1   Acetylation-Dependent Recruitment of the FACT Complex and Its Role in

2   Regulating Pol II Occupancy Genome-Wide in *Saccharomyces cerevisiae.*

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ABSTRACT

Histone chaperones, chromatin remodelers, and histone modifying complexes play a critical role in alleviating the nucleosomal barrier for DNA-dependent processes. Here, we have examined the role of two highly conserved yeast (*Saccharomyces cerevisiae*) histone chaperones, FACT and Spt6, in regulating transcription. We show that the H3 tail contributes to the recruitment of FACT to coding sequences in a manner dependent on acetylation. We found that deleting a H3 HAT Gcn5 or mutating lysines on the H3 tail impairs FACT recruitment at *ADH1* and *ARG1* genes. However, deleting the H4 tail or mutating the H4 lysines failed to dampen FACT occupancy in coding regions. Additionally, we show that FACT-depletion reduces Pol II occupancy in the 5' ends genome-wide. In contrast, Spt6-depletion leads to reduction in Pol II occupancy towards the 3' end, in a manner dependent on the gene-length. Severe transcription and histone eviction defects were also observed in a strain that was impaired for Spt6 recruitment (*spt6Δ202*) and depleted of FACT. Importantly, the severity of the defect strongly correlated with WT Pol II occupancies at these genes, indicating critical roles of Spt6 and Spt16 in promoting high-level transcription. Collectively, our results show that both FACT and Spt6 are important for transcription globally and may participate during different stages of transcription.

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66 **INTRODUCTION**

67   The nucleosome is the fundamental unit of chromatin and is composed of ~ 147 bp of

68 DNA wrapped around a histone octamer consisting of two copies of histones H2A, H2B, H3 and

69 H4. Nucleosomes pose a significant impediment to all steps of transcription, including the steps

70 of initiation and elongation of RNA polymerase II (Pol II) through the coding sequences (CDS)

71 (LI *et al.* 2007). There are two principle mechanisms suggested to alleviate nucleosomal barrier:

72 I) removal of the H2A-H2B dimer to generate hexamers that can be readily overcome by

73 elongating polymerases (KIREEVA *et al.* 2002), and II) complete removal of histone octamers

74 leading to reduced nucleosomal density across the transcribing genes (LEE *et al.* 2004; DION *et*

75 *al.* 2007).

76 Various factors and enzyme complexes have been implicated in removing the nucleosomal

77 barriers *in vivo* (LI *et al.* 2007). For example, acetylation of histone tails facilitates histone

78 eviction by weakening histone-DNA interactions (GOVIND *et al.* 2007; GOVIND *et al.* 2010) and

79 by promoting recruitment of ATP-dependent remodelers, such as RSC and SWI/SNF (HASSAN *et*

80 *al.* 2001; DECHASSA *et al.* 2010; SPAIN *et al.* 2014). While nucleosome disassembly is important

81 for transcription, it is also important to restore the chromatin structure in the wake of

82 transcription. Histone chaperones, many of which are cotranscriptionally recruited to actively

83 transcribing genes, are implicated in performing this function (GURARD-LEVIN *et al.* 2014).

84   Two such chaperones, the FACT complex (Spt16/SSRP1 in humans and Spt16/Pob3 in

85 yeast) and Spt6, are enriched in transcribed coding regions (ANDRULIS *et al.* 2000; KAPLAN *et al.*

86 2000; KROGAN *et al.* 2002; MASON AND STRUHL 2003; MAYER *et al.* 2010; FORMOSA 2012;

87 BURUGULA *et al.* 2014), consistent with a role for these factors in regulating transcription and in

88 maintaining chromatin integrity. Human FACT recognizes and displaces one of the H2A/H2B

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89   dimers from the nucleosome, and promotes transcription on a chromatin template, *in vitro*. In

90   addition, it can assemble all four histones on the DNA (BELOTSERKOVSKAYA *et al.* 2003). Yeast

91   (*S. cerevisiae*) FACT also interacts with all four histones and displays a strong affinity toward

92   intact nucleosomes (FORMOSA *et al.* 2001; VANDEMARK *et al.* 2008). Multiple domains within

93   the FACT subunits Spt16 and Pob3 are implicated in binding and chaperoning histones

94   (HONDELE AND LADURNER 2011; FORMOSA 2012). The N-terminal domain (peptidase-like) of *S.*

95   *pombe* Spt16, for example, binds to both core histones as well as histone N-terminal tails

96   (STUWE *et al.* 2008), and the M-domain of *Chaetomium thermophilum* Spt16 recognized

97   H2A/H2B with affinity similar to that observed with full-length Spt16 (HONDELE *et al.* 2013).

98   However, in *S. cerevisiae*, the C-terminal regions of both Spt16 and Pob3 were defined as

99   H2A/H2B binding domains (VANDEMARK *et al.* 2008; KEMBLE *et al.* 2015). Whether these

100   different domains are required for interacting with nucleosomes in a context-dependent manner

101   remains to be seen.

102   FACT is shown to promote reassembly of the displaced H3/H4 in the *ADH1*, *ADH2* and

103   *STE3* coding regions (JAMAI *et al.* 2009) implicating FACT in restoring chromatin behind

104   elongating Pol II. The role of FACT (and Spt6) in regulating histone occupancy has been

105   examined at a genome-wide scale (VAN BAKEL *et al.* 2013; JERONIMO *et al.* 2015). Impairing

106   FACT function leads to reduced histone occupancy and also results in aberrant incorporation of a

107   histone variant H2AZ (JERONIMO *et al.* 2015). Additionally, recruitment of FACT to the *HO*

108   promoter is shown to promote histone eviction (TAKAHATA *et al.* 2009). Likewise, FACT helps

109   in evicting H2A/H2B from the promoter of the *PHO5* gene, upon induction (RANSOM *et al.*

110   2009).

111   Gene-specific studies have shown a role for FACT in regulating transcription. For

112   example, FACT mutants impaired Pol II and TBP recruitment at the *GAL1* promoter, implicating

113   a role for FACT in regulating transcription at the initiation step (BISWAS *et al.* 2006; FLEMING *et*

114   *al.* 2008). However, moderate reductions in Pol II occupancy were observed in the 3' ORFs of

115   *GAL1* and *PHO5* but not in *LacZ* or *YAT1* genes expressed on plasmids under the control of

116   *GAL1* promoter (JIMENO-GONZALEZ *et al.* 2006). Likewise, transcription defects were observed

117   only at *ADH1* and not at *ADH2* or *STE3*, despite all three genes showing a histone reassembly

118   defect (JAMAI *et al.* 2009), making it unclear if FACT is generally required for transcription. In

119   addition to transcription initiation, FACT is shown to participate in promoting Pol II processivity

120   and elongation rate at *GAL1* gene, in conjunction with the H2B ubiquitination (FLEMING *et al.*

121   2008).

122   FACT shares many functional similarities with another histone chaperone, known as

123   Spt6, which interacts with H3/H4 (KAPLAN *et al.* 2003; MAYER *et al.* 2010), and also with

124   H2A/H2B (MCCULLOUGH *et al.* 2015). Loss of Spt6 function leads to reduced histone occupancy

125   over transcribed regions, suggesting a role for Spt6 in cotranscriptional histone reassembly

126   (IVANOVSKA *et al.* 2011; PERALES *et al.* 2013; VAN BAKEL *et al.* 2013; JERONIMO *et al.* 2015).

127   Spt6, along with the FACT complex, is also implicated in preventing spurious incorporation of

128   H2AZ in coding regions (JERONIMO *et al.* 2015) and thereby helps in restricting H2AZ to

129   promoter nucleosomes (BILLON AND COTE 2013). Therefore, Spt6 and FACT play important

130   roles in maintaining chromatin integrity. Consistent with this, widespread aberrant transcription

131   was observed in the cells deficient of Spt6 or FACT (CHEUNG *et al.* 2008; VAN BAKEL *et al.*

132   2013). Altered histone modifications, and reduced transcription genome-wide has been observed

133   in Spt6 mutant (DEGENNARO *et al.* 2013; KATO *et al.* 2013; PERALES *et al.* 2013). Spt6 possesses

134   a tandem SH2 (tSH2) domain at its C-terminus that interacts with phosphorylated Pol II CTD, *in*

135   *vitro* (DENGL *et al.* 2009; CLOSE *et al.* 2011; LIU *et al.* 2011) and this domain is implicated in

136   promoting Spt6 recruitment to transcribed genes, *in vivo* (MAYER *et al.* 2010; MAYER *et al.*

137   2012; BURUGULA *et al.* 2014).

138   In this study, we show that the acetylated histone H3 tail contributes to efficient

139   recruitment of FACT to transcribed coding sequences in *S. cerevisiae*. Depleting Spt16 subunit

140   of FACT elicits a greater reduction in Pol II occupancies in the 5'ends of the genes, suggesting a

141   potential role for FACT in the early elongation steps of transcription. In contrast, Pol II

142   occupancies were reduced more towards the 3' end in Spt6-depleted cells, suggestive of

143   processivity defects. Significant reductions in Pol II occupancies were also observed in a strain

144   defective for Spt6 recruitment. Our study reveals that both FACT and Spt6 promotes

145   transcription genome-wide and perhaps participate in different stages of transcription.

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147   **MATERIALS AND METHODS**

148   *Yeast strains and growth conditions*

149   The yeast strains used in this study are listed in Table S1. The cells were grown to absorbance

150   A_{600} of 0.5-0.6 in synthetic complete (SC) media lacking isoleucine/valine, and treated with

151   sulfometuron methyl (SM; 0.6 μg/ml) for 30 minutes to induce Gcn4 targets. Esa1 in the

152   *gcn5Δ/esa1ts* strain was inactivated by shifting the cultures grown at 25°C to 37°C for ~1.5

153   hours prior to induction by SM. Spt16 and Spt6 were depleted in *SPT16-TET* and *SPT6-TET*

154   (HUGHES *et al.* 2000; MNAIMNEH *et al.* 2004) cells by growing these strains in the presence of 10

155   μg/ml doxycycline overnight, and sub-culturing the overnight cultures in 100 ml of synthetic

156   complete (SC) media with 10 μg/ml doxycycline to an OD_{600} of 0.6.

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158   *Coimmunoprecipitation Assay*

159   The coimmunoprecipitation experiments were performed as described previously (GOVIND *et al.*

160   2010). The HA-tagged H2B or Spt16-Myc tagged WT and *H3Δ1-28* strains were resuspended in

161   500 µl of lysis buffer (50 mM Tris-HCl [pH-7.5], 50 mM HEPES-KOH [pH 7.9], 10 mM

162     $MgSO_4$, 100 mM $(NH_4)_2SO_4$, 12.5 mM KOAc, 0.01% NP-40, 20% Glycerol, 1ug/ml Pepstatin

163   A, 100 mM PMSF, 1ug/ml Leupeptin;) and 500 µl of glass beads, and disrupted by vortexing (18

164   seconds x 8 times, and 150 seconds on ice between each agitation cycle). Whole cell extracts

165   were incubated overnight with magnetic beads that were pre-conjugated to anti-Myc or anti-HA

166   antibodies in 100 µl 4X MTB buffer (200 mM HEPES-KOH [pH 7.9], 800 mM KOAc, 54 mM

167   MgOAc2, 40% Glycerol, 0.04% NP-40, 400 mM PMSF, 4 ug/ml Pepstatin, 4ug/ml Leupeptin),

168   and washed 5 times with the wash buffer (50 mM Tris-HCl (pH-8.0), 0.3% NP-40, 500 mM

169   NaCl, 10% Glycerol, 1mM PMSF, 1ug/ml Leupeptin, and 1ug/ml Pepstatin).

170   Immunoprecipitates were analyzed by western blot using the following antibodies: anti-Myc

171   (Roche), anti-HA (Roche), anti-Spt16 and anti-Spt6 antibodies (kindly provided by Tim

172   Formosa). The signal intensities were quantified using Image Studio lite version 5.2 (LI-COR

173   Biosciences).

174

175   *ChIP and ChIP-chip*

176   The cultures were crosslinked with formaldehyde and processed for chromatin

177   immunoprecipitation as described previously (GOVIND *et al.* 2012). ChIPs were performed using

178   antibodies, anti-Myc (Roche), anti-Rpb3 (Neoclone), and anti-H3 (Abcam). ChIP DNA and the

179   related input DNA was amplified using the primers against specific regions. 5µl of ChIP dye

180   (15% Ficol, 0.25% bromophenol blue in 1X TBE) and SYBR green dye were added in the PCR

181   products, resolved on 8% TBE gels and visualized on a phosphorimager and quantified using

182   ImageQuant 5.1 software. The fold enrichments were determined by taking the ratios of the ChIP

183   signal for gene of interest, and the signal obtained for the *POL1* used as an internal control and

184   dividing by the ratios obtained for the related input samples (ChIP/Input fold enrichment: ChIP

185   intensities [*ARG1/POL1*]) / input intensities [*ARG1/POL1*]). The ChIP experiments were

186   performed using at least three independent cultures, and PCR reactions were conducted at least

187   in duplicates. The error bars represent standard error of mean (SEM).

188   For ChIP-chip experiments, ChIP and related input DNA samples were amplified, from at least

189   two biological replicates, using the GenomePlex complete whole genome amplification (WGA)

190   kit (Sigma, cat # WGA2), according to the manufacturer's instructions. The amplified ChIP

191   DNA and input DNA were purified by using PCR cleanup kit (Qiagen Cat # 28104) and the

192   DNA was quantified by NanoDrop. The samples were hybridized on Agilent 4x44 arrays

193   (G4493A) after labeling the ChIP and input DNA with Alexa555 and Alexa647 fluorescent dyes,

194   respectively, as per the manufacturer's instructions at genomic core facility at Michigan State

195   University. The arrays were scanned using Agilent scanner, and data was extracted with the

196   Feature Extraction software (Agilent) as described previously (SPAIN *et al.* 2014).

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198   *Bioinformatics Analysis*

199   The data extracted with the Feature Extraction software (Agilent) was normalized using Limma

200   package from Bioconductor, as described previously (VENKATESH *et al.* 2012). The genes were

201   divided into 10 equal sized bins, with the two bins assigned to the region 500 bp upstream of the

202   transcription start site (TSS) and two bins to the 500 bp to the region downstream of the

203   transcription end site (TES). The average probe enrichment values were assigned to the closest

204   bin according to the probe location, and a 10 bin matrix was generated using a PERL script.

205   Genes corresponding to the majority of dubious ORFs, tRNA genes, small nuclear RNA genes as

206   well as autonomously replicating sequences (ARS) were removed from the dataset. The

207   enrichments in the 6 bins between TSS and TES were averaged to obtain an average ORF

208   occupancy. The genes for analysis were selected on the basis of ORF enrichment. The genes

209   <500 bp in length were removed from the analyses. The versatile aggregate profiler (BRUNELLE

210   *et al.* 2015) was used to generate gene-average profile. The genes were split in the middle, and

211   the probe intensities were aligned to the TSS for the first half and to the TES for the second half

212   of the genes.

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214   *Box-plots*

215   Center lines show the median and box limits indicate the 25th and 75th percentiles as determined

216   by R software. Whiskers extend 1.5 times the interquartile range from the 25th and 75th

217   percentiles and outliers are represented by dots.

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219   *Data Availability*

220   Strains generated in this study, and the sequences of primers used for ChIP analysis are available

221   upon request. The Gene Expression Omnibus accession number for the ChIP-chip data reported

222   in this paper is GSE69642.

223

224   **RESULTS**

225   **Histone H3 N-terminal tails facilitate FACT interaction with chromatin *in vivo***

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226   While Spt16 is enriched in coding regions of actively transcribing genes (MASON AND

227   STRUHL 2003; MAYER *et al.* 2010), the mechanisms by which it is recruited remains to be

228   established. Given that the FACT complex interacts with nucleosomes *in vitro*

229   (BELOTSERKOVSKAYA *et al.* 2003), it can be recruited through nucleosome interactions.

230   Moreover, Spt16 interaction was greatly reduced with the nucleosomes lacking the histone N-

231   terminal tails (NTTs) implicating histone tails in promoting Spt16-nucleosome interactions

232   (STUWE *et al.* 2008; VANDEMARK *et al.* 2008). Significantly, removal of the H3 and H4 tails, but

233   not of the H2A-H2B tails, abolished Spt16-histone interactions *in vitro* (WINKLER *et al.* 2011).

234   The H3 or H4 tails therefore could facilitate recruitment/retention of the FACT complex. To test

235   this possibility, we examined yeast Spt16 occupancy by ChIP in histone mutants lacking the H3

236   or H4 N-terminal tail. Spt16 occupancy in the *ARG1* 5' and 3' ORFs was reduced by ~50% in

237   the H3 mutants lacking 1-20 (*H3Δ1-20*) or 1-28 (*H3Δ1-28*) N-terminal residues (Figure 1A). In

238   comparison, in the H4 tail mutant (*H4Δ1-16*), only a small (~20%) reduction was observed in the

239   *ARG1* 3' ORF (Figure 1A). A substantial reduction in Spt16 occupancy (~80%) was also

240   observed at a constitutively expressed *ADH1* gene in the H3 mutants (Figure 1B), but not in the

241   H4 mutant (Figure S1A). Although, FACT/Spt16 interacts with both H3 and H4 tail peptides, *in*

242   *vitro,* (STUWE *et al.* 2008; VANDEMARK *et al.* 2008) it appears that the H3 tail may help in

243   recruiting or retaining FACT to its target genes, *in vivo*. Consistent with this idea, reduced

244   occupancy of Spt16 was also observed in the coding regions of *PYK1*, *PMA1* and *GLY1* genes

245   (Figure 1C). In contrast to the impaired Spt16 occupancy in the H3 mutant, for most genes, Pol II

246   occupancies were comparable in WT and the H3 mutants, except for the *GLY1* gene, which

247   showed a small reduction in Pol II occupancy (Figure S1B).

248   To further examine the role of the H3 tails in promoting FACT association with

249   chromatin, we performed coimmunoprecipitation assay. The HA-tagged histone H2B (H2B-HA)

250   efficiently pulled-down Spt16 from the whole cell extracts (WCEs) prepared from HA-tagged

251   WT cells but not from untagged cells (Figure 1D, left). We also observed a reduced Spt16 pull-

252   down from the *H3Δ1-28* WCEs (~50 %; Figure 1D, right). No such reduction in Spt16

253   occupancy was seen in the H4 tail deletion mutant (*H4Δ1-16*; Figure S1C), supporting the idea

254   that the H3 tail promotes Spt16 association with chromatin *in vivo*. However, the extent to which

255   the H3 tail contributes in this process may be variable, as observed by the differences of Spt16

256   occupancies at the different genes in the H3 mutant (Figure 1A-1C).

257

258   **Acetylation of the H3 tail promotes FACT occupancy at *ADH1* and *ARG1* genes**

259   After observing that the H3 tail contributes to Spt16, we examined the role for

260   posttranslational modifications on the H3 tail in regulating FACT localization to transcribed

261   genes. The H3 tail lysines, K4 and K36, are methylated by Set1 and Set2, respectively (BRIGGS

262   *et al.* 2001; STRAHL *et al.* 2002). To examine, whether the reduction in Spt16 occupancy in the

263   H3 tail deletion mutant is due to the loss of H3 methylation, we measured Spt16 enrichment in

264   the *set1Δ/set2Δ* double mutant. Comparable occupancies of Spt16 were observed in the ORFs of

265   *ARG1* and *ADH1* genes in WT and the *set1Δ/set2Δ* mutant (Figure 2A). Furthermore, we found

266   very similar signal for coimmunoprecipitation of Pol II with Spt16 in WT and *set1Δ/set2Δ* WCEs

267   (Figure 2B), suggesting that H3 methylation is likely dispensable for maintaining WT level of

268   FACT occupancy, at least, at these two genes. The H3 and H4 tails are also acetylated by Gcn5-

269   containing SAGA and Esa1-containing NuA4 histone acetyltransferase (HAT) complexes,

270   respectively. Accordingly, a *gcn5Δ/esa1ts* double mutant elicits strong reductions in H3 and H4

271   acetylation (GINSBURG *et al.* 2009). The *gcn5Δ/esa1ts* mutant, grown at the permissive

272   temperature 25°C (represented as *gcn5Δ*) or at the non-permissive temperature 37°C to inactivate

273   Esa1 (*gcn5Δ/esa1ts*), produced comparable reductions in Spt16 occupancy in the *ARG1* and

274   *ADH1* ORFs (Figure 2C and Figure S2, right). In contrast, both WT and the HAT mutant

275   displayed comparable Pol II occupancies at *ARG1* and *ADH1* (Figure 2D, and Figure S2, left).

276   To examine whether FACT occupancy correlates to H3 acetylation levels, we determined Spt16

277   enrichment at *ARG1* in a histone deacetylase mutant, *rpd3Δ/hos2Δ*. Interestingly, Spt16

278   occupancy was not increased in the histone deacetylase mutant *rpd3Δ/hos2Δ* (Figure 2E). This

279   is surprising given that H3 acetylation levels were shown to be elevated in this mutant (GOVIND

280   *et al.* 2010), and that our current results reveal diminished FACT occupancy in the HAT mutant.

281   It is possible that the WT level of histone acetylation is sufficient for normal FACT occupancy,

282   suggesting that histone acetylation, but not deacetylation, plays a role in FACT

283   recruitment/retention in coding regions. As such, any further increase does not necessarily

284   increase FACT occupancy. Taken together, these results suggest a role for the acetylated H3 tail

285   in promoting FACT occupancy in the transcribed coding regions.

286   To provide additional proof for the role of histone acetylation, we examined Spt16

287   occupancy in the H3 and H4 tail point mutants. The H3 mutant (K4, K9, K14, K18 substituted to

288   alanine; H3K→A) displayed reduced Spt16 occupancy in the ORFs of both *ARG1* and *ADH1*

289   genes (Figure 2F). However, only minimal changes in Spt16 occupancy were observed in the H4

290   mutant (K5, K8, K12 and K16 substituted to arginine; H4K→R, or to glutamine H4K→Q)

291   (Figure 2G; H4K→A mutant exhibits a lethal phenotype). While Spt16 interacts with both H3

292   and H4 N-terminal tails, and the histone tail mutants impair FACT function (BISWAS *et al.* 2006;

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293   VANDEMARK *et al.* 2008), our results suggest that acetylation of H3 tail makes a greater

294   contribution to the FACT occupancy in the coding regions.

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296   **FACT and Spt6 are required for transcription genome-wide**

297   Microarray analyses examining transcription defects have revealed that the loss of Spt16

298   function leads to aberrant transcription at many genomic locations, including within coding

299   regions (CHEUNG *et al.* 2008; VAN BAKEL *et al.* 2013). While it is evident that Spt16 functions to

300   suppress wide-spread cryptic and anti-sense transcription, the role of FACT in regulating Pol II

301   occupancy in coding regions is not well understood at a genome-wide scale. Gene-specific

302   studies have suggested that FACT regulates transcription at the initiation step (BISWAS *et al.*

303   2006; JIMENO-GONZALEZ *et al.* 2006; DUINA *et al.* 2007). Additionally, FACT, in cooperation

304   with H2B ubiquitination, is important for restoration of chromatin in the wake of Pol II

305   elongation (FLEMING *et al.* 2008). As mentioned earlier, similar to the FACT complex, Spt6 (a

306   H3/H4 chaperone) is localized to the coding regions of strongly transcribed genes (MAYER *et al.*

307   2010; IVANOVSKA *et al.* 2011; PERALES *et al.* 2013; BURUGULA *et al.* 2014) and is also important

308   for suppressing aberrant transcription (KAPLAN *et al.* 2003; CHEUNG *et al.* 2008; VAN BAKEL *et*

309   *al.* 2013). To compare the impact of FACT and Spt6 on transcription, we utilized strains in

310   which the expression of *SPT16* or *SPT6* was under the control of a tetracycline repressible

311   promoter (*SPT16-TET* and *SPT6-TET*). These promoters can be repressed by growing cells in the

312   presence of doxycycline (dox).

313   To rule out unexpected consequences of replacing the endogenous promoter with the

314   TET-promoter, we first compared Spt16 and Spt6 protein levels in TET-strains and BY4741 (*S.*

315   *cerevisiae* WT strain). Spt16 and Spt6 protein levels in the untreated (no dox; ND) *SPT16-TET*

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316   and *SPT6-TET*, respectively, were very similar to those detected in the BY4741 cells (Figure 3A,

317   top panel). As expected, treating *SPT16-TET* and *SPT6-TET* cells with dox led to reduced

318   expression of Spt16 and Spt6, respectively (Figure 3A, bottom panel). We noted that Spt16 was

319   depleted to a greater extent than Spt6 upon dox-treatment. Since Spt16 mutants have been shown

320   to cause cell cycle defects (PRENDERGAST *et al.* 1990), we also measured the level of budded and

321   unbudded cells in BY4741, *SPT16-TET* and *SPT6-TET* dox-treated cells. We did not find any

322   significant increase in the number of budded and unbudded cells under Spt6 or Spt16 depleted

323   conditions (Figure S3A), suggesting that depleting Spt16 or Spt6, under the experimental

324   conditions employed, elicit minimal cell cycle defects. Moreover, the TET-strains grown in the

325   presence or absence of dox, exhibited similar viability (Figure 3B). Altogether, these results

326   indicate that the untreated *SPT16-TET* and *SPT6-TET* cells behave similar to BY4741.

327   To examine the effects of depleting Spt16 and Spt6 on transcription, we determined Rpb3

328   occupancy in untreated cells (*SPT16-TET*; referred to as WT hereafter), and dox-treated *SPT16-*

329   *TET* (*spt16*) and *SPT6-TET* (*spt6*) cells by ChIP-chip. We observed a strong correlation (Pearson

330   correlation, r= 0.93) between the Pol II occupancies in the dox-untreated *SPT16-TET* and

331   BY4741 strains, genome-wide, which further indicated that replacing the endogenous *SPT16*

332   promoter with the TET-promoter does not adversely affect transcription. We also determined

333   Spt16 occupancy genome-wide by ChIP-chip and found that Spt16 occupancy in coding regions

334   strongly correlated with Pol II occupancy (Pearson correlation, r=0.85), in agreement with

335   previous studies (MAYER *et al.* 2010).

336   The heat-maps depicting changes in Rpb3 enrichment (*spt16*/WT) showed diminished

337   ratios in coding regions of the genes displaying the greatest Rpb3 enrichments in WT cells

338   (Figure 3C). Consistent with a strong correlation between Spt16 and Rpb3 occupancies, the

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339   genes with the highest Spt16 enrichments showed greatest Rpb3 reductions (Figure 3D). We also

340   noticed that depletion of Spt16 (and Spt6, described later) also revealed an increase in Pol II

341   occupancies at those genes, which otherwise show very poor enrichment ratios in WT cells.

342   Given that our ChIP-chip normalization was performed without spike-in control, it is difficult to

343   ascertain the apparent increase in Rpb3 occupancy as biologically relevant.

344   To further analyze the impact of depleting Spt16 on Pol II occupancy, we selected the top

345   25% genes showing greatest Rpb3 occupancy in WT cells (n=1246). Nearly identical profiles for

346   Rpb3 occupancy were observed in WT and BY4741 at the metagene comprised of these

347   transcribed genes (Figure 3E). In contrast, Spt16 depletion evoked reduction in Pol II occupancy

348   in the coding region of these sets of genes. Given that Gcn4 target genes are activated under the

349   growth conditions used (see Materials and Methods), we additionally analyzed the effect of

350   depleting Spt16 on transcription of Gcn4 targets. 130 Gcn4-regulated genes were enriched

351   among the top 1246 transcribed genes. Reduced Pol II occupancies were evident in coding

352   regions of these genes as well in Spt16-depleted cells (Figure 3F). Further, we identified 226

353   genes showing reduction in Pol II occupancy ≥0.5 $\log_2$ ratio (ChIP/input) in Spt16-depleted cells

354   (Figure 3G). Interestingly, these genes were enriched among the top 10% transcribed genes (p-

355   value=10^{-117}), suggesting that depletion of Spt16 imparts a significant effect on Pol II occupancy

356   at highly expressed genes.

357   We noticed a small increase in Pol II occupancy in the Spt16-depleted cells at the 3' ends

358   of genes, analyzed above (Figure 3E). This increase may reflect increased cryptic transcription

359   events that accumulate Pol II from the 5' to 3' end. Such an explanation would be consistent with

360   the established role of histone chaperones in suppressing cryptic transcription (KAPLAN *et al.*

361   2003; CHEUNG *et al.* 2008; VAN BAKEL *et al.* 2013). A previous study utilizing microarray

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362   predicted 960 and 1130 genes to have cryptic transcription in *spt6-1004* and *spt16-197* mutants

363   (CHEUNG *et al.* 2008). We found that only 154 genes, predicted to express cryptic transcripts in

364   the previous study, were among the 1246 genes exhibiting high-levels of Pol II occupancy

365   (Figure S3B). Replotting Pol II occupancy data after excluding these 154 genes (Figure S3C)

366   displayed profiles similar to that observed in Figure 3E. Pol II pausing and queuing in the 3' end

367   superimposed on elongation defects at the very 5' end could potentially explain an apparent

368   increase in Pol II occupancy towards the 3' end on depleting Spt16. Regardless, our results

369   showing reduction in Pol II occupancy across the coding regions is consistent with previous

370   studies indicating a role for FACT in transcription initiation or early stages of Pol II elongation.

371

372   Next, we analyzed Pol II occupancy in the Spt6 depleted cells. Interestingly, at the top

373   25% of Pol II-occupied genes, Spt6-depletion elicited a greater reduction in Rpb3 occupancy

374   towards the 3' end (Figures 3C (right) and 3E), consistent with previous studies showing the

375   greatest Spt6 enrichments in the 3' ends of transcribed genes (PERALES *et al.* 2013; BURUGULA *et*

376   *al.* 2014). A 5' to 3' bias in Pol II occupancy was also evident at the Gcn4-targets (Figure 3F)

377   and at 238 genes, which showed a reduction in Pol II occupancy $\geq$ 0.5 log$_2$ ratio (ChIP/input)

378   (Figure S3D). A progressive reduction in Pol II occupancy in the 5' to 3' direction, in Spt6-

379   depleted cells, suggests that Spt6 may regulate Pol II processivity. Alternately, given the role of

380   Spt6 activity in the 3'—mRNA processing, diminished Spt6 levels could also result in reduced

381   Pol II occupancy at the 3' end (KAPLAN *et al.* 2005). To distinguish between these two

382   possibilities, we analyzed Rpb3 occupancy at the top 25% transcribing genes based on their gene

383   length. All three groups of genes, long (> 2 kb), medium (1-2 kb) and short (0.5-1 kb), showed a

384   5' to 3' bias in Pol II occupancies (Figure 3H). Interestingly, however, long and medium genes

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385   displayed a greater reduction in the 3' end compared to the short genes (0.5-1 kb) (Figure 3H and

386   S3E). A simpler explanation for this observation is that Spt6 promotes Pol II processivity, in

387   agreement with previous studies (ENDOH *et al.* 2004; ARDEHALI *et al.* 2009; PERALES *et al.*

388   2013). A 3'—mRNA processing defect (KAPLAN *et al.* 2005) would be expected to produce

389   similar reductions in Pol II occupancies at the 3' end irrespective of gene length. Collectively,

390   our data suggest that loss of FACT and Spt6 functions produces distinct effects on Pol II

391   occupancy. However, the role of FACT and Spt6 in suppressing aberrant transcription, to some

392   extent, could have an effect on Pol II occupancy under depletion conditions.

393

394   **FACT and Spt6 differentially impact transcription and histone occupancy**

395   To further address the functional overlap between FACT and Spt6, we examined genes,

396   which showed a reduction in Pol II occupancy ($\leq$ -0.5 $\log_2$ ratio) upon depleting these factors.

397   We found a significant overlap between the genes exhibiting Pol II fold-change $\log_2 \geq 0.5$ upon

398   depleting either Spt16 or Spt6 (p-value = 5.1 x 10^{-76}, n= 111) (Figure 4A). The genes showing

399   Pol II occupancy defects upon depleting either Spt16 or Spt6 (common; n=111) exhibited, on

400   average, higher Pol II occupancy (in WT cells) than those genes which showed defects only after

401   depleting either Spt16 or Spt6 (unique) (Figure 4B). This observation suggests that strongly

402   transcribed genes may need full functions of Spt6 and Spt16 for a high-level of transcription. It is

403   also interesting to note that while Spt6 depletion was less efficient compared to that of Spt16, it

404   nonetheless evoked very similar Pol II occupancy defects on these genes (Figure 4C). Rpb3

405   profiles at the genes uniquely affected either by Spt6 or Spt16 depletion showed expected

406   profiles (Figures 4D and 4E). Considering that Spt6 exhibits higher occupancy towards the 3'

407   ends   (MAYER *et al.* 2010; PERALES *et al.* 2013; BURUGULA *et al.* 2014), we analyzed

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408   distribution of the gene-length in the three classes of genes. The longer genes were enriched in

409   the Spt6-unique and common genes, whereas Spt16-unique genes were shorter in comparison

410   (Figure S4A and S4B). Thus, it appears that the differential effect of Spt16 and Spt6 depletion

411   could partly be due to differences in their localization patterns over the coding sequences and

412   transcription level in WT conditions.

413   We further examined the differential effect of depletion by determining Rpb3 ChIP

414   occupancy at four genes, which showed comparable Spt6, and Spt16 occupancy in our ChIP-chip

415   experiments. Pol II occupancies in the ORFs of *GLY1*, *HAC1*, *BDF2*, and *PHM8* were

416   substantially reduced (~2-5 folds) upon Spt16 depletion (Figure 4F). By contrast, only a

417   moderate to negligible reduction was observed after depleting Spt6. For example, Rpb3 was

418   reduced by less than 1.5 folds at *GLY1*, *HAC1*, *PMH8* upon Spt6 depletion. Similarly, we found

419   that histone H3 occupancy was more severely reduced in 5' ORFs of these genes upon depleting

420   Spt16 than upon Spt6 (Figure 4G). Reduced H3 occupancies upon depletion of Spt16 and Spt6

421   are consistent with their role in histone reassembly (IVANOVSKA *et al.* 2011; PERALES *et al.*

422   2013; JERONIMO *et al.* 2015). Bigger reductions in Pol II and H3 occupancies were not due to

423   higher Spt16 occupancies at these genes compared to that of Spt6 (Figure 4H). These analyses

424   illustrate the importance for both FACT and Spt6 in regulating transcription globally. The

425   differences observed on a subset of genes under the conditions of diminished FACT or Spt6

426   function might be a reflection of their apparent role in regulating different aspects of

427   transcription, as well as on their different localization patterns across the gene.

428

429   **FACT cooperates with Spt6 to promote transcription**

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430   To further investigate whether Spt6 and FACT cooperate in promoting transcription, we

431   deleted the Spt6 tandem SH2 domain (tSH2; 202 residues from C-terminus), which mediates

432   Spt6 recruitment, genome-wide (MAYER *et al.* 2010; BURUGULA *et al.* 2014), in the *SPT16-TET*

433   background (*SPT16*/*spt6Δ202*). As expected, treating the *SPT16*/*spt6Δ202* mutant with dox

434   resulted in reduced Spt16 protein levels (Figure S5A). We then determined Rpb3 occupancies by

435   ChIP-chip in untreated (S*PT16*/*spt6Δ202*) and dox-treated (*spt16*/*spt6Δ202*) cells, and compared

436   the occupancies with that of the untreated *SPT16-TET* cells (WT). The changes in Rpb3

437   occupancy in untreated *SPT16*/*spt6Δ202* were significantly anti-correlated with the Rpb3

438   occupancy in the WT cell (r= -0.81) (Figure 5A), indicating a strong requirement of Spt6 in

439   transcription, genome-wide. The changes in Pol II occupancies in the dox-treated

440   *SPT16*/*spt6Δ202* showed very similar reductions as observed for the untreated cells. However, a

441   modest but statistically significant reduction in Rpb3 occupancy was observed in dox-treated

442   (*spt16*/*spt6Δ202)* cells compared to the untreated cells only for the first decile (p =5.2 x 10^{-59})

443   representing the most highly transcribed genes (Figure 5B). This is consistent with the idea that

444   both Spt6 and Spt16 are necessary to elicit high transcription levels.

445   Rpb3 profiles in *spt16*/*spt6Δ202* mutant revealed greatly diminished occupancy across

446   the coding regions of the top 25% Pol II-occupied genes (Figure S5B). It appeared that untreated

447   *SPT16*/*spt6Δ202* elicited a greater reduction in Pol II occupancy than observed upon depleting

448   Spt6 (compare Figures S5B and 3E). An incomplete depletion of Spt6 (Figure 3A), or different

449   experimental conditions (no dox in *spt6Δ202* vs dox-treatment for Spt6 depletion) could account

450   for this observation. Nonetheless, these results indicate the importance of Spt6 in stimulating

451   high-level transcription, genome-wide. However, depleting Spt16 in *SPT16*/*spt6Δ202* cells

452   produced only modest reductions in Pol II occupancy at the top 25% Pol II occupied (Figure

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453   S5B). This observation raises a possibility that FACT may require certain aspects of Spt6

454   function to stimulate transcription. Such an explanation is consistent with the observation that

455   Spt16 depletion elicited reduced Pol II occupancy in otherwise WT strain (Figures 3D-G).

456   To further address functional cooperation between Spt16 and Spt6, we focused on the

457   genes eliciting greater reduction in dox-treated *spt16/spt6Δ202* than in untreated cells. The top

458   200 genes showing the greatest Pol II occupancy defect revealed that depleting Spt16 in

459   *spt6Δ202* background significantly reduced Rpb3 occupancy across the coding region (Figure

460   5C). 137 of these 200 genes were among the top 25% expressed genes (n=1246) in WT cells.

461   The double mutant exhibited greater reduction in Pol II occupancy than observed in *spt6Δ202* or

462   *spt16* cells at these 137 genes (Figure S5C). These observations support the idea that both FACT

463   and Spt6 are needed for eliciting high-level of transcription. At the 63 lowly expressed genes

464   among the 200 genes, significant reduction in Pol II occupancy was seen only in the double

465   mutant *spt16/spt6Δ202* (Figure 5D). This could suggest that FACT acts redundantly with Spt6 in

466   promoting transcription of a subset of lowly expressed genes. More sensitive methods will be

467   required to fully comprehend the extent to which Spt6 and FACT coordinate transcription of

468   lowly expressed genes, especially considering the technical challenges in accurately measuring

469   Pol II occupancies at genes expressed at very low levels.

470

471   **DISCUSSION**

472   In this study, we have examined the role of two highly conserved histone chaperones,

473   Spt16 and Spt6, in regulating genome-wide transcription, under amino acid starvation conditions.

474   Spt6 recruitment to coding regions is stimulated by the phosphorylated Pol II CTD and by

475   HDACs Rpd3 and Hos2 (MAYER *et al.* 2012; BURUGULA *et al.* 2014). However, the mechanism

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476   by which the FACT complex associates with chromatin is not well understood. Our results

477   showing diminished occupancy of Spt16 at the ORFs of several genes and reduced interaction

478   with nucleosomes in the H3 tail mutant (Figure 1), suggests a role for the H3 tail in promoting

479   FACT association with transcribed regions. Our results additionally suggest that histone

480   acetylation enhances the ability of the FACT complex to associate with transcribed ORFs, since

481   deleting Gcn5 (a H3 HAT) or mutating the H3 tail lysine residues significantly dampened Spt16

482   enrichment in coding sequences of *ARG1* and *ADH1* (Figures 2C and 2F). These results provide

483   *in vivo* evidence for the previous studies showing impaired FACT binding to the

484   nucleosomes/histones lacking N-terminal tails (VANDEMARK *et al.* 2008; WINKLER *et al.* 2011).

485   Currently, however, which regions of Spt16 are required for histone tail interactions is unclear.

486   While Spt16 N-terminal domain of *S. pombe* was shown to be important for interacting with H3

487   and H4 tails (STUWE *et al.* 2008), in *S. cerevisiae*, this domain was found to be dispensable

488   (VANDEMARK *et al.* 2008).

489   Considering that Spt16 exhibits similar affinity towards the acetylated and unacetylated

490   histone peptides *in vitro* (STUWE *et al.* 2008), it is plausible that acetylation-mediated changes to

491   the nucleosome structure allow FACT to stably bind chromatin at transcribing loci. Consistent

492   with this idea, Gcn5 promotes histone eviction, Pol II elongation, and stimulates recruitment of

493   bromodomain-containing chromatin remodelers, RSC and SWI/SNF (GOVIND *et al.* 2007;

494   DUTTA *et al.* 2014; SPAIN *et al.* 2014). Additional contacts with core domains of H3/H4 and with

495   H2A/H2B through its C-terminal domain (WINKLER *et al.* 2011; KEMBLE *et al.* 2015) could

496   further stabilize FACT-chromatin interactions, thereby maintaining chromatin in an accessible

497   conformation to promote transcription, and concomitantly aiding in reassembly of evicted

498   histones in the wake of transcription (JAMAI *et al.* 2009). Additional factors may act

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499   cooperatively with other factors to enhance FACT enrichment. For instance, FACT interacts with

500   the Paf1 complex, and chromatin remodeler Chd1, both of which are enriched in transcribed

501   regions (KROGAN *et al.* 2002; SQUAZZO *et al.* 2002; SIMIC *et al.* 2003).

502   Enrichment of FACT in coding regions (MAYER *et al.* 2010) (*data not shown*), and its

503   ability to promote Pol II transcription through the nucleosomal templates, *in vitro*,

504   (BELOTSERKOVSKAYA *et al.* 2003; HSIEH *et al.* 2013), strongly suggests a role for FACT in the

505   elongation step of transcription. FACT is linked to the reestablishment of the disrupted

506   chromatin structure in the wake of transcription (JAMAI *et al.* 2009), and consequently, in

507   suppressing aberrant transcription by preventing utilization of cryptic promoters (KAPLAN *et al.*

508   2003; CHEUNG *et al.* 2008; VAN BAKEL *et al.* 2013). Our data indicate that FACT is globally

509   required for promoting transcription in the coding regions. Spt16 deficiency reduced Pol II

510   occupancy in coding regions of highly transcribed genes, including of the ribosomal protein

511   genes and Gcn4 targets (Figure 3). It was previously reported that histone mutants which perturb

512   association of Spt16 (shift in occupancy towards the 3' end) also reduced Pol II occupancy at the

513   5' end of *PMA1* and *FBA1* genes (NGUYEN *et al.* 2013). Thus, it seems that impairing FACT

514   activity, through depletion of Spt16 or by altering FACT association in the coding region

515   (NGUYEN *et al.* 2013), elicit greater defects in transcription in the early transcribed regions.

516   These results raise a possibility that FACT may help polymerases in negotiating the nucleosomal

517   barrier downstream of the TSS. Such an idea is consistent with biochemical studies showing that

518   FACT relieves Pol II pauses well within the nucleosomes and acts primarily to overcome

519   tetramer-DNA contacts (BONDARENKO *et al.* 2006). Considering that the rate of elongation in the

520   early transcribed regions is slower than that observed in the mid or 3' end regions (DANKO *et al.*

521   2013), our findings suggest that FACT might help polymerases to overcome the nucleosomal

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522   barrier at the 5' ends of the transcribed genes. Although, nucleosomes are expected to pose a

523   similar block to elongating Pol II irrespective of their position along coding sequences,

524   nucleosomal impediment to transcription at the distal end may be additionally relieved by

525   factors, such as chromatin remodelers RSC and SWI/SNF, which are also enriched in the ORFs

526   of many genes (DUTTA *et al.* 2014; SPAIN *et al.* 2014). Moreover, post-translational histone

527   modifications, which are not uniform across coding regions, may have a differential effect on the

528   FACT activity to stimulate transcription. In support of such a possibility, it was shown that the

529   FACT complex cooperates with H2B ubiquitination to regulate transcription both *in vitro* and *in*

530   *vivo* (PAVRI *et al.* 2006; FLEMING *et al.* 2008).

531   In contrast to FACT, Spt6-depletion diminished Pol II occupancy at the 3' end (Figures

532   3E-F and 3H). Lower Pol II occupancy towards the 3' end could result from a processivity defect

533   and/or from a specific defect at the 3' end. Our analyses, based on gene-length, favor the idea

534   that Spt6 enhances Pol II processivity, possibly through stimulating nucleosome eviction, or by

535   preventing premature dissociation during Pol II traversal through coding regions. Such an

536   interpretation is consistent with previous studies showing reduced Pol II occupancy at the 3' end

537   of exceptionally long-genes (PERALES *et al.* 2013). Spt6 has been also shown to enhance the rate

538   of Pol II elongation at heat-shock genes in *Drosophila* (ARDEHALI *et al.* 2009). Therefore,

539   reduced Pol II occupancy near the 3' ends of yeast genes suggests that Spt6 may function to help

540   elongating polymerase traverse coding regions through multiple mechanisms.

541   Pol II occupancy in the Spt6 mutant lacking the C-terminal tandem SH2 domain was

542   strongly correlated with Pol II occupancy in the WT cells, indicating a strong requirement for

543   Spt6 in stimulating transcription genome-wide. Even though depleting Spt16 in otherwise WT

544   cells evoked reduction in Pol II occupancy from highly transcribed genes, this surprisingly had

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545 only a minor impact on Pol II occupancy at the majority of transcribed genes in *spt6Δ202* cells.

546 This suggests that both factors might have overlapping roles and that FACT may rely on the

547 certain aspects of Spt6 functions in promoting transcription. It makes sense considering that

548 FACT, and Spt6 are recruited to transcribed regions (MAYER *et al.* 2010; BURUGULA *et al.*

549 2014), and that both factors can interact with H2A-H2B and H3-H4 (MCCULLOUGH *et al.* 2015).

550 Despite interacting with nucleosomes as well as with histones, unlike Spt6, FACT can reorganize

551 the nucleosome structure in a manner that increases DNA accessibility. This distinct ability of

552 FACT could explain reduction in Pol II occupancy observed in a subset of transcribed genes in

553 *spt16/spt6Δ202* double mutant, but not in the single mutant (Figure 5C). Overall, our study

554 strongly implicates both chaperones in promoting transcription genome-wide.

555

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Legends to the Figures:

Figure 1: The H3 N-terminal tail promotes FACT recruitment.

A-B) ChIP occupancy of Myc-tagged Spt16 in WT and mutants lacking the H3 N-terminal tail residues 1-20 (*H3Δ1-20*) or 1-28 (*H3Δ1-28*) and 1-16 residues of H4 (*H4Δ1-16*) at *ARG1* (A) and in H3 mutants at *ADH1* (B). Graphs show mean and SEM. * represents a p-value < 0.01, ** represents a p-value < 0.001, and *** represents a p-vlaue <0.0001.

C) ChIP occupancies of Myc-tagged Spt16 at the indicated genes in the WT and the H3 tail mutant, *H3Δ1-28*. * represents a p-value < 0.01, ** represents a p-value < 0.001.

D) Whole-cell extracts prepared from HA-tagged H2B WT and *H3Δ1-28* strains were pulled-down with the anti-HA beads, and the immunoprecipitates were subjected to western blot using antibodies against Spt16 and HA. Untagged WT was used as a control. The representative blot is shown on the left, and the quantified data on the right.

798

Figure 2: Role of the acetylated H3 tail in modulating Spt16 occupancy .

A) ChIP enrichment of Spt16-Myc at *ARG1* in WT and histone methyltransferase mutant *set1Δ/set2Δ*. * represents a p-value < 0.001, and ** represents a p-vlaue <0.0001.

B) Whole-cell extracts prepared from Spt16-Myc tagged WT and *set1Δ/set2Δ* strains were immunoprecipitated with anti-Myc antibodies, and the immunoprecipitates were analyzed by western blot to detect signals for Myc and Rpb3. Untagged WT was used as a control. Rpb3 band in immunoprecipitated samples is shown by an arrow and the IgG heavy chain by an asterisk (*).

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807 C) Spt16-Myc ChIP occupancy in WT and *gcn5Δ/esa1* histone acetyletransferase (HAT)
808 mutant. Spt16-Myc occupancies were measured by ChIP at *ARG1* and *ADH1* in a *gcn5Δ/esa1ts*
809 strain grown either at 25°C (represented as *gcn5Δ*) or at 37°C to inactivate Esa1 (*gcn5Δ/esa1*).
810 Graphs show mean and SEM. * represents a p-value < 0.001, and ** represents a p-vlaue
811 <0.0001. Spt16 occupancy differences between *gcn5Δ* and *gcn5Δ/esa1ts* were not significnat
812 (N.S).

813 D) Rpb3 occupancies in WT and *gcn5Δ/esa1ts* at *ARG1* and *ADH1*.

814 E) Spt16 occupancies in WT and HDAC mutant *hos2Δ/rpd3Δ* at *ARG1*.

815 F-G) Spt16-Myc enrichments at *ARG1* and at *ADH1* in histone H3 (F) and H4 tail mutants (G).
816 H3 K4, K9, K14, K18 substituted to alanine, H3K→A; H4 K5, K8, K12 and K16 substituted to
817 arginine; H4K→R, or to glutamine H4K→Q. Graphs show mean and SEM. ** represents a p-
818 vlaue <0.0001.

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820 **Figure 3: Effect of depleting Spt16 and Spt6 on Pol II occupancy genome-wide.**

821 A) *BY4741*, *SPT16-TET* and *SPT6-TET* cells were grown in SC media and induced by SM.
822 Western blots show Spt16 and Spt6 protein levels in BY4741, *SPT16-TET* and *SPT6-TET* strains
823 without dox-treatment (top panel). The Spt16 and Spt6 levels in untreated (ND) and dox-treated
824 TET-strains are shown (bottom panel).

825 B) *SPT16-TET* and *SPT6-TET* cells grown and subcultured in SC media, with and without
826 doxycycline (dox), were collected, serially 10-fold diluted, and spotted on SC plates. Growth for
827 the two cultures of each *SPT16-TET* (left) and *SPT6-TET* (right) with and without dox (no-dox)
828 treatment are shown.

829 C) Heat-maps depicting genome-wide Rpb3 (Pol II) enrichment in *SPT16-TET* (without dox;
830 WT) (left), and changes in Rpb3 occupancies in dox-treated *SPT16-TET* (*spt16*/WT) (middle)
831 and *SPT6-TET* (*spt6*/WT) (right). Genes were sorted from highest to lowest ORF Rpb3
832 enrichment in WT cells.

833 D) Box-plot showing the changes in Rpb3 occupancy according to the Spt16 enrichment in
834 deciles. The average ORF occupancy of Rpb3 in *SPT16-TET* dox-treated and untreated cells was
835 determined, and the change in Rpb3 occupancy on depleting Spt16 (dox/no dox) was calculated
836 for each gene. Genes were then grouped into deciles according to the Spt16 occupancy in WT
837 cells. The first decile shows the highest Spt16 occupancy and the 10th shows the lowest.

838 E) Rpb3 occupancy profiles for the top 25% Pol II-occupied genes (n=1246) in untreated
839 *BY4741* and *SPT16-TET* (WT), and in dox-treated *SPT16-TET* (*spt16*) and *SPT6-TET* (*spt6*) cells
840 are shown.

841 F) Rpb3 occupancy profiles in untreated *SPT16-TET* (WT), and dox-treated *SPT16-TET* (*spt16*)
842 and *SPT6-TET* (*spt6*) cells at the Gcn4 targets genes enriched in the top 25% Pol II-occupied
843 genes.

844   G) Rpb3 occupancy profile at the genes eliciting reduction in Rpb3 occupancy ≥ 0.5 $\log_2$ ratio
845   (ChIP/input) on depleting Spt16 in WT and *spt16*.

846   H) The top 25% Pol II-occupied genes were grouped on the basis of their gene-length and Rpb3
847   occupancy profiles for the long (>2 kb), medium (1-2 kb), and short (0.5-1 kb) are shown in the
848   WT and Spt6-depleted cells.

849

850   **Figure 4: FACT promotes nucleosome reassembly.**

851   A) Venn diagram showing overlap among the genes which displayed Rpb3 occupancy defect
852   >0.5 $\log_2$ ratio (ChIP/Input) in Spt16 and Spt6 depleted cells. p-value for the overlap is shown.

853   B) Box-plot showing Rpb3 average ChIP-chip enrichments at Spt16-unique, Spt6-unique, and
854   Spt16 and Spt6 common genes in WT, Spt16-depleted and Spt6-depleted cells.

855   C-E) Metagene analysis for changes in Pol II enrichment under Spt16 and Spt6 depleted cells,
856   for genes showing reduction > 0.5 $\log_2$ ratio (ChIP/Input) under Spt16 or Spt6 depleted condition
857   (C; common), only under Spt16 depletion (D) only under Spt6 depletion (E).

858   F-H) ChIP occupancies of Rpb3 (F), histone H3 (G), and of Spt6 and Spt16 in 5' ORFs of
859   indicated genes in *SPT16-TET* and *SPT6-TET* untreated (ND) or dox-treated (dox) cells. *
860   represents a p-value < 0.05, and ** represents a p-vlaue <0.001.

861

862   **Figure 5: Spt6 promotes histone eviction and transcription.**

863   A) The changes in Rpb3 occupancies ($\log_2$ ratio, ChIP/input) in the untreated (no dox) and
864   treated (dox) *spt16/spt6Δ202* cells relative to occupancies in the untreated *SPT16-TET* (WT) are
865   plotted against the Rpb3 ORF occupancies in WT. Pearson correlations are shown.

866   B) Box-plot showing changes in Rpb3 occupancy in *SPT16/spt6Δ202* (dox and no dox) relative
867   to the *SPT16-TET* (no dox) WT cells, at the genes grouped in deciles on the basis of the average
868   ORF Rpb3 occupancies observed in WT cells. The first decile shows the highest Rpb3
869   occupancy and the 10th shows the lowest.

870   C) Top 200 genes showing the greatest reduction in Pol II occupancy in dox-treated
871   *SPT16/spt6Δ202* relative to the untreated cells were selected based on the averge Rpb3
872   enrichments in coding regions. Pol II occupancy profile for these genes is plotted for WT,
873   *SPT16/spt6Δ202* and *spt16/spt6Δ202*.

874   D) Box-plot showing average Rpb3 enrichment ($\log_2$ ratio, ChIP/input) for 67 genes of the 200
875   genes. These genes were not among the top 25% genes showing greatest Rpb3 enrichment in WT
876   cells. p-values are shown.

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879 **Legends to the supplemental figures:**

880 **Figure S1: Role of the H3 tail in FACT recruitment.**

881 A) Spt16 ChIP occupancy at *ADH1* in a H4 tail mutant lacking the first 16 residues (*H4Δ1-16*).

882 B) Spt16 and Rpb3 ChIP occupancy in the mutant was normalized against the WT occupancy at
883 each gene and the normalized data is presented for the indicated genes.

884 C) Anti-HA beads were used to pull down HA-tagged H2B from the whole cell extracts prepared
885 from the untagged WT, the HA-tagged WT and *H4Δ1-16* cells. The immunoprecipitates were
886 subjected to western blot using anti-HA and anti-Spt16 antibodies. The representative blot is
887 shown on the left, and the quantified data on the right.

888

889 **Figure S2: Role of histone acetylation in recruiting Spt16.**

890 ChIP occupancies of Rpb3 (left) and Spt16 (right) at *ARG1* in WT, *H3K→A,* and *gcn5Δ* mutants
891 were determined by real-time (RT) PCR. ChIP occupancy in the *ARG1* 3' ORF in WT were
892 normalized to *POL1* signals from the same ChIP sample, and normalized again with the
893 *ARG1*/*POL1* signals for the related input samples. Average data with SEM for three independent
894 experiments is shown.

895

896 **Figure S3: Rpb3 occupancy in WT, and Spt16 and Spt6 depleted cells.**

897 A) A bar-graph showing the bud counts in *SPT16-TET* and *SPT6-TET* cells in the presence or in
898 the absence of doxycycline (dox). The cells were grown exactly as they were for the ChIP-chip
899 experiments. The cells were briefly sonicated before counting budded and unbudded cells under
900 the microscope. The error bars represent standard error of the mean.

901 B) Venn diagram showing overlap between the top 25% Pol II-occupied genes and those genes
902 which were identified to express cryptic transcripts in *spt16-197* and *spt6-1004* (Cheung et al.
903 2008).

904 C) Overlapping genes, identified to express cryptic transcripts and were among the top 25% Pol
905 II occupied genes (n=154), were removed from the list of 1246 highly expressed genes. Pol II
906 occupancy profile for the remaining 1092 genes in WT, spt16 and spt6 cells is shown.

907 D) Rpb3 occupancy profile of WT and *spt6* cells at the genes eliciting reduction in Rpb3
908 occupancy $\geq$ 0.5 log$_2$ ratio (ChIP/input) upon depleting Spt6.

909 E) The top 25% Pol II-occupied genes were grouped on the basis of their gene-length and
910 changes in Rpb3 occupancy profiles (*spt6*/WT) for the long (>2 kb), medium (1-2 kb), and short
911 (0.5-1 kb) are shown in the WT and Spt6-depleted cells.

912

913 **Figure S4: Gene-length analyses for the genes most affected by depletion of Spt6 or Spt16.**

914 A) The genes which showed more than 0.5 $\log_2$ reduction in Pol II occupancy upon depleting
915 either Spt6 or Spt16 were analyzed, and were classified as Spt16-unique, Spt6-unique and Spt16-
916 Spt6-common depending on whether they were enriched only upon depleting Spt16, Spt6 or
917 both, respectively. Frequency distribution of these classes of genes based on their gene-length is
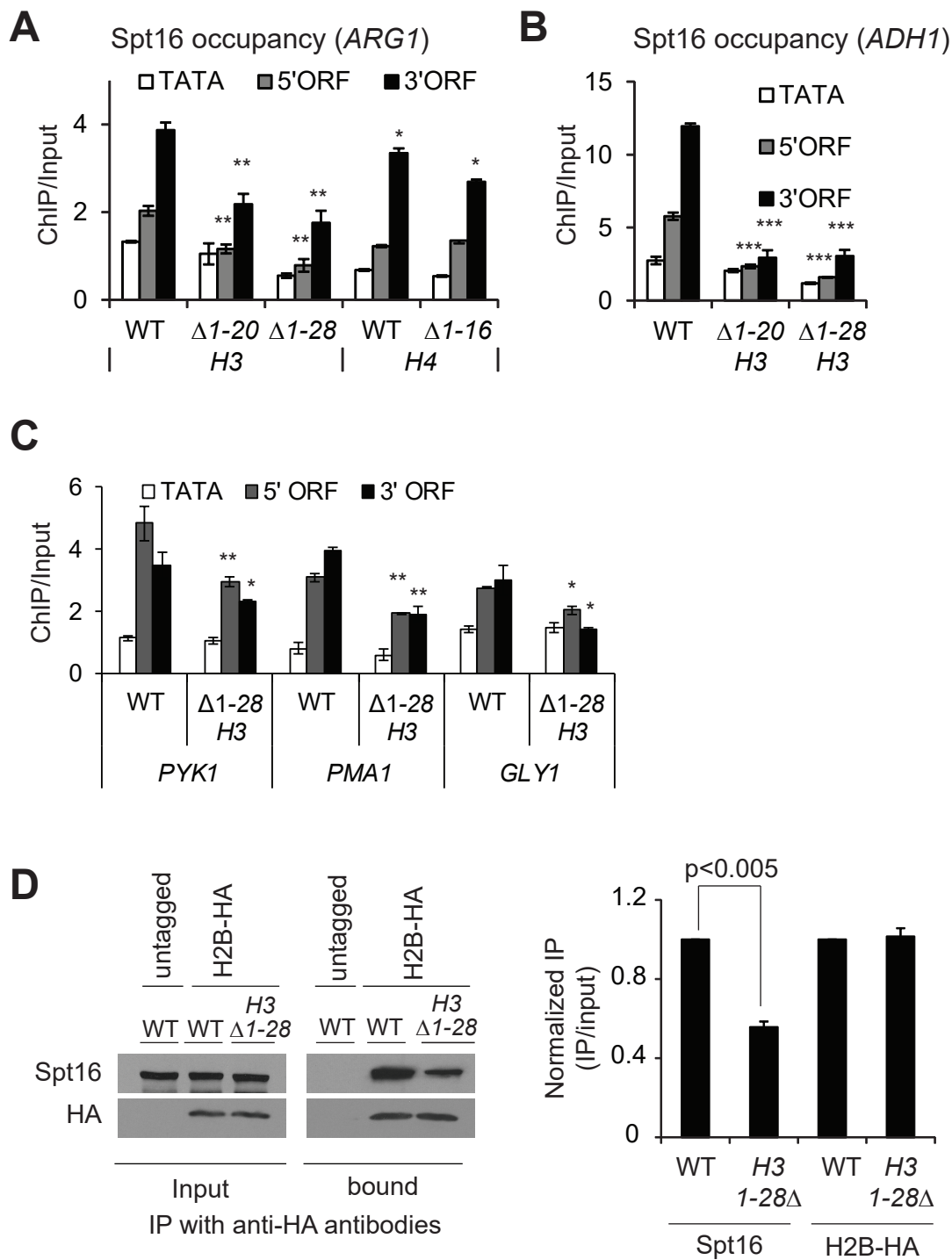918 plotted as a histogram.

919 B) Gene-length for the three classes of genes is shown in the box plot. Center lines show the
920 medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers
921 extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are
922 represented by dots. 115, 217 and 111 genes were present in the Spt16-unique, Spt6-unique and
923 common gene-sets, respectively.

924

925 **Figure S5: Pol II occupancy in *spt16/spt6Δ202* cells.**

926 A) Western blot showing Spt16 protein levels in untreated and dox-treated *Spt16/spt6Δ202* cells.

927 B) Rpb3 occupancy profiles for the top 25% Pol II-occupied genes (n=1246) in untreated
928 *SPT16/spt6Δ202* and dox-treated (*spt16/spt6Δ202*) cells.

929 C) Box-plot showing Rpb3 enrichment ($\log_2$ ratio, ChIP/input) for the 137 highly expressed
930 genes among the 200 genes showing greatest reduction in *Spt16/spt6Δ202* dox-treated compared
931 to untreated cells. Pol II occupancies are shown for the untreated WT and *SPT16/spt6Δ202*, and
932 dox-treated *SPT16-TET* (*spt16*) and *SPT6-TET* (*spt6*) and *SPT16/spt6Δ202* (*spt16/spt6Δ202*)
933 cells.

Pathak_Figure 1

Pathak_Figure 2

A Genes with reduced Pol II occupancy $\log_2$ (mutant / WT) $\leq$ -0.5
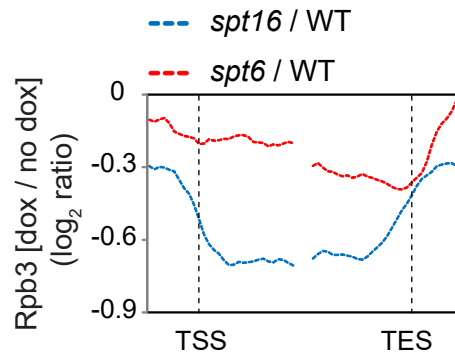
B Genes with reduced Pol II occupancy $\leq$ -0.5 in

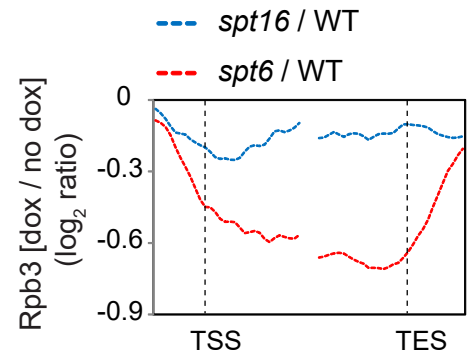**C** Genes with reduced Pol II occupancy $\leq$ -0.5 in both *spt16 and spt6* cells
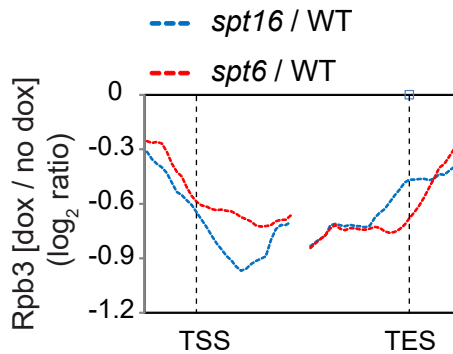
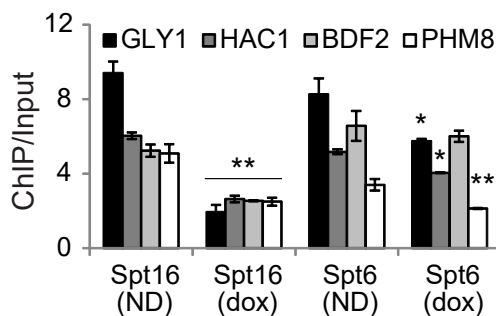**D** Genes with reduced Pol II occupancy $\leq$ -0.5 only in *spt16* cells

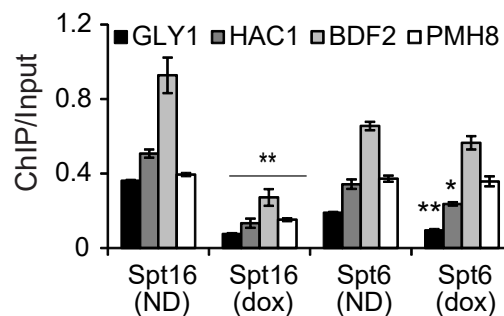**E** Genes with reduced Pol II occupancy $\leq$ -0.5 only in *spt6* cells
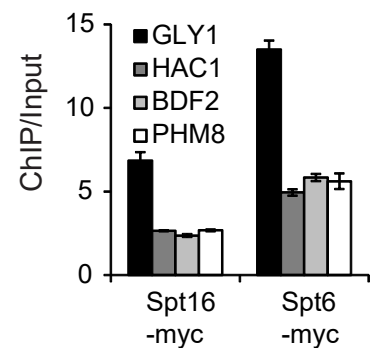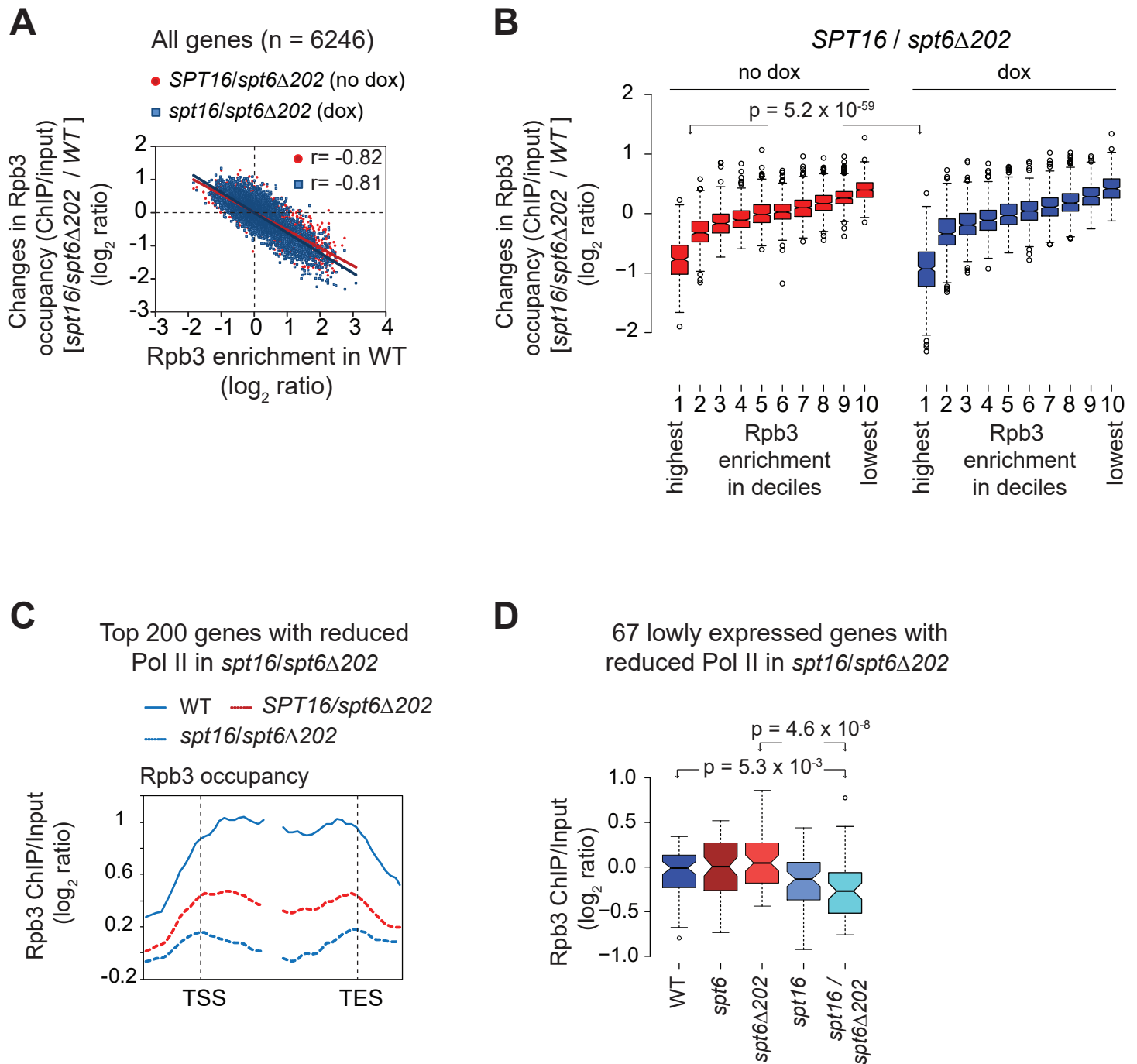
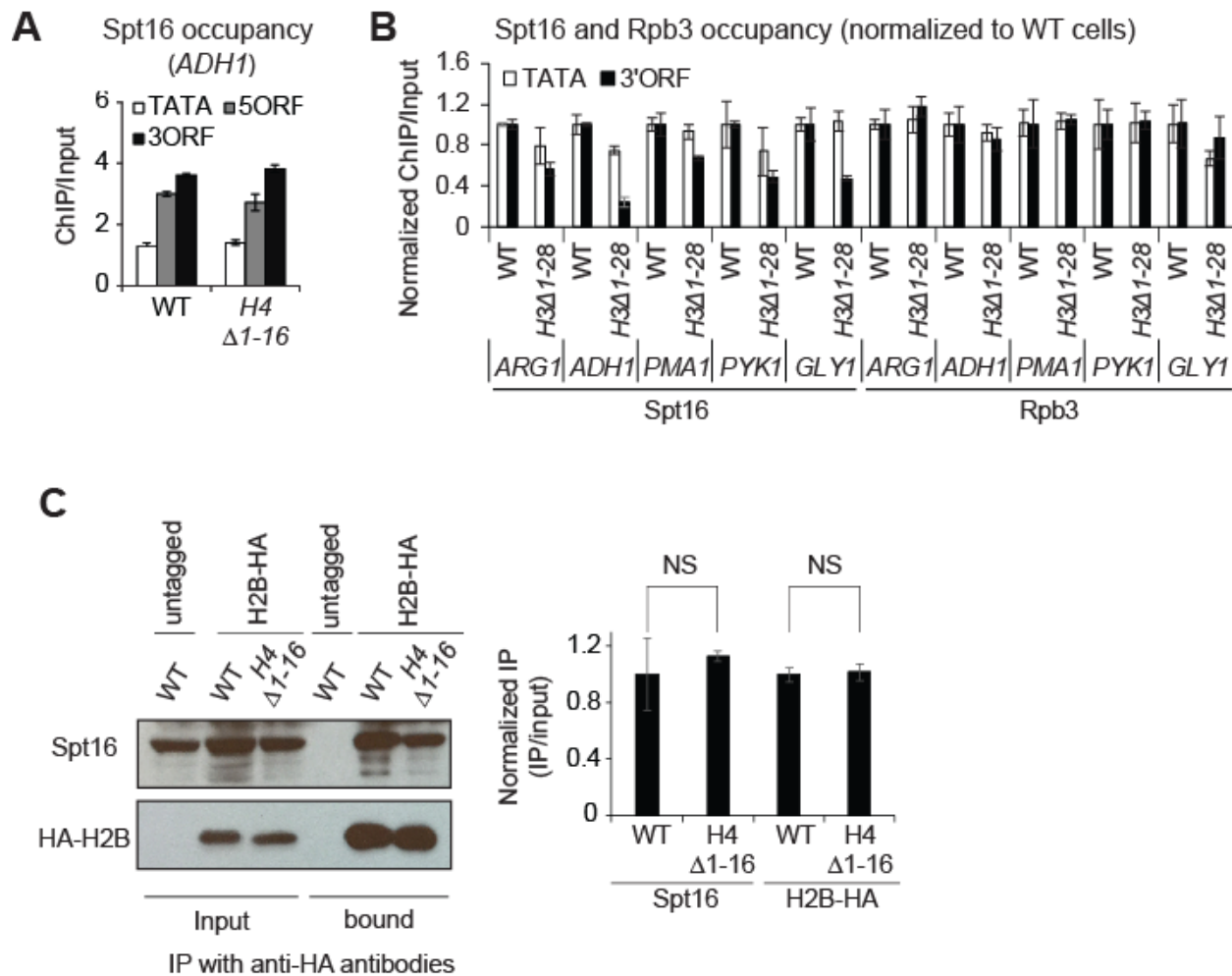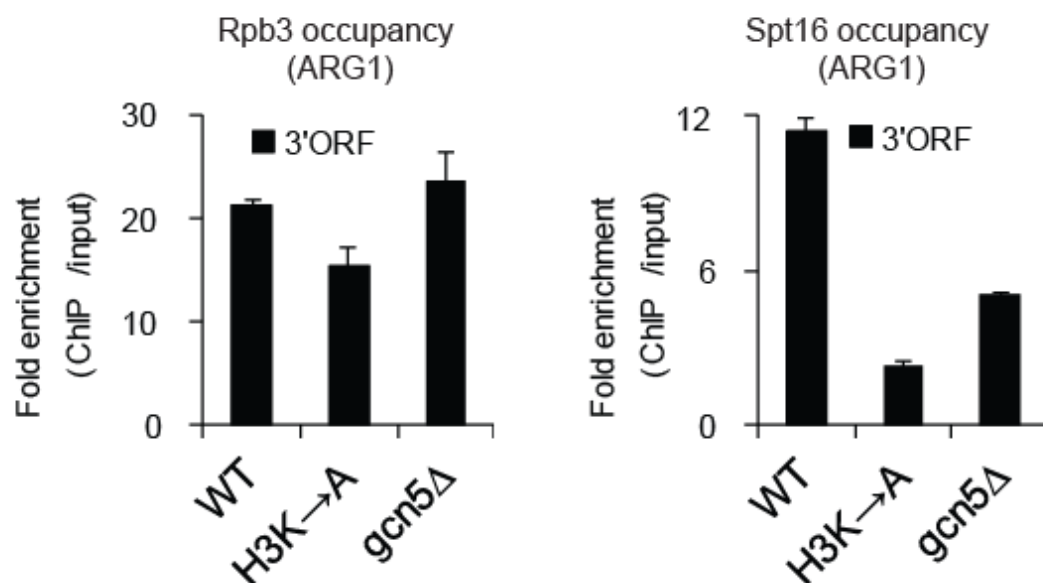**F** Rpb3 occupancy

G H3 occupancy

H Spt16 and Spt6 occupancy

Figure S1: Role of the H3 tail in FACT recruitment.

A) Spt16 ChIP occupancy at *ADH1* in a H4 tail mutant lacking the first 16 residues (*H4Δ1-16*). B) Spt16 and Rpb3 ChIP occupancy in the mutant was normalized against the WT occupancy at each gene and the normalized data is presented for the indicated genes. C) Anti-HA beads were used to pull down HA-tagged H2B from the whole cell extracts prepared from the untagged WT, the HA-tagged WT and *H4Δ1-16* cells. The immunoprecipitates were subjected to western blot using anti-HA and anti-Spt16 antibodies. The representative blot is shown on the left, and the quantified data on the right.

**Figure S2: Role of histone acetylation in recruiting Spt16.**

ChIP occupancies of Rpb3 (left) and Spt16 (right) at *ARG1* in WT, *H3K→A,* and *gcn5Δ* mutants were determined by real-time (RT) PCR. ChIP occupancy in the *ARG1* 3' ORF in WT were normalized to *POL1* signals from the same ChIP sample, and normalized again with the *ARG1/POL1* signals for the related input samples. Average data with SEM for three independent experiments is shown.
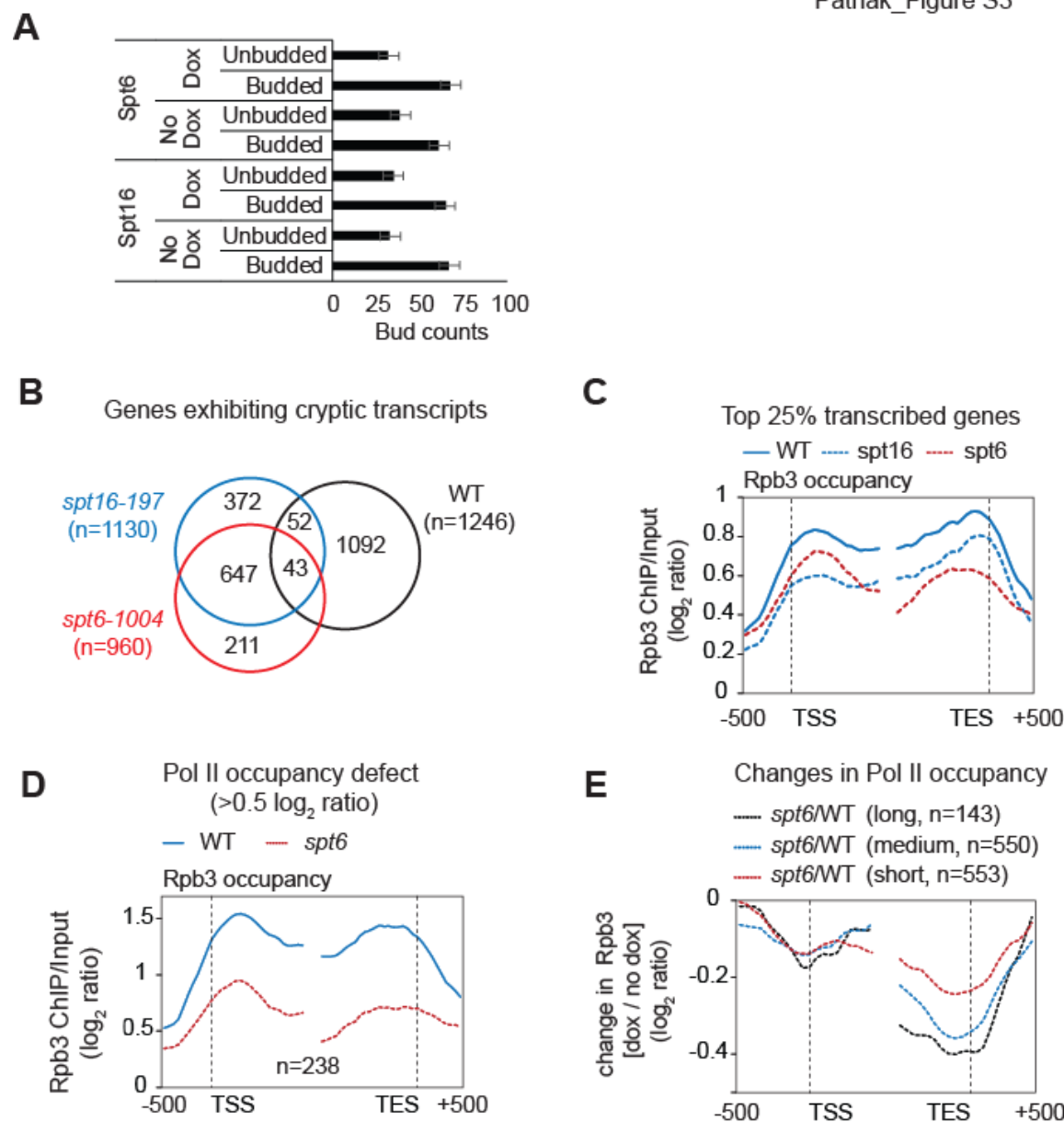
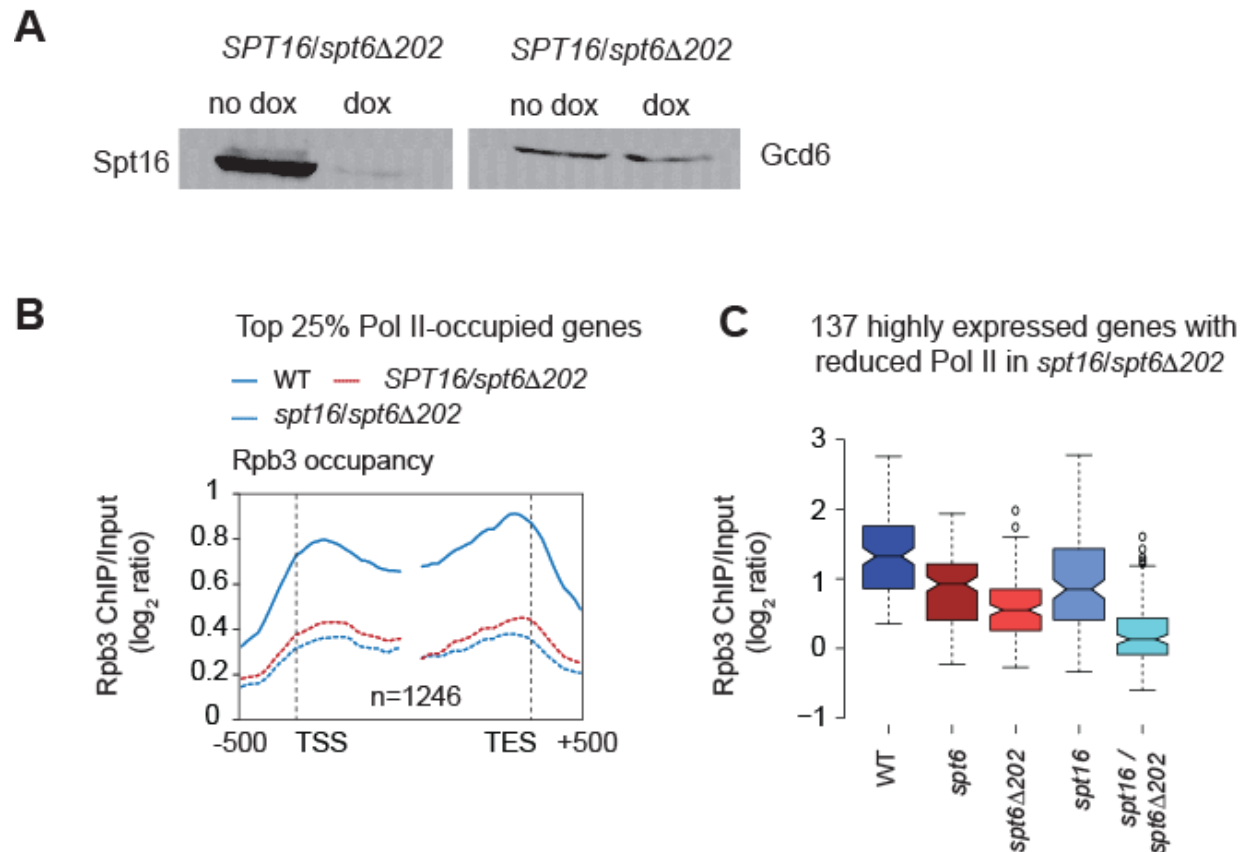**Figure S3: Rpb3 occupancy in WT, and Spt16 and Spt6 depleted cells.**

A) A bar-graph showing the bud counts in *SPT16-TET* and *SPT6-TET* cells in the presence or in the absence of doxycycline (dox). The cells were grown exactly as they were for the ChIP-chip experiments. The cells were briefly sonicated before counting budded and unbudded cells under the microscope. The error bars represent standard error of the mean. B) Venn diagram showing overlap between the top 25% Pol II-occupied genes and those genes which were identified to express cryptic transcripts in *spt16-197* and *spt6-1004* (Cheung et al. 2008). C) Overlapping genes, identified to express cryptic transcripts and were among the top 25% Pol II occupied genes (n=154), were removed from the list of 1246 highly expressed genes. Pol II occupancy profile for the remaining 1092 genes in WT, spt16 and spt6 cells is shown. D) Rpb3 occupancy profile of WT and *spt6* cells at the genes eliciting reduction in Rpb3 occupancy ≥ 0.5 log2 ratio (ChIP/input) upon depleting Spt6. E) The top 25% Pol II-occupied genes were grouped on the basis of their gene-length and changes in Rpb3 occupancy profiles (*spt6*/WT) for the long (>2 kb), medium (1-2 kb), and short (0.5-1 kb) are shown in the WT and Spt6-depleted cells.
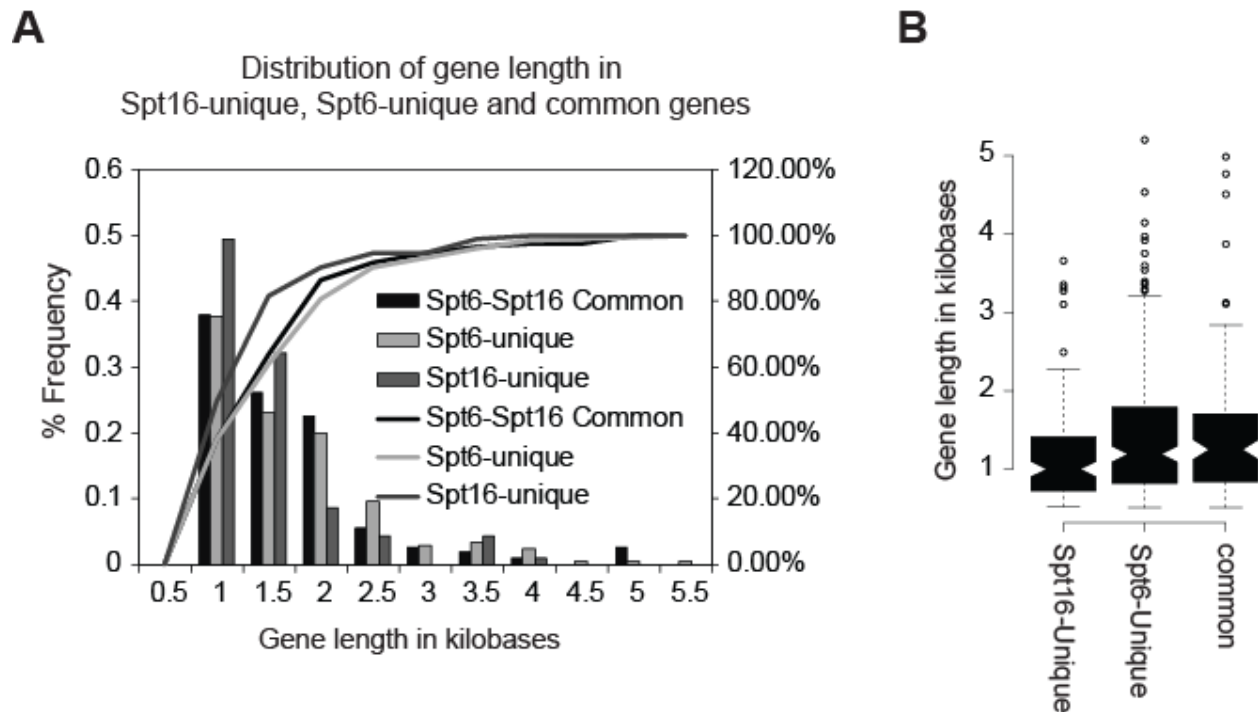
Figure S4: Gene-length analyses for the genes most affected by depletion of Spt6 or Spt16.

A) The genes which showed more than 0.5 $\log_2$ reduction in Pol II occupancy upon depleting either Spt6 or Spt16 were analyzed, and were classified as Spt16-unique, Spt6-unique and Spt16-Spt6-common depending on whether they were enriched only upon depleting Spt16, Spt6 or both, respectively. Frequency distribution of these classes of genes based on their gene-length is plotted as a histogram. B) Gene-length for the three classes of genes is shown in the box plot. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. 115, 217 and 111 genes were present in the Spt16-unique, Spt6-unique and common gene-sets, respectively.

A

SPT16/spt6Δ202   SPT16/spt6Δ202
no dox   dox   no dox   dox

Spt16   Gcd6

B   Top 25% Pol II-occupied genes

— WT — SPT16/spt6Δ202
— spt16/spt6Δ202

Rpb3 occupancy

Rpb3 ChIP/Input ($\log_2$ ratio)

n=1246

-500 TSS   TES +500

C   137 highly expressed genes with reduced Pol II in spt16/spt6Δ202

Rpb3 ChIP/Input ($\log_2$ ratio)

WT — spt6 — spt6Δ202 — spt16 — spt16 / spt6Δ202

Figure S5: Pol II occupancy in *spt16/spt6Δ202* cells.

A) Western blot showing Spt16 protein levels in untreated and dox-treated *Spt16/spt6Δ202* cells. B) Rpb3 occupancy profiles for the top 25% Pol II-occupied genes (n=1246) in untreated *SPT16/spt6Δ202* and dox-treated (*spt16/spt6Δ202*) cells. C) Box-plot showing Rpb3 enrichment ($\log_2$ ratio, ChIP/input) for the 137 highly expressed genes among the 200 genes showing greatest reduction in *Spt16/spt6Δ202* dox-treated compared to untreated cells. Pol II occupancies are shown for the untreated WT and *SPT16/spt6Δ202*, and dox-treated *SPT16-TET* (*spt16*) and *SPT6-TET* (*spt6*) and *SPT16/spt6Δ202* (*spt16/spt6Δ202*) cells.

Table S1: Yeast strains used in this study

Strain	Parent	Genotype	Source
DG154	BY4741	*MATa his3Δ leu2Δ met15Δ ura3Δ gcn5Δ::kanMX4 esa1L254P*	(Ginsburg et al., 2009)
F729	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Qiu et al., 2009)
WT HDACs	BY 4705	*MATα ade2Δ::hisG HIS3Δ200 LEU2Δ0 ura3Δ0 trp1Δ63 LYS2Δ0 met15Δ0*	(Sharma et al., 2007)
hos2Δ/rpd3Δ	BY 4705	*MATα ade2Δ::hisG HIS3Δ200 LEU2Δ0 ura3Δ0 trp1Δ63 LYS2Δ0 met15Δ0 rpd3::LEU2hos2::LYS2*	(Sharma et al., 2007)
FH62(WT H3)	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]-URA3*	Thermo Scientific
FH63(WT H4)	S288C	*MATa his3_200 leu2_0 lys2_0 trp1_63 ura3_0 met15_0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]-URA3*	Thermo Scientific
FH59(Δ1-28 H3)	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts (Δ1-28)-HHFS]-URA3*	Thermo Scientific
FH58(Δ1-2 H3)	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts (Δ1-20)-HHFS]-URA3*	Thermo Scientific
FH60(Δ1-16 H4)	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-hhfs (Δ1-16)]-URA3*	Thermo Scientific
FH46((H3K4, K9, K14,K18-A)	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts K4,9,14,18A-HHFS]-URA3*	Thermo Scientific
FH47	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-hhfs K5,8,12,16R]-URA3 SPT16-13myc::TRP1*	Thermo Scientific
FH48	S288C	*MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-hhfs K5,8,12,16Q]-URA3 SPT16-13myc::TRP1*	Thermo Scientific
SPT16-TET	R1158	*pSPT16::kanR-tet07-TATA URA3::CMV-tTA MATa his3Δ1 leu2Δ0 met15Δ0*	Thermo Scientific

SPT6-TET	R1158	*pSPT6::kanR-tet07-TATA URA3::CMV-tTA MAT*a *his3Δ1 leu2Δ0 met15Δ0*	Thermo Scientific
RP4	BY 4741	*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPT16-13myc::HIS3*	This Study
RP6	1257	*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 set1Δ::hphMX4 set2Δ::kanMX4 SPT16-13myc::HIS3*	This Study
RP8	BY 4705	*MAT*α *ade2Δ::hisG HIS3Δ200 LEU2Δ0 ura3Δ0 trp1Δ63 LYS2Δ0 met15Δ0 SPT16-13myc::HIS3*	This Study
RP9	BY 4705	*MAT*α *ade2Δ::hisG HIS3Δ200 LEU2Δ0 ura3Δ0 trp1Δ63 LYS2Δ0 met15Δ0 rpd3::LEU2hos2::LYS2 SPT16-13myc::HIS3*	This Study
RP10	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]-URA3 SPT16-13myc::TRP1*	This Study
RP11	S288C	*MAT*a *his3_200 leu2_0 lys2_0 trp1_63 ura3_0 met15_0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]-URA3, SPT16-3myc::TRP1*	This Study
RP12	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts (Δ1-28)-HHFS]-URA3 SPT16-13myc::TRP1*	This Study
RP13	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts (Δ1-20)-HHFS]-URA3 SPT16-13myc::TRP1*	This Study
RP14	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-hhfs(Δ1-16)]-URA3SPT16-13myc::TRP1*	This Study
RP15	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts* K4,9,14,18A*-HHFS]-URA3 SPT16-13myc::TRP1*	This Study
RP16	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-hhfs* K5,8,12,16R]*-URA3 SPT16-13myc::TRP1*	This Study
RP17	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-hhfs* K5,8,12,16Q]*-URA3 SPT16-13myc::TRP1*	This Study
RP18	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]-URA3 H2B-HA::TRP1*	This Study
RP19	S288C	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[hhts (Δ1-28)-HHFS]-URA3 H2B-HA::TRP1*	This Study

| RP20 | DG154 | *MATa his3Δ leu2Δ met15Δ ura3Δ gcn5Δ::kanMX4 esa1L254P SPT116-myc13::HIS3* | This Study |
| RP21 | R1158 | *pSPT16::kanR-tet07-TATA URA3::CMV-tTA MATa his3Δ1 leu2Δ0 met15Δ0 SPT6Δ202::HIS3* | This Study |