Supplemental Information

Short repeat RNA reduces cytotoxicity by preventing the aggregation of TDP-43 and its 25 kDa carboxy-terminal fragment

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Figure S1. Interaction strength in the presence of electrostatic interaction inhibitors.
(a) Relative cross-correlation amplitude (RCA) of PC and NC in the presence of 100–250 mM KCl. The dashed line indicates zero. (b) RCA of PC and NC in the presence of 0–15 mM $MgCl_2$. The dashed line indicates zero. (c) RCA of the mixture of GFP-tagged TDP-43/TDP25 and G4, A4, or UG RNA in the presence of represented concentration of heparin. Significance: $*p<0.05$ and $**p < 0.01$. NS: $p \geq 0.05$.
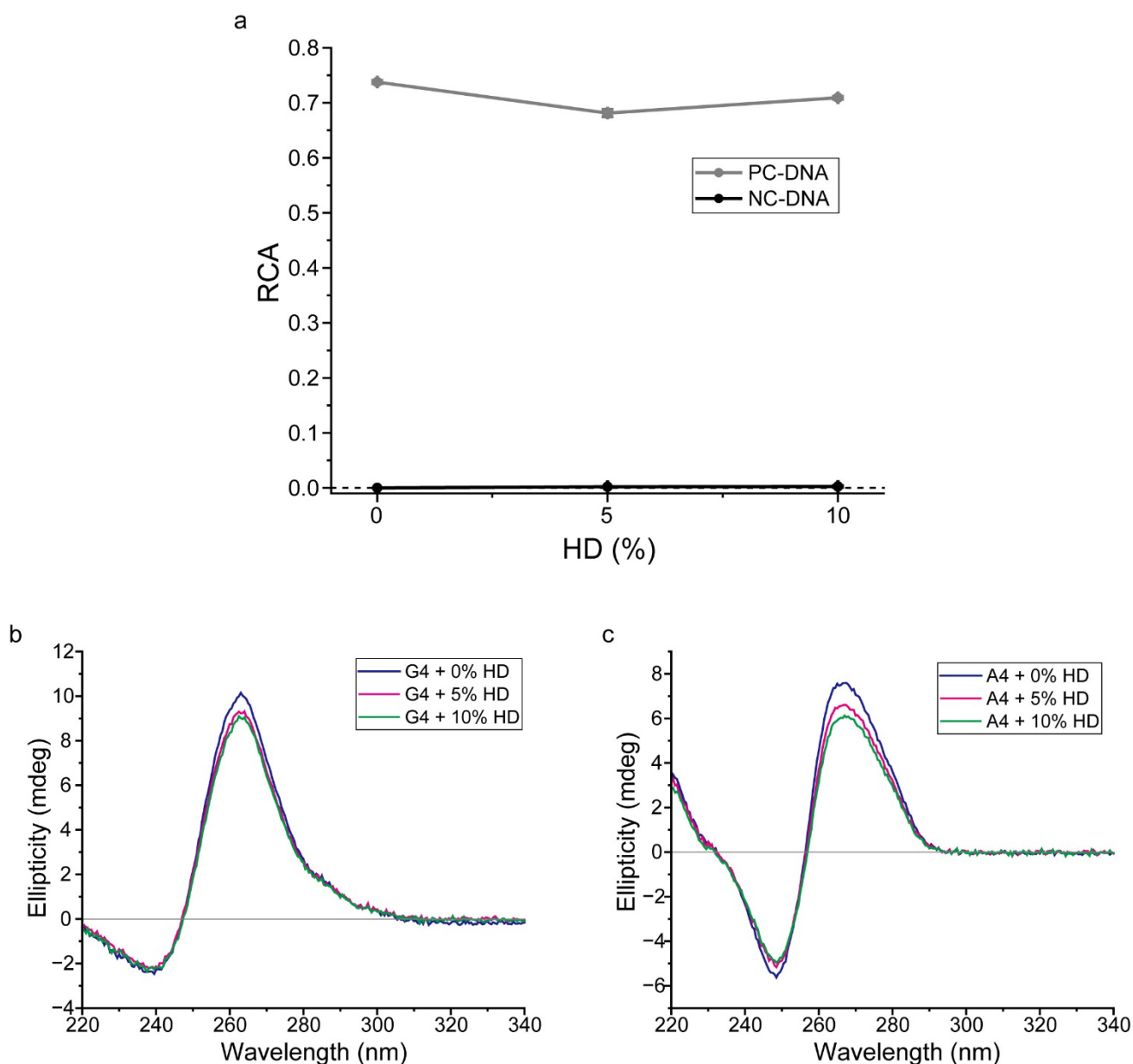
Figure S2. Controls of FCCS measurement and RNA conformation when adding 1,6-hexanediol. (a) Relative cross-correlation amplitude (RCA) of PC and NC in the presence of 0, 5, and 10% 1,6-hexanediol (HD). The dashed line indicates zero. (b and c) Circular dichroism (CD) spectra of G4-RNA (b) and A4-RNA (c) with 100 mM KCl in the presence of 0, 5, and 10% HD. The gray line indicates zero (baseline). A slight decrease in the local maximum ellipticity but no significant changes in the conformation of G4/A4-RNA were observed upon the addition of HD.
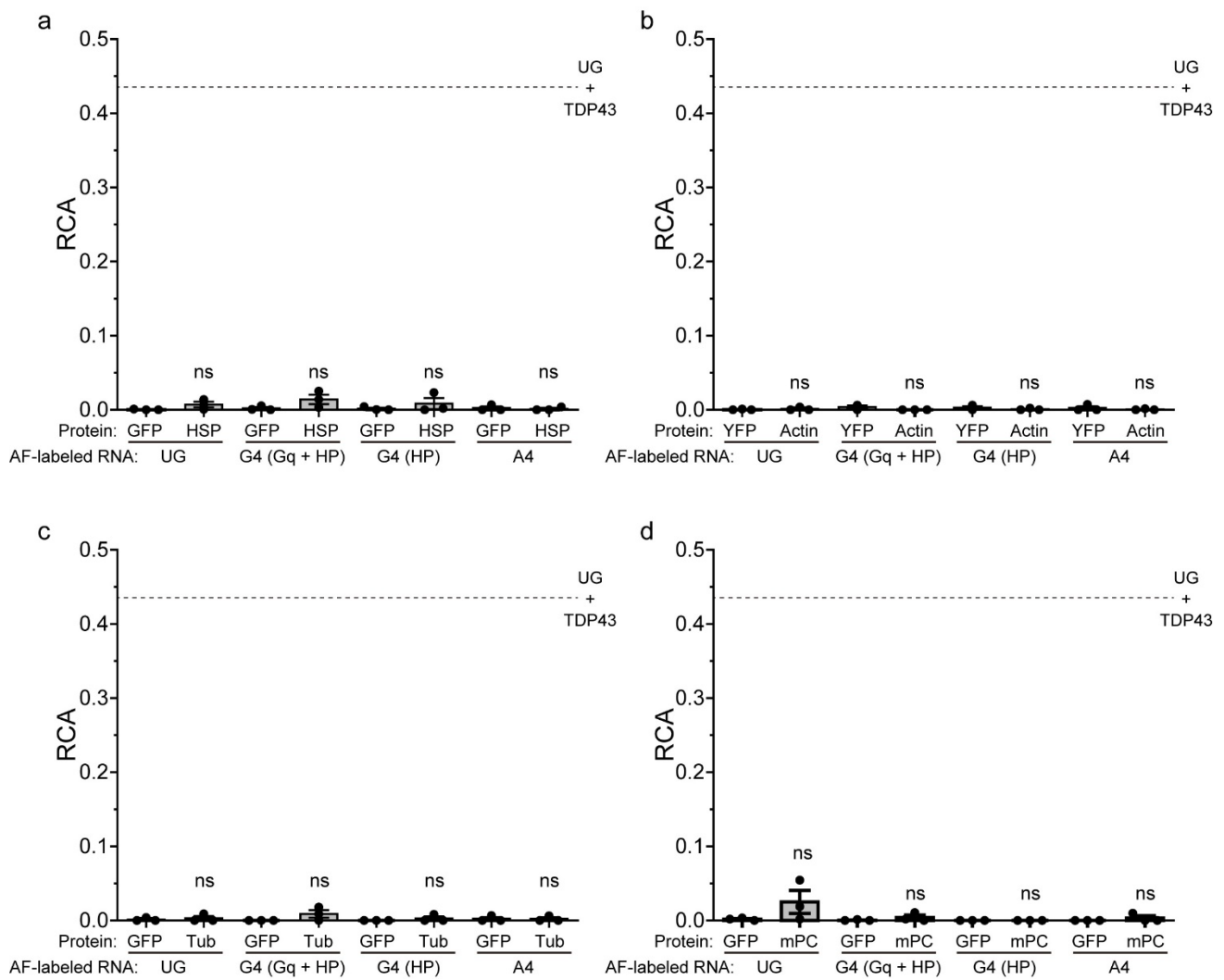
Figure S3. Binding of AF-G4/A4 to the proteins co-purified with GFP-TDP-43/TDP-25.
The binding of G4/A4-RNA to the proteins identified in Fig. 2a that were co-purified with GFP-TDP43 or GFP-TDP25 (HSP70 (HSP) (a), β-actin (Actin) (b), α-tubulin (Tub) (c), and mitochondrial pyruvate carboxylase (mPC) (d)) was examined using fluorescence cross-correlation spectroscopy (FCCS). The relative cross-correlation amplitude (RCA) values when Alexa Fluor 647-labeled RNAs (UG, G4, and A4) that were folded in 100 mM KCl (Gq + HP) or 100 mM LiCl (HP) were mixed in lysates of Neuro2a cells expressing GFP- or YFP-tagged proteins with 100 mM KCl (n = 3; dots and bars indicate independent trials and mean ± SE). GFP or YFP monomers were used as binding negative controls. The dotted line indicates the mean RCA values when UG was mixed with GFP-TDP43 as a positive control. Significance: ns ($p \geq 0.05$).
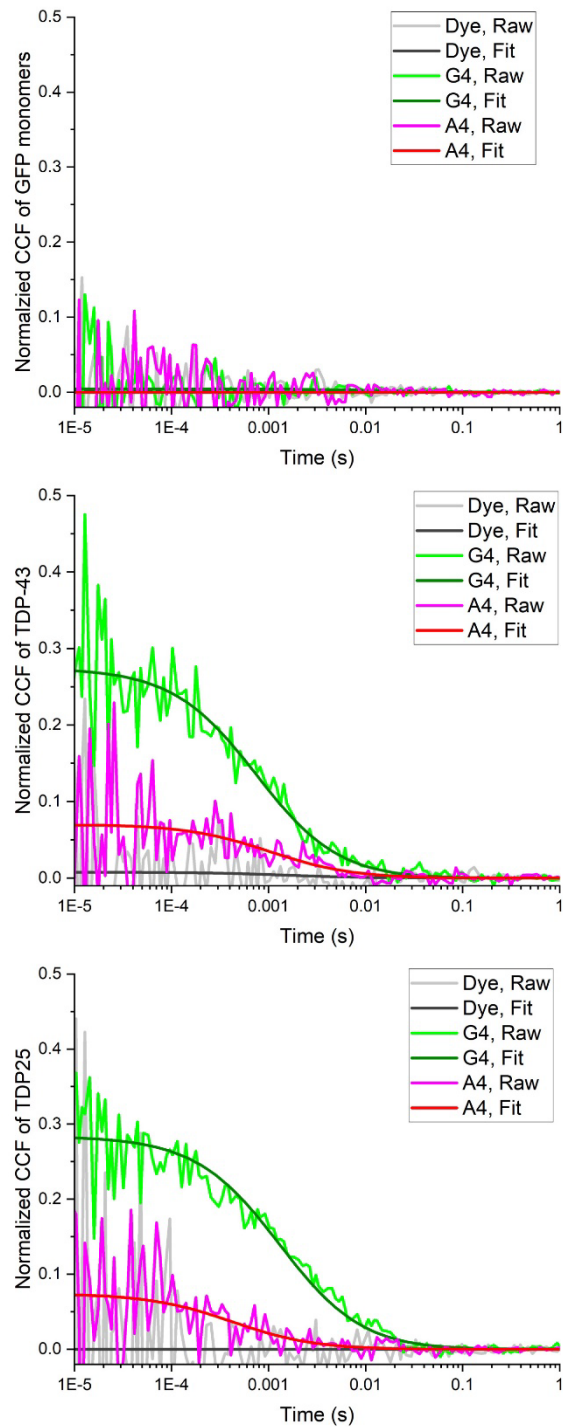
Figure S4. Typical normalized cross-correlation functions (CCFs) when purified proteins were mixed with AF-labeled RNA. The purified GFP monomers (*top*), GFP-TDP-43 (*middle*), and GFP-TDP25 (*bottom*) were mixed with Alexa Fluor647 (Dye) or Alexa Fluor647-labeled G4 or A4. Raw CCFs (Raw) and fitted curves (Fit) were represented.
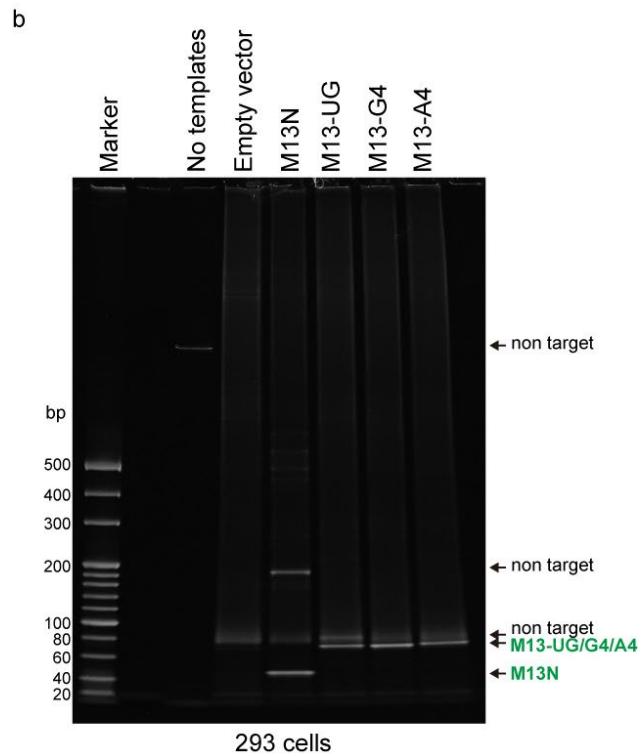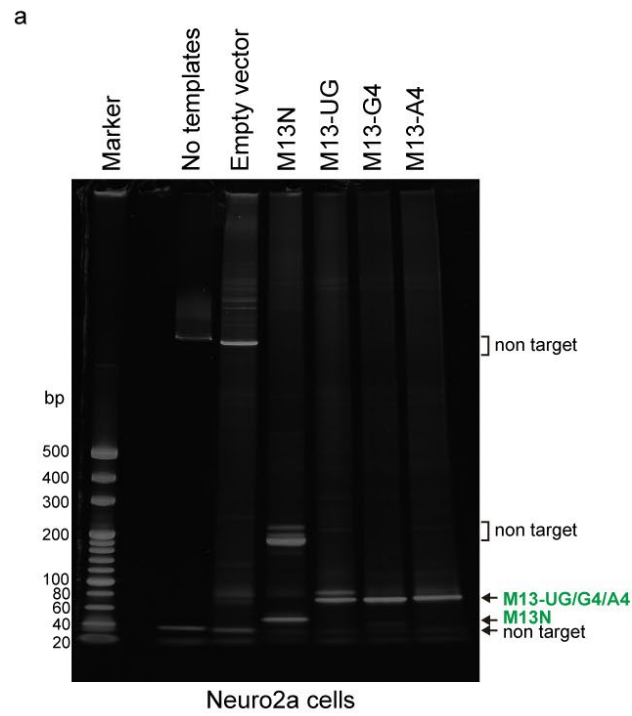
Figure S5. Confirmation of the expression levels of M13-tagged RNA using RT-PCR. (a & b) The amplified DNA was analyzed on the gel derived from Neuro2a (a) and Flp-In T-Rex 293 cells (b). The green letters indicate the bands of target product (M13N, M13-UG, M13-G4 and -A4). The values shown on the left of the gel images indicate the size of the molecular weight markers for double-stranded DNA.
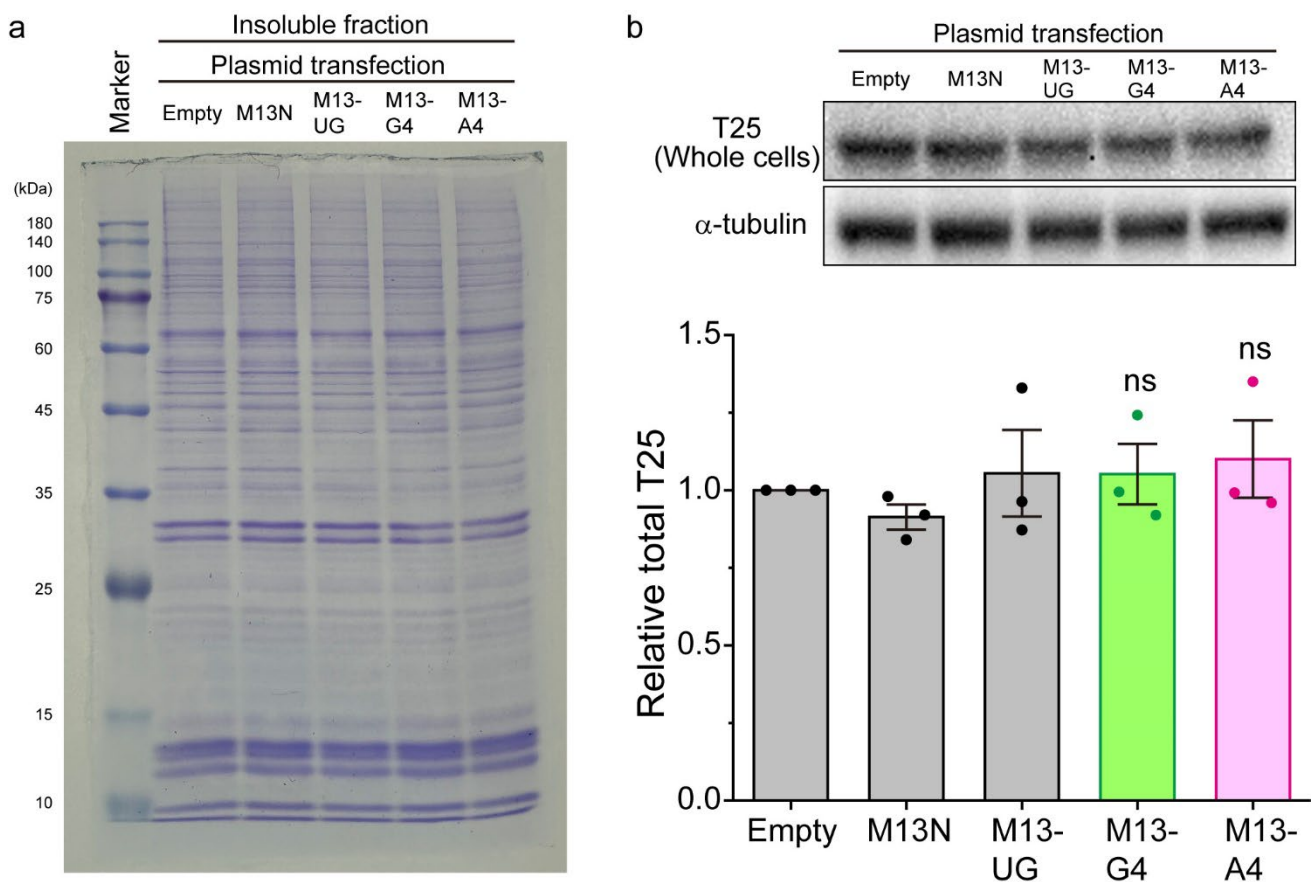
Figure S6. Total GFP-TDP-25 abundance in cell lysate when expressing M13-G4/A4.
(a) SDS-PAGE gel images of the insoluble fraction of cell lysates that are represented in Fig. 4b, stained with Coomassie brilliant blue R-250. The numbers on the left side of the gel images show the molecular weight of the protein marker standard (Loaded in the lane for 'Marker'). The insoluble fraction of Neuro2a cells expressing GFP-TDP-25 (T25) when an empty vector (Empty) or M13-tagged RNA was expressed. (b) Western blotting of T25 and α-tubulin as a loading control in the whole cell lysates when expressing T25 (*top*). The quantified intensity ratio of total T25 in the whole cell lysates compared with that in the empty vector transfection (the number of trials = 3; mean ± SE) (*bottom*). Significance: ns ($p \geq 0.05$).
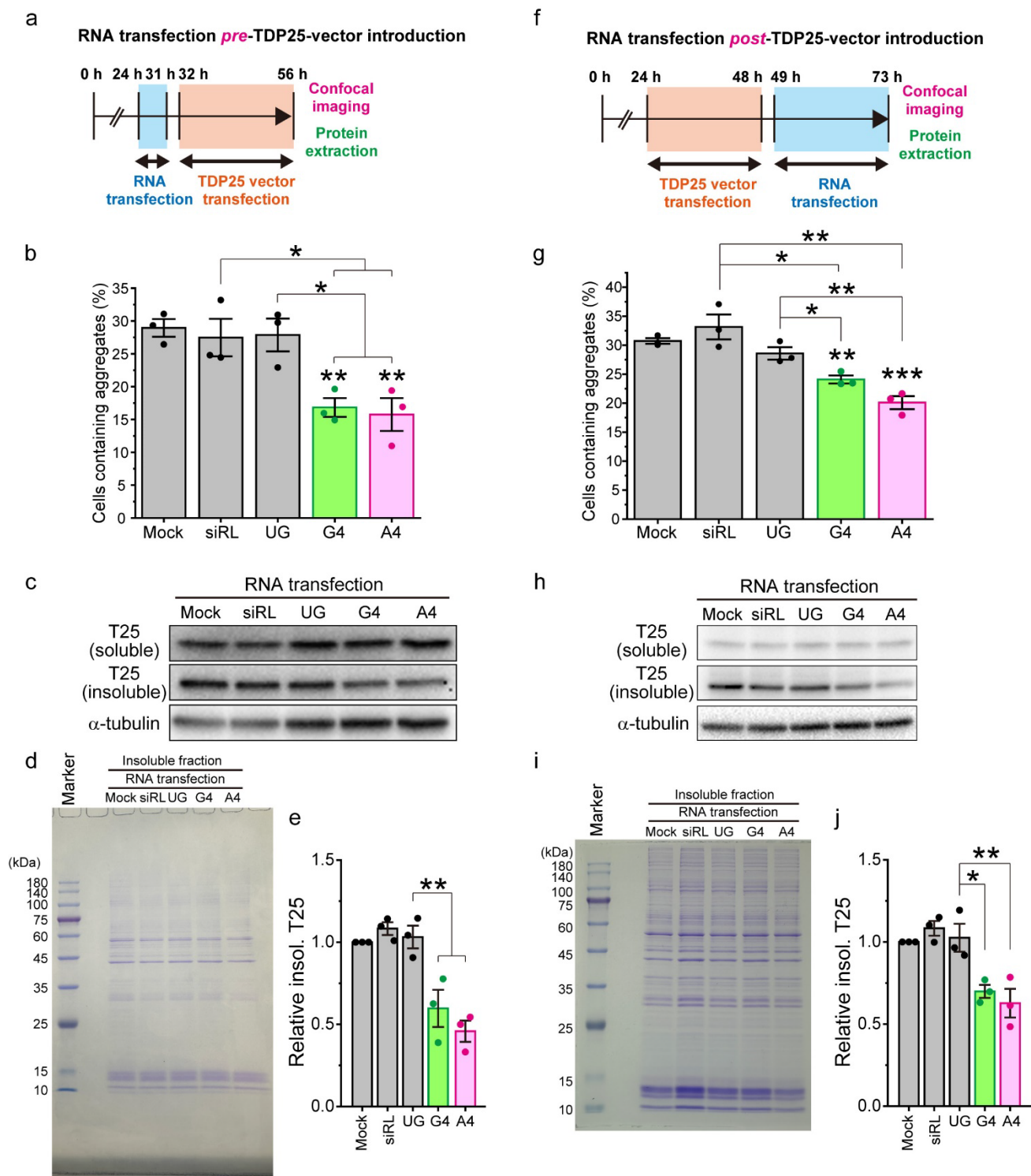
Figure S7. Reduced aggregates of GFP-TDP-25 in Neuro2a cells when synthetic RNA was directly introduced.

(a) Scheme of RNA transfection pre-TDP-25-vector introduction. (b) The percentage of cells containing cytoplasmic aggregates of GFP-TDP-25 (T25) when synthetic RNA (Mock, siRL, UG, G4, or A4) was transfected ($n = 3$; mean ± SE). Significance: $*p < 0.05$ and $**p < 0.01$. (c) Western blotting of Neuro2a cells expressing T25 when synthetic RNA (Mock, siRL, UG, G4, or A4) was transfected.

(d) SDS-PAGE gel images of the insoluble fractions of cell lysates that are represented in (c), stained with Coomassie brilliant blue R-250. The numbers on the left side of the gel images show the molecular weight of the protein marker standard (Loaded in the lane for 'Marker'). (e) The quantified intensity ratio of T25 in the insoluble fraction compared with that in the empty vector transfection (the number of trials = 3; mean ± SE). (f) Scheme of RNA transfection post-TDP-25-vector introduction. (g) The percentage of cells containing cytoplasmic aggregates of T25 when synthetic RNA (Mock, siRL, UG, G4, or A4) was transfected (n = 3; mean ± SE). Significance: *$p < 0.05$ and **$p < 0.01$. h) Western blotting of Neuro2a cells expressing T25 when synthetic RNA (Mock, siRL, UG, G4, or A4) was transfected. (i) SDS-PAGE gel images of the insoluble fraction of cell lysates that are represented in (h), stained with Coomassie brilliant blue R-250. The numbers on the left side of the gel images show the molecular weight of the protein marker standard (Loaded in the lane for 'Marker'). (j) The quantified intensity ratio of T25 in the insoluble fraction compared with that in the empty vector transfection (the number of trials = 3; mean ± SE).

a

Neuro2a cells

b
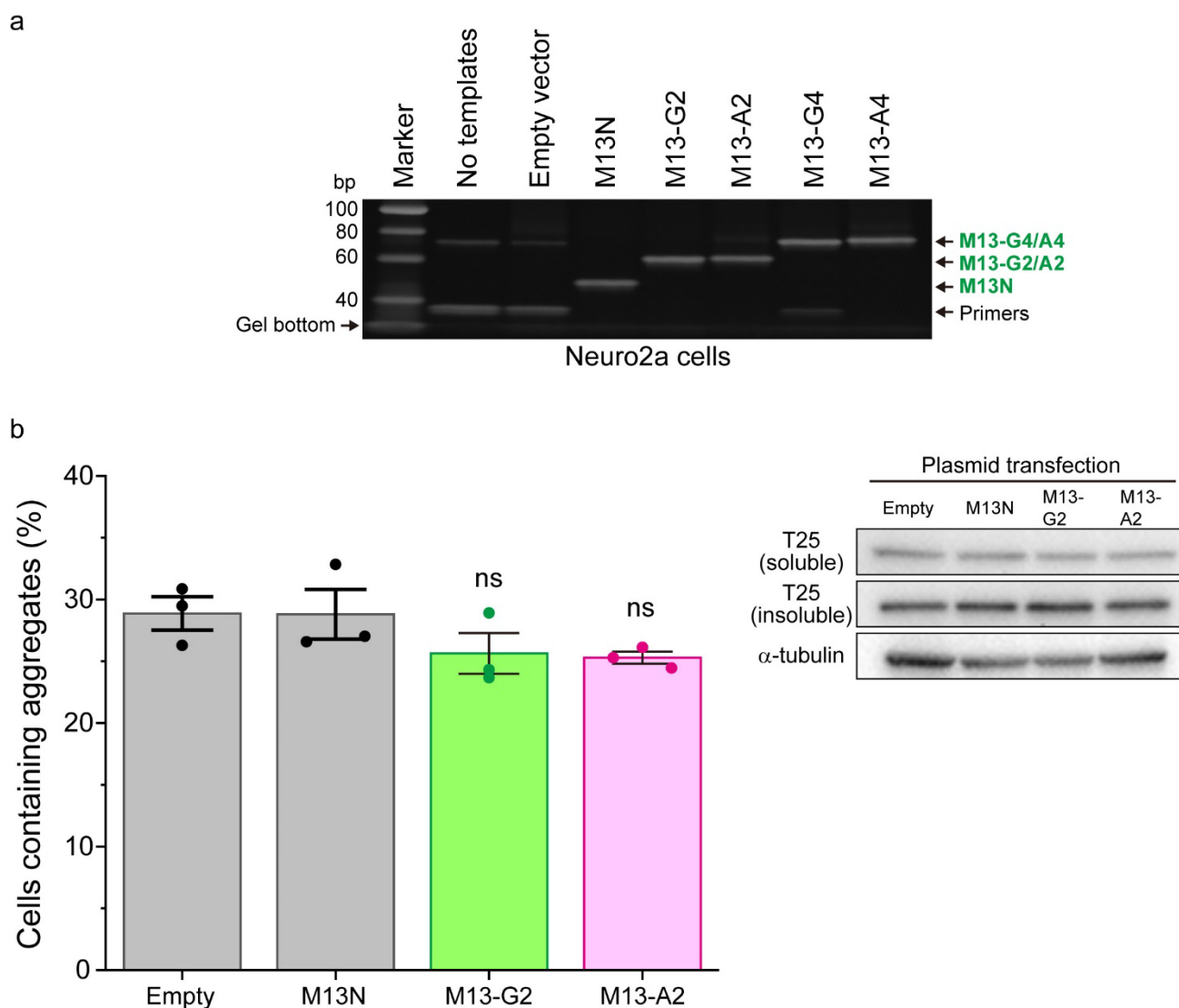
Figure S8. Aggregate formation of GFP-TDP-25 in Neuro2a cells expressing M13-G2/A2. (a) Confirmation of the expression levels of M13-G2, -A2, -G4, and -A4. M13N is a tag control. Green letters indicate the bands of target product. Unreacted/remained primers were indicated using arrows. (b) The percentage of cells containing cytoplasmic aggregates of GFP-TDP-25 (T25) when an empty vector (empty) or M13N, M13-G2, and M13-A2 was expressed (*left*) (n = 3; mean ± SE). Significance: ns ($p \geq 0.05$). Western blotting of Neuro2a cells expressing T25 when M13-tagged RNAs were expressed (*right*).
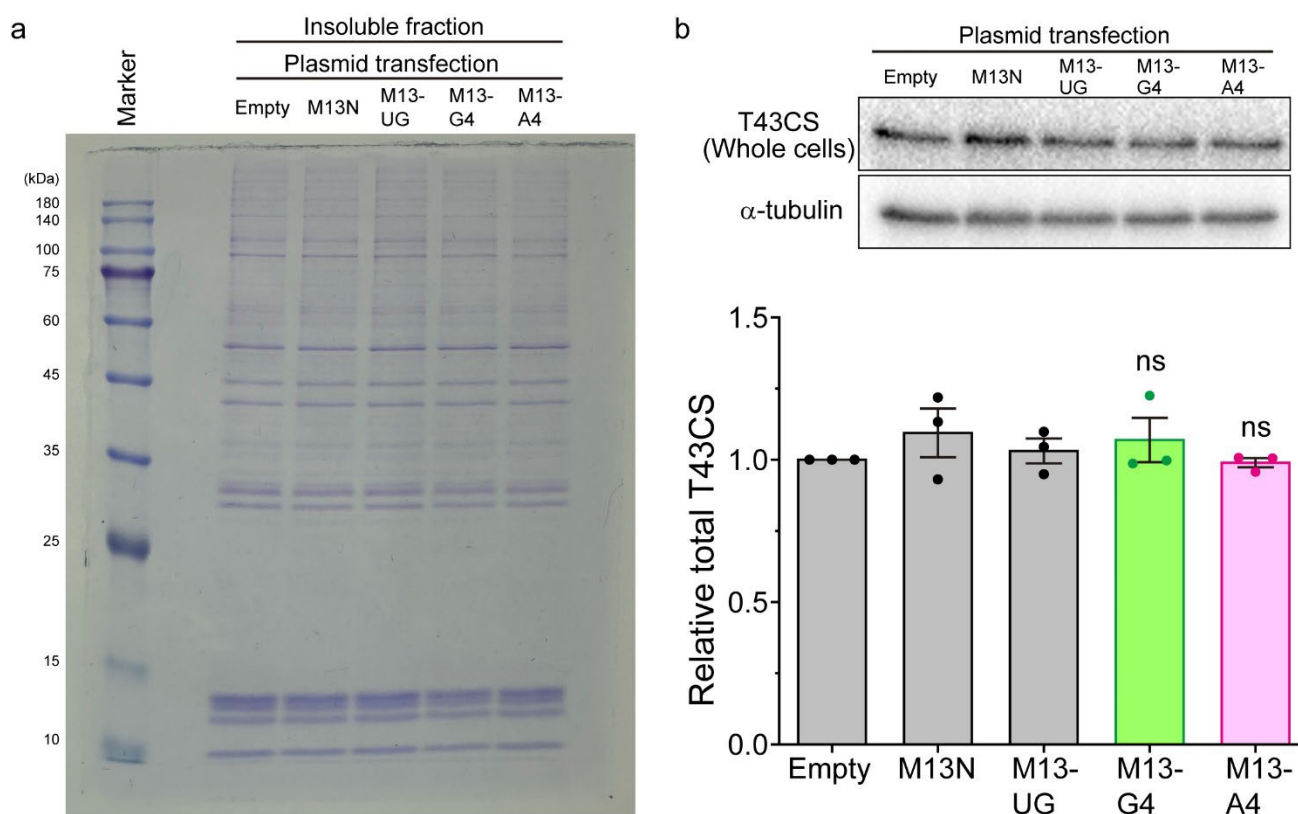
Figure S9. Total GFP-TDP-43CS abundance in cell lysate when expressing M13-G4/A4.
(a) SDS-PAGE gel images of the insoluble fraction of cell lysates that are represented in Fig. 5b, stained with Coomassie brilliant blue R-250. The numbers on the left side of the gel images show the molecular weight of the protein marker standard (Loaded in the lane for 'Marker'). The insoluble fraction of Flp-In T-Rex 293 cells expressing GFP-TDP-43CS (T43CS) when an empty vector (Empty) or M13-tagged RNA was expressed. (b) Western blotting of T43CS and α-tubulin as a loading control in the whole cell lysates when expressing T43CS (*top*). The quantified intensity ratio of total T43CS in the whole cell lysates compared with that in the empty vector transfection (the number of trials = 3; mean ± SE) (*bottom*). Significance: ns ($p \geq 0.05$).

Table S1. cDNA sequence for plasmid DNA

Name	cDNA sequence (5' to 3')
TDP-43CS-mNLS/mNES	AAGCTTATGTCTGAATATATTCGGGTAACCGAAGATGAGAACGATGAGCCCATTGAAATACCATCGGAAGACGATGGGACGGTGCTGCTCTCCACGGTTACAGCCCAGTTTCCAGGGGCGTGTGGGCTTCGCTACAGGAATCCAGTGTCTCAGTGTATGAGAGGTGTCCGGCTGGTAGAAGGAATTCTGCATGCCCCAGATGCTGGCTGGGGAAATCTGGTGTATGTTGTCAACTATCCAAAAGATAACGCCGCTGCCATGGATGAGACAGATGCTTCATCAGCAGTGGCCGTGGCCGCTGCAGTCCAGAAAACATCCGATTTAATAGTGTTGGGTCTCCCATGGAAAACAACCGAACAGGACCTGAAAGAGTATTTTAGTACCTTTGGAGAAGTTCTTATGGTGCAGGTCAAGAAAGATCTTAAGACTGGTCATTCAAAGGGGTTTGGCTTTGTTCGTTTTACGGAATATGAAACACAAGTGAAAGTAATGTCACAGCGACATATGATAGATGGACGATGGTCCGACTCCAAACTTCCTAATTCTAAGCAAAGCCAAGATGAGCCTTTGAGAAGCAGAAAAGTGTTTGTGGGGCGCTGTACAGAGGACATGACTGAGGATGAGCTGCGGGAGTTCTTCTCTCAGTACGGGGATGTGATGGATGTCTTCATCCCCAAGCCATTCAGGGCCTTTGCCTTTGTTACATTTGCAGATGATCAGGCCGCGCAGTCTGCCTGTGGAGAGGACGCCGCTGCCAAAGGAATCAGCGTTCATATATCCAATGCCGAACCTAAGCACAATAGCAATAGACAGTTAGAAAGAAGTGGAAGATTTGGTGGTAATCCAGGTGGCTTTGGGAATCAGGGTGGATTTGGTAATAGCAGAGGGGGTGGAGCTGGTTTGGGAAACAATCAAGGTAGTAATATGGGTGGTGGGATGAACTTTGGTGCGTTCAGCATTAATCCAGCCATGATGGCTGCCGCCCAGGCAGCACTACAGAGCAGTTGGGGTATGATGGGCATGTTAGCCAGCCAGCAGAACCAGTCAGGCCCATCGGGTAATAACCAAAACCAAGGCAACATGCAGAGGGAGCCAAACCAGGCCTTCGGTTCTGGAAATAACTCTTATAGTGGCTCTAATTCTGGTGCAGCAATTGGTTGGGGATCAGCATCCAATGCAGGGTCGGGCAGTGGTTTTAATGGAGGCTTTGGCTCAAGCATGGATTCTAAGTCTTCTGGCTGGGGAATGAATCACGCGGATCC
M13N	GGATCCCCGTAAAACGACGGCCAGTGAGCTCACCTGTGTGAAATTGTTATCCGCTCTTTTTAAGCTT
M13-UG	GGATCCCCGTAAAACGACGGCCAGTGGGGCCGGGGCCGGGGCCGGGGCCGAGCTCACCTGTGTGAAATTGTTATCCGCTCTTTTTAAGCTT
M13-G4	GGATCCCCGTAAAACGACGGCCAGTTGTGTGTGTGTGTGTGTGTGTGGAGCTCACCTGTGTGAAATTGTTATCCGCTCTTTTTAAGCTT
M13-A4	GGATCCCCGTAAAACGACGGCCAGTAAAATTAAAATTAAAATTAAAATTGAGCTCACCTGTGTGAAATTGTTATCCGCTCTTTTTAAGCTT
M13-G2	GGATCCCCGTAAAACGACGGCCAGTGGGGCCGGGGCCGAGCTCACCTGTGTGAAATTGTTATCCGCTCTTTTTAAGCTT
M13-A2	GGATCCCCGTAAAACGACGGCCAGTAAAATTAAAATTGAGCTCACCTGTGTGAAATTGTTATCCGCTCTTTTTAAGCTT

Table S2. Synthetic RNA sequence

Name	Synthetic RNA sequence (5' to 3')
UG	UGUGUGUGUGUGUGUGUGUGUGUG
G4	GGGGCCGGGGCCGGGGCCGGGGCC
A4	AAAAUUAAAAUUAAAAUUAAAAUU
G2	GGGGCCGGGGCC
A2	AAAAUUAAAAUU
siRL sense	GCCAAGAAGUUUCCUAAUATT
siRL antisense	UAUUAGGAAACUUCUUGGCTT

Table S3. DNA sequences for FCCS controls.

Name	DNA sequence (5' to 3')
PC-DNA	GATGAGTTCGTGTCCGTACAACTGGCGTAATCATGGCCCTTCGGGGCCATTGTTTCTCTGT GGAGGAGTCCATGACGAAAGATGAACTGATTGCCCGTCTCCGCTCGCTGGGTGAACAACTG AACCGTGATGTCAGCCTGACGGGGACGAAAGAAGAACTGGCGCTCCGTGTGGCAGAGCTGA AAGAGGAGCTTGATGACACGGATGAAACTGCCGGTCAGGACACCCCTCTCAGCCGGGAAAA TGTGCTGACCGGACATGAAAATGAGGTGGGATCAGCGCAGCCGGATACCGTGATTCTGGAT ACGTCTGAACTGGTCACGGTCGTGGCACTGGTGAAGCTGCATACTGATGCACTTCACGCCA CGCGGGATGAACCTGTGGCATTTGTGCTGCCGGGAACGGCGTTTCGTGTCTCTGCCGGTGT GGCAGCCGAAATGACAGAGCGCGGCCTGGCCAGAATGCAATAACGGGAGGCGCTGTGGCTG ATTTCGATAACC
ATTO647N-λDNA-F	GATGAGTTCGTGTCCGTACAACT
ATTO488-λDNA-R	GGTTATCGAAATCAGCCACAGCG

Table S4. Transcripts that exhibited changes upon expression of G4- or A4-RNA in 293 cells

mRNA

	Fold change	p value	G4/A4
DENND11	3.0	0.0006	A4
MAGED4B	2.9	0.0071	G4
NTAN1	2.4	0.0316	A4
ZNF251	2.2	0.0345	A4
PARN	2.1	0.0019	A4
TRPV2	2.1	0.0077	A4
SLC27A1	2.0	0.0023	G4
SKIV2L	2.0	0.0340	G4
NFASC	0.8	0.0359	A4
COL24A1	0.5	0.0133	A4
RETSAT	0.5	0.0283	G4
FAM120B	0.5	0.0163	G4
CHCHD10	0.4	0.0274	G4
PLEKHM1	0.3	0.0091	G4
ASMT	0.3	0.0019	A4
DHRS4	0.3	0.0282	A4
RAB11FIP3	0.3	0.0437	A4

ncRNA

	Fold change	p value	G4/A4
LOC100506472	2.1	0.0019	G4
UXT-AS1	0.5	0.0413	G4
FAM106A	0.4	0.0042	A4