Supplemental information

Characterization of the upstream and intron promoters of the gene encoding TAR DNA-binding protein.

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Figure S1

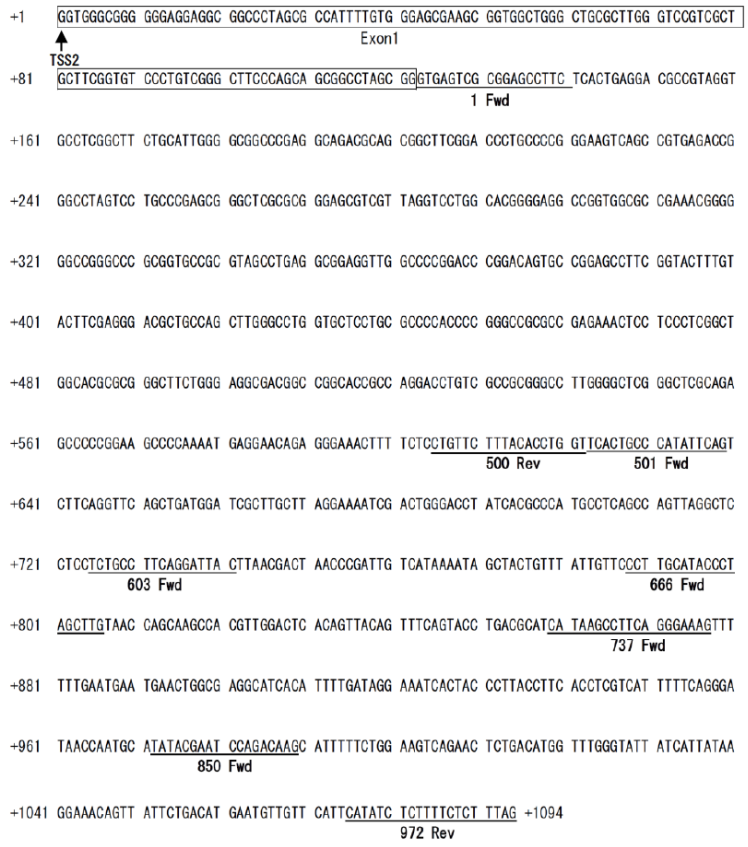

Figure S1. *TARDBP* exon 1 and intron 1 sequences and primers. *TARDBP* noncoding exon 1 (+1 to +122), and all 972 nucleotides of *TARDBP* intron 1, (+123 to +1094), with respect to TSS2. The highlighted sequences indicate exon 1. Underlining denotes primer sequences. Fwd; forward primer, Rev; reverse primer.

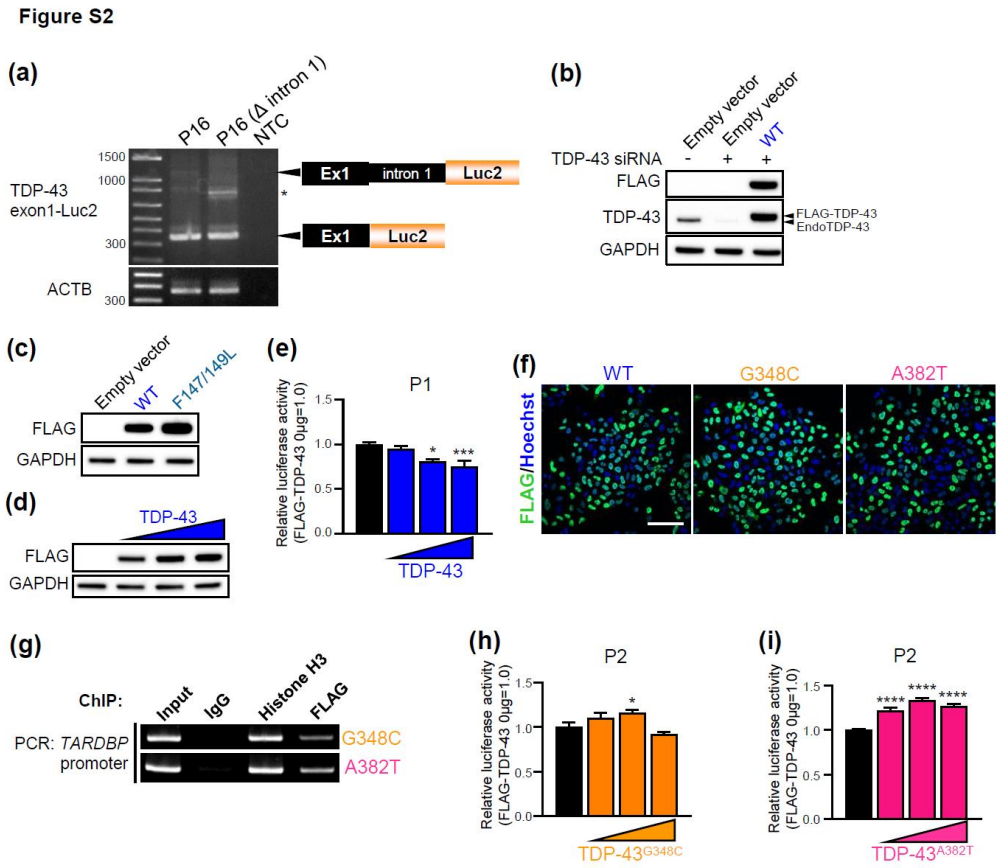

Figure S2. TDP-43 affects the activity of the *TARDBP* upstream and intron 1 promoters. (**a**) RT-PCR analysis of *TARDBP* exon1 to Luc2 and actin-beta mRNA expression in HeLaS3 cells overexpressing P16 or P16 (Δintron 1). Forward and reverse primers are set at *TARDBP* exon1 and Luc2 to detect intron 1 splicing. Amplicon length are following; non-spliced product (exon1-intron 1-Luc2); 1294bp and spliced product (exon1-Luc2); 322bp, respectively. Asterisk indicates non-specific band. (**b-d**) Immunoblot analysis of endogenous TDP-43 KD and exogenous FLAG-WT TDP-43 overexpression (**b**), FLAG-WT TDP-43 or mutant TDP-43 (F147/149L) overexpression (**c**) and FLAG-WT TDP-43 dose-dependent overexpression (**d**) in HeLaS3 cells. Mock siRNA and/or Empty vector was transfected as the negative control. (**e**) Luciferase assay of P1 (upstream promoter) in HeLaS3 cells overexpressing dose-dependent FLAG-WT TDP-43. n=9 replicates; one-way ANOVA with *post hoc* Dunnett's test relative to 0μg of TDP-43/pFLAG-CMV2. (**f**) Immunocytochemistry images of FLAG-TDP-43 (WT, G348C or A382T) overexpressing HeLaS3 cells after 24hr from transfection. Nuclei were stained with Hoechst (blue). Scale bar: 100 μm. (**g**) ChIP assay of overexpressed 2 pathological mutant TDP-43 (G348C or A382T) binding to the *TARDBP* promoter in HEK293T cells. Immunoprecipitation was performed using anti-DDDDK (FLAG) antibody, then PCR was conducted using primer detecting

TARDBP -721 to -431 (290bp). Control IgG and Histone H3 antibody are used for negative or positive control for immunoprecipitation. (**h, i**) Luciferase assay of P2 (intron 1 promoter) in HeLaS3 cells overexpressing dose-dependent FLAG-TDP-43 (G348C) (**h**) and FLAG-TDP-43 (A382T) (**i**). n=6 replicates; one-way ANOVA with *post hoc* Dunnett's test relative to 0µg of TDP-43/pFLAG-CMV2. All samples transfected 2 µg of reporter vector and 2 µg of pFLAG-CMV2 vector (empty vector + TDP-43). All error bars indicate the means ± SEMs. **P*<0.05, ****P*<0.001, **** *P*<0.0001.

Figure S3

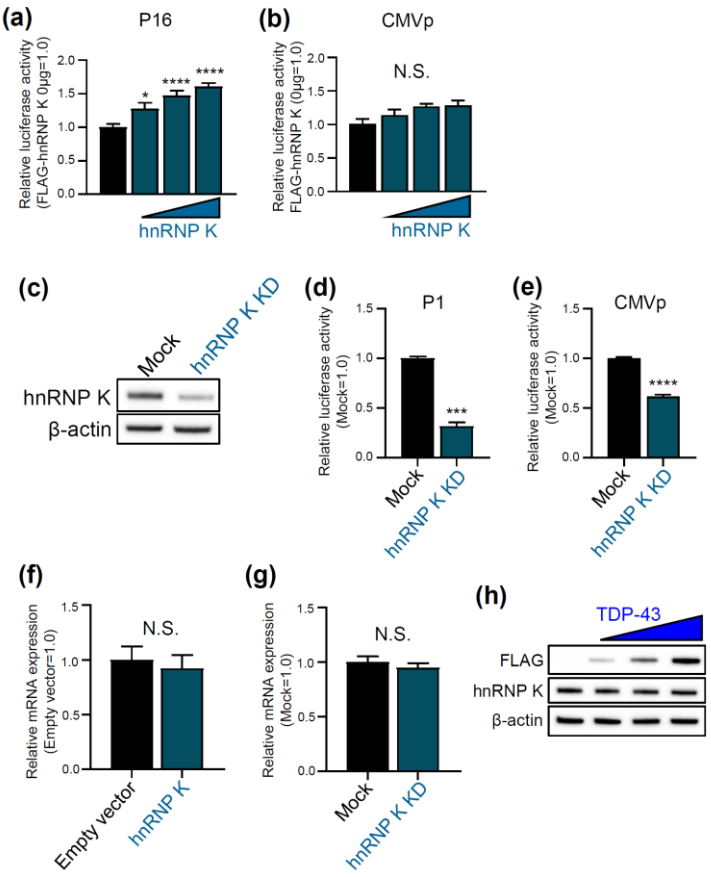

Figure S3. hnRNP K does not affect intron 1 or CMV promoter activity. (**a, b**) Dose-dependent effects of hnRNP K on P16 (**a**) or the CMV promoter (**b**). n=9 (**a**), n=3 (**b**) replicates, one-way ANOVA with *post hoc* Dunnett's test relative to 0μg of hnRNP K/pFLAG-CMV2. All samples transfected 2 μg of reporter vector and 2 μg of pFLAG-CMV2 vector (empty vector + hnRNP K). (**c**) Immunoblot analysis of hnRNP K in HeLaS3 cells. Mock and KD indicate transfected negative control or hnRNP K siRNA, respectively. (**d, e**) Luciferase assay of P1 (**d**) or CMV promoter (**e**) activity with hnRNP K KD. n=3 (P1) (**d**), n=4 (CMVp) (**e**) replicates; Welch's t-test relative to Mock. (**f, g**) mRNA expression of endogenous TDP-43 with FLAG-hnRNP K overexpression (**f**) or hnRNP K KD (**g**). n=3 replicates, Welch's t-test relative to Empty vector (**f**) or Mock (**g**). (**h**) Immunoblot analysis of hnRNP K with dose-dependent overexpression of FLAG-WT TDP-43. All error bars indicate the means ± SEMs. **P*<0.05, ****P*<0.001, **** *P*<0.0001.

Figure S4

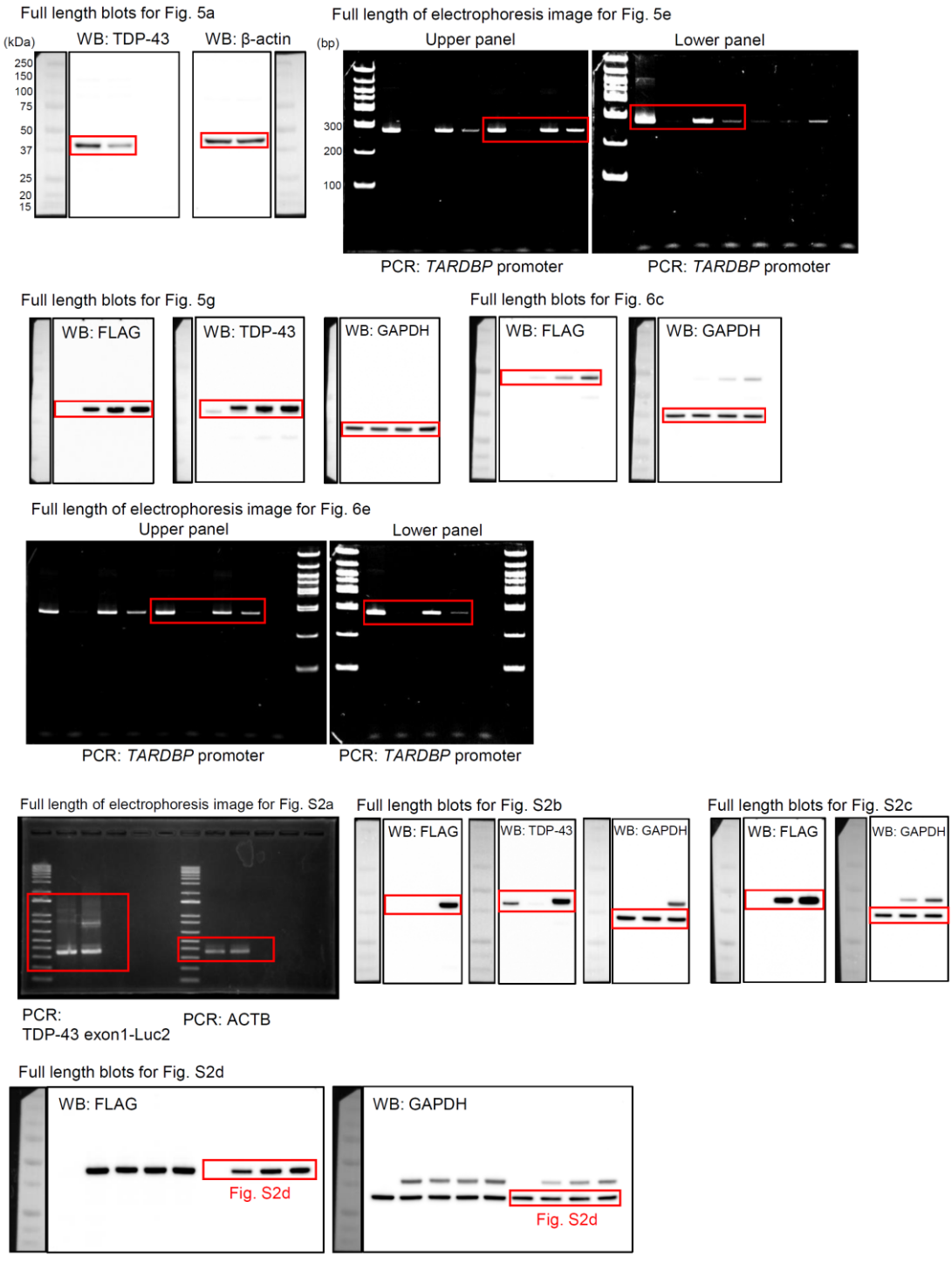
Full length blots for Fig. 5a
(kDa)
WB: TDP-43
WB: β-actin
250
150
100
75
50
37
25
20
15
Full length of electrophoresis image for Fig. 5e
(bp)
Upper panel
Lower panel
300
200
100
PCR: TARDBP promoter
PCR: TARDBP promoter
Full length blots for Fig. 5g
WB: FLAG
WB: TDP-43
WB: GAPDH
Full length blots for Fig. 6c
WB: FLAG
WB: GAPDH
Full length of electrophoresis image for Fig. 6e
Upper panel
Lower panel
PCR: TARDBP promoter
PCR: TARDBP promoter
Full length of electrophoresis image for Fig. S2a
PCR: TDP-43 exon1-Luc2
PCR: ACTB
Full length blots for Fig. S2b
WB: FLAG
WB: TDP-43
WB: GAPDH
Full length blots for Fig. S2c
WB: FLAG
WB: GAPDH
Full length blots for Fig. S2d
WB: FLAG
Fig. S2d
WB: GAPDH
Fig. S2d

Figure S4

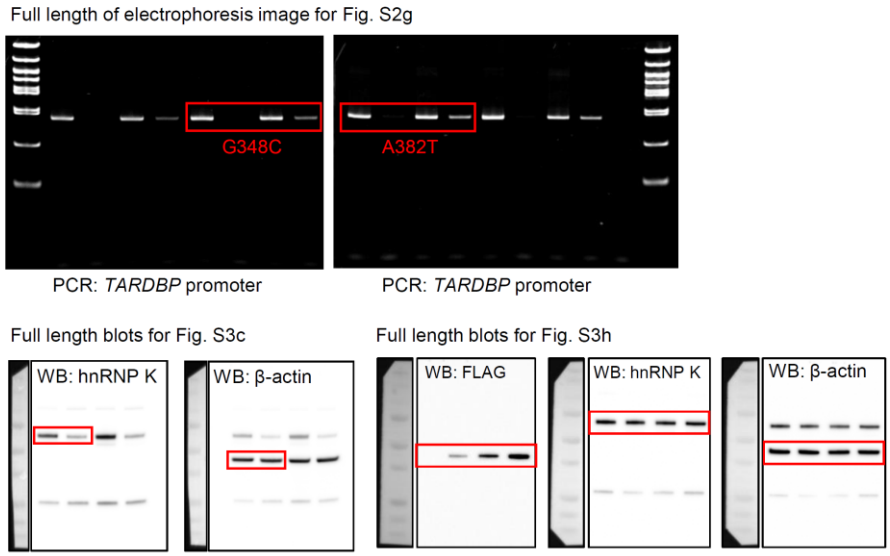

Figure S4. Full length blots or PCR images used to generate main and supplemental figures.

Table S1. siRNA sequences for TDP-43 KD experiments.

siRNA	siRNA Sequence
TDP-43 siRNA passenger	5′-GGUUUCUCCUGUAAUAUUUUA-3′
TDP-43 siRNA guide	5′-AAAUAUUACAGGAGAAACCUU-3′
Negative control siRNA passenger	5′-GUAAACGGCCACAAGUUCAGC-3′
Negative control siRNA guide	5′-UGAACUUGUGGCCGUUUACGU-3′

Table S2. Primers used for construction of TDP-43 tagged with N-terminal FLAG.

For production of TDP-43 tagged with N-terminal FLAG	
Gene	**Primer Sequence**
FLAG-TDP-43 (WT)	Forward: 5′-CAAGCTTTCTGAATATATTCGGGTAAC-3′ Reverse: 5′-GGGTACCTTACATTCCCCAGCCAGAAGAC-3′
FLAG-TDP-43 (F147/149L)	Forward: 5′-AGGGGCTGGGCCTGGTTCGTTTTACGGAATATG-3′ Reverse: 5′-GAACCAGGCCCAGCCCCTTTGAATGACCAGTCT-3′
FLAG-TDP-43 (G348C)	Same with TDP-43 (WT)
FLAG-TDP-43 (A382T)	Same with TDP-43 (WT)
For production of TDP-43 tagged with C-terminal Venus driven by CMV	
TDP-43	Forward: 5′- TGGGCCCCCACCATGTCTGAATATATTCGGGTAAC-3′ Reverse: 5′-GGGTACCCATTCCCCAGCCAGAAGAC-3′
Venus	Forward: 5′-CGGATCCGTGAGCAAGGGCGAGG-3′ Reverse: 5′- CGGATCCGTGAGCAAGGGCGAGG -3′
For production of TDP-43 tagged with C-terminal Venus driven by P1	
TDP-43	Forward: 5′-TTGCGGCCGCCCACCATGTCTGAATATATTCGG-3′ Reverse: 5′-GGGTACCCATTCCCCAGCCAGAAGAC-3′
Venus	Forward: 5′-CGGATCCGTGAGCAAGGGCGAGG-3′ Reverse: 5′-CAAGCTTTTACTTGTACAGCTCGTCCATG-3′

Table S3. Primers used for construction of *TARDBP* upstream and intron fragments fused with Luc2.

Cloning *TARDBP* upstream -721/-1	
721/-412	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TAGTGCGTCTTCTCCTCCCT-3′
-431/-281	Forward: 5′-AGGGAGGAGAAGACGCACTA-3′ Reverse: 5′-GAGAGTAGCACAGAGTCTCG-3′
-300/-1	Forward: 5′-CGAGACTCTGTGCTACTCTC-3′ Reverse: 5′-TTGCGGCCGCGGCGTCCTCTCCCACCGGTTG-3′
***TARDBP* upstream promoter region**	
Name	**Primer Sequence**
P1 **(-721/-1)**	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TTGCGGCCGCGGCGTCCTCTCCCACCGGTTG-3′
P4 **(-300/-1)**	Forward: 5′-GTCAATTGCGAGACTCTGTGCTACTCTC-3′ Reverse: 5′-TTGCGGCCGCGGCGTCCTCTCCCACCGGTTG-3′
P5 **(-721/-223)**	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TTGCGGCCGCCGGCCGACCAACCGCCGGCC-3′
P6 **(-721/-281)**	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TTGCGGCCGCGAGAGTAGCACAGAGTCTCG-3′
P7 **(-527/-223)**	Forward: 5′-GTCAATTGTGTGAATACTCAGAAAGTACCTGGC-3′ Reverse: 5′-TTGCGGCCGCCGGCCGACCAACCGCCGGCC-3′
P8 **(-527/-281)**	Forward: 5′-GTCAATTGTGTGAATACTCAGAAAGTACCTGGC-3′ Reverse: 5′-TTGCGGCCGCGAGAGTAGCACAGAGTCTCG-3′
P9 **(-527/-327)**	Forward: 5′-GTCAATTGTGTGAATACTCAGAAAGTACCTGGC-3′ Reverse: 5′-TTGCGGCCGCCCTGGAGGGGGCGGGGCTCGGGATC-3′
P17 **(*del* -371/-307)**	Forward1: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse1: 5′-GCTCGAGGCAGGATAAGGCCTGGAC-3′ Forward2: 5′-CCTCGAGCTGGCCGAGACTCTGTGC-3′ Reverse2: 5′-TTGCGGCCGCGGCGTCCTCTCCCACCGGTTG-3′
***TARDBP* intron1 (+123/+1094, total 972 nucleotides)**	
P2 **(1/972)**	Forward: 5′-GCCAATTGGTGAGTCGCGGAGCCTTC-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
P10 **(1/500)**	Forward: 5′-GCCAATTGGTGAGTCGCGGAGCCTTC-3′ Reverse: 5′-TTGCGGCCGCACCAGGTGTAAAGAACAG-3′
P11 **(501/972)**	Forward: 5′-GTCAATTGTCACTGCCCATATTCAG-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′

P12 (603/972)	Forward: 5′-GCCAATTGTCTGCCTTCAGGATTAC-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
P13 (666/972)	Forward: 5′-GCCAATTGCCTTGCATACCCTAGCTTG-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
P14 (737/972)	Forward: 5′-GTCAATTGCATAAGCCTTCAGGGAAAG-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
P15 (850/972)	Forward: 5′-GTCAATTGTATACGAATCCAGACAAG-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
***TARDBP* -721/intron1 (-721/+1094, total 1815 nucleotides)**	
P16	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
P16 (Δexon 1)	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TTGCGGCCGCGGCGTCCTCTCCCACCGGTTG-3′ Forward: 5′-TTGCGGCCGCGTGAGTCGCGGAGCCTTC-3′ Reverse: 5′-TTGCGGCCGCCTAAAGAGAAAAGAGATATG-3′
P16 (Δintron 1)	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-TTGCGGCCGCCCGCTAGGCCGCTGCTGG-3′
***TARDBP* intron2 (+1345/+4222, total 2878 nucleotides)**	
P3 (+1345/+4222)	Forward: 5′-GCCAATTGGTTTGTTACCATTTGG-3′ Reverse: 5′-TTGCGGCCGCCTAAATAAAACGAGCAG-3′
Luciferase reporter	
Luc2	Forward: 5′-ATTTGCGGCCGCCCACCATGGAAGATGCCAAAAAC-3′ Reverse: 5′-GGGGTACCTTACACGGCGATCTTGCC-3′

Table S4. Primers used for RT-PCR analysis.

***TARDBP* exon 1 to Luc2**	Forward: 5′-CAAGCTTGGTGGGCGGGGGGAGGAG-3′ Reverse: 5′-CTTCATAGCTTCTGCCAGCC-3′
Endogenous TDP-43	Forward: 5′-GTTACAGCCCAGTTTCCAG-3′ Reverse: 5′-CAGTCATGTCCTCTGTACA-3′
ACTB	Forward: 5′-TCACCATGGATGATGATATC-3′ Reverse: 5′-CTGGGTCATCTTCTCGCGG-3′

Table S5. Primers used for ChIP assay.

***TARDBP* -721 to -431**	Forward: 5′-GTCAATTGCGCCTACCGCGTTCAAGCAATTC-3′ Reverse: 5′-GAGAGTAGCACAGAGTCTCG-3′