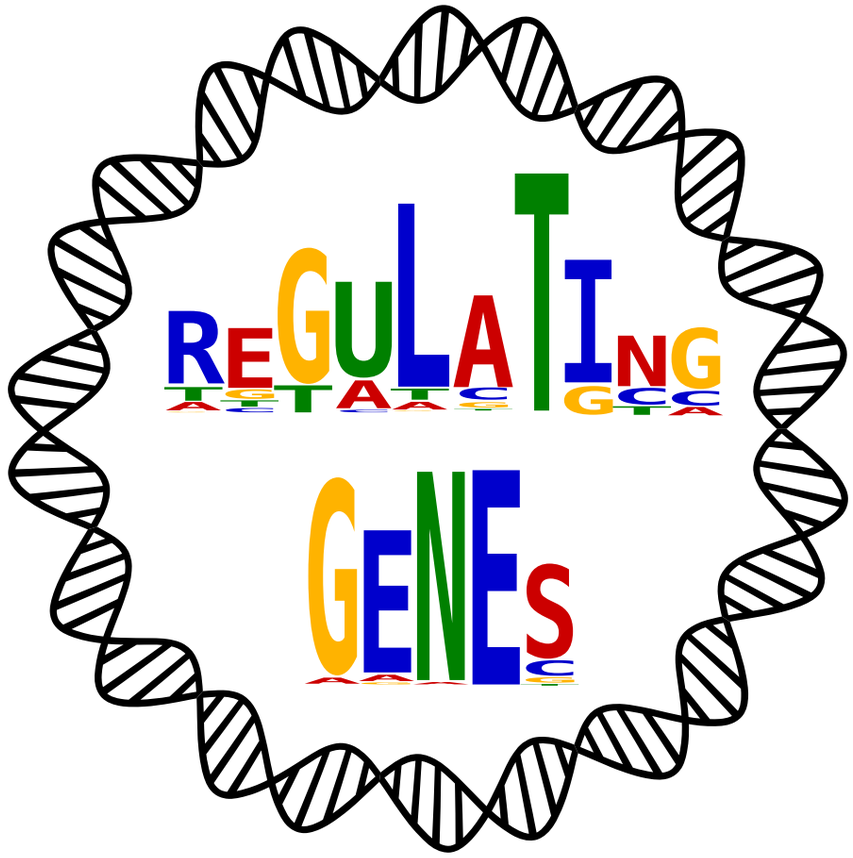
1) Molecular tracking of differentiation cascades;
2) Mechanisms of direct repression by a transcription activator



UCONN
HEALTH

Genetics and Genome Sciences Department

Center for Cell Analysis & Modeling

GUERTIN LAB

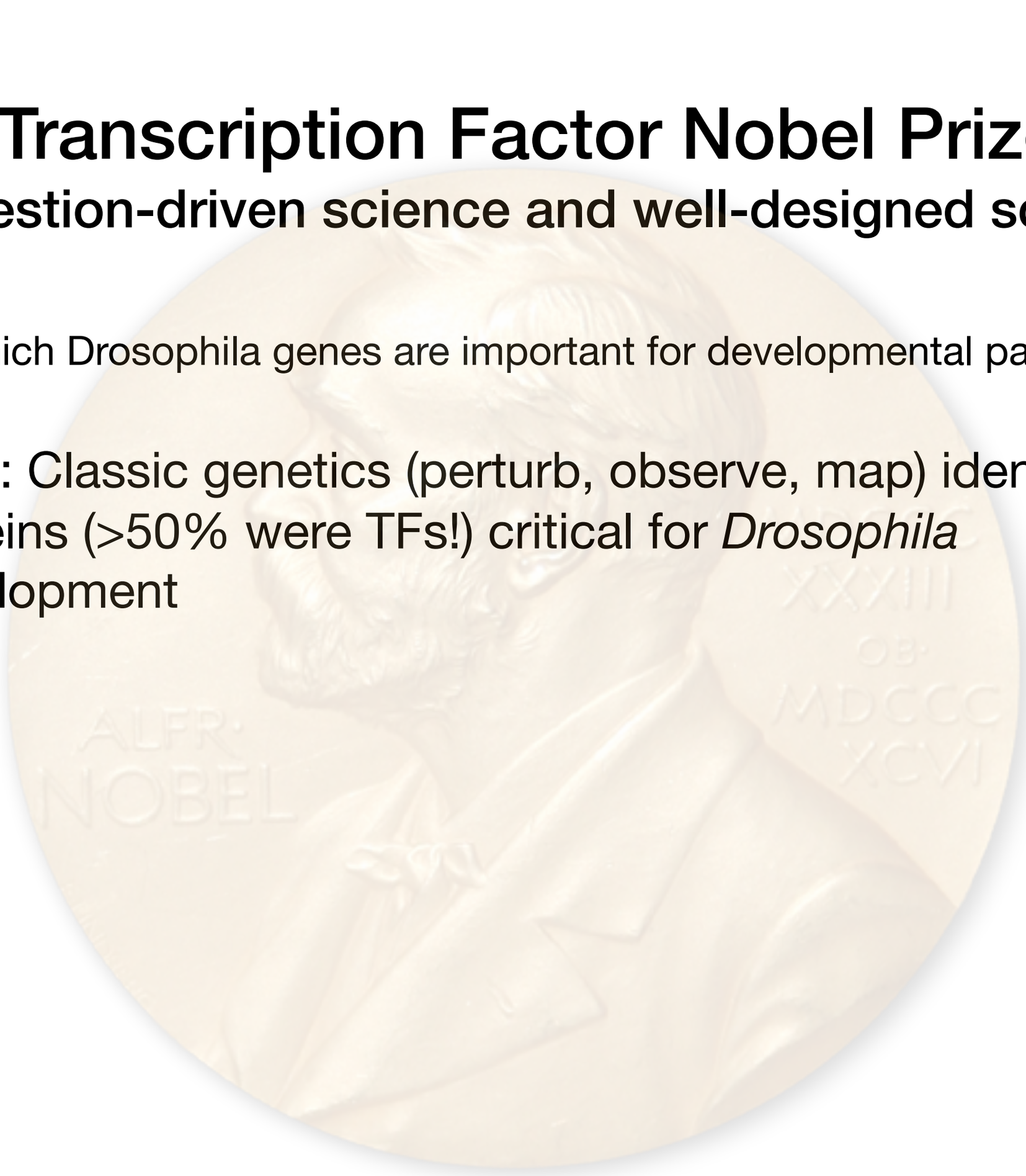
Michael Guertin

Transcription Factor Nobel Prizes

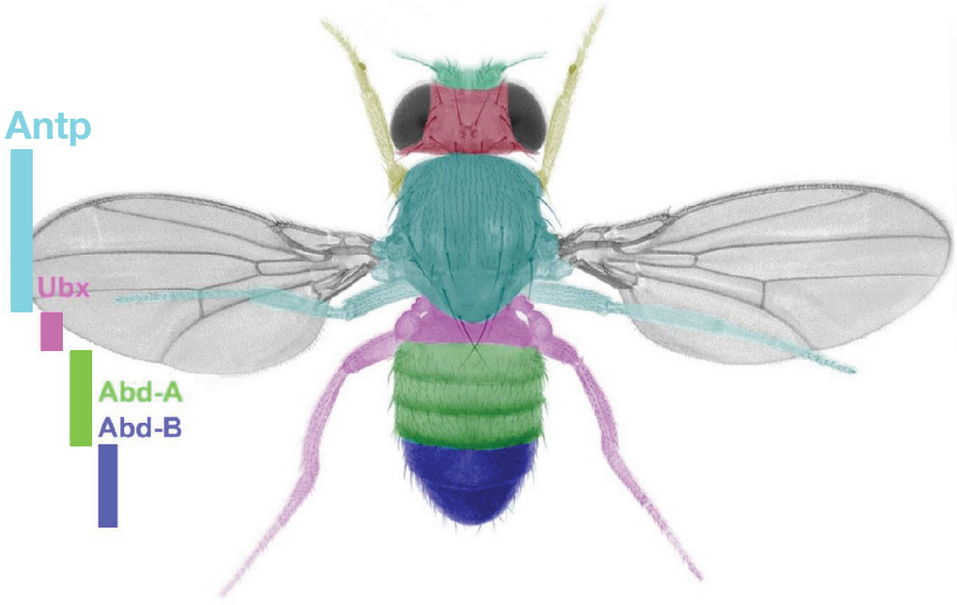
(Question-driven science and well-designed screens)

Q: Which Drosophila genes are important for developmental patterning?

1995: Classic genetics (perturb, observe, map) identifies proteins (>50% were TFs!) critical for *Drosophila* development

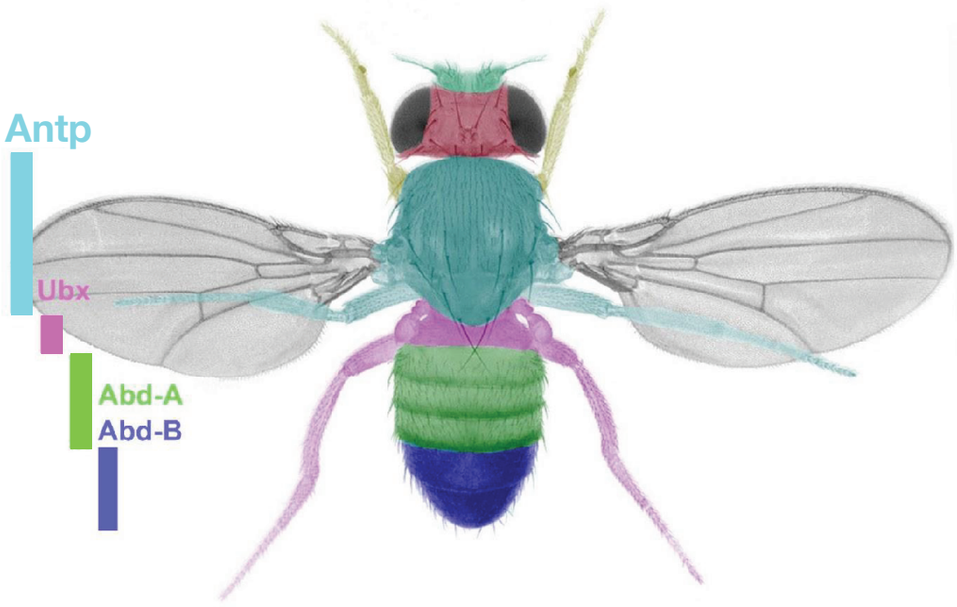


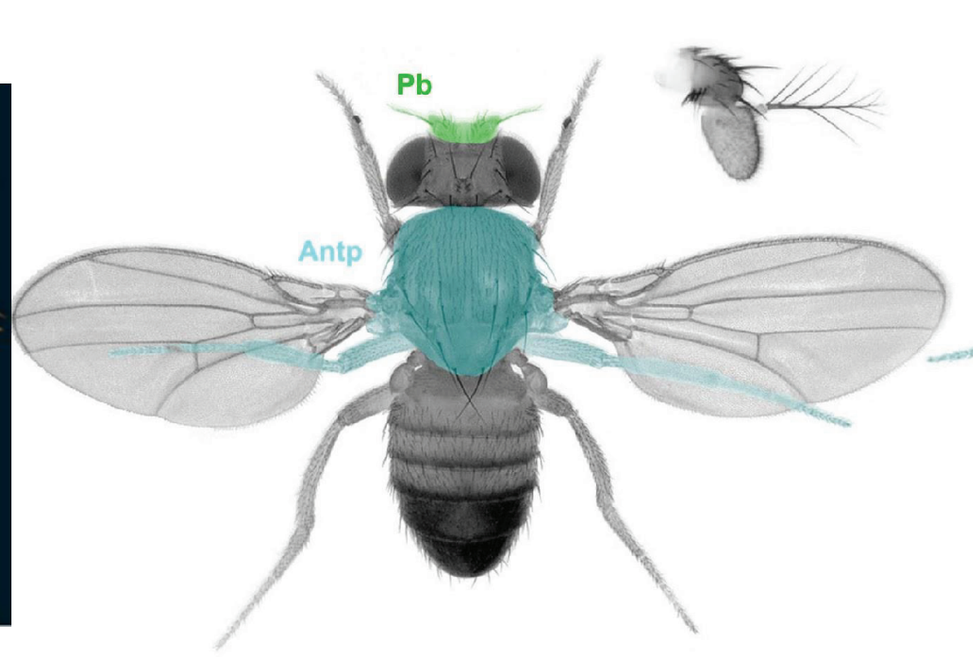
Transcription dysregulation alters developmental patterning



pseudocolored flies: Justin Crocker, Ed Lewis, Nicolas Gompel, and Welcome Bender

Classic Genetics: Perturb and Map





pseudocolored flies: Justin Crocker, Ed Lewis, Nicolas Gompel, and Welcome Bender

pseudocolored SEM heads: Jürgen Berger

Classic genetics (**perturb, observe, map**) found that Transcription Factors control developmental patterning

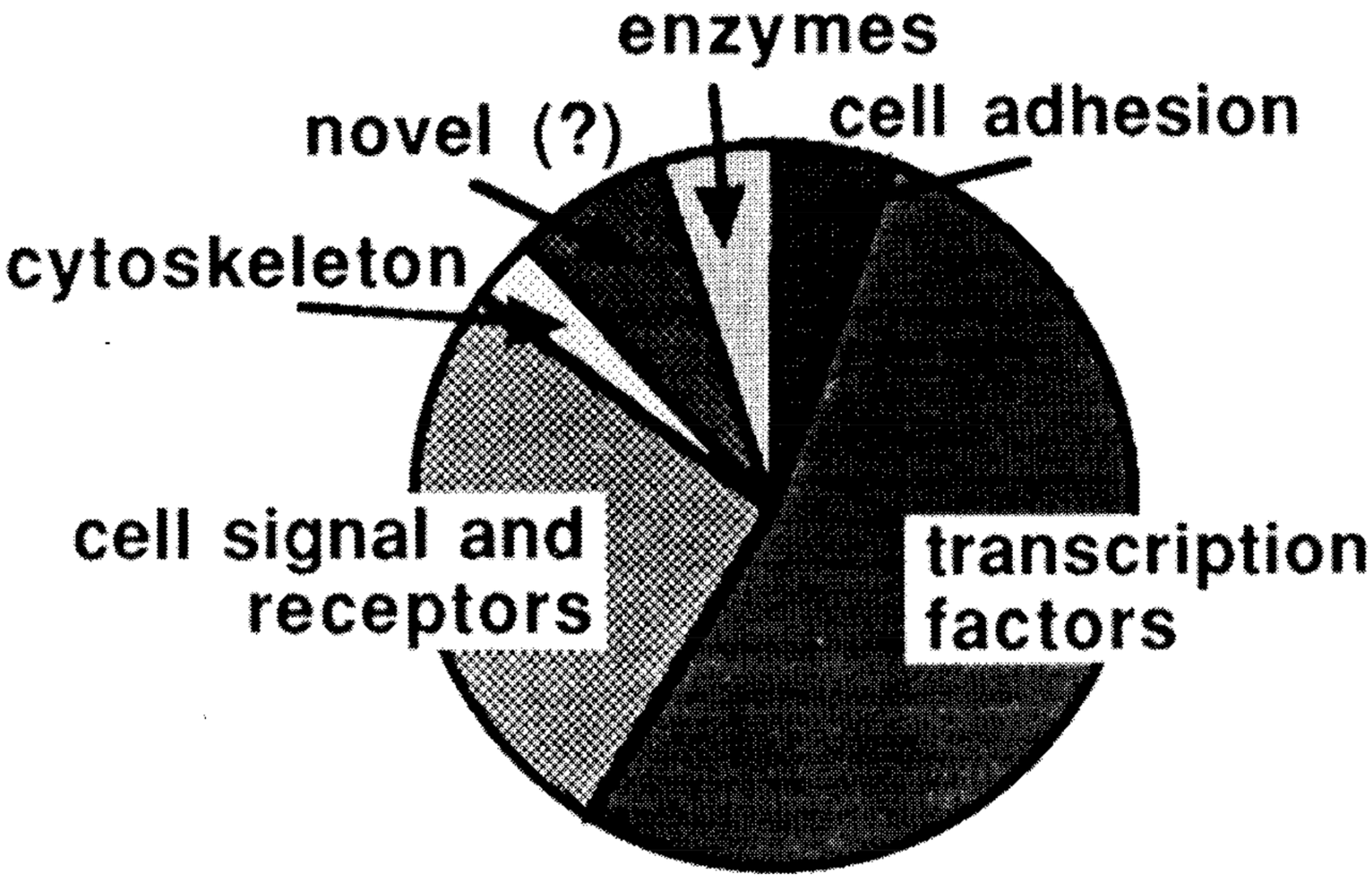


Figure 3. *Cellular Function of Heidelberg Mutations.* Based on the sequence of 75 cloned genes, most of the loci identified in Heidelberg encode transcription factors, or cell signals and receptors.

Eric Weischaus, Nobel Lecture 1995
Prize shared with Christiane Nüsslein-Volhard & Ed Lewis

Transcription Factor Nobel Prizes
(Question-driven science and well-designed screens)

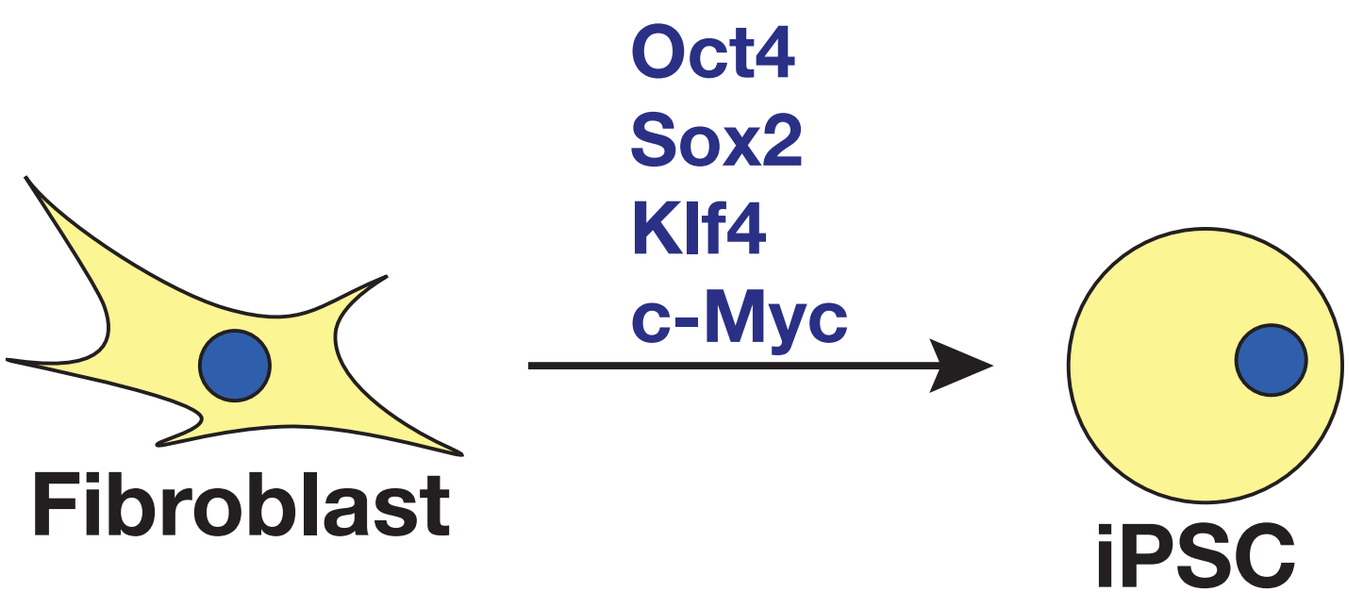
Q: Which Drosophila genes are important for developmental patterning?

1995: Classic genetics (perturb, observe, map) identifies proteins (>50% were TFs!) critical for *Drosophila* development

Q: Can we reprogram differentiated cells to a pluripotent state?

2012: Brute force screen with candidate genes identified from expression microarrays. Transduce 24 genes that are specifically expressed in embryonic stem cells into fibroblasts. Systematically narrow down the list: Oct3/4, Klf4, Sox2, and c-Myc (all TFs!)

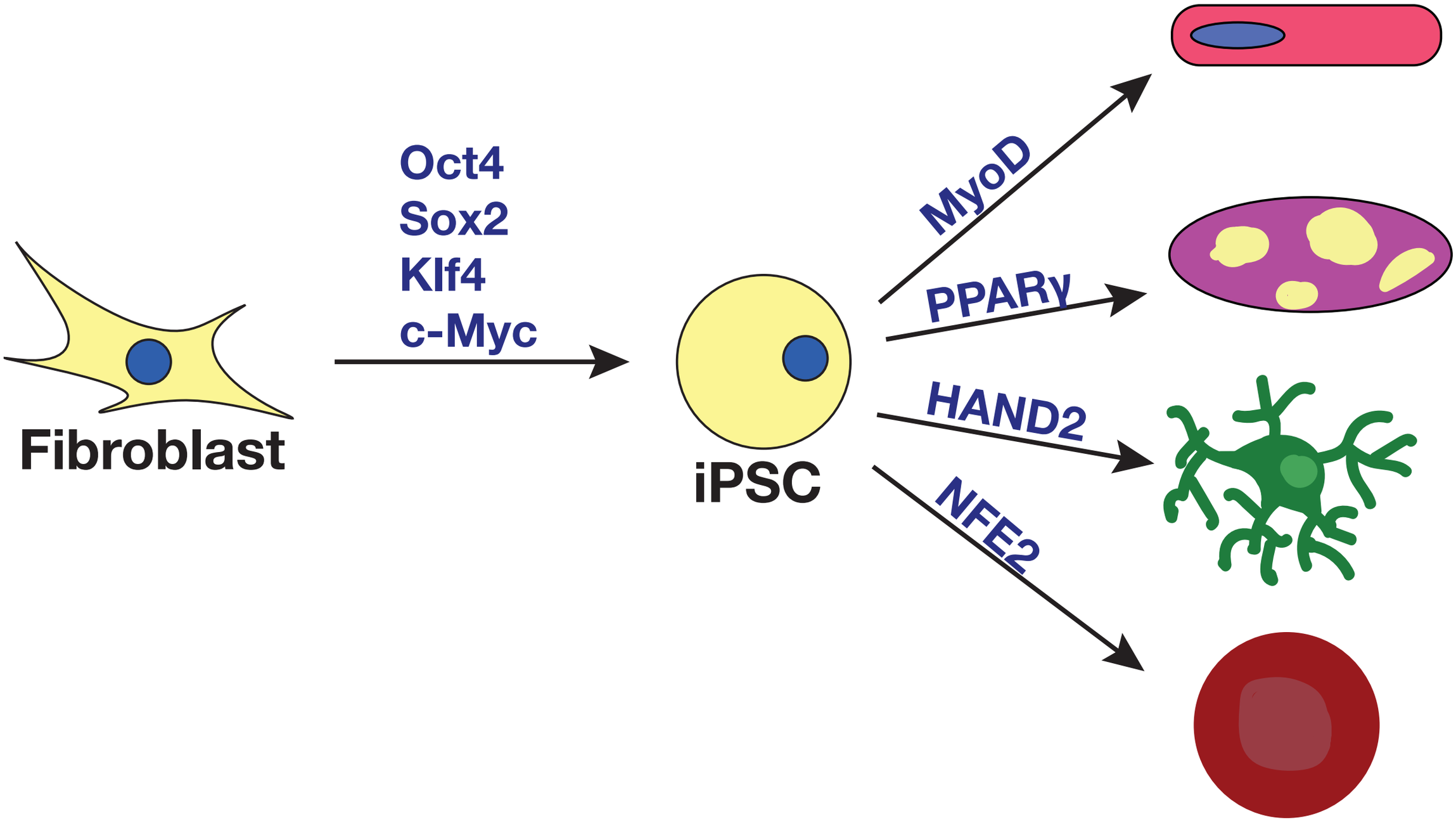
Transcription factors drive changes in cell identity



Brute force screening approach:
express many genes in combination until we get iPSCs

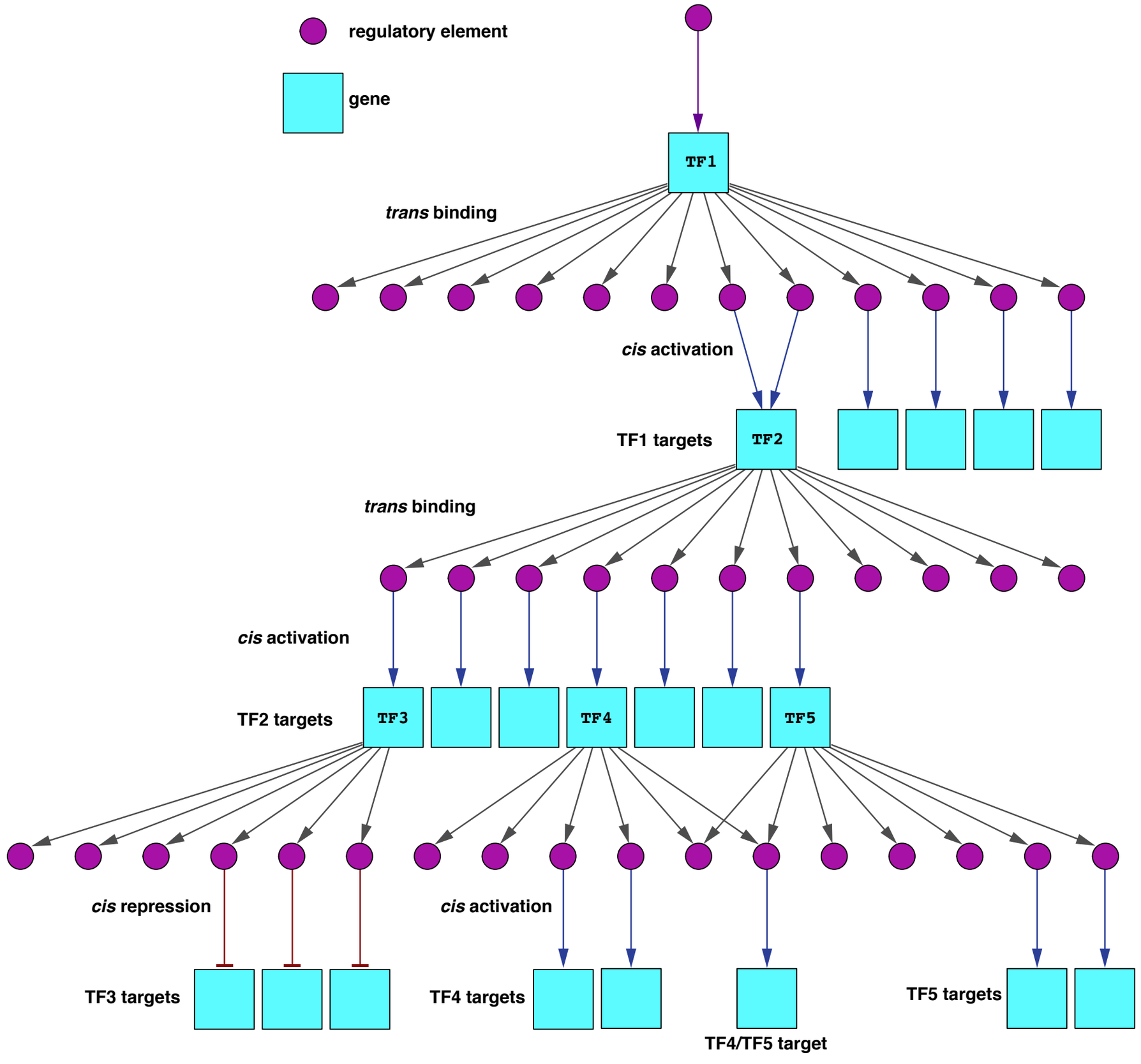
Takahashi & Yamanaka, Cell 2006
2012 Nobel Prize shared with John Gurdon

Transcription factors drive changes in cell identity



Takahashi & Yamanaka, Cell 2006
2012 Nobel Prize shared with John Gurdon

Which TFs and regulatory elements are important in regulatory cascades?



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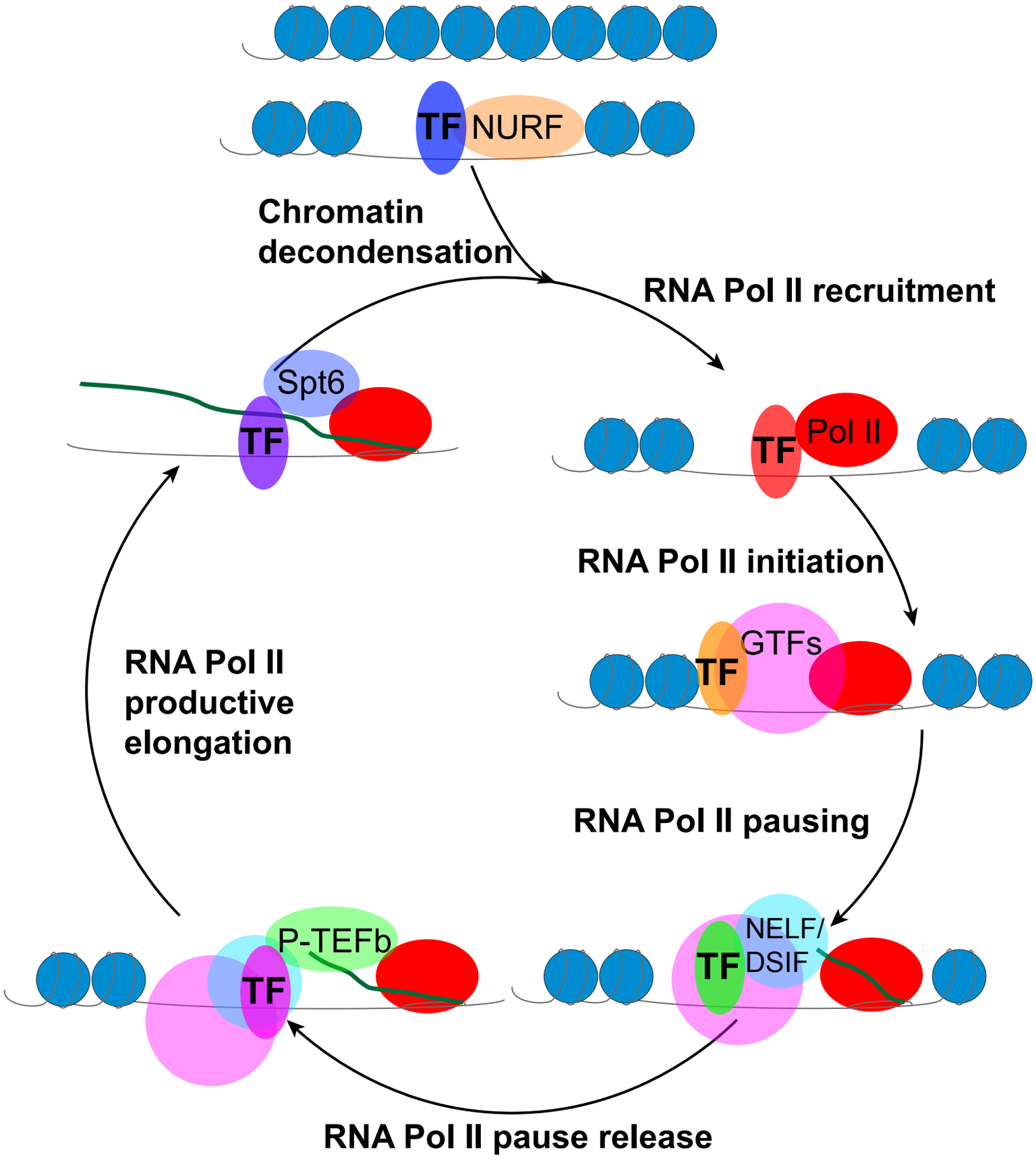
- Which time points do we choose?

- Which models can we genetically manipulate to validate candidate TFs and elements?

What are the step(s) in transcription that each key TF regulates?



Sequence-specific TFs specialize and work together

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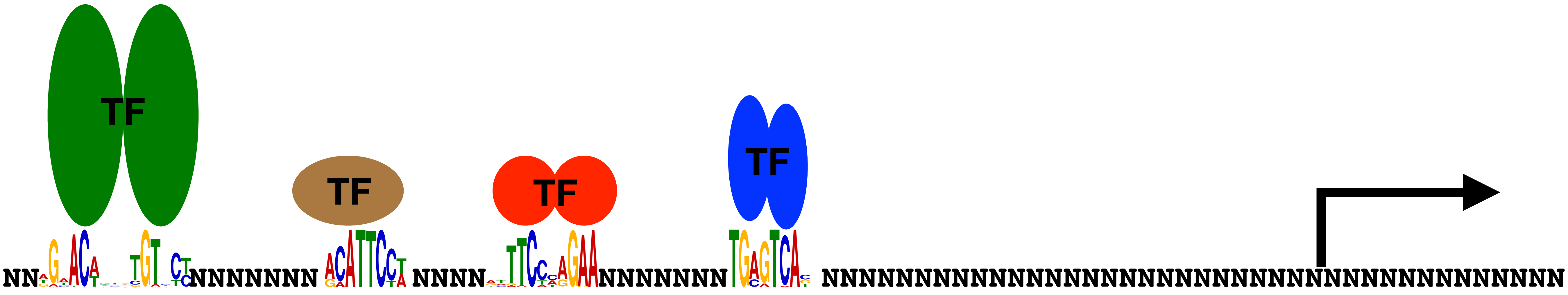
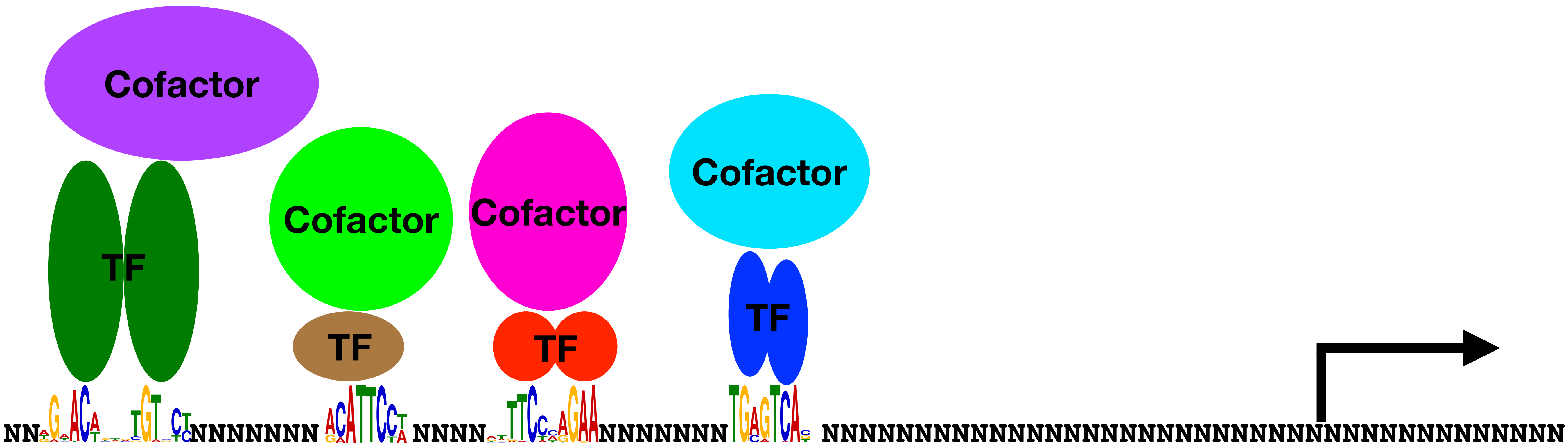


Sequence-specific TFs specialize and work together



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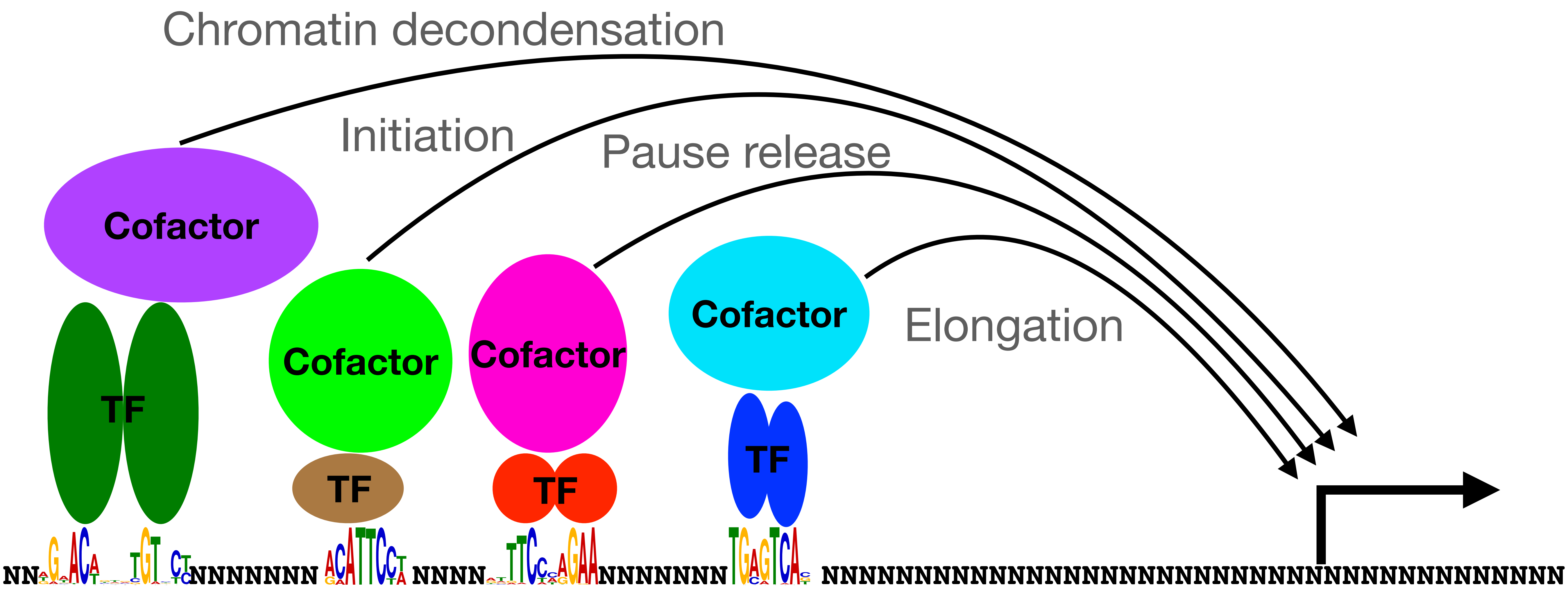

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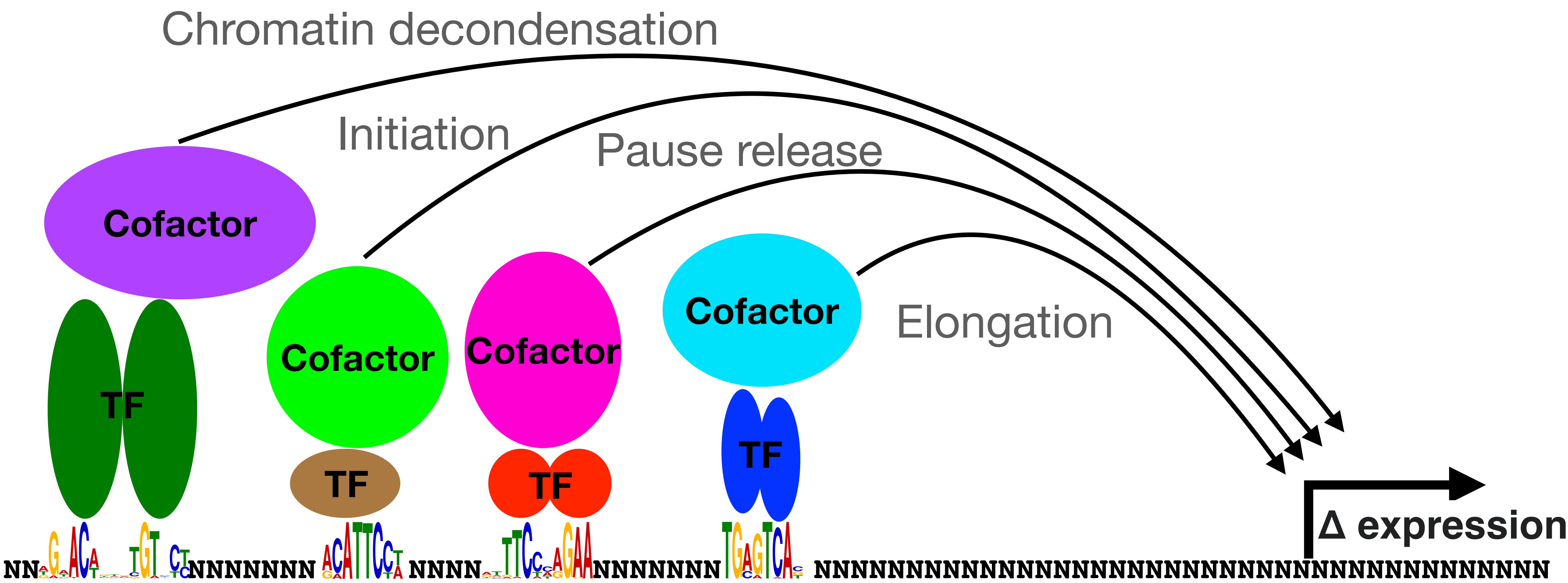
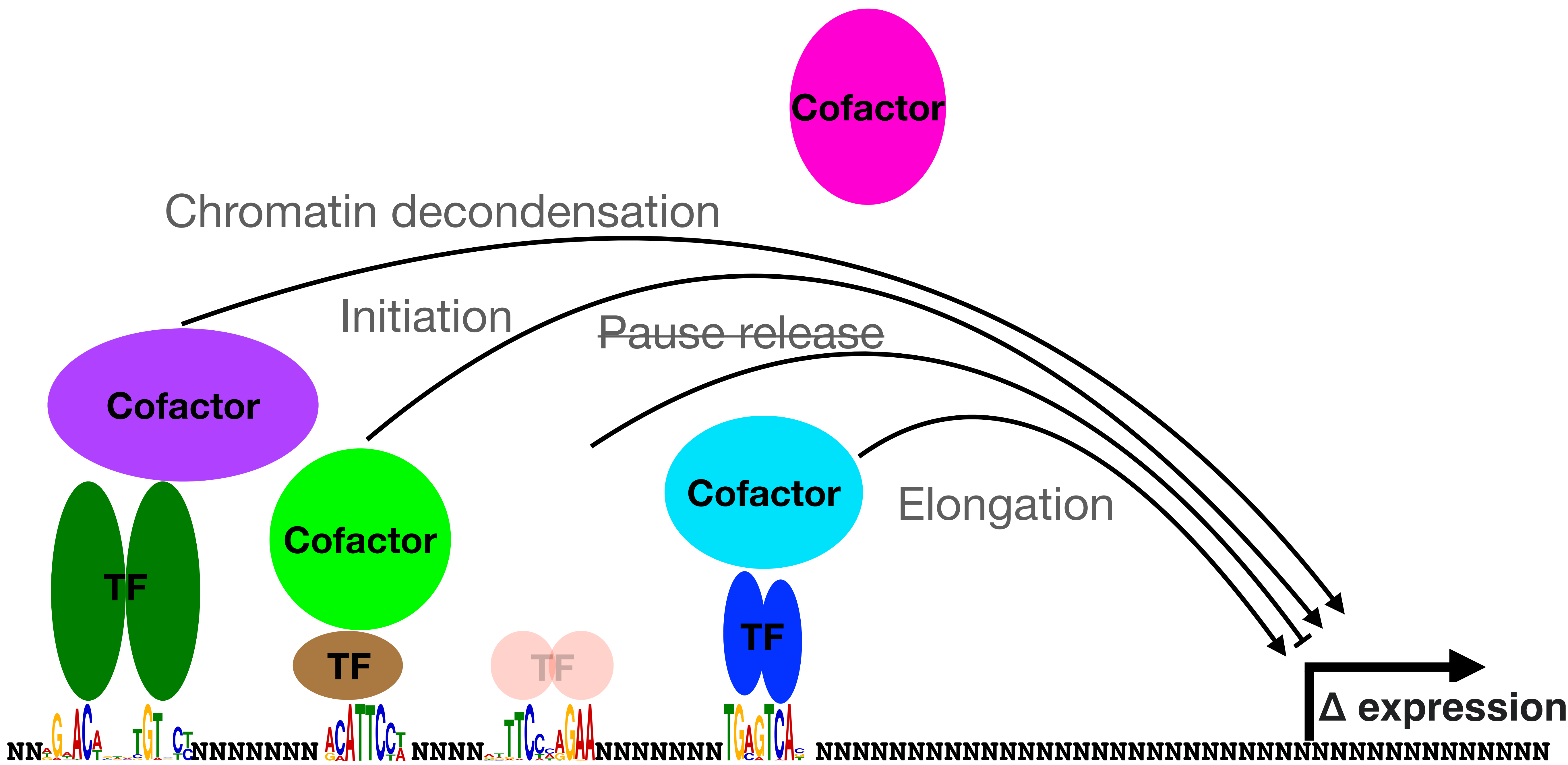


Approach: rapidly degrade red TF and quantify changes in each step



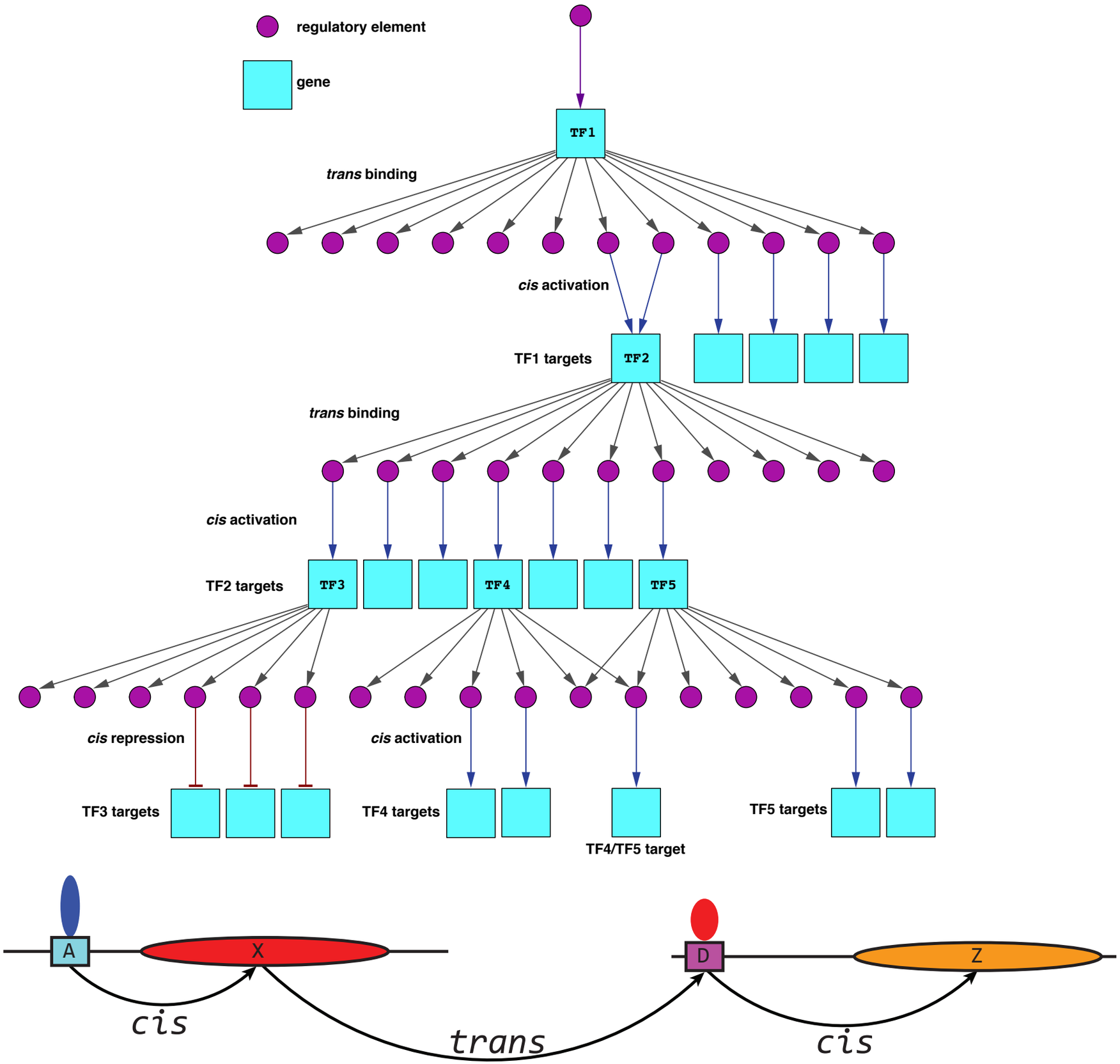
Outline

- Question-driven science and well-designed studies

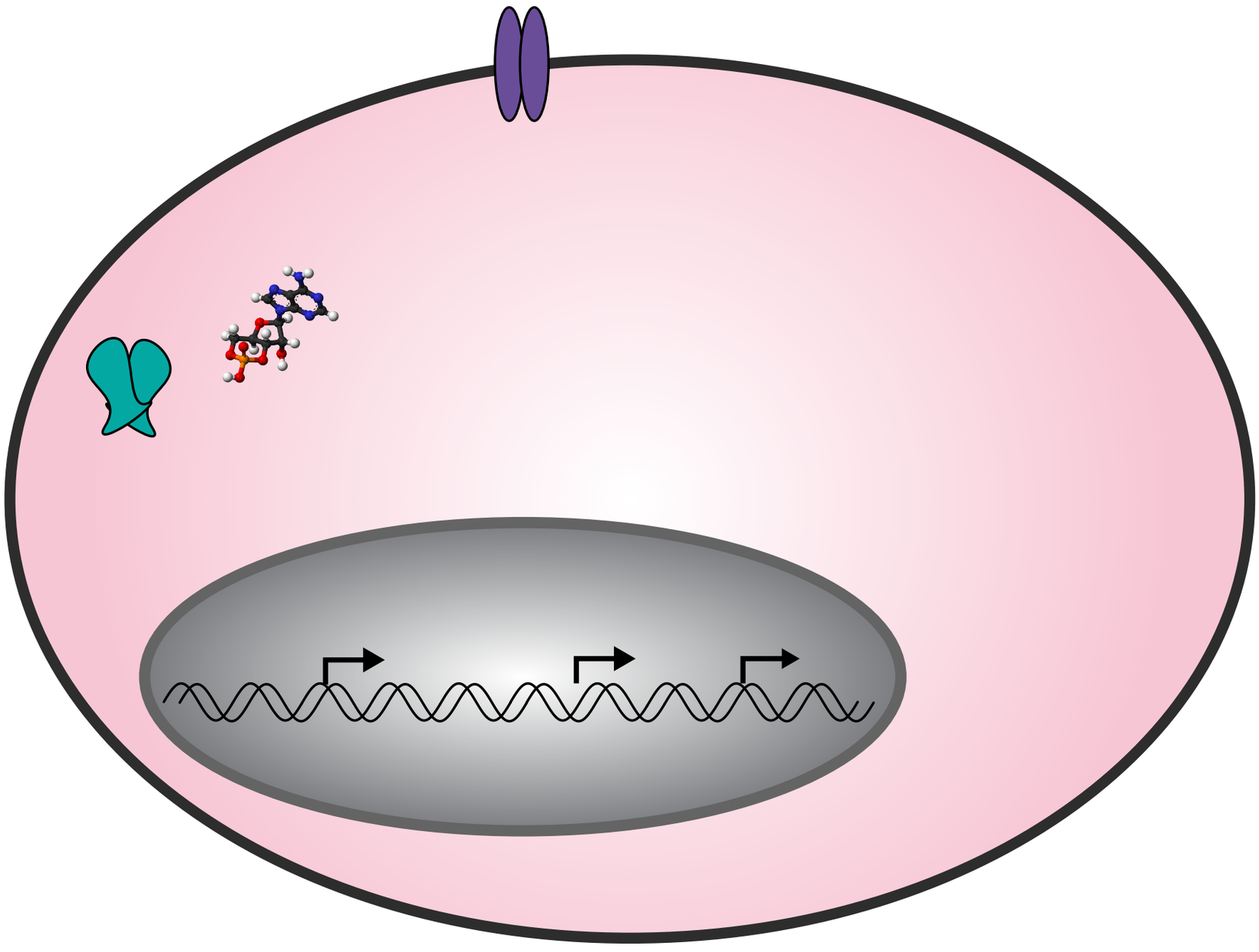
- Mechanistic gene regulatory networks

- Rapid depletion of the activator ZNF143 identifies unexpected direct repression of ZNF143 target genes

Molecular tracking of differentiation cascades



Adipogenesis of 3T3-L1 cells



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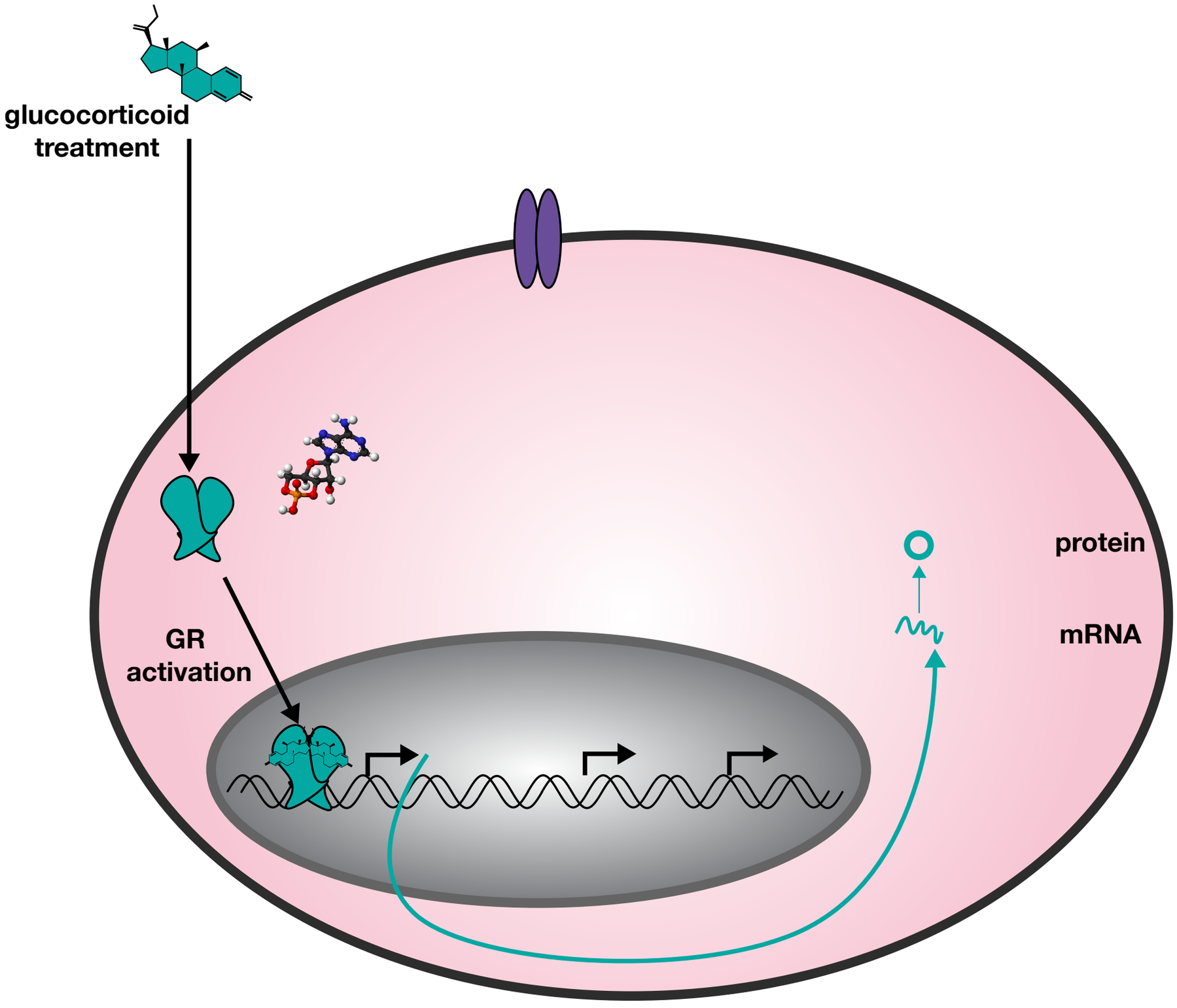

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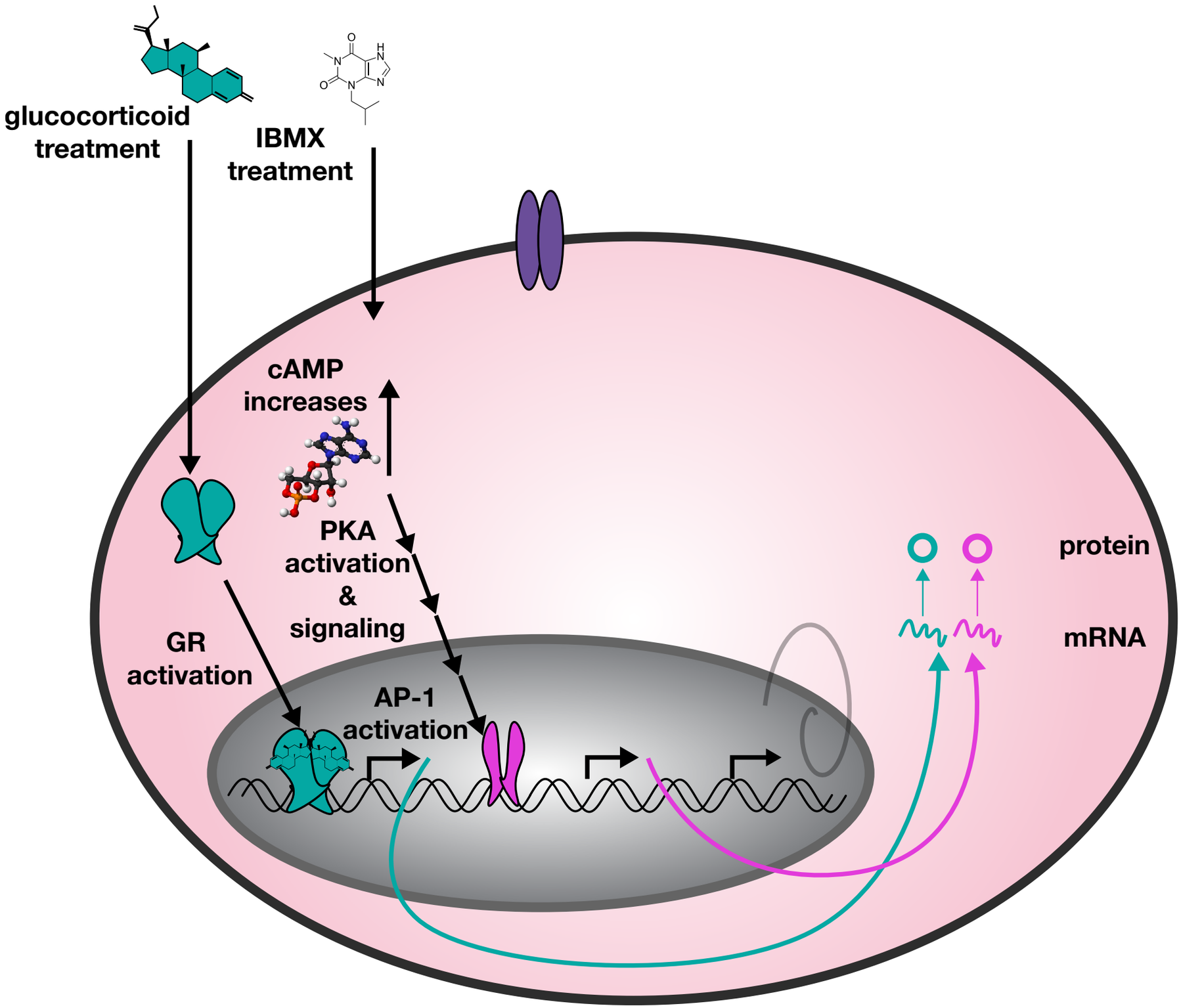

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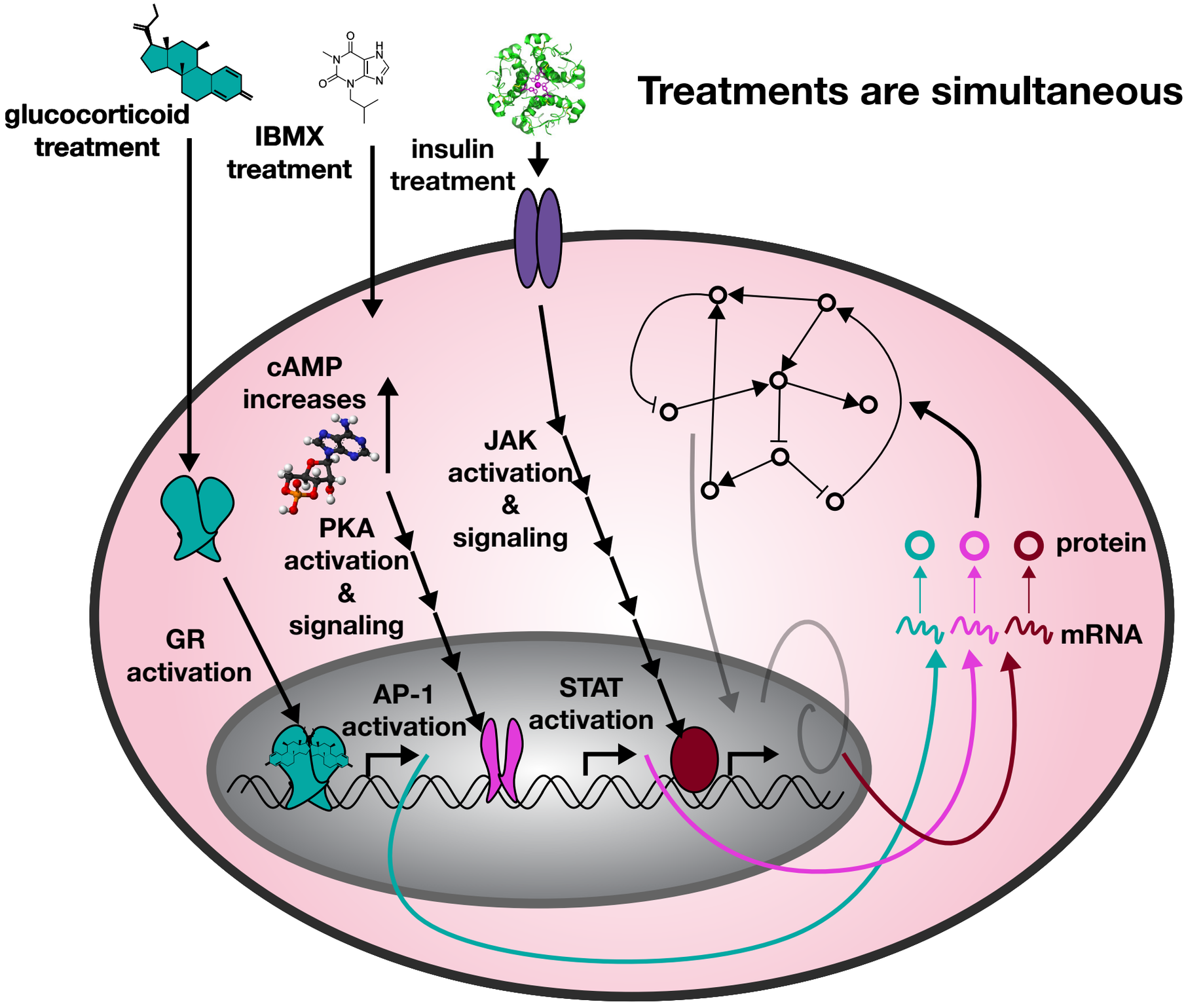
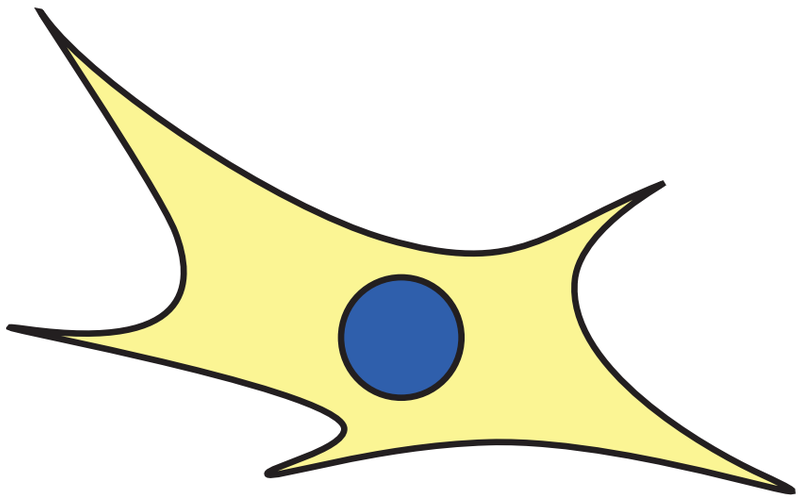
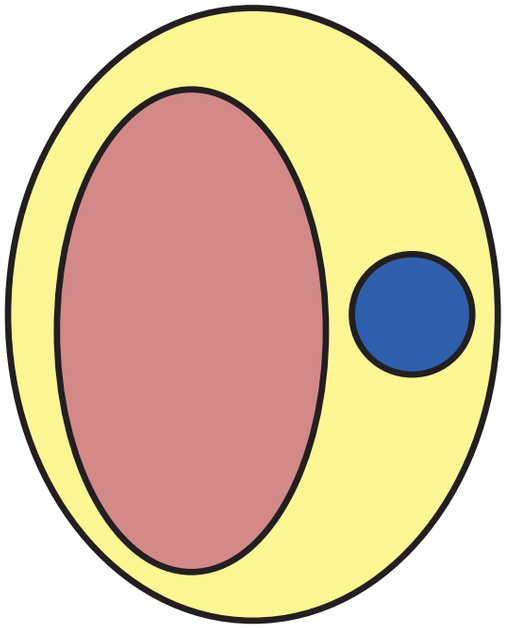


Experimental Design



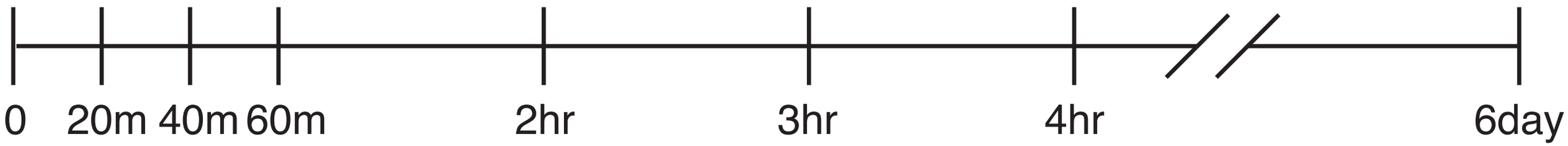
Glucocorticoid receptor agonist
cAMP agonist
Insulin receptor agonist

6 days



3T3-L1 preadipocyte fibroblast

Mature adipocycte



Time points for ATAC-seq and PRO-seq

Transposase Hypersensitivity (ATAC-seq)

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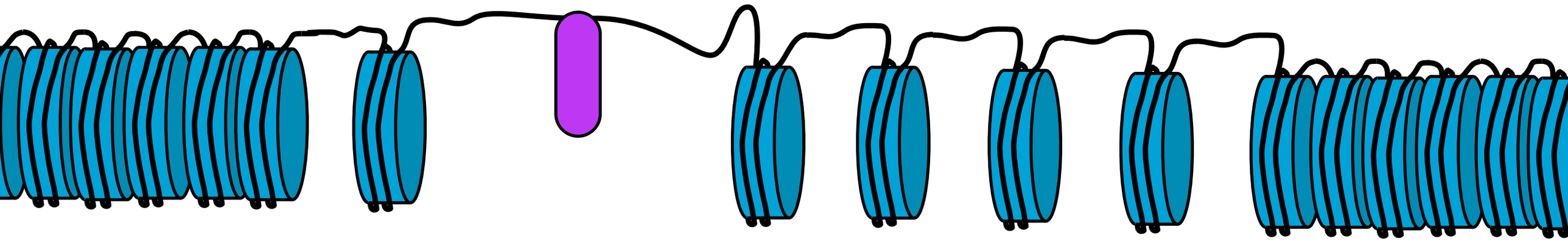
- A general measure of chromatin structure.

Transposase Hypersensitivity (ATAC-seq)

- A general measure of chromatin structure.
  - ATAC peaks and DNA motifs can be used to infer TF binding (i.e. if chromatin is accessible and contains a binding sequence, then a TF may be bound)

Transposase Hypersensitivity (ATAC-seq)



Buenrostro, et. al. Nature Methods, 2013

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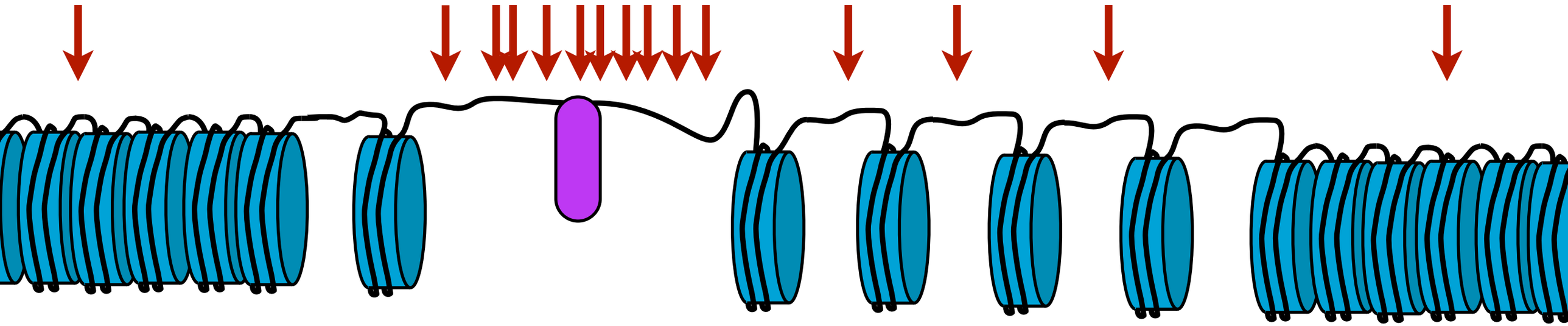

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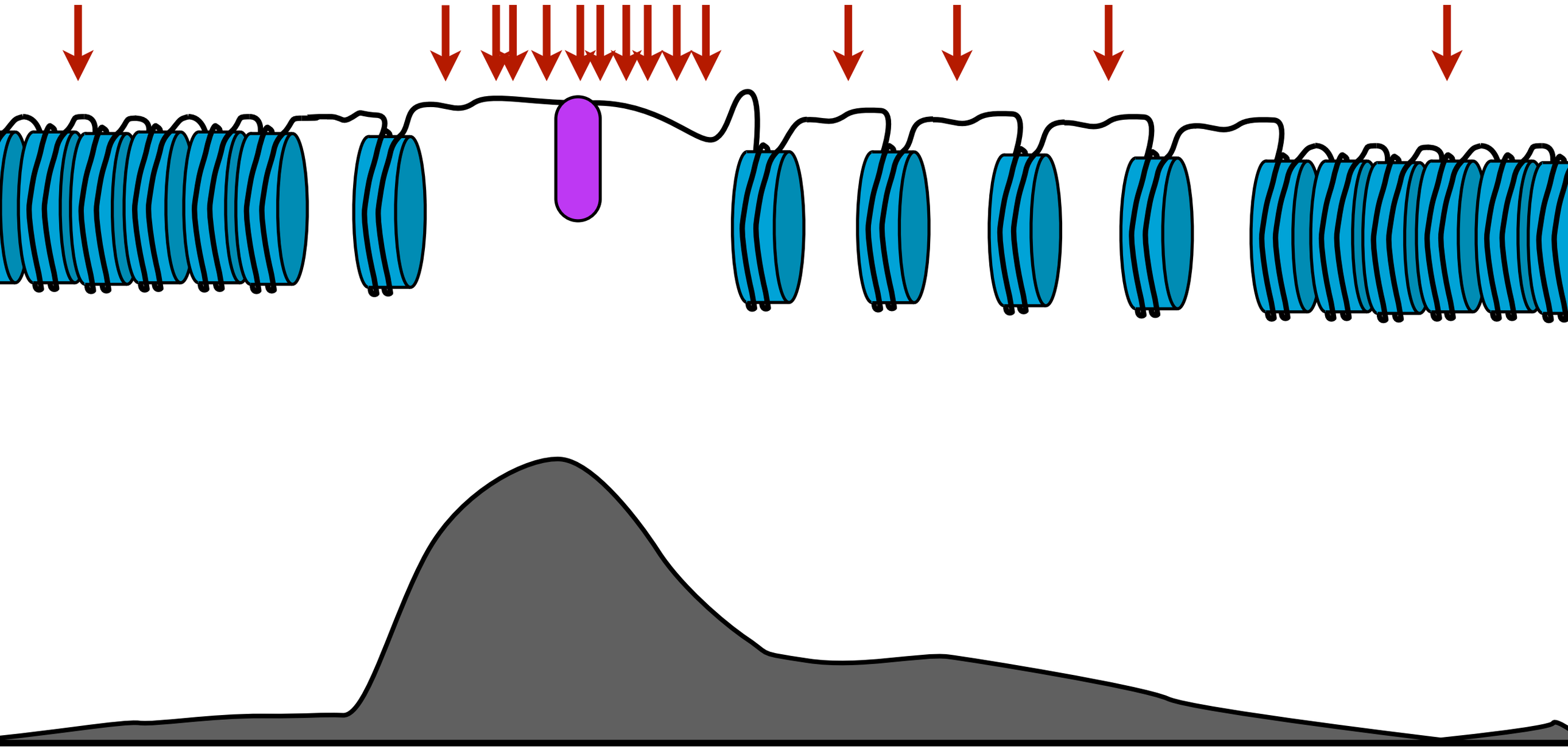
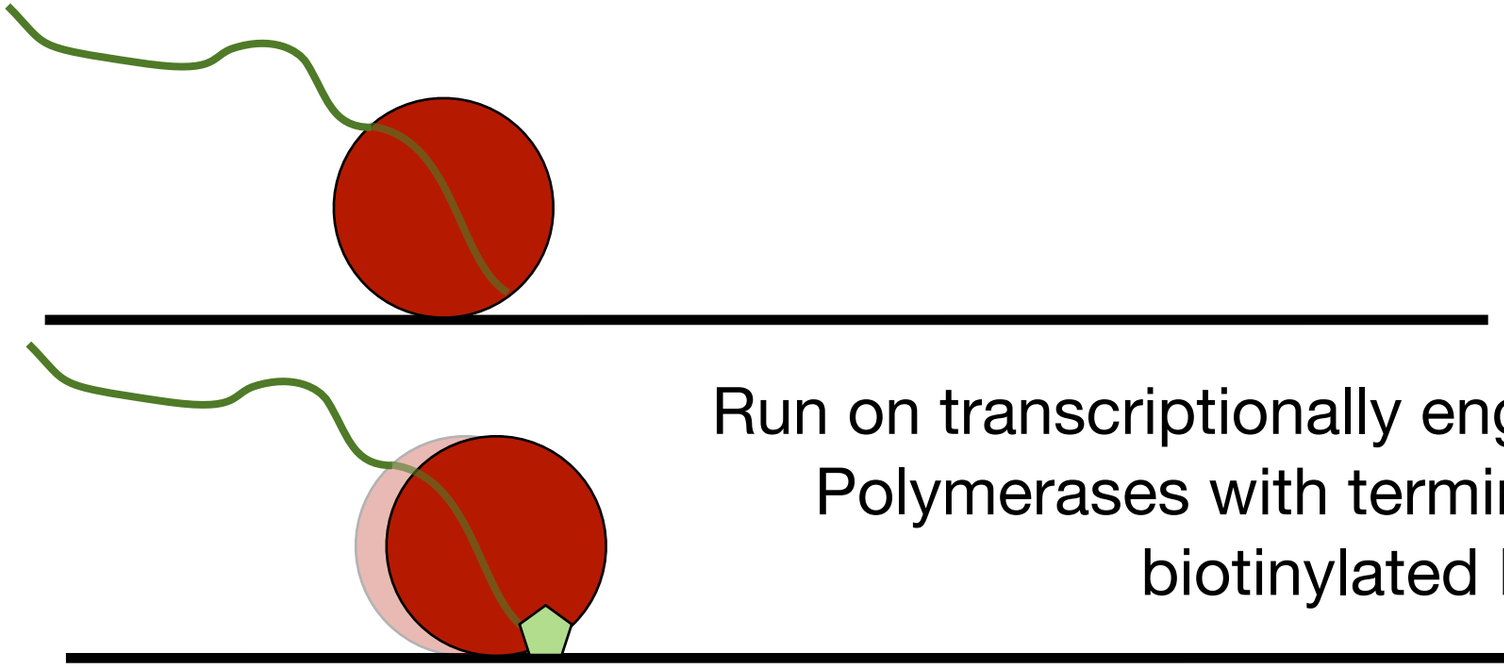


Buenrostro, et. al. Nature Methods, 2013

PRO-seq detects nascent RNA

Kwak, et. al. Science, 2013

Precision Genomic-Run On (PRO-seq)



Run on transcriptionally engaged Polymerases with terminating biotinylated NTPs.

Kwak, et. al. Science, 2013

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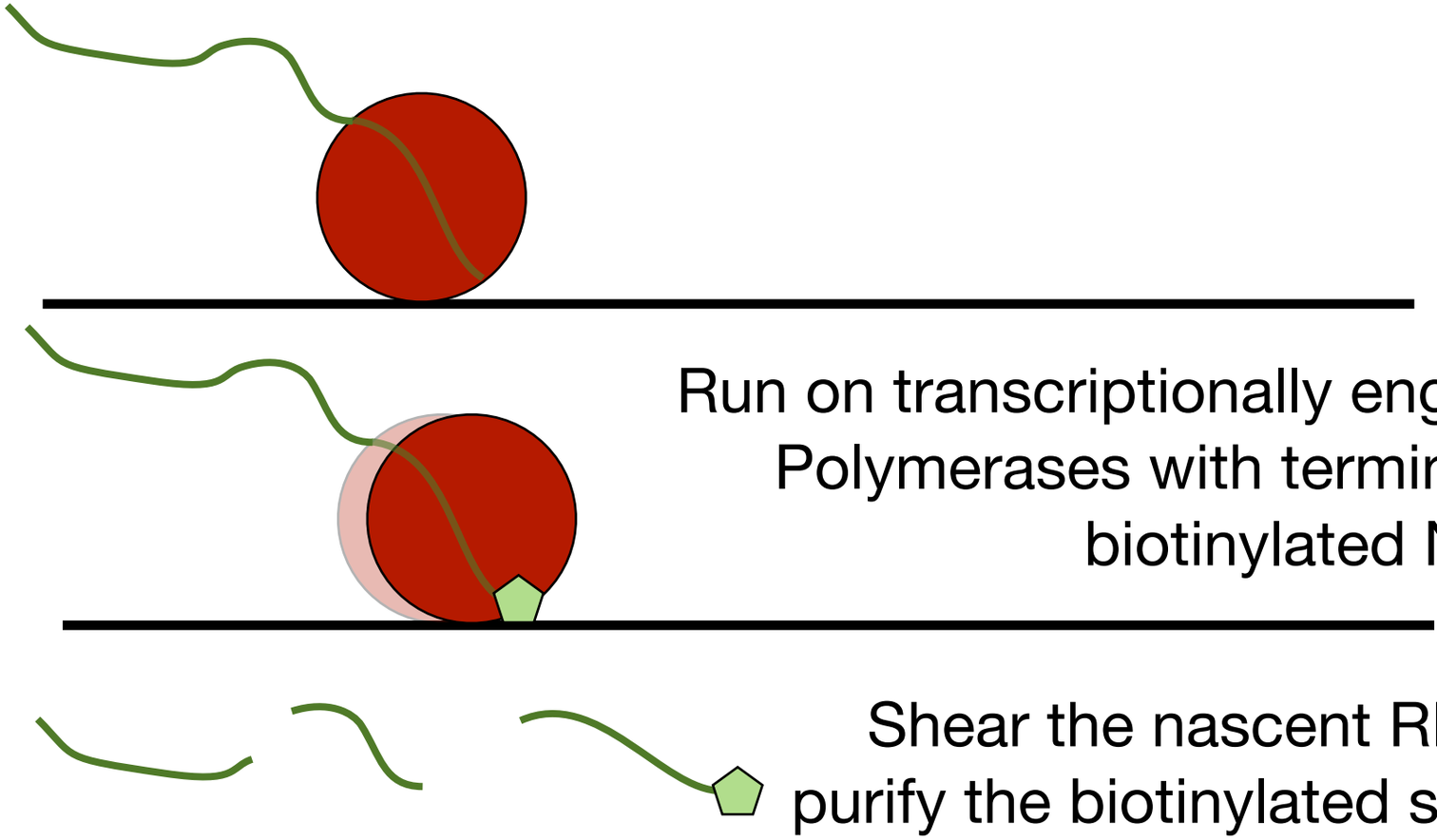
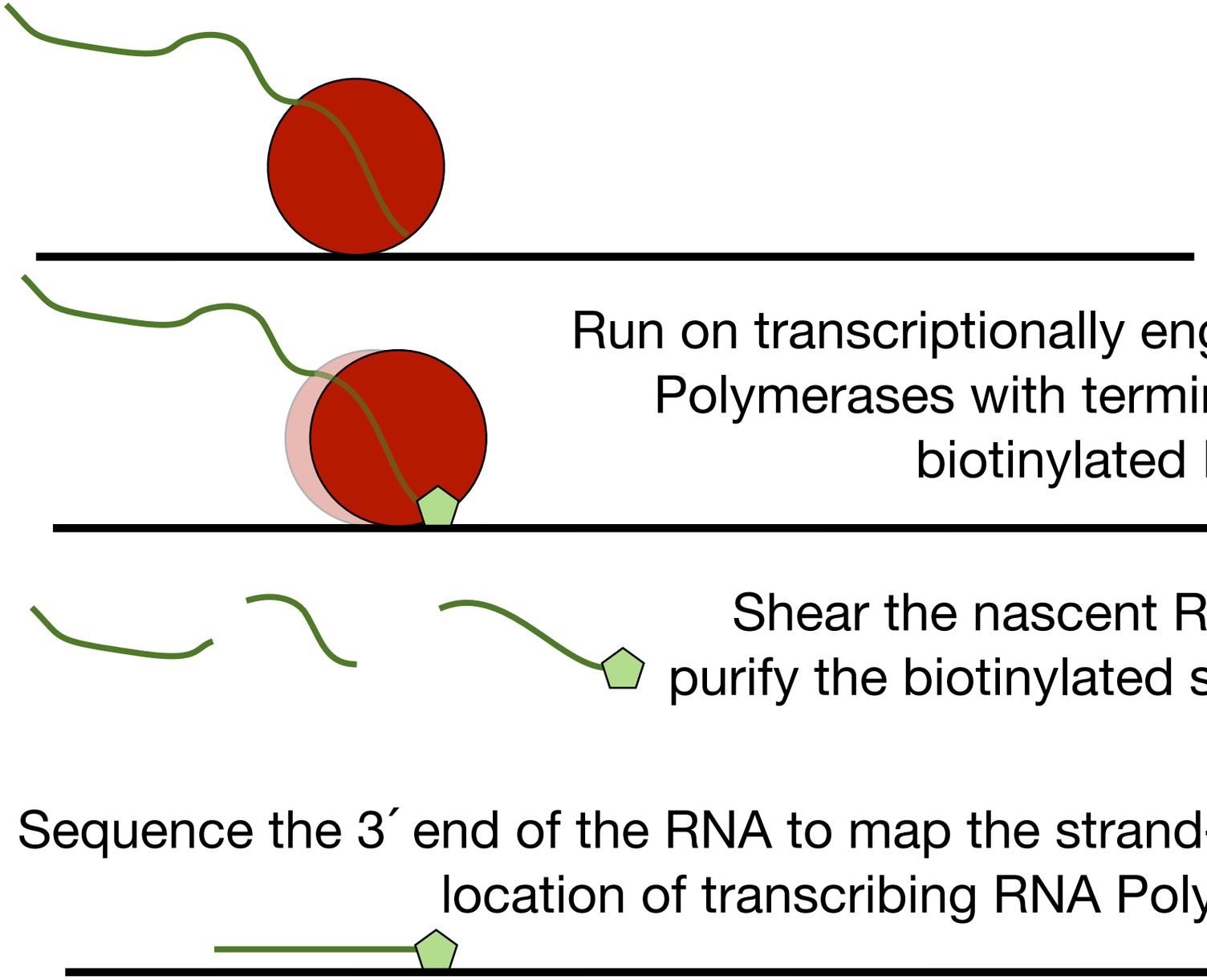


Run on transcriptionally engaged Polymerases with terminating biotinylated NTPs.

Shear the nascent RNA and purify the biotinylated species.

Kwak, et. al. Science, 2013

Precision Genomic-Run On (PRO-seq)



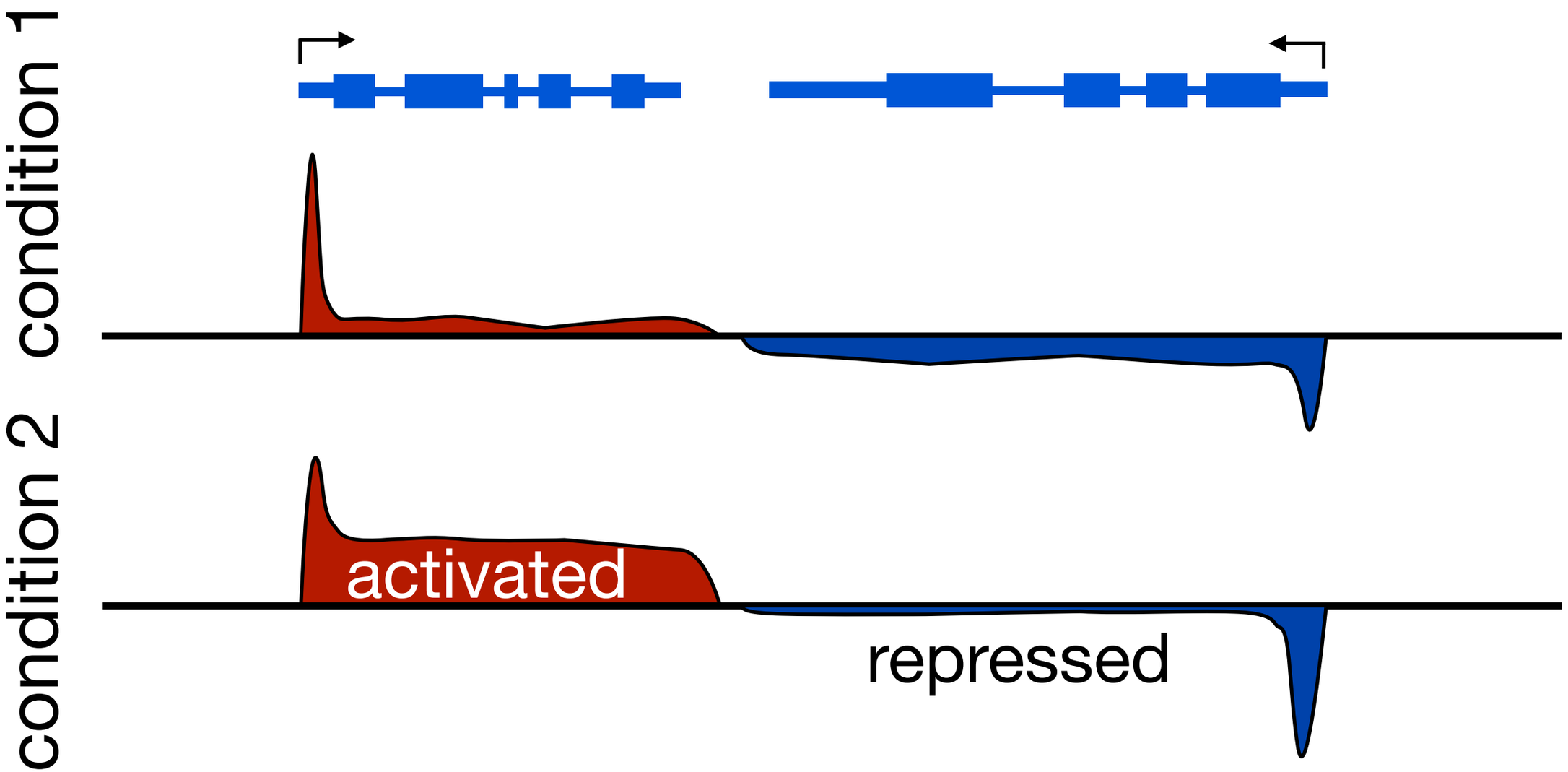
Run on transcriptionally engaged Polymerases with terminating biotinylated NTPs.

Shear the nascent RNA and purify the biotinylated species.

Sequence the 3´ end of the RNA to map the strand-specific location of transcribing RNA Polymerase.

Kwak, et. al. Science, 2013

PRO-seq measures immediate responses



~200,000 peaks

ATAC signal

t0
20
40
60
120
180
240
6day

~30,000 genes

PRO signal

t0_plus
t0_minus
20min_plus
20min_minus
40min_plus
40min_minus
60min_plus
60min_minus
2hr_plus
2hr_minus
3hr_plus
3hr_minus
4hr_plus
4hr_minus
6day_plus
6day_minus

~200,000 peaks

ATAC signal

t0
20
40
60
120
180
240
6day

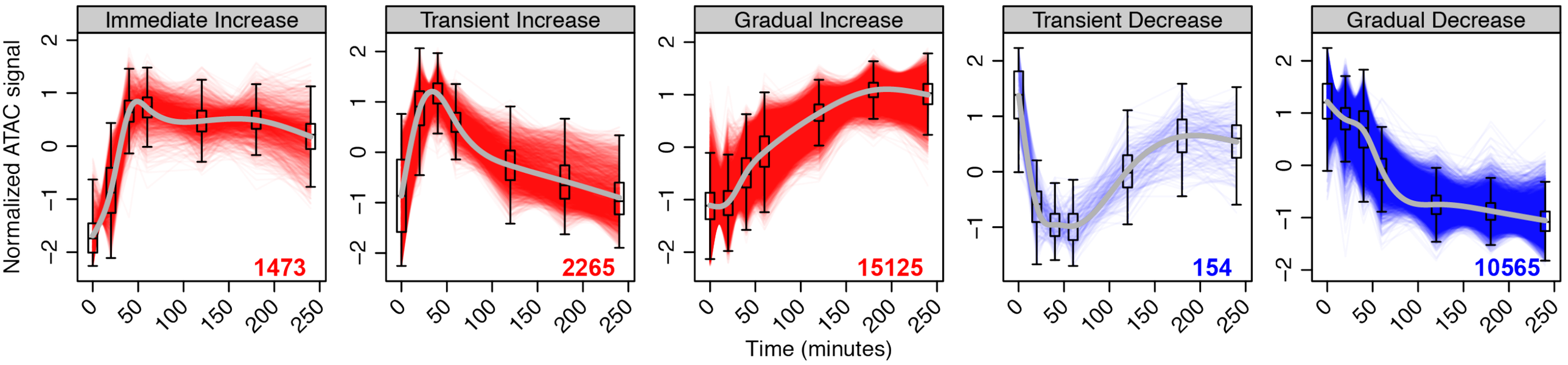
x2

t0_plus
t0_minus
20min_plus
20min_minus
40min_plus
40min_minus
60min_plus
60min_minus
2hr_plus
2hr_minus
3hr_plus
3hr_minus
4hr_plus
4hr_minus
6day_plus
6day_minus

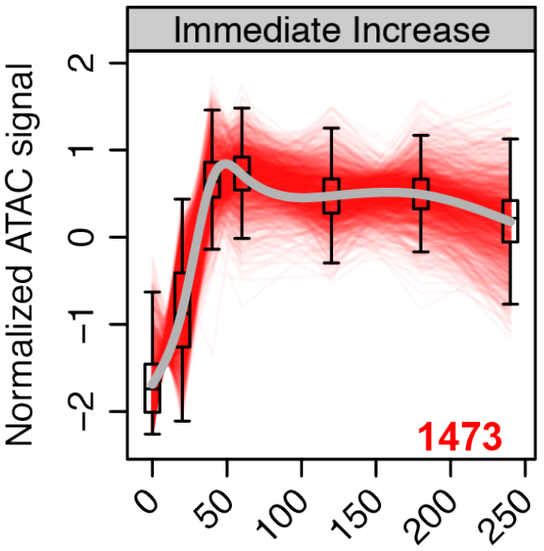
PRO signal

~30,000 genes

Interesting ATAC-seq peaks are dynamic over the time course



de novo motif analysis identifies enriched sequence elements within dynamic ATAC peaks



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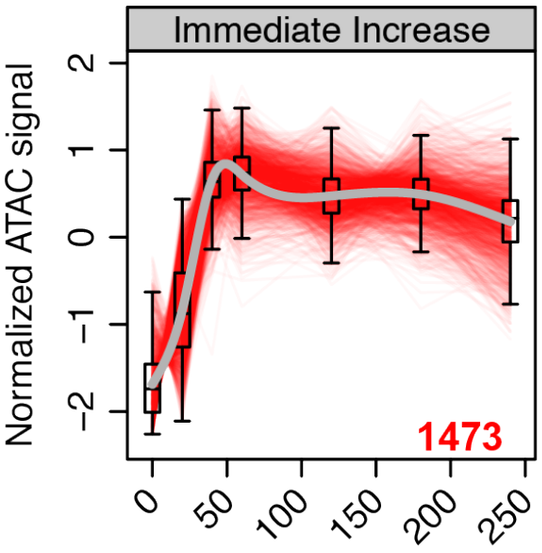

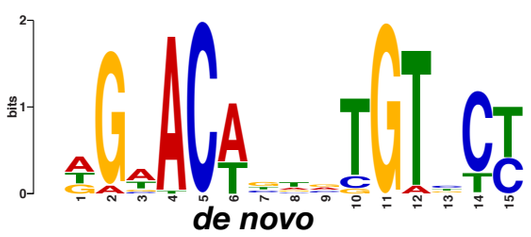

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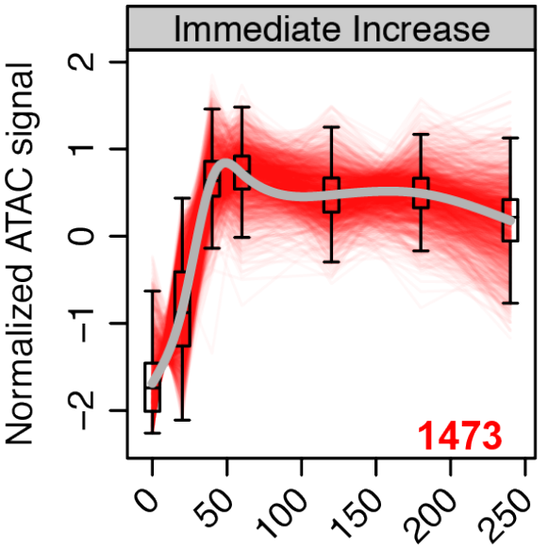
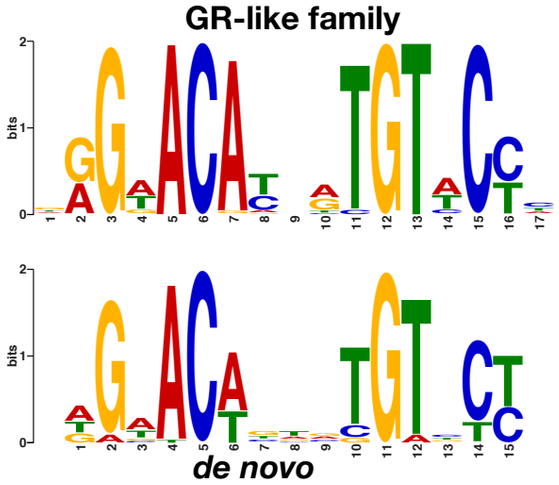
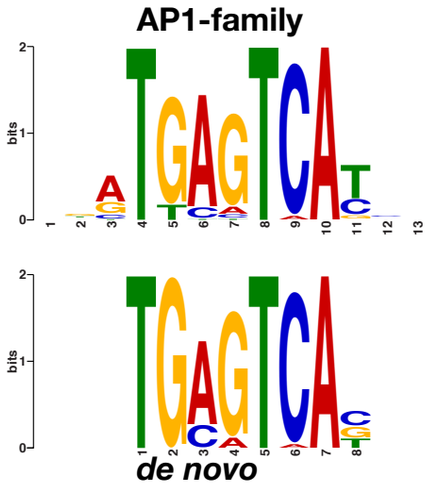
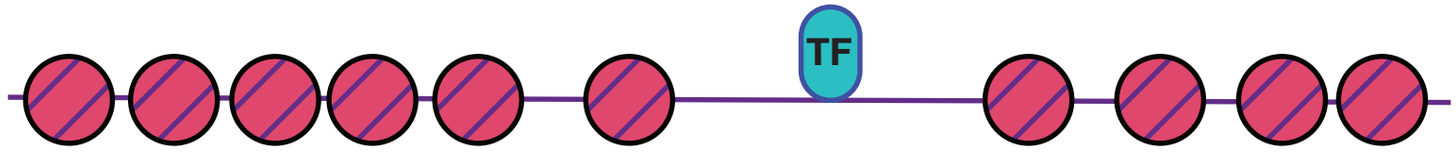




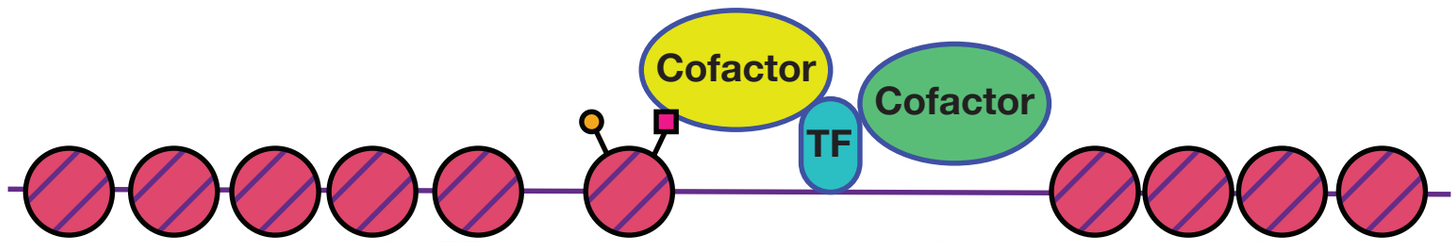


Bidirectional Transcription is a hallmark of regulatory regions

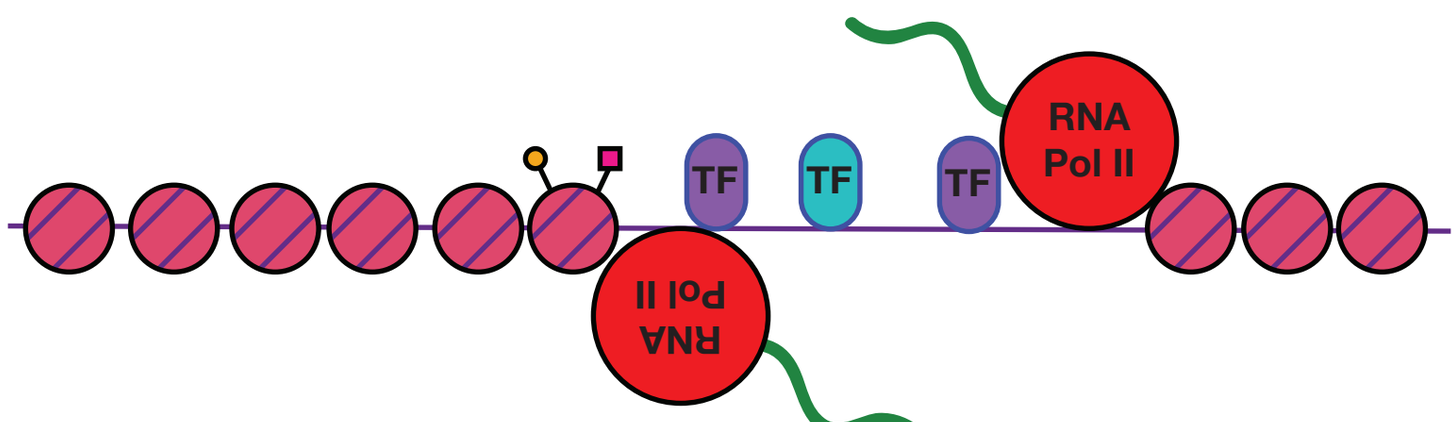
DNA sequence influences nucleosome positions/dynamics and chromatin compaction



DNA sequence directs Transcription Factor (TF) binding

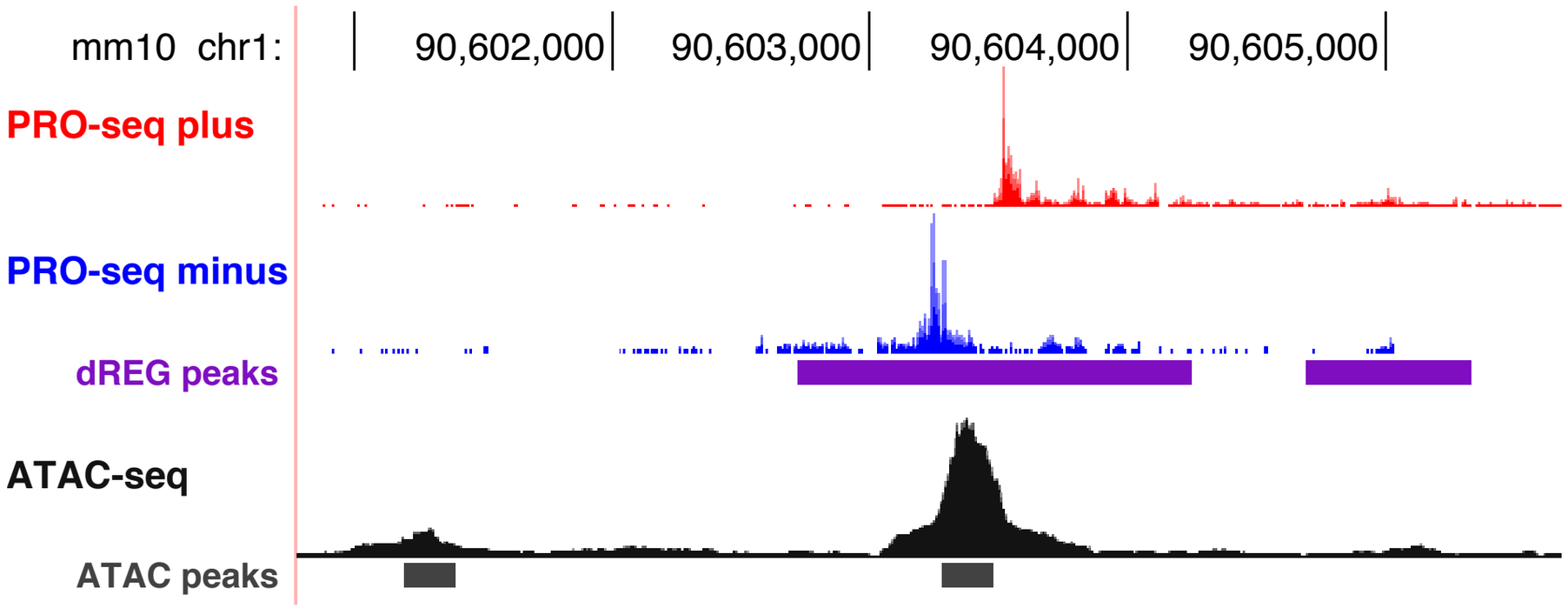


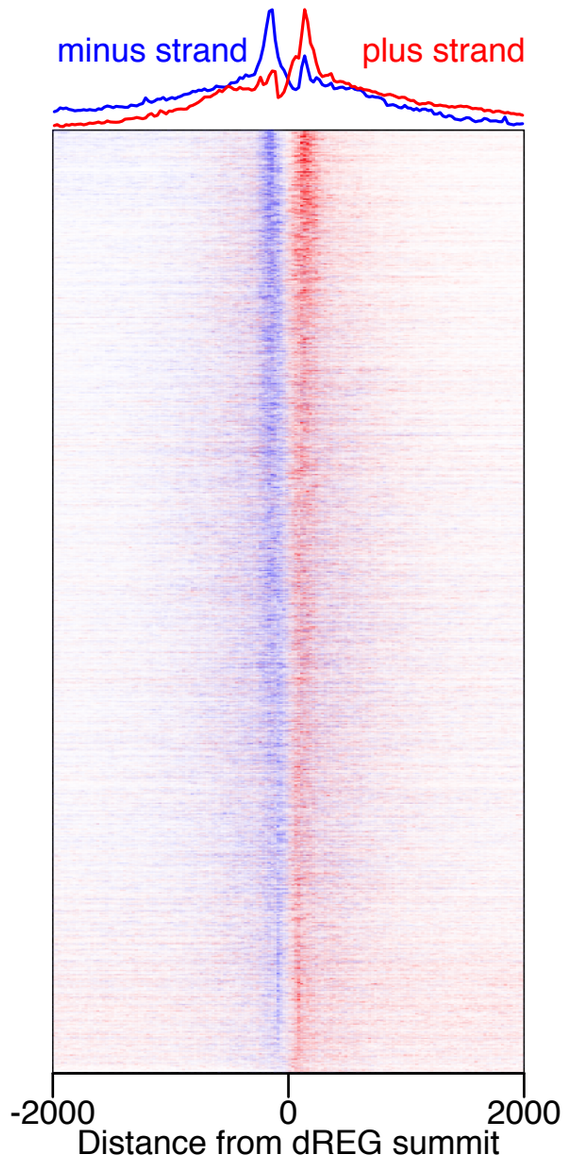
sequence-specific TFs recruit chromatin modifying/remodeling factors



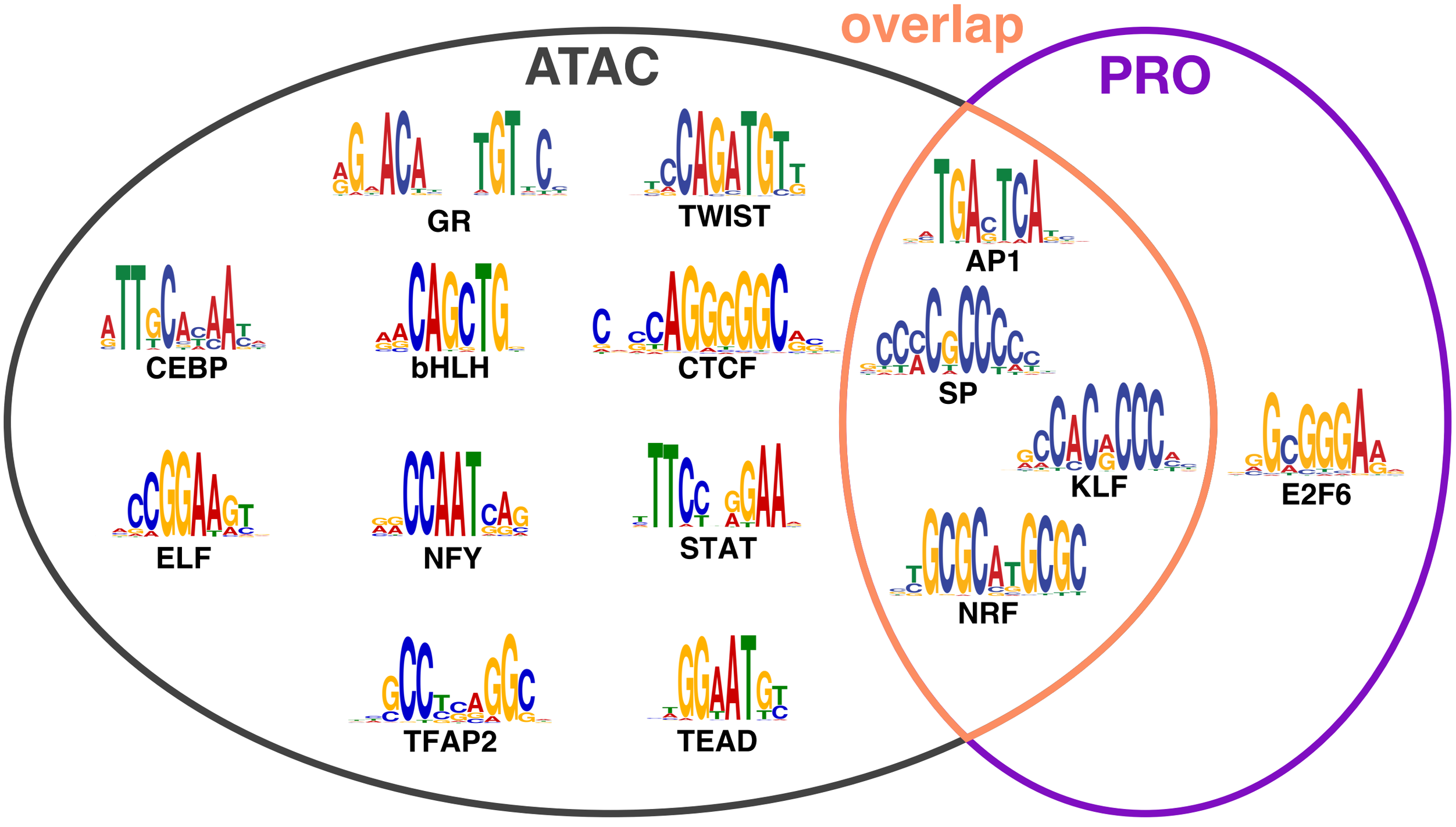
sequence-specific TFs recruit initiation factors and RNA Polymerase

Bidirectional transcription signatures from PRO-seq independently identifies putative regulatory regions

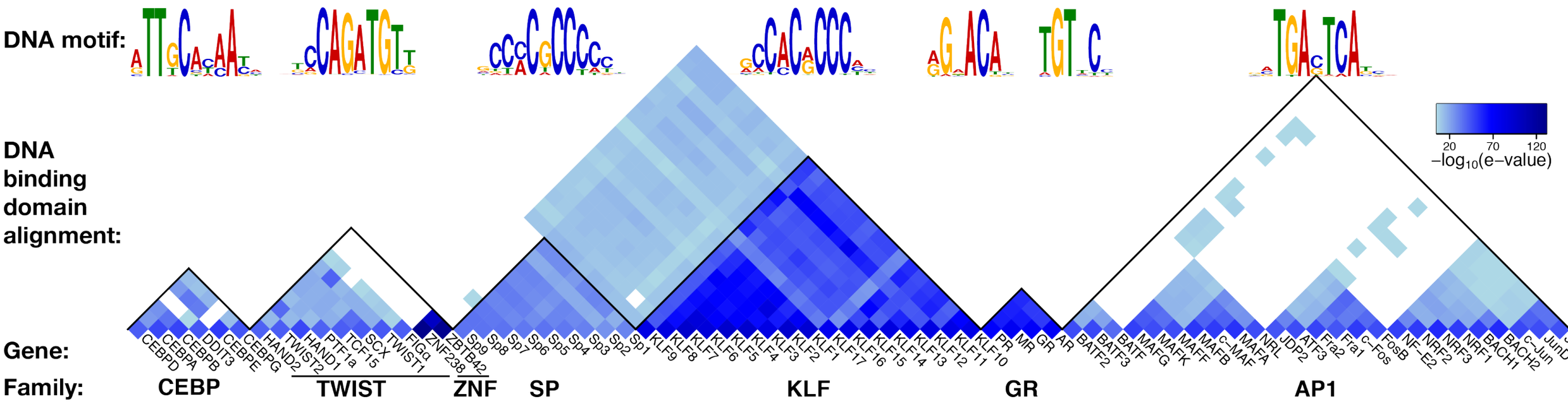




Motifs enriched within dynamic ATAC and bidirectional PRO peaks



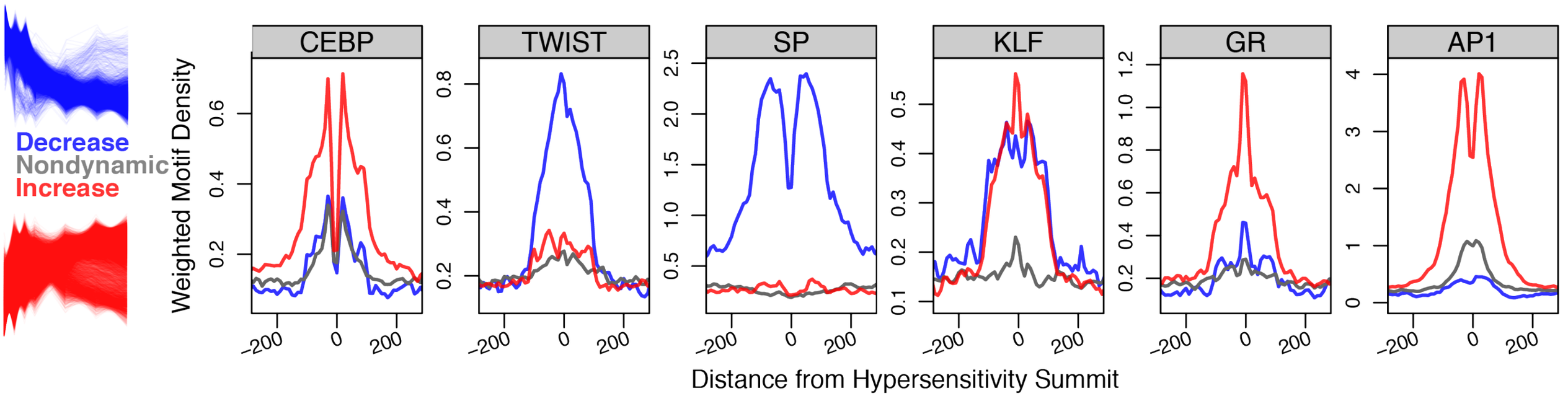
14 TF-family motifs (top 6 shown) drive early changes in chromatin and transcription



One motif ≠ One TF

Paralogous TF DBD families can recognize each motif

CEBP, KLF, GR, and AP1 motifs associate w/ increasing chromatin accessibility
TWIST and SP associate w/ decreasing chromatin accessibility



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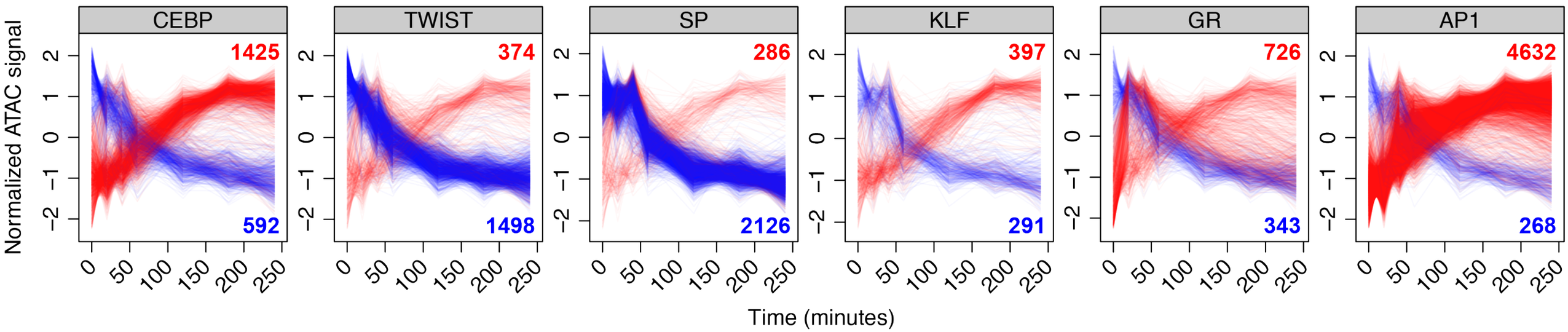
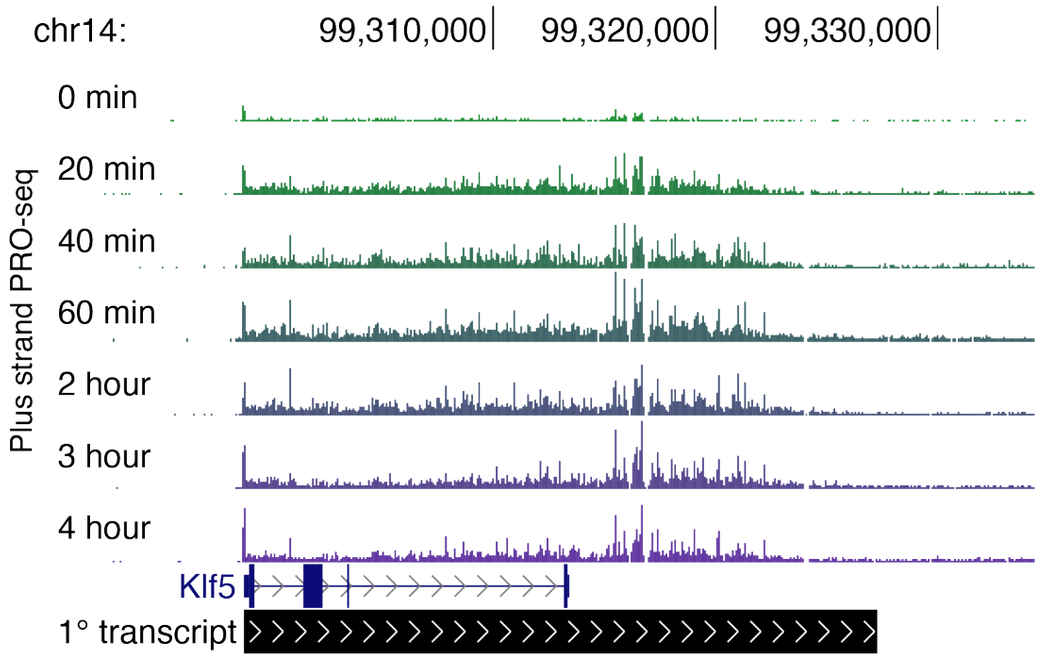
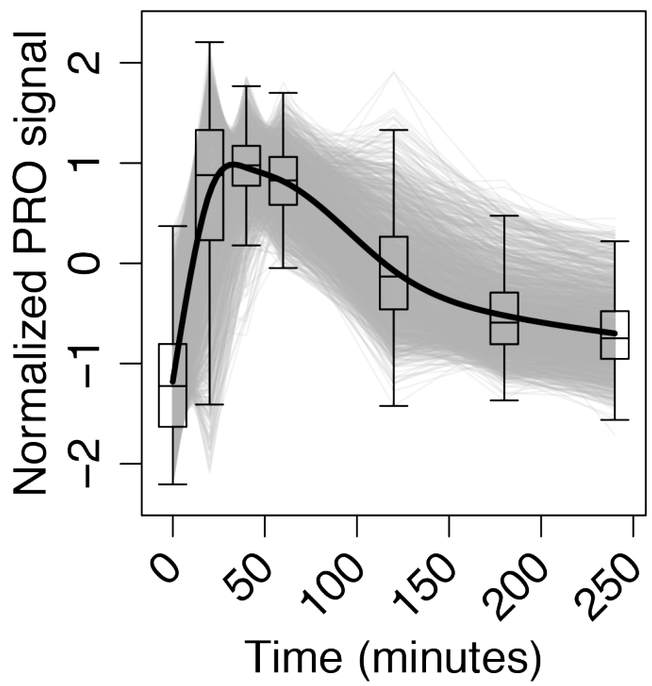
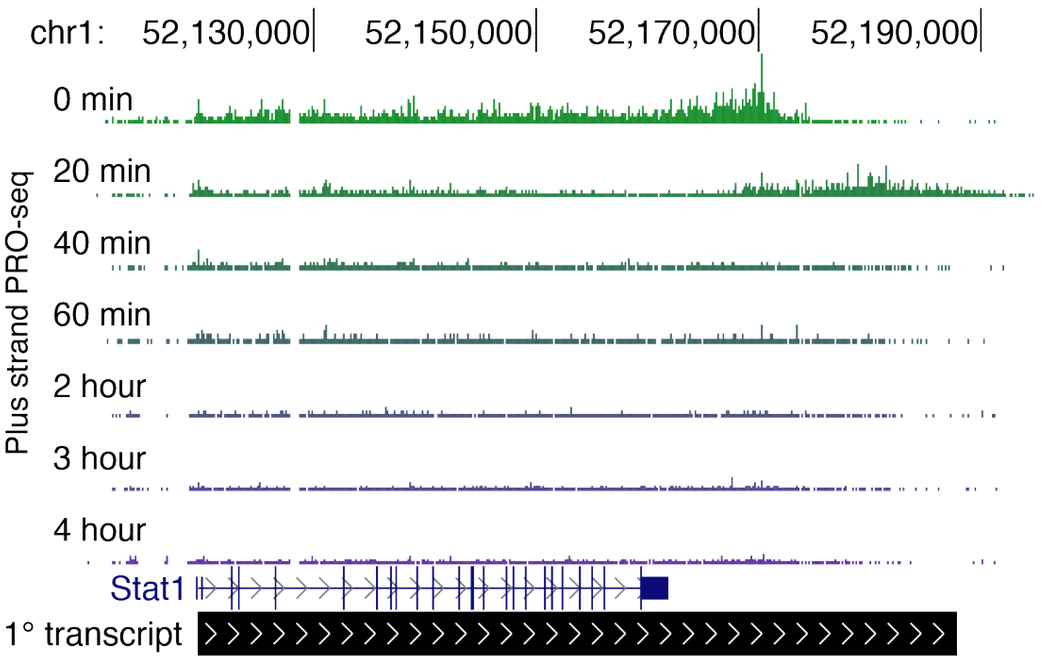
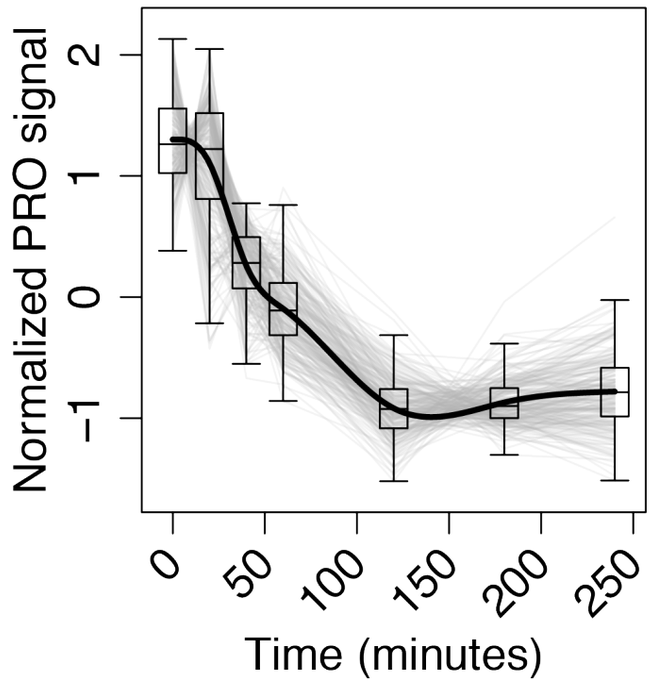


Genes also have distinct activation and repression kinetics



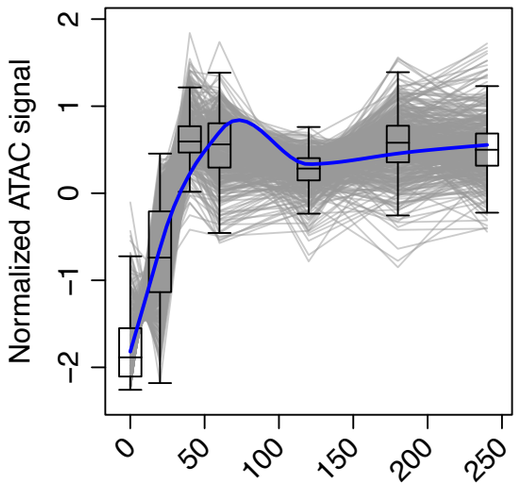






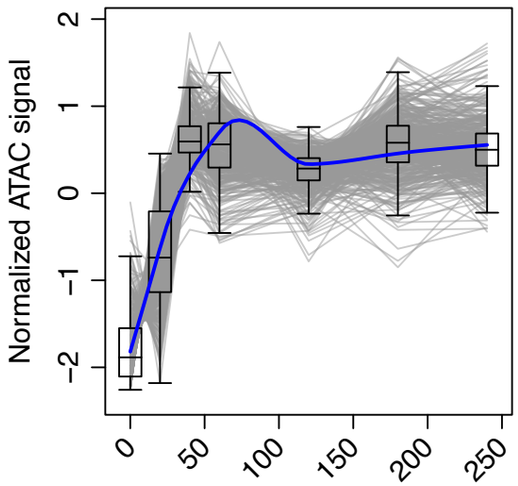
Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)



&

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)



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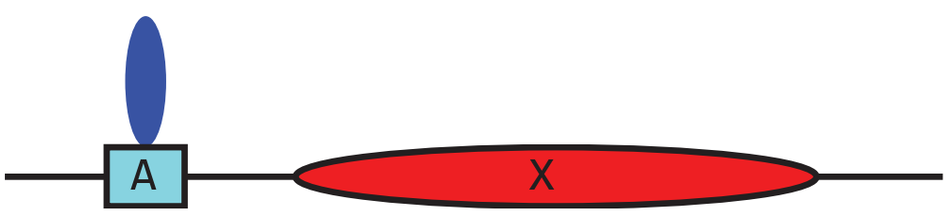
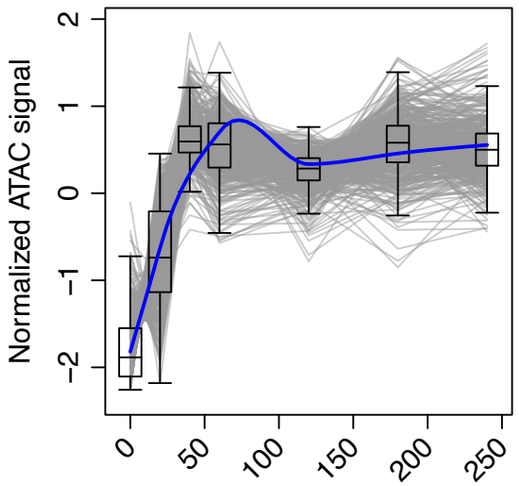
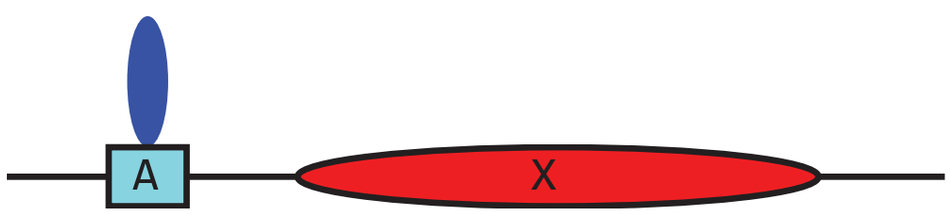


Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)





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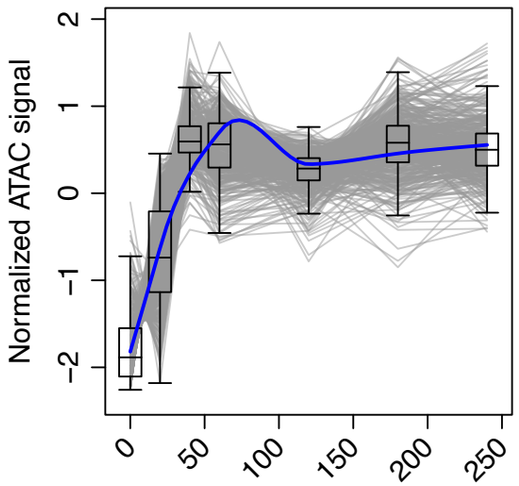
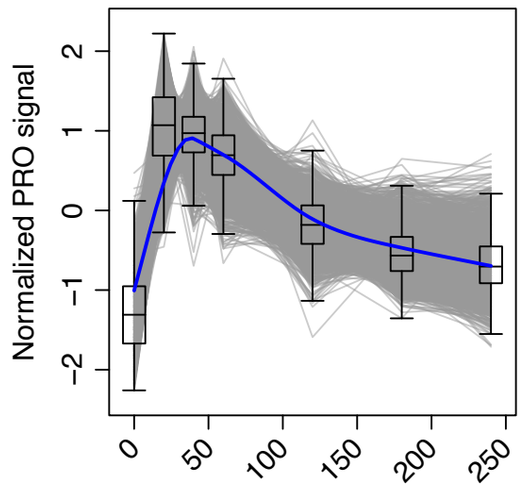
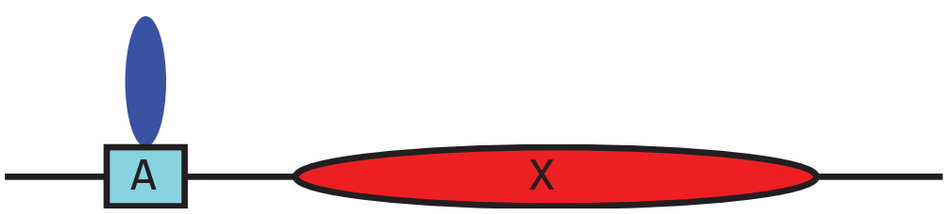




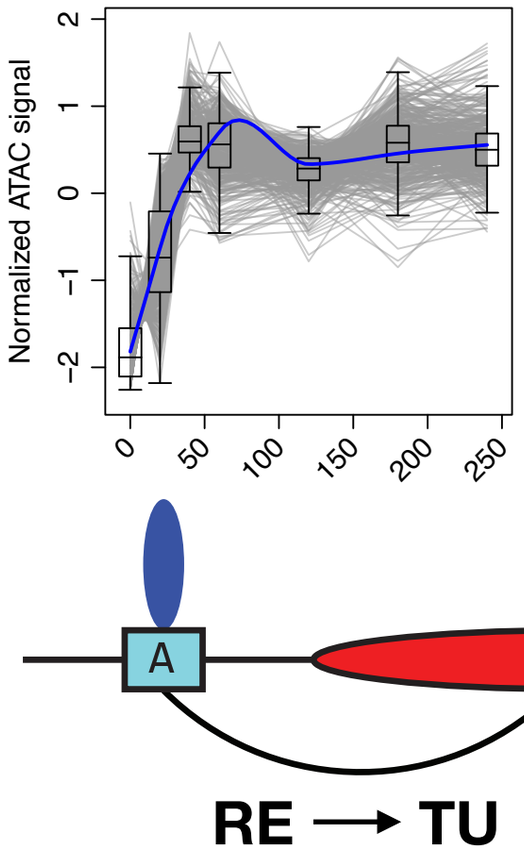


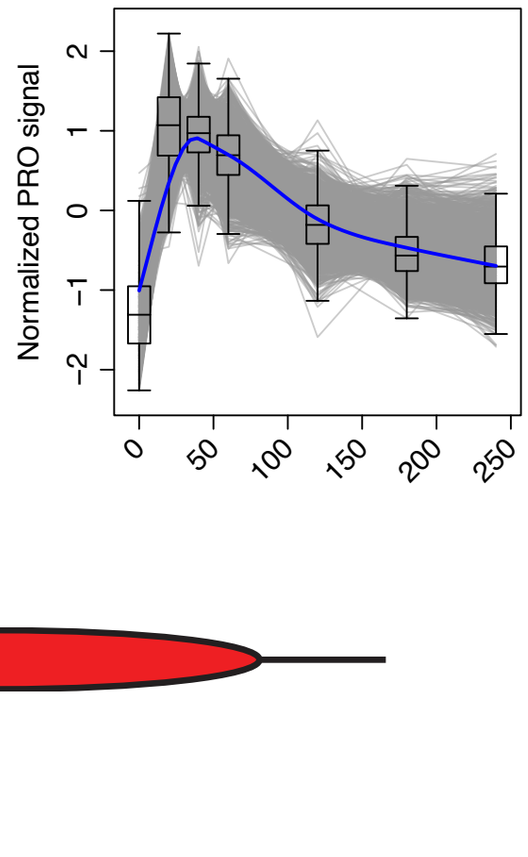
ATAC peak ↑
gene PRO ↑

&

peak and gene are nearby

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)





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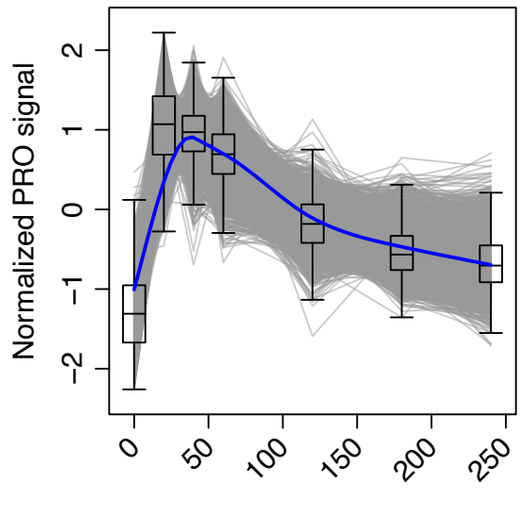
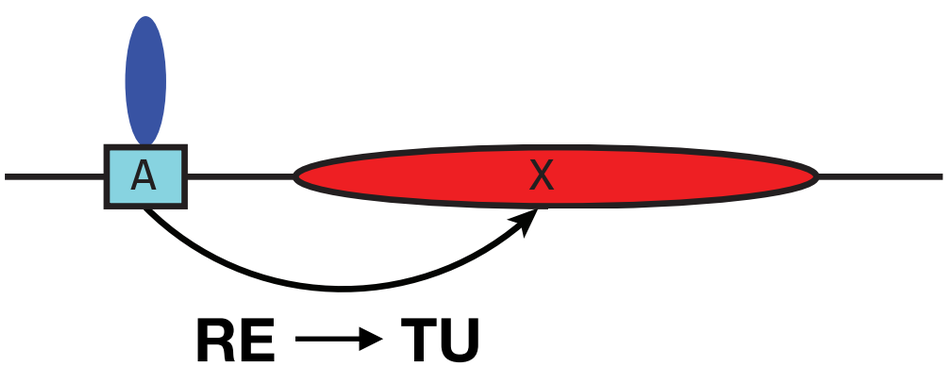
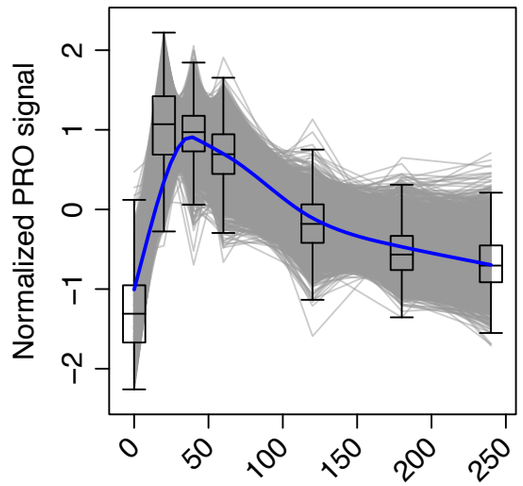
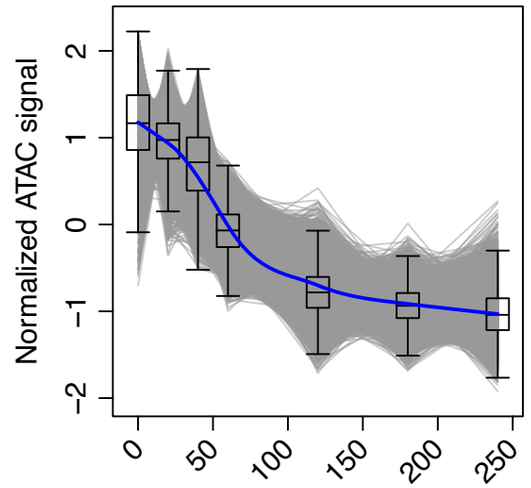
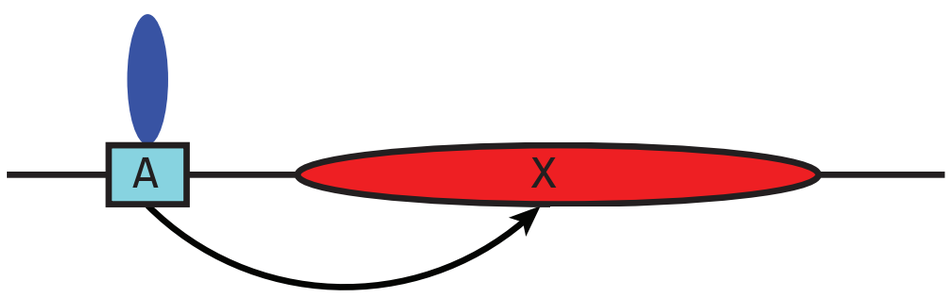




Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)







RE → TU

gene PRO ↑
ATAC peak ↓
(repressor binding)

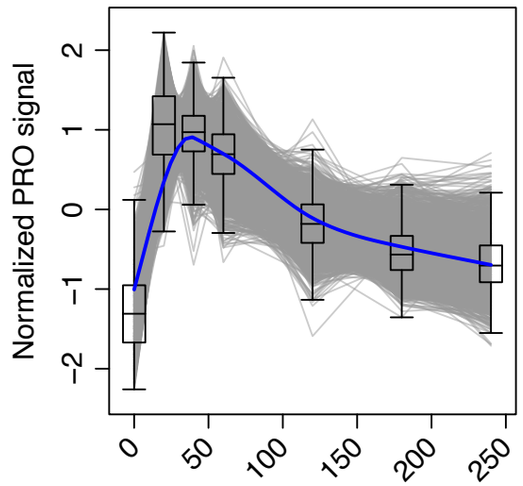
&

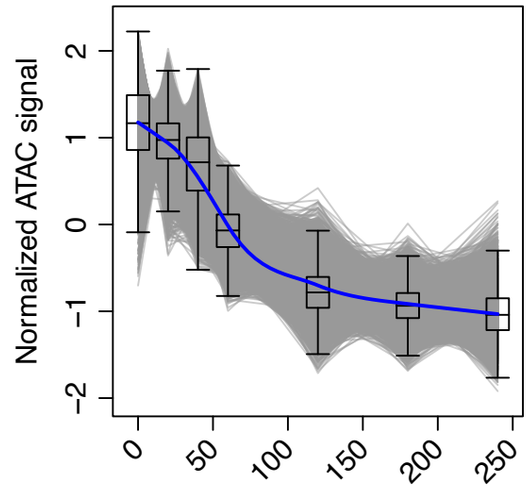
time delay
(PRO prior ATAC)

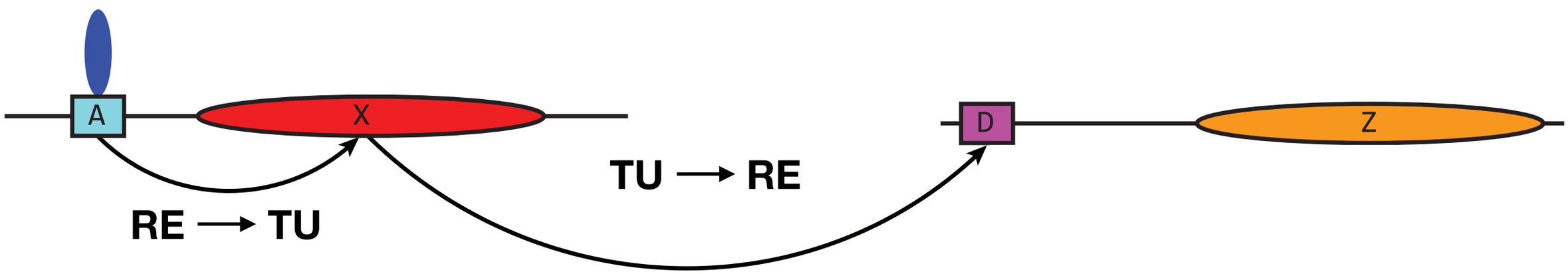
&

gene's motif within peak

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)

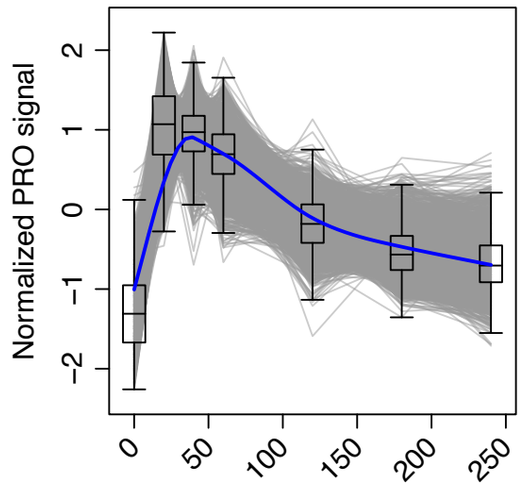


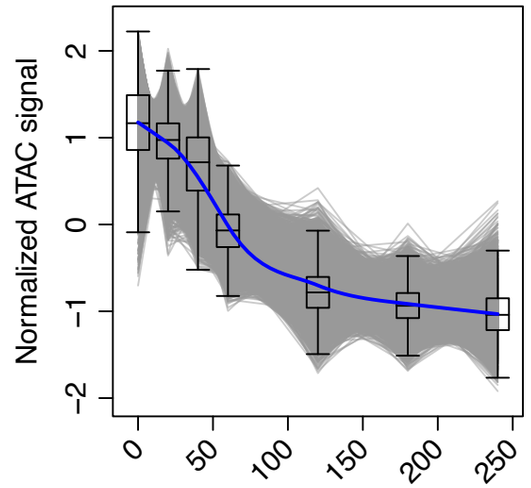


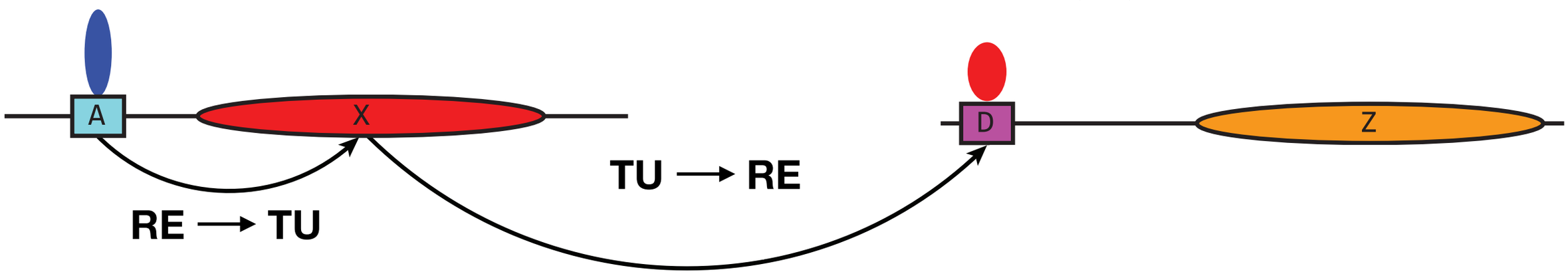


gene PRO ↕ ATAC peak (repressor binding) **&** **time delay (PRO prior ATAC)** **&** **gene's motif within peak**

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)

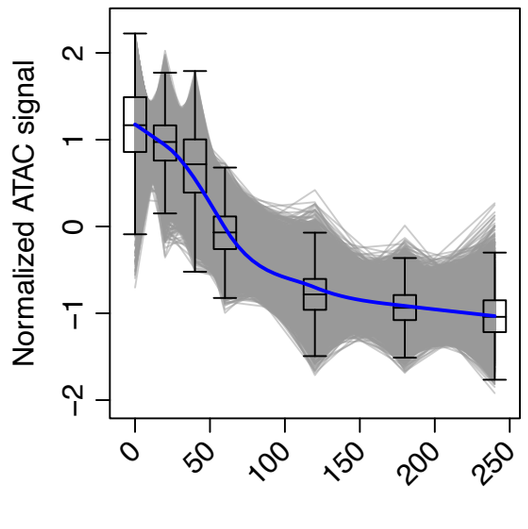


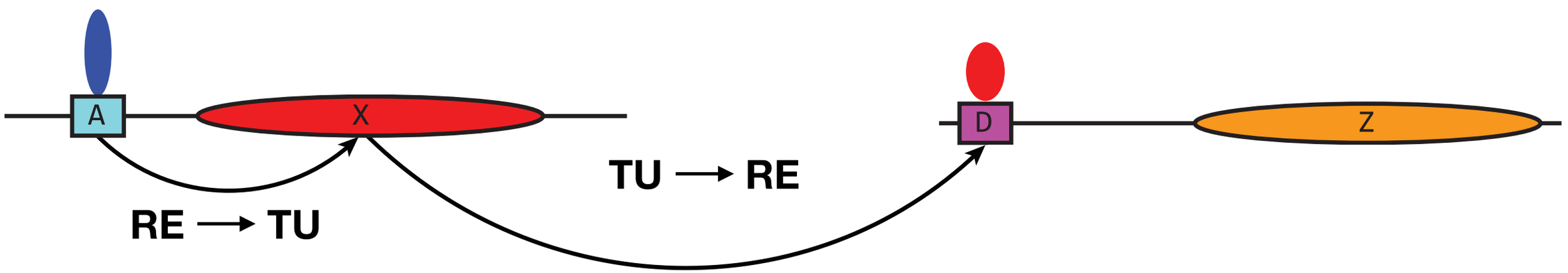




gene PRO ↕ ATAC peak (repressor binding) **&** **time delay (PRO prior ATAC)** **&** **gene's motif within peak**

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)





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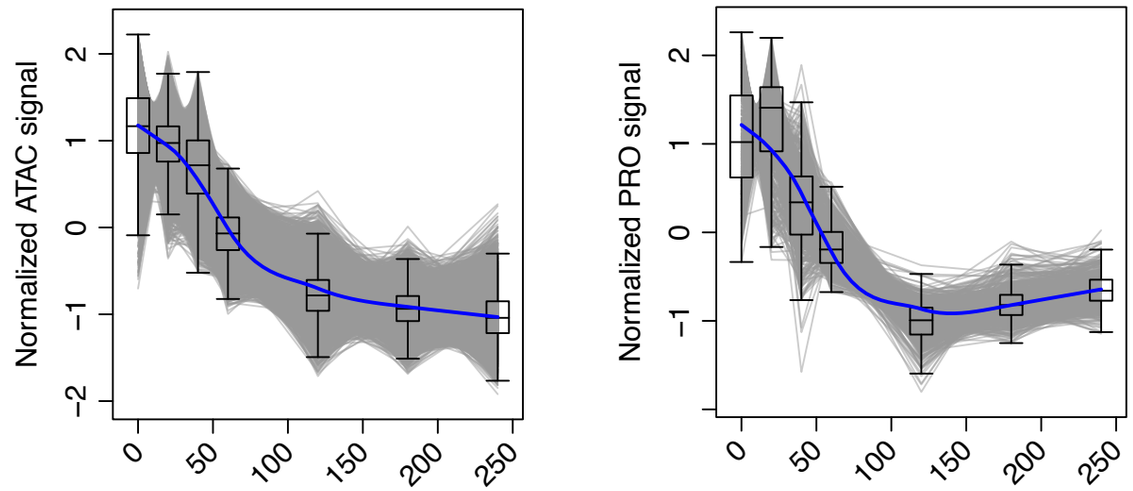
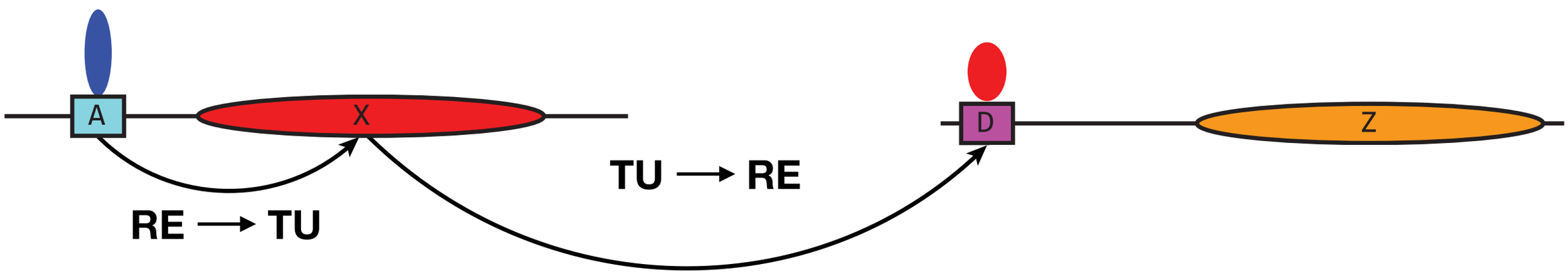
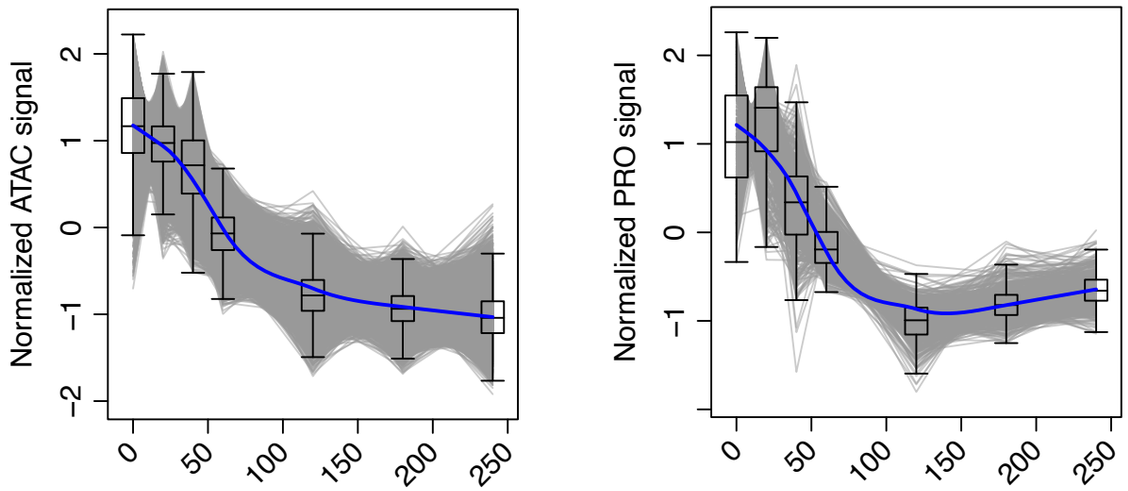


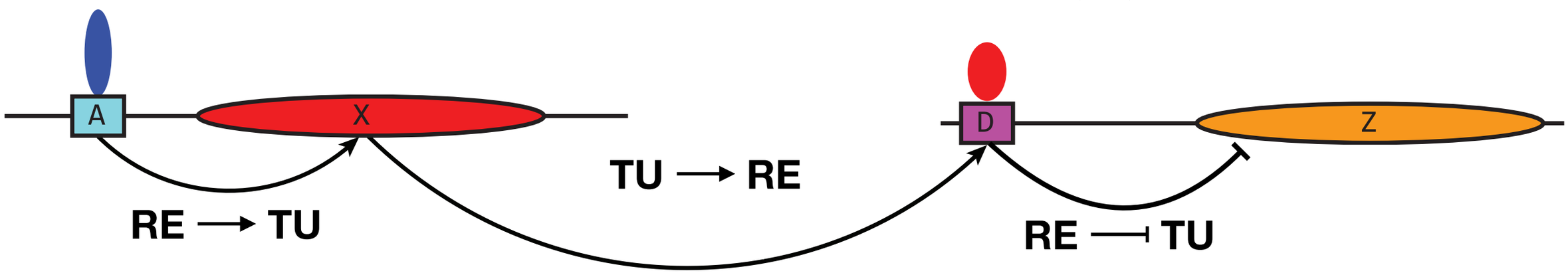


ATAC peak ↓ & peak and gene are nearby

gene PRO ↓

Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)





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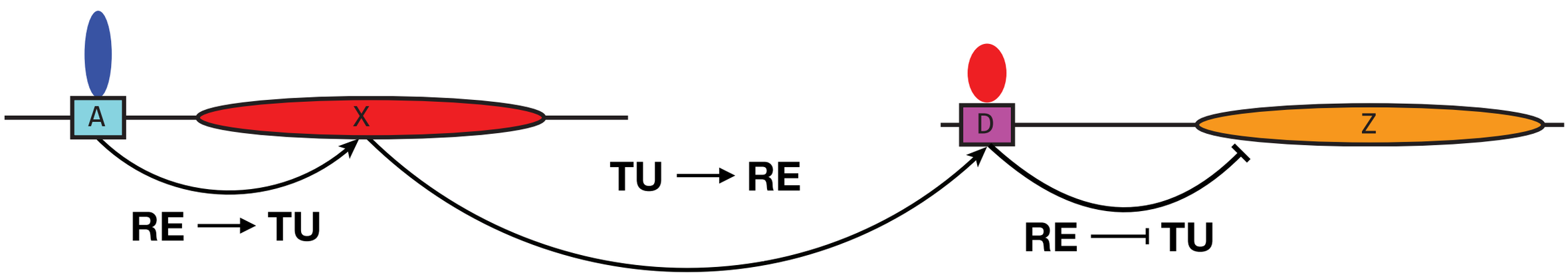
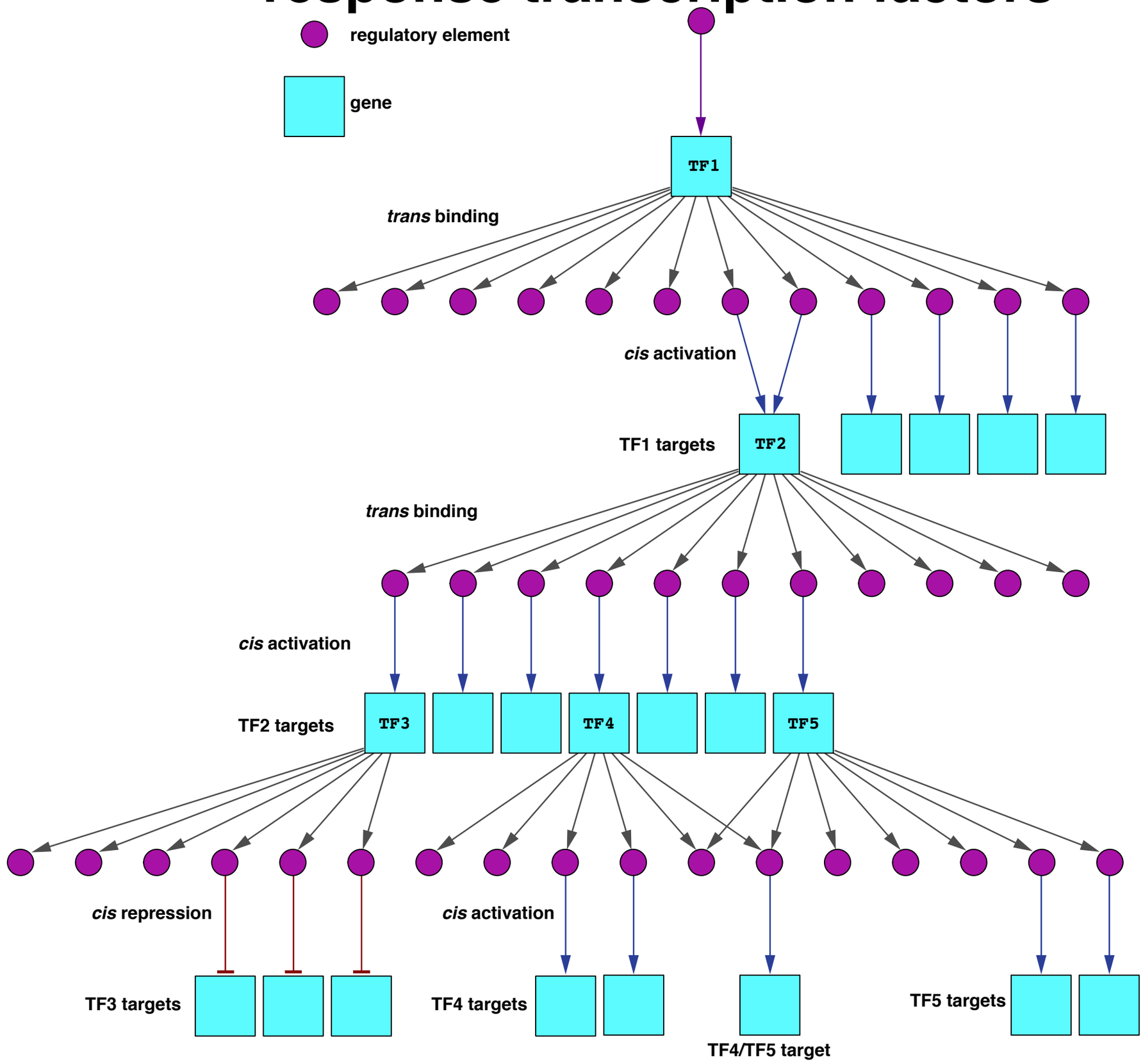
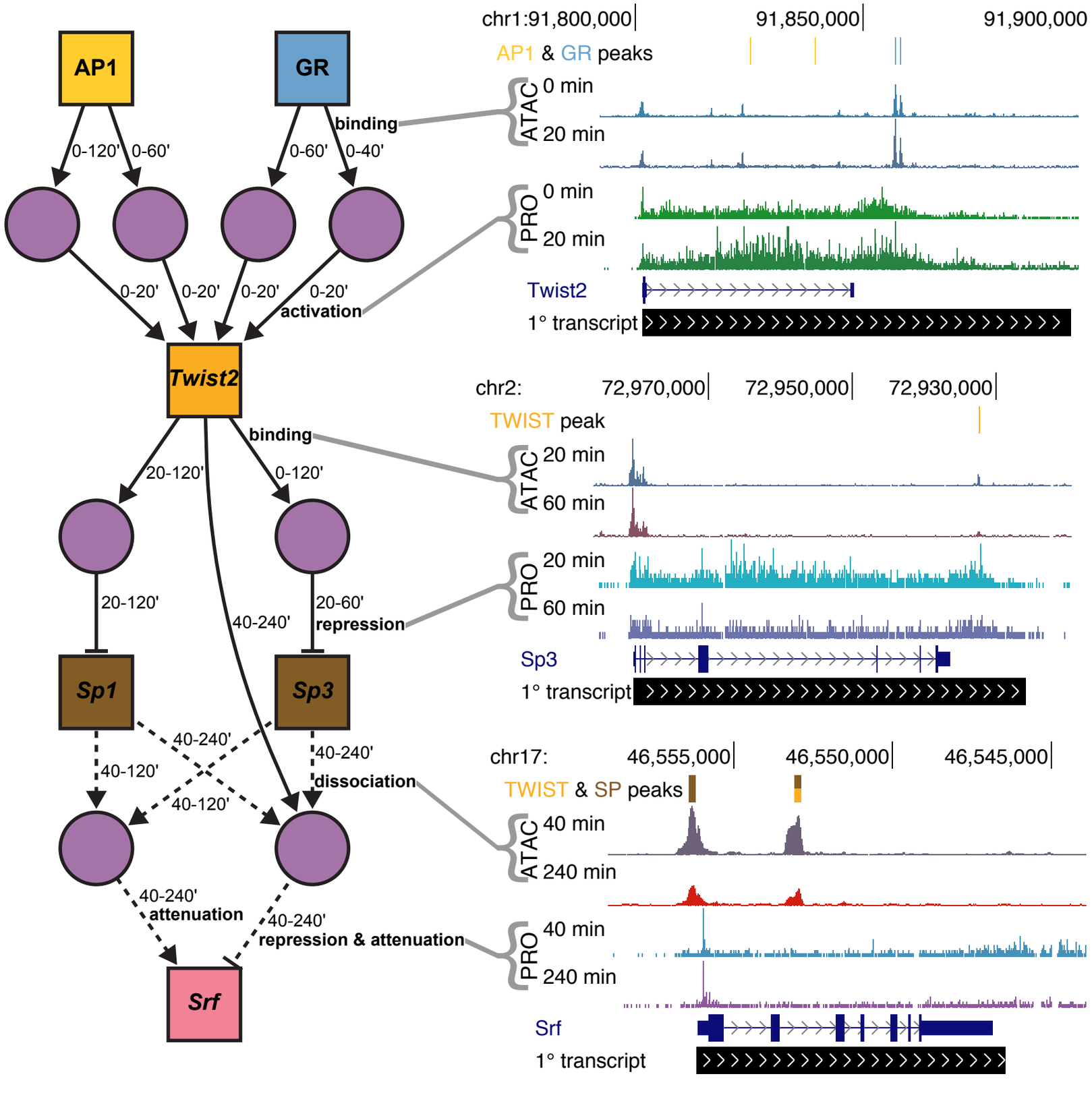
Linking direct functional interactions between Regulatory Elements (RE) and Genes (TU)



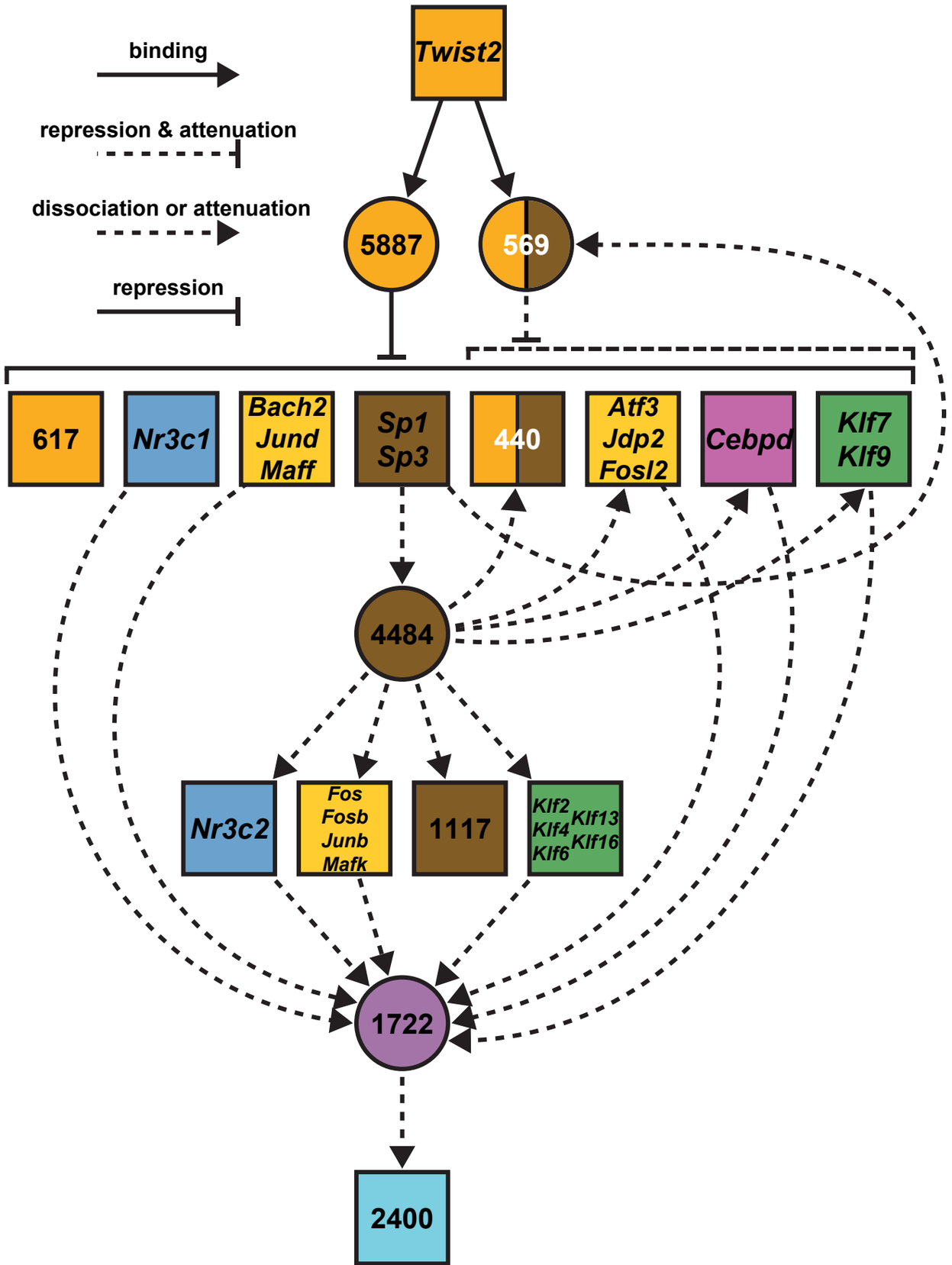
We are interested in highly connected early response transcription factors



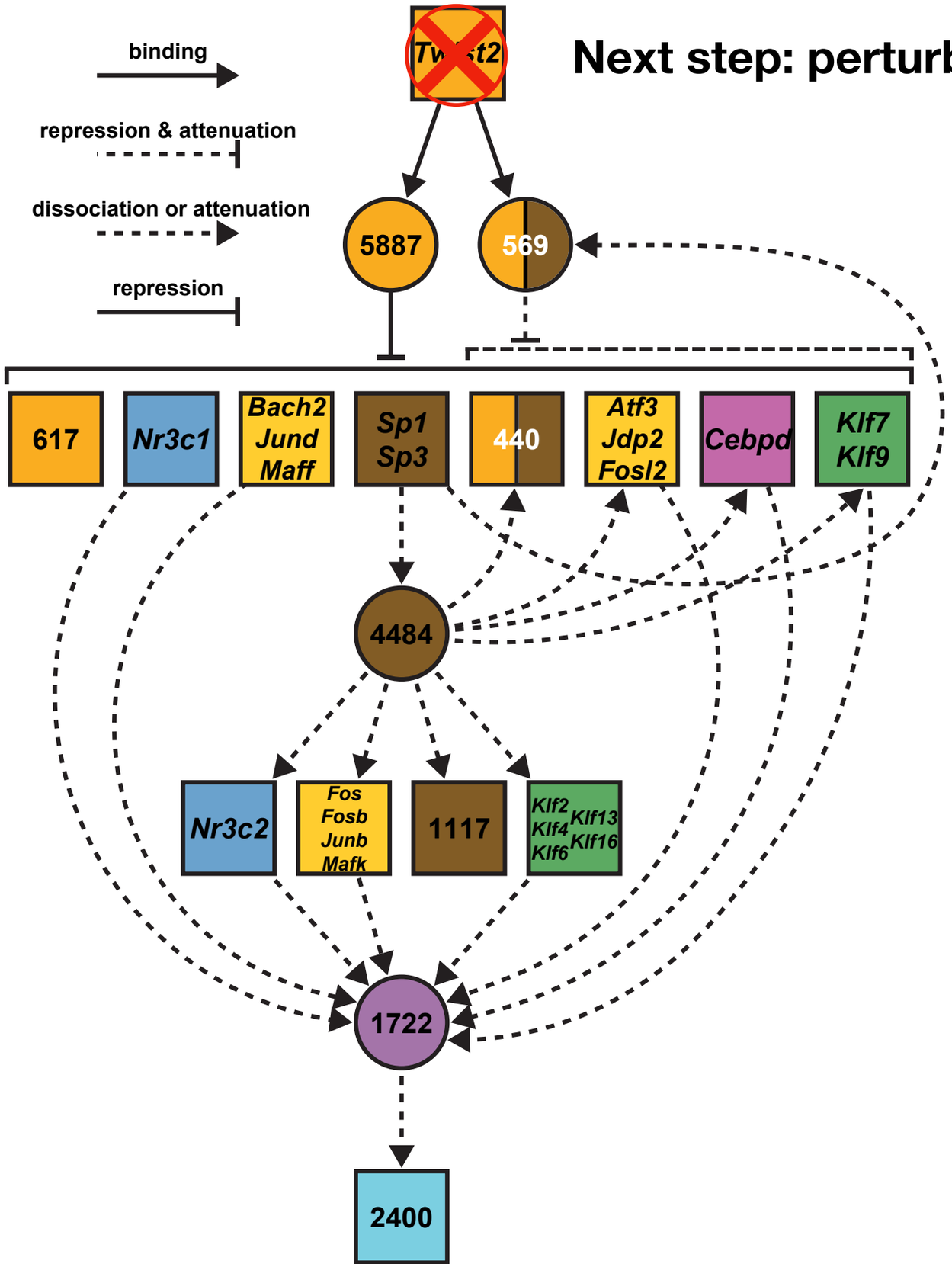
TWIST2 is active early and transiently



TWIST2 is a highly connected early response gene

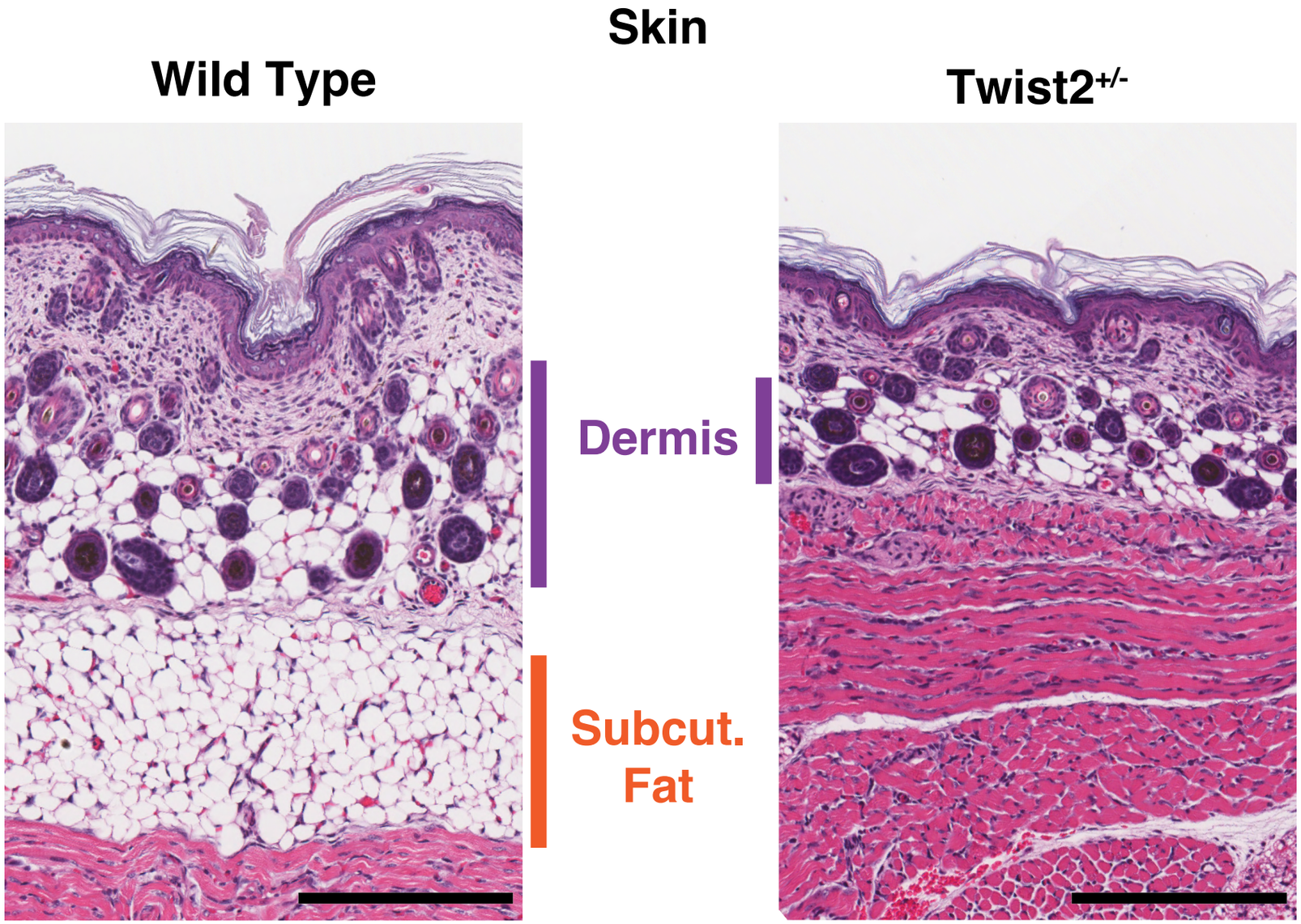


TWIST2 is a highly connected early response gene

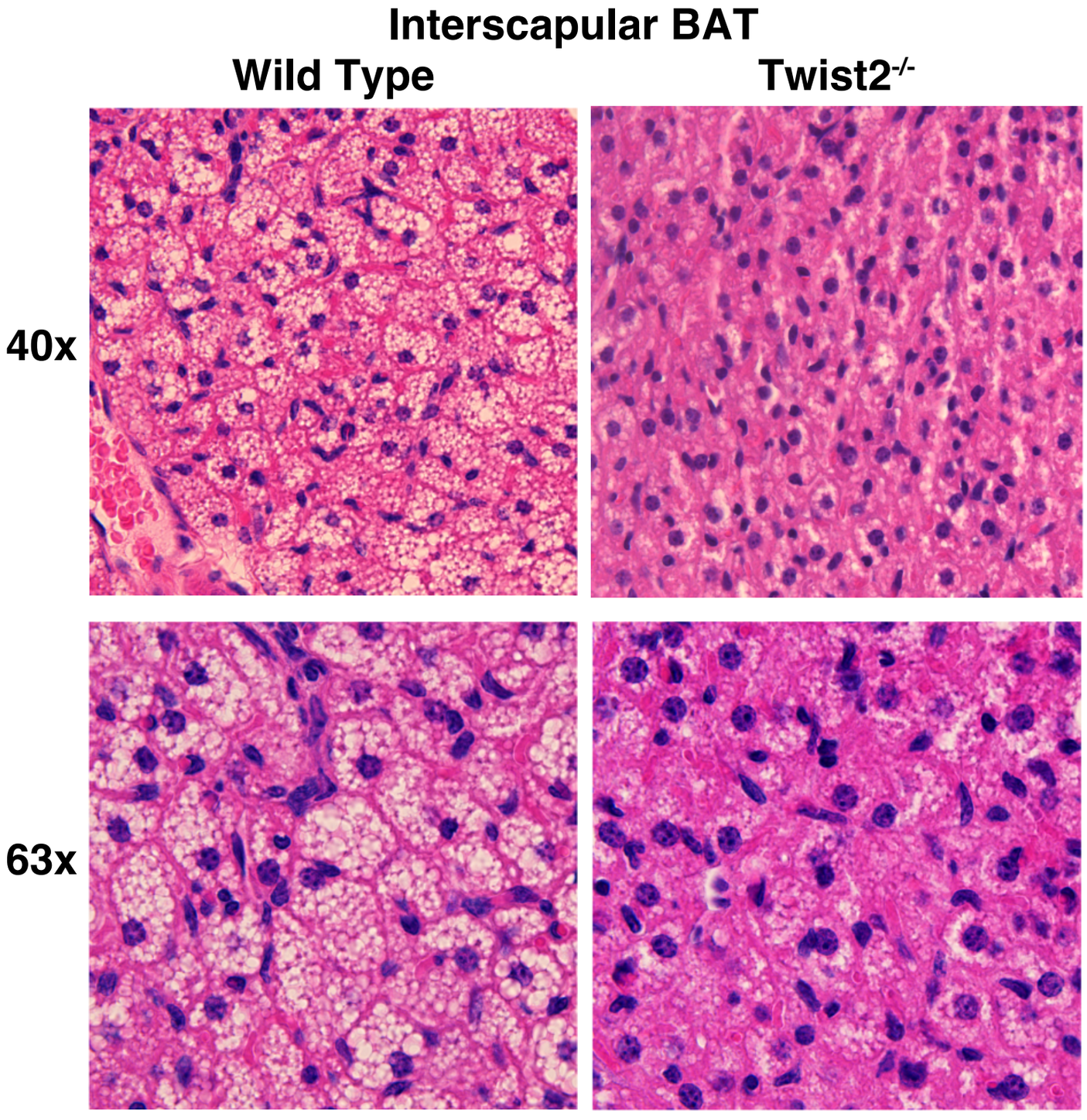


Next step: perturb and observe

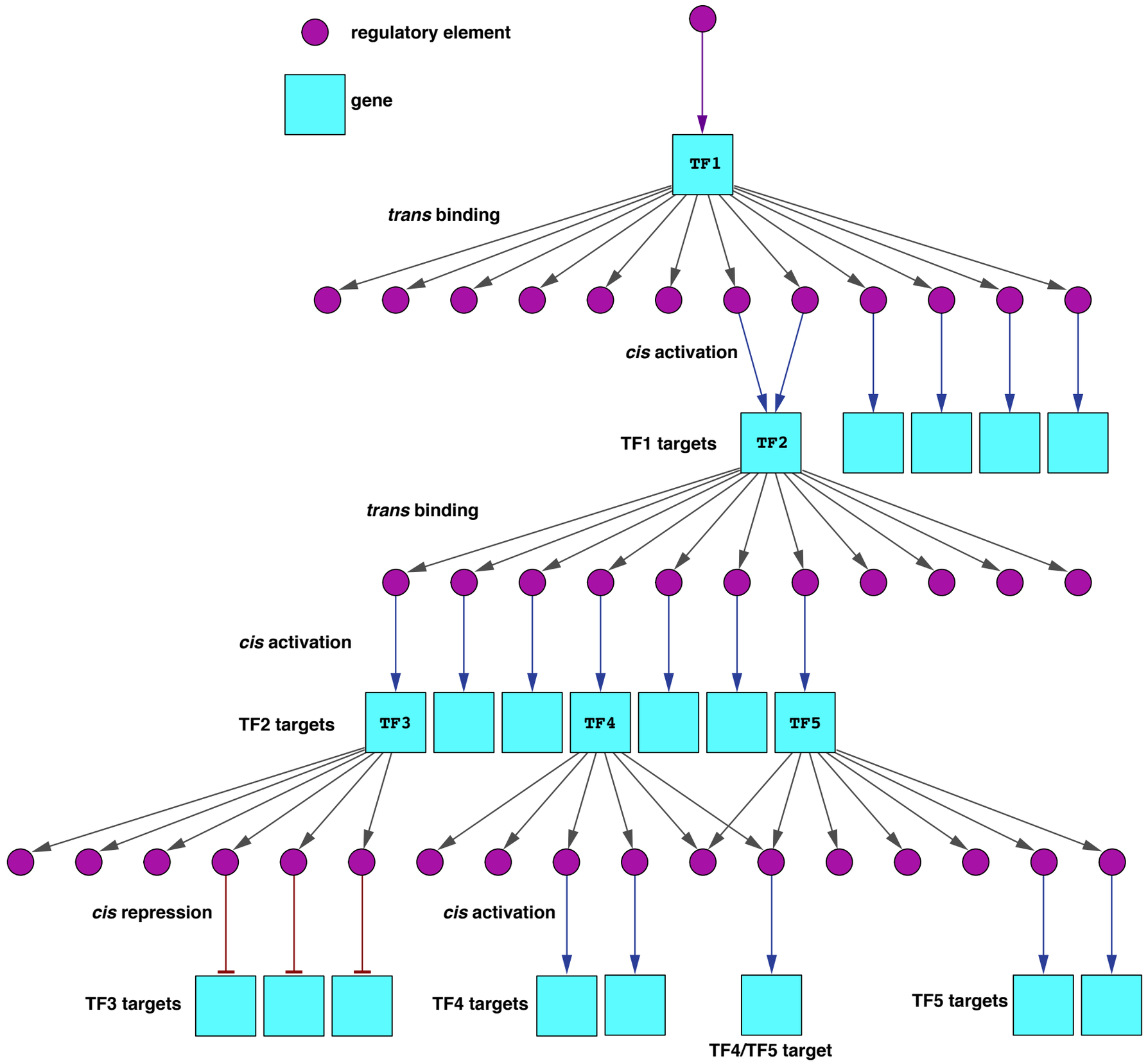
Twist2$^{+/-}$ mice have a near absence of subcutaneous fat



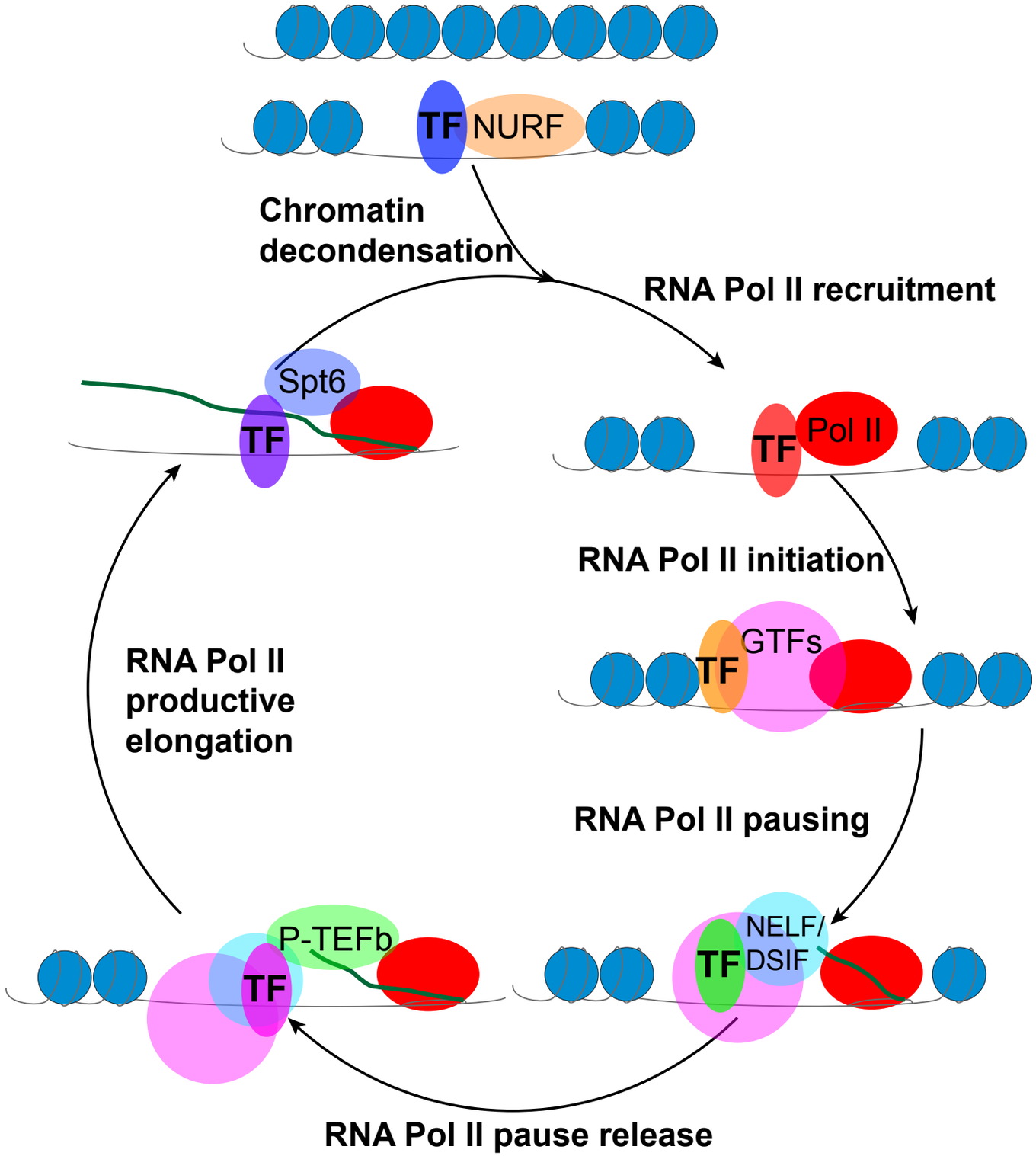
Twist2$^{-/-}$ mice have reduced lipid droplets in brown fat



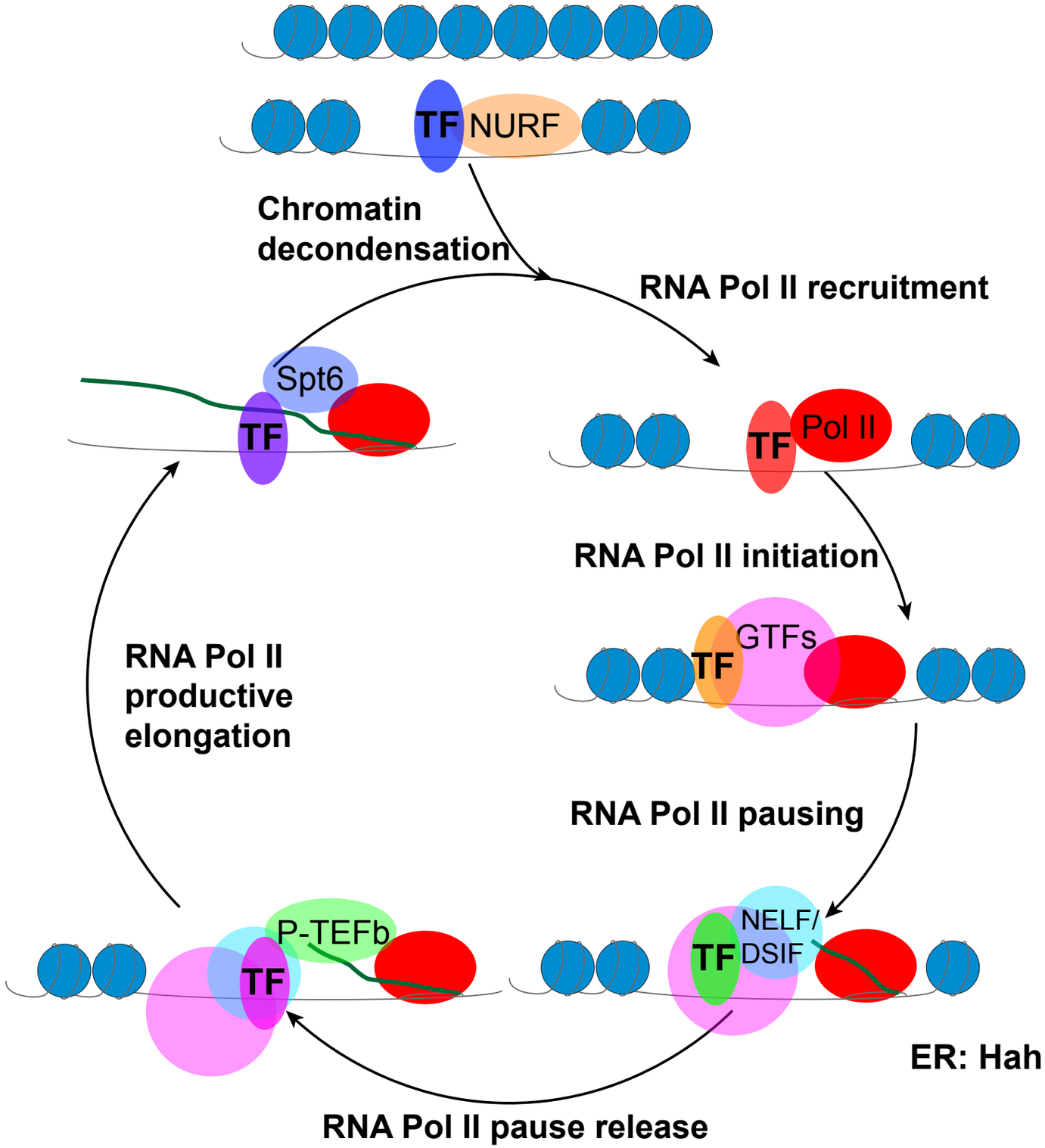
What are the molecular functions of these key TFs?

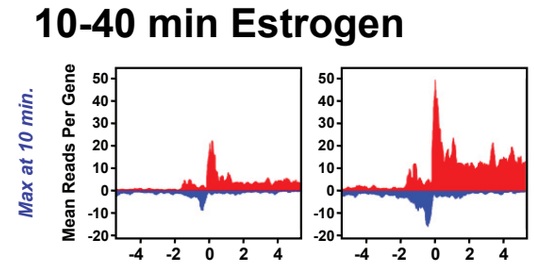


Coupling rapidly inducible systems to nascent transcript profiling informs on TF function



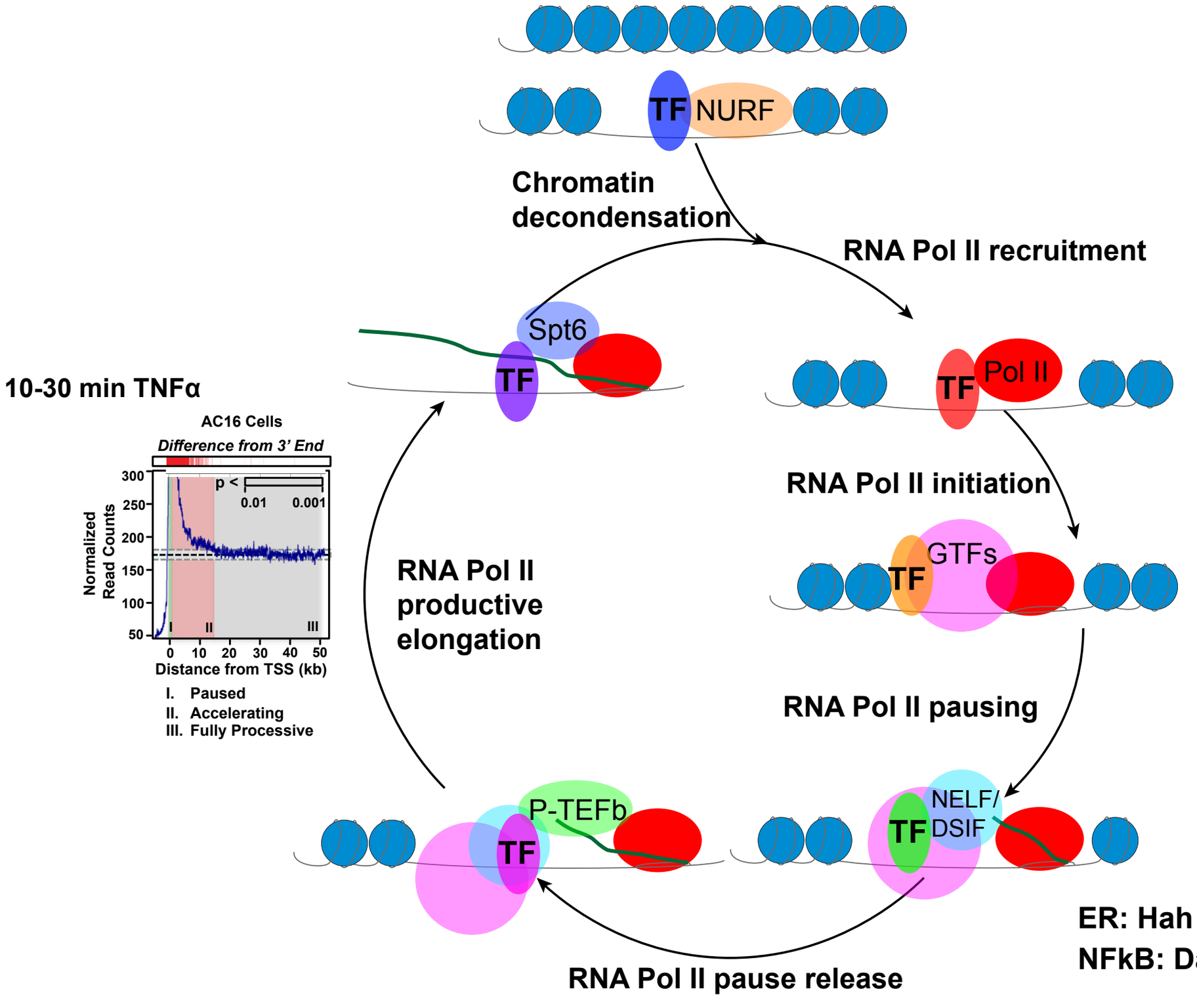
Coupling rapidly inducible systems to nascent transcript profiling informs on TF function





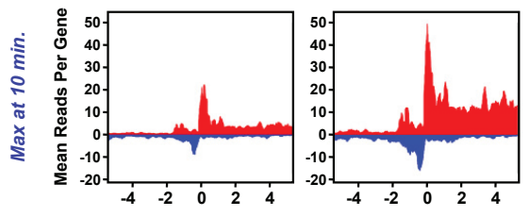
ER: Hah et. al., Cell, 2011

Coupling rapidly inducible systems to nascent transcript profiling informs on TF function



10-30 min TNFα

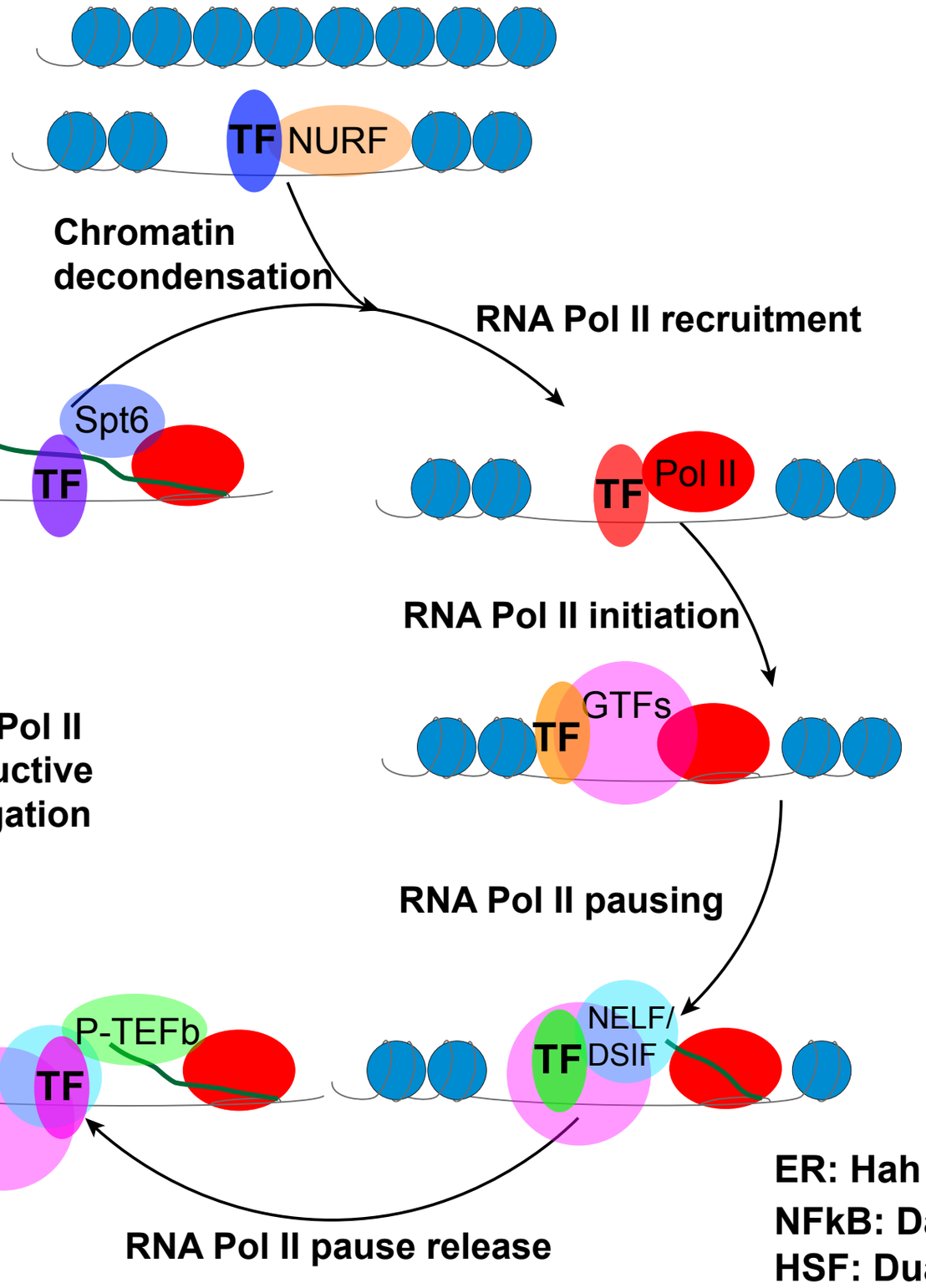
10-40 min Estrogen



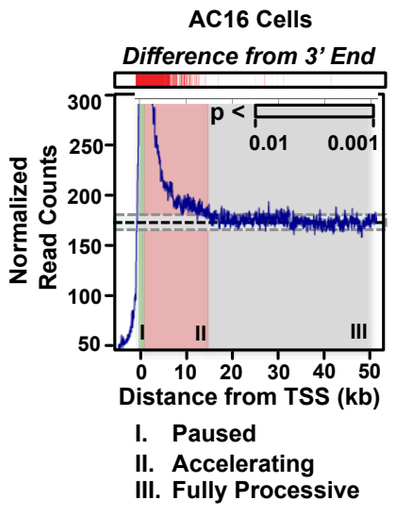
ER: Hah et. al., Cell, 2011

NFkB: Danko, et. al. Mol. Cell, 2014

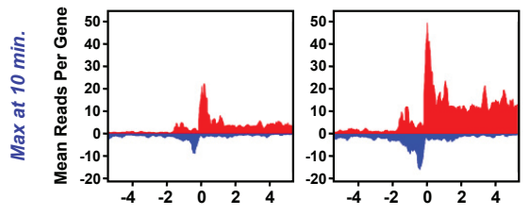
Coupling rapidly inducible systems to nascent transcript profiling informs on TF function



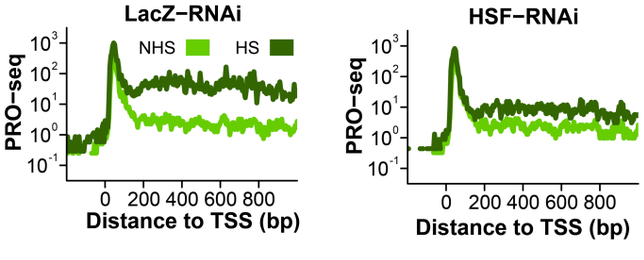
10-30 min TNFα



10-40 min Estrogen



10-20 min heat shock

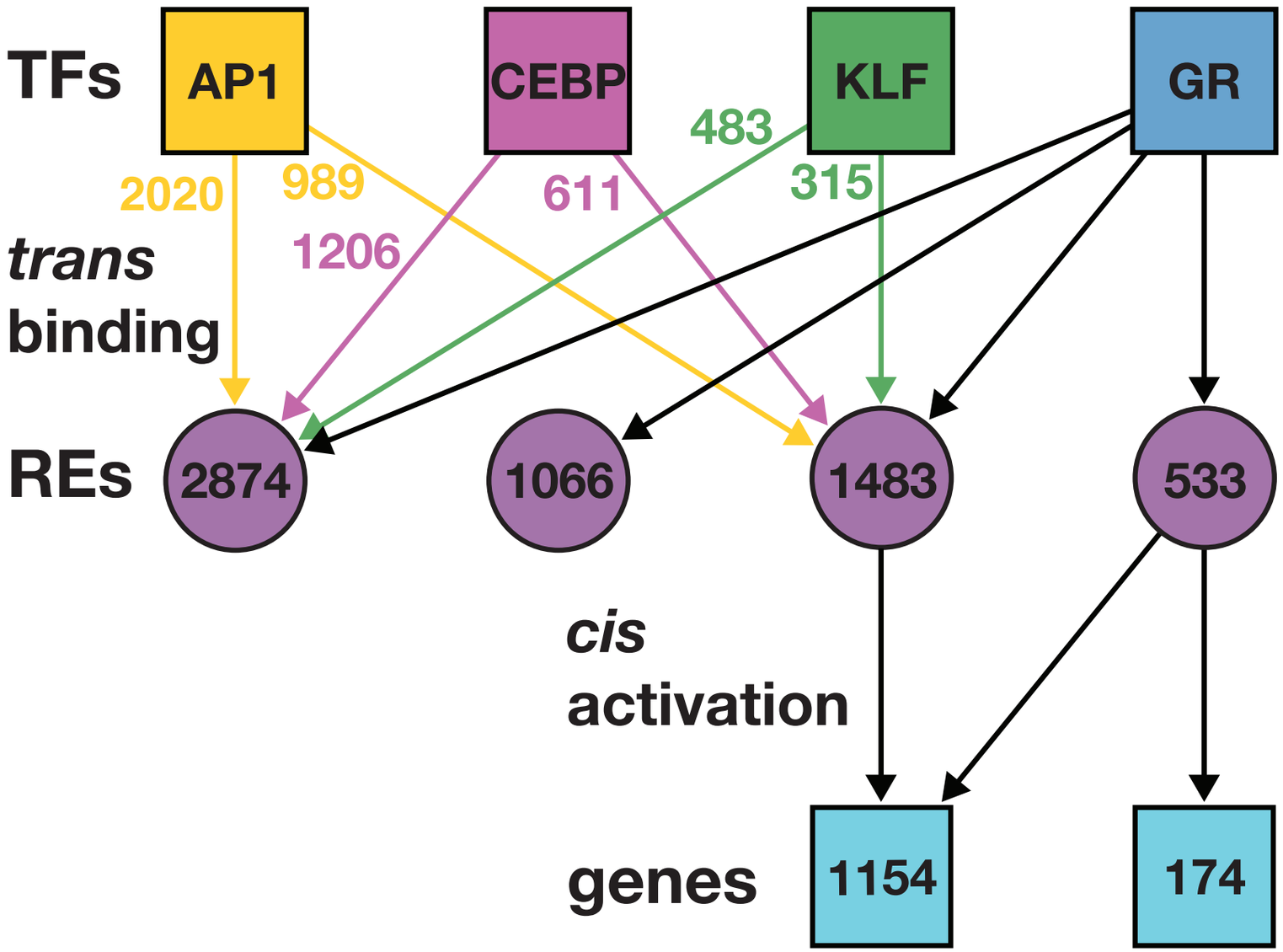


ER: Hah et. al., Cell, 2011

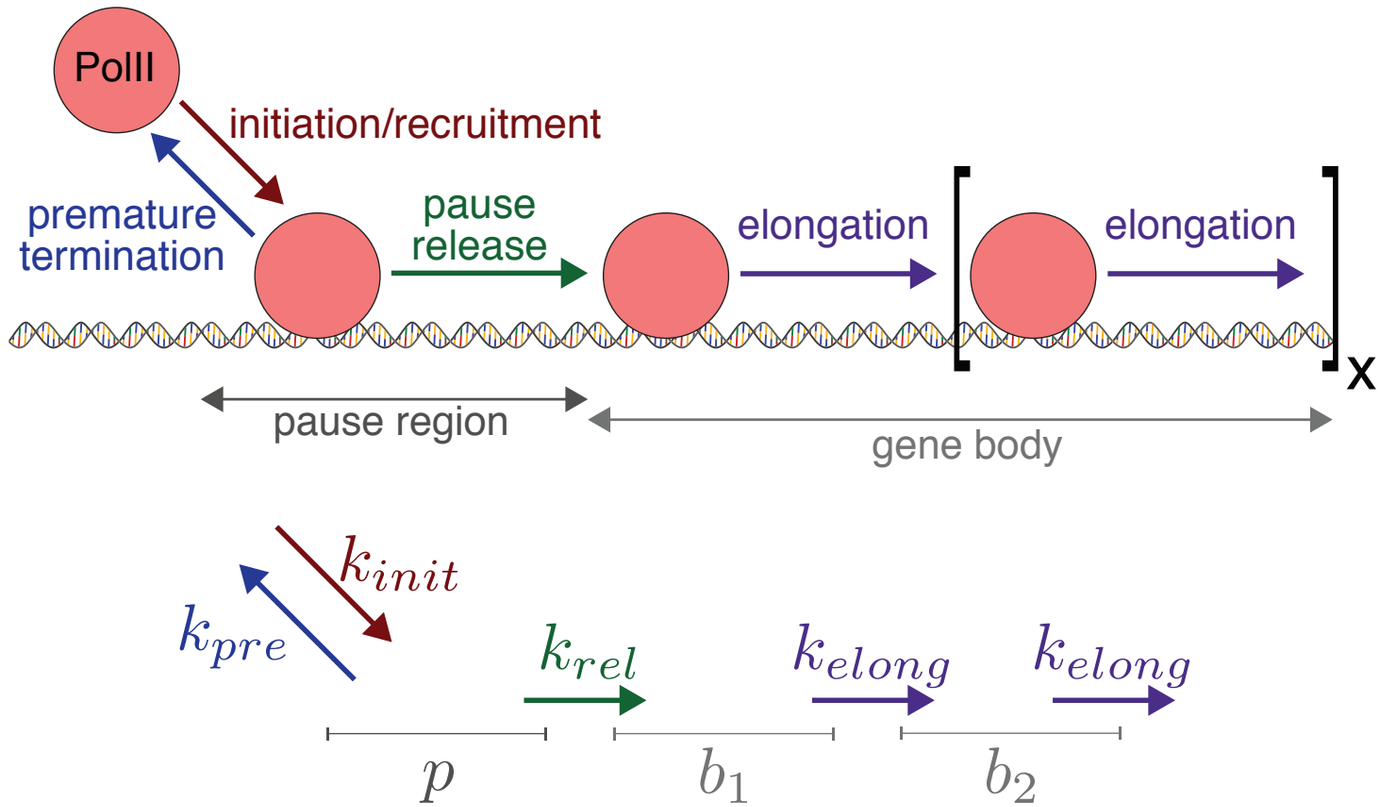
NFkB: Danko, et. al. Mol. Cell, 2014

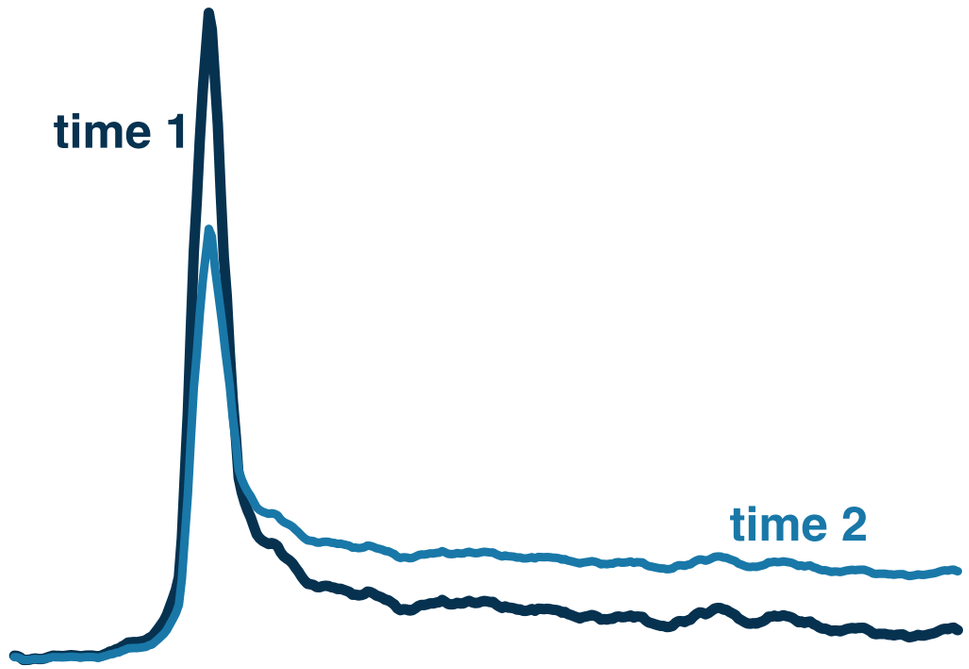
HSF: Duarte et. al., G&D, 2016

Simplified networks identify genes that are primarily regulated by a single factor



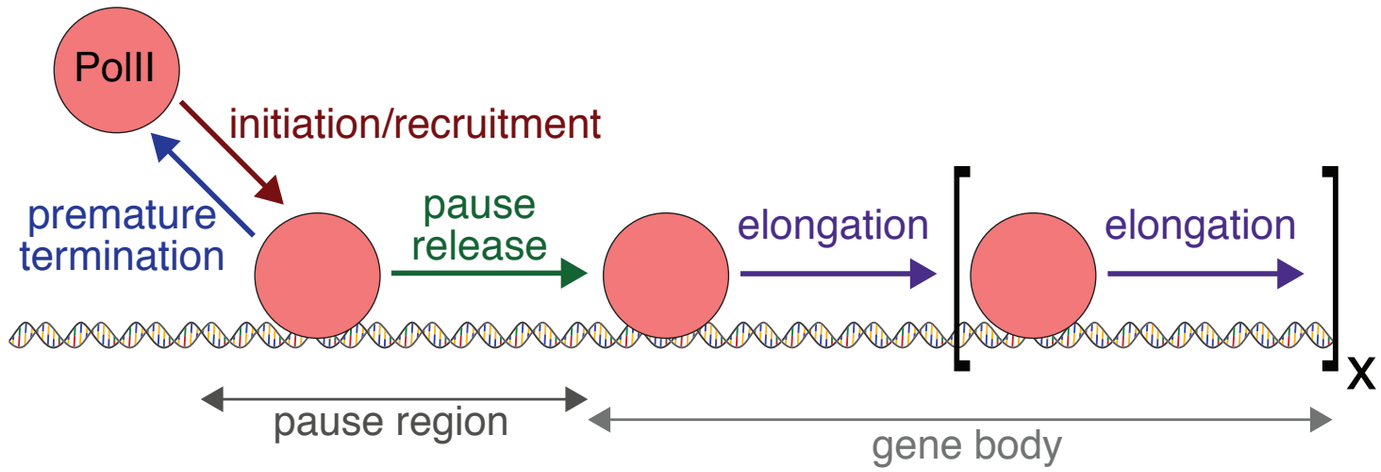
Estimating rates with a model that considers RNA Polymerase density within the pause region and gene body

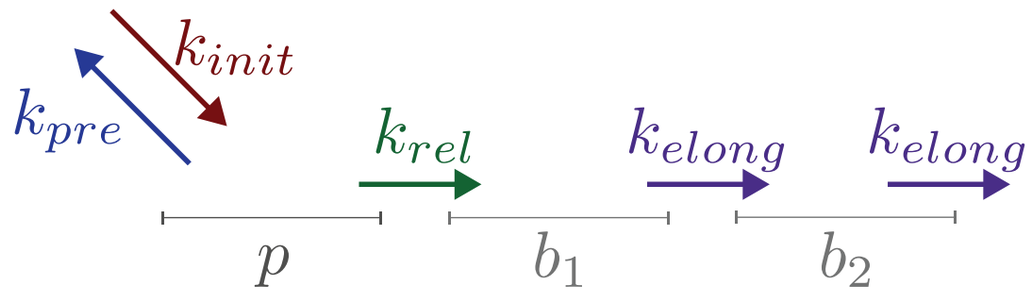




RNA polymerase composite profile

We can determine the step(s) that a TF regulates by quantifying RNA polymerase density changes in genic regions





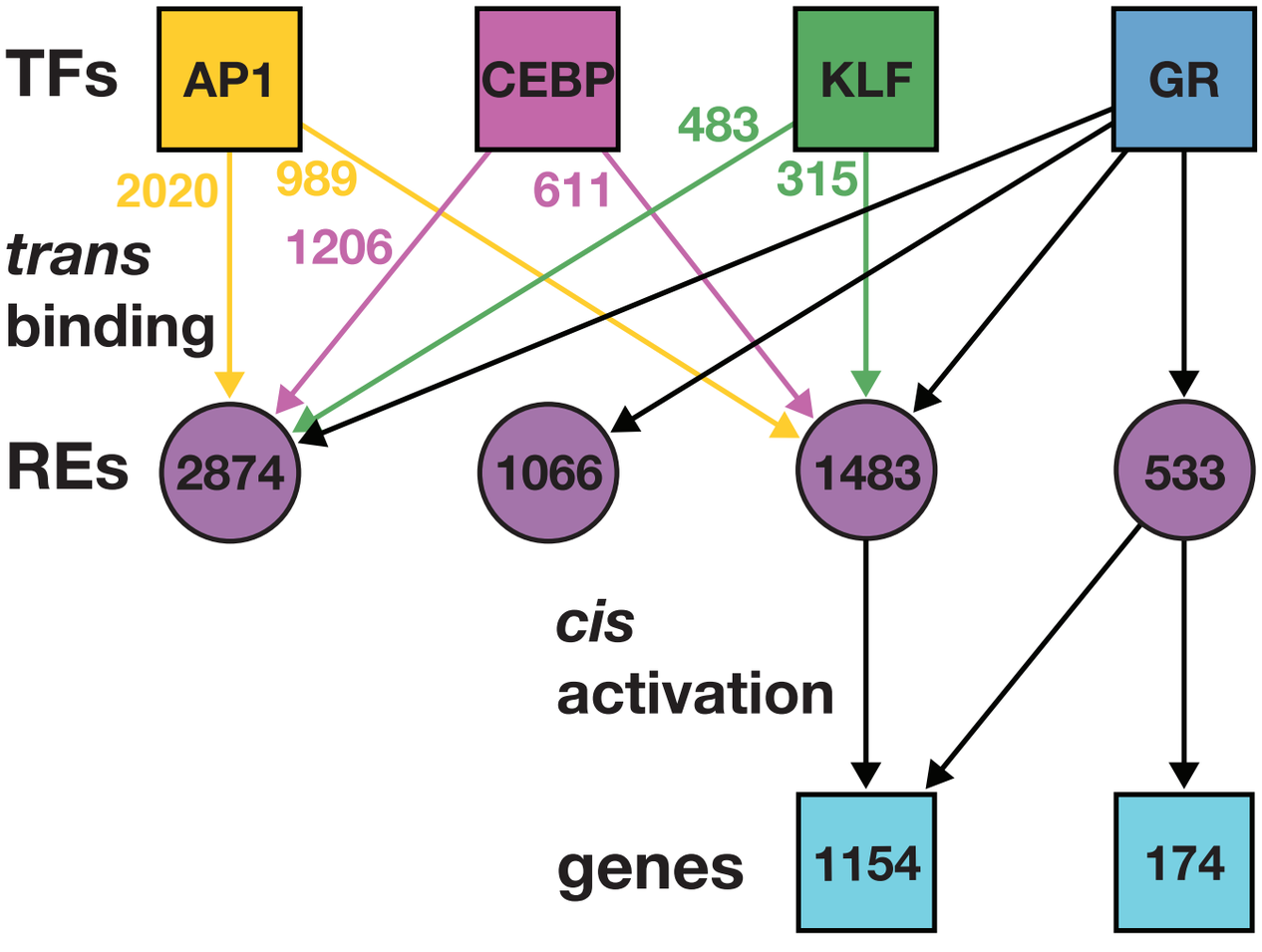
$$\frac{dp}{dt} = k_{init} - (k_{pre} + k_{rel})\, p$$

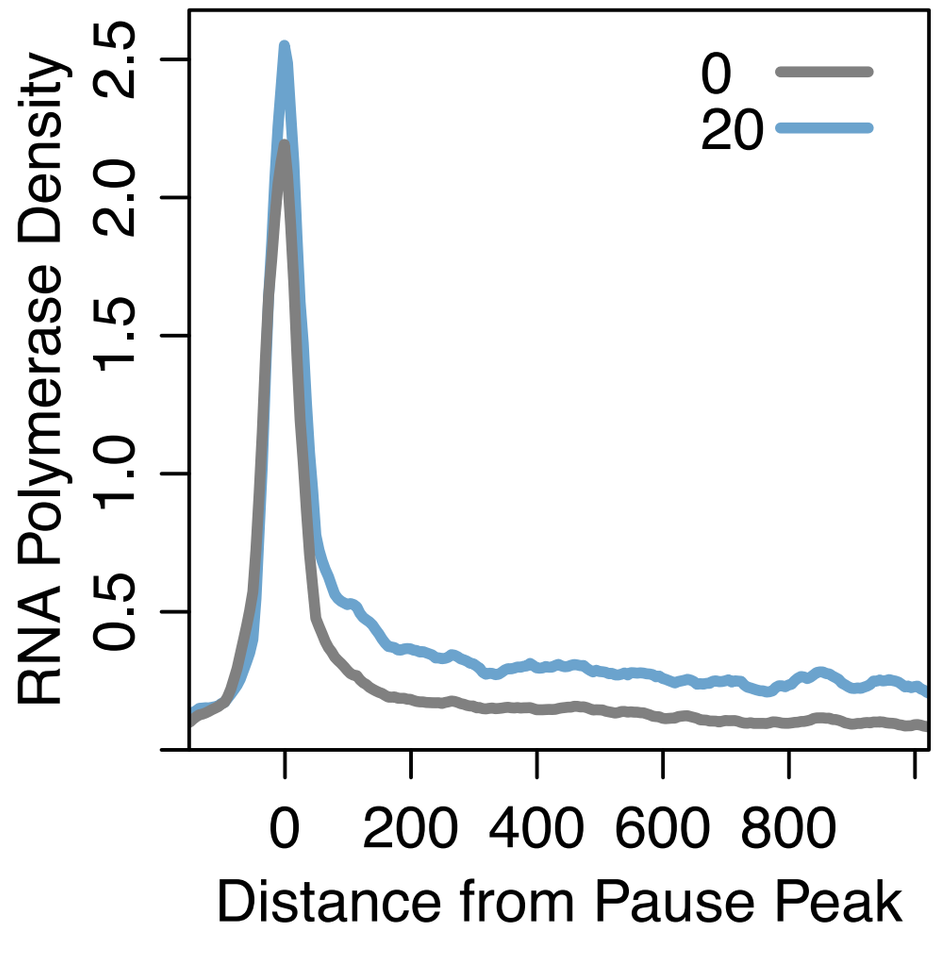
$$\frac{db_1}{dt} = k_{rel}\, p - k_{elong}\, b_1$$

$$\frac{db_2}{dt} = k_{elong}\, b_1 - k_{elong}\, b_2$$

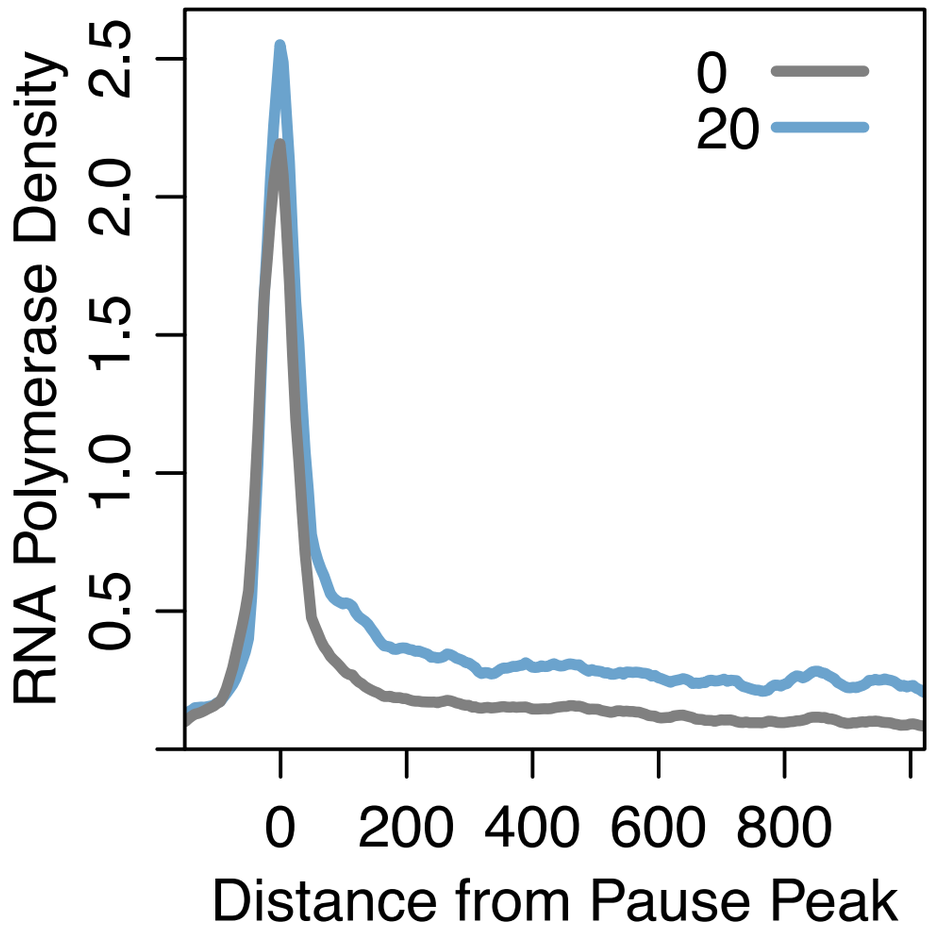
$$\frac{db_x}{dt} = k_{elong}\, b_{x-1} - k_{elong}\, b_x$$

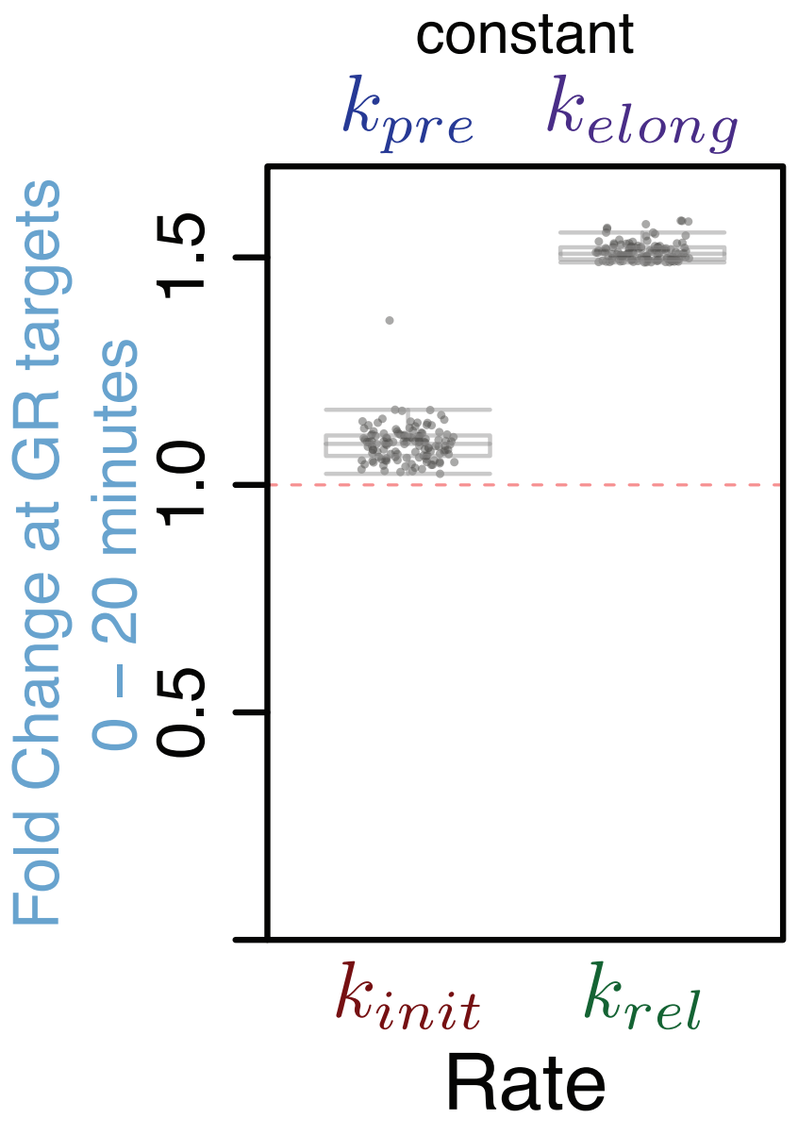
Simplified networks identify genes that are primarily regulated by a single factor





GR preferentially regulates pause release





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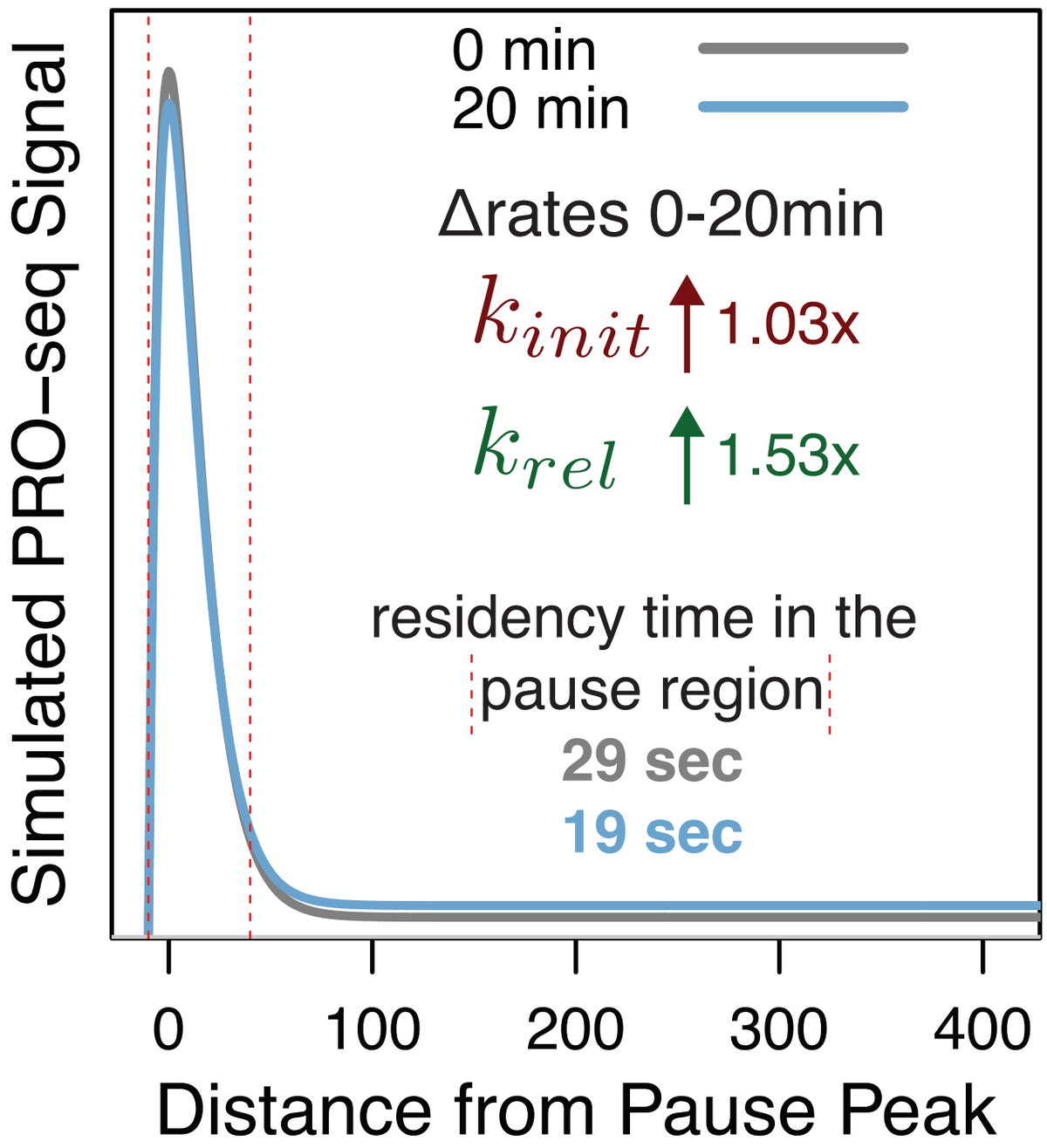
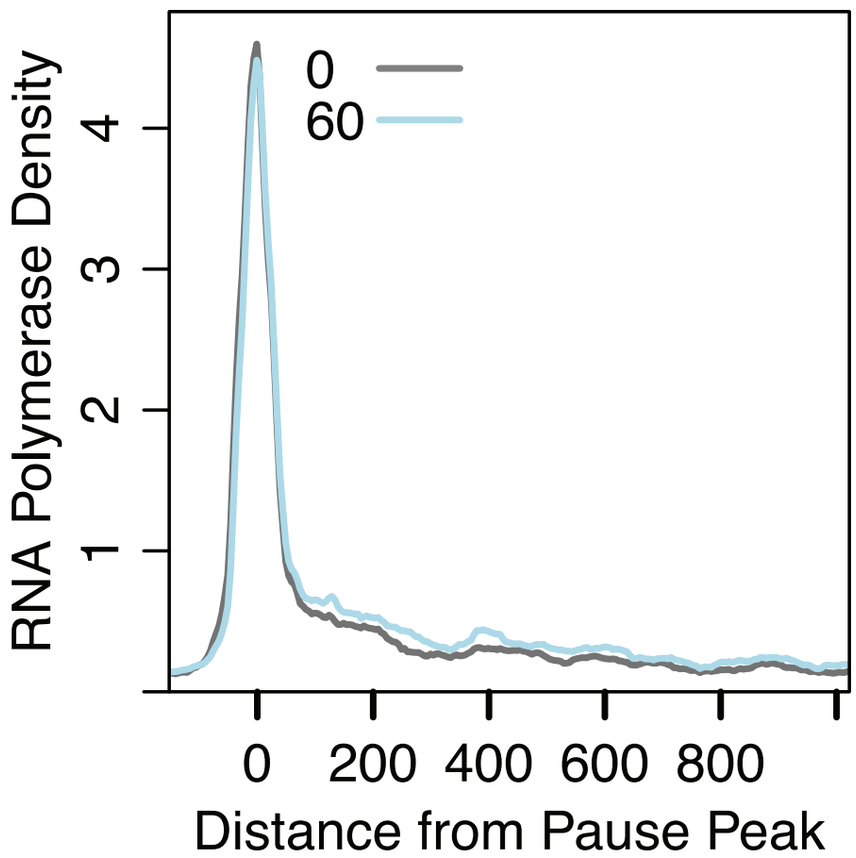
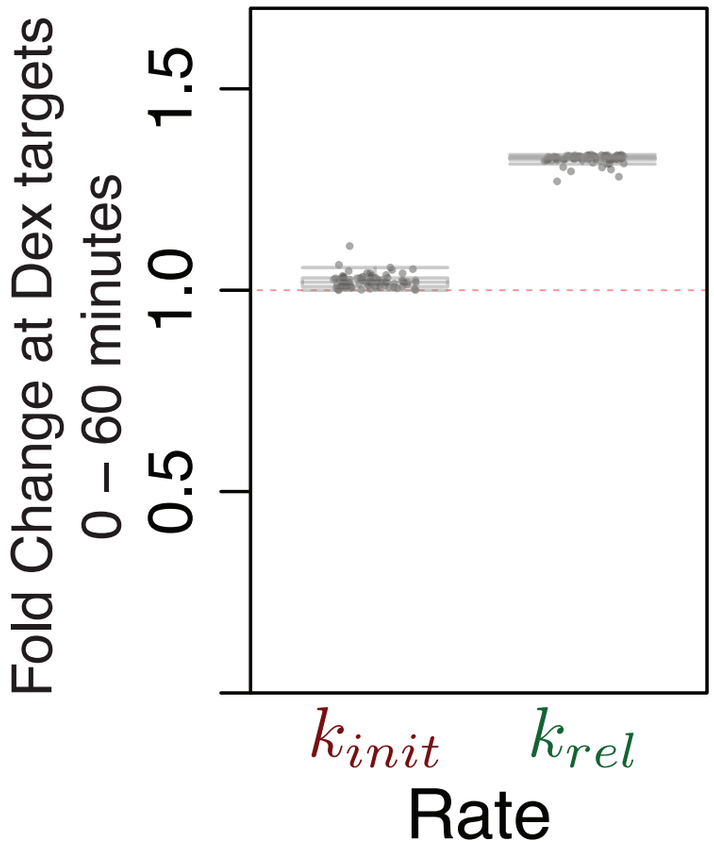
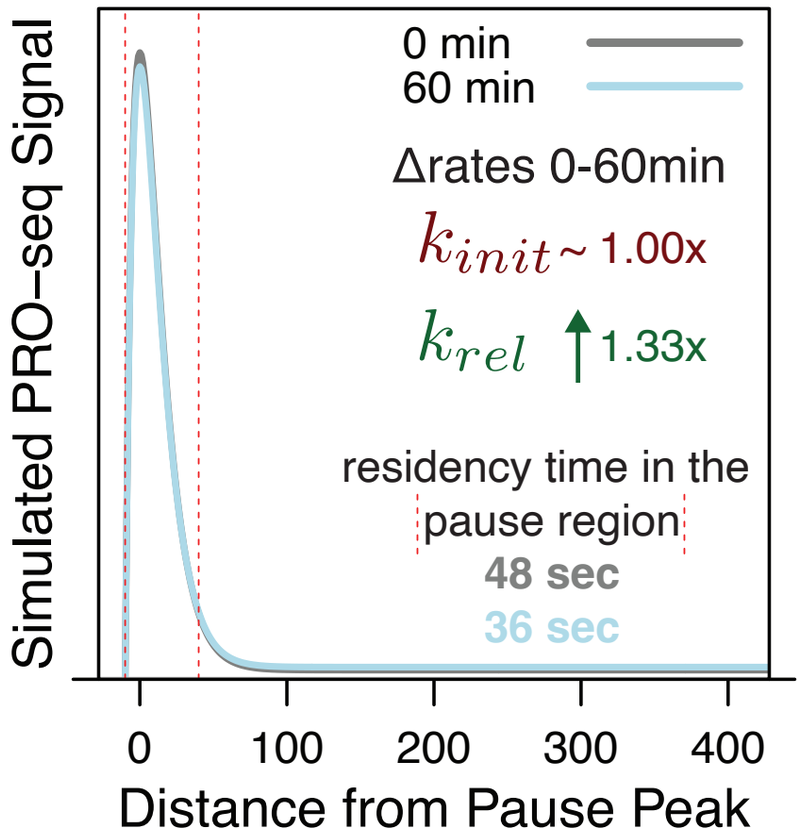


GR preferentially regulates pause release in another system: Leukemia cells treated with dexamethasone for 1 hour







Adipogenesis Network Conclusions

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- Rapidly inducible systems are necessary to provide these mechanistic insight.

TWIST2 Future

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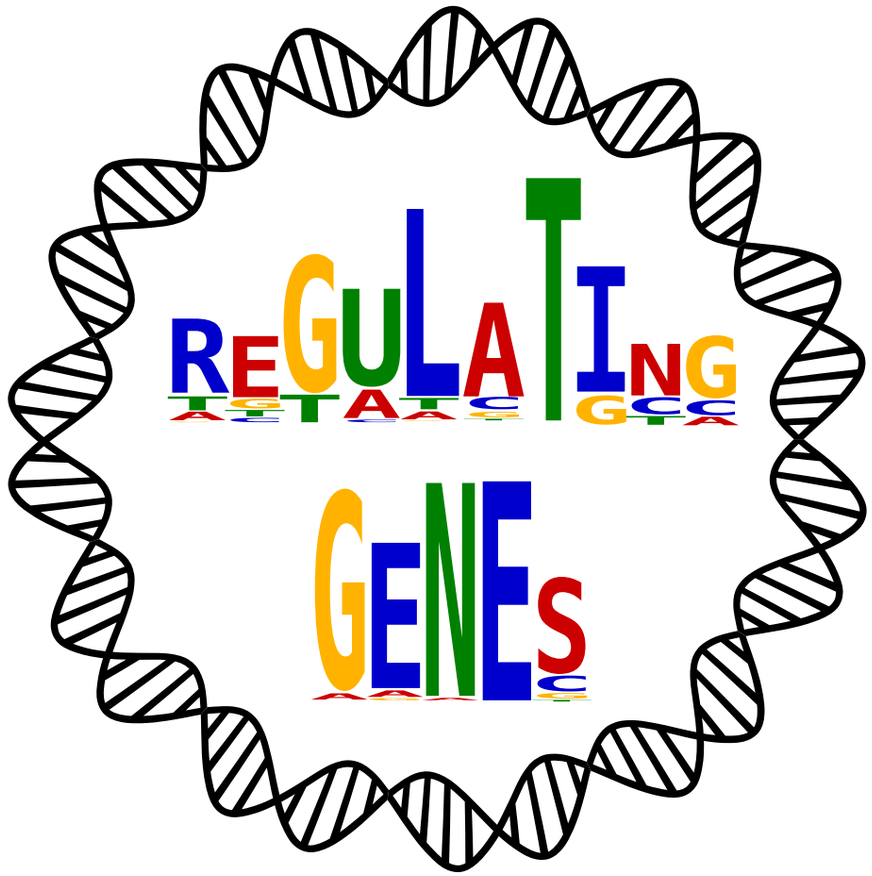
- Isolate the effect of Twist2 in proadipocytes and perform organismal phenotyping (glucose tolerance/insulin sensitivity)
- Molecular mechanisms of Twist1 and Twist2 coopertivity and antagonism

2) Mechanisms of direct *cis* Repression by a Transcription Activator



A history of rapidly inducible TFs we study.
A new era and democratizing the study of TFs.

Inherent rapid inducibility

- 2010-2021: Heat Shock Factor in Drosophila, yeast, and human cells
- 2012-2023: Estrogen Receptor in human cells
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- 2024+: SPDEF, GATA3…

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cannot rapidly induce or inhibit;
essential;
implicated in chromatin looping;
regulates spliceosomal U RNAs;
Our preliminary analyses suggested a role in Estrogen signaling

Functional perturbation experiments:
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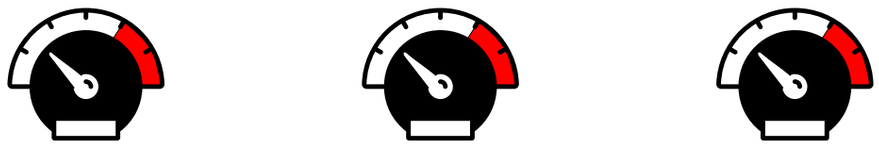
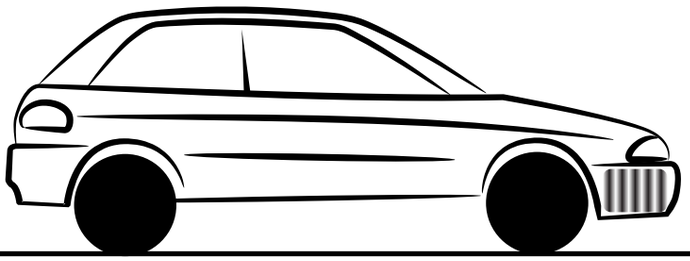
- Degron tagging is an attractive alternative

What does a car’s radiator do?

Speed **Tire Pressure** **Temperature**



Poke small holes in radiator (3 days to fully deplete fluid)



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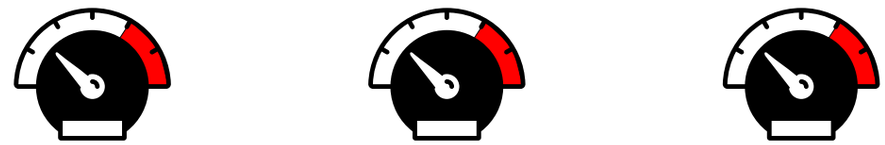
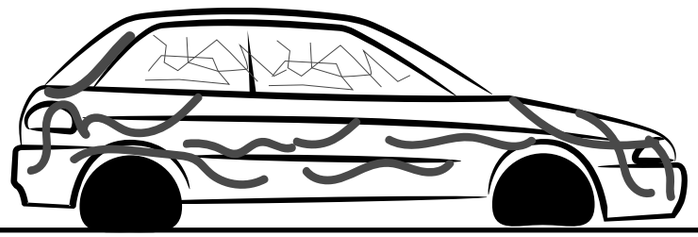
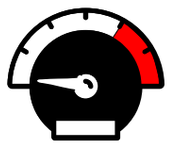


Control

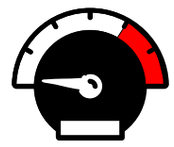
What does a car’s radiator do?



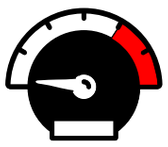
Speed



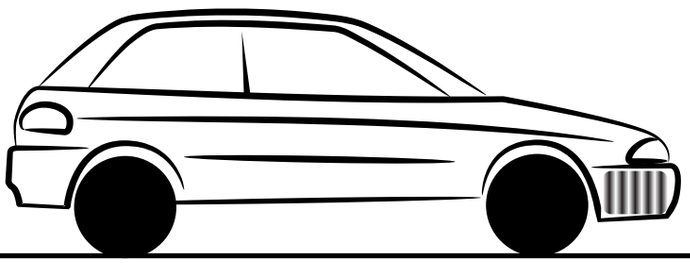
Tire Pressure



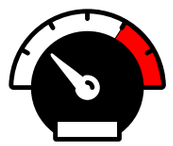
Temperature



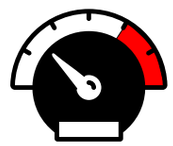
At some point on day 3



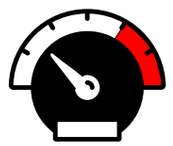
Speed



Tire Pressure

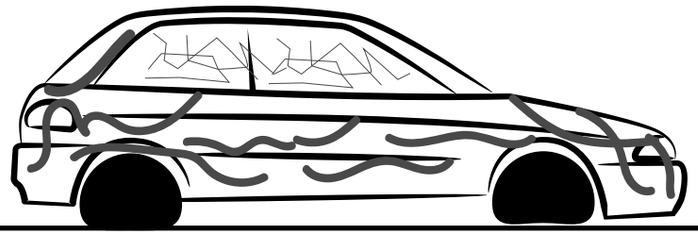


Temperature

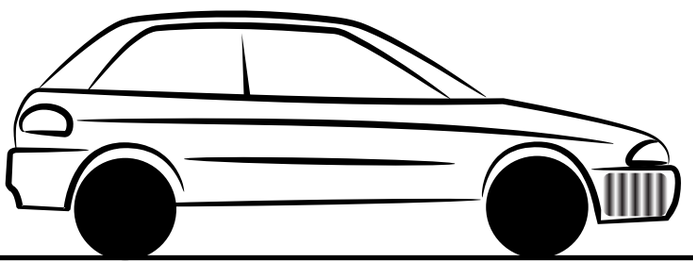


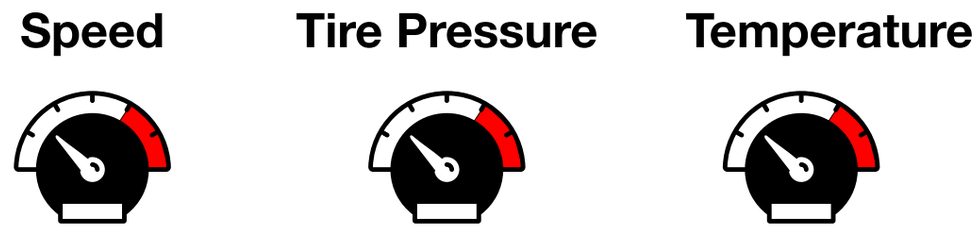
Control

What does a car’s radiator do?



Assess gauges and car phenotypes on day 4

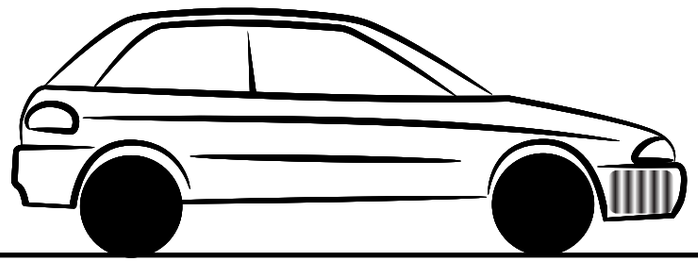




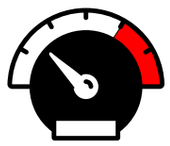
Control

The radiator keeps the car healthy;
The radiator affects the starter, speed, tire pressure, and temperature

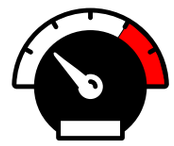
What does a car’s radiator do?



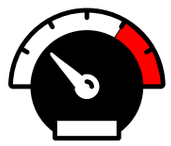
Speed



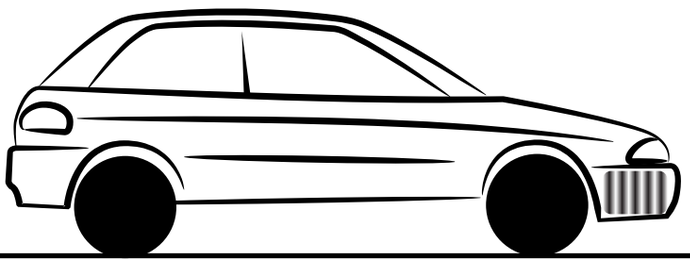
Tire Pressure



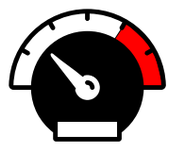
Temperature



Rip out the radiator while driving



Speed



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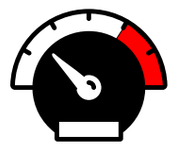
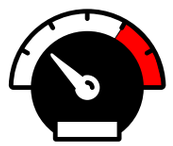
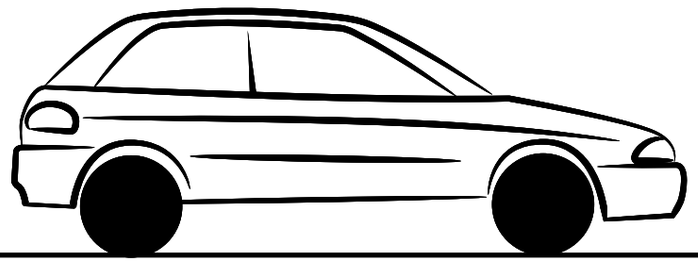


Temperature

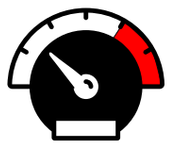


Control

What does a car’s radiator do?



Speed



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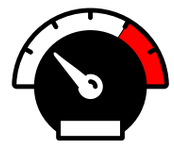
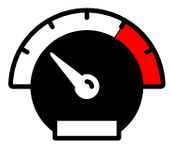


Temperature



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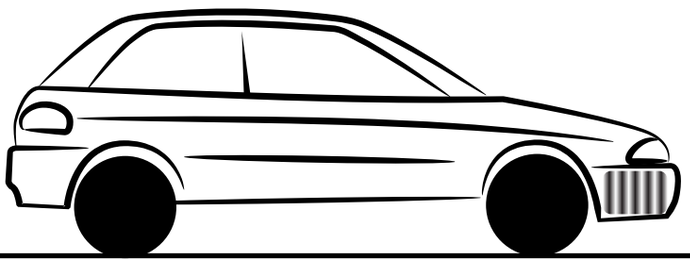
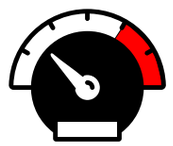


Speed



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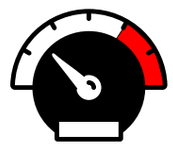

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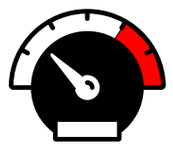
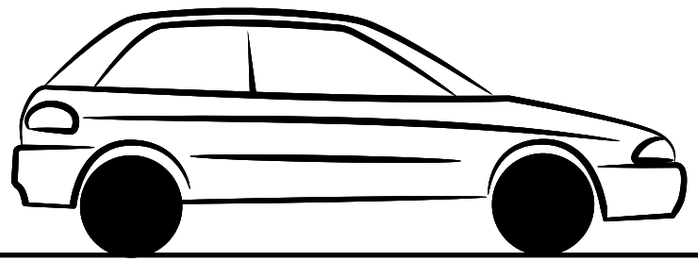
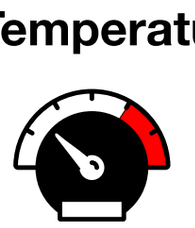


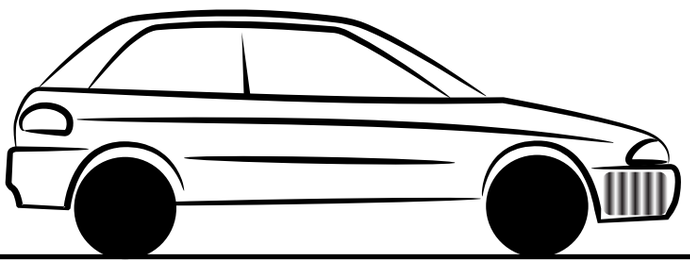
Control

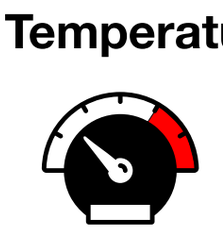
What does a car’s radiator do?





Monitor gauges and car phenotypes continually





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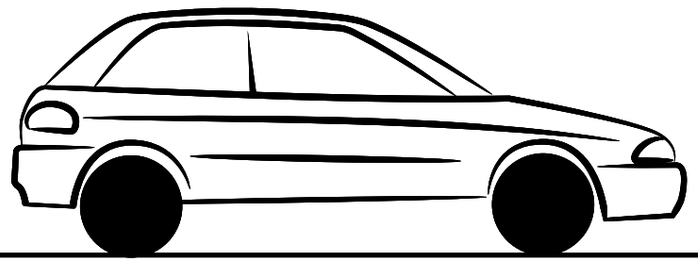

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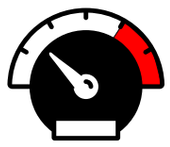
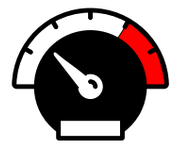
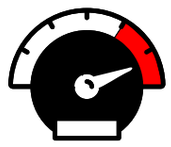


Tire Pressure



Temperature



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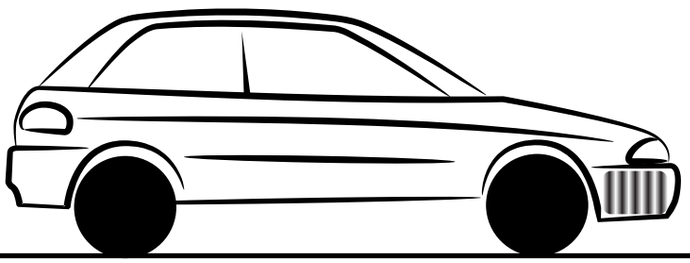

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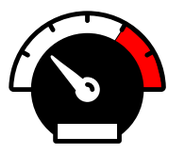

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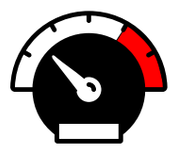

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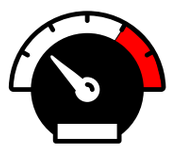
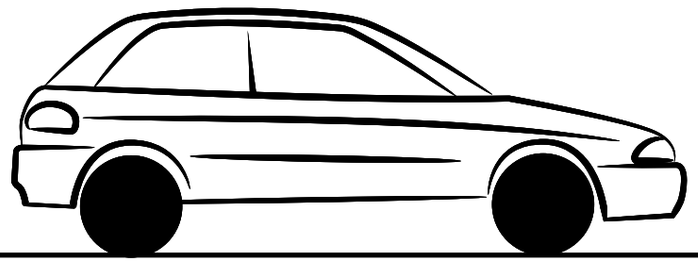
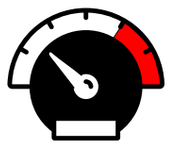


Control

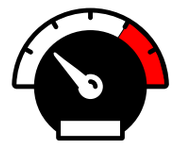
What does a car’s radiator do?



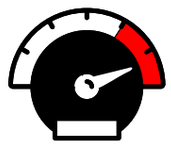
Speed



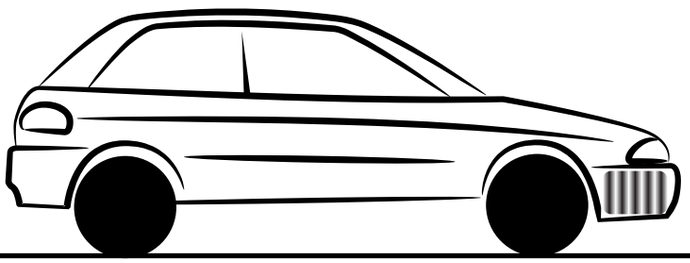
Tire Pressure



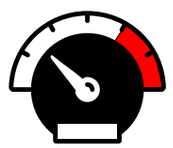
Temperature



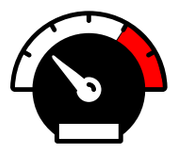
Monitor gauges and car phenotypes continually



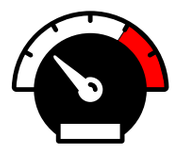
Speed



Tire Pressure



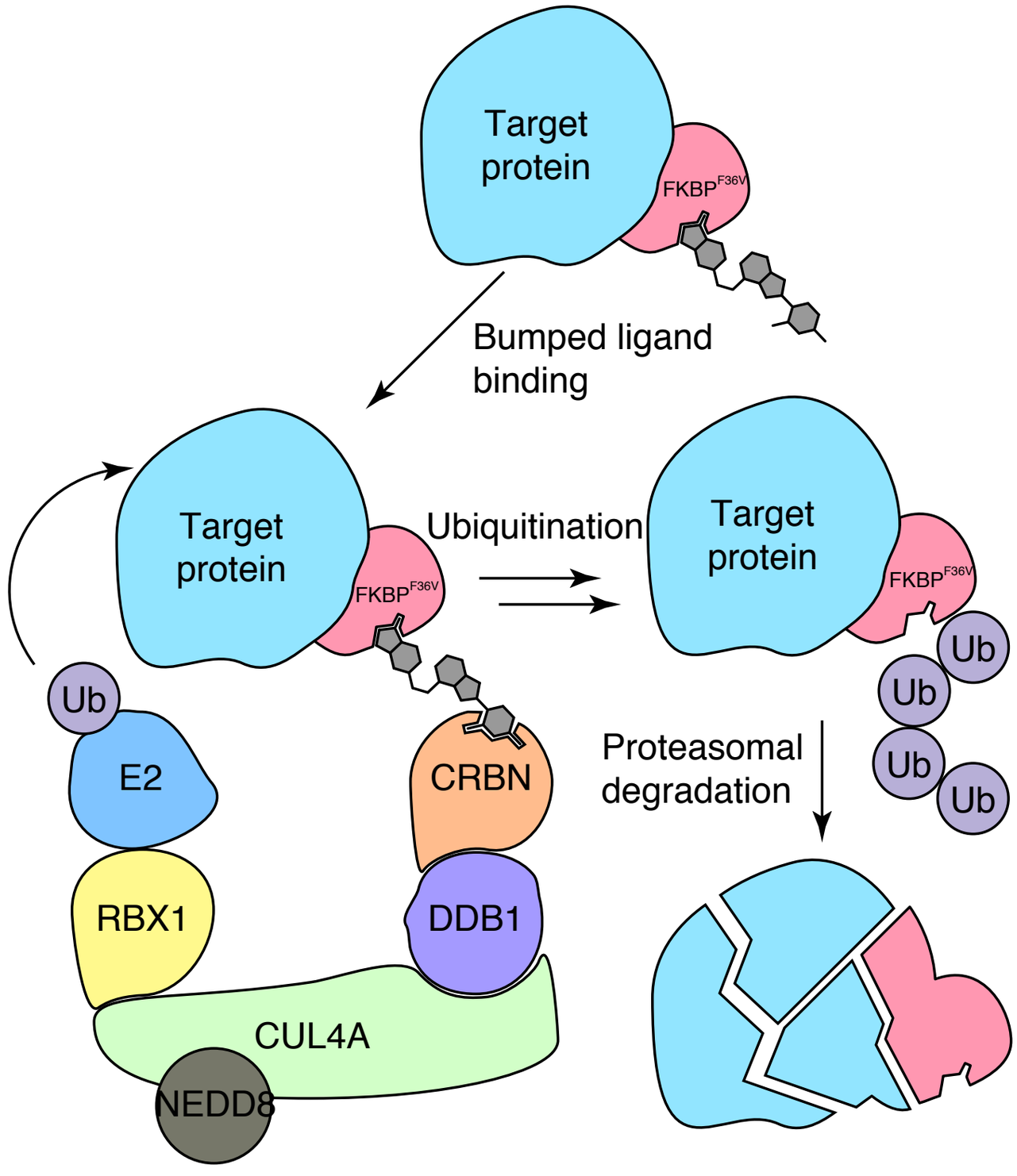
Temperature



Control

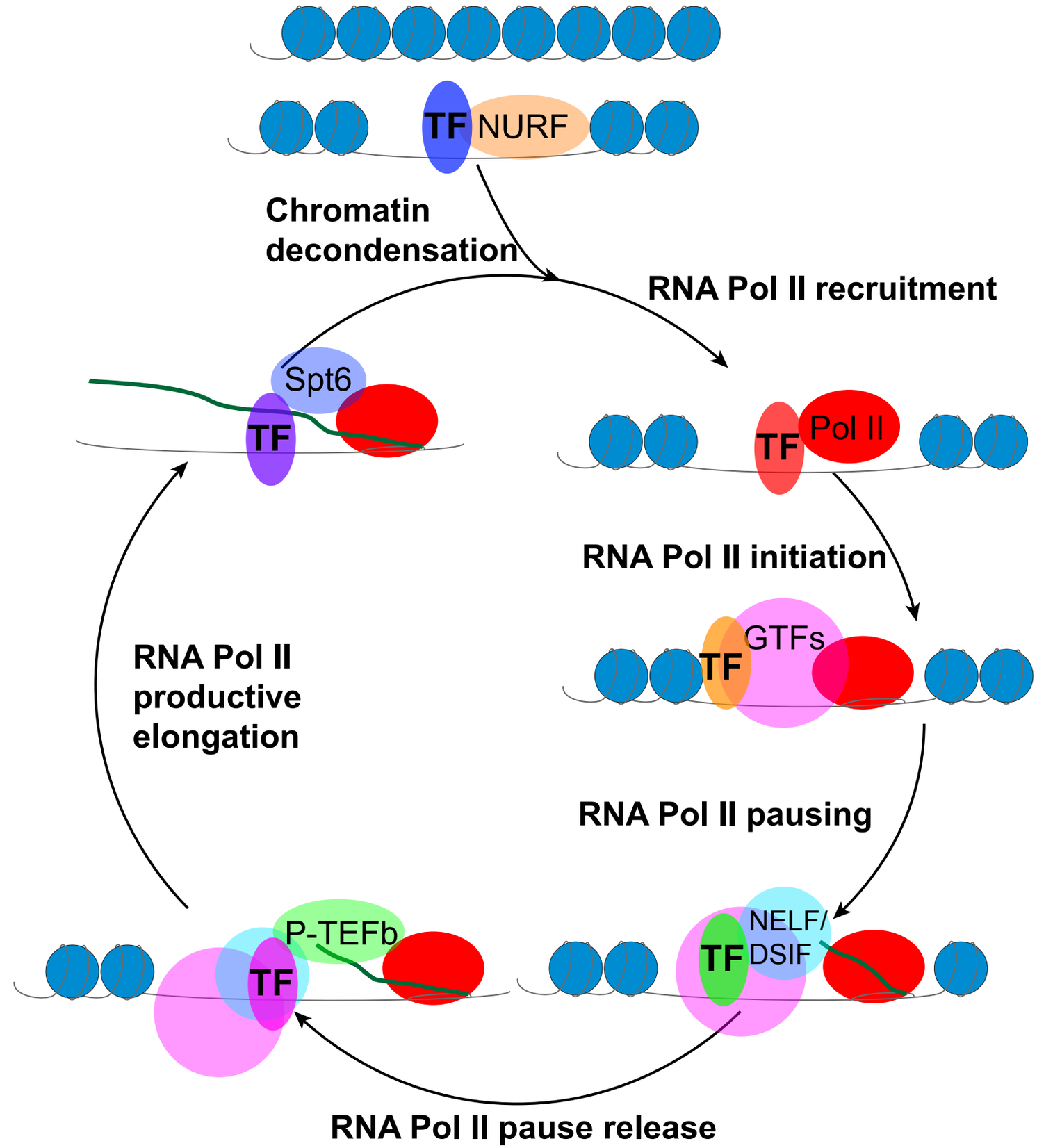
The radiator directly regulates temperature;
all other effects are indirect effects of the car catching fire.

dTAG: rapid and specific protein degradation

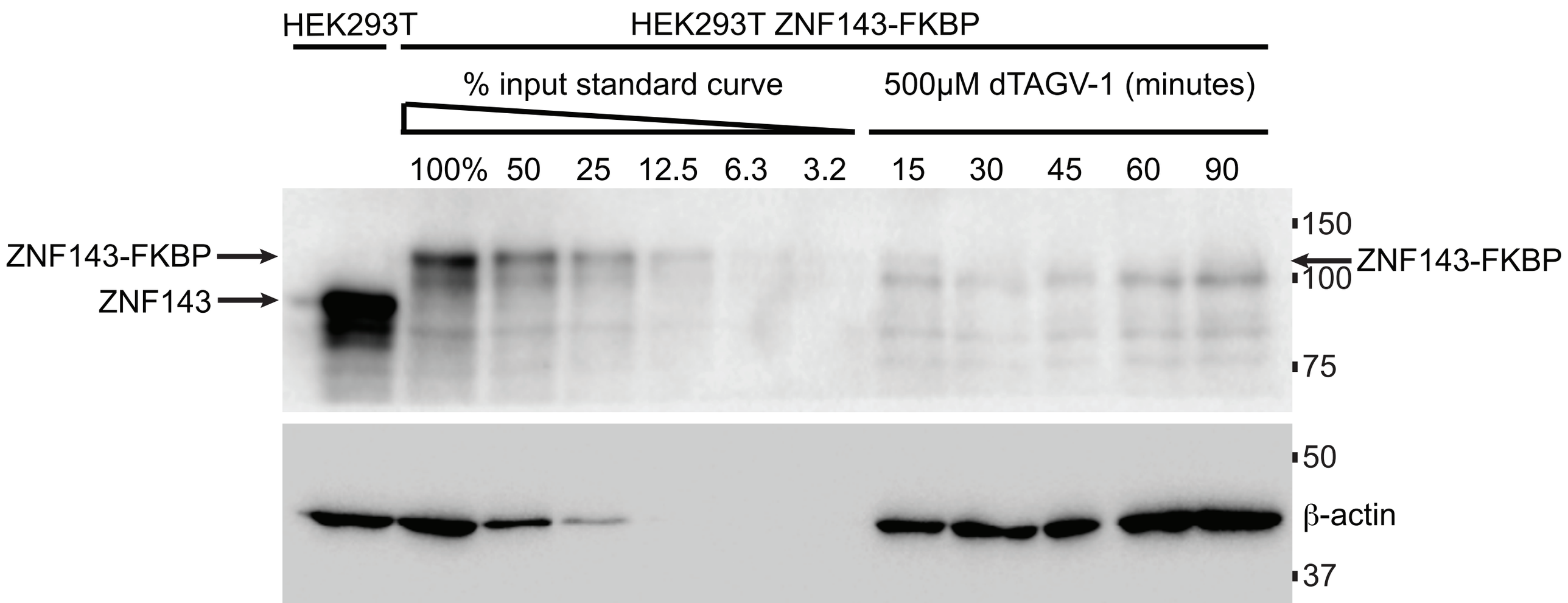


Nabet, et. al., Nat Chem Biol. 2018

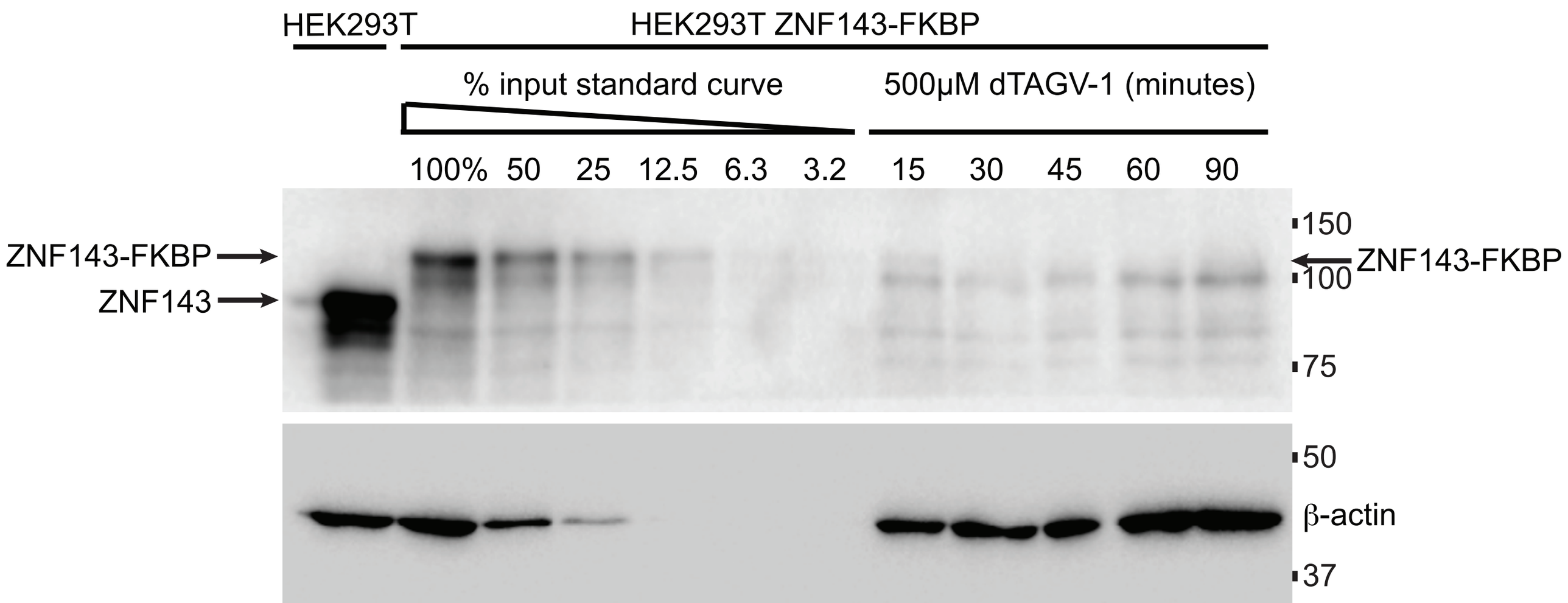
What is/are the molecular function(s) of ZNF143?



Degron tagged ZNF143 is degraded at 30 minutes

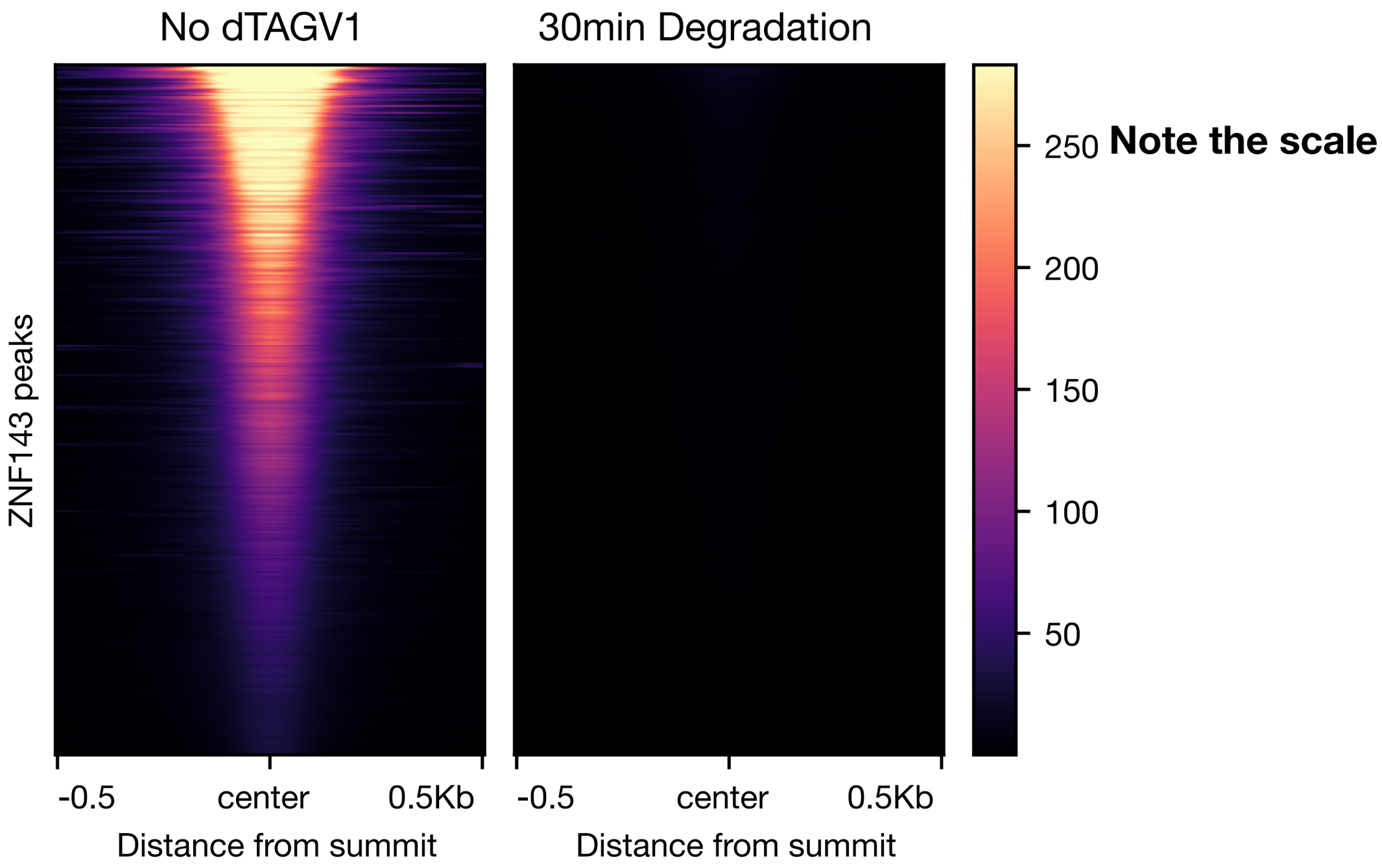


Degron tagged ZNF143 is degraded at 30 minutes

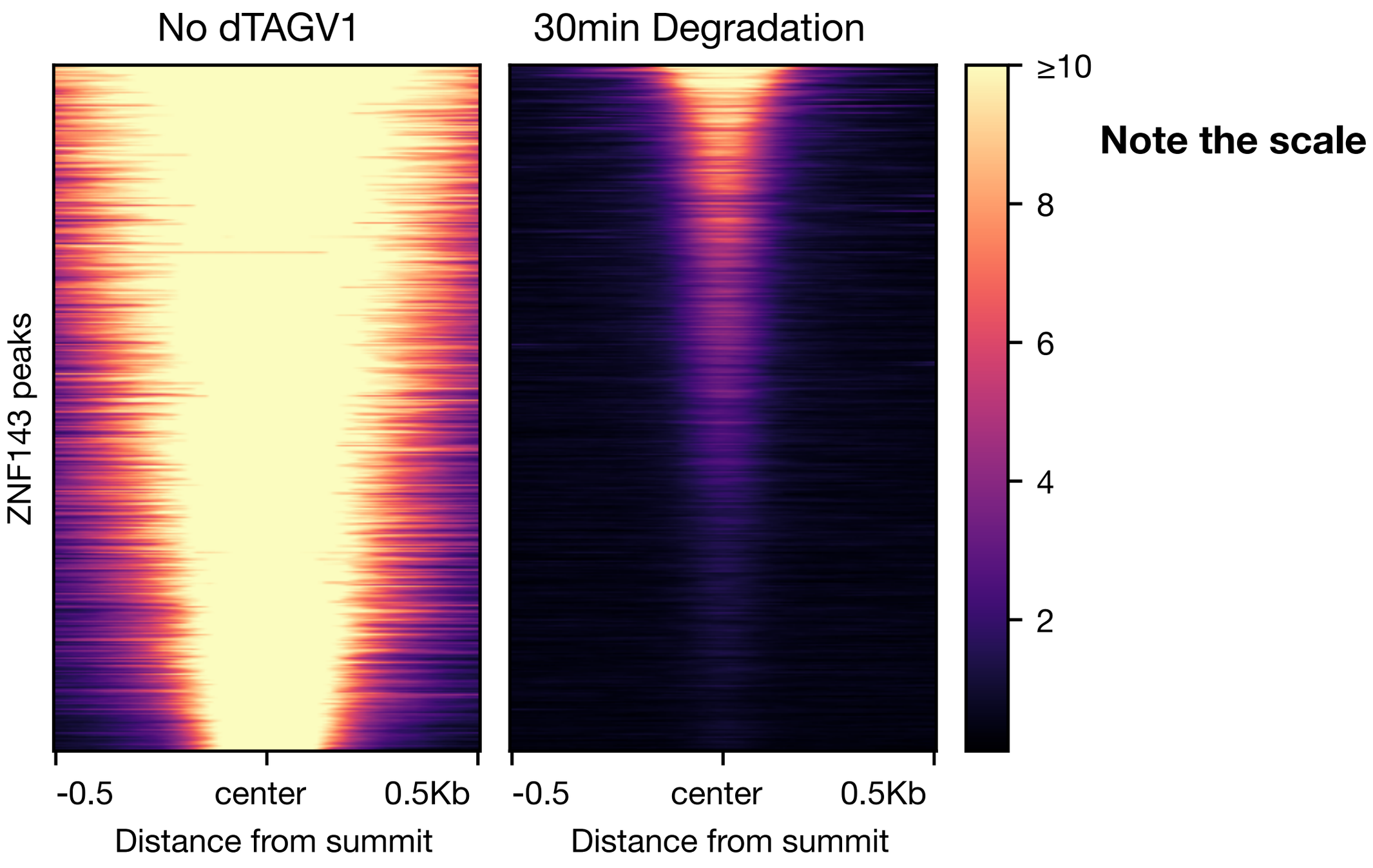


****All degradation data in this presentation are after 30min****

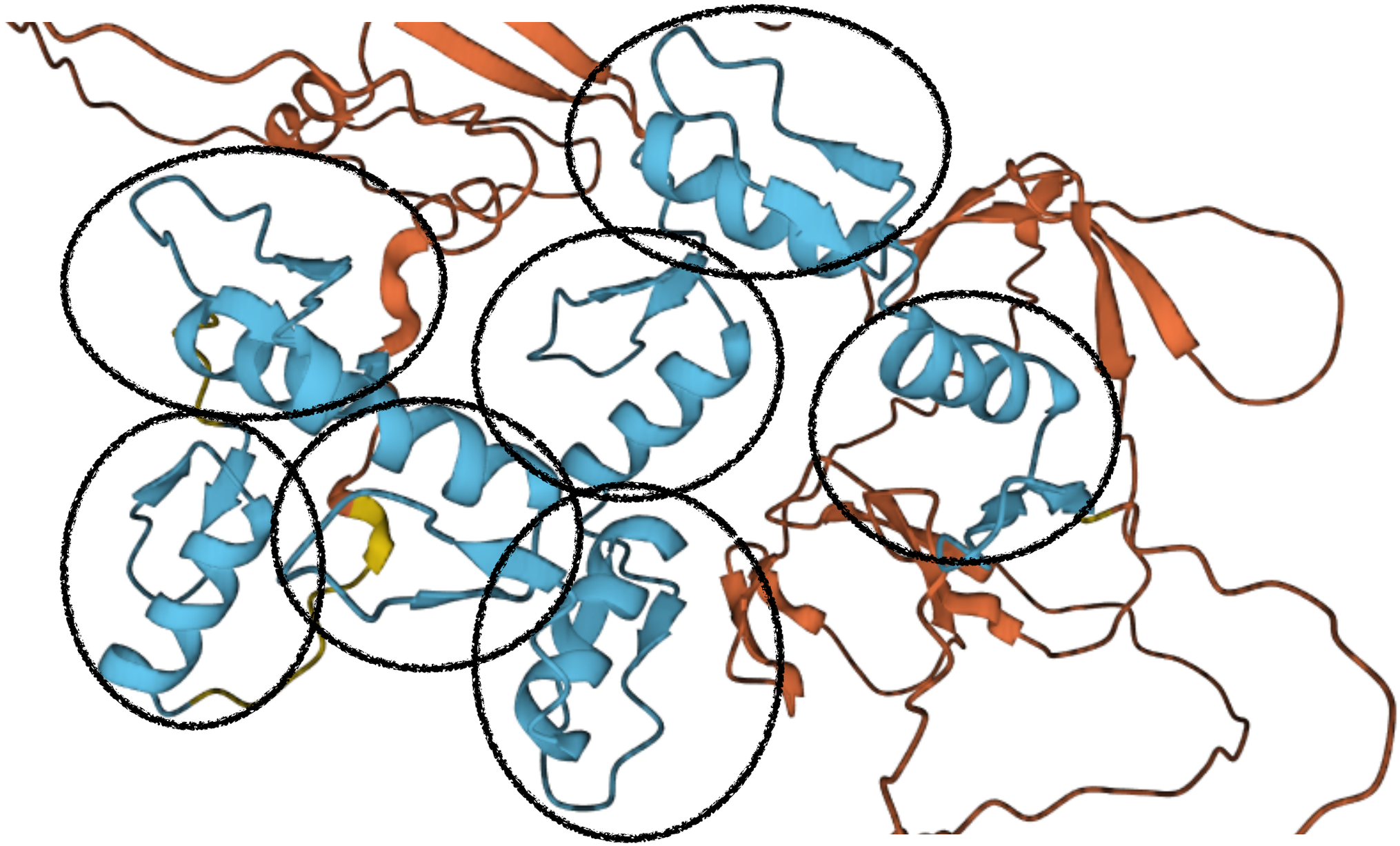
ZNF143 is off chromatin at 30 minutes



ZNF143 is off chromatin at 30 minutes

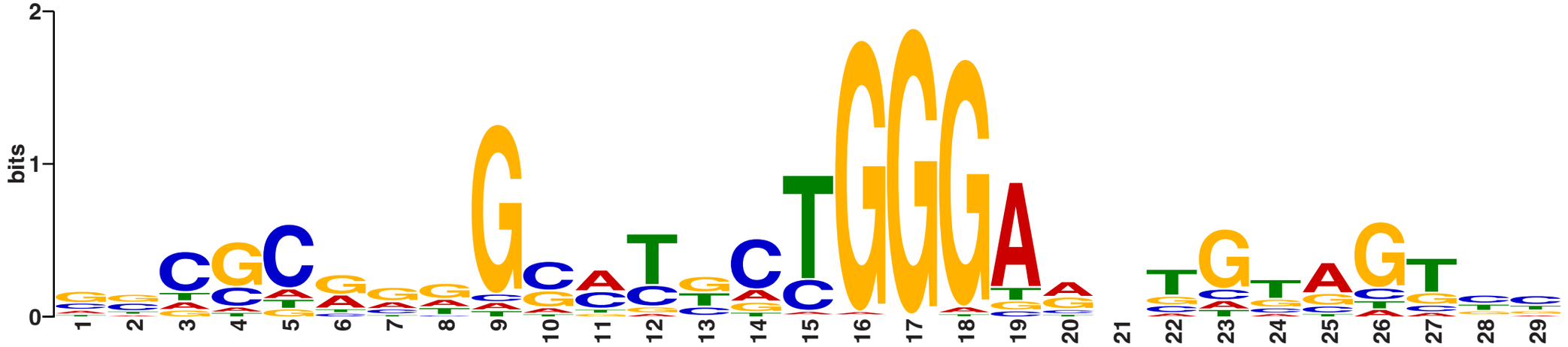


ZNF143 has 7 blue Zn Fingers



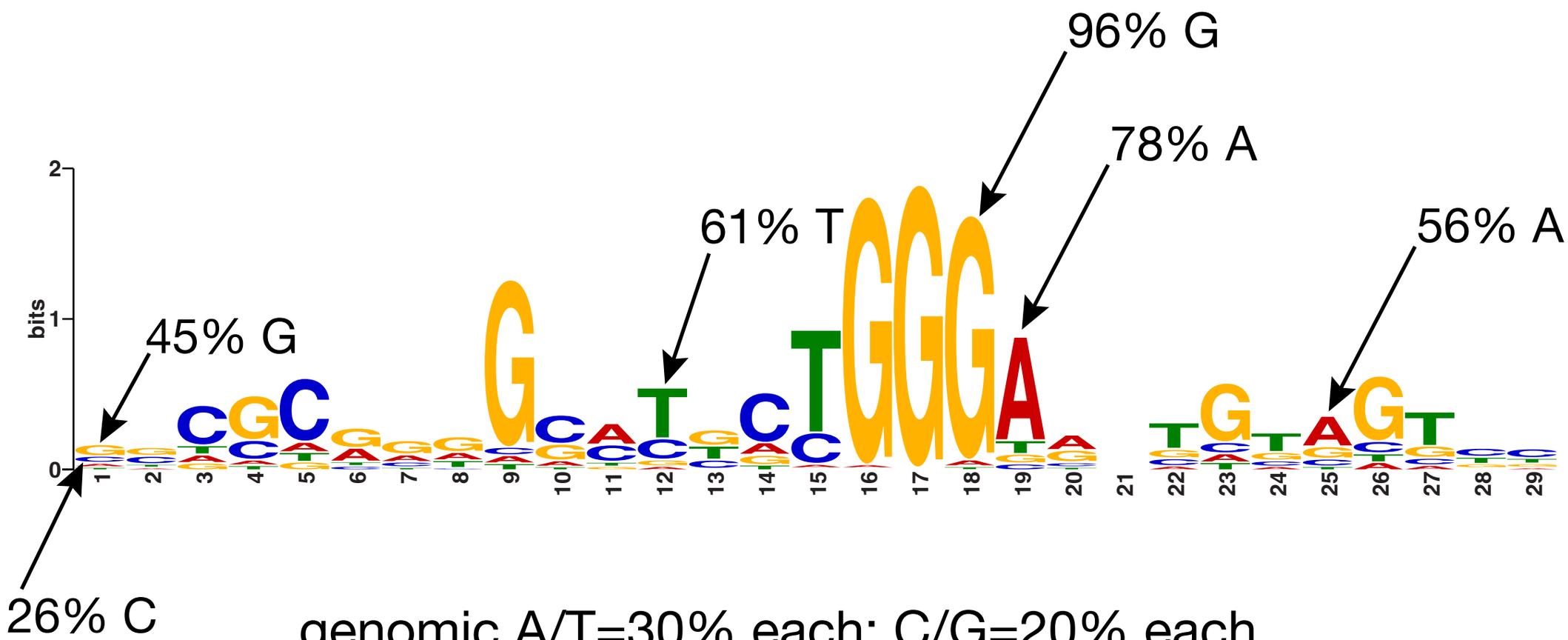
AlphaFold

ZNF143’s 7 Zn fingers span a 29 base degenerate motif



All ZNF143 ChIP peaks have a ZNF143 motif

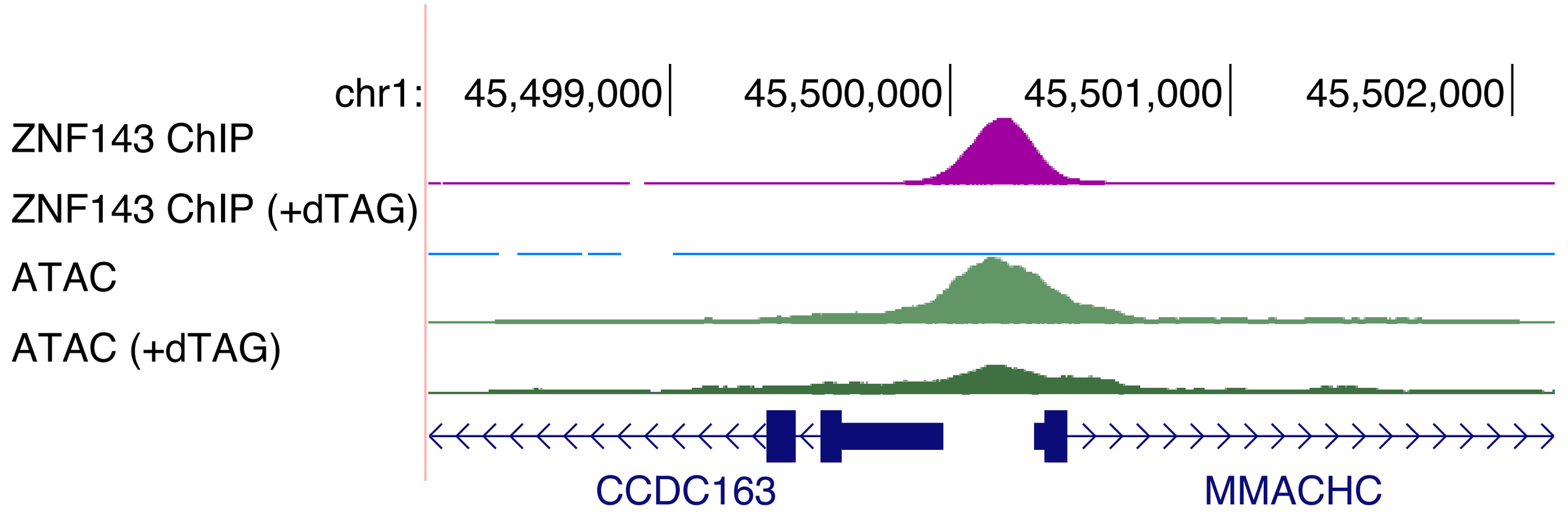
ZNF143's 7 Zn fingers span a 29 base degenerate motif



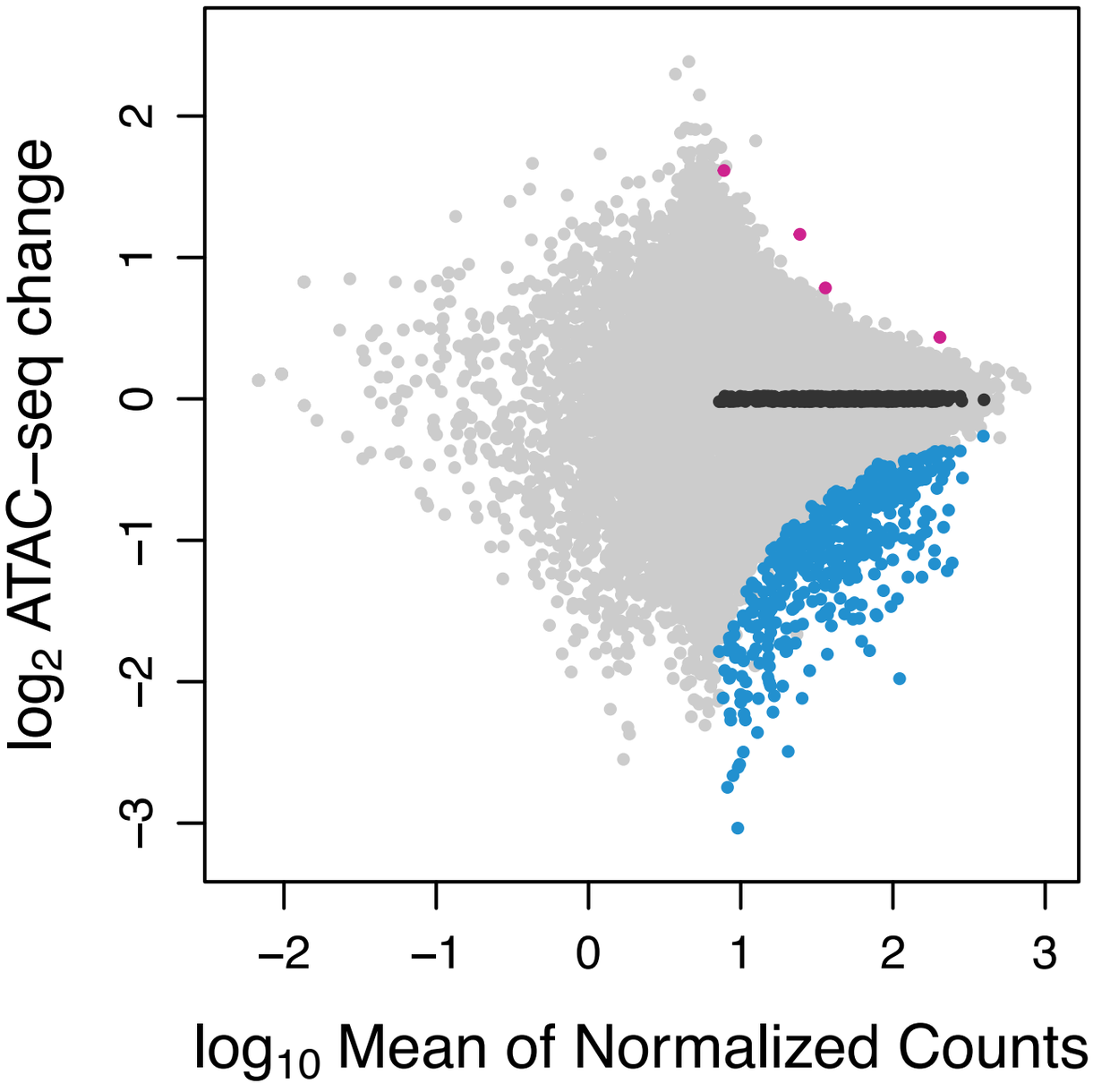
genomic A/T=30% each; C/G=20% each

All ZNF143 ChIP peaks have a ZNF143 motif

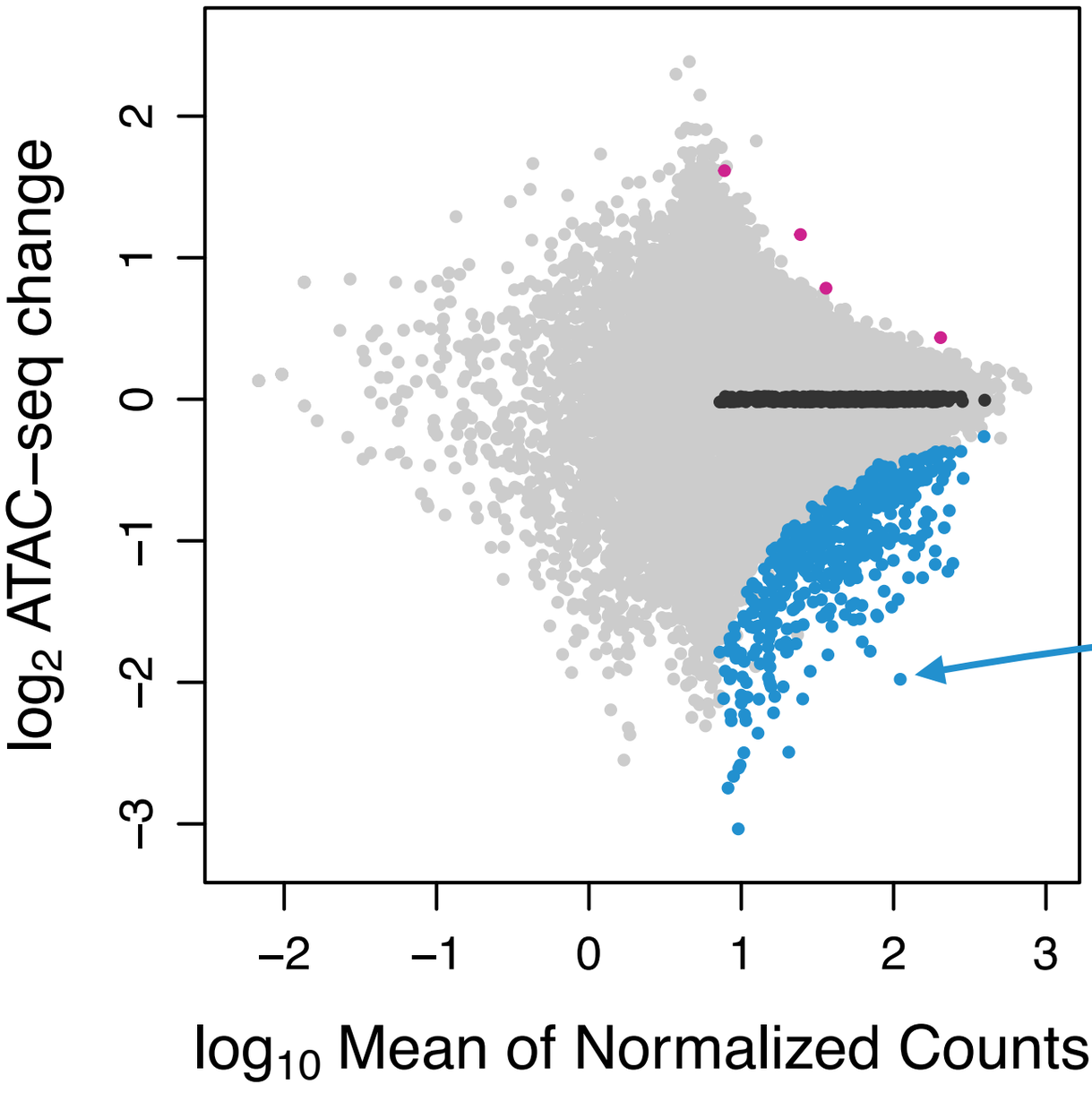
Use ATAC-seq to quantify changes in chromatin

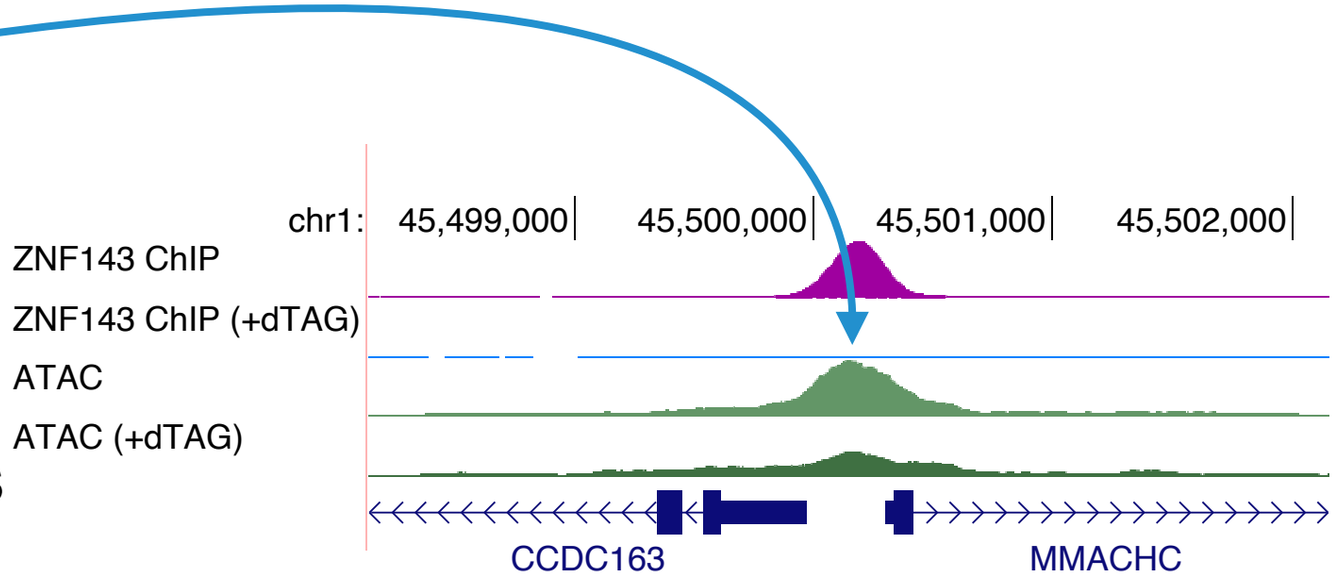


30 min of ZNF143 depletion causes decreased chromatin accessibility

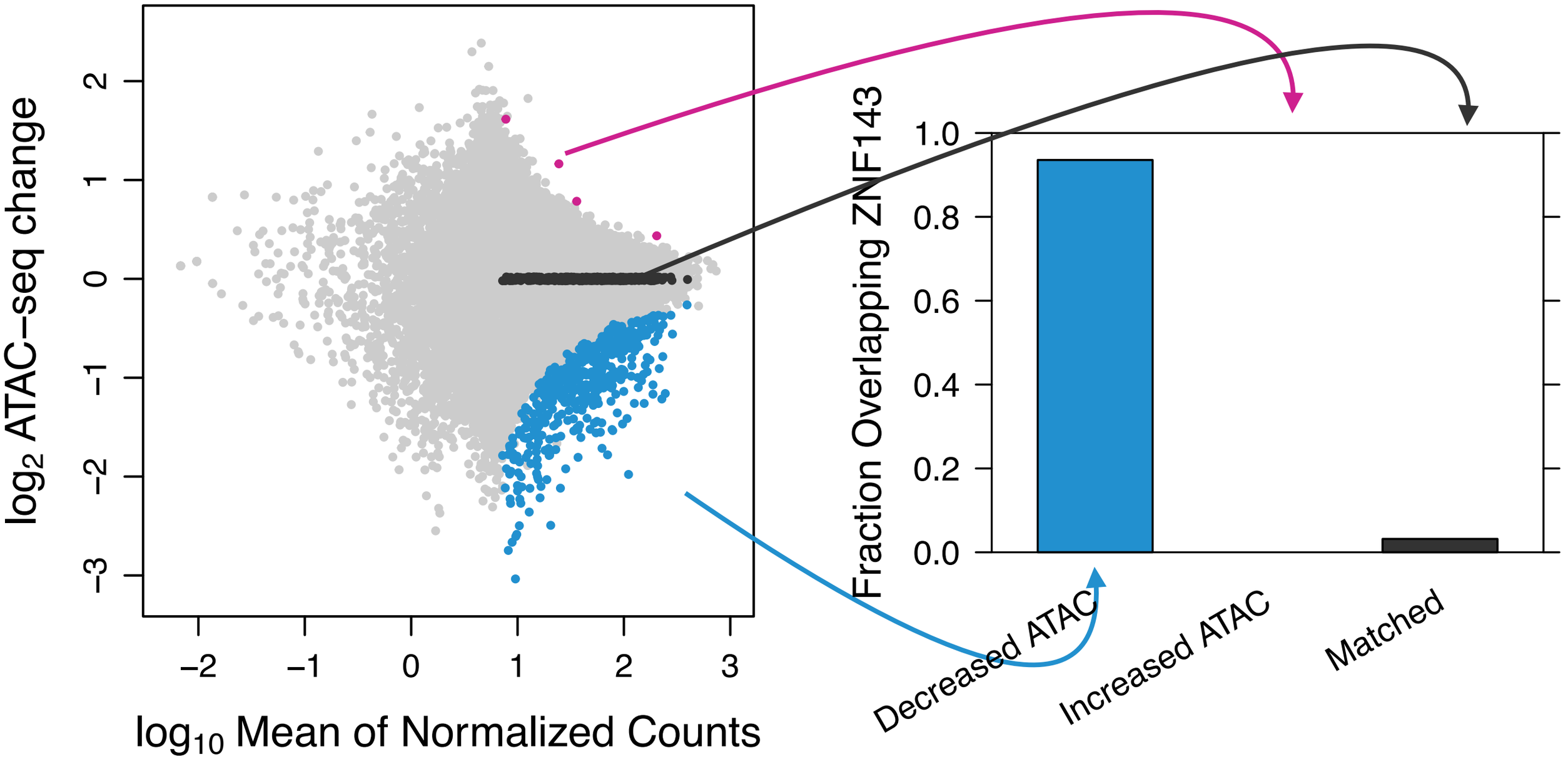


ZNF143 maintains open chromatin structure

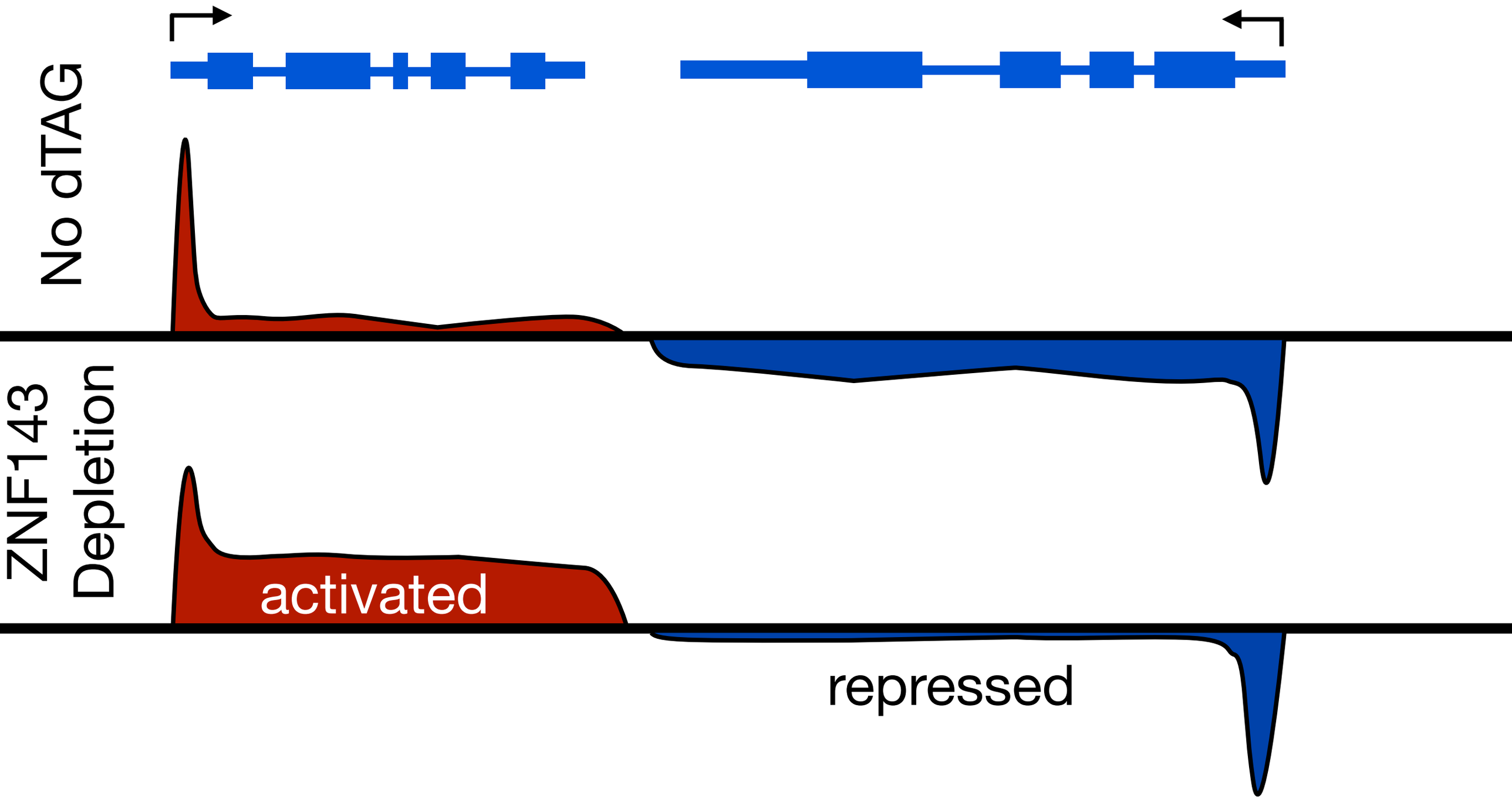




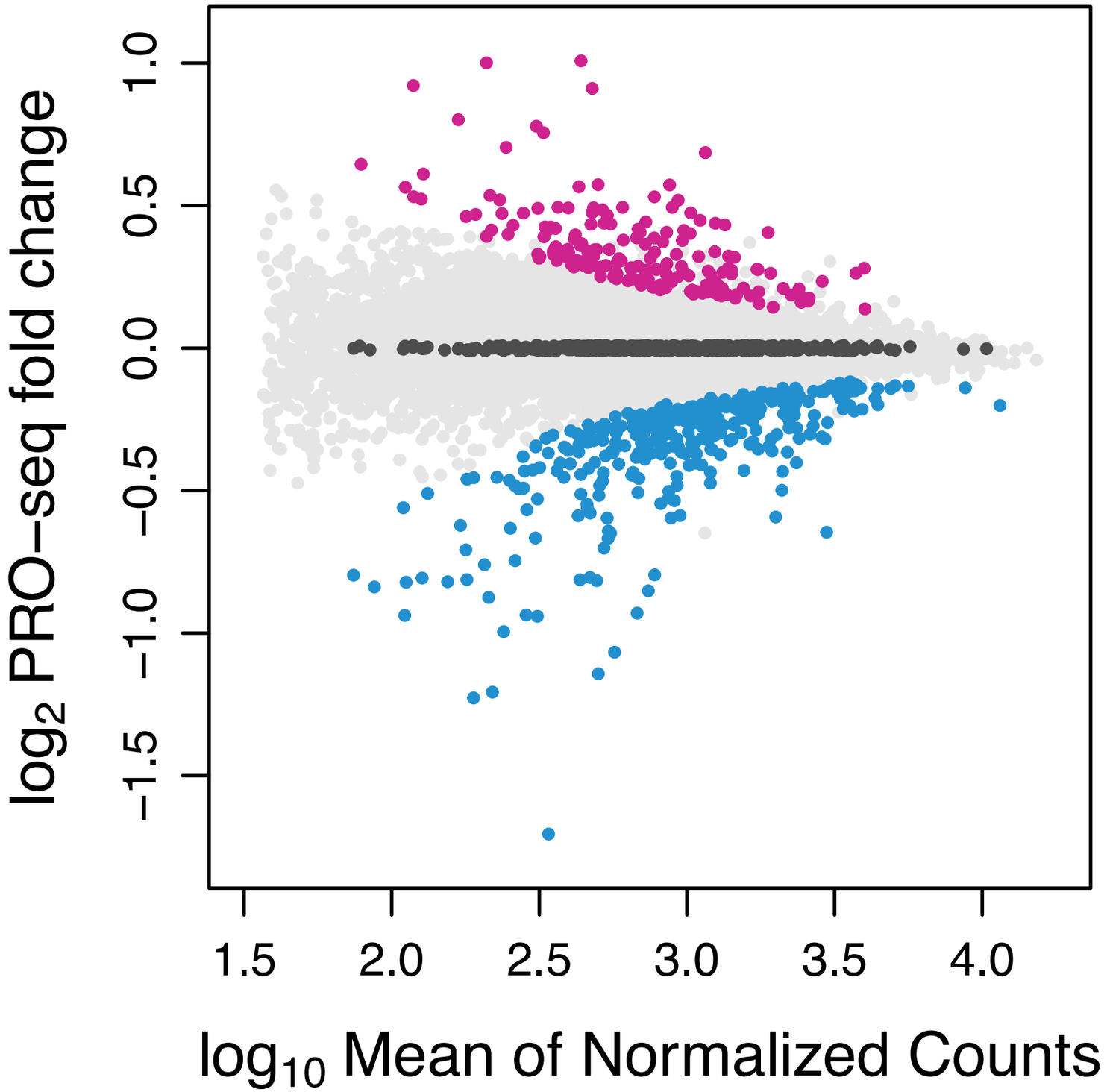
94% of decreased ATAC peaks overlap ZNF143; ZNF143 degradation leads to decreased accessibility



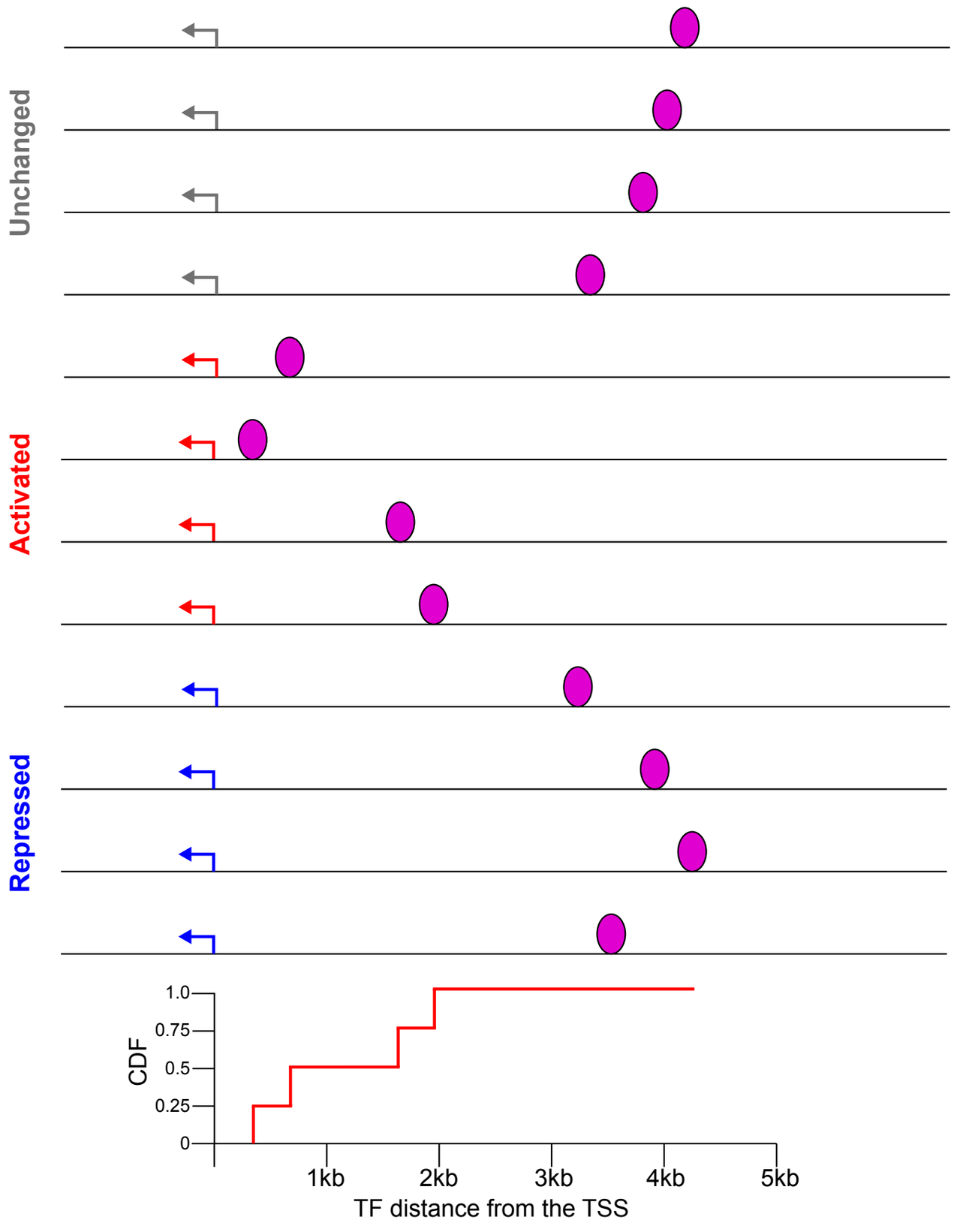
Use PRO-seq to quantify changes in gene expression



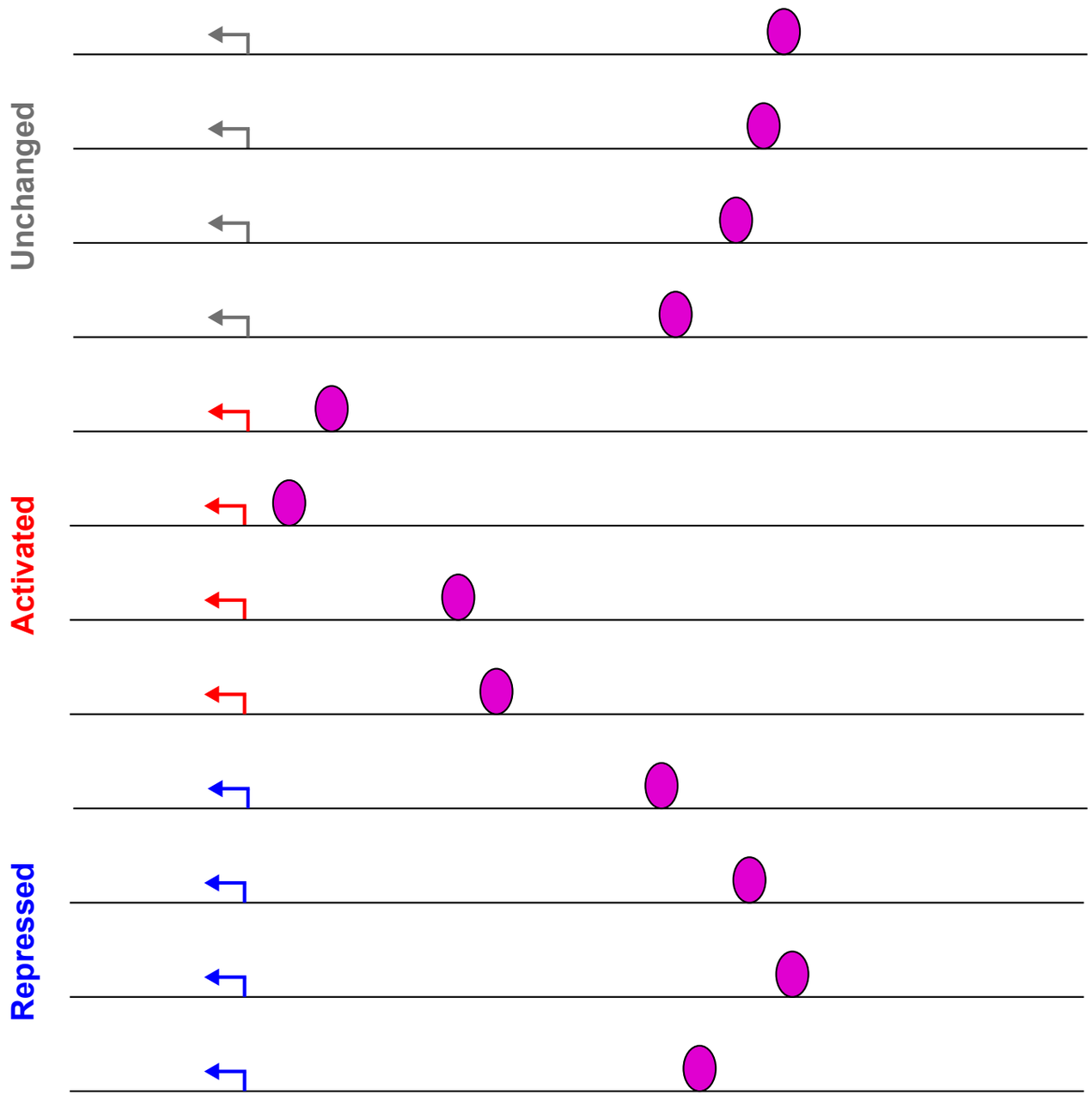
Gene expression goes up and down after ZNF143 degradation



Interpreting TF binding site/TSS cumulative distributions

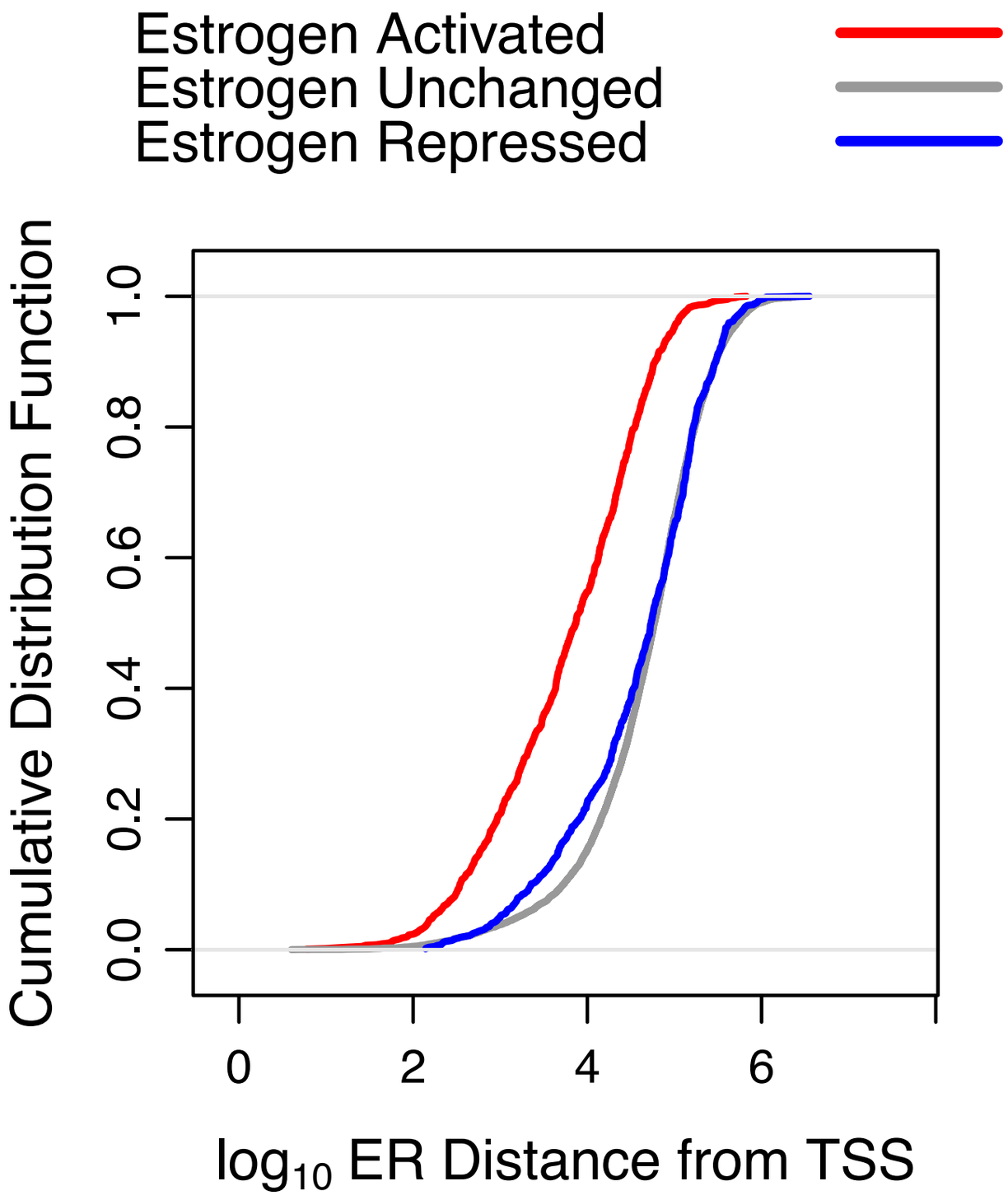


Left shifted CDF indicates that the TF regulates that gene set

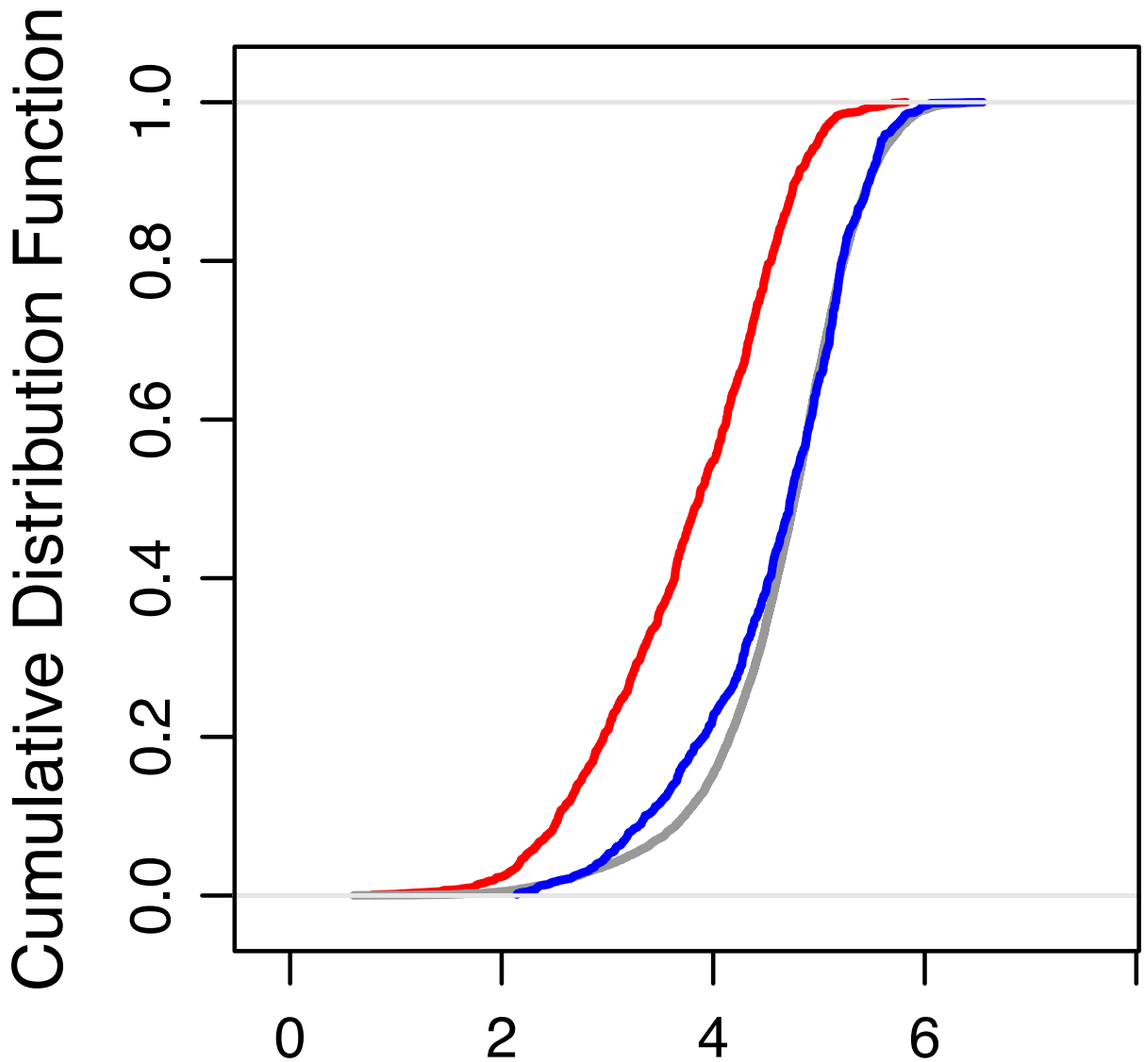


Left-shift indicates that the activated genes tend to be closer to pink TF binding sites.

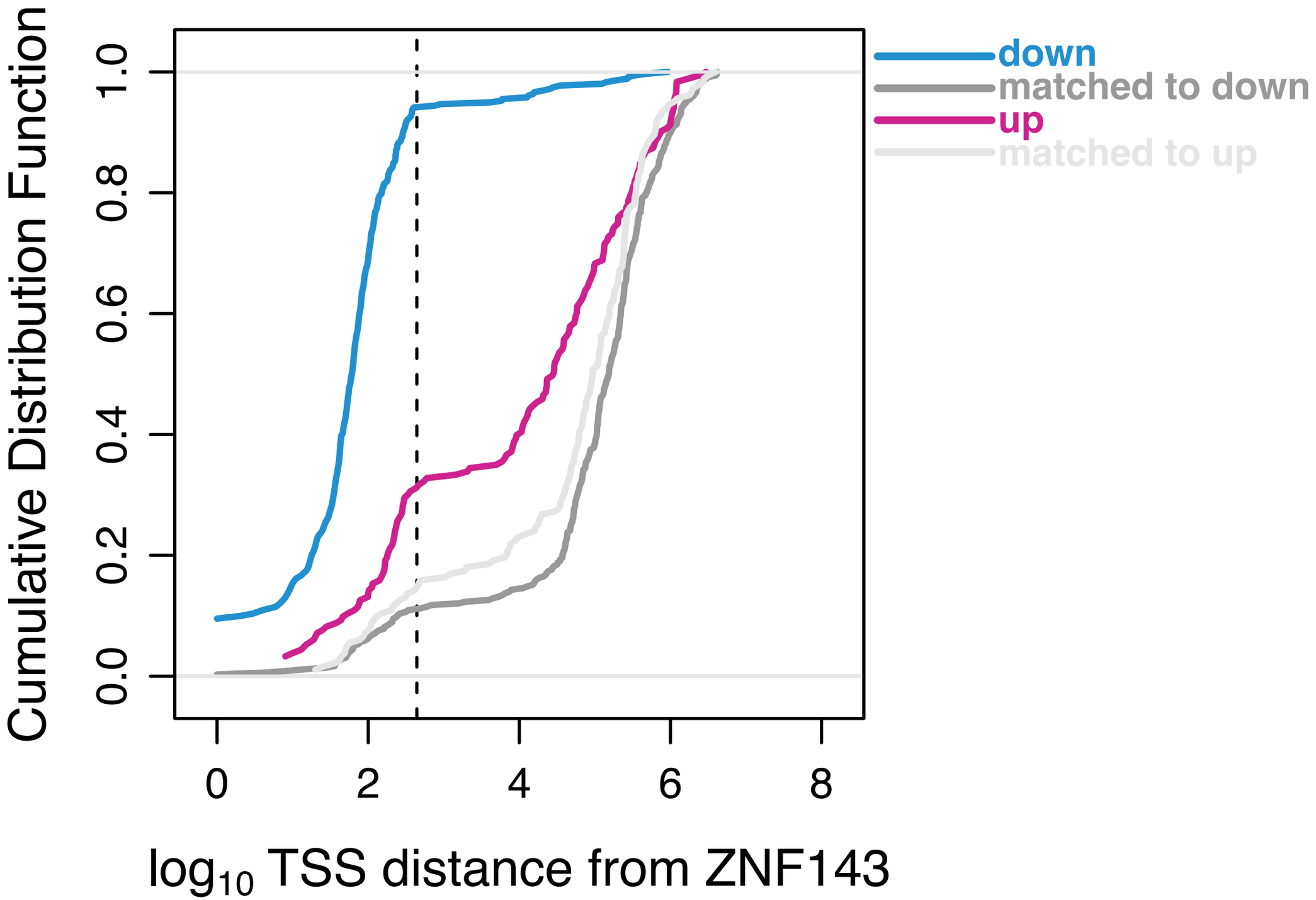
Immediate estrogen activated genes are close to inducible Estrogen Receptor binding; ER is an activator



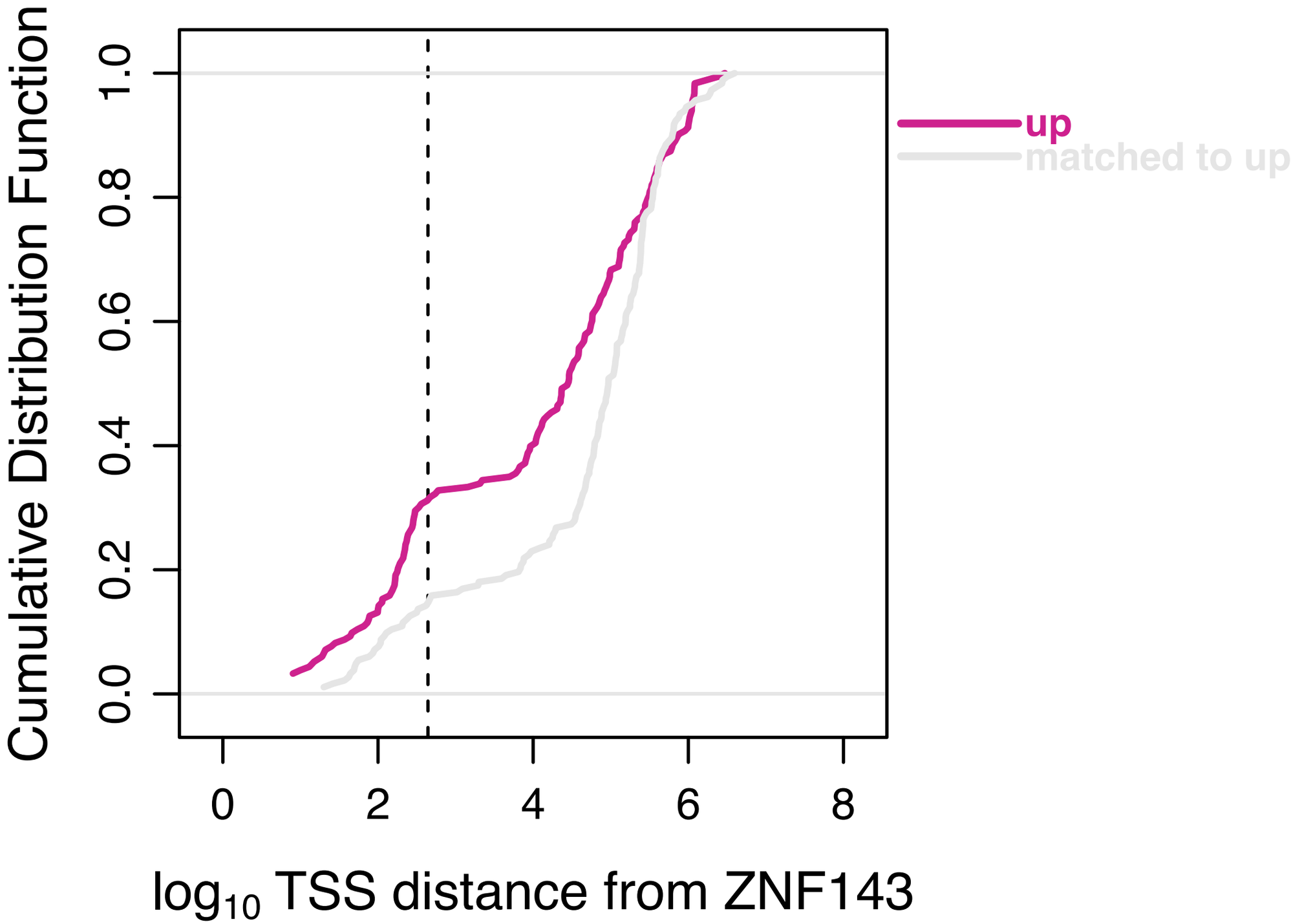
The shift left of either activated or repressed genes
is what I observed/confirmed previously for HSF, ER, GR, and AR
(not both!)



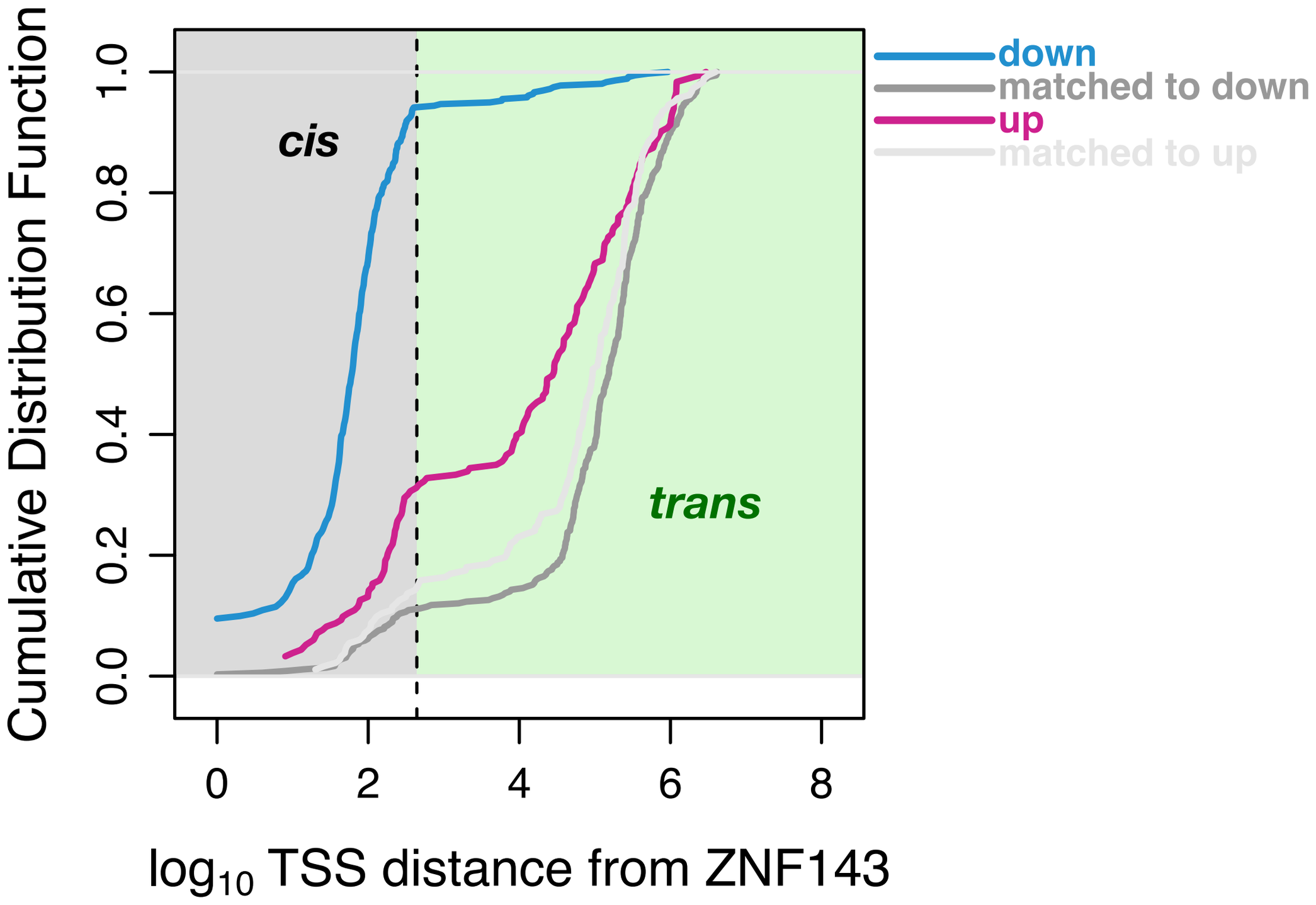
94% of down and 31% of up are within 440 bases of ZNF143



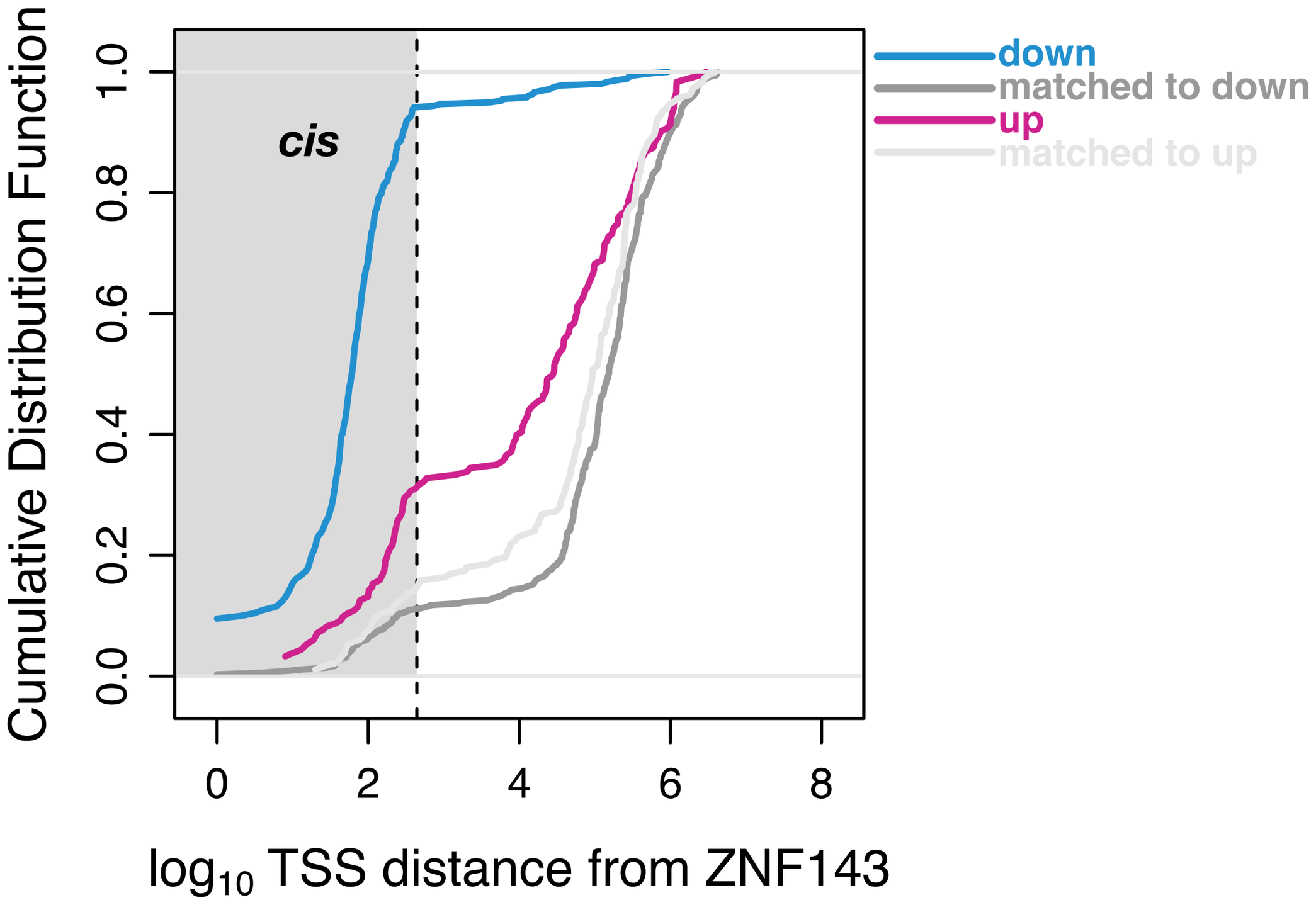
Up genes significantly closer to ZNF143 challenged my TF world view



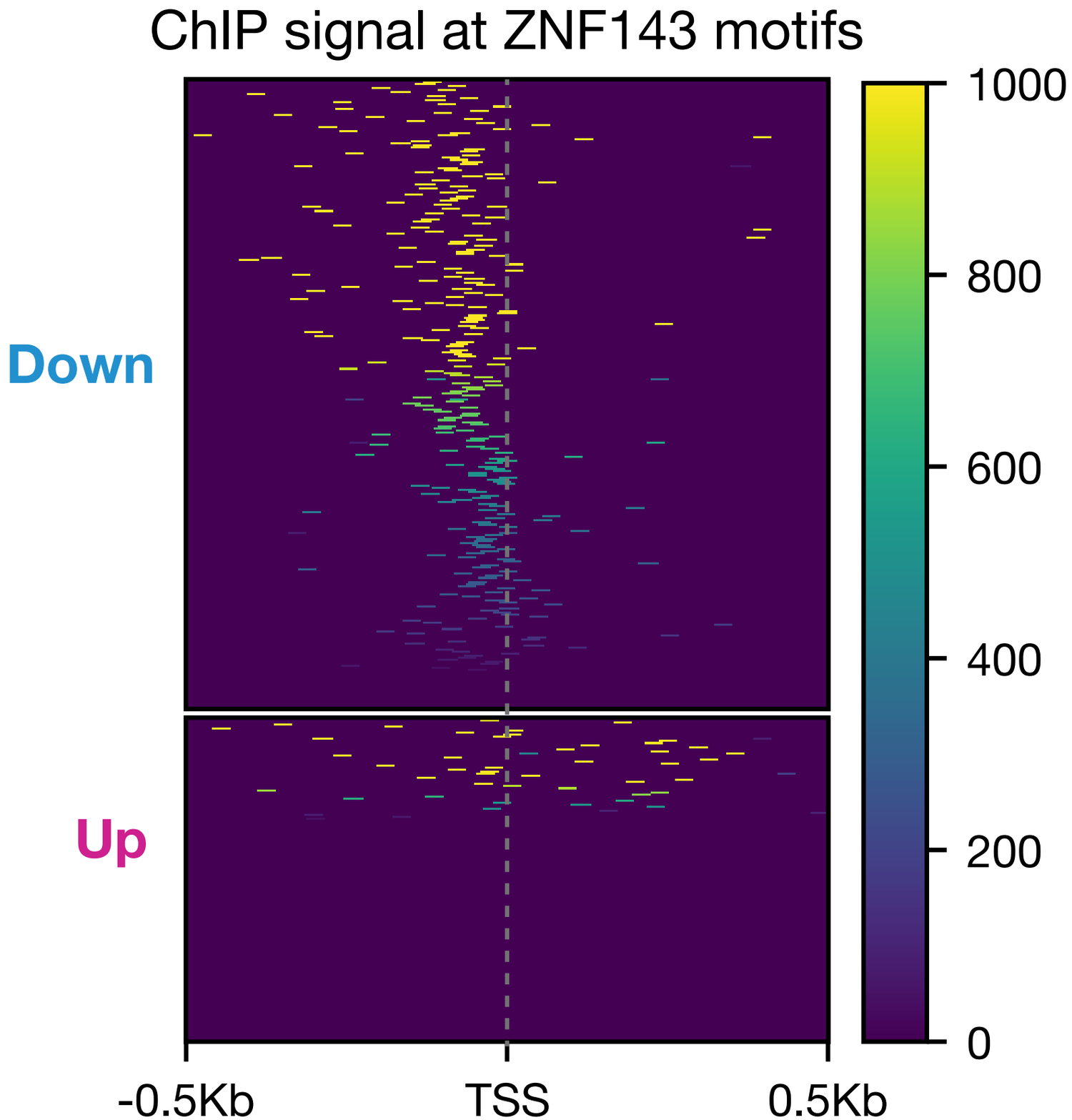
Within 440 bases is considered ZNF143 *cis* targets



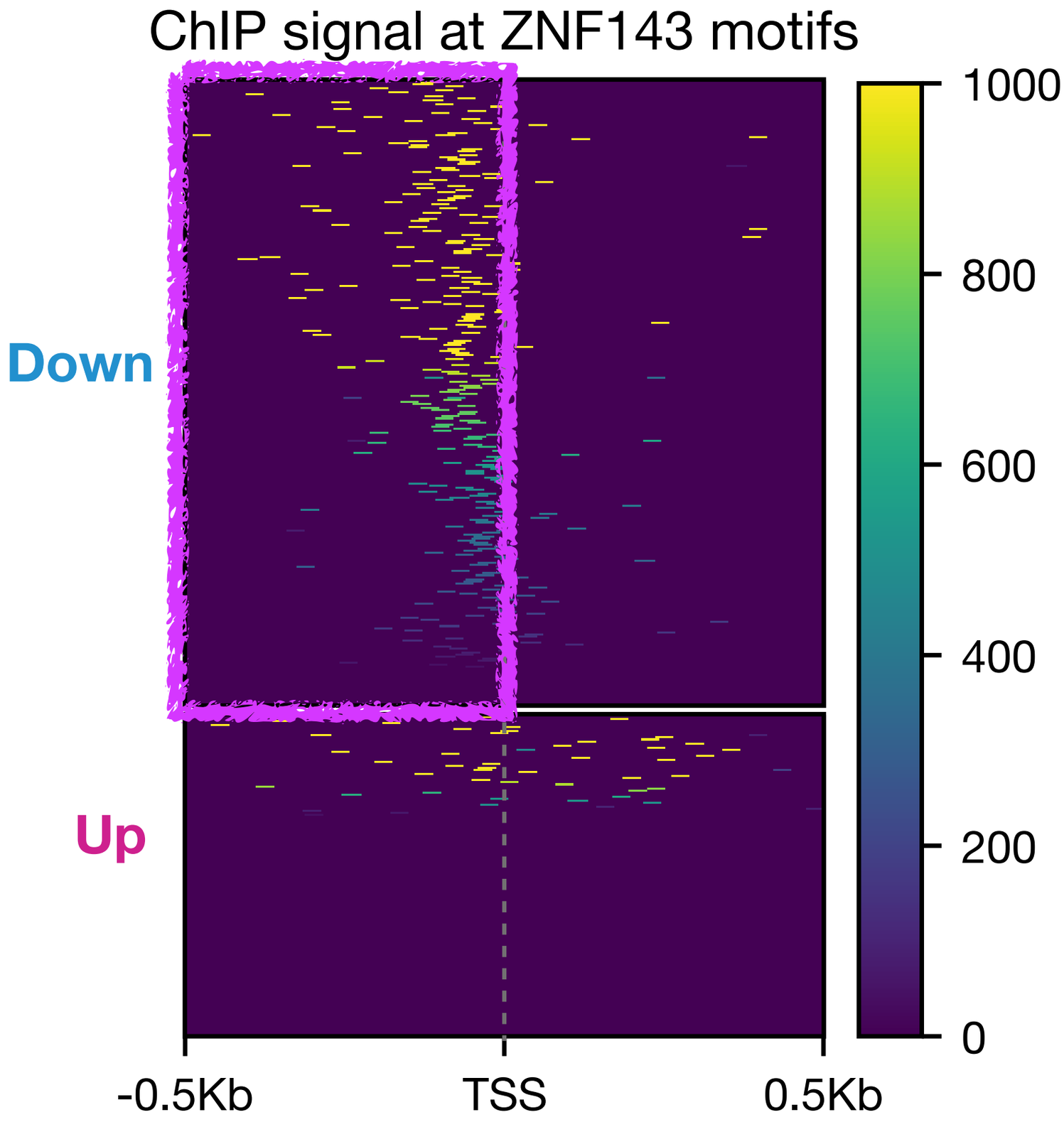
How does ZNF143 activate gene targets?



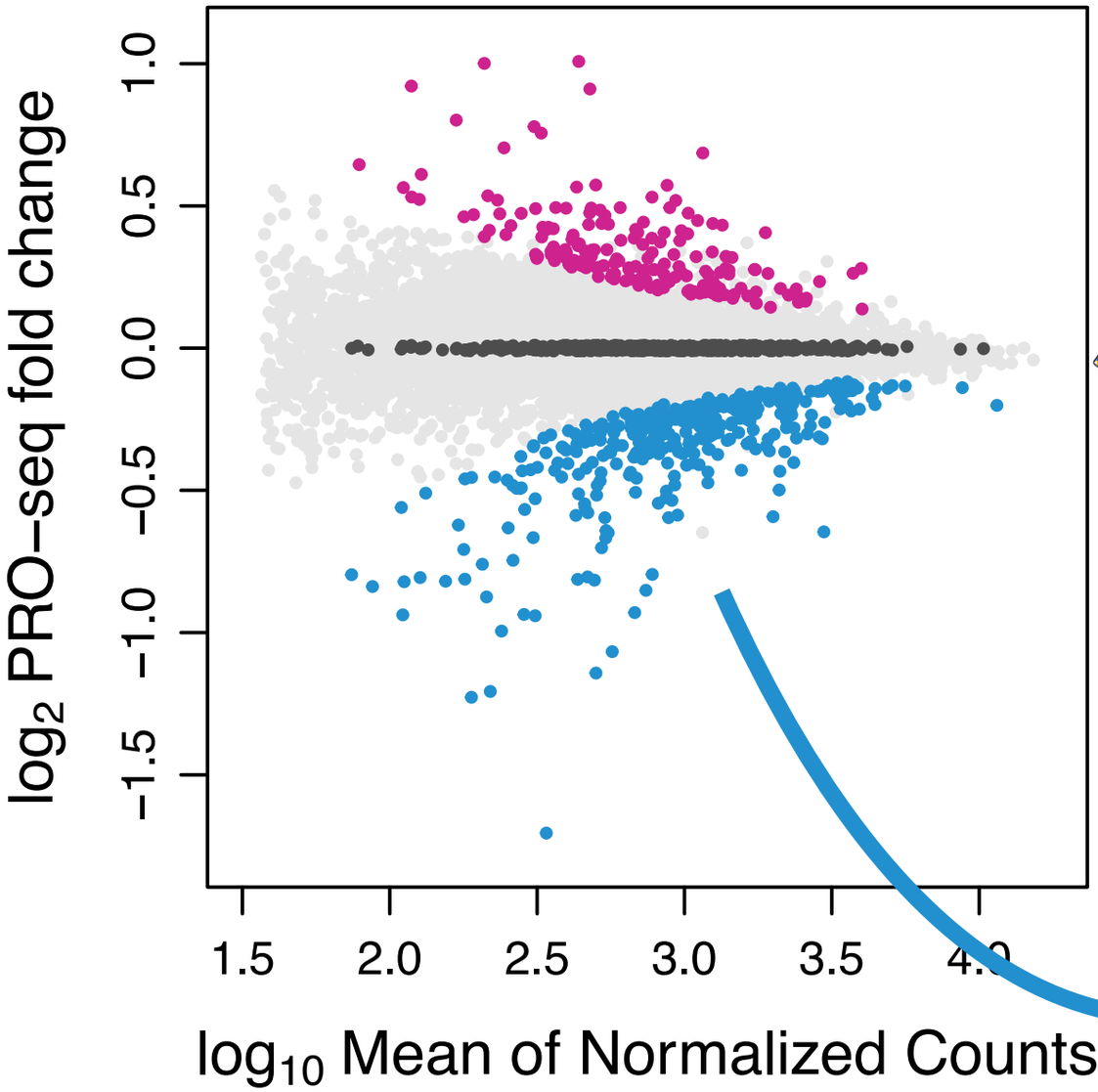
ZNF143 binding sites distribution relative to TSSs

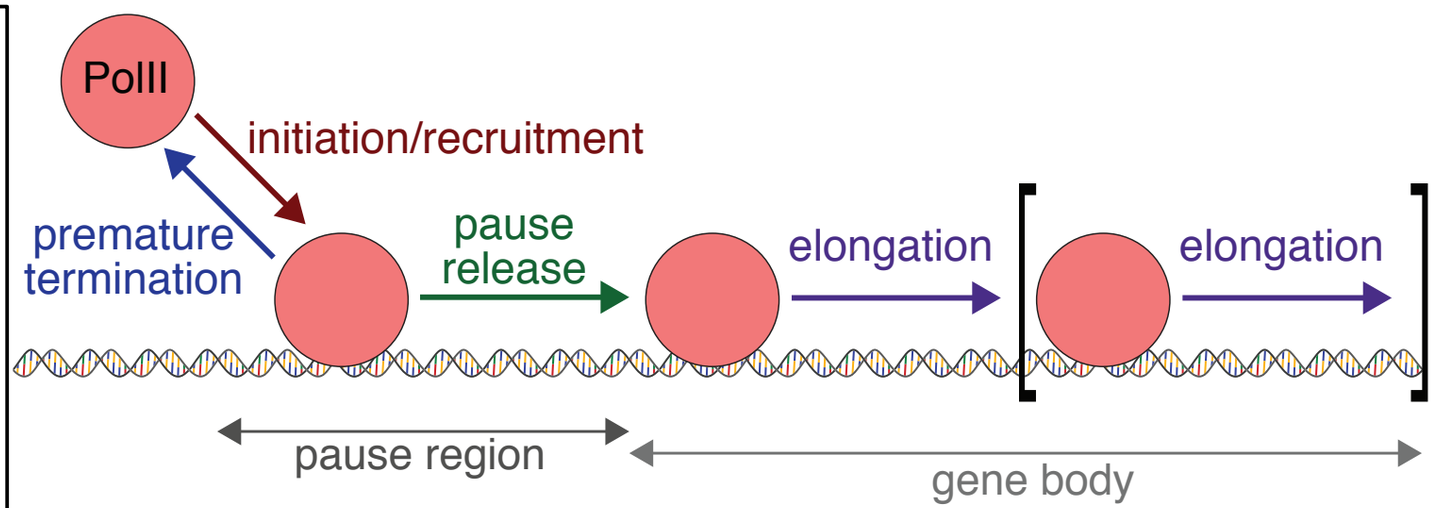


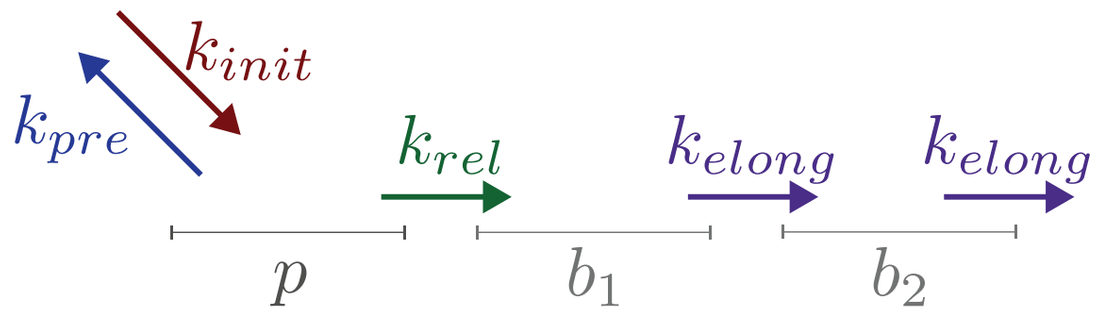
ZNF143 stimulates transcription from the promoter



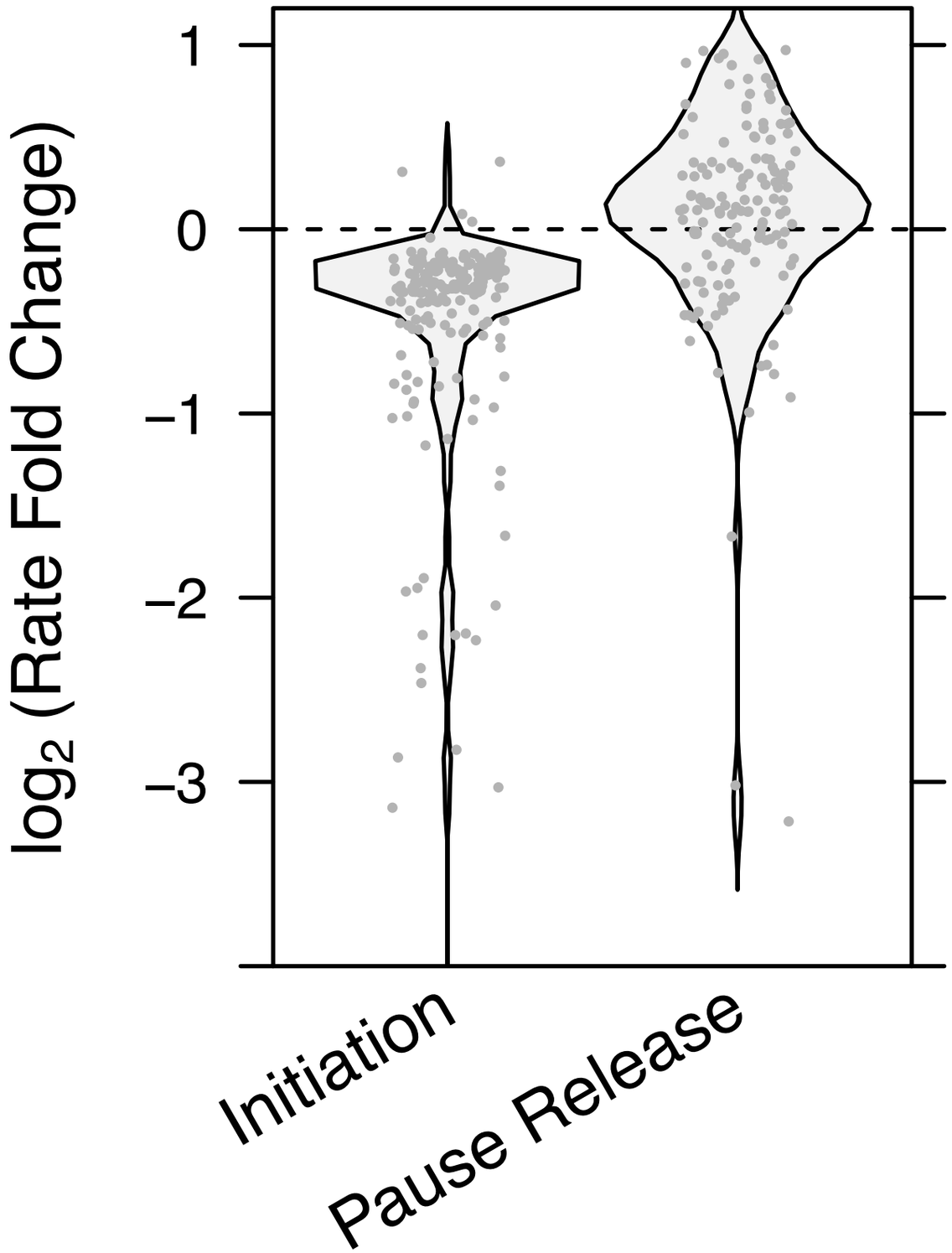
Compartment modeling on each down gene



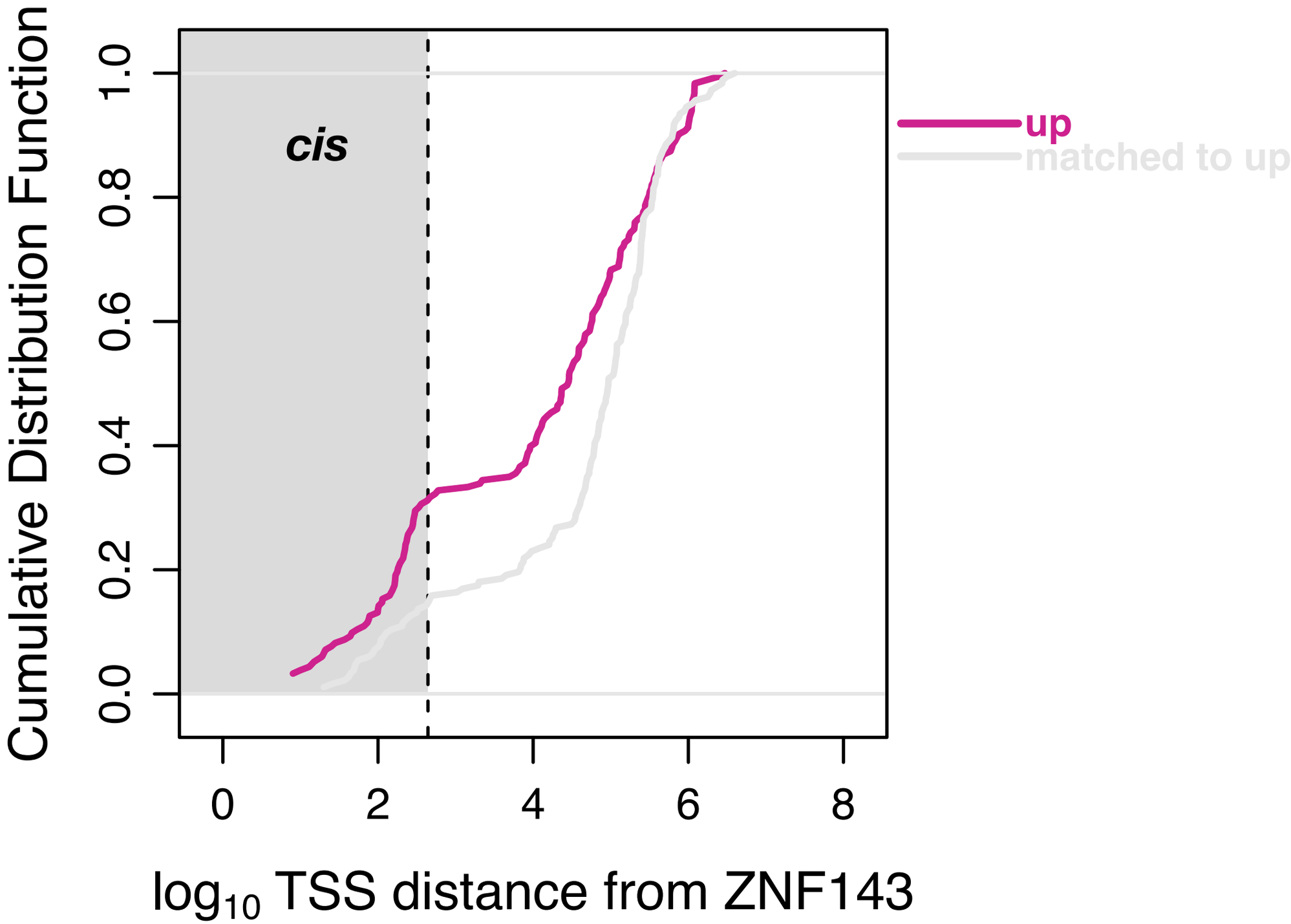




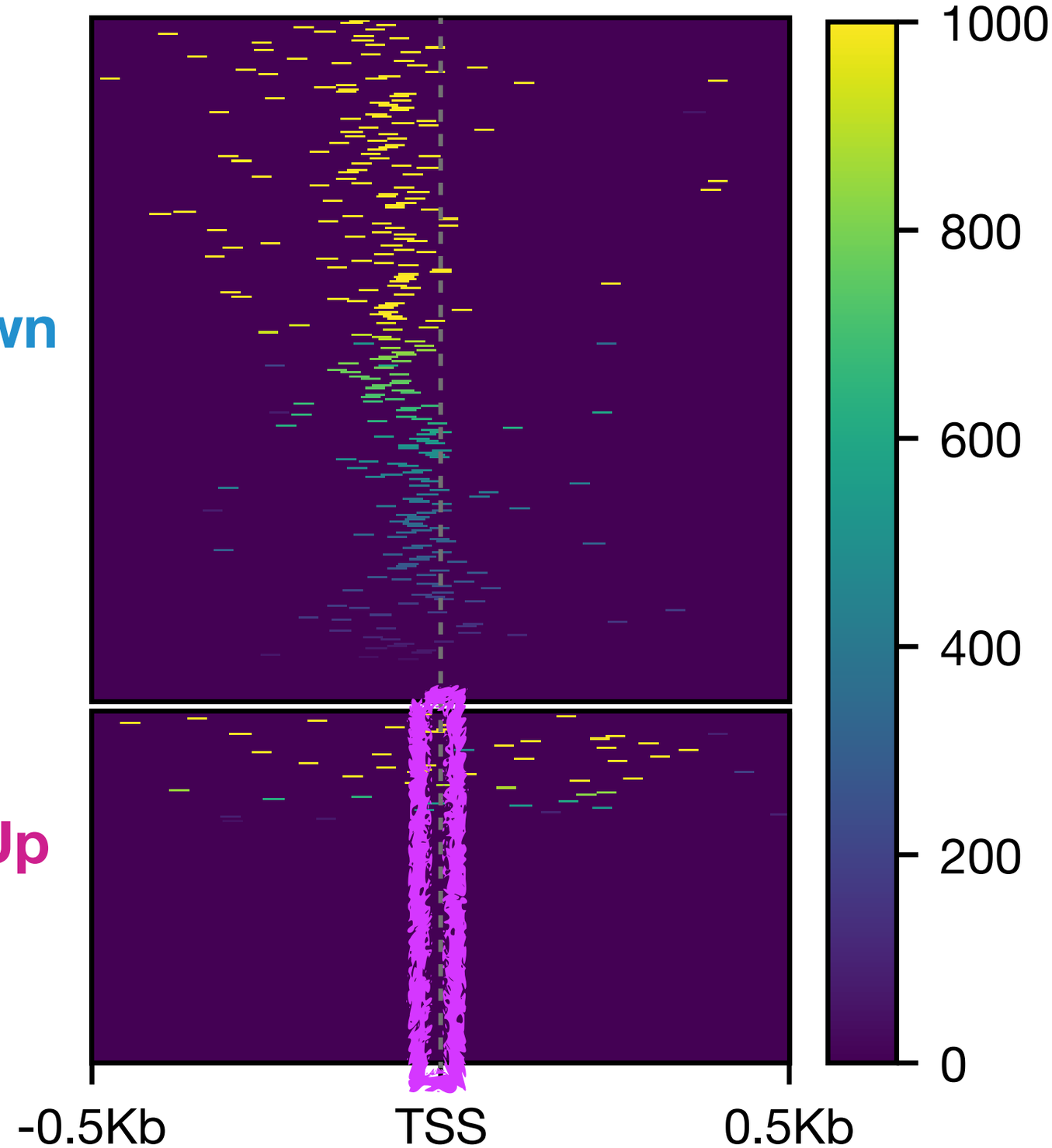
ZNF143 predominantly regulates initiation



How does ZNF143 repress gene targets?

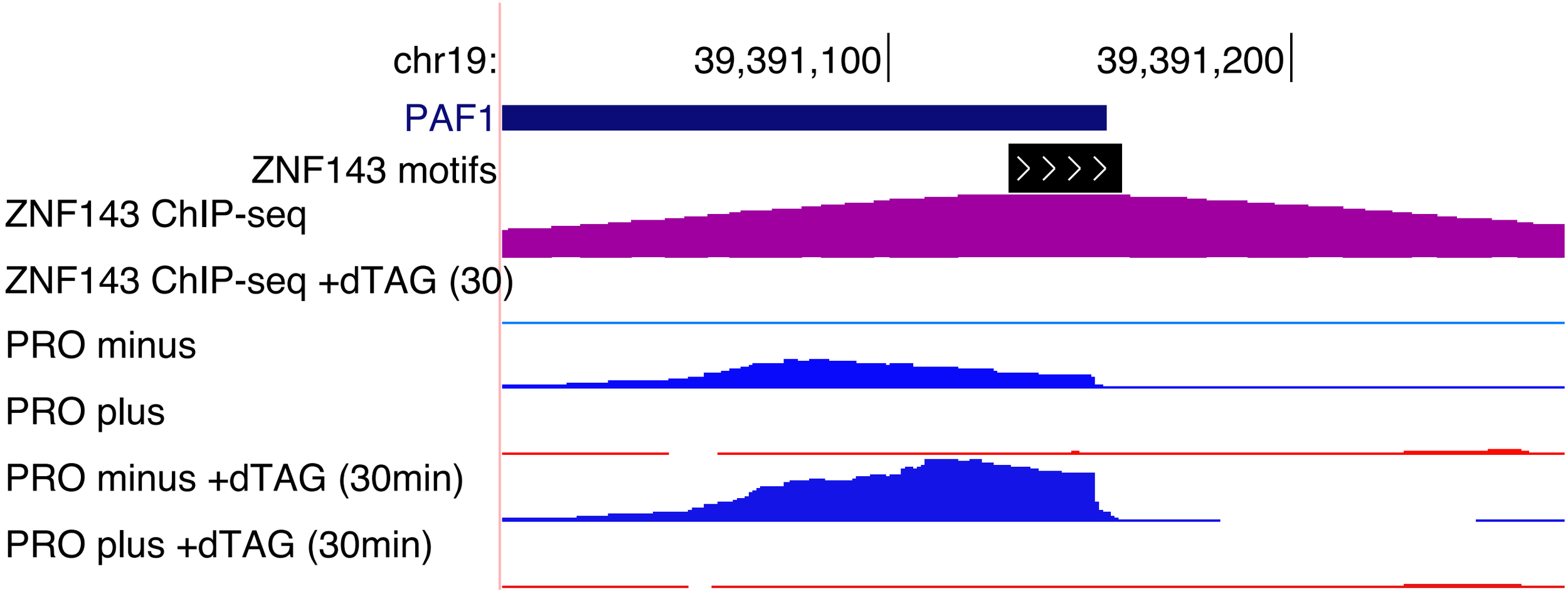


What explains TSS-bound ZNF143 depletion increasing gene expression?

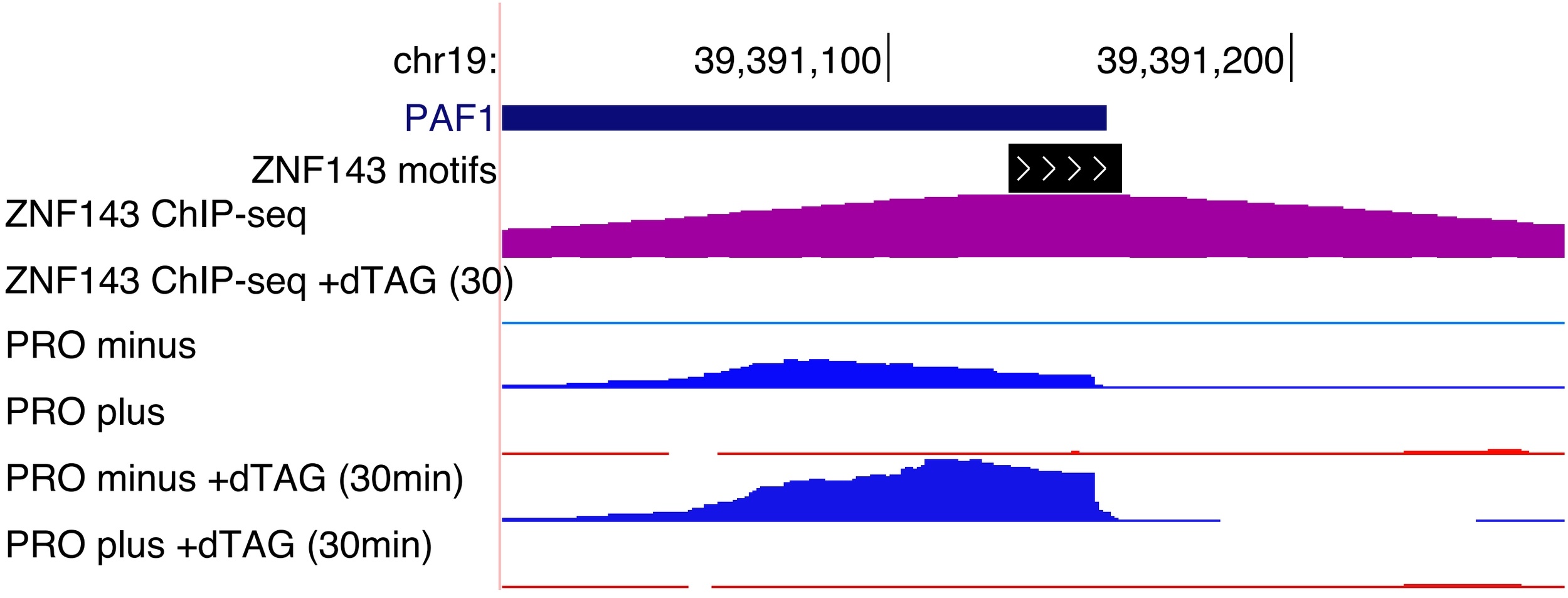


183 up
57 in *cis*
5 over the TSS

ZNF143 binding at the TSS may be an impediment at 5 up genes

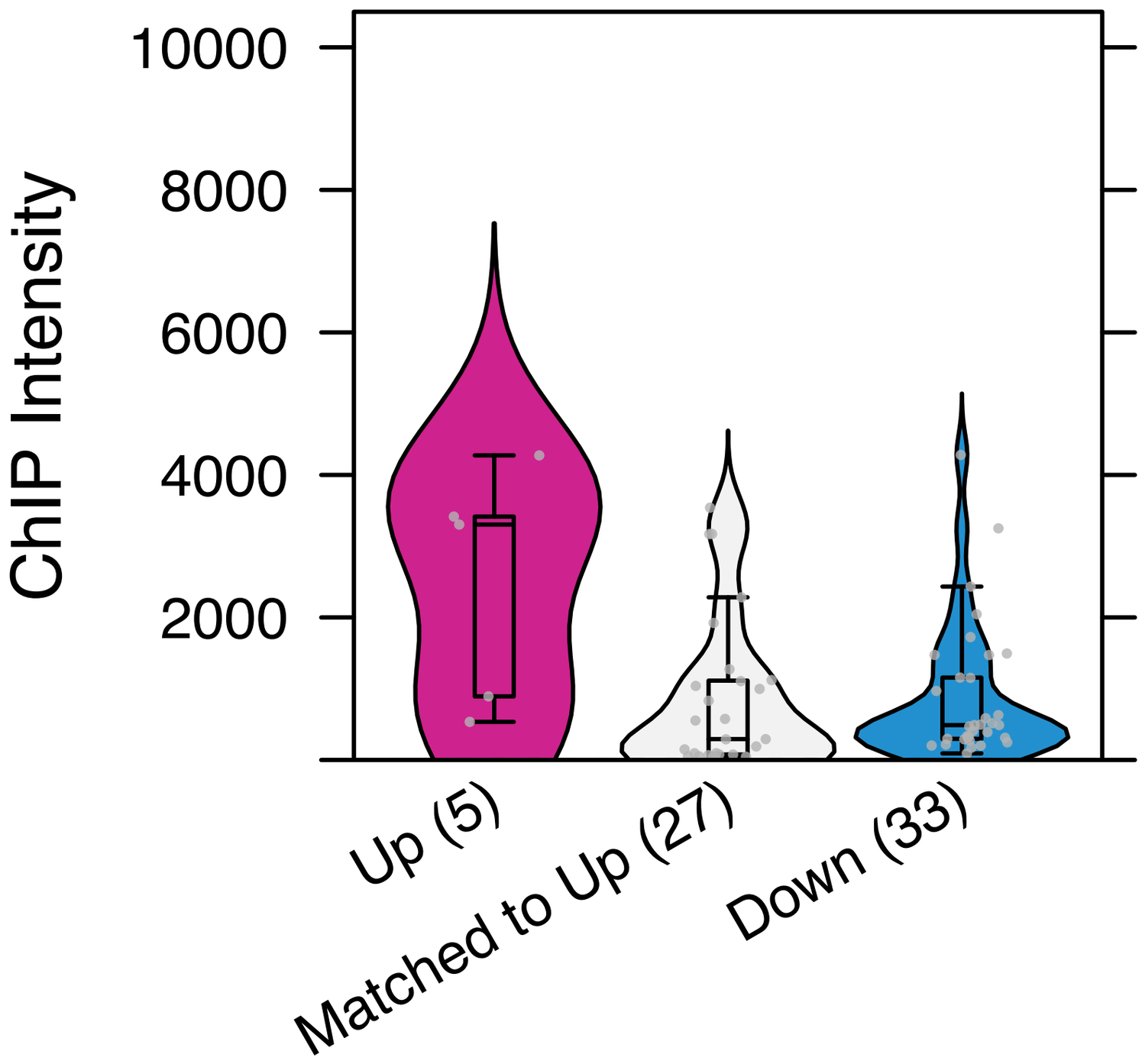


ZNF143 binding at the TSS may be an impediment at 5 up genes

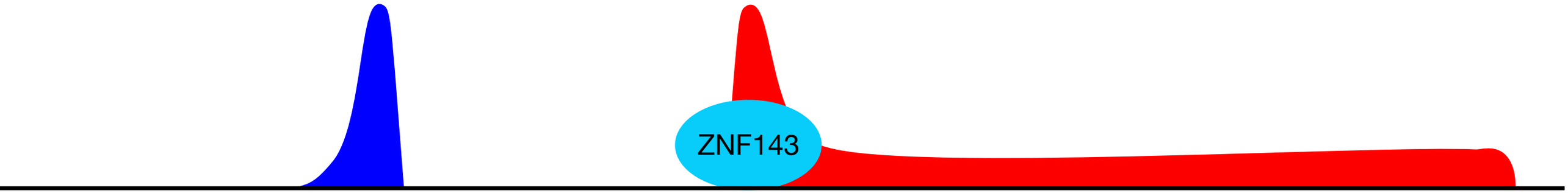


The typical convincing functional experiment:
degrade ZNF143 rapidly and measure expression by PRO at these genes

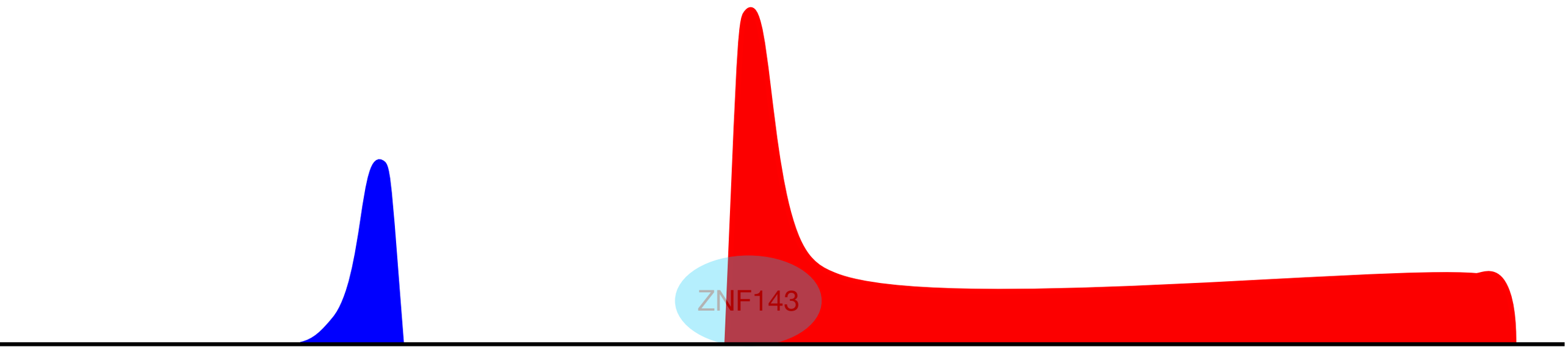
ZNF143 binding is relatively strong at the 5 TSS-bound up genes



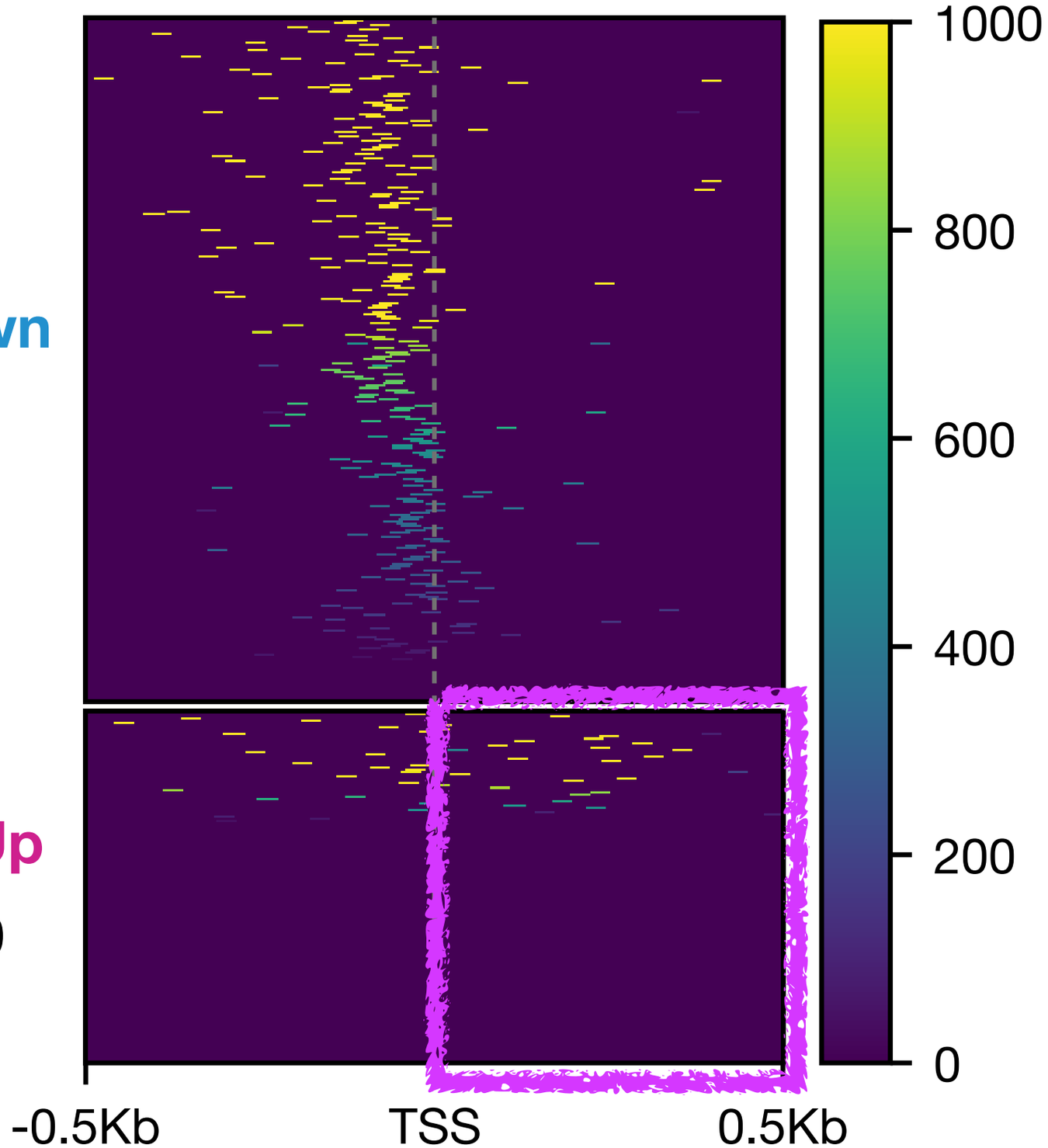
ZNF143 blocks the TSS to attenuate transcription



ZNF143 blocks the TSS to attenuate transcription

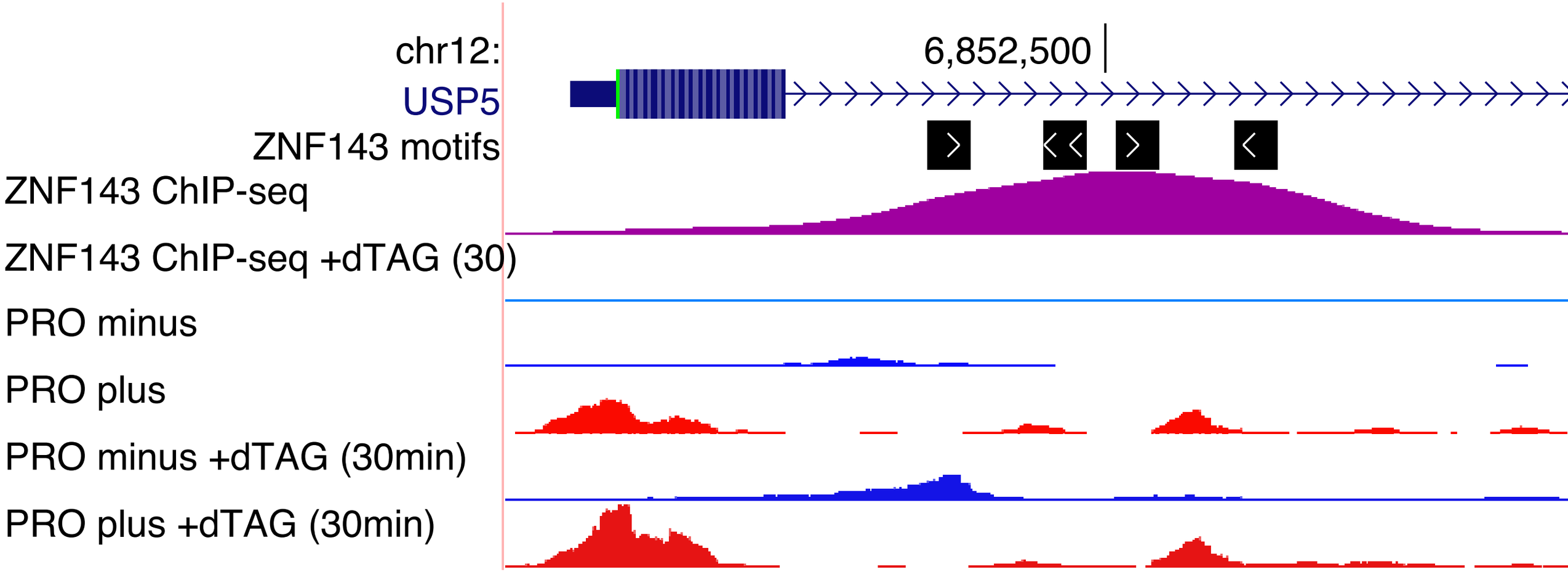


What explains downstream-bound ZNF143 depletion increasing gene expression?

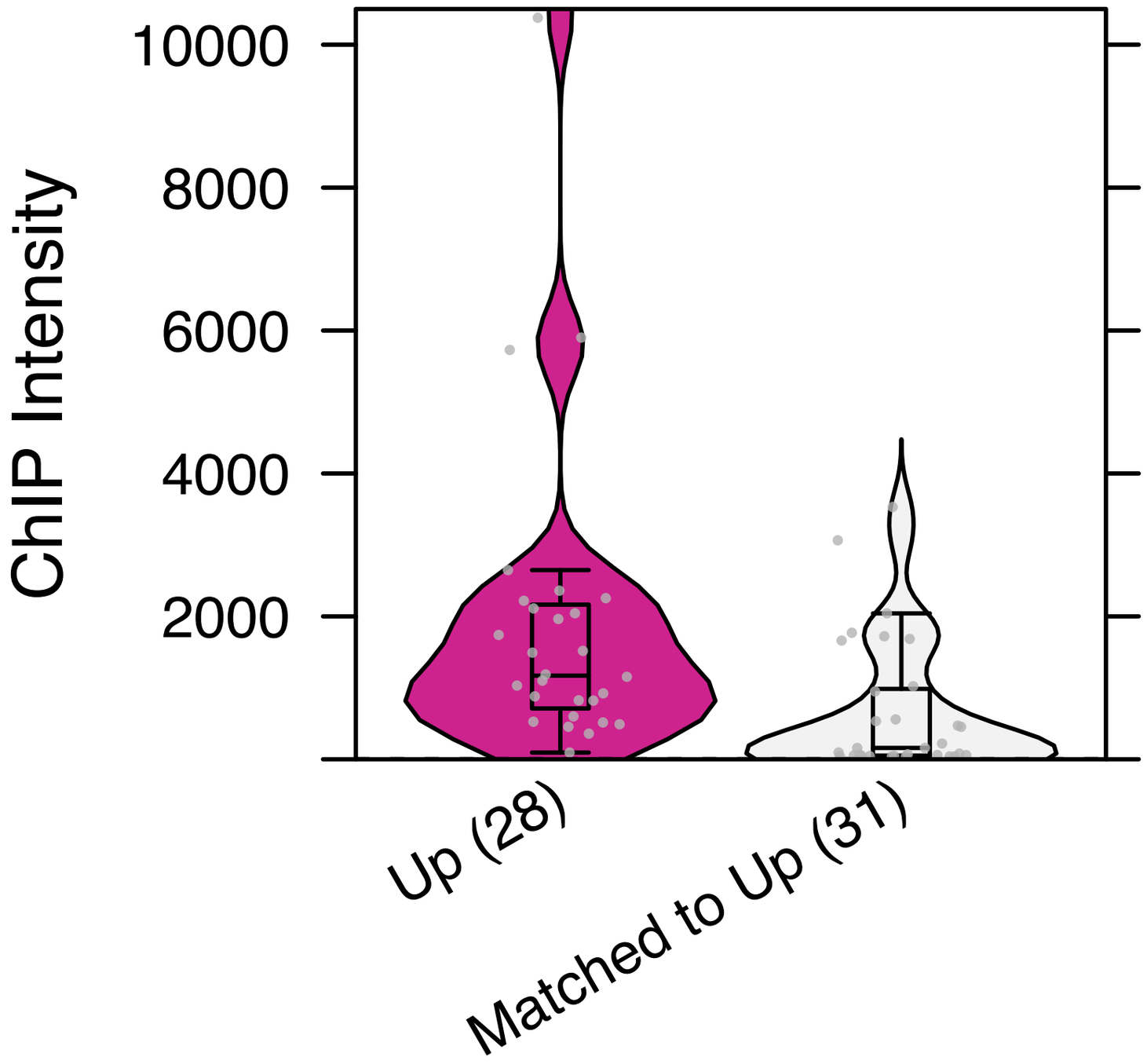


183 up
57 in *cis*
28 genic w/in 440

ZNF143 binding at the strongest ZNF143 peak may act as a roadblock

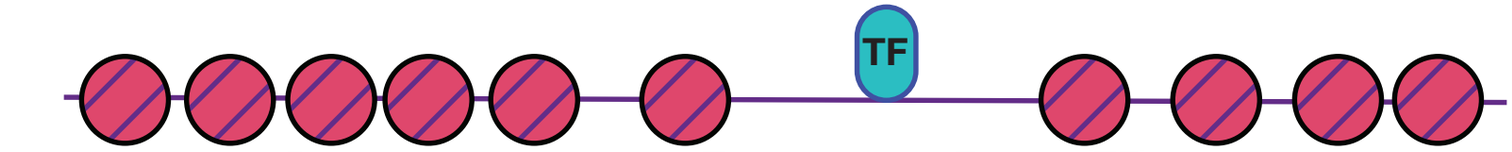


Binding within up genes is relatively strong and may act as a road block

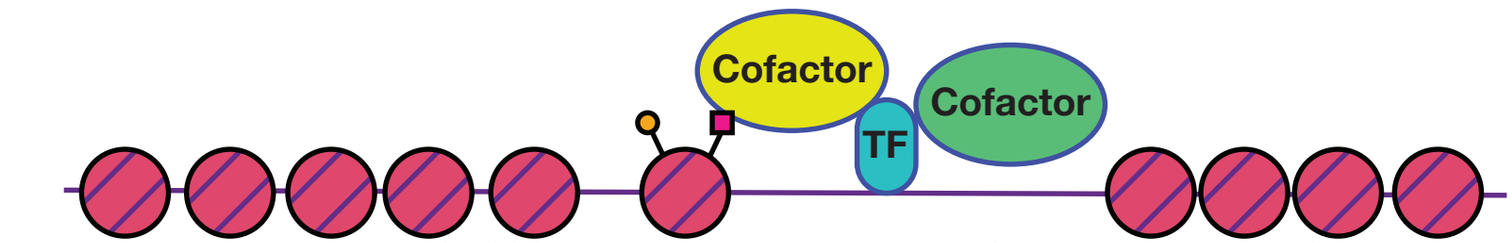


Recall: Bidirectional Transcription is a hallmark of regulatory regions

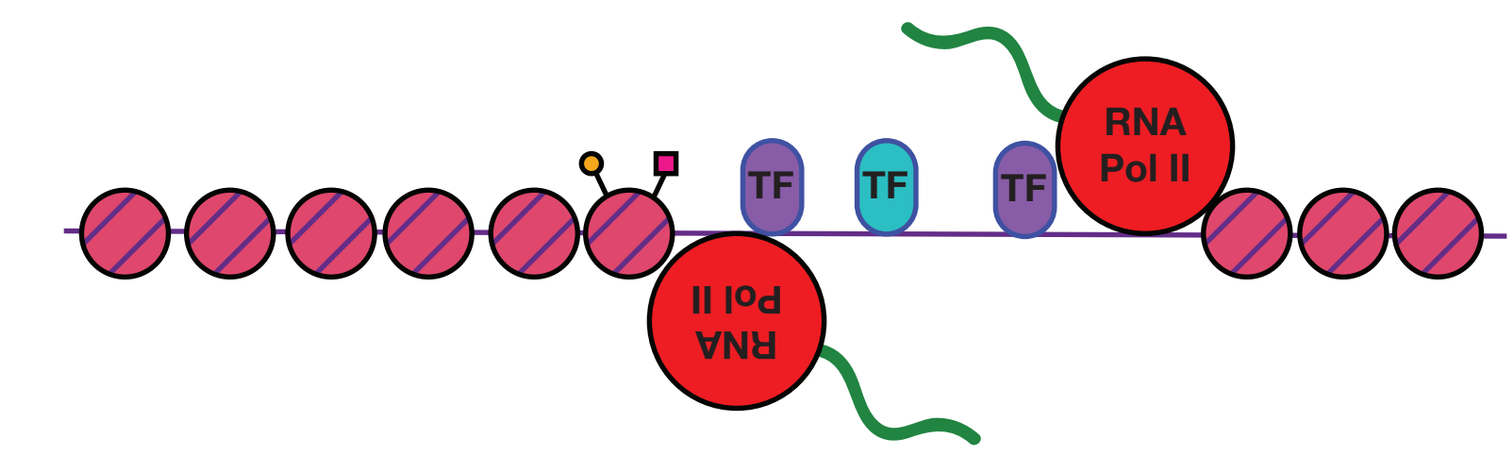
DNA sequence influences nucleosome positions/dynamics and chromatin compaction



DNA sequence directs Transcription Factor (TF) binding

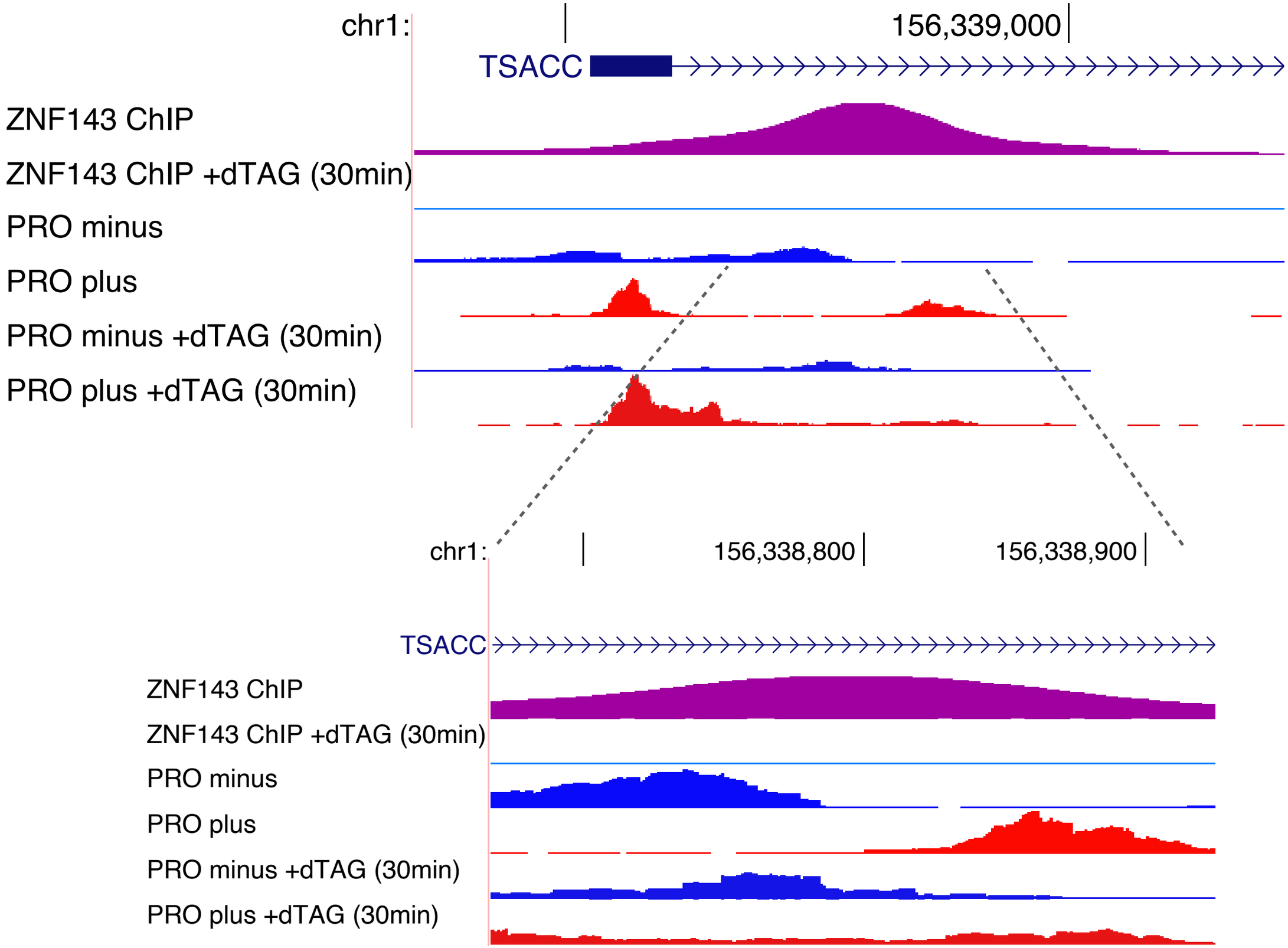


sequence-specific TFs recruit chromatin modifying/remodeling factors

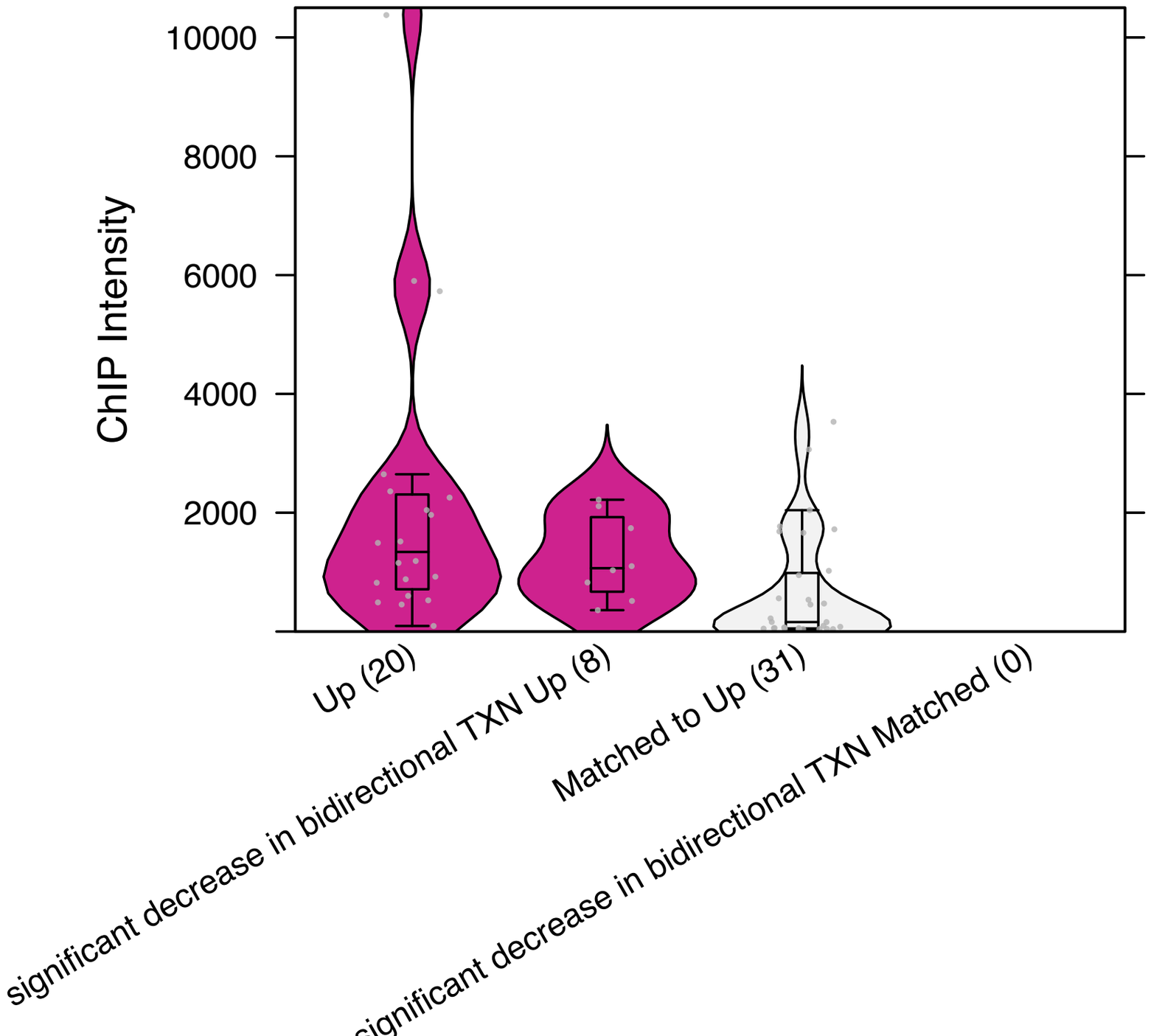


sequence-specific TFs recruit initiation factors and RNA Polymerase

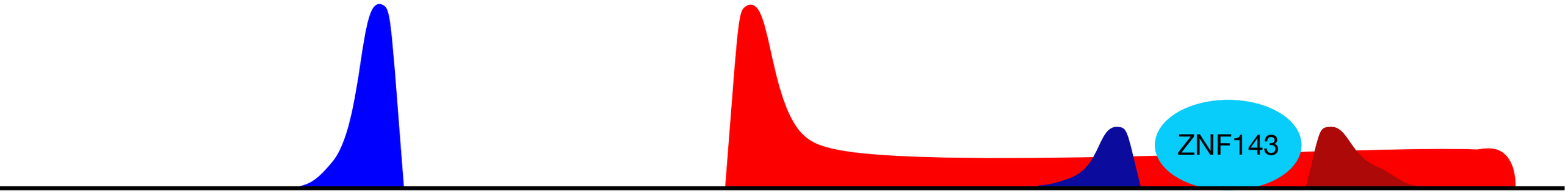
Binding within genes stimulates bidirectional transcription that may act as a road block



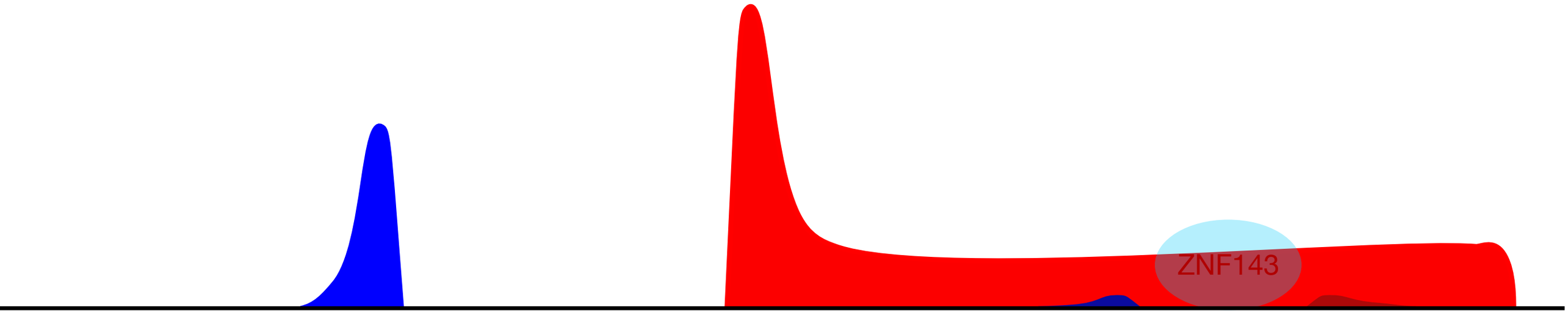
Bidirectional transcription within genes may act as a road block



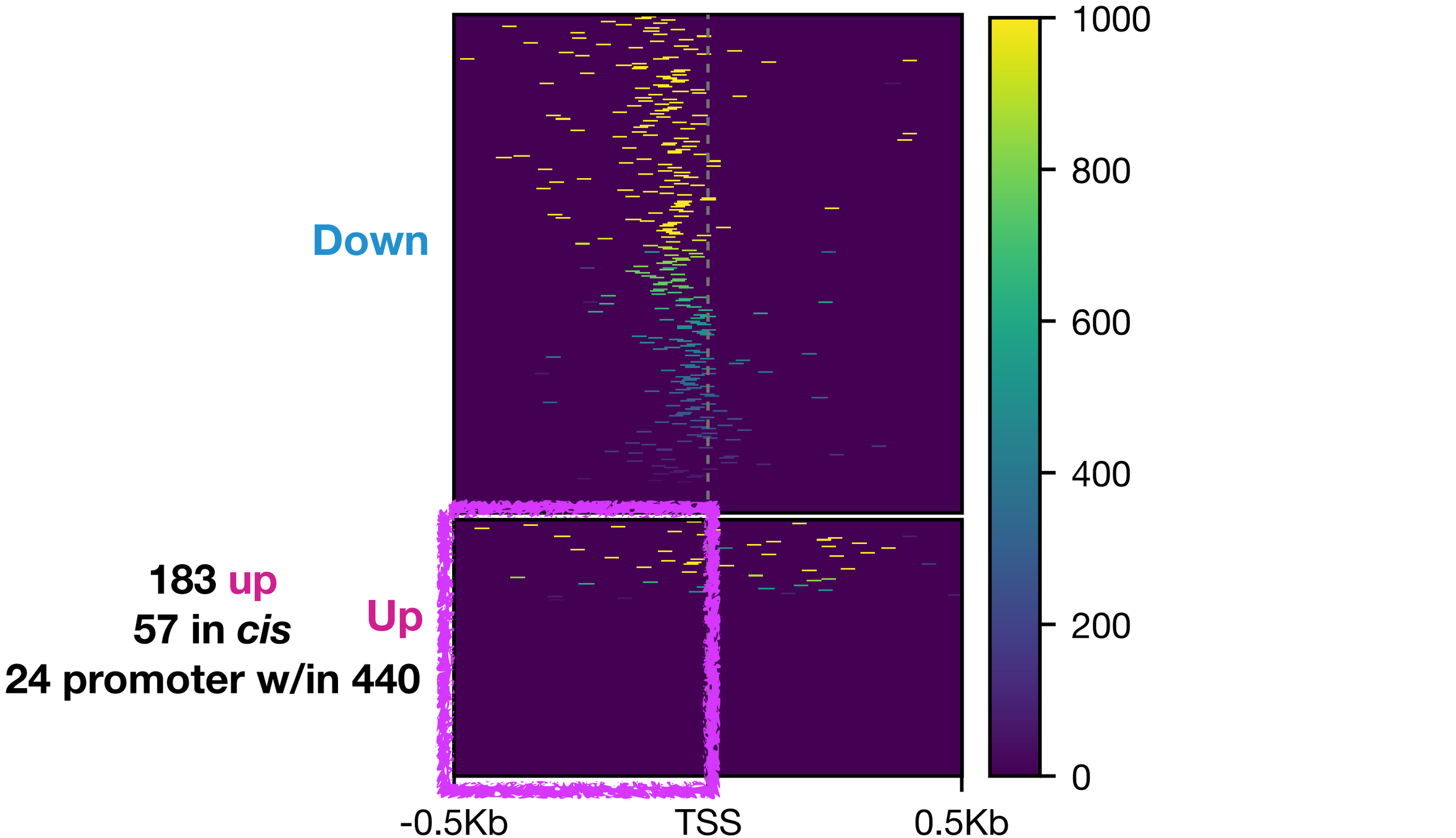
ZNF143 and/or ZNF143-stimulated bidirectional TXN may act as physical barriers



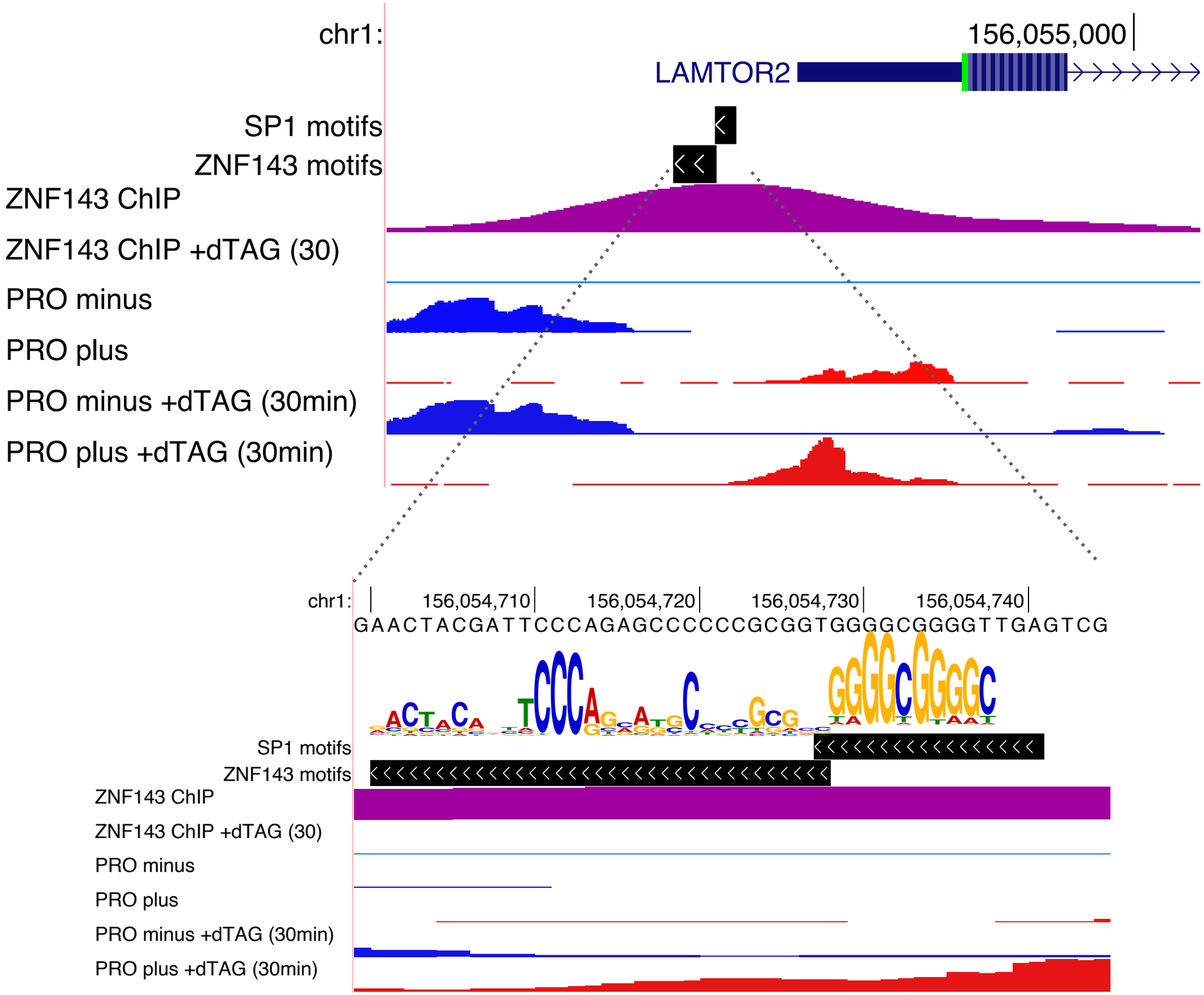
ZNF143 and/or ZNF143-stimulated bidirectional TXN may act as physical barriers



What explains upstream-bound ZNF143 depletion increasing gene expression?



SP motifs overlap promoter ZNF143 sites at up genes



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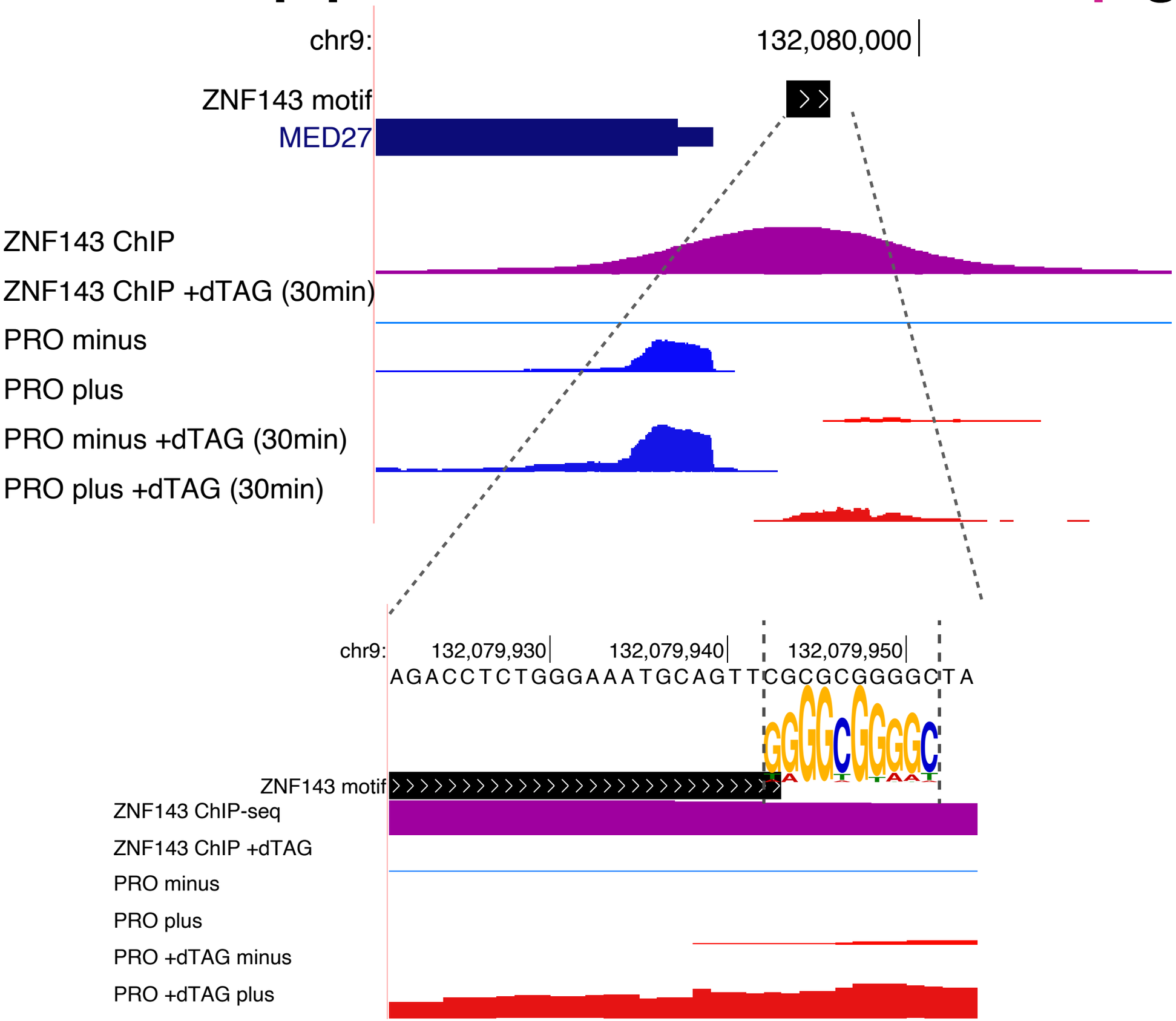
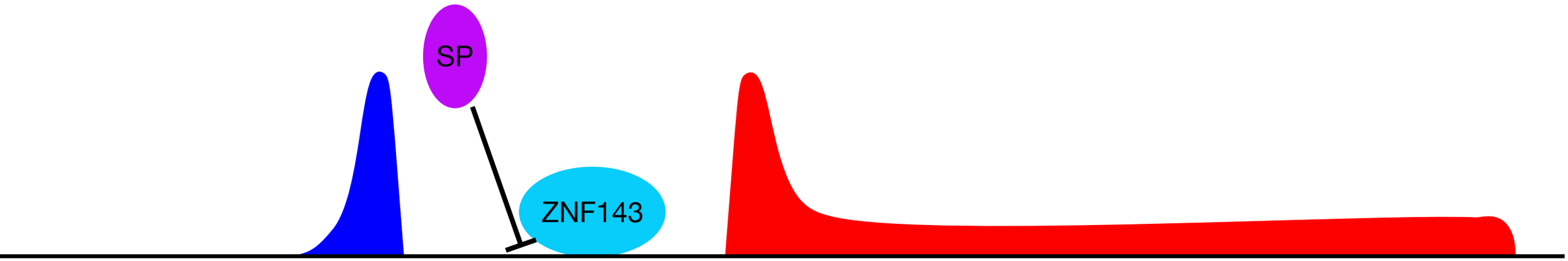


SP motifs overlap promoter ZNF143 sites at 46% (11/24) of up genes

ZNF143 may outcompete better activators such as SP/KLF factors for promoter access



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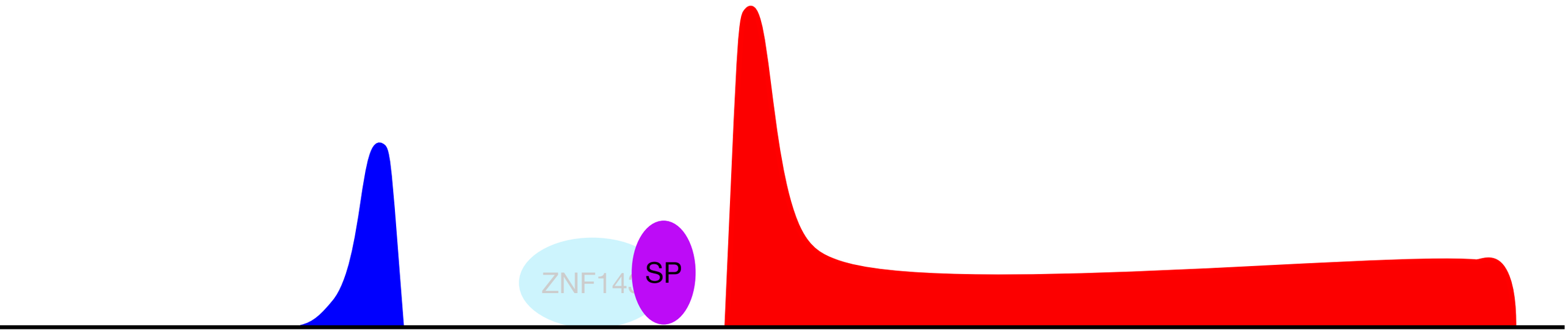
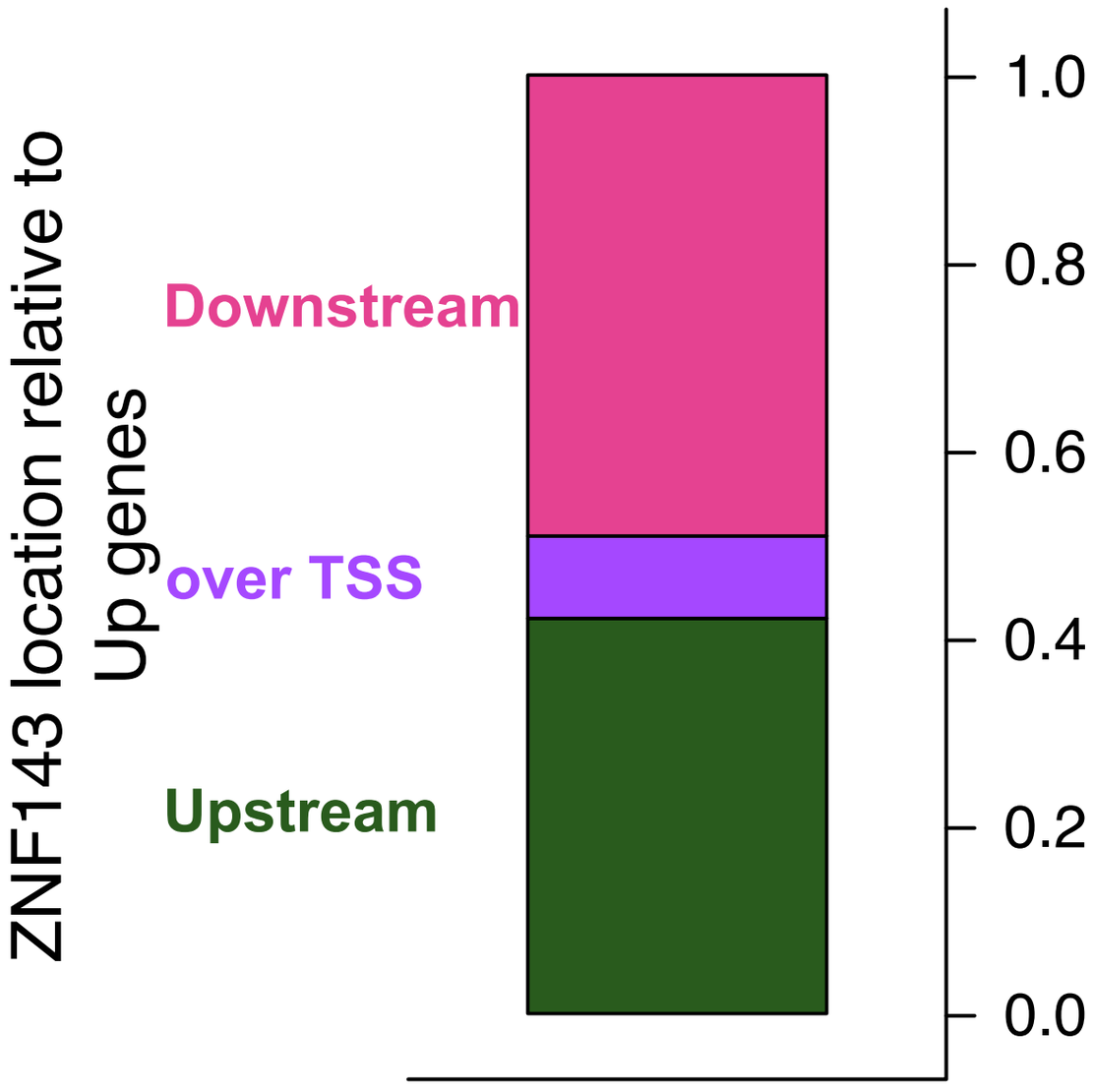


Proposed repressive mechanisms of an Activator



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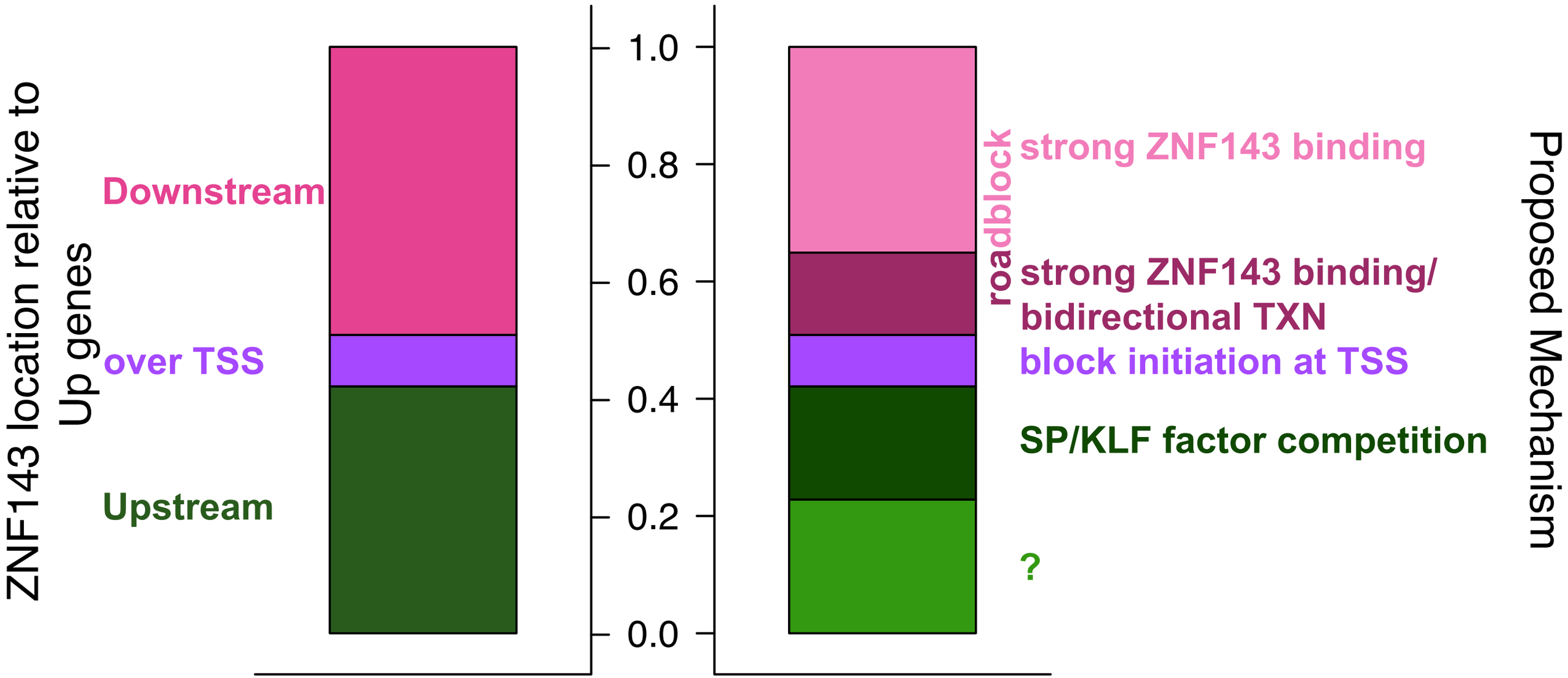

ZNF143 Conclusions/Future

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    1. Bind DNA

    2. Stimulate RNA polymerase initiation

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    1. Bind DNA

    2. Stimulate RNA polymerase initiation

- The molecular functions do not change when ZNF143 represses target genes.

- To do:

    1. ChIP-seq SP/KLF factors to test redistribution

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Arun Dutta (adipogenesis)

Jinhong Dong (ZNF143 degron)

Rudradeep Mukherjee (compartment modeling & Twist1/2)

Tommy Scott (Estrogen response & TRPS1 degradation)

Siyu Sun (GATA3 degron & Estrogen response)

UCONN
SCHOOL OF MEDICINE

National Institute
of General Medical
Sciences

National **Heart**
Lung and Blood Institute

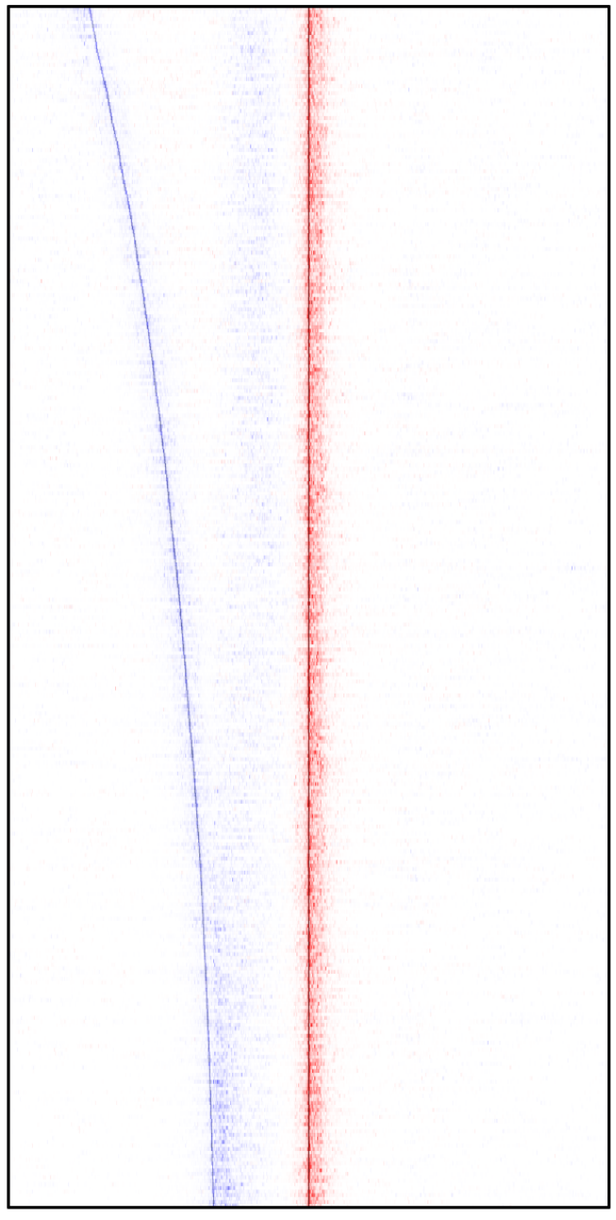
SP motifs are enriched within promoters

5´ end of PRO-seq reads

Antisense

Sense

Bidirectional Promoters



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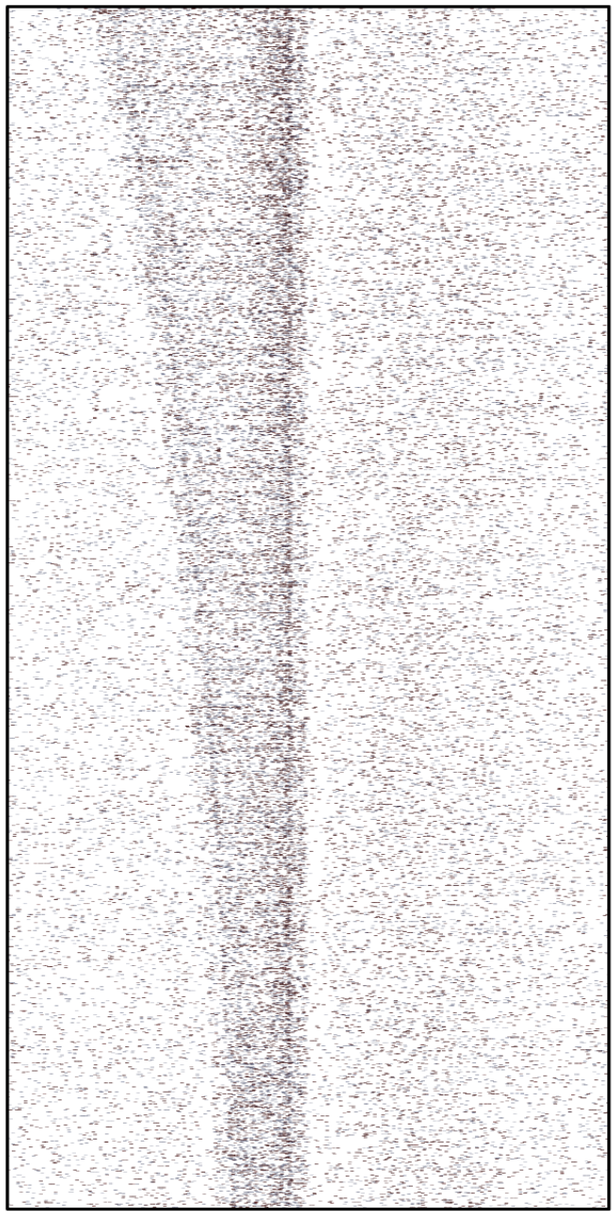
SP1 motif



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