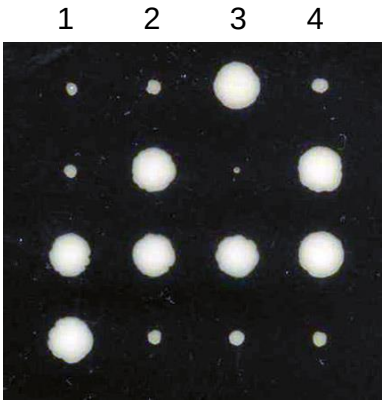
Figure S1

A


1
2
3
4

B

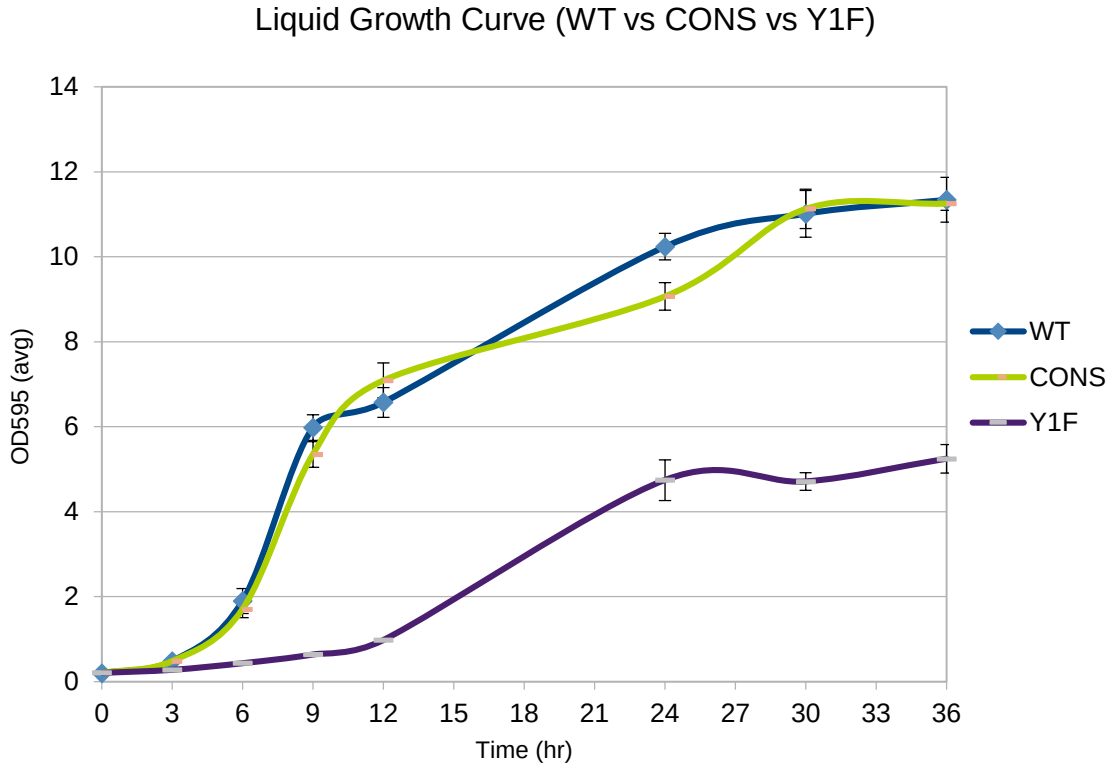
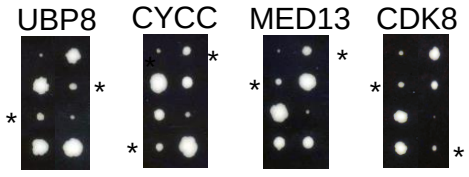
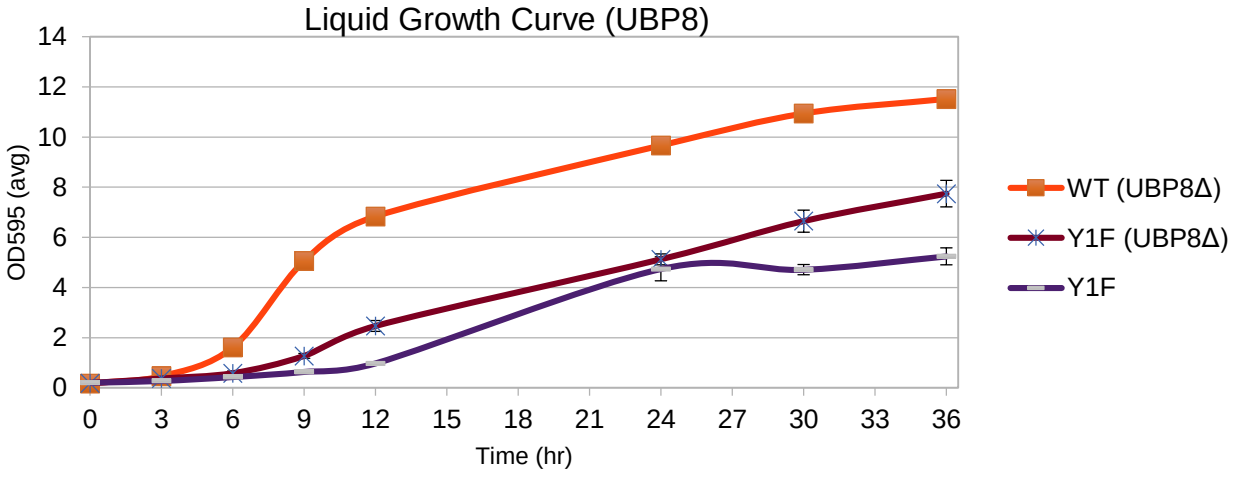

Liquid Growth Curve (WT vs CONS vs Y1F)
OD595 (avg)
Time (hr)
0
2
4
6
8
10
12
14
0
3
6
9
12
15
18
21
24
27
30
33
36
WT
CONS
Y1F

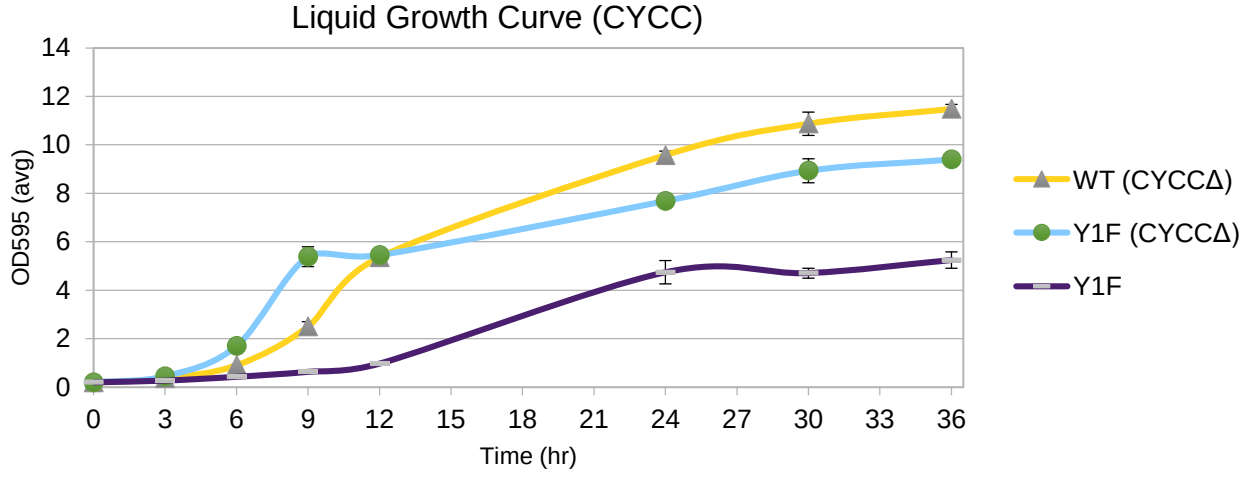
Figure S2

A


UBP8
CYCC
MED13
CDK8

B


Liquid Growth Curve (UBP8)
OD595 (avg)
Time (hr)
WT (UBP8Δ)
Y1F (UBP8Δ)
Y1F


Liquid Growth Curve (CYCC)
OD595 (avg)
Time (hr)
WT (CYCCΔ)
Y1F (CYCCΔ)
Y1F

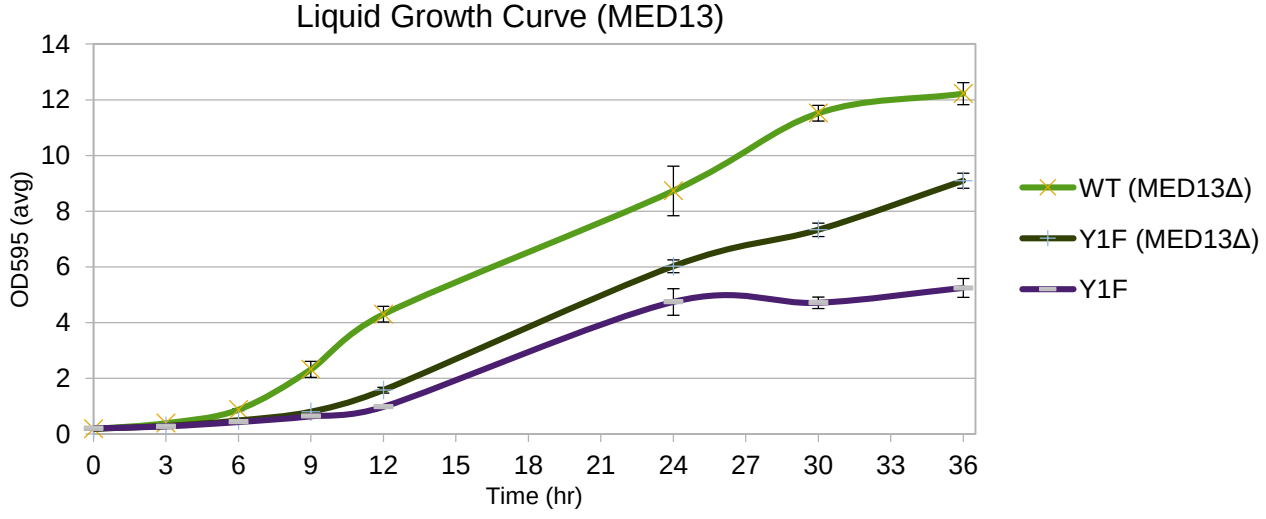
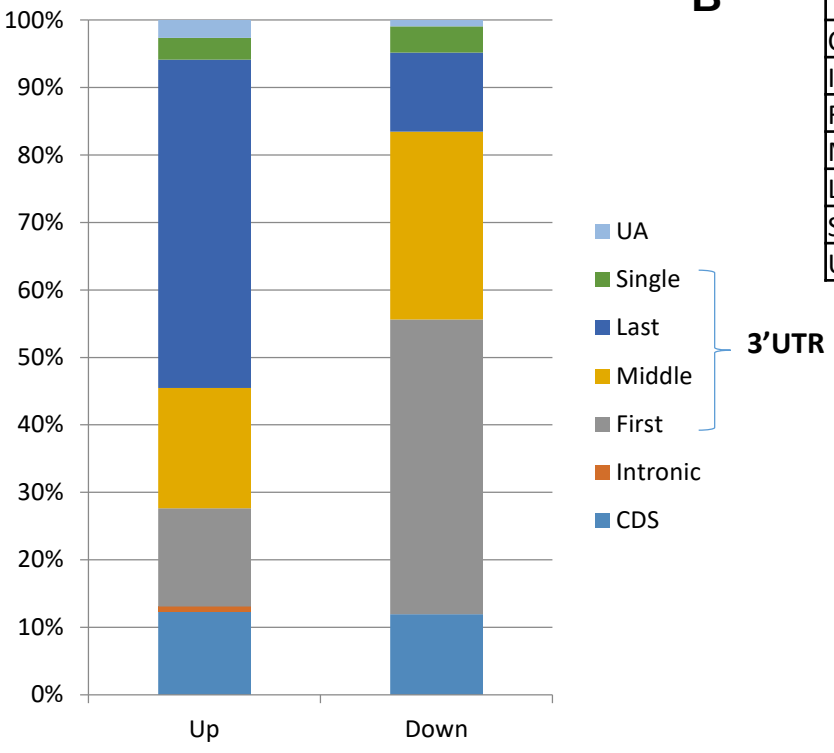

Liquid Growth Curve (MED13)
OD595 (avg)
Time (hr)
WT (MED13Δ)
Y1F (MED13Δ)
Y1F

Figure S3

A



B

	Up	Down	No_sign
CDS	65	52	3052
Intronic	4	0	23
First	77	190	3089
Middle	94	121	4326
Last	257	51	3048
Single	17	17	325
UA	14	4	951

C

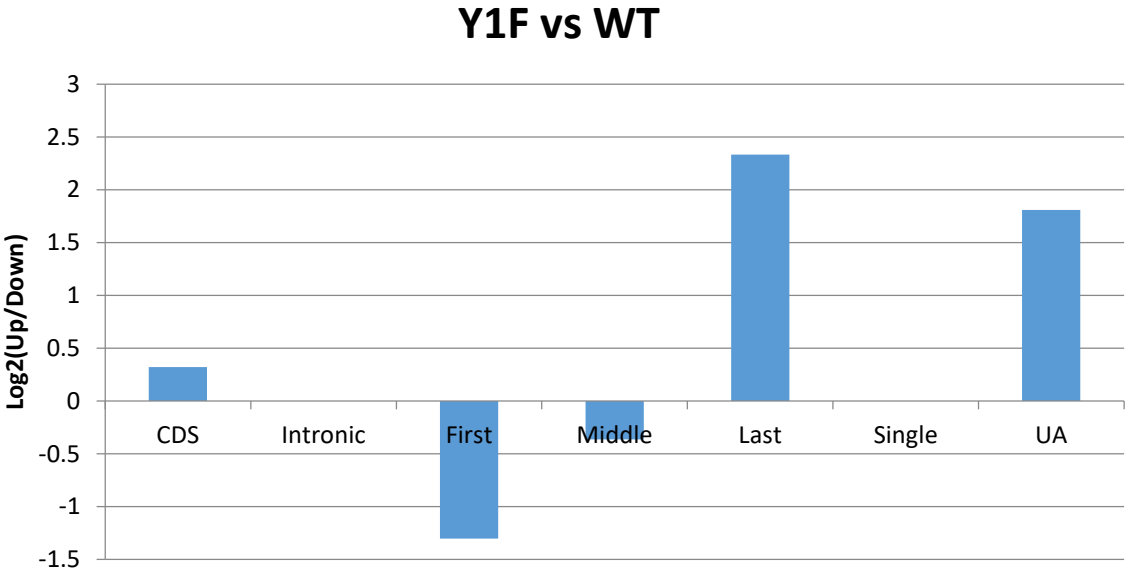


Figure S4

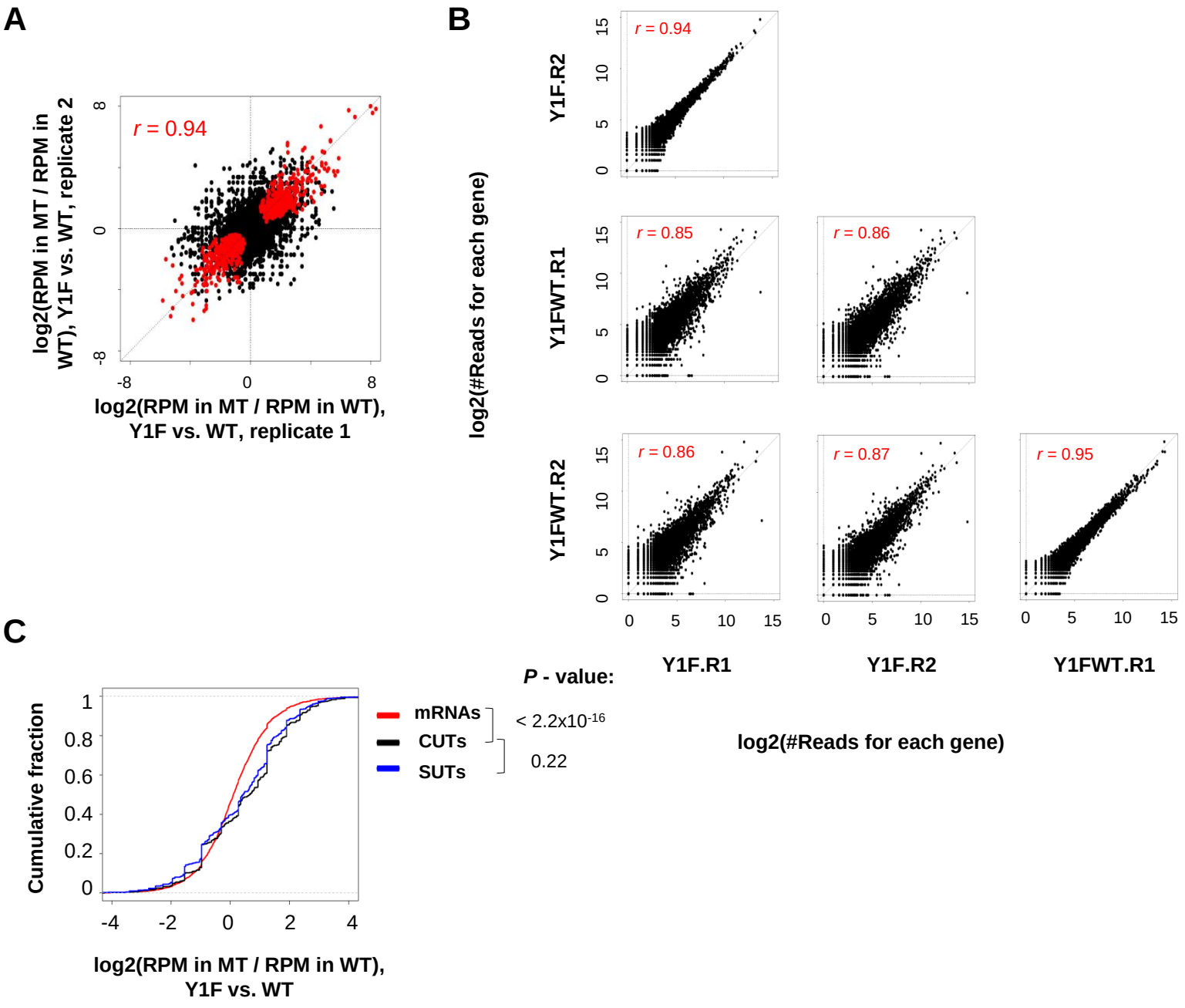
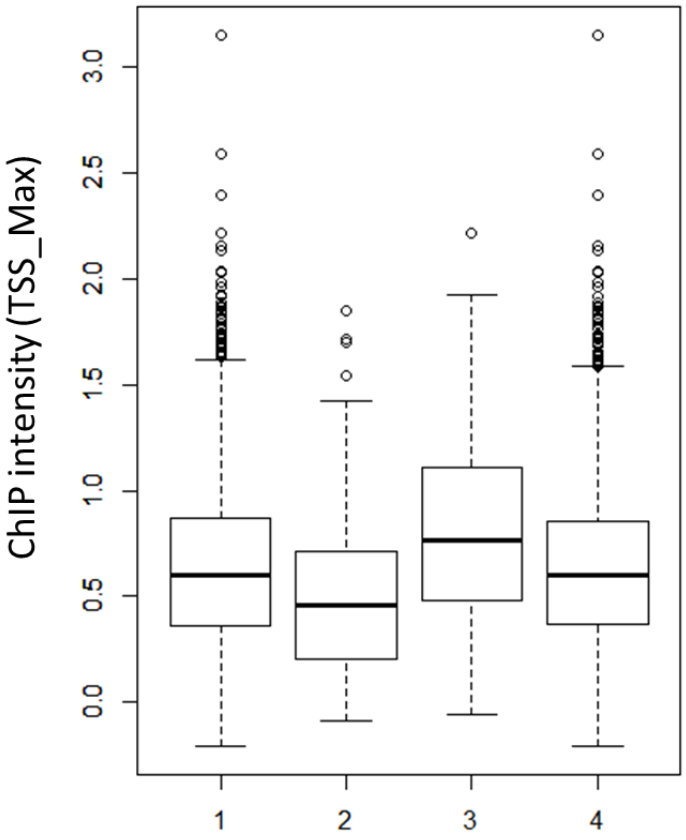

A
r = 0.94
log2(RPM in MT / RPM in WT), Y1F vs. WT, replicate 2
log2(RPM in MT / RPM in WT), Y1F vs. WT, replicate 1
B
log2(#Reads for each gene)
r = 0.94
r = 0.85
r = 0.86
r = 0.86
r = 0.87
r = 0.95
Y1F.R2
Y1FWT.R1
Y1FWT.R2
Y1F.R1
Y1F.R2
Y1FWT.R1
log2(#Reads for each gene)
C
Cumulative fraction
mRNAs
CUTs
SUTs
P - value:
< 2.2x10-16
0.22
log2(RPM in MT / RPM in WT), Y1F vs. WT

Figure S5

Summary of Tyr1P ChIP intensity in each gene set



1. All genes (4530)
2. Up-regulated genes (222)
3. Down-regulated genes (276)
4. No-changed genes (4032)

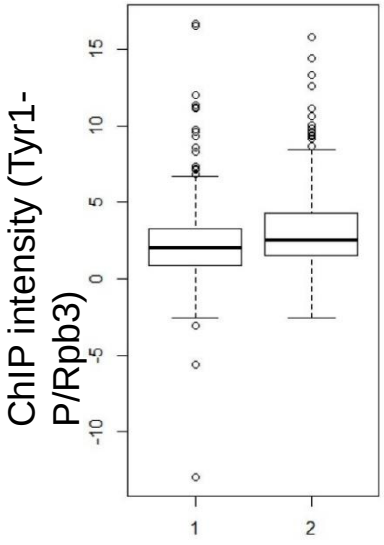
TSS_Max	Group1	Group2	Group3	Group4
Min. :	-0.2034	-0.08644	-0.05819	-0.2034
1st Qu.:	0.3649	0.20549	0.48645	0.3697
Median :	0.6005	0.45741	0.76779	0.6000
Mean :	0.6332	0.48432	0.80010	0.6300
3rd Qu.:	0.8666	0.70770	1.10623	0.8557
Max. :	3.1474	1.84801	2.21684	3.1474

1. Tyr1P ChIP signal in Up-regulated gene set is weaker than No-changed gene set.
   (Welch two sample t-test, p-value = 4.449e-08)
2. Tyr1P ChIP signal in Down-regulated gene set is stronger than No-changed gene set.
   (Welch two sample t-test, p-value = 6.603e-10)

Figure S6

A

Summary of normalized Tyr1P/Rpb3 ChIP intensity in each gene set



1. Up-regulated genes (217)
2. Down-regulated genes (272)

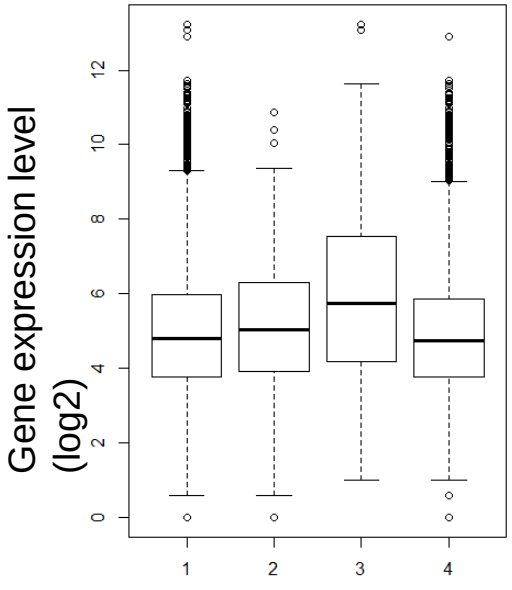
Extreme values (samples with very low Rpb3-ChIP signal: -0.015< Rpb3 < 0.015) were removed from each gene set.
(5 Up-regulated and 4 Down-regulated genes were discarded.

Rpb3	Group1	Group2
Min. :	-12.980	-2.546
1st Qu.:	0.898	1.504
Median :	2.045	2.554
Mean :	2.553	3.296
3rd Qu.:	3.265	4.326
Max. :	16.721	15.795

Tyr1P/Rpb3 ChIP signal in the Down-regulated gene set is stronger than in the Up-regulated gene set. (Welch two sample t-test, p-value = 0.004854)

B

Summary of expression level in each gene set



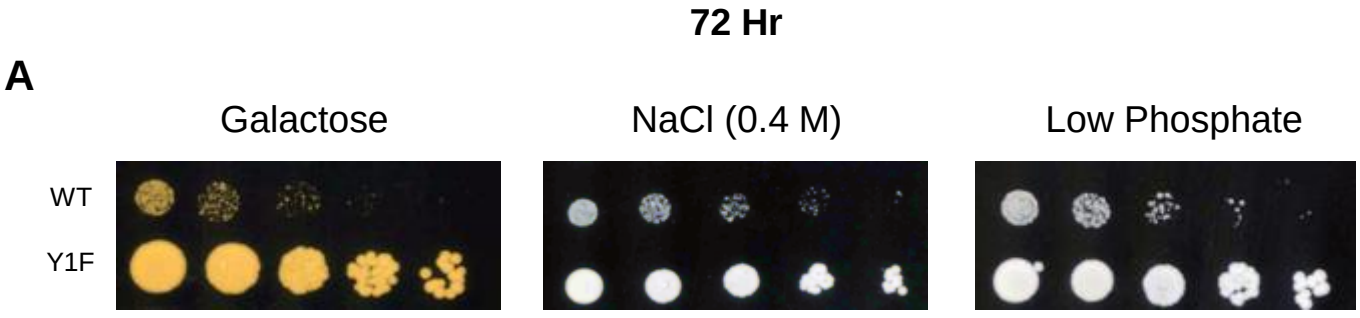
1. All genes (4530)
2. Up-regulated genes (222)
3. Down-regulated genes (276)
4. No-changed genes (4032)

Exp	Group1	Group2	Group3	Group4
Min.:	0.000	0.000	1.000	0.000
1st Qu. :	3.755	3.907	4.180	3.755
Median :	4.781	5.022	5.728	4.728
Mean :	5.003	5.155	5.964	4.928
3rd Qu.:	5.977	6.313	7.535	5.858
Max. :	13.223	10.880	13.223	12.898
NA's :	543	21	30	492

1, Expression level of Up-regulated gene set is slightly stronger than No-changed gene set.
(Welch two sample t-test, p-value = 0.08967)

2, Expression level of Down-regulated gene set is stronger than No-changed gene set.
(Welch two sample t-test, p-value = 1.534e-10)

Figure S7


72 Hr
A
Galactose
NaCl (0.4 M)
Low Phosphate
WT
Y1F

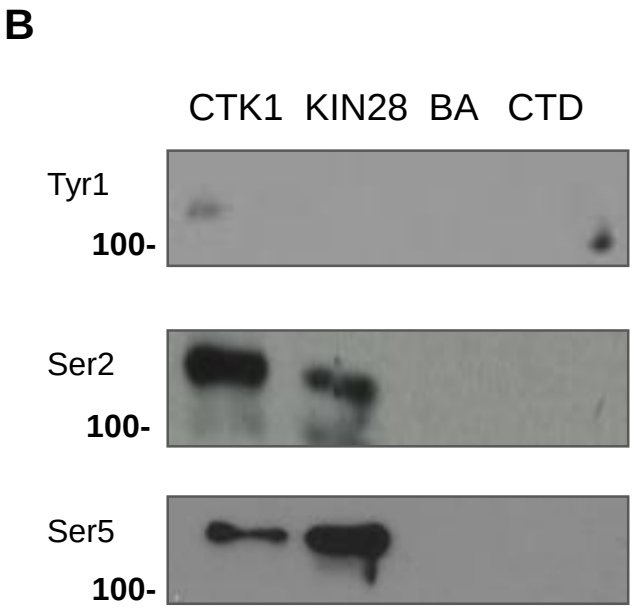

B
CTK1 KIN28 BA CTD
Tyr1
100-
Ser2
100-
Ser5
100-

Table S1

Strain	Genotype
BY4741	MATa his3del1 leu2del0 met15del0 ura3del0
BY4742	MATalpha his3del1 leu2del0 lys2del0 ura3del0
BY4743	(Diploid) MATa/MATalpha his3del1/his3del1 leu2del0/leu2del0 lys2del0/+ met15del0/+ ura3del0/ura3del0
The following strains are haploids derived from BY4743	
NYYM203A	rpb1::rpb1-CTD(Y1F)26-NatR (Y1F 1)
NYYM204D	rpb1::rpb1-CTD(Y1F)26-NatR (Y1F 2)
NYYM205A	rpb1::rpb1-CTD(CONS)26-NatR
NYYM208A	ygl178cdel::kanMX rpb1::rpb1-CTD(Y1F)26-NatR
NYYM209A	ygl178cdel::kanMX
NYYM210C	ubp8del::kanMX rpb1::rpb1-CTD(Y1F)26-NatR
NYYM211A	ubp8del::kanMX
NYYM212B	ssn3del::kanMX rpb1::rpb1-CTD(Y1F)26-NatR
NYYM213A	ssn3del::kanMX
NYYM214A	ssn8del::kanMX rpb1::rpb1-CTD(Y1F)26-NatR
NYYM215A	ssn8del::kanMX
NYYM216B	ssn2del::kanMX rpb1::rpb1-CTD(Y1F)26-NatR
NYYM217A	ssn2del::kanMX
NYYM218D	SSN8::3HA HIS3+ rpb1::rpb1-CTD(Y1F)26-NatR
NYYM219A	SSN8::3HA HIS3+
NYYM220B	SSN3::3HA HIS3+ rpb1::rpb1-CTD(Y1F)26-NatR
NYYM221A	SSN3::3HA HIS3+
NYYM222A	SLT2::3HA HIS3+ rpb1::rpb1-CTD(Y1F)26-NatR
NYYM223A	SLT2::3HA HIS3+
NYYM224A	slt2del::kanMX
NYYM225C	slt2del::kanMX [pRS315-slt2(K54R)-3HA] LEU2+
NYYM226A	HOG1::3HA HIS3+
NYYM227A	CTK1::3HA HIS3+
NYYM228A	KIN28::3HA HIS3+
NYYM229A	hog1del::kanMX
NYYM230A	slt2del::kanMX hog1del::NatR
NYYM231C	NRD1::3HA HIS3+ rpb1::rpb1-CTD(Y1F)26-NatR
NYYM232A	NRD1::3HA HIS3+
NYYM233B	RTT103::3HA HIS3+ rpb1::rpb1-CTD(Y1F)26-NatR
NYYM234A	RTT103::3HA HIS3+
The following strains were derived from the S288C background	
Y7092	can1del::STE2pr-SpHis5 his3del1 leu2del0 ura3del0 met15del0 lyp1del LYS2+
NYYM206A	rpb1::rpb1-CTD(Y1F)26-NatR [pADH::RPB1/URA3]
NYYM207A	rpb1::rpb1-CTD(CONS)26-NatR [pADH::RPB1/URA3]

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Tetrad dissection and liquid growth of WT and Y1F strains. Related to Figure 1. **(A)** Tetrad dissection of heterozygous diploid Y1F CTD strain (Y1F). Y1F CTD in haploid tetrads was marked with NAT resistance and was present in all small-size tetrads. **(B)** Liquid growth curve for WT and Y1F strains in rich medium over 48 hours. Results are averages of three biological replicates for each strain.

Figure S2: Tetrad dissection and liquid growth of SGA suppressor-containing strains. Related to Figure 2. **(A)** Sample tetrad dissection results for SGA genes UBP8, CYCC, MED13, and CDK8. Asterisk (*) indicates NAT-/G418-resistant double mutant containing both gene deletion (G418 resistance) and Y1F polymerase (NAT resistance). **(B)** Liquid growth curve for suppressors of Y1F strains (UBP8, CYCC, MED13) in rich medium over 48 hours (growth of Y1F from Figure S1B is shown for comparison). Results are averages of three biological replicates for each strain.

Figure S3: Analysis of poly(A) site usage in WT and Y1F strains by 3'READS. Related to Figure 3. **(A)** Up- and down-regulation of poly(A) site usage in Y1F strain compared to WT. Labels "First" through "Single" are found in 3' UTR of genes. **(B)** Number of poly(A) site changes from (A) in table. **(C)** Log2 values for ratio of up-/down-regulated poly(A) sites in Y1F vs. WT.

Figure S4: Correlation data for RNA sequencing analysis. Related to Figure 3. **(A)** Correlation of significant genes (up-regulated/down-regulated) between WT and Y1F replicates. **(B)** Correlation for number of reads of genes in samples. **(C)** Analysis of CUT and SUT expression changes between WT and Y1F.

Figure S5: Comparison of P-Tyr1 ChIP microarray data with RNA transcript levels in Y1F vs WT strains. Related to Figure 3. Tyr1P ChIP signal intensities from Mayer et al. (2012) on up- and down-regulated genes, and genes with no expression changes in Y1F compared to WT cells were determined.

Figure S6: Comparison of P-Tyr1 ChIP microarray data with RNA polymerase II levels in Y1F vs WT strains. Related to Figure 3. **(A)** Tyr1P ChIP signal intensities from Mayer et al. (2012), normalized to Rpb3 levels from Mayer et al. (2010), on up- and down-regulated genes in Y1F compared to WT cells were determined. **(B)** Expression levels in WT yeast, determined by RNA-seq data from Nagalakshmi et al. (2008), of up-regulated, down-regulated, and genes with no expression changes in Y1F cells, as analyzed in Figures S5 and S6A.

Figure S7: Effects of additional stresses on Y1F growth and in vitro kinase assays with Ctk1 and Kin28. Related to Figure 4. **(A)** Media containing galactose as the sole carbon source, or 0.4 M NaCl, or media depleted of phosphate were used to determine Y1F and WT growth compared to rich media at the indicated time points. **(B)** Both 3HA-tagged kinases were extracted from yeast and following IP incubated with GST-CTD as in Figure 4. Blots with Tyr1p- (3D12), Ser2p- (3E10), and Ser5p- (3E8) antibodies are shown. Lanes are Ctk1-HA (CTK1), Kin28-HA (KIN28), beads and antibody control (BA), and GST-CTD alone (CTD).

SUPPLEMENTARY TABLES

Table S1: List of strains used for this study. Related to STAR*METHODS.

Table S2: Full list of suppressor mutants from the Y1F suppressor SGA. Related to Figure 2. See STAR*METHODS for Mendeley database link.

Table S3: Complete results from Y1F RNA sequencing analysis (separated by tabs). Related to Figure 3. See STAR*METHODS for Mendeley database link