

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. **Clearing of endogenous nuclear TDP-43.** Double-label immunofluorescence of QBI-293 cells 24-h (**A-C**) or 72-h (**D-F**) after transfection with myc-TDP-43- Δ NLS2 (Δ NLS2), or 24-h (**G-I**) or 72-h (**J-L**) after transfection with myc-TDP-43- Δ NLS1/2 (Δ NLS1/2) immunostained with anti-TDP-43 (red) and anti-myc (green) antibodies. Nuclei were labeled with DAPI stain (blue). Merge images (**C and I**) show colocalization of TDP-43 and myc immunoreactivity in cytoplasm of transfected cells. Note the presence of endogenous nuclear TDP-43 in all cells at 24 h, but subsequent clearing of endogenous nuclear TDP-43 in cells expressing myc-TDP-43- Δ NLS mutants (**F and L**), as compared to non-transfected cells (asterisks). Scale bar; 20 μ m.

Figure S2. **Cytoplasmic expression of TDP-43 does not promote the nuclear clearance of other RNA-binding proteins.** **A-F**, Double-label immunocytochemistry of QBI-293 cells 72 h after transfection of myc-TDP-43- Δ NLS1/2 (Δ NLS1/2) stained with (**A and D**) anti-TDP-43 (red), (**B and C**) anti-hRNP A1 (green, top panel), or anti-hRNP C1/C2 (green, bottom panel) antibodies. Note clearing of endogenous nuclear TDP-43 in cells expressing myc-TDP-43- Δ NLS1/2 (**A and D**), but the presence of A1 and C1/C2 immunoreactivity in the nuclei of all cells (**B and C**).

Figure S3. **TDP-43 fragments are N-terminally truncated.** QBI-293 cells transfected with TDP-43- Δ NLS1 (Δ NLS1), or myc-TDP-43- Δ NLS2 were sequentially extracted with RIPA (R) and urea buffer (U). Immunoblotting was conducted with antibodies raised against (**A**) the C-terminus of TDP-43, (**B**) the N-terminus of TDP-43 and an anti-Myc antibody. Immunoblot demonstrates the presence of a high Mr smear (***) and C-terminal fragments (*) in the urea fractions of myc-TDP-43-NLS mutants in transfected cells. Note that N-terminal TDP-43 or anti-Myc antibodies do not detect C-terminal fragments.

Figure S4. **TDP-43 forms punctuate cytoplasmic aggregates.** Double-labeled immunofluorescence of myc-TDP-43- Δ NLS1 (Δ NLS1) transfected cells extracted with 0.2 % Triton-X 100 prior to fixation and stained with (**A**) anti-TDP-43 (red) and (**B**) anti-myc (green) antibodies. Merge image (**C**) shows colocalization of punctate TDP-43 and myc immunoreactivity in the cytoplasm of transfected cells. Scale bar; 20 μ m.







