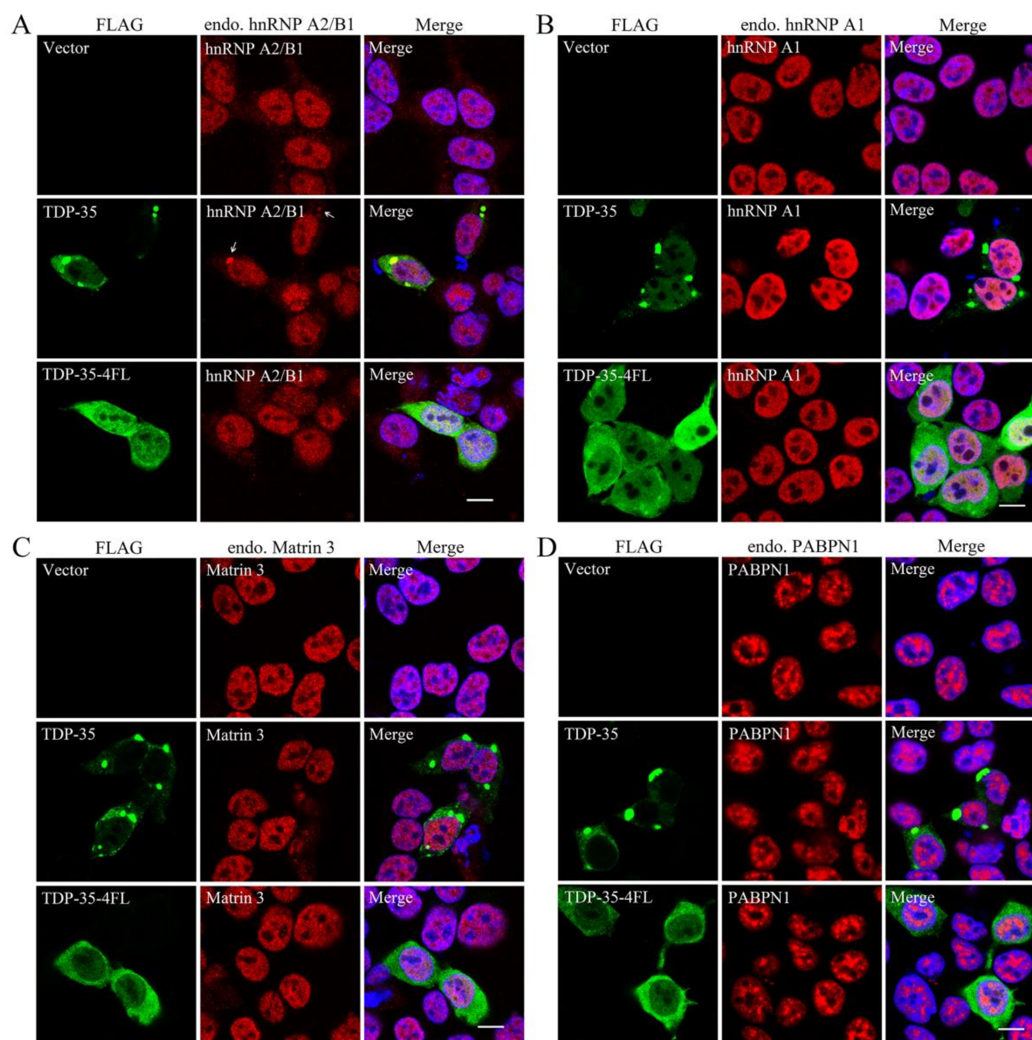

Fig. S1. Characterization of the RBPs sequestered by the cytoplasmic TDP-35 inclusions by immunofluorescence imaging

(**A**) Identification of hnRNP A2B1 that co-localizes with the inclusions. (**B**, **C**, **D**) hnRNP A1 (B), Matr3 (C) or PABPN1 (D) that does not co-localize with the inclusions. The HEK 293T cells expressing FLAG vector, FLAG-TDP-35 or FLAG-TDP-35-4FL were imaged, and the endogenous RBPs were examined with the indicated antibodies. TDP-35 and its mutant were stained with anti-FLAG antibody (green), RBPs were stained with indicated antibodies (red), and the nuclei were stained with Hoechst (blue). Scale bar = 10 μm.

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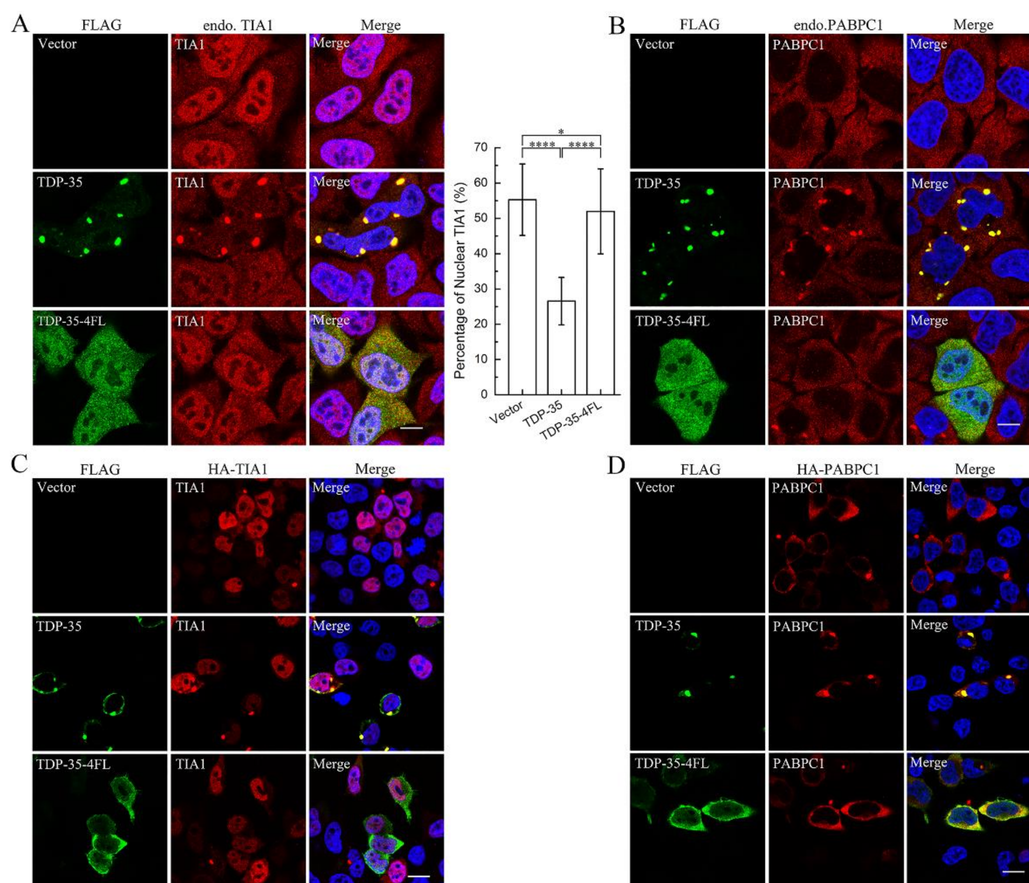

Fig. S2. Confirmation of TIA1 and PABPC1 co-localized with the cytoplasmic TDP-35 inclusions by immunofluorescence imaging

(**A**, **B**) Co-localization of endogenous TIA1 and PABPC1 with the cytoplasmic TDP-35 inclusions in HeLa cells. The HeLa cells over-expressing FLAG vector, FLAG-TDP-35 or FLAG-TDP-35-4FL were imaged, and the endogenous TIA1 (A) or PABPC1 (B) was shown to be co-localized with the cytoplasmic TDP-35 inclusions. FLAG-TDP-35-4FL was set as a control. The inset graph shown in (A) is the percentage of nuclear TIA1 in the cells with TDP-35 inclusions. The amounts of nuclear and total TIA1 were obtained from the pixel intensities of individual cell image, and the data were statistically analyzed by one-way ANOVA and presented as Means ± SEM (Vector, n=36; TDP-35, n=35; TDP-35-4FL, n=24). *, $p < 0.05$, ****, $p < 0.0001$. **(C**, **D)** Co-localization of exogenous TIA1 or PABPC1 with the cytoplasmic TDP-35 inclusions in HEK 293T cells. HA-TIA1 (C) or HA-PABPC1 (D) was co-transfected with FLAG-TDP-35 in HEK 293T cells, and the cells were visualized by immunofluorescence imaging. TDP-35 and its mutant were stained with anti-FLAG antibody (green). Endogenous TIA1 or PABPC1 was stained with antibody anti-TIA1 or anti-PABP antibody (red), while exogenous HA-tagged TIA1 or PABPC1 was stained with anti-HA antibody (red). The nuclei were stained with Hoechst (blue). Scale bar = 10 μm.

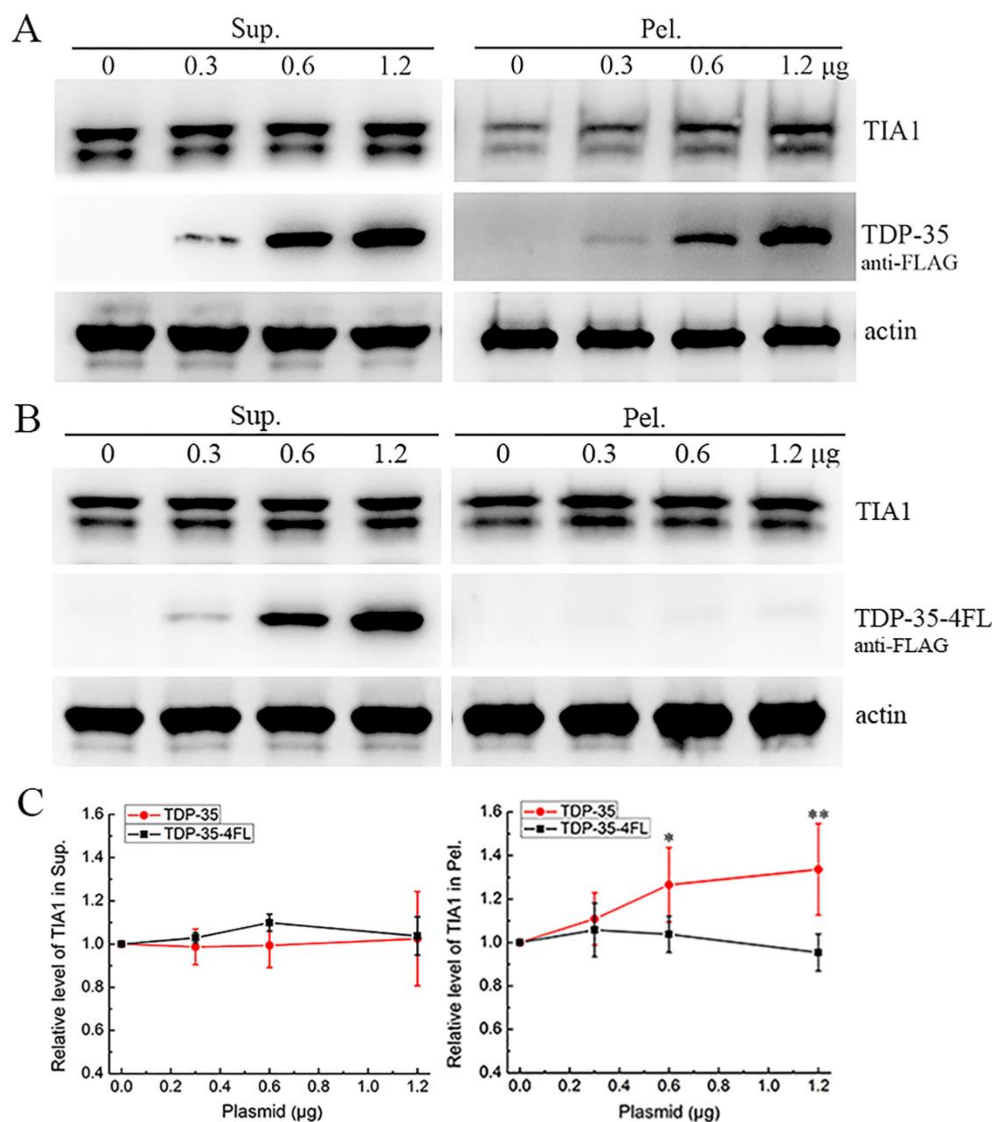

Fig. S3. Dose-dependent experiment for characterizing the sequestration of endogenous TIA1 by the TDP-35 aggregates

(**A**) Sequestration of endogenous TIA1 by TDP-35. (**B**) By TDP-35-4FL as a control. HeLa cells were transfected with different dose of the FLAG-TDP-35 (A) or FLAG-TDP-35-4FL (B) plasmid, and the cell lysates were subjected to supernatant/pellet fractionation and Western blotting analysis for TIA1. The proteins were detected by using anti-FLAG, anti-TIA1 and anti-actin antibodies. (**C**) Quantification of the amounts of TIA1 in supernatant and pellet fractions. Data are shown as Means ±SEM (n=3). *, $p<0.05$; **, $p<0.01$. Sup., supernatant; Pel., pellet.

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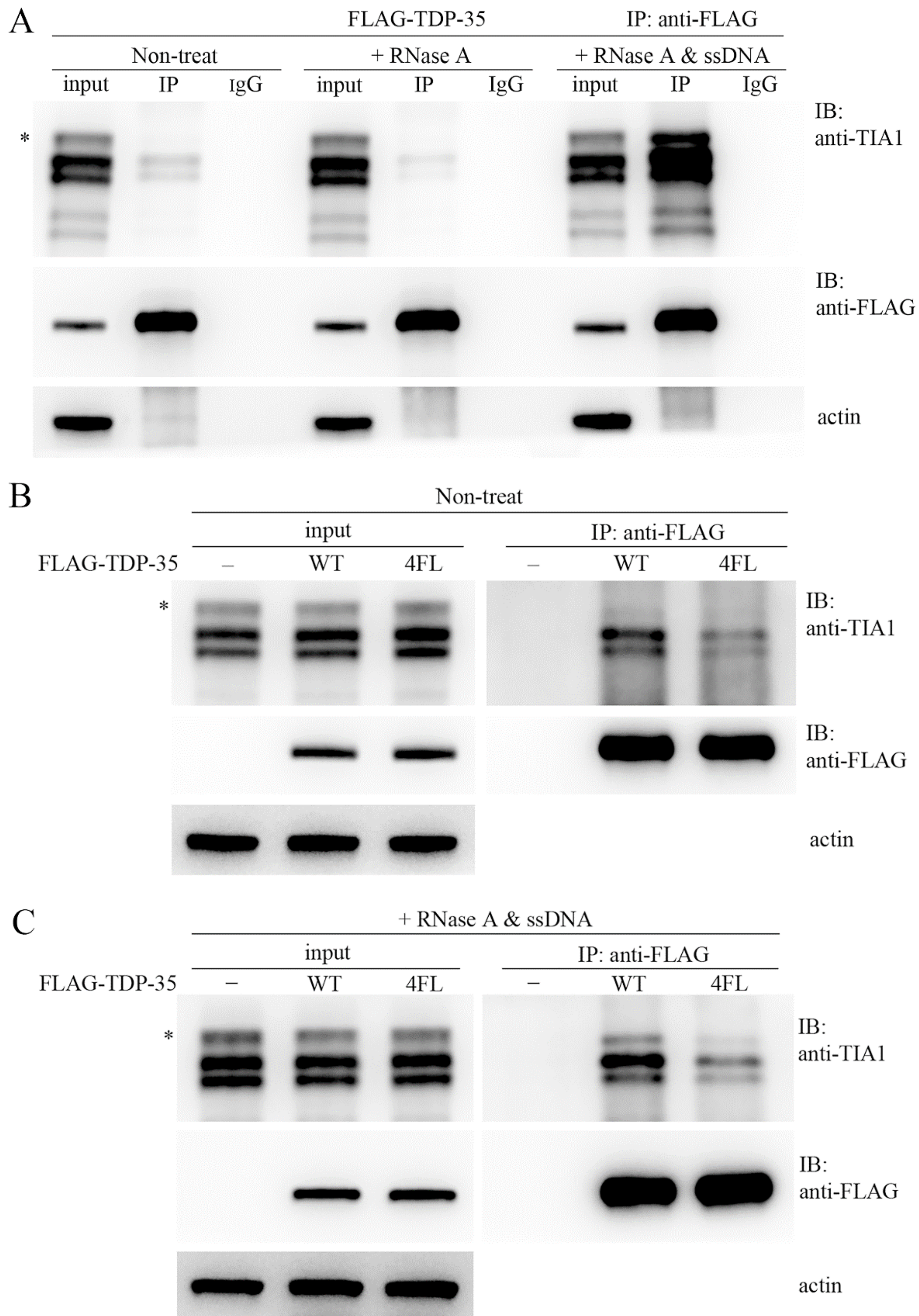

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Fig. S4. TDP-35 associates with endogenous TIA1 mediated by sequence-specific RNA.

(**A**) Immunoprecipitation experiment examining the effects of RNase and ssDNA treatments on the association of FLAG-TDP-35 with endogenous TIA1. The cell lysates were immunoprecipitated with protein A/G beads plus FLAG antibody under various conditions including non-treat, RNase treatment, and RNase A plus ssDNA treatment. IgG, a control without FLAG antibody; IP, with anti-FLAG antibody. The immunoblotting was carried out with an antibody against either TIA1 or FLAG. ssDNA, TG+TC. *, non-specific band. (**B**) Immunoprecipitation for association of FLAG-TDP-35 or FLAG-TDP-35-4FL with endogenous TIA1. The lysates were immunoprecipitated with protein A/G beads plus FLAG antibody under the condition of non-treat and then immunoblotted with an antibody against either TIA1 or FLAG. (**C**) Immunoprecipitation for ssDNA-assisted association of FLAG-TDP-35 with endogenous TIA1. FLAG-TDP-35-4FL was set as a control. The lysates were immunoprecipitated with protein A/G beads plus FLAG antibody and subjected to RNase and ssDNA (TG+TC) treatments, and then immunoblotted with the indicated antibodies. In all IP experiments, about 8% loading of the sample was applied for input.

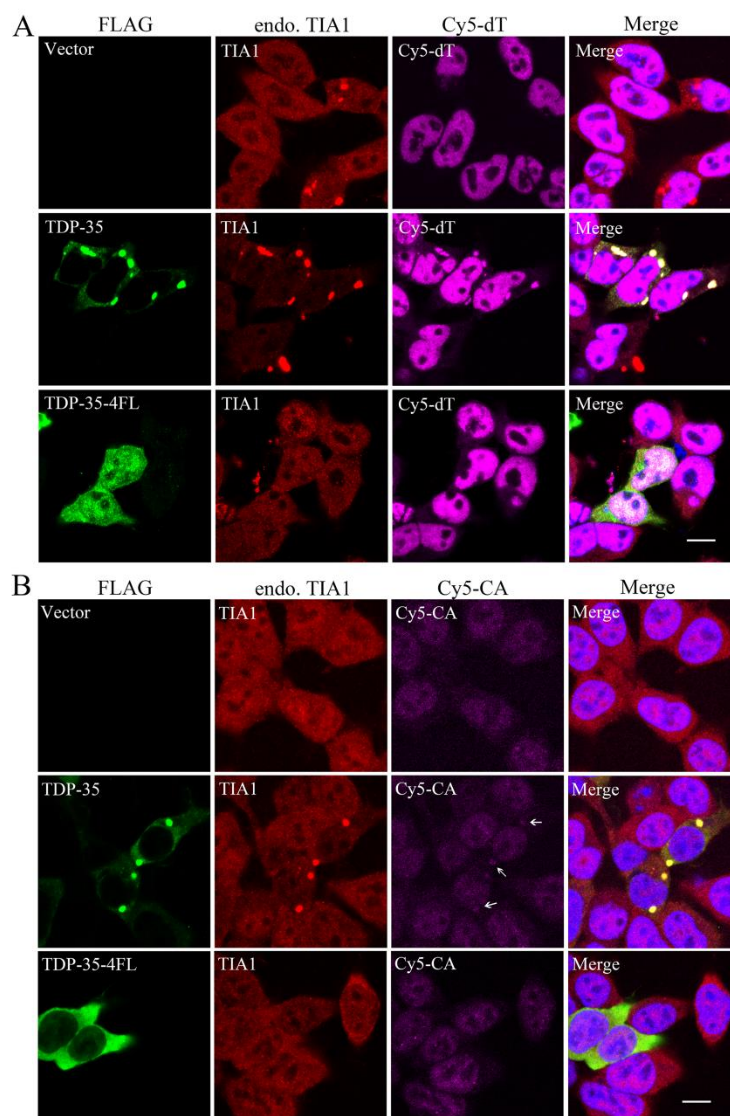

Fig. S5. Combined FISH and IF experiment for detecting specific RNAs enriched in the TDP-35 inclusions in HEK 293T cells.

(**A**) Imaging with the Cy5-oligo-dT probe showing poly(A)-containing RNAs enriched in the cytoplasmic TDP-35 inclusions co-localized with endogenous TIA1. (**B**) Imaging with the Cy5-CA probe showing UG-repeat RNAs in the inclusions co-localized with endogenous TIA1. FLAG-TDP-35 and FLAG-TDP-35-4FL are in green, TIA1 is in red, RNAs are in pink, while the nuclei are in blue (DAPI). Scale bar = 10 μm.

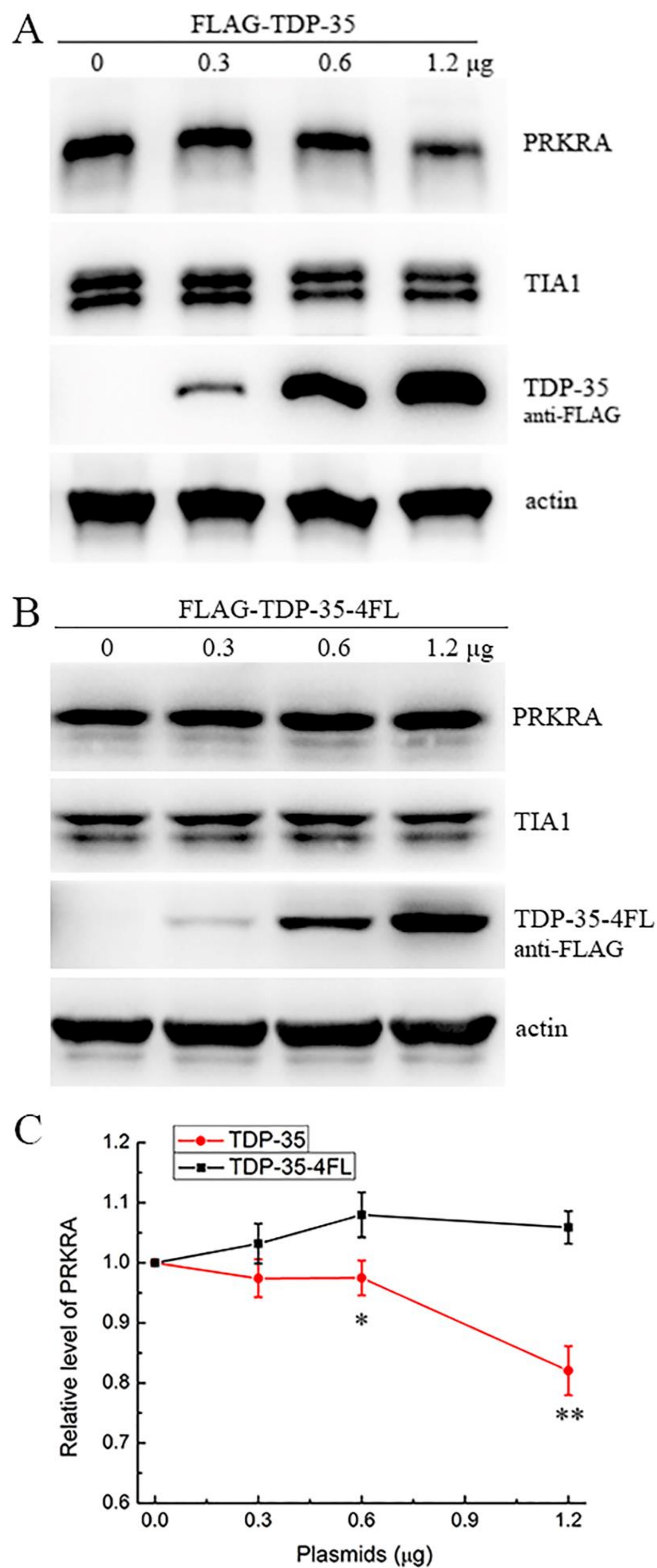

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Fig. S6. Dose-dependent experiment for characterizing the effect of TDP-35 on the protein level of PRKRA.

(**A**) Effect of TDP-35 over-expression on the PRKRA level. (**B**) TDP-35-4FL as a control. (**C**) Qualification of the alteration of the PRKRA level. HeLa cells were transfected with each indicated plasmid, the cell lysates were centrifuged, and then the supernatant was subjected to Western blotting analysis. Data are shown as Means ± SEM (n=3). *, $p<0.05$; **, $p<0.01$.

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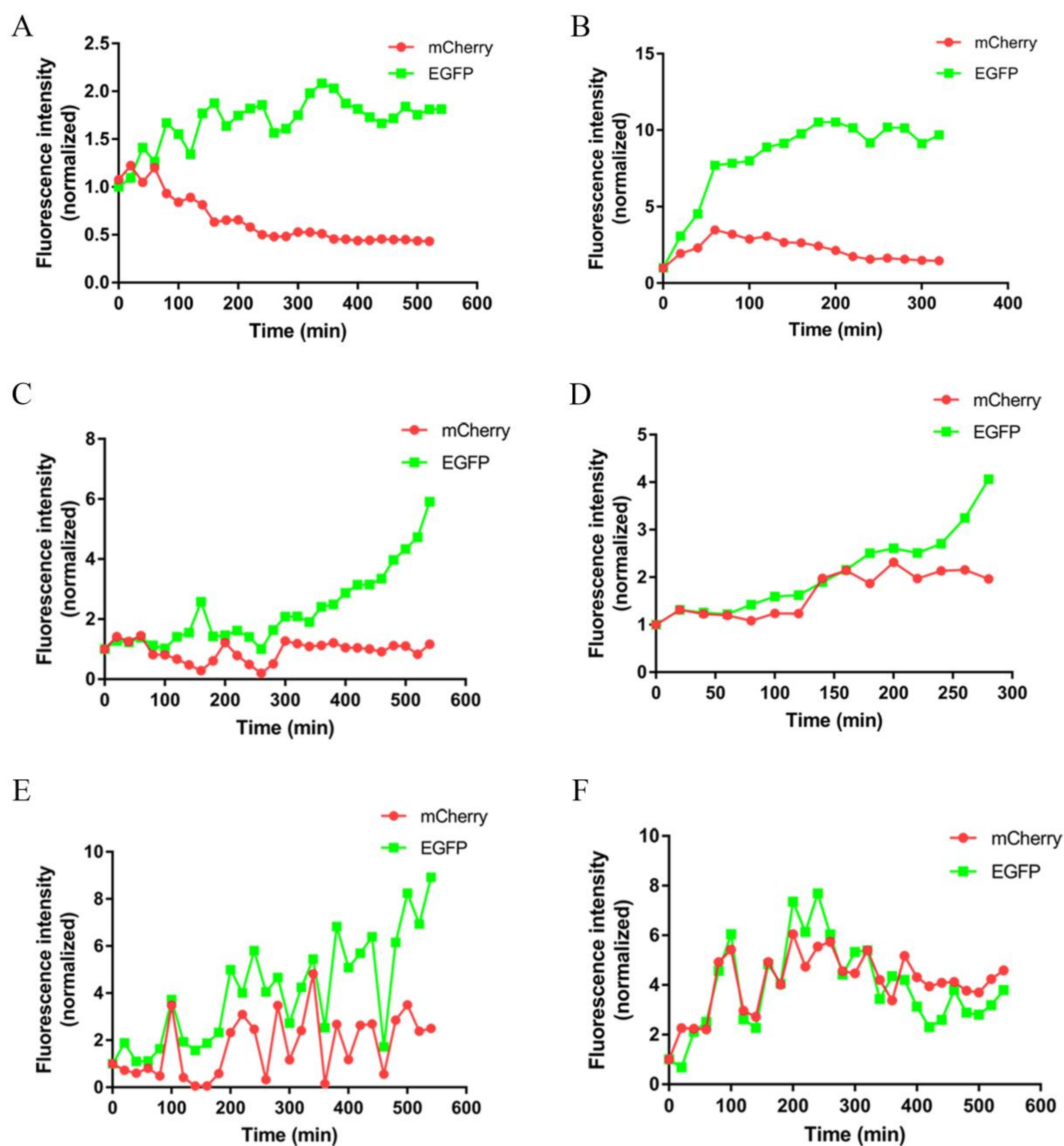

Fig. S7. Quantification of the fluorescence intensities of mCherry and EGFP for individual inclusions in a time-lapse movie.

Six graphs are extracted from the movie and each stand for an individual inclusion. The intensity data are normalized to that of the 0-min point.

Table S1. List of the constructs applied in this study.

Constructs	Vectors	Restriction Enzyme sites	Additional
FLAG-TDP-35	FLAG-pcDNA3.1	BamH I / Xho I	TDP-35, residues 90-414 of TDP-43
mCherry-TDP-35	FLAG-pcDNA3.1	Hind III / BamH I	TDP-35, N-terminally tagged mCherry
TDP-35-mCherry	FLAG-pcDNA3.1	BamH I / Xho I	TDP-35, C-terminally tagged mCherry
FLAG-TDP-35-4FL	FLAG-pcDNA3.1	BamH I / Xho I	TDP-35, RRM mutant: F147/149/229/231L
HA-TIA1	HA-pcDNA3.0	BamH I / Xho I	Full-length TIA1, isoform 1
HA-PABPC1	HA-pcDNA3.0	BamH I / Xho I	Full-length PABPC1
Cas9-sgRNA(TDP-43)-mCherry EGFP-TDP-43	pX330 FLAG-pcDNA3.1	Bpi I BamH I / Xho I	For constructing an EGFP-TDP-43 cell line by CRISPR/Cas9

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Table S2. List of the antibodies used in this study.

Antibody	Source	Catalog Number	Dilution
Anti-FLAG (mouse)	Sigma-Aldrich	F1804	WB: 1:1000 IF: 1:200 IP: 1:1000
Anti-FLAG (rabbit)	Proteintech	20543-1-AP	WB: 1:1000 IF: 1:200
Anti-HA (mouse)	Sigma-Aldrich	H9658	WB: 1:1000 IF: 1:200
Anti-TIA1 (mouse)	Santa Cruz Biotechnology	sc-166247	WB: 1:1000 IF: 1:100
Anti-TIA1 (rabbit)	Proteintech	12133-2-AP	WB: 1:3000 IF: 1:200
Anti-PABP (rabbit, for PABPC1)	Abcam	ab21060	IF: 1:100
Anti-Matrin 3 (rabbit)	Abcam	ab151714	IF: 1:200
Anti-PABPN1 (rabbit)	Abcam	ab75855	IF: 1:200
Anti- hnRNP A2/B1 (rabbit)	Proteintech	14813-1-AP	IF: 1:200
Anti-hnRNP A1 (mouse)	Santa Cruz Biotechnology	sc-32301	IF: 1:100
Anti-PACT (mouse, for PRKRA)	Santa Cruz Biotechnology	sc-377103	WB: 1:1000
Anti-β-actin (mouse)	Proteintech	6008-1-Ig	WB: 1:5000

WB, Western blotting; IF, immunofluorescence. IP, immunoprecipitation.

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Table S3. Nucleotide sequences of ssDNAs, FISH probes and RT-PCR primers applied in this study.

ssDNA/probe /primer	Sequence
TG (58 nt)	5'-TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT GTGTGTGTGTGTG-3'
TC (30 nt)	5'-TTTTTACTCCCCTCATTTTTTTTTTACTCC-3'
TG+TC (78 nt)	5'-TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT GTGTTTTTACTCCCCTCATTTTTTTTTTACTCC-3'
Cy5-oligo dT	Cy5-5'-TTTTTTTTTTTTTTTTTTTTTTTTT-3'
Cy5-CA	Cy5-5'- CACACACACACACACACACACACA -3'
Cy5-NN	Cy5-5'- NNNNNNNNNNNNNNNNNNNNNNNNN-3'
GAPDH-forward GAPDH-reverse	5'-GGAGCGAGATCCCTCCAAAAT-3' 5'-GGCTGTTGTCATACTTCTCATGG-3'
PRKRA-forward PRKRA-reverse	5'-GTGAAGGTACAAGTAAGAAGCTGGCG-3' 5'-AATGAACCAATAGGATTAAGCTGGTTCTTT-3'

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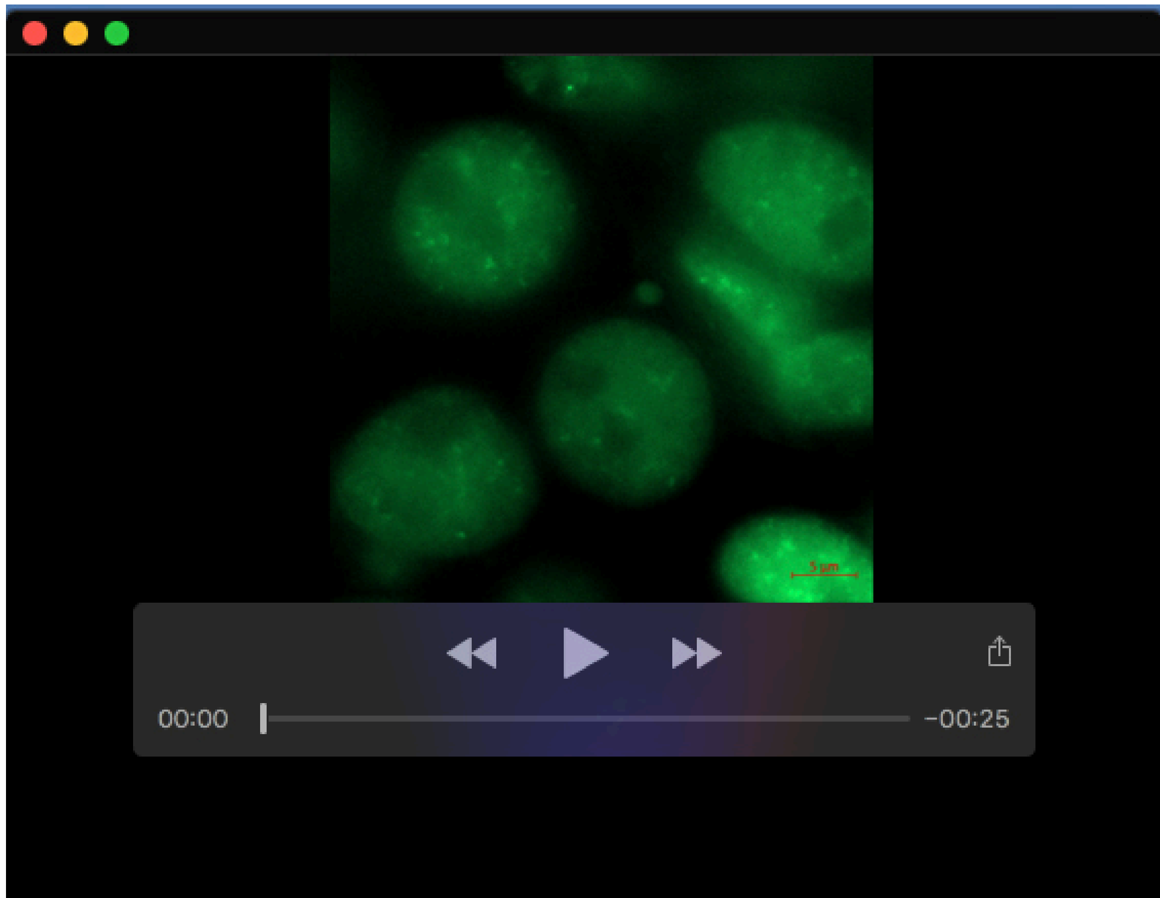
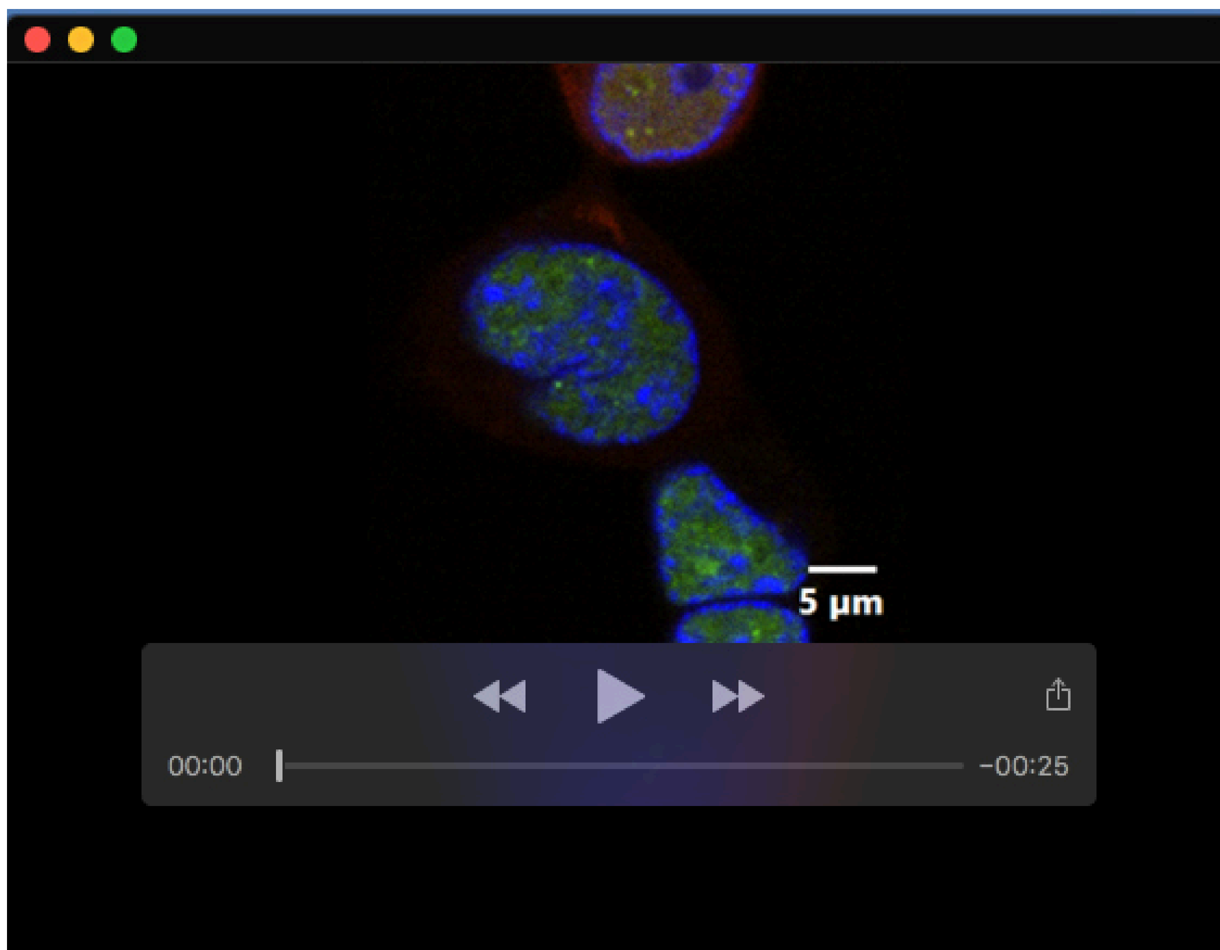


Movie 1. Time-lapse movie for sequestration of TDP-43 over-expressing FLAG-TDP-35. Movie related to Fig. 6B.

The movie was imaged with 20-min intervals in the EGFP-TDP-43 cell line transfected with FLAG-TDP-35. The imaging began at c.a. 12 hrs after transfection with FLAG-TDP-35. TDP-43 was indicated from the fluorescence of its fused EGFP (green). The live-cell images were obtained by Zeiss Celldiscoverer 7 (Zeiss). Scale bar, 5 µm.



Movie 2. Time-lapse movie for sequestration of TDP-43 over-expressing TDP-35-mCherry. Movie related to Fig. 7A.

The movie was imaged with 20-min intervals in the EGFP-TDP-43 cell line transfected with TDP-35-mCherry. The imaging began at c.a. 12 hrs after transfection with TDP-35-mCherry. TDP-43 was indicated from the fluorescence of its fused EGFP (green), while TDP-35 was lighted up by the fluorescence of its fused mCherry (red), and nuclei were stained with Hoechst (blue). The live-cell images were obtained by Olympus SpinSR (Olympus), Scale bar = 5 µm.

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