

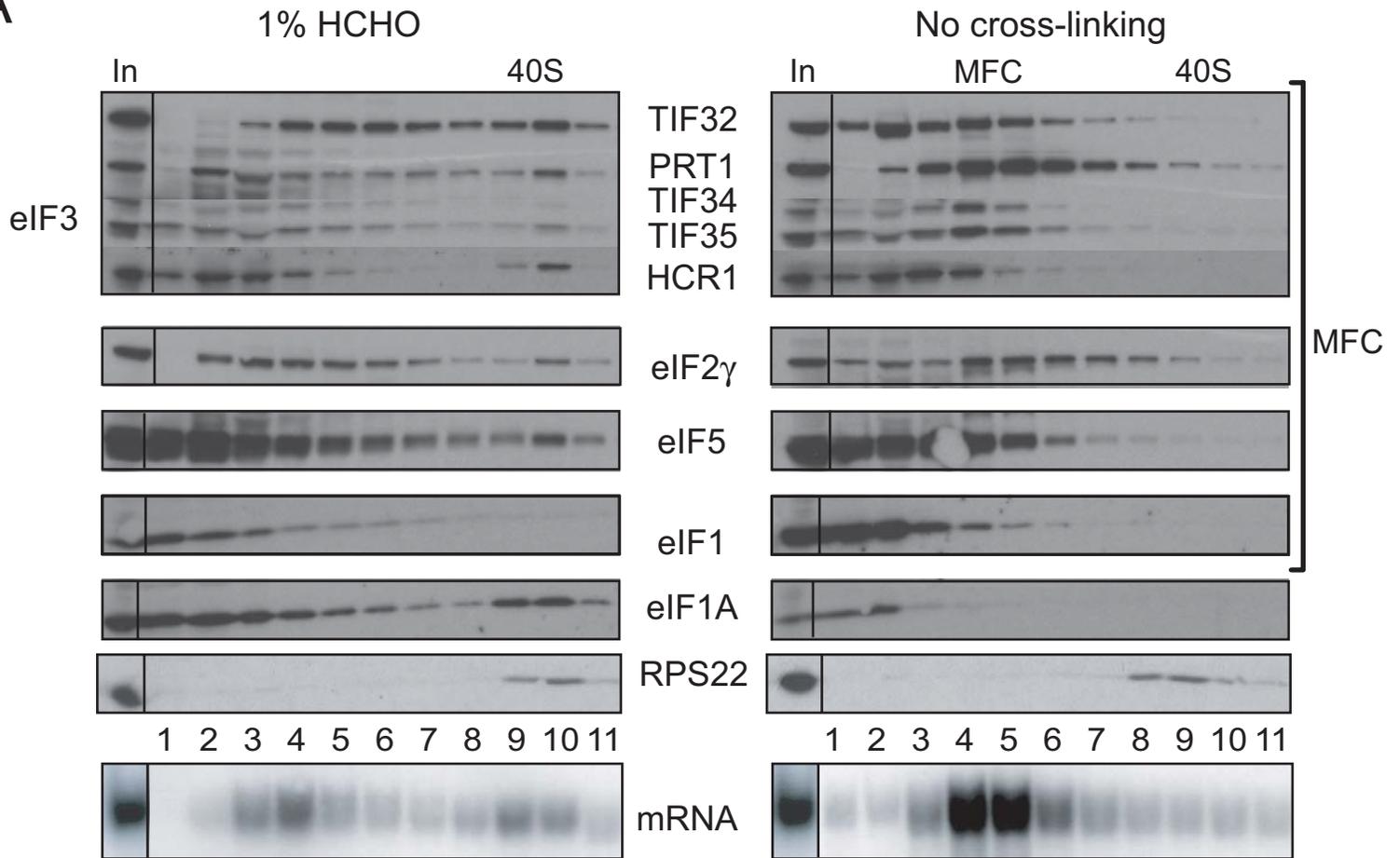
Supplementary figure legends

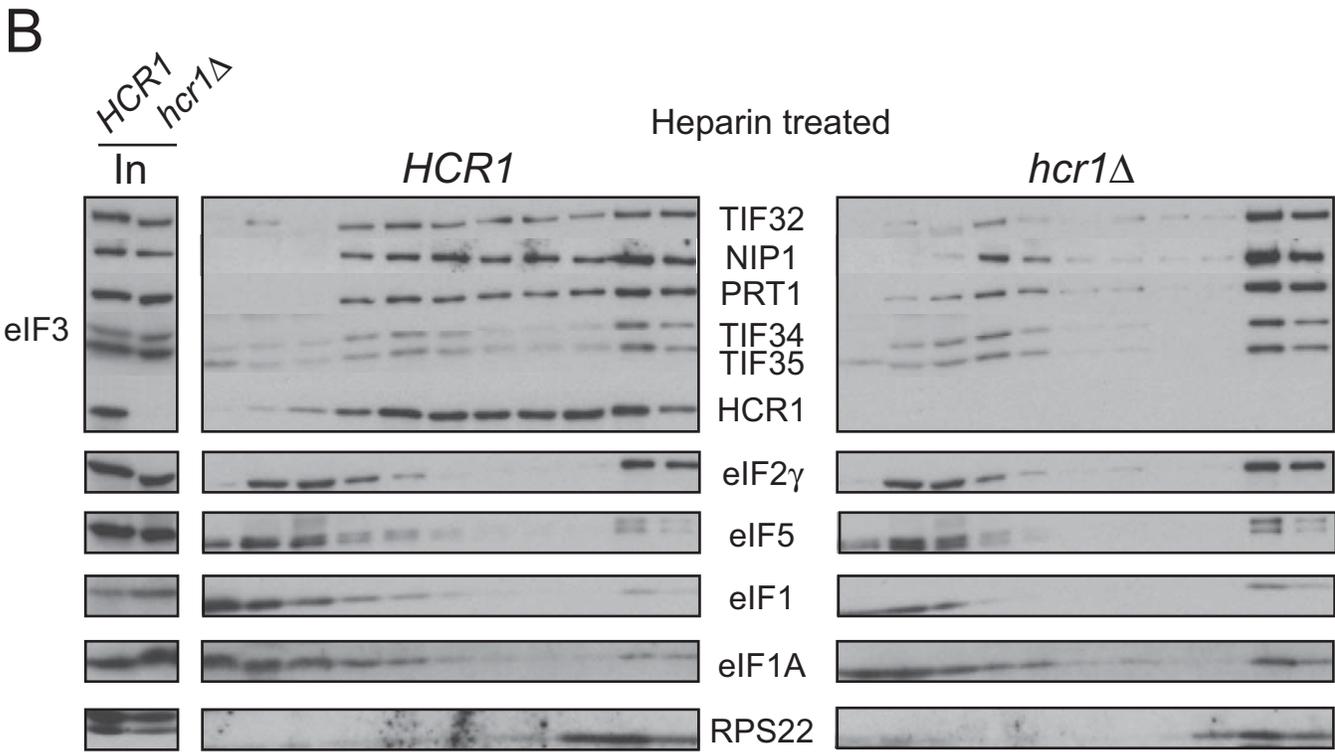
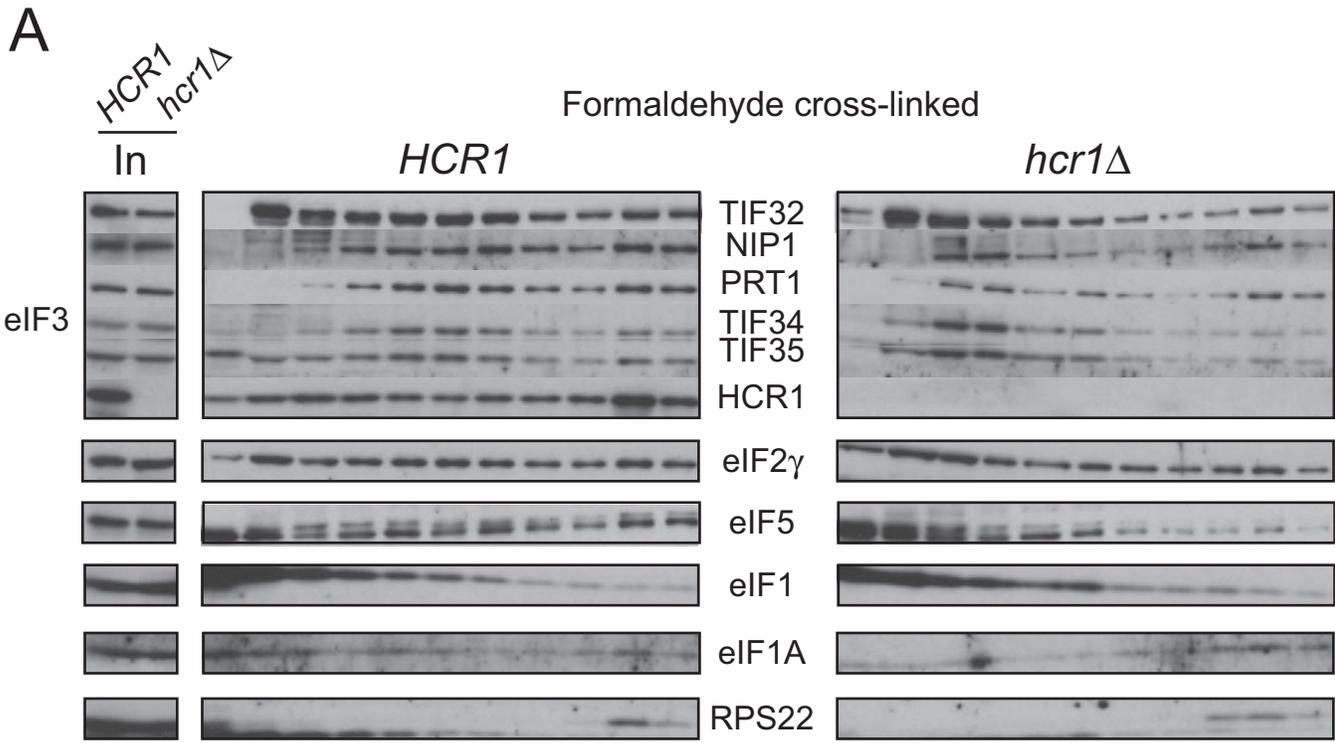
Fig. S1. Formaldehyde (HCHO) cross-linking stabilization of 43S- and 48S- PICs during the sedimentation through a sucrose gradient. *PRT1* cells from strain H2879 were grown in YPD medium at 30°C and at OD₆₀₀ of ~1.0 one-half of the culture was cross-linked with 1% HCHO for 1 h and then processed for WCE, while the other half was processed directly for WCE. The WCEs were separated by velocity sedimentation on sucrose gradients. The gradient fractions (numbered from the top) were subjected to Western and Northern analysis, respectively, using antibodies against the indicated proteins and a radioactive probe against *RPL41A* mRNA, as indicated.

Fig. S2. Analysis of the effects of *hcr1Δ* on 40S-binding of initiation factors in vivo using HCHO cross-linking of yeast cells (**A**) or heparin treated extracts of non-cross-linked cells (**B**). *HCR1* cells from strain H2879 or *hcr1Δ* cells from H3676 were grown in YPD medium at 30°C and at OD₆₀₀ of ~1.0 one-half of the culture was cross-linked with 1% HCHO for 1 h and then processed for WCE, while the other half had cyclohexamide added just prior to harvesting and heparin was added to the breaking buffer and then processed for WCE. The WCEs were separated by velocity sedimentation on sucrose gradients. The gradient fractions (numbered from the top) were subjected to Western analysis using antibodies against the indicated proteins

Fig. S3. Overexpression of HCR1-R215I and TC confers additive suppression of the Ts⁻ phenotype and defective 40S-binding of eIF3, eIF5 and eIF1 in *prt1-rnp1* cells. (**A**) Serial dilutions of *PRT1* (H2879) and *prt1-rnp1* (H3674) cells harboring vector YEp24, YEp24-*HCR1-R215I* (p4471), YEp24-TC-*HCR1-R215I* (p4472) or YEp24-TC (p3000) were spotted on SC-Ura

and incubated at 25°C or 33°C for 2 days. **(B and D)** Analysis of 40S-bound eIFs in HCHO cross-linked cells of *prt1-rnp1* strain H3674 containing empty vector or YEp24-TC (p3000) was conducted and quantified essentially as described in Figs. 2A-B, based on 5 independent experiments. **(C and E)** same as (B and D) but for *prt1-rnp1* (H3674) cells containing empty vector or YEp24-TC-*HCR1-R215I* (p4472), based on 3-5 independent experiments.

A



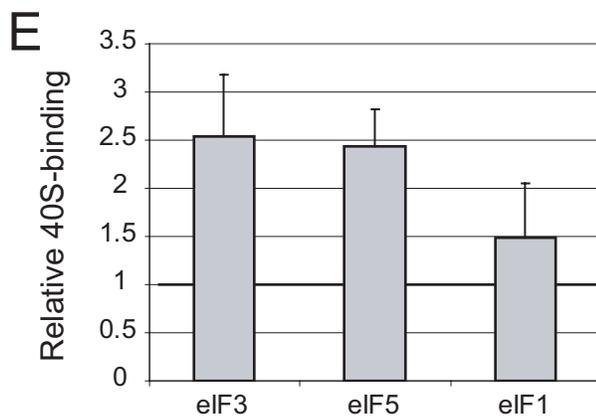
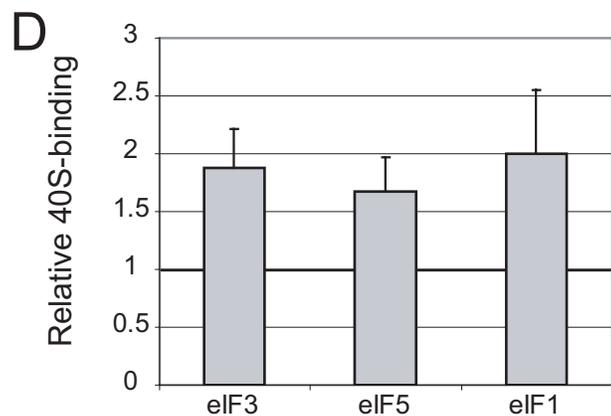
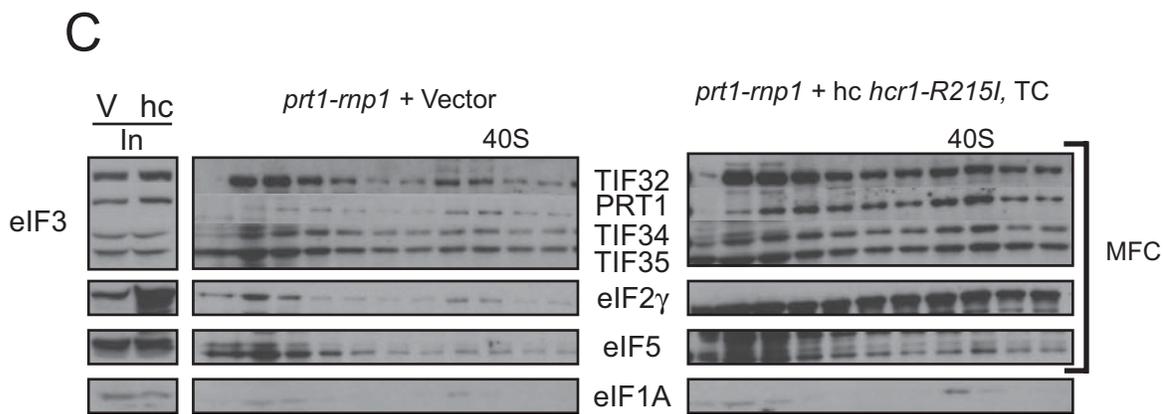
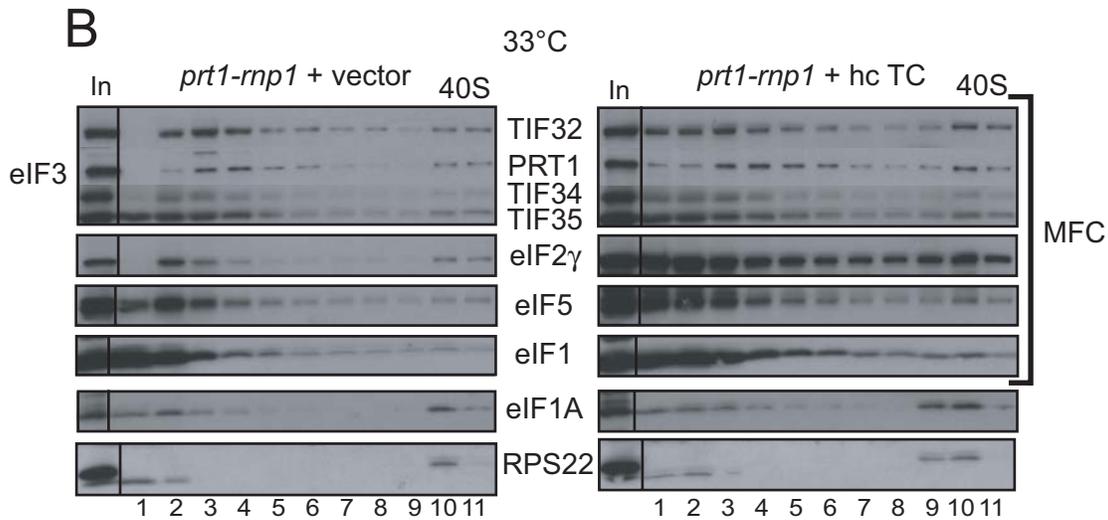
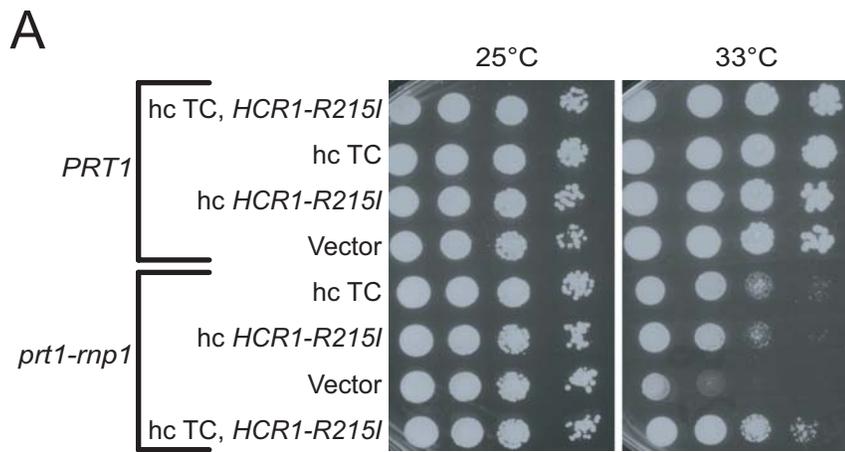


Fig.S3