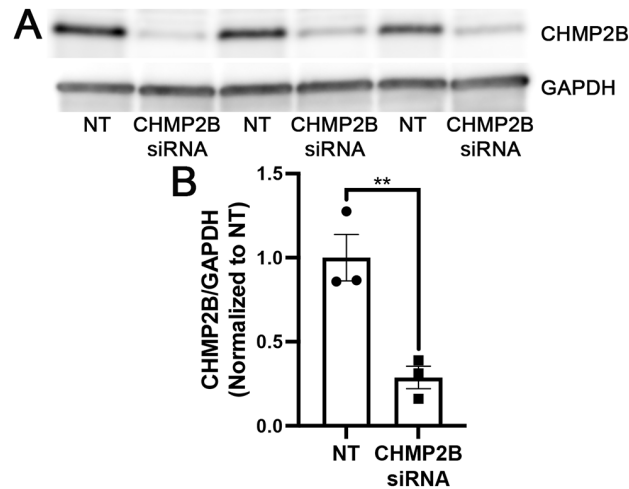
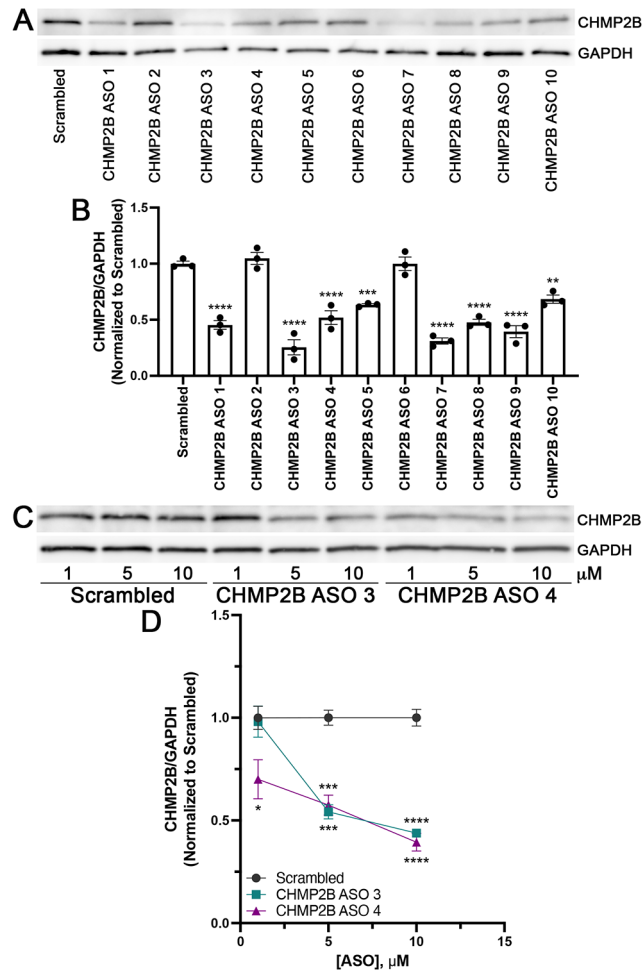


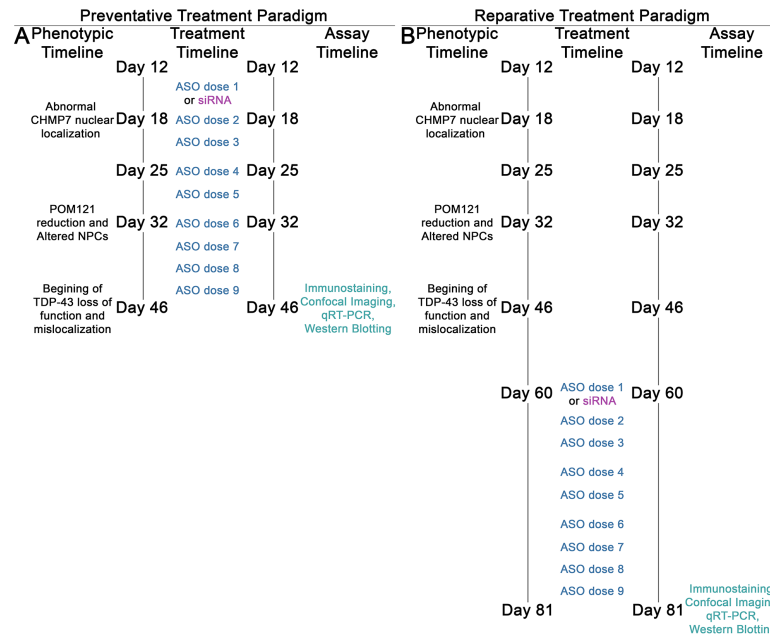


Supplemental Figure 1: siRNA mediated knockdown of CHMP2B in iPSNs. (A) Western blot for CHMP2B expression 3 weeks following nucleofection of CHMP2B or non-targeting (NT) control siRNAs. siRNA as indicated on bottom, antibody for western blot as indicated on right. (B) Quantification of CHMP2B protein expression in iPSN lysates 3 weeks following the nucleofection of CHMP2B or NT control siRNAs. GAPDH was used for normalization. n = 3 control iPSC lines. Student's t-test was used to calculate statistical significance. ** p < 0.01.

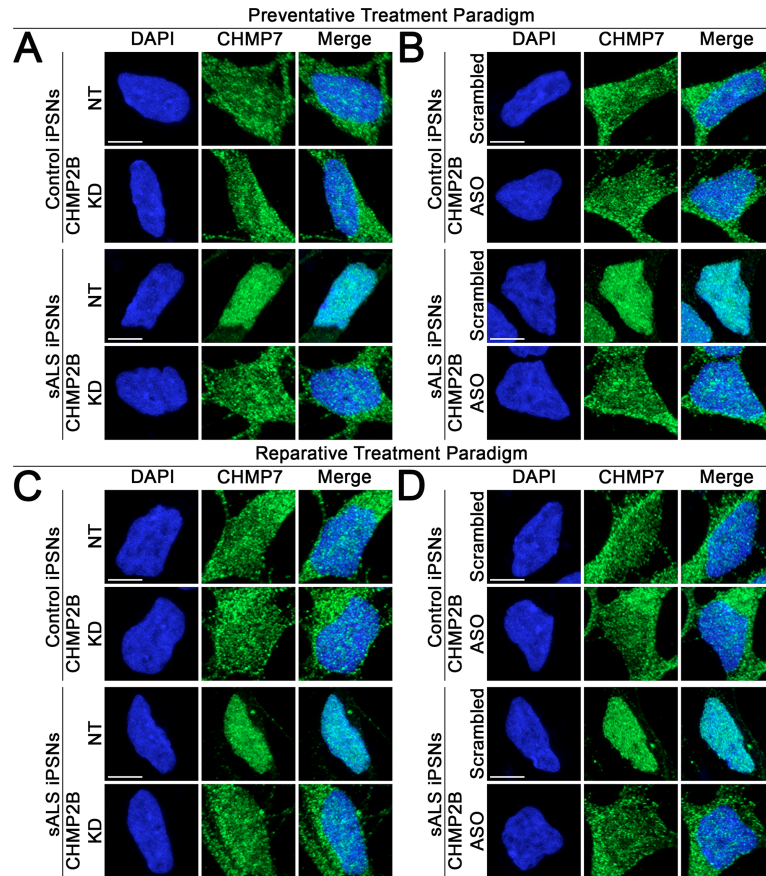


Supplemental Figure 2: ASO mediated knockdown of CHMP2B in iPSNs. (A) Western blot for CHMP2B expression following 3 weeks of treatment with 5 μ M scrambled control or CHMP2B targeting ASOs. ASO as indicated on bottom, antibody for western blot as indicated on right. (B) Quantification of CHMP2B protein expression in iPSN lysates following 3 weeks of treatment with scrambled control or CHMP2B targeting ASOs. GAPDH was used for normalization. $n = 3$ control iPSC lines. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (C) Western blot for CHMP2B expression following 3 weeks of treatment with 1, 5, and 10 μ M scrambled control or CHMP2B targeting ASOs. ASO and concentration as indicated on bottom, antibody for western blot as indicated on right. (D) Quantification of CHMP2B protein expression in iPSN lysates following 3 weeks of treatment with scrambled control or CHMP2B targeting

ASOs. GAPDH was used for normalization. n = 3 control iPSC lines. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. CHMP2B ASO 3 significance compared to scrambled control as indicated on top, CHMP2B ASO 4 significance compared to scrambled control as indicated on bottom ** p < 0.01, *** p < 0.001, **** p < 0.0001.

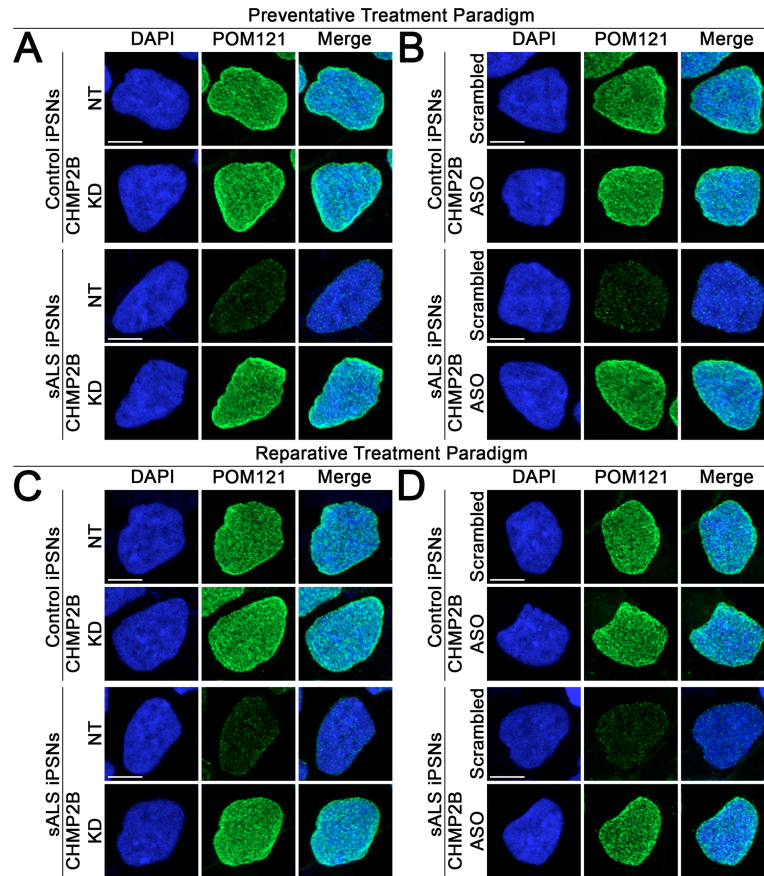


Supplemental Figure 3: Schematic of preventative and reparative siRNA and ASO treatment paradigms. (A) Schematic of pathologic events in NPC injury cascades, preventative treatment paradigm, and experimental time points used in this study. **(B)** Schematic of pathologic events in NPC injury cascades, reparative treatment paradigm, and experimental time points used in this study.



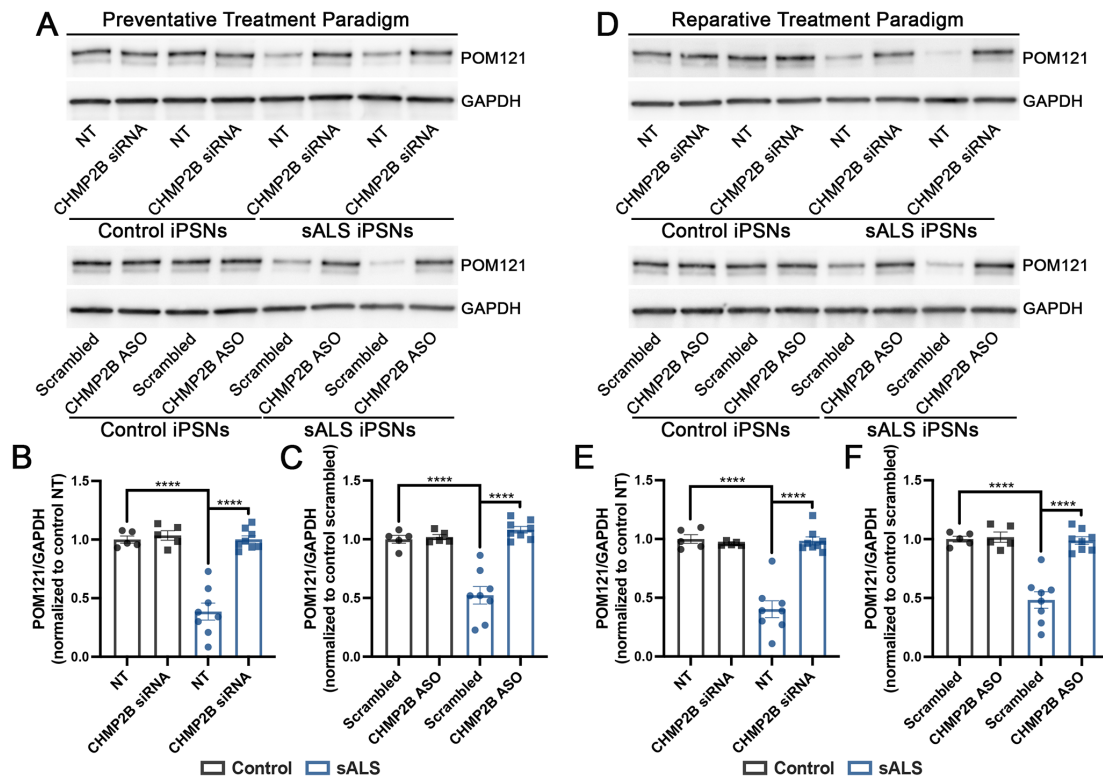
Supplemental Figure 4: High magnification images of CHMP7 in control and sALS iPSNs following knockdown of CHMP2B. (A) Maximum intensity projections from immunostaining and confocal imaging of CHMP7 in control and sALS iPSNs on day 46 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). siRNA and genotype as indicated on left, antibodies for immunostaining as indicated on top. Knockdown was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (B) Maximum intensity projections from immunostaining and confocal imaging of CHMP7 in control and sALS iPSNs on day 46 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO and genotype as indicated on left, antibodies for immunostaining as indicated on top. ASO treatment was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (C) Maximum intensity projections from immunostaining and

confocal imaging of CHMP7 in control and sALS iPSNs on day 81 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). siRNA and genotype as indicated on left, antibodies for immunostaining as indicated on top. Knockdown was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. **(D)** Maximum intensity projections from immunostaining and confocal imaging of CHMP7 in control and sALS iPSNs on day 81 of differentiation following 3 weeks of treatment with scrambled control or CHMP2B targeting ASOs. ASO and genotype as indicated on left, antibodies for immunostaining as indicated on top. ASO treatment was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. Scale bar = 5 μ m.



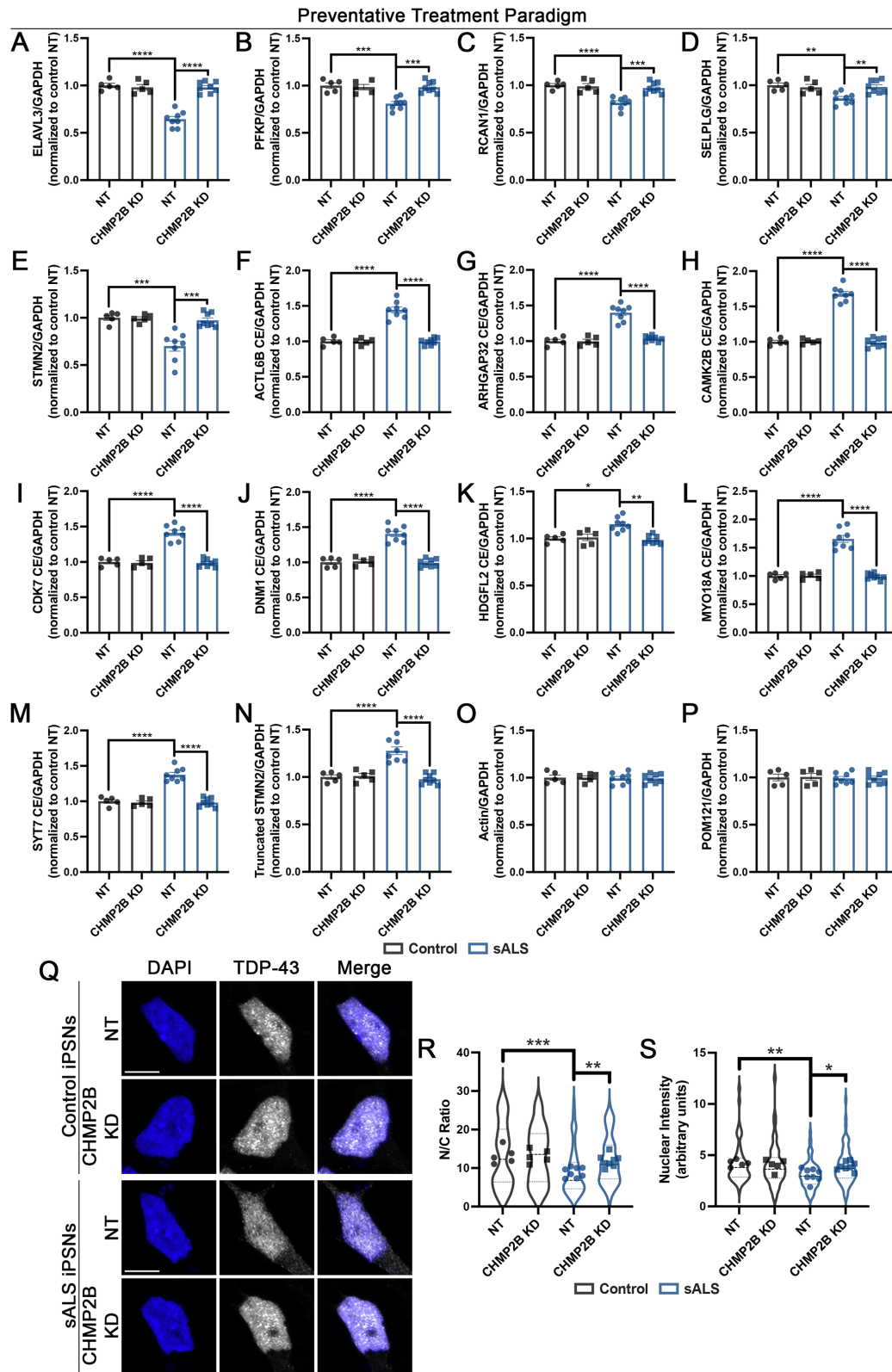
Supplemental Figure 5: High magnification images of POM121 in control and sALS iPSNs following knockdown of CHMP2B. (A) Maximum intensity projections from immunostaining and confocal imaging of POM121 in control and sALS iPSNs on day 46 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). siRNA and genotype as indicated on left, antibodies for immunostaining as indicated on top. Knockdown was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (B) Maximum intensity projections from immunostaining and confocal imaging of POM121 in control and sALS iPSNs on day 46 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO and genotype as indicated on left, antibodies for immunostaining as indicated on top. ASO treatment was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (C) Maximum intensity projections from immunostaining and

confocal imaging of POM121 in control and sALS iPSNs on day 81 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). siRNA and genotype as indicated on left, antibodies for immunostaining as indicated on top. Knockdown was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. **(D)** Maximum intensity projections from immunostaining and confocal imaging of POM121 in control and sALS iPSNs on day 81 of differentiation following 3 weeks of treatment with scrambled control or CHMP2B targeting ASOs. ASO and genotype as indicated on left, antibodies for immunostaining as indicated on top. ASO treatment was initiated at day 61 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. Scale bar = 5 μ m.



Supplemental Figure 6: siRNA and ASO mediated knockdown of CHMP2B restores levels of POM121 in sALS iPSN nuclei. (A) Western blot for POM121 expression in nuclei isolated on day 46 of differentiation 3 weeks following nucleofection of CHMP2B or non-targeting (NT) control siRNAs or following 3 weeks of treatment with 5 μ M scrambled control or CHMP2B targeting ASOs. Genotype as indicated on top, siRNA and ASO as indicated on bottom, antibody for western blot as indicated on right. siRNA nucleofection or ASO treatment was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (B-C) Quantification of POM121 protein expression in iPSN nuclei lysates 3 weeks following the nucleofection of CHMP2B or NT control siRNAs (B) or following 3 weeks treatment with scrambled control or CHMP2B targeting ASOs (C). GAPDH was used for normalization. n = 5 control and 8 sALS iPSC lines. Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. (D) Western blot for POM121 expression in nuclei isolated on day 81 of differentiation 3 weeks following nucleofection of CHMP2B or non-targeting (NT) control siRNAs or following 3 weeks of treatment with 5 μ M

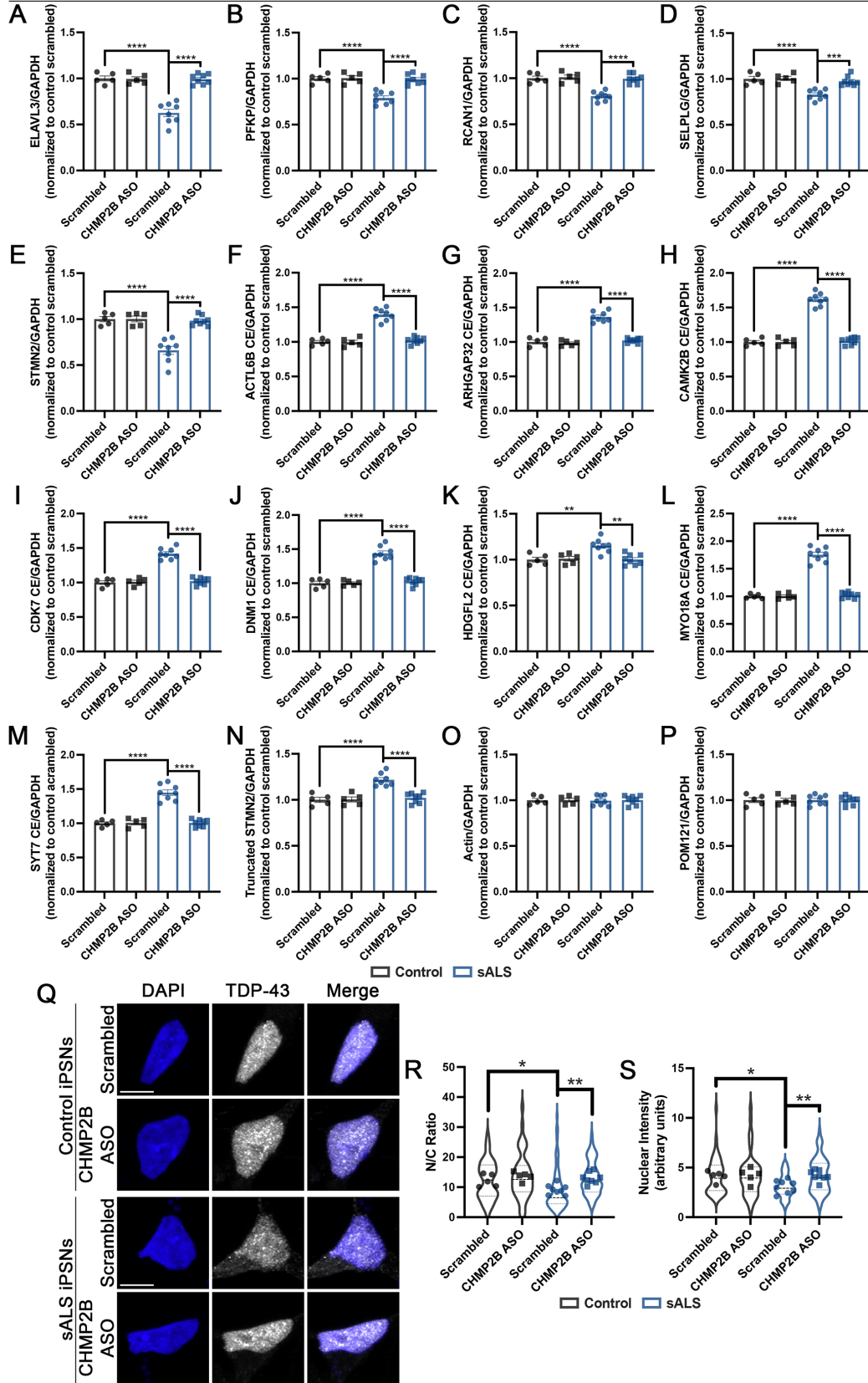
scrambled control or CHMP2B targeting ASOs. Genotype as indicated on top, siRNA and ASO as indicated on bottom, antibody for western blot as indicated on right. siRNA nucleofection or ASO treatment was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. **(E-F)** Quantification of POM121 protein expression in iPSN nuclei lysates 3 weeks following the nucleofection of CHMP2B or NT control siRNAs **(E)** or following 3 weeks treatment with scrambled control or CHMP2B targeting ASOs **(F)**. GAPDH was used for normalization. n = 5 control and 8 sALS iPSC lines. Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001.



Supplemental Figure 7: Early siRNA mediated knockdown of CHMP2B restores TDP-43 function in sALS iPSNs. (A-P) qRT-PCR for *ELAVL3* (A), *PFKFB3* (B), *RCAN1* (C), *SELPLG* (D),

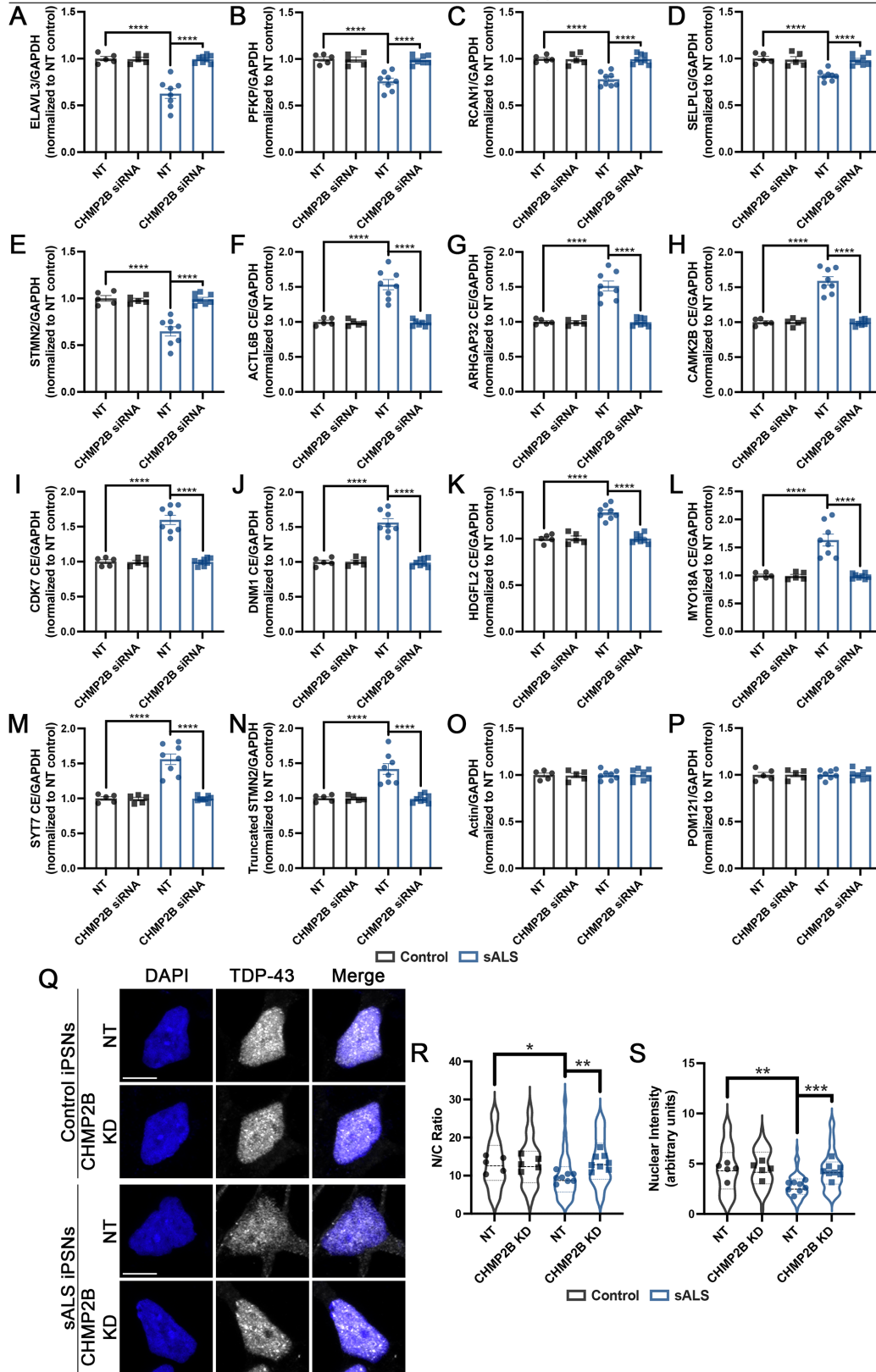
and *STMN2* (**E**), *ACTL6B* (**F**), *ARHGAP32* (**G**), *CAMK2B* (**H**), *CDK7* (**I**), *DNM1* (**J**), *HDGFL2* (**K**), *MYO18A* (**L**), and *SYT7* (**M**) cryptic exon (CE) containing mRNA, and Truncated *STMN2* (**N**), *Actin* (**O**), and *POM121* (**P**) mRNA in control and sALS iPSNs on day 46 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). Knockdown was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). GAPDH was used for normalization. *Actin* and *POM121* were used as negative control mRNAs not known to be regulated by TDP-43. n = 5 control and 8 sALS iPSC lines. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. (**Q**) Maximum intensity projections and confocal imaging of TDP-43 in control and sALS iPSNs on day 46 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). siRNA and genotype as indicated on left, antibodies for immunostaining as indicated on top. Knockdown was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (**R**) Quantification of the nuclear to cytoplasmic ratio of TDP-43. n = 5 control and 8 sALS iPSC lines, 100 Map2+ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. (**S**) Quantification of TDP-43 nuclear intensity. n = 5 control and 8 sALS iPSC lines, 100 Map2+ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01, *** p < 0.001. Scale bar = 5 μ m.

Preventative Treatment Paradigm



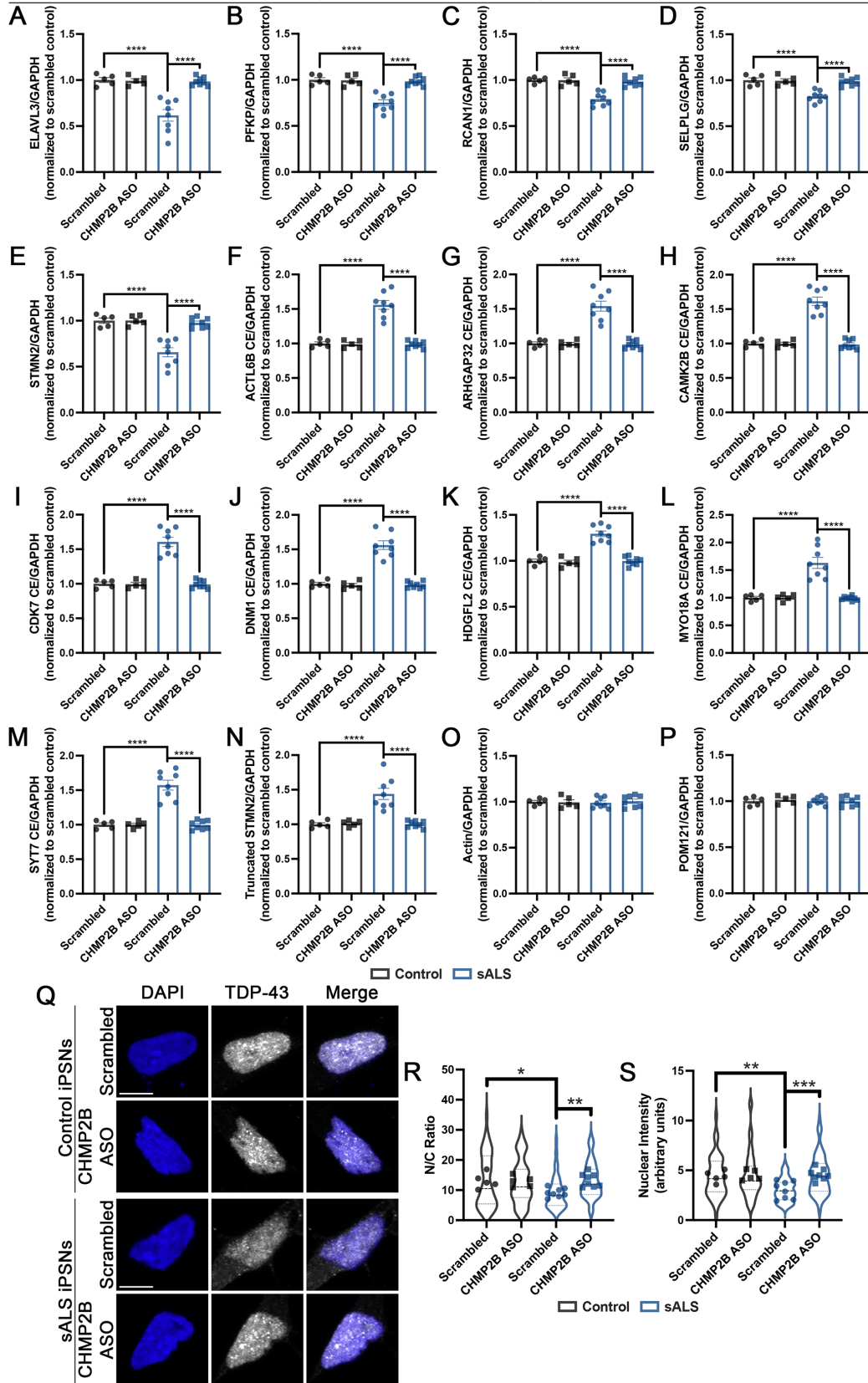
Supplemental Figure 8: Early ASO mediated knockdown of CHMP2B restores TDP-43 function in sALS iPSNs. (A-P) qRT-PCR for *ELAVL3* (A), *PFKP* (B), *RCAN1* (C), *SELPLG* (D), and *STMN2* (E), *ACTL6B* (F), *ARHGAP32* (G), *CAMK2B* (H), *CDK7* (I), *DNM1* (J), *HDGFL2* (K), *MYO18A* (L), and *SYT7* (M) cryptic exon (CE) containing mRNA, and Truncated *STMN2* (N), *Actin* (O), and *POM121* (P) mRNA in control and sALS iPSNs on day 46 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO treatment was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). GAPDH was used for normalization. *Actin* and *POM121* were used as negative control mRNAs not known to be regulated by TDP-43. n = 5 control and 8 sALS iPSC lines. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. ** p < 0.01, **** p < 0.0001. (Q) Maximum intensity projections and confocal imaging of TDP-43 in control and sALS iPSNs on day 46 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO and genotype as indicated on left, antibodies for immunostaining as indicated on top. ASO treatment was initiated at day 15 of differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS iPSNs [20]). (R) Quantification of the nuclear to cytoplasmic ratio of TDP-43. n = 5 control and 8 sALS iPSC lines, 100 Map2+ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. (S) Quantification of TDP-43 nuclear intensity. n = 5 control and 8 sALS iPSC lines, 100 Map2+ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01. Scale bar = 5 μ m.

Reparative Treatment Paradigm

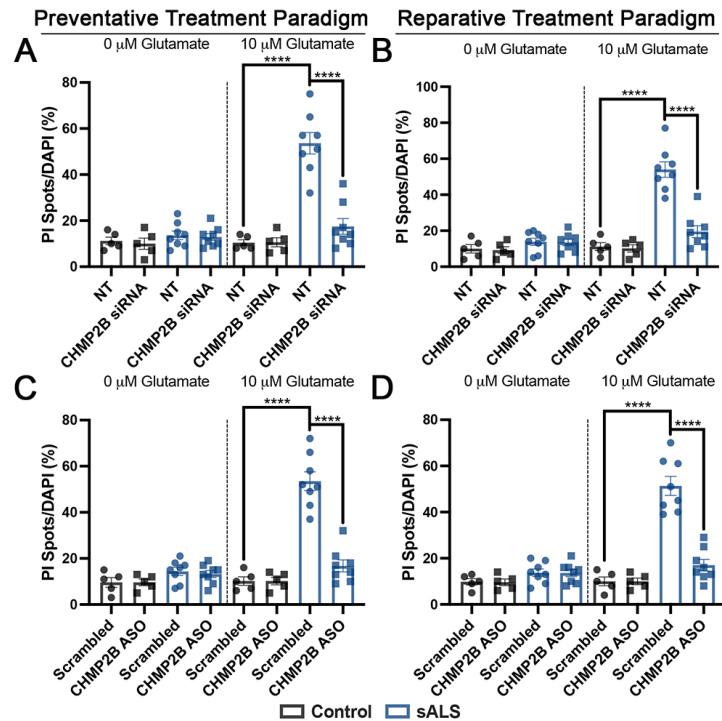


Supplemental Figure 9: Late stage siRNA mediated knockdown of CHMP2B restores TDP-43 function in sALS iPSNs. (A-P) qRT-PCR for *ELAVL3* (A), *PFKP* (B), *RCAN1* (C), *SELPLG* (D), and *STMN2* (E), *ACTL6B* (F), *ARHGAP32* (G), *CAMK2B* (H), *CDK7* (I), *DNM1* (J), *HDGFL2* (K), *MYO18A* (L), and *SYT7* (M) cryptic exon (CE) containing mRNA, and Truncated *STMN2* (N), *Actin* (O), and *POM121* (P) mRNA in control and sALS iPSNs on day 81 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). Knockdown was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. GAPDH was used for normalization. *Actin* and *POM121* were used as negative control mRNAs not known to be regulated by TDP-43. n = 5 control and 8 sALS iPSC lines. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. (Q) Maximum intensity projections and confocal imaging of TDP-43 in control and sALS iPSNs on day 81 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). siRNA and genotype as indicated on left, antibodies for immunostaining as indicated on top. Knockdown was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. (R) Quantification of the nuclear to cytoplasmic ratio of TDP-43. n = 5 control and 8 sALS iPSC lines, 100 Map2⁺ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. (S) Quantification of TDP-43 nuclear intensity. n = 5 control and 8 sALS iPSC lines, 100 Map2⁺ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01, *** p < 0.001. Scale bar = 5 μm.

Reparative Treatment Paradigm



Supplemental Figure 10: Late stage ASO mediated knockdown of CHMP2B restores TDP-43 function in sALS iPSNs. (A-P) qRT-PCR for *ELAVL3* (A), *PFKP* (B), *RCAN1* (C), *SELPLG* (D), and *STMN2* (E), *ACTL6B* (F), *ARHGAP32* (G), *CAMK2B* (H), *CDK7* (I), *DNM1* (J), *HDGFL2* (K), *MYO18A* (L), and *SYT7* (M) cryptic exon (CE) containing mRNA, and Truncated *STMN2* (N), *Actin* (O), and *POM121* (P) mRNA in control and sALS iPSNs on day 81 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO treatment was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. GAPDH was used for normalization. *Actin* and *POM121* were used as negative control mRNAs not known to be regulated by TDP-43. n = 5 control and 8 sALS iPSC lines. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. ** p < 0.01, **** p < 0.0001. (Q) Maximum intensity projections and confocal imaging of TDP-43 in control and sALS iPSNs on day 81 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO and genotype as indicated on left, antibodies for immunostaining as indicated on top. ASO treatment was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. (R) Quantification of the nuclear to cytoplasmic ratio of TDP-43. n = 5 control and 8 sALS iPSC lines, 100 Map2+ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. (S) Quantification of TDP-43 nuclear intensity. n = 5 control and 8 sALS iPSC lines, 100 Map2+ cells per line/knockdown. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01, *** p < 0.001. Scale bar = 5 μ m.



Supplemental Figure 11: siRNA and ASO mediated reduction of CHMP2B alleviates

glutamate induced neuronal death in sALS iPSCs. (A) Quantification of the percentage of

propidium iodide (PI) positive spots normalized to DAPI positive nuclei following 4 hour

exposure to 0 or 10 μ M glutamate on day 46 of differentiation (3 weeks following nucleofection

of CHMP2B or non-targeting (NT) siRNAs). Knockdown was initiated at day 15 of differentiation

(the time point where nuclear localization of CHMP7 begins to increase in sALS iPSCs [20]). n =

5 control and 8 sALS iPSC lines. Data points represent the average cell death across 10 images

per well for each line/siRNA. Two-way ANOVA with Tukey's multiple comparison test was used

to calculate statistical significance. **** p < 0.0001. (B) Quantification of the percentage of

propidium iodide (PI) positive spots normalized to DAPI positive nuclei following 4 hour

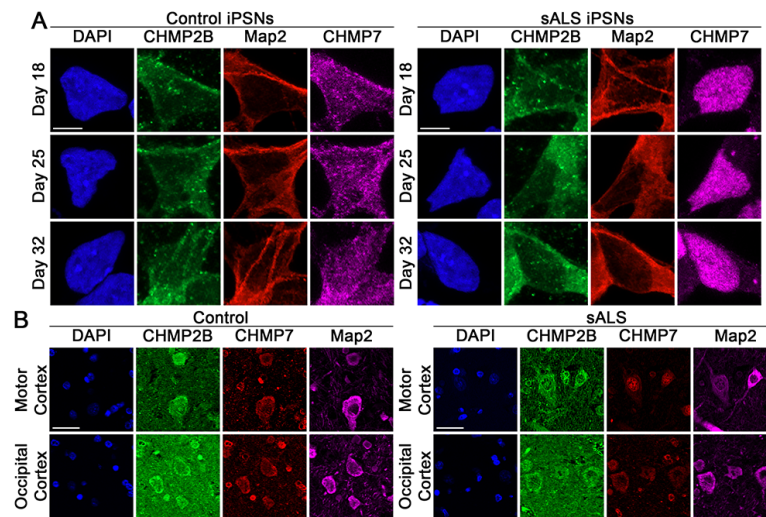
exposure to 0 or 10 μ M glutamate on day 46 of differentiation following 3 weeks of treatment

with scrambled control of CHMP2B targeting ASOs. ASO treatment was initiated at day 15 of

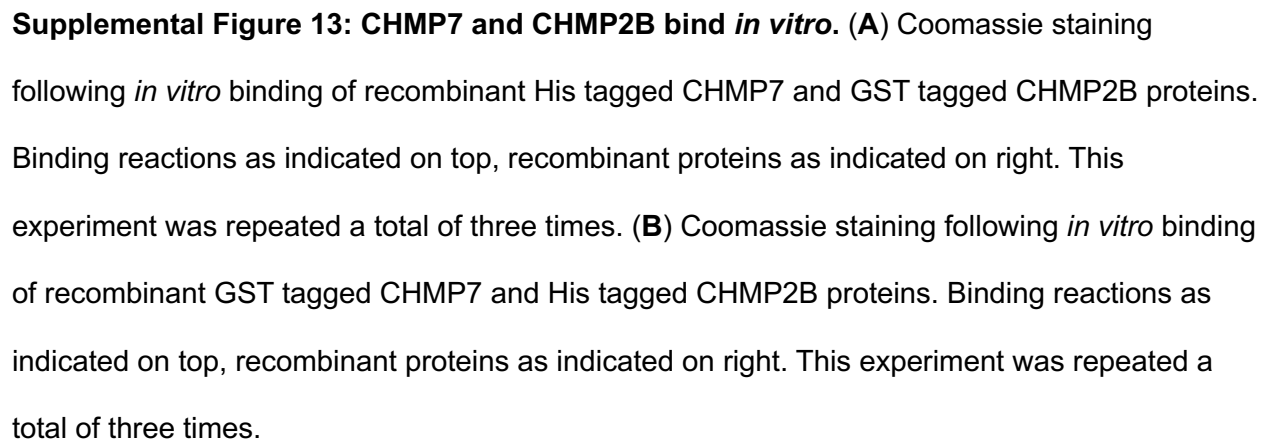
differentiation (the time point where nuclear localization of CHMP7 begins to increase in sALS

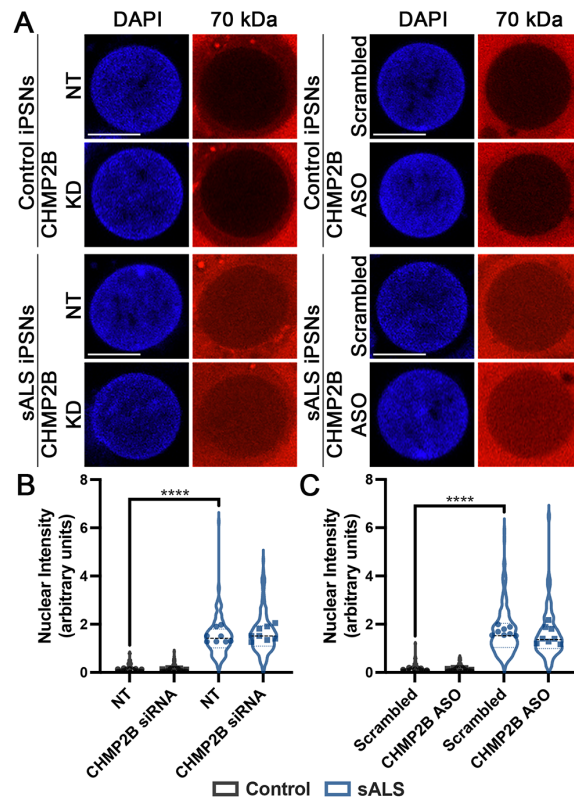
iPSCs [20]). n = 5 control and 8 sALS iPSC lines. Data points represent the average cell death

across 10 images per well for each line/ASO. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$. **(C)** Quantification of the percentage of propidium iodide (PI) positive spots normalized to DAPI positive nuclei following 4 hour exposure to 0 or 10 μ M glutamate on day 81 of differentiation (3 weeks following nucleofection of CHMP2B or non-targeting (NT) siRNAs). Knockdown was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. $n = 5$ control and 8 sALS iPSC lines. Data points represent the average cell death across 10 images per well for each line/siRNA. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$. **(D)** Quantification of the percentage of propidium iodide (PI) positive spots normalized to DAPI positive nuclei following 4 hour exposure to 0 or 10 μ M glutamate on day 46 of differentiation following 3 weeks of treatment with scrambled control of CHMP2B targeting ASOs. ASO treatment was initiated at day 60 of differentiation following the emergence of NPC injury and TDP-43 loss of function and mislocalization [20, 82]. $n = 5$ control and 8 sALS iPSC lines. Data points represent the average cell death across 10 images per well for each line/ASO. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$.



Supplemental Figure 12: Localization of CHMP2B and CHMP7 in control and sALS iPSNs and postmortem human tissues. (A) Maximum intensity projections from immunostaining and confocal imaging of CHMP7 and CHMP2B in control and sALS iPSNs. Time points as indicated on left, antibody for immunostaining and genotype as indicated on top. (B) Immunostaining and apotome based fluorescent imaging of CHMP7 and CHMP2B in control and sALS postmortem human tissues. Brain region as indicated on left, antibody for immunostaining and genotype as indicated on top.





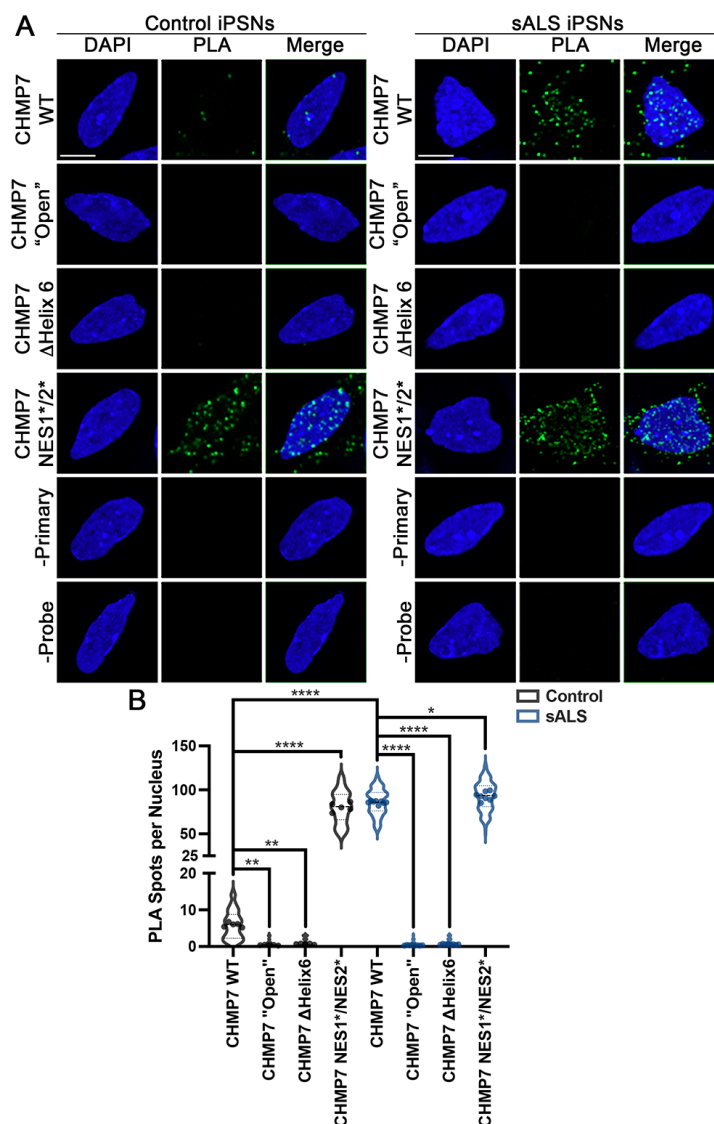
Supplemental Figure 14: Knockdown of CHMP2B does not restore NPC permeability

barrier integrity in sALS iPSNs. (A) Single z sections from confocal imaging of fluorescently

tagged 70 kDa dextrans in digitonin permeabilized control and sALS iPSNs at day 46 of differentiation following 3 weeks of treatment with non-targeting or CHMP2B siRNAs (left) or scrambled control or CHMP2B targeting ASOs (right). Genotype and treatment as indicated on left, dextran as indicated on top. DAPI was used to define the nucleus. **(B)** Quantification of

nuclear intensity of 70 kDa dextran in non-targeting and CHMP2B siRNA treated control and sALS iPSNs. n = 5 control and 8 sALS iPSC lines, at least 50 nuclei per line/dextran. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. **(C)** Quantification of nuclear intensity of 70 kDa dextran in scrambled control and

CHMP2B targeting ASO treated control and sALS iPSNs. n = 5 control and 8 sALS iPSC lines, at least 100 nuclei per line/dextran. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** p < 0.0001. Scale bar = 10 μ m.



Supplemental Figure 15: CHMP7 “Open” and CHMP7 ΔHelix 6 mutants do not associate with CHMP2B in iPSNs. (A) Maximum intensity projections from confocal imaging of Flag – CHMP2B PLA signals in control and sALS iPSNs. CHMP7 plasmid as indicated on left, genotype as indicated on top. Note: Anti-Flag antibodies were used to detect Flag tagged CHMP7 proteins. (B) Quantification of number of PLA signals per nucleus. n = 5 control and 8 sALS iPSC lines, at least 100 nuclei per line/plasmid. Two-way ANOVA with Tukey’s multiple comparison test was used to calculate statistical significance. * p < 0.05, ** p < 0.01, **** p < 0.0001. Scale bar = 5 μm.