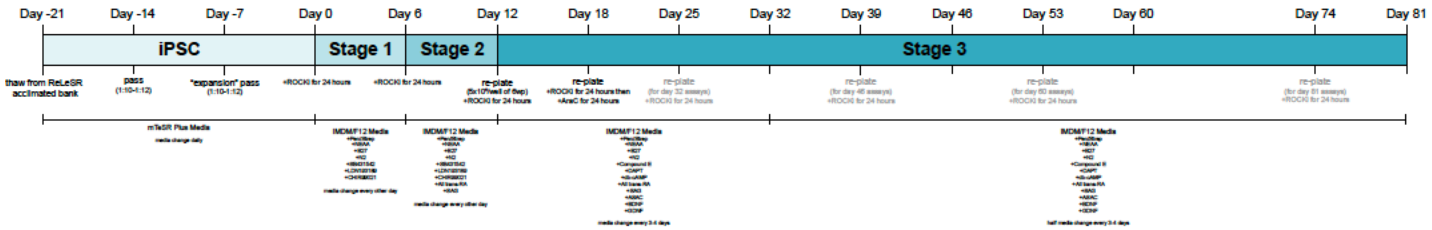
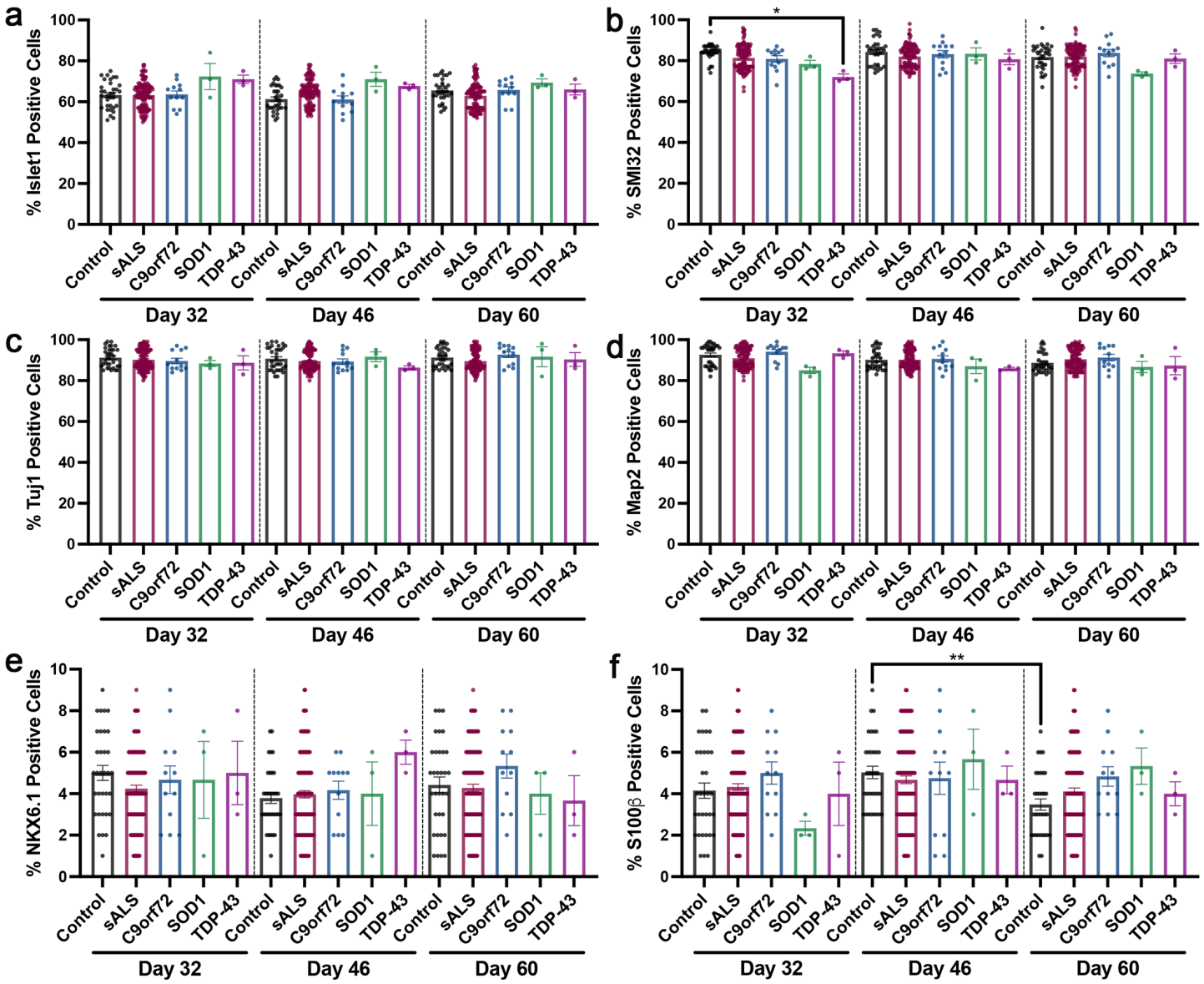
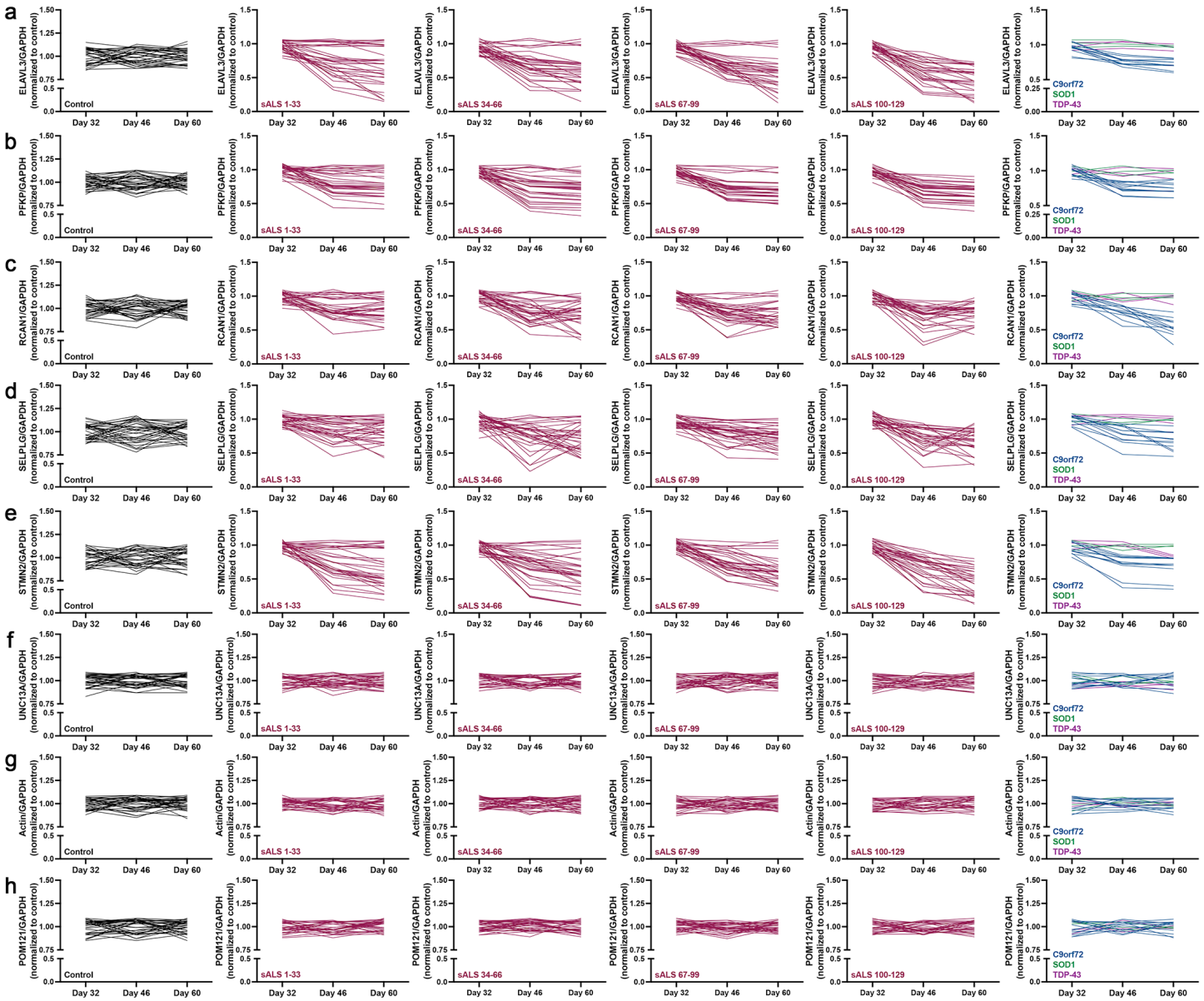


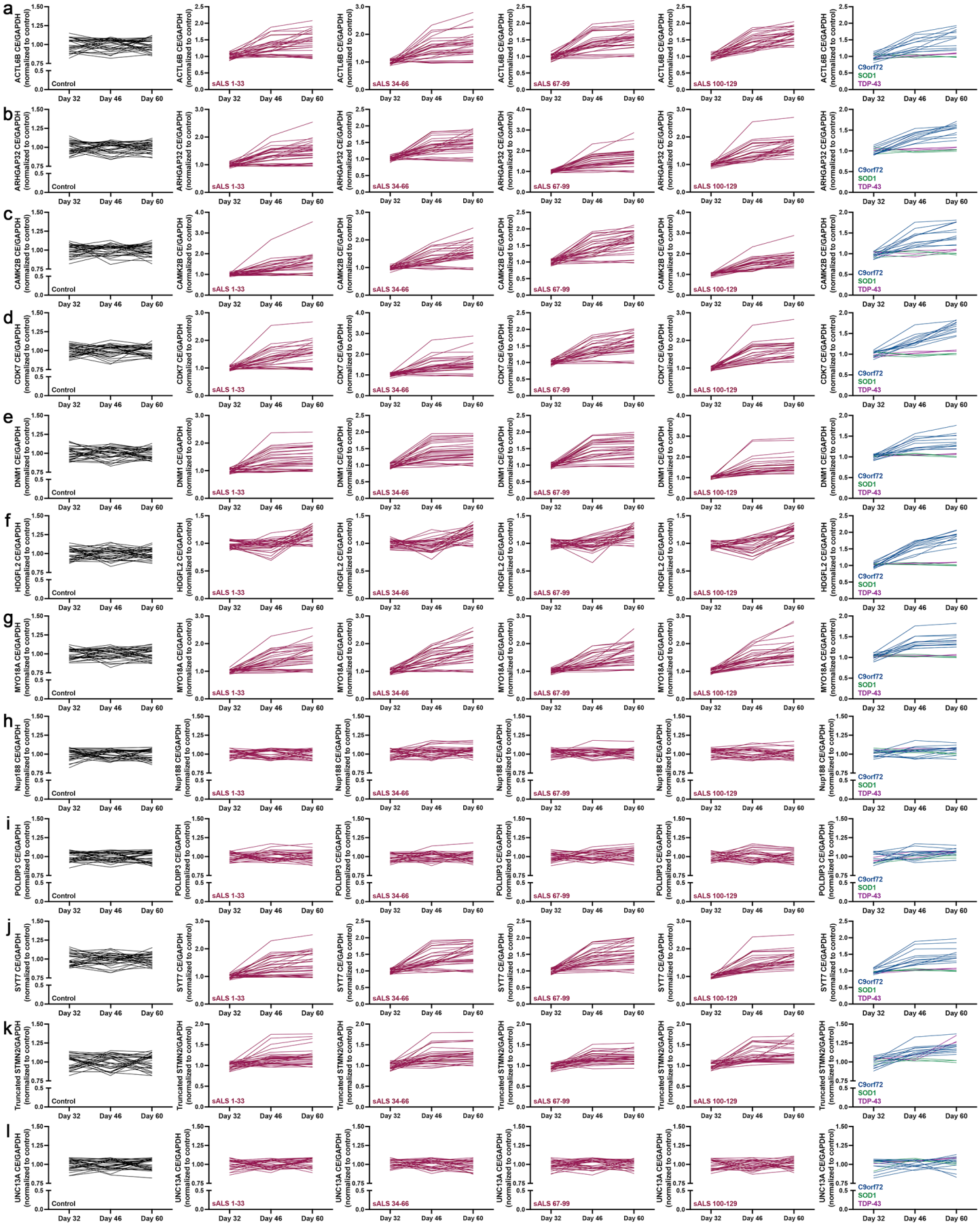
Supplementary Figure 1: Schematic depiction of modified diMNs differentiation protocol used in this study. Graphic depiction of modified direct induced motor neuron (diMNs) differentiation protocol.



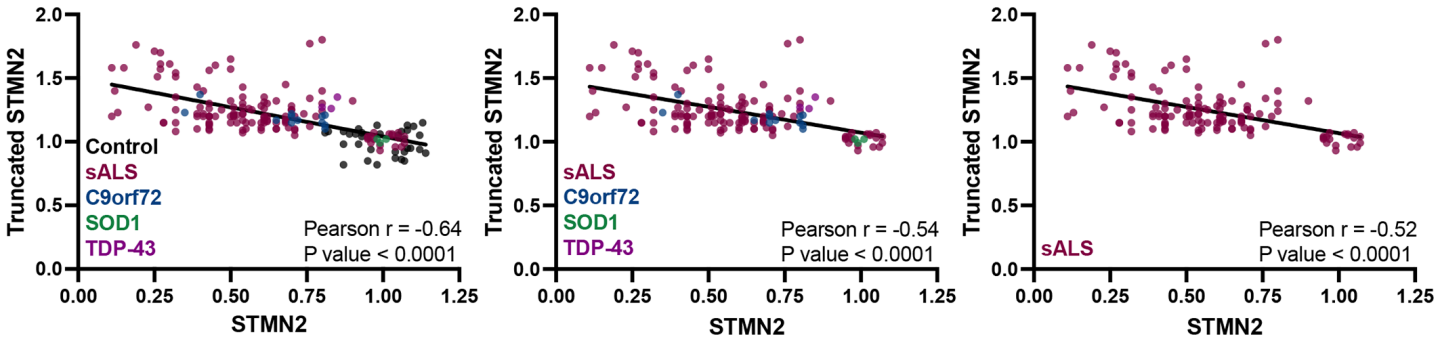
Supplementary Figure 2: The cell type composition of spinal neuron cultures generated with the modified diMNs protocol is consistent amongst iPSC lines and over time and is enriched for motor neurons. (**a-f**) Percentage of cells positive for Islet1 (**a**), SMI32 (**b**), Tuj1 (**c**), Map2 (**d**), NKX6.1 (**e**), and S100β (**f**) in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points for each line represent the average value across 3 independent differentiations. Data are presented as mean values +/- SEM. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * $p < 0.05$, ** $p < 0.01$.



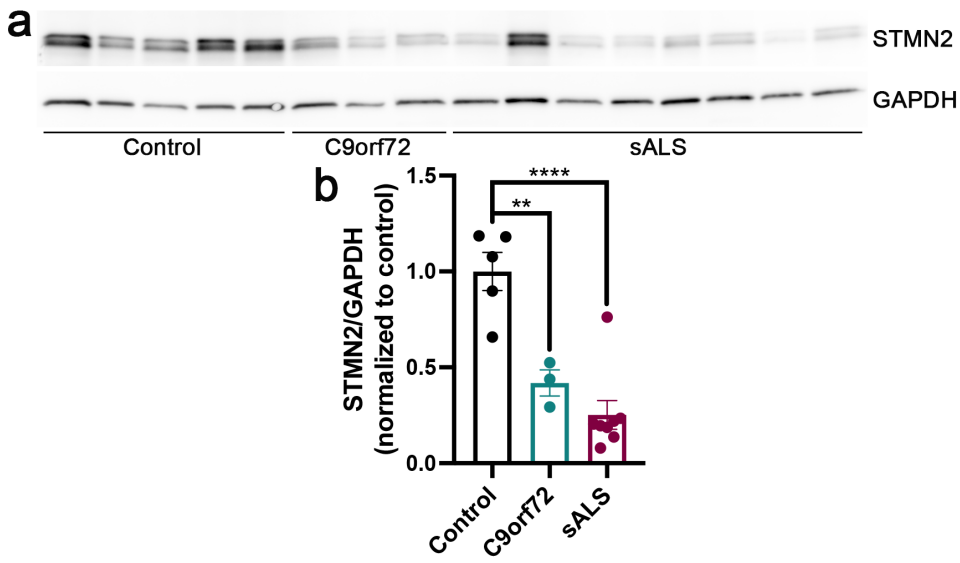
Supplementary Figure 3: Time dependent and patient specific profiles of TDP-43 loss of function associated changes in target gene expression in sALS, C9orf72, SOD1, and TDP-43 iPSNs. (**a-h**) Line graphs from qRT-PCR for *ELAVL3* (**a**), *PFKP* (**b**), *RCAN1* (**c**), and *SELPLG* (**d**), *STMN2* (**e**), *UNC13A* (**f**), *ACTIN* (**g**), and *POM121* (**h**) mRNA in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 32, 46, and 60 of differentiation. *ACTIN* and *POM121* represent negative control mRNAs not known to be regulated by TDP-43. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. For each line and time point, data points represent the average value across 3 independent differentiations.


a
b
c
d
e
f
g
h
i
j
k
l
ACTL6B CE/GAPDH (normalized to control)
ARHGAP32 CE/GAPDH (normalized to control)
CAMK2B CE/GAPDH (normalized to control)
CDK7 CE/GAPDH (normalized to control)
DNM1 CE/GAPDH (normalized to control)
HDGFL2 CE/GAPDH (normalized to control)
MYO18A CE/GAPDH (normalized to control)
Nup188 CE/GAPDH (normalized to control)
POLDIP3 CE/GAPDH (normalized to control)
SYT7 CE/GAPDH (normalized to control)
Truncated STMN2/GAPDH (normalized to control)
UNC13A CE/GAPDH (normalized to control)
Control
sALS 1-33
sALS 34-66
sALS 67-99
sALS 100-129
C9orf72
SOD1
TDP-43
Day 32
Day 46
Day 60

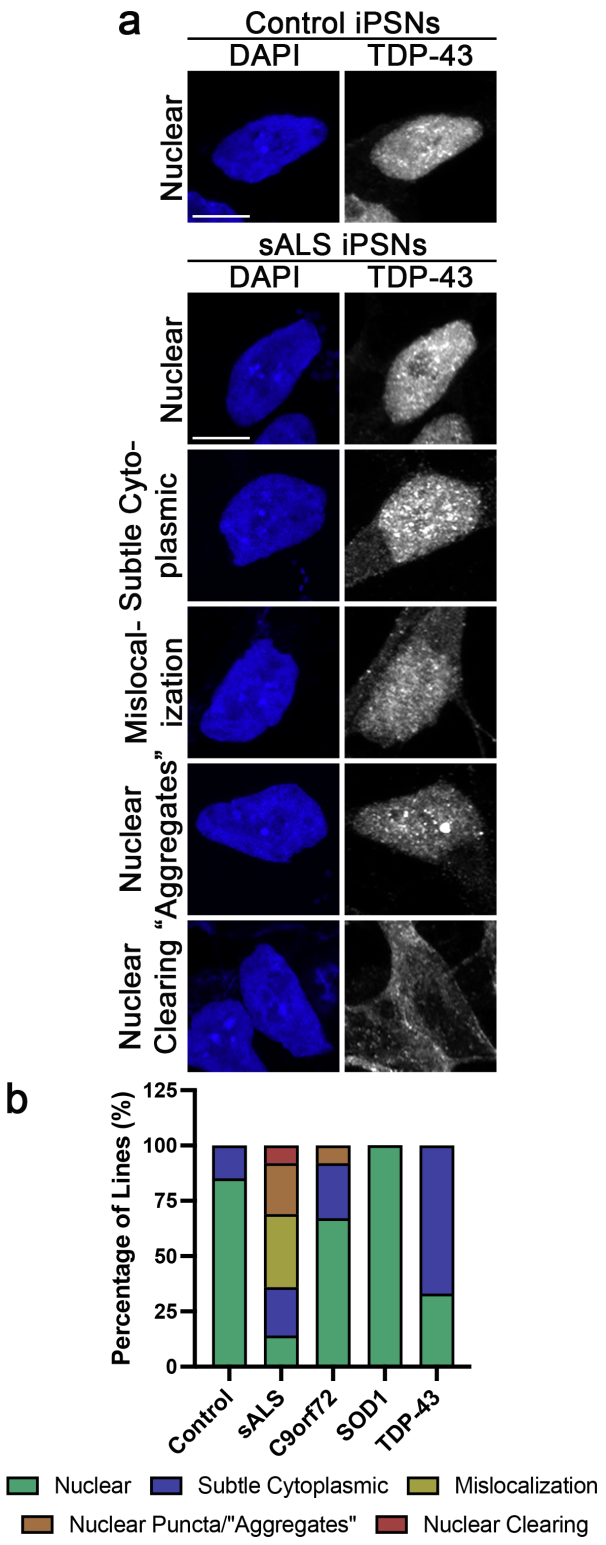
Supplementary Figure 4: Time dependent and patient specific profiles of TDP-43 loss of function associated changes in target mRNA splicing in sALS, C9orf72, SOD1, and TDP-43 iPSNs. Line graphs from qRT-PCR for *ACTL6B* cryptic exon containing (**a**), *ARHGAP32* cryptic exon containing (**b**), *CAMK2B* cryptic exon containing (**c**), *CDK7* cryptic exon containing (**d**), *DNM1* cryptic exon containing (**e**), *HDGFL2* cryptic exon containing (**f**), *MYO18A* cryptic exon containing (**g**), *NUP188* cryptic exon containing (**h**), *POLDIP3* cryptic exon containing (**i**), *SYT7* cryptic exon containing (**j**), truncated *STMN2* (**k**), and *UNC13A* cryptic exon containing (**l**) mRNA in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. For each line and time point, data points represent the average value across 3 independent differentiations.



Supplementary Figure 5: Decrease in STMN2 gene expression moderately correlates with increased abundance of truncated STMN2 transcripts in patient iPSNs. Correlation of *STMN2* gene expression with abundance of *truncated STMN2* transcripts in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines.

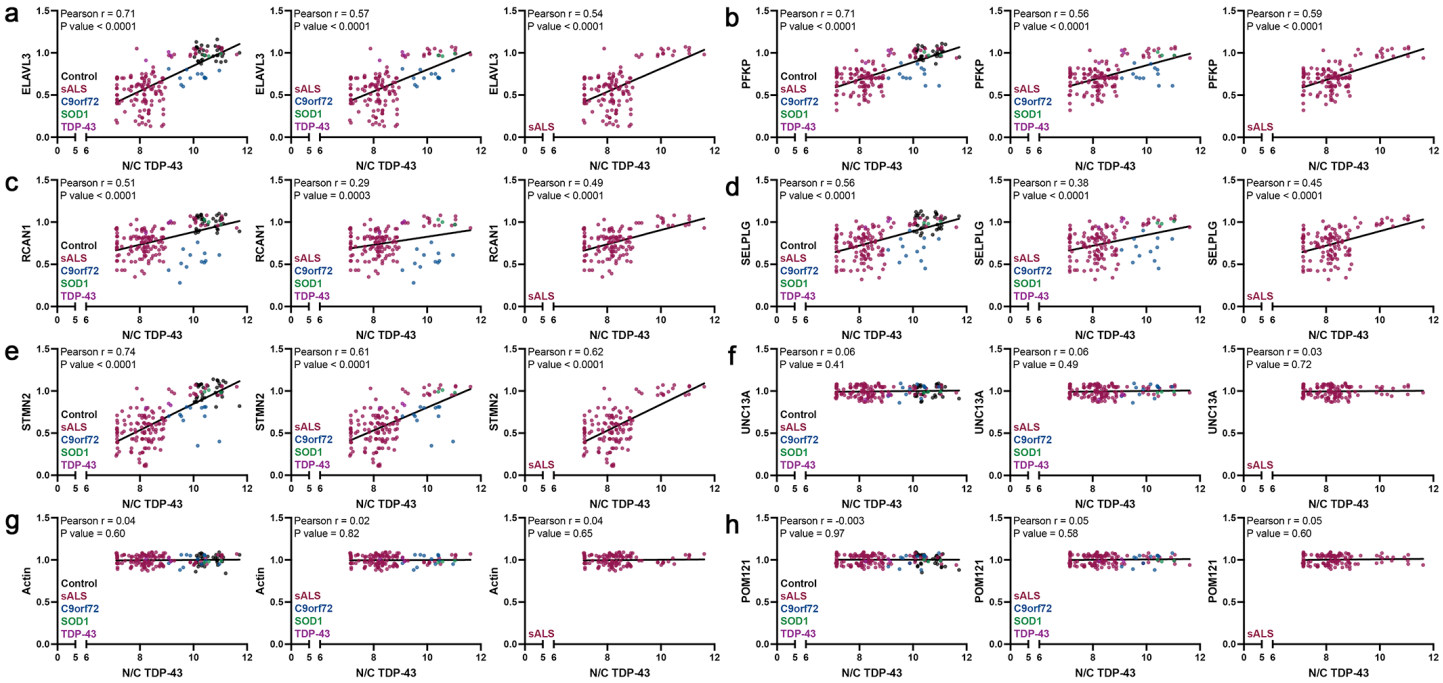


Supplementary Figure 6: STMN2 protein is reduced in C9orf72 and sALS patient iPSNs. (**a**) Western blot for STMN2 protein in control, C9orf72, and sALS iPSNs at day 60 of differentiation. Antibody as indicated on right, genotype as indicated on bottom. (**b**) Quantification of STMN2 protein expression. GAPDH was used as a loading control for normalization. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. ** $p < 0.01$, **** $p < 0.0001$.

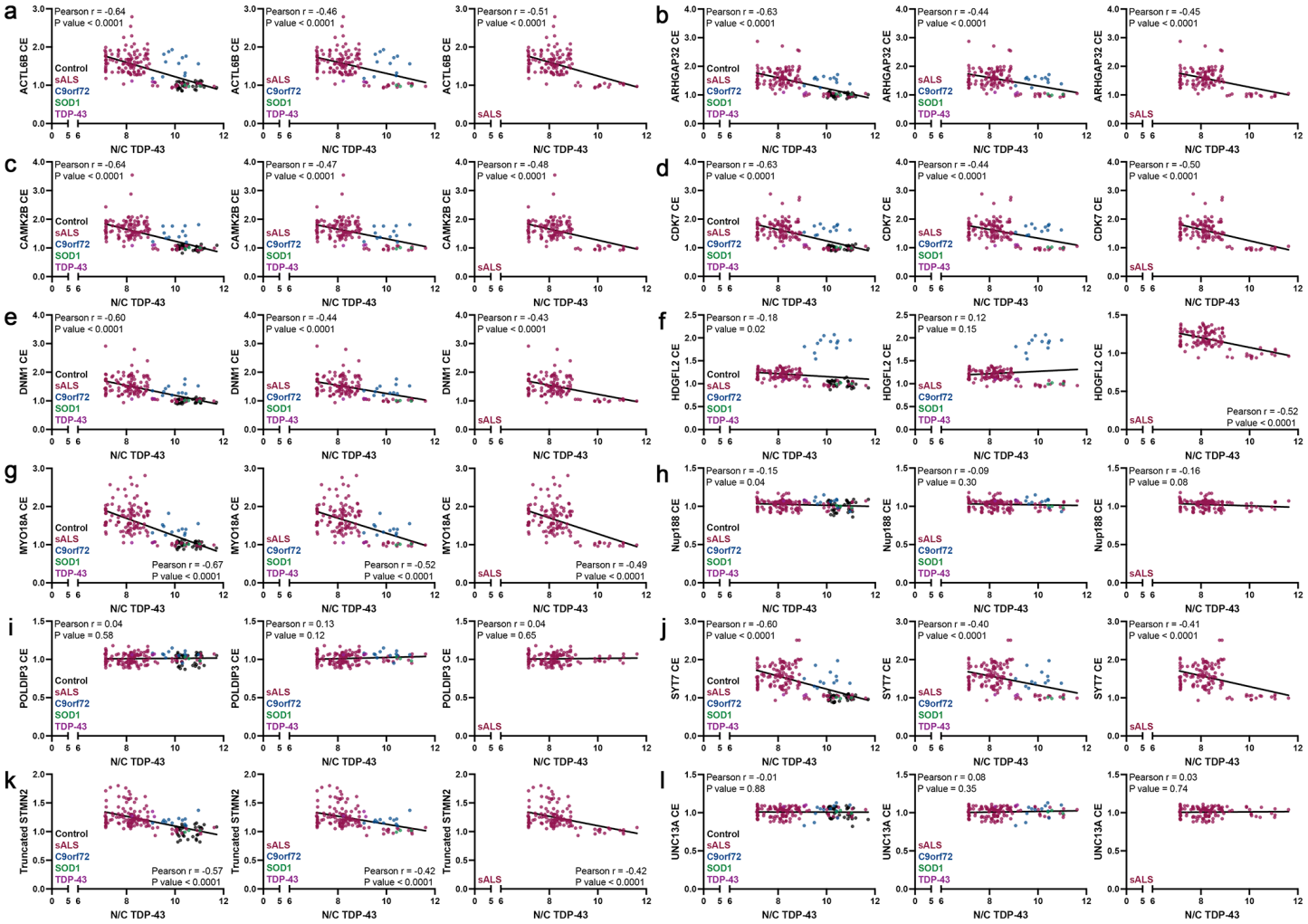


Supplementary Figure 7: Heterogeneity of TDP-43 localization in ALS patient iPSNs. (**a**) Immunostaining and confocal imaging for TDP-43 in control and sALS iPSNs at day 60 of differentiation. Genotype and antibody/stain as indicated on top, histologic pattern of TDP-43 distribution as indicated on left. Scale bar = 5 μm. (**b**) Quantification of percentage of iPSC lines where at least 75% of cells displayed specified histologic

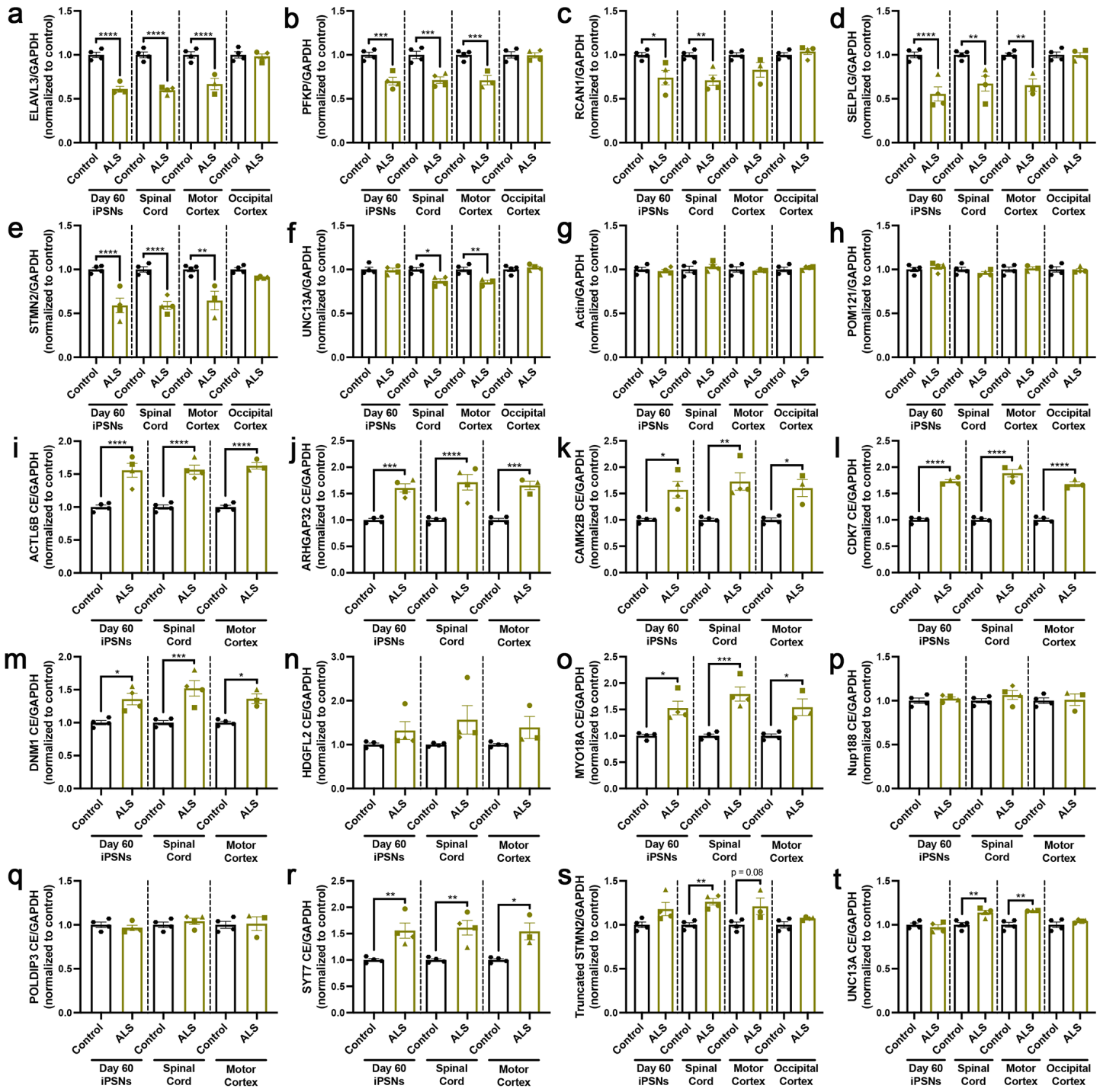
patterns of TDP-43 distribution. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines, at least 100 Map2+ cells per line. Data are presented as mean values.



Supplementary Figure 8: Correlation of TDP-43 localization with TDP-43 loss of function associated changes in gene expression in iPSNs. (**a-h**) Correlation between nuclear/cytoplasmic distribution of TDP-43 (obtained from immunostaining and confocal imaging at day 60 of differentiation) and qRT-PCR for *ELAVL3* (**a**), *PFKP* (**b**), *RCAN1* (**c**), and *SELPLG* (**d**), *STMN2* (**e**), *UNC13A* (**f**), *ACTIN* (**g**), and *POM121* (**h**) mRNA in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations.

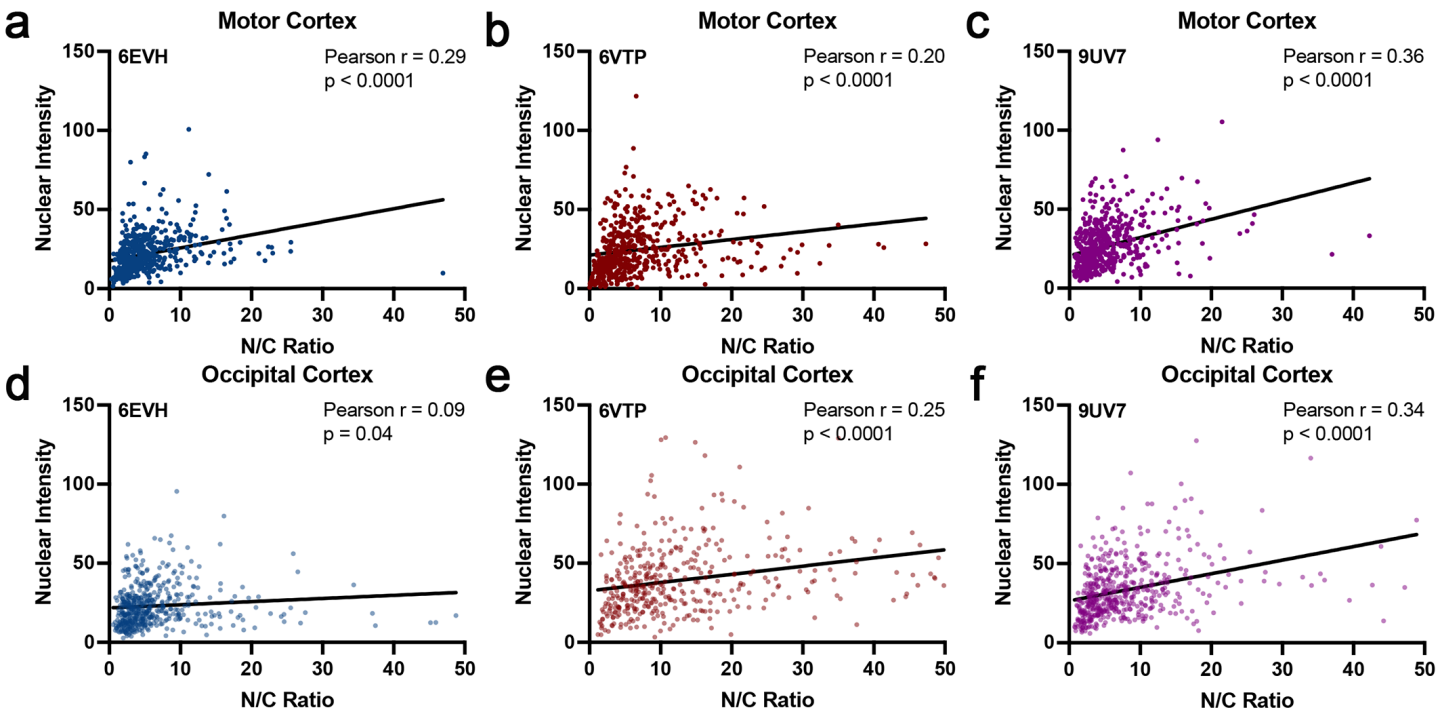


Supplementary Figure 9: Correlation of TDP-43 localization with TDP-43 loss of function associated changes in mRNA splicing in iPSNs. (**a-l**) Correlation between nuclear/cytoplasmic distribution of TDP-43 (obtained from immunostaining and confocal imaging at day 60 of differentiation) and qRT-PCR for *ACTL6B* cryptic exon containing (**a**), *ARHGAP32* cryptic exon containing (**b**), *CAMK2B* cryptic exon containing (**c**), *CDK7* cryptic exon containing (**d**), *DNM1* cryptic exon containing (**e**), *HDGFL2* cryptic exon containing (**f**), *MYO18A* cryptic exon containing (**g**), *NUP188* cryptic exon containing (**h**), *POLDIP3* cryptic exon containing (**i**), *SYT7* cryptic exon containing (**j**), truncated *STMN2* (**k**), and *UNC13A* cryptic exon containing (**l**) mRNA in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations.

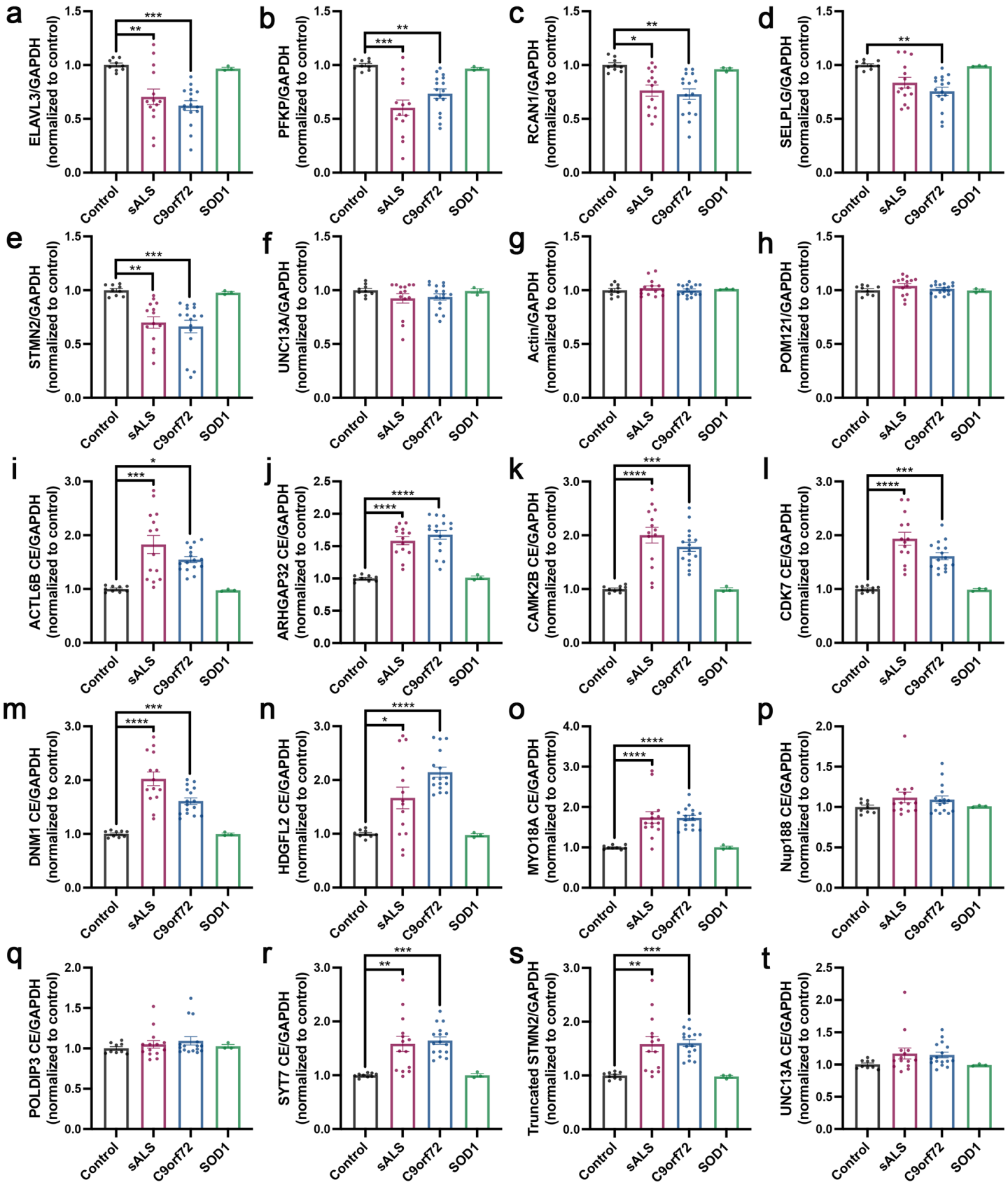


Supplementary Figure 10: Molecular signatures of TDP-43 loss of function in spinal iPSN cultures are highly similar to those observed in end-stage patient motor cortex and spinal cord tissues. (**a-t**) qRT-PCR for *ELAVL3* (**a**), *PFKP* (**b**), *RCAN1* (**c**), and *SELPLG* (**d**), *STMN2* (**e**), *UNC13A* (**f**), *ACTIN* (**g**), and *POM121* (**h**) mRNA and *ACTL6B* cryptic exon containing (**i**), *ARHGAP32* cryptic exon containing (**j**), *CAMK2B* cryptic exon containing (**k**), *CDK7* cryptic exon containing (**l**), *DNM1* cryptic exon containing (**m**), *HDGFL2* cryptic exon containing (**n**), *MYO18A* cryptic exon containing (**o**), *NUP188* cryptic exon containing (**p**), *POLDIP3* cryptic exon containing (**q**), *SYT7* cryptic exon containing (**r**), truncated *STMN2* (**s**), and *UNC13A*

cryptic exon containing (**t**) mRNA in control, sALS, and C9orf72 iPSNs at day 60 of differentiation and postmortem motor and occipital cortex and spinal cord tissue. GAPDH was used as the reference gene for normalization. Graphs lacking occipital cortex data indicate that cryptic exon containing RNAs were not detected. n = 4 control, 3 sALS, and 1 C9orf72. sALS and C9orf72 samples are grouped as “ALS”. Note: one sALS patient did not have motor cortex tissue available for analysis. Data points for motor cortex samples represent the average value across 3 samples of tissue across the motor strip. Data are presented as mean values +/- SEM. Two-way ANOVA with Tukey’s multiple comparison test was used to calculate statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

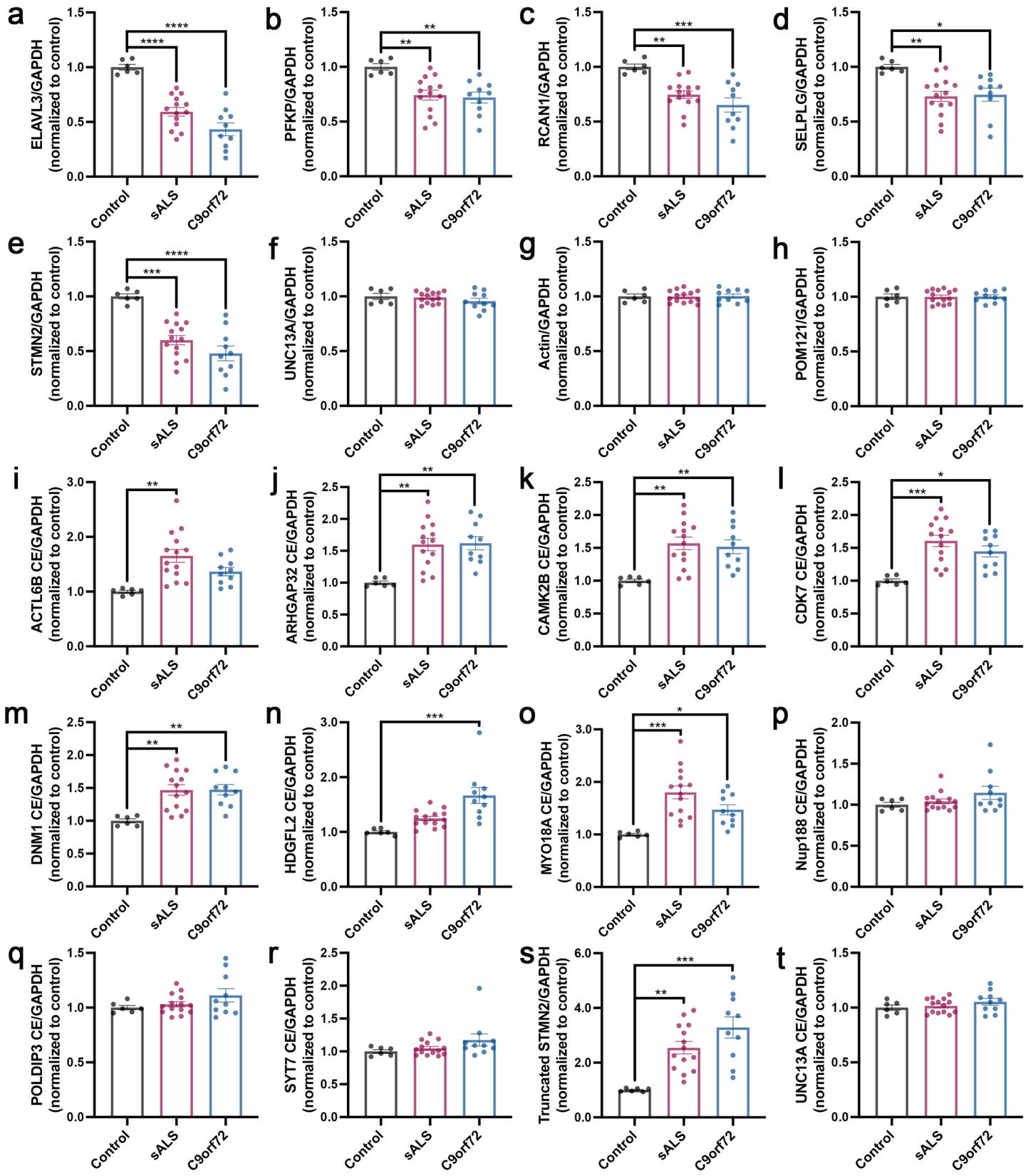


Supplementary Figure 11: Correlation of TDP-43 nuclear/cytoplasmic distribution and nuclear intensity in postmortem sALS patient brain tissue. (**a-c**) Nuclear/cytoplasmic distribution of TDP-43 compared to nuclear intensity of TDP-43 in postmortem sALS patient motor cortex. Deidentified sALS patient code as indicated in upper left. (**d-f**) Nuclear/cytoplasmic distribution of TDP-43 compared to nuclear intensity of TDP-43 in postmortem sALS patient occipital cortex. Deidentified sALS patient code as indicated in upper left.



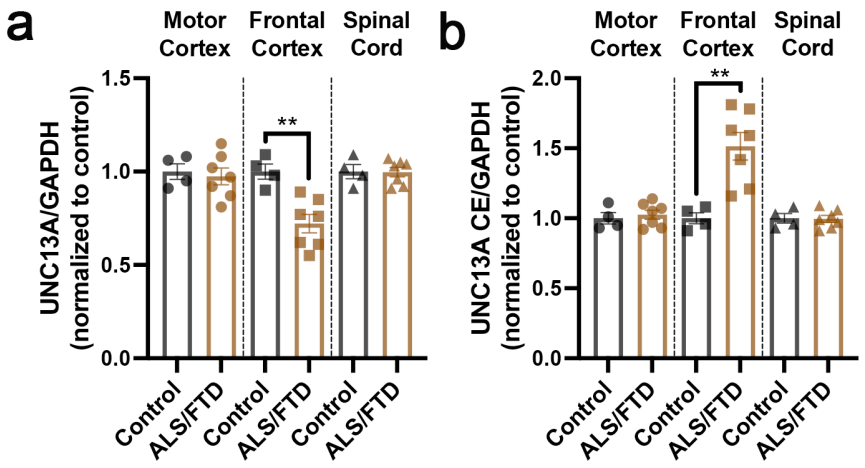
Supplementary Figure 12: Detection of molecular signatures of TDP-43 loss of function in postmortem patient motor cortex. (**a-t**) qRT-PCR for *ELAVL3* (**a**), *PFKP* (**b**), *RCAN1* (**c**), and *SELPLG* (**d**), *STMN2* (**e**), *UNC13A* (**f**), *ACTIN* (**g**), and *POM121* (**h**) mRNA and *ACTL6B* cryptic exon containing (**i**), *ARHGAP32* cryptic

exon containing (**j**), *CAMK2B* cryptic exon containing (**k**), *CDK7* cryptic exon containing (**l**), *DNM1* cryptic exon containing (**m**), *HDGFL2* cryptic exon containing (**n**), *MYO18A* cryptic exon containing (**o**), *NUP188* cryptic exon containing (**p**), *POLDIP3* cryptic exon containing (**q**), *SYT7* cryptic exon containing (**r**), truncated *STMN2* (**s**), and *UNC13A* cryptic exon containing (**t**) mRNA in control, sALS, C9orf72 ALS, and SOD1 postmortem patient motor cortex tissue. n = 9 control, 14 sALS, 16 C9orf72 ALS, and 3 SOD1 patients. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

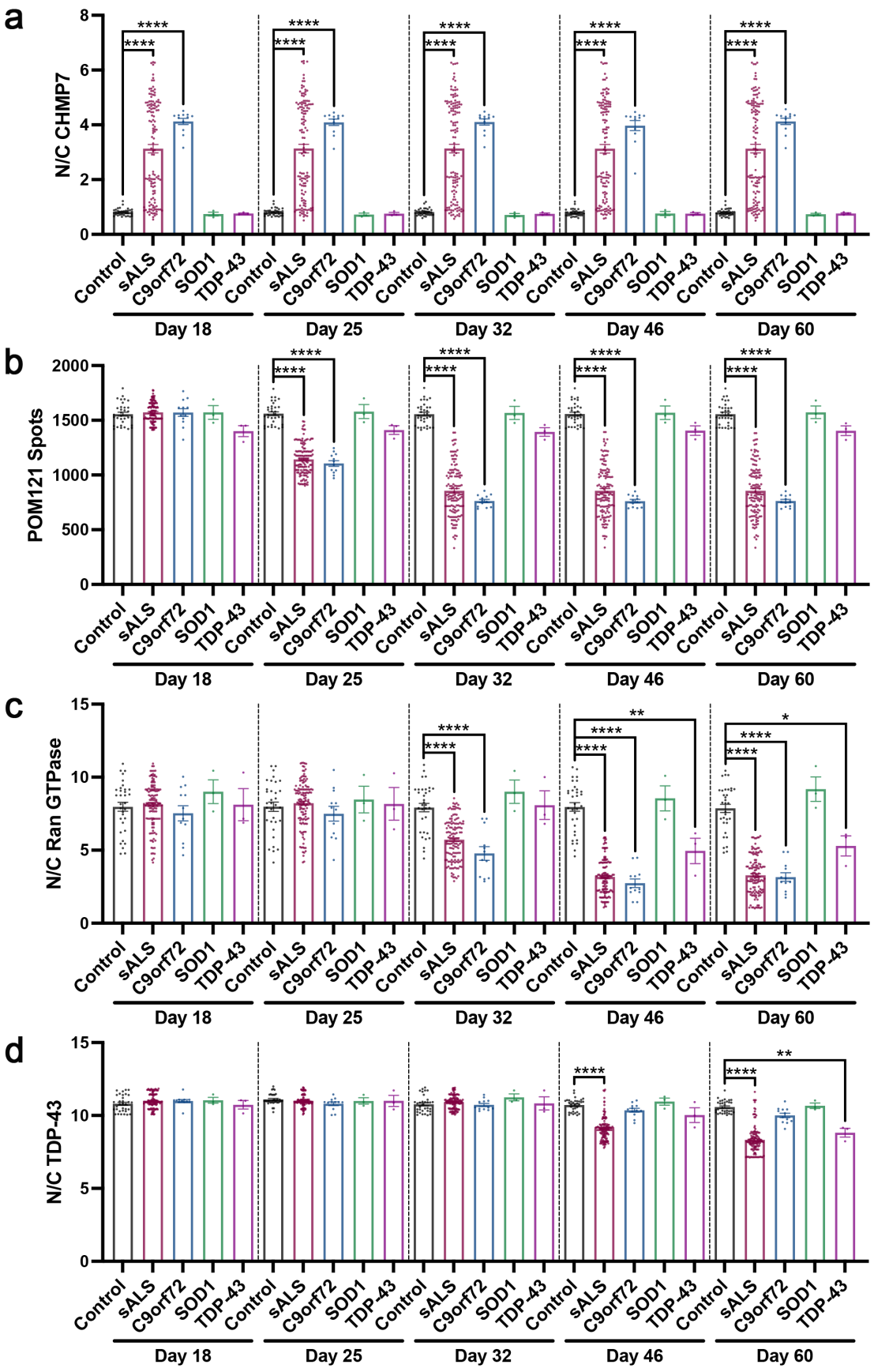


Supplementary Figure 13: Detection of molecular signatures of TDP-43 loss of function in postmortem patient spinal cord. (**a-t**) qRT-PCR for *ELAVL3* (**a**), *PFKP* (**b**), *RCAN1* (**c**), and *SELPLG* (**d**), *STMN2* (**e**), *UNC13A* (**f**), *ACTIN* (**g**), and *POM121* (**h**) mRNA and *ACTL6B* cryptic exon containing (**i**), *ARHGAP32* cryptic exon containing (**j**), *CAMK2B* cryptic exon containing (**k**), *CDK7* cryptic exon containing (**l**), *DNM1* cryptic exon

containing (**m**), *HDGFL2* cryptic exon containing (**n**), *MYO18A* cryptic exon containing (**o**), *NUP188* cryptic exon containing (**p**), *POLDIP3* cryptic exon containing (**q**), *SYT7* cryptic exon containing (**r**), truncated *STMN2* (**s**), and *UNC13A* cryptic exon containing (**t**) mRNA in control, sALS, and C9orf72 ALS postmortem patient motor cortex tissue. n = 6 control, 14 sALS, and 10 C9orf72 ALS patients. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

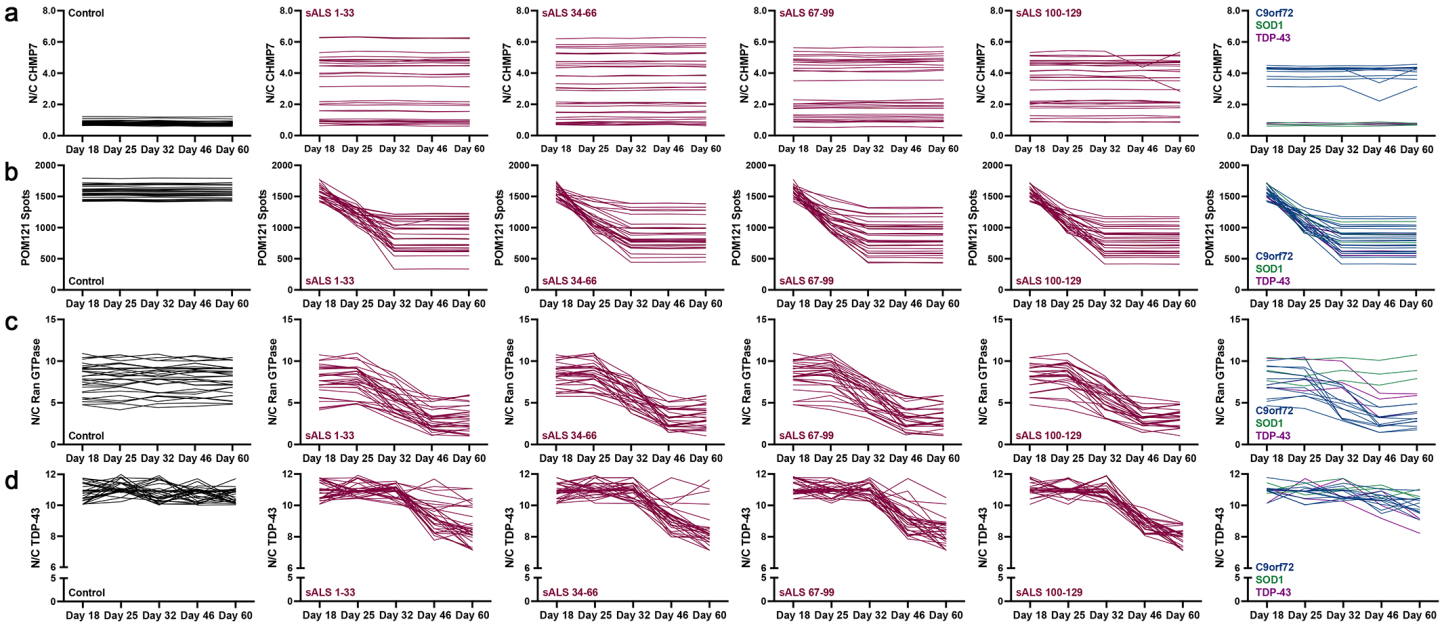


Supplementary Figure 14: Detection of *UNC13A* reduction and *UNC13A* cryptic exon inclusion in ALS/FTD patient postmortem frontal cortex. (**a-b**) qRT-PCR for *UNC13A* (**a**) and *UNC13A* cryptic exon containing (**b**) mRNA in control and ALS/FTD postmortem patient motor cortex, frontal cortex, and spinal cord tissue. n = 4 control and 7 ALS/FTD patients. Data are presented as mean values +/- SEM. Student's t-test was used to calculate statistical significance. ** $p < 0.01$.

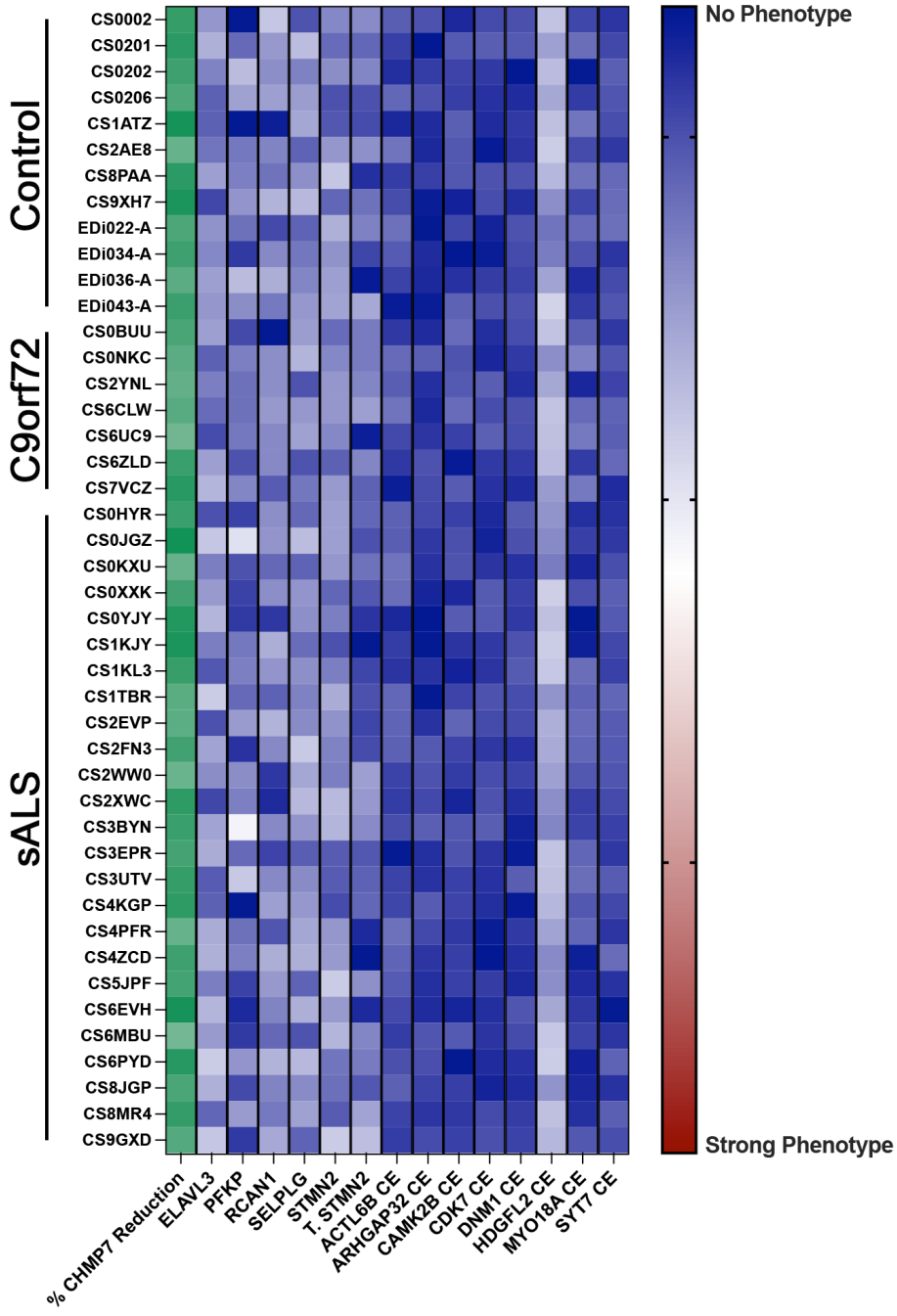


Supplementary Figure 15: Quantifiable measure of pathophysiologic events related to nuclear pore complex injury in sALS, C9orf72, SOD1, and TDP-43 iPSNs. (**a**) Quantification of nuclear/cytoplasmic

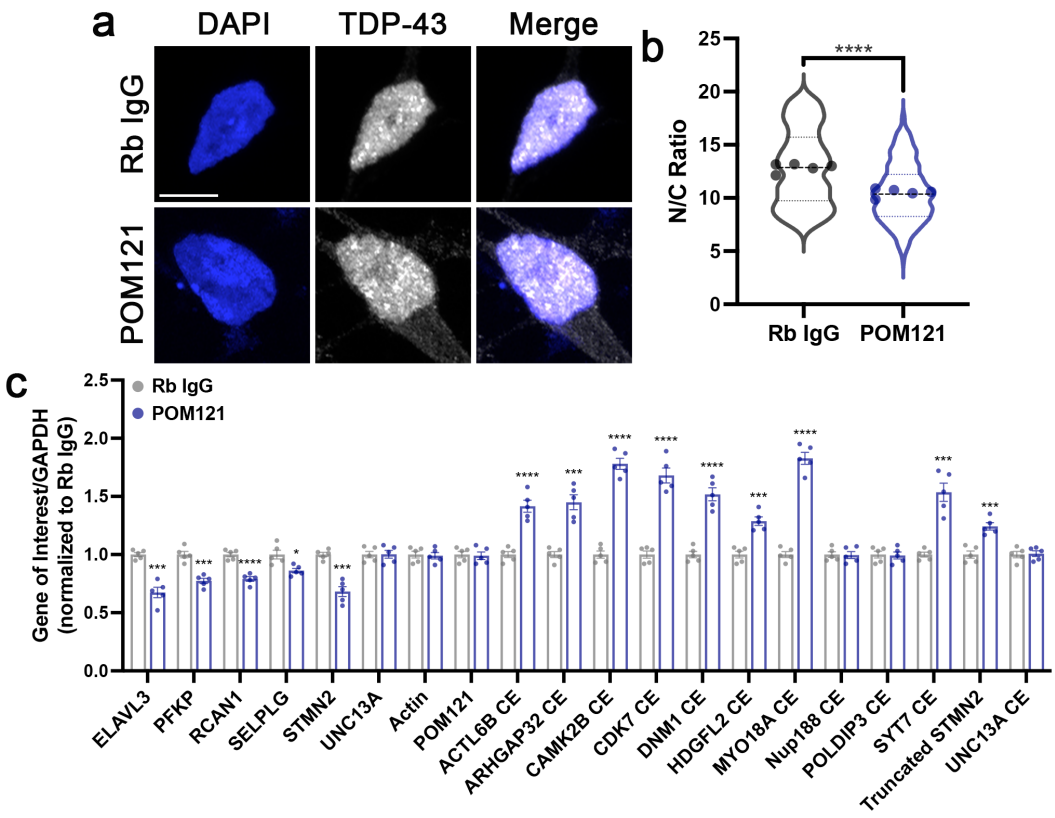
distribution of CHMP7 in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$. (**b**) Quantification of the number of POM121 spots detected in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$. (**c**) Quantification of nuclear/cytoplasmic distribution of Ran GTPase in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. (**d**) Quantification of nuclear/cytoplasmic distribution of TDP-43 in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. Data are presented as mean values +/- SEM. One-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. ** $p < 0.01$, **** $p < 0.0001$.



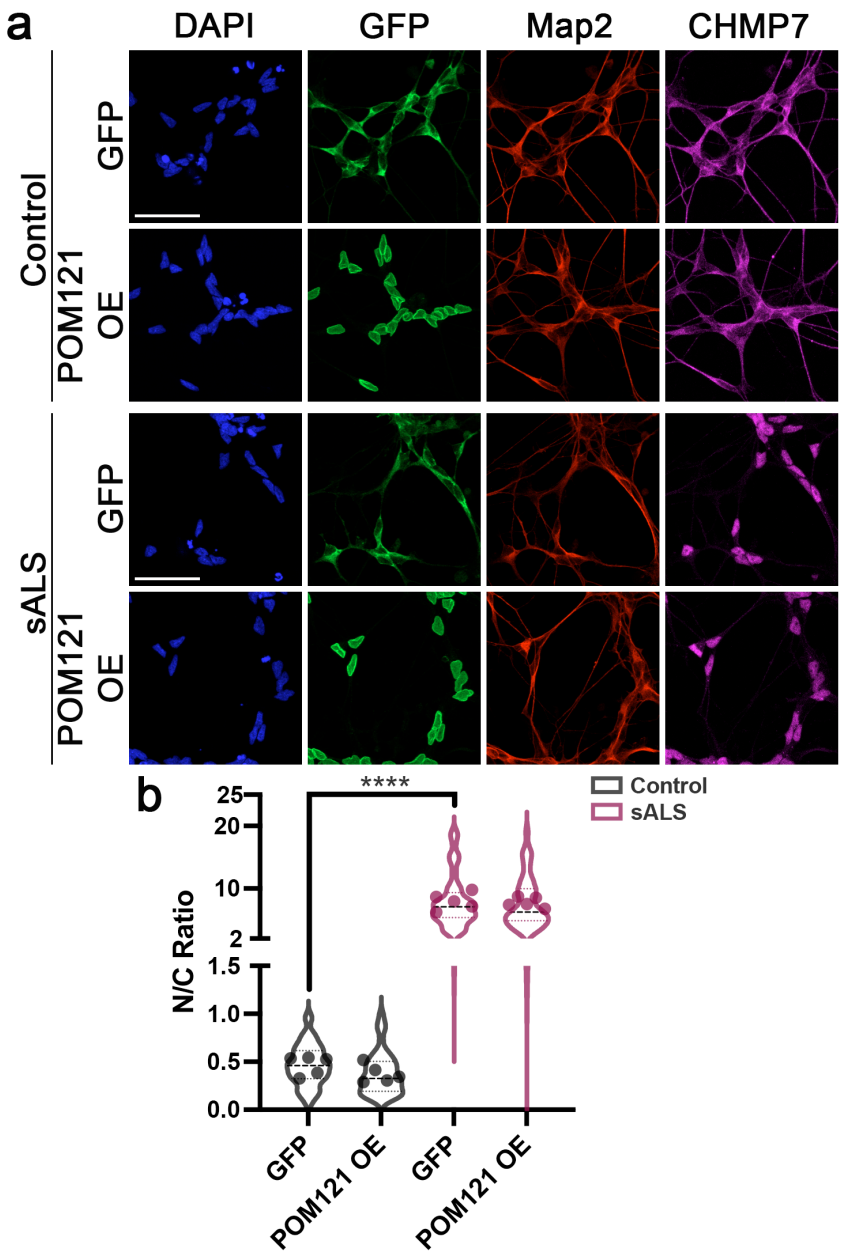
Supplementary Figure 16: Time dependent sequential emergence of pathophysiologic events related to nuclear pore complex injury in sALS, C9orf72, SOD1, and TDP-43 iPSNs. (**a**) Line graphs of nuclear/cytoplasmic distribution of CHMP7 in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. (**b**) Line graphs of the number of POM121 spots detected in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. (**c**) Line graphs of nuclear/cytoplasmic distribution of Ran GTPase in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations. (**d**) Line graphs of nuclear/cytoplasmic distribution of TDP-43 in control, sALS, C9orf72, SOD1, and TDP-43 iPSNs at day 18, 25, 32, 46, and 60 of differentiation. n = 33 control, 129 sALS, 12 C9orf72, 3 SOD1, and 3 TDP-43 iPSC lines. Data points represent the average value across 3 independent differentiations.



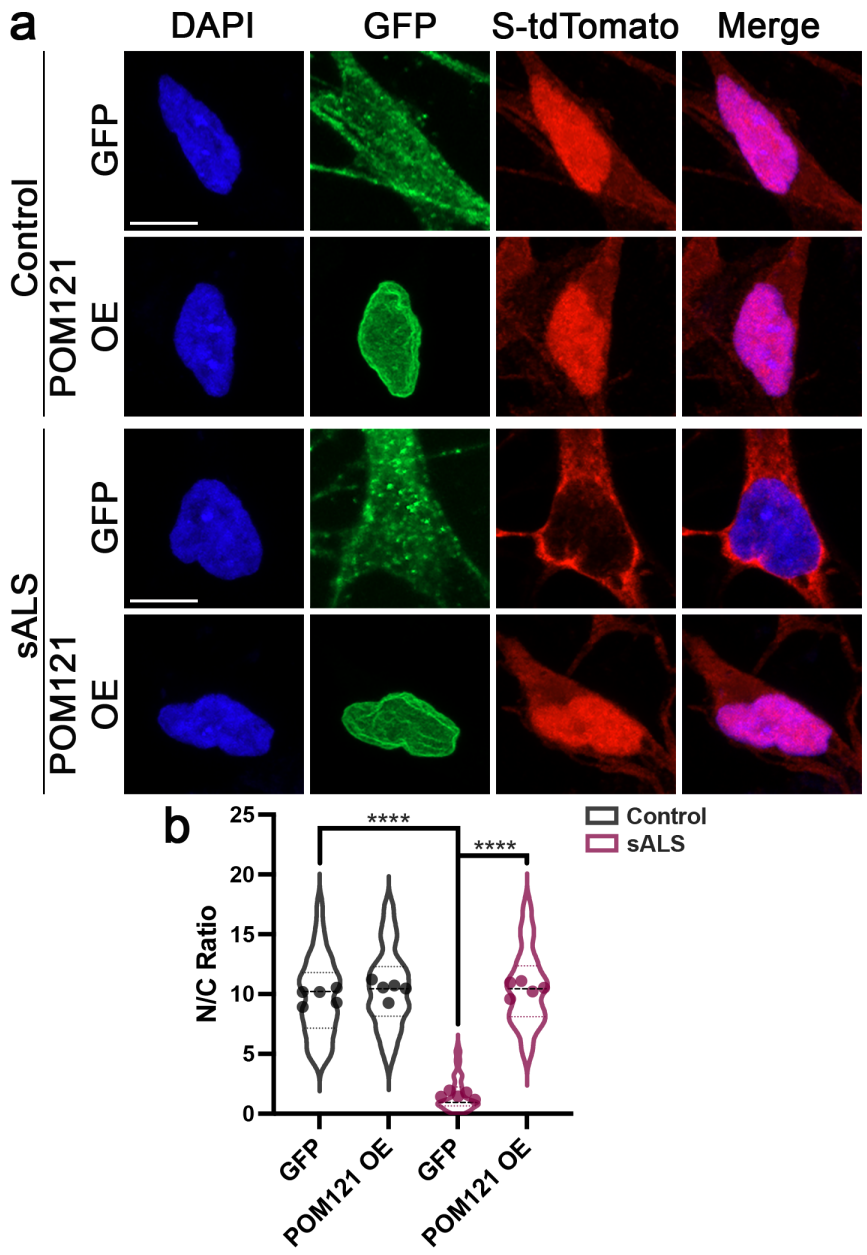
Supplementary Figure 17: ASO mediated reduction of CHMP7 restores TDP-43 function in C9orf72 ALS/FTD and sALS patient iPSNs. Heat map depicting percentage of CHMP7 protein reduction (obtained from western blot) and TDP-43 loss of function associated alterations in target gene expression and mRNA splicing (obtained from qRT-PCR) at day 81 of differentiation following 3 weeks of treatment with 5μM CHMP7 ASO. CHMP7 ASO treatment was initiated on day 60 *following* the detection of molecular signatures of TDP-43 dysfunction. n = 12 control, 7 C9orf72, and 25 sALS iPSC lines. Boxes represent the average value across 3 independent differentiations.



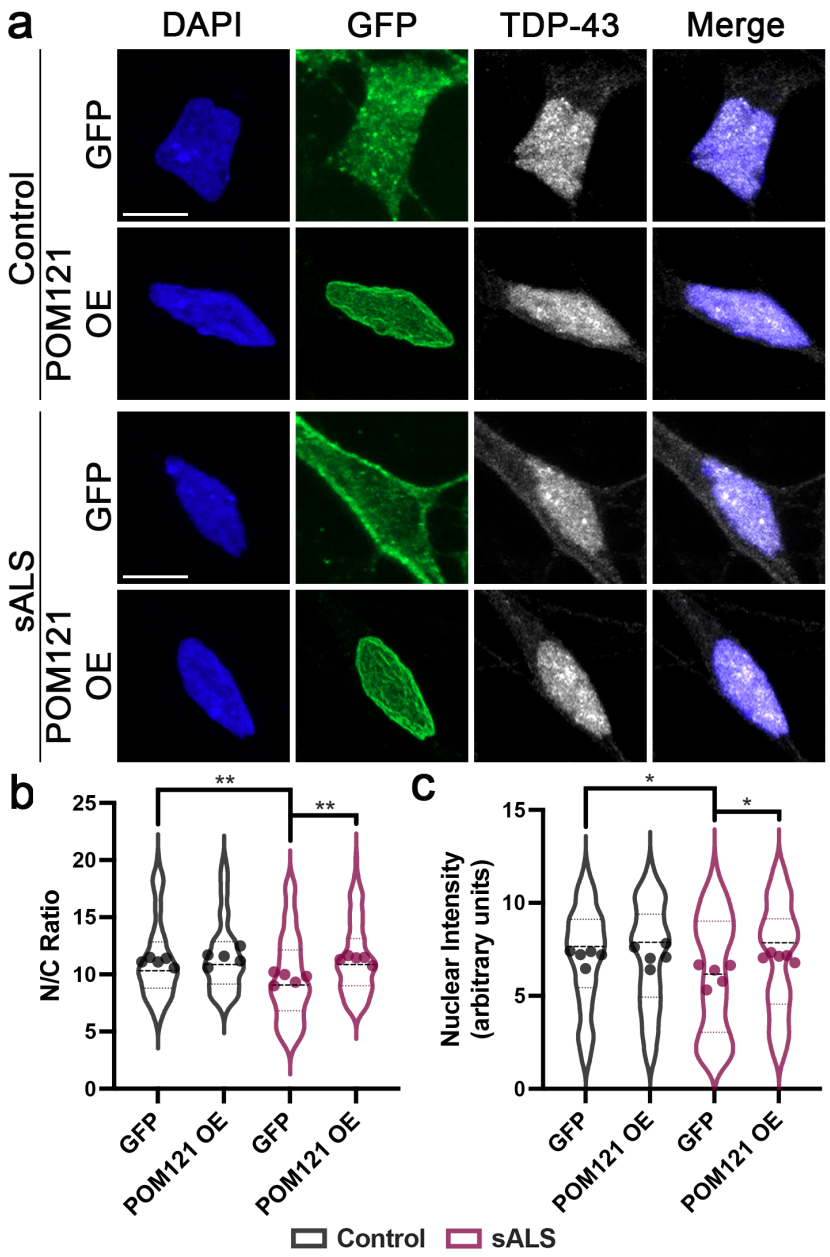
Supplementary Figure 18: Knockdown of transmembrane Nup POM121 is sufficient to initiate TDP-43 loss of function associated changes in gene expression and mRNA splicing in wildtype iPSNs. (**a**) Immunostaining and confocal imaging for TDP-43 in control iPSNs 7 days following rapid degradation of endogenous POM121. Antibody for Trim Away as indicated on left, antibody/stain for immunostaining as indicated on top. Scale bar = 5μm. (**b**) Quantification of nuclear/cytoplasmic distribution of TDP-43 in control iPSNs 7 days following rapid degradation of endogenous POM121. n = 5 control iPSC lines, at least 100 Map2+ cells per line/knockdown. Data are presented as median values with 25th and 75th percentiles. Individual data points represent average of all cells analyzed per iPSC line. Student's t-test was used to calculate statistical significance. **** $p < 0.0001$. (**c**) qRT-PCR for *ELAVL3*, *PFKP*, *RCAN1*, *SELPLG, STMN2*, *UNC13A*, *ACTIN*, *POM121*, *ACTL6B* cryptic exon containing, *ARHGAP32* cryptic exon containing, *CAMK2B* cryptic exon containing, *CDK7* cryptic exon containing, *DNM1* cryptic exon containing, *HDGFL2* cryptic exon containing, *MYO18A* cryptic exon containing, *NUP188* cryptic exon containing, *POLDIP3* cryptic exon containing, *SYT7* cryptic exon containing, truncated *STMN2*, and *UNC13A* cryptic exon containing mRNA in control iPSNs 7 days following rapid degradation of endogenous POM121. n = 5 control iPSC lines. Data are presented as mean values +/- SEM. Student's t-test was used to calculate statistical significance. *** $p < 0.001$, **** $p < 0.0001$.



Supplementary Figure 19: Overexpression of POM121 does not impact nuclear accumulation of CHMP7 in sALS iPSNs. (**a**) Immunostaining and confocal imaging for CHMP7 in control and sALS iPSNs following 2 weeks of GFP or POM121 overexpression. POM121 overexpression was initiated at day 46 of differentiation following the detectable initiation of TDP-43 dysfunction in sALS iPSNs. Antibody for immunostaining as indicated on top, genotype and overexpression as indicated on left. Scale bar = 50 μm. (**b**) Quantification of nuclear/cytoplasmic distribution of CHMP7. n = 5 control and 5 sALS iPSC lines, 100 Map2+ neurons per line. Data are presented as median values with 25th and 75th percentiles. Individual data points represent average of all cells analyzed per iPSC line. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$.

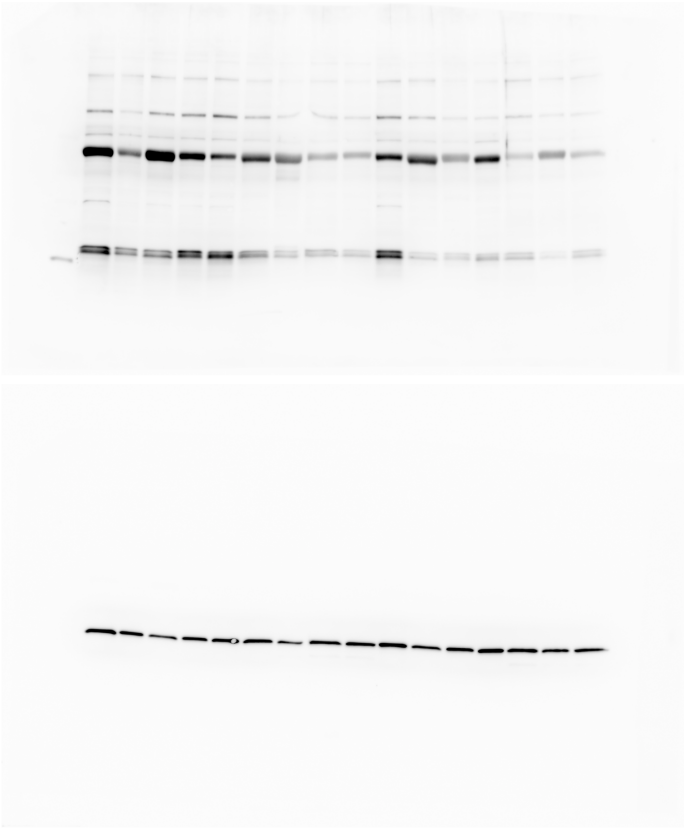


Supplementary Figure 20: Overexpression of POM121 restores the nuclear import of the S-tdTomato NCT reporter in sALS iPSNs. (**a**) Confocal imaging for the S-tdTomato NCT reporter in control and sALS iPSNs following 2 weeks of GFP or POM121 overexpression. POM121 overexpression was initiated at day 46 of differentiation following the detectable initiation of TDP-43 dysfunction in sALS iPSNs. Stain and fluorescent protein marker as indicated on top, genotype and overexpression as indicated on left. Scale bar = 5μm. (**b**) Quantification of nuclear/cytoplasmic distribution of the S-tdTomato NCT reporter. n = 5 control and 5 sALS iPSC lines, 100 neurons per line. Data are presented as median values with 25th and 75th percentiles. Individual data points represent average of all cells analyzed per iPSC line. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. **** $p < 0.0001$.



Supplementary Figure 21: Overexpression of POM121 reverses subtle alterations in nuclear/cytoplasmic distribution of TDP-43 in sALS iPSNs. (**a**) Immunostaining and confocal imaging TDP-43 in control and sALS iPSNs following 2 weeks of GFP or POM121 overexpression. POM121 overexpression was initiated at day 46 of differentiation following the detectable initiation of TDP-43 dysfunction in sALS iPSNs. Antibody for immunostaining as indicated on top, genotype and overexpression as indicated on left. Scale bar = 5μm. (**b**) Quantification of nuclear/cytoplasmic distribution of TDP-43. n = 5 control and 5 sALS iPSC lines, 100 Map2+ neurons per line. Data are presented as median values with 25th and 75th percentiles. Individual data points represent average of all cells analyzed per iPSC line. Two-way ANOVA with Tukey's multiple comparison test was used to calculate statistical significance. * $p < 0.05$, ** $p < 0.01$.

Supplemental Figure 6



Supplementary Figure 22: Uncropped western blots.