Supplementary Material

Reduced C9orf72 Expression Exacerbates polyGR Toxicity in Patient Induced Pluripotent Stem Cell-Derived Motor Neurons and a Type I Protein Arginine Methyltransferase Inhibitor Reduces that Toxicity

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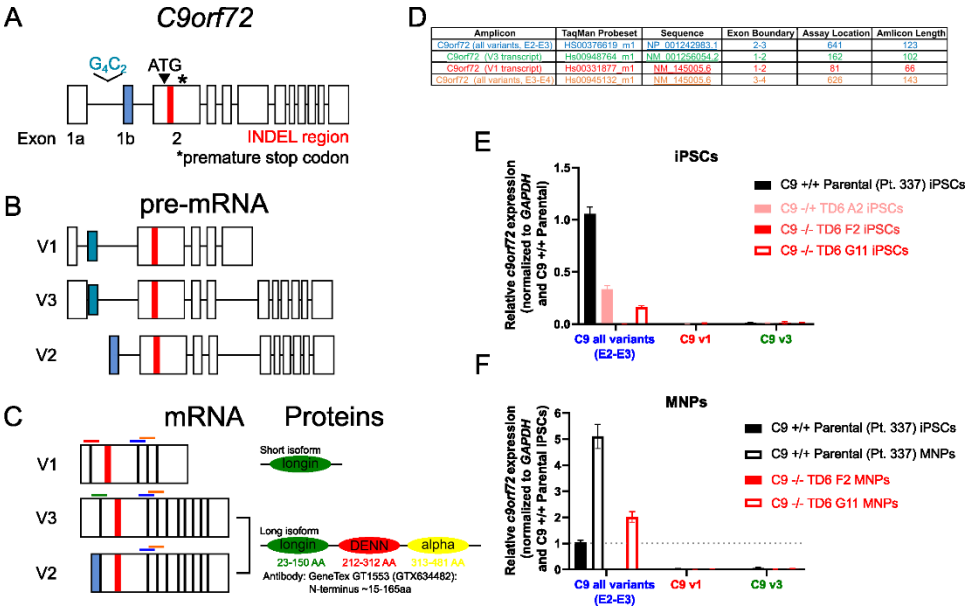
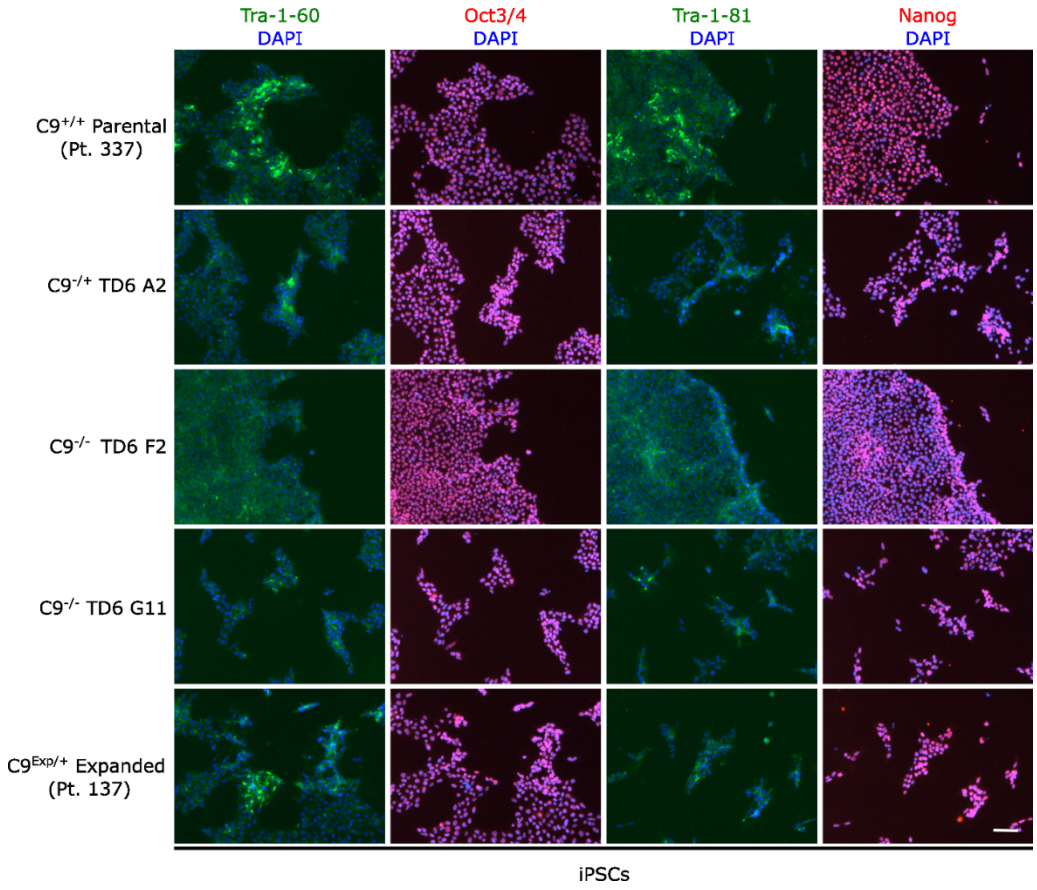
1 Supplementary Figures



Amplicon	TaqMan Probeset	Sequence	Exon Boundary	Assay Location	Amlicon Length
C9orf72 (all variants, E2-E3)	Hs00376619_m1	NP_001242983.1	2-3	641	123
C9orf72 (V3 transcript)	Hs00948764_m1	NM_001256054.2	1-2	162	102
C9orf72 (V1 transcript)	Hs00331877_m1	NM_145005.6	1-2	81	86
C9orf72 (all variants, E3-E4)	Hs00945132_m1	NM_145005.6	3-4	628	143

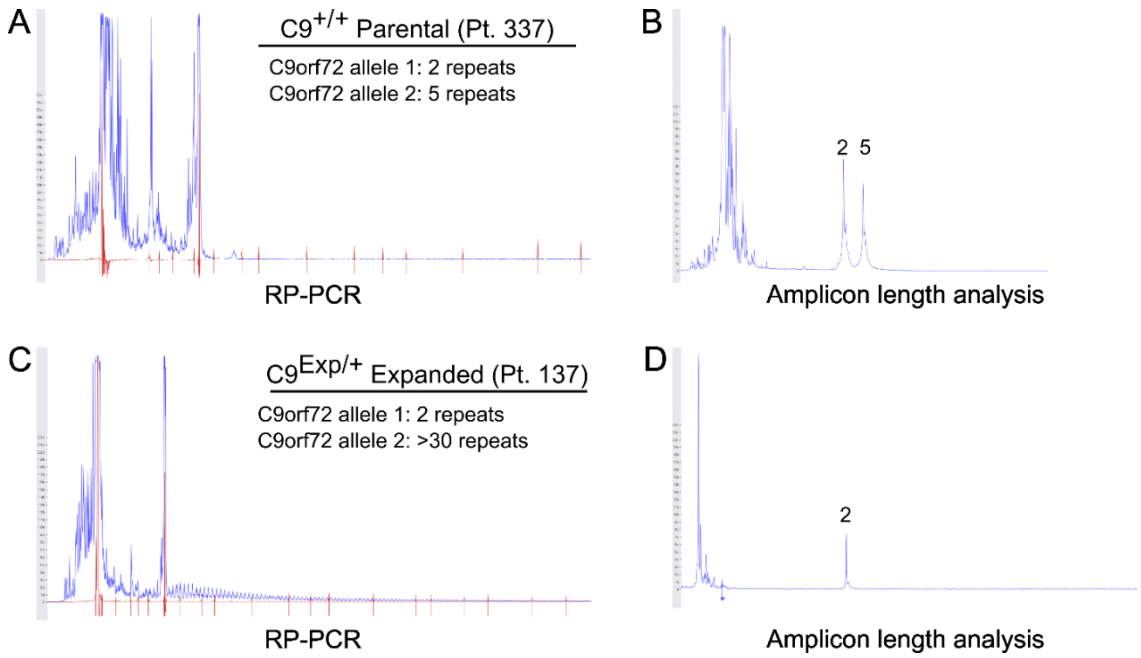
Supplementary Figure 1: Expression of *C9orf72* Splice Variants

(**A-C**) Schematic representation of the *C9orf72* gene, pre-mRNA, mRNA, and C9orf72 protein. CRISPR-Cas9 targeted region of exon 2 highlighted in red introduces a downstream premature stop codon. Antibody used in Fig. 1 binds the N-terminus of C9orf72. Modified from Liu et al. 2021 and Xiao et al. 2016. (**D**) Table of TaqMan probe sets used in E-F. (**E**) Expression of total *C9orf72*, transcript v1, and transcript v3 in iPSCs normalized to C9+/+ parental (Pt. 337). (**F**) Expression of *C9orf72* in motor neuron progenitors normalized to C9+/+ parental (Pt. 337) iPSCs.



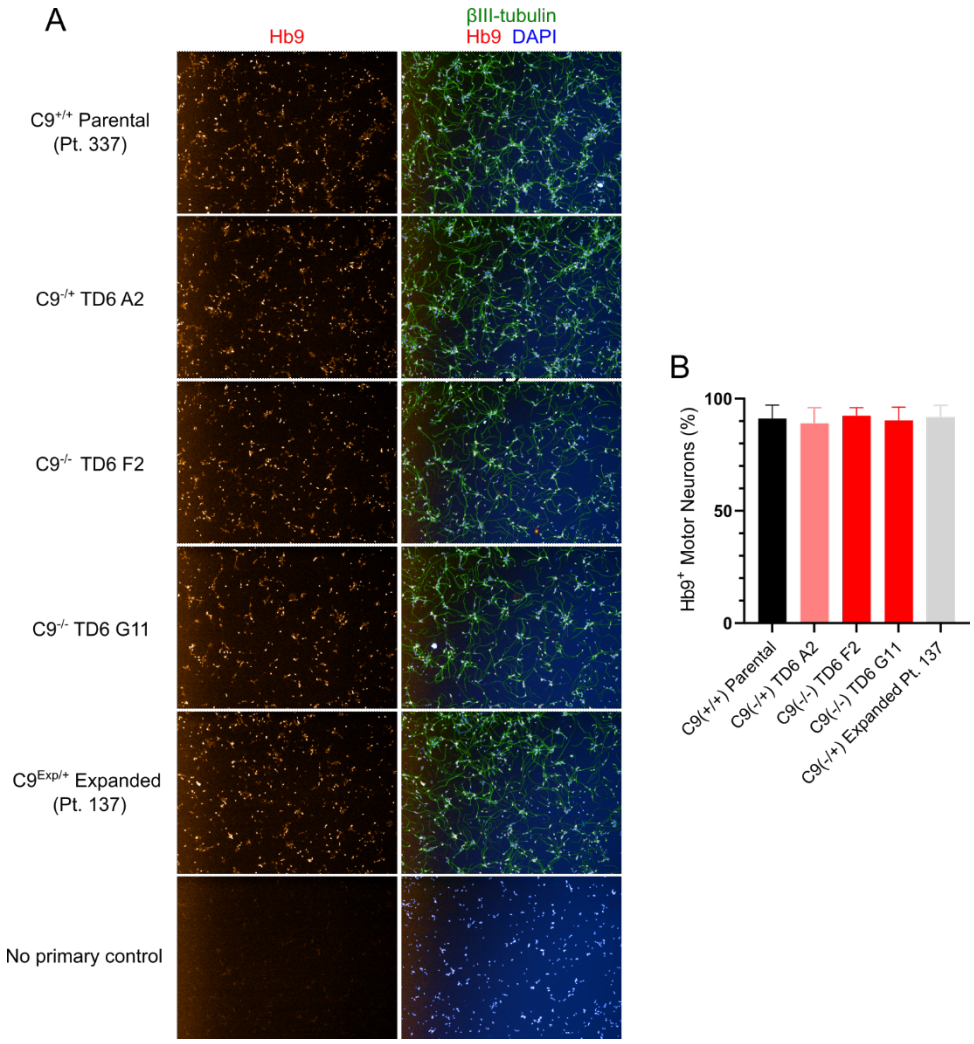
Supplementary Figure 2: *C9orf72* Knockout iPSCs Maintain Pluripotency Marker Expression

Representative images of immunocytochemistry for pluripotency markers Tra-1-60, Oct3/4, Tra-1-81, and Nanog.



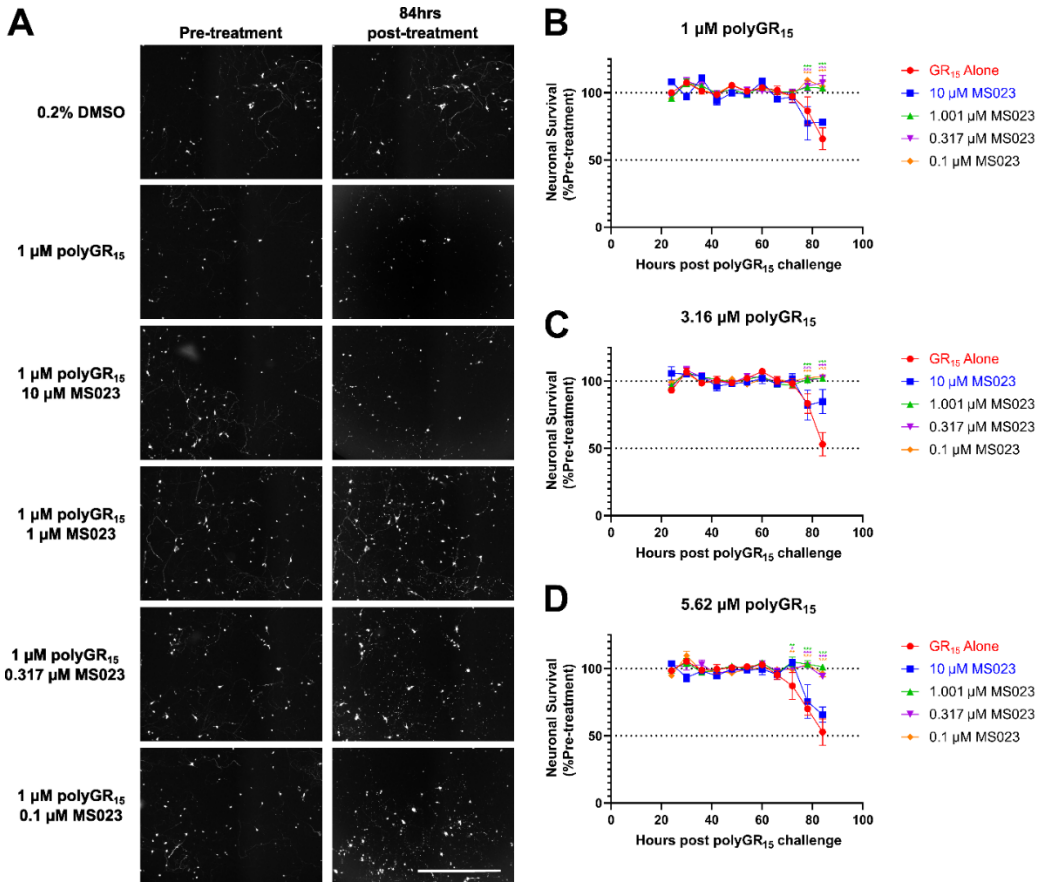
Supplementary Figure 3: *C9orf72*-HRE Genotyping by Repeat-Primed PCR

(**A**) Repeat-primed PCR electropherograms from patient 337 fibroblast genomic DNA. (**B**) Amplicon length analysis electropherograms from patient 337 fibroblast genomic DNA. (**C**) Repeat-primed PCR electropherograms from patient 137 fibroblast genomic DNA. Note the sawtooth pattern characteristic of long C9-HRE expansions. (**D**) Amplicon length analysis electropherograms from patient 137 fibroblast genomic DNA.



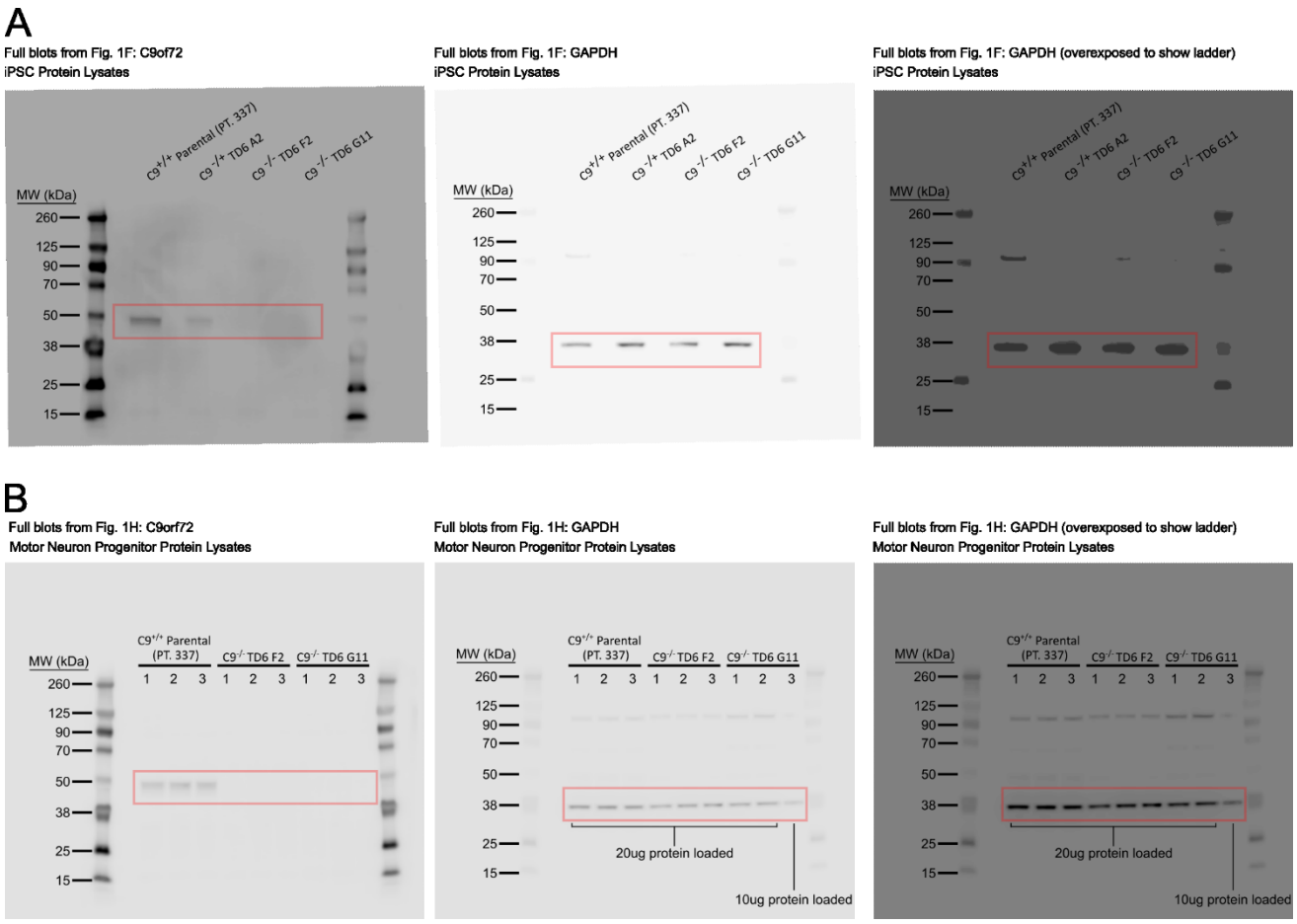
Supplementary Figure 4: Spinal Motor Neuron Differentiation Efficiency

(A) Representative images of iPSC-MNs stained on day 25 of differentiation for the pan-neuronal marker βIII-tubulin (Covance MRB-435P, 1:500) and the spinal motor neuron specific transcription factor Hb9 (*MNX1*, DSHB 815.c10c, 1;100). **(B)** Quantification of the percentage of $Hb9^{+}$ cells. No significant difference was observed between cell lines.



Supplementary Figure 5: Type I PRMT Inhibition Preserves Neuronal Survival

(**A**) Representative images of day 12 VACHT-tdTomato neurons before and after $polyGR_{15}$ challenge with or without MS023 co-treatment. Scale bar = 2000 µm. (**B-D**) Quantification of the number of $tdTomato^{+}$ MNs measured every 6 hours beginning 24 hours post treatment. For each well, the neuron count was normalized to the same wells previous time point. * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$ (comparing MS023 co-treated groups to $polyGR_{15}$ alone by two-way ANOVA analysis of variance followed by Dunnett's test). Data are presented as mean values ± standard deviations (error bars).



Supplementary Figure 6: Full Uncropped Western Blot Images from Figure 1

(A) Full blots for C9orf72 and GAPDH from iPSC lysates shown in Fig. 1F. **(B)** Full blots for C9orf72 and GAPDH from iPSC lysates shown in Fig. 1H. Only 10ug of total protein was loaded for lane 3 of $C9^{-/-}$ TD6 G11 due to insufficient sample volume.