

Supplemental Material

for the manuscript

Transcription-dependent enrichment of the yeast FACT complex influences nucleosome dynamics on the RNA polymerase III-transcribed genes

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I Supplementary information

IA. Spt16 ChIP-seq data analysis

The quality control results showed high reproducibility among the biological replicates of the acquired ChIP-seq data (Supplemental Figs. S1A, B, C). The plotPCA function of deeptools (2.3.2) was used to generate the panels S1B and S1C. In the PCA plot (Fig. S1B), there is a strong effect of “genomic positions” on the principal component 1 (PC1). However stacking of majority of samples on the same vertical line suggest this effect is similar across samples. The treatment conditions have greater influence on PC2 in this case. The result (Fig. S1C) shows the first 2 or 3 principal components give maximum amount of variability in the data (>90%). The rest of principal components account for a small proportion of the variability and are trivial. Overall, panels S1B and S1C show robust clustering of the data (experimental conditions) and least variability between the replicates. Moreover, despite resolution differences, the Spt16-TAP occupancy profile on the pol II-transcribed ORFs normalized to Input/mock IP (ChIP-seq, this study) is similar and comparable (Supplemental Fig. S1D) to the earlier reported Input-normalized profile obtained by using untagged cells as controls (ChIP-chip, Mayer et al. 2010). Normalization with Input instead of the mock returned the same occupancy profile showing highest Spt16 levels at the 5' end of ORFs (Fig. 2A and Supplemental Fig. S2D) and in the DS region on the tRNA genes (Supplemental Fig. S1E). The differences in peak heights on the tRNA genes after normalizations (Supplemental Fig. S1E) could be related to the differences in the Input and mock values at the positions around the tRNA genes (Supplemental Fig. S1F). These comparisons also show that the US peak is not due to the Spt16 enrichment there, as it could not be validated by the ChIP and Real Time PCR measurements as well (Fig. 2C). However, as mock is a better control (Flensburg et al. 2014) and we could get good quality mock data, it was used for all tRNA ChIP normalizations.

Similar to the tRNA genes, input- or mock-normalized occupancy profiles on the non-tRNA *SNR6* were found largely similar to each other (Supplemental Fig. S2F). In contrast, we found that normalization with either mock (Supplemental Figs. S2G) or input (Fig. 2D) gave significantly different Spt16 occupancy profiles for the pol III-transcribed non-tRNA genes *RPR1* and *SCR1*. This could be due to the abrupt decrease in the mock signal causing large differences in the profiles of the two normalizers for these genes (Supplemental Fig. S2H, I) at the marked positions. As ChIP-Real Time PCR measurements

matched the input-normalized Spt16 profiles better, all the data for the three non-tRNA genes were normalized with input for further use.

IB. High enrichment of Spt16 on tRNA genes

This study has found an unexpected enrichment of the yeast FACT subunit Spt16 at the region downstream (DS) of the 3'- ends of the pol III- transcribed genes. An earlier genome-wide study had reported "hyper-ChIPability" of 238 sites including 145 tRNA genes and genes highly transcribed by Pol II as listed in the Table S1 of Teytelman et al. (2013). According to the authors, this may not be due to a particular tag or protein and may have more to do with a step in ChIP sample preparation and processing. Though the real cause is not clear, the clear association of hyper-ChIPability with highly transcribed regions suggests involvement of open chromatin structure found on such regions. Since our mappings have been made on pol III-transcribed genes, we made some checks on our data in the context of hyper-ChIPability, high transcription and open chromatin structure of these genes and found the following.

(1) We had earlier reported the nucleosomal arrangement on the pol III-transcribed loci (Kumar and Bhargava, 2013), which showed that all tRNA genes reside in nucleosome-free regions (NFRs) of different lengths. We did not find any correlation of hyper-ChIPable tRNA genes with either the longest or any other specific NFR length. Grouping of all genes according to their NFR lengths found all hyper-ChIPable genes distributed among all the groups. This means open chromatin structure may not be the cause of hyper-ChIPability of the yeast tRNA genes.

(2) We had earlier mapped genome-wide yeast pol III occupancy (Kumar and Bhargava 2013) and found pol III on >90 pol II-transcribed genes (unpublished data). As pol III is a protein known to occupy the tRNA genes, we checked whether these pol II genes were listed as hyper-ChIPable. Interestingly, we could find only 7 of these pol III-occupied genes in the list of hyper-ChIPable pol II-transcribed genes. Moreover, all of the seven genes were not the highly transcribed ones, suggesting the pol III occupancy on pol II-transcribed genes may not be related to their highly transcribed nature.

(3) We did not find any difference in Spt16 distribution over the tRNA genes classified as hyper-ChIPable or otherwise (Supplemental Fig. S2E). Spt16 occupancy also did not show a correlation with transcription from individual tRNA genes as measured by the nascent RNA levels (Fig. 3A, Supplemental Fig. S3A, B, C).

(4) In our genome-wide data on Spt16 occupancy, average Spt16 levels on the tRNA gene bodies were found similar to the highest average levels of Spt16 at the 5' end of the pol II-transcribed genes (cf. Figs. 2A and 2B). If Spt16 was associated with tRNA genes due to their hyper-ChIPability, we should not have seen it on the tRNA gene bodies at levels similar to levels on all those tRNA genes, which do not show hyperChIPability (Supplemental Fig. S2E) or to average levels on pol II-transcribed genes.

(5) All tRNA genes are similarly occupied by pol III and highly transcribed. Therefore, not finding all of them in the list of hyperChIPable regions may have some other reasons, not specific for tRNA genes.

(6) Out of seven genes used for validations by ChIP and Real Time qPCR in this study, three are found in the list of hyperChIPable tRNA genes. One of the seven, *tE(UUC)EI* is not in the list of hyperChIPable genes but shows highest Spt16 occupancy levels; higher than *tY(GUA)J2*, *tV(AAC)MI* and *tQ(CUG)M* which are found in the list.

All these observations make us believe that despite the earlier report on hyper-ChIPability of the tRNA genes, our Spt16 occupancy data represent a specific association profile of Spt16 on the pol III-transcribed genes.

IC. The tRNA genes are not the Phantom binding sites for Spt16

The "phantom" binding sites have been reported in other systems, including IgG pulldowns of TAP-tagged proteins, which we used for Spt16 here (Park et al. 2013; Jain et al. 2015). We did not use "No tag" control but included other control (mock) like Protein A/G sepharose in place of IgG sepharose beads for the TAP tag. We have used Input as background control and used mock for normalization of the data. Fig. S2F shows our mock and input data as compared to ChIP data without normalization.

Earlier Mayer et al. (2010) had used Spt16-TAP-tagged cells for their ChIP-chip experiment and BY4741 untagged cells for mock IP. Within the resolution limits of the two methods used, analysis of their data normalized with their mock shows similar profile on ORFs as reported by us (Supplemental Fig. S1D). We have also used a polyclonal Spt16-specific antibody, in our ChIP Real Time PCR quantifications, along with mock immunoprecipitation involving protein A/G sepharose and non-specific antibody IgG. These measurements did not find Spt16 enrichment in the mock, again suggesting that Spt16 associations found in our data represent specific enrichments. Moreover, both the methods showed a similar trend of variations among different genes and showed highest Spt16

occupancy on the *tE(UUC)EI* gene which is not in the list of hyperChIPable tRNA genes. We have noticed that in most of the measurements, tRNA genes behave in a gene-specific manner, giving a non-uniform level of quantitative changes. Thus, the measured effects may appear small on some of the tested genes but there are other genes in the same estimation, which show larger changes. These observations are reflected in the trend seen in the wild type Spt16 DS occupancy values in the Figs 2C (using TAP tag) and the Fig. 6B (using Spt16-specific antibody) for the corresponding genes. High enrichment in the DS region as well as gene-specific trend of levels, regardless of using TAP-tag or Spt16-specific antibody for the ChIP was consistently seen.

ID. The myc tag at the Rpc160 C-terminus is masked and Rpc160 protein is not degraded in the *spt16-197* cells

Rpc160-myc ChIP was comparatively very low at 30⁰C or 37⁰C in the mutant cells (Supplemental Fig. S3E). We noticed, despite confirmation of the myc tag insertion at the right genomic location by sequencing, the Rpc160-myc protein shows highly reduced myc signal in the mutant cells at both the temperatures (Supplemental Fig. S3F). As a pronounced myc signal is seen in the wild type cells, the negligible signal in the mutant cells appears to be *spt16* mutation-specific and not temperature- or tag- related. This implied either *degradation of Rpc160* or a *masking of the myc tag*, making it non-available for immuno-detection in western or immuno-capture in ChIP from the mutant cells. *For the same reason, further checking on both the possibilities could not be done.*

RNA levels in the tagged cells (not shown) were found similar to those in the Figs. 3B and S3D, which may not be possible if Rpc160 (the largest core subunit of pol III) is as extensively degraded as suggested by the anti-myc probings in the Fig. S3F. Recent studies have reported that the pre-tRNAs in the wild type cells are made in excess, of which more than 50% are degraded without undergoing maturation (Wlotzka et al. 2011; Gudipati et al. 2012; Schneider et al. 2012). On the other hand, the mutant cells may be able to produce pre-tRNAs enough for meeting only the cellular requirements of tRNAs. Thus, finally both cell types may produce similar steady-state tRNA levels. In this context, we did not find any degradation of the loading control Pgk1 (Supplemental Fig. S3F) or a general protein degradation (not shown) in the Rpc160-tagged *spt16-197* cells.

Myc tagging of Rpc160 gene does not cause any growth defect (Supplemental Fig. S3G). We ruled out the loss of Myc tag coding sequence from the genomic DNA by repeated

genomic locus sequencings after several times sub-culturing of the positive clones having tagged Rpc160 gene. Considering all the above-mentioned checks, it appears that Myc tag is not detected in our probings/ ChIP because of its masking by some cellular component and not because of the degradation of the Rpc160 protein. Although our further preliminary checks on this were encouraging, further probings are beyond the scope of this manuscript. Finally, since the normal growth of the mutant (Malone et al. 1991) may not be possible if pol III levels are severely compromised, we inferred that the Rpc160 levels are similar in both cell types, which is consistent with the similar cellular levels of total tRNAs in the wild type and *spt16-197* cells (Fig. 3B, Supplemental Fig. S3D).

II Supplementary References

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III Supplementary Tables

Table S1. Spt16 interacts with pol III, TFIIIC and TFIIIB.

Prey	Bait						
FACT subunits	All	Pol III (Rpc128)		TFIIIC (Tfc6)		TFIIIB (Brf1)	
	Control	Active SSC sum	Repressed SSC sum	Active SSC sum	Repressed SSC sum	Active SSC sum	Repressed SSC sum
Spt16	0	13	18	2	4	0	14
Pob3	0	2	5	2	0	7	47
Nhp6	0	5	6	22	22	4	3
Total	0	20	29	26	26	11	64

FACT complex associates with the pol III TC in both active and repressed states. Data from the AP-MS/MS on the pol III TC interaction with FACT complex (Bhalla et al. 2019a) are given. Sums of SSCs (Specific Spectral Counts) for each subunit of the FACT complex in the IP and control (mock) samples of the three baits are shown.

Table S2 List of the Yeast strains used in this study

Strain	Genotype	Source
SPT16-TAP	<i>MATa leu2-3 112 ura3-1 his3-1115 trp1-1 ade2-1 can1-100 SPT16-TAP::TRP1</i>	Open Biosystem
TFC1-FLAG SPT16-HA	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 TFC1-3xFLAG::kanMX4 SPT16-6xHA::HphMX</i>	This study

BRF1- FLAG SPT16-HA	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 BRF1-3xFLAG::kanMX4 SPT16-6xHA::HphMX</i>	This study
RPC160-MYC SP16-HA	<i>MATα his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 RPC160-9xMyc::HIS3 SPT16-6xHA::HphMX</i>	This study
RH932.5A	<i>MATα bar1-1 trp1-1</i>	Jamai et al. 2009
AJ001E	<i>MATα bar1-1 spt16-197 ura3-52</i>	Jamai et al. 2009
<i>spt16-197</i> RPC160-MYC	<i>MATα bar1-1 spt16-197 ura3-52 RPC160-9xMyc::kanMX4</i>	This study
YM1729	<i>MATα his3Δ0 leu2Δ0 ura3Δ0 lys2Δ0</i>	Meneghini et al. 2003
YM1729 RPC160-MYC	<i>MATα his3Δ0 leu2Δ0 ura3Δ0 lys2Δ0 RPC160-9xMyc::HIS3</i>	This study
MW1067	<i>MATα ade2- 101 lys2-801 leu2-Δ1 his3-Δ200 ura3-52 trp1- Δ63 rpcl60-Δ1::HIS3 pC160-249 (URA3 CEN4 rpcl60-112-3xHA)</i>	Dieci et al. 1995
MW671	<i>MATα ade2- 101 lys2-801 leu2-Δ1 his3-Δ200 ura3-52 trp1- Δ63 rpcl60-Δ1::HIS3 pC160-240 (URA3 CEN4 RPC160-3xHA)</i>	Dieci et al. 1995
SJY25	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1::kanMX4 SPT16-18xMyc</i>	Jimeno-González et al. 2006

Table S3 List of the Primers used in this study

Primer Name	Forward Primer	Reverse Primer	Used for
tL(CAA)A-UP	TTCCCATTAACAATGCCGAAT	TCTTTCTGAAGAGACACCC AAA	ChIP
tL(CAA)A-Down	AGTCCGCTTCCTACGGTTT	AAGAAAACGCTGATGCAGAA	ChIP
tN(GUU)L-UP	ACTCCAACAATAGCCGTTACT	TCAAATAGTTTTGAAGAAT CAGCG	ChIP
tN(GUU)L-Down	ACTTAGCCACATTCGGGCTT	CGAGTTTCGGATGGGATCAA	ChIP
tQ(CUG)M-UP	TAGCCCAAAGTCATGAGGAGAGC	TTTAACAATATCGCAAGCAATGGA	ChIP
tQ(CUG)M-Down	ACAGATACAATCGCTCATCTCGA	AGGACTTGTCTGTACACACGC	ChIP
tE(UUC)E1-UP	TCTATCGGCTGGTGTTCGTA CT	TGGGTACTTTGCTAGGTTGAG	ChIP
tE(UUC)E1-Down	GGCTGAATCCTGATAGTTTGAG	GGTAAAAACGATGGAGTGCAAGAG	ChIP
tV(AAC)M1-UP	CCCACATATATCTCACCTTCTTAC	TCGTTGATAGTTAGGTTTTCGTGT	ChIP
tV(AAC)M1-Down	GTGAATAGTCTGGGTAGGTTGAAA	TGCCATCGGTGTCATTTAGG	ChIP
tY(GUA)J2-UP	TGGTCATGATGTCGCTATTTCT	CTAAAGCTAAGGGATGGAA GAGA	ChIP
tY(GUA)J2-Down	CCCGGGAGATTTTTTTTGT	AAAAGAGGCTACAAGAGTT CGTTAAT	ChIP

ORF-Free 1	GGCTGTCAGAATATGGGGC CGTAGTA	CACCCCGAAGCTGCTTTCA CAATAC	ChIP
ORF-Free 2	GCAATCAACATCTGAAGAA AAGA	CATAATCTGCGTAAAAATG GCGT	ChIP
TELVIR	GCGTAACAAAGCCATAATG CCTCC	CTCGTTAGGATCACGTTCG AATCC	ChIP
SCR1 A1	CAGTCTTATTTGATGATTCA CACATTCCTTG	GCCTAGCACAAATTGGAATA AACTTTTCG	ChIP
SCR1 A2	TGTAATGGCTTTTCTGGTGGG ATG	AGCTCTGCCCAGGACAAAT TTAC	ChIP, RNA
SCR1 A3	TAAATTTGTCCTGGGCAGAG CTGT	TGACGCTGGATAAAACTCC CCTAA	ChIP
SCR1 A4	ATAGCACATATCAGTCGGAT AATT	ATCCCTCATTGGGACGGTT AATTAG	ChIP
SCR1 A5	ATTAACCGTCCCAATGAGGG ATGT	TTATCCTTCTTATATTCCGC TGCCG	ChIP
SNR6 Up 5'-end	CGCTGATACTTTAGAGTTTC ATTGC	CTTCGCGAACACATAGTTG C	ChIP
SNR6 Down 3'-end	GTACTTATGTGCTTTATGAA TGTG	CCTTTCTCTTCTGTTTGACA	ChIP
RPR1 A	CGTGTGTTTTATATGTCTCTT ATCTAAG	CGTGTGTTTTATATGTCTCT TATCTAAG	ChIP
RPR1 B	CAGAAGGATCCCCACCTATG	CGACATTAACCCGGAGGAC	ChIP, RNA
RPR1 C	AAATTCACGGAGTTCGGCCT	GGCGACAAGTCAAACGGA AC	ChIP
RPR1 D	CCAGCCCATATCCAACTTCC A	CCATATCTAATCTAACCAA ATCCAAAC	ChIP
SNR14 (U4snRNA)	CACGGGAAATACGCATATC AGTGA	CCGAATTGACCATGAGGAG ACG	RNA
tE(UUC)E1	TGTAACGGCTATCACATCAC GC	GGAGTCGAACCCCGGTCT	RNA
tM(CAU)E	AGTGGAAGCGCGCAG	TAGCGCCGCTCGGTTT	RNA
tP(UGG)O3*	GCGATCCCCCTGATCAAA	GGGCGAGCTGGGAATT	RNA
tQ(UUG)H	ACTTTCGGTTTTGATCCGGA	AAAGGTCCTACCCGGATTC G	RNA
tR(CCG)L	GCTCCTCTAGTGCAATGG	TCGAACCCGGATCACAG	RNA
tT(CGU)K	CAAGTGGTAAGGCATCG	GCCCTCTGTGGGAATT	RNA
tRNA Glu(UUC)	GGAGTCGAACCCCGGTCT		Northern
tRNA Asn(GUU)	GAACTCACGATCTTGCGATTAAC		Northern
tRNA Phe(GAA)	GCGCTCTCCCAACTGAGCT		Northern
tRNA iMet(CAU)	GTTTCGATCCGAGGACATCAG		Northern
tRNA Leu(CAA)	GACCGCTCGGCCAAACAAC		Northern

SNR14 (U4snRNA)	GGTTTATAATTAAATTTCAACC	Northern
SNR6 (U6 snRNA)	TCTCTTTGTAAAACGGTTCATCCT	Northern

* marks the primers unique for the isogene.

Table S4 Summary of Datasets retrieved from different sources and analysed in this study.

Data type	Data source	Genome wide data for	Shown in the Figure(s)
ChIP-chip ChIP-seq	GSE39566 (Kumar and Bhargava, 2013)	Nucleosomes and RNA pol III occupancy	2B, 7C, S2B, S6E, S6G, S7A
ChIP-Chip	E-TABM-1033 (Mayer et al. 2010)	Spt16 and Pol II occupancy in the wild type cells	S1D, S2C
ChIP-chip	GSE58859 (Jordan-Pla et al. 2015)	RNA pol III nascentome (BioGRO)	3A, S3B, S3C
ChIP-seq	GSE80235 (True et al. 2016)	Nucleosome (H3) occupancy in wild type and spt16-197	4A, 4E, 4F, S4E, S4F
MNase-seq	GSE18530 (Weiner et al. 2010)	Total nucleosomes occupancy	2A, S1D
ChIP-seq	SRP055441 (Feng et al. 2016)	Spt16 occupancy in wild type cells	S2A
ChIP-seq	SRP018874 (Foltman et al. 2013)	Spt16 occupancy in wild type cells	S2A
ChIP-seq	SRP133951 (Martin et al. 2018)	Spt16 occupancy in wild type cells	2A, S2A
ChIP-seq	SRP073244 (True et al. 2016)	Spt16 occupancy in wild type cells	2A, S2A
ChIP-exo	SRP106497 (Vinayachandran et al. 2018)	Spt16 occupancy in wild type cells	S2A
ChIP-seq	SRP036647 (Wong et al. 2014)	Spt16 occupancy in wild type cells	S2A

The listed datasets were analysed for extracting the unpublished information on tRNA genes or ORFs, available in them.

IV Supplementary Figure Legends

Figure S1. Quality checks and analyses of the Spt16 ChIP-seq data

Results from the genome-wide mapping data of Spt16 in the budding yeast. Quality and reproducibility checks of our data from Spt16 ChIP-seq experiment showing **(A)** Mapping statistics **(B)** Principal component analysis (PCA) plot, which shows samples variability between experimental conditions and between the replicates of a condition. The x and y axis show the eigenvalues of the top two principal components. **(C)** Scree plots showing the eigenvalues for each principal component ordered from largest to the smallest. The red line displays cumulative variance explained by each principal component. **(D)** On the pol II-transcribed ORFs, Spt16 is found enriched at the +1 nucleosome position. Similar profiles are obtained by Spt16-TAP ChIP-seq (this study) and earlier published ChIP-chip (Mayer et al. 2010). Average tag counts (per million reads) from MNase-seq data of total nucleosomes (Weiner et al. 2010) are compared with average Spt16 Tag counts (per million reads) from our ChIP-seq data; all aligned to the TSS. **(E)** ChIP data normalized with either mock or input show DS enrichment of Spt16 on the tRNA genes. Average tag counts from all the genes in a window of -1 kb to +1 kb positions from the TSS of all the genes are plotted bin-wise. The US and gene body peaks in the input-normalized data appear to be at the background level, suggesting absence of Spt16 there. **(F)** Control measurements. Average tag counts (normalized to per million reads) of all tRNA genes for Input, mock and ChIP samples are plotted together for comparison.

Figure S2. Spt16 levels are high at the 3'-end of pol III-transcribed genes

(A) Correlation plot of Spt16 occupancy data from different studies. Previously published Spt16 ChIP-seq and ChIP-exo data sets downloaded from Sequence Read Archive (SRA) projects database (Table S4) were used to calculate Pearson coefficients of correlation with this study. The multiBigwigSummary function from deeptools (Ramírez et al. 2014) was used in the BED-file mode to compute the average scores for each of the files on pol II ORF regions. The plotCorrelation function from deeptools was then used for data visualization. **(B)** DS Spt16 is co-localized with the DS nucleosome on the tRNA genes. Average Spt16 occupancy profile is shown with the average nucleosome profile of all the tRNA genes (Kumar and Bhargava 2013) in the background to mark the US and DS nucleosome positions. An asterisk marks the Spt16 peak at the +40 bp on tRNA gene body in the NFR, where occupancy is above the US levels and similar to that at the 5'-ends of the pol II-

transcribed genes. **(C)** Pol II occupancy near tRNA genes. ChIP-chip data (Mayer et al. 2010) was analysed to obtain Rpb3 (representing pol II) occupancy in the flanking regions of the tRNA genes. Average occupancy on all the genes is compared with those not having any ORF within 300 bp US/DS of the tRNA gene ends (filtered genes). **(D)** DS Spt16 enrichment is not related to the presence of Pol II-transcribed genes in the close vicinity of the tRNA genes. The average occupancy profiles of Spt16 (Tag counts per million reads) are indistinguishable on the 275 (Spt16_All) and those filtered (Spt16_Filtered) for not having pol II-transcribed gene-ends within 150 (238 genes) or 300 (129 genes) bp in both sides of the tRNA gene body. **(E)** Average Spt16 occupancy profiles of 274 tRNA genes (all) and the genes not reported as hyperChIPable (Normal) are identical and overlapping. The list of hyperChIPable tRNA genes was obtained from the Table S1 of Teytelman et al. (2013). **(F)** and **(G)** Spt16 occupancy on the non-tRNA pol III-transcribed genes. Asterisks mark the US and DS peaks of Spt16 on the genes. Occupancy is given as the normalized Tag Counts per million reads. **(F)** Occupancy in a window of -600 bp to +1000 bp with respect to the *SNR6* TSS. Similar to tRNA genes, either mock- or input- normalized Spt16 profiles are indistinguishable. **(G)** Mock-normalized Spt16 occupancy on the non-tRNA pol III-transcribed *SCR1* and *RPR1* genes, in a window of -900 bp to +1000 bp with respect to the gene TSS. **(H)** and **(I)** Mock-normalized occupancy profiles (panel G) differ from the Input-normalized profiles (given under the Fig. 2D), due to the striking differences in the mock and input read counts profiles near both the genes, specially in the regions demarcated with yellow lines for **(H)** *SCR1* and **(I)** *RPR1* genes.

Figure S3. The *spt16-197* mutation does not affect tRNA levels

(A) Spt16 occupancy on pol II-transcribed genes correlates with transcription activity. Pol II ORFs were divided into five categories (color code on top) based on transcript abundance (Holstege et al. 1998). Averaged Spt16 occupancy (this study) within 1 kb upstream and 1 kb downstream of the TSS of the five gene categories is shown. **(B)** and **(C)** Spt16 occupancy on the tRNA genes (this study) and pol III bound to their nascent transcripts (Jordán-Pla et al., 2015) are not correlated on (B) individual genes or (C) the tRNA genes grouped families-wise. **(D)** Quantifications of total RNA isolated from the wild type and *spt16-197* mutant cells was performed as detailed under the Methods section. RNA was visualized on the agarose gel by ethidium bromide staining and band intensities in the gel images were quantified using the Bio-Rad Image Lab software version 6.0. The 5S rRNA levels were used as normalizer.

The averages with scatter from minimum three biological replicates are plotted. * $p=0.0003$. **(E)** Pol III (Rpc160-Myc) occupancy was measured in the *spt16-197* cells by the ChIP-Real Time PCR method. Occupancies at multiple genes were first calculated by the % Input method and then normalized with the occupancy at the TELVIR region. None of the occupancies showed a statistically significant change with growth at the non-permissive temperature. **(F)** Western analysis of the Rpc160-myc protein in the wild type and *spt16-197* mutant cells. One typical western blot is shown. Whole cell lysates from both cell types, grown at 30°C or 37°C were prepared multiple times. TCA precipitated, equivalent total proteins per lane were resolved by SDS-PAGE, western transferred and probed with anti-Myc and anti-Pgk1 antibodies. Myc band is barely visible in the lanes 3 and 4, probably because of its masking due to some unknown effect of the *spt16-197* mutation. The band in the lane M represents the 180 kDa size marker. **(G)** Five fold serial dilutions of the freshly grown cultures of the *spt16-197* cells at 0.2 OD_{600nm}/ml were spotted on YEPD plates and incubated for 2 days at 30°C before photographing. Growth of cells carrying the myc-tagged (T) Rpc160 gene is not different from the untagged (U) cells.

Figure S4. Spt16 protein total levels and Nucleosome occupancies on the pol III-transcribed tRNA and non-tRNA genes.

(A) Exposure to higher temperature does not affect the nucleosome occupancy in the DS region of the tRNA genes. Measurements by ChIP and Real Time PCR method for the histone H3 are shown. The dots represent non-significant changes. Comparison of relative H3 occupancies in the DS region of selected tRNA genes at 37°C, normalized with the respective H3 levels at 30°C in the *spt16-197* cells. The observed gene-specific changes are statistically not significant. All p values are >0.09 . **(B)** Total Spt16 protein levels in the wild type and *spt16-197* mutant cells were measured by western analyses. Whole cell lysates from both cell types, grown at 30°C or 37°C were prepared. TCA precipitated, total proteins were resolved by SDS-PAGE, western transferred and probed with anti-Spt16 and anti-Pgk1 antibodies. Two biological replicates of each condition are shown. **(C)** Quantifications from multiple blots similar to the one in the panel (B) were carried out using the Image Guage (Fuji) software. Averages with scatter for three or more biological replicates are plotted. The dot represents non-significant change ($p=0.067$) whereas the asterisk marks the significant change ($p=0.0059$). **(D)** Occupancies of Spt16 in the DS region of the tRNA genes in the wild type and mutant cells as measured by the ChIP and Real Time PCR (% Input) method

do not differ when grown at 30⁰C. **(E)** and **(F)** Low nucleosome levels are found on the body of the pol III-transcribed non-tRNA genes. Nucleosome occupancy from the ChIP-seq data (True et al. 2016) for occupancy of H3 (Tag counts per million reads) on the **(E)** *RPR1* gene and **(F)** *SCR1* gene, in the 600 bp upstream and 1 kb downstream regions, with respect to the TSS. Shaded bar represents the gene body.

Figure S5. The DS Spt16 levels on the non-tRNA genes reduce under starvation

Similar to tRNA genes, Spt16 occupancy (Tag counts per million reads) in the DS region reduces under starvation on the non-tRNA pol III-transcribed genes, as seen in our ChIP-seq data on the **(A)** *SNR6* gene **(B)** *RPR1* gene and **(C)** *SCR1* gene. Additionally, there is complete Spt16 loss in the *SCR1* US region and on the *RPR1* gene body (both marked with an asterisk) under the nutrient stress condition. The increase in Spt16 levels further upstream of -400 bp positions on the *SCR1* and *RPR1* genes may be related to genomic features upstream and not to these genes.

Figure S6. Spt16 enrichment requires normal pol III transcription on the genes.

Cells harboring 3XHA-tagged either the wild type (control) or mutated *RPC160* gene (*rpc160-112*) were used to measure the relative pol III and Spt16 occupancies. **(A)** Pol III occupancy in the DS region of the tRNA genes were measured by the ChIP and Real Time PCR method and enrichments were calculated by the % Input method. The p values are 0.017545, 0.085043, 0.002285, 0.041463 and 0.742805 for the **tE(UUC)EI*, *tL(CAA)A*, ***tN(GUU)L*, **tQ(CUG)M* and *tV(AAC)MI* genes respectively. Dots denote non-significant changes. **(B)** and **(C)** Total Rpc160 and Spt16 protein levels in the wild type (W) and *rpc160-112* mutant (M) cells were measured by western analyses. **(B)** Whole cell lysates from both type of cells grown at 28⁰C, were prepared. TCA precipitated total proteins were resolved by SDS-PAGE, western transferred and probed with anti-HA, anti-Spt16 and anti-Pgk1 antibodies. Three biological replicates of each are shown. **(C)** The quantifications of the Rpc160 and Spt16 normalized with Pgk1 levels. Bands on the western blots were quantified using the Bio-Rad Image Lab software version 6.0. Average and scatter from the three biological replicates on the same blot are plotted. *p=0.0367. **(D)** Relative Spt16 occupancy in the US and 5'-end region of the tRNA genes was measured by the ChIP and Real Time PCR method and enrichments were calculated by the % Input method. ORF-free, used as the negative control, represents the background levels. The p values are 0.002523, 0.006326, 0.000064, 0.000104 and 0.001724 for **tE(UUC)EI*, **tL(CAA)A*, ****tN(GUU)L*,

*****tQ(CUG)M*** and ****tV(AAC)MI*** genes respectively. **(E)** Comparison of ChIP-seq data (tag counts per million reads) for occupancies of pol III (Kumar and Bhargava, 2013) and Spt16 (this study) on the *SCR1* gene (shaded bar), in the 600 bp upstream and 1 kb downstream regions, with respect to the TSS. Green bars mark the positions of the five amplicons used in Real Time PCR measurements of occupancies. **(F)** Lower than wild type processivity of the mutant pol III. Pol III level at each gene body location in control and pol III mutant strains from the panel B was normalized to the respective 5'-most (A1) position. A5 was not included as it is not on the gene body. Ratios of the values obtained for pol III mutant strain at each position to that for the corresponding position in the control strain are plotted. **(G)** Comparison of ChIP-seq data (tag counts per million reads) for occupancies of pol III (Kumar and Bhargava, 2013) and Spt16 (this study) on the *RPR1* gene (shaded bar), in the 600 bp upstream and 1 kb downstream regions, with respect to the TSS. Green bars mark the positions of the four amplicons used in the Real Time PCR measurements of occupancies. Blue (pol III) and red (Spt16) asterisks mark the highest peaks, falling close to the amplicons B and C in the 3'-half of the *RPR1* gene.

Figure S7. Total Spt16 levels and occupancies on the tRNA genes increase under stress conditions.

(A) Spt16 (this study) and pol III (Kumar and Bhargava, 2013) ChIP-seq occupancies on the tRNA genes correlate weakly in the active or repressed (nutrient starvation) states. The R programming script was used to calculate the Pearson's coefficients of correlations and generate the heat map. **(B)** For the experiment under the Figure 7A/B, SJY25 cells carrying a myc-tagged *SPT16* gene were used. Total levels of the Spt16-Myc protein do not change in presence of the pol III inhibitor for up to 2 h. Cells were grown in the absence (DMSO) or presence of inhibitor (dissolved in DMSO) as described under the legends of the Figure 7A/B. Whole cell extracts were prepared as described under the methods section. Proteins were estimated in the whole cell extracts and equal proteins loaded per lane were resolved by the SDS-PAGE, western transferred and probed with anti-Myc antibody. Pgk1 was used as a loading control. M marks the lane having molecular size marker. **(C)** Quantifications using the blots similar to the panel B. Average and scatter of Spt16 protein values normalized against Pgk1 from minimum three biological replicates of each sample are plotted. **(D)** At the non-permissive temperature 37⁰C, total Spt16 protein levels increase in the wild type

($p < 0.0001$) and the *spt16-197* ($p < 0.0041$) cells. This graph represents the data from the Fig. S4C re-plotted differently.

V Supplementary Figures.

Figure S1

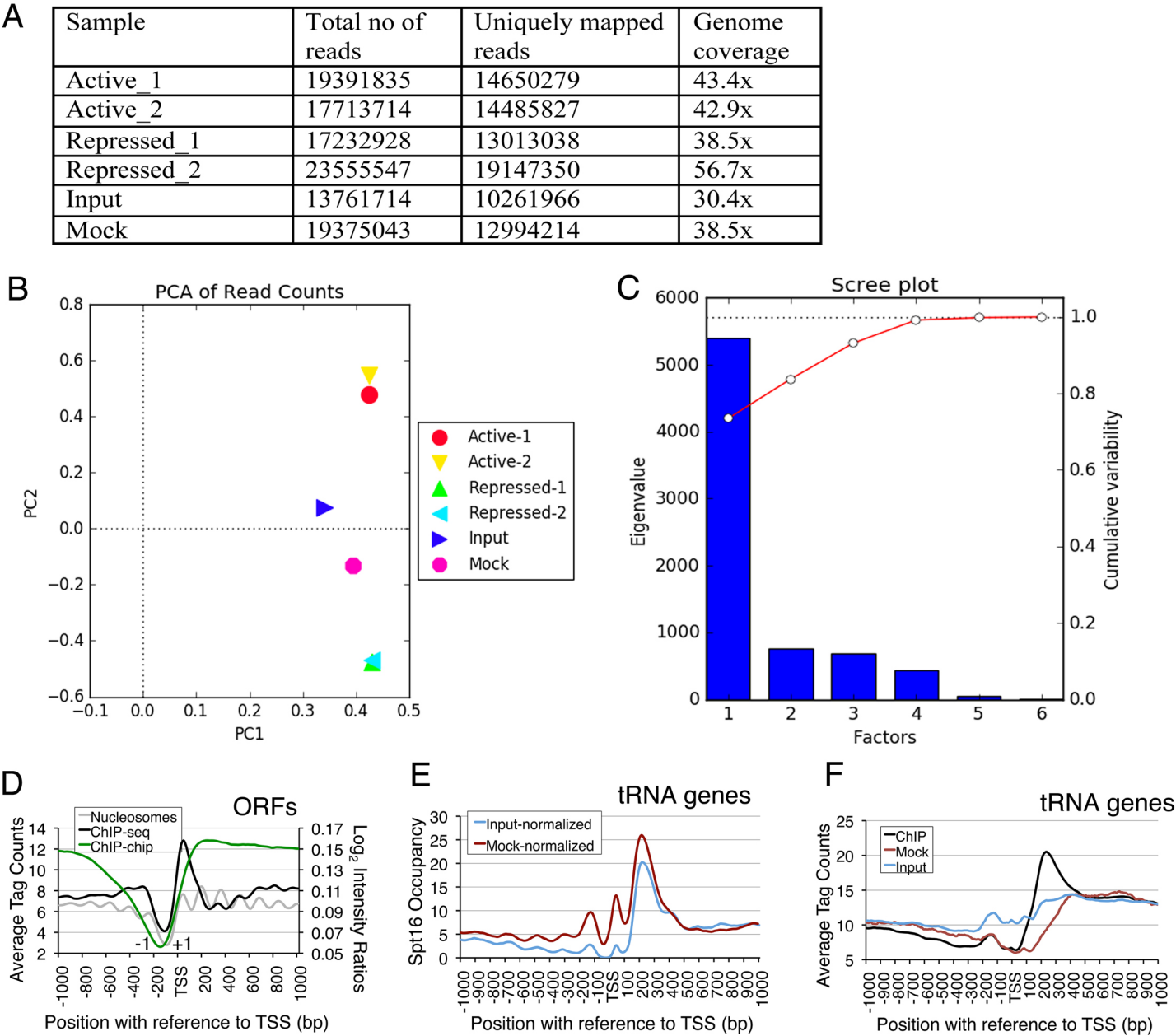


Figure S2

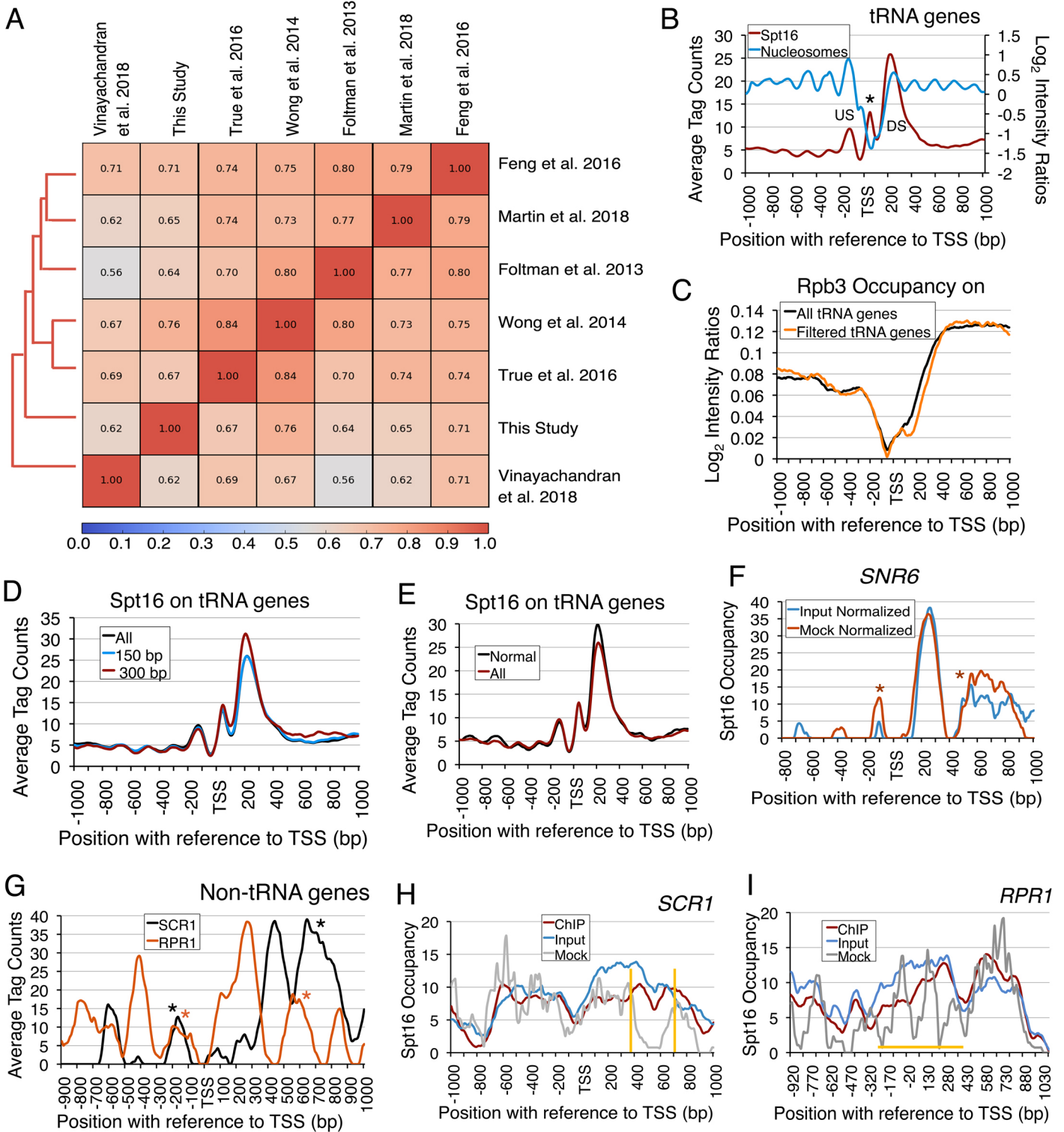


Figure S3

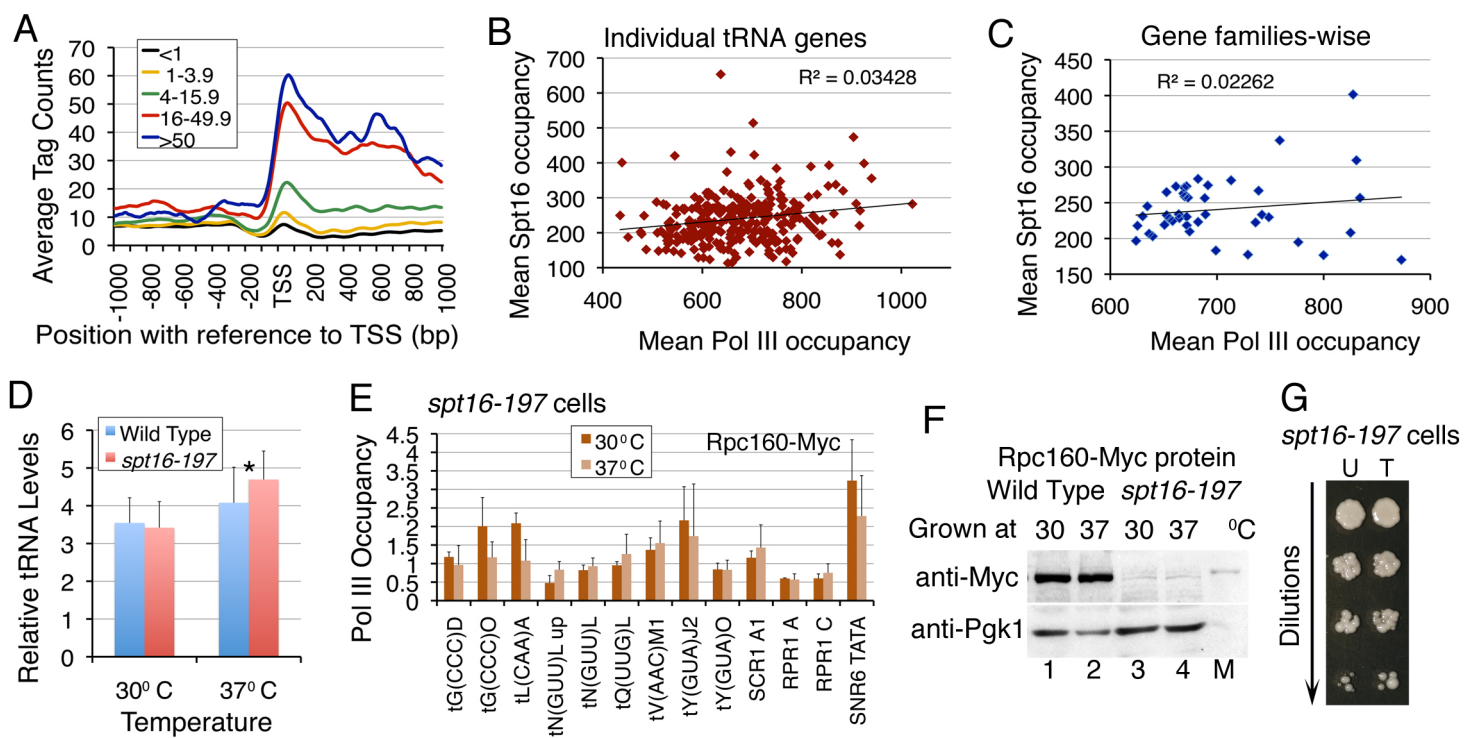


Figure S4

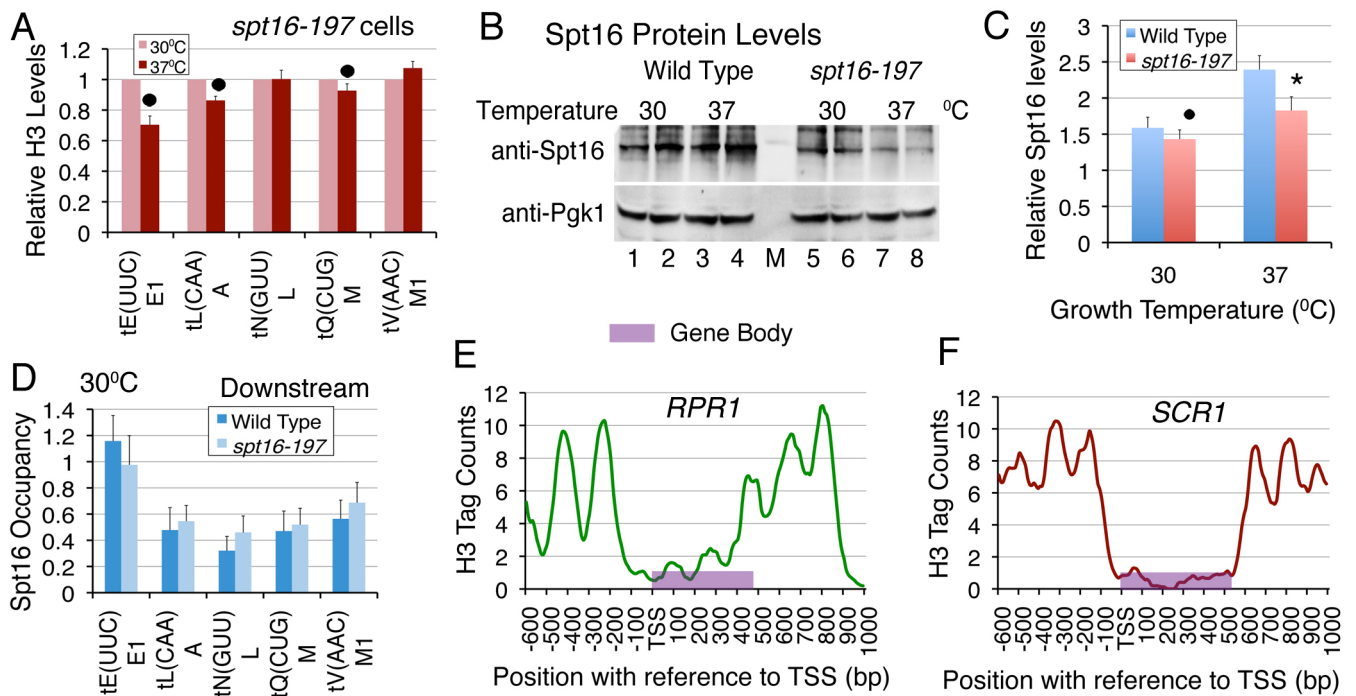


Figure S5

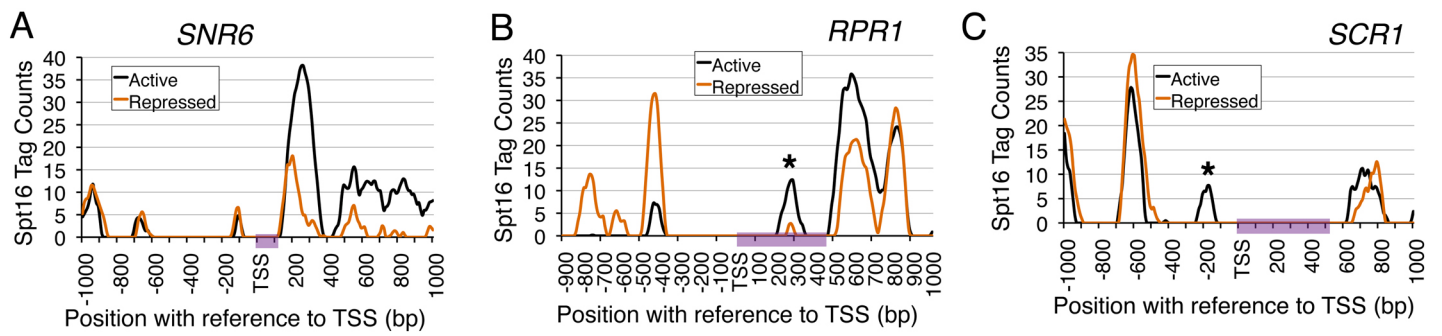


Figure S6

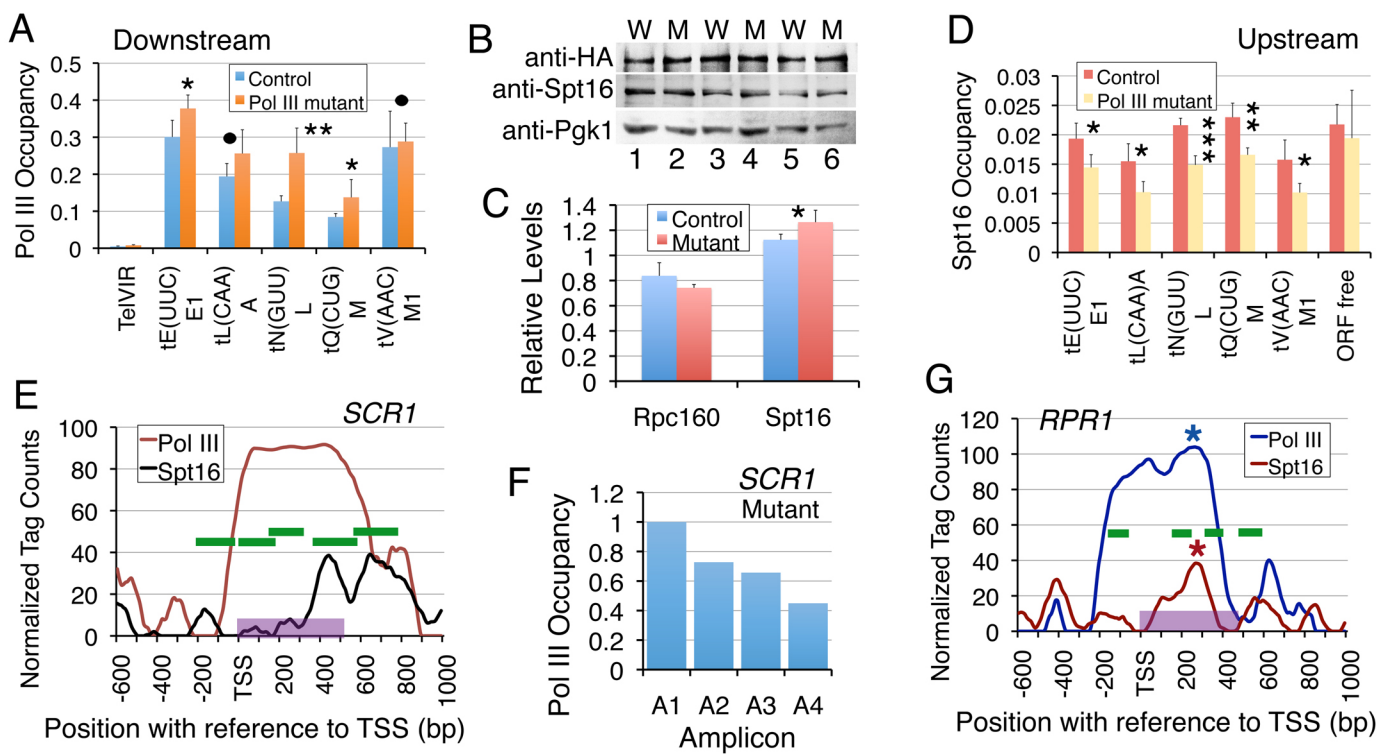


Figure S7

