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Supplemental information

Homozygous ALS-linked mutations in TARDBP/TDP-43 lead to hypoactivity and synaptic abnormalities in human iPSC-derived motor neurons

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I. Supplemental Figures

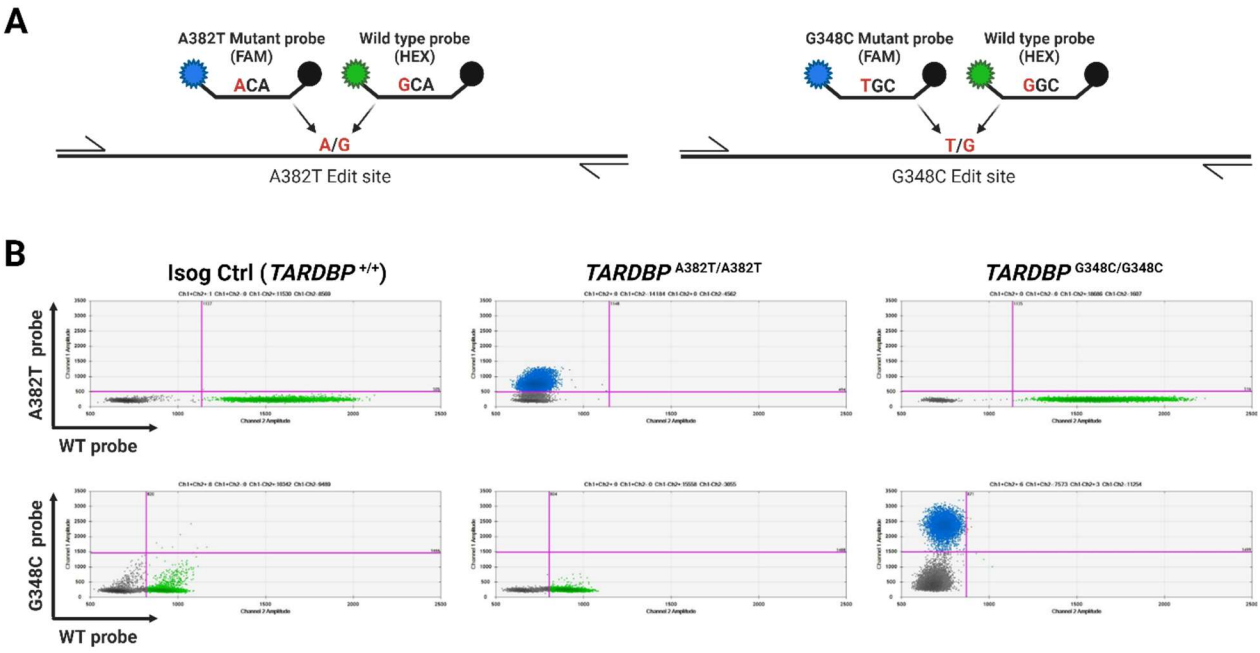
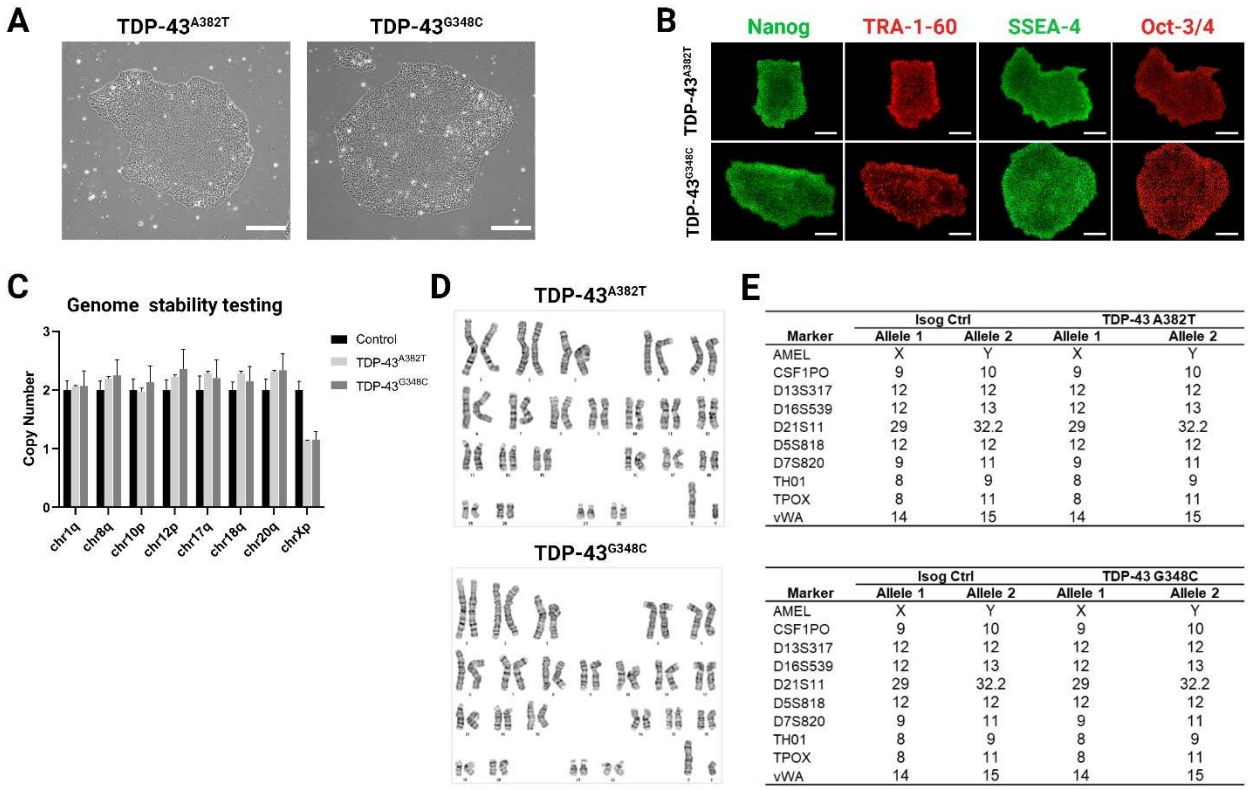


Figure S1. Validation of CRISPR/Cas9 gene editing by ddPCR. Related to Figure 1.

(A and B) Pairs of mutant (FAM, blue) and wild-type (HEX, green) probes designed to target the edited or wild-type alleles, respectively (A). ddPCR scatter plots confirming correct gene editing and homozygosity of iPSC lines (B).



Marker	Isog Ctrl		TDP-43 A382T	
	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X	Y	X	Y
CSF1PO	9	10	9	10
D13S317	12	12	12	12
D16S539	12	13	12	13
D21S11	29	32.2	29	32.2
D5S818	12	12	12	12
D7S820	9	11	9	11
TH01	8	9	8	9
TPOX	8	11	8	11
vWA	14	15	14	15

Marker	Isog Ctrl		TDP-43 G348C	
	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X	Y	X	Y
CSF1PO	9	10	9	10
D13S317	12	12	12	12
D16S539	12	13	12	13
D21S11	29	32.2	29	32.2
D5S818	12	12	12	12
D7S820	9	11	9	11
TH01	8	9	8	9
TPOX	8	11	8	11
vWA	14	15	14	15

Figure S2. Characterization of *TARDBP* knock-in iPSCs. Related to Figure 1.

(A) Representative phase-contrast images of *TARDBP* knock-in iPSCs. Scale bar, 250 μm.
(B) Representative images of *TARDBP* knock-in iPSCs subjected to immunocytochemistry for pluripotency-associated markers Nanog, TRA1-60, SSEA-4, and OCT-3/4. Scale bar, 250 μm.
(C and D) Genomic stability analyses. *TARDBP* knock-in iPSC lines have normal chromosome copy numbers, as assessed by qPCR. Data shown as mean ± SEM of technical triplicates. The control used here was provided by the manufacturer of the genome stability testing kit (C). Edited iPSC lines display normal G-band karyotypes (D).
(E) STR analysis confirming the isogeneity of *TARDBP* knock-in iPSCs with the parental control line (AIW002-02).

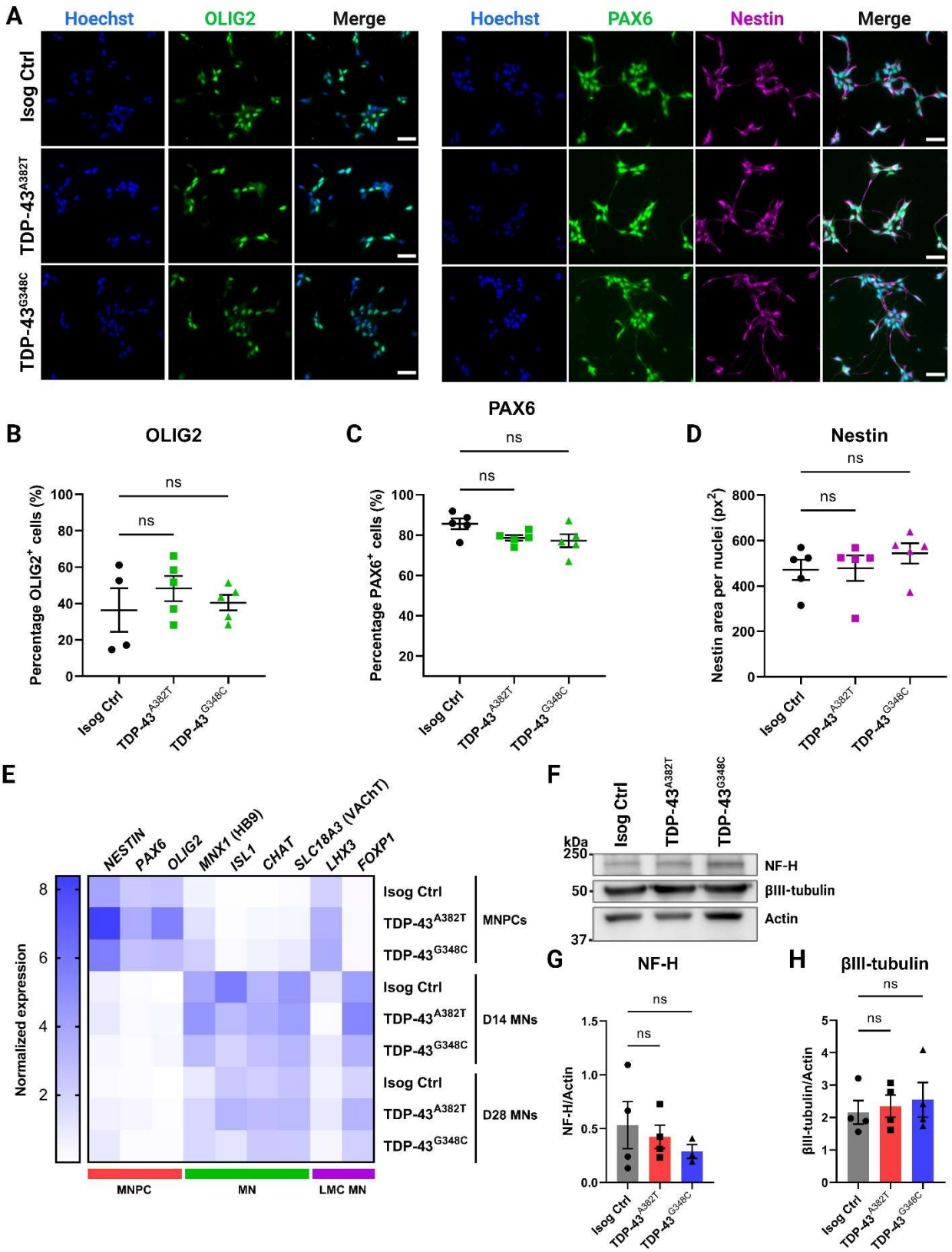
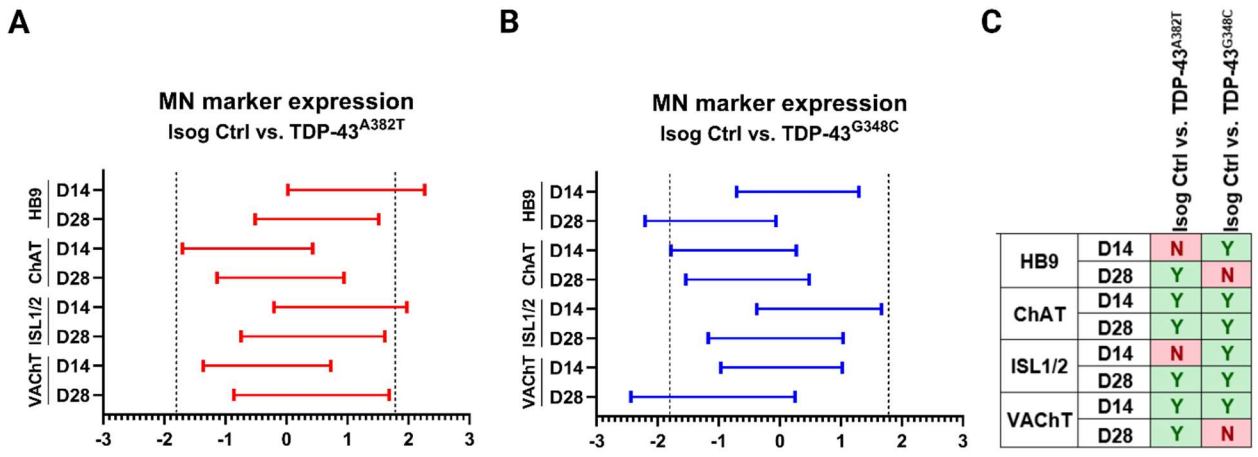
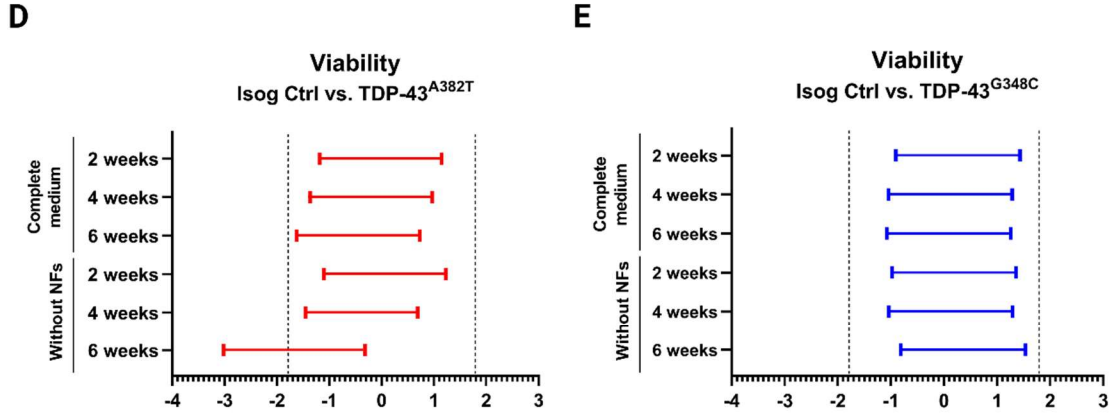

A
Hoechst
OLIG2
Merge
Hoechst
PAX6
Nestin
Merge
Isog Ctrl
TDP-43A382T
TDP-43G348C
B
OLIG2
Percentage OLIG2+ cells (%)
ns
ns
C
PAX6
Percentage PAX6+ cells (%)
ns
ns
D
Nestin
Nestin area per nuclei (px2)
ns
ns
E
NESTIN
PAX6
OLIG2
MNX1 (HB9)
ISL1
CHAT
SLC18A3 (VAChT)
LHX3
FOXP1
Normalized expression
Isog Ctrl
TDP-43A382T
TDP-43G348C
MNPCs
D14 MNs
D28 MNs
MNPC
MN
LMC MN
F
kDa
250
50
37
NF-H
βIII-tubulin
Actin
G
NF-H
NF-H/Actin
ns
ns
H
βIII-tubulin
βIII-tubulin/Actin
ns
ns

Figure S3. Characterization of iPSC-derived MNPCs and MNs. Related to Figures 1 and 2.

(A-D) Representative images (A) and quantification of MNPCs differentiated from iPSCs subjected to immunocytochemistry for common MNPC markers OLIG2 (B), PAX6 (C), and Nestin (D). Scale bar, 100 μm. n=5 replicates from at least 2 independent inductions from iPSCs. Data shown as mean ± SEM.
(E) qPCR heatmap showing normalized transcripts levels of MNPC (red), MN (green), and LMC (magenta) markers during differentiation of MNPCs into MNs. Mean plotted. n=3 independent experiments.
(F-H) Immunoblot (F) and quantification of total levels of neurofilament heavy (NF-H) (G) and βIII-tubulin (H). Actin was used as loading control. Extractions were performed in MNs harvested after 6 weeks post-plating. n=4 independent experiments. Data shown as mean ± SEM. Ordinary one-way.





F

		Isog Ctrl vs. TDP-43A382T	Isog Ctrl vs. TDP-43G348C
Complete	2 weeks	Y	Y
	4 weeks	Y	Y
	6 weeks	Y	Y
Without NFs	2 weeks	Y	Y
	4 weeks	Y	Y
	6 weeks	N	Y

Figure S4. Equivalence testing with confidence intervals. Related to Figures 1 and 2.

(A-C) 90% confidence intervals with equivalence bounds Δ_L = -1.8 and Δ_U = 1.8 for comparisons of MN marker expression by immunocytochemistry in TDP-43^{A382T} MNs (A) or TDP-43^{G348C} MNs (B) compared with isogenic control MNs after 2-weeks (D14) and 4-weeks (D28) of final differentiation, with results summary (Equivalence Yes/No) (C).
(E-F) 90% confidence intervals with equivalence bounds Δ_L = -1.8 and Δ_U = 1.8 for comparisons of MN viability in TDP-43^{A382T} MNs (E) or TDP-43^{G348C} MNs (F) compared with isogenic control MNs at 2-. 4- and

6-weeks of final differentiation in medium with or without neurotrophic factor (NF) supplementation, with results summary (Equivalence Yes/No) (F).

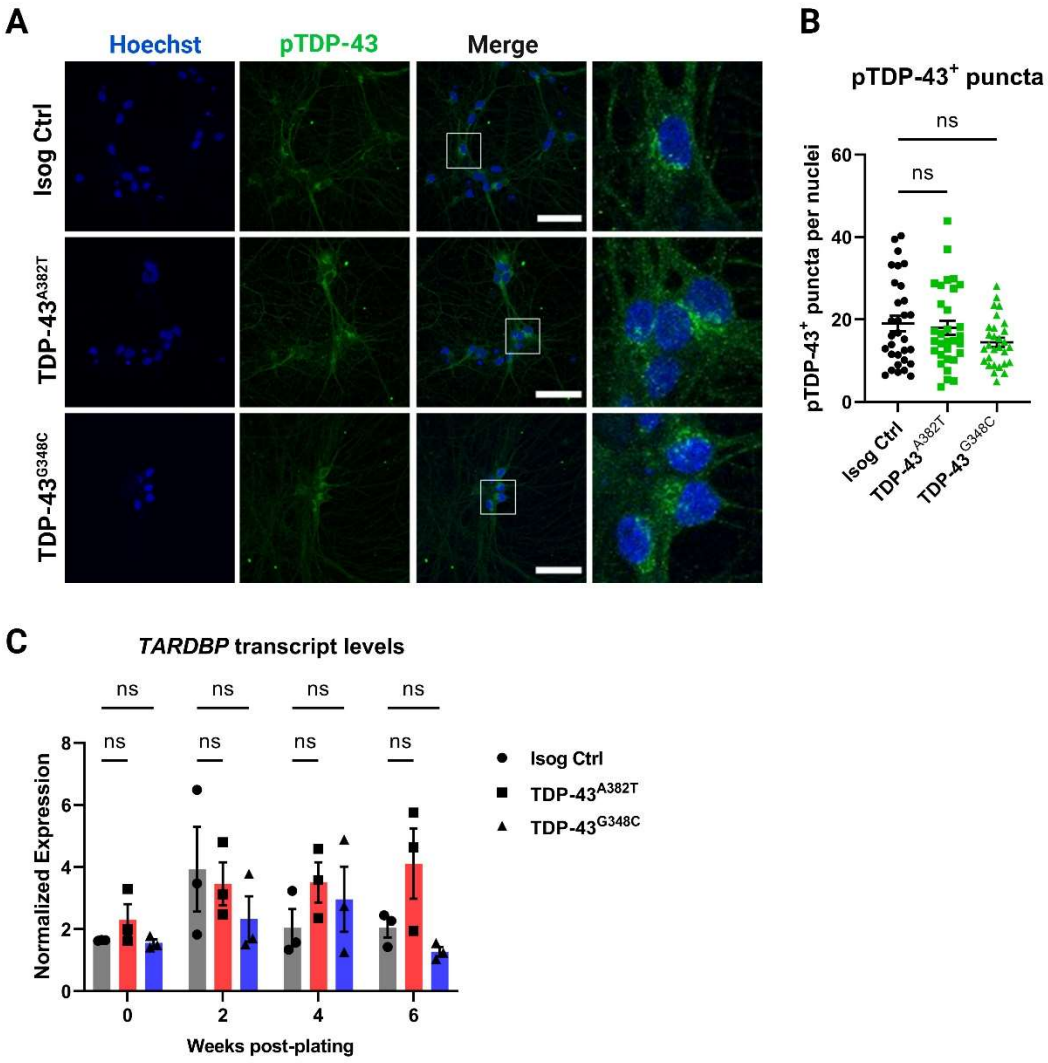


Figure S5. Mutant MNs do not accumulate *TARDBP* transcripts or phosphorylated TDP-43. Related to Figure 3.

(A) Representative images MNs differentiated for 6 weeks subjected to immunocytochemistry for phosphorylated TDP-43 (Ser409/410) showing punctate cytosolic staining. Scale bar, 50 µm.
(B) Quantification of pTDP-43⁺ puncta. Individual data points represent per-frame mean values from 5 independent experiments. Ordinary one-way ANOVA.
(C) Relative transcript levels of *TARDBP* at several timepoints determine by qPCR. n=3 independent experiments. Two-way ANOVA.
All data shown as mean ± SEM.

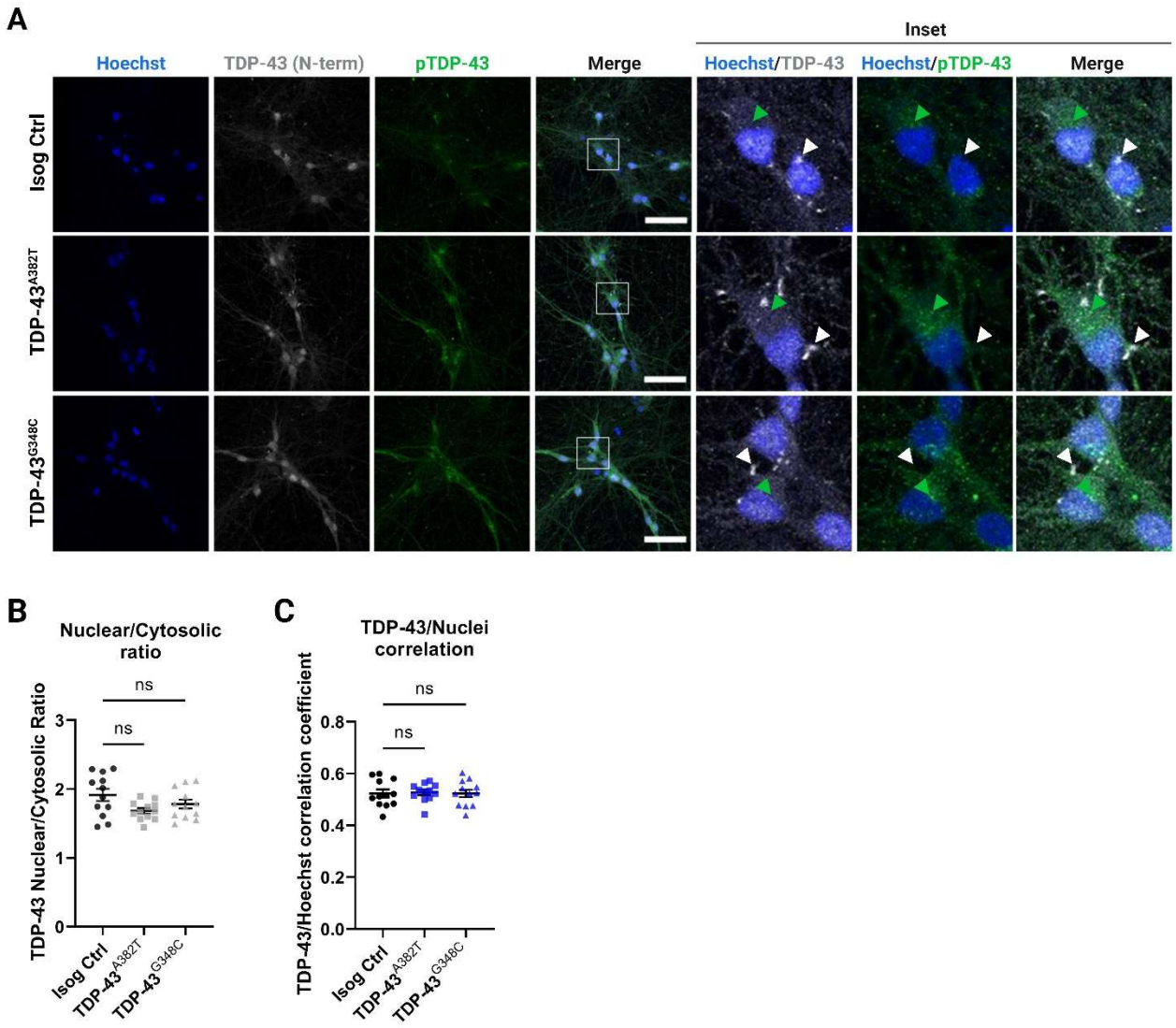


Figure S6. TDP-43 variants do not exhibit changes in nucleocytoplasmic localization. Related to Figure 4.

(A) Representative immunostainings of MNs differentiated for 6 weeks with an antibody targeted to the N-terminus of TDP-43 showing TDP-43 subcellular distribution and cytosolic TDP-43^{+} puncta. TDP-43^{+} puncta (white arrows) do not colocalize with pTDP-43^{+} puncta (green arrows). Scale bar, 50 μm.
(B and C) Quantification of TDP-43 distribution using the nuclear/cytosolic ratio of TDP-43 fluorescence signal intensity (B) and the TDP-43/Hoechst correlation coefficient (C). Individual data points represent per-frame mean values from 2 independent experiments. Data shown as mean ± SEM. Ordinary one-way ANOVA.

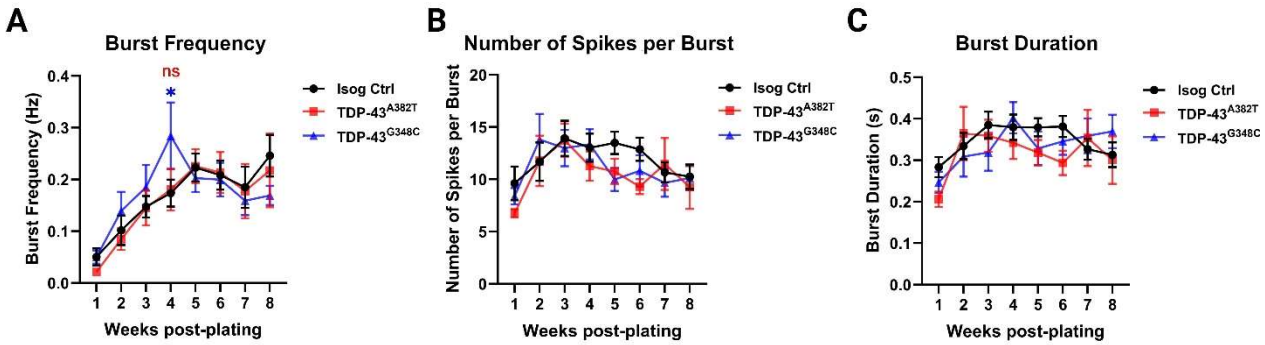


Figure S7. Supplemental neuronal activity measurements recorded using multielectrode array. Related to Figure 5.

(A-C) Longitudinal changes in burst frequency (A), number of spikes per burst (B), and burst duration (C) of MN cultures recorded weekly over a span of 8 weeks. n=11 independent experiments. All data shown as mean ± SEM. *p<0.05. Two-way ANOVA.

Supplemental Tables

Table S1. Overview of iPSC lines used. Related to STAR Methods.

Cell line ID	ALS mutation	Sex	Age	Ethnicity	Primary cell line	Reprogramming method
AIW002-02	None	Male	37	Caucasian	PBMC	Sendai virus
TARDBP A382T/AIW002-02	p.A382T	Male	37	Caucasian	Knock-in	n/a
TARDBP G348C/AIW002-02	p.G348C	Male	37	Caucasian	Knock-in	n/a

* PBMC, peripheral blood mononuclear cell; n/a, not applicable.

Table S2. Sequences of sgRNAs and ssODNs used in making of *TARDBP* knock-in iPSC lines. Related to STAR Methods.

Cell line ID	gRNA	ssODN template
TARDBP A382T/AIW002-02	UCUAAUUCUGGUGCAGCAAU	GGCCTTCGGTTCTGGAAATAACTCTTATAGTGGCTCT AATTCTGGTGCAACAATCGGTTGGGGATCAGCATCC AATGCAGGGTCGGGCAGTGGTTTTAATGG
TARDBP G348C/AIW002-02	GCCAGCCAGCAGAACCAGUC	GAGCAGTTGGGGTATGATGGGCATGTTAGCCAGCC AGCAGAACCAGTCATGCCCATCGGGTAATAACCAAA ACCAAGGCAACATGCAGAGGGAGCCAAACCAGG

Table S3. List of primers and affinity probes used for ddPCR or Sanger sequencing. Related to STAR Methods.

	***TARDBP* A382T/AIW002-02**	***TARDBP* G348C/AIW002-02**
probe-HEX (WT)*	ATT+G+C+TG+CA+CC	AGTC+A+G+GC+CC
probe-FAM (Mutant)*	ATT+G+T+TG+C+AC+CA	AG+TC+A+T+GC+CCAT
ddPCR primer-F	CTTCGGTTCTGGAAATAACTCTTATAG	TTAGCCAGCCAGCAGAA
ddPCR primer-R	CCCAGCCAGAAGACTTAGAA	GTTATTTCCAGAACCGAAGGC
Sanger seq primer-F	GCTTTGGGAATCAGGGTGGA	GCTTTGGGAATCAGGGTGGA
Sanger seq primer-R	ACTCCACACTGAACAAACCA	ACTCCACACTGAACAAACCA

* "+" signs in front of nucleotides indicate the location of Locked Nucleic Acids (LNA®).

Table S4. List of TaqMan probes.
Related to STAR Methods.

Gene	Reference
ACTB	Hs01060665_g1
GAPDH	Hs02786624_g1
NES	Hs04187831_g1
PAX6	Hs01088114_m1
OLIG2	Hs00377820_m1
MNX1/HB9	Hs00907365_m1
ISL1	Hs00158126_m1
CHAT	Hs00758143_m1
SLC18A3/VAChT	Hs00268179_s1
LHX3	Hs01033412_m1
FOXP1	Hs00212860_m1
TARDBP	Hs00606522_m1
DLG4/PSD95	Hs01555373_m1
SYN1	Hs00199577_m1
SYP	Hs00300531_m1