Supplementary information

Legends

Supplementary Figure S1. Insertion mutagenesis and suppressors screening.

Supplementary Figure S2. *DAS* suppressors. **A.** The *das* mutants suppress *dst1Δ* sensitivity to 6AU and MPA. Serial dilutions of the indicated isogenic mutants after three days of growth on SC-URA plates in the presence of 6AU or MPA at the indicated concentration. All the strains were BY4741 derivatives. **B.** Effect of 6AU (25 μg/ml) on the doubling times (d.t.) of the liquid cultures of the wild type and the indicated mutants, as described in the Materials and Methods. Note that unlike the microcolony assay, the proliferation of the wild type in the liquid culture was affected by 6AU.

Supplementary Figure S3. Deletion of *SFP1* suppresses the sensitivity of *dst1Δ* to 6AU in a microcolony assay. **A.** Alginate microcapsules containing the representative microcolonies of the experiments described in Figure 1A. **B.** Comparison of the size distributions of the microcolonies developed by the isogenic *dst1Δ* and *dst1Δsfp1Δ* cells incubated in the presence of 25 µg/ml 6AU for 20 h. See Figure 1 for additional details.

Supplementary Figure S4.
Quantification of the Northern blots showing the effect of 6AU (100 μg/ml, 90 min) on the mRNA levels of *IMD2* in the wild type and the indicated isogenic mutants. A fold induction (+6AU/-6AU) is indicated for each strain.

Supplementary Figure S5. *sch9Δ* suppresses *imd2Δ* sensitivity to MPA. Serial dilutions of the indicated isogenic mutants after 3 days of growth on SC-URA plates in the presence of MPA (2 μg/ml). All the strains were BY4741 derivatives.

Supplementary Figure S6. Mutations affecting the regulators of RP genes suppress *dst1Δ* sensitivity to MPA. **A.** Serial dilutions of the indicated *RAP1* partial deletion mutants (**A**) or the *tpk2Δ* mutant (**B**) after 3 days of growth on SC-URA plates in the

presence of MPA (10 μg/ml). The deleted region of each *RAP1* mutant is shown in the diagram below.

Supplementary Figure S7. TFIIS binding to RNA pol I- (**A**) and RNA pol II-dependent genes (**B**) can be detected by ChIP. The ChIP experiments were performed utilising anti-HA antibodies and were quantified in relation to the input material. No-tag controls confirm the specificity of the HA-TFIIS signals. The six amplicons for rDNA were located as shown in Figure 2. The results of an untranscribed intergenic region (Chromosome V, coordinates 9716-9863) are also provided. Error bars indicate standard deviation.

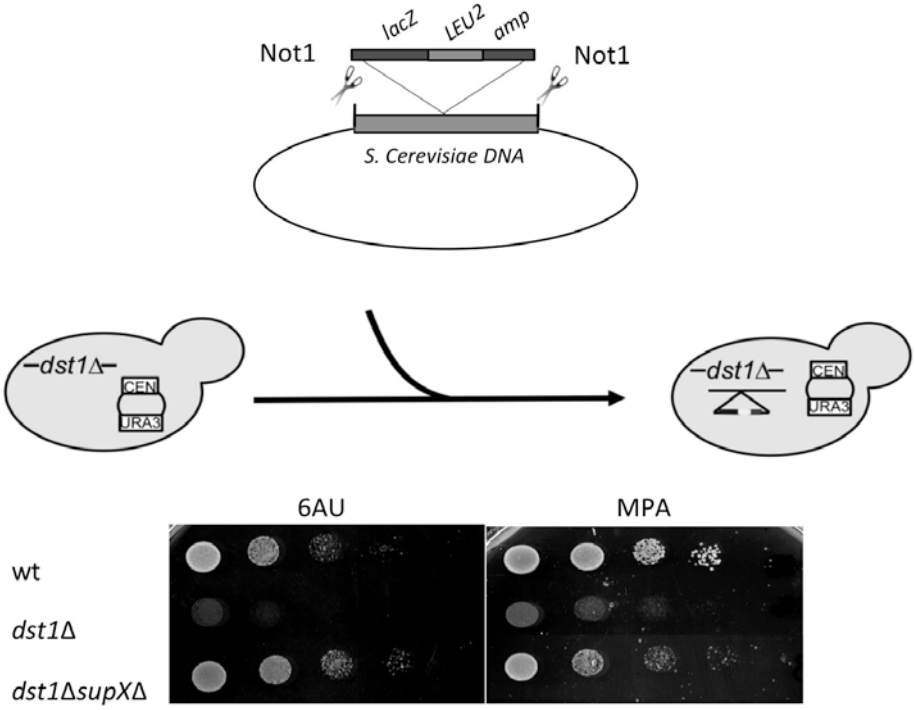
Supplementary Figure S8. TFIIS and RNA polymerase II occupancy in response to 6AU. Changes in HA-TFIIS (**A**) and Rpb3 (**B**) binding to *RRPA43* and *RRP12* in response to 6AU (100 μg/ml). All the values represent the average of three independent experiments at three different amplicons distributed along the genes. Samples were taken from the same extracts to analyse HA-TFIIS and Rpb3 in parallel. ChIP signals were quantified in relation to the input material. The results of an untranscribed intergenic region (Chromosome V, coordinates 9716-9863) are also shown. Error bars indicate standard deviation. **C.** Changes in the TFIIS/RNA polymerase II ratios upon 6AU (100 µg/ml) addition, as measured by ChIP experiments utilising antibodies against HA-TFIIS and Rpb3. All the values represent the average of three independent experiments at three different amplicons (shown in Figure 3A and B).

Supplementary Figure S9. Changes in RNA polymerase II occupancy upon 6AU addition (100 μg/ml) to the wild type and the indicated isogenic strains. All the values represent the average of three independent experiments at three different amplicons distributed along the genes. ChIP signals were quantified in relation to the input material. The results of an untranscribed intergenic region (Chromosome V, coordinates 9716-9863) are also shown. Error bars indicate standard deviation.

Supplementary Figure S10. Variation in the levels of the active RNA polymerases sitting on the indicated genes, as measured by transcriptional run-on, caused by

6AU addition (100 µg/ml) to the wild type and to an isogenic *dst1Δ* strain in both the presence (**A**) and absence of Sfp1 (**B**). All the values represent the average of three independent experiments normalised to the signal given by the same gene at time 0. Error bars indicate standard deviation.

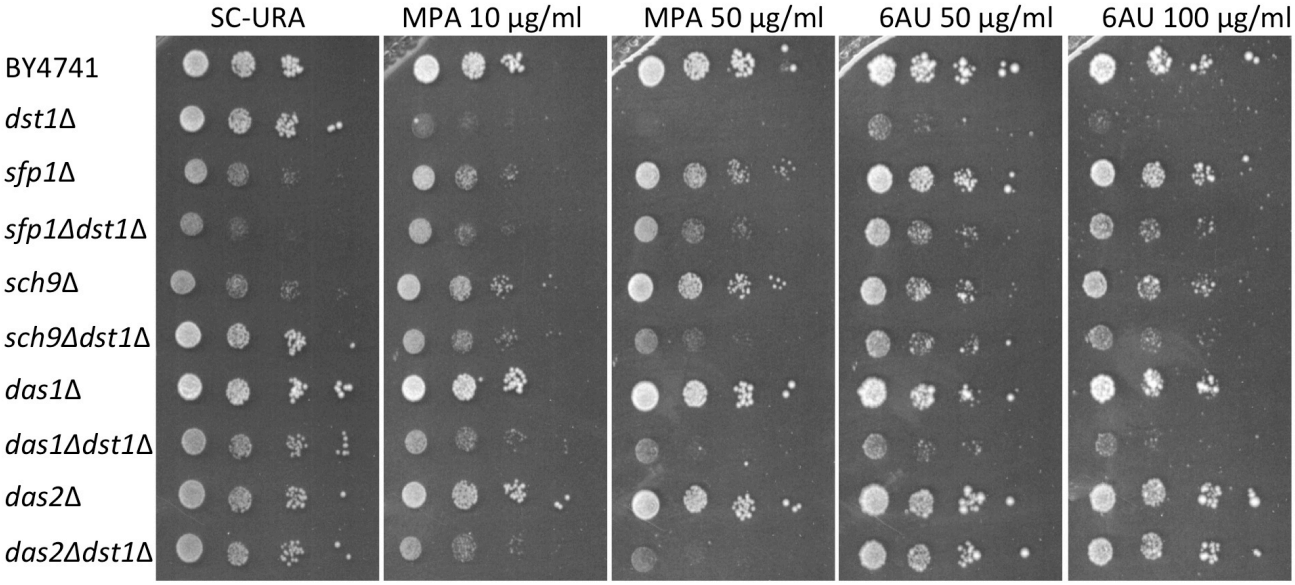
Figure S1



Round	[6AU] µg/ml	Number of transformants	6AU^r	MPA^r	Unic insertion	Insertion ligated phenotype
1	25	7600	2	2	2	2
2	10	7641	7	3	2	3
3	20	4382	5	3	3	1

Candidate	Disrupted ORF	BY4741 suppression	Plasmid complementation	Suppressor
1.1	*YDR020c*	Yes	-	*DAS2*
1.2	*SFP1*	Yes	-	*DAS4*
2.9	*SCH9*	Yes	-	*DAS5*
2.20	*RPL40A*	No	Yes	*DAS3*
3.8	*YJL149w*	Yes	-	*DAS1*

Figure S2


SC-URA
MPA 10 µg/ml
MPA 50 µg/ml
6AU 50 µg/ml
6AU 100 µg/ml
BY4741
dst1Δ
sfp1Δ
sfp1Δdst1Δ
sch9Δ
sch9Δdst1Δ
das1Δ
das1Δdst1Δ
das2Δ
das2Δdst1Δ

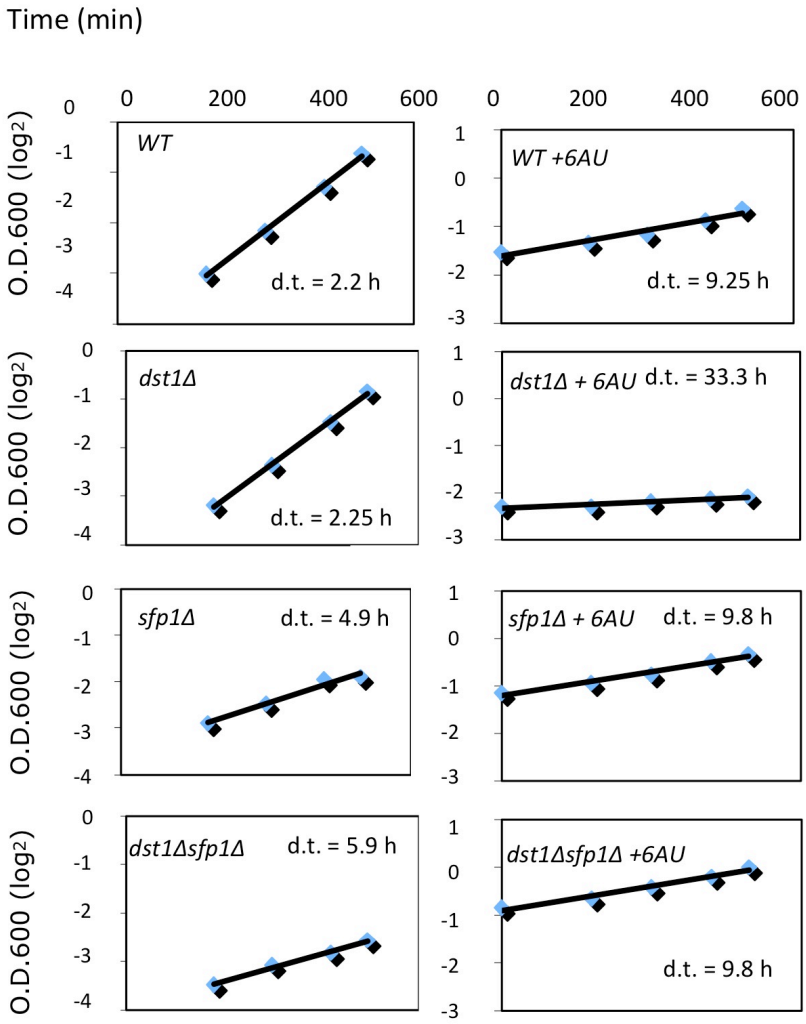
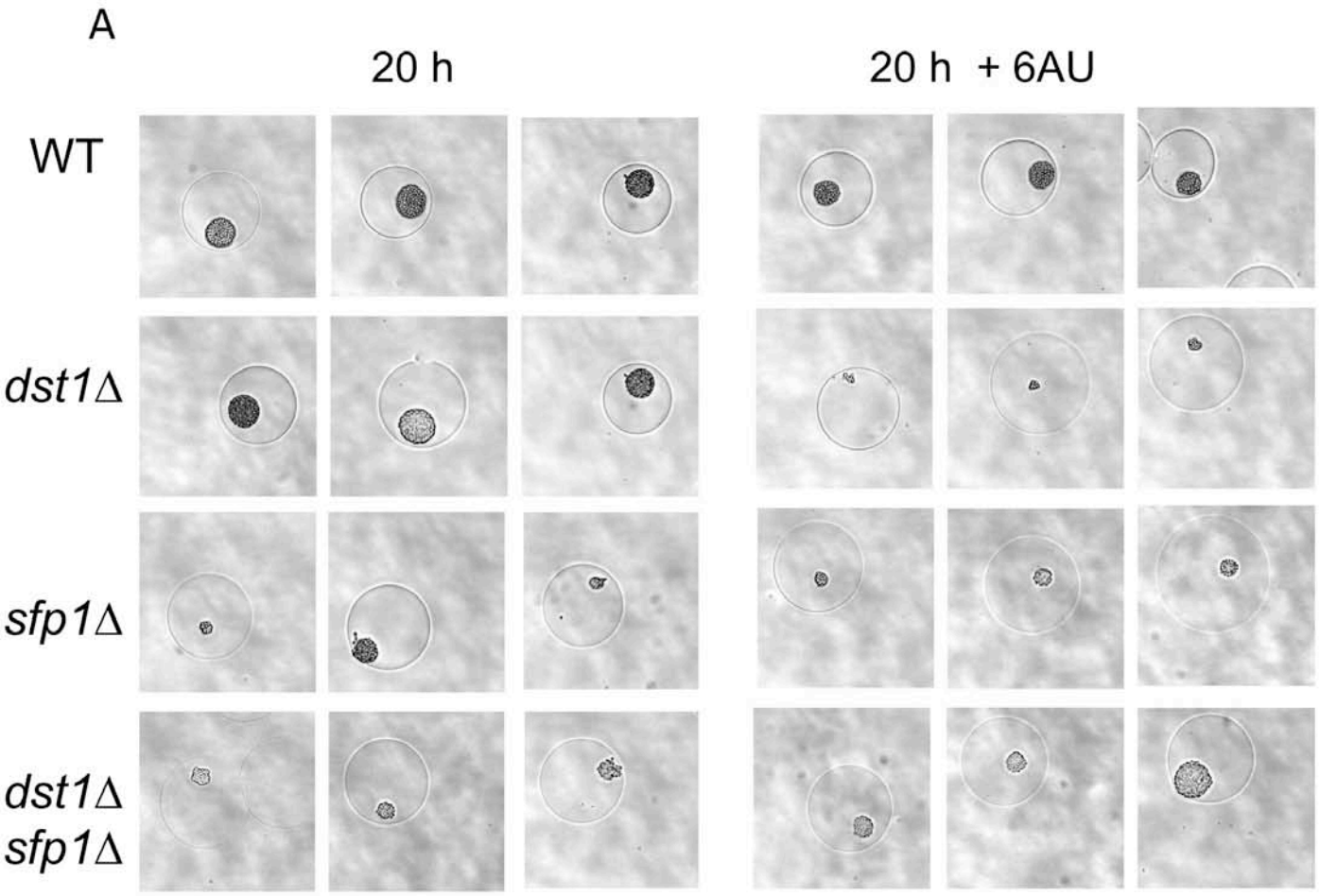

Time (min)
O.D.600 (log2)
WT
d.t. = 2.2 h
WT +6AU
d.t. = 9.25 h
dst1Δ
d.t. = 2.25 h
dst1Δ + 6AU
d.t. = 33.3 h
sfp1Δ
d.t. = 4.9 h
sfp1Δ + 6AU
d.t. = 9.8 h
dst1Δsfp1Δ
d.t. = 5.9 h
dst1Δsfp1Δ +6AU
d.t. = 9.8 h

Figure S3


A
20 h
20 h + 6AU
WT
dst1Δ
sfp1Δ
dst1Δ
sfp1Δ

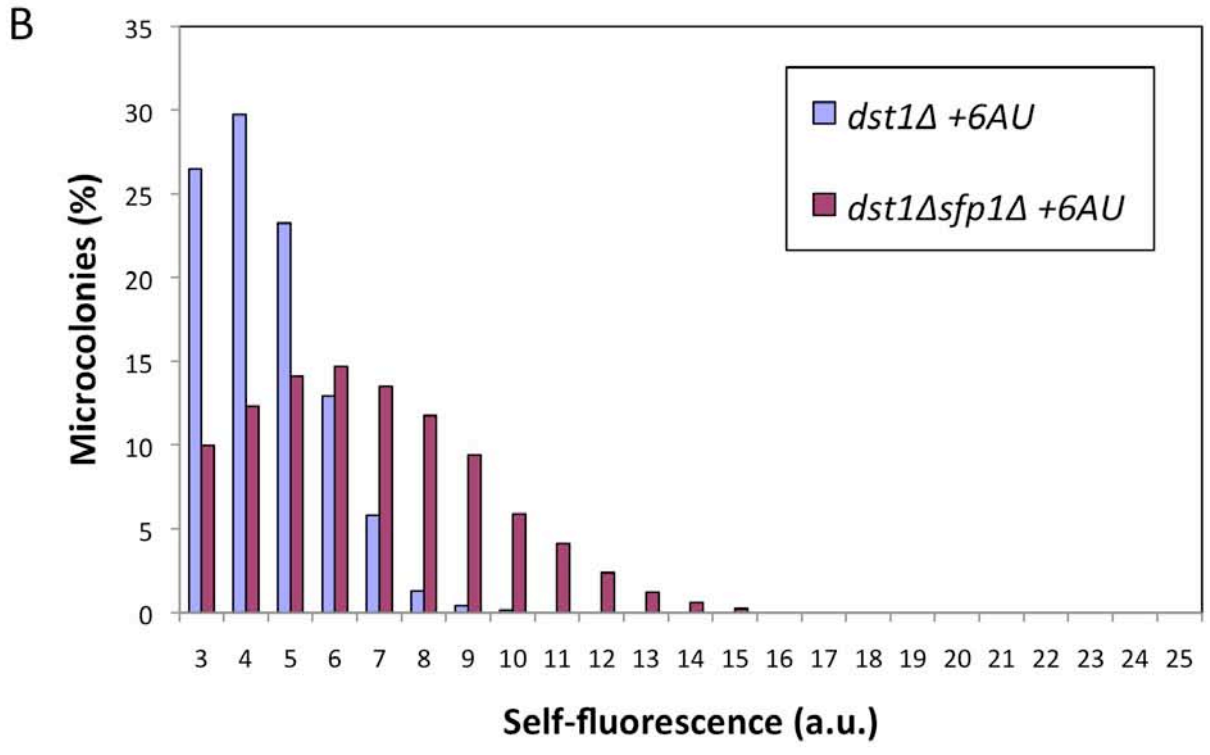

B
Microcolonies (%)
Self-fluorescence (a.u.)
dst1Δ +6AU
dst1Δsfp1Δ +6AU
0
5
10
15
20
25
30
35
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

Figure S4

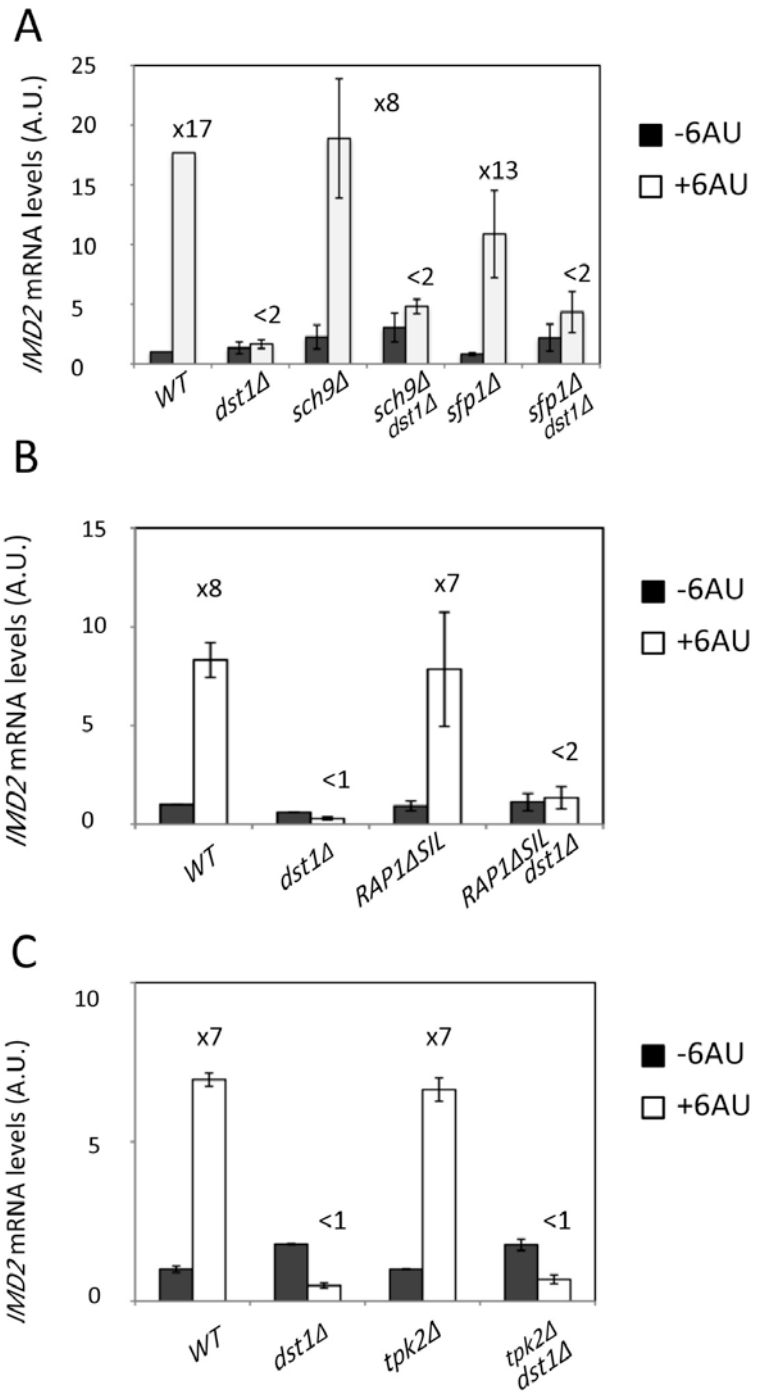

A
IMD2 mRNA levels (A.U.)
25
20
15
10
5
0
x17
<2
x8
<2
x13
<2
WT
dst1Δ
sch9Δ
sch9Δ dst1Δ
sfp1Δ
sfp1Δ dst1Δ
-6AU
+6AU
B
IMD2 mRNA levels (A.U.)
15
10
5
0
x8
<1
x7
<2
WT
dst1Δ
RAP1ΔSIL
RAP1ΔSIL dst1Δ
-6AU
+6AU
C
IMD2 mRNA levels (A.U.)
10
5
0
x7
<1
x7
<1
WT
dst1Δ
tpk2Δ
tpk2Δ dst1Δ
-6AU
+6AU

Figure S5

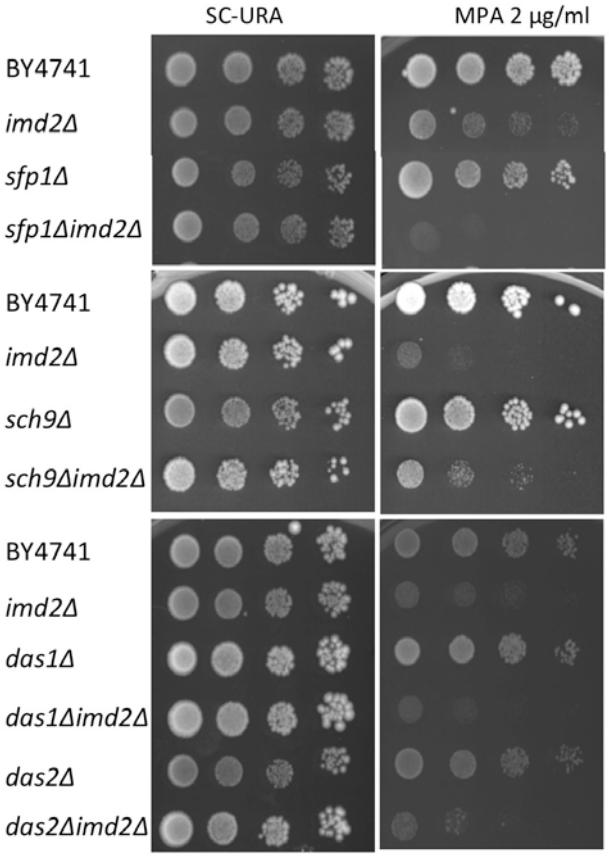
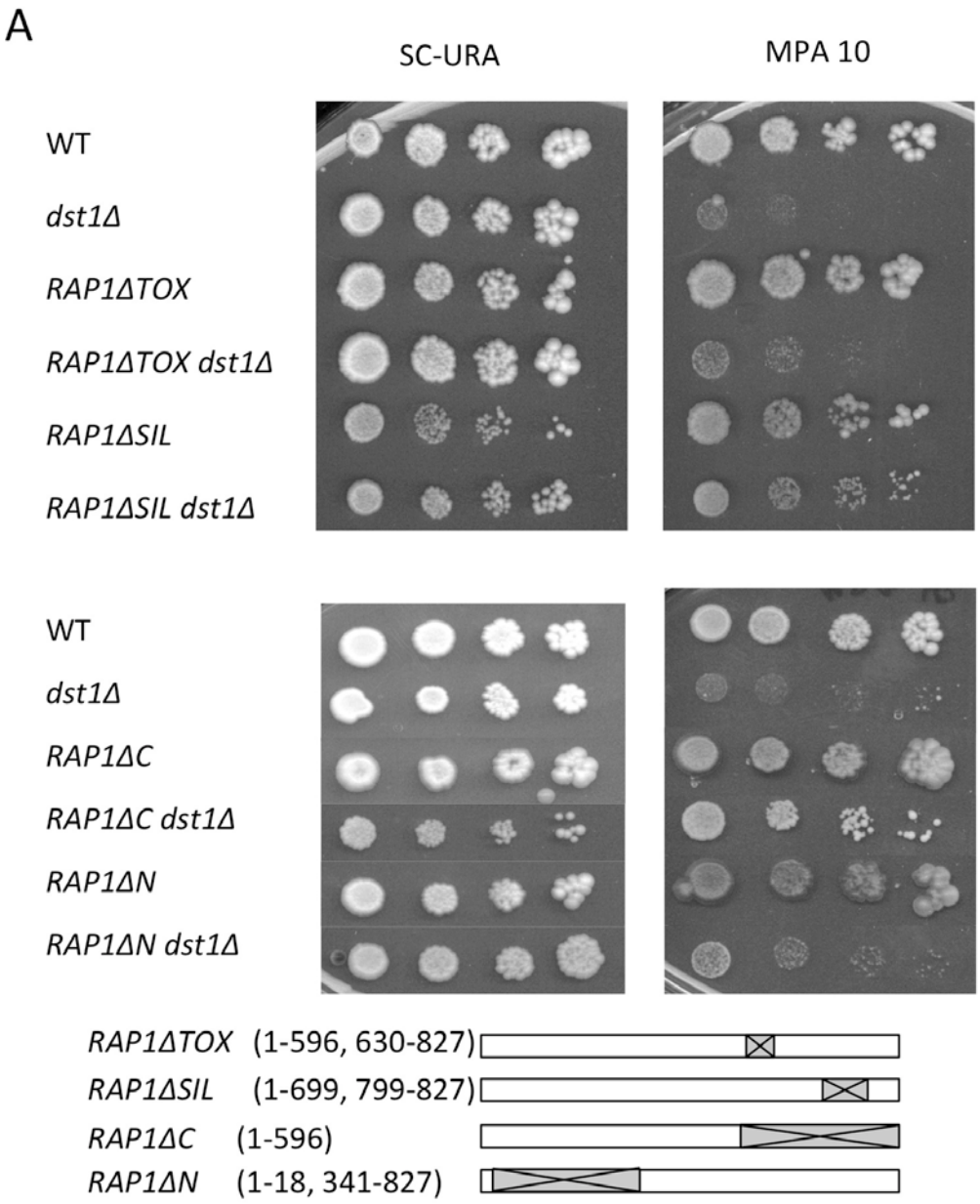

SC-URA
MPA 2 µg/ml
BY4741
imd2Δ
sfp1Δ
sfp1Δimd2Δ
BY4741
imd2Δ
sch9Δ
sch9Δimd2Δ
BY4741
imd2Δ
das1Δ
das1Δimd2Δ
das2Δ
das2Δimd2Δ

Figure S6


A
SC-URA
MPA 10
WT
dst1Δ
RAP1ΔTOX
RAP1ΔTOX dst1Δ
RAP1ΔSIL
RAP1ΔSIL dst1Δ
WT
dst1Δ
RAP1ΔC
RAP1ΔC dst1Δ
RAP1ΔN
RAP1ΔN dst1Δ
RAP1ΔTOX (1-596, 630-827)
RAP1ΔSIL (1-699, 799-827)
RAP1ΔC (1-596)
RAP1ΔN (1-18, 341-827)

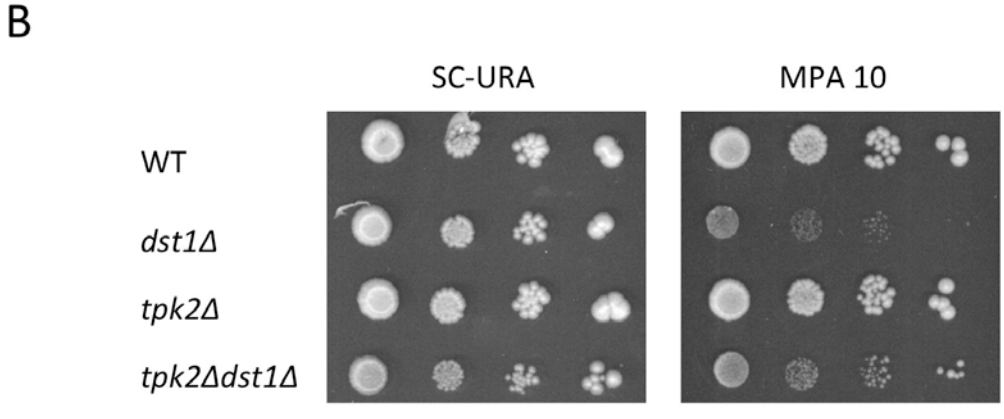

B
SC-URA
MPA 10
WT
dst1Δ
tpk2Δ
tpk2Δdst1Δ

Figure S7

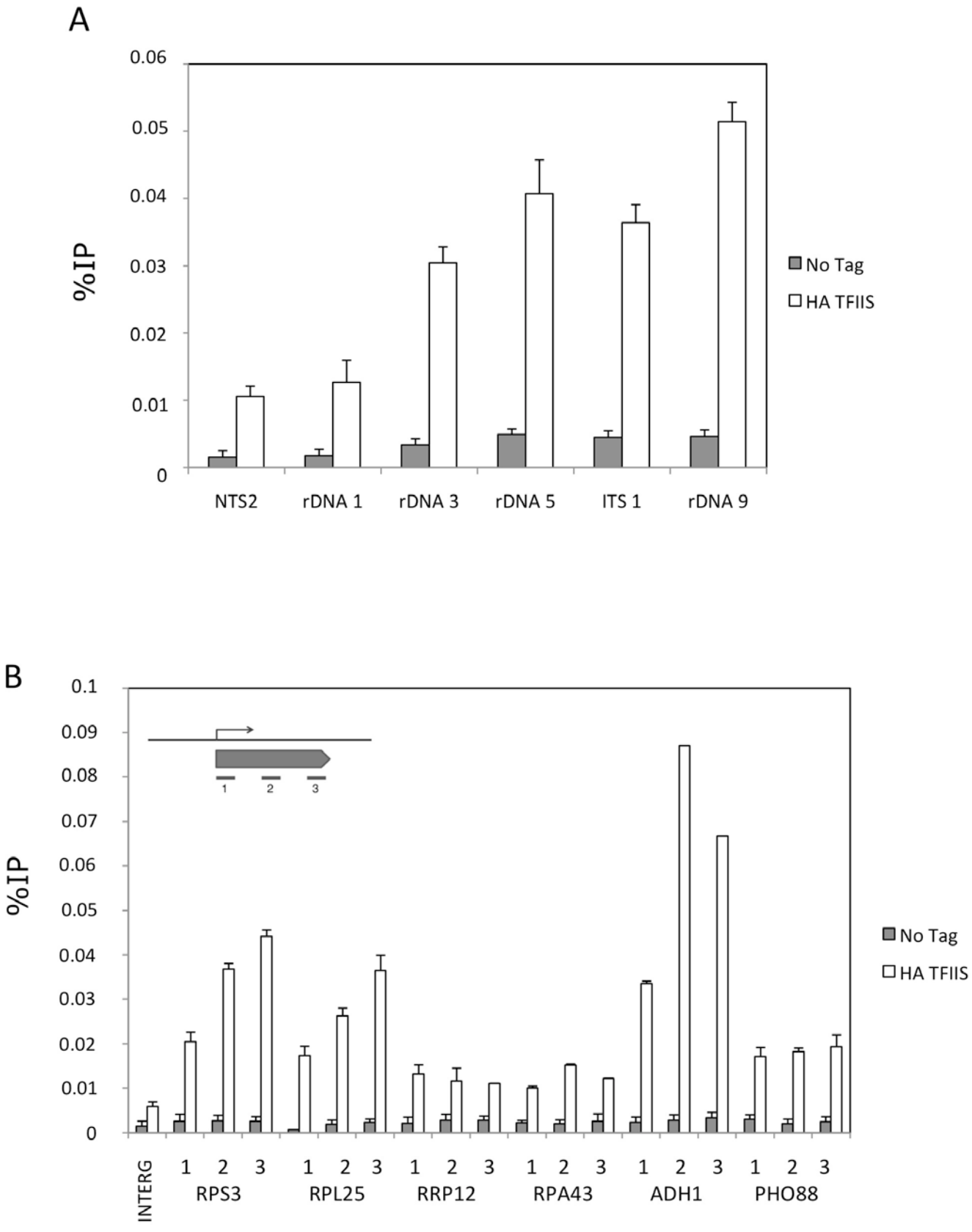

A
%IP
0.06
0.05
0.04
0.03
0.02
0.01
0
NTS2
rDNA 1
rDNA 3
rDNA 5
ITS 1
rDNA 9
No Tag
HA TFIIS
B
%IP
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1
2
3
INTERG
RPS3
RPL25
RRP12
RPA43
ADH1
PHO88
No Tag
HA TFIIS

Figure S8

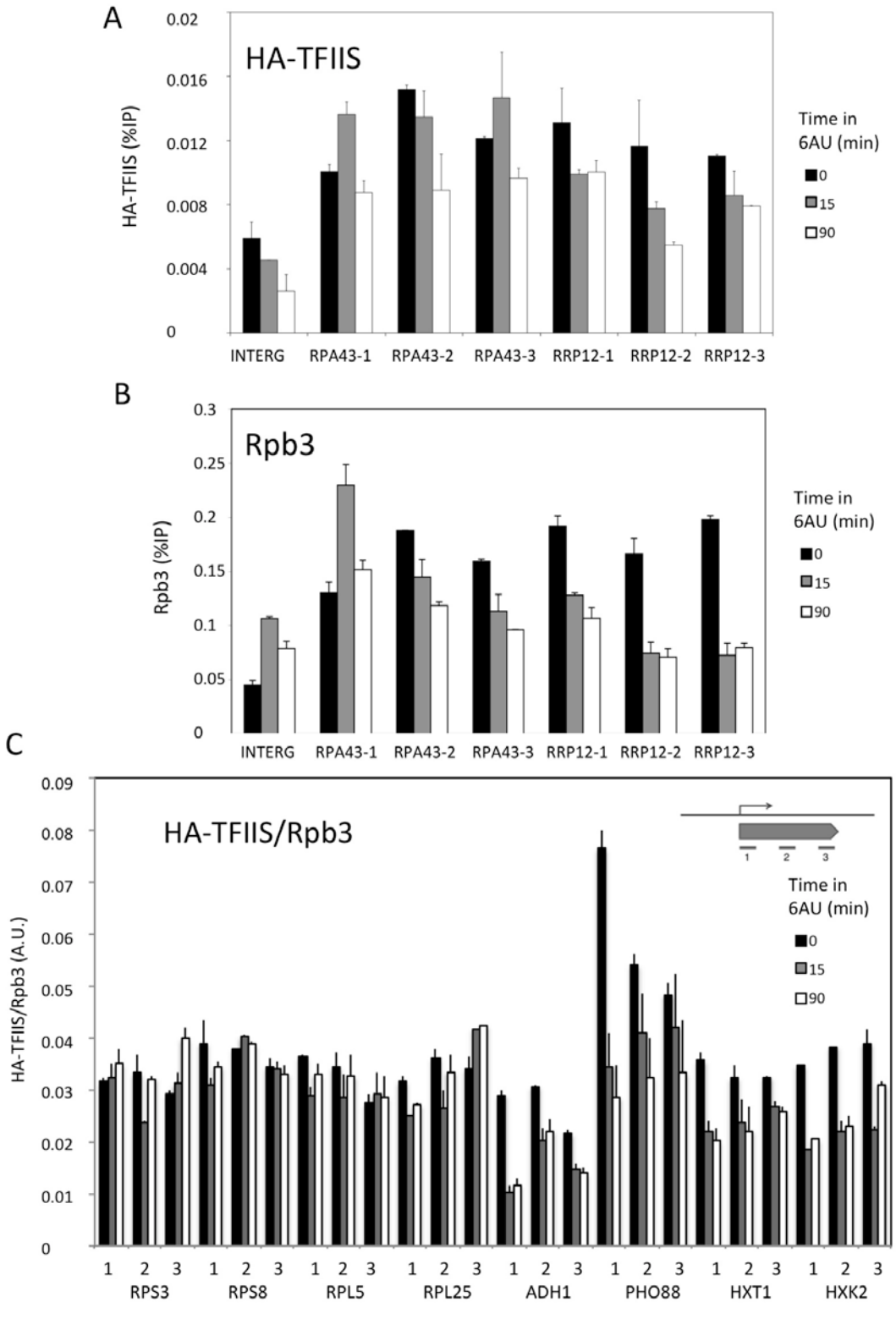

A
HA-TFIIS
HA-TFIIS (%IP)
0.02
0.016
0.012
0.008
0.004
0
INTERG
RPA43-1
RPA43-2
RPA43-3
RRP12-1
RRP12-2
RRP12-3
Time in 6AU (min)
0
15
90
B
Rpb3
Rpb3 (%IP)
0.3
0.25
0.2
0.15
0.1
0.05
0
INTERG
RPA43-1
RPA43-2
RPA43-3
RRP12-1
RRP12-2
RRP12-3
Time in 6AU (min)
0
15
90
C
HA-TFIIS/Rpb3
HA-TFIIS/Rpb3 (A.U.)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1
2
3
RPS3
RPS8
RPL5
RPL25
ADH1
PHO88
HXT1
HXK2
Time in 6AU (min)
0
15
90

Figure S9

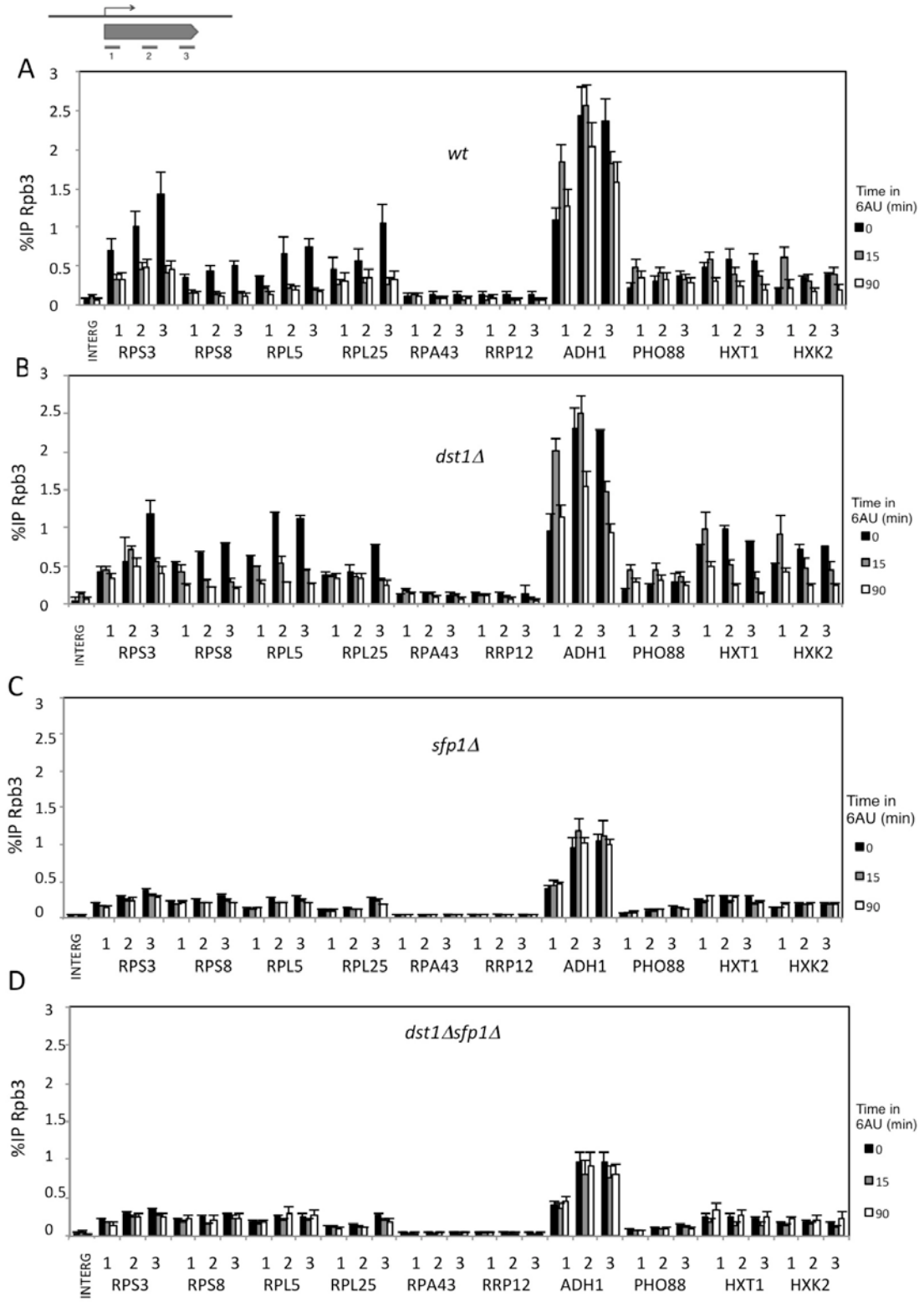

A
B
C
D
wt
dst1Δ
sfp1Δ
dst1Δsfp1Δ
%IP Rpb3
Time in 6AU (min)
0
15
90
INTERG
RPS3
RPS8
RPL5
RPL25
RPA43
RRP12
ADH1
PHO88
HXT1
HXK2

Fig S10

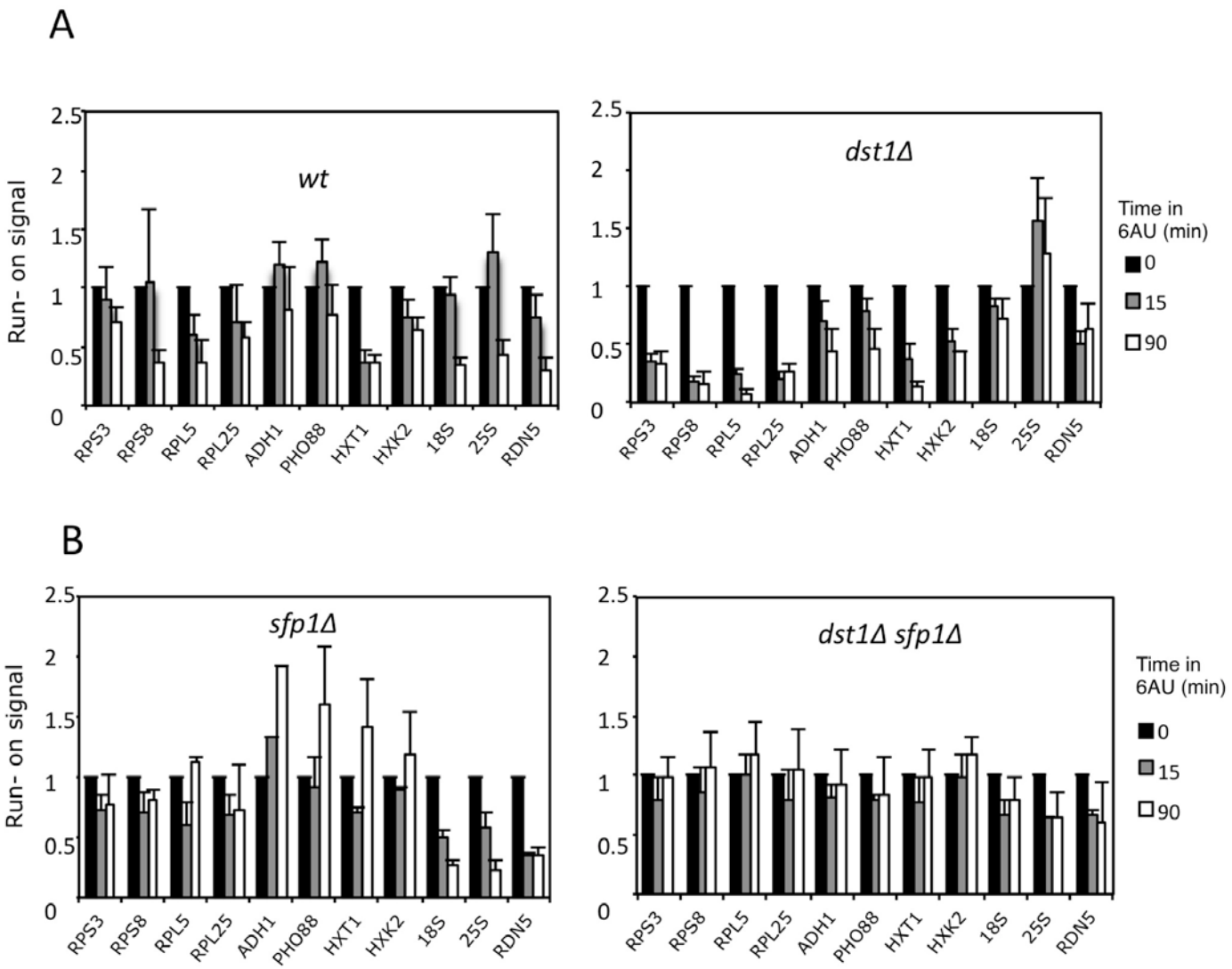

A
wt
dst1Δ
Run- on signal
2.5
2
1.5
1
0.5
0
RPS3
RPS8
RPL5
RPL25
ADH1
PHO88
HXT1
HXK2
18S
25S
RDN5
Time in 6AU (min)
0
15
90
B
sfp1Δ
dst1Δ sfp1Δ

Table S1. Yeast strains.

Yeast strain	Relevant genotype	Reference
SchY66.9D	MAT α *ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1 dst1Δ*	This work
SchY66.4B	MAT a *ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1 dst1Δ*	This work
BY4741	MAT a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
W303-1A	MAT a *ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1*	Thomas et al, 1989
MMY40	SchY66.9D *rpl40A::mTnlacZLEU2*	This work
MMY9.2	BY4741 *dst1::kanMX4*	This work
MMY9.1	BY4742 *dst1::kanMX4*	This work
yMT2291	BY4741 *sch9ΔnatR*	Jorgensen et al, 2002
FGY48.1B	BY4742 *dst1::kanMX4 sch9ΔnatR*	This work
Y05312	BY4741 *sfp1::kanMX4*	Euroscarf
Y15312	BY4742 *sfp1::kanMX4*	Euroscarf
FGY43.1B	BY4741 *dst1::kanMX4 sfp1::kanMX4*	This work
Y01276	BY4741 *das1::kanMX4*	Euroscarf
Y03959	BY4741 *das2::kanMX4*	Euroscarf
MMY27.1	BY4742 *dst1::kanMX4 das2::kanMX4*	This work
FGY41.41	BY4741 *imd2::hphMX4*	This work
FGY54.4C	BY4741 *imd2::hphMX4 sch9ΔnatR*	This work
FGY60.2A	BY4741 *imd2::hphMX4 sfp1::kanMX4*	This work
SCR101	MAT a *ade2-1 trp1-1 leu2 his3-11 ura3Δ1 can1-100 GAL1::RAP1*	Graham et al, 1999
FGY52.13c	*GAL1::RAP1 dst1::kanMX4*	This work
FGY52.14b	*GAL1::RAP1 dst1::kanMX4*	This work
Y01089	BY4741 *tpk2::kanMX4*	Euroscarf
LMY1.3B	BY4741 *dst1::kanMX4 tpk2::kanMX4*	This work
Y07202	BY4741 *trp1::kanMX4*	Euroscarf
LMY5.2	Y07202 *TRP1::pADH::3HA::DST1*	This work
ARG6	BY4741 *RPA190·3XHA::HIS3MX6*	This work
ARG7	BY4741 *RPA190·3XHA::HIS3MX6 dst1::kanMX4*	This work

Table S2. Isolation of the *das* mutants.

Round	[6AU] µg/ml	Number of transformants	$6AU^r$	MPA^r	Unic insertion	Insertion ligated phenotype
1	25	7600	2	2	2	2
2	10	7641	7	3	2	3
3	20	4382	5	3	3	1

Table S3. Oligonucleotides

Primer	Sequence 5'-3'	Use
3-D/A_2	GACTCTCCATCTCTTGTCTTCTTG	Northern probe
5' A_0	GGTCTCTCTGCTGCCGG	Northern probe
5.8S	TTTCGCTGCGTTCTTCATC	Northern probe
5S	GGTCACCCACTACACTACTCGG	Northern probe
A_2/A_3	TGTTACCTCTGGGCCC	Northern probe
C_1/C_2	GAACATTGTTCGCCTAGA	Northern probe
E/C_2	GGCCAGCAATTTCAAGTTA	Northern probe
PUR5 up	TGGCCGCCATTAGAGACTAC	Northern probe
PUR5 lo	ACGCCACCTTCTAGTTGAGC	Northern probe
ADH1 up	TCCAAAGCCAAAGGCCAACGA	Northern/ Run-on probes
ADH1 lo	TTGGCACCAGCTGGCATACCG	Northern/ Run-on probes
RPS3 up	AAGAGATTCAAGTACGCTCCAGGT	Northern/ Run-on probes
RPS3 lo	AAACCGTCAGCAAATTTCATAGC	Northern/ Run-on probes
RPS8 up	ATTCTCGTCACAAAAGATCC	Northern/ Run-on probes
RPS8 lo	GTCTGGAAGAGATACAAGCG	Northern/ Run-on probes
RPL5 up	ATGGCTTTCCAAAAAGACGC	Run-on probes
RPL5 lo	GATCTTGGCAGCAACACGAG	Run-on probes
RPL25 up	GCTACTGCCGCTAAGAAAGC	Run-on probes
RPL25 lo	TAGCAATGTCCAAAGCATCG	Run-on probes
PHO88 up	TGGTCATGATGCAACTCTCC	Run-on probes
PHO88 lo	TACCGGCTCTTTCAGCTTCT	Run-on probes
HXK2 up	TGCCAAAGGAATTGATGCAA	Run-on probes
HXK2 lo	TGAGGTTTGAGTCCAGCCGTA	Run-on probes
HXT1 up	GGCCATGAATACTCCAGAAGGT	Run-on probes
HXT1 lo	GCGCCTCTCTTGGATACTGG	Run-on probes
RDN5 up	GGTTGCGGCCATATCTACCA	Run-on probes
RDN5 lo	AGATTGCAGCACCTGAGTTT	Run-on probes
NTS2 up	AGTGAGGAACTGGGTTACCCG	Q-PCR
NTS2 lo	TTTCTTTTGCCCTCTCTGTCG	Q-PCR
rDNA 1 up	AATTGAAGTTTTTCTCGGCGA	Q-PCR
rDNA 1 lo	ATGAAGTACCTCCCAACTACTTTTCC	Q-PCR
rDNA 2 up	AACAGTCTCATCGTGGGCA	Q-PCR
rDNA 2 lo	TGAGAGGAGGTTACACTTGAAGAAT	Q-PCR
rDNA 3 up	CAATAGCGTATATTAAAGTTGTTGCAGTT	Q-PCR
rDNA 3 lo	AAAGTCCTGGTTCGCCAAGAG	Q-PCR
rDNA 4 up	AACATTCTGTTTGGTAGTGAG	Q-PCR
rDNA 4 lo	AGTATAAAAAAAGATTAGCCG	Q-PCR
rDNA 5 up	TAACAGCTTATCACCCCGGAA	Q-PCR
rDNA 5 lo	CGGTTATCAGTACGACCTGGC	Q-PCR
REF up	TGTTCCTTTAAGAGGTGATGGTGAT	Q-PCR
REF lo	GTGCGCAGTACTTGTGAAAACC	Q-PCR
ADH1 5' up	GCACGGTGACTGGCCATT	Q-PCR
ADH 5' lo	TCTTCCAGCCCTTAACGTTTTC	Q-PCR
ADH1 C up	TGCTAACTTGATGGCCGGTC	Q-PCR
ADH1 C lo	AGACTCTGTAACCCATAGCCTTGG	Q-PCR
ADH1 3' up	ACGTCGGTAACAGAGCTGACAC	Q-PCR
ADH1 3' lo	TTCTGGCAAGGTAGACAAGCC	Q-PCR
PHO88 5' up	CATGTTGGTCATGATGCAACTCT	Q-PCR
PHO88 5' lo	GTTGGGTCCTCCATGTCAATG	Q-PCR
PHO88 C up	GGTAATGCTATGTCCGGCGA	Q-PCR
PHO88 C lo	TCTGACGGTAGTAACTTGCAGCTT	Q-PCR
PHO88 3' up	CCTCTTCGGTAAGCCTGCAA	Q-PCR
PHO88 3' lo	TGGAGCCTTGAATGGTCTCTTC	Q-PCR
RPL25 5' up	CACGAGAAAATTGAGAGGAAGATAGA	Q-PCR

RPL25 5’ lo	CGTGTGGTTTTCGTATCTCATCA	Q-PCR
RPL25 C up	AGCAACGTAATTATCGGGCTCA	Q-PCR
RPL25 C lo	AACTTCCACAGCATCAACGCT	Q-PCR
RPL25 3’ up	TGGTTTTCCAAGTTTCCATGAA	Q-PCR
RPL25 3’ lo	AACTTCGTATAATTCCTTGACGGC	Q-PCR
RPS3 5’ up	TTTTTGAACATTGTTTTGATAACTGAAA	Q-PCR
RPS3 5’ lo	AGAGATTAAAGCGACCATTTTTGTAGT	Q-PCR
RPS3 C up	AAGAGATTCAAGTACGCTCCAGGT	Q-PCR
RPS3 C lo	GCGGACAAACCACGGTCT	Q-PCR
RPS3 3’ up	GGTTGTGAAGTTGTTGTTTCCG	Q-PCR
RPS3 3’ lo	AAACCGTCAGCAAATTTCATAGC	Q-PCR
HXK2 5’ up	TGCCAAAGGAATTGATGCAA	Q-PCR
HXK2 5’ lo	CAATTCGGAAATGAAGTGCTTG	Q-PCR
HXK2 C up	GGCCATCAACTGTGAATACGG	Q-PCR
HXK2 C lo	GGCCTGGTCTTGGAGATTCTTC	Q-PCR
HXK2 3’ up	GTCCGTTTGTGGTATTGCTGC	Q-PCR
HXK2 3’ lo	TGAGGTTTGAGTCCAGCCGTA	Q-PCR
HXT1 5’ up	GGCCATGAATACTCCAGAAGGT	Q-PCR
HXT1 5’ lo	GAAACCACCGAAAGCAACCA	Q-PCR
HXT1 C up	CGTTATTTGGTTGAAGCTGGC	Q-PCR
HXT1 C lo	AAGATGCAGTACCAGCGGCT	Q-PCR
HXT1 3’ up	TCATGGGCTGTATGGTTTTCG	Q-PCR
HXT1 3’ lo	GCGCCTCTCTTGGATACTGG	Q-PCR
RPS8 5’ up	CGTCACAAAAGATCCGCTACC	Q-PCR
RPS8 5’ lo	TCTTGGTGTTGGCTGGTTGA	Q-PCR
RPS8 C up	CCAAGAAGACCAGAATTGCTGG	Q-PCR
RPS8 C lo	AGCATCAATTTGGACAATGGC	Q-PCR
RPS8 3’ up	GCTGCTTCTGCCAAGATCG	Q-PCR
RPS8 3’ lo	CGGATTGACCTGGTCTGGAA	Q-PCR
RPL5 5’ up	CCAAAAAGACGCTAAGTCCTCTG	Q-PCR
RPL5 5’ lo	CTTGGCCTTGTGTTGGGTG	Q-PCR
RPL5 C up	TGGGTTTGGACGAAACTTACAA	Q-PCR
RPL5 C lo	AGAGCACCGAAAACTCTGGC	Q-PCR
RPL5 3’ up	CACTTCTGCTCACGAAGCTATCA	Q-PCR
RPL5 3’ lo	GATCTTGGCAGCAACACGAG	Q-PCR