Title: *De novo* [*PSI*$^{+}$] prion formation involves multiple pathways to form infectious oligomers

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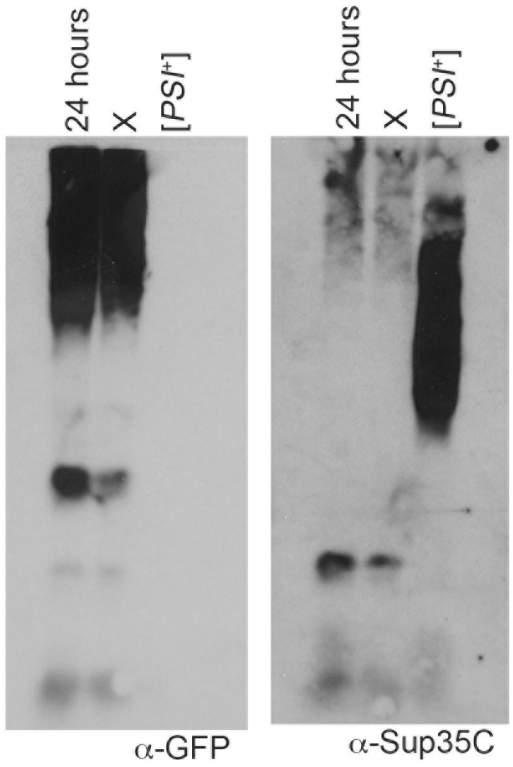
Supplementary Information Figure Legends

Supplementary Figure 1. Video - 4D Live cell imaging of the pathway I during *de novo* prion induction. Timelapse movies of the four cells shown in figure 2a. Movies are 10 frames per second, with each second representing 4 minutes and 30 seconds of time. Movie images were captured in 3D, deconvolved and collapsed.

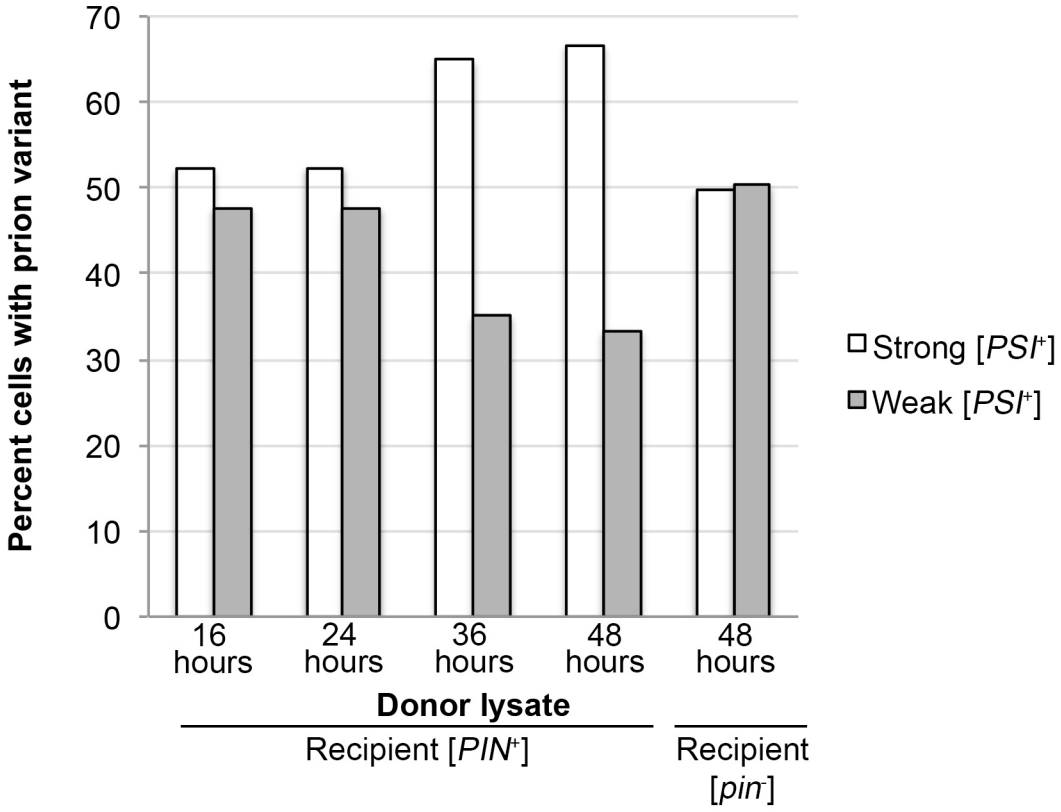
Supplementary Figure 2. Video - 4D Live cell imaging of the pathway II during *de novo* prion induction.

Supplementary Figure 3. Video - 4D Live cell imaging of the pathway III during *de novo* prion induction.

Supplementary Figure 4. Video - 4D Live cell imaging of the pathway IV during *de novo* prion induction.



Supplementary Figure 5. Overexpressed Sup35PrD-GFP and endogenous Sup35p co-migrate as high molecular weight assemblies in culture induced for 24 hours. Lysates from 24 hour induced cultures in a [*psi*$^-$][*PIN*$^+$] background were treated with SDS without boiling. Samples were run on the same SDD-AGE gel (approximately 2%) and separated for immunoblotting. The blots were incubated with either anti-GFP (left) or anti-Sup35C antibody (right). Lysates from [*PSI*$^+$] cultures were run as a control. X indicates a sample unrelated to this study.



Supplementary Figure 6. Weak [*PSI*$^+$] variants predominate over strong [*PSI*$^+$] variants when transfected with lysates from cultures with long Sup35PD-GFP overexpression times. Cells transfected with lysates from figure 5A were further scored for the presence of strong (white bars) and weak (gray bars) [*PSI*$^+$].