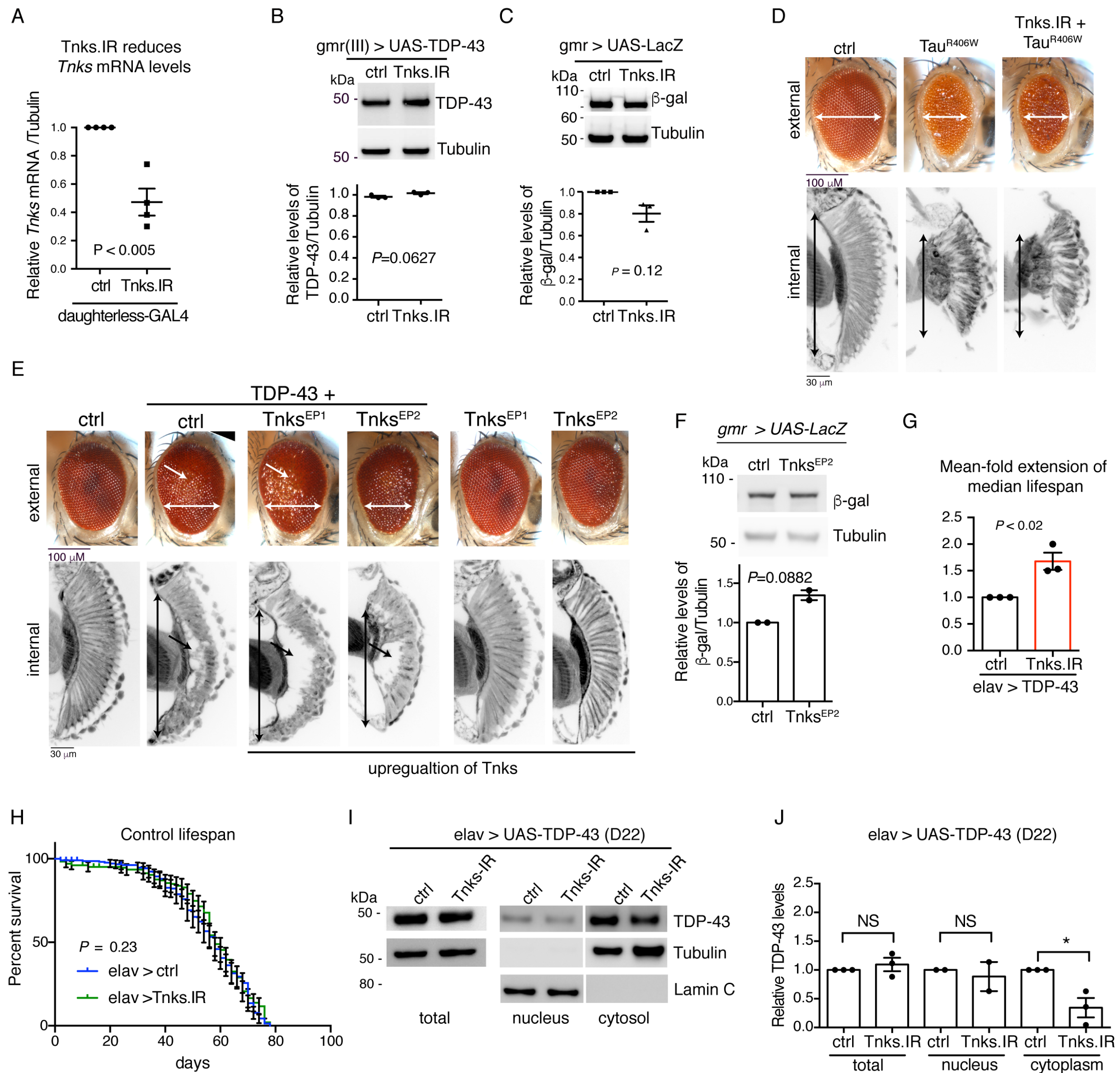

Supplementary Figure 1 (Related to Figure 1): Tankyrase is a genetic modifier of TDP-43.

(A) Expression of the *UAS-tankyrase* RNAi transgene (Tnks.IR) in fly larvae with the *daughterless-GAL4* (*da-GAL4*) driver leads to a significant reduction in *tankyrase* (*Tnks*) mRNA compared to control (ctrl). The graph represents the mean (± s.e.m) of 4 independent experiments. An unpaired and two-tailed T test was used to determine significance (P < 0.005). Control (ctrl) genotype is *daugtherless-GAL4/+* and the Tnks.IR genotype is *daugtherless-GAL4/UAS-Tnks-IR*$^{4179R\text{-}4}$*; +/+*.

(B) Reduction of *tankyrase* mRNA (Tnks.IR) had no effect on TDP-43 protein levels. The Tnks.IR transgene was co-expressed with TDP-43 in the fly eye with the *gmr-GAL4* driver and total protein extract was isolated from heads of day-1-old female flies. The mean (± s.e.m.) of TDP-43 protein

levels relative to Tubulin was calculated from three independent experiments. An unpaired and two-tailed T test revealed that no significant difference was detected in the total levels of TDP-43 between the experimental and control genotype. The control (ctrl) genotype is *UAS-TDP-43 (37M)/+; gmr-GAL4 (III)/+* and the Tnks.IR genotype: *UAS-TDP-43 (37M)/ UAS-Tnks-IR*$^{4179R\text{-}4}$; *+/+*.

(C) Reduction of *tankyrase* mRNA (Tnks.IR) had no effect on the GAL4 binary system. The Tnks.IR transgene and the *UAS-LacZ* reporter transgene were co-expressed in the fly eye with the *glass multiple repeat-GAL4* (*gmr-GAL4*) driver. Total protein was isolated from the heads of female flies at day 1 and the levels of the *lacZ* protein, β-galactosidase (β-gal), and Tubulin were measured by immunoblot. The graph represents the mean (± s.e.m.) of three independent experiments. An unpaired T test was performed to reveal that β-gal levels were not statistically different between the control and experimental genotype. Control (ctrl) genotype: *gmr-GAL4 (II*), *UAS-LacZ*/*UAS-mCD8-GFP*; +/+ and the experimental Tnks-IR genotype: *gmr-GAL4 (II), UAS-LacZ / UAS-Tnks-IR*$^{4179R\text{-}4}$*; +/+*.

(D) Reduction of *Tankyrase* has no effect on the toxicity of the Alzheimer's and frontotemporal degeneration-associated protein Tau. White arrowheads indicate width of external retina and black arrowheads indicate the length of the internal retina. The control (ctrl) genotype is *gmr-GAL4/UAS-mCD8-GFP; +/+*, the Tau R406W genotype is *gmr-GAL4/UAS-mCD8-GFP; UAS-Tau R406W/+*, and the Tau R406RW+ Tnks.IR genotype is *gmr-GAL4/ Tnks-IR*$^{4179R\text{-}4}$*; UAS-Tau R406W/+*.

(E) Upregulation of *Tnks* enhances TDP-43-associated toxicity in the fly eye, while upregulation of *Tnks* alone in the fly eye has no effect on the external (upper panel) or internal retina (lower panel). White arrowheads indicate width of external retina, white arrows point to roughening of the external eye, black arrowheads indicate the length of the internal retina and black arrows indicate vacuolization of the internal retina. Genotypes are ctrl: *UAS-mCD8-GFP/+*; *gmr-GAL4* (III*)/+:* TDP-43 + ctrl is *UAS-TDP-43(M)*/*UAS-mCD8-GFP*; *gmr-GAL4* (*III*)*/+: TDP-43 + Tnks*^{EP1} is *UAS-TDP-43(M)*/+; *gmr-GAL4* (*III*)/*Tnks [EPg]Tnks*^{HP37069}*:* TDP-43 + Tnks^{EP2} is *UAS-TDP-43(M)/+*; *gmr-GAL4* (*III*)/*Tnks [EP]Tnks*^{EP3476}*:* Tnks^{EP1} *is +/+; gmr-GAL4* (*III*)/*Tnks [EPg]Tnks*^{HP37069} and Tnks^{EP2} is +/+; *gmr-GAL4* (*III*)/*Tnks [EP]Tnks*^{EP3476}.

(F) Upregulation of Tankyrase in the eye with the *glass multiple repeat-GAL4* (*gmr-GAL4*) had no effect on the GAL4 system. Total protein was isolated from the heads of female flies at day 1 and the levels of the *lacZ* protein, β-galactosidase (β-gal), and Tubulin were measured by immunoblot. The graph represents the mean (±s.e.m.) of two independent experiments. A one-way ANOVA was performed to reveal that β-gal levels were not statistically different between the control (ctrl) and experimental genotype. Genotypes ctrl is *gmr-GAL4 (II*), *UAS-LacZ*/*UAS-mCD8-GFP*; *+/+*: Tnks^{EP2} is *gmr-GAL4 (II*), *UAS-LacZ*/+; +/ *Tnks [EP]Tnks*^{EP3476}.

(G) Reduction of *Tnks* (Tnks.IR) in the nervous system of the fly extends the median lifespan of TDP-43 flies. Data represent the mean-fold extension of the median lifespan (± s.e.m.) of elav > TDP-43 compared to elav > TDP-43+Tnks.IR, n=3 independent lifespan assays. An unpaired T test with equal variance was performed. More than 150 male flies were followed per genotype, and

this was performed three independent times. The elav > TDP-43 + ctrl genotype is *UAS-mCD8-GFP/+*; *elav3A-GAL4, UAS-TDP-43 (S)/ +* and elav > TDP-43+Tnks.IR genotype is *UAS-Tnks-IR*$^{4179R-4}$/+; *elav3A-GAL4, UAS-TDP-43 (S)*/+.

(H) Reduction of *tankyrase* (Tnks.IR) in the nervous system in the fly has no effect on the lifespan of the fly compared to the elav control. More than 214 male flies were followed throughout the experiment per genotype. A log-rank test for trend was performed $P > 0.2$. Genotypes: elav > control (ctrl) is *UAS-mCD8-GFP/+*; *elav3A-GAL*4*/+* and elav >Tnks.IR is *UAS-Tnks.IR/+; elav3A-GAL4/+*.

(I) Reduction of *tankyrase* (Tnks.IR) in the nervous system of the fly has no effect on the total TDP-43 protein levels (aged to 22 days). Reduction of *Tnks* (Tnks.IR) significantly decreases cytoplasmic TDP-43 protein levels. Control (ctrl) genotype is *UAS-mCD8-GFP/+*; *elav3A-GAL4, UAS-TDP-43 (S)/+* and elav > TDP-43 + Tnks.IR genotype is *UAS-Tnks-IR*$^{4179R-4}$/+; *elav3A-GAL4, UAS-TDP-43 (S)*/+.

(J) TDP-43 protein levels were quantified from total protein, nuclear extract and cytosolic extract. The mean (± s.e.m.) of TDP-43 levels relative to Tubulin/LaminC from three independent experiments is presented. A one-way ANOVA ($P = 0.0088$) followed by a Tukey's test was performed to reveal significance (asterisk), NS: not significant.

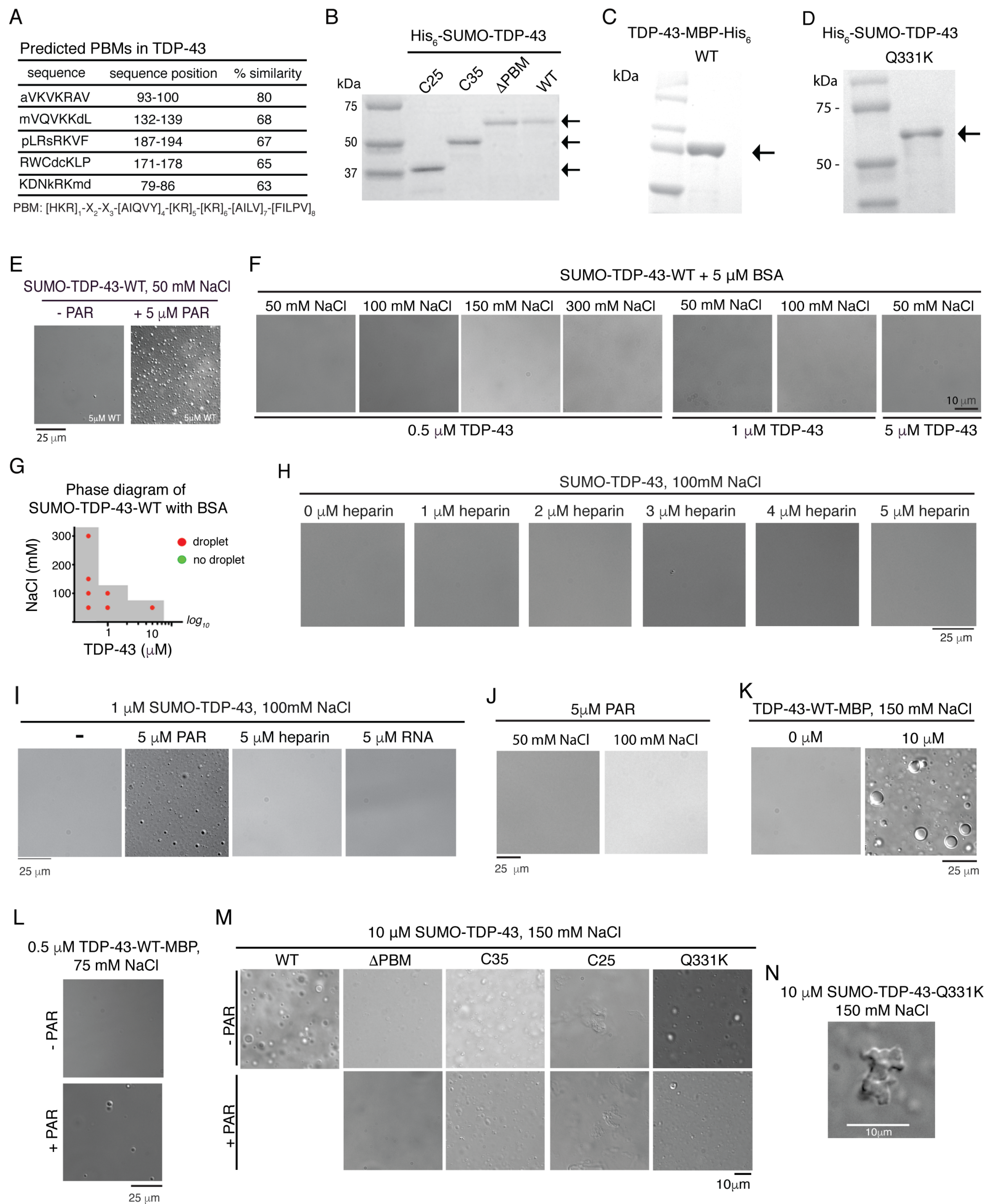

Figure S2 (Related to Figures 2, 3 and 4): PAR nucleates LLPS of TDP-43.

(A) The PAR-binding motif (PBM) consensus was aligned to TDP-43 using the PATTINPROT search engine (Combet et al., 2000). Table lists all regions (and sequence similarity to the PBM) identified in TDP-43.

(B) Expression and purification of HIS_6-SUMO-TDP-43-WT, -ΔPBM, -C35 and -C25 led to pure protein of the correct size (arrows).

(C) Expression and purification of TDP-43-WT-MBP-His_6 led to pure protein of the correct size (arrow).

(D) Expression and purification of HIS_6-SUMO-TDP-43-Q331K led to pure protein of the correct size (arrow).

(E) 5 µM ADP-ribose equivalents of PAR nucleated LLPS of 5 µM SUMO-TDP-43-WT in 50 mM NaCl. This was repeated twice from 1 preparation of protein.

(F) 5µM of BSA did not nucleate SUMO-TDP-43-WT LLPS. This was performed on one preparation of protein.

(G) BSA did not nucleate SUMO-TDP-43-WT. Protein and salt concentration pairs are plotted for the presence (green) or absence (red) of protein droplet formation.

(H) Addition of increasing amounts of heparin (1-5 µM ADP-ribose equivalents) did not promote SUMO-TDP-43-WT LLPS. This was performed on one preparation of protein.

(I) The addition of PAR, stimulated LLPS of 5 µM SUMO-TDP-43-WT in 100 mM NaCl whereas the addition of heparin, yeast total RNA or mono (ADP-ribose) (MAR) at 5 µM ADP-ribose equivalents did not promote SUMO-TDP-43-WT LLPS.

(J) PAR did not undergo LLPS. 5 µM ADP-ribose equivalents of PAR was incubated in 50mM or 100mM NaCl. This was performed on one sample of PAR.

(K) 10 µM TDP-43-MBP in 150 mM NaCl underwent LLPS.

(L) 5 µM ADP-ribose equivalents of PAR nucleated LLPS of 0.5 µM TDP-43-WT-MBP in 75 Mm NaCl.

(M) Addition of 5 µM ADP-ribose equivalents of PAR did not nucleate LLPS of 10 µM SUMO-TDP-43-ΔPBM, SUMO-TDP-43-C35, SUMO-TDP-43-C25 or SUMO-TDP-43-Q331K in 150mM NaCl. This was repeated twice from one protein prep.

(N) Example of solid structure formed by TDP-43-Q331K. At lower NaCl concentrations (150 mM, 100 mM and 50 mM) TDP-43-Q331K formed the occasional solid aggregate.

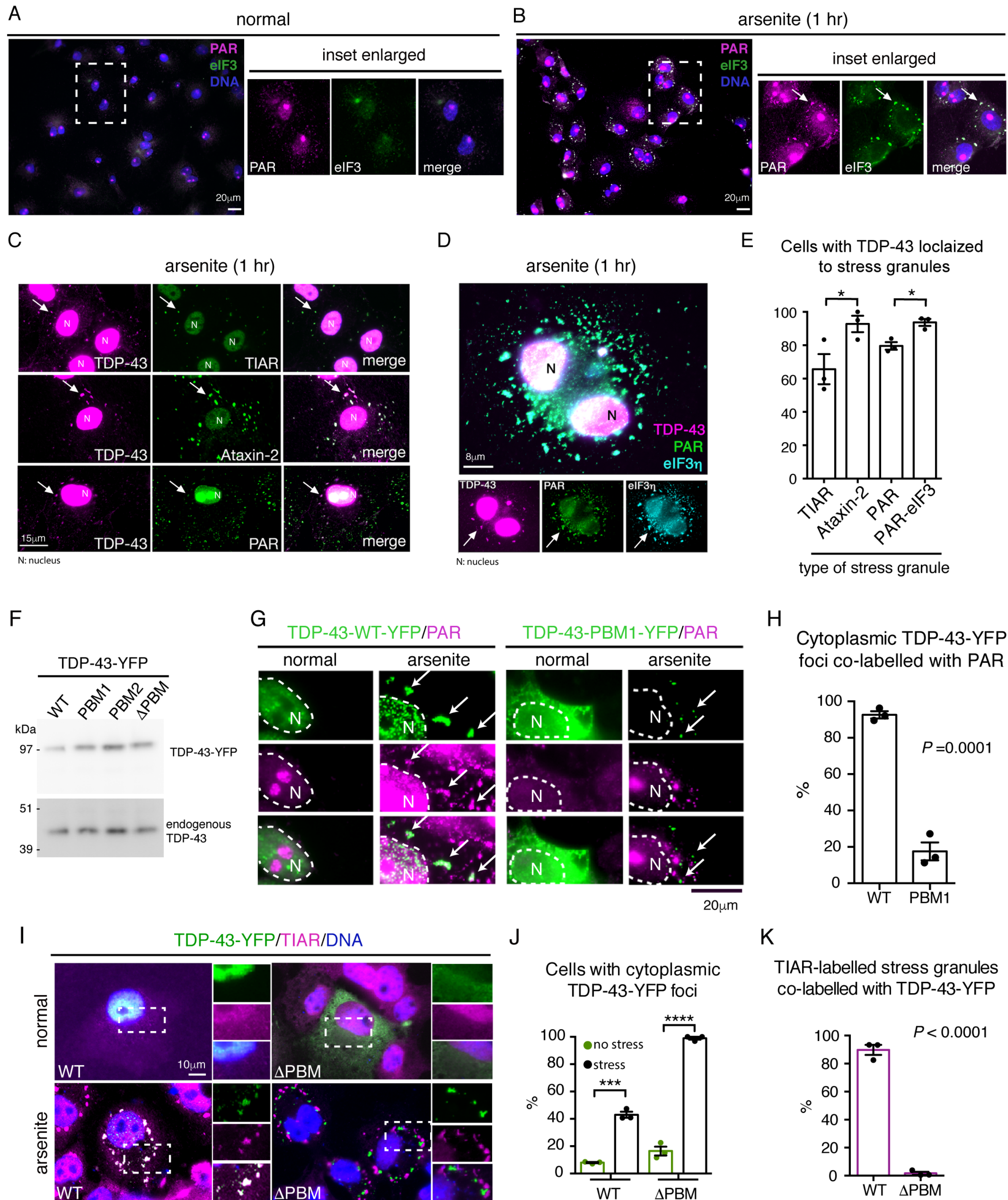

Figure S3 (Related to Figure 5): TDP-43 localization to PAR-containing stress granules is dependent on functional PBMs embedded in the nuclear localization sequence.

(A-B) Stress induces the formation of PAR-containing stress granules (insets and arrows). Cells were immunostained for eiF3η and PAR (Tulip, 10H) and counterstained with Hoescht. 100% of eIF3-positve stress granules contained PAR. 5 images at 40X magnification and ~25-100 cells and 400 or more eIF3η-positive stress granules were quantified. This experiment was repeated three independent times. Arrows indicate cytoplasmic PAR foci. Scale bar: 30μm.

(C) After treatment with 0.5mM sodium arsenite (1 hr), endogenous TDP-43 localized to stress granules that were immunoreactive for TIAR, Ataxin-2, and PAR. Arrows indicate cytoplasmic TDP-43 foci. Scale bar: 15μm.

(D) Upon treatment with 0.5mM sodium arsenite (1 hr) endogenous TDP-43 localized to stress granules immunolabelled with both PAR (Tulip, 10H) and eIF3η. Arrows indicate cytoplasmic TDP-43 foci. Scale bar: 8 μm.

(E) The percentage of cells with arsenite-induced stress granules labelled with TIAR, Ataxin-2, PAR or PAR-eIF3η were quantified for colocalization with endogenous TDP-43. Five images at 40X magnification, per condition, were quantified. The graph represents the mean (±s.e.m.) of three independent experiments. One-way ANOVA ($P < 0.05$) and a Tukey's test was performed. Asterisks: significant pairs.

(F) Expression of TDP-43-WT-YFP and TDP-43-ΔPBM-YFP in mammalian cells leads to the production of full-length protein at comparable levels. Blots were immunoblotted for TDP-43, and endogenous TDP-43 is presented as the internal control. Note TDP-43-PBM2-YFP was not presented in this study but was included in the figure so that gel was uninterrupted.

(G) Stress induces the formation of cytoplasmic foci of TDP-43-WT-YFP that co-label for PAR. Stress induced the formation of TDP-43-PBM1-YFP foci in the cytoplasm that do not co-label for PAR. Arrows indicate cytoplasmic TDP-43-YFP foci.

(H) The cytoplasmic and stress-induced foci of TDP-43-YFP were quantified for colocalization with PAR. Mutation in the PBM led to a significant reduction in the amount of TDP43-YFP foci that colocalized with PAR.

(I) In mammalian cells, stress-induced foci of TDP-43-WT-YFP localize to TIAR-labelled stress granules whereas stress-induced foci of TDP-43-ΔPBM-YFP are excluded from TIAR-labelled stress granules. Cells were exposed to 0.5mM sodium arsenite for 1 hr, immunolabelled for TIAR and counterstained with Hoescht.

(J) Cells were quantified for the presence of cytoplasmic foci of TDP-43-YFP. The mean (± s.e.m.) was calculated from three independent experimental repeats. Two-way ANOVA ($P < 0.0001$) and a Tukey's test was performed. Asterisks: significant pairs and NS: not significant.

(K) Cells were quantified for the localization of TDP-43 to TIAR-labelled stress granules. The mean (±s.e.m.) from three independent experiments is presented. An unpaired and two-tailed T Test was performed ($P < 0.0001$).

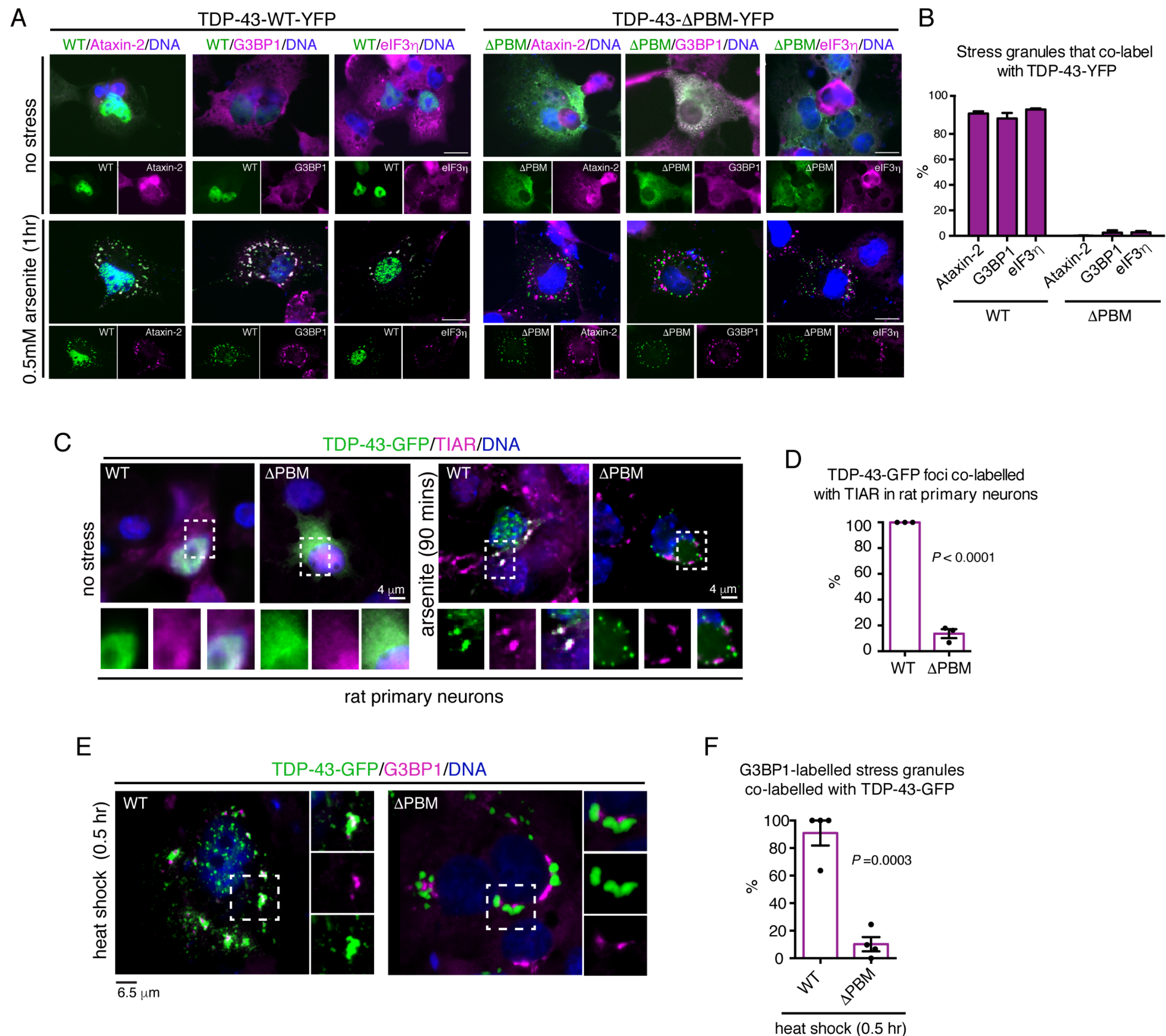

Figure S4 (Related to Figure 5): Mutation of the PAR-binding motifs prevents the recruitment of TDP-43 into stress granules.

(A) Upon treatment with 0.5mM sodium arsenite (1hr), TDP-43-WT-YFP and TDP-43-PBM-YFP formed cytoplasmic foci. TDP-43-WT was localized to stress granules whereas TDP-43-ΔPBM formed foci that were excluded from stress granules. Cells were immunostained with antibodies directed to Ataxin-2, G3BP1 and eIF3η. Cells were counterstained with Hoescht. Scale bars: 20μm.

(B) Cells with both foci of TDP-43 and stress granules were quantified for colocalization. One coverslip was examined per condition. At least 300 stress granules from 5 non-overlapping images at 40X magnification were quantified from each condition. Graph represents the mean (± s.e.m.) of 5 images per condition.

(C) In rat cortical neurons, TDP-43-WT-GFP is nuclear, whereas TDP-43-∆PBM-GFP is cytoplasmic. In rat cortical neurons, stress-induced foci of TDP-43-WT-GFP localized to TIAR-labelled stress granules, whereas stress-induced foci of TDP-43-∆PBM-GFP were excluded from TIAR-labelled stress granules. Neurons were exposed to 0.5mM sodium arsenite for 90 min, immunolabelled with TIAR and counterstained with Hoescht.

(D) Rat cortical neurons with both TIAR-labelled stress granules and cytoplasmic foci of TDP-43 were quantified for colocalization. The mean (±s.e.m.) calculated from three independent experiments is presented. An unpaired and two-tailed T test was performed ($P < 0.0001$).

(E) Upon treatment with heat shock (43oC for 30 mins) TDP-43-WT-GFP localized to stress granules whereas TDP-43-∆PBM-GFP formed foci that were largely excluded from stress granules. Cells were immunostained with an antibody directed to G3BP1 and counterstained with Hoescht.

(F) G3BP1-labelled stress granules were quantified for co-labelling with TDP-43-GFP. A mean (± s.e.m.) from four independent experiments is presented. An unpaired and two tailed T-test was used to test for significance.

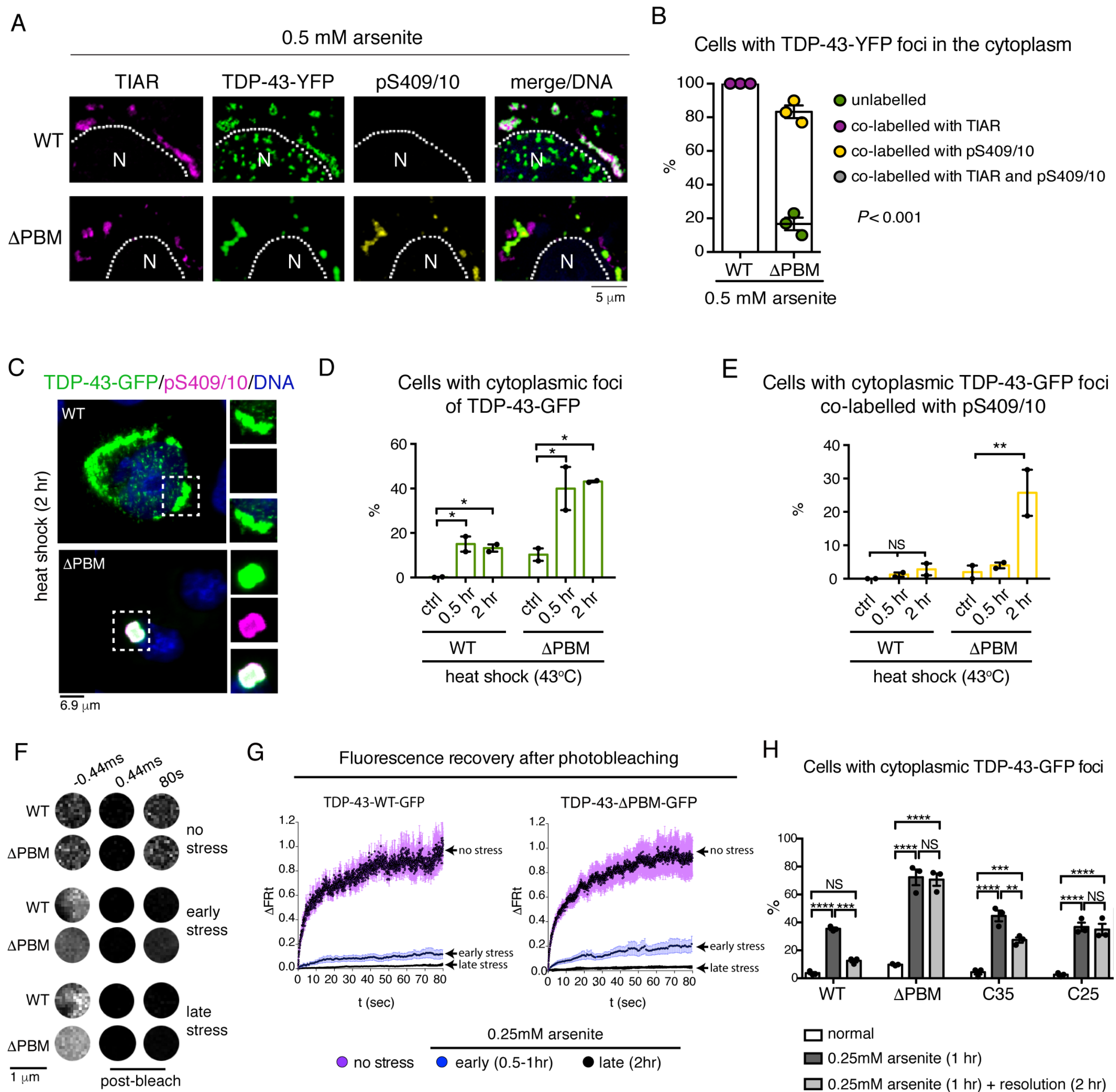

Figure S5 (Related to Figure 6): Exclusion from stress granules leads to phosphorylation of TDP-43 foci that fail to resolve after the removal of stress.

(A) Stress-induced foci of TDP43-WT-YFP recruited to TIAR-labelled stress granules were not co-labelled with pS409/10. Stress-induced foci of TDP-43-ΔPBM-YFP excluded from TIAR-labelled stress granules were co-labelled with pS409/10. Cells were immunolabelled with TIAR and pS409/10, counterstained with Hoescht and imaged by confocal microscopy. N: nucleus and hatched line is the nuclear/cytoplasm boundary.

(B) Cells were quantified for the presence of cytoplasmic TDP-43-YFP foci that were unlabeled, labelled with TIAR, pS409/10 or both TIAR and pS409/10. The mean (±s.e.m.) of three independent experiments is presented. Two-way ANOVA ($P < 0.0001$) was performed.

(C) Heat shock (43°C) induced the phosphorylation of TDP-43-ΔPBM-GFP foci and not TDP-43-WT-GFP foci. Cells were immunostained with an antibody directed to pS409/10 and counterstained with Hoescht.

(D) Cells with cytoplasmic TDP-43-GFP foci were quantified. The graph represents the mean (± s.e.m.) of two independent experiments. A one-way ANOVA ($P < 0.05$) and a Fishers LSD test was used to test for significance. Asterisks: significant pairs.

(E) Cells with cytoplasmic TDP-43-GFP foci co-labelled with pS409/10 were quantified. The graph represents the mean (± s.e.m.) of two independent experiments. A one-way ANOVA ($P < 0.05$) and a Fishers LSD test was used to test for significance. Asterisks: significant pairs. NS: not significant.

(F) The mobility of TDP-43-WT-GFP and TDP-43-ΔPBM-GFP was assessed by fluorescence recovery after photobleaching (FRAP) in live cells. Under normal conditions the mobility of diffuse cytoplasmic TDP-43 was assessed. Under stress the mobility of TDP-43 in cytoplasmic foci was measured during the early stage of treatment with 0.25mM sodium arsenite (30 mins to 1 hr), and during the late stage of stress (2 hrs onwards). Shown are the 1 μm regions of interest (ROIs) that were bleached.

(G) The rate of fluorescence recovery after photobleaching was calculated for each condition. The graph (s.e.m.) represents one experiment for each condition (see arrows in graph). For the normal conditions, more than 7 ROIs, each from a different cell, are plotted. For the early stress more than 12 ROIs, each from an individual foci and cell, is plotted, and for the late stress, more than 6 ROIs, each from an individual foci and cell, are plotted. These experiments were repeated more than three independent times and each time the fluorescence recovery was similar.

(H) Cells were exposed to 0.25mM sodium arsenite (1 hr), the cells were then left to recover for 2 hrs. Cells were quantified for the presence of TDP-43-GFP foci from normal conditions, stress conditions and after resolution. Stress-induced TDP-43-WT-GFP cytoplasmic foci resolved 2 hrs after the removal of stress. However, stress-induced foci of TDP-43-ΔPBM-GFP, TDP-43-C35-GFP and TDP-43-C25-GFP did not resolve after 2 hrs of recovery. Transfected cells in 5 non-overlapping images (20X magnification) were quantified for the presence of TDP-43-GFP cytoplasmic foci. Graph represents the mean (± s.e.m.) of three independent experiments. Two-way ANOVA ($P < 0.0001$) and a Tukey's test identified significance (asterisks). NS: not significant

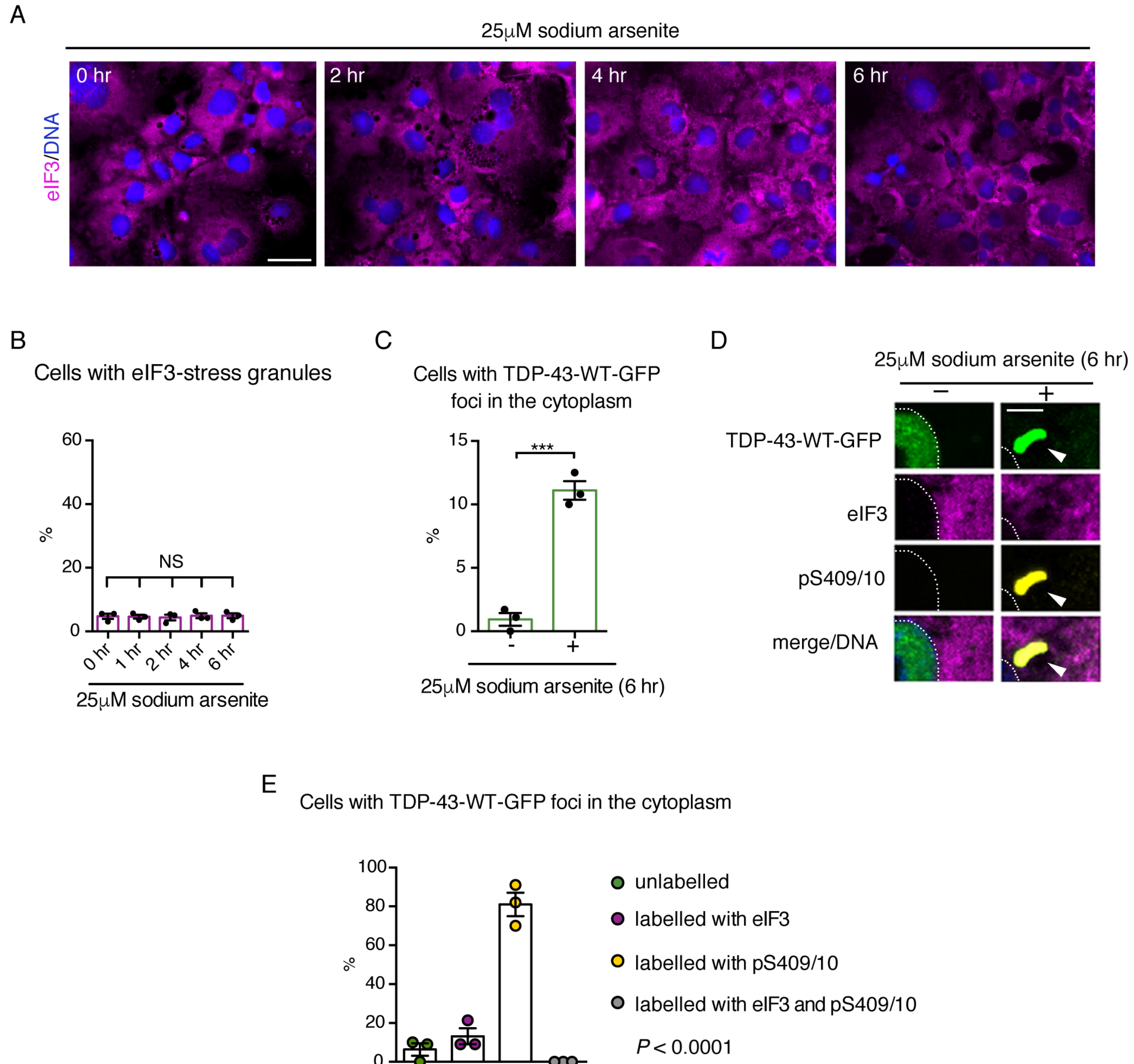

Figure S6 (Related to Figure 7): TDP-43 foci that form under low levels of prolonged stress are phosphorylated.

(A) Treatment with 25μM sodium arsenite for 6 hrs did not lead to the formation of eIF3-labelled stress granules. Cells were immunolabelled for eIF3 and counterstained for Hoescht. Scale bar: 35 μm.

(B) Cells were quantified for the presence of eIF3-labelled stress granules. Four non-overlapping images taken at 20X magnification were quantified from each condition. Graph represents the mean (± s.e.m.) of three independent experiments. NS: not significant.

(C) Cells were quantified for cytoplasmic TDP-43-WT foci. The mean (± s.e.m.) of three independent experiments is presented. See methods for details. One-way ANOVA ($P = 0.0003$) was performed.

(D) Exposure to 25μM sodium arsenite for 6 hrs resulted in cytoplasmic TDP-43-WT foci that lacked eIF3 and were co-labelled for pS409/10 (arrowheads indicate TDP-43-WT foci that are labelled with pS409/10). Cells were labelled for eIF3, pS409/10 and Hoescht, and imaged by confocal microscopy. Scale bar: 3.5 μm.

(E) Cells with cytoplasmic TDP-43-WT foci were quantified as unlabeled, labelled with eIF3, pS409/10 or both. After 6 hrs of treatment with 25μM sodium arsenite there was an increase in the percentage of cells with phosphorylated (pS409/10) TDP-43-WT foci and a decrease in the percentage of cells with TDP-43-WT foci co-labelled with eIF3. One-way ANOVA ($P < 0.0001$) and a Tukey's test was performed.

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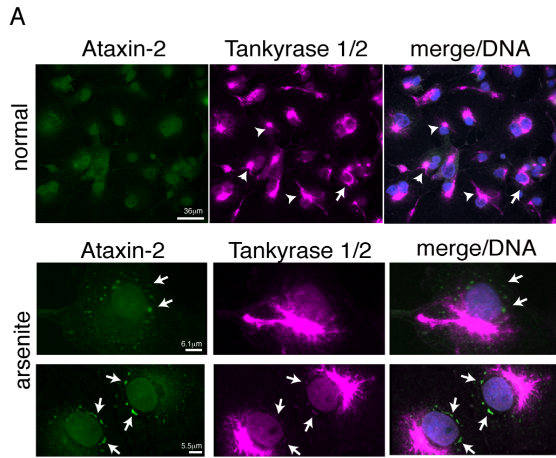

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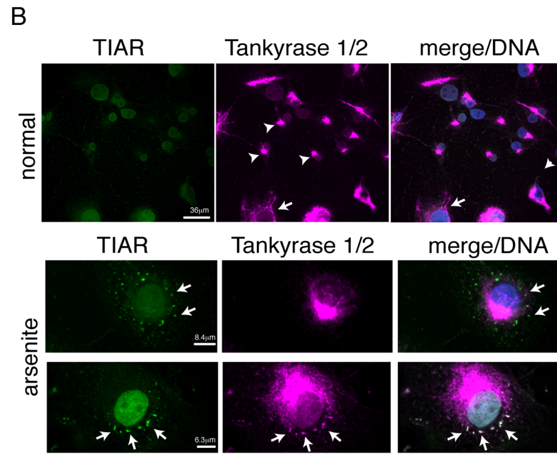

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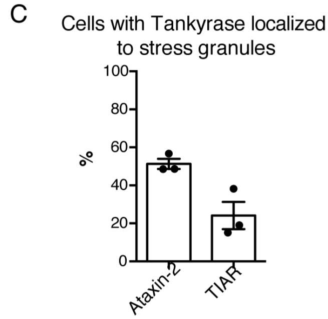

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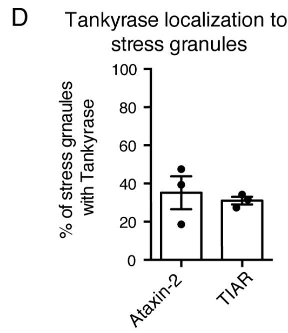

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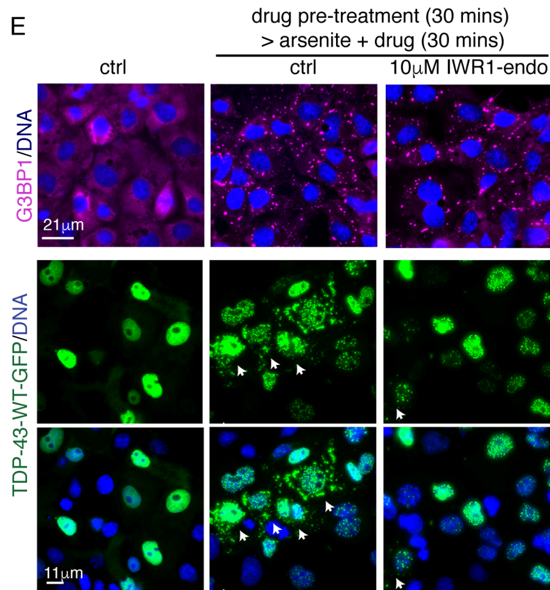

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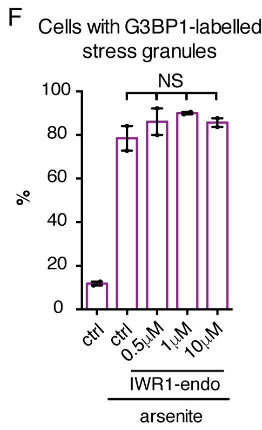

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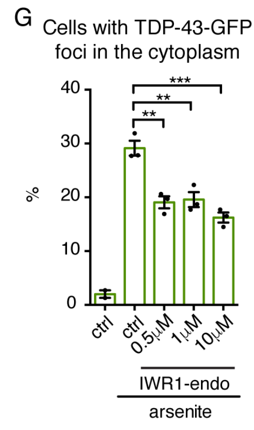

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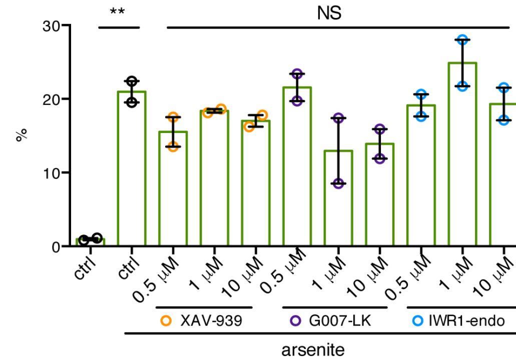

I

	IC50 (μM)			
	XAV-939	G007-LK	IWR1-endo	citation
PARP-1	2.194	no effect	>18.75	Huang et al., 2009
PARP-2	0.114	not tested	>18.75	Voronkov et al., 2013
TNKS1	0.0011	0.046	1.897	Huang et al., 2009
TNKS2	0.004	0.025	0.78	Huang et al., 2009

Figure S7 (related to Figure 7): Small-molecule inhibitors of Tankyrase reduces stress-induced TDP-43 foci without altering the percentage of cells with G3BP1-labelled stress granule formation.

(A) Tankyrase 1/2 and Ataxin-2 immunolocalization in mammalian cells under normal and stress conditions. Arrow in the top panel indicate immunostaining of the nuclear periphery, arrowheads in the top panel indicate example cells with cytoplasmic Tankyrase 1/2, arrows in the middle panel indicate Tankyrase-negative stress granules and arrows in the lower panel indicate Tankyrase 1/2-positive stress granules. Cells were extracted with 0.5% Triton-X100 for 30 secs prior to fixation in paraformaldehyde, then immunostained for Ataxin-2 and Tankyrase 1/2, and counterstained with Hoescht.

(B) Tankyrase 1/2 and TIAR immunolocalization in mammalian cells under normal and stress conditions. Arrow in the top panel indicate immunostaining of the nuclear periphery, arrowheads in the top panel indicate example cells with cytoplasmic Tankyrase 1/2, arrows in the middle panel indicate Tankyrase-negative stress granules and arrows in the lower panel indicate Tankyrase 1/2-positive stress granules. Cells were extracted with 0.5% Triton-X100 for 30 secs prior to fixation in paraformaldehyde, then immunostained for TIAR and Tankyrase 1/2, and counterstained with Hoescht.

(C) Cells were quantified for the presence of Tankyrase 1/2 labelling of Ataxin-2 and TIAR labelled stress granules. Cells with one or more Tankyrase 1/2 containing stress granules were counted. The graph represents the mean (± s.e.m.) of three independent experiments.

(D) Ataxin-2 and TIAR-labelled stress granules were quantified for the presence of Tankyrase 1/2 labeling of stress granules. The graph represents the mean (± s.e.m.) of three independent experiments

(E) Cells expressing TDP-43-WT-GFP were either left untreated or treated with 0.5mM sodium arsenite (30 mins) either in the absence of or presence of the Tankyrase 1/2 inhibitor (IWR1-endo). Cells were pretreated with the indicated amount of inhibitor for 30 mins prior to stress. Cells were immunostained for G3BP1 and counterstained with Hoescht. Arrows indicate cells with cytoplasmic TDP-43-WT-GFP foci.

(F) Cells were quantified for the presence of G3BP1-labeleld stress granules. The graph represents the mean (± s.e.m.) from two independent experiments, a one-way ANOVA ($P < 0.0001$) and Tukey's test was used to test for significance. NS: not significant.

(G) Cells were quantified for the presence of cytoplasmic TDP-43-GFP foci. The mean (± s.e.m.) from three independent experiments is presented. A one-way ANOVA ($P < 0.0001$) followed by Tukey's test was used to test for significance. Asterisks: significant pairs.

(H) The Tankyrase inhibitors were tested for an effect on the formation of TDP-43-GFP foci in the cytoplasm without a 30-min pre-treatment prior to the onset of arsenite treatment. The graph represents the mean of two independent experiments. A one-way ANOVA followed by a Tukey's test revealed that there was no significant difference in the control treated cells compared to drug treated cells. These data indicate that a pre-treating the cells with inhibitor is necessary to reduce the formation of stress-induced TDP-43 foci in the cytoplasm. Asterisk: significant pair and NS: not significant.

(I) The reported IC50 of XAV-939, G007-LK and IWR1-endo (Huang et al., 2009; Voronkov et al., 2013).