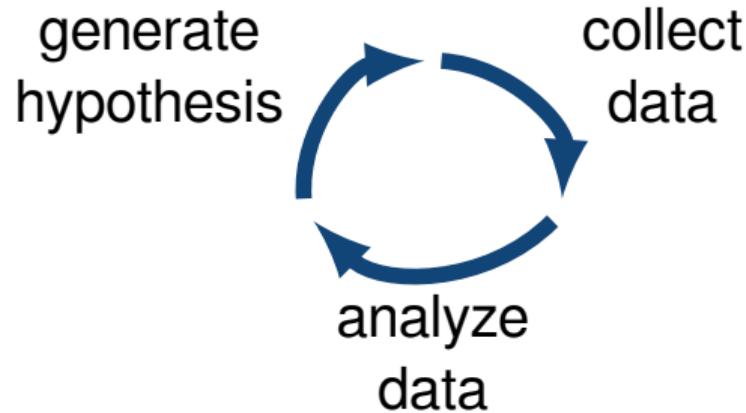


Genomic analyses of transcription elongation factors and intragenic transcription

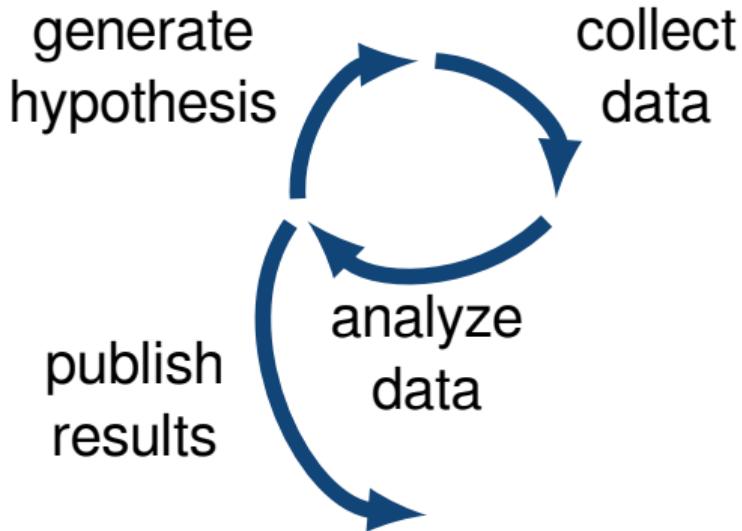
James Chuang

June 19, 2019

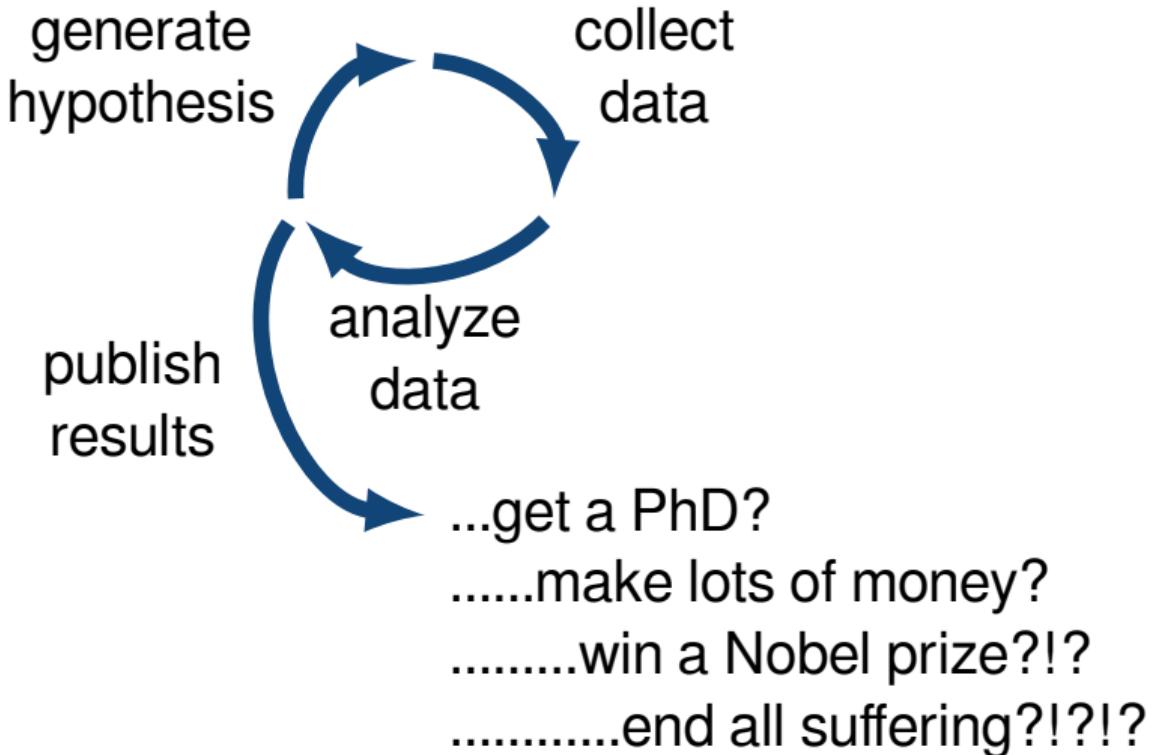
The scientific process

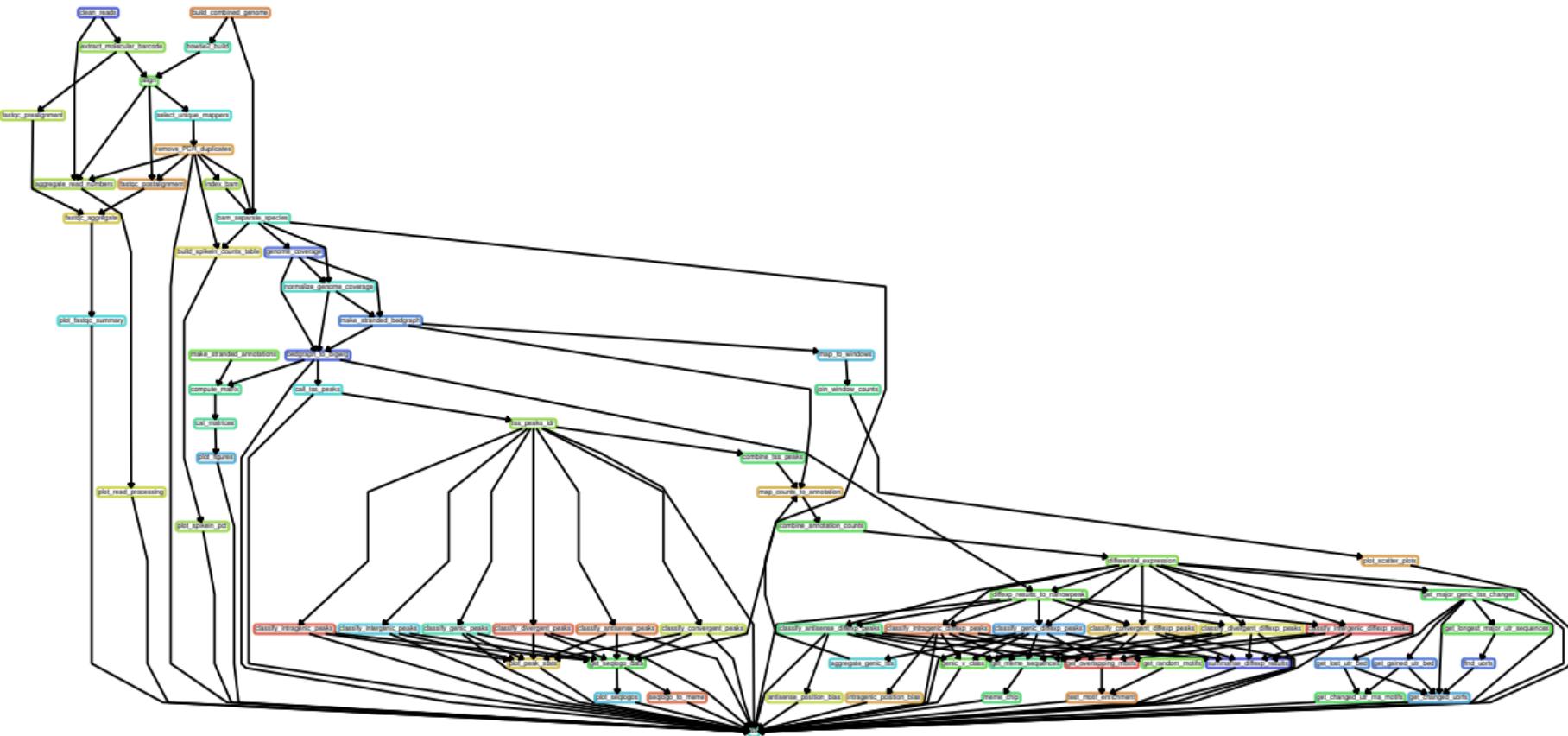


The scientific process



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)

IP Chuang James

Other(s)

Churchman, L. Stirling

Researcher(s)

Doris, Stephen M.; Viktorovskaya, Olga; Murawska, Małgorzata

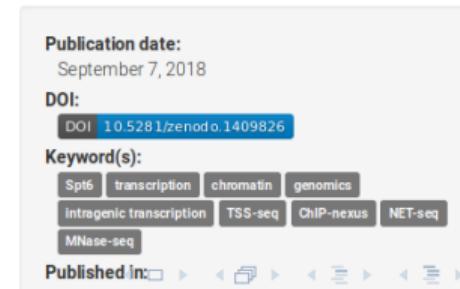
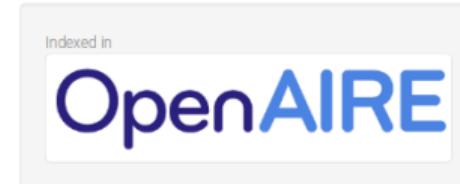
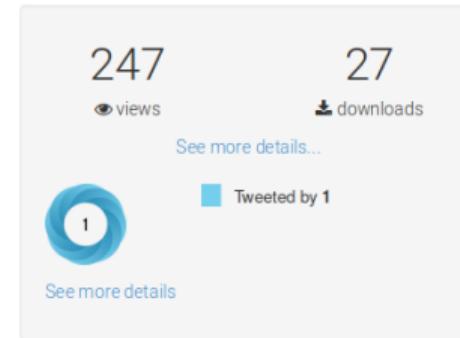
All data analyses supporting our publication "Spt6 is required for the fidelity of promoter selection". Reproduce the figures of the paper starting from raw data, as well as thousands of figures and analyses that aren't shown.

See README.md for instructions.

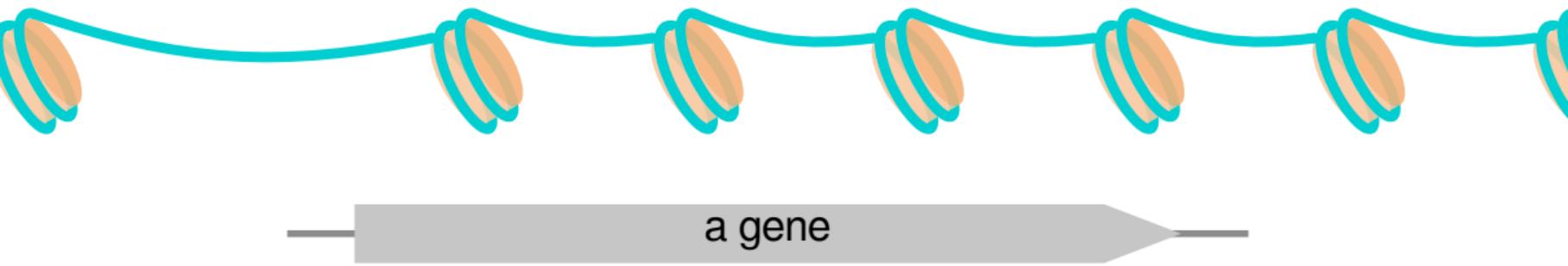
For updated versions of the pipelines used, see our [github page](#)

[Files \(47.2 GB\)](#)

Name	Size	
spt6_2018.tar.gz	47.2 GB	 Download

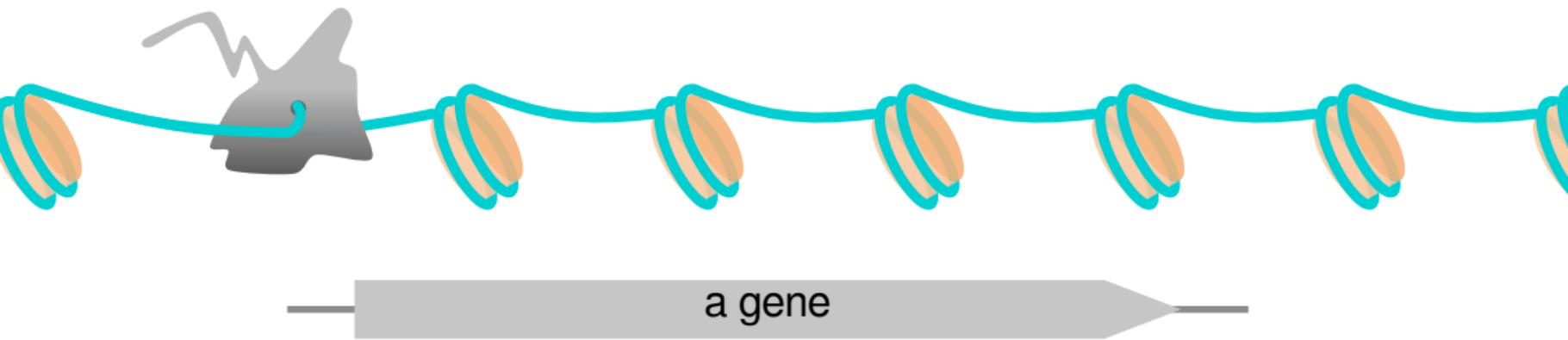


An introduction to transcription

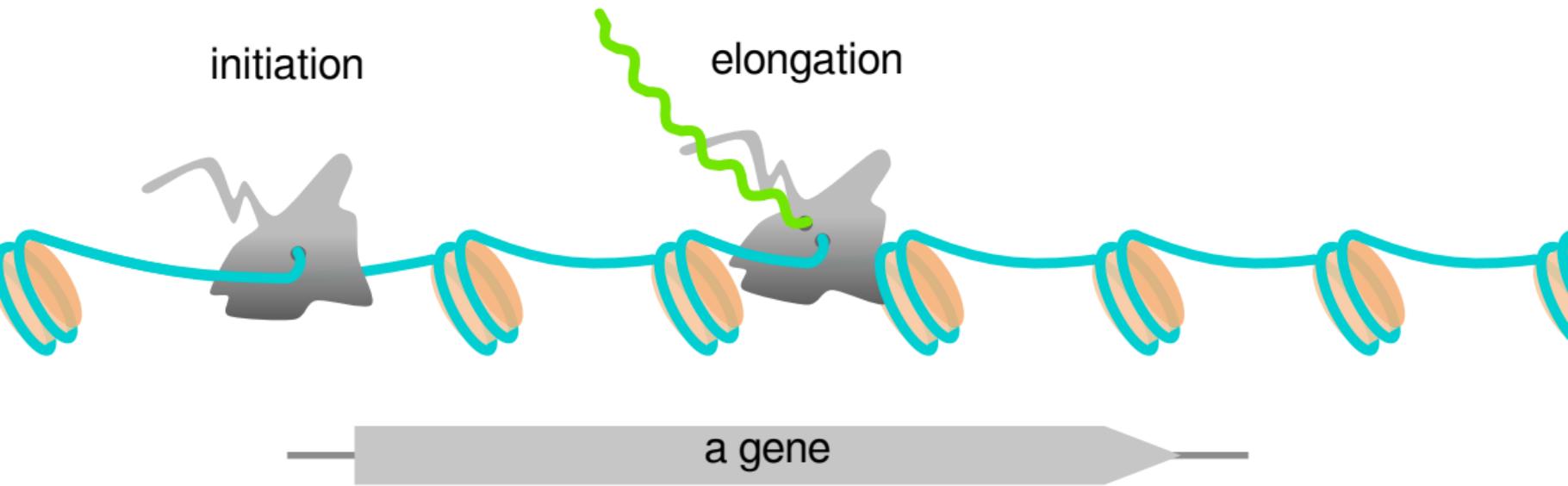


An introduction to transcription

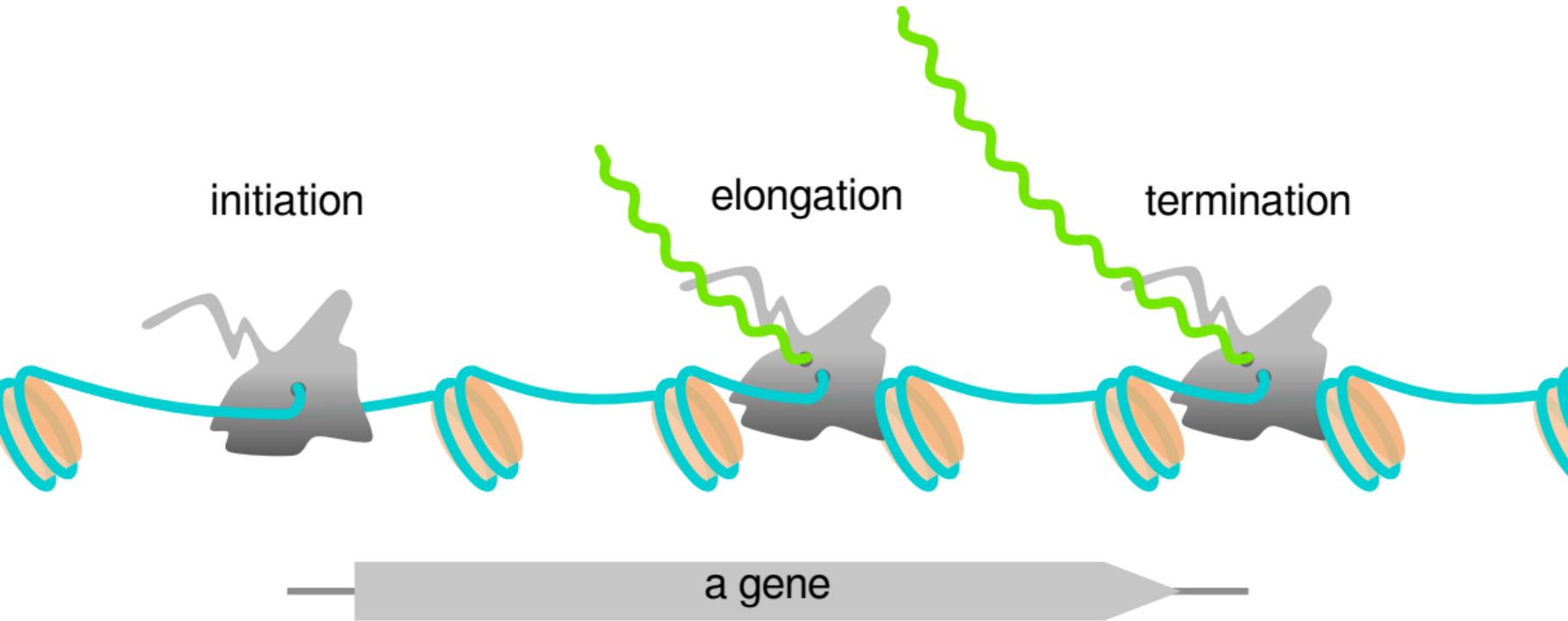
initiation



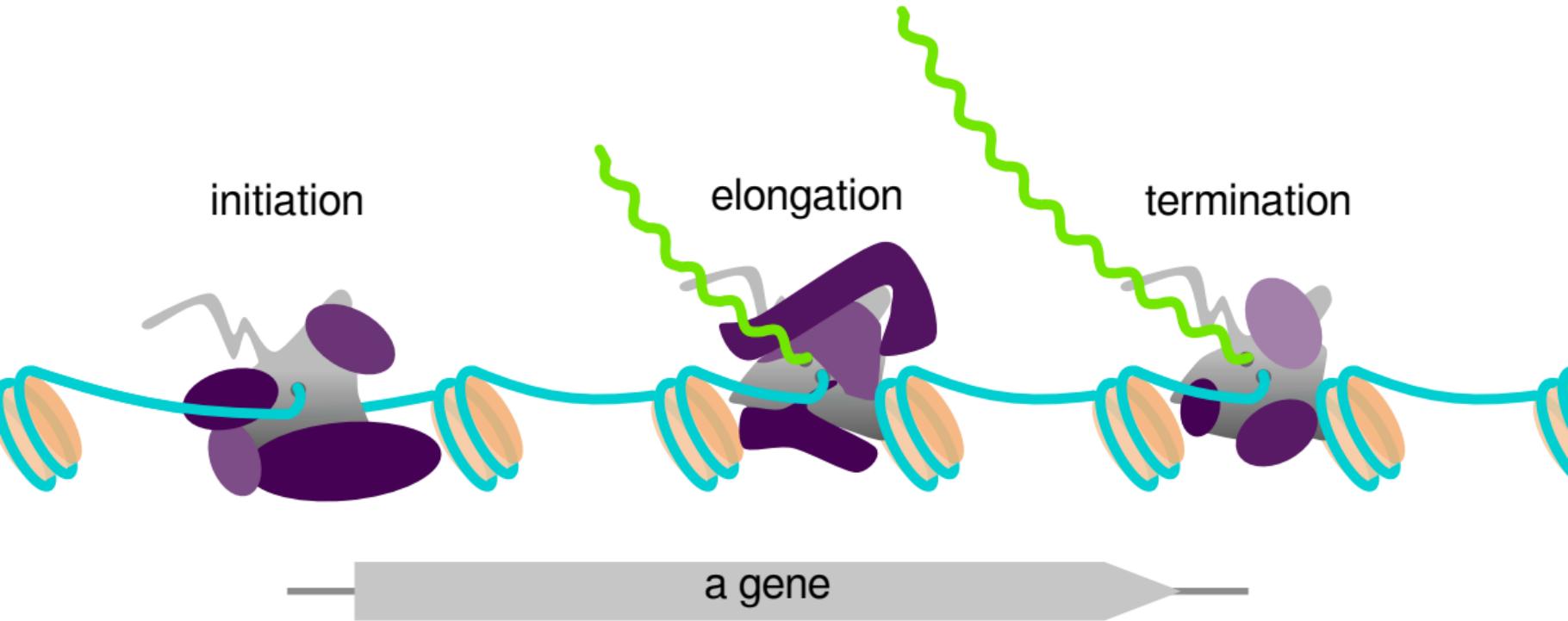
An introduction to transcription

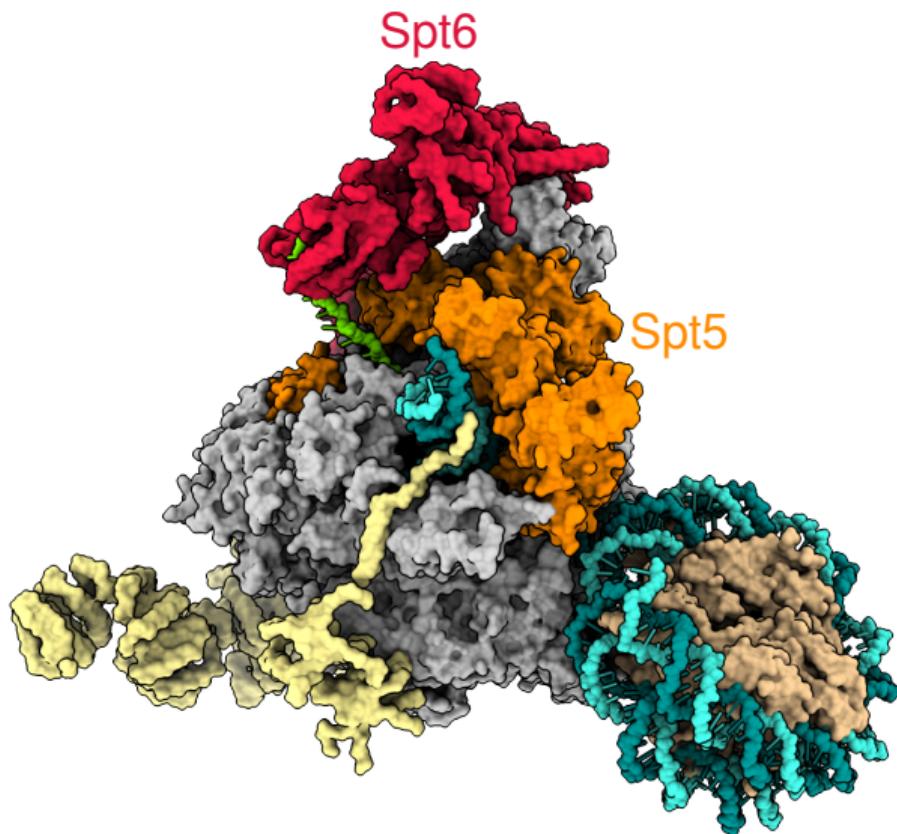


An introduction to transcription

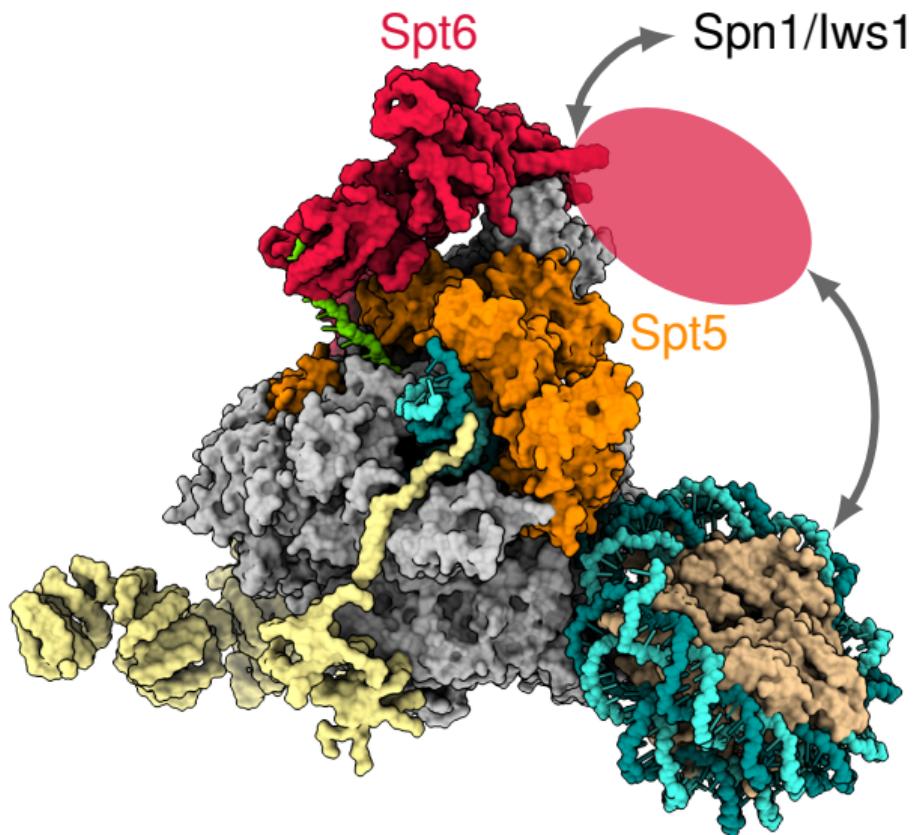


An introduction to transcription





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

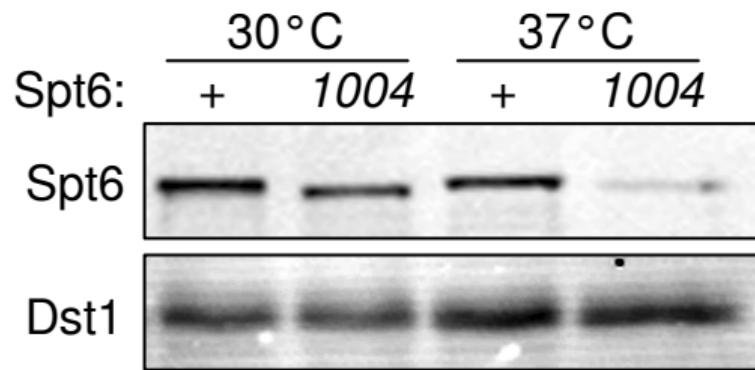
Steve Doris TSS-seq and ChIP-nexus

Olga Viktorovskaya MNase-seq

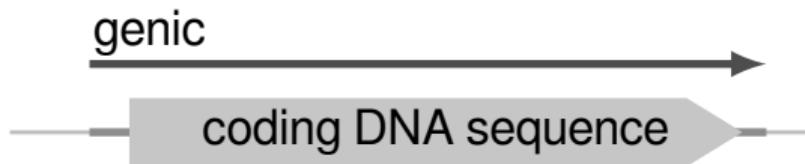
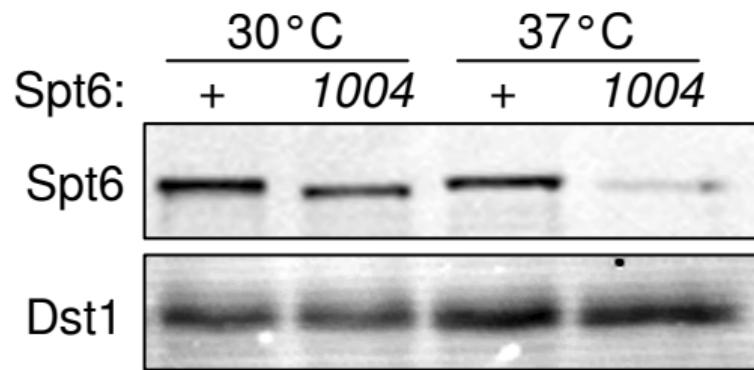
Magdalena Murawska NET-seq

Dan Spatt Northern, Western, and ChIP experiments

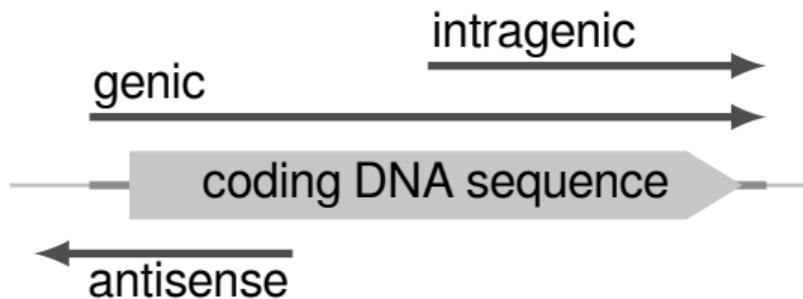
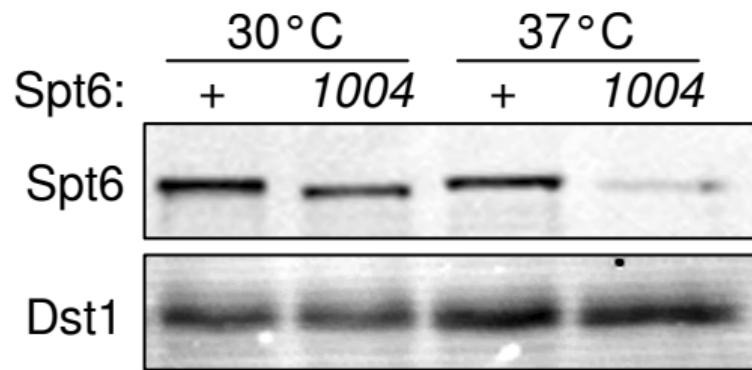
The *spt6-1004* mutant expresses intragenic transcripts

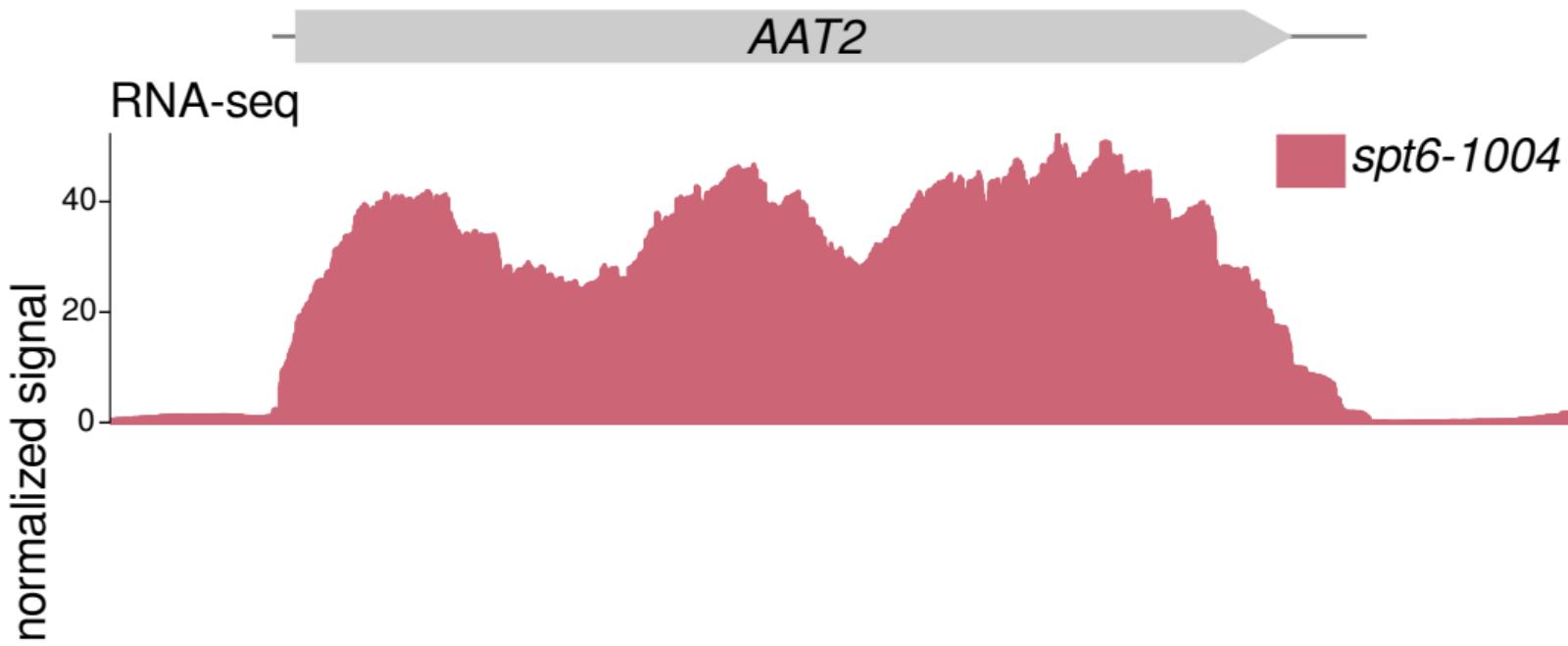


The *spt6-1004* mutant expresses intragenic transcripts

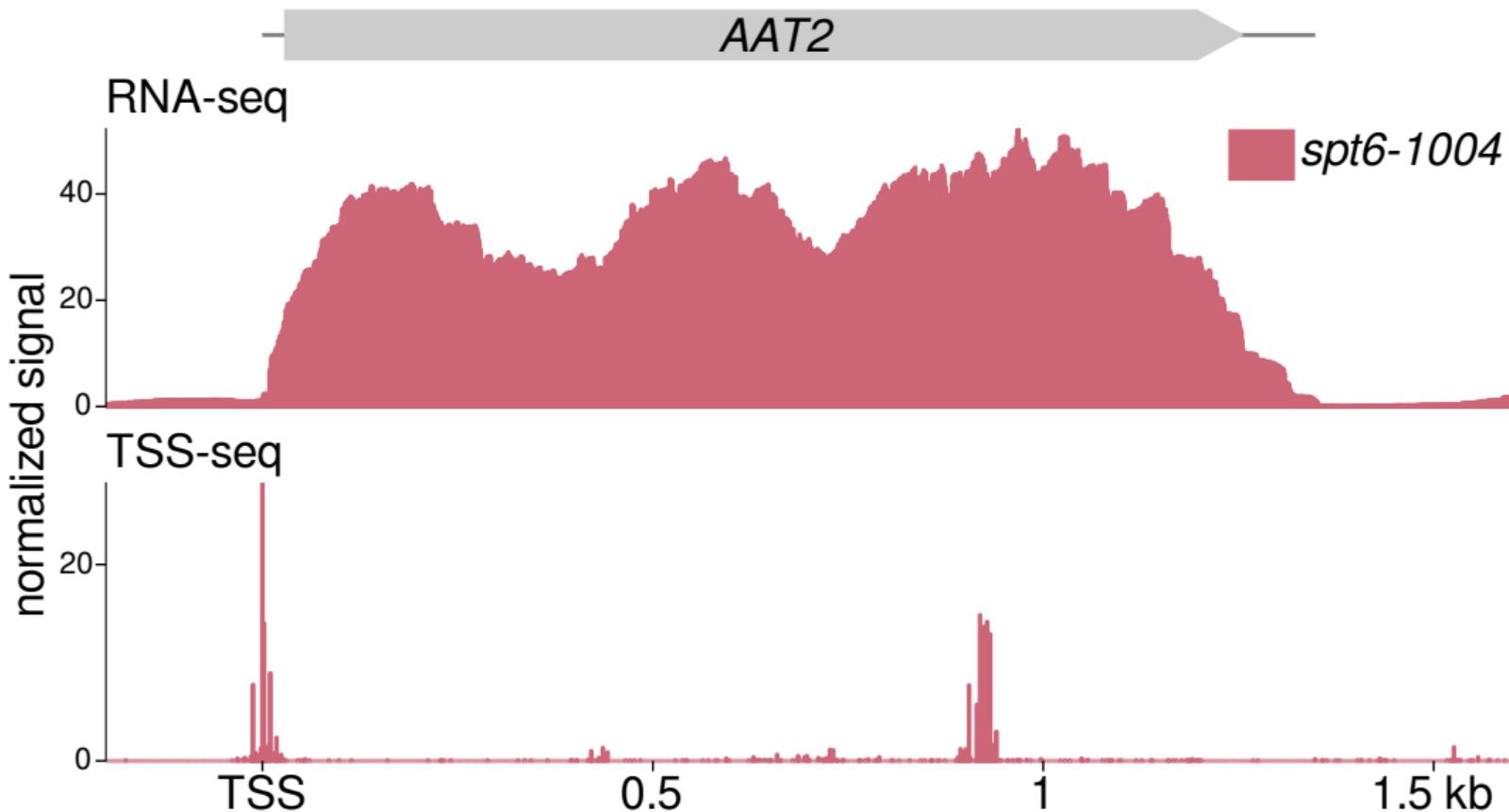


The *spt6-1004* mutant expresses intragenic transcripts



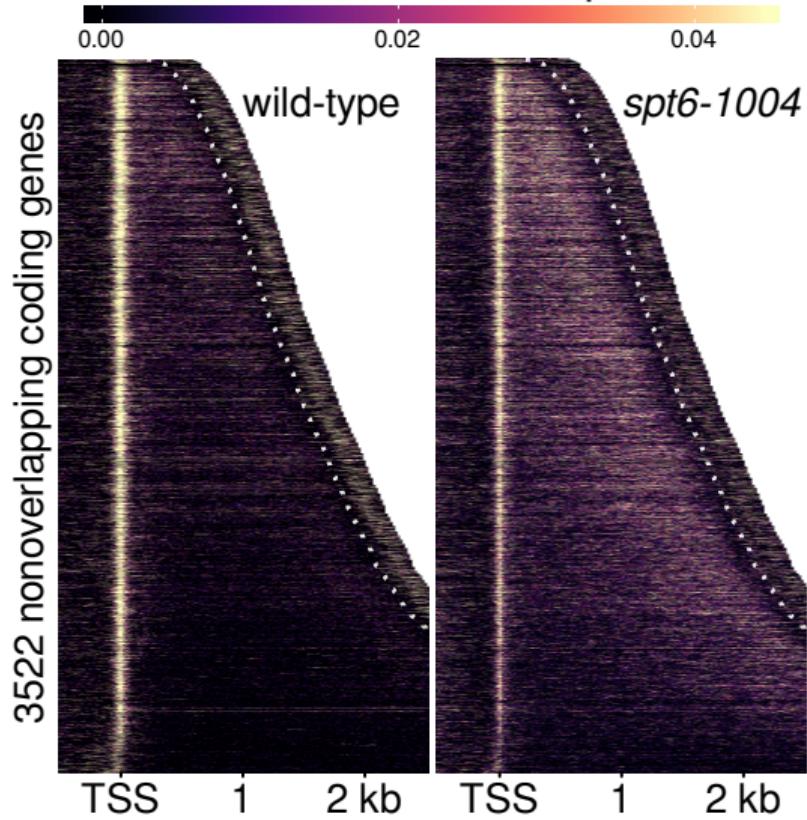


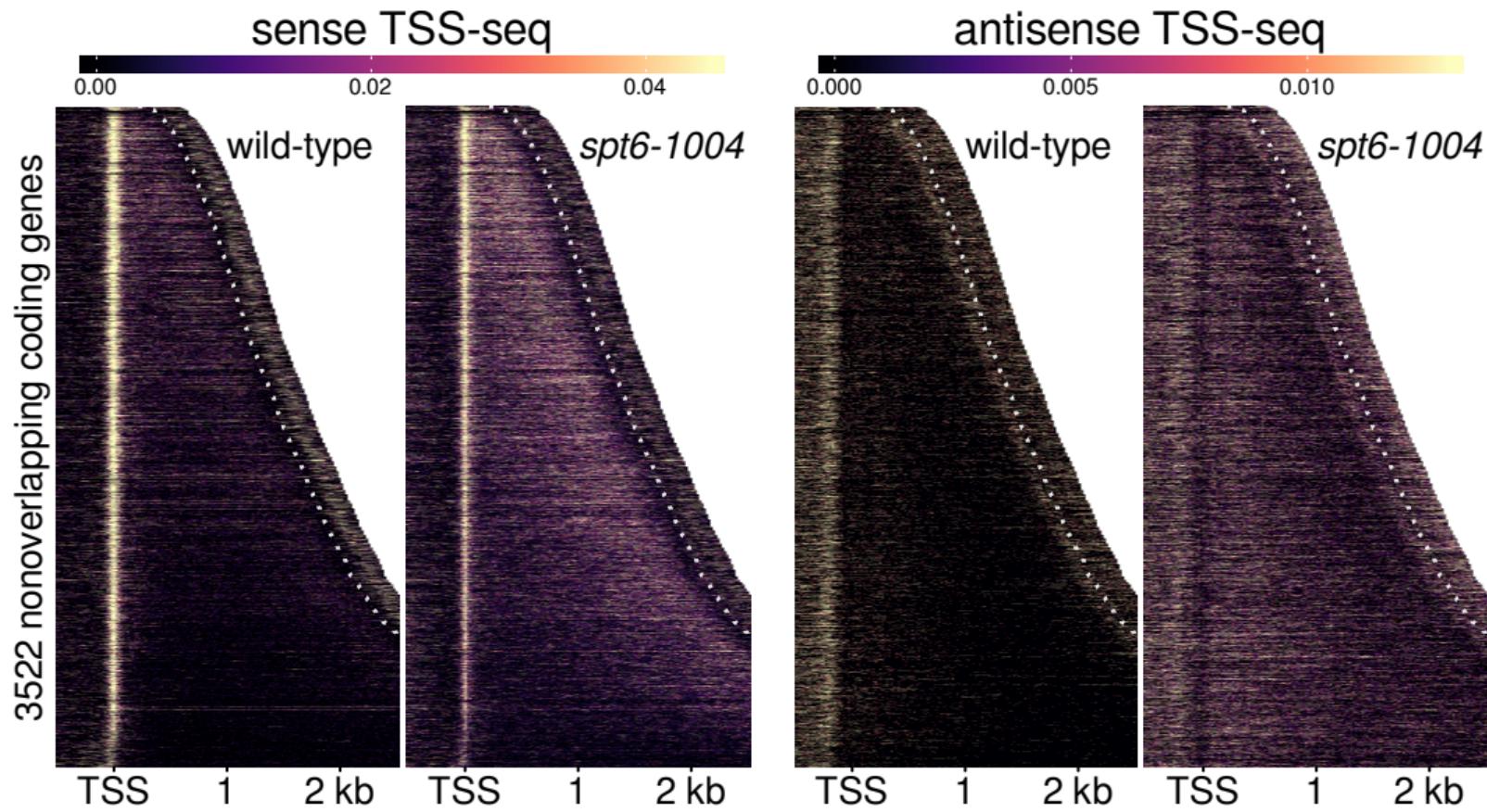
RNA-seq: Uwimana *et al.* (2017). *Nucleic Acids Res.*



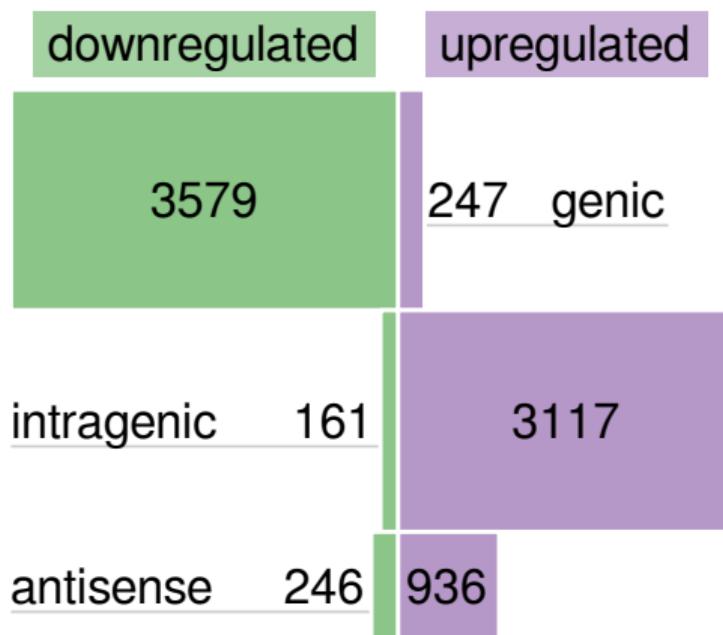
RNA-seq: Uwimana *et al.* (2017). *Nucleic Acids Res.*

sense TSS-seq

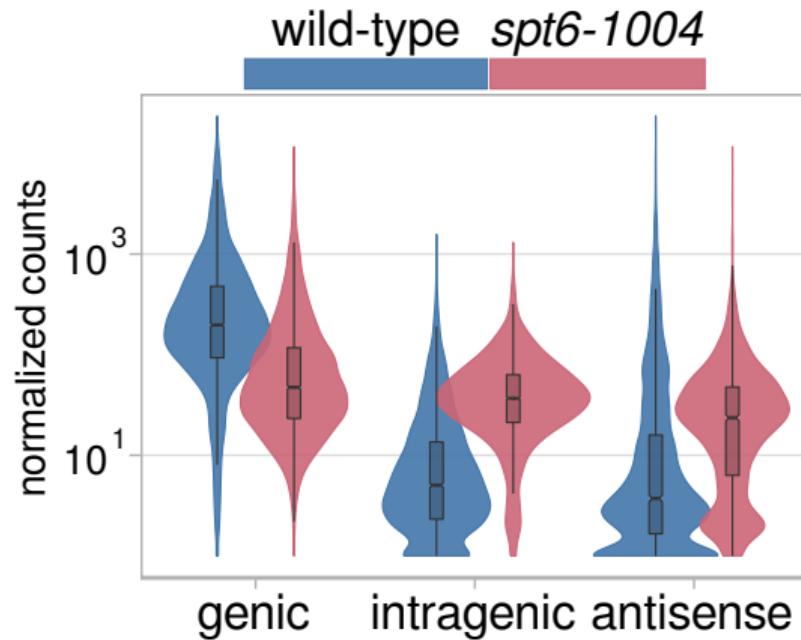
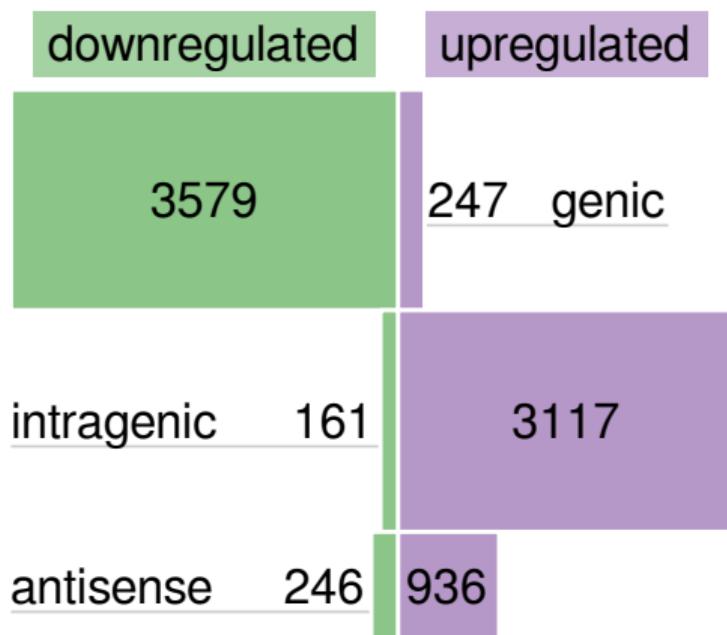




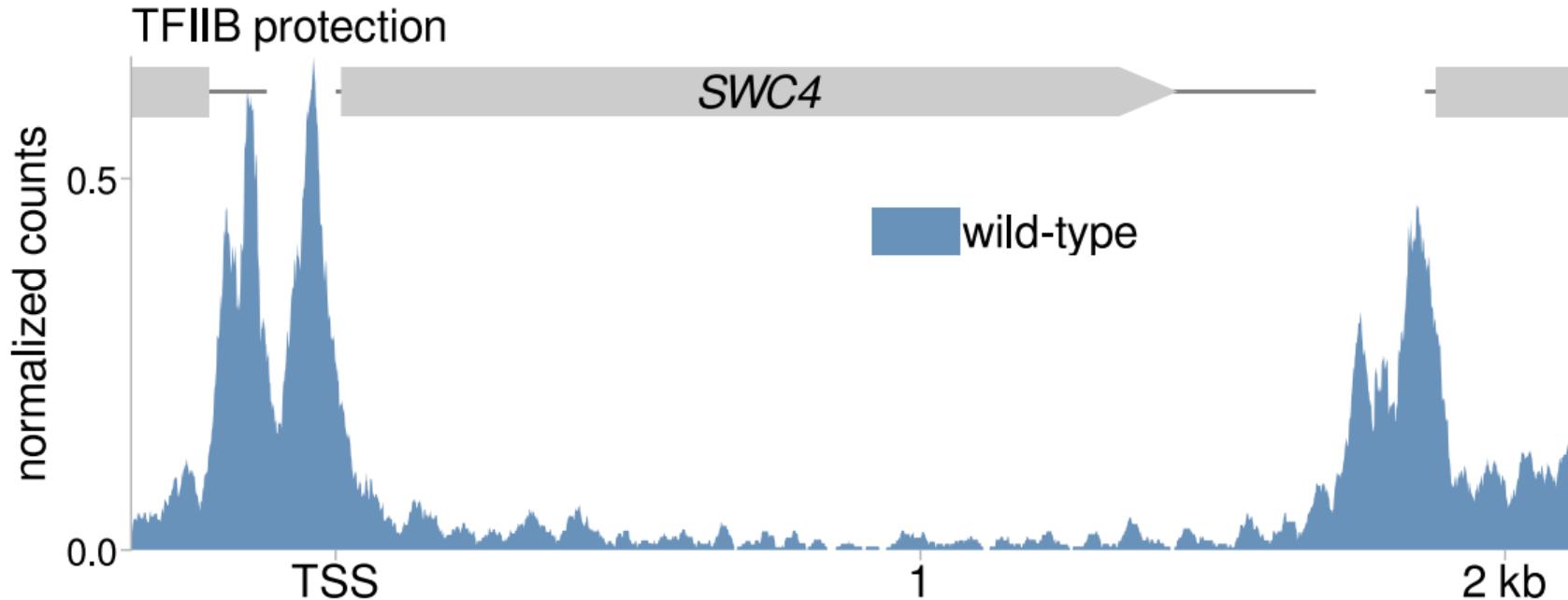
Genic transcript abundances are reduced



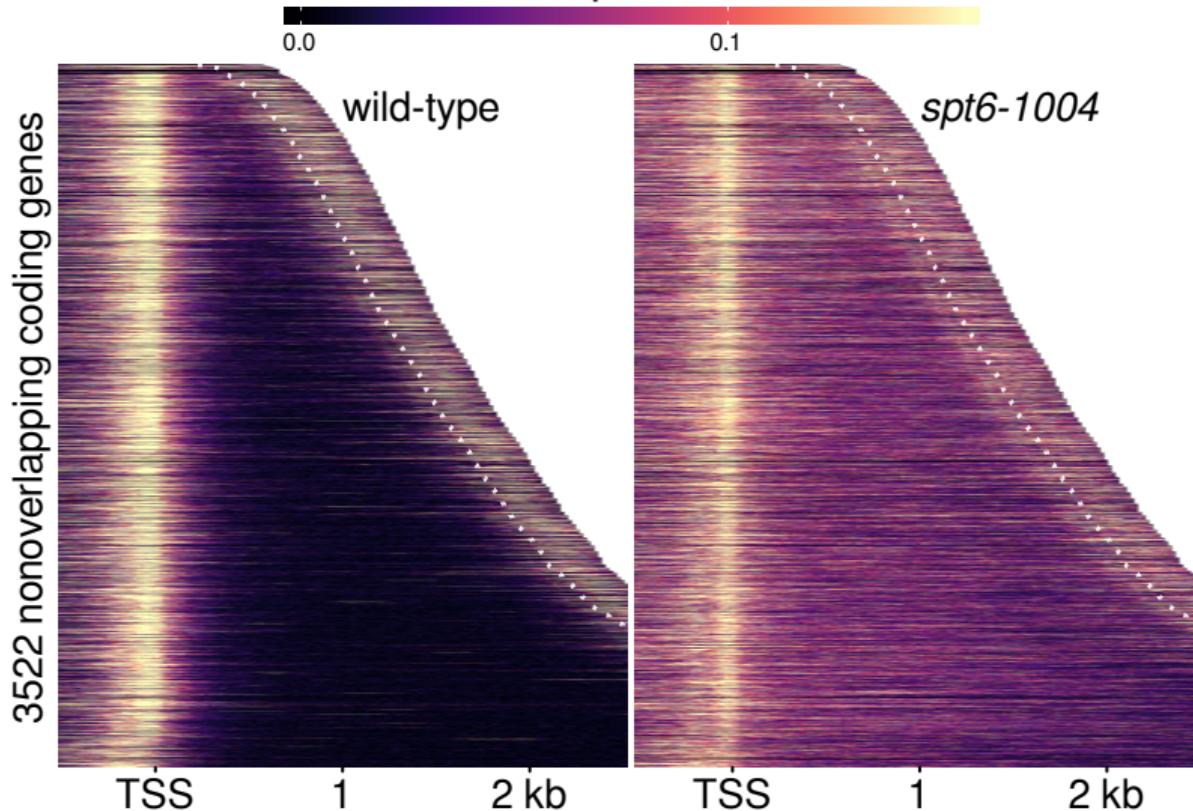
Genic transcript abundances are reduced



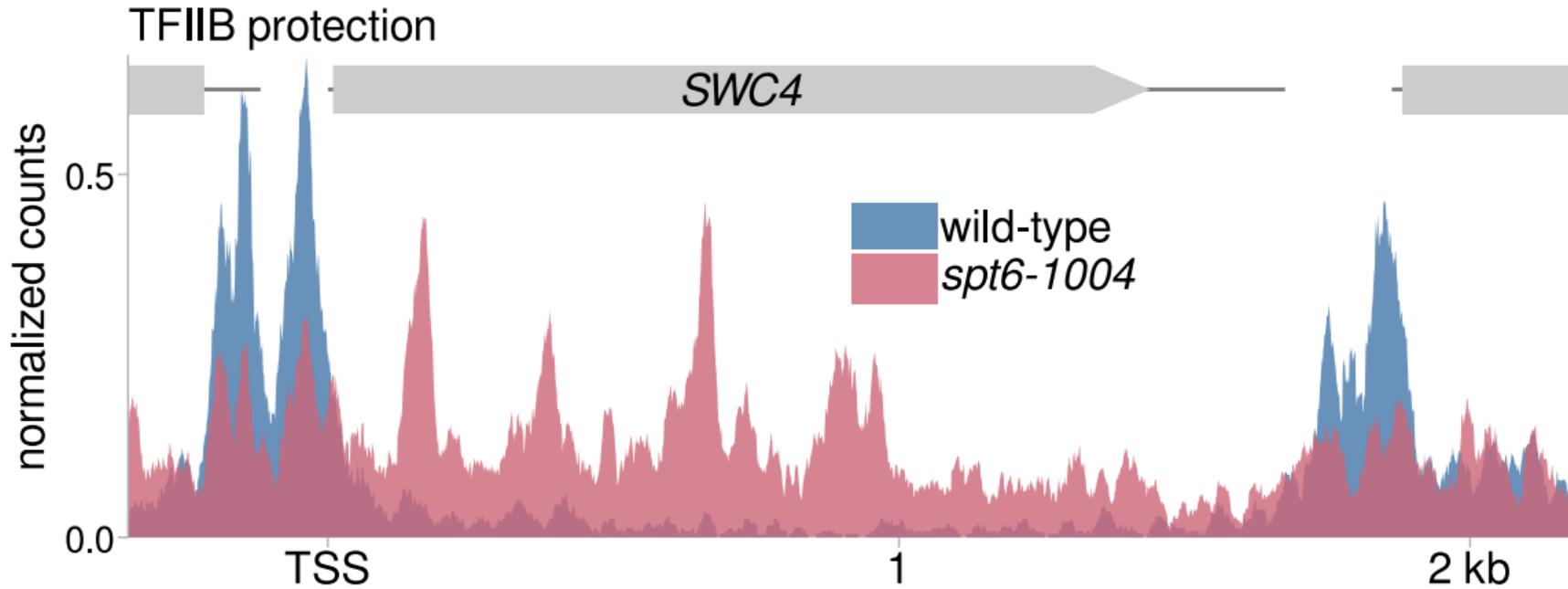
TFIIB ChIP-nexus measures transcription initiation



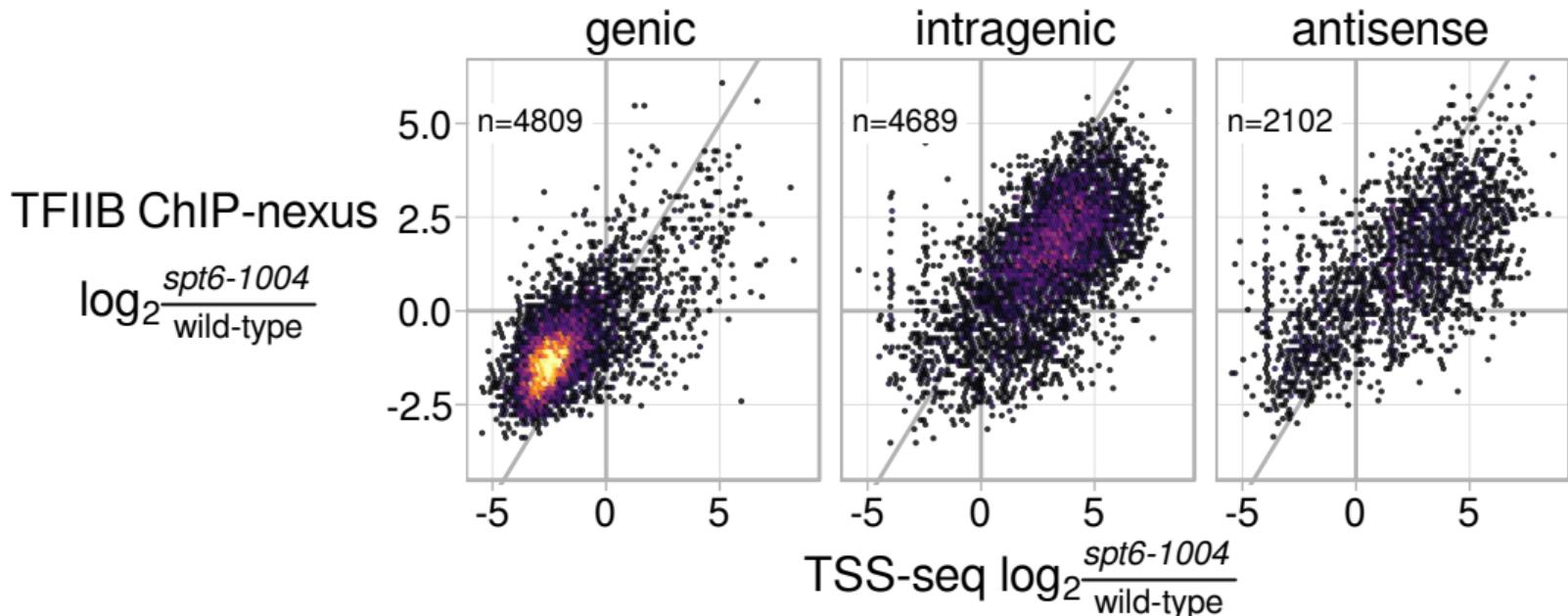
TFIIB protection



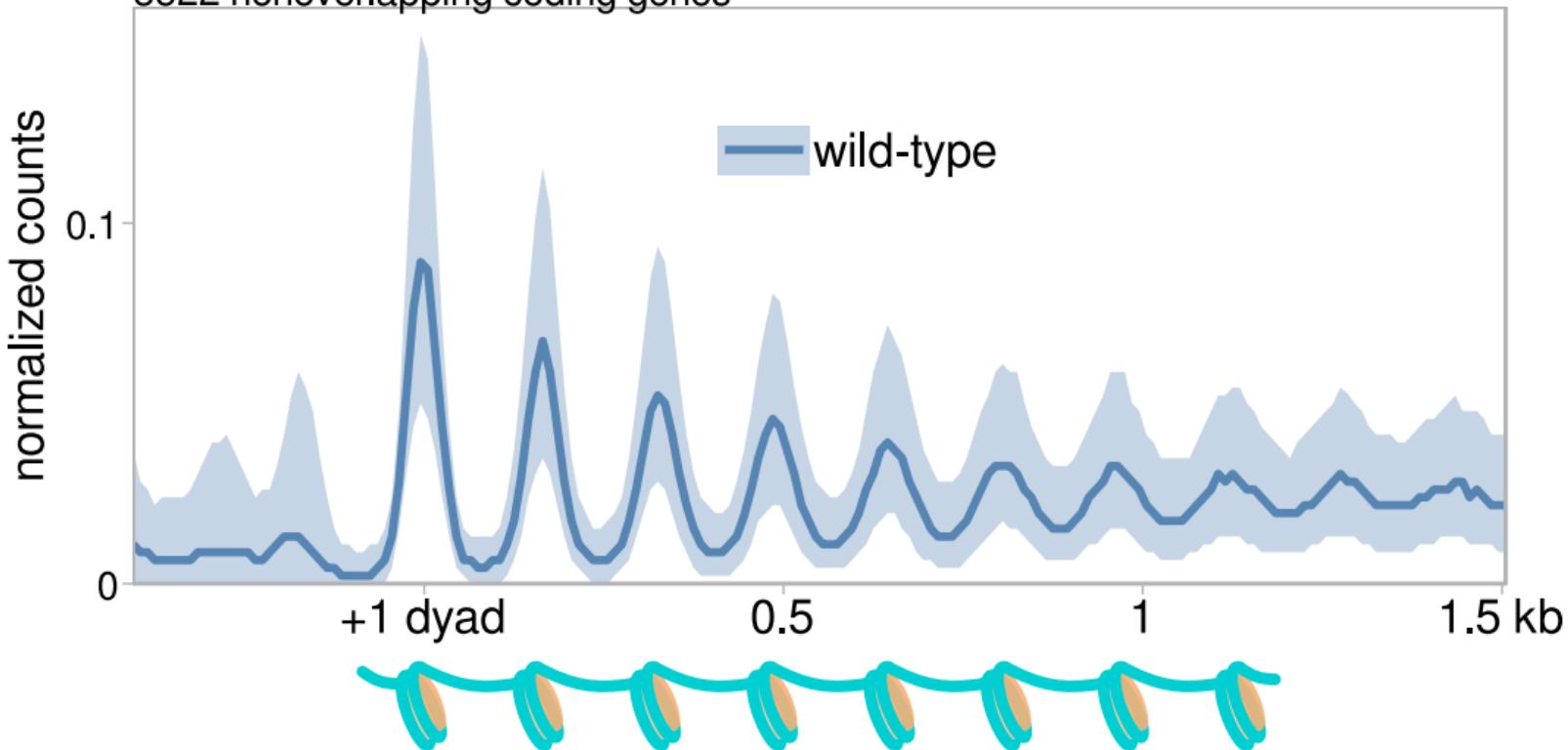
TFIIB binding changes dramatically in *spt6-1004*:



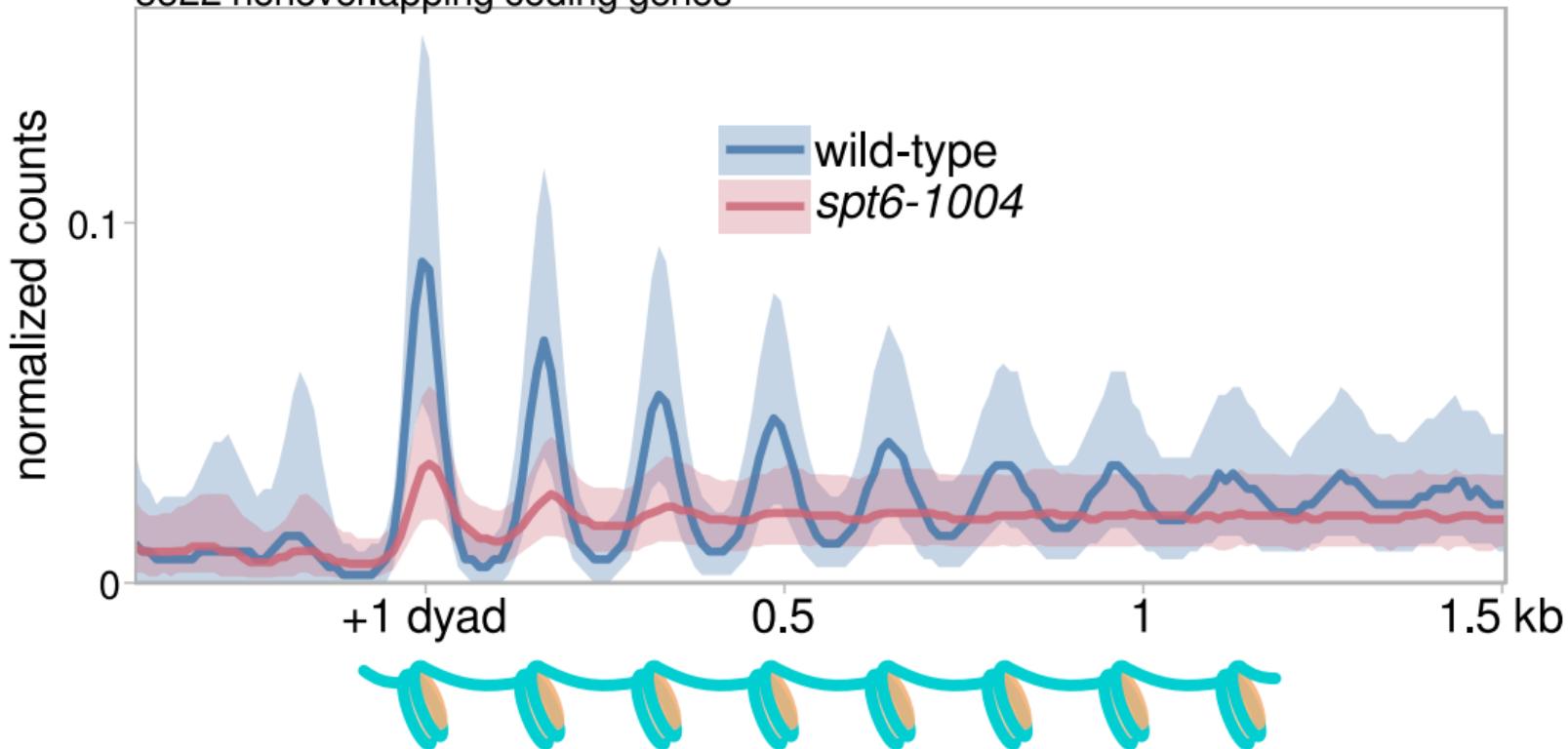
Transcriptomic changes are mostly explained by changes in initiation



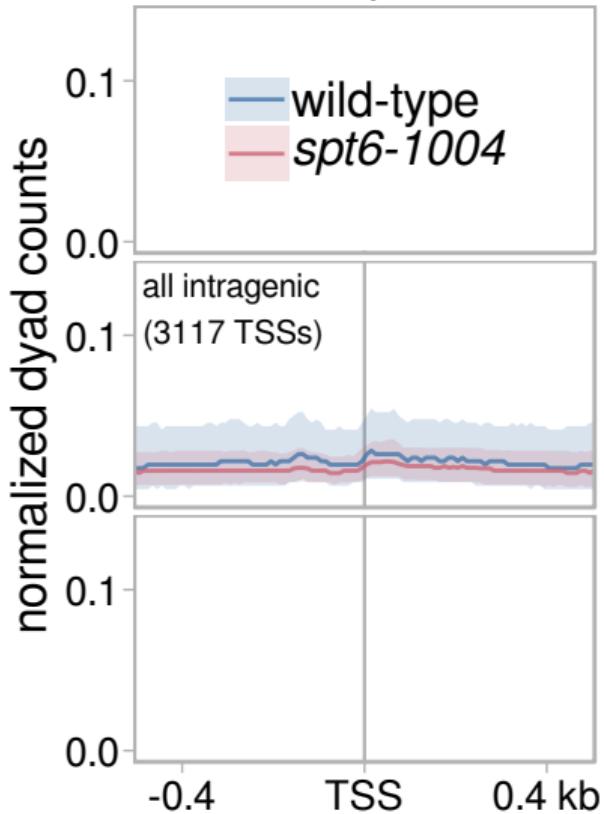
MNase-seq dyad signal
3522 nonoverlapping coding genes



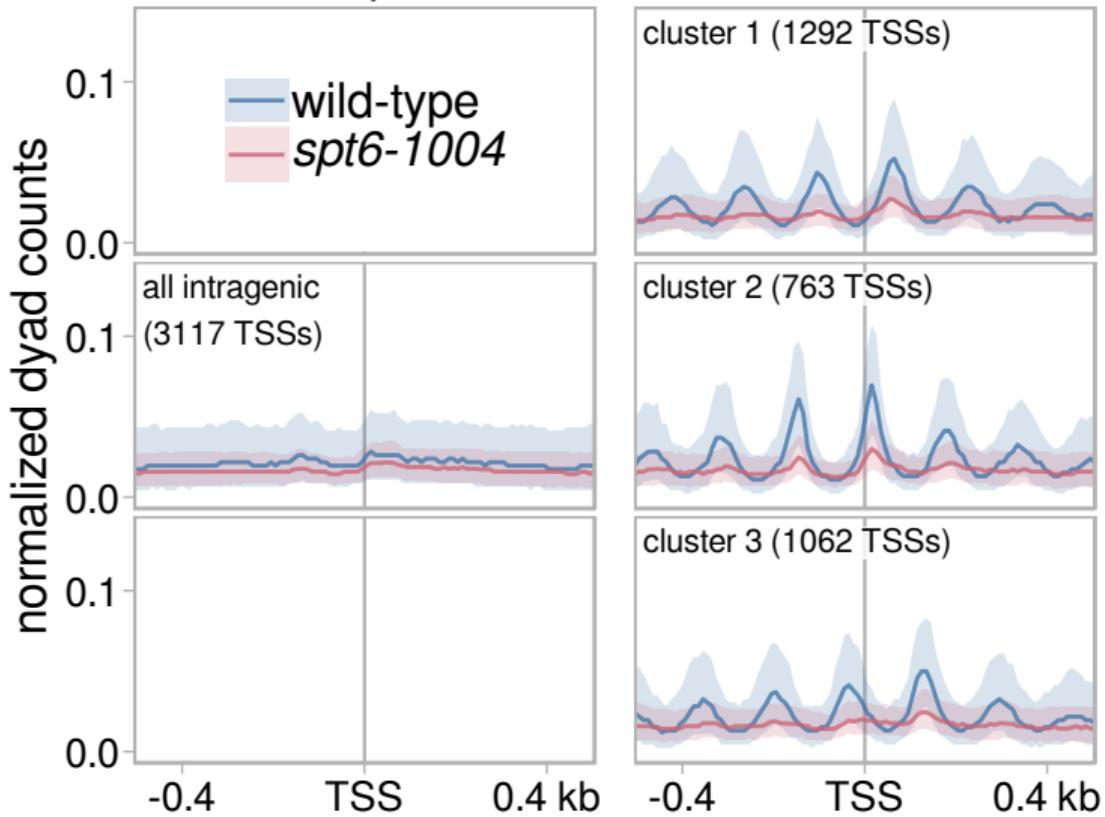
MNase-seq dyad signal
3522 nonoverlapping coding genes



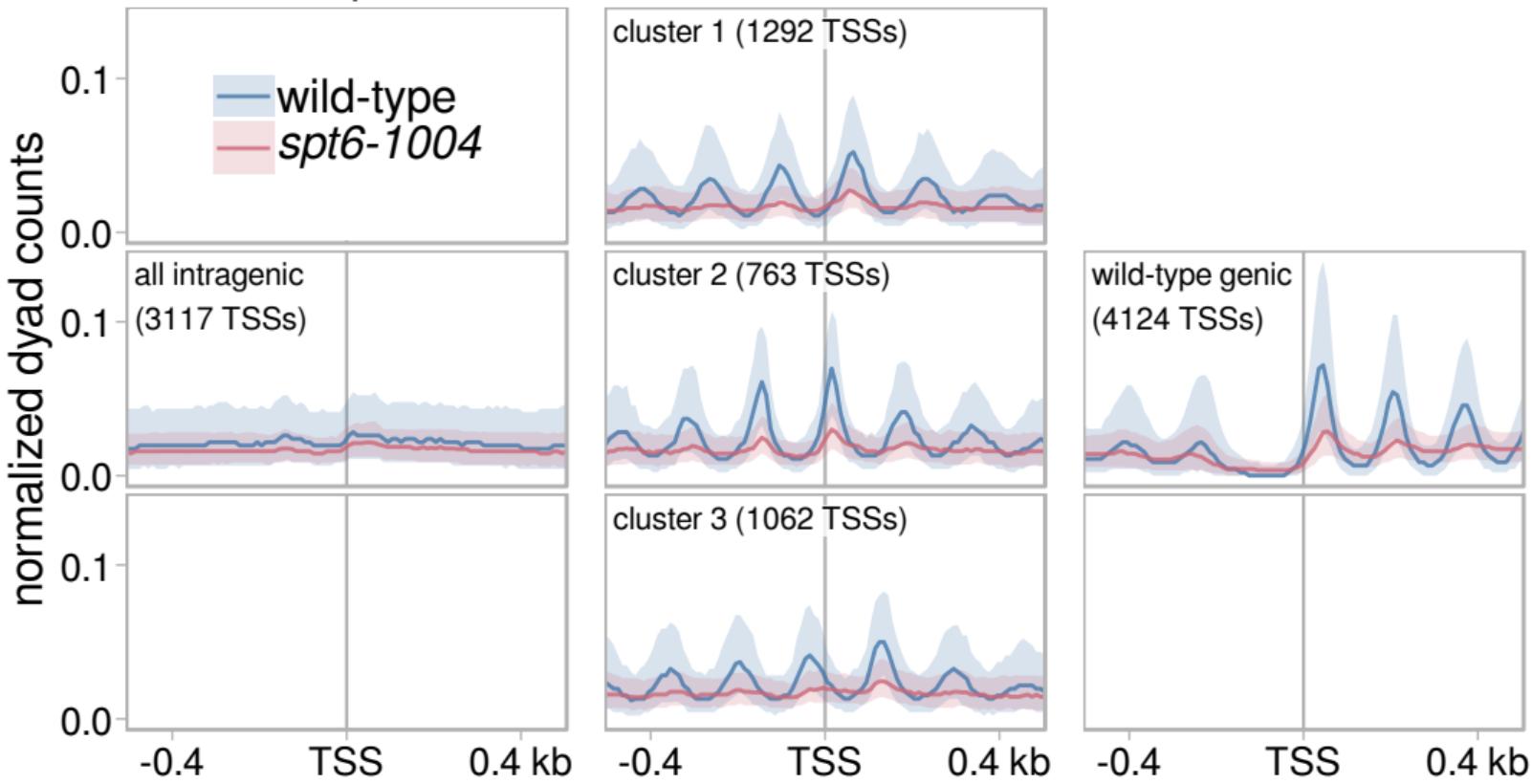
MNase-seq



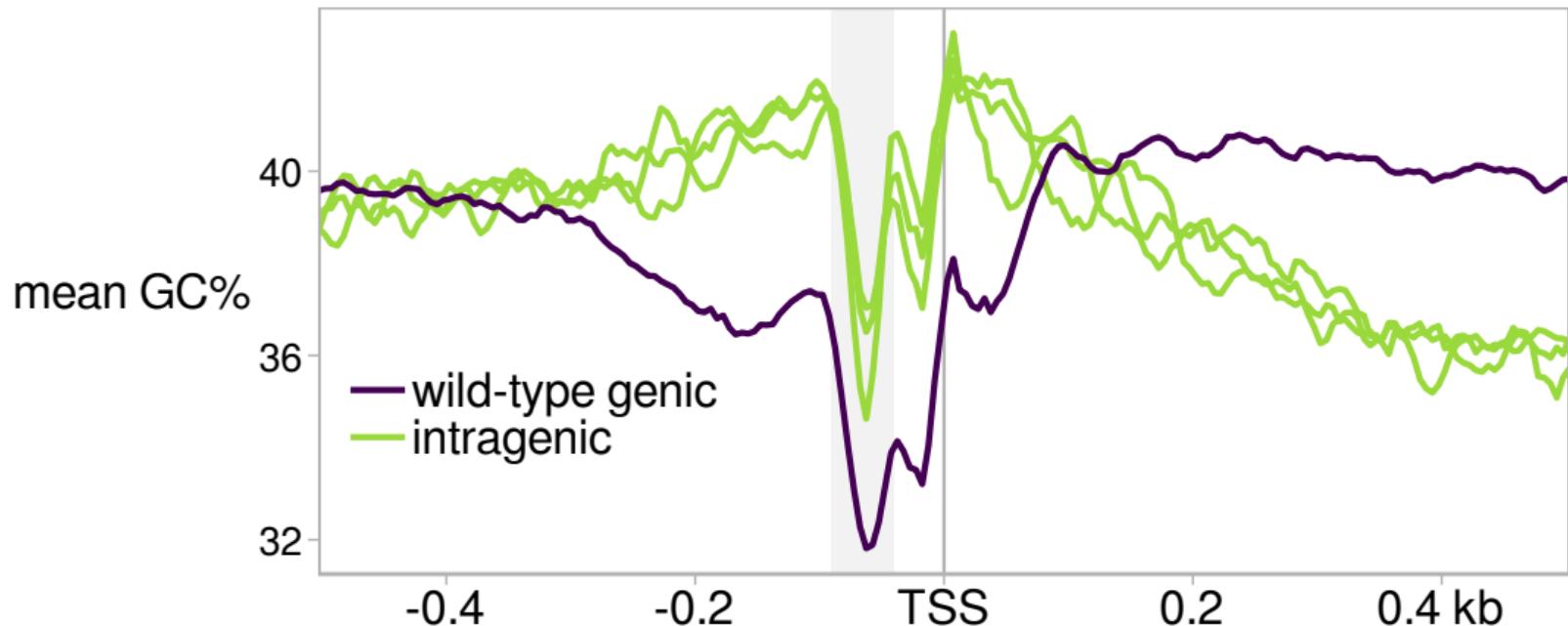
MNase-seq



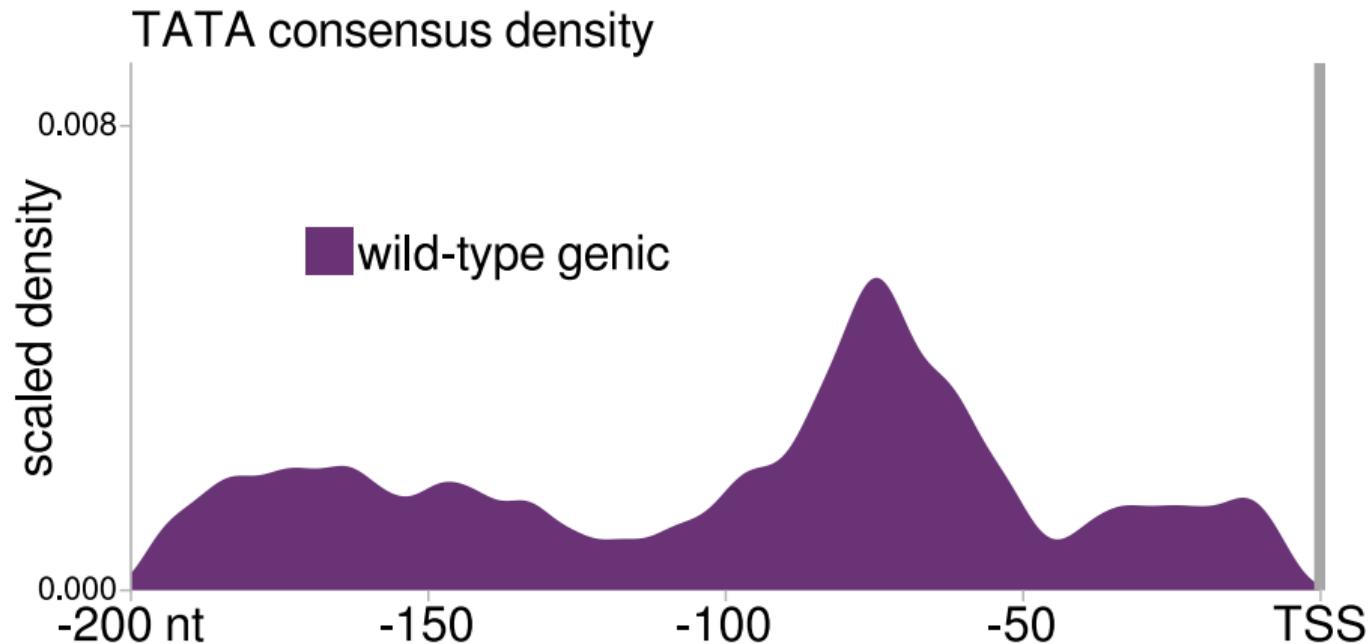
MNase-seq



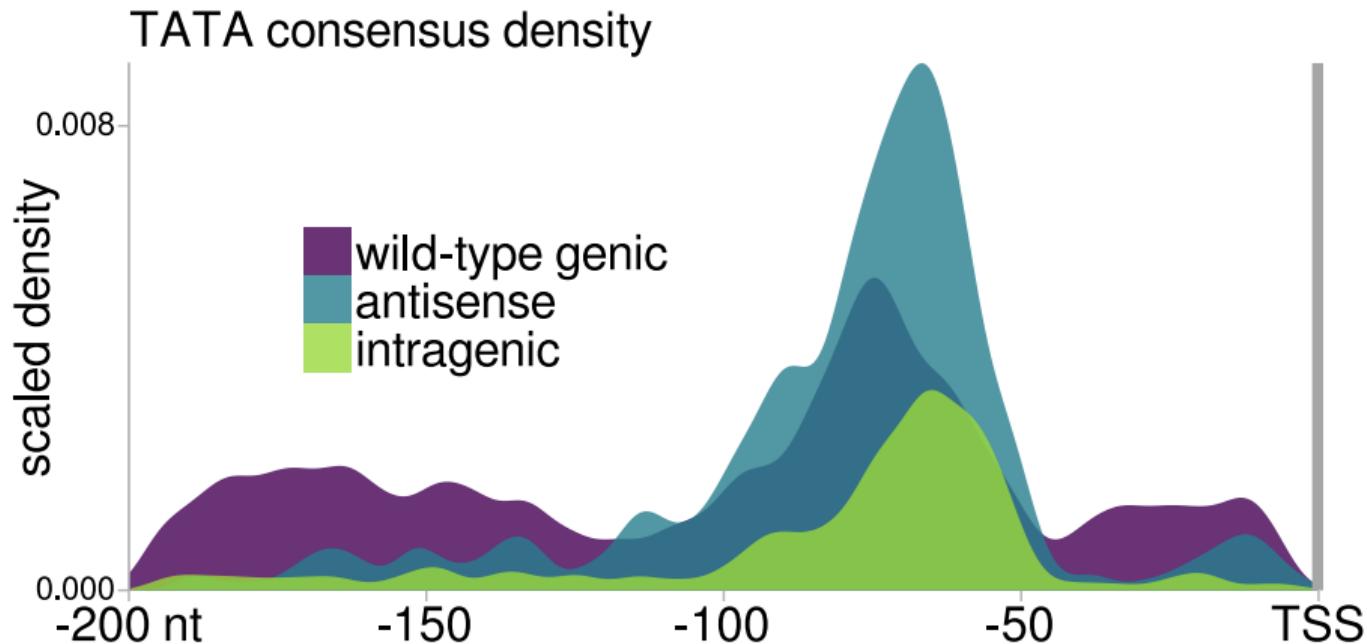
Intragenic promoters have features of genic promoters



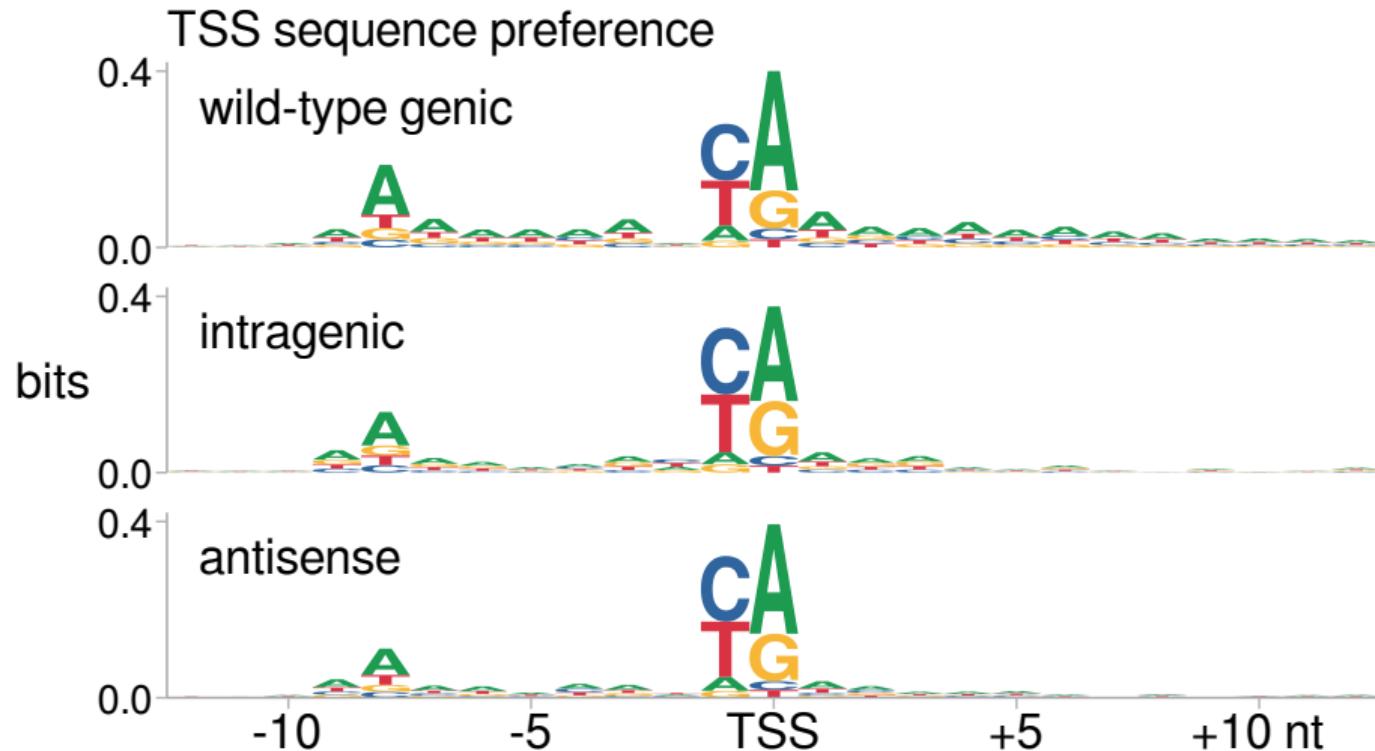
Intragenic promoters have features of genic promoters

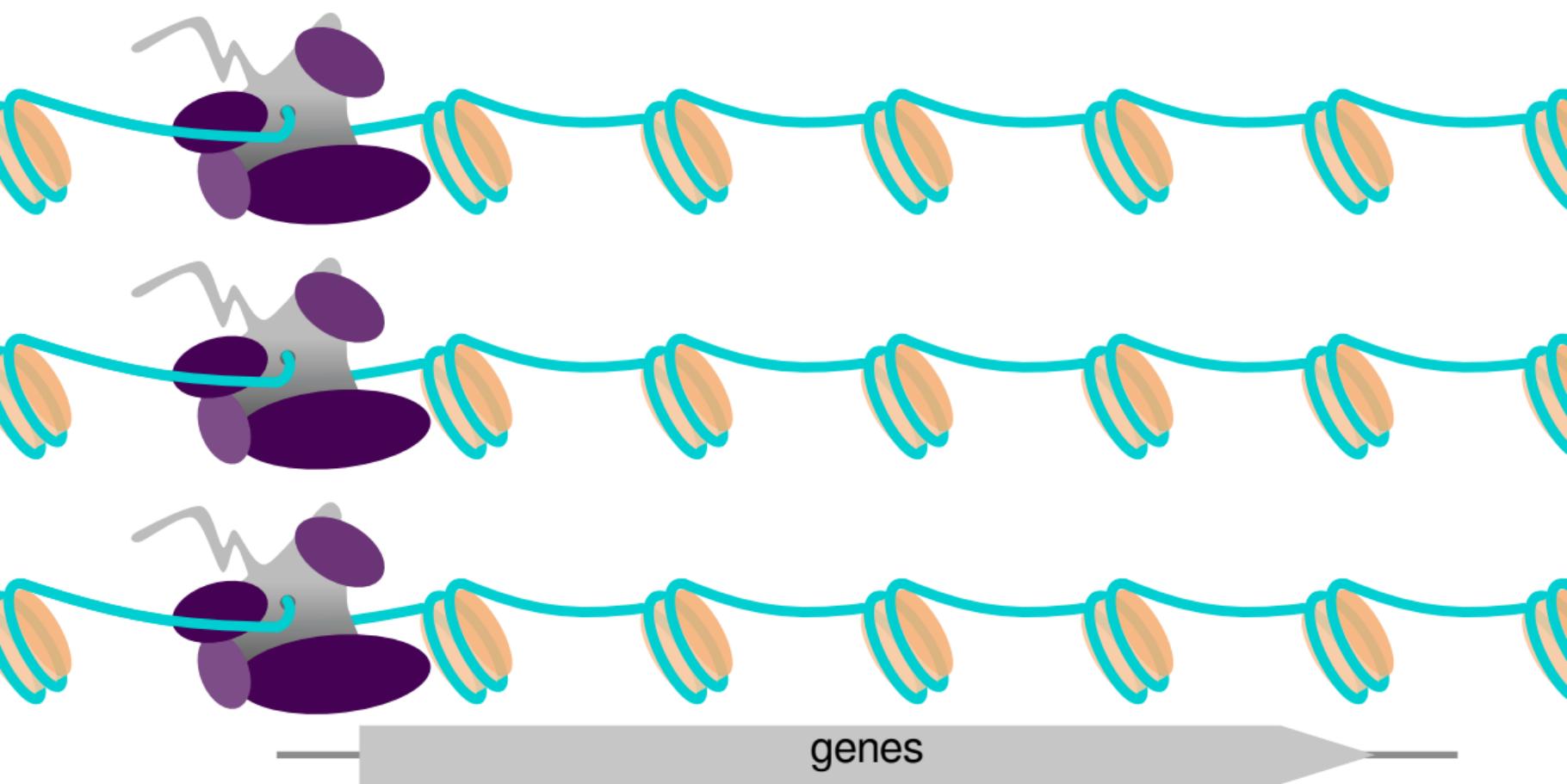


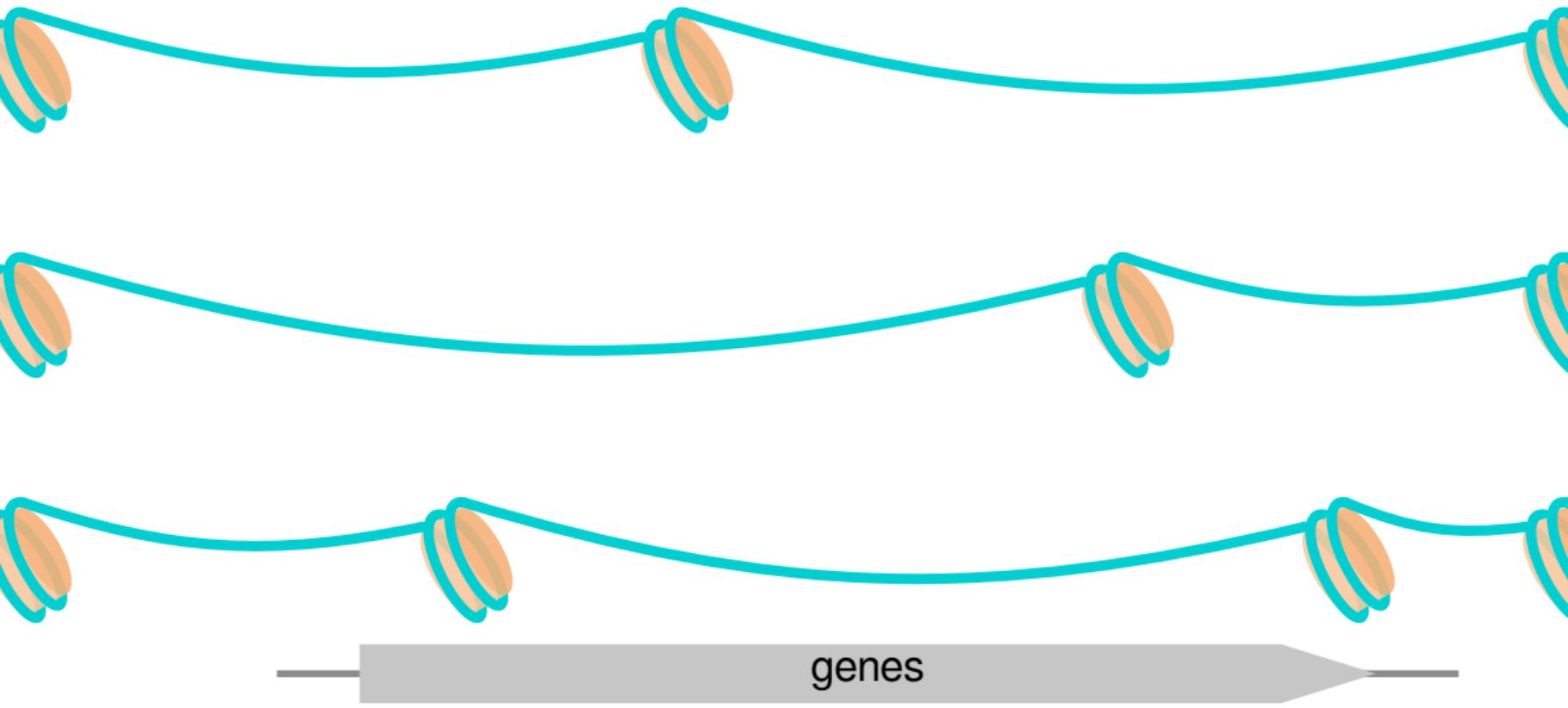
Intragenic promoters have features of genic promoters

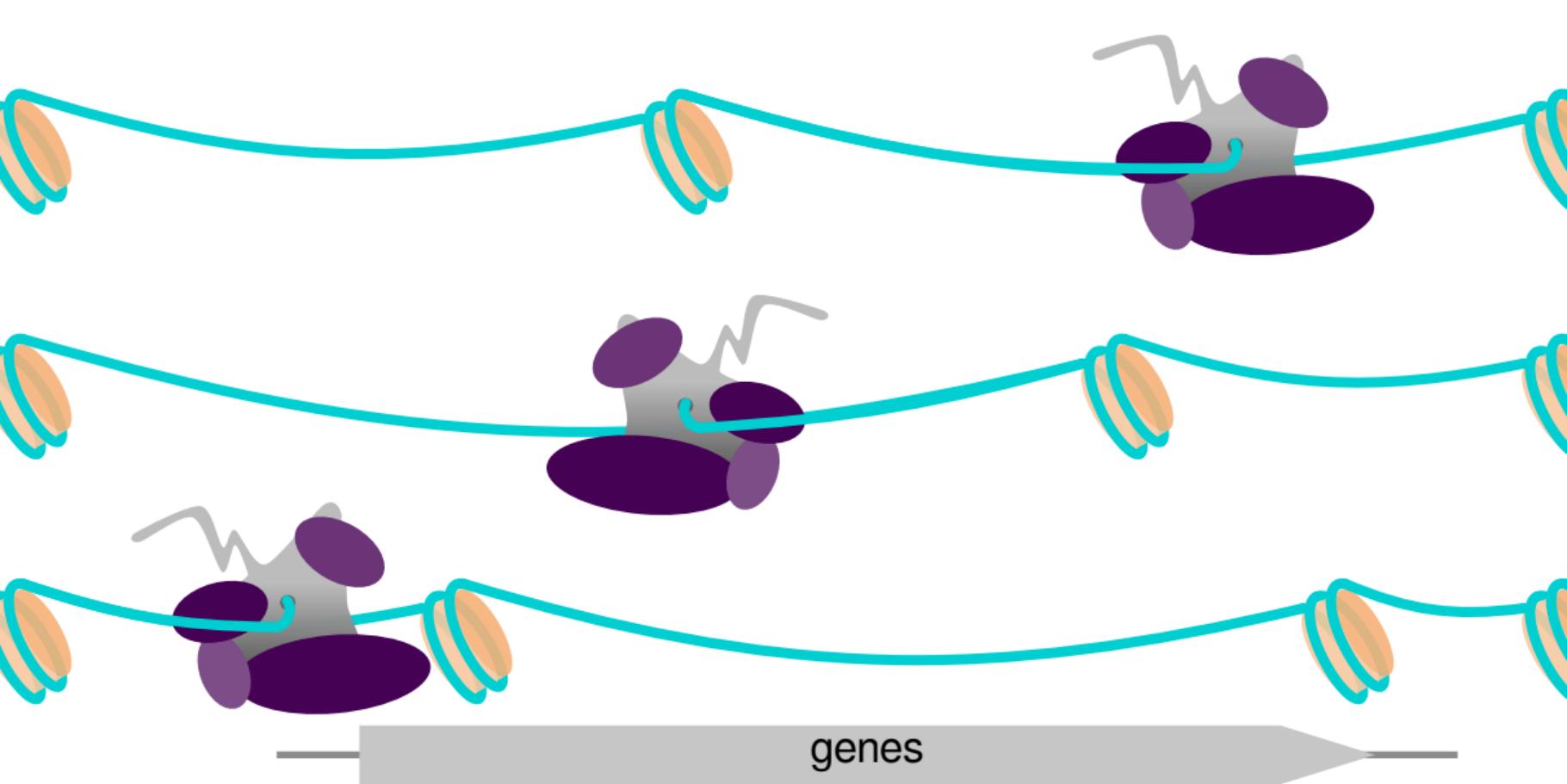


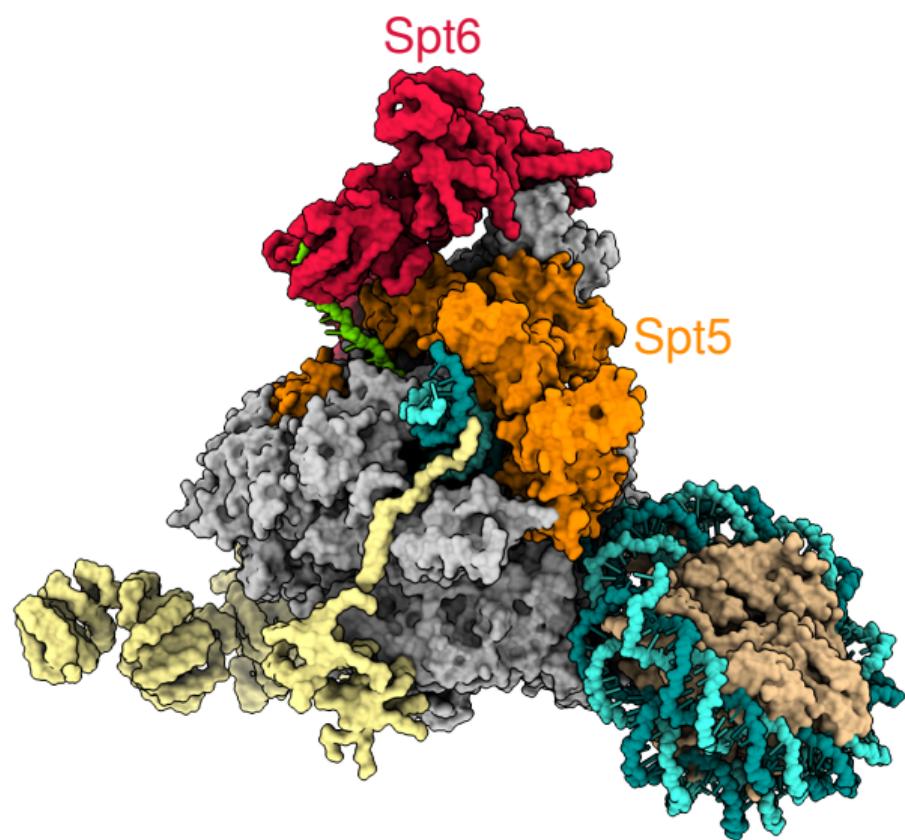
Intragenic promoters have features of genic promoters



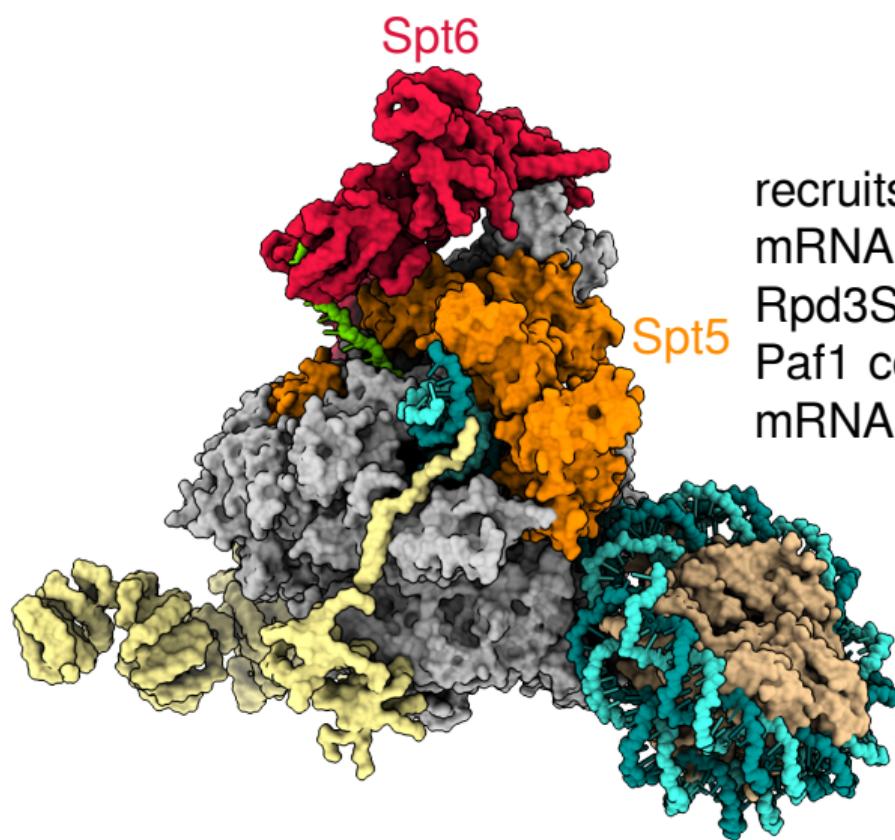








Vos *et al.* (2018). *Nature*
Farnung *et al.* (2018). *Nat. Commun.*



recruits:

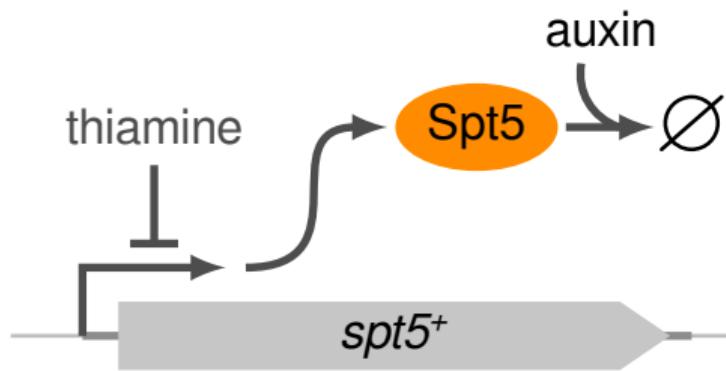
- mRNA capping enzyme
- Rpd3S HDAC complex
- Paf1 complex
- mRNA 3'-end processing factors

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

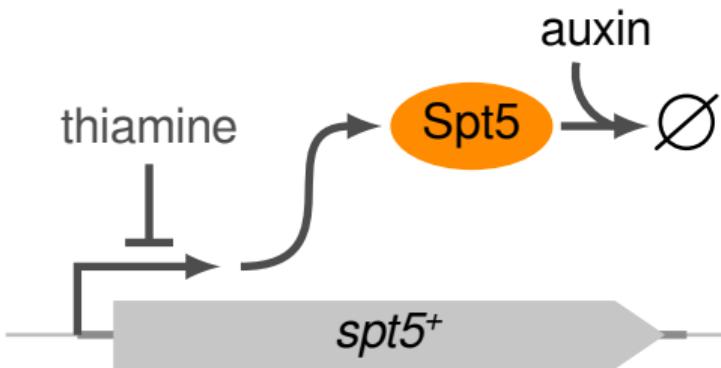
Spt5 project collaborators

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

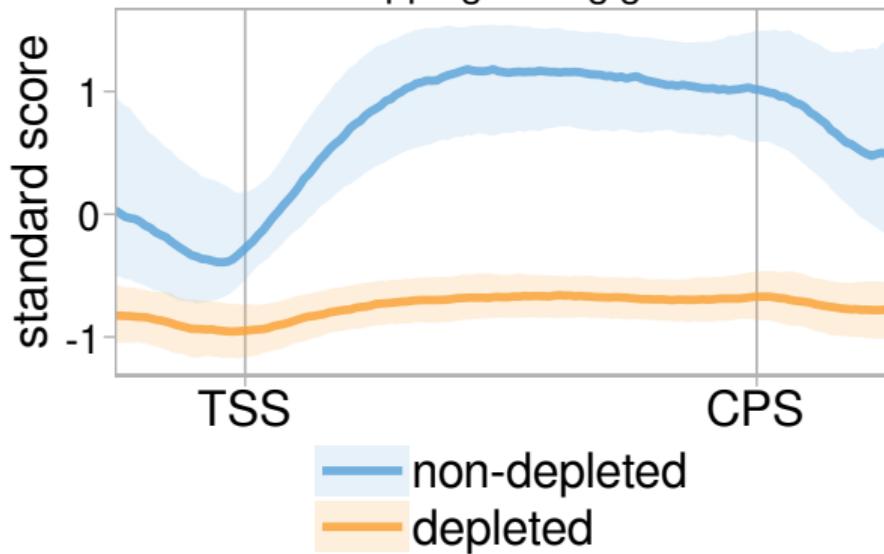
Spt5 depletion system



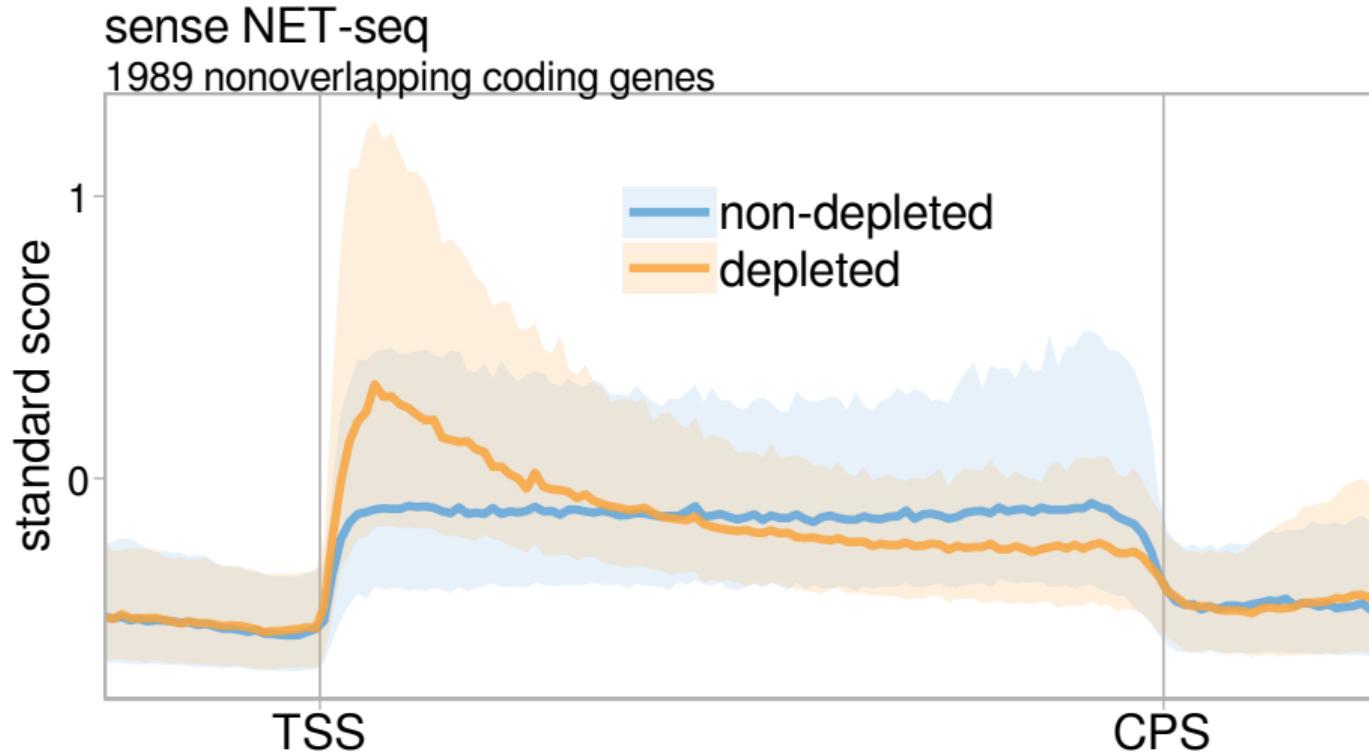
Spt5 depletion system



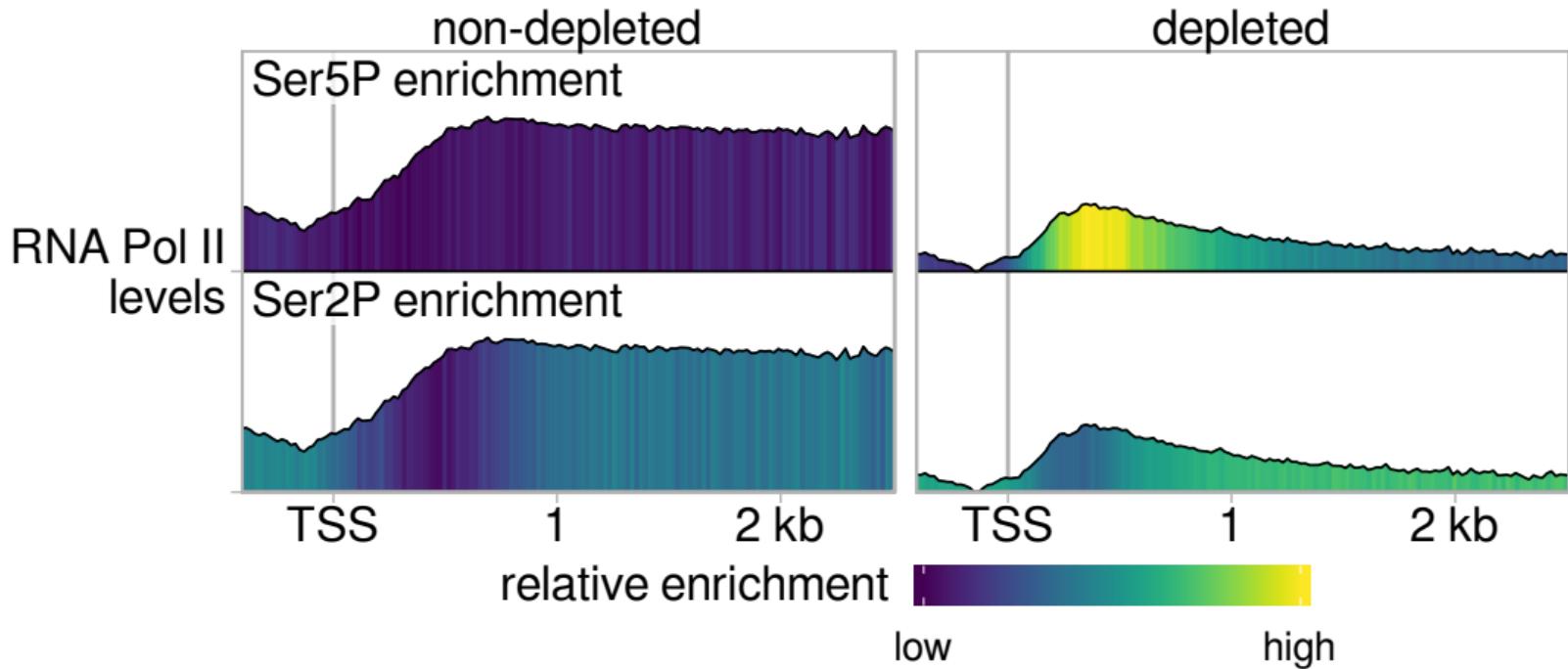
Spt5 ChIP-seq
1989 nonoverlapping coding genes



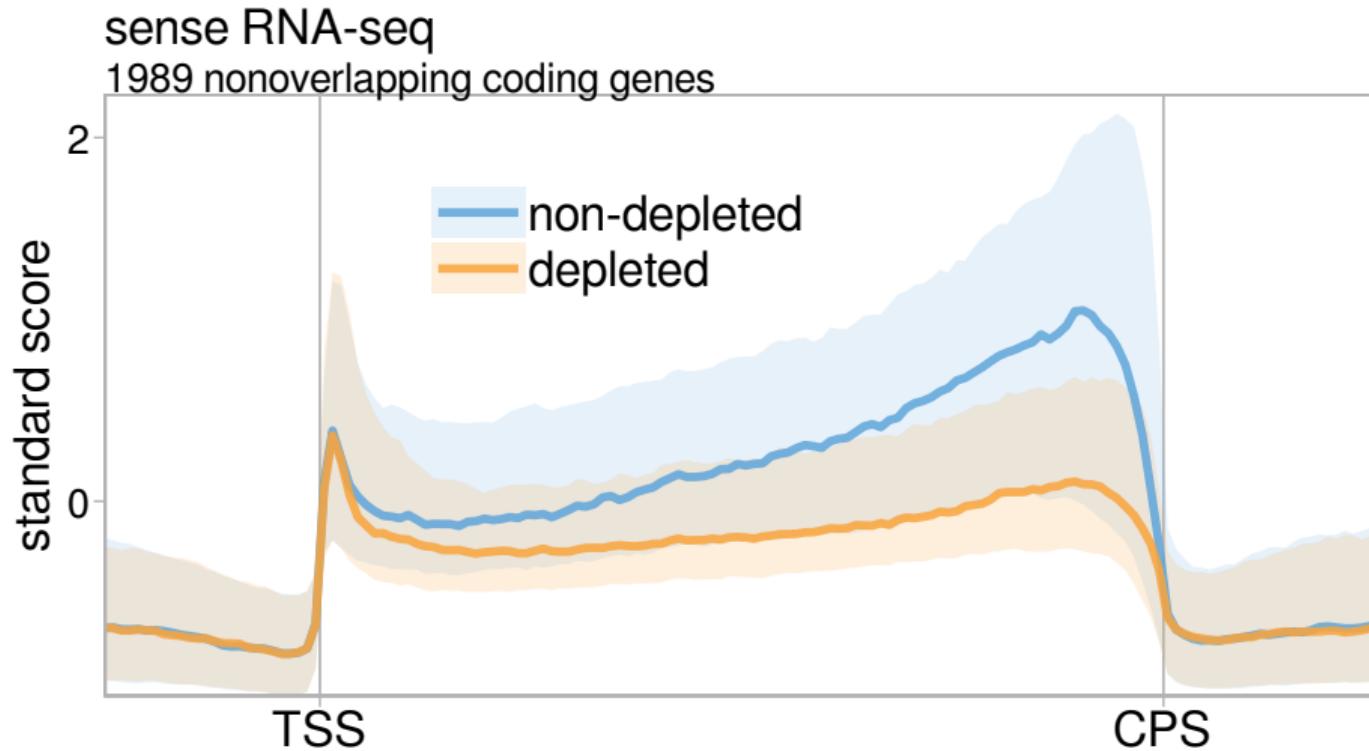
Elongation defects upon Spt5 depletion



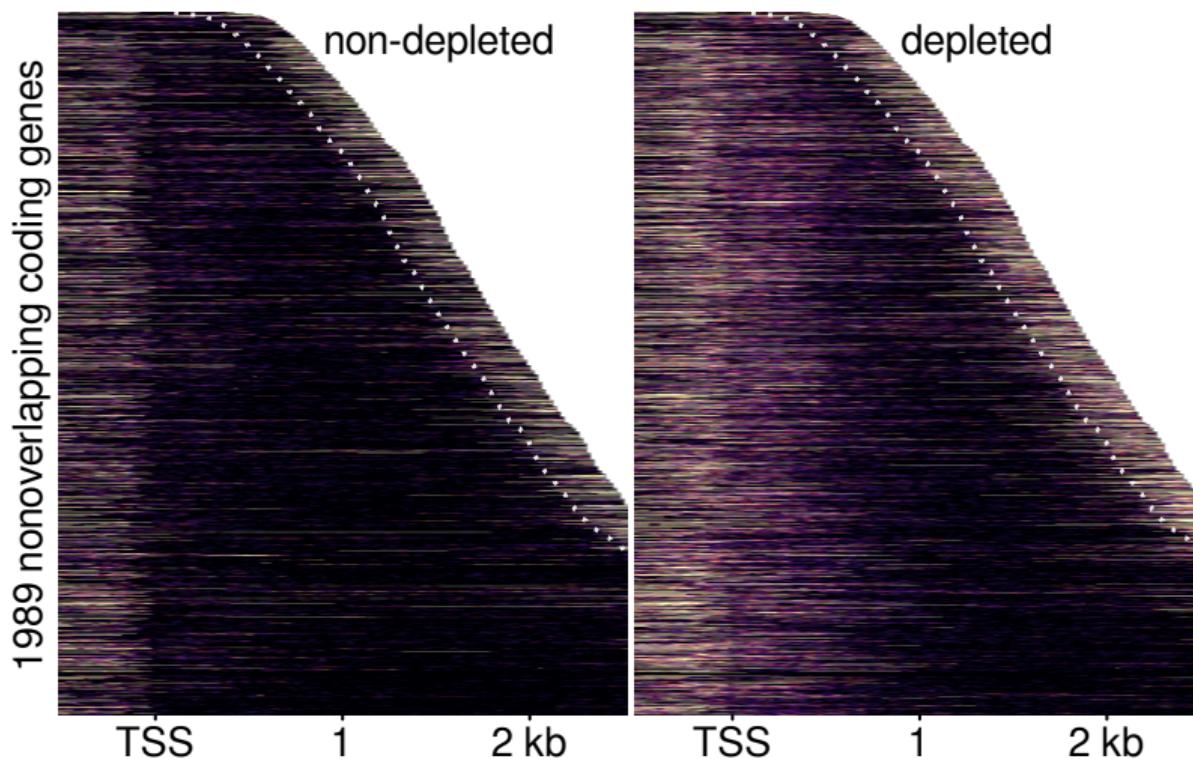
Trapped Pol II is enriched for CTD serine 5 phosphorylation

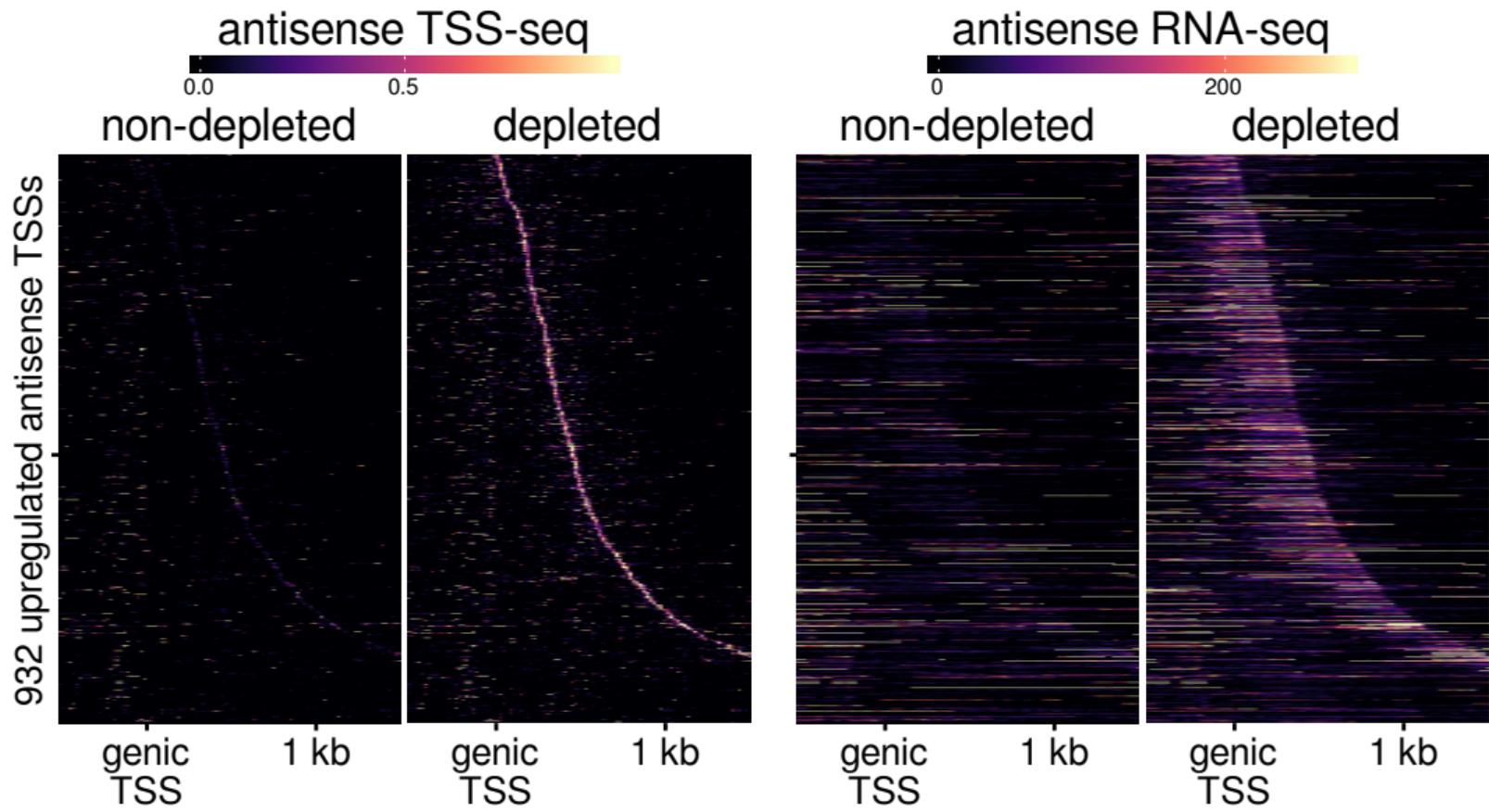


Evidence for premature termination upon Spt5 depletion

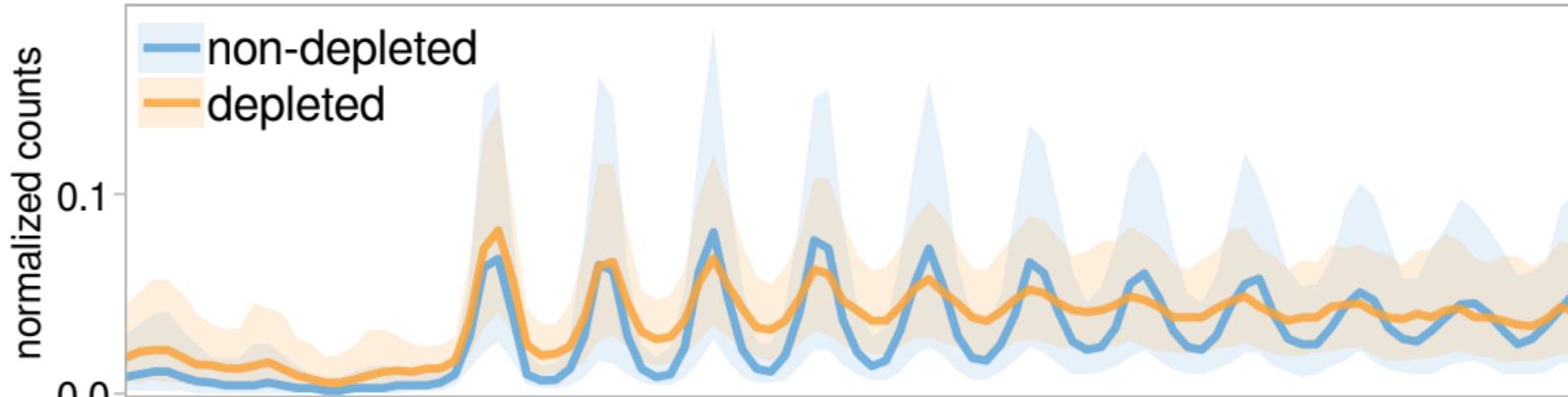


antisense RNA-seq

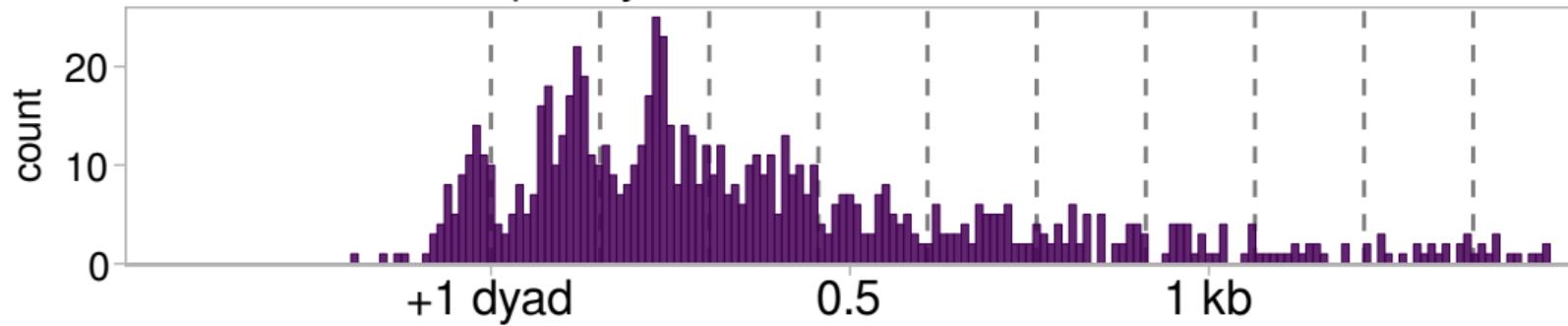




MNase-seq dyad signal

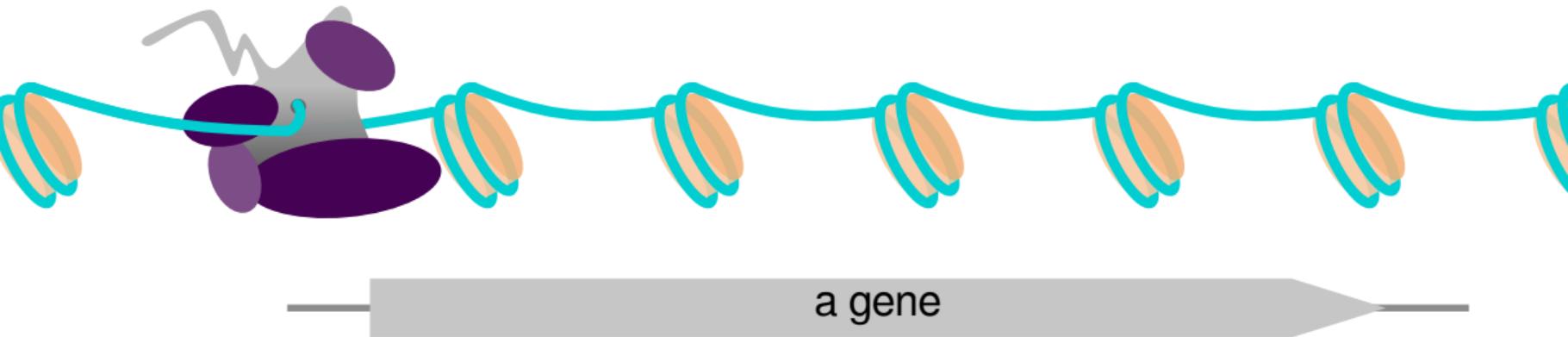


antisense TSS frequency

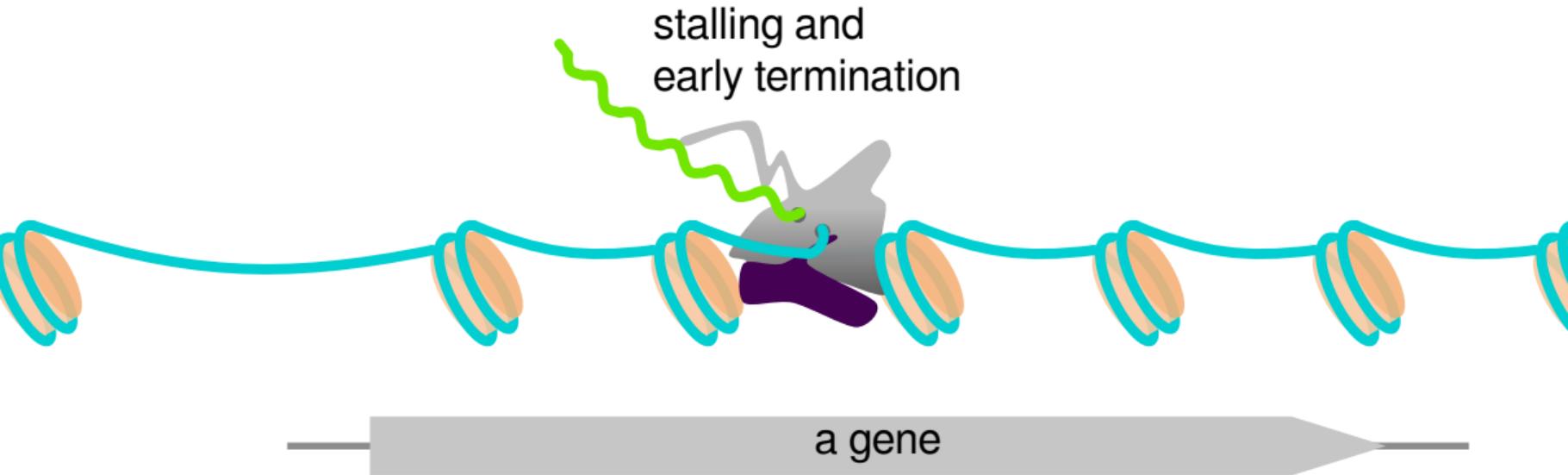


Transcription upon Spt5 depletion:

initiation

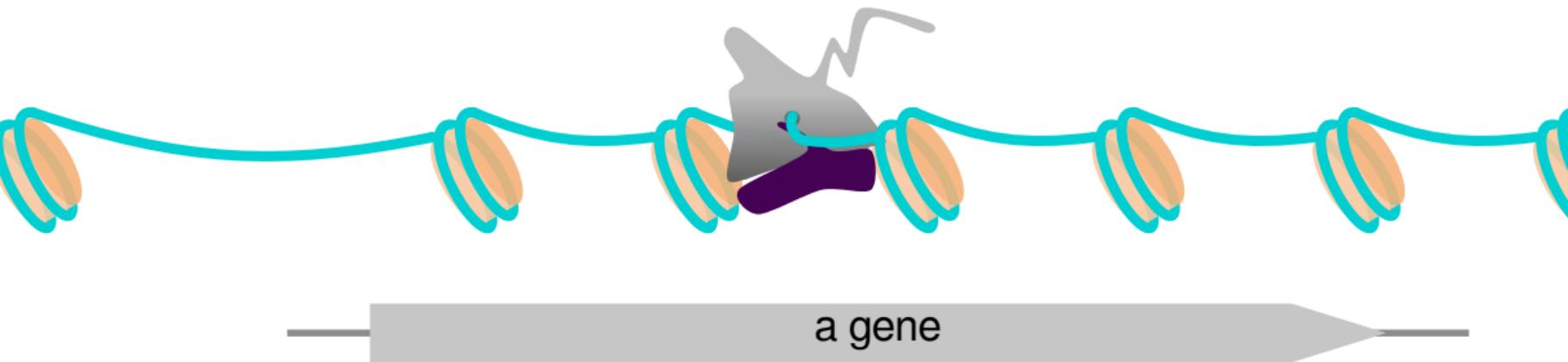


Transcription upon Spt5 depletion:



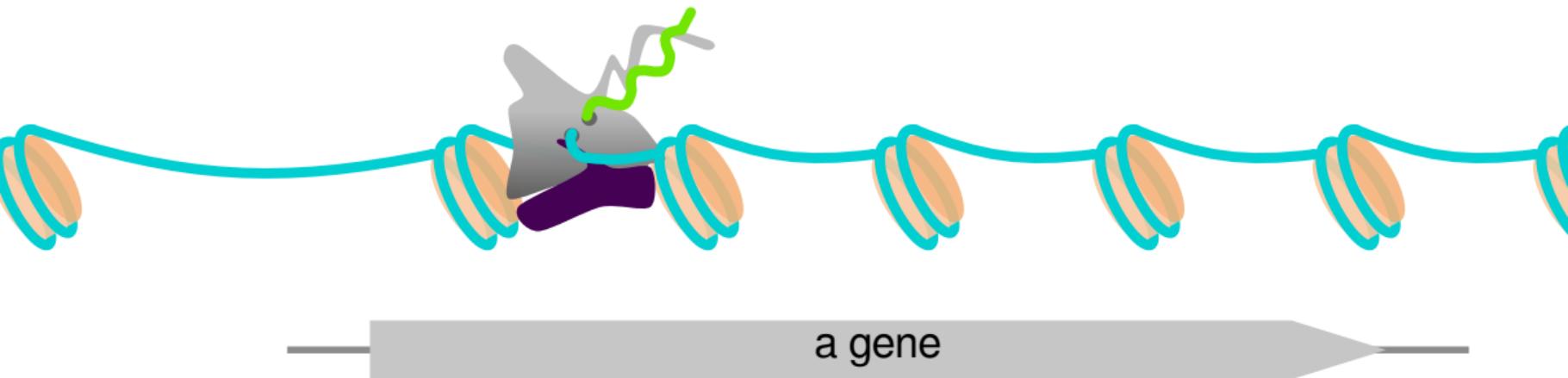
Transcription upon Spt5 depletion:

possible antisense re-initiation

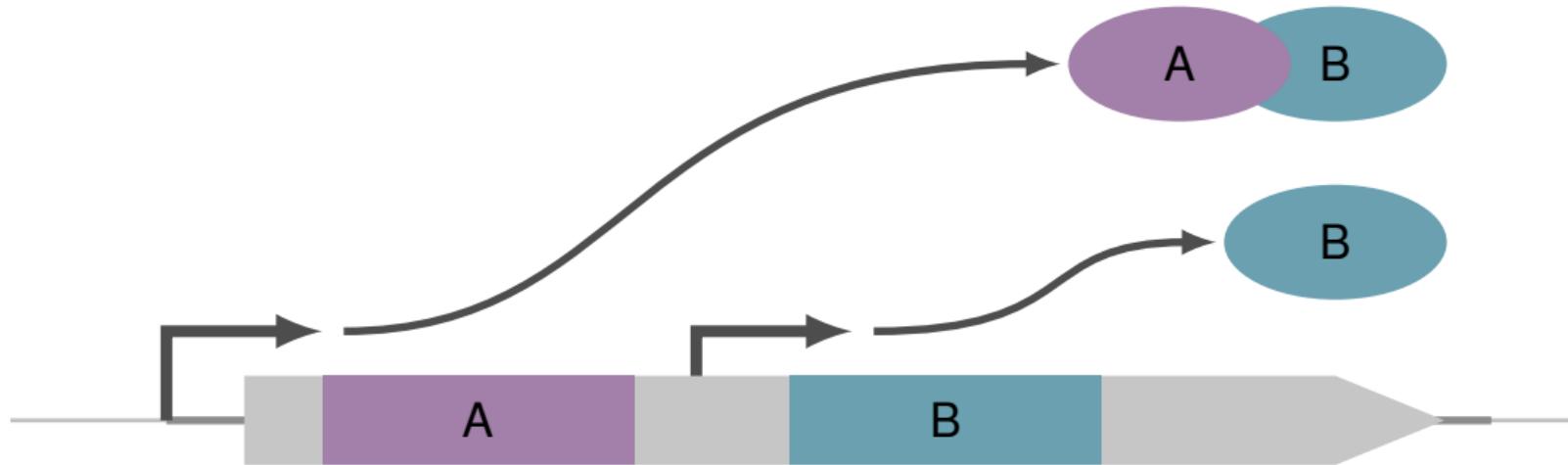


Transcription upon Spt5 depletion:

antisense transcription



Intragenic transcription in wild-type cells



project collaborators

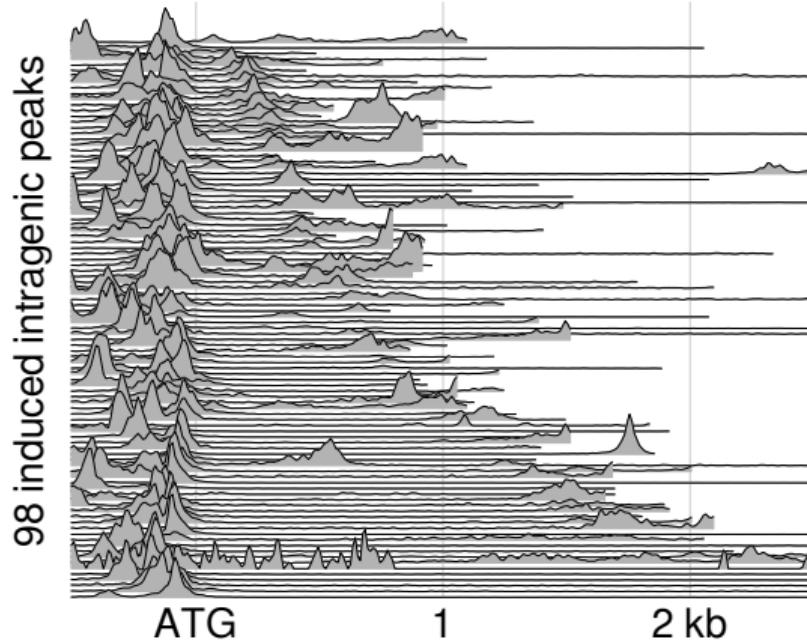
Steve Doris TSS-seq and ChIP-nexus

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

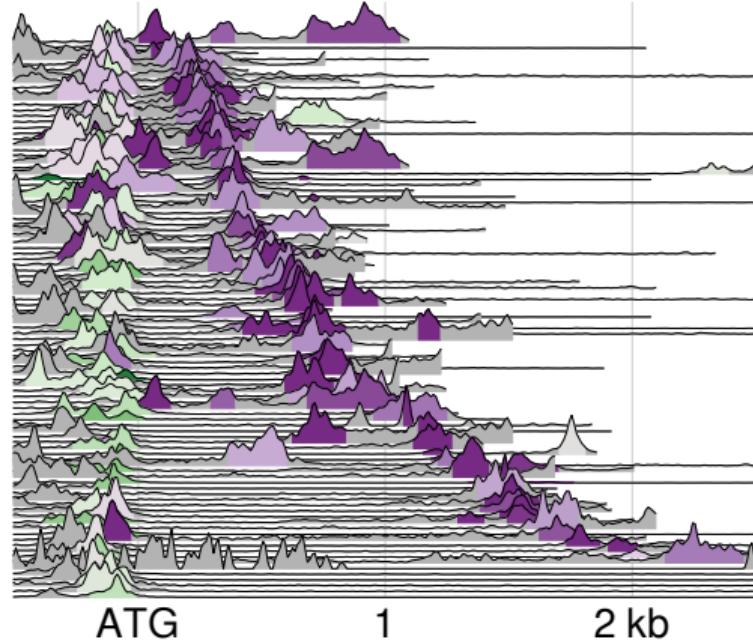
James Warner Northern blots

relative TFIIB protection

unstressed

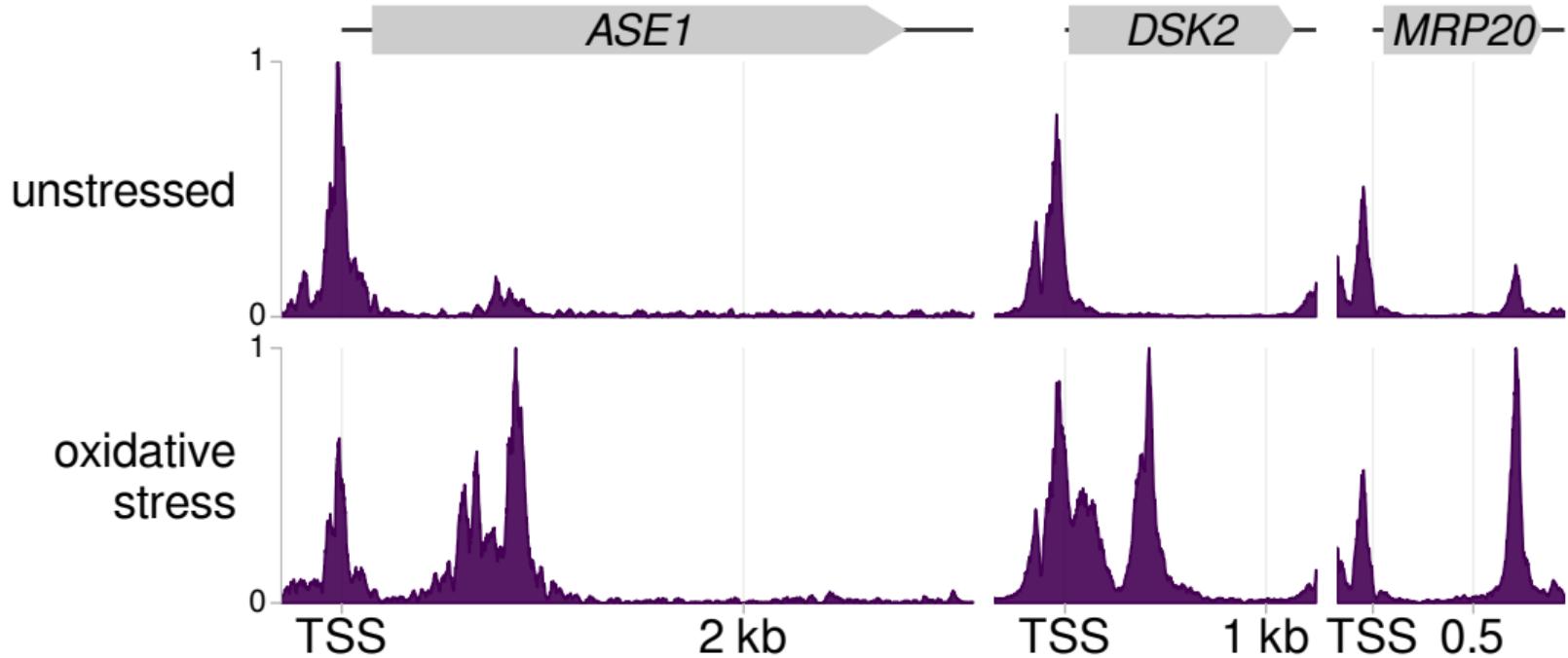


oxidative stress



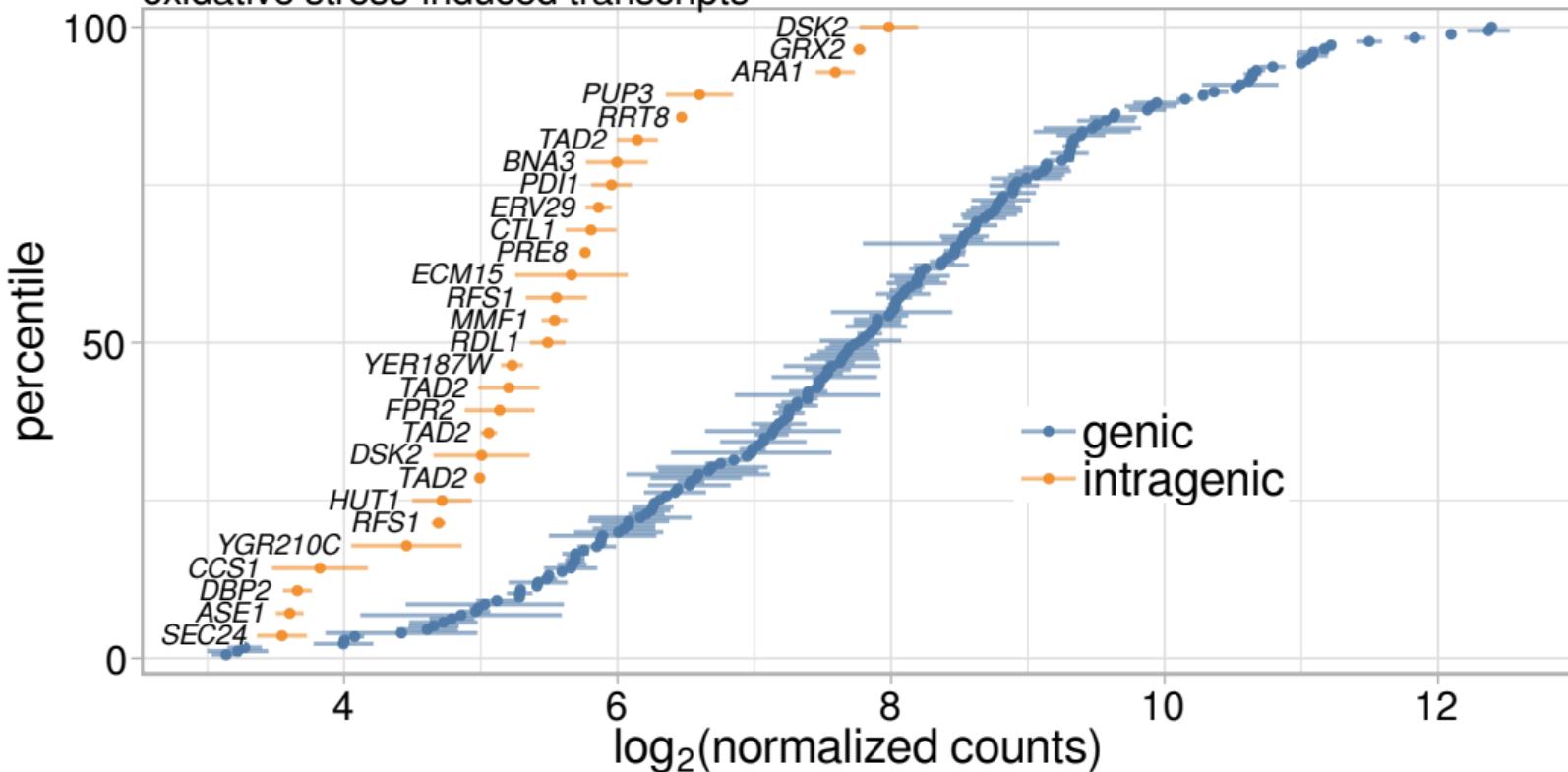
\log_2 oxidative stress
unstressed ≤ -2 -1 0 1 ≥ 2

relative TFIIB protection

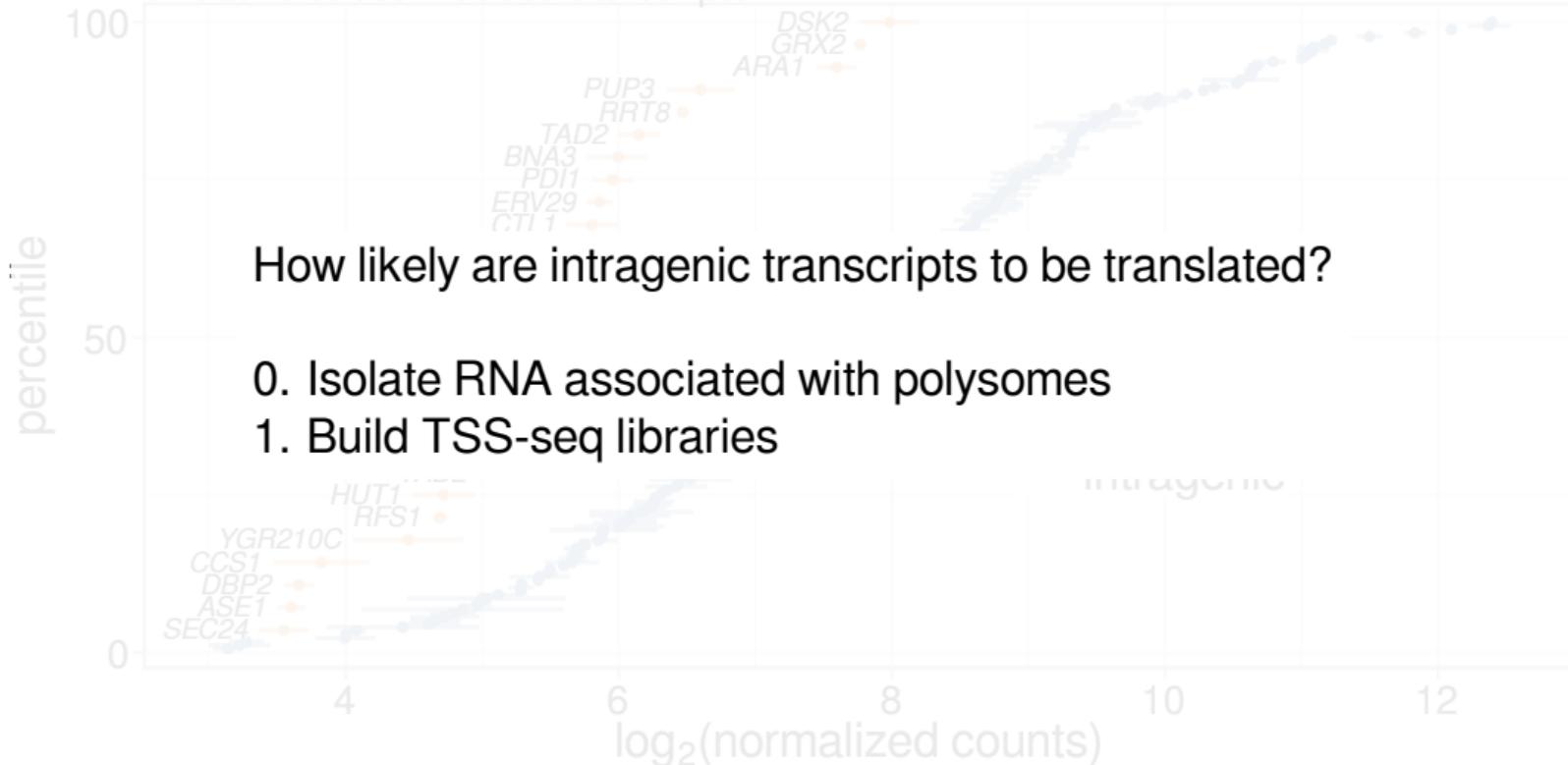


transcript abundances in oxidative stress

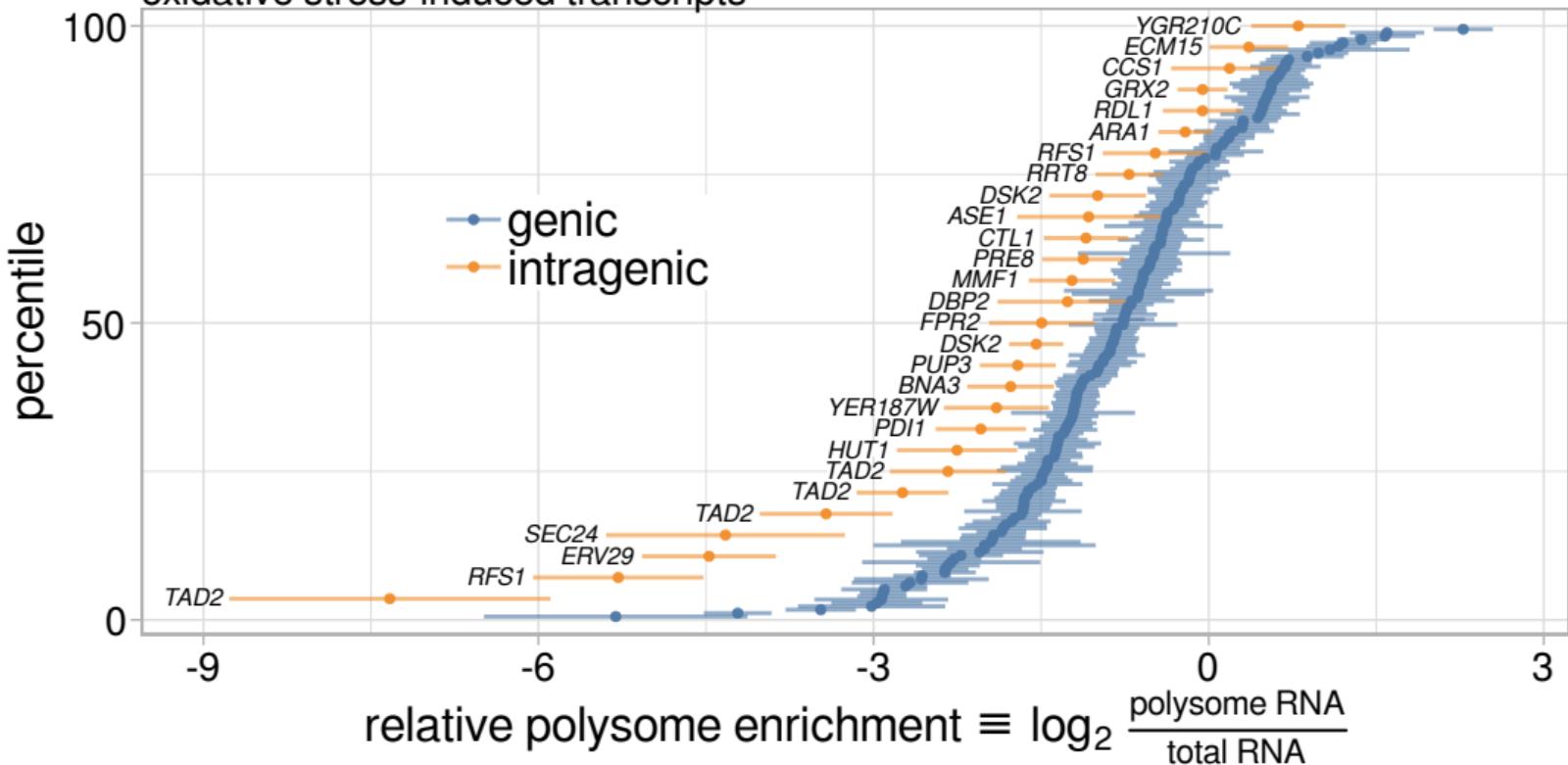
oxidative stress-induced transcripts



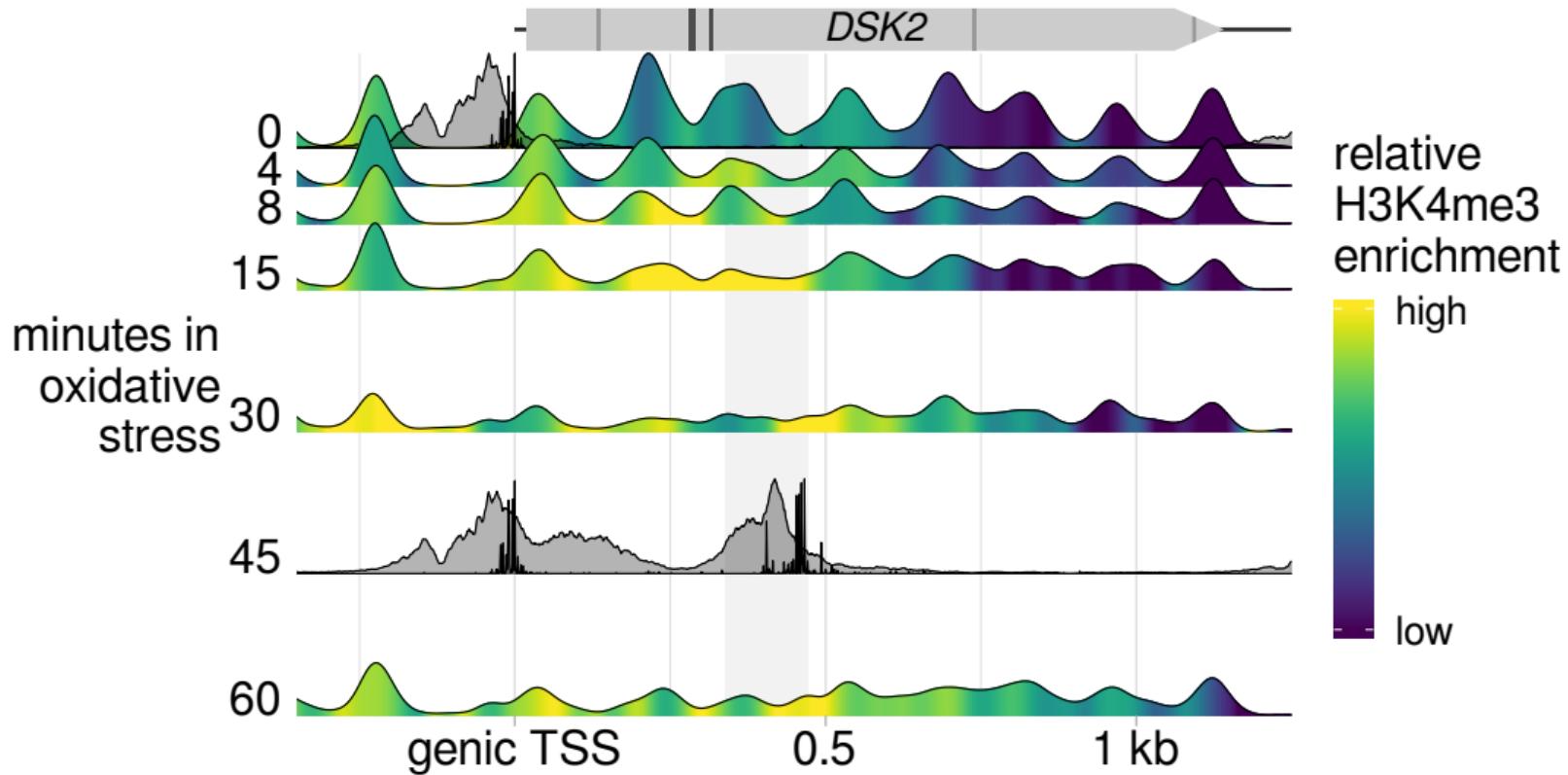
transcript abundances in oxidative stress oxidative stress-induced transcripts



polysome enrichment in oxidative stress oxidative stress-induced transcripts

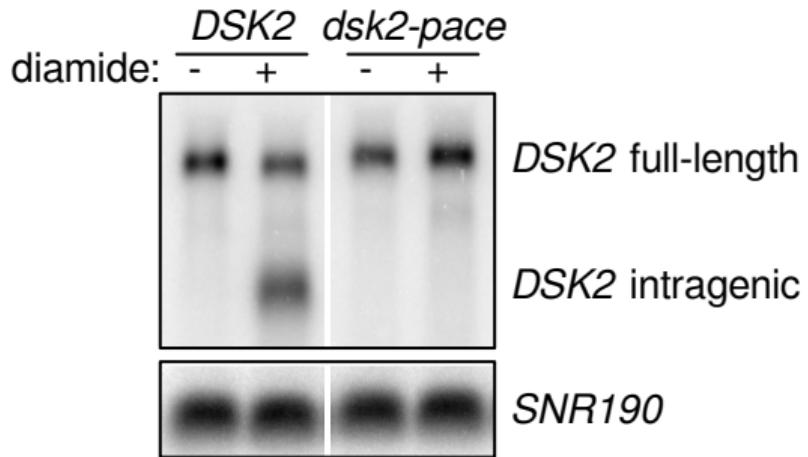


MNase dyads, TFIIB protection, and sense TSSs

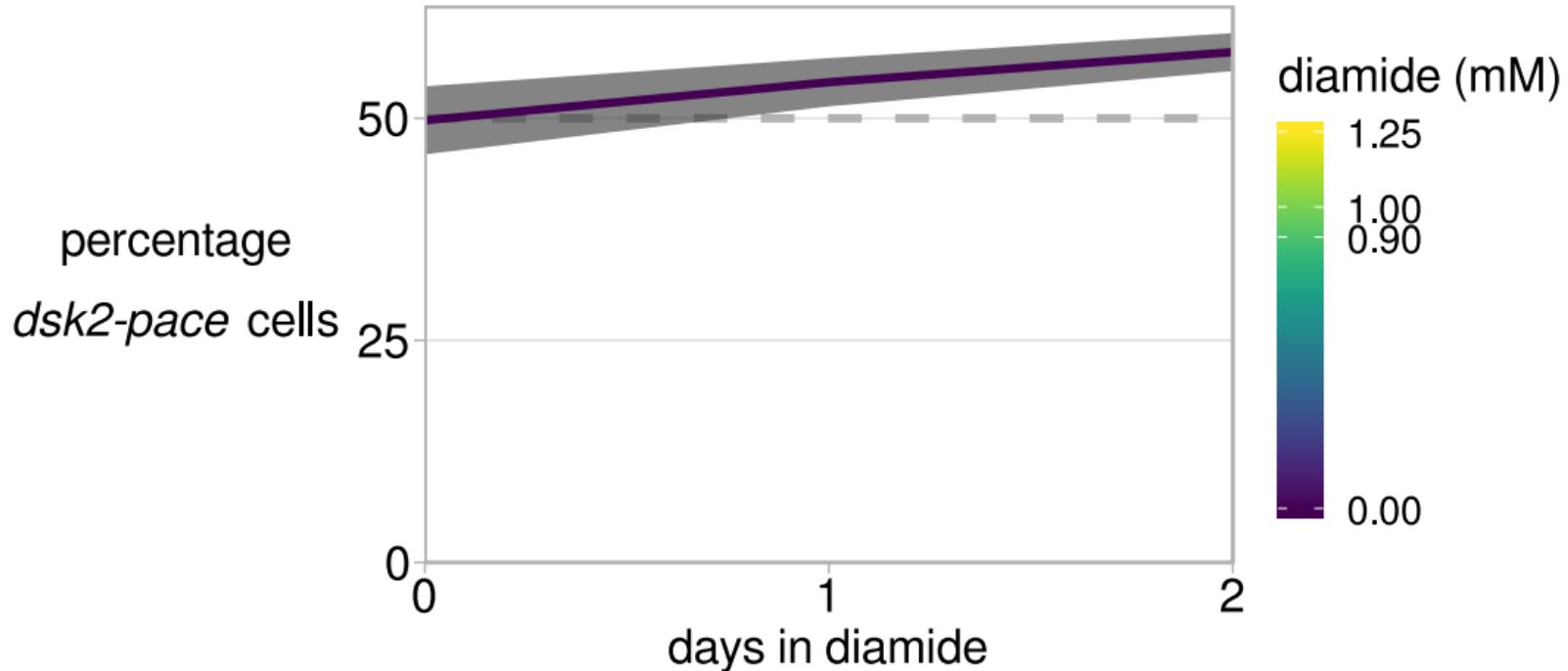


MNase-ChIP-seq: Weiner *et al.* (2015). *Mol. Cell*

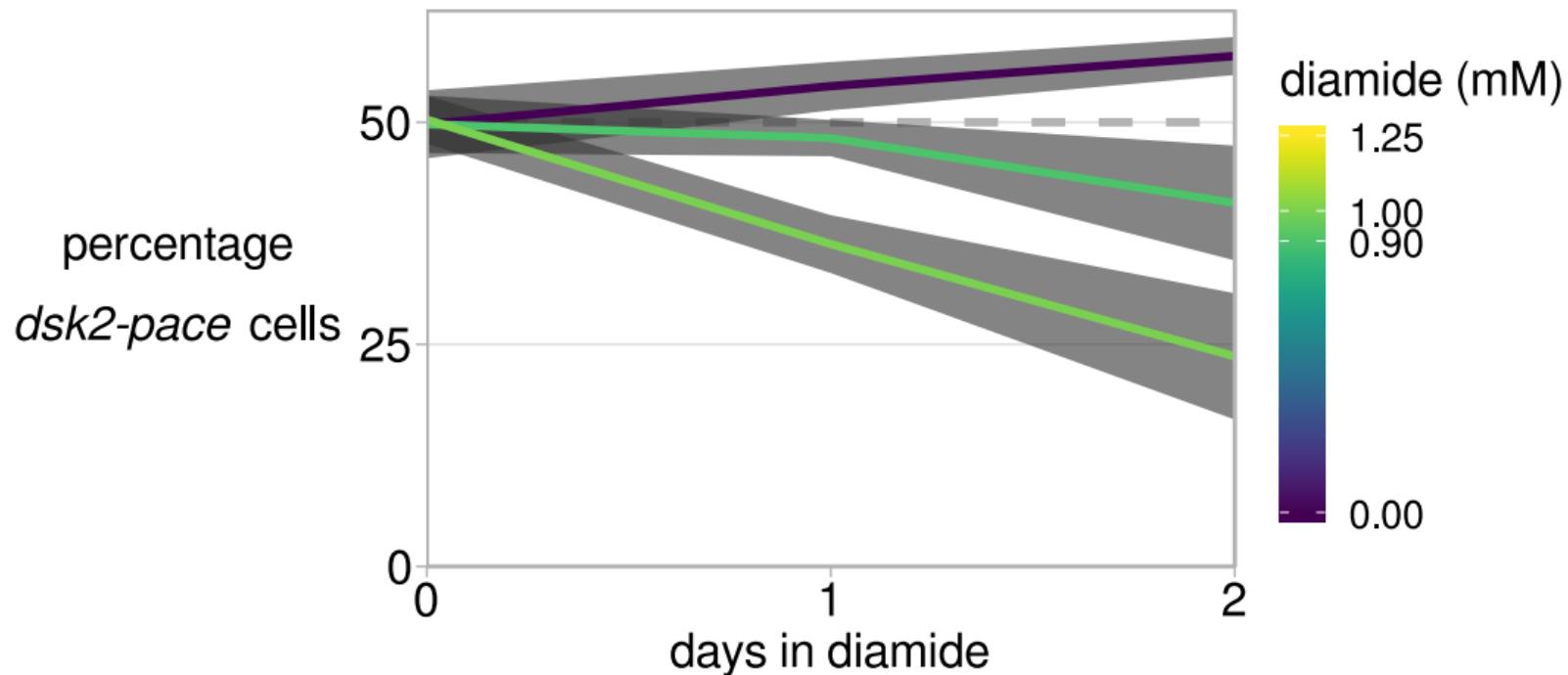
intragenic *DSK2* expression requires PACE elements



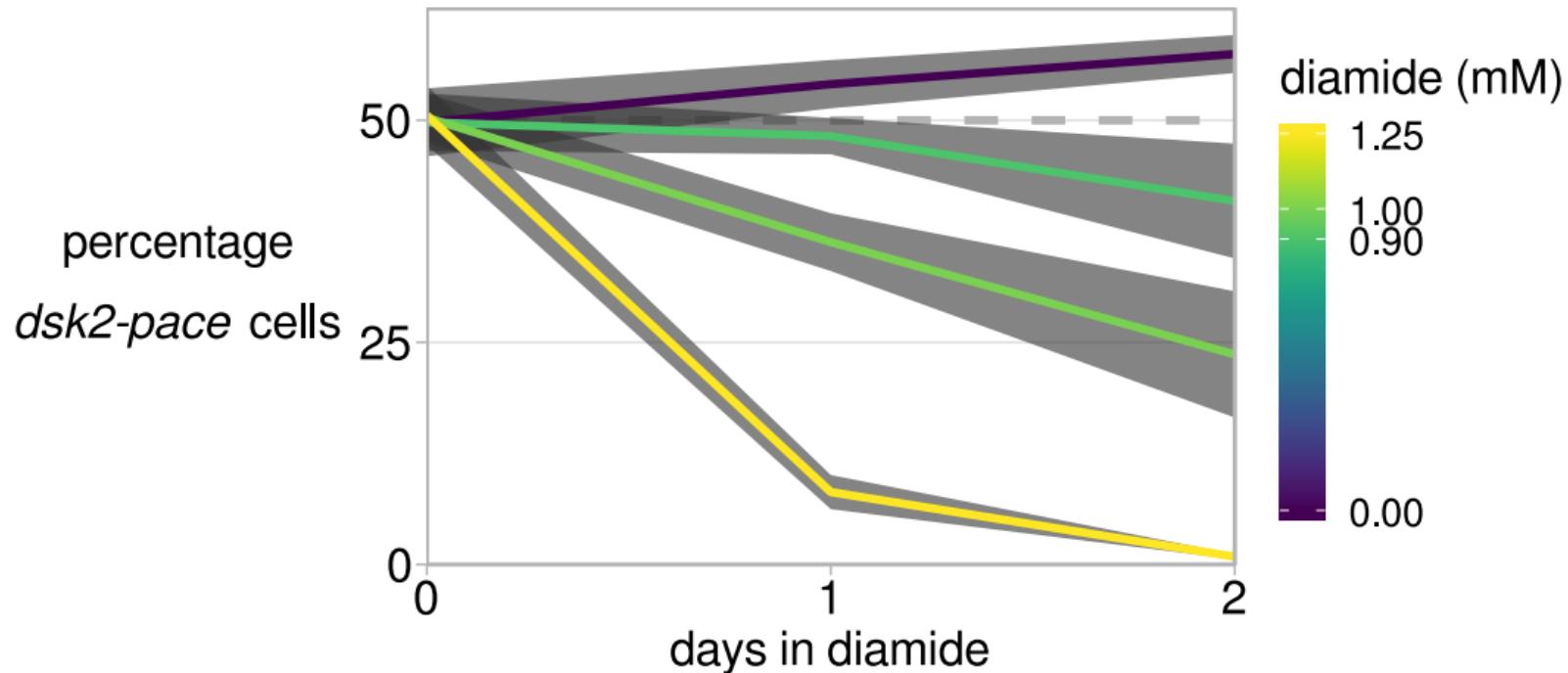
dsk2-pace cells respond poorly to oxidative stress



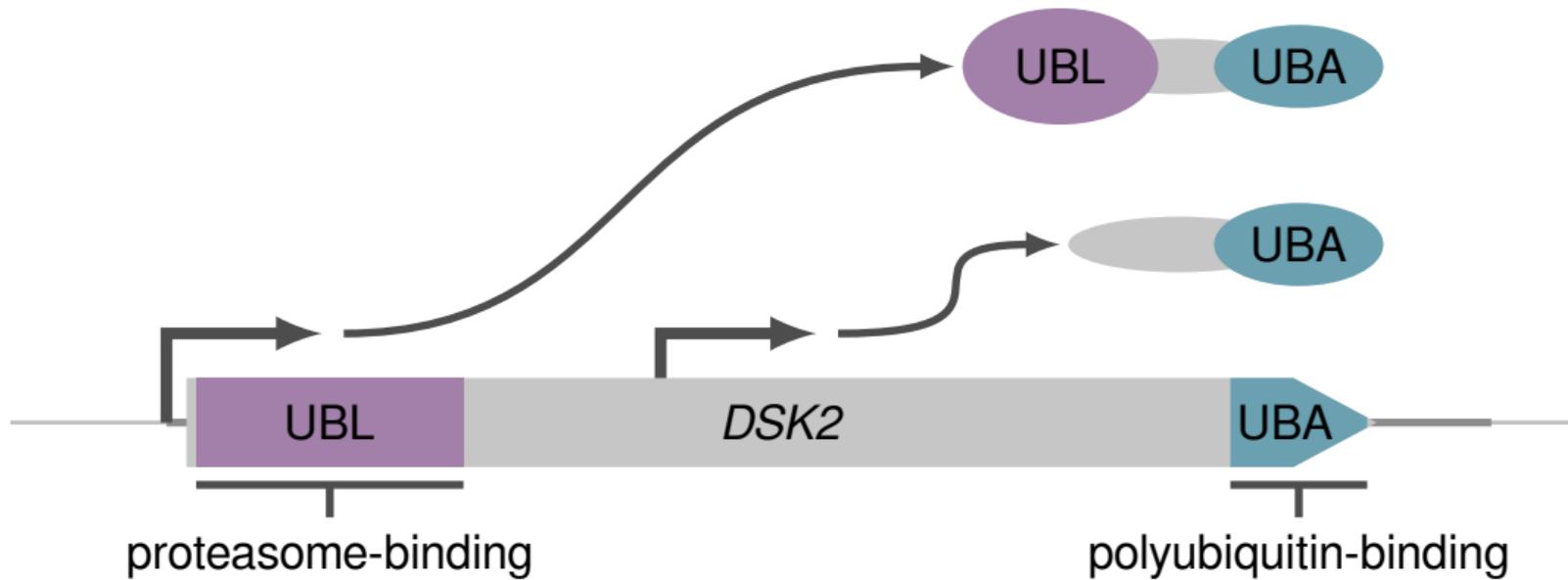
dsk2-pace cells respond poorly to oxidative stress



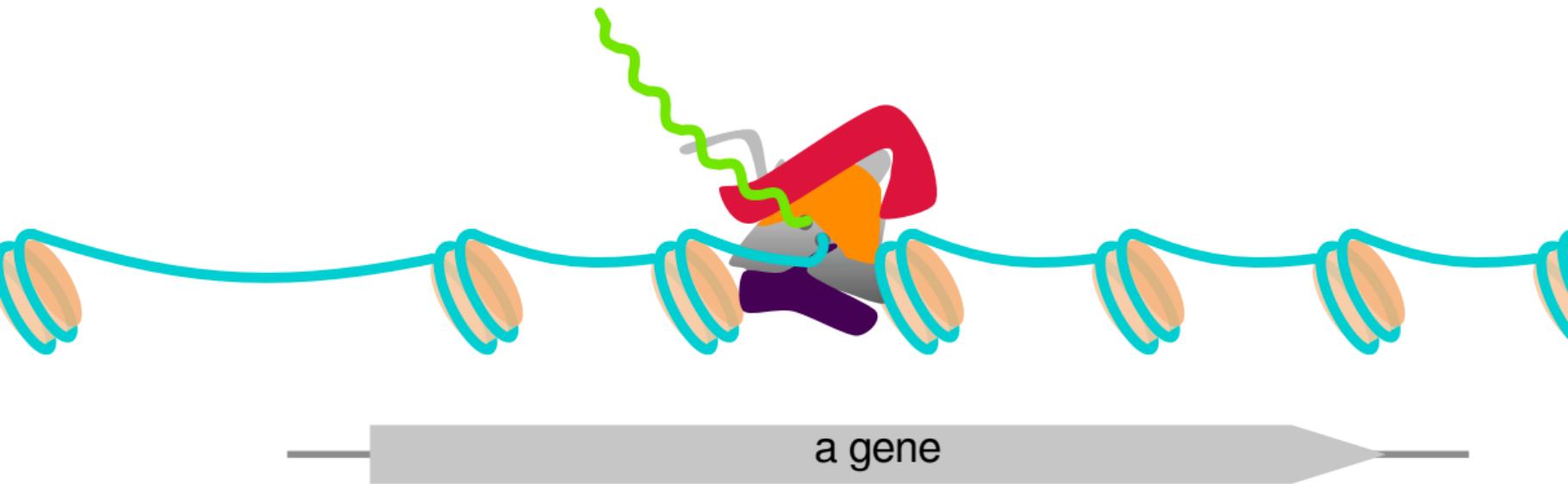
dsk2-pace cells respond poorly to oxidative stress



One possible mechanism for intragenic *DSK2* function



Summary



Acknowledgements

Winston lab

Fred Winston
Ameet Shetty
Steve Doris
Olga Viktorovskaya
Magdalena Murawska
Dan Spatt
Natalia Reim
Rajaraman Gopalakrishnan
Francheska Lopez Rivera
Katie Weiner
James Warner
Mallory Rice

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Fred Winston
Mo Khalil
Stirling Churchman
John Ngo
Wilson Wong

HMS Research Computing

github.com/winston-lab

github.com/james-chuang