Neuron

Supplemental Information

Cytoplasmic TDP-43 de-mixing independent of stress granules drives inhibition of nuclear import, loss of nuclear TDP-43, and cell death

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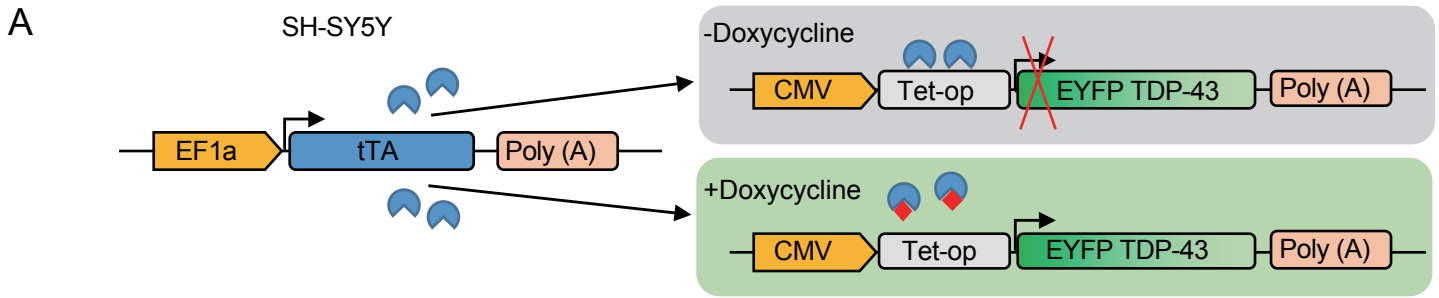

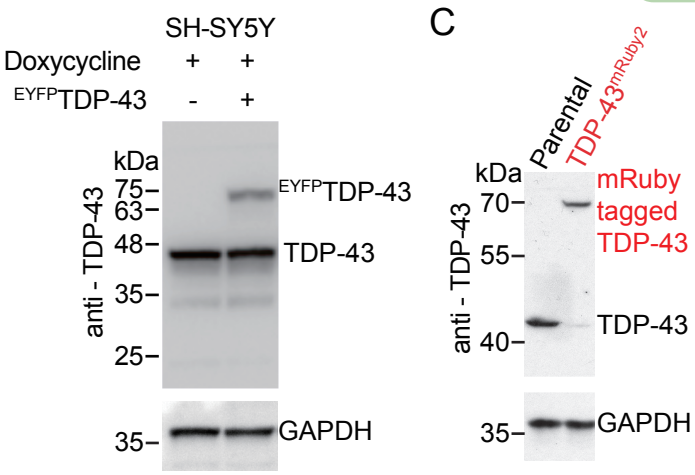

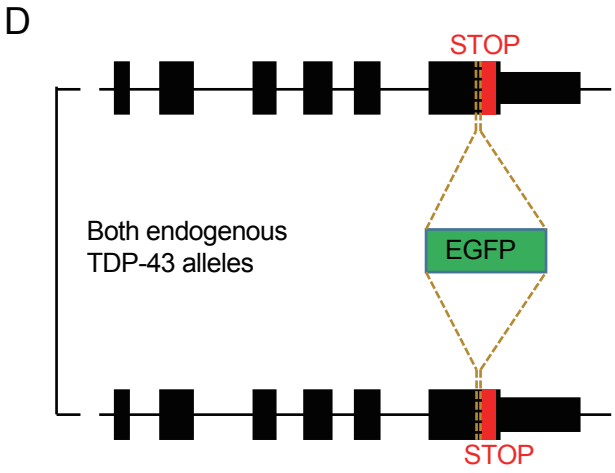

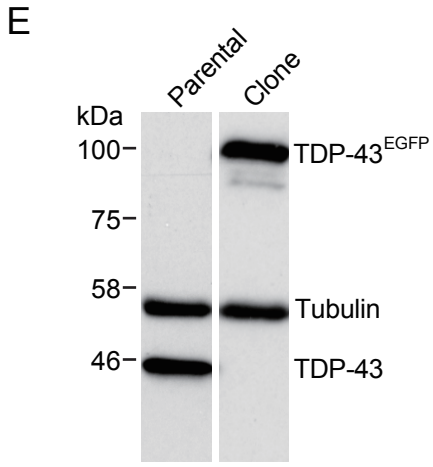

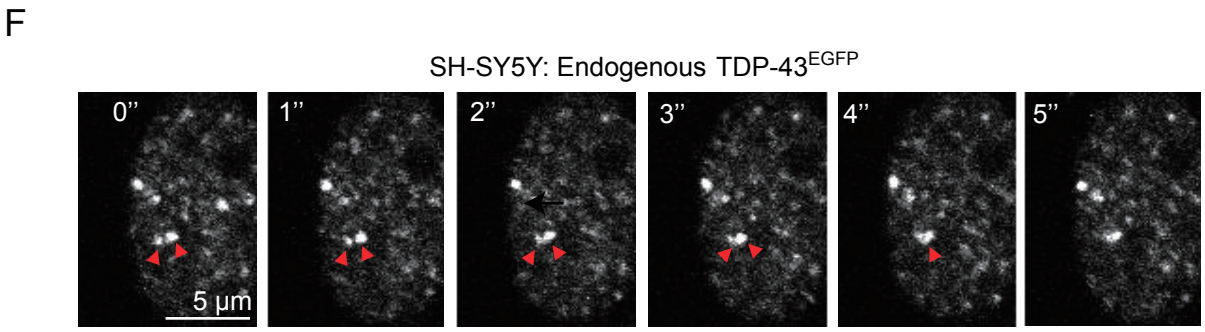

Figure S1. Expression of fluorescently-tagged TDP-43 at physiological levels in SH-SY5Y cells. Related to Figure 1

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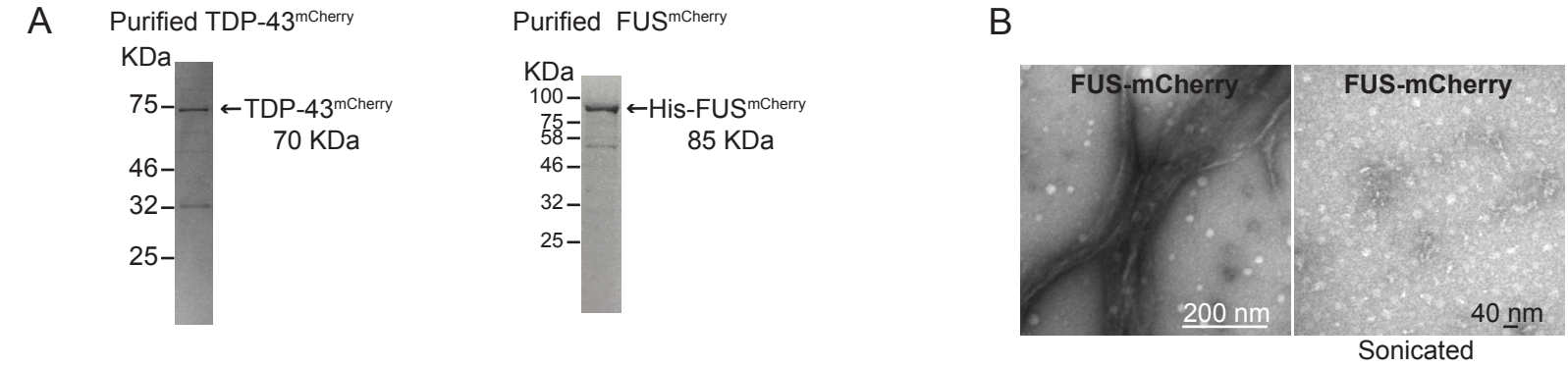
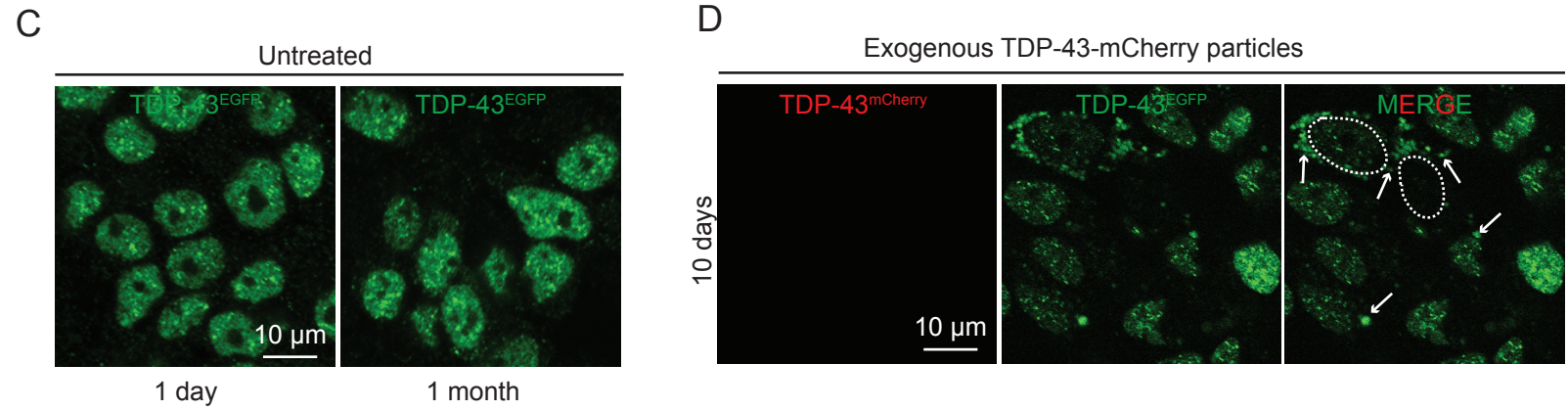
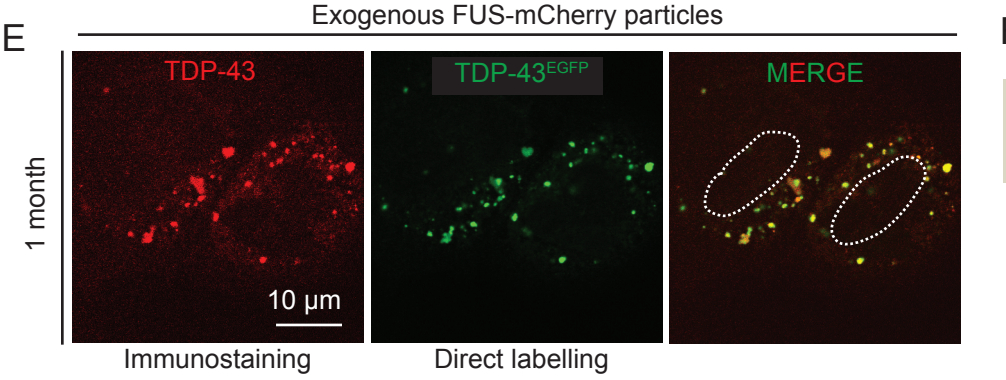
(A) Scheme outlining doxycycline inducible expression of TDP-43 with N-terminally tagged EYFP (^{EYFP}TDP-43) in SH-SY5Y cells. (B) Immunoblot showing ^{EYFP}TDP-43 levels in SH-SY5Y cells after 72 hours of induction using a TDP-43 antibody. GAPDH antibody was used as loading control. (C) Immunoblotting of TDP-43 levels in U2OS cells expressing TDP-43^{mRuby2} upon doxycycline induction compared to endogenous TDP-43. GAPDH was used as loading control. (D) Scheme illustrating the genome editing of TDP-43 locus to introduce EGFP in both TDP-43 alleles before the stop codons in SH-SY5Y cells. (E) Immunoblot showing that TDP-43^{EGFP} replaces endogenous TDP-43 with TDP-43 total level being maintained (compared to the parental untagged cells) using TDP-43 antibody. Tubulin antibody was used as loading control. (F) Representative fusion event of endogenous TDP-43^{EGFP} droplets in SH-SY5Y cells. Red arrowheads indicate the fusing droplets.







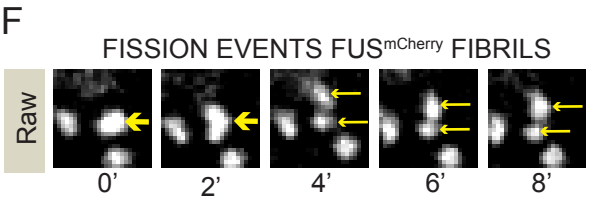


Figure S2. After 10 days of TDP-43^{mCherry} particles treatment TDP-43 starts to mislocalize in the cytoplasm and TDP-43 LLPS presents fission events. Related to Figure 2

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(A) Coomassie blue staining of recombinant TDP-43^{mCherry} protein purified from bacteria at the expected size of 70 KDa (left panel), and FUS^{mCherry} protein at the expected size of 85 KDa (right panel). (B) Electron micrograph of amyloid-like fibrils of FUS^{mCherry} recombinant protein purified from bacteria. Right panel illustrates the FUS^{mCherry} fibrils after sonication before inoculating them into cell media. Scale bars, 200 nm and 40nm (sonicated fibrils). (C) Representative images using confocal microscopy of endogenous nuclear TDP-43^{EGFP} in non-fibril-treated SH-SY5Y cells at day 1 (left) and after 1 month of culture (right). Scale bar, 10 μm. (D) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells inoculated with sonicated His-TDP-43^{mCherry} particles at a final concentration of 200nM and further imaged for TDP-43^{mCherry} fibrils (red) and TDP-43^{EGFP} (green) at 10 days. Media was changed after 3 days. White arrows indicate cytoplasmic particles containing mislocalized endogenous TDP-43^{EGFP} (green). Dashed white line outlines nuclei. Scale bar, 10 μm. (E) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells inoculated with sonicated FUS^{mCherry} fibrils and immunostained with TDP-43 antibody (red) with direct GFP fluorescence signal from TDP-43^{EGFP} (green). Scale bar, 10 μm. (F) A representative fission event of cytoplasmic TDP-43^{EGFP} LLPS is shown with thick yellow arrow pointing to the particle that is divided into two new particles (two smaller arrows) at minute 4.

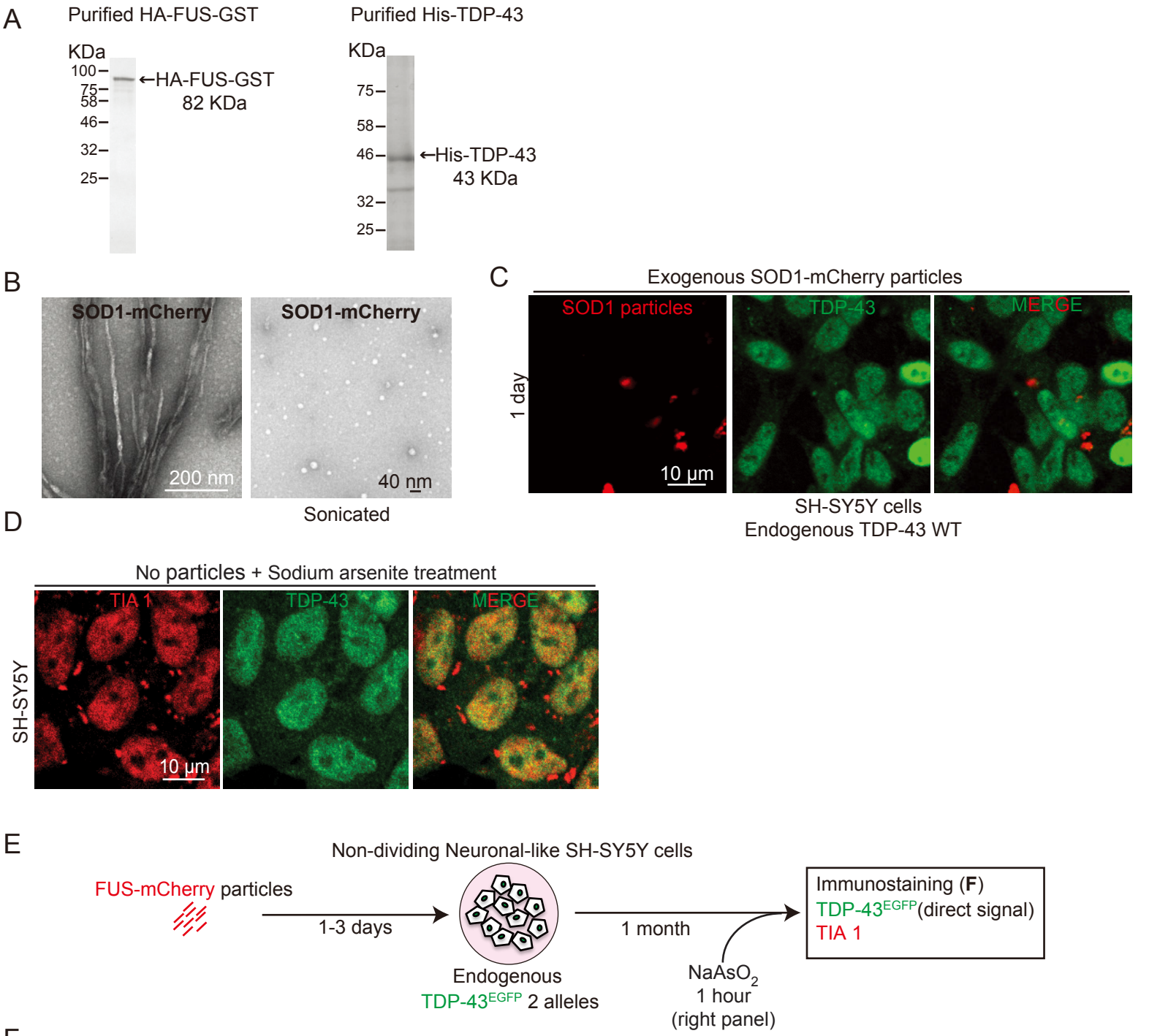

Figure S3. TDP-43 droplets are induced by FU_S particles, but not SOD1 particles and are independent of stress granules. Related to Figure 3

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(A) Coomassie blue staining of recombinant HA-FUS-(TEV cleavage site)-GST protein purified from bacteria at the expected size of 82 KDa (left panel), and His-TDP-43 protein at the expected size of 43 KDa (right panel). (B) Electron micrograph of amyloid-like fibrils of $SOD1^{mCherry}$ recombinant protein purified from bacteria. Right panel illustrates the $SOD1^{mCherry}$ fibrils after sonication before inoculating them into cell media. Scale bars, 200 nm and 40nm (sonicated fibrils). (C) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells inoculated with sonicated $SOD1^{mCherry}$ particles (red) and immunostained after 1 day TDP-43 antibody. Scale bar, 10 μm. (D) Confocal representative images of neuronal-like cells treated with $NaAsO_2$ (500 μM) for 1 hour and immunostained with TIA1 (red) and TDP-43 (green) antibodies to induce TIA1 positive stress granules. (E) Scheme of the experimental design to assess stress granule dependency of endogenously EGFP tagged TDP-43 in non-cycling SH-SY5Y cells (knock-in in both alleles), which were incubated with fluorescently labelled $FUS^{mCherry}$ particles and visualized over time by live-imaging or immunofluorescence. (F) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells inoculated with sonicated $FUS^{mCherry}$ particles in absence (left panel) or presence of $NaAsO_2$ for 1 hour (right panel) and further immunostained with stress granules marker TIA1 (red) and cytoplasmic $TDP\text{-}43^{EGFP}$ (green) after 1 month of fibril treatment. Scale bar, 10 μm.

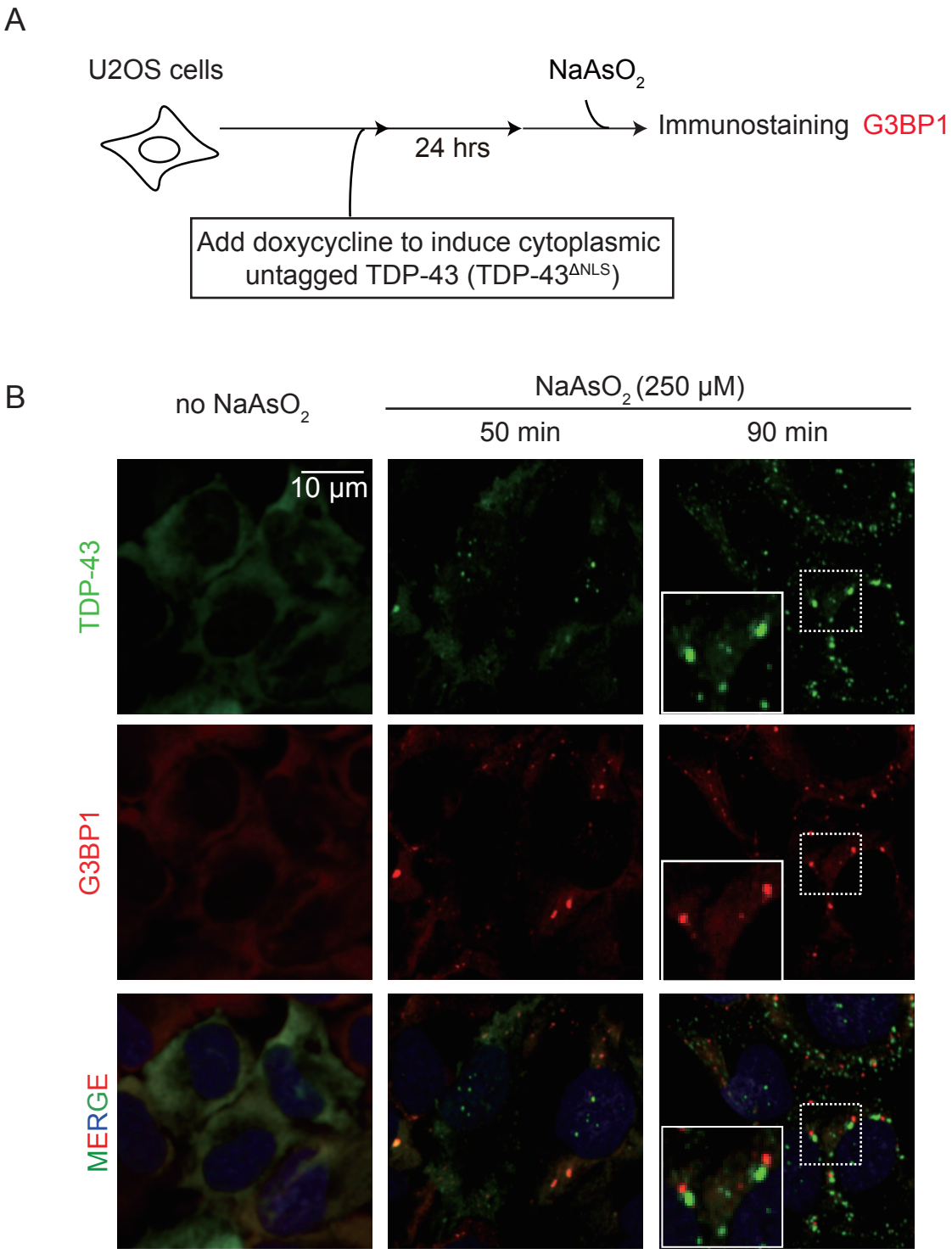

Figure S4. Untagged TDP-43$^{\Delta NLS}$ forms stress granule independent particles. Related to Figure 4

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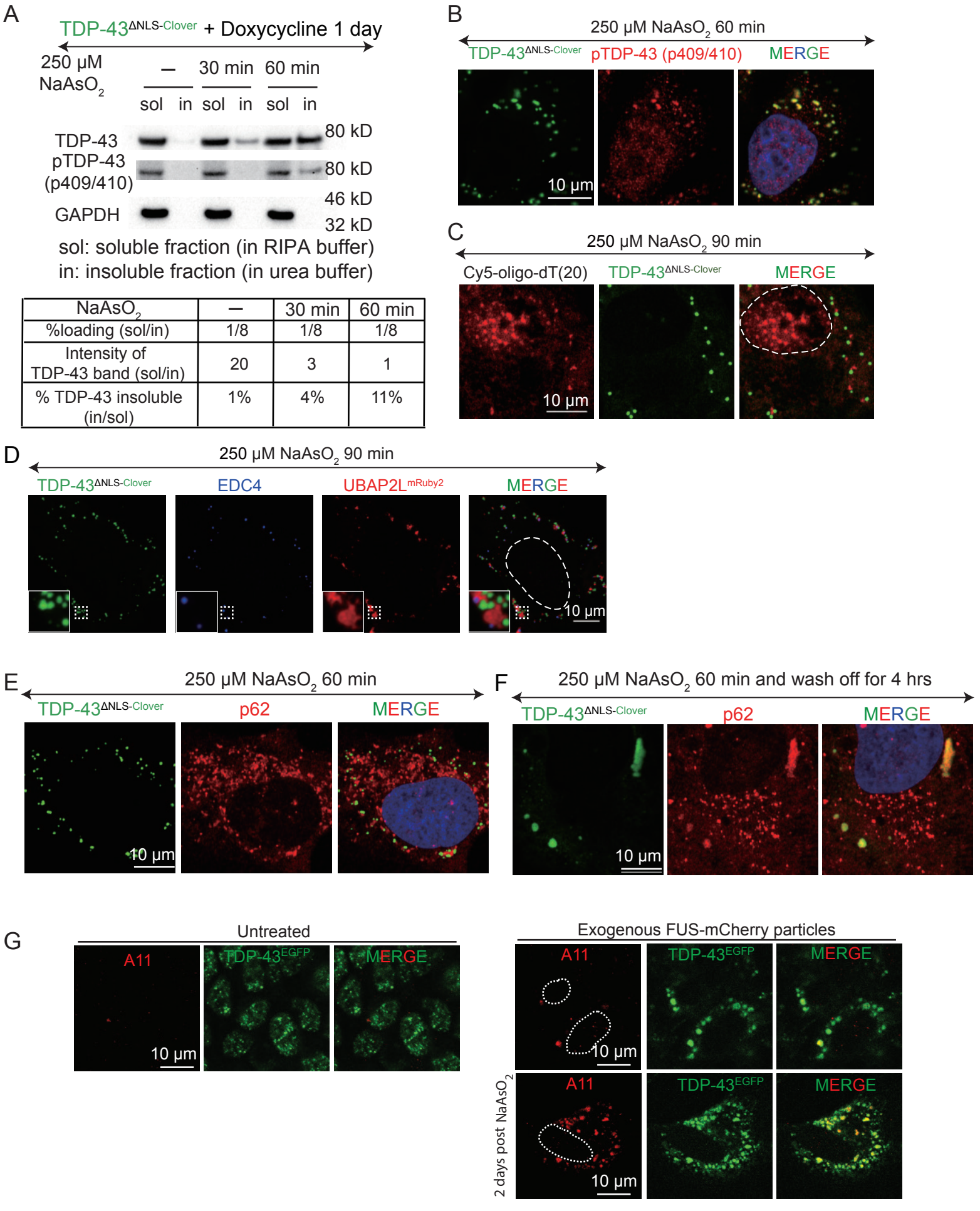
(A) Experimental design and (B) immunostaining of untagged cytoplasmic TDP-43 forming stress granule-independent particles after 250 μM sodium arsenite treatment of neuronal-like SH-SY5Y cells. G3BP1 was used as a stress granule marker. Scale bar, 10 μm.



$NaAsO_2$	—	30 min	60 min
%loading (sol/in)	1/8	1/8	1/8
Intensity of TDP-43 band (sol/in)	20	3	1
% TDP-43 insoluble (in/sol)	1%	4%	11%

Figure S5. Stress induces the formation of detergent-insoluble TDP-43 inclusions with gel-like particles exhibiting amyloid-like features. Related to Figure 5

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(A) Western blot analysis of the proportion of TDP-43 and phospho-TDP-43 in the soluble (RIPA buffer) and insoluble (Urea buffer) fractions without or with 30 min or 60 min of 250 μM sodium arsenite treatment. GAPDH was used as loading control. (B) Representative image of phospho-TDP-43 immunostaining (red) in neuronal-like SH-SY5Y cells that accumulate cytoplasmic TDP-43$^{\Delta NLS\text{-}Clover}$ particles (green) after 60 min of 250 μM sodium arsenite treatment. Scale bar, 10 μm. (C) Representative image of mRNA (red) using FISH and cytoplasmic TDP-43$^{\Delta NLS\text{-}Clover}$ particles (green) after 90 min of 250 μM sodium arsenite treatment (red). Scale bar, 10 μm. (D) Representative image of EDC4 (P body) immunostaining (blue) in neuronal-like SH-SY5Y cells that form stress granule-independent cytoplasmic TDP-43$^{\Delta NLS\text{-}Clover}$ particles (green) and stress granules indicated by UBAP2L^{mRuby2} (red) after 90 min of 250 μM sodium arsenite treatment. Scale bar, 10 μm. (E-F) Representative image of p62 immunostaining (red) with cytoplasmic TDP-43$^{\Delta NLS\text{-}Clover}$ particles after 60 min of 250 μM sodium arsenite treatment (E) or in the cells with remaining cytoplasmic TDP-43$^{\Delta NLS\text{-}Clover}$ particles after a four hour of wash off of 250 μM sodium arsenite (F). Scale bar, 10 μm. (G) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells inoculated with sonicated FUS^{mCherry} particles in absence or presence of $NaAsO_2$ for 30 minutes (lower panel) and further immunostained with amyloid-oligomers antibody A11 (red) and cytoplasmic TDP-43^{EGFP} (green) after 1 month of fibril treatment or in absence of fibril treatment (left panel). Scale bar, 10 μm.

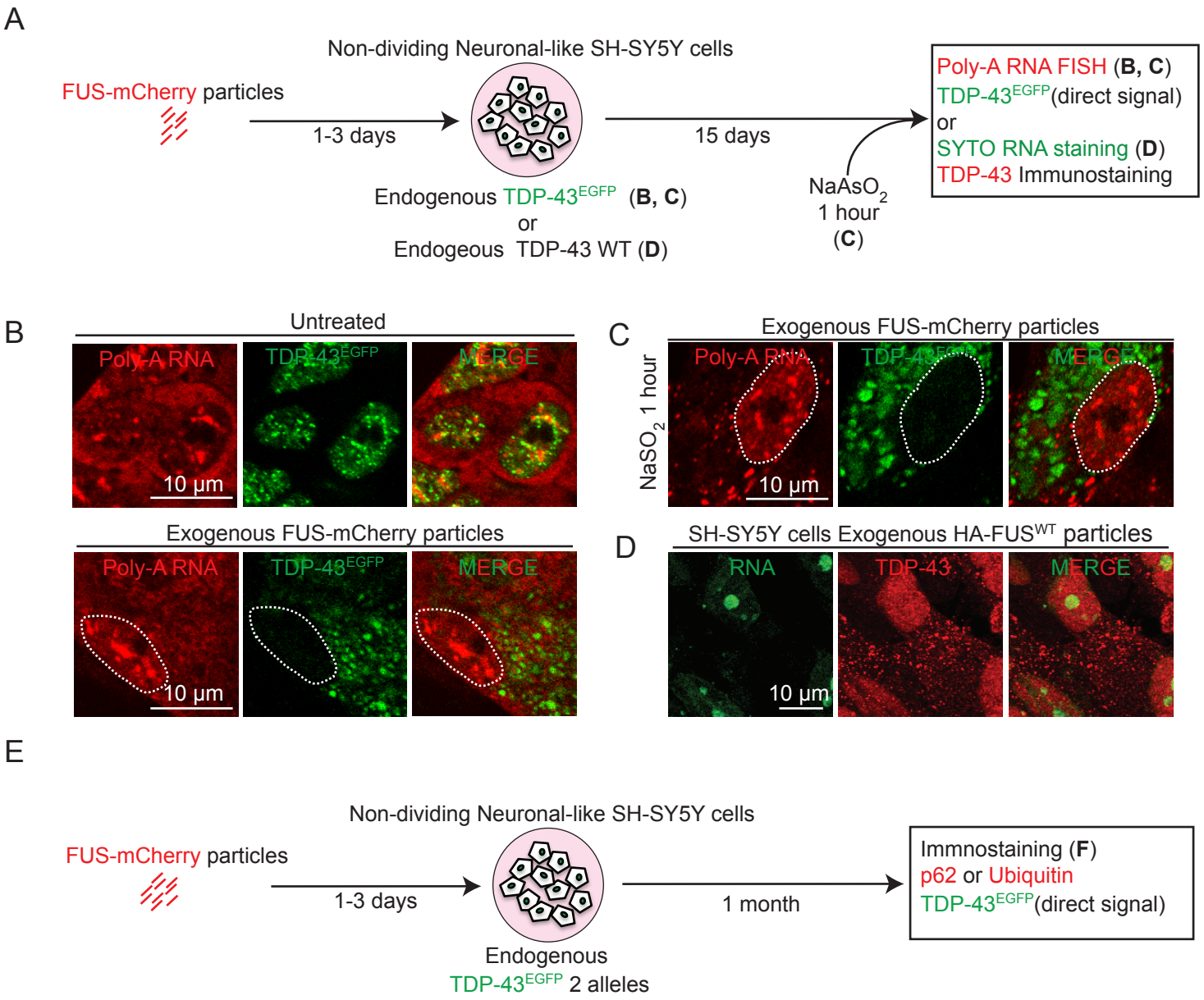

Figure S6. Cytoplasmic TDP-43 de-mixed droplets remain liquid for long periods and do not co-localize with polyA-containing RNAs. Related to Figure 7

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(A) Scheme of the experimental design to assess LLPS properties of endogenous TDP-43^{EGFP} in cells and determine recruitment of RNA within the droplets (B-C) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells (endogenously EGFP tagged TDP-43) 1 month after inoculation of sonicated FUS^{mCherry} particles revealing Poly-A-RNA using fluorescence in situ hybridization (FISH) (grey) and cytoplasmic TDP-43^{EGFP} (green) or (C) subsequently treated for one hour of $NaAsO_2$ (0.5 mM). (D) Neuronal-like SH-SY5Y cells 1 month after inoculation of sonicated FUS^{mCherry} fibrils further stained with SYTO RNA (green) and TDP-43 (red). Scale bar, 10 μm. (E) Scheme of the experimental design to assess LLPS properties of endogenous TDP-43^{EGFP} in cells and determine LLPS features. (F) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells 1 month after inoculation of sonicated FUS^{mCherry} fibrils and further immunostained with p62 (upper panel) or ubiquitin (lower panel) (red) and cytoplasmic TDP-43^{EGFP} (green). Left panel illustrates cells in absence of fibril treatment. Scale bar, 10 μm.

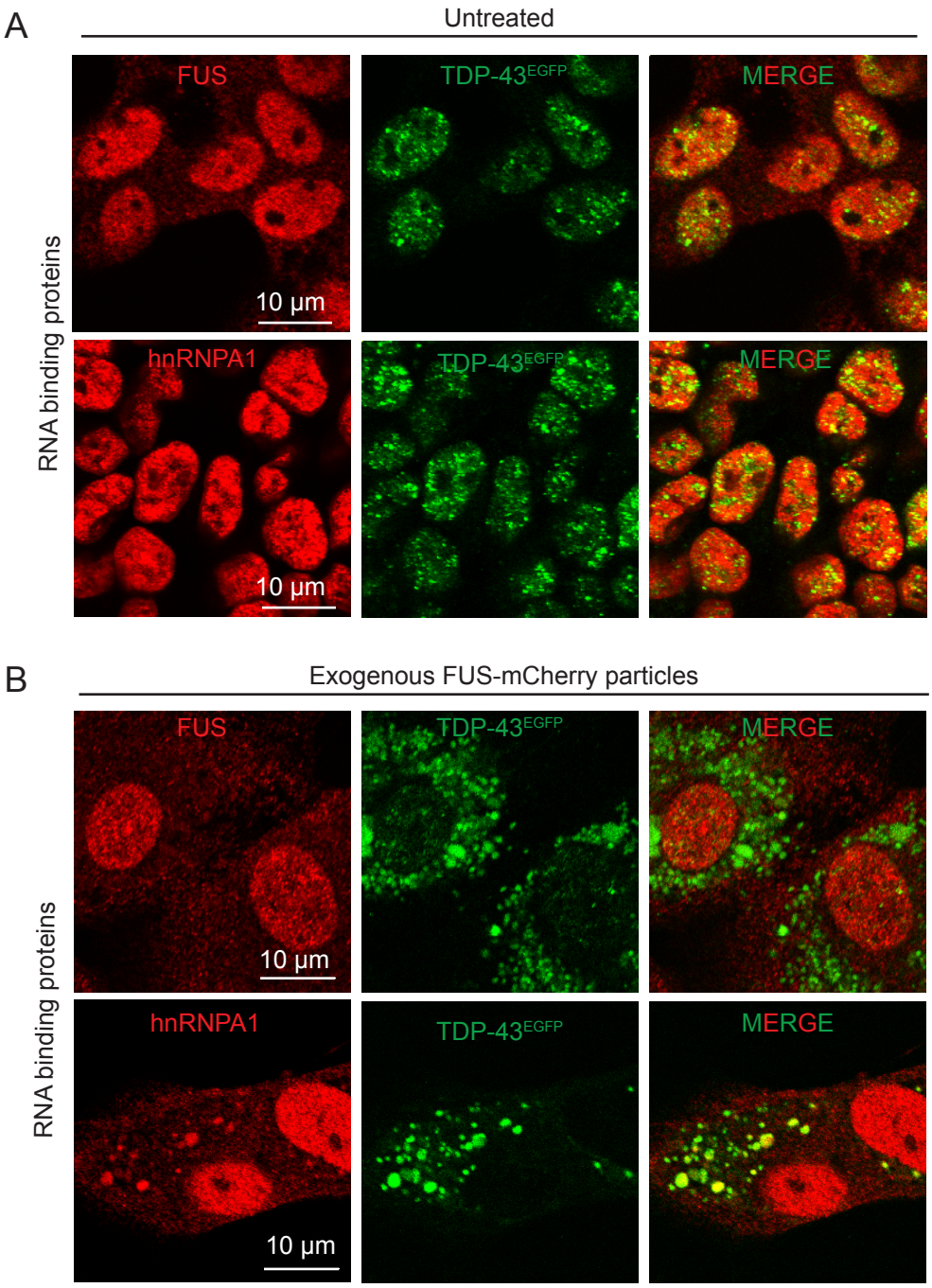

Figure S7. TDP-43 LLPS induces mislocalization of hnRNPA1 and FUS into the cytoplasm due to nuclear import defects. Related to Figure 8

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(A-B) Representative images using confocal microscopy of neuronal-like SH-SY5Y cells in absence of fibril treatment (A) or 1 month after inoculation of sonicated $FUS^{mCherry}$ fibrils (B) and further immunostained with FUS (upper panel), hnRNPA1 (lower panel) (red) and cytoplasmic $TDP\text{-}43^{EGFP}$ (green). Scale bar, 10 μm.