SUPPLEMENTARY FIG. 1. **ATP and domain requirements for Hsp70 inhibition of fibril assembly.** Assembly reactions containing monomeric full-length α-Syn (72 μM) were incubated in the presence of either Hsp70 or BSA in assembly buffer containing an ATP-regeneration system (see Experimental Procedures). To examine the effects of nucleotide binding on assembly inhibition, ATP (2mM) was added to reactions where indicated, and ThT fluorescence quantified at various timepoints (***A***). ATP and the regeneration system did not affect the progression of assembly when added in the absence of Hsp70. Addition of Hsp70 virtually eliminated assembly as detected by ThT. Reactions containing ATP showed detectable increases at 72 h, although this was far lower than values observed without chaperone. ***B***, ThT fluorescence was measured in assembly reactions containing 7.2 μM wildtype Hsp70 (WT) or Hsp70 mutants lacking either the N-terminal ATPase portion ($Hsp70^{386-640}$) or extreme C-terminal ($Hsp70^{1-611}$). Control reactions contained BSA at the same concentration.

SUPPLEMENTARY FIG. 2. **Hsp70 inhibits seeded α-Syn assembly.** Reactions containing full-length α-Syn (72 μM) with or without preformed fibrils (1-4%, w/w) were incubated with agitation for 0, 24, 48, or 96 h. BSA (***A***) or Hsp70 (***B***) were included in reactions (7.2μM) which were monitored at various time points by ThT fluorescence. Addition of pre-formed fibrils (PFF) greatly accelerated the rate of fibril assembly in a concentration-dependent manner while no significant ThT signal was detected in unseeded reactions in the presence of Hsp70. Seeded reactions displayed significantly lower ThT values in the presence of Hsp70, although a small rise in ThT signal could be detected, suggesting some extension of existing mature fibrils.

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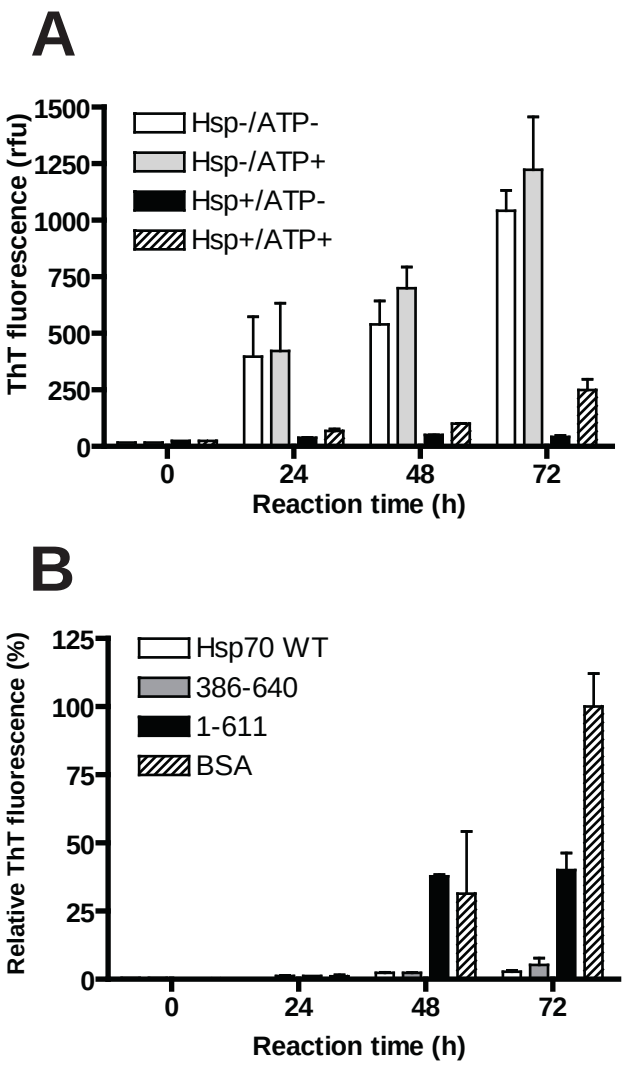

Luk et al, Supplementary Figure 2