

Figure S1 (related to Fig 1): Motor neuron differentiation of iPS cells from human fibroblasts. A) Schematic representation of the MN differentiation protocol and B) immunostaining for motor neuron markers SMI-32, ChAT, Islet-1 and Tuj1 to confirm successful differentiation of motor neurons. C) Efficiency of the differentiation protocol was determined as 80.5% SMI-32 positive cells, of which 94.4% are ChAT⁺ (n>653 cells counted per differentiation, from at least 3 independent differentiations). No significant differences are seen in differentiation efficiency among the genotypes. Scale bar = 20 μm.

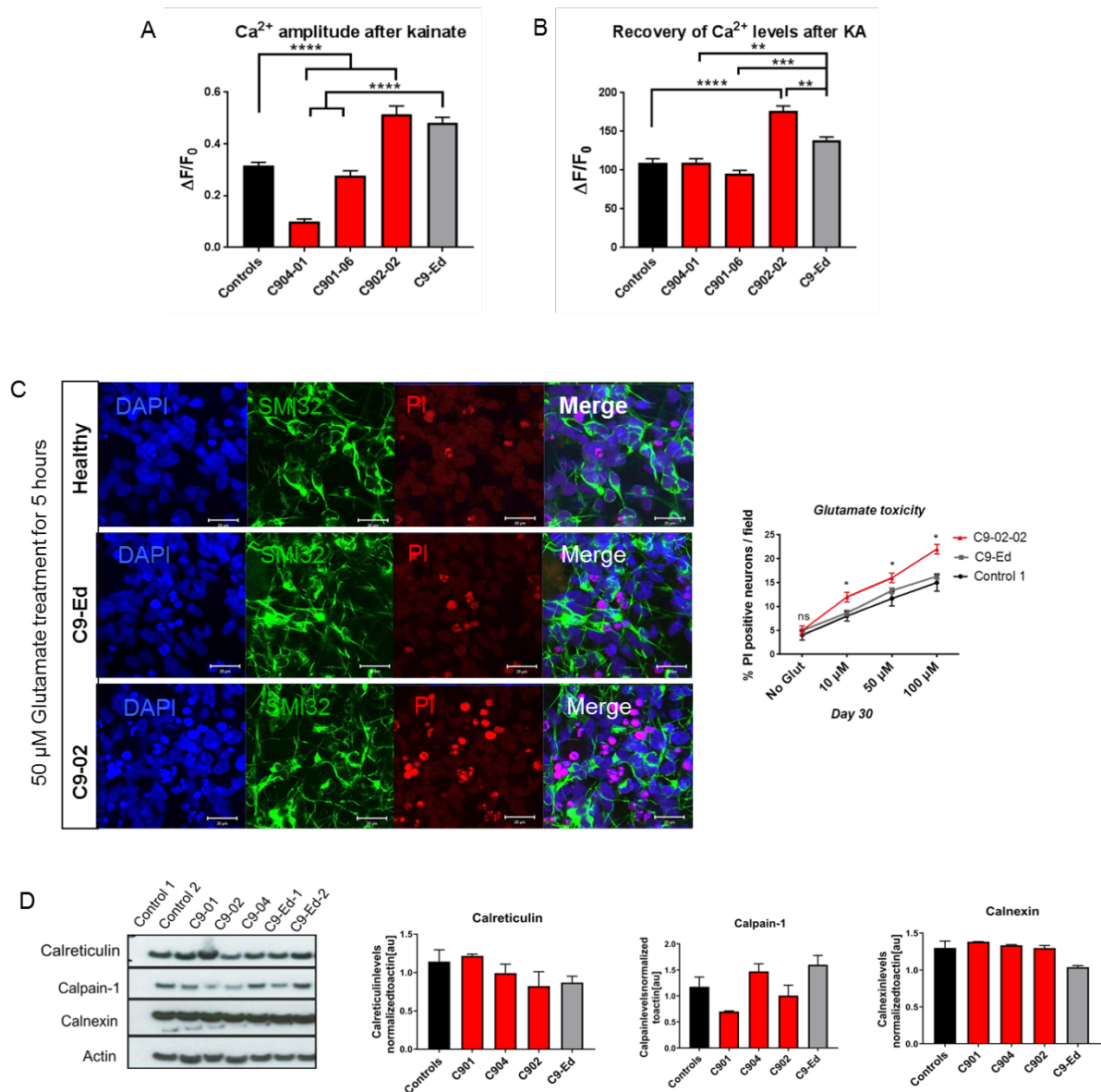


Figure S2. Kainate and glutamate induced toxicity in C9orf72 MNs (related to Fig 2). A) Stimulation of iPS-derived MNs with KA induces various amplitudes in the 5 lines. B) Recovery to baseline Ca^{2+} levels after KA stimulation is increased in C902-02 and this is rescued in C9-Ed. C) Treatment with glutamate induces significantly higher cell death in C9-02 patient line compared to healthy controls and C9-Ed (One-Way ANOVA with Dunnett's post hoc); $n > 100$ cells counted per experiment from 3 independent differentiations. D) Immunoblotting for calreticulin, calpain-1 and calnexin shows no significant differences between C9orf72, healthy controls and genome edited lines. Data are shown as mean \pm SEM.

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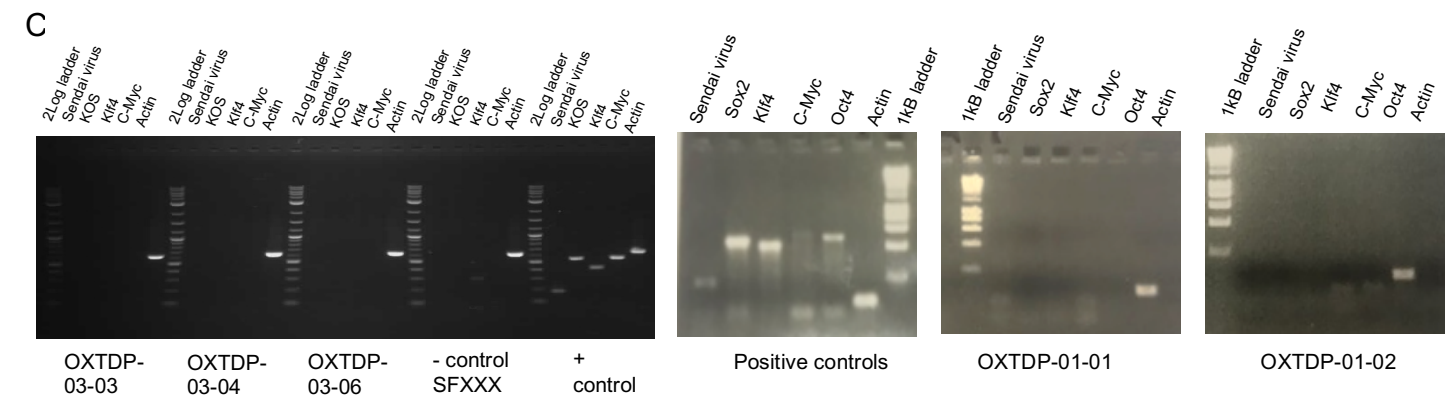
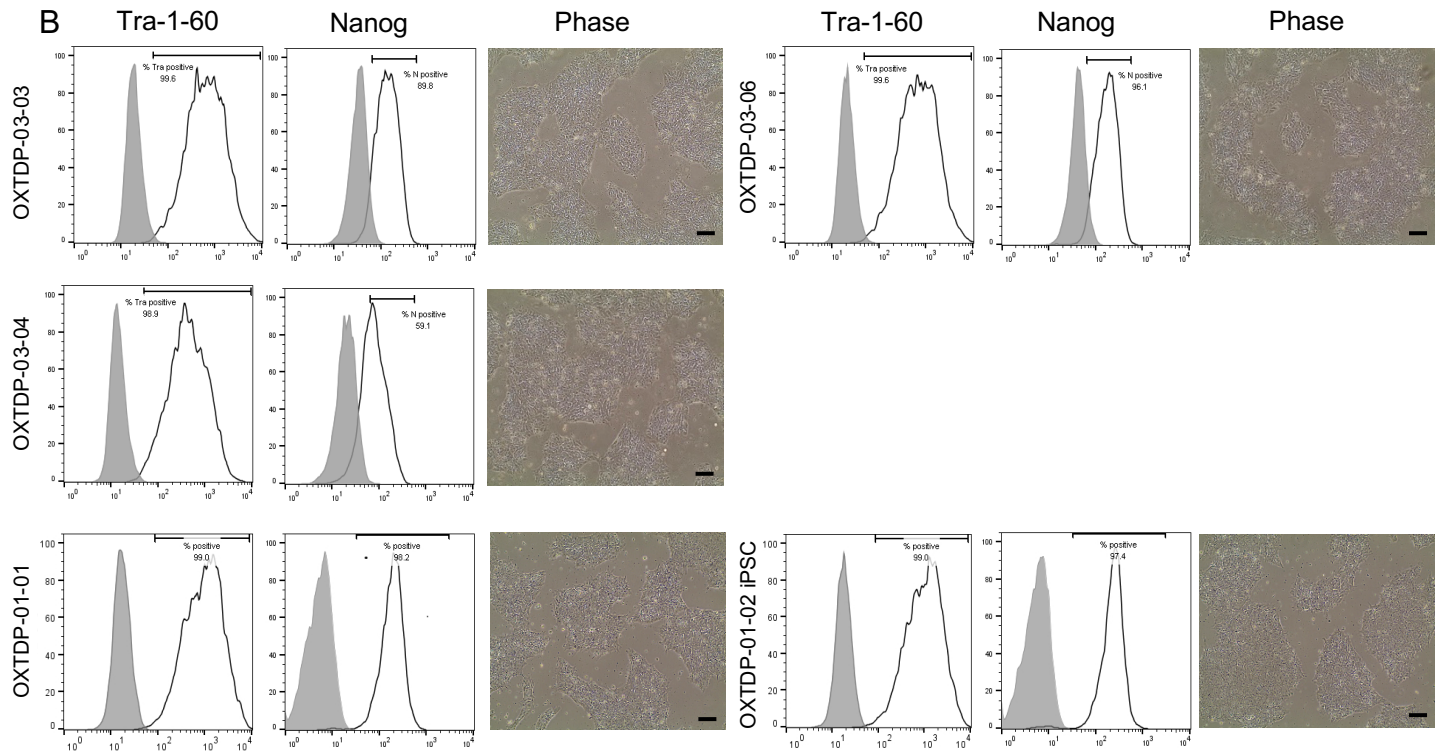


Figure S3: Quality control of iPSC lines used in this study (related to Experimental procedures, Generation of hiPSC lines). iPS cell lines OXDP-03-03, -04 and -06 and OXDP-01-01 and -02 are newly

described in this manuscript. (A) Genome integrity of newly derived lines, assessed by Illumina Human CytoSNP12v2.1 or OmniExpress24 SNP array. Karyograms (KaryoStudio, Illumina) show amplifications (green)/deletions (orange)/LOH regions (grey) alongside the relevant chromosome (NB single copy sex chromosomes annotated orange). (B) FACS analysis confirms expression of pluripotency markers Tra-1-60 and Nanog; open black plot represents antibody, filled grey plot is isotype control; Right-hand panel shows expected iPSC colony morphology (phase-contrast microscopy), Scale bar, 100 μ m. (C) Clearance of Sendai vectors from iPSC lines: OXTDP-03 clones, Cytotune v.2 (Log2 ladder; Product sizes: SeV 181bp; KOS 528bp; SeV-Klf 410bp; SeV-Myc 532bp; Actin 623bp; - control uninfected fibroblasts; + control is fibroblasts infected with Cytotune v.2 5 d previously); OXTDP-01 clones, Cytotune v.1 (SeV 181bp; SeV-Sox 451bp; SeV-Klf 410bp; SeV-Myc 532bp; SeV-Oct 483bp; Actin 92bp, 1kb ladder).

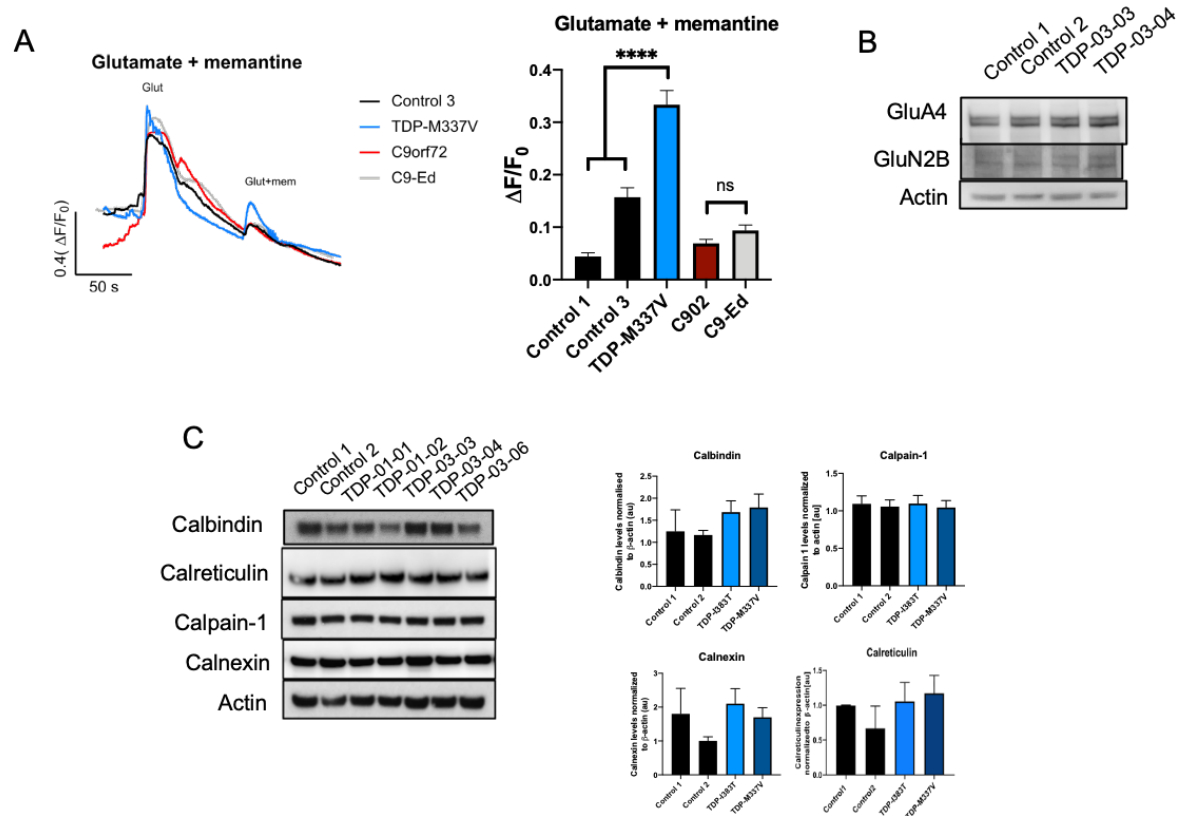


Figure S4: Calcium responses in TDP-43^{M337V} and C9orf72 MNs following memantine and expression of calcium-binding proteins in TDP-43 MNs (related to Figs 3 and 4). A) Ca^{2+} traces in MNs stimulated with 10 μM memantine and 100 μM glutamate show significantly higher Ca^{2+} influx in TDP^{M337V} compared to healthy controls ($***p < 0.001$, ANOVA with Sidak's post hoc); $n > 20$ neurons per iPS clone analysed from 2 independent differentiations; B) Immunoblotting for AMPA and NMDA receptors in TDP^{M337V} iPS clones; C) Immunoblotting for calbindin, calreticulin, calpain-1 and calnexin shows no significant differences between TDP-43^{M337V}, TDP-43^{I383T} and healthy controls. Data are shown as mean \pm SEM with One-Way ANOVA tests and Dunnett's *post hoc* tests. $N = 3-5$ independent differentiations for all iPS clones.

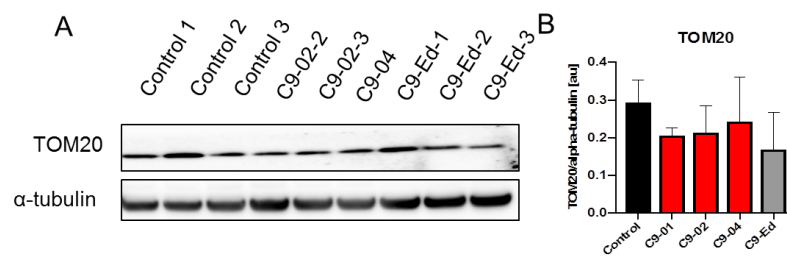


Figure S5 (related to Fig 5). Immunoblotting for the mitochondrial marker TOMM20 shows no differences between lines.

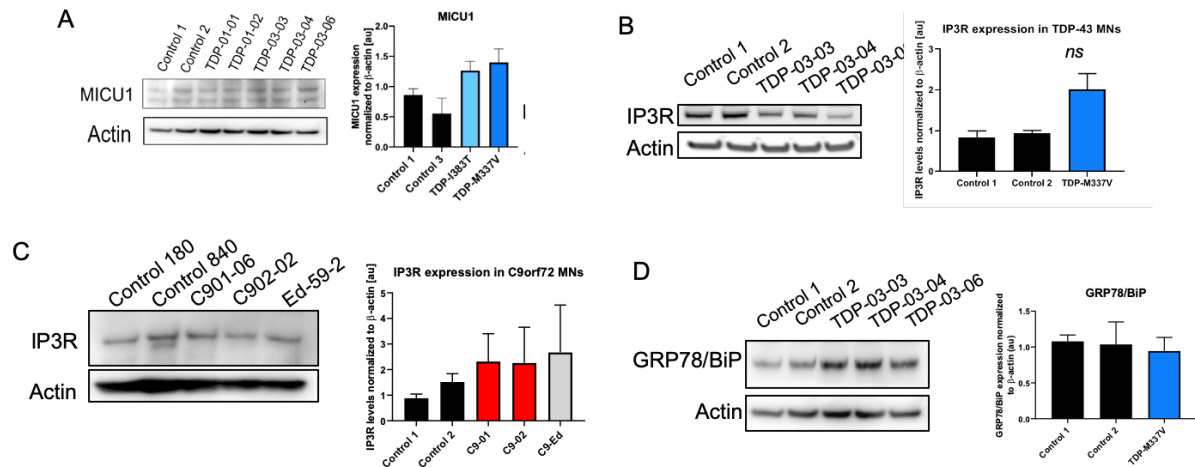


Figure S6. Immunoblotting for mitochondrial MICU1 and Ca^{2+} channels in the ER (related to Fig 6 and 7). A) Immunoblotting for MICU1 in TDP-43^{M337V} and TDP-43^{I383T}; B) IP3R in TDP-43 and C) C9orf72 MNs shows no significant difference between patient lines and healthy controls. C) Immunoblotting for GRP78/BiP in TDP-43 MNs shows no significant differences between patient lines and healthy controls. Data are shown as mean \pm SEM.

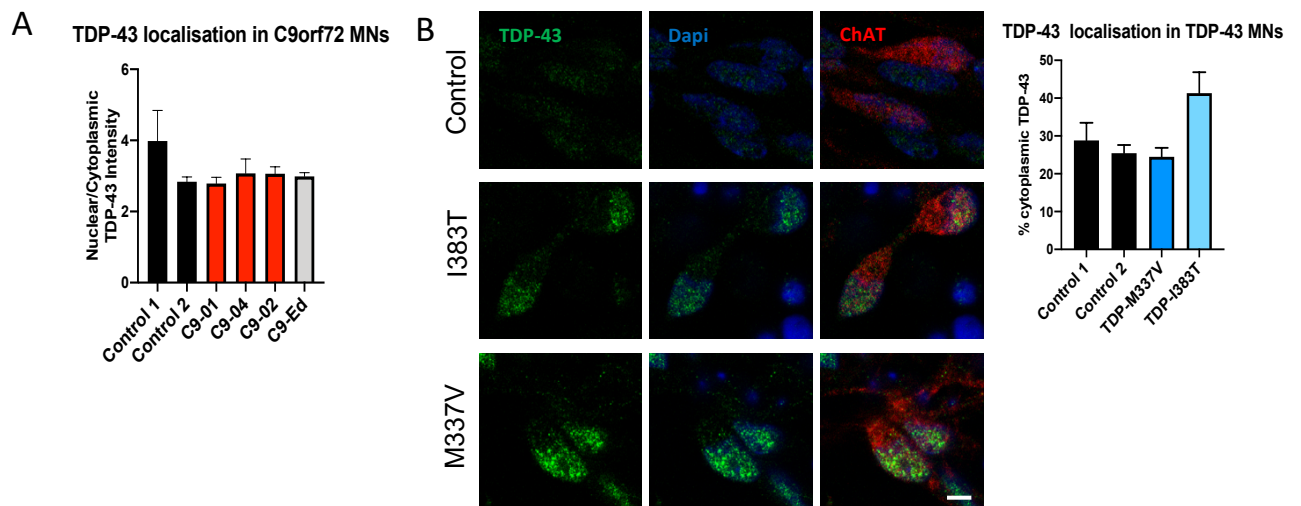


Figure S7. TDP-43 mislocalisation is not observed in C9orf72 or TDP-43 iPS-derived MNs (related to Fig 7). A) Quantification from immunostaining for TDP-43 shows no differences between nuclear/cytoplasmic distribution of TDP-43 in C9orf72 patient lines, healthy controls, C9-Ed (n>30 MNs per differentiation from up to 4 independent differentiations) or B) TDP-43 and healthy controls (n>20 MNs per differentiation from 2 independent differentiations). Data are shown as mean±SEM.

Table S1: Primer sequences used for qPCR.

Target	Primers used	Sequence
GAPDH	GAPDH_fwd	TGCACCACCACCTGCTTAGC
	GAPDH_rev	GGCATGGACTGTGGTCATGAG
ATP5B	ATP5B-2_F	GCATTTGGGTGAGAGCACAG
	ATP5B-2_R	TGGTGCACCAGAATCCAGTAC
TOP1	TOP1_F	GTCCAAGCATAGCAACAGTGAAC
	TOP1_R	GCTCGAACCTTTTCCTCTTTTGG
GRIA1	L_GRIA1_1_F	AGGTGCCAATTTCCCAACA
	L_GRIA1_1_R	GGTCATCTCAAAGCTGTTCGC
GRIA2	GRIA2-1_24.1_F	AGCTTCGCAGTCACTAATGC
	GRIA2-1_24.1_R	ATGAAGGAGACGTGGAGTGTTC
GRIA3	GRIA3-2_F	GGAAGTGCTGGAGACTGCTT
	GRIA3-2_R	CGAGAGCCACTGACTTTCATT
GRIA4	L_GRIA4_1_F	TCTGGATTTTGGGGACTCGC
	L_GRIA4_1_R	TAAAAGGAGCTTCCGACGCA
GRIN1	L_GRIN1_1_F	TCGAGATTGCCTACAAGCGG
	L_GRIN1_1_R	TCTTGAAGCTGGAAGCCAGG
GRIN2A	GRIN2A-2_24.1_F	CCCTTCAGAAAGGCTCTCCT
	GRIN2A-2_24.1_R	GCCATGTTGTCAATGTCCAG
GRIN2B	L_GRIN2B_2_F	CATCTCAACCCACACCGTCA
	L_GRIN2B_2_R	TCCCTGAAGTAGCGCTTGTG
GRM2	L_GRM2-1_F	CCGCGATGCAAGTATGTTGG
	L_GRM2-1_R	CCAGATGATGCAGGTGGTGT
GRM3	GRM3-1_24.1	CTTCTCATTGCAGGGGTCATTG
	GRM3-1_24.1	GCGACTTATCACTGAGTTTGGC
GRM4	L_GRM4-1_F	TGTGTGTGGTTTGTGGTGGA
	L_GRM4-1_R	TCATTGAAGGTCTCGGGCAC
MICU1	L_MICU1_1_F	GCATCATTCGCTCCCAAACC
	L_MICU1_1_R	AGGGTCATGGCGTTCAAAC
MICU2	MICU2_1_F	CGGGATGGCAGTTTTACAGTC
	MICU2_1_R	TGCTTACGAAGAGACGGTTTCC

Table S2: Demographics of healthy controls and patients used in this study.

Patient/control	iPS lines	Mutation	Age	Gender
SFC 180 (control 1)	01-01	-	60	Female
SFC 840 (control 2)	03-03	-	67	Female
SFC 841 (control 3)	03-01	-	36	Male
C901	06 and 07	C9orf72 (~970 G ₄ C ₂ repeats)	72	Male
C902	02, 03 and 10	C9orf72 (~1000 G ₄ C ₂ repeats)	58	Female
C904	01, 09, 12	C9orf72 (>500 G ₄ C ₂ repeats)	39	Male
TDP-03	03, 04 and 06	M337V in TARDBP	57	Male
TDP-01	01 and 02	I383T in TARDBP	60	Male
C9-Ed	59-1, 59-2, 59-3	CRISPR/Cas9 isogenic control of C902	-	-