Supplemental Figure

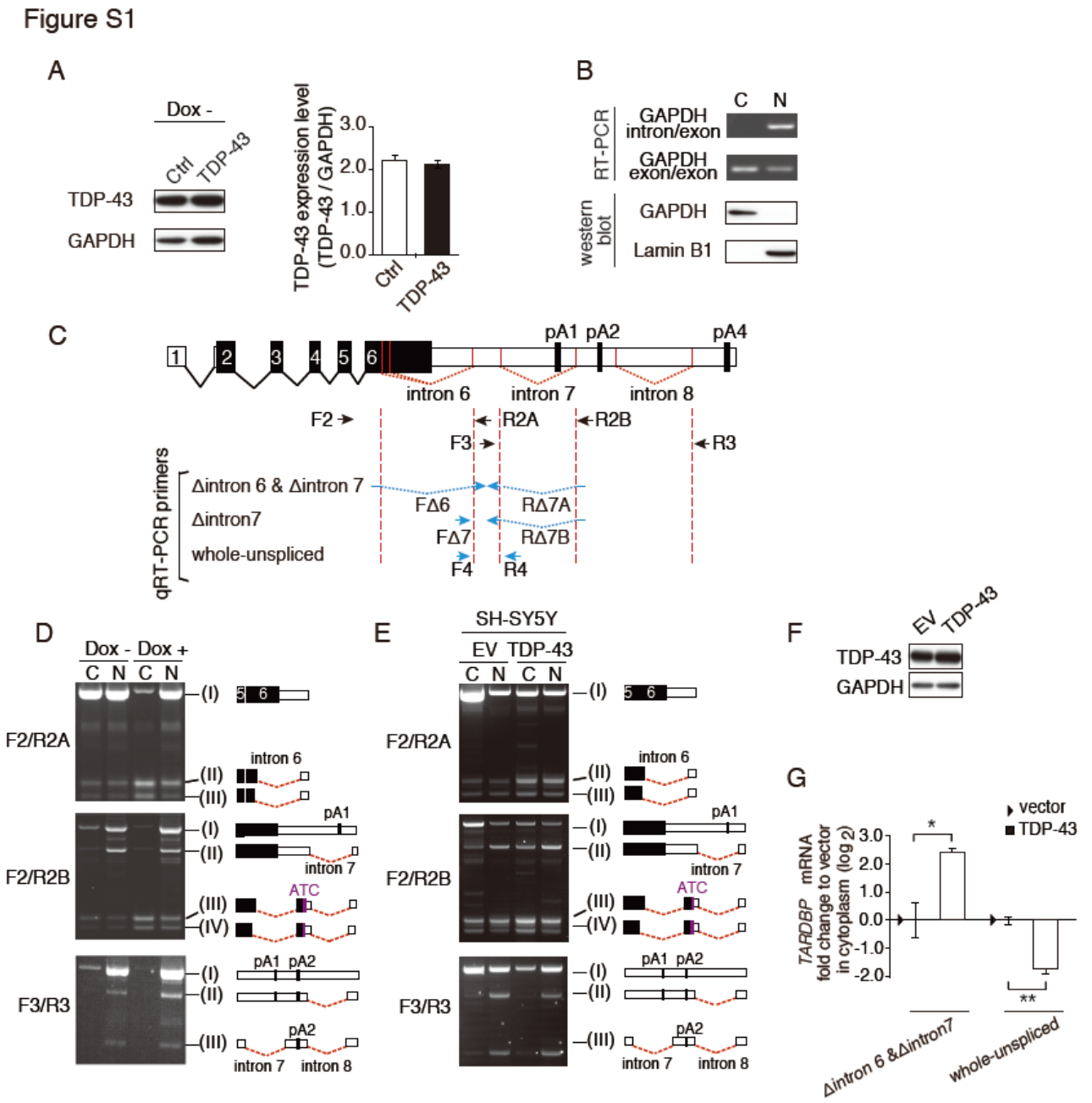

Figure S1. *TARDBP* mRNA splicing induced by ectopic expression of TDP-43. (A) The left panel shows Western blot analysis of Flp-In T-REx™HEK293 cells in which TDP-43 complementary DNA with a myc sequence or aprataxin complementary DNA as a control (Ctrl) were inserted, using an anti-TDP-43 antibody and an anti-GAPDH antibody. The right panel shows a quantitative analysis of TDP-43 without doxycycline (*n* = 5). (B) Separation of the cytoplasm and nucleus. RT-PCR analysis of GAPDH pre-mRNA (upper panel) using primers located in an intron and an exon (Table S2). Western blot analysis using an anti-GAPDH or anti-Lamin B1 antibody (lower panel). C: materials from the cytoplasmic fraction; N: materials from the

nuclear fraction. (C) Schematic representation of *TARDBP* gene and primers. Boxes represent exons; white boxes represent noncoding regions, and black boxes represent coding exons. The arrows represent the primers for RT-PCR (black arrow) and for qPCR (blue arrow) (primer sequences are listed in Table S2). The diagonal dashed lines within arrows indicate the spanning exon. pA: polyadenylation signals. (D) RT-PCR analysis of endogenous *TARDBP* mRNAs extracted from cytoplasmic (C) or nuclear (N) fractions of Flp-In T-REx^{TM} HEK293 with each primer pair. Without doxycycline, isoform I is the most abundant isoform in the cytoplasm as well as in the nucleus in F2/R2A products. In contrast, two of four isoforms in F2/R2B products (isoforms I and II) and all three isoforms in F3/R3 products were abundant in the nucleus. These results indicate that isoforms using the pA2 or pA4 predominantly exist in the nucleus. After induction of ectopic TDP-43, the abundance of the canonical isoform I in the cytoplasm was markedly decreased, whereas its abundance in the nucleus did not decrease. In contrast, the abundances of isoforms lacking both intron 6 and intron 7 increased in the cytoplasm. (E) RT-PCR analysis of endogenous *TARDBP* mRNAs extracted from cytoplasmic (C) or nuclear (N) fractions with each primer pair in SHSY5Y cells. With empty vactor transfection, isoform I is the most abundant isoform in the cytoplasm as well as in the nucleus in F2/R2A products. In ectopic TDP-43 expression, the abundance of the isoform I in the cytoplasm was markedly decreased, whereas its abundance in the nucleus did not decrease. In contrast, the abundances of isoforms lacking both intron 6 and intron 7 increased in the cytoplasm. (F) Western blot analysis of SH-SY5Y cells in which empty vector or TDP-43 complementary DNA were transfected, using an anti-TDP-43 antibody and an anti-GAPDH antibody. (G) The qRT-PCR analysis of the *TARDBP* mRNA isoform lacking both intron 6 and intron 7 (Δintron 6 and Δintron 7) and total *TARDBP* mRNA (whole-unspliced) from the SH-SY5Y cells transfected with the empty vector or TDP-43 expression vector. Data are expressed as fold change (log_2) relative to empty vector (mean ± SEM, n = 3). (*p<0.05, **p<0.01, ***p<0.001, t-test)

Figure S2

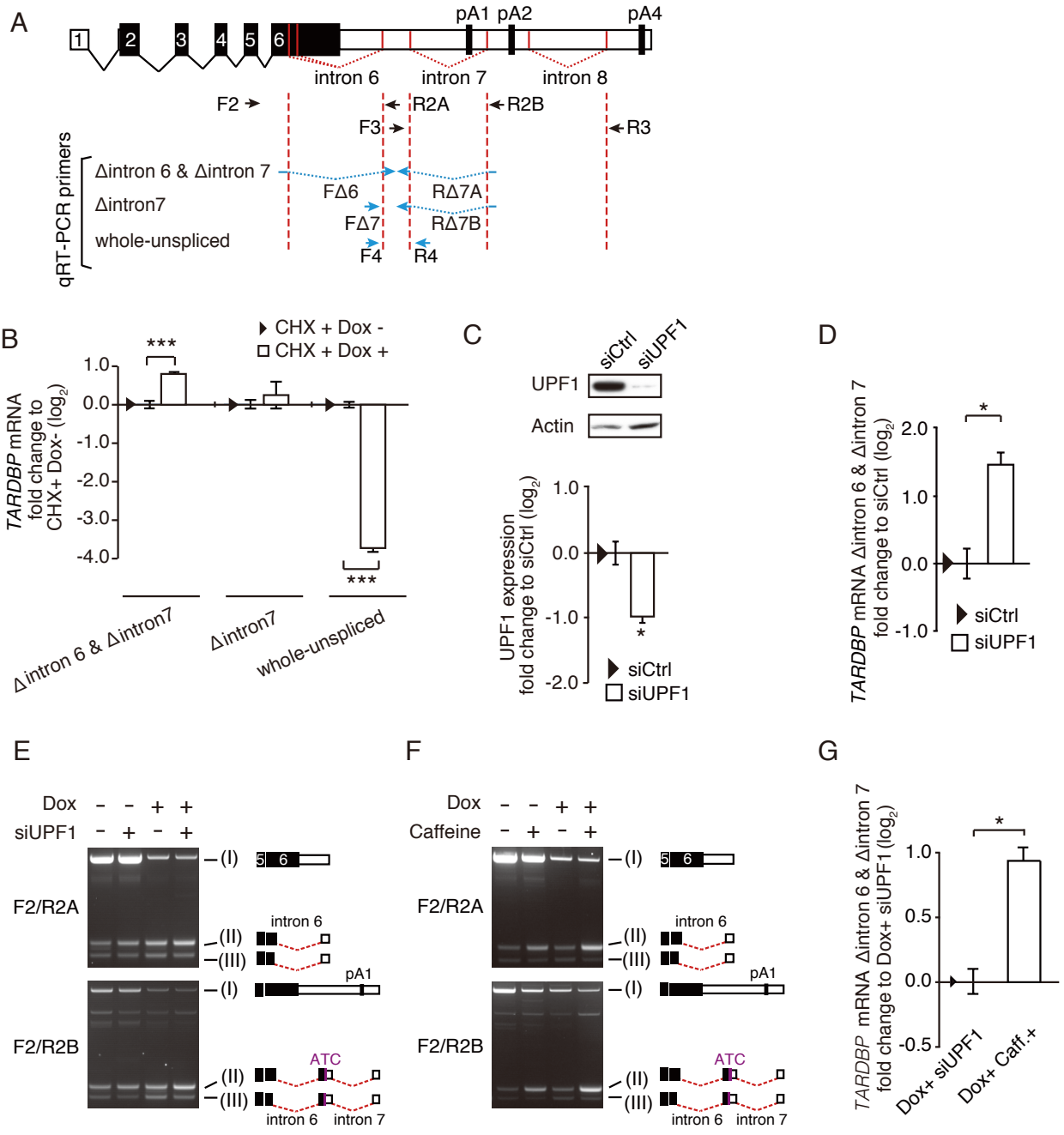

Figure S2. *TARDBP* mRNA lacks both intron 6 and intron 7 and is sensitive to nonsense-mediated mRNA decay. (A) Schematic representation of *TARDBP* gene and primers. Boxes represent exons; white boxes represent noncoding regions, and black boxes represent coding exons. Arrows indicate primers. The arrows represent the primers for RT-PCR (black arrow) and for qPCR (blue arrow) (primer sequences are listed in Table S2). The diagonal dashed lines within arrows indicate the spanning exon. pA: polyadenylation signals. (B) qRT-PCR analysis of *TARDBP* mRNA isoforms from the cytoplasmic fraction of TDP-43 tet-inducible Flp-

In T-REx™ HEK293 cells under cyclohexamide treatment (CHX). Data are expressed as fold change (log_2) of the values without doxycycline (n = 3). (***P < 0.001, Tukey's multiple comparison test). (C) Western blot analysis (upper panel) and qRT-PCR analysis of UPF1 (lower panel) in HEK293T cells with UPF1 siRNA (siUPF1) or control siRNA (siCtrl). (D, G) The isoform levels lack both intron 6 and intron 7 under depletion of UPF1 (D) or with caffeine (G). Data are expressed as fold change (log_2) of control siRNA transfection (n = 3). Data information: Data are presented as mean ± SEM. Asterisks indicate statistically significant (P < 0.05, t-test) differences. (E, F) RT-PCR analysis of endogenous *TARDBP* mRNAs extracted from Flp-In T-REx™HEK293 cells in which TDP-43 complementary DNA were inserted with each primer pair. (E) In the presence of doxycycline, isoform I decreased and isoform II increased with a depletion of UPF1. (F) The increase in isoform II with caffeine is more prominent than that in a UPF1 depletion (also see Fig S2G).

Figure S3

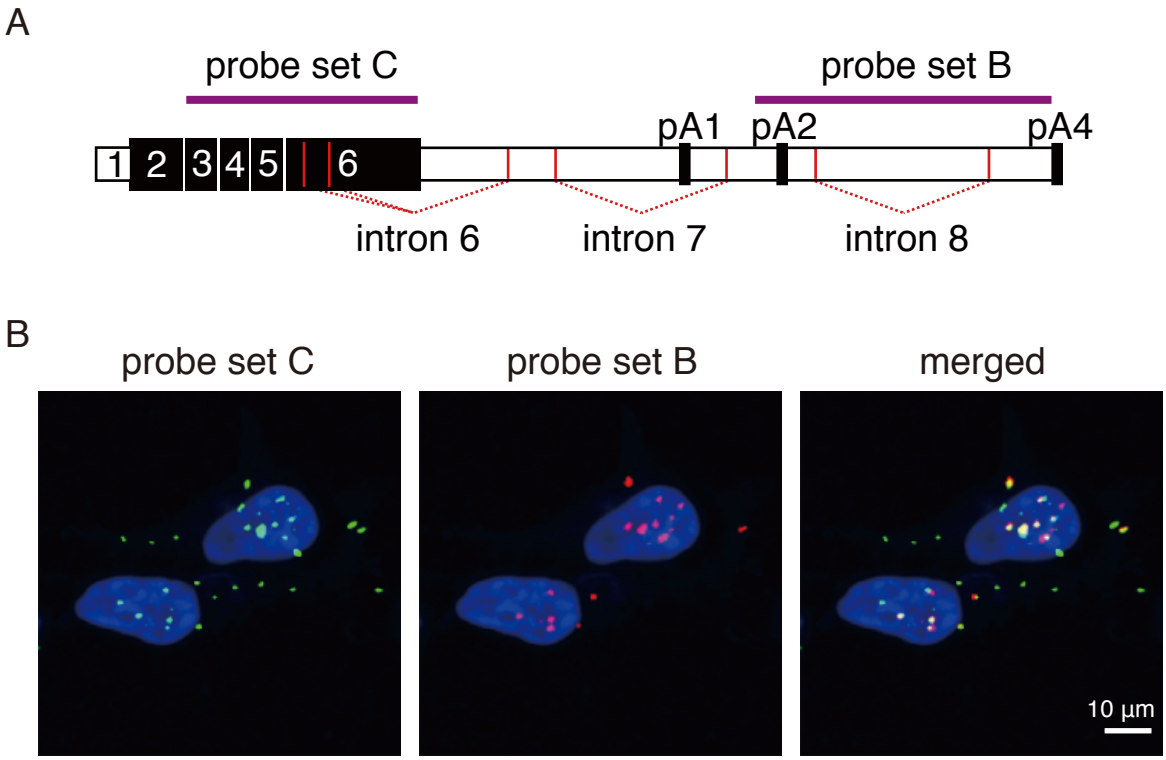

Figure S3. Evaluation of specificity for *in situ* hybridization probes. (A) The location of the probe set of QuantiGene® ViewRNA for *in situ* hybridization. Purple lines represent probes. Probe set C: targeting nucleotides 373-1327 of the human *TARDBP* mRNA(NM_007375). Probe set B: targeting nucleotides 2795–4198 (NM_007375)). Boxes indicate exons. Black boxes indicate coding region. Vertical red lines indicate the splicing sites. The diagonal red dashed lines indicate the splicing between exons. Vertical bold black lines indicate the polyadenylation signal (pA). (B) *In situ* hybridization with probe set C (green) or probe set B (red) for the HEK293T cells. The yellow spots in merged images have both introns. As expected, probe set C detected spots in both the cytoplasm and nucleus, whereas probe set B detected spots preferentially in the nucleus. Importantly, most of the spots detected by probe set B were concomitantly detected by probe set C. The nuclei were stained with Hoechst 33342 (blue). Scale bar: 10 µm.

Figure S4

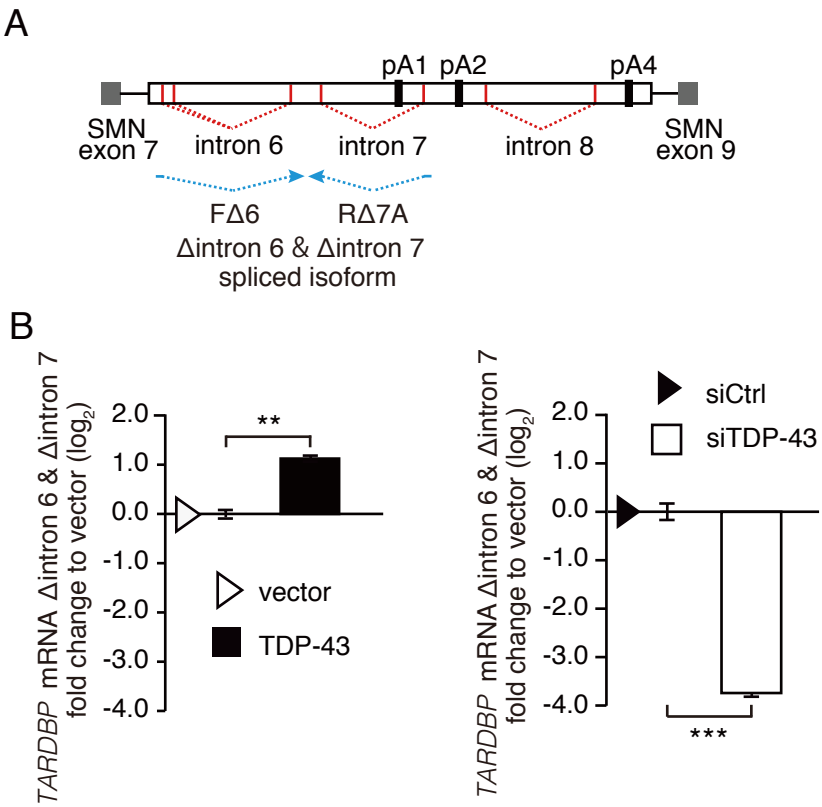

Figure S4. The depletion of TDP-43 inhibits the splicing of exon 6 of *TARDBP* mRNA. (A) Schematic representation of a minigene containing exon 6 of the human *TARDBP* gene and location of the primers for the spliced isoform (Δintron 6 and Δintron 7). Gray boxes represent exons of the *SMN* gene. The lines represent introns. Vertical red lines indicate the splicing sites. The diagonal red dashed lines indicate the splicing between exons. The arrows represent the primers and the diagonal dashed lines within arrows indicate the spanning exon. Vertical bold black lines indicate the polyadenylation signal (pA). (B) The qRT-PCR analysis of the *TARDBP* mRNA isoform lacking both intron 6 and intron 7 (Δintron 6 and Δintron 7 spliced isoform) from the cytoplasmic fraction of HEK293T cells transfected with the minigene and TDP-43 expression vector (left panel) or TDP-43 siRNA (right panel). Data are expressed as fold change (log_2) relative to control (mean ± SEM, n = 3). (**p<0.01, ***p<0.001, t-test)

Figure S5

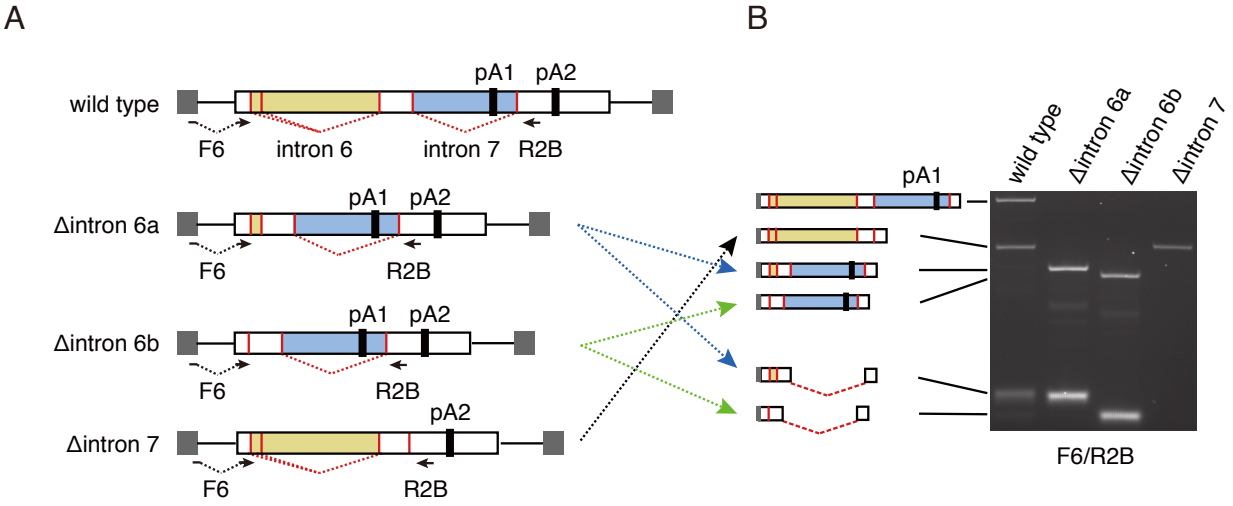

Figure S5. Intron 7 is necessary for the splicing of intron 6. (A) Schematic representation of the minigene constructs containing wild-type exon 6 or exon 6 with deletion of intron 6 or intron 7 of the human *TARDBP* gene and the location of the primers for amplification. Gray boxes represent exons of the *SMN* gene. White boxes represent a part of exon 6 of the *TARDBP* gene. Yellow boxes represent intron 6, and blue boxes represent intron 7. The arrows represent the primers (Table S2), and the diagonal dashed line within the arrows indicates the spanning exon. Vertical red lines indicate the splicing sites. The diagonal red lines indicate splicing between exons. Vertical bold black lines indicate the polyadenylation signal (pA). The Δintron 6a minigene construct contained a deletion of intron 6 at the 833 donor site. The Δintron 6b construct contained a deletion of intron 6 at the 769 donor site. The Δintron 7 minigene construct contained a deletion of intron 7. (B) The results of RT-PCR analysis of RNAs derived from HEK293T cells co-transfected with minigene and TDP-43 cDNA expression vector. The results of the RT-PCR analysis are shown in the right panel, and the left panel shows a schematic representation of each PCR product. Dashed arrows between A and B indicate the correlation between the constructs and their products.

Figure S6

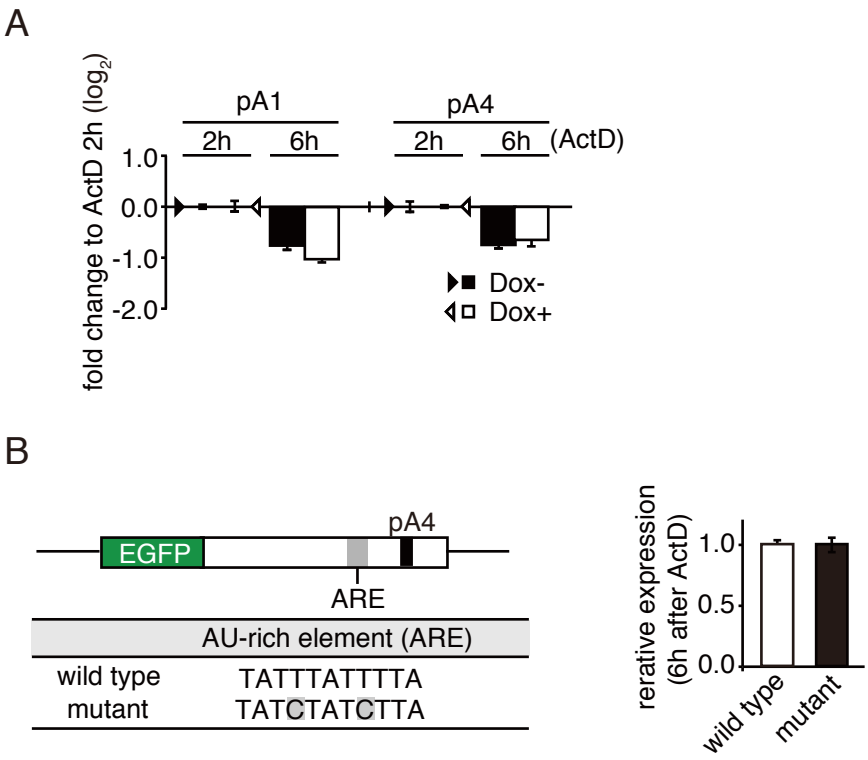

Figure S6. The 3´-UTR sequence of *TARDBP* mRNA does not contribute its stability. (A) The stability of each mRNA isoform (pA1 and pA4) after actinomycin D (ActD) treatment. TDP-43 tet-inducible Flp-In T-REx™ HEK293 cells cultured in the absence or presence of doxycycline (Dox) were treated with ActD for 2 to 6 hours. The levels of each isoform at 6 hours were compared to those at 2 hours by qRT-PCR. Data (mean ± SEM, n = 3) are expressed as fold change ($\log_2$) of the levels of each isoform at 2 hours. There was no statistical difference between the levels of pA1 and pA4 isoforms. (B) The stability of EGFP-*TARDBP*-3´-UTR fused RNA with or without an AU-rich element. (left panel). We fused the TDP-43 3´-UTR from pA2 to pA4 to the C-terminus of EGFP. We then introduced a nucleotide substitution in the putative adenylate uridylate-rich element (ARE: gray box; mutant) (right panel). These constructs were transfected into HEK293T cells, which were then treated with ActD for 6 hours. The levels of fused mRNA derived from the mutant minigene were compared to those derived from the wild-type minigene. Data (mean ± SEM, n = 3) are expressed as fold change to wild-type. No statistical difference was shown by a t-test.

Figure S7

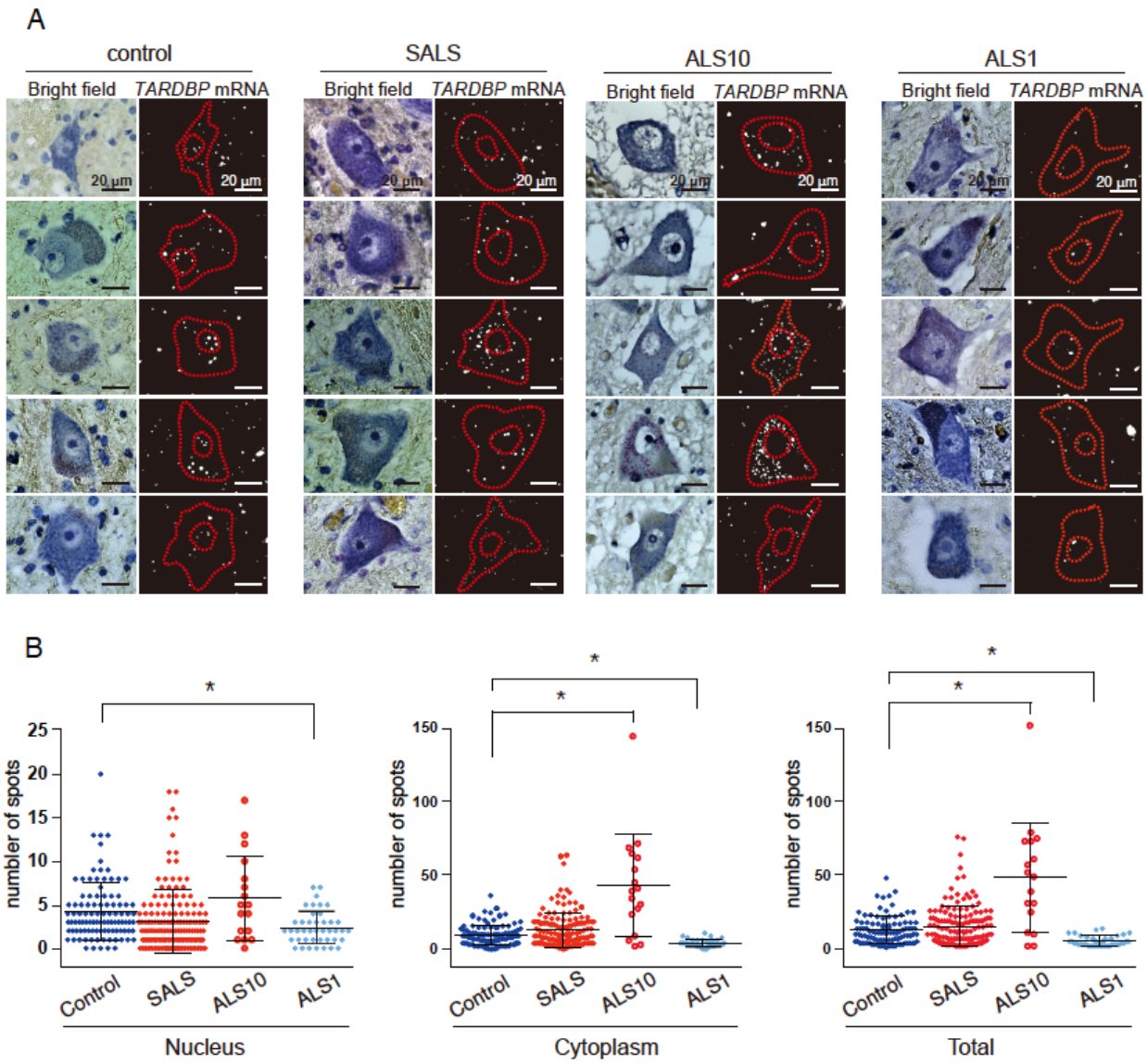

Figure S7. Alteration of TARDBP mRNA intracellular distribution in spinal cord motor neurons of ALS. (A) Quantitative in situ hybridization for the spinal motor neurons from lumbar spinal cord of controls, SALS cases, a familial ALS case carrying a mutation in *TARDBP* (ALS10: p.Gln343Arg) (1), and familial ALS cases carrying mutations in *SOD-1* (ALS1: p.Asp101Tyr and p.Ala4Thr) (2-4). Table S5 shows details of the patients and controls included in this study. Left panels: Bright field image of hematoxylin staining. Right panels: In situ hybridization detection for TARDBP mRNA (white). Red dashed lines represent outlines of the displayed motor neurons and its nucleus. Scale bar, 20 μm. (B) Scatter gram of the number of spots for *TARDBP* mRNA in spinal motor neurons from control patients (n = 100), sporadic ALS patients (n = 154), ALS10 patients (n = 17), and ALS1 patients (n = 42). The lines and error bars represent the mean ± SEM.

Asterisks indicate statistically significant ($P < 0.01$, Steel-Dwass nonparametric multiple comparison test following Kruskal-Wallis nonparametric ANOVA) differences.

1. Yokoseki, A., Shiga, A., Tan, C.F., Tagawa, A., Kaneko, H., Koyama, A., Eguchi, H., Tsujino, A., Ikeuchi, T., Kakita, A. *et al.* (2008) TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann. Neurol.*, **63**, 538-542.
2. Takahashi, H., Makifuchi, T., Nakano, R., Sato, S., Inuzuka, T., Sakimura, K., Mishina, M., Honma, Y., Tsuji, S. and Ikuta, F. (1994) Familial amyotrophic lateral sclerosis with a mutation in the Cu/Zn superoxide dismutase gene. *Acta Neuropathol.*, **88**, 185-188.
3. Tan, C.F., Eguchi, H., Tagawa, A., Onodera, O., Iwasaki, T., Tsujino, A., Nishizawa, M., Kakita, A. and Takahashi, H. (2007) TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. *Acta Neuropathol.*, **113**, 535-542.
4. Tan, C.F., Piao, Y.S., Hayashi, S., Obata, H., Umeda, Y., Sato, M., Fukushima, T., Nakano, R., Tsuji, S. and Takahashi, H. (2004) Familial amyotrophic lateral sclerosis with bulbar onset and a novel Asp101Tyr Cu/Zn superoxide dismutase gene mutation. *Acta Neuropathol.*, **108**, 332-336.

Table S1. TDP-43 polyadenylation signal sites and splicing sites.

Polyadenylation signal sites

Polyadenylation site	Position
polyA 1	c.2615–2620
polyA 2	c.2754–2759
polyA 4	c.4059–4064

Splicing sites

	Alternative splice site		Intron consensus sequence
	Donor site	Acceptor site	
Intron 6	c.769	c.1783	at-ag
	c.833	c.1783	gt-ag
	c.842	c.1783	gt-ag
Intron 7	c.1921	c.2654	gt-ag
Intron 8	c.2927	c.3700	gt-ag

The number of nucleotides +1 in the coding DNA reference sequence is the A of the ATG translation initiation codon.

Table S2. List of PCR primers and siRNA probe sequences

Constructs

Construct name	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)
Flp-In TDP-43-myc wild-type	AAAGGATCCATGTCTGAATATATTCGGGTA	TTTCTCGAGCTACAGATCCTCTTCTGAGATGAGTTTTTGTTCCATTC CCCAGCCAGAAGACTT
Minigene (pA2)	GGGCCTAGGCTGTGTTGTCTAGGCTGCCAG	GGGCCTAGGCTTCCAACAGATGTGGATGTC
Minigene2 (pA4)	GGGCCTAGGCTGTGTTGTCTAGGCTGCCAG	GGGCCTAGGACTGTGAGTCTTGGCCTCATTTCTCTTGTGCATATG
EGFP-fused minigene	GGGCTCGAGATAATTCCCCCACCACC	GGGAAGCTTTTAATCAAGAAGAGTTACCC
Δintron 6a minigene	CACCAAATCTTCCACTTCTTTC	TTCATCTCATTTCAAATGTTTATGGAAG
Δintron 6b minigene	ATGAACGCTGATTCCTTTAATGATC	TTCATCTCATTTCAAATGTTTATGGAAG
Δintron 7 minigene	CGTCAAAGACGCGGCCTGTG	CCCTGAATGCAAAGAATTCATAGTAG
EGFP-fused AU-rich mutant	CATAGATACGGGTATCTATCTTACCCTAAG	ATACCCGTATCTATGCAGTACCATA
Intron 6 donor (769) mutant	GAATCAGCGTTCATCCATCCAATGCCGAAC	GTTCGGCATTGGATGGATGAACGCTGATTC
Intron 6 donor (833/842) mutant	GTGGAAGATTTGGTGCCAATCCAGCCGGCTTTGGGAATC	GATTCCCAAAGCCGGCTGGATTGGCACCAAATCTTCCAC
Intron 6 acceptor (1783) mutant	ATTTAATCTCTGCCCTTCATCTCATTTC	GAAATGAGATGAAGGGCAGAGATTAAAT
Intron 7 donor (1921) mutant	CCGCGTCTTTGACGGGGGGTGTCCCATTTT	AAAATGGGACACCCCCCGTCAAAGACGCGG

Northern blotting

Probe name	Sense primer sequence (5′ to 3′)	Antisense primer sequence (5′ to 3′)
Northern probe A	AATTAACCCTCACTAAAGGGCGGGTAACCGAAGATGAGAA	TAATACGACTCACTATAGGGCAGTCACACCATCGTCCATC
Northern probe B	AATTAACCCTCACTAAAGGGGGGTTGTGGTTGGTTGGTAT	TAATACGACTCACTATAGGGGATCCATATGGTGGGGTTCAA

Northern probe C	AATTAACCCTCACTAAAGGGCTATGAACGCAAGGCTGTGA	TAATACGACTCACTATAGGGGGACACAGAACTGCAGCAAA
Northern probe for isoform III	AATTAACCCTCACTAAAGGGTTCATCTCATTTCAAATGTTTATG	TAATACGACTCACTATAGGGCATTTTAGGTGCGGTCAC
Northern minigene	AATTAACCCTCACTAAAGGGGGGAGACCCAAGCTTATGAA	TAATACGACTCACTATAGGGGGATTACCACCAAATCTTCCA
Northern GAPDH	AATTAACCCTCACTAAAGGGACACCCACTCCTCCACCTTT	TAATACGACTCACTATAGGGTGGTTGAGCACAGGGTACTTT

RT-PCR

Name of primer	Primer sequence (5′ to 3′)	Appears in article
F1	ATCGACTGGGACCTATCACG	Fig. 1A, 1C
R1	ATGGGCTCATCGTTCTCATC	Fig. 1A, 1C
F2	GCGCTGTACAGAGGACATGA	Fig. 1A, 1C, S1C, S1D, S1E, S2A, S2E, S2F
R2A	GCCTGTGATGCGTGATGA	Fig. 1A, 1C, S1C, S1D, S1E, S2A, S2E, S2F
GAPDH intron F	AAGGGCCCTGACAACTCTTT	Fig. 1C, S1B
GAPDH exon R	TGGTTGAGCACAGGGTACTTT	Fig. 1C, S1B
GAPDH exon F	ACACCCACTCCTCCACCTTT	Fig. S1B
R2B	AGTTCCATCTCAAAAGGGTC	Fig. S1C, S1D, S1E, S2A, S2E, S2F, 4G, 4H, S5A, S5B
F3	ATTCGTCATCACGCATCACA	Fig. S1C, S1D, S1E
R3	ATGAATGCATTTAGATTGACCA	Fig. S1C, S1D, S1E
FΔ6	AAAGAAGTGGAAGATTTGGTGTTC	Fig. S1C, S1G, S2A, S2B, S2D, S2G, S4A, S4B, S4C, 7A, 7B
RΔ7A	TCTTTGCATTCAGGGCGTC	Fig. S1C, S1G, S2A, S2B, S2D, S2G, S4A, S4B, S4C, 7A, 7B
FΔ7	TCATGGTGTCACAGTGTTTGG	Fig. S2A, S2B,
RΔ7B	TTGCATTCAGGGCGTCA	Fig. S2A, S2B
F4	TGTCACAGTGTTTGGTTCTTTTG	Fig. S1C, S1G, S2A, S2B, 2A, 2D, 5A, 5B, 5C
R4	AGCGGATAAAAATGGGACAC	Fig. S1C, S1G, S2A, S2B, 2A, 2D, 5A, 5B, 5C
RPLP1 F and R	Purchased (Takara bio) primer set ID: HA067802	Fig. S1G, S2B, S2C, S2D, S2G, S4B, S6A 6B, 6C, 7B

RPLP2 F and R	Purchased (Takara bio) primer set ID: HA067804	Fig. S1G, S2B, S2C, S2D, S2G, S4B, S6A, 6B, 6C
UPF1 F and R	Purchased (Takara bio) primer set ID: HA115956	Fig. S2C
F5	CCAAACCCTGTACCATCTGAA	Fig. 2A, 2D
R5	TCTTTGGAGGTCCCCGAGTA	Fig. 2A, 2D
F6	CATACTGGCTATTATATGATTG	Fig. 4G, 4H, S5A, S5B
TDP-43 ex5/ex6 F	CTGCGGGAGTTCTTCTCTCA	Fig. 4B, 4H, 7A, 7B
TDP-43 ex5/ex6 R	CGCAATCTGATCATCTGCAA	Fig. 4B, 4H, 7A, 7B
P7-t25-vn	CAAGCAGAAGACGGCATACGAGATTTTTTTTTTTTTTTTTTTTTTTTTVN	Fig. 5A, 5B, 5C
F7	TTTGCTGCAGTTCTGTGTCC	Fig. 5A, 5B, 5C, 5D, S6A, 6B
R7A	CAAGCAGAAGACGGCATACG	Fig. 5A, 5B, S6A, 6B
F8	ATCTCCATTGGGAAGTCATGC	Fig. 5A, 5B, S6A, 6B
R8	CCCAAACTAGGAAGGTGCTG	Fig. 5A, 5B, S6A, 6B
F9	GCAGGAGGACTTGAAGCAGA	Fig. 5C, 5D
R9	CAGAACTGCAGCAAACAGCA	Fig. 5C, 5D
R7B	AAAAAGGGGAATTAACTGCTATGAA	Fig. 5C, 5D
pA4 primer F	TTAGGGTGGGTTGTCTGTCTG	Fig. 6A
pA4 primer R	AGGCCACTCAACATTGAAAGC	Fig. 6A
F10	CCCACTGCTTAACTGGCTTATC	Fig. 6C
R10	GCATCAGCATCATCAAGAGAATC	Fig. 6C

siRNA

Name of siRNA	Sequence (5’ to 3’)
siTDP-43	CAAGAAAGAUCUUAAGACU
siControl 1	Purchased (negative control siRNA, catalog no. S20C0600; Cosmobio)

siUPF-1	Purchased (FlexiTube siRNA UPF-1, catalog no. GS5976; Qiagen)
siControl 2	Purchased (AllStar negative control siRNA, catalog no. 1027281; Qiagen)

Table S3. Neurons without *TARDBP* mRNA in the nucleus

	Neurons without *TARDBP* mRNA in the nucleus, %	Neurons without *TARDBP* mRNA in the nucleus, no.	Neurons with *TARDBP* mRNA in the nucleus, no.
Control	5.00	5	95
SALS	20.78	32	122
ALS10	5.88	1	16
ALS1	16.67	7	35

The proportion of neurons without *TARDBP* mRNA in the nucleus in each group was compared using Ryan's multiple comparison test. The proportions of neurons without *TARDBP* mRNA in the nucleus were significantly increased in SALS cells compared to controls. $p = 0.00046$.

Table S4. Correlation analysis of soma area and the levels of nuclear or cytoplasmic *TARDBP* mRNA

Control

	Pearson correlation coefficient		
	Soma area (μm^2)	Nuclear *TARDBP* mRNA	Cytoplasmic *TARDBP* mRNA
Soma area (μm^2)	—	0.490**	0.513**
Nuclear *TARDBP* mRNA	0.490**	—	0.536**
Cytoplasmic *TARDBP* mRNA	0.513**	0.536**	—

**Correlation is significant at $p < 0.01$ (2-tailed).

SALS

	Pearson correlation coefficient		
	Soma area (μm^2)	Nuclear *TARDBP* mRNA	Cytoplasmic *TARDBP* mRNA
Soma area (μm^2)	—	0.191*	0.418**
Nuclear *TARDBP* mRNA	0.191*	—	0.471**
Cytoplasmic *TARDBP* mRNA	0.418**	0.471**	—

**Correlation is significant at $p < 0.01$ (2-tailed).
*Correlation is significant at $p < 0.05$ (2-tailed).

ALS10

	Pearson correlation coefficient		
	Soma area (μm^2)	Nuclear *TARDBP* mRNA	Cytoplasmic *TARDBP* mRNA
Soma area (μm^2)	—	0.534*	0.494*
Nuclear *TARDBP* mRNA	0.534*	—	0.278
Cytoplasmic *TARDBP* mRNA	0.494*	0.278	—

*Correlation is significant at $p < 0.05$ (2-tailed).

ALS1

	Pearson Correlation Coefficient		
	Soma area (μm^2)	Nuclear *TARDBP* mRNA	Cytoplasmic *TARDBP* mRNA
Soma area (μm^2)	—	−0.016	0.270
Nuclear *TARDBP* mRNA	−0.016	—	0.140
Cytoplasmic *TARDBP* mRNA	0.270	0.140	—

Table S5. Clinical data for samples used for qRT-PCR analysis

	Disease	Age, years	Sex	PMI, hours
ALS	ALS	83	M	12.5
	ALS	61	M	4
	ALS	63	M	3
	ALS	61	F	4
	ALS	83	F	2
	ALS	76	M	1.5
	ALS	45	F	6.5
Control	Adult T cell Leukemia	55	F	4
	Gastrointestinal bleeding	76	M	3.5
	Myasthenia gravis	82	F	4.5
	Cushing syndrome	75	F	1.5
	Renal failure	64	M	2
	Abdominal hemorrhage	80	M	3

PMI: postmortem interval; M: male; F: female.

Table S6. Clinical data for samples used for *in situ* hybridization analysis

PMI: postmortem interval; M: male; F: female.

		Disease	Age, years	Sex	PMI, hours
SALS	A1	ALS	76	M	4
	A2	ALS	76	M	2.5
	A3	ALS	77	F	1.5
	A4	ALS	75	M	1.5
	A5	ALS	57	M	2
	A6	ALS	58	M	2
	A7	ALS	61	M	2.5
	A8	ALS	73	M	2
	A9	ALS	74	F	3
	A10	ALS	84	F	15
Control	C1	Cerebral infarction	70	F	4
	C2	Intracerebral hemorrhage	49	F	2
	C3	Gastrointestinal bleeding	76	M	3.5
	C4	Abdominal hemorrhage	80	M	3
	C5	Myotonic dystrophy	66	M	4
	C6	Facioscapulohumeral muscular dystrophy	68	F	4
ALS10 (1, 3)	A10-1	ALS10 (p.Gln343Arg)	77	F	9.5
ALS1 (2, 4)	A1-1	ALS1 (p.Asp101Tyr)	59	M	5
	A1-2	ALS1 (p.Ala4Thr)	61	M	12

1. Yokoseki, A., Shiga, A., Tan, C.F., Tagawa, A., Kaneko, H., Koyama, A., Eguchi, H., Tsujino, A., Ikeuchi, T., Kakita, A. *et al.* (2008) TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann. Neurol.*, **63**, 538-542.
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3. Tan, C.F., Eguchi, H., Tagawa, A., Onodera, O., Iwasaki, T., Tsujino, A., Nishizawa, M., Kakita, A. and Takahashi, H. (2007) TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. *Acta Neuropathol.*, **113**, 535-542.
4. Tan, C.F., Piao, Y.S., Hayashi, S., Obata, H., Umeda, Y., Sato, M., Fukushima, T., Nakano, R., Tsuji, S. and Takahashi, H. (2004) Familial amyotrophic lateral sclerosis with bulbar onset and a novel Asp101Tyr Cu/Zn superoxide dismutase gene mutation. *Acta Neuropathol.*, **108**, 332-336.