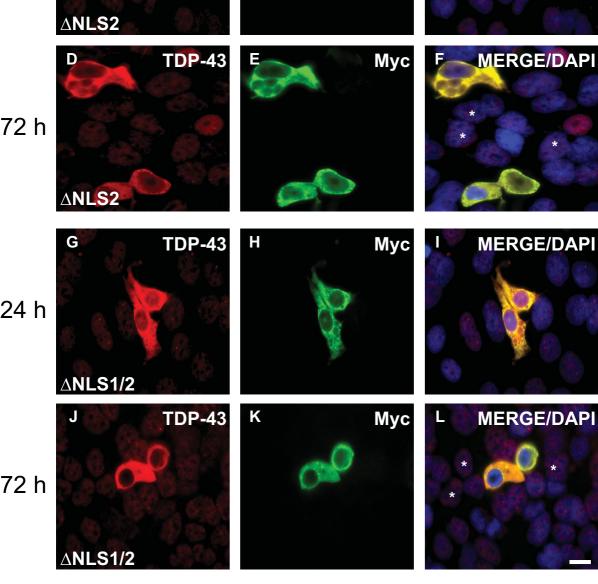
## SUPPLEMENTARY FIGURE LEGENDS

- 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-ΔNLS2 (ΔNLS2), or 24-h (G-I) or 72-h (J-L) after transfection with myc-TDP-43-ΔNLS1/2 (ΔNLS1/2) 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-ΔNLS mutants (F and L), as compared to non-transfected cells (asterisks). Scale bar; 20 μm.
- Figure S2. Cytoplasmic expression of TDP-43 does not promote the nuclear clearance of other RNA-biding proteins. A-F, Double-label immunocytochemistry of QBI-293 cells 72 h after transfection of myc-TDP-43-ΔNLS1/2 (ΔNLS1/2) 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-ΔNLS1/2 (**A** and **D**), but the presence of A1 and C1/C2 immunreactivity 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-ΔNLS1 (ΔNLS1), or myc-TDP-43-ΔNLS2 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-ΔNLS1 (ΔNLS1) 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 μm.



**TDP-43** 

24 h

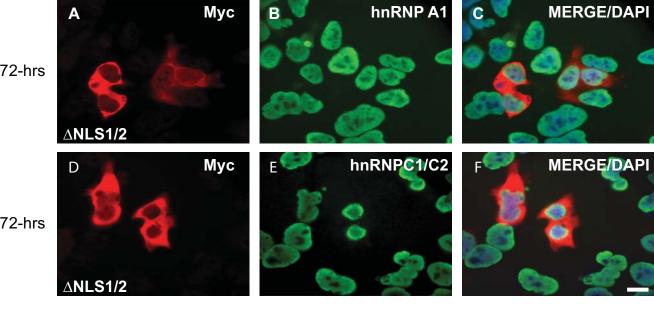
В

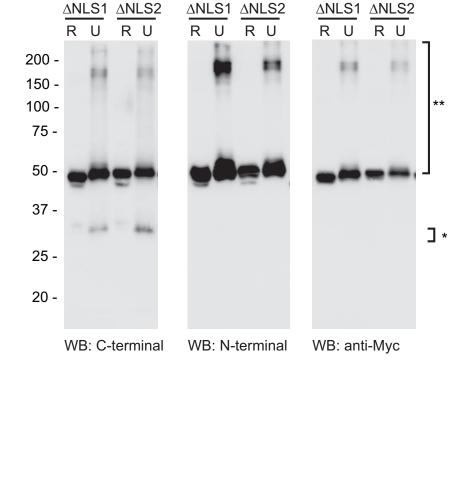
Мус

C

**MERGE/DAPI** 

Winton et al. SUPPLEMENTARY FIGURE 1





C

В

Α

## Winton et al. SUPPLEMENTARY FIGURE 3

