Jack *et al.* Supporting Information

Materials and Methods

Yeast strains and media

Yeast deletion strains (Table S1) were created by standard methods: deletion cassettes were amplified from the pFA6a plasmid series (1, 2), pFA6a-*LEU2* or pFA6a-*URA3* (3) using the primers listed in Table S2. Plasmid construction was by standard methods, details are given in Table S3. Cells were grown at 30° on synthetic media (0.69% w/v YNB with ammonium sulfate, amino acids, 2% w/v sugar). Media components were purchased from Formedium. Rapamycin (SCBT) was used at 25nM, nicotinamide (Sigma) at 5mM. For 5 FOA selection, synthetic media containing the appropriate amino acids was supplemented with 1 mg/ml 5 FOA (Formedium) and 50 µg/ml uracil. Growth curves were acquired using a BMG Fluostar system, cells were inoculated in liquid media at OD0.05, maintained at 30° and OD_{600} measured every 30 min for 1-3 days with orbital shaking at 500 rpm between reads.

PFGE determination of rDNA copy number

Cells were streaked on four successive plates (~60 generations) except where stated otherwise and grown in liquid media to stationary phase. PFGE assays were performed as described (4) using 0.8% w/v agarose gels, and probed for rDNA intergenic spacer 2. Where multiple samples are shown these represent independent plasmid transformants. Copy number distribution plots were obtained by quantifying each lane using the AIDA software (FUJI), normalising for the total signal in each lane and then averaging the profile for each experimental condition.

qPCR analysis of rDNA copy number

Genomic DNA was extracted from cells on plates by re-suspension in 200µl FLB (2% v/v Triton-X100, 1% w/v SDS, 100mM NaCl, 1mM EDTA, 10mM Tris pH8) with 200µl phenol:chloroform and 50µl glass beads, and 5 minutes vortexing. The aqueous phase was then ethanol precipitated and re-suspended in 100µl TE. 5µl of DNA was digested with *Eco*RI (4U) and 0.2µg RNase A in 10µl then purified using QIAQuick PCR Purification columns into 30µl. qPCR reactions were performed on 1µl DNA in 10µl using Maxima Probe Mix (Thermo Scientific) with 0.1µM probes and 0.3µM each primer pair (see Table S5). Thermal cycling conditions were 95°C 10 min, then 40 cycles of 95°C 15 seconds, 60°C 30 seconds with plate read. qPCR plates were run on Bio Rad CFX96 machines and analysed using Bio Rad CFX Manager v2. Prior to analysis the baseline threshold of each fluorophore was set to 50.

Northern analysis

RNA was extracted using the GTC-phenol method, glyoxylated, separated on a 1.2% agarose gel and probed with a random primed PCR product to ITS1 (made by PCR of GGCAAGAGCATGAGAGCTTTTA and CCAGTTACGAAAATTCTTGTT on genomic DNA) in Church Hyb at 60° as described (5), then washed 2x 20 min with 0.5x SSC 0.1% w/v SDS at 60°.

qPCR analysis of *PNC1* expression

1µg RNA was treated with 1µg RNase-free DNase (Thermo), purified by phenol:chloroform extraction and reverse transcribed with Superscript III (Life Technologies) according to manufacturer's instructions. cDNA was diluted 1:2.5-1:25 and 1µl analysed per 10µl qPCR reaction using Maxima SYBR Mix (Thermo) with 0.3µM primers. Thermal cycling conditions were 95°C 10 min, then 40 cycles of 95°C 15 seconds, 60°C 30 seconds with plate read, plates were run on Bio Rad CFX96 machines and analysed using Bio Rad CFX Manager v2.

Figures

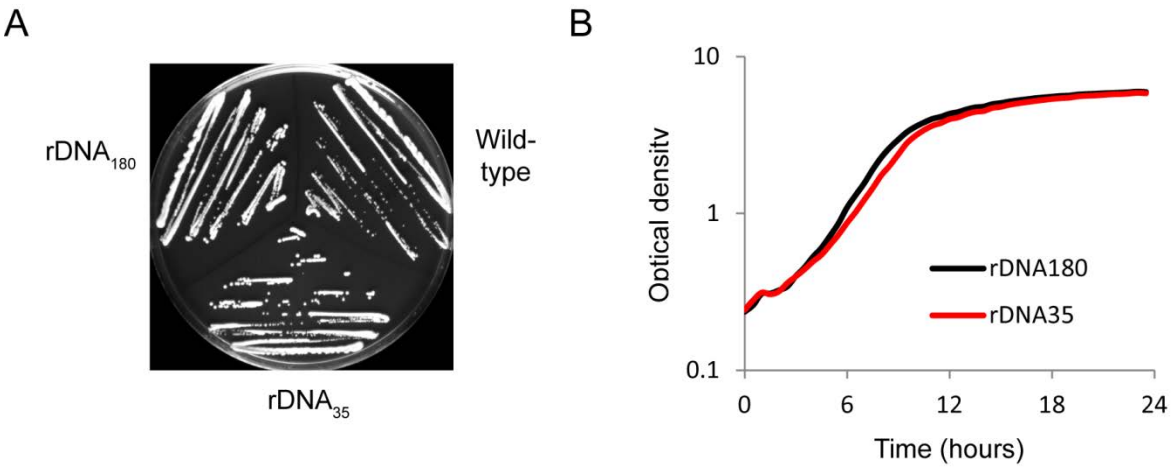

Fig. S1: Growth of $rDNA_{35}$ and $rDNA_{180}$ cells**.** A) Growth of $rDNA_{35}$ cells compared to isogenic $rDNA_{180}$ cells on a YPD plate, with a BY4741 wild-type control. B) Growth of $rDNA_{35}$ and $rDNA_{180}$ cells in liquid synthetic media at 30°, data shows averages of seven cultures from three biological replicates of each genotype. Doubling times at mid-log: $rDNA_{180}$ – 1:58 hours, $rDNA_{35}$ – 2:04 hours. Over the 60 generations used for experiments in this work, this difference is insufficient to explain more than a minimal selectivity towards the cells with higher rDNA copy number.

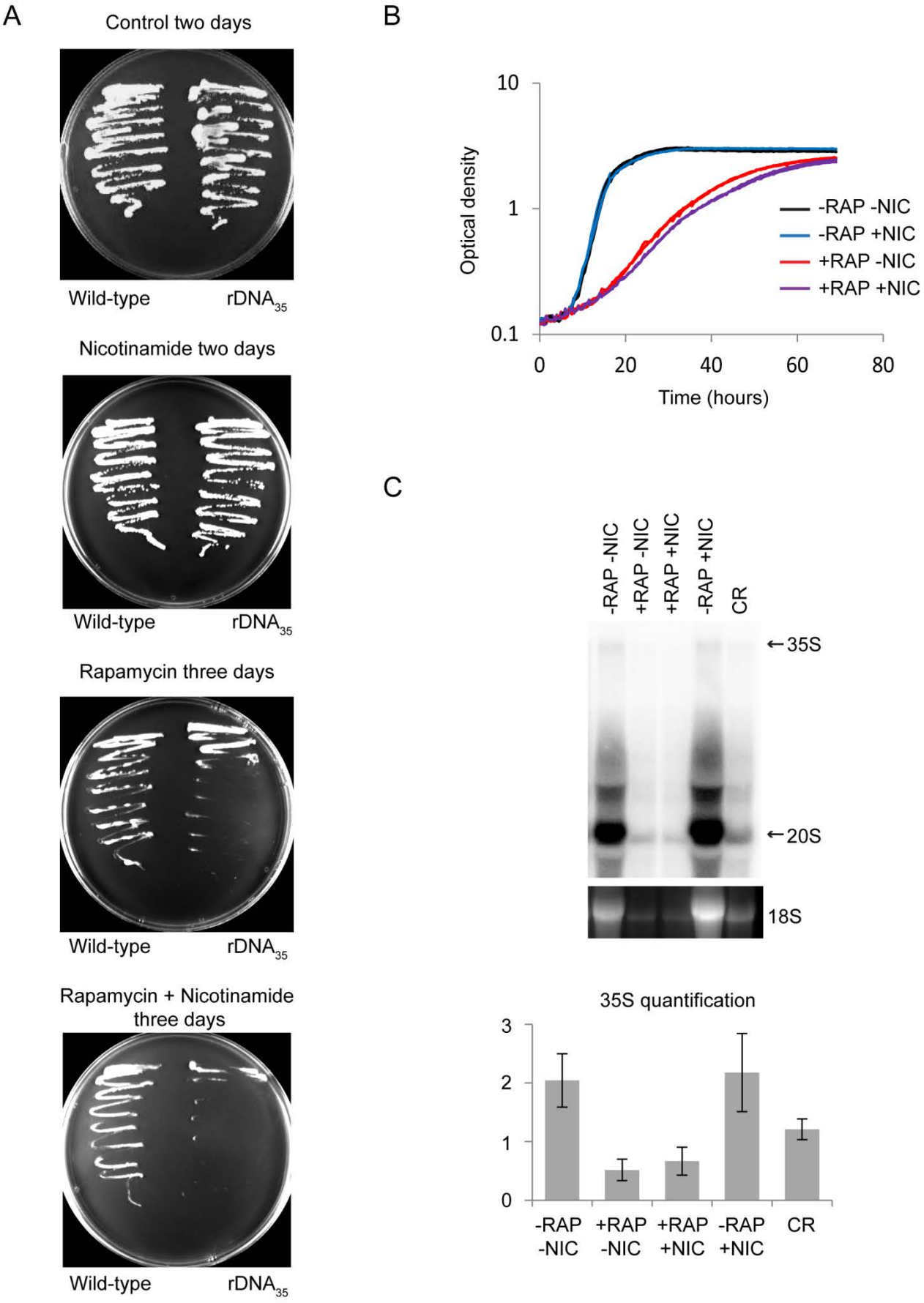
A
Control two days
Wild-type
rDNA35
Nicotinamide two days
Wild-type
rDNA35
Rapamycin three days
Wild-type
rDNA35
Rapamycin + Nicotinamide three days
Wild-type
rDNA35
B
Optical density
10
1
0.1
0
20
40
60
80
Time (hours)
-RAP -NIC
-RAP +NIC
+RAP -NIC
+RAP +NIC
C
-RAP -NIC
+RAP -NIC
+RAP +NIC
-RAP +NIC
CR
←35S
←20S
18S
35S quantification
3
2
1
0
-RAP -NIC
+RAP -NIC
+RAP +NIC
-RAP +NIC
CR

Fig. S2: Growth and transcriptional effects of nicotinamide treatment. A) Growth of wild-type and $rDNA_{35}$ cells on synthetic media plates in the presence of 25nM rapamycin (RAP) and/or 5mM nicotinamide (NIC). Plates were grown at 30°. B) Growth of $rDNA_{35}$ cells in liquid synthetic complete media at 30° containing 25nM rapamycin (RAP) and/or 5mM nicotinamide (NIC). Each curve represents an average of eight cultures, two biological replicates were performed for each drug combination (both are shown but are inseparable over this long time-course). C) Northern analysis of pre-rRNA species in $rDNA_{35}$ under the same drug combinations as in A/B. Caloric restriction (CR), growth in 0.05% w/v glucose, is also included for comparison with data in Fig. 4 and Fig. S6. Each lane contains RNA from 1.0±0.2 ODs of cells, 35S and 20S were detected using a probe to rDNA intergenic spacer 1, 18S is shown by ethidium staining. For quantification of 35S, error bars represent 95% CI, n=3.

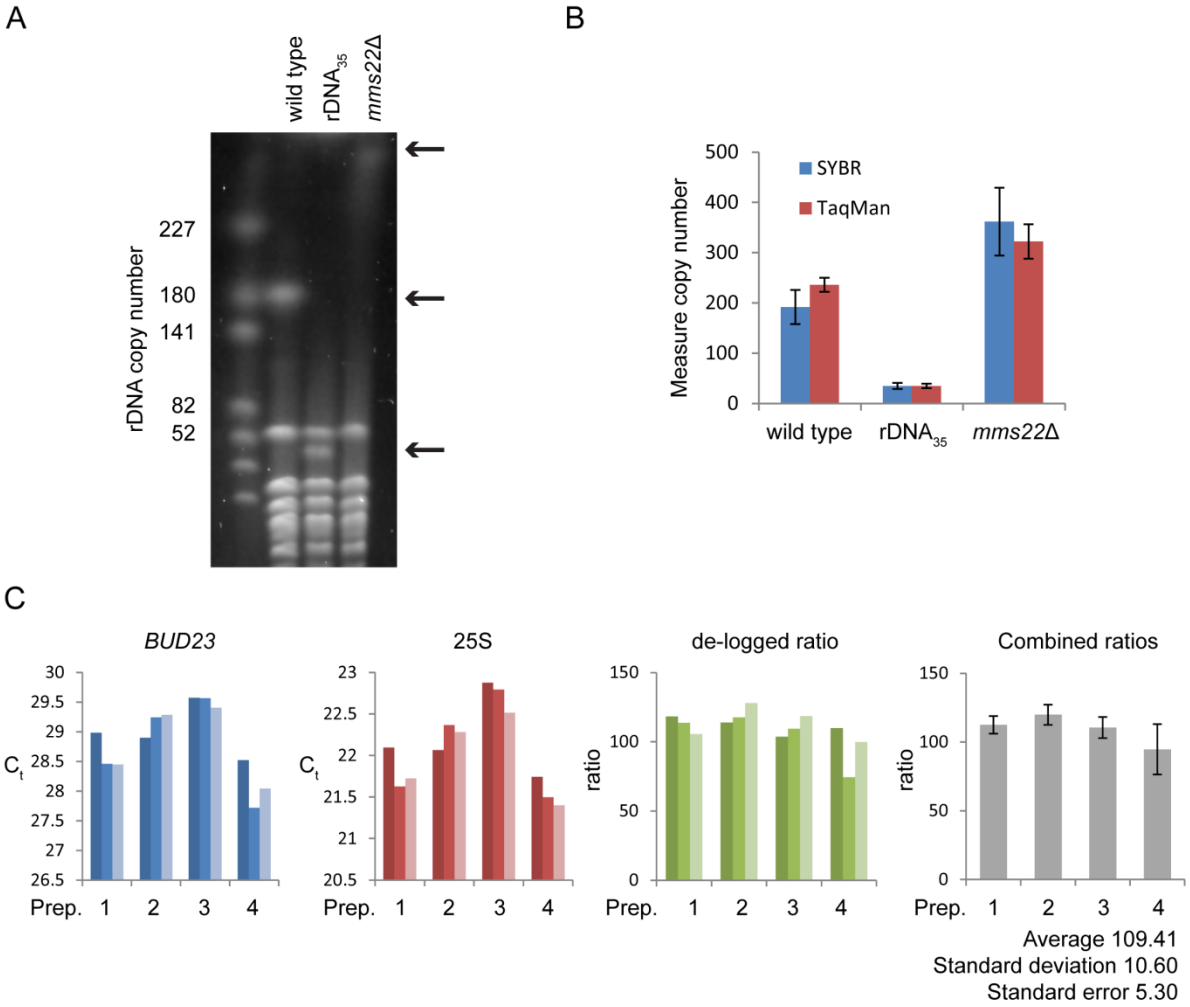
A
wild type
rDNA35
mms22Δ
rDNA copy number
227
180
141
82
52
B
Measure copy number
SYBR
TaqMan
wild type
rDNA35
mms22Δ
C
BUD23
25S
de-logged ratio
Combined ratios
Ct
ratio
Prep. 1 2 3 4
Average 109.41
Standard deviation 10.60
Standard error 5.30
D
ratio
Plate 1 2 3

Fig. S3: Development of a qPCR assay for rDNA copy number. qPCR assays for the measurement of rDNA copy number changes are challenging as the changes are often less than two-fold. We therefore set up a multiplex assay for copy number measurements using multiple technical replicates to improve accuracy and multiple clones to allow statistical analysis. A) PFGE analysis of rDNA copy number in test strains wild type BY4741, $rDNA_{35}$ and *mms22*Δ, arrows indicate the chromosome XII bands for the three strains. B) Comparison of measured rDNA copy number in these strains by SYBR green and TaqMan multiplex qPCR, both using primer sets in the rDNA 25S region and the single copy *BUD23* gene. Measured copy numbers have been normalised to 35 repeats for the $rDNA_{35}$ strain. For each strain, four qPCR reactions of each type were performed on a single DNA sample, error bars ±1 s.d. The measured copy number by PFGE does differ from the copy number measured by qPCR, however, this difference is relatively small and should affect all samples equally within one experiment. Note that the s.d. is much lower in the TaqMan samples. C) Variability across DNA preparation and qPCR. Four DNA preparations were performed from cells on the same plate, and three qPCR reactions were performed for each. Individual C_t values are shown for each primer set, along with the ratio of de-logged C_t values. A single qPCR reaction from preparation 4 deviates slightly from the others. Average ratio and SEM for each DNA preparation were calculated; note that preparation 4 gives a lower value with higher standard error. A single average ratio was then calculated from these four individual preparations; despite the variability of the individual measurements, the standard error of the calculated ratio is small (less than 5%). Different DNA preparations used in this work derived from different clones or at least different cell populations, so large standard errors indicate variability between cells/clones rather than technical variability in copy number measurement. D) Variability between plates. We noticed that although measured ratios were highly consistent within a single plate, they could vary greatly between plates, and particularly between plates run on different (identical) qPCR machines. Here, twelve DNA preparations were made from wild type cells on the same YPD plate and assayed on three different qPCR plates (each bar represents a different DNA preparation and the shown value is an average of three qPCR reactions with SEM). Set 3 deviates markedly from sets 1 and 2, though within a plate the variability is low. We therefore ensured that experiments were performed on a single plate where possible. Where replicate experiments were run on different plates, all calculated ratios were divided by the median ratio for that plate before plates were combined. When more samples needed to be assayed than could fit on one plate (notably in Fig. 2), a selection of samples were run on all the plates and the median of these samples used to normalise the other ratios. Note that the ratios calculated by qPCR are not a direct readout of copy number, instead each experiment needs to be normalised such that the rDNA copy number of the parental strain matches the value measured by PFGE.

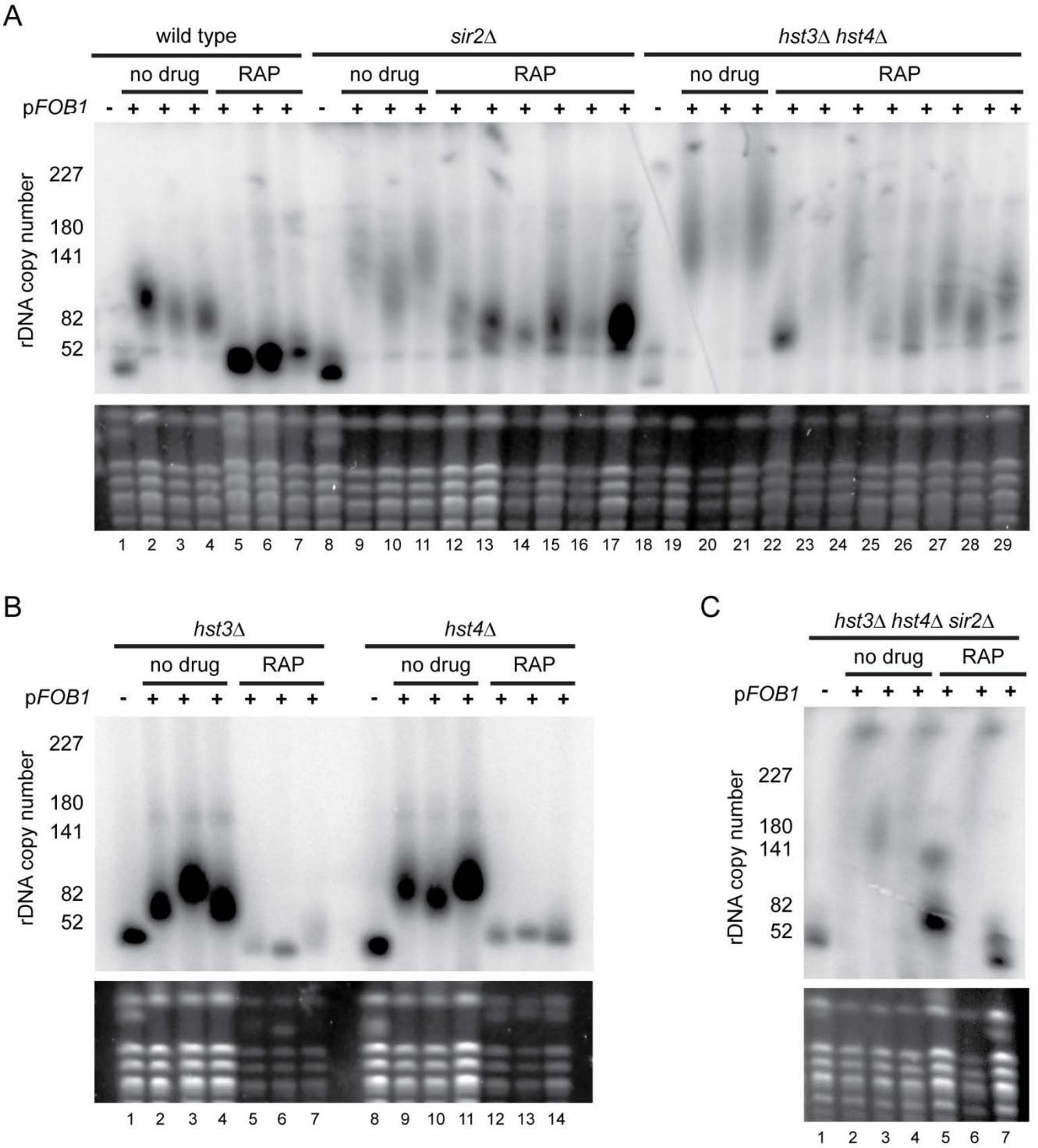

Fig. S4: Effects of HDAC mutations on rDNA expansion. A) rDNA copy number analysis of HDAC mutants in the presence or absence of rapamycin. Experiment is identical to Fig. 2, but a wider set of independent p*FOB1* transformants are shown for *sir2*Δ and *hst3*Δ *hst4*Δ cells treated with rapamycin. Note the massive variation between different clones of $rDNA_{35}$ *hst3*Δ *hst4*Δ on rapamycin despite identical treatment, reflected in the large confidence interval for these cells in quantification for Fig. 2. B) rDNA copy number analysis of $rDNA_{35}$ *hst3*Δ and $rDNA_{35}$ *hst4*Δ cells in the absence or presence of rapamycin; experiments were performed as in Fig. 2. C) rDNA copy number analysis of $rDNA_{35}$ *hst3*Δ *hst4*Δ *sir2*Δ in the presence or absence of rapamycin, revealing levels of clone-to-clone variation that precluded further analysis.

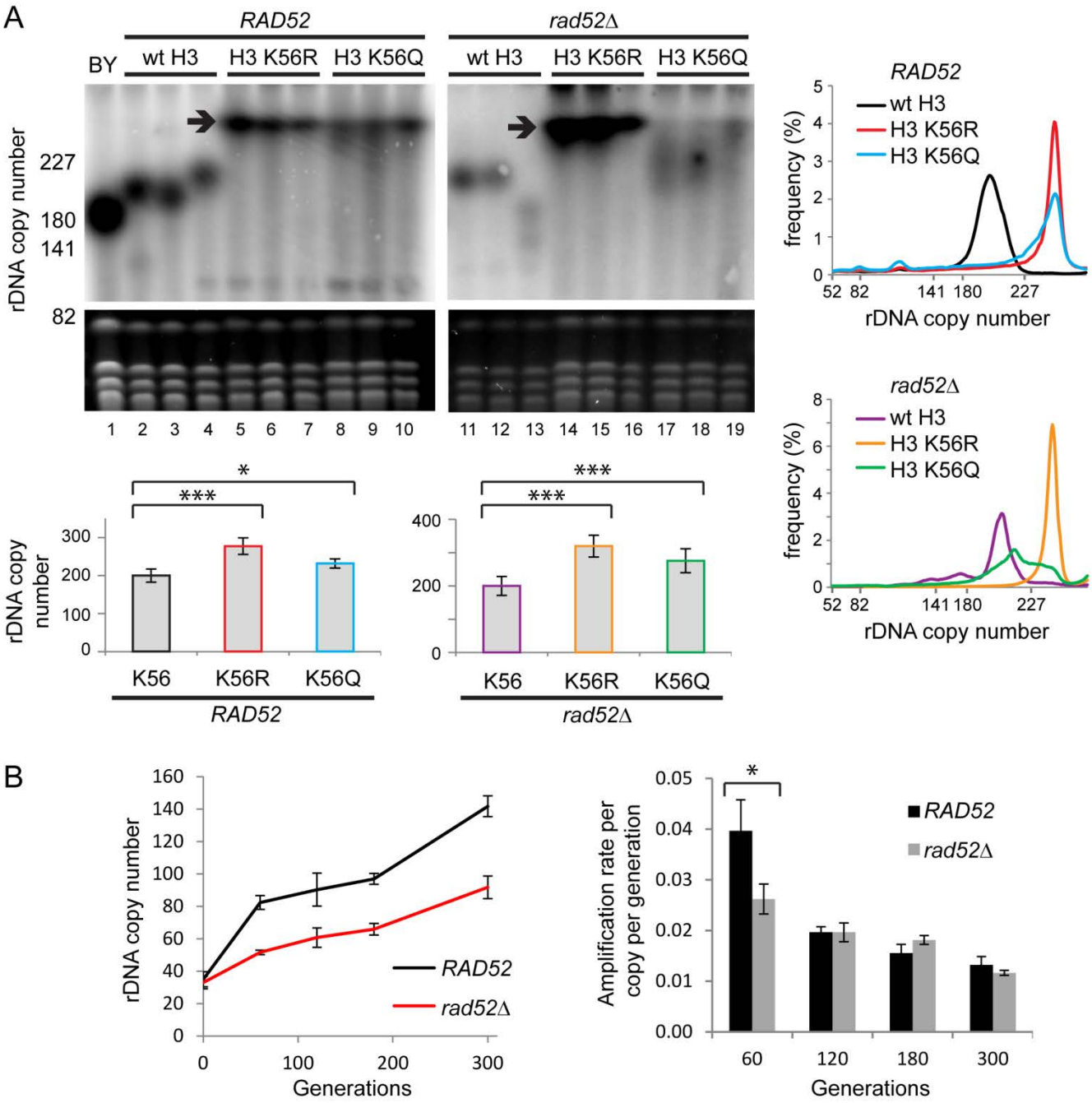

Fig. S5: rDNA amplification in the presence and absence of Rad52. A) *RAD52* was deleted in cells expressing H3 only from a counter-selectable plasmid, then a plasmid shuffle was performed to exchange the H3 plasmid for one expressing wild-type H3 (lanes 2-4 & 11-13) or mutant H3 K56 (lanes 5-10 & 14-19) (6). Three clones from each plasmid transformation were analysed, DNA from BY4741 wild-type cells is shown in lane 1 for comparison. The clone in lane 13 has formed a split population during culture post-transformation. Many further clones were analysed by qPCR due to considerable variability in copy number, but the trend visible by PFGE was always observed. For quantification, error bars represent 95% CI, *- $P < 0.05$, ***- $P < 0.01$ by one-way ANOVA, n=15 for *RAD52* and n=9 for *rad52*Δ, B) rDNA copy number measured by qPCR of $rDNA_{35}$ and $rDNA_{35}$ *rad52*Δ cells, monitored over ~240 generations after p*FOB1* transformation, error bars represent 95% CI (left). Rate of rDNA amplification per repeat per generation calculated from the same data (right). For quantification, error bars represent 95% CI, *- $P < 0.05$ by Student's *t*-test, n=3.

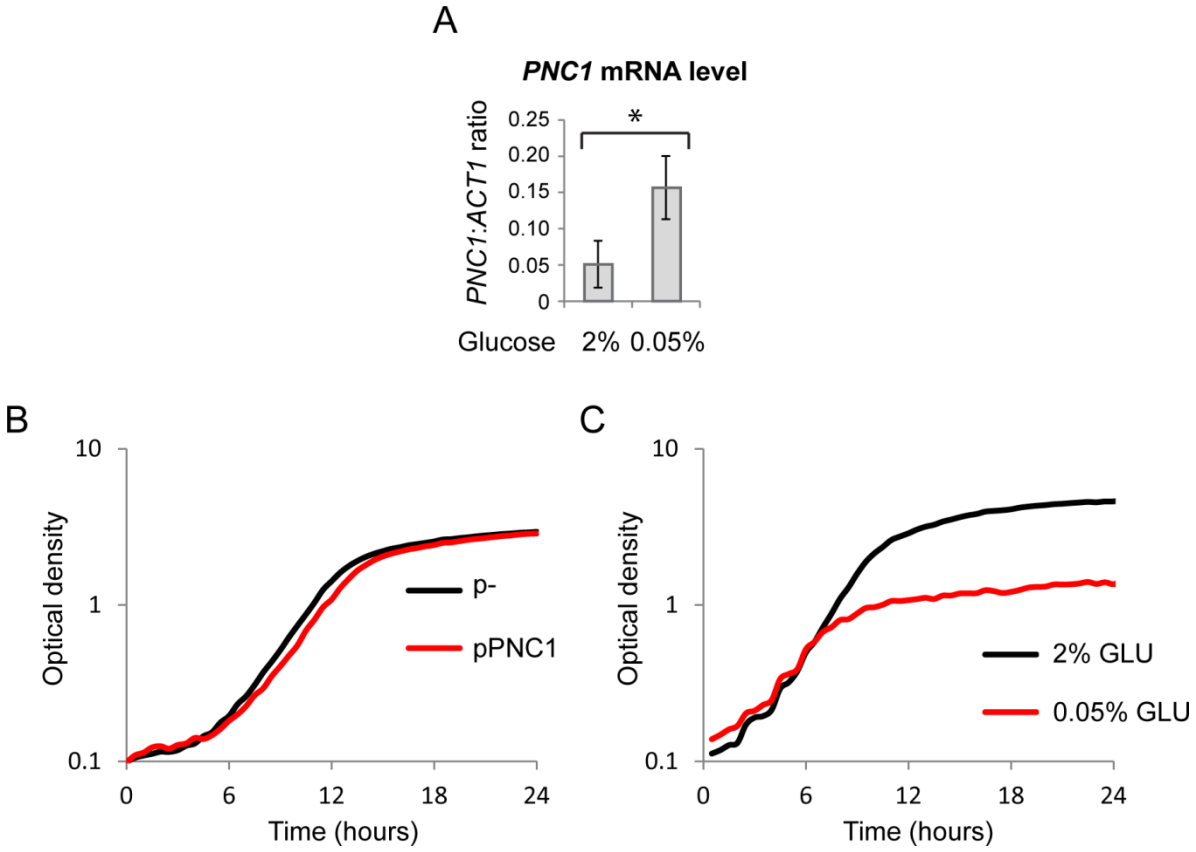

Fig. S6: Relationships between *PNC1* expression, glucose concentration and growth rate A) Expression of *PNC1* relative to *ACT1* in $rDNA_{35}$ cells grown in 2% or 0.05% w/v glucose measured by qPCR. Error bars represent 95% CI, *- $P < 0.05$ by Student's *t*-test, n=7 for 2% glucose, n=3 for 0.05% glucose. B) Growth of $rDNA_{35}$ cells carrying an empty vector or a high-copy *PNC1* plasmid in liquid synthetic media at 30°, data shows averages of seven cultures from three biological replicates of each genotype. C) Growth of $rDNA_{35}$ cells in liquid synthetic media containing 2% or 0.05% w/v glucose at 30°, data shows averages of eight cultures from three biological replicates of each genotype.

Tables

Table S1: Strains used in this study

Note that strains transformed with p*FOB1* are not listed. We do not routinely keep these strains as copy number changes during freeze-thaw, and always work with fresh plasmid transformants.

Strain number	Strain Name	Genotype	Source
YJH2	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	EUROSCARF
YCJ084	AKY1968	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 pPK189*	(6)
YCJ085	AKY1968 pMP3	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 pMp3*	(6)
YCJ086	AKY1968 pAK965	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 pAK965*	(6)
YCJ090	AKY1968 pAK973	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 pAK973*	(6)
YCJ104	AKY1968 *rad52*Δ pMP3	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 rad52::NatMX6 plus pMP3*	This study
YCJ100	AKY1968 *rad52*Δ pAK965	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 rad52::NatMX6 pAK965*	This study
YCJ102	AKY1968 *rad52*Δ pAK973	*hht1-hhf1Δ::LEU2 hht2 hhf2Δ::HIS3 rad52::NatMX6 pAK973*	This study
YCJ083	NOY1071	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 (~35 rDNA repeats)*	(7)
YCJ091	NOY1071 *rad52*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 rad52::NatMX6*	This study
YCJ292	NOY1071 *dun1*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 dun1::TRP1*	This study
YCJ305	NOY1071 *dun1*Δ *rad52*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 dun1::TRP1 rad52::LEU2 pRS316 FOB1*	This study
YCJ247	NOY1071 *sir2*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 sir2::LEU2*	This study
YCJ270	NOY1071 *hst4*Δ *hst3*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 hst4::LEU2 hst3::HygMX6*	This study
YCJ271	NOY1071 *hst4*Δ *hst3*Δ *sir2*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1Δ::HIS3 hst4::LEU2 hst3::HygMX6 sir2::NatMX6*	This study
YCC408	NOY1071 *hst3*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1::HIS3 hst3::KanMX6*	This study
YCJ264	NOY1071 *hst4*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1::HIS3 hst4::LEU2*	This study
YCC405	NOY1071 *hst1*Δ	*MATa ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11 can1-100 fob1::HIS3 hst1::LKanMX6*	This study

Table S2: Oligonucleotides used in this study

Primer number	Primer Name	Sequence	Primer use
OCJ11	HST4 DN45	TGCATTAATTTTATCTCCAACCTTTTTTGGTAGGACAA ATACTTTGAATTCGAGCTCGTTTAAAC	HST4 deletion
OCJ12	HST4 UP45	CAACTGTATTTTAAAACTGTAAATAATACTAAGCAGAA CCACAAACGGATCCCCGGGTTAATTAAG	HST4 deletion
OCJ124	HST3 UP45	AAGGGATTAATTTACATACAACTAGATCCATCTTTCTC AAAAATGCGGATCCCCGGGTTAATTAAG	HST3 deletion
OCJ125	HST3 DN45	TGCCTCGATTATTTATCGTTAACTCAATTTTAATAGTTA AGTTTAGAATTCGAGCTCGTTTAAAC	HST3 deletion
OJH195	RAD52 UP45	TTGCCAAGAACTGCTGAAGGTTCTGGTGGCTTTGGTG TGTTGTTGCGGATCCCCGGGTTAA	RAD52 deletion
OJH196	RAD52 DN45	TAAATAATGATGCAAATTTTTTATTTGTTTCGGCCAGG AAGCGTTCGATGAATTCGAGCTCGTT	RAD52 deletion
OJH248	SIR2 UP45	AAGGGCGTGTATGTCGTTACATCAGATGAACATCCCA AAACCCTCCGGATCCCCGGGTTAATTAA	SIR2 deletion
OJH249	SIR2 DN45	ATTGATATTAATTTGGCACTTTTAAATTATTAAATTGCC TTCTACGAATTCGAGCTCGTTTAAAC	SIR2 deletion
OJH68	DUN1 UP45	AGTAAAGGGGCTTAACATACAGTAAAAAAGGCAATTA TAGTGAAGCGGATCCCCGGGTTAATTAAG	DUN1 deletion
OJH69	DUN1 DN45	AAAATCCAGATTCAAACAATGTTTTTGAAATAATGCTT CTCATGTGAATTCGAGCTCGTTTAAAC	DUN1 deletion
OJH107	HST1 UP45	CACTTCTCTTCTTTTTTGTTGTTTTTGTGAGAAAAAAAA ATCTAACGGATCCCCGGGTTAATTAA	HST1 deletion
OJH108	HST1 DN45	TACATGAATGAAATGCTCGAATATATGCAATAGCAGC GGTATACTGAATTCGAGCTCGTTTAAAC	HST1 deletion
OCC236	PNC1 F5	actgactgacGGATCCCCCTACGATCCTCCTTATATGAA	PNC1 cloning
OCC237	PNC1 R5	actgactgacCTCGAGGGCGAGAAATAATGTCGGTAT	PNC1 cloning

Table S3: Plasmids used in this study

Plasmid number	Plasmid name	Description	Reference
PCJ02	pPK189	HHT2/HHF2 ARS/CEN/URA3	(6)
CJ03	pAK973	H3 K56Q H4 ARS/CEN/TRP1	(6)
pCJ04	pPAK965	H3 K56R H4 ARS/CEN/TRP1	(6)
pCJ05	pMP3	HHT2 HHF2 ARS/CEN/TRP1	(8)
pJH113	pRS316 FOB1	*Bam*HI-*Bam*HI *FOB1* fragment from pTAK101 (9) cloned into *Bam*HI site of pRS316	This study
pRH3	pRS424 PNC1	PCR OCC236-OCC237 on genomic DNA, cloned into pRS424 using *Bam*HI and *Xho*I	This study

Table S4: qPCR primers and probes used in this study

Primer number	Primer Name	Sequence
OJH579	BUD23 F1	GACCATGTGTGGTGTGGTTT
OJH578	BUD23 R1	CCCATATCCTGCAACATCAA
OJH626	KK 25S F	GAATCCATATCCAGGTTCCG
OJH627	KK 25S R	GACGTGGGTTAGTCGATCCT
OCJ155	BUD23 Probe	[6FAM]CCTCCAGCTCTCACTAAGACCGG[BHQ1]
OCJ156	25S Probe	[HEX]AGGCCACCATCGAAAGGGAA[BHQ1]
OJH475	ACT1 1 F	TCCTACGTTGGTGATGAAGC
OJH474	ACT1 1 R	TTTCCATATCGTCCCAGTTG
OCC268	PNC1 F8	TGTCGACAAGGGTTTCTTGA
OCC269	PNC1 R8	CTCCAAAGCTACACCGACAA

References

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