Supplementary Figure legends

Figure 1. Diagrammatic representation of the rDNA locus on yeast chromosome XII and the processing pathway from 35S full length nascent rRNA to mature 25S, 18S and 5.8S rRNAs. Note that internal transcribed sequence 1 (ITS1) is rapidly removed from the primary transcript.

Figure 2. Rpa43p localisation in cells released from metaphase block. A wildtype yeast strain carrying an epitope tagged copy of Rpa43p (*RPA43-9MYC)* was liberated from a metaphase block. Samples were collected at the times indicated and the localization of Rpa43p with respect to the nucleus (stained with PI) was analysed by indirect immunofluorescence. Nucleolar localization of Rpa43p was not observed during anaphase (top graph third column), instead during nuclear segregation Rpa43p becomes dispersed throughout the nucleoplasm (bottom graph first column). Representative micrographs of Rpa43p nuclear staining pattern during metaphase (metaphase arrest; nucleolar localisation) and anaphase (30 min after release; nuclear or dispersed localisation) are shown. Nucleus in red and Rpa43-9myc in green. Scale bar 5 μm.

Figure 3. Cell cycle progression, primary rRNA transcript levels and Cdc14p nucleolar release in cells liberated from a metaphase block (in Supplementary Fig. 2). A reduction in rRNA transcript amount that coincided with the nucleolar release (and activation) of Cdc14p is observed during anaphase. Quantitative RT-PCR using primers to the ITS1 region were used to determine levels of intact 35S rRNA transcripts. Cdc14p release was monitored *in vivo* using a GFP tagged copy of Cdc14p as the sole source of Cdc14p in the cell.

Figure 4. Quantification of experiment shown in Fig. 2c. Cells carrying epitope tagged Rpa43p (Rpa43-6HA) and Cfi1p (Cfi1-GFP) and either wildtype *CDC14* or a temperature sensitive mutant of Cdc14p, *cdc14-1*, and a stabilised *CLB2* (*CLB2-DK*)

under the galactose promoter were arrested in metaphase (nocodazole) in raffinose media. Galactose was added (during the arrest) to induce *CLB2-DK* expression. Note that expression of *CLB2-DK* causes a mitotic arrest where Cdc14p is released (and activated) from the nucleolus. Nuclear vs nucleolar localisation of Rpa43p was scored before and after expression of *CLB2-DK* using DAPI and Cfi1p staining as a nucleolar guide.

Figure 5. Quantification of nuclear vs nucleolar localisation of the RNA Pol I subunits Rpa43p and Rpa190p was scored before and after expression of *CLB2-DK* as in Supplementary Fig 4. In contrast to Rpa43p, Rpa190p does not become delocalized from the nucleolus after expression of *CLB2-DK*.

Figure 6. a, *In vivo* analysis of Rpa43p dephosphorylation by Cdc14p. Cells carrying epitope tagged Rpa43p (Rpa43-6HA) and wildtype *CDC14* under the galactose promoter were arrested in metaphase (nocodazole) on raffinose media. Galactose was added (during the arrest) to induce *CDC14* expression. Western blot analysis of Rpa43p before (raffinose) and after (galactose) *CDC14* expression showed the disappearance of an intermediate phospho-band of Rpa43p (indicated with an arrow *) in a *CDC14*-dependent manner. Tubulin levels were used as a loading control in the experiment. **b,** *In vitro* analysis of Rpa43p dephosphorylation by Cdc14p. Immunoprecipitated Rpa43p (Rpa43-6HA) was incubated in the absence or presence of λ-phosphatase, purified Cdc14p (Cdc14p) or purified phosphatase-dead Cdc14p (Cdc14-C/Sp) as indicated. A partial reduction in the intensity of Rpa43p phospho-bands is observed in the presence of wildtype Cdc14p, demonstrating that Cdc14p has specificity for some, but not all, phosphorylation sites. Treatment with λ-phosphatase removes all phospho-bands in Rpa43p.

Figure 7. a, Diagramme showing C-teminal truncations of Rpa43p used. **b,** Quantitative RT-PCR using primers to the ITS1 region were used to determine levels of intact 35S rRNA transcripts in cells carrying different C-terminal truncations of Rpa43p before and after (1.5hrs) expression of *CLB2-DK* from the galactose promoter. Cells were arrested in metaphase (nocodazole) in raffinose media. Galactose was added (during the arrest) to induce *CLB2-DK* expression. Like full-

length Rpa43p, most truncations showed inhibition of rRNA transcription dependent on Cdc14p activation. **c,** Nuclear vs nucleolar localisation of wildtype Rpa43p and C-terminal truncations of Rpa43p before and after expression of *CLB2-DK* as shown in b. In contrast to full-length Rpa43p, most truncations did not become fully delocalized from the nucleolus after expression of *CLB2-DK*.

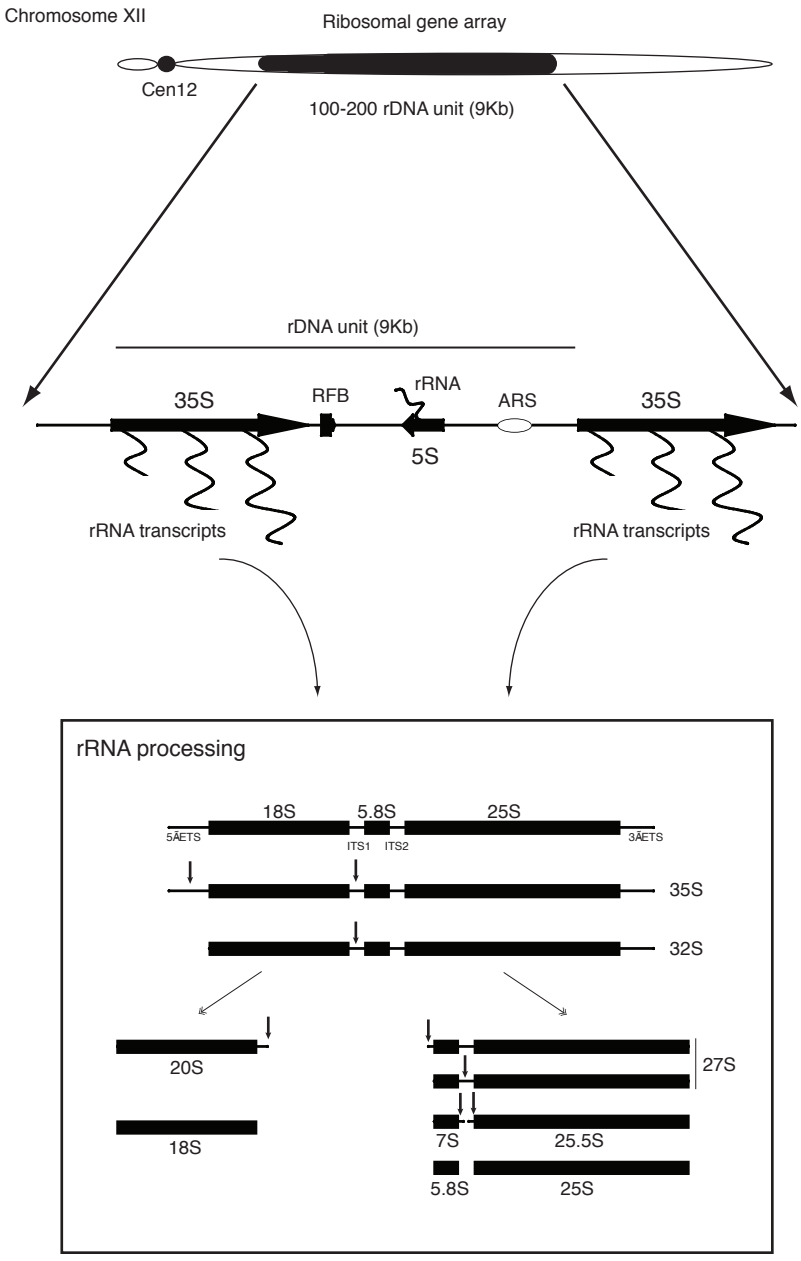
Figure 8. qRT-PCR analysis was used to determine levels of primary 35S transcripts in cells carrying phospho-mimicking mutations in the four phosphorylation sites mapped for Rpa43p (Rpa43-S208/220/262-263/285Dp) before and after expression of *CLB2-DK*. In contrast to full-length Rpa43p, inhibition of rRNA transcripts was defective in cells carrying Rpa43-S208/220/262-263/285Dp.

Figure 9. Analysis of *Aspergillus oryzae* ribonuclease RntA expression in budding yeast cells. A *cdc14-1* mutant strain expressing a myc-tagged RntA containing a nuclear localisation signal (NLS) under the galactose promoter (G*AL-NLS-RntA-MYC)* was arrested in metaphase in medium containing raffinose. Cells were released from metaphase in the presence of glucose or galactose at 37°C. Samples were collected 120 min after release and the expression and localisation of RntA in yeast cells was analysed by western analysis and indirect immunofluorescence. Nucleus is shown in red and RntA in green. Scale bar 5 μm. The effect of RntA expression on total RNA was also investigated. Cells were treated as described and total RNA was extracted and visualised in ethidium bromide stained gels after 60, 120 and 180 minutes of galactose or glucose addition. The expression of RntA induced degradation of all cellular RNA species.

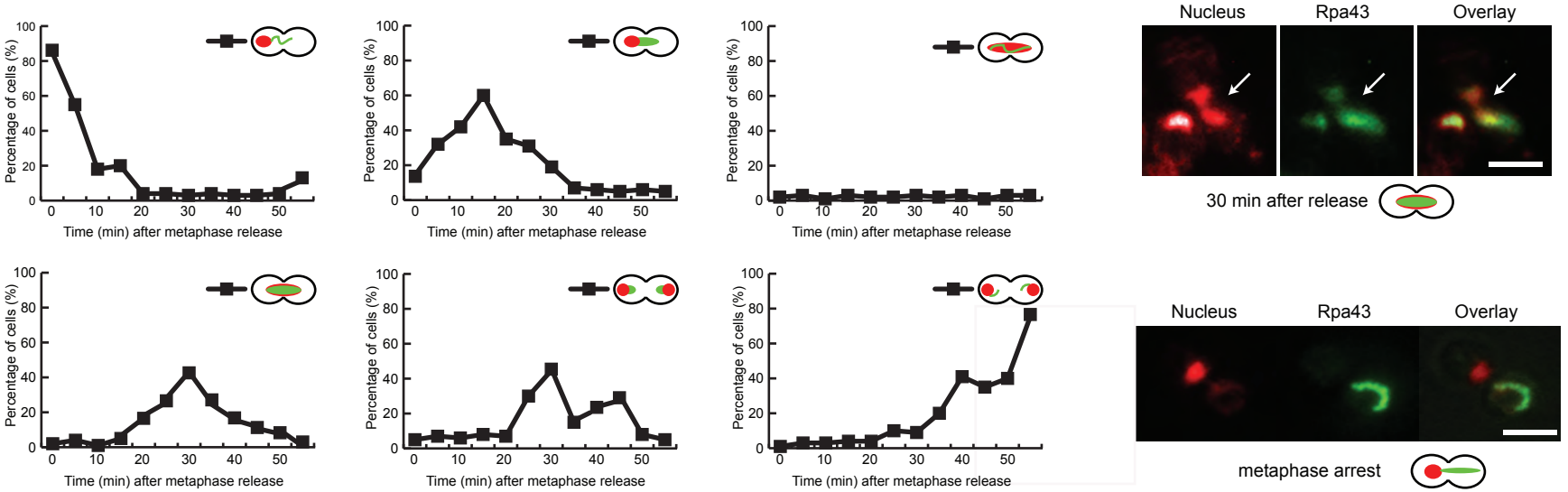
Figure 10. *cdc14-1* temperature sensitive mutant cells carrying a *tet* operator chromosome tag on the distal flank of the rDNA array on chromosome XII and either G*AL-NLS-RntA-MYC* in a plasmid (+RntA) or an empty plasmid (-RntA) were grown in the presence of raffinose. Cells were arrested in G1 with α-factor before being released at 37°C for 2.5 h to reach *cdc14-1* anaphase arrest. Cultures were then transferred to media containing galactose at 37°C for 4 hours. Samples were collected at the indicated times and cells were scored for separation of chromosome tags. Representative micrographs of samples collected in the time course are shown.

Nucleus is shown in red and chromosome tags in green. Scale bar 5 μm. Expression of RntA causes segregation of chromosome tags in *cdc14-1* mutant cells.

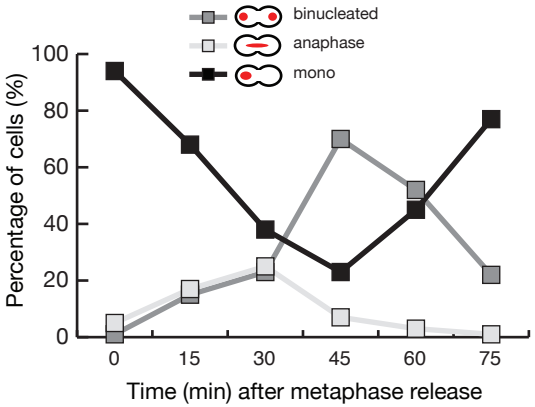
Supplementary Figure 1

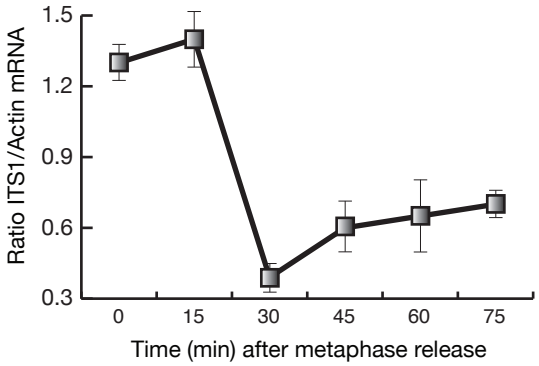


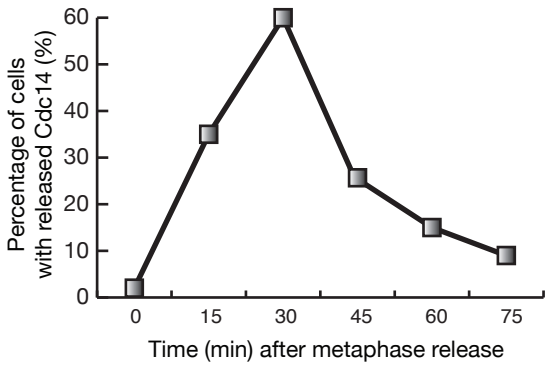
Supplementary Figure 2



Supplementary Figure 3







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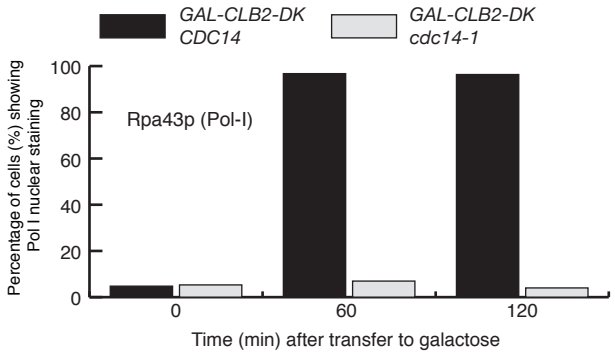

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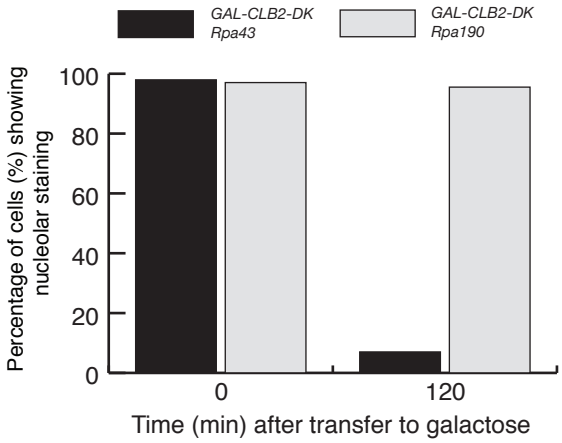

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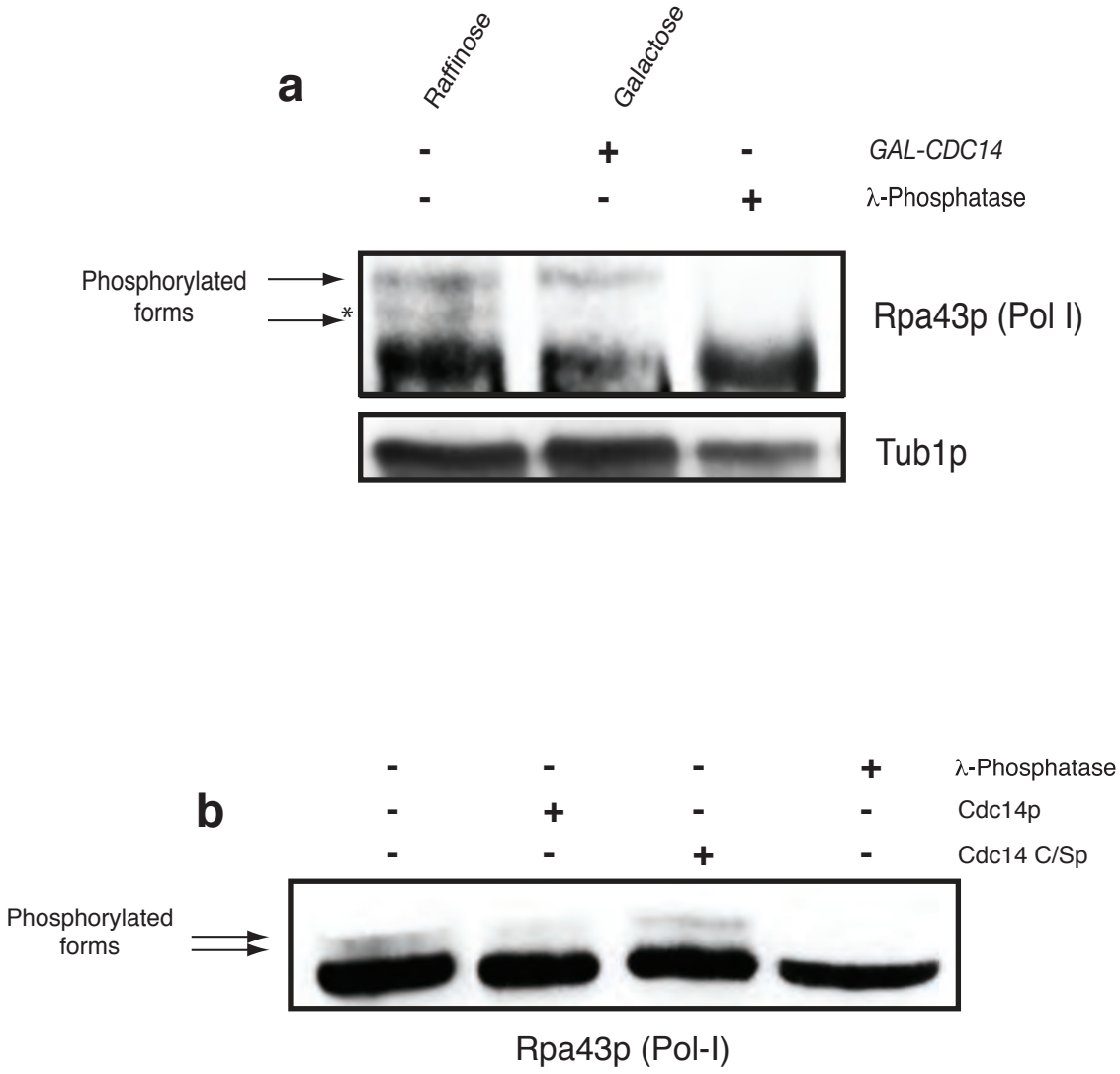

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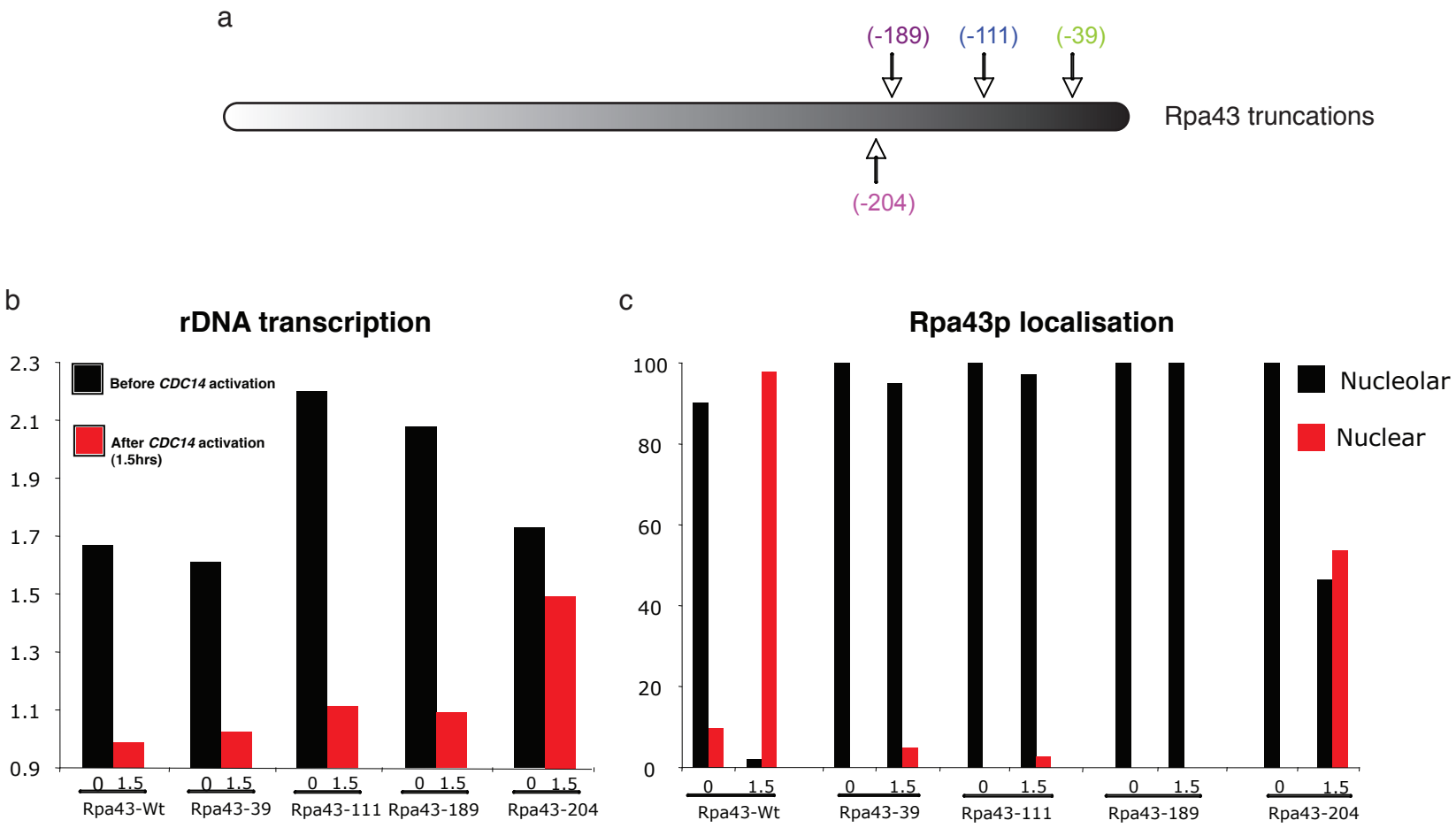

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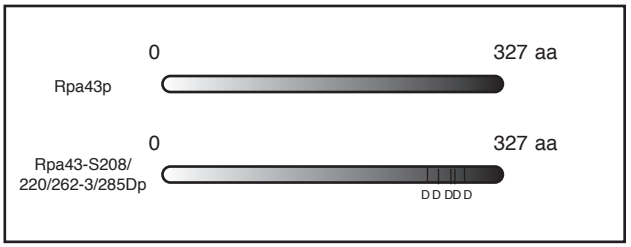

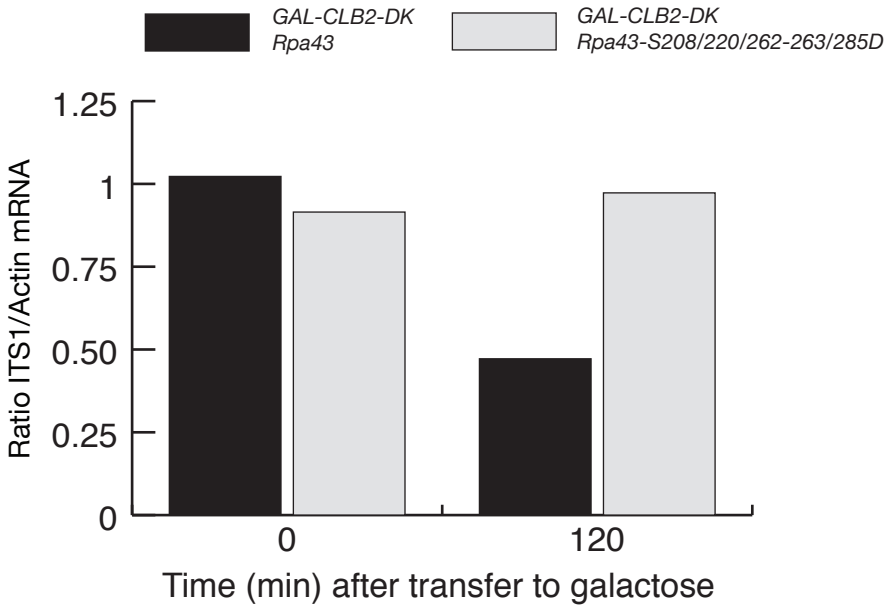

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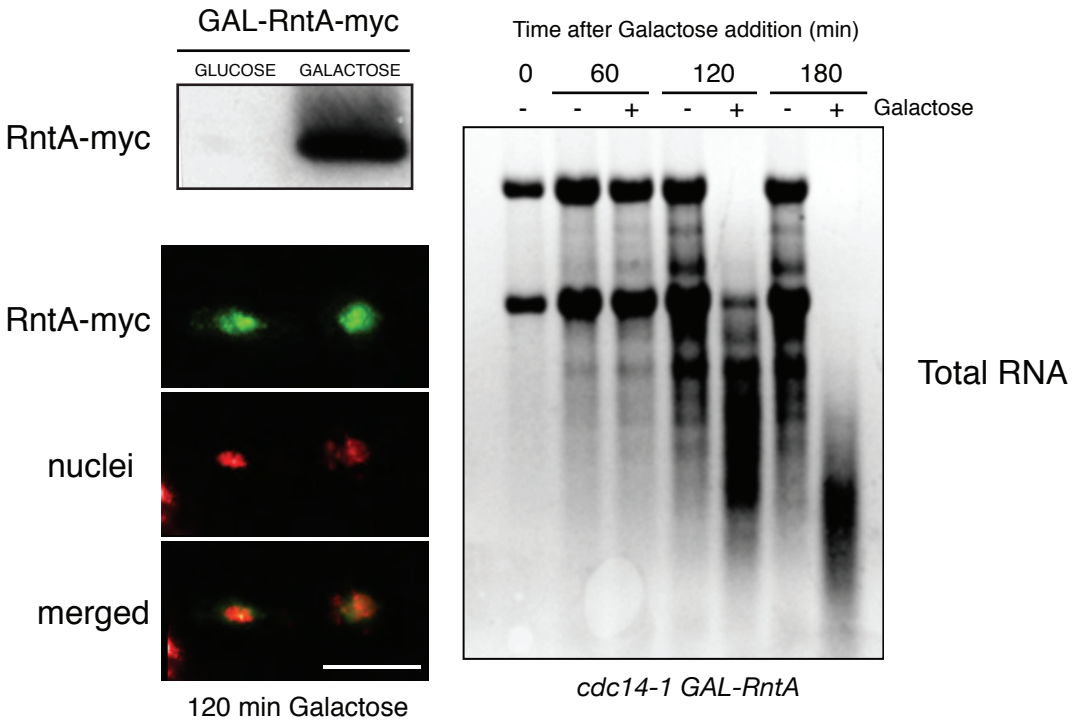

Supplementary Figure 10

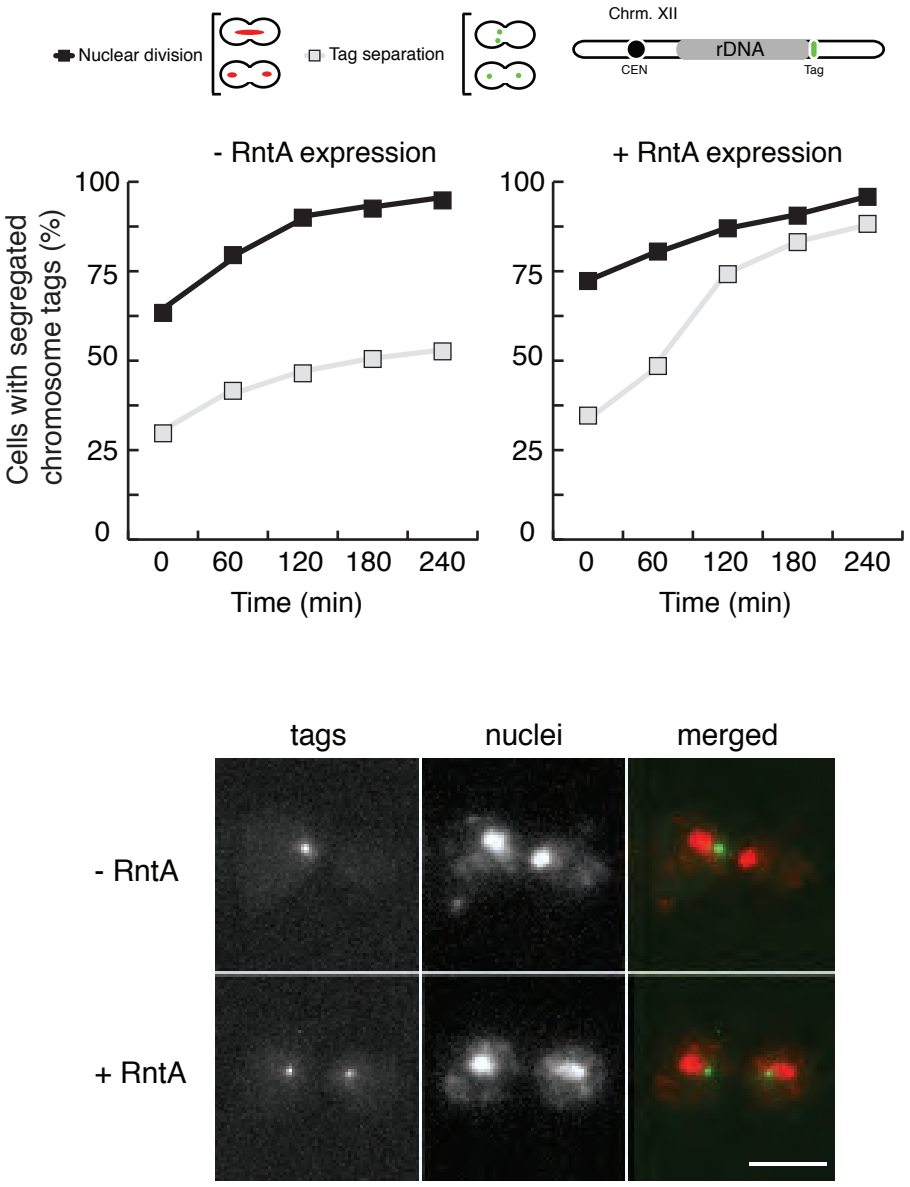

Table1. Yeast strains used in this study

Strain	Relevant genotype	Reference
CCG1412	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 bar1::natMX4*	L. Aragón
CCG1679	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 TetR-YFP ADE2 TetO(5.6Kb):487Kb ChrXII HIS3; Cdc14-1:9myc TRP1*	L. Aragón
CCG1835	*Mata bar1:hisG ura3-1 trp1-1 leu2-3,112 his3-11 ade2-1 can1-100 GAL+ cdc15-2*	L. Aragón
CCG2703	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 TetR-YFP ADE2 TetO(5.6Kb):487Kb ChrXII HIS3; Cdc14-1:9myc TRP1; URA3:GAL-CDC14*	This study
CCG3605	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 TetR-YFP ADE2 TetO(5.6Kb):487Kb ChrXII HIS3; NET1-CFP KanMX4; Cdc14-1:9myc:TRP [pGAL-TOP2 URA3]*	This study
CCG4000	*MATa bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4*	L. Aragón
CCG5473	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 RPA43-9MYC natMX4*	This study
CCG5476	*Mata bar1:hisG ura3-1 trp1-1 leu2-3,112 his3-11 ade2-1 can1-100 GAL+ cdc15-2 RPA43-9MYC natMX4*	This study
CCG5491	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 RPA43-9MYC NatMX4*	This study
CCG5500	*Mata bar1Δ leu2-3,112 ura3-52 his3-D200 trp1-D63 ade2-1 lys2-801 pep4; CDC14-GPF Kan; TUB4-CFP TRP; NET1-CFP HygB GAL-CDC20 natMX4 RPA43-9MYC HIS*	This study
CCG5549	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 TetR-YFP ADE2 TetO(5.6Kb):487Kb ChrXII HIS3; Cdc14-1:9myc TRP1 GAL-NLS-RntA-MYC URA3*	This study
CCG5570	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 bar1::natMX4 SMC4-GFP KanMX4 GAL-NLS-RntA-MYC URA3*	This study
CCG6043	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 TetR-YFP ADE2 TetO(5.6Kb):487Kb ChrXII HIS3; Cdc14-1:9myc TRP1 ura3::GAL-CDC14 URA3*	This study
CCG6046	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 TetR-YFP ADE2 TetO(5.6Kb):487Kb ChrXII HIS3; Cdc14-1:9myc TRP1 ura3::GAL-CDC14-C283S/R289A URA3*	This study
CCG6144	*MATa, ura3, trp1, leu2, his3, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1*	A. Amon
CCG6145	*MATa, ura3, trp1, leu2, his3, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1 GAL-CDC14 URA3*	A. Amon
CCG6146	*MATa , ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1, brn1-60 GAL-CDC14 URA3*	This study
CCG6147	*MATa , ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1, brn1-60*	A. Amon

		This study
CCG6462	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 RPA43-9MYC NatMX4 pMet::CDC20 TRP GAL-CDC14 URA3*	
		This study
CCG6464	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 RPA43-9MYC NatMX4 pMet::CDC20 TRP GAL-CDC14-C283S/R289A URA3*	
		This study
CCG6522	*MATa, ura3, trp1, leu2, his3, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1 GAL-NLS-RntA-MYC HIS3*	
		This study
CCG6523	*MATa, ura3, trp1, leu2, his3, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1 GAL-NLS-Top2Chlv TRP*	
		This study
CCG6525	*MATa , ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, pURA3::tetR::GFP::LEU2, RDN1::tetOx112::487Kb URA3, mcd1-1, brn1-60 GAL-NLS-RntA-MYC HIS3*	
		This study
CCG6578	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 RPA43-9MYC NatMX4 GAL-CLB2-DK URA3*	
		This study
CCG6579	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 RPA43-9MYC natMX4 GAL-CLB2-DK URA3*	
		This study
CCG6636	*Mata leu2 ura3 his3 trp1 ade2 RPA43-6HA natMX4 Ne1-GFP::Leu2 GAL-CLB2-DK URA3*	
		This study
CCG6638	*Mata cdc14-1 leu2 ura3 his3 trp1 ade2 RPA43-6HA natMX4 Ne1-GFP::Leu2 GAL-CLB2-DK URA3*	
		This study
CCG6680	*Mata bar1Δ leu2-3,112 ura3-52 his3-Δ200 trp1-Δ63 ade2-1 lys2-801 pep4 RPA43□C-6Ha NatMX4 GAL-CLB2-DK URA3*	
		This study
CCG6981	*Mata rpa43::LEU2 ade2-101 ura3-52 lys2-801 trp1-D63 his3-D200 leu2-D1 pRS314-rpa43-S208/220/262/263/285D (TRP1) GalCLB2::URA3*	