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Supplemental Information

Unique and Shared Roles for Histone H3K36 Methylation States in Transcription Regulation Functions

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	Caffeine Sensitivity	Rapamycin Sensitivity	Phleomycin Sensitivity	6AU Resistance	*bur1*Δ Bypass	*spt16-11* Bypass	Cryptic Initiation
Wild-type	-	-	-	-	-	-	-
***set2*Δ**	+	+	+	+	+	+	+
set2-Y149F	-	-	-	-	-	-	-
set2-F234Y	-	-	-	+/-	-	-	-
set2-H199L	+	+	+	+	+	+	+

Table S1. Summary of *set2* mutant phenotypes, Related to Figure 3
A minus (-) indicates wild-type phenotype and a plus (+) indicates *set2*Δ phenotype. A plus/minus (+/-) indicates an intermediary phenotype.

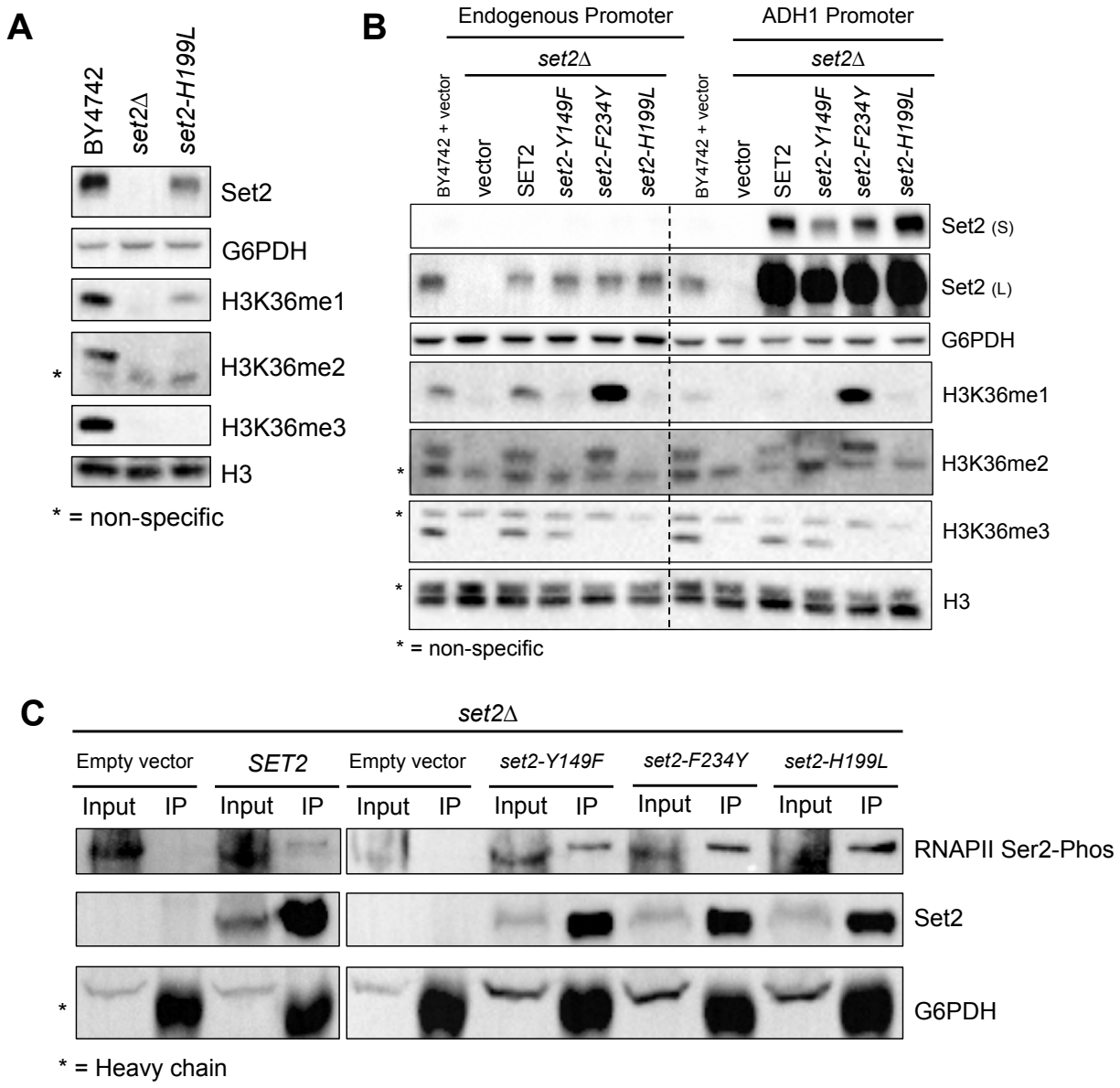


Figure S1. *set2* Mutants Differentially Methylate H3K36 and Interact with RNAPII, Related to Figures 2 and 3

A. Western blots of indicated strains probed with Set2 and different H3K36 methylation antibodies. H3 and G6PDH served as loading controls B. Western blots of indicated strains transformed with plasmids that expressed *SET2* from its native promoter or overexpressed *SET2* from the *ADH1* promoter and probed with Set2 and different H3K36 methylation antibodies. Loading controls were H3 and G6PDH. C. Co-immunoprecipitation showing interaction of *set2* mutants and serine 2 phosphorylated form of RNAPII. Set2 was immunoprecipitated by anti-HA antibody. G6PDH served as a loading control.

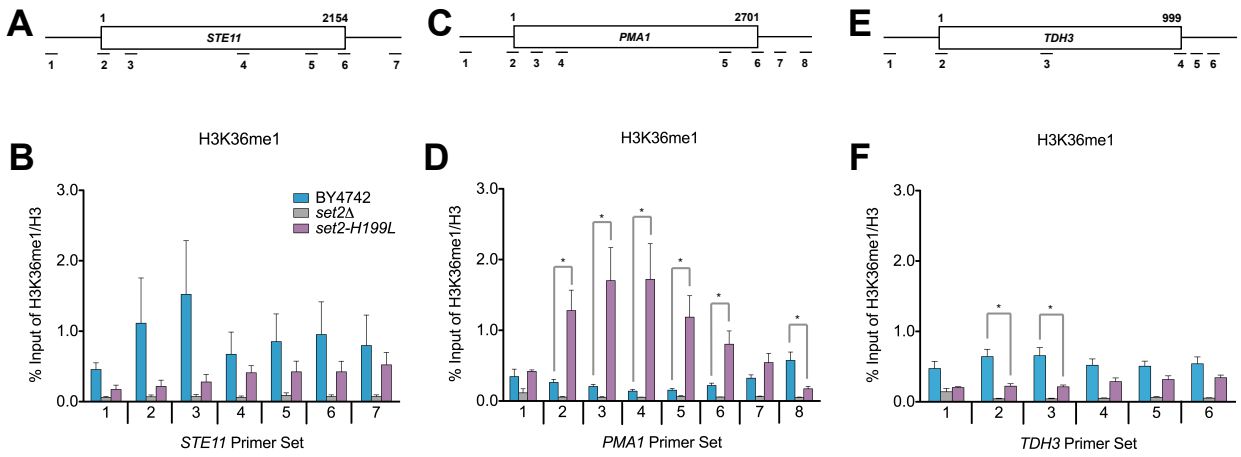
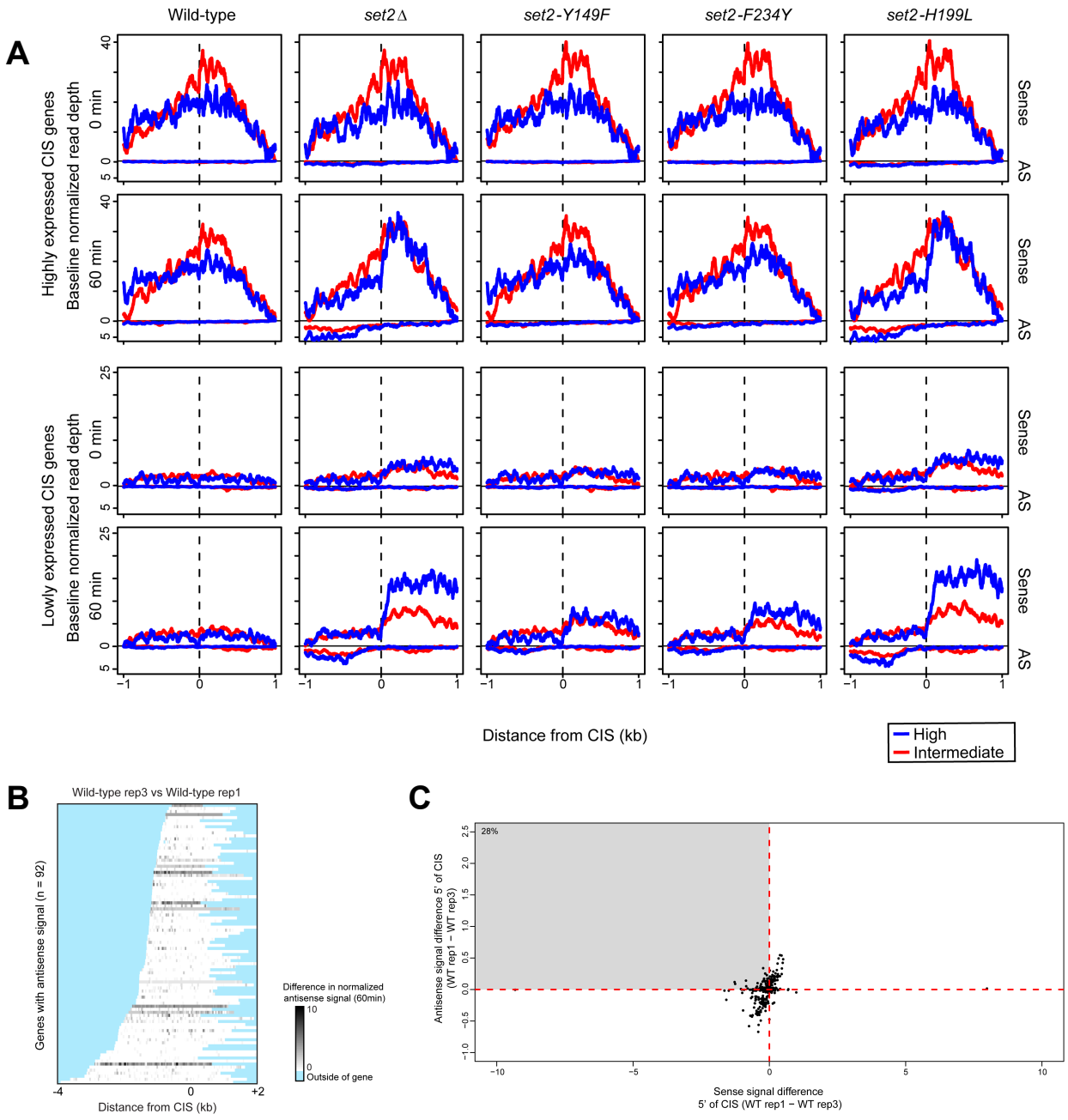


Figure S2. H3K36me1 is Deposited within or near Transcribed Regions of Genes, Related to Figure 3

A. Schematic of *STE11* with amplicons indicated below. B. ChIP analysis of H3K36me1 across *STE11* in the indicated strains. C. Schematic of *PMA1* with amplicons indicated below. D. ChIP analysis of H3K36me1 across *PMA1* in the indicated strains. E. Schematic of *TDH3* with amplicons indicated below. F. ChIP analysis of H3K36me1 across *TDH3* in the indicated strains. Data represented as mean ± SEM of three independent biological replicates. Student's t-test was used to obtain p-values. Asterisks indicate significance (* $p<0.05$); non-significant comparisons are not shown. All qPCR primers are listed in Table S3.



D

Motif	-log$_{10}$ p-value	%obs	%bkg
TTCAGTTC	32	62.0	27.3

Figure S3. Activity of H3K36 Methylation States in Cryptic Transcription Regulation, Related to Figure 5

A. Sense and antisense normalized transcriptional signal (reads per million mapped) across 439 high- (blue) and intermediate- (red) confidence cryptic initiation sites (CIS) defined previously, separated by high (top) or low (bottom) gene expression. Signal was averaged across three

independent biological replicates and plotted for 0 min (top) and 60 min (bottom) following nutrient deprivation for each genetic model. High or low expression was defined as the top or bottom 25% of genes based on their expression in wild-type. The minimum value for each line was adjusted to zero (y axis) to adjust for subtle differences in baseline expression and focus on the position and range in magnitude of signal, as in Mcdaniel et al., 2017. B. Heatmap of anti-sense transcription, plotted as the difference in antisense signal between two wild-type replicates (WT rep3–WT rep1) at 60 min following nutrient deprivation. Normalized signal is plotted for the 92 genes that were previously shown to have antisense transcription between the CIS and transcription start site. Darker gray indicates more anti-sense signal in WT rep3 compared to WT rep1. Regions outside of the gene body are masked blue. C. Scatterplot of sense and antisense signal differences (WT replicate 1– WT replicate 3) in the gene regions between the CIS and transcription start site, for 439 high- and intermediate-confidence CIS. Each point represents the CIS of a given gene; points that extend into the upper-left quadrant indicate decrease in sense transcription (relative to wild-type) with a concomitant increase in antisense transcription. The percentage of all 439 genes falling in this quadrant is supplied in the top left. D. Significantly enriched sequence motifs discovered in the 100 bp surrounding 439 randomly-selected low-confidence CIS, requiring at least 2-fold enrichment relative to local background sequence.

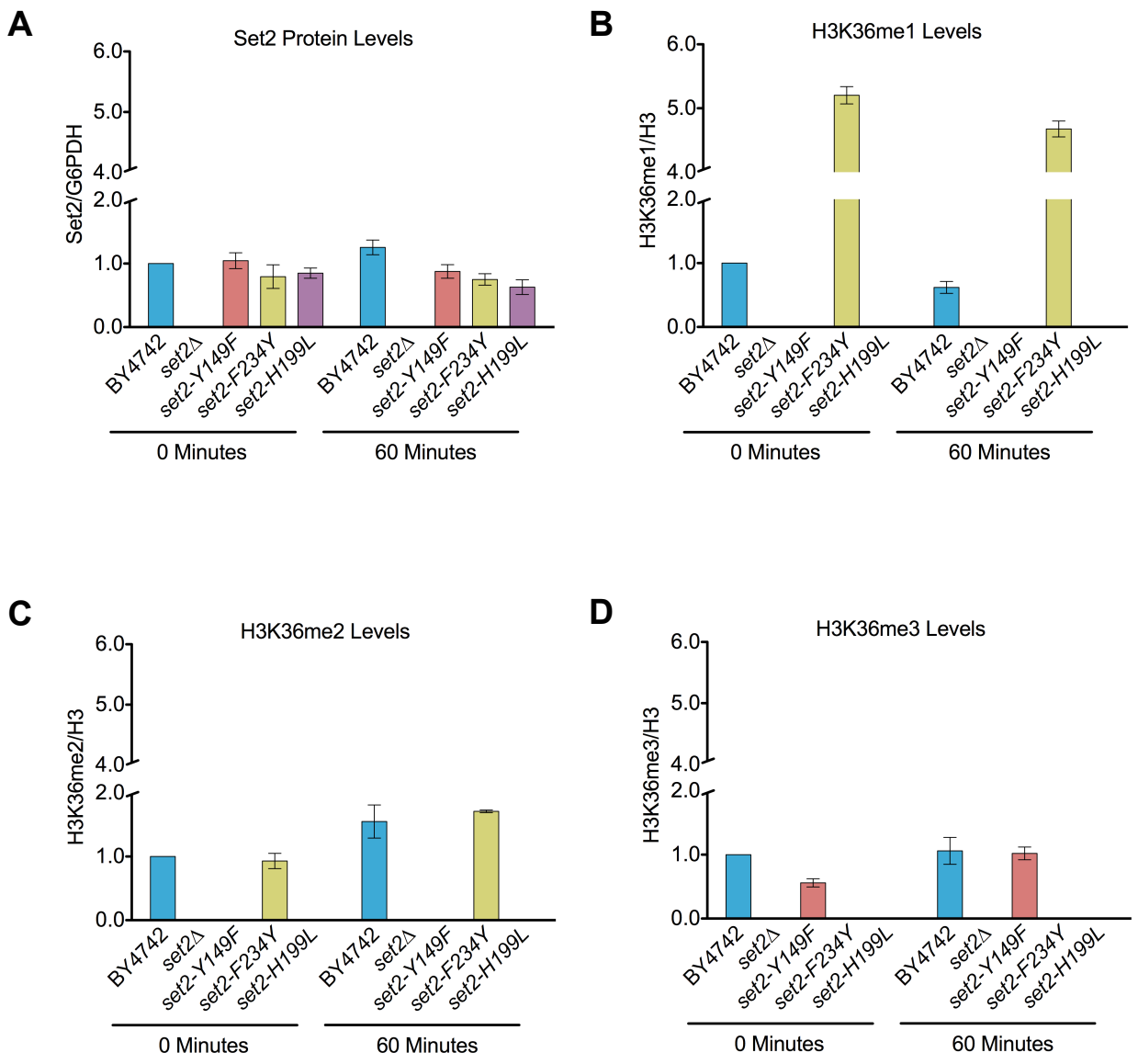
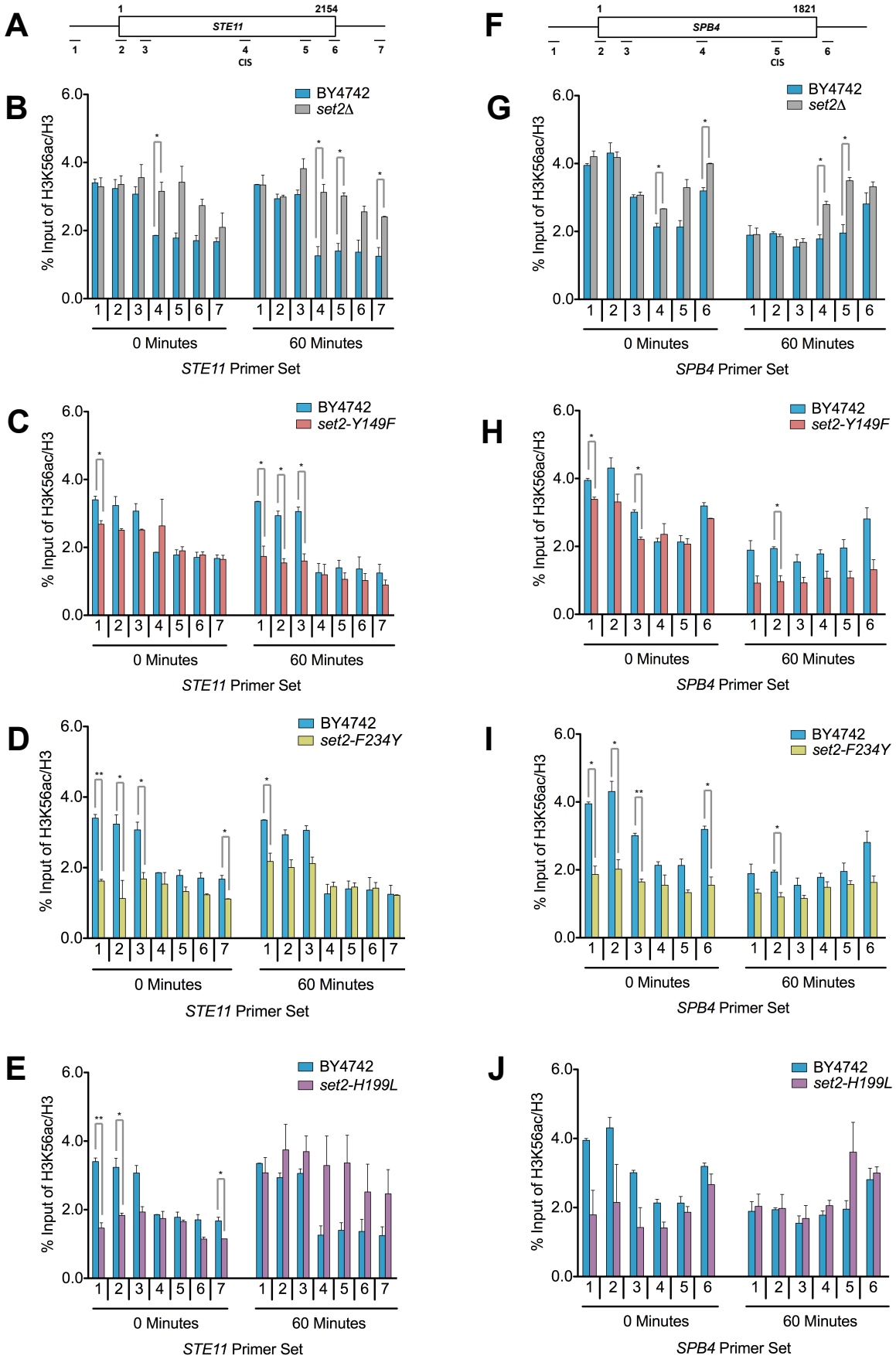


Figure S4. Set2 and H3K36 Methylation Levels are Similar at 0 and 60 Minutes after Nutrient Deprivation, Related to Figure 6

A. Quantification of Set2 normalized to G6PDH in indicated strains. All strains were quantified relative to BY4742 at 0 minutes. B-D. Quantification of H3K36me1 (B), H3K36me2 (C), and H3K36me3 (D) normalized to H3 in the indicated strains. H3K36me1 was quantified using the short exposure Western blot. All strains were quantified relative to BY4742 at 0 minutes. Each bar graph is representative of two or more independent biological replicates with a representative replicate shown in Figure 6.


A
1
2154
STE11
1
2
3
4
CIS
5
6
7
F
1
1821
SPB4
1
2
3
4
5
CIS
6
B
BY4742
set2Δ
% Input of H3K56ac/H3
6.0
4.0
2.0
0.0
1 2 3 4 5 6 7 1 2 3 4 5 6 7
0 Minutes
60 Minutes
STE11 Primer Set
G
BY4742
set2Δ
% Input of H3K56ac/H3
1 2 3 4 5 6 1 2 3 4 5 6
0 Minutes
60 Minutes
SPB4 Primer Set
C
BY4742
set2-Y149F
% Input of H3K56ac/H3
1 2 3 4 5 6 7 1 2 3 4 5 6 7
0 Minutes
60 Minutes
STE11 Primer Set
H
BY4742
set2-Y149F
% Input of H3K56ac/H3
1 2 3 4 5 6 1 2 3 4 5 6
0 Minutes
60 Minutes
SPB4 Primer Set
D
BY4742
set2-F234Y
% Input of H3K56ac/H3
1 2 3 4 5 6 7 1 2 3 4 5 6 7
0 Minutes
60 Minutes
STE11 Primer Set
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BY4742
set2-F234Y
% Input of H3K56ac/H3
1 2 3 4 5 6 1 2 3 4 5 6
0 Minutes
60 Minutes
SPB4 Primer Set
E
BY4742
set2-H199L
% Input of H3K56ac/H3
1 2 3 4 5 6 7 1 2 3 4 5 6 7
0 Minutes
60 Minutes
STE11 Primer Set
J
BY4742
set2-H199L
% Input of H3K56ac/H3
1 2 3 4 5 6 1 2 3 4 5 6
0 Minutes
60 Minutes
SPB4 Primer Set

Figure S5. H3K36me1/2 and H3K36me3 are Important for Proper H3K56ac Localization, Related to Figure 7

A. Schematic of *STE11* with amplicons indicated below. Predicted cryptic initiation site (CIS) located within primer set 4. B-E. ChIP analysis of H3K56ac across *STE11* in the indicated strains and time points. F. Schematic of *SPB4* with amplicons indicated below. Predicted cryptic initiation site (CIS) located within primer set 5. G-J. ChIP analysis of H3K56ac across *SPB4* in the indicated strains and time points. Data represented as mean ± SEM of two independent biological replicates. Student's t-test was used to obtain p-values. Asterisks indicate significance (* $p<0.05$; ** $p<0.01$); non-significant comparisons are not shown. All qPCR primers are listed in Table S3.

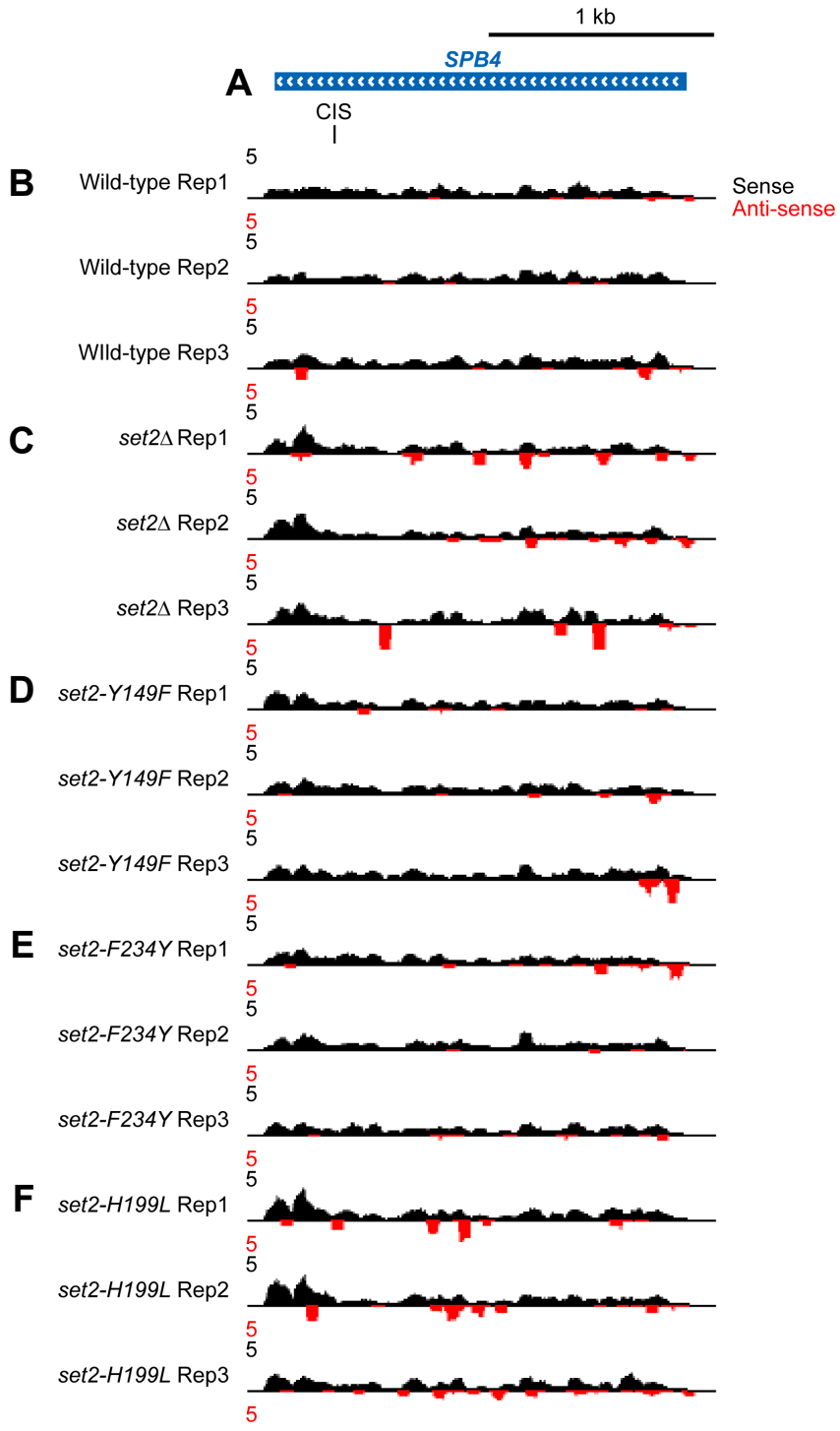

Figure S6. H3K36me1/2 and H3K36me3 are Important for Repressing Cryptic Antisense Transcription at *SPB4* after Nutrient Deprivation, Related to Figure 7

A. Schematic of *SPB4* and predicted cryptic initiation site (CIS). B. Genome browser tracks of wild-type replicates 1-3. C. Genome browser tracks of *set2*Δ replicates 1-3. D. Genome browser tracks of *set2-Y149F* replicates 1-3. E. Genome browser tracks of *set2-F234Y* replicates 1-3. F. Genome browser tracks of *set2-H199L* replicates 1-3. All data is from 60 minutes after nutrient deprivation, with black representing sense signal and red representing antisense signal.