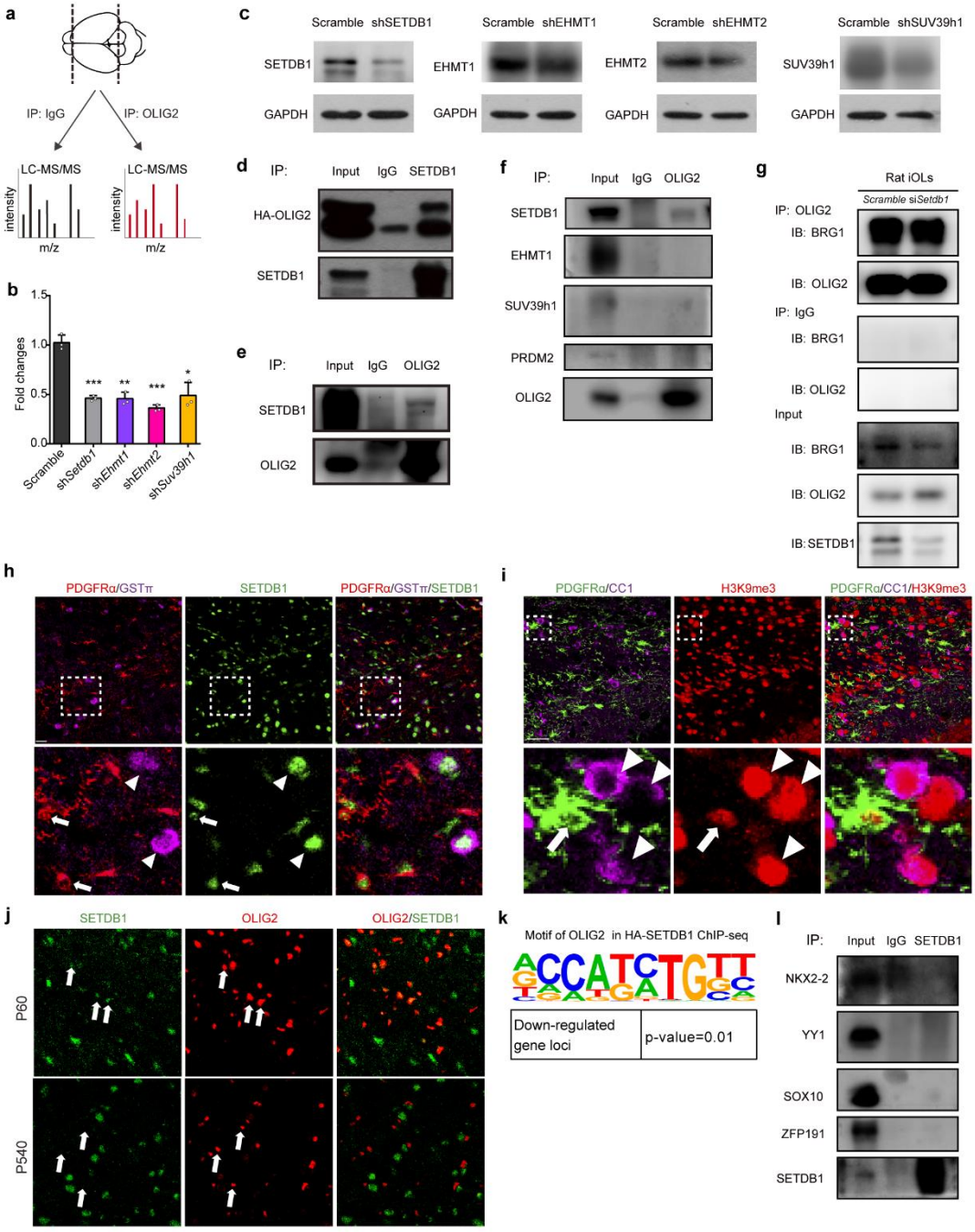
Supplementary Information



Supplementary Figure 1. The repressor role of OLIG2 is essential for oligodendrocyte differentiation

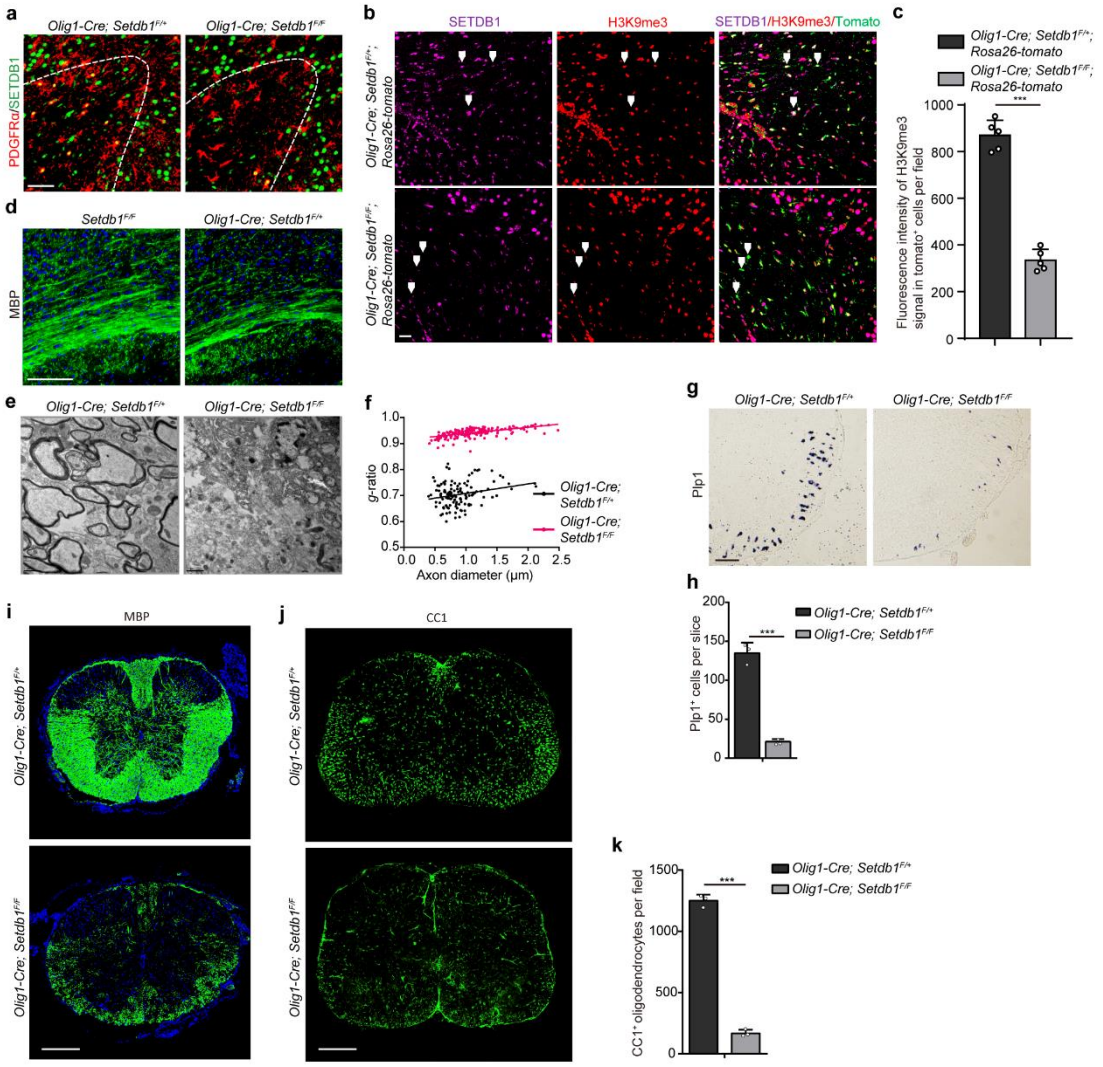
(a) Box plots showing the percentage of downregulated genes, bound by OLIG2 in rat iOLs, during OPC differentiation or oligodendrocyte maturation. (**b**) Schematic diagram showing the strategy that we transfected OLIG2 or OLIG2-VP64 in rat OPCs. (**c**) Pie chart showing number of genes that were significantly upregulated or downregulated in OLIG2-VP64 transfected rat iOLs. (**d**) Real-time PCR analysis of *Mag* and *Mbp* in rat OPCs under differentiation condition following transfection with vehicle, *Olig2-VP64* or *VP64*. Error bars indicate SEM (***p<0.001. one-way ANOVA). (**e**) Schematic diagram showing the retrovirus that we injected in mouse brain.



Supplementary Figure 2. OLIG2 assembles a repressor complex to dominant oligodendrocyte differentiation

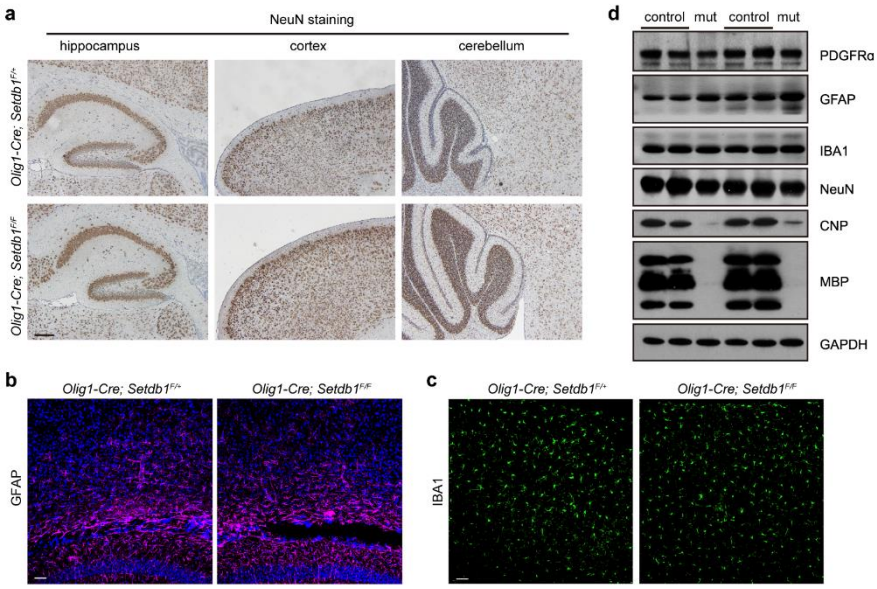
(a) Schematic illustration of OLIG2 binding protein screening. (**b-c**) Efficiency of H3K9me transferases (Setdb1, Ehmt1, Ehmt2 and Suv39h1) knockdown in rat OPCs was examined by quantitative real-time PCR (b) Error bars indicate SEM (***p<0.001; sh*Ehmt1*, p=0.0062; sh*Suv39h1*, p=0.015 one-way ANOVA) and Western Blotting (c). n=3 independent experiments. (**d-e**) Co-immunoprecipitation (Co-IP) of HA-OLIG2 with endogenous SETDB1 from transiently transfected 293T cells (d) or brain tissue lysate of P5 pups (e). n=3 independent experiments. (**f**) Co-IP of OLIG2 with HMTs from rat iOLs. n=3 independent experiments. (**g**) Co-IP of OLIG2 with BRG1 from control or *Setdb1* knock-down rat iOLs. n=3 independent experiments. (**h**) Brain at P14 was immunostained with GSTπ, PDGFRα and SETDB1. Boxed areas in upper panels are shown at

a high magnification in corresponding lower panels. Arrow indicates the co-expression of SETDB1 with PDGFRα, and arrowhead indicates the co-expression of SETDB1 with GSTπ. n=3 different mice. (**i**) Brain at P14 was immunostained with PDGFRα, CC1 and H3K9me3. Boxed areas in upper panels are shown at a high magnification in lower panels. Arrow indicates the co-expression of H3K9me3 with PDGFRα, and arrowhead indicates the co-expression of H3K9me3 with CC1. n=3 different mice. (**j**) Brain at P60 or P540 was immunostained with SETDB1 and OLIG2. n=3 different mice. (**k**) OLIG2 binding motif was identified by HOMER analysis within HA-SETDB1 peaks among loci of genes downregulated upon rat OPC differentiation. Letter size indicates nucleotide frequency at each position of OLIG2 binding motif. (**l**) Co-IP of indicated transcriptional factors with SETDB1 from rat iOLs. n=3 independent experiments. Scale bars: 50μm in (**g-i**).



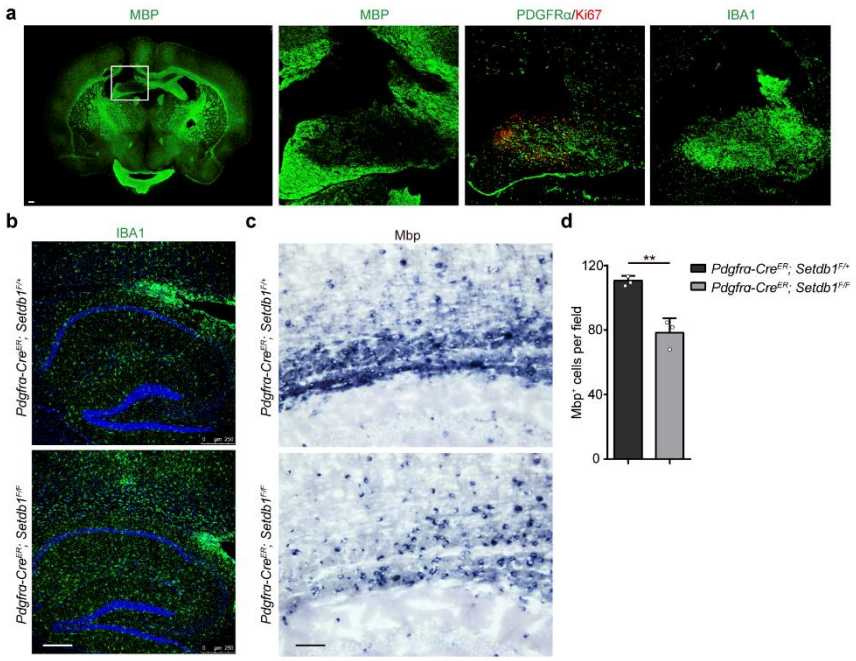
Supplementary Figure 3. SETDB1 is required for oligodendrocyte differentiation in spinal cord

(**a**) Brain sections from *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P14 were immunostained with PDGFRα and SETDB1. n=3 control and mutant mice. (**b**) Immunostaining of SETDB1, H3K9me3 and Rosa26-tomato (OPC lineage cells) in the corpus callosum of *Olig1-Cre*; *Setdb1*$^{F/+}$; Rosa26-tomato and *Olig1-Cre*; *Setdb1*$^{F/F}$; Rosa26-tomato mice. n=3 control and mutant mice. (**c**) Quantification of H3K9me3 in OPC lineage cells in corpus callosum of control and mutant mice. Error bars indicate SEM ($p<0.001$. two-tailed unpaired Student's t test). (**d**) Immunostaining of MBP in the corpus callosum of *Setdb1*$^{F/F}$ and *Olig1-Cre*; *Setdb1*$^{F/+}$ mice at P14. n=3 control and mutant mice. (**e**) Electron micrographs of spinal cord sections from P14 *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice. n=3 control and mutant mice. (**f**) Quantification of myelin sheath thickness (*g*-ratio) in spinal cord. (**g**) In situ hybridization of Plp1 in the spinal cord of *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P7. (**h**) Quantification of Plp1 cells in the spinal cord. Error bars indicate SEM ($p<0.001$. two-tailed unpaired Student's t test). n=3 control and mutant mice. (**i-j**) Immunolabeling of MBP (i) or CC1 (j) in the spinal cord of *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P14. n=3 control and mutant mice. (**k**) Quantification of CC1^{+} cells in the spinal cord. Error bars indicate SEM ($p<0.001$. two-tailed unpaired Student's t test). Scale bars: 50μm in (**a, b** and **d**); 1μm in (**e**); 50μm in (**g** and **i-j**).

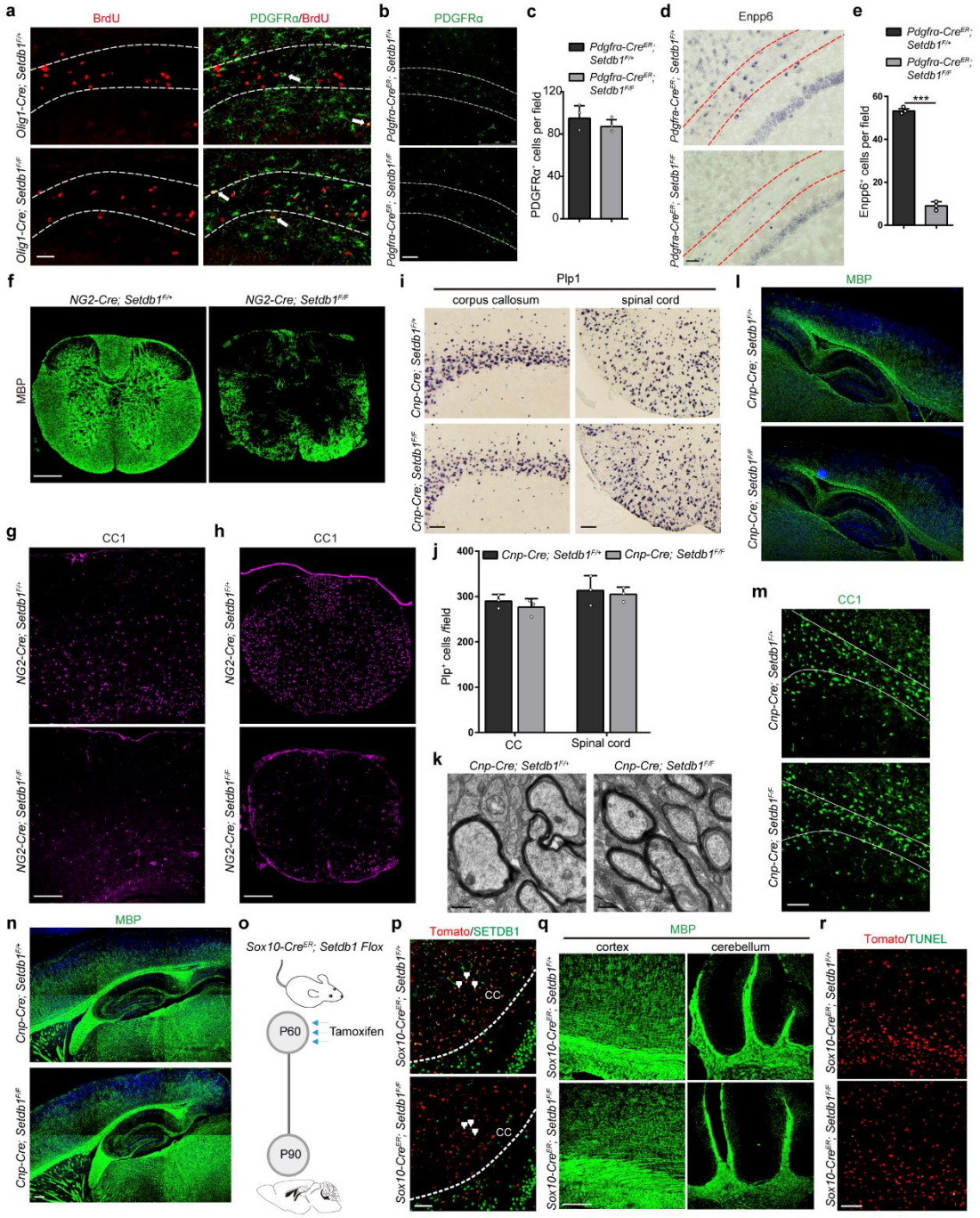


Supplementary Figure 4. SETDB1 ablation in oligodendrocyte lineage does not affect other cell types in brain

(**a**) Immunohistochemical staining of NeuN in the brain of *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P14. n=3 control and mutant mice. (**b**-**c**) Immunostaining of GFAP (b) or IBA1 (c) in the corpus callosum from P14 *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice. n=3 control and mutant mice. (**d**) Western blot analysis of markers of various cells in central nervous system. n=3 independent experiments. Scale bars: 100μm in (**a**); 50μm in (**b**-**c**).



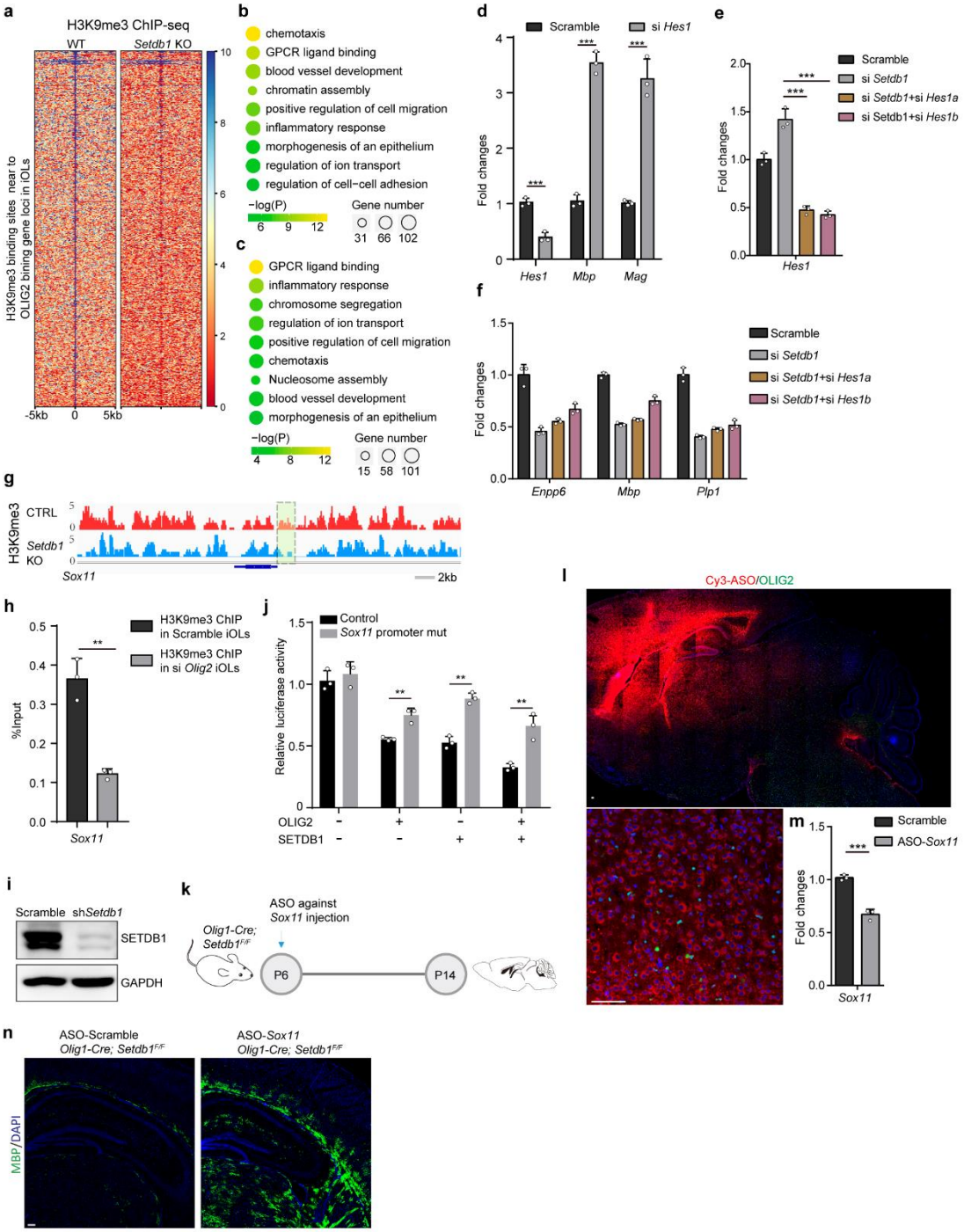
Supplementary Figure 5. SETDB1 is critical for proper myelin repair after demyelination
(**a**) MBP, PDGFRα, Ki67, IBA1 staining shows the LPC lesion pattern in the brain at 7 Dpl. n=3 different mice. (**b**) The IBA1 aggregation scar shows the LPC lesion region in *Pdgfrα-Cre*^{ER}; *Setdb1*$^{F/+}$ and *Pdgfrα-Cre*^{ER}; *Setdb1*$^{F/F}$ mice at 7 Dpl. n=3 control and mutant mice. (**c**-**d**) In situ hybridization and quantification of Mbp^{+} cell in the lesion regions from *Pdgfrα-Cre*^{ER}; *Setdb1*$^{F/+}$ and *Pdgfrα-Cre*^{ER}; *Setdb1*$^{F/F}$ mice 14 Dpl. Error bars indicate SEM (p=0.0043. two-tailed unpaired Student's t test). n=3 control and mutant mice. Scale bars: 250μm in (**a**-**b**); 100μm in (**c**).



Supplementary Figure 6. OLIG2-SETDB1 repressor complex functions at the onset of OPC differentiation

(**a**) Immunostaining for BrdU and PDGFRα in the corpus callosum from *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P14. n=3 control and mutant mice. (**b-c**) Immunostaining and quantification of PDGFRα^{+} cell in the lesion regions from *Pdgfrα-Cre*^{ER}; *Setdb1*$^{F/+}$ and *Pdgfrα-Cre*^{ER}; *Setdb1*$^{F/F}$ mice 14 Dpl. n=3 control and mutant mice. (**d-e**) In situ hybridization and quantification of Enpp6^{+} cell in the corpus callosum from *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P14. Error bars indicate SEM ($p<0.001$. two-tailed unpaired Student's t test). n=3 control and mutant mice. (**f**) Immunostaining of MBP in the spinal cord from *NG2-Cre*; *Setdb1*$^{F/+}$ and *NG2-Cre*; *Setdb1*$^{F/F}$ mice at P14. n=3 control and mutant mice. (**g-h**) Immunostaining of CC1 in the corpus callosum (g) and spinal cord (h) from *NG2-Cre*; *Setdb1*$^{F/+}$ and *NG2-Cre*; *Setdb1*$^{F/F}$

mice at P14. n=3 control and mutant mice. (**i-j**) In situ hybridization and quantification of $Plp1^{+}$ cell in the corpus callosum (left panel) and spinal cord (right panel) of *Cnp-Cre*; $Setdb1^{F/+}$ and *Cnp-Cre*; $Setdb1^{F/F}$ mice at P14. n=3 control and mutant mice. (**k**) Electron micrographs of transverse optic nerve sections from P14 *Cnp-Cre*; $Setdb1^{F/+}$ and *Cnp-Cre*; $Setdb1^{F/F}$ mice. n=3 control and mutant mice. (**l-m**) Immunolabeling of MBP (l) or CC1 (m) in the brain of *Cnp-Cre*; $Setdb1^{F/+}$ and *Cnp-Cre*; $Setdb1^{F/F}$ mice at P14. n=3 control and mutant mice. (**n**) Immunolabeling of MBP in the brain of *Cnp-Cre*; $Setdb1^{F/+}$ and *Cnp-Cre*; $Setdb1^{F/F}$ mice at P60. n=3 control and mutant mice. (**o**) Schematic diagram showing tamoxifen administration to *Sox10-*Cre^{ER}; *Setdb1 Flox* mice in 3 consecutive days at P60, followed by tissue collection at P90. (**p-q**) Immunolabeling of SETDB1 (p) and MBP (q) in the brain white matter of *Sox10-*Cre^{ER}; $Setdb1^{F/+}$; Rosa26-tomato and *Sox10-*Cre^{ER}; $Setdb1^{F/F}$; Rosa26-tomato mice at P90. n=3 control and mutant mice. (**r**) TUNEL assay in the corpus callosum of *Sox10-*Cre^{ER}; $Setdb1^{F/+}$; Rosa26-tomato and *Sox10-*Cre^{ER}; $Setdb1^{F/F}$; Rosa26-tomato mice at P90. n=3 control and mutant mice. Scale bars: 100µm in (**a, b**, **d** and **i**); 250µm in (**f**, **l**, **g**, **h, m**, **n** and **p-r**); 0.5µm in (**k**).



Supplementary Figure 7. OLIG2 recruits SETDB1 to silent inhibitors of oligodendrocyte differentiation

(**a**) Heatmap of H3K9me3 enrichment signals from control and *Setdb1* mutant mouse iOLs around genes occupied with OLIG2 and downregulated from rat OPCs to iOLs. (**b-c**) Representative GO analysis of the significantly upregulated genes between iOLs from *Olig1-Cre*; *Setdb1*$^{F/+}$ and *Olig1-Cre*; *Setdb1*$^{F/F}$ brain cortex (b) and between OPCs under differentiation condition following transfection with *Olig2* and *Olig2-VP64* (c). (**d**) Real-time PCR analysis of indicated genes in rat iOLs transfected with Scramble or siRNA against *Hes1*. Error bars indicate SEM ($p<0.001$. two-tailed unpaired Student's t test). (**e**) Efficiency of Hes1 knockdown in rat OPCs. Error bars indicate

SEM (***p<0.001. one-way ANOVA). (**f**) Expression of *Enpp6*, *Mbp* and *Plp1* was measured in rat OPCs under differentiation condition following transfection with siRNA against *Setdb1* and *Hes1*. (**g**) Genome browser visualization of H3K9me3-targeting regions on the gene loci of *Sox11* in control and *Setdb1* mutant iOLs. (**h**) H3K9me3 ChIP-qPCR assay on Sox11 promoter in in control and *Olig2* knock-down rat iOLs. Error bars indicate SEM (p=0.0052. two-tailed unpaired Student's t test). (**i**) Efficiency of Setdb1 knockdown in 293T was examined by Western Blot. n=3 independent experiments. (**j**) Luciferase activity in 293T cells transfected with luciferase reporter driven by wild-type or mutant *Sox11* promoter together with OLIG2 or SETDB1. Error bars indicate SEM (OLIG2+, p=0.0012; SETDB1+, p=0.0029; OLIG2+ SETDB1+, p=0.0046; two-tailed unpaired Student's t test). (**k**) Schematic diagram showing modified ASO to *Sox11* injected into *Olig1-Cre*; *Setdb1*$^{F/F}$ pups at P6 and followed by tissue collection at P14. (**l**) Immunolabeling of OLIG2 and Cy3 fluorescence tagged ASO showed the efficiency in mouse forebrain. n=3 different mice. (**m**) Efficiency of *Sox11* knockdown by ASO in the whole forebrain as shown. Error bars indicate SEM (p<0.001. two-tailed unpaired Student's t test). (**n**) Immunolabeling for MBP on the corpus callosum from ASO-Scramble, *Olig1-Cre*; *Setdb1*$^{F/F}$ and ASO-si *Sox11*, *Olig1-Cre*; *Setdb1*$^{F/F}$ mice at P14. n=3 control and mutant mice. Scale bars: 50μm in (**l**); 100μm in (**n**).