Aggregation of the nucleic acid–binding protein TDP-43 occurs via distinct routes that are coordinated with stress granule formation

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Supporting Information:

Figure S1: Mature TDP-43 aggregates modulate FMRP phosphorylation status.

Figure S2: Cytoplasmic TDP-43 accumulation in primary mouse neurons treated with an HDAC6 inhibitor.

Figure S3: HDAC6 1-840 lacking the SE14 and C-terminal domains shows impaired clearance of TDP-43 despite co-localization to cytoplasmic TDP-43 inclusions.

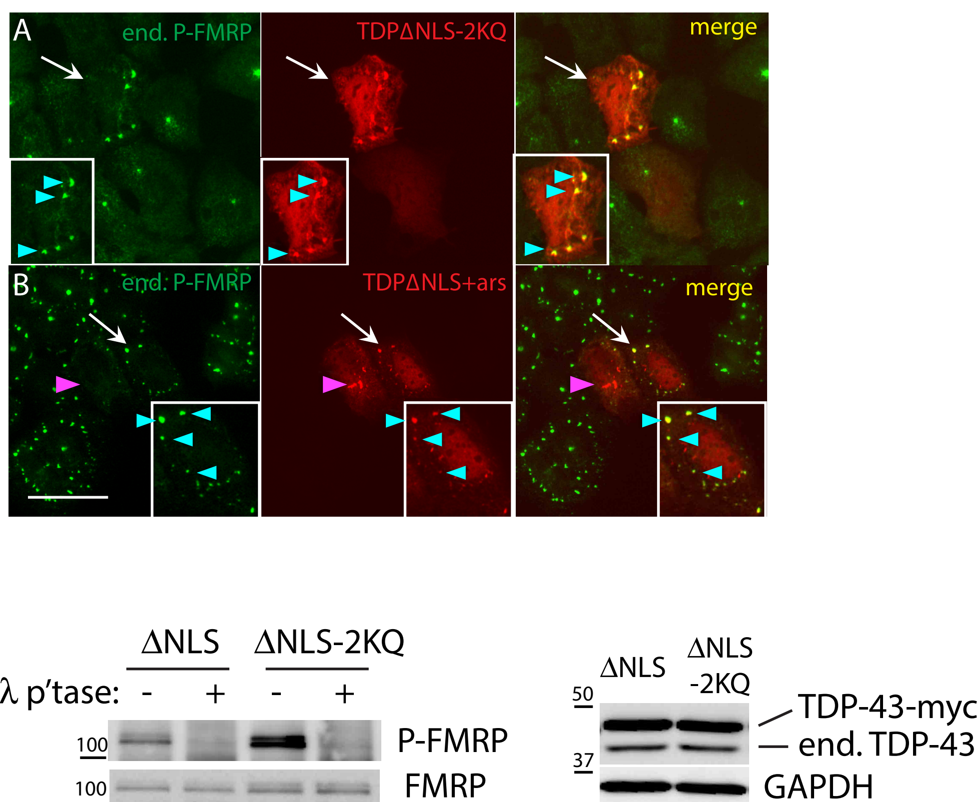


Figure S1: Mature TDP-43 aggregates modulate FMRP phosphorylation status.
(A-B) TDP-43 aggregates were generated in QBI-293 cells by either transfection with TDP-43-ΔNLS and exposure to arsenite (as described in the Fig. 1 legend) or transfection with the TDP-43-ΔNLS-2KQ mutant, either of which generates amorphous cytoplasmic inclusions. Fixed cells were analyzed by immunofluorescence microscopy using antibodies detecting myc-tagged TDP-43 and double-labeled with phosphorylated FMRP at residue S499. Scale bar = 50 μm. White arrows highlight cells that are magnified in insets, cyan arrowheads highlight endogenous SGs, and magenta arrowheads highlight TDP-43 inclusions. (C) Co-transfected cells expressing TDP-43-ΔNLS or TDP-43-ΔNLS-2KQ along with FMRP-GFP were analyzed by immunoprecipitation and immunoblotting. FMRP-protein A/G complexes were isolated by immunoprecipitation, followed by λ-phosphatase treatment, where indicated, and the resulting lysates were immunoblotted using P-FMRP (S499) and total FMRP antibodies (left panel). Total inputs and loading controls from transfected cells were confirmed by immunoblotting with total TDP-43 (detecting myc-tagged and endogenous TDP-43 proteins) and GAPDH antibodies (right panel).

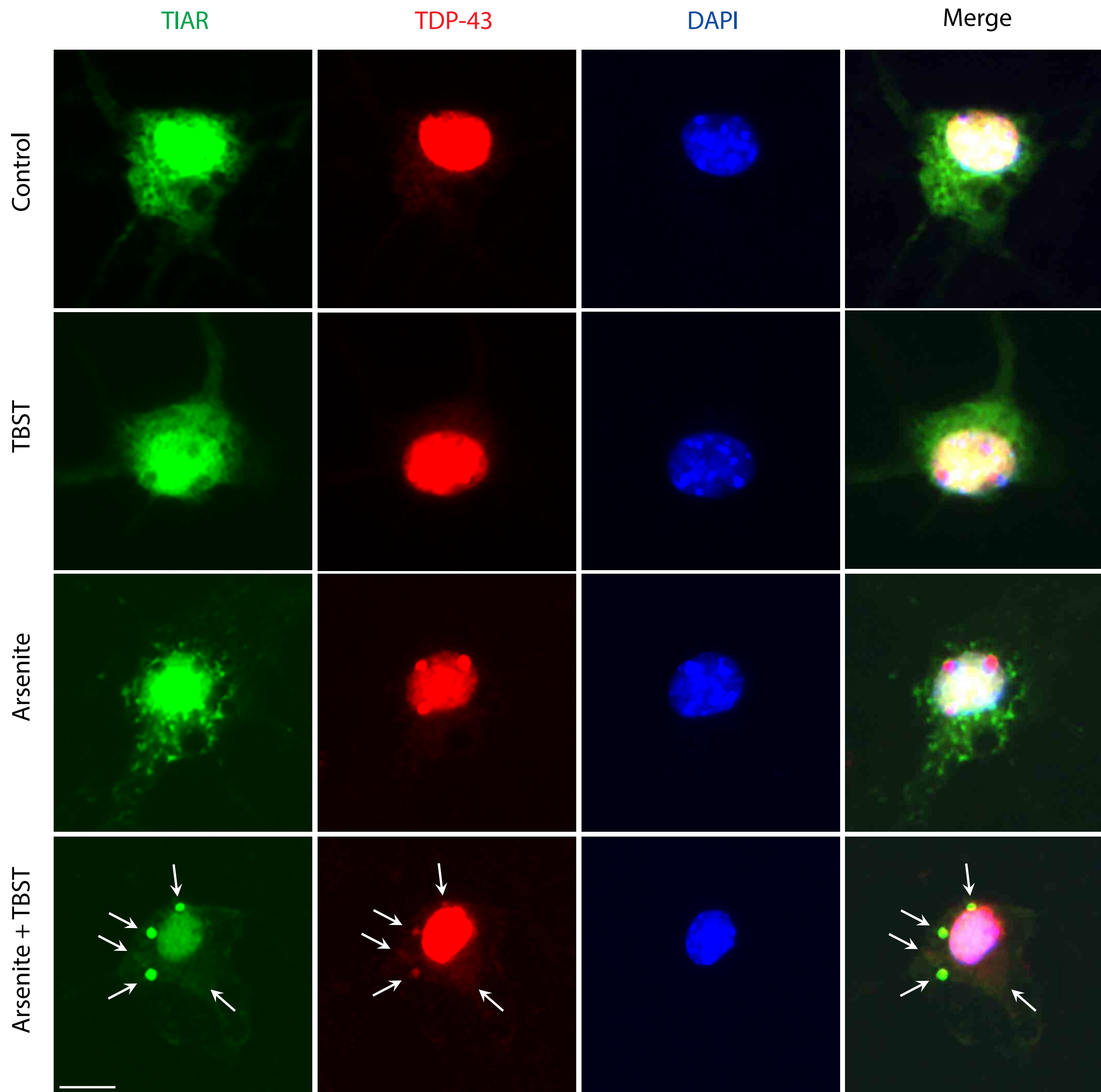
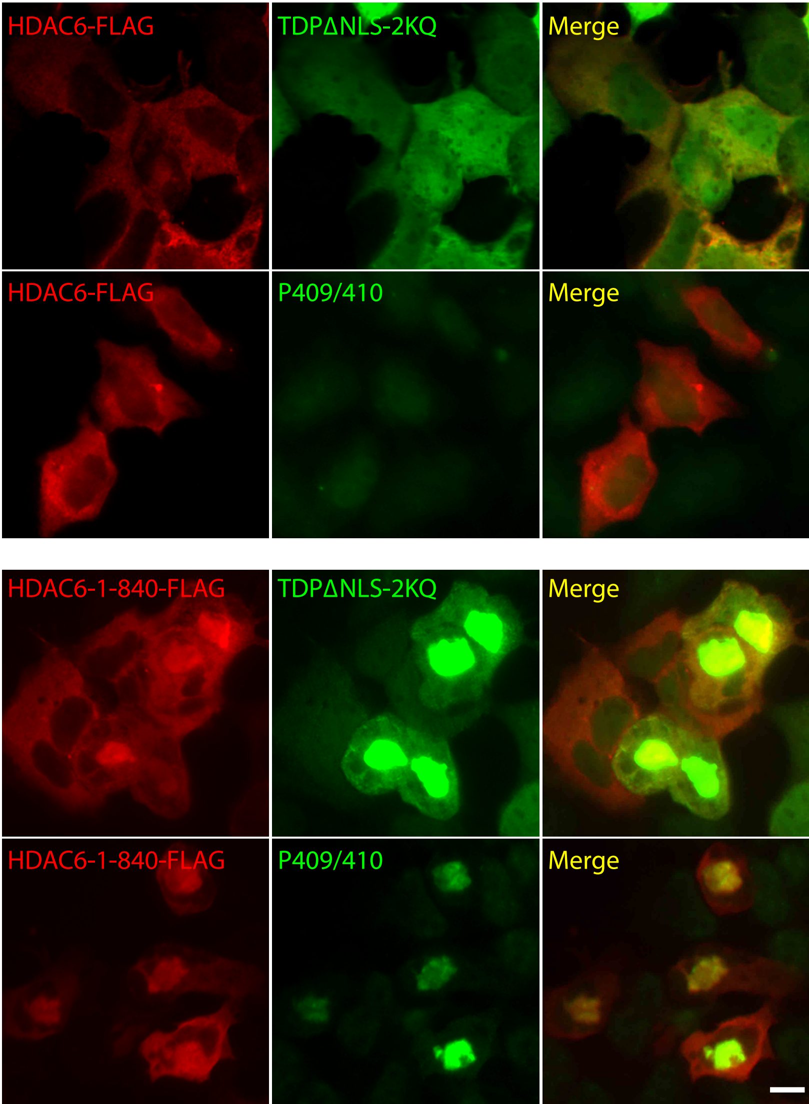


Figure S2: Cytoplasmic TDP-43 accumulation in primary mouse neurons treated with an HDAC6 inhibitor.
Primary mouse cortical neurons were cultured for 11 days in vitro (DIV) and either left untreated or pre-treated with 5 μM tubastatin A (TBST) for 1 hr, followed by treatment with 50 μM sodium arsenite for 80 min, where indicated. Fixed neurons adhered to coverslips were analyzed by double-labeling immunofluorescence using antibodies that detect SGs (TIAR, green) and total TDP-43 (red), while nuclei were counterstained with DAPI (blue). Scale bar = 10 μm.

A



B

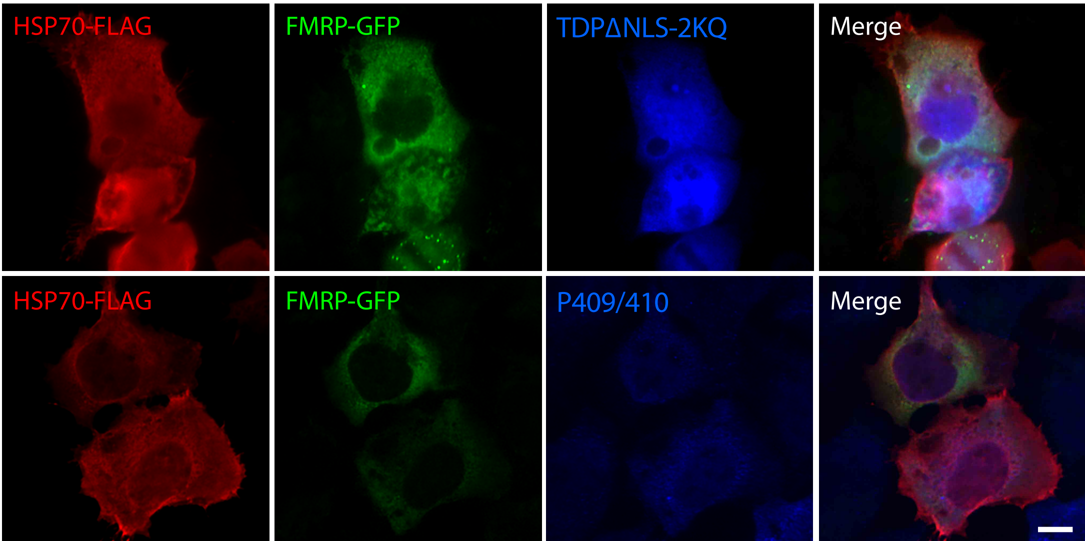


Figure S3: HDAC6 1-840 lacking the SE14 and C-terminal domains shows impaired clearance of TDP-43 despite co-localization to cytoplasmic TDP-43 inclusions.

(A) QBI-293 cells were co-transfected with TDP-43-ΔNLS-2KQ in the presence of wild-type HDAC6 or an HDAC6 1-840 C-terminal truncation mutant lacking the SE14 and C-terminal BUZ domain. Fixed cells were analyzed by immunofluorescence microscopy using antibodies detecting FLAG-tagged HDAC6 (red) and either phosphorylated TDP-43 (P-409/410) or myc-tagged TDP-43 (green). **(B)** Immunofluorescence analysis similar to **A** above was performed in QBI-293 cells co-transfected with FLAG-tagged Hsp70 (red), GFP-tagged FMRP (green), and myc-tagged TDP-43-ΔNLS-2KQ (blue). Scale bar = 10 μm.