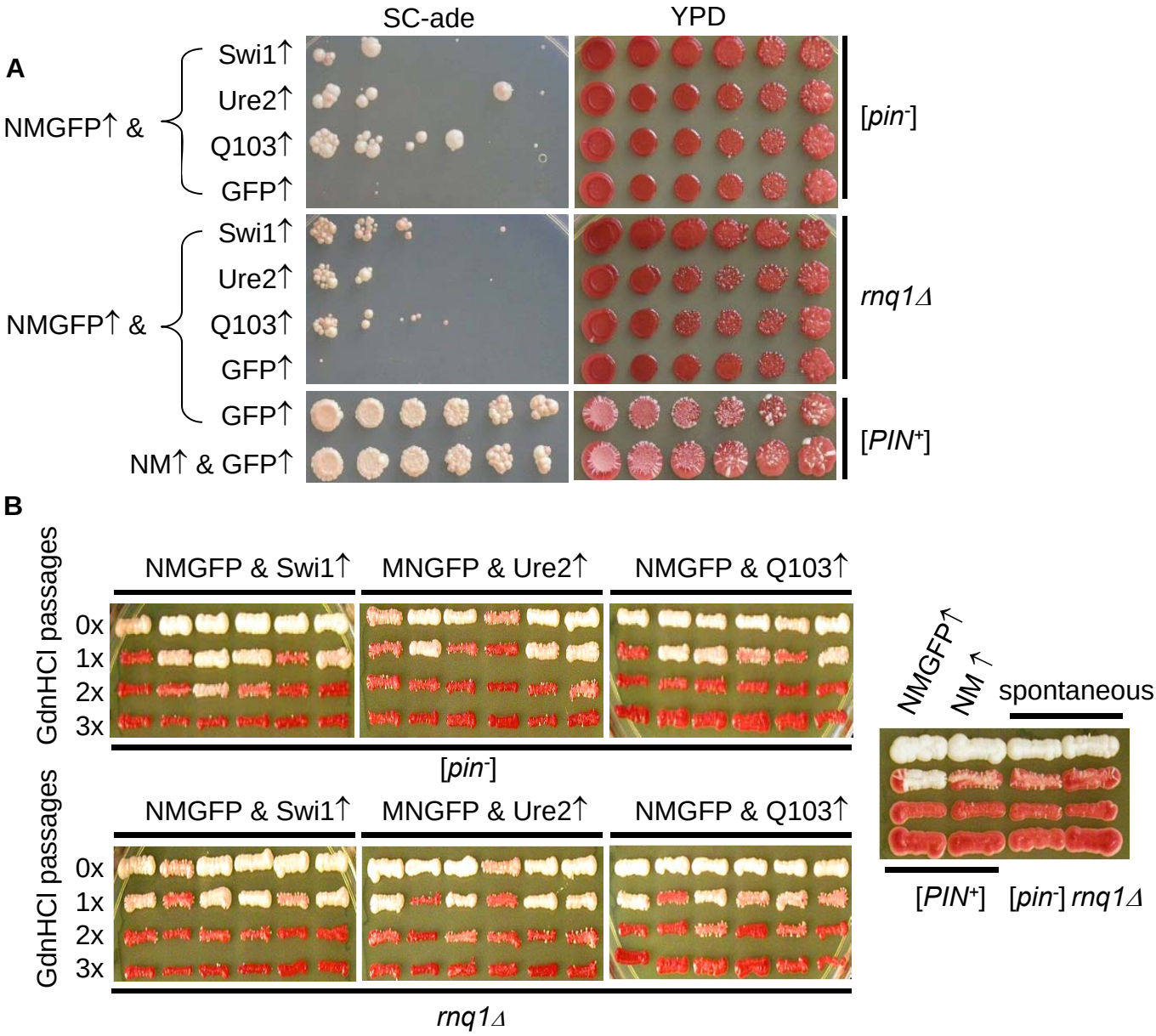

Figure S1 **A**. Yeast cells with indicated strain background were transformed with plasmid *p413CUP1-NMGFP (*NMGFP↑) or *pRS413CUP1-NM* (NM↑), and also one of the following plasmids: *p426GPD-SWI1* (Swi1↑), *p416GPD-URE2NPDGFP (*Ure2↑), *p426GPD-Q103GFP* (Q103↑) or *p426GPD-GFP* (GFP↑). [*PSI*$^+$] induction was carried out as described in Materials and Methods. After 48 hours of expression upon addition of 100 μ*M* $CuSO_4$, cultures were spotted onto the indicated plates. Note: The appearance of Ade+ colonies by Swi1, Ure2, or Q103 overproduction in [*pin*$^-$] cells was delayed ~2 to 3 days when compared to that obtained from [*PIN*$^+$]. **B**. Randomly selected [*PSI*$^+$] isolates shown in panel A were replica-plated onto GdnHCl-containing plates for up to three times (3x) and then patched back to YPD plates to see the curability and to determine [*PSI*$^+$] variants. Spontaneously formed [*PSI*$^+$] isolates were also included. Shown are representative results. Note: overproduction of NM and NMGFP gave similar [*PSI*$^+$] induction efficiency.

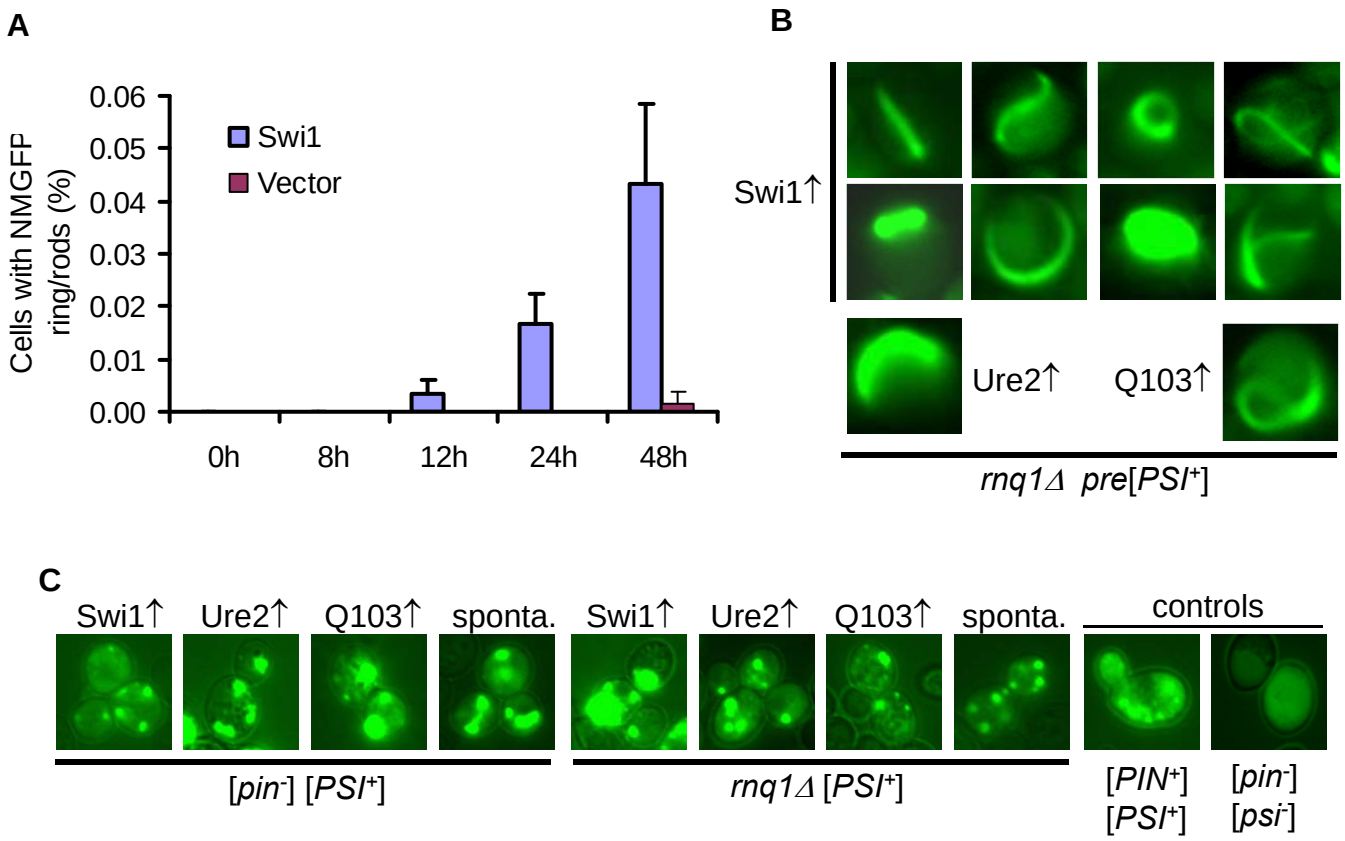

Figure S2 **A**. Sup35NMGFP and Swi1 were co-overproduced in a non-prion strain containing the plasmids *p413CUP1-NMGFP* and *p426GPD-SWI1 (*blue) in the presence of 100 μM CuSO4. The frequency of Sup35NMGFP ring/rod-like aggregates was analyzed at the indicated times. Cells containing *p413CUP1-NMGFP* and *p426GPD* were treated under identical conditions as the vector control (red). **B**. Representative images of ring/rod Sup35NMGFP aggregates in pre-mature [*PSI*$^+$] cells generated by co-overproduction of Sup35NMGFP and one of the indicated proteins. This experiment was carried out in a *rnq1*Δ strain with conditions similar to what described for Figure S1A. Images were taken after 24h induction. **C.** Sup35NMGFP aggregates of mature [*PSI*$^+$] isolates obtained in experiments shown in Figure S1A-1B. Ure2: Ure2$_{1\text{-}65}$-GFP; Q103: polyQ103-GFP; and sponta.: spontaneously formed [*PSI*$^+$] isolates.

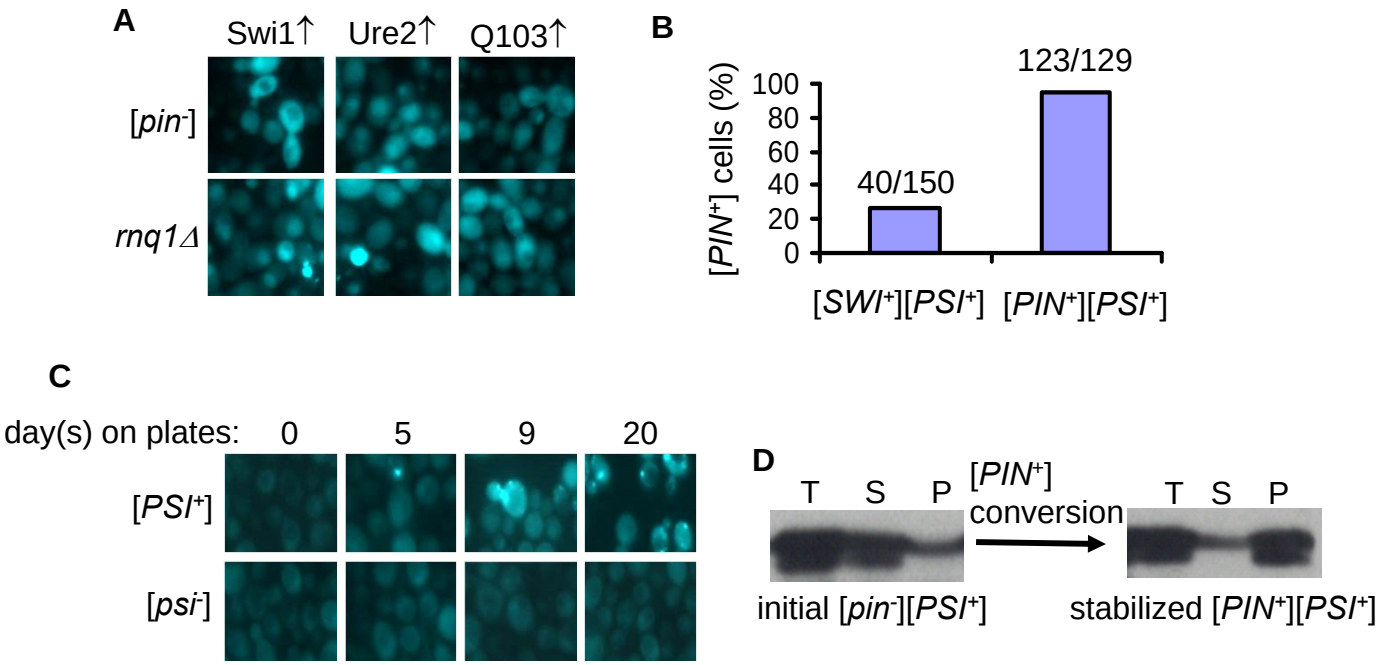

Figure S3 **A**. Majority [*PSI*$^+$] isolates obtained by co-production of Sup35NMGFP and Swi1 (Swi1 ↑), Ure2$_{1\text{-}65}$-GFP (Ure2 ↑) or polyQ103-GFP (Q103↑) remained [*pin*$^-$]. As shown, Rnq1CFP remained diffused in stabilized [*PSI*$^+$] cells obtained under the indicated co-overproduction conditions. **B**. Heritable Rnq1CFP aggregates (indicative of [*PIN*$^+$]) were analyzed after eliminating the plasmid *pRS413CUP1-NMGFP* and *p426GPD-SWI1*. Numbers shown are [*PIN*$^+$] isolates versus total examined isolates. **C.** A [*PSI*$^+$] isolate acquired by co-overproduction of Sup35NMYFP and Swi1 was compared with an isogenic [*pin*$^-$][*psi*$^-$] strain for their ability in promoting spontaneous [*PIN*$^+$] conversion after incubation at 4^{o}C for the indicated days. The method used in this study is similar to that described in an earlier paper (Derkatch ***et al***., 2001, cell 106: 171-182). Shown are Rnq1CFP signals. **D.** The acquired Rnq1CFP aggregate-containing [*PSI*$^+$] cells (20 days in panel C) were further stabilized by passages and compared with the initial [*PSI*$^+$] strain (0 day cells) using a centrifugation assay. T, total lysate; S, supernatant; P, pellet. An anti-Rnq1 polyclonal antibody was used in the Western Blotting. Note: all results shown in this figure were obtained using cells carry an integrated copy of *TEF1-RNQ1CFP.*

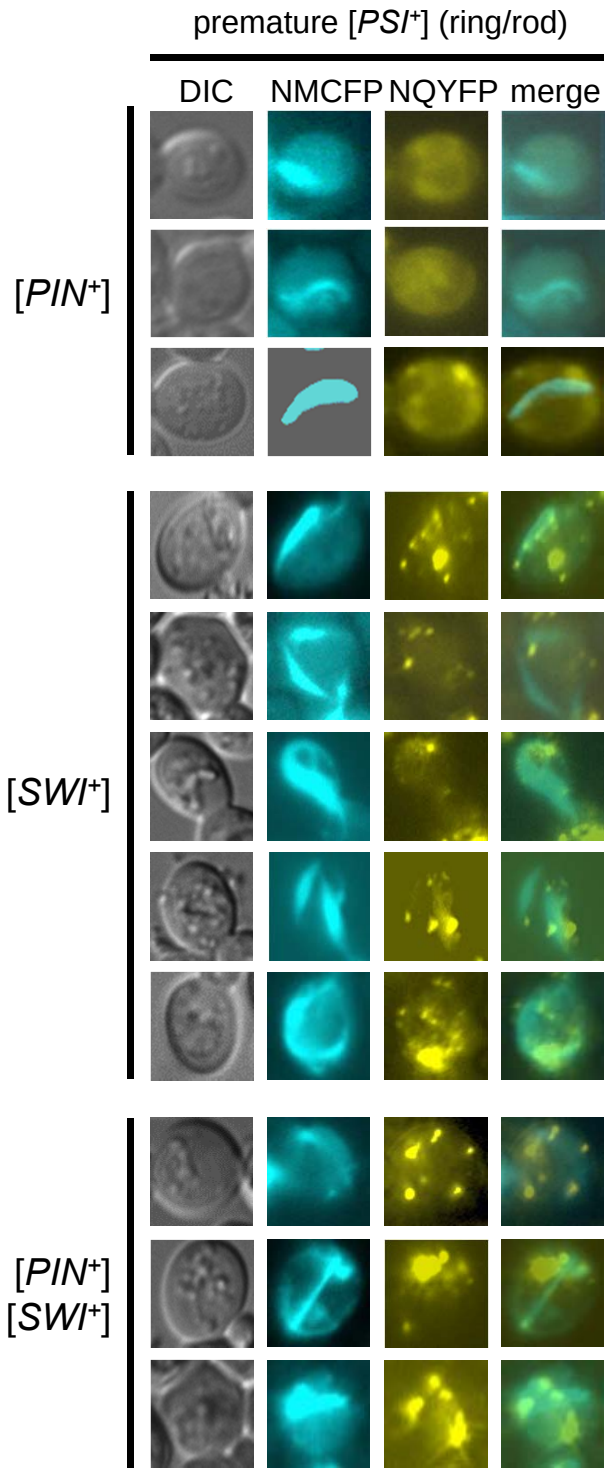

Figure S4 Representative images of Swi1-NQYFP and Sup35NM-CFP fluorescence patterns in pre-mature [*PSI*$^+$] cells. Sup35NMYFP was overproduced under the *CUP1* promoter. In strains containing the indicated prion(s), the *SWI1-NQYFP* was expressed under the *TEF1* promoter.