Supporting Information

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SI Materials and Methods

Maf1-Cka2 Coimmuoprecipitation. Cka2 was HA-tagged in YPH499 strain as described previously (1). The sequences of primers used are available upon request. Cells expressing HA epitope-tagged Cka2 were grown in rich glucose medium (YPD) to exponential phase and transferred to a nonfermentable glycerol medium (YPGly) and incubated for 3 h at 30 °C. Cells were pelleted and frozen in liquid nitrogen. Pellets corresponding to 50 mL of culture were thawed on ice and resuspended in 0.5 mL of IP buffer (50 mM Hepes-KOH pH 7.5, 250 mM NaCl, 1 mM EDTA, 0.05% Nonidet P-40, 0.5 mM DTT, 10% glycerol, O-Complete protease inhibitor; Roche). Cells were broken with glass beads using Vibramax disruptor (IKA) at maximum speed for 30 min at 4 °C. Cellular debris was removed by centrifugation at 11,000 × *g* for 10 min at 4 °C. Protein concentration was determined with the Bio-Rad protein assay. Fifty microliters of suspension of Dynabeads PanMouse IgG magnetic beads (Invitrogen), washed three times with 0.5 mL PBS containing 0.5% BSA, was incubated for 1 h with mouse monoclonal anti-HA 16B12 antibody at 4 °C. After extensive washing in PBS containing 0.5% BSA and then in IP buffer, the beads were incubated overnight with equal amounts (usually 2 mg) of protein extracts with gentle shaking at 4 °C, then washed five times with IP buffer. Immunoprecipitated proteins were eluted by boiling for 3 min in SDS gel electrophoresis loading buffer and analyzed by 8% SDS/PAGE followed by Western blotting.

Maf1-Rpc160 Coimmuoprecipitation. Yeast cells expressing HA epitope-tagged Rpc160 (strain MW4415) were grown in rich glucose medium (YPD) to exponential phase and transferred to a nonfermentable glycerol medium (YPGly). Following 3 h incubation at 30 °C, the culture was split in half; one part was transferred to YPD with 2% (vol/vol) DMSO and the other to YPD with 100 μM TBBt in DMSO. Cultures were grown for 1 h at 30 °C, then pelleted and frozen in liquid nitrogen. Further steps were the same as described above except that protein extract was incubated with beads for 4 h and the composition of IP buffer was: 50 mM Hepes-KOH pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.05% Nonidet P-40, 0.5 mM DTT, 5% glycerol, O-Complete protease inhibitor (Roche).

Chromatin Immunoprecipitation (ChIP). Overnight yeast cultures were used to inoculate fresh YPD medium to OD_{600} of 0.2. The cultures were grown to OD_{600} of 0.6–0.8 and fixed with formaldehyde at a final concentration of 1% for 20 min at room temperature. The fixing was stopped by the addition of glycine to 340 mM and incubation for 5 min. The fixed cells (usually 50 mL) were harvested by centrifugation, washed twice with cold PBS, frozen and stored at −80 °C for further use.

The cell pellets were thawed, resuspended in 700 μL of lysis buffer (50 mM Hepes-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 1 mM PMSF, O-Complete protease inhibitor; Roche) and transferred to 2-mL Eppendorf tubes. Glass beads (~0.2 mL, 0.4–0.6 mm; Sigma) were then added to each tube and the cells were lysed at 4 °C for 45 min using an Vibramax disruptor (IKA) at maximum speed. Each tube was pierced at the bottom and the cell lysate was collected by centrifugation in a fresh tube. The lysate was then sonicated eight times for 30 s with 30-s intervals in a Bioruptor (Diagenode) set to maximum power. The sonication resulted in chromatin fragments of 0.2–1 kb with a mean size of 450 bp as determined by gel electrophoresis. After 20 min of centrifugation in 1.5-mL Eppendorf tubes at 15,000 rpm, the supernatant (whole cell extract, WCE) was transferred to another tube on ice. The preparation of magnetic beads, immunoprecipitation (anti-myc 9E10), elution from beads, reversal of crosslinking, and DNA purification were done exactly as described (2).

Real-Time PCR Quantification of Immunoprecipitated DNA Fragments. For ChIP experiments, the input and immunoprecipitated samples were assayed by quantitative PCR to assess the extent of protein occupancy at different genomic regions. PCR reactions contained 2 μL of DNA template, 300 or 200 nM primer pairs and 5 μL of 2× SYBR Green reaction mix (Sigma-Aldrich). Quantitative PCR was performed on an Applied Biosystem 7300 unit or Roche LightCycler 480 using a 3-min soak at 95 °C, followed by 45 cycles of 15 s at 95 °C, 30 s at 50 °C, and 30 s at 72 °C (with a plate read after each cycle). A melting curve analysis was performed for each sample after PCR amplification to ensure that a single product of expected melting curve characteristics was obtained. Occupancy values (in arbitrary units) were calculated by determining the apparent immunoprecipitation efficiency (the amount of PCR product in the immunoprecipitated sample divided by the amount of PCR product in the input sample) and dividing it by the apparent cross-linking efficiency of a control DNA segment (35S rDNA or TEL15 gene). The sequence of primers used are available upon request.

Northern Blot Analysis. Cells (50 mL of liquid culture) were harvested by centrifugation and resuspended in 50 mM sodium acetate, pH 5.3, 10 mM EDTA. Total RNA was isolated by heating and freezing the cells in the presence of SDS and phenol as described previously (3). RNA (5 μg per sample) was resolved by electrophoresis in 8 M urea, 10% PAGE, transferred to Hybond-N^+ membrane (Amersham) with 0.5 × TBE by electroblotting and crosslinked by UV radiation (1,200 mJ/cm^2). The membrane was prehybridized in phosphate buffer (7% SDS; 0.5 M sodium phosphate pH 7.2; 1 mM EDTA pH 7.0; 1% BSA) and hybridized at 37 °C in the same solution with oligonucleotide probes labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase (New England Biolabs). The probes were: 5′-TATTCCCACAGTTAACTGCGG-3′ for $tRNA^{Leu}$(CAA) and 5′-GGATTGCGGACCAAGCTAA-3′ for U3 snoRNA. After hybridization, the blots were washed 2 × 10 min with 1× SSC, 1% SDS and 3 × 10 min with 0.5× SSC, 0.1% SDS at 37 °C, exposed to a phosphoimager screen (Fujifilm). RNA was quantified using a FLA-7000 phosphoimager (Fujifilm). Band intensities were quantified using MultiGauge v3.0 software (Fujifilm).

Purification of Native CK2 Holoenzyme. Purification of native CK2 holoenzyme was carried out essentially as described previously (4) until TEV protease cleavage. Briefly, yeast cells with TAP-tagged Cka1 (SC1820) were grown in 16l of YPD medium at 30 °C until OD_{600} = ~3. Cells were harvested and resuspended in 500 mL of ice-cold water, harvested again, resuspended in 80 mL of lysis buffer (1 mM DTT, 40 mM Hepes pH 8, 250 mM NaCl, 2 mM PMSF, O-Complete protease inhibitor; Roche) and frozen in liquid nitrogen. Yeast were broken in a laboratory blender with dry ice and the obtained homogenate was clarified by centrifugation in a JA 20 Beckman rotor for 20,000 rpm for 20 min at 4 °C and afterward in a refrigerated 35Ti rotor at 32,000 rpm for 90 min at 4 °C. Obtained extract was then dialyzed against buffer D (1 mM DTT, 40 mM Hepes pH 8, 150 mM NaCl, 1 mM PMSF, 20% glycerol), supplemented with reduced

Triton X-100 (final concentration 0.1%) and incubated with 200 μL of IgG Sepharose (Pharmacia) for 2 h at 4 °C under rotation. The resin was washed two times with IPP150 buffer (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% reduced Triton X-100) and two times with TEV cleavage buffer (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5 mM EDTA, 1 mM DTT). TEV protease cleavage was carried out in 200 μL of TEV cleavage buffer with 100 units of TEV protease for 2 h at room temperature.

The eluate was subjected to ion-exchange chromatography on ResourceQ column (GE Healhtcare) using AKTA purifier FPLC machine under NaCl gradient (0.15–1 M). CK2 holoenzyme was eluted from the colum at ~450 mM NaCl. The purity and composition of the eluted protein was analyzed by SDS/PAGE and Coomassie blue staining.

Identification of Maf1 Phosphorylation by Mass Spectrometry. Recombinant, in vitro phosphorylatyed Maf1, was digested by addition of trypsin (sequencing grade modified trypsin; Promega V5111) directly to the reaction mixture and incubation overnight at 37 °C. Immoprecipitated Maf1-myc was digested on the beads. Magnetic beads were washed with 25 mM ammonium bicarbonate and digested by trypsin as described above.

Isolation of phosphopeptides was performed as described by Thingholm at al. (5). Briefly, peptides obtained after trypsin digestion were mixed with loading buffer (80% acetonitrile, 5% TFA, 1 M phthalic acid) and incubated with titanium dioxide slurry (GL Sciences), in proportion of 0.6 mg of the titanium dioxide per 100 μg of peptides for 10 min and centrifuged. Titanium beads were washed with Washing Buffer I (80% acetonitrile, 1% TFA) and with Washing Buffer II (20% acetonitrile, 0.05% TFA). Phosphopeptides were eluted with 30 μL water alkalized by ammonia to pH 10.5 and immediately after elution acidified with a mixture of 2 μL 10% TFA and 5 μL 100% FA.

Peptide mixtures were separated by liquid chromatography before molecular mass measurements on Orbitrap Velos mass spectrometer (Thermo Electron).

Peptide mixture was applied to RP-18 precolumn (nanoACQUITY Symmetry C18; Waters no. 186003514) using water containing 0.1% TFA as mobile phase and then transferred to nano-HPLC RP-18 column (nanoACQUITY BEH C18; Waters no. 186003545) using an acetonitrile gradient (0–60% AcN in 120 min) in the presence of 0.05% formic acid with the flow rate of 150 nL/min. Column outlet was directly coupled to the ion source of the spectrometer working in the regime of data dependent MS to MS/MS switch. A blank run ensuring lack of cross contamination from previous samples preceded each analysis.

Acquired raw data were processed by Mascot distiller followed by database search with the Mascot program (Matrix Science, 8-processor on-site license) against *Saccharomyces* Genome Database (http://www.yeastgenome.org/). Search parameters for precursor and product ions mass tolerance were 20 ppm and 0.4 Da, respectively, without accepting missed trypsin cleavage sites, and the following allowed variable modifications: cysteine and lysine carbamidomethylation, methionine oxidation, serine, threonine, and tyrosine phosphorylation. Peptides with Mascot score exceeding the threshold value corresponding to <5% false positive rate, calculated by Mascot procedure, were considered to be positively identified. Data were next submitted to an additional Mascot database search focusing of Maf1 sequence in an error tolerant option with enzyme specificity changed to semitrypsin and one missed trypsin cleavage site allowed. Then, all peptides identified in the Mascot search runs as phosphorylated were subjected to the confirmation procedure on the basis of visual inspection of the fragmentation spectra corresponding to the modified (and unmodified, when detected) peptide and identification of a significant fraction of expected product ions.

Purification of Recombinant Maf1 Protein. To construct expression plasmid coding for His-tagged Maf1, a PCR product containing the *MAF1* sequence was cloned into pET15-b vector (Novagene) using BamHI sites. The 6× His tag was thus placed at the N terminus of the expected recombinant protein in the plasmid called pET-MAF. The sequences of primers used are available upon request. The His-tagged Maf1 protein was purified using the QIAexpress protocol (Qiagen). *Escherichia coli* (BL21) was transformed with the pET-MAF construct and grown to OD_{600} of about 0.8. The synthesis of the recombinant protein was induced with 1 mM IPTG. After 4 h of incubation, cells were collected and sonicated. After centrifugation the lysate was loaded onto an equilibrated Ni-NTA agarose column (Qiagen). The resin was washed with washing buffer and the protein was eluted with elution buffer containing 100 mM imidazole. The obtained fractions were analyzed by SDS/PAGE.

Construction of Maf1 Mutants. Amino acid substitutions in pRS315-*MAF1* were made using QuikChange site-directed mutagenesis kit (Stratagene). The sequences of primers used are available upon request.

1. Longtine MS, et al. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast* 14:953–961.
2. Ren B, et al. (2000) Genome-wide location and function of DNA binding proteins. *Science* 290:2306–2309.
3. Towpik J, Graczyk D, Gajda A, Lefebvre O, Boguta M (2008) Derepression of RNA polymerase III transcription by phosphorylation and nuclear export of its negative regulator, Maf1. *J Biol Chem* 283:17168–17174.
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6. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122:19–27.
7. Hanna DE, Rethinaswamy A, Glover CV (1995) Casein kinase II is required for cell cycle progression during G1 and G2/M in Saccharomyces cerevisiae. *J Biol Chem* 270: 25905–25914.
8. Oficjalska-Pham D, et al. (2006) General repression of RNA polymerase III transcription is triggered by protein phosphatase type 2A-mediated dephosphorylation of Maf1. *Mol Cell* 22:623–632.
9. Soutourina J, et al. (2006) Rsc4 connects the chromatin remodeler RSC to RNA polymerases. *Mol Cell Biol* 26:4920–4933.
10. Bansal PK, Mishra A, High AA, Abdulle R, Kitagawa K (2009) Sgt1 dimerization is negatively regulated by protein kinase CK2-mediated phosphorylation at Ser361. *J Biol Chem* 284:18692–18698.

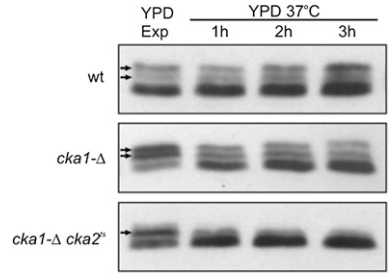

Fig. S1. CK2 mutations affect Maf1 phosphorylation. Control strains (WT and *cka1-Δ*) and isogenic thermosensitive double *cka1-Δ cka2*^{ts} mutant (1) were grown to exponential phase in rich glucose medium (YPD Exp) at 30 °C, transferred to prewarmed rich glucose medium (YPD), incubated at 37 °C, and harvested as indicated. Crude extracts were prepared from cells by TCA precipitation and analyzed using SDS/PAGE with a modified acrylamide-bisacrylamide ratio followed by immunoblotting with polyclonal anti-Maf1 antibodies. Arrows indicate phosphorylated forms of Maf1.

1. Hanna DE, et al. (1995) Casein kinase II is required for cell cycle progression during G1 and G2/M in Saccharomyces cerevisiae. *J Biol Chem* 270:25905–25914.

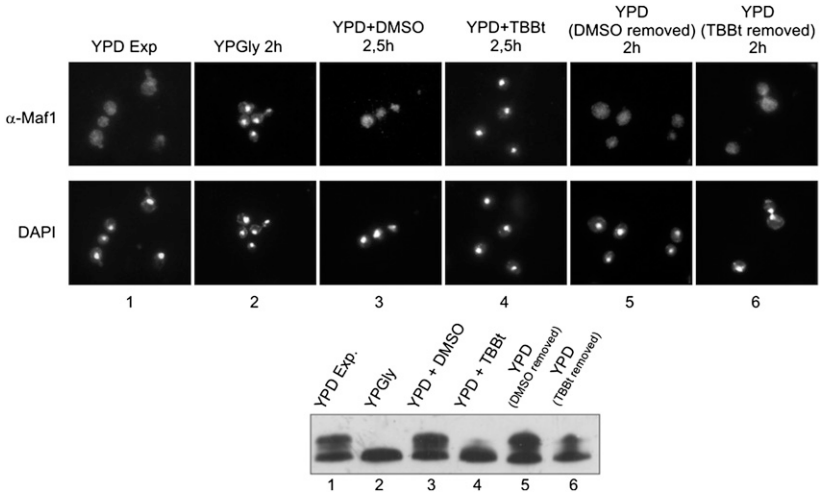

Fig. S2. CK2 inhibition by TBBt precludes Maf1 export to cytoplasm after transferring cells from respiratory conditions to glucose. Wild-type cells (YPH500) grown in rich glucose medium (YPD) to exponential phase were transferred to a nonfermentable glycerol medium (YPGly). Following 2-h incubation at 30 °C, the culture was split in half; one part was transferred to YPD with 2% (vol/vol) DMSO (control) and the second, to YPD with 100 μM TBBt in DMSO. Cultures were grown for 2.5 h at 30 °C, followed by TBBt and DMSO removal and further 2-h incubation. Polyclonal antibodies were used to monitor Maf1 cellular localization by immunofluorescence (*Top*) and Maf1 phosphorylation by immunoblotting (*Bottom*). Nuclei were stained with DAPI (*Middle*). Maf1-specific signal was uniformly distributed throughout the cell in YPD and concentrated in the nuclear compartment in YPGly, which was accompanied by Maf1 dephosphorylation (compare lanes 1 and 2). Upon transfer of the cells back to YPD, supplemented with DMSO for control, Maf1 was rephosphorylated and relocated to the cytoplasm (lane 3) but remained in the dephosphorylated state in the nucleus when TBBt was added (lane 4). The effect of TBBt was reversible because Maf1 was rephosphorylated and relocated out of the nucleus following TBBt removal (lane 6).

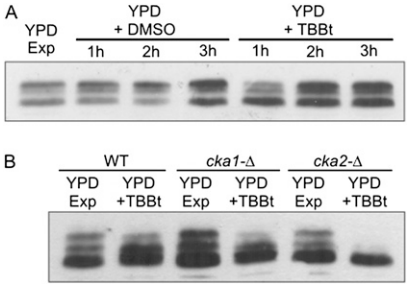

Fig. S3. Effect of TBBt and mutants in CK2 on Maf1 phosphorylation in exponential glucose cells. Yeast extracts were examined by Western blot using anti-Maf1 antibody. Cells were grown to exponential phase in rich glucose medium (YPD Exp), transferred to YPD containing 4% vol/vol DMSO or 200 μM TBBt. (*A*) Wild-type cells were incubated with TBBt at 30 °C and harvested as indicated. (*B*) Wild-type cells and isogenic mutants *cka1-Δ*, *cka2-Δ*, and *cka1-Δ cka2*^{ts} were incubated with TBBt at 30 °C for 1 h. Deletion of a single catalytic subunit of CK2 did not significantly change Maf1 phosphorylation, indicating a functional redundancy of the two catalytic subunits. Specificity of TBBt toward the Cka1 and Cka2 catalytic subunits varied; TBBt precluded phosphorylation of Maf1 only in *cka2-Δ* cells, suggesting that Cka1 is more sensitive to this inhibitor.

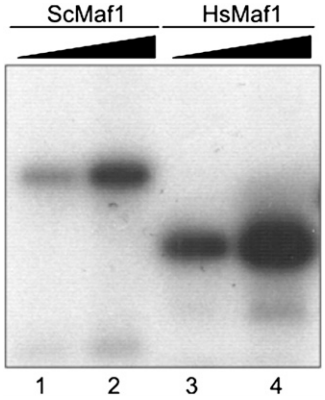

Fig. S4. Recombinant human Maf1 is phosphorylated by human CK2 in vitro. Recombinant human Maf1 (44.7 kDa) was expressed in insect cells (baculovirus system) and recombinant yeast Maf1 (28.9 kDa) was expressed in *Escherichia coli*. Both were purified by HIS-tag isolation. Autoradiogram shows [γ^{32}P]-ATP–labeled products of reactions containing 50 ng of α-subunit of human CK2 kinase and 0.5 μg yeast Maf1 (lane 1), 2.5 μg yeast Maf1 (lane 2), 0.5 μg human Maf1 (lane 3), and 2.5 μg human Maf1 (lane 4).

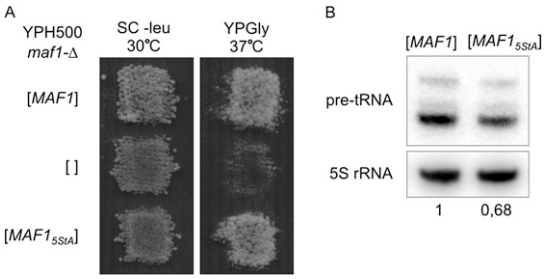

Fig. S5. Effect of inactivated CK2-phosphorylation sites on Maf1 function in 5StA mutant (S159A, S160A, S161A, S162A, and S388A). (*A*) 5StA mutant complements growth phenotype of *maf1-Δ* on glycerol medium YPH500 *maf1-Δ* cells, transformed with empty vector, pRS315-MAF1, and pRS315-MAF1-5StA were grown on SC −leu and replicated to YPGly before incubation at the indicated temperatures. (*B*) Pol III activity is slightly decreased in 5StA mutant. YPH500 *maf1-Δ* cells, transformed with pRS315-MAF1 and pRS315-MAF1-5StA grown on SC −leu were analyzed by Northern blotting. Amount of newly synthesized $tRNA^{Leu}$ is normalized to 5S rRNA.

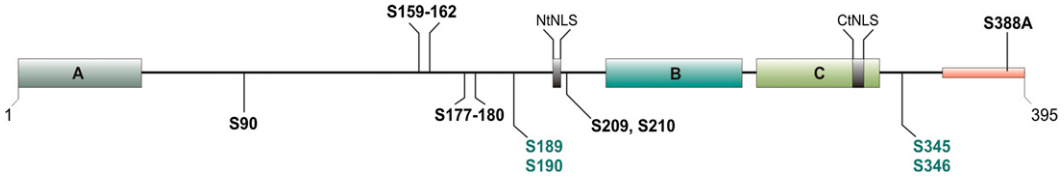

Fig. S6. Maf1 phosphorylation sites. Linear representation of yeast Maf1 with marked A and BC conserved regions, C-terminal acidic tail and two nuclear localization signals (NtNLS, CtNLS). Phosphorylation sites that have been found in MS analysis are marked: CK2 phosphorylation sites: above, scheme in black; PKA/Sch9 sites: below scheme in black; other, below scheme in gray.

Table S1. Maf1 phosphorylation sites identified by mass spectrometry

Experiment no.	Recombinant Maf1 phosphorylated by purified CK2
1	S159–162, S388
2	S159–162
3	S155, S157, S159–162*, S388
	Maf1 immunopurified from exponential glucose cells
4	S159–162*, S177–180, S189–190, S345–346
5	S90, S118–119, S159–162*, S189–190, S209–210, S345–346
6	S90, S159–162, S345–346

Samples (experiments 1–6) were measured by mass spectrometry twice, with and without phosphopeptide enrichment on titanium oxide resin (*SI Materials and Methods*). On average peptide fragments derived from recombinant Maf1 phosphorylated by purified yeast TAP tagged CK2 without titanium enrichment were identified with 70–80% of the total protein sequence coverage, whereas peptides derived from Maf1 immunopurified from yeast exponentially growing in glucose medium, with 40–60% coverage. Following titanium oxide enrichment the coverage of protein sequence by peptides was lower; however, the number of phosphorylated peptides was higher. Asterisks indicate serine tracts where both one and two phosphomoieties were detected.

Table S2. Yeast strains used in this study

Strain	Genotype	Source	Figures
YPH250	*MAT***a** *ura3-52 lys2-801 ade2-101 trpl-Δ1 his3-Δ200 leu2-Δ1*	(6)	Fig. 1; Fig. S1
YDH6	YPH250; *cka1::HIS3 cka2::TRP1* [*CEN6/ARSH4 LEU2 CKA2*]	(7)	Fig. 1; Fig. S1
YDH13	YPH250; *cka1*::HIS3 *cka2*::*TRP1* [*CEN6/ARSH4 LEU2 cka2-13*]	(7)	Figs. 1, 5B; Fig. S1
YDH13 *maf1-Δ*	YPH13; *maf1::KanMX4*	This study	Fig. 5*B*
YPH500	*MAT*α *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1*	(6)	Figs. 1*B*, 5*A*; Figs. S2, S3*A*
YPH500 *maf1-Δ*	YPH500; *maf1::KanMX4*	(8)	Figs. 5A, 3B, Fig. S5
YPH499	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1*	(6)	Fig. 2
YPH499 Cka2-HA	YPH499; *CKA2-HA:KanMX6*	This study	Fig. 2
MW4415	*Rpc160-HA:KanMX6*	(9)	Fig. 4*B*
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf	Fig. S3*B*
BY4741 *cka1-Δ*	BY4741; *cka2::KanMX4*	Euroscarf	Fig. S3*B*
BY4741 *cka2-Δ*	BY4741; *cka2::KanMX4*	Euroscarf	Fig. S3*B*
SC1820	*MAT***a** *ade2 arg4 leu2-3,112 trp1-289 ura3-52 CKA1-TAP*	Euroscarf	Fig. 3*A*
Y1734	*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 CKA1-13Myc:HIS3*	(10)	Fig. 6
Y1734 *maf1-Δ*	Y1734; *maf1::KanMX4*	This study	Fig. 6
yCC10	YPH500; *MAF1-13Myc:KanMX6*	This study	Fig. 4*A*

Table S3. Plasmids generated and used in this study

Plasmid	Vector insert
pET-MAF	pET15-b-MAF1
pRS-MAF	pRS315-MAF1
pRS-MAF-1StA	pRS315-MAF1$_{S388A}$
pRS-MAF-4StA	pRS315-MAF1$_{S159A,\ S160A,\ S161A,\ S162A}$
pRS-MAF-5StA	pRS315-MAF1$_{S159A,\ S160A,\ S161A,\ S162A,\ S388A}$