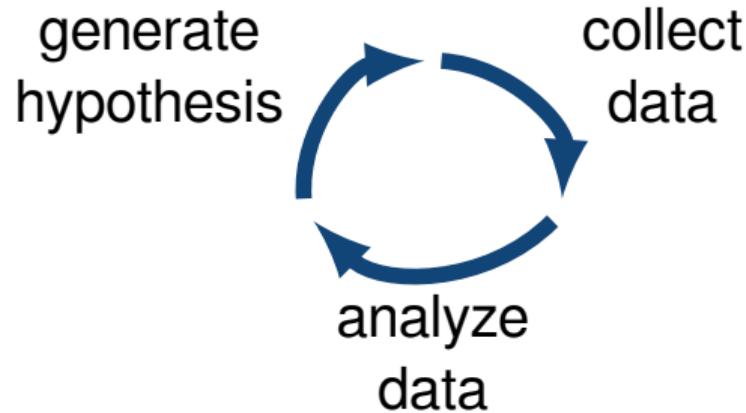


# Genomic analyses of transcription elongation factors and intragenic transcription

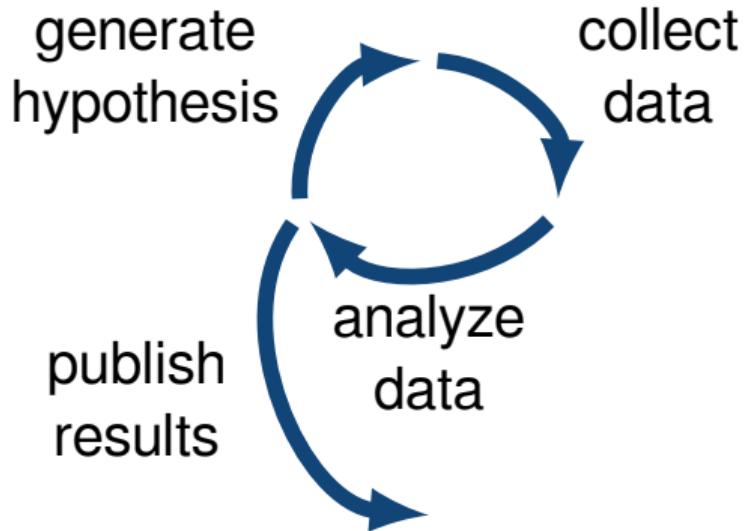
James Chuang

June 19, 2019

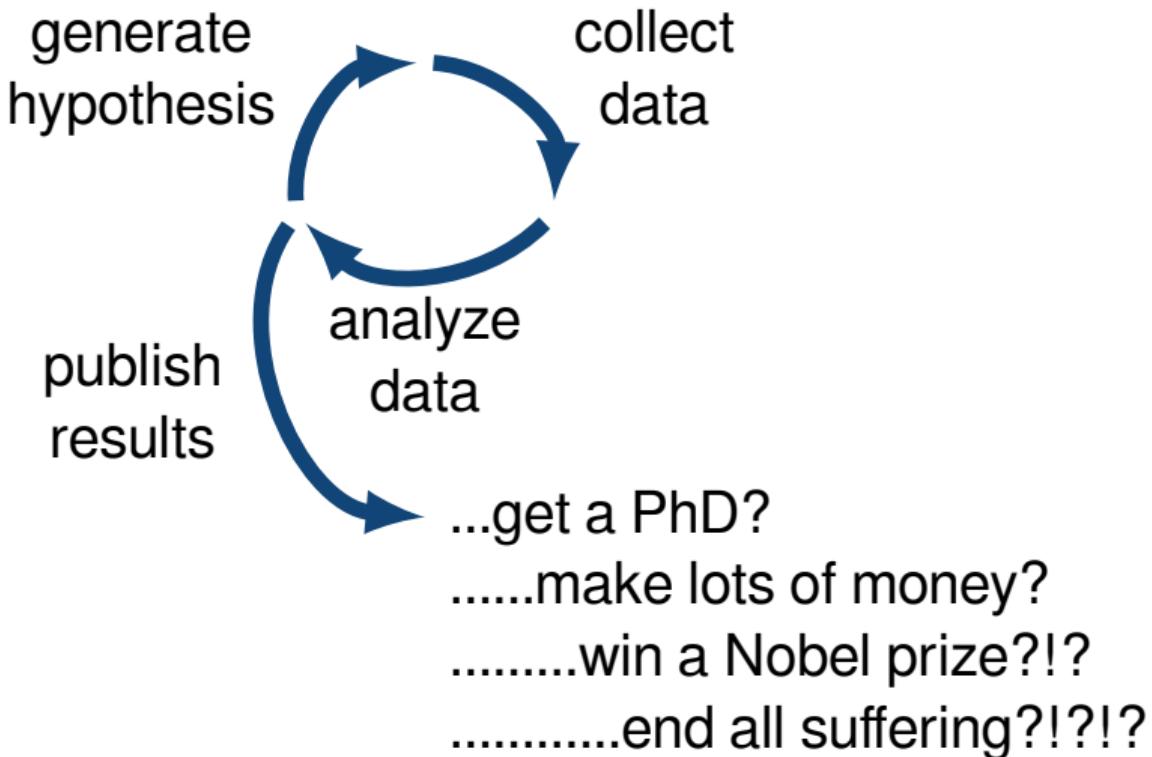
# The scientific process

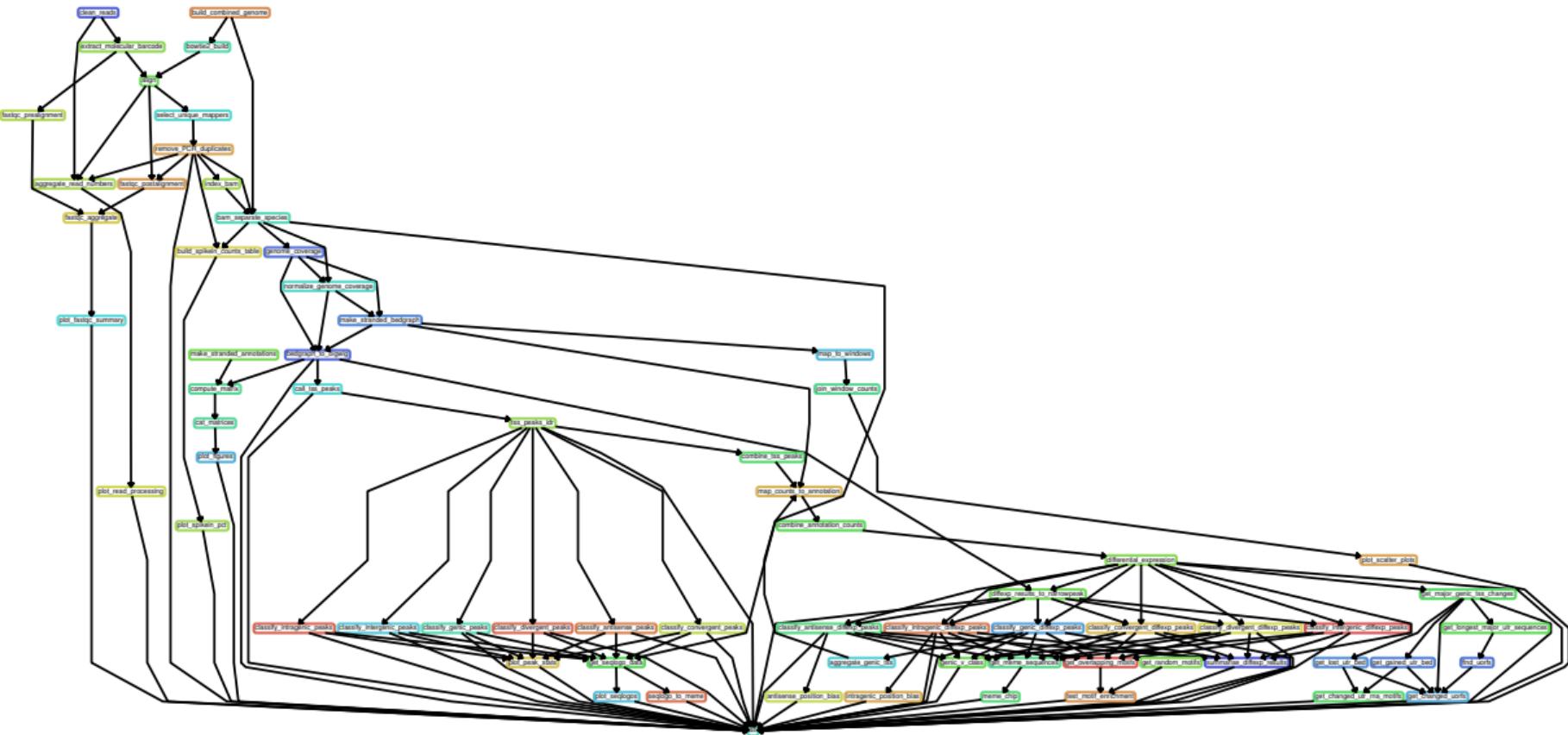


# The scientific process



# The scientific process







# an example Snakemake rule:

```
rule foobar:
    input: 'input.txt'
    output: 'output.txt'
    params: species='cerevisiae'
    conda: 'environment.yaml'
    script: 'make_output_from_input.py'
```





September 7, 2018

Journal article

Open Access

Spt6 is required for the fidelity of promoter selection

Doris, Stephen M.;  Chuang, James;  Viktorovskaya, Olga; Murawska, Magdalena; Spatt, Dan;  Churchman, L.; Stirling;  Winston, Fred

Contact person(s)

Winston, Fred

### Data collector(s)

Spatt Dan

### Data manager(s)

Chuang, James

Other(s)

Churchman | Stirling

**Researcher(s)**

Doris Stephen M.;  Viktorovskaya Olga; Murawska Małgorzata

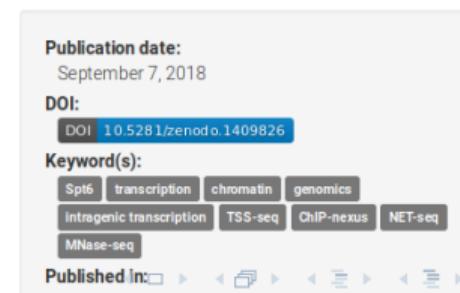
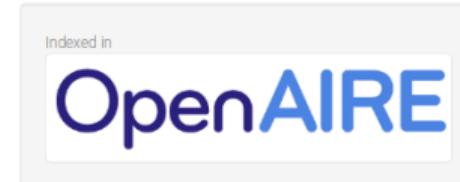
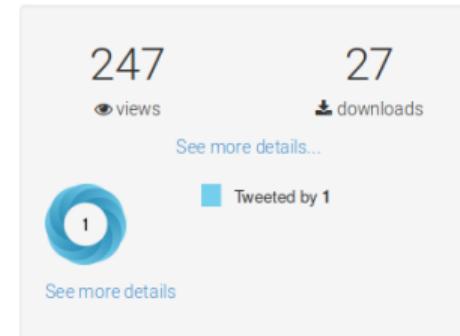
All data analyses supporting our publication "Spt6 is required for the fidelity of promoter selection". Reproduce the figures of the paper starting from raw data, as well as thousands of figures and analyses that aren't shown.

See README.md for instructions

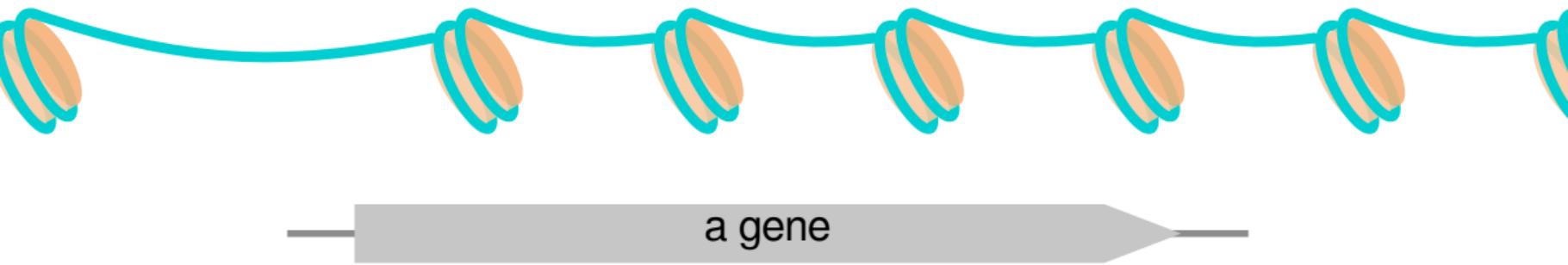
For updated versions of the pipelines used, see our [github page](#).

Files (47.2 GB)

Name	Size	
spt6_2018.tar.gz	47.2 GB	 Download

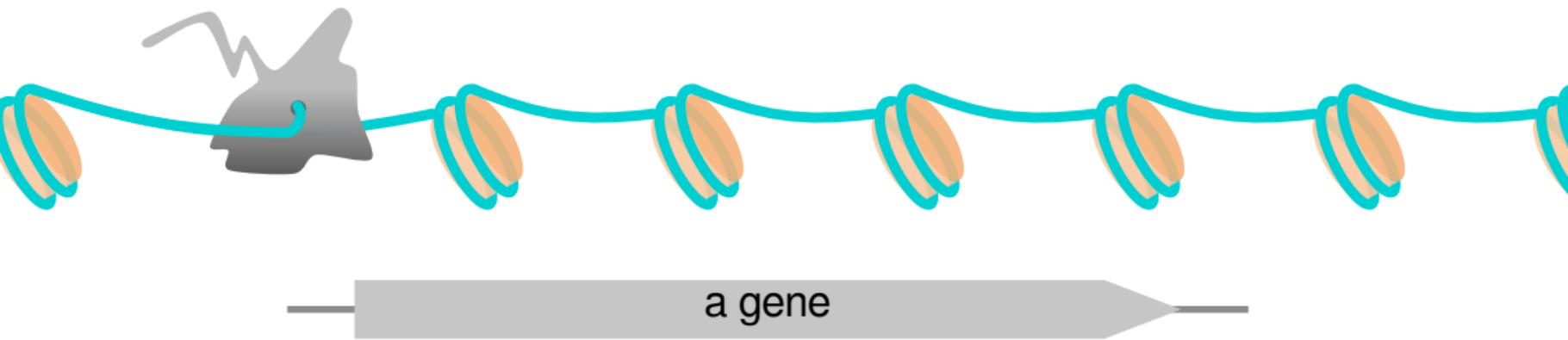


# An introduction to transcription

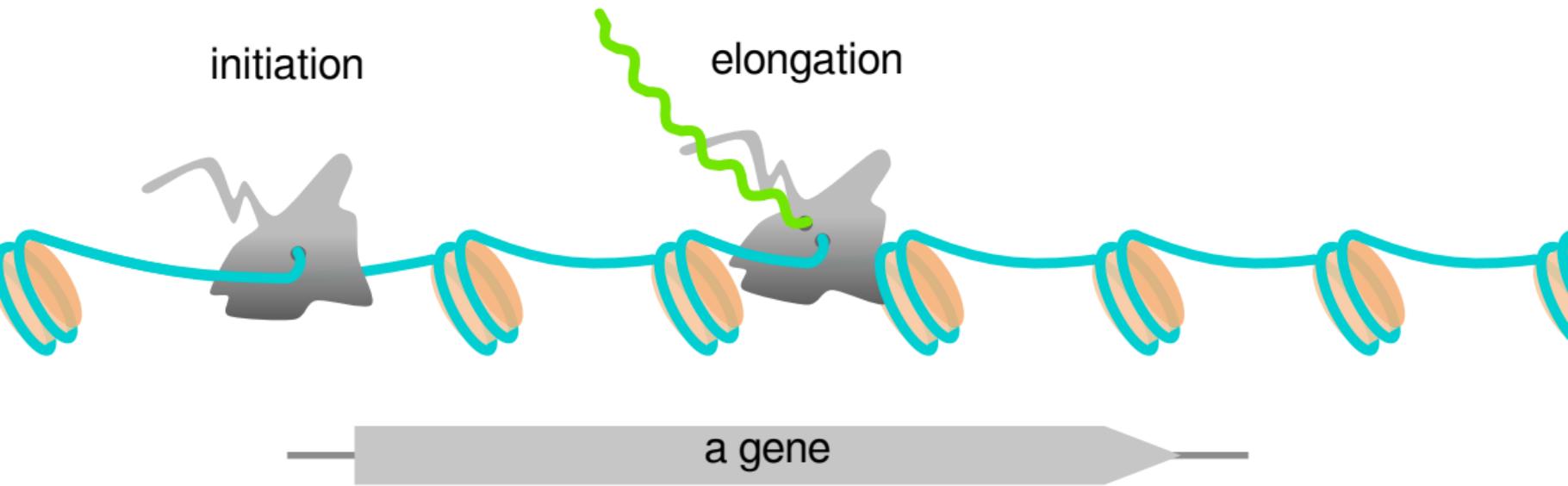


# An introduction to transcription

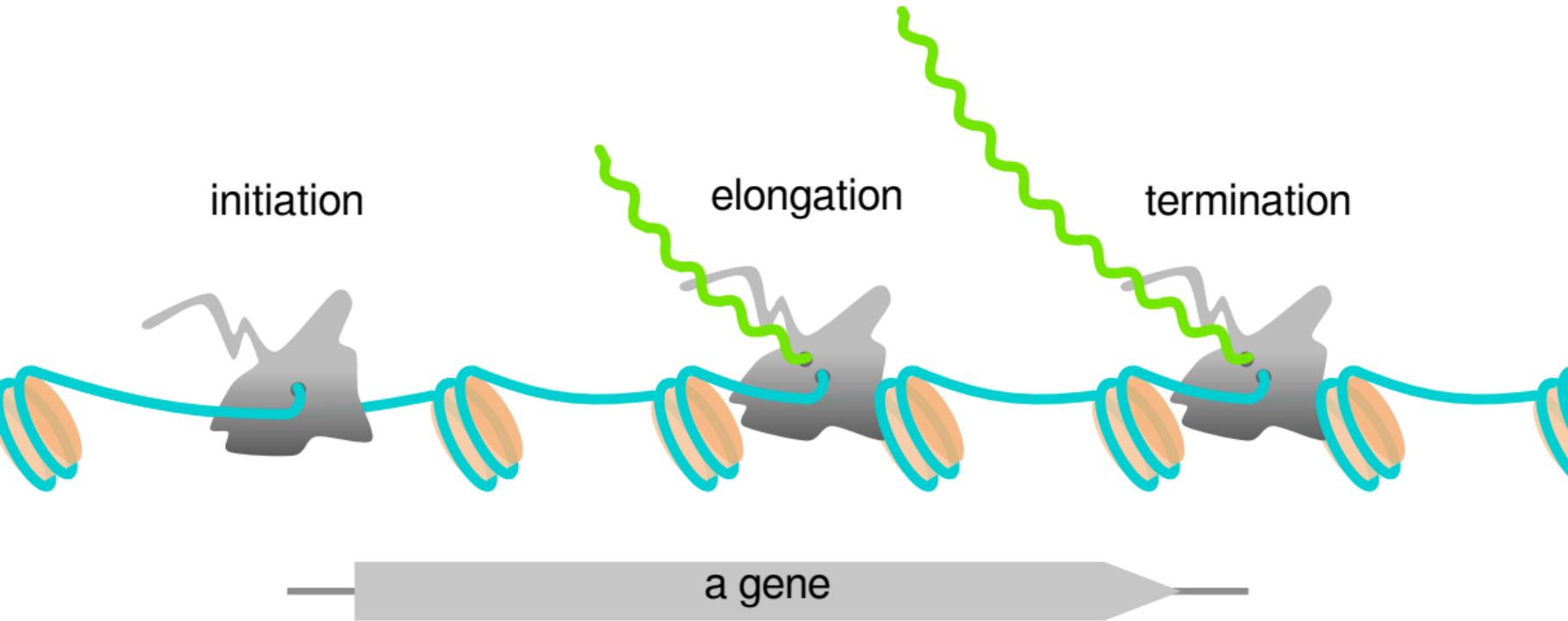
initiation



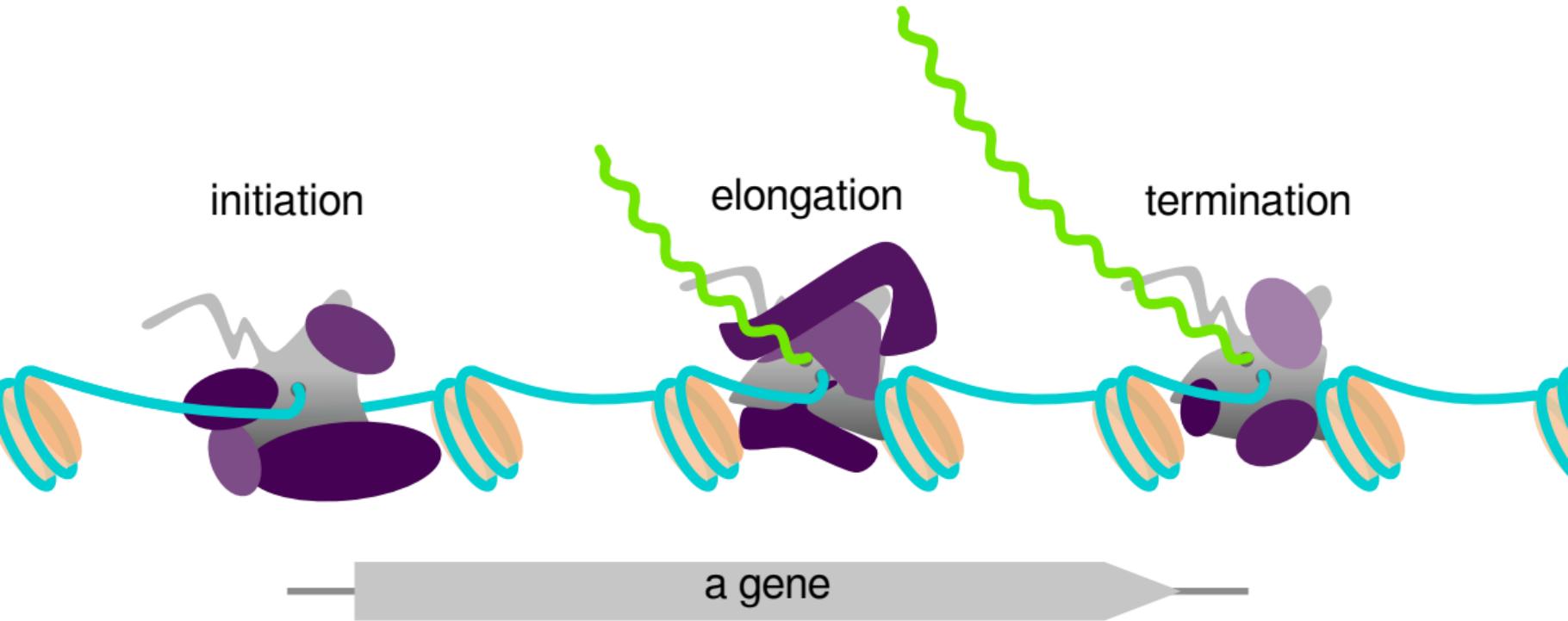
# An introduction to transcription

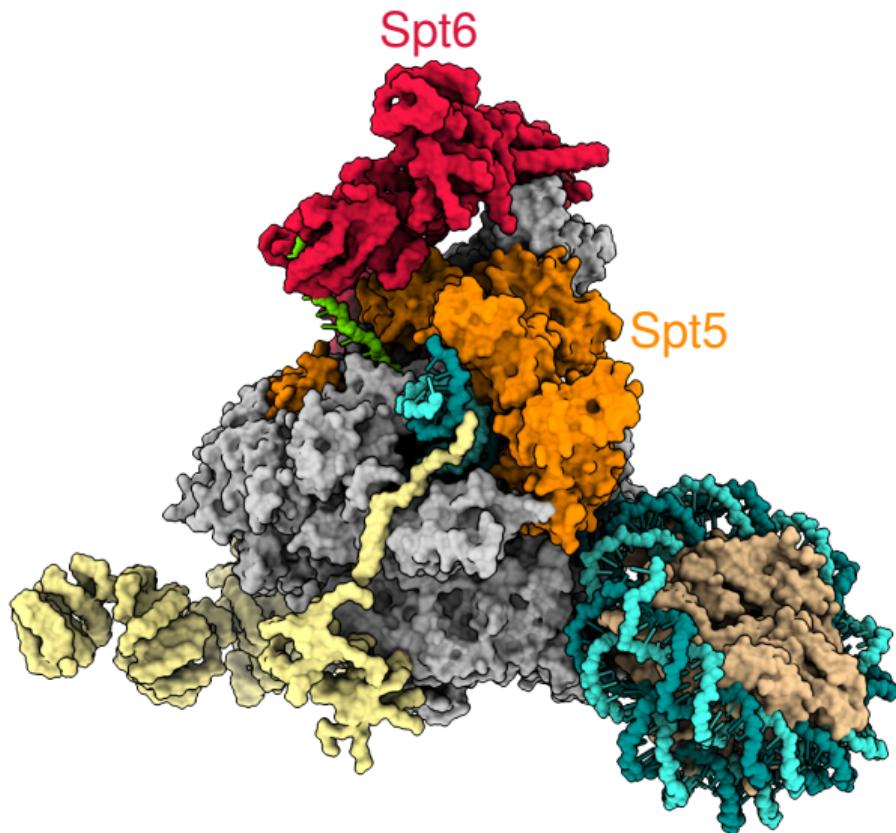


# An introduction to transcription

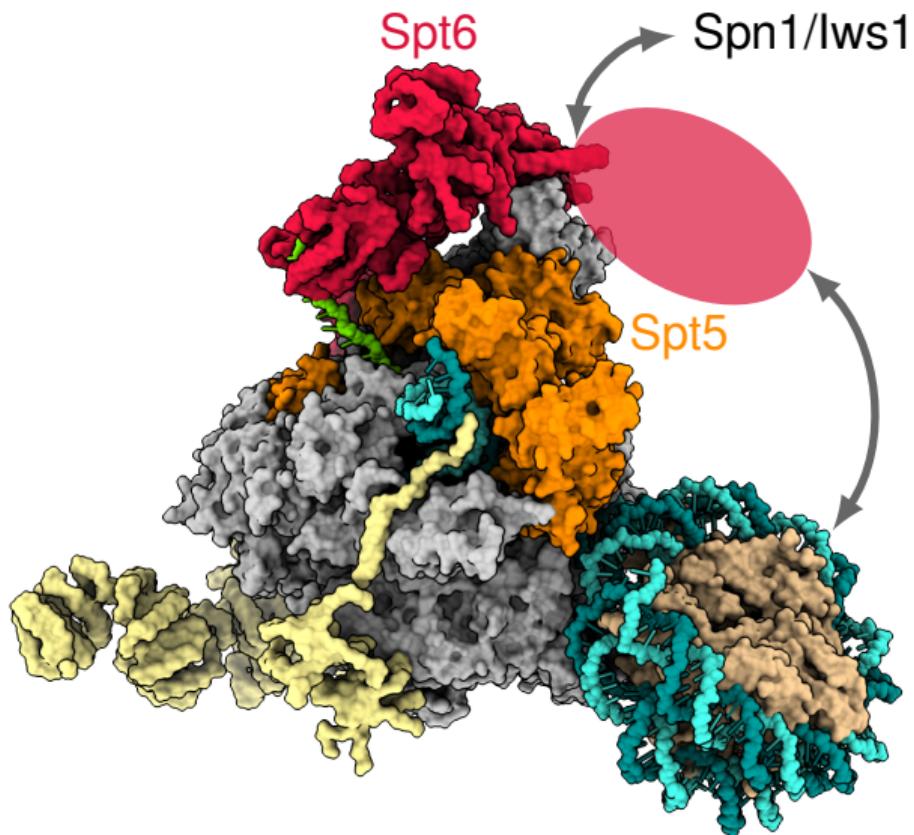


# An introduction to transcription





Vos *et al.* (2018). *Nature*  
Farnung *et al.* (2018). *Nat. Commun.*



Vos *et al.* (2018). *Nature*  
Farnung *et al.* (2018). *Nat. Commun.*

# Spt6 project collaborators

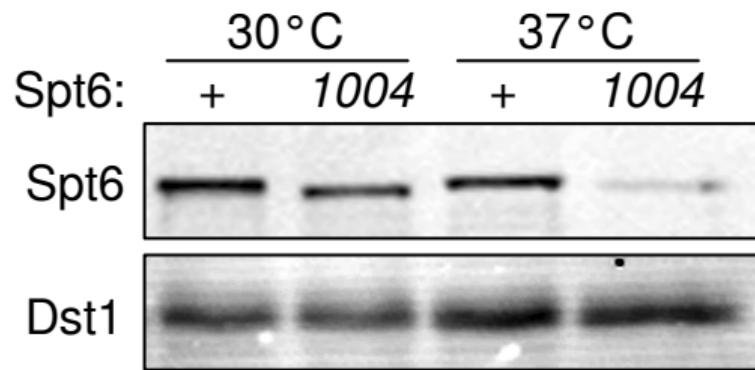
**Steve Doris** TSS-seq and ChIP-nexus

**Olga Viktorovskaya** MNase-seq

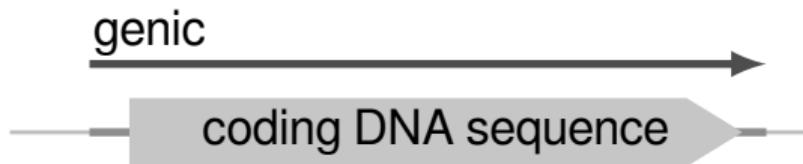
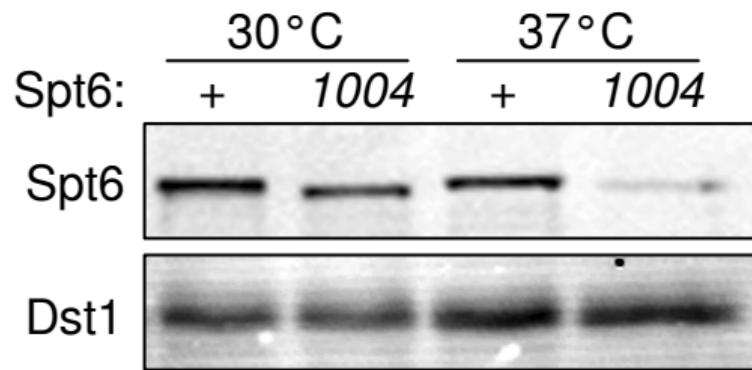
**Magdalena Murawska** NET-seq

**Dan Spatt** Northern, Western, and ChIP experiments

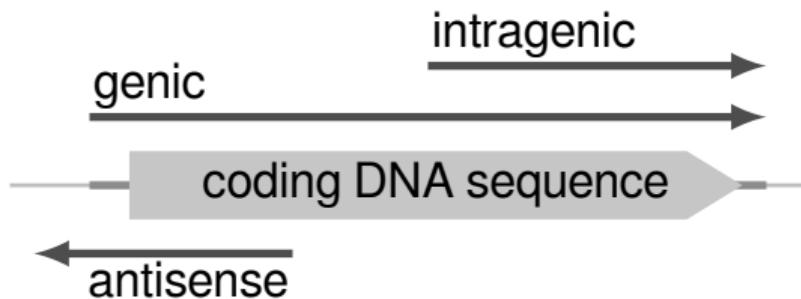
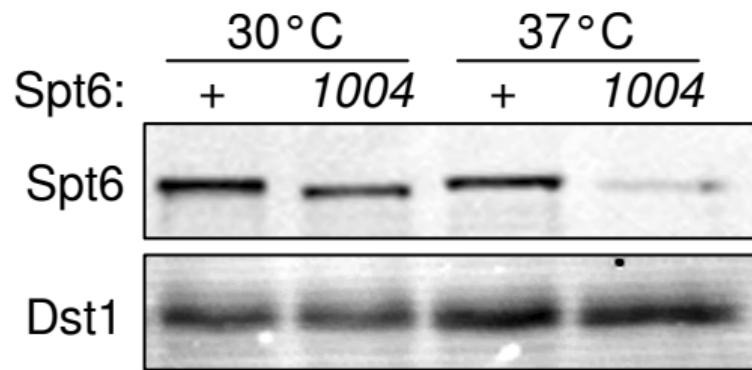
# The *spt6-1004* mutant expresses intragenic transcripts

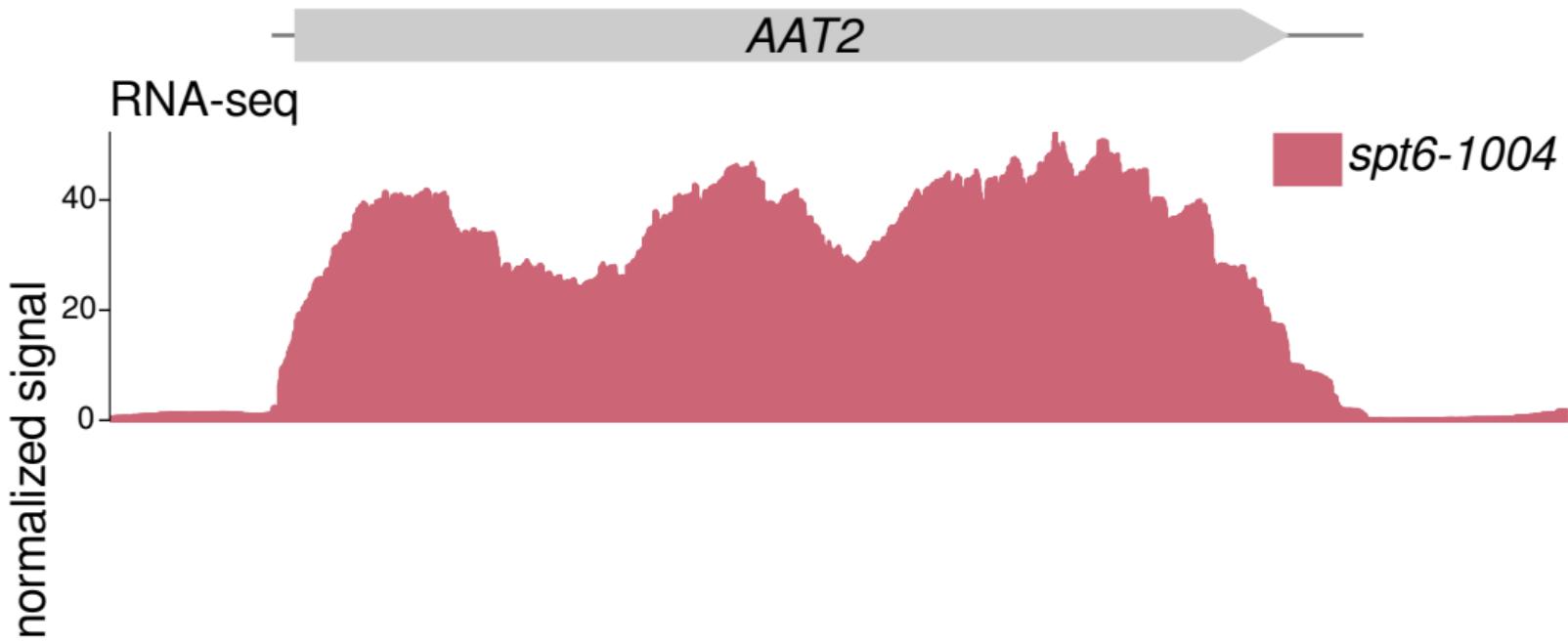


# The *spt6-1004* mutant expresses intragenic transcripts

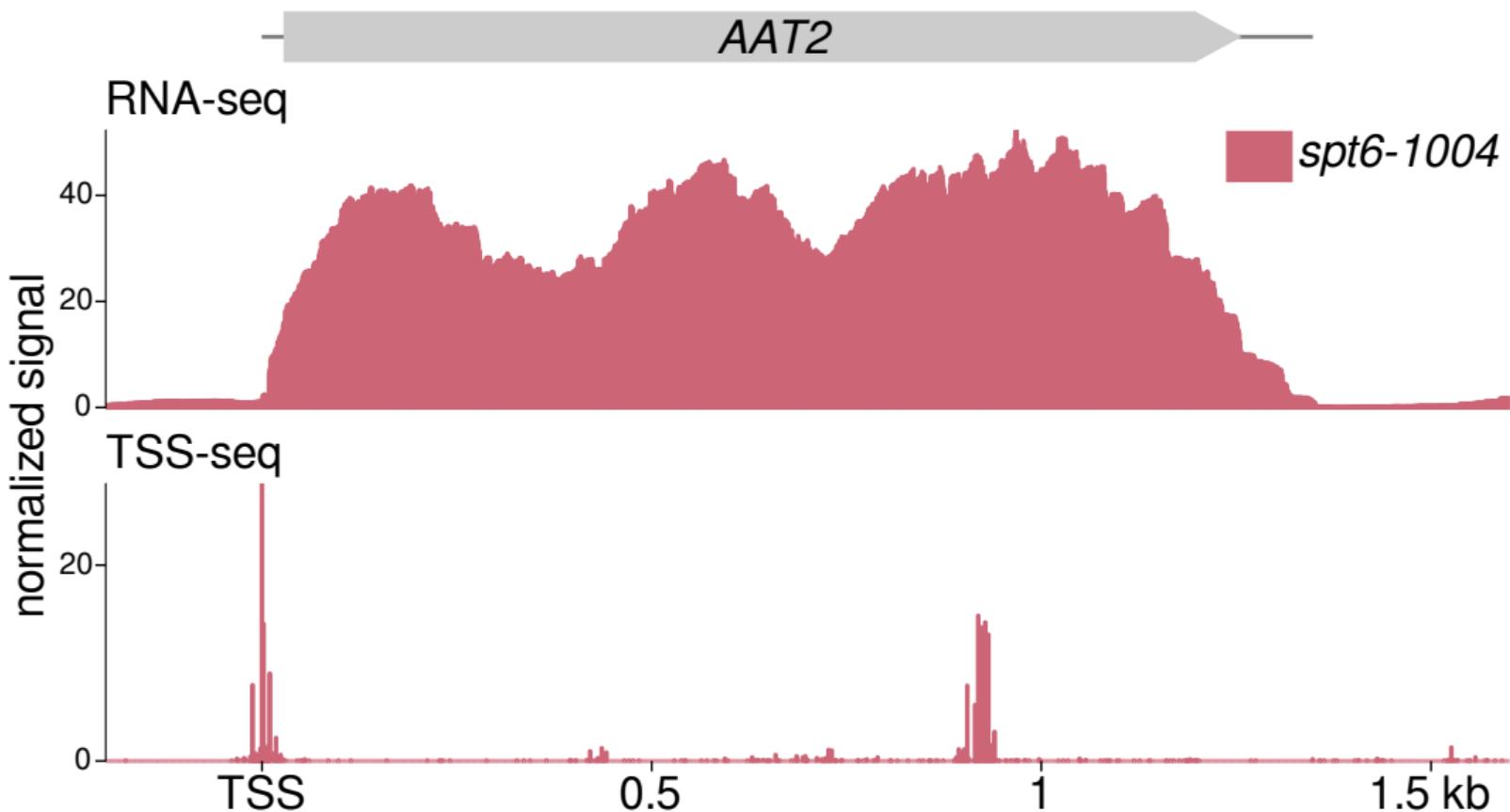


# The *spt6-1004* mutant expresses intragenic transcripts



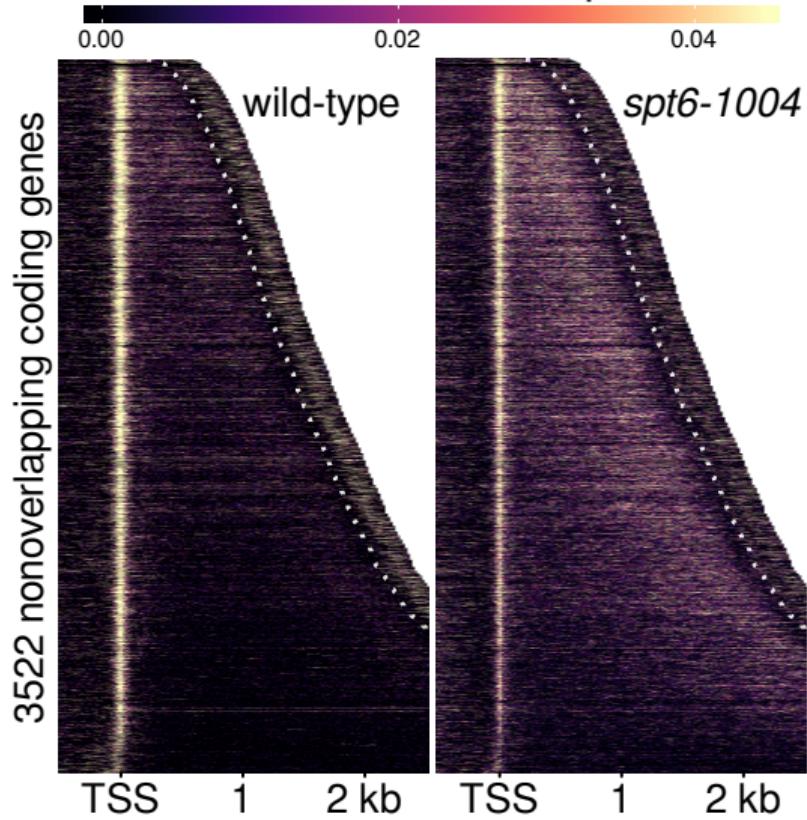


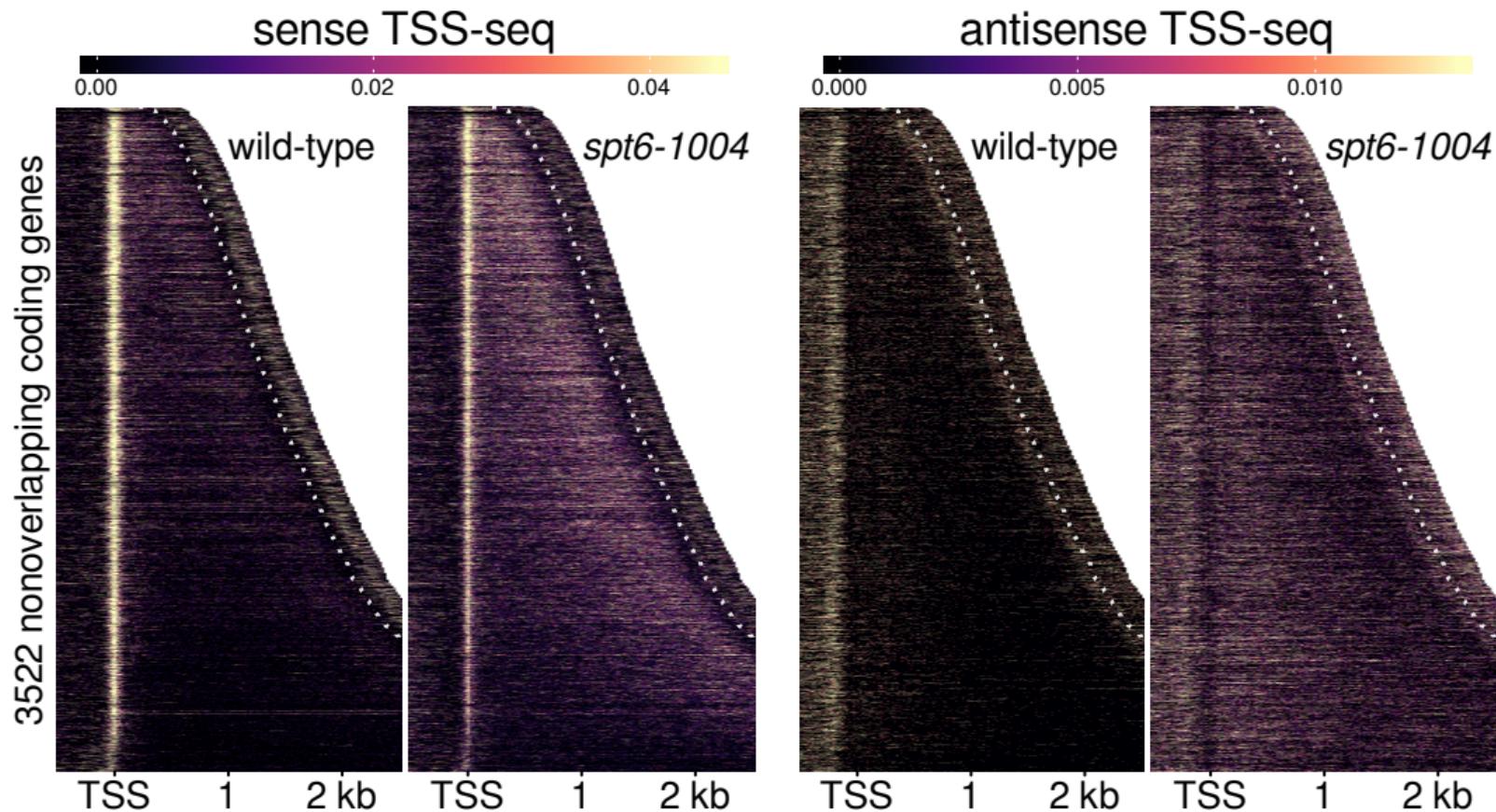
RNA-seq: Uwimana *et al.* (2017). *Nucleic Acids Res.*



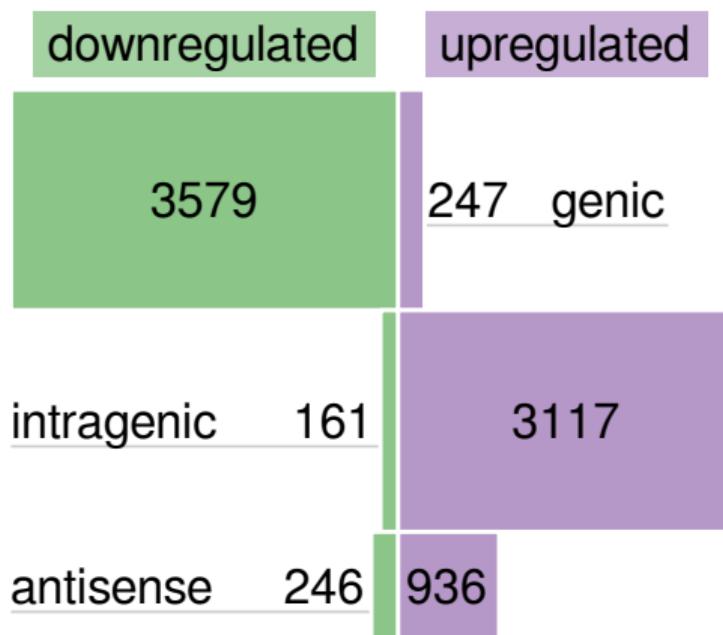
RNA-seq: Uwimana *et al.* (2017). *Nucleic Acids Res.*

# sense TSS-seq

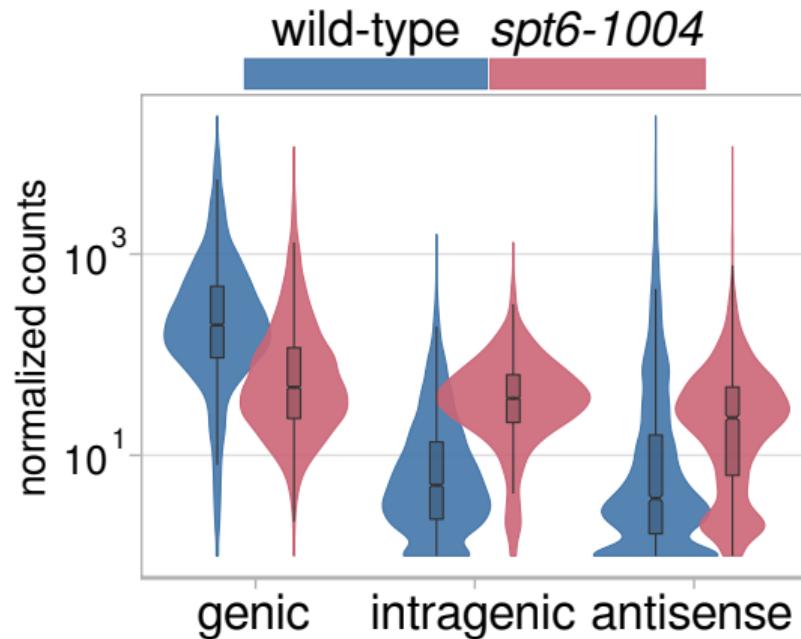
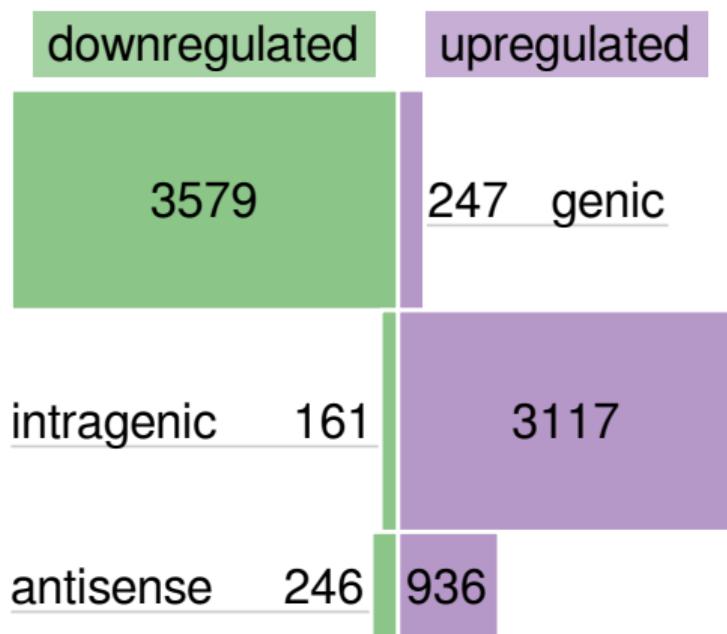




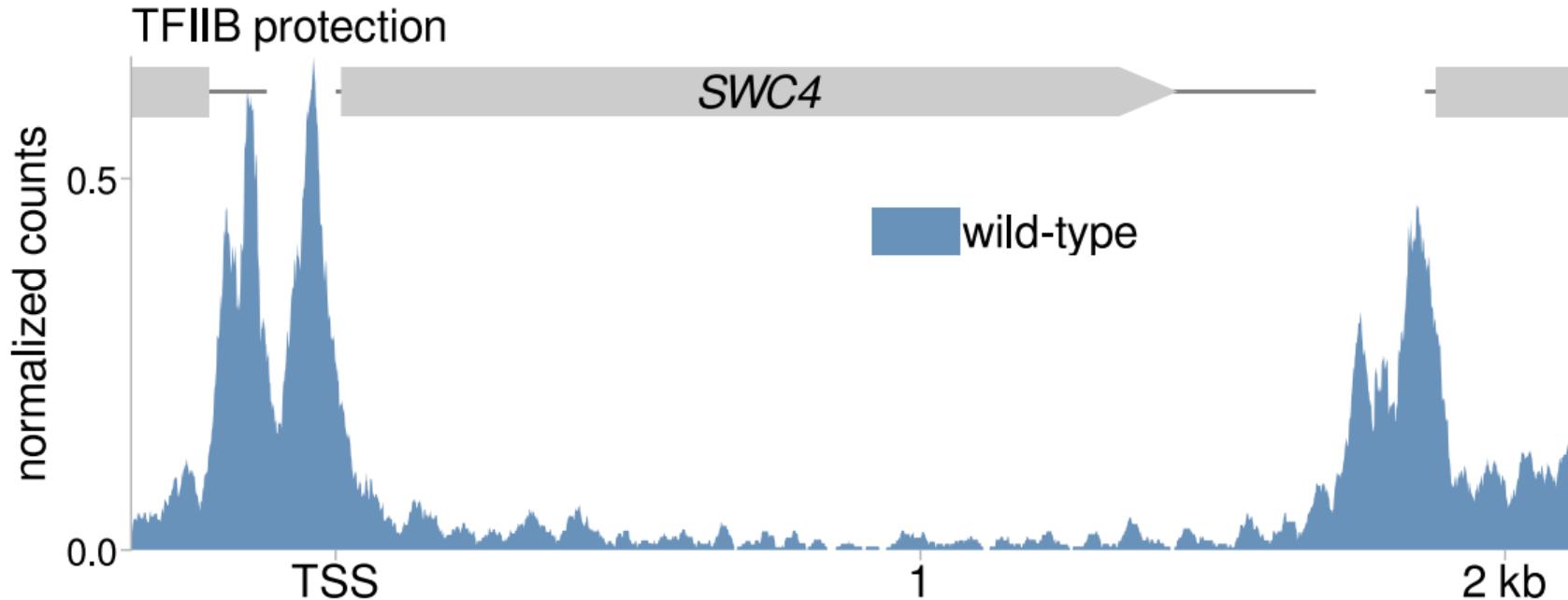
## Genic transcript abundances are reduced



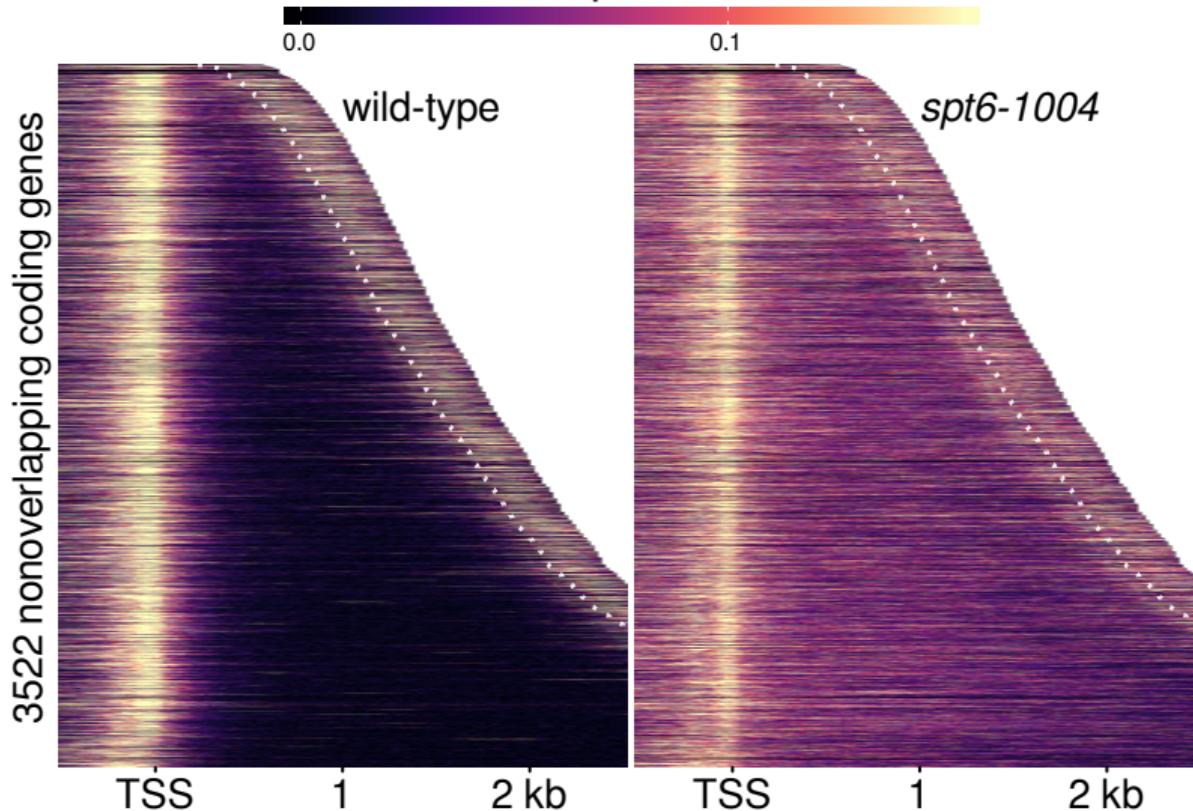
## Genic transcript abundances are reduced



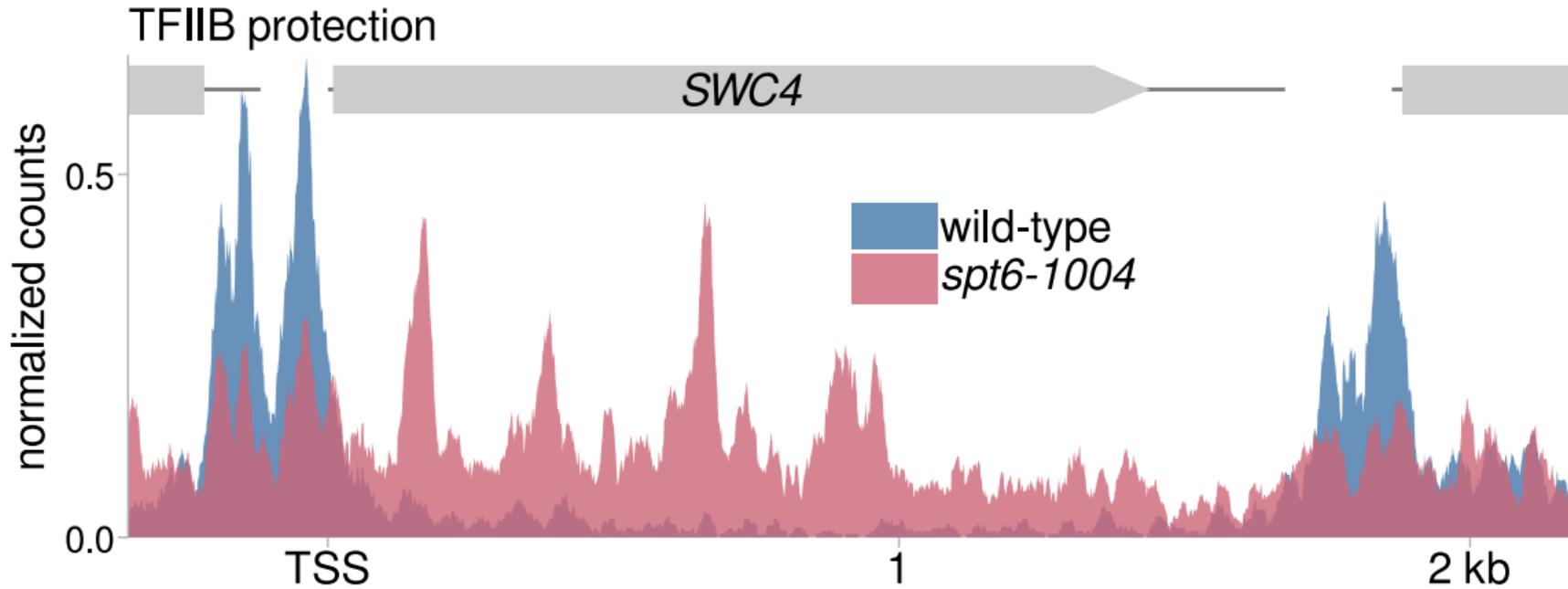
# TFIIB ChIP-nexus measures transcription initiation



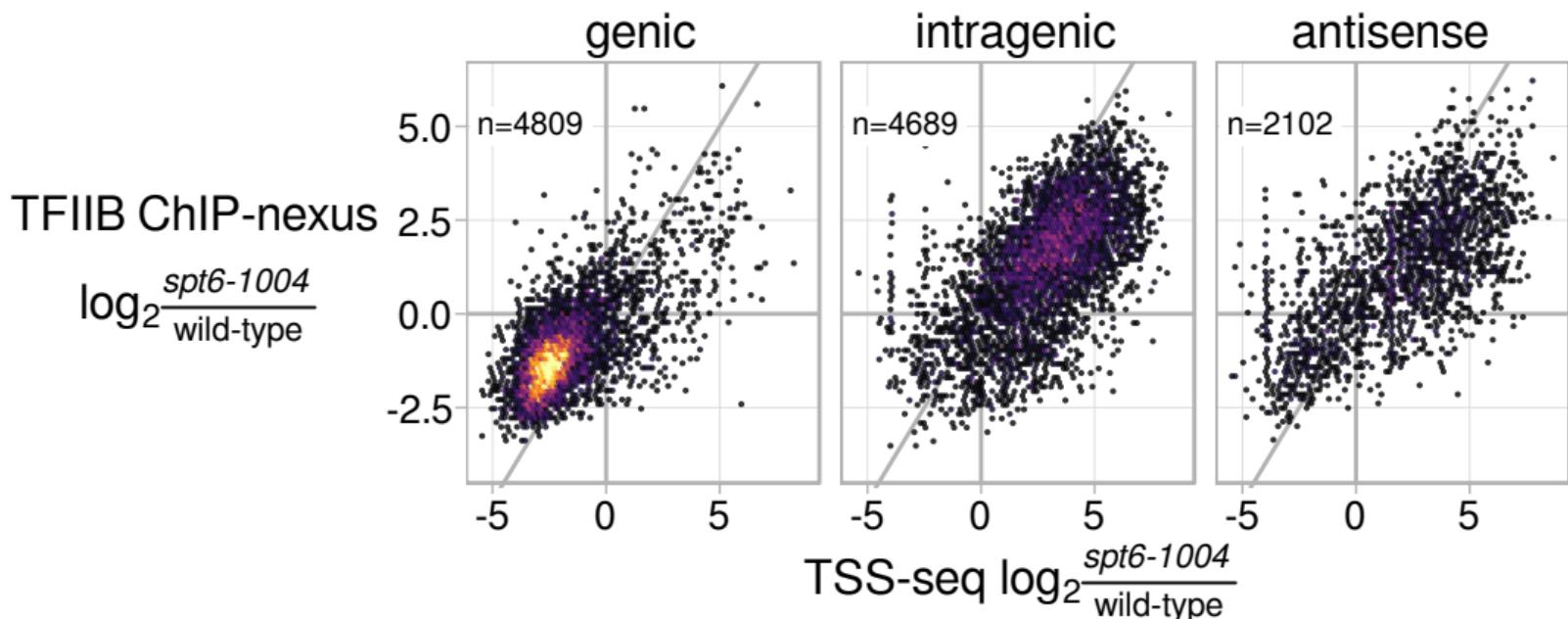
# TFIIB protection



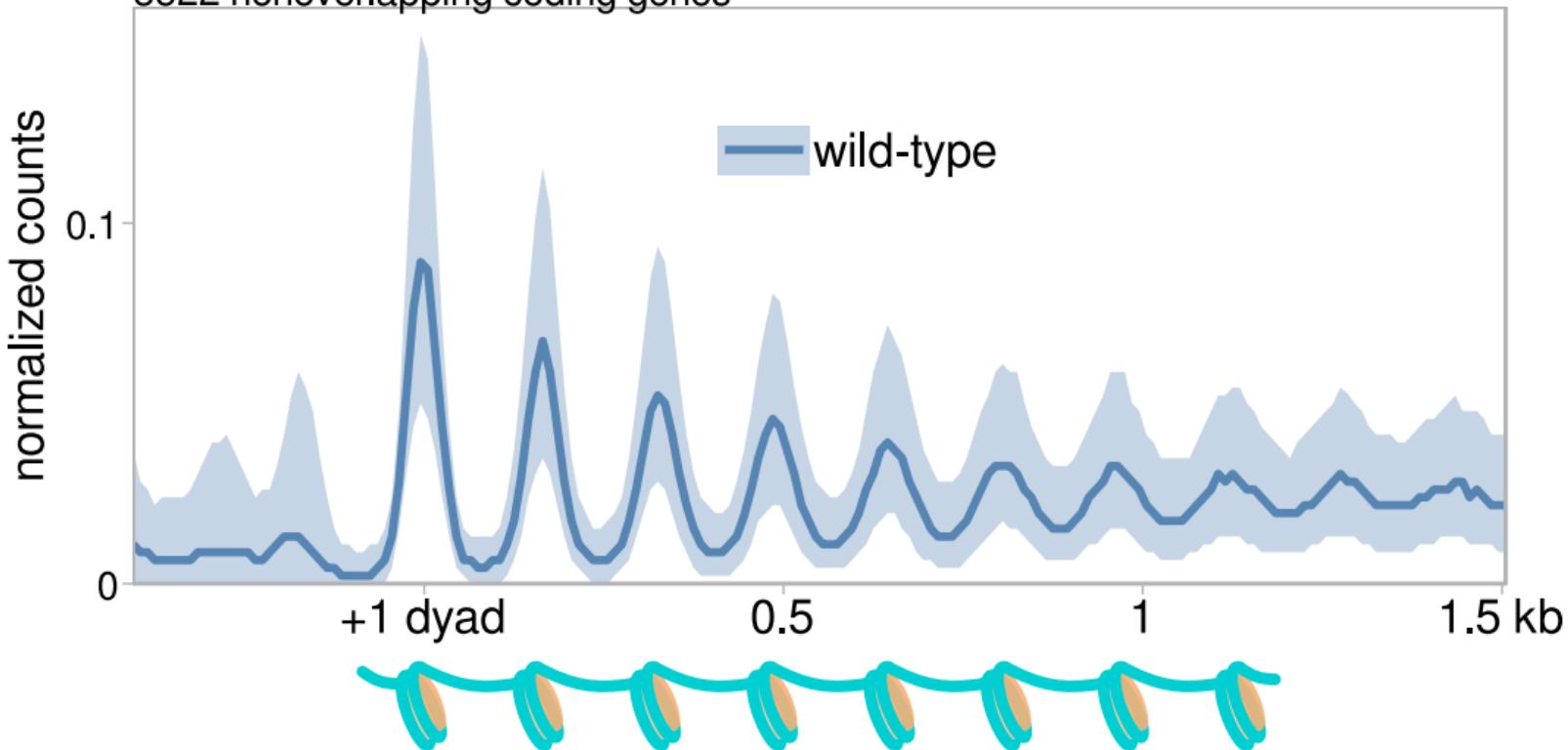
# TFIIB binding changes dramatically in *spt6-1004*:



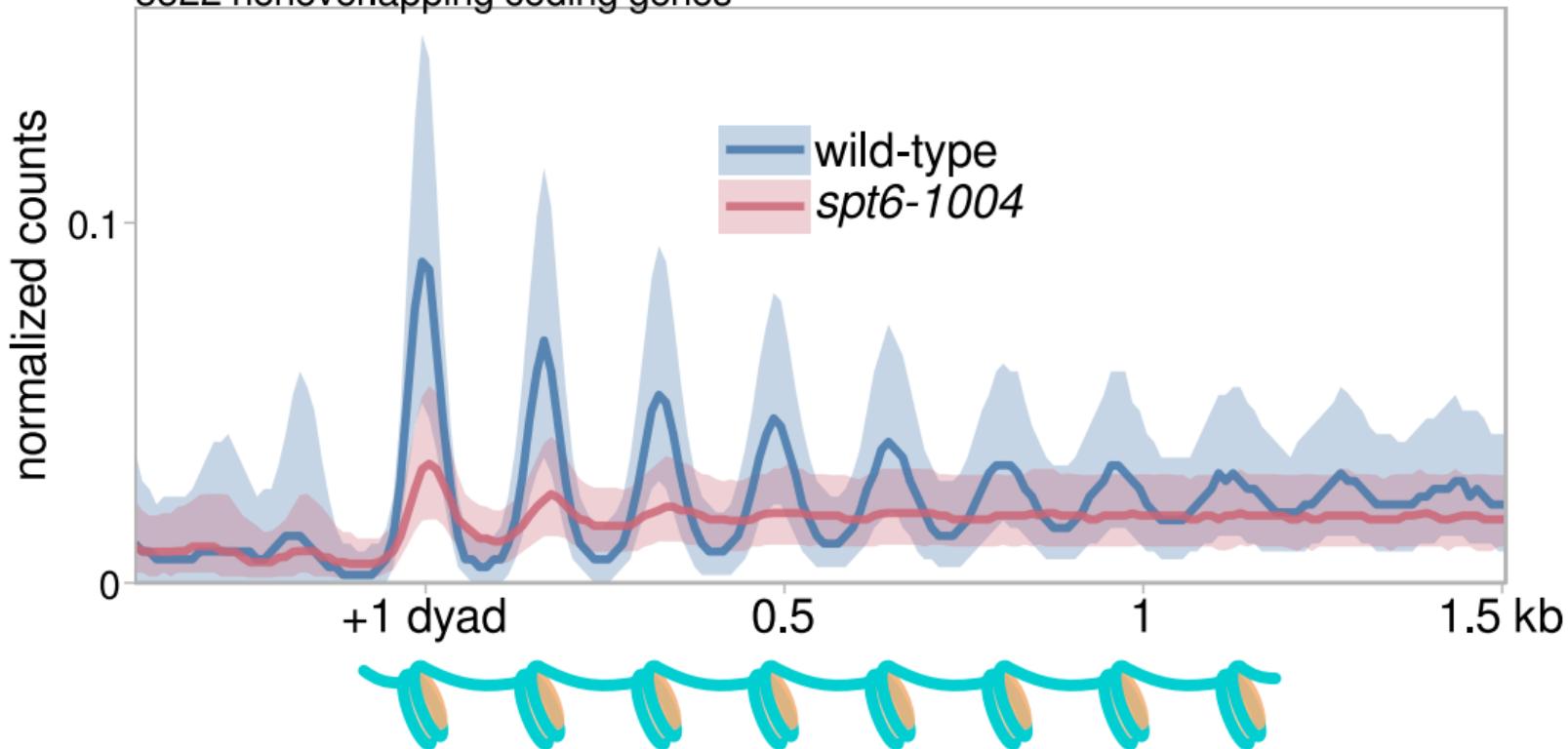
# Transcriptomic changes are mostly explained by changes in initiation



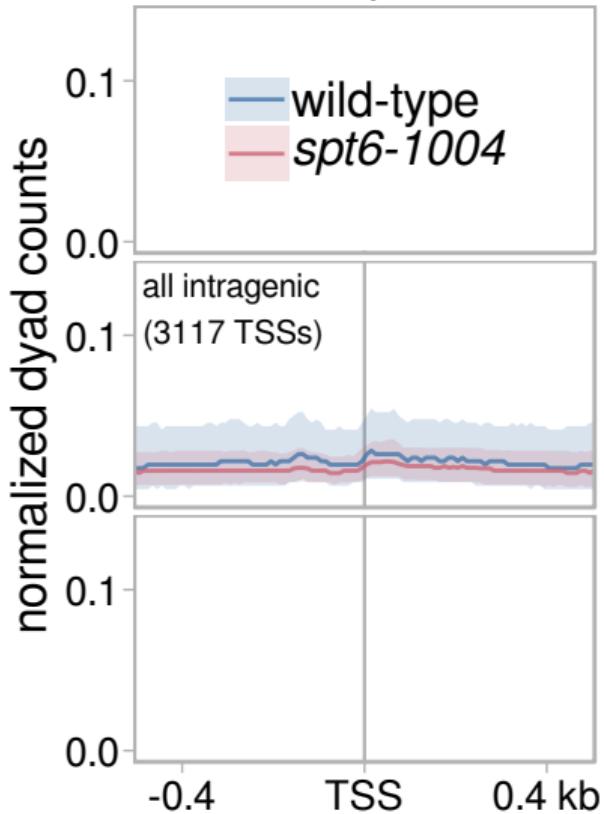
MNase-seq dyad signal  
3522 nonoverlapping coding genes



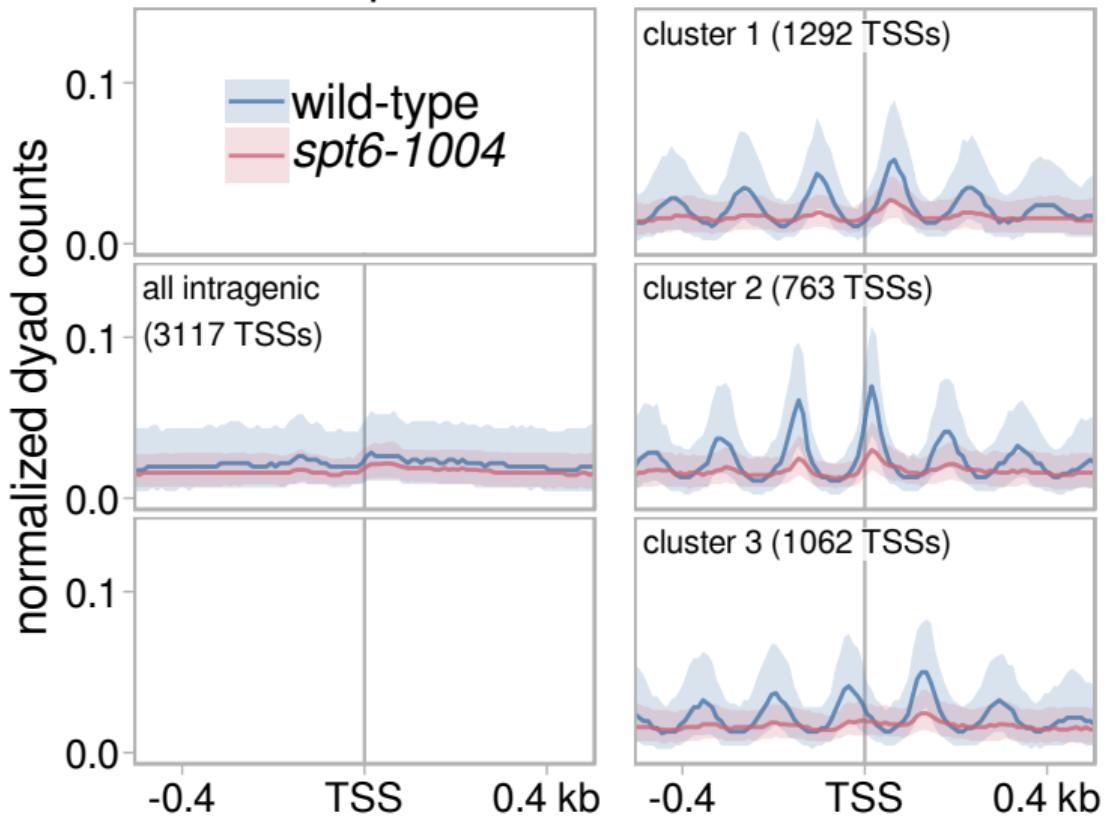
MNase-seq dyad signal  
3522 nonoverlapping coding genes



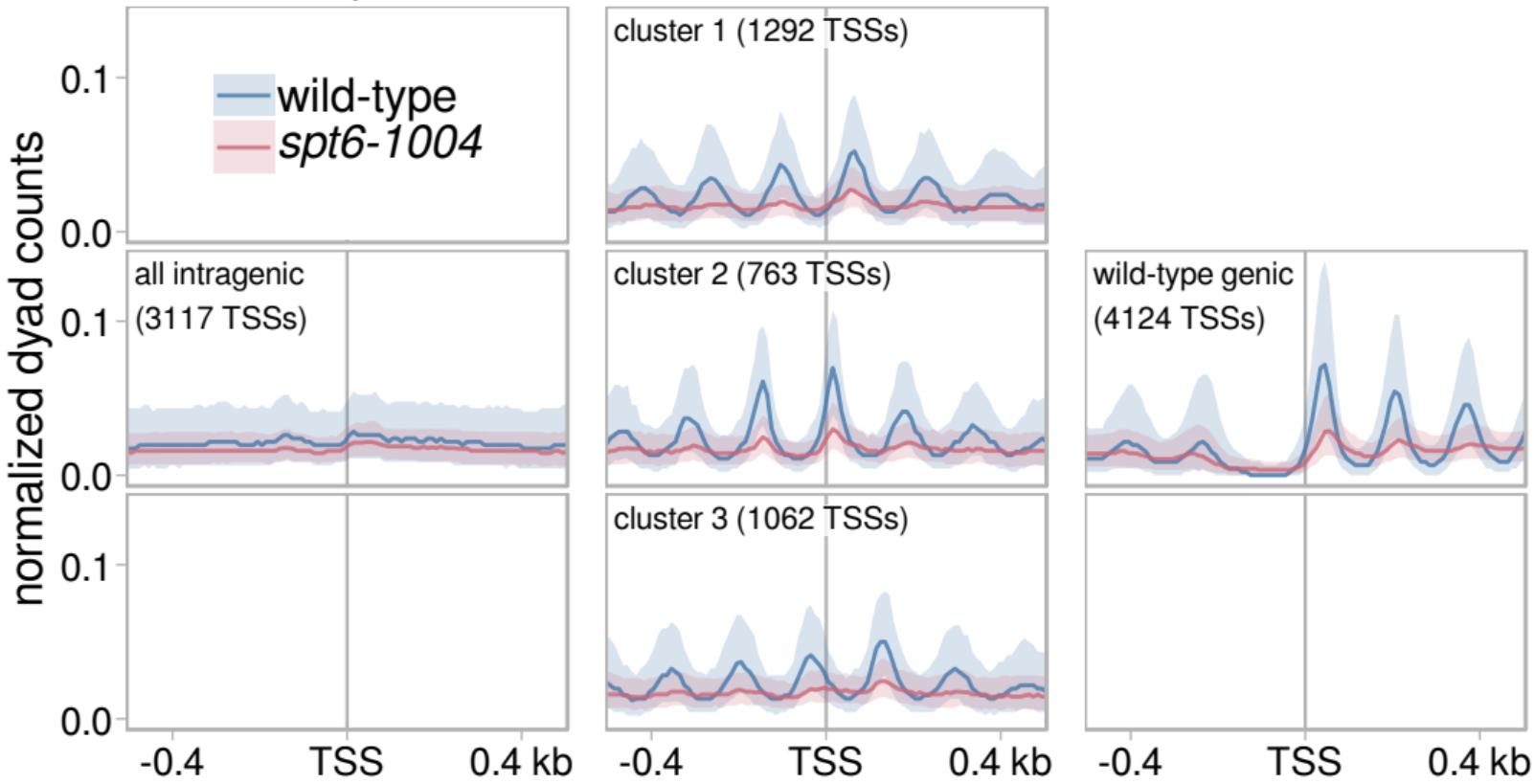
# MNase-seq



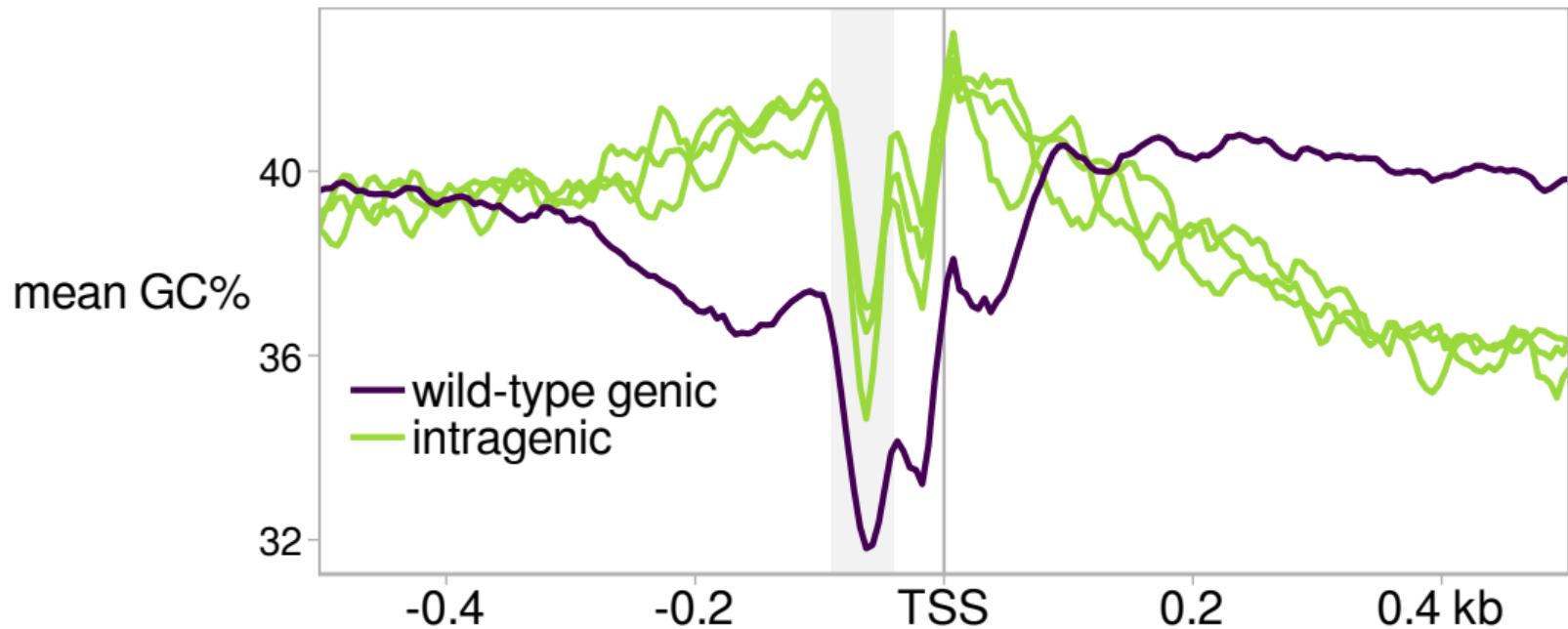
# MNase-seq



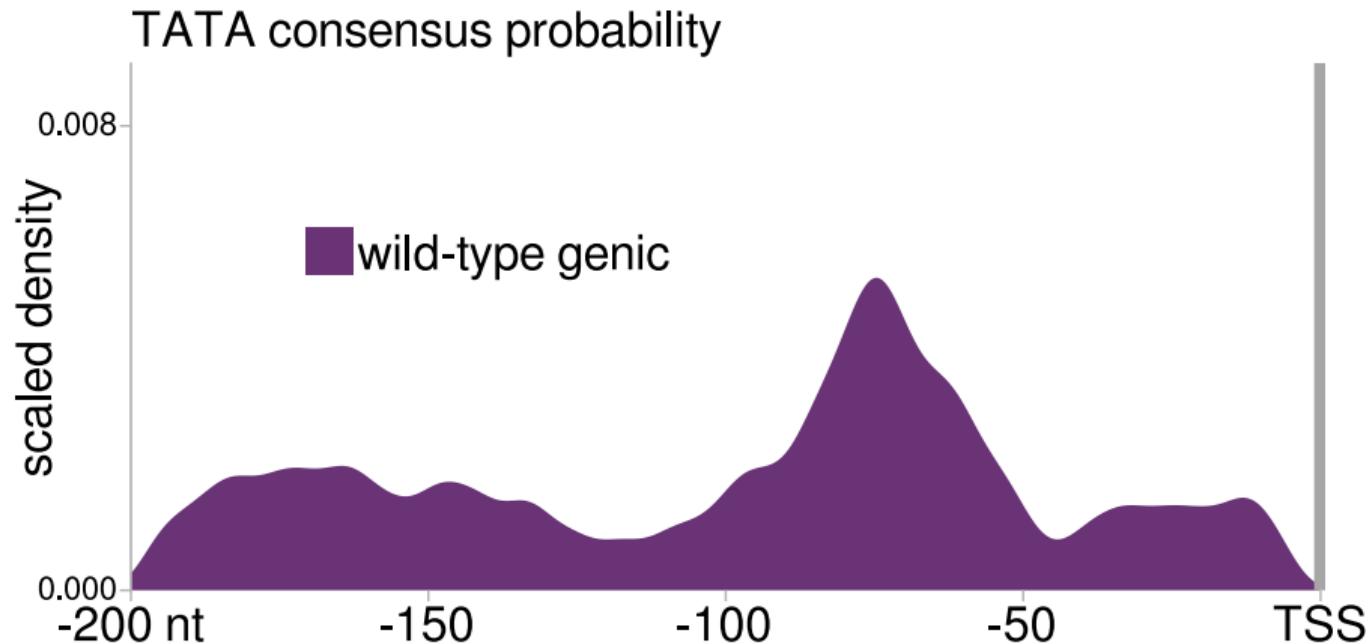
# MNase-seq



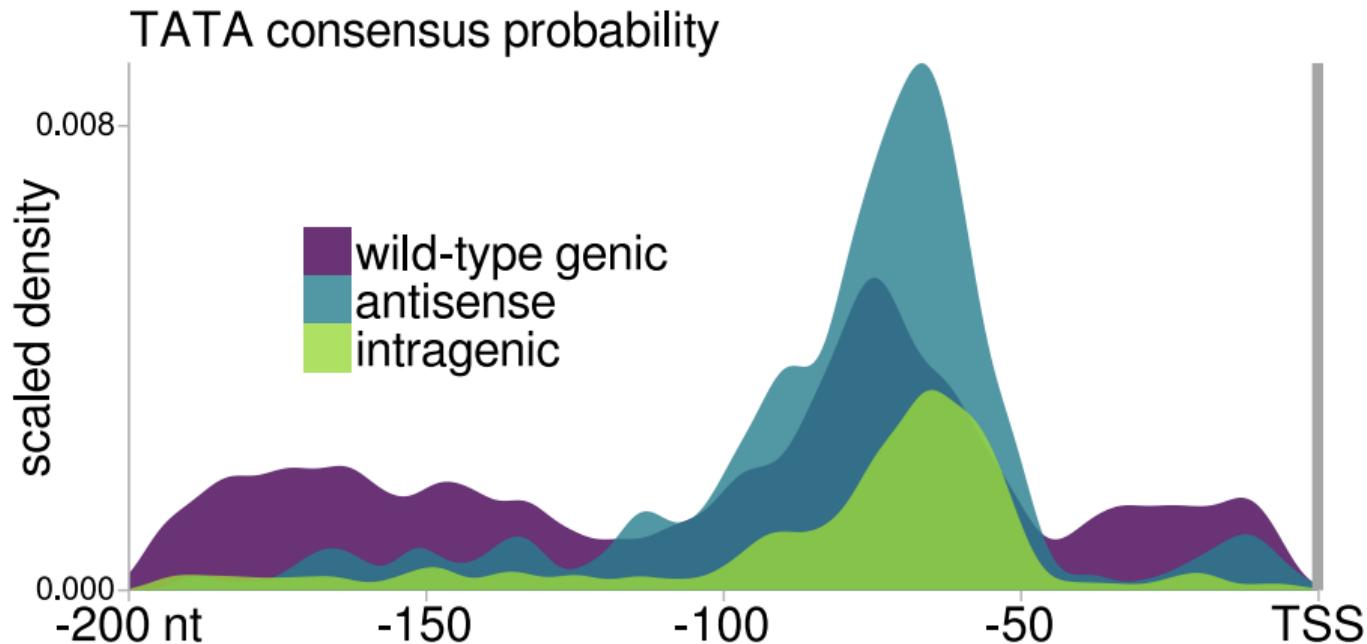
## Intragenic promoters have features of genic promoters



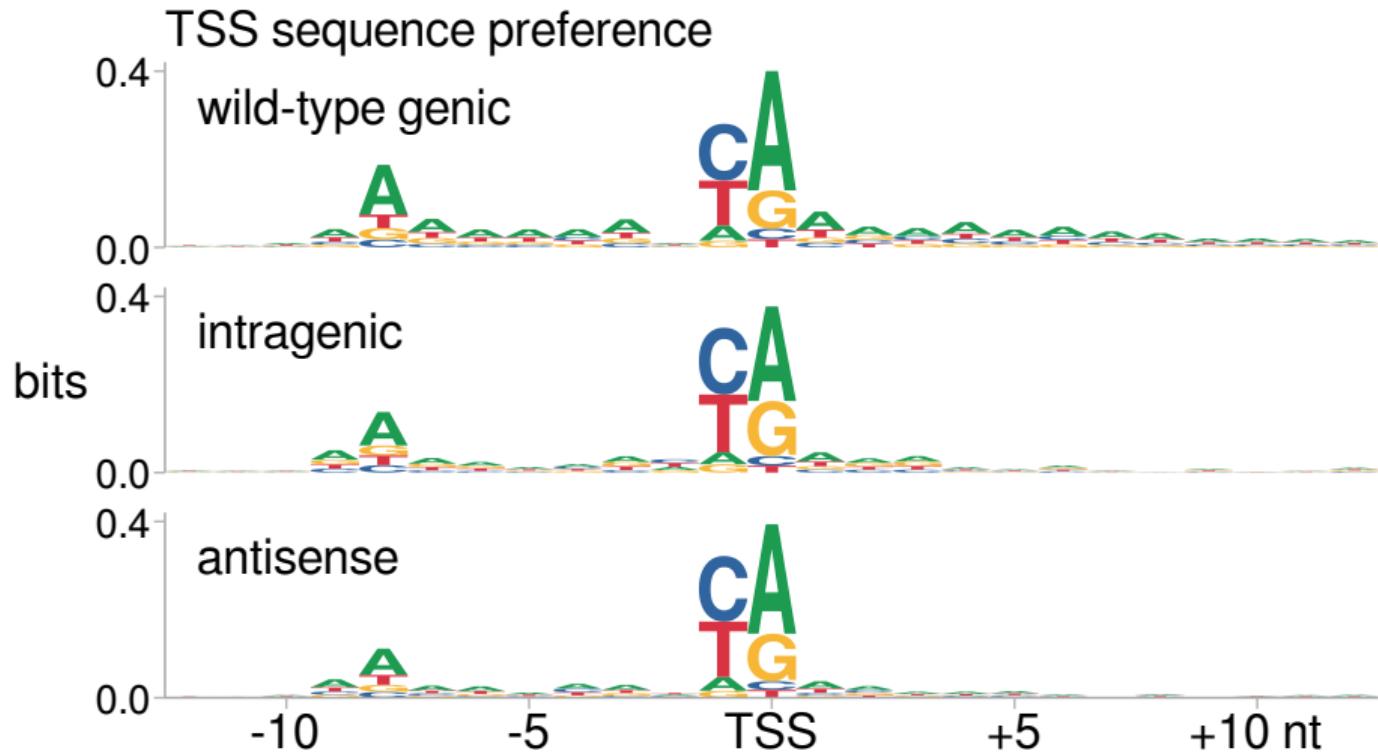
# Intragenic promoters have features of genic promoters

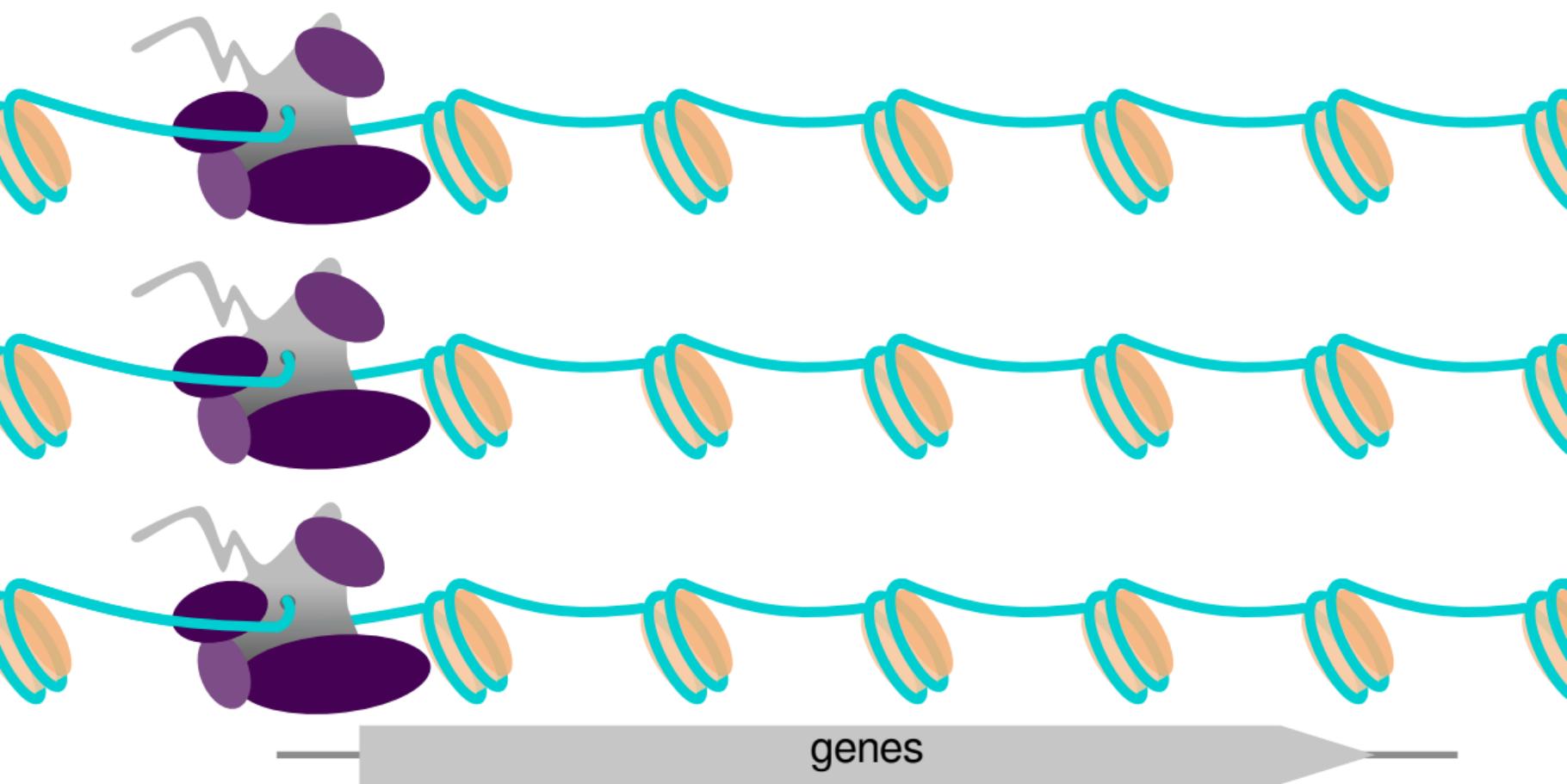


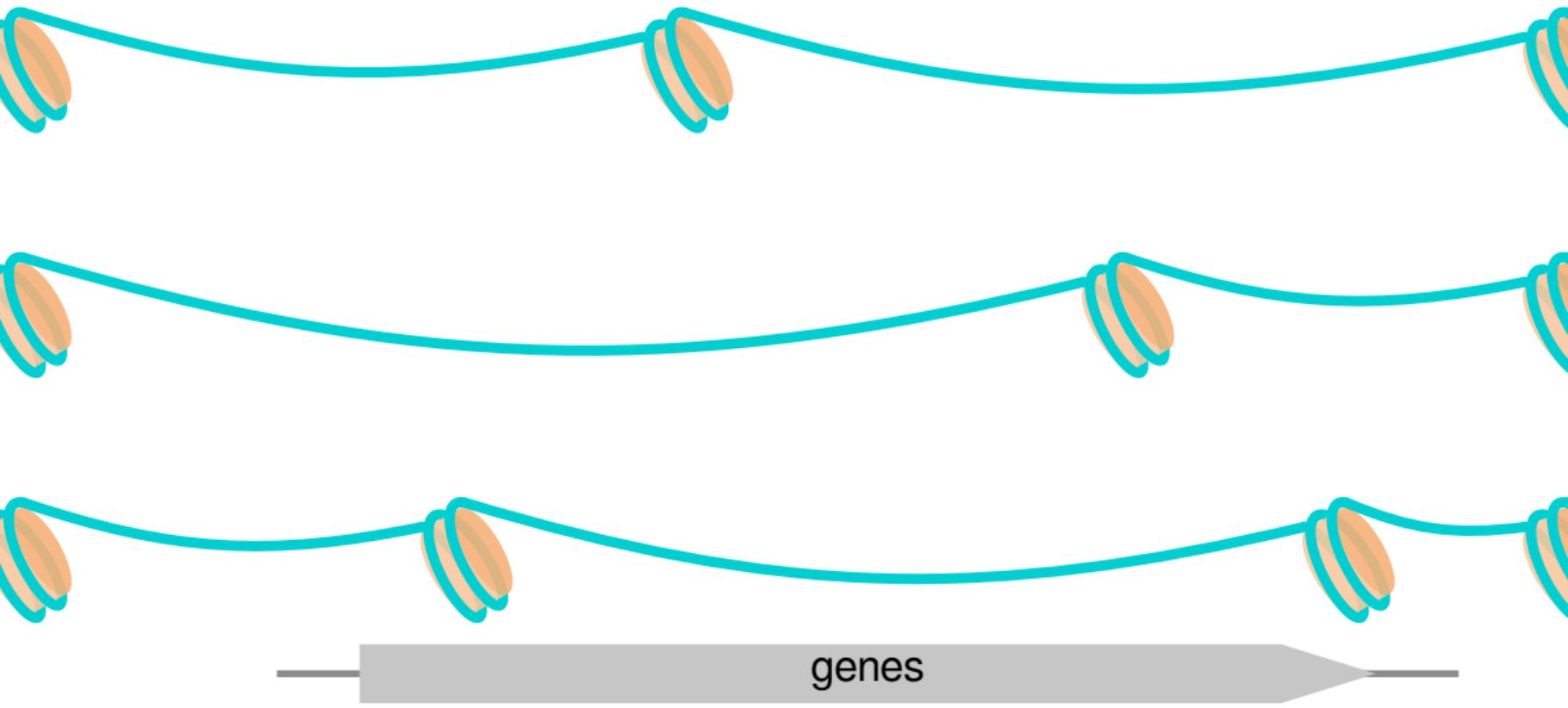
# Intragenic promoters have features of genic promoters

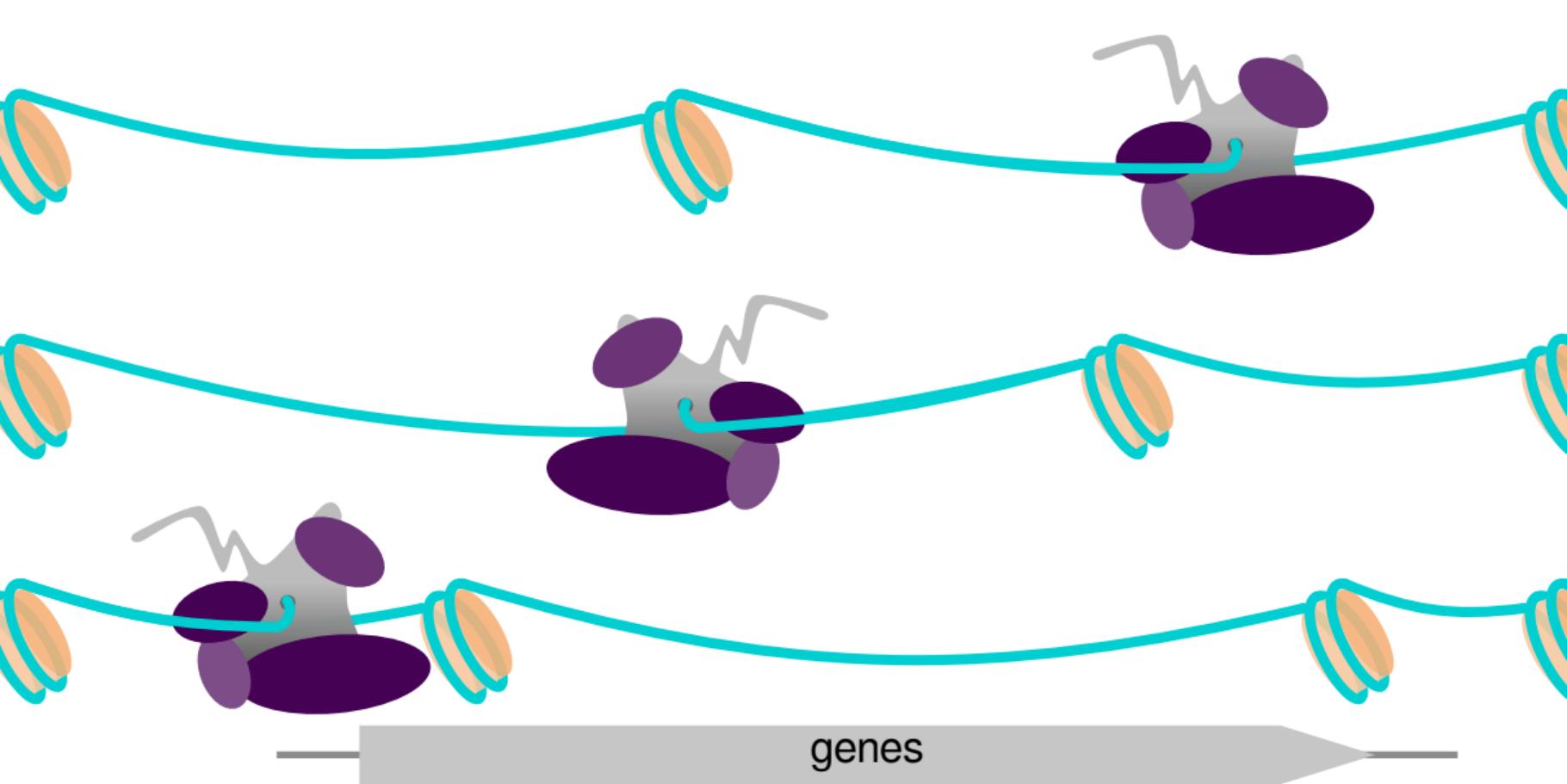


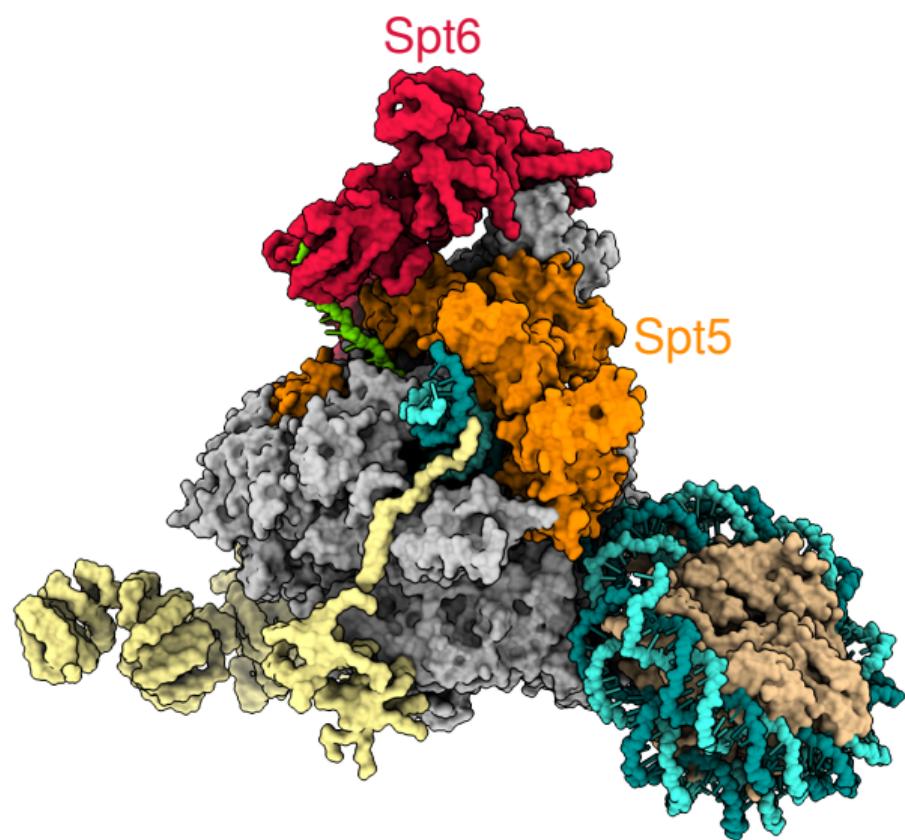
# Intragenic promoters have features of genic promoters



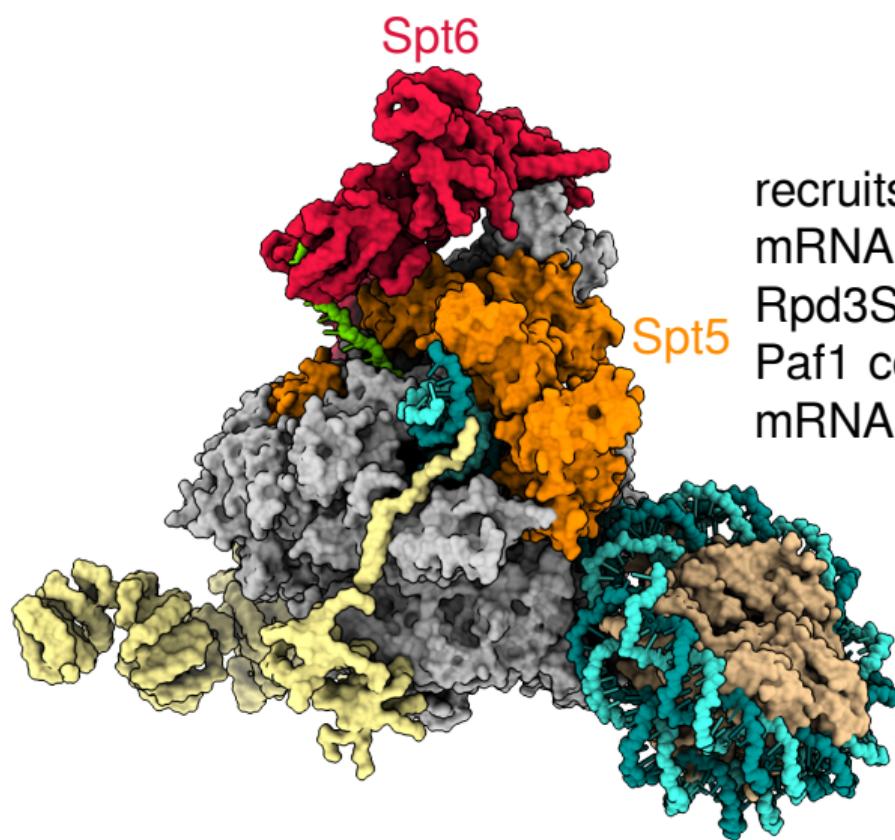








Vos *et al.* (2018). *Nature*  
Farnung *et al.* (2018). *Nat. Commun.*



recruits:

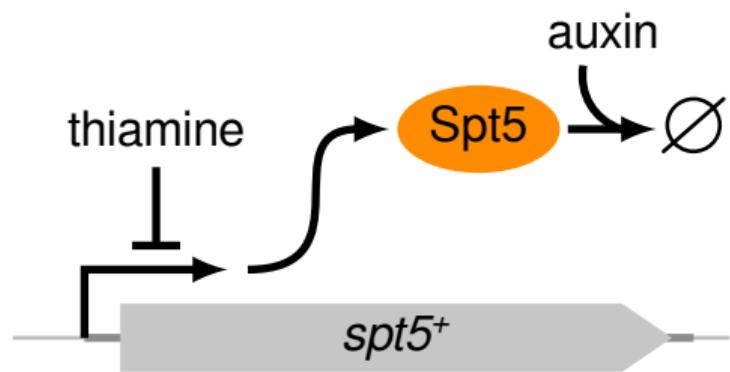
- mRNA capping enzyme
- Rpd3S HDAC complex
- Paf1 complex
- mRNA 3'-end processing factors

Vos *et al.* (2018). *Nature*  
Farnung *et al.* (2018). *Nat. Commun.*

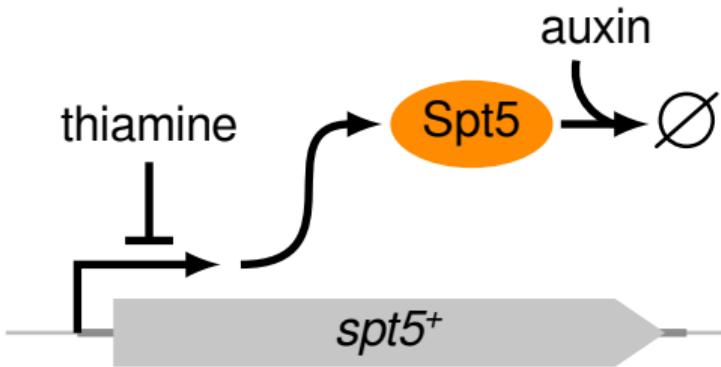
# Spt5 project collaborators

**Ameet Shetty** NET-seq,  
ChIP-seq,  
RNA-seq,  
TSS-seq,  
MNase-seq,  
etc.

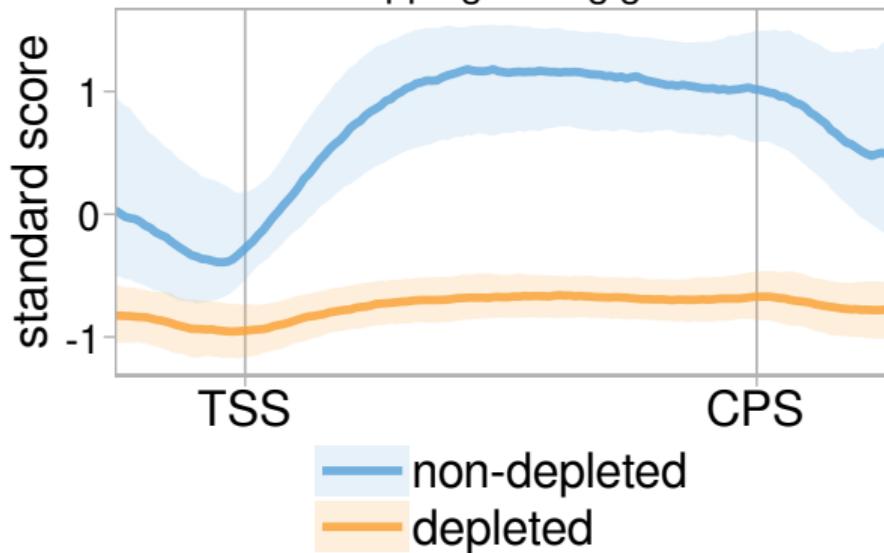
# Spt5 depletion system



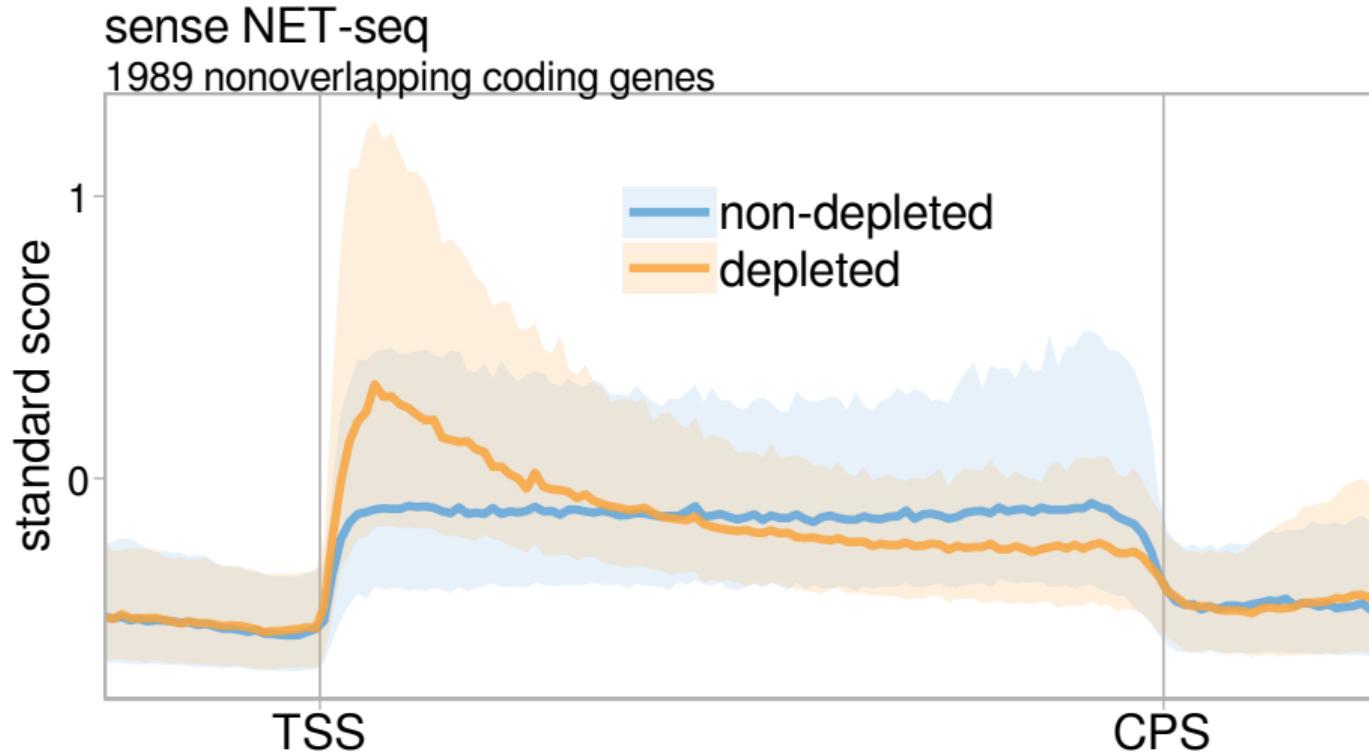
# Spt5 depletion system



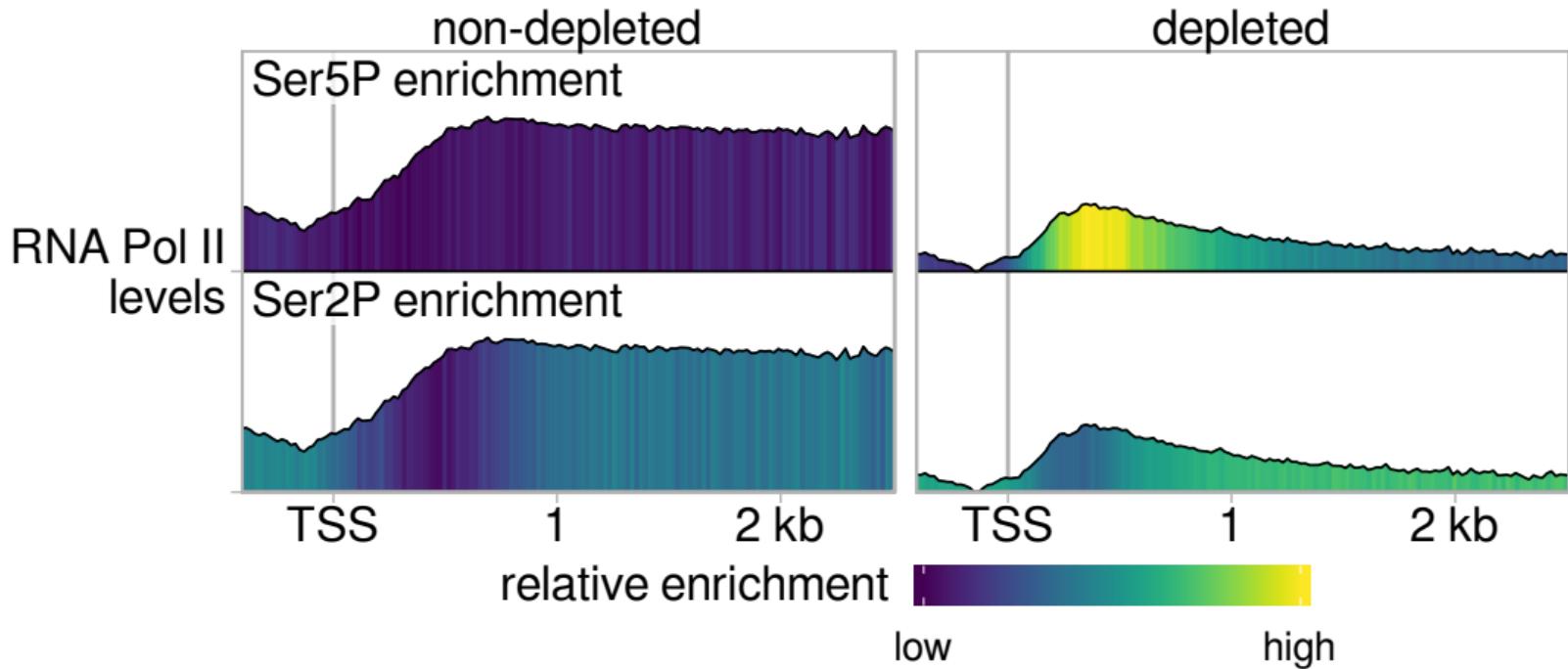
Spt5 ChIP-seq  
1989 nonoverlapping coding genes



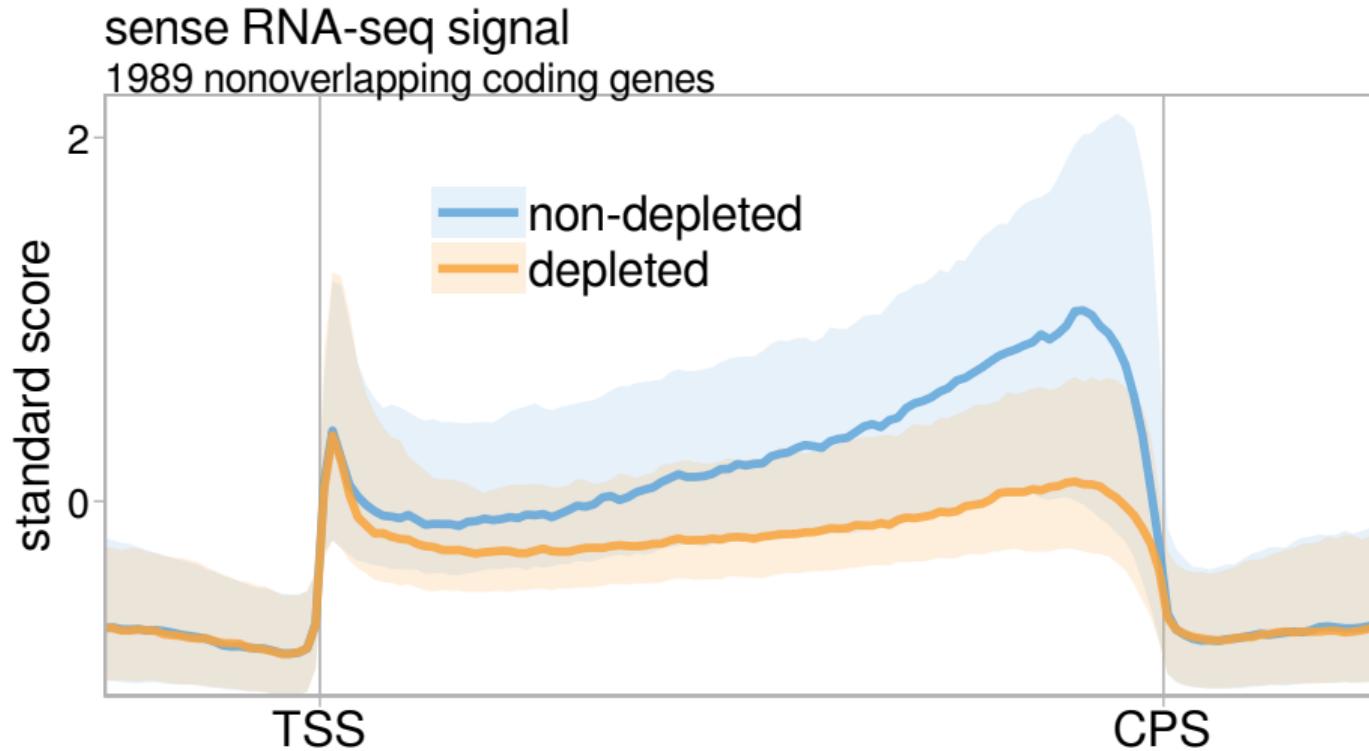
# Elongation defects upon Spt5 depletion



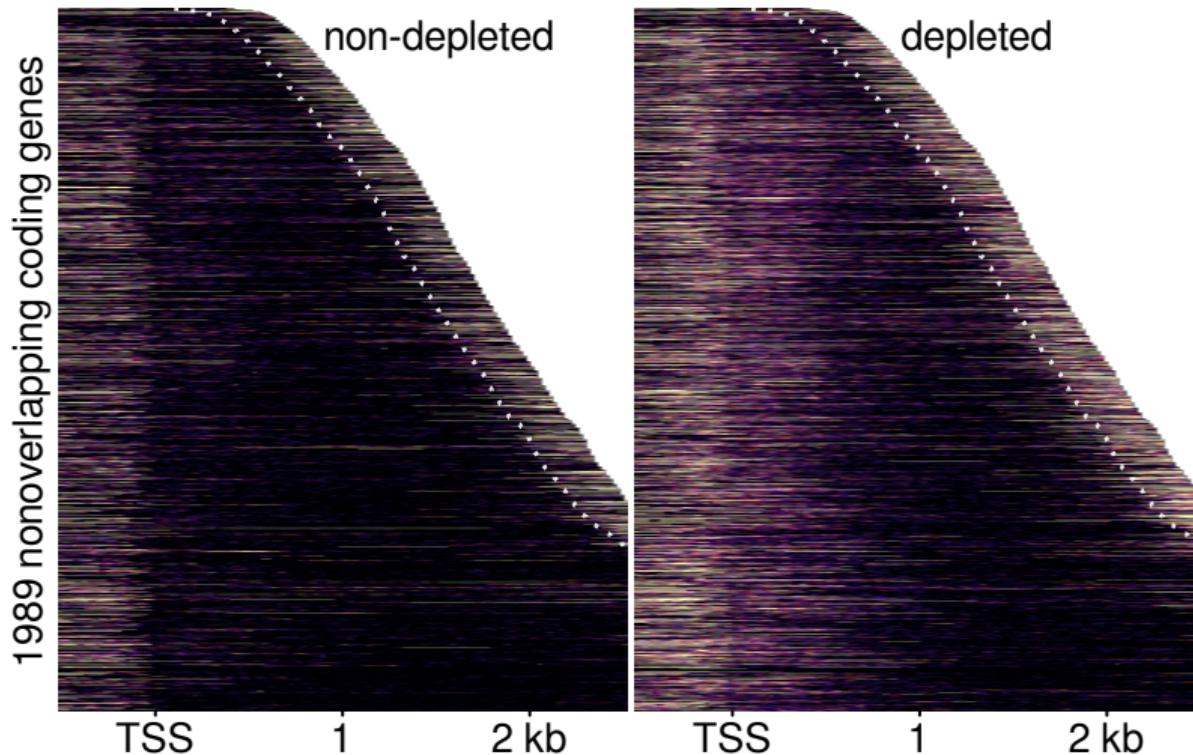
# Trapped Pol II is enriched for CTD serine 5 phosphorylation

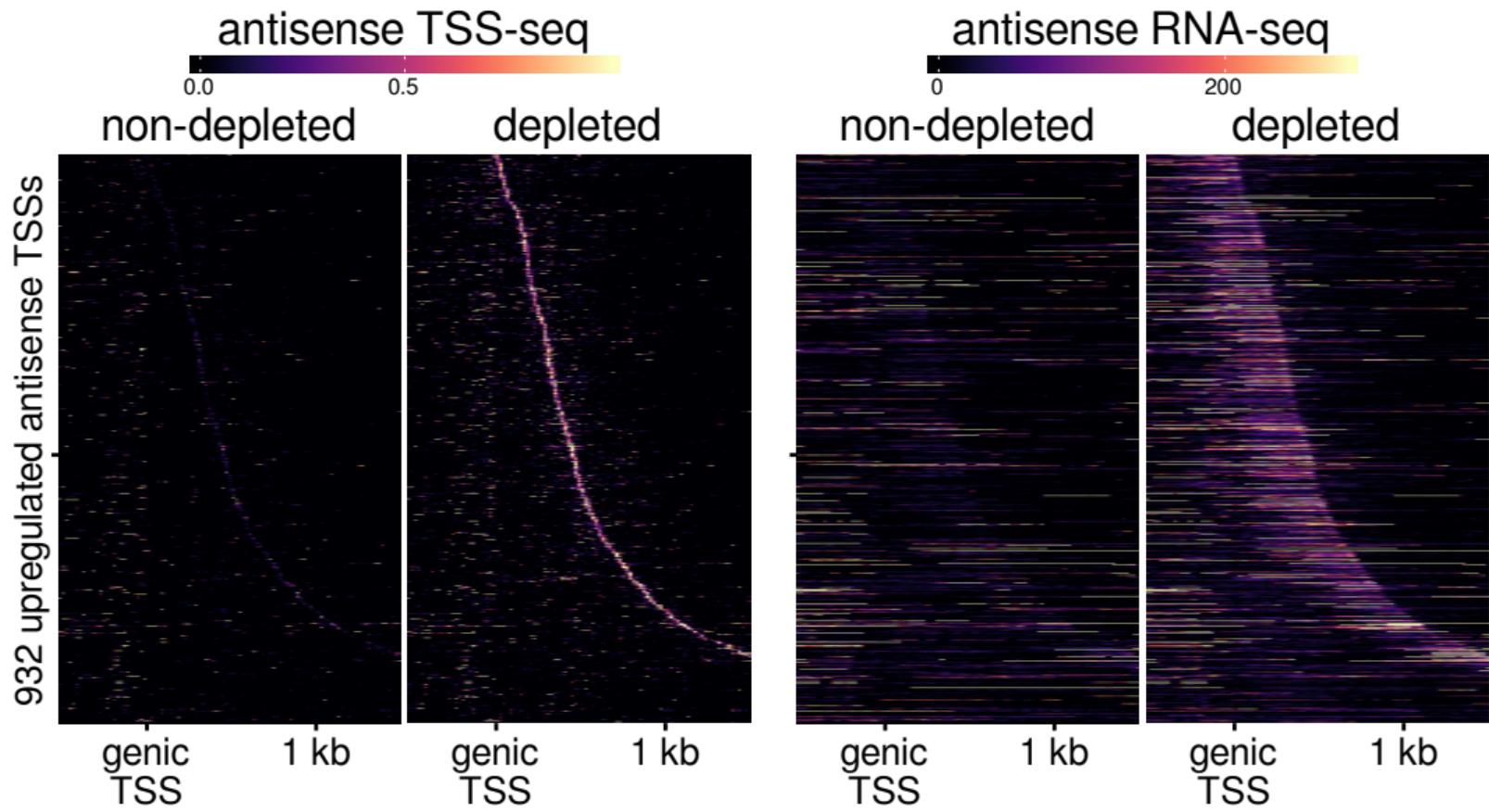


# Evidence for premature termination upon Spt5 depletion

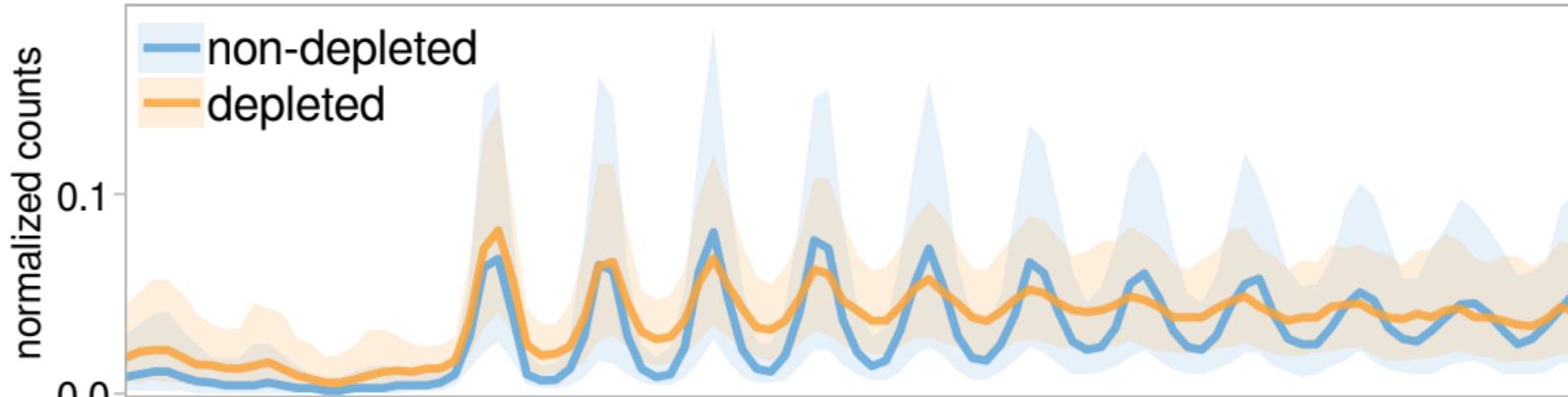


# antisense RNA-seq signal

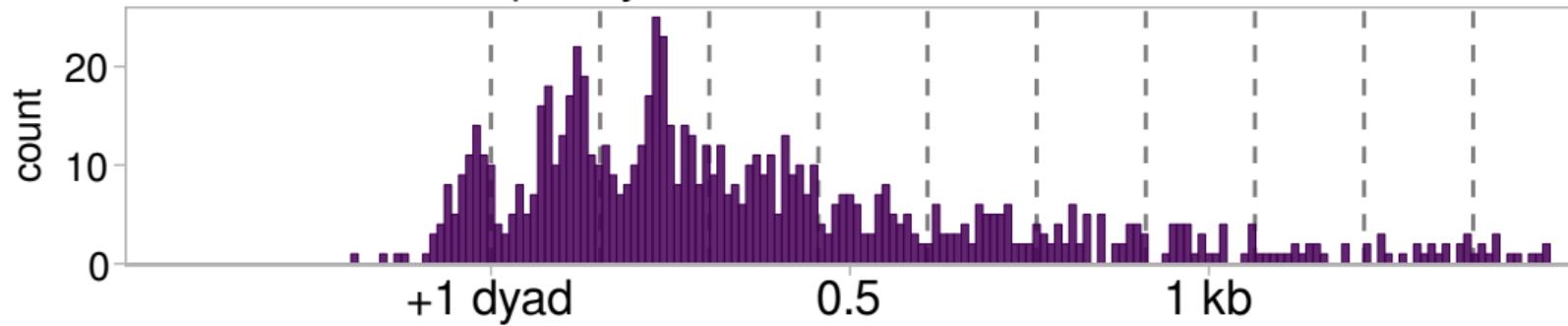




# MNase-seq dyad signal

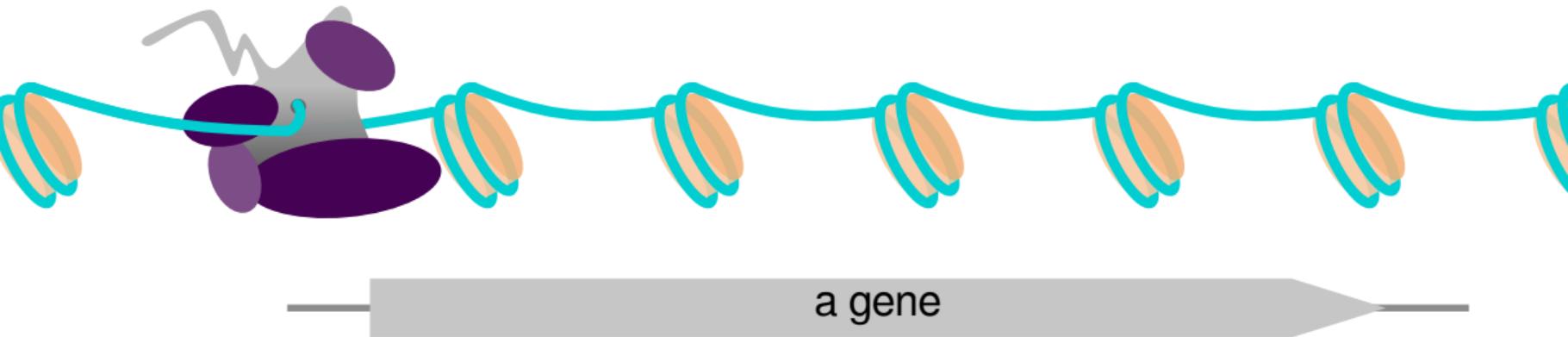


## antisense TSS frequency

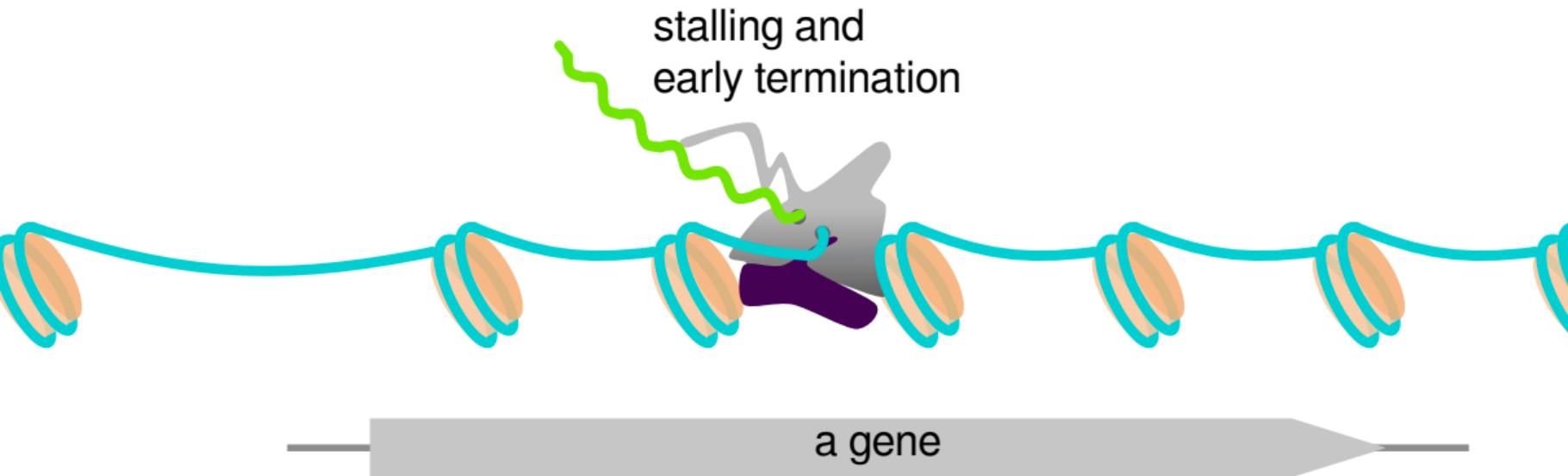


# Transcription without Spt5

initiation

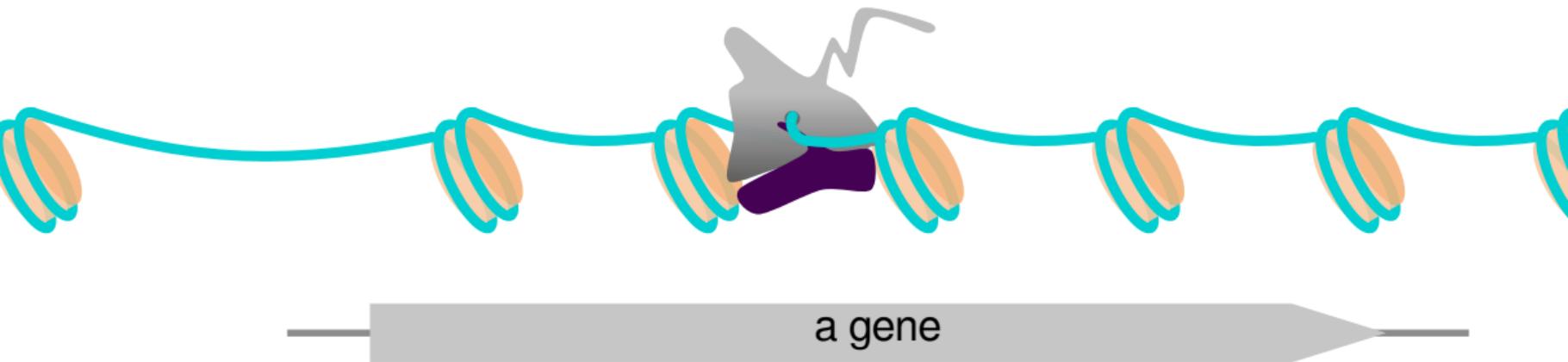


## Transcription without Spt5



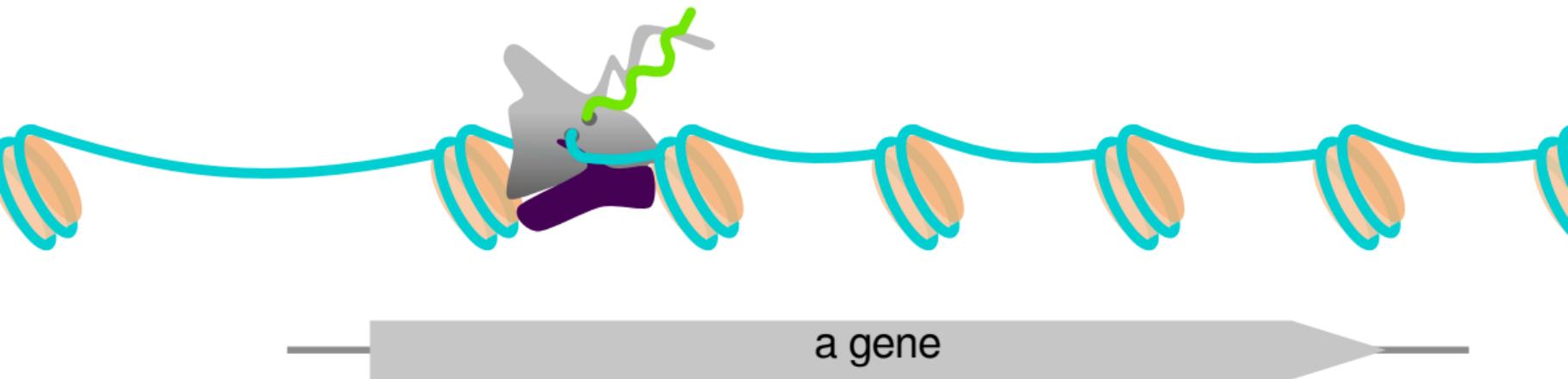
# Transcription without Spt5

possible antisense re-initiation



# Transcription without Spt5

antisense transcription



# WT intragenic transcription

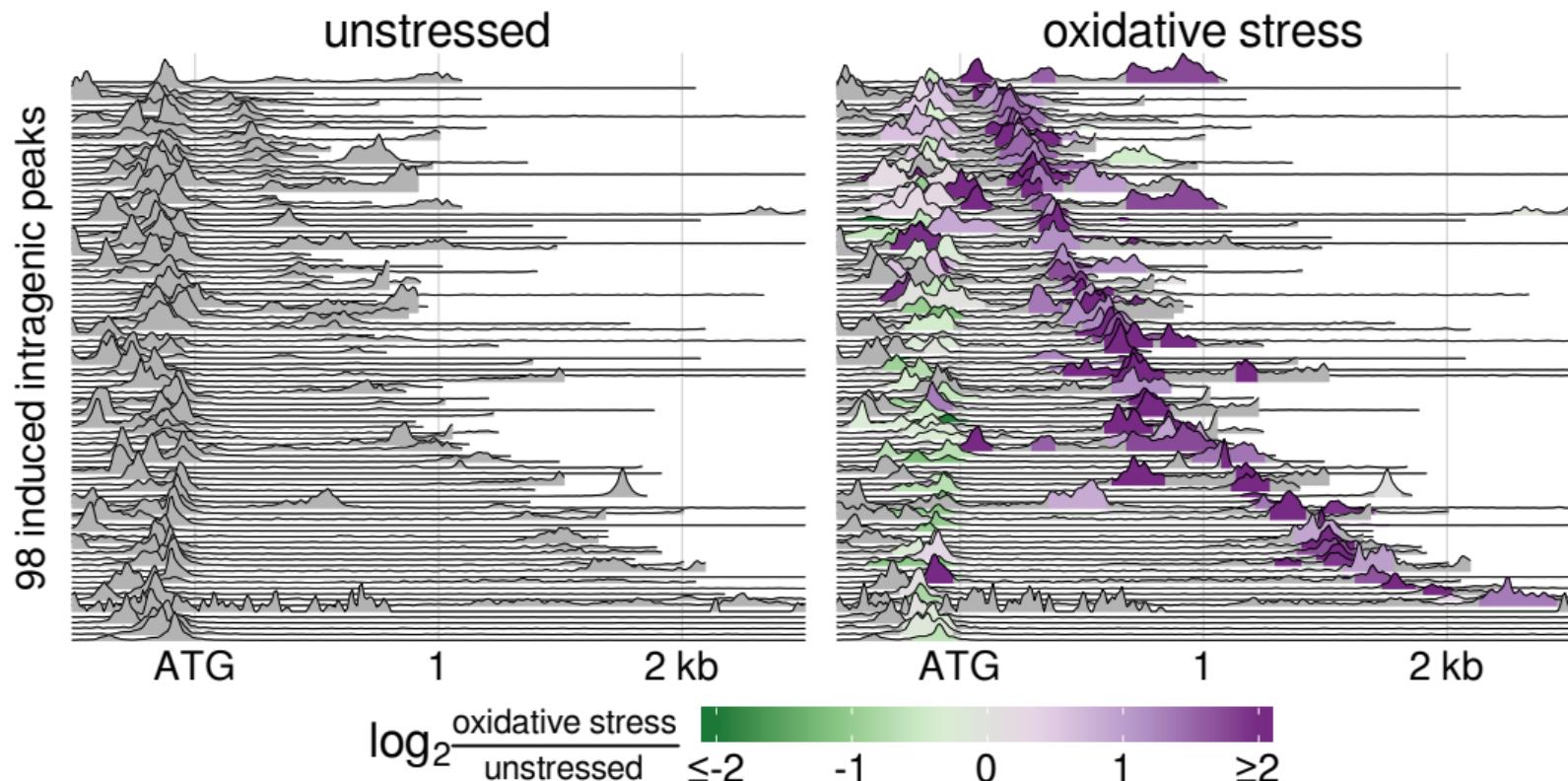
## project collaborators

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

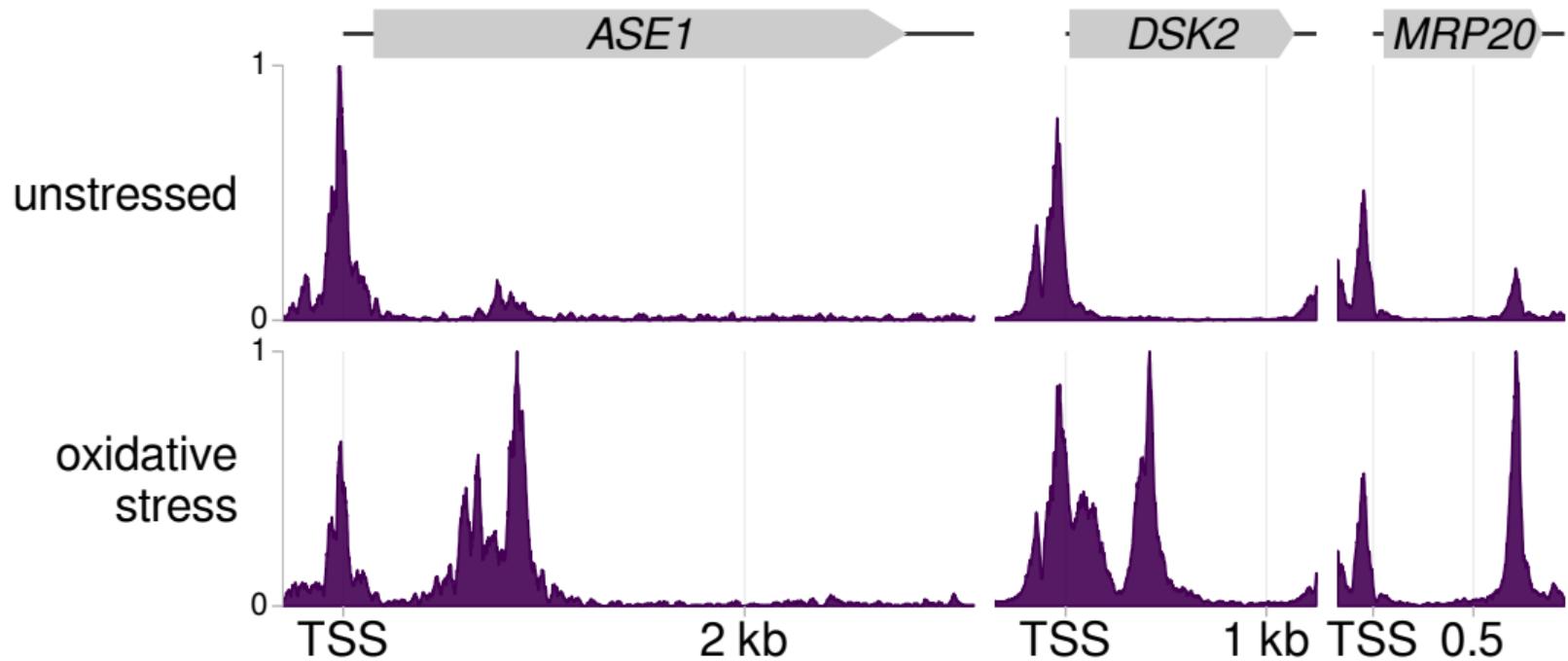
**Dan Spatt** polyribosome fractionation,  
competitive growth assays,  
and Northern blots

**James Warner** Northern blots

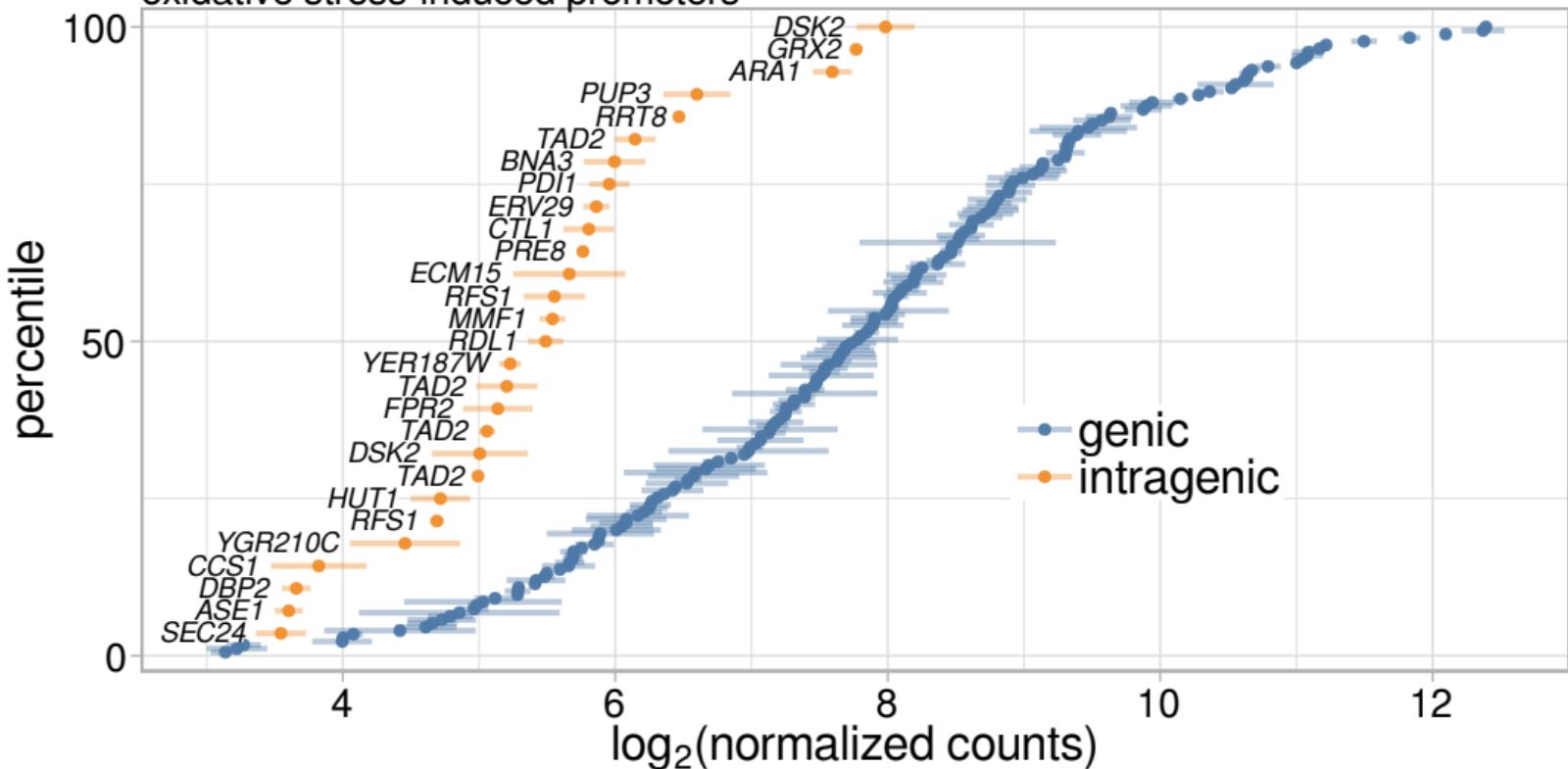
## relative TFIIB ChIP-nexus protection



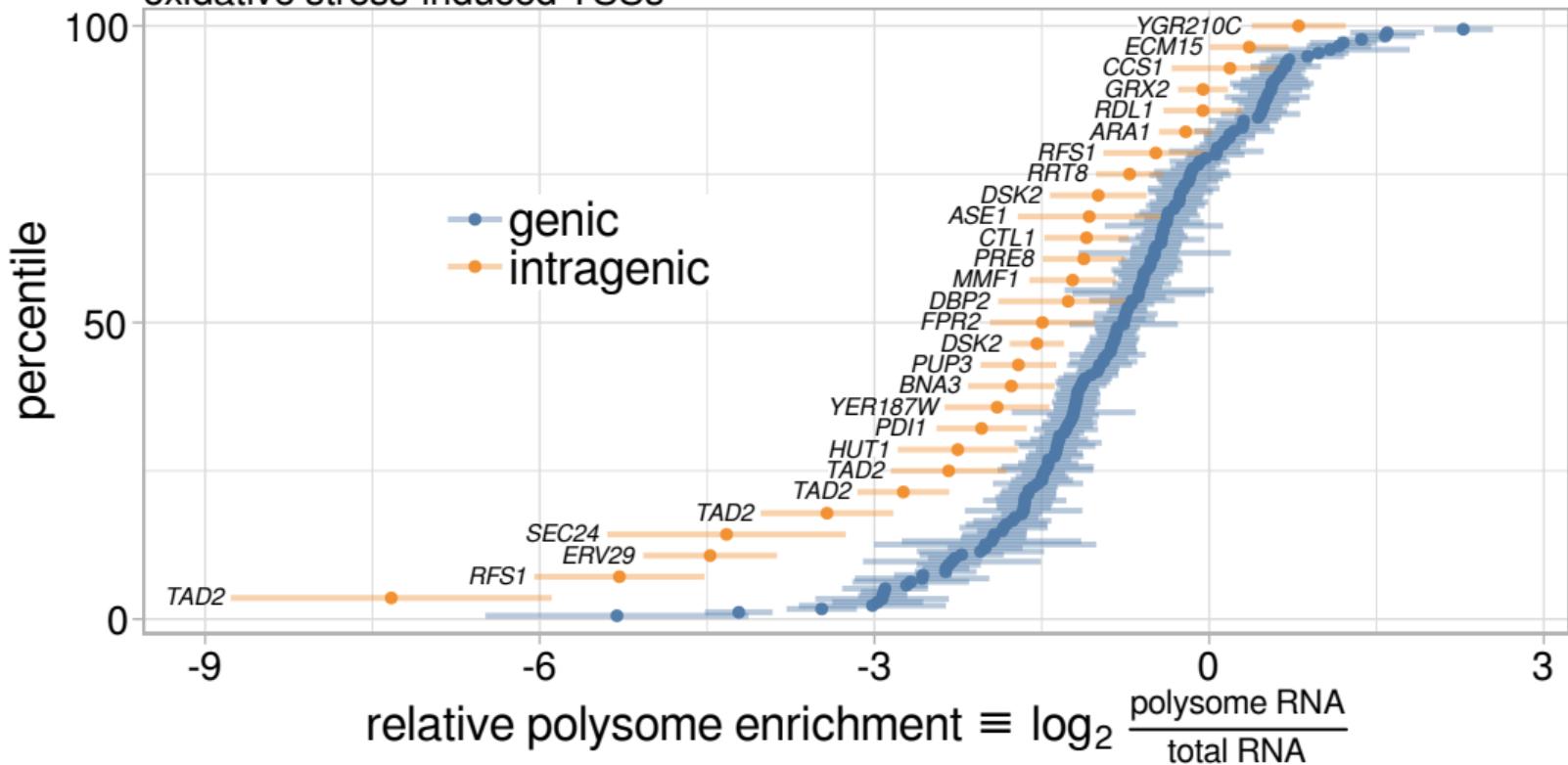
### relative TFIIB ChIP-nexus protection



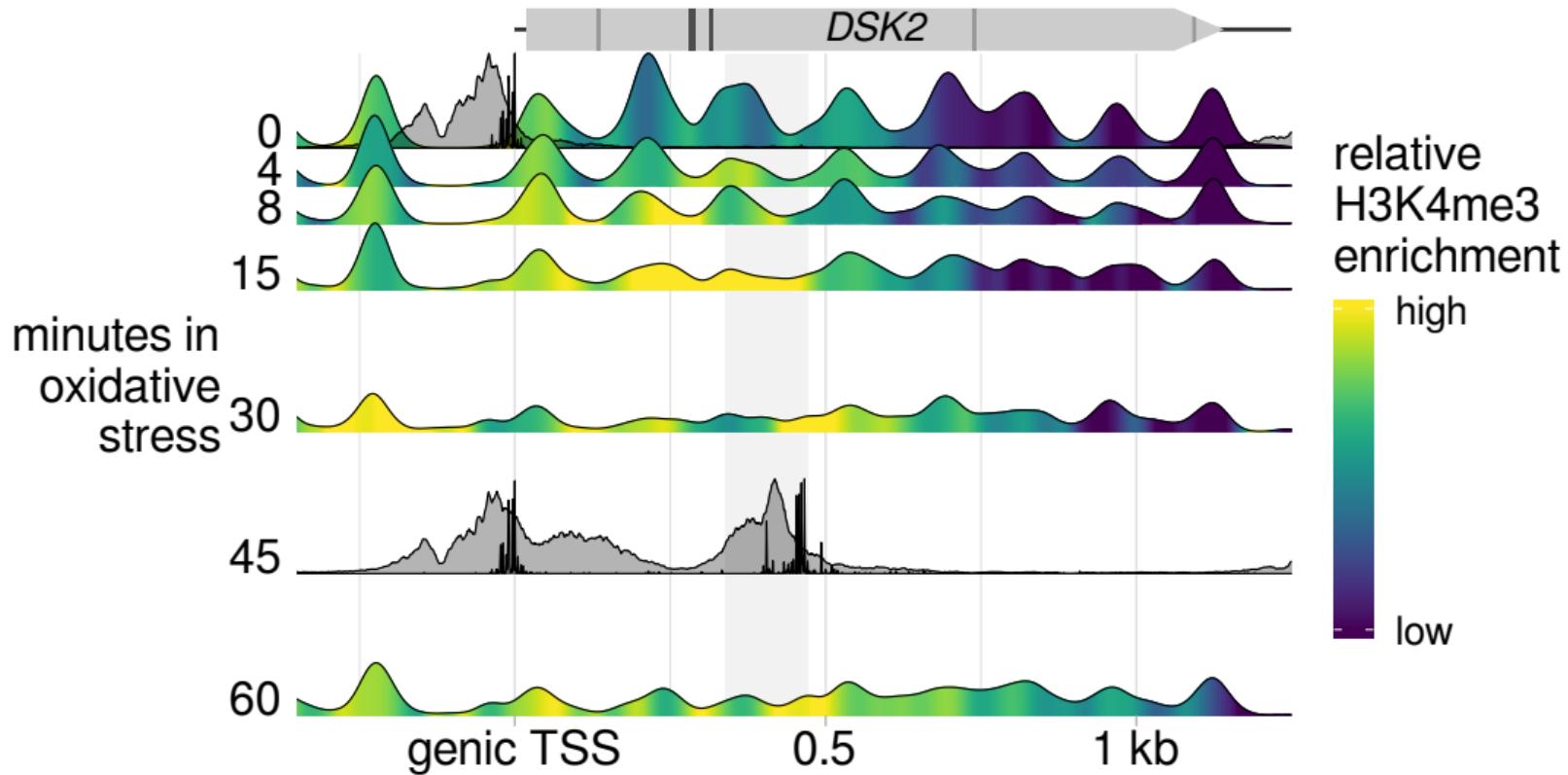
# TSS expression levels in oxidative stress oxidative stress-induced promoters



## polysome enrichment in oxidative stress oxidative stress-induced TSSs

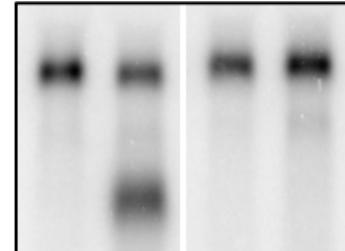


# MNase dyads, TFIIB protection, and sense TSSs



MNase-ChIP-seq: Weiner *et al.* (2015). Mol. Cell

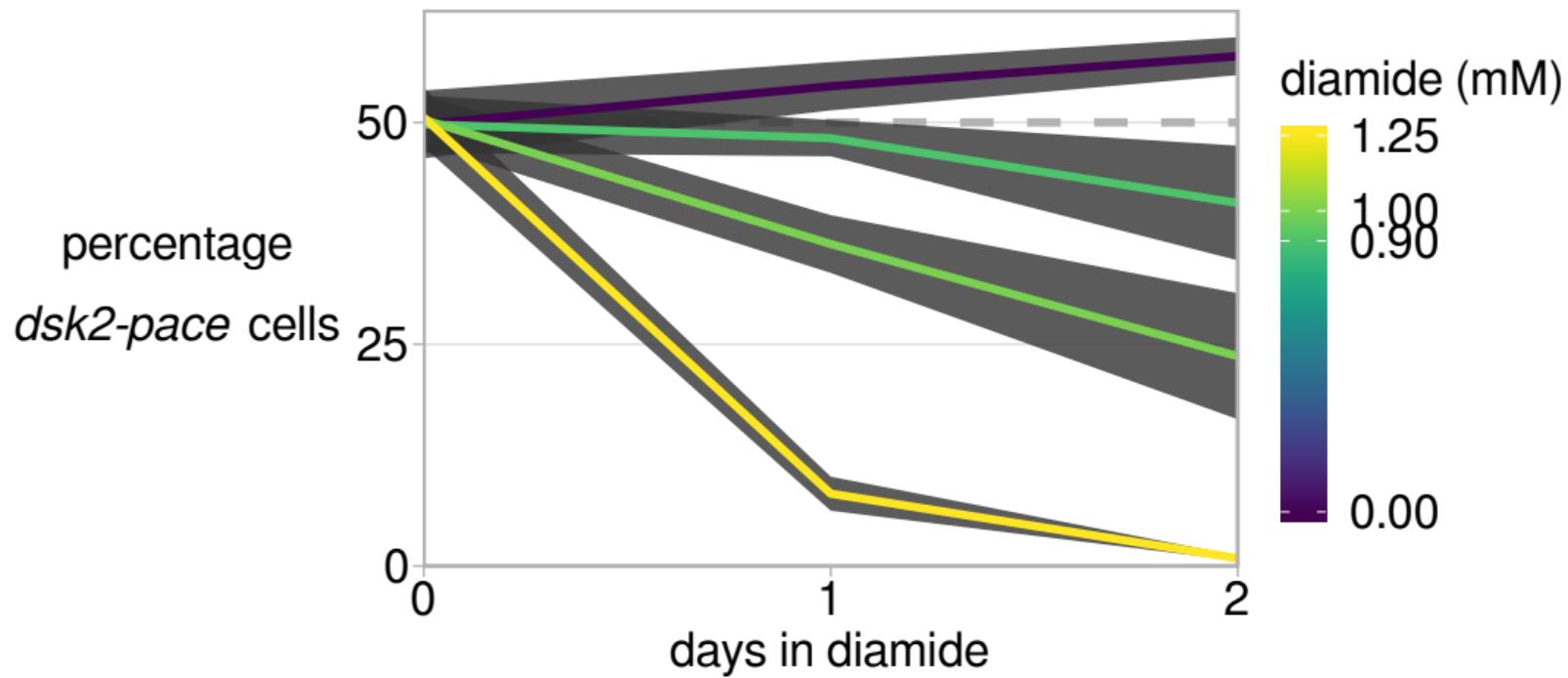
diamide:  $\frac{DSK2}{- +}$   $\frac{dsk2\text{-pace}}{- +}$



*DSK2* full-length

*DSK2* intragenic

*SNR190*



# Summary

# Acknowledgements