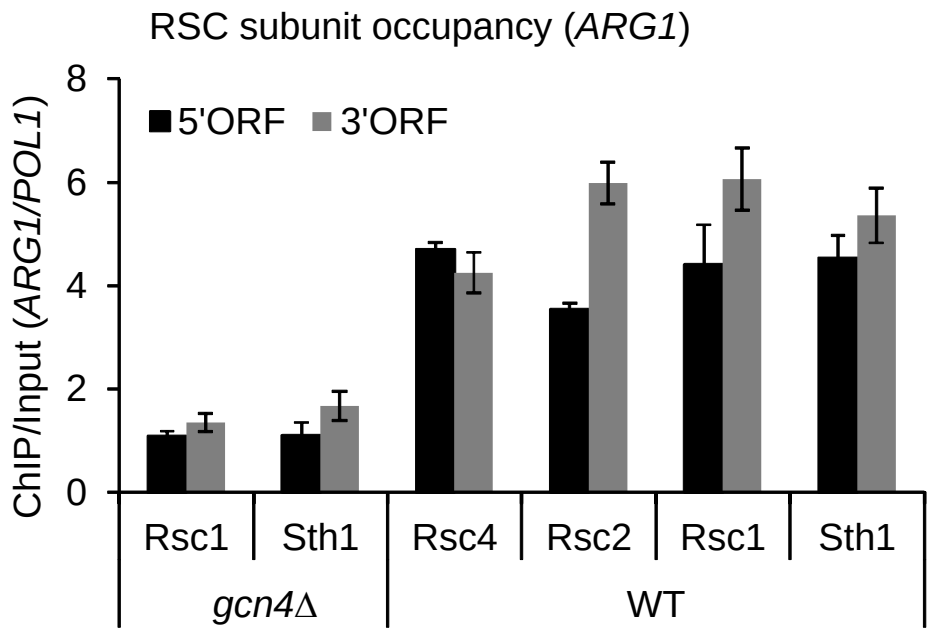

Figure S1 (Related to Figure 1): RSC localizes to the *ARG1* ORF.

Chromatin samples were prepared by sonicating (Branson Sonifier 450) whole cell extracts prepared from formaldehyde-crosslinked cells expressing Myc-tagged Sth1, Rsc1, Rsc2 or Rsc4, and were subjected to chromatin immunoprecipitation (ChIP) analysis. The immunoprecipitates were reverse cross-linked, treated with proteinase-K, and DNA was extracted by phenol:chroloform, and subjected to PCR using primers designed against the 5' open reading frame (5' ORF) and 3' ORF of *ARG1*. The relative enrichment of Myc-tagged RSC subunits were determined by taking the ratio of immunoprecipitated *ARG1*/*POL1* (primer against *POL1* used as an internal control for each PCR reaction) over the *ARG1*/*POL1* ratio obtained for the input DNA samples. The ChIP occupancies of Myc-tagged Sth1, Rsc1, Rsc2 and Rsc4 at *ARG1* 5' and 3' ORFs are shown for WT. Rsc1-Myc and Sth1-Myc occupancy at the *ARG1* ORF are also shown for *gcn4*Δ. The error bars represent SEM.

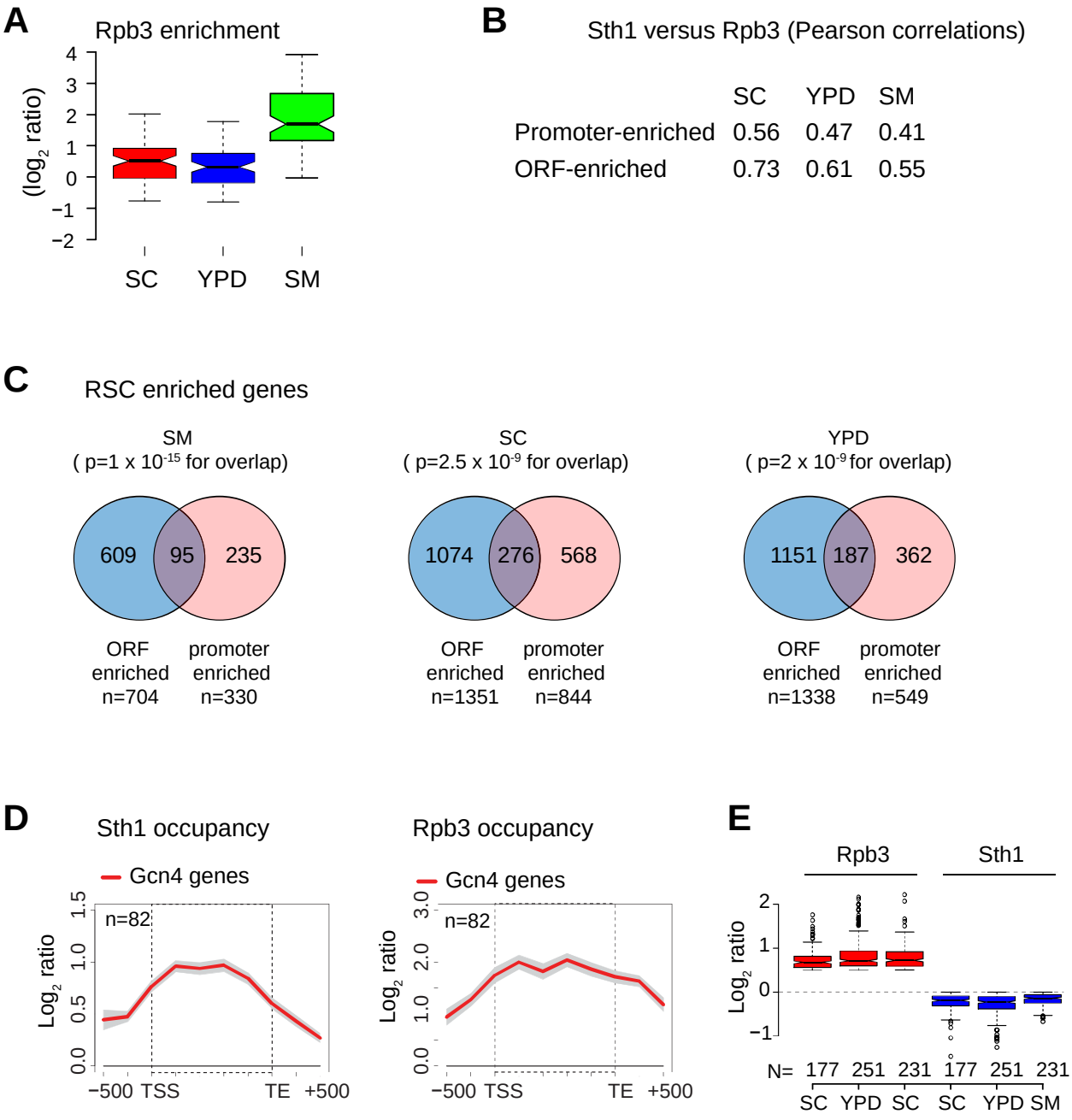

Figure S2 (Related to Figure 2): Analysis of Sth1 binding in three different growth conditions.

A) The Gcn4 regulated genes identified in (Harbison et al., 2004) displaying an average ORF enrichment of Sth1 of $\log_2$ ratio of >0.5 in cells induced with SM were selected. The average ORF occupancies of Rpb3 at these genes in cells grown in YPD, SC or induced with SM are

represented in the box plot. B) The average enrichment of Sth1 in the promoter region (500 bp upstream of transcription start site, TSS) or in the coding region (ORF) was determined for each gene. The gene displaying enrichment of Sth1 greater than 0.5 $\log_2$ ratio (ChIP/Input) in the promoter region or in the coding region were classified as promoter-enriched or ORF-enriched, respectively. The Pearson correlation between Sth1 and Rpb3 occupancies at the promoter-enriched and ORF-enriched genes in SC, YPD and SM are shown. C) Venn diagrams showing the overlap between the ORF-enriched and promoter enriched classes of genes in SM (left), SC (middle) and YPD (right). The p-value for the overlap obtained by hypergeometric analysis is shown at the top for each set. D) The gene averaged profiles of Sth1 (right) and Rpb3 binding (left) at the Gcn4 regulated genes enriched for Sth1 (>0.5 log2 ratio) during growth in SM are shown. The gray shaded area represents SEM. E) The box plot showing Rpb3 and Sth1 ORF occupancy of the genes which have relatively high Rpb3 occupancy (> 0.5 $\log_2$ ratio) and are depleted for Sth1 binding in the coding regions (<0 $\log_2$ ratio). The number of such genes identified under each growth conditions (YPD, SC and SM) is indicated. Notches in the box-plots represent the 95% confidence interval for each Median.

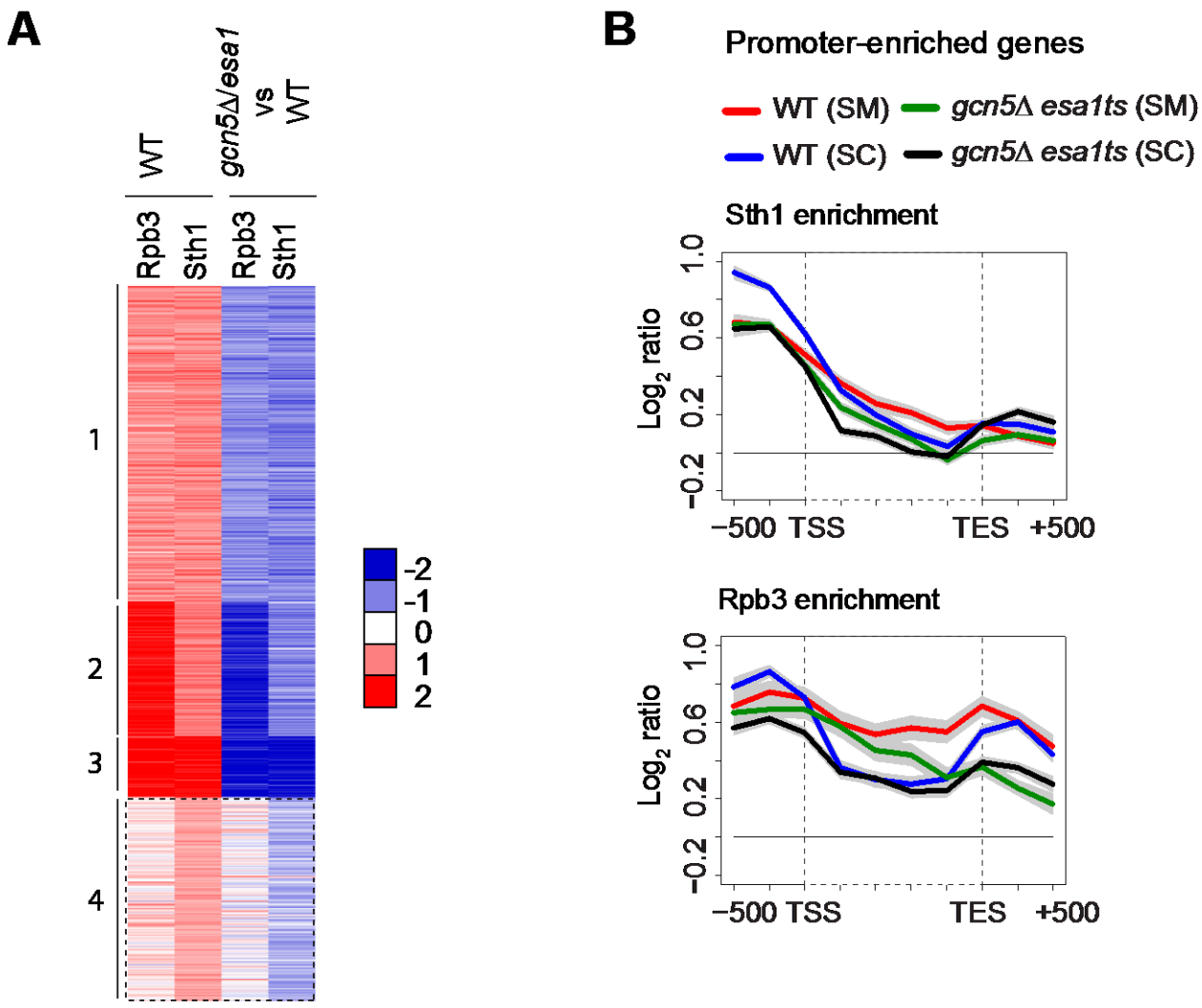

Figure S3 (Related to Figure 3): HAT mutants impair RSC binding to the coding regions of low transcribed genes.

A) The ORF-enriched genes (>0.5 $\log_2$ Sth1 enrichment in the coding regions) were selected and the average ORF binding $\log_2$ values of Sth1 and Rpb3 in the WT cells along with the changes in Sth1 and Rpb3 binding (*gcn5*Δ/*esa1ts* versus WT) were subjected to k-means clustering analysis (k=4). After clustering, the heat-map was generated using TreeView, and the 4th cluster showing a reduction in Sth1 occupancy without significant changes in Rpb3 binding is shown by dotted lines. B) The $\log_2$ enrichment of Sth1 (top) and of Rpb3 (bottom) along the metagene comprised of promoter-enriched genes classified on the basis of the average Sth1 occupancy >0.5 $\log_2$ ratio in WT cells cultured in SC or induced with SM. The enrichments are shown for WT and *gcn5*Δ/*esa1ts*. The gray shading represents SEM.

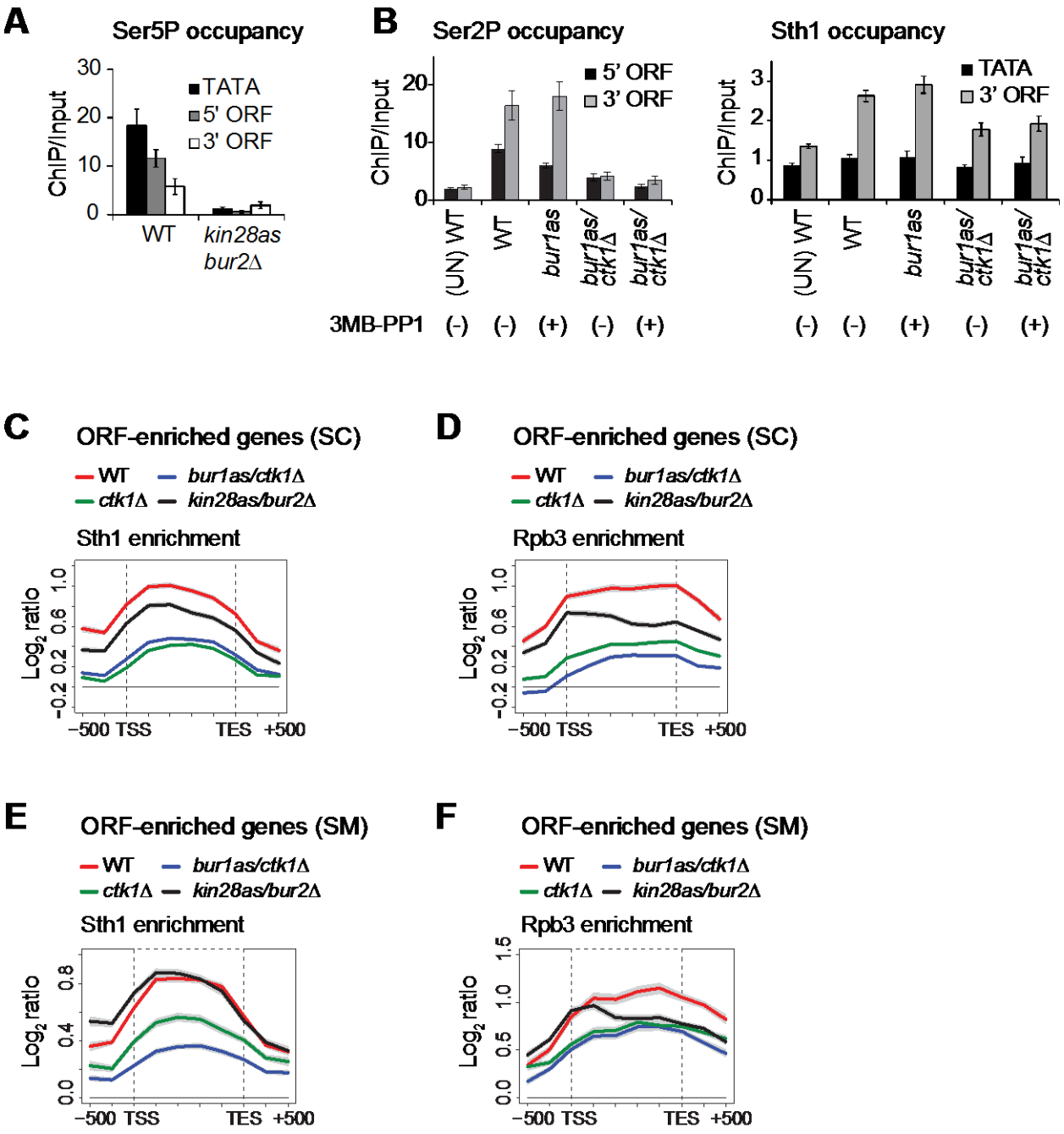

Figure S4 (Related to Figure 4): Sth1 occupancy in Ser2 and Ser5 CTD kinase mutants.

A) The Sth1-Myc tagged *kin28as*/*bur2*Δ strain was treated with NA-PP1 for 15 minutes, to inactivate kin28 kinase activity, prior to induction of Gcn4 target genes by SM, and occupancy of Pol II phosphorylated at Ser5 of the C-terminal domain (Ser5P) at the *ARG1* TATA, 5' ORF and 3' ORF was determined by ChIP using anti-Ser5P antibodies (Covance). Ser5P occupancy at *ARG1* in WT and NA-PP1-treated *kin28as*/*bur2*Δ is shown. B) The chromatin extracts prepared

from the Sth1-Myc tagged strains in the indicated background were subjected to ChIP analysis using antibodies against phosphorylated Pol II at Ser2 (Ser2P; Bethyl Laboratories)) or against the Myc-tag. The Bur1 kinase activity in the *bur1as* and *bur1as*/*ctk1*Δ cells was inactivated by treating the cells with 3MB-PP1 for 60 minutes prior to induction of Gcn4 genes by SM for 20 minutes. Ser2P (left) and Sth1 (right) occupancy, at the *ARG1* TATA and 3' ORF regions, is shown for the untagged WT (UN), WT, *bur1as*/*ctk1*Δ without 3MB-PP1 treatment, and for *bur1as* and *bur1as*/*ctk1*Δ after analog treatment. The error bars represent SEM. C-F) The kinase activities of Kin28 and Bur1 were inactivated as described above and Sth1-Myc and Rpb3 occupancies in WT, *kin28as*/*bur2*Δ, and *bur1as*/*ctk1*Δ (with and without 3MB-PP1 treatment) were determined by ChIP-chip analysis using Agilent 4x44K arrays as described in the Materials and Methods. The average Sth1 occupancy in WT cells over the coding region was determined for each gene and the genes exhibiting ORF occupancies greater than 0.5 $\log_2$ ratio (ORF-enriched) in WT cells were selected. The gene averaged Sth1 (C) and Rpb3 (D) enrichments over the metagene comprised of the ORF-enriched genes in the indicated strains grown in SC are shown. Gene averaged profiles Sth1 (E) and Rpb3 (F) at the ORF-enriched genes in indicated strains grown in SC and induced with SM are shown. The *bur1as*/*ctk1*Δ strain without 3MB-PP1 treatment is depicted as *ctk1*Δ. The gray shading represents SEM.

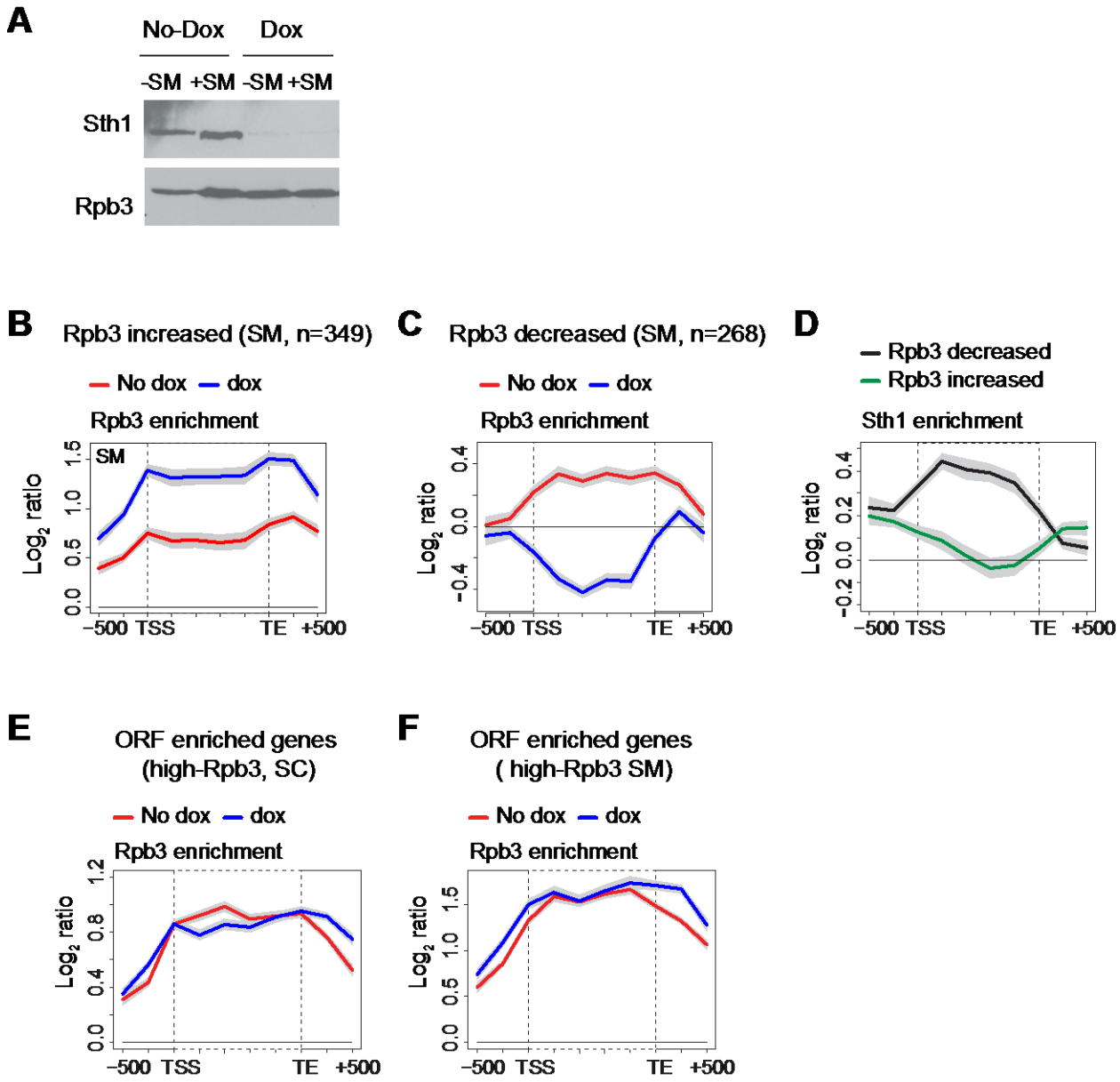

Figure S5 (Related to Figure 5): Sth1 depletion alters Pol II occupancy.
A) The Sth1 protein was depleted by utilizing a conditional knock-down strain (*STH1-TET*) (Hughes et al., 2000) in which the endogenous promoter was replaced by a tetracycline titratable promoter. Culturing cells in the presence of doxycycline resulted in a robust depletion of Sth1 in *STH1-TET* cells in both Gcn4 non-inducing (-SM) and inducing (+SM) conditions. B-C) Genome-wide Rpb3 occupancy was determined in *STH1-TET* cells grown in the presence (dox) and absence (No dox) of doxycycline under SM-inducing conditions. The average ORF occupancy of Rpb3 was determined for each gene, and the change in average ORF occupancy of Rpb3 (Rpb3 in dox versus Rpb3 in No dox) was calculated for individual genes. The genes

displaying an increase in log2 ratio > 0.5 were selected and classified as 'Rpb3 increased' (n=349). Similarly, the genes displaying a reduction in Rpb3 > -0.5 log2 ratio were classified as 'Rpb3 decreased' (n= 268). The gene average profile of Rpb3 binding is shown for Rpb3 increased (B) and Rpb3 decreased (C). D) Sth1 binding data from SM-induced cells is plotted at the genes displaying altered Rpb3 occupancy under Sth1-depleting conditions (increased and decreased Rpb3). E-F) Gene averaged Rpb3 occupancy profile at the ORF-enriched genes (Sth1 occupancy > 0.5 $\log_2$ ratio) with an Rpb3 occupancy > 0.5 $\log_2$ in Sth1-depleted (dox) and – undepleted (No dox) *STH1-TET* cells grown in SC (E) or induced with SM (F). The gray shading represents SEM.

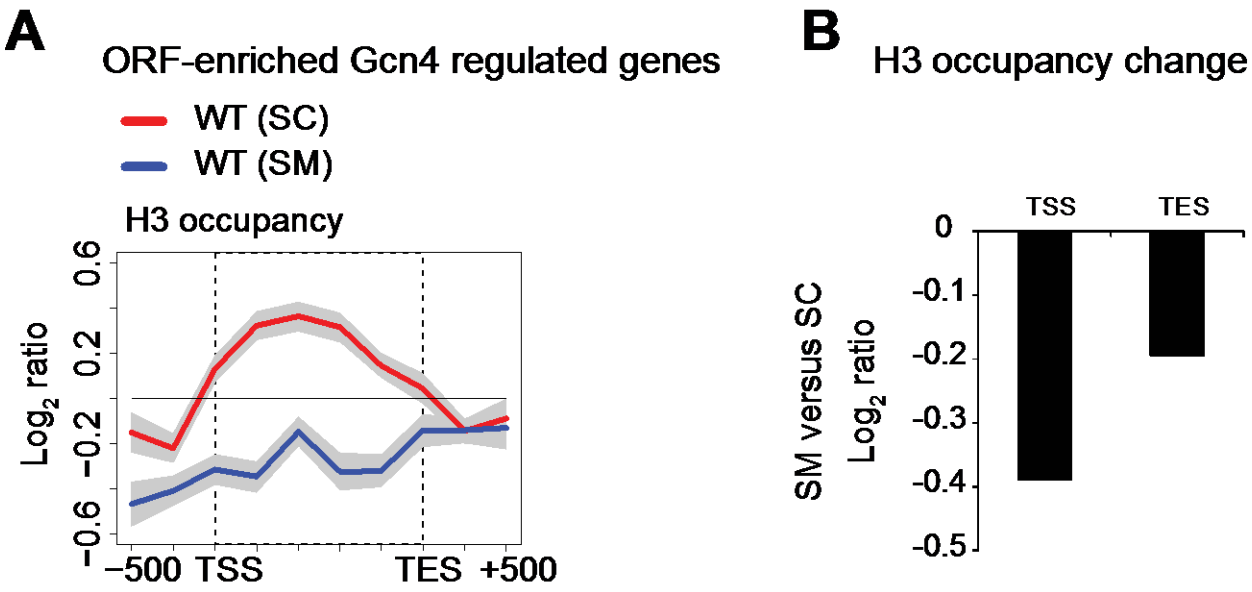

Figure S6 (Related to Figure 6): Histone H3 occupancy at RSC occupied Gcn4 regulated genes.

(A-B) *STH1-TET* cells were induced with SM to activate Gcn4 regulated genes and histone H3 occupancy was determined by ChIP-chip. Gene averaged profile for H3 binding at the Gcn4 regulated genes, which show RSC enrichment in the ORF ($log_2 > 0.5$), is shown under non-inducing (SC) and inducing (SM) conditions (A). The gray shading represents SEM. The change in H3 occupancy in SC versus SM was calculated $\pm$ 250 base pairs around the transcription start site (TSS) and the transcription end site (TES) and is shown in the graph (B).

TABLE S1: List of Strains, Related to Figures 1-6

Name	Parent	Genotype	Reference
CGY 1	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ STH1-myc13::HIS3*	This Study
CGY 7	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ RSC1-myc13::HIS3*	This Study
CGY 12	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ RSC2-myc13::HIS3*	This Study
CGY 17	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ RSC4-myc13::HIS3*	This Study
HQY469	HQY459	*MATa his3Δ leu2Δ met15Δ ura3Δ gcn4Δ::hisG STH1-myc13::HIS3*	Ginsburg, et al., 2009
DG271	DG154	*MATa his3Δ leu2Δ met15Δ ura3Δ gcn5Δ::kanMX4 esa1L254P STH1-myc13::HIS3*	(Ginsburg et al., 2009)
MSY1	HQY957	*MATa his3Δ leu2Δ met15Δ ura3Δ kin28::kanMX4 pHQ1430 [KIN28HA, LEU2] STH1-myc13::HIS3*	This study
MSY2	HQY958	*kin28Δ::kanMX4 pHQ1431 [LEU2 kin28HA-ts16] STH1-myc13:HIS3*	This study
MSY3	HQY959	*kin28Δ::kanMX4 gcn4Δ::kanMX4 pHQ1430 [LEU2 KIN28HA] STH1-myc13:HIS3*	This study
MSY4	yFR762	*MATalpha, ade::higG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0, STH1-myc13::HIS3*	This study
MSY5	yFR912	*MATalpha, ade::higG, his3-Δ200, leu2-Δ0, lys2-Δ0,met15-Δ0, trp1-Δ63, ura3-Δ0,kin28::kin28-L83G,bur2Δ::Leu2, [pSH579, ARSCEN URA3 kin28-L83G]STH1-myc13::HIS3*	This study
MSY6	HQY1219	*MATa his3Δ leu2Δ met15Δ ura3Δ bur1as STH1-myc13::HIS3*	This study
MSY7	HQY1038	*MATa his3Δ leu2Δ met15Δ ura3Δ bur2Δ::kanMX4 STH1-myc13::HIS3*	This study
MSY8	HQY1236	*MATa his3Δ leu2Δ met15Δ ura3Δ bur1as ctk1Δ::kanMX4 STH1-myc13::HIS3*	This study
MSY9	HQY1238	*MATa his3Δ leu2Δ met15Δ ura3Δ ctk1Δ::kanMX4 STH1-myc13::HIS3*	This study
yFR762	S288C	*MATalpha, ade::higG, his3Δ200, leu2Δ0, lys2Δ0, met15Δ0, trp1Δ63, ura3Δ0*	(Bataille et al., 2012)
yFR912	S288C	*MATalpha, ade::higG,his3Δ200, leu2Δ0, lys2Δ0,met15Δ0, trp1Δ63, ura3Δ0,kin28::kin28-L83G, [pSH579,ARS CEN URA3 kin28-L83G]*	(Bataille et al., 2012)
HQY1219	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ bur1as*	(Qiu et al., 2009)
HQY1038	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ bur2Δ::kanMX4*	(Qiu et al., 2009)
HQY1236	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ bur1as ctk1Δ::kanMX4*	(Qiu et al., 2009)
HQY1238	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ ctk1Δ::kanMX4*	(Qiu et al., 2009)
STH1-TET	BY4741	*pSTH1::kanR-tet07-TATA URA3::CMV-tTA MATa*	(Hughes et al.,

		his3Δ1 leu2Δ0 met15Δ0	2000)
MSY10	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ STH1-HA6::HIS3*	This study
MSY11	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ STH1-HA6::HIS3*	This study

TABLE S2: List of primers used for ChIP analysis, Related to Figure 1

Gene	**Location**	**Forward Primers**
ARG1	UAS	ACGGCTCTCCAGTCATTTAT
ARG1	TATA	TAATCTGAGCAGTTGCGAGA
ARG1	5' ORF	TGGCTTATTCTGGTGGTTTAG
ARG1	3' ORF	TTCTGGGCAGATCTACAAAGA
POL I	ORF	GACAAAATGAAGAAAATGCTGATGCACC
Gene	**Region**	**Reverse Primers**
ARG1	UAS	GCAGTCATCAATCTGATCCA
ARG1	TATA	ATGTTCCTTATCGCTGCACA
ARG1	5' ORF	ATCCACACAAACGAACTTGCA
ARG1	3' ORF	AAGTCAACTCTTCACCTTTGG
POL I	ORF	TAATAACCTTGGTAAAACACCCTG

Extended Experimental Procedures

Yeast strain generation

Yeast strains used in this study are listed in Table S1. Myc tagged strains were generated as described in (Govind et al., 2012). Briefly, the plasmids pFA6a-13Myc-His3MX6 (for Myc-tag) and pFA6a-6HA-His3MX6 (for HA-tag) were used as templates to PCR-amplify the 13Myc or 6HA using primers containing homologous sequences flanking the stop codon of the gene of interest (*RSC1*, *RSC2*, *RSC4* and *STH1*). The amplified DNA was used to transform the strains and the tagging was verified by western blot for the expression of the tagged protein.

Growth conditions

For chromatin immunoprecipitation (ChIP), the cells were grown in synthetic complete (SC) or YPD media to an optical density (OD) at 600 nm of ~0.7. For Gcn4 induction, cells were grown in SC to an OD_{600} of ~0.6, treated with 0.65 μM of sulfometuron methyl (SM; Chemservice, cat# N-13254) for 20 minutes and processed for ChIP analysis. To inhibit the kinase activity of Kin28, *kin28as* and *kin28as*/*bur2*Δ strains grown to an OD_{600} of 0.6 were treated with the ATP analog NA-PP1 (6 μM; Cayman chemicals, cat#10954) for 35 minutes, and for inhibiting Bur1 kinase activity, *bur1as* and *bur1as*/*ctk1*Δ cells grown to an OD_{600} of 0.6 were treated with the ATP analog 3MB-PP1 (12 μM; EMD chemicals, cat#52958) for 60 minutes before being processed for ChIP analysis. To inactivate Esa1, the *gcn5*Δ*esa1ts* cells were grown at 25°C to an O.D of 0.5 and moved to 37°C for 1.5-2 hr. To deplete Sth1, the *STH1-TET* cells were grown in the presence of 50 μg/ml doxycycline for ~ 6 hr and processed for ChIP as described below.

Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation was performed as described previously (Govind et al., 2012). Briefly, 100 ml of yeast cultures grown to OD_{600} of ~0.6-0.8 were cross-linked by adding 11 ml cross-linking solution (50 mM HEPES-KOH [pH 7.5], 1 mM EDTA, 100 mM NaCl, 11% formaldehyde) and incubated for 20 minutes at room temperature with intermittent mixing. Cross-linking was quenched by adding 15 ml of 2.5 M glycine. The cells were washed twice with ice cold Tris-buffered saline, collected by centrifugation and stored at -70 °C until further use. The cell pellets were thawed on ice and resuspended in pre-chilled 500 µl of FA-lysis buffer (50 mM HEPES-KOH [pH 7.5], 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate) containing protease inhibitors and ~500 µl of acid-washed glass beads were added to the cell pellets, and disrupted for 45 minutes at 4 °C. The cell extracts were collected and the beads were washed once with 500 µl of FA-lysis buffer. The cell extracts were sonicated using a Branson Sonifier 450 with the output of ~1.8 with 60% duty cycle for 30 seconds. The sonication steps were repeated 10 times, and the samples were cooled on ice for 30 seconds between each sonication step. The soluble chromatin was obtained by centrifuging the sonicated extracts for 30 minutes at 13000 rpm at 4 °C. Using these steps, the chromatin was fragmented to an average size of ~ 200-300 bp. A 50 µl aliquot of chromatin extract was used for immunoprecipitation (IP) using anti-Myc (Roche), anti-Rpb3 (Neoclone), anti-Ser5P (H14; Covance), anti-Ser2P (Bethyl) or anti-H3 (Abcam, ab1791) antibodies, and an identical aliquot was reserved as input. Depending on the specificity of the antibody used for the immunoprecipitation, either 50 µl of anti-mouse (for Myc and Rpb3), 35 µl of anti-rabbit (for H3 and Ser2P) or 40 µl of anti-IgM (for Ser5P) magnetic beads were washed with PBS/BSA (5 mg/ml) and incubated for 3 hr with 1 µl of antibody in 150 µl of PBS/BSA. Unbound antibody was removed by washing twice with 1 ml

of PBS/BSA, and 50 ul of chromatin extracts, 50 ul of FA-lysis buffer and 30 µl of PBS/BSA were added to the antibody-conjugated beads and incubated for an additional 2 hr with continuous mixing at 4 °C. The beads were then washed once with PBS/BSA, twice with wash buffer II (50mM HEPES-KOH, 500 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Triton-X 100), twice with wash buffer III (10mM Tris-Hcl (pH8.0), 250mMLiCl, 1mM EDTA, 0.5% sodium deoxycholate, 0.5% NP-40 substitute) and once with 1X TE. The immunoprecipitated complexes were eluted at 65 °C once by elution buffer I (50mM Tris-HCl, 10mM EDTA, 1% SDS) for 10 minutes and then by elution buffer II (10mM Tris-HCl, 1mM EDTA, 0.67% SDS) for 15 minutes at 65 °C. The input samples were similarly treated with elution buffers and were left overnight in a water bath (65 °C) to reverse the cross-linking. The next day, the samples were treated with proteinase K (20mg/ml, Ambion, cat# AM2548) and DNA was extracted from the samples twice with phenol:chloroform:isoamyl alcohol (IAA), once with chloroform:IAA, and precipitated by ethanol overnight at -70 °C. The DNA was resuspended in 50 µl TE/RNAse (10 µg/ml) and used for conventional ChIP PCRs or ChIP-chip (described below). ChIP DNA as well as the corresponding input DNA was amplified using the oligonucleotides listed in Table S2. PCR reactions were resolved on 6% TBE gels and the amplified DNA was visualized with a phosphoimager after the addition of SYBR green dye and quantified using ImageQuant 5.1 software. The fold enrichments were determined by taking the ratios of the signal for gene of interest (for example, *ARG1*) and the signal obtained for the *POL1* (used as internal control) and dividing by the ratios obtained for the input samples. The equation used is: (ChIP ratio [*ARG1*/*POL1*]) / (Input ratio [*ARG1*/*POL1*]). The ChIP experiments were performed using at least three independent cultures, and PCR reactions were conducted at least in duplicates. The error bars for ChIP experiments represent SEM.

ChIP-chip Experiments

Chromatin immunoprecipitated DNA as well as corresponding input DNA samples were amplified using the GenomePlex complete whole genome amplification (WGA) kit (Sigma, cat # WGA2). 10 ng of IP DNA and Input chromatin were separately amplified per the manufacturer instructions except that the initial fragmentation step was eliminated. If one amplification reaction did not provide enough material for array hybridization two amplification reactions were pooled. For hybridization to the Affymetrix tiling arrays (P/N 520055), the PCR amplified samples were fragmented to generate the 30-70bp fragments. This was achieved by treating amplified DNA samples with DNase I (1u/50ul reaction) for 5 minutes at 37 °C and quickly transferring to a boiling water bath for 15 minutes to inactivate the enzyme. Fragments were biotin labeled with the Gene ChIP WT Double Stranded DNA Terminal Labeling Kit (Sigma) per the manufacturer's instructions. Hybridization to Affymetrix tiling arrays (P/N 520055) was performed at the genomic core facility (Wadsworth Center, New York) as per the manufacturer instructions. For hybridization to the Agilent 4x44K arrays (G4493A), the ChIP and input DNA were amplified as described above for the Affymetrix arrays. The ChIP and input samples were labeled with Cy3 and Cy5 (or Cy5 and Cy3) using the BioPrime Array CGH Genomic Labeling Module (Invitrogen) and hybridized to 4x44 K arrays as per manufacturer instructions at the core facility at Wadsworth Center, New York. Data from the replicates (at least duplicate) were combined and analyzed as described below.

Bioinformatics analysis

Data from Affymetrix tiling arrays were processed with Affymetrix GCOS software and analyzed using Affymetrix Tiling Analysis Software (TAS)

(http://www.affymetrix.com/support/developer/downloads/TilingArrayTools/index.affx), and the BPMAP file 2006Feb_S288c_All_BothStrands_7G.bpmap (http://www-sequence.stanford.edu/S288c/bpmap.html). A two-sample analysis was conducted using two independently derived ChIP samples as the "treatment" group and two corresponding input samples (Lee et al., 2007) as the "control" group for both wild type and *gcn4*Δ cells induced with SM. Data were normalized using built-in quantile normalization and probe-level analysis with both perfect match and mismatch (PM/MM) probes and run with a bandwidth of 30. Occupancy profiles were visualized with Affymetrix Integrated Genome Browser (IGB) (http://www.affymetrix.com/support/developer/tools/download_igb.affx). The signal values of all probes falling within each ORF's coordinates were summed and averaged to obtain average Sth1 occupancy in the coding regions. Thirty-nine ORFs had missing probes within their coordinate boundaries and were ignored.

For the analysis of Agilent arrays, the arrays were scanned with Agilent DNA microarray scanner and extracted with the Feature extraction software (Agilent). The data were read into R software and the data normalization was performed using Limma package from Bioconductor as described previously (Venkatesh et al., 2012). In brief, the occupancies of Rpb3, Sth1 or H3 were calculated by averaging the normalized $\log_2$ values (IP/input) for the probes over the coding regions (ORF occupancy) or in the region 500 bp upstream of the transcription start sites (TSS) (promoter occupancy). Genes with an average Sth1 occupancy of > $\log_2$ 0.5 ratio in coding regions were considered to be "ORF-enriched". Similarly, genes with an average Sth1 occupancy > log2 0.5 ratio in the intergenic regions were considered to be "promoter-enriched". Intergenic regions which were not uniquely assignable to a particular gene were excluded while analyzing

the promoter-enriched genes. As such, 2590 promoter regions were analyzed to compute promoter-enriched genes.

The gene averaged profiles were generated in the following manner. The genes were divided into 10 equal sized bins. Two bins were assigned to the region 500 bp before the transcription start site (TSS) and two bins to the 500 bp region after the transcription end site (TES). The region encompassing the TSS and TES were assigned 6 bins. The average probe enrichment values were assigned to the closest bin according to the probe location, and a 10 bin matrix was generated using a PERL script. About 1321 sequences belonging to the genes not regulated by Pol II, tRNA genes, majority of dubious ORFs, small nuclear RNA genes as well as autonomously replicating sequences (ARS) were filtered out from the analysis. The metagene profiles (Figures 2-6 and S2-S5) were generated by R software using modified matrices corresponding to a particular set of genes. The standard error of mean was calculated in R software and is included as grey shade around the average plot line.

Coimmunoprecipitation Experiments

Co-IP experiments were performed as described previously (Govind et al., 2010) with the following modifications. Cells expressing Sth1-Myc, Sth1-HA and Rsc4-HA were grown to an OD_{600} of ~1.5-2. Cells were collected by centrifugation and were disrupted in lysis buffer (50 mM Tris-HCl, pH-7.5,50 mM HEPES-KOH, pH 7.9, 10 mM MgSo4, 100 mM $(NH_4)_2SO_4$, 12.5 mM KOAc, .01% NP-40, 20% Glycerol, 1ug/ml Pepstatin A, 100 mM PMSF, 1ug/ml Leupeptin; 4X MTB: 200 mM HEPES-KOH pH 7.9, 800 mM KOAc, 54 mM MgOAc2, 40% Glycerol, 0.04% NP-40, 400 mM PMSF, 4 ug/ml Pepstatin, 4ug/ml Leupeptin) by vortexing with glass beads for 18 seconds followed by 1.5 minutes on ice. This step was repeated 10 times. Anti-

mouse or anti-rabbit IgG conjugated beads were pre-incubated with primary antibody (anti-Myc or anti-HA) for three hours. After washing twice with PBS/BSA (5%), whole cell extracts were added to the antibody-bead complexes, and incubated for 2 hr at 4°C on a tube rotator. Post-incubation, beads were washed once with 1 ml of lysis buffer, and 3 times with wash buffer (50 mM Tris-HCl (pH-8.0), 0.1% NP-40, 150 mM NaCl, 10% Glycerol, 1mM PMSF, 1ug/ml Leupeptin, and 1ug/ml Pepstatin). Immunoprecipitates were analyzed by western blot using anti-myc (Roche), anti-HA 3F10 (Roche), anti-Ser5P (H14; Covance) and anti-Ser2P (Bethyl) antibodies. The experiments were performed using three independent cultures.

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