

Expanded View Figures

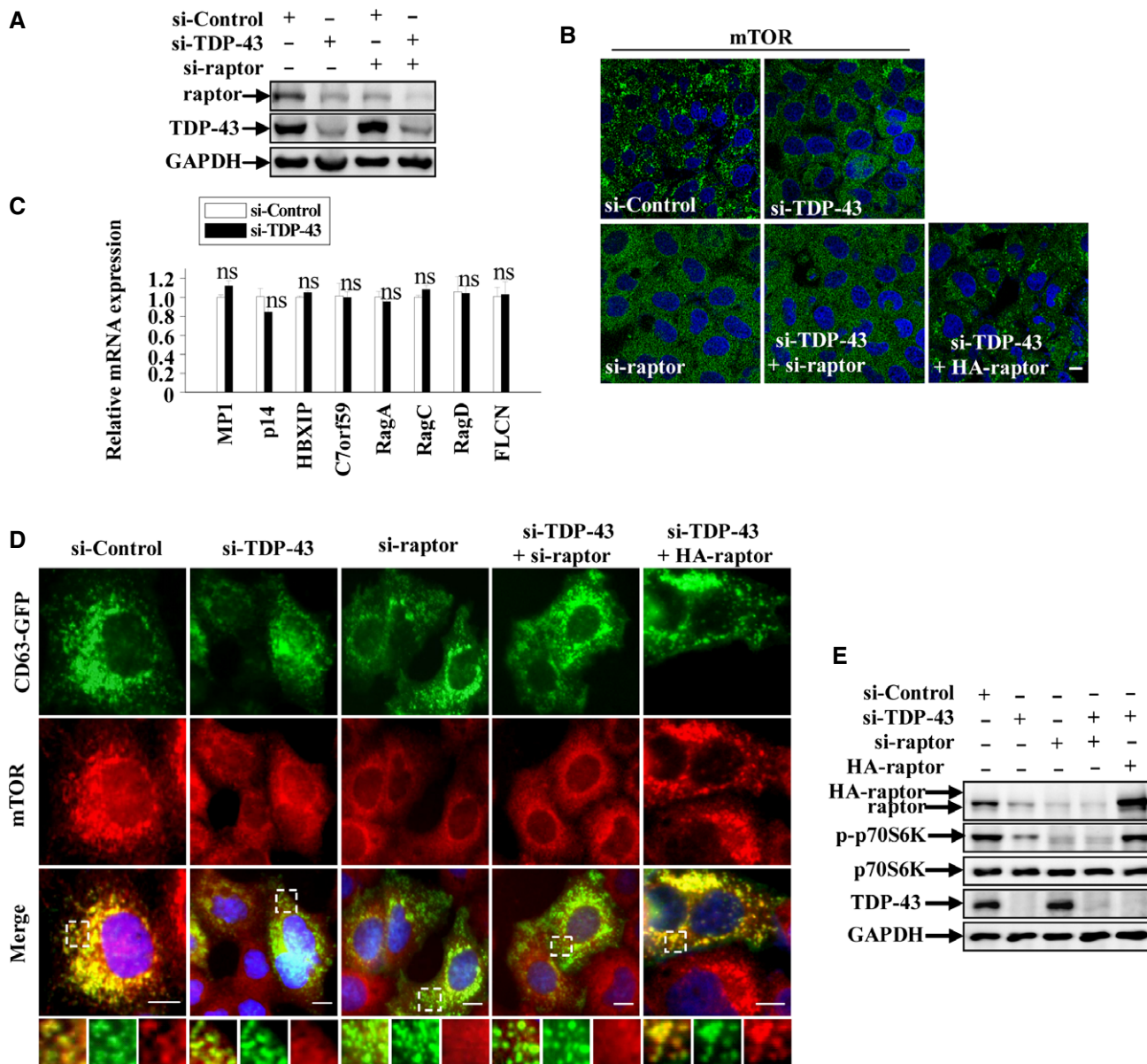


Figure EV1. TDP-43 regulates mTOR lysosomal localization and mTORC1 activity in a raptor-dependent manner.

- A HeLa cells were transfected with the indicated siRNAs. After 72 h, cell lysates were subjected to immunoblot analysis using anti-TDP-43, -raptor, and -GAPDH antibodies.
- B HeLa cells were transfected with the indicated siRNAs. After 72 h, the cells were stained with anti-mTOR (green) antibody and DAPI (blue). Cells were fixed and visualized using confocal microscopy. Scale bar, 5 μ m.
- C Similar transfection as in (A) was performed, and transfected cells were processed for qRT–PCR analysis. The level of MP1, p14, HBXIP, C7orf59, RagA, RagC, RagD, and FLCN mRNA was quantified and normalized relative to GAPDH. The data from three independent experiments are presented as means \pm S.E.M.; ns, not significantly different; one-way ANOVA.
- D HeLa cells were transfected with the indicated siRNAs. After 48 h, the cells were transfected with CD63-GFP (green), with or without HA-raptor for 24 h. The cells were stained with anti-mTOR (red) antibody and DAPI (blue). Cells were fixed and visualized using microscope IX71. Regions within the dotted boxes are magnified in the insets. Scale bar, 5 μ m.
- E Similar transfection as in (D) was performed, and transfected cells were subjected to immunoblot analysis using anti-TDP-43, -p70S6K, -p-p70S6K, -raptor, and -GAPDH antibodies.

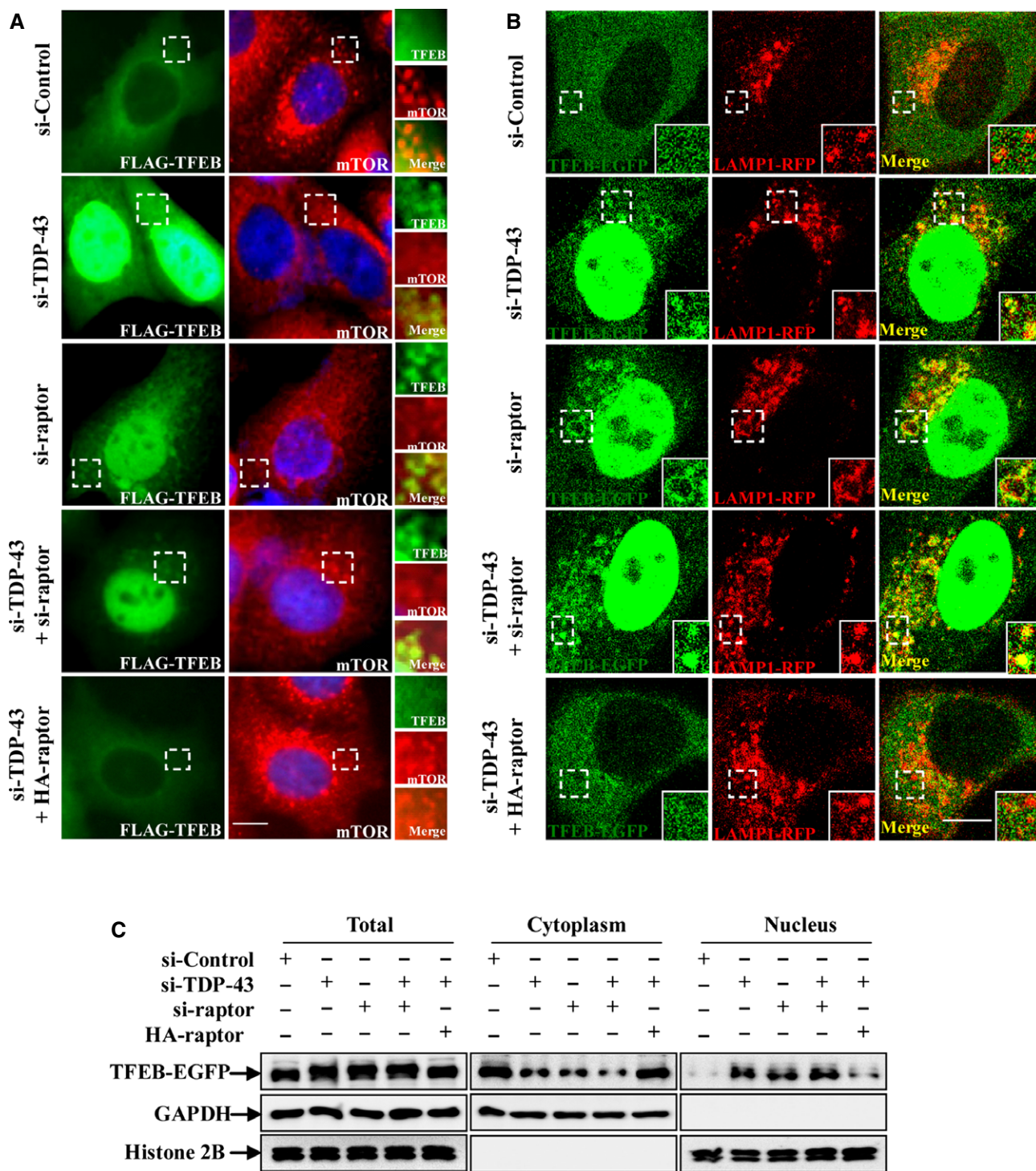


Figure EV2. TDP-43 regulates TFEB cellular localization by targeting raptor.

A HeLa cells were transfected with the indicated siRNAs. After 48 h, the cells were transfected with FLAG-TFEB, with or without HA-raptor for 24 h. The cells were stained with anti-FLAG (green) and -mTOR (red) antibodies and DAPI (blue), and then the stained cells were visualized using microscope IX71. Regions within the dotted boxes are magnified in the insets. Scale bar, 5 μm.

B HeLa cells were transfected with the indicated siRNAs. After 48 h, the cells were transfected with TFEB-EGFP (green) and LAMP1-RFP (red), with or without HA-raptor for 24 h. Cells were fixed and visualized using confocal microscopy. Regions within the dotted boxes are magnified in the insets. Scale bar, 5 μm.

C HeLa cells were similarly transfected as in (B). Lysates from the cells were separated into cytoplasmic and nuclear fractions and then subjected to immunoblot analysis using anti-GFP, -GAPDH, and -histone 2B antibodies.

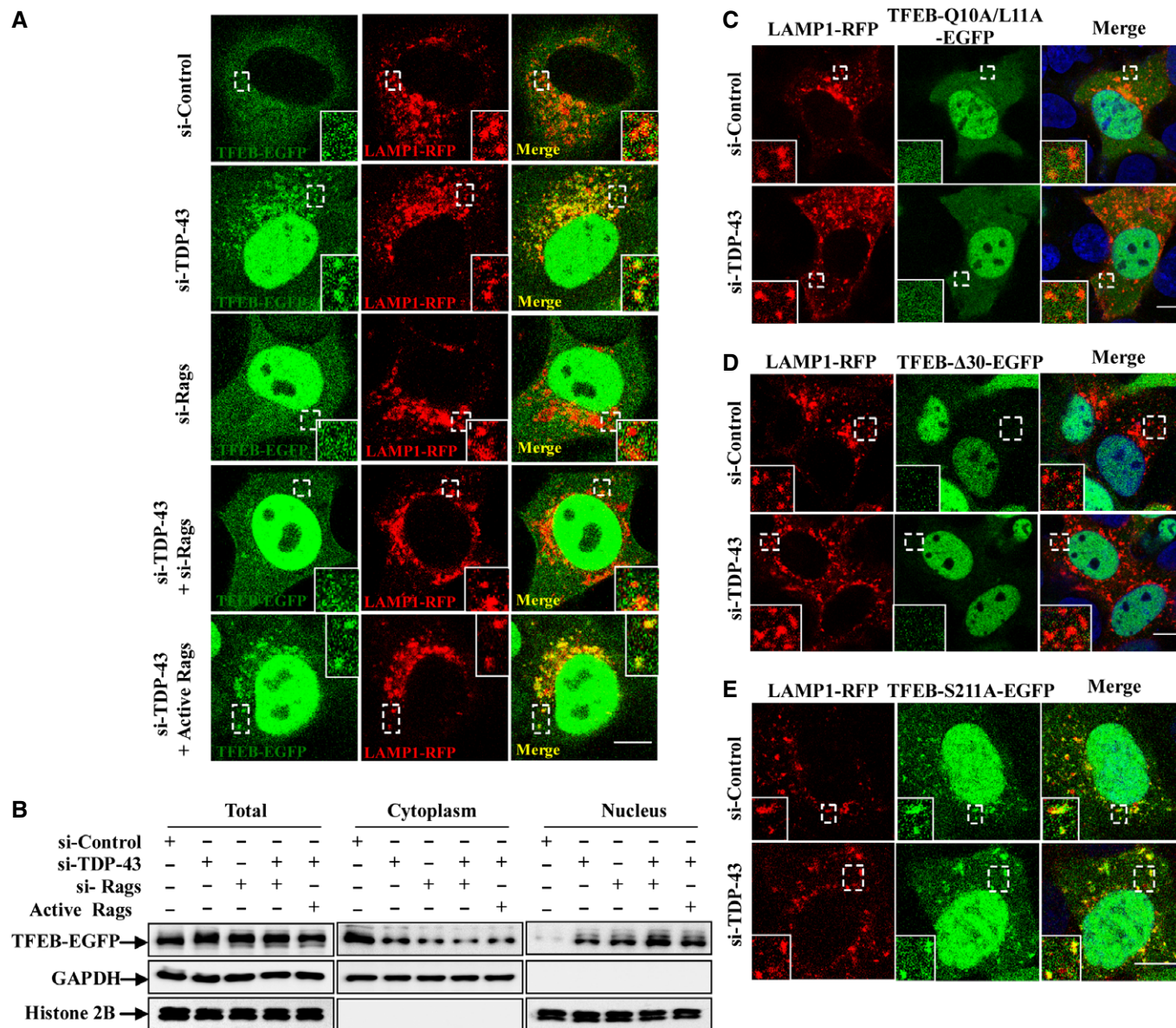


Figure EV3. TDP-43 does not regulate TFEB cellular localization through Rag GTPases, and effects of TDP-43 on TFEB mutants.

A HeLa cells were transfected with the indicated siRNAs. Note that si-Rags indicates a combination of siRNAs targeting RagA and RagB. After 48 h, the cells were transfected with TFEB-EGFP (green) and LAMP1-RFP (red), with or without constitutively active HA-GST-tagged Rag GTPase mutants (RagA Q66L + RagC S75L = RagA^{GTP} + RagC^{GDP}) for 24 h. Cells were fixed and visualized using confocal microscopy. Regions within the dotted boxes are magnified in the insets. Scale bar, 5 μm.

B HeLa cells were similarly transfected as in (A). Lysates from the cells were separated into cytoplasmic and nuclear fractions and then subjected to immunoblot analysis using anti-GFP, -GAPDH, and -histone 2B antibodies.

C–E HEK 293 cells were transfected with the indicated siRNAs. After 48 h, the cells were re-transfected with EGFP-tagged TFEB-Q10A/L11A, TFEB-Δ30, or TFEB-S211A, along with LAMP1-RFP for 24 h. The cells were stained with DAPI (blue), and then the stained cells were visualized using confocal microscopy. Regions within the dotted boxes are magnified in the insets. Scale bar, 5 μm.

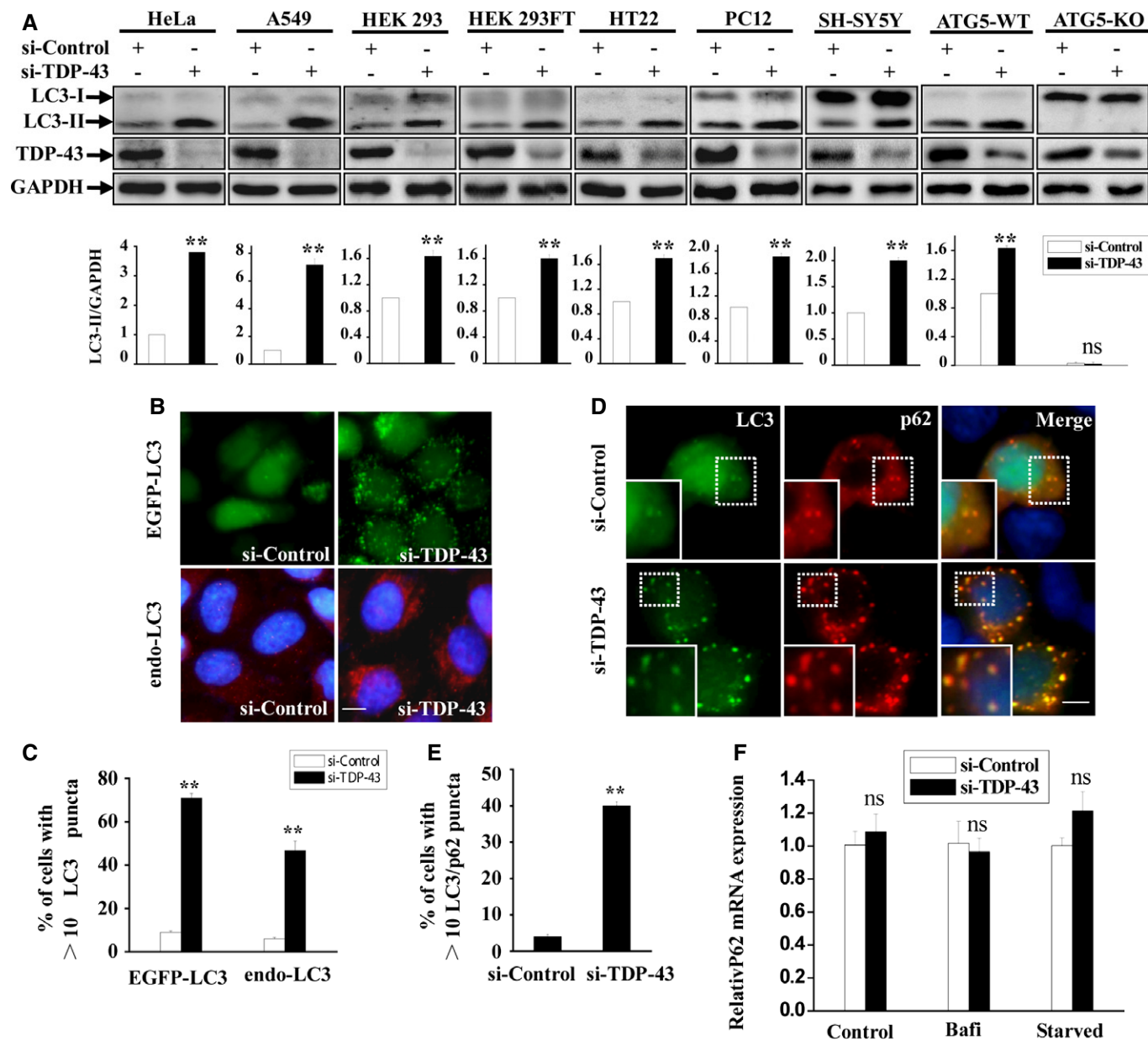


Figure EV4. TDP-43 influences autophagosome formation.

A HeLa, A549, HEK 293, HEK 293FT, HT22, PC12, SH-SY5Y, ATG5-WT, and ATG5-KO cells were transfected with the indicated siRNAs. After 72 h, lysates from the cells were subjected to immunoblot analysis using anti-TDP-43, LC3, and GAPDH antibodies. The relative densities of LC3-II to GAPDH are shown in the lower panel. Data from three independent experiments represented as means ± S.E.M.; ns, not significantly different; **, $P < 0.01$; one-way ANOVA.

B, C HeLa cell line stably expressing EGFP-LC3 or HeLa cells was transfected with the indicated siRNAs. After 72 h, the cells were stained with anti-rabbit Rhodamine (Santa Cruz Biotechnology) following treatment with anti-LC3 (red) antibody and DAPI (blue), and then, the stained cells were visualized using microscope IX71. Scale bar, 5 μm. The quantification data of punctate LC3 are shown in Figure EV4C. Data from three independent experiments represented as means ± S.E.M.; **, $P < 0.01$; one-way ANOVA.

D, E HEK 293 cells were transfected with the indicated siRNAs. After 48 h, the cells were re-transfected with EGFP-LC3 and FLAG-p62 for 24 h. The cells were stained with anti-FLAG (red) antibody and DAPI (blue), and then, the stained cells were visualized using microscope IX71. Regions within the dotted boxes are magnified in the insets. Scale bar, 5 μm. The quantification data of punctate LC3/p62 are shown in (E). Data from three independent experiments represented as means ± S.E.M.; **, $P < 0.01$; one-way ANOVA.

F HeLa cells were transfected with the indicated siRNAs for 48 h. For bafilomycin A1 treatment, the cells were then incubated with bafilomycin A1 (100 nM) for 24 h. For starvation treatment, the cells were cultured for another 22 h and then treated with Earle's balanced salt solution for 2 h. The cells were subjected to qRT-PCR analysis. The mRNA level of p62 was quantified and normalized relative to GAPDH. The data from three independent experiments are presented as means ± S.E.M.; ns, not significantly different.

Figure EV5. TDP-43 regulates gene expression of DCTN1, which encodes dynactin 1, and influences lysosome positioning.

- A HeLa cells were transfected with the indicated siRNAs. After 72 h, the transfected cells were processed for qRT–PCR analysis. The mRNA levels of autophagosome/endosome/lysosome fusion-related proteins were quantified and normalized relative to GAPDH. The data from three independent experiments are presented as means \pm S.E.M.; *, $P < 0.05$; one-way ANOVA.
- B Similar experiments as in (A) were performed in SH-SY5Y cells. The mRNA levels of autophagosome/endosome/lysosome fusion-related proteins were quantified and normalized relative to GAPDH. Data from three independent experiments represented as means \pm S.E.M.; **, $P < 0.01$; one-way ANOVA.
- C Three representative distribution patterns of lysosome in cells: compact perinuclear, perinuclear, or dispersed. HeLa cells were transfected with LAMP1-RFP for 24 h. DAPI (blue) was used for nuclear staining. Cells were visualized using confocal microscopy. Scale bar, 5 μ m.
- D, E HeLa cells were transfected with the indicated siRNAs. After 72 h, the cells were subjected to immunoblot analysis using anti-DHC, dynactin 1, and GAPDH antibodies.
- F Similar transfection as in (E) was performed. After 72 h, the transfected cells were processed for qRT–PCR analysis. The mRNA level of KIF5B was quantified and normalized relative to GAPDH. The data from three independent experiments are presented as means \pm S.E.M.; **, $P < 0.01$; one-way ANOVA.
- G, H HeLa cells were transfected with the indicated siRNAs. After 48 h, the cells were re-transfected with LAMP1-RFP (red) for 24 h. And then, the cells were incubated with Earle's balanced salt solution (starvation) for 2 h. DAPI (blue) was used for nuclear staining. Cells were fixed and visualized using confocal microscopy. Scale bar, 5 μ m. The quantification of lysosomes from the nucleus boundary is shown in Figure EV5H. Data are from three independent experiments, means \pm S.E.M.; ns, not significantly different; **, $P < 0.01$; one-way ANOVA.

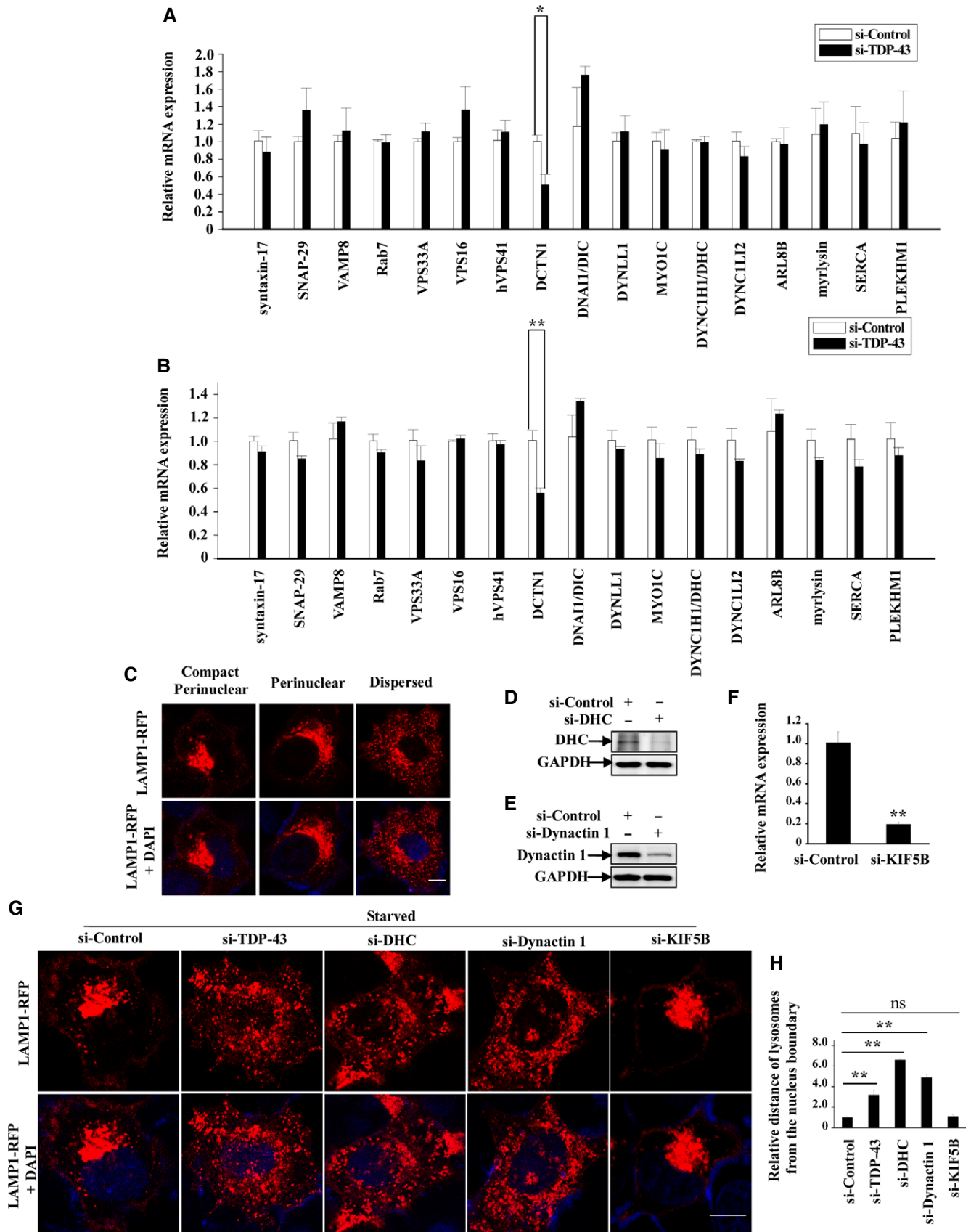


Figure EV5.