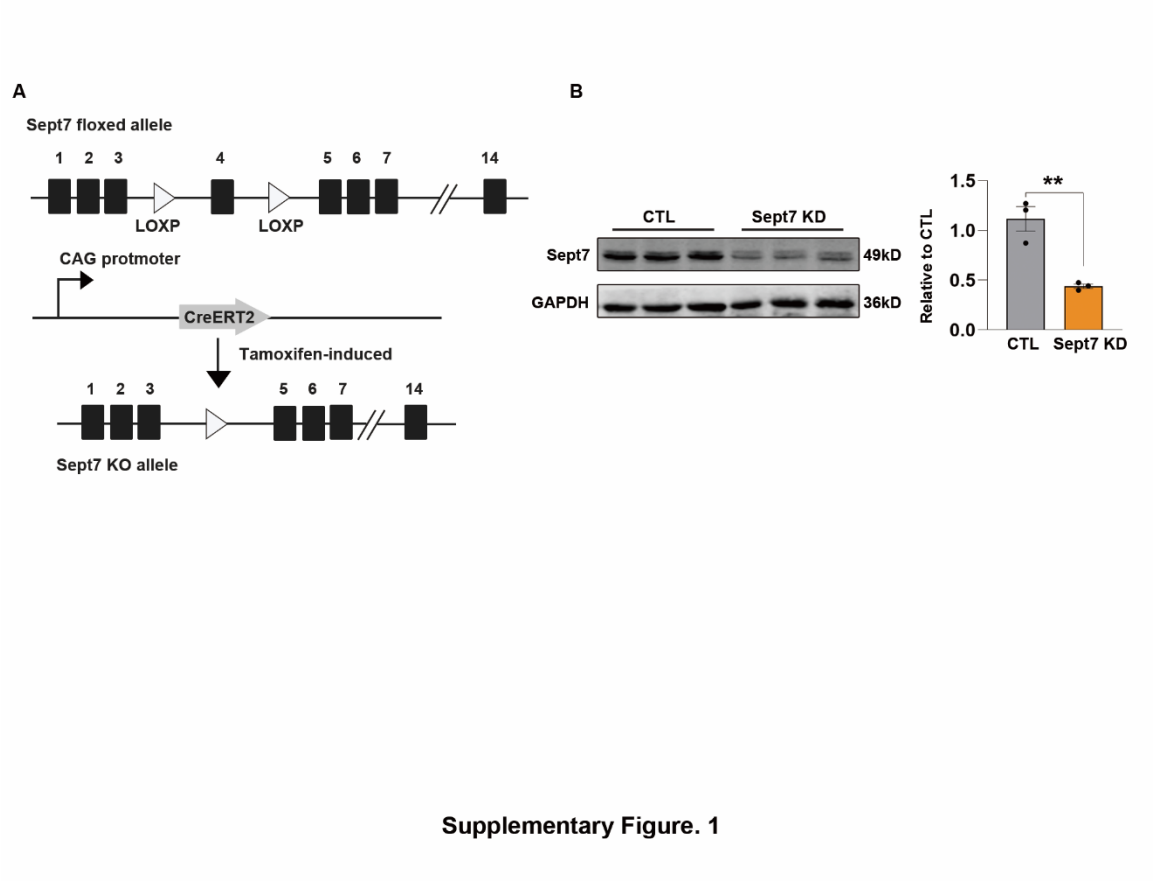
Supplementary Material 1:

Supplementary Figures and legends



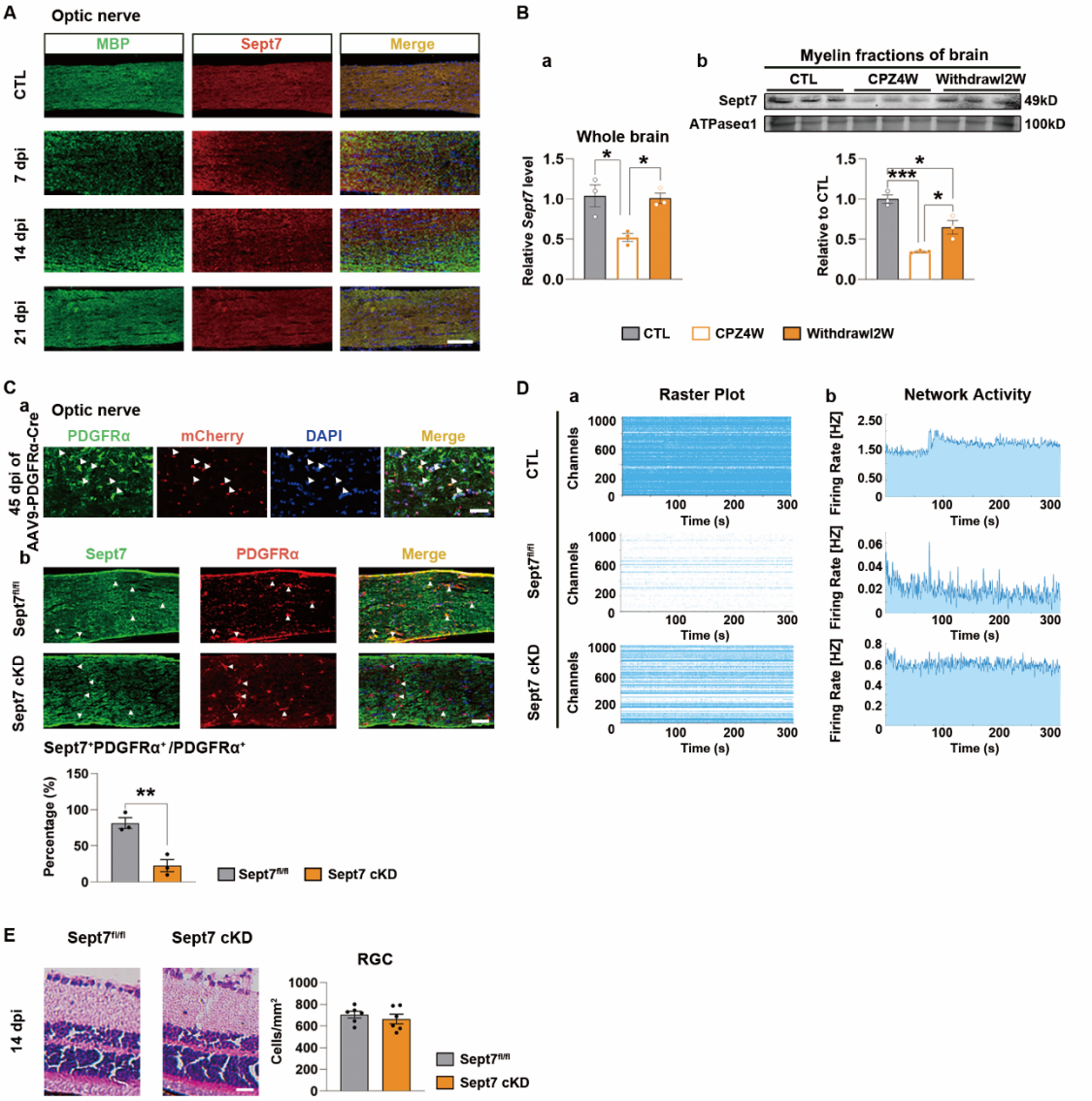
Supplementary Figure. 1

Supplementary Figure 1. Sept7 knockdown efficiency.

(A) Schematic representation of the constructs used to create the transgenic mice.

(B) WB analysis of Sept7 expression in the brain. (n = 3/group).

Data are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined by Student's unpaired t-test (**B**). $^{**}P < 0.01$. WB: western blot.



Supplementary Figure. 2

Supplementary Figure 2. Sept7 is essential for remyelination.

(A) Immunostaining of MBP and Sept7 in the ON at 7, 14, and 21 dpi. Scale bar = 100 μm.

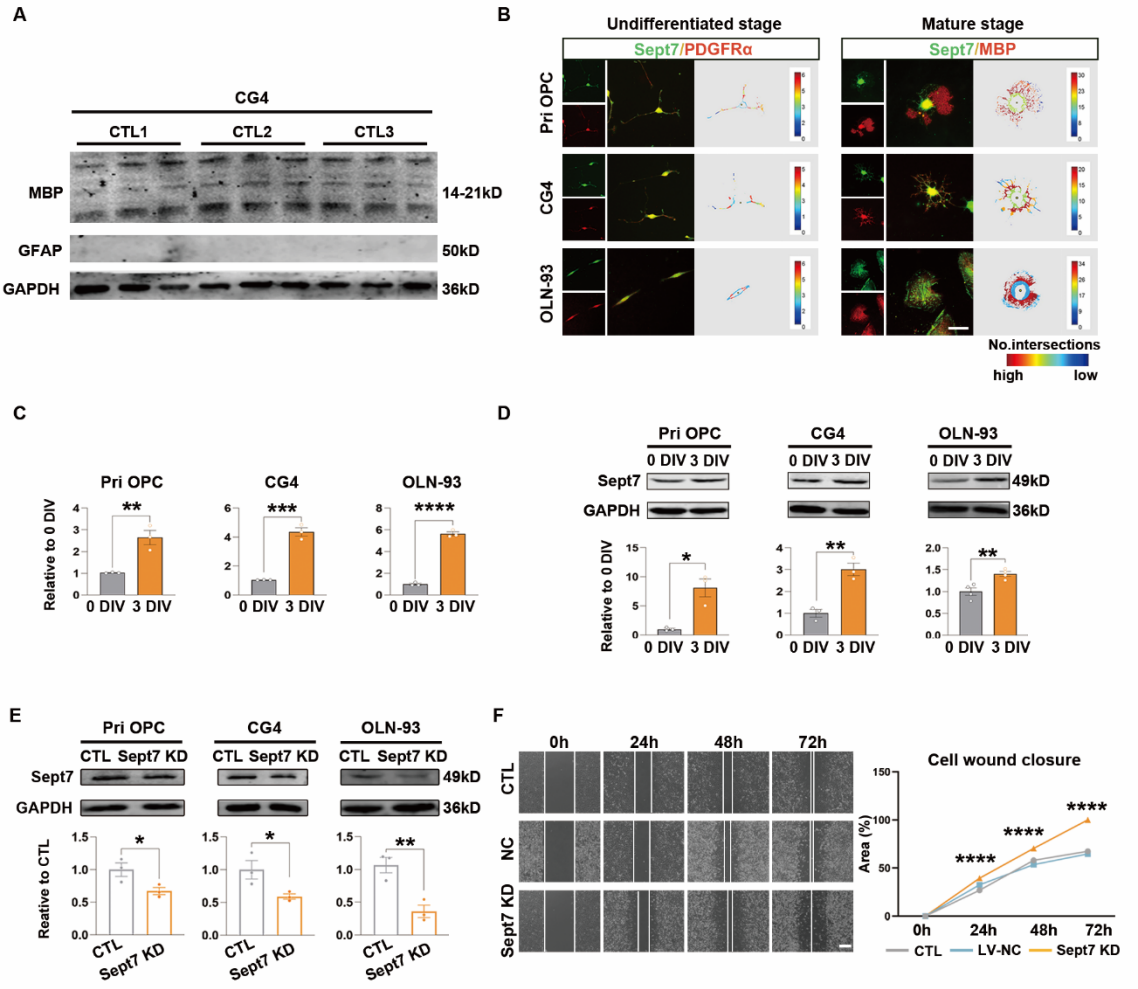
(B) Sept7 expression in the CPZ-induced model**. (a)** qPCR and **(b)** WB analyses of Sept7 expression in the brain and myelin fractions of the brain. (*n* = 3/group).

(C) Confirmation of AAV9-PDGFRα-Cre-mCherry (AAV9-PDGFRα-Cre) transfection into PDGFRα^{+} OPCs. **(a)** Immunostaining of PDGFRα and mCherry in the ON. Scale bar = 25 μm. **(b)** Immunostaining of Sept7 and PDGFRα in the ON of Sept7$^{fl/fl}$ and Sept7 cKD mice. Scale bar = 50 μm. (*n* = 3/group).

(D) *In vitro* retinal electrophysiological recording analysis of light-evoked electrophysiological responses of RGC at 21 dpi**. (a)** Raster plot over a total duration of 300 s at 21 dpi. **(b)** Corresponding firing rates over the same duration. (*n* = 3/group).

(E) H&E staining of the retina at 14 dpi. Representative images and quantification are shown. Scale bar = 25 μm. (*n* = 6/group).

Immunostaining for Sept7 in this figure was performed using a rabbit antibody (unlike the mouse antibody used in Fig. 1A). Data are expressed as the mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Tukey's post-test **(B)** and Student's unpaired *t*-test **(C** and **E)**. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. ON: optic nerve; dpi: days post-injection; CPZ: cuprizone; qPCR: Quantitative real-time PCR; WB: western blot; s: seconds; OPC: oligodendrocyte precursor cell; cKD: conditional knockdown; RGC: retinal ganglion cell; H&E: hematoxylin & eosin.



Supplementary Figure. 3

Supplementary Figure 3. Sept7 participates in OPC proliferation, migration, and differentiation *in vitro.*

(A) WB analysis of the mature OL marker MBP and the astrocyte marker GFAP after CG4 differentiation.

(B) Representative images of Sept7 expression in PDGFRα^{+} OPCs and MBP^{+} mature OLs in Pri OPCs, CG4, and OLN-93 cell lines. Scale bar = 25 μm.

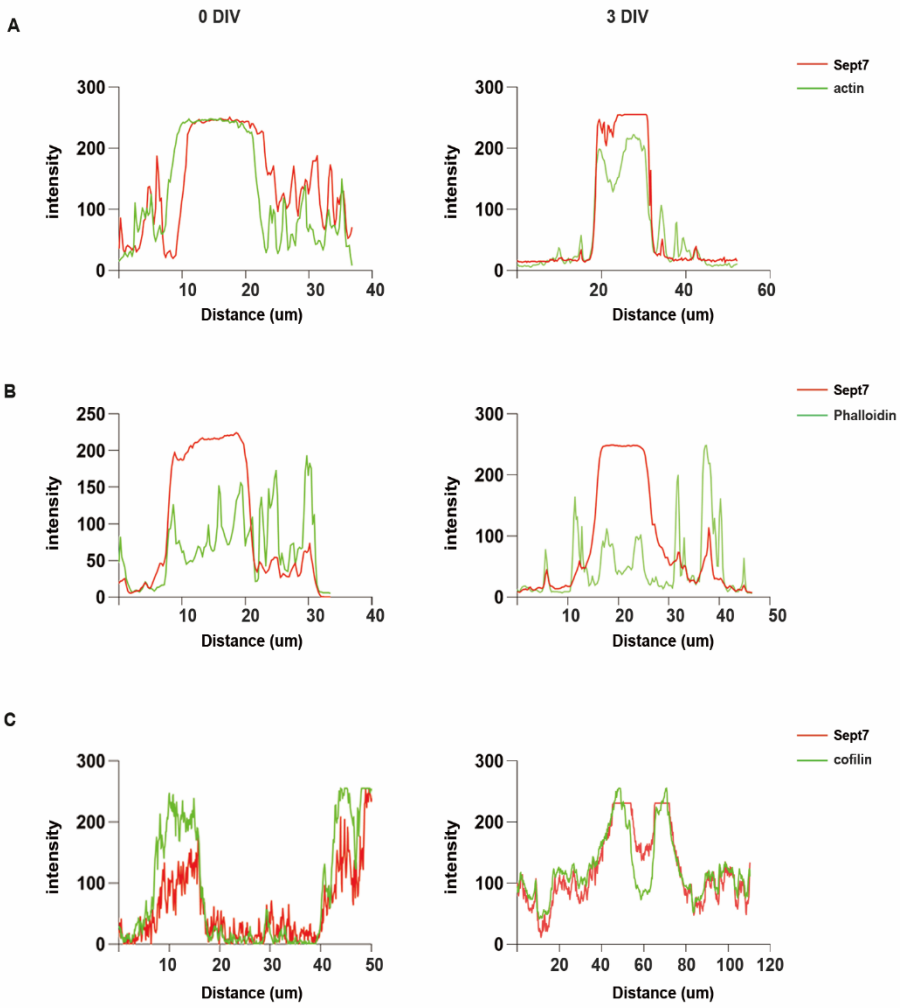
(C) qPCR analysis of Sept7 mRNA expression (n = 3/group).

(D) WB analysis of Sept7 protein expression. (n = 3/group).

(E) WB analysis of Sept7 KD efficiency in Pri OPCs, CG4, and OLN-93 cell lines after LV-shRNA transfection. (n = 3/group).

(F) Scratch wound healing assay of OLN-93 cells. (n = 3/group; CTL vs. Sept7 KD).

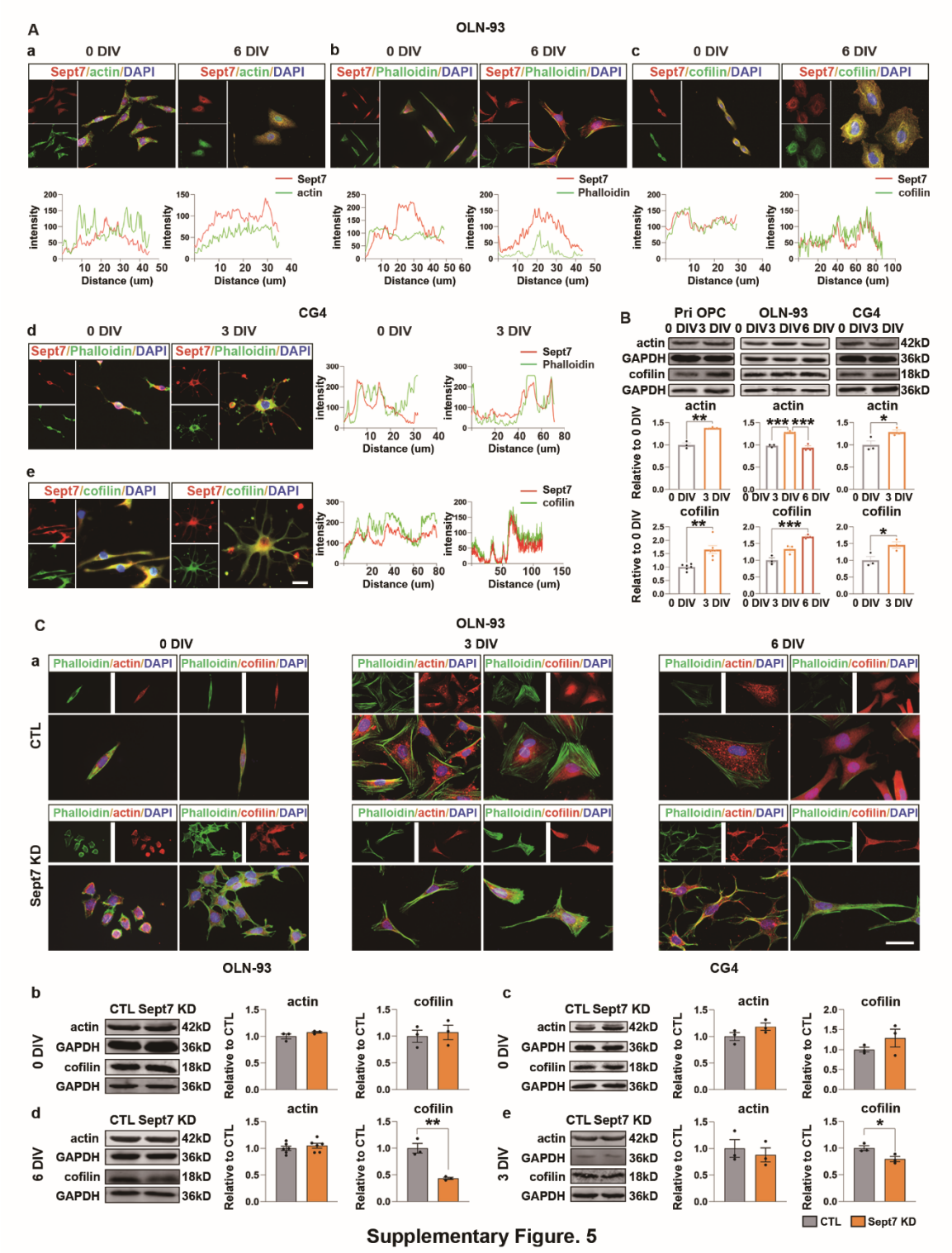
Data are expressed as the mean ± SEM. Statistical significance was determined by Student's unpaired t-test **(C-E)** and two-way ANOVA followed by Tukey's post-test **(F)**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. qPCR: Quantitative real-time PCR; WB: western blot; OL: oligodendrocyte; OPC: oligodendrocyte precursor cell; CTL: control; KD: knockdown; Pri: primary; LV: lentivirus.



Supplementary Figure. 4

Supplementary Figure 4. Correlative line scans of Sept7 with microfilaments and cofilin.

(A–C) Correlative line scan analyses showing the spatial correlation between Sept7 (red) and **(A)** actin (green), (**B**) F-actin (phalloidin, green), or **(C)** cofilin (green) at 0 and 3 DIV. DIV: days *in vitro*.



Supplementary Figure. 5

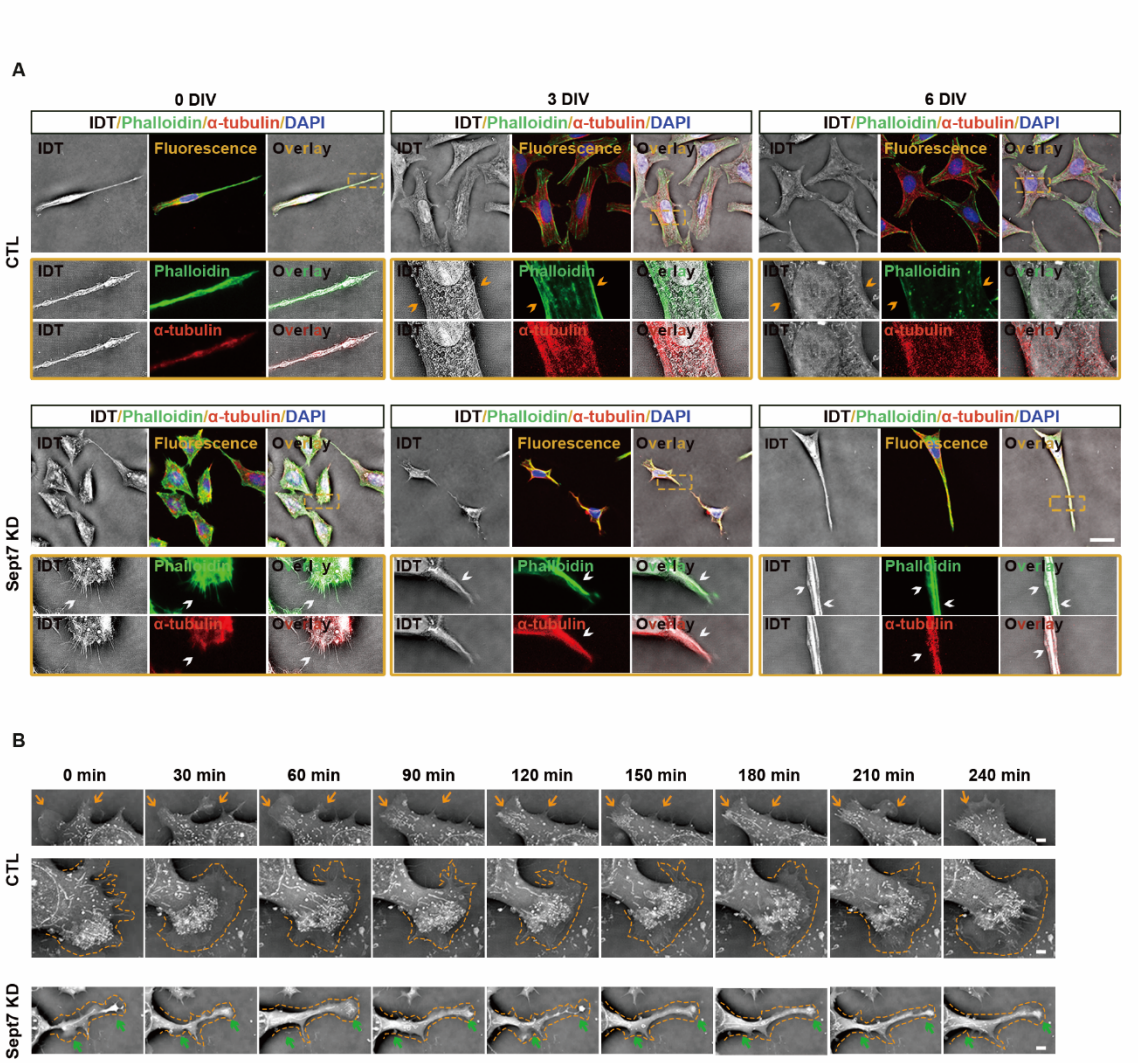
Supplementary Figure 5. Sept7 deficiency impairs OPC differentiation through the disruption of actin dynamic organization.

(A) Immunostaining of Sept7 with **(a)** actin, **(b, d)** F-actin (phalloidin), and **(c, e)** cofilin in undifferentiated and mature OLN-93 and CG4 cells, with correlative line scan analyses. Scale bar = 25 μm.

(B) WB analysis of actin and cofilin expression during Pri OPCs, OLN-93, and CG4 cell lines differentiation. (n = 3-6/group).

(C) Representative images and quantifications of actin, cofilin, and F-actin (phalloidin) expression. **(a)** Immunostaining and phalloidin staining of actin (red), cofilin (red), and F-actin (phalloidin, green) in OLN-93 cells. Scale bar = 25 μm. **(b-e)** WB analysis of actin and cofilin expression in **(b**, **c)** undifferentiated and **(d, e)** mature OLN-93 and CG4 cells. (n = 3-6/group).

Data are expressed as the mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Tukey's post-test **(B,** OLN-93**)** and Student's unpaired t-test **(B,** Pri OPCs and CG4; **C)**. *P < 0.05, **P < 0.01, ***P < 0.001. OPC: oligodendrocyte precursor cell; DIV: days *in vitro*; WB: western blot; Pri: primary.



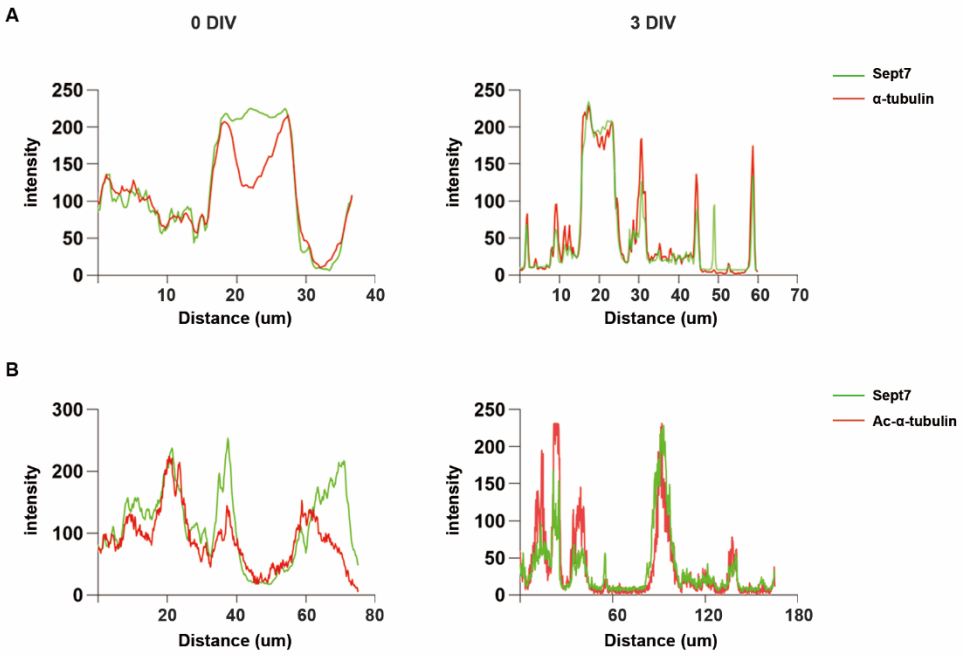
Supplementary Figure. 6

Supplementary Figure 6. Live-cell IDT analysis during OLN-93 cell differentiation.

(A) Representative images of IDT, F-actin (phalloidin, green), and α-tubulin (red) in OLN-93 cells at 0, 3, and 6 DIV. Boxed regions are shown at higher magnification in the lower panels. At 0 DIV, Sept7 KD induced filopodial protrusions that colocalized with

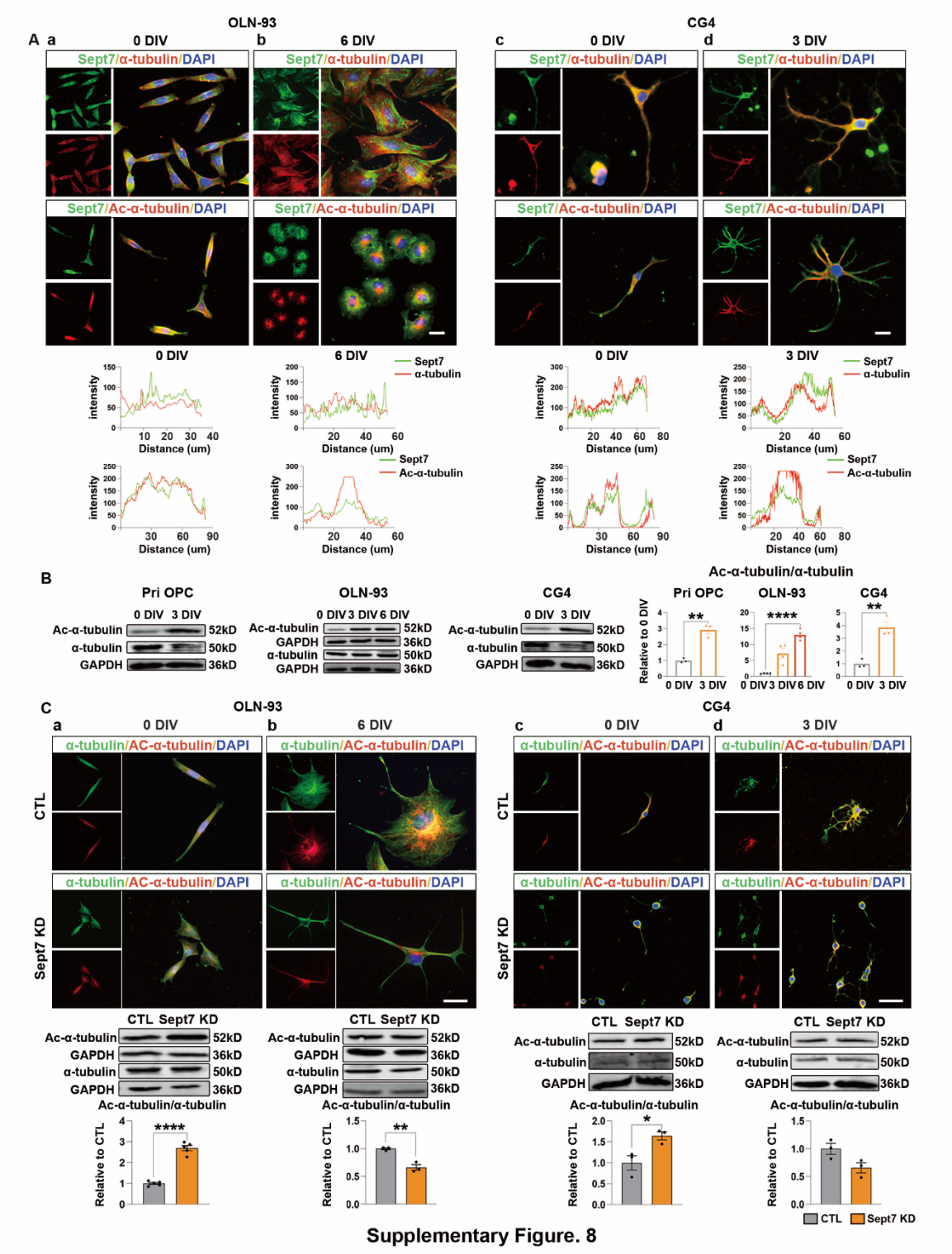
phalloidin-stained F-actin (white arrowheads). At 3 and 6 DIV, CTL cells expanded into flattened lamellar membranes (yellow arrowheads), whereas Sept7 KD cells remained constricted and filopodia-rich, with accumulations of α-tubulin that colocalized with F-actin (white arrowheads). Scale bar = 20 μm.

(B) Live-cell IDT imaging of CTL and Sept7 KD OLN-93 cells at 3 DIV for 4 h. In the CTL cell insets, yellow arrows point to a lamellipodia undergoing membrane fusion. The membrane, which remained stable during overall growth, is outlined by a yellow dotted line. In the Sept7 KD cell insets, protrusions that remained stable are outlined by a yellow dotted line, and green arrows point to lamellipodia that converted into filopodia. Scale bar = 3 μm. IDT: Intensity diffraction tomography; DIV: days *in vitro*; CTL: control; KD: knockdown.



Supplementary Figure. 7

Supplementary Figure 7. Correlative line scans of Sept7 with α-tubulin and Ac-α-tubulin. (A and B) Correlative line scan analyses showing the spatial correlation between Sept7 (green) and **(A)** α-tubulin (red) or **(B)** Ac-α-tubulin (red) at 0 and 3 DIV. AC: acetylated; DIV: days *in vitro.*



Supplementary Figure. 8

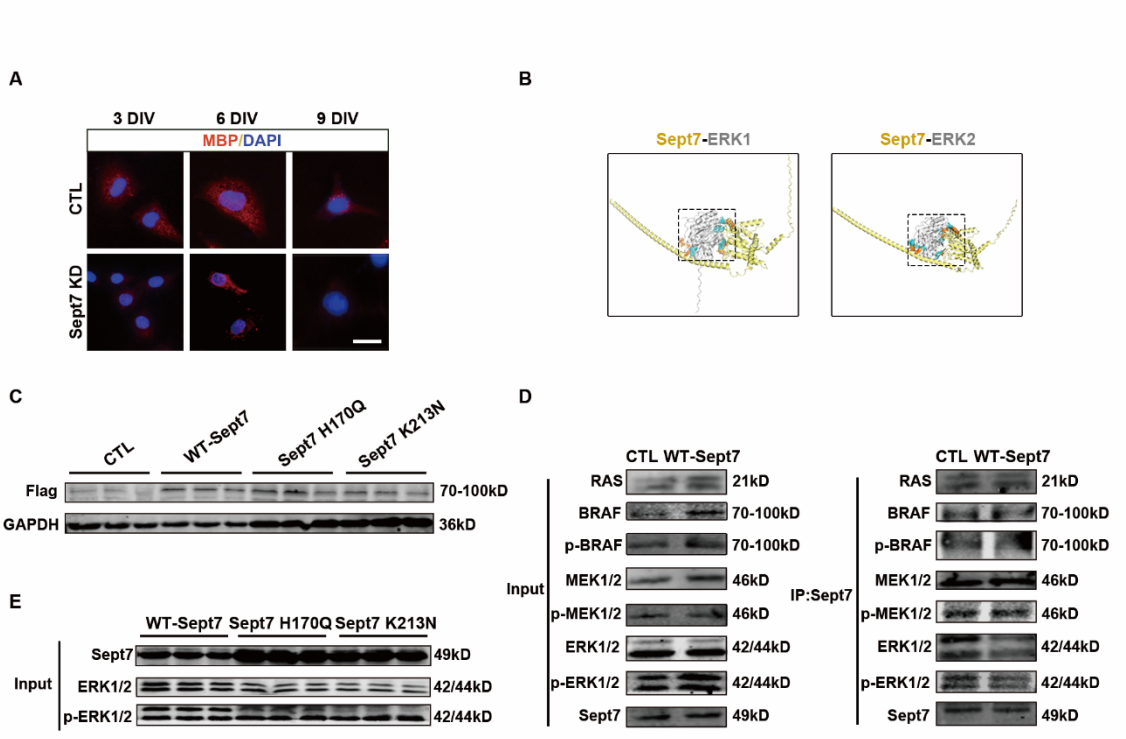
Supplementary Figure 8. Ablation of Sept7 impairs microtubule plasticity in OLN-93 and CG4 cell lines.

(A) Immunostaining of Sept7 (green) with α-tubulin (red) and Ac-α-tubulin (red) in undifferentiated and mature **(a, b)** OLN-93 and **(c, d)** CG4 cells, with correlative line scan analyses. Scale bar = **(a, b)** 20 μm and **(c, d)** 25 μm.

(B) WB analysis of Ac-α-tubulin/α-tubulin expression during Pri OPCs, OLN-93, and CG4 cell lines differentiation. (n = 3-4/group).

(C) Representative images and quantifications of α-tubulin and Ac-α-tubulin expression. Immunostaining of α-tubulin (green) and Ac-α-tubulin (red) in **(a**, **b** upper panels**)** OLN-93 and **(c**, **d** upper panels**)** CG4 cells. WB analysis of Ac-α-tubulin/α-tubulin expression in (**a, b** lower panels**)** OLN-93 and **(c**, **d** lower panels**)** CG4 cells. Scale bar = 25 μm (OLN-93 cells), 50 μm (CG4 cells). (n = 3-5 group).

Data are expressed as the mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Tukey's post-test **(B,** OLN-93**)** and Student's unpaired t-test **(B,** Pri OPCs and CG4; **C)**. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$. AC: acetylated; OPC: oligodendrocyte precursor cell; WB: western blot; Pri: primary.



Supplementary Figure. 9

Supplementary Figure 9. Sept7 associates with the multiple components of the MAPK/ERK1/2 pathway.

(A) Immunostaining of MBP at 3, 6, and 9 DIV in CTL and Sept7 KD OLN-93 cells. Scale bar = 25 μm.

(B) Structural model generated by AlphaFold3 showing the interaction between Sept7 and ERK1/2. The images in **Fig. 8C** correspond to the dotted rectangle in **Supplementary Fig. 9B**, and are shown at a higher magnification. The ERK1/2 structure (gray) is shown with its amino acids (blue sticks); the Sept7 structure (yellow) is shown with its amino acids (yellow sticks).

(C) WB analysis of Flag expression. (*n* = 3/group).

(D) Co-IP analysis of Sept7 association with the entire MAPK/ERK1/2 pathway in CTL and WT-Sept7 cells. (*n* = 3/group). Co-IP assays were performed on multiple independent membranes, and representative blots are shown. Full input blots with GAPDH loading controls (dotted lines) and IP membranes are provided in Supplementary Material 2.

(E) Input of Sept7, ERK1/2, and p-ERK1/2 in OLN-93 cells. (*n* = 3/group). Full input blots are provided in Supplementary Material 2.

CTL: control; KD: knockdown; Co-IP: co-immunoprecipitation; WB: western blot; DIV: days *in vitro*.