Supplementary Figures

Title: Elevated nuclear TDP-43 induces constitutive exon skipping

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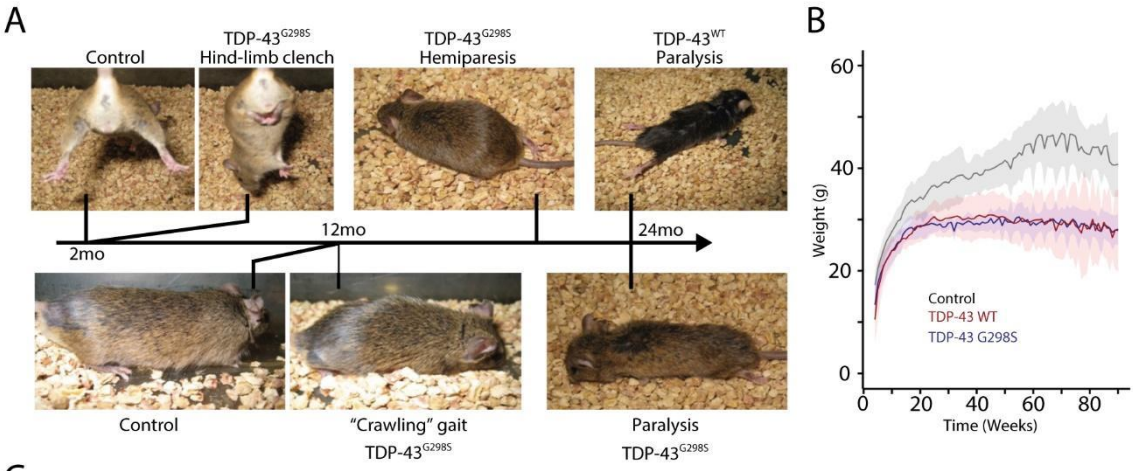
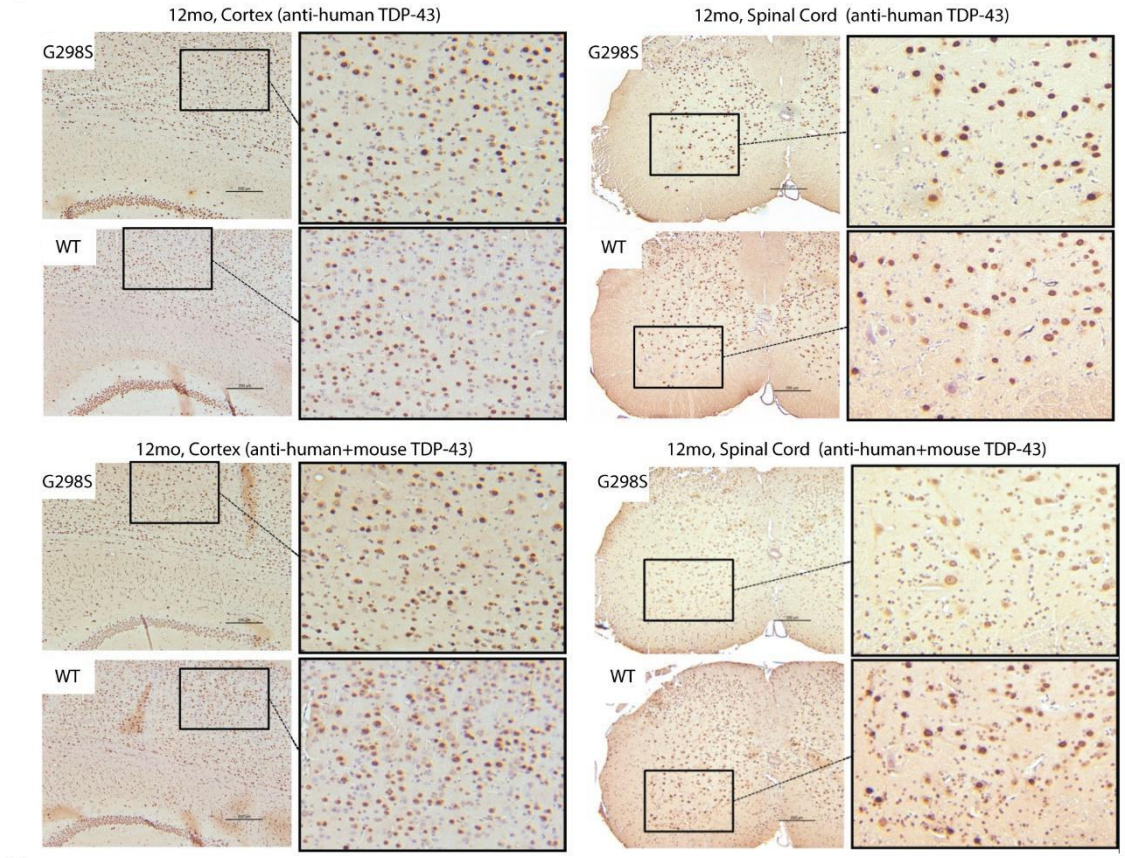
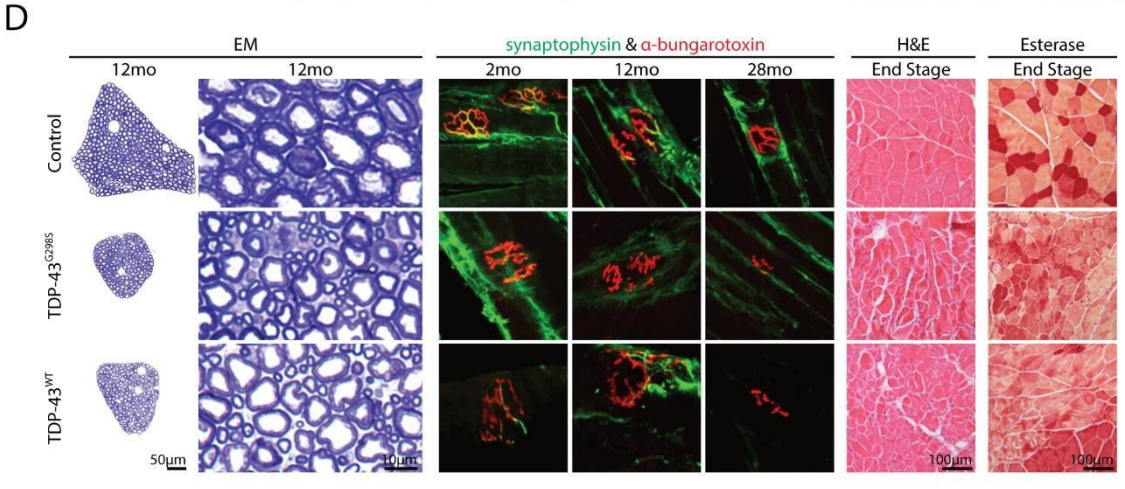
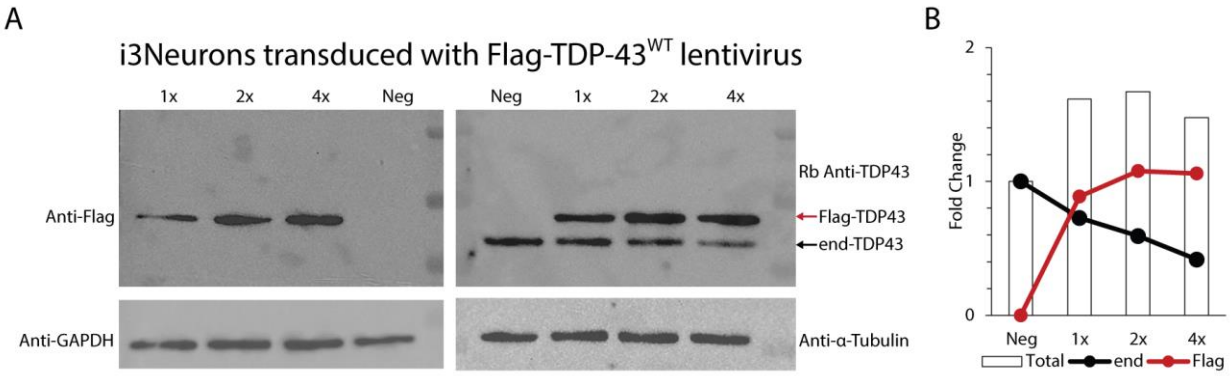

A
Control
TDP-43G298S Hind-limb clench
TDP-43G298S Hemiparesis
TDP-43WT Paralysis
2mo
12mo
24mo
Control
"Crawling" gait TDP-43G298S
Paralysis TDP-43G298S
B
Weight (g)
Time (Weeks)
Control
TDP-43 WT
TDP-43 G298S


C
12mo, Cortex (anti-human TDP-43)
12mo, Spinal Cord (anti-human TDP-43)
G298S
WT
12mo, Cortex (anti-human+mouse TDP-43)
12mo, Spinal Cord (anti-human+mouse TDP-43)


D
EM
12mo
12mo
synaptophysin & α-bungarotoxin
2mo
12mo
28mo
H&E
End Stage
Esterase
End Stage
Control
TDP-43G298S
TDP-43WT
50μm
10μm
100μm
100μm

Supplementary Figure 1. Pathological findings in TDP-43^{WT} and TDP-43^{G298S} transgenic models. (**A**) Animals exhibited marked motor disabilities starting with hind-limb clench at 2 months of age, progressing through a crawling gait at 12 months and continue with hemiparesis at 18 months, finally leading to end stage paralysis at 24 months. (**B**) Reduction in motor capabilities was accompanied by a decrease in weight compared to controls. Immunohistochemical images showed that TDP-43 was confined to the nucleus of cortical and spinal cord cells. No cytoplasmic inclusions or TDP-43 clearance was observed. (**C**) Immunohistochemistry using an antibody against human-specific TDP-43 (top panel) and immunohistochemistry using an antibody against both human and mouse TDP-43 (bottom panel). (**D**) In order to assess the lumbar L3 ventral roots of both wild-type (WT) and G298S transgenic mice at 12 months, Toluene-stained Epon thick sections were utilized. The L3 roots from transgenic mice exhibited smaller ventral roots than the non-transgenic animals. Further examination of the electron microscopy thick sections of the ventral roots in G298S and WT transgenic mice revealed a reduced number of large myelinated axons, resulting in the increased visibility of smaller axons. Staining of synaptophysin (green) and α-bungarotoxin (red) on gastrocnemius muscles revealed a progressive denervation of neuromuscular junctions in both transgenic mice. While a significant number of junctions appeared well-innervated in the 2-month-old transgenic mice, denervated junctions were notably observed in the 12-month-old transgenic mice. At the end-stage of symptoms, transgenic mice experienced muscle degeneration to the extent that it was difficult to detect end plates by α-bungarotoxin-staining. We analyzed quadriceps muscle at their end-stage because the motor neurons innervating quadriceps muscle are in the L1 to L3 region of the spinal cord in mice. As the transgenic mice aged, H&E staining showed a progressively increased disorganization and angulation. Additionally, esterase staining indicated denervated fibers (darker staining) with the fiber muscle architecture completely altered in transgenic animals.



Supplementary Figure 2. i3Neuron transduction with human TDP-43. (**A**) i3Neurons were transduced with lentivirus containing an N-terminal Flag-tagged wild-type TDP-43 at 1MOI, 2MOI, and 4MOI, respectively. Consistent increase of exogenous TDP-43 (left) is seen accompanied with endogenous TDP-43 autoregulation (right). (**B**) Quantification of the right immunoblot, shows ~1.6 fold increase of total TDP-43 (endogenous TDP43 + transduced Flag-TDP43^{WT}).

A

Mouse

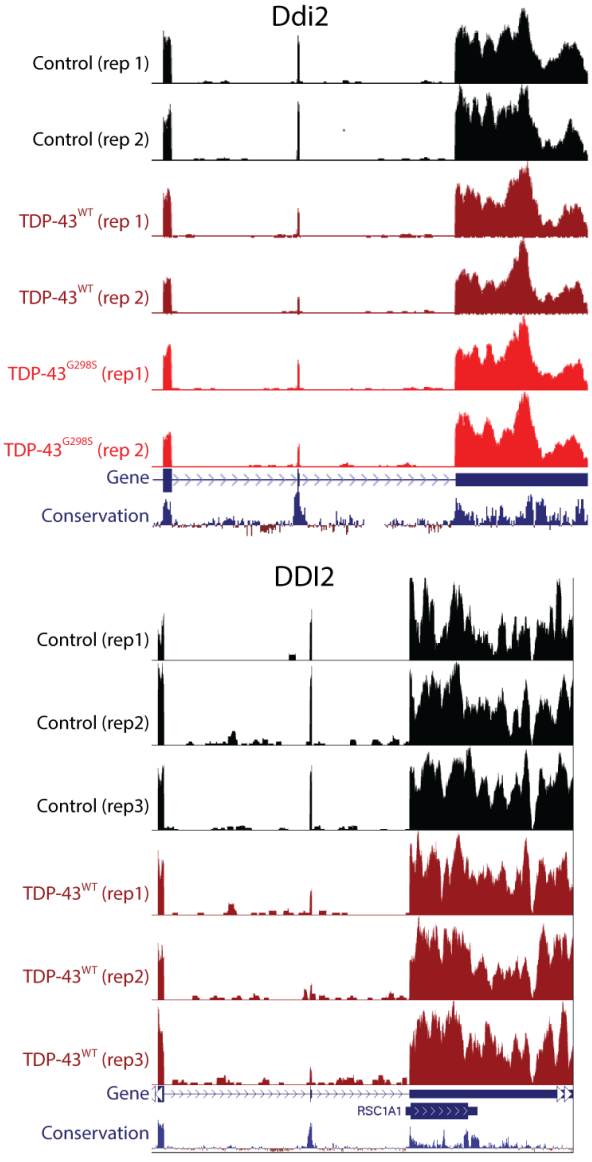
Gene	Control_1	Control_2	G298S_1	G298S_2	TDP43WT_1	TDP43WT_2
Arhgap44	89	78	31	28	13	8
Ddi2	100	96	52	48	64	42
Psme3	90	92	51	46	45	39
Dgkq	100	100	63	55	55	49
Mrpl45	99	99	62	61	49	49
Psmd14	100	100	58	62	53	60
Hjurp	96	95	49	44	75	64
Cops4	90	85	54	56	52	49
Tiam1	71	49	30	21	15	44
Creld1	100	100	72	66	70	61
Inpp4a	67	77	45	41	37	38
Shank1	59	57	27	27	31	27
Pakap	66	61	28	27	36	43
Arhgef11	94	76	71	70	40	43
Osbpl6	64	81	36	51	61	33
Pik3cb	93	100	72	80	71	54
Pdzd4	78	82	59	43	51	62
Dst	37	28	4	8	9	3
Herc2	100	91	76	80	64	58
Golga4	40	33	14	10	11	7
Stk24	56	50	42	23	21	21
Ube3c	100	97	75	76	75	64
C530008M17Rik	64	57	39	33	31	36
Tub	100	80	66	72	56	71
Atp2b1	67	65	41	46	45	38

B

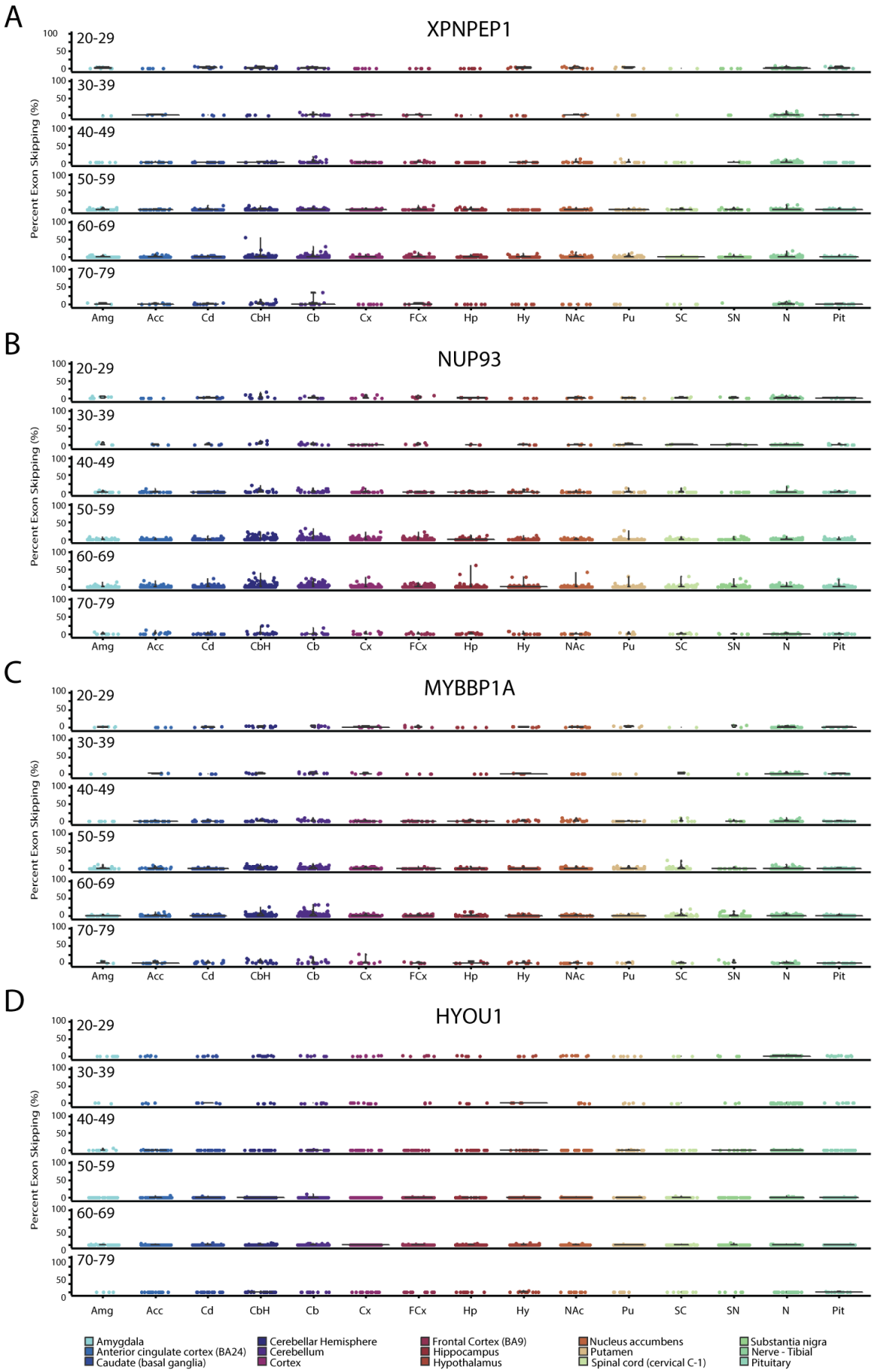
Human

Gene	CONTROL1	CONTROL2	CONTROL3	TDP43WT1	TDP43WT2	TDP43WT3
ADAM23	83	63	64	7	2	23
AMT	100	95	89	11	5	29
ATP9B	100	96	100	-1	44	13
BMPR1A	100	100	100	20	19	26
BRI3BP	100	100	100	42	20	60
CANX	99	100	99	46	37	49
CCDC126	100	79	100	26	36	44
CERT1	70	73	72	17	10	20
CLASP2	94	92	96	28	27	31
COQ5	100	99	99	27	30	19
CSDE1	98	96	97	10	8	11
CSPP1	100	94	100	22	17	38
DDI2	86	96	100	29	13	60
DPY19L1P1	-1	100	100	20	-1	40
DRGX	93	87	100	42	29	45
ELP1	88	88	98	21	31	56
ELP2	96	96	97	27	29	41
ENSG00000256591	100	100	100	40	40	42
ERMAD	100	100	100	27	31	47
FAM102A	100	100	95	-1	-1	-1
FAM66C	73	53	76	0	22	-1
FANCG	83	57	88	14	14	13
FAT1	88	91	79	11	11	20
FBRS	100	98	98	34	25	39
FBXO22	95	89	89	25	28	26
FRAS1	100	100	100	29	38	30
GART	90	94	93	15	10	18
HARS2	98	97	92	27	22	32
HCFC2	100	88	100	31	17	18
HECW1	59	73	75	24	8	9
HIRA	100	99	100	45	41	45
HYOU1	100	100	100	18	16	21
IL17RB	-1	91	71	0	2	0
INSYN1 INSYN1-AS1	-1	75	86	17	20	29
INTS10	84	91	87	26	17	25
ITGB1	100	100	100	7	5	14
KCNMA1	74	59	66	-1	0	7
LGI4	88	85	97	27	20	50
LINC00680-GUSBP4	100	92	69	4	0	0
MYBBP1A	91	86	95	14	14	10
NEBL	-1	100	100	40	-1	33
NHLRC3	-1	100	100	-1	-1	-1
NICN1	85	83	94	29	25	31
NIFK	90	97	98	22	44	52
NIPAL3	59	86	91	2	0	6
NQO2	100	100	100	9	10	44
NRCAM	91	86	93	32	24	27
NUP88	100	93	91	35	36	25
NUP93	100	99	100	32	28	34
PLXNB1	95	97	97	27	21	21
PPA1	100	96	99	29	29	37
PTS	91	86	92	16	11	17
RABGGTB	98	89	96	10	12	23
RCHY1	94	91	93	24	17	13
RHOT2	100	97	91	50	30	38
RNASET2	100	71	89	22	50	20
RNF114	97	94	95	33	17	31
SBF1	81	81	76	15	8	14
SCN3A	100	99	100	51	29	31
SCN9A	100	100	99	33	32	55
SEC61A2	100	100	99	41	39	37
SESN3	100	100	97	53	25	47
SLC17A5	100	86	80	11	11	27
SLC35A5	100	100	100	32	40	26
SPRK2	86	65	84	21	13	29
STC2	100	100	-1	23	29	-1
SUN1	100	96	95	0	4	0
TESK1	100	94	99	34	26	45
TLCD3A	100	92	95	38	29	28
TMEM263	71	59	52	0	14	0
TNR	100	95	95	24	32	31
TP53BP2	75	80	80	24	9	35
TRIM16	100	100	100	-1	-1	-1
TSPAN11	100	94	97	19	16	20
TTBK2	-1	100	100	25	33	57
VARS2	98	98	99	20	14	19
WDR41	88	89	93	4	4	19
WRAP73	100	100	100	38	29	38
WSCD1	100	100	100	45	28	34
XPNPEP1	100	99	100	24	23	26
ZMYND11	78	64	63	2	6	12
ZNF767P	82	88	85	21	20	8

C



Supplementary Figure 3. Differences in exon skipping targets between mouse and human. (**A**) We conducted a comparative analysis of RNA-seq datasets obtained from transgenic lines that overexpressed either TDP-43^{G298S} or TDP-43^{WT} and control littermates. Our analysis revealed that both transgenic lines showed similar profiles of exon repression when compared to the control group. (**B**) Moreover, the comparative analysis of RNA-seq data from i3N human neurons revealed that the set of repressed exons observed in mice differed from those observed in humans. (**C**) Notably, only one target, *Ddi2*, was found to have a repressed exon by TDP-43 overexpression in both mouse and human samples, as shown in the UCSC genome browser plots (Supplemental Data file).


A
XPNPEP1
B
NUP93
C
MYBBP1A
D
HYOU1
20-29
30-39
40-49
50-59
60-69
70-79
Percent Exon Skipping (%)
0
50
100
Amg
Acc
Cd
CbH
Cb
Cx
FCx
Hp
Hy
NAc
Pu
SC
SN
N
Pit
Amygdala
Anterior cingulate cortex (BA24)
Caudate (basal ganglia)
Cerebellar Hemisphere
Cerebellum
Cortex
Frontal Cortex (BA9)
Hippocampus
Hypothalamus
Nucleus accumbens
Putamen
Spinal cord (cervical C-1)
Substantia nigra
Nerve - Tibial
Pituitary

Supplementary Figure 4. Exon skipping events are found in human brain samples. Aging is the main risk factor for developing neurodegenerative diseases. We show that skipping events appear normally in the CNS, but we wanted to explore whether skipping events correlate with aging. We queried the splice junction archive, Snaptron, and analyzed publicly available human RNA-seq datasets from GTEx. We show the percent of exon skipping in four genes at ages ranging from 20-29, 30-39, 40-49, 50-59, 60-69, and 70-79. Skipping events were found in most of the different brain areas analyzed, with a higher frequency in the cerebellum and cortex.