



Zhengmz Lab

Dynamic 3D Structure and Gene Regulation of *Drosophila* under Heat Stress

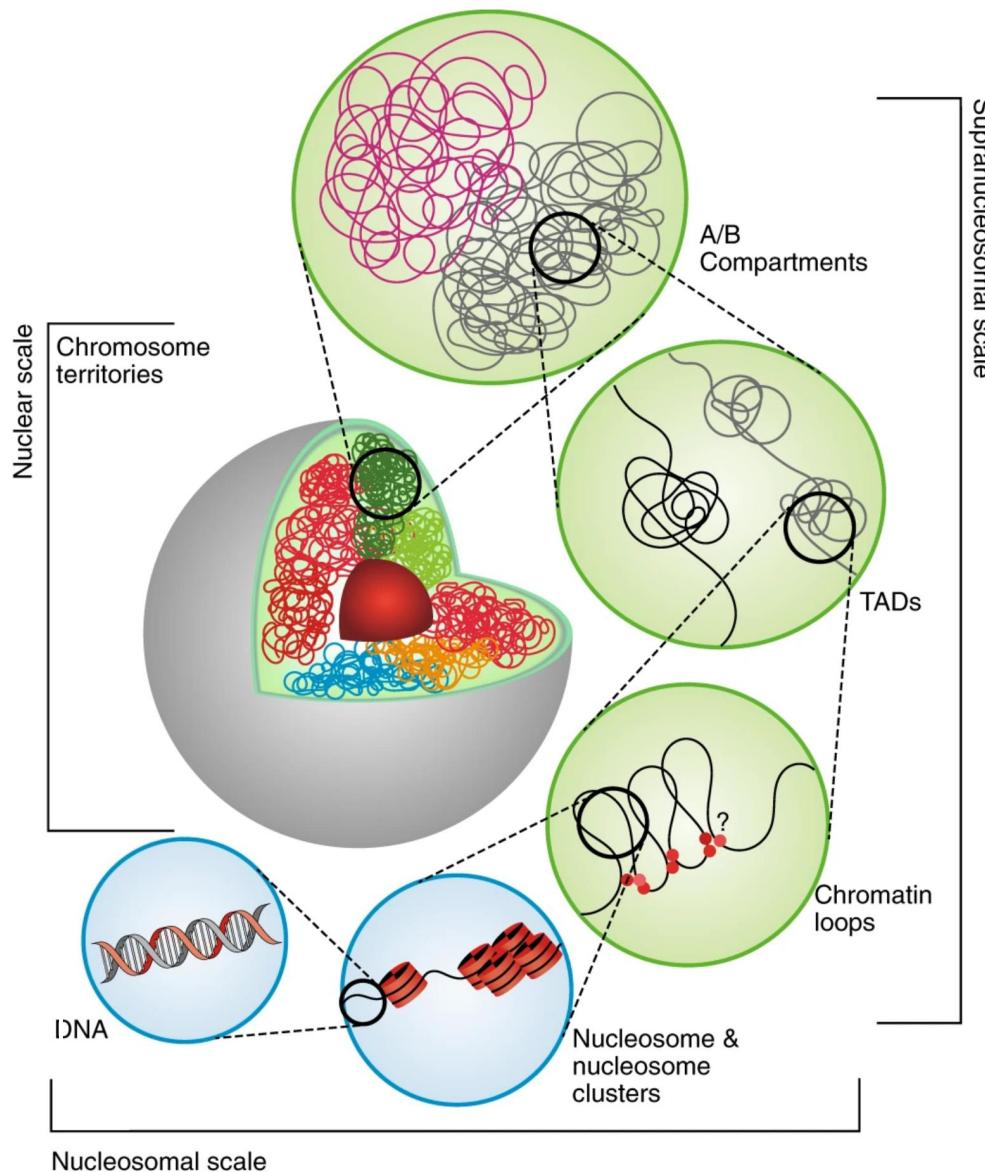
Presenter: Yin Pengfei
Instructor: Zheng Meizhen
Time: 2024 May 13th

Content

- Background
- Project design
- Data analysis
- Results

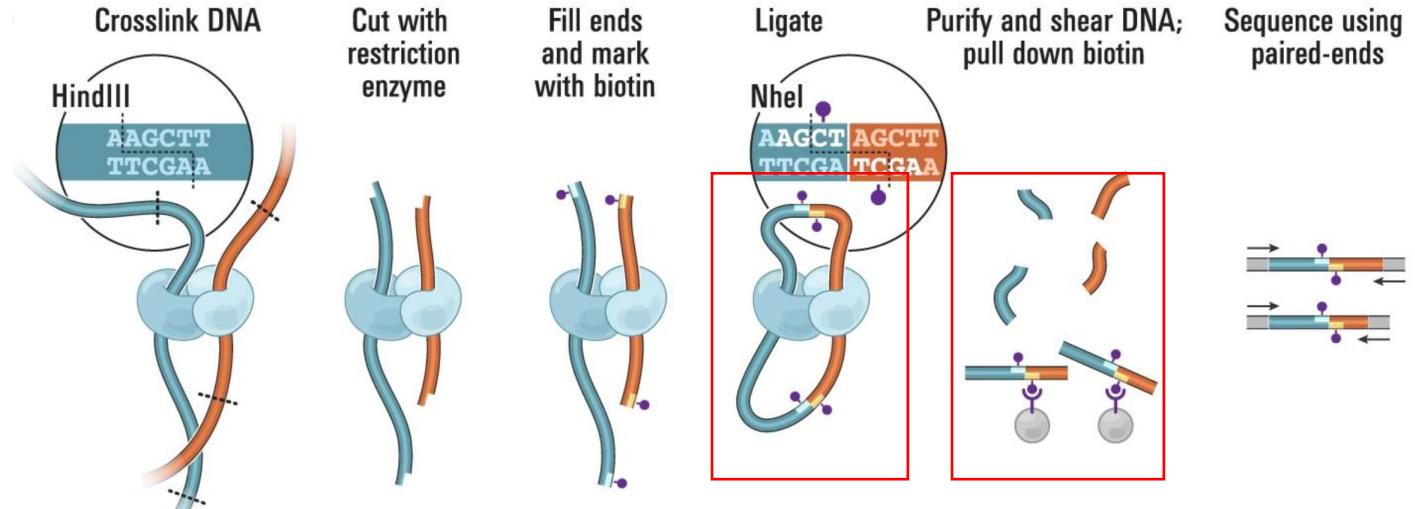
Background

- Brief introduction to 3D genomics
- Brief introduction to heat stress
- Dynamic changes of 3D structure under heat stress



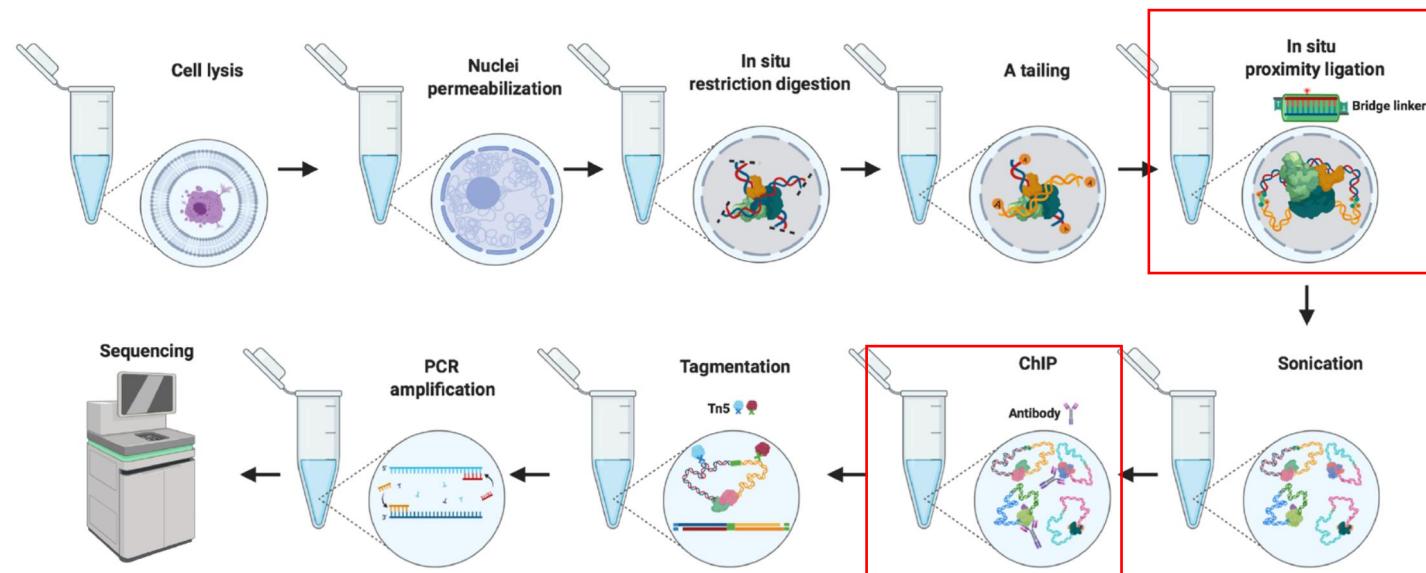
The essence: how the 3D genome is organized into multiple layers and how this organization influences gene expression and regulation.

Doğan et al., Nature Plants. 2018



Erez et al., *Science*. 2009

Hi-C

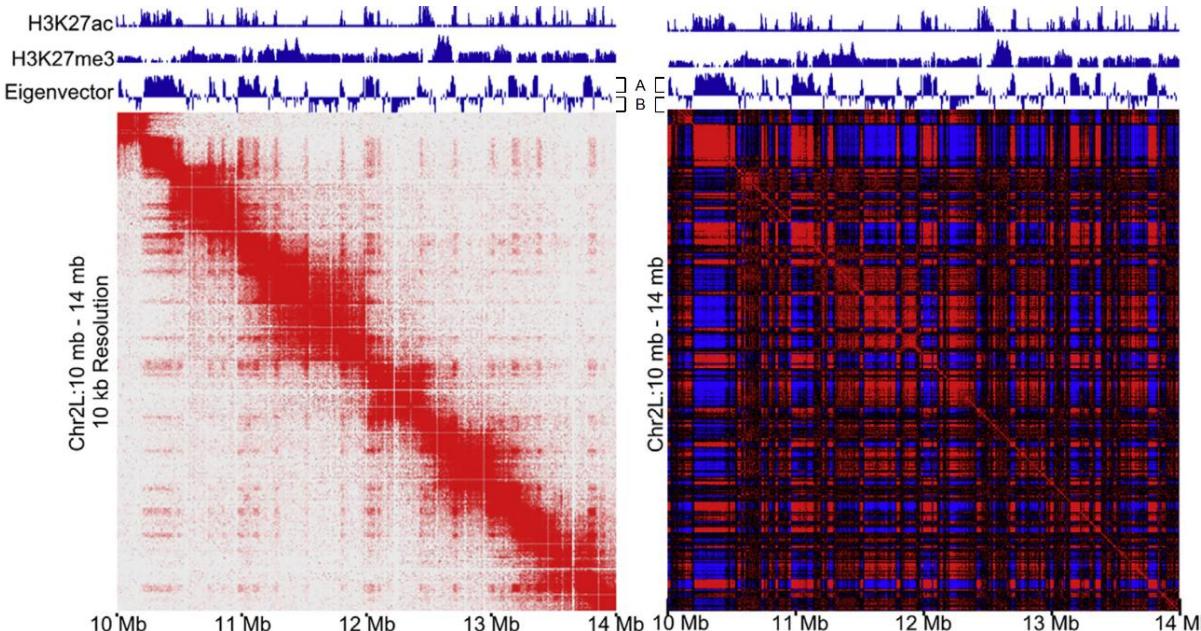


Wang et al., *Curr Protoc*. 2021

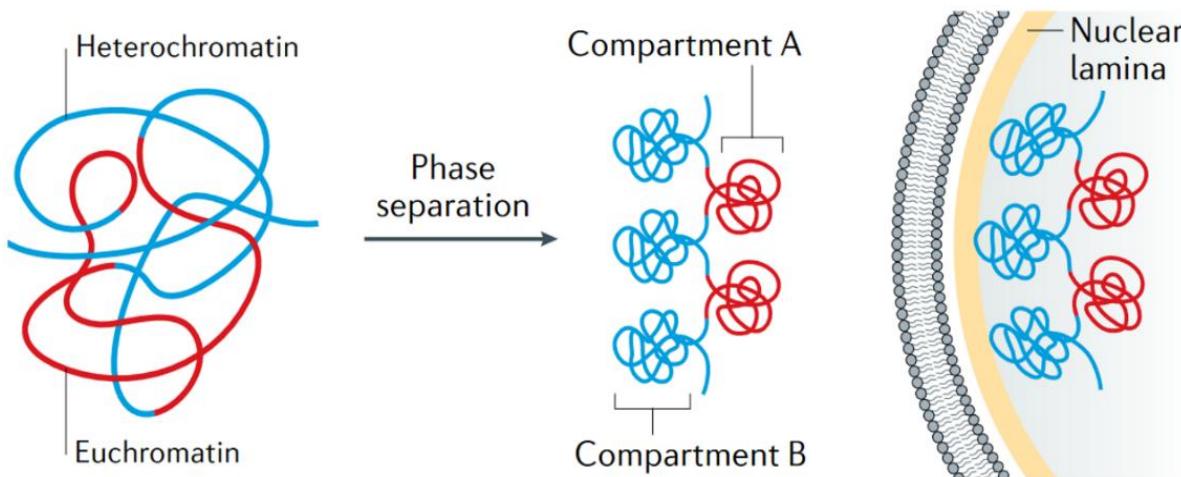
in situ ChIA-PET

Hi-C captures all the interactions, while ChIA-PET captures the interactions mediated by some factor.

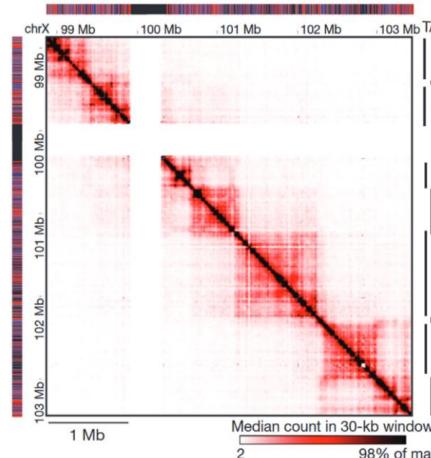
Rusk et al., *Nature Methods*. 2009



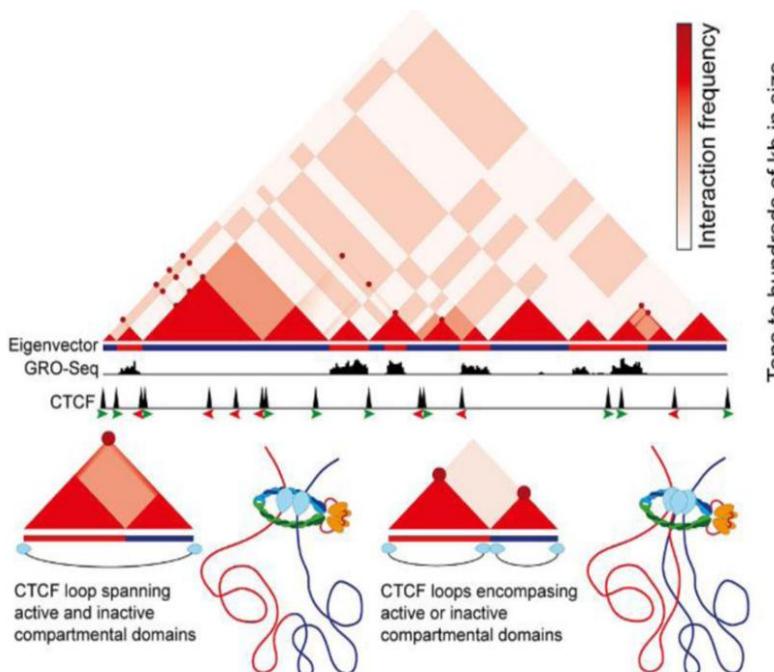
Adapted from Rowley et al., Molecular cell. 2017



1. A/B compartments show as a 'checkerboard' pattern.
2. A compartments are prone to interact with A compartments, while B compartments are prone to interact with B compartments.
3. A compartments correspond to euchromatin located inside the nucleus, while B compartments correspond to heterochromatin, primarily located at the nuclear periphery and around the nucleolus.
4. Phase separation (related to epigenetic modifications) is an important driving force for chromatin compartmentalization.

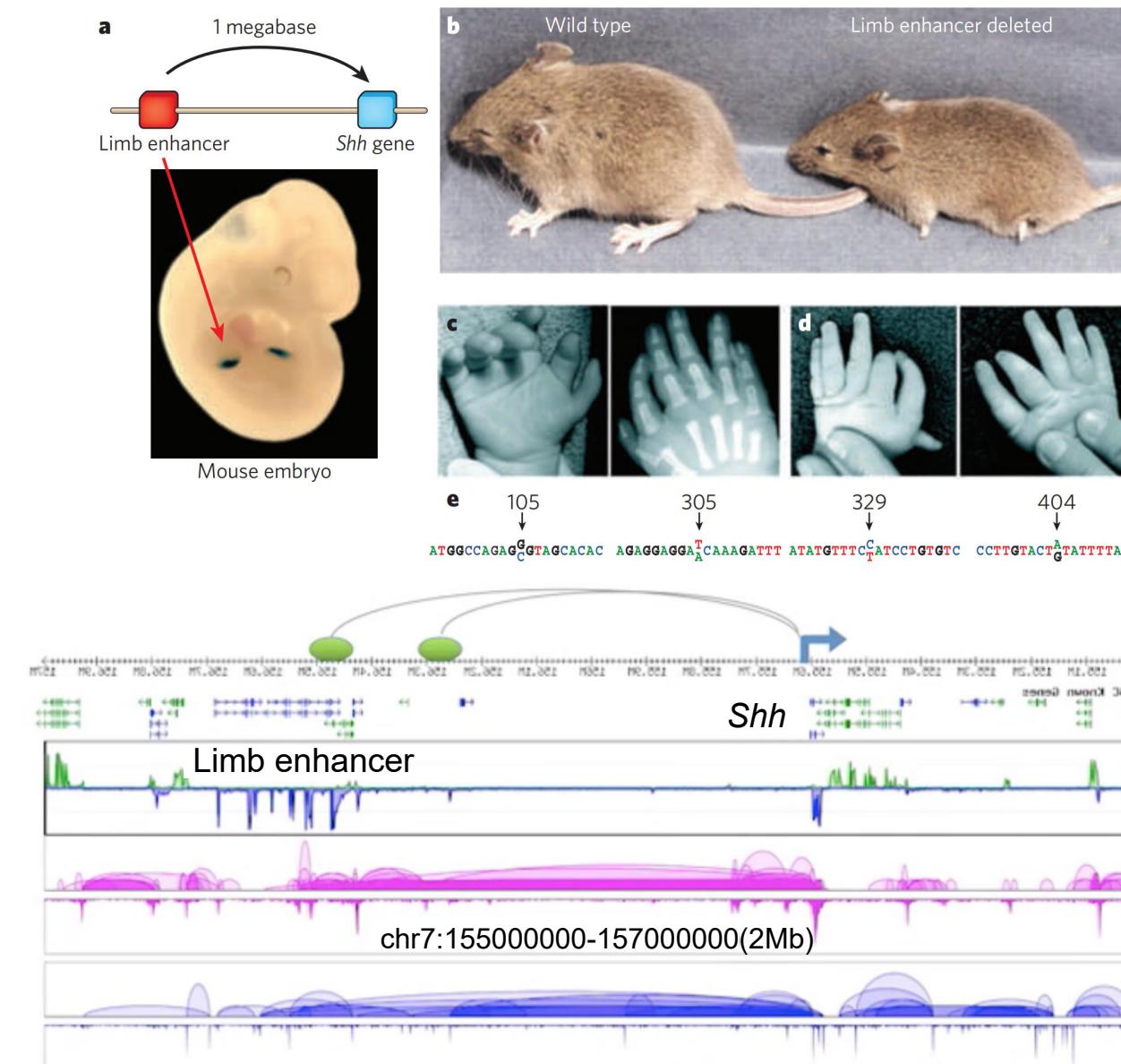


Nora et al., Nature. 2012



Jordan et al., Nature Reviews Genetics. 2018

- 1. TADs are the square regions located on the diagonal, with higher interaction frequency inside the square regions.**
- 2. TAD boundaries in mammalian cells enrich convergent CTCF binding sites. (TAD boundaries in *Drosophila* enrich BEAF-32/CP190).**
- 3. Loop extrusion is a promising mechanism to explain the formation of TADs**
- 4. TADs and A/B compartments are distinct chromosomal structures; however, they share some interrelated features (overlapped and nested) that are currently under investigation.**



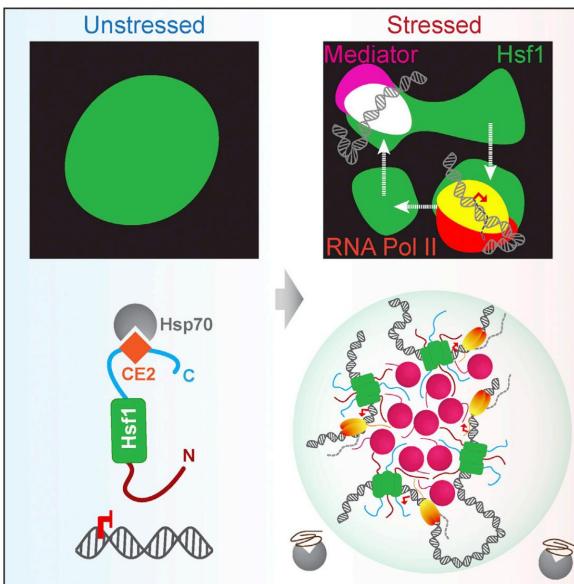
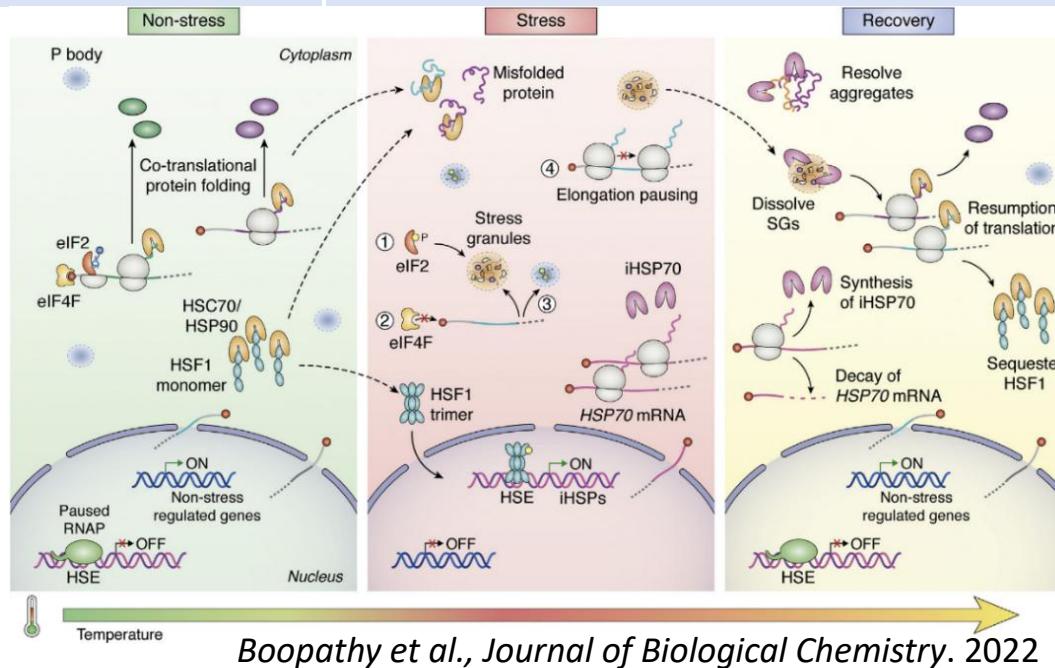
Visel et al., Nature. 2009

Limb enhancer and *shh* gene are spatially close

From Yijun Lab

Background

Heat stress



1. Heat shock can have some typical genes highly expressed like Hsp70 in *Drosophila*.

Li et al., Molecular Cell. 2015

2. After heat shock, there are thousands of up-regulated and down-regulated genes in *Drosophila*.

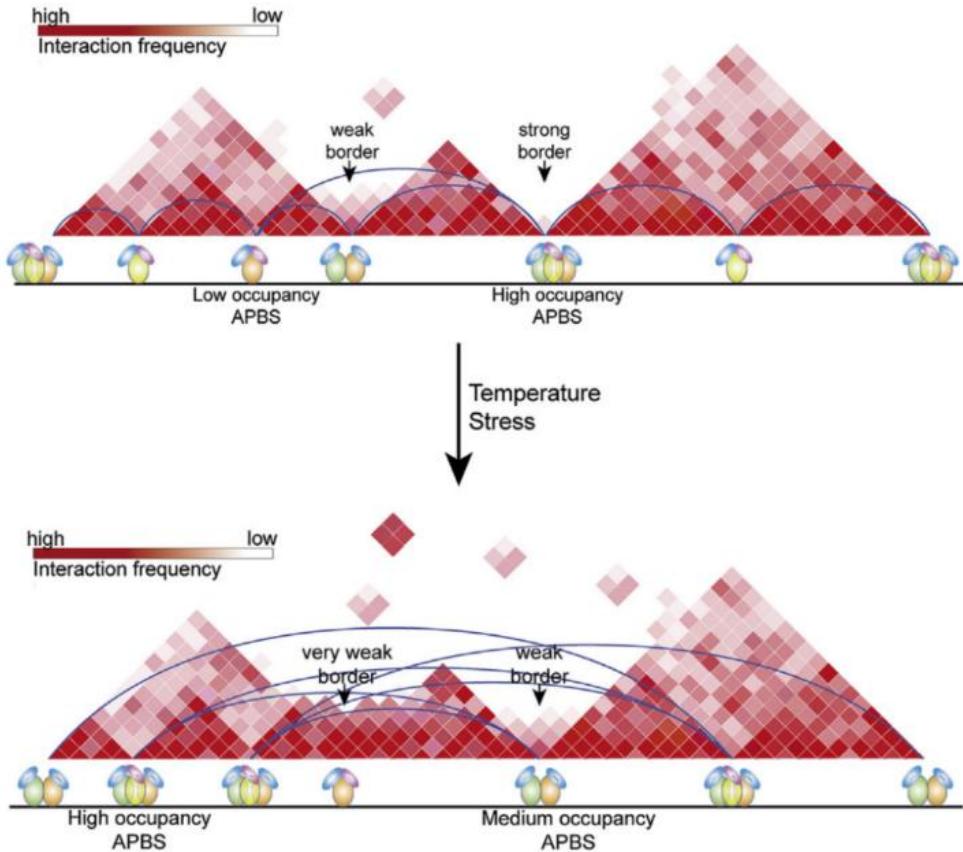
Hieda et al., Chromosome Res. 2005

Teves et al., Genes Dev. 2011

3. Heat shock can result in the increased release of bound RNAPII from DNA.

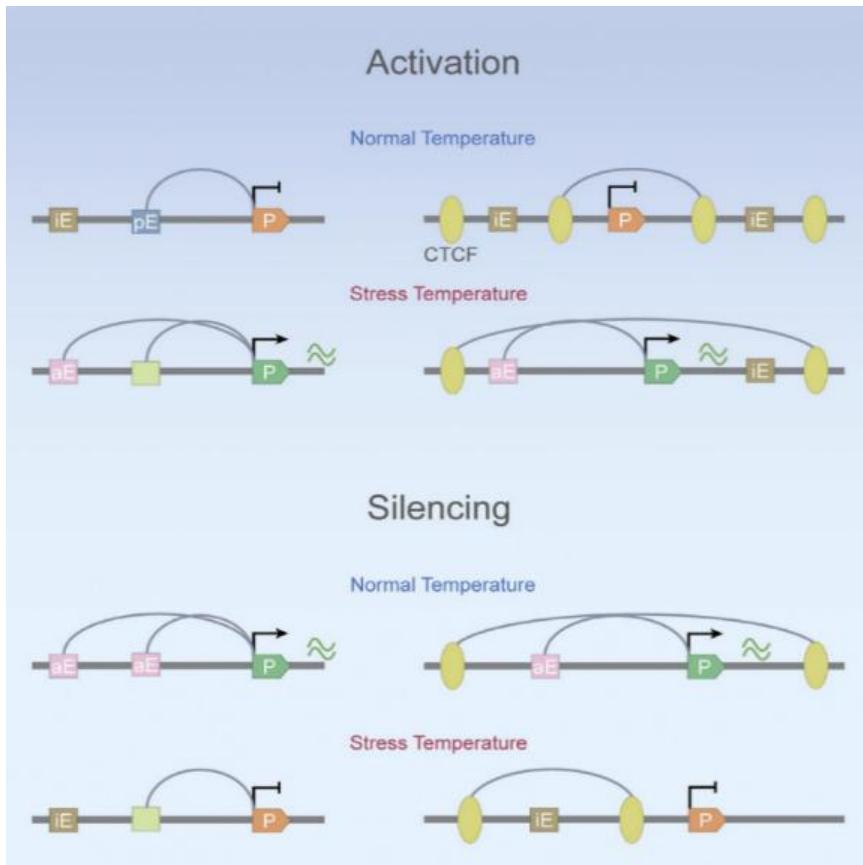
4. Heat shock can induce biomolecular condensates.

5. Hsf1 (usually located in H3K27ac regions), together with Mediator and Pol II, forms dynamic transcriptional condensates to drive 3D genome reorganization during stress



After HS, **architecture proteins (APs)** redistribute from the **TAD boundaries** to the **inside of TADs**. **TAD boundaries weaken** and the proportion of loops with a large span increases.

Li et al., Molecular Cell. 2015



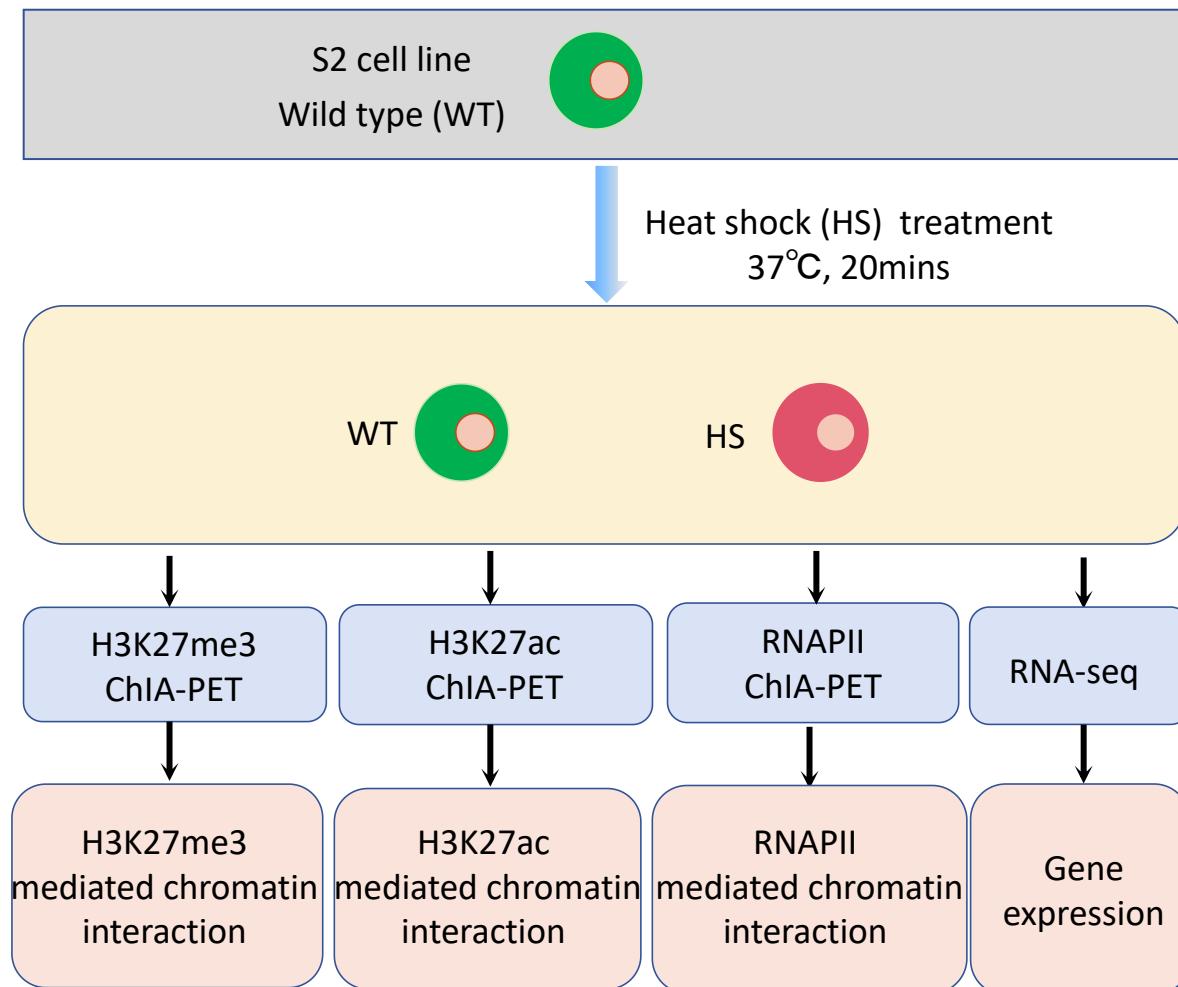
After HS, loops can randomly extrude to regulate the gene expression

Lyu et al., Molecular Cell. 2018

Project design

Project design

Question: How active and inactive regions of genome are functionally changed in 3D chromatin architecture in response to heat stress?

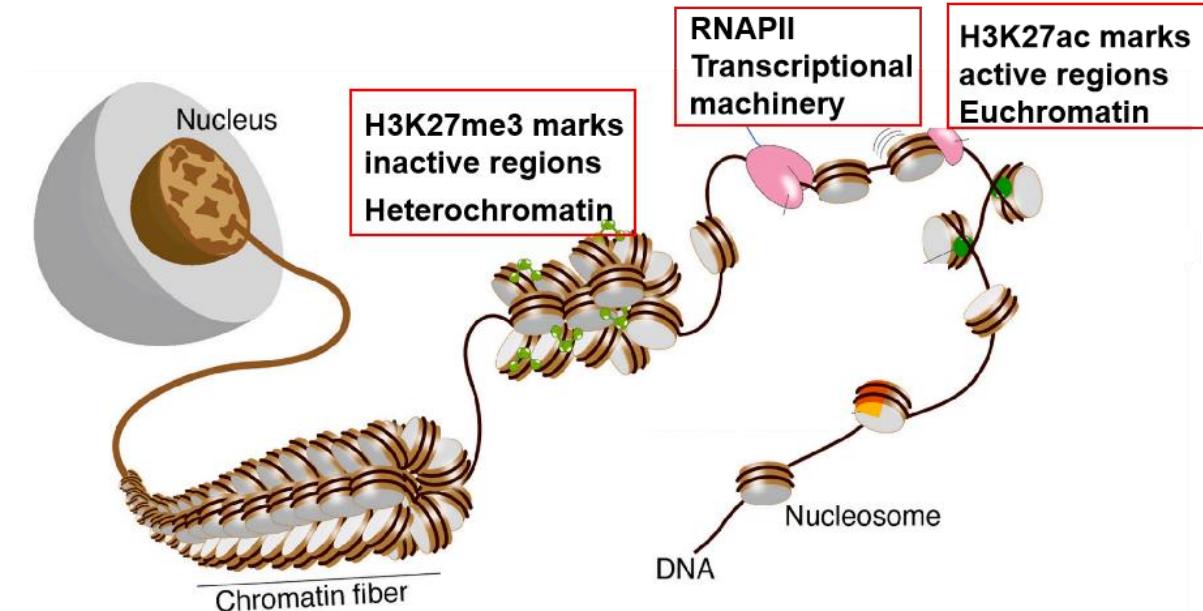


H3K27me3: inactive histone mark

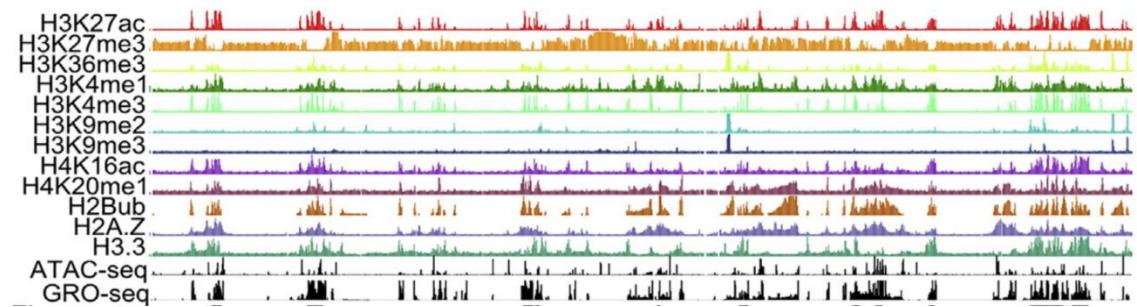
H3K27ac: active histone mark

RNAPII: transcriptional machinery protein

RNA-seq: mark the gene expression level



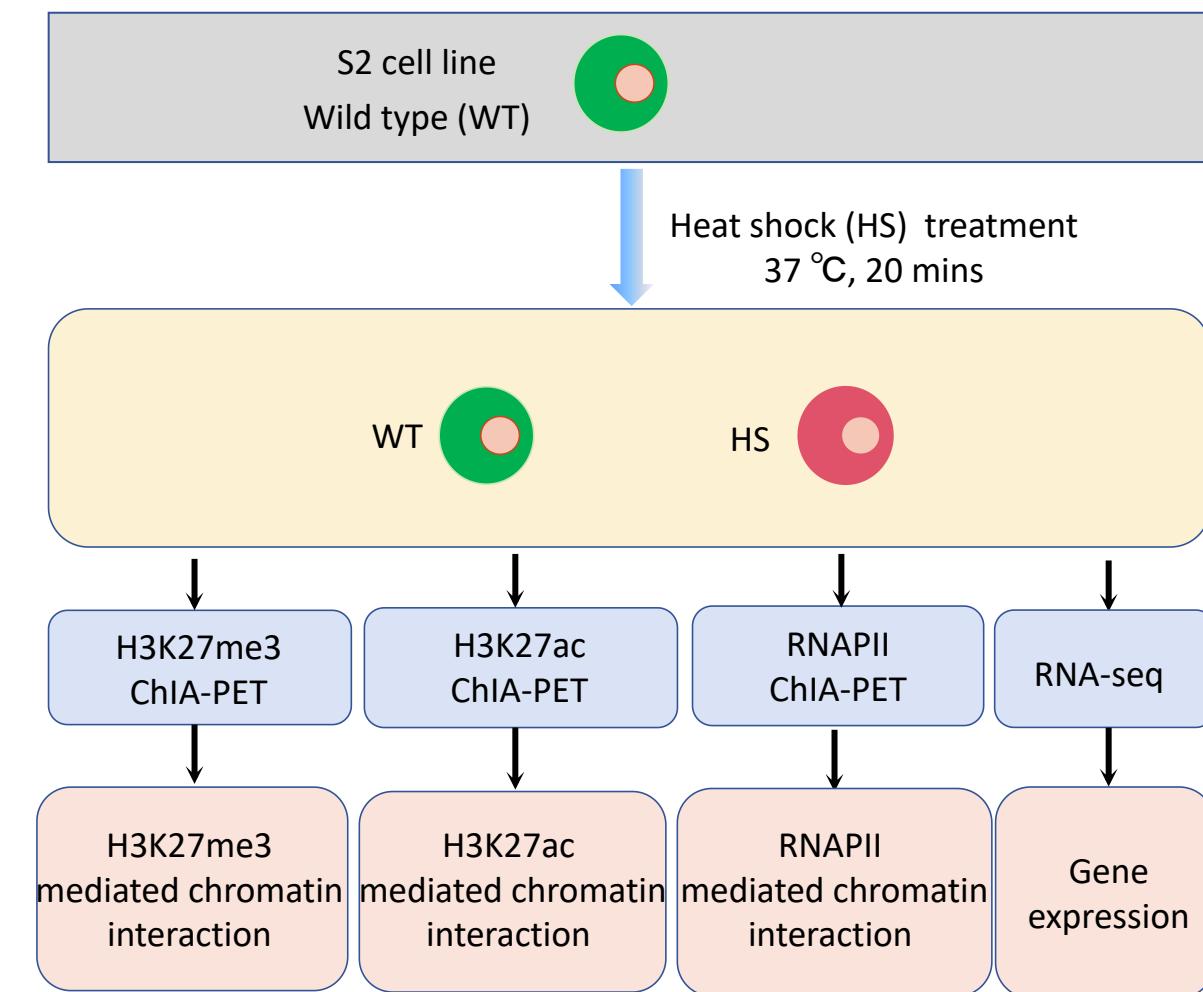
Adapted from Takizawa et al., BBA - Gene Regulatory Mechanisms . 2022



Rowley et al., Molecular Cell. 2017

in situ ChIA-PET: Duo Ning, Yang Yang, Xu yewen, Chen Gengzhan, Gao Tong
RNA-seq: Novogen

Project design

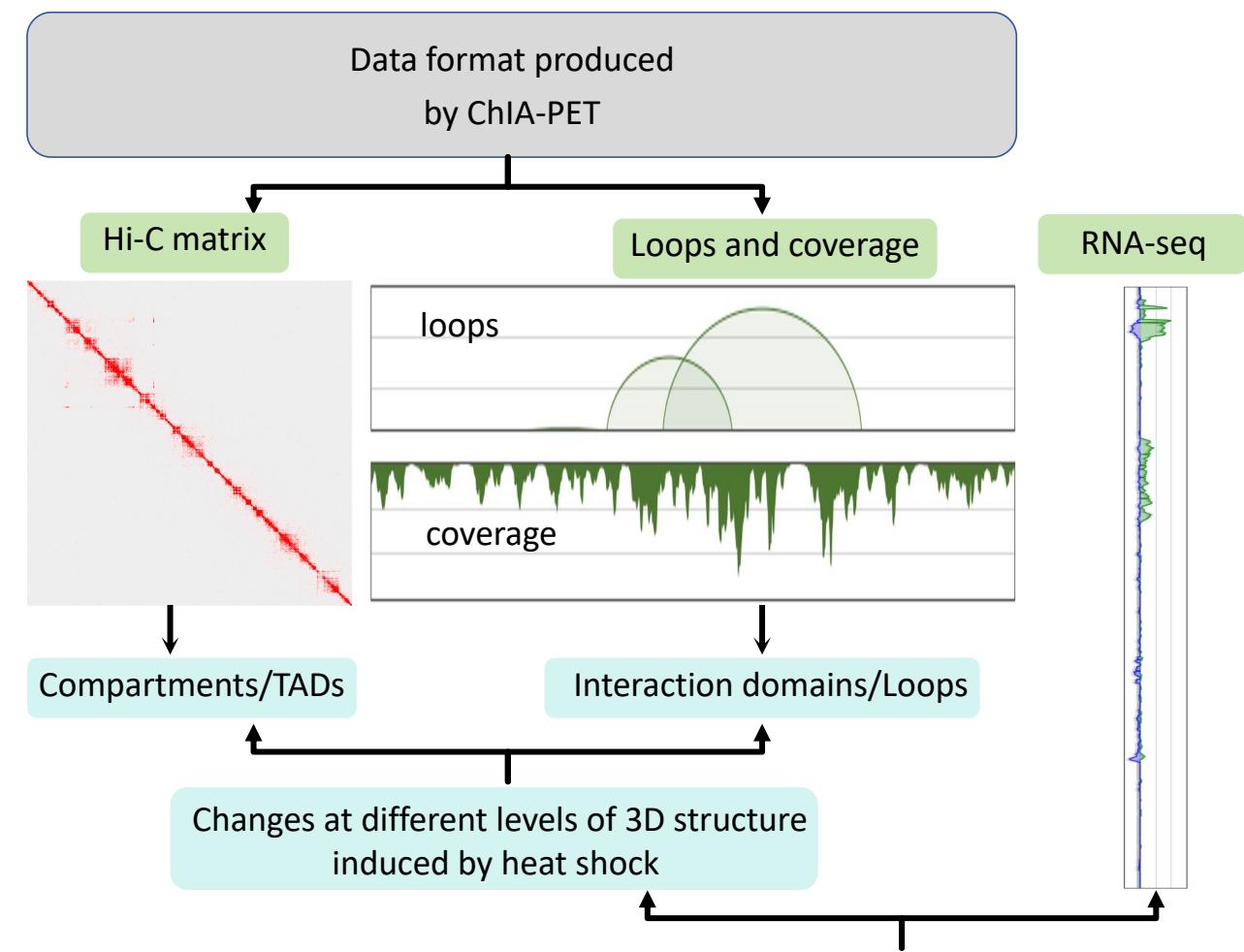


H3K27me3: inactive histone mark

H3K27ac: active histone mark

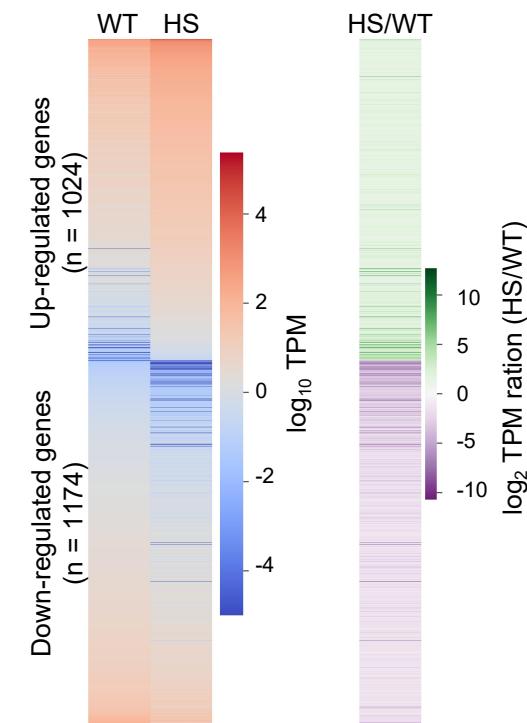
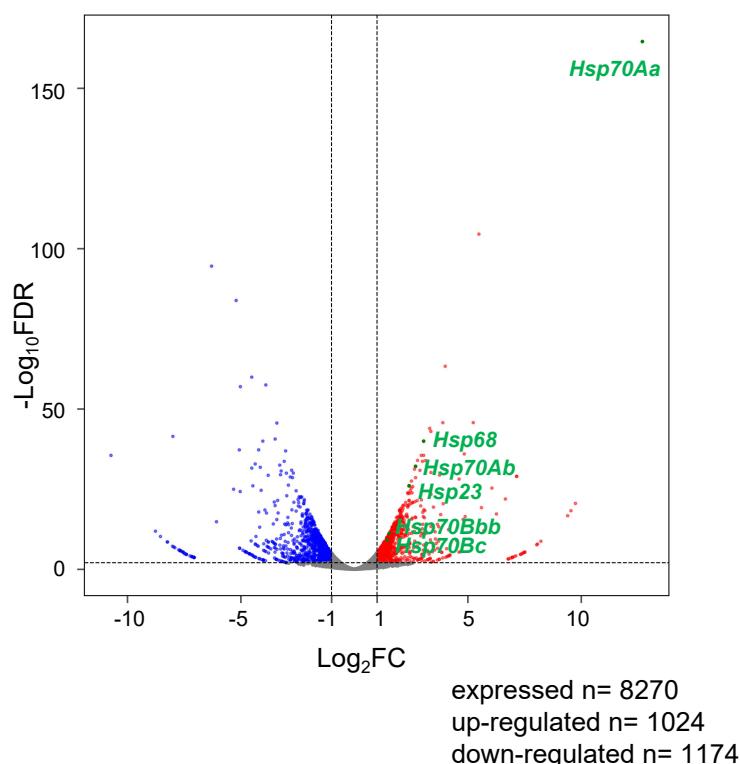
RNAPII: transcriptional machinery protein

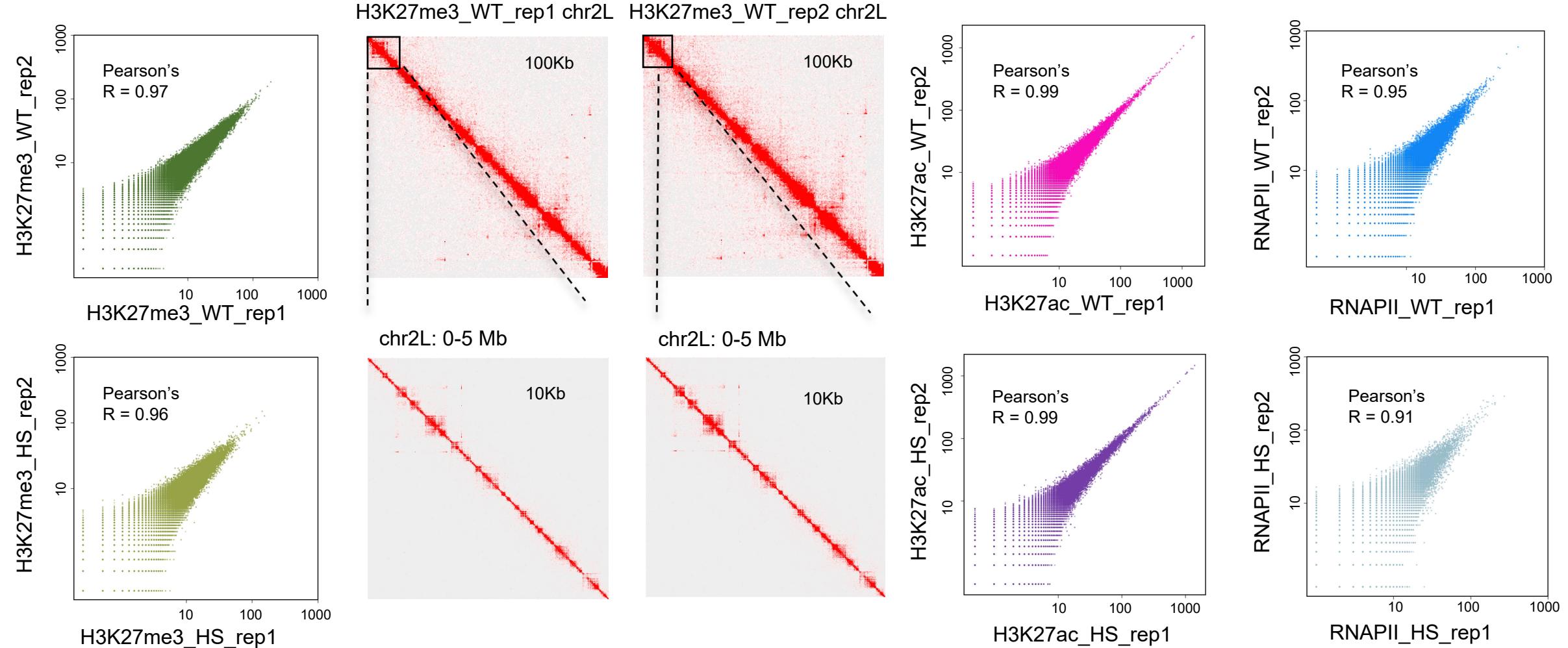
RNA-seq: mark the gene expression level

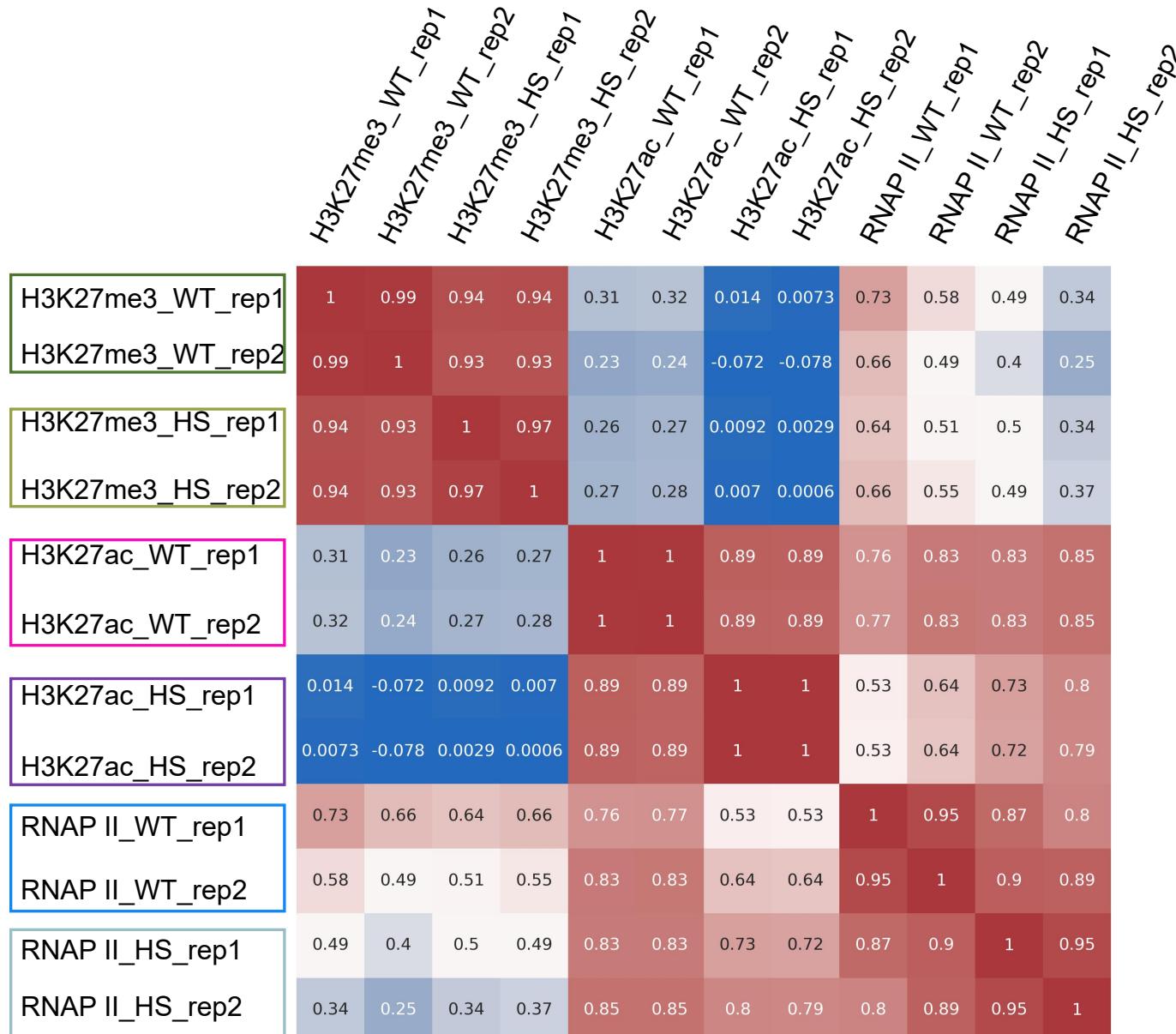


Data analysis

- Data quality: successful heat shock treatment and high reproducibility of ChIA-PET libraries.
- Describe changes of feature induced by heat shock and their relationship with gene regulation at different levels: loops, A/B compartments, and TADs.





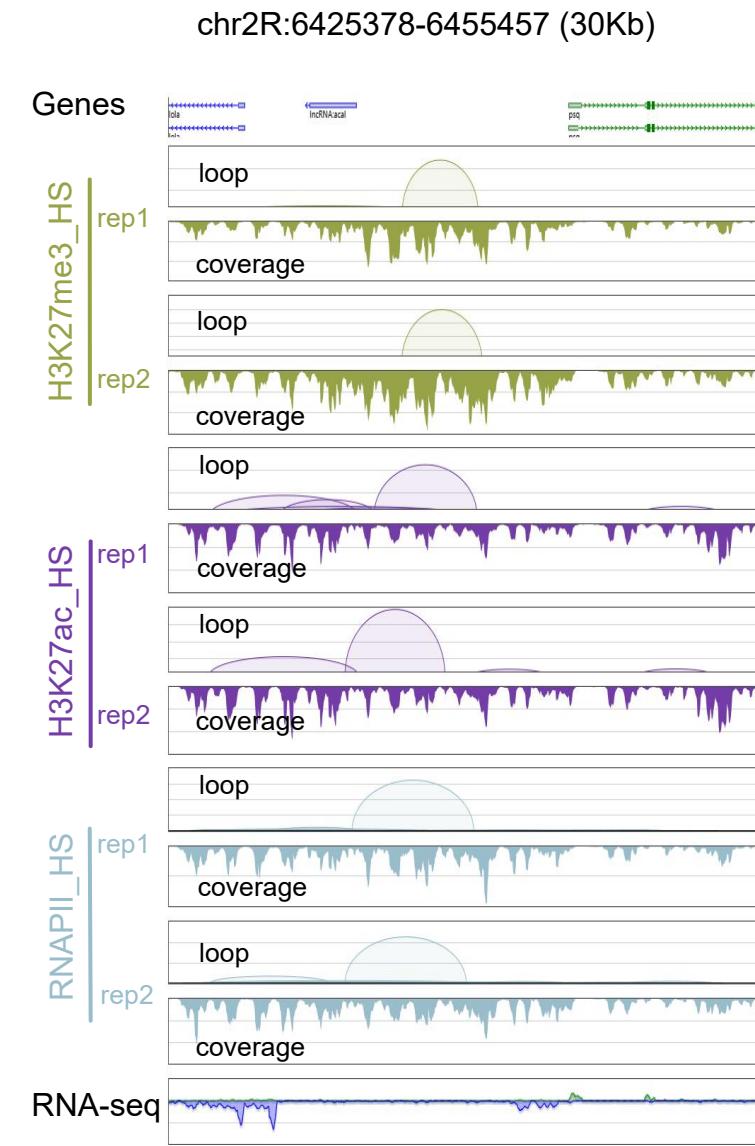
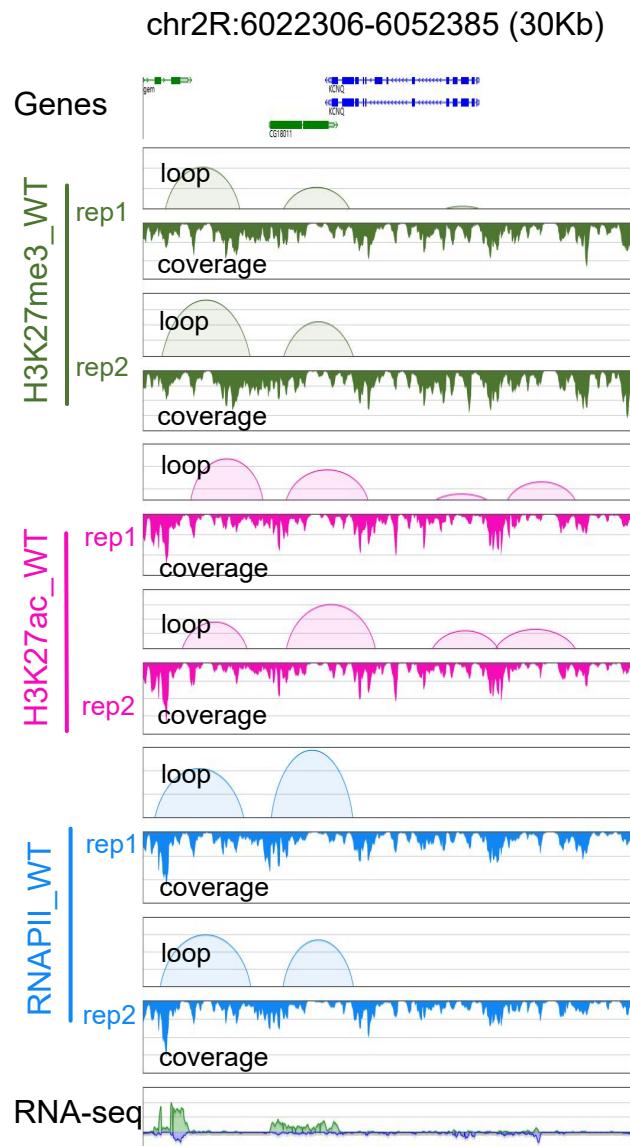


Heatmap of stratum adjusted correlation coefficient (SCC)

The SCC between the WT and its corresponding HS is smaller than that of two replicates;

The SCC between H3K27ac and RNAPII is larger than that between H3K27me3 and RNAPII.

(SCC is called using HiCRep.)
Yang et al., Genome Res. 2017

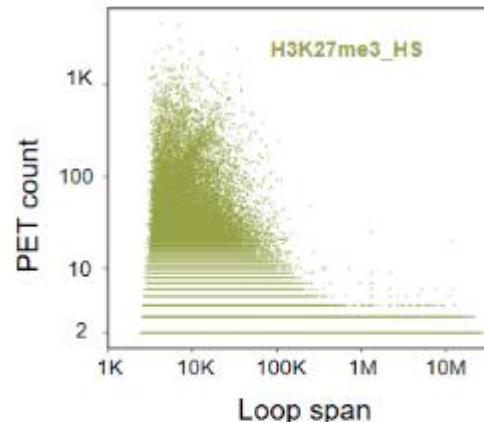
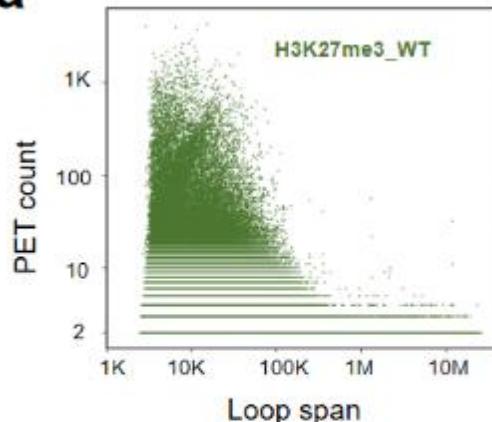
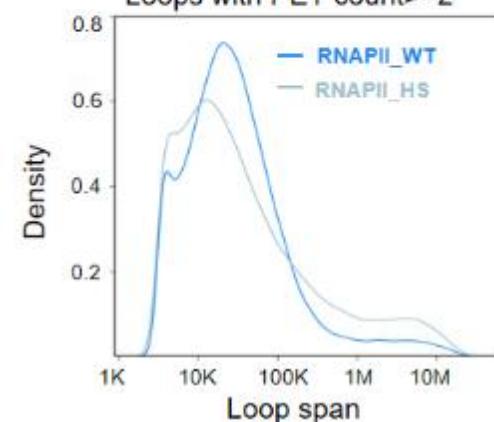
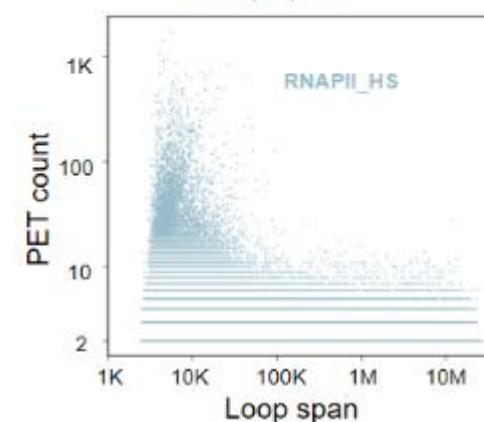
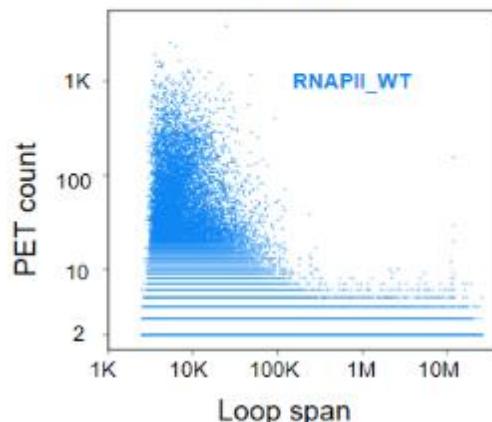
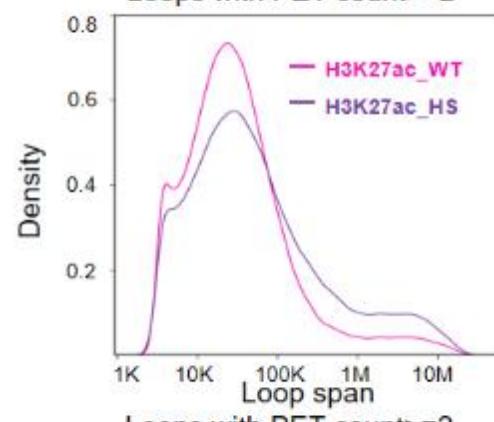
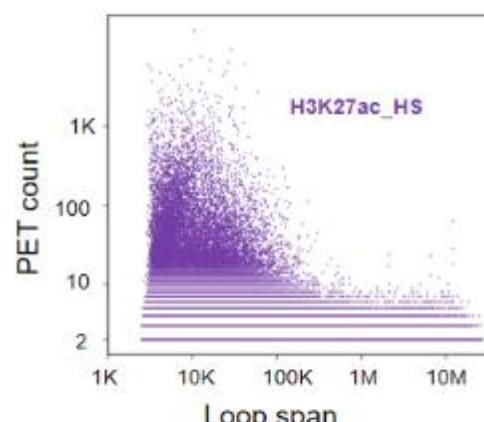
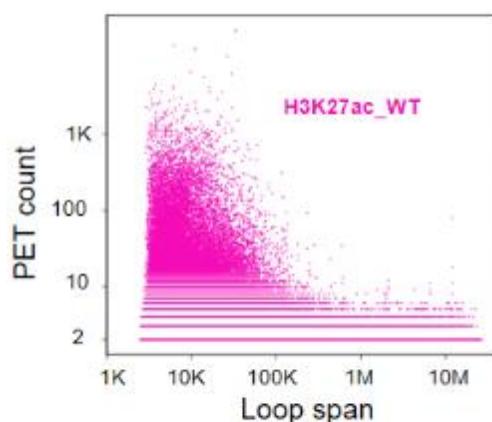
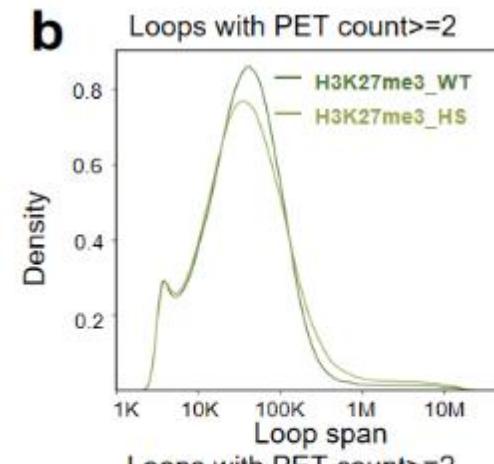
High reproducibility of *in situ* ChIA-PET (loops and coverage)

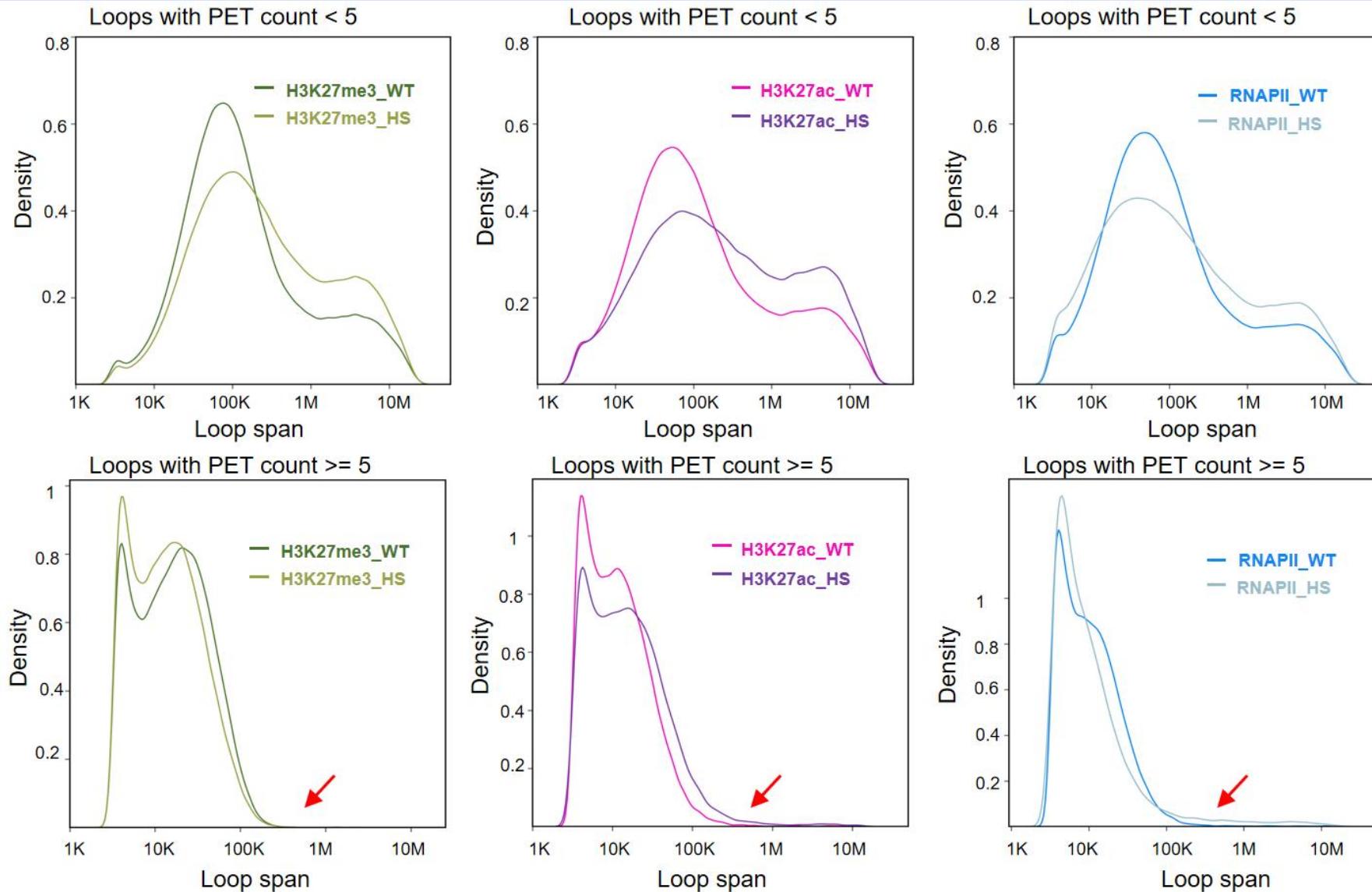
Summary of ChIA-PET libraries **WT and its corresponding HS have similar number of uniquely-mapped PETs**

Factor	Condition	Replicate	Total PETs	Uniquely-mapped PETs	Non-redundant PETs	Self-ligation PETs	Inter-ligation PETs	Intra-chrom cluster	Inter-chrom clusters
H3K27me3	WT	Combined	54,196,067	11,754,993	8,255,144	1,905,070	6,350,074	5,871,420	478,654
		Replicate 1	31,196,067	7,842,702	5,218,198	1,226,086	3,992,112	3,695,348	296,764
		Replicate 2	23,000,000	3,912,291	3,037,084	679,092	2,357,992	2,176,100	181,892
H3K27me3	HS	Combined	53,581,951	12,475,429	8,971,369	1,788,053	7,183,316	6,576,555	606,761
		Replicate 1	29,022,879	6,258,437	4,441,052	815,232	3,992,112	3,695,348	296,764
		Replicate 2	24,559,072	6,216,992	4,530,443	972,918	3,557,525	3,288,767	268,758
H3K27ac	WT	Combined	58,556,384	15,846,412	6,692,692	2,089,351	4,603,341	4,211,408	391,933
		Replicate 1	31,468,415	9,048,737	2,648,285	662,048	1,986,237	1,804,554	181,683
		Replicate 2	27,464,789	6,616,512	3,094,846	966,519	2,128,327	1,943,330	184,997
H3K27ac	HS	Combined	56,947,753	15,597,158	5,414,052	1,373,305	4,040,747	3,667,067	373,680
		Replicate 1	29,980,516	9,048,737	2,648,285	662,048	1,986,237	1,804,554	181,683
		Replicate 2	26,967,237	6,548,421	2,768,647	712,217	2,056,430	1,864,269	192,161
RNAPII	WT	Combined	65,554,144	15,807,711	5,729,916	1,985,975	3,743,941	3,472,699	271,242
		Replicate 1	27,879,478	7,140,240	2,985,833	970,671	2,015,162	1,875,146	140,016
		Replicate 2	37,674,666	8,667,471	2,744,406	1,015,604	1,728,802	1,597,576	131,226
RNAPII	HS	Combined	61,542,728	15,270,275	3,291,274	950,879	2,340,395	2,140,003	200,392
		Replicate 1	23,451,727	5,820,051	1,306,565	326,043	980,522	900,508	80,014
		Replicate 2	38,091,001	9,450,224	1,984,759	624,883	1,359,876	1,239,498	120,378

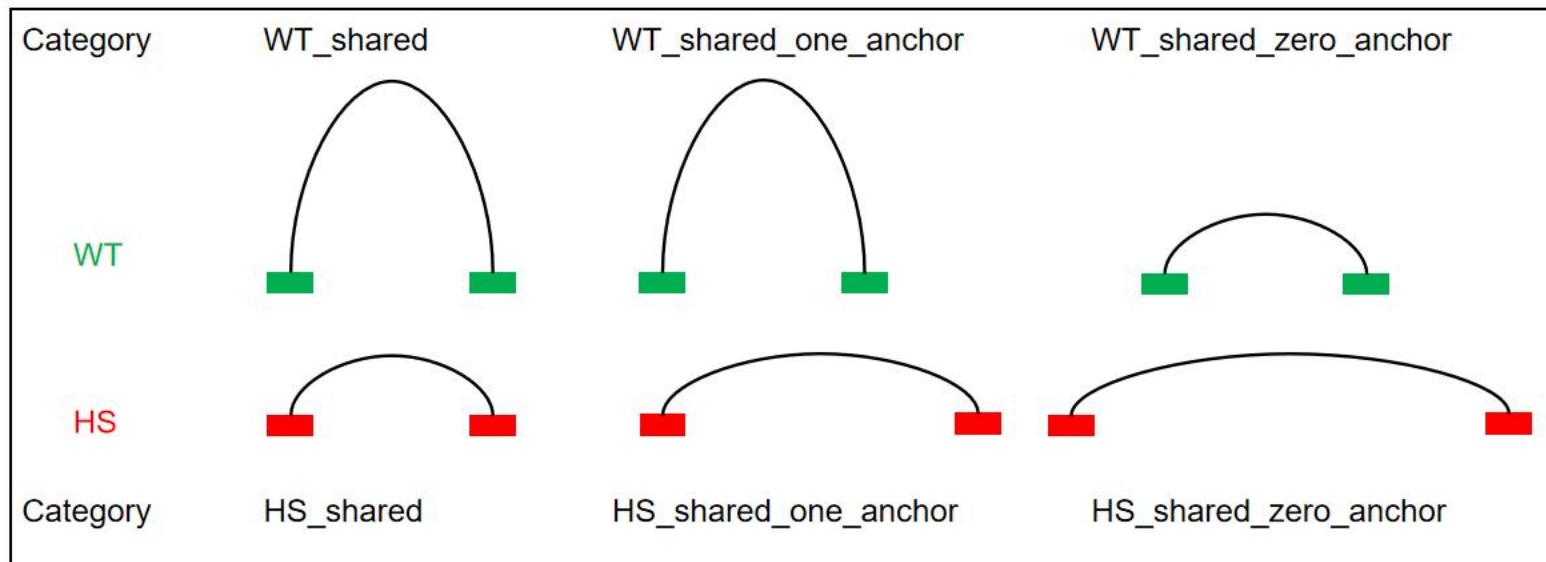
The ChIA-PET datasets of S2 cell line in the above table were mapped to the reference genome dm3.

in situ ChIA-PET: Duo Ning, Yang Yang, Xu yewen, Chen Gengzhan, Gao Tong
 RNA-seq: Novogen
 ChIA-PIPE: Deng Yuqing

a**b**

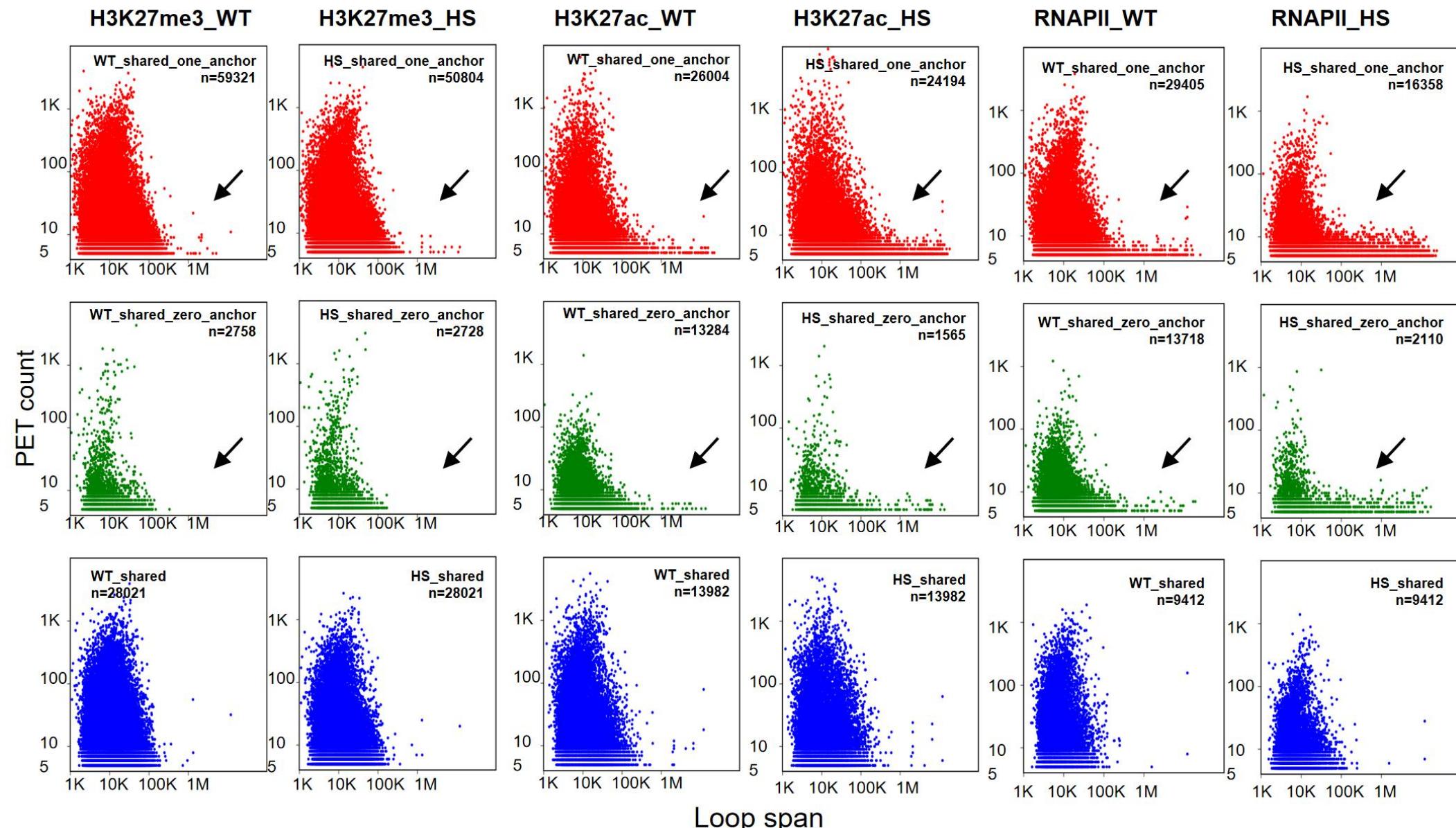


After HS, proportion of loops with large loop span increase in H3K27ac_ChIA-PET and RNAP II_ChIA-PET



Loop level

Features of chromatin loops enriched by different factors before and after heat shock

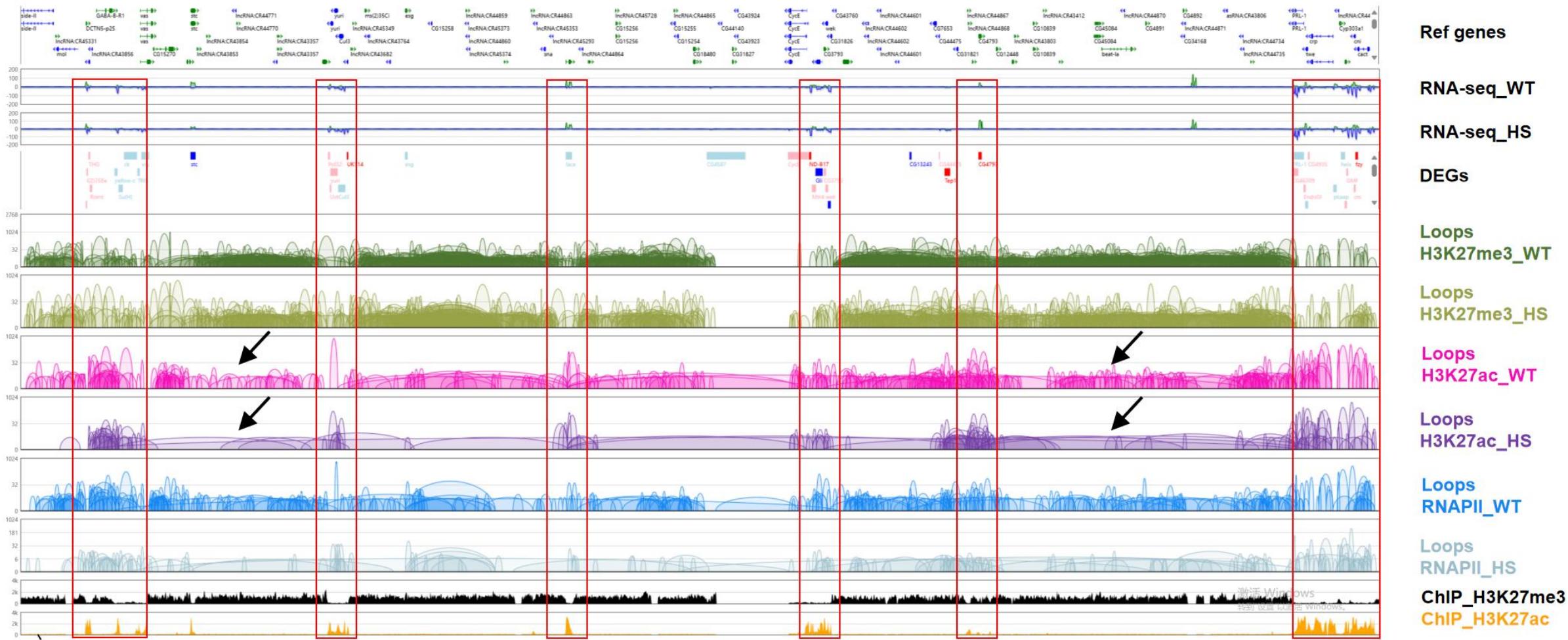


After HS, proportion of loops with large loop span and low PET count increase in H3K27ac_ChIA-PET and RNAP II_ChIA-PET

Loop level

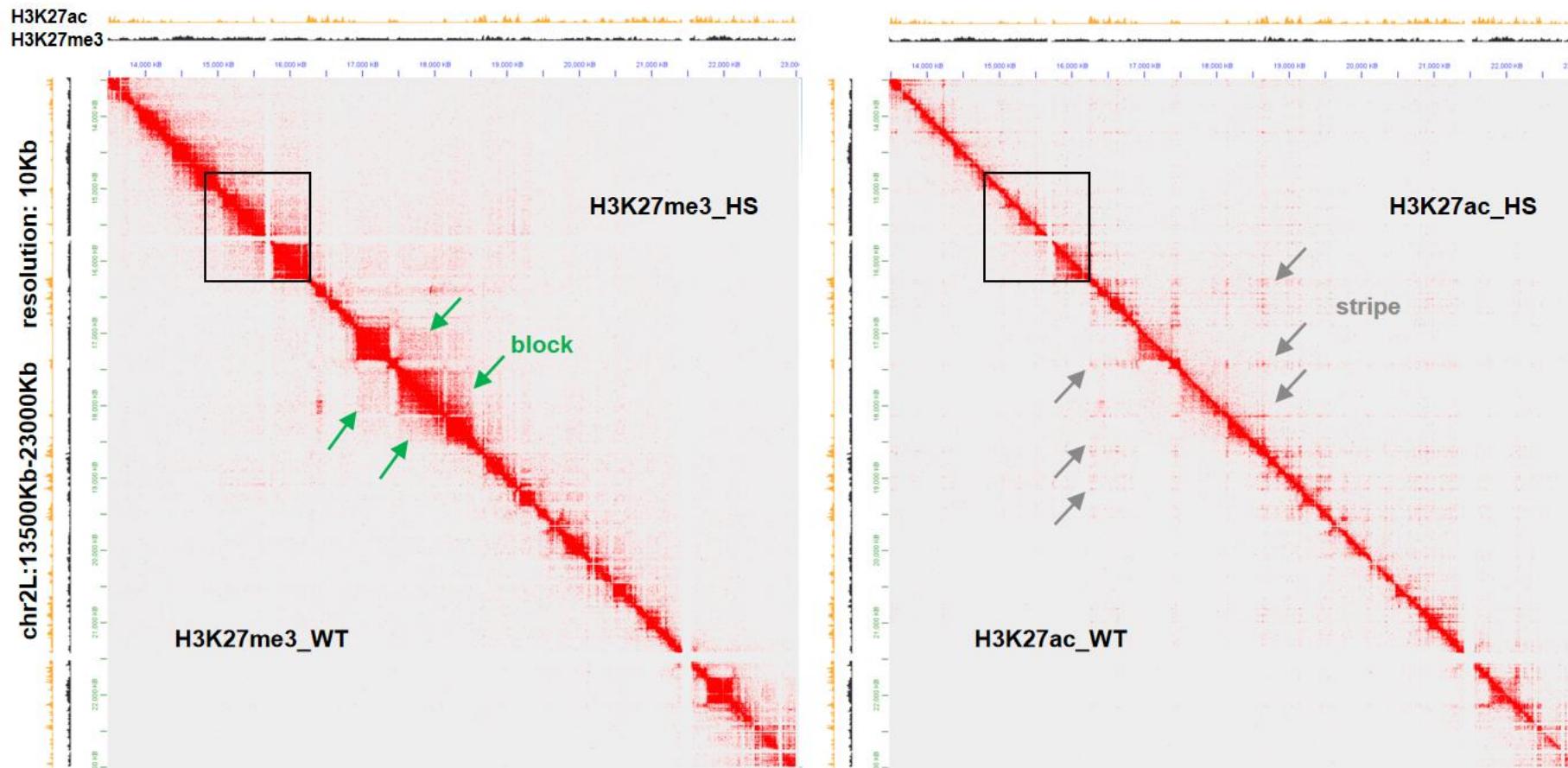
Loops induced by heat shock with low PET counts and large loop span connecting different H3K27ac-dominant regions

chr2L:14899489-16346700



Loop level

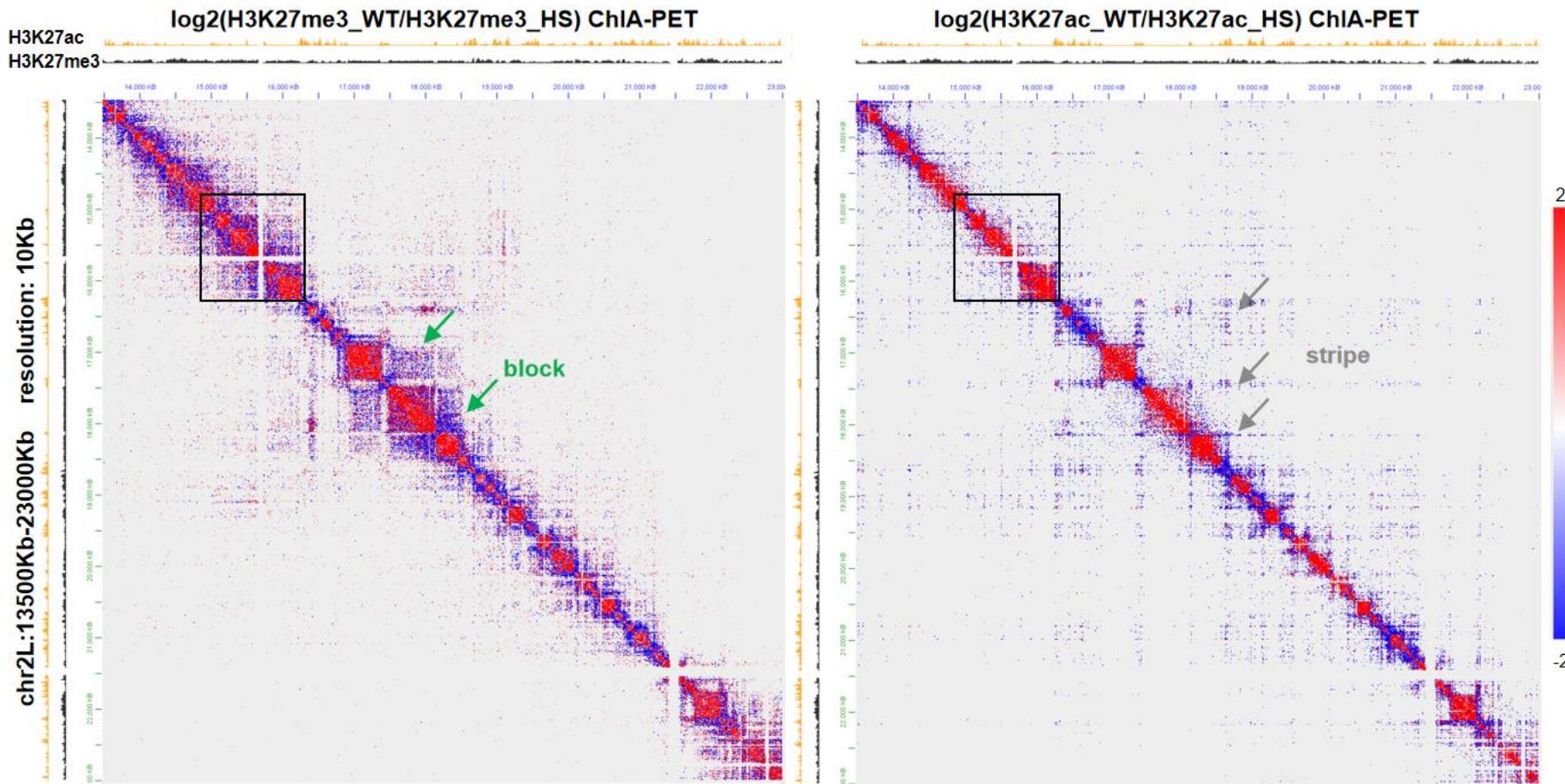
Loops induced by heat shock with low PET counts and large loop span connecting different H3K27ac-dominant regions



1. Blocks in H3K27me3_ChIA-PET reflect the interaction between adjacent H3K27me3-dominant regions, while stripes in H3K27ac_ChIA-PET reflect the interaction among multiple H3K27ac-dominant regions
2. After heat shock, blocks in H3K27me3_ChIA-PET and stripes in H3K27ac_ChIA-PET are more obvious.

Loop level

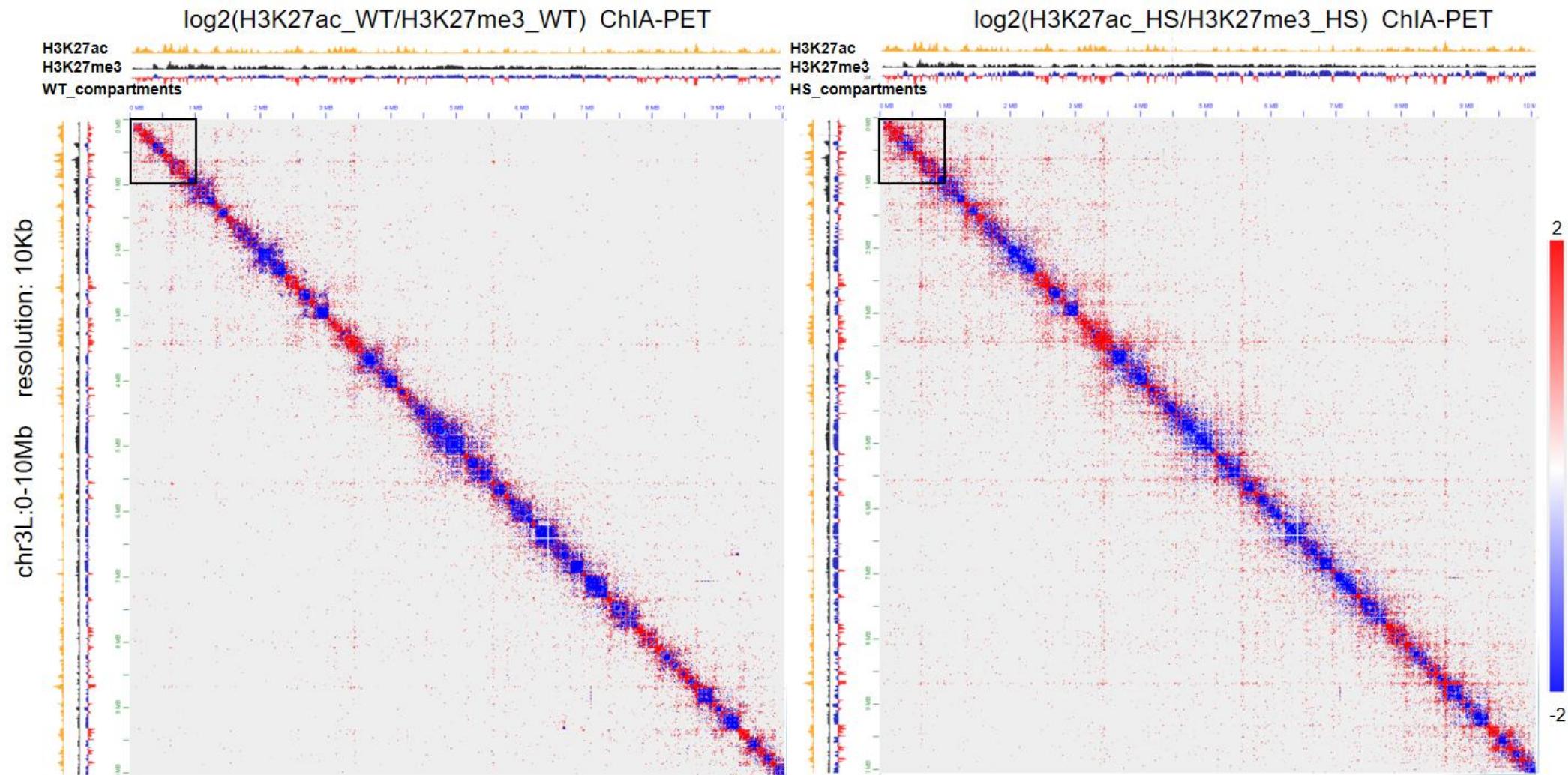
Loops induced by heat shock with low PET counts and large loop span connecting different H3K27ac-dominant regions



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Compartment level

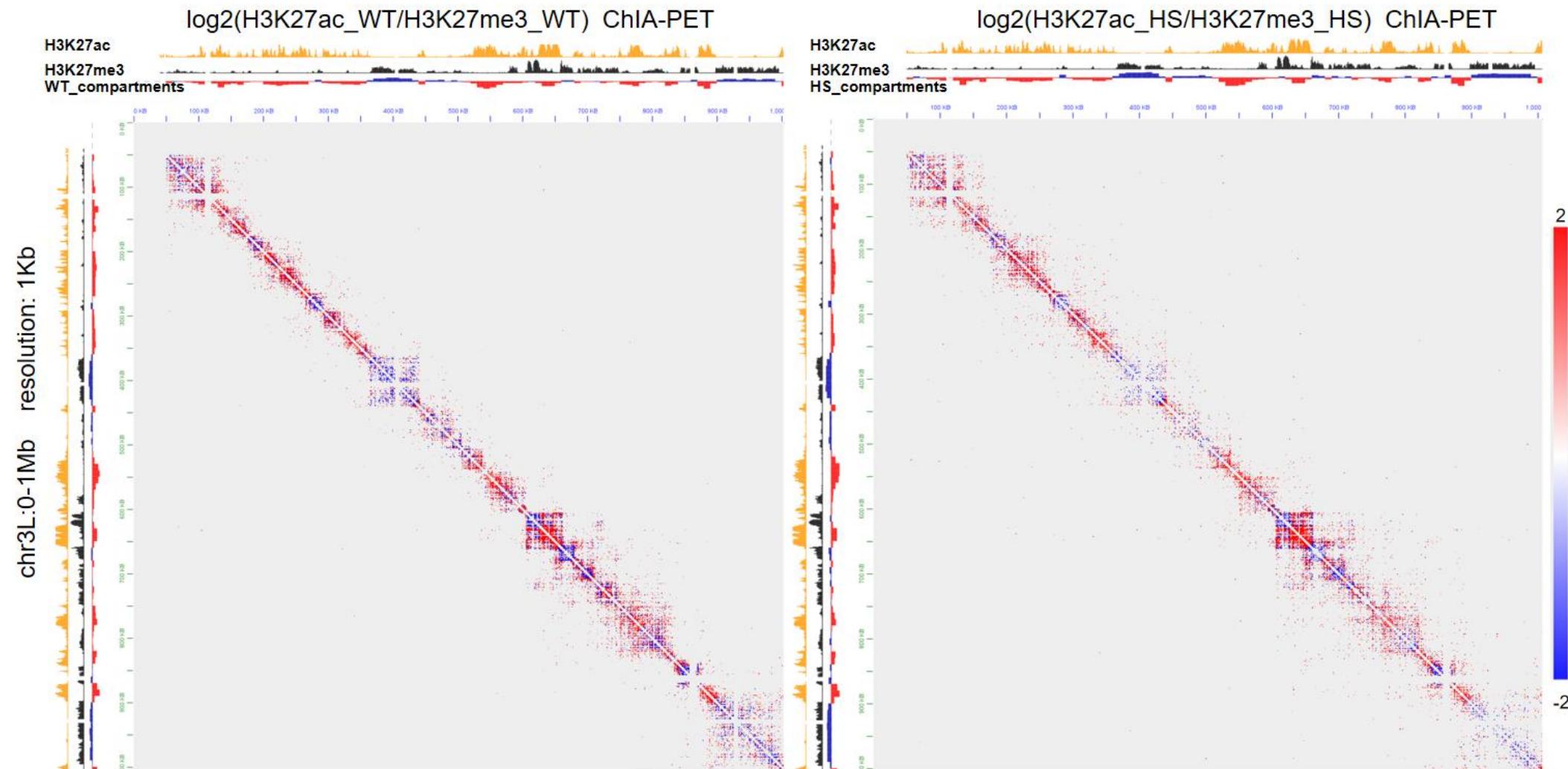
Log₂(H3K27ac/H3K27me3) and can be used to call H3K27ac-dominant and H3K27me3-dominant regions



Compartment score = $\log_2(\text{H3K27ac}/\text{H3K27me3})$, if it's positive, assign the bin as 'A', else assign the 'bin' as 'B'. Its absolute value denotes the extent of compartmentalization

Compartment level

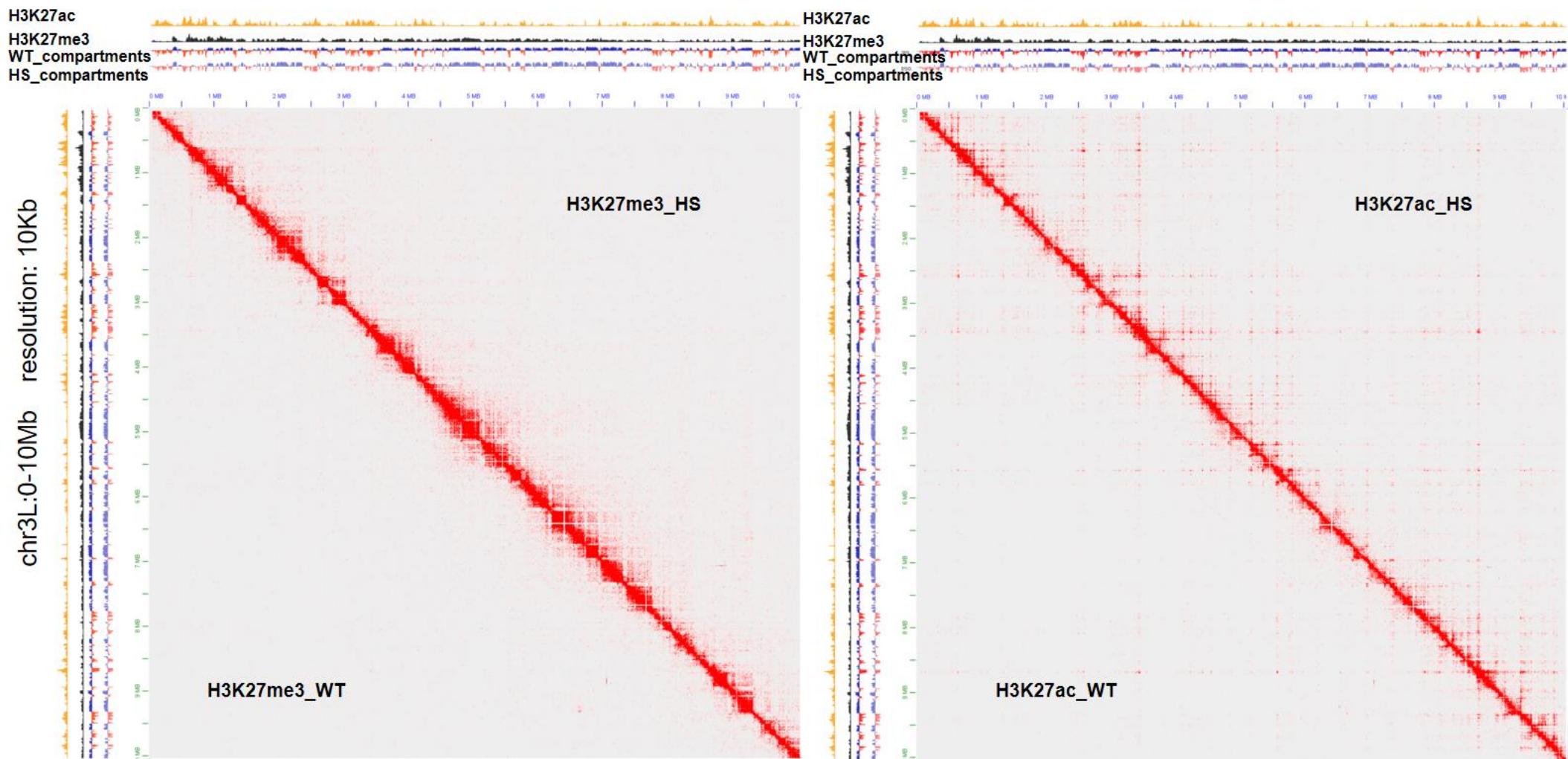
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Compartment level

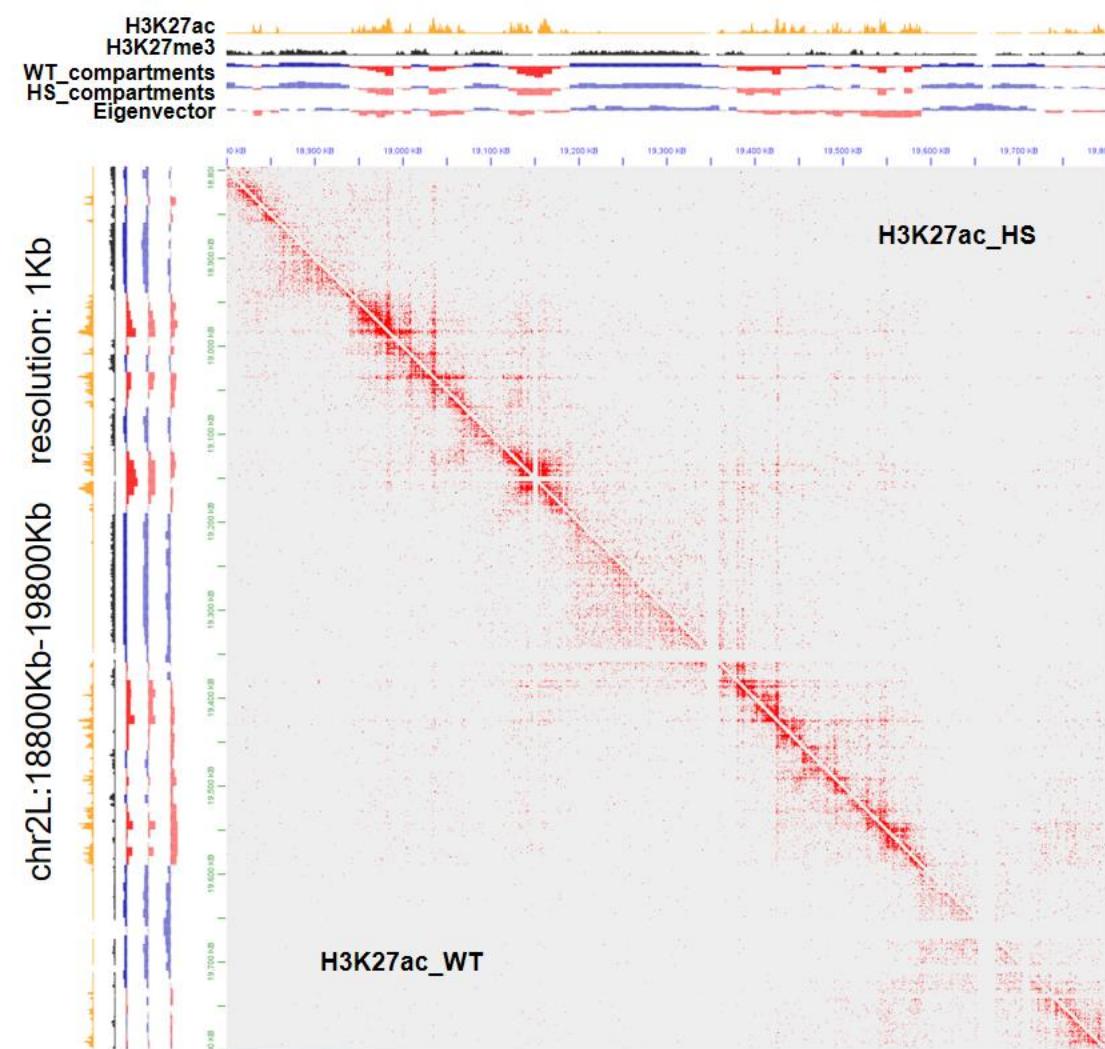
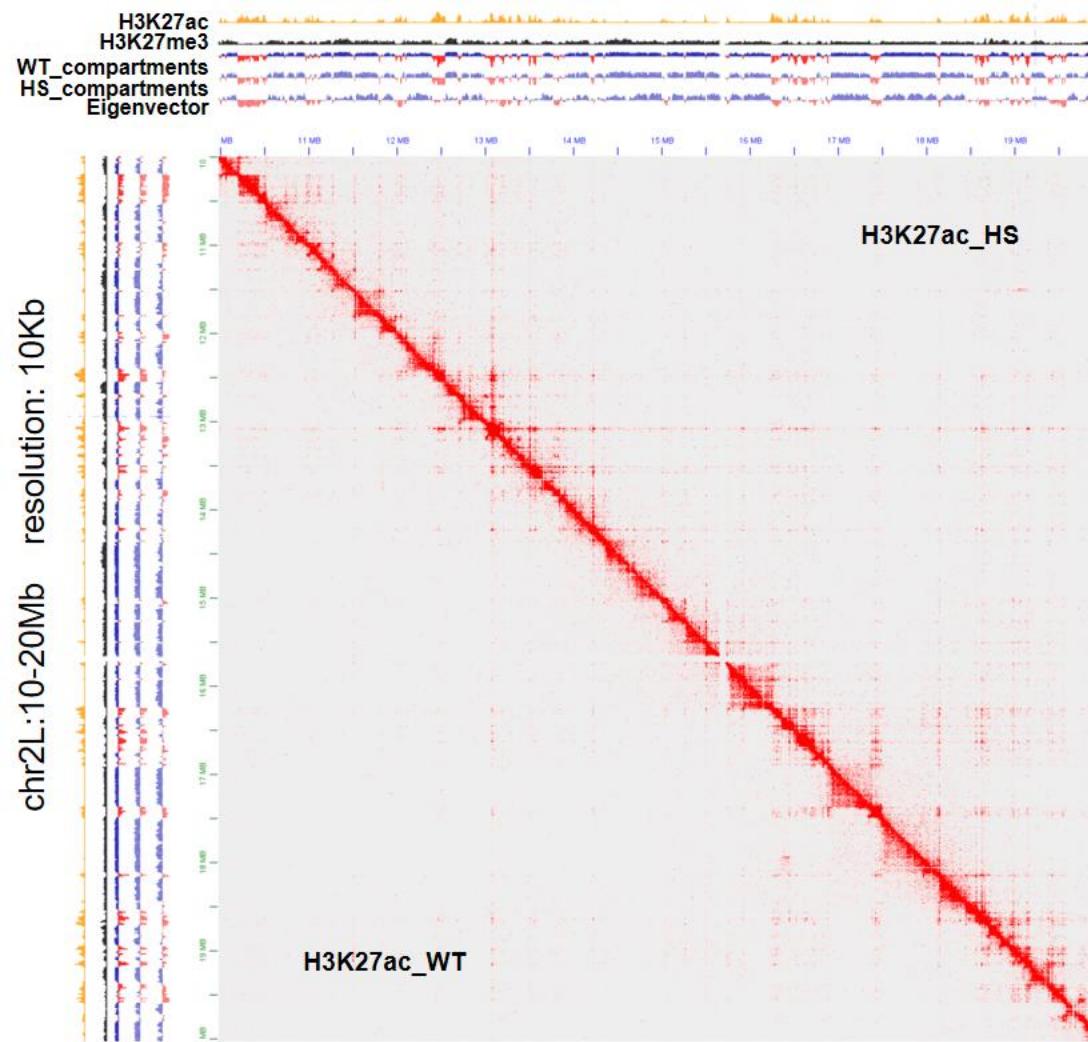
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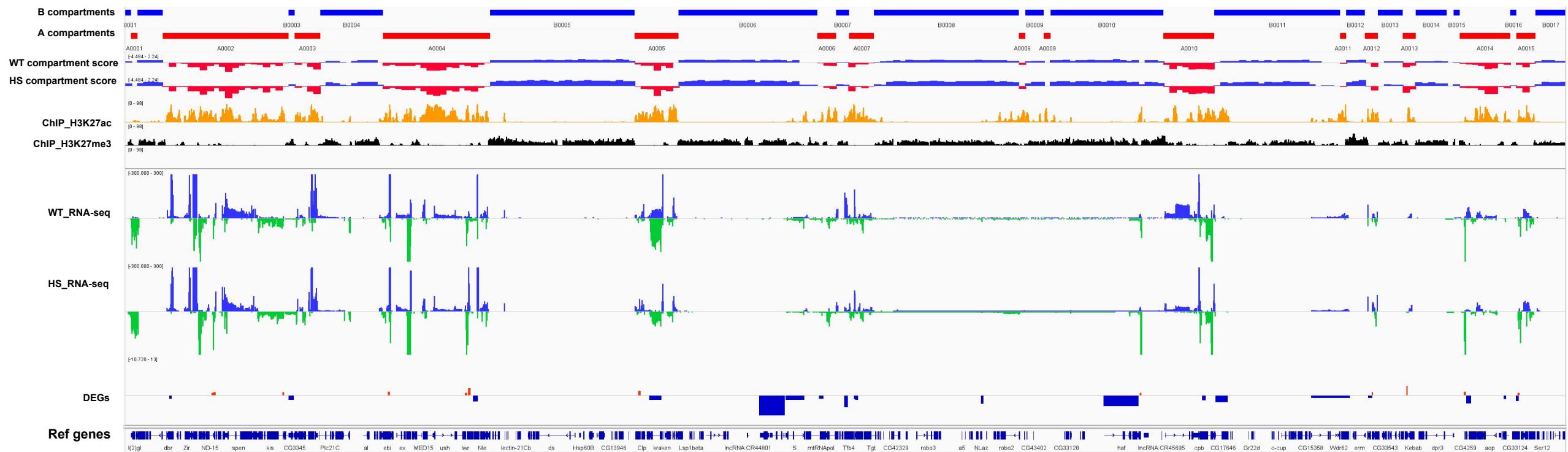
Compartment score = log₂(H3K27ac/H3K27me3), if it's positive, assign the bin as 'A', else assign the 'bin' as 'B'. Its absolute value denotes the extent of compartmentalization

Compartment level

H3K27ac-dominant and H3K27me3-dominant regions align with A and B compartments



Eigenvectors are called using Hi-C data of Drosophila in WT condition at 10 Kb resolution (*Ray et al., PNAS. 2019*)
Eigenvectors are called using PCA (*Erez et al., Science. 2009*)



For DEGs track, red denotes 'up-regulated', while blue denotes 'dn-regulated'. Its height denotes log2FC

$$\text{switch percent} = \frac{AB + BA}{AB + BA + AA + BB}$$

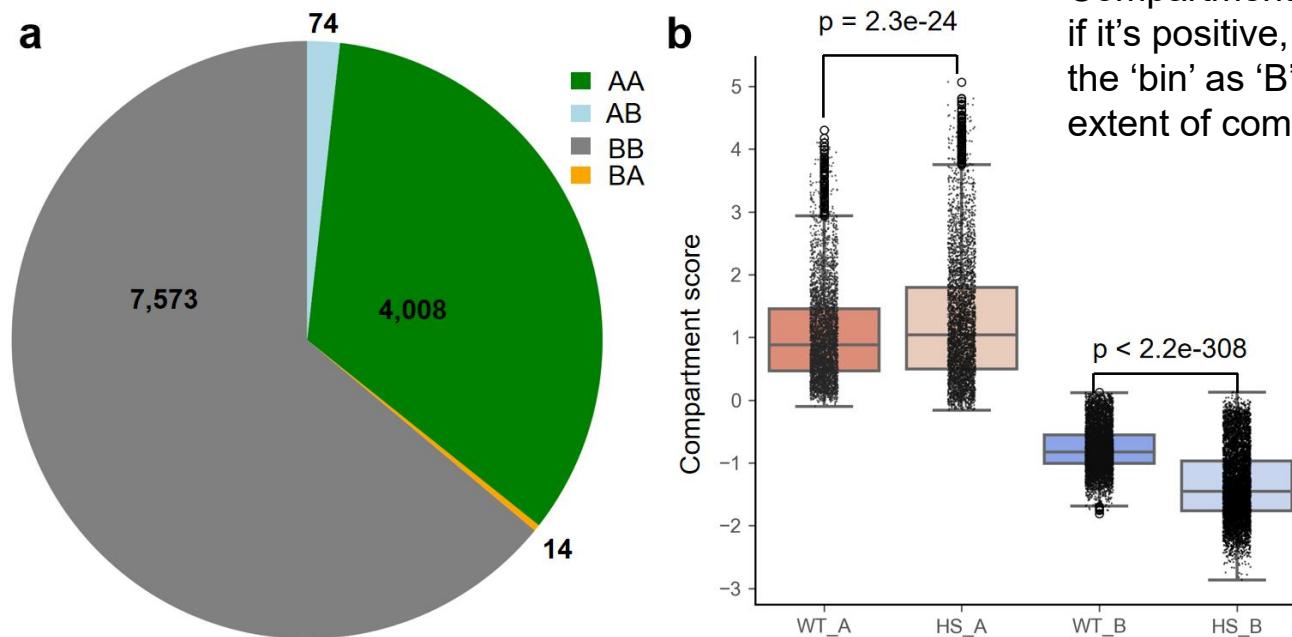
$$A \text{ switch percent} = \frac{AB}{AB + AA}$$

$$B \text{ switch percent} = \frac{BB}{BB + BA}$$

$$\text{switch percent} = 0.8\%$$

$$A \text{ switch percent} = 1.8\%$$

$$B \text{ switch percent} = 0.2\%$$

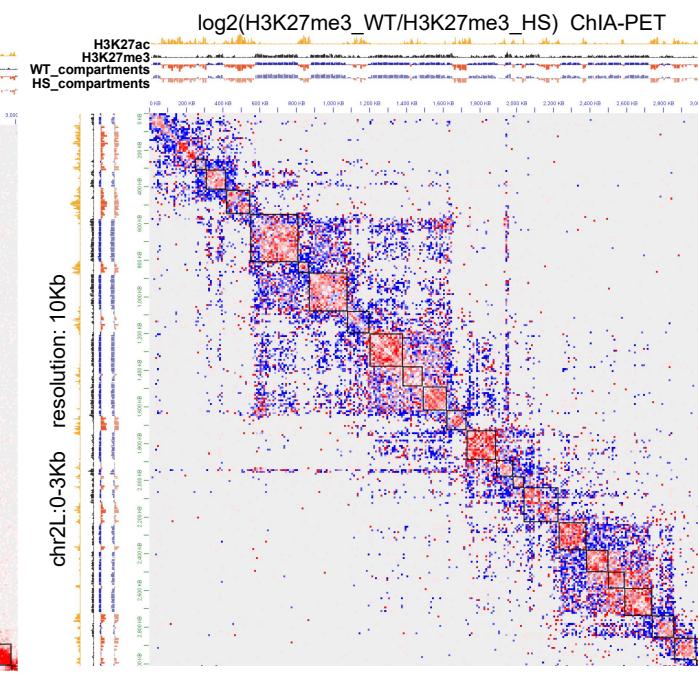
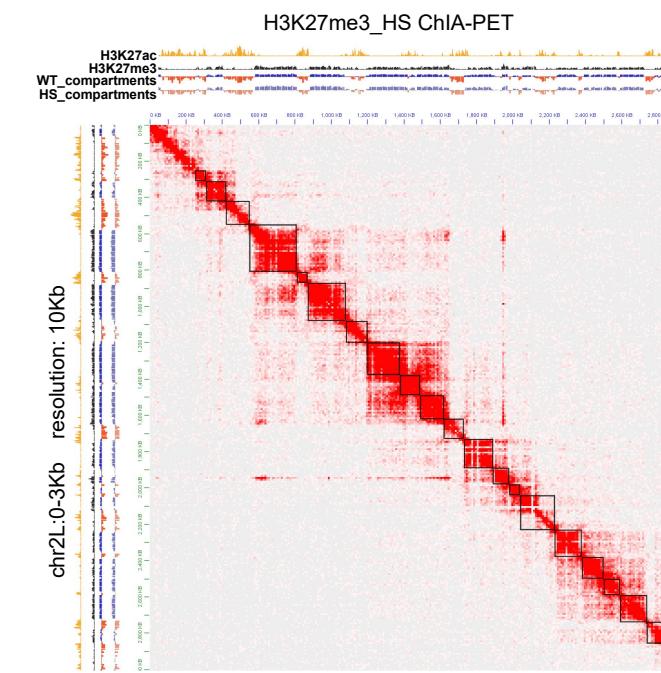
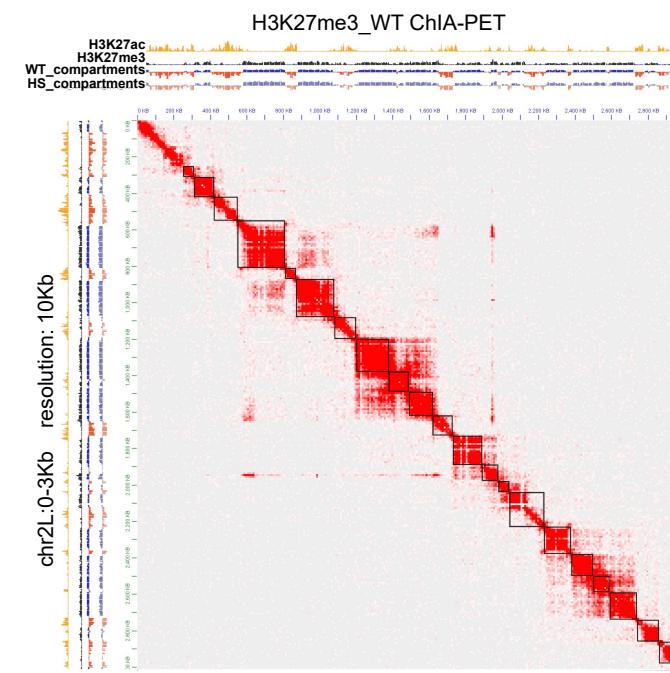


Compartment score = $\log_2(H3K27ac/H3K27me3)$, if it's positive, assign the bin as 'A', else assign the 'bin' as 'B'. Its absolute value denotes the extent of compartmentalization.

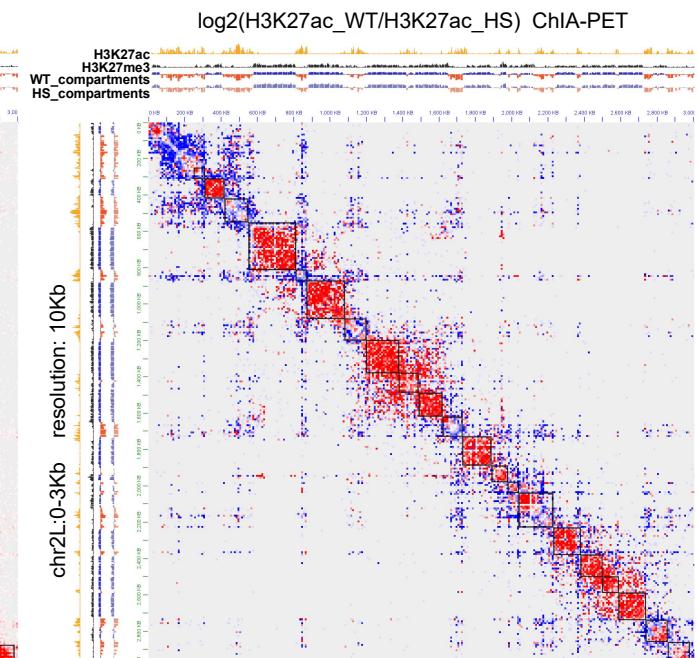
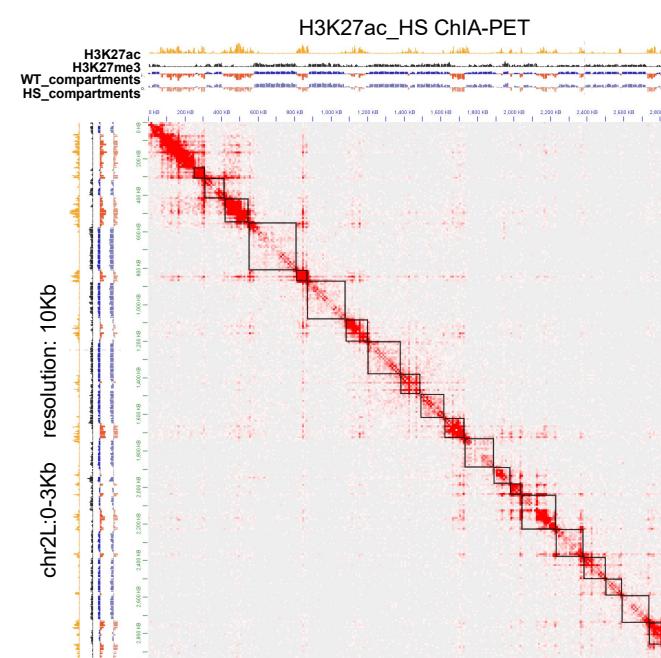
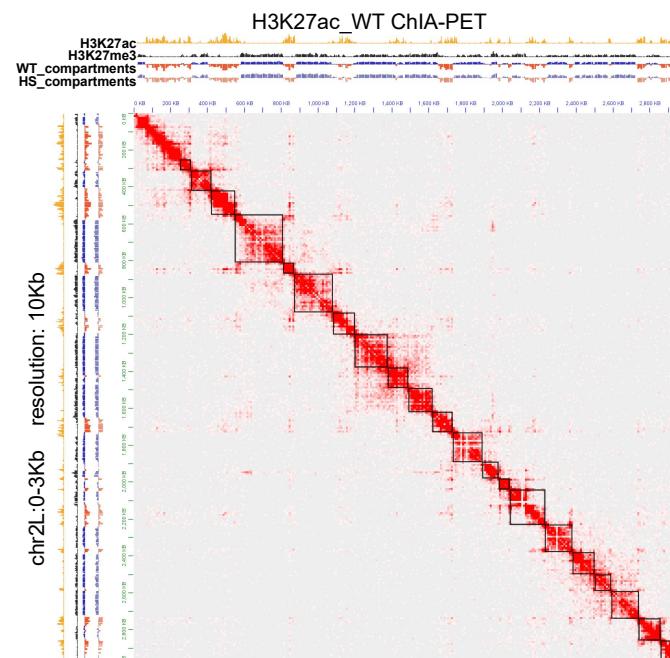
After heat shock, A/B compartment intervals almost do not change, while signals of A/B compartments increase. Because A/B compartment intervals do not change, it's convenient to compare them before and after heat shock.

Compartment level

Dynamic A/B compartments



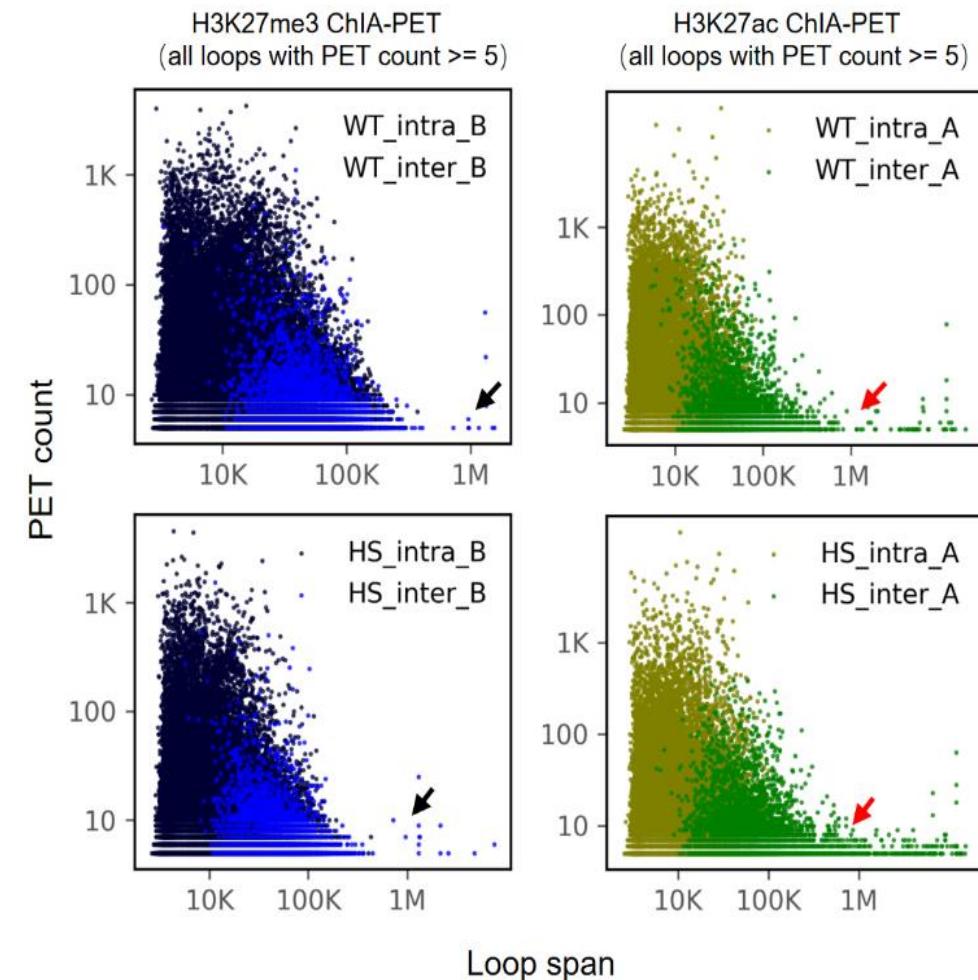
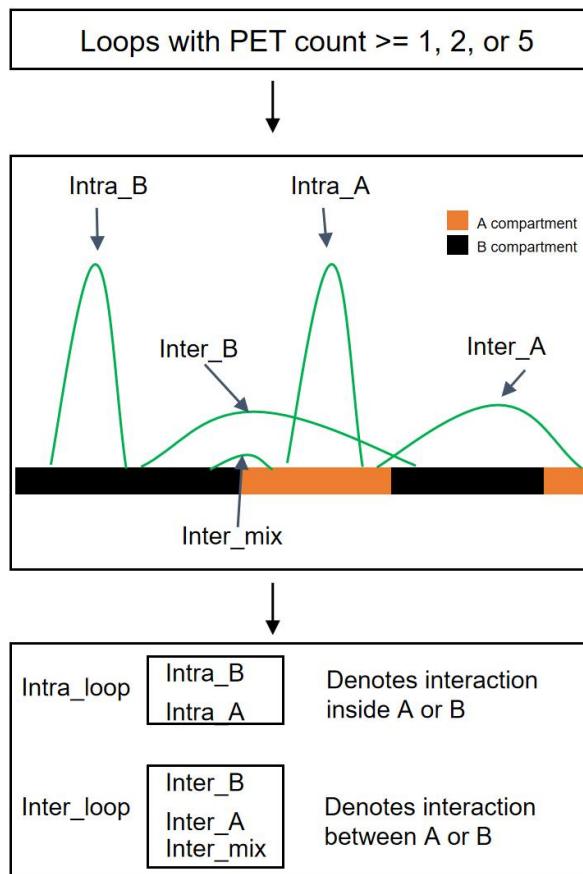
The black square is the TADs called using hic matrix.

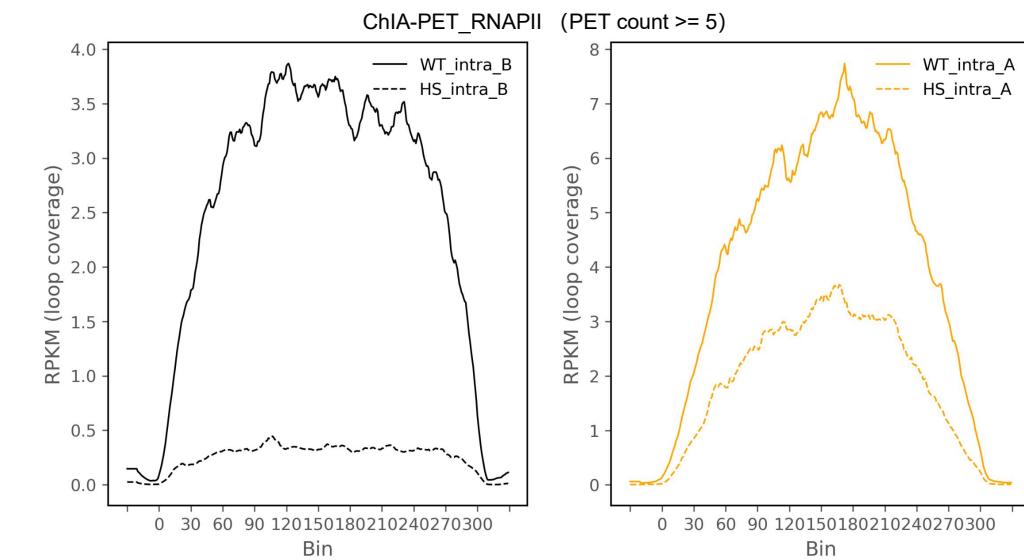
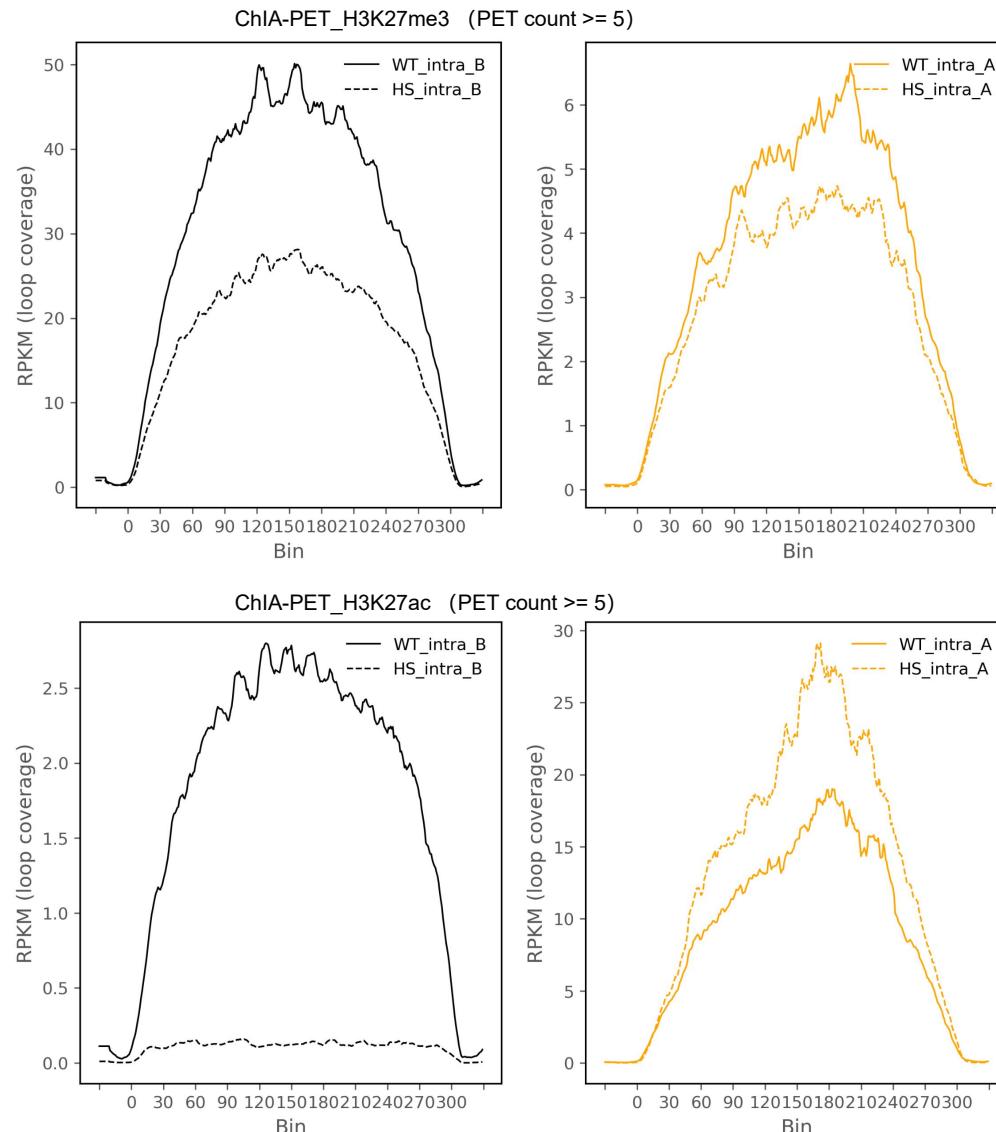


After heat shock, interaction inside A compartments increases, interaction inside B compartments decreases.

Interaction between adjacent B compartments increases, and interaction among multiple A compartments increases.

Goal: quantitatively describe it

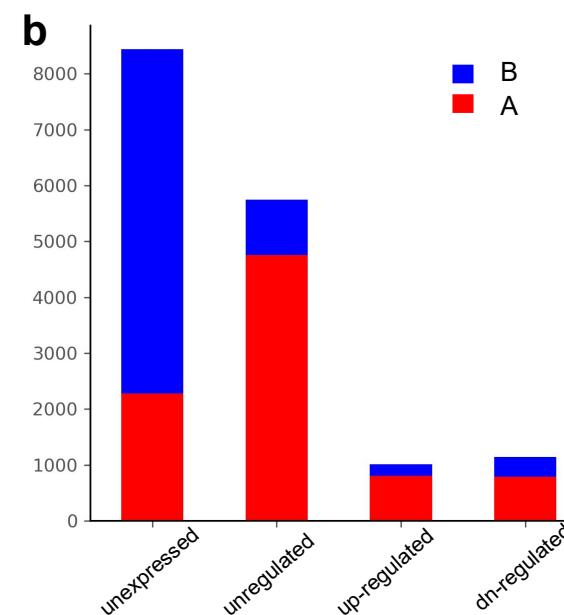
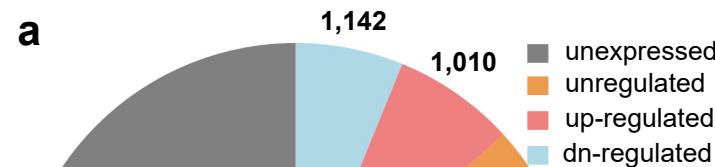




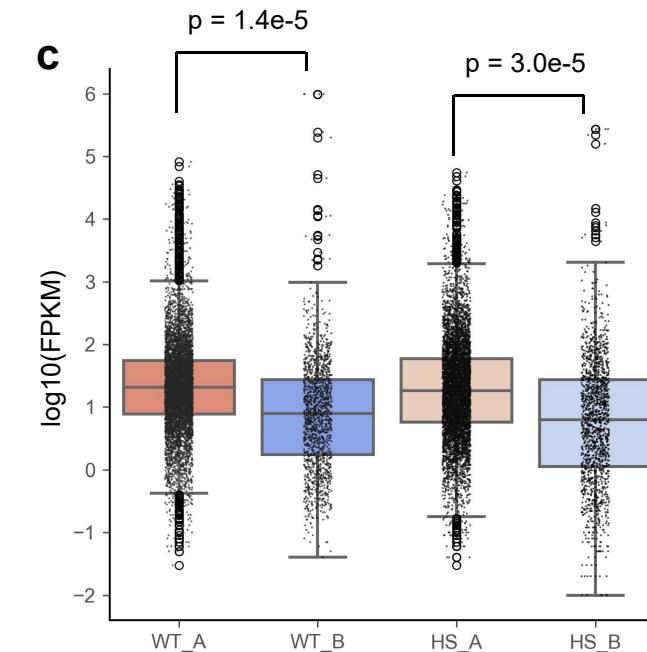
After heat shock, interaction strength mediated by RNAPII drops more in B compartments than A compartments.

While interaction (mediated by H3K27me3) strength inside B compartments decreases after heat shock, interaction (mediated by H3K27ac) strength inside A compartments increases.

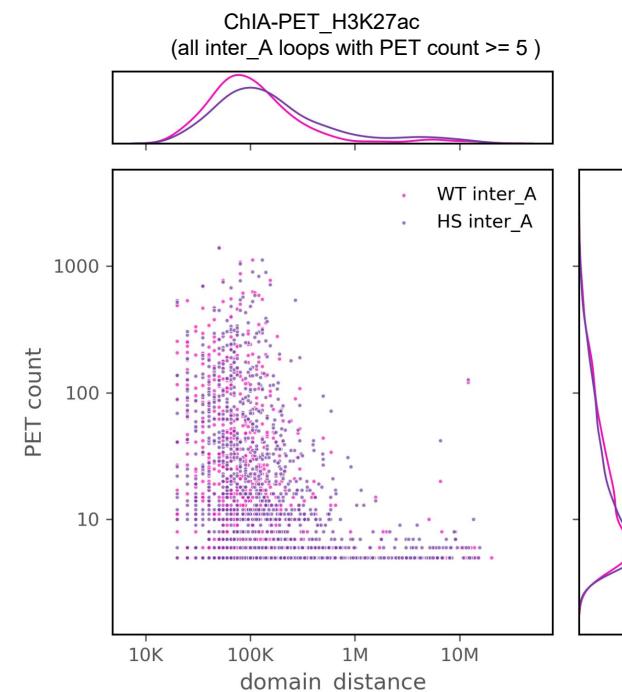
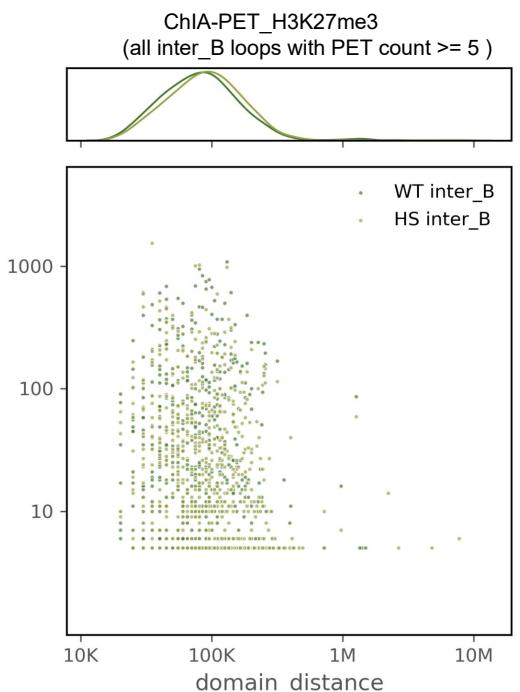
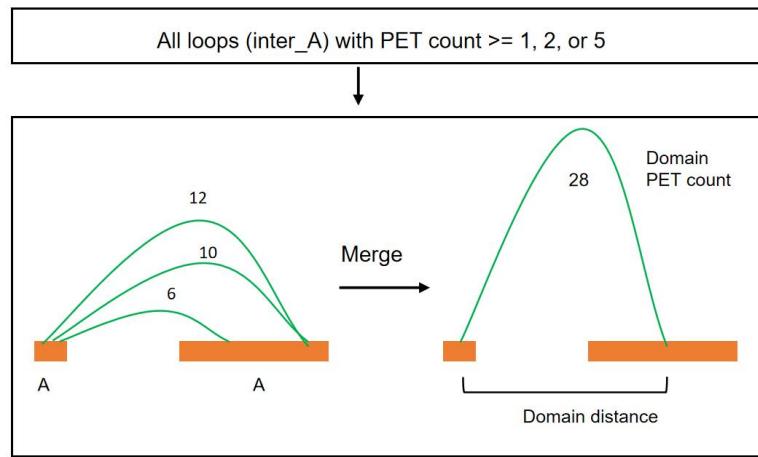
After heat shock, Pol II occupancy has an overall drop.
Teves et al., Genes & Development. 2011



	A	B	Row Totals
up-regulated	808	202	1010
dn-regulated	794	346	1140
Column Totals	1602	548	2150(Grand Total)



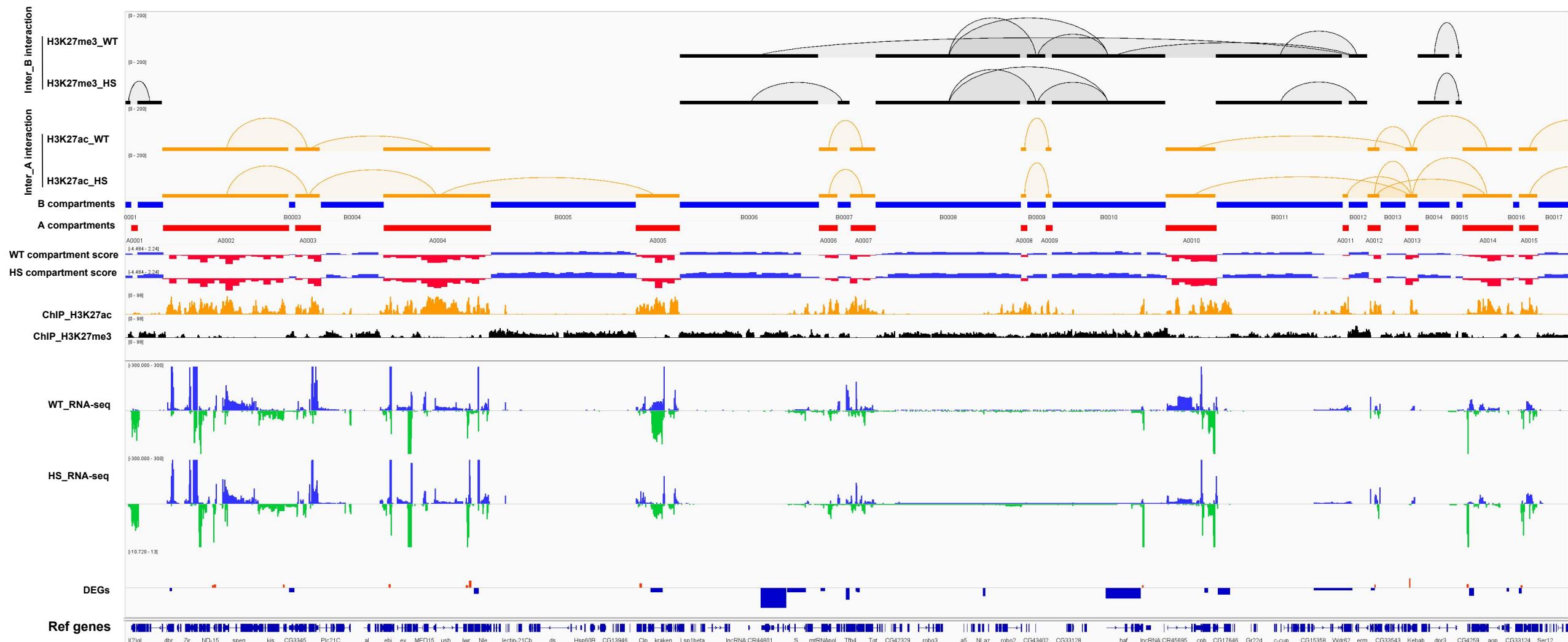
1. Genes located in A compartments have higher expression level than those located in B compartments
2. Down-regulated DEGs are more likely to locate in B compartments (chi-square test, $p = 5.12e-08$). Its consistent with the fact that interaction strength mediated by RNAPII drops more in B compartments than A compartments after heat shock.

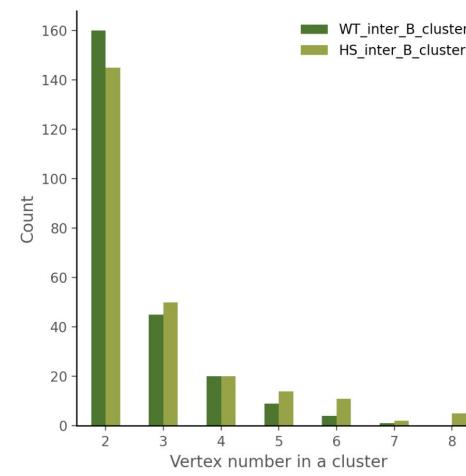
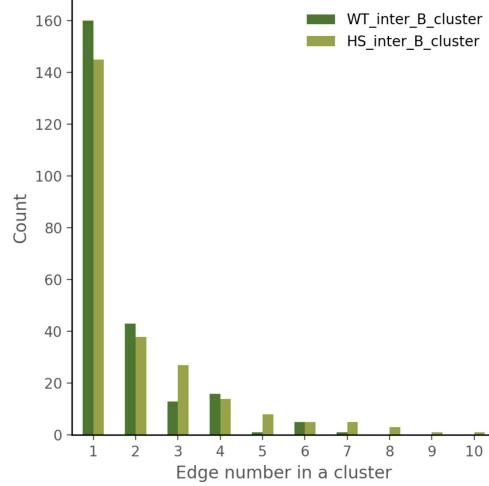
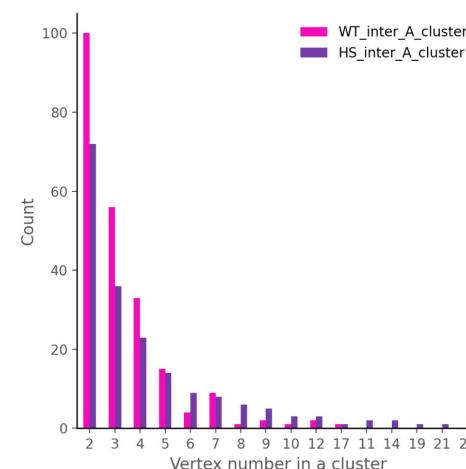
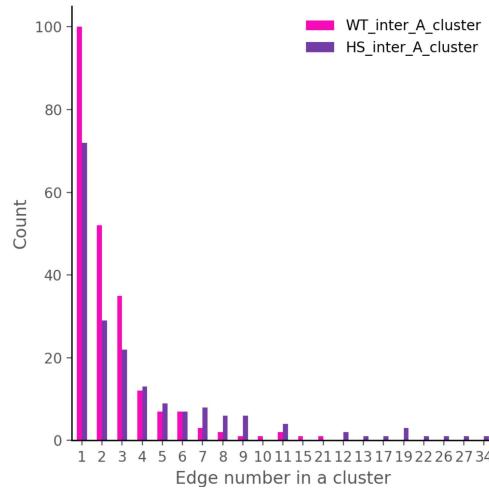


The data format is almost the same as that of ChIA-PET loops, except that the anchor is a compartment.

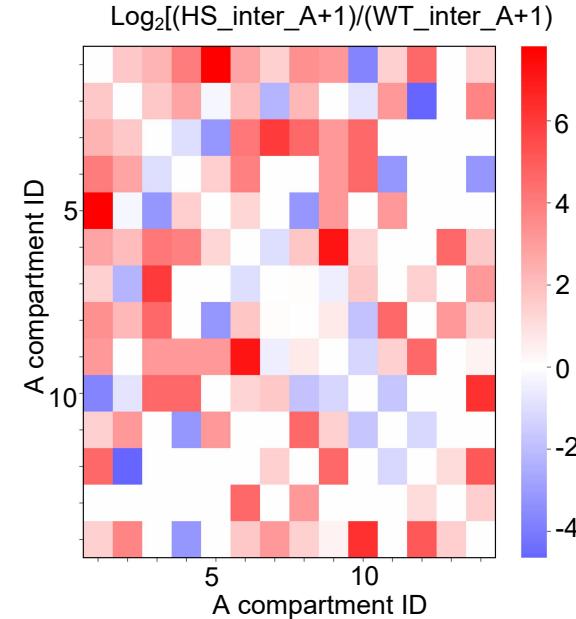
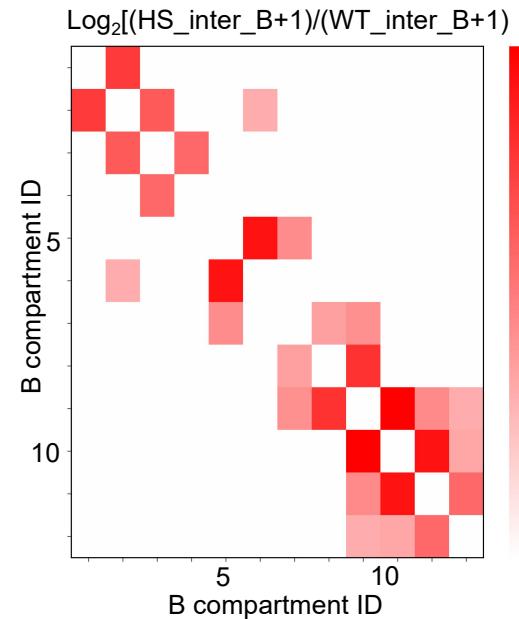
Compartment level

Dynamic compartment network and gene expression and regulation

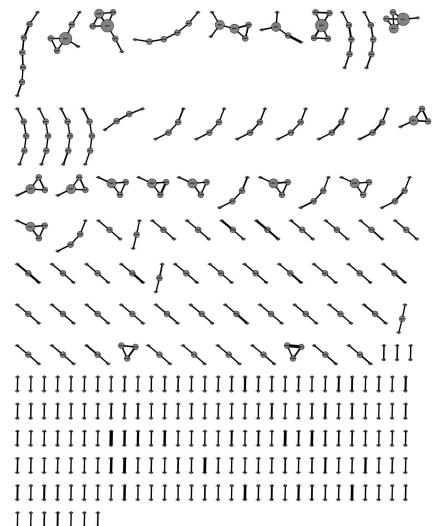


a**b**

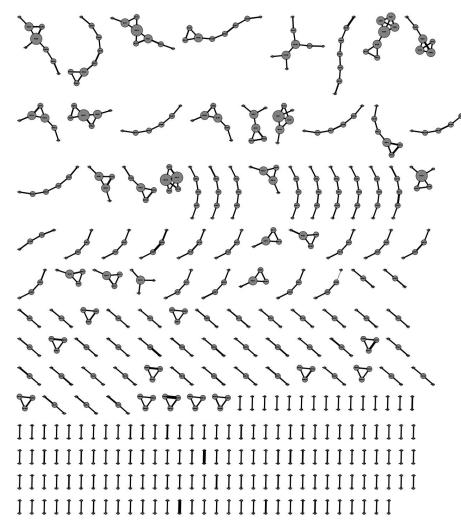
After heat shock, while adjacent B compartments have stronger interaction, A compartments in hubs become even closer or form new larger hubs. So, we can construct a network of A compartments before and after heat shock, and we can also explore the dynamic network and its relationship with gene expression and regulation.



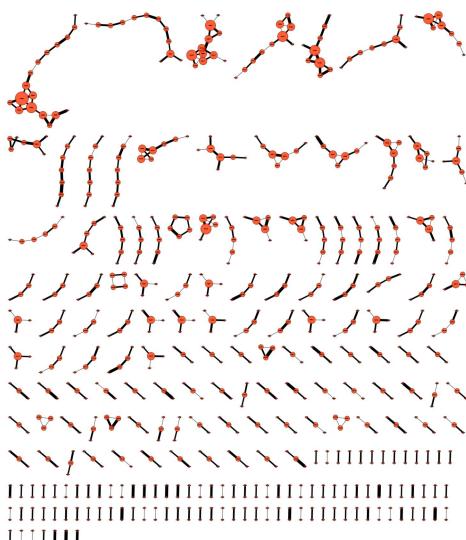
While inter_B interaction usually increases between two adjacent B compartments, inter_A interaction increases among multiple A compartments.



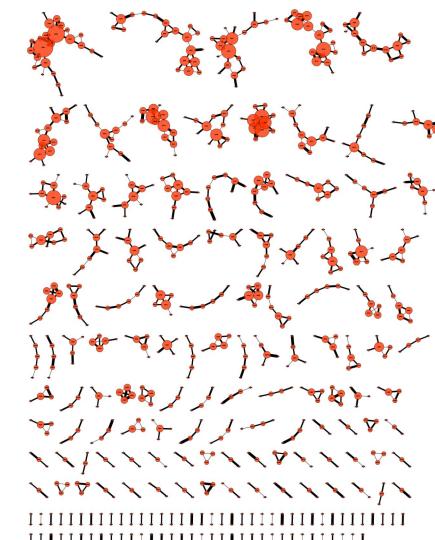
B compartments cluster captured
by H3K27me3_WT



B compartments cluster captured
by H3K27me3_HS



A compartments cluster captured
by H3K27ac_WT



A compartments cluster captured
by H3K27ac_HS

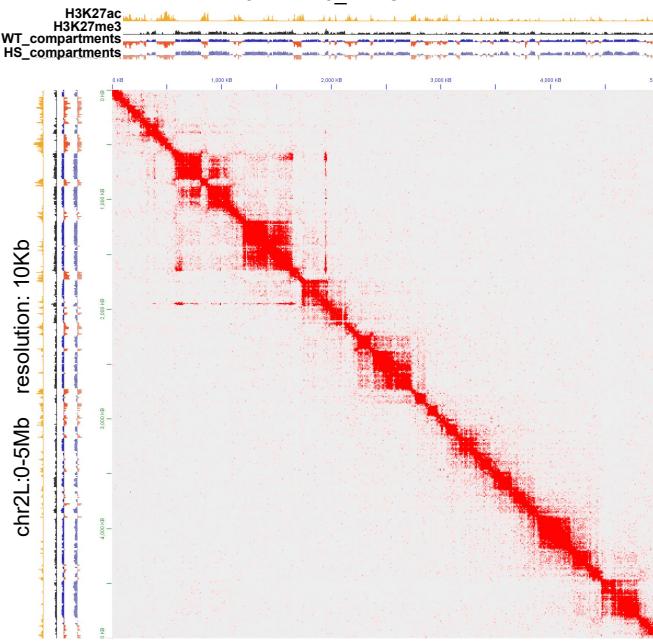
After heat shock, while adjacent B compartments have stronger interaction, A compartments in hubs become even closer or form new larger hubs. So, we can construct a network of A compartments before and after heat shock, and we can also explore the dynamic network and its relationship with gene expression and regulation.

Is the change of the compartment network related to gene expression and regulation ?
Its relationship with gene expression and regulation is still under exploration

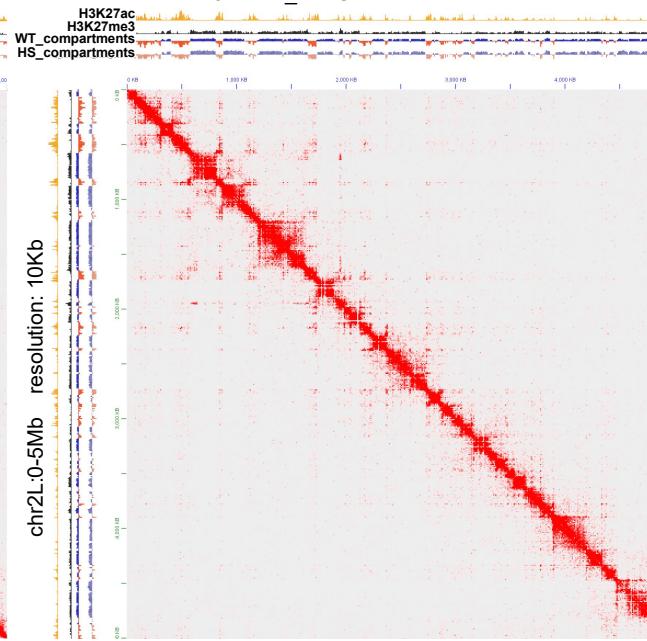
TAD level

Sum of H3K27me3_ChIA-PET and H3K27ac_ChIA-PET

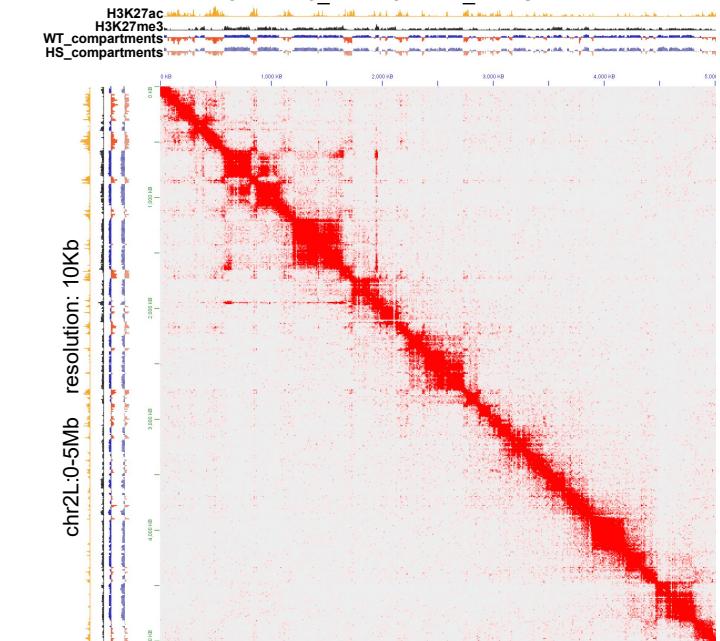
H3K27me3_WT ChIA-PET



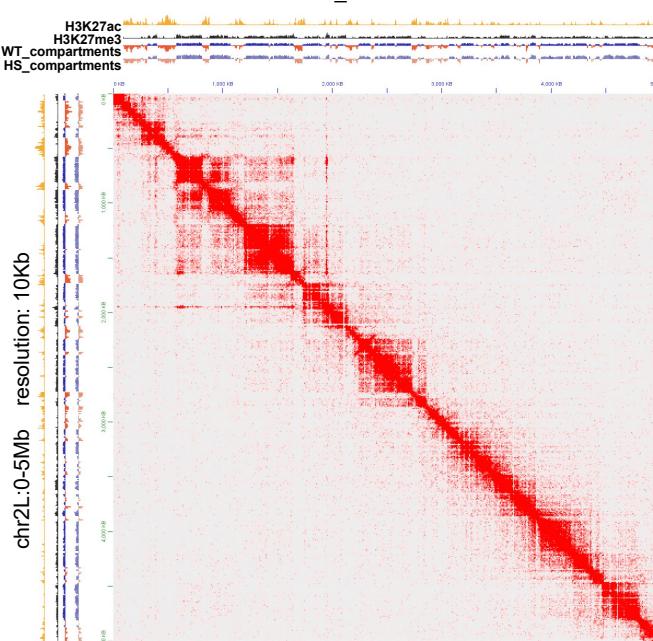
H3K27ac_WT ChIA-PET



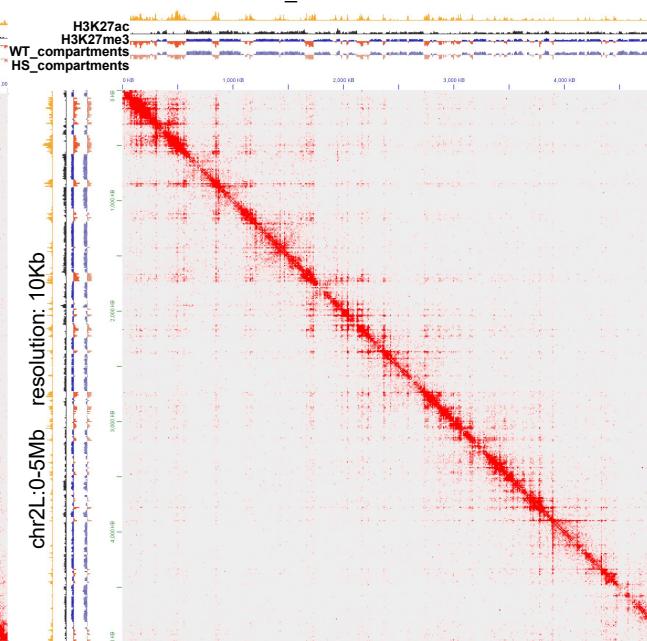
H3K27me3_WT+H3K27ac_WT ChIA-PET



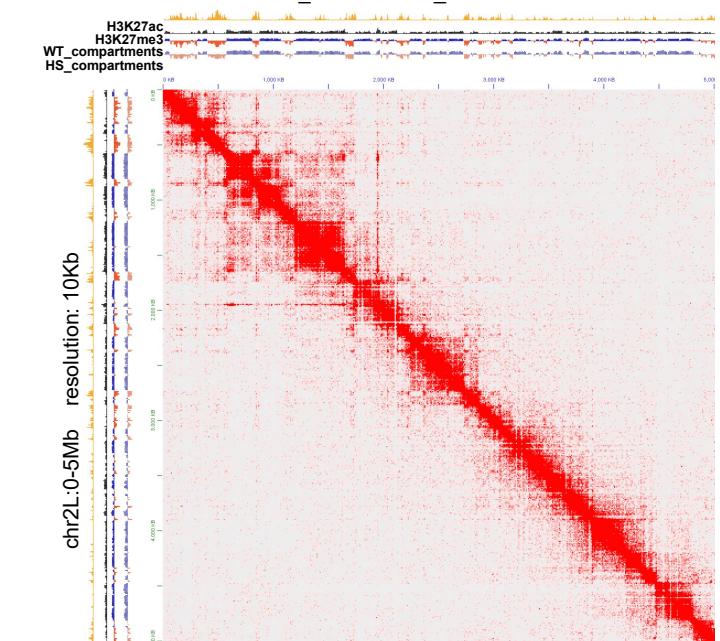
H3K27me3_HS ChIA-PET



H3K27ac_HS ChIA-PET



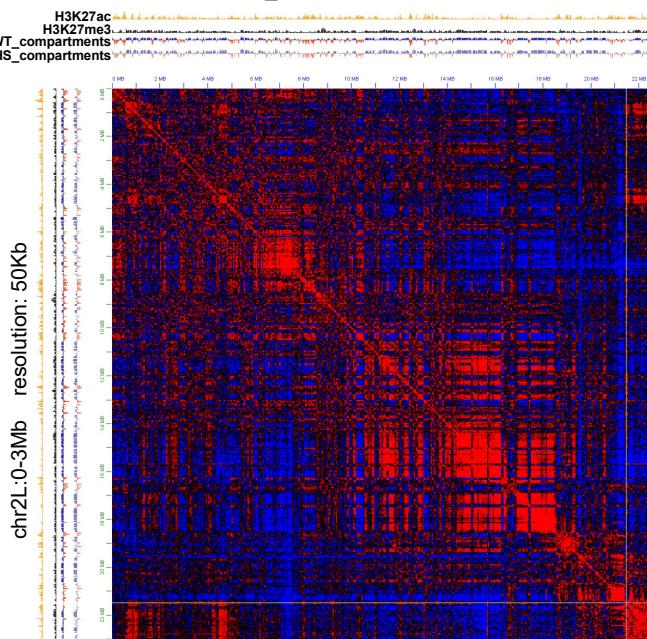
H3K27ac_HS/H3K27ac_HS ChIA-PET



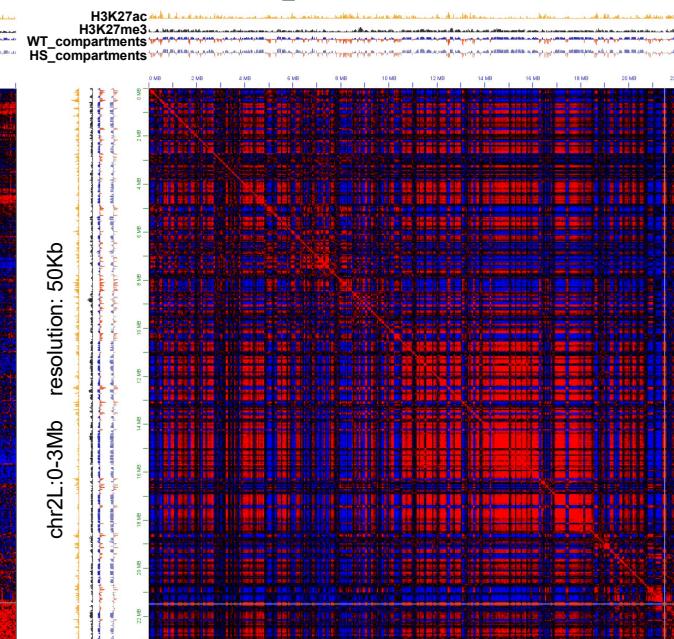
TAD level

Sum of H3K27me3_ChIA-PET and H3K27ac_ChIA-PET

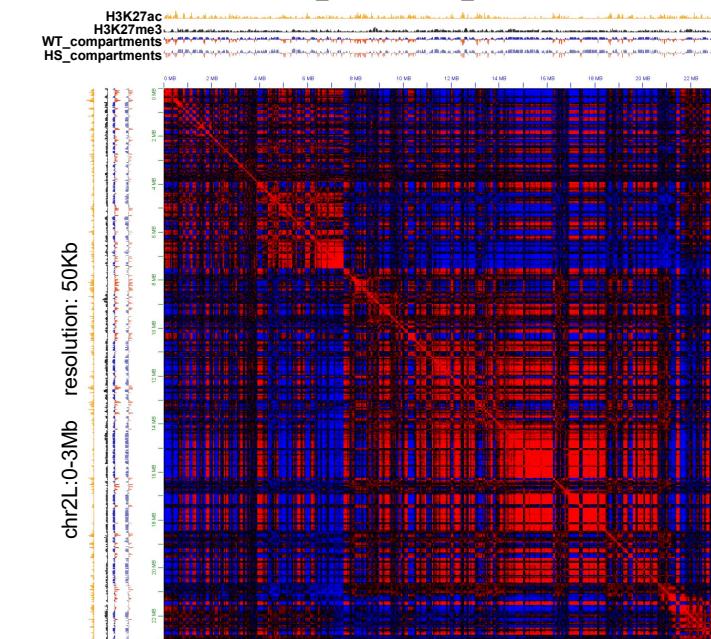
H3K27me3_WT ChIA-PET



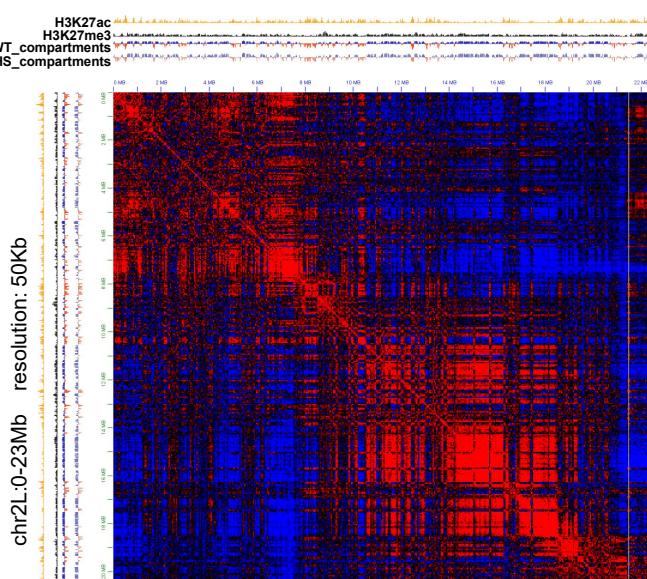
H3K27ac_WT ChIA-PET



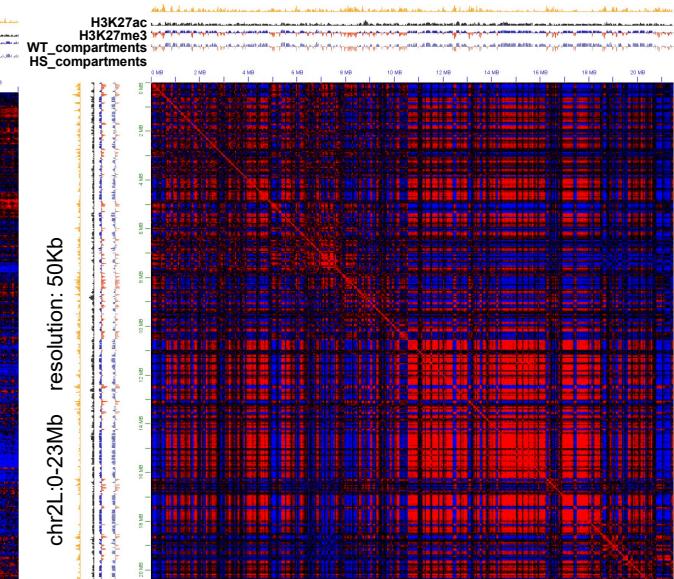
H3K27me3_WT+H3K27ac_WT ChIA-PET



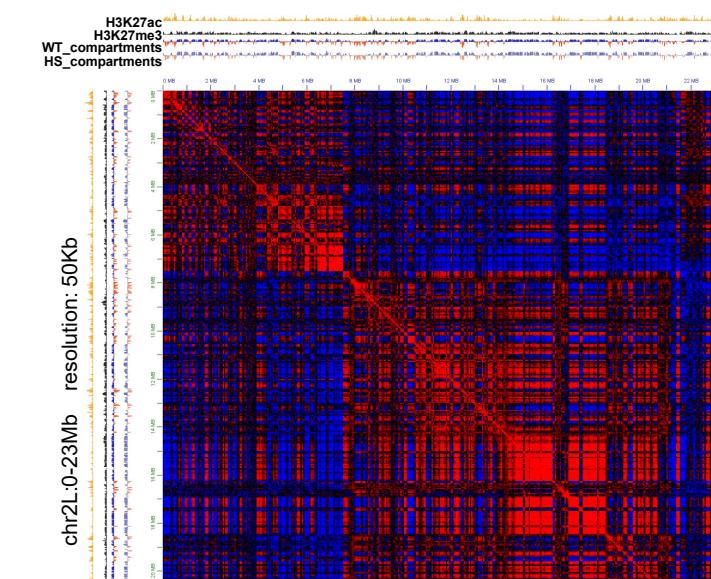
H3K27me3_HS ChIA-PET



H3K27ac_HS ChIA-PET



H3K27ac_HS+H3K27ac_HS ChIA-PET



TAD level

Sum of H3K27me3_ChIA-PET and H3K27ac_ChIA-PET is approximate to Hi-C

H3K27me3_ChIA-PET and H3K27ac_ChIA-PET is approximate to Hi-C in Drosophila.

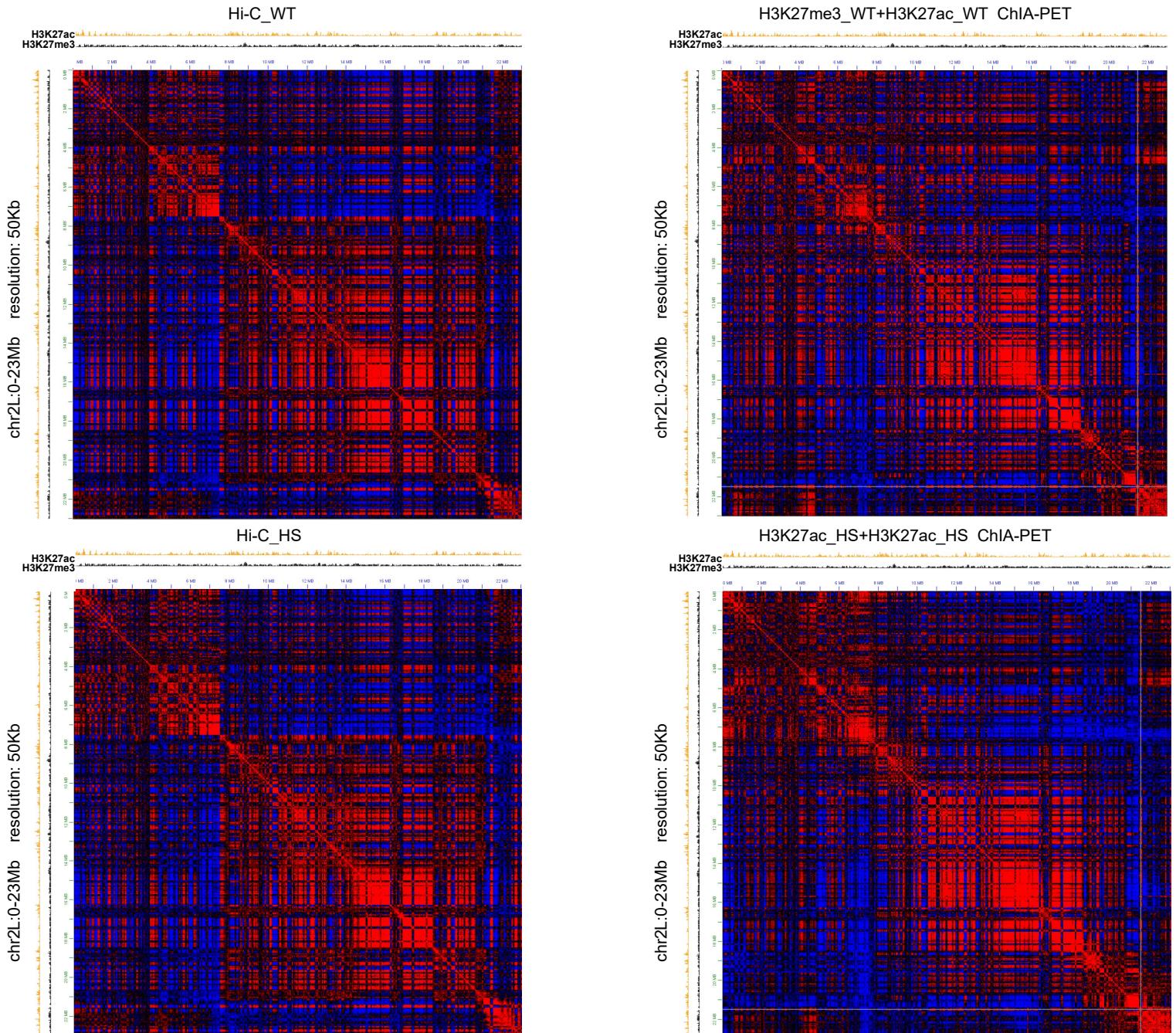
Nichols et al., Cell Reports. 2021

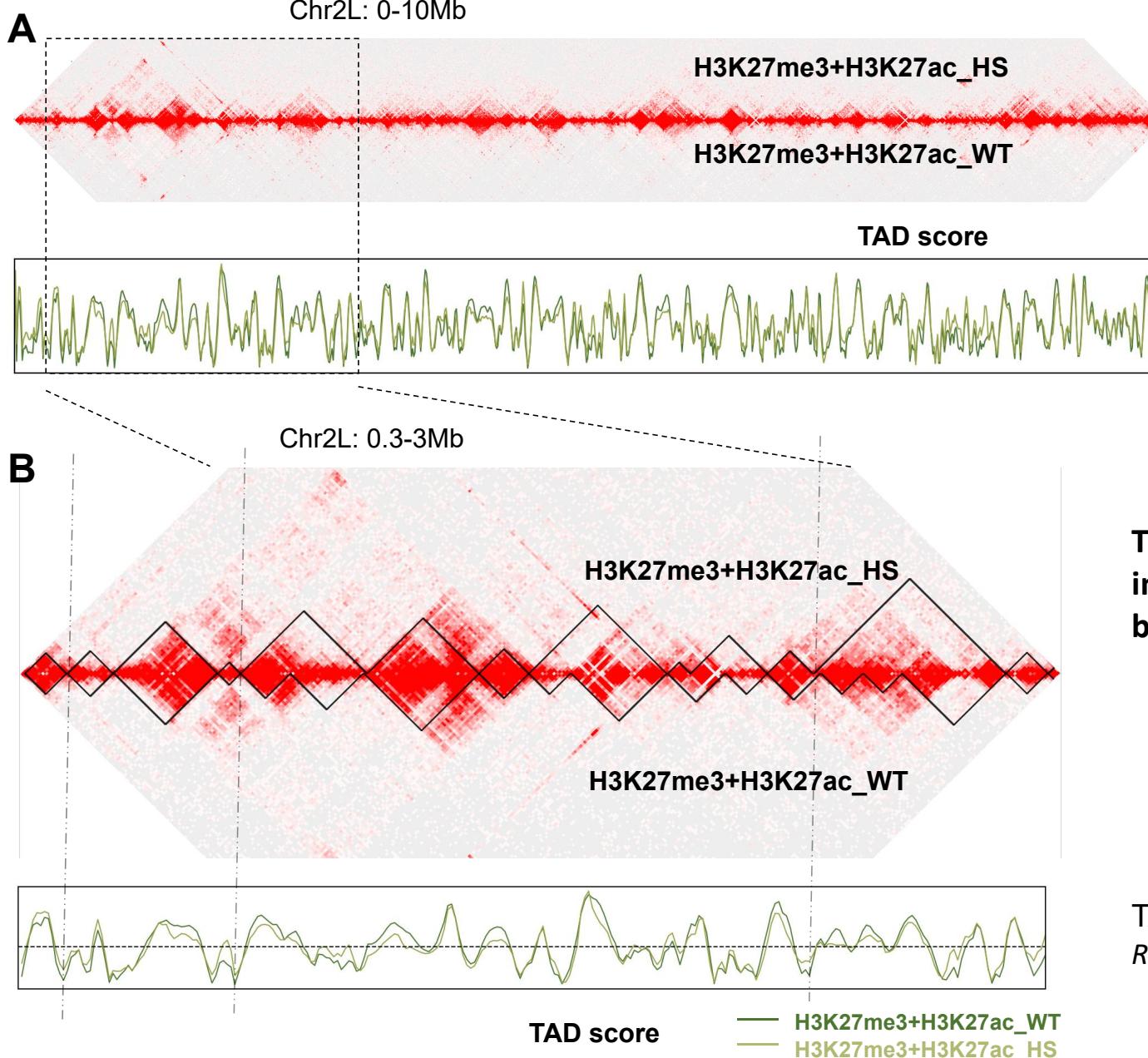
ChIA-PET is approximate to Hi-C plus the enrichment of some factor

(H3K27me3_WT+H3K27ac_WT) ChIA-PET and (H3K27me3_HS+H3K27ac_HS) ChIA-PET can be used to study the dynamic TADs induced by heat shock.

RNAPII_WT ChIA-PET and RNAPII_HS ChIA-PET can be used to study dynamic RNAPII and TAD boundaries under heat shock.

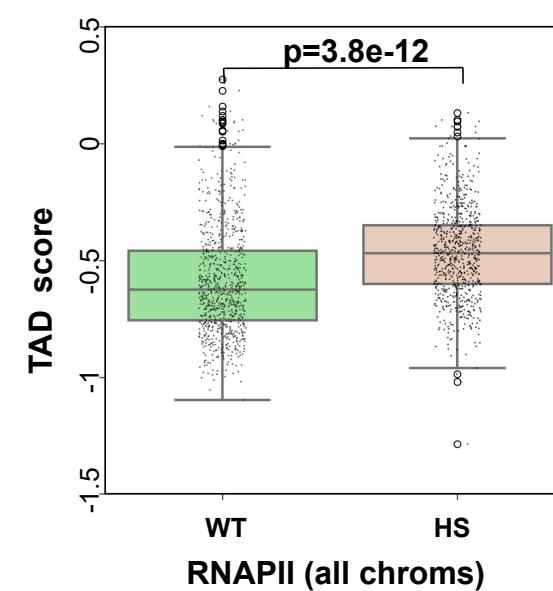
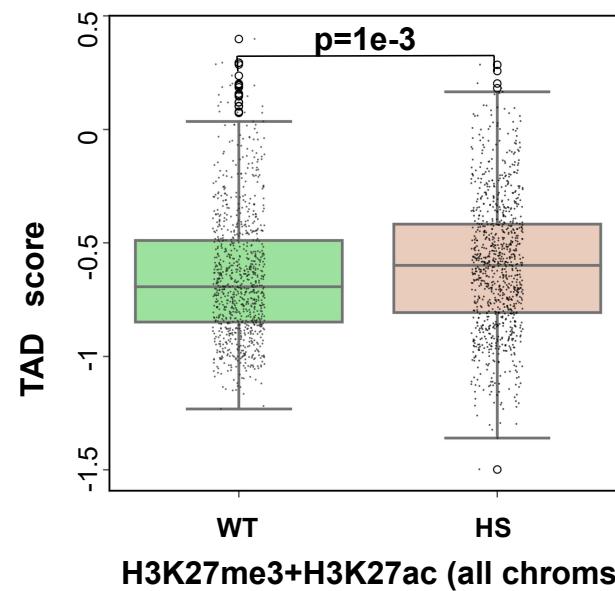
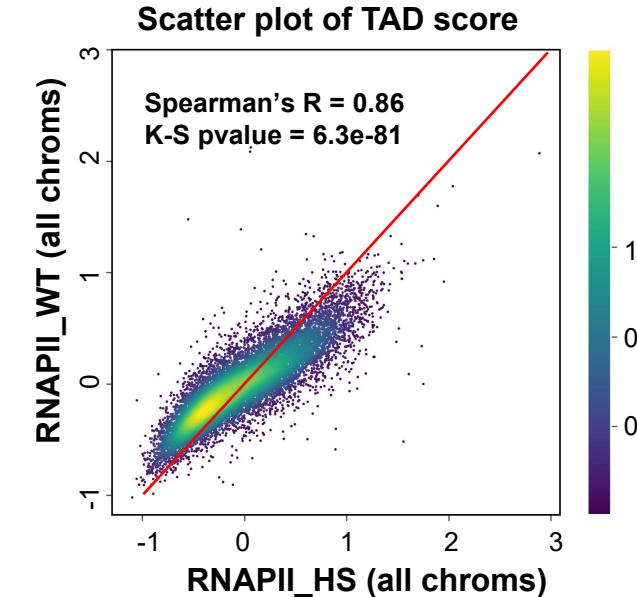
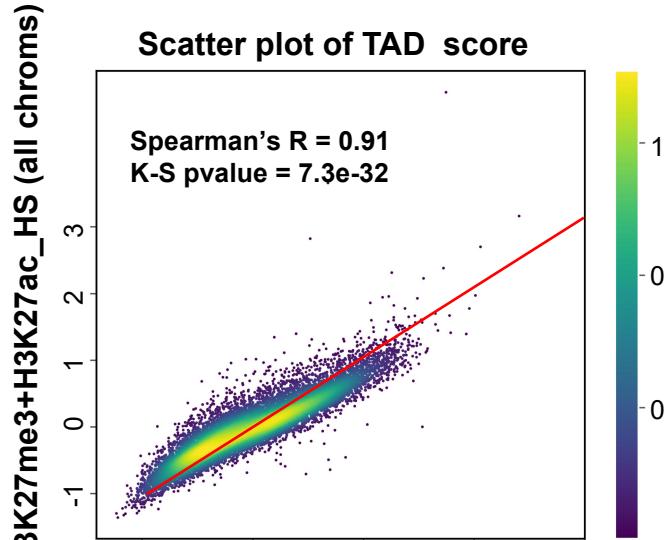
Hi-C data comes from *Ray et al., PNAS. 2019*



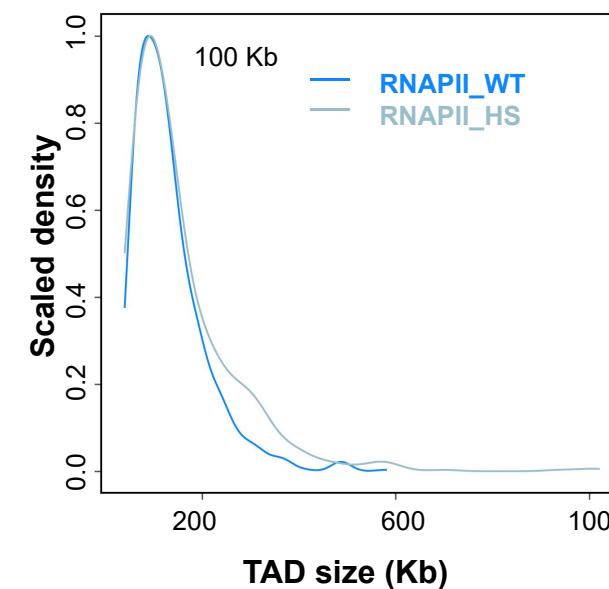
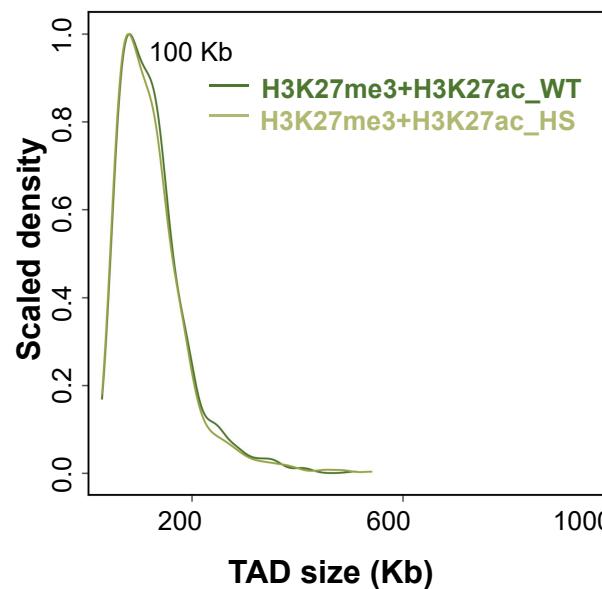
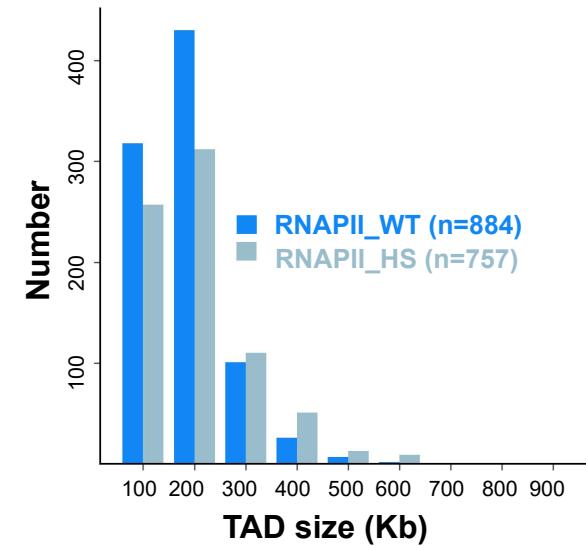
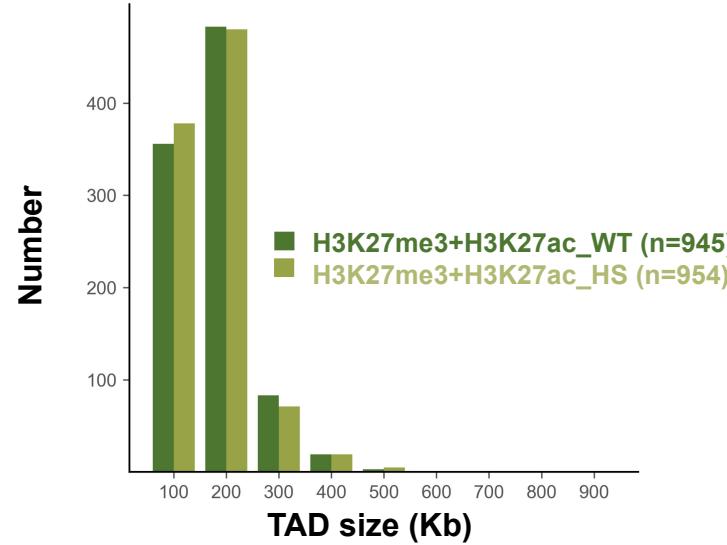


The local minimal of TAD score (TAD separation score) increases after heat shock, indicating that the TAD boundaries weaken after heat shock.

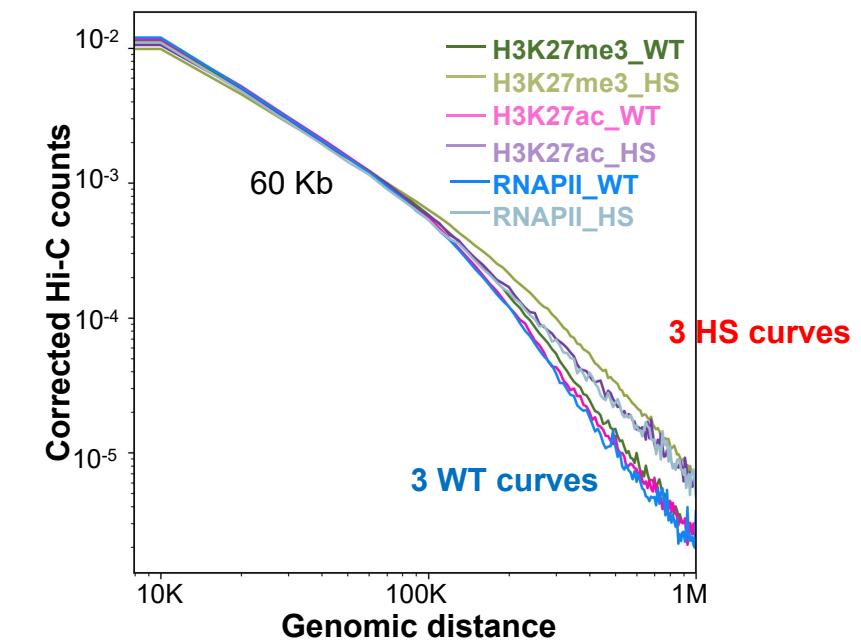
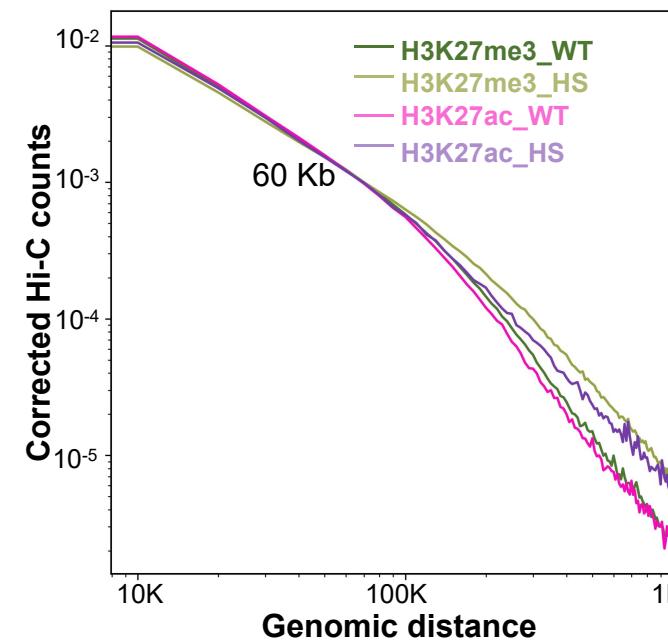
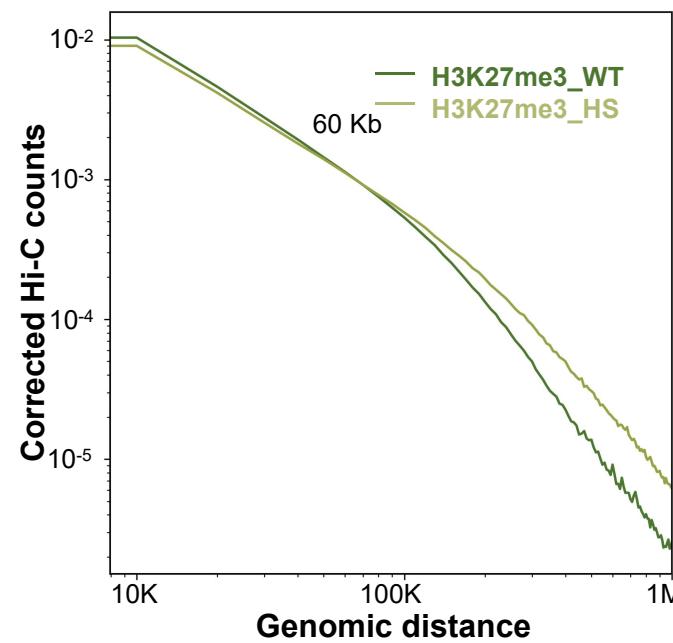
TADs at 10Kb resolution is called by hicexplorer
Ramirez et al., Nature Communications. 2018



TAD boundaries are weaker after heat shock (Mann-Whitney U test).



After heat shock, TAD number and TAD size in H3K27me3+H3K27ac ChIA-PET almost keep, while TAD number reduces and TAD size increases in RNAPII



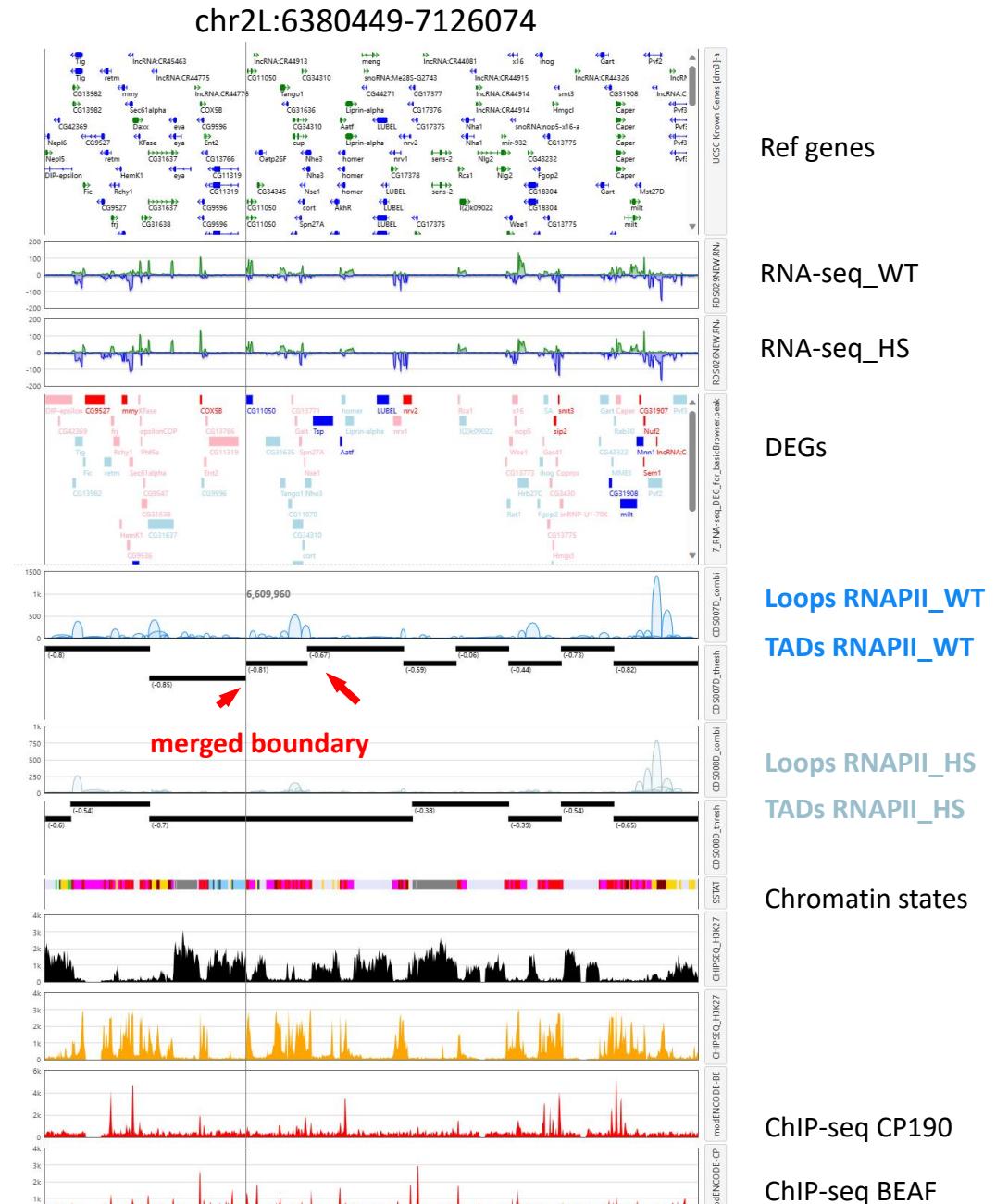
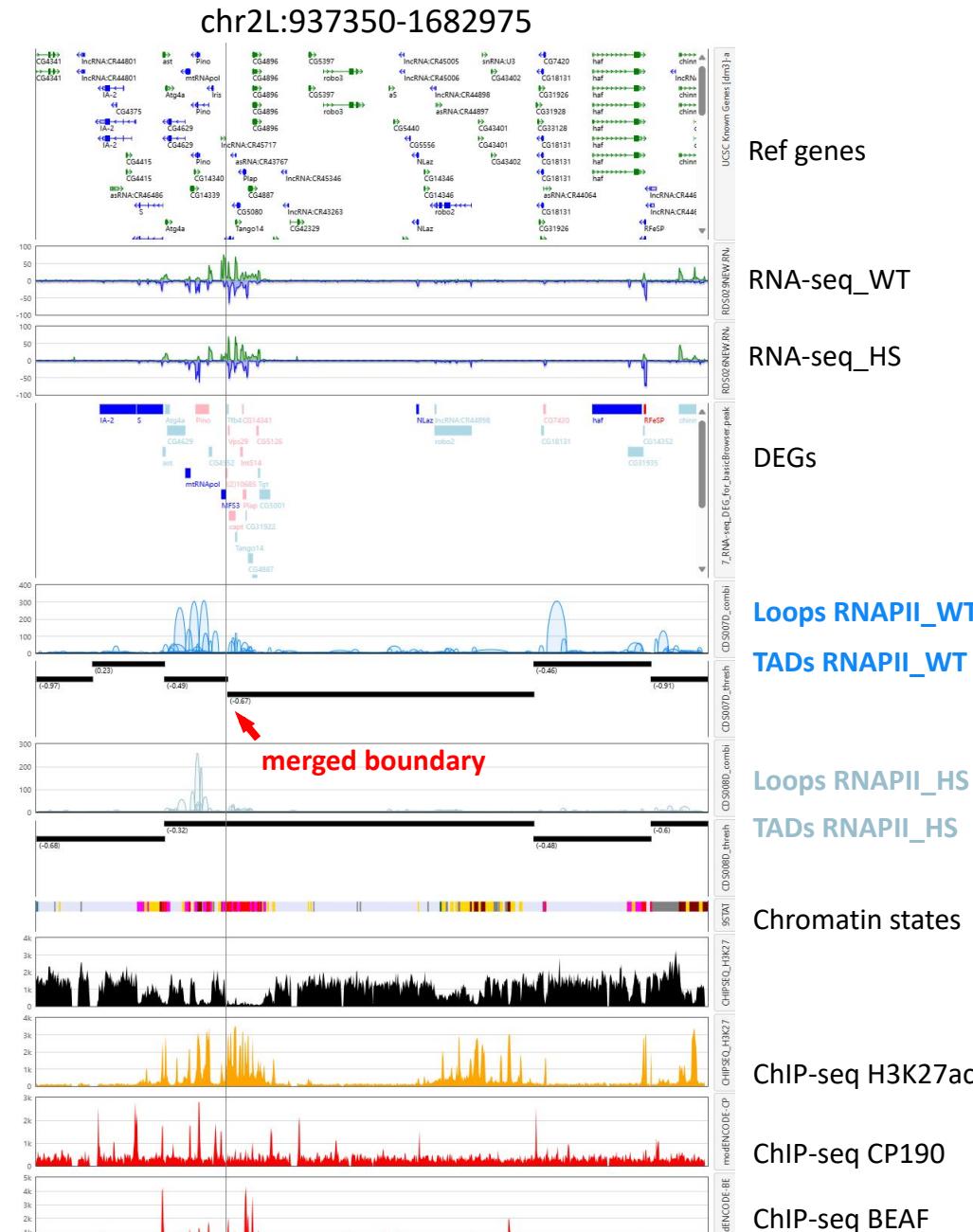
K-S test	H3K27me3_WT	H3K27me3_HS	H3K27ac_WT	H3K27ac_HS	RNAPII_WT	RNAPII_HS
H3K27me3_WT	1					
H3K27me3_HS	8.9e-06	1				
H3K27ac_WT	0.97	4.2e-07	1			
H3K27ac_HS	2.5e-04	0.59	3.6e-05	1		
RNAPII_WT	0.59	7.9e-08	0.91	9.3e-07	1	
RNAPII_HS	2.5e-04	0.59	1.8e-05	1	2.0e-06	1

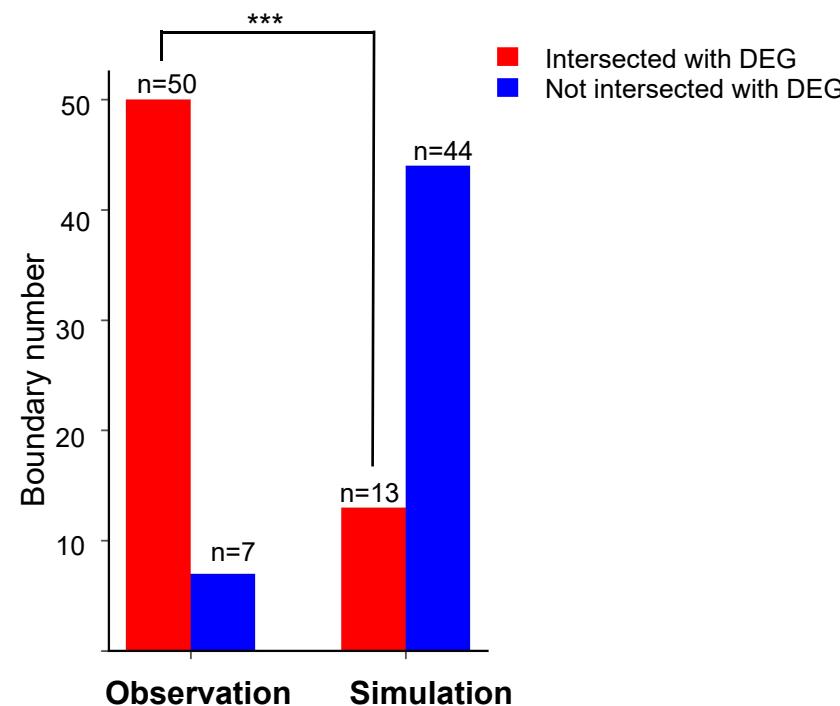
Genomic distance increases after heat shock;

Curves between different factors have no significant difference, but curves between WT and HS have significant difference (K-S test).

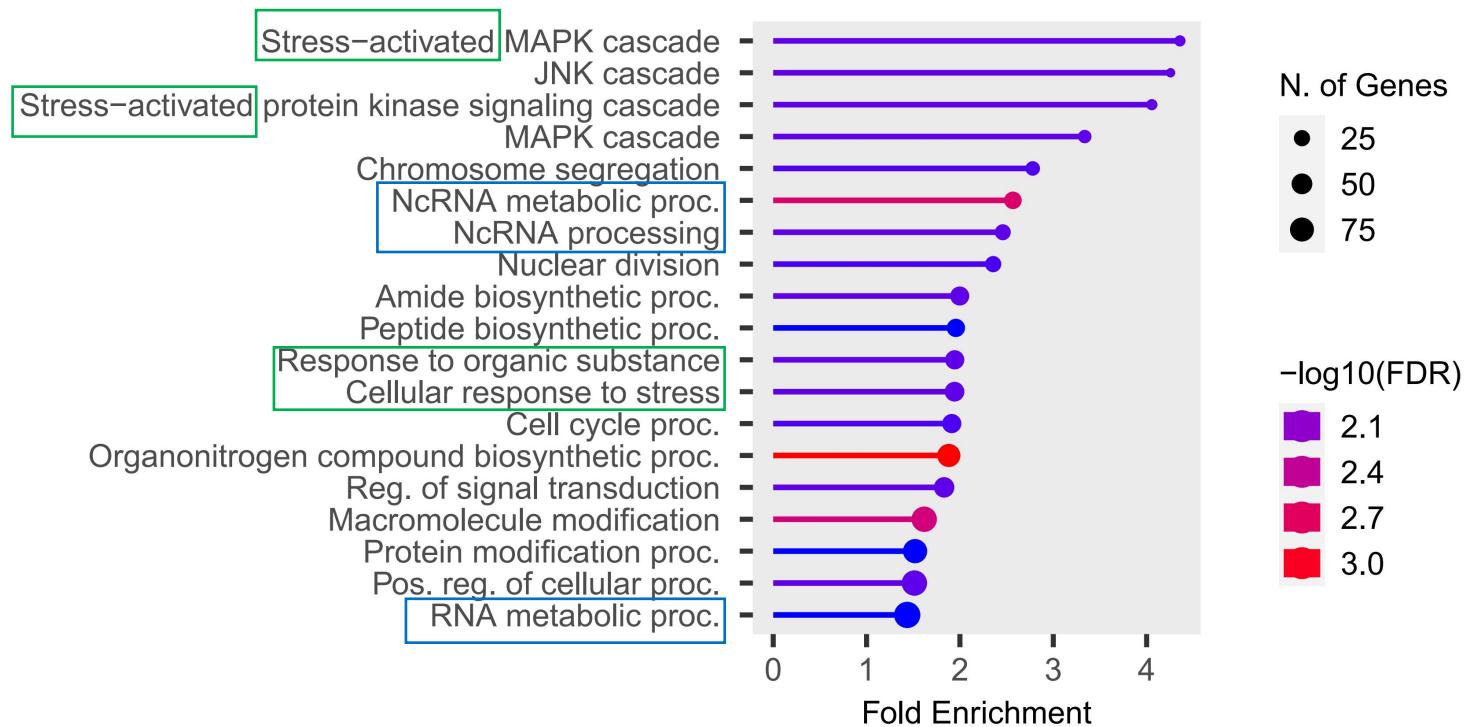
TAD level

Merged boundaries appeared in RNAP2_HS are highly correlated with DEGs



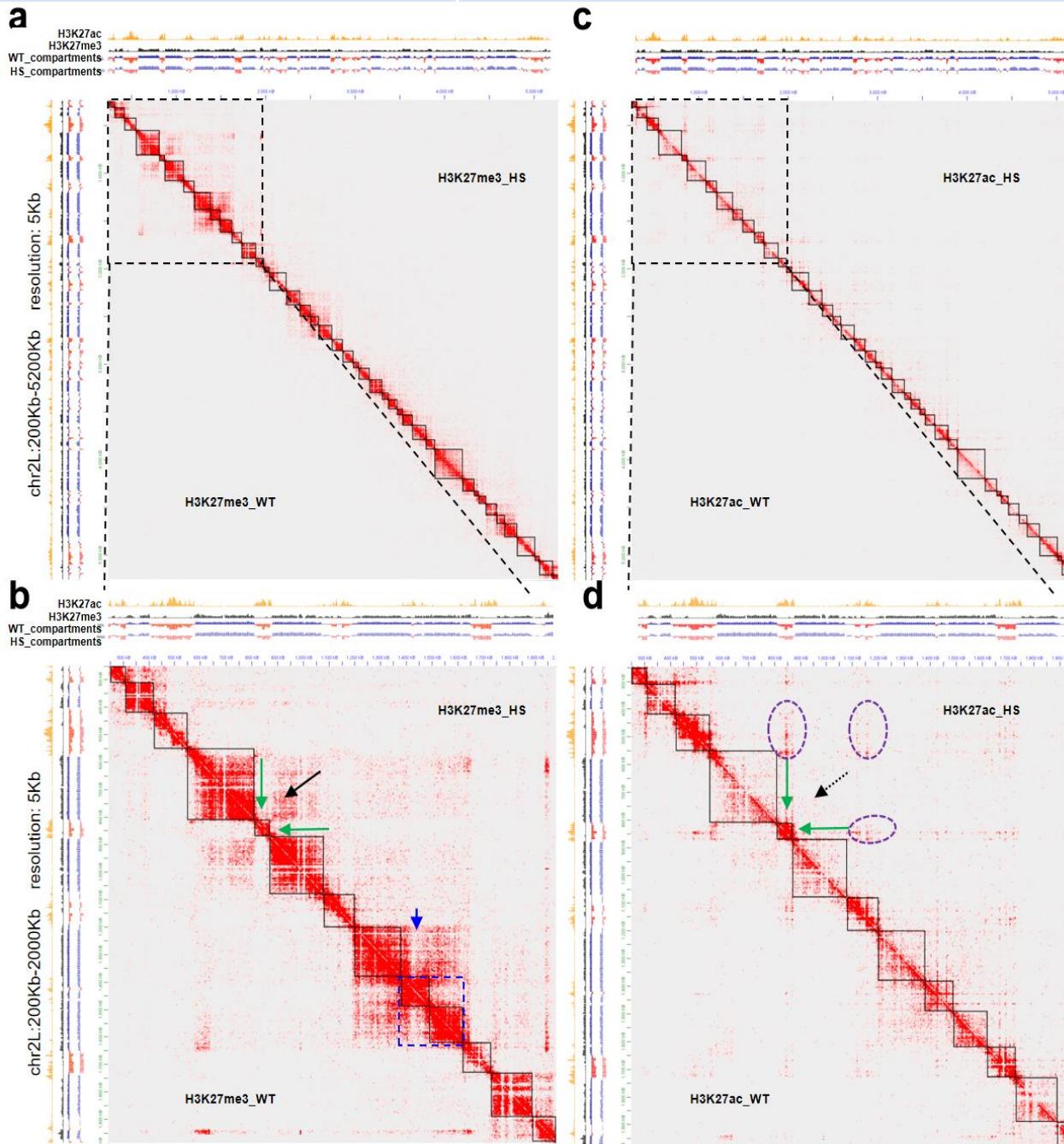


For chr2L, there are 57 merged boundaries: 50 intersected with DEGs, 7 are not, the observed $p=0.88$. With the DEGs' positions set, and randomly throwing the TAD boundary intervals, the expected probability of intersecting with a DEG, P is 0.23. We can treat it as a binomial test, and calculate the z score as 11.25, whose p value is close to 0. The conclusion still holds for all chromosomes.



Go analysis of 492 DEGs located in merged boundaries of all chromosomes

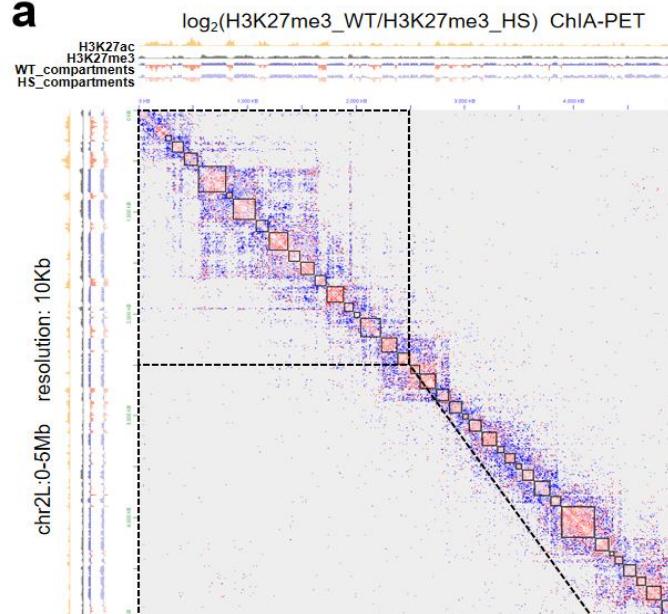
Compartments and TADs



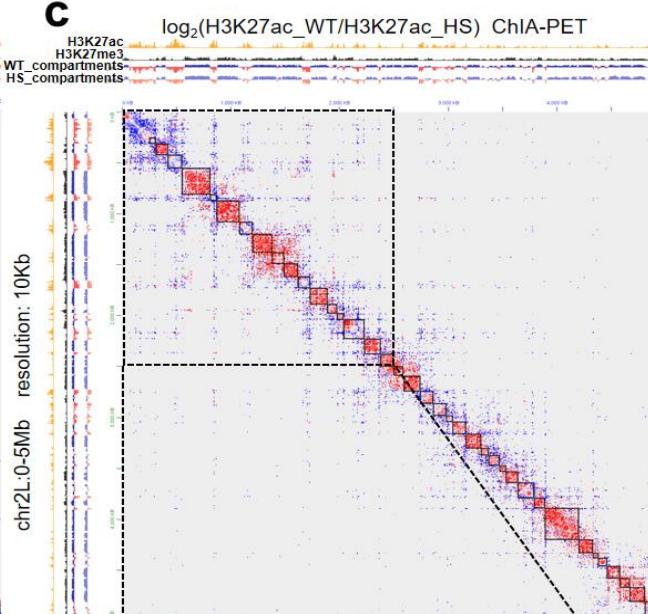
B compartments usually correspond to larger TADs, while A compartments usually correspond to smaller TADs. Still, there are some TADs consisting of B compartments and their adjacent A compartments. Or, a compartment consist of severral TADs.

Compartments and TADs

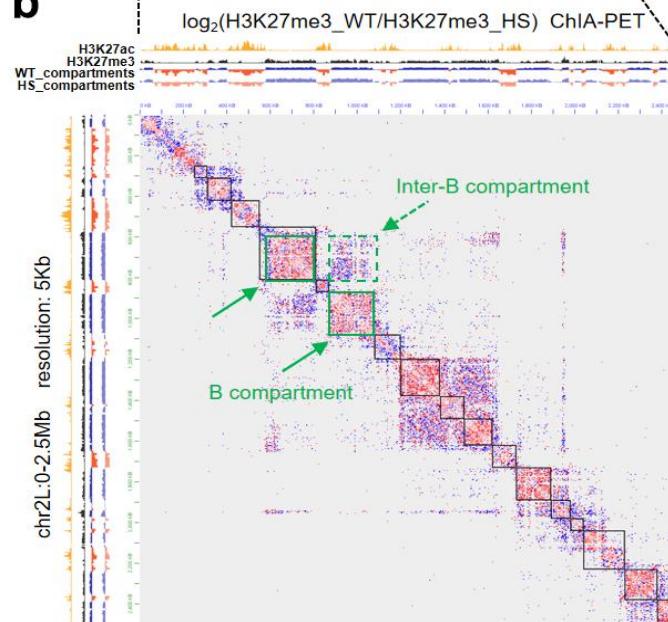
a



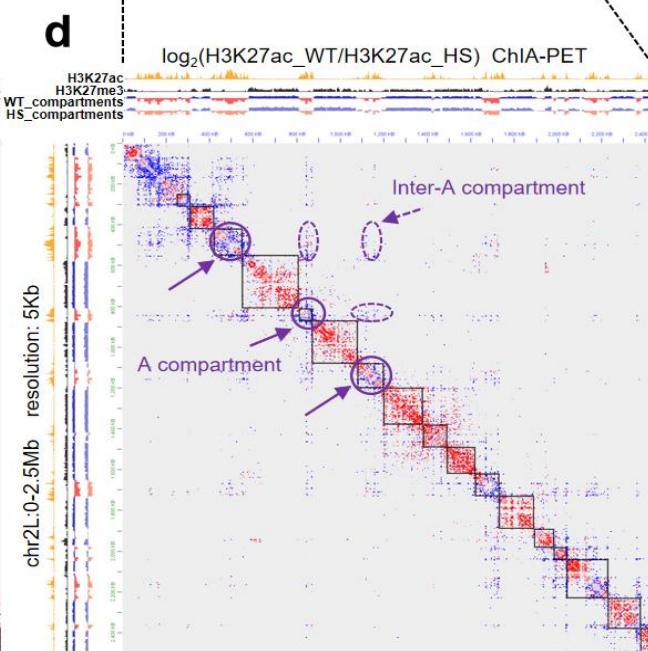
c



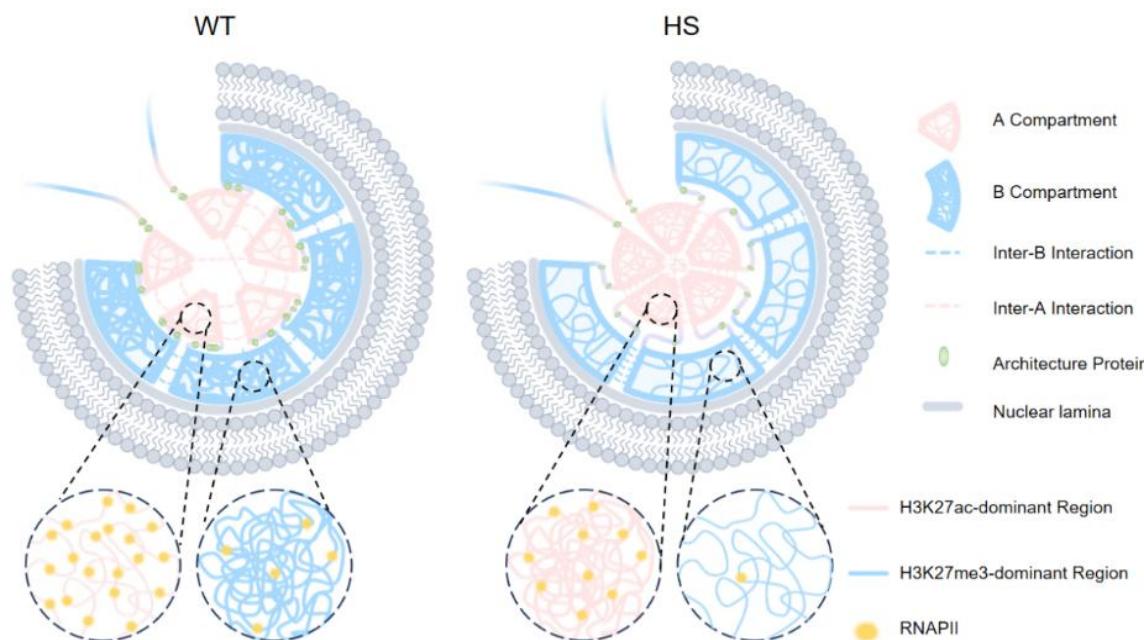
b



d



After HS, interaction strength inside A compartments increase, while interaction strength inside B compartments decrease. Besides, interaction strength between adjacent B compartments increase, and clustered A compartments come even closer.



1. After HS, interaction inside TADs and B compartments decrease, while interaction inside A compartments increase. Besides, interaction between adjacent TADs and adjacent B compartments both increases. Combined with the fact that some TADs share the boundaries with B compartments, we can guess that the interaction's decrease insides B compartments may share the mechanism with TADs, that after HS, architecture proteins (APs) redistribute from the TAD boundaries to the inside of TADs and TAD boundaries weaken.

Li et al., Molecular Cell. 2015

2. After HS, H3K27ac_ChIA-PET produce more obvious stripes, while H3K27me3_ChIA-PET produce more obvious block between adjacent B compartments. Combined with that fact that A compartments correspond to euchromatin located inside the nucleus, while B compartments correspond to heterochromatin, primarily located at the nuclear periphery and around the nucleolus. We can reasonably speculate that the increase of inter-B is caused by the redistribution of APs, while the increase of inter-A is caused by the closer spatial distance of clustered A compartments.

Oudelaar et al., Nature Reviews Genetics. 2021

3. Interaction mediated by RNAPII drops more inside B compartments than that inside A compartments. Combined with the fact that heat shock can result in the increased release of bound RNAPII from DNA, we can reasonably speculate that after heat shock larger proportion of RNAPII in B compartments releases from DNA.

Hieda et al., Chromosome Res. 2005

Teves et al., Genes Dev. 2011

Green denotes the observation from our data, while purple denotes some conclusions from other publications.

1. Our study showed that heat shock can induce lots of loops with low PET counts and large loop span connecting different H3K27ac-dominant regions. Further analysis demonstrated that H3K27ac-dominant and H3K27me3-dominant regions highly align with A and B compartments respectively.
2. After heat shock, while interaction strength inside A compartments increases, interaction strength inside B compartments decreases. Besides, interaction mediated by RNAPII drops more inside B compartments than that inside A compartments, which aligns with the finding that down-regulated genes are more likely to be found in B compartments.
3. After heat shock, A compartments in hubs become even closer or form new larger hubs, while adjacent B compartments have stronger interaction. ([Its relationship with gene expression and regulation is still under exploration](#))
4. After heat shock, TAD boundaries weaken while TAD number and TAD size remain largely unchanged. Proportion of loops with large loop span increases. Changes observed at the TAD level support the findings presented in Li's 2015 Molecular Cell paper, while the conclusions drawn in Ray's PNAS paper diverge from these findings. Besides, dynamic TADs called using RNAPII_ChIA-PET correlate with DEGs, and these DEGs are involved in RNA metabolism and the cellular response to stress.
5. In summary, we propose a model of dynamic 3D structures at the levels of A/B compartments and TADs, along with gene regulation patterns, in response to heat stress in *Drosophila*.



Zhengmz Lab

Acknowledgement

Meizhen Zheng

Zhongyuan Tian

Duo Ning

Yang Yang

Yewen Xu

Kai Jing

Guangyu Huang

Yuqing Deng

Gengzhan Chen

Tong Gao

Wenxin Wang

Zhimin Zhang