Super-Resolution Imaging of Nano-Scale Chromatin Organization

Journal Club 17th August 2021

Alexandra Bentrup

Cell Reports
Article

Super-Resolution Imaging of Higher-Order Chromatin Structures at Different Epigenomic States in Single Mammalian Cells

Jianquan Xu,[1] Hongqiang Ma,[1] Jingyi Jin,[1,2] Shikhar Uttam,[3] Rao Fu,[1,4] Yi Huang,[5] and Yang Liu[1,6,*]

2018

New Results

Chemo-Mechanical Cues Modulate Nano-Scale Chromatin Organization in Healthy and Diseased Connective Tissue Cells

Su-Jin Heo, Shreyasi Thakur, Xingyu Chen, Claudia Loebel, Boao Xia, Rowena McBeath, Jason A. Burdick, Vivek B. Shenoy, Robert L. Mauck, Melike Lakadamyali

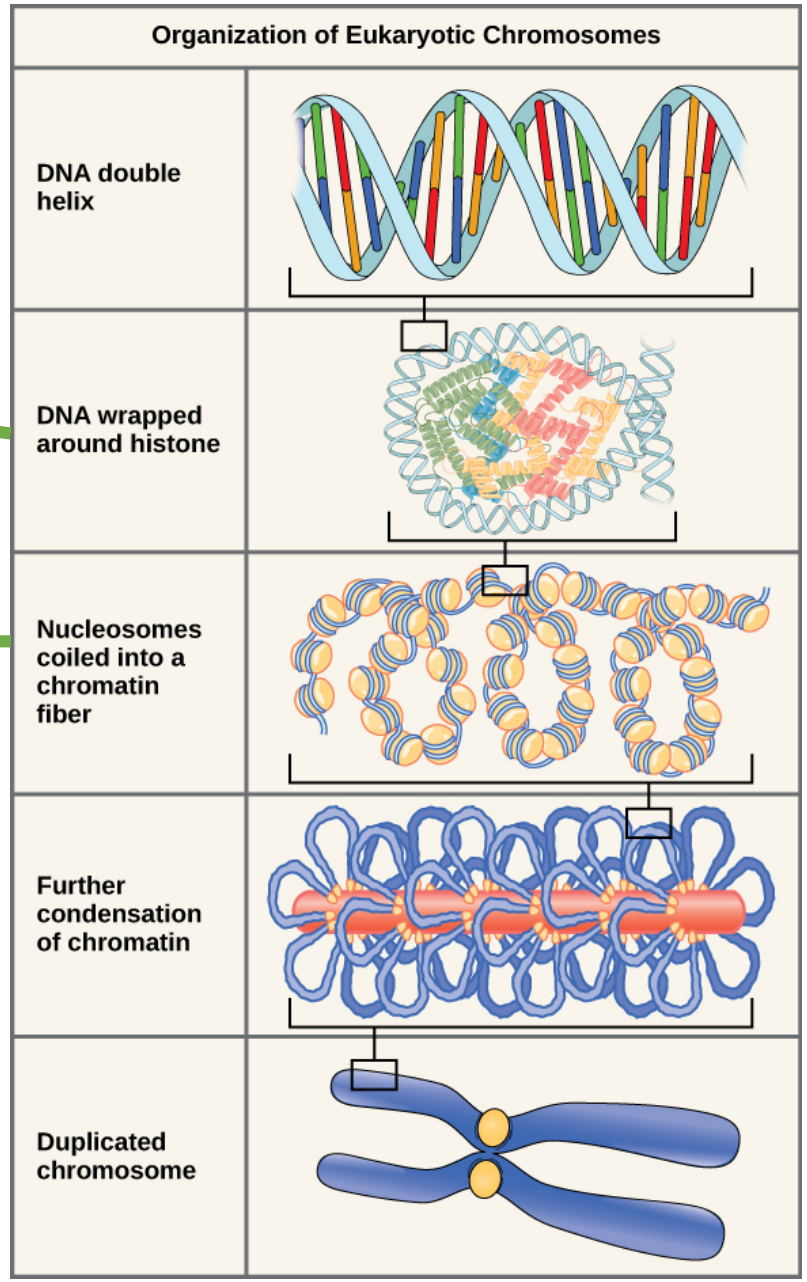
doi: https://doi.org/10.1101/2021.04.27.441596

2021

Chromatin Organization

Nucleosome:
147 bp of DNA wrapped around an octamer of four core histone proteins: H2A, H2B, H3, and H4

10-nm ‘‘beads-on-string’’ chromatin fiber



https://courses.lumenlearning.com/wmopen-biology1/chapter/chromosomes-and-dna-packaging/

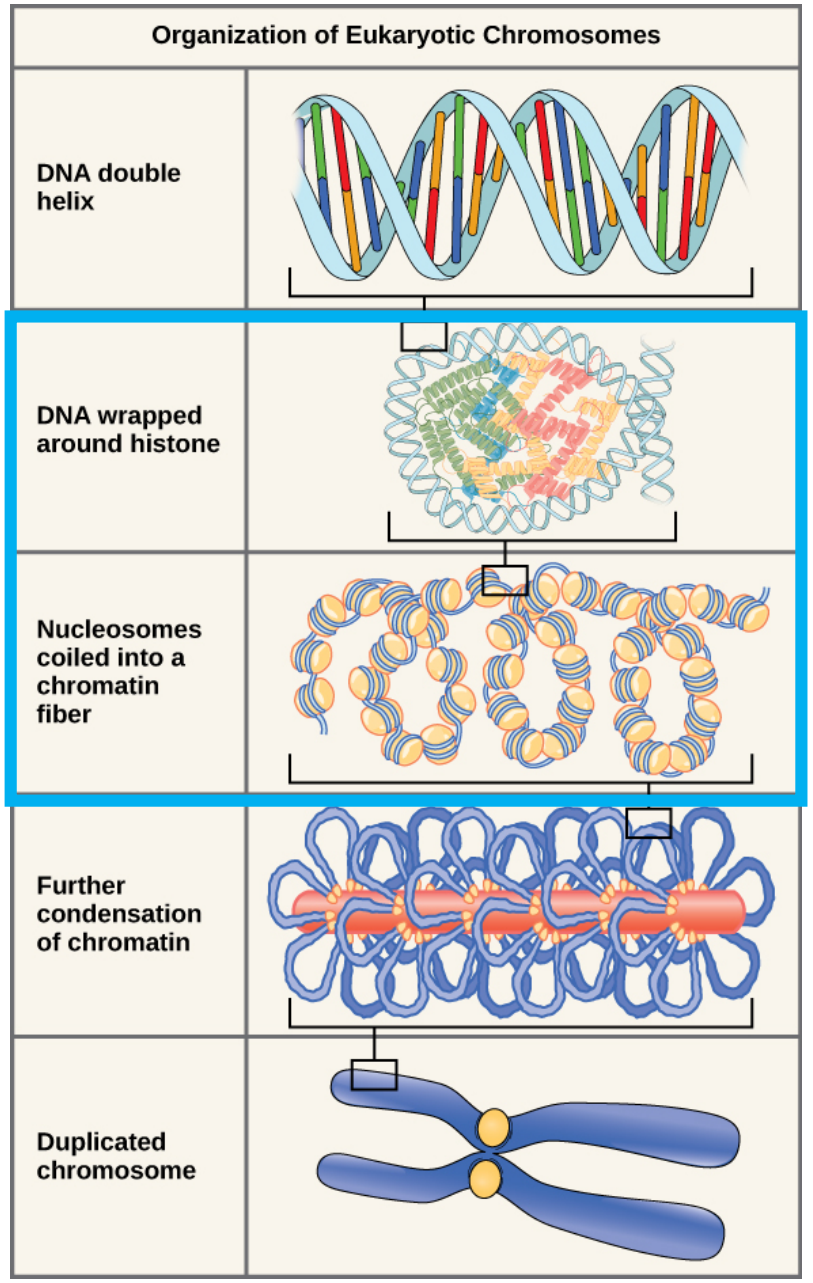
Compaction of DNA into DNA-protein assemblies to fit nucleus

- Regulated by chemical modifications
- Mainly N-terminal tail of histone core proteins
- Acetylation, methylation,…
- Depending on modification, compactation is increased or decreased
  →DNA replication, cell division, DNA repair,…

Chromatin Organization

Folding and unfolding for transcriptional activity

But how? Where? When? Why?



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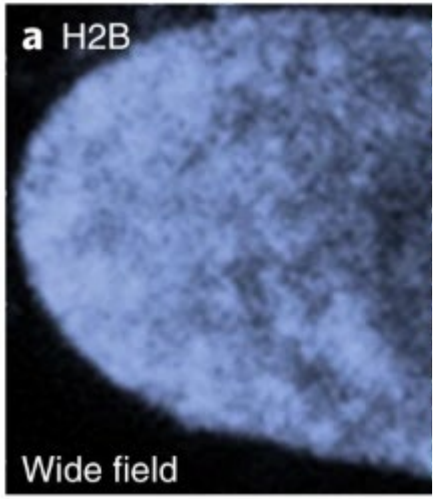
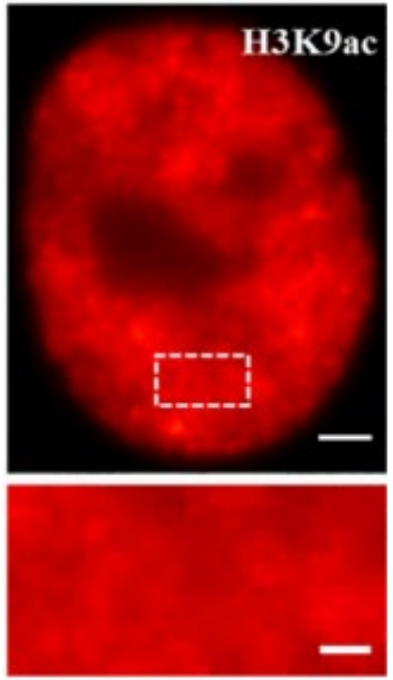
Lakadamyali & Cosma 2020

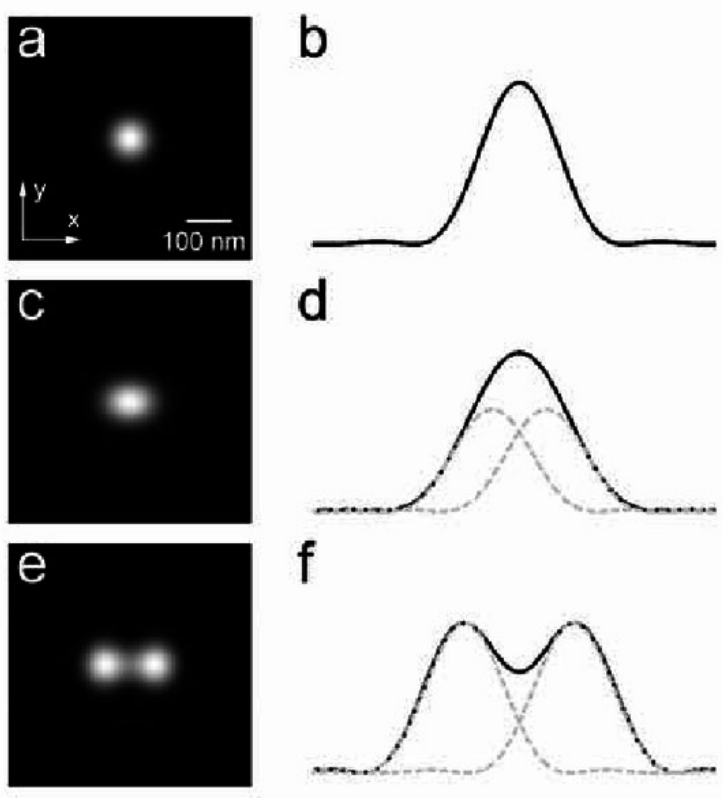




Xu 2018

Widefield image of nucleus:

- Heterogenous staining
  → local hotspots
- Optical diffraction limit
  → structures too small to visualize with classic light microscopy



Bertocchi 2013

Chromatin Organization

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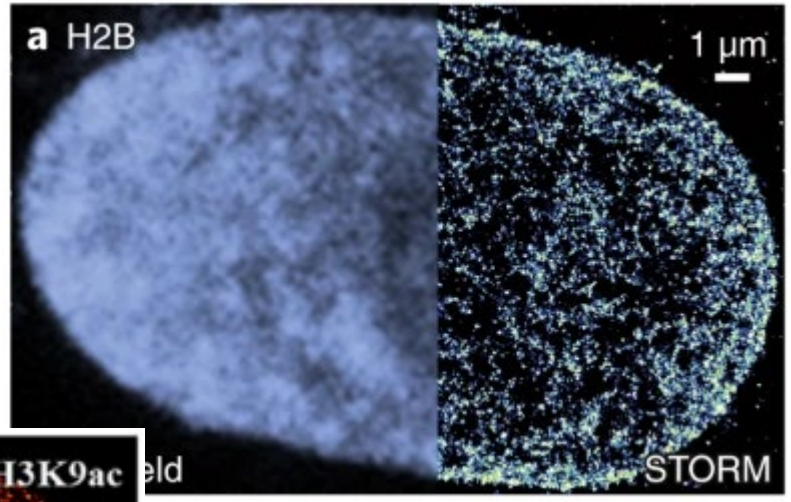
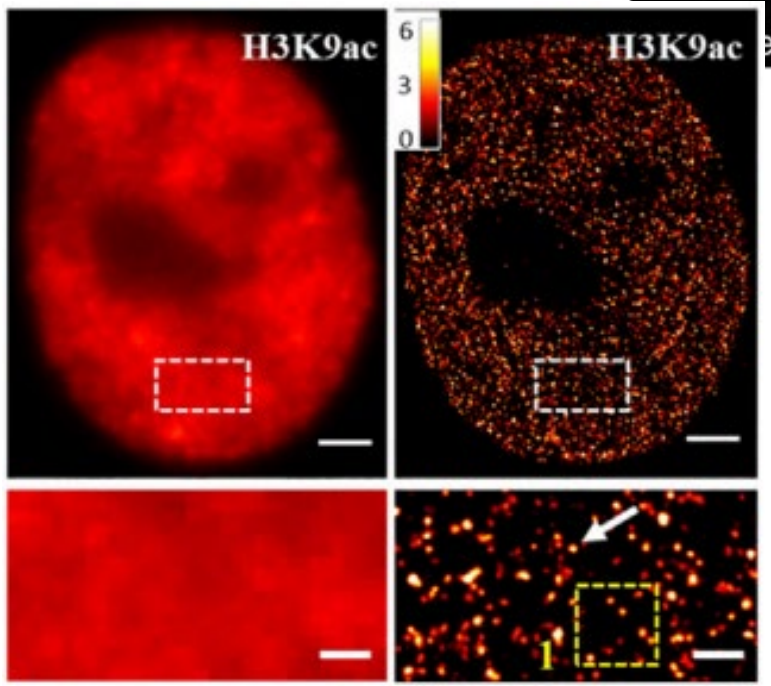
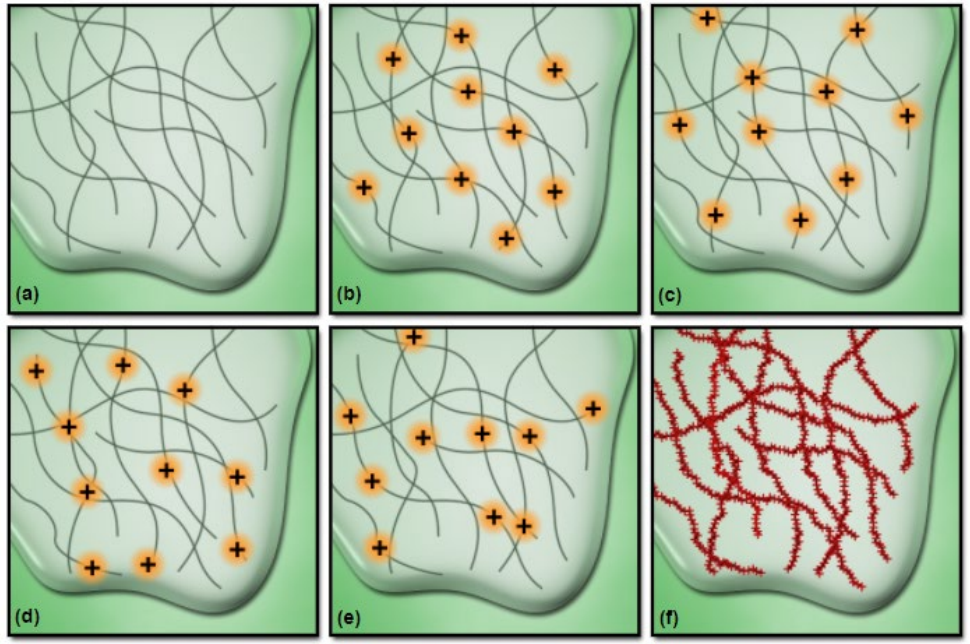




Xu 2018

STORM imaging:
Stochastic Optical Reconstruction Microscopy



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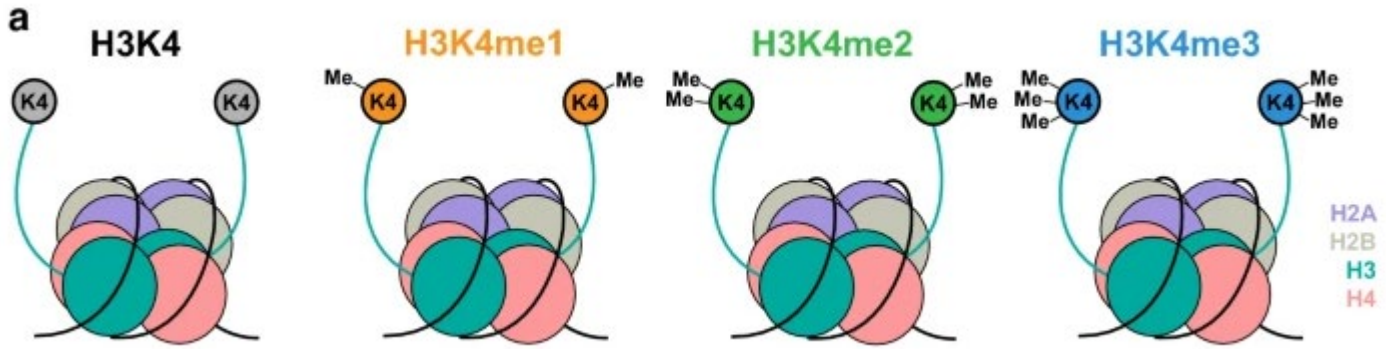
Genome-wide histone marks that structure DNA

A. **Transcriptionally active** histone **acetylation** marks: H3K9ac, H3K27ac, H3ac, and H4ac

B. **Transcriptionally active** histone **methylation** marks: H3K4me1, H3K4me2, H3K4me3, and H3K36me3

C. **Transcriptionally repressive** histone **methylation** marks: H3K27me3 and H3K9me3



H3 lysine 4

H3 lysine 4 mono-methylation

H3 lysine 4 dimethylation

H3 lysine 4 trimethylation

Collins 2019

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Euchromatin

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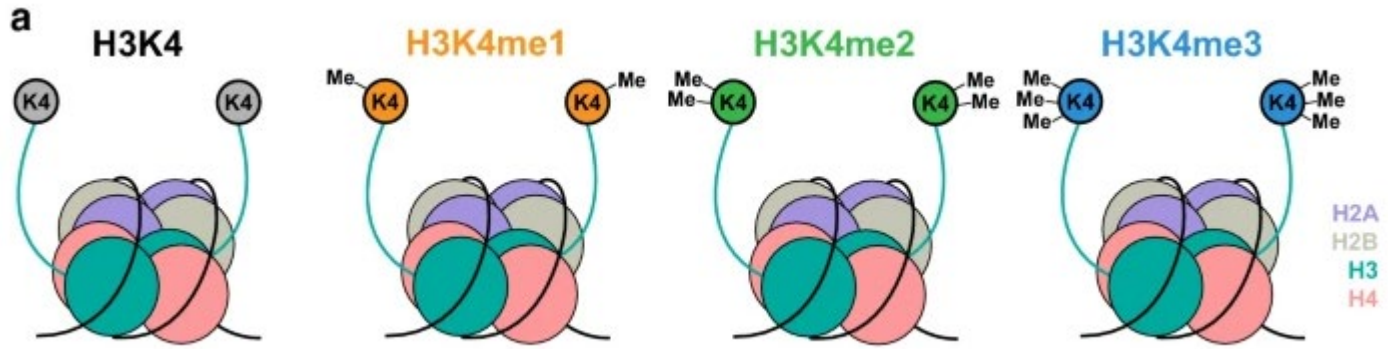
Heterochromatin



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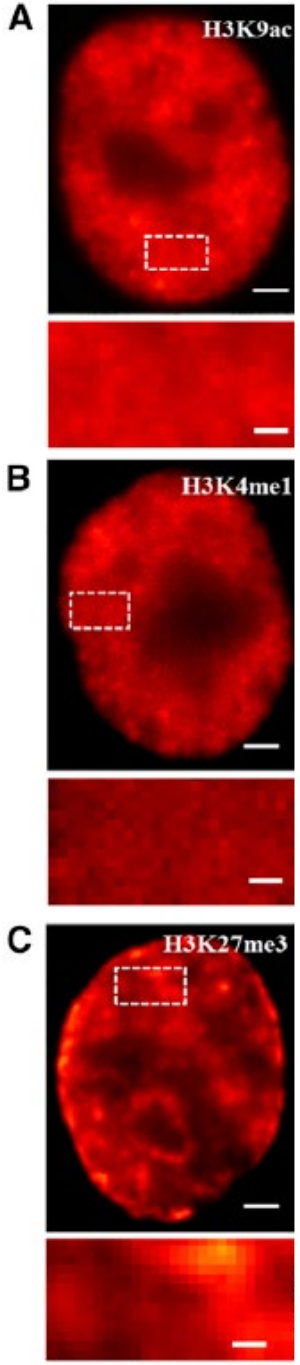

Widefield images are not very informative

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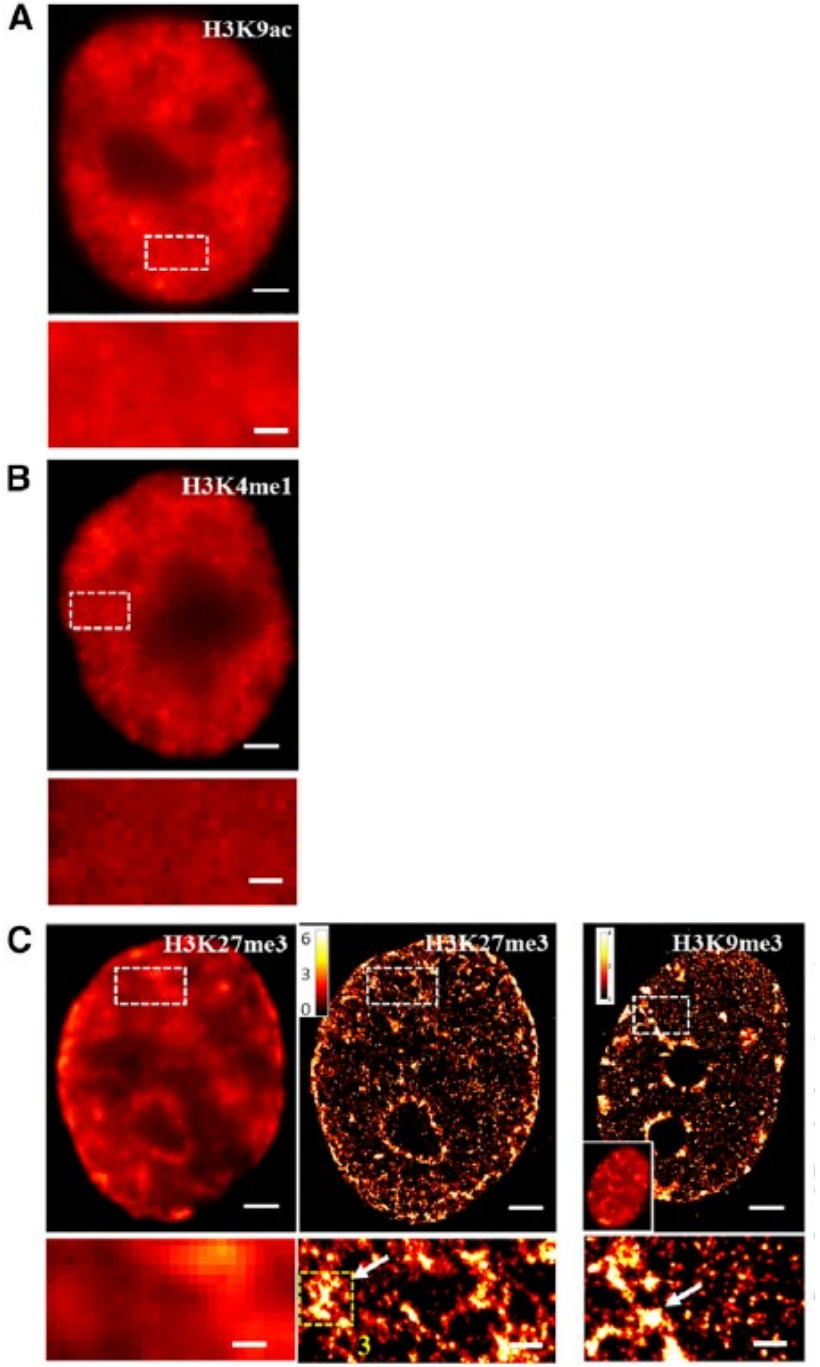

Highly condensed large clumps (100s nm to µm) enriched at the periphery of the nucleus & nucleolus → heterochromatin (existing EM evidence)

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Spatially segregated and discrete nucleosome nanoclusters of **similar size**

B. Transcriptionally active histone methylation marks: H3K4me1, H3K4me2, H3K4me3, and H3K36me3

Highly heterogeneous and spatially dispersed nucleosome nanodomains

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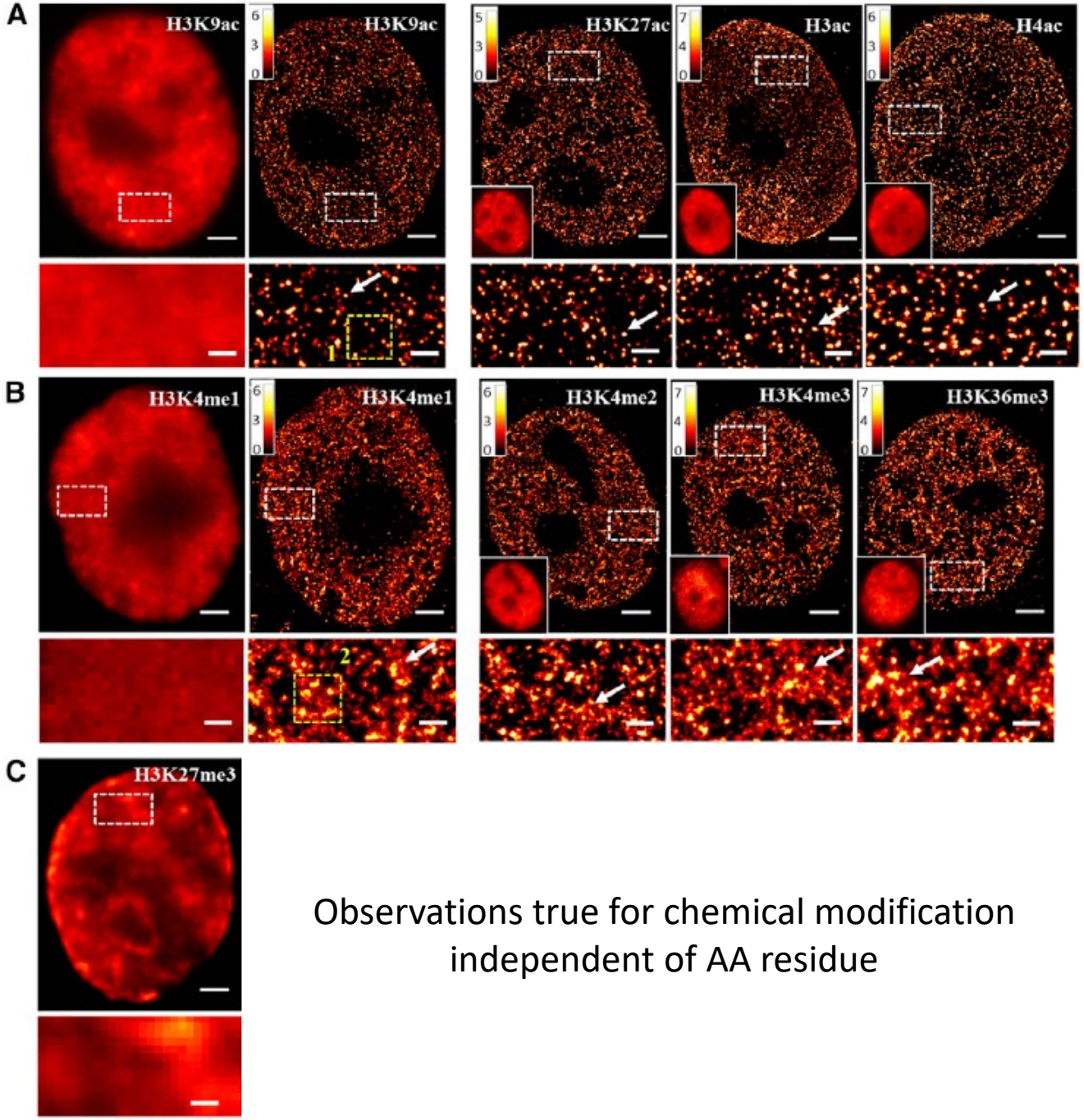

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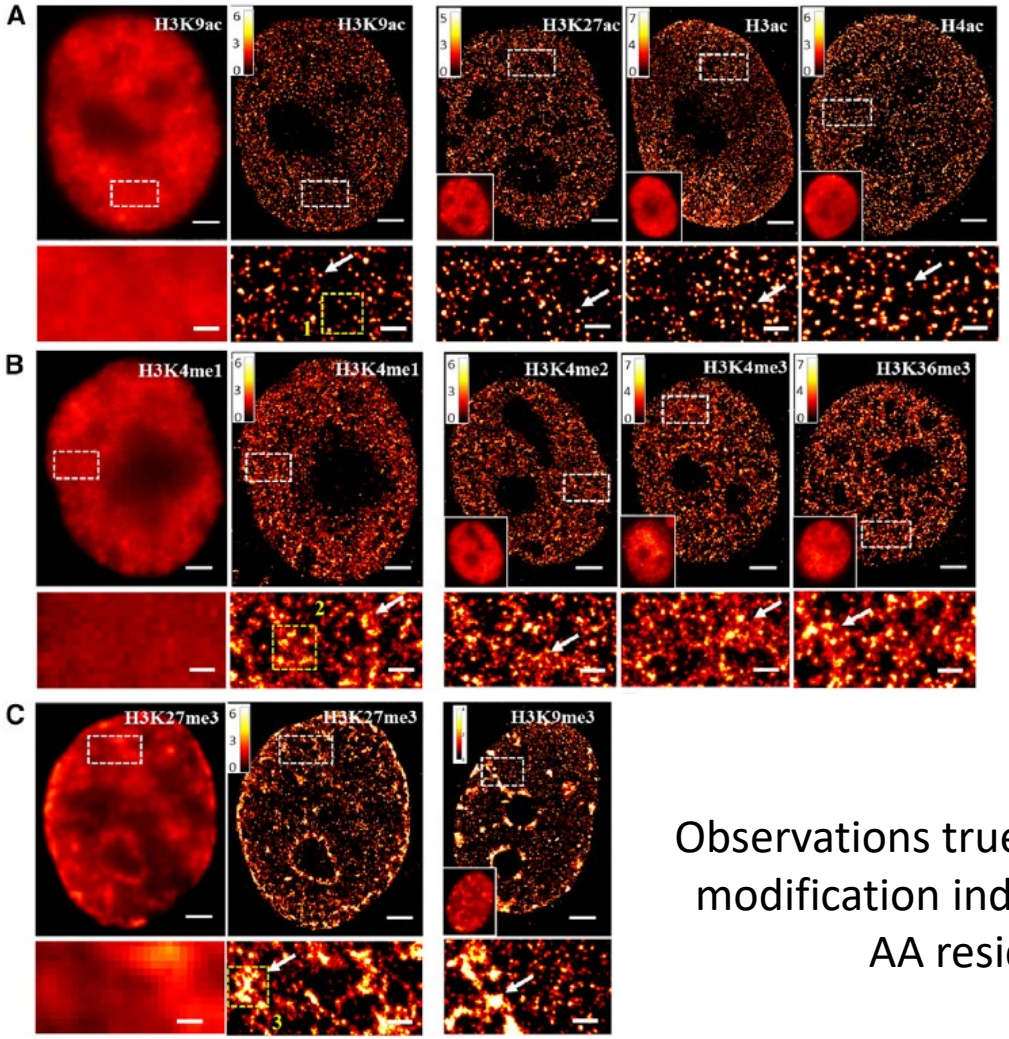

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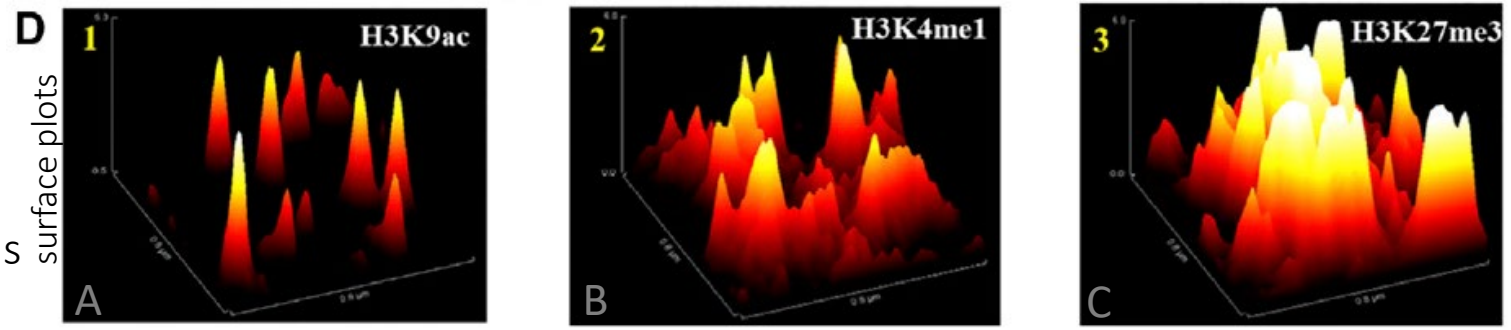

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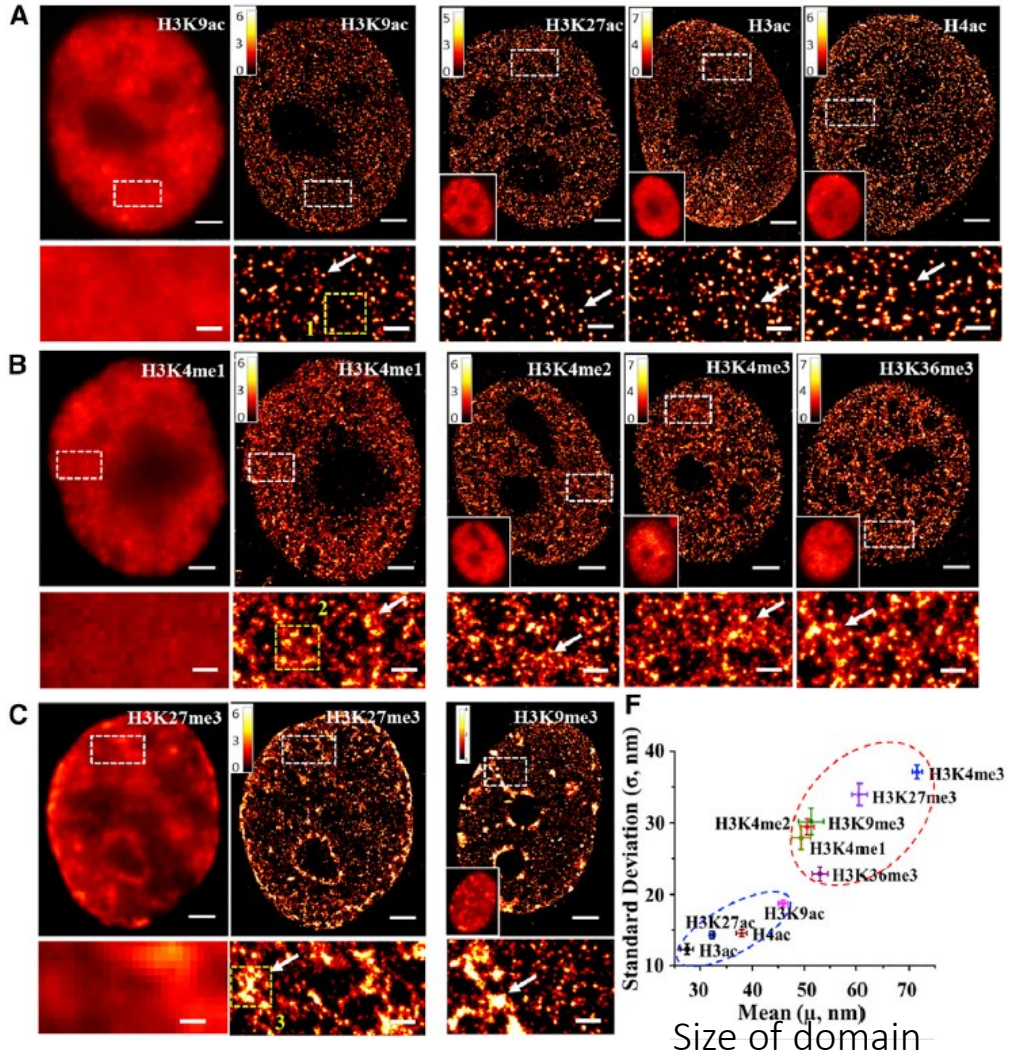

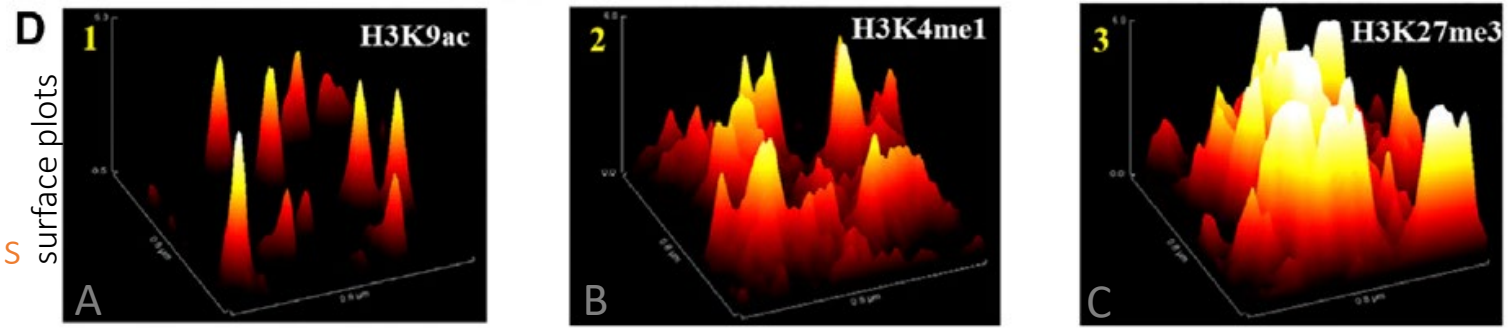

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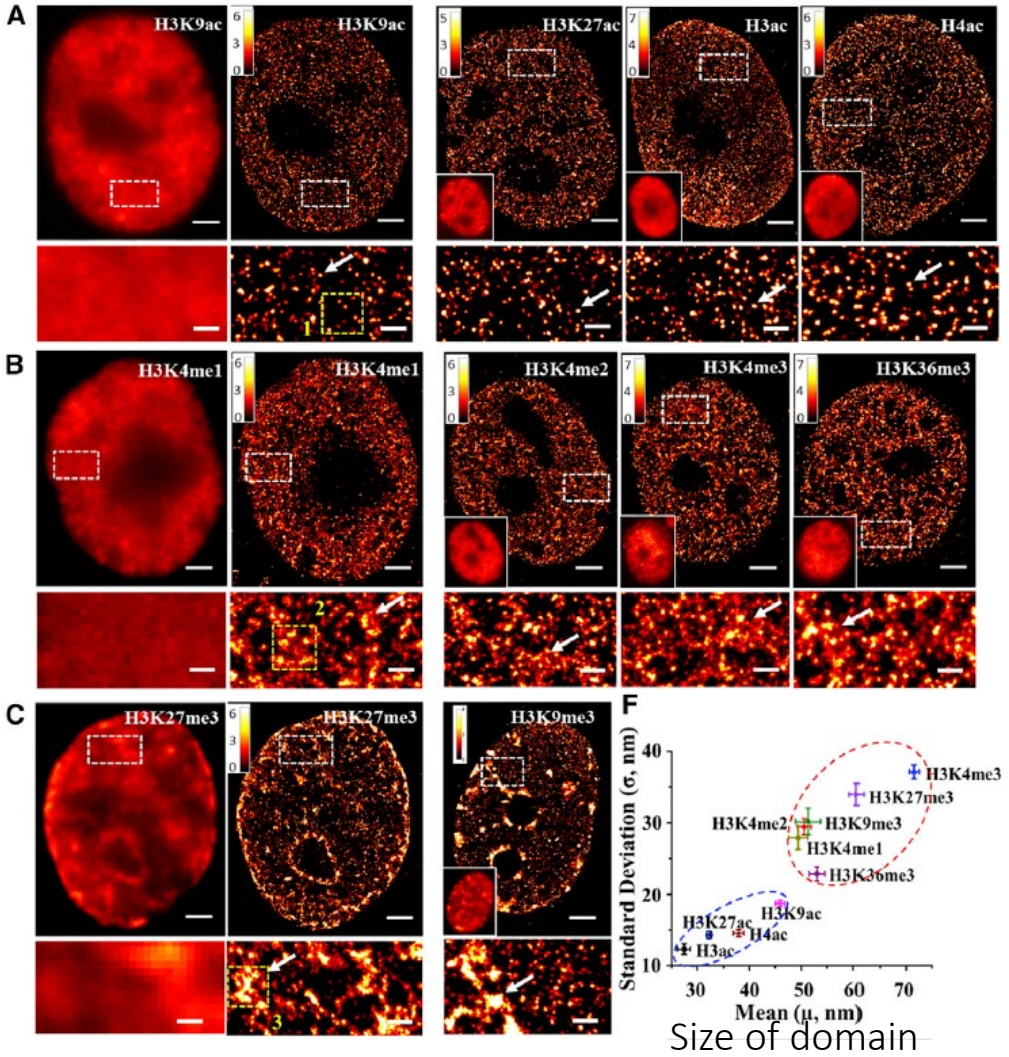

Size of domain

→ Three Distinct Structural Characteristics of Higher-Order Chromatin Structure Formed by Histone Marks in the Interphase Nuclei

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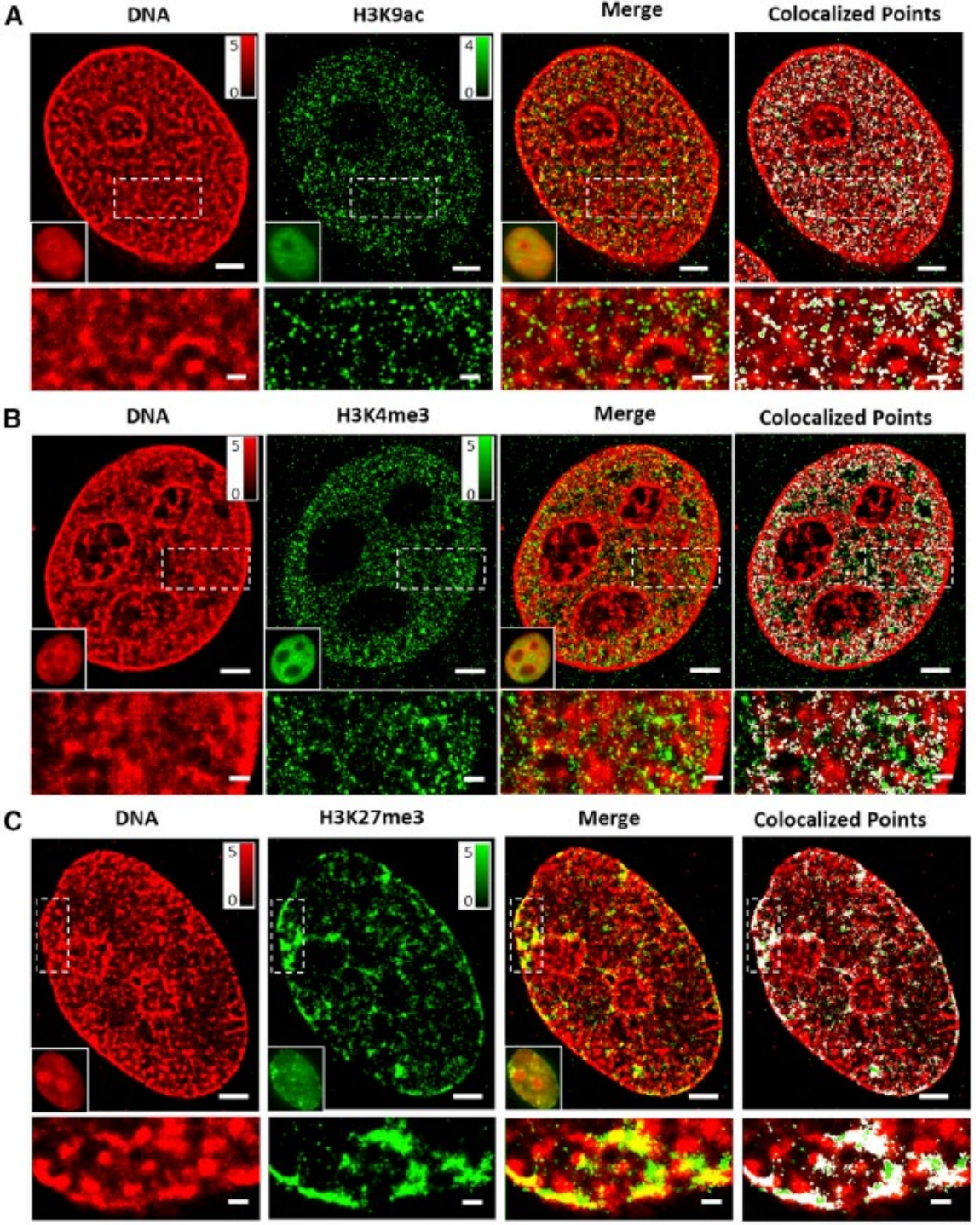
→ heterochromatin

DNA much more compact in some regions of nucleus

H3K9ac & H3K4me3 (transcriptionally active marks) can be found in regions with less DNA signal → less condensation

H3K27me3 (repressive methylation) colocalizes with compact DNA
→ heterochromatin



transcription activities

Active transcription detected by (P)-RNA polymerase II

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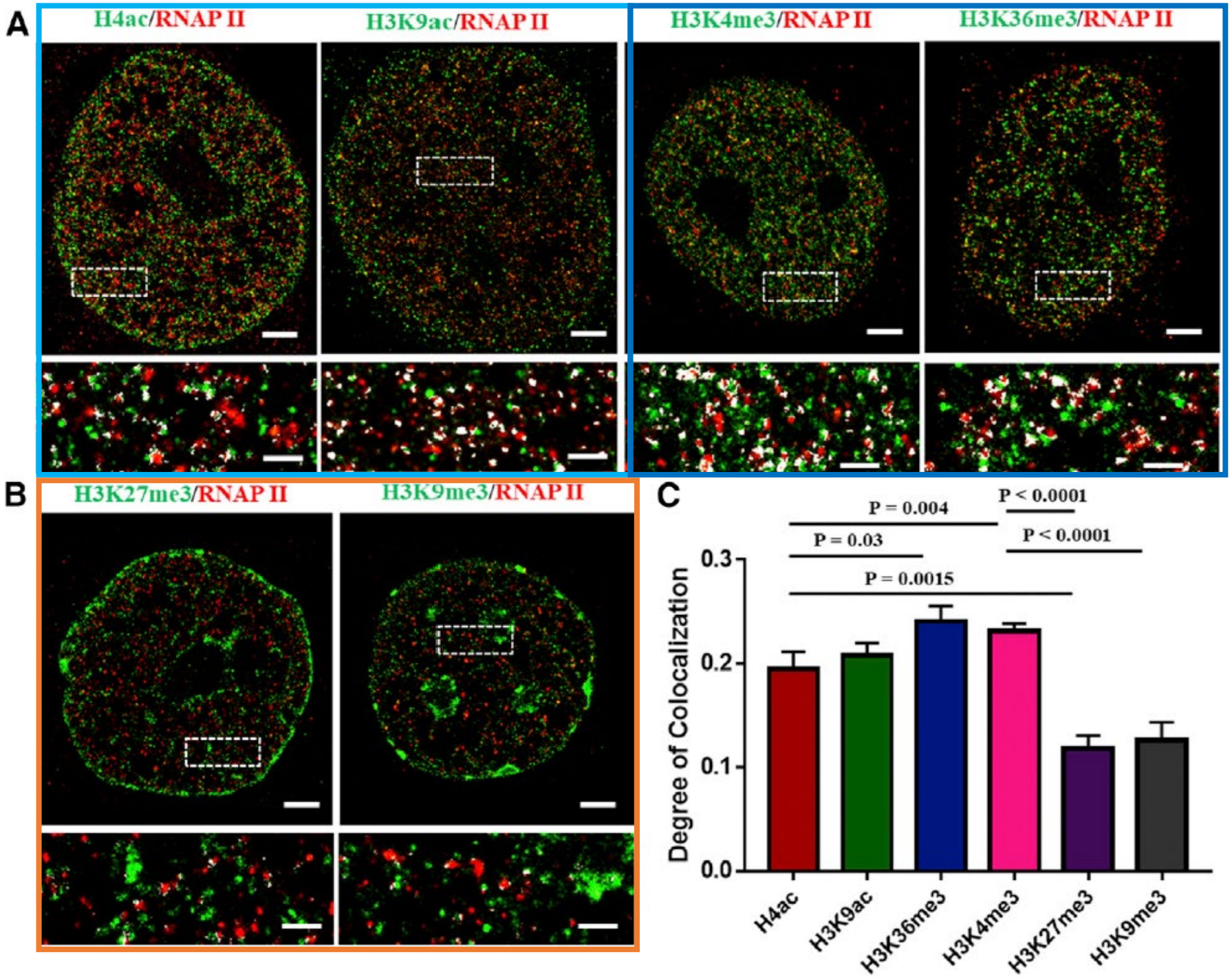
Transcriptionally repressive histone methylation marks: **H3K27me3** and **H3K9me3C**

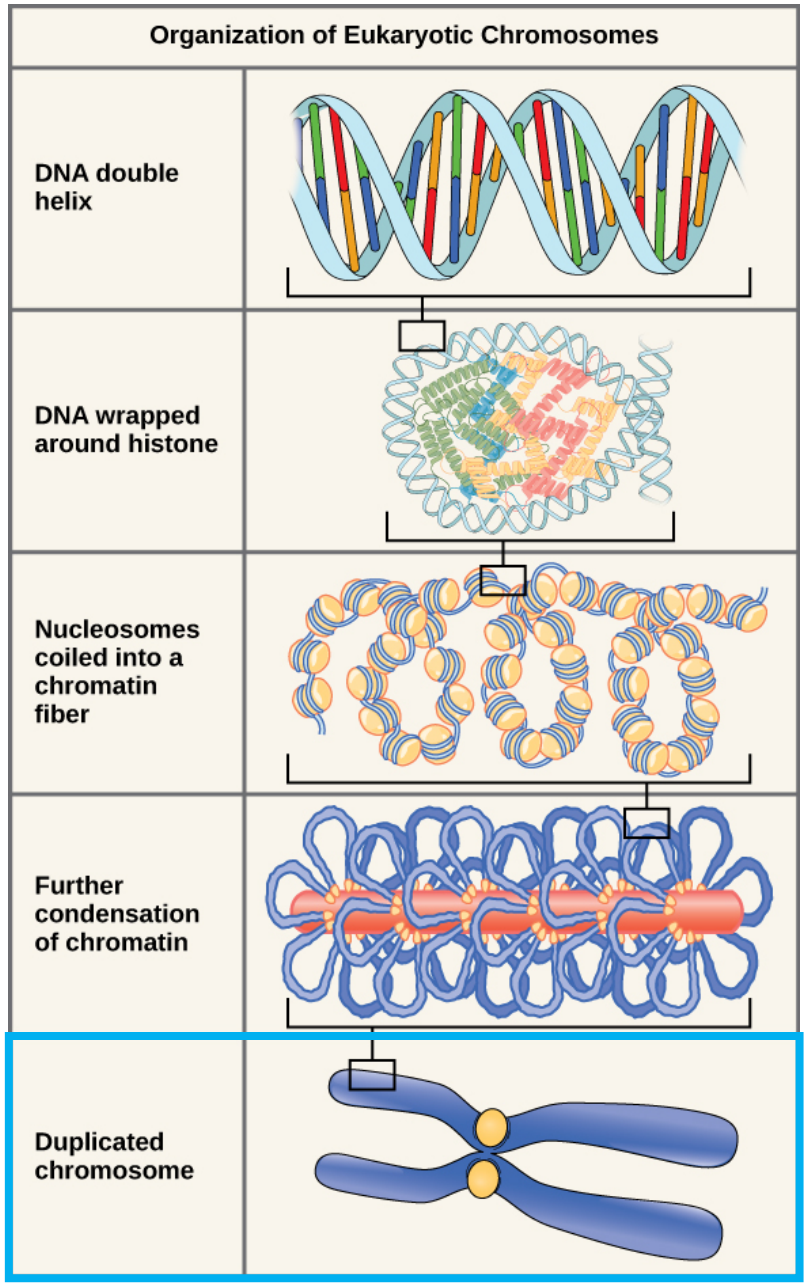
→ RNA polymerase II colocalizes with active histone marks, but less with repressive ones



Mitosis

- Observations of the 3 types of structures have been made in interphase cells
  - → transcriptional activity
  - → accessibility for enzymes
  - → chromatin is not extremely compact

- Mitosis: peak-compactation needed



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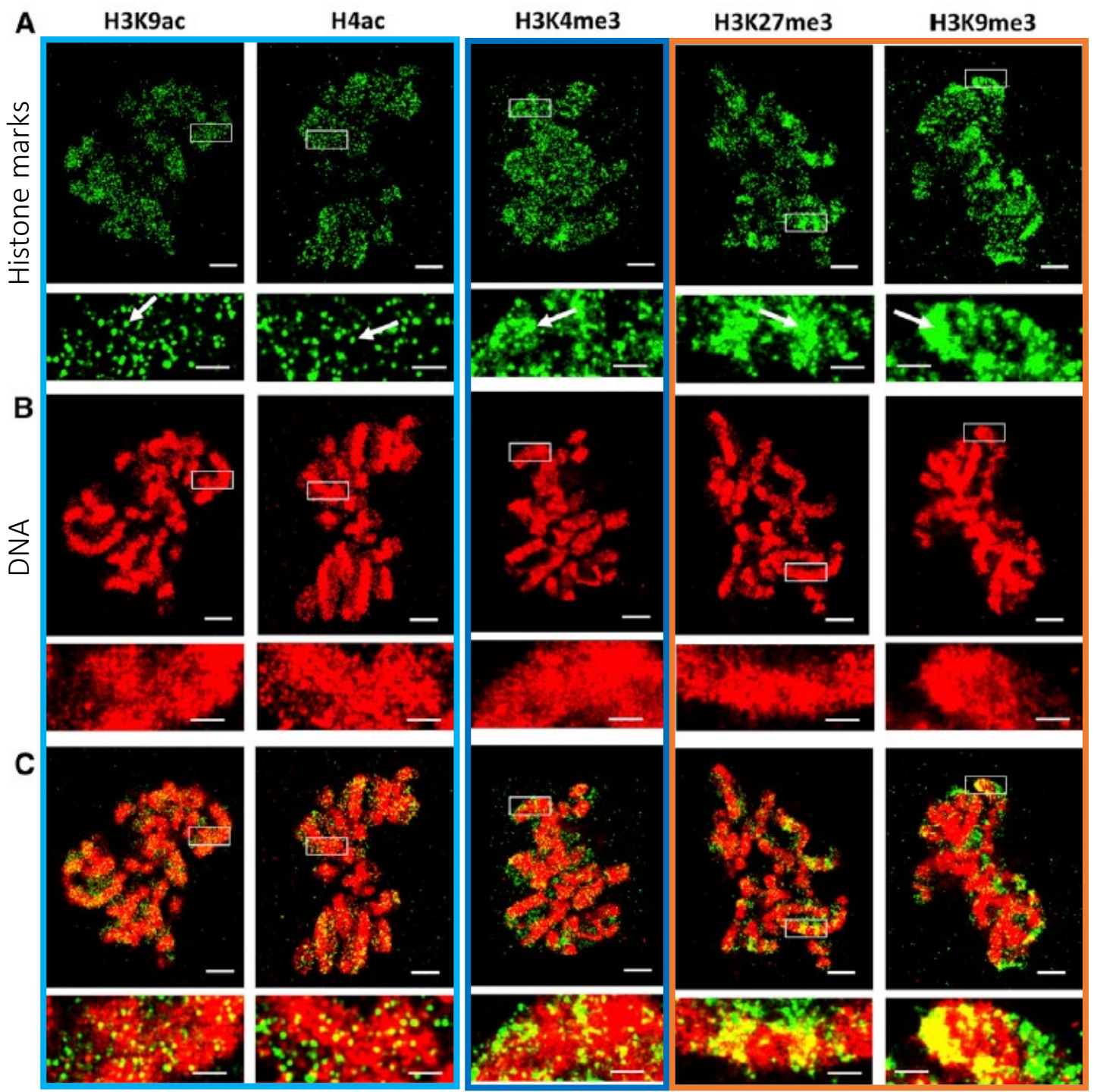


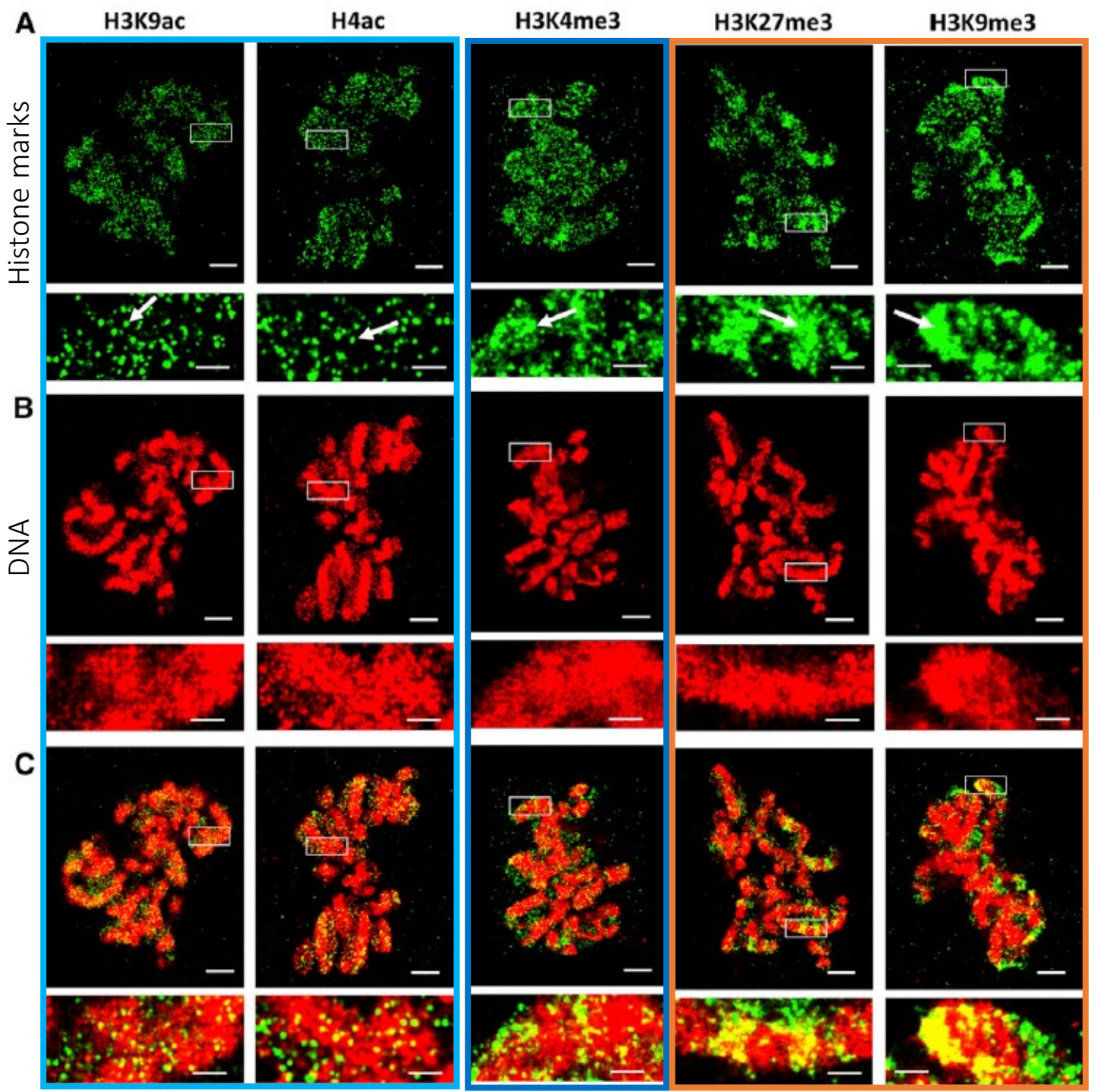
Mitosis

Transcriptionally active histone acetylation marks: **H3K9ac**, H3K27ac, H3ac, and **H4ac** — discrete

Transcriptionally active histone methylation marks: H3K4me1, H3K4me2, **H3K4me3**, and H3K36me3 — dispersed

Transcriptionally repressive histone methylation marks: **H3K27me3** and **H3K9me3** — large clumps

- → Despite highly condensed state of DNA:
  - ➢ all markers can be found
  - ➢ again very discrete pattern for active acetylation marks
- → Same pattern of domain-forming in mitosis and interphase
- → Conservation of important patterns during cell division?



Spatial proximity between different histone marks

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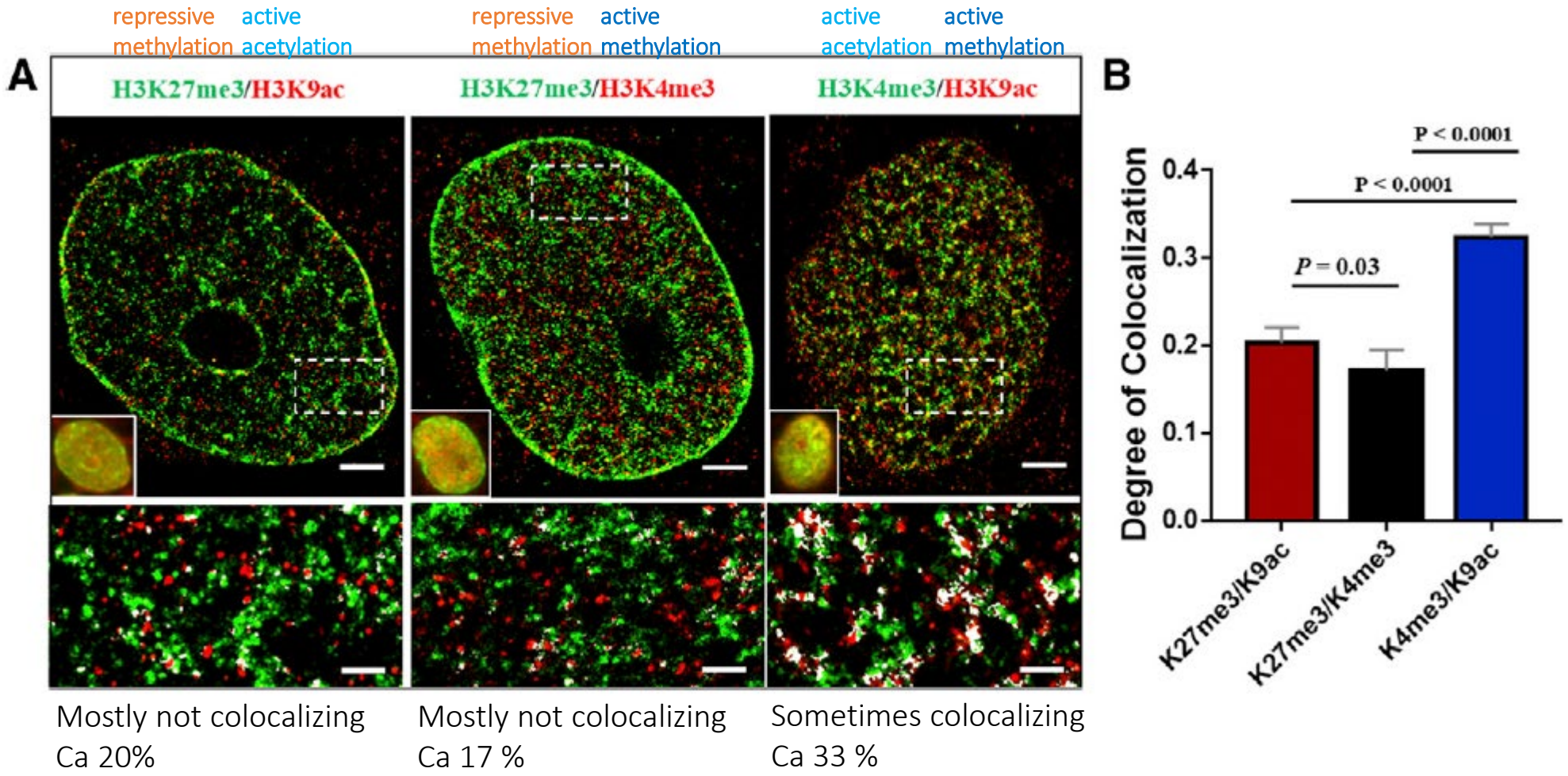

Mostly not colocalizing
Ca 20%

Mostly not colocalizing
Ca 17 %

Sometimes colocalizing
Ca 33 %

→ The 2 active chemical modifications colocalize best
→ However, not perfectly
→ Distinct domains?

Conclusions

- resolution of ~20–30 nm in the nucleus of single mammalian cells

- genome-wide higher-order chromatin structure at distinct epigenomic states
  - 2 active sites
  - 1 «silenced» site

  → Little overlap

- preserved features during mitosis → inheritance of epigenomic modifications

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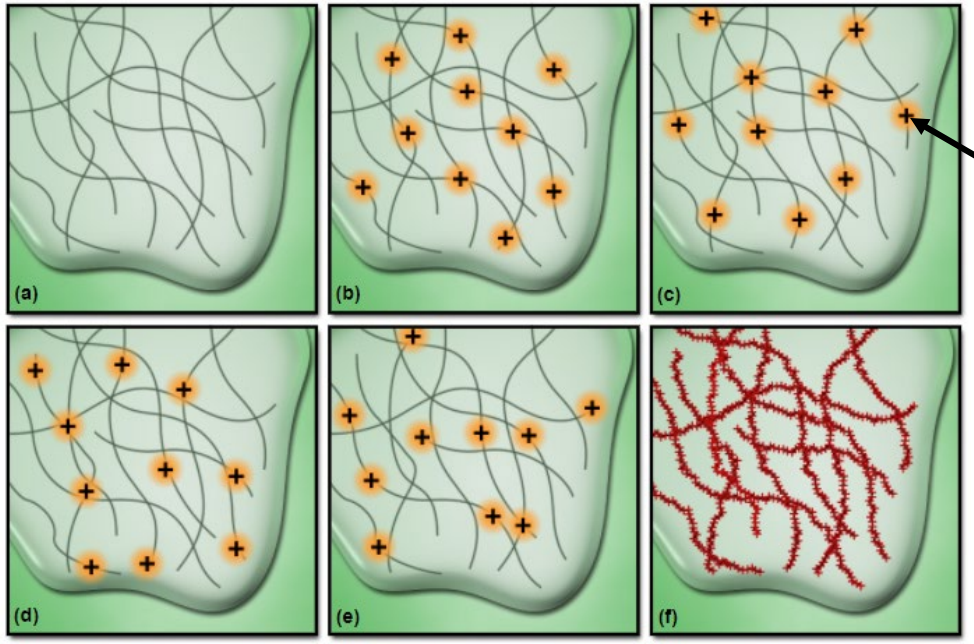
Chromatin re-organization as response to environment?

- Chromatin re-organization as cue for transcriptional changes
  → Changes in phenotype & behaviour of cells

- Physiology: stem cells in different tissues
- Pathology: changes in ECM, cell composition,…
- Medicine: artificial materials like protheses
- Lab: cell culture dishes and coatings
- …

Chromatin re-organization as response to environment?

- Quantification: Voronoi tessellation-based image segmentation



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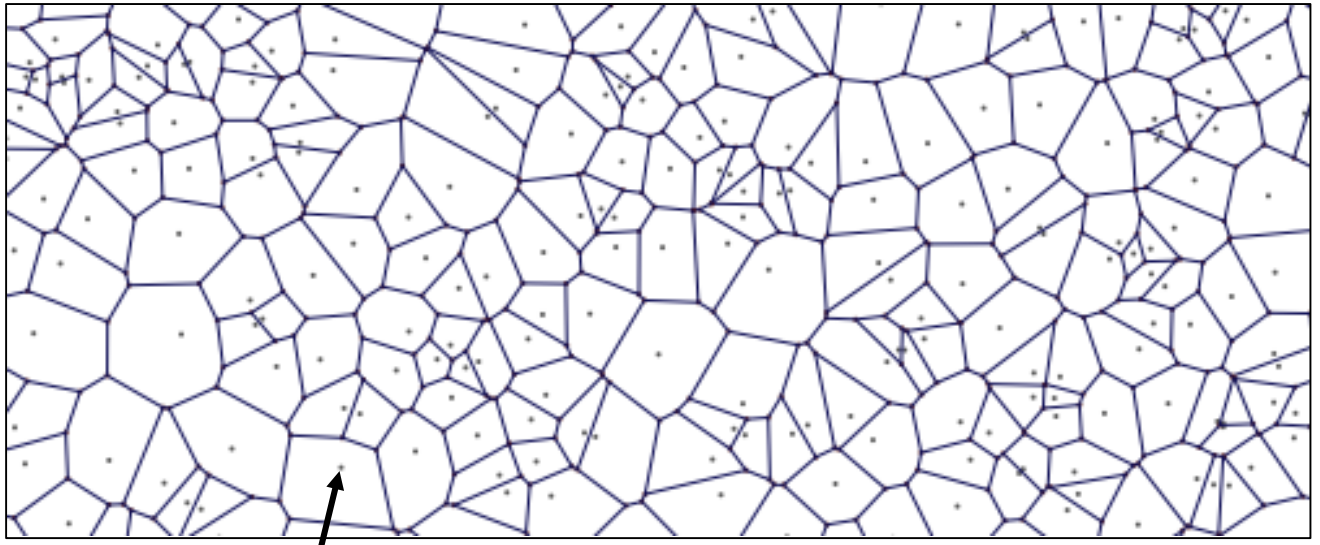

Polygons surrounding each signal

https://philogb.github.io/blog/2010/02/12/voronoi-tessellation/

Center of point spread function

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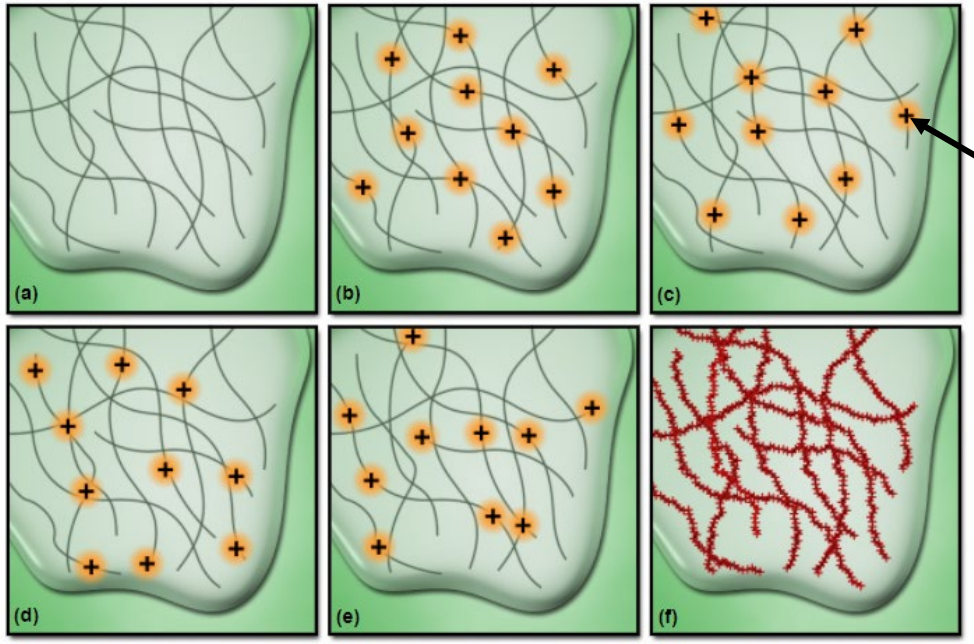

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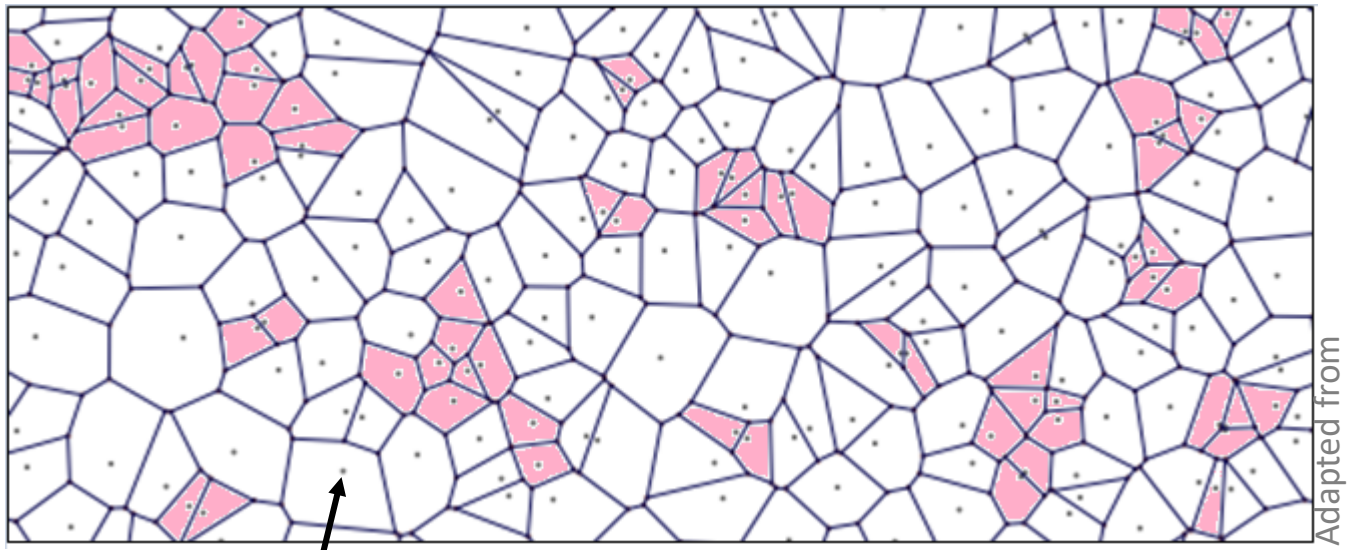

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Polygons surrounding each signal
→Threshold for only small polygons

Center of point spread function

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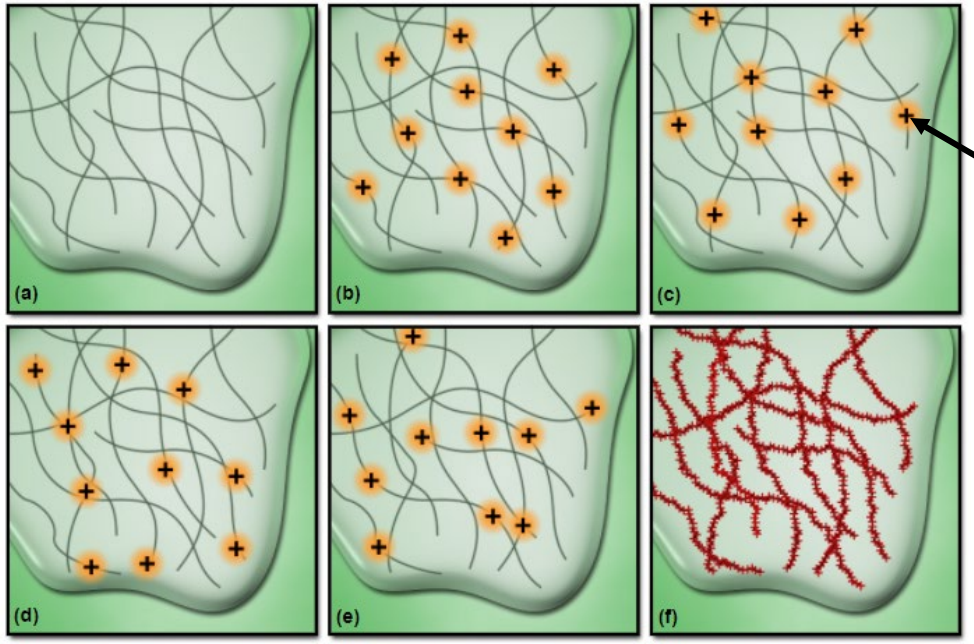

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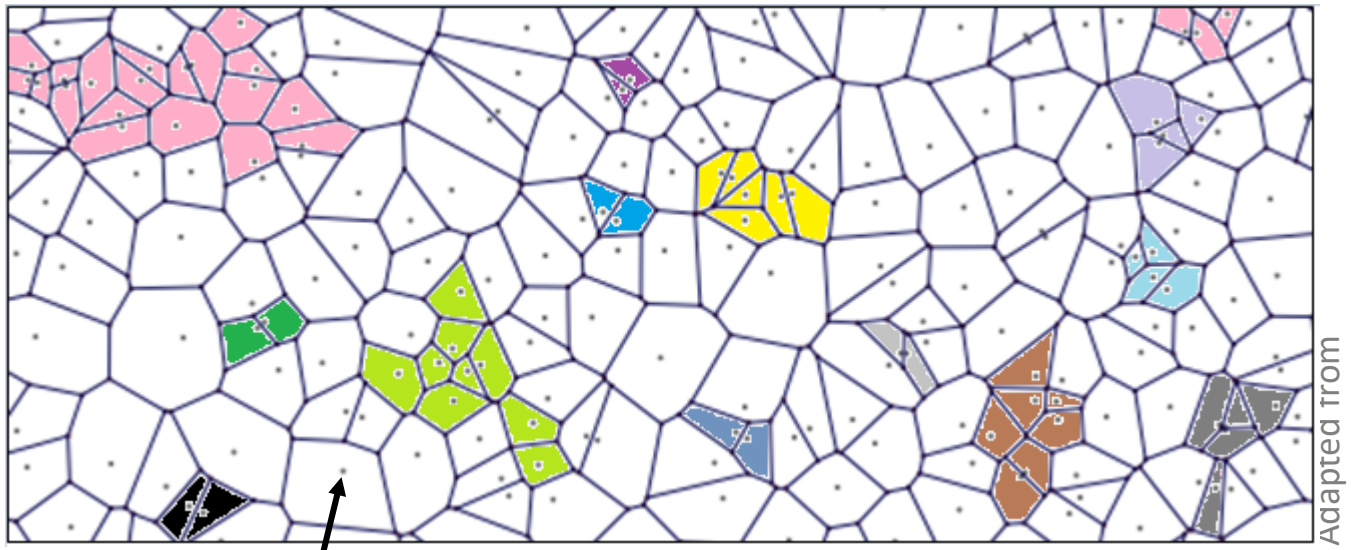

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Center of point spread function

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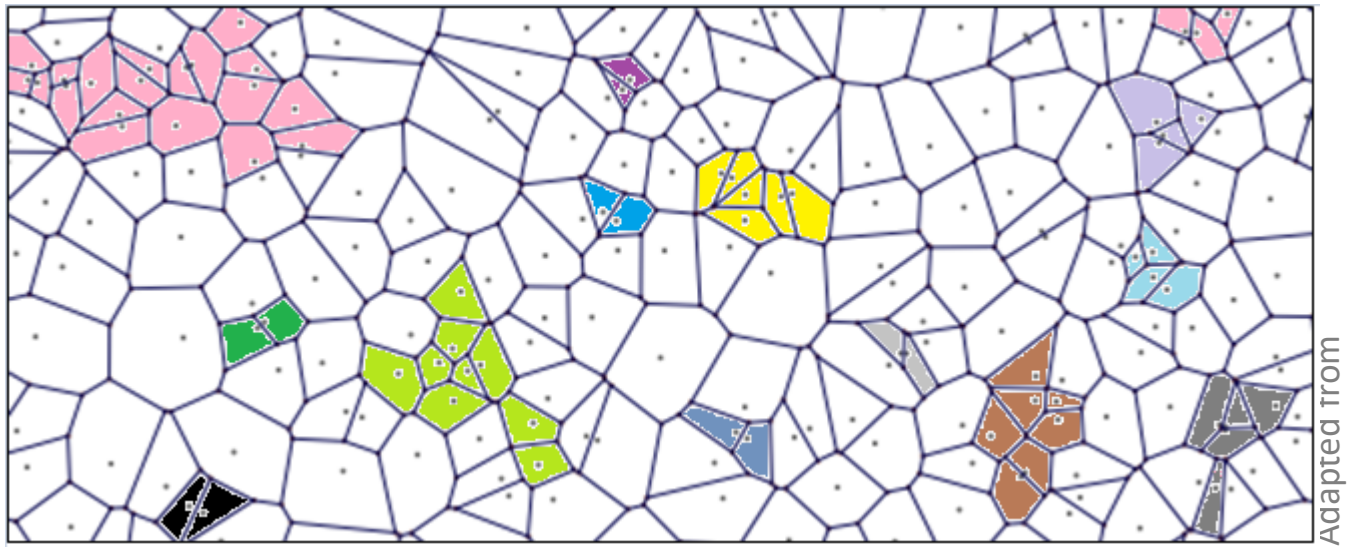

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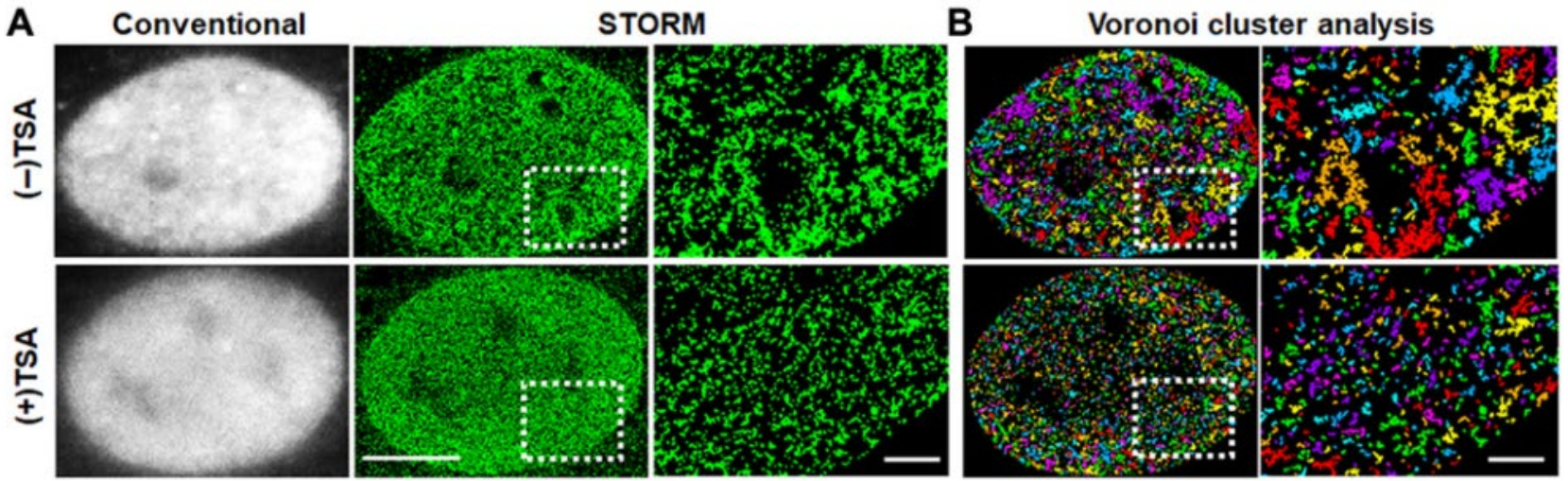

Heo 2020

Chromatin re-organization as response to environment?

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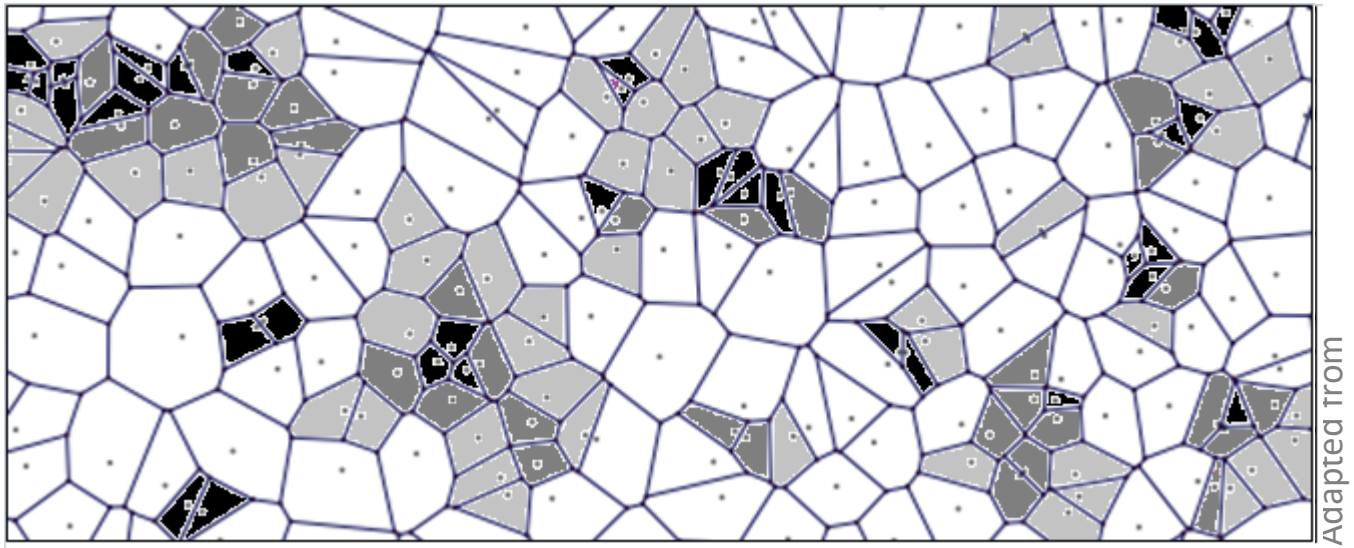
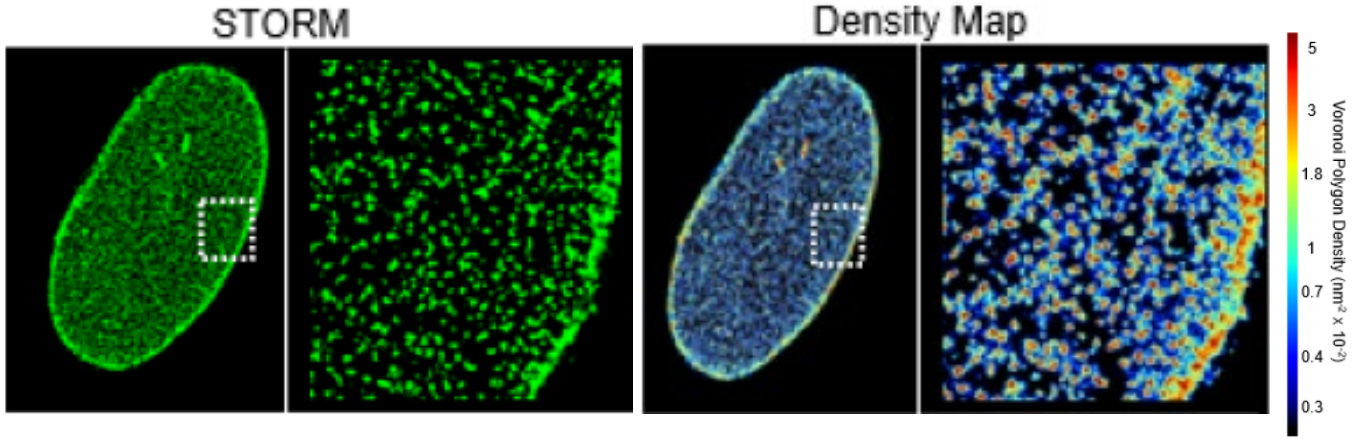


Adapted from https://philogb.github.io/blog/2010/02/12/voronoi-tessellation/

Polygons surrounding each signal

→Small polygons = polygon density ↑

→Heatmap of density

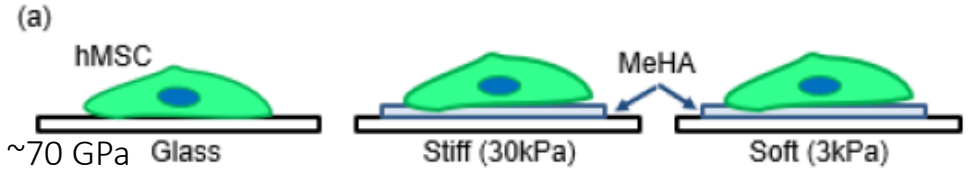


Heo 2021

Small polygon = Dense

Large polygons = Not dense

Influence of substrate stiffness on nano-scale chromatin spatial organization



hMSC: human mesenchymal stem cell
MeHA: methacrylated hyaluronic acid hydrogels

Influence of substrate stiffness on nano-scale chromatin spatial organization

- Histone 2B changes localization depending on substrate the cell is growing on
  - Overall signal / number of localizations stays the same
  - softer substrate, = more peripheral H2B localization
    = heterochromatin
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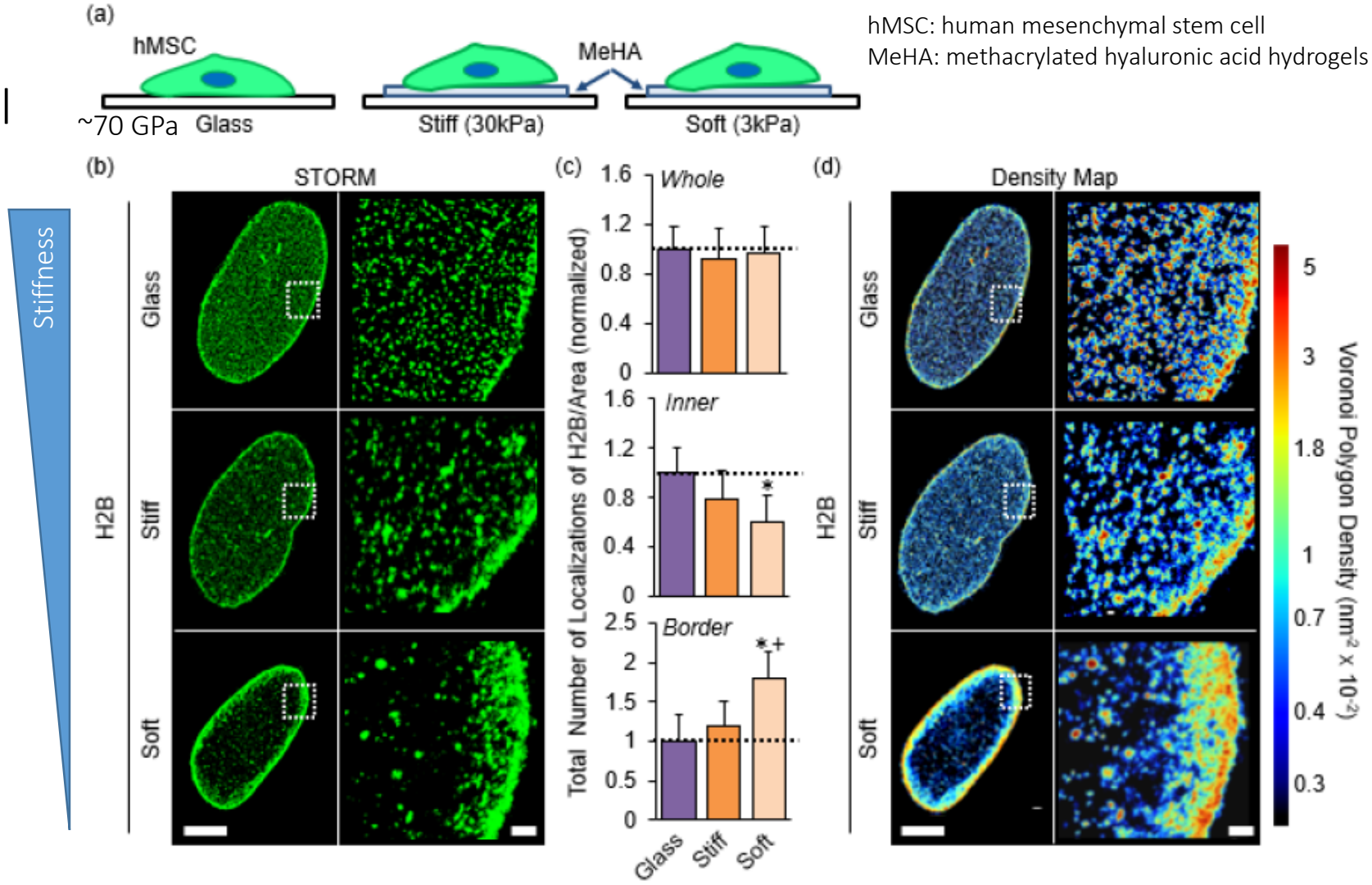

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- Condensed chromatin mainly (but not only!) found in periphery of nuclei in the cells, especially on soft substrate

- Chromatin is more compact on soft than stiff hydrogel, independent of nuclear localization

hMSC: human mesenchymal stem cell
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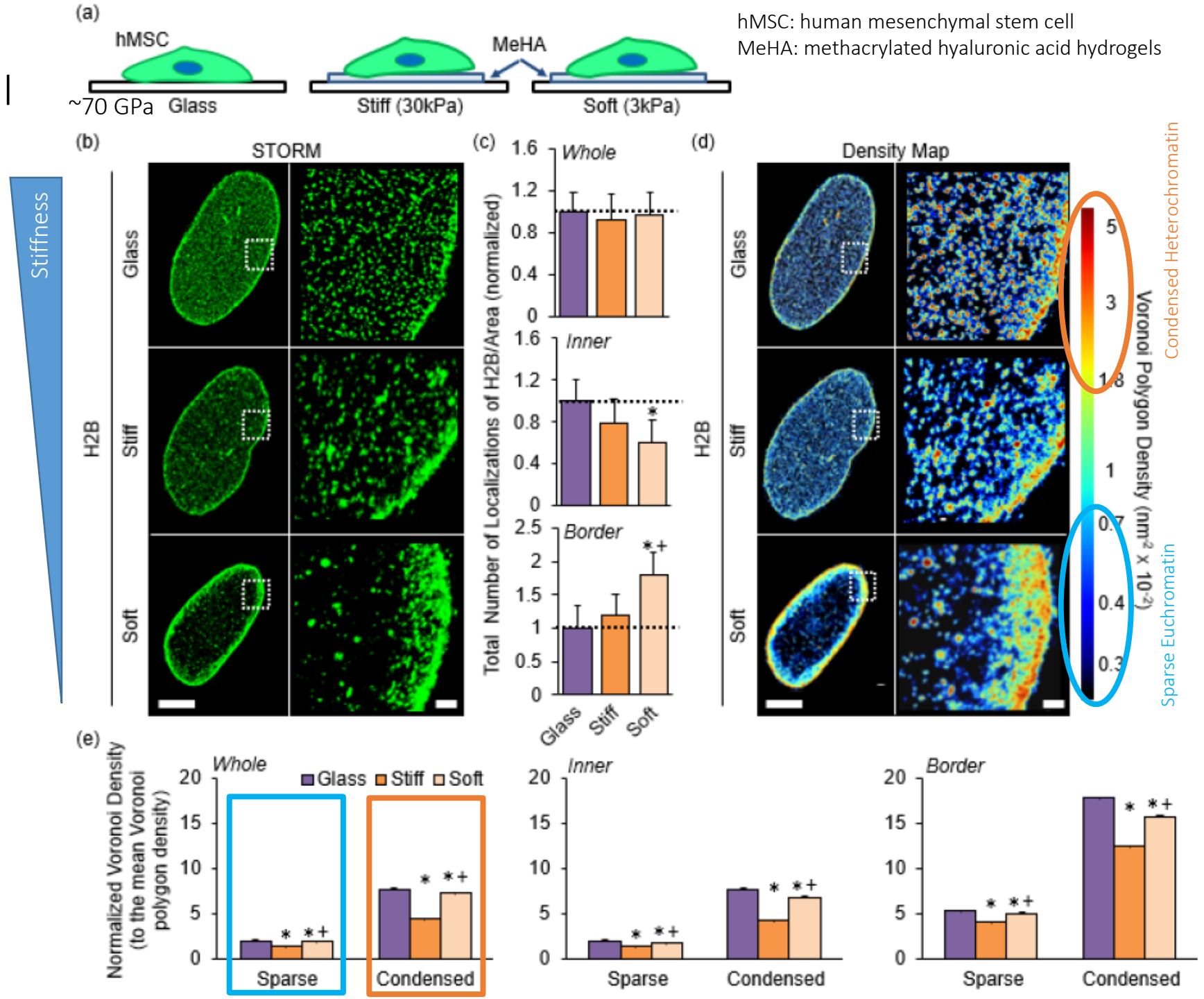

Influence of substrate stiffness on specific chromatin domains → transcriptional activity

Compared to glass:

- Active histone mark (H3K4me3):
  ↑ on stiff substrate
  ↓ on soft substrate
- Repressive mark (H3K27me3):
  ↓ on stiff substrate (mainly nucl. centre)
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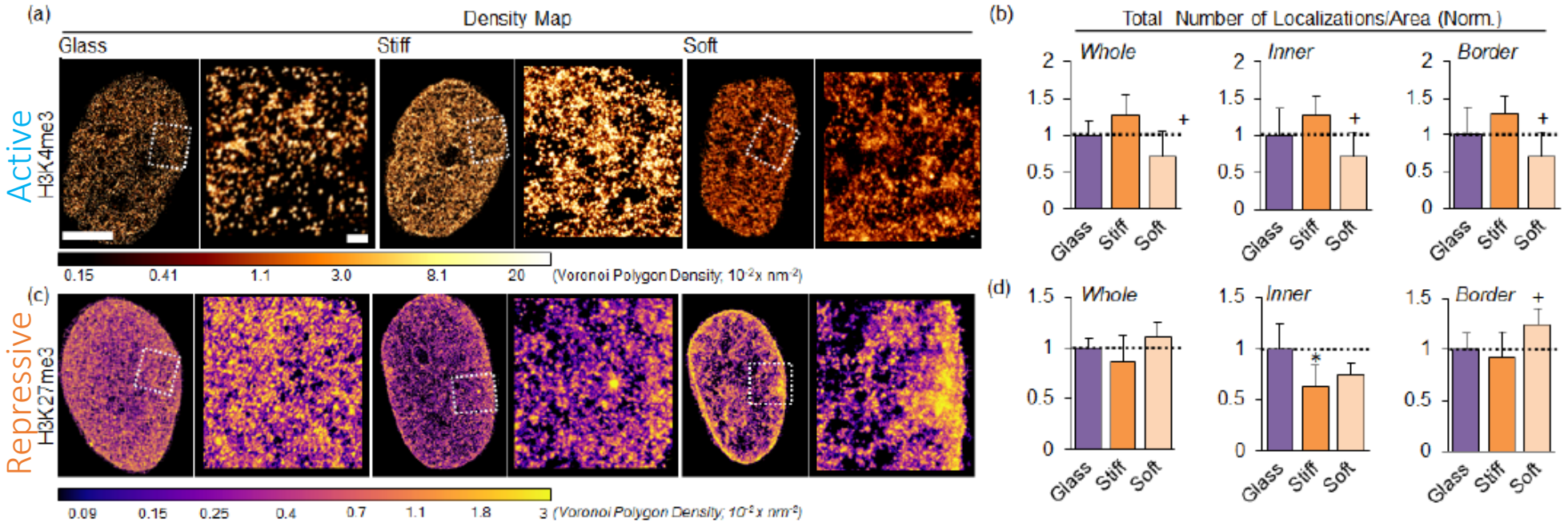

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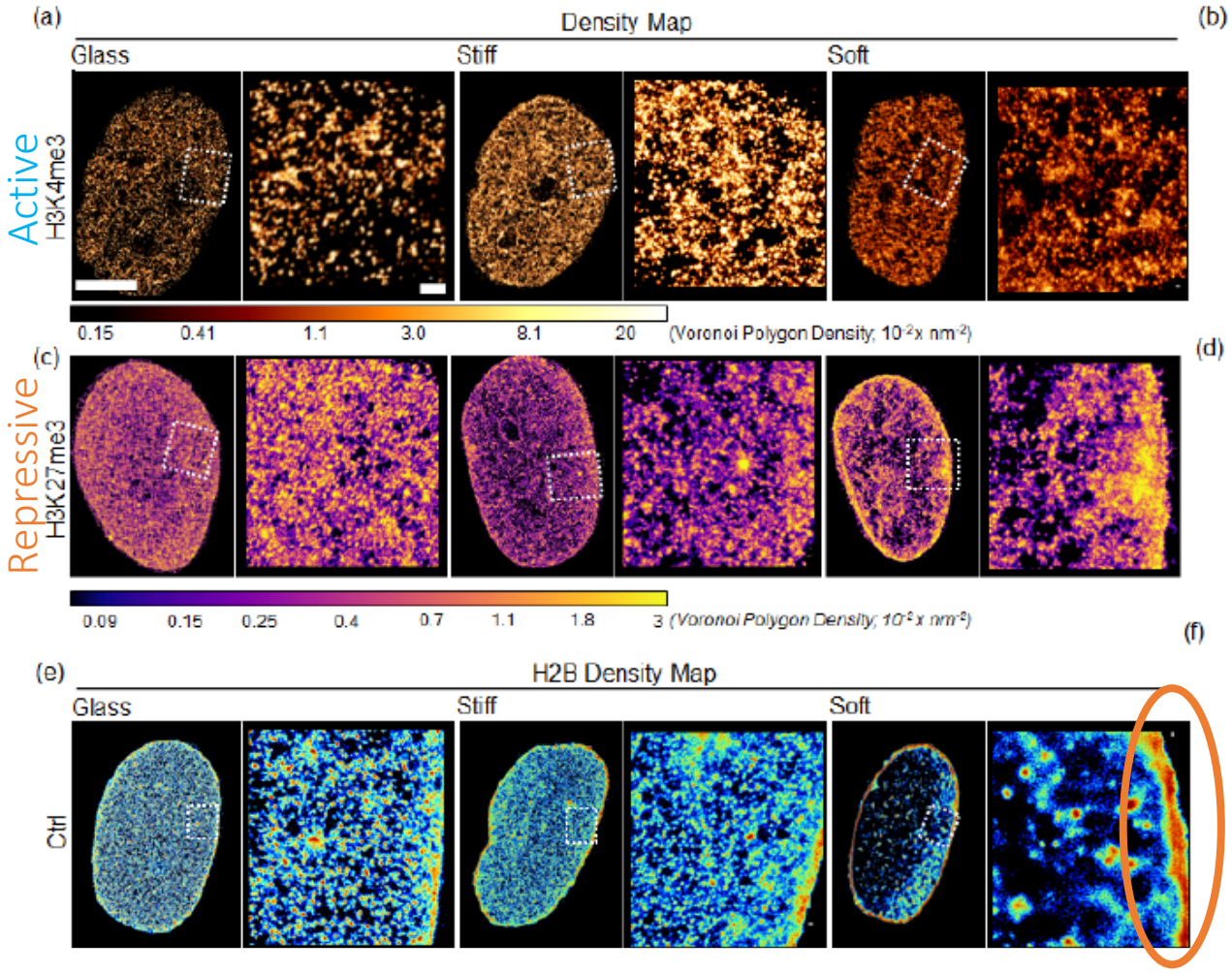

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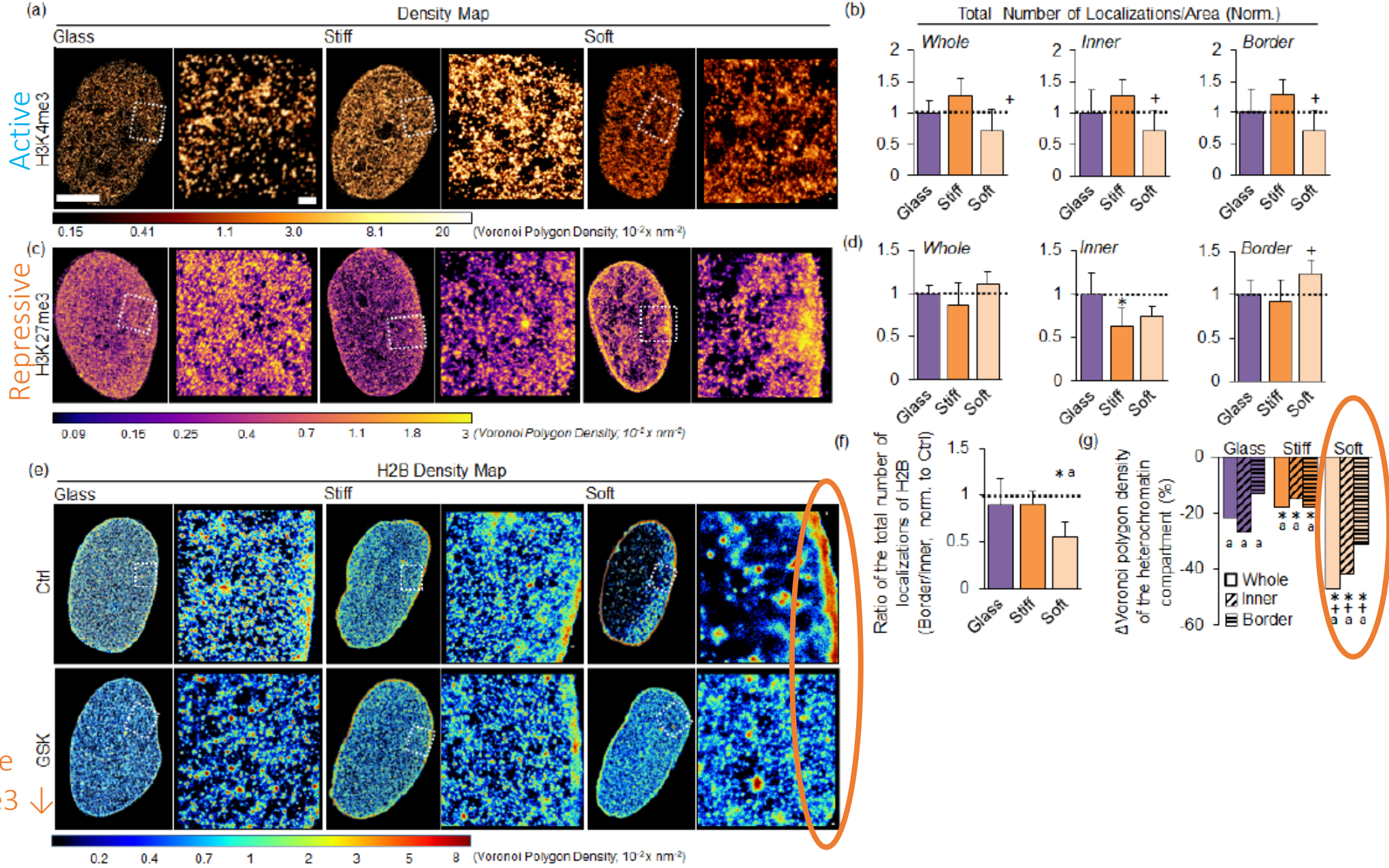

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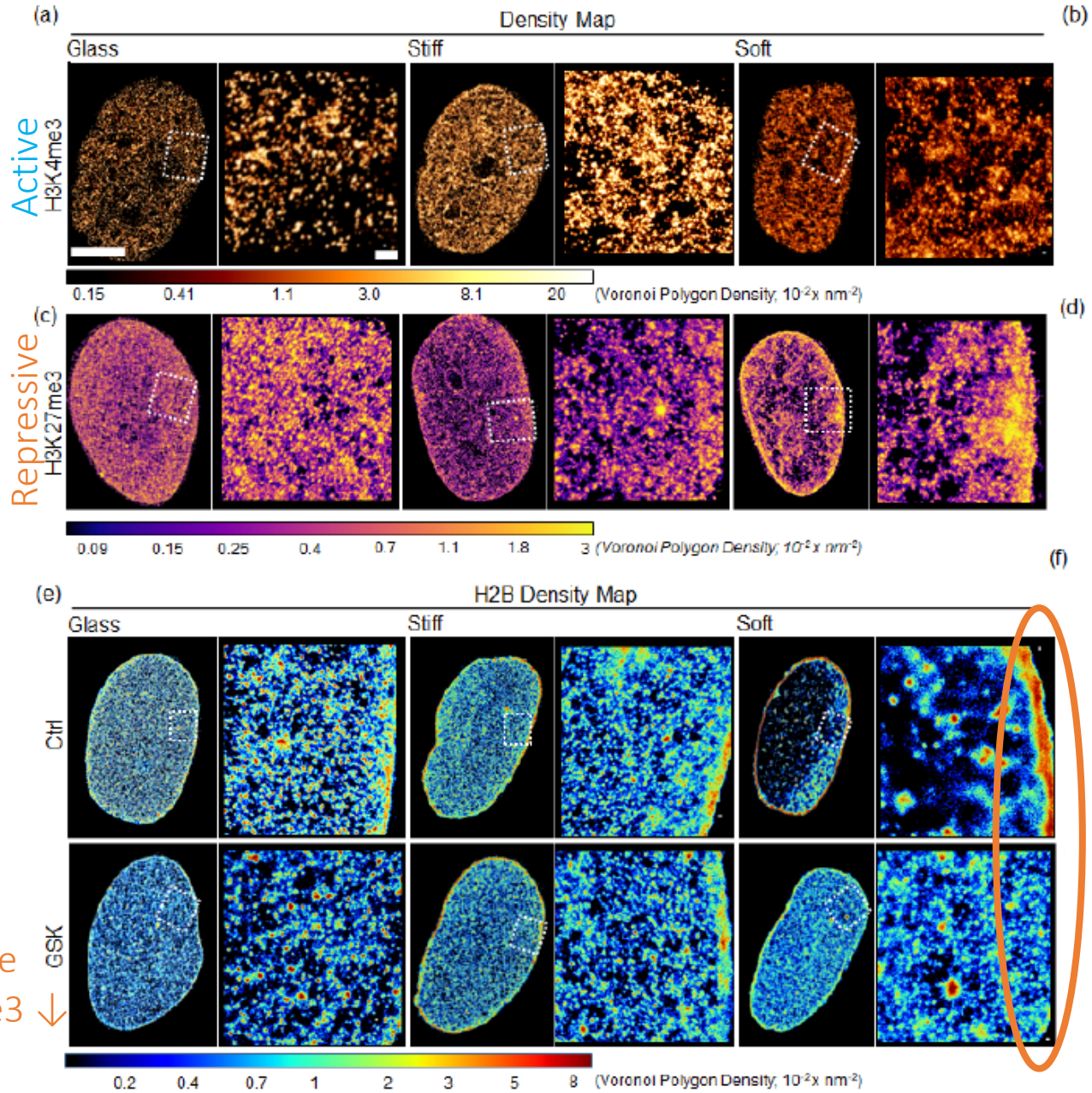
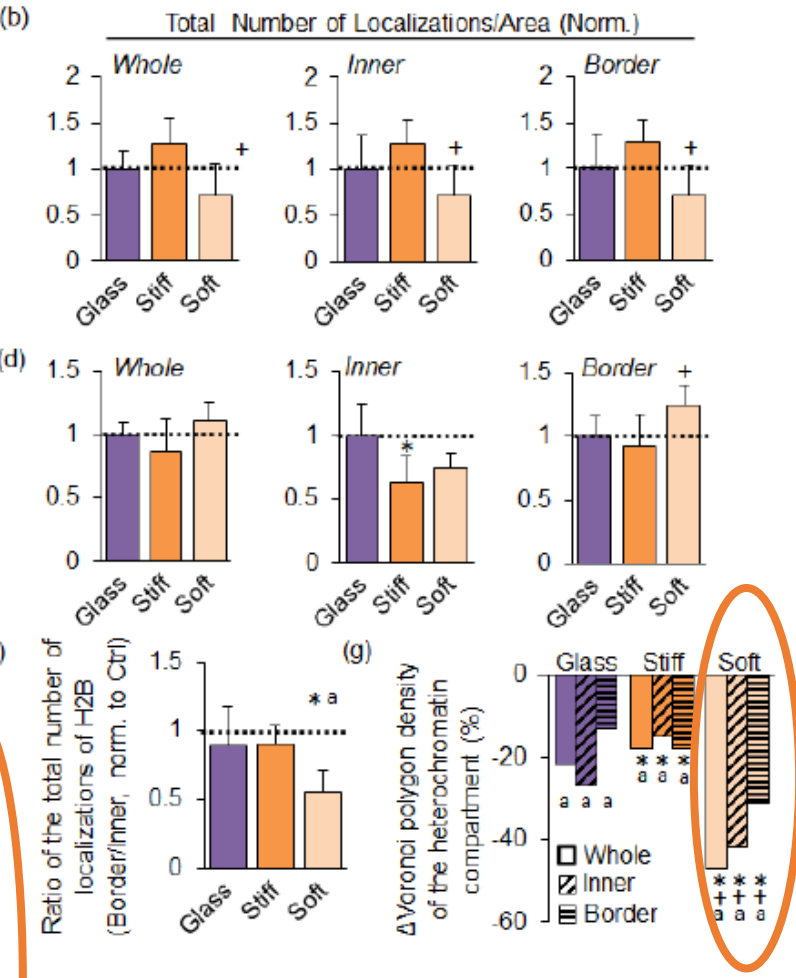


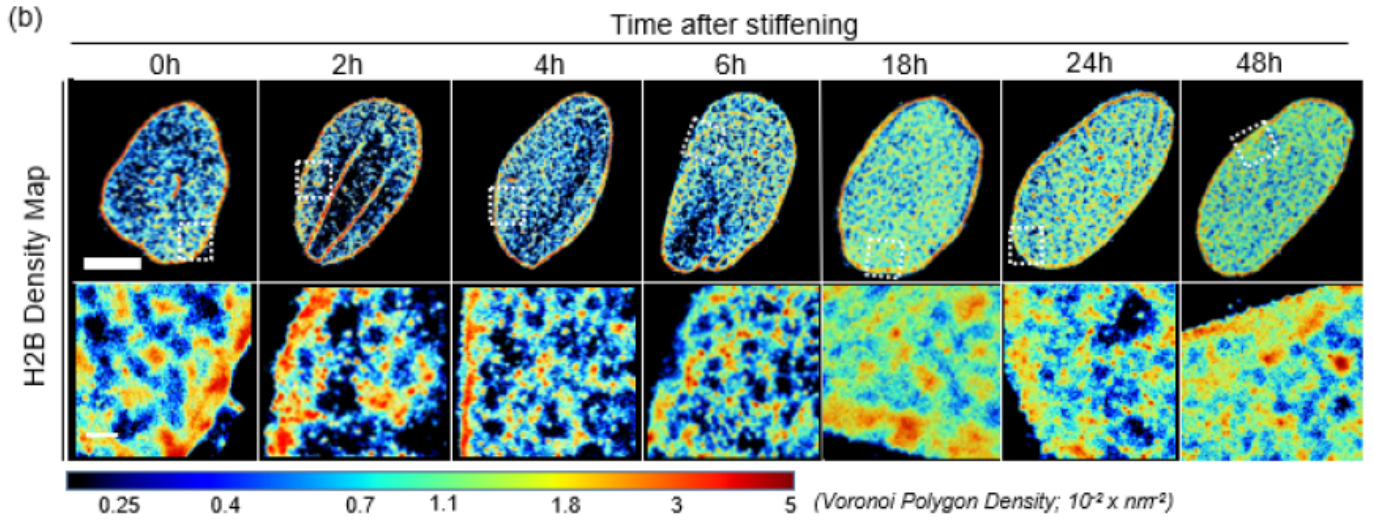


Hypothesis:
Substrate stiffness ↓
→ Methyltransferase EZH2 activity ↑
→ chromatin condensation and accumulation in periphery

Hypothesis:
Substrate stiffness ↑
→ Methyltransferase EZH2 activity ↓
→ chromatin condensation
and relocalization to centre

} Dynamics?

stiffening hydrogel system:
from a soft (~3kPa) to a stiff (~30kPa) mechanical state



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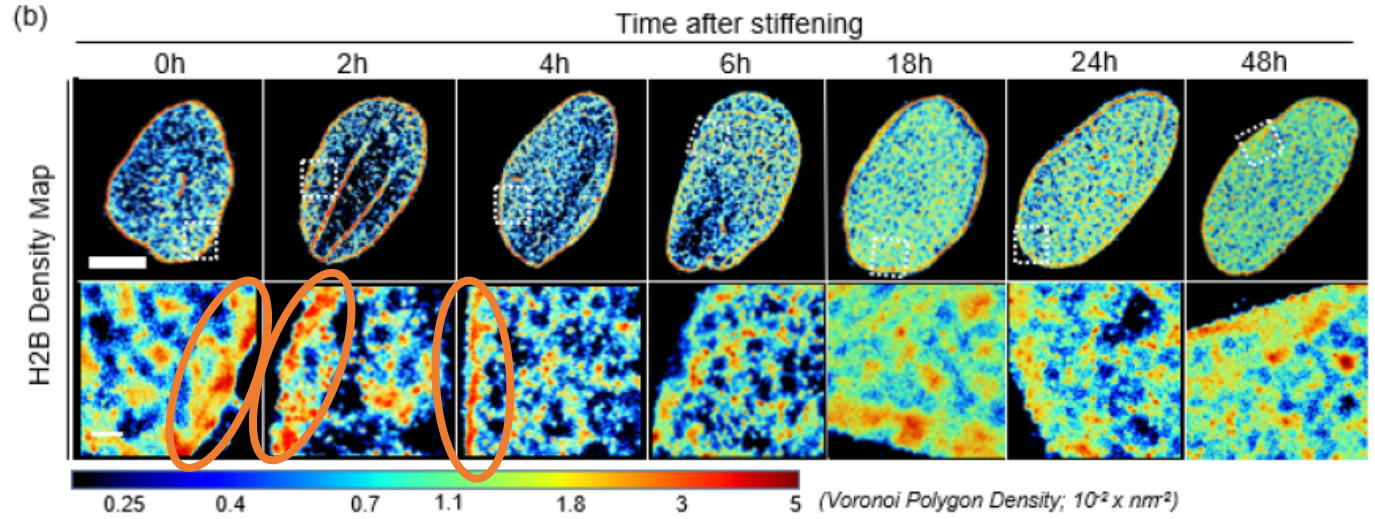
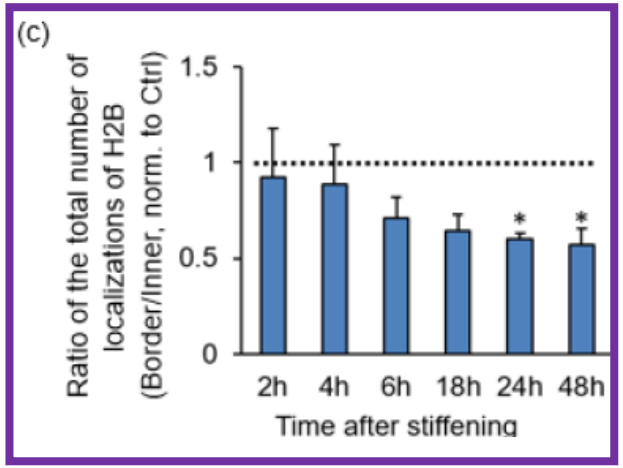
First 4-6 h: most chromatin in nucleus periphery
Then slow redistribution also into nucleus centre





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Dynamics?

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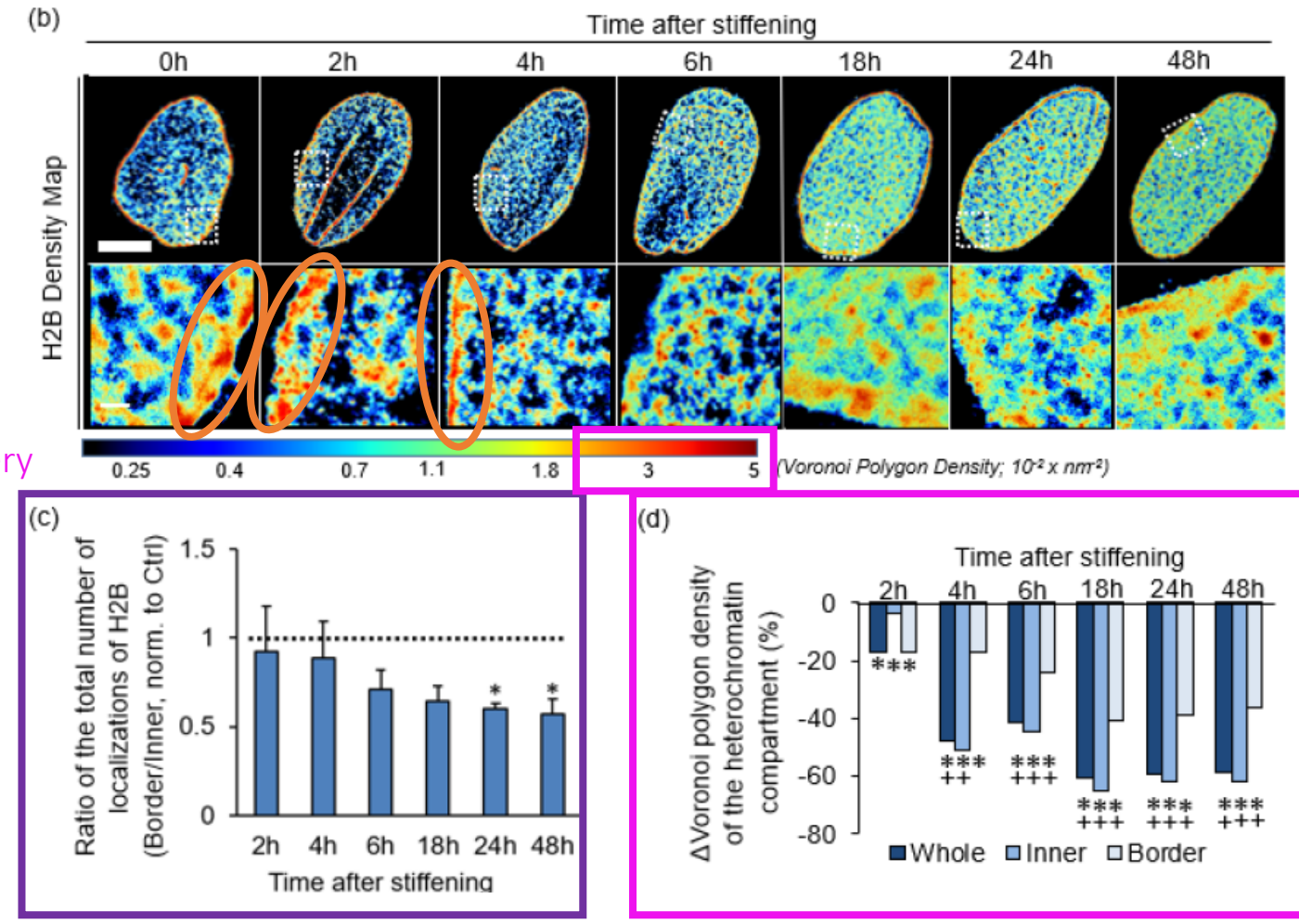
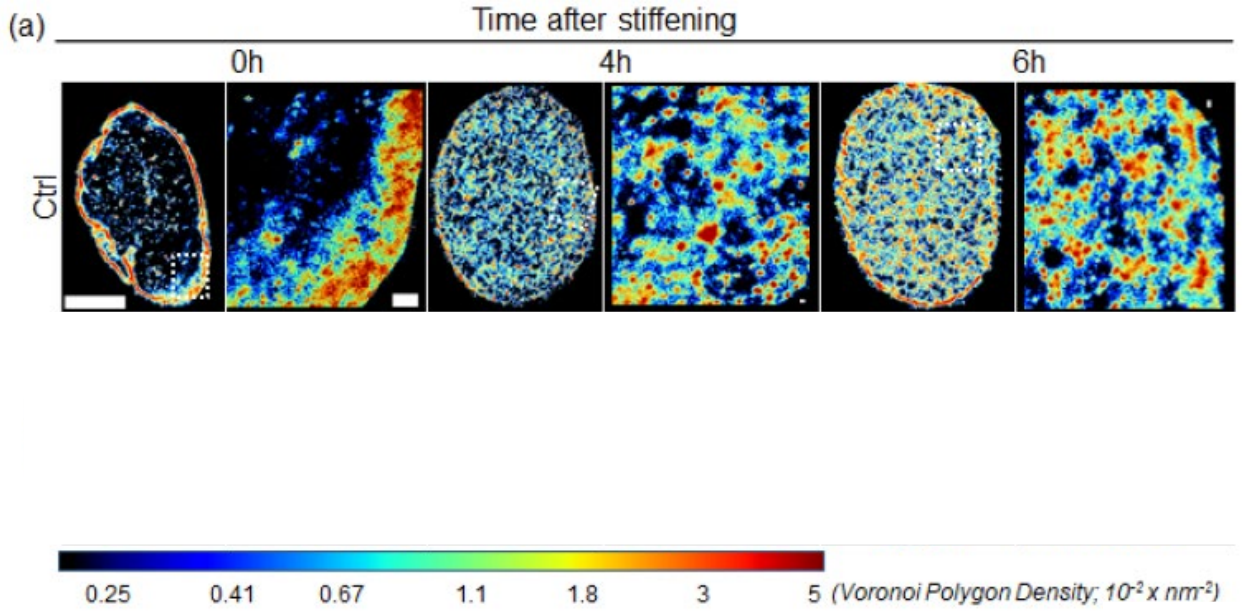
First 4-6 h: most chromatin in nucleus periphery
Then slow redistribution also into nucleus centre

Heterochromatin condensation decreases in centre & periphery
& precedes changes in redistribution (starting at 2h)



The role of cell contractility

Hypothesis:
Substrate stiffness ↑
→ Methyltransferase EZH2 activity ↓
→ chromatin condensation ↓
→ and relocalization to centre ↑



The role of cell contractility

Hypothesis:
Substrate stiffness ↑
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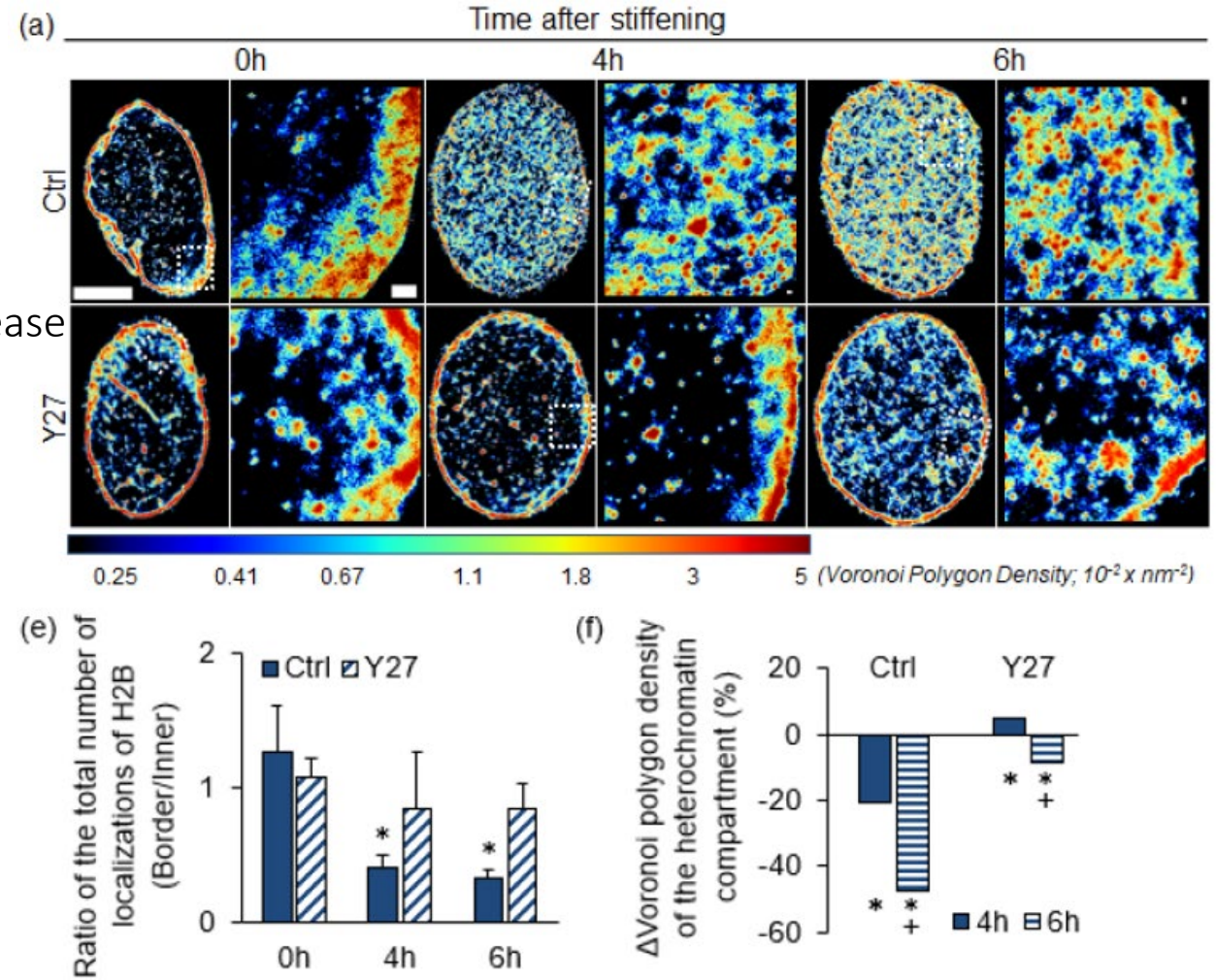
Cell Contractility

Inhibiting cellular contractility
→ Relocalization from border to centre ↓
→ Heterochromatin density does not decrease



Y27632 = ROCK inhibitor
= decreases cell contractility

The role of cell contractility

Hypothesis:
Substrate stiffness ↑
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→ and relocalization to centre ↑
} requires actomyosin based cellular contractility

Inhibiting cellular contractility
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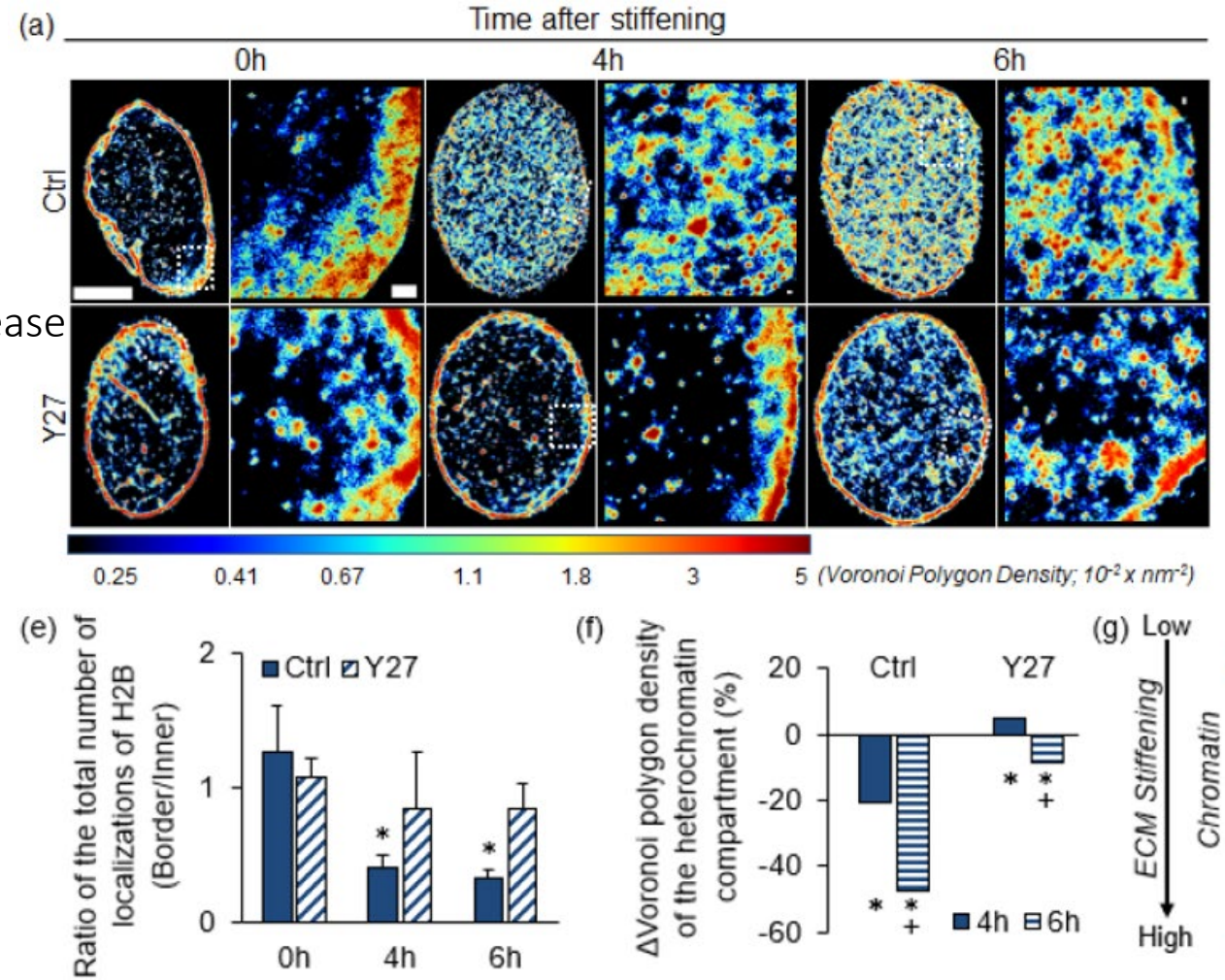

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What about other environmental cues?

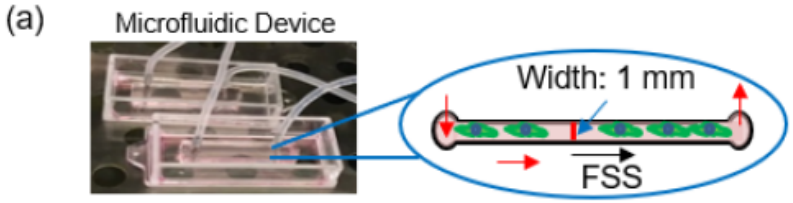
fluid-flow induced shear stress (FSS)

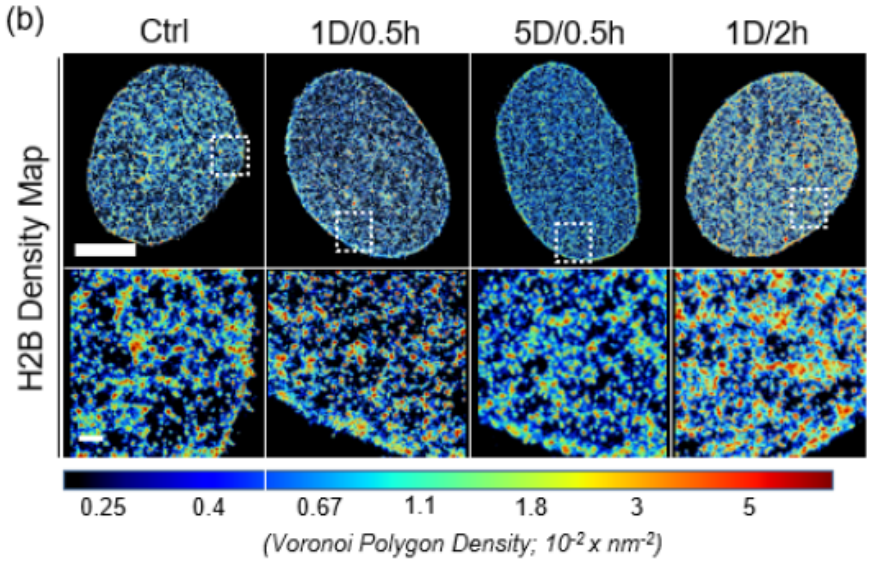
varying magnitude (1 - 5 dyne/cm$_2$) and duration (0.5 - 2 hours)

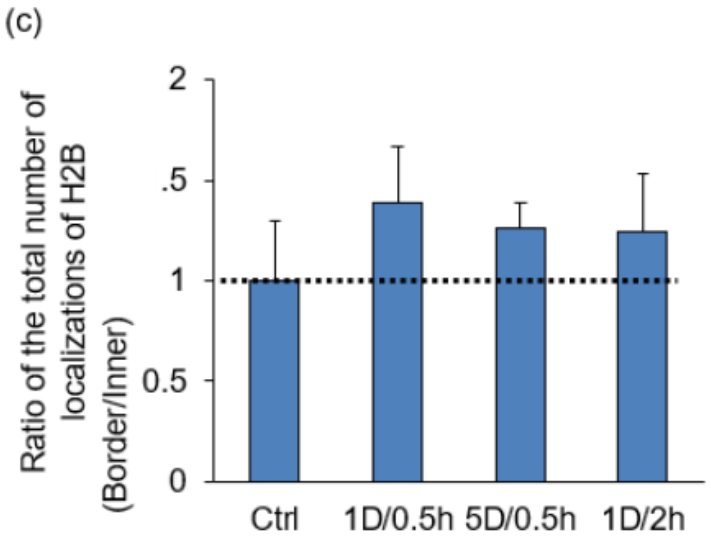
(c)
Shear stress leads to:
→ chromatin relocalization to border

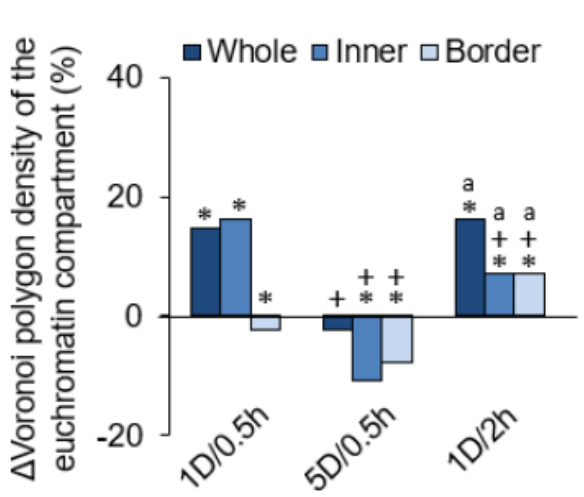
(d) + (e)
Low shear stress
→ Chromatin compactation
High shear stress
→ Less condensation

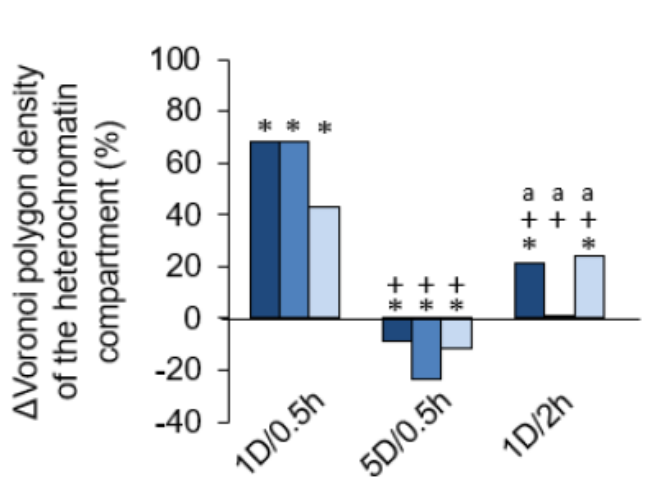
→ Very rapid changes within 30 mins











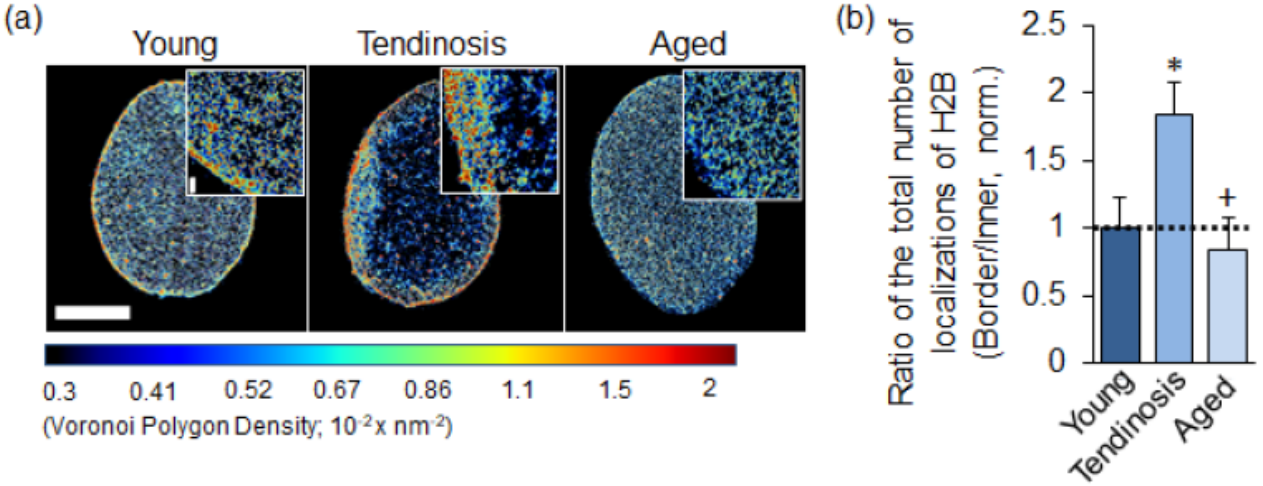
Chromatin structure in aging and diesease

isolated tenocytes → cultured on glass for 48 h

- young healthy: distribution over nucleus, some condensed domains
- young tendinosis: more condensed H2B signal, & higher in periphery
- Aged, healthy: distribution over nucleus, few condensed domains

→ Possible reasons for differences?

→ Changes in ECM, hypoxia, inflammation



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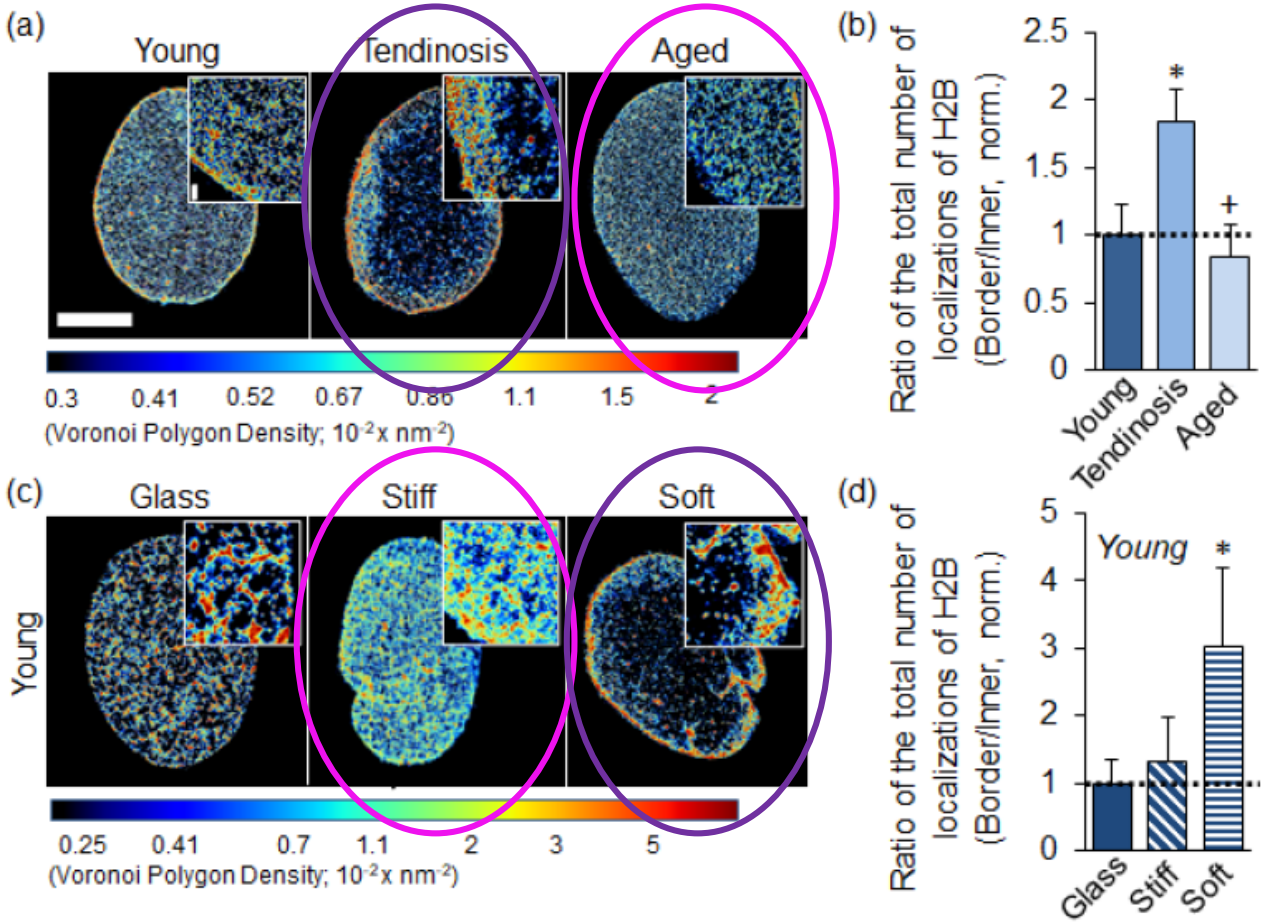

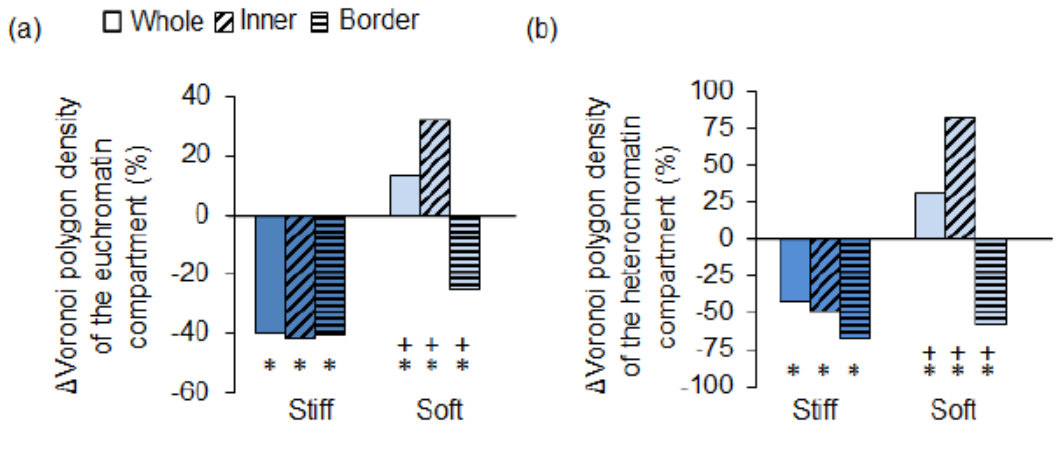

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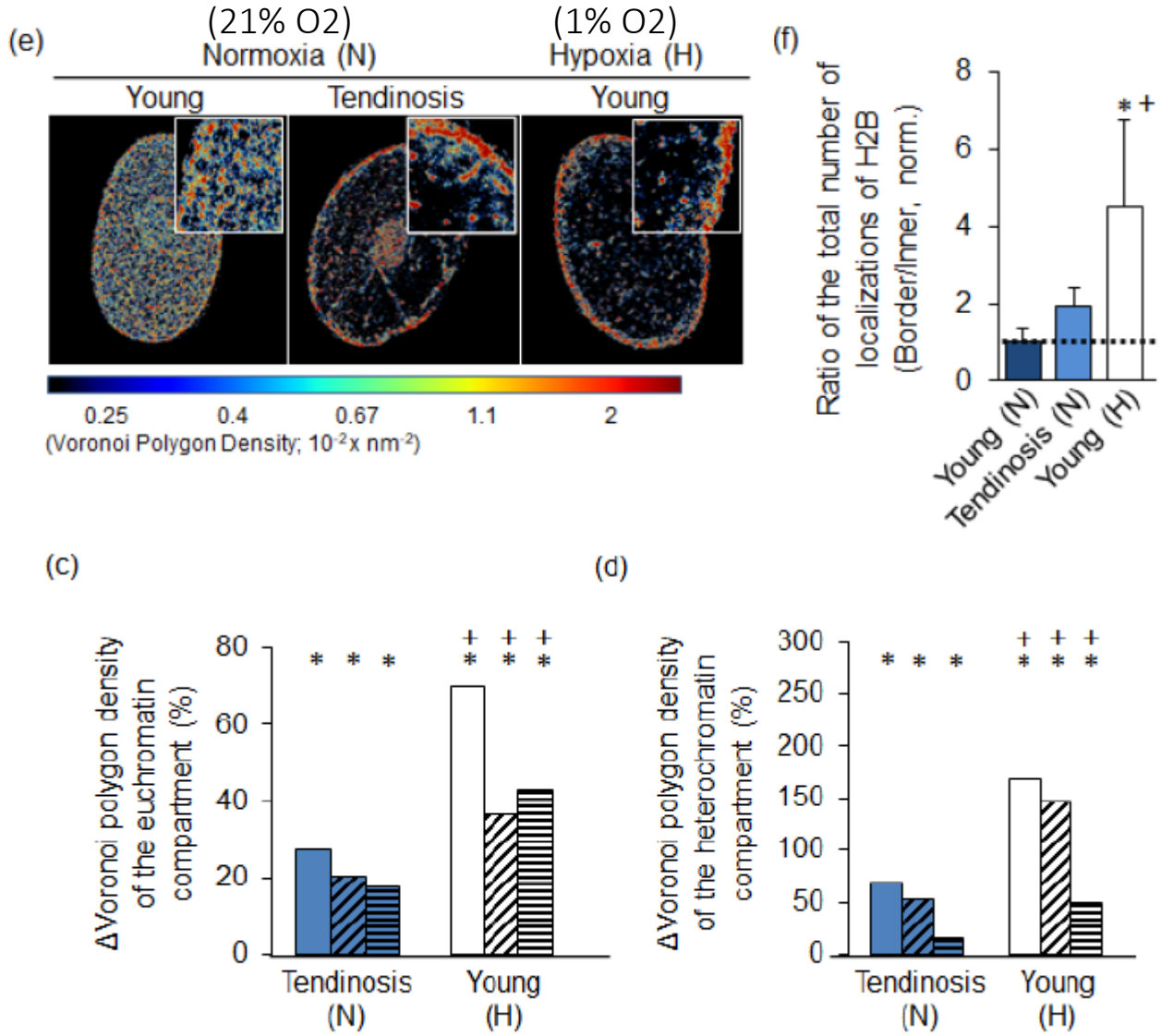
→ Possible reasons for differences?

→ Changes in ECM, **hypoxia**, inflammation

4 days under controlled oxygen levels

- ➢ Young healthy tenocytes resemble diseased tenocytes
  - Condensation ↑↑↑
  - Even more relocalization to border
- ➢ Young diseased tenocytes did not (fully) recover under normoxia



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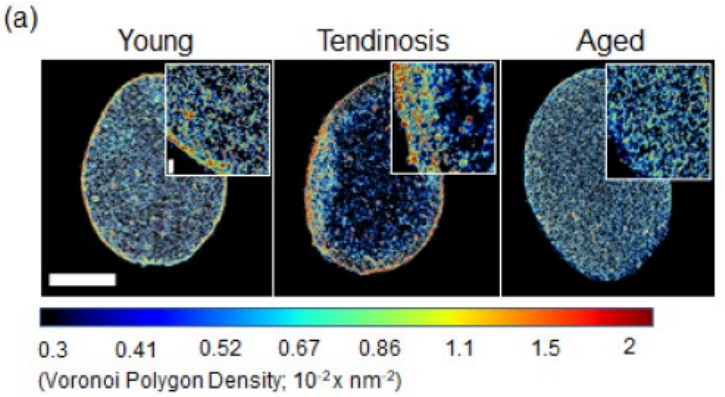
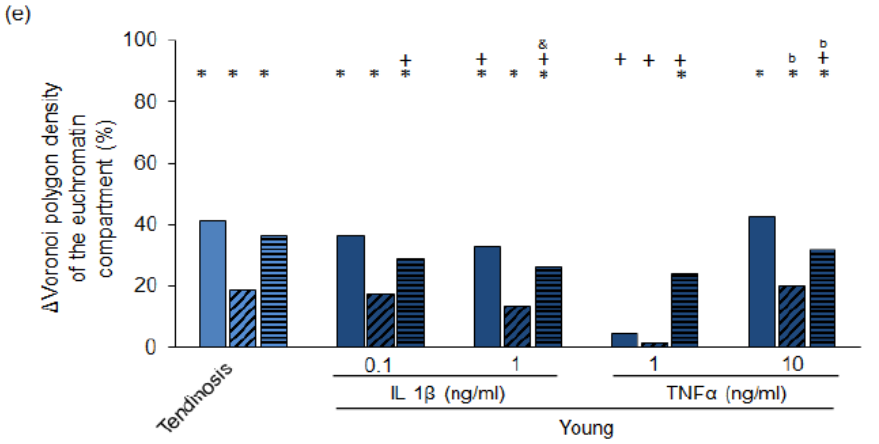
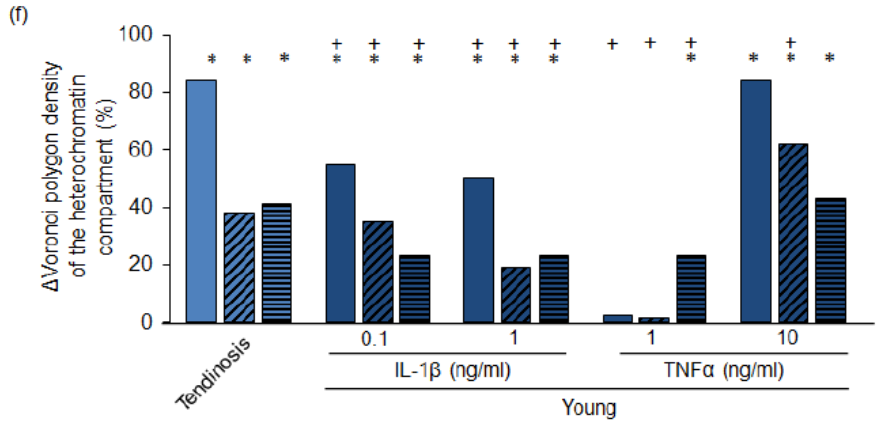
→ Possible reasons for differences?
→ Changes in ECM, hypoxia, **inflammation**

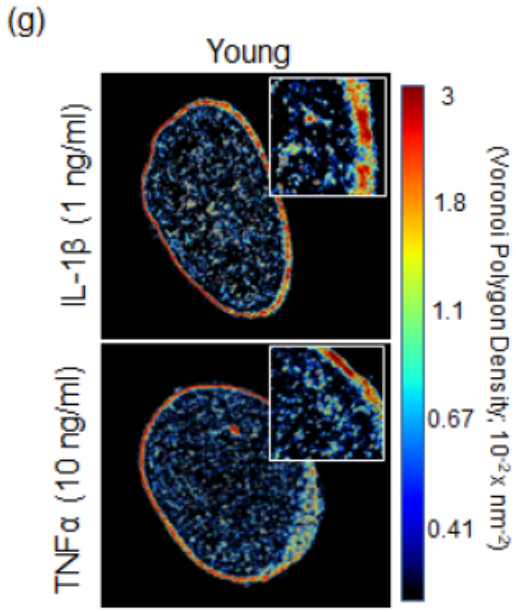
proinflammatory cytokines promote tendon inflammation processes in early tendon repair

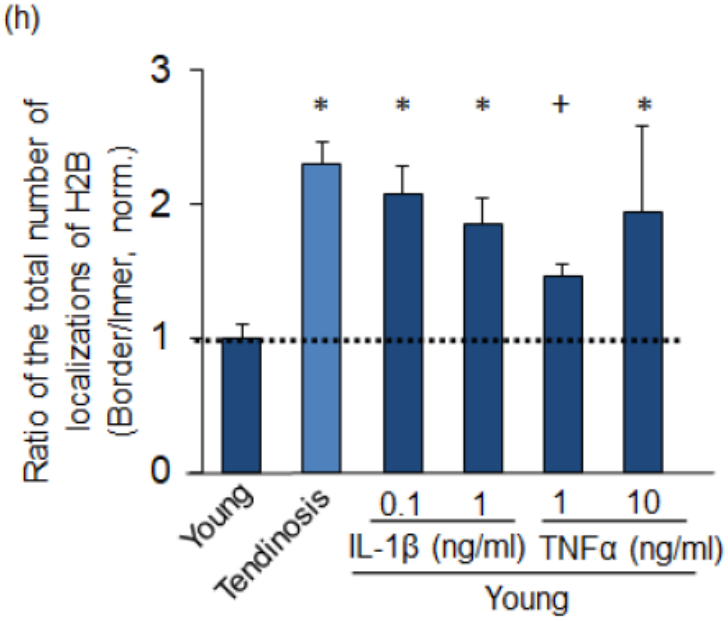


24 h of cytokine treatment on healthy young tenocytes
→ Condensation of chromatin and re-localization to periphery as in tendinosis









Cellular reactivity to changes in environment in age and disesae

How well can aged / diseased tenocytes adapt to a change of the environment?

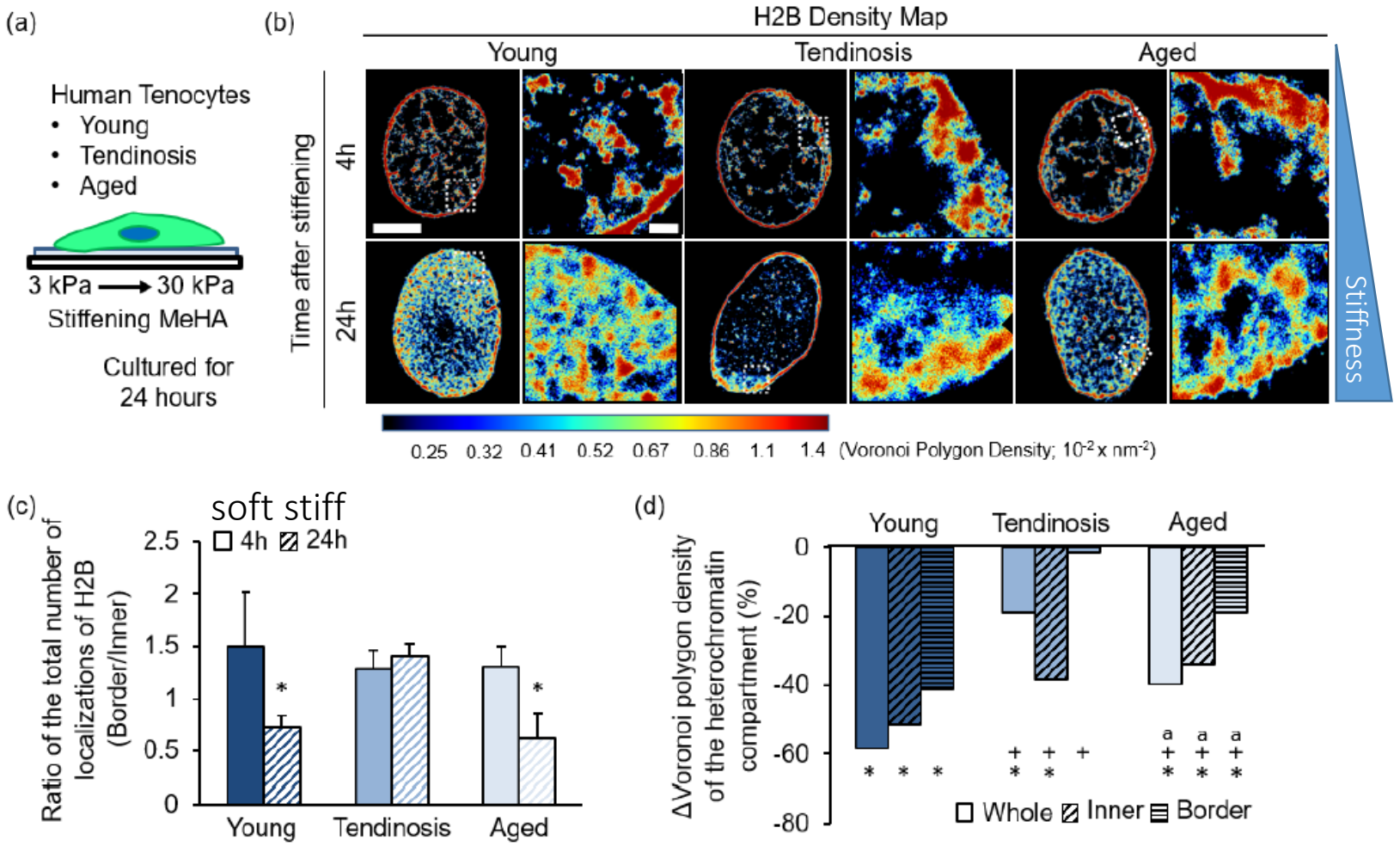
→ Change in stiffness

→ Young helathy tenocytes show drastic changes:
- ➢ dispersion
- ➢ de-compaction

→ Aged tenocytes show same behaviour but less drastic

→ Diseased (tendinosis) tenocytes
- ➢ Did not relocalize
- ➢ No de-condensation at border

→ loss of mechanical sensitivity with degeneration



Conclusions

- Chemical and physical cues in the environment influence the pattern of histone modification and subsequently chromatin organization
- In age and disease the adaptation is impaired
- The chromatin organization in aged and diseased cells may be altered due to changes in their microenvironment (oxygen levels, stiffness, inflammation)

Cell Reports
Article

Super-Resolution Imaging of Higher-Order Chromatin Structures at Different Epigenomic States in Single Mammalian Cells

Jianquan Xu,[1] Hongqiang Ma,[1] Jingyi Jin,[1,2] Shikhar Uttam,[3] Rao Fu,[1,4] Yi Huang,[5] and Yang Liu[1,6,*]

2018

New Results

Chemo-Mechanical Cues Modulate Nano-Scale Chromatin Organization in Healthy and Diseased Connective Tissue Cells

Su-Jin Heo, Shreyasi Thakur, Xingyu Chen, Claudia Loebel, Boao Xia, Rowena McBeath, Jason A. Burdick, Vivek B. Shenoy, Robert L. Mauck, Melike Lakadamyali

doi: https://doi.org/10.1101/2021.04.27.441596

2021