

**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 (*RDN1-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 1.53×10$^{-3}$, 0.57×10$^{-3}$, 4.68×10$^{-3}$, and 4.84×10$^{-3}$, 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*E161Q,E262Q 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 μg/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 μg/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 μg/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 μg/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 sir2Δ cells with or without treatment with 100 μg/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 1.45×10⁻³, 0.64×10⁻³, 4.68×10⁻³, and 4.21×10⁻³, 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 1.53×10⁻³, 1.39×10⁻³, 4.68×10⁻³, and 4.62×10⁻³, 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 1.48×10^{-3}, 0.40×10^{-3}, 2.07×10^{-3}, 1.28×10^{-3}, 1.01×10^{-3}, 1.05×10^{-3}, and 1.35×10^{-3}, respectively. Asterisks indicate *P*<0.05, compared with WT cells (two-tailed Student’s *t*-test).

**Supplementary Figure S7.** The lack of Gas1 β-1,3-glucanosyltransferase activity and the treatment of Congo red decrease the *in vivo* activity of PKA. (A) The absence of Gas1 β-1,3-glucanosyltransferase activity decreases the *in vivo* activity of PKA. Total protein was extracted from WT and gas1Δ cells harboring pRS423-pr^{CUP-6×MYC-cki1^{2-200(S125/130A)}} and containing an empty, WT GAS1 and gas1^{E161Q,E262Q} 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^{CUP-6×MYC-cki1^{2-200(S125/130A)}} 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^{S582D, S620D, S625D, S633D}.
Supplementary Data

Supplementary Table S1. Yeast strains used in this study

| Strain      | Genotype                                                                 | Source                  |
|-------------|---------------------------------------------------------------------------|-------------------------|
| BY4741      | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0                                         | Open Biosystems         |
| DMY2798     | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3          | [6]                     |
| DMY2804     | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3   | [6]                     |
| HY0245      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 sir2Δ::TRP1 | This study              |
| HY0291      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 sir2Δ::TRP1 | This study              |
| HY1164      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 | This study              |
| HY1165      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1 | This study              |
| HY1167      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 sir2Δ::HIS3 | This study              |
| HY1168      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 gas1Δ::TRP1 sir2Δ::HIS3 | This study              |
| DMY3010     | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5Δ+ with RDN1::ADE2 | [6]                     |
| HY1185      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5Δ+ with RDN1::ADE2 gas1Δ::TRP1 | This study              |
| HY0236      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5Δ+ with RDN1::ADE2 sir2Δ::TRP1 | This study              |
| HY1448      | MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5Δ+ with RDN1::ADE2 gas1Δ::LEU2 sir2Δ::TRP1 | This study              |
| HY1170      | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6                      | This study              |
| HY1171      | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MSN2-GFP-HIS3MX6 gas1Δ::LEU2            | This study              |
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**HY1337**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MN2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1\textsubscript{E161Q,E262Q}-GFP]} 
*This study*

**HY1402**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 [pRS415]} 
*This study*

**HY1403**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 gas1Δ::URA3 [pRS415]} 
*This study*

**HY1404**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-GFP]} 
*This study*

**HY1405**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PNC1-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1\textsubscript{E161Q,E262Q}-GFP]} 
*This study*

**HY1338**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 [pRS415]} 
*This study*

**HY1341**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415]} 
*This study*

**HY1342**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-GFP]} 
*This study*

**HY1343**  
\texttt{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1\textsubscript{E161Q,E262Q}-GFP]} 
*This study*

**HY1350**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 [pRS413]} 
*This study*

**HY1353**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 RDNI-NTS1::mURA3 [pRS413]} 
*This study*

**HY1356**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 [pRS413]} 
*This study*

**HY1357**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 [pRS413 GAS1-GFP]} 
*This study*

**HY1358**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas1Δ::TRP1 [pRS413 gas1\textsubscript{E161Q,E262Q}-GFP]} 
*This study*

**HY1359**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 RDNI-NTS1::mURA3 gas1Δ::TRP1 [pRS413]} 
*This study*

**HY1360**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 RDNI-NTS1::mURA3 gas1Δ::TRP1 [pRS413 GAS1-GFP]} 
*This study*

**HY1361**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 RDNI-NTS1::mURA3 gas1Δ::TRP1 [pRS413 gas1\textsubscript{E161Q,E262Q}-GFP]} 
*This study*

**HY1344**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 RAD5\textsuperscript{+} with RDNI::ADE2 [pRS415]} 
*This study*

**HY1347**  
\texttt{MATa ade2-1 ura3-1 trpl-1 leu2-3,112 his3-11 can1-100 RAD5\textsuperscript{+} with RDNI::ADE2 gas1Δ::URA3 [pRS415]} 
*This study*
This study

**HY1348**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5}^+ \text{ with } \text{RDN1::ADE2 gas1Δ::URA3 [pRS415 GAS1-GFP]} \]

This study

**HY1349**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5}^+ \text{ with } \text{RDN1::ADE2 gas1Δ::URA3 [pRS415 gas}^{E66Q,E262Q}\text{-GFP]} \]

This study

**HY1202**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6} \]

This study

**HY1203**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3} \]

This study

**HY1398**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 [pRS415]} \]

This study

**HY1399**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3 [pRS415]} \]

This study

**HY1400**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3 [pRS415 GAS1-TAP]} \]

This study

**HY1401**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TPK1-GFP-HIS3MX6 gas1Δ::URA3 [pRS415 gas1}^{E66Q,E262Q}\text{-TAP]} \]

This study

**HY1497**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 HIS3MX6::P}_{\text{RPL7B}}\text{-HA-BCY1} \]

This study

**HY1498**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 HIS3MX6::P}_{\text{RPL7B}}\text{-HA-BCY1 gas1Δ::URA3} \]

This study

**HY1499**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 HIS3MX6::P}_{\text{RPL7B}}\text{-HA-BCY1 mpk1Δ::LEU2} \]

This study

**HY1500**

\[\text{MAT}^a \text{his3Δ1 leu2Δ0 met15Δ0 HIS3MX6::P}_{\text{RPL7B}}\text{-HA-BCY1 mpk1Δ::LEU2 gas1Δ::URA3} \]

This study

**HY1390**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas2Δ::TRP1} \]

This study

**HY1391**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDNI-NTSI1::mURA3 gas2Δ::TRP1} \]

This study

**HY1392**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas3Δ::TRP1} \]

This study

**HY1393**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDNI-NTSI1::mURA3 gas3Δ::TRP1} \]

This study

**HY1394**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas4Δ::TRP1} \]

This study

**HY1395**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDNI-NTSI1::mURA3 gas4Δ::TRP1} \]

This study

**HY1396**

\[\text{MAT}^a \text{ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 gas5Δ::TRP1} \]

This study
| HY1397  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1::mURA3 gas5Δ::TRP1 | This study |
| HY1388  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 bgl2Δ::TRP1 | This study |
| HY1389  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 bgl2Δ::TRP1 | This study |
| HY1444  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5+ with RDN1::ADE2 gas2Δ::TRP1 | This study |
| HY1445  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5+ with RDN1::ADE2 gas3Δ::TRP1 | This study |
| HY1446  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5+ with RDN1::ADE2 gas4Δ::TRP1 | This study |
| HY1447  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5+ with RDN1::ADE2 gas5Δ::TRP1 | This study |
| HY1443  | MATα ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5+ with RDN1::ADE2 bgl2Δ::TRP1 | This study |
**Supplementary Table S2.** Oligonucleotide primers used for ChIP assays in this study

| Locus                  | Forward Primer             | Reverse Primer             |
|------------------------|-----------------------------|----------------------------|
| rDNA-25S               | CGACTAACCACGTCCAACT         | CCGAATGAACCTGCGCTGAA       |
| rDNA-NTS1              | TCCCCACTGTTACTGTTCA         | AGGGCTTTCAAAGCTTCC         |
| rDNA-NTS2/18S          | AAGATGCCACGATGAGACT         | GGGAGGTACTTCATGCAGAA       |
| rDNA-18S               | CCAGAACGTCATAAGGACATC       | CTCACCAGGTCCAGACACAA       |
| CUP1                   | TGAAGGTCATGAGTGCAAT         | TTCGTTTCATTTCCCAGAGCA      |
| PNC1 promoter          | GATCAAGGTCACACAGGG          | ATACATAGTGCCAAACGG         |
| ACT1                   | TGACTGACTACTTGATGAA         | ACAGAAGGTGGAAACAAGC        |
Supplementary Figure S1

A

Cells

| leu2::mURA3 | NTS1::mURA3 |
| WT          |            |
| sir2Δ       |            |
| gas1Δ       |            |
| gas1Δ sir2Δ |            |

B

Relative mURA3 transcript level (NTS1/leu2)

WT     | gas1Δ | sir2Δ | gas1Δ sir2Δ

C

% marker loss

WT     | gas1Δ | sir2Δ | gas1Δ sir2Δ
Supplementary Figure S2

| No tag | WT   | gas1Δ | msn2Δ/4Δ | gas1Δ/ msn2Δ/4Δ |
|--------|------|-------|----------|-----------------|
|        |      |       |          |                 |

Western Blot

Sir2-TAP

Actin

1.0 1.0 1.0 1.1
Supplementary Figure S6

A

Cells

|          | SC          | SC-Ura      |
|----------|-------------|-------------|
| leu2::mURA3 | NTS1::mURA3 | WT          |
| gas1Δ     |             |             |
| gas2Δ     |             |             |
| gas3Δ     |             |             |
| gas4Δ     |             |             |
| gas5Δ     |             |             |
| bgl2Δ     |             |             |

B

% marker loss

WT  gas1Δ  gas2Δ  gas3Δ  gas4Δ  gas5Δ  bgl2Δ
Supplementary Figure S7

A

WT  gas1Δ  WT  gas1Δ  gas1Δ
Vector  Vector  GAS1  gas1ΔER10,E220

Cki1-P  Cki1
1.0  0.2  1.2  0.4  1.3  0.4

B

Congo red

-  +

Cki1-P  Cki1
1.0  0.2
Supplementary Figure S8

SC-Ura

leu2::mURA3 + Vector
leu2::mURA3 + MSN2
leu2::mURA3 + msn2^{S582D, S620D, S625D, S633D}
NTS1::mURA3 + Vector
NTS1::mURA3 + MSN2
NTS1::mURA3 + msn2^{S582D, S620D, S625D, S633D}

gas1Δ msn2Δ

Cells  SC  SC-Ura