SUPPLEMENTARY MATERIAL

Supplementary Table 1. Yeast strains used in this study

Strain	Genotype	Source
W303a	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100*	(16)
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
BY4742	*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Open Biosystems
HY0008	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SIR2-TAP::HIS3MX6*	This study
HY0026	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 SIR2-TAP::TRP1*	This study
HY0240	*MAT***a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 lys2Δ0/LYS2 ura3Δ0/ura3Δ0 SIR2-GFP::His3MX6/NOP56-RFP::KanMX6*	This study
DMY2798	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3*	(35)
DMY2804	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3*	(35)
HY0245	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 sir2Δ::TRP1*	This study
HY0291	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 sir2Δ::TRP1*	This study
DMY3010	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RAD5^{+} with RDN1::ADE2*	(35)
HY0616	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 pnc1Δ::LEU2*	This study
HY0561	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 SIR2-TAP::TRP1 pnc1Δ::LEU2*	This study
HY0618	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 pnc1Δ::LEU2* [p416GPD]	This study
HY0569	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 SIR2-TAP::TRP1 pnc1Δ::LEU2* [p416GPD]	This study
HY0619	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 pnc1Δ::LEU2* [p416GPD-PNC1-GFP]	This study

HY0570	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 SIR2-TAP::TRP1 pnc1*Δ*::LEU2* [p416GPD-PNC1-GFP]	This study
HY0565	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 sir2*Δ*::HIS3* [p416GPD]	This study
HY0566	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 sir2*Δ*::HIS3* [p416GPD-SIR2-TAP]	This study
HY0022	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 NET1-TAP::TRP1*	This study
HY0239	*MAT***a**/α *his3*Δ*1/his3*Δ*1 leu2*Δ*0/leu2*Δ*0 met15*Δ*0/MET15 lys2*Δ*0/LYS2 ura3*Δ*0/ura3*Δ*0 NET1-GFP::His3MX6/NOP56-RFP::KanMX6*	This study
HY0796	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 net1*Δ*::URA3*	This study
HY0771	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 SIR2-TAP::TRP1, net1*Δ*::URA3*	This study
HY0769	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 NET1-TAP::TRP1, pnc1*Δ*::URA3*	This study
HY0363	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 NET1-3HA::HIS3MX6*	This study
HY0390	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 NET1-3HA::HIS3MX6 SIR2-Myc::KlURA3*	This study
HY0777	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 NET1-3HA::HIS3MX6, pnc1*Δ*::LEU2*	This study
HY0779	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 NET1-3HA::HIS3MX6 SIR2-Myc::KlURA3, pnc1*Δ*::LEU2*	This study
HY1012	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 rpl22a*Δ*::KanMX6 SIR2-TAP::HIS3MX6*	This study
HY1013	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 rpl35b*Δ*::KanMX6 SIR2-TAP::HIS3MX6*	This study
HY1014	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 rpl13a*Δ*::KanMX6 SIR2-TAP::HIS3MX6*	This study
HY1024	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 sir2*Δ*::KanMX6*	This study
HY1025	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 tor1*Δ*::KanMX6*	This study
HY1026	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 tor1*Δ*::KanMX6 sir2*Δ*::URA3*	This study
HY1027	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 leu2::mURA3 tor1*Δ*::TRP1*	This study
HY1028	*MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 RDN1-NTS1::mURA3 tor1*Δ*::TRP1*	This study

SUPPLEMENTARY FIGURE LEGENDS

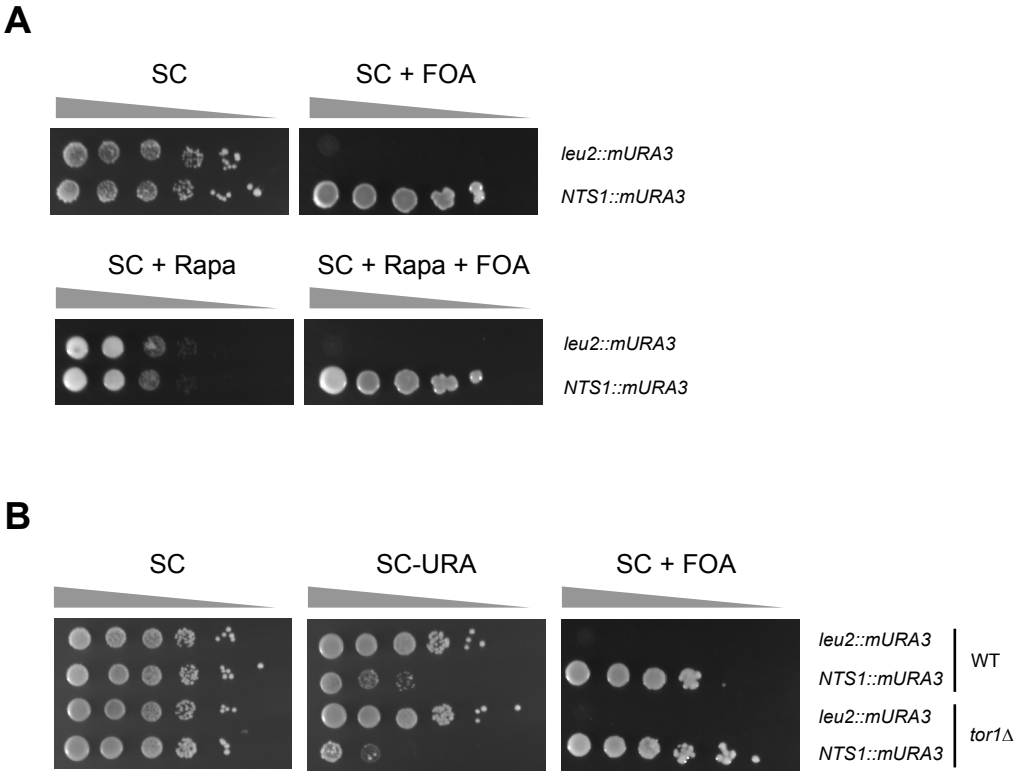
Supplementary Figure 1. Rapamycin treatment or deletion of *TOR1* enhances rDNA silencing. (A) Rapamycin increases silencing of the *mURA3* reporter gene at the NTS1 region of the rDNA locus. Silencing was assayed by monitoring the growth of 10-fold serial dilutions of cells plated on SC medium or SC medium containing 2 mg/ml of 5-fluoroorotic acid (SC + FOA), each with or without 10 ng/ml rapamycin (Rapa), and incubated at 30°C for 3 days. (B) Deletion of *TOR1* promotes silencing of the *mURA3* reporter gene at the NTS1 region of the rDNA locus. Silencing was assayed by monitoring the growth of 10-fold serial dilutions of cells plated on SC, SC-Ura, or SC containing 2 mg/ml of 5-fluoroorotic acid (SC + FOA) medium and incubated at 30°C for 3 days.

Supplementary Figure 2. Nicotinamide inhibits the enzymatic activity of Sir2 and abolishes silencing of the *mURA3* reporter gene at the rDNA locus. (A) Nicotinamide inhibits the histone deacetylase activity of Sir2 *in vitro*. TAP-tagged Sir2 was purified from yeast cells using Calmodulin Sepharose 4B (17-0529-01; GE Healthcare) and assayed for deacetylase activity using a HDAC Fluorescent Activity Assay/Drug Discovery Kit (AK-500; BIOMOL Research Laboratories). Reactions consisted of 5 μg of Sir2, 250 μM acetylated histone substrate, 1 mM dithiothreitol, and 200 μM NAD^+, and the following treatments: 5 mM nicotinamide for 30 min (NAM), 5 mM nicotinamide for 15 min and then 200 ng/ml of rapamycin for 15 min (NAM→Rapa). Reactions were carried out at 30°C for 30 min. Fluorescence was measured on a fluorescent plate reader (EnVision; PerkinElmer) with excitation set at 360 nm and emission detection set at 460 nm. Values represent the average of three independent experiments and error bars indicate standard deviations. (B) Nicotinamide efficiently abolishes silencing of the *mURA3* reporter gene at the NTS1 region of the rDNA locus. Silencing was assayed by monitoring the growth of 10-fold serial dilutions of cells plated on SC-Ura medium with or without 5 mM nicotinamide (NAM) and incubated at 30°C for 3 days. SC medium was used as a plating control. (C) Nicotinamide abolishes silencing of the *mURA3* reporter gene at rDNA whether it was treated before or after rapamycin. *mURA3* transcript levels were measured by quantitative real-time reverse transcription-PCR after the following treatments: 200 ng/ml rapamycin for 1 hr (Rapa), 5 mM nicotinamide for 1 hr (NAM), 5 mM nicotinamide for 1 hr and then 200 ng/ml rapamycin for 1 hr (NAM→Rapa), 200 ng/ml rapamycin for 1 hr and then 5 mM nicotinamide for 1 hr

(Rapa→NAM). For control, cells were treated with DMSO only. Relative *mURA3* transcript levels were calculated as described in Figure 2A legend. Values represent the average of three independent experiments and error bars indicate standard deviations.

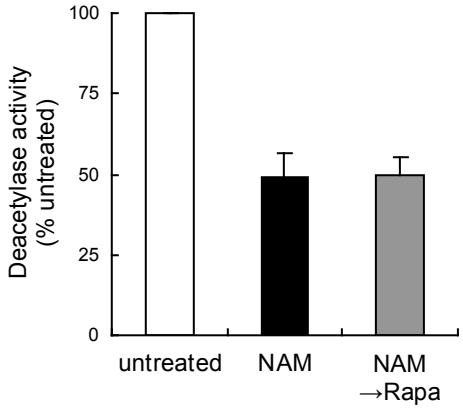
Supplementary Figure 3. Rapamycin increases replicative lifespan additively with deletion of *FOB1*. The *P*-values for wild-type cells treated with 10 ng/ml rapamycin (WT+Rapa), *fob1*Δ, and *fob1*Δ cells treated with 10 ng/ml rapamycin (*fob1*Δ+Rapa) versus wild-type cells (WT) are 4.8×10^{-4}, 6.5×10^{-5}, and 8.5×10^{-9}, respectively. Replicative lifespan was determined by scoring the number of daughter cells produced by each mother cell. Mean lifespans are shown in parentheses.

Supplementary Figure 4. Depletion of 60S subunit ribosomal proteins does not significantly enhance association of Sir2 with rDNA. The degree of Sir2 binding to rDNA was measured using the ChIP assay in wild-type, *rpl22a*Δ, *rpl35b*Δ, and *rpl13a*Δ cells. Values represent the average of three independent experiments and error bars indicate standard deviations.

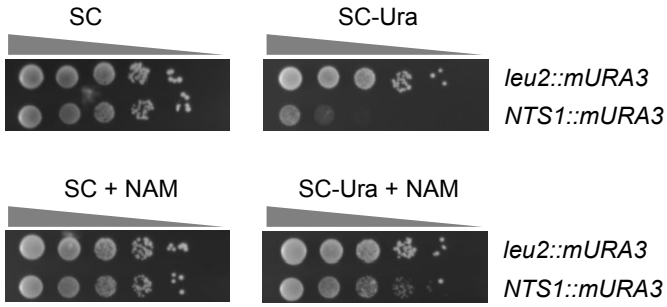


Supplementary Figure 1

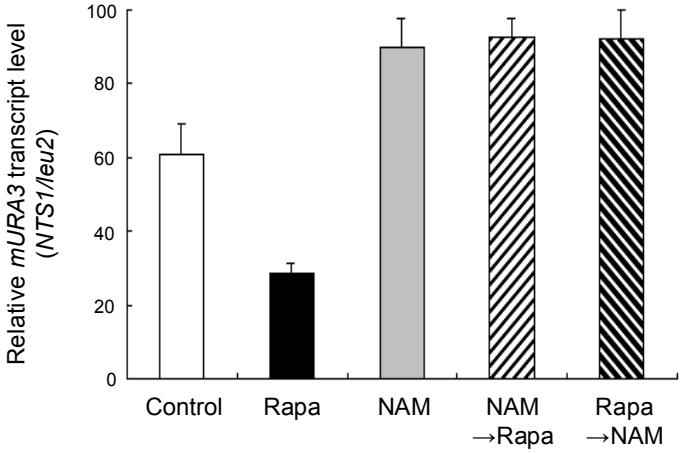
A



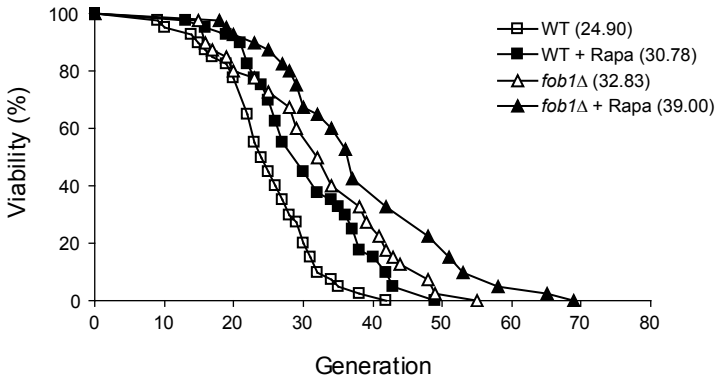
B



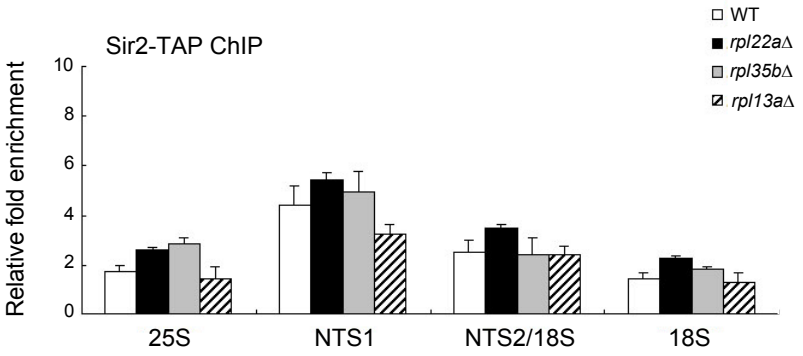
C



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4