SUPPLEMENTARY INFORMATION

Supplementary Materials and Methods

Supplementary Figure S1

Additional ChIP analyses supporting phosphorylation of Thr4 on RNAP II-transcribed genes

Supplementary Figure S2

RT-PCR analysis validation of RNA-seq data showing elevated expression of PHO gene RNAS in T4V strain

Supplementary Figure S3

Scatterplot comparing RNA-seq analyses of WT versus T4V and CONS strains

Supplementary Figure S4

Complete results for T4V SGA analysis.

Supplementary Figure S5

Analysis of Htz1 eviction in CONS strain

Supplementary Tables S1, S2 and S3

RNA seq statistics for Datasets S1 (Table S1) and S2 (Table S2); List of yeast strains used in this study (Table S3)

Datasets S1 and S2

Full RNA sequencing results for CONS vs T4V analysis (Dataset S1; RNA-seq) and CONS vs WT analysis (Dataset S2; 3′READS), as Excel spreadsheets

MATERIALS AND METHODS

Yeast strains and growth media.

Yeast strains used are listed in Table S3. T4V and CONS strains were constructed by PCR amplification of corresponding CTDs generated in preparation for our previous study (1), followed by transformation into haploid S288C or W303 strains. WT, CONS, and T4V strains were marked with NAT-MX cassette 3′ of the *RPO21* gene. Low P_i medium was prepared by dissolving 10 g/L yeast extract, 20 g/L peptone and 2.45 g/L magnesium sulfate in water, then adding drop-wise 8 mL/L ammonium hydroxide while mixing vigorously. The medium was allowed to sit for several hours on a bench to allow salts to precipitate, which were then filtered twice by vacuum filtration (0.22 μm). Glucose was then added to 2% (w/v), the pH was adjusted to ~7.0 with hydrochloric acid, and the medium was autoclaved. For solid medium, agar was added after pH adjustment. A yeast strain lacking *PHO4* was unable to grow specifically on the low P_i medium confirming it was successfully depleted of phosphate (Figure 2C). For liquid growth on low P_i medium, samples that had grown overnight in SC medium were washed then placed in low P_i medium for 5-6 hours before analysis. For liquid growth in galactose medium, samples were first grown in SC plus raffinose (2% w/v), then galactose was added to 5% (w/v) for one hour before samples were analyzed. SGA screen, yeast spot assays and media used were as previously described (2, 3).

ChIP, RNA and protein analyses.

Chromatin fractionation was performed as previously described (4) in strain W303a. For ChIP analyses, 50-mL cultures were grown and cells were lysed by bead-beating three times for one min each with one min in between in a cold room, but, otherwise, samples were processed and analyzed, and data quantified, as previously described (3). Western blot analyses and semi-

quantitative PCR were as previously described (2, 3, 5). Random hexamer oligonucleotides were used with total RNA for the RT-PCR analyses. Where statistical analysis was performed for ChIP, a two-tailed Student's *t*-test was used, with *P*-values indicated above bars in the graph. Error bars in graphs represent standard deviations of at least three experiments. Antibodies used for ChIP and western blotting were HA (abm), GAPDH (Sigma), T4p (Novus), and Rpb1 (y-80; Santa Cruz). Primer sequences used for cloning, ChIP and RT-PCR analyses are available upon request.

RNA sequencing.

For RNA sequencing analyses, the integrity of total RNA was measured by Agilent Bioanalyzer. RNA samples with an integrity number >8.0 were used for further processing. For T4V vs WT samples, total RNA was subjected to 2 rounds of poly(A) selection using oligo-dT magnetic beads (NEB) followed by fragmentation using Ambion's RNA fragmentation kit at 70°C for 5 min. Fragmented RNA was purified by ethanol precipitation followed by dephosphorylation using recombinant shrimp alkaline phosphatase (NEB) and then phosphorylation using T4 kinase (NEB). Phosphorylated RNA was then purified by RNeasy kit (Qiagen) and was sequentially ligated to a 5′-adenylated 3′ adapter (5′-/5rApp/TGGAATTCTCGGGTGCCAAGG/3ddC/) with the truncated T4 RNA ligase 2 (NEB) and to a 5′ adapter (5′-GUUCAGAGUUCUACAGUCCGACGAUC) with T4 RNA ligase 1 (NEB). The resultant RNA was reverse-transcribed to cDNA with Superscript III (Invitrogen), and the cDNA was amplified by 12 cycles of PCR with Phusion high fidelity polymerase (NEB). Amplified cDNAs were separated on 8% polyacrylamide gels. cDNAs containing an inset size ~100 nt were purified from the gel based on the size. The quality and quantity of the cDNA library were evaluated by Agilent Bioanalyzer and qPCR analysis. The strand-specific cDNA

libraries generated by this protocol were sequenced on an Illumina Genome Analyzer IIx. RNA sequencing of CONS and WT samples in SC or XY media (Dataset S2) was performed using 3′READS, as previously described (6). Both RNA sequencing methods produced equivalent results, as determined by comparison of T4V vs WT samples analyzed by both methods.

REFERENCES

1. Hsin JP, Sheth A & Manley JL (2011) RNAP II CTD phosphorylated on threonine-4 is required for histone mRNA 3' end processing. *Science* 334(6056): 683-6.

2. Rosonina E, Duncan SM & Manley JL (2010) SUMO functions in constitutive transcription and during activation of inducible genes in yeast. *Genes Dev* 24(12): 1242-1252.

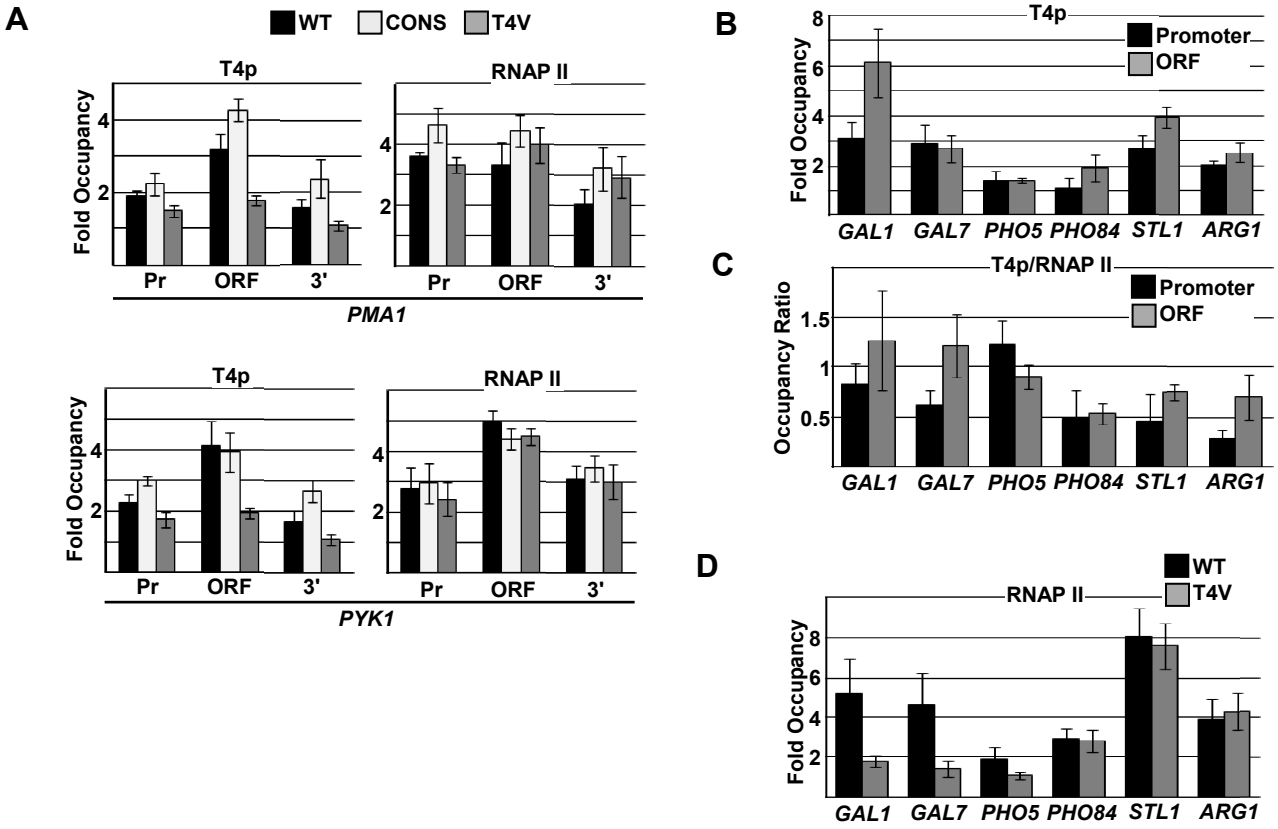
3. Rosonina E, Willis IM & Manley JL (2009) Sub1 functions in osmoregulation and in transcription by both RNA polymerases II and III. *Mol Cell Biol* 29(8): 2308-2321.

4. Svejstrup JQ, Petrakis TG & Fellows J (2003) Purification of elongating RNA polymerase II and other factors from yeast chromatin. *Methods Enzymol* 371: 491-8.

5. Rosonina E, Duncan SM & Manley JL (2012) Sumoylation of transcription factor Gcn4 facilitates its Srb10-mediated clearance from promoters in yeast. *Genes Dev* 26(4): 350-355.

6. Hoque M, *et al* (2013) Analysis of alternative cleavage and polyadenylation by 3' region extraction and deep sequencing. *Nat Methods* 10(2): 133-139.

Supplementary Figure S1.



Thr4 is phosphorylated on RNAP II-transcribed genes.

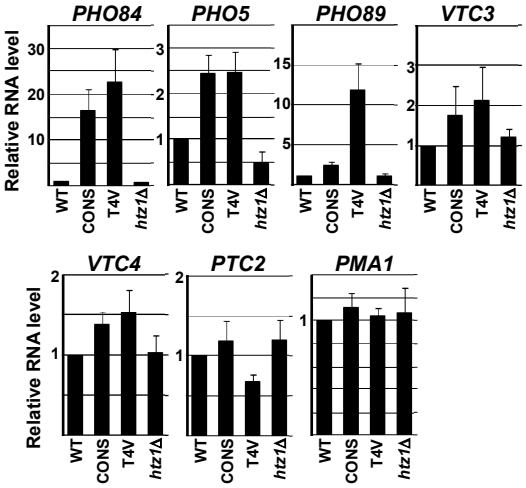
(A) ChIP analysis of phosphorylated Thr4 on CTD repeats (T4p) or RNAP II in WT, CONS, or T4V strains at promoter-proximal (Pr), ORF, or 3' regions of constitutively expressed *PMA1* and *PYK1* genes. Data are represented as mean +/- SD of three independent experiments.

(B) ChIP analysis of phosphorylated Thr4 on CTD repeats (T4p) on promoter-proximal or ORF regions of indicated genes under their respective induced conditions. Data are represented as mean +/- SD of three independent experiments.

(C) ChIP values from *B* were normalized to RNAP II levels, determined in the same samples at the same positions with an Rpb1 antibody. Data are represented as mean +/- SD of three independent experiments.

(D) ChIP analysis of RNAP II occupancy at promoters of indicated genes under their respective induced conditions in WT or T4V cells. Data are represented as mean +/- SD of three independent experiments.

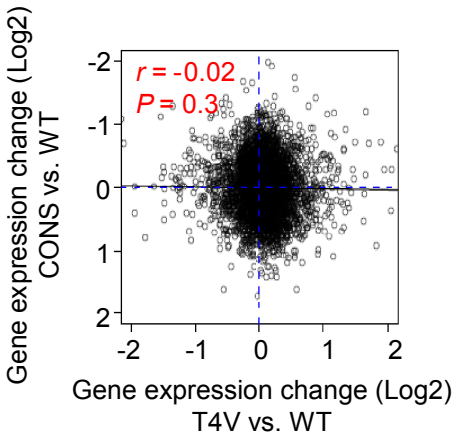
Supplementary Figure S2.



Elevated expression of PHO gene RNAs in T4V

RT-PCR validation of selected up- or down-regulated genes, with *PMA1* control, in WT, CONS, T4V, and *htz1*Δ strains. Total RNA from samples grown in normal (SC) medium was analyzed by semi-quantitative RT-PCR using random hexamer oligonucleotides for cDNA synthesis.

Supplementary Figure S3.



(4,610 genes with average read # >=20)

Comparison of RNA sequencing in CONS vs WT and T4V vs WT analyses.

Scatterplot comparing gene expression changes from CONS vs WT (Dataset S1) and T4V vs WT (Dataset S2) RNA sequencing analyses.

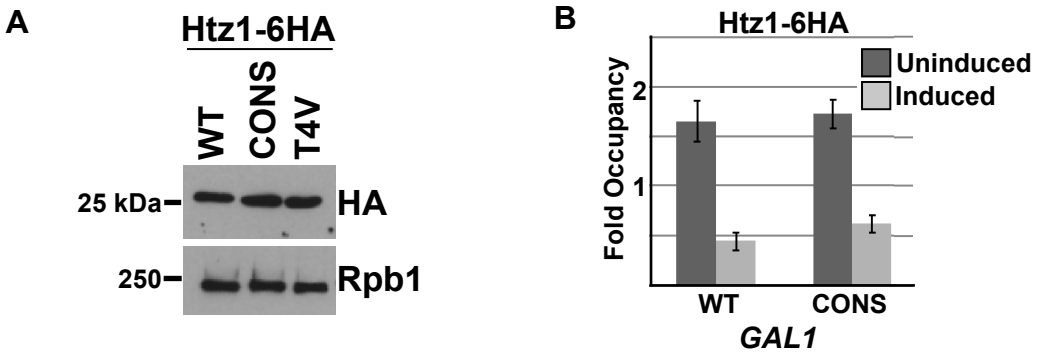
Supplementary Figure S4.

RNAP II TRANSCRIPTION			
	YHR041C	*SRB2*	Subunit of the RNA polymerase II mediator complex
	YHR167W	*THP2*	Subunit of the THO and TREX complexes
	YML062C	*MFT1*	Subunit of the THO complex
	YIL128W	*MET18*	DNA repair and TFIIH regulator
	YOL051W	*GAL11*	Subunit of the RNA polymerase II mediator complex
	YLR226W	*BUR2*	Cyclin for Bur1 Rpb1 CTD kinase
Chromatin Modification/Organization:			
*	YAL011W	*SWC3*	Component of the SWR1 complex
	YBR175W	*SWD3*	Subunit of the COMPASS (Set1C) histone methylation complex
*	YBR231C	*SWC5*	Component of the SWR1 complex
	YBR289W	*SNF5*	Subunit of the SWI/SNF chromatin remodeling complex
*	YDR334W	*SWR1*	Swi2/Snf2-related ATPase that is the structural component of the SWR1 complex
	YGL058W	*RAD6*	Ubiquitin-conjugating enzyme (E2); ubiquitylates histone H2B at K123
	YGL244W	*RTF1*	Subunit of RNAPII-associated chromatin remodeling Paf1 complex
*	YML041C	*VPS71*	Nucleosome-binding component of the SWR1 complex
	YNL215W	*IES2*	Protein that associates with the INO80 chromatin remodeling complex
	YNL097C	*PHO23*	Probable component of the Rpd3 histone deacetylase complex
**	YOL012C	*HTZ1*	Histone variant H2AZ, exchanged for histone H2A in nucleosomes by the SWR1 complex
	YPL055C	*LGE1*	Gene requried for H2B ubiquitination and H3 K4 methylation (REF: Hwang WW .. Madhani HD Mol Cell 2003)
OTHER			
	YBR275C	*RIF1*	Telomere maintenance
	YER122C	*GLO3*	ADP-ribosylation factor GTPase activating protein
	YER151C	*UBP3*	Ubiquitin-specific protease involved in transport and osmotic response; interacts with Bre5p
	YHR043C	*DOG2*	2-deoxyglucose-6-phosphate phosphatase
	YIL006W	*YIA6*	Mitochondrial NAD+ transporter
	YKL204W	*EAP1*	eIF4E-associated protein
	YMR198W	*CIK1*	Kinesin-associated protein required for both karyogamy and mitotic spindle organization
	YNL248C	*RPA49*	RNA polymerase I subunit A49
	YNR027W	*BUD17*	Putative pyridoxal kinase
	YNR047W	*FPK1*	Ser/Thr protein kinase that regulates the putative phospholipid translocases Lem3p-Dnf1p/Dnf2p
	YNR051C	*BRE5*	Ubiquitin protease cofactor, forms deubiquitination complex with Ubp3
	YPR101W	*SNT309*	Member of the NineTeen Complex (NTC) s

List of genes strongly interacting genetically with T4V mutation in SGA screens

Two SGA analyses were performed with WT and T4V strains. Stains expressing T4V and each of ~4700 deleted non-essential genes were inspected for growth defects compared to the same gene deletion in WT strains. Gene deletions causing the most severe growth defect in combination with T4V in both screens are indicated with locus name, gene name and brief description.

Supplementary Figure S5.



Htz1 is eviction from the activated *GAL1* promoter is unaffected in the CONS strain.

(A) Western blot analysis of WT, CONS, and T4V strains expressing 6x HA tagged Htz1. Levels of Htz1-6HA and RNAP II (Rpb1) in the same samples are shown.

(B) ChIP analysis of Htz1-6HA occupancy at the *GAL1* promoter before and after induction with galactose in WT or CONS strains.

Supplementary Table S1. RNA-seq statistics for WT and T4V analysis.

Sample	No. of raw reads	No. of uniquely mapped reads
T4V replicate 1	4,216,055	3,601,290
T4V replicate 2	5,374,604	4,580,668
WT replicate 1	5,048,318	4,333,368
WT replicate 2	4,502,798	3,897,106

Supplementary Table S2. RNA-seq statistics for WT and CONS analysis.

Sample	**No. of raw reads**	**No. of uniquely mapped reads**
SC medium, CONS ("26p"), replicate 1	**8,974,658**	**2,804,521**
SC medium, CONS ("26p"), replicate 2	**10,825,427**	**3,353,291**
SC medium, WT, replicate 1	**6,436,125**	**1,813,413**
SC medium, WT, replicate 2	**6,778,954**	**1,935,444**
XY (rich) medium, CONS ("26p"), replicate 1	**9,905,275**	**3,015,817**
XY (rich) medium, CONS ("26p"), replicate 2	**4,563,665**	**1,416,412**
XY (rich) medium, WT, replicate 1	**10,244,166**	**3,249,571**
XY (rich) medium, WT, replicate 2	**8,221,832**	**2,357,730**

Supplementary Table S3. Yeast Strains Used in this Study
All derivative strains were constructed for this study.

Strain	Genotype
W303a	*MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
The following strains were derived from W303a.	
ERYM745A	*RPB1::RPB1-NatR*
ERYM746A	*rpb1::rpb1-CTD(CONS)$_{26}$-NatR*
ERYM747B	*rpb1::rpb1-CTD(T4V)$_{24}$-NatR*
ERYM748A	*htz1Δ ::kanMX*
ERYM779A	*ino80Δ900::kanMX HTZ1-6HA::Kl TRP1*
ERYM749A	*RPB1::RPB1-NatR HTZ1-6HA::Kl TRP1*
ERYM750A	*rpb1::rpb1-CTD(CONS)$_{26}$-NatR HTZ1-6HA::Kl TRP1*
ERYM751B	*rpb1::rpb1-CTD(T4V)$_{24}$-NatR HTZ1-6HA::Kl TRP1*
NYYM152A	*ybr184wΔ::kanMX*
NYYM153A	*ybr184wΔ::kanMX rpb1::rpb1-CTD(T4V)$_{24}$-NatR*
NYYM145A	*swr1Δ::kanMX*
NYYM146A	*swr1Δ::kanMX rpb1::rpb1-CTD(T4V)$_{24}$-NatR*
NYYM147A	*swc5Δ::kanMX*
NYYM148A	*swc5Δ::kanMX rpb1::rpb1-CTD(T4V)$_{24}$-NatR*
The following strains were derived from the BY4172 background.	
Y7092	*can1Δ::STE2pr-SpHis5 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lyp1Δ LYS2+*
NYYM115A	*rpb1::rpb1-CTD(CONS)$_{26}$-NatR* (derived from Y7092)
NYYM116C	*rpb1::rpb1-CTD(T4V)$_{24}$-NatR* (derived from Y7092)