

Supplementary Information

Human iPSC-derived motor neurons with TDP-43 nuclear loss present early ALS pathological phenotypes

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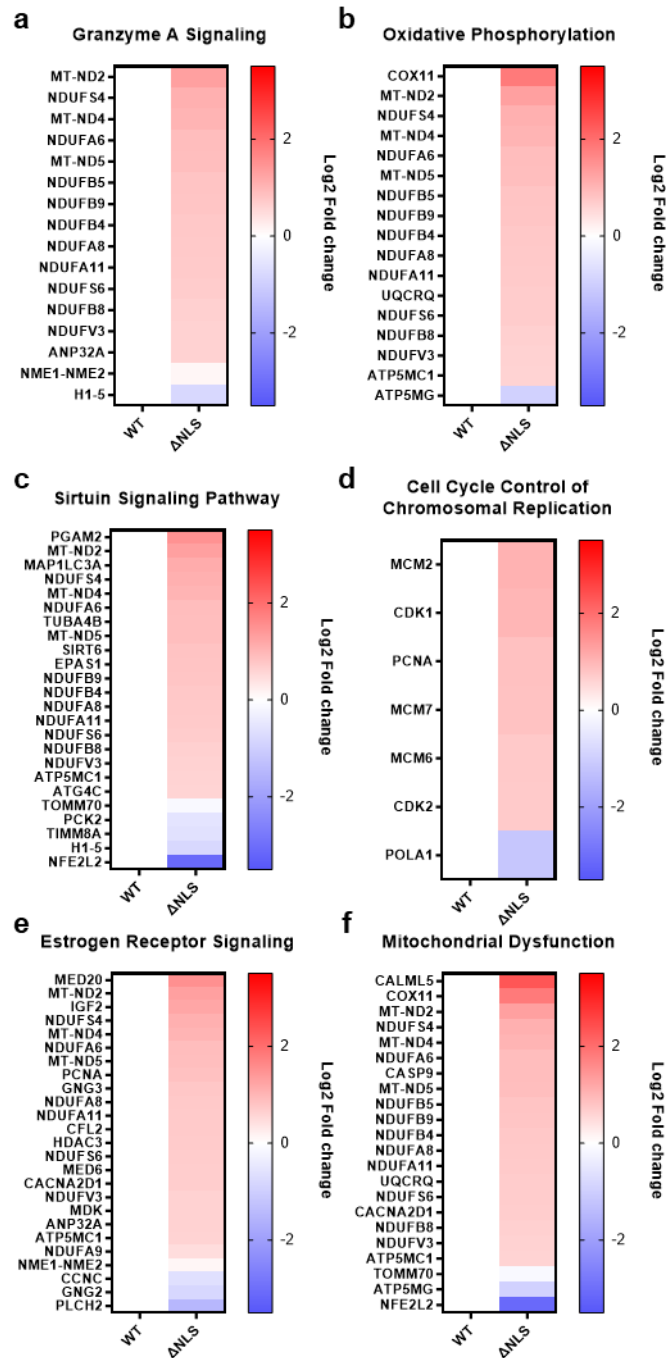
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MSD International GmbH (Singapore Branch)

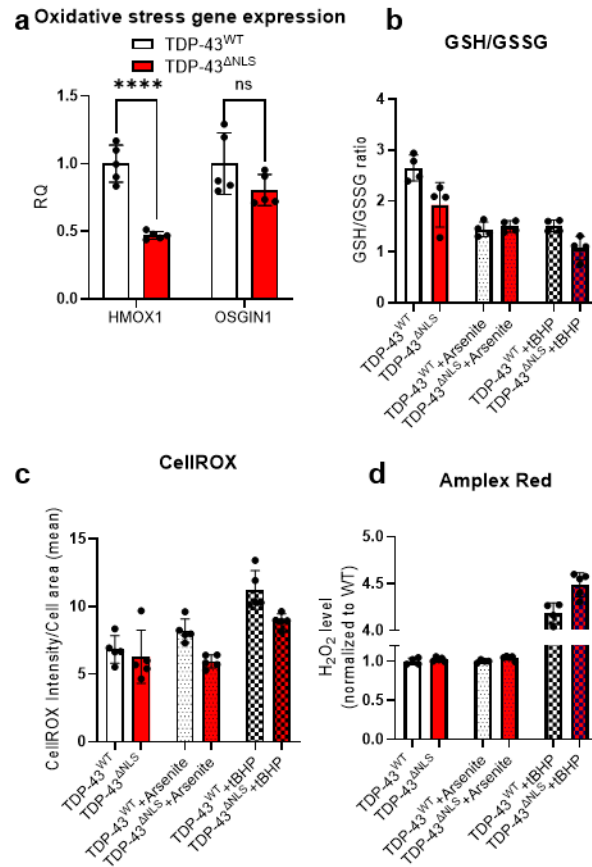
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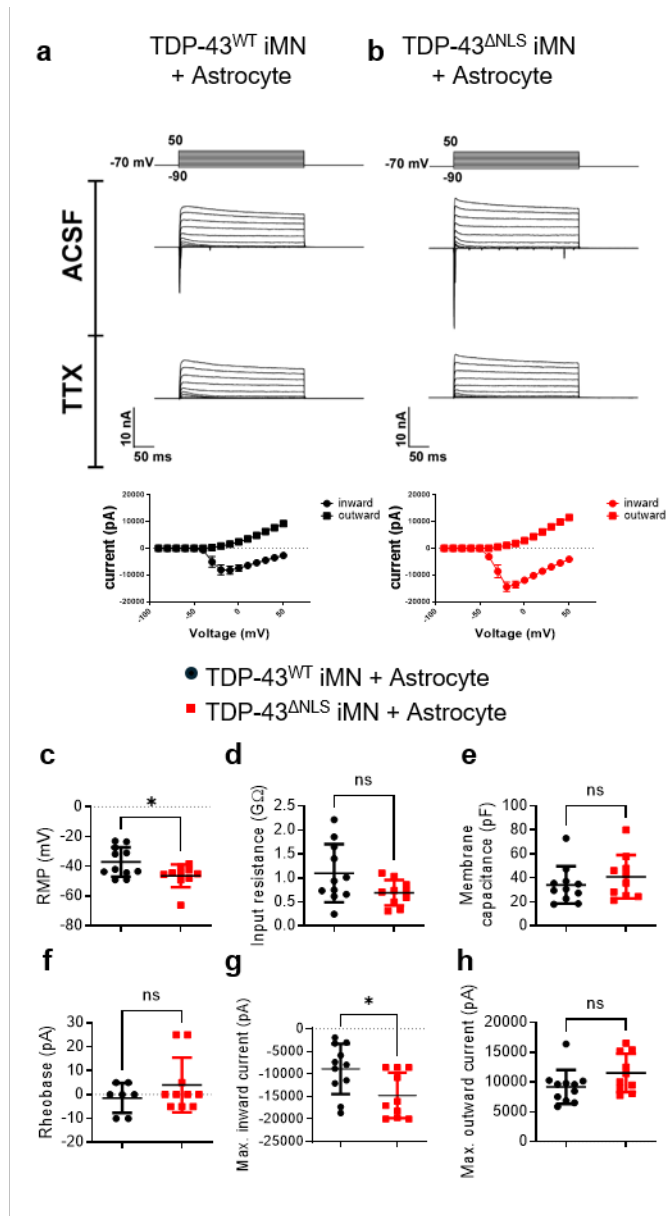
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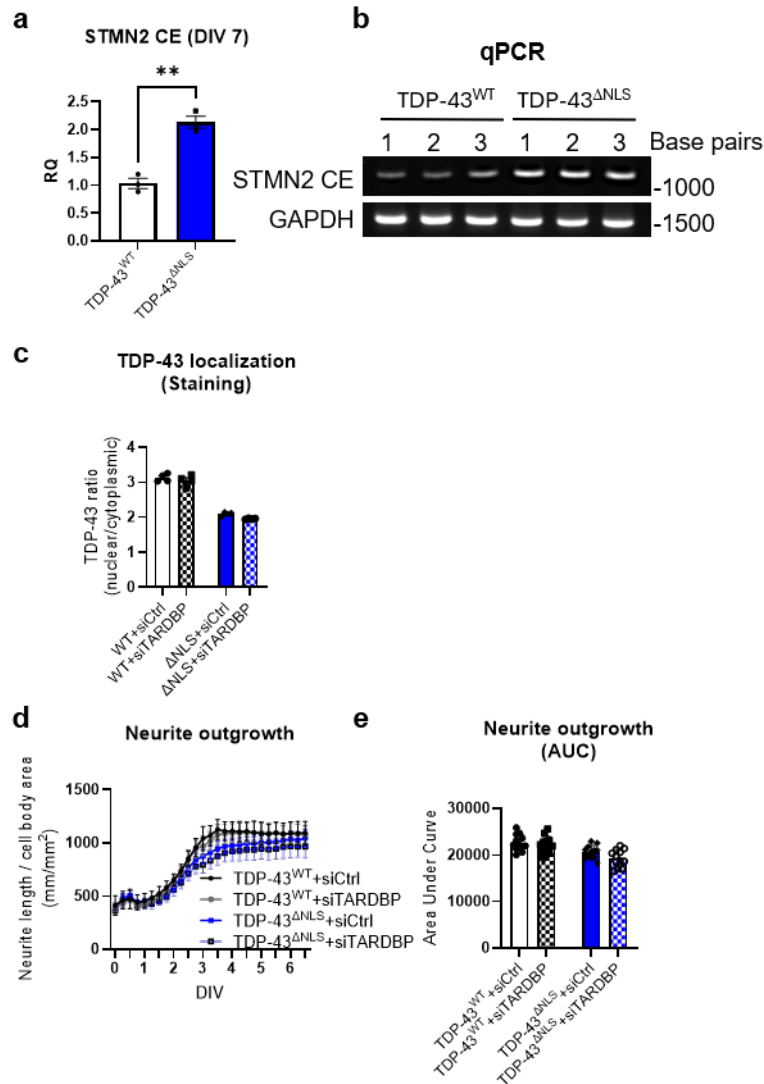
Supplementary Figure 1. Heatmap displaying the protein levels associated with the top 6 dysregulated pathways in TDP-43^{ANLS} iMNs compared to TDP-43^{WT} iMNs. a-f Proteins involved in Granzyme A signaling, oxidative phosphorylation, sirtuin signaling pathway, cell cycle control of chromosomal replication, estrogen receptor signaling and mitochondrial dysfunction. All proteins were normalized to protein levels from TDP-43^{WT} iMNs and presented as log2-fold changes.



Supplementary Figure 2. Antioxidant gene expression and oxidative stress markers in TDP-43^{ΔNLS} iMNs compared to TDP-43^{WT} iMNs. **a** RT-qPCR quantification of *HMOX1* and *OSGIN1* gene expression (N=3, n=4). **b** Quantification of GSH/GSSG ratio in response to sodium arsenite and tBHP stressor treatment (N=2, n=4). **c** CellROX to quantify cellular ROS level. **d** Amplex Red to quantify H₂O₂ production (N=2, n=4). Bar graphs show mean ± SD with individual data points representing independent replicate. For RT-qPCR, each data point is average of 4 technical replicates. For immunostaining, each data point is the average of at least 25 fields per well. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001 indicate significant difference from respective control. One-way ANOVA with Tukey's post-hoc test (**a-d**). N = number of independent experiments; n = number of wells.



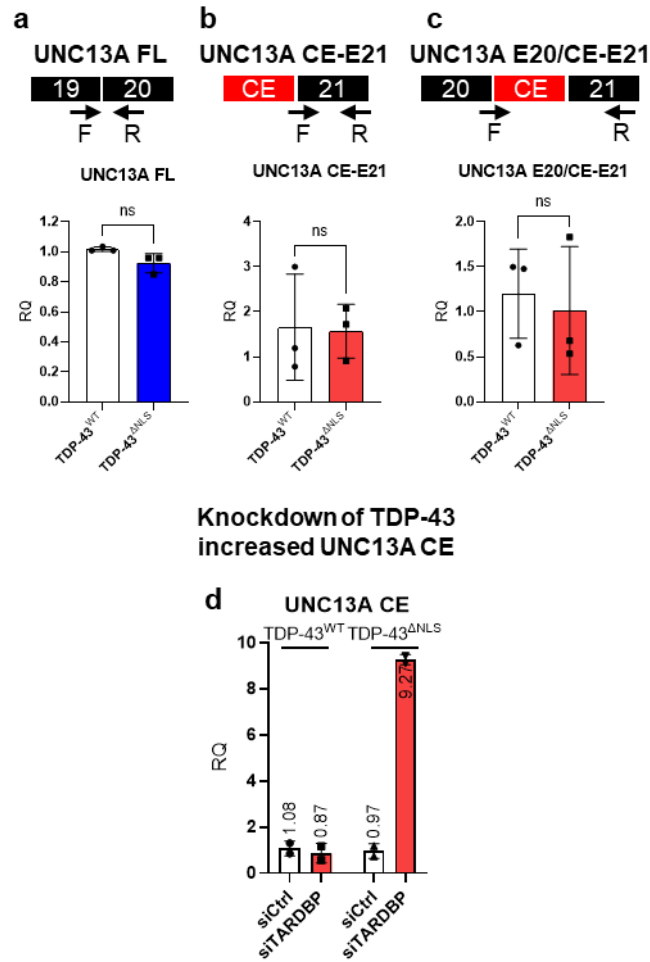
Supplementary Figure 3. Other parameters of electrophysiology analysis. **a-b** Recording of voltage-dependent membrane current in iMN. Top, exemplar traces of a TDP-43^{WT} iMN or TDP-43^{ΔNLS} iMN co-cultured with WT iAstrocyte showing the voltage-sensitive current evoked from holding potential of -70 mV to different test potential ranging from -90 mV to 50 mV with 10 mV increments first with normal ACSF solution then with TTX. Bottom. Current-voltage profile of the inward and outward current (N=3, n=5-10). **c** Resting membrane potential (RMP) defined as the membrane potential measured with 0 pA current injection. **d** Input resistance calculated in response to a -20 pA current injection. **e** Membrane capacitance measured from the RC waveform produced by a -20 pA current injection. **f** Rheobase, defined as the minimal current injection necessary to evoke an action potential. **g,h** The maximal outward and inward current was derived from Supp Fig. 3a and b. Scatter dot plots show mean $\pm$ SD with individual data points representing independent replicate. * $P < 0.05$ indicates significant difference from respective control. Unpaired t-test (**c-h**). N = number of independent experiments; n = number of cells.



Supplementary Figure 4. STMN2 CE expression on DIV 7 and effects of TDP-43 KD on different readouts. a, b STMN2 CE expression on DIV 7 quantified using RT-qPCR (N=3, n=3) and qPCR (N=2). **c** Effect of *TARDBP* knockdown (KD) on TDP-43 localization (N=2, n=4). **d** Neurite outgrowth quantified using live cells imaging software Incucyte, images taken every 6 hours for 7 days after *TARDBP* KD (N=2, n=6-12). **e** Area under curve (AUC) of neurite outgrowth curve from Supp Fig. 4d. Bar graphs or scatter dot plots show mean $\pm$ SD with individual data points representing independent replicate. For RT-qPCR, each data point is averaged of 4 technical replicates. For Incucyte, each data point is averaged of 9 fields per well. For immunostaining, each data point is the average of at least 25 fields per well. ** $P < 0.01$ indicates significant difference from respective control. Unpaired t-test (a). N = number of independent experiments; n = number of wells.

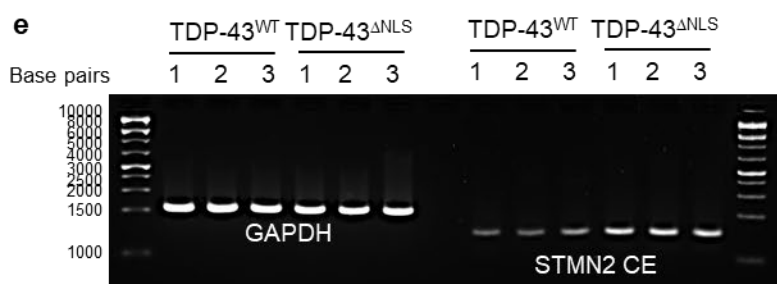
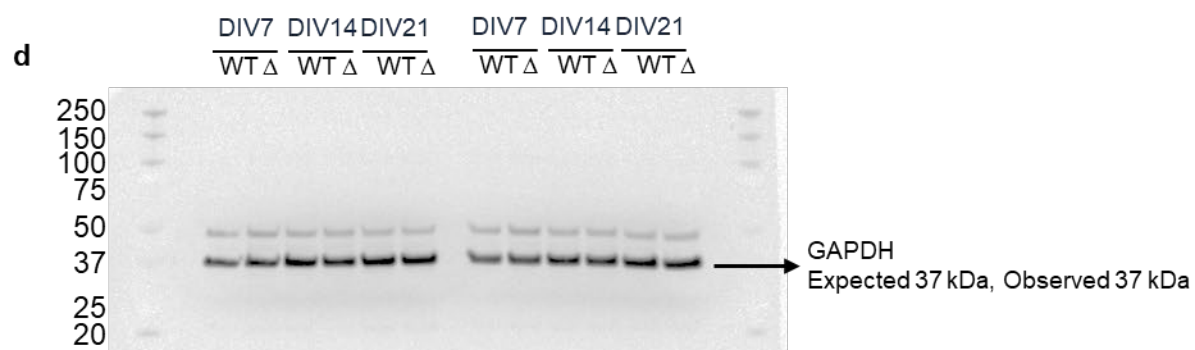
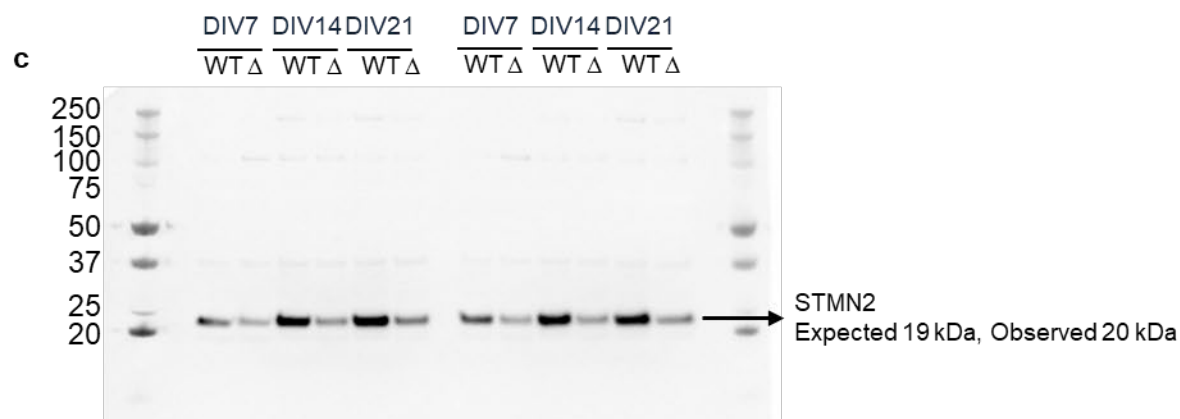
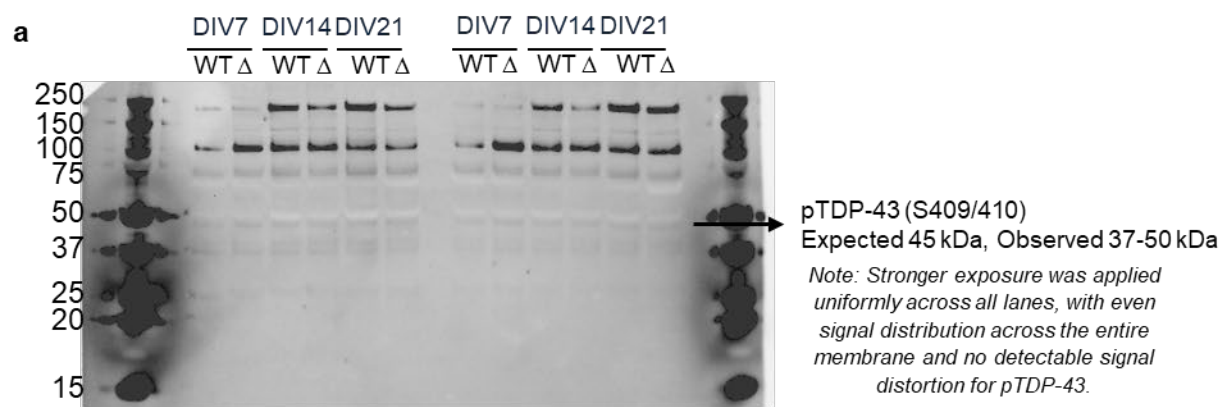
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ASO10 (23 nt)	GCACACATGCTCACACAGAGAGC

[illegible]



Knockdown of TDP-43
increased UNC13A CE

Supplementary Figure 6. Splicing of UNC13A in TDP-43^{ΔNLS} iMNs. **a** RT-qPCR to quantify the gene expression of *UNC13A* full-length (FL) expression (N=3, n=3), or **b-c** *UNC13A* cryptic exon (CE) expression in TDP-43^{WT} and of TDP-43^{ΔNLS} iMNs (N=3, n=3), primers targeting *UNC13A* CE-Exon 21 junction and primers targeting *UNC13A* Exon20-CE junction and Exon 21. **d** RT-qPCR to quantify the *UNC13A* cryptic exon (CE) expression in TDP-43^{ΔNLS} after *TARDBP* KD using siRNA (N=2, n=2). Bar graphs show mean $\pm$ SD with individual data points representing independent replicate. For RT-qPCR, each data point is averaged of 4 technical replicates. Unpaired t-test (**a-c**). N = number of independent experiments; n = number of wells.



Supplementary Figure 7. Uncropped immunoblot and DNA gel images. a-b Immunoblots from Figure 2g. **c-d** Immunoblots from Figure 6b. **e** Agarose gel images from Supplementary Figure 4b.

Supplementary Table 1. Antibodies used for IHC staining

Antibody	Dilution	Catalogue Number
GFAP	1:500	Cell Signaling Technology, 3670
ChAT	1:500	Synaptic systems, 297015
HB9/MNX1	1:500	Novus Biologicals, NBP2-24691
Islet-1	1:200	R&D System, AF1837
TDP-43	1:400	Proteintech, 10782-2-AP
pTDP-43 (Ser409/410)	1:400	Proteintech, 22309-1-AP
G3BP1	1:500	Abcam, ab56474
Tubulin	1:500	Abcam, ab6161
Neurofilament	1:500	Millipore, MAB5262
PGP9.5	1:500	Genetex, GTX109637
STMN2	1:1000	Novus Biologicals, nbp1-49461
ALDH1L1	1:1000	Abcam, ab190298
Synaptophysin	1:1000	Abcam, ab8049
NeuN	1:100	Cell Signaling Technology, 24307S
vGlut1	1:500	Millipore, AB5905
Tyrosine Hydrolase	1:500	Abcam, ab137869
GAD67	1:100	Millipore, MAB5406
S100 β	1:300	Abcam, ab52642

Supplementary Table 2. Antibodies used for Western blot

Antibody	Dilution	Catalogue Number
TDP-43	1:2000	Proteintech, 10782-2-AP
TDP-43 [3H8]	1:2000	Abcam Ab104223
pTDP-43 (Ser409/410)	1:1000	Proteintech, 22309-1-AP
STMN2	1:2500	Proteintech, 10586-1-AP
GAPDH	1:1000	Santa Cruz, sc-47724
Goat anti-Rabbit IgG (H+L) HRP	1:10 000	Invitrogen, 31460
Goat anti-Mouse IgG (H+L) HRP	1:10 000	Invitrogen, 31430

Supplementary Table 3. Primer sequences used for DNA sequencing and qPCR and RT-PCR analysis

Primer name	FWD / REV	Sequence	Usage
TDP-43 NLS Set 1	FWD	GGGAGAGATTGTAATCCGAAA	DNA sequencing
TARDBP	FWD	TCATCCCCAAGCCATTGAGG	RT-qPCR
TARDBP	REV	TGCTTAGGTTTCGGCATTGGA	
GAPDH (1)	FWD	GTTTCGACAGTCAGCCGCATC	
GAPDH (1)	REV	GGAATTTGCCATGGGTGGA	
GAPDH (2)	FWD	GAAGGTGAAGGTCGGAGTC	
GAPDH (2)	REV	GAAGATGGTGATGGGATTTC	
STMN2 Full Length	FWD	AGCTGTCCATGCTGTCACTG	
STMN2 Full Length	REV	GGTGGCTTCAAGATCAGCTC	
STMN2 Cryptic Exon	FWD	GGACTCGGCAGAAGACCTTC	
STMN2 Cryptic Exon	REV	GCAGGCTGTCTGTCTCTCTC	
UNC13A Full Length	FWD	GGACGTGTGGTACAACCTGG	
UNC13A Full Length	REV	GTGTACTGGACATGGTACGGG	
UNC13A CE-E21	FWD	CAAGCGAACTGACAAATCTGCCGTGTCG	
UNC13A CE-E21	REV	GGCATCGTCACCCTTGGCATCTGG	
UNC13A E20/CE-21	FWD	ACATCAGTGTGGAGATCAAAGG	
UNC13A E20/CE-21	REV	GAACAGGTTCCATCTCTCCATC	
STMN2 exon 1	FWD	GACTCAGTGCCTTATTCAGT	qPCR
STMN2 exon 2	REV	GTTGATGTTGCGAGGTTCCG	qPCR
STMN2 exon 2a	REV	TTTCTCTCGAAGGTCTTCTG	qPCR

Supplementary Table 4. TaqMan Probe for qPCR analysis

Material	Assay ID	Catalog #	Dye
Human NQO1 TaqMan probe	Hs01045993_g1	#4331182	FAM-MGB
Human TBP TaqMan probe (Control)	Hs00427620_m1		
Human GAPDH TaqMan probe (Control)	Hs02786624_g1		

Supplementary Table 5. Isotope-labeled metabolite standards for targeted metabolomic

Isotope-labeled metabolite standards	Catalogue Number
L-glutamic acid d3	Sigma Aldrich 49449
L-serine ¹⁵ N	Sigma Aldrich 609005
Acetylcholine-d9 chloride	Sigma Aldrich 771767
Dopamine d4	Sigma Aldrich 655651
4-Aminobutyric acid-2- ¹³ C	Sigma Aldrich 905526
L-Arginine- ¹³ C ₆ ¹⁵ N ₄ hydrochloride	Sigma Aldrich 608033

Supplementary Table 6. siRNA for target knockdown

Material	Manufacturer	Catalog ID
Accell Non-targeting Control Pool	Dharmacon	D-001910-10-50
Accell Human TARDBP siRNA SMARTPool	Dharmacon	E-012394-00-0050
Accell Human STMN2 siRNA SMARTPool	Dharmacon	E-019342-00-0050
Accell Human UNC13A siRNA SMARTPool	Dharmacon	E-031249-00-0050