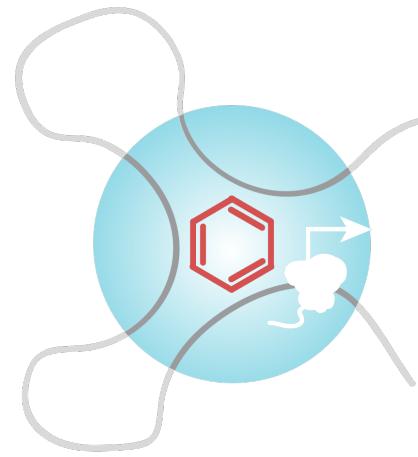


Epigenetics and 3D chromatin

Berkley E. Gryder, Ph.D.

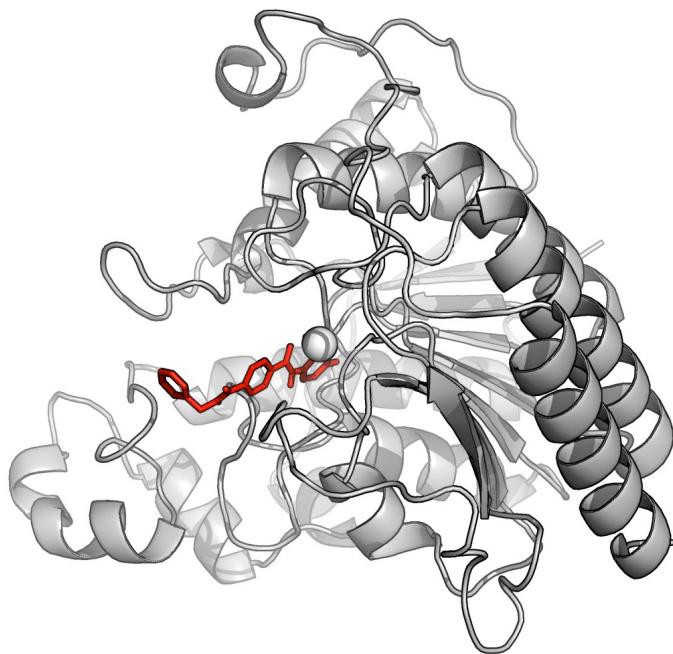
Assistant Professor, Case Western Reserve University, School of Medicine
Member, Angie Fowler AYA and Case Comprehensive Cancer Center



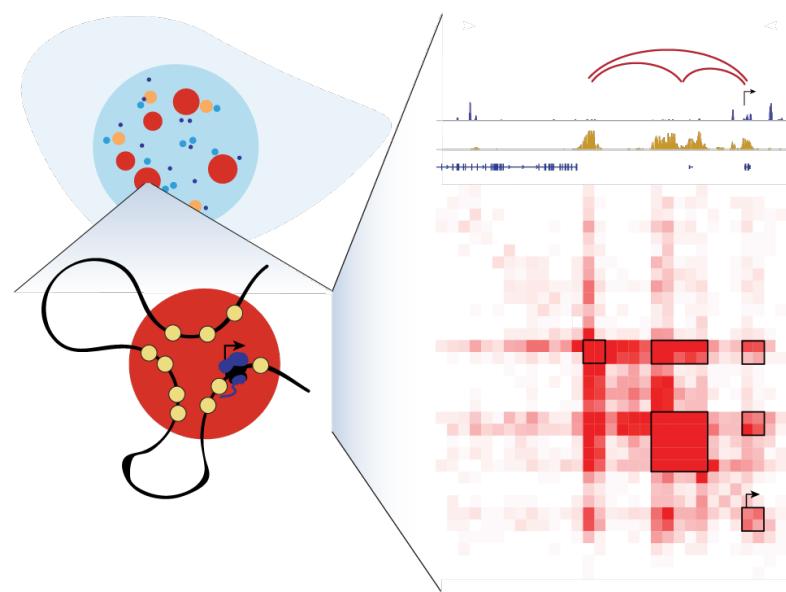


gryderlab

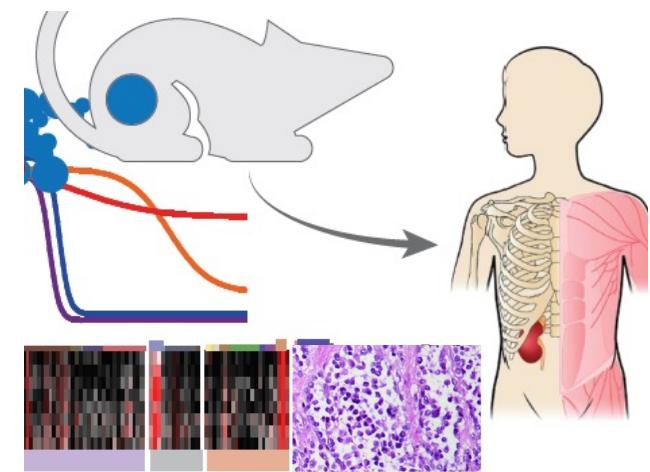
Chemical Biology
Transcription Factors and
Epigenetic Regulators



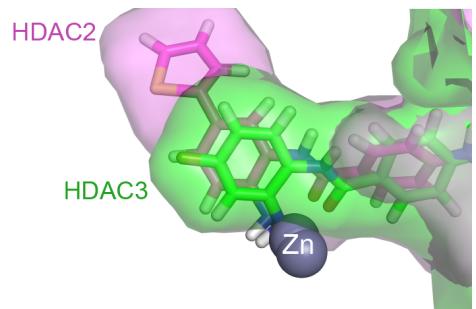
3D Systems Biology
Transcriptional
Condensates



Cancer Biology and Therapy
Childhood Sarcomas, Prostate
Cancer, many others



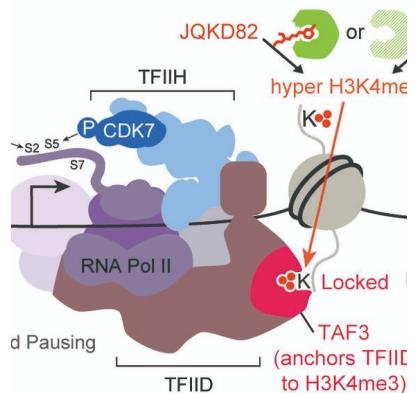
HDACs & H3K27ac



New mechanism to explain HDAC inhibition

Nature Comm., 2019
Nature Genetics, 2019

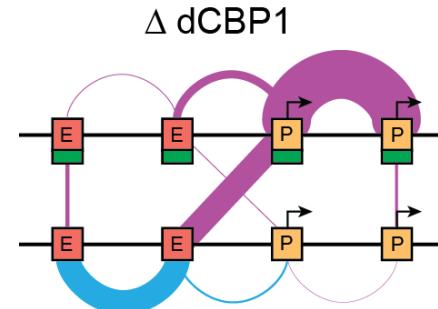
KDM5A & H3K4me3



New mechanism to explain KDM5A inhibition

Blood Cancer Discovery, 2021

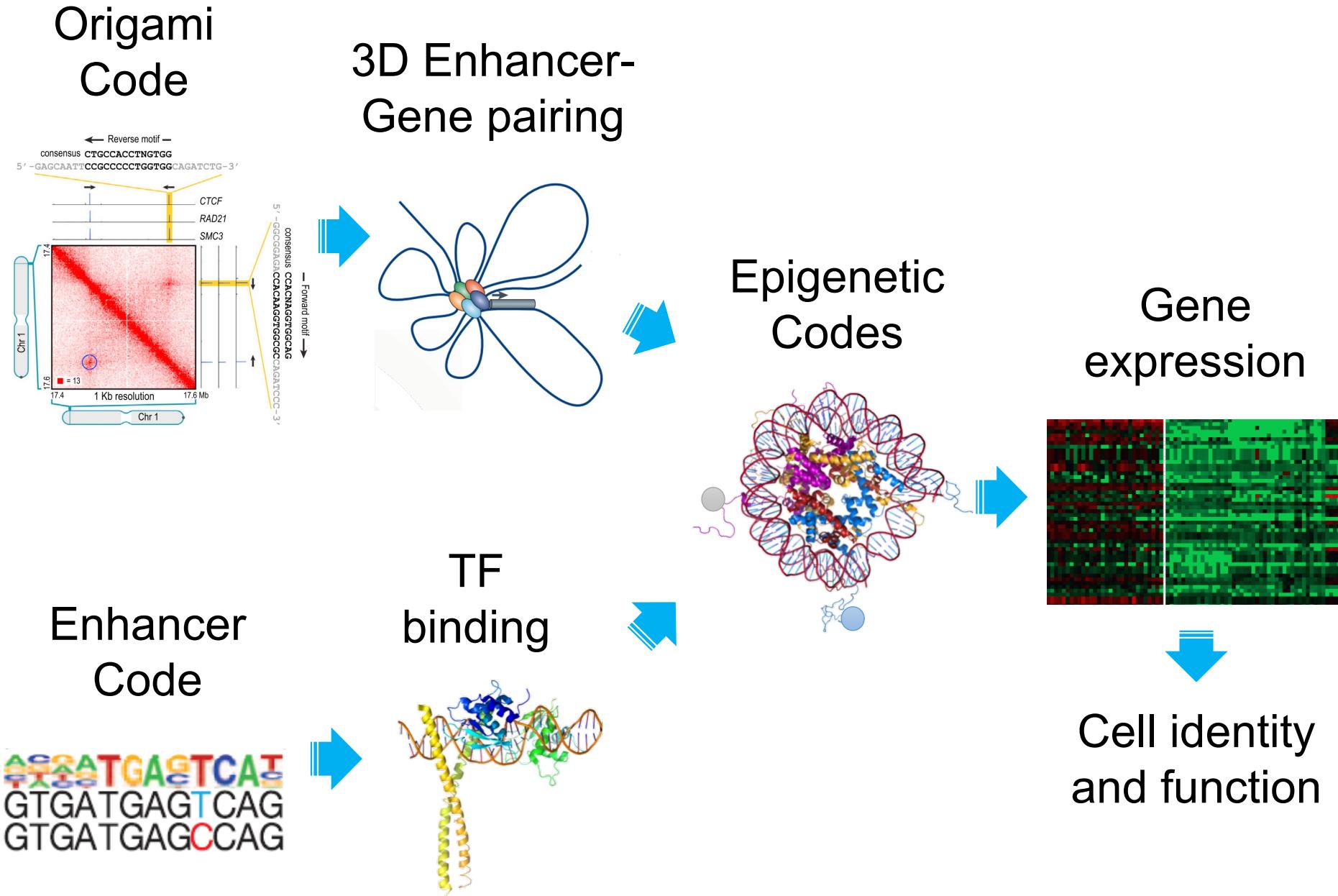
HATs & RNA Pol 2



Pol2 cluster collapse

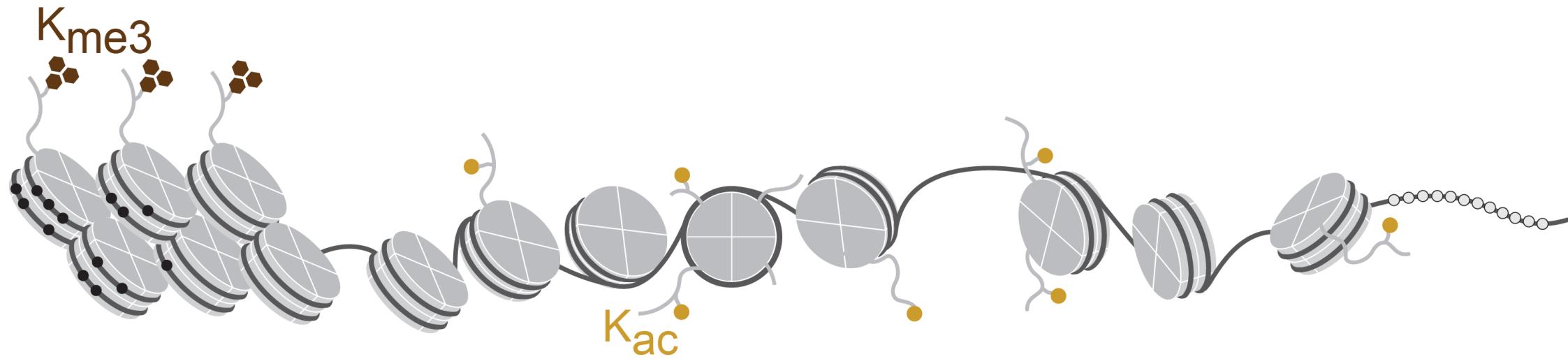
Unpublished

Non-Protein-Coding DNA Drives Epigenetics

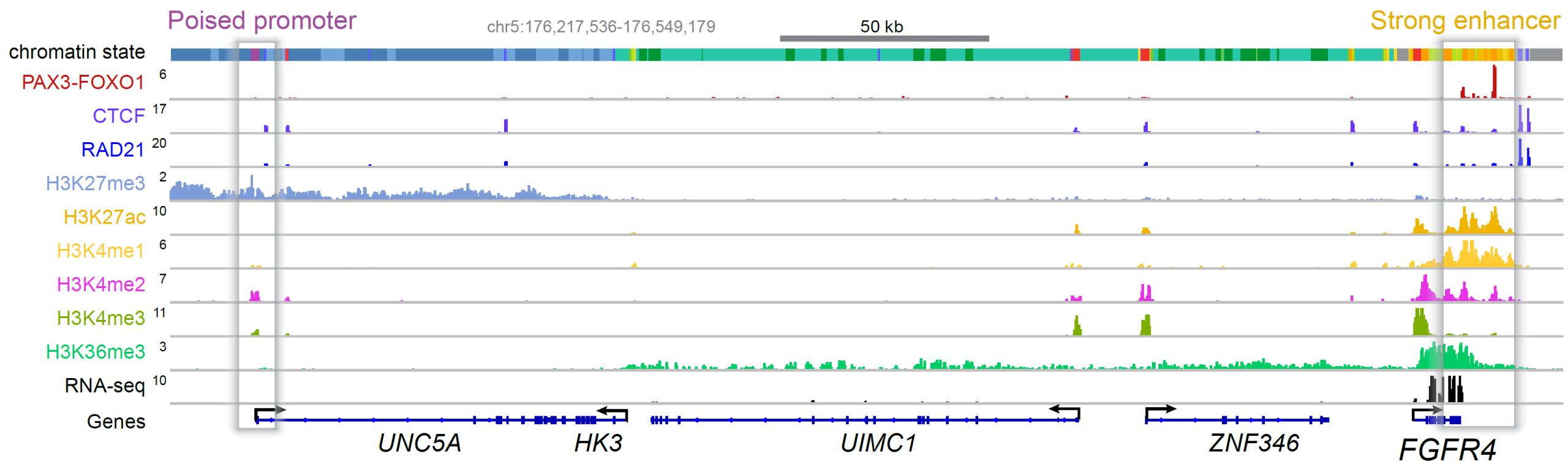
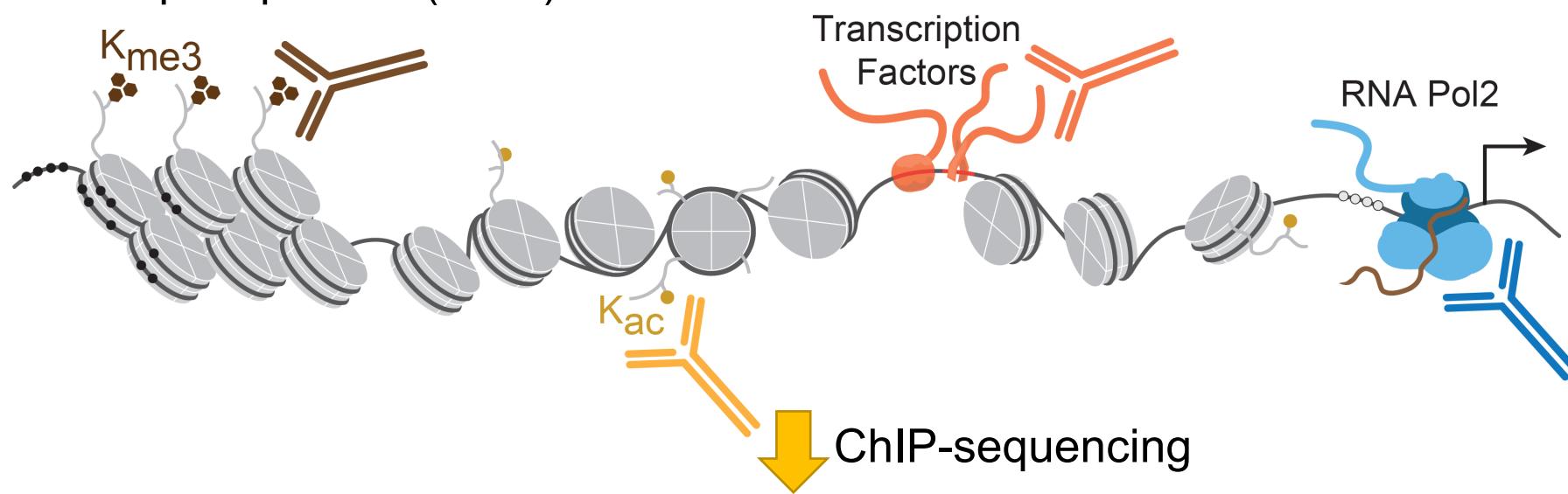


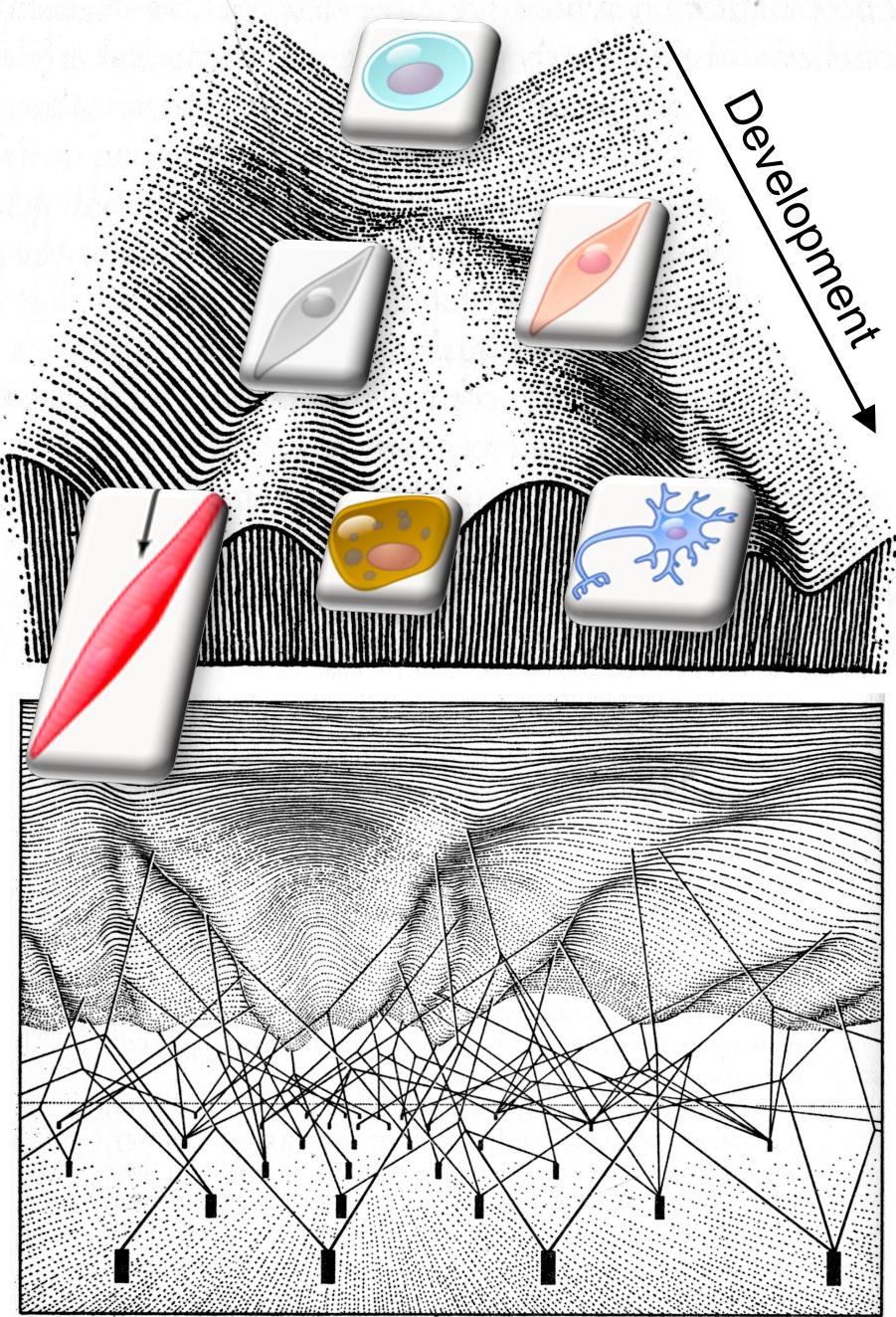
2 Epigenetic “Codes”

- The histone modification code
- DNA methylation code



Chromatin Immunoprecipitation (ChIP)



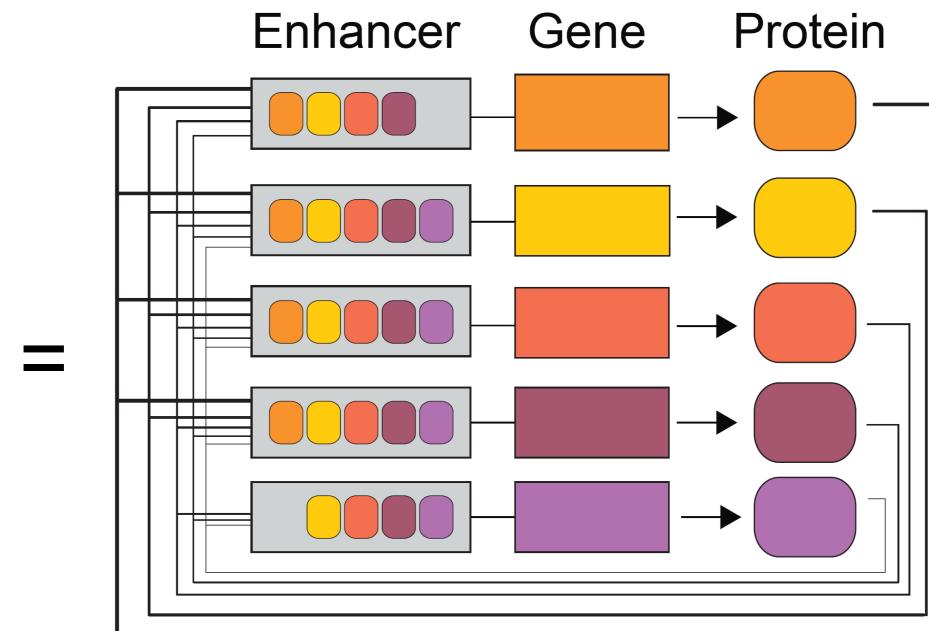


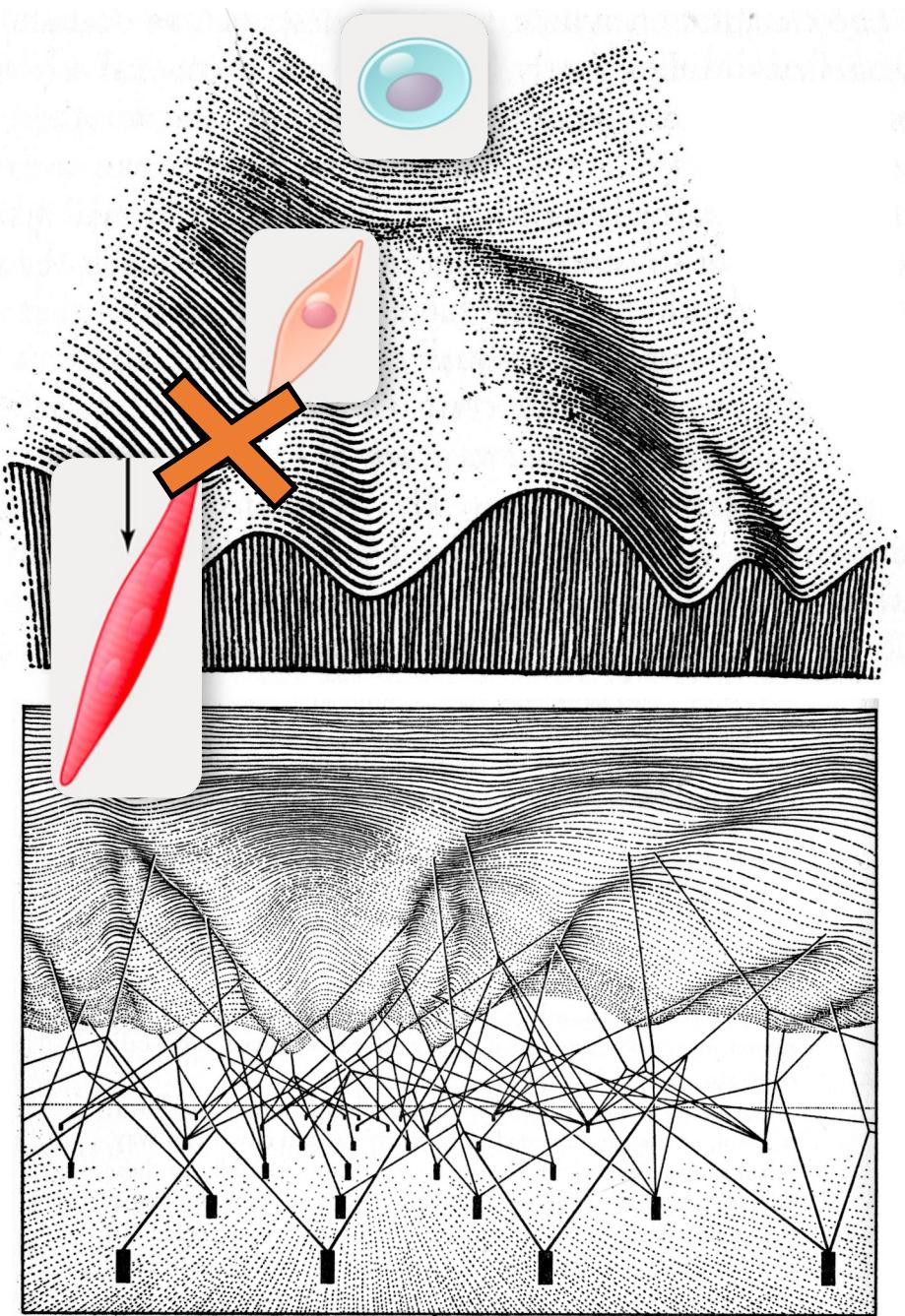
1957, Conrad Waddington

"to account for the fact that development is channeled into separate, distinct pathways: if the product of the reaction makes the reaction go faster... autocatalytic... self regulating... In cell differentiation we must be dealing with **chemical cybernetic systems**"

1953, Conrad Waddington

Core Regulatory Transcription Factors

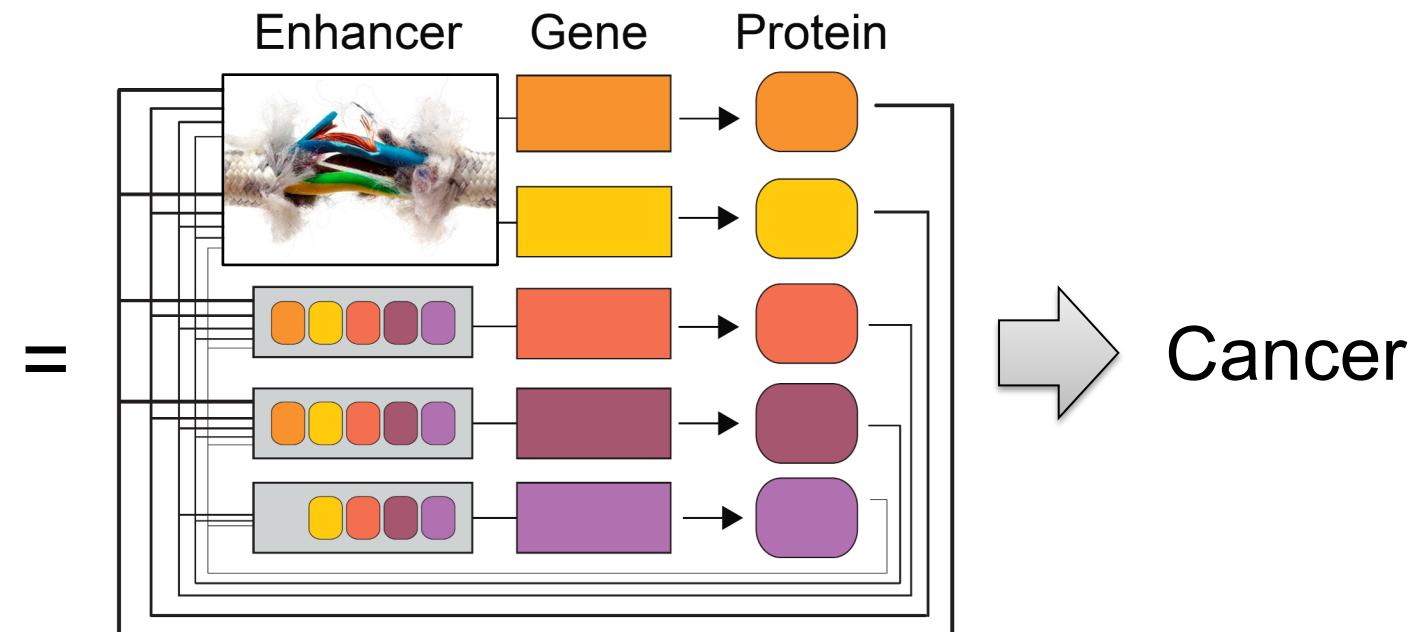




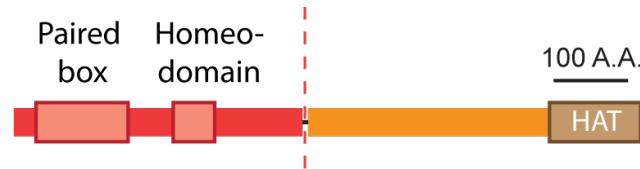
1957, Conrad Waddington

Core Regulatory Transcription Factors are:

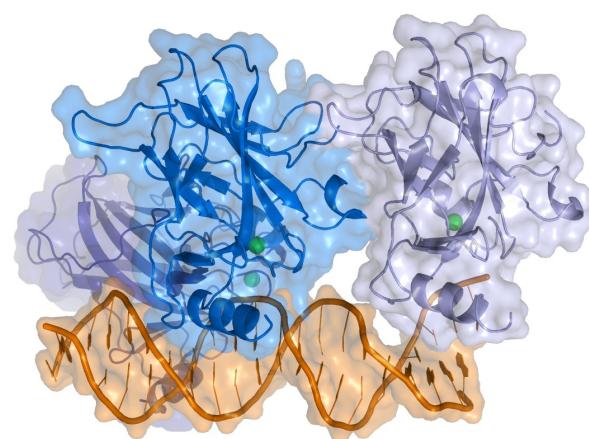
- Logical Circuits
- Essential in cancer
- Druggable



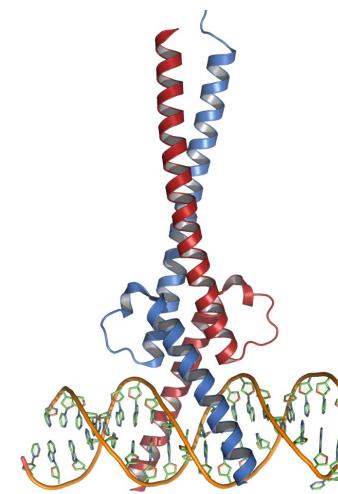
Kasey Altman



PAX3-FOXO1
Rare translocation



p53
mutation

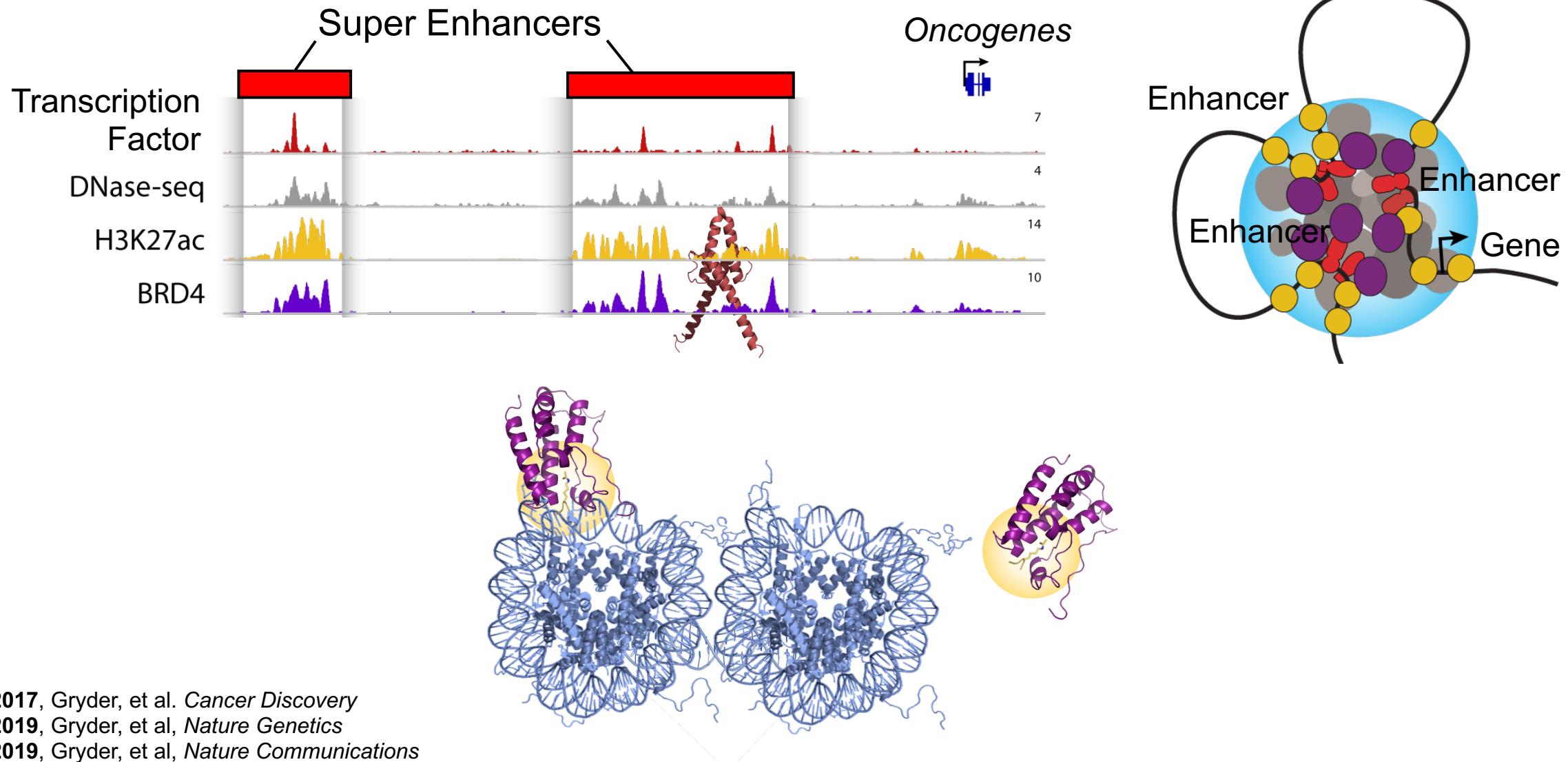


MYCN
amplification



www.instagram.com/kasey.altman

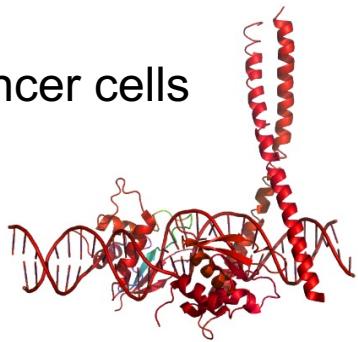
Core Reg. TFs build Super Enhancers



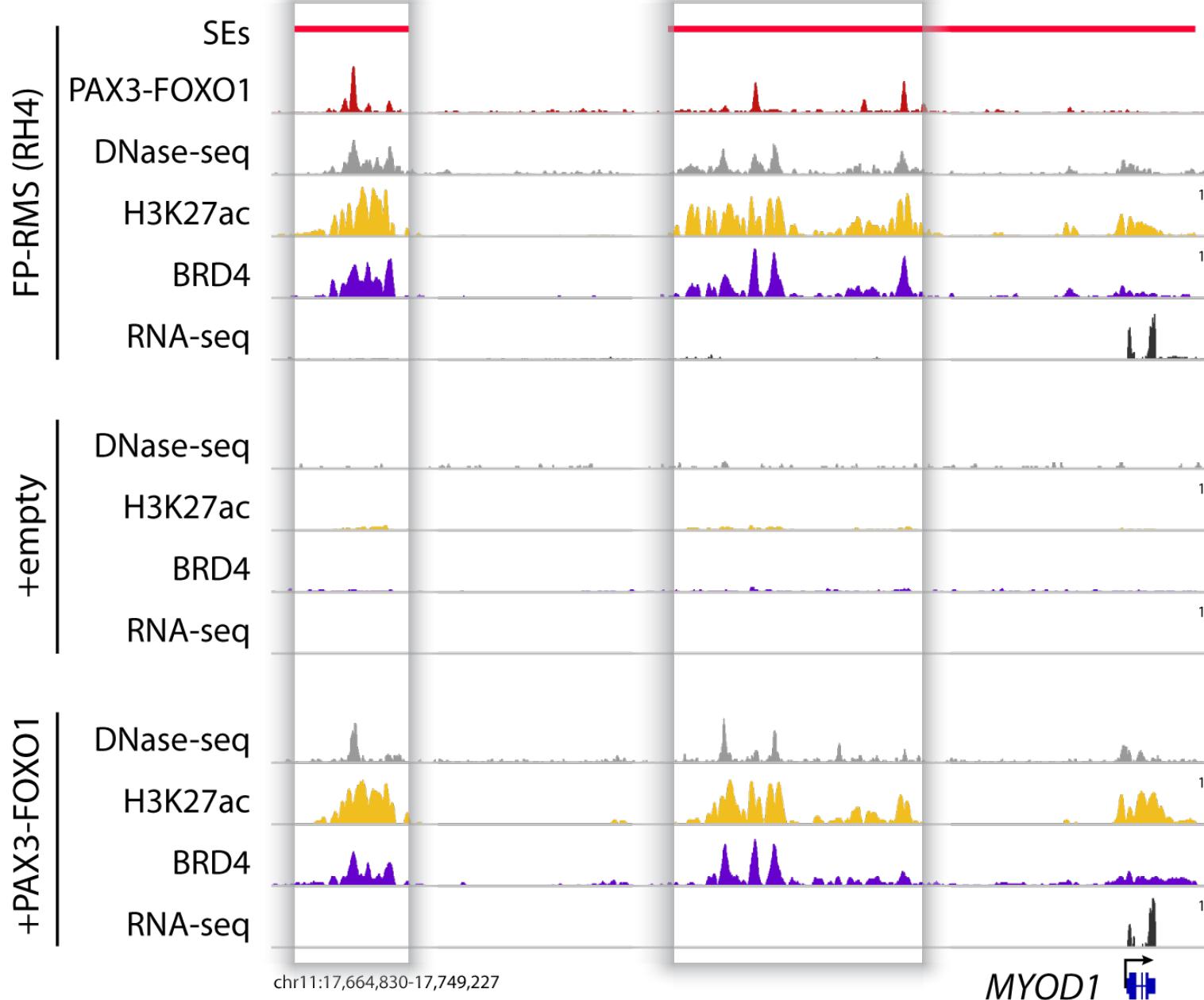
2017, Gryder, et al. *Cancer Discovery*
2019, Gryder, et al. *Nature Genetics*
2019, Gryder, et al. *Nature Communications*
2020, Gryder, et al. *Nature Protocols*
2021, Gryder, et al. *iScience*
2021, Laubscher, Gryder, *Nature Comm.*

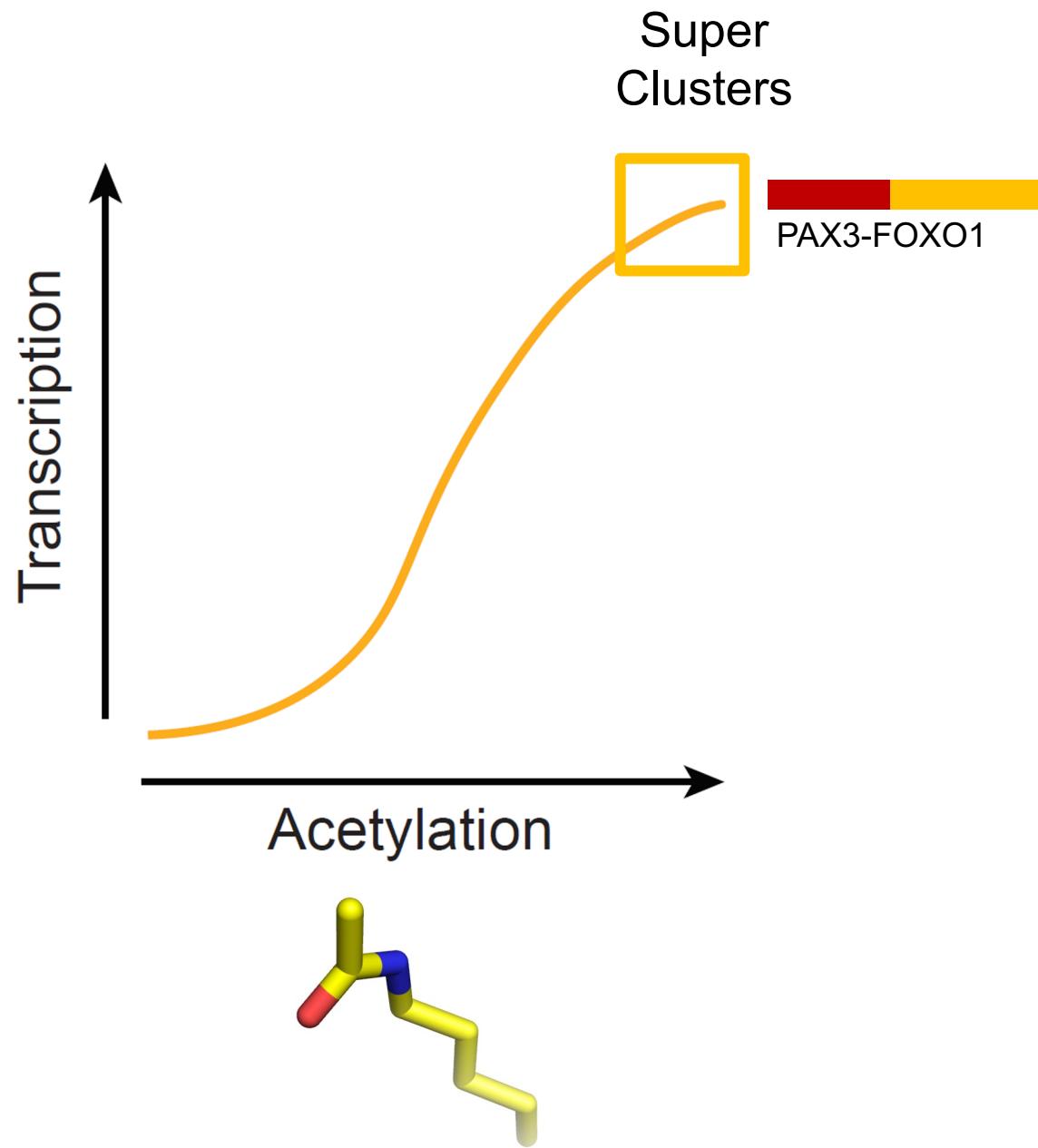
Where PAX3-FOXO1 goes, **Acetylation follows**

Cancer cells



Fibroblasts





Transcription Factors

PAX3-FOXO1
MYOD1
MYOG
MYCN
SOX8

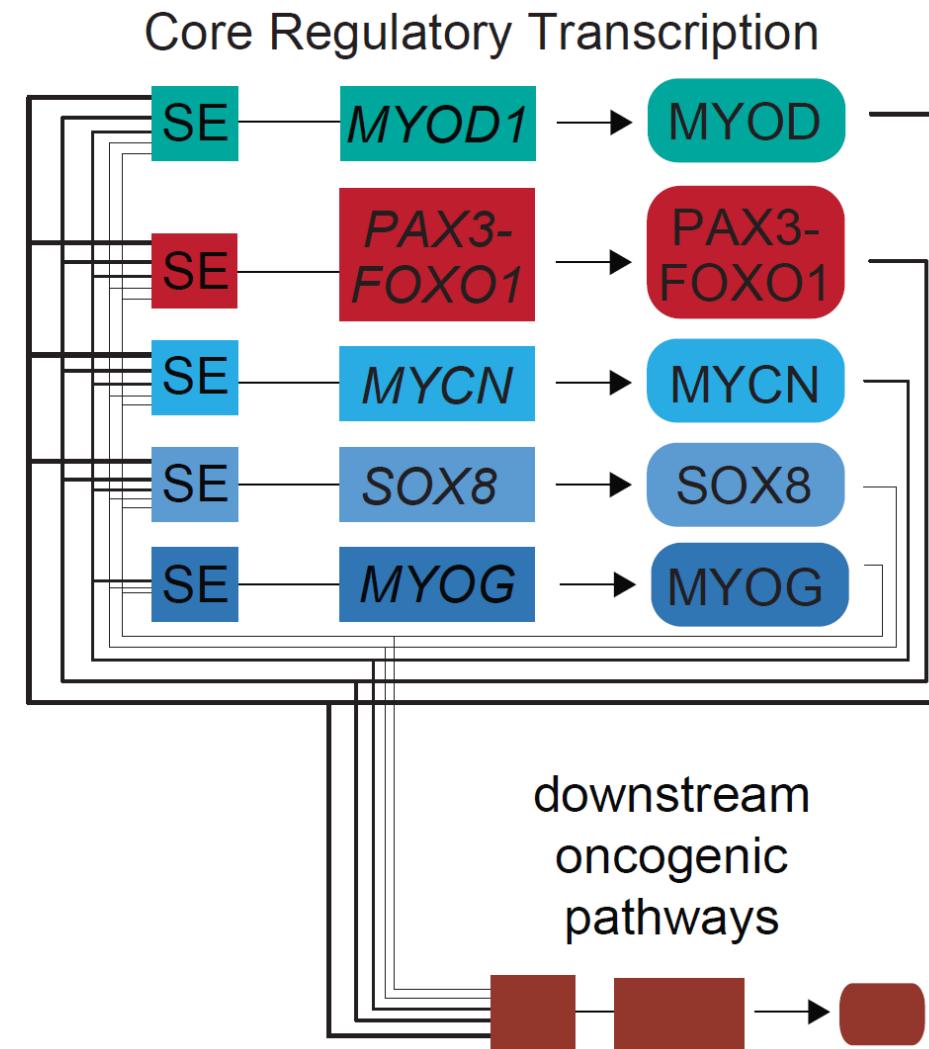
Oncogenes

FGFR4
IGF2
ALK

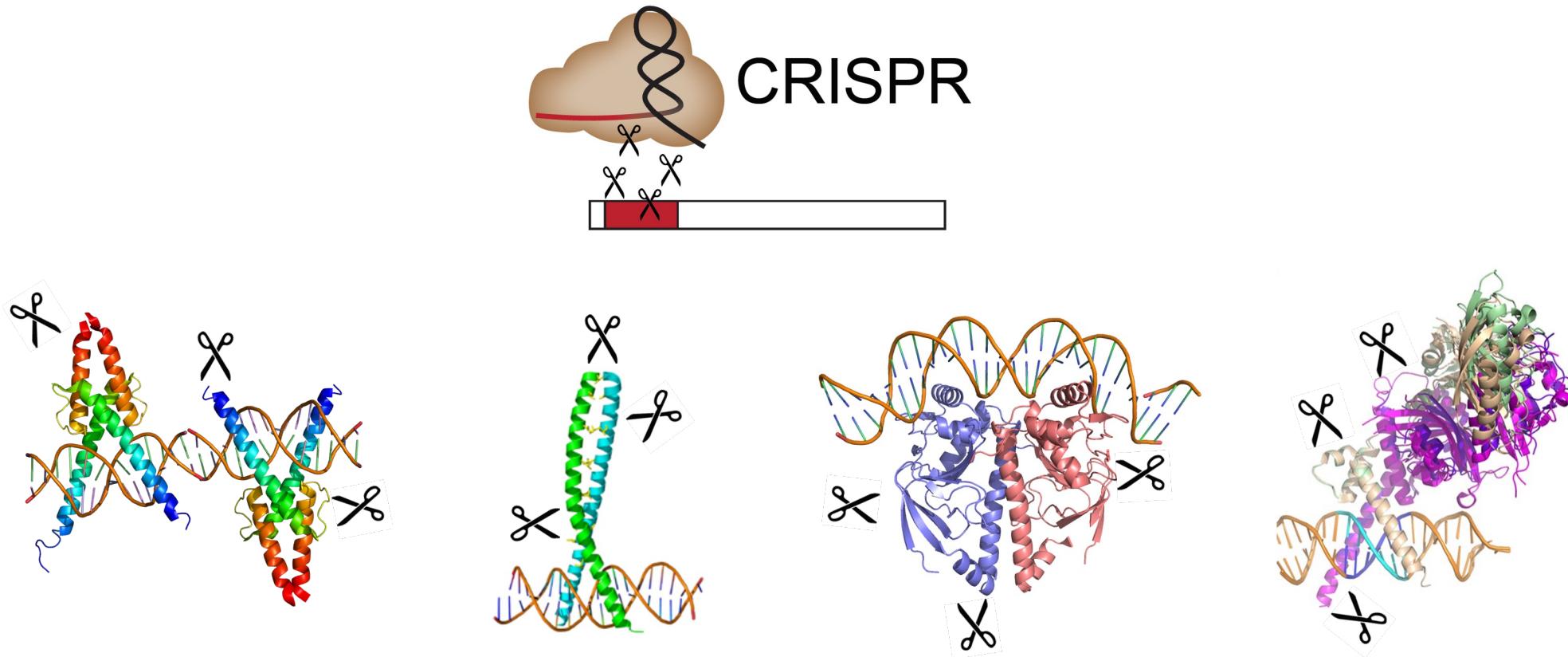
Core Regulatory TFs Bind Each others SEs



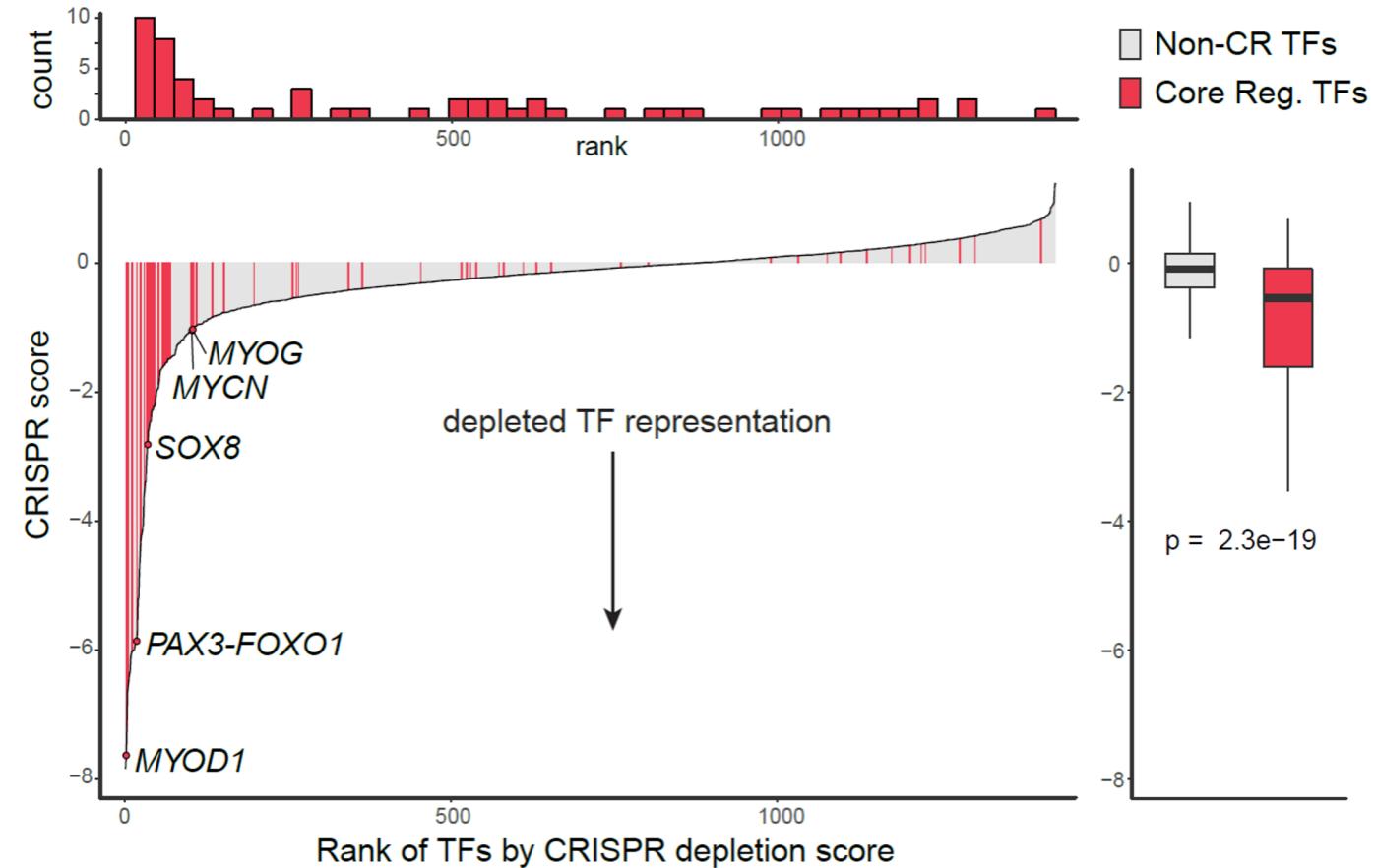
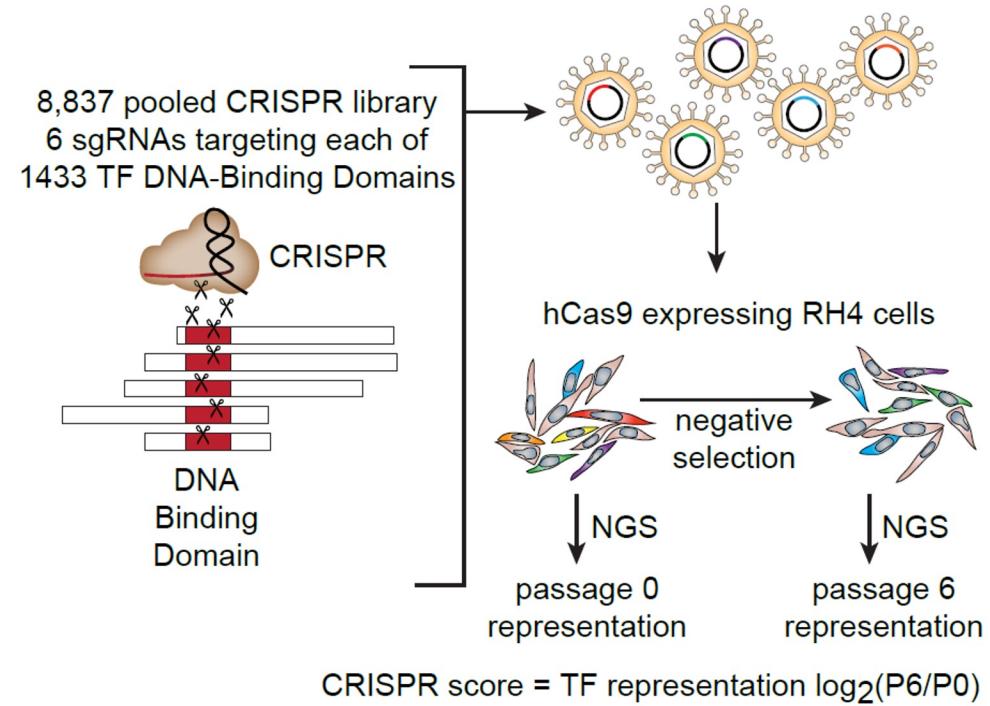
Core Regulatory TF Networks



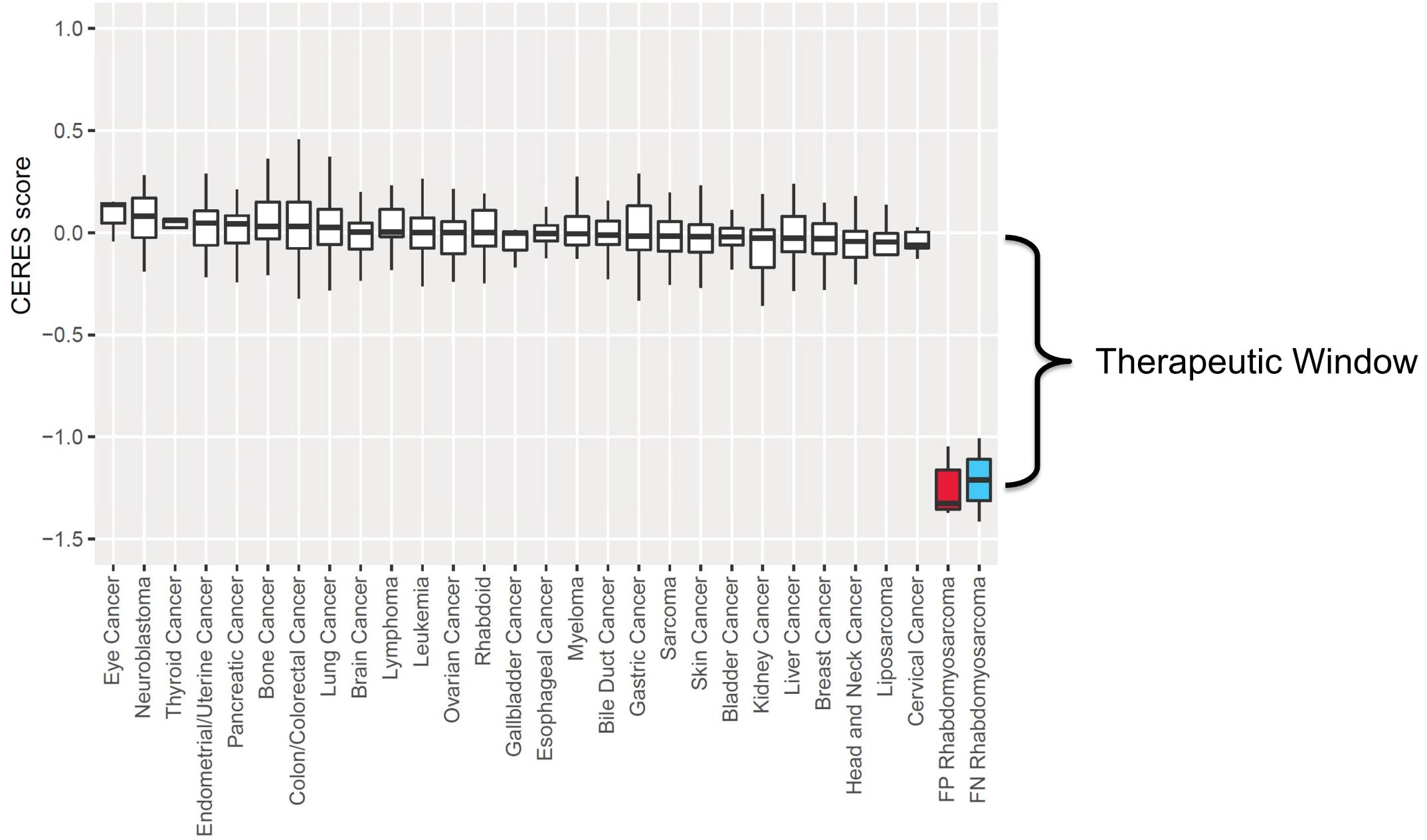
1. Are Core Regulatory TFs essential for cancer growth?
2. Are they functionally redundant?



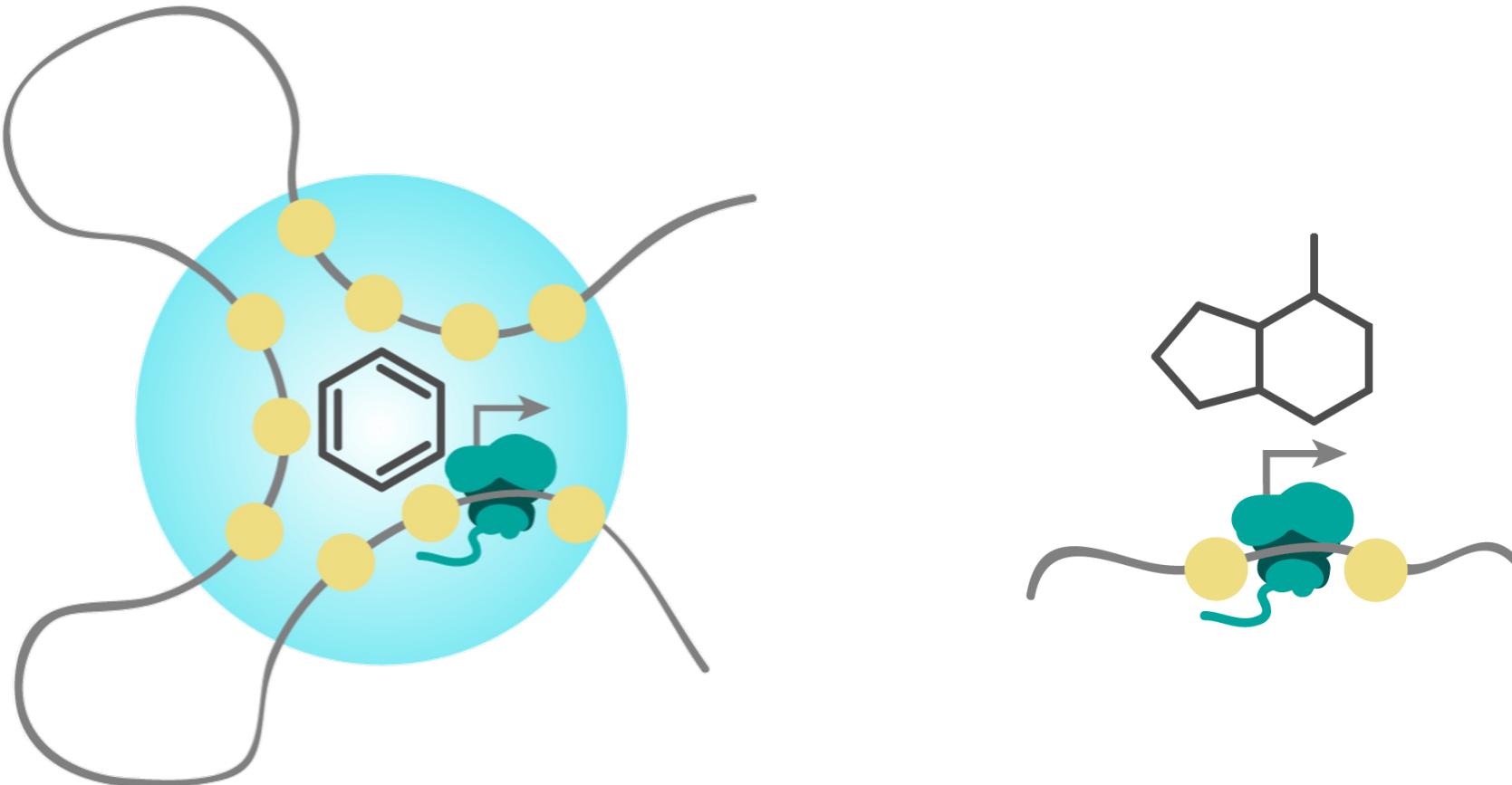
1. Are Core Regulatory TFs essential for cancer growth? YES
2. Are they functionally redundant? NO



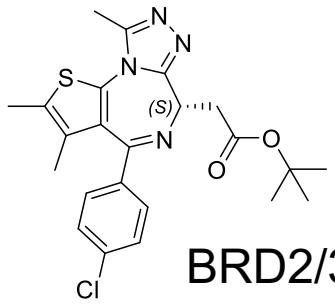
MYOD1



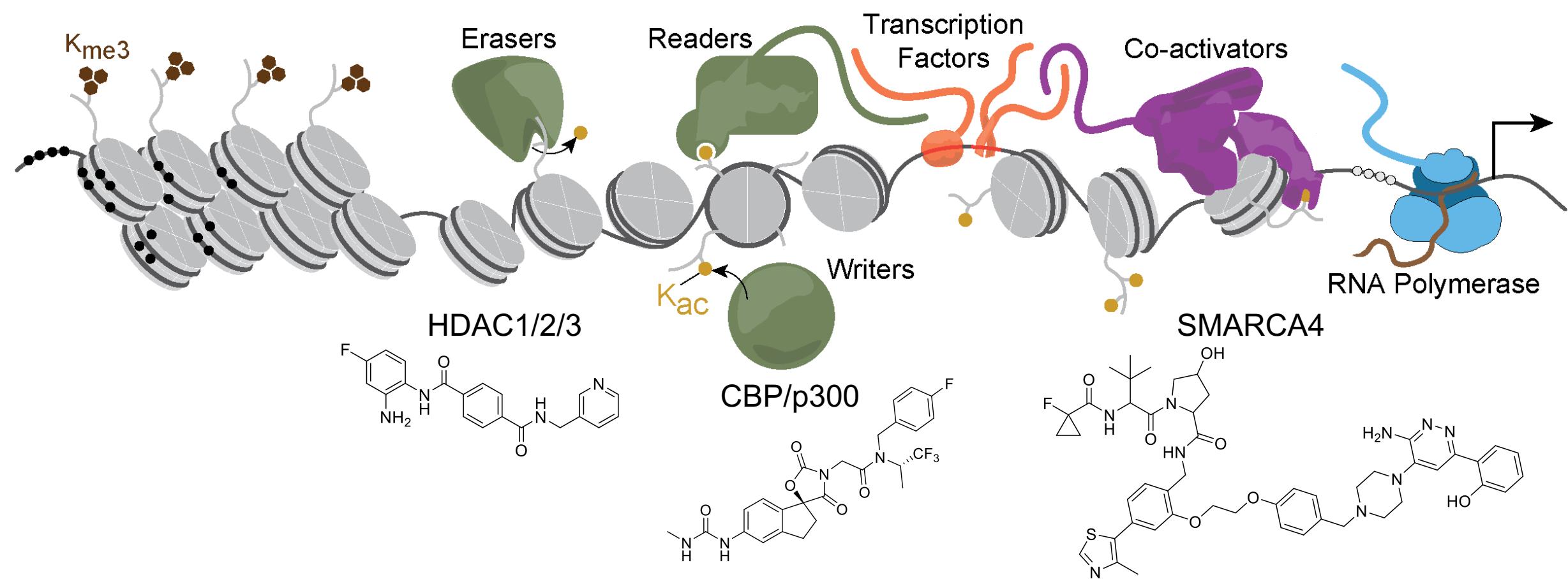
How do we drug core regulatory TFs?



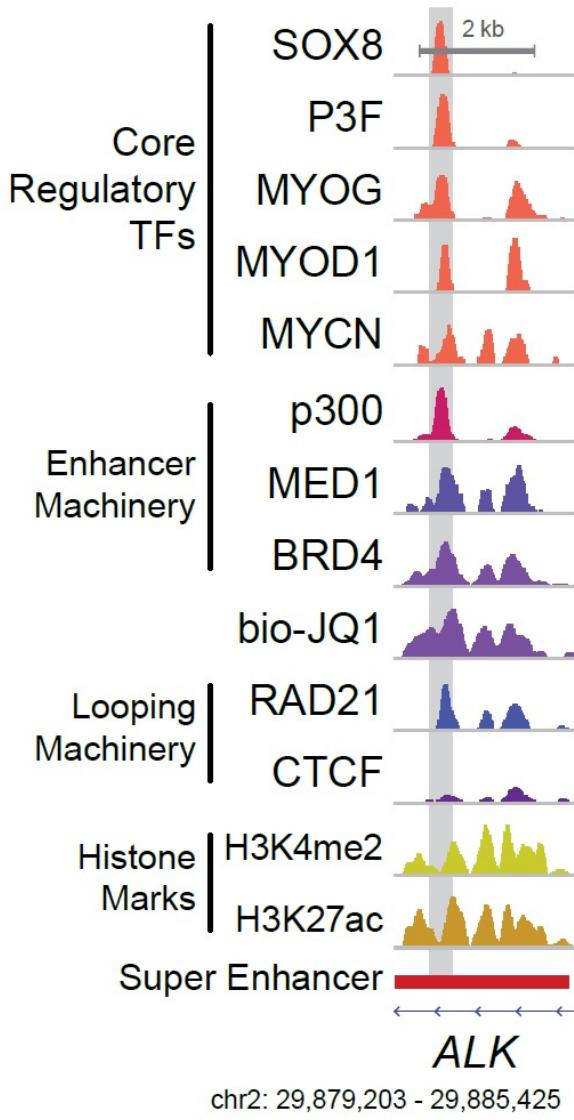
Heterochromatin



Euchromatin

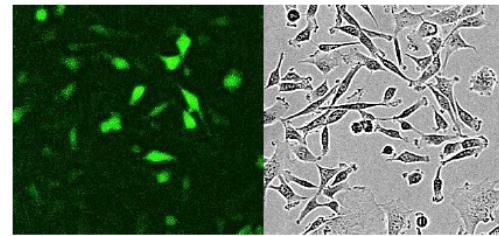


16 top PAX3-FOXO1
bound Super Enhancers

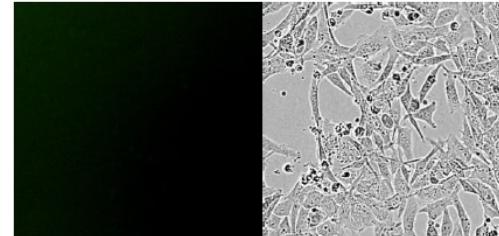


ALK-SE-pGF1

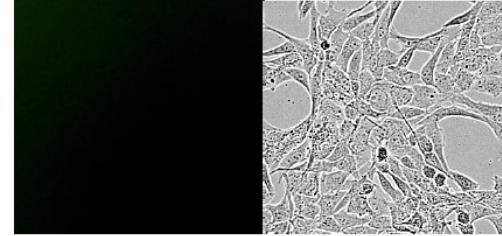
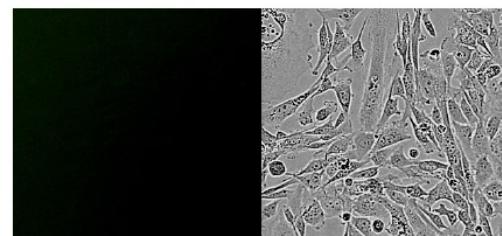
PAX3-FOXO1
RH41 cells



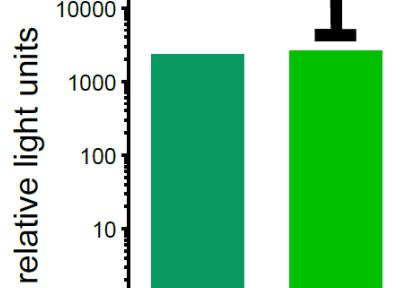
fusion negative
RD cells



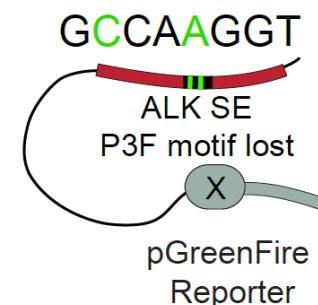
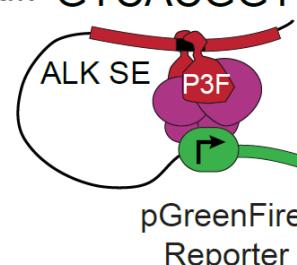
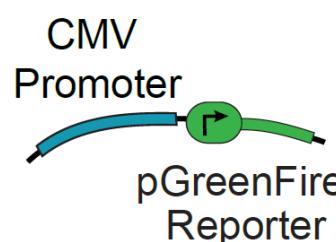
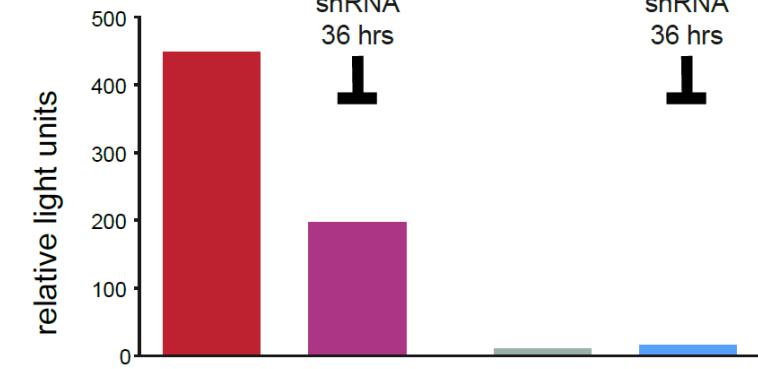
empty-pGF1

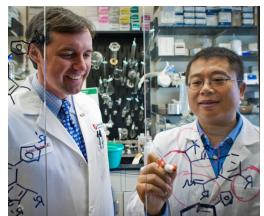


PAX3-
FOXO1
shRNA
36 hrs

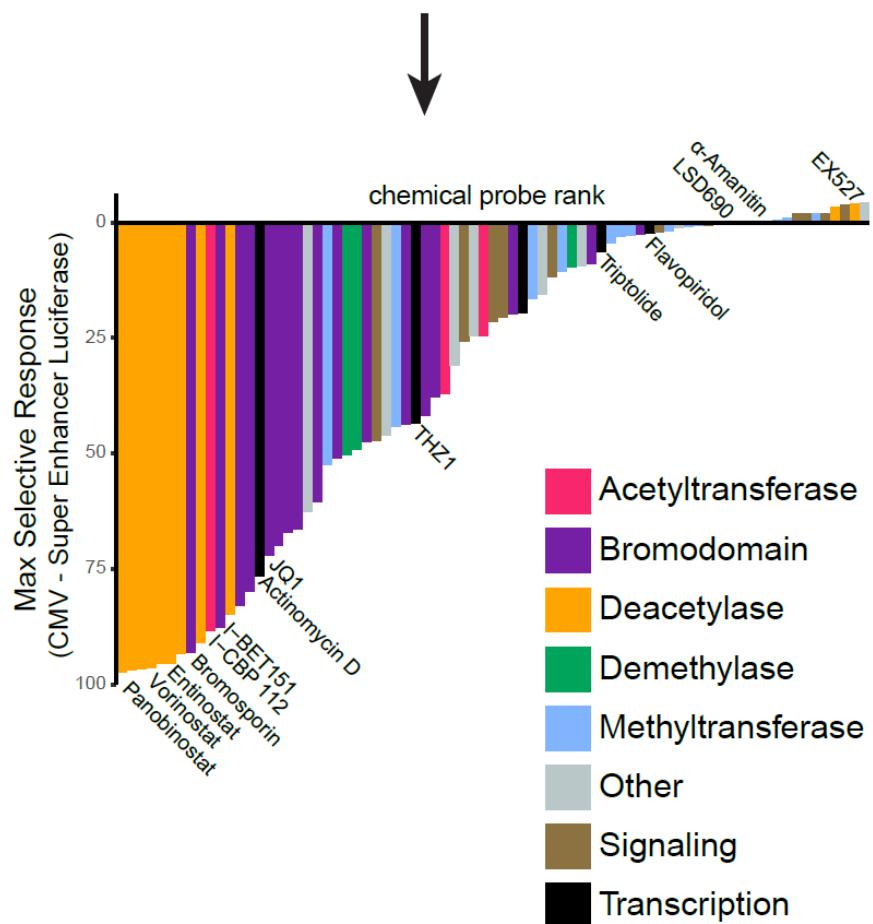


PAX3-
FOXO1
shRNA
36 hrs

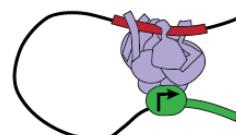




100 Chemical probes
11-point dose response
(technical quadruplicate, biological duplicate)



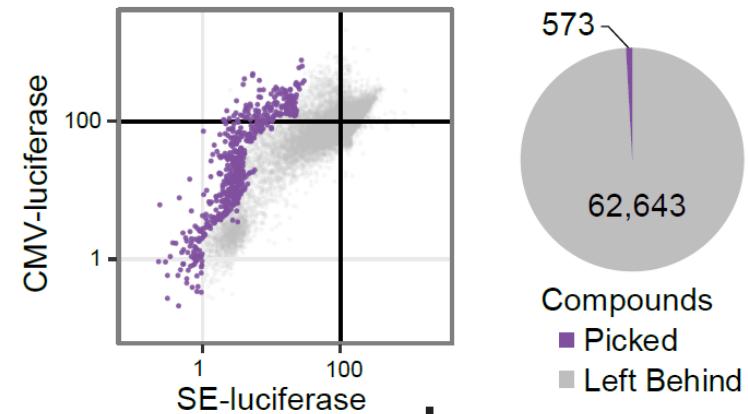
Super-Enhancer driven



v.
Promoter driven

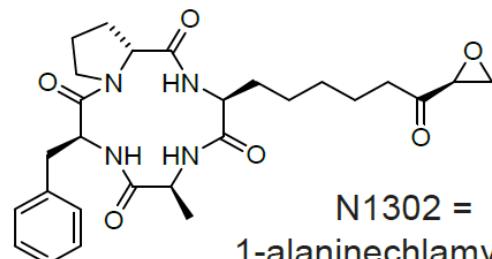


63,000 pure compound library:
single 10 µM dose



573 hits: dose response followup

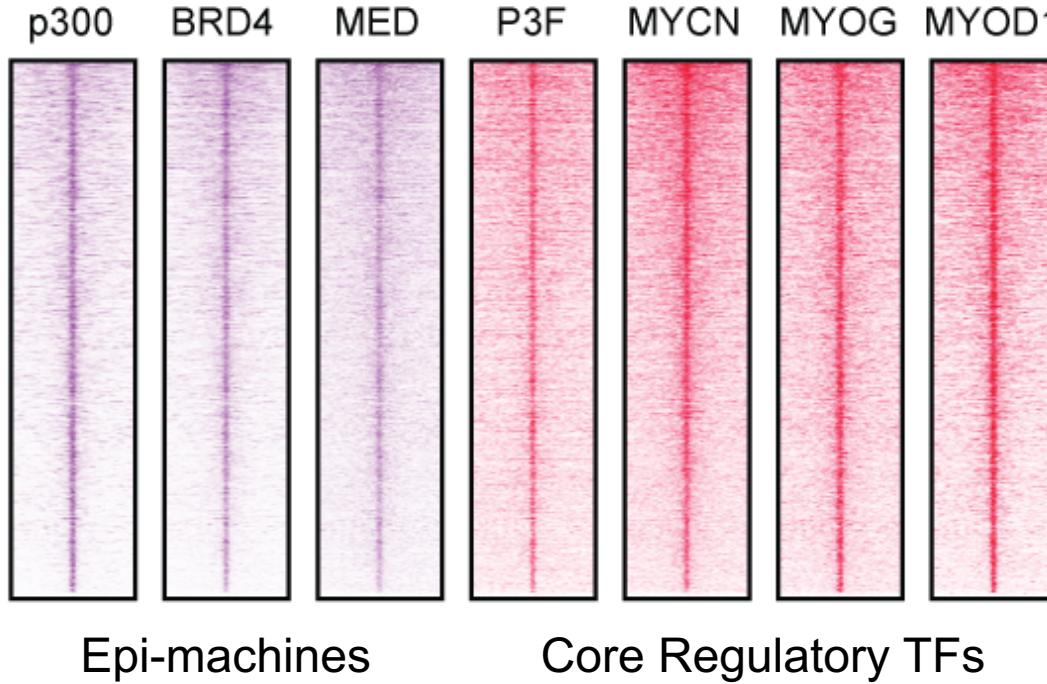
15 leads: RNA-seq



N1302 =
1-alaninechlamydocin

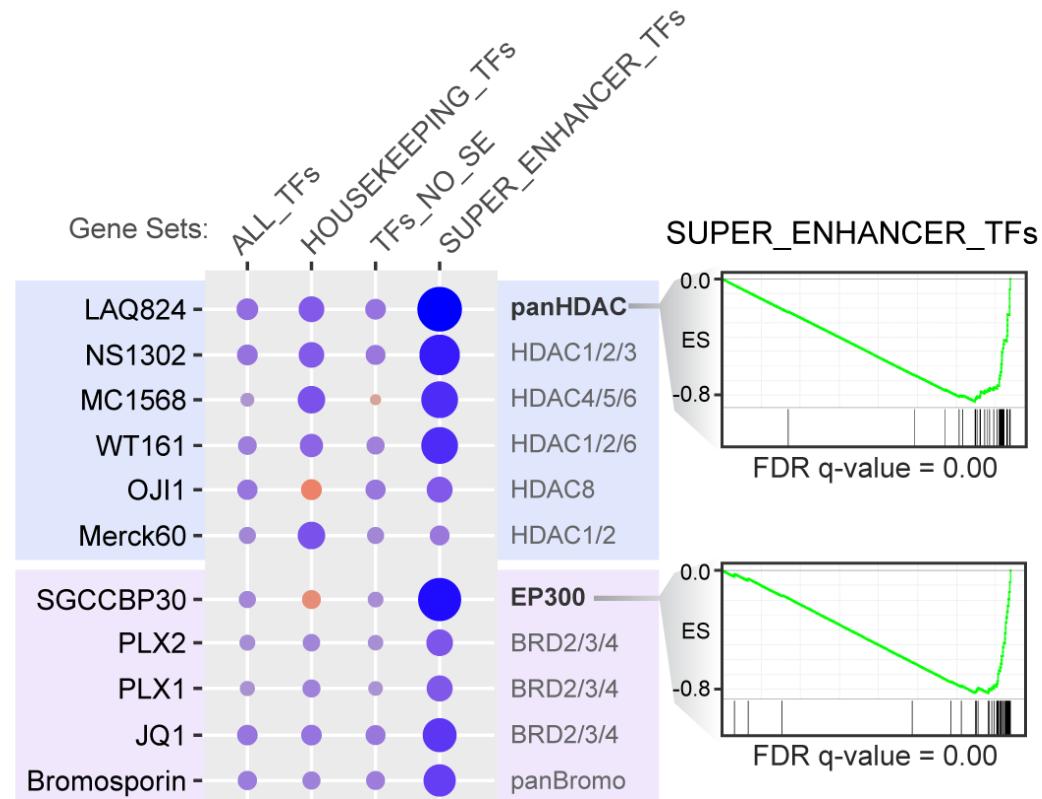
Histone Deacetylase inhibitor

Drugging CR TF transcription



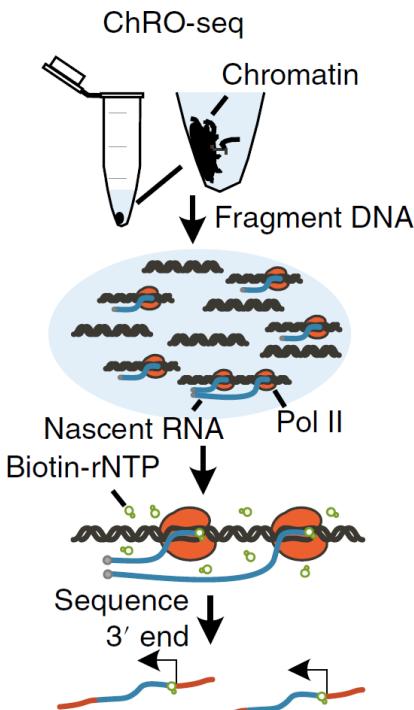
HDAC
(Histone Deacetylase)

Bromodomain

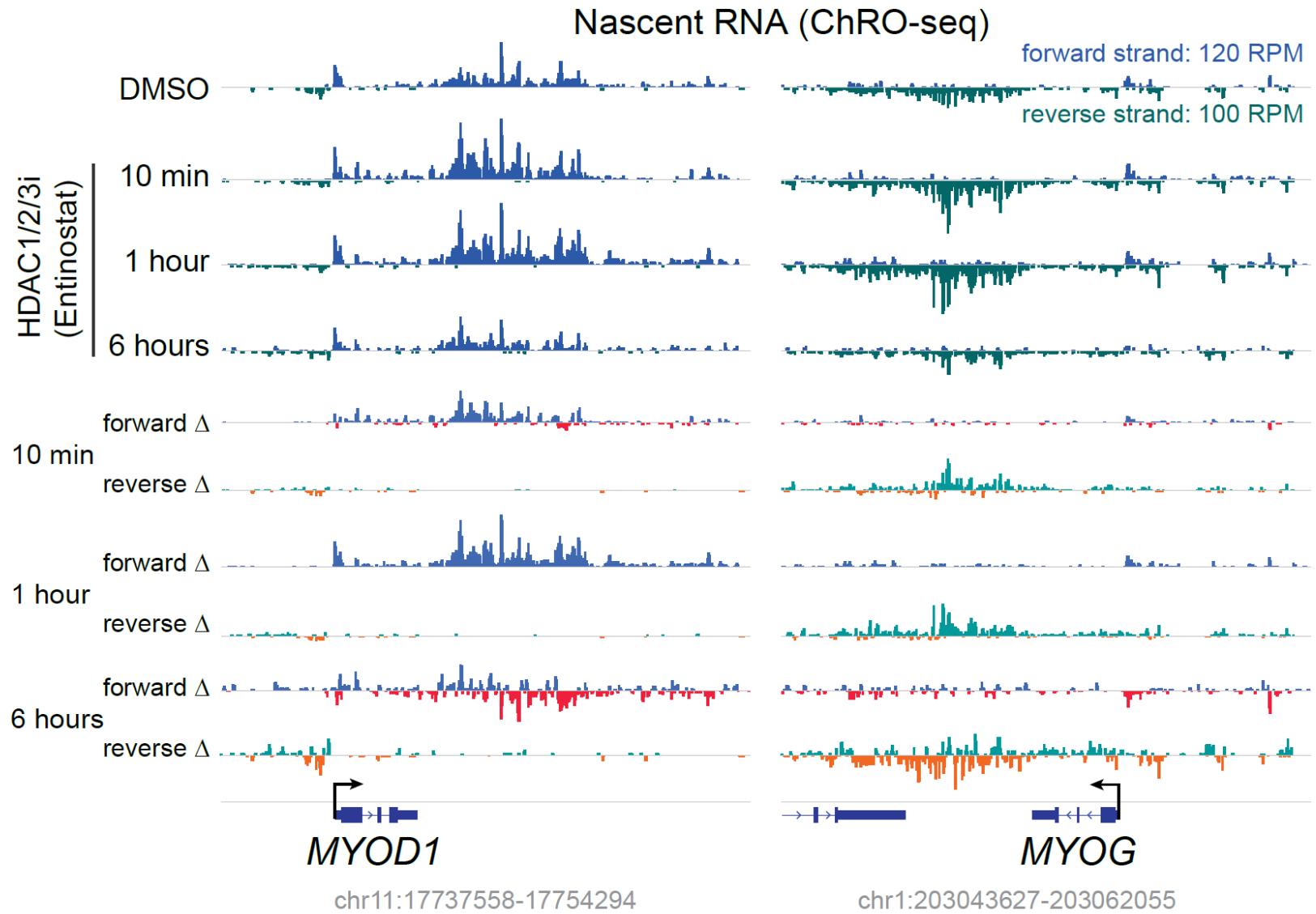


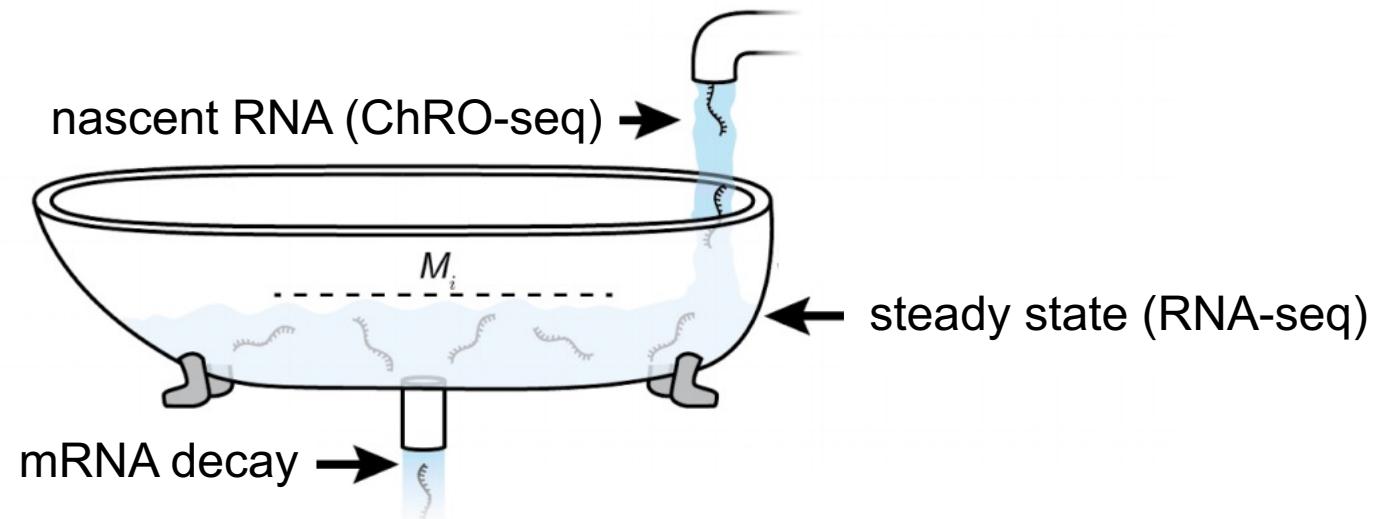
Transcription of CR TFs

could nascent transcription
reveal different CR TF
transcription kinetics?



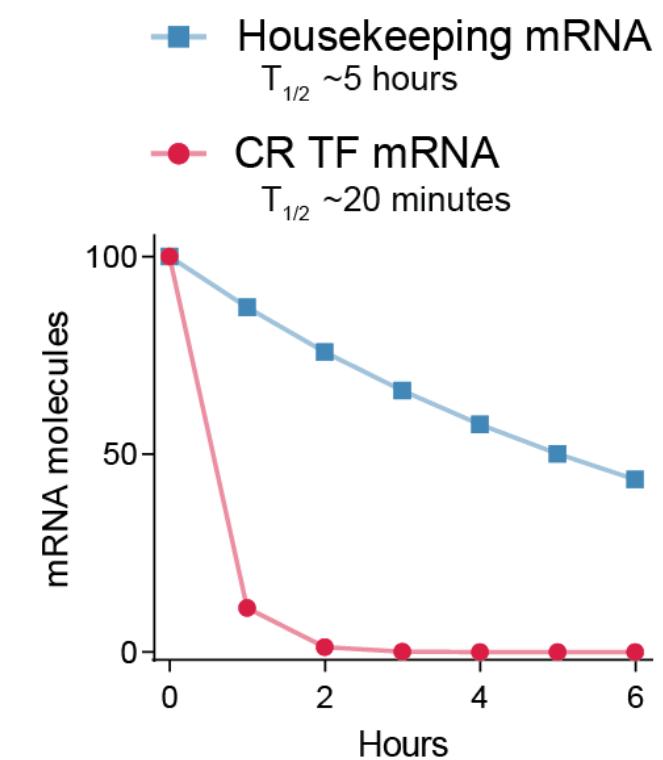
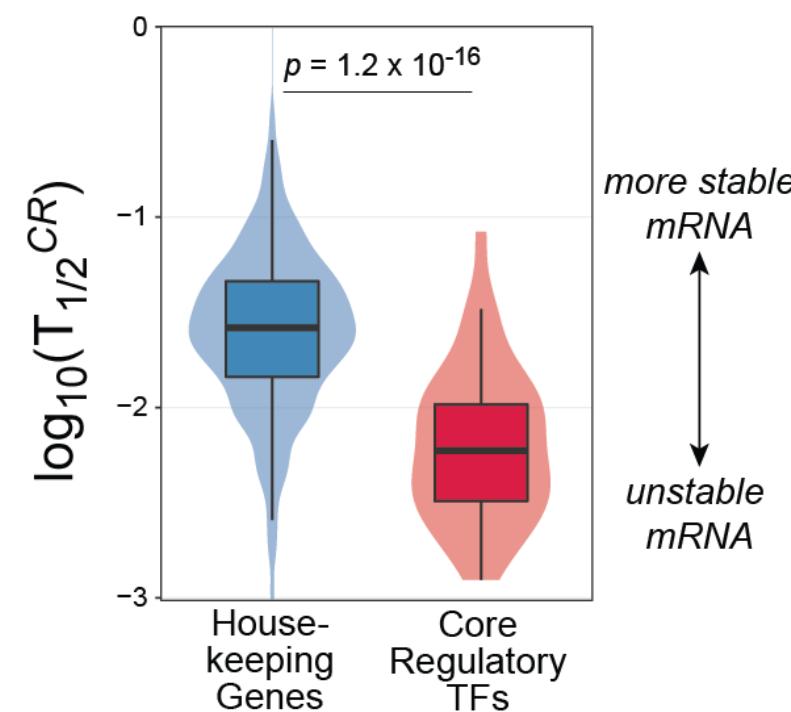
2018, *Nature Genetics*, Chu et al





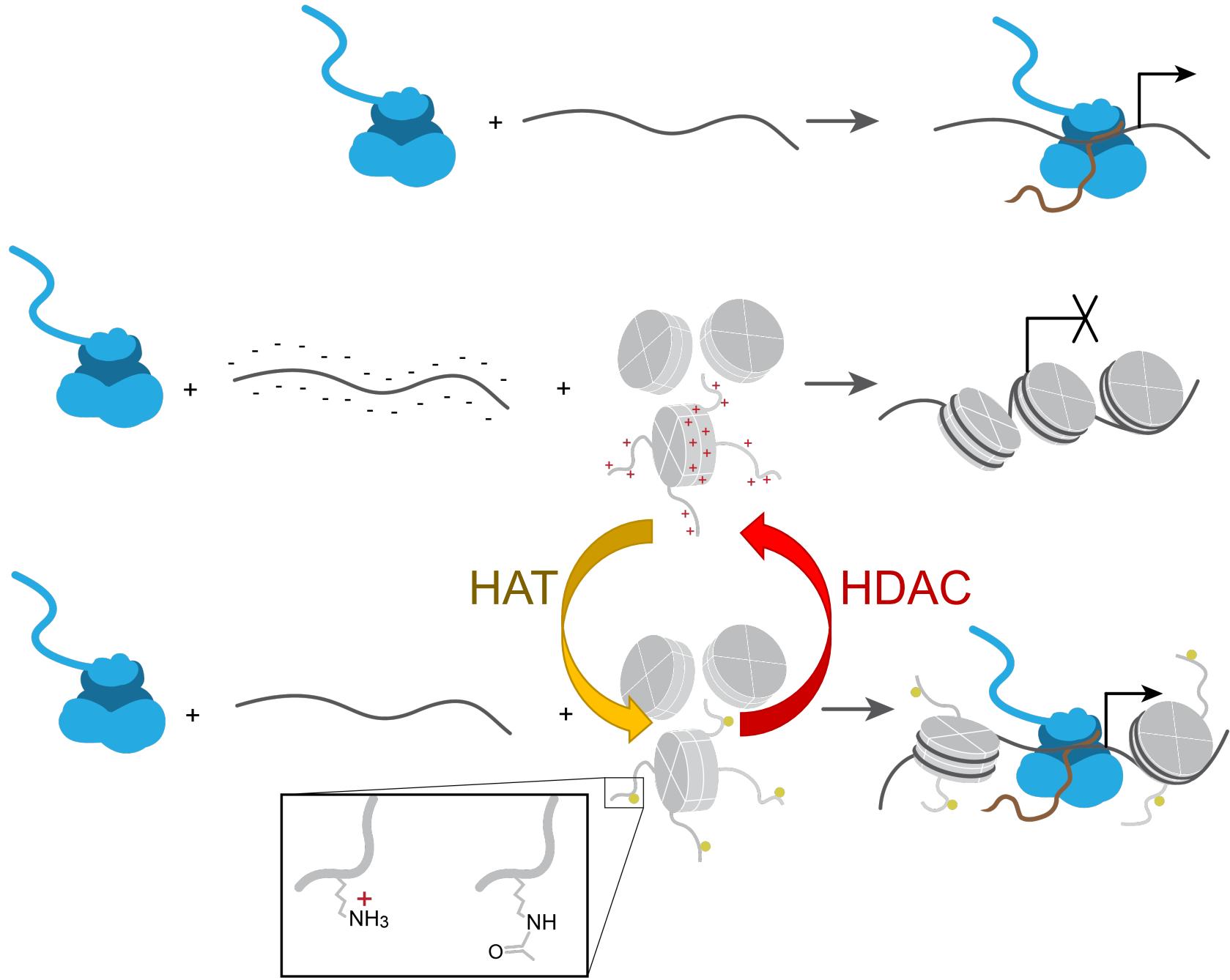
$$\text{mRNA decay} \propto \frac{\text{RNA production} \quad (\text{ChRO-seq})}{\text{RNA steady-state} \quad (\text{RNA-seq})}$$

$$T_{1/2}^{CR} \propto \frac{\ln(2)}{\text{mRNA decay}}$$

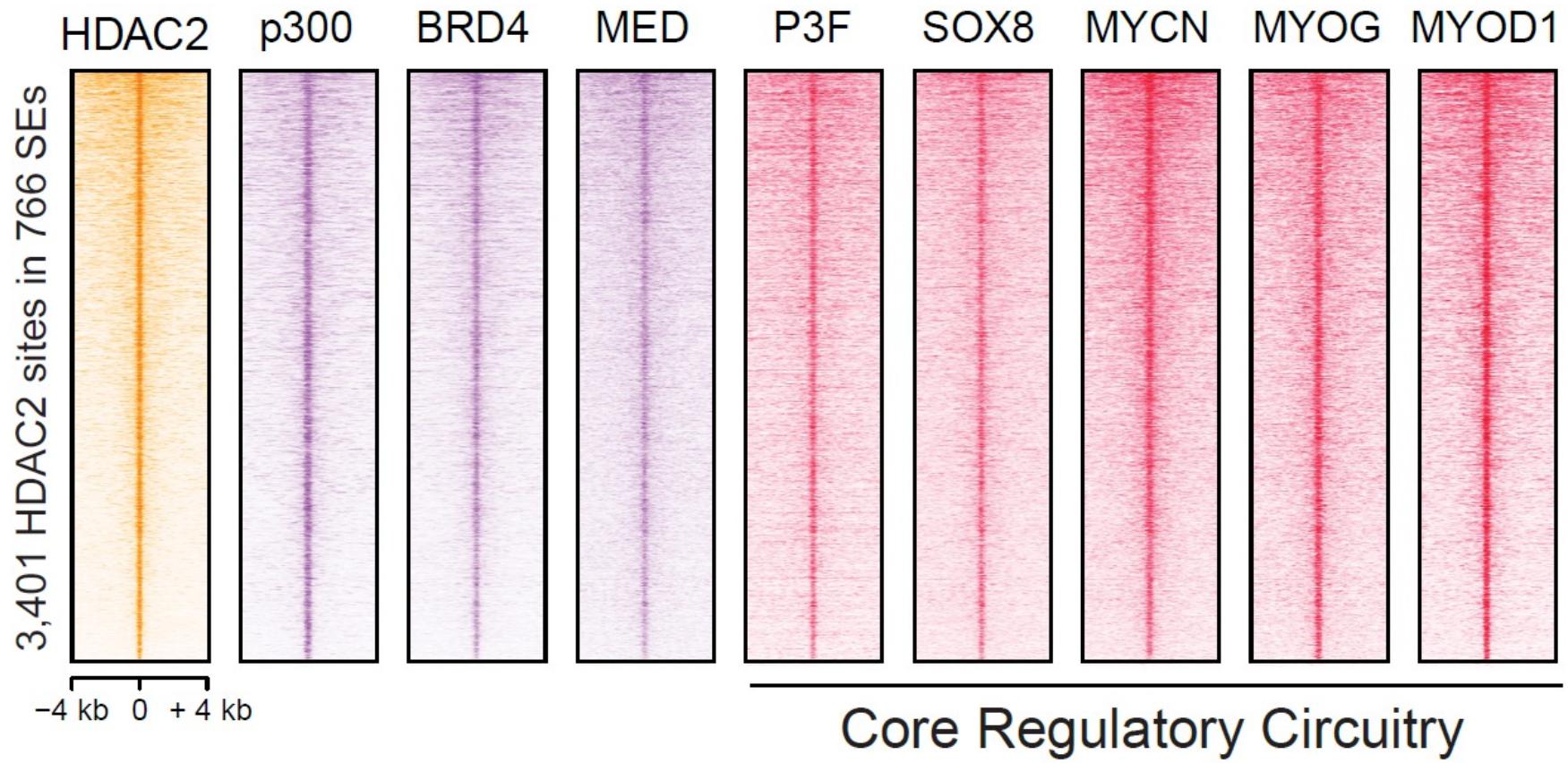
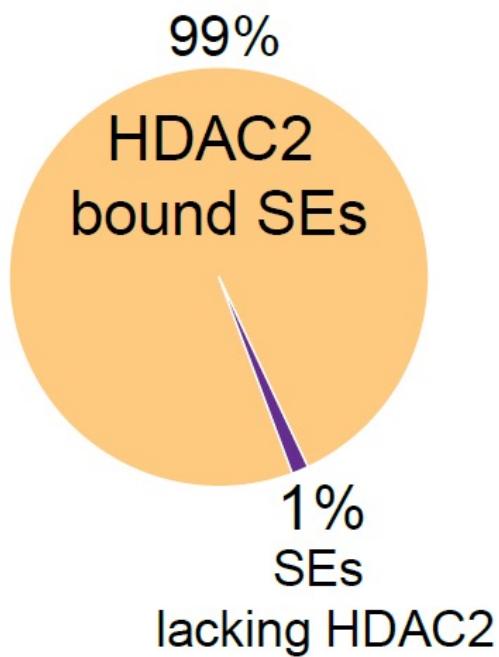


Theory: Super enhancers are required for core regulatory TFs because

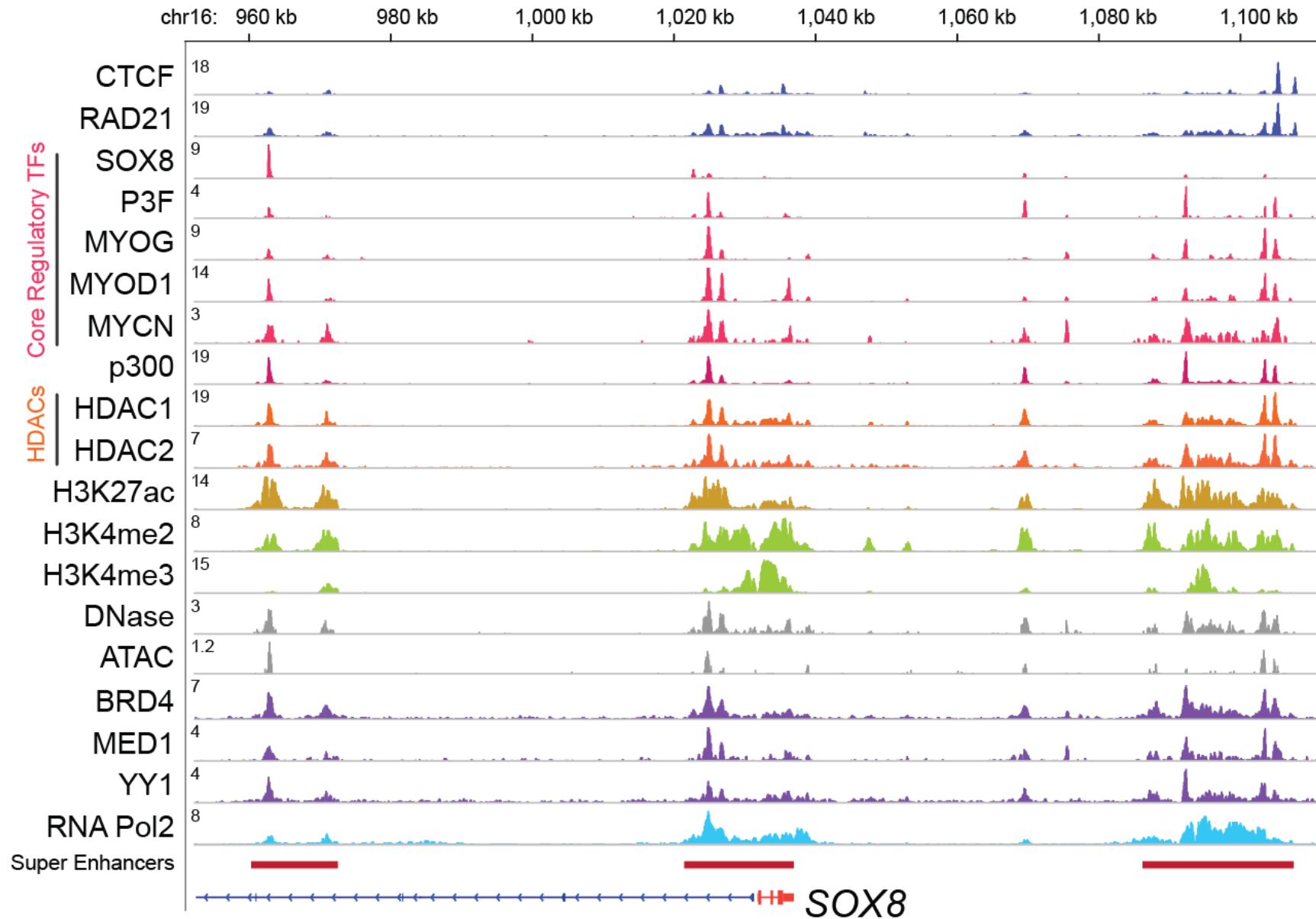
- (1) CR TFs are encoded by unstable mRNA transcripts, requiring unusually high levels of continuous transcription
- (2) SEs are difficult to turn on, ensuring lineage restriction



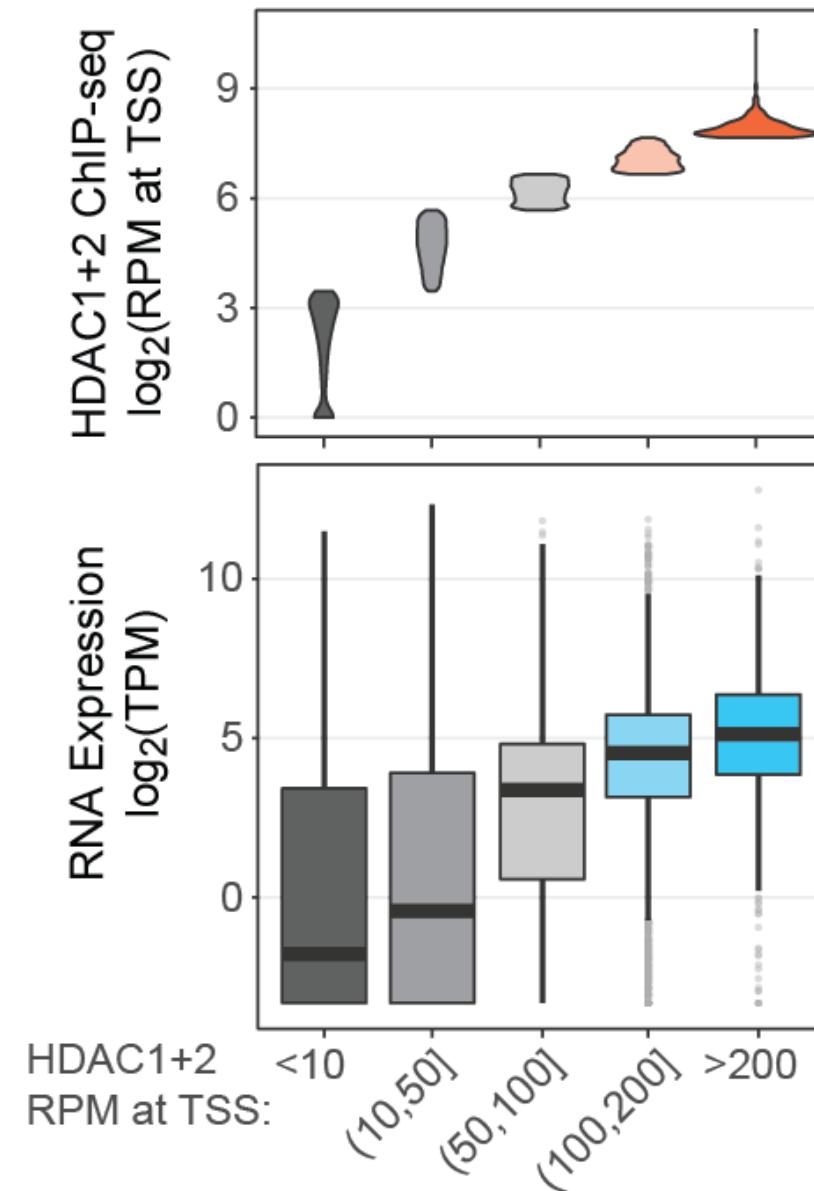
CR TF co-bind with HDACs

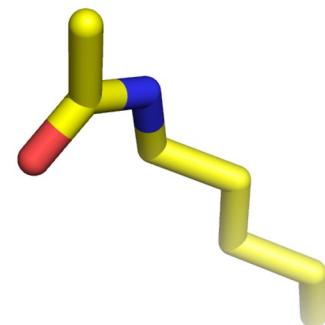
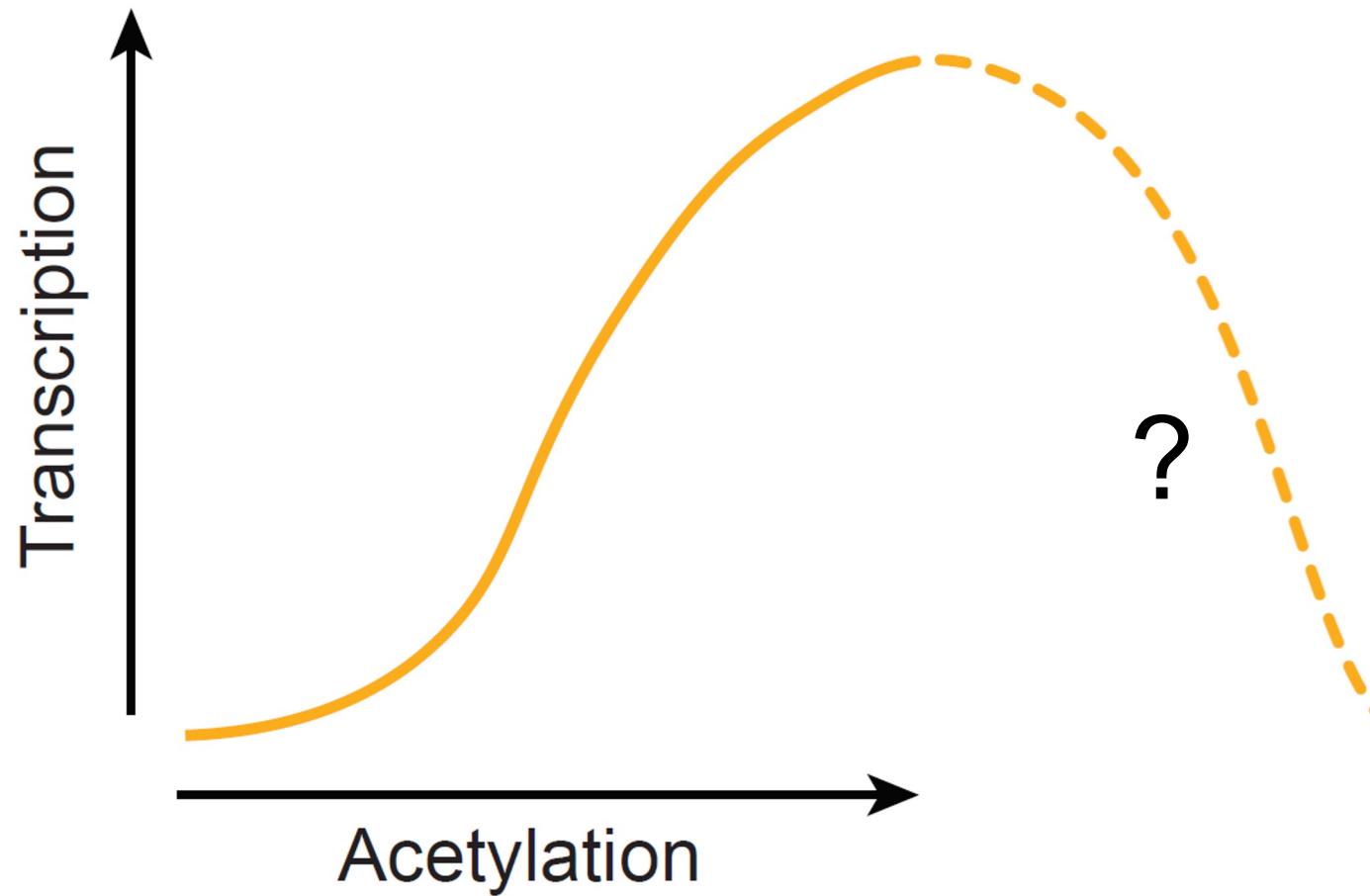


CR TF co-bind with HDACs



More HDAC \propto more transcription

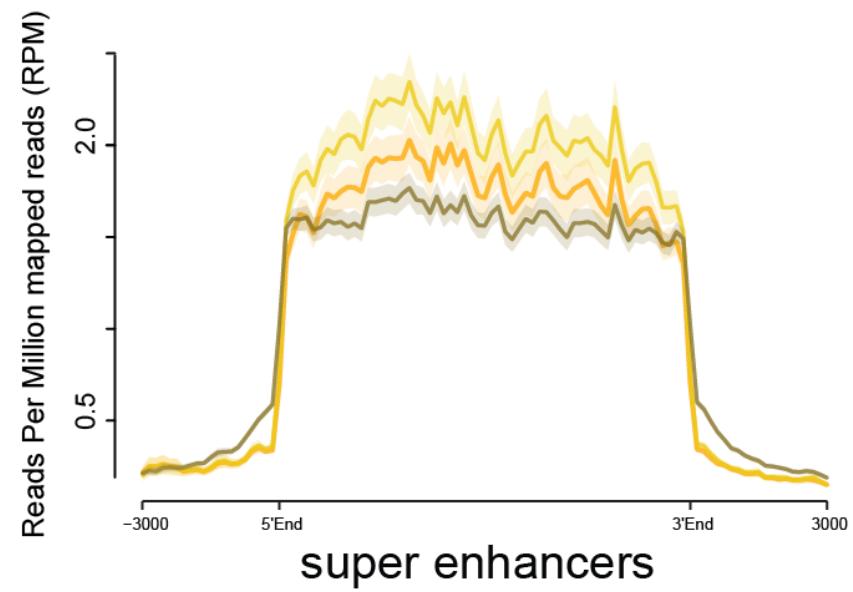




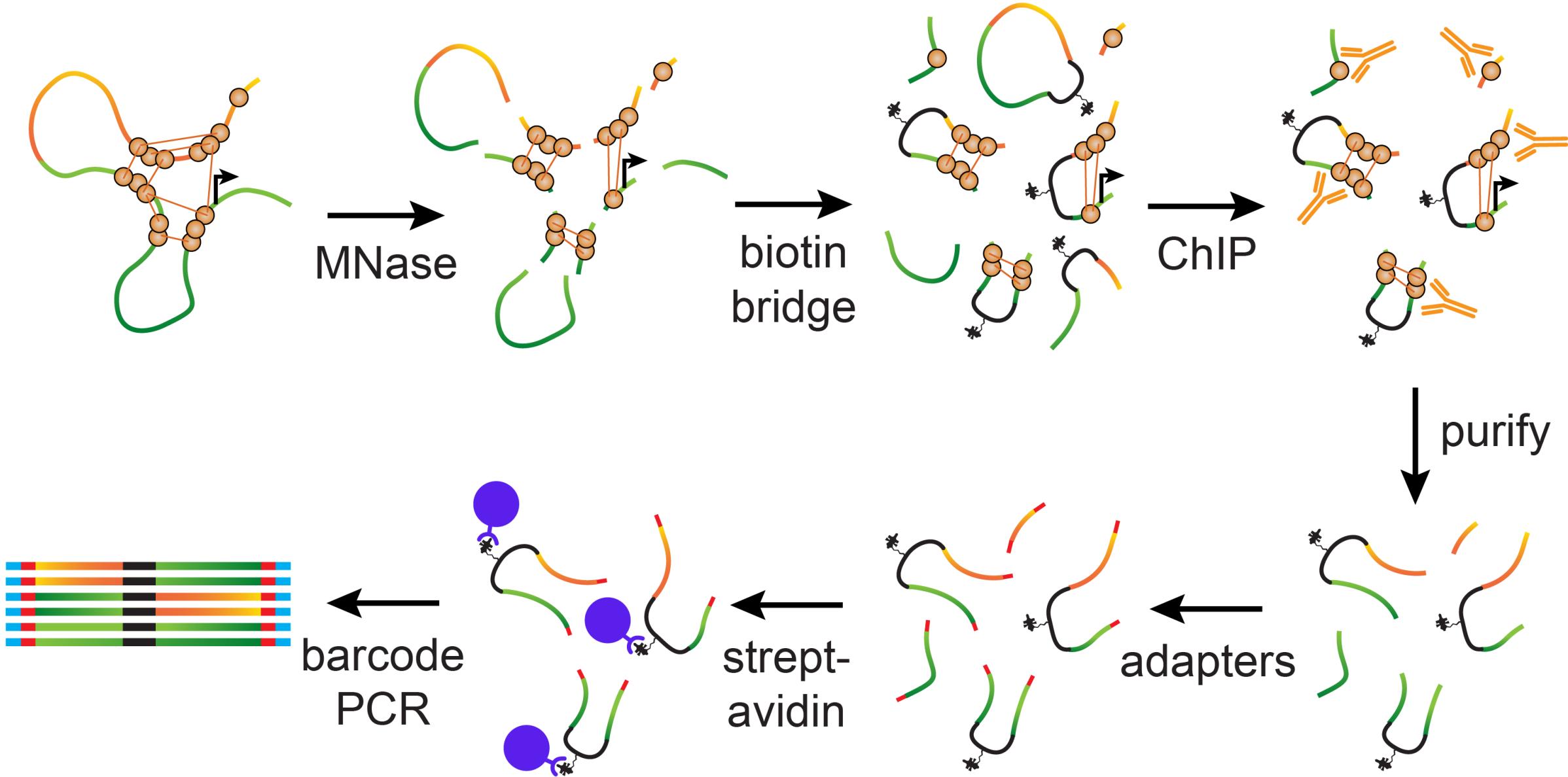
Spike in ChIP-seq: ChIP-Rx

H3K27ac

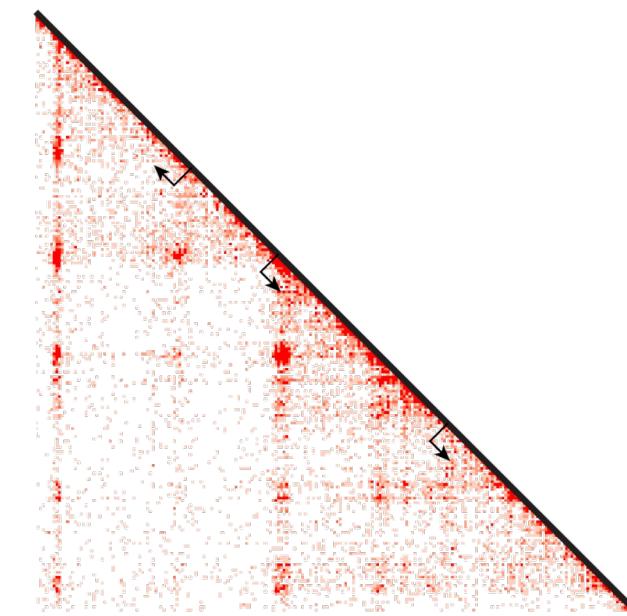
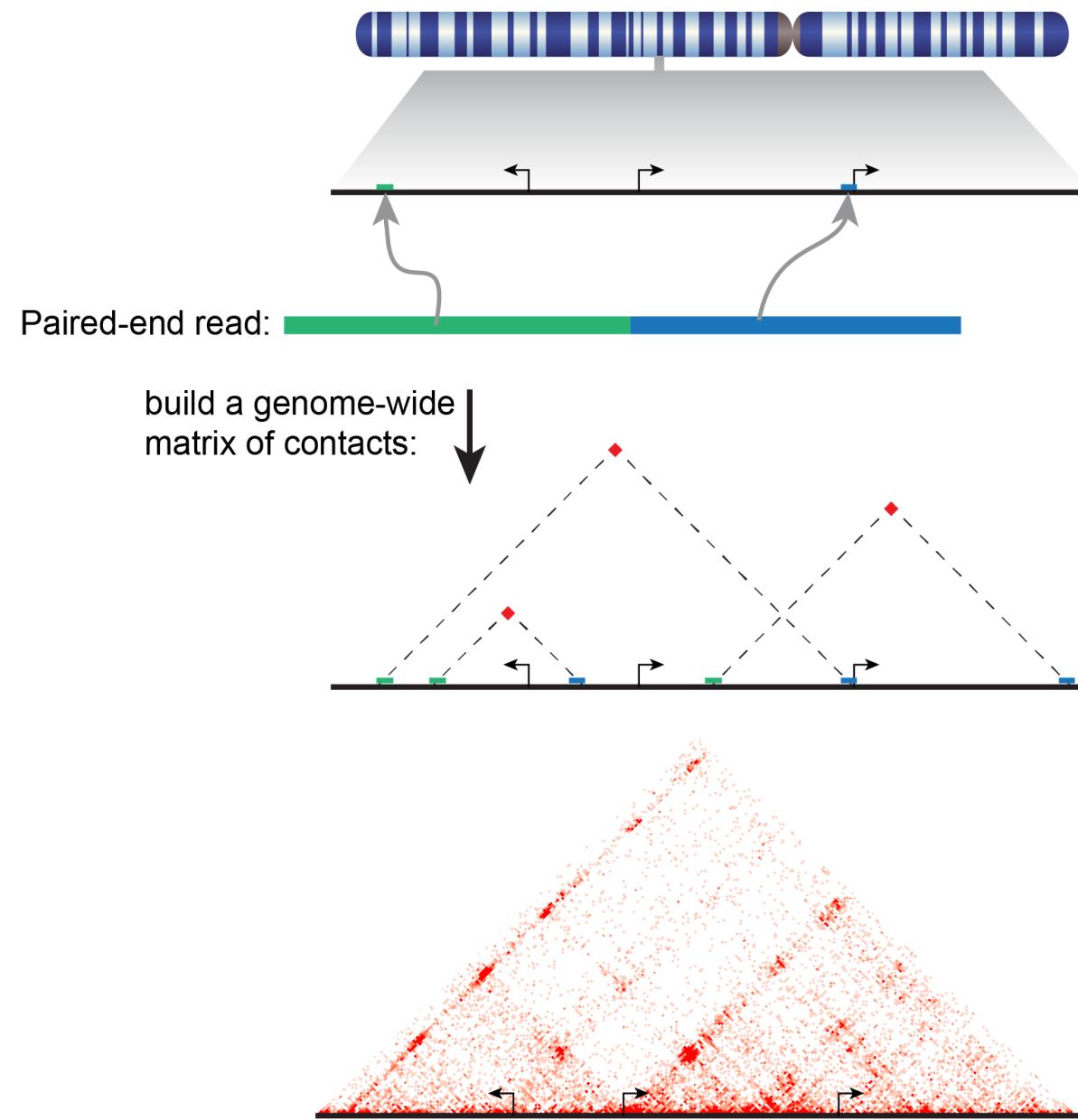
- DMSO
- Entinostat 1 hrs
- Entinostat 6 hrs



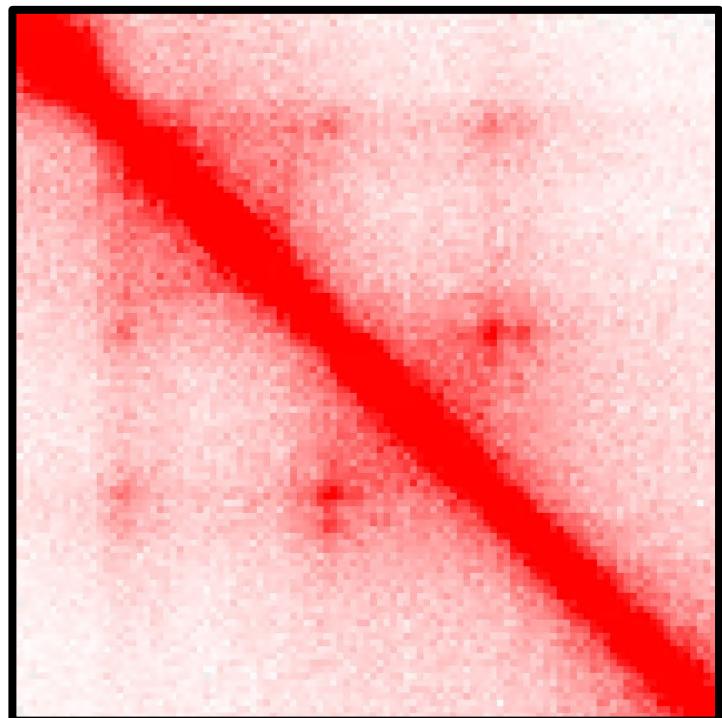
How can we measure genome architecture?



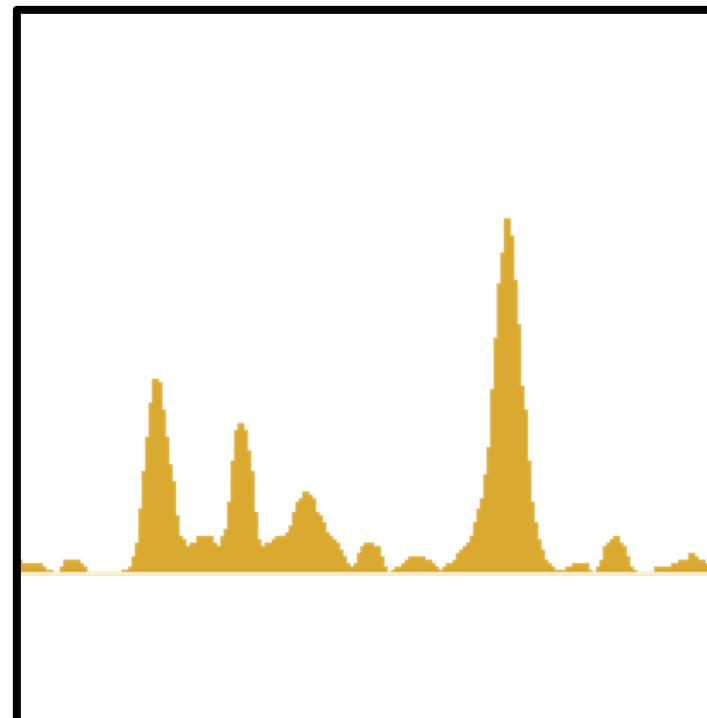
How can we measure genome architecture?



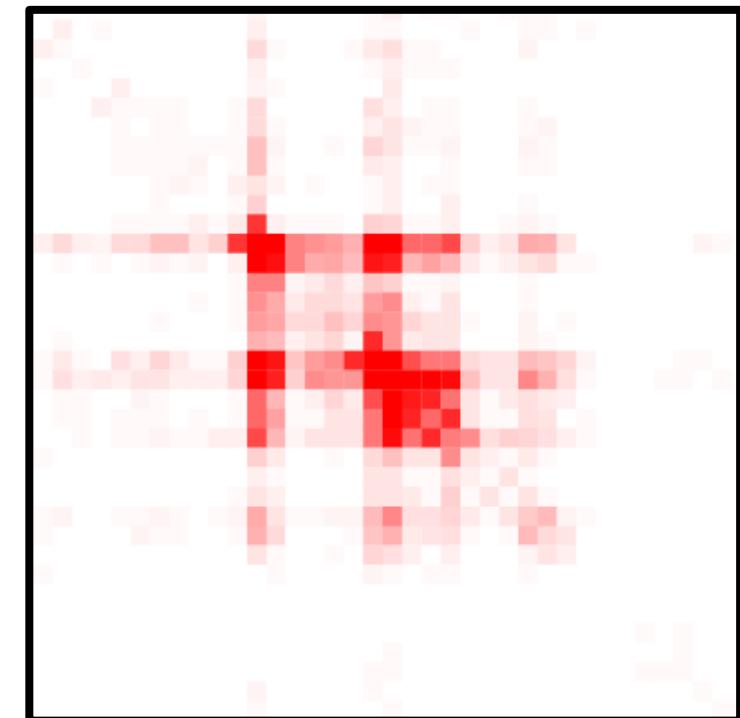
HiC



ChIP-seq



HiChIP



+

=

2009, *Nature*, Fullwood ... Ruan: **ChIA-PET**

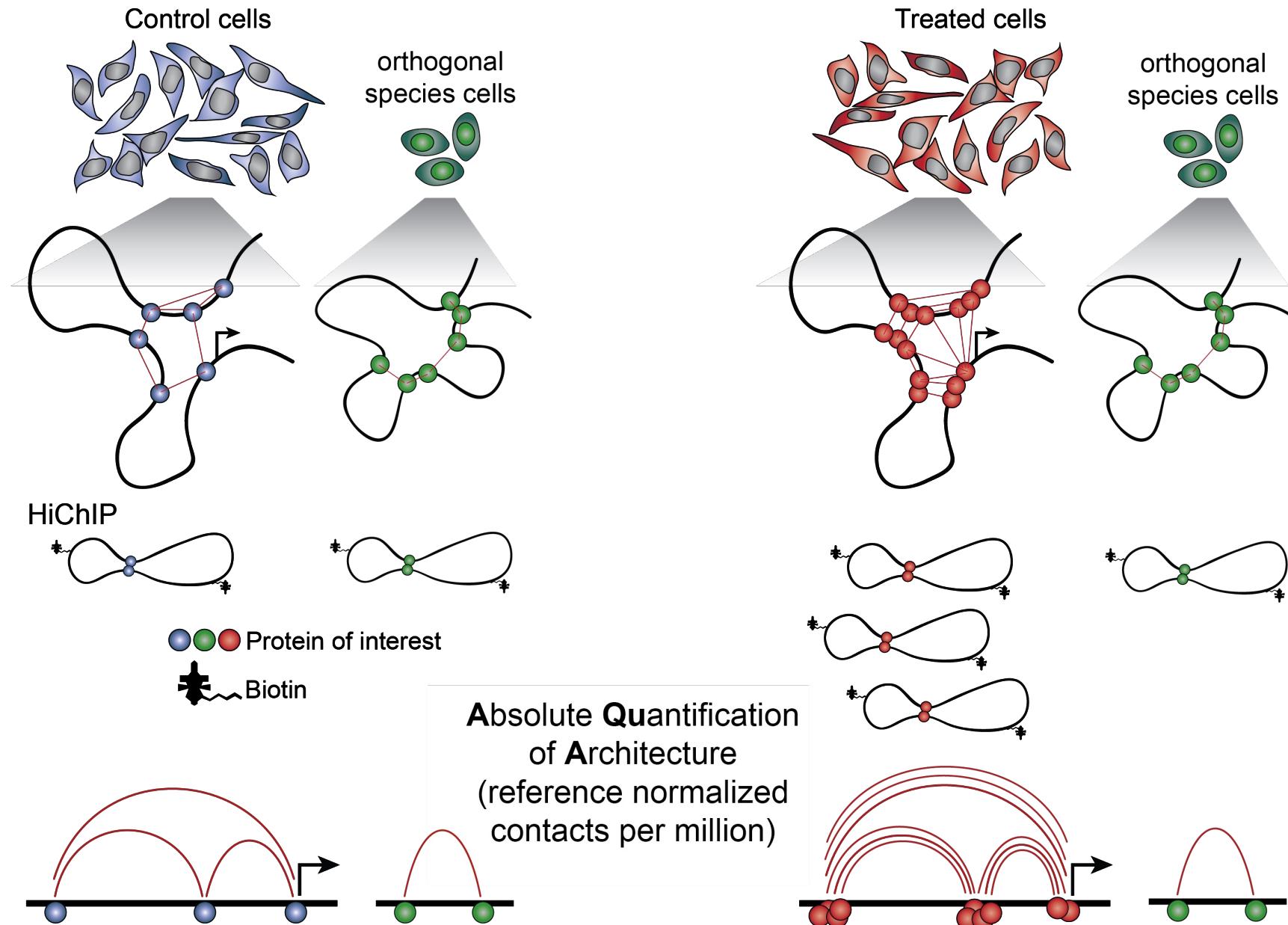
2016, *Nat Meth*, Mumbach ... Greenleaf, Chang: **HiChIP**

2016, *Cell Res*, Fang ... Ren: **PLAC-seq**

Measurement of differential chromatin interactions with absolute quantification of architecture (AQuA-HiChIP)

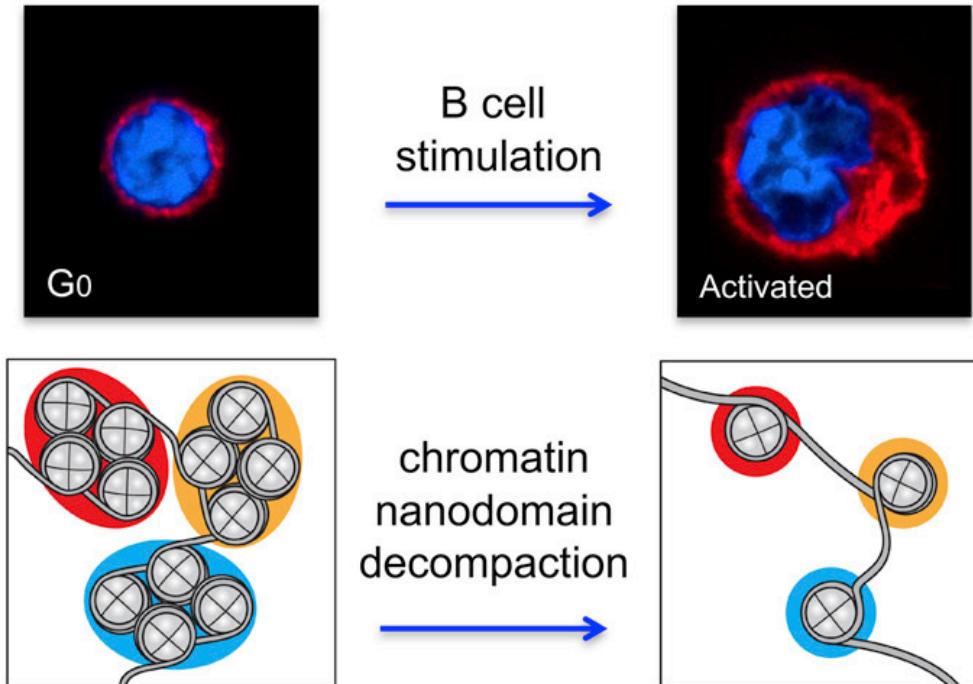
Berkley E. Gryder^{1,3*}, Javed Khan^{1*} and Benjamin Z. Stanton^{2,3*}

How can we see global changes in architecture?



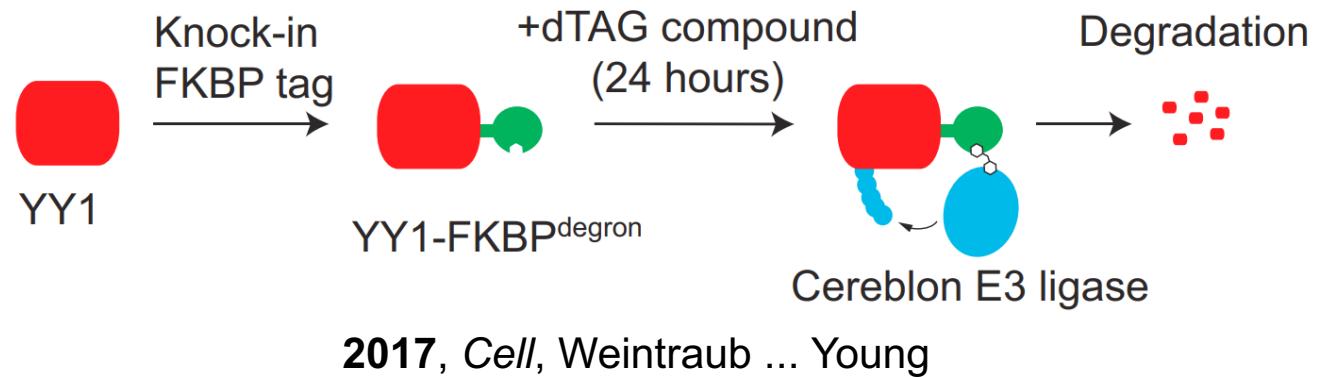
When to use AQuA?

global changes in chromatin compaction

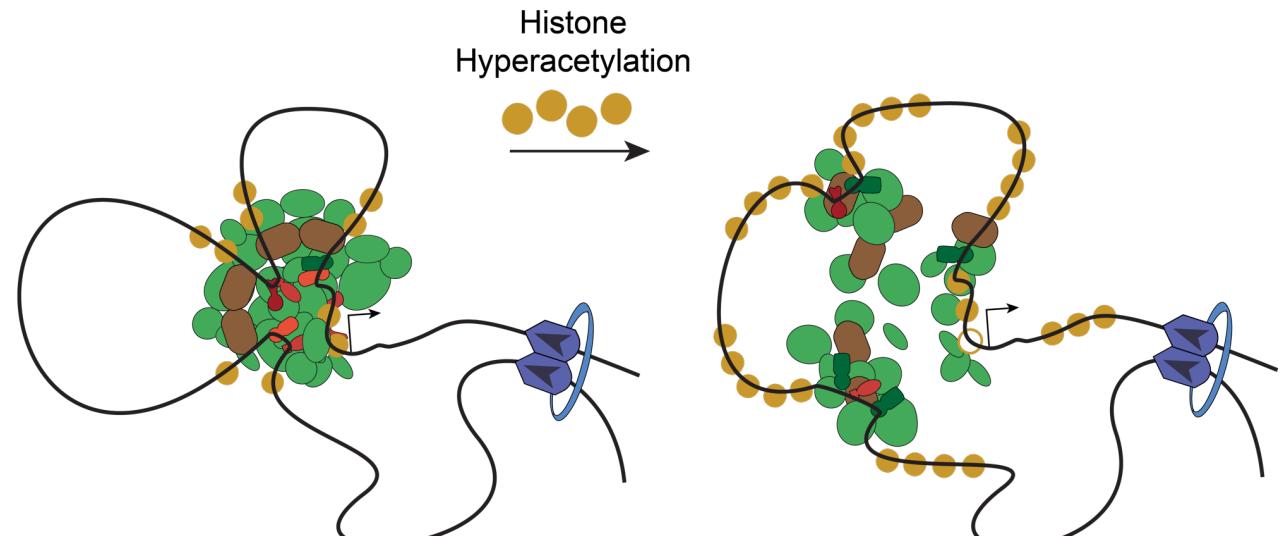


2017, *Molecular Cell*, Kieffer-Kwon ... Casellas

global changes in ChIP-target protein abundance



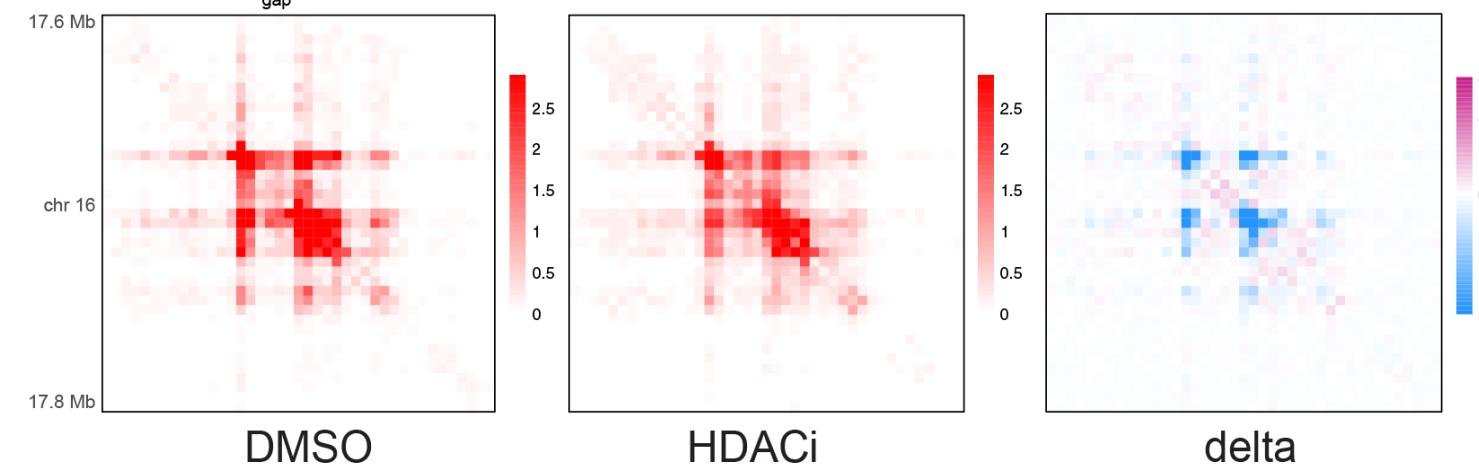
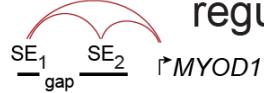
2017, *Cell*, Weintraub ... Young



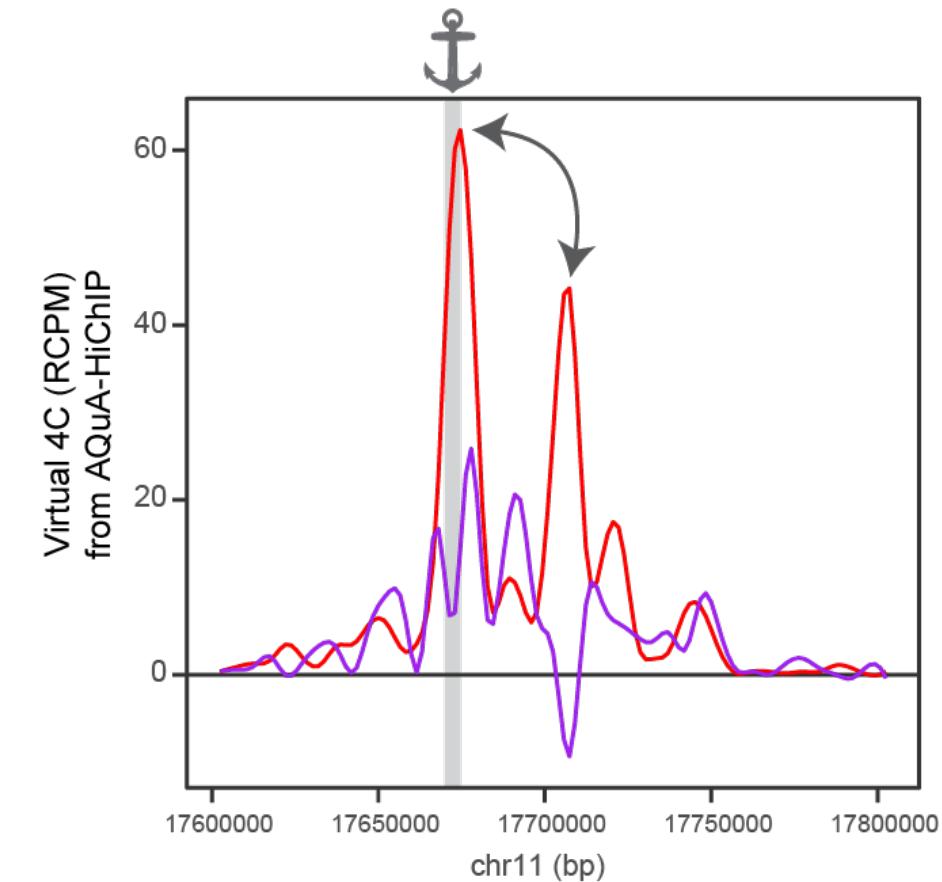
2019, *Nature Genetics*, Gryder ... Khan

H3K27ac

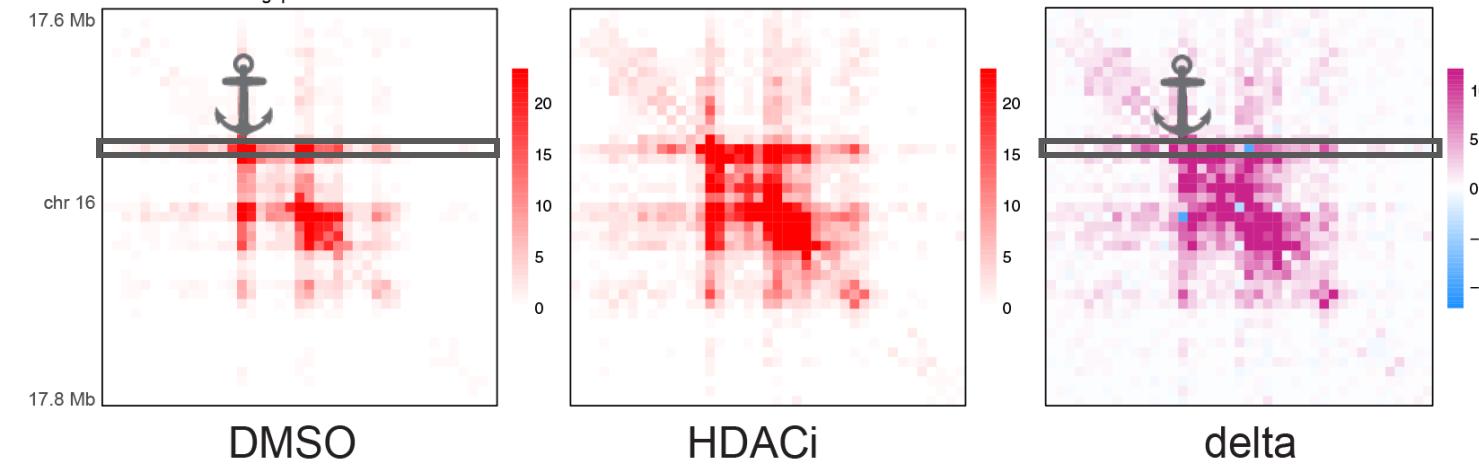
regular HiChIP (contacts per million)

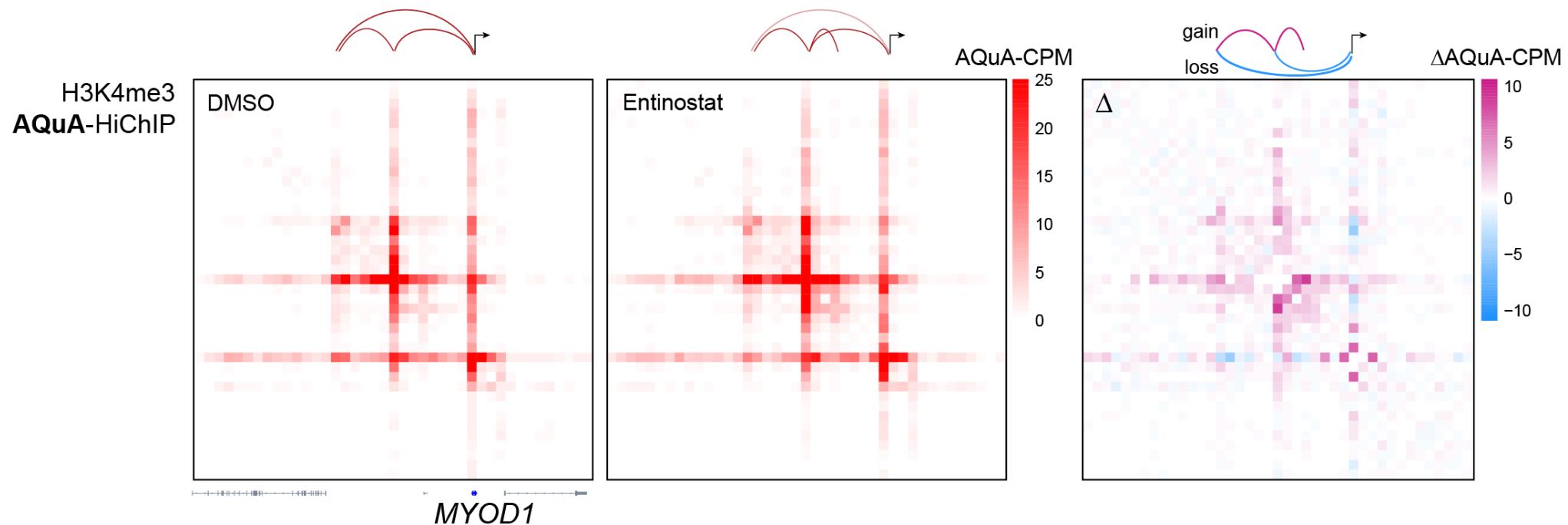
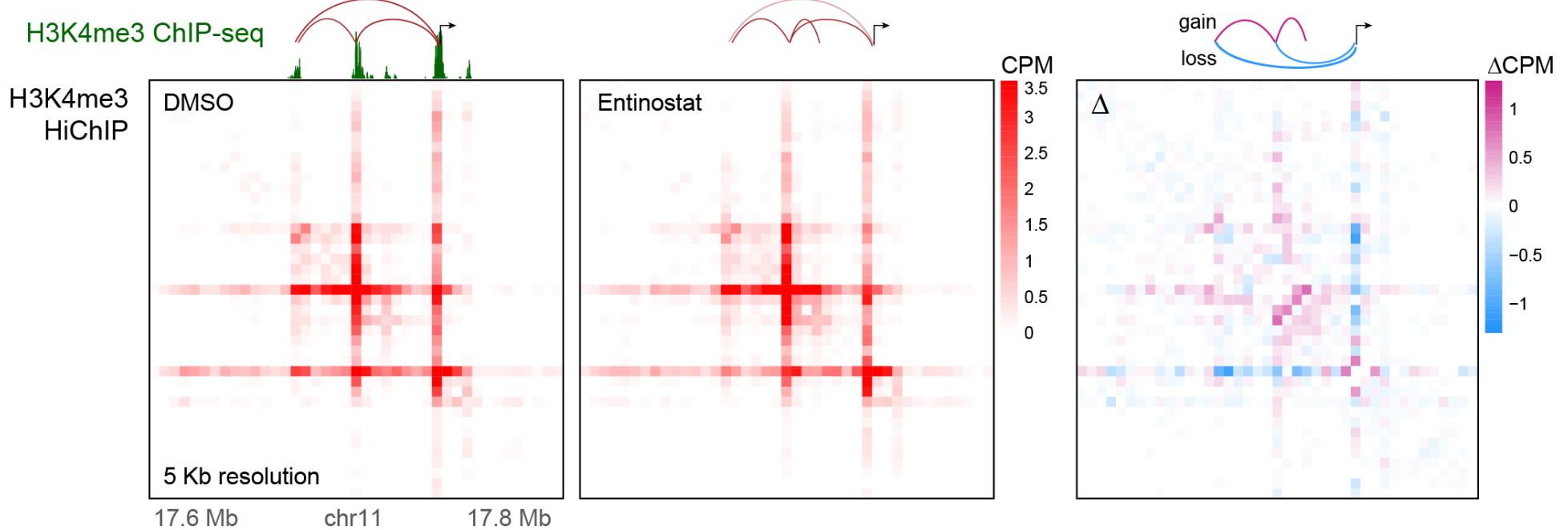


— DMSO
— delta (Entinostat - DMSO)



AQuA-HiChIP (reference normalized CPM)





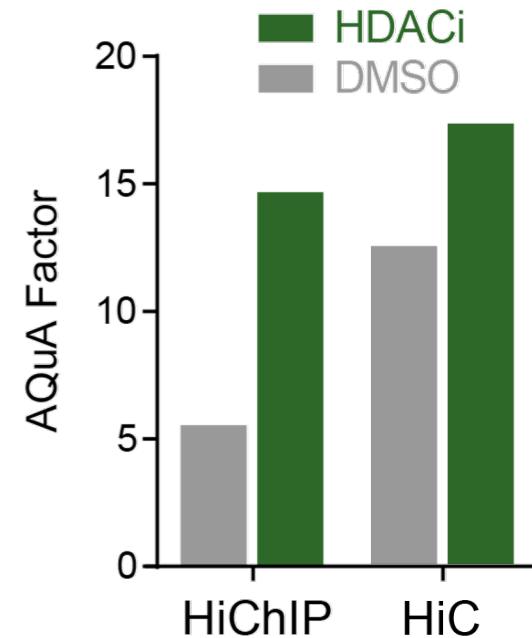
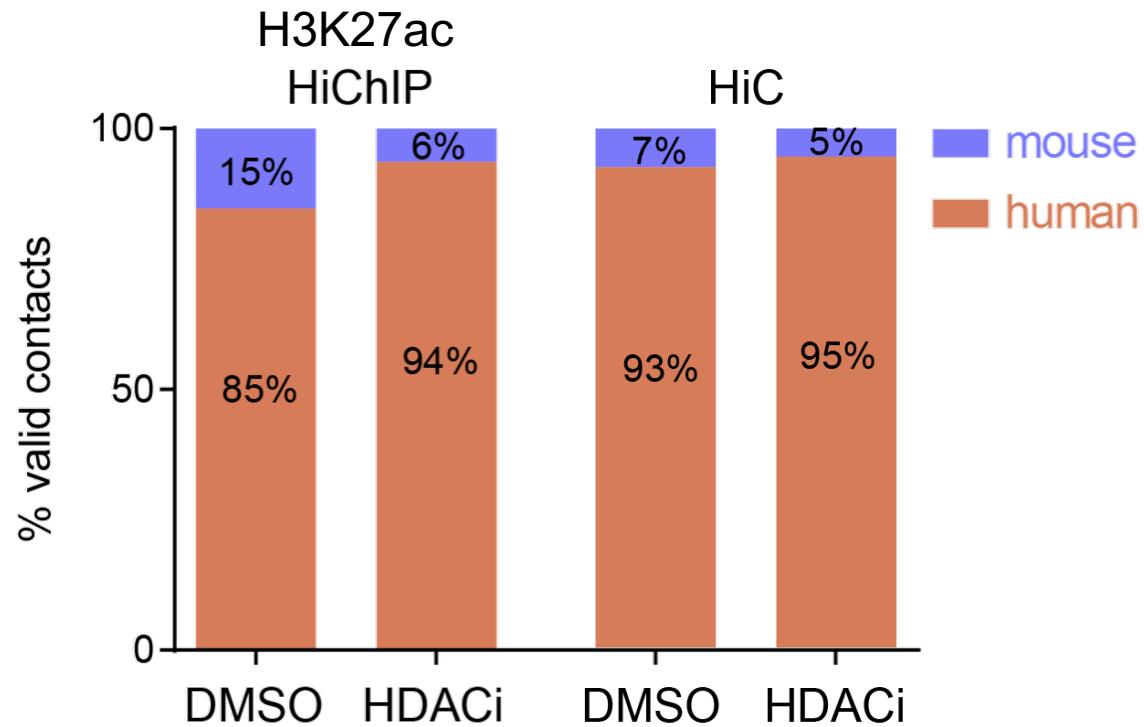
AQuA normalization strategy

$$(1) \text{ [Matrix]}_{\text{Contacts Per Million}} = \text{[Matrix]}_{\text{Raw contact counts}} \cdot \frac{1,000,000}{\text{total (hg+mm) contacts}}$$

$$(2) \text{ AQuA Factor} = \frac{\text{human unique valid contacts}}{\text{mouse unique valid contacts}}$$

$$(3) \text{ [Matrix]}_{\text{Reference normalized Contacts Per Million}} = \text{[Matrix]}_{\text{Contacts Per Million}} \cdot \text{AQuA Factor}$$

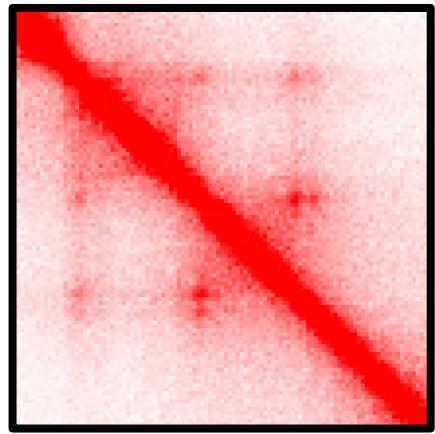
AQuA normalization strategy



(all experiments RH4 cells +/- Entinostat, 6 hours)

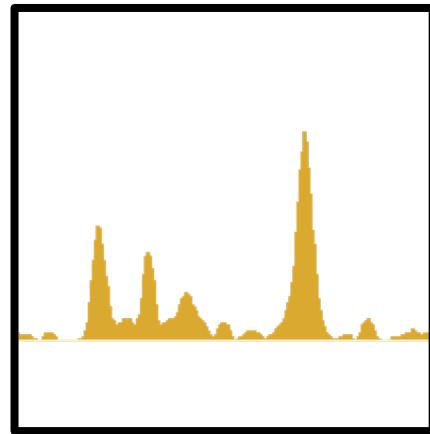
AQuA normalization strategy

AQuA
HiC



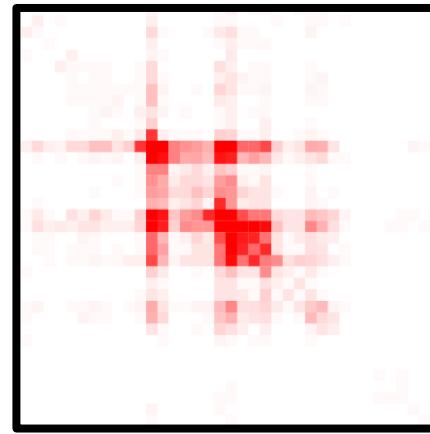
1.43x
AQuA
impact

ChIP-Rx

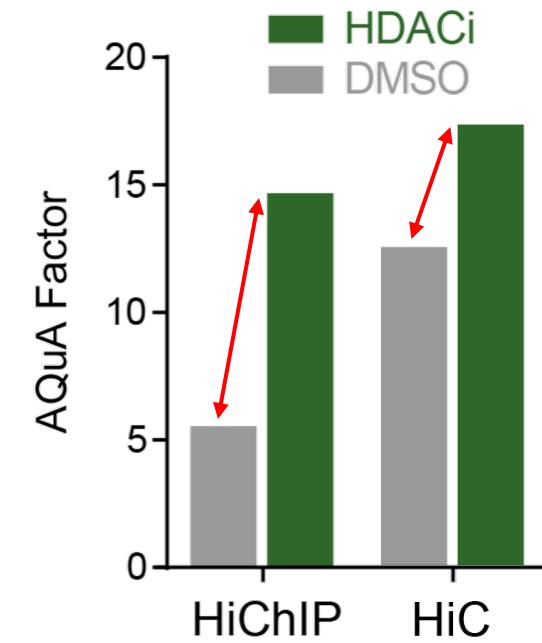


1.44x
Rx
impact

AQuA
HiChIP

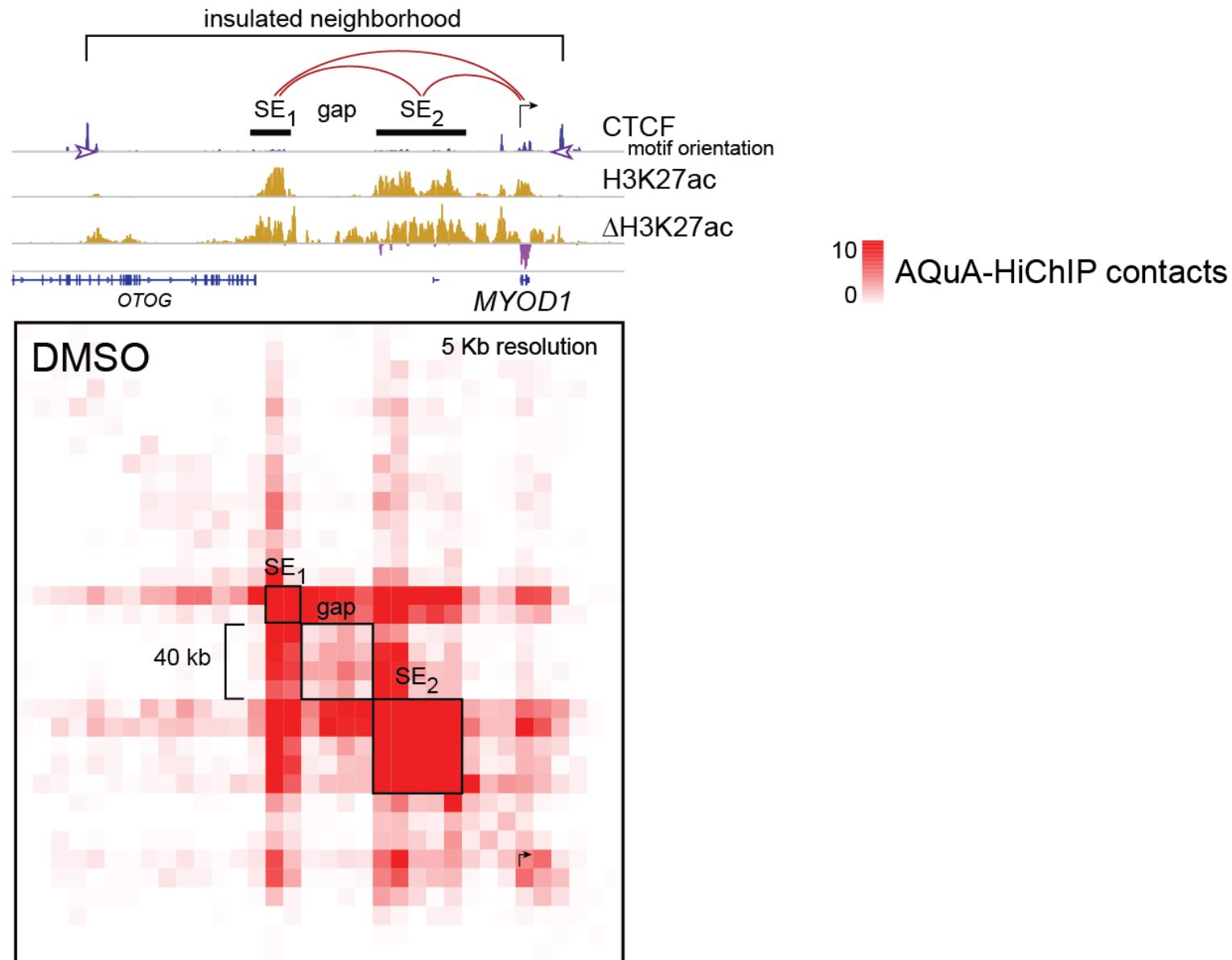


2.76x
AQuA
impact

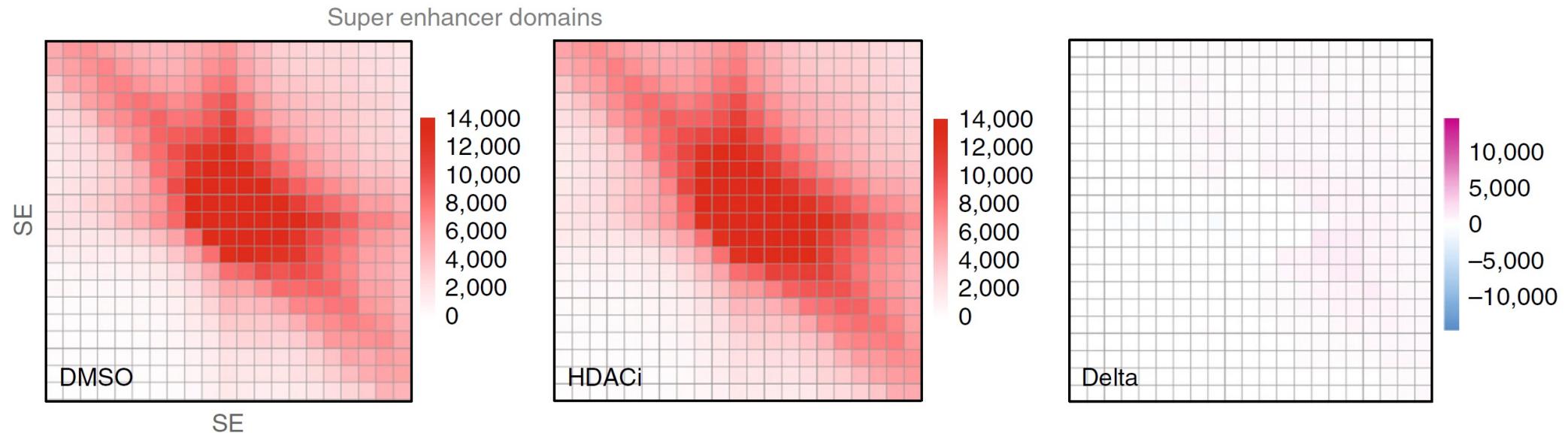


(all experiments RH4 cells +/- Entinostat, 6 hours)

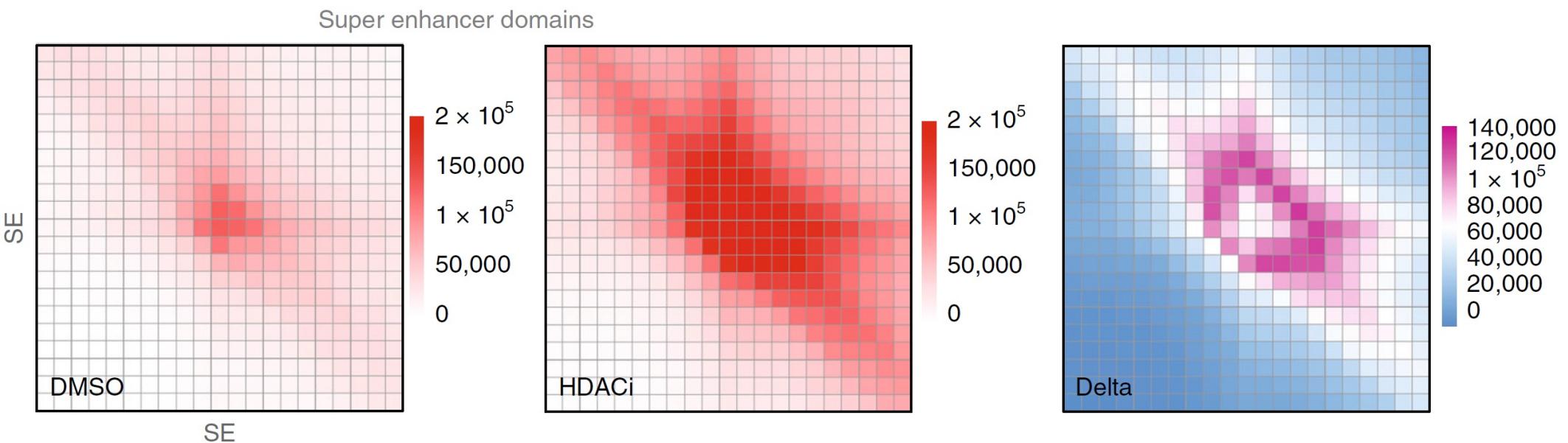
Super Enhancers must Mind the Gap



HiChIP
(H3K27ac)

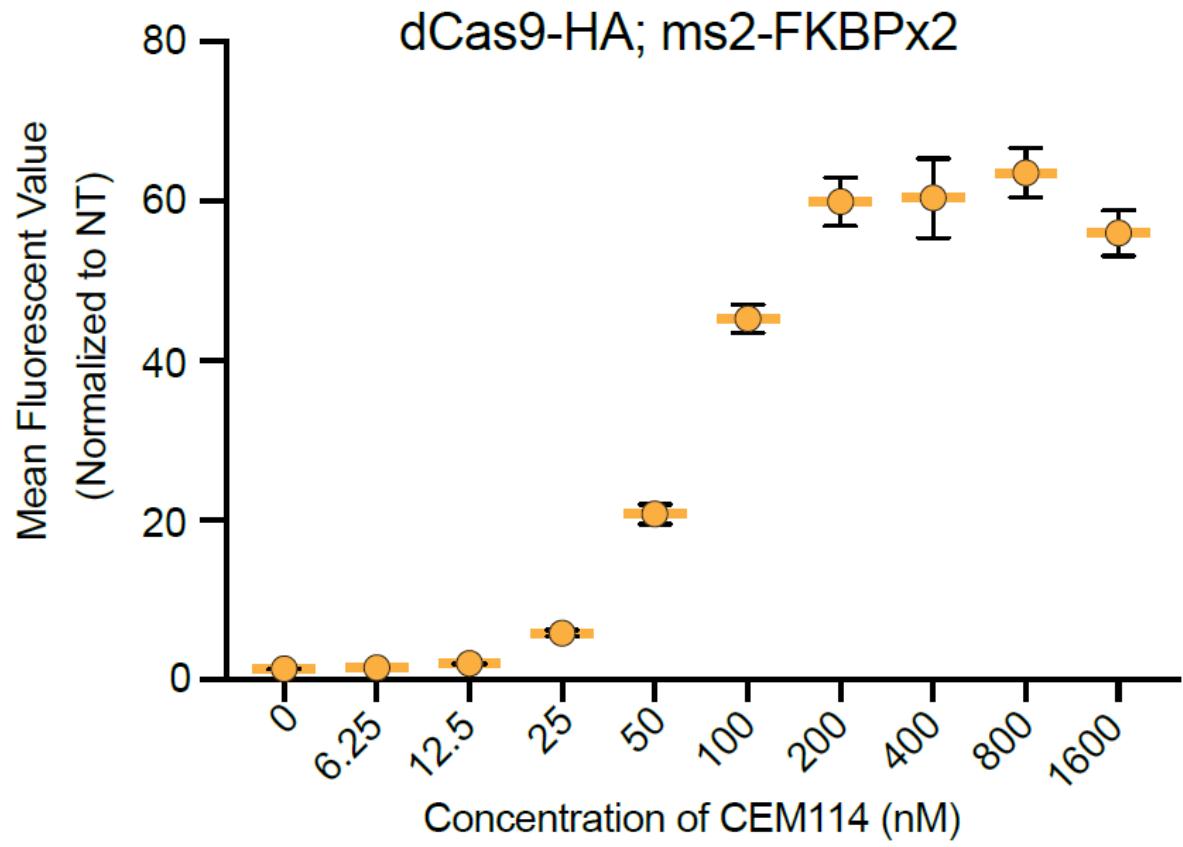
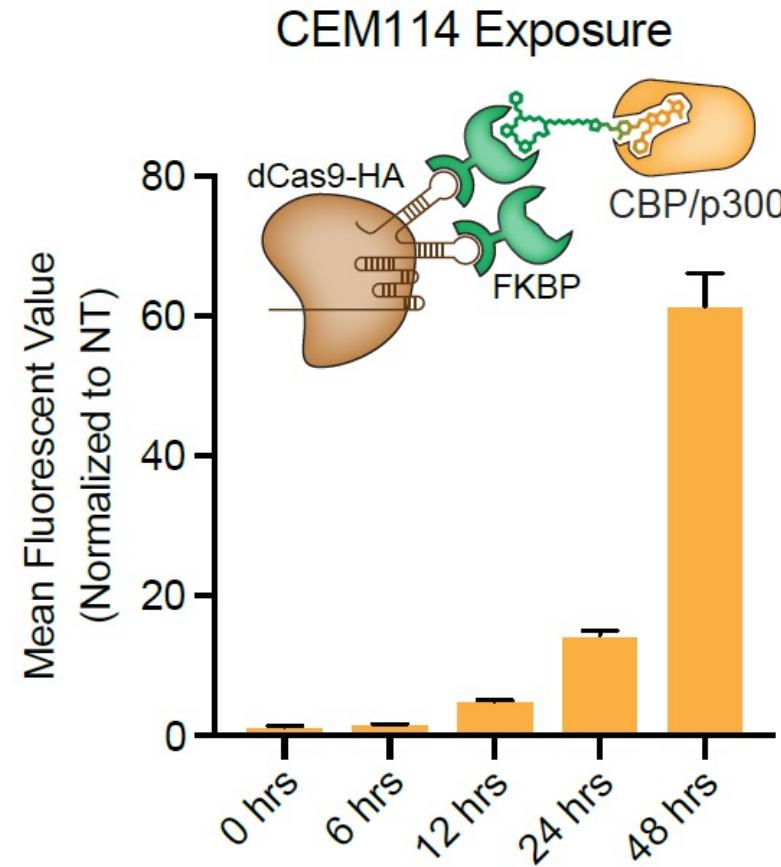


AQuA
HiChIP
(H3K27ac)

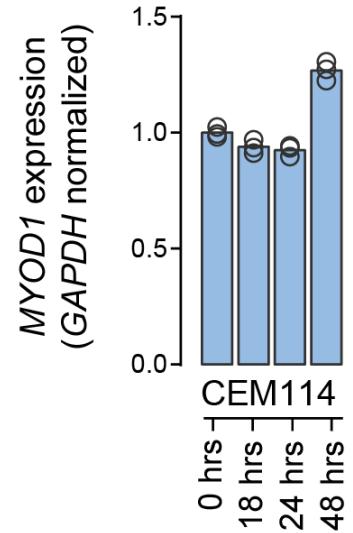
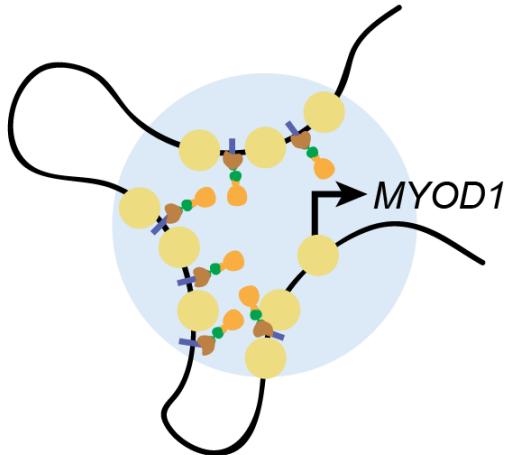
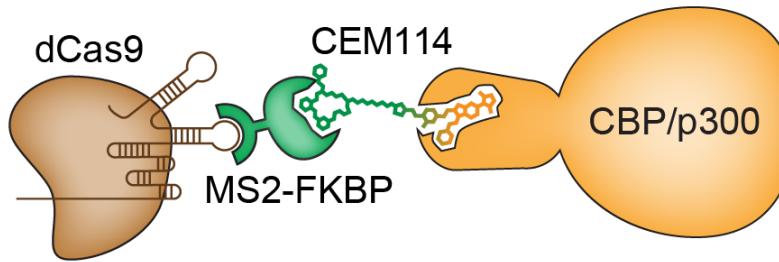


Can hyper-acetylating the boundaries of
1 super cluster phenocopy the effects of
small molecule HDAC inhibition?

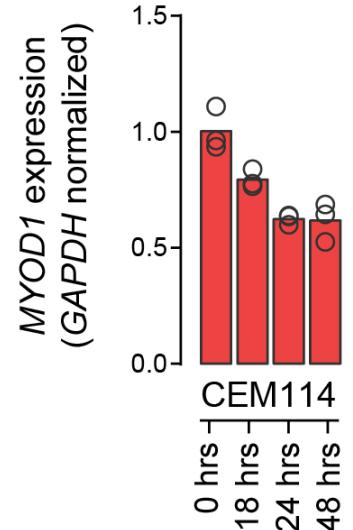
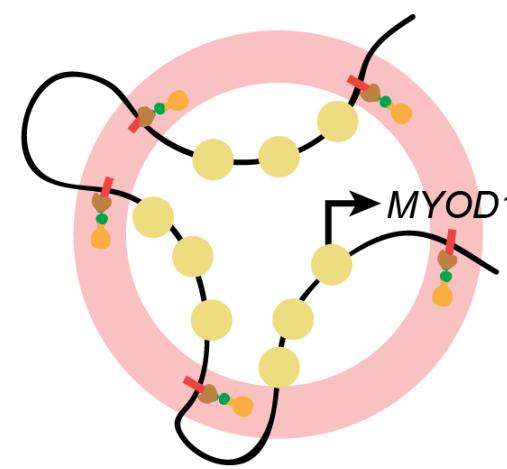
Recruitment of HAT by dCas9-FKPB



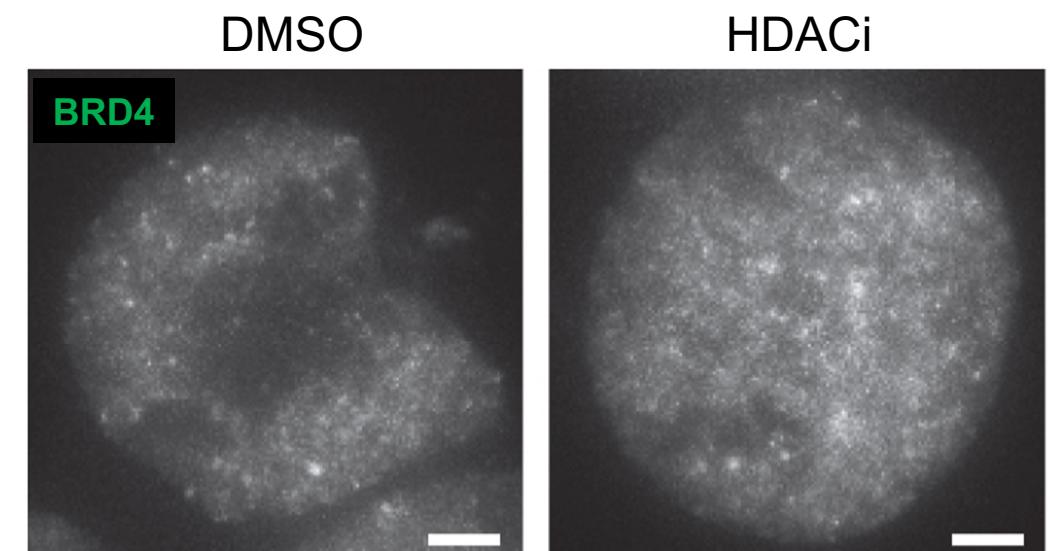
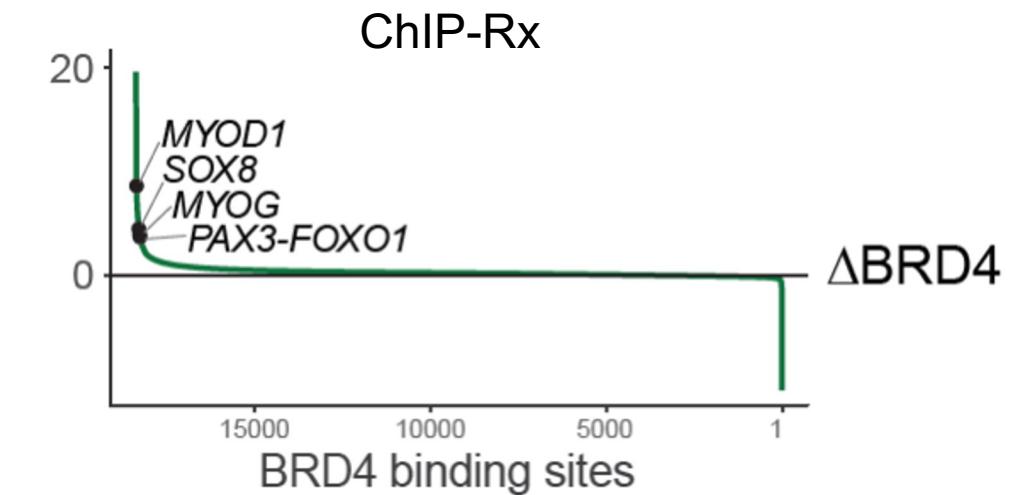
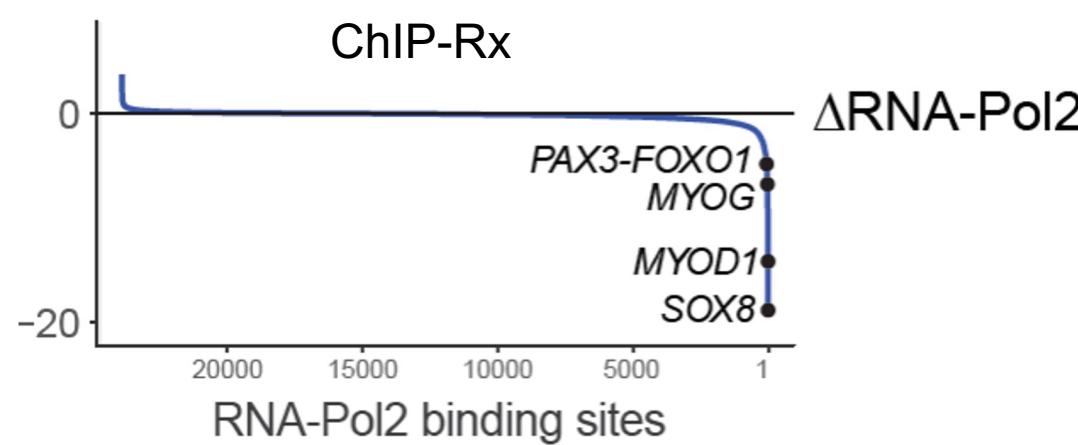
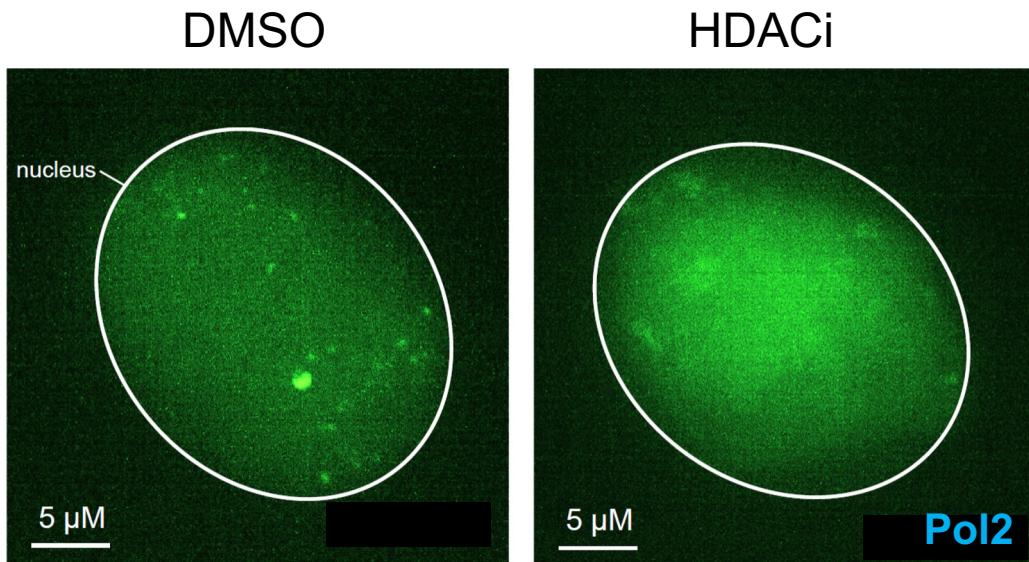
Recruitment of HAT by dCas9-FKPB



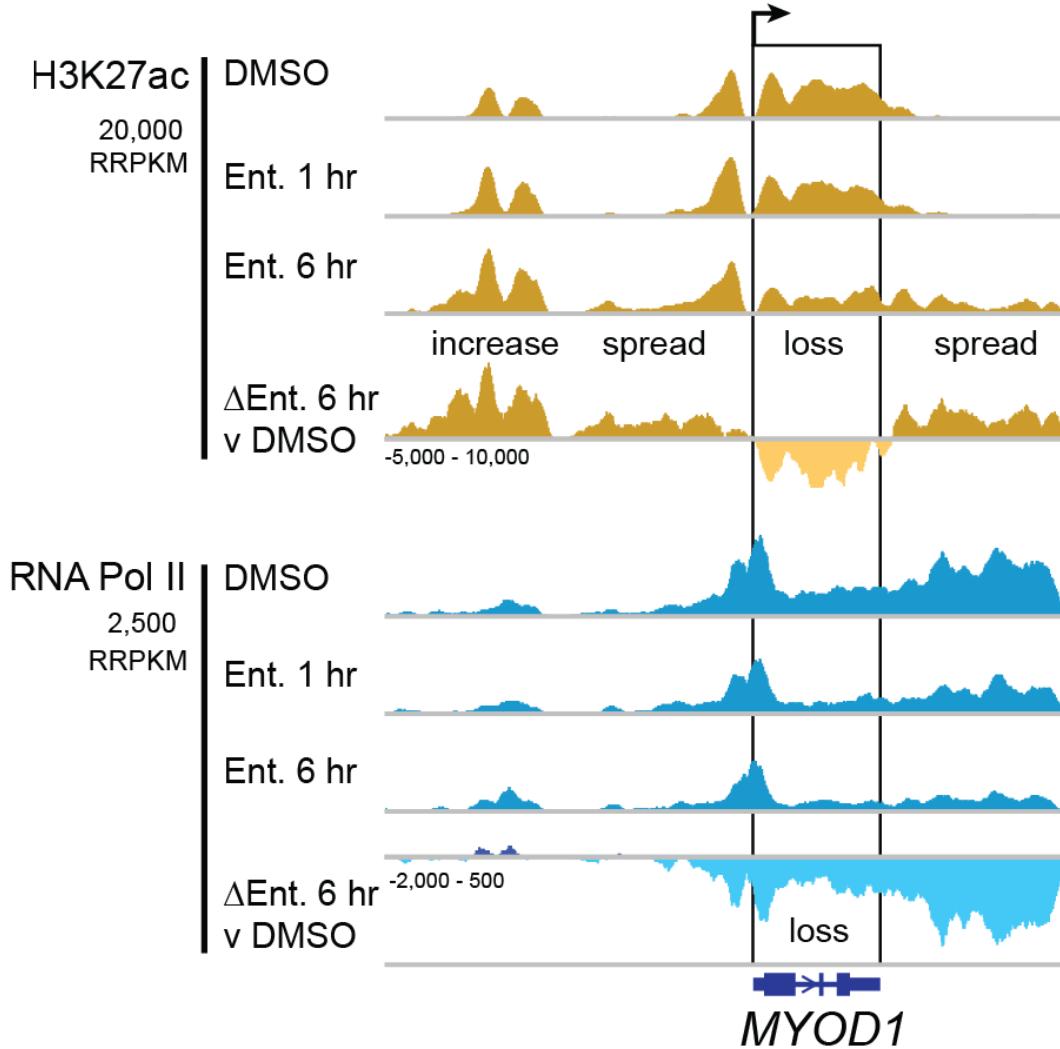
MYOD1



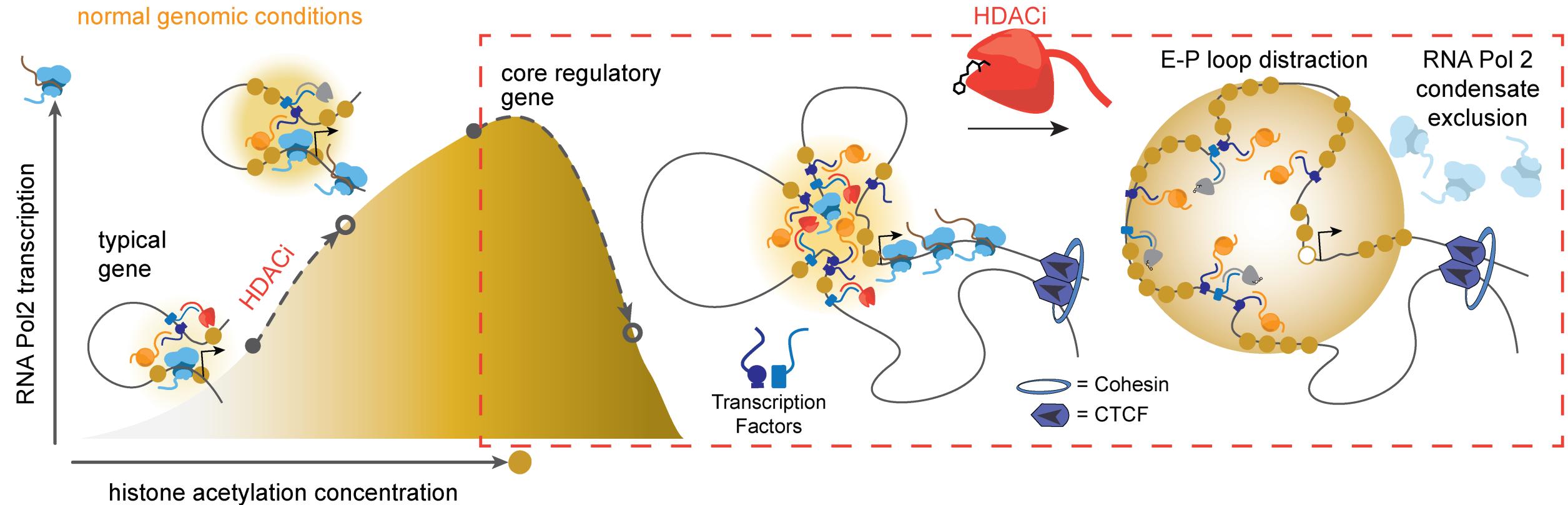
HDACi disrupts Pol2 but not BRD4 puncta



HDACi removes Pol2 at SEs

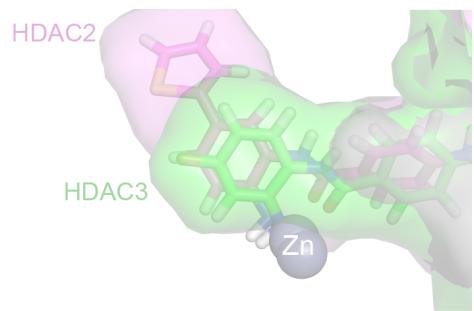


Histone Acetylation Paradox Explained by Dynamic Opposition



~1000-3000 histone lysine + charges lost per CR TF loci

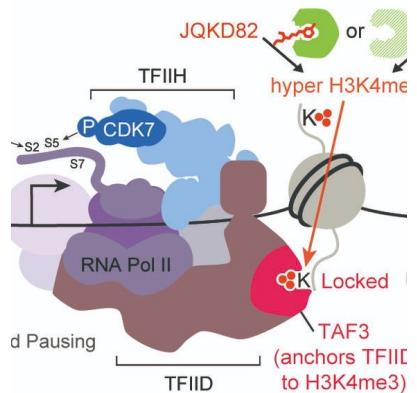
HDACs & H3K27ac



New mechanism to explain HDAC inhibition

Nature Comm., 2019
Nature Genetics, 2019

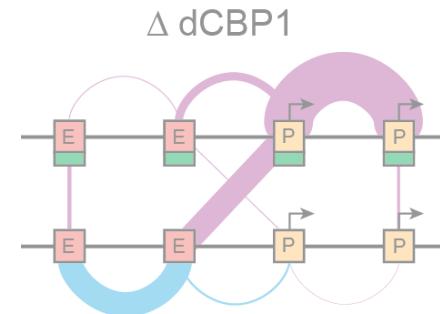
KDM5A & H3K4me3



New mechanism to explain KDM5A inhibition

Blood Cancer Discovery, 2021

HATs & RNA Pol 2



Pol2 cluster collapse

Unpublished



Lysine Demethylase 5A is Required
for MYC-Driven Transcription
in Multiple Myeloma

Blood Cancer Discovery, 2021



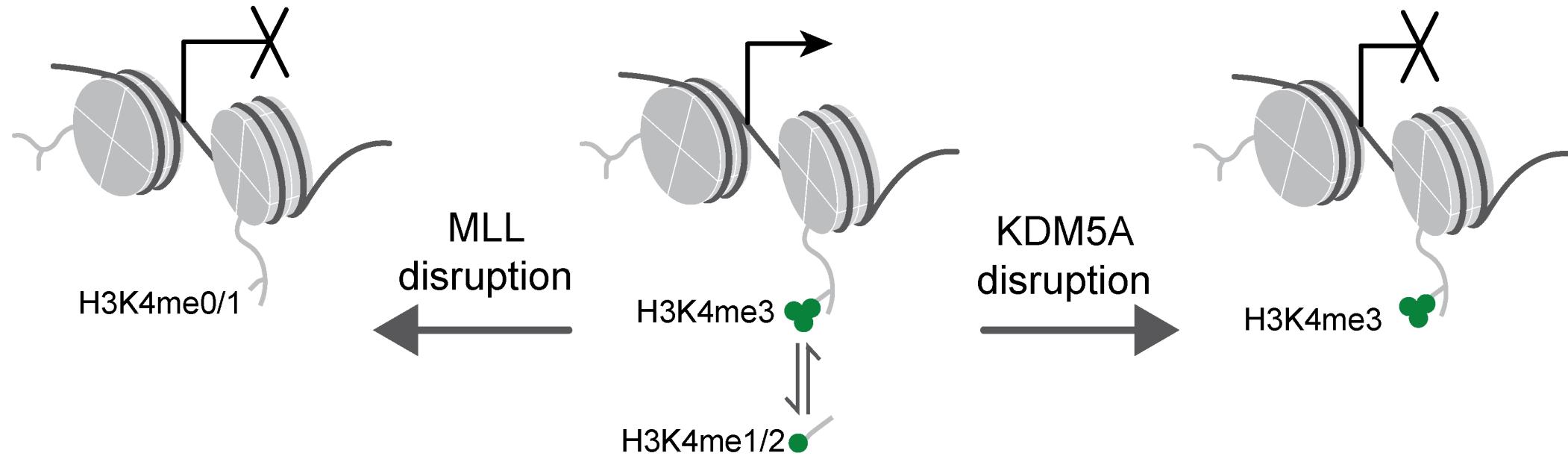
Hiroto
Ohguchi



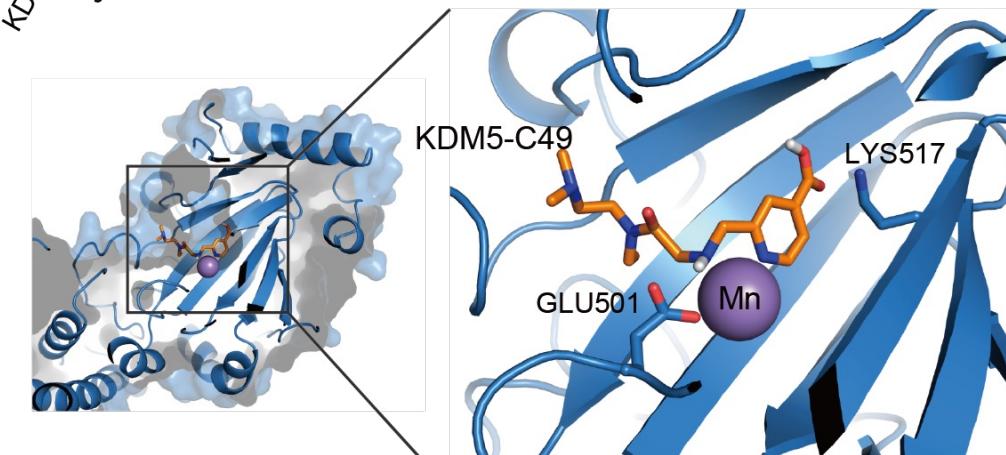
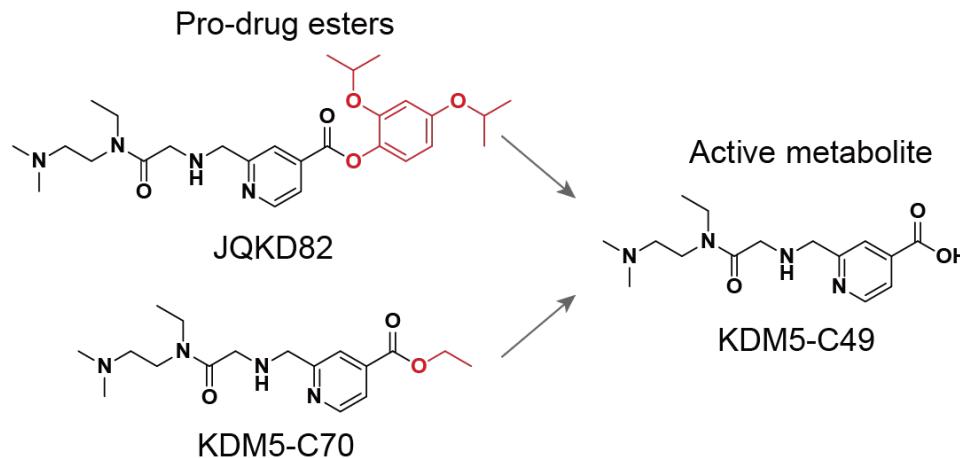
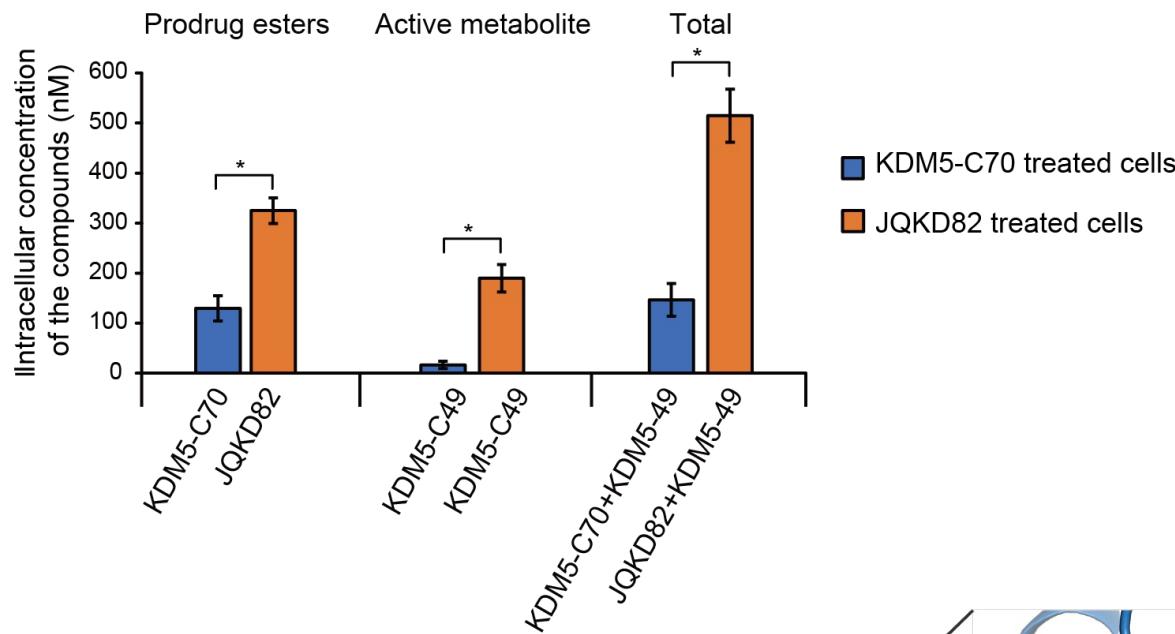
Jun
Qi

Rotary
Evaporator

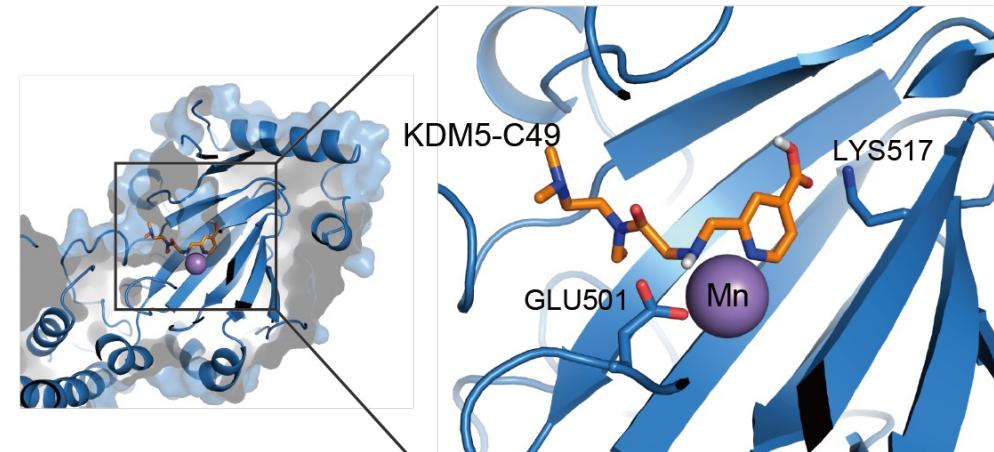
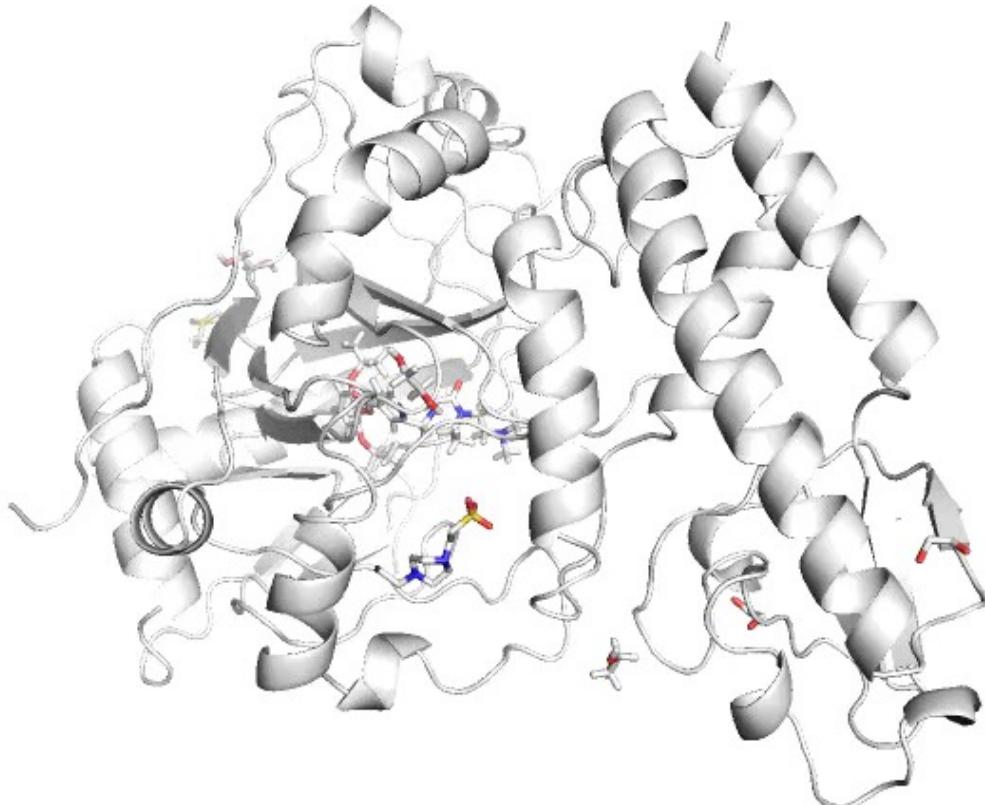
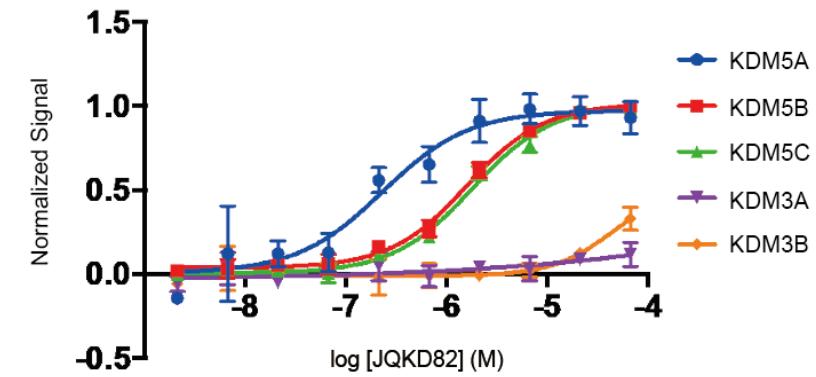
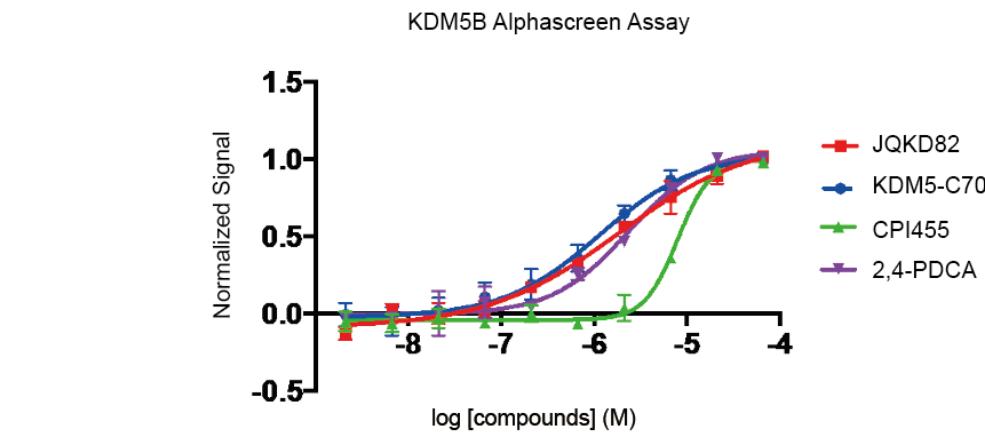
KDM5A erases H3K4me3



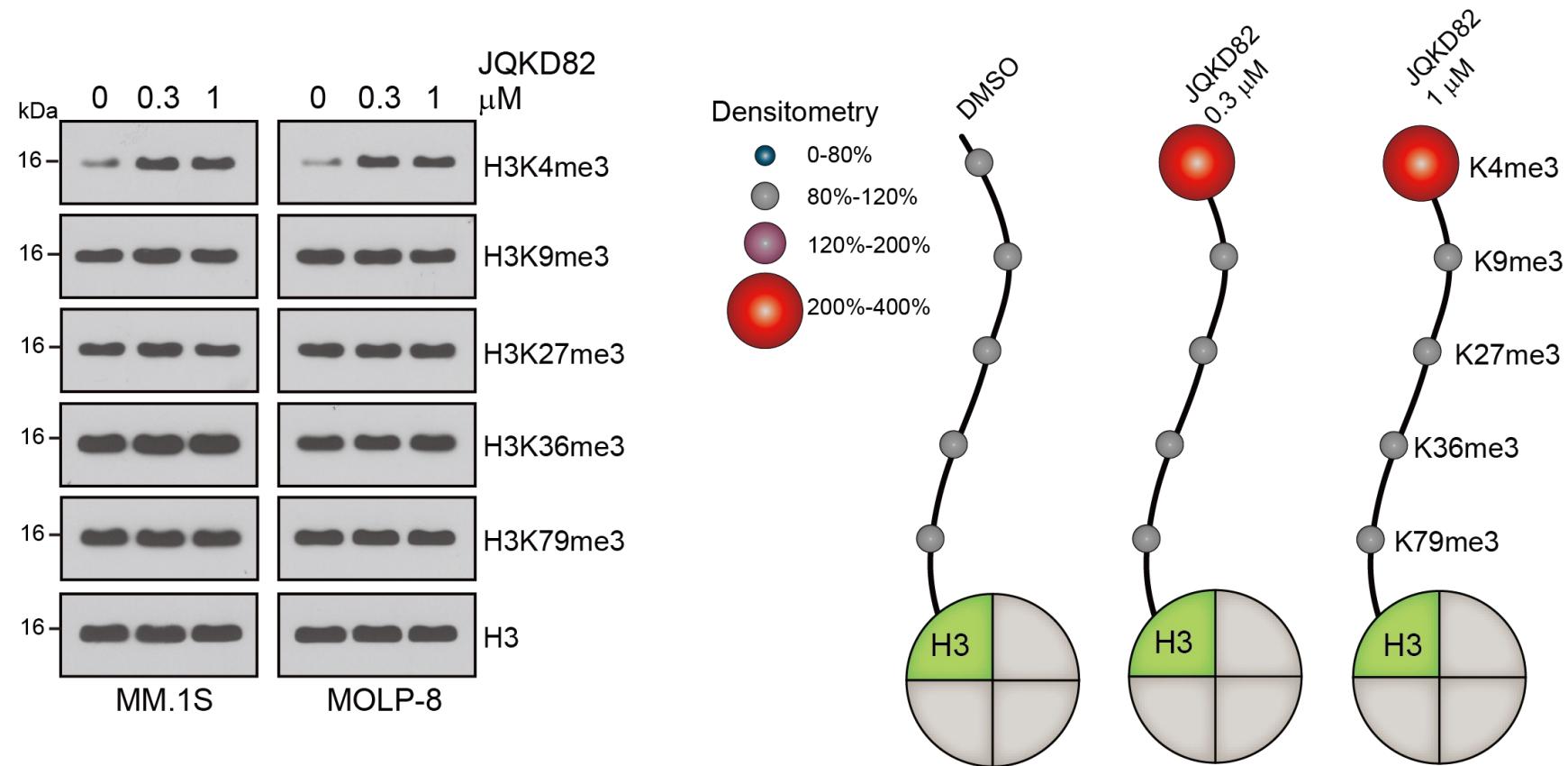
Designing our way through cell membranes, counting on metabolism

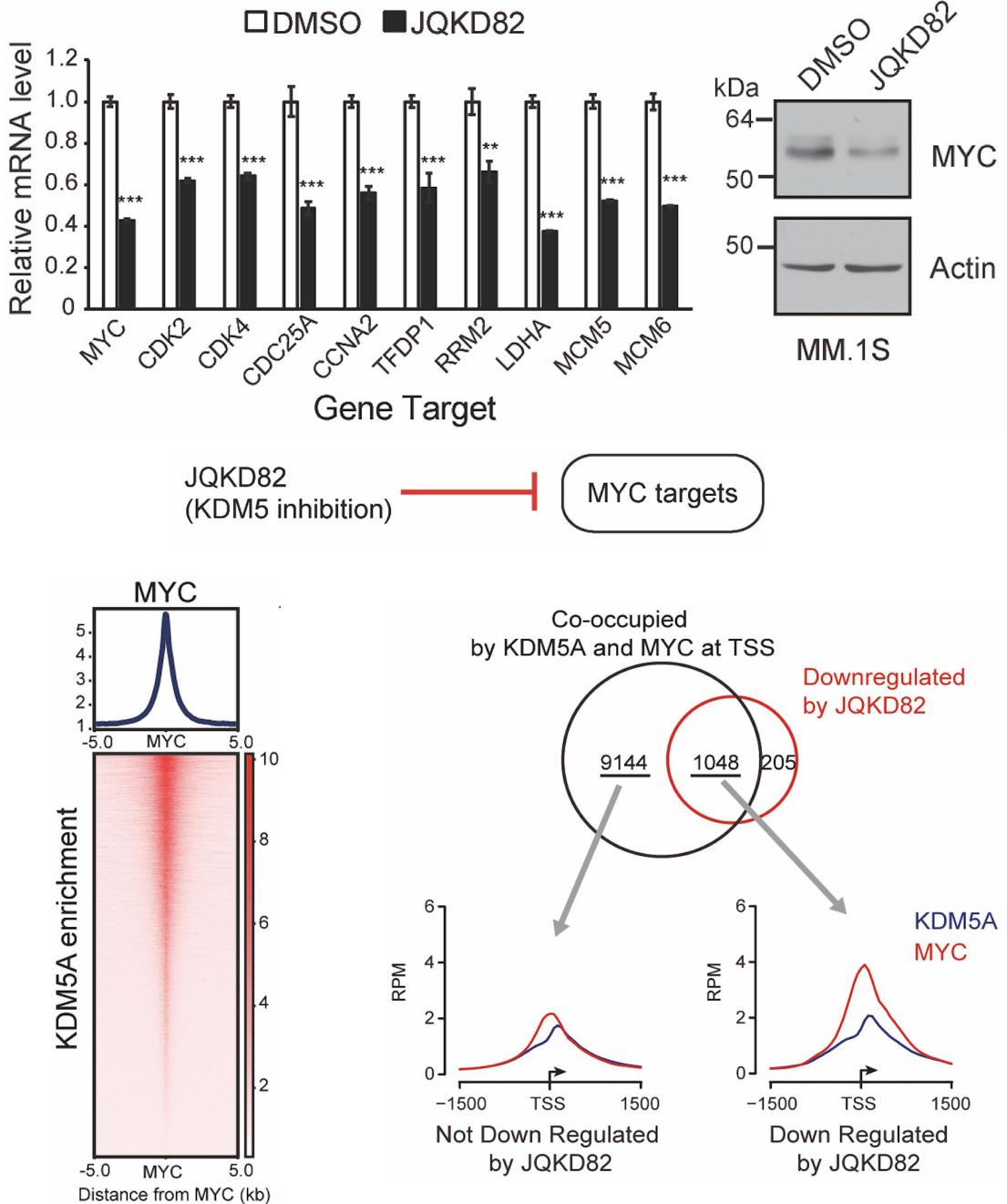
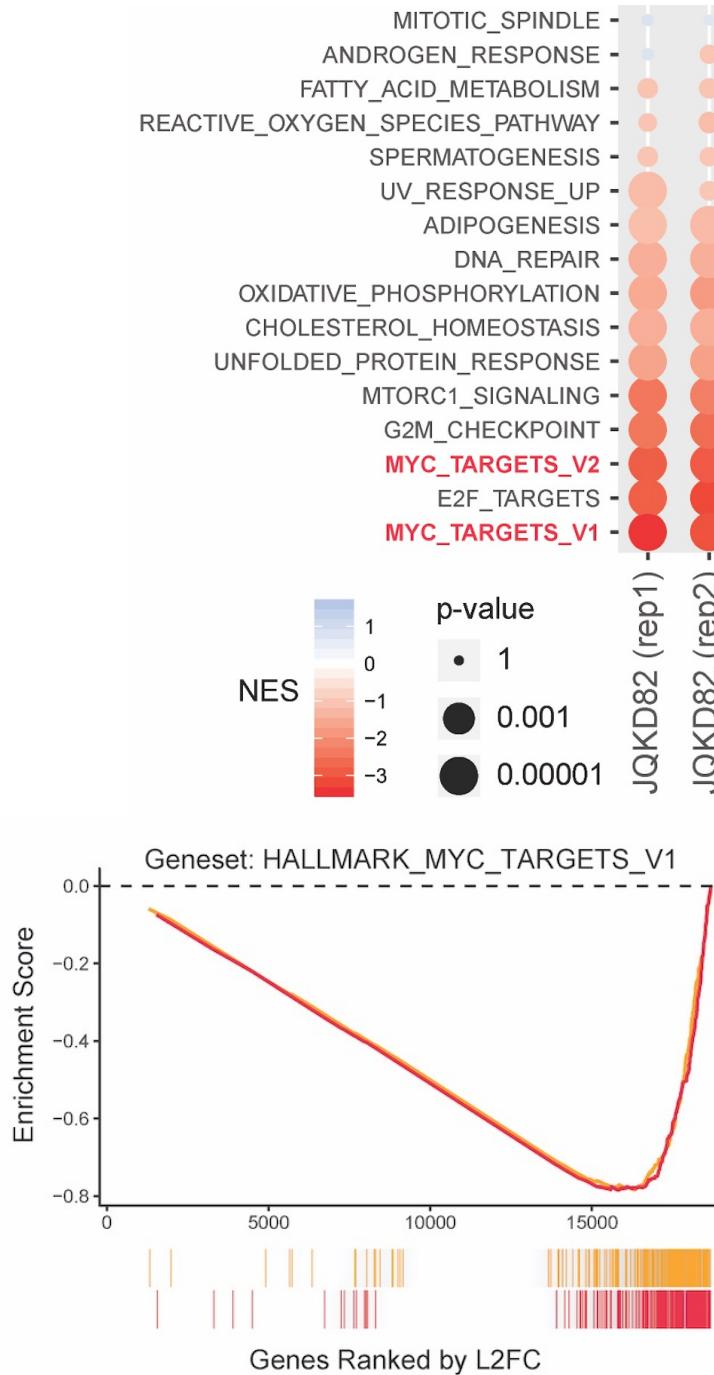


KDM5A inhibition

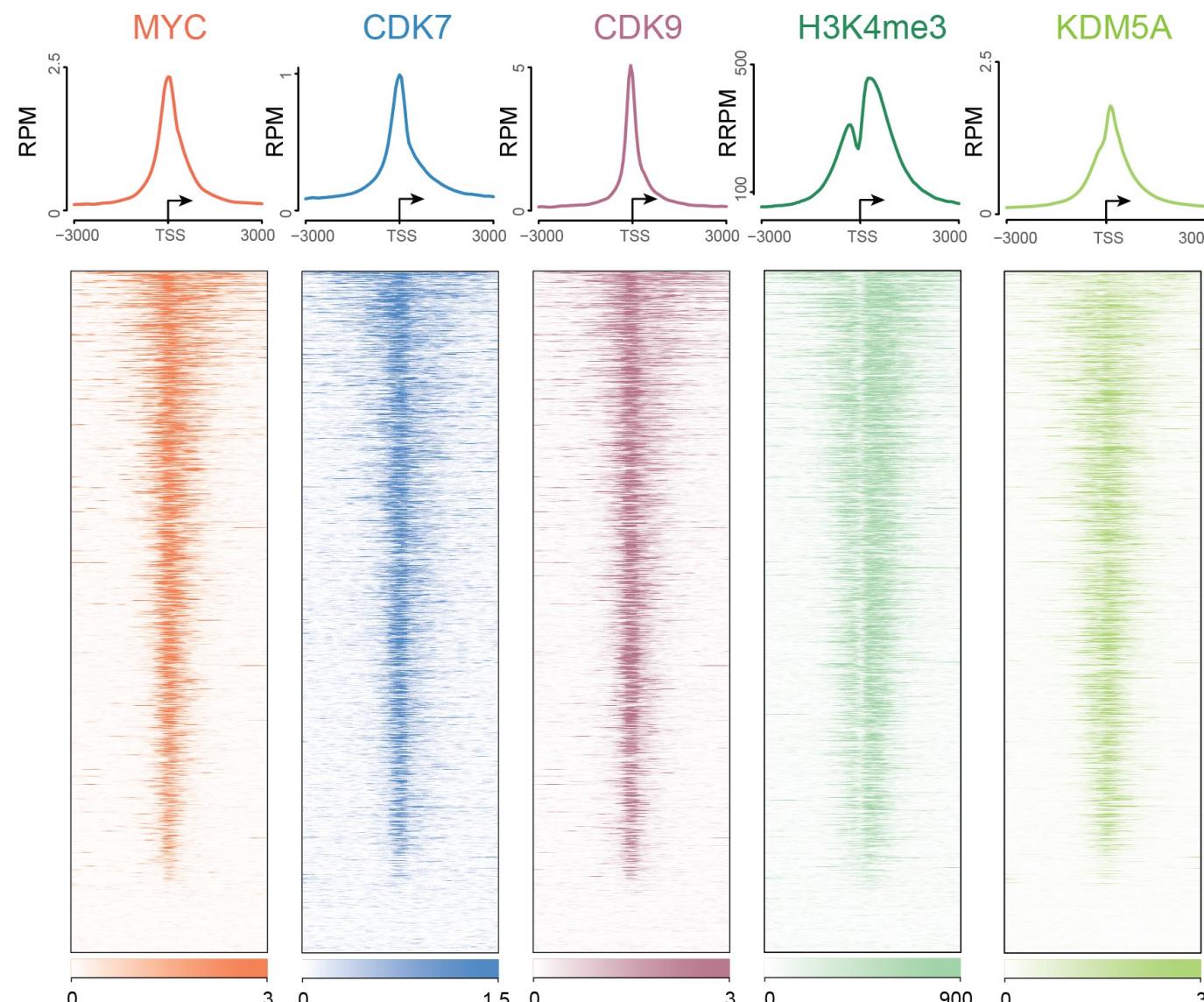


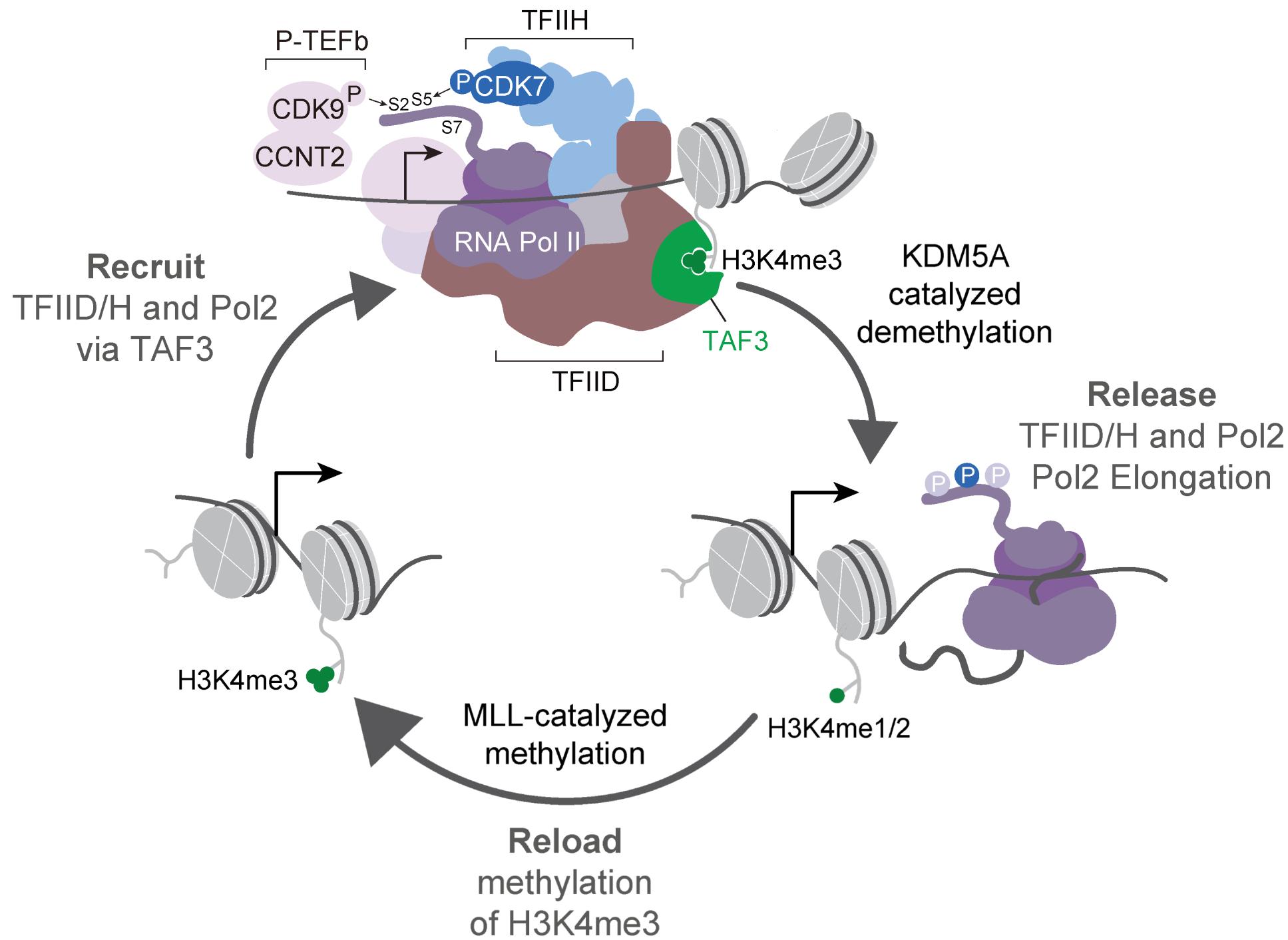
Lysine demethylase selectivity → Histone selectivity



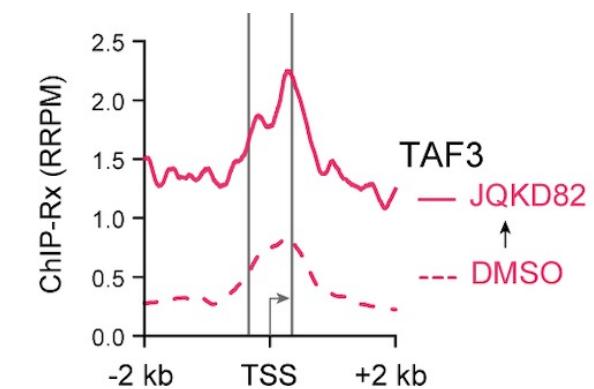
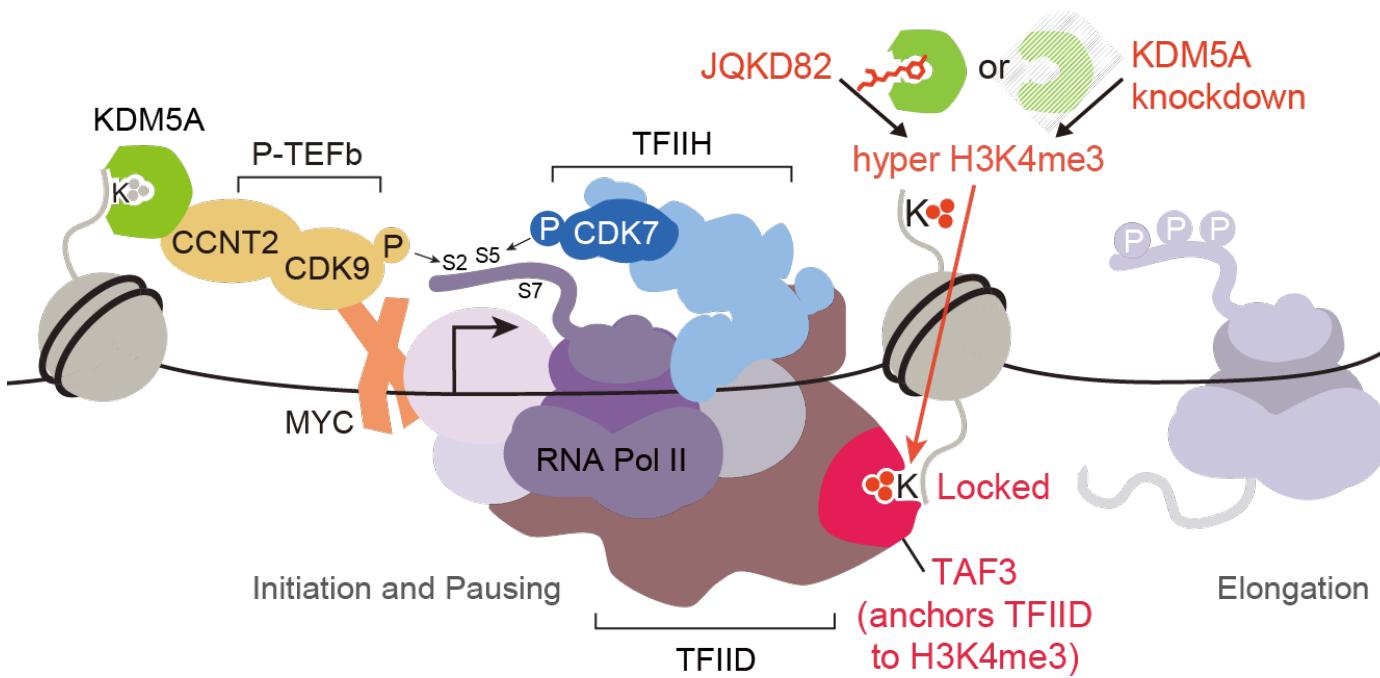
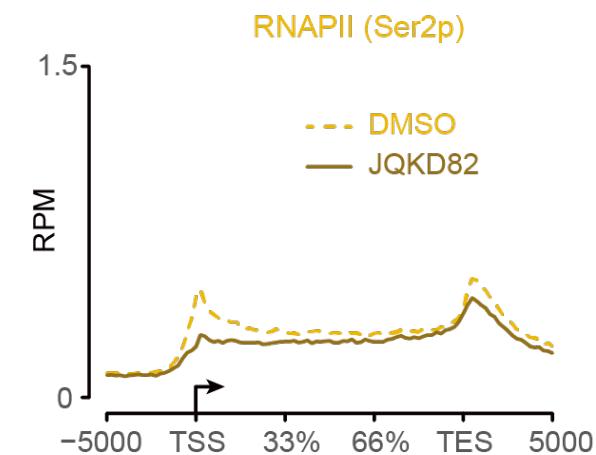
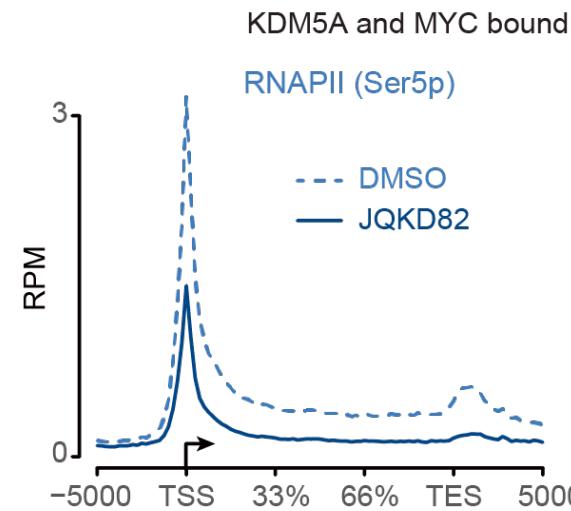
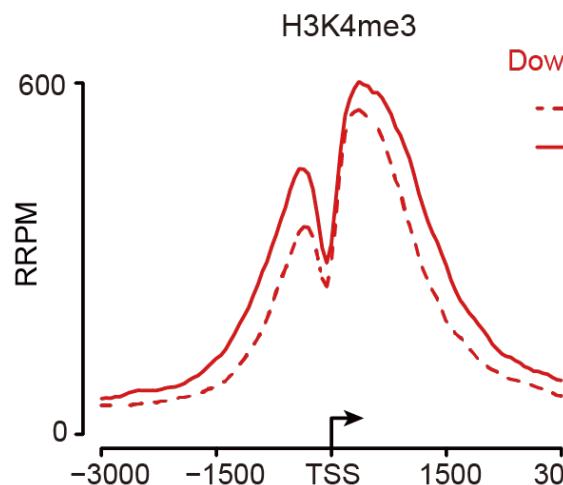


KDM5A co-binds with CDK7/9 and MYC at H4K4me3 rich promoters

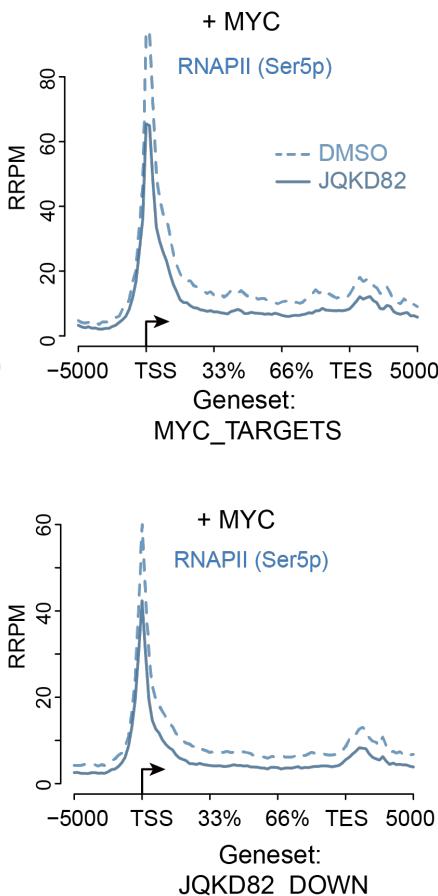
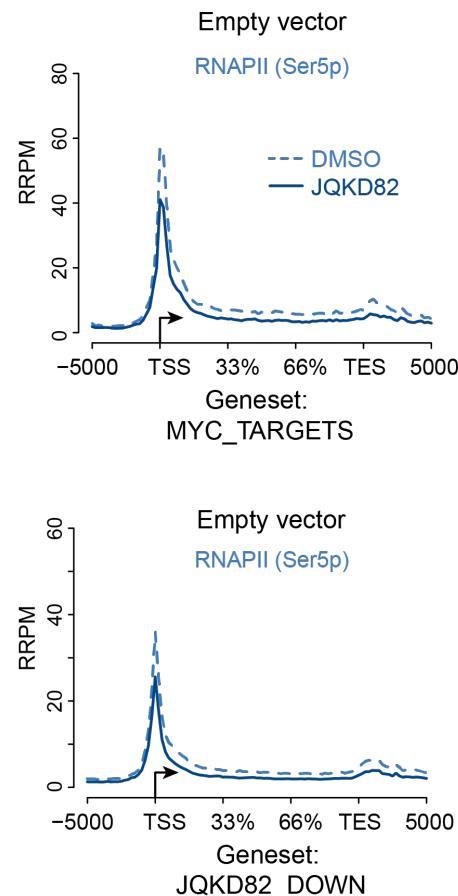
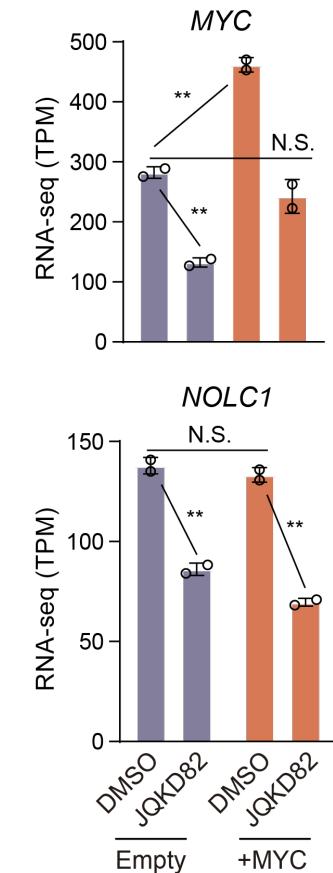
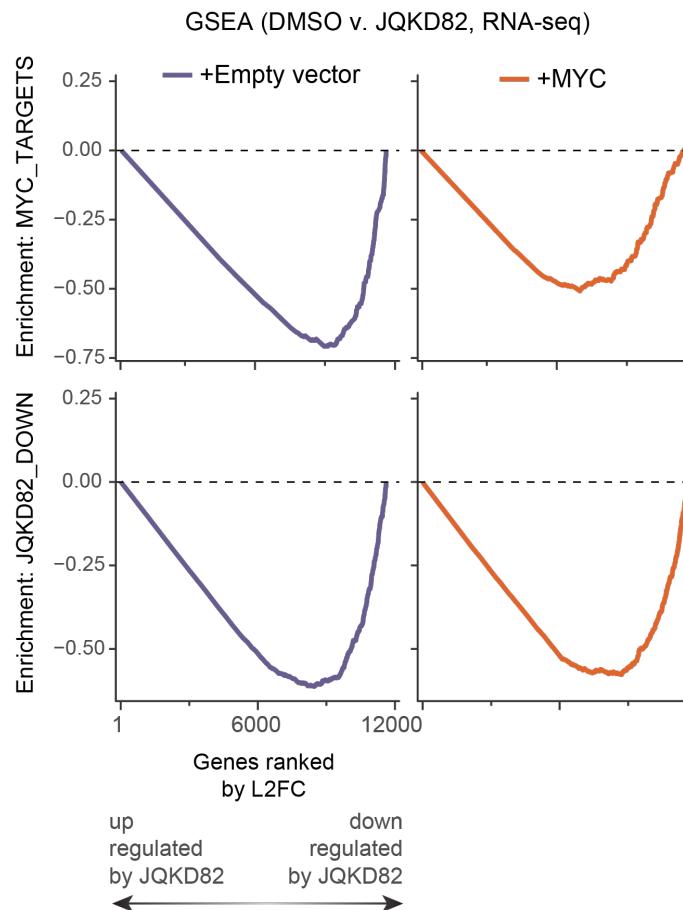
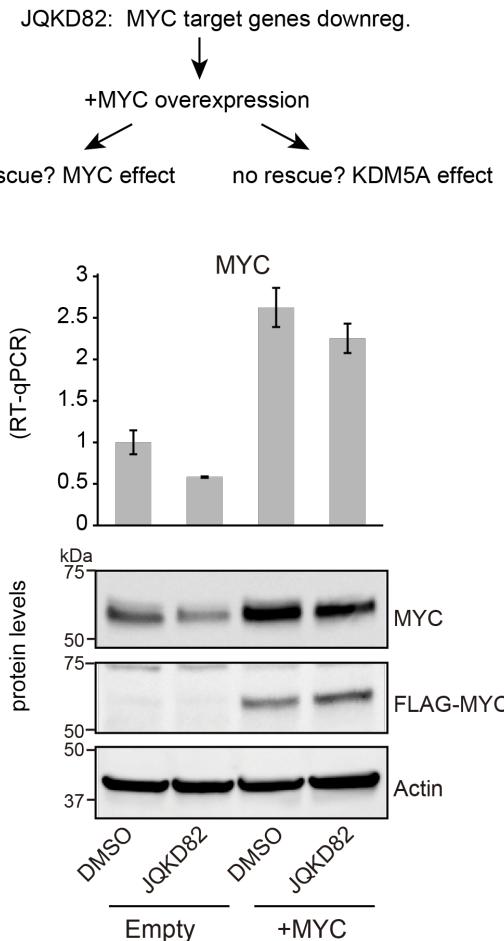




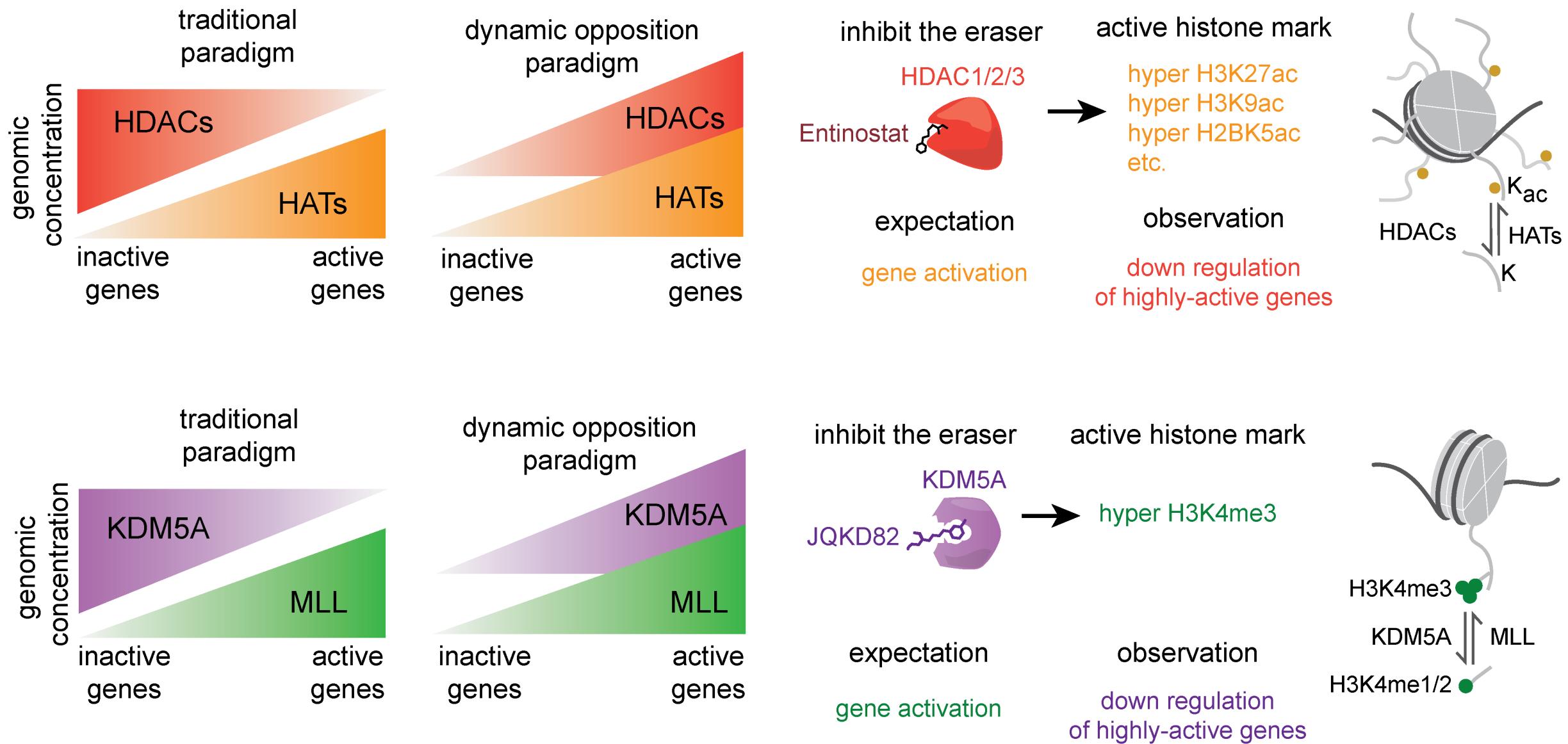
Resolving the H3K4me3 conundrum



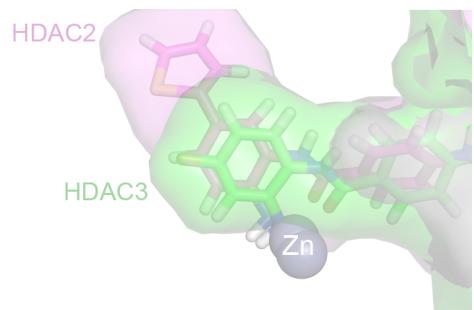
Rescue attempt: distinguish direct v. indirect KDM5A effect



Dynamic Opposition of Histone Marks



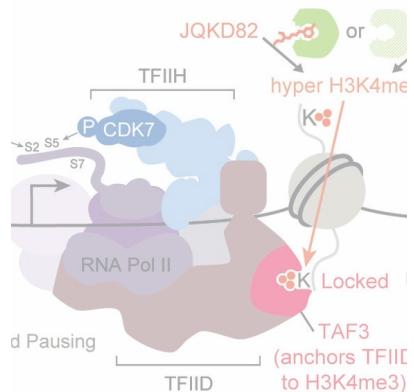
HDACs & H3K27ac



New mechanism to explain HDAC inhibition

Nature Comm., 2019
Nature Genetics, 2019

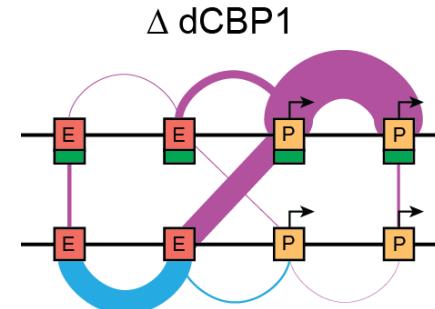
KDM5A & H3K4me3



New mechanism to explain KDM5A inhibition

Blood Cancer Discovery, 2021

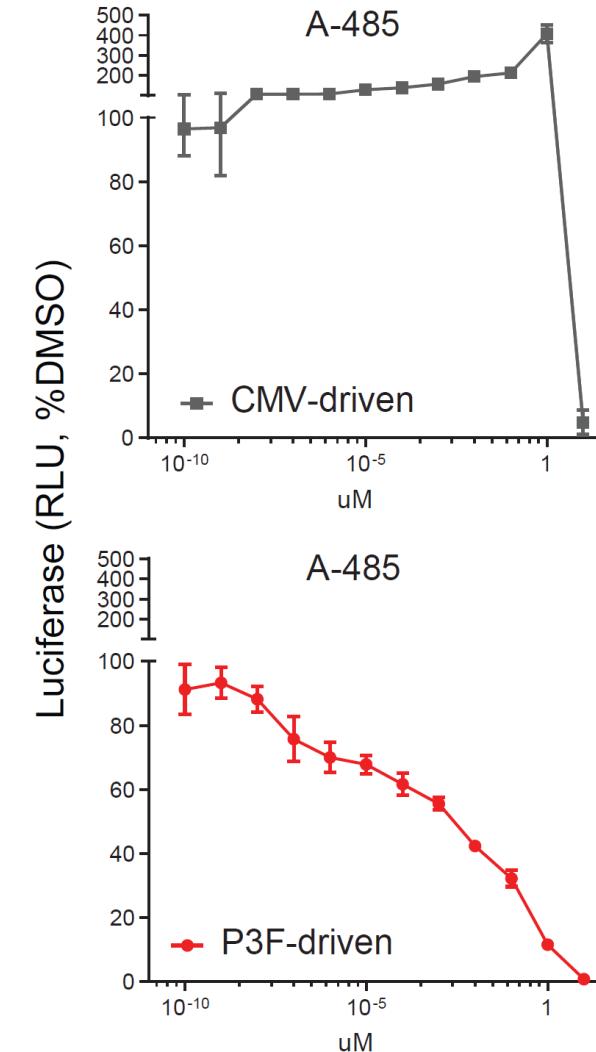
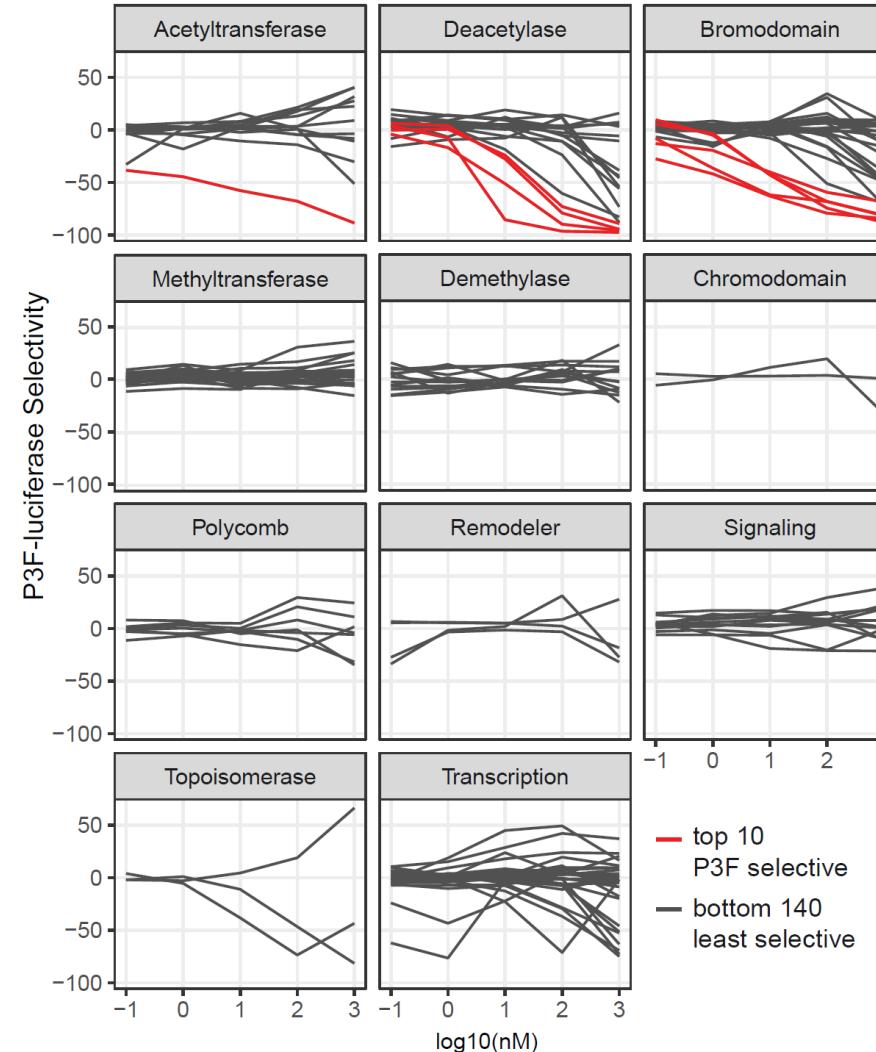
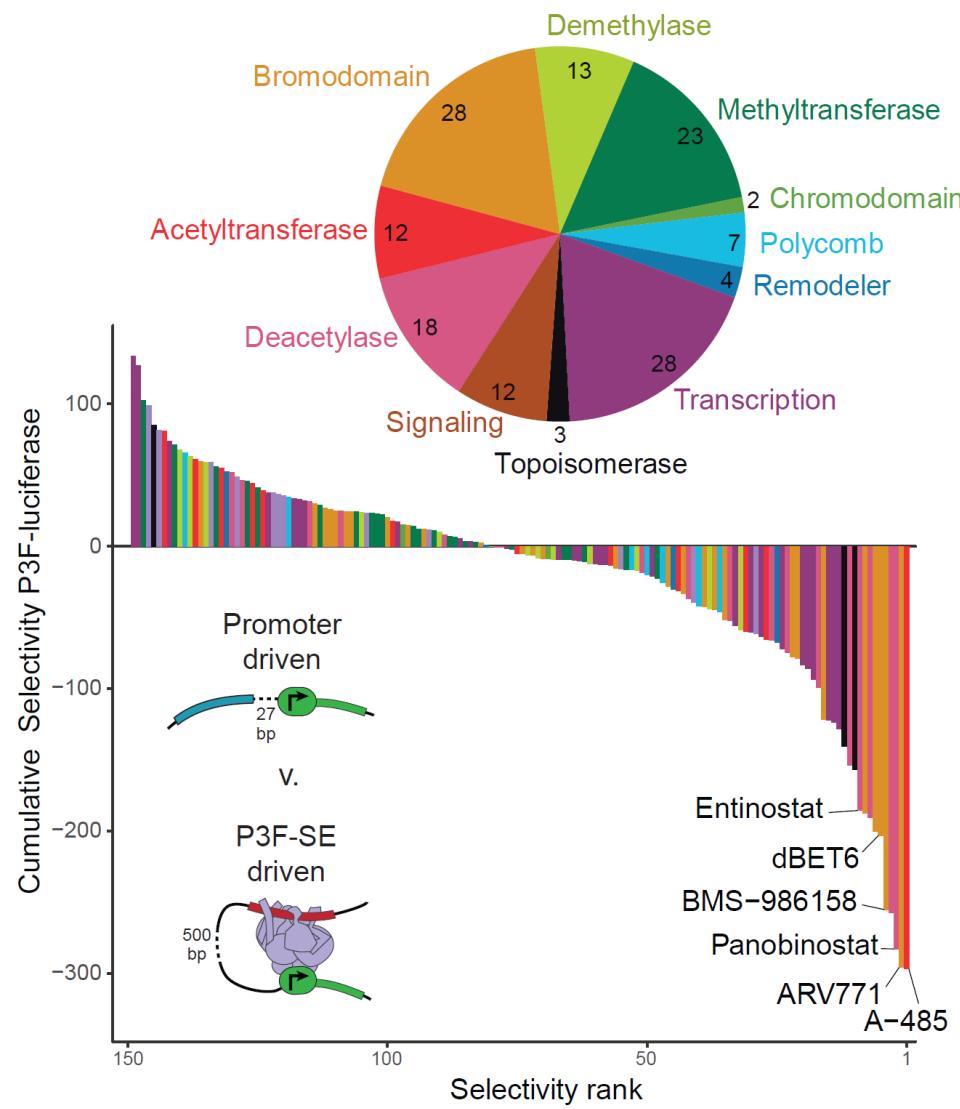
HATs & RNA Pol 2



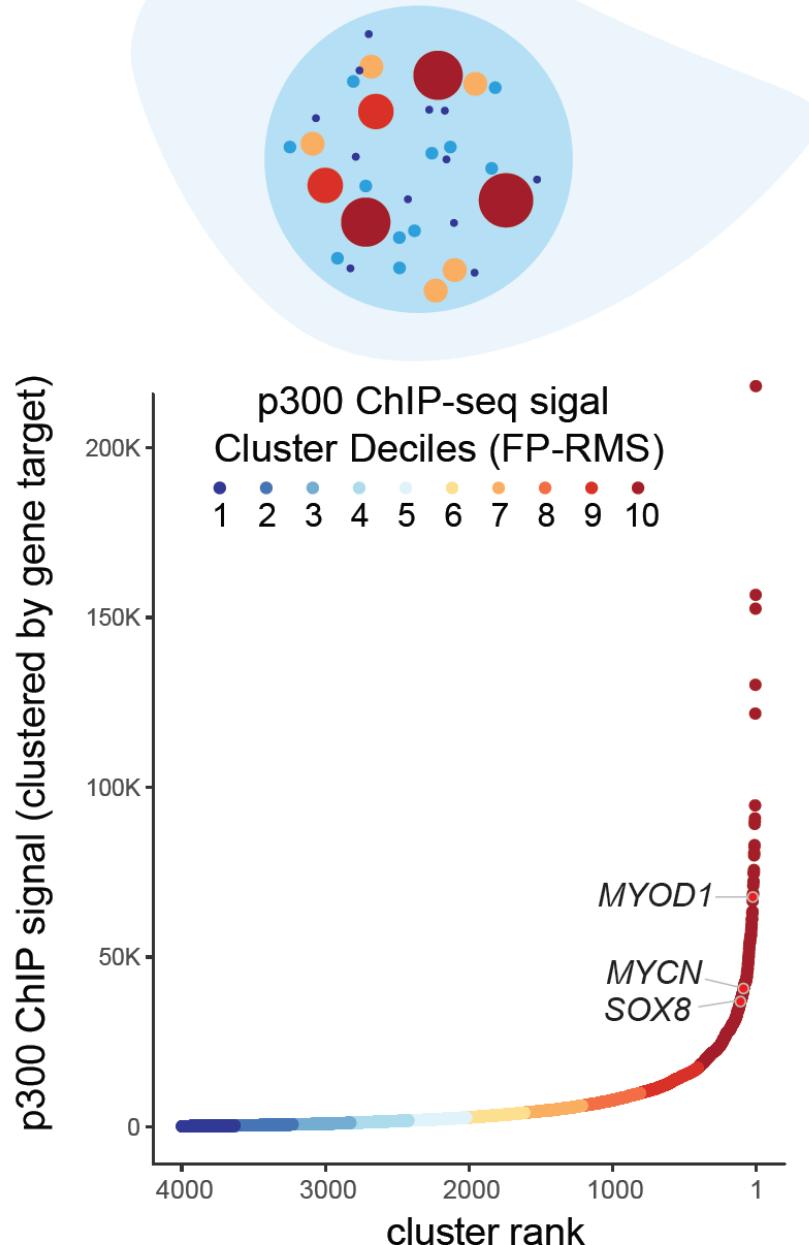
Pol2 cluster collapse

Unpublished

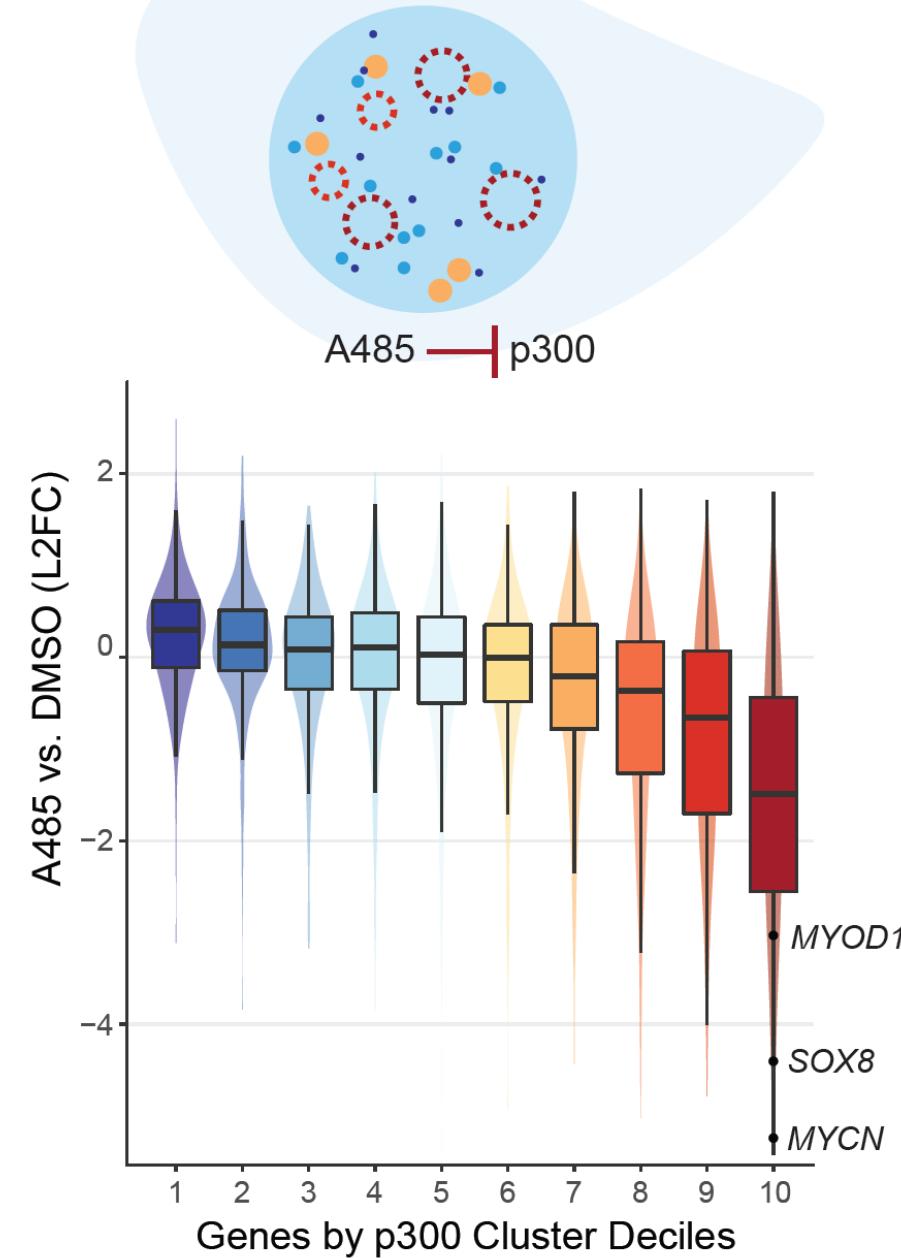
Drugging Super Cluster Driven Transcription



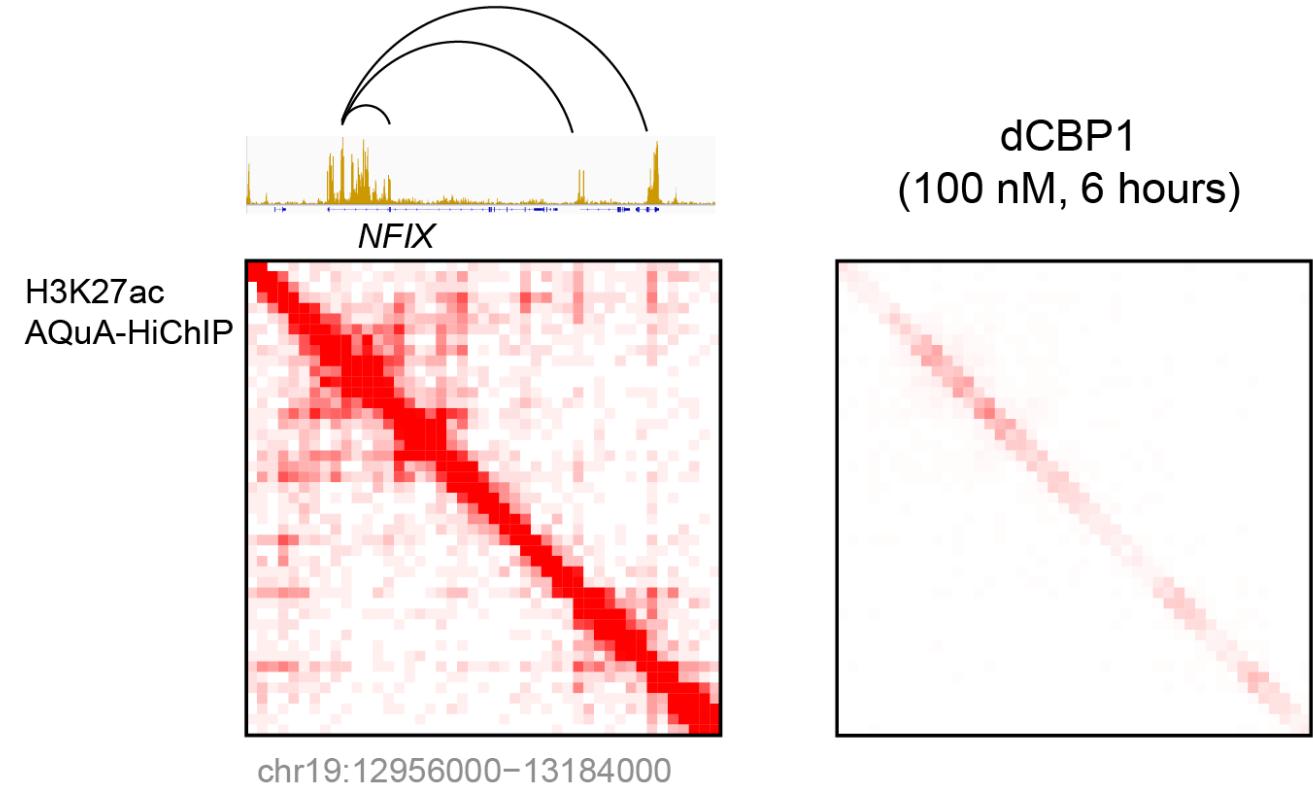
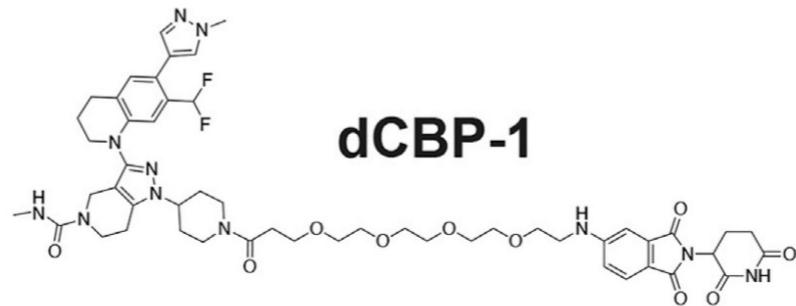
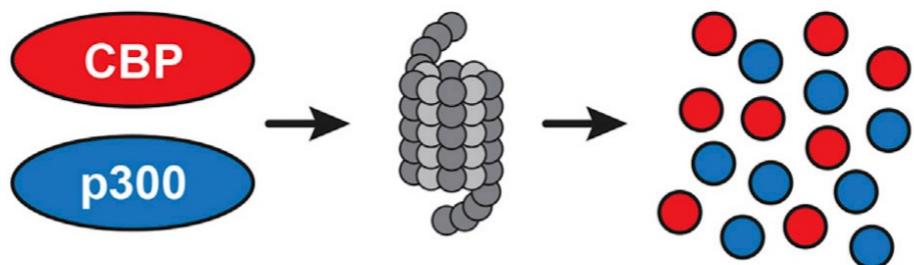
super-clusters of active enhancers



disruption of super-cluster driven oncogenes



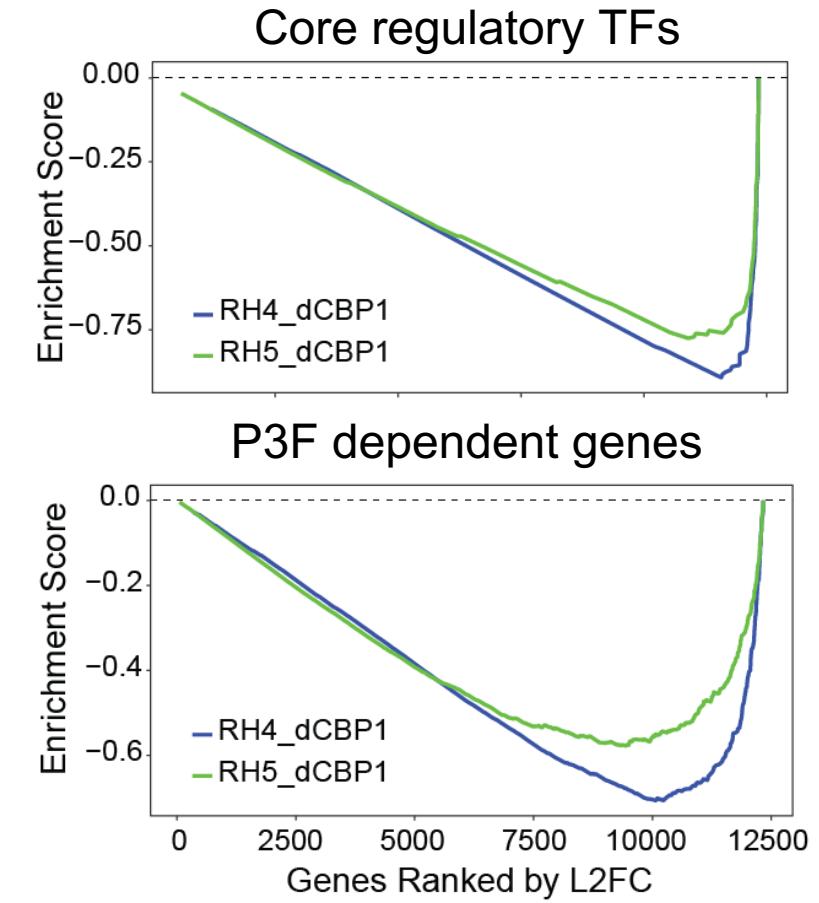
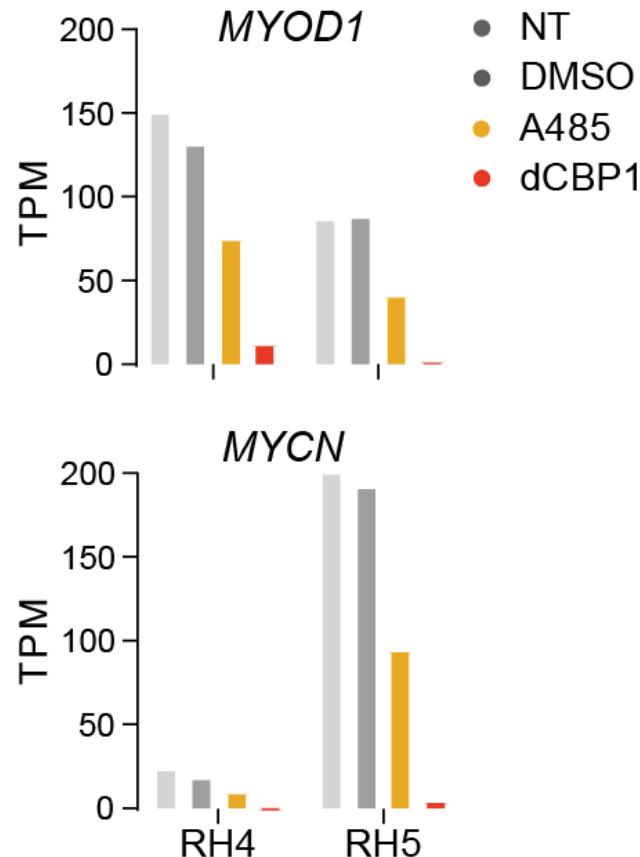
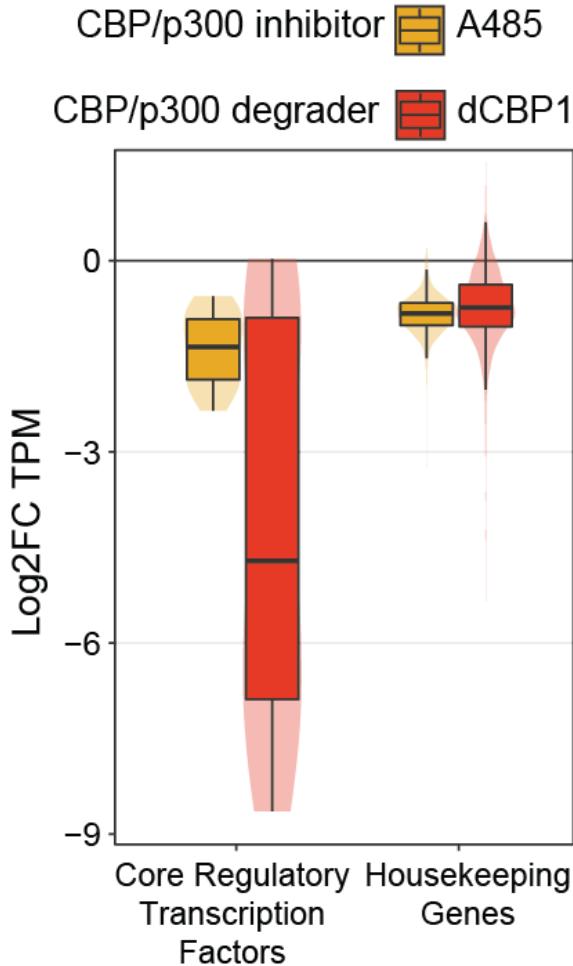
New chemical tool to study H3K27ac loop removal in short time frames



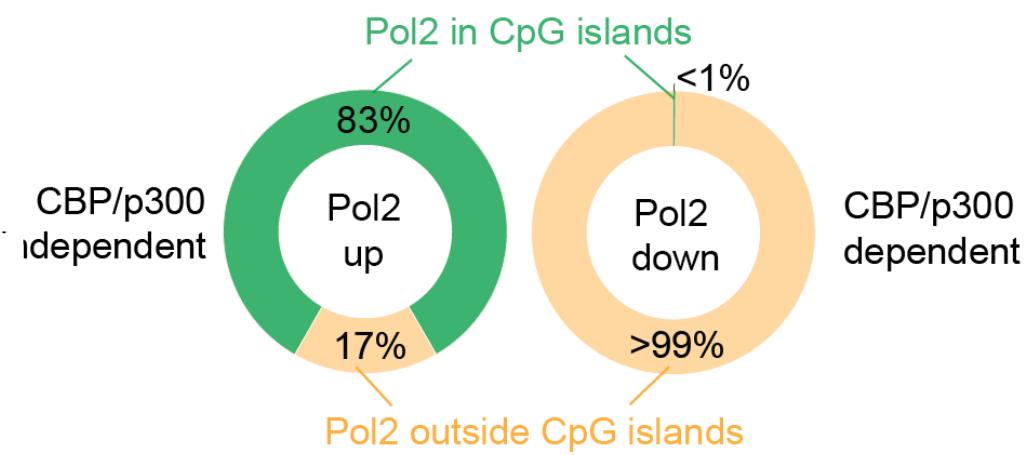
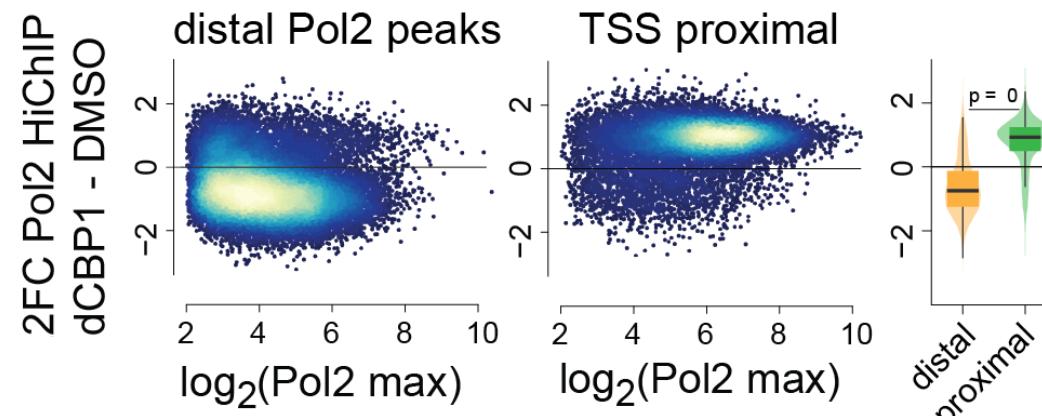
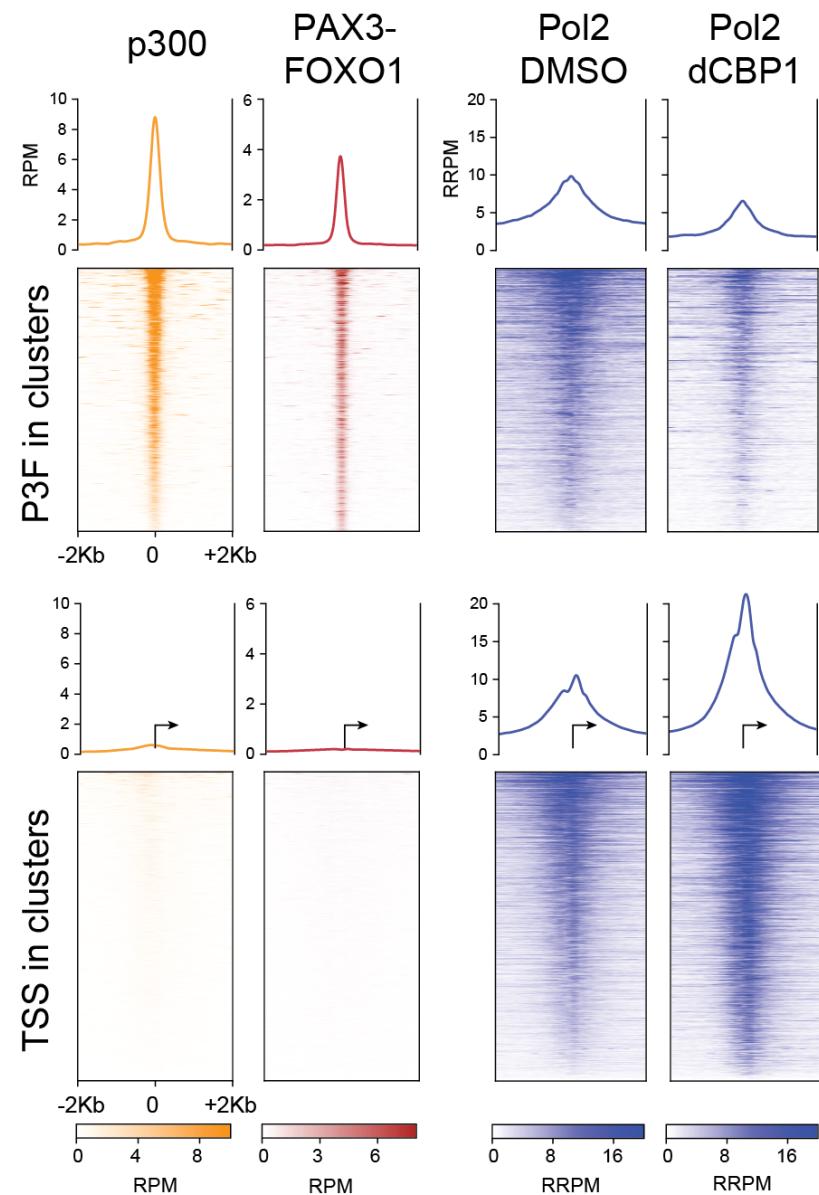
2021, *Cell Chemical Biology*,
Vannam et al, Ott lab

unpublished

Core Regulatory TF Network requires CBP/p300

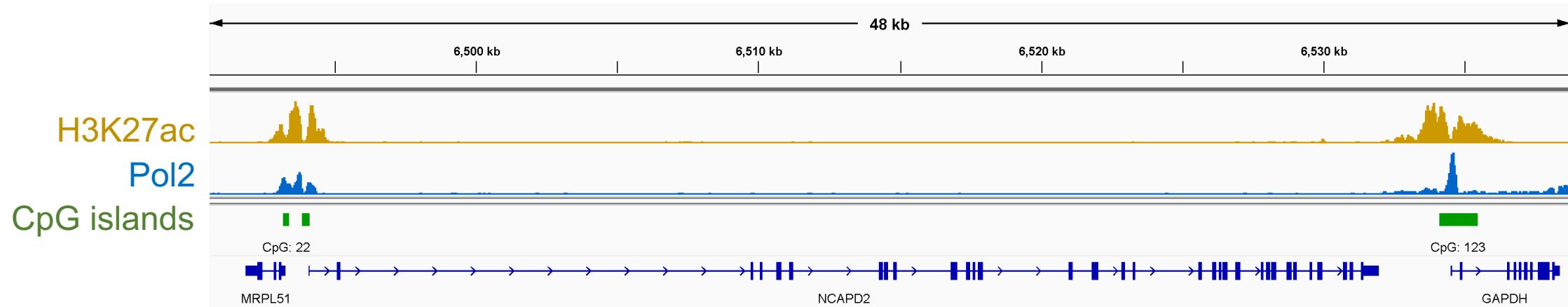


dCBP1 rapidly secludes Pol2 away from P3F enhancers, and into CpG islands

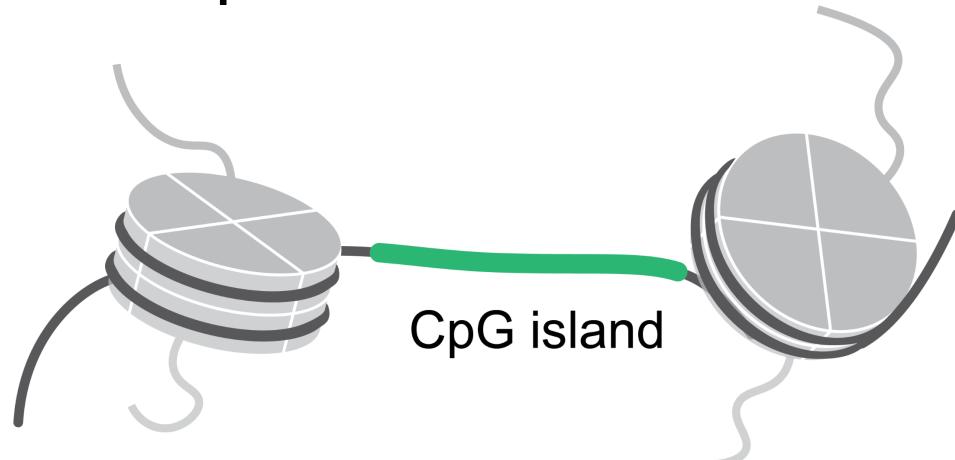


Why would Pol2 move to CpG islands after HAT inhibition?

CpG islands promote open “landing pads” for Pol2 transcription

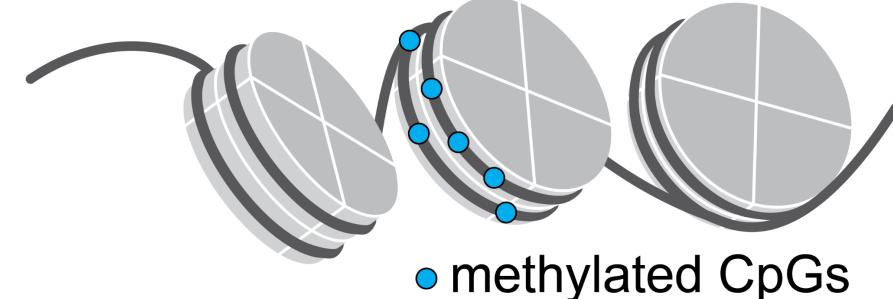


CpG rich sequences **cannot**
wrap around histones

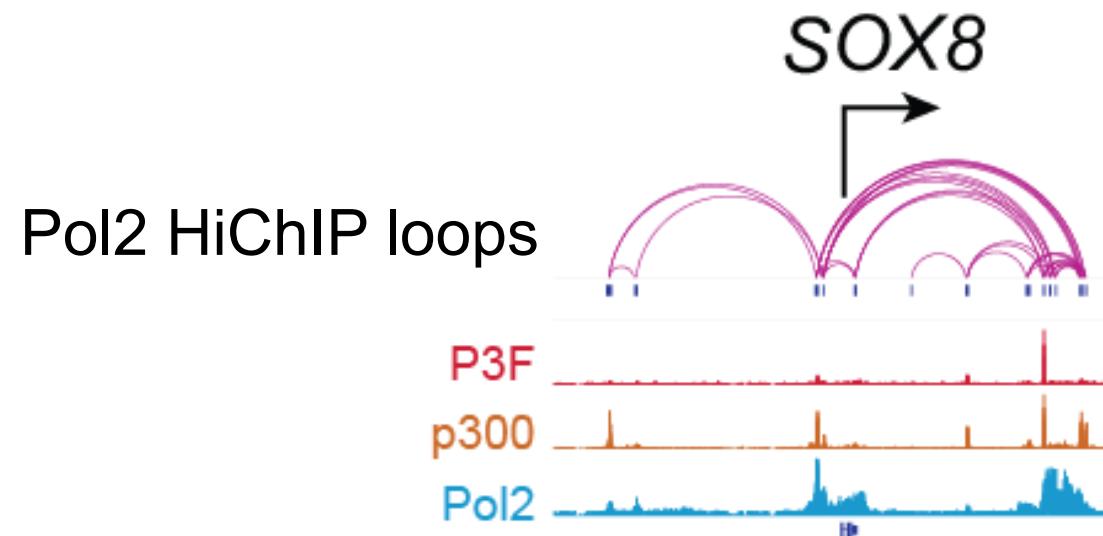


They are always open for Pol2 binding

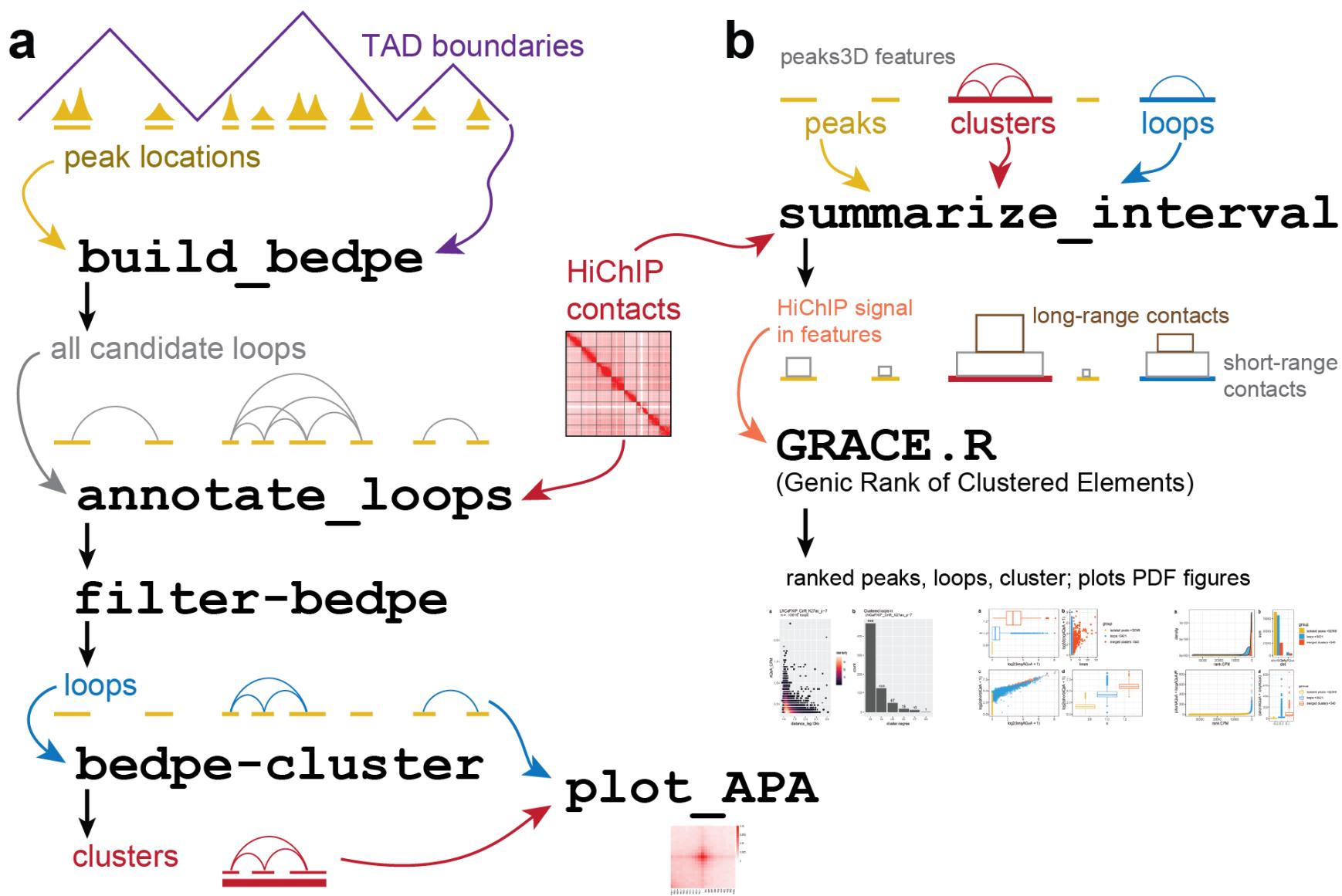
Methylated CpG rich sequences
can wrap around histones



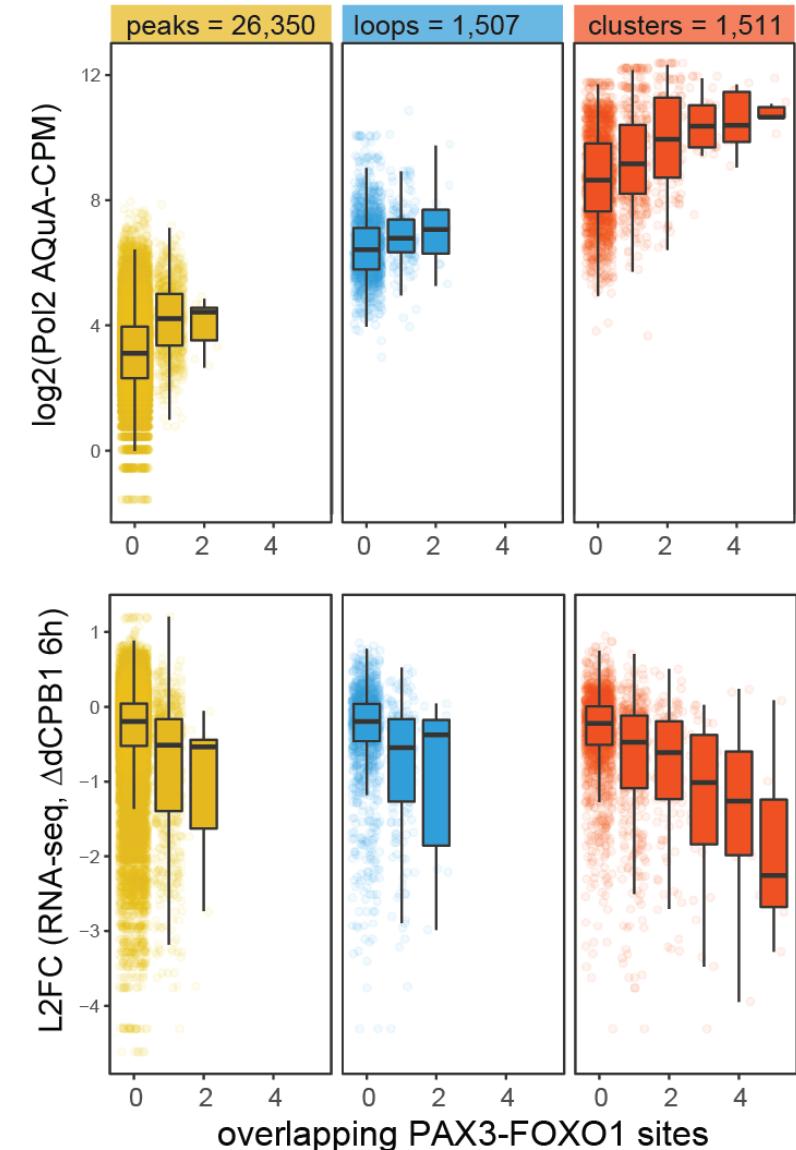
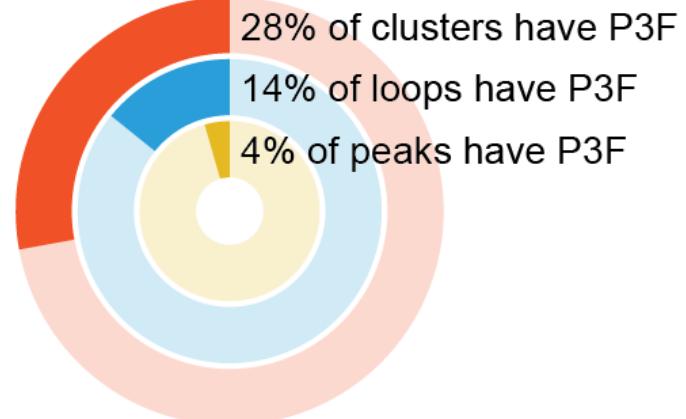
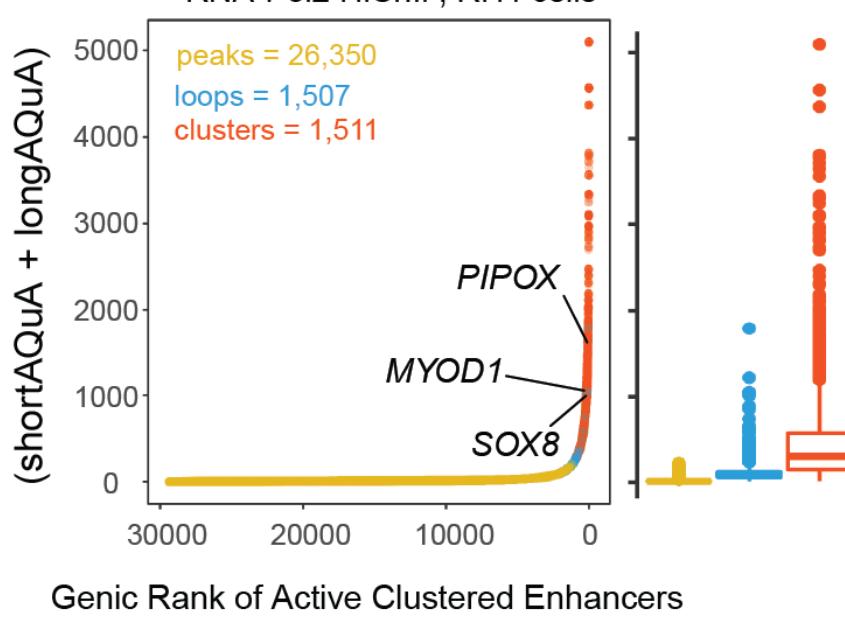
DNA methylation causes gene silencing

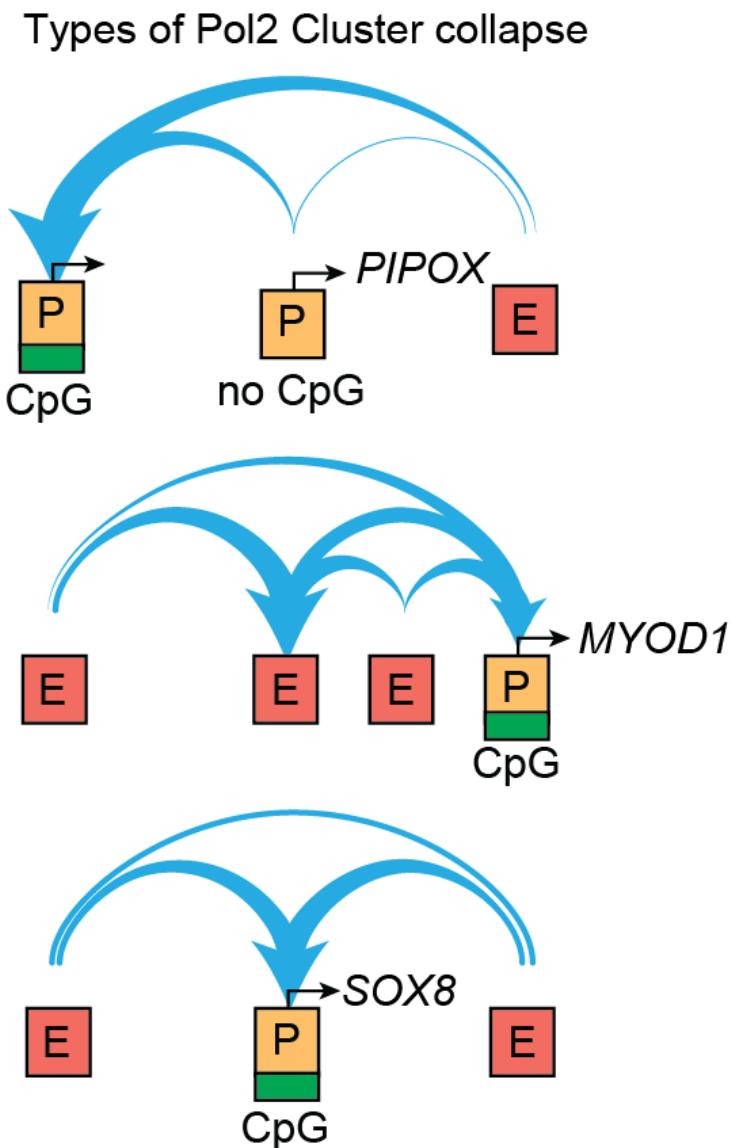
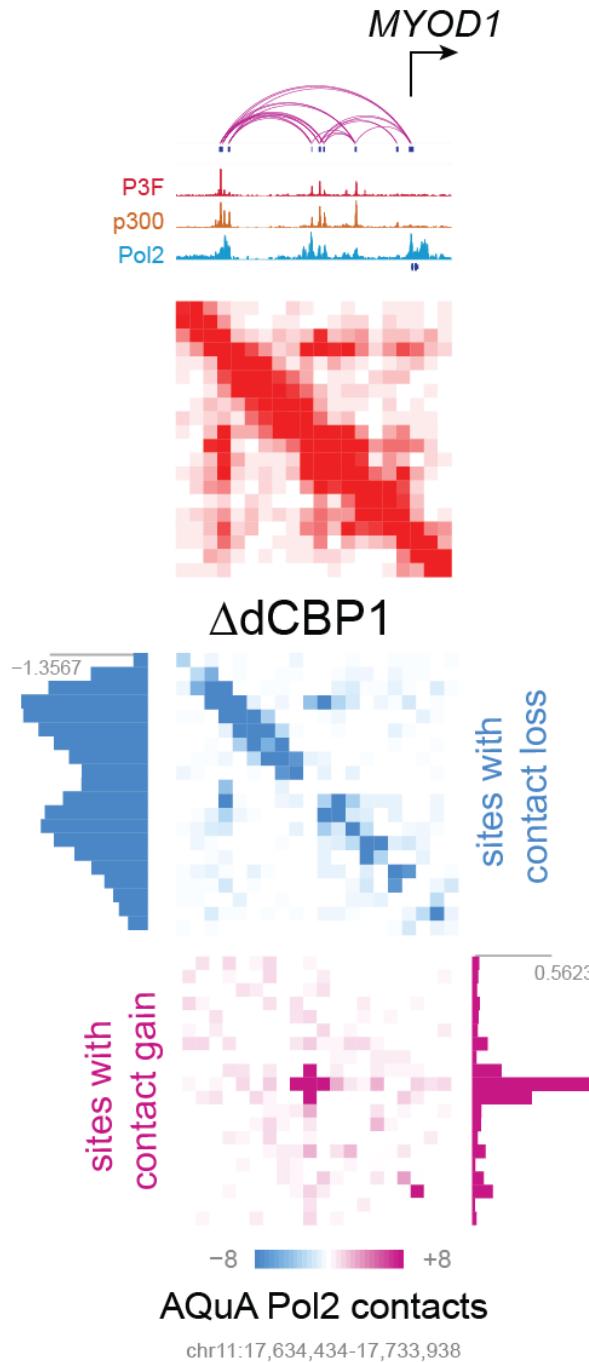
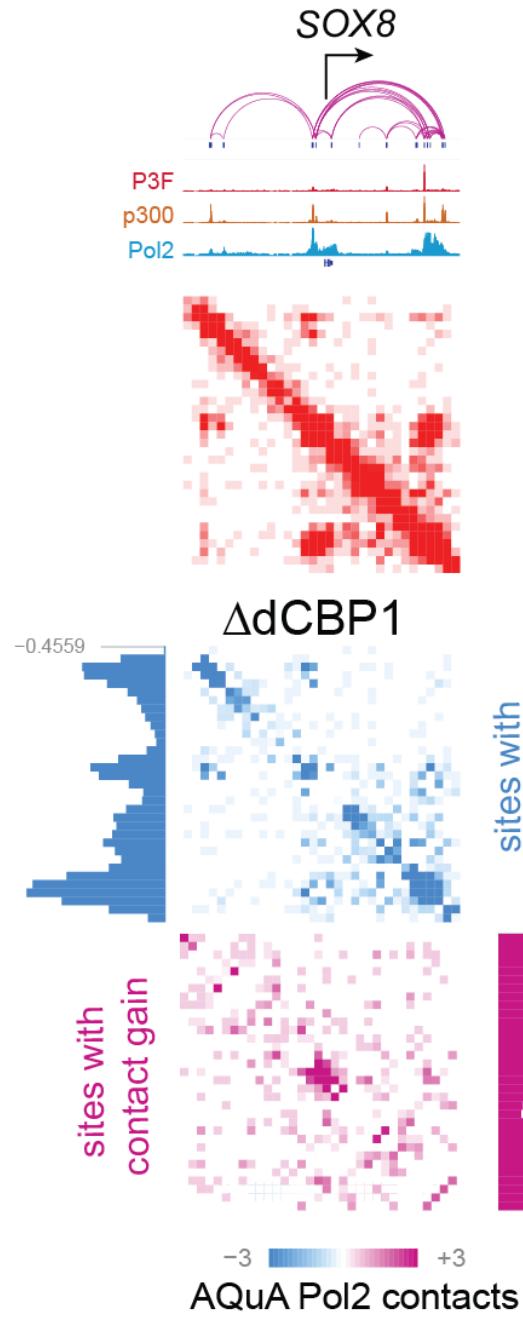


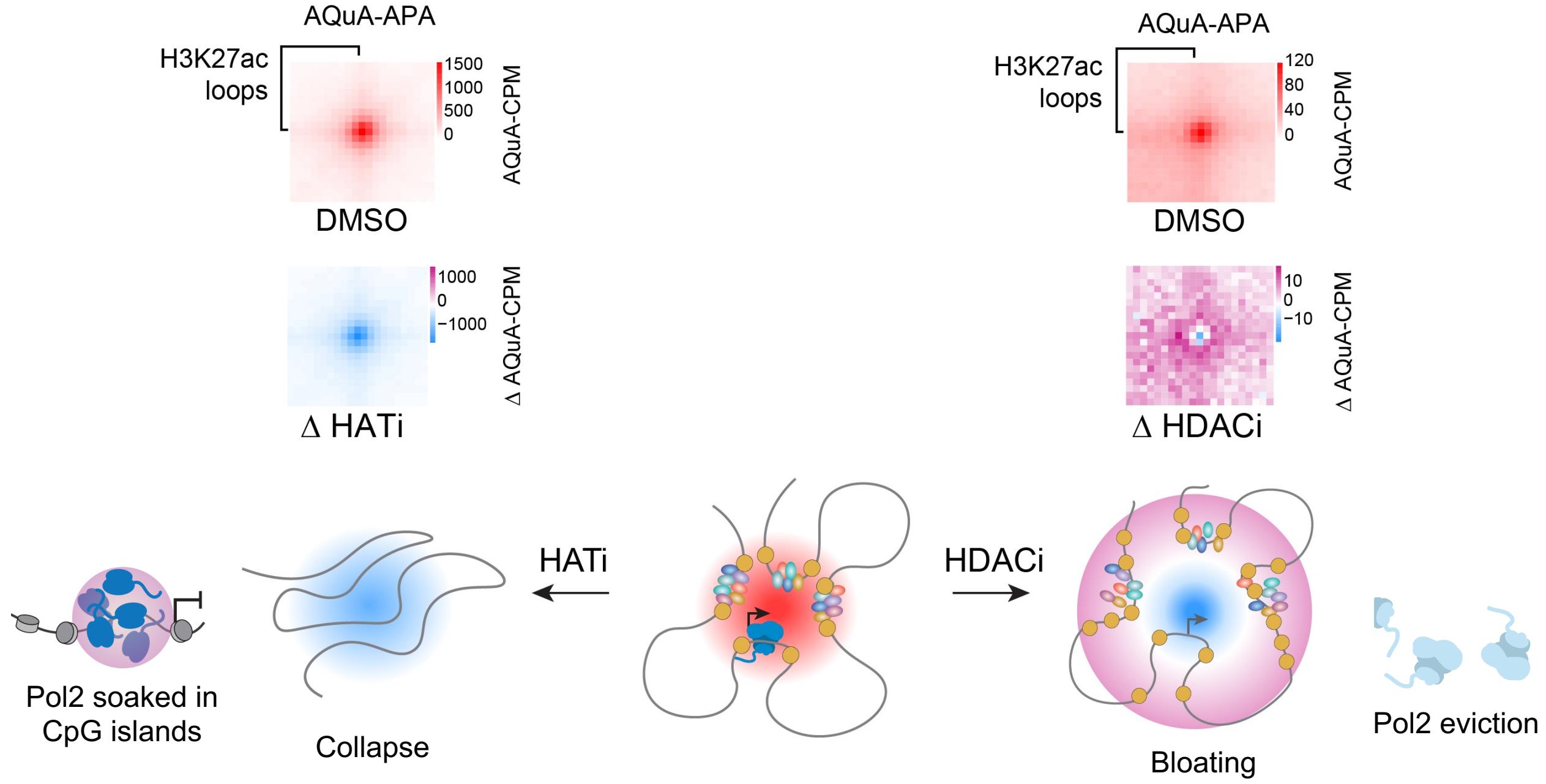
Tools to classify loops and clusters: github.com/GryderLab/peaks3d

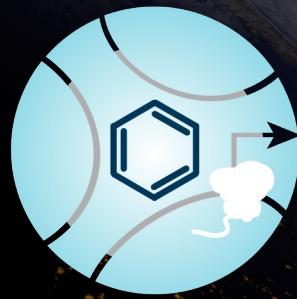


PAX3-FOXO1 occupies the strongest Pol2 clusters









gryderlab

Care. Collaborate. Create.



Collaborator labs:



gryderlab.com

Special thanks to:
Ben Stanton
Javed Khan
Marielle Yohe



Jun Qi

