



Zhengmz Lab

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

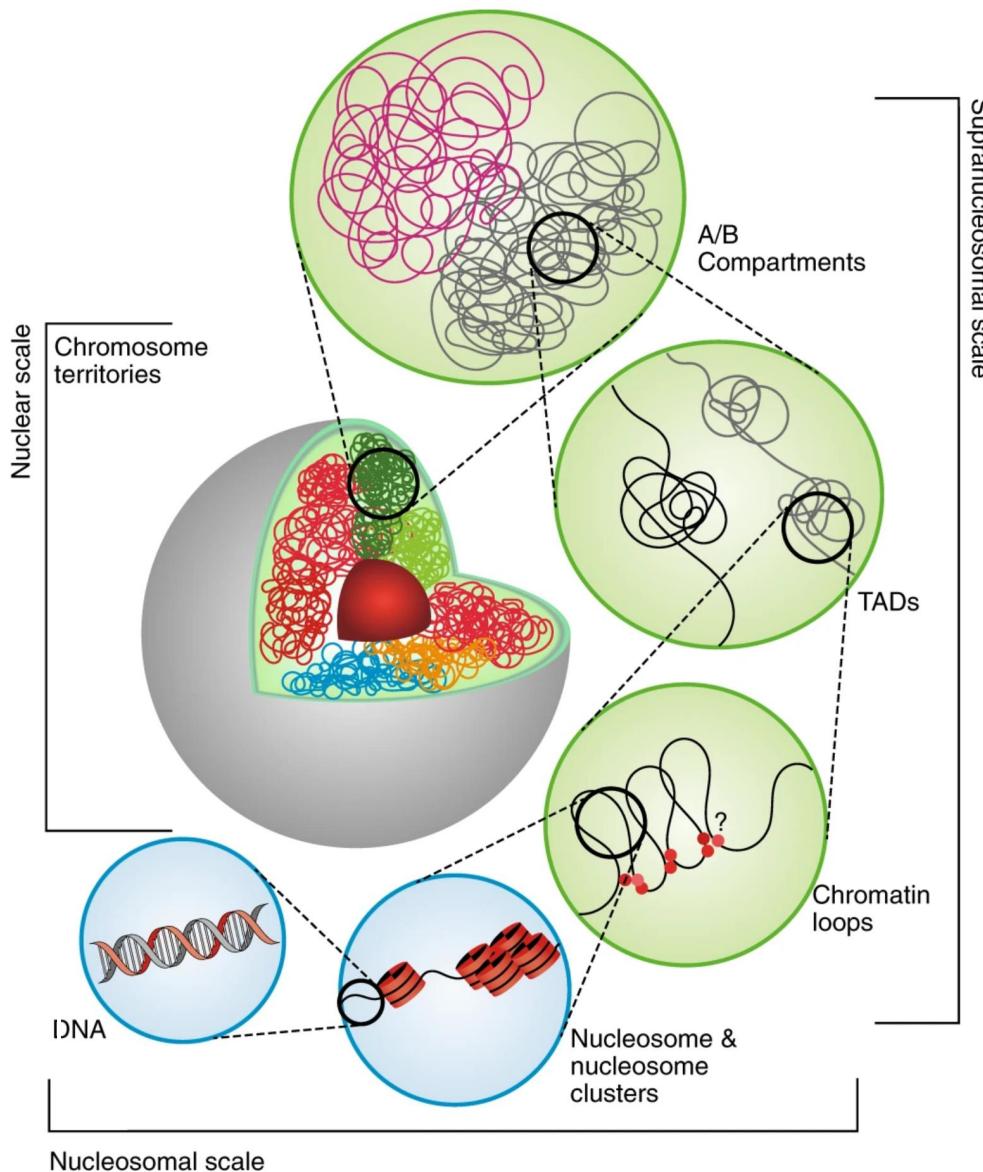
Presenter: Yin Pengfei
Instructor: Zheng Meizhen

Content

- Background
- Project design
- Data analysis
- Results

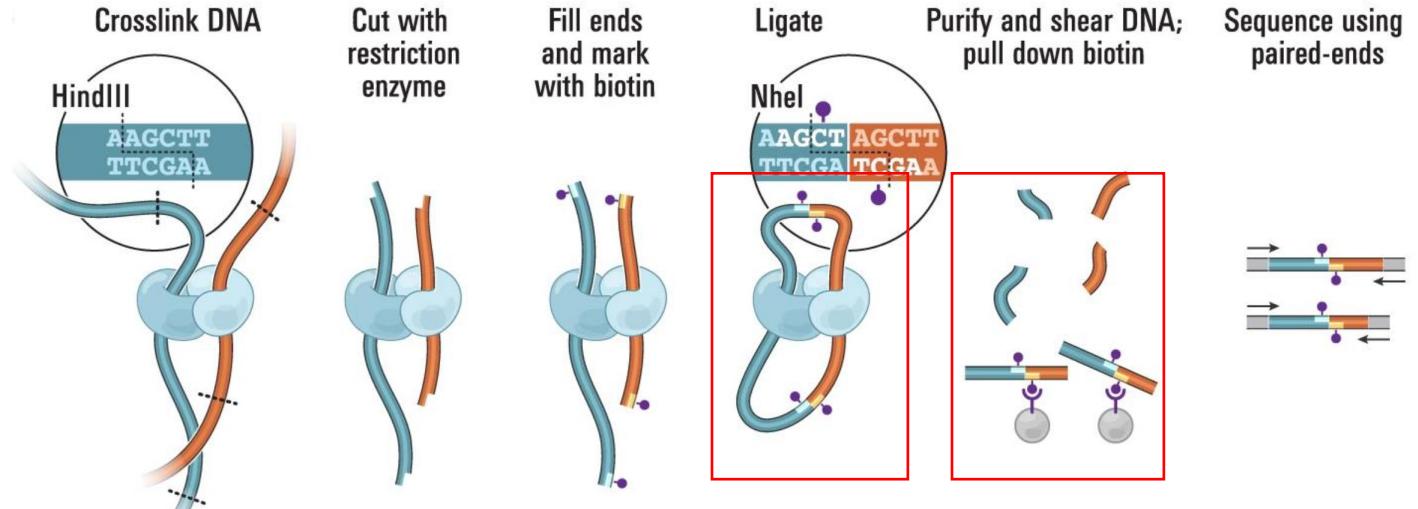
Background

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



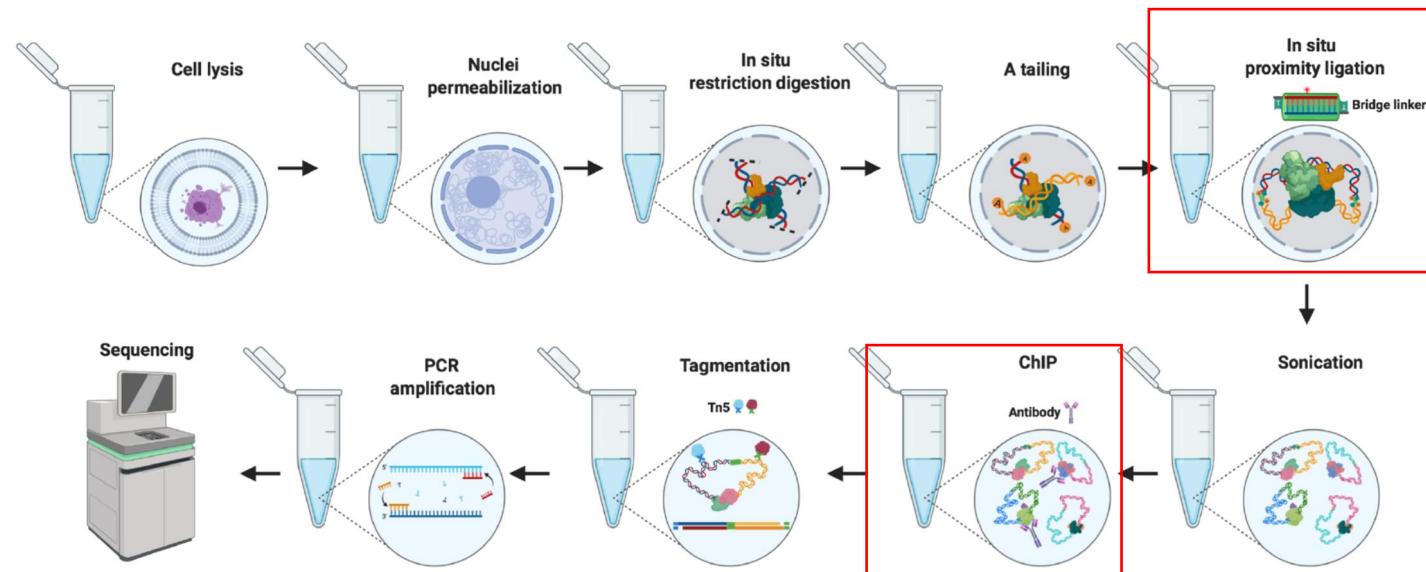
The essence: how the 3D genome is organized into multiple layers and how this organization influences gene expression and regulation.

Doğan et al., Nature Plants. 2018



Erez et al., *Science*. 2009

Hi-C

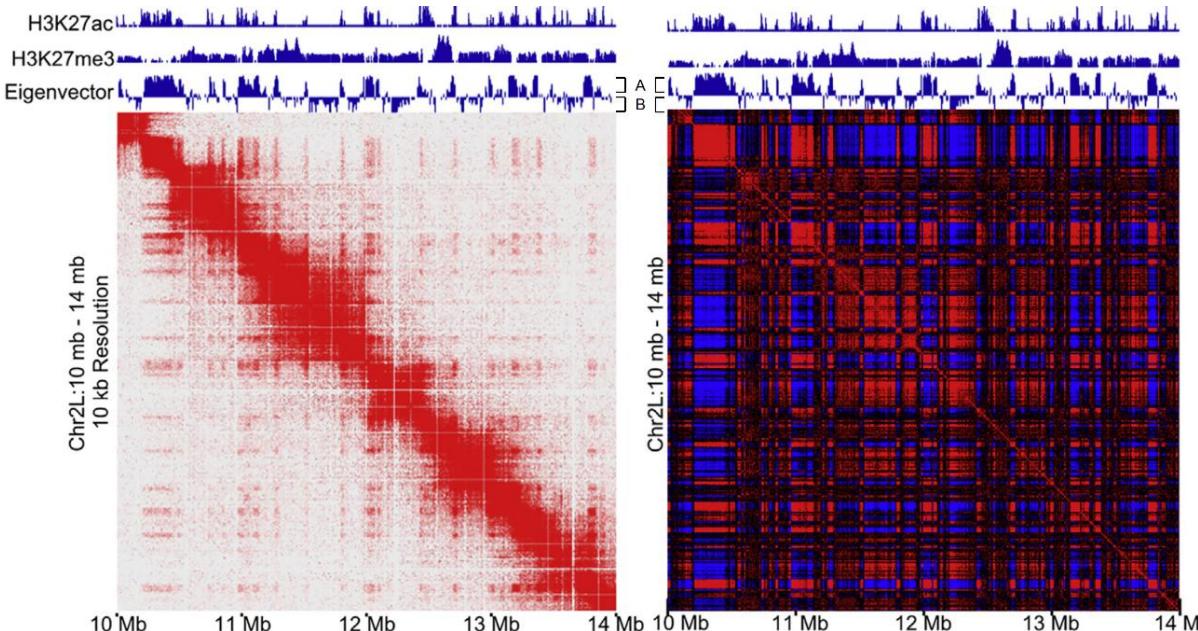


Wang et al., *Curr Protoc*. 2021

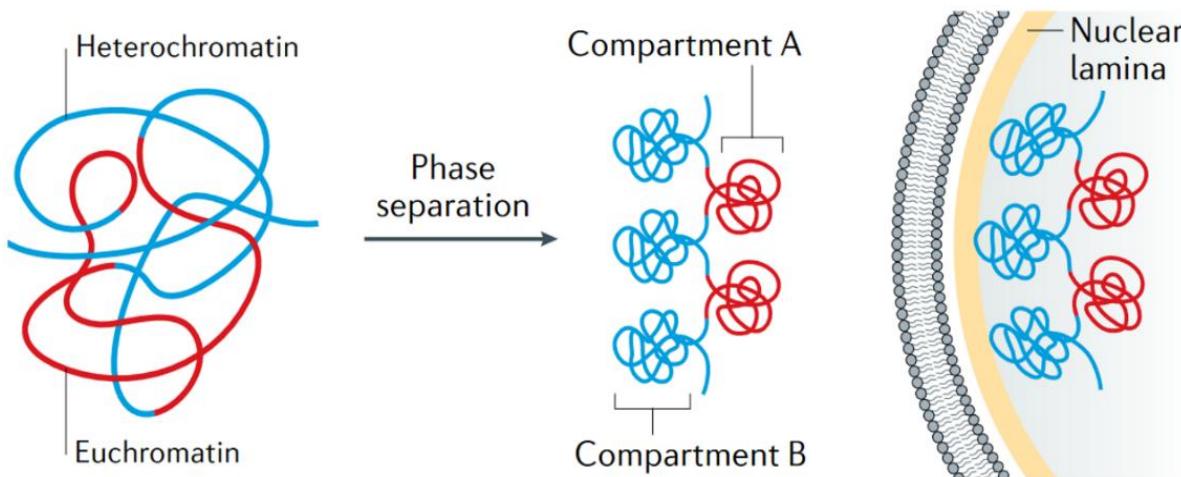
in situ ChIA-PET

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

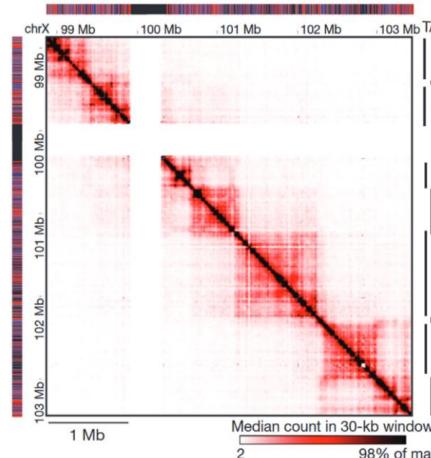
Rusk et al., *Nature Methods*. 2009



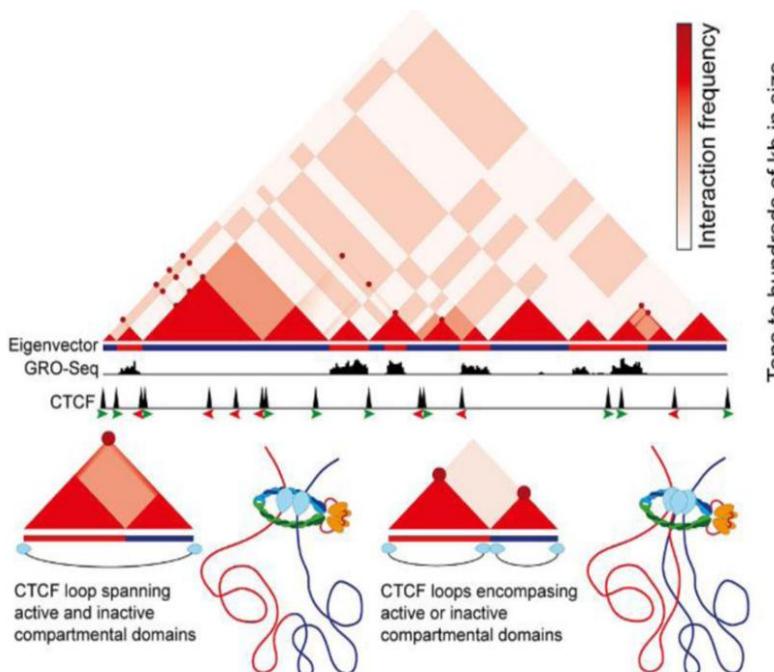
Adapted from Rowley et al., Molecular cell. 2017



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.

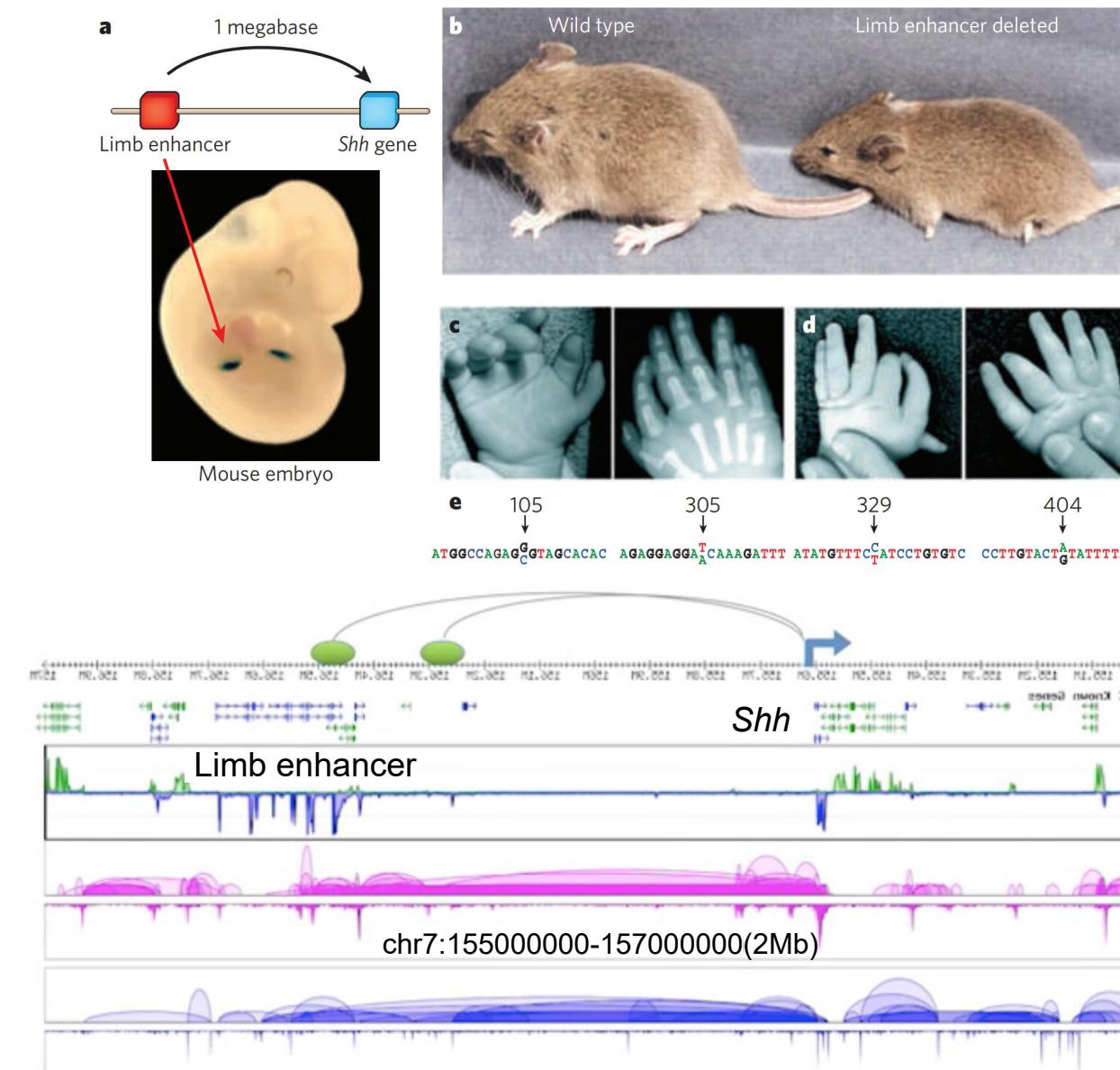


Nora et al., Nature. 2012



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.**



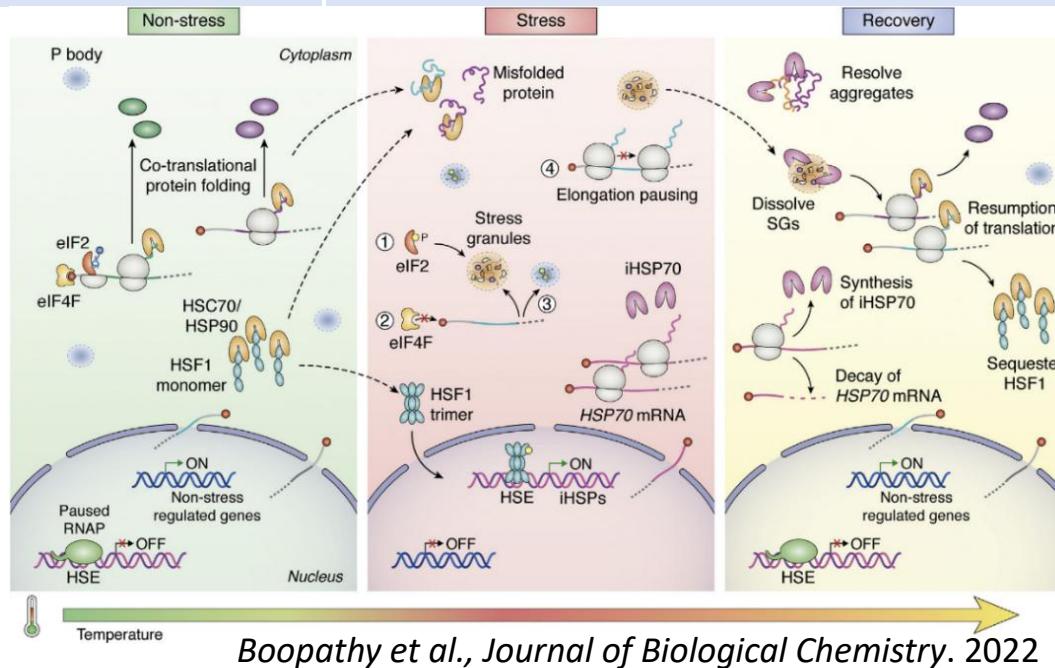
Visel et al., Nature. 2009

Limb enhancer and *shh* gene are spatially close

From Yijun Lab

Background

Heat stress



Boopathy et al., Journal of Biological Chemistry. 2022

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

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

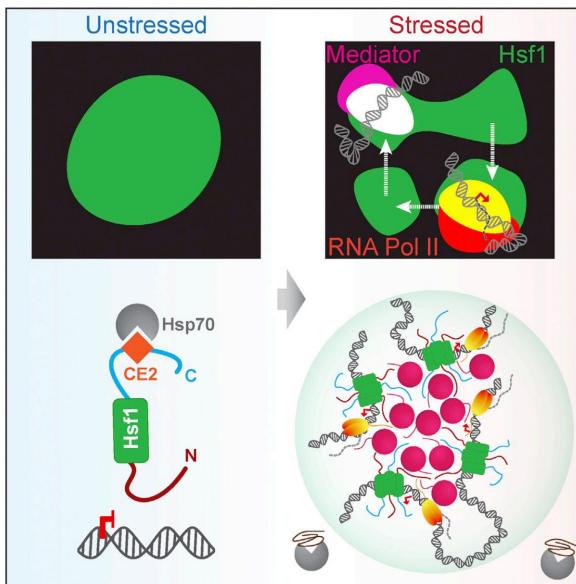
Li et al., Molecular Cell. 2015

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

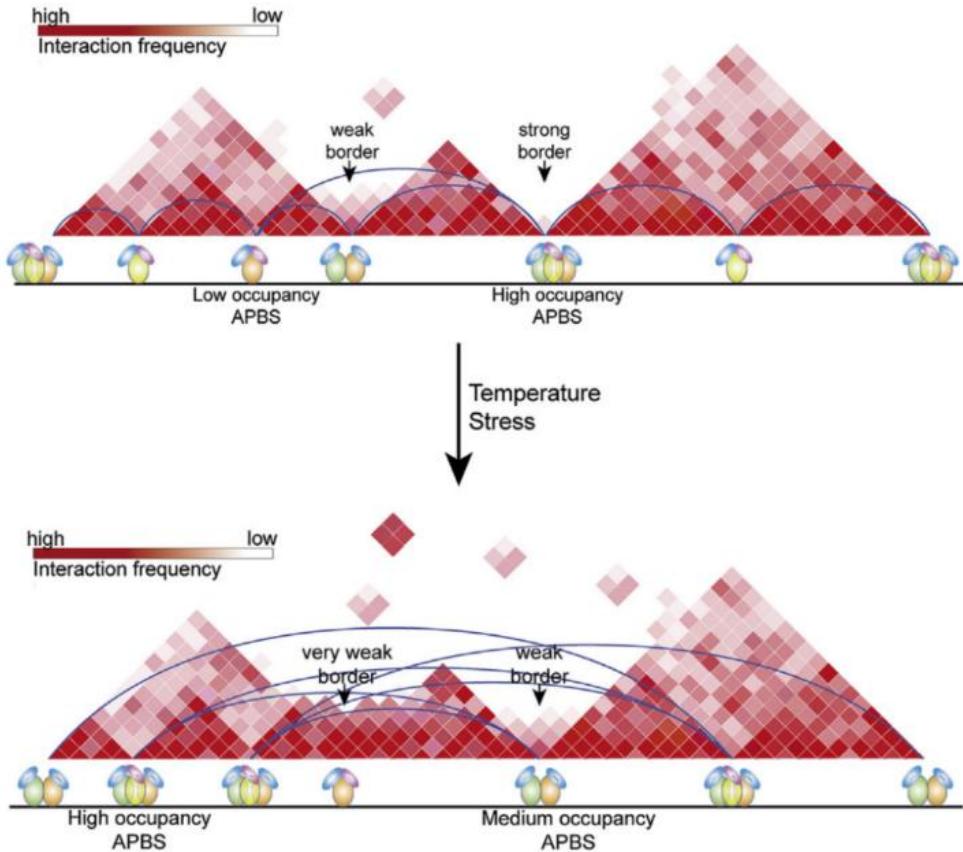
Hieda et al., Chromosome Res. 2005
Teves et al., Genes Dev. 2011

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

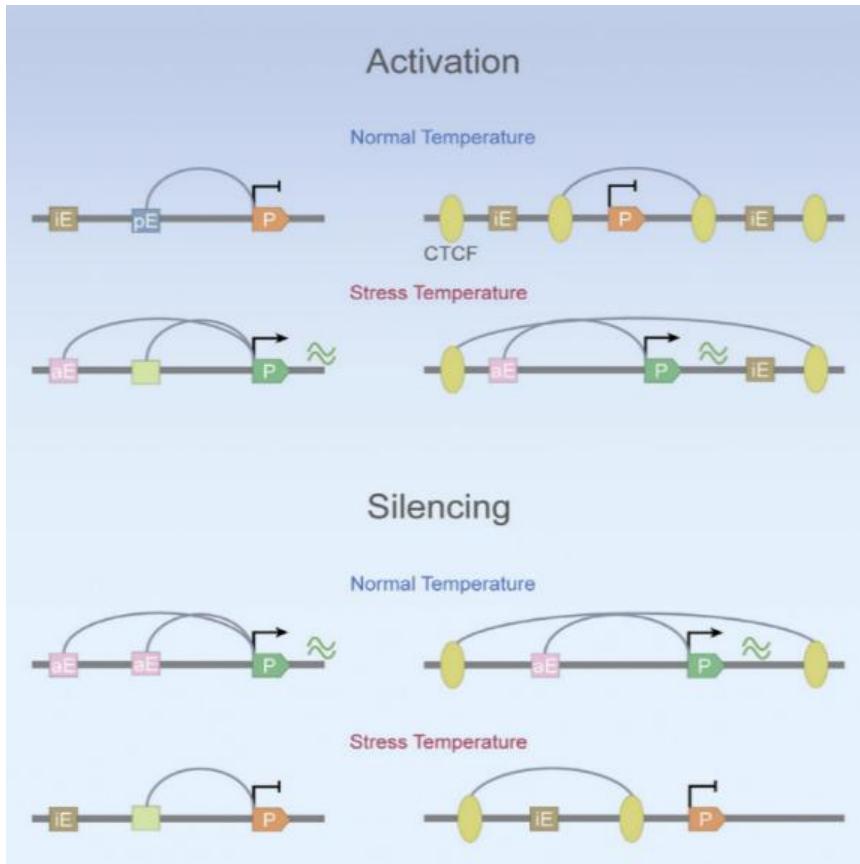


Chowdhary et al., Molecular Cell. 2022



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



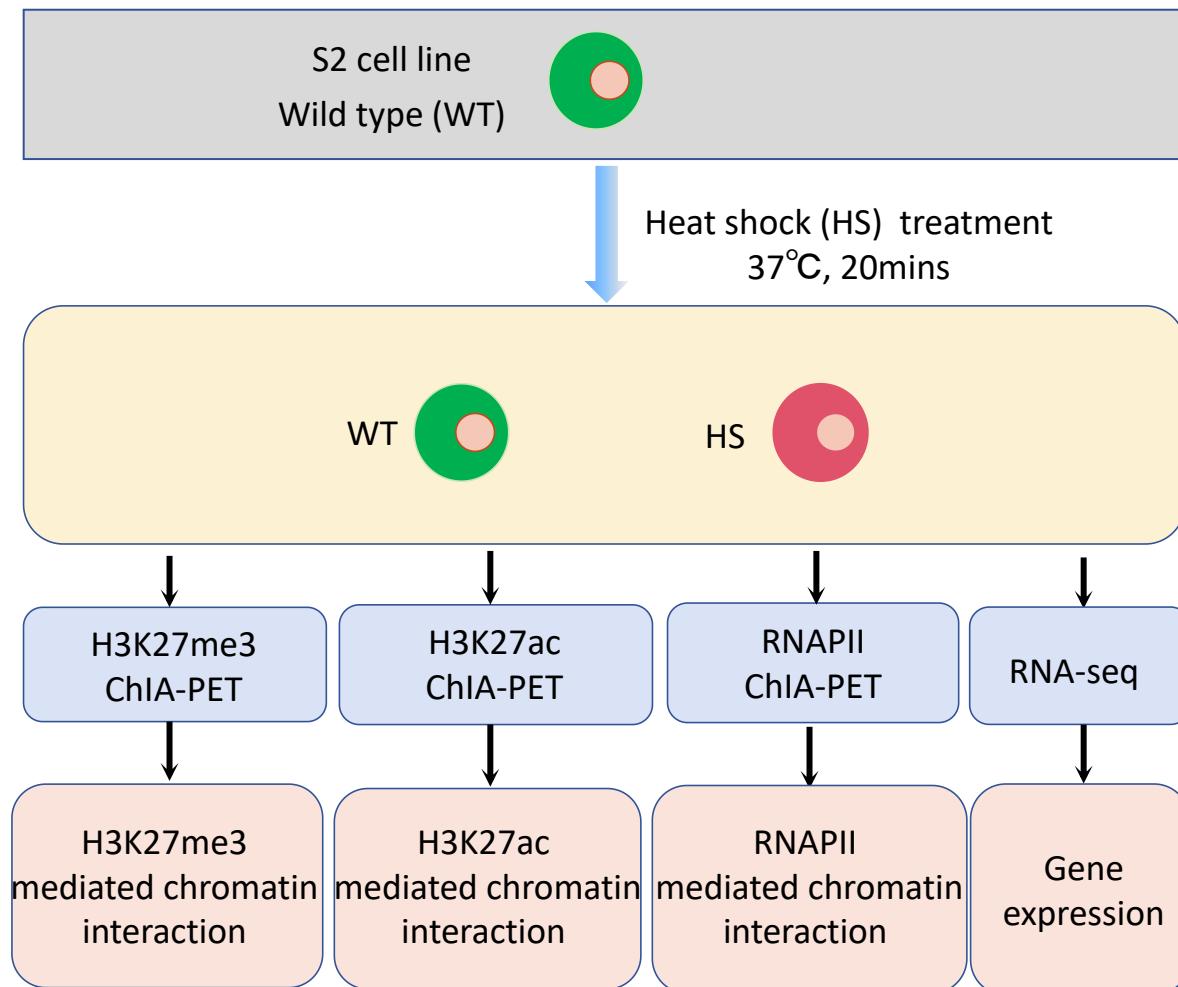
After HS, loops can randomly extrude to regulate the gene expression

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?

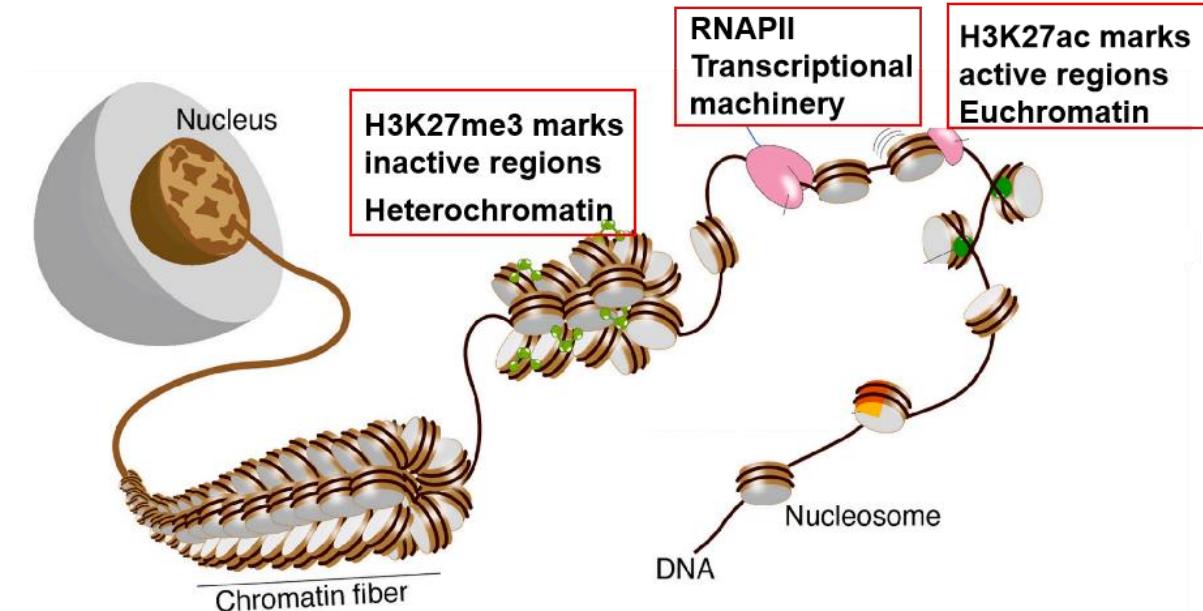


H3K27me3: inactive histone mark

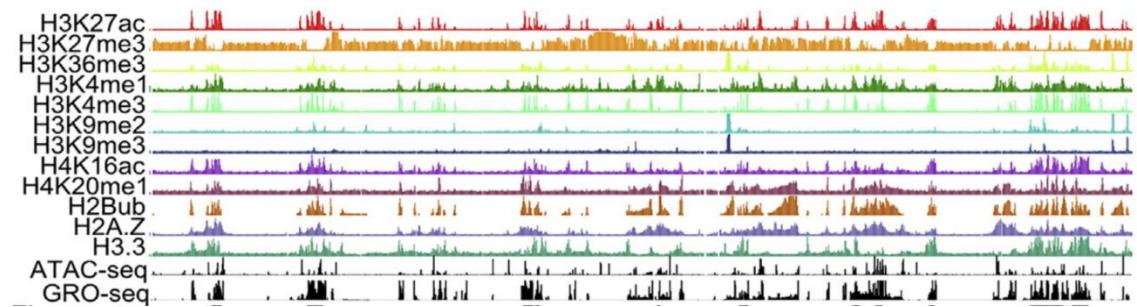
H3K27ac: active histone mark

RNAPII: transcriptional machinery protein

RNA-seq: mark the gene expression level



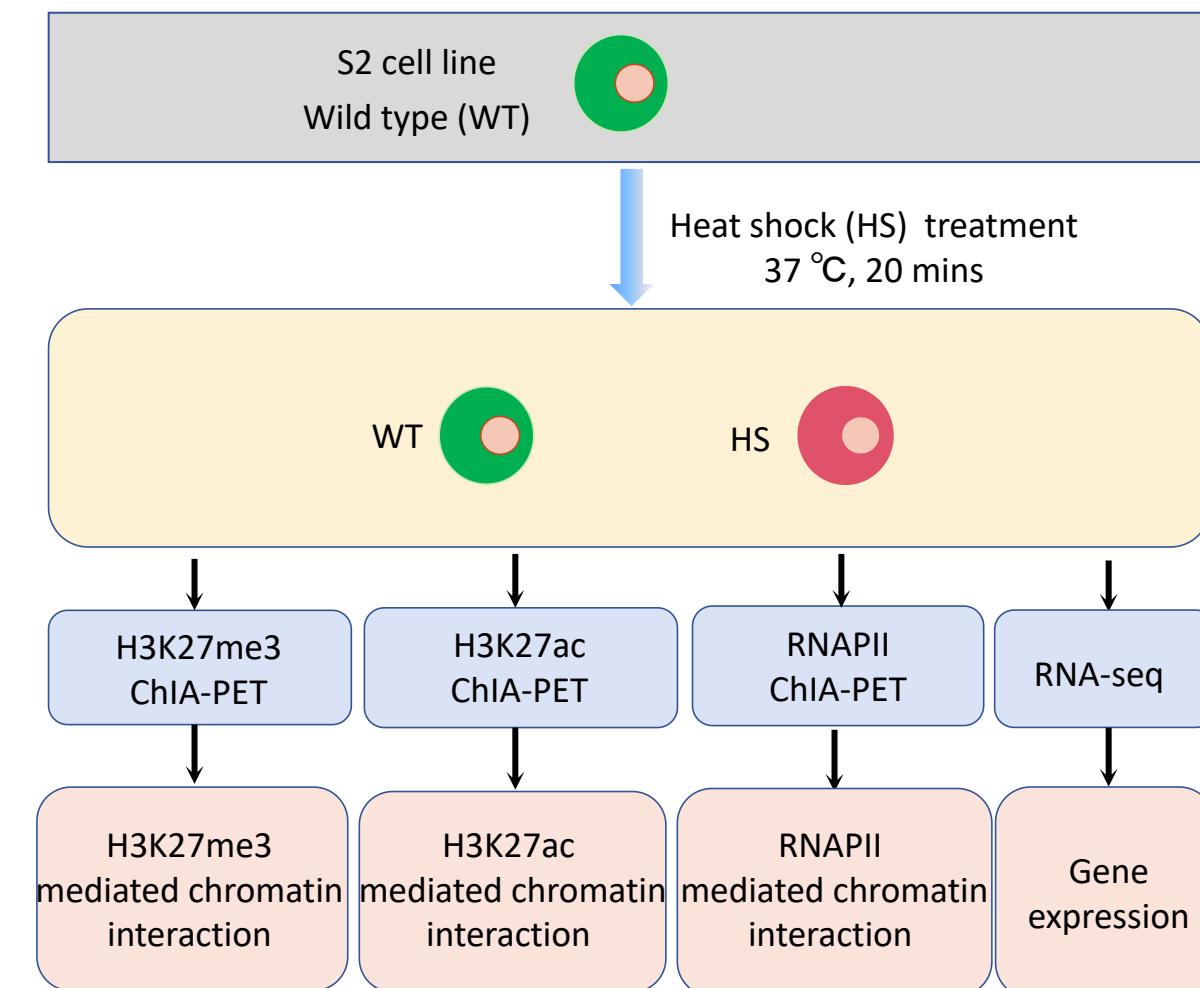
Adapted from Takizawa et al., BBA - Gene Regulatory Mechanisms . 2022



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

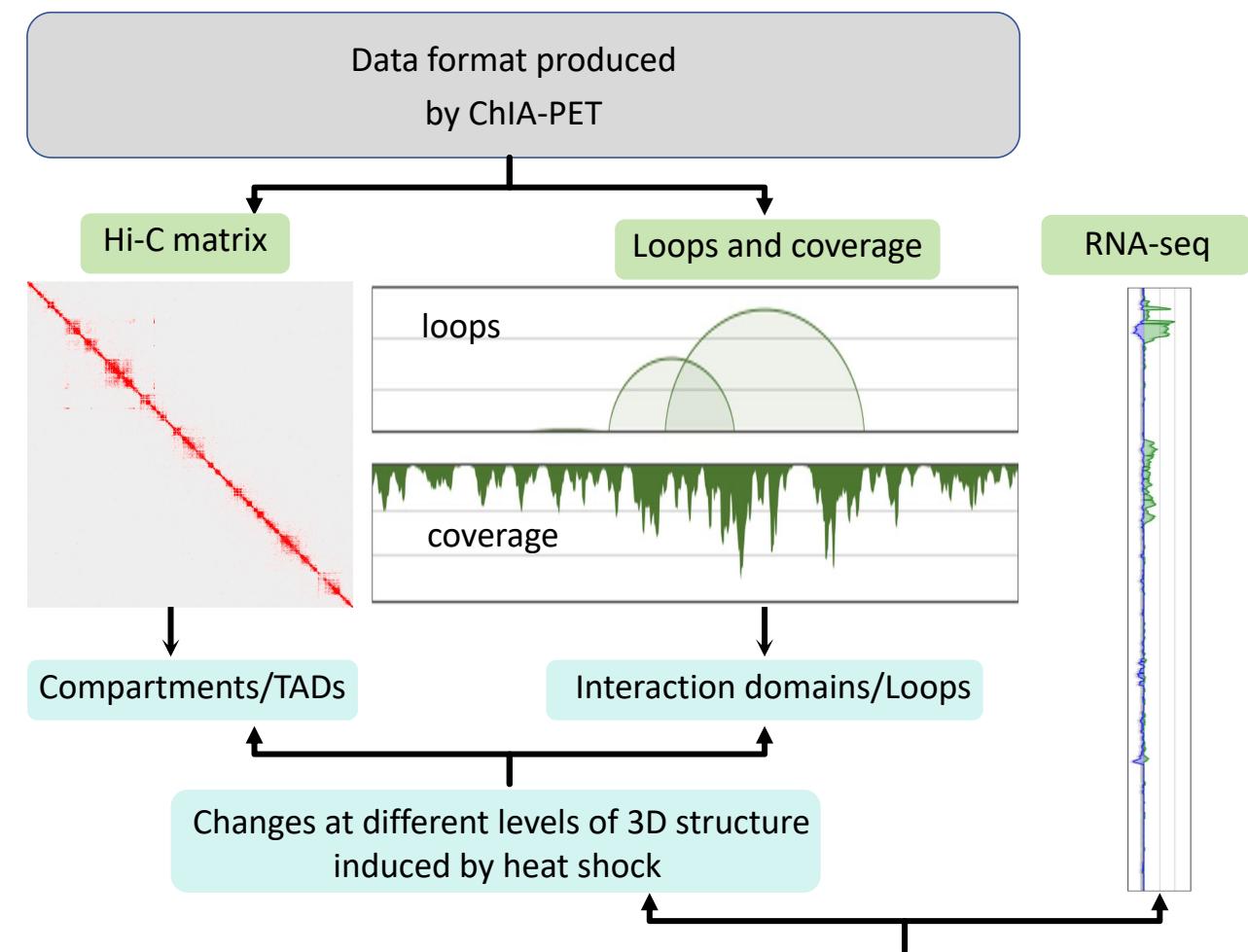


H3K27me3: inactive histone mark

H3K27ac: active histone mark

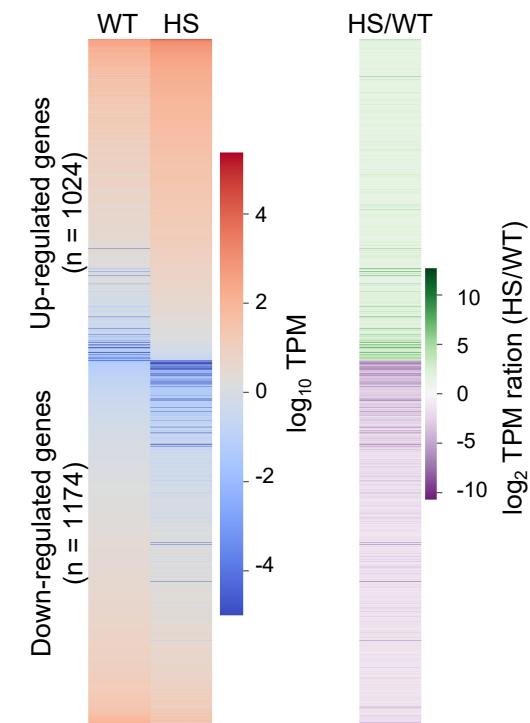
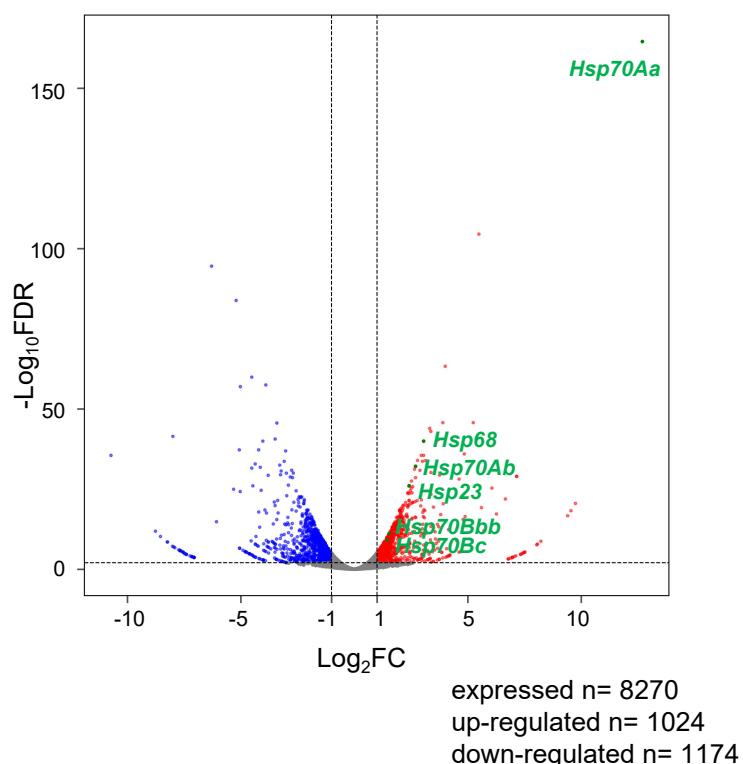
RNAPII: transcriptional machinery protein

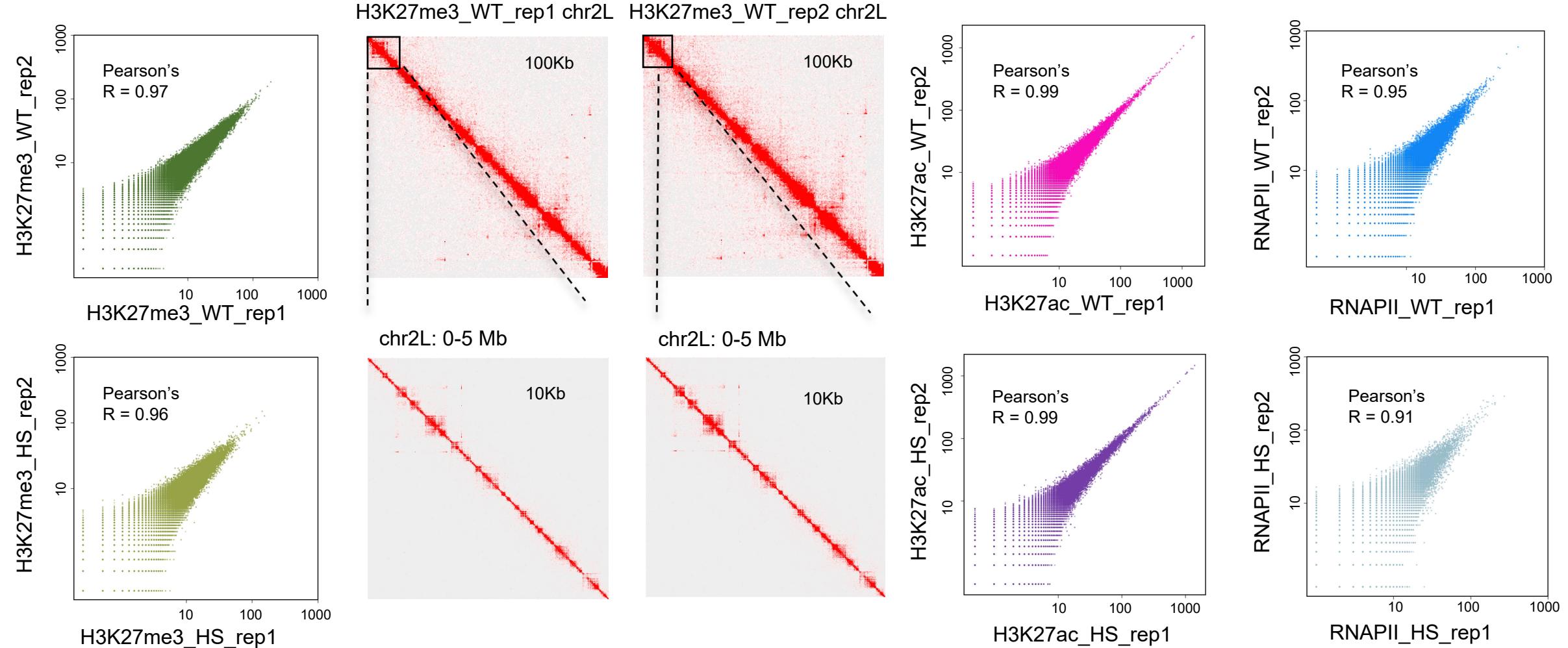
RNA-seq: mark the gene expression level

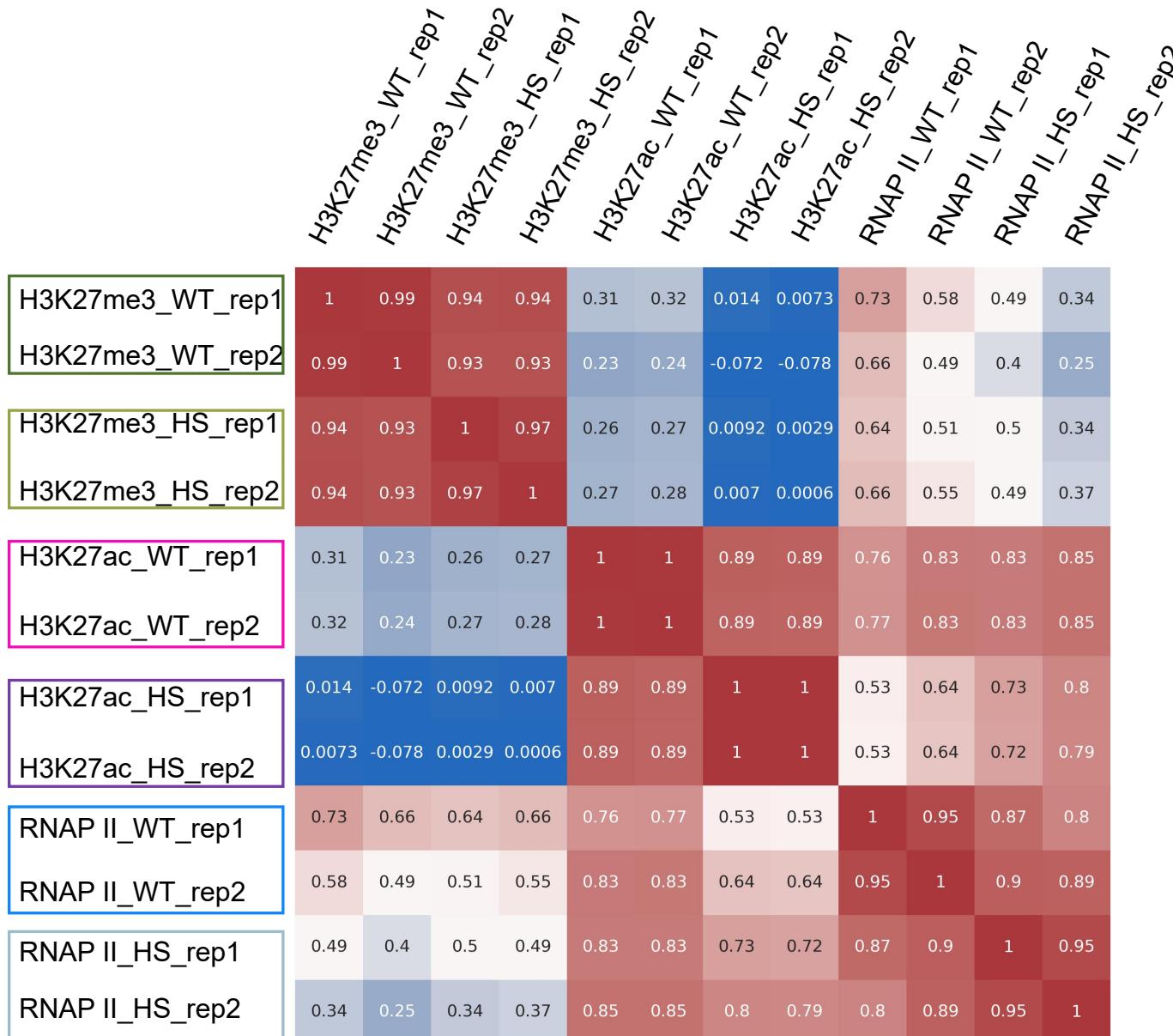


Data analysis

- 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.





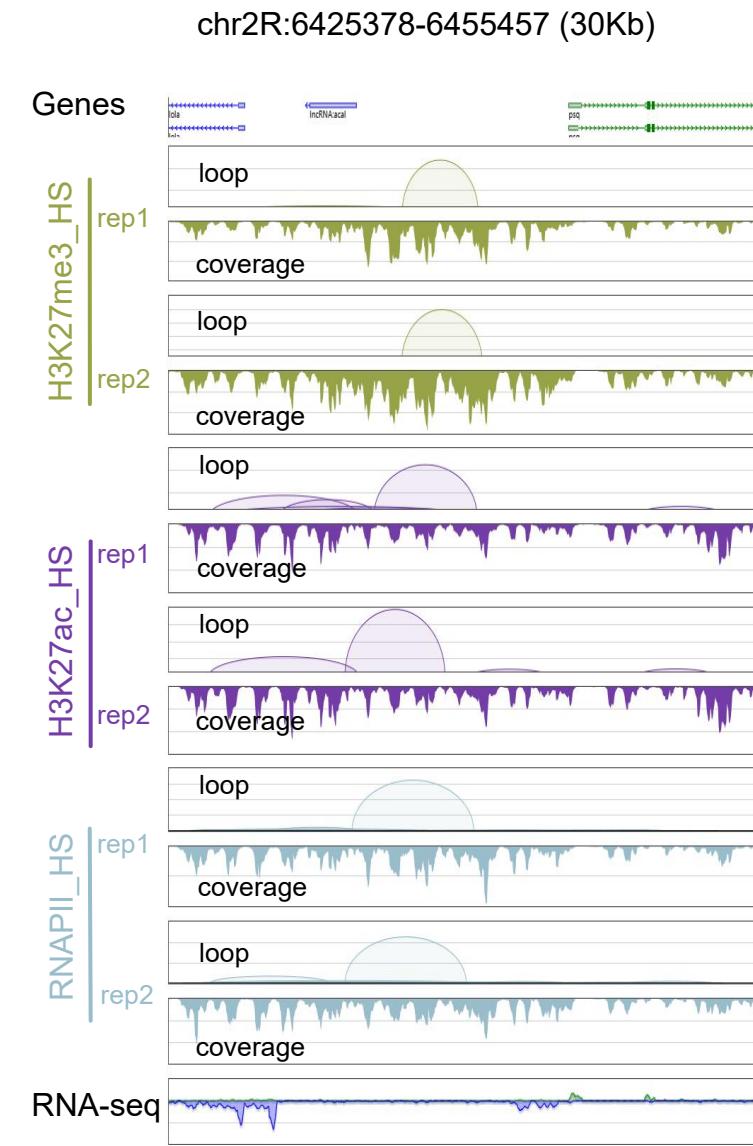
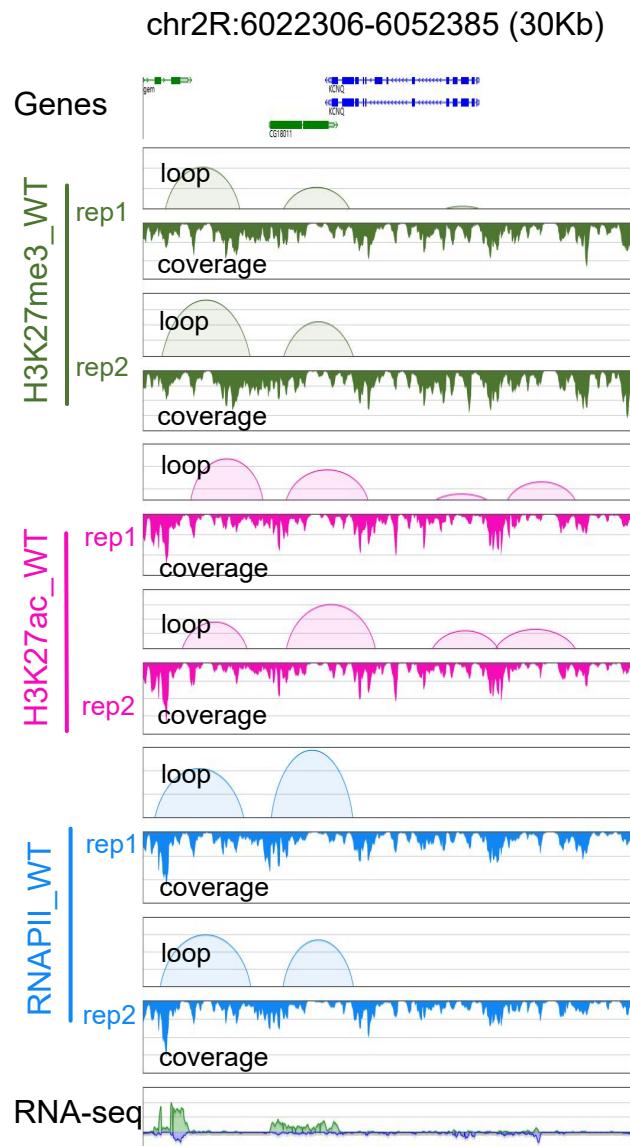


Heatmap of stratum adjusted correlation coefficient (SCC)

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

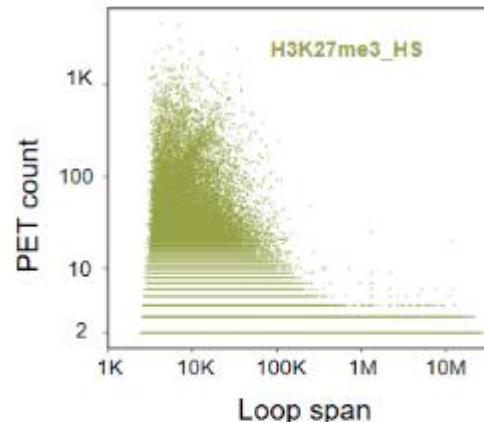
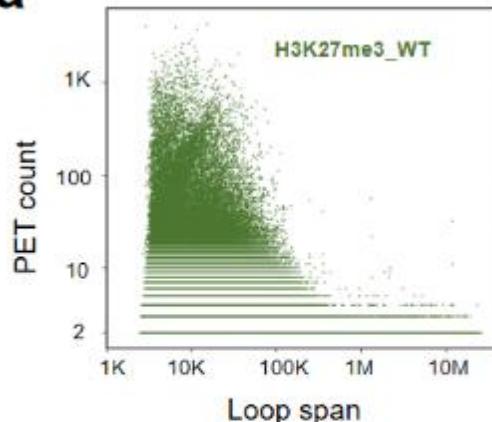
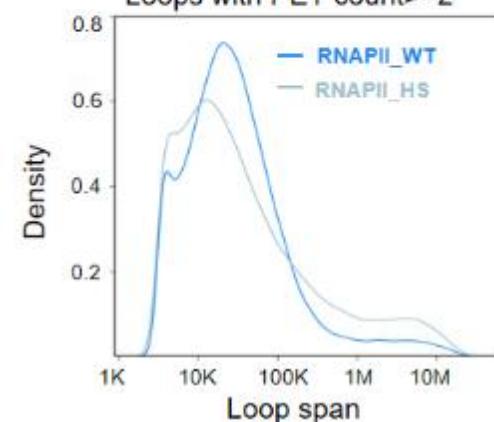
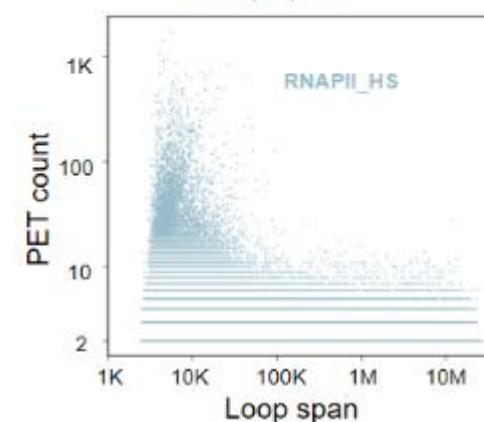
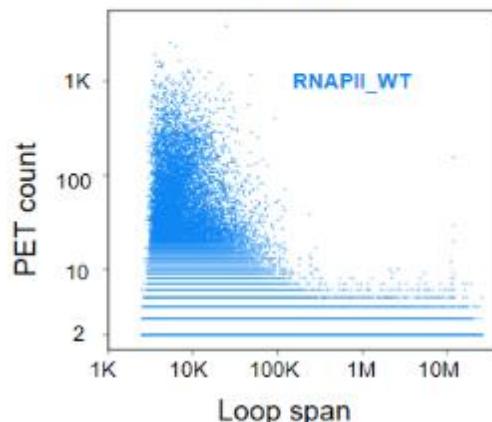
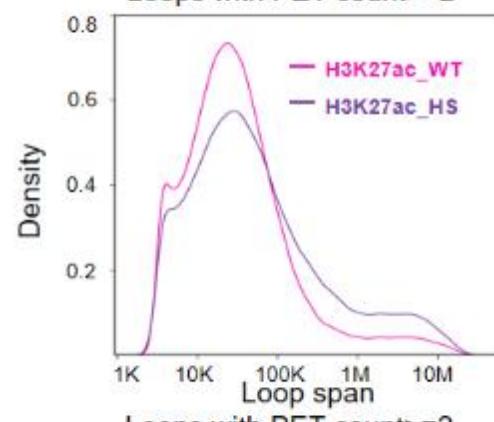
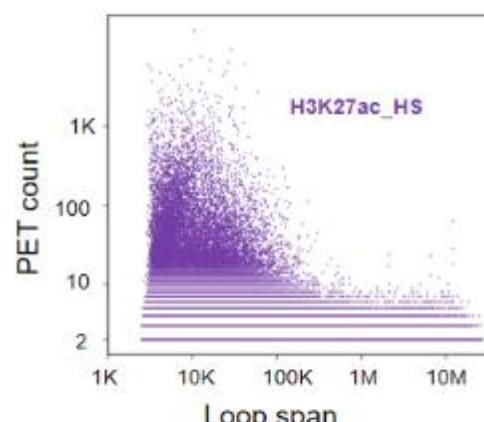
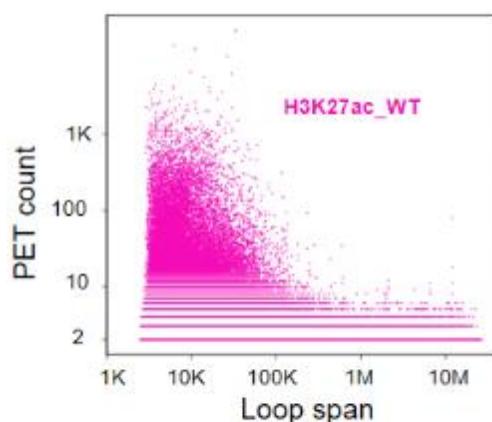
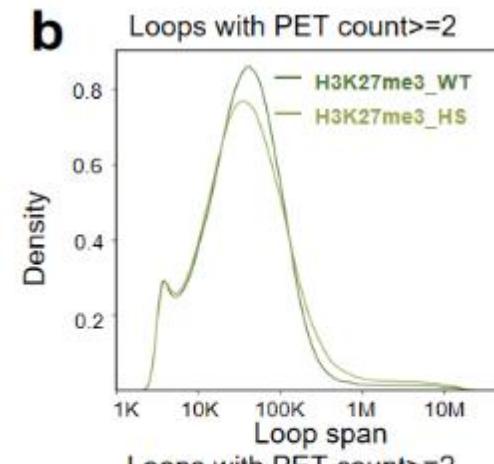
High reproducibility of *in situ* ChIA-PET (loops and coverage)

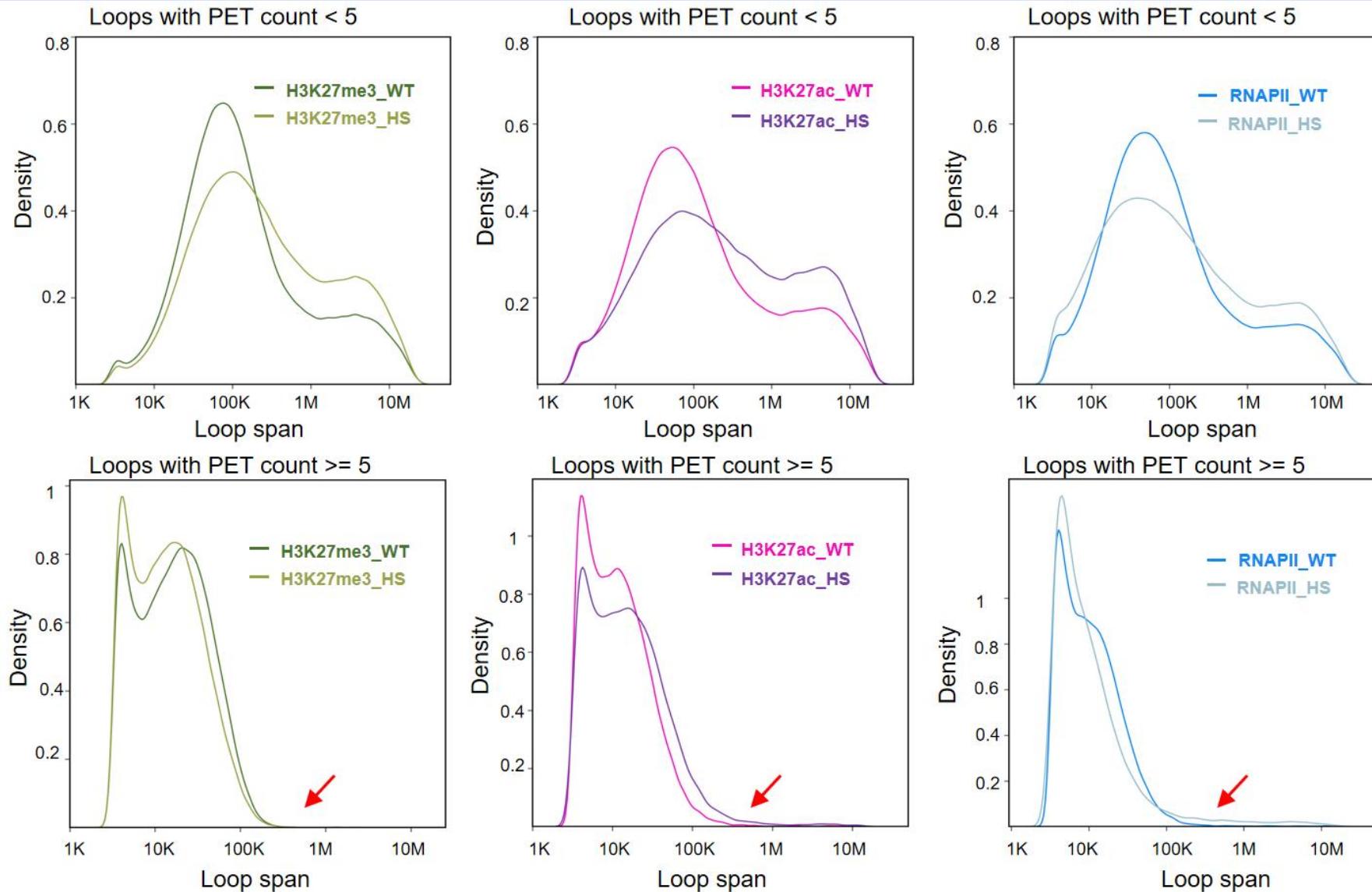
Summary of ChIA-PET libraries **WT and its corresponding HS have similar number of uniquely-mapped PETs**

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

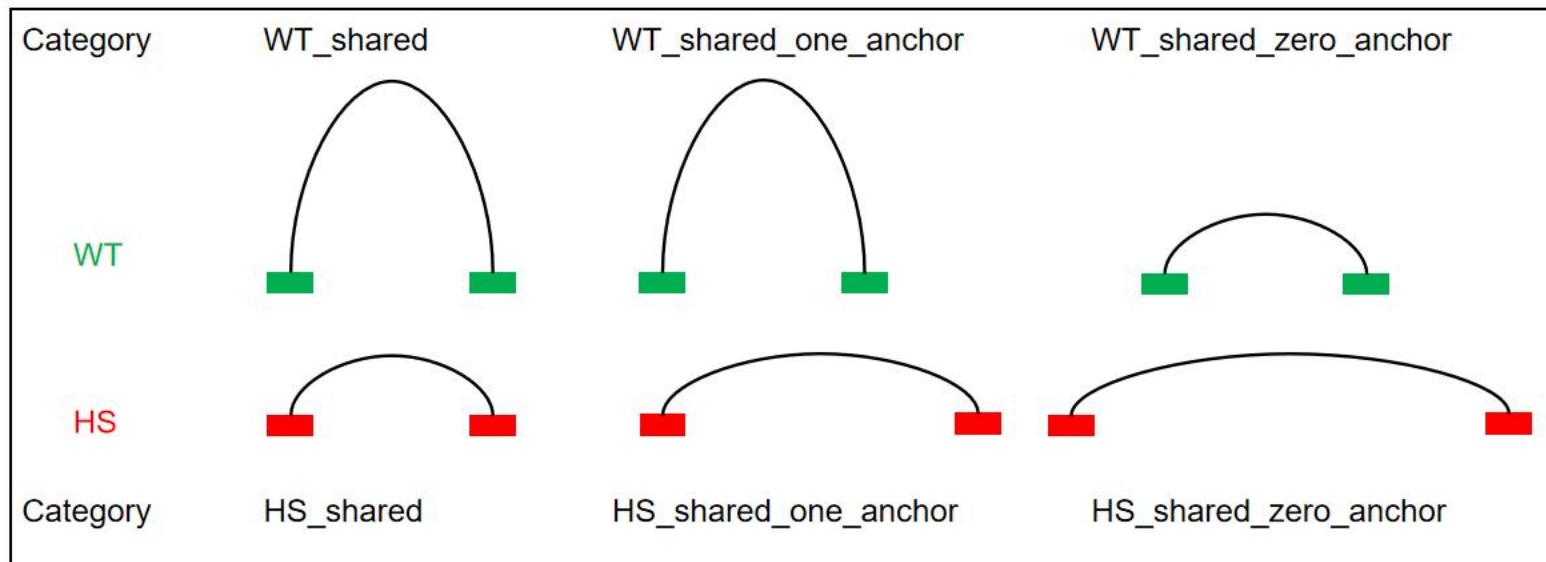
The ChIA-PET datasets of S2 cell line in the above table were mapped to the reference genome dm3.

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

a**b**

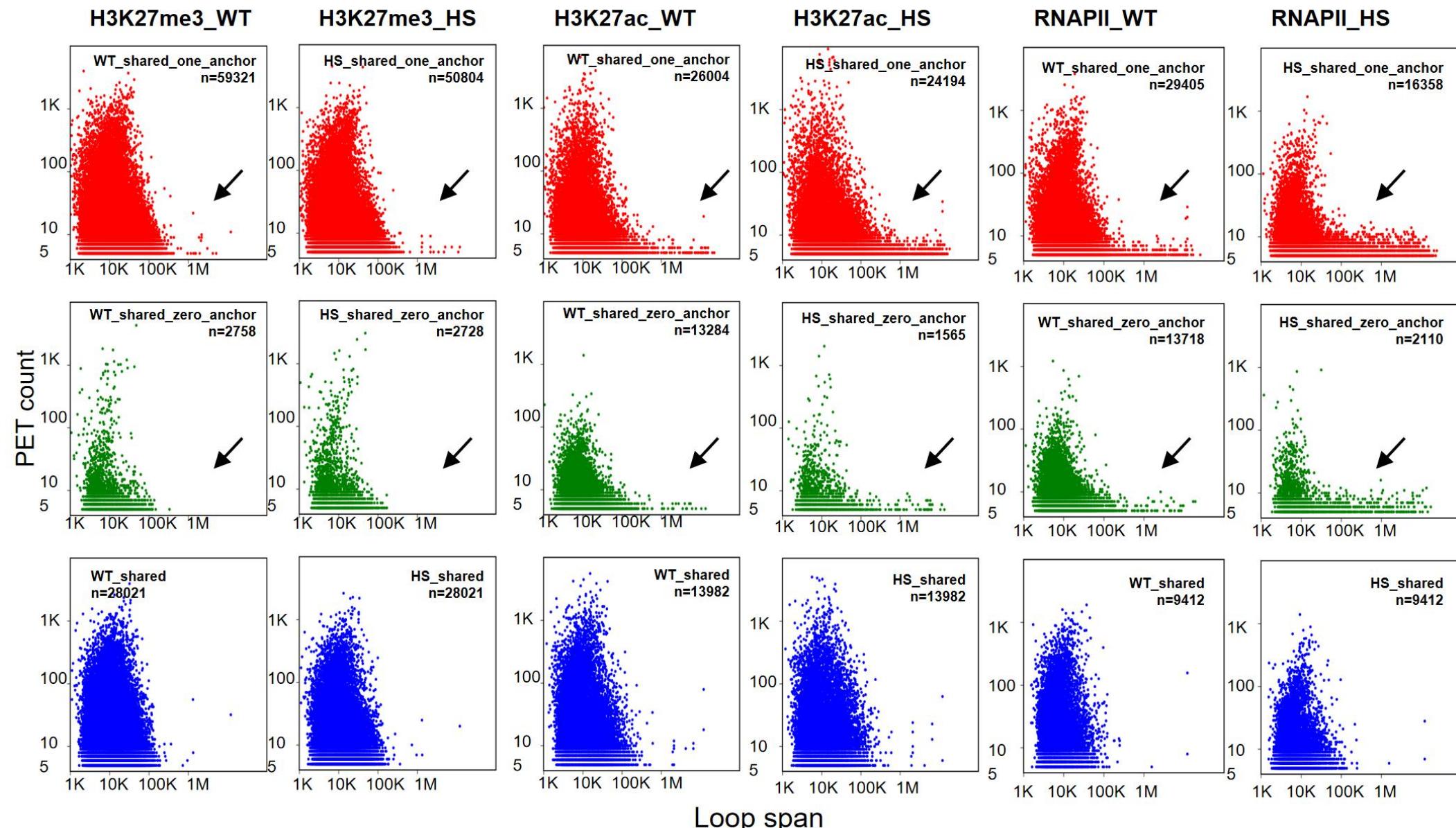


After HS, proportion of loops with large loop span increase in H3K27ac_ChIA-PET and RNAP II_ChIA-PET



Loop level

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

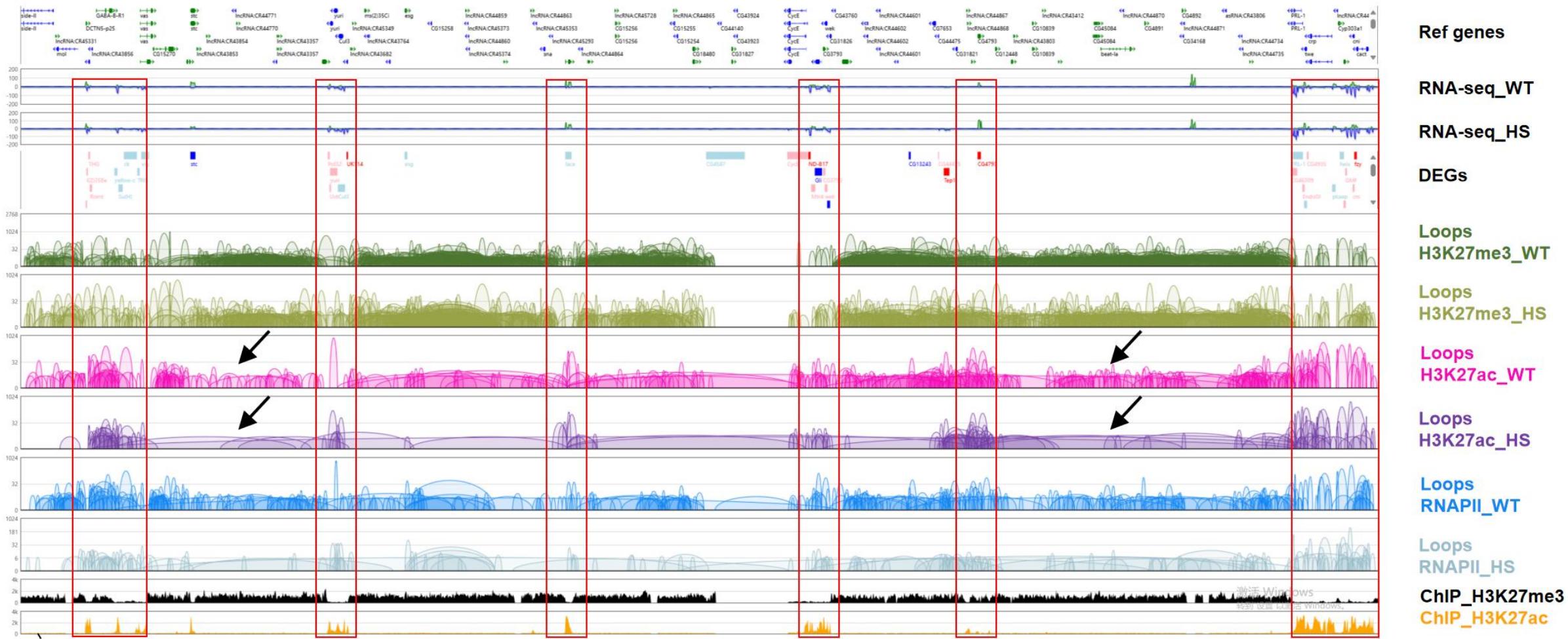


After HS, proportion of loops with large loop span and low PET count increase in H3K27ac_ChIA-PET and RNAP II_ChIA-PET

Loop level

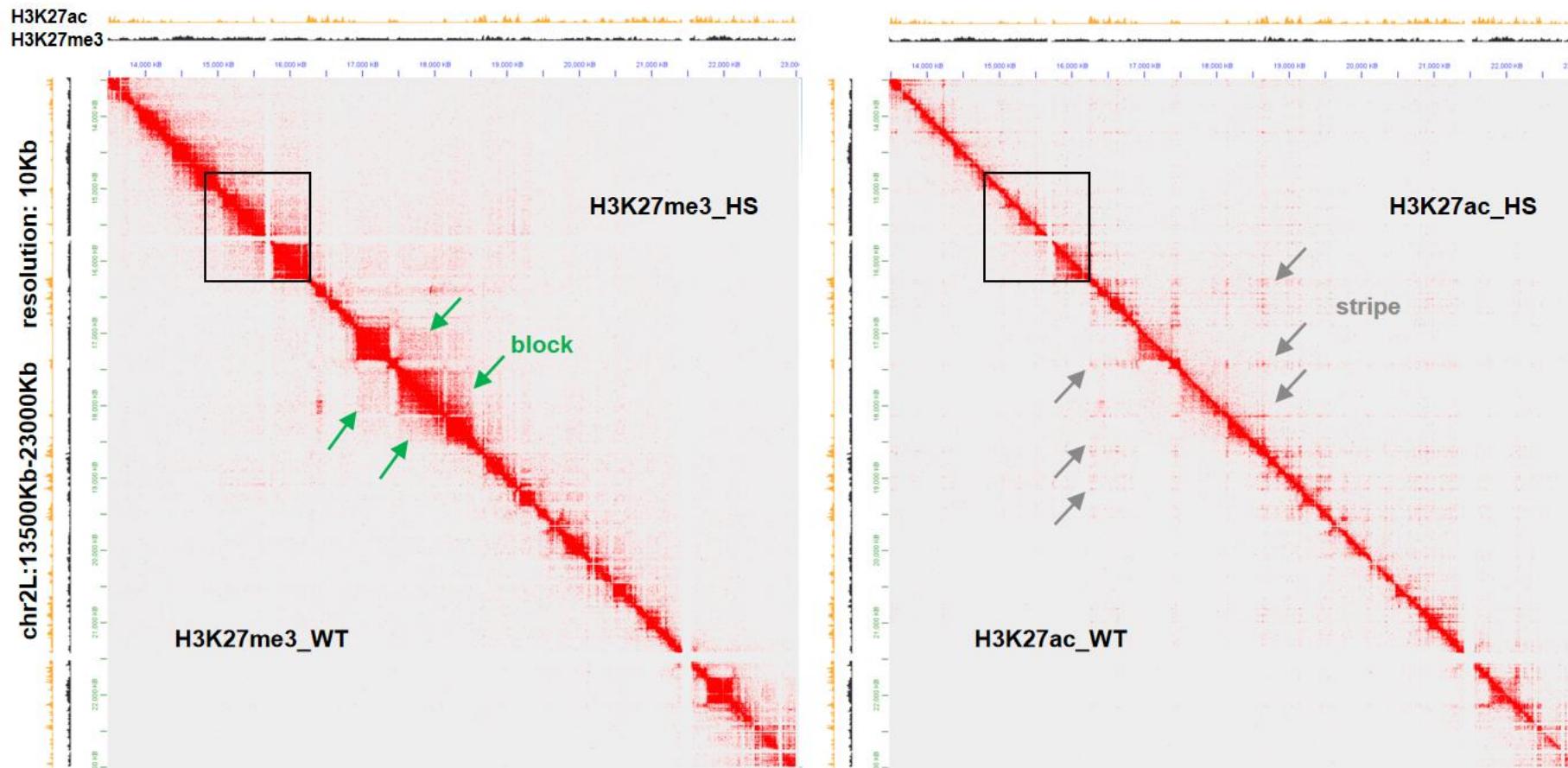
Loops induced by heat shock with low PET counts and large loop span connecting different H3K27ac-dominant regions

chr2L:14899489-16346700



Loop level

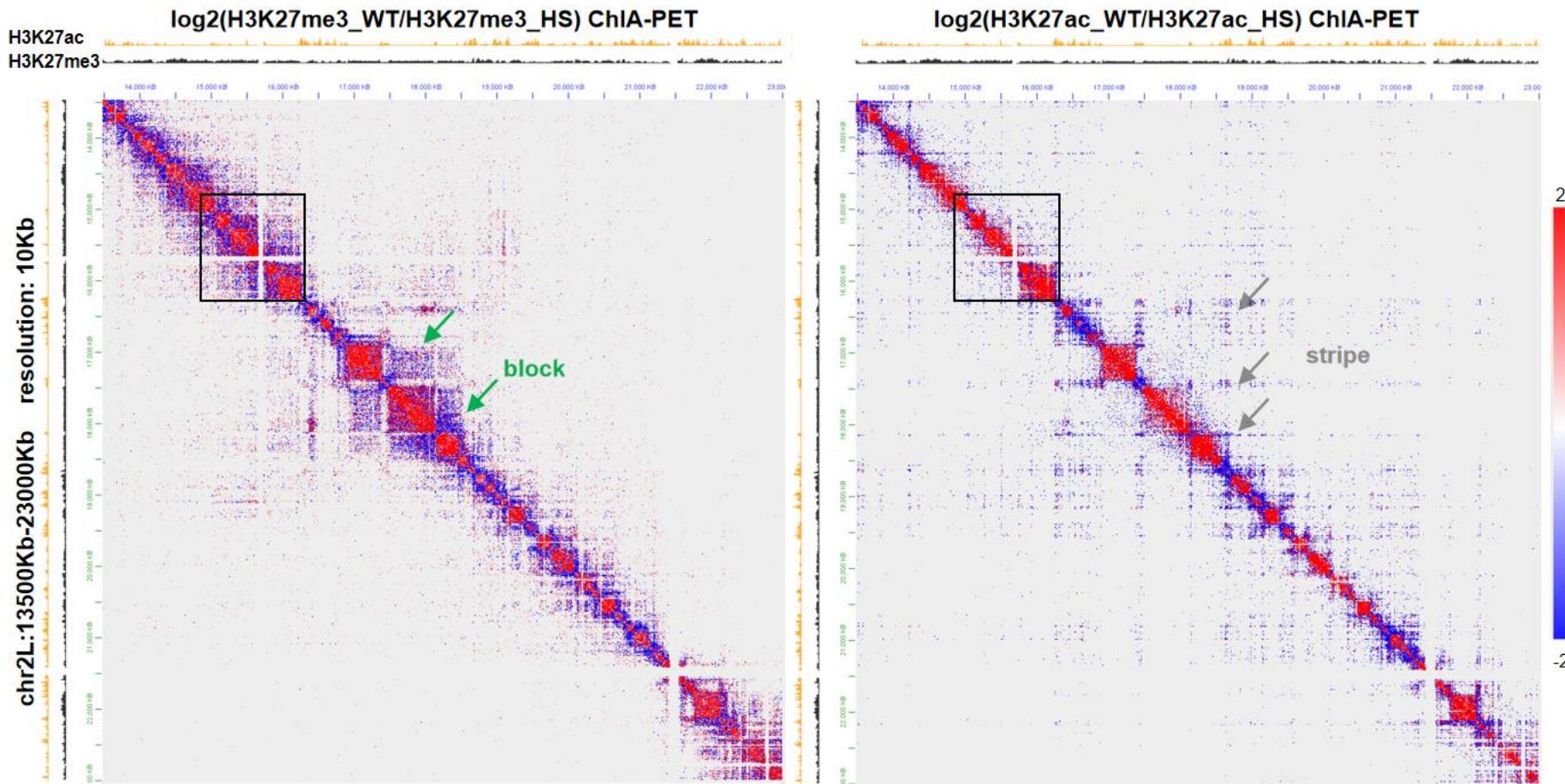
Loops induced by heat shock with low PET counts and large loop span connecting different H3K27ac-dominant regions



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

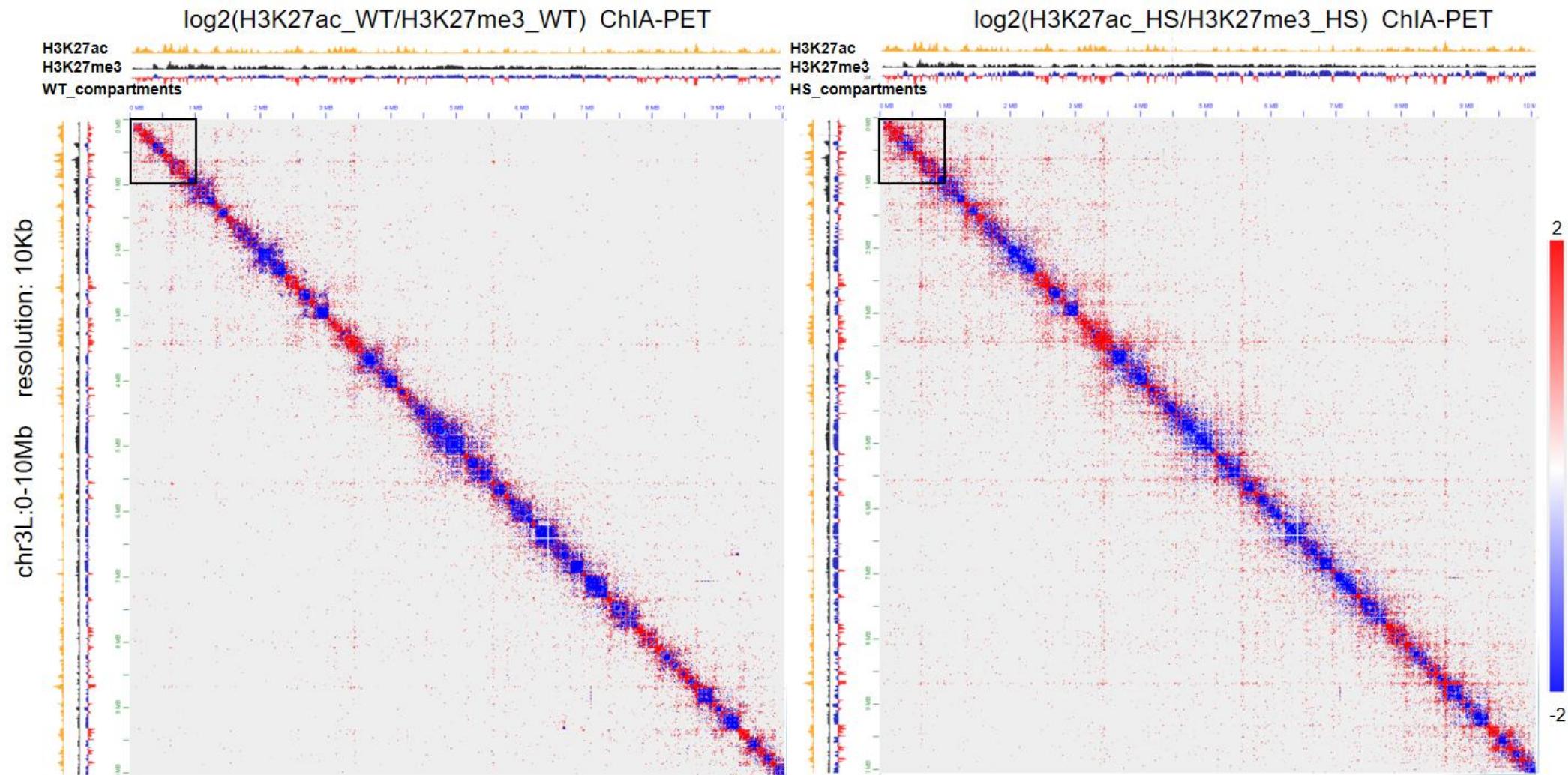
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Compartment level

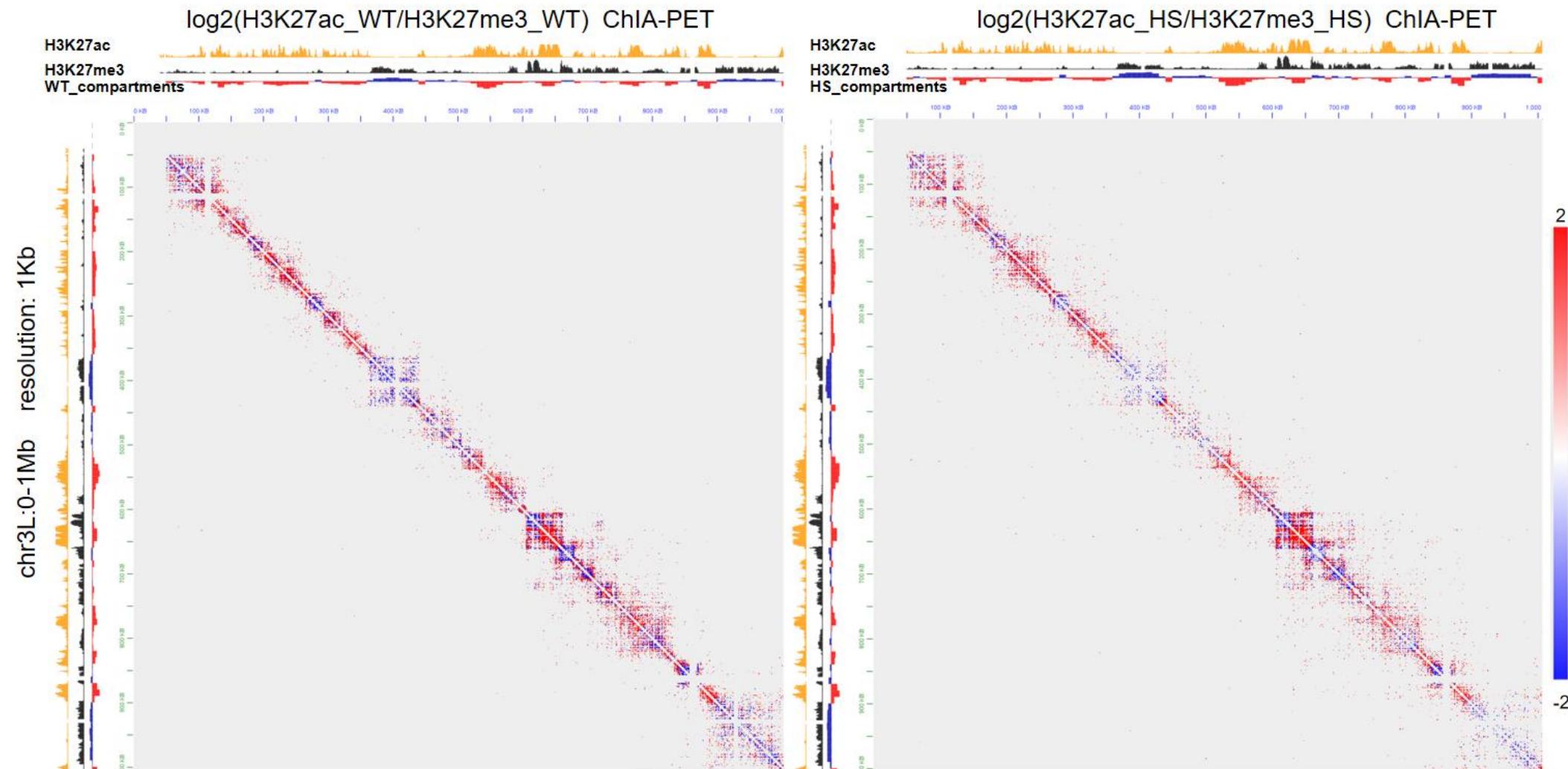
Log₂(H3K27ac/H3K27me3) and can be used to call H3K27ac-dominant and H3K27me3-dominant regions



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

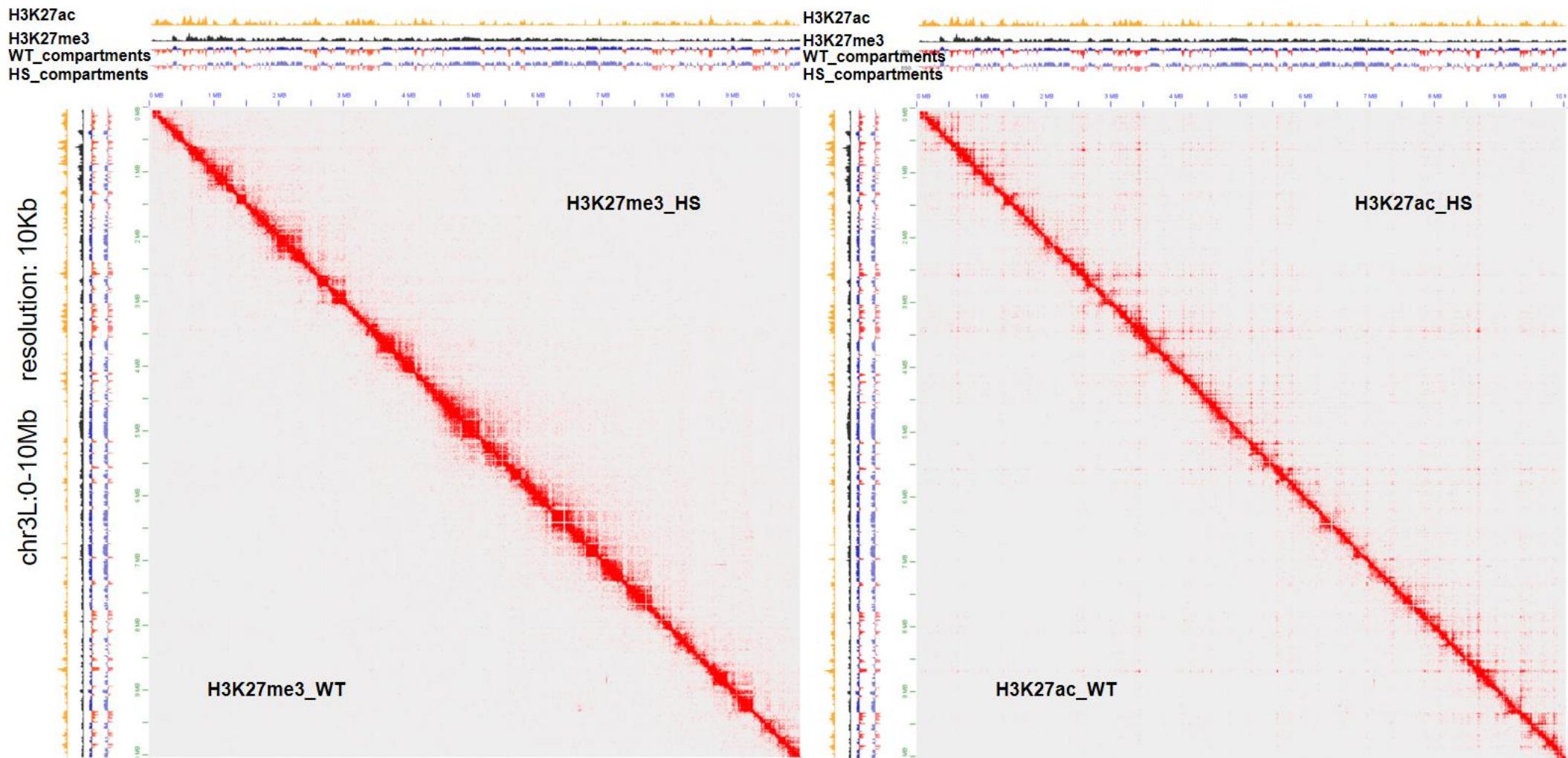
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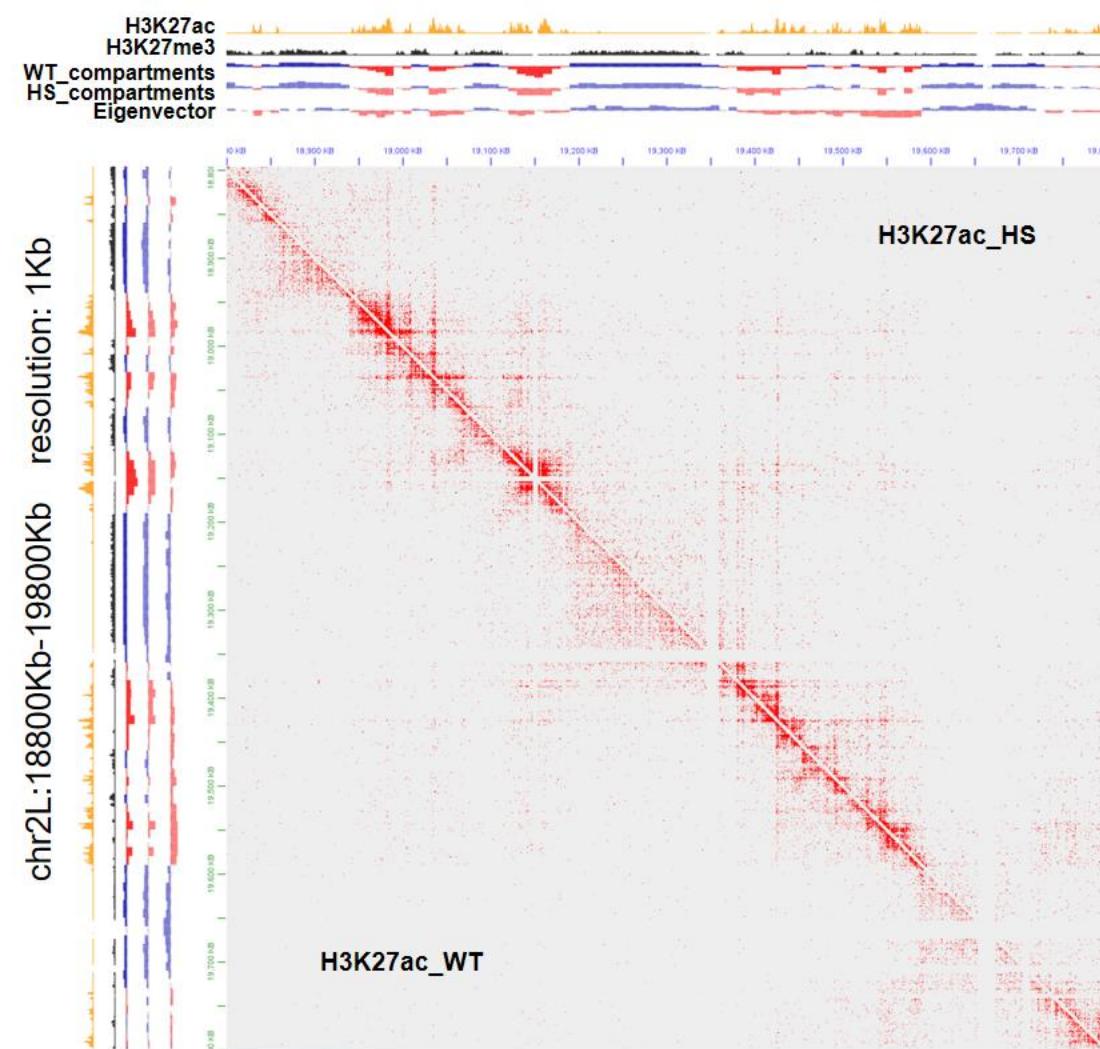
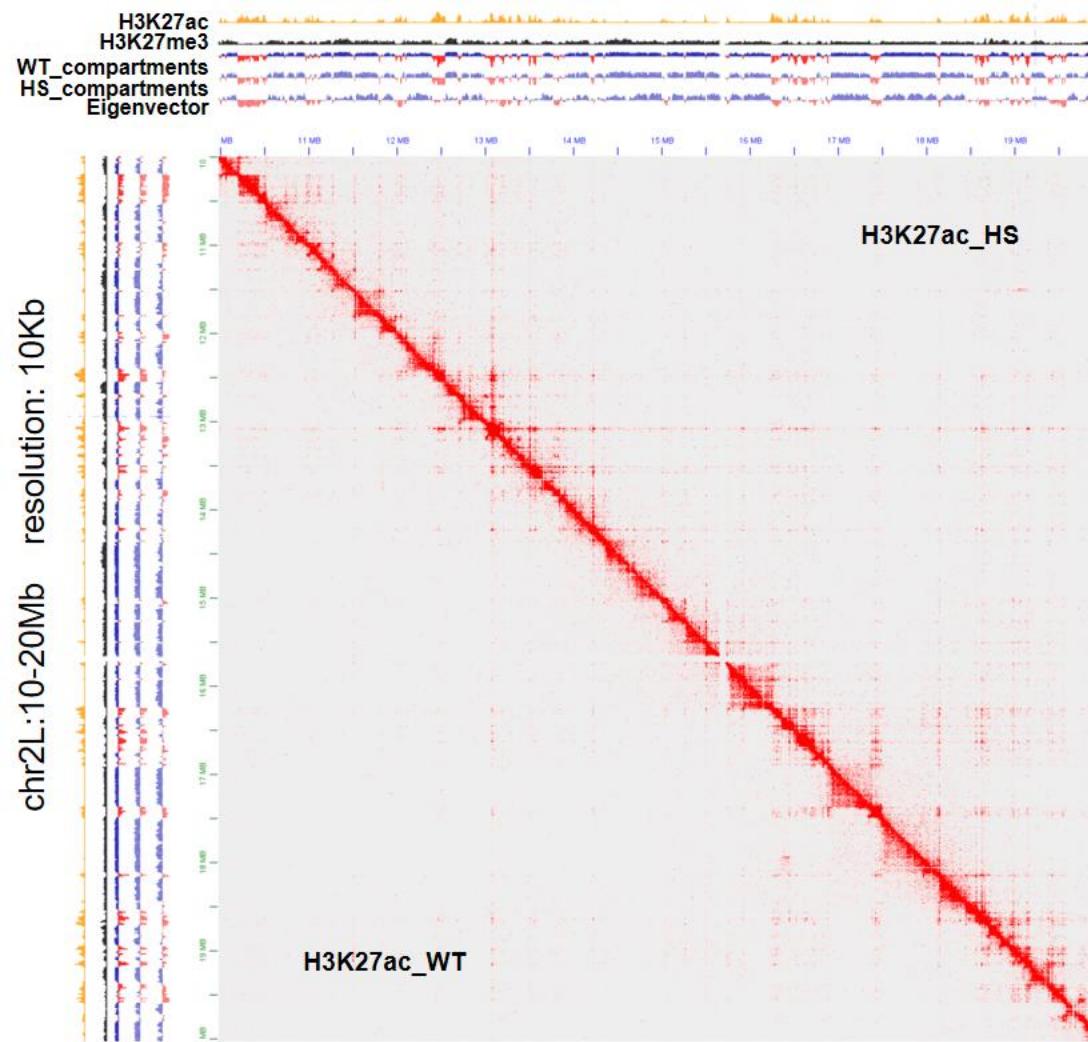
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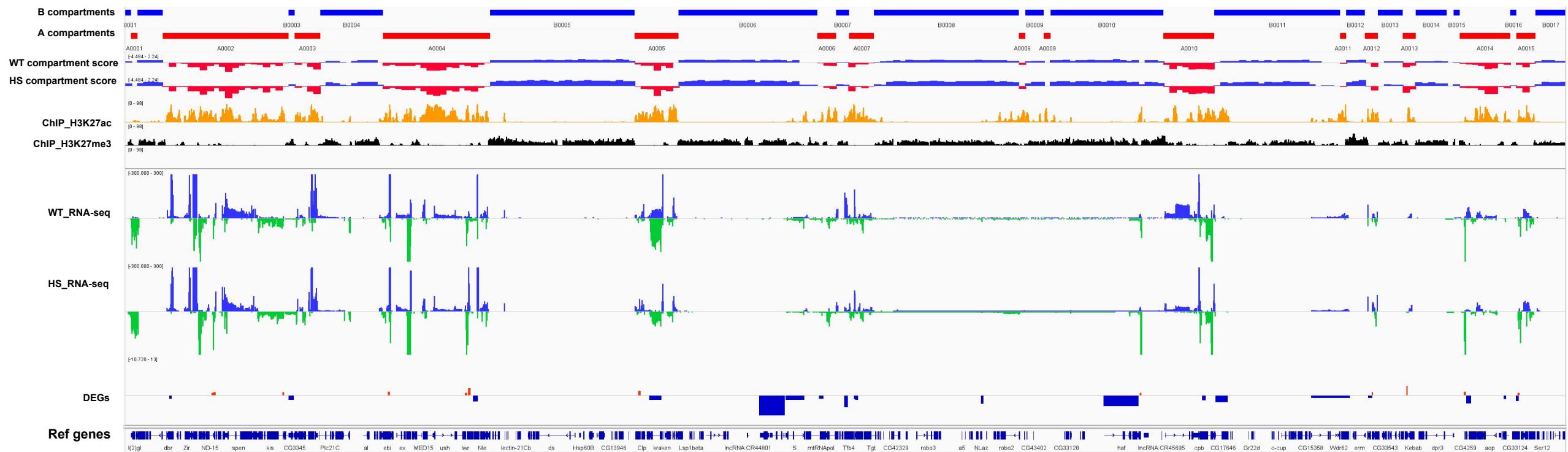
Compartment score = log₂(H3K27ac/H3K27me3), if it's positive, assign the bin as 'A', else assign the 'bin' as 'B'. Its absolute value denotes the extent of compartmentalization

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

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

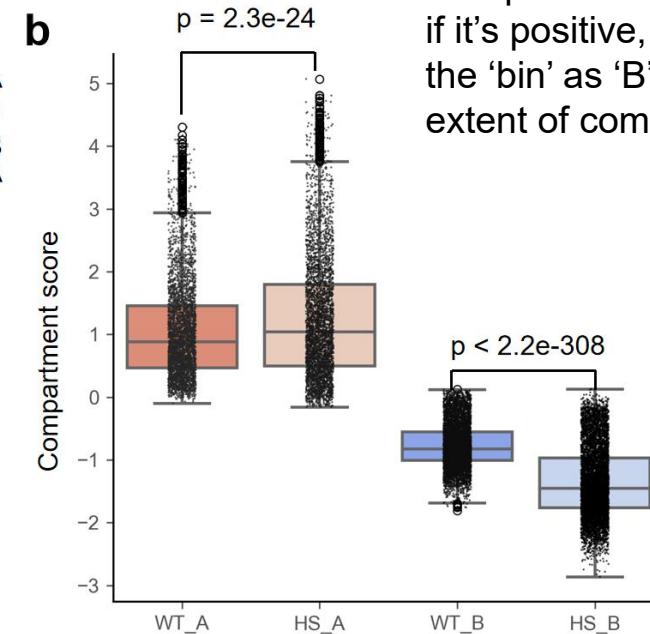
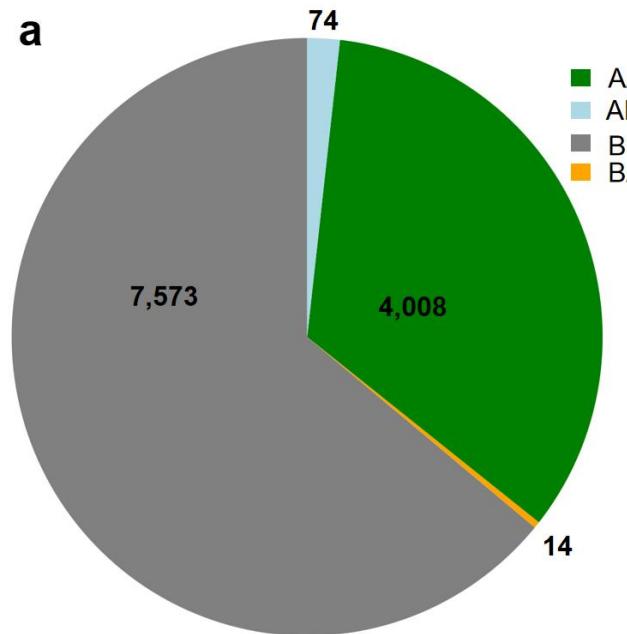
$$A \text{ switch percent} = \frac{AB}{AB + AA}$$

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

switch percent = 0.8%

A switch percent = 1.8%

B switch percent = 0.2%

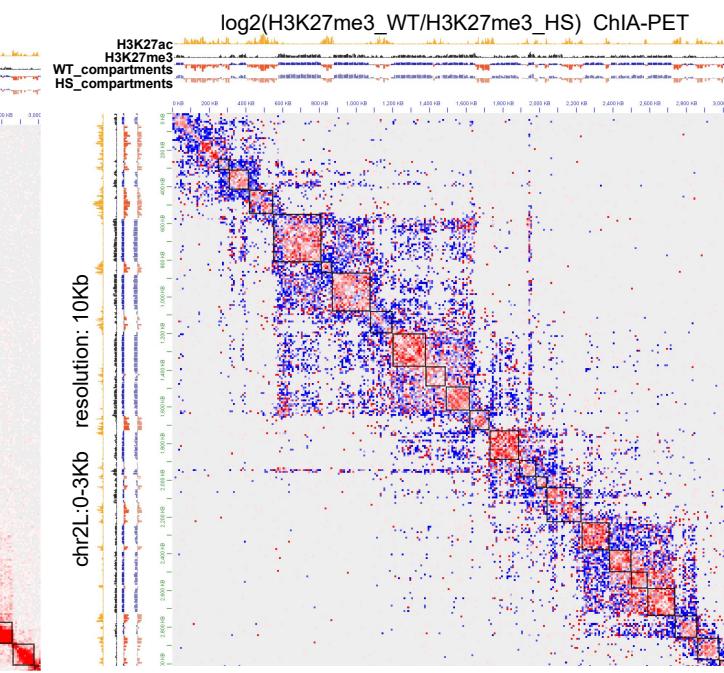
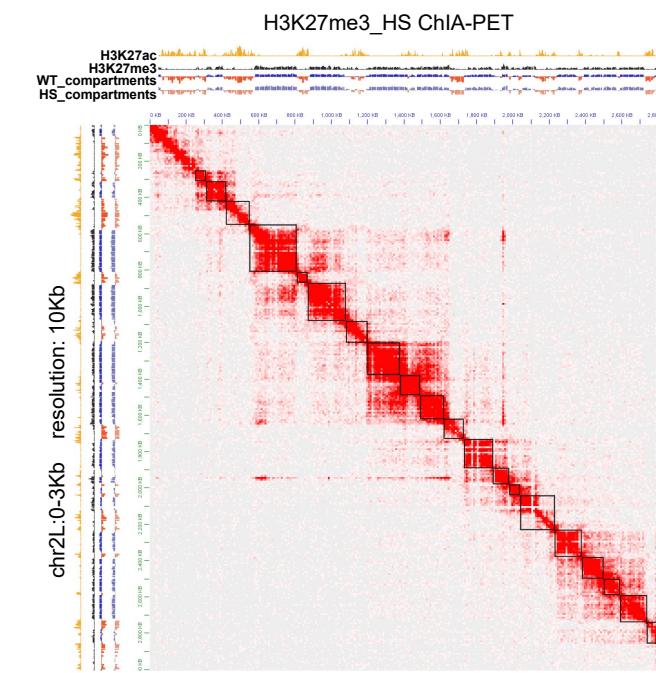
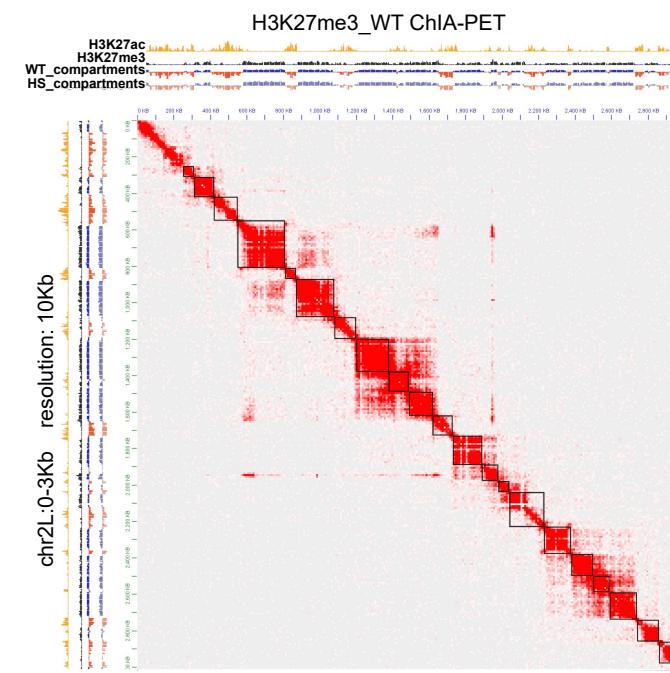


Compartment score = $\log_2(H3K27ac/H3K27me3)$, if it's positive, assign the bin as 'A', else assign the 'bin' as 'B'. Its absolute value denotes the extent of compartmentalization.

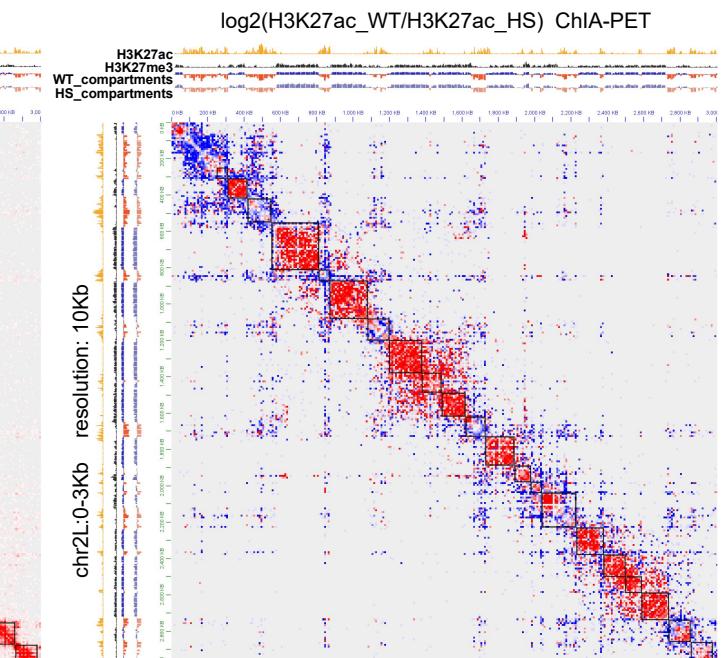
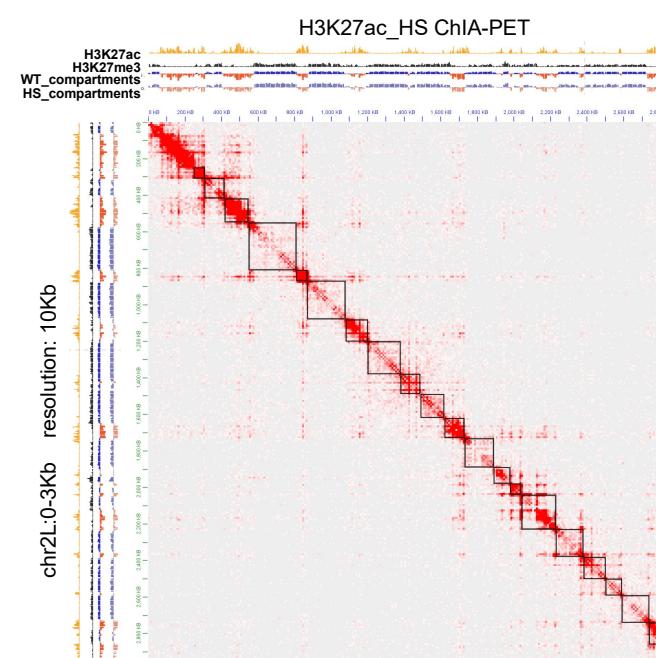
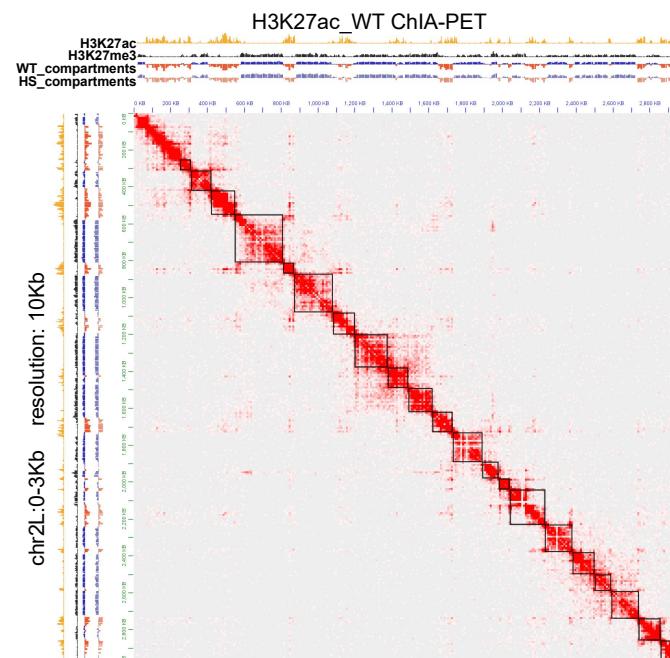
After heat shock, A/B compartment intervals almost do not change, while signals of A/B compartments increase. Because A/B compartment intervals do not change, it's convenient to compare them before and after heat shock.

Compartment level

Dynamic A/B compartments



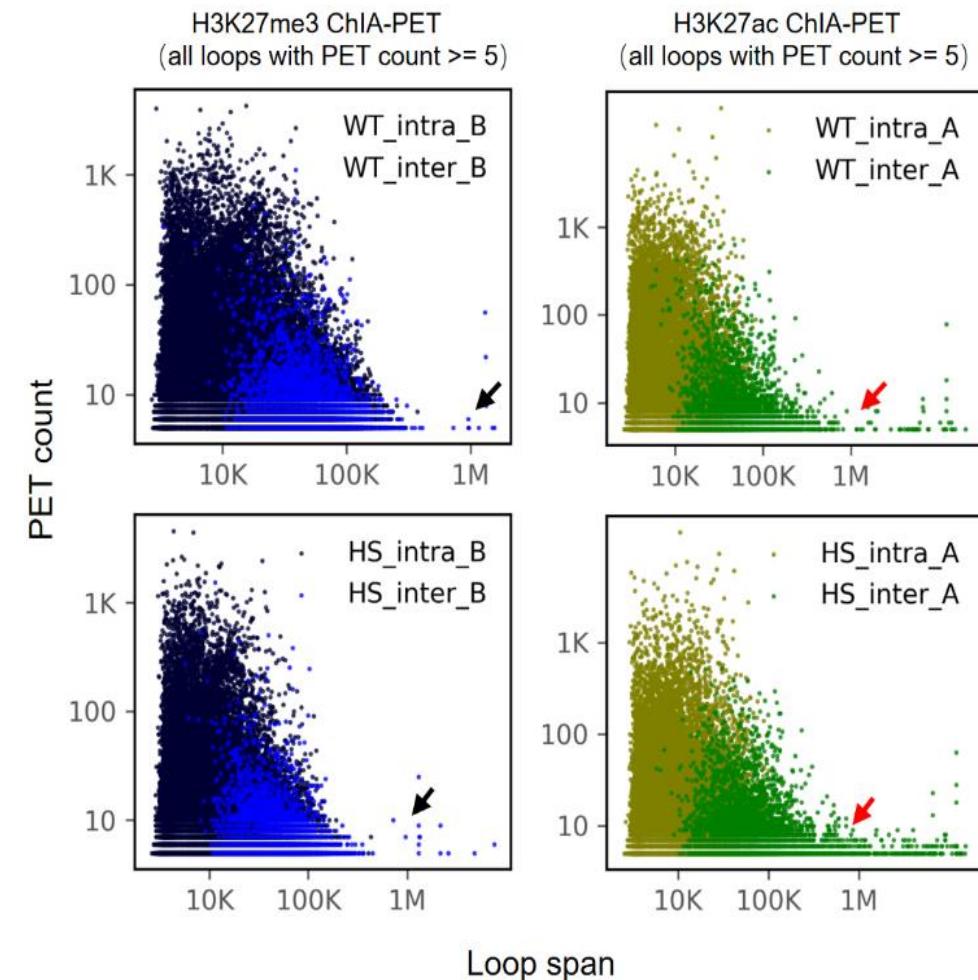
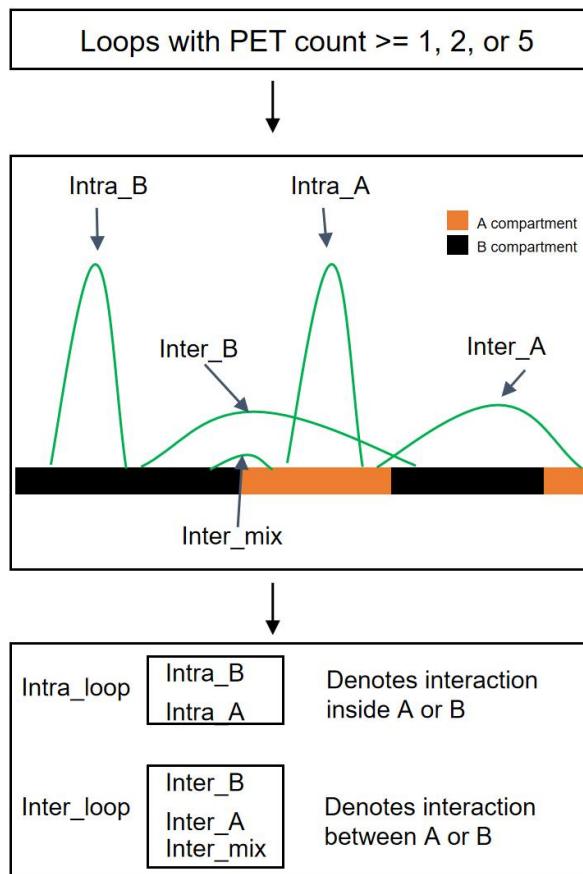
The black square is the TADs called using hic matrix.

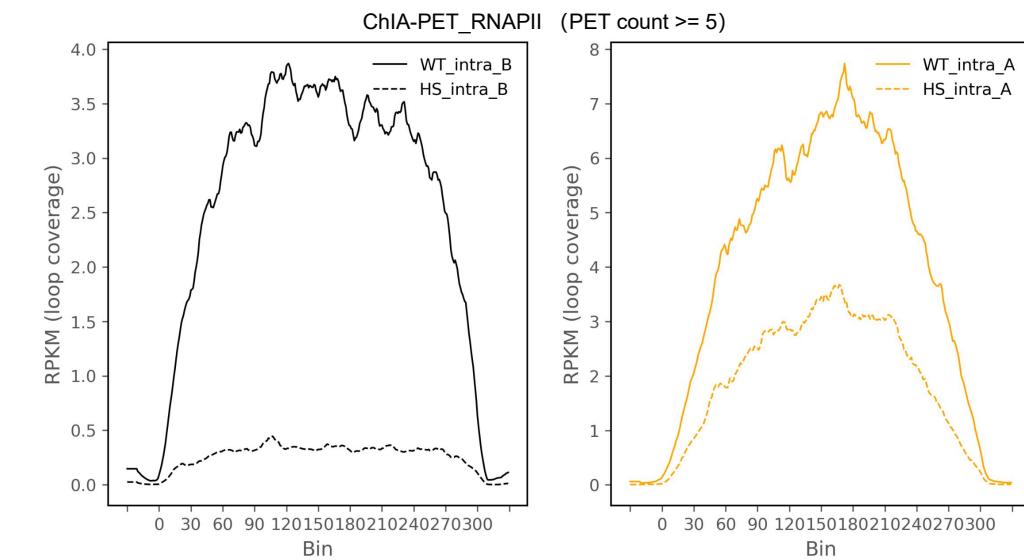
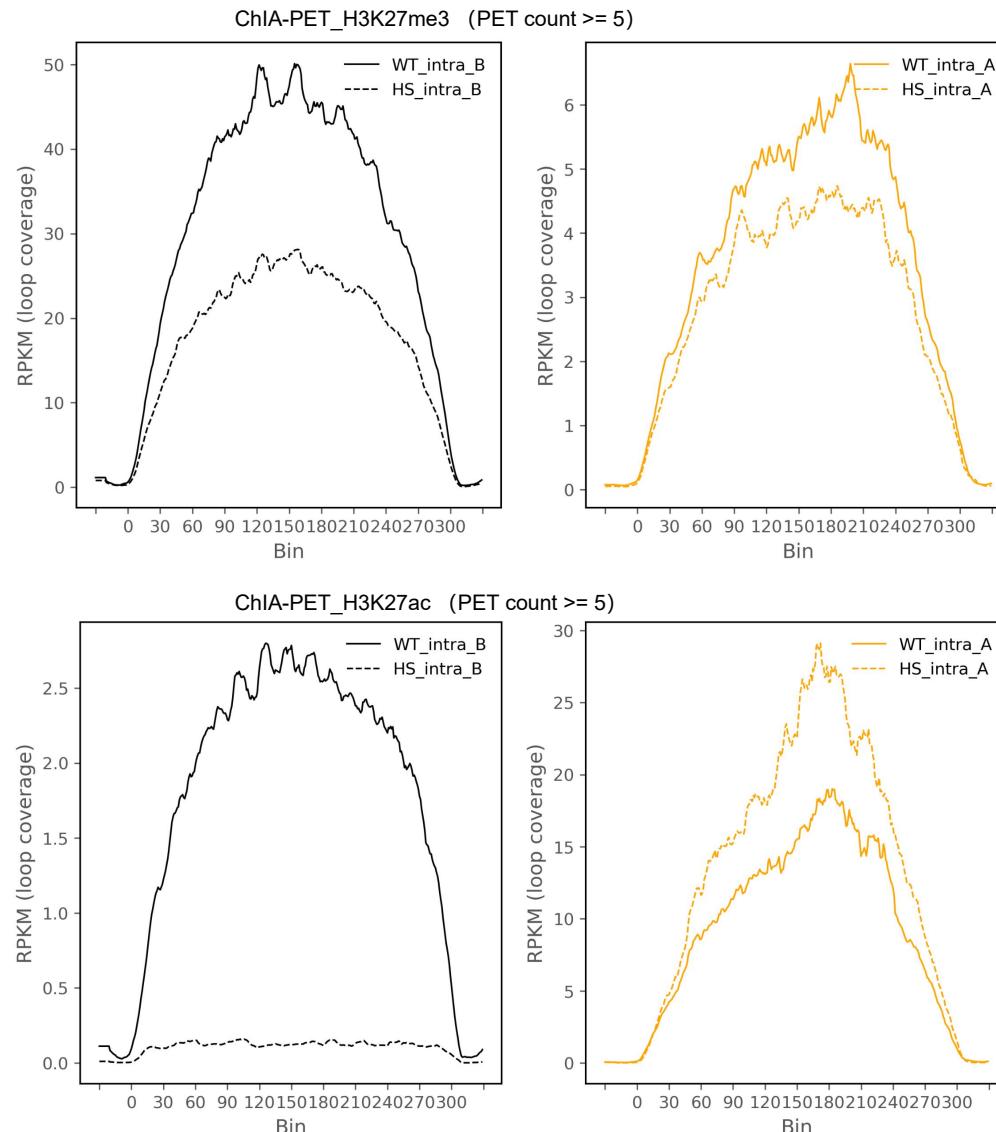


After heat shock, interaction inside A compartments increases, interaction inside B compartments decreases.

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

Goal: quantitatively describe it

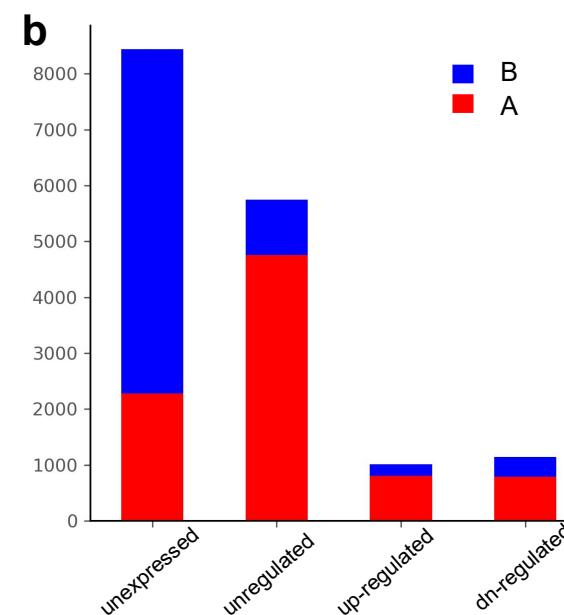
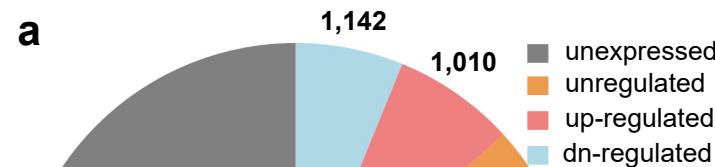




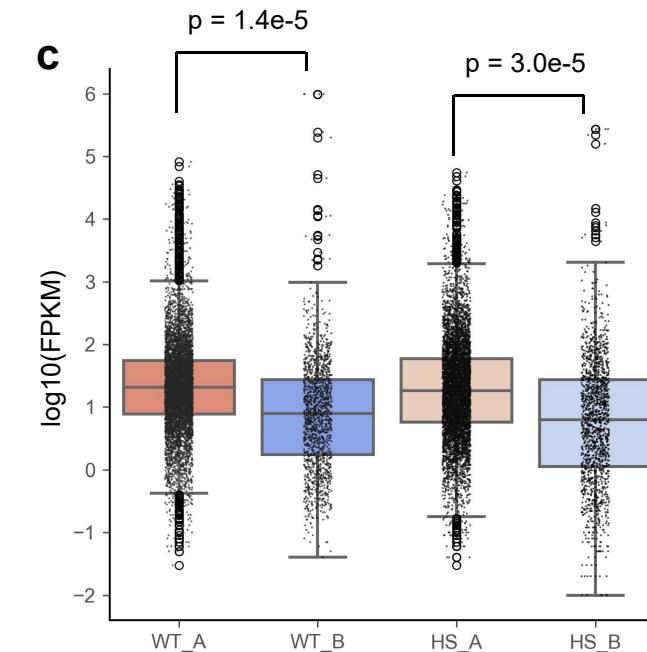
After heat shock, interaction strength mediated by RNAPII drops more in B compartments than A compartments.

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

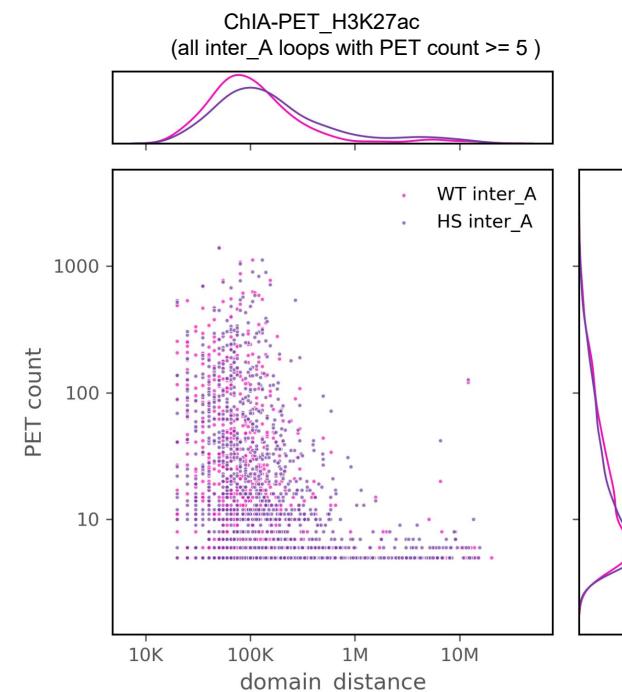
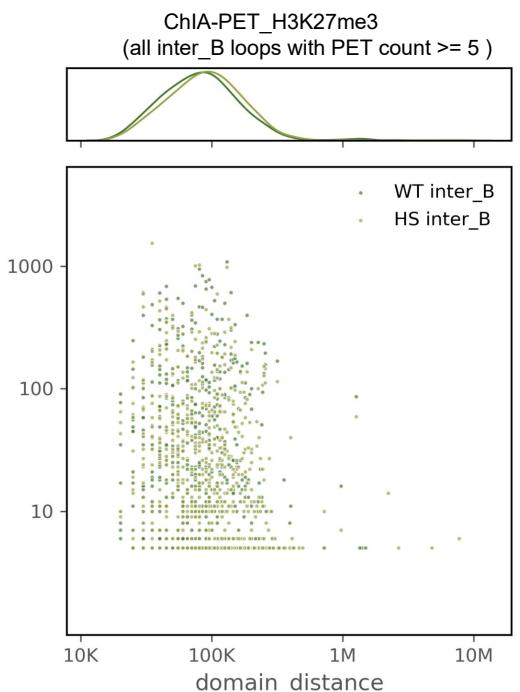
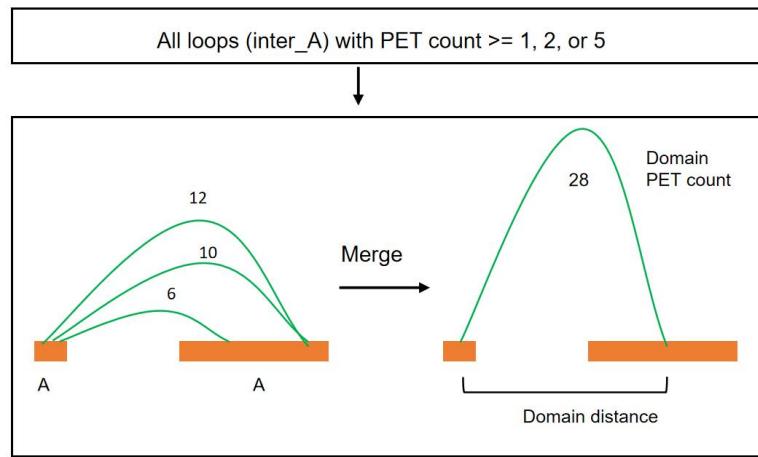
After heat shock, Pol II occupancy has an overall drop.
Teves et al., Genes & Development. 2011



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



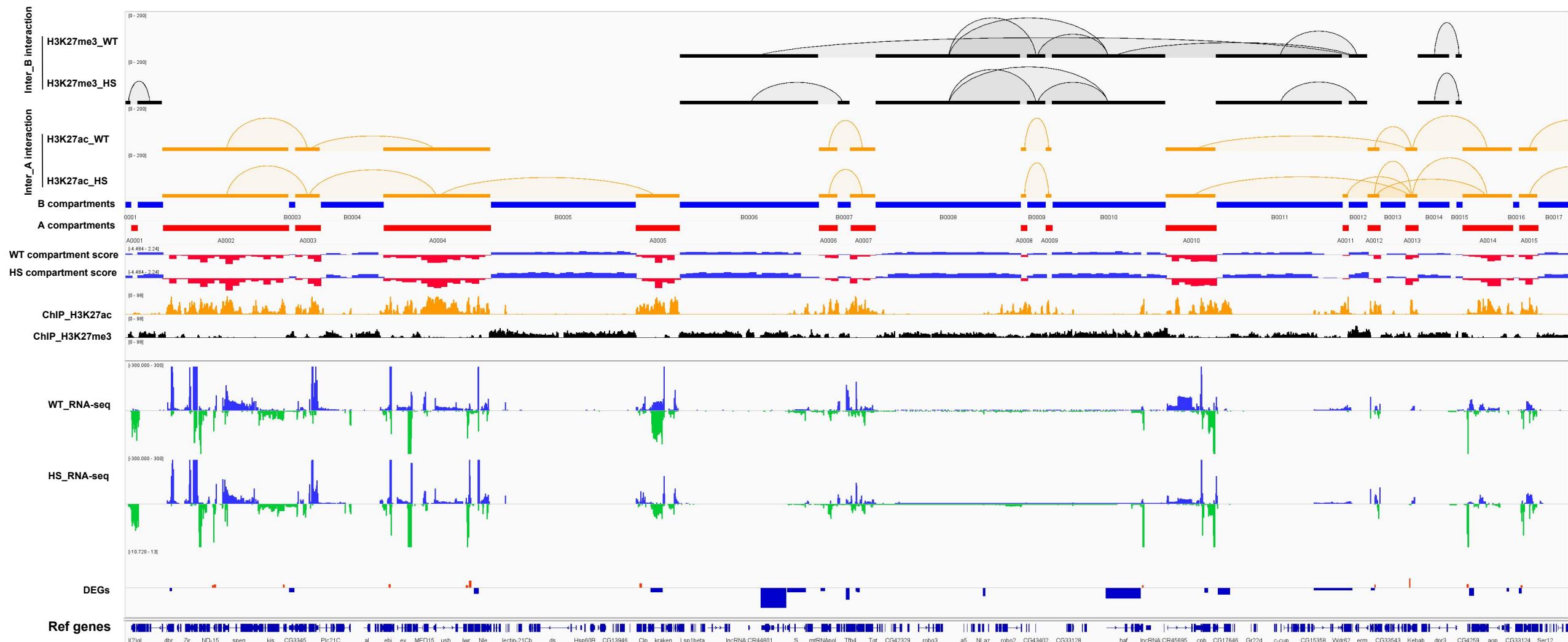
1. Genes located in A compartments have higher expression level than those located in B compartments
2. Down-regulated DEGs are more likely to locate in B compartments (chi-square test, $p = 5.12e-08$). Its consistent with the fact that interaction strength mediated by RNAPII drops more in B compartments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

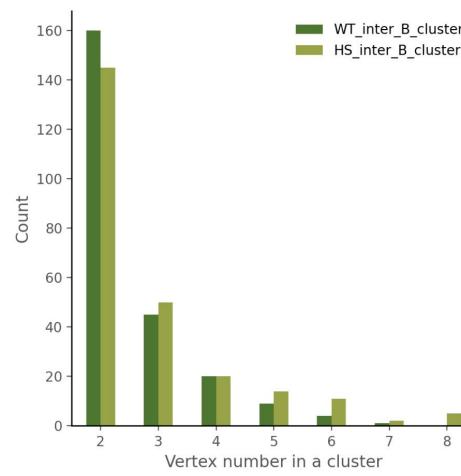
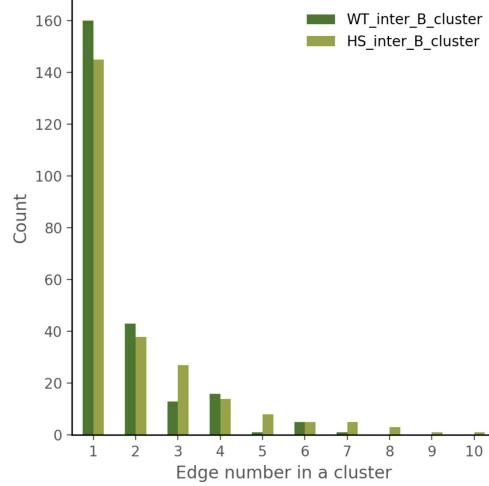
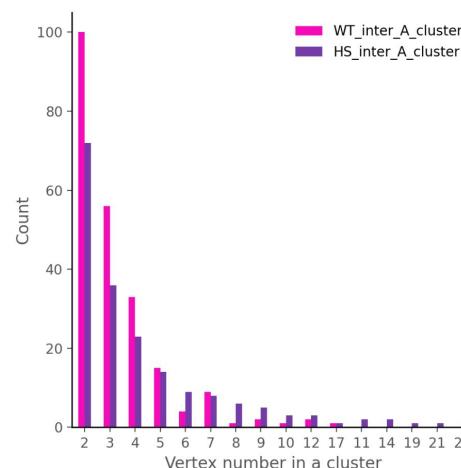
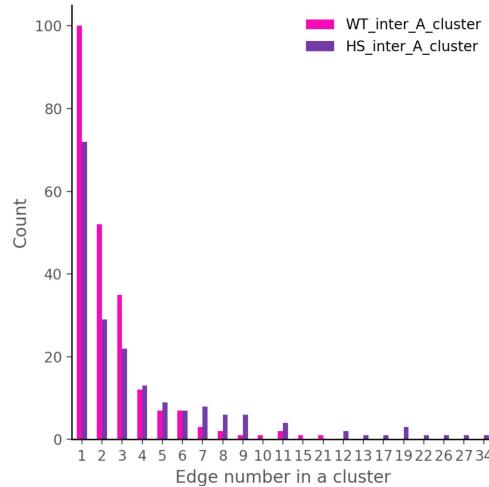


The data format is almost the same as that of ChIA-PET loops, except that the anchor is a compartment.

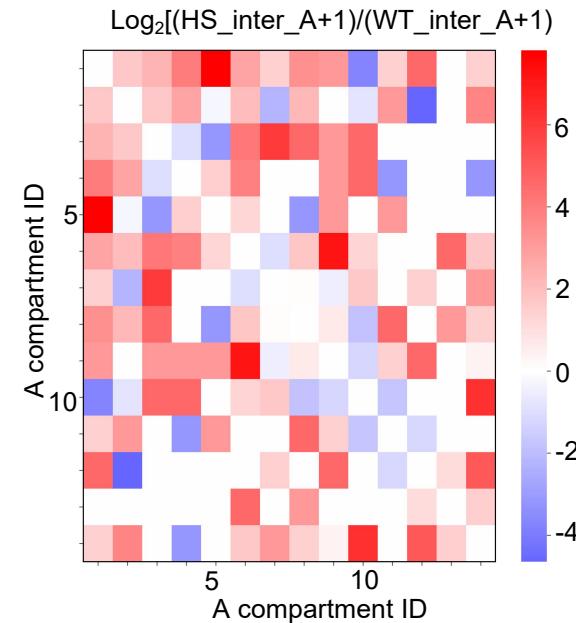
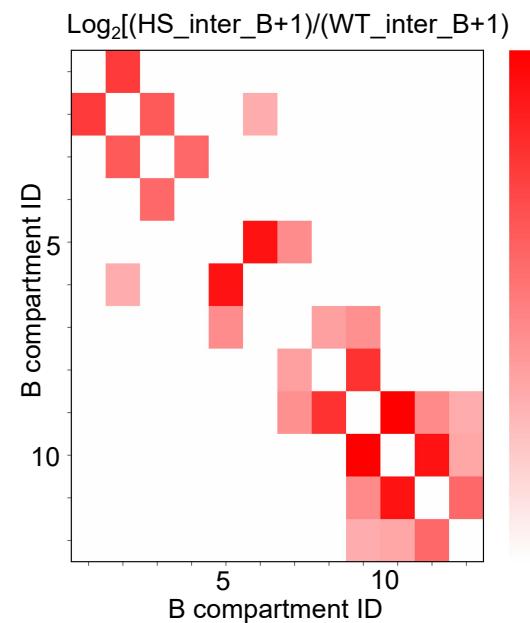
Compartment level

Dynamic compartment network and gene expression and regulation

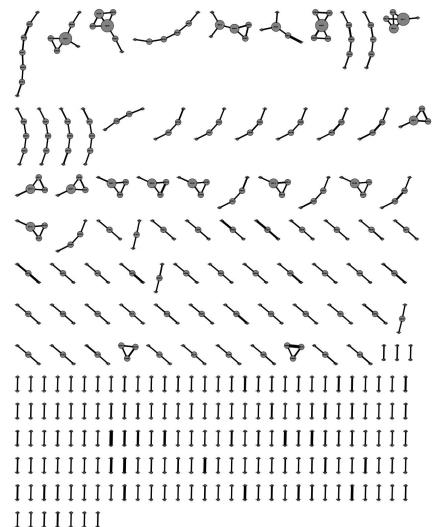


a**b**

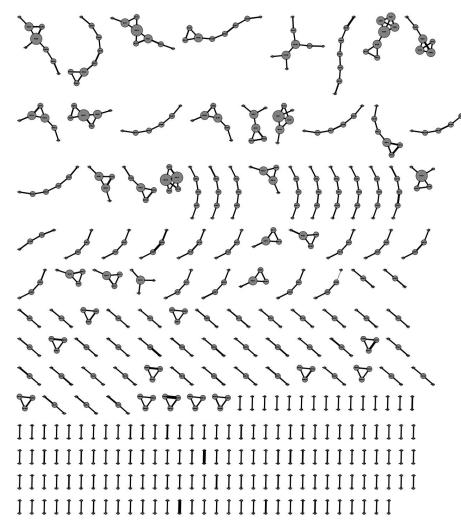
After heat shock, while adjacent B compartments have stronger interaction, A compartments in hubs become even closer or form new larger hubs. So, we can construct a network of A compartments before and after heat shock, and we can also explore the dynamic network and its relationship with gene expression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



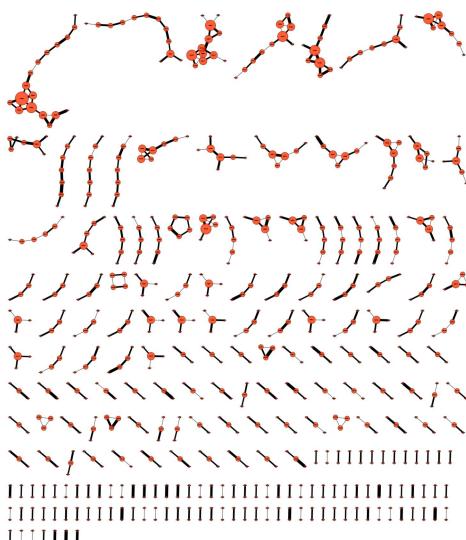
While inter_B interaction usually increases between two adjacent B compartments, inter_A interaction increases among multiple A compartments.



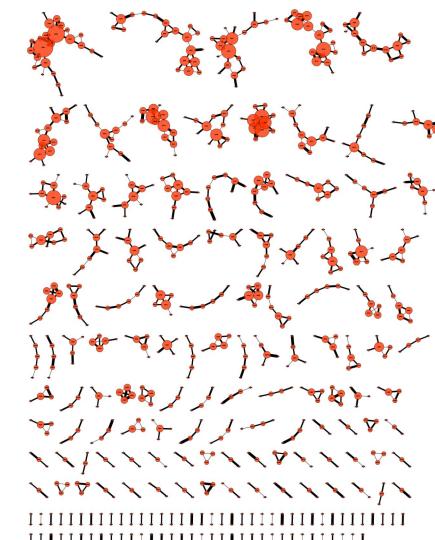
B compartments cluster captured by H3K27me3_WT



B compartments cluster captured by H3K27me3_HS



A compartments cluster captured by H3K27ac_WT



A compartments cluster captured by H3K27ac_HS

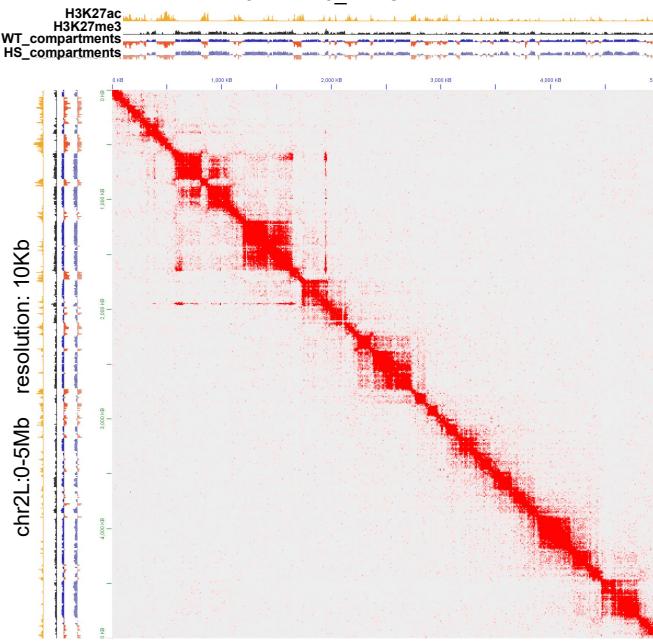
After heat shock, while adjacent B compartments have stronger interaction, A compartments in hubs become even closer or form new larger hubs. So, we can construct a network of A compartments before and after heat shock, and we can also explore the dynamic network and its relationship with gene expression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

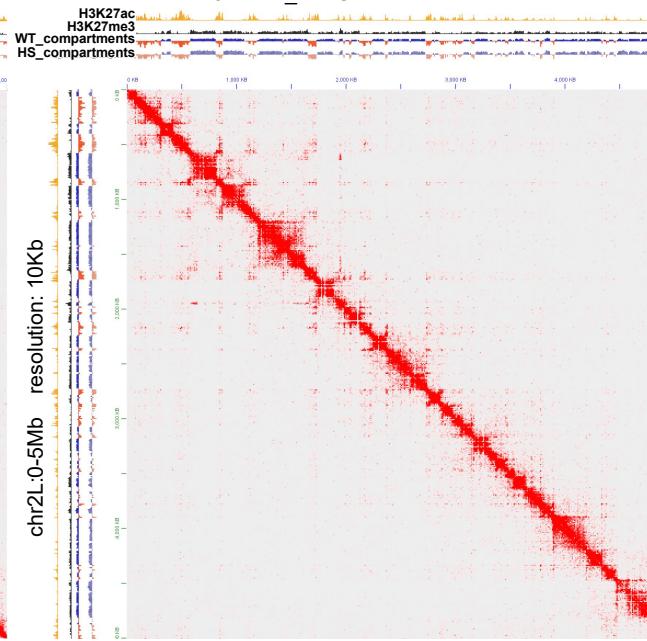
TAD level

Sum of H3K27me3_ChIA-PET and H3K27ac_ChIA-PET

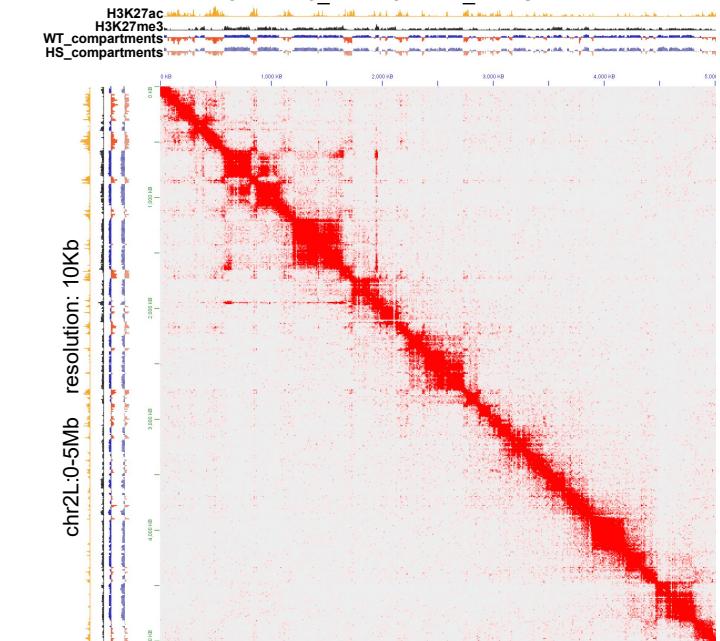
H3K27me3_WT ChIA-PET



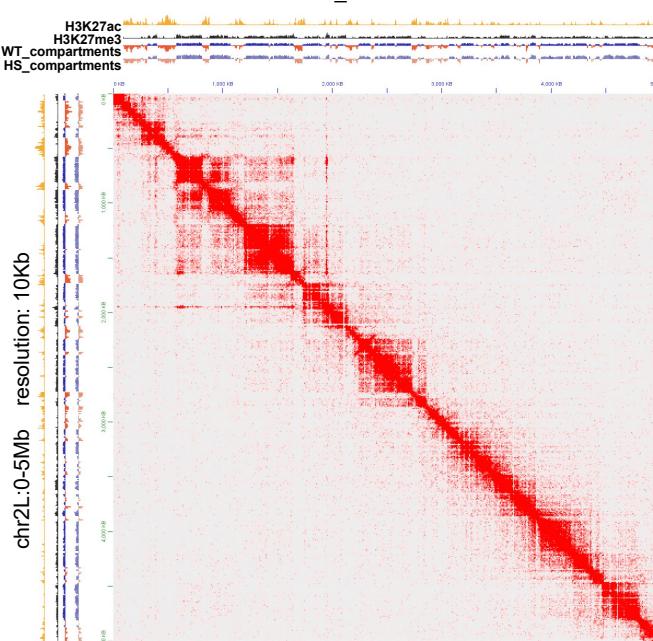
H3K27ac_WT ChIA-PET



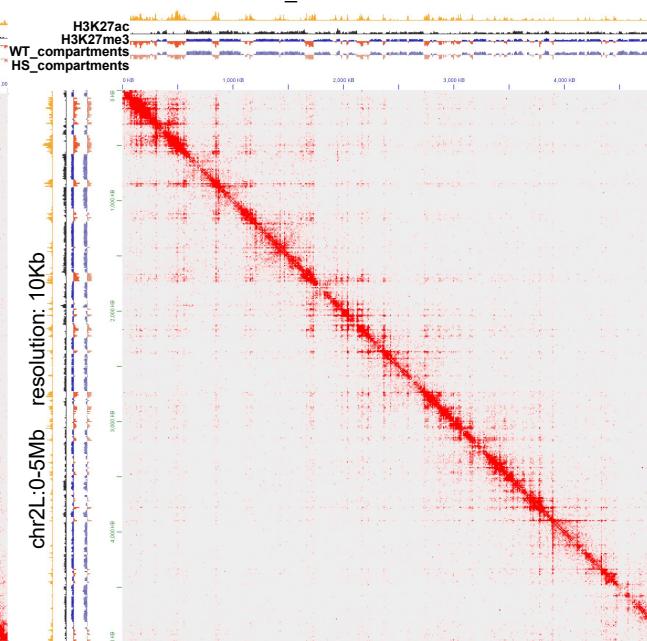
H3K27me3_WT+H3K27ac_WT ChIA-PET



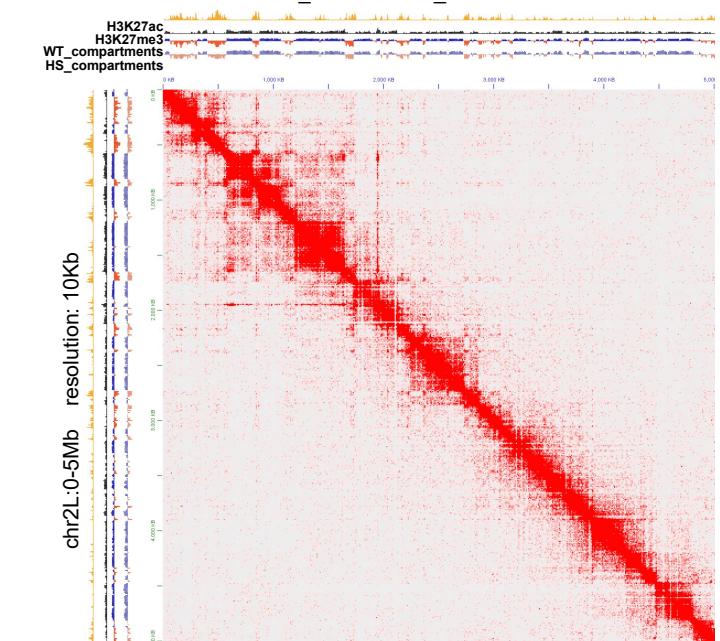
H3K27me3_HS ChIA-PET



H3K27ac_HS ChIA-PET



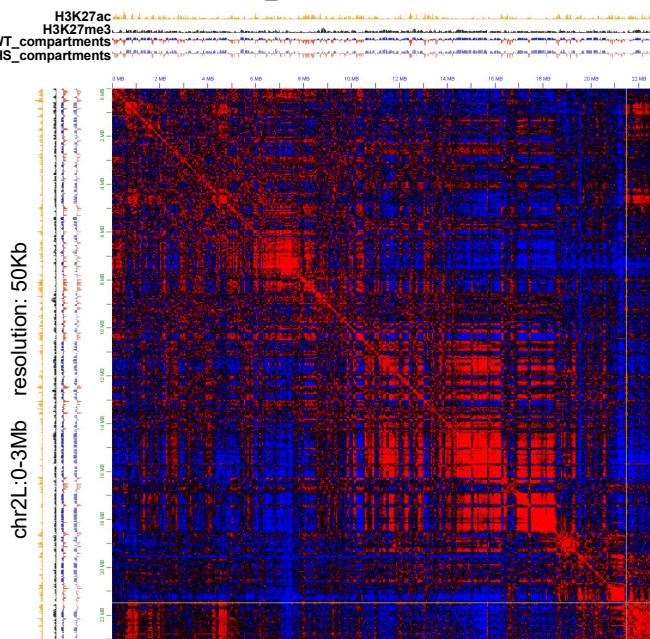
H3K27ac_HS/H3K27ac_HS ChIA-PET



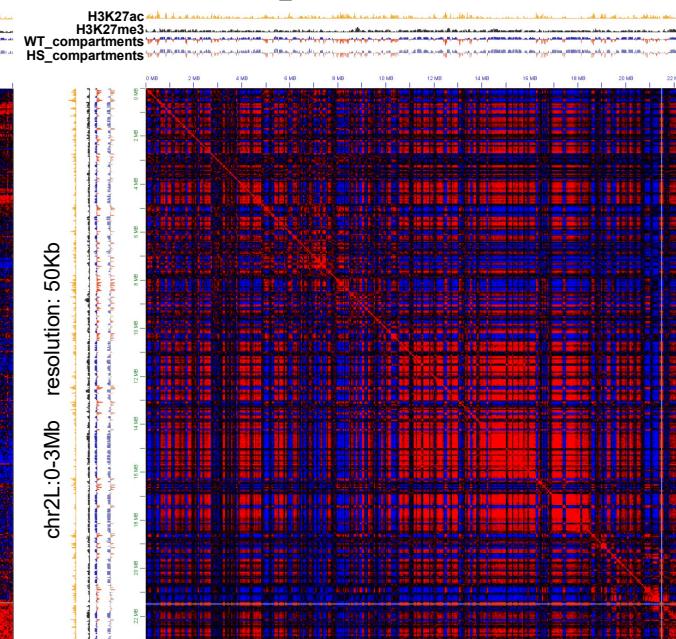
TAD level

Sum of H3K27me3_ChIA-PET and H3K27ac_ChIA-PET

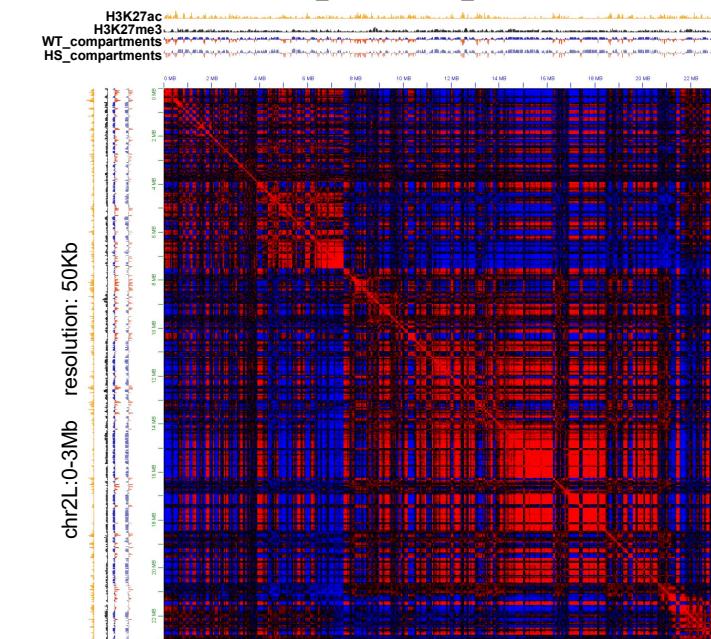
H3K27me3_WT ChIA-PET



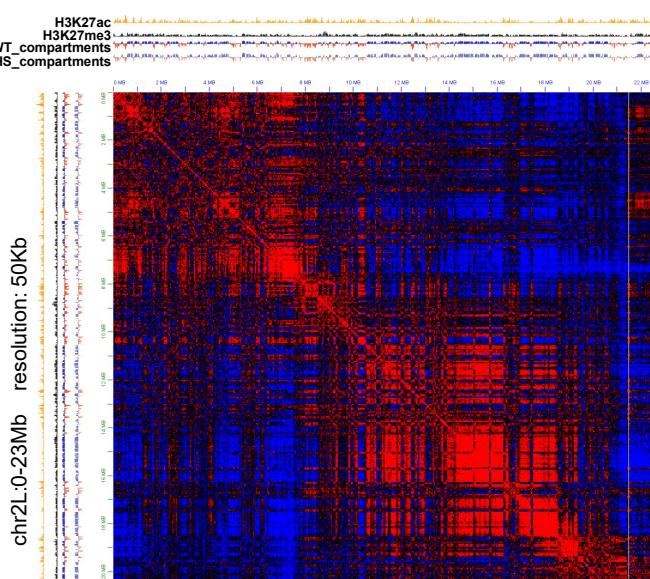
H3K27ac_WT ChIA-PET



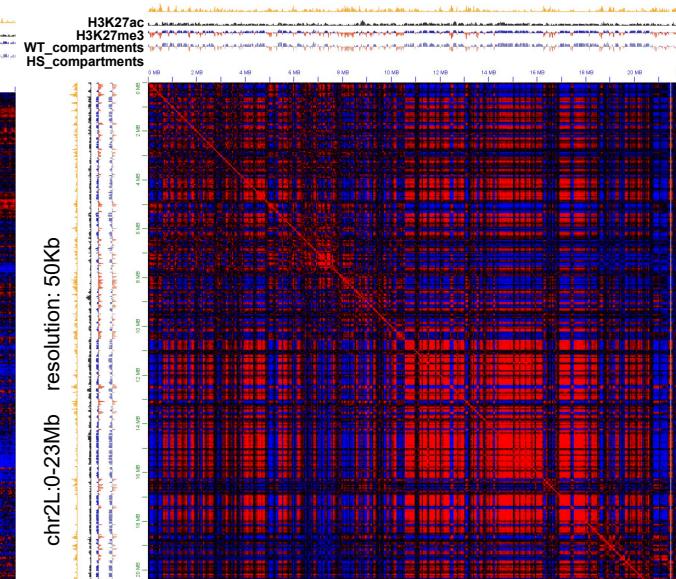
H3K27me3_WT+H3K27ac_WT ChIA-PET



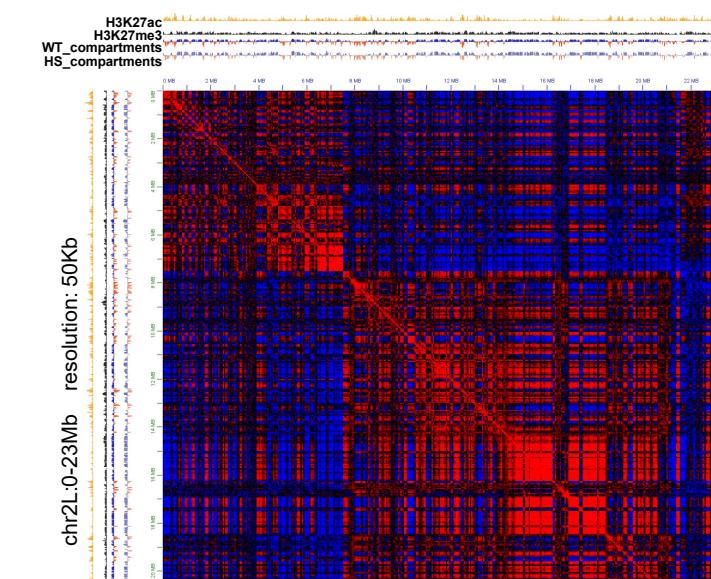
H3K27me3_HS ChIA-PET



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.

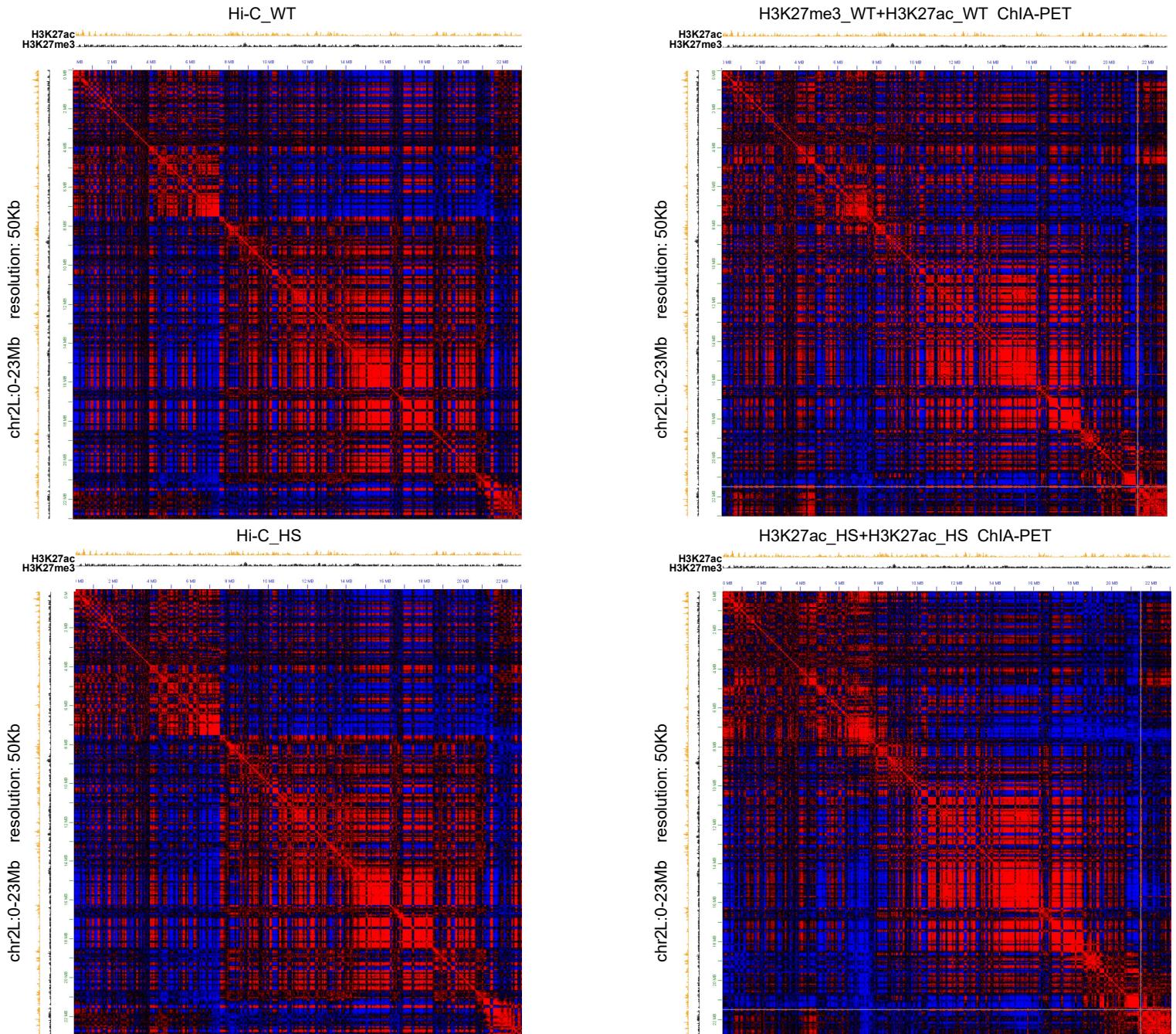
Nichols et al., Cell Reports. 2021

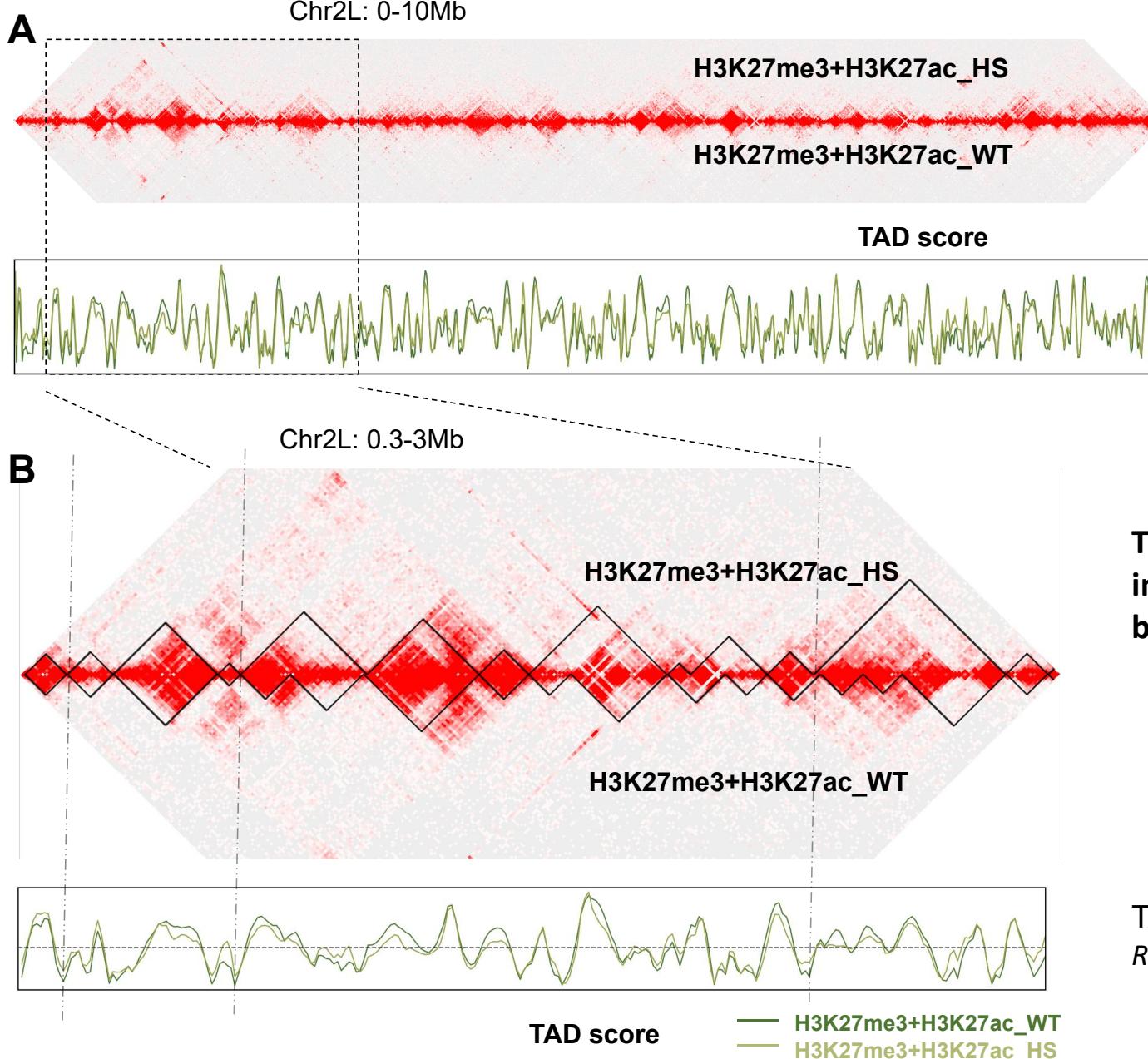
ChIA-PET is approximate to Hi-C plus the enrichment of some factor

(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.

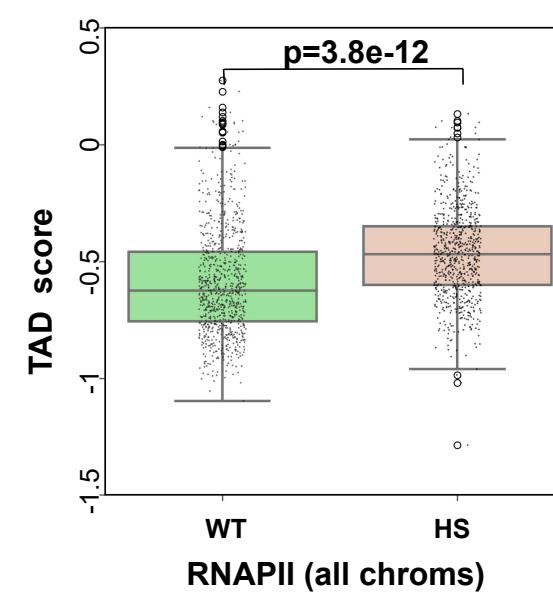
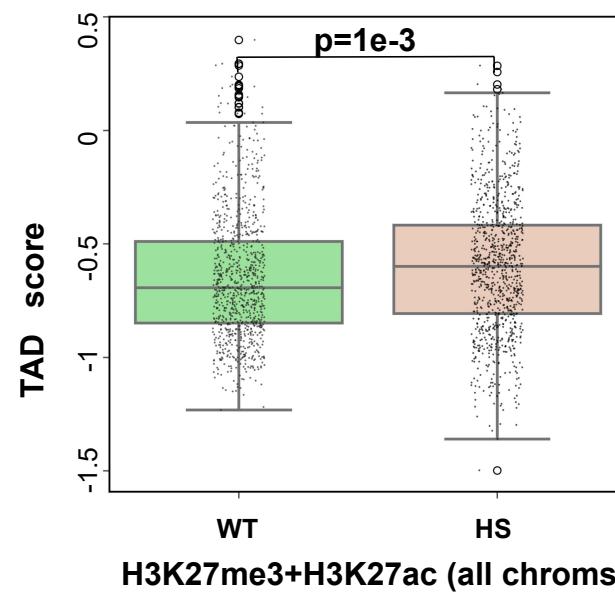
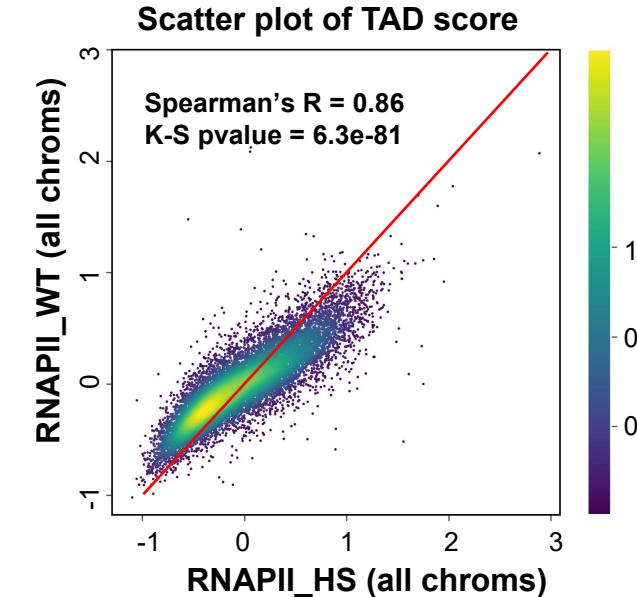
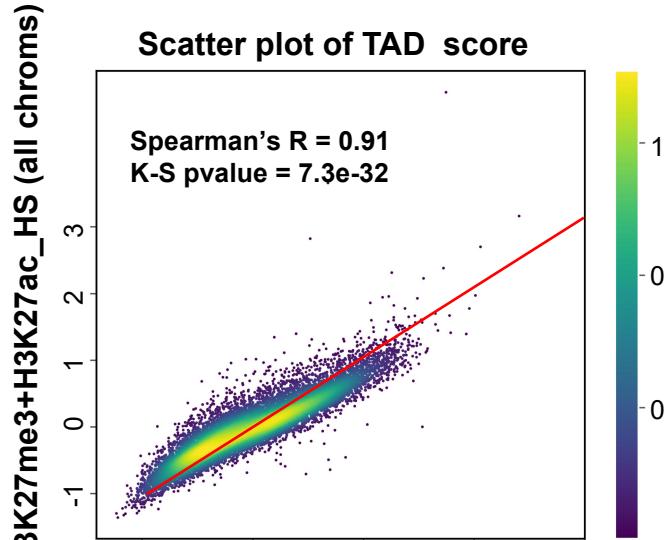
Hi-C data comes from *Ray et al., PNAS. 2019*



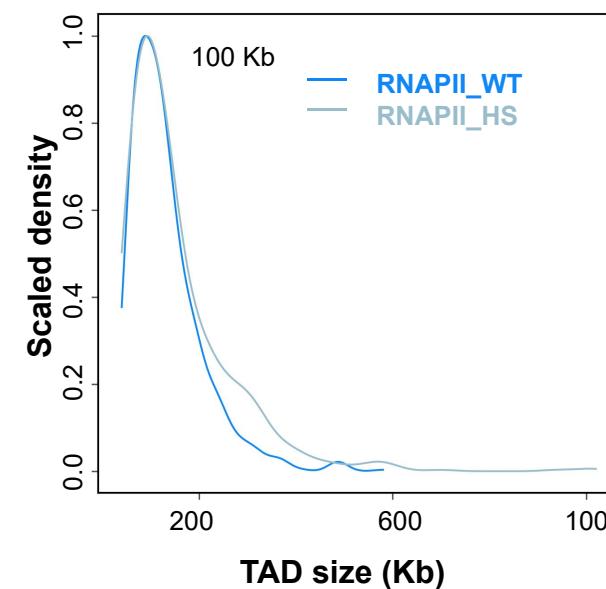
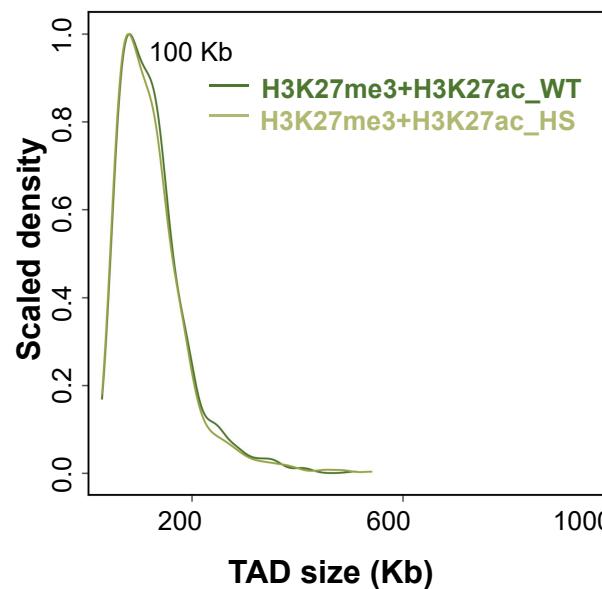
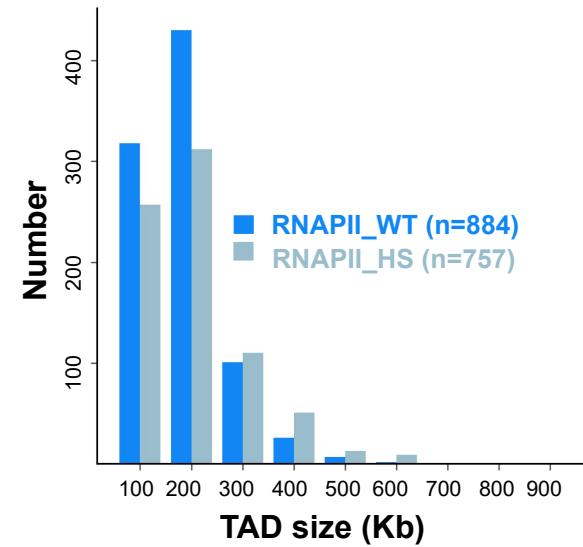
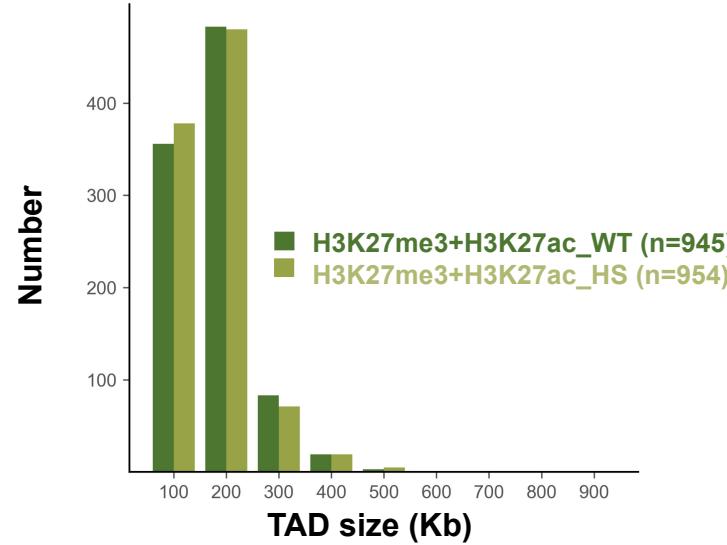


The local minimal of TAD score (TAD separation score) increases after heat shock, indicating that the TAD boundaries weaken after heat shock.

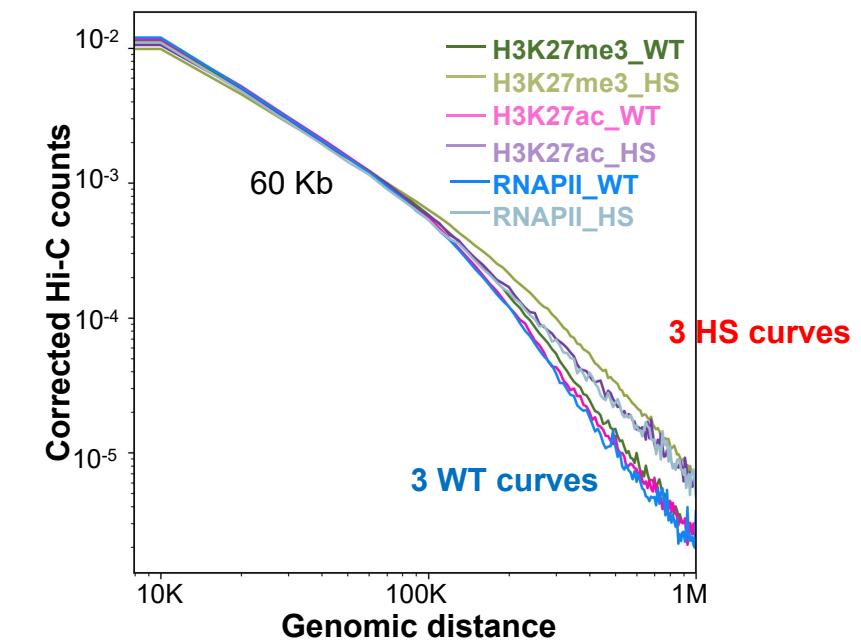
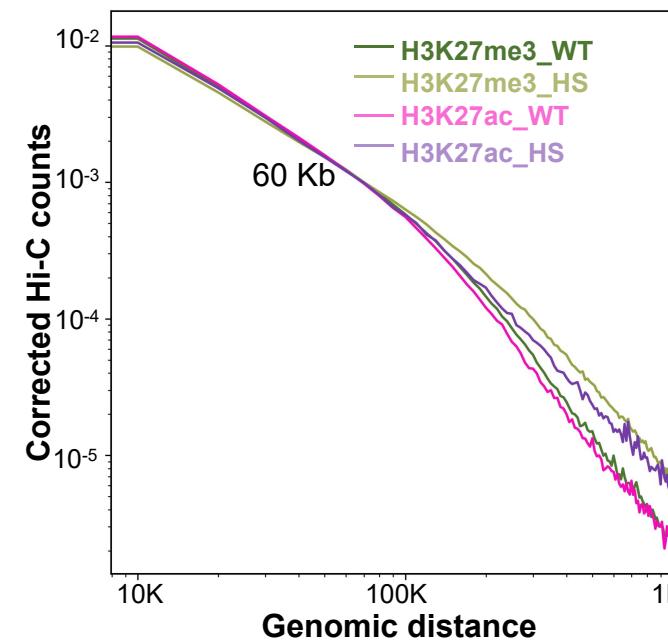
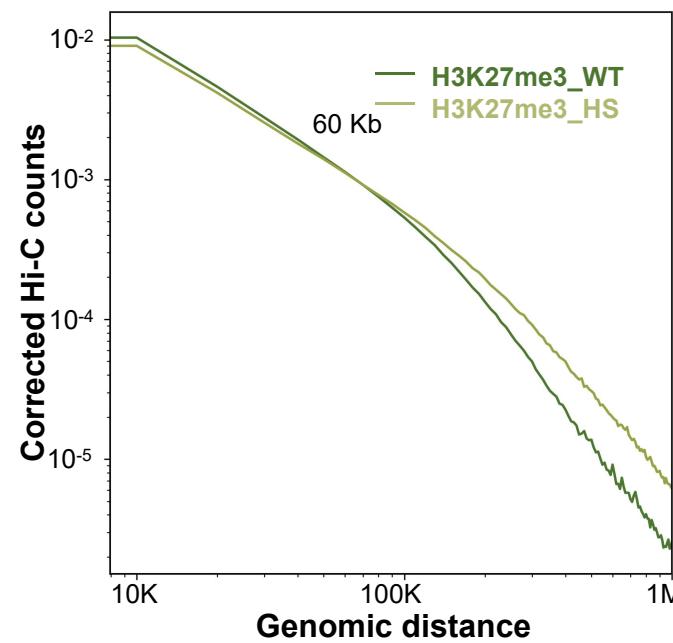
TADs at 10Kb resolution is called by hicexplorer
Ramirez et al., Nature Communications. 2018



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



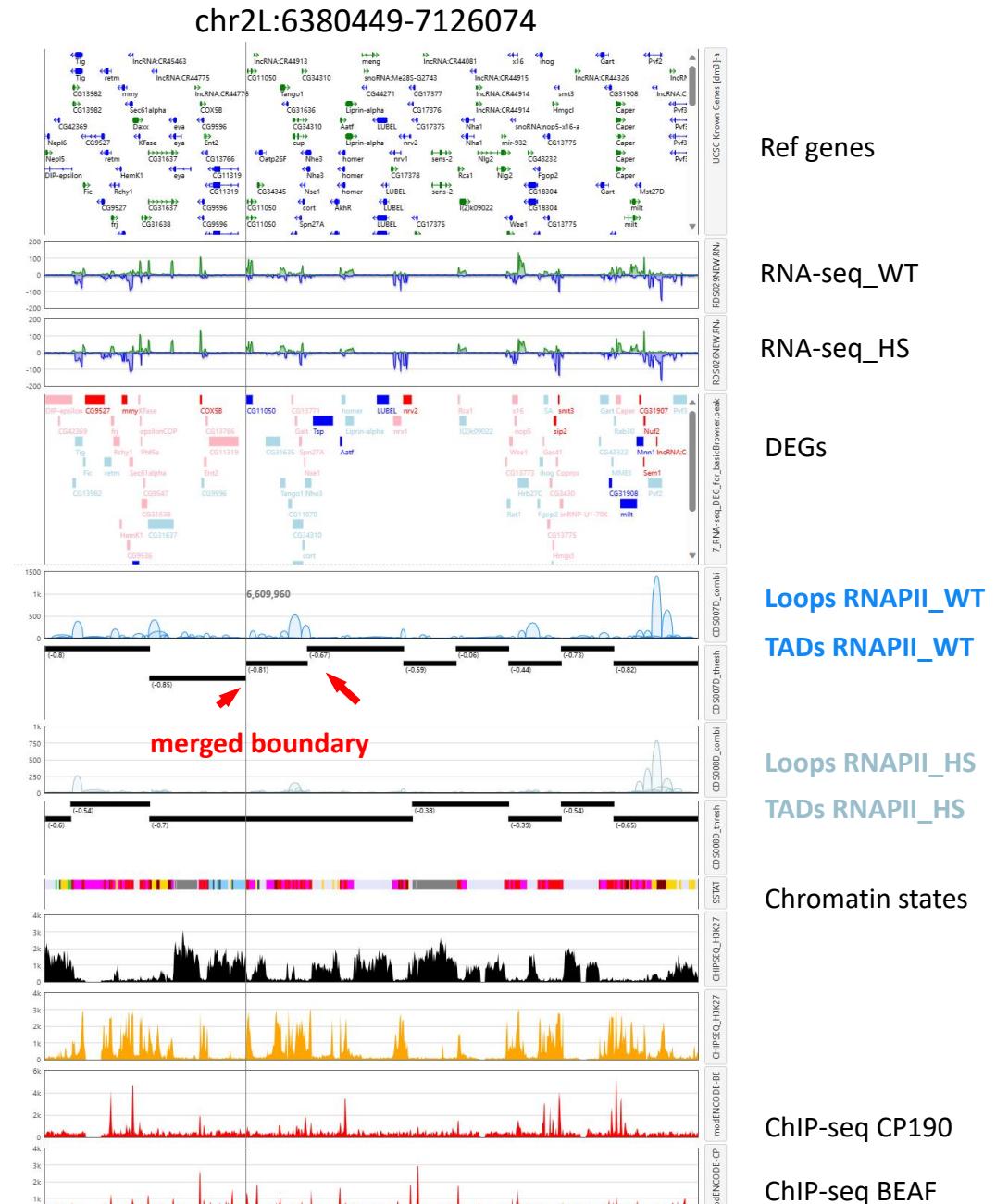
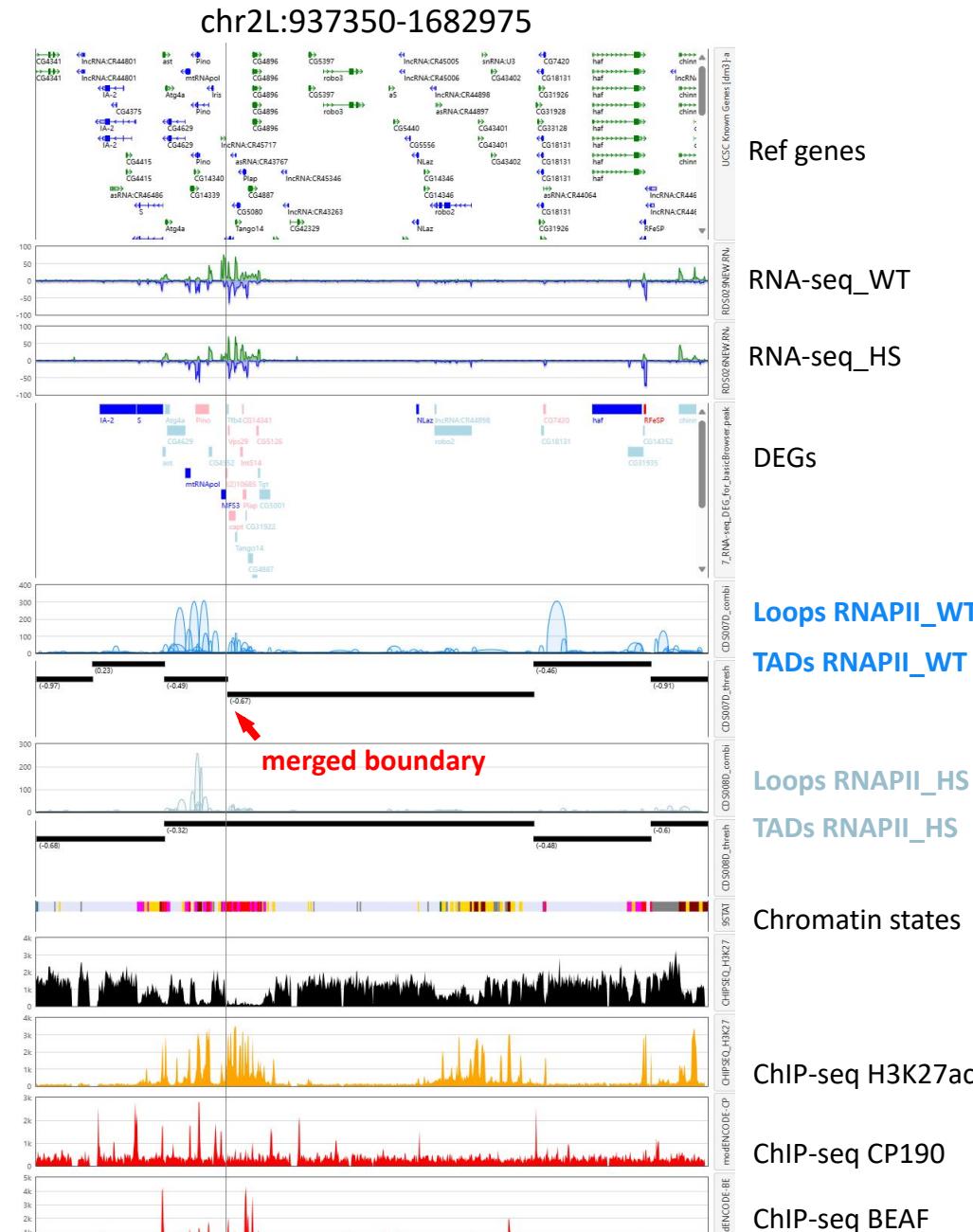
K-S test	H3K27me3_WT	H3K27me3_HS	H3K27ac_WT	H3K27ac_HS	RNAPII_WT	RNAPII_HS
H3K27me3_WT	1					
H3K27me3_HS	8.9e-06	1				
H3K27ac_WT	0.97	4.2e-07	1			
H3K27ac_HS	2.5e-04	0.59	3.6e-05	1		
RNAPII_WT	0.59	7.9e-08	0.91	9.3e-07	1	
RNAPII_HS	2.5e-04	0.59	1.8e-05	1	2.0e-06	1

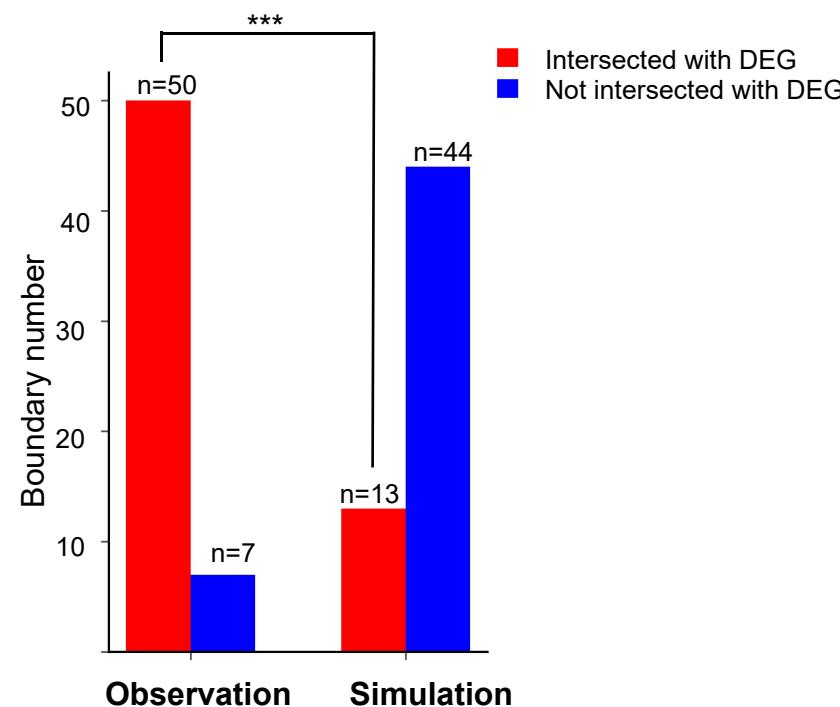
Genomic distance increases after heat shock;

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

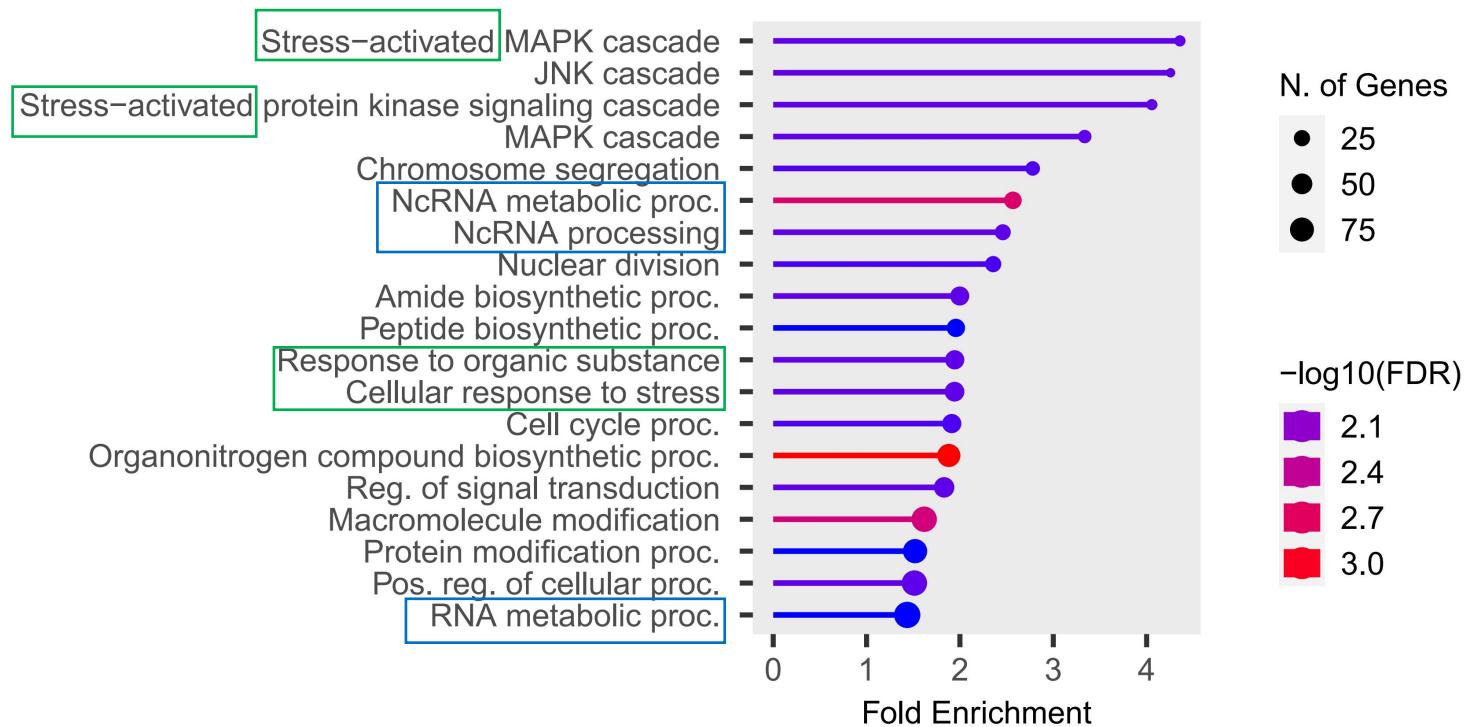
TAD level

Merged boundaries appeared in RNAP2_HS are highly correlated with DEGs



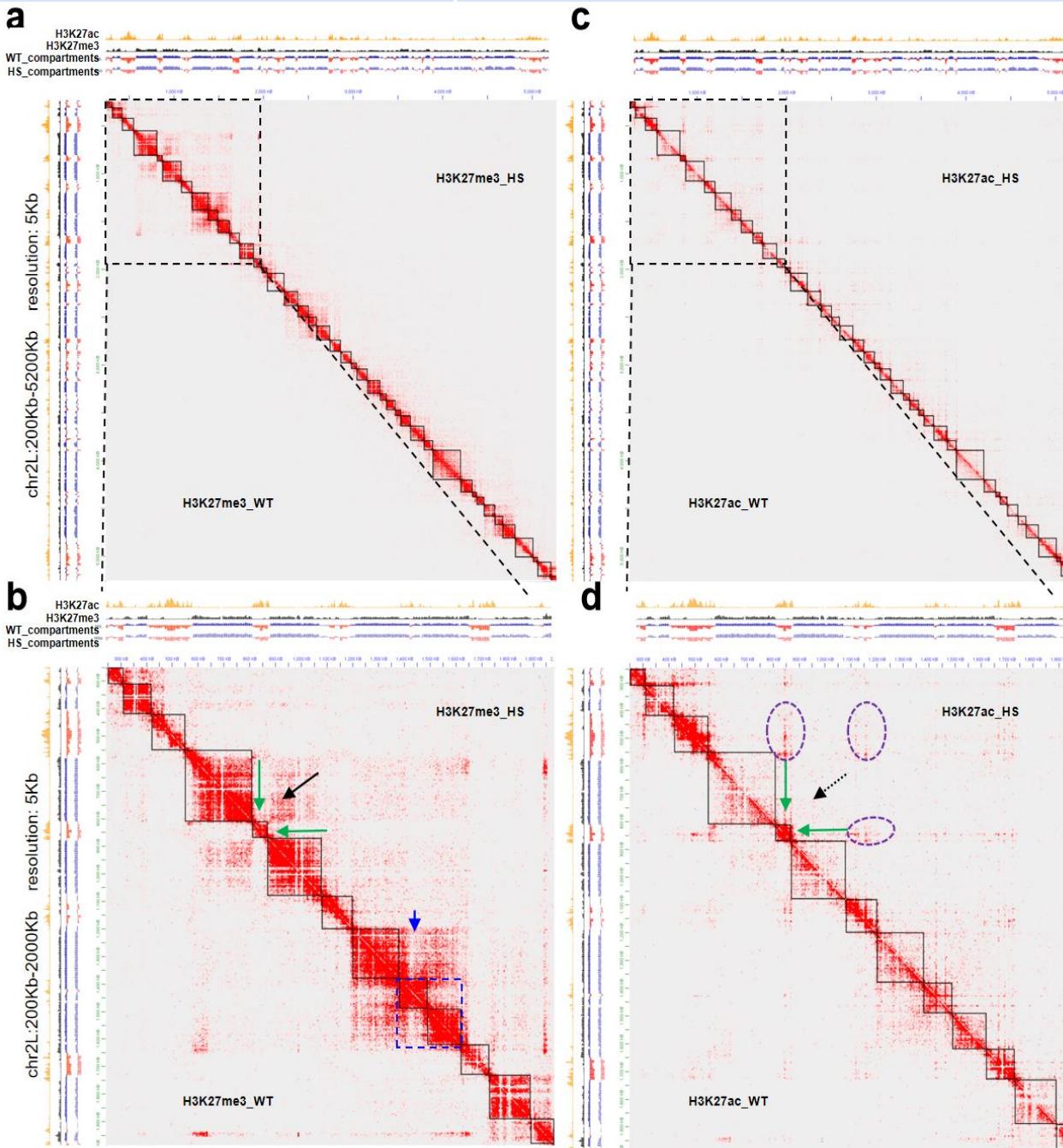


For chr2L, there are 57 merged boundaries: 50 intersected with DEGs, 7 are not, the observed $p=0.88$. With the DEGs' positions set, and randomly throwing the TAD boundary intervals, the expected probability of intersecting with a DEG, P is 0.23. We can treat it as a binomial test, and calculate the 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

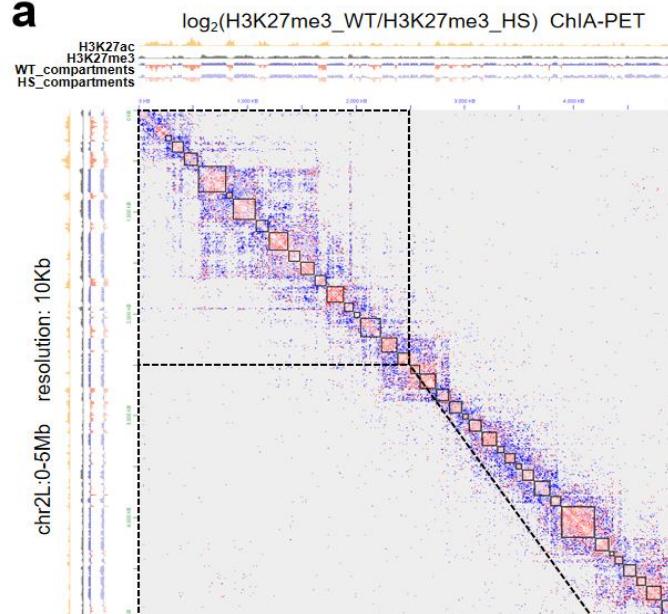
Compartments and TADs



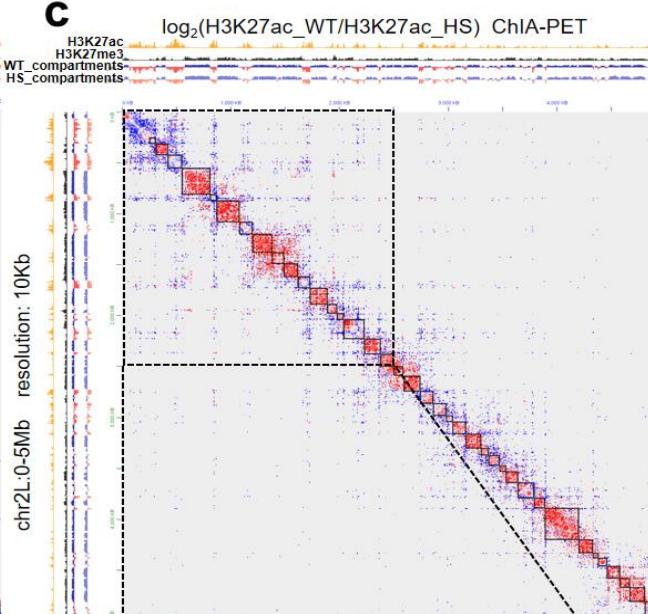
B compartments usually correspond to larger TADs, while A compartments usually correspond to smaller TADs. Still, there are some TADs consisting of B compartments and their adjacent A compartments. Or, a compartment consist of severral TADs.

Compartments and TADs

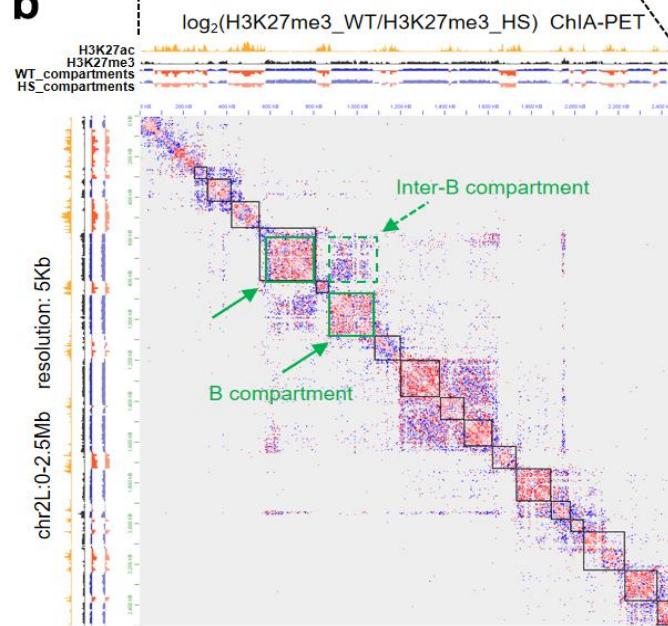
a



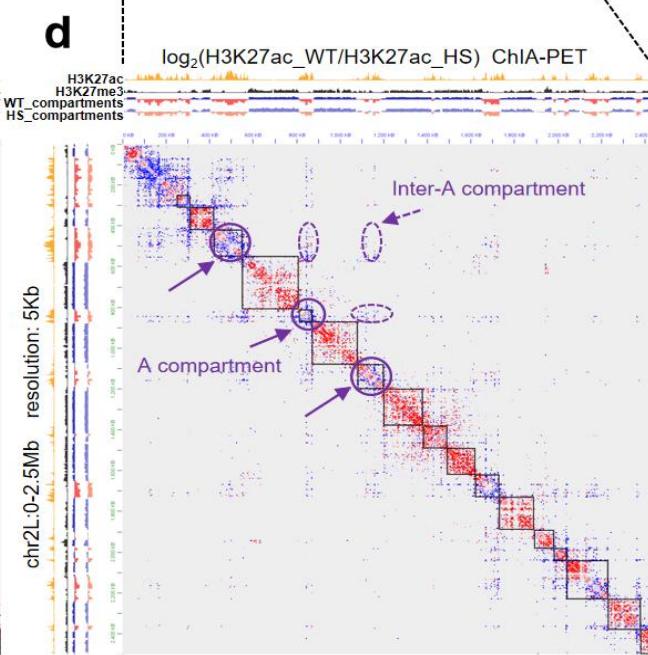
c



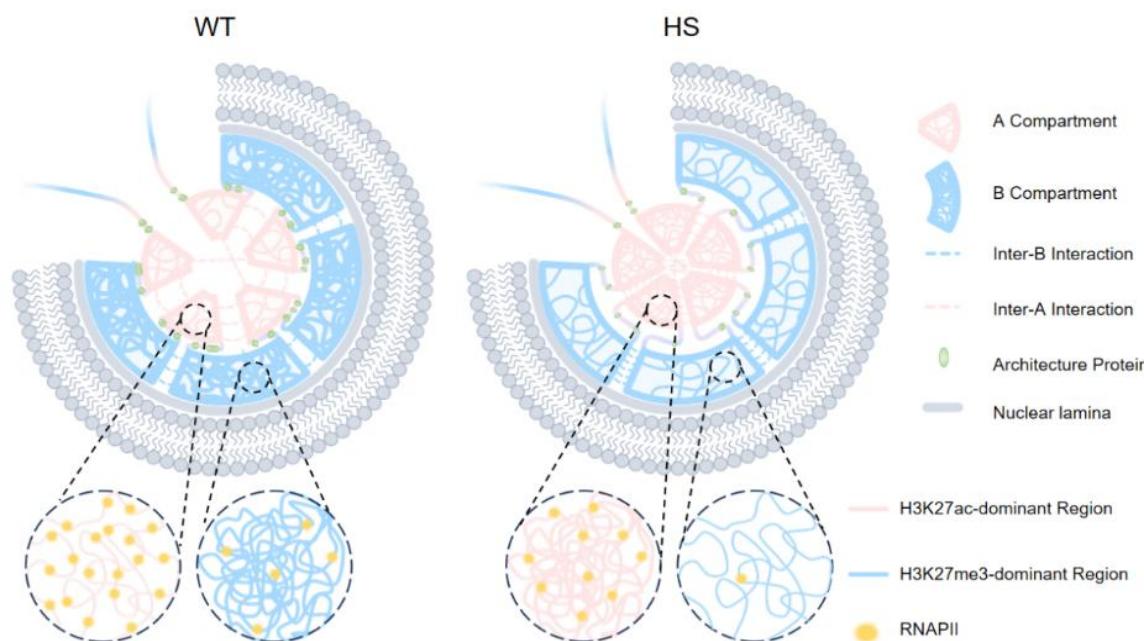
b



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*.

审稿人1

评阅结论：C

是否推荐优秀论文：否

对论文的综合评价：该研究利用 $in situ$ ChIA-PET方法，对果蝇S2细胞在热应激前后的染色质长程相互作用进行了捕获，并结合RNA-seq数据，阐明了染色质三维结构和基因调控的变化。分析结果表明，热休克处理后，H3K27ac和RNAPII介导的交互网络中低PET count的长loop比例明显增多，这表明H3K27ac主导区域之间的相互作用增强。另外，热休克处理后A/B区室的位置未发生变化，但信号增强。热休克处理前后，A区室内基因的表达水平均高于B区室。同时还发现，热休克处理后，A区室内部相互作用增强，而B区室内部相互作用减弱。RNAPII在A/B区室内介导的相互作用均减弱，但在B区室中减弱的程度更大，这与下调的DEGs更可能出现在B区室相符。此外，A区室间会进一步靠近，形成更大的簇，在接触矩阵热图上形成的条纹会明显。B区室间并不会形成更大的簇，但是邻近B区室间的相互作用会增强，在接触矩阵热图上形成的区块会更明显。研究提出了热休克引起果蝇基因组三维结构动态变化的初步模型。总体而言，这篇论文通过综合分析染色质相互作用、基因表达和RNA-seq数据，系统地研究了果蝇基因组在热应激下的三维结构变化及其与基因调控的关系。研究方法可行，结果较详实。这些发现为进一步了解遗传调控机制提供了重要线索，对该领域的研究具有一定的推动作用。然而，在撰写论文过程中，我希望作者能对实验设计、数据分析和结果解释等方面进行更详细的描述和解释，同时对图表进行修订和优化，以提高论文的可读性和科学性。

学位论文存在的不足或主要问题：论文基于实验室其他人产生的数据和数据库下载的数据进行了相关生物信息学的分析，论文需要仔细修改完善，主要问题如下：1. 摘要需要重新写，因为目前的内容太少，没有准确总结研究的重要结论。您需要明确指出研究的关键结论和相关的基因表达调控模式，并提及相应的模型。比如“这些变化与基因表达相关”，与什么基因表达相关？什么基因表达调控模式？相应的模型是什么？这些关键结论在摘要里都没有体现。2. 关键词太多，请将其浓缩为约5个左右。3. 论文中的图片大小偏小，质量和展示效果也很差。请检查图片的清晰度和大小，可能需要调整。特别是请确保图片中的字体大小合适。4. 所有基因名称应使用斜体字体，在全文中检查并修改。5. 将“本课题的研究目的和意义”改为“研究目的与意义”。6. 建议将“2.1 课题总体设计”部分放到“1.5 研究技术路线”，并重新绘制技术路线图。7. 在“2.4 节 RNA-seq数据处理”中，提到了使用了dm3作为参考基因组。请添加参考文献或下载地址。8. 第三章的“结果与分析”部分存在问题，对结果的分析不够详细，很多小节内容描述过于简洁，建议进行补充。9. 部分小节标题过长，比如“3.2.2”。请考虑简化标题，使其更加简洁明了。10. 论文中存在过多口语化的描述，例如在讨论部分使用了“我们专门分析”、“我们发现”、“我们想把”、“我们试了”、“我们意识到”等表达方式。请全文修改，使写作更加正式和客观。11. 论文中未体现实验工作量，包括前期使用了他人实验产生的数据，后续也未进行验证实验。请在论文中充分体现实验工作的重要性和具体内容。以上建议旨在提供建设性的意见来改善您的论文，希望作者针对以上建议逐条修改完善。

感谢审稿人1如此具体的建议，下面是对审稿意见的逐一回复。

1. 摘要需要重新写，因为目前的内容太少，没有准确总结研究的重要结论。您需要明确指出研究的关键结论和相关的基因表达调控模式，并提及相应的模型。比如“这些变化与基因表达相关”，与什么基因表达相关？什么基因表达调控模式？相应的模型是什么？这些关键结论在摘要里都没有体现。

答复：已经重新书写摘要并具体描述了相关基因及模型。基因表达部分增加了“由RNAPII介导的相互作用在B区域内的下降幅度比在A区域内的要大，这与下调基因更有可能出现在B区域的发现相一致”，以及“合并的TAD边界与涉及RNA代谢和细胞对压力反应的差异表达基因相关联”。相应的模型表述为“并提出了热应激下果蝇基因组三维结构在A/B区室层面变化的模型”，模型的更具体内容对应于摘要的第三段的部分结论

“热休克后，A区域内部的相互作用强度增加，而B区域内部的相互作用强度减少。并且，由RNAPII介导的相互作用在B区域内的下降幅度比在A区域内的要大，这与下调基因更有可能出现在B区域的发现相一致。此外，聚集的A区室会进一步靠近或者形成更大的簇，而相邻B区室之间的相互作用会增强。”

2. 关键词太多，请将其浓缩为约5个左右。

答复：关键词已经缩减为5个。由原来的“热应激；果蝇；*in situ* ChIA-PET；H3K27me3；H3K27ac；RNAPII；三维结构；A/B区室；拓扑相关结构域；基因表达”缩减为现在的“热应激；果蝇；*in situ* ChIA-PET；三维结构；基因调控”。

3. 论文中的图片大小偏小，质量和展示效果也很差。请检查图片的清晰度和大小，可能需要调整。特别是请确保图片中的字体大小合适。

答复：P27，已经将清晰度差的图片替换为清晰度高的图片，字体大小也调整到与正文接近（原图3-1，图3-2，图3-3，图3-5，图3-6，图3-7，图3-8，图3-9，图3-11，图3-12，图3-15）；展示不好的图片也重新排版（原图3-1拆分为两张图）。

4. 所有基因名称应使用斜体字体，在全文中检查并修改。

答复：P28，基因均改为斜体，并且全文检查修改。

5. 将“本课题的研究目的和意义”改为“研究目的与意义”。

答复：P15，已修改为“研究目的与意义”。

6. 建议将“2.1 课题总体设计”部分放到“1.5 研究技术路线”，并重新绘制技术路线图。

答复：P17，已经将“2.1 课题总体设计”部分放到“1.5 研究技术路线”，并重新绘制技术路线图，技术图做了局部的调整。

7. 在“2.4 节 RNA-seq数据处理”中，提到了使用了dm3作为参考基因组。请添加参考文献或下载地址。

答复：P21，已经添加dm3参考基因组及其基因注释的下载地址。

8. 第三章的“结果与分析”部分存在问题，对结果的分析不够详细，很多小节内容描述过于简洁，建议进行补充。

答复：P28，已经对部分小节重新描述，并在方法中增加了具体的操作细节，同时在图注中也补充了一定的内容。

9. 部分小节标题过长，比如“3.2.2”。请考虑简化标题，使其更加简洁明了。

答复：P29，已经简化3.2.1和3.2.2。将3.2.1的“热休克处理后H3K27ac和RNAPII介导的交互网络中低PET count的长loop的比例明显增多”修改为“热休克诱导低频长程染色质环的产生”，将3.2.2的“热休克处理后H3K27ac和RNAPII中增加的低PET count的长loop对应着增加的H3K27ac主导区域之间的相互作用”修改为“热休克增加H3K27ac主导区域之间的相互作用”。

10. 文论中存在过多口语化的描述，例如在讨论部分使用了“我们专门分析”、“我们发现”、“我们想把”、“我们试了”、“我们意识到”等表达方式。请全文修改，使写作更加正式和客观。

答复：P54，已经全文修改，去除类似口语化和含有主观色彩的词汇。

11. 论文中未体现实验工作量，包括前期使用了他人实验产生的数据，后续也未进行验证实验。请在论文中充分体现实验工作的重要性和具体内容。

答复：P18，已经在附表B-1添加了对应的建库人员，同时在材料和方法中添加了“2.2实验方法”用于具体描述实验。目前本项目并没有进行实验证，我们也正在考虑如何进行合理的验证。

审稿人2

评阅结论：B

是否推荐优秀论文：是

对论文的综合评价：本研究利用 $in situ$ ChIA-PET技术，探究了热休克条件下果蝇S2细胞染色质的三维结构变化。重点关注了组蛋白修饰H3K27ac、H3K27me3以及RNA聚合酶RNAPII的调控区段的变化，以及与基因转录相关的变化。通过交叉比较以上数据，阐明了热休克如何影响染色质的三维结构动态变化，从而调控基因表达。论文的数据量大，分析手段多样，说明了作者对研究课题的深入理解和全面把握。整体而言，这篇毕业论文展现了高水平的学术研究水平，为果蝇S2细胞染色质结构在热休克条件下的动态变化提供了重要的新见解。

学位论文存在的不足或主要问题：论文的不足主要集中在两个方面：书写方面：三维基因组学领域的专有名词和术语较多，这使得论文难以理解。建议作者在描述时尽量避免过多使用专业术语，而是采用简单易懂的语言来描述研究内容。另外，论文多处出现中英夹杂的叙述方式，降低了文本的流畅度。建议仅对一些难以理解或容易产生歧义的词汇，标注英文原文，简单的语境和叙述应尽量使用中文（如限制性内切酶无需英文释义）。内容方面：论文中某些关键的数据分析流程和参数，以及一些主要的结果和图片描述过于简洁。建议作者增加对数据处理和结果具体数值、图表的细节描述，提高论文的科学性和研究结果的说服力。其它具体修改建议：1. 前言中增加热应激与组蛋白修饰的相关研究进展。2. 图3-2、3-4、3-10、3-20图注过于简洁。所有图中如果存在箭头、方框等有针对性的强调作用的符号，应予以说明。3. 2.2.1数据来源的阐述中，是否需要详细说明每个数据是由哪位同学或老师产生的？不清楚学校对论文的统一要求，请仔细斟酌。另外，本部分主要描述实验的材料与方法，应尽量详细的阐述清楚实验材料、培养过程和实验方法。4. 2.3数据处理及可视化，建议按照数据处理和分析的流程从客观视角逐步撰写，分析过程清晰，可重复，关键性参数应给出，语言简洁明了即可。5. 2.4 参考基因组dm3具体是什么？应给出网站或者相关参考基因组版本的参考文献。尽管数据分析参照GeneLab的转录组分析流程，也应简要描述一下数据分析的过程，尤其是用到的软件和程序。语言上，尽量减少中英夹杂的叙述方式。6. 课题总体设计的第二段，关于“果蝇基因组12组表观修饰的ChIP-seq数据”相关研究建议挪至前言中。7. 2.2.2 公共数据描述的“Oryza sativa 细胞”是水稻细胞吗？“S2细胞热休克先后的Hi-C数据……”直至本段结束的内容是否出现在结果分析的章节更合理？8. 摘要中对染色体修饰变化引发的基因表达变化进行简单总结，而不是“有趣的是，这些变化与基因表达相关。”9. 缩略此表中Fold Change翻译为差异倍数更符合语义；FDR应翻译为“错误发现率”；10. 在写法上，学位论文应避免使用“我们”这样的说法，比如第21页材料与方法“这样我们就可以得到每个bin的值”、“这样我们便可以得到每个 bin 的 compartment score 以及区室的类型”、“同样的，我们也可以用log2”等等。。

评审专家意见回复

感谢审稿人2如此具体的建议，下面是对审稿意见的逐一回复。

1. 前言中增加热应激与组蛋白修饰的相关研究进展。

答复：P12，已将在“1.2 热应激概述”补充了热应激与组蛋白修饰的相关研究进展。

2. 图3-2、3-4、3-10、3-20图注过于简洁。所有图中如果存在箭头、方框等有针对性的强调作用的符号，应予以说明。

答复：P28，将图3-2、3-4、3-10、3-20图注逐一补充，对箭头、图形等标注也进行了说明。此外，也对其它图（原图3-5、3-6、3-11、3-13、3-14）也进行必要的补充说明。

3. 2.2.1数据来源的阐述中，是否需要详细说明每个数据是由哪位同学或老师产生的？不清楚学校对论文的统一要求，请仔细斟酌。另外，本部分主要描述实验的材料与方法，应尽量详细的阐述清楚实验材料、培养过程和实验方法。

答复：P18，已经在附表B-1添加了对应的建库人员，同时在材料和方法中添加了“2.2实验方法”用于具体描述实验材料、培养过程和实验方法。

4. 2.3数据处理及可视化，建议按照数据处理和分析的流程从客观视角逐步撰写，分析过程清晰，可重复，关键性参数应给出，语言简洁明了即可。

答复：P21，已经补充了关键的参数，可以用于重复。例如将“转化为特定分辨率的cool文件”改为“分辨率 为 10 Kb 的cool文件”。

5. 2.4 参考基因组dm3具体是什么？应给出网站或者相关参考基因组版本的参考文献。尽管数据分析参照GeneLab的转录组分析流程，也应简要描述一下数据分析的过程，尤其是用到的的软件和程序。语言上，尽量减少中英夹杂的叙述方式。

答复：P21，已经添加dm参考基因组及其基因注释的下载地址。也对GeneLab的转录组分析流程进行了简要描述。除了常用专用术语，已经在全文范围内进行了检查和更正中英夹述。

6. 课题总体设计的第二段，关于“果蝇基因组12组表观修饰的ChIP-seq数据”相关研究建议挪至前言中。

答复：P16，“果蝇基因组12组表观修饰的ChIP-seq数据”相关研究移至前言中的“1.4 研究目的和意义”。

7. 2.2.2 公共数据描述的“Oryza sativa 细胞”是水稻细胞吗？“S2细胞热休克先后的Hi-C数据.....”直至本段结束的内容是否出现在结果分析的章节更合理？

答复：P18，已经将这些内容从“2.2.2 公共数据”中删除。将Oryza sativa 细胞已经修改为水稻细胞。“S2细胞热休克先后的Hi-C数据.....”在结果分析以及讨论章节中有描述。

8. 摘要中对染色体质修饰变化引发的基因表达变化进行简单总结，而不是“有趣的是，这些变化与基因表达相关。”

答复：已经重新书写摘要并具体描述了相关基因及模型。基因表达部分增加了“由RNAPII介导的相互作用在B区域内的下降幅度比在A区域内的要大，这与下调基因更有可能出现在B区域的发现相一致”，以及“合并的TAD边界与涉及RNA代谢和细胞对压力反应的差异表达基因相关联”。相应的模型表述为“并提出了热应激下果蝇基因组三维结构在A/B区室层面变化的模型”，模型的更具体内容对应于摘要的第三段的部分结论“热休克后，A区域内部的相互作用强度增加，而B区域内部的相互作用强度减少。并且，由RNAPII介导的相互作用在B区域内的下降幅度比在A区域内的要大，这与下调基因更有可能出现在B区域的发现相一致。此外，聚集的A区室会进一步靠近或者形成更大的簇，而相邻B区室之间的相互作用会增强”。

9. 缩略此表中Fold Change翻译为差异倍数更符合语义；FDR应翻译为“错误发现率”；

答复：已将Fold Change修改为差异倍数，FDR应翻译为“错误发现率”。

10. 在写法上，学位论文应避免使用“我们”这样的说法，比如第21页材料与方法“这样我们就可以得到每个bin的值”、“这样我们便可以得到每个 bin 的 compartment score 以及区室的类型”、“同样的，我们也可以用 \log_2 ”等等。

答复：P22，已将这类表述全部更正，同时也全文范围内更正中英夹述。



Zhengmz Lab

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Zhengmz Lab

**Thank you for your attention and
suggestions!**