

## Supplementary Figure Legends

**Supplementary Figure 1.** The loss of Gas1 enhances rDNA silencing and rDNA stability in the presence of Sir2. (A) The absence of Gas1 increases rDNA silencing. Silencing within rDNA was assessed by monitoring the growth of cells (10-fold serial dilutions) plated on SC medium without uracil. SC medium was used as a plating control. (B) The loss of Gas1 promotes transcriptional silencing of the *mURA3* reporter gene at the rDNA locus in a Sir2-dependent manner. Total RNA was extracted from wild-type (WT), *gas1Δ*, *sir2Δ*, and *gas1Δ sir2Δ* cells. Quantitative real-time reverse transcription-PCR analysis was performed to measure the transcript levels of the *mURA3* reporter gene inserted inside (*RDNI-NTS1::mURA3*) or outside the rDNA array (*leu2::mURA3*). Amplification efficiencies were validated and normalized against *ACT1*. Relative *mURA3* transcript levels were calculated as the ratio of the normalized transcript level of the *mURA3* reporter gene inside the NTS1 region to that outside the rDNA array. The values were the mean of three independent experiments and error bar indicates standard deviations. Asterisks indicate  $P < 0.05$ , compared with WT cells (two-tailed Student's *t*-test). (C) The loss of Gas1 represses rDNA recombination in a Sir2-dependent manner. rDNA recombination is represented by the rate of loss of the *ADE2* marker gene integrated at the rDNA locus in WT, *gas1Δ*, *sir2Δ*, and *gas1Δ sir2Δ* cells. Values represent the average of three independent experiments, and error bars indicate the standard deviation. Mean values of the recombination rates for WT, *gas1Δ*, *sir2Δ*, and *gas1Δ sir2Δ* cells are  $1.53 \times 10^{-3}$ ,  $0.57 \times 10^{-3}$ ,  $4.68 \times 10^{-3}$ , and  $4.84 \times 10^{-3}$ , respectively. Asterisks indicate  $P < 0.05$ , compared with WT cells (two-tailed Student's *t*-test).

**Supplementary Figure S2.** The protein level of Sir2 is not changed in the absence of Gas1 or Msn2/4. Total protein was extracted from wild-type (WT), *gas1Δ*, *msn2Δ msn4Δ*, and *gas1Δ msn2Δ msn4Δ* cells, and immunoblotting was performed using an HRP-conjugated anti-mouse IgG antibody for the detection of TAP-tagged protein. Actin was used as a loading control. The relative ratio of Sir2 to actin, normalized against that of WT cells, is shown below each lane. Data are representative of at least three independent experiments.

**Supplementary Figure S3.** The protein level of Gas1 is not changed in the lack of Gas1 β-1,3-glucanosyltransferase activity. Total protein was extracted from WT and *gas1Δ* cells containing an empty vector and *gas1Δ* cells expressing WT *GAS1* and *gas1*<sup>E161Q, E262Q</sup> on the

pRS413 vector, and immunoblotting was performed using a mouse anti-GFP antibody for the detection of GFP-tagged protein. Hexokinase was used as a loading control. The relative ratio of Gas1 to hexokinase, normalized against that of WT cells, is shown below each lane. Data are representative of at least three independent experiments.

**Supplementary Figure S4.** Congo red treatment promotes Sir2-mediated rDNA silencing.

(A) The association of Msn2/4 with the *PNC1* promoter region is enhanced under Congo red treatment. The degree of association of Msn2-TAP (left panel) and Msn4-TAP (right panel) with the *PNC1* promoter region was measured using a ChIP assay with or without treatment with 100  $\mu\text{g/ml}$  Congo red for 1 h. Cells without Congo red treatment were used as a control. Values represent the average of three independent experiments, and error bars indicate the standard deviation. Asterisks indicate  $P < 0.05$ , compared with untreated control cells (two-tailed Student's *t*-test). (B) The protein level of Pnc1 increases under Congo red treatment. Total protein was extracted from cells with or without treatment with 100  $\mu\text{g/ml}$  Congo red for 1 h, and immunoblotting was performed using an HRP-conjugated anti-mouse IgG antibody for the detection of TAP-tagged protein. Actin was used as a loading control. The relative ratio of Pnc1 to actin, normalized against that of untreated control cells, is shown below each lane. Data are representative of at least three independent experiments. (C) The association of Sir2 with rDNA is enhanced under Congo red treatment. The degree of Sir2 binding to four representative regions in the rDNA locus (25S, NTS1, NTS2/18S, and 18S regions) was measured using a ChIP assay with or without treatment with 100  $\mu\text{g/ml}$  Congo red for 1 h. Values represent the average of three independent experiments, and error bars indicate the standard deviation. Asterisks indicate  $P < 0.05$ , compared with untreated control cells (two-tailed Student's *t*-test). (D) Congo red increases rDNA silencing in the presence of Sir2. Silencing within the rDNA region was assessed by monitoring the growth of cells (10-fold serial dilutions) plated on SC medium without uracil in the presence or absence of 100  $\mu\text{g/ml}$  Congo red. SC medium was used as a plating control. (E) Congo red promotes transcriptional silencing of the *mURA3* reporter gene at the rDNA locus in a Sir2-dependent manner. Total RNA was extracted from WT and *sir2* $\Delta$  cells with or without treatment with 100  $\mu\text{g/ml}$  Congo red for 1 h. Quantitative real-time reverse transcription-PCR analysis was performed as in Supplementary Figure 1B. The values were the mean of three independent experiments and error bar indicates standard deviations. Asterisks indicate  $P < 0.05$ , compared with untreated WT cells (two-tailed Student's *t*-test). (F) Congo red suppresses rDNA

recombination in a Sir2-dependent manner. rDNA recombination is represented by the rate of loss of the *ADE2* marker gene integrated at the rDNA locus in WT and *sir2Δ* cells plated on SC medium with or without 100 μg/ml Congo red. Values represent the average of three independent experiments, and error bars indicate the standard deviation. Mean values of the recombination rates for WT (control), WT (Congo red), *sir2Δ* (control), and *sir2Δ* (Congo red) cells are  $1.45 \times 10^{-3}$ ,  $0.64 \times 10^{-3}$ ,  $4.68 \times 10^{-3}$ , and  $4.21 \times 10^{-3}$ , respectively. Asterisks indicate  $P < 0.05$ , compared with untreated WT cells (two-tailed Student's *t*-test).

**Supplementary Figure S5.** Cell wall stress agents, such as calcofluor white, SDS, vanadate, and caffeine, do not affect rDNA silencing. (A) Calcofluor white (CFW) does not promote rDNA silencing. Silencing within the rDNA region was assessed by monitoring the growth of cells (10-fold serial dilutions) plated on SC medium without uracil in the presence or absence of 50 μg/ml CFW. SC medium was used as a plating control. (B) CFW does not promote transcriptional silencing of the *mURA3* reporter gene at the rDNA locus. Total RNA was extracted from wild-type and *sir2Δ* cells with or without treatment with 50 μg/ml CFW for 1 h. Quantitative real-time reverse transcription-PCR analysis was performed to measure the transcript levels of the *mURA3* reporter gene inserted inside (*RDNI-NTS1::mURA3*) or outside the rDNA array (*leu2::mURA3*). Values represent the average of three independent experiments, and error bars indicate the standard deviation. (C) CFW does not promote rDNA stability. rDNA recombination is represented by the rate of loss of the *ADE2* marker gene integrated at the rDNA locus in wild-type and *sir2Δ* cells plated on SC medium with or without 50 μg/ml CFW. Values represent the average of three independent experiments, and error bars indicate the standard deviation. Mean values of the recombination rates for WT (control), WT (CFW), *sir2Δ* (control), and *sir2Δ* (CFW) cells are  $1.53 \times 10^{-3}$ ,  $1.39 \times 10^{-3}$ ,  $4.68 \times 10^{-3}$ , and  $4.62 \times 10^{-3}$ , respectively. (D) SDS, vanadate, and caffeine do not promote transcriptional silencing of the *mURA3* reporter gene at the rDNA locus. Total RNA was extracted from cells with or without treatment with 0.01% SDS, 5 mM vanadate, or 12 mM caffeine for 1 h. Quantitative real-time reverse transcription-PCR analysis was performed as described above. Values represent the average of three independent experiments, and error bars indicate the standard deviation.

**Supplementary Figure S6.** Gas1 paralogs are not involved in rDNA silencing. (A) The absence of Gas1 paralogs does not increase rDNA silencing. The spot assay was performed in

wild-type (WT), *gas1Δ*, *gas2Δ*, *gas3Δ*, *gas4Δ*, *gas5Δ*, and *bgl2Δ* cells. Silencing within rDNA was assessed by monitoring the growth of cells (10-fold serial dilutions) plated on SC medium without uracil. SC medium was used as a plating control. (B) The absence of Gas1 paralogs does not significantly contribute to rDNA stability. rDNA recombination is represented by the rate of loss of the *ADE2* marker gene integrated at the rDNA locus in WT, *gas1Δ*, *gas2Δ*, *gas3Δ*, *gas4Δ*, *gas5Δ*, and *bgl2Δ* cells. Values represent the average of three independent experiments, and error bars indicate the standard deviation. Mean values of the recombination rates for WT, *gas1Δ*, *gas2Δ*, *gas3Δ*, *gas4Δ*, *gas5Δ*, and *bgl2Δ* cells are  $1.48 \times 10^{-3}$ ,  $0.40 \times 10^{-3}$ ,  $2.07 \times 10^{-3}$ ,  $1.28 \times 10^{-3}$ ,  $1.01 \times 10^{-3}$ ,  $1.05 \times 10^{-3}$ , and  $1.35 \times 10^{-3}$ , respectively. Asterisks indicate  $P < 0.05$ , compared with WT cells (two-tailed Student's *t*-test).

**Supplementary Figure S7.** The lack of Gas1  $\beta$ -1,3-glucanosyltransferase activity and the treatment of Congo red decrease the *in vivo* activity of PKA. (A) The absence of Gas1  $\beta$ -1,3-glucanosyltransferase activity decreases the *in vivo* activity of PKA. Total protein was extracted from WT and *gas1Δ* cells harboring pRS423-pr<sup>CUP</sup>-6×MYC-*cki1*<sup>2-200(S125/130A)</sup> and containing an empty, WT *GAS1* and *gas1*<sup>E161Q, E262Q</sup> on the pRS415 vector. Immunoblotting was performed using a mouse anti-Myc antibody. The relative ratio of phosphorylated (Cki1-P) to non-phosphorylated (Cki1) forms of Cki1, normalized against that of WT cells, is shown below each lane. Data are representative of at least three independent experiments. (B) The Congo red treatment decreases the *in vivo* activity of PKA. Total protein was extracted from the cells harboring pRS423-pr<sup>CUP</sup>-6×MYC-*cki1*<sup>2-200(S125/130A)</sup>, and immunoblotting was performed using a mouse anti-Myc antibody. The relative ratio of phosphorylated (Cki1-P) to non-phosphorylated (Cki1) forms of Cki1, normalized against that of untreated cells, is shown below each lane. Data are representative of at least three independent experiments.

**Supplementary Figure S8.** The lack of PKA-dependent phosphorylation of Msn2 abolishes the effect of *gas1Δ* on rDNA silencing in *gas1Δ* cells. Silencing within the rDNA region was assessed by monitoring the growth of cells (10-fold serial dilutions) plated on SC medium without uracil. SC medium was used as a plating control. The spot assay was performed with WT and *gas1Δmsn2Δ* cells containing an empty vector and *gas1Δmsn2Δ* cells expressing WT *MSN2* and *msn2*<sup>S582D, S620D, S625D, S633D</sup>.

## Supplementary Data

**Supplementary Table S1.** Yeast strains used in this study

Strain	Genotype	Source
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Open Biosystems
DMY2798	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3</i>	[6]
DMY2804	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3</i>	[6]
HY0245	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 sir2Δ::TRP1</i>	This study
HY0291	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 sir2Δ::TRP1</i>	This study
HY1164	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1</i>	This study
HY1165	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1</i>	This study
HY1167	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 sir2Δ::HIS3</i>	This study
HY1168	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1 sir2Δ::HIS3</i>	This study
DMY3010	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2</i>	[6]
HY1185	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas1Δ::TRP1</i>	This study
HY0236	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 sir2Δ::TRP1</i>	This study
HY1448	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas1Δ::LEU2 sir2Δ::TRP1</i>	This study
HY1170	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6</i>	This study
HY1171	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6 gas1Δ::LEU2</i>	This study

HY1172	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-TAP-HIS3MX6</i>	This study
HY1173	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-TAP-HIS3MX6 gas1Δ::URA3</i>	This study
HY1174	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-GFP-HIS3MX6</i>	This study
HY1175	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-GFP-HIS3MX6 gas1Δ::URA3</i>	This study
HY1176	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ PNC1-GFP-HIS3MX6 msn2Δ::KanMX4 msn4Δ::LEU2</i>	This study
HY1177	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ PNC1-GFP-HIS3MX6 msn2Δ::KanMX4 msn4Δ::LEU2 gas1Δ::URA3</i>	This study
HY1178	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6</i>	This study
HY1179	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3</i>	This study
HY1180	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ SIR2-TAP-HIS3MX6 msn2Δ::KanMX4 msn4Δ::LEU2</i>	This study
HY1181	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ SIR2-TAP-HIS3MX6 msn2Δ::KanMX4 msn4Δ::LEU2 gas1Δ::URA3</i>	This study
HY1186	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 msn2Δ::URA3 msn4Δ::LEU2</i>	This study
HY1187	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 msn2Δ::URA3 msn4Δ::LEU2 gas1Δ::TRP1</i>	This study
HY1320	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6 [pRS415]</i>	This study
HY1323	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6 gas1Δ::URA3 [pRS415]</i>	This study
HY1324	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-TAP]</i>	This study
HY1325	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1<sup>E161Q, E262Q</sup>-TAP]</i>	This study
HY1332	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-TAP-HIS3MX6 [pRS415]</i>	This study
HY1335	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415]</i>	This study
HY1336	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-GFP]</i>	This study

HY1337	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1<sup>E161Q, E262Q</sup>-GFP]</i>	This study
HY1402	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 [pRS415]</i>	This study
HY1403	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 gas1Δ::URA3 [pRS415]</i>	This study
HY1404	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-GFP]</i>	This study
HY1405	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1<sup>E161Q, E262Q</sup>-GFP]</i>	This study
HY1338	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 [pRS415]</i>	This study
HY1341	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415]</i>	This study
HY1342	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-GFP]</i>	This study
HY1343	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1<sup>E161Q, E262Q</sup>-GFP]</i>	This study
HY1350	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 [pRS413]</i>	This study
HY1353	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 [pRS413]</i>	This study
HY1356	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 [pRS413]</i>	This study
HY1357	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 [pRS413 GAS1-GFP]</i>	This study
HY1358	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 [pRS413 gas1<sup>E161Q, E262Q</sup>-GFP]</i>	This study
HY1359	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1 [pRS413]</i>	This study
HY1360	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1 [pRS413 GAS1-GFP]</i>	This study
HY1361	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1 [pRS413 gas1<sup>E161Q, E262Q</sup>-GFP]</i>	This study
HY1344	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 [pRS415]</i>	This study
HY1347	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas1Δ::URA3 [pRS415]</i>	This study

HY1348	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas1Δ::URA3 [pRS415 GAS1-GFP]</i>	This study
HY1349	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas1Δ::URA3 [pRS415 gas1<sup>E161Q, E262Q</sup>-GFP]</i>	This study
HY1202	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6</i>	This study
HY1203	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3</i>	This study
HY1398	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 [pRS415]</i>	This study
HY1399	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3 [pRS415]</i>	This study
HY1400	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-TAP]</i>	This study
HY1401	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1<sup>E161Q, E262Q</sup>-TAP]</i>	This study
HY1497	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HIS3MX6::P<sub>RPL7B</sub>-HA-BCY1</i>	This study
HY1498	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HIS3MX6::P<sub>RPL7B</sub>-HA-BCY1 gas1Δ::URA3</i>	This study
HY1499	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HIS3MX6::P<sub>RPL7B</sub>-HA-BCY1 mpk1Δ::LEU2</i>	This study
HY1500	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HIS3MX6::P<sub>RPL7B</sub>-HA-BCY1 mpk1Δ::LEU2 gas1Δ::URA3</i>	This study
HY1390	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas2Δ::TRP1</i>	This study
HY1391	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas2Δ::TRP1</i>	This study
HY1392	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas3Δ::TRP1</i>	This study
HY1393	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas3Δ::TRP1</i>	This study
HY1394	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas4Δ::TRP1</i>	This study
HY1395	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas4Δ::TRP1</i>	This study
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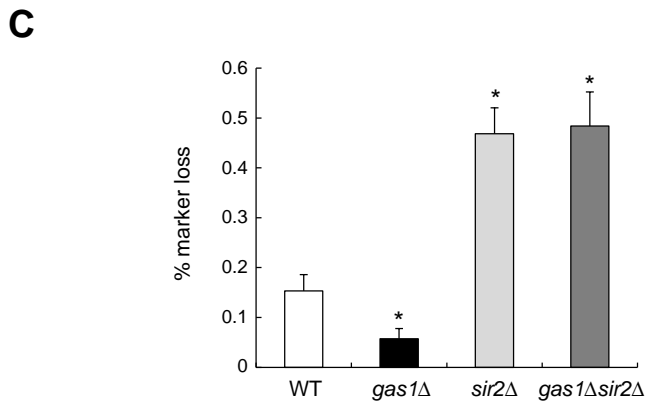
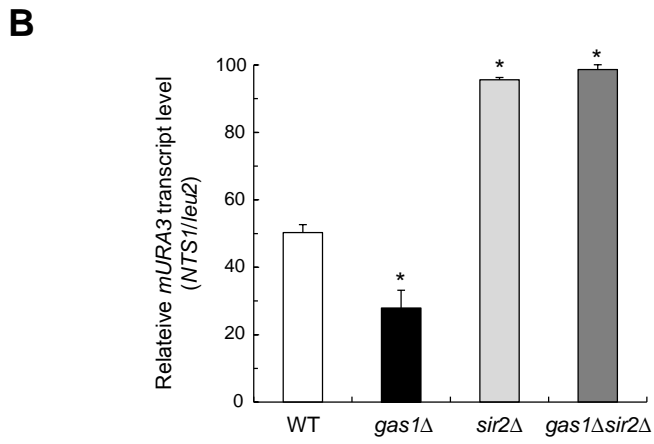
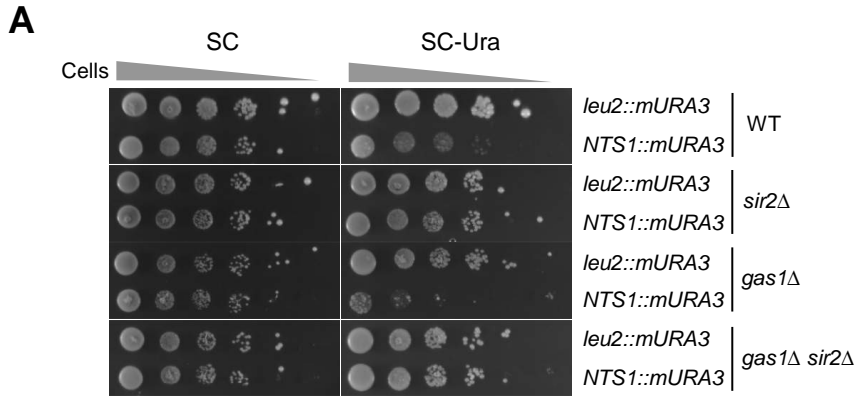
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HY1388	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 bgl2Δ::TRP1</i>	This study
HY1389	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 bgl2Δ::TRP1</i>	This study
HY1444	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas2Δ::TRP1</i>	This study
HY1445	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas3Δ::TRP1</i>	This study
HY1446	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas4Δ::TRP1</i>	This study
HY1447	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 gas5Δ::TRP1</i>	This study
HY1443	<i>MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5<sup>+</sup> with RDN1::ADE2 bgl2Δ::TRP1</i>	This study

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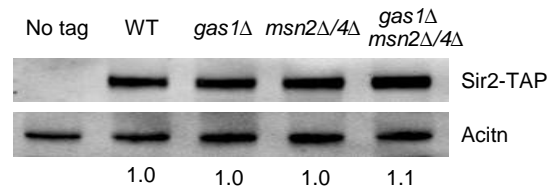
**Supplementary Table S2.** Oligonucleotide primers used for ChIP assays in this study

<b>Locus</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
rDNA-25S	CGACTAACCCACGTCCAAC	CCGAATGAACTAGCCCTGAA
rDNA-NTS1	TCCCCACTGTTCACTGTTCA	AGGGCTTTCACAAAGCTTCC
rDNA-NTS2/18S	AAGATGCCCACGATGAGACT	GGGAGGTACTTCATGCGAAA
rDNA-18S	CCAGAACGTCTAAGGGCATC	CTCACCAGGTCCAGACACAA
<i>CUP1</i>	TGAAGGTCATGAGTGCCAAT	TTCGTTTCATTTCCAGAGCA
<i>PNC1</i> promoter	GATCAAGGTGGCACACAGGG	ATACATAGTGGGCCAAACGG
<i>ACT1</i>	TGACTGACTACTTGATGAAG	ACAGAAGGATGGAACAAAGC

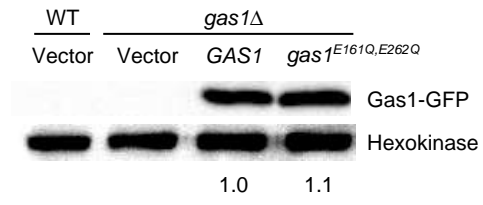
# Supplementary Figure S1



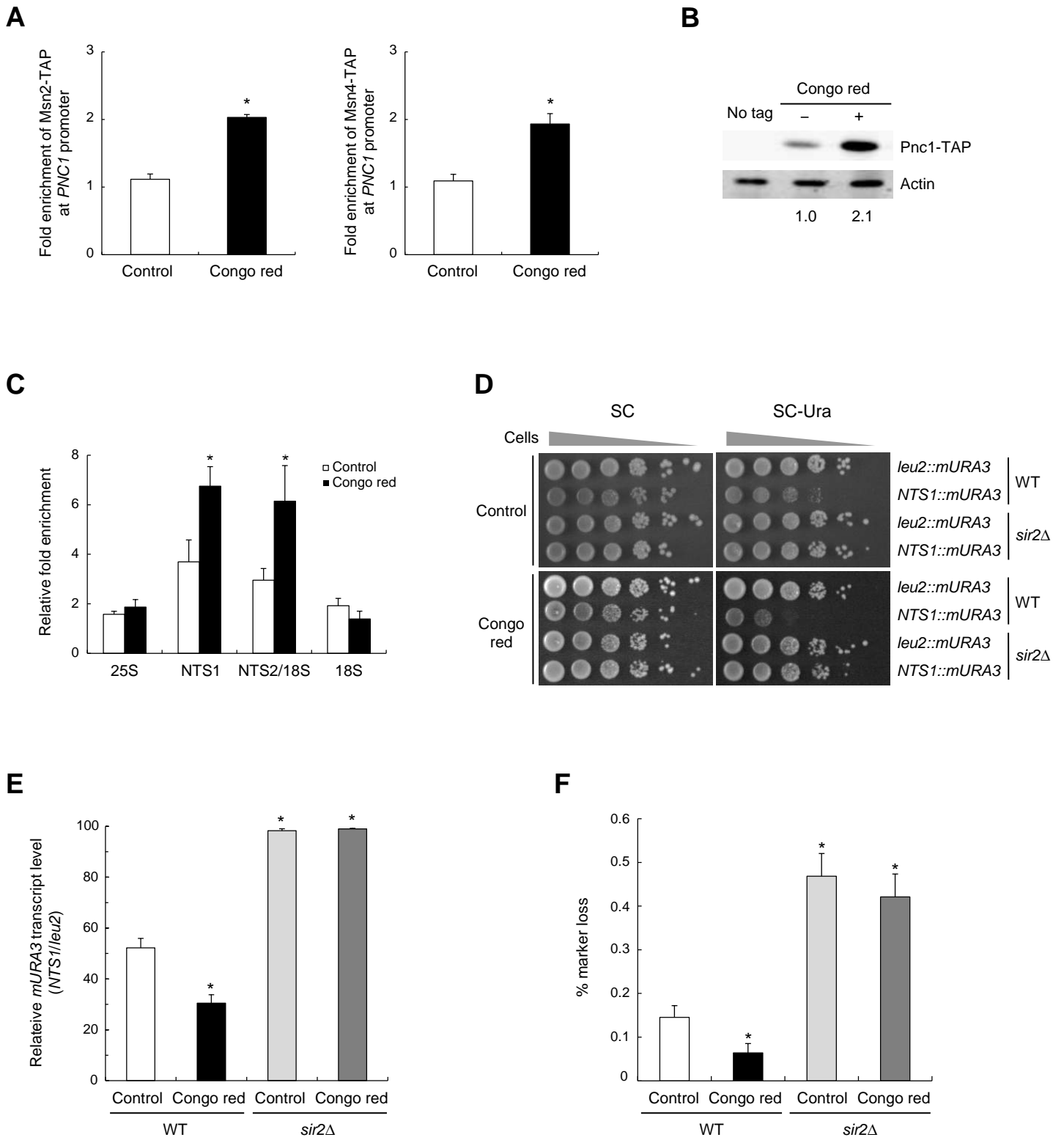
## Supplementary Figure S2



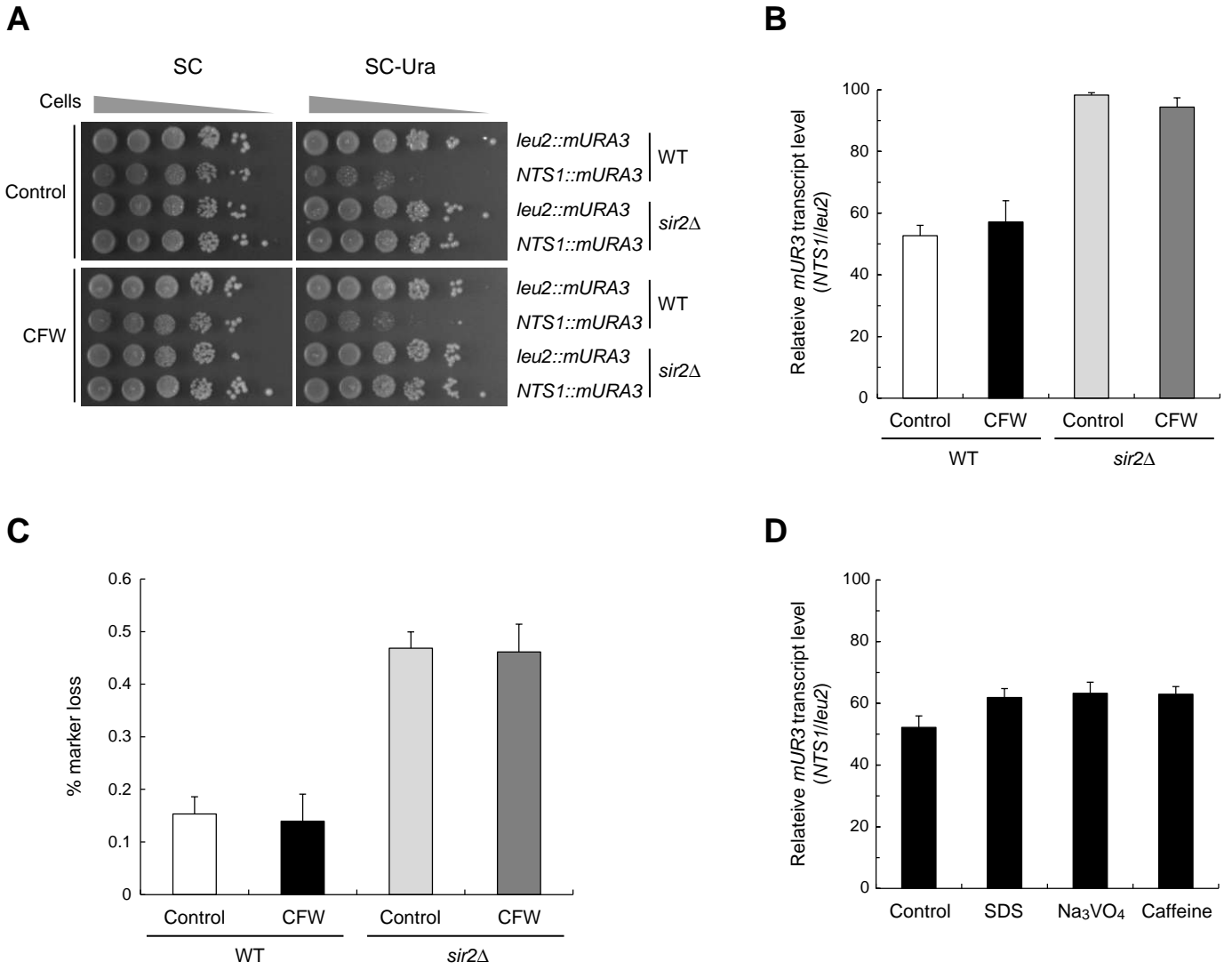
# Supplementary Figure S3



# Supplementary Figure S4

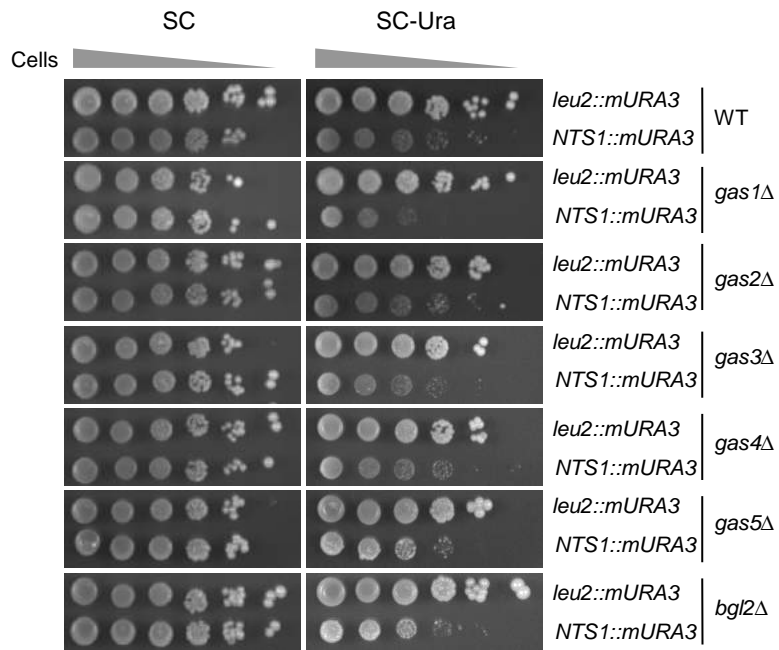


# Supplementary Figure S5

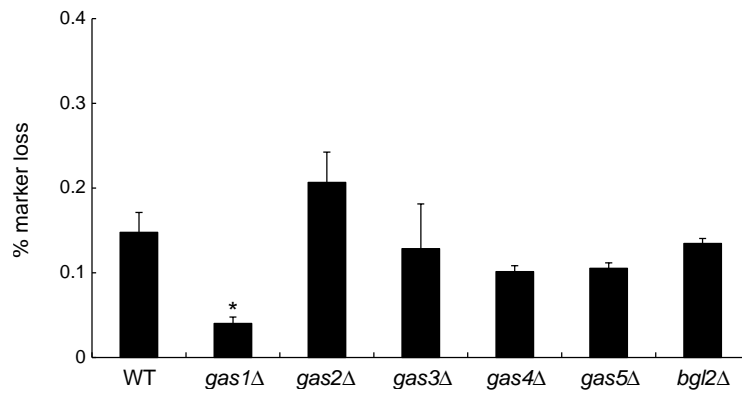


# Supplementary Figure S6

**A**



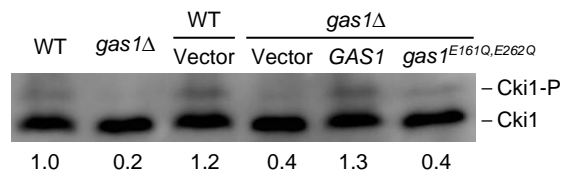
**B**



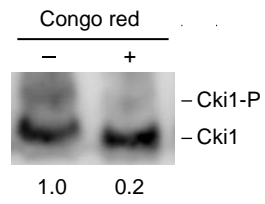


# Supplementary Figure S7

**A**



**B**



# Supplementary Figure S8

