

SUPPLEMENTARY MATERIALS

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Condensin is required for condensation of rDNA array during starvation

(A) Loss of condensin blocks rDNA condensation during starvation. Early log phase WT, *brn1-9*, *ycs4-2* and *ycs5-1* cells in synthetic complete (SC) medium (+N) were shifted from 23 °C to 37 °C or remained at 23 °C for 2 hrs, and then changed to the nutrient starvation (-N) medium or fresh SC medium (+N) and incubated for 30 min. rDNA structure was analyzed by FISH with a 25S rDNA probe (Red). The boxed cell is enlarged on the right. Nuclear DNA was stained with DAPI (Blue).

(B) Quantification of cells with different rDNA morphologies from the Fig. 1A experiment. Open rDNA structure (O, restriction ratio ≥ 0.3); condensed rDNA (C, restriction ratio ≤ 0.15). N = 100.

(C) Condensin is required for rDNA condensation during rapamycin treatment. Early log phase cultures of WT, *brn1-9*, *ycs4-2* and *ycs5-1* cells in YPD medium were shifted from 23 °C to 37 °C or remained at 23 °C for 2 hrs, which was followed by incubation without or with 200 nM rapamycin for 30 min. rDNA and nuclei was analyzed by FISH (Red) and DAPI (Blue), respectively.

(D) Quantification of cells in the Fig. 1C experiment (N = 100).

(E) Condensin is required for rapamycin-induced nucleolar contraction. WT and condensin mutants grown at 23 or 37°C were treated as described in Fig 1C. Nucleolar structure was analyzed by IF with a Nop1 antibody (Red). Nuclear DNA was stained with DAPI (Blue).

(F) Condensin is required for maintaining the condensed nucleolar structure in the presence of rapamycin. Early log phase WT and *ycs4-2* cells were treated with 200 nM rapamycin for 1 hr at 23°C to induce nucleolar contraction, and then shifted to 37°C. Cells were withdrawn at different times and analyzed for nucleolar structure.

Supplementary Figure 2 Rapamycin causes rapid relocation of condensin into the nucleolus and loading of condensin to rDNA array

(A) Rapamycin causes Ycs4 to rapidly relocate into the nucleolus. Early log phase cultures of untagged (BLY03) and Ycs4-Myc12 chromosomally tagged (ZW206) cells in YPD were treated with or without 200 nM rapamycin for 1 hr at 23°C. Nucleolar structure and distribution of Ycs4-Myc12 were examined by IF with a monoclonal Nop1 antibody (Red) and the A14 rabbit polyclonal anti-Myc antibody (Green), respectively. The nuclei were stained with DAPI (Blue).

(B) Rapamycin does not affect the level of Ycs4-Myc12. The samples in Fig 2A is analyzed for Ycs4-Myc12 level by Western blot with a Myc antibody. Tubulin is used as a loading control.

(C) Rapamycin causes rapid relocation of Smc2/4 into the nucleolus. Early log phase cells expressing Smc2-HA6 or Smc4-HA6 in YPD were treated with or without 200 nM rapamycin for 1 hr. Nucleolar structure was examined by IF staining with a monoclonal Nop1 antibody (Red). The distribution of Smc2/4-HA6 was determined by IF with a rabbit polyclonal anti-HA antibody (Green). The yeast nuclei were visualized by DAPI staining (Blue).

(D) The structural organization of a yeast genomic rDNA unit and the PCR primers used for the ChIP assays. NTS, non-transcribed spacer.

Supplementary Figure 3 Rpd3 is required for rapamycin-induced nucleolar contraction and condensin loading to rDNA

(A) Rpd3 is required for rapamycin induction of condensin relocation to the nucleolus and nucleolar contraction. WT and *rpd3Δ* cells expressing Ycs4-Myc9 were treated with 200 nM rapamycin for 1 hr. Nucleolar structure and Ycs4-Myc9 localization were analyzed by indirect immunofluorescence (IF) staining with Nop1 (Red) and Myc antibodies (green), respectively. Nuclear DNA was stained with DAPI (Blue).

(B) Quantification of the ChIP results from Figure 3B.

(C) The *rpd3Δ* mutation does not affect Ycs4-Myc9 protein level.

Supplementary Figure 4 Histone deacetylation at H4 K5,12 is necessary and sufficient to cause condensin enrichment to the nucleolus and rDNA array

(A) Deacetylation at H4 K5,12 is necessary and sufficient to relocalize Ycs4-Myc9 to the nucleolus. WT, H4 K5,12G and H4 K5,12R strains carrying Ycs4-Myc9 are treated without or with rapamycin for 1 hr, and analyzed for Ycs4-Myc9 localization by IF with a Myc antibody. The nucleolus and nucleus are marked by IF with a Nop1 antibody and DAPI staining, respectively.

(B) Quantification of the ChIP results from Figure 4B.

(C) The histone H4 mutations do not affect Ycs4-Myc9 protein level.

Supplementary Figure 5 Rapamycin treatment in the absence of condensin causes the fragmented nucleolus phenotype as a result of ERC formation

(A) Rapamycin treatment of condensin mutant causes fragmented nucleolus phenotype. Early log phase WT and *ycs4-2* cells in YPD were shifted from 23 °C to 37 °C for 2 hrs, followed by incubation with 200 nM rapamycin (+Rap) or a drug carrier (-Rap) for 0, 1, 3, and 6 hrs. Nucleolar structure was visualized by IF with a Nop1 antibody (red). Shown are merged images with DAPI staining. Boxed cells were enlarged and shown below for better visualization.

(B) *top1Δtop2-4* cells show similar fragmented nucleolus phenotype and distribution of rDNA throughout the entire nucleus. *top1Δtop2-4* cells cultured at 23°C were analyzed for nucleolar and rDNA structures by IF and FISH, respectively. Shown are merged images with DAPI staining.

(C) Rapamycin treatment in the absence of condensin causes rDNA to distribute throughout the entire nucleus. Early log phase *ycs4-2* cells in YPD were shifted from 23°C to 37°C for 2 hrs, followed by incubation with 200 nM rapamycin (+Rap) or drug carrier (-Rap) for 6 hrs. rDNA structure was visualized by FISH with a 25S rDNA probe (red). Shown are merged images with DAPI staining. The arrow head points to a cell containing rDNA distributed throughout the entire nucleus.

Supplementary Figure 6 Condensin maintains rDNA stability during transcriptional repression

(A) *rpa190-1/3* mutations cause fragmented nucleolus phenotype. Early log phase WT and *rpa190-1/3* cells were shifted from 23 °C to 37 °C in YPD for different times. Shown are merged images of the nucleolus with the nucleus.

(B) *rpa190-1/3* mutations cause accumulation of cells with rDNA distributed throughout the entire nucleus. *rpa190-1/3* cells were incubated at 37°C for 5 hrs, and then analyzed for the nucleolar and rDNA structures by IF and FISH, respectively. Shown are typical images of cells containing fragmented nucleolus or high levels of ERCs.

(C) Rapamycin treatment suppresses the ERC formation in Pol I mutants. Early log phase WT and *rpa190-1/3* cells were shifted from 23 °C to 37 °C for 2.5 hrs to inactivated Pol I, followed by incubation without or with rapamycin for different times. rDNA was analyzed by FISH. The upper right panel shows the experimental strategy. Boxed cells were enlarged for better visualization.

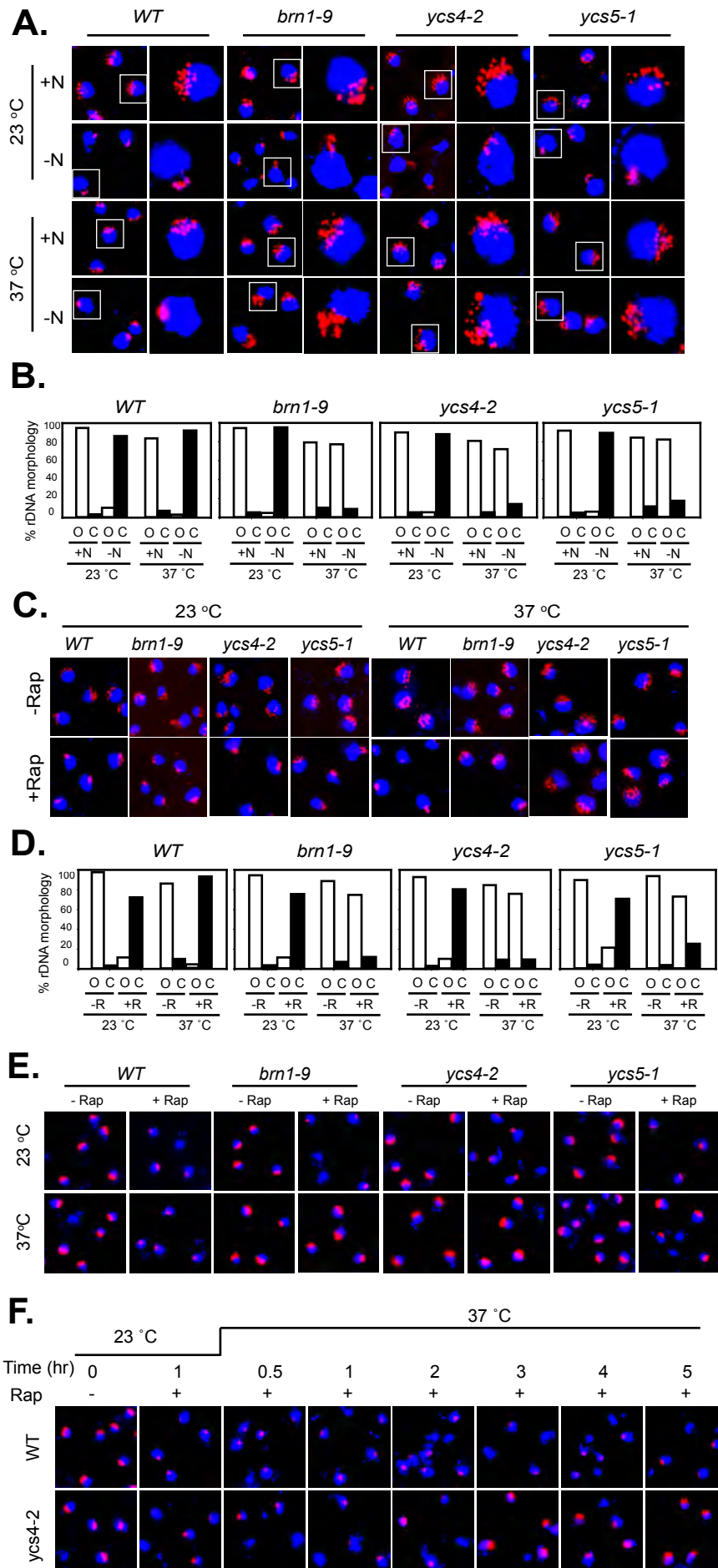
(D) Quantification of the results in Fig 7C experiment.

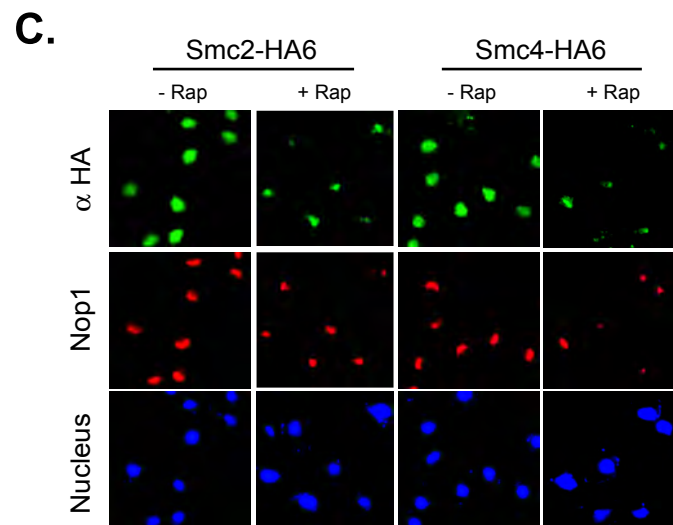
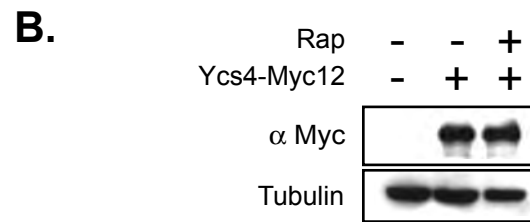
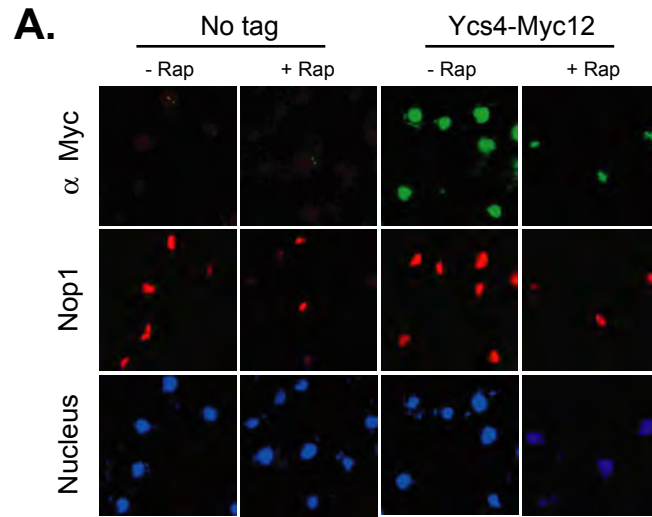
(E) Condensin is required for suppression of ERC formation in Pol I mutant in the presence of rapamycin. Single mutant (*rpa190-1 YCS4*) and double mutant (*rpa190-1 ycs4-2*) were shifted from 23 °C to 37 °C for 2.5 hrs to inactivate Pol I and condensin, followed by incubation without or with rapamycin for 3 hrs. Shown are merged images of the nucleolus with the nucleus.

(F) Quantification of the results in Fig 7E experiment.

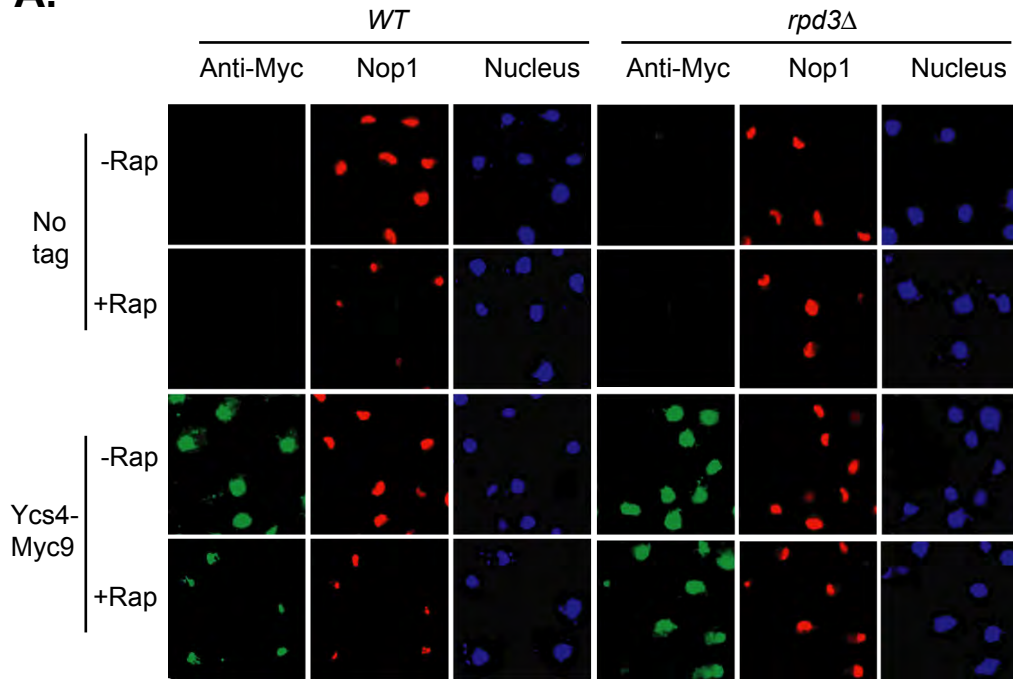
Supplementary Table 1: Yeast strains

Strain	Genotype	Source
BLY03	<i>MATa bar1 ura3 leu2 trp1 ade2 his3Δ200 gall can1 YCG1:KAN</i>	Brigitte Lavoie
BLY04	<i>MATa bar1 ura3 leu2 trp1 ade2 his3Δ200 gall can1 ycg1-1-HA3:his5+</i>	Brigitte Lavoie
FM392	<i>MATα hisΔ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Research Genetics
MYA-1404	<i>MATa ura3-52 leu2 trp1-63 brn1-9::TRP1</i>	ATCC
NBY514	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3 top1Δ::KAN top2-4</i>	Andrew Murray
NOY259	<i>MATα rpa190-1 trp1-Δ1 his4-Δ401 leu2-3,112 ura3-52 can1</i>	Masayasu Nomura
NOY260	<i>MATα RPA190 trp1-Δ1 his4-Δ401 leu2-3,112 ura3-52 can1</i>	Masayasu Nomura
NOY265	<i>MATα rpa190-3 trp1-Δ1 his4-Δ401 leu2-3,112 ura3-52 can1</i>	Masayasu Nomura
SZY1033	<i>MATα hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 rpd3Δ::KanMX</i>	Research Genetics
SZY1593	<i>MATα ura3-52 ade2-101 lys2-801 YCS4-MYC9::URA3</i>	This study
SZY1605	<i>MATα hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 rpd3Δ::KanMX YCS4-MYC9::URA3</i>	This study
SZY1610	<i>MATα ura3-52 lys2-Δ201 leu2-3 112 YCS4-MYC9::URA3 (hht1 hhf1)Δ (hht2 hhf2) pMS386[LEU2 HHT1 hhf1-15]</i>	This study
SZY1614	<i>MATa ade2-101 his3-Δ201 lys2-801 trp1-Δ901 ura3-52 YCS4-MYC9::URA3 (hht1 hhf1)::LEU2 (hht2 hhf2)::HIS3plus pK512G (CENT4 ARS1 TRP1 hht2-Δ430, hhf2-K5, 12G)</i>	This study
SZY1617	<i>MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 SMC2-HA6::HIS3</i>	This study
SZY1618	<i>MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 SMC4-HA6::HIS3</i>	This study
SZY1643	<i>MATα ade5 his7-2 leu2-3 leu2-112 trp1-289amber ura3-52 syc1-8 YCS4-MYC9::URA3</i>	This study
SZY1780	<i>MATa trp1-Δ1 leu2 ura3-52 trp1 rpa190-1 HIS3:yca4-2-MYC12</i>	This study
YCC95	<i>MATα ade5 his7-2 leu2-3 leu2-112 trp1-289amber ura3-52 syc1-8</i>	John Carbon
ZW206	<i>MATa bar1 ura3-52 leu2 his3Δ-200 trp1 ade2-101 HIS3:yca4-2-MYC12</i>	Brigitte Lavoie

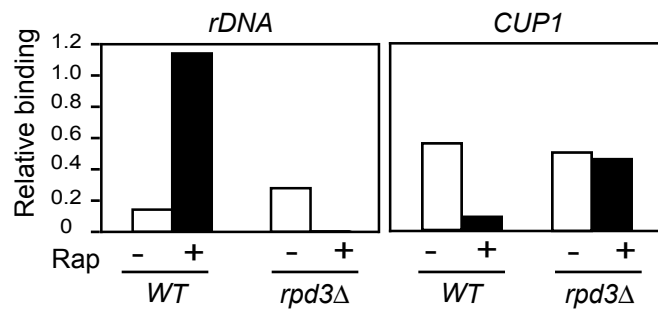




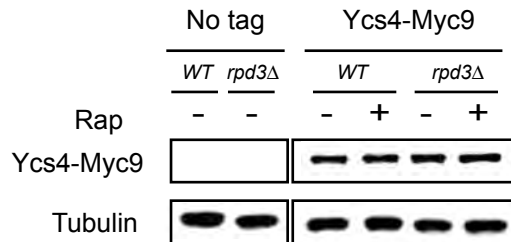
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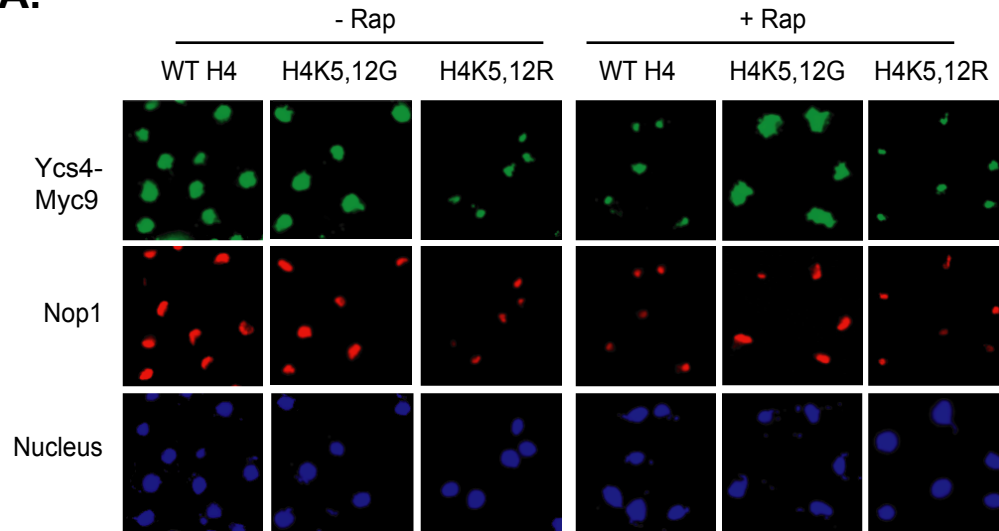
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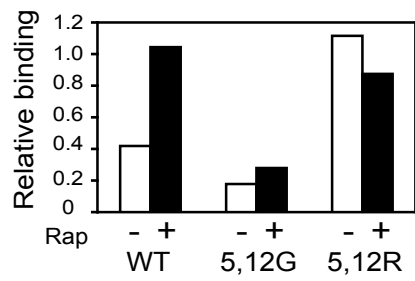
C.



A.



B.



C.

