

## Supporting material

Title: Actin impacts the late stages of prion formation and prion propagation

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**Supplemental Figure 1. Fixation and phalloidin-rhodamine staining alters the physical appearance of Sup35PrD-GFP aggregates.** Wildtype cells overexpressing Sup35PrD-GFP for approximately 24 hours were fixed and stained with rhodamine-phalloidin. Shown is an example of a ring structure that is jagged, likely an artifact of fixation/rhodamine-phalloidin treatment.

**Supplemental Figure 2. Generation of *act1-122* sibling isolates.** The original cytoduced high [*PIN*<sup>+</sup>] *act1-122* strain was streaked consecutively for single colony two times (approximately 30-50 generations) to generate sibling isolates M254 and M257. A second isolation, in which M231 was streaked for single colony consecutively six times (approximately 100-150 generations) was performed to generate sibling isolates M314 and M315.

**Supplemental Figure 3. Live cell imaging of wildtype cells expressing Rnq1-GFP.** Wildtype strains (M266) were mated to D163 containing a copper inducible Rnq1-GFP plasmid. Diploids were grown in SD-Leu overnight, and the Rnq1-GFP plasmid was induced for 4-6 hours. Cultures were time-lapse imaged for approximately 10 seconds. Movies are 15 frames per second, with each second representing 3.5 seconds of time.

**Supplemental Figure 4. Live cell imaging of *act1-122* cells expressing Rnq1-GFP.** *act1-122* (M257) were mated to D163 and heterozygous diploids (*ACT1/act1-122* [*PIN*<sup>+</sup>]) were induced and imaged similar to Supplemental Figure. Movies are 15 frames per second, with each second representing 3.5 seconds of time.

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