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Supplementary Materials for

A quantitative biology approach correlates neuronal toxicity with the largest inclusions of TDP-43

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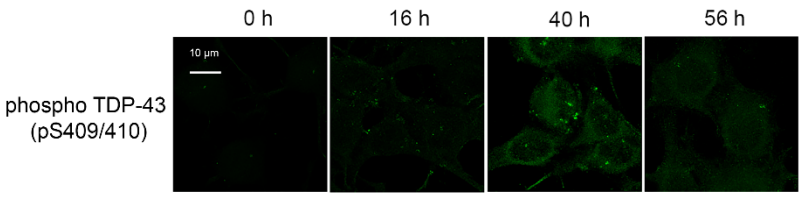

Fig. S1. TDP-43 inclusions are phosphorylated at S409/410. Representative confocal scanning microscopy images showing TDP-43 phosphorylated at S409/410 in NSC-34 cells (n=3) transfected with 20 µg of pCI-neo plasmid expressing human TDP-43 and analysed at 0, 16, 40 and 56 h after transfection.

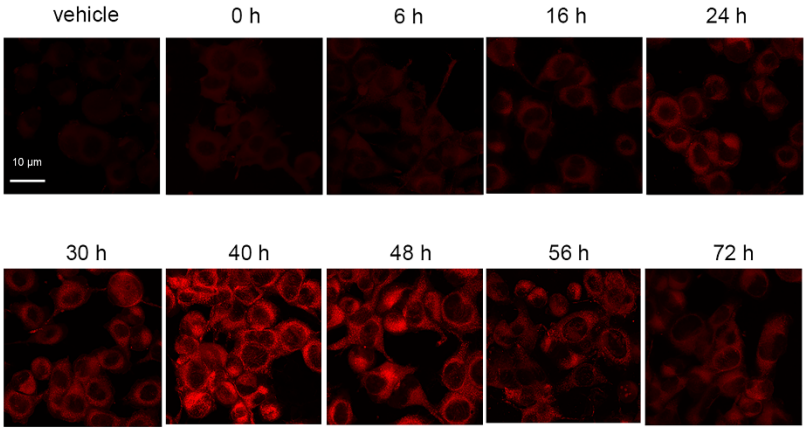

Fig. S2. Both nuclear depletion and cytoplasmic accumulation of TDP-43 are relevant for neuronal dysfunction. Representative confocal scanning microscopy images showing mitochondrial superoxide production detected with MitoSOX probe in living NSC-34 cells (n=3) transfected with vehicle or 20 µg of pCI-neo plasmid expressing human TDP-43 and analysed at different lengths of time after transfection. Red fluorescence: MitoSOX staining.

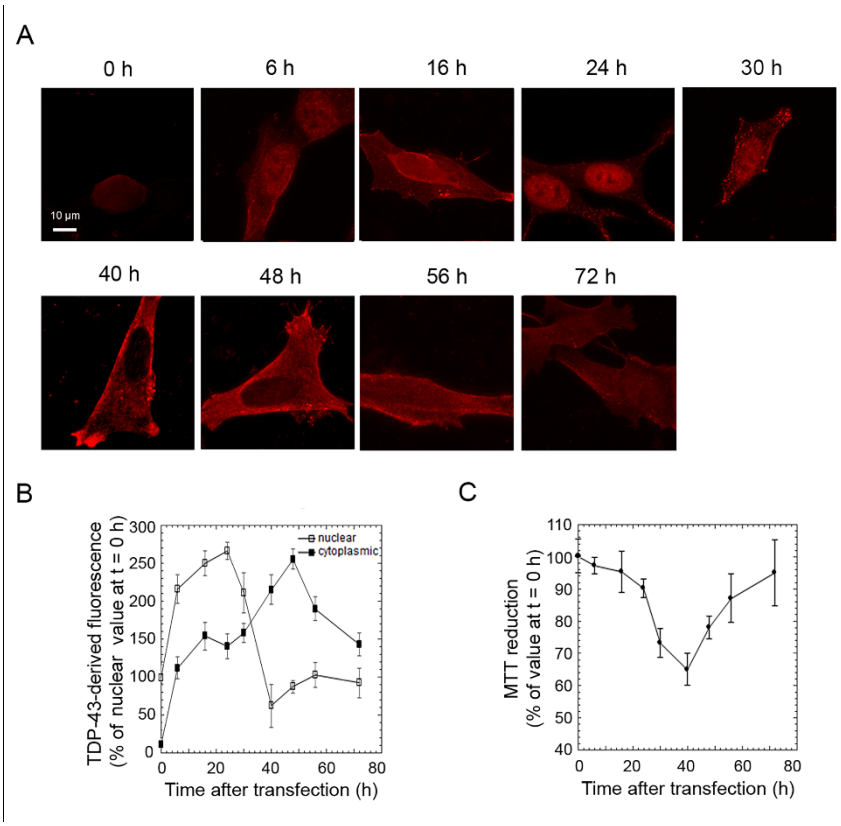

Fig. S3. Overexpression of TDP-43 in SH-SY5Y cells and related cytotoxicity (A) Representative STED microscopy images of SH-SY5Y cells (n=3) transfected with 20 µg of pCI-neo plasmid expressing human TDP-43 and analyzed at different lengths of time after transfection. Red fluorescence: total TDP-43 (endogenous and exogenous). (**B**) Time courses of nuclear and cytoplasmic TDP-43-derived fluorescence determined over 200-250 SH-SY5Y cells and expressed as a percentage of the value for cells transfected for 0 h, taken as 100% (n=3). Experimental errors are S.E.M. (**C**) Time course of MTT reduction (n=3) in SH-SY5Y cells treated as in panel A. Experimental errors are S.E.M.

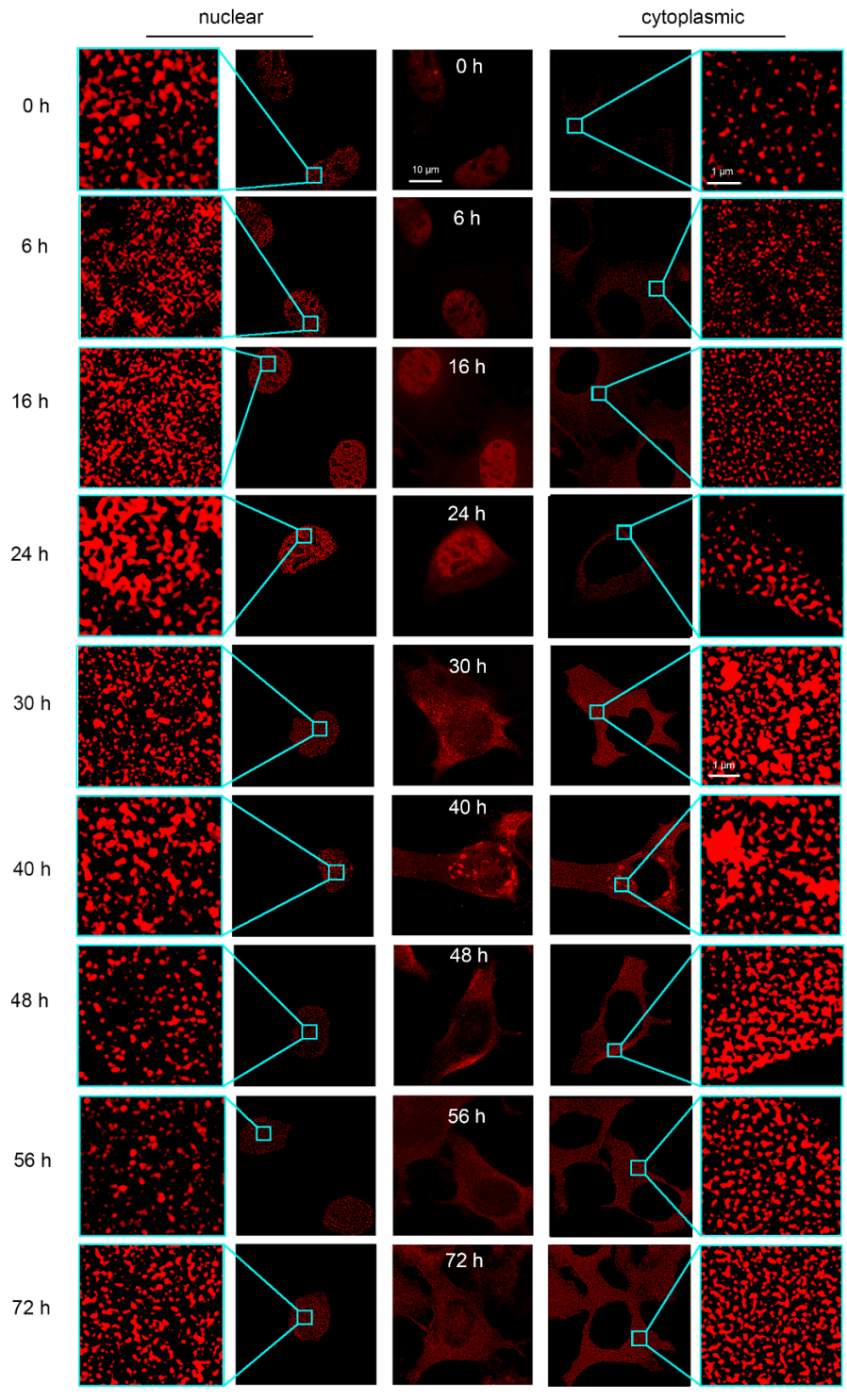

Fig. S4. Time courses of different size classes of TDP-43 inclusions. Approach used to identify different size classes of TDP-43 inclusions: representative STED microscopy images of NSC-34 cells (n=3) transfected with 20 µg of pCI-neo plasmid expressing human TDP-43 at the indicated time points (middle, third column) were treated to exclude the nucleus area, after background subtraction, to obtain a better definition of the cytoplasmic inclusions (fourth column) and then magnified to identify the various inclusions (zoom on the right, fifth column) and count them, as described in *Materials and Methods*. The same original STED images (middle, third column) were also treated to exclude the cytoplasmic area, after background subtraction, to obtain a better definition of the nuclear aggregates (second column) and then magnified to identify the various nuclear inclusions (zoom on the left, first column).

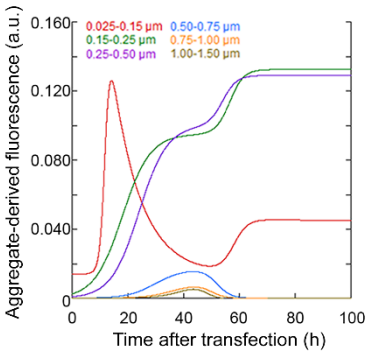

Fig. S5. Identification of the mechanism of TDP-43 inclusion formation and toxic inclusions by a quantitative analytical approach. Analytical kinetic traces (theoretical time courses) for each size class of TDP-43 inclusions, derived from the experimental values reported in Fig. 3.

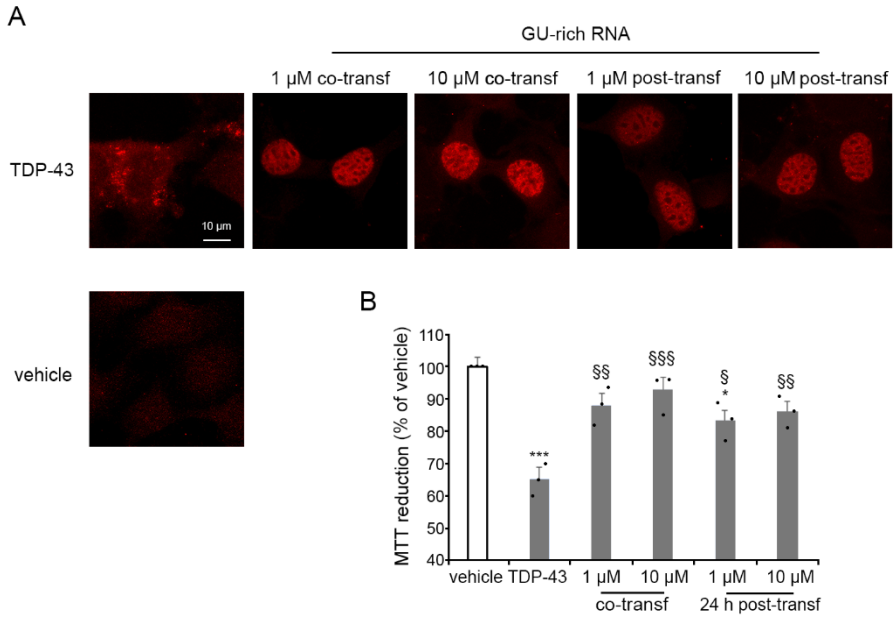

Fig. S6. GU-rich RNA prevents nucleus-to-cytoplasm redistribution of TDP-43 in NSC-34 cells and its related cytotoxicity (A) Representative STED microscopy images of NSC-34 cells (n=3) transfected with 20 µg of pCI-neo plasmid expressing human TDP-43 in the absence or presence of a GU-rich RNA oligonucleotide (1 µM and 10 µM), either co-transfected with the pCI-neo plasmid (co-transf) or transfected 24 h later (post-transf). The images were acquired 40 h after pCI-neo plasmid transfection. Cells transfected with vehicle were also showed. Red fluorescence: total TDP-43 (endogenous and exogenous). (**B**) MTT reduction (n=3) in NSC-34 cells treated as in panel A. Experimental errors are S.E.M. *** and *: P<0.001 and P<0.05 relative to vehicle, respectively. §§§, §§ and §: p<0.001, p<0.01 and p<0.05 relative to TDP-43 expression without RNA, respectively.

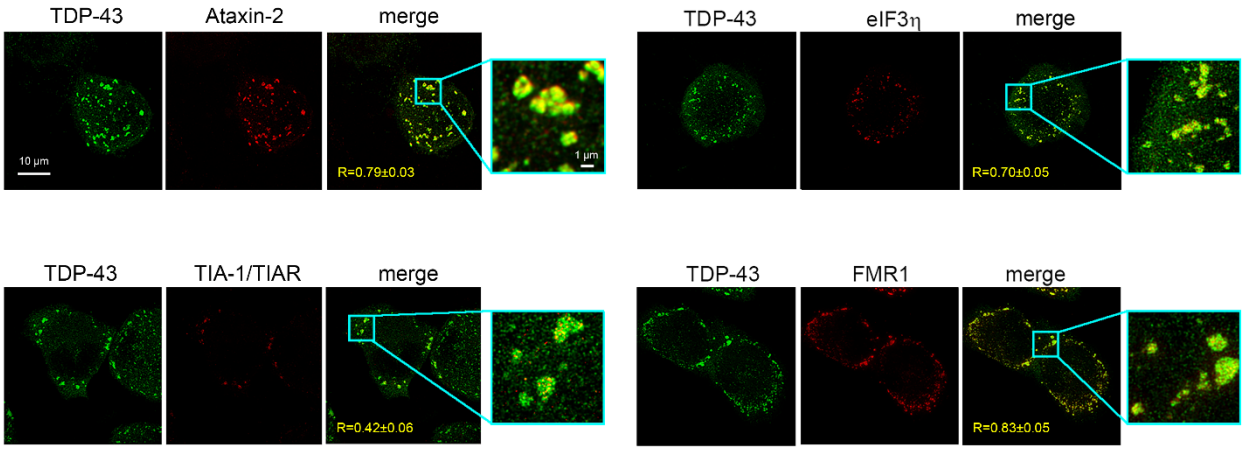

Fig. S7. Endogenous TDP-43 is recruited to arsenite-induced stress granules. Representative STED microscopy images of untransfected NSC-34 cells (n=3) treated with 0.5 mM sodium arsenite for 1 h. The green and red fluorescence indicate total TDP-43 (endogenous) and the indicated SG markers, respectively. The magnification boxes indicate areas with TDP-43 assemblies with a high degree of colocalization with SG markers. R values are indicated in merge images.