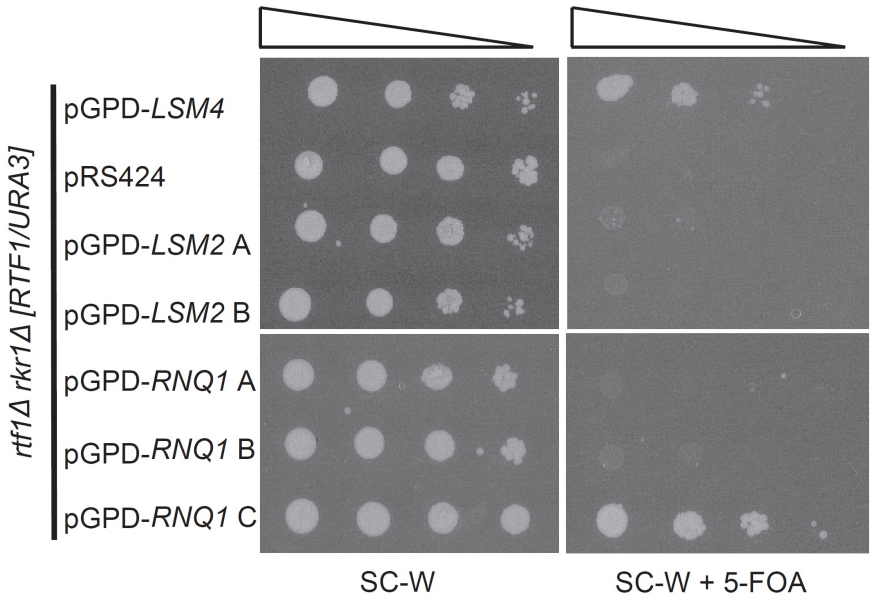

Figure S1 Effect of *LSM2 or RNQ1* overexpression on *rtf1Δ rkr1Δ* synthetic lethality. An *rtf1Δ rkr1Δ* strain (KY2205) carrying a *URA3*-marked *RTF1* plasmid was transformed with 2μ *TRP1*-marked p*GPD-LSM4*, p*GPD-LSM2*, p*GPD-RNQ1*, or empty vector. Ten-fold serial dilution assays were performed on SC-W or SC-W + 5-FOA media and plates were incubated at 30^{o}C for 2 days. Overexpression of *RNQ1* resulted in 9 out of 23 transformants that showed rescue of *rtf1Δ rkr1Δ* lethality (representative transformants depicted as A, B, or C). No variation in the lack of rescue of *rtf1Δ rkr1Δ* lethality by overexpression of *LSM2* was observed in 25 transformants (representative transformants depicted as A or B).

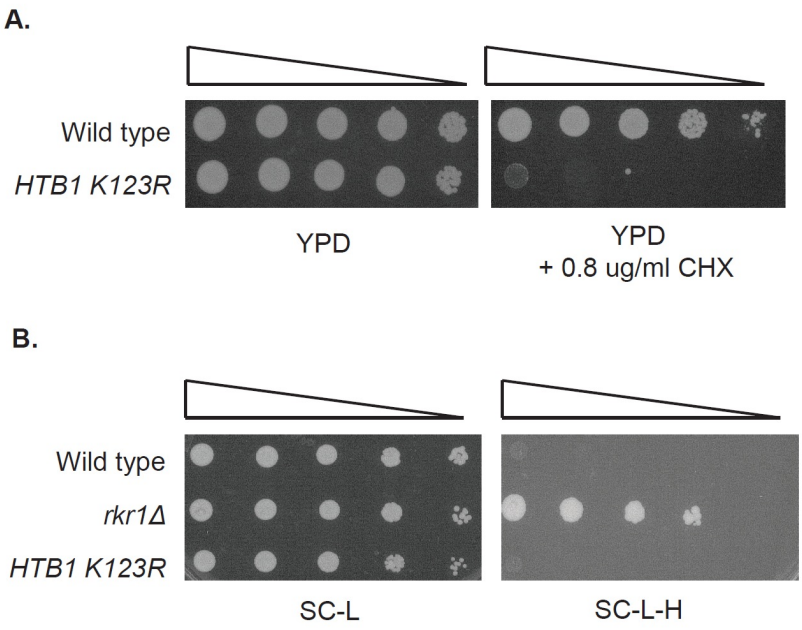

Figure S2 An H2B K123R mutant strain is sensitive to cycloheximide but does not exhibit increased expression of a nonstop reporter. (A) *HTB1* (KY2043) and *htb1-K123R* (KY2044) strains were grown to saturation and diluted to 3 x 10^8 cells/ml. Ten-fold serial dilutions were performed on YPD or YPD containing 0.8 µg/ml cycloheximide (CHX) and incubated at 30°C for 2 or 6 days, respectively. (B) *HTB1* (KY2043) and *htb1-K123R* (KY2044) strains were transformed with a *LEU2*-marked plasmid containing a *his3* nonstop reporter. Strains were then grown to saturation and diluted to 1 x 10^8 cells/ml. Ten-fold serial dilutions were performed on SC-L or SC-L-H and plates were incubated at 30°C for 3 days.

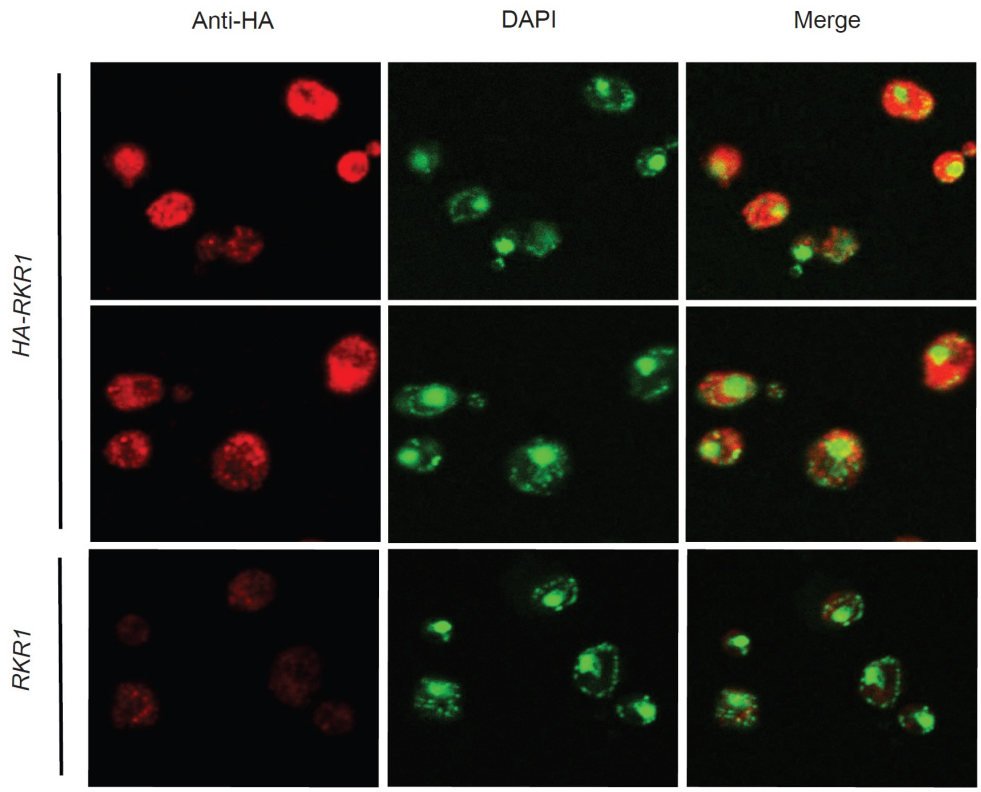

Figure S3 Localization of HA-Rkr1 is predominantly cytoplasmic. A *rkr1Δ* strain (KY2289) was transformed with either an H*A-RKR1* (pMB11) or untagged *RKR1* (pPC65) plasmid and grown to mid log phase before fixation and incubation with anti-HA antibody primary (Roche) and Alexa Red 647 secondary (Molecular Probes). Cells were mounted with ProLong DAPI stain (Invitrogen) and visualized using confocal microscopy.

Table S1 ***Saccharomyces cerevisiae*** **strains**

Strain	Genotype
KY307	*MAT*α *his3Δ200 ura3-52 trp1Δ63 lys2Δ202*
KY453	*MAT*α *rtf1Δ100::URA3 his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-173R2*
KY564	*MAT*α *rtf1Δ102::ARG4 his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-173R2 arg4-12*
KY607	*MAT*α *rtf1Δ101::LEU2 his3Δ200 leu2Δ1 ura3-52 lys2-128δ*
KY761	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY958	*MAT*α *rtf1Δ101:LEU2: his3Δ200 leu2Δ1 ura3Δ0 trp1Δ63 lys2-128δ*
KY1030	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY1404	*MAT*α *htz1Δ::KanMX his3Δ200 leu2Δ0 ura3Δ0 lys2Δ0*
KY1663	*MAT*α *rtf1Δ102::ARG4 rkr1Δ::KanMX leu2Δ1 ura3-52 arg4-12 [pKA69: RTF1/URA3/C/A]*
KY1898	*MAT*α *chl1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY1899	*MAT***a** *chl1Δ::KanMX his3Δ200 leu2Δ1 ura3-52*
KY2043	*MAT***a** *hta2-htb2Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2044	*MAT***a** *HTA1-htb1K123R hta2-htb2Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2202	*MAT***a** *rkr1Δ::HIS3 his3Δ200 leu2Δ1 ura3-52*
KY2203	*MAT***a** *hta1-htb1Δ::LEU2 hta2-htb2Δ::TRP1 his3Δ200 leu2Δ1 ura3-52 trp1Δ63* [*URA3/HTA1-HTB1/C/A*] [*HIS3/HTA1-FLAG-htb1-K123R/C/A*]
KY2204	*MAT***a** *hta1-htb1Δ::LEU2 hta2-htb2Δ::TRP1 rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63* [*URA3/HTA1-HTB1/C/A*] [*HIS3/HTA1-FLAG-htb1-K123R/C/A*]
KY2205	*MAT***a** *rtf1Δ102::ARG4 rkr1Δ::KanMX leu2Δ1 ura3-52 arg4-12 trp1Δ63 [pKA69: RTF1/URA3/C/A]*
KY2206	*MAT***a** *chl1Δ::KanMX rtf1Δ101::LEU2 rkr1Δ::HIS3 his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2207	*MAT***a** *chl1Δ::KanMX rtf1Δ101::LEU2 his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2208	*MAT*α *chl1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63 arg4-12*
KY2209	*MAT*α *rtf1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63* [*psi*$^-$]
KY2210	*MAT***a** *rtf1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ1 trp1Δ63* [*psi*$^-$]
KY2211	*MAT*α *rtf1Δ::KanMX his3Δ200 leu2Δ1 ura3Δ0 trp1Δ63*
KY2212	*MAT***a** *ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2213	*MAT***a** *rtf1Δ101::LEU2 ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2214	*MAT*α *chl1Δ::KanMX ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2215	*MAT*α *chl1Δ::KanMX rtf1Δ101::LEU2 ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2236	*MAT*α *rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2286	*MAT*α *rtf1Δ101::LEU2 rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 ade1-14* [*psi*$^-$]
KY2289	*MAT***a** *rkr1Δ::HIS3 his3Δ200 leu2Δ1 trp1Δ63 lys2-128δ*
KY2292	*MAT***a** *rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 ade1-14* [*psi*$^-$]
KY2306	*MAT***a** *rkr1Δ::KanMX sup35-Y351C his3Δ200 leu2Δ1 ura3-52 trp1Δ63 ade1-14* [*psi*$^-$]
KY2309	*MAT***a** *rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 ade1-14* [*psi*$^-$]
L2261	*MAT***a** *kar1 ura2 leu2 his* [*PIN*$^+$]
L2265	*MAT***a** *kar1 ura2 leu2 his* [*PSI*$^+$]