SUPPLEMENTARY FIGURES AND LEGENDS

Supplementary Information includes seven figures (Figures S1 to S7).

Figure S1 shows the effect of whole-body knockout of Seh1 on mouse myelination.

Figure S2 shows that Seh1 is required for oligodendrocyte maturation in brain.

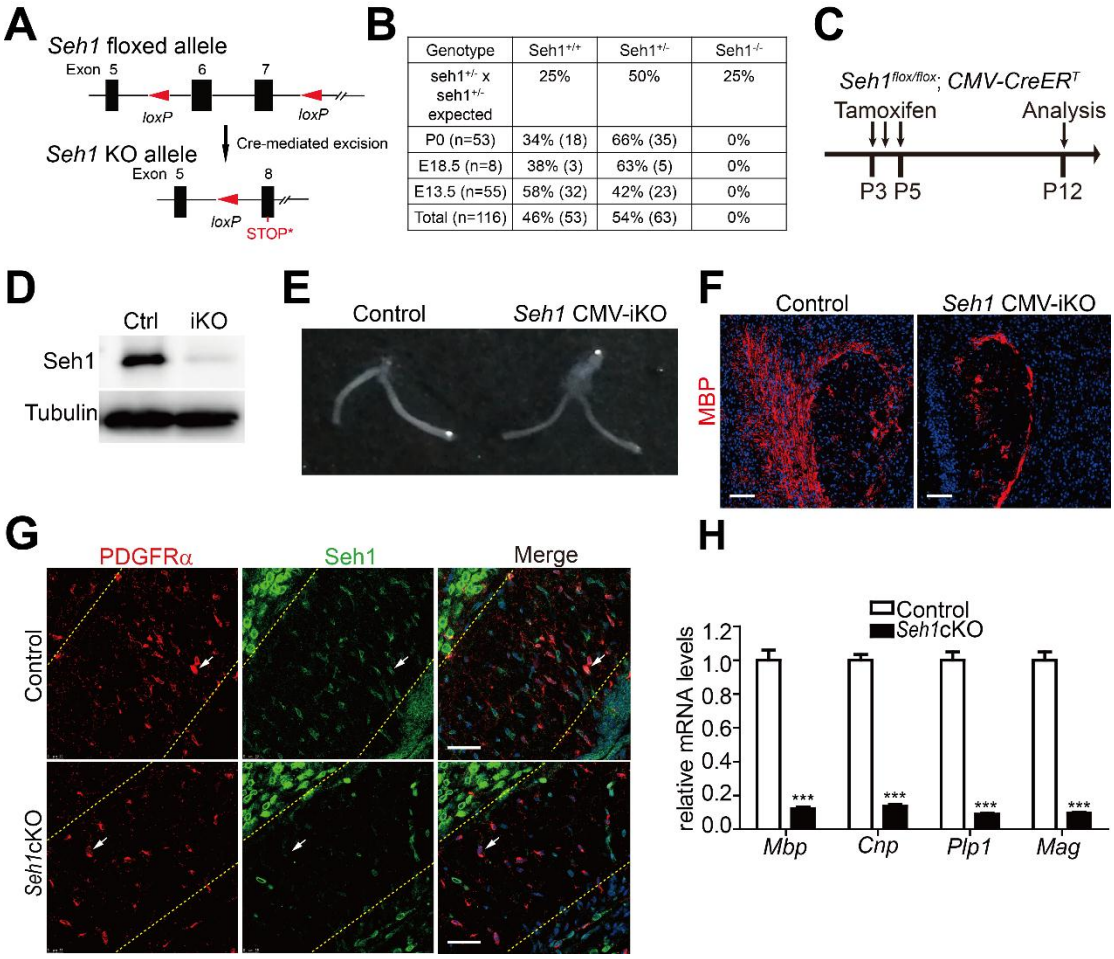
Figure S3 shows that Seh1 is required for remyelination after demyelination in spinal cord.

Figure S4 shows that the phenotypes upon depletion of Seh1 are not attributed to significant alterations in nucleocytoplasmic transport or cellular signaling.

Figure S5 shows that Seh1 regulates a core network of genes.

Figure S6 shows that Seh1 interacts with Olig2 and the interaction is not regulated by protein phosphorylation.

Figure S7 shows that intact Seh1 recruit Olig2 and Brd7 at nuclear pheriphery.



Genotype	Seh1$^{+/+}$	Seh1$^{+/-}$	Seh1$^{-/-}$
seh1$^{+/-}$ x seh1$^{+/-}$ expected	25%	50%	25%
P0 (n=53)	34% (18)	66% (35)	0%
E18.5 (n=8)	38% (3)	63% (5)	0%
E13.5 (n=55)	58% (32)	42% (23)	0%
Total (n=116)	46% (53)	54% (63)	0%

Figure S1. Seh1 Is Critical for Myelination in the CNS, Related to Figure 1.

(**A**) Schematic diagram depicting the strategy used to generate *Seh1*$^{LoxP/LoxP}$ mice and Cre-mediated excision of the floxed *Seh1*. LoxP sites flanking exon 6 and 7 of *Seh1* are indicated.

(**B**) Genotype distribution of offspring from Seh1$^{+/-}$ intercross.

(**C**) Diagram showing tamoxifen administration from P3 to P5 and analysis at P12 on the inducible mouse models.

(**D**) Immunoblotting validation of knockout efficiency of Seh1 in tail protein lysate from control and *Seh1*CMV-iKO mice at P12 after tamoxifen administration from P3 to P5.

(**E**) Representative images of optic nerves from control and *Seh1*CMV-iKO mice at

P12.

(**F**) Immunostaining of MBP in the corpus callosum of control and *Seh1*CMV-iKO at P12. Scale bars=100 μm.

(**G**) Immunostaining of PDGFRα and Seh1 in the corpus callosum of control and *Seh1*cKO mice at P14. Arrows indicate the PDGFRα$^+$ cells. Scale bars=50 μm.

(**H**) qRT-PCR analysis of myelin-associated genes in the spinal cord of control and *Seh1*cKO mice at P14. (*n*= 3 for control and *n*=3 for mutant mice. Mbp, ***p=0.0001, Cnp, ***p<0.0001, Plp1, ***p<0.0001, Mag, ***p<0.0001)

Data are represented as mean ± SEM; ***p<0.001; two-tailed unpaired Student's *t* test.

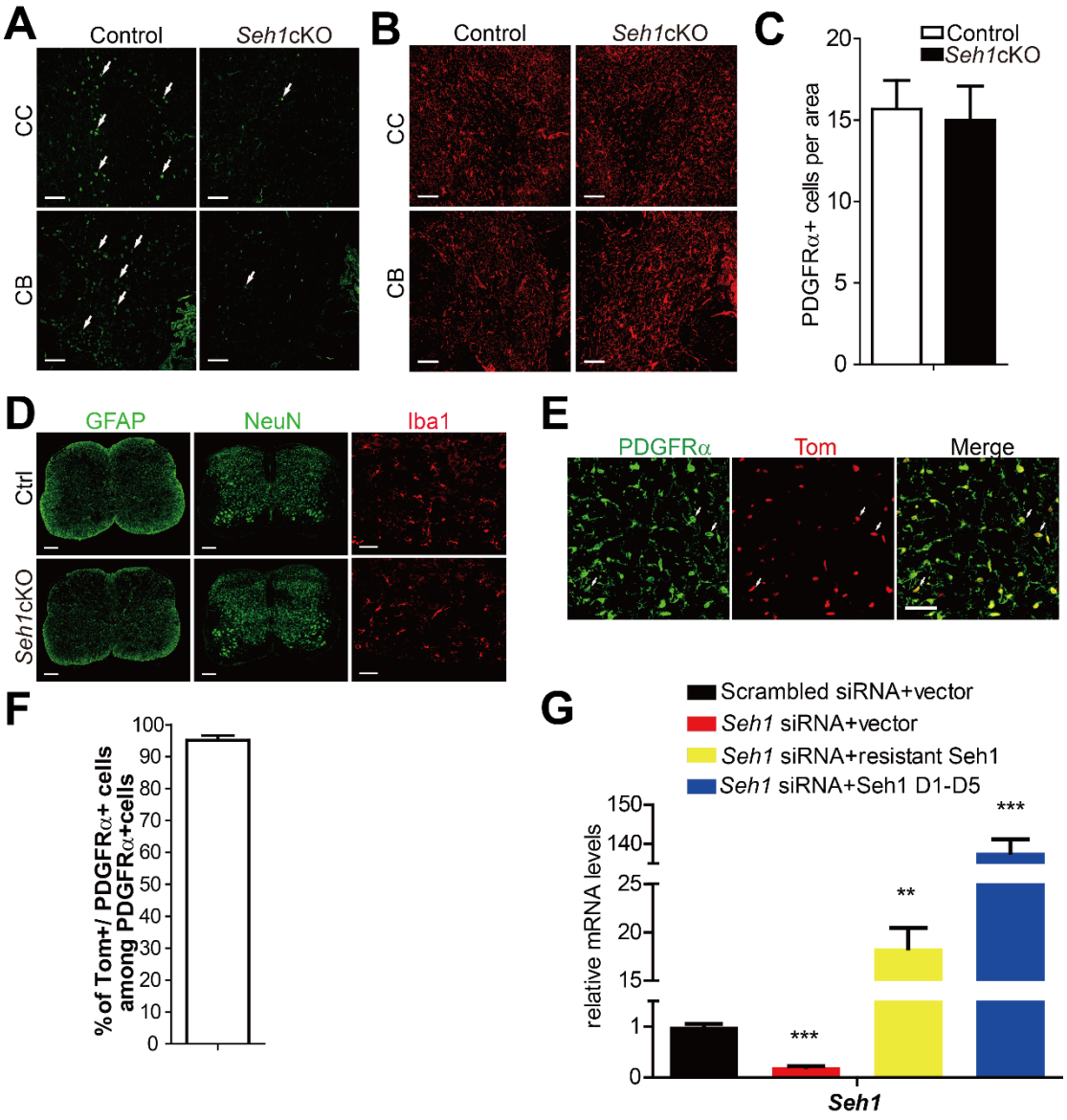


Figure S2. Seh1 Is Required for Oligodendrocyte Maturation in Brain and Seh1 Deletion in the OL Lineage Does not Affect the Development of other Neural Cell Types in the Brain, Related to Figure 2.

(**A**-**B**) Immunostaining of CC1 (**A**) and PDGFRα (**B**) in the corpus callosum (CC) and cerebellum (CB) of control and *Seh1*cKO at P14. Arrows indicate CC1$^+$cells. Scale bars=100 μm.

(**C**) Quantification of PDGFRα$^+$ OPCs per 0.04mm^2 in the corpus callosum of control

and *Seh1*cKO at P14 (*n*= 3 for control and *n*=3 for mutant mice).

(**D**) Immunostaining of astrocyte marker GFAP, neuron marker NeuN and microglial marker Iba1 in the spinal cord of control and *Seh1*cKO at P14. Scale bars=200 μm.

(**E**) Immunostaining of PDGFRα and tdTomato on the corpus callosum of *Sox10-CreER*^{T}:*Seh1*$^{LoxP/+}$:*Rosa26*^{Tom} mice at P4 after tamoxifen administration at P3. Arrows indicate PDGFRα^{+}/tdTomato^{+} cells. Scale bars=50 μm.

(**F**) Quantification of Tom^{+}/ PDGFRα^{+} cells among PDGFRα^{+} cells in the corpus callosum of *Sox10-CreER*^{T}:*Seh1*$^{LoxP/+}$:*Rosa26*^{Tom} mice at P4 after tamoxifen administration at P3 (*n*=3 mice).

(**G**) qRT-PCR analysis of Seh1 in primary rat OPCs following treatments with siRNA and expression construct as indicated. (*n*=3 independent experiments from 6 rats. Seh1 siRNA, ***p=0.0002, Seh1 siRNA+resistant Seh1, **p=0.0012, Seh1 siRNA+Seh1 D1-D5, ***p<0.0001)

Data are represented as mean ± SEM; **p<0.01, ***p<0.001, two-tailed unpaired Student's *t* test.

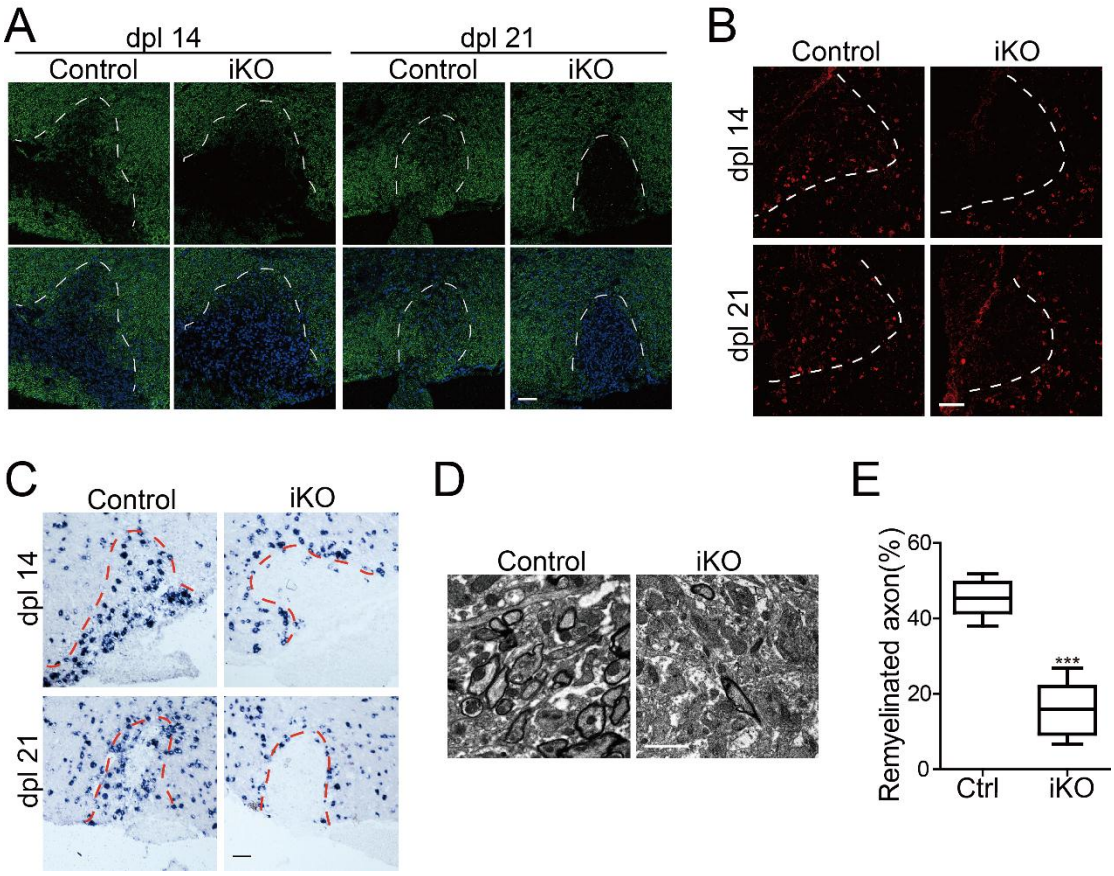


Figure S3. Seh1 Is Required for CNS Remyelination after Demyelination, Related to Figure 3.

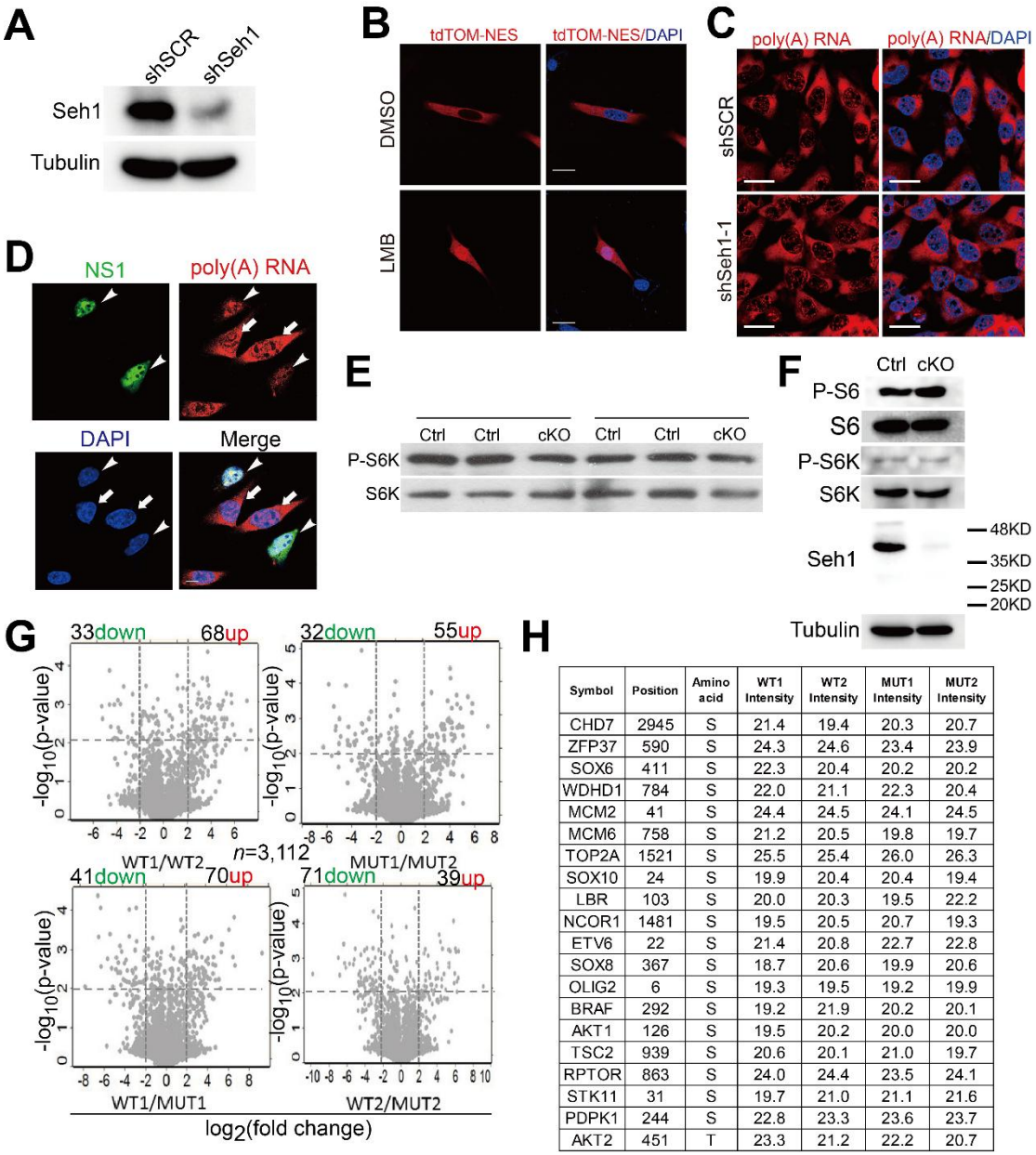
(**A**-**B**) Immunostaining of MBP (**A**) and CC1 (**B**) in spinal cord lesions of control and *Seh1 OPC*-iKO mice at Dpl 14 and 21. Scale bars=50 μm.

(**C**) *In situ* hybridization of *Plp1* in the spinal cord LPC lesions of control and *Seh1 OPC*-iKO mice at Dpl 14 and 21. Scale bars=100 μm.

(**D**) Electron microscopy of LPC lesions from control and *Seh1 OPC*-iKO spinal cord at Dpl 14. Scale bars=2 μm

(**E**) Quantification of remyelinated axons in LPC-induced lesion of control and *Seh1 OPC*-iKO spinal cord at Dpl 14 (*n*= 3 for control and *n*=3 for *Seh1*-iKO mice at 14dpl. ***p=0.0009).

Data are represented as mean ± SEM; ***p<0.001, two-tailed unpaired Student's *t* test.



Symbol	Position	Amino acid	WT1 Intensity	WT2 Intensity	MUT1 Intensity	MUT2 Intensity
CHD7	2945	S	21.4	19.4	20.3	20.7
ZFP37	590	S	24.3	24.6	23.4	23.9
SOX6	411	S	22.3	20.4	20.2	20.2
WDHD1	784	S	22.0	21.1	22.3	20.4
MCM2	41	S	24.4	24.5	24.1	24.5
MCM6	758	S	21.2	20.5	19.8	19.7
TOP2A	1521	S	25.5	25.4	26.0	26.3
SOX10	24	S	19.9	20.4	20.4	19.4
LBR	103	S	20.0	20.3	19.5	22.2
NCOR1	1481	S	19.5	20.5	20.7	19.3
ETV6	22	S	21.4	20.8	22.7	22.8
SOX8	367	S	18.7	20.6	19.9	20.6
OLIG2	6	S	19.3	19.5	19.2	19.9
BRAF	292	S	19.2	21.9	20.2	20.1
AKT1	126	S	19.5	20.2	20.0	20.0
TSC2	939	S	20.6	20.1	21.0	19.7
RPTOR	863	S	24.0	24.4	23.5	24.1
STK11	31	S	19.7	21.0	21.1	21.6
PDPK1	244	S	22.8	23.3	23.6	23.7
AKT2	451	T	23.3	21.2	22.2	20.7

Figure S4. Depletion of Seh1 Does not Cause Major Defects in Nuclear Transport or cellular Signaling, Related to Figure 4.

(**A**) Immunoblotting validation of knockdown efficiency of Seh1 in Oli-neu cells. shSCR, scrambled shRNA; shSeh1, *Seh1* shRNA.

(**B**) Immunofluorescence of tdTomato-NES signals in Oli-neu cells treated with DMSO or Leptomycin B (LMB). NES, nuclear export signal. Nuclei were counterstained with

DAPI as blue color. Scale bars=15 μm.

(**C**) *In situ* hybridization of poly (A) RNA with oligo-dT probe in Oli-neu cells transduced with scrambled or *Seh1* shRNA. Scale bars=25 μm.

(**D**) *In situ* hybridization of poly (A) RNA with oligo-dT probe in Oli-neu cells transfected with NS1 plasmid. Scale bars=10 μm.

(**E**) Immunoblotting of P-T389-S6K and S6K in spinal cord lysate of control and *Seh1*cKO at P7. Ctrl: control; cKO: *Seh1*cKO.

(**F**) Immunoblotting of indicated proteins in OPCs from control and *Seh1*cKO mice.

(**G**) Volcano plots showing changes in phosphopeptides in wild-type1/ wild-type2, mutant1/mutant2, wild-type1/ mutant1 and wild-type2/ mutant2 comparisons. Up, up-regulated; Down, down-regulated; WT, wild-type; MUT, mutant. The absolute value of $\log_2$(fold change) >= 2 and -$\log_{10}$(p-value) > 2 were considered as significantly changed.

(**H**) Intensities of specific phosphosites of myelination regulators in wild-type1, wild-type2, mutant1 and mutant2. The number represent $\log_2$(label free quantification values).

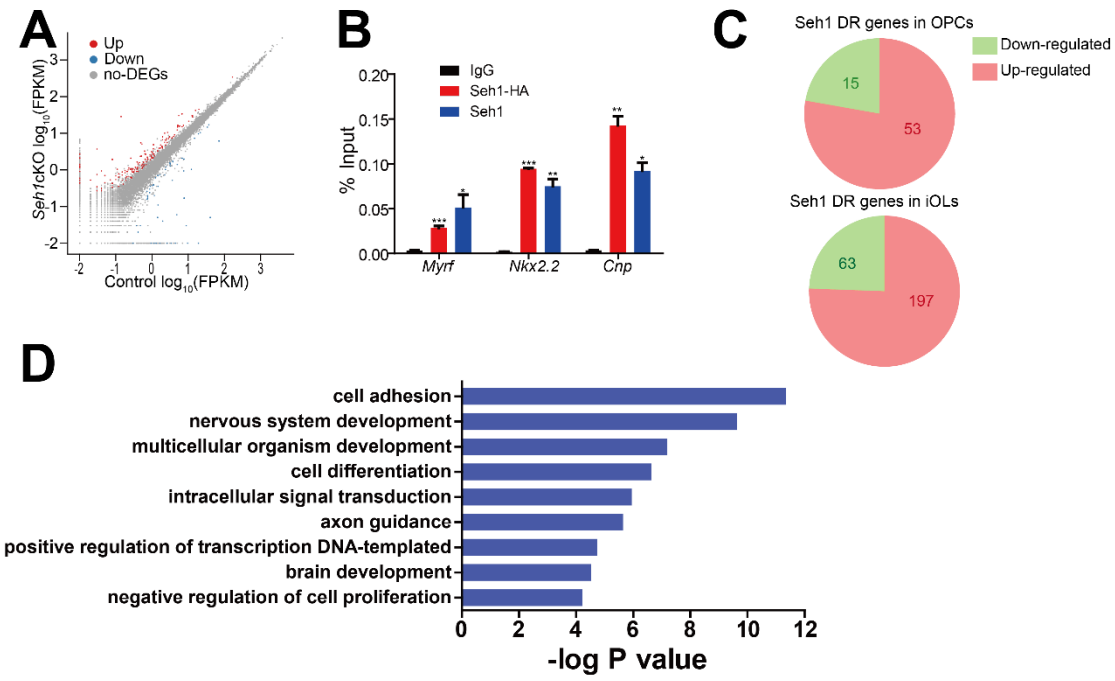


Figure S5. Seh1 Regulates a Core Network of Genes, Related to Figure 5.

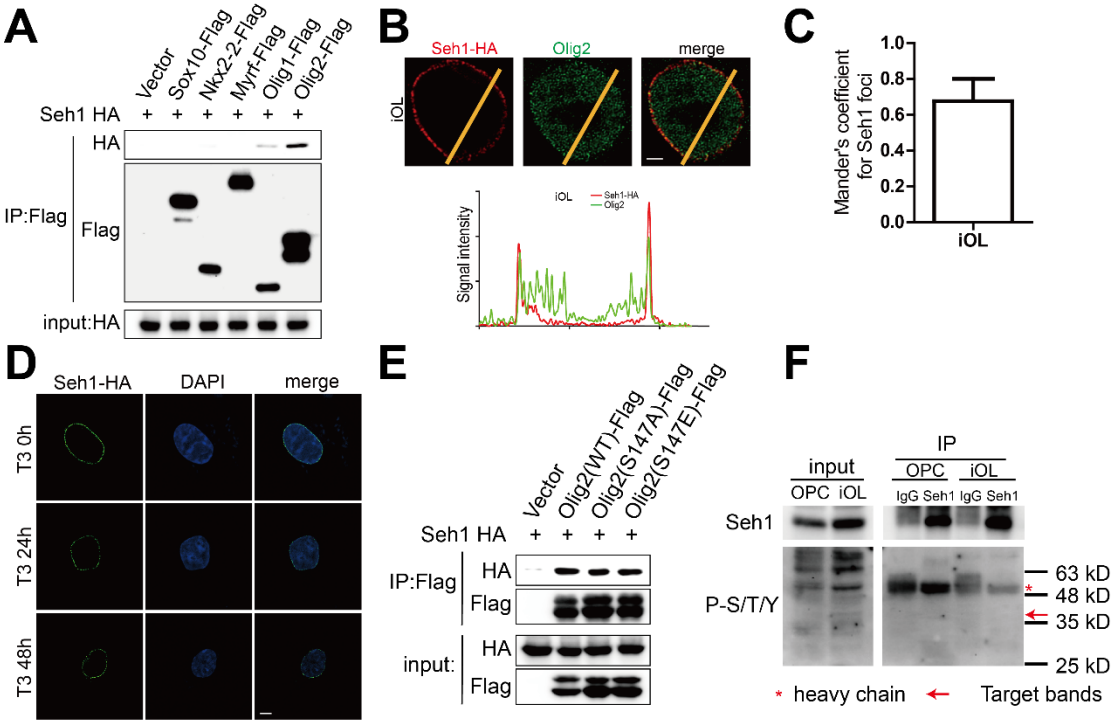
(**A**) Differentially expressed transcripts (highlighted in color; fold change >2, false discovery rate<0.001) between control and *Seh1*cKO OPCs.

(**B**) Enrichment of Seh1 and Seh1-HA at promoter regions of oligodendrocyte genes in iOLs (*n*= 3 independent experiments from 6 rats. *Myrf*, ***p=0.0007, *p=0.02; *Nkx2.2*, ***p<0.0001, **p=0.0012; *Cnp*, **p=0.0052, *p=0.0101).

(**C**) Fraction of down-regulated and up-regulated by Seh1 in the Seh1-DR genes. DR, directly regulated.

(**D**) Gene ontology (GO) analysis of the CO loci.

Data are represented as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001; two-tailed unpaired Student's *t* test.



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Symbol	Protein name	Accession
Nup98	Nucleoporin 98	P49793
Nup85	Nucleoporin 85	Q4QQS8
Sec13	Protein SEC13 homolog	Q5XFW8
Nup160	Nucleoporin 160	D3ZBL6
Nup155	Nucleoporin 155	F1LS02
Nup43	Nucleoporin 43	M0R9Y3
Nup133	Nucleoporin 133	D3Z8B2
Nup107	Nucleoporin 107	P52590
Nup50	Nucleoporin 50	G3V7R1
Nup214	Nucleoporin 214	D4ACK1
Nup37	Nucleoporin 37	G3V6M8
Nup188	Nucleoporin 188	F1LRC6
Nup93	Nucleoporin 93	Q66HC5
BRD7	Bromodomain containing 7	D3ZXX7
CHD2	Chromodomain helicase DNA binding protein 2	D4AD08
SMARCA2	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin a2	Q6DUH4

Figure S6. Identification of Seh1-Binding Proteins, Related to Figure 6.

(**A**) Co-immunoprecipitation of Seh1-HA with Sox10-Flag, Nkx2-2-Flag, Myrf-Flag, Olig1-Flag and Olig2-Flag from transiently transfected 293T cells. It shows that Seh1 does not interact with Sox10, Nkx2-2, Myrf and weakly binds Olig1.

(**B**) Top panel: the fluorescence intensities of Seh1 and Olig2 at the nuclear periphery were observed by quantifying the intensity along a line drawn through a cell cross-section in iOLs. Bottom panel: the representative intensity graphs show associated

Seh1 signals with Olig2 signals at nuclear periphery. Scale bars=5 μm.

(**C**) Quantitative analysis of association of Seh1 and Olig2 signals by Mander's coefficient analyzing the ratio of Seh1-positive foci associated with Olig2-positive foci.

(**D**) Super-resolution images of Seh1-HA in rat OPCs treated with T3 for 0, 24, 48hours. Scale bars=5 μm.

(**E**) Co-immunoprecipitation of Seh1-HA with Olig2-Flag, Olig2(S147A)-Flag, and Olig2(S147E)-Flag from transiently transfected 293T cells. It shows that the interaction between Seh1 and Olig2 was not affected by phosphorylation status at serine 147 site.

(**F**) Immunoblotting with phospho-serine/threonine/tyrosine antibody following immunoprecipitation of endogenous Seh1 in rat OPCs or iOLs.

(**G**) A subset list of proteins that were specifically identified in anti-Seh1 IP from primary rat differentiating iOLs.

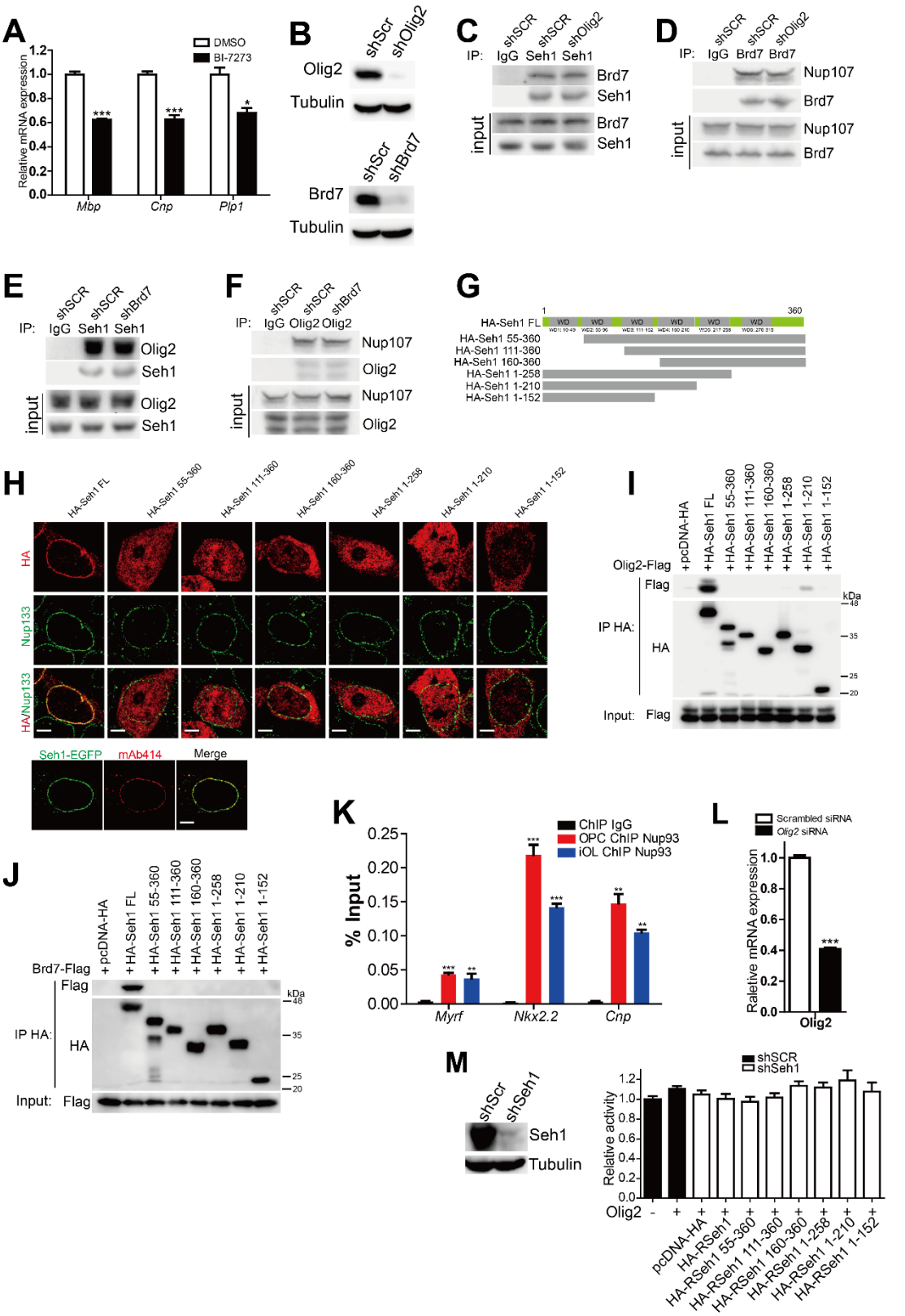
A
DMSO
BI-7273
Relative mRNA expression
Mbp
Cnp
Plp1
B
shScr
shOlig2
Olig2
Tubulin
shScr
shBrd7
Brd7
Tubulin
C
shSCR
shSCR
shOlig2
IP: IgG Seh1 Seh1
Brd7
Seh1
input
Brd7
Seh1
D
shSCR
shSCR
shOlig2
IP: IgG Brd7 Brd7
Nup107
Brd7
input
Nup107
Brd7
E
shSCR
shSCR
shBrd7
IP: IgG Seh1 Seh1
Olig2
Seh1
input
Olig2
Seh1
F
shSCR
shSCR
shBrd7
IP: IgG Olig2 Olig2
Nup107
Olig2
input
Nup107
Olig2
G
HA-Seh1 FL
HA-Seh1 55-360
HA-Seh1 111-360
HA-Seh1 160-360
HA-Seh1 1-258
HA-Seh1 1-210
HA-Seh1 1-152
H
HA-Seh1 FL
HA-Seh1 55-360
HA-Seh1 111-360
HA-Seh1 160-360
HA-Seh1 1-258
HA-Seh1 1-210
HA-Seh1 1-152
HA
Nup133
HA/Nup133
Seh1-EGFP
mAb414
Merge
I
+pcDNA-HA
+HA-Seh1 FL
+HA-Seh1 55-360
+HA-Seh1 111-360
+HA-Seh1 160-360
+HA-Seh1 1-258
+HA-Seh1 1-210
+HA-Seh1 1-152
Olig2-Flag
IP HA:
Flag
HA
kDa
Input:
Flag
J
+pcDNA-HA
+HA-Seh1 FL
+HA-Seh1 55-360
+HA-Seh1 111-360
+HA-Seh1 160-360
+HA-Seh1 1-258
+HA-Seh1 1-210
+HA-Seh1 1-152
Brd7-Flag
IP HA:
Flag
HA
kDa
Input:
Flag
K
% Input
ChIP IgG
OPC ChIP Nup93
iOL ChIP Nup93
Myrf
Nkx2.2
Cnp
L
Scrambled siRNA
Olig2 siRNA
Raletive mRNA expression
Olig2
M
shScr
shSeh1
Seh1
Tubulin
shSCR
shSeh1
Relative activity
Olig2
pcDNA-HA
HA-RSeh1
HA-RSeh1 55-360
HA-RSeh1 111-360
HA-RSeh1 160-360
HA-RSeh1 1-258
HA-RSeh1 1-210
HA-RSeh1 1-152

Figure S7. Intact Seh1 Is Required for its Binding to Olig2 and Brd7 and its Nuclear Pore Localization and Inhibitor of Brd7 (BI-7273) impairs myelination-associated gene induction, Related to Figure 7.

(**A**) qRT-PCR analysis of myelination-associated genes in primary rat OPCs under differentiation conditions following treatments with BI-7273 (10 μM). The data are presented as mean±SEM. (*n*= 3 independent experiments from 3 rats. *Mbp*, ***p=0.0001; *Cnp*, ***p=0.0009; *Plp1*, *p=0.0103)

(**B**) Immunoblotting validation of knockdown efficiency of Olig2 and Brd7 in Oli-neu cells. shSCR, scrambled shRNA; shOlig2, *Olig2* shRNA; shBrd7, Brd7 shRNA.

(**C**) Co-immunoprecipitation of Brd7 with Seh1 from Oli-neu cells transfected with indicated shRNA construct. SCR, scrambled.

(**D**) Co-immunoprecipitation of Nup107 with Brd7 from Oli-neu cells transfected with indicated shRNA construct. SCR, scrambled.

(**E**) Co-immunoprecipitation of Olig2 with Seh1 from Oli-neu cells transfected with indicated shRNA construct. SCR, scrambled.

(**F**) Co-immunoprecipitation of Nup107 with Olig2 from Oli-neu cells transfected with indicated shRNA construct. SCR, scrambled.

(**G**) The scheme of Seh1 truncation constructs used in the mapping experiments.

(**H**) Top panel, Immunofluorescence of Seh1 variants and Nup133 signals in 293T cells. Seh1 variants were transfected in 293T cells. 48hours later, cell staining were performed. Endogenous nup133 serves as nuclear pore marker. Scale bars=5 μm. Bottom panel, Immunofluorescence of Seh1-EGFP and mAb414 signals in 293T cells.

Seh1-EGFP was transfected in 293T cells. 48hours later, cell staining were performed.

(**I** and **J**) The interaction of Seh1 variants and Olig2 (**I**) or Brd7 (**J**) analyzed by immunoprecipitation in 293T cells.

(**K**) Enrichment of Nup93 at promoter regions of oligodendrocyte genes in OPCs and iOls (*n*= 3 independent experiments from 6 rats. *Myrf*, ***p=0.0002, **p=0.0069; *Nkx2.2*, ***p=0.0003, ***p<0.0001; *Cnp*, **p=0.0089, **p=0.0015).

(**L**) qPCR validation of knockdown efficiency of *Olig2* in primary rat OPCs following treatments with scrambled or *Olig2* siRNAs, respectively. (*n*= 3 independent experiments from 3 rats. ***p<0.0001).

(**M**) Left: Immunoblotting validation of knockdown efficiency of *Seh1* in 293T cells. shSCR, scrambled shRNA; shSeh1, *Seh1* shRNA. Right: Luciferase activity of *interferon-*β promoter-driven reporters in control (shSCR, scrambled) or *Seh1* knockdown (shSeh1) 293T cells transfected with Olig2 and indicated Seh1 overexpressing constructs. (*n*= 3 independent experiments).

Data are represented as mean±SEM; *p<0.05, **p<0.01, ***p<0.001; two-tailed unpaired Student's *t* test.