Supplemental Materials

Molecular Biology of the Cell

Owen *et al*.

Supplementary Figure 1. Phospho-specific antibodies show non-specific bands by Western Blot. A- Phospho-specific antibodies against Serine 57, Threonine 71, and Serine 96 within the FUS prion-like domain recognize the phosphorylated and not the unmodified peptide as shown by dot blot. Serine 57 peptide dilution was probed with a non-phosphospecific antibody (Pan-Ser57) to confirm proper peptide loading. B- Western Blots of serial diluted phospho-FUS peptides. Each peptide dilution was probed with each of the phospho-specific antibodies (pSer26, pSer30, pSer57, pThr71, or pSer96). Serine 57 blot probed with Pan-Ser57 was used as a peptide loading control. C,D **-** Full Western Blots from H4 cells with or without FUS knockdown treated with DMSO or 50 nM Calicheamicin (C) or sodium arsenite or sorbitol (D). Blots were probed with anti-FUS (pSer30, pSer57, pThr71, or pSer96(red) and commercial FUS (SC373698) (green)).

Supplementary Figure 2: Torin 2 inhibits the PIKK family kinases. A- Schematic of known targets of the PIKK family kinases. Replication stress (RS) or double stranded breaks (DSB) cause activation of the PIKK family kinases. (Schematic adapted from (Ashley and Kemp, 2018)). B– Western blot (right panels) of H4 cells placed under various stressors to activate the PIKK family kinases**.** Cells were pretreated with torin 2 (200 uM) for 1 hour followed by treatment with 2 mM hyroxyurea for 1 hour to activate ATR, UV radiation for 90 minutes to activate ATM, or irradiated with ~20 Gy to activate DNA-PK. B– Quantification (lower panels) of nuclear and cytoplasmic FUS(pT71) and FUS(pS96) fluorescence following cellular stress with or without torin 2.

Supplementary Figure 3. FUS phosphorylated at non-PIKK consensus sites localizes to cytoplasmic granules. H4 cells treated with either Sodium Arsenite or Sorbitol for one hour were analyzed using confocal microscopy. Both FUS and pFUS (pThr71 and pSer96) are found in cytoplasmic granules.

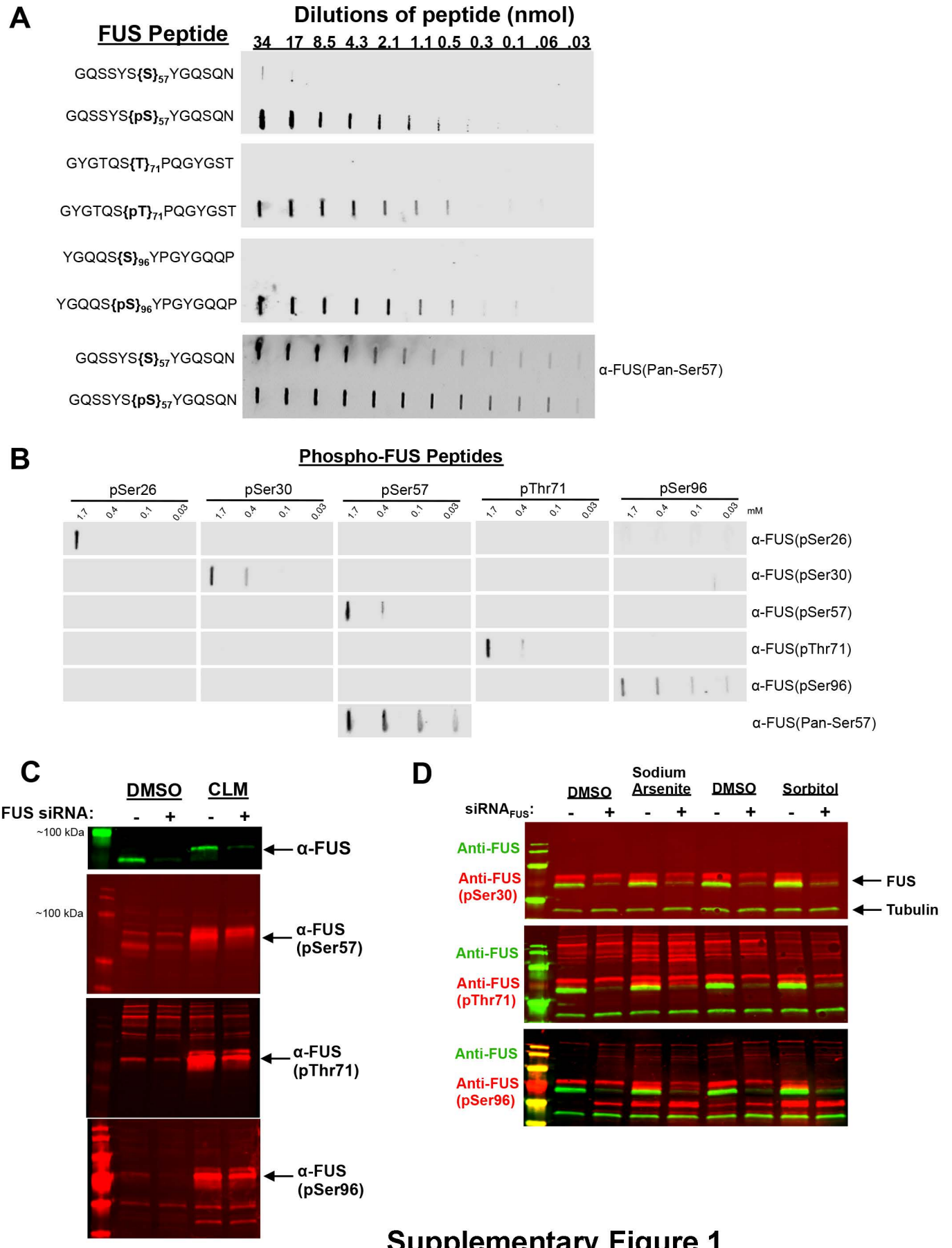
Supplementary Figure 4. Phosphorylated FUS localizes to TIA1+ cytoplasmic stress granules. H4 cells were treated for 1 hour with sorbitol or sodium arsenite and probed for TIA1 and phospho-FUS (pSer30, pThr71, or pSer96). Cells were analyzed by confocal microcopy.

Supplementary Figure 5. Cytoplasmic mutant FUS is phosphorylated at multiple sites regardless of PIKK kinase inhibition. A- H4 cells were transfected with GFP-FUS(495X) and immunoprecipitated (IP) using anti-GFP antibodies and dynabeads at various time points post transfection (PT). Western blots of IP products were probed with phospho-FUS (pSer26, pSer30, pThr71, and pSer96) and anti-GFP antibodies. B-H4 cells were transfected with FUS(494)-GFP. 6-hours post-transfection cells were treated with torin2. At 8 hours post-transfection cells were fixed and imaged using confocal microscopy.

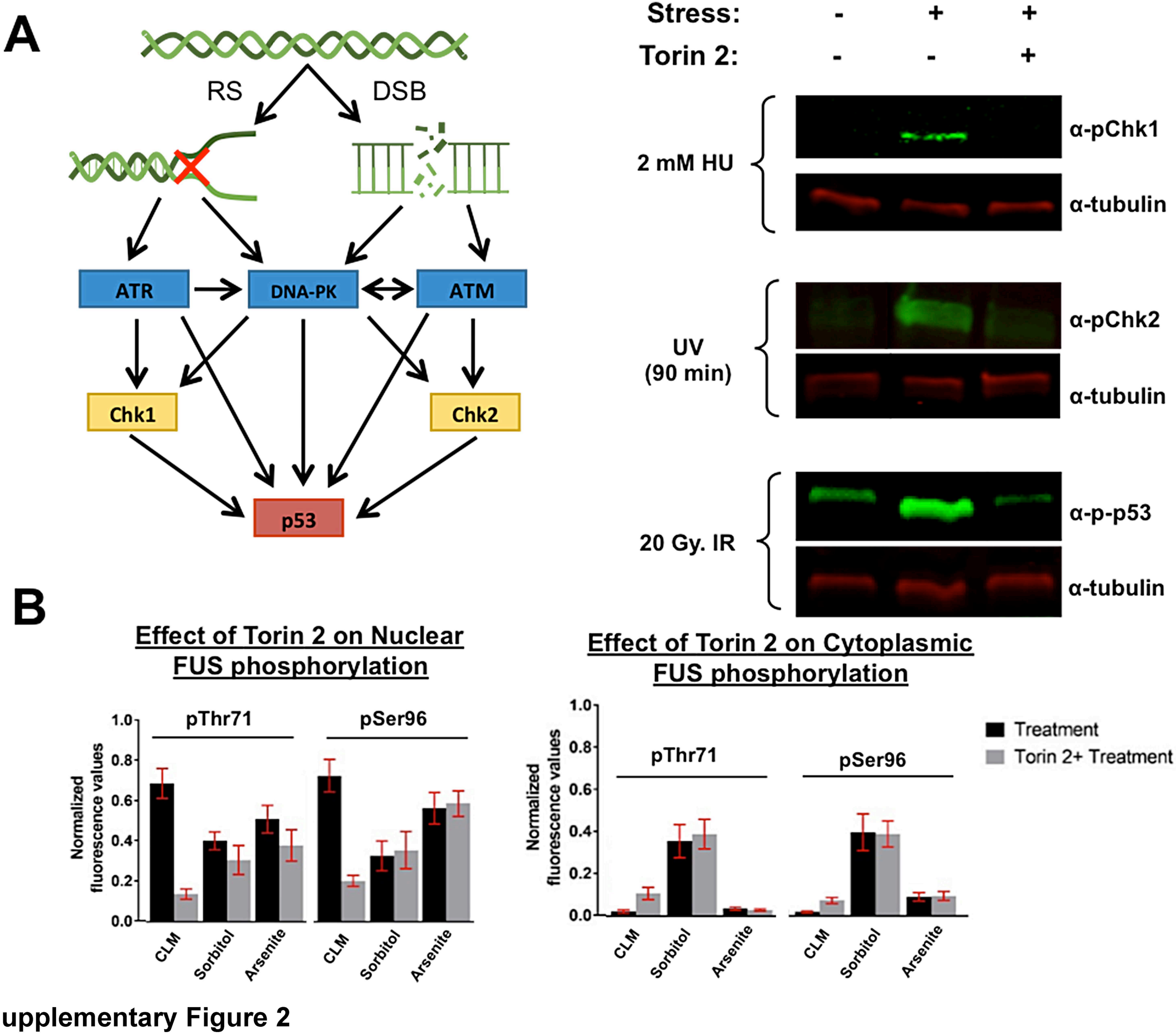
Supplementary Figure 6. FUS phosphomimetic substitution do not alter liquid like dynamics of arsenite induced stress granules. FRAP half times of arsenite-induced stress granules containing FUS(494)-GFP phosphomimetic constructs 24 hours post-transfection; error bars represent 95% CI; (n=30).

Supplementary Figure 7. FUS Phosphomimetic construct expression in yeast. A – Western blot from yeast lysate showing expression of all phosphomimetic constructs used. Blots were probed with anti-FUS and anti-PSTAIRE (loading control) antibodies. B- All variants with phosphomimetic substitutions in the prion-like domain rescue FUS toxicity in yeast. C – Colony areas from experiments shown in Figure 8B were quantified using ImageJ and normalized to 12E area (n=12). Student t test was used for statistical analysis (All P values < 0.0001).

Supplementary Figure 8. Phosphomimetic FUS variants form spherical droplets with liquid-like characteristics that persist over 48 hours. A- Wild-type FUS amorphous aggregates form with 6 hours of agitation, while the phosphomimetic variants form small spherical droplets. By 48 hours, FUS has formed large aggregates and the phosphomimetic variants remain in droplets. B- Phosphomimetic FUS droplets, but not wild-type FUS aggregates, dissolve following treatment with10% 1,6-hexanediol.



Supplementary Figure 1



Supplementary Figure 2

α-FUS (pThr71)

α-FUS

DAPI

Merge

Vehicle

Sorbitol

Sodium Arsenite

α-FUS (pSer96)

α-FUS

DAPI

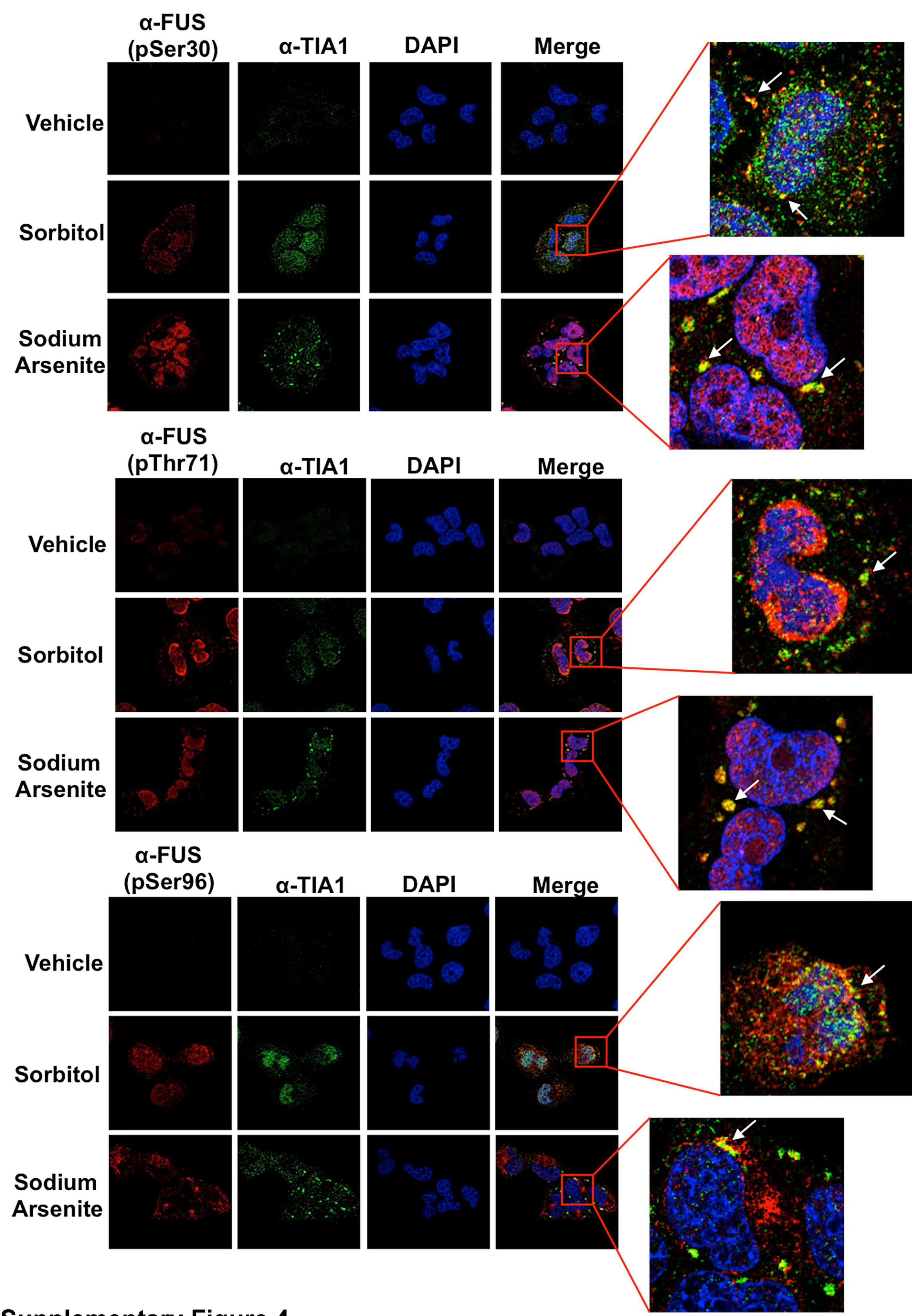
Merge

Vehicle

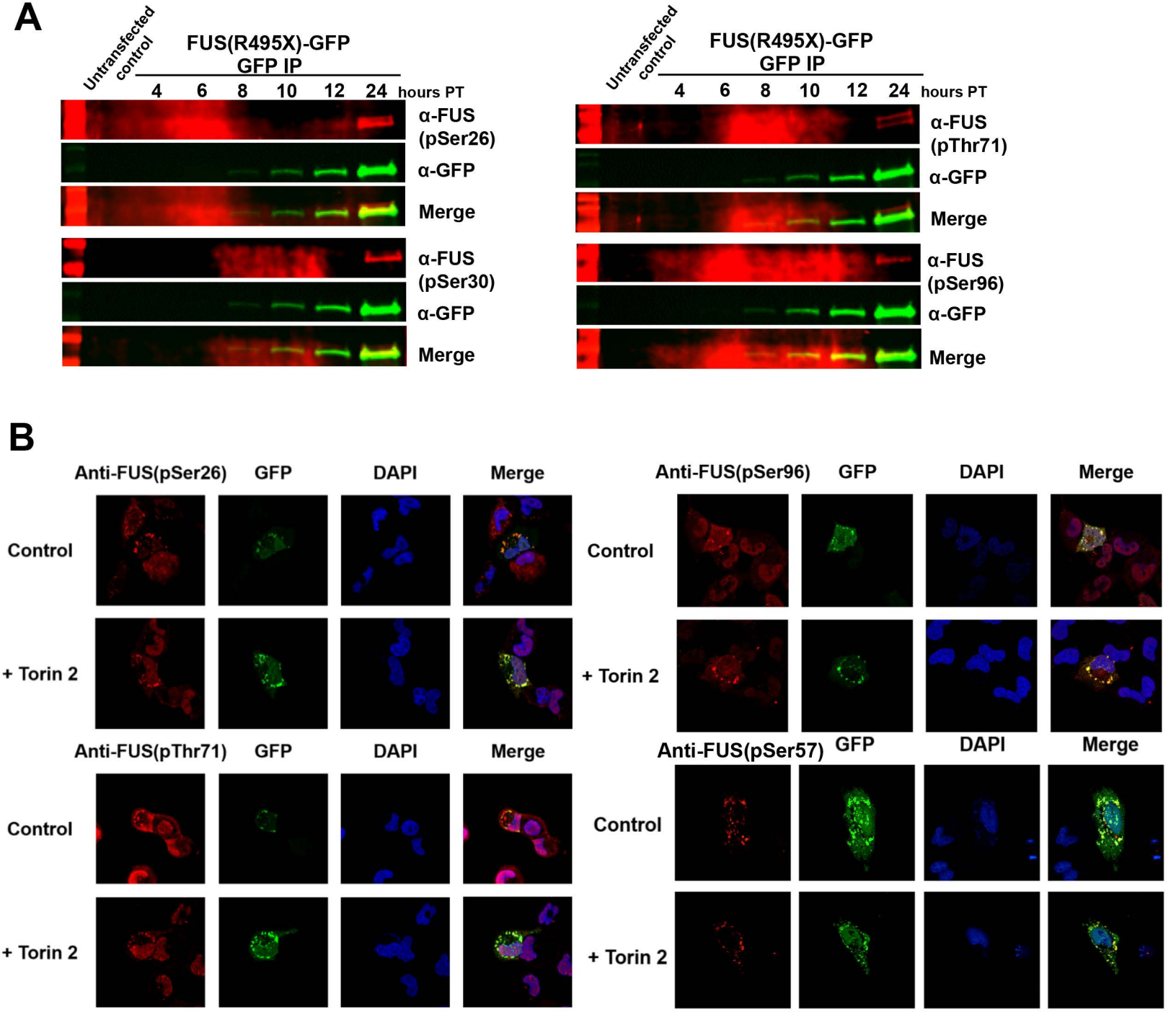
Sorbitol

Sodium Arsenite

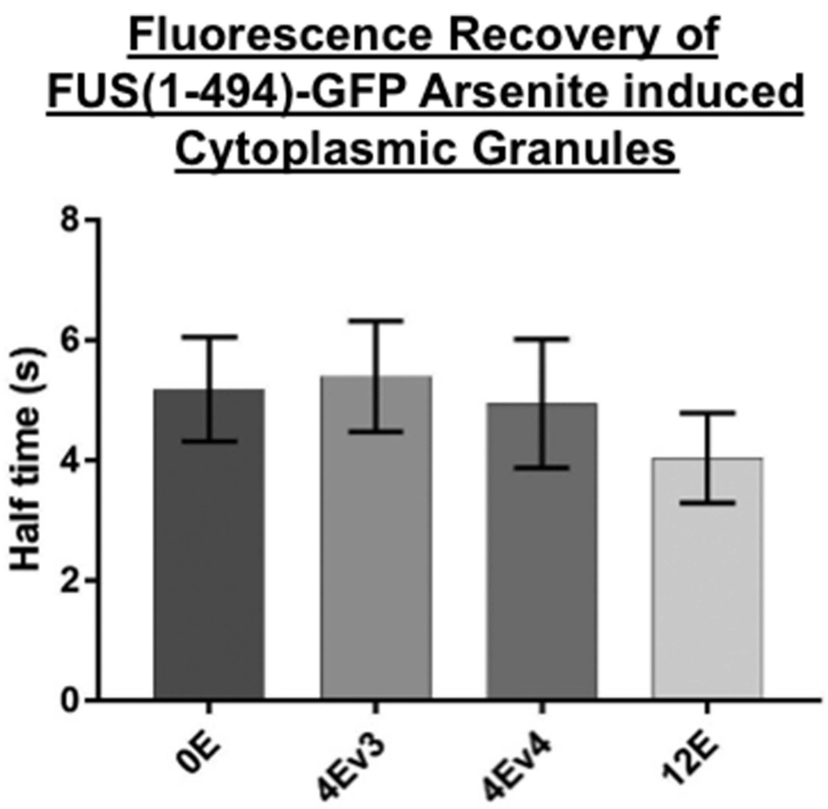
Supplementary Figure 3



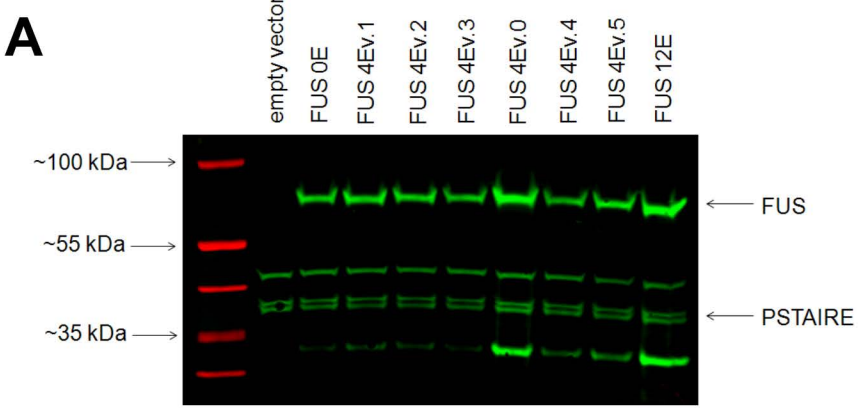
Supplementary Figure 4

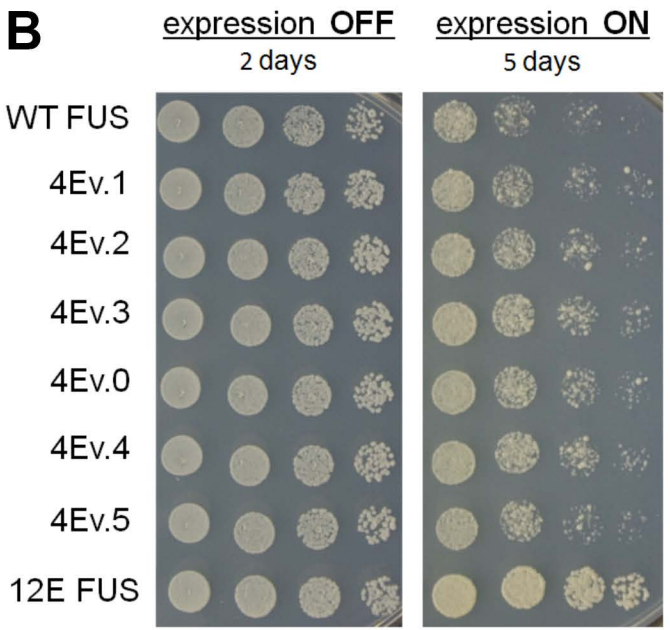


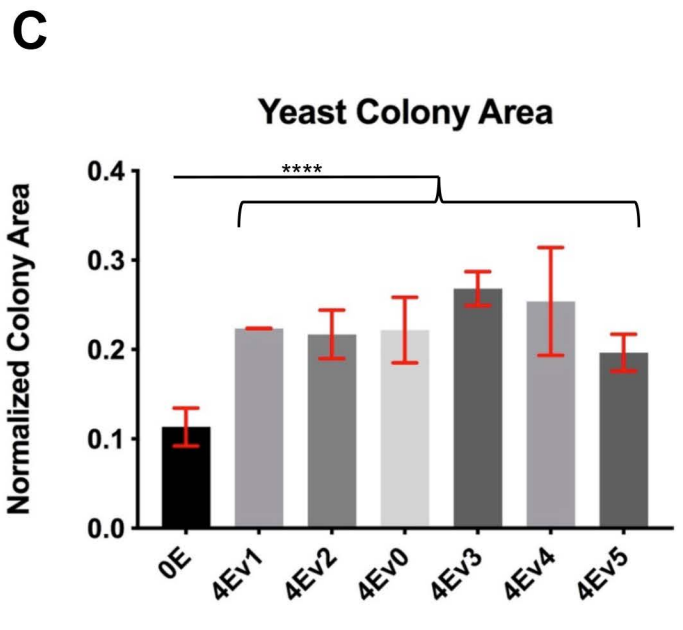
Supplementary Figure 5



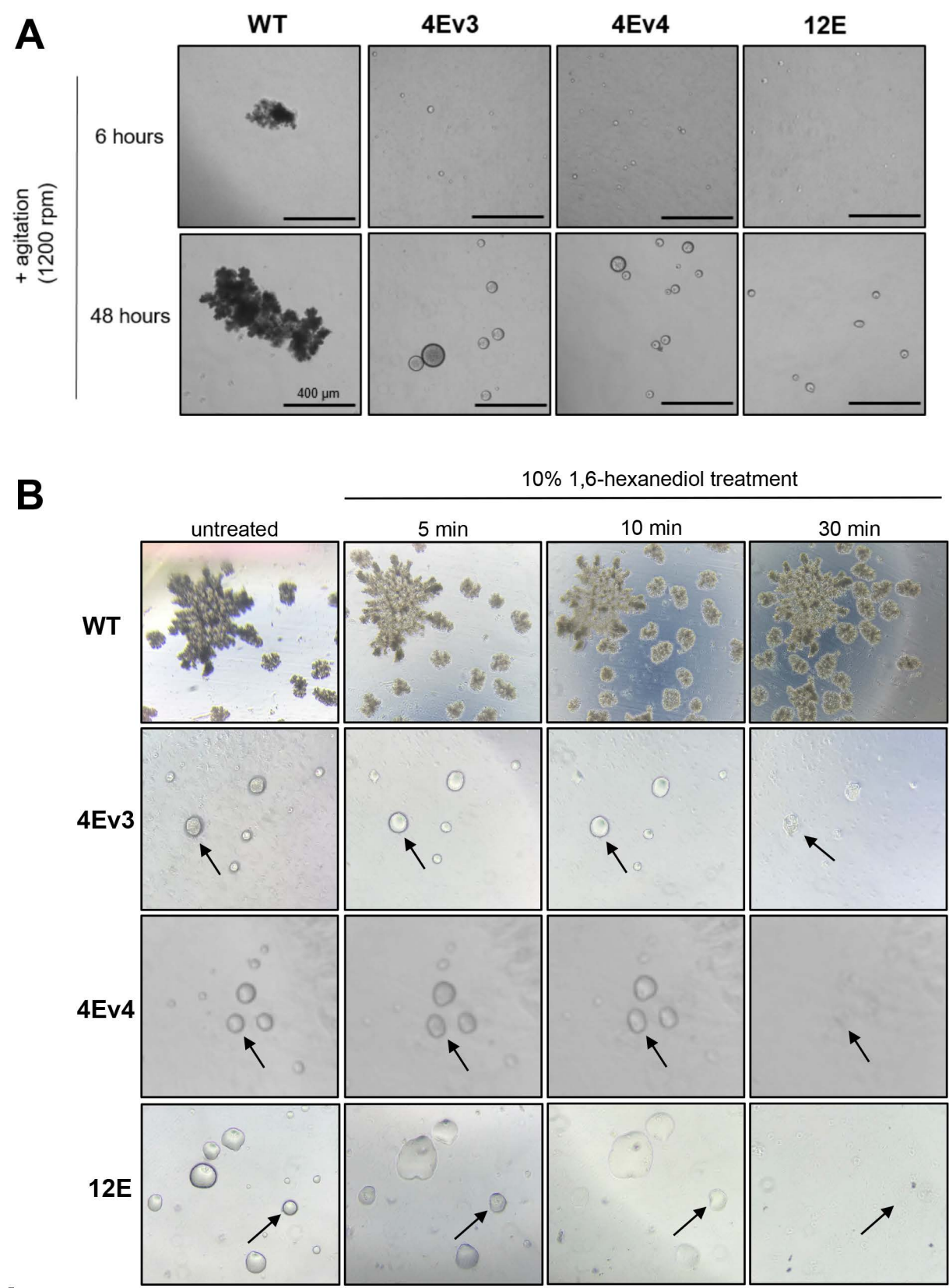
Supplementary Figure 6







Supplementary Figure 7



Supplementary Figure 8