Suppression of spt16 phenotypes by set2.

An spt16 mutation displays synthetic defects with mutations in either the GCN5 or the ELP3 histone acetyltransferase genes (Formosa et al., 2002). Strains with either a spt16 or a gcn5 mutation grow well at 30°C (Fig S1A). However, the spt16 gcn5 double mutant strain grows poorly at 25°C and is lethal at 30°C. Importantly, this spt16 gcn5 lethality can be suppressed by deletion of SET2 in this strain. Similarly, the spt16 elp3 double mutant cannot grow at 34°C, but set2 suppresses this defect (Fig S1B). An spt16 nhp6a nhp6b triple mutant also shows synthetic growth defects (Formosa et al., 2001), and this can also be suppressed by set2 (Fig S1C).

The lys2-173R2 allele confers a Lys+ phenotype in wild type cells, but some spt mutants with the lys2-173R2 allele are Lys- (Winston et al., 1984). A gcn5 lys2-173R2 strain is Lys-, an Spt- phenotype, while the gcn5 set2 lys2-173R2 strain is Lys+, showing suppression of the Spt- phenotype (Fig S1D). Thus, deletion of Set2 can alter the process of selecting transcription initiation sites that is defective in a gcn5 mutant, even though it does not ameliorate the defect in this process caused by spt16-11.
Supplemental Figure S1. A set2 mutation suppress various phenotypes.

A. A set2 mutation suppresses the spt16 gcn5 synthetic growth defect. Strains DY150, DY8780, DY8821, DY8155, and DY8820 were plated on complete medium at 25°C for 3 days or at 30°C for 2 days.

B. A set2 mutation suppresses the spt16 elp3 synthetic growth defect. Dilutions of strains DY150, DY8780, DY8153, DY8837, and DY8833 were plated on complete medium at 30°C for 2 days or at 34°C for 4 days.

C. A set2 mutation suppresses the spt16 nhp6ab synthetic growth defect. Strains DY8779, DY8808, DY8810, and DY7588 were plated on completed medium at the indicated temperature for 2 days.

D. A set2 mutation reverses the Spt- phenotype of a gcn5 mutant. Dilutions of strains DY7467 (his4-917Δ lys2-173R2), DY9413 (set2 his4-917Δ lys2-173R2), DY6077 (gcn5 lys2-173R2), and DY9412 (gcn5 set2 lys2-173R2), were plated at 30°C on complete medium for 4 days or on medium lacking lysine for 3 days.
Figure S1

A. 25°C
-wild type
- set2
- spt16
- gcn5

30°C
-wild type
- set2
- spt16
- gcn5

B. 30°C
-wild type
- set2
- spt16
- elp3
- elp3

34°C
-wild type
- set2
- spt16
- elp3

C. 25°C
-spt16
- set2
- nhp6ab

33°C
-spt16
- set2
- nhp6ab

D. complete
-wild type
-set2
-gcn5
-gcn5
-set2

-Lys
-wild type
-set2
-gcn5
-gcn5
-set2
Specificity of set2 suppression.

Fig S2A shows the phenotypes of double mutant strains constructed with set1 and set2 and mutations in paf1, cdc73, dst1, spt4 or elp3, as well as spt16. PAF1 and CDC73 encode subunits of the Paf1 complex, previously thought to be elongation factors but recently shown to also be involved in mRNA 3’ end processing (Penheiter et al., 2005; Sheldon et al., 2005). The paf1 set2 double mutant shows a synthetic growth defect (Fig S2B), as described previously (Krogan et al., 2003). In contrast, set1 suppresses, as the paf1 set1 double mutant strain grows at 32°C while the paf1 single does not (Fig S2B). We find set1 suppresses the temperature sensitive growth defect of cdc73 (Fig S2C); this result disagrees with that of Krogan et al. (2003) who reported a synthetic growth defect in the cdc73 set1 double mutant. Testing a different phenotype, we do see a synthetic defect with the cdc73 set1 double mutant on 6-AU, and suppression by set2 (Fig S2D). Thus, the temperature sensitivity and 6-AU sensitivity caused by cdc73 appear to arise from distinct mechanisms, and are affected in opposite ways by deletion of SET1. DST1 encodes the TFIIS elongation factor, and dst1 mutants are 6-AU sensitive (Exinger and Lacroute, 1992). We find that both the dst1 set1 and dst1 set2 double mutants show greater 6-AU sensitivity than the single mutants (Fig S2E), as has been observed previously for dst1 set2 (Li et al., 2003). SPT4 and SPT5 encode the yeast version of the DSIF elongation factor (Hartzog et al., 1998). spt4 mutants are temperature sensitive, but this can be suppressed by an set2 mutation (Fig S2F). Additionally, the spt4 set1 double mutant shows enhanced 6-AU sensitivity compared to either single mutant (Fig S2F). ELP3 encodes a histone acetyltransferase that is part of the elongator complex (Wittschieben et al., 1999). elp3 mutants show mild temperature sensitivity, but there a synthetic growth defect in elp3 set2 double mutants at 37°C (Fig S2G).
Supplemental Figure S2. Genetic interactions of set1 and set2 with various mutations.

A. Table summarizing phenotypes of mutants.

B. A paf1 mutant is suppressed by set1. Dilutions of strains DY3398 (wild type), DY7014 (paf1), DY8917 (set1), DY8919 (set2), DY8911 (paf1 set1), and DY8913 (paf1 set2), were plated on complete medium at 25°C for 3 days or at 32°C for 2 days.

C. A cdc73 mutant is suppressed by set1. Dilutions of strains DY3398 (wild type), DY8870 (cdc73), DY8917 (set1), and DY8923 (cdc73 set1), were plated on complete medium at 25°C for 3 days or at 32°C for 2 days.

D. The 6-AU sensitivity of a cdc73 mutant is oppositely affected by set1 and set2 mutations. Dilutions of strains DY3398 (wild type), DY8870 (cdc73), DY8917 (set1), DY8919 (set2), DY8923 (cdc73 set1), and DY8925 (cdc73 set2), were plated at 30°C on complete medium for 2 days or on medium lacking uracil containing 100 ug/ml 6-azauracil for 6 days.

E. The 6-AU sensitivity of a dst1 mutant is exacerbated by a set1 or a set2 mutation. Dilutions of strains DY3398 (wild type), DY8872 (dst1), DY8917 (set1), DY8919 (set2), DY8930 (dst1 set1), and DY8933 (dst1 set2), were plated at 25°C on complete medium for 2 days or on medium lacking uracil containing 25 ug/ml 6-azauracil for 4 days.

F. Phenotypes of a spt4 mutant are affected by a set1 or a set2 mutation. Dilutions of strains DY3398 (wild type), DY8917 (set1), DY8919 (set2), DY9050 (spt4), DY9051 (spt4 set1), and DY9052 (spt4 set2), were plated on complete medium for 2 days, at 37°C on complete medium for 3 days, or on medium lacking uracil containing 50 ug/ml 6-azauracil for 4 days.

G. An elp3 mutation synthetic lethal with set2. Dilutions of strains DY150 (wild type), DY8690 (set2), DY8156 (elp3), and DY8837 (elp3 set2), were plated on complete medium at the indicted temperature for 4 days.
## Figure S2

### A.

<table>
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<td>6-AU sensitivity</td>
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<td>set2</td>
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<td>no change</td>
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<td>Ts+</td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

### B.

#### 25°C
- wild type
- paf1
- set1
- set2
- paf1 set1
- paf1 set2

#### 32°C

### C.

#### 30°C
- wild type
- cdc73
- set1
- cdc73 set1

#### 37°C

### D.

#### complete
- wild type
- cdc73
- set1
- set2
- cdc73 set1
- cdc73 set2

#### 6-AU

### E.

#### complete
- wild type
- dst1
- set1
- set2
- dst1 set1
- dst1 set2

#### 6-AU

### F.

#### wild type
- set1
- set2
- spt4
- spt4 set1
- spt4 set2

#### 25°C

#### 37°C

#### 6-AU

### G.

#### wild type
- set2
- elp3
- elp3 set2

#### 30°C

#### 37°C
Effect of *pob3* and *set2* mutations at other promoters.

To test the generality of the results obtained with the GAL1 promoter, we used DNA microarrays to compare transcript levels throughout the genome for WT, *set2*, *spt16*, and *spt16 set2* mutants. We found a number of genes whose expression was reduced in an *spt16* mutant, and this transcriptional defect was partially restored in the *spt16 set2* strain. We used S1 nuclease protection assays to verify these results. As shown in Fig S3A, RNA levels for *YJL097w*, *PPA2*, *SUT2*, *PMA1*, and *RIP1* are reduced in either a *spt16* or *pob3* mutant, and a *set2* mutation partially suppresses these deficiencies. We also examined TBP binding to promoters by ChIP assays (Fig S3B). TBP binding at *PMA1* and *RIP1* is reduced in the *pob3* mutant, and increased TBP binding is seen in the *pob3* *set2* strain. These results suggest that yFACT and Set2 have positive and negative roles, respectively, in regulating TBP binding to some promoters.
Fig S3. Reduced expression and TBP binding for some genes in a pob3 mutant and suppression by set2.

A. RNA was isolated from strains DY150 (wild type), DY8690 (set2), DY8107 (spt16), DY8777 (spt16 set2), DY8881 (pob3), and DY8877 (pob3 set2), and mRNA levels for specific genes measured by S1 nuclease protection.

B. TBP binding to promoters from strains DY150 (wild type), DY8690 (set2), DY8881 (pob3), and DY8877 (pob3 set2), were measured by ChIP. Error bars show variance among replicate PCRs.
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Figure S3

A.

- YJL097w
- PPA2
- SUT2

B.

- PMA1
- RIP1
No defect in elongation at *GAL1- YLR454w* in pob3 mutants.

We used the *GAL1-YLR454w* reporter to assess the rate of pol II elongation in wild type and mutant strains. We took RNA samples at intervals following galactose induction, and used probes for S1 nuclease protection assays specific to the 5’ and 3’ ends of the gene (Fig S4). The time between appearance of mRNA sequences corresponding to the 5’ and 3’ ends gives an indication of how long it takes for pol II to traverse the 8 kb gene. We find that in a wild type strain pol II takes about 8 min. to traverse the gene, consistent with previous work (Mason and Struhl, 2005). Importantly, this rate is not altered by the set2 mutation, the pob3 mutation, or the pob3 set2 double mutation.

A different assay was performed to examine elongation rates at *GAL1-YLR454w* in wild type and mutant cells. In this experiment cells are grown in galactose so that *GAL1-YLR454w* is expressed, and then glucose is added to stop new transcription. At one min. intervals following glucose addition, samples are taken to measure pol II occupancy at positions either 1 kb or 8 kb down the ORF (Fig S5). The idea is to follow Pol II occupancy during the last wave of transcription down the gene (Mason and Struhl, 2005). In wild type cells it takes about 3 min. to travel the 7 kb distance from the 1 kb to 7.8 kb landmarks. The data with the pob3 mutant shows a great deal more scatter, presumably because there is much less pol II associated with the gene. While it is hard to get an exact elongation rate from the data from the pob3 mutant, it is clear that the elongation rate in the pob3 mutant is not significantly different from that seen for wild type. A slower elongation rate would result in an increased interval between the 1 kb curve and the 7.8 kb curve, and this is clearly not seen.

Thus both of these assays fail to find an effect of the pob3 mutation on elongation rate at *GAL1-YLR454w*. This is a surprising result since earlier reports have suggested that yFACT has a positive role in transcriptional elongation. It is possible that a yFACT mutation affects transcriptional elongation at other genes.
**Fig S4. RNA accumulation at GAL1-YLR454w following galactose induction.**

Strains DY9591 (wild type), DY9976 (set2), DY9972 (pob3), and DY9974 (pob3 set2), all containing *GAL1-YLR454w*, were grown in 2% raffinose media to mid-log, and galactose was added to 2%. Samples were taken at intervals, and RNA was isolated and used for S1 nuclease protection assays using probes specific to the 5’ (black) and 3’ (red) ends of the gene. S1 oligos corresponding to nt 511–581 and nt 7816–7871, respectively, of the YLR454w ORF, are listed in Supplemental Table 3. For the wild type and set2 strains the experiment was conducted once, with samples taken at ten min. intervals. For the pob3 and pob3 set2 strains, with the lower RNA levels, the induction experiment was repeated three times, with samples taken at five min. intervals. The graph shows the average and standard deviation of the three independent experiments.
Figure S4

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**wild type**

![Graph showing YLR454w mRNA levels over time for wild type.]

- 5' mark at 8 min.

**set2**

![Graph showing YLR454w mRNA levels over time for set2.]

- 8 min.

**pob3**

![Graph showing YLR454w mRNA levels over time for pob3.]

- 6 min.

**pob3 set2**

![Graph showing YLR454w mRNA levels over time for pob3 set2.]

- 7 min.

Time after addition of galactose (min.)
Fig S5. RNA II occupancy at \textit{GAL1-YLR454w} following glucose shut down.

Strains DY9591 (wild type), DY9976 (set2), DY9972 (pob3), and DY9974 (pob3 set2), all containing \textit{GAL1-YLR454w}, were grown in 2% galactose media to mid-log, and glucose was added to 2%. Samples were taken at one min. intervals and processed for ChIP to measure pol II binding at 1 kb (black) and at 7.8 kb (red). Oligos amplifying the 1 kb region (+945 to +1147) and the 7.8 kb region (7701 to +7850) are listed in Supplemental Table 3. Values were normalized so that the zero time point was 1.0, and the point with the least pol II binding was zero.
Figure S5

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![Graphs showing Pol II ChIP Ratio over time after addition of glucose for wild type, set2, pob3, and pob3 set2 cases.](image-url)
REFERENCES


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