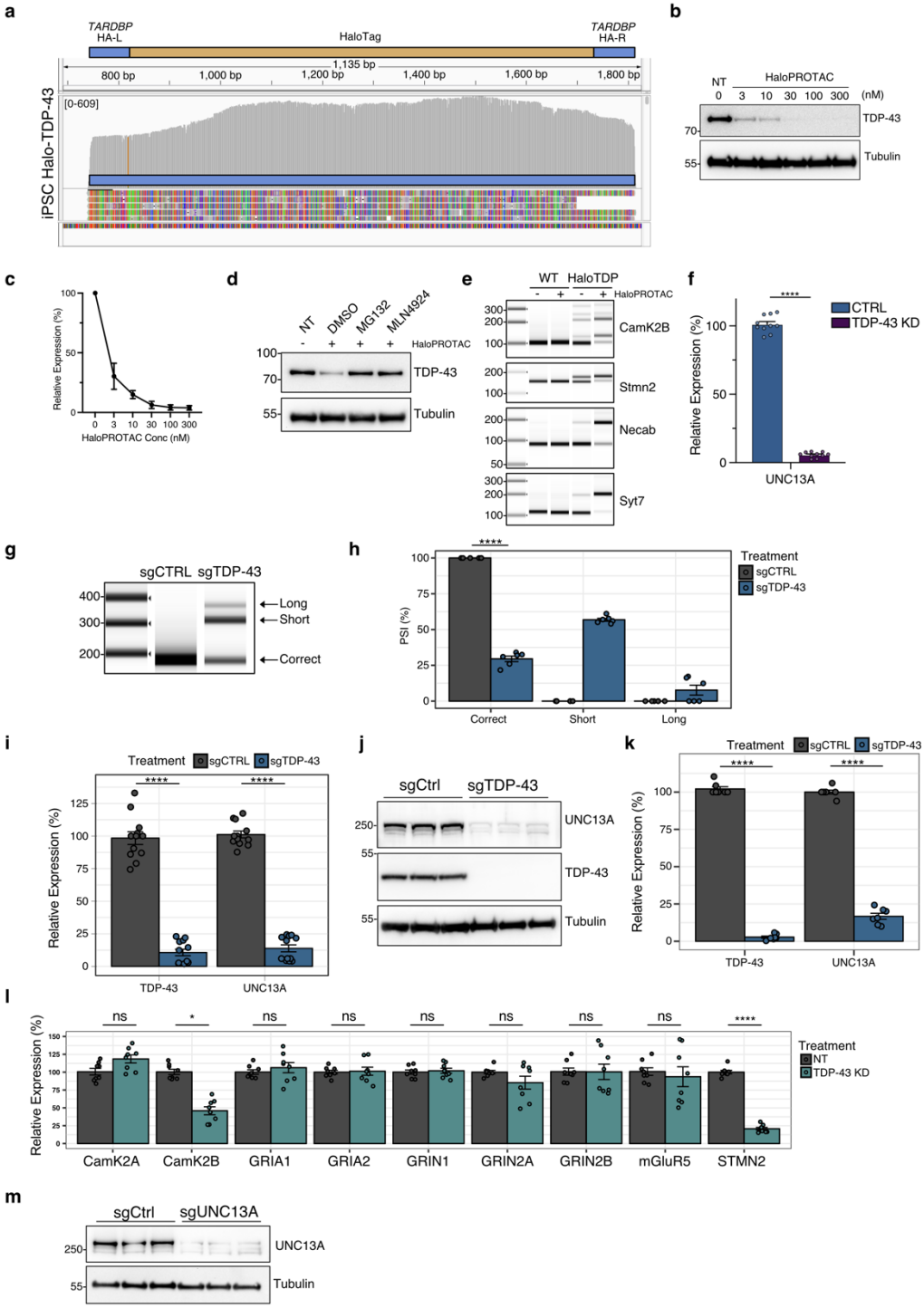
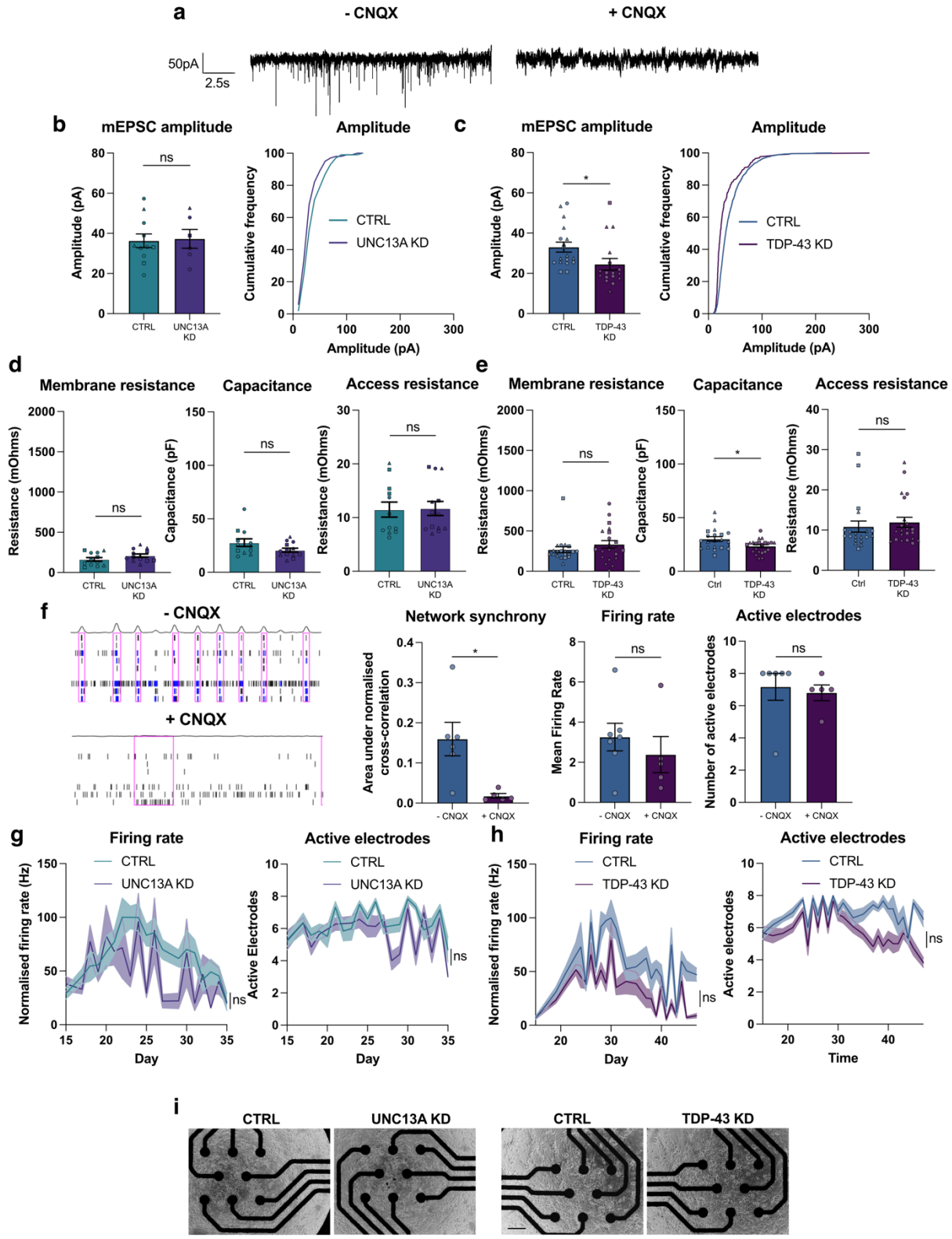
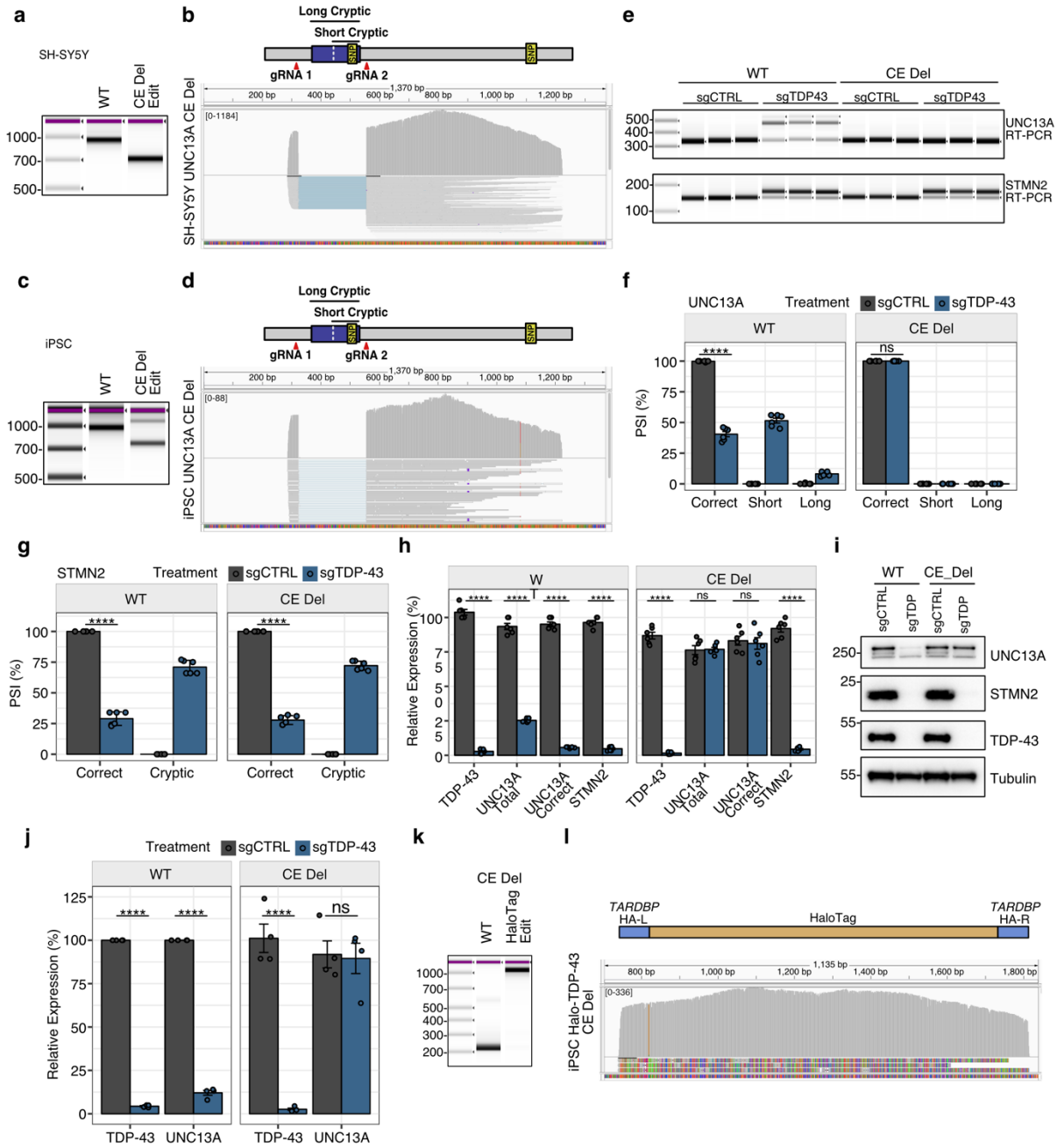
Extended Data Figures and Table



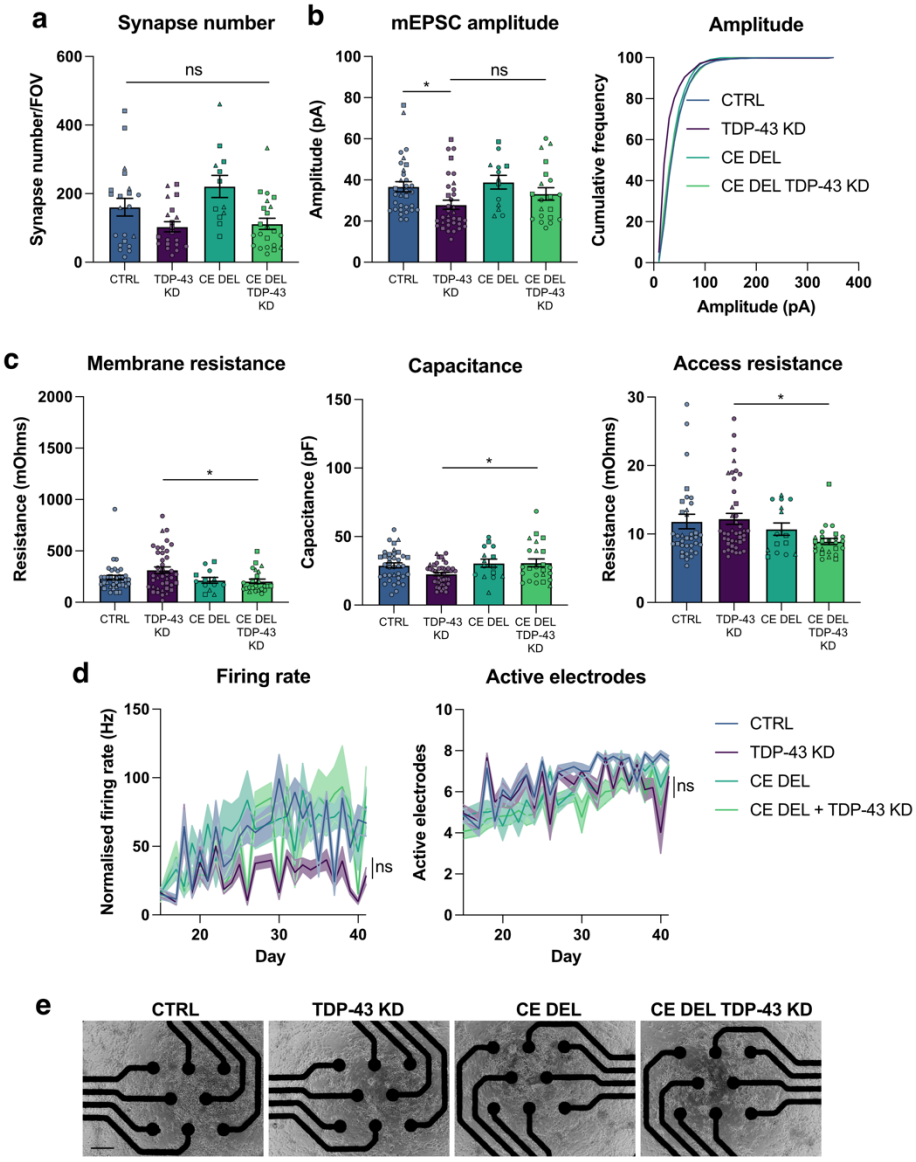
Extended Data Figure 1 | CRISPRi mediated knockdown of TDP-43 in iNeurons. a, Amplicon sequencing of PCR products from Fig. 1A indicates correct HaloTag editing of *TARDBP*. **b**, HaloTDP iNeurons were treated with the indicated concentration of HaloProtac for 4 days and processed for western blot analysis indicating a dose response curve of HaloProtac on TDP-43 knockdown. **c**, Quantification of western blots in (**b**) *n*=3 biological replicates. **d**, HaloTDP iNeurons were treated with 30 nM HaloProtac for 3 hours in the presence of DMSO, proteasome inhibitor MG132, or NEDD8 E1 inhibitor MLN4924. **e**, RT-PCR analysis of WT and Halo-iNeurons shows the Halo tag induces a mild loss of TDP-43 function and can induce cryptic exon inclusion in the indicated transcripts. **f**, RT-qPCR analysis of correctly spliced *UNC13A* at exon 20-21 junction. *n*=10 biological replicates from 3 experiments. **g-k**, Analysis of control and CRISPRi-mediated TDP-43 knockdown in DIV 28 iNeurons. (**g**) RT-PCR analysis of *UNC13A* splicing at exon junction 20-21 shows the inclusion of a long and short cryptic exon after TDP-43 KD. (**h**) Quantification of results in (**g**) *n*=6 from 3 experiments. (**i**) RT-qPCR analysis shows a reduction of correctly spliced *UNC13A* at exon 20-21 junction following TDP-43 KD. (**j**) Western blot analysis indicates a dramatic decrease in UNC13A protein after TDP-43 KD. (**k**) Quantification of results in (**j**) *n*=7 from 3 experiments. **l**, RT-qPCR analysis of DIV 38 HaloTDP iNeuron cultures indicates similar expression of glutamate receptors. A cryptic exon in *CamK2B* and cryptic polyadenylation in *STMN2* results in reduction of mRNA transcripts. *n*=8 from 2 experiments. **m**, Western blot analysis of control and CRISPRi-mediated UNC13A knockdown in iNeurons. Graphs in (**f**) (**h**) (**i**) (**k**) and (**l**) represent mean ± s.e.m. Statistics are two-sided Student's *t* test with additional Bonferroni adjustment for multiple comparisons in (**l**) **P* < 0.05; ** *P* <0.01; *** *P* <0.001; **** *P* < 0.0001; ns (not significant).



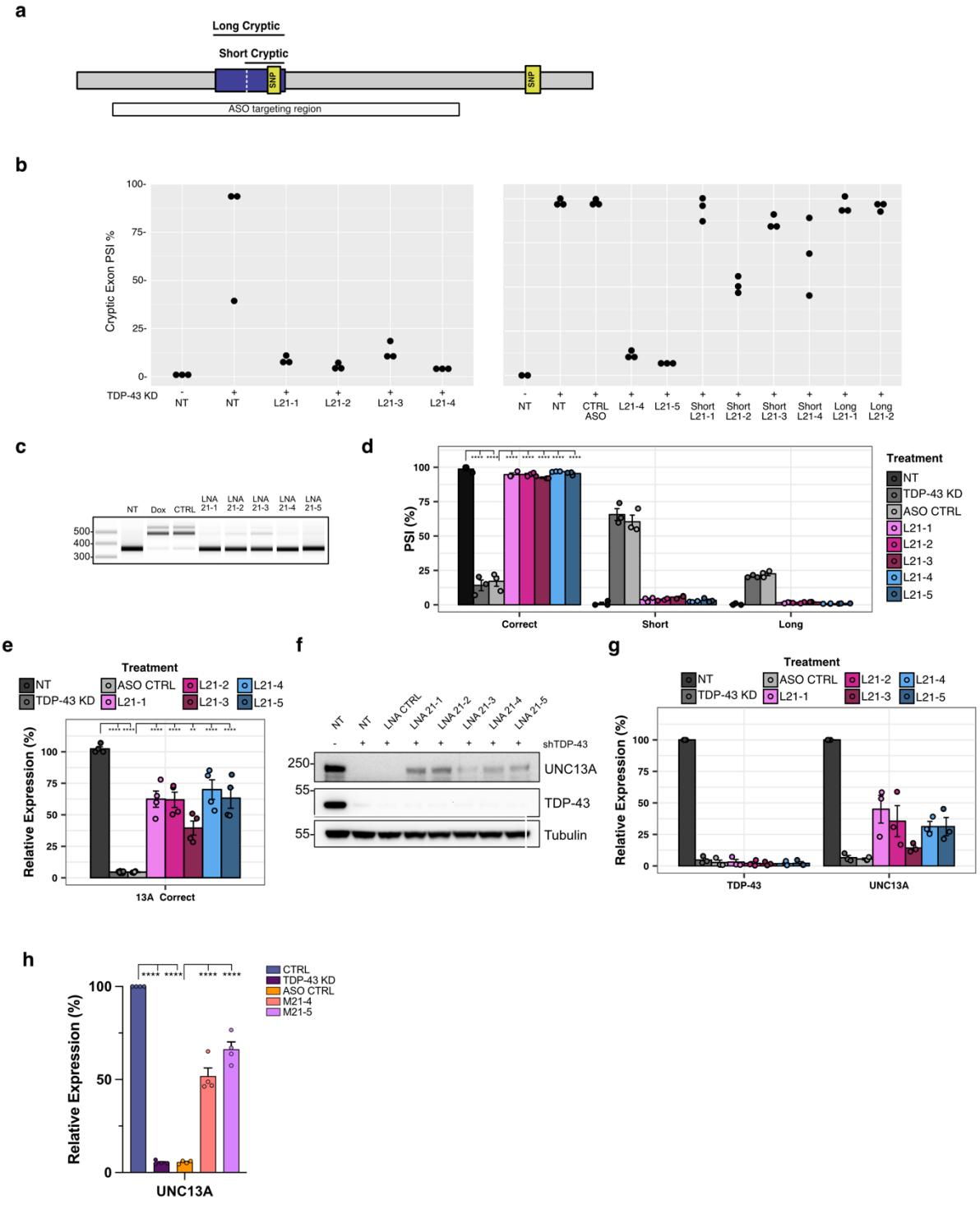
Extended Data Figure 2 | Additional electrophysiological parameters following UNC13A and TDP-43 knockdown. a, AMPAR blockade using CNQX abolishes mEPSC activity. **b**, mEPSC amplitude from control (n=12) and UNC13A depleted (n=13) iNeurons pooled from 3 experiments. **c**, mEPSC amplitude from control (n=20) and TDP-43 knockdown (n=22) HaloTDP iNeurons from 3 experiments. **d**, Quantification of passive membrane properties for UNC13A knockdown iNeurons. **e**, Quantification of passive membrane properties for TDP-43 knockdown iNeurons. **f**, AMPAR blockade using CNQX abolishes network synchrony on multielectrode array recordings, with minimal effects on firing rate and number of active electrodes. **g**, Quantification of firing rate and number of active electrodes following UNC13A knockdown. **h**, Quantification of firing rate and number of active electrodes following TDP-43 knockdown. **i**, Phase contrast images showing cell coverage on multielectrode array plates. Scale bar = 350 µm. Graphs for (**b**) (**c**) (**d**) (**e**) (**f**) represent mean ± s.e.m. Statistics for (**b**) (**c**) (**d**) and (**f**) are two-sided Student's t tests. Statistics for (**g**) and (**h**) are paired t tests. *$P < 0.05$; ns (not significant).



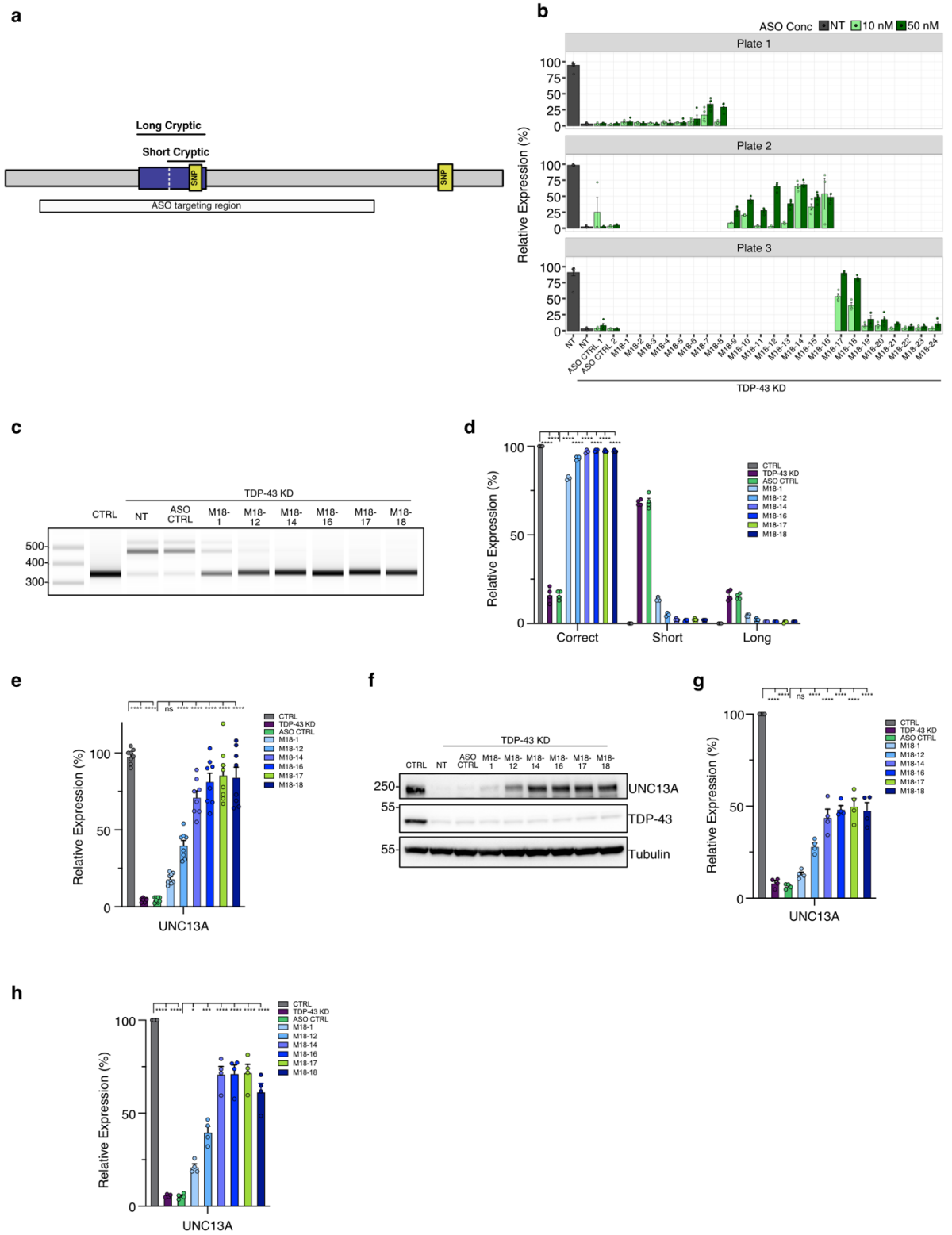
Extended Data Figure 3 | *UNC13A* cryptic exon deletion rescues *UNC13A* splicing/expression following TDP-43 knockdown. a, PCR from genomic DNA of WT SH-SY5Y cells and CE Del SH-SY5Y cells indicated successful deletion of the genomic region of *UNC13A* containing the sequence corresponding to the exon 20-21 cryptic exon. **b**, Amplicon sequencing of PCR products in (**a**). **c**, PCR from genomic DNA of WT iPSCs and CE Del iPSCs indicated successful deletion of the genomic region of *UNC13A* containing the sequence corresponding to the exon 20-21 cryptic exon. **d**, Amplicon sequencing of PCR products in (**c**) **e-j**, Analysis of WT and CE Del iNeurons following CRISPRi knockdown with CTRL or TDP-43 sgRNAs. (**e**) RT-PCR analysis shows *UNC13A* cryptic deletion rescues *UNC13A* exon 20-21 splicing following TDP-43 knockdown, but not cryptic polyadenylation of *STMN2* (**f**). Quantification of *UNC13A* splicing in figure (**e**) n=6 biological replicates from 2 experiments. (**g**) Quantification of *STMN2* splicing in figure (**e**) n=6 biological replicates from 2 experiments. (**h**) RT-qPCR analysis shows *UNC13A* CE Del prevents the loss of *UNC13A* transcripts but not *STMN2* transcripts in iNeurons after TDP-43 knockdown. *UNC13A* correct RT-qPCR assay detects correct splicing at *UNC13A* exon 20-21 junction. n = 6 biological replicates from 2 experiments. (**i**) Western blot analysis shows *UNC13A* CE Del rescues the loss of UNC13A protein following TDP-43 knockdown. (**j**) Quantification of western blots in (**i**) n=4 biological replicates from 2 experiments. **k**, PCR of genomic DNA of *UNC13A* CE Del iPSCs with primers flanking exon 1 of *TARDBP* indicates successful edit of *TARDBP* with HaloTag. **l**, Amplicon sequencing of HaloTag PCR product in (**k**). Graphs for (**f**) (**g**) (**h**) and (**j**) represent mean ± s.e.m. Statistics are One-way ANOVA with Tukey multiple comparison test. *P < 0.05; ** P <0.01; *** P <0.001; **** P < 0.0001; ns (not significant).



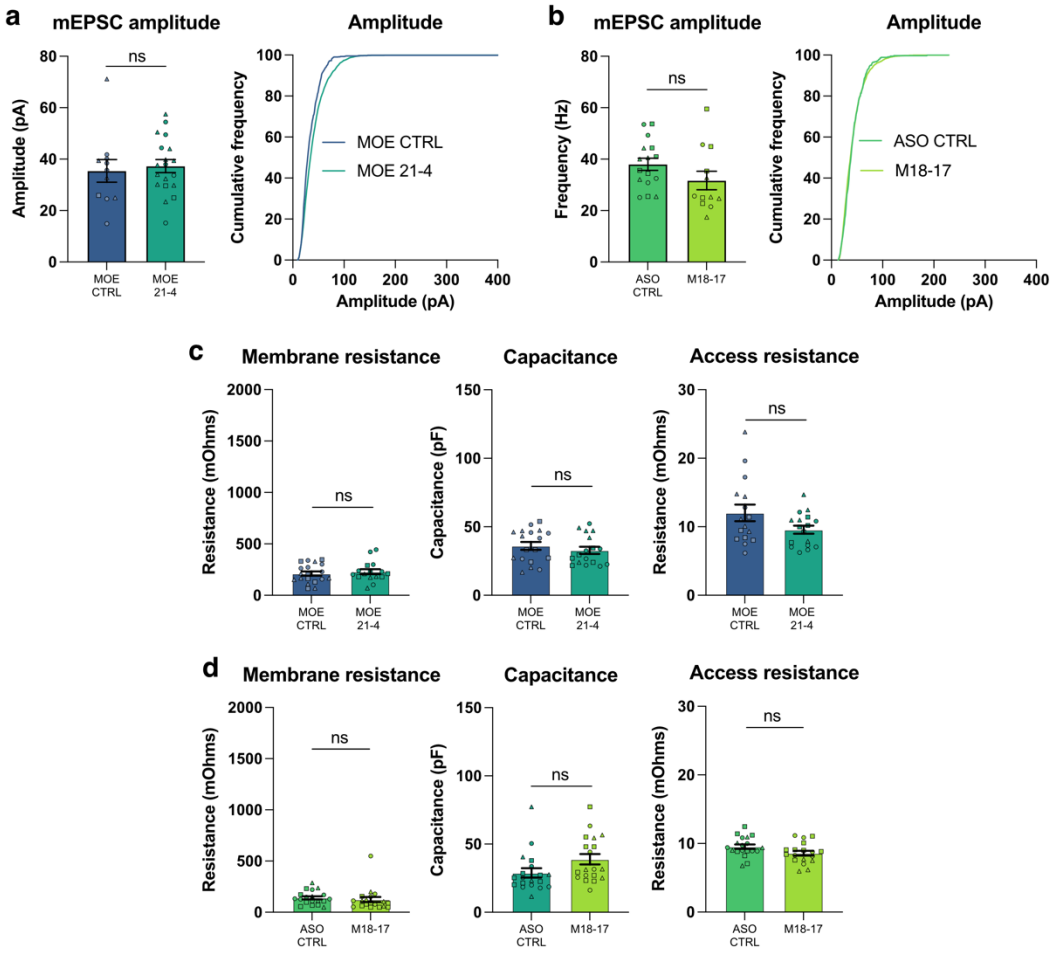
Extended Data Figure 4 | Additional synaptic and electrophysiological parameters following genomic deletion of *UNC13A* CE. a, Immunofluorescence quantification of synapse number and size based on synapsin labelling in control n=21, TDP-43 KD n= 20, CE Del n=12 and CE Del TDP-43 KD n=22 fields of view from 3 experiments. **b,** mEPSC amplitude from control n=17, TDP-43 knockdown n=22, CE Del n=15, CE Del TDP-43 knockdown n=28 iNeurons pooled from 3 experiments. **c,** Quantification of passive membrane properties. **d,** Quantification of multielectrode array mean firing rates and number of active electrodes for control, TDP-43 KD, CE Del, CE Del TDP-43 KD conditions n=18 wells from 3 experiments. **e,** Phase contrast images showing cell coverage on multielectrode array plates. Scale bar = 350 µm. Graphs for (**a**) (**b**) (**c**) (**d**) represent mean ± s.e.m. Statistics are One-way ANOVA with Dunnet's multiple comparison test. *P < 0.05; ns (not significant).



Extended Data Figure 5 | ASOs targeting the *UNC13A* cryptic exon rescues *UNC13A* splicing/expression following TDP-43 knockdown. **a**, Schematic of *UNC13A* cryptic exon. **b**, Quantification of RT-PCR products following treatment of SK-N-BE(2) cells with indicated ASOs. **c**, RT-PCR analysis of *UNC13A* splicing in SH-SY5Y cells shows ASOs prevent cryptic splicing after TDP-43 knockdown. **d**, Quantification of results in (C) n=3 biological replicates from 2 experiments. **e**, RT-qPCR analysis of *UNC13A* shows an ASO mediated rescue of *UNC13A* RNA after TDP-43 KD in SH-SY5Y cells. n=4 biological replicates from 2 experiments. **f**, Western blot analysis shows rescue of UNC13A protein following ASO treatment after TDP-43 KD in SH-SY5Y cells. **g**, Quantification of blots from (**f**). n=3 biological replicates from 3 experiments. **h,** RT-qPCR analysis of *UNC13A* shows an ASO mediated rescue of *UNC13A* RNA after TDP-43 KD in HaloTDP iNeurons. n=4 biological replicates from 2 experiments. Graphs for (**d**) (**e**) (**g**) and (**h**) represent mean ± s.e.m. Statistics are One-way ANOVA with Tukey multiple comparison test. *P < 0.05; ** P <0.01; *** P <0.001; **** P < 0.0001; ns (not significant).



Extended Data Figure 6 | Improved ASOs targeting the *UNC13A* cryptic exon rescues *UNC13A* splicing/expression following TDP-43 knockdown. **a**, Schematic of *UNC13A* cryptic exon. **b**, Quantification of RT-PCR products following treatment of SH-SY5Y cells with indicated ASOs. **c**, RT-PCR analysis of *UNC13A* splicing in SH-SY5Y cells shows ASOs prevent cryptic splicing after TDP-43 knockdown. **d**, Quantification of results in (**c**) *n*=3 biological replicates from 2 experiments. **e**, RT-qPCR analysis of *UNC13A* shows an ASO mediated rescue of *UNC13A* RNA after TDP-43 KD in SH-SY5Y cells. *n*=4 biological replicates from 2 experiments. **f**, Western blot analysis shows rescue of UNC13A protein following ASO treatment after TDP-43 KD in SH-SY5Y cells. **g**, Quantification of blots from (**f**). *n*=3 biological replicates from 3 experiments. **h**, RT-qPCR analysis of *UNC13A* shows an ASO mediated rescue of *UNC13A* RNA after TDP-43 KD in Halo-iNeurons. *n*=4 biological replicates from 2 experiments. Graphs for (**d**) (**e**) (**g**) and (**h**) represent mean ± s.e.m. Statistics are One-way ANOVA with Tukey multiple comparison test. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns (not significant).



Extended Data Figure 7 | Additional electrophysiological parameters following ASO treatments. a, Quantification of mEPSC amplitude in TDP-43 KD iNeurons treated with MOE CTRL ASO (*n*=17) and MOE 21-4 ASO (*n*=18) from 3 experiments. **b,** Quantification of mEPSC amplitude in TDP-43 KD iNeurons treated with CTRL ASO (*n*=19) and M18-17 ASO (*n*=19) from 3 experiments. **c,** Passive membrane properties for TDP-43 KD iNeurons treated with MOE CTRL ASO (*n*=17) and MOE 21-4 ASO (*n*=18) from 3 experiments. **d,** Passive membrane properties in TDP-43 KD iNeurons treated with CTRL ASO (*n*=19) and M18-17 ASO (*n*=19) from 3 experiments. Graphs for (**a**) (**b**) (**c**) and (**d**) represent mean ± s.e.m. Statistics are two-sided Student's *t* test. ns (not significant).

Extended Data Table 1 | Key Reagents

Reagent	Supplier	Catalog #
XAV939	Cambridge Bioscience	SM38-10
LDN-193189 hydrochloride	Cambridge Bioscience	19396-5mg-CAY
SB431542	Cambridge Bioscience	SM33-50
Recombinant Cas9	IDT	Alt-R® S.p. Cas9 Nuclease V3
Recombinant Cas12	IDT	Alt-R® A.s. Cas12a (Cpf1) Ultra
HDR Enhancer V2	IDT	NA
Trans-IT 293	Mirius	MIR2700
dibutyryl cAMP	Merck Sigma	D0627-100MG
Doxycycline Hyclate	Merck Sigma	D9891
L-Ascorbic Acid	Merck Sigma	A0278-25G
Poly-D-lysine hydrobromide >300,000 MW	Merck Sigma	P7405
Poly-L-ornithine hydrobromide 30,000-70,000 MW	Merck Sigma	P3655
Puromycin	Merck Sigma	P8833
BDNF	PeproTech	450-02
GDNF	PeproTech	450-10
Accutase	ThermoFisher	A1110501
cultureOne	ThermoFisher	A3320201
Geltrex	ThermoFisher	A14133-01
Laminin	ThermoFisher	23017015
RevitaCell	ThermoFisher	A2644501
TrypLE	ThermoFisher	12605010
Versene	ThermoFisher	15040066
ROCK inhibitor (Y-27632)	Tocris	1245/10
Halo-Protac E	University of Dundee DSTT	HALO-PROTAC-E active
Tetrodotoxin	Tocris	1069
Gabazine	Tocris	1262

Extended Data Table 2 | DNA Oligonucleotides sequences

Name	Sequence 5'-3'	Use	Supplier	Catalog Number
UNC13A_For	CAGACGATCATTGAGGTGCG	RT-PCR	IDT	NA
UNC13A_Rev	ATACTTGGAGGAGAGGCAGG	RT-PCR	IDT	NA
STMN2_For	GCTCTCTCCGCTGCTGTAG	RT-PCR	IDT	NA
STMN2_Rev	CGAGGTTCCGGGTAAAAGCA	RT-PCR	IDT	NA
STMN2_Cryptic_Rev	CTGTCTCTCTCTCTCGCACA	RT-PCR	IDT	NA
UNC13A_Total_For	TGATGTTGACCTCGATGAACG	RT-qPCR	IDT	Hs.PT.58.1883136
UNC13A_Total_Rev	TCTGTCCATGTTGAGCTGTTC	RT-qPCR	IDT	Hs.PT.58.1883136
UNC13A_Total_Probe	/56-FAM/AGCCACCAC/ZEN/TTTCACTGTGACCTT/3IABkFQ/	RT-qPCR	IDT	Hs.PT.58.1883136
UNC13A_Correct_For	GGACAAGCGAACTGACAAATC	RT-qPCR	IDT	NA
UNC13A_Correct_Rev	ACAGGTTCTCATGCAGACAG	RT-qPCR	IDT	NA
UNC13A_Correct_Probe	/5SUN/ATCAAAGGC/ZEN/GAGGAGAAGGTGGC/3IABkFQ/	RT-qPCR	IDT	NA
Gapdh-Jun	NA	RT-qPCR	Thermo	4485713
CamK2A_For	TCAATCAGCTGCTCTGTCAC	RT-qPCR	IDT	Hs.PT.56a.28027747
CamK2A_Rev	CCAGTTCCAGCGTTCAGTT	RT-qPCR	IDT	Hs.PT.56a.28027747
CamK2B_For	TCTTGGCTGCATACTCATGG	RT-qPCR	IDT	Hs.PT.56a.20211778.g
CamK2B_Rev	CTTCACCGACGAGTACCAG	RT-qPCR	IDT	Hs.PT.56a.20211778.g
GRIA1_For	CTTAATCGAGTTCTGCTACAAATCC	RT-qPCR	IDT	Hs.PT.58.15517507
GRIA1_Rev	GTATGGCTTCGTTGATGGTTG	RT-qPCR	IDT	Hs.PT.58.15517507
GRIA2_For	GGTACGACAAAGGAGAGTGC	RT-qPCR	IDT	Hs.PT.58.25075751
GRIA2_Rev	CCCGACAAGGATGTAGAATACTC	RT-qPCR	IDT	Hs.PT.58.25075751
GRIN1_For	CTCCTGGAAGATTCAGCTCAA	RT-qPCR	IDT	Hs.PT.58.39141804
GRIN1_Rev	GTGGATGGCTAACTAGGATGG	RT-qPCR	IDT	Hs.PT.58.39141804
GRIN2A_For	CAAGAAGTAATGGCACCGTCT	RT-qPCR	IDT	Hs.PT.58.26949410
GRIN2A_Rev	GCAGAAACAATGAGCAGCATC	RT-qPCR	IDT	Hs.PT.58.26949410
GRIN2B_For	CTTCATAGAGACAGGCATCAGT	RT-qPCR	IDT	Hs.PT.58.40419546
GRIN2B_Rev	CATCACAAACATCATCACCCATAC	RT-qPCR	IDT	Hs.PT.58.40419546
mGluR5_For	TGTGAGAAAGGCCAGATCAAG	RT-qPCR	IDT	Hs.PT.58.40025787
mGluR5_Rev	TGCCTTGCATGTGTACTCATC	RT-qPCR	IDT	Hs.PT.58.40025787
STMN2_For	CCACGAACTTTAGCTTCTCCA	RT-qPCR	IDT	Hs.PT.58.5075784
STMN2_Rev	GCCAATTGTTTCAGCACCTG	RT-qPCR	IDT	Hs.PT.58.5075784

Extended Data Table 3 | Antibodies

Antibody	Supplier; Catalog Number	Use	Dilution
Rabbit anti UNC13A	Synaptic Systems; 126 103	WB	1:2,000
Rabbit anti TDP-43	ProteinTech; 10782-2-AP	WB	1:2,000
Mouse anti Tubulin	ProteinTech; 66031-1-Ig	WB	1:2,000
Rabbit anti STMN2	ProteinTech; 10586-1-AP	WB	1:2,000
Goat anti Mouse IGG-HRP conjugate	BioRad; 1706516	WB	1:10,000
Goat anti Rabbit IGG-HRP conjugate	BioRad; 1706515	WB	1:10,000
Mouse anti TDP-43	Abcam; ab104223	IF	1:1,000
Mouse anti Synapsin	Synaptic Systems; 106 011	IF	1:1,000
Guinea Pig anti UNC13A	Synaptic Systems; 126 104	IF	1:500
Chicken anti MAP2	Abcam; ab5392	IF	1:10,000
Goat anti Mouse IgG (H+L) AlexaFluor 488	ThermoFisher; A-11029	IF	1:1,000
Goat anti Guinea Pig IgG (H+L) AlexaFluor 647	ThermoFisher; A-21450	IF	1:1,000
Goat anti Chicken IgY (H+L) AlexaFluor 647	ThermoFisher; A-21449	IF	1:1,000

Extended Data Table 4 | Software

Software	Source
FIJI/ImageJ v2.14	https://imagej.net/software/fiji/
Prism v10	GraphPad
R v4.2.2	https://www.r-project.org/
Clampex v10.6	Molecular Devices
Axon Multiclamp Commander v10.4	Molecular Devices
Clampfit 10.7	Molecular Devices
MATLAB R2021a	Mathworks
ImageLab Touch Software v1.0.0.15	Bio-Rad
IGV browser v2.8.2	UC San Diego and Broad Institute of MIT and Harvard
Minimap2 v2.26-r1175	https://github.com/lh3/minimap2
QIAxcelR	https://github.com/Delayed-Gitification/QIAxcelR
TapeStation Systems Software v3.2	Agilent
QuantStudio Design & Analysis v1.5.2	ThermoFisher Scientific
Maestro Pro – AxIS Navigator v3.7.2	Axion Biosystems
Neural Metric Tool v4.0.5	Axion Biosystems
Zen Blue v3.3	Carl Zeiss AG