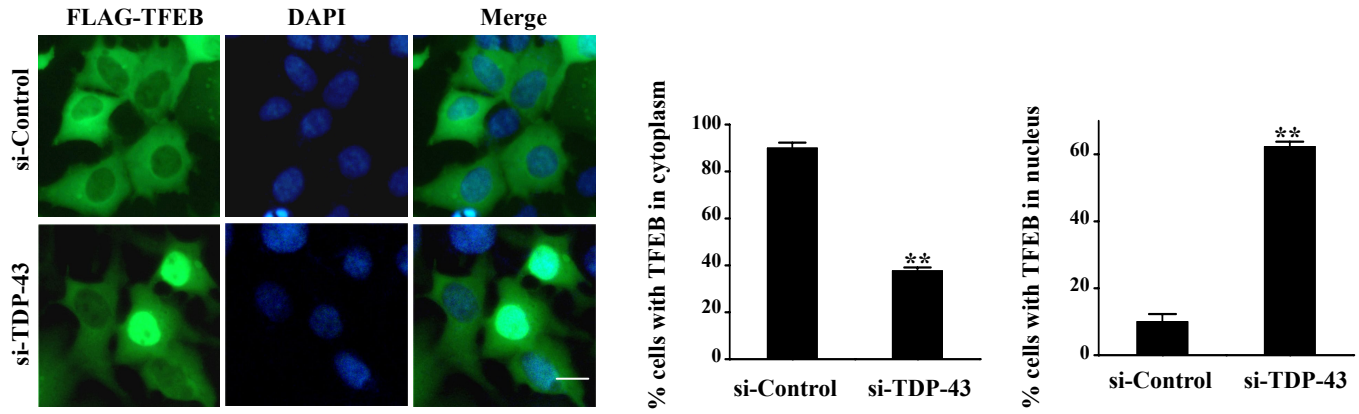
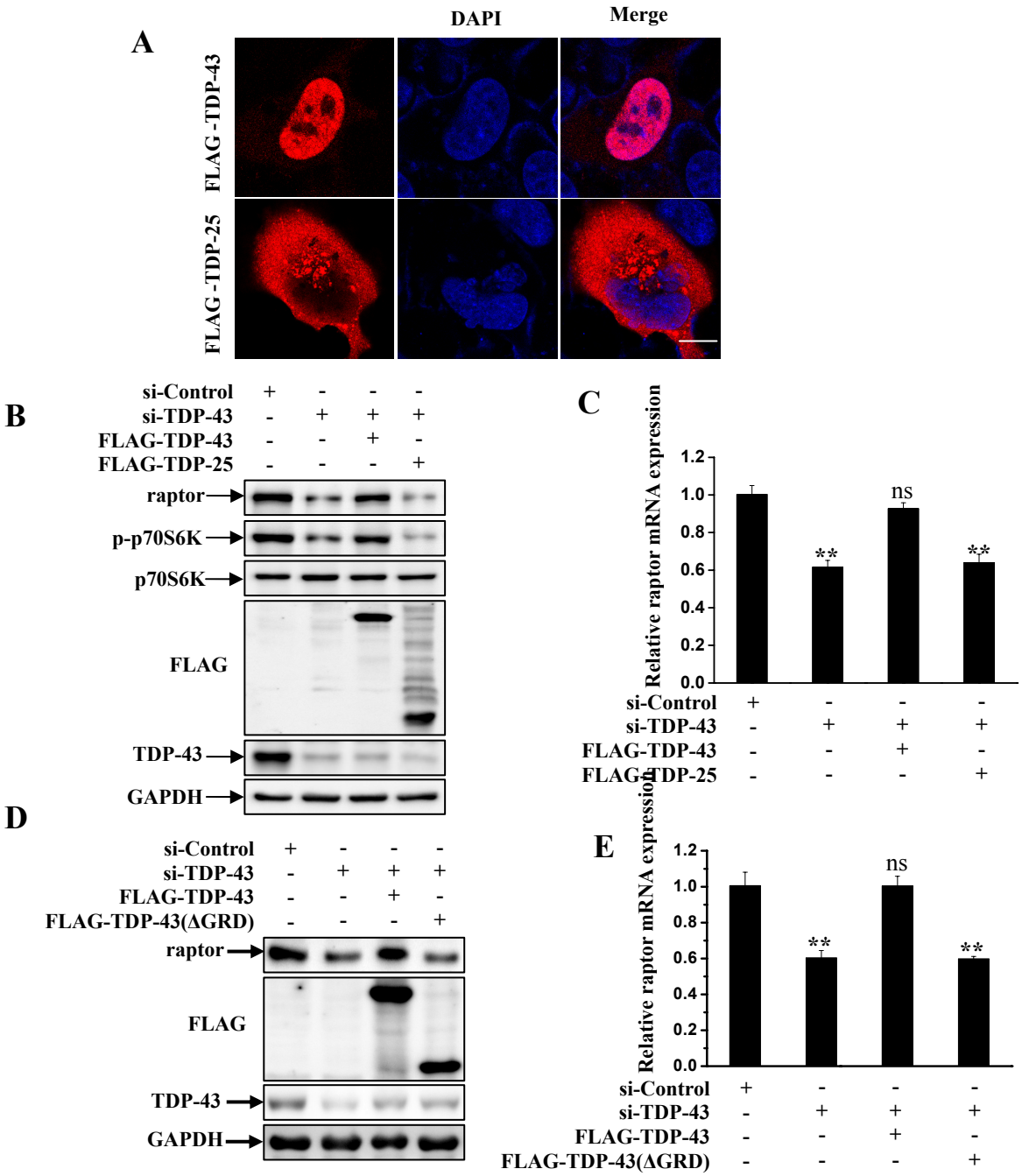


Appendix Figure S1: Effect of TDP-43 on TFEB nuclear translocation.

HEK 293 cells were transfected with the indicated siRNAs for 48 h, and then were re-transfected with FLAG-TFEB. After 24 h, the cells were fixed, stained with antibody against FLAG (green). DAPI (blue) was used for nuclear staining, and then the stained cells were visualized using microscope IX71. Scale bar, 5 μm. The quantifications of TFEB localization in cytoplasm and nucleus are shown on the right side. The data from three independent experiments indicated the means ± S.E.M., **, $p<0.01$, one-way ANOVA.



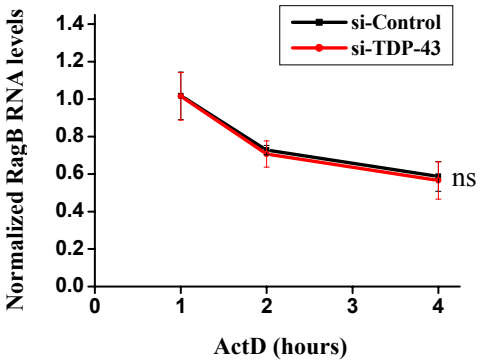
Appendix Figure S2: TDP-43 mutants fail to restore raptor mRNA and protein levels in TDP-43 depleted cells.

(A) HeLa cells were transfected with FLAG-TDP-43 or FLAG-TDP-25. After 24 h, the cells were stained with anti-FLAG (red) antibody and DAPI (blue). Cells were fixed and visualized using confocal microscopy. Scale bar, 5 µm.

(B-E) HeLa cells were transfected with the indicated siRNAs. After 48 h, the cells were re-transfected with FLAG tagged wild type or mutant TDP-43 for 24 h.

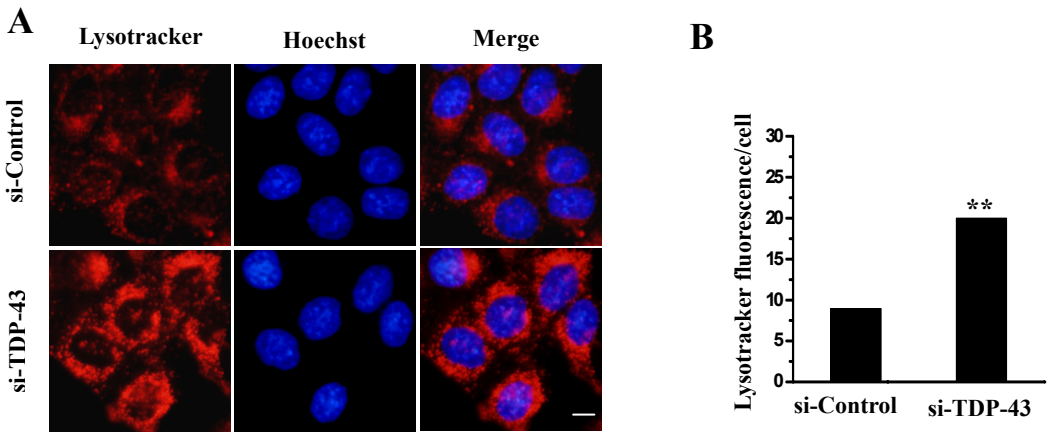
(B, D) Cell lysates were subjected to immunoblot analysis using anti-raptor, p70S6K, p-p70S6K, FLAG, TDP-43 and GAPDH antibodies.

(C, E) Cells were processed for qRT–PCR analysis. The level of raptor mRNA was quantified and normalized relative to GAPDH. The data from three independent experiments are presented as means ± S.E.M., ns, not significantly different; **, $p<0.01$, one-way ANOVA.



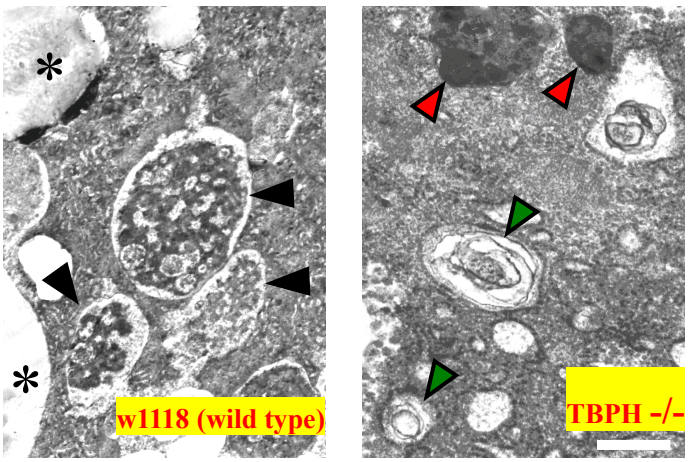
Appendix Figure S3: TDP-43 does not affect the stability of RagB mRNA.

HeLa cells were transfected with the indicated siRNAs for 72 h. Then the cells were treated with ActD and processed for qRT–PCR analysis. The level of RagB mRNA was quantified and normalized relative to GAPDH. Data from three independent experiments represented as means ± S.E.M., ns, not significantly different, one-way ANOVA.

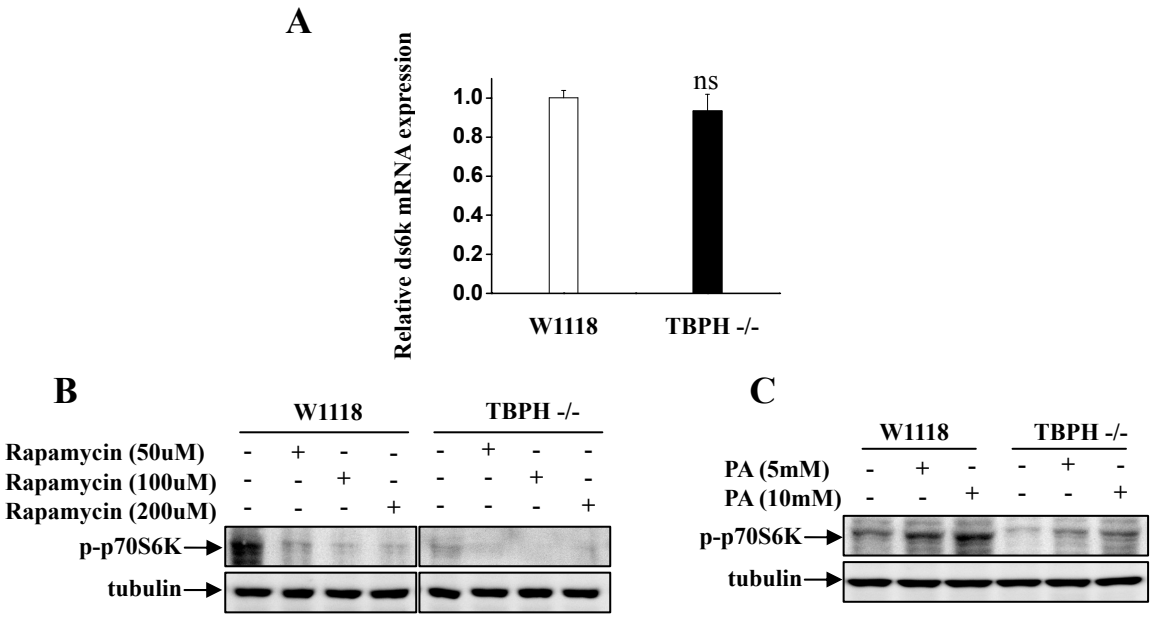


Appendix Figure S4: TDP-43 regulates lysosomal biogenesis.

(A, B) HeLa cells were transfected with the indicated siRNAs. After 72 h, the cells were stained with Lysotracker (red). Hoechst (blue) was used for nuclear staining. The stained cells were visualized using microscope IX71. Scale bar, 5 μm. The quantification of the fluorescent intensity of Lysotracker is shown in Appendix Figure S4B. The data from three independent experiments indicated the means ± S.E.M., **, $p<0.01$, one-way ANOVA.



Appendix Figure S5: EM analysis of autophagosome-lysosome fusion in TBPH-/- flies.
The third instar larvae of W1118 and TBPH-/- were transferred to a 20% sucrose solution for starvation. After 4 h, the fat bodies of the larvae were dissected, and then were subjected to EM analysis. Black arrowheads: autolysosome; red arrowheads: lysosome; green arrowheads: autophagosome; asterisks: lipid droplet. Scale bar, 1μm.



Appendix Figure S6: Expression and activity of ds6k (fly p70S6K) in untreated, rapamycin-treated and PA-treated flies

(A) Total RNA was prepared from W1118 and TBPH-/- larvae and subjected to qRT–PCR analysis. The level of ds6k was quantified and normalized relative to RPL32. Data from three independent experiments represented as means ± S.E.M., ns, not significantly different.

(B) The first instar TBPH-/- larvae were treated with increasing amount of (50uM, 100uM, 200uM) Rapamycin for 72 h. Then the third instar larvae of W1118 and TBPH-/- were subjected to immunoblot analysis using anti- p-p70S6K and tubulin antibodies.

(C) Similar experiments as in (B) were performed, but using 5mM or 10mM PA instead of Rapamycin.