Figure S1

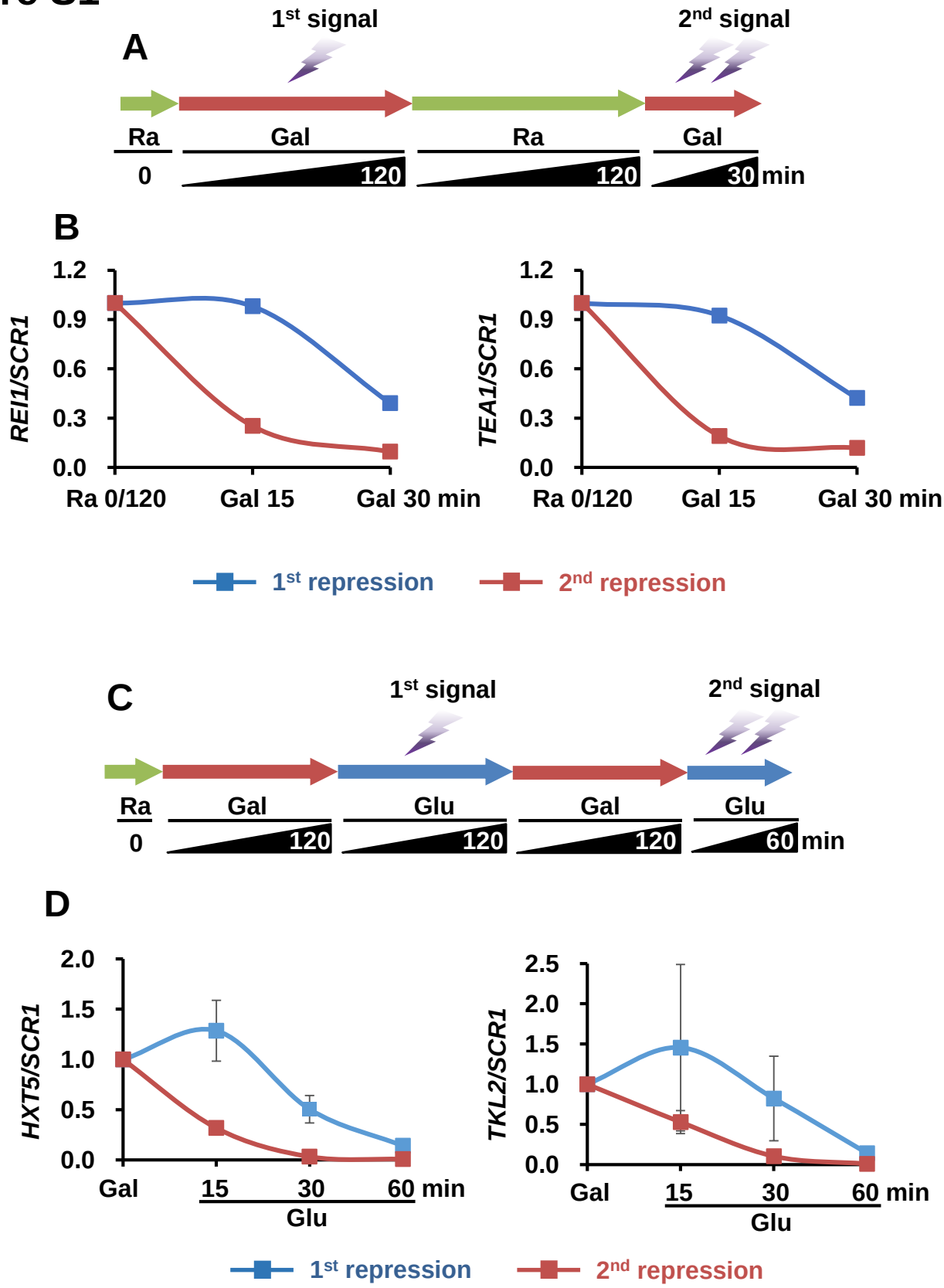

A
1st signal
2nd signal
Ra
Gal
Ra
Gal
0
120
120
30
min
B
REI1/SCR1
TEA1/SCR1
1.2
0.9
0.6
0.3
0.0
Ra 0/120
Gal 15
Gal 30 min
1st repression
2nd repression
C
1st signal
2nd signal
Ra
Gal
Glu
Gal
Glu
0
120
120
120
60
min
D
HXT5/SCR1
TKL2/SCR1
2.0
1.5
1.0
0.5
0.0
2.5
Gal
15
30
60 min
Glu
1st repression
2nd repression

Figure S1

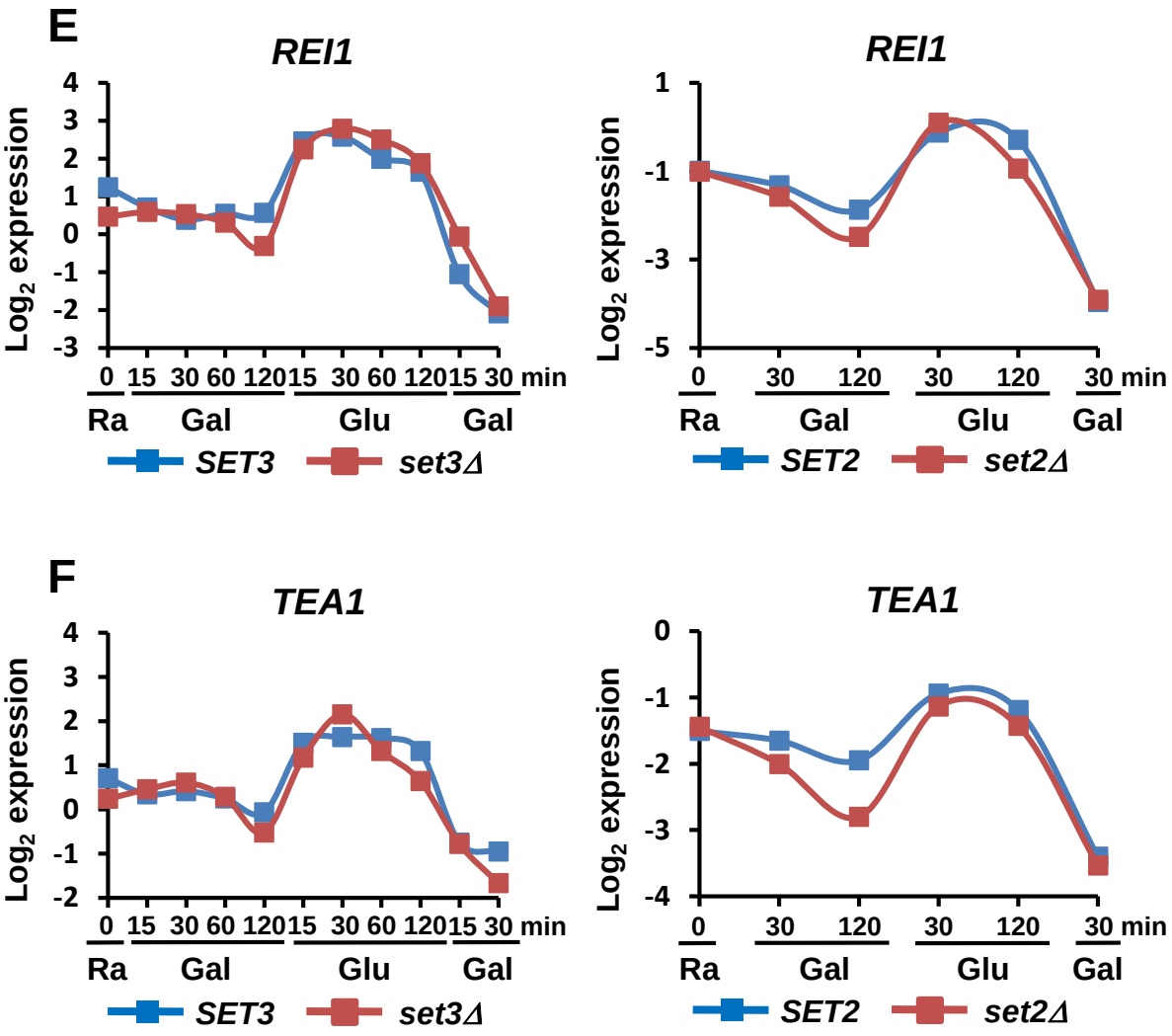

E
REI1
Log2 expression
4
3
2
1
0
-1
-2
-3
0 15 30 60 120 15 30 60 120 15 30 min
Ra
Gal
Glu
Gal
SET3
set3Δ
REI1
Log2 expression
1
-1
-3
-5
0 30 120 30 120 30 min
Ra
Gal
Glu
Gal
SET2
set2Δ
F
TEA1
Log2 expression
4
3
2
1
0
-1
-2
0 15 30 60 120 15 30 60 120 15 30 min
Ra
Gal
Glu
Gal
SET3
set3Δ
TEA1
Log2 expression
0
-1
-2
-3
-4
0 30 120 30 120 30 min
Ra
Gal
Glu
Gal
SET2
set2Δ

Figure S2

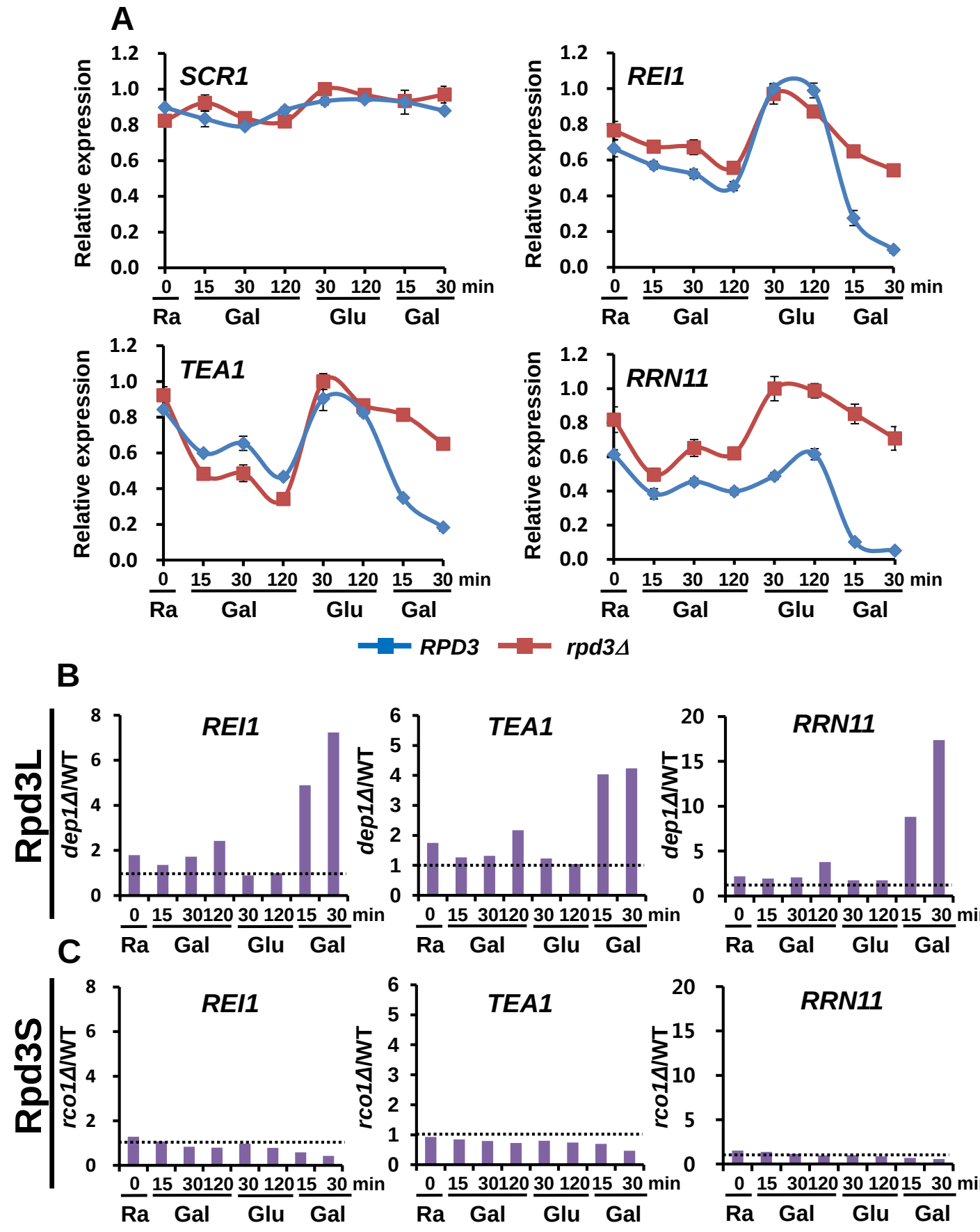

A
SCR1
REI1
TEA1
RRN11
Relative expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0 15 30 120 30 120 15 30 min
Ra Gal Glu Gal
RPD3
rpd3Δ
B
Rpd3L
dep1Δ/WT
REI1
TEA1
RRN11
0 2 4 6 8
0 1 2 3 4 5 6
0 5 10 15 20
C
Rpd3S
rco1Δ/WT
REI1
TEA1
RRN11
0 2 4 6 8
0 1 2 3 4 5 6
0 5 10 15 20

Figure S3

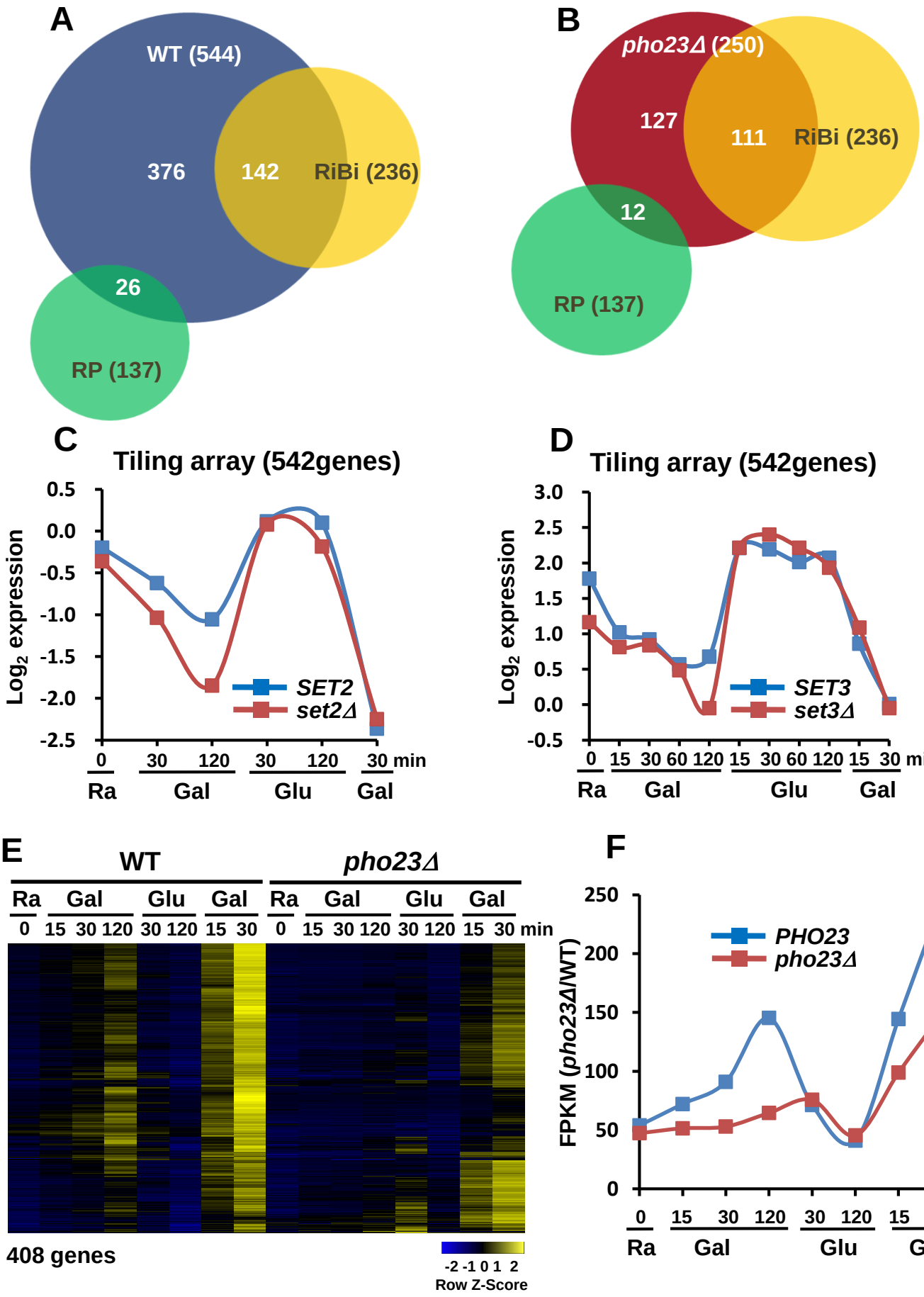

A
WT (544)
376
142
RiBi (236)
26
RP (137)
B
pho23Δ (250)
127
111
RiBi (236)
12
RP (137)
C
Tiling array (542genes)
Log2 expression
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
SET2
set2Δ
0 30 120 30 120 30 min
Ra Gal Glu Gal
D
Tiling array (542genes)
Log2 expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
SET3
set3Δ
0 15 30 60 120 15 30 60 120 15 30 min
Ra Gal Glu Gal
E
WT
pho23Δ
Ra Gal Glu Gal Ra Gal Glu Gal
0 15 30 120 30 120 15 30 0 15 30 120 30 120 15 30 min
408 genes
-2 -1 0 1 2
Row Z-Score
F
FPKM (pho23Δ/WT)
250
200
150
100
50
0
PHO23
pho23Δ
0 15 30 120 30 120 15 30 min
Ra Gal Glu Gal

Figure S4

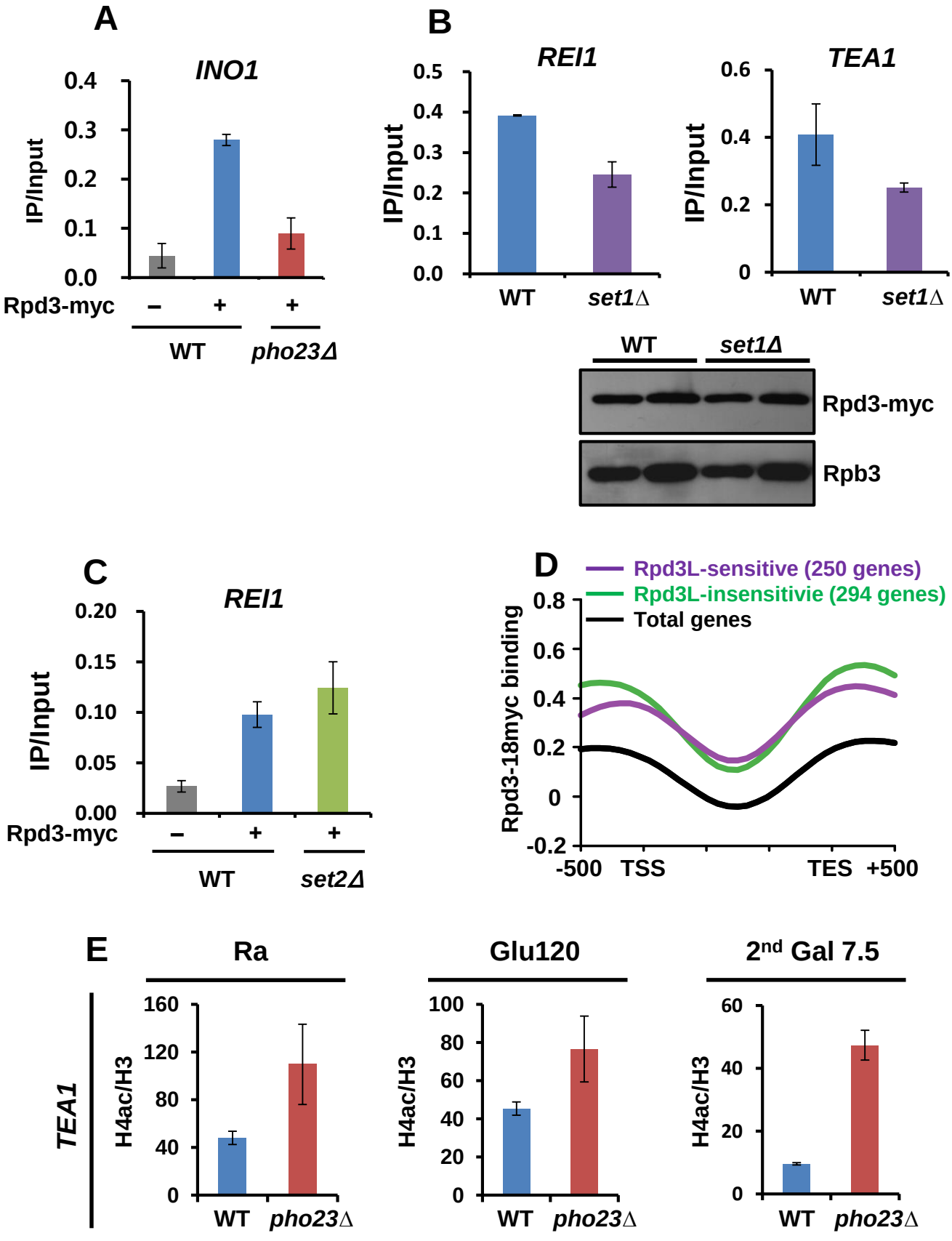
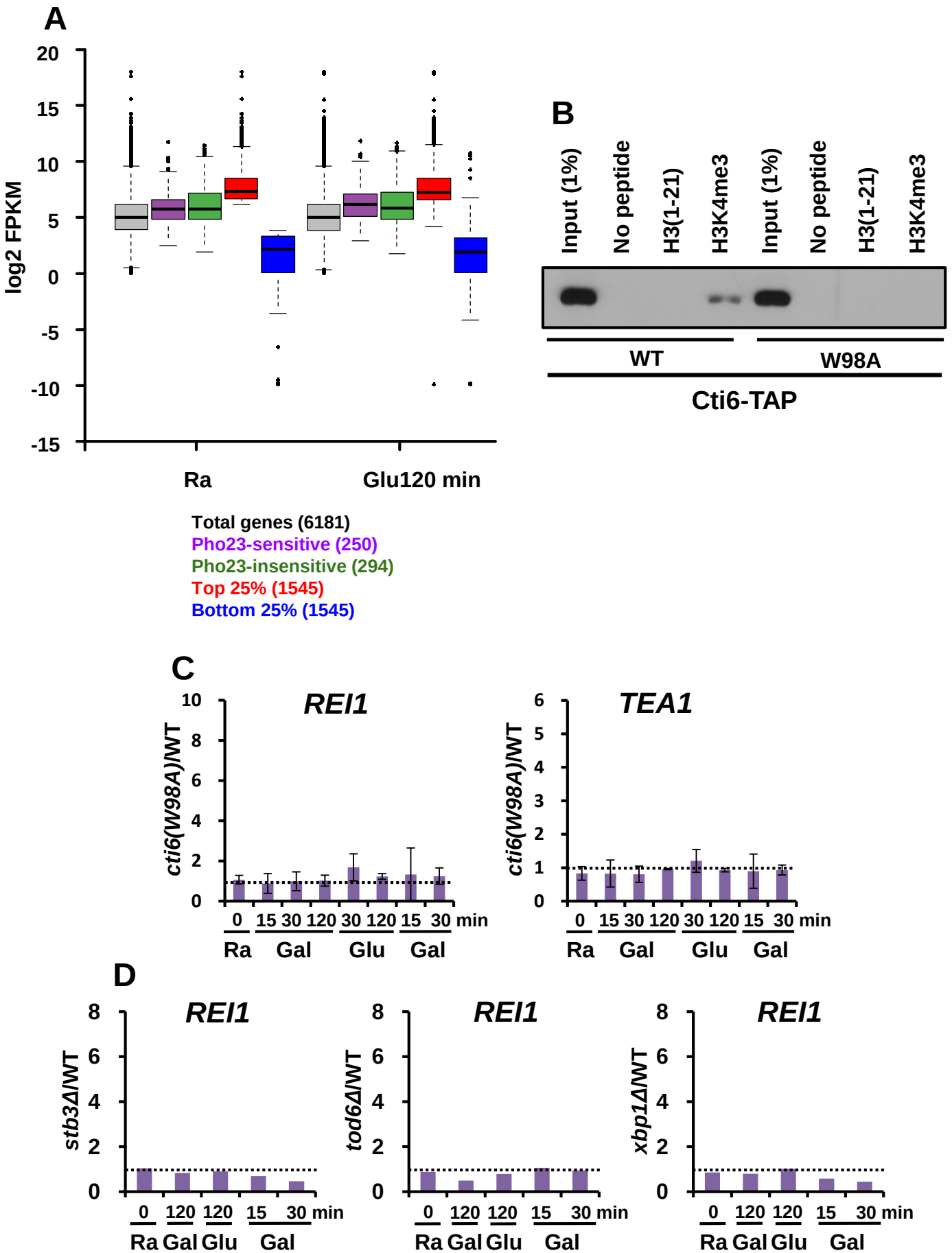

A
INO1
IP/Input
0.4
0.3
0.2
0.1
0.0
Rpd3-myc
–
+
+
WT
pho23Δ
B
REI1
IP/Input
0.5
0.4
0.3
0.2
0.1
0.0
WT
set1Δ
TEA1
IP/Input
0.6
0.4
0.2
0
WT
set1Δ
WT
set1Δ
Rpd3-myc
Rpb3
C
REI1
IP/Input
0.20
0.15
0.10
0.05
0.00
Rpd3-myc
–
+
+
WT
set2Δ
D
Rpd3L-sensitive (250 genes)
Rpd3L-insensitivie (294 genes)
Total genes
Rpd3-18myc binding
0.8
0.6
0.4
0.2
0
-0.2
-500
TSS
TES
+500
E
TEA1
Ra
Glu120
2nd Gal 7.5
H4ac/H3
160
120
80
40
0
100
80
60
40
20
0
60
40
20
0
WT
pho23Δ

Figure S5


A
log2 FPKM
20
15
10
5
0
-5
-10
-15
Ra
Glu120 min
Total genes (6181)
Pho23-sensitive (250)
Pho23-insensitive (294)
Top 25% (1545)
Bottom 25% (1545)
B
Input (1%)
No peptide
H3(1-21)
H3K4me3
Input (1%)
No peptide
H3(1-21)
H3K4me3
WT
W98A
Cti6-TAP
C
REI1
cti6(W98A)/WT
0 15 30 120 30 120 15 30 min
Ra Gal Glu Gal
TEA1
cti6(W98A)/WT
0 15 30 120 30 120 15 30 min
Ra Gal Glu Gal
D
REI1
stb3Δ/WT
0 120 120 15 30 min
Ra Gal Glu Gal
REI1
tod6Δ/WT
0 120 120 15 30 min
Ra Gal Glu Gal
REI1
xbp1Δ/WT
0 120 120 15 30 min
Ra Gal Glu Gal

Supplementary Figure Legends

Supplementary Figure 1. Transcriptional repression memory **(A)** Schematic representation of the time course experiments to monitor changes in transcript levels under an alternative carbon-source shift. **(B)** Wild type cells were grown in SC-raffinose medium and then sequentially shifted to SC media containing the indicated carbon sources for the times specified in Supplementary Figure S1A. *REI1* and *TEA1* mRNA levels were determined by RT-PCR with two independent RNA samples. *SCR1* was used as an internal control. **(C)** Schematic representation of the time course experiments to monitor changes in transcript levels of glucose-repressed genes. **(D)** Wild type cells were grown in SC-raffinose medium and then sequentially shifted to SC media containing the indicated carbon sources for the times specified in Supplementary Figure S1C. *HXT5* and *TKL2* mRNA levels were determined by RT-PCR with two independent RNA samples. *SCR1* was used as an internal control. **(E)** Set3 HDAC did not affect the kinetics of *REI1* or *TEA1* repression. Log_2 expression levels of *REI1* or *TEA1* during carbon-source shifts from Kim et al (2012). **(F)** Set2 was not involved in *REI1* or *TEA1* repression. Log_2 expression levels of *REI1* or *TEA1* during carbon source shifts from Kim et al (2016).

Supplementary Figure 2. Rpd3L, but not Rpd3S, is required for TREM **(A)** The original data for Figure 2B. *RPD3* and *rpd3Δ* cells were grown in SC-raffinose medium and then sequentially shifted to SC media containing the indicated carbon sources for the times specified in Figure 2A. Transcript levels were determined by RT-PCR with two independent RNA samples. **(B)** Deletion of *DEP1* delayed TREM. Transcript levels of TREM genes were analyzed as in Figure 2A. **(C)** Loss of Rco1, a subunit of Rpd3S, had no effect on TREM. Transcript levels of TREM genes were analyzed as in Figure 2A.

Supplementary Figure 3. Rpd3L-mediated regulation of TREM. **(A)** The number of RiBi (yellow circle) and RP (green circle) genes that showed TREM during carbon-source shifts. **(B)** RiBi (yellow circle) and RP (green circle) genes that were regulated by Pho23. **(C)** Loss of Set2 increased the rate of gene repression during the first galactose pulse. Averaged expression profiles of 542 TREM genes in *SET2* and *set2Δ* cells from Kim et al (2016). **(D)** Set3 had no effect on repression of TREM genes. Averaged expression profiles of 542 TREM genes in *SET3* and *set3Δ* cells from Kim et al (2012). **(E)** 408 genes that displayed faster induction kinetics during the second galactose incubation. These genes showed memory of their previous active states. **(F)** Average expression profiles of 408 genes from Supplementary Figure S4E. Deletion of *PHO23* significantly delayed the rate of gene induction.

Supplementary Figure 4. Rpd3L binding to TREM genes **(A)** Rpd3 binding to *INO1* gene. PCR analysis of the precipitated DNA was carried out on the *INO1* promoter as in Figure 4A. **(B)** Deletion of *SET1* slightly reduced Rpd3 binding to the promoters of TREM genes. ChIP was done with the indicated strains expressing Rpd3-myc protein as in Figure 4A. The bottom panel shows Rpd3 proteins levels in the indicated strains. Rpb3 was used a loading control. **(C)** Set2 was not required for Rpd3 binding. ChIP was performed with the indicated strains expressing Rpd3-myc as in Figure 4A. **(D)** Rpd3 occupancy of TREM genes was analyzed as in Figure 4C. The graph shows the average occupancy of Rpd3 for total genes (black), for 250 Rpd3L-sensitive genes (purple), and for 294 Rpd3L-insensitive genes (green). **(E)** Loss of Pho23 delayed deacetylation at the *TEA1* promoter upon the second galactose incubation. Histone acetylation at the indicated time points was analyzed as in Figure 4E.

Supplementary Figure 5. The Cti6 PHD finger and gene specific repressors do not affect TREM. **(A)** Averaged expression profiles of total genes (6181), Rpd3L-sensitivie genes

(250), Rpd3L-insensitive genes (294), top 25% highly transcribed genes (1545), and bottom 25% inactive genes (1545). **(B)** The Cti6 PHD finger binds to H3K4me3. Histone peptide binding assays were performed with whole-cell extracts expressing Cti6-TAP or a PHD finger mutant, cti6 W98A-TAP, and 1μg of the indicated histone peptides immobilized on magnetic beads. Precipitated Cti6 protein was analyzed by immunoblot with anti-TAP antibody. **(C)** The interaction between the Cti6 PHD finger and H3K4me3 was not required for TREM. Transcript levels from the indicated cells were determined by RT-PCR as in Figure 2B. The ratios of transcript levels in *cti6* W98A over those in *CTI6* strains were plotted. **(D)** Wild type and the indicated mutant strains were grown in SC-raffinose medium and then sequentially shifted to SC media containing the indicated carbon sources for the times specified in the upper panel. mRNA levels were measured by RT-PCR with two independent RNA samples. The ratios of the transcript levels in the mutants over those in wild type were plotted.

Table S1. Strains used in this study.

Strain	Genotype	Source or Reference
BY4741	MATa, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	Saccharomyces Genome Deletion Project
YSB787	MATa, bur1Δ::HIS3, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2Δ202 (*pRS316-BUR1*)	Keogh et al., 2005
YSB1002	MATa, bur1Δ::HIS3, ura3-52 or ura3Δ0, leu2Δ1 or leu2Δ0, trp1Δ63, his3Δ200 or his3Δ1, lys2Δ202 or LYS2, met15Δ0 or MET15, set2Δ::KanMX (*pRS316-BUR1*)	M. Keogh
YSB1064	MATa, bur1Δ::HIS3, ura3-52 or ura3Δ0, leu2Δ1 or leu2Δ0, trp1Δ63, his3Δ200 or his3Δ1, lys2Δ202 or LYS2, met15Δ0 or MET15, set3Δ::KanMX (*pRS316-BUR1*)	M. Keogh
YSB1850	MATa, bur1Δ::HIS3, ura3-52 or ura3Δ0, leu2Δ1 or leu2Δ0, trp1Δ63, his3Δ200 or his3Δ1, lys2Δ202 or LYS2, met15Δ0 or MET15, rpd3Δ::KanMX (*pRS316-BUR1*)	M. Keogh
YSB1319	MATa, bur1Δ::HIS3, ura3-52 or ura3Δ0, leu2Δ1 or leu2Δ0, trp1Δ63, his3Δ200 or his3Δ1, lys2Δ202 or LYS2, met15Δ0 or MET15, pho23Δ::KanMX *(pRS316-BUR1)*	S. Buratowski
YSB1849	MATa, bur1Δ::HIS3, ura3-52 or ura3Δ0, leu2Δ1 or leu2Δ0, trp1Δ63, his3Δ200 or his3Δ1, lys2Δ202 or LYS2, met15Δ0 or MET15, rco1Δ::KanMX (*pRS316-BUR1*)	M. Keogh
YSB1321	MATa, bur1Δ::HIS3, ura3-52 or ura3Δ0, leu2Δ1 or leu2Δ0, his3Δ200 or his3Δ1, lys2Δ202 or LYS2, met15Δ0 or MET15, dep1Δ::KanMX *(pRS316-BUR1)*	S. Buratowski
YTK84, YTK85	MATa, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, pho23(W305A)	This study
YTK302, YTK303	MATa, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, cti6(W98A)	This study
YF2111	MATa, RPD3::18MYC::TRP1, ura3-52, leu2::hisG, trp1::hisG	Bumgarner et al., 2009
YTK282,	MATa, pho23Δ::KanMX, RPD3::18MYC::TRP1, ura3-52, leu2::hisG, trp1::hisG	This study

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YTK331	MATa, set1Δ::KanMX, RPD3::18MYC::TRP1, ura3-52, leu2::hisG, trp1::hisG	This study
YTK299	MATa, set2Δ::KanMX, RPD3::18MYC::TRP1, ura3-52, leu2::hisG, trp1::hisG	This study
YTK304	MATa, leu2Δ0, his3Δ1, met15Δ0, trp1::ura3	This study
YTK339	MATa, PHO23::18MYC::TRP1, leu2Δ0, his3Δ1, met15Δ0, trp1::ura3	This study
YTK378	MATa, pho23(W305A)::18MYC::TRP1, leu2Δ0, his3Δ1, met15Δ0, trp1::ura3	This study
YTK363	MATa, CTI6::TAP::HIS3, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	This study
YTK364	MATa, cti6(W98A)::TAP::HIS3, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	This study
YTK222	MATa, stb3Δ::KanMX, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	This study
YTK224	MATa, tod6Δ::KanMX, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	This study
YTK252	MATa, xbp1Δ::KanMX, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	This study

Keogh et al. (2005) Cotranscriptional Set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell. 123, 593-605.

Bumgarner et al. (2009) Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. PNAS. 106, 18321-18326.

Table S2. Oligonucleotides used in this study.

Gene	Sequences
SCR1	(F) GAAGTGTCCC GGCTATAATA AA (R) GACGCTGGATAAAACTCCCC
REI1	(F) GAGCACAATAAGCACTTCAAAGA (R) ACAAATGTTCCCAAGACCAATC
RRN11	(F) CATAACTGGTCGCTTGCATATAA (R) TTGACACATCCATTGTAAGAAGT
TEA1	(F) AGGACTAAACGATGATAATCCCA

	(R) TTCTTGGAGTGATTGAAGAATCC
TKL2	(F) GAAGACTGTTGTGGAACCCG (R) TTCGGTAAATGCTTTTCCCAAC
HXT5	(F) CAAACTCTGTCCAATGGAGG (R) TAGCTCTTGCTAAGGACCGC
STL1	(F) CCAAATGATG AGGAAGTTAT AAC (R) AAATTGCGTT GAAGCTGCAA TC
TEL VI	(F) TTTCGATCAAACTGCGTCAGG (R) CGAGACAACGACAGGAAATAC
REI1 (prom.)	(F) TAACATTATTACCACGGCGA (R) CGTATTGTTACTGATATGCT
TEA1 (prom.)	(F) CAAAAATGGAAAGTAGGTATGGG (R) TTCATTCTTGTTTGGCCACA
RRN11 (prom.)	(F) GCATCGCTAACATCTAAAAAAGT (R) AGGGACTTCAAACATGGTTTAT
INO1 (prom.)	(F) GATGCGGAATCGAAAGTGTT (R) AAATTAACATTGCCGCCAAC
PHO23(W305)-CORE	(F) TGGCATACGGGGAAATGGTGGGGTGTGATGGCGCAGACTGTGAGCTAGAA GAGCTCGTTTTCGACACTGG (R) CACTTGCCCTTAGGTAGAGTTTCGAGTCCAATACATGGCAAATGGAACCA TCCTTACCATTAAGTTGATC
PHO23(W305A)	(F) TGGCATACGGGGAAATGGTGGGGTGTGATGGCGCAGACTGTGAGCTAGAAGCCTTCCATTTGCCATGTATTGGACTCGAAACTCTACCTA AGGGCAAGTG
CTI6(W98)-CORE	(F) ATACTCCAGATGACTCCGGATTTTTCATTCAATGTGAGCAGTGCAGTTCC GAGCTCGTTTTCGACACTGG (R) TTATCAGGTGCGTTGTCTTGAGTAATACTCACACAATAACCGTGCTGCCA TCCTTACCATTAAGTTGATC

CTI6(W98A)	(F) ATACTCCAGATGACTCCGGATTTTTCATTCAATGTGAGCAGTGCAGTTCCGCACAGCACGGTTATTGTGTGAGTATTA CTCAAGACAACGCACCTGATAA
Pho23-Cterm-myc	(F) CTACCTAAGGGCAAGTGGTATTGCGACGACTGCAAAAAAAAAACTGTCCGGTTCTGCTGCTAGTGGT (R) ATTTAGTAGATATTTCCTTAAGGCGATATTCGCCCGAGAGCTATTCCTCGAGGCCAGAAGACTAAG