## Supplementary Figure Legends

**Figure S1: APA events in SH-SY5Y cells and hESC-derived MNs upon TDP-43 knock-down**
(**A**) Table of top 10 APA events in SH-SY5Y cells treated with control siRNA or TARDBP siRNA for 96 hrs. (**B**) Depiction of ΔPDUI for all significant APA genes in TDP-43 depleted SH-SY5Y cells. (**C**) Overlapping APA genes between siRNA-treated SH-SY5Y cells and doxycycline-induced TARDBP knockdown SH-SY5Y cells, filtering for $p < 0.05$ and $|\Delta PDUI| \geq 0.1$. (**D**) Table of top 10 APA events in hESC-derived motor neurons (ESC-MNs) treated with control siRNA or TARDBP siRNA. Note that TARDBP siRNA treated ESC-MNs displayed only a partial (~60%) reduction in TDP-43 protein levels. (**E**) Depiction of ΔPDUI for all significant APA genes in TDP-43 KD ESC-MNs.

**Figure S2: Genes affected by APA function in ALS-relevant pathways**
Gene ontology (GO) analysis of biological processes (BP) and molecular functions (MF) of coding APA genes with $p < 0.05$ and $|\Delta PDUI| \geq 0.1$ in (**A, B**) SH-SY5Y cells, (**C, D**) SK-N-BE(2) cells, and (**E**) i$^3$Neurons (note that GO MF terms were not significantly enriched in i$^3$Neurons). Analysis was performed using ShinyGO v0.77.

**Figure S3: APA genes are bound by TDP-43 outside of the 3'UTR**
Venn diagram illustrating the intersection of APA events between SH-SY5Y cells, SK-N-BE(2) cells, and i$^3$Neurons depleted of TDP-43, along with evidence for direct TDP-43 binding in regions outside of the 3'UTR, in which eCLIP-seq data show TDP-43 binding within the 5'UTR, in introns, or in exons of APA genes.

**Figure S4: RNA-sequencing tracks depict APA for the *CNPY3* and *SMC1A* genes in the presence or absence of TDP-43 knock-down**
RNA-seq tracks for *CNPY3* (**A**) and *SMC1A* (**B**) in i$^3$Neurons, SH-SY5Y cells, and SK-N-BE(2) cells in the presence or absence of TDP-43 knock-down. The location of the TDP-43 3'UTR binding site is depicted in green (*CNPY3* at chr6: 42,935,715 - 42,935,762; *SMC1A* at chrX: 53,379,722 - 53,379,785).

**Figure S5: Isoform specific quantification of CNPY3 APA in the presence or absence of TDP-43 knockdown.**
qRT-PCR quantification of CNPY3 variant 1 and variant 2 in (**A**) SH-SY5Y cells or (**B**) i$^3$Neurons in the presence of absence of TDP-43 knockdown. $^{**}p < 0.01$; unpaired two-tailed t-test; n = 3 biological replicates. Data are presented as mean values ± s.e.m.

**Figure S6: Validation of TDP-43 knockdown in i³Neurons and iPSC-MNs.**

Immunoblot of i³Neurons (**A**) and iPSC-MNs (**B**) in which TDP-43 was knocked down with shRNAs for 10 days. ****p<0.0001, one-way ANOVA (**A**), unpaired two-tailed t-test (**B**); n=3 biological replicates. Error bars = s.e.m.

**Figure S7: Gene ontology (GO) analysis of coding APA events in ALS/FTD postmortem neuronal nuclei depleted of TDP-43.**

GO analysis of biological processes enriched for coding APA events with $p < 0.05$ and $|\Delta PDUI| \geq 0.1$ highlights genes that function in nucleocytoplasmic transport and synapse assembly.

**Figure S8: The MARK3 3'UTR contains two conserved RBP binding motifs**

(**A**) Predicted RBP binding motifs in the MARK3 3'UTR with $p < 0.001$ and Z-score > 3.5 for at least one motif (RBPmap). (**B**) Graphical representation of select conserved cis-regulatory elements in the MARK3 3'UTR.

**Figure S9**: **Quantification of MARK3 APA in ALS/FTD and FTLD-TDP frontal cortex.**

qRT-PCR quantification of (**A**) the short 3'UTR MARK3 isoform, (**B**) the long-3'UTR MARK3 isoform, and (**C**) the ratio of long:short 3'UTR isoforms in postmortem frontal cortex tissue from controls or from ALS/FTD and FTLD-TDP patients with confirmed inclusion of the *UNC13A* CE (as described in **Fig. 3E**). *p<0.05; unpaired two-tailed t-test; n = 11 (control), n = 30 (*UNC13A* CE). Data are presented as mean values ± s.e.m.

**Figure S10: TDP-43 knock-down in iPSC-MNs corresponds with a trend towards increased tau S262 phosphorylation.**

(**A**) Immunoblot of S262 phosphorylated tau and total tau in iPSC-MNs transduced with lentivirus encoding control shRNA or shRNA TARDBP for 10 days. Statistical significance was determined by one sample t-test; n=3 biological replicates. (**B**) Immunoblot of MARK3, S262 phosphorylated tau, and total tau in iPSC-MNs transduced with lentivirus encoding either control shRNA, MARK3 shRNA, GFP (empty vector), or MARK3-V5 for 10 days. Statistical significance was determined by unpaired two-tailed t-test; n=3 biological replicates. *p<0.05, ***p<0.001; unpaired two-tailed t-test; n = 3 biological replicates. Error bars = s.e.m. (**C**) Immunoblot of MARK3 in iPSC-MNs transduced with lentivirus encoding control shRNA, TARDBP shRNA, or MARK3 shRNA for 10 days. Statistical significance was determined by unpaired two-tailed t-test; n=3 biological replicates. Error bars = s.e.m.

**Figure S11: TDP-43 knock-down does not significantly increase MARK3 or MARK4 protein levels in i³Neurons or in postmortem ALS/FTD or FTLD-TDP patient tissue.**

(**A**) Immunoblot of MARK3 in i³Neurons depleted of TDP-43 with shRNA for 10 days. n=3 biological replicates. Statistical significance was determined by one-way ANOVA; p=n.s. Note that the lower molecular weight portion of this blot is shown in Figure S6; thus, the same total protein blot was used for quantification. (**B**) Quantification of immunoblots for MARK3 protein in the frontal cortex of healthy controls (n=5) or ALS/FTD and FTLD-TDP patients (n=11). Statistical significance was determined by unpaired two-tailed t-test; p=n.s. (**C**) Immunoblot of MARK4 in i³Neurons depleted of TDP-43 with shRNAs for 10 days. Statistical significance was determined by one sample t-test against a value of 1; p=n.s. Error bars = s.e.m.

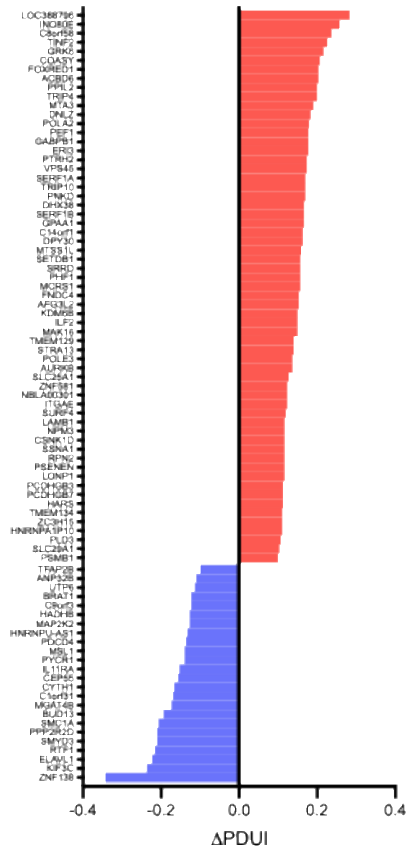
**A**

| Gene | PDUI CTL | PDUI KD | ΔPDUI | p-value | Adj. p-value | TDP-43 binding |
|---|---|---|---|---|---|---|
| ILF2 | 0.71 | 0.86 | 0.15 | 7.10E-10 | 1.22E-06 | N/A |
| RPN2 | 0.883 | 1 | 0.117 | 8.77E-10 | 1.22E-06 | Intron |
| HNRNPA1P10 | 0.75 | 0.86 | 0.11 | 1.26E-09 | 1.40E-06 | Exon |
| SMC1A | 0.28 | 0.0733 | -0.207 | 1.44E-06 | 0.000616 | Intron **3'UTR** |
| PLD3 | 0.783 | 0.89 | 0.107 | 5.60E-05 | 0.0195 | N/A |
| COASY | 0.78 | 0.987 | 0.207 | 0.00012 | 0.0381 | N/A |
| SSNA1 | 0.883 | 1 | 0.117 | 0.00015 | 0.0477 | N/A |
| ACBD6 | 0.72 | 0.923 | 0.203 | 0.00024 | 0.0659 | Intron |
| PNKD | 0.817 | 0.987 | 0.17 | 0.00028 | 0.0754 | N/A |
| STRA13 | 0.757 | 0.897 | 0.14 | 0.00045 | 0.104 | N/A |

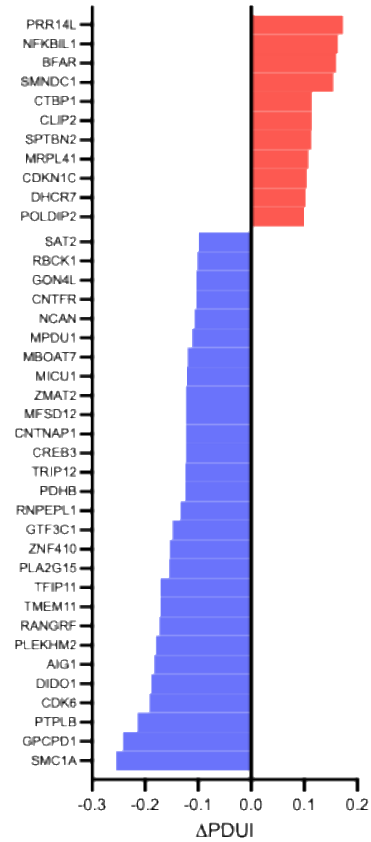
**D**

| Gene | PDUI CTL | PDUI KD | ΔPDUI | p-value | Adj. p-value | TDP-43 binding |
|---|---|---|---|---|---|---|
| SMC1A | 0.722 | 0.467 | -0.255 | 0.0064 | 1 | Intron 3'UTR |
| GPCPD1 | 0.783 | 0.542 | -0.242 | 0.0055 | 1 | N/A |
| PTPLB | 0.907 | 0.692 | -0.215 | 0.011 | 1 | N/A |
| CDK6 | 0.972 | 0.780 | -0.192 | 0.0053 | 1 | Intron Exon 3'UTR |
| DIDO1 | 0.515 | 0.327 | -0.188 | 0.043 | 1 | Intron |
| AIG1 | 0.835 | 0.652 | -0.183 | 0.033 | 1 | Intron |
| PLEKHM2 | 0.718 | 0.538 | -0.180 | 0.030 | 1 | N/A |
| RANGRF | 0.957 | 0.783 | -0.173 | 0.0009 | 0.55 | N/A |
| TMEM11 | 0.937 | 0.765 | -0.172 | 0.0004 | 0.37 | N/A |
| TFIP11 | 0.912 | 0.740 | -0.172 | 0.0093 | 1 | N/A |

**B**



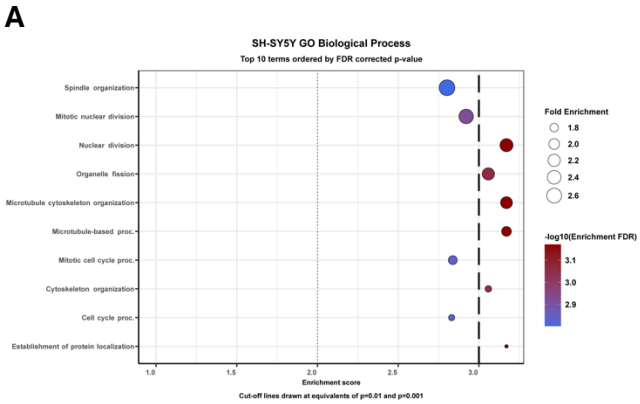

**E**

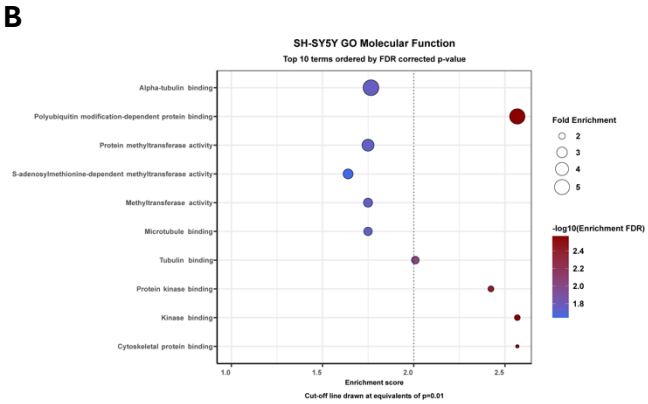


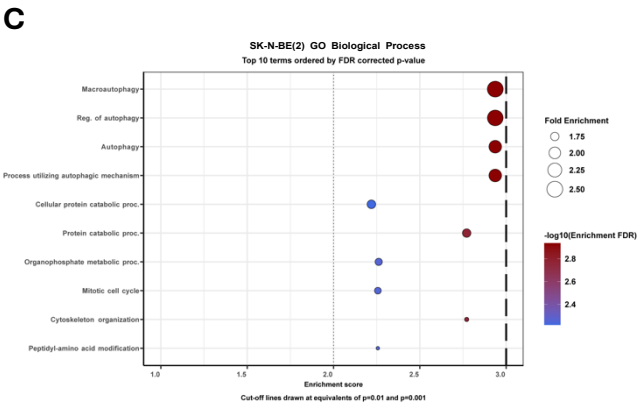

**C**

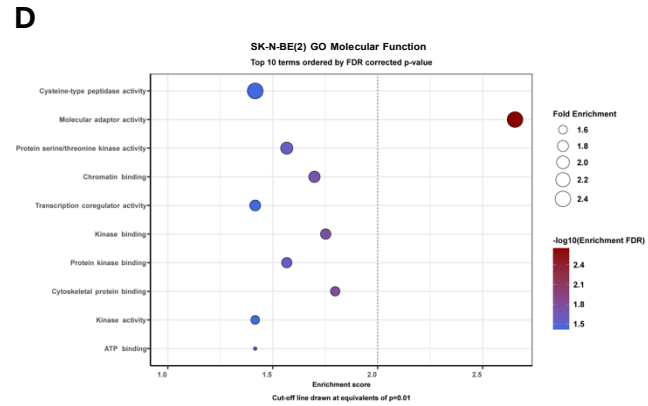
| Overlap with Brown et. al, 2022 |
|---|
| ACBD6 |
| ELAVL1 |
| GRK6 |
| INO80E |
| NPM3 |
| PPP2R2D |
| PTRH2 |
| SMC1A |
| ZNF138 |

Figure S1


A
SH-SY5Y GO Biological Process
Top 10 terms ordered by FDR corrected p-value
Spindle organization
Mitotic nuclear division
Nuclear division
Organelle fission
Microtubule cytoskeleton organization
Microtubule-based proc.
Mitotic cell cycle proc.
Cytoskeleton organization
Cell cycle proc.
Establishment of protein localization
1.0
1.5
2.0
2.5
3.0
Enrichment score
Cut-off lines drawn at equivalents of p=0.01 and p=0.001
Fold Enrichment
1.8
2.0
2.2
2.4
2.6
-log10(Enrichment FDR)
3.1
3.0
2.9


B
SH-SY5Y GO Molecular Function
Top 10 terms ordered by FDR corrected p-value
Alpha-tubulin binding
Polyubiquitin modification-dependent protein binding
Protein methyltransferase activity
S-adenosylmethionine-dependent methyltransferase activity
Methyltransferase activity
Microtubule binding
Tubulin binding
Protein kinase binding
Kinase binding
Cytoskeletal protein binding
1.0
1.5
2.0
2.5
Enrichment score
Cut-off line drawn at equivalents of p=0.01
Fold Enrichment
2
3
4
5
-log10(Enrichment FDR)
2.4
2.2
2.0
1.8


C
SK-N-BE(2) GO Biological Process
Top 10 terms ordered by FDR corrected p-value
Macroautophagy
Reg. of autophagy
Autophagy
Process utilizing autophagic mechanism
Cellular protein catabolic proc.
Protein catabolic proc.
Organophosphate metabolic proc.
Mitotic cell cycle
Cytoskeleton organization
Peptidyl-amino acid modification
1.0
1.5
2.0
2.5
3.0
Enrichment score
Cut-off lines drawn at equivalents of p=0.01 and p=0.001
Fold Enrichment
1.75
2.00
2.25
2.50
-log10(Enrichment FDR)
2.8
2.6
2.4


D
SK-N-BE(2) GO Molecular Function
Top 10 terms ordered by FDR corrected p-value
Cysteine-type peptidase activity
Molecular adaptor activity
Protein serine/threonine kinase activity
Chromatin binding
Transcription coregulator activity
Kinase binding
Protein kinase binding
Cytoskeletal protein binding
Kinase activity
ATP binding
1.0
1.5
2.0
2.5
Enrichment score
Cut-off line drawn at equivalents of p=0.01
Fold Enrichment
1.6
1.8
2.0
2.2
2.4
-log10(Enrichment FDR)
2.4
2.1
1.8
1.5

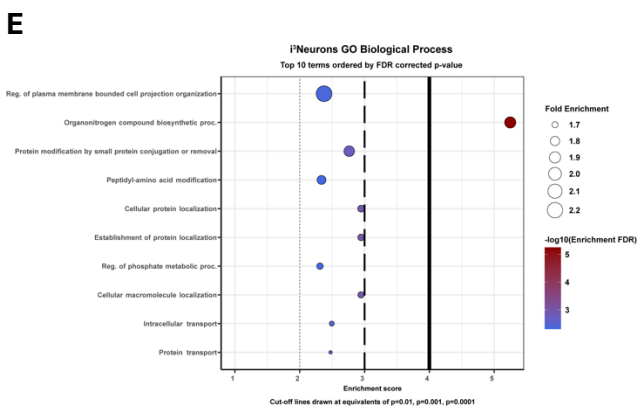

E
i³Neurons GO Biological Process
Top 10 terms ordered by FDR corrected p-value
Reg. of plasma membrane bounded cell projection organization
Organonitrogen compound biosynthetic proc.
Protein modification by small protein conjugation or removal
Peptidyl-amino acid modification
Cellular protein localization
Establishment of protein localization
Reg. of phosphate metabolic proc.
Cellular macromolecule localization
Intracellular transport
Protein transport
1
2
3
4
5
Enrichment score
Cut-off lines drawn at equivalents of p=0.01, p=0.001, p=0.0001
Fold Enrichment
1.7
1.8
1.9
2.0
2.1
2.2
-log10(Enrichment FDR)
5
4
3

Figure S2

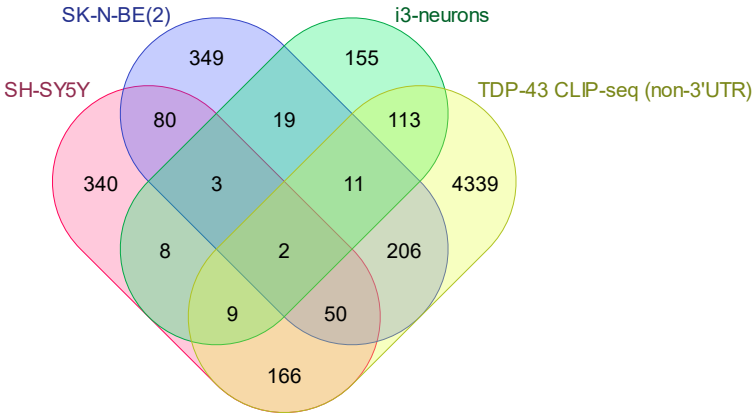

SK-N-BE(2)
i3-neurons
SH-SY5Y
TDP-43 CLIP-seq (non-3'UTR)
349
155
80
19
113
340
3
11
4339
8
2
206
9
50
166

Figure S3

A

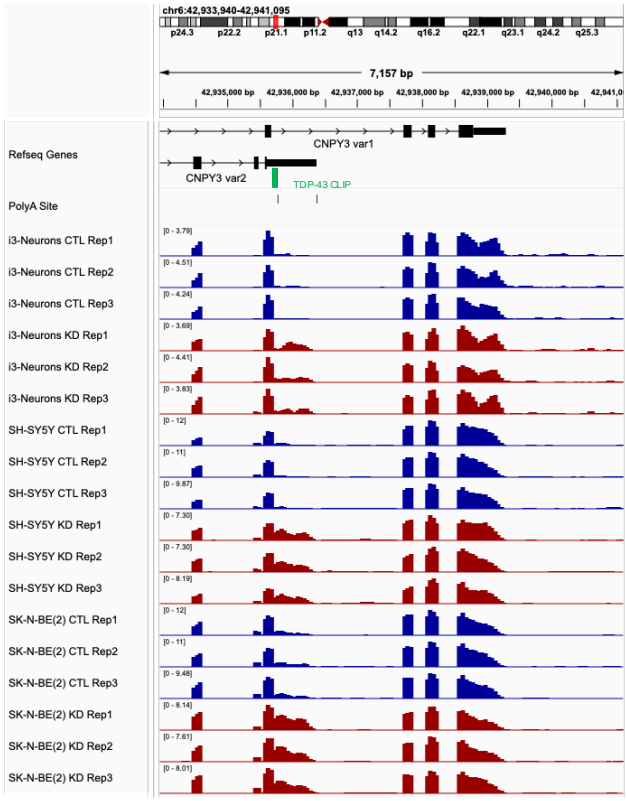
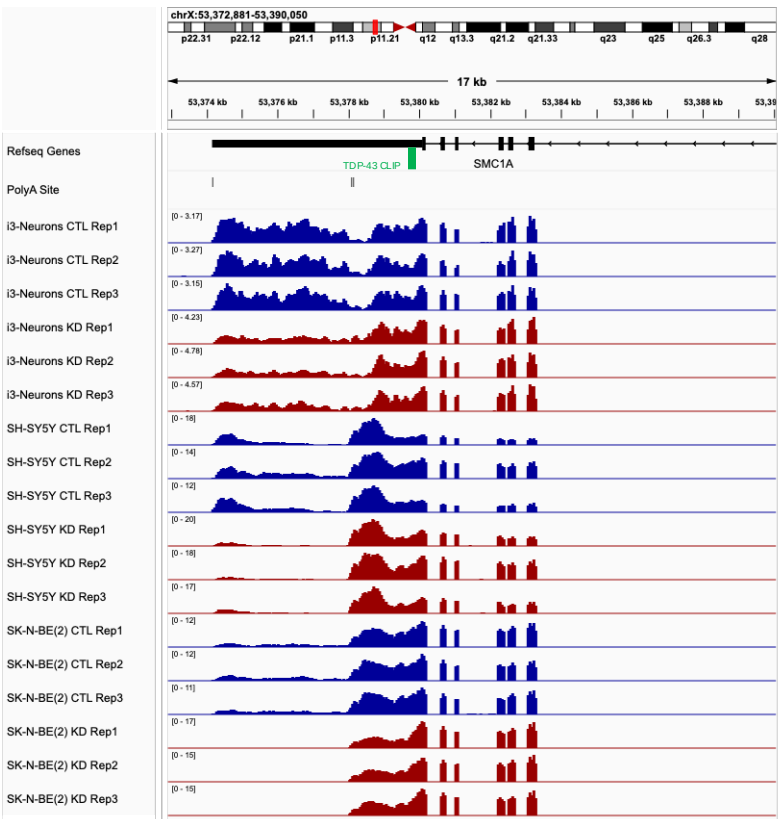

chr6:42,933,940-42,941,095
7,157 bp
Refseq Genes
CNPY3 var1
CNPY3 var2
TDP-43 CLIP
PolyA Site
i3-Neurons CTL Rep1
i3-Neurons CTL Rep2
i3-Neurons CTL Rep3
i3-Neurons KD Rep1
i3-Neurons KD Rep2
i3-Neurons KD Rep3
SH-SY5Y CTL Rep1
SH-SY5Y CTL Rep2
SH-SY5Y CTL Rep3
SH-SY5Y KD Rep1
SH-SY5Y KD Rep2
SH-SY5Y KD Rep3
SK-N-BE(2) CTL Rep1
SK-N-BE(2) CTL Rep2
SK-N-BE(2) CTL Rep3
SK-N-BE(2) KD Rep1
SK-N-BE(2) KD Rep2
SK-N-BE(2) KD Rep3

Figure S4

B


chrX:53,372,881-53,390,050
17 kb
Refseq Genes
TDP-43 CLIP
SMC1A
PolyA Site
i3-Neurons CTL Rep1
i3-Neurons CTL Rep2
i3-Neurons CTL Rep3
i3-Neurons KD Rep1
i3-Neurons KD Rep2
i3-Neurons KD Rep3
SH-SY5Y CTL Rep1
SH-SY5Y CTL Rep2
SH-SY5Y CTL Rep3
SH-SY5Y KD Rep1
SH-SY5Y KD Rep2
SH-SY5Y KD Rep3
SK-N-BE(2) CTL Rep1
SK-N-BE(2) CTL Rep2
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SK-N-BE(2) KD Rep2
SK-N-BE(2) KD Rep3

**A**

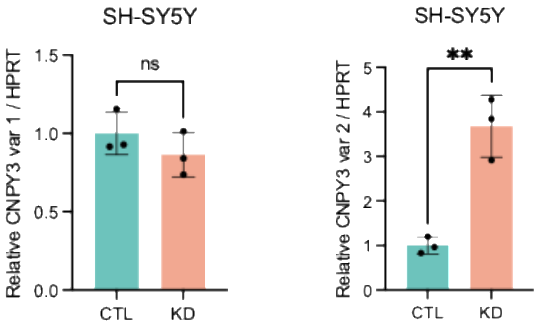
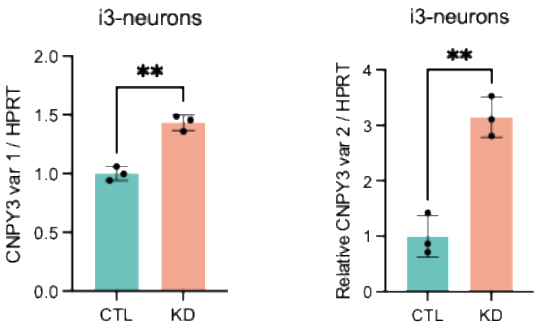

SH-SY5Y
ns
Relative CNPY3 var 1 / HPRT
1.5
1.0
0.5
0.0
CTL
KD
SH-SY5Y
**
Relative CNPY3 var 2 / HPRT
5
4
3
2
1
0
CTL
KD

Figure S5

**B**


i3-neurons
**
CNPY3 var 1 / HPRT
2.0
1.5
1.0
0.5
0.0
CTL
KD
i3-neurons
**
Relative CNPY3 var 2 / HPRT
4
3
2
1
0
CTL
KD

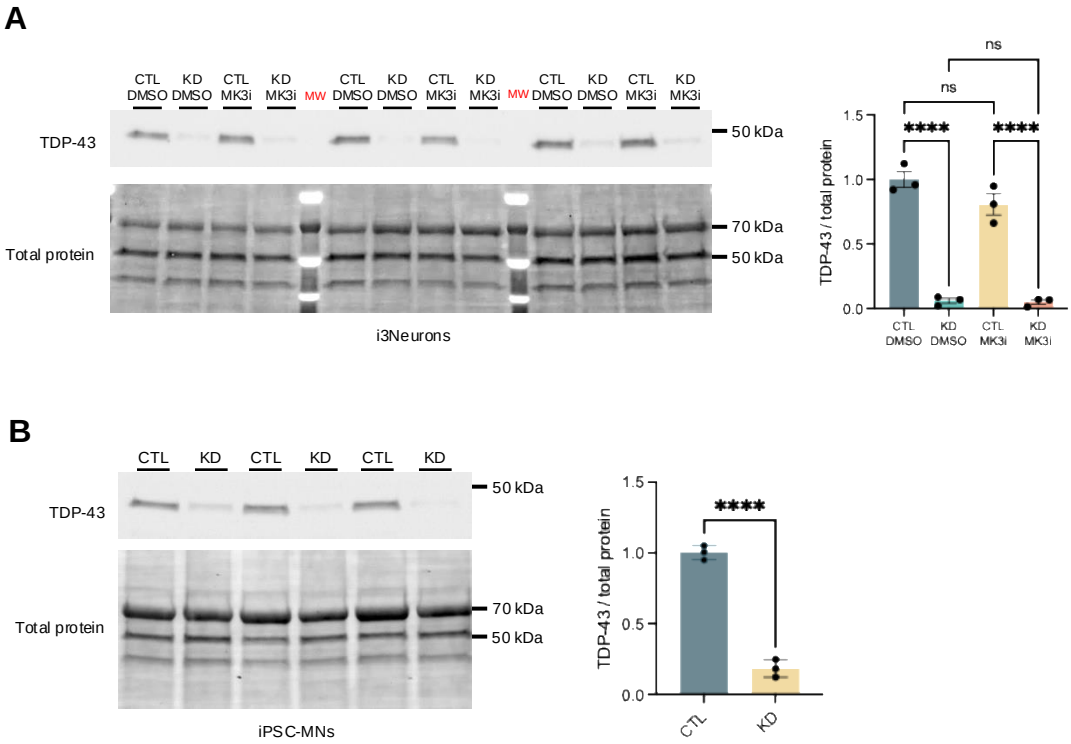

A
CTL DMSO
KD DMSO
CTL MK3i
KD MK3i
MW
TDP-43
Total protein
50 kDa
70 kDa
50 kDa
i3Neurons
ns
ns
****
****
TDP-43 / total protein
1.5
1.0
0.5
0.0
CTL DMSO
KD DMSO
CTL MK3i
KD MK3i
B
CTL
KD
TDP-43
Total protein
50 kDa
70 kDa
50 kDa
iPSC-MNs
****
TDP-43 / total protein
1.5
1.0
0.5
0.0
CTL
KD

Figure S6

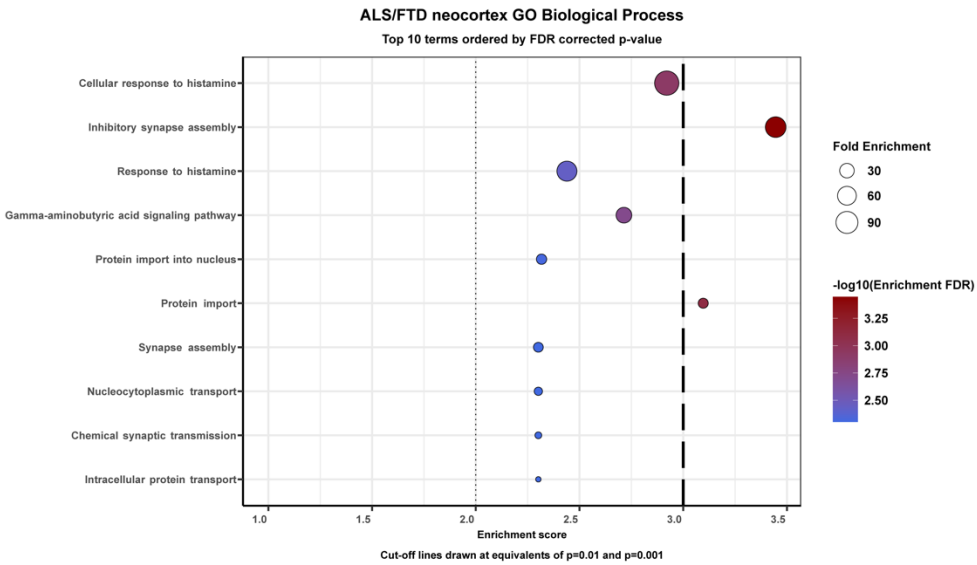
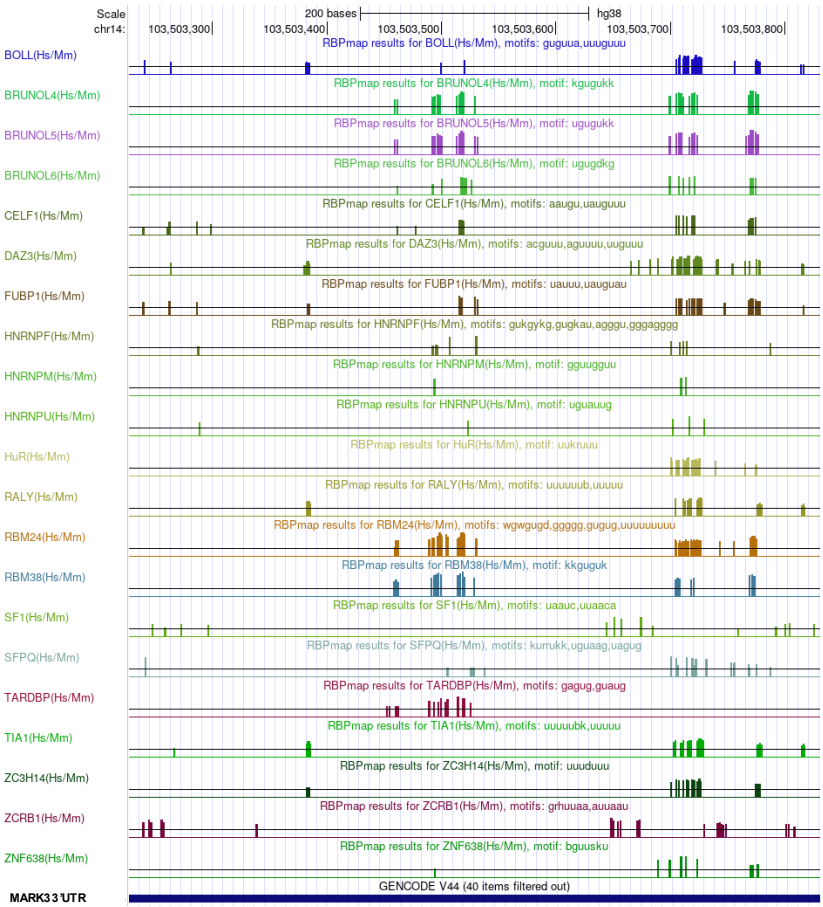

ALS/FTD neocortex GO Biological Process
Top 10 terms ordered by FDR corrected p-value
Cellular response to histamine
Inhibitory synapse assembly
Response to histamine
Gamma-aminobutyric acid signaling pathway
Protein import into nucleus
Protein import
Synapse assembly
Nucleocytoplasmic transport
Chemical synaptic transmission
Intracellular protein transport
1.0
1.5
2.0
2.5
3.0
3.5
Enrichment score
Cut-off lines drawn at equivalents of p=0.01 and p=0.001
Fold Enrichment
30
60
90
-log10(Enrichment FDR)
3.25
3.00
2.75
2.50

Figure S7

**A**


Scale
chr14:
200 bases
hg38
103,503,300
103,503,400
103,503,500
103,503,600
103,503,700
103,503,800
BOLL(Hs/Mm)
RBPmap results for BOLL(Hs/Mm), motifs: guguua,uuuguuu
BRUNOL4(Hs/Mm)
RBPmap results for BRUNOL4(Hs/Mm), motif: kgugukk
BRUNOL5(Hs/Mm)
RBPmap results for BRUNOL5(Hs/Mm), motif: uguguk
BRUNOL6(Hs/Mm)
RBPmap results for BRUNOL6(Hs/Mm), motif: ugugdkg
CELF1(Hs/Mm)
RBPmap results for CELF1(Hs/Mm), motifs: aaugu,uauguuu
DAZ3(Hs/Mm)
RBPmap results for DAZ3(Hs/Mm), motifs: acguuu,aguuuu,uuguuu
FUBP1(Hs/Mm)
RBPmap results for FUBP1(Hs/Mm), motifs: uauuu,uauguau
HNRNPF(Hs/Mm)
RBPmap results for HNRNPF(Hs/Mm), motifs: gukgykg,gugkau,agggu,gggagggg
HNRNPM(Hs/Mm)
RBPmap results for HNRNPM(Hs/Mm), motif: gguugguu
HNRNPU(Hs/Mm)
RBPmap results for HNRNPU(Hs/Mm), motif: uguauug
HuR(Hs/Mm)
RBPmap results for HuR(Hs/Mm), motif: uukruuu
RALY(Hs/Mm)
RBPmap results for RALY(Hs/Mm), motifs: uuuuuub,uuuuu
RBM24(Hs/Mm)
RBPmap results for RBM24(Hs/Mm), motifs: wgwgugd,ggggg,gugug,uuuuuuuuu
RBM38(Hs/Mm)
RBPmap results for RBM38(Hs/Mm), motif: kkguguk
SF1(Hs/Mm)
RBPmap results for SF1(Hs/Mm), motifs: uaauc,uuaaca
SFPQ(Hs/Mm)
RBPmap results for SFPQ(Hs/Mm), motifs: kurrukk,uguaag,uagug
TARDBP(Hs/Mm)
RBPmap results for TARDBP(Hs/Mm), motifs: gagug,guaug
TIA1(Hs/Mm)
RBPmap results for TIA1(Hs/Mm), motifs: uuuuubk,uuuuu
ZC3H14(Hs/Mm)
RBPmap results for ZC3H14(Hs/Mm), motif: uuuduuu
ZCRB1(Hs/Mm)
RBPmap results for ZCRB1(Hs/Mm), motifs: grhuuaa,auuaau
ZNF638(Hs/Mm)
RBPmap results for ZNF638(Hs/Mm), motif: bguusku
MARK3 3'UTR
GENCODE V44 (40 items filtered out)

**B**

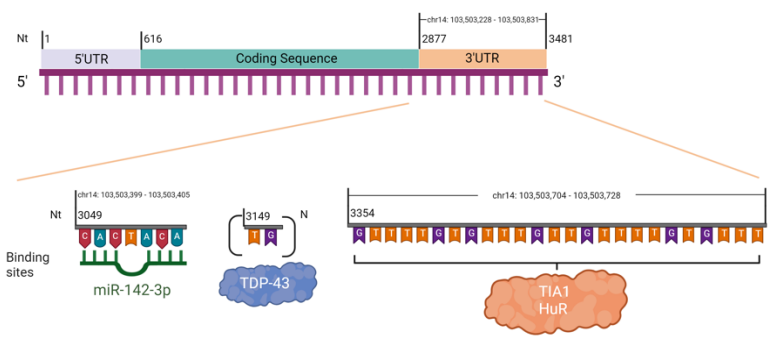

Nt
1
616
chr14: 103,503,228 - 103,503,831
2877
3481
5'UTR
Coding Sequence
3'UTR
5'
3'
chr14: 103,503,399 - 103,503,405
Nt
3049
Binding sites
miR-142-3p
3149
N
TDP-43
chr14: 103,503,704 - 103,503,728
3354
TIA1
HuR

Figure S8

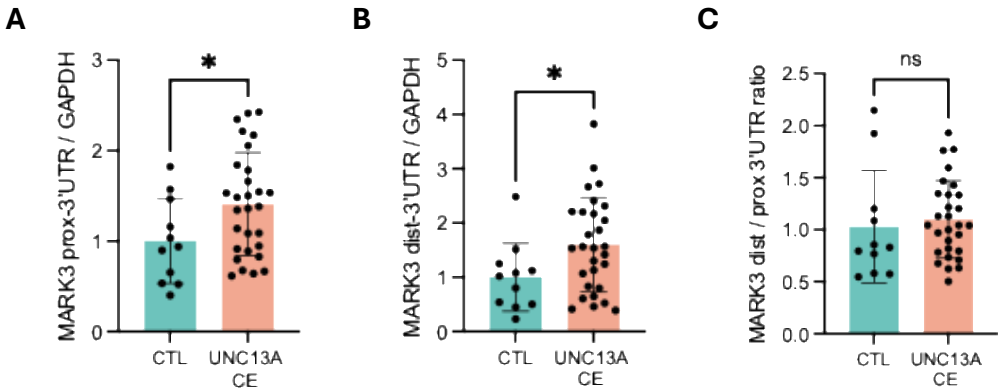

A
B
C
MARK3 prox-3'UTR / GAPDH
MARK3 dist-3'UTR / GAPDH
MARK3 dist / prox 3'UTR ratio
*
*
ns
0
1
2
3
4
5
0.0
0.5
1.0
1.5
2.0
2.5
CTL
UNC13A CE

Figure S9

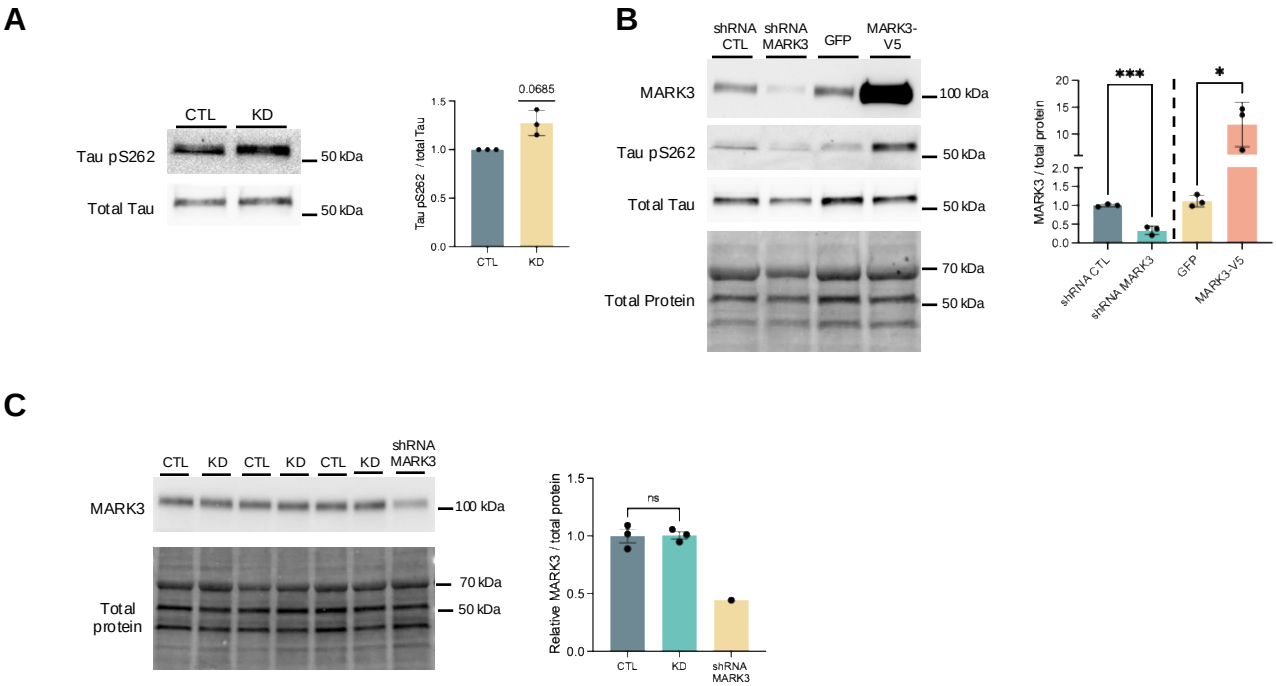

A
CTL
KD
Tau pS262
50 kDa
Total Tau
50 kDa
0.0685
Tau pS262 / total Tau
1.5
1.0
0.5
0.0
CTL
KD
B
shRNA CTL
shRNA MARK3
GFP
MARK3-V5
MARK3
100 kDa
Tau pS262
50 kDa
Total Tau
50 kDa
Total Protein
70 kDa
50 kDa
***
*
MARK3 / total protein
20
15
10
2.0
1.5
1.0
0.5
0.0
shRNA CTL
shRNA MARK3
GFP
MARK3-V5
C
CTL
KD
CTL
KD
CTL
KD
shRNA MARK3
MARK3
100 kDa
Total protein
70 kDa
50 kDa
ns
Relative MARK3 / total protein
1.5
1.0
0.5
0.0
CTL
KD
shRNA MARK3

Figure S10

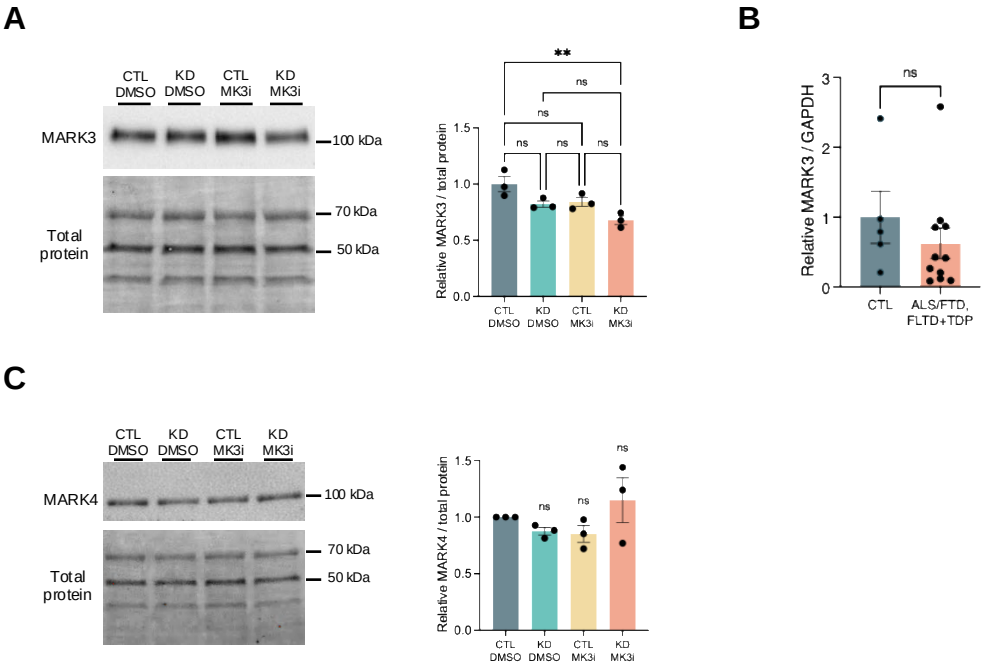

A
CTL DMSO
KD DMSO
CTL MK3i
KD MK3i
MARK3
100 kDa
Total protein
70 kDa
50 kDa
**
ns
Relative MARK3 / total protein
1.5
1.0
0.5
0.0
B
Relative MARK3 / GAPDH
3
2
1
0
CTL
ALS/FTD, FLTD+TDP
C
MARK4
Relative MARK4 / total protein

Figure S11