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COLLEGE OF ENGINEERING

Dissertation

**THE TITLE IS WASDA**

by

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B.S., Johns Hopkins University, 2013  
M.S., Boston University, 2018

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requirements for the degree of  
Doctor of Philosophy

2019

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## Acknowledgments

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James Chuang

# **THE TITLE IS WASDA**

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## **ABSTRACT**

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# **Chapter 1**

## **Introduction**

### **1.1 A brief introduction to transcription**

In eukaryotic cells, transcription of protein-coding genes is carried out by the protein complex RNA polymerase II

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## **1.2 Transcription elongation factors Spt6 and Spt5**

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## **1.3 Reproducible data analysis for genomics**

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## Chapter 2

### Genomics of transcription elongation factor Spt6

#### 2.1 Collaborators

**Steve Doris** optimized TSS-seq and ChIP-nexus protocols  
generated TSS-seq and ChIP-nexus libraries

**Olga Viktorovskaya** generated MNase-seq libraries

**Magdalena Murawska** generated NET-seq libraries

**Dan Spatt** various experiments for publication

#### 2.2 Introduction to Spt6 and intragenic transcription

Studies in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have previously examined the requirement for Spt6 in normal transcription (Cheung et al., 2008; DeGennaro et al., 2013; Kaplan et al., 2003; Pathak et al., 2018; Uwimana et al., 2017; van Bakel et al., 2013). As Spt6 is essential for viability in *S. cerevisiae*,

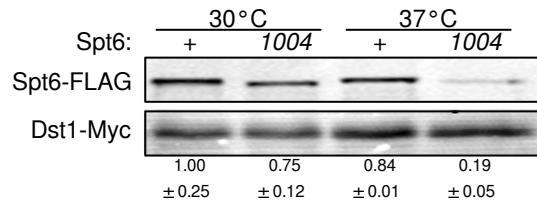
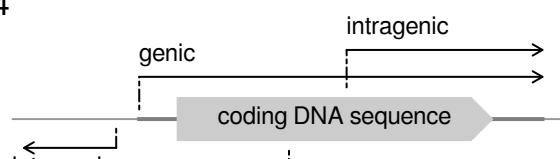


Figure 2.1: Western blot showing Spt6 protein levels in wild-type and *spt6-1004* cells, at 30°C and after 80 minutes at 37 °C. Immunoblotting was performed using  $\alpha$ -FLAG antibody to detect Spt6 and  $\alpha$ -Myc antibody to detect Dst1 from a spike-in strain. The quantification shown is the mean  $\pm$  standard deviation of three blots.



many of these studies use the same temperature-sensitive *spt6* mutant used in this project, ***spt6-1004***, which encodes an in-frame deletion of a helix-hairpin-helix domain within Spt6 (Kaplan et al., 2003). When *spt6-1004* cells are shifted from 30°C to 37°C for 80 minutes, bulk Spt6 protein levels are depleted to about 20% of wild-type levels (Figure 2.1). The most notable phenotype of the *spt6-1004* mutant is the appearance of **intragenic transcripts**, transcripts which appear to arise from within protein-coding sequences, in both sense and antisense orientations relative to the coding gene (Figure 2.2) (Cheung et al., 2008; DeGennaro et al., 2013; Kaplan et al., 2003; Uwimana et al., 2017).

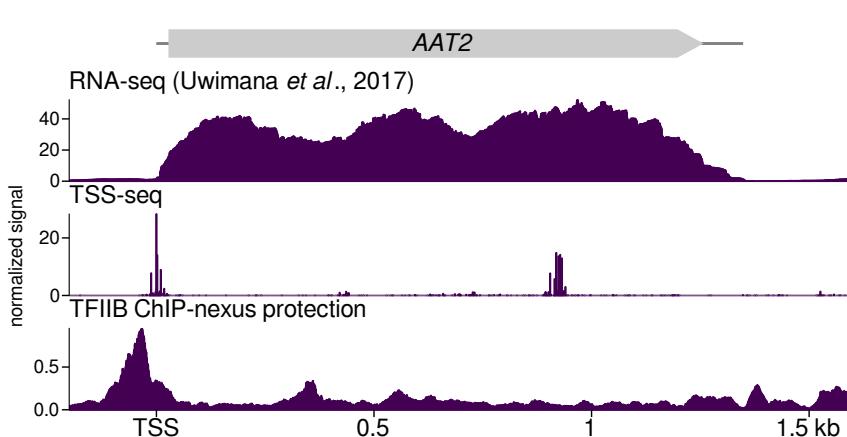


Figure 2.3: Sense strand RNA-seq signal, sense strand TSS-seq signal, and TFIIB ChIP-nexus protection at the *AAT2* gene, in *spt6-1004* after 80 minutes at 37°C.

Previous genome-wide measurements of transcript levels in *spt6-1004* relied on tiled microarrays (Cheung et al., 2008) and RNA sequencing (Uwimana et al., 2017). Studying intragenic transcription is difficult with these methods, since the signal for an

intragenic transcript in the same orientation as the gene it overlaps is convoluted with the signal from the full-length ‘genic’ transcript (Figure 2.3) (Cheung et al., 2008; Lickwar et al., 2009). Therefore, these methods can only discover intragenic transcripts which are highly expressed relative to the corresponding genic transcript, and are likely to find many false positives. Additionally, these methods are assays of steady-state RNA levels, which makes them unable to distinguish whether the intragenic transcripts observed in *spt6-1004* result from: A) new intragenic transcription initiation in the mutant, B) reduced decay of intragenic transcripts which are rapidly degraded in wild-type, or C) processing of full-length protein-coding RNAs.

To address these challenges to studying intragenic transcription, we applied two genomic assays to *spt6-1004*: transcription start-site sequencing (**TSS-seq**), and **ChIP-nexus of TFIIB**, a component of the RNA polymerase II pre-initiation complex (PIC). TSS-seq sequences the 5' end of capped and polyadenylated RNAs (Arribere and Gilbert, 2013; Malabat et al., 2015), allowing separation of intragenic from genic RNA signals and identification of intragenic transcript starts with single-nucleotide resolution (Figure 2.3). ChIP-nexus is a high-resolution chromatin immunoprecipitation technique, in which the immunoprecipitated DNA is exonuclease digested up to the bases crosslinked with the protein of interest before sequencing (He et al., 2015). When applied to the PIC component TFIIB, ChIP-nexus reports where transcription initiation is occurring, thus allowing us to determine if intragenic transcripts in *spt6-1004* result from new transcription initiation.

## **2.3 Data analysis pipelines for TSS-seq and ChIP-nexus**

### **2.3.1 TSS-seq peak calling**

### **2.3.2 A note on ChIP-nexus peak calling**

## **2.4 TSS-seq and TFIIB ChIP-nexus results for *spt6-1004***

To study the relationship between Spt6 and transcription, TSS-seq and TFIIB ChIP-nexus libraries were prepared from wild-type and *spt6-1004* cells, both shifted from 30°C to 37°C for 80 minutes. In wild-type cells, TSS-seq and TFIIB ChIP-nexus recapitulate their expected distributions over the genome: Most TSS signal is restricted to annotated genic TSSs, while most TFIIB signal is localized just upstream of the TSS (Figures 2.4, 2.5). In *spt6-1004*, the signal for both assays infiltrates gene bodies, already suggesting that new transcription initiation does contribute to the intragenic transcription phenotype. Notably, sense strand TSS-seq signal in *spt6-1004* tends to occur towards the 3' end of genes, while antisense strand TSS-seq signal tends to occur towards the 5' end of genes.

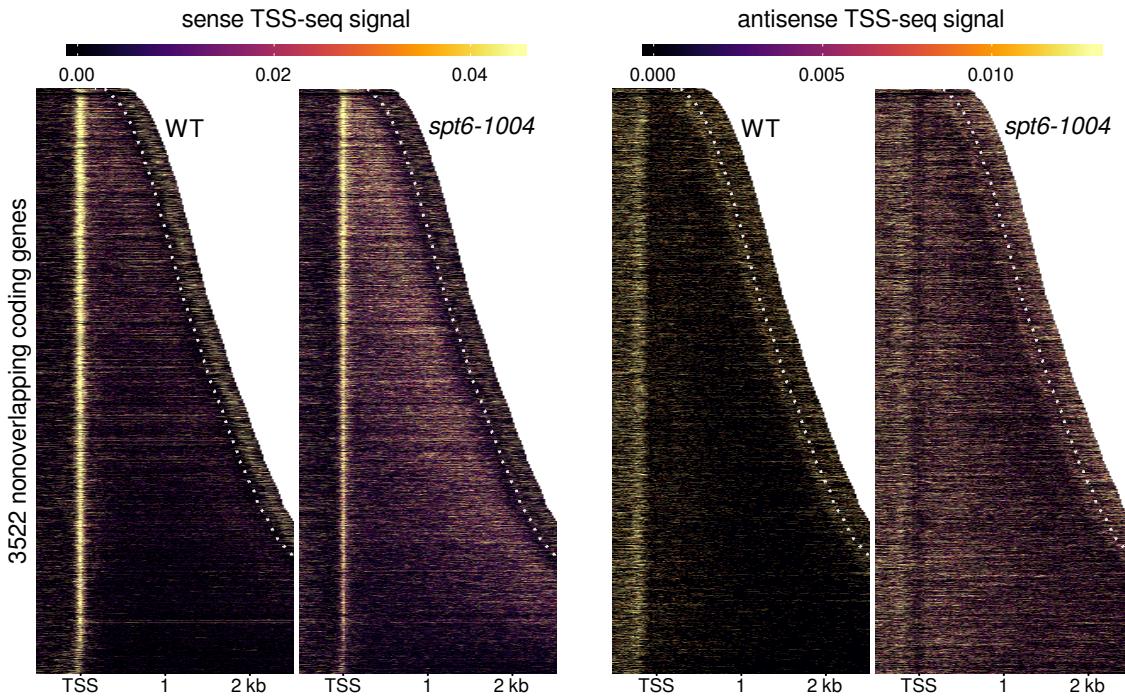


Figure 2.4: Heatmaps of sense and antisense TSS-seq signal from wild-type and *spt6-1004* cells, over 3522 non-overlapping genes aligned by wild-type genic TSS and sorted by annotated transcript length. Data are shown for each gene up to 300 nucleotides 3' of the cleavage and polyadenylation site (CPS), indicated by the white dotted line. Values are the mean of spike-in normalized coverage in non-overlapping 20 nucleotide bins, averaged over two replicates. Values above the 92nd percentile are set to the 92nd percentile for visualization.

The TSS-seq data were quantified by peak calling and differential expression analysis, and classified into genomic categories based on their position relative to coding genes. As suggested by the heatmap visualization (Figure 2.4), we detect significant induction of over 4000 intragenic and antisense TSSs in *spt6-1004* (Figure 2.6). Compared to previous studies identifying *spt6-1004* intragenic transcription by tiled microarray and RNA-seq, we identify intragenic transcription at over 1000 additional genes (Figure 2.7) and have the exact start sites of all identified TSSs.

The TSS-seq data also revealed an unexpected downregulation of most genic TSSs: In this experiment, we detected a significant downregulation to levels below 67% of wild-type levels at 75% (3579/4792) of genic TSSs (Figure 2.6). As a result of intra-genic/antisense induction and genic repression, expression levels in *spt6-1004* of all classes of transcripts become similar to one another (Figure 2.8).

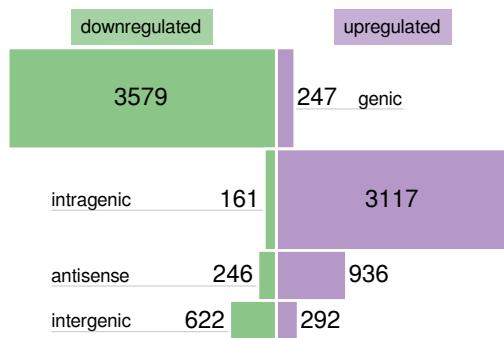


Figure 2.6: Bar plots of the number of TSS-seq peaks differentially expressed in *spt6-1004* after 80 minutes at 37°C versus wild-type after 80 minutes at 37°C. The height of each bar is proportional to the total number of peaks in the category, including those not found to be significantly differentially expressed.

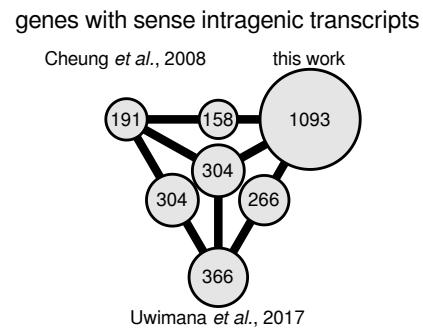


Figure 2.7: Set diagram of the number of genes reported to have *spt6-1004*-induced intragenic transcripts using tiled arrays (Cheung *et al.*, 2008), RNA-seq (Uwimana *et al.*, 2017), and TSS-seq (this work).

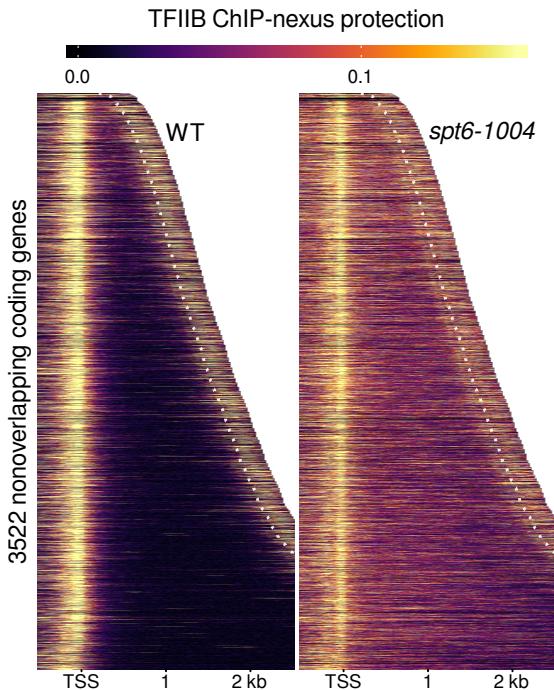


Figure 2.5: Heatmaps of TFIIB binding measured by ChIP-nexus, over the same regions shown in Figure 2.4. Values are the mean of library-size normalized coverage in non-overlapping 20 bp bins, averaged over two replicates. Values above the 85th percentile are set to the 85th percentile for visualization.

The changes in transcript levels in *spt6-1004* observed by TSS-seq correspond with substantial differences in the pattern of TFIIB binding on the genome. In contrast to the discrete peaks in promoter regions seen in wild-type, TFIIB in *spt6-1004* binds much more promiscu-

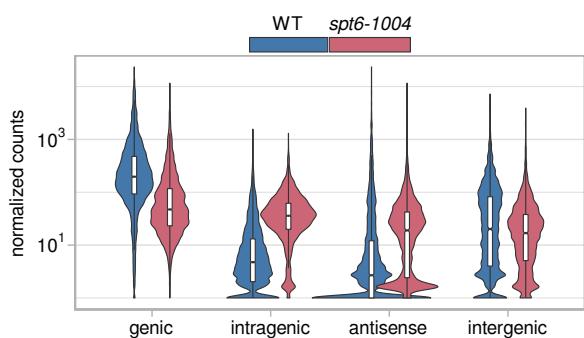


Figure 2.8: Violin plots of expression level distributions for genomic classes of TSS-<sup>10</sup>seq peaks in wild-type and *spt6-1004*, both after 80 minutes at 37°C. Normalized counts are the mean of spike-in size factor

ously, with many loci having TFIIB signal spread over broad regions of the genome (Figure 2.9). This difference in binding pattern makes peak calling ineffective for quantifying TFIIB signal in this case: ChIP-seq peak callers generally use different algorithms for calling ‘narrow’ peaks (e.g. for sequence-specific transcription factors) and ‘broad’ peaks (e.g. for histone modifications), meaning that a single algorithm is unable to call peaks that are meaningful for differential binding analyses between wild-type and *spt6-1004*. Therefore, to see if changes in transcript levels in *spt6-1004* correspond to changes in transcription initiation, we compared the change in TSS-seq signal at TSS-seq peaks in *spt6-1004* to the change in TFIIB ChIP-nexus signal in the window extending 200 bp upstream of the TSS-seq peak. Changes in TSS-seq signal in *spt6-1004* are associated with a change in TFIIB signal of the same sign at over 82% of TSSs of any genomic class, indicating that the increase in intragenic transcript levels and decrease in genic transcript levels observed in *spt6-1004* are in large part explained by changes in transcription initiation.

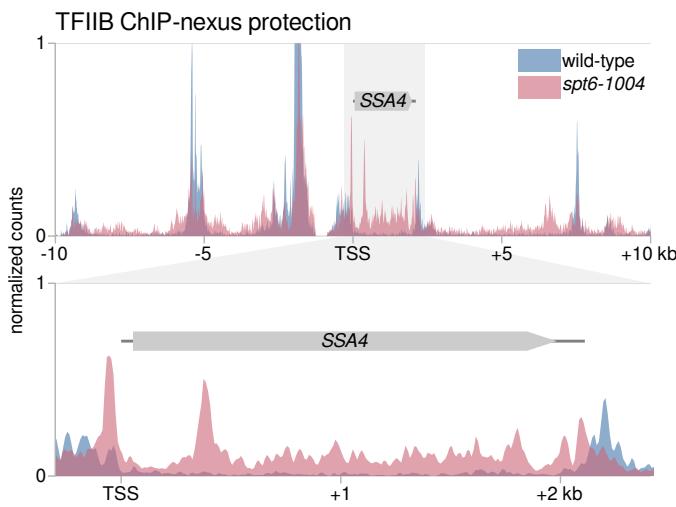


Figure 2.9:

- top) TFIIB ChIP-nexus protection in wild-type and *spt6-1004*, over 20 kb of chromosome II flanking the *SSA4* gene.
- bottom) Expanded view of TFIIB protection over the *SSA4* gene.

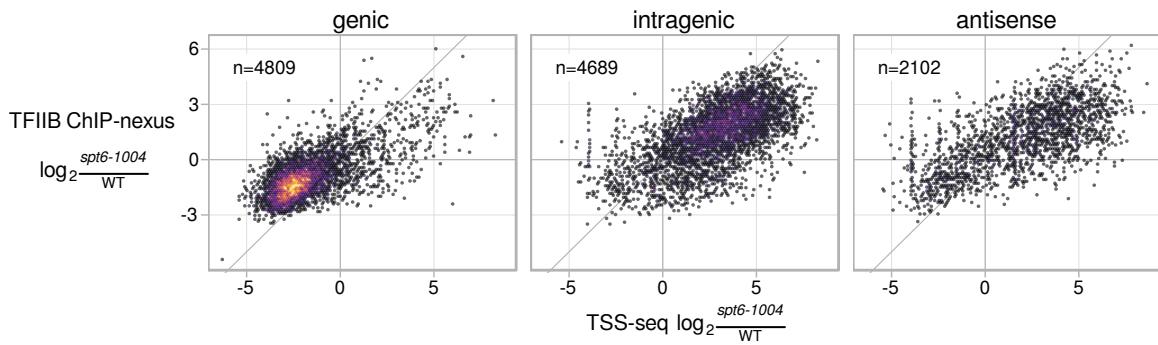


Figure 2.10: Scatterplots of fold-change in *spt6-1004* over wild-type, comparing TSS-seq and TFIIB ChIP-nexus. Each dot represents a TSS-seq peak paired with the window extending 200 bp upstream of the TSS-seq peak summit for quantification of TFIIB ChIP-nexus signal. Fold-changes are regularized fold-change estimates from DESeq2, with size factors determined from the *S. pombe* spike-in (TSS-seq), or *S. cerevisiae* counts (ChIP-nexus).

## 2.5 MNase-seq results from *spt6-1004*

Because a primary function of Spt6 is to act as histone chaperone that reassembles nucleosomes in the wake of transcription (Duina, 2011), it is reasonable to expect that the transcriptional changes seen in *spt6-1004* would be associated with changes in chromatin structure. The requirement for Spt6 in maintaining normal chromatin structure has been demonstrated in previous studies (Bortvin and Winston, 1996; Ivanovska et al., 2011; Jeronimo et al., 2015; Kaplan et al., 2003; Perales et al., 2013; van Bakel et al., 2013). To re-examine this requirement in higher resolution, we assayed nucleosome protection genome-wide using micrococcal nuclease digestion of chromatin followed by sequencing (MNase-seq).

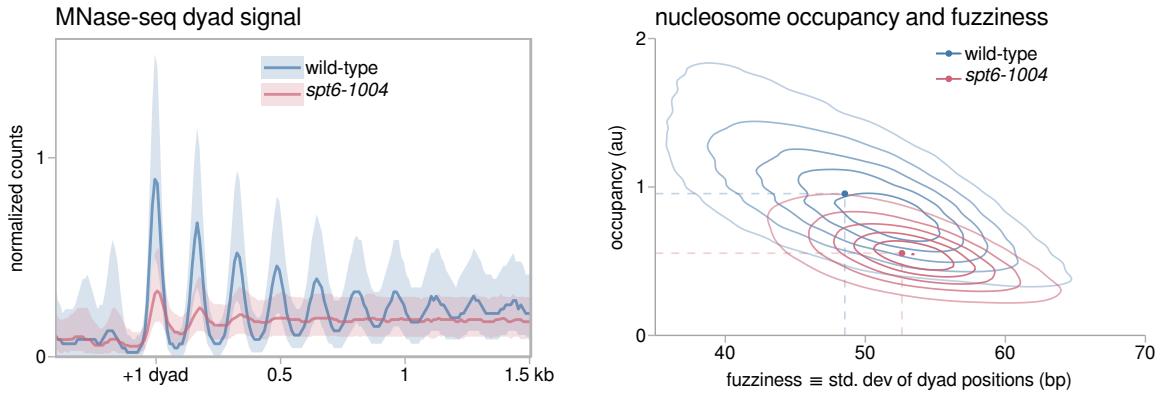


Figure 2.11: Average MNase-seq dyad signal in wild-type and *spt6-1004*, over 3522 non-overlapping genes aligned by wild-type +1 nucleosome dyad. Values are the mean of spike-in normalized coverage in non-overlapping 20 bp bins, averaged over two replicates (*spt6-1004*) or one experiment (wild-type). The solid line and shading are the median and the inter-quartile range.

Figure 2.12: Contour plot of the global distributions of nucleosome occupancy and fuzziness in wild-type and *spt6-1004*. Dashed lines indicate median values.

In wild-type, the MNase-seq data recapitulate the expected signature over genes, with a nucleosome-depleted region upstream of a strongly positioned ‘+1’ nucleosome, and a regularly phased array of nucleosomes over the gene body (Figure 2.11). In *spt6-1004*, nucleosome signal is severely reduced at canonical nucleosome positions and spreads into inter-nucleosome regions. Changes in aggregate nucleosome signal such as those observed in Figure 2.11 are the combination of changes to nucleosome occupancy (the number of reads assigned to a nucleosome), fuzziness (the standard deviation of read positions for a nucleosome), and position (the coordinate with the maximum reads for a nucleosome) (Chen et al., 2013). Using DANPOS2

(Chen et al., 2013), we called nucleosome positions and quantified these metrics for wild-type and *spt6-1004*. Wild-type nucleosomes span a relatively wide range of occupancy and fuzziness space, with highly occupied nucleosomes tending to be less fuzzy (i.e., more well-positioned) (Figure 2.12). In *spt6-1004*, the population of nucleosomes is much more homogeneous: nucleosome occupancy is decreased globally, and nucleosome fuzziness is restricted to the high end of the wild-type distribution.

Previous studies observed two trends: 1) In wild-type cells, nucleosome positioning is weaker over highly transcribed genes than over moderately transcribed genes (Shivaswamy et al., 2008), and 2) In *spt6-1004* cells, the decrease in nucleosome occupancy is greater for highly transcribed genes (Ivanovska et al., 2011). To re-examine these trends, we looked at the MNase-seq data in the context of NET-seq data, which reports the position of actively transcribing RNAPII and reflects a gene's level of transcription (Figure 2.13) (Churchman and Weissman, 2012). The data support the first trend: in wild-type, genes with the strongest NET-seq signal have decreased MNase-seq signal. However, there is no obvious relationship between transcription level and the nucleosome changes observed in *spt6-1004* (Figure 2.13). The apparent discrepancy might be explained by the improved resolution and breadth of MNase-seq versus the MNase and microarray of chromosome III used in the previous study (Ivanovska et al., 2011).

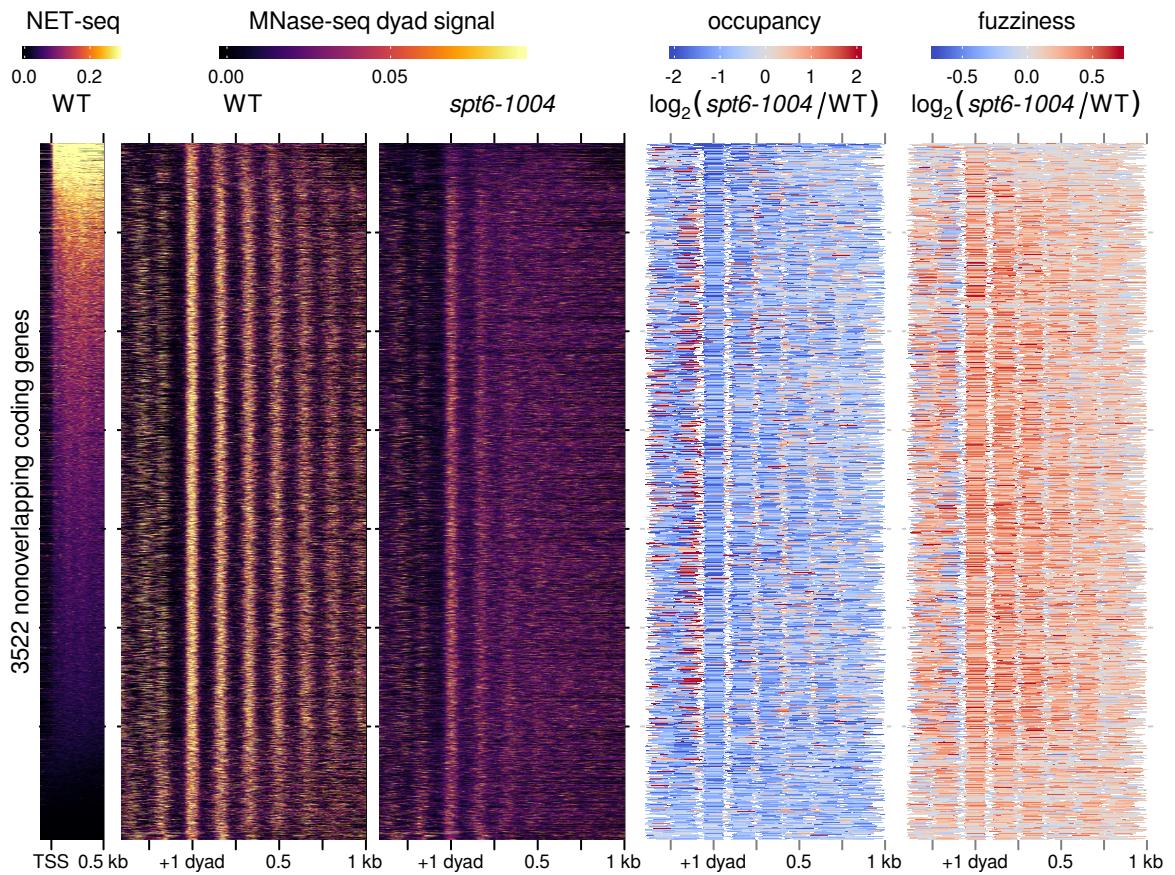
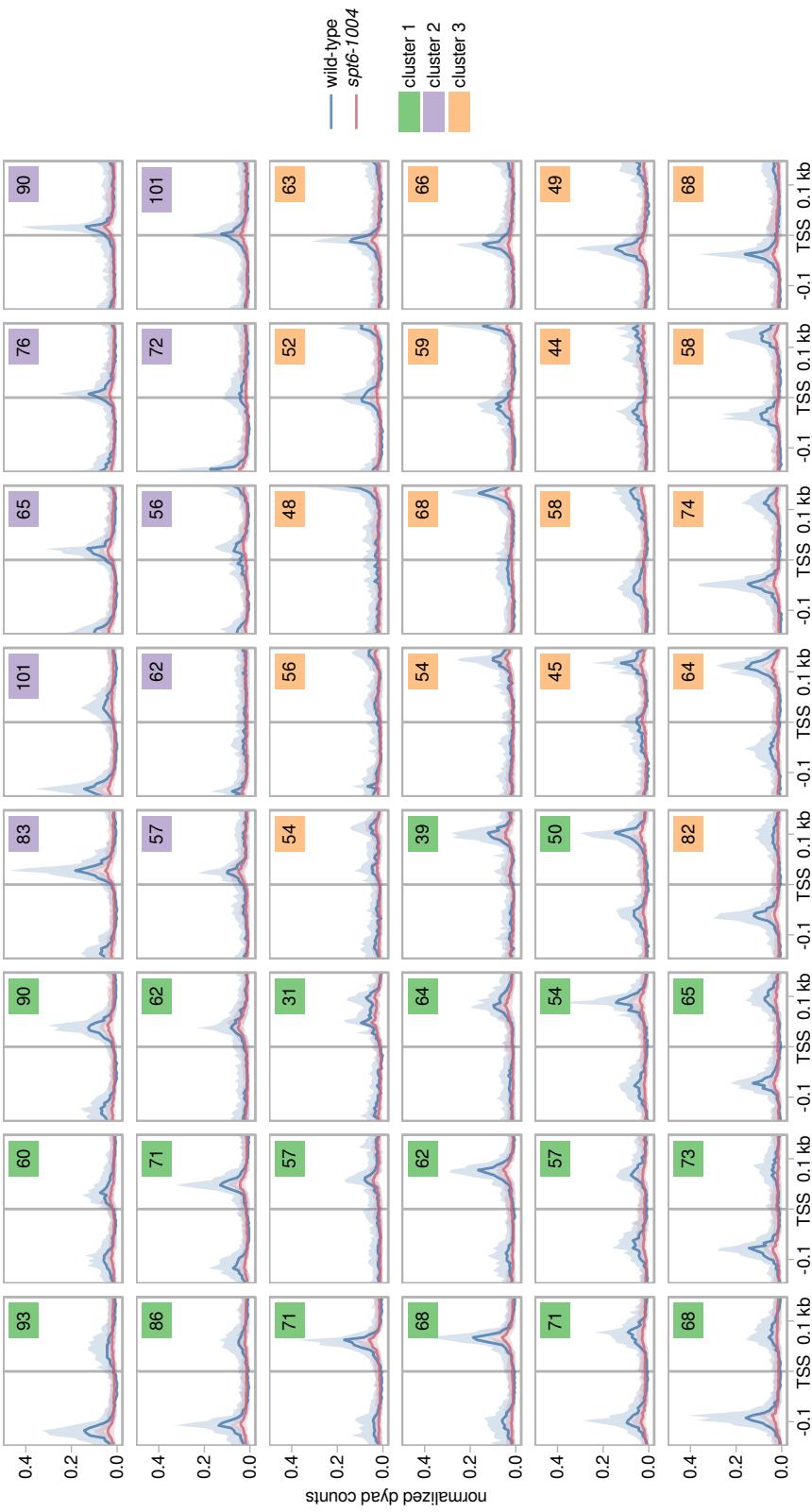


Figure 2.13:

- left) Heatmap of sense strand NET-seq signal for 3522 non-overlapping genes, aligned by genic TSS and sorted by total sense strand NET-seq signal in the window extending 500 nt downstream from the genic TSS. Values are the mean of library-size normalized coverage in non-overlapping 20 nt bins, averaged over two replicates.
- middle) Heatmaps of MNase-seq dyad signal in wild-type and *spt6-1004* for the same genes, aligned by wild-type +1 nucleosome dyad and arranged by sense NET-seq signal as in the leftmost panel. Values are the mean of spike-in normalized coverage in non-overlapping 20 bp bins, averaged over two replicates (*spt6-1004*) or one experiment (wild-type).
- right) Heatmaps of fold-change in nucleosome occupancy and fuzziness for the same genes, aligned by wild-type +1 nucleosome dyad and arranged by sense NET-seq signal as in the leftmost panel.

### 2.5.1 Clustering of MNase-seq profiles at *spt6-1004*-induced intragenic TSSs

The aggregate MNase-seq dyad signal around all *spt6-1004* intragenic TSSs is aperiodic (Figure 2.15, top left panel), which occurs as a result of destructive interference from offset nucleosome phasing patterns. To discover these phasing patterns, we used the wild-type and *spt6-1004* MNase-seq data flanking intragenic TSSs to train a self-organizing map to assign TSSs with similar MNase-seq patterns to nearby nodes in a rectangular grid (Figure 2.14). This allowed us to see that, although there is considerable diversity in the nucleosome pattern surrounding intragenic TSSs, most intragenic TSSs occur in areas between the positions of nucleosome dyads. By hierarchically clustering the nodes of the self-organizing map, we further grouped intragenic TSSs into three major clusters differing primarily by the phasing of the nucleosome array relative to the TSS, as shown in Figure 2.15.



**Figure 2.14: Average MNase-seq dyad signal around all *spt6-1004*-induced intragenic TSSs, grouped by assignment to nodes of a 6x8 super-organizing map (SOM).** The number of TSSs assigned to each node is shown in the upper right of each panel, and is shaded by the node's assignment to a cluster determined by agglomerative hierarchical clustering of the nodes. The solid line and shading are the median and inter-quartile range of the mean spike-in normalized coverage over two replicates (*spt6-1004*) or one experiment (wild-type), in non-overlapping 5 bp bins.

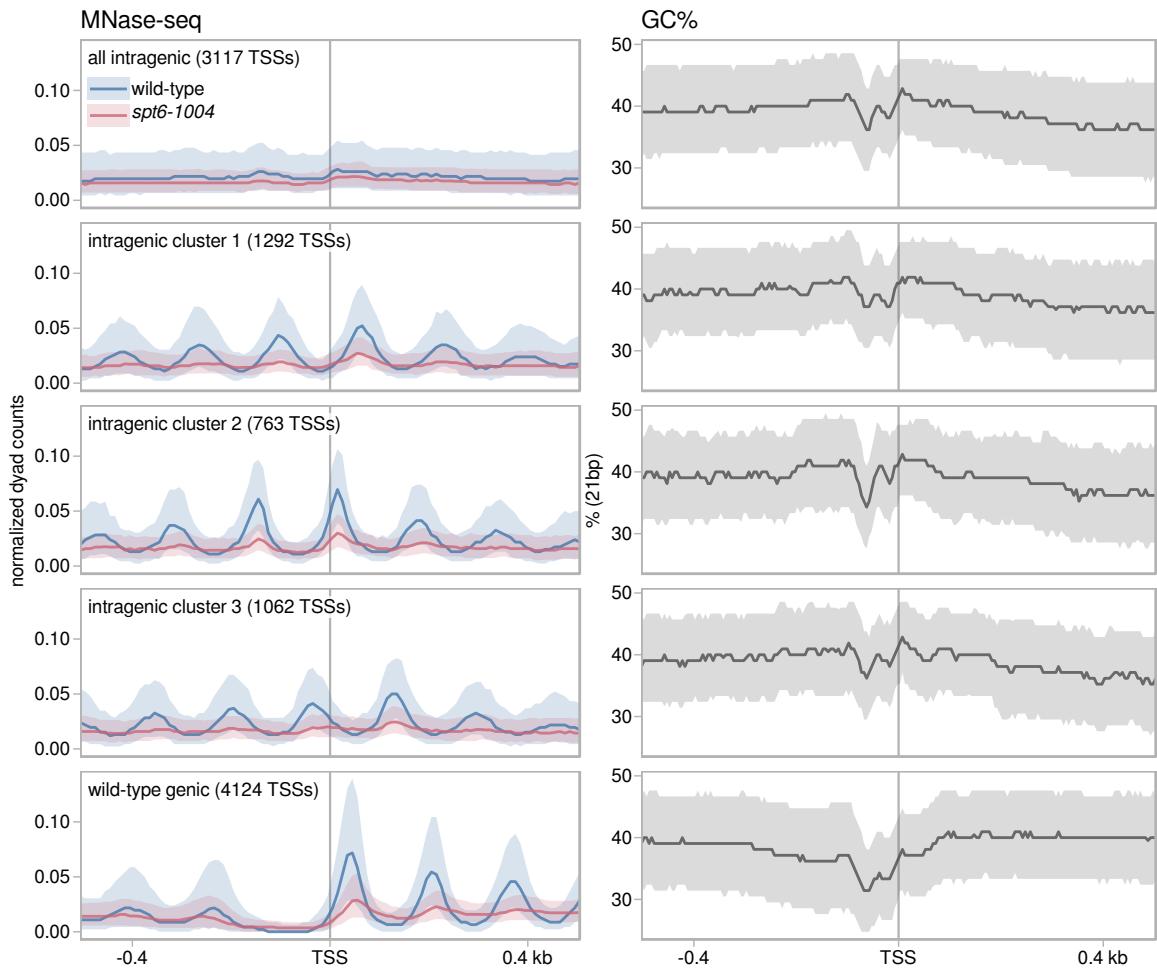


Figure 2.15:

- left column) Average MNase-seq dyad signal for *spt6-1004* intragenic TSSs, both aggregated and grouped into three clusters by the wild-type and *spt6-1004* MNase-seq dyad signal flanking the TSS, as well as all genic TSSs detected in wild-type. Values are the mean of spike-in normalized dyad coverage in non-overlapping 10 bp bins, averaged over two replicates (*spt6-1004*) or one experiment (wild-type). The solid line and shading are the median and inter-quartile range.
- right column) Average GC content of the DNA sequence in a 21 bp window, as above.

## 2.6 Other features of *spt6-1004* intragenic promoters

The resolution with which we were able to identify intragenic TSSs allowed us to closely examine their sequence features and compare them to genic TSSs.

### 2.6.1 Information content and sequence preference of intragenic TSSs

To examine the DNA sequence preference of intragenic and genic TSSs in *spt6-1004*, we aligned the sequences of all TSS-seq reads overlapping TSS-seq peaks of each class, and calculated the information content and sequence distribution for each class (Figure 2.16). Intragenic TSSs have a sequence preference almost identical to previously observed sequence preference of genic TSSs (Malabat et al., 2015), suggesting that RNA polymerase initiates transcription similarly at genic and intragenic TSSs, and that the lack of intragenic initiation in wild-type is due to inaccessibility of the initiation sequence, possibly due to histones.

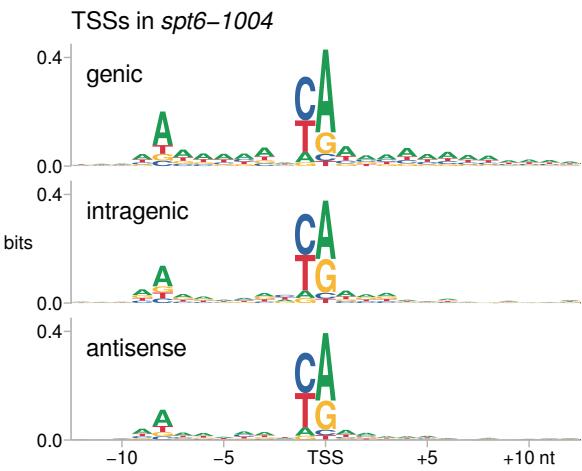


Figure 2.16: Sequence logos depicting information content and sequence preference of TSS-seq reads overlapping genic and intragenic TSS-seq peaks in *spt6-1004*.

### 2.6.2 Sequence motifs enriched at intragenic TSSs

To examine whether sequence-specific transcription factors contribute to the expression of intragenic transcripts in *spt6-1004*, we looked for enrichment or depletion of

the DNA sequence motifs associated with these factors upstream of intragenic TSSs. Exact matches to the TATA element consensus sequence TATAWAWR are enriched upstream between 100 and 150 nt upstream of intragenic TSSs, in the same position but to a lesser degree than the TATA enrichment observed upstream of genic TSSs (Figure 2.17). This further supports the model that *spt6-1004* intragenic promoters are sequences similar to canonical genic promoters, which become accessible for transcription initiation when the normal chromatin state is disturbed.

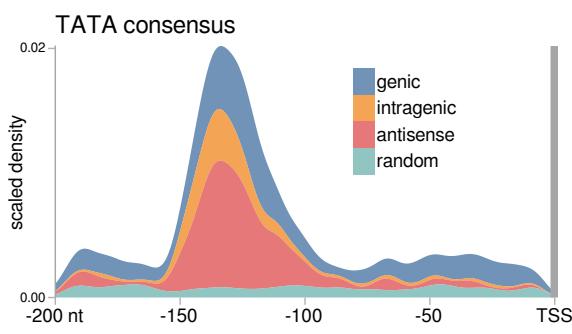


Figure 2.17: Scaled density of occurrences of exact matches to the motif TATAWAWR upstream of TSSs. For each category, a Gaussian kernel density estimate of the positions of motif occurrences is scaled by the number of motif occurrences per region.

## 2.7 Discussion

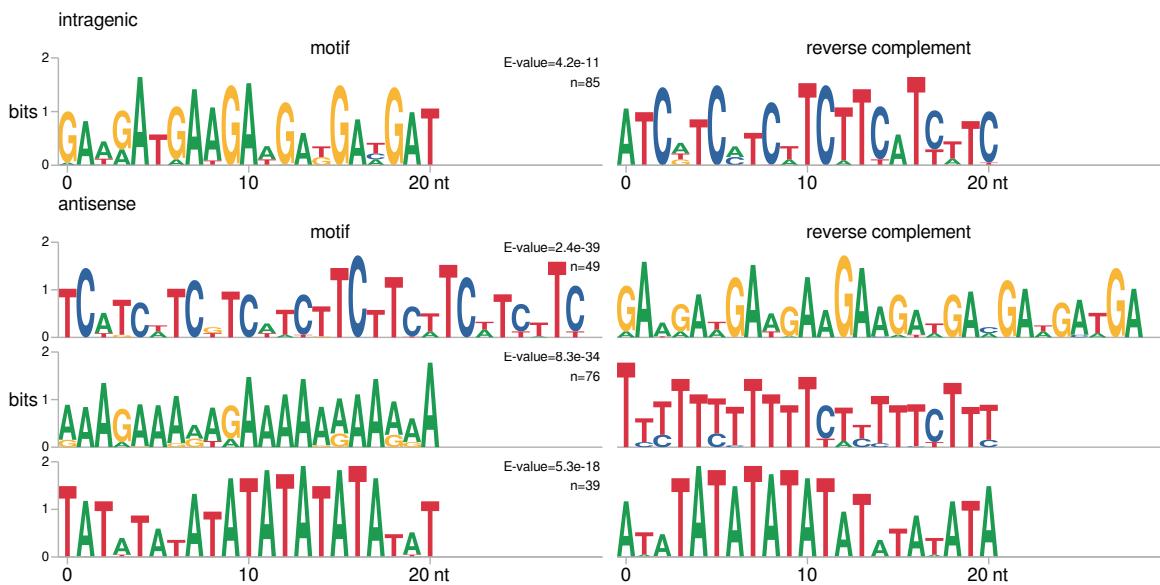


Figure 2.18: Sequence logos of motifs discovered by MEME (Bailey et al., 2015) in the window -100 to +30 bp relative to *spt6-1004* intragenic and antisense TSSs. The number of motif occurrences and the E-value, indicating the expected number of motif occurrences if the input sequences were scrambled, are shown for each motif.

## 2.8 Methods

### 2.8.1 Yeast strain construction and growth conditions

All yeast strains were constructed by standard yeast transformation or crosses. The *spt6-1004* and wild-type strains were grown as previously described (Cheung et al., 2008): Cells were grown in YPD at 30 °C to a concentration of approximately  $1 \times 10^7$  cells/ml ( $\text{OD}_{600} = 0.6$ ), at which point an equal volume of YPD medium pre-warmed to 44 °C was added, and the cultures were shifted to 37 °C for 80 minutes.

### 2.8.2 Sequencing library preparation (TSS-seq, ChIP-nexus, MNase-seq, NET-seq)

All library preparation methods are detailed in Doris et al. (2018).

### 2.8.3 Genome builds

The genome build used for *S. cerevisiae* was R64-2-1. The genome build used for *S. pombe* was ASM294v2.

### 2.8.4 TSS-seq data analysis

An up-to-date version of the Snakemake (Köster and Rahmann, 2012) workflow used to process TSS-seq libraries is maintained at [github.com/winston-lab/tss-seq](https://github.com/winston-lab/tss-seq). At the time of writing, removal of adapter sequences and random hexamer sequences from the 3' end of the read and 3' quality trimming were performed using cutadapt (Martin, 2011). The random hexamer molecular barcode on the 5' end of the read was then removed and processed using a custom Python script (adapted from ?). Reads were aligned to the combined *S. cerevisiae* and *S. pombe* reference genomes using Tophat2 (Kim et al., 2013) without a reference transcriptome, and uniquely mapping

reads were selected using SAMtools (?). Reads mapping to the same location as another read with the same molecular barcode were identified as PCR duplicates and removed using a custom Python script (adapted from ?). Coverage of the 5'-most base, corresponding to the TSS, was extracted using bedtools genomecov (Quinlan and Hall, 2010) and normalized to the total number of uniquely mapping, non-duplicate *S. pombe* reads. Quality statistics of raw, cleaned, non-aligning, and uniquely aligning non-duplicate reads were assessed using FastQC (?).

The pipeline additionally performs TSS-seq peak calling, differential expression, classification of peaks into genome categories, gene ontology analysis, motif enrichment analysis, *de novo* motif discovery, sequence logo visualization, and data visualization with the option to separate data into clusters of similar signal.

#### **2.8.4.1 Reannotation of *S. cerevisiae* TSSs using TSS-seq data**

TSS-seq coverage from two replicates of a wild-type *S. cerevisiae* strain grown at 30°C in YPD was averaged and used to adjust the 5' ends of an annotation of major transcript isoforms based on TIF-seq data (?). The 5' end of the original annotation was changed to the position of maximum TSS-seq signal in a window  $\pm$  250 nt of the original 5' end if the maximum TSS-seq signal was greater than the 95th percentile of all non-zero TSS-seq signal.

#### **2.8.4.2 TSS-seq peak calling**

TSS-seq data representing transcription from a single promoter tends to occur as a cluster of signal distributed over a range of positions, rather than a single nucleotide (Arribere and Gilbert, 2013; Malabat et al., 2015). It is reasonable to consider such a cluster of TSS-seq signal as a single entity, because the signals within the cluster

are usually highly correlated to one another across different conditions. Therefore, to identify TSSs from TSS-seq data and quantify them for downstream analyses such as differential expression, it is necessary to annotate these groups of TSS-seq signal by using the data to perform peak-calling.

At the time of writing, TSS-seq peak calling for a given experimental group was performed by 1-D watershed segmentation of the data for each sample in the group, followed by filtering for reproducibility within the group by the Irreproducible Discovery Rate (IDR) method (Li et al., 2011). First, a smoothed version of the TSS-seq coverage is generated for each sample using an adaptive two-stage kernel density estimation with a discretized Gaussian kernel (?). For a given nucleotide, the adaptive kernel bandwidth,  $\sigma_{\text{adaptive}}$ , is given by

$$\sigma_{\text{adaptive}} = \sigma_{\text{pilot}} \left( \frac{\rho_{\text{pilot}}}{g} \right)^{-\alpha},$$

where  $\sigma_{\text{pilot}}$  is the standard, fixed bandwidth of a Gaussian kernel used to calculate the pilot signal density  $\rho_{\text{pilot}}$  at that nucleotide,  $g$  is the geometric mean of  $\rho_{\text{pilot}}$  over the whole genome, and  $\alpha$  is a parameter in  $[0, 1]$  that determines the degree to which the pilot density  $\rho_{\text{pilot}}$  affects  $\sigma_{\text{adaptive}}$ . The adaptive kernel adjusts the kernel bandwidth to be smaller in regions of high signal density and larger in regions of lower signal density, allowing the smoother to better accommodate both ‘sharp’ TSSs where the signal is distributed over a relatively small window, as well as ‘broad’ TSSs where the signal is more dispersed. For all analyses in this document, adaptive smoothing was performed with  $\sigma_{\text{pilot}} = 10$  and  $\alpha = 0.2$ .

Following smoothing, an initial set of peaks is formed by assigning all nonzero signal in the original, unsmoothed coverage to the nearest local maximum of the smoothed coverage, and taking the minimum and maximum genomic coordinates of

the original coverage as the peak boundaries for each local maximum of the smoothed coverage. Peaks are then trimmed to the smallest genomic interval that includes 95% of the original coverage, and the probability of the peak begin generated by noise is estimated by a Poisson model where  $\lambda$ , the expected coverage, is the maximum of the expected coverage over the chromosome and the expected coverage in the 2 kb window upstream of the peak (à la the ChIP-seq peak caller MACS2 (Zhang et al., 2008)). Finally, peaks are ranked by their significance under the Poisson model, and a final list of peaks for the group is generated using the IDR method (IDR = 0.1) (Li et al., 2011). In brief, IDR compares ranked lists of regions in order to set a cutoff, beyond which the regions are no longer consistent between replicates.

The python script used for 1-D watershed segmentation of TSS-seq data is [available as part of the TSS-seq pipeline](#), and the IDR implementation used in the pipeline is also [available on GitHub](#).

#### 2.8.4.3 TSS differential expression analysis

For TSS-seq differential expression analysis, TSS-seq peak-calling was performed as described above for both *S. cerevisiae* and the *S. pombe* spike-in. The read counts for each peak in each condition were used as the input to differential expression analysis by DESeq2 (Love et al., 2014), with the alternative hypothesis  $|\log_2(\text{fold-change})| > 1.5$  and a false discovery rate of 0.1. To normalize by spike-in, the size factors of the *S. pombe* spike-in counts were used as the size factors for *S. cerevisiae*, although we note that due to the median of ratios normalization used in DESeq2, the major TSS-seq results of this work are still observed when *S. cerevisiae* size factors are used.

#### **2.8.4.4 Classification of TSS-seq peaks into genomic categories**

TSS-seq peaks were assigned to genomic categories based on their position relative to the transcript annotation described above and an annotation of all verified open reading frames (ORFs) and blocked reading frames in *S. cerevisiae* (??). First, ‘genic’ regions were defined as follows: If a gene was present in both the transcript and ORF annotations, the genic region was defined as the interval (annotated TSS - 30 nt, start codon). If gene was present in the transcript annotation but not the ORF annotation, the genic region was defined as the interval (annotated TSS - 30 nt, annotated TSS + 30 nt). If a gene was present only in the ORF annotation, the genic region was defined as the interval (start codon - 30 nt, start codon). For the purposes of peak classification, regions were considered overlapping if they had at least one base of overlap. TSS-seq peaks were classified as genic if they overlapped a genic region on the same strand. Peaks were classified as intragenic if they were not classified as a genic peak, and their summit position overlapped an open or closed reading frame on the same strand. Peaks were classified as antisense if their summit position overlapped a transcript on the opposite strand. Finally, peaks were classified as intergenic if they did not overlap a transcript, reading frame, or genic region on either strand.

#### **2.8.4.5 TSS information content and sequence composition**

TSS-seq alignments were pooled for all replicates in a condition, and the DNA sequence flanking the position of every read overlapping TSS-seq peaks of a particular genomic category was extracted using SAMtools (?) and bedtools (Quinlan and Hall, 2010). The information content and sequence composition was quantified using WebLogo (?), with the zeroth-order Markov model of the *S. cerevisiae* genomic se-

quence as the background composition. Sequence logos were plotting using helper functions from ggseqlogo (?).

### 2.8.5 ChIP-nexus data analysis

An up-to-date version of the Snakemake (Köster and Rahmann, 2012) workflow used to process ChIP-nexus libraries is maintained at [github.com/winston-lab/chip-nexus](https://github.com/winston-lab/chip-nexus). At the time of writing, filtering for reads containing the constant region of the adapter on the 5' end of the read, 3' adapter removal, and 3' quality trimming were performed using cutadapt (Martin, 2011). The random pentamer molecular barcode on the 5' end of the read was then removed and processed using a custom Python script modified from ?. Reads were aligned to the combined *S. cerevisiae* and *S. pombe* genomes using Bowtie2 (Langmead and Salzberg, 2012), and uniquely mapping reads were selected using SAMtools (?). Reads mapping to the same location as another read with the same molecular barcode were identified as PCR duplicates and removed using a custom Python script modified from ?. Coverage of the 5'-most base, corresponding to the point of crosslinking, was extracted using bedtools genomecov (Quinlan and Hall, 2010). The median fragment size estimated by MACS2 (Zhang et al., 2008) over all samples was used to generate coverage of factor protection and fragment midpoints, by extending reads to the fragment size, or by shifting reads by half the fragment size, respectively. Coverage was normalized to the total number of reads uniquely mapping to *S. cerevisiae*. Quality statistics of raw, cleaned, non-aligning, and uniquely aligning non-duplicate reads were assessed using FastQC ?.

### **2.8.5.1 A note on ChIP-nexus peak calling**

### **2.8.5.2 TFIIB ChIP-nexus differential binding analysis**

For TFIIB ChIP-nexus differential binding analysis, TFIIB peaks were called by MACS2 and IDR filtering as described above. A non-redundant list of peaks called in the condition and control groups being compared was generated using bedtools multiinter (Quinlan and Hall, 2010), and the counts of fragment midpoints for each peak in each sample were used as the input to differential binding analysis by DESeq2 (Love et al., 2014), with the alternative hypothesis  $|\log_2(\text{fold-change})| > 1.5$  and a false discovery rate of 0.1. For estimation of change in TFIIB binding upstream of TSS-seq peaks, TFIIB fragment midpoint counts in the window extending 200 bp upstream of the TSS-seq peak summit were used as the input to DESeq2. *S. cerevisiae* counts were used for size factor calculation.

### **2.8.5.3 Classification of TFIIB ChIP-nexus peaks into genomic categories**

As for TSS-seq peaks, TFIIB ChIP-nexus peaks were assigned to genomic categories based on their position relative to the transcript annotation described above, an annotation of all verified open reading frames (ORFs) and blocked reading frames (??), and an annotation of ‘genic’ regions derived from the transcript and ORF annotations. TFIIB ChIP-nexus peaks were classified as genic if they overlapped a genic region. Peaks were classified as intragenic if they were not classified as a genic peak, and the entire peak overlapped an open or closed reading frame. Finally, peaks were classified as intergenic if they did not overlap a transcript, reading frame, or genic region.

## **2.8.6 MNase-seq data analysis**

### **2.8.6.1 Nucleosome quantification**

### **2.8.6.2 Clustering of MNase-seq signal at *spt6-1004* intragenic TSSs**

## **2.8.7 Motif enrichment**

## **2.8.8 *De novo* motif discover motif discovery**

## 2.9 Bibliography

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- Zhang, Y., Liu, T., Meyer, C. A., Eeckhoute, J., Johnson, D. S., Bernstein, B. E., Nusbaum, C., Myers, R. M., Brown, M., Li, W., and Liu, X. S. (2008). Model-based analysis of chip-seq (macs). *Genome Biology*, 9(9):R137. 2.8.4.2, 2.8.5

## Chapter 3

### Genomics of transcription elongation factor Spt5

#### 3.1 Collaborators

**Ameet Shetty** generated TSS-seq, MNase-seq, NET-seq, RNA-seq, and ChIP-seq libraries

#### 3.2 Introduction to Spt5 and prior work

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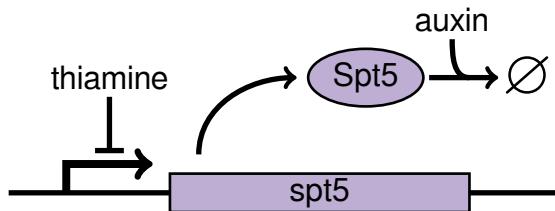


Figure 3.1: Diagram of the dual-shutoff system used to deplete Spt5 from *S. pombe*. Spt5 is expressed from a thiamine-repressible promoter, and tagged with an auxin-inducible degron tag for specific degradation upon addition of auxin.

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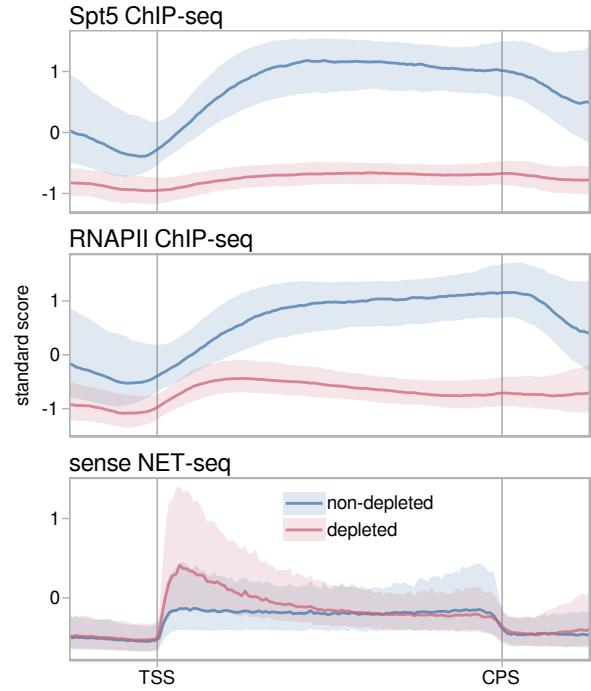
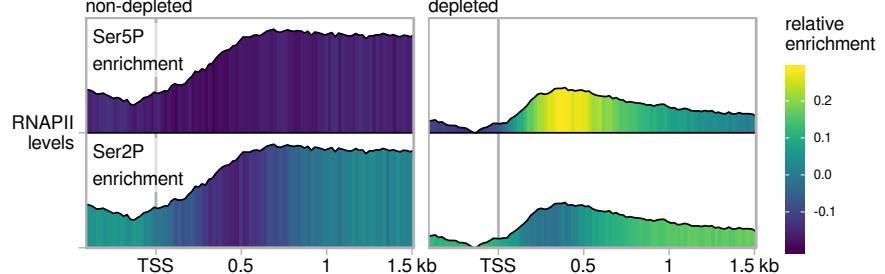


Figure 3.2: Average Spt5 ChIP-seq, RNAPII ChIP-seq, and sense NET-seq signal in Spt5 non-depleted and depleted cells, over 1989 non-overlapping coding genes scaled from TSS to CPS. The solid



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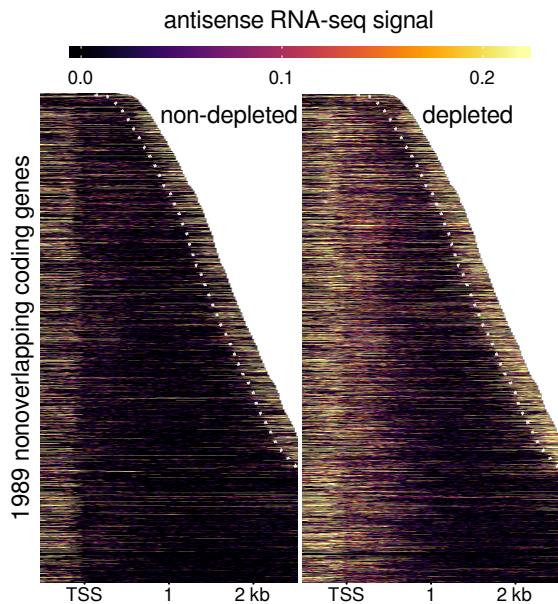


Figure 3.4: Caption wsdasdr zzzz.

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### 3.3 An aside on spike-in normalization for ChIP-seq

### 3.4 TSS-seq results from Spt5 depletion

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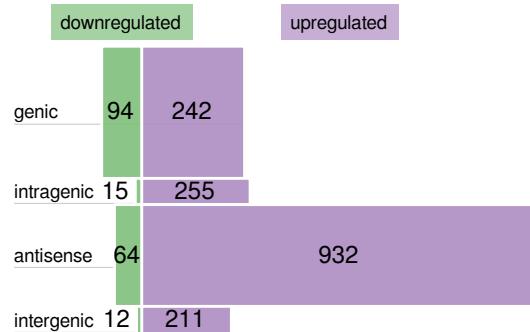


Figure 3.5: Caption wsdasdr zzzz.

Figure 3.6: Caption wsdasdr zzzz.

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### 3.5 MNase-seq results from Spt5 depletion

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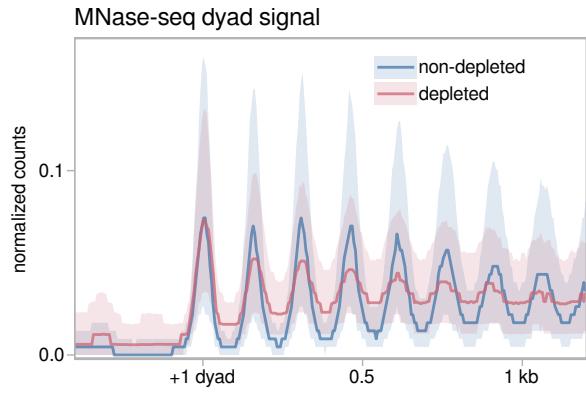


Figure 3.7: Caption wsdasdr zzzz.

Figure 3.8: Caption wsdasdr zzzz.

Figure 3.9: Caption wsdasdr zzzz.

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### **3.5.1 MNase-seq profile at Spt5-depletion-induced antisense TSSs**

## **3.6 Sequence motifs enriched at antisense TSSs**

## **3.7 Discussion**

## **3.8 Methods**

### **3.9 Bibliography**

## **Chapter 4**

### **Stress-responsive intragenic transcription**

#### **4.1 Collaborators**

**Steve Doris** generated TSS-seq and ChIP-nexus libraries

**Dan Spatt** polyribosome fractionation, fitness competitions,  
and other experiments

**James Warner** fitness competitions and other experiments

#### **4.2 Possible functions for intragenic transcription in wild-type cells**

#### **4.3 Discovery of stress-induced intragenic promoters by TFIIB ChIP-nexus and TSS-seq**

#### **4.4 Chromatin landscape of oxidative-stress-induced promoters.**

#### **4.5 Polysome enrichment of oxidative-stress-induced intragenic transcripts**

#### **4.6 TSS-seq analysis of oxidative stress in *Saccharomyces sensu stricto* species**

#### **4.7 Functions of intragenic DSK2 expression in oxidative stress**

#### **4.8 Discussion**

#### **4.9 Methods**

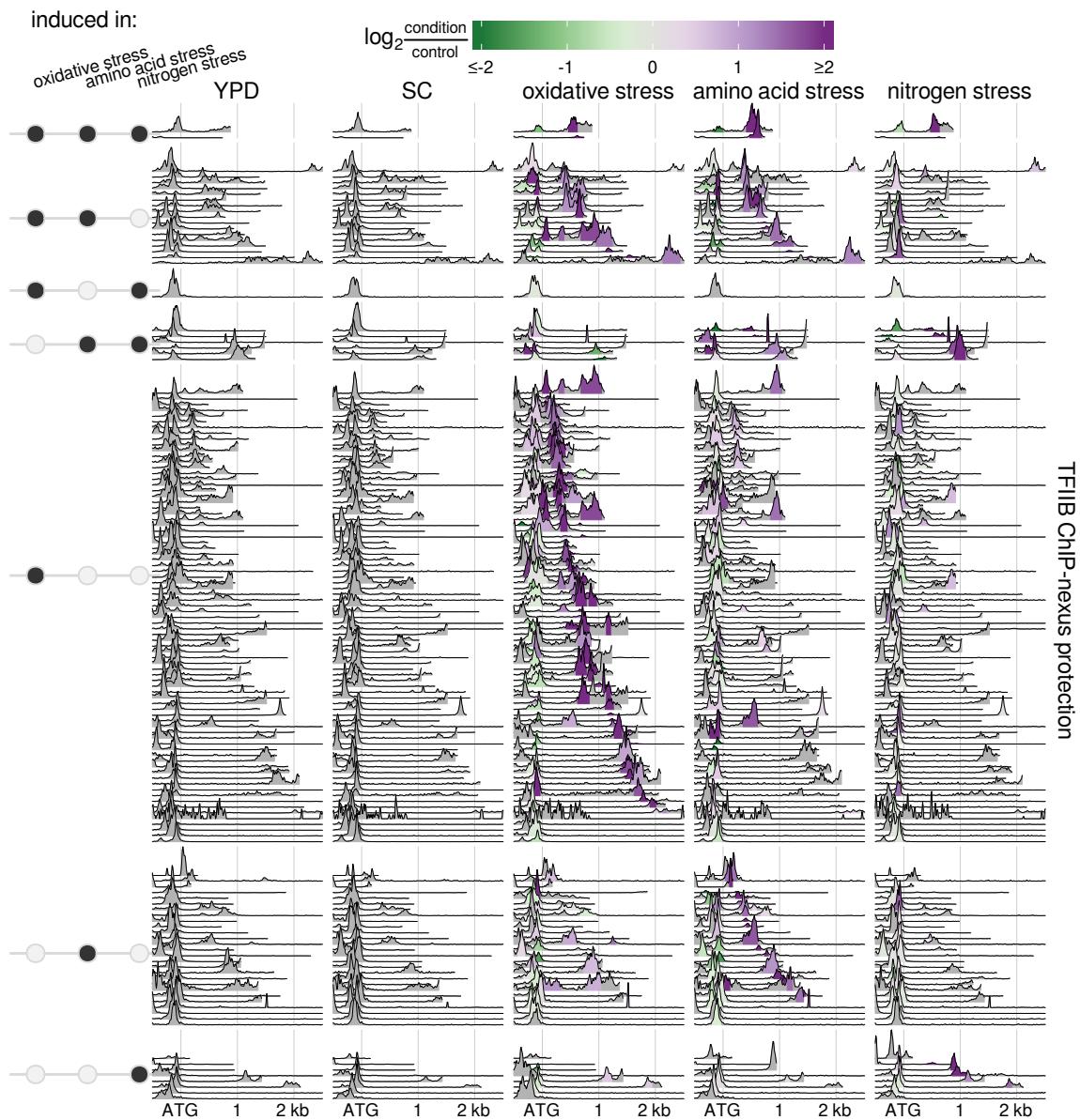


Figure 4.1: Relative TFIIB ChIP-nexus protection over all genes with an intragenic TFIIB peak significantly induced in one or more of the stress conditions tested, as depicted in the left panel. Genes are aligned by start codon, and are sorted within each group by the distance from the start codon to the summit of the induced intragenic TFIIB peak. Data are shown for each gene up to the stop codon of the gene. Regions where TFIIB peaks are called are shaded in the stress conditions according to the fold-change of the peak relative to the corresponding control condition.

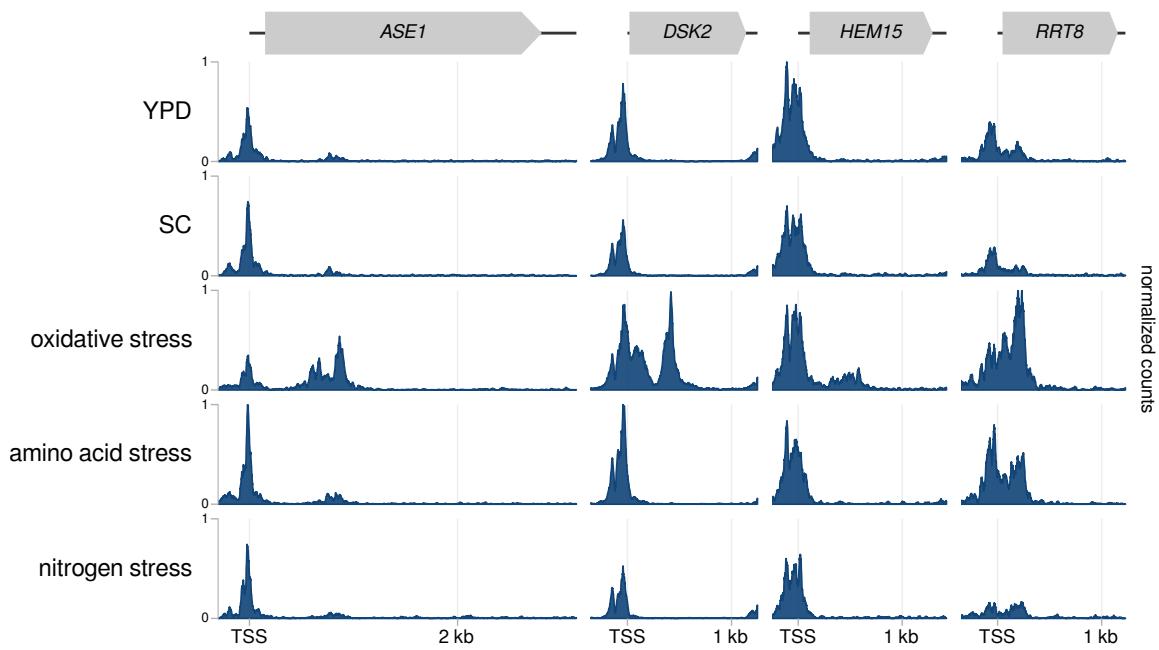


Figure 4.2: Caption asdflkj asldkfjlkj.

## 4.10 Bibliography

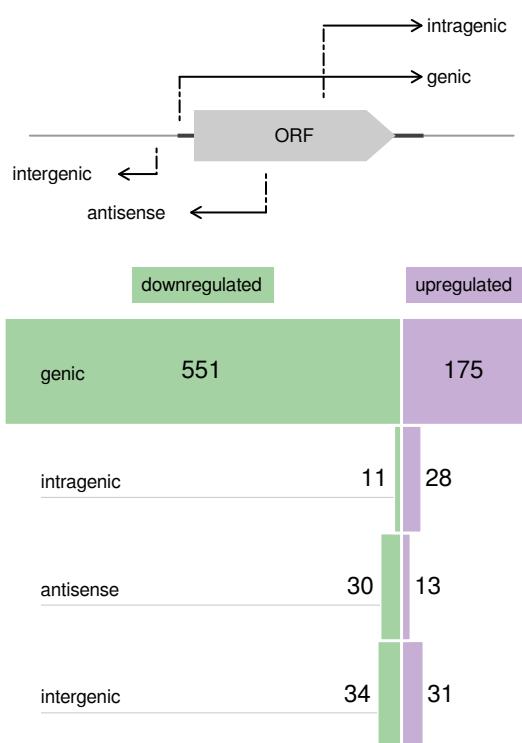


Figure 4.3: Caption dsafklj asldkfjlkj.

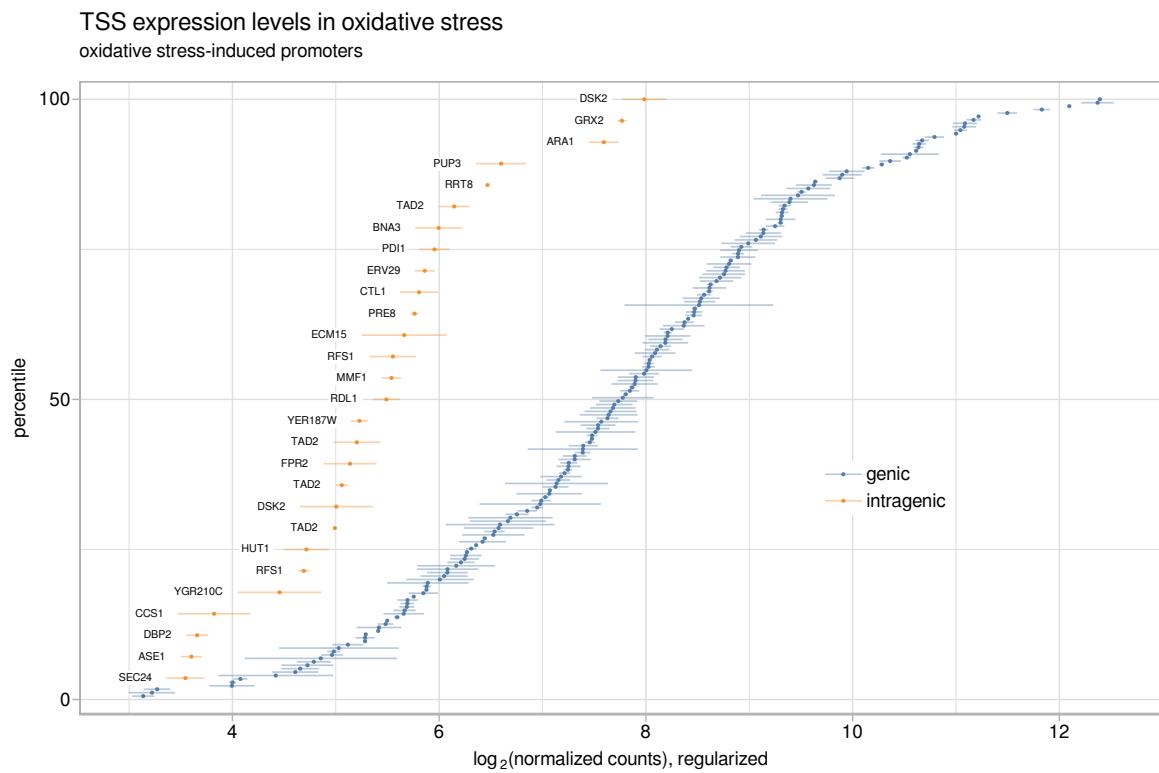


Figure 4.4: Caption dsafklj zzzz.

Figure 4.5: Caption dsafklj .

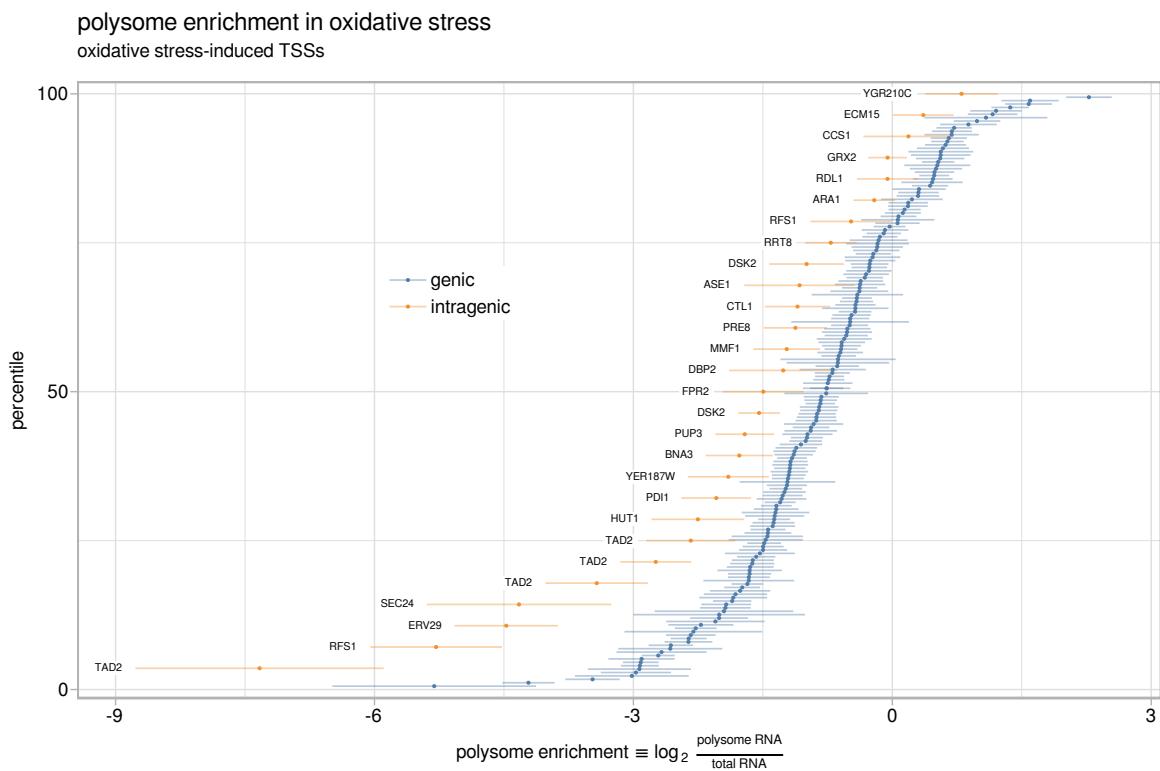


Figure 4.6: Caption wsadasdr zzzz.

Figure 4.7: Caption dsafklj .

Figure 4.8: Caption dsafklj .

Figure 4.9: Caption dsafklj .

Figure 4.10: Caption dsafklj .

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## Vita

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Sed commodo posuere pede. Mauris ut est. Ut quis purus. Sed ac odio. Sed vehicula hendrerit sem. Duis non odio. Morbi ut dui. Sed accumsan risus eget odio. In hac habitasse platea dictumst. Pellentesque non elit. Fusce sed justo eu urna porta tincidunt. Mauris felis odio, sollicitudin sed, volutpat a, ornare ac, erat. Morbi quis dolor. Donec pellentesque, erat ac sagittis semper, nunc dui lobortis purus, quis congue purus metus ultricies tellus. Proin et quam. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos hymenaeos. Praesent sapien turpis, fermentum vel, eleifend faucibus, vehicula eu, lacus.