UNIVERSITÀ
DEGLI STUDI
DI TRIESTE

DIPARTIMENTO DI
SCIENZE DELLA VITA

Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

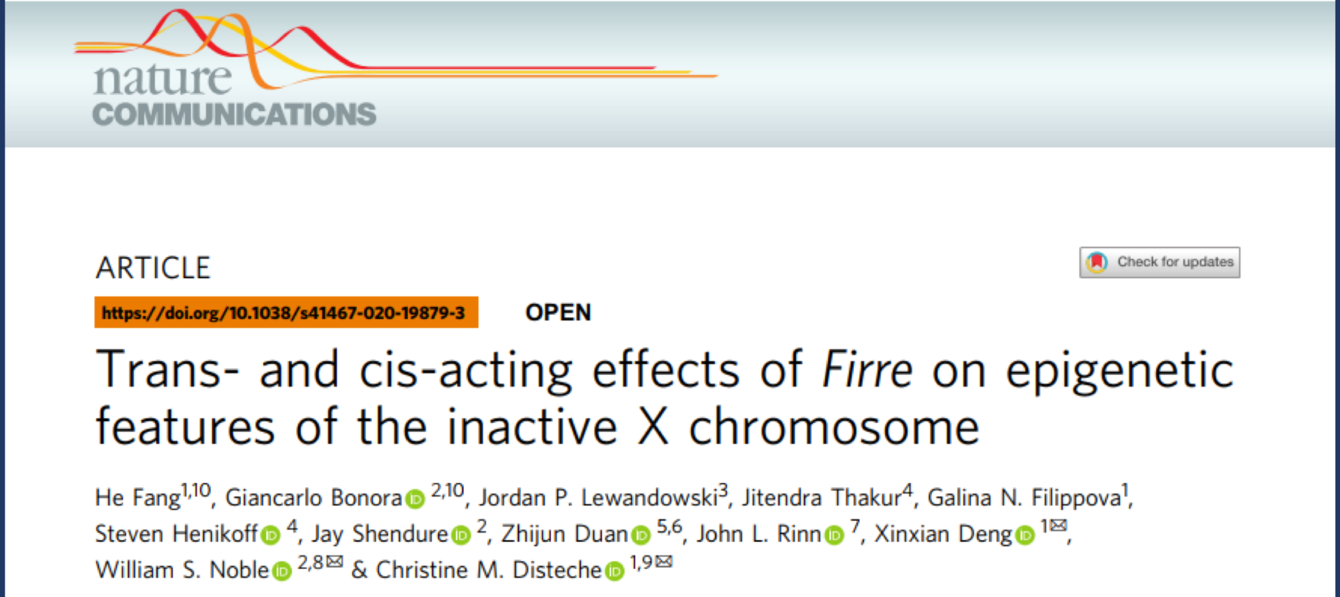
Raphaëlle IMAN - Léna SCHLEICH – Elie THOMAS

Outline

I. Introduction to the X chromosome inactivation

II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

III. Discussion



nature COMMUNICATIONS

ARTICLE

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Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

He Fang[1,10], Giancarlo Bonora[2,10], Jordan P. Lewandowski[3], Jitendra Thakur[4], Galina N. Filippova[1], Steven Henikoff[4], Jay Shendure[2], Zhijun Duan[5,6], John L. Rinn[7], Xinxian Deng[1✉], William S. Noble[2,8✉] & Christine M. Disteche[1,9✉]

I. Introduction to the X chromosome inactivation

- → General introduction
- → Importance of the genome 3D structure
- → TADs modulators : *Xist*, CTCF, and *Dxz4*
- → Focus on *Firre* lncRNA, another TADs modulator
- → Study models of XCI

Mammalian sex chromosome

X chromosome = ~900-1500 genes

Y chromosome = ~70 genes

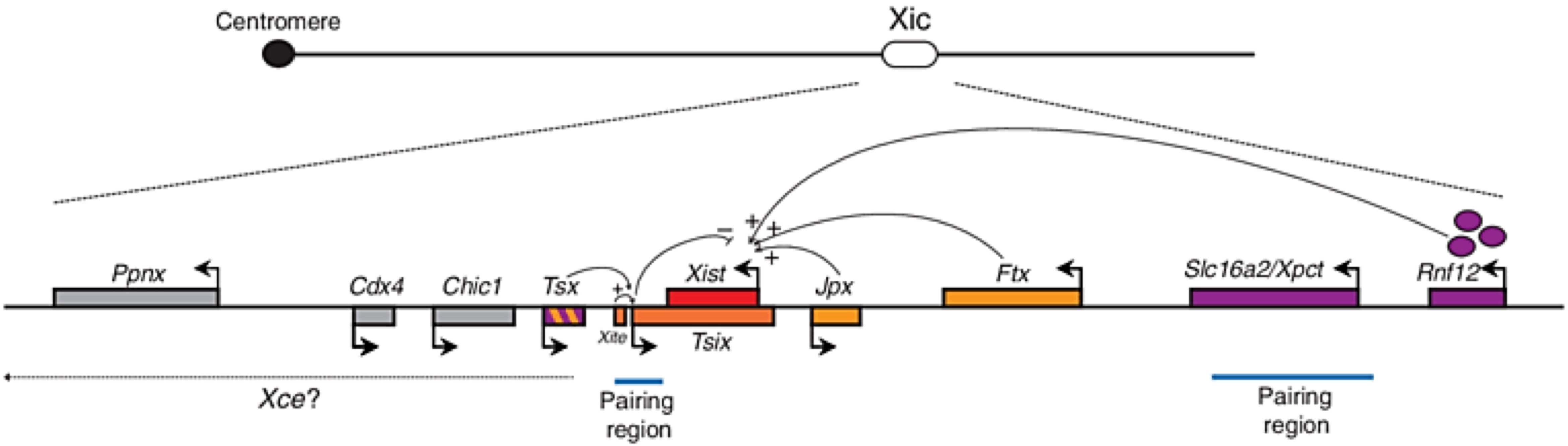
Gene dosage mechanism needed

- ✓ Higher regulation of genes located on the X chromosomes
- ✓ **X chromosome inactivation (XCI), only in female (XX)**

XCI: random silencing *of one of the female X chromosomes beginning in the early embryogenesis and maintained throughout somatic cells in an organism*

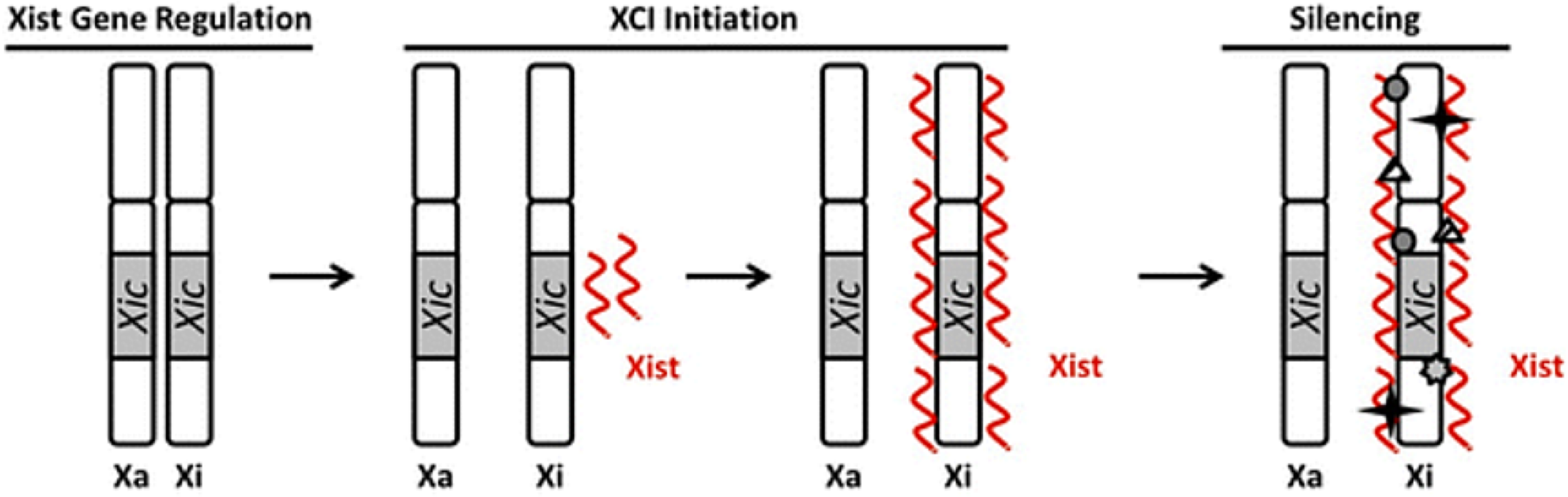
X chromosome-inactivation center (Xic)

- XCI depends on the **X chromosome-inactivation center (Xic),** essential for X inactivation
- Xic contains antisense pair of **lncRNAs**, encoded by ***Xist*** and *Tsix*, among others



Gendrel and Heard, Development (2011)

X inactivation steps implicate Xist lncRNA



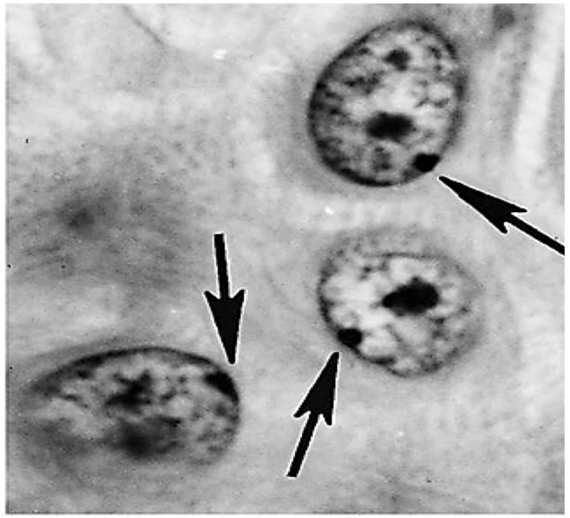
Tattermush et al. 2011

Active X chromosome (Xa)

Inactive X chromosome (Xi)

Xist and *Tsix* are transcribed from all active X chromosomes (Xa) in each male and female cell until inactivation is initiated.

Condensed Xi chromosome forms the **Barr body** in the perinucleus

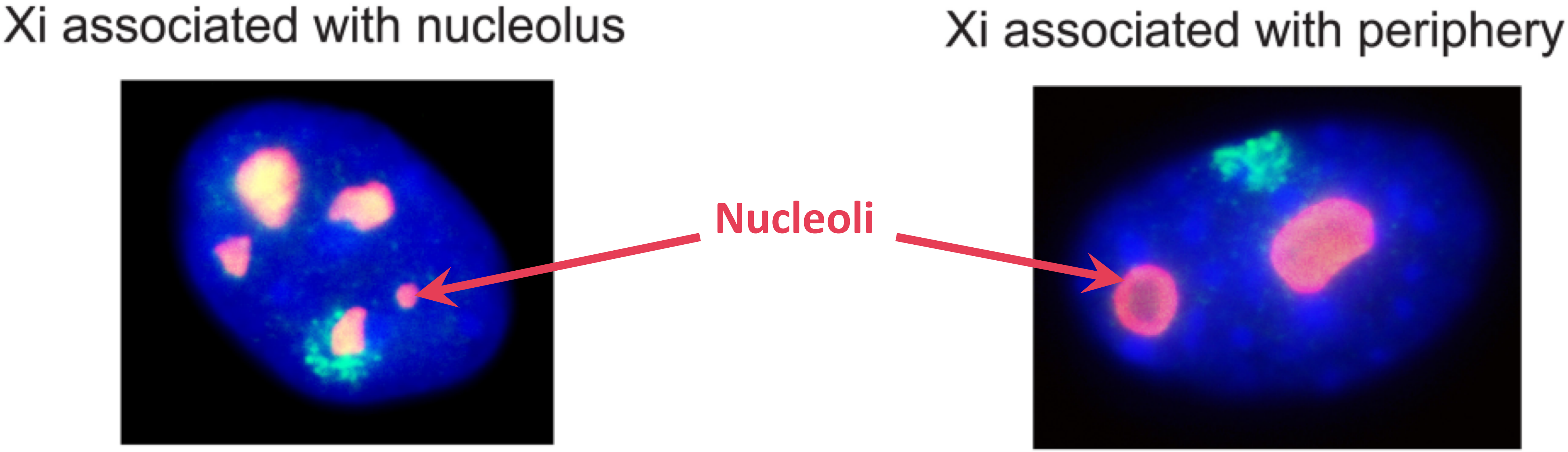


Nason and Dehaan, Biological World (1973)

Nuclear localization of the Xi is important for the XCI

- **Nucleolar location of the Xi** important for its **repressive epigenetic state**

- Xi preferentially localized **near the nuclear periphery or the nucleolus**
  → Factors unrelated to XCI may control positioning



Xi associated with nucleolus

Xi associated with periphery

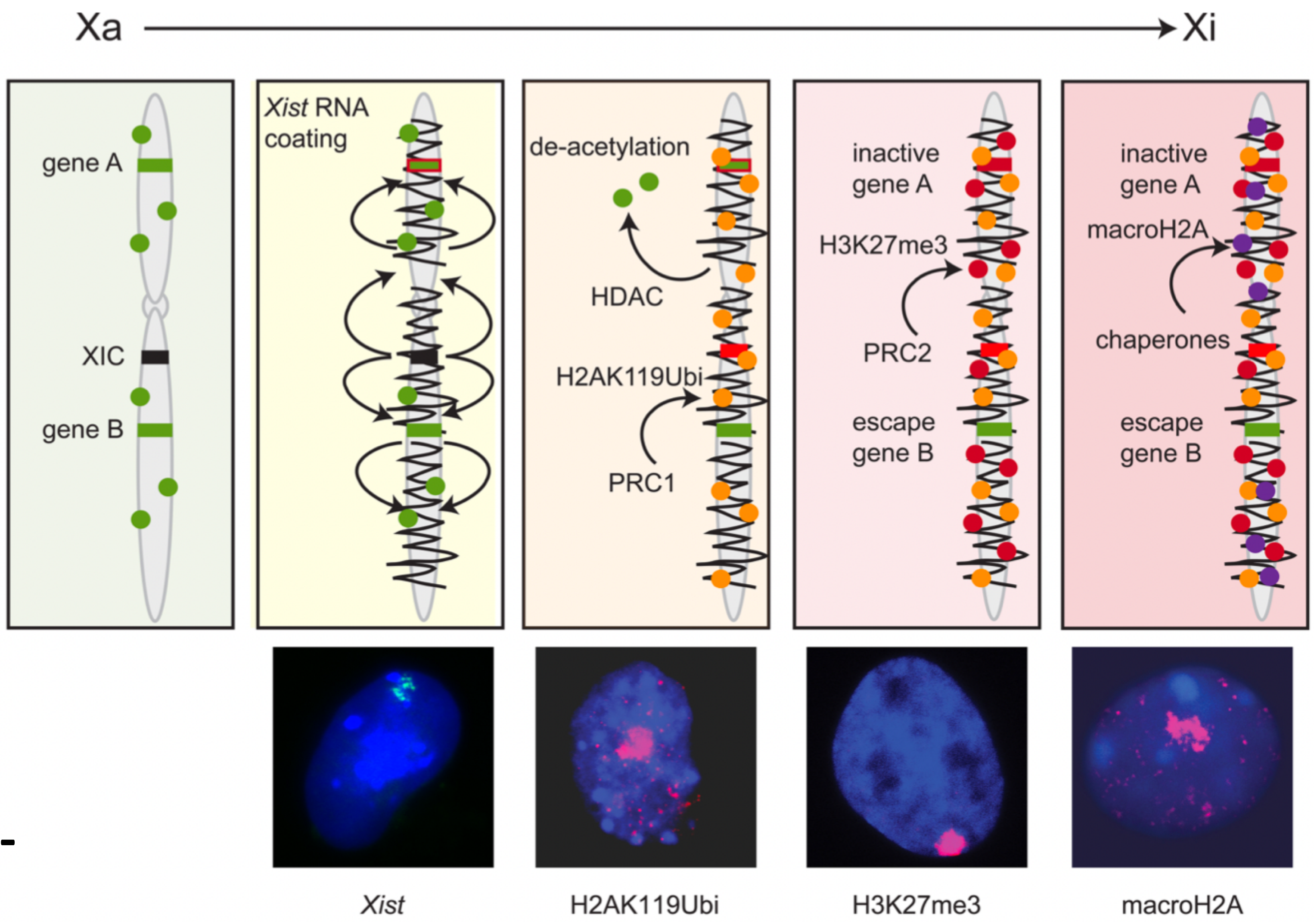
Nucleoli

Fang et al. 2019, Cell and Developmental Biology

Xist lncRNA recruits other factors for X silencing

XCI steps:

1. *Cis*-coating of the future Xi by the lncRNA *Xist*
2. *Xist* recruits **repressive factors** (PRC1, PRC2…) to stabilize its transcriptional silencing of Xi
3. Changes of 3D structure and location of the Xi, depending on **X-linked lncRNA loci: *Dxz4, Firre*…**



Fang et al., Cell and Developmental Biology (2019)

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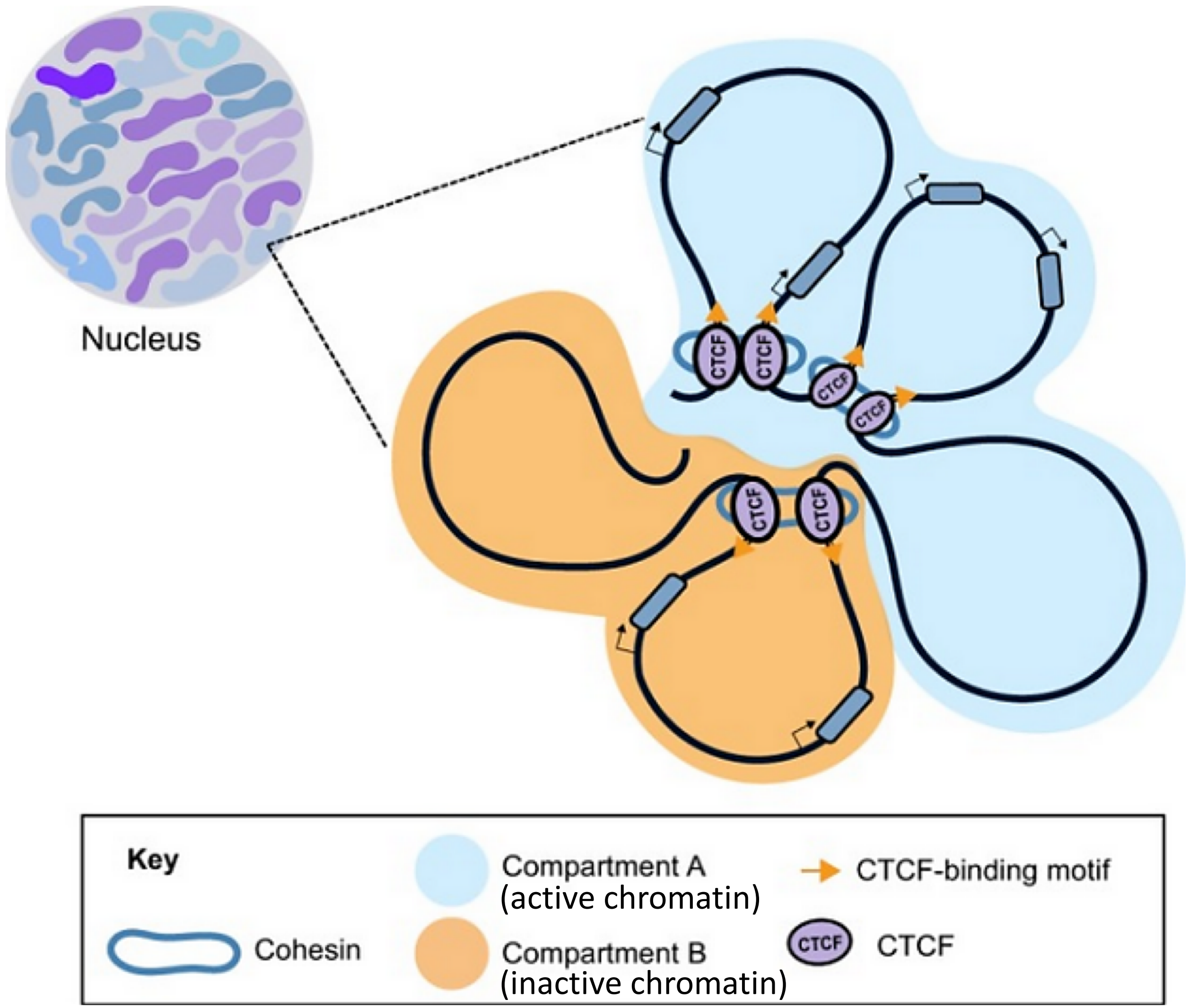
General 3D structure of the genome

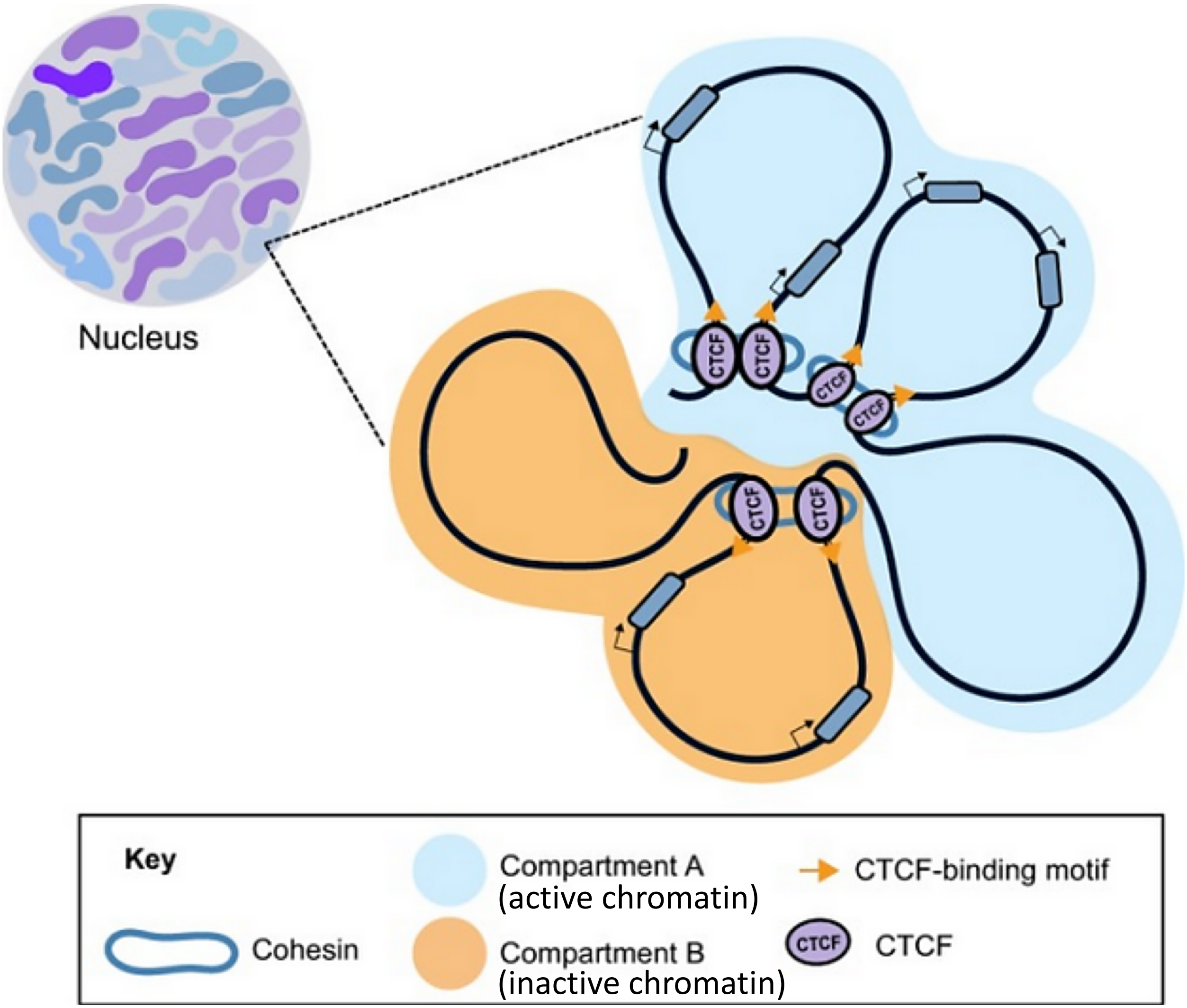


Arzate-Mejía et al., Development (2018)

Compartment A (actively transcribed euchromatic regions) and **compartment B** (heterochromatic regions) self-interact

Topologically associated domains (TADs) = regions with frequent intrachromosomal interactions

General 3D structure of the genome



Arzate-Mejía et al., Development (2018)

TADs with similar:

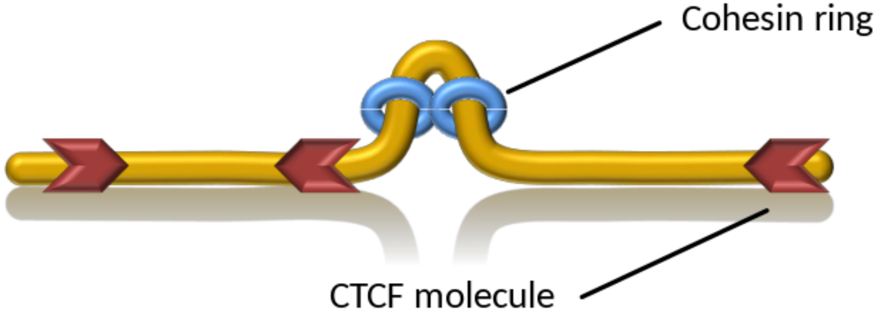
- ✓ Histone modifications
- ✓ Transcription level
- ✓ DNA replication timing

Boundaries surrounding TADs:

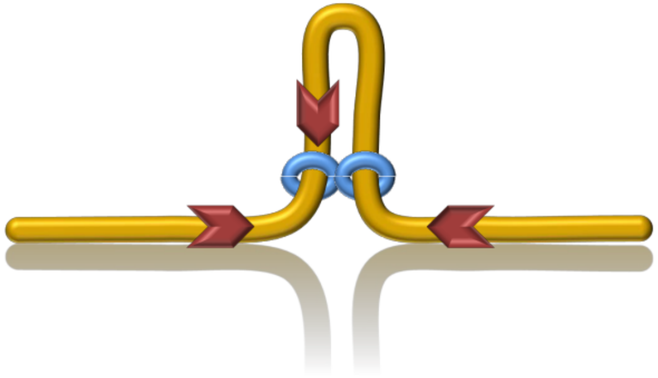
- ✓ Conserved across species
- ✓ Enriched with **CTCF binding factor and cohesin binding sites**
  - → **Loops extrusion**

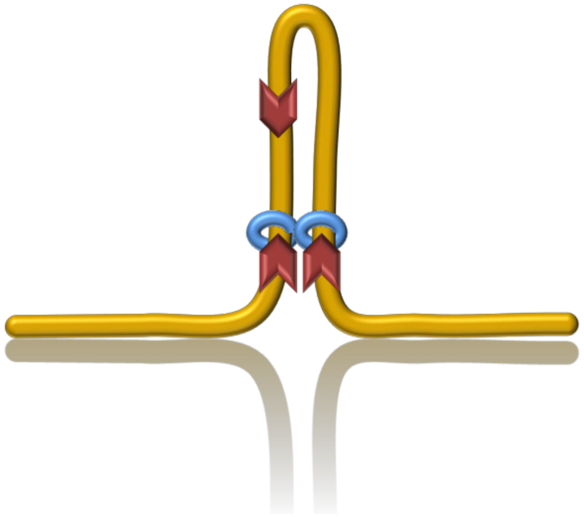
Chromatin loop extrusion driven by cohesin rings

1. Cohesin rings bind to DNA



2. The cohesin rings slide over the **outward directed CTCF sequence**
→ Loop continues to grow.



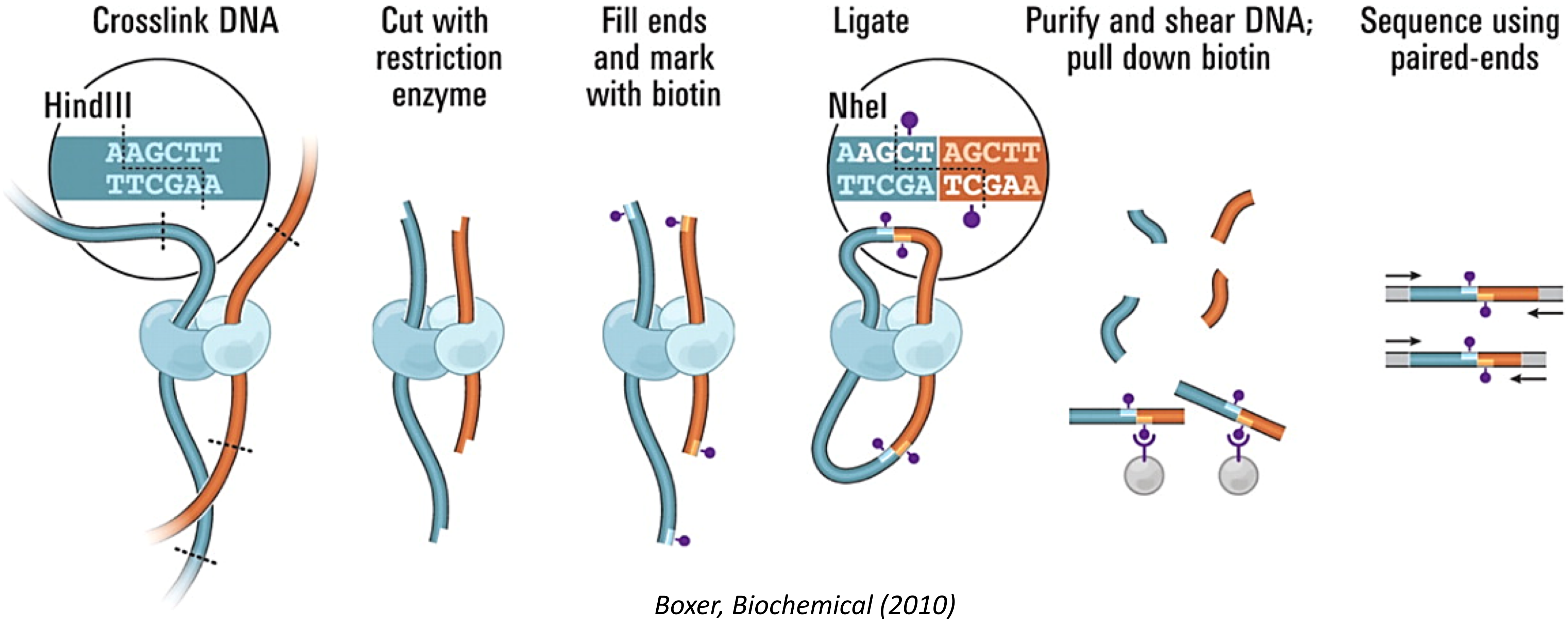


3. Each of the ring has reached an **inward directed CTCF sequence**
→ Loop formation stops

✓ **CTCF binding sites stabilize chromatin loops**

High-throughput chromosome conformation capture (Hi-C)

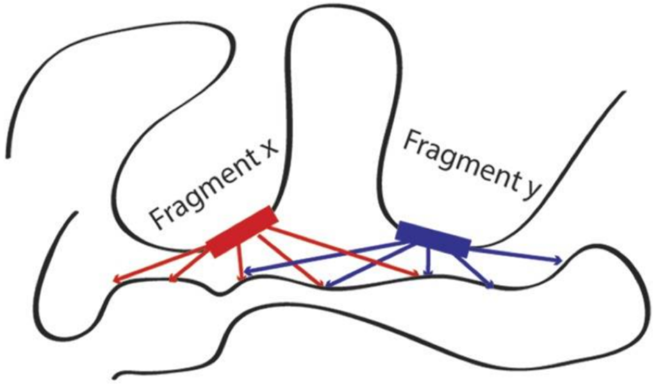
***Hi-C** = **high-throughput sequencing** and can identify genome-wide long-range interactions (compartmentations, TADs, and chromatin loops)*

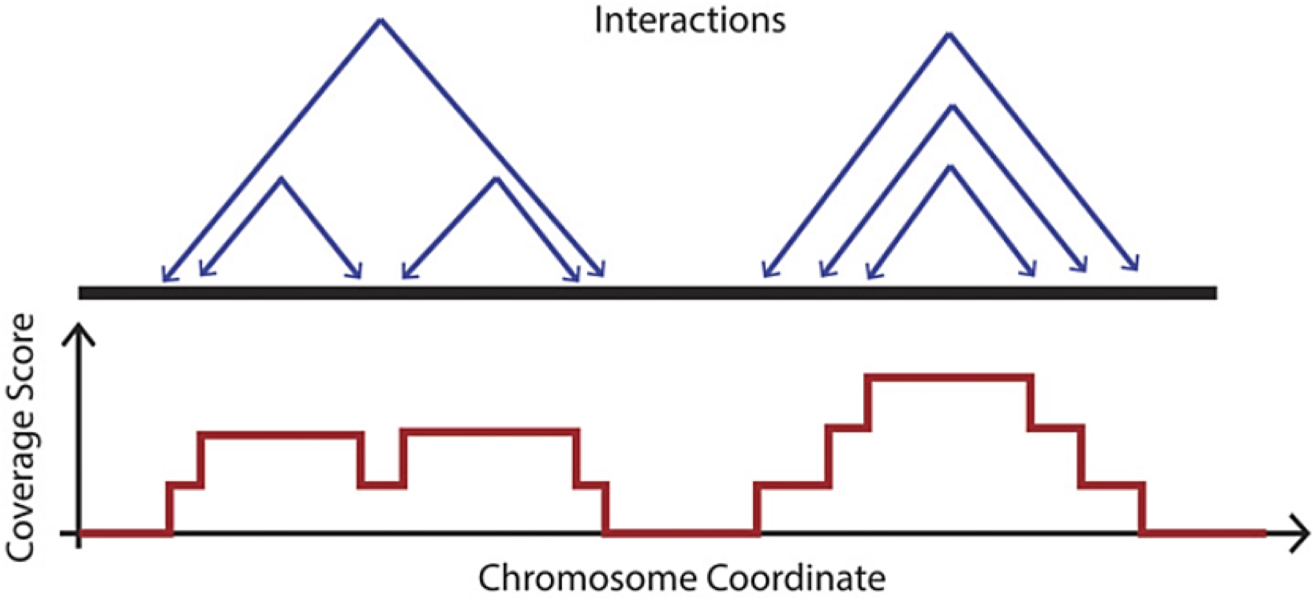


Boxer, Biochemical (2010)

Hi-C data analysis to study TADs

***Hi-C** = **high-throughput sequencing** and can identify genome-wide long-range interactions (compartmentations, TADs, and chromatin loops)*



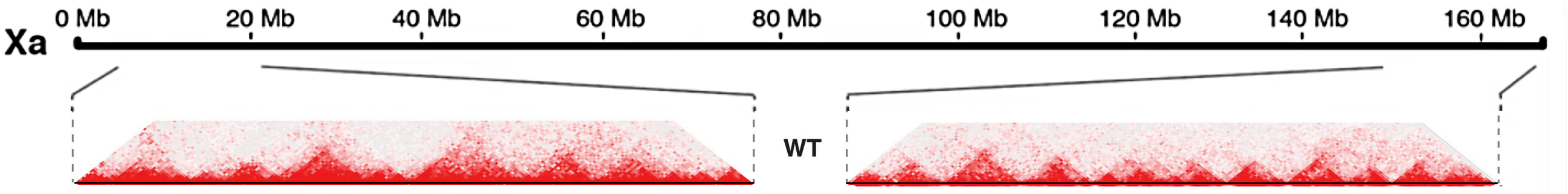


- Highly covered loci at the center
- Poorly covered loci in boundary regions

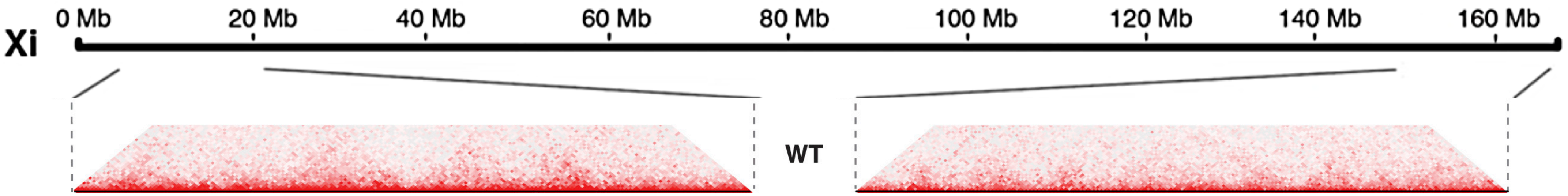
Eser, Umut et al., PNAS of the USA (2017)

Xi chromosome shows attenuated formation of TADs

Xa chromosome similar to autosomes:



Xi chromosome with weakened TADs through *Xist* spreading:



Modified from Colognori, Sunwoo et al., Molecular Cell (2019)

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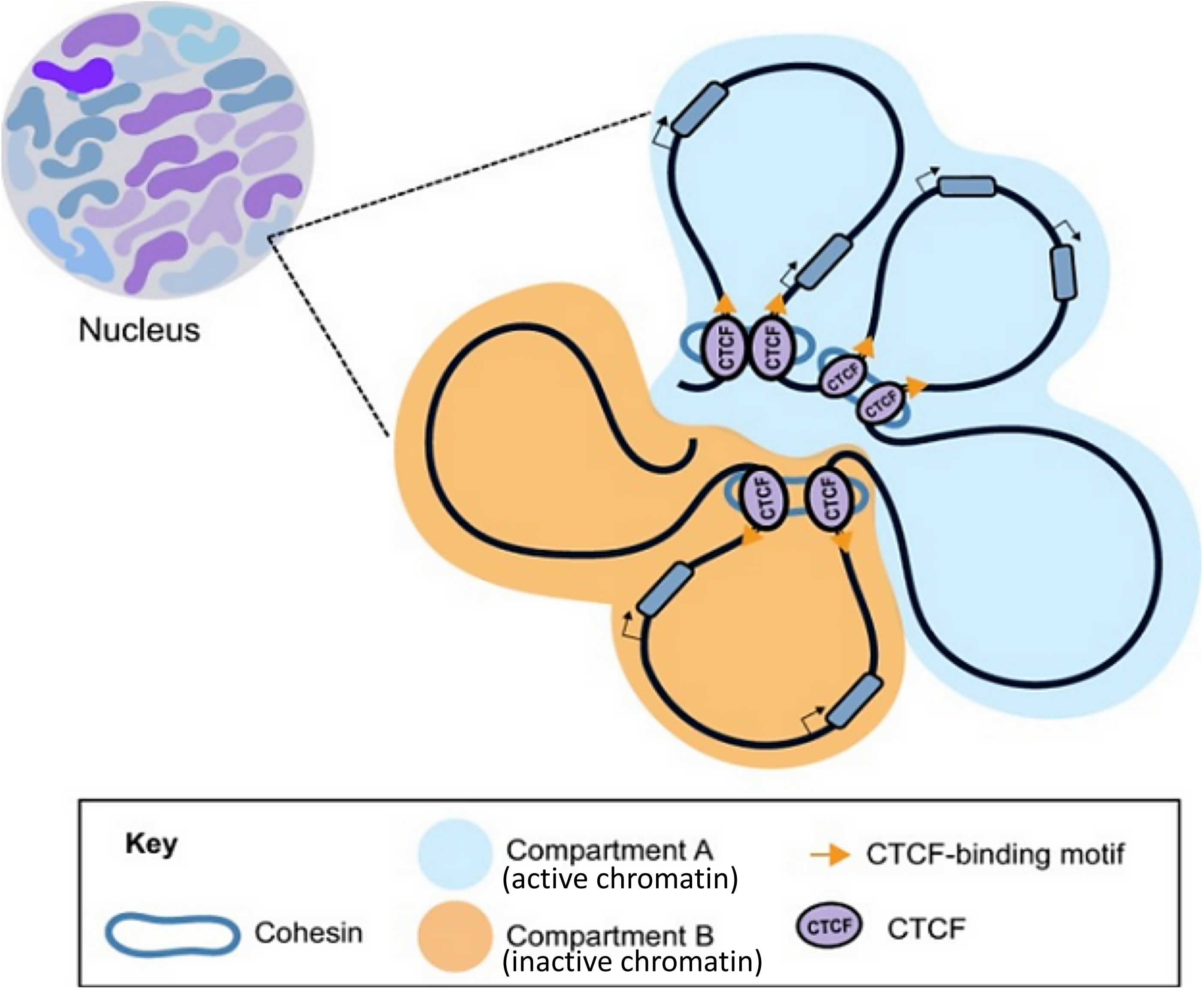
→ **Study models of XCI**

Xist lncRNA is implicated in the TADs weakening of Xi

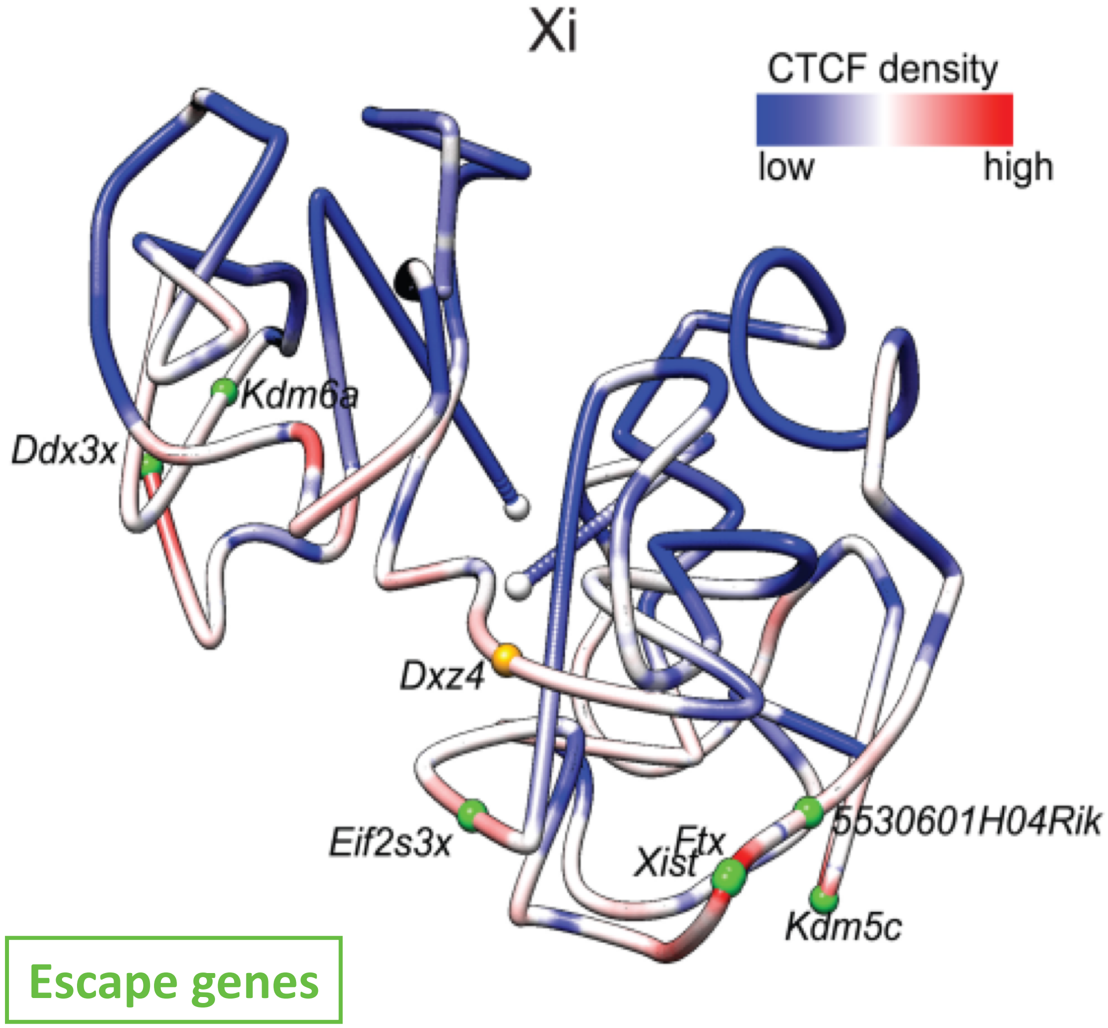
- *Xist* and *Tsix* promoters lie in **adjacent regions of TADs**
  ➔ important for the proper **initiation of XCI**

- ***Xist* lncRNA reduces cohesin loops** of TAD boundaries across Xi
  ➔ TADs merging



Arzate-Mejía et al., Development (2018)

Some genes escape the inactivation of the Xi chromosome



Fang et al., Cell and Developmental Biology (2019)

- 15% of **escapee genes** on the human Xi

  → Protected from the repressive chromatin modifications of X inactivation

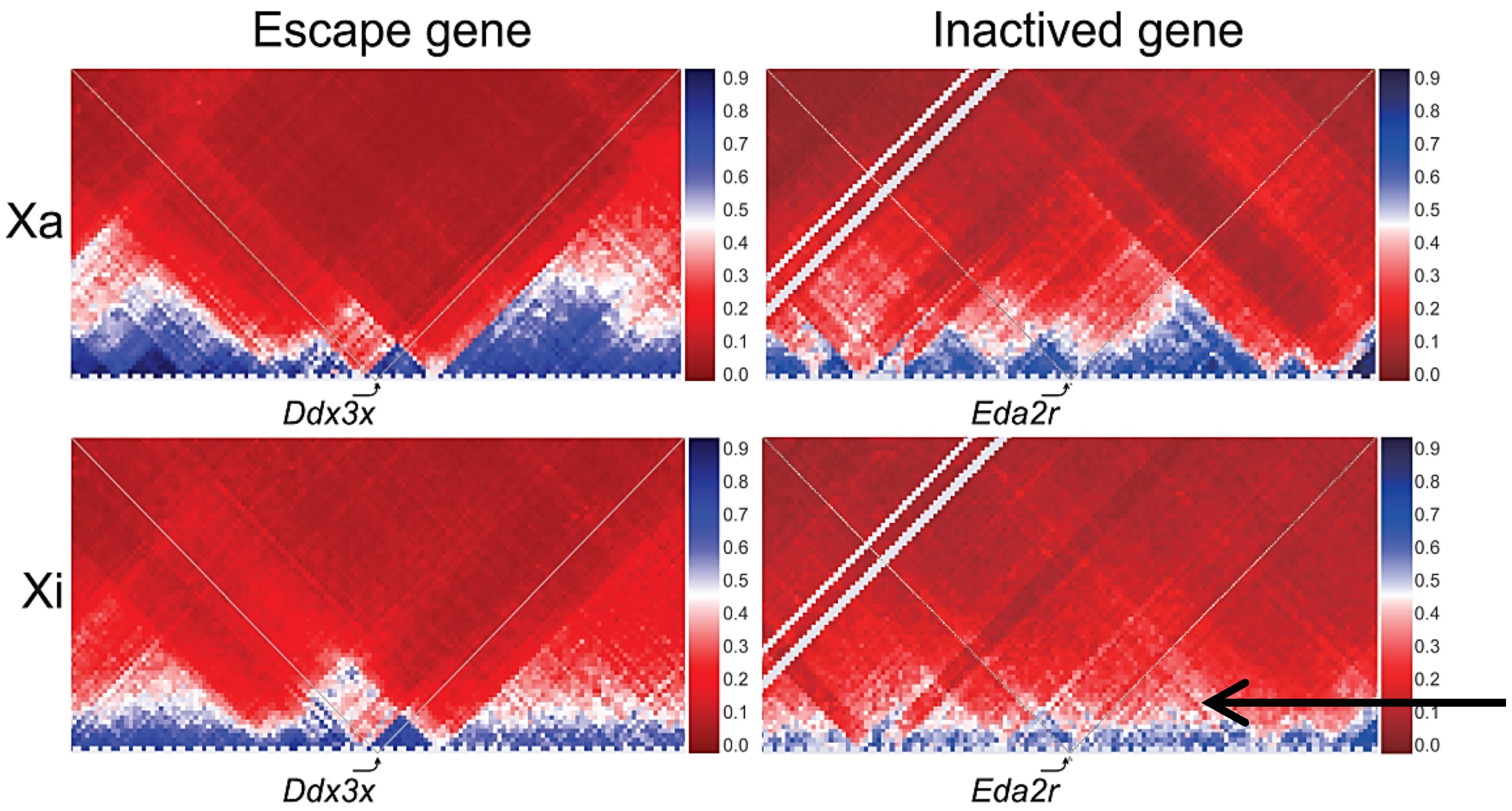
- TADs reduced in Xi = **CTCF reduced in Xi**

- Escape genes colocalize with **clusters of CTCF binding and TADs outside of the Xi 3D structure**

  ➔ Local Xi compartmentalization

Escape genes from the Xi do not present weakened TADs

Partial Hi-C contact maps (4mb resolution)

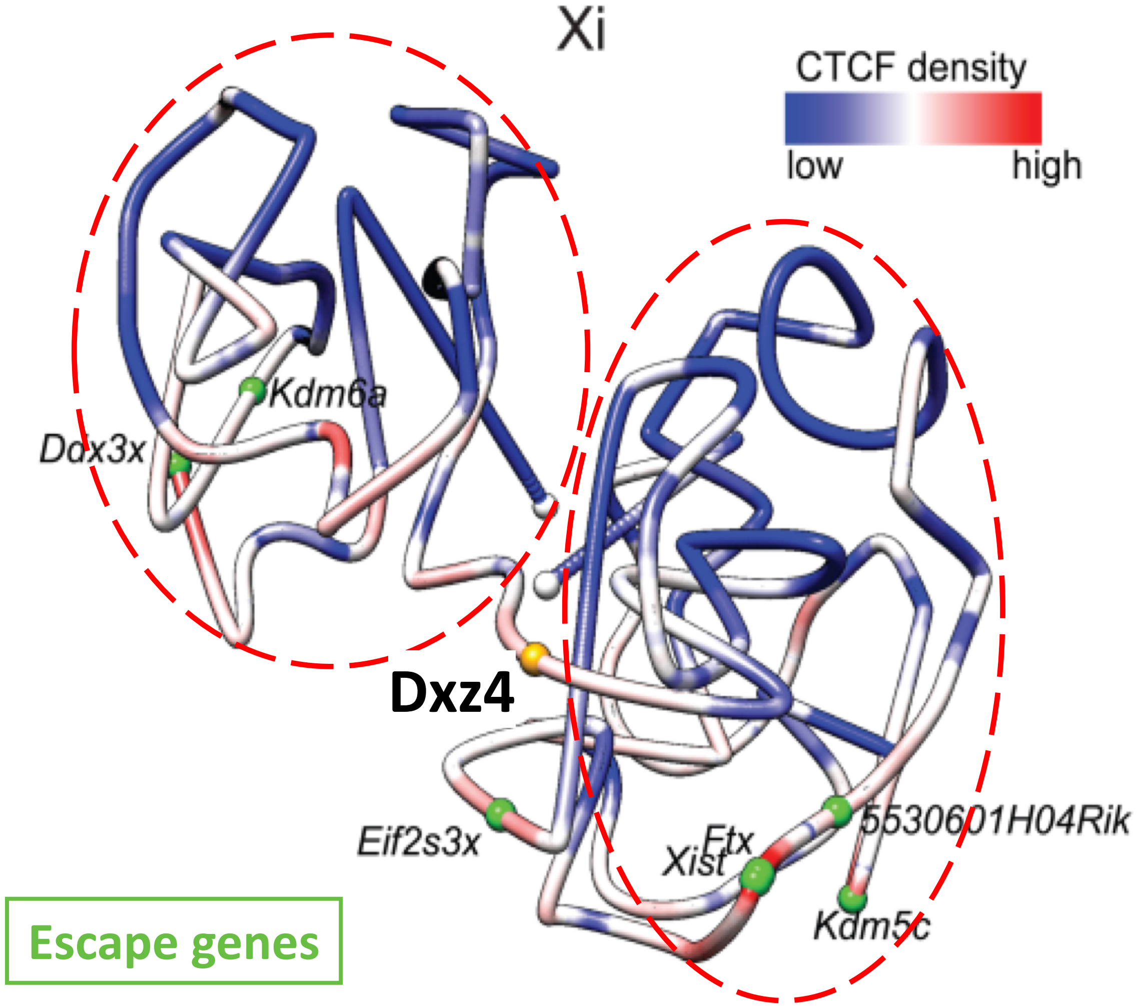


TAD attenuation

Fang et al., Cell and Developmental Biology (2019)

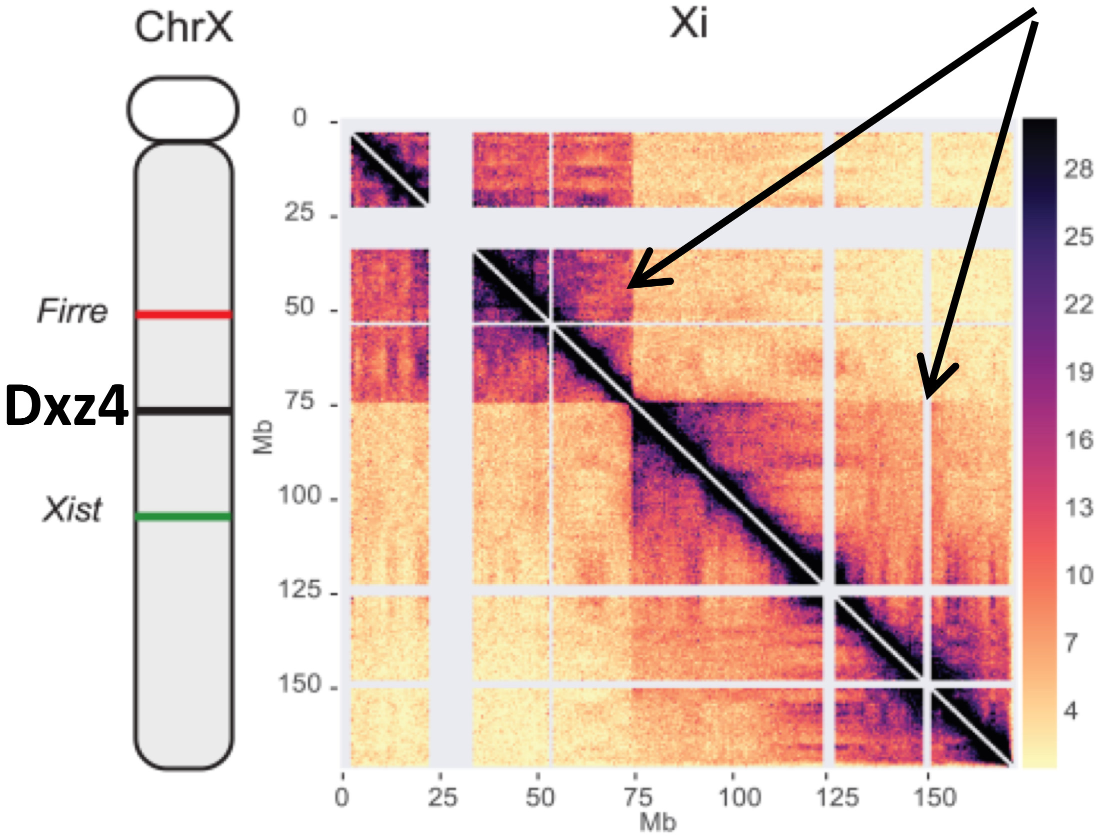
Two superdomains of chromatin condensation are separated by *Dxz4*

Dxz4 = escape gene



Escape genes

Bipartite structure

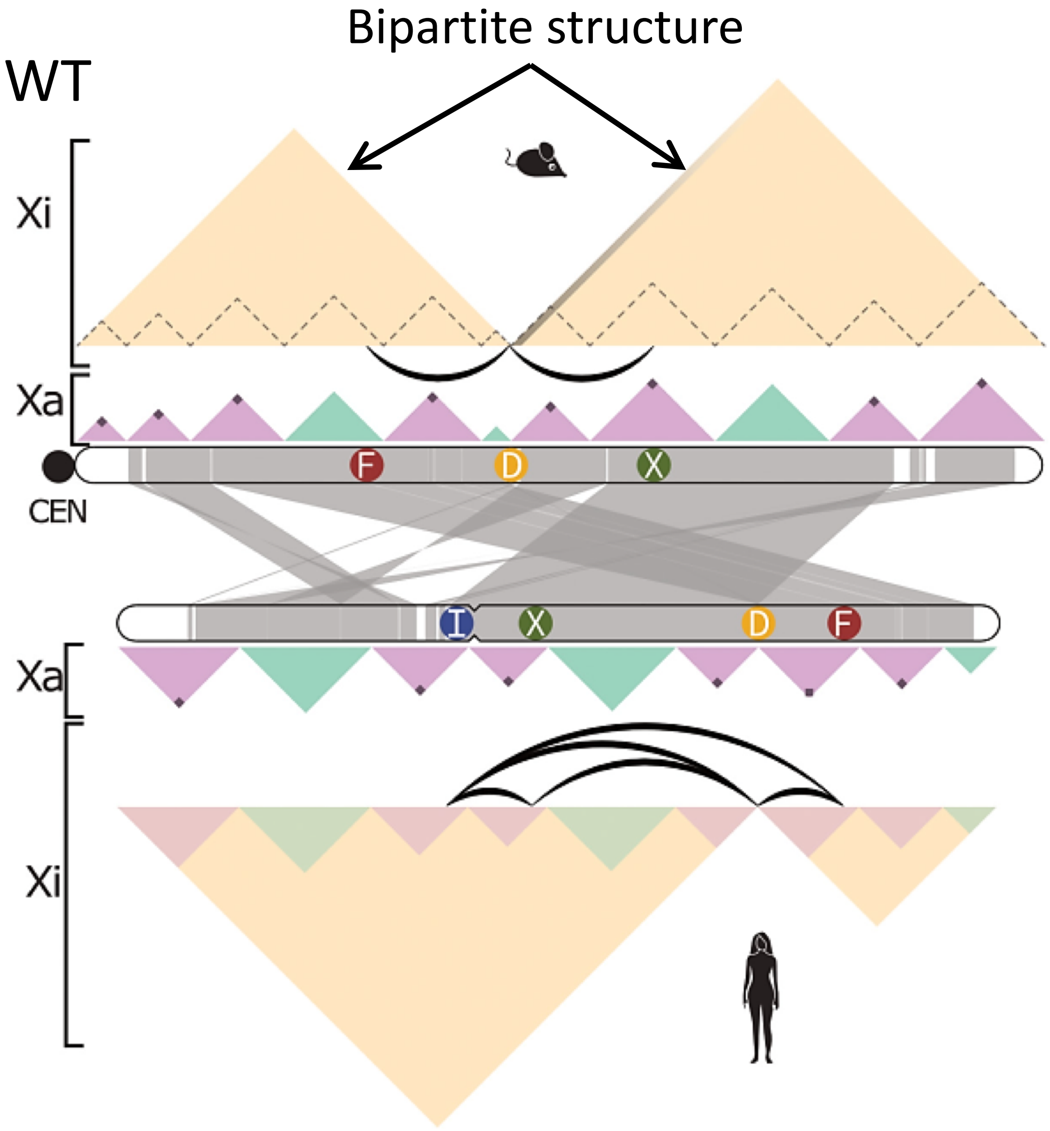


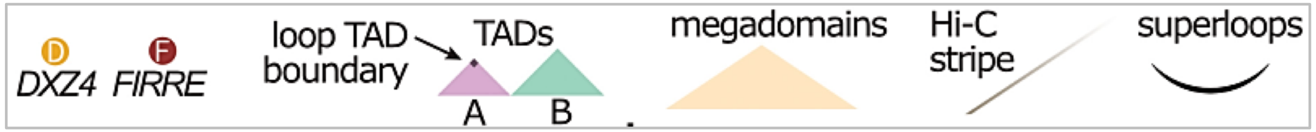
Hi-C contact map of the Xi

Fang et al., Cell and Developmental Biology (2019)

Bipartite structure of Xi on mouse and human

Schematic Hi-C contact map



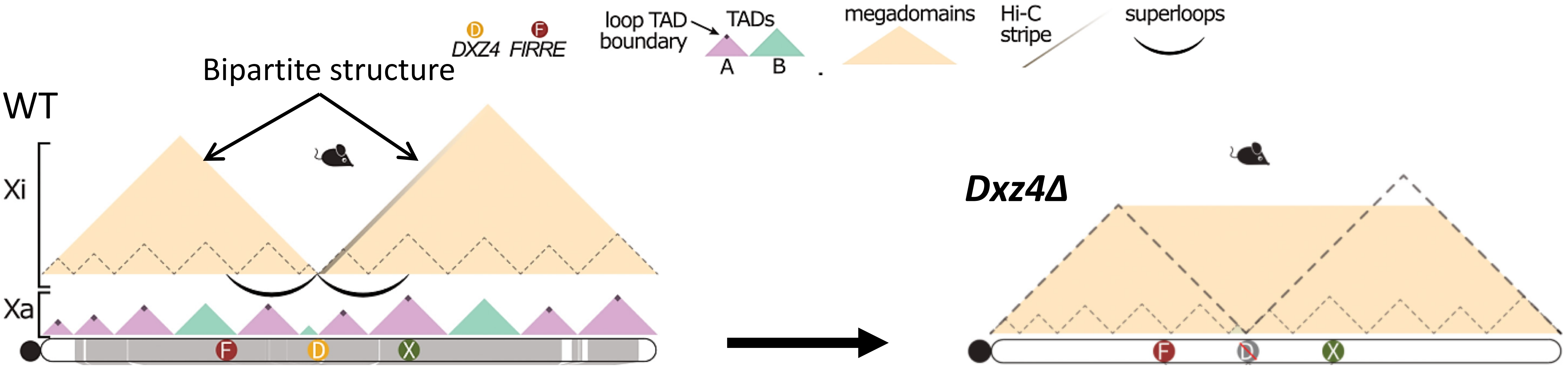


TADs observed on the Xa are **lost** (mouse) or **attenuated** (human) on the Xi

Bansal, Prakhar et al., Frontiers in cell and developmental biology (2020)

LncRNA *Dxz4* is responsible for the bipartite structure of Xi

Schematic Hi-C contact map in differentiated mESC



Bansal, Prakhar et al., Frontiers in cell and developmental biology (2020)

Bipartite structure disrupted:

No effects on gene expression or mouse phenotype

➔ **Bipartite structure function is yet unknown**

But Dxz4 and the bipartite structure are conserved between human and mouse

➔ **Preservation of function**

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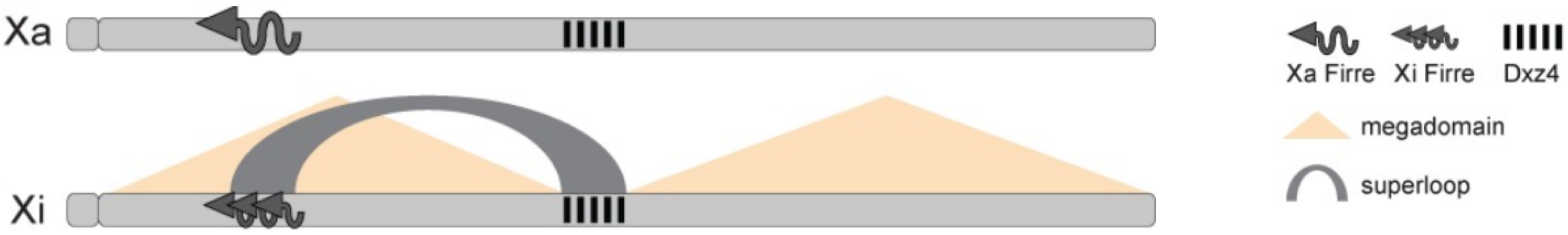
LncRNA *Firre* roles during the XCI

- ✓ ***Firre* X-linked-lncRNA locus** was originally identified as an escape gene of Xi

FALSE

→ **Predominantly transcribed from the Xa chromosome**



Andergassen et al., eLife (2019)

Firre locus escapes random XCI resulting in a **full-length transcript from Xa** and **multiple short isoforms from Xi**

- ✓ *Firre* RNA interacts with many genomic regions and recruits:
  - chromatin organizers (**CTCF…**)
  - the nuclear matrix protein **hnRNPU**

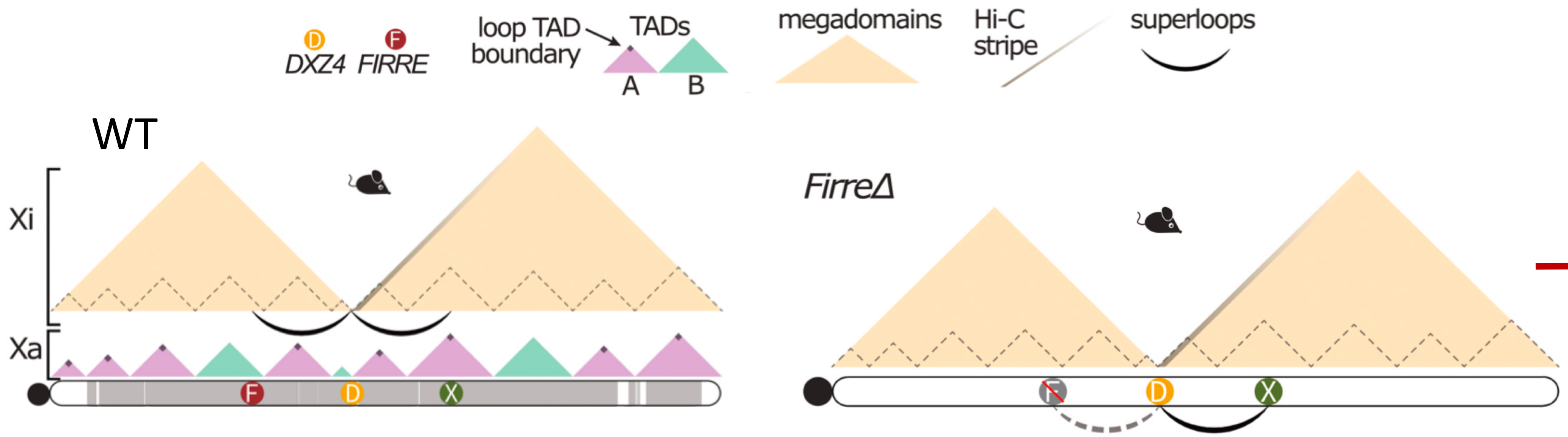
FIRRE/Firre locus is implicated in the XCI

- ✓ Functions: **epigenetic features and 3D structure of the Xi, and possible maintenance of Xi location within the nucleus**
- ✓ LncRNA *Firre* **anchors Xi to the nucleolus** by binding CTCF to maintain H3K27me3

LncRNAs from *FIRRE*/*Firre* locus **regulate autosomal genes**
→ primarily in the **hematopoietic system**

- ✓ **Association of *FIRRE/Firre* locus and HNRNPU** → Autosomal targets function in RNA splicing processing and transport

Deletion of *Firre* : *ΔFirre*



Firre-Dxz4 superloop disrupted

Bansal, Prakhar et al., Frontiers in cell and developmental biology (2020)

- Xi-linked superloop depends only on **its own pair of attaches** and on the presence of the **DXZ4/dxz4 center**
- Long-range contacts between loci may help isolate the Xi in a nucleus specific compartment (near the nuclear periphery) or nucleus phase (near the nucleolus)

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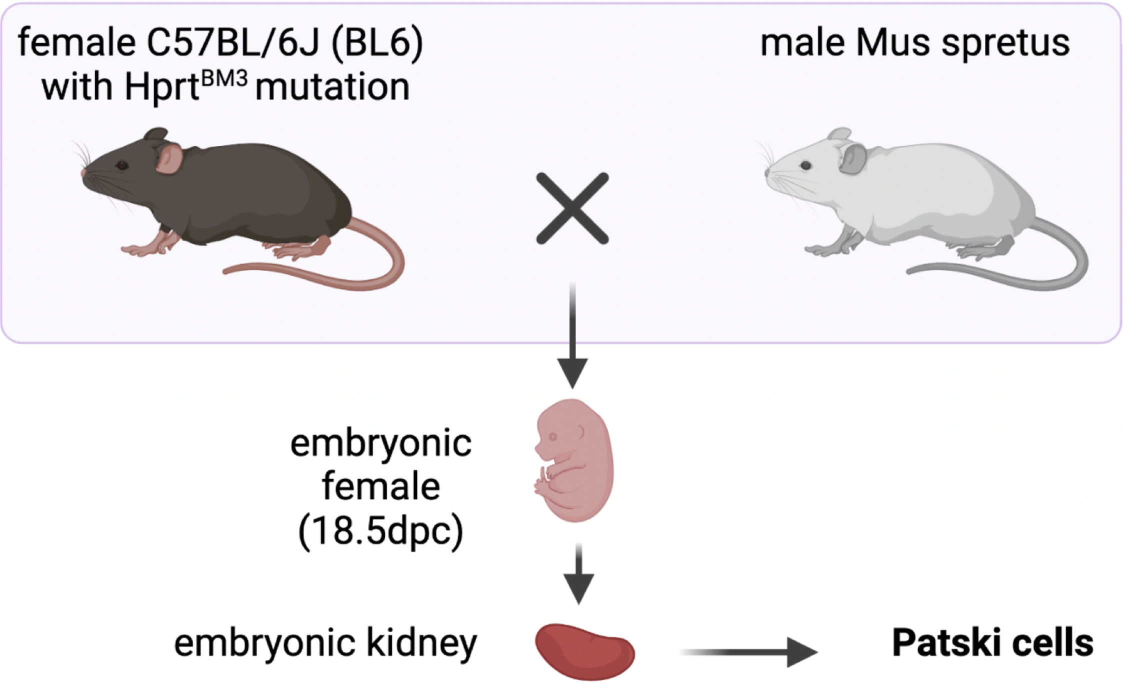
Study models of XCI

Patski cells

mouse Embryonic Stem Cells (mESC)



female C57BL/6J (BL6) with Hprt^{BM3} mutation

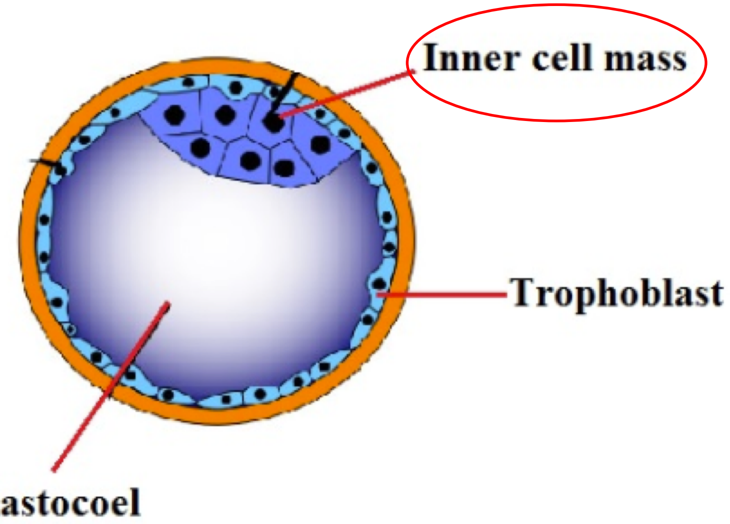
male Mus spretus

embryonic female (18.5dpc)

embryonic kidney

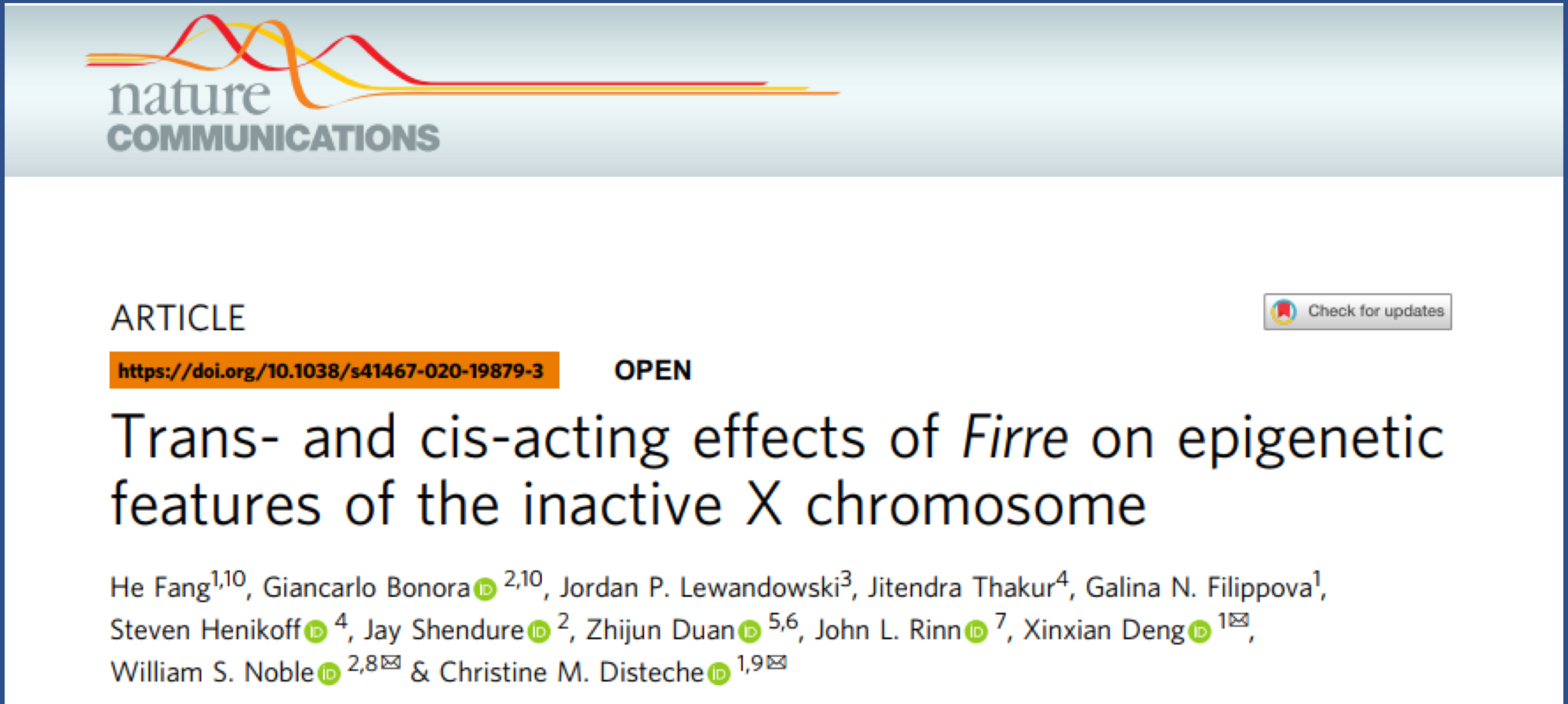
Patski cells

Known origin: X inactivation always on the X chromosome from BL6 mouse



Unknown origin: 2 active chromosomes with random XCI during *in vitro* differenciation

II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome



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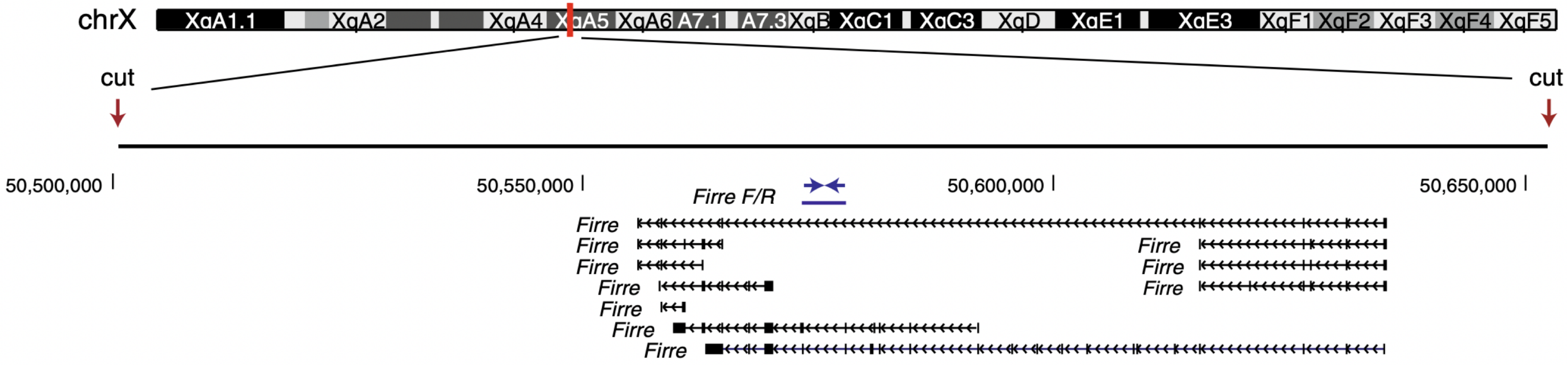
II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

- → Transcription of *Firre* from the Xa
- → Impact of the trans-acting effect of *Firre* on epigenetic features of the Xi
- → Impact of the trans-acting effect of *Firre* on the nuclear location of Xi
- → Trans-acting effect of *Firre* RNA on gene expression
- → Impact of *Firre* RNA on Xi and Xa structure

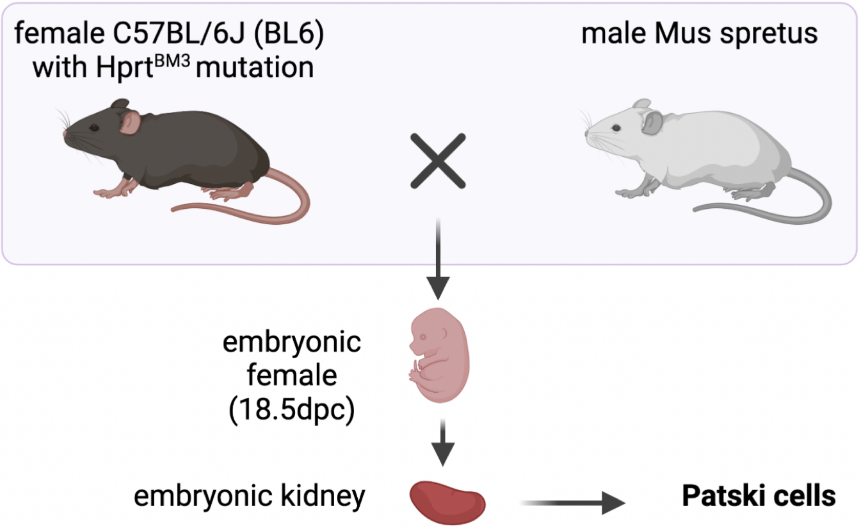
Model and method of study of *Firre* expression



Allele-specific CRISPR/Cas9 editing of *Firre* region on Patski cells

Isolation of editing single-cell clones with either a ***ΔFirre*Xa**, ***ΔFirre*Xi** and an **Inv-*Firre*Xi**

Target the Xi from BL6 and the Xa from *M. spetrus*



female C57BL/6J (BL6) with Hprt^{BM3} mutation

male Mus spretus

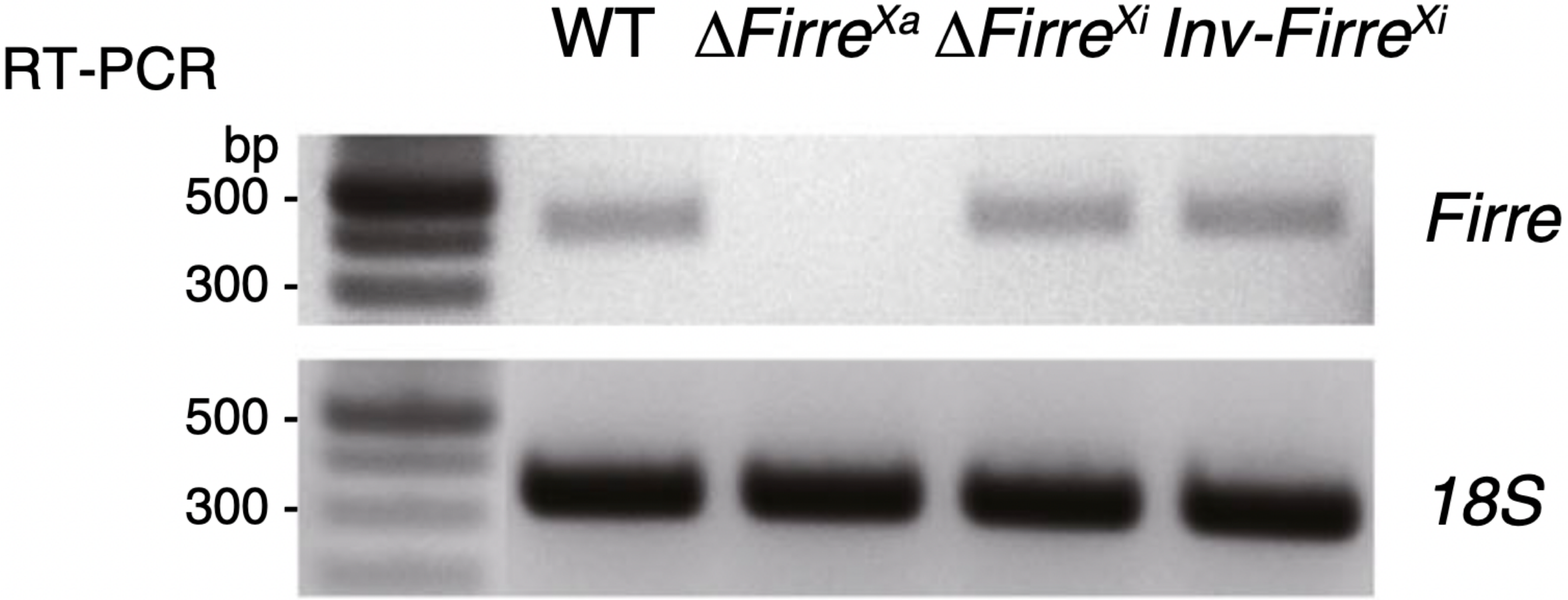
embryonic female (18.5dpc)

embryonic kidney

Patski cells

Firre RNA is transcribed from the Xa

Test of *Firre* expression:



- ✓ ***Firre* is predominantly expressed from the Xa**
- ✓ Previous studies show that there is a low remaining expression of *Firre* RNA from the Xi

II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

→ Transcription of *Firre* from the Xa

→ Impact of the trans-acting effect of *Firre* on epigenetic features of the Xi

→ Impact of the trans-acting effect of *Firre* on the nuclear location of Xi

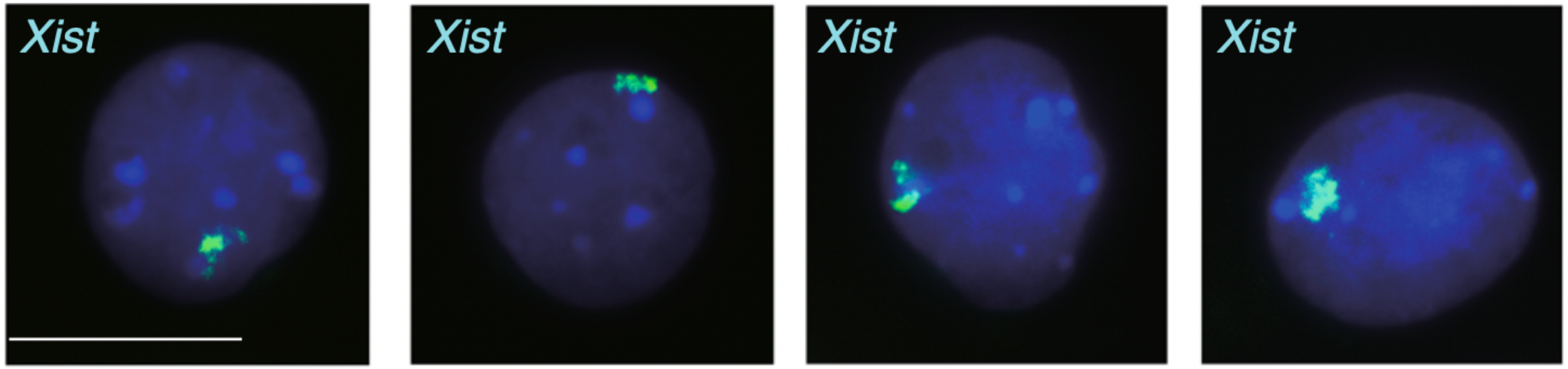
→ Trans-acting effect of *Firre* RNA over gene expression

→ Impact of *Firre* RNA on Xi and Xa structure

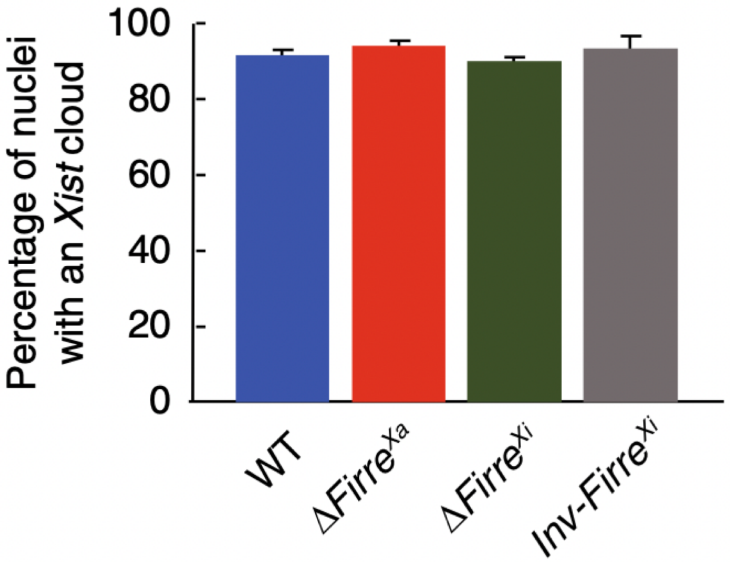
Does *Firre* RNA influence epigenetic marks on the Xi?

Firre RNA doesn't impact *Xist* RNA coating

Xist RNA-FISH → Location of Xi



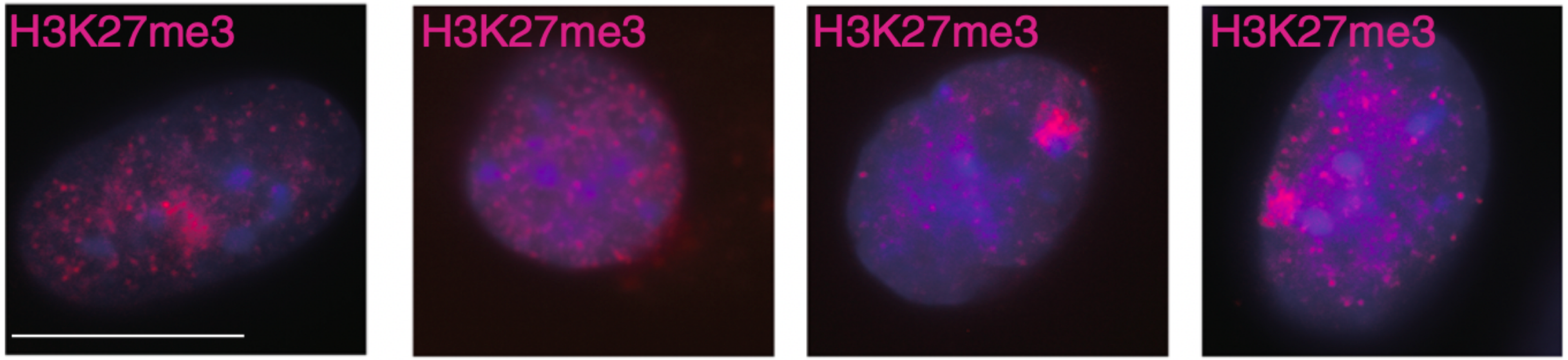
WT Δ*Firre*^{Xa} Δ*Firre*^{Xi} *Inv-Firre*^{Xi}



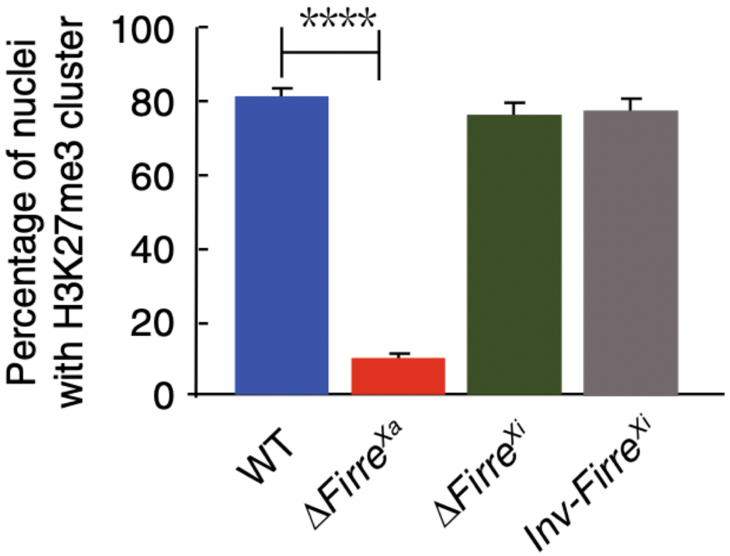
The majority of the nuclei had one *Xist* cloud in all edited cell lines → **No disruption of *Xist* RNA coating**

Firre RNA from the Xa has a trans-effect on H3K27me3 on the Xi

Immunostainings of H3K27me3



WT $\quad$ $\Delta Firre^{Xa}$ $\quad$ $\Delta Firre^{Xi}$ $\quad$ $Inv\text{-}Firre^{Xi}$



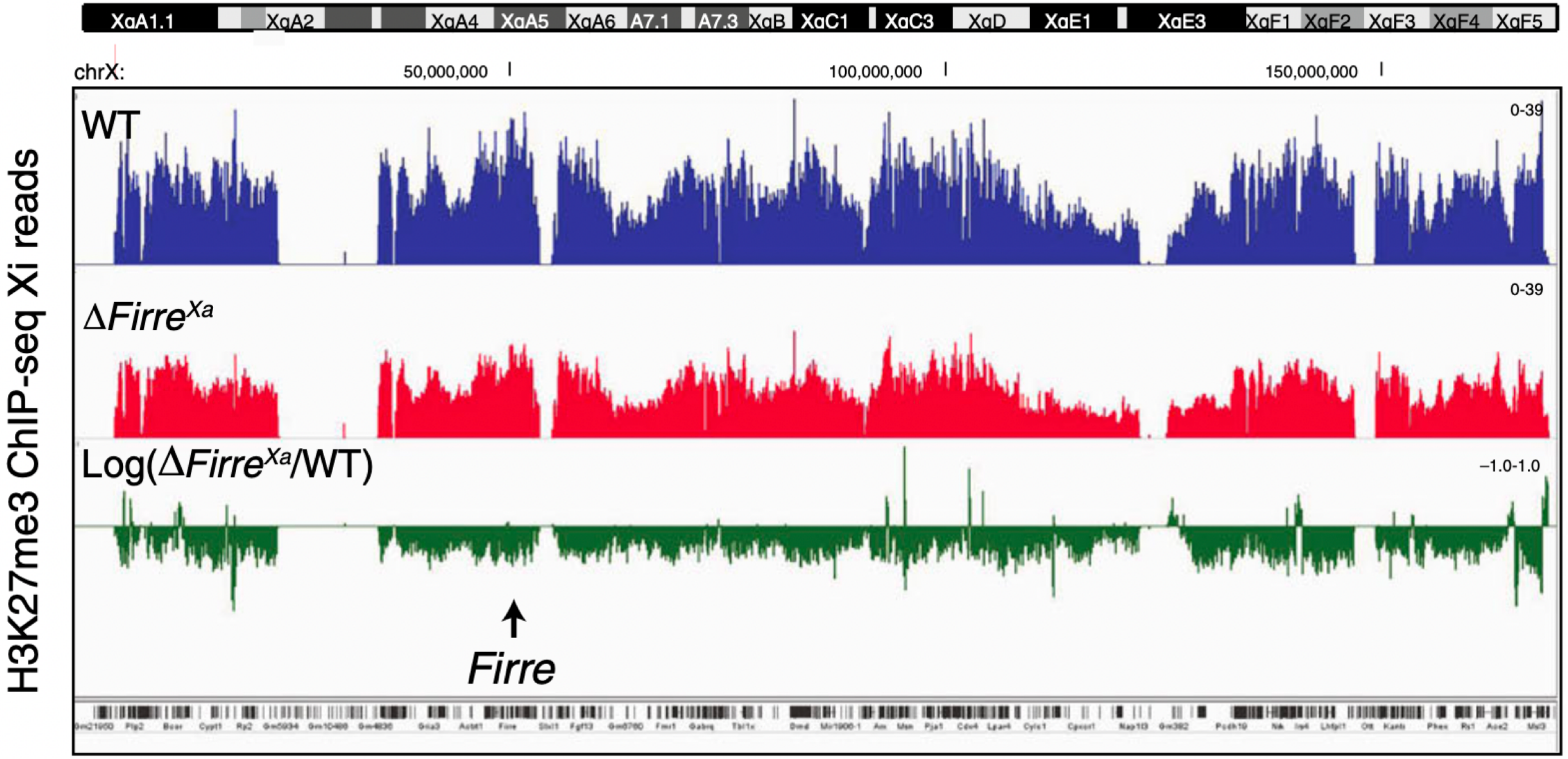
Strong H3K27me3 cluster

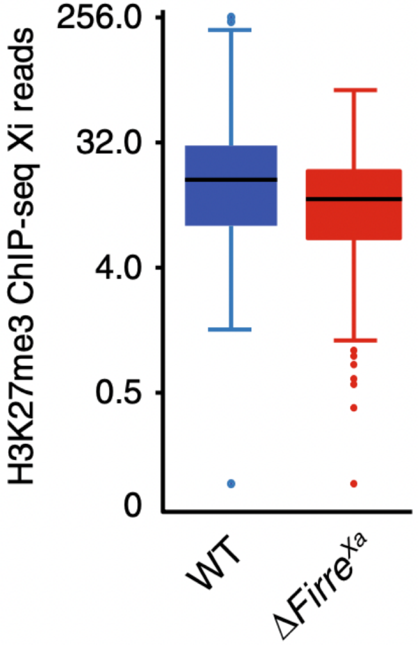
➔ Retention of *Firre* RNA

High decrease of nuclei with H3K27me3 cluster

Firre RNA from the Xa has a trans-effect on H3K27me3 on the Xi

ChIP-seq → Allele-specific profile of H3K27me3

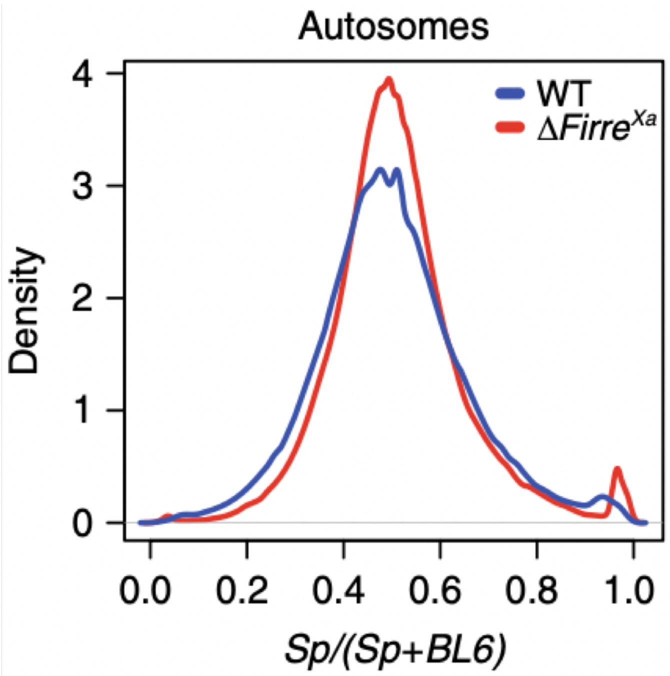


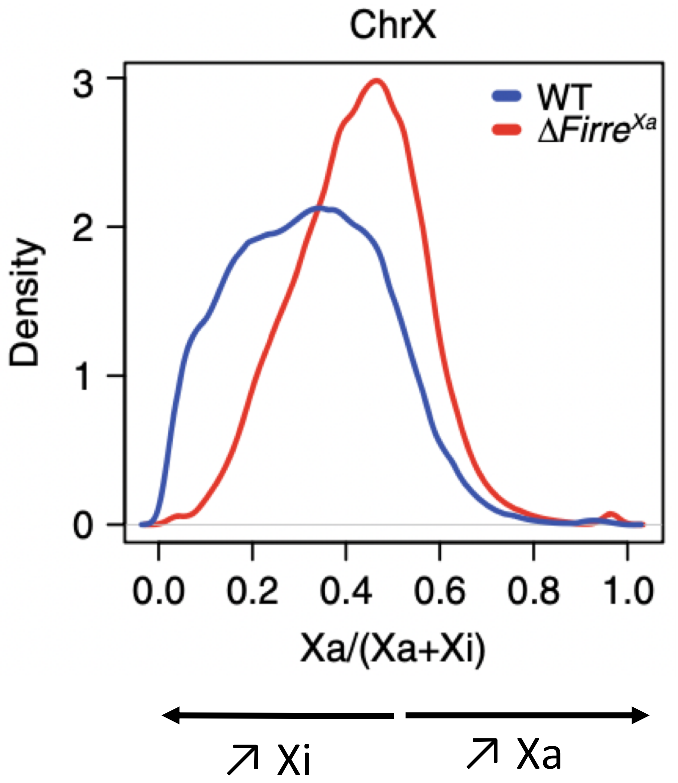


- **WT → H3K27me3 enrichment on the Xi**
- ***ΔFirre*$^{\mathbf{Xa}}$ → chromosome-wide decrease on the Xi**

H3K27me3 decrease on the Xi in Δ*Firre*Xa

Density histograms → Distribution of H3K27me3 allelic proportions





Centered close to the 0.5

Reflecting similar enrichment between alleles

at 0.35 in WT

H3K27me3 enrichment on the Xi

at 0.5 in Δ*Firre*Xa

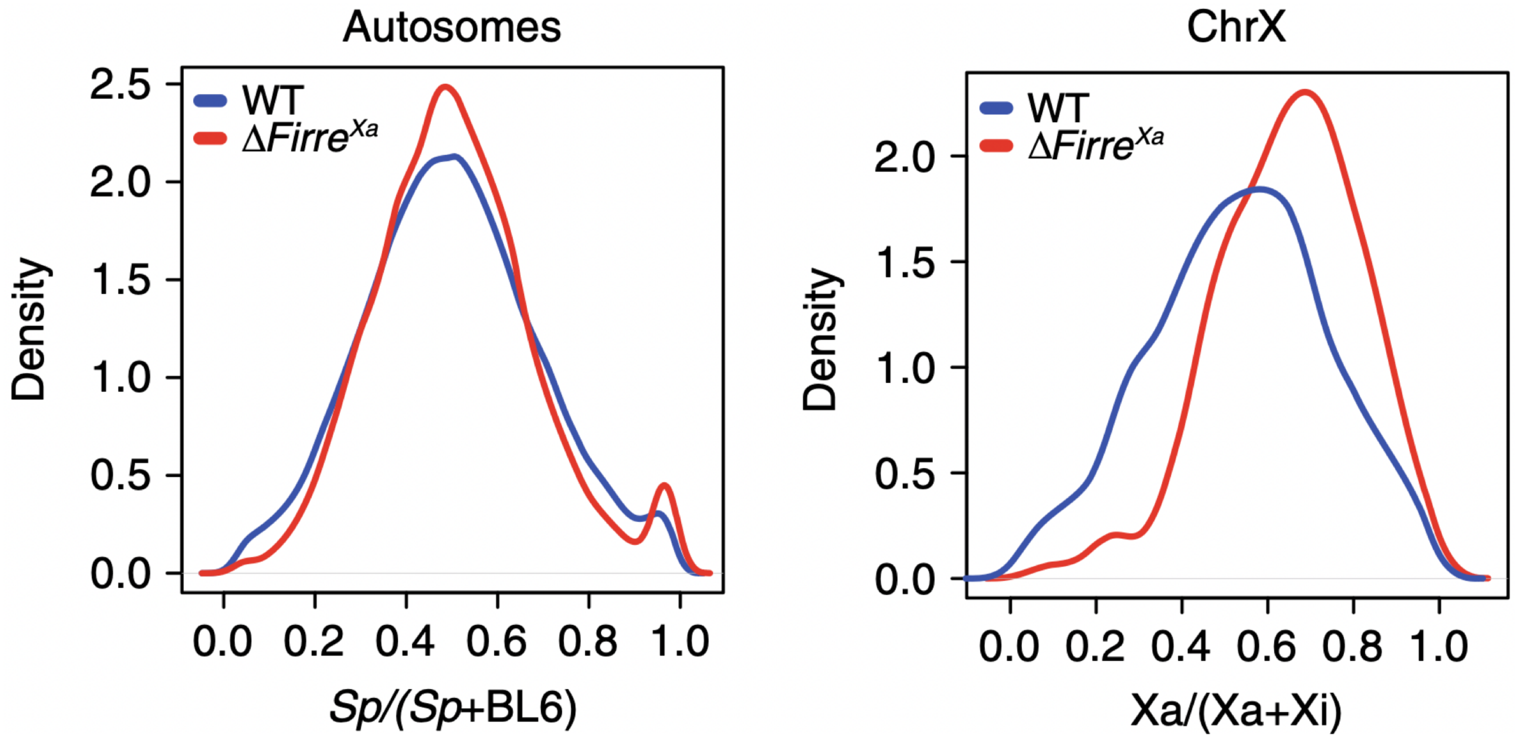
Decrease of H3K27me3 on the Xi

Do PRCs have a role on the epigenetic marks regulation of Xi mediated by *Firre* Xa transcript?

Trans-acting role of *Firre* RNA to recruit PRC components to the Xi

CUT&RUN → *Investigation of SUZ12, a subunit of the PRC2 complex* → Density histograms

Distribution of SUZ12 allelic proportions:



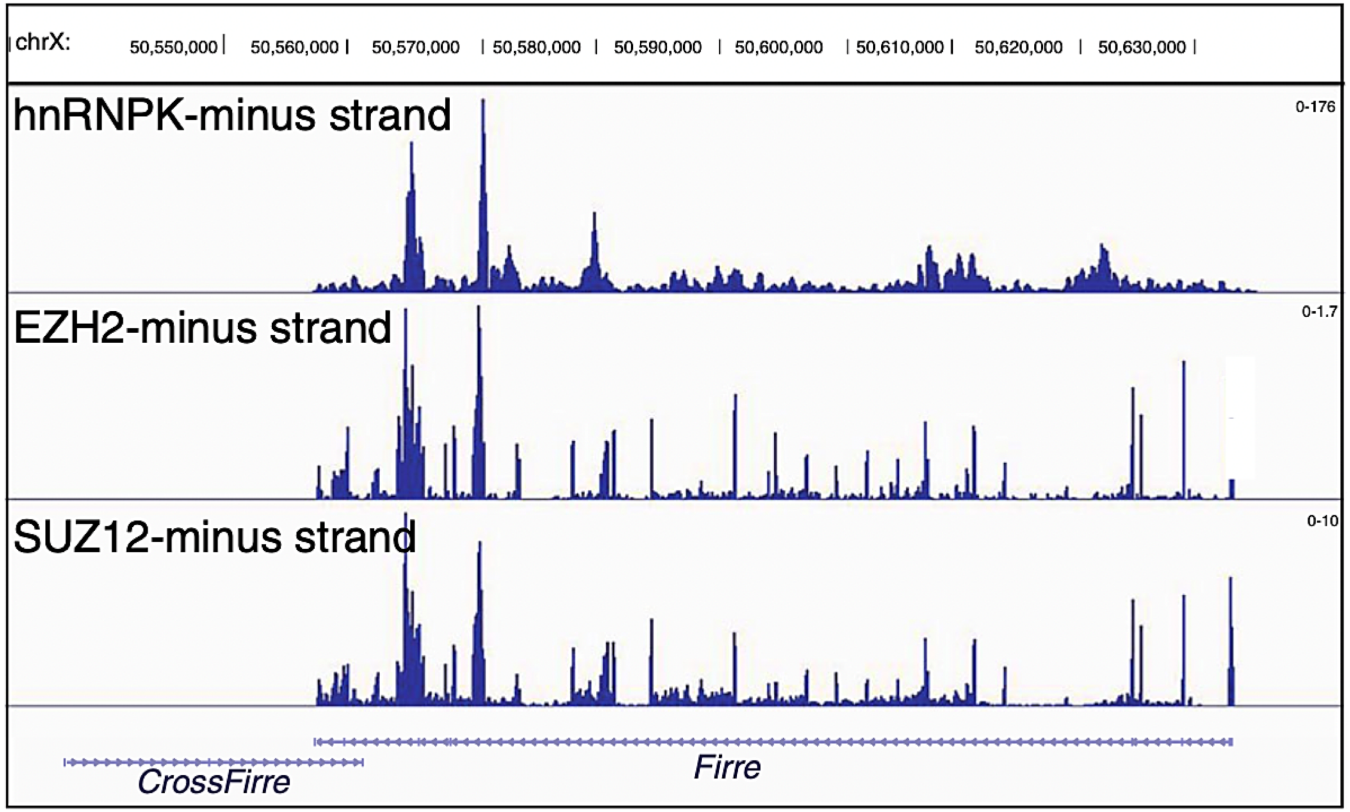
No change

Pronouced shift toward higher values after deletion

SUZ12 decrease on the Xi in Δ*Firre*^{Xa}

Trans-acting role of *Firre* RNA to recruit PRC components to the Xi

Data from previous studies:



Trans-acting effect of *Firre* RNA transcribed from the Xa targets PRC1 and PRC2 complexes to the Xi

→

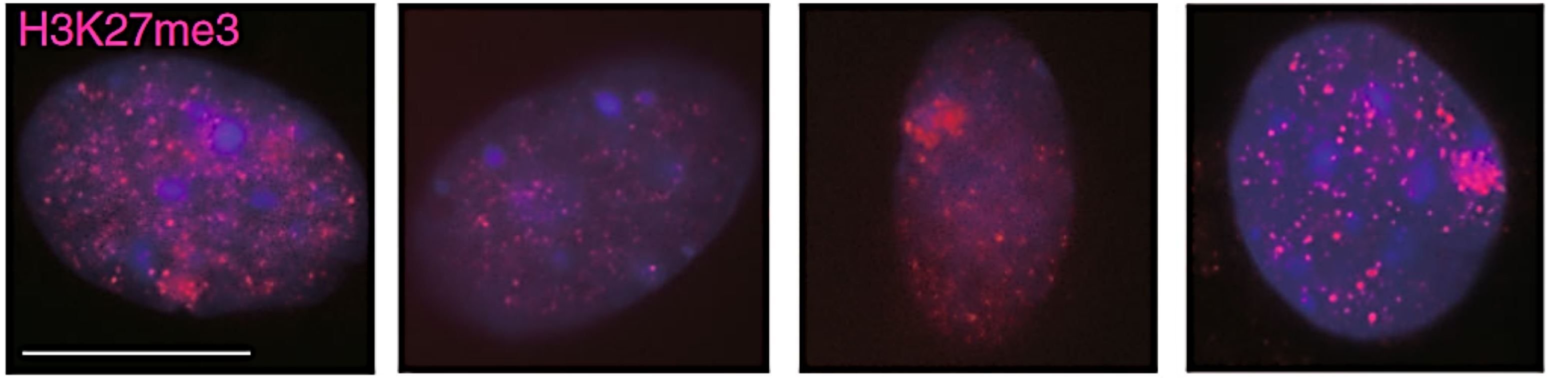
Maintenance of H3K27me3

Does *Firre* have a causative role on H3K27me3 enrichment on the Xi?

Ectopic expression of *Firre* rescued the presence of H3K27me3 cluster

Transfection of *ΔFirreXa* cells with a **mouse or a human *Firre* cDNA transgene**

Ectopic expression of *Firre*

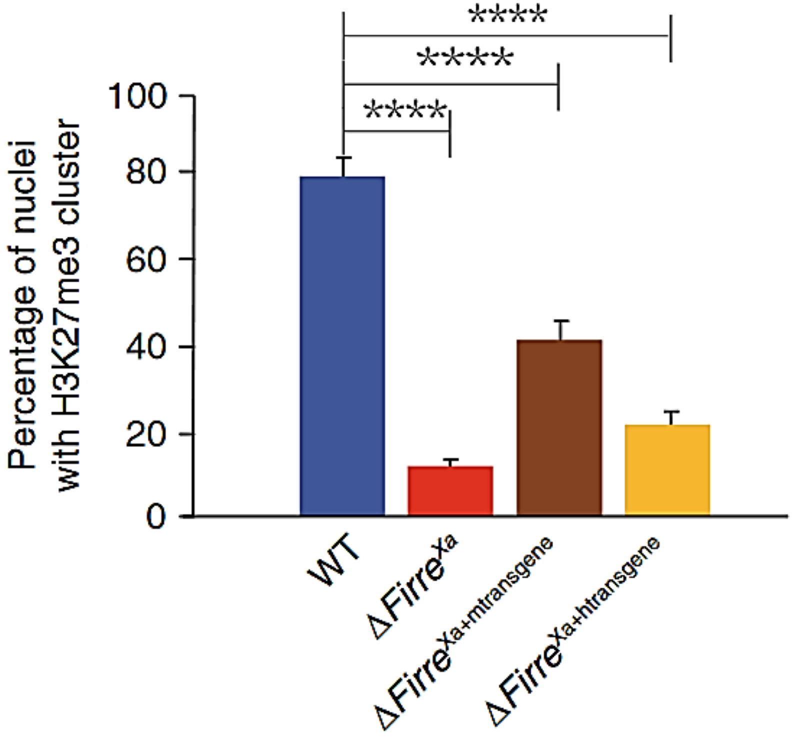


WT

$\Delta \mathit{Firre}^{Xa}$

$\Delta \mathit{Firre}^{Xa}$
+mouse cDNA
transgene

$\Delta \mathit{Firre}^{Xa}$
+human cDNA
transgene



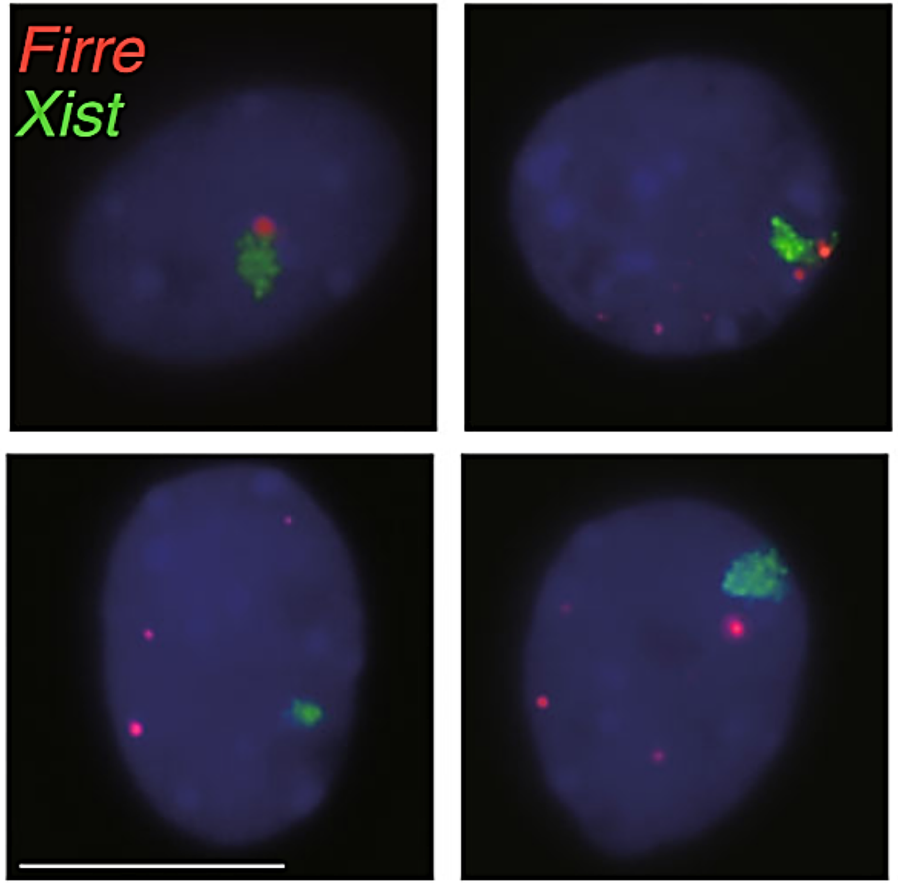
H3K27me3 cluster on the Xi is partially rescued

Functional compatibility between species

Trans-acting role of *Firre*

Ectopic expression of *Firre* partially rescued its association with *Xist* cloud

Transfection of *ΔFirreXa* cells with a **mouse *Firre* cDNA transgene** → *Xist* and *Firre* RNA-FISH in the nuclei



ΔFirre^{Xa}
+mouse cDNA
transgene

Association of Firre to the Xist cloud in 15% of cells

Partially rescued

***So, Firre* RNA transcribed from Xa or from an ectopic cDNA transgene has a trans effect on the epigenetic features of the Xi**

II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

→ **Transcription of *Firre* from the Xa**

→ **Impact of the trans-acting effect of *Firre* on epigenetic features of the Xi**

→ **Impact of the trans-acting effect of *Firre* on the nuclear location of Xi**

→ **Trans acting effect of *Firre* over gene expression**

→ **Impact of *Firre* RNA on Xi and Xa structure**

Nuclear location of Xi is important for its repressive epigenetic state

⇩

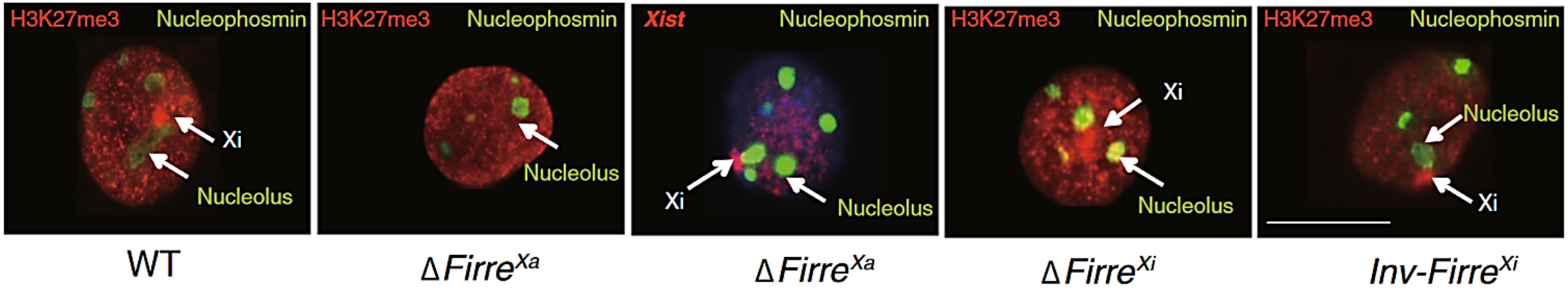
Does *Firre* RNA have an impact on Xi location within the nucleus?

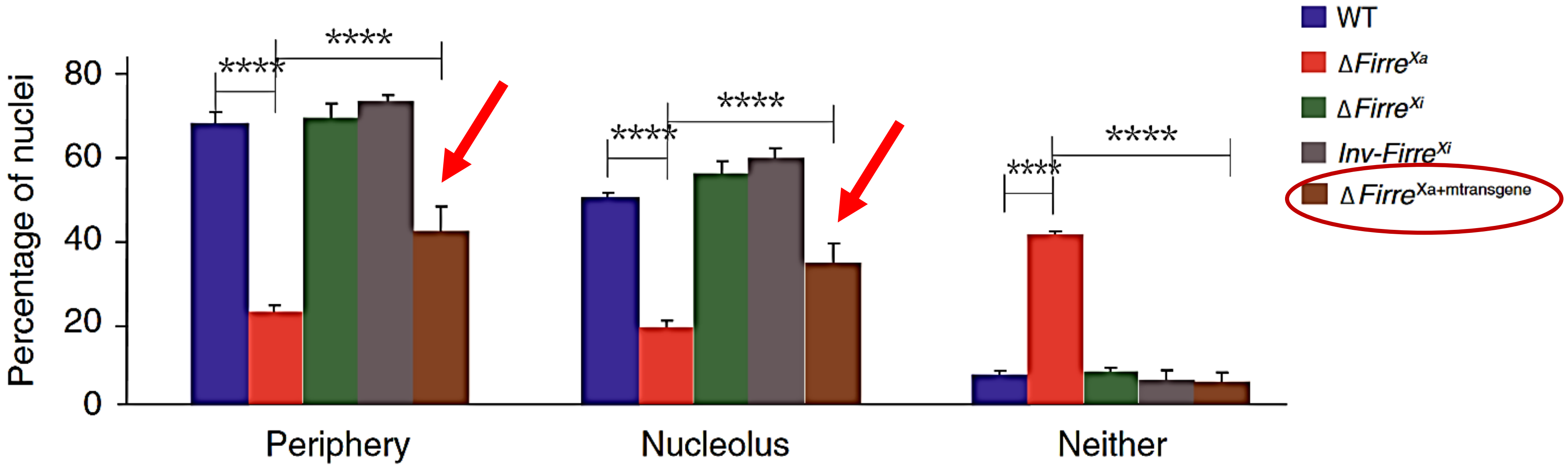
Firre influences in *trans* the Xi location within the nucleus

Allelic *Firre* mutations →

H3K27me3 (Xi) and nucleophosmin (nucleoli) immunostaining

+ Xist RNA-FISH (Xi) immunostaining in *ΔFirreXa*





Loss of Firre RNA from Xa reduces the periphery and nucleolus association of Xi

↓

Partially rescued

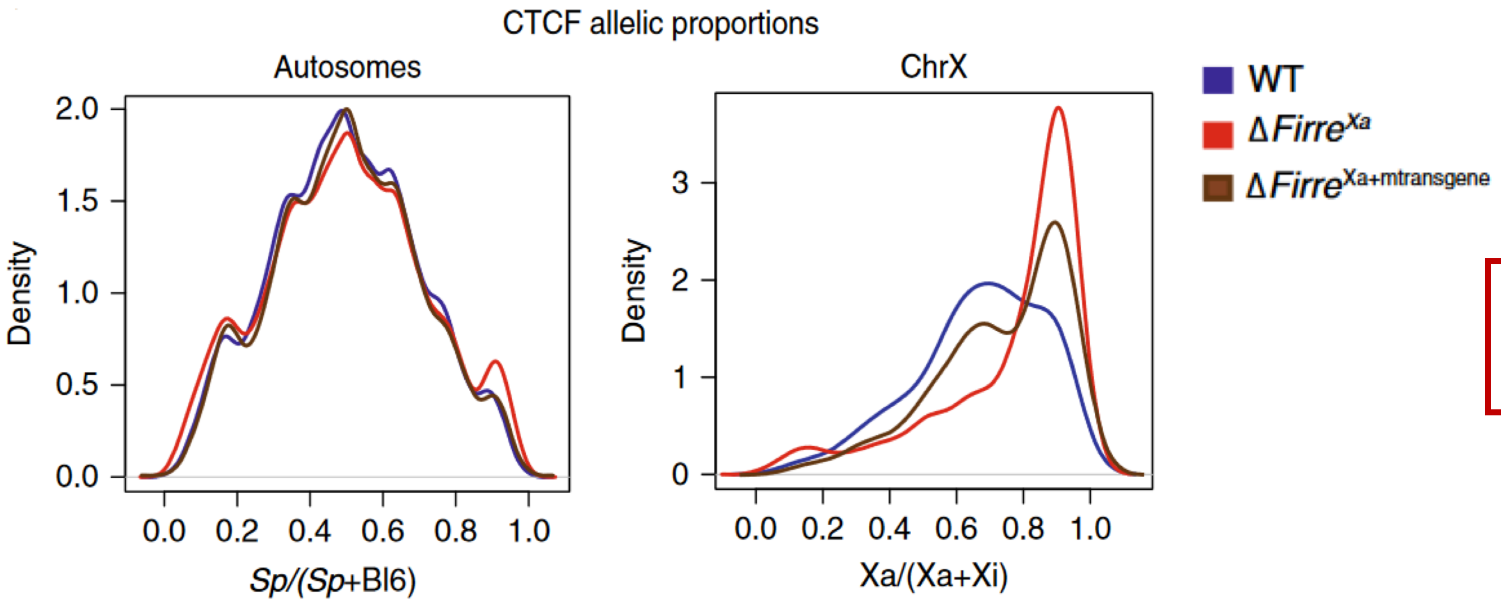
CTCFs have been implicated in Xi nucleolus association in Patski cells

↓

Is CTFC implicated in the maintenance of Xi location through *Firre* RNA function?

Potential cooperation between *Firre* and CTCF in the maintenance of Xi location

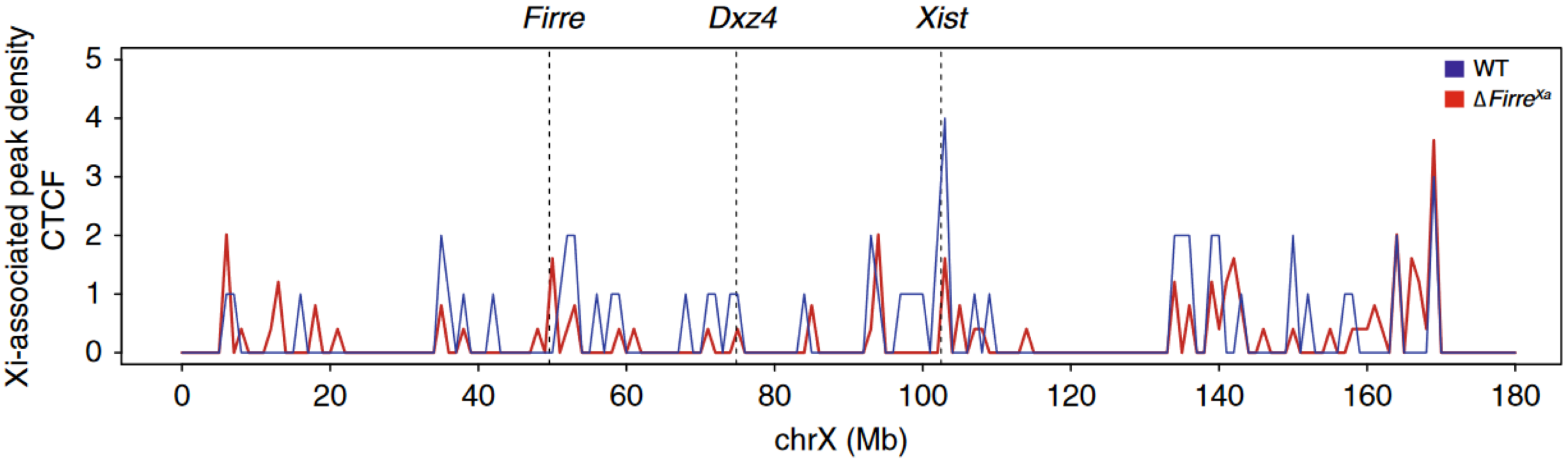
CUT&RUN → Allelic CTCF binding profile + Xi-associated CTCF peak density



Loss of CTCF binding on the Xi in ΔFirreXa

↓

Partial restoration



II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

→ Transcription of *Firre* from the Xa

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→ Impact of the trans-acting effect of *Firre* on the nuclear location of Xi

→ Trans-acting effect of *Firre* RNA over gene expression

→ Impact of *Firre* RNA on Xi and Xa structure

Is there a trans-effect of *Firre* RNA on X-linked genes and autosomes?

Firre is expressed genome-wide

Sequencing of RNA-seq libraries for Patski cells

↓

RNA seq reads mapped to refSeq BL6 mouse transcriptome

↓

Mapped RNA seq reads performed on those mouse were assigned to RefSeq reads and counted

↓

Genes with an expression > 1 TPM (Transcript per million) kept

Δ*Firre*^{Xa} cells:

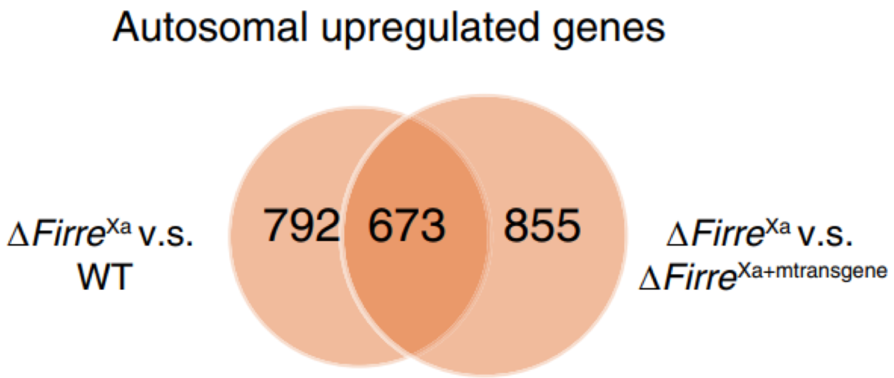
- **14% of X-linked genes** are up or down regulated
  - 40% rescued with ΔFirre$^{Xa+mtransgene}$
- **11% of autosomal genes** are up or down regulated
  - 46% rescued ΔFirre$^{Xa+mtransgene}$

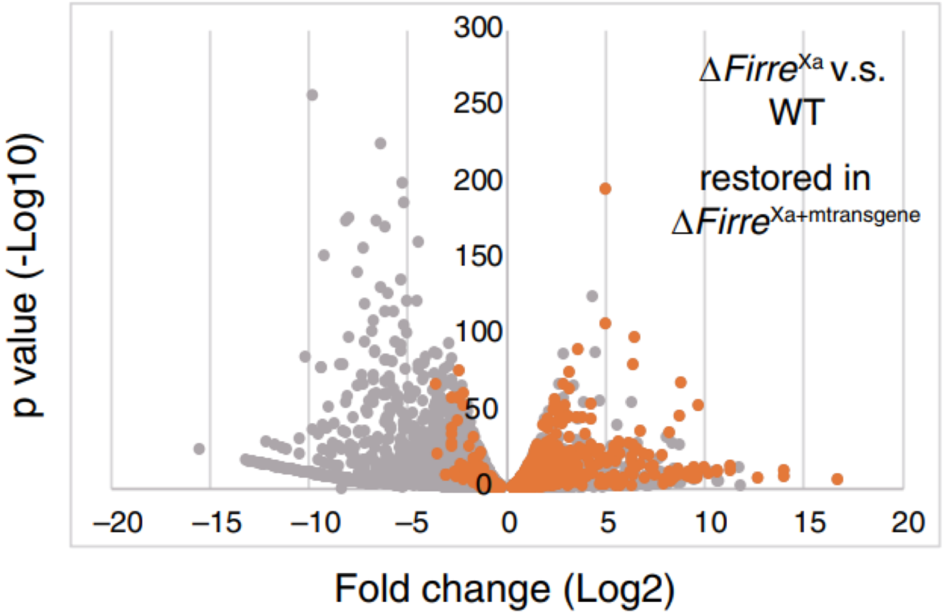
Firre lncRNA is linked to the expression of many genes across the genomes, not only X-linked genes

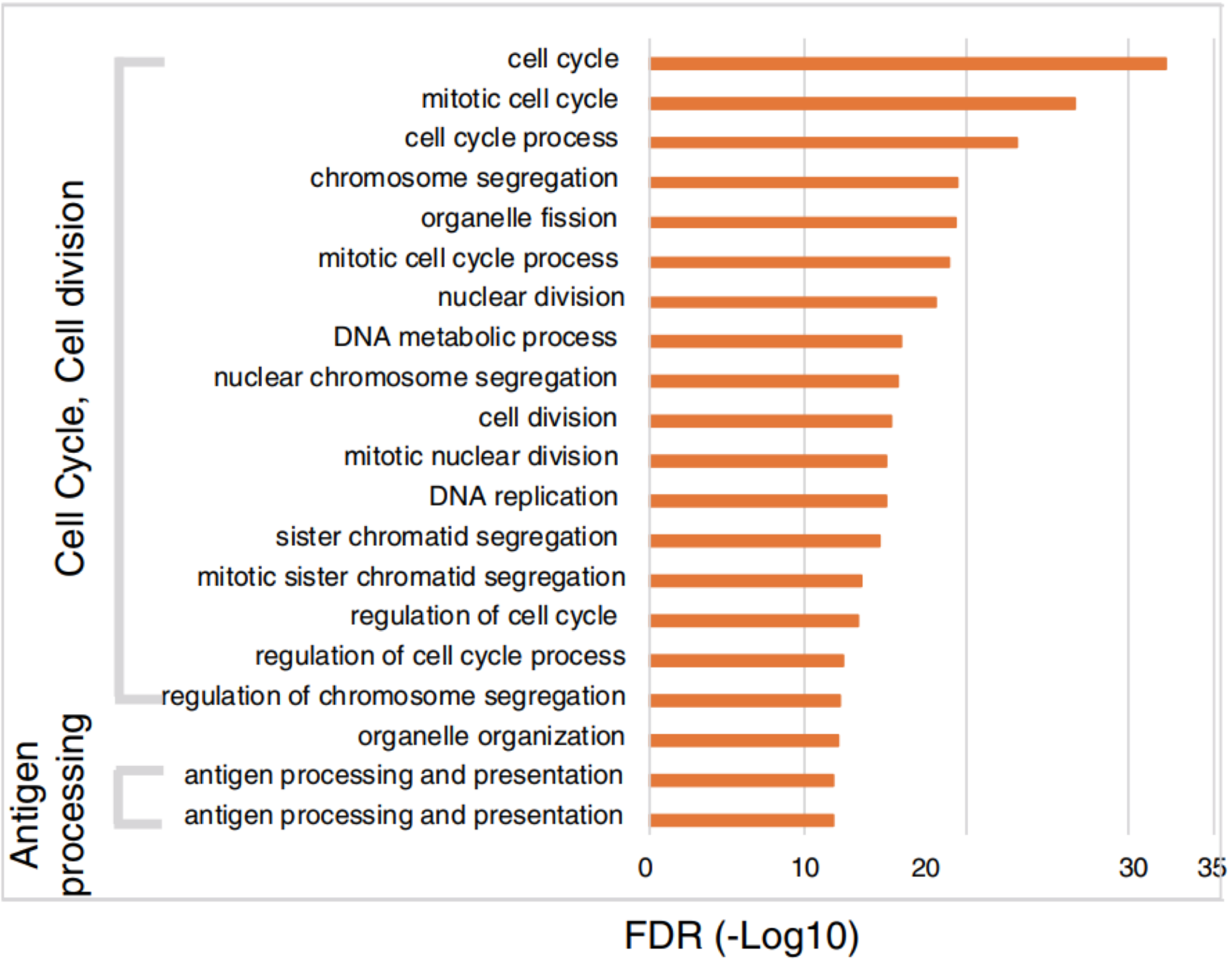
What are the biological functions linked to *Firre* RNA trans-effect?

Firre is linked to various biological functions

Gene ontology analysis

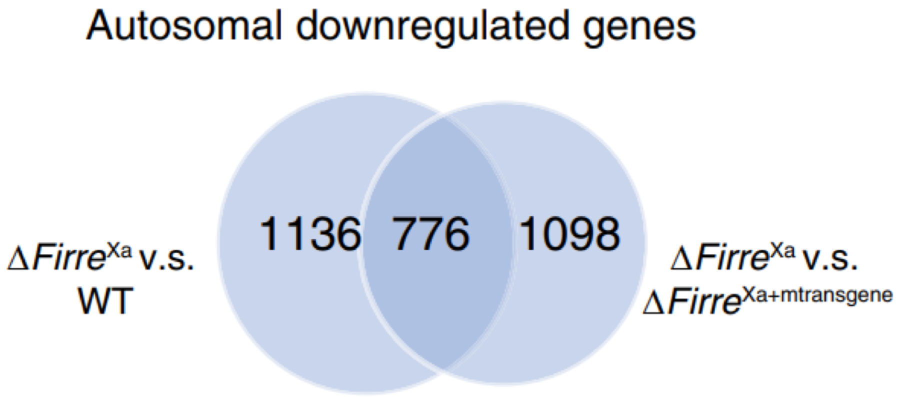


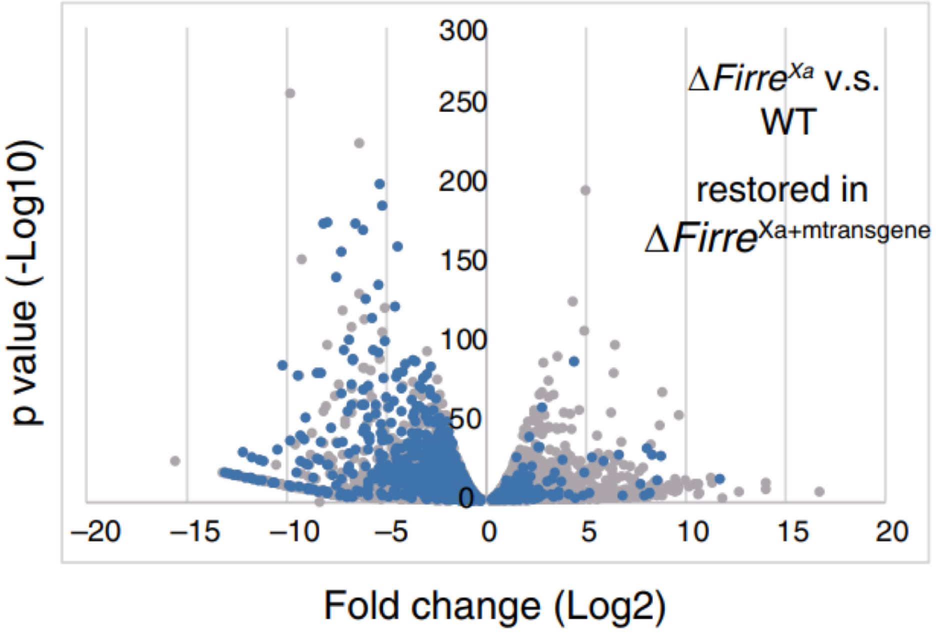


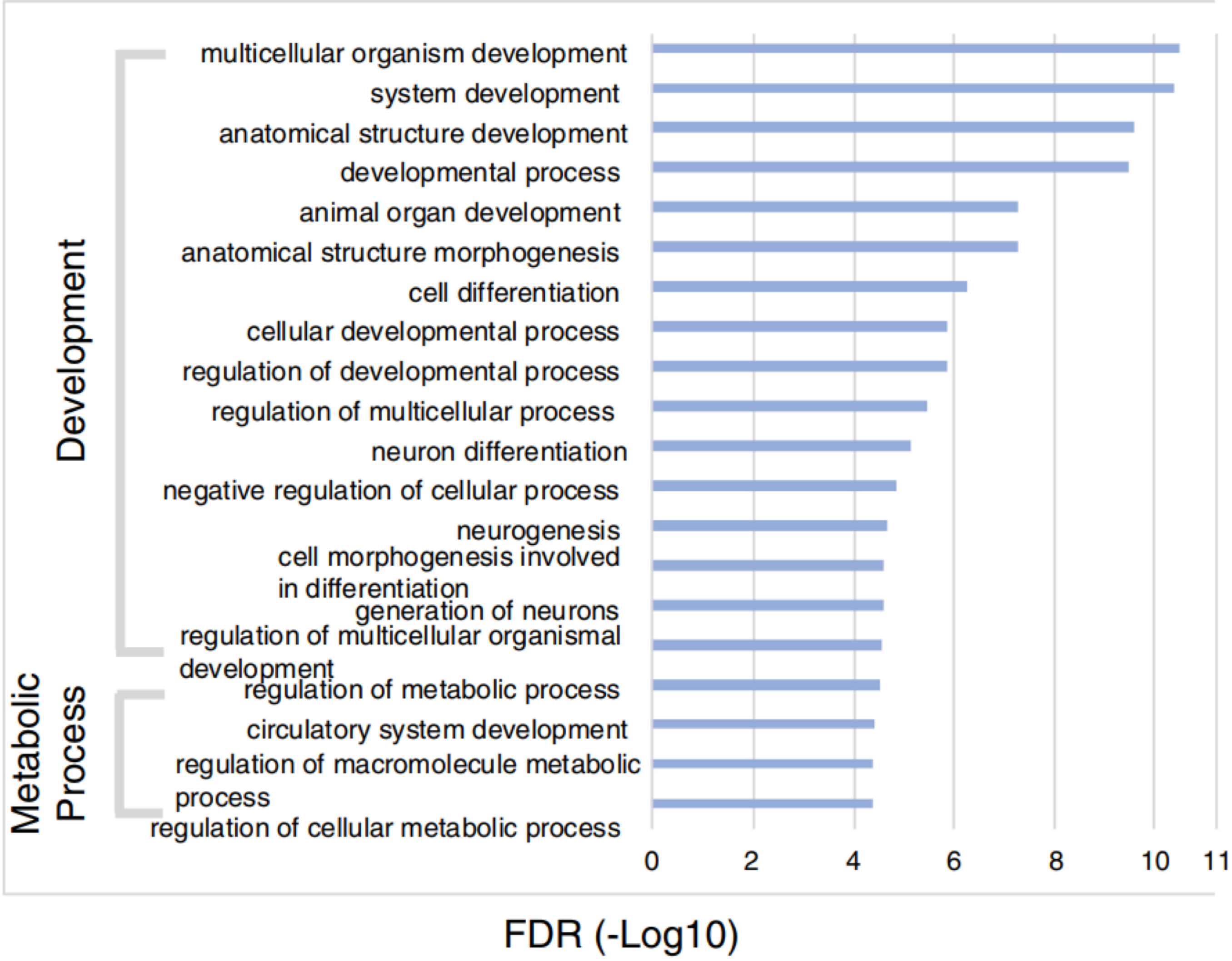


Firre is linked to various biological functions

Gene ontology analysis



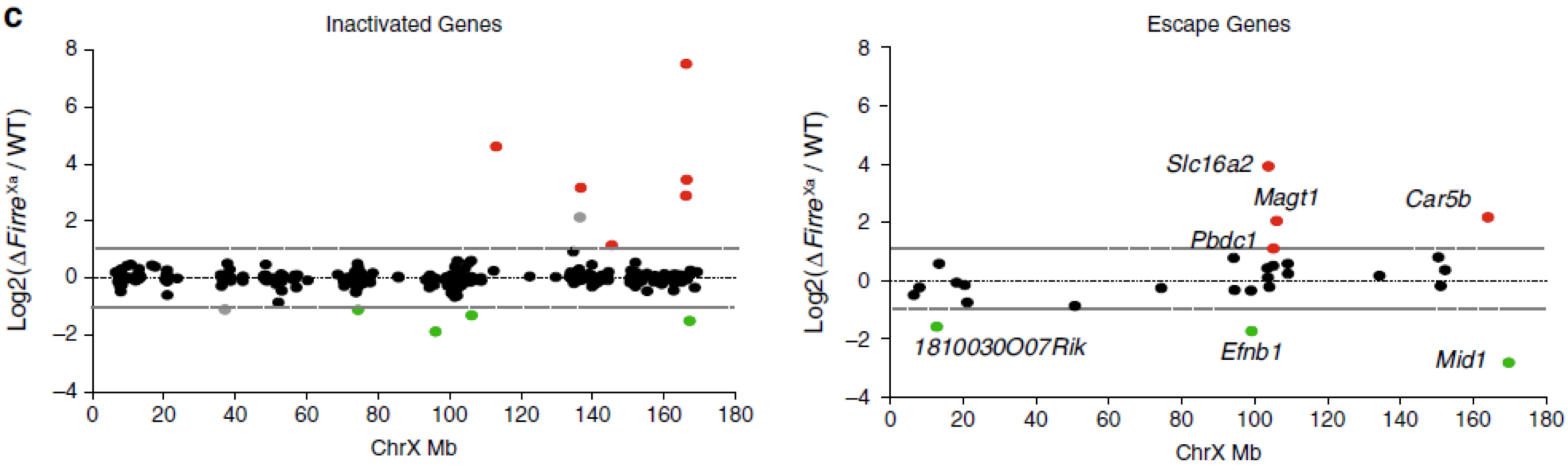




Does *Firre* RNA from the Xa affect gene activity of the Xi?

Gene expression is disrupted on the Xi upon the loss of *Firre* RNA

Study of allelic gene expression (*ΔFirreXa* / WT):



➔ **Minor Xi reactivation**

➔ **No changes for escape genes**

> 352 genes subject to XCI

-> 6 of them were **upregulated in cells with Firre depletion (4 of them located on telomeres)**

H3K27me3 clusters are only one of the many layers that control XCI gene expression

Trans acting effect of *Firre* RNA on gene expression

***Firre* RNA are linked to:**

→ the expression of many autosomal and X-linked genes

→ one of the many layers of repression of the Xi gene expression

II. Trans- and cis-acting effects of *Firre* on epigenetic features of the inactive X chromosome

→ Transcription of *Firre* and *CrossFirre* from the Xa

→ Impact of the trans-acting effect of *Firre* on epigenetic features of the Xi

→ Impact of the trans-acting effect of *Firre* on the nuclear location of Xi

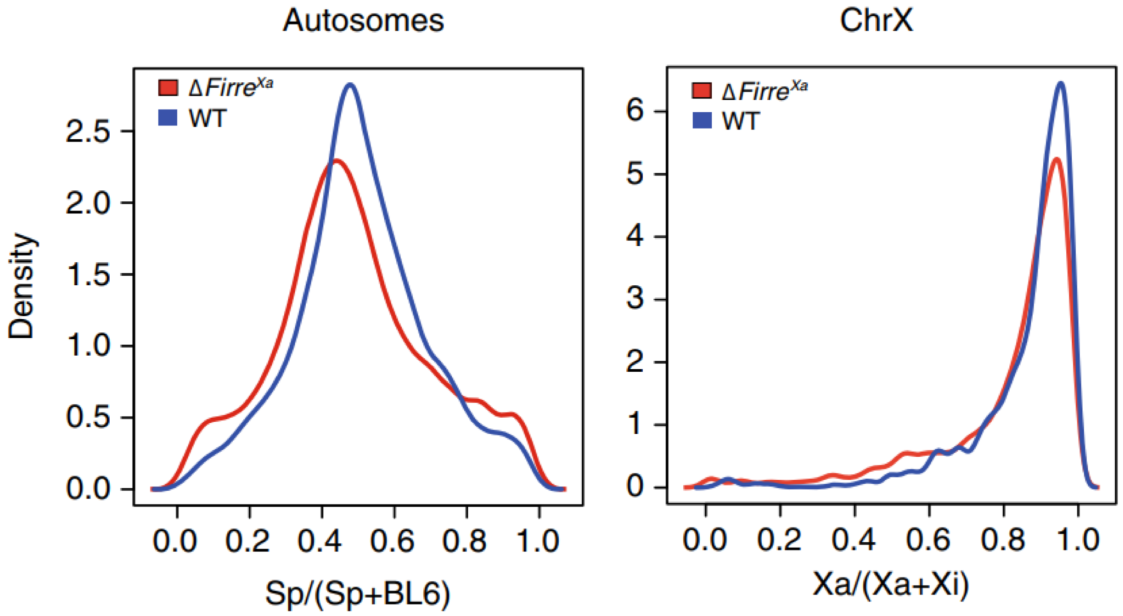
→ Trans acting effect of Firre RNA on gene expression

→ Impact of the *Firre* RNA on Xi and Xa structures

Does *Firre* RNA have an impact on the Xi chromatine accessibility ?

Firre locus on the Xa impacts Xi structure

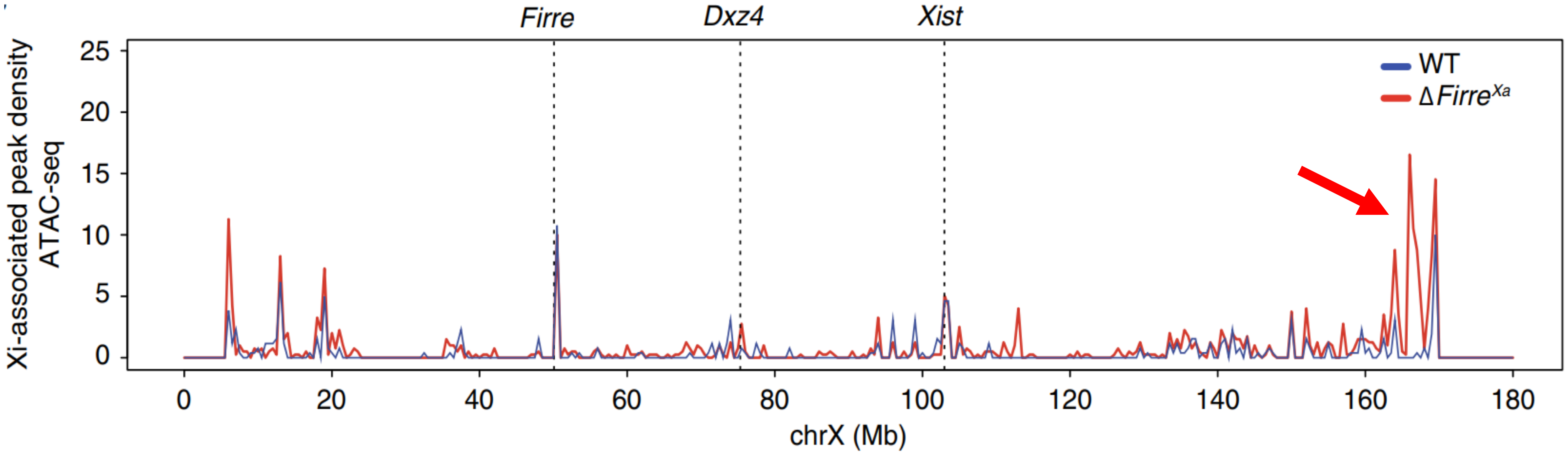
ATAC (CTCF) allelic proportions



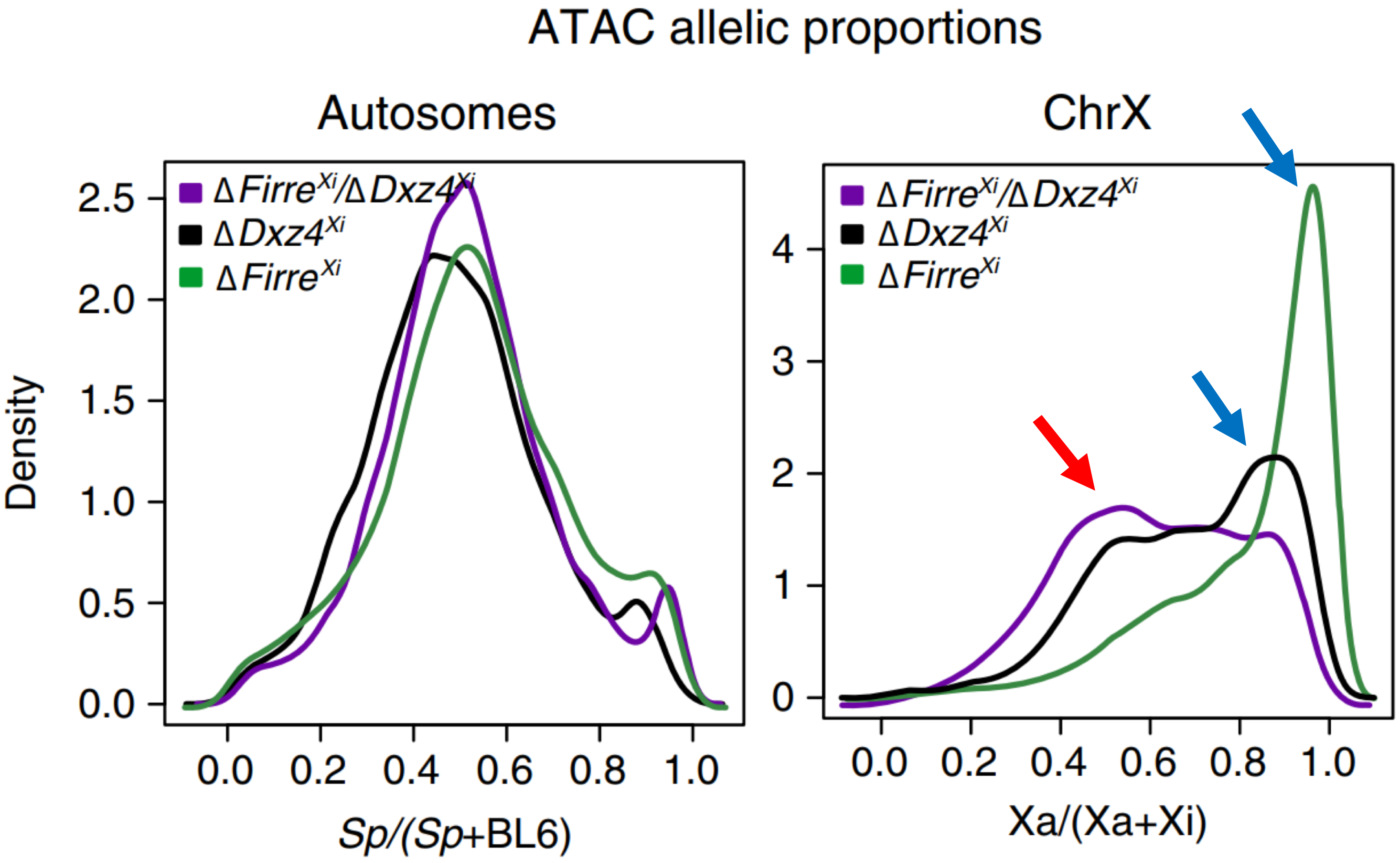
→ No shift in chromatine accessibility

HOWEVER…..

→ Some genes were reactivated in the telomeric region



Firre locus on the Xa impacts Xi structure

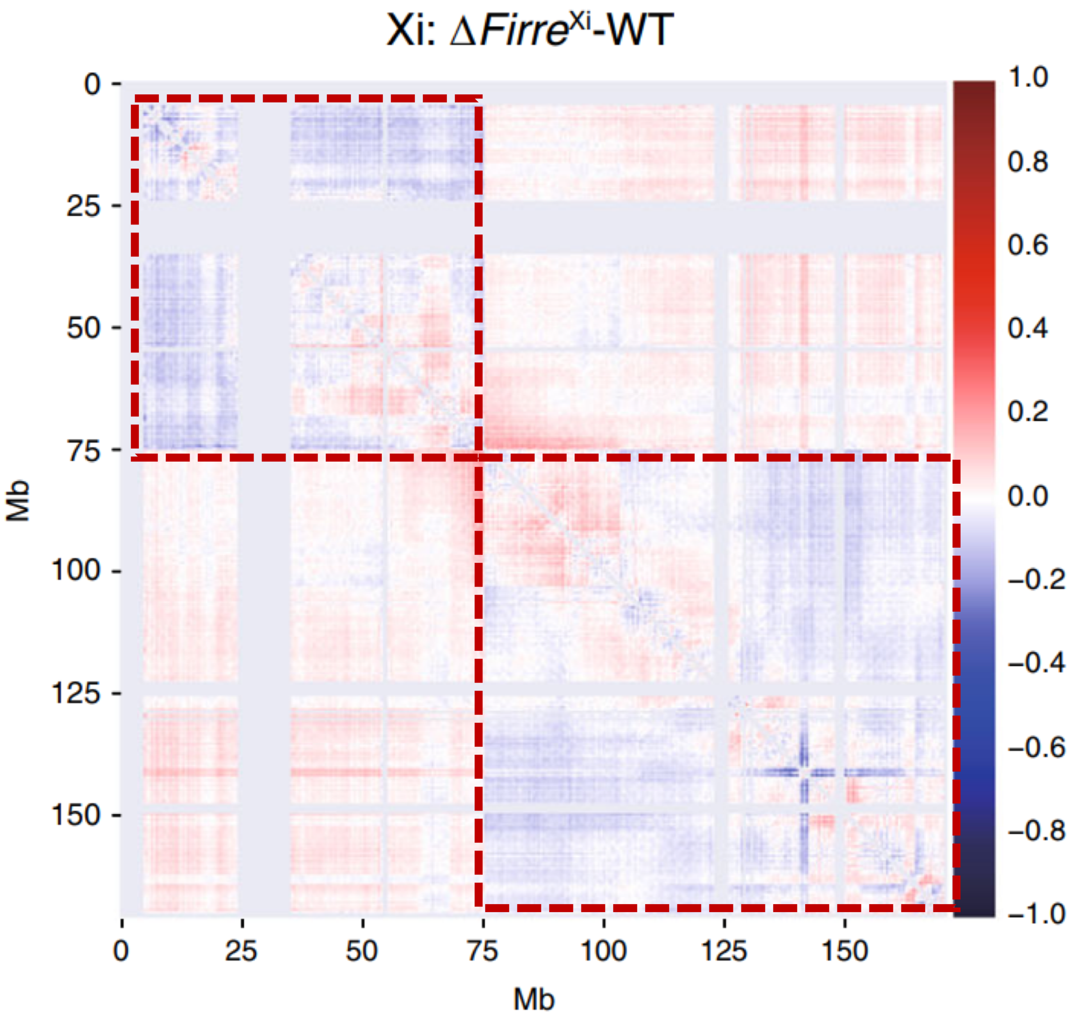


Double-mutant of lncRNAs *Firre* and *Dxz4* compared to simple mutants

- ✓ Increased of chromatin accessibility on the Xi for the double mutant
- ✓ Possible cooperation between Firre and Dxz4 in the repression of chromatin accessibility on the Xi

Does *Firre* RNA from the Xi impact its 3D structure by cis-effect?

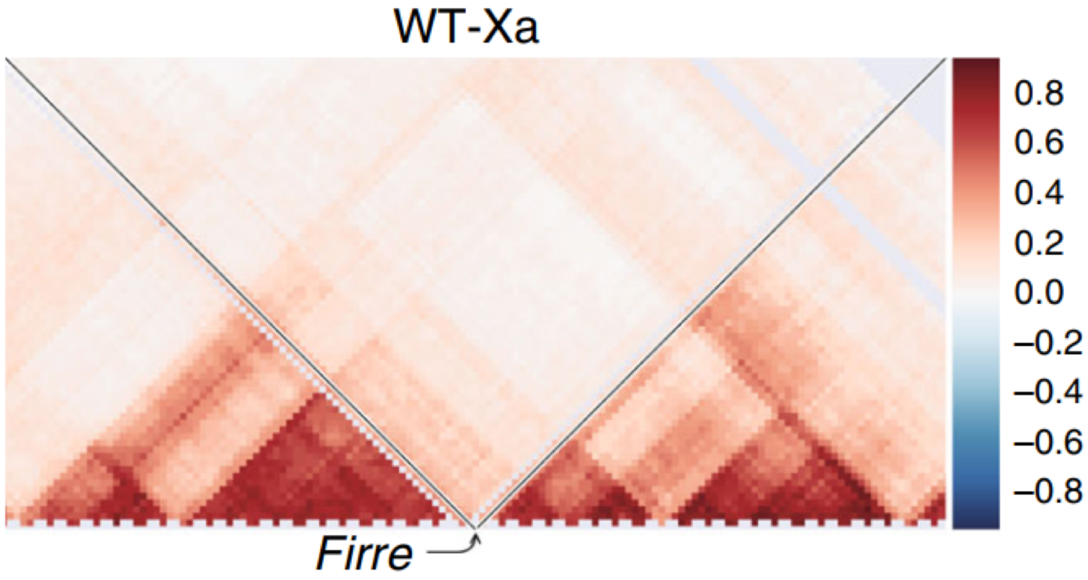
Firre RNA shows a cis-effect on Xi structure

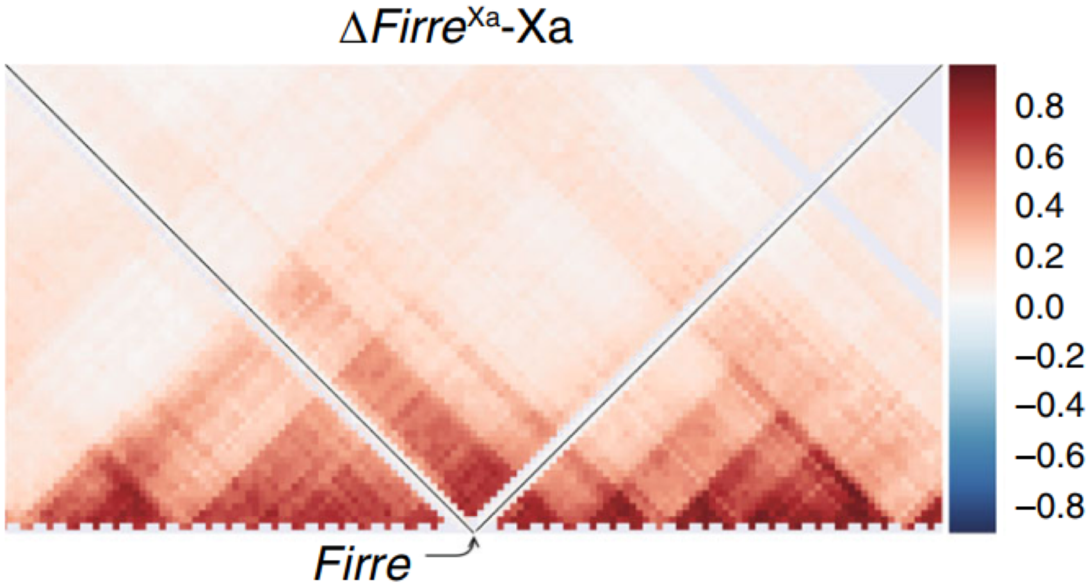


Contacts **increased between the superdomains** and **decreased within each superdomain.**

Does *Firre* RNA from the Xa have a cis-effect on its structure?

Firre RNA transcribed from Xa impacts its chromosome structure





→ Loss of the strong boundary between TADs on *Firre* locus

Contact changes on the Xa, including loss of the boundary at or close to the locus

Impact of *Firre* RNA on Xi and Xa structure

- Possible cooperation between *Firre* and *Dxz4* for repression of gene expression on the Xi
- *Firre* RNA from the Xi exerts a cis-effects on the Xi 3D structure
- *Firre* RNA from the Xa exerts cis-effect on the Xa structure

III. Discussion

What’s next?

Firre acts in *trans* in different cell lines, and **no loss of H3K27me3 was observed on the Xi** of different cell types (brain, kidney, and liver)

↓

Such loss is detected in immune cells (such as Common Lymphoid Progenitors)

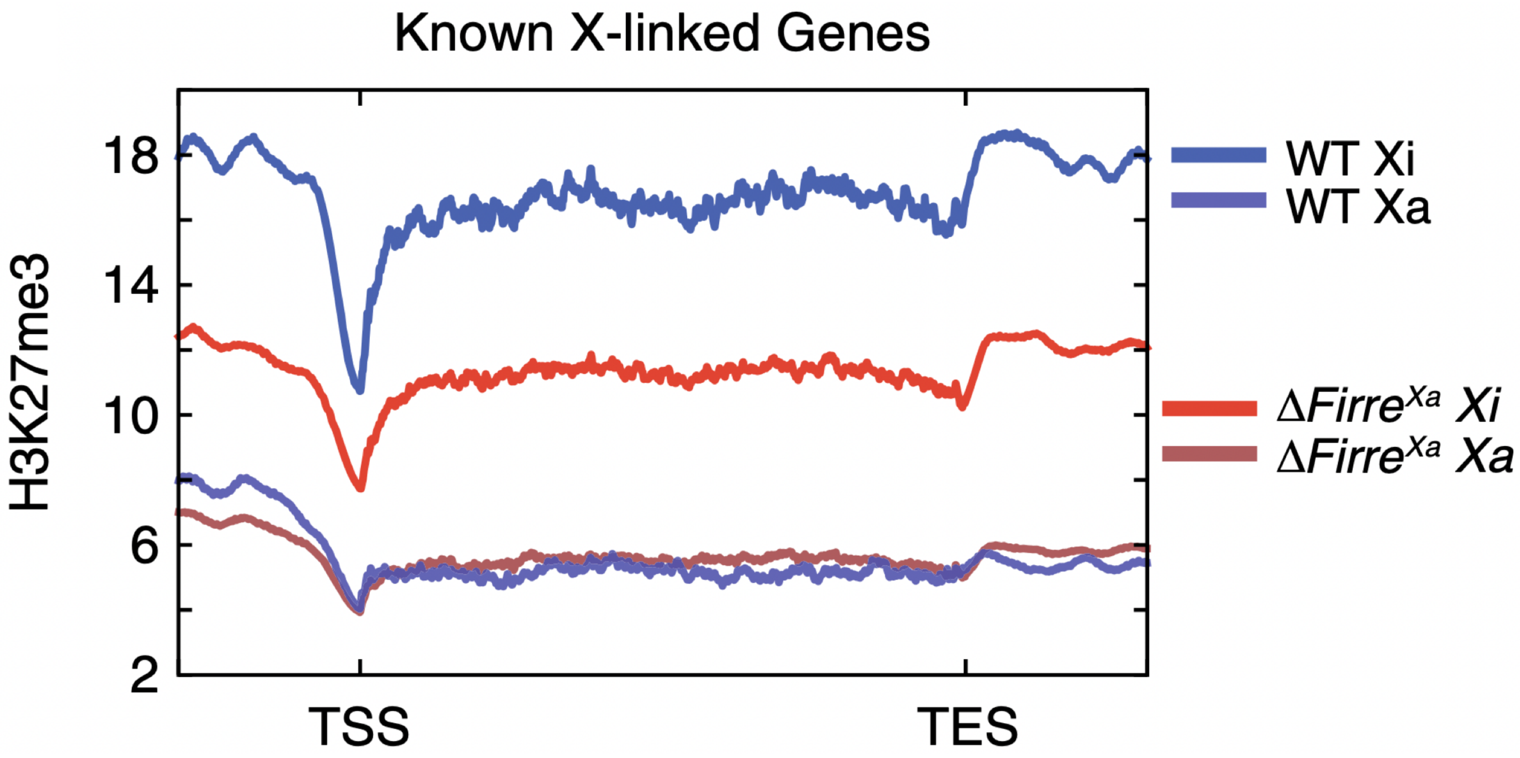
Observed Xi reactivation on female lymphocytes and
cis-effect of *Firre* RNA in immune cells

→ *Does it improve Firre expression from the Xi in those cells?*

Need to better understand *Firre* local effect on immune cells to further application on X-linked disease

THANK YOU FOR YOUR ATTENTION

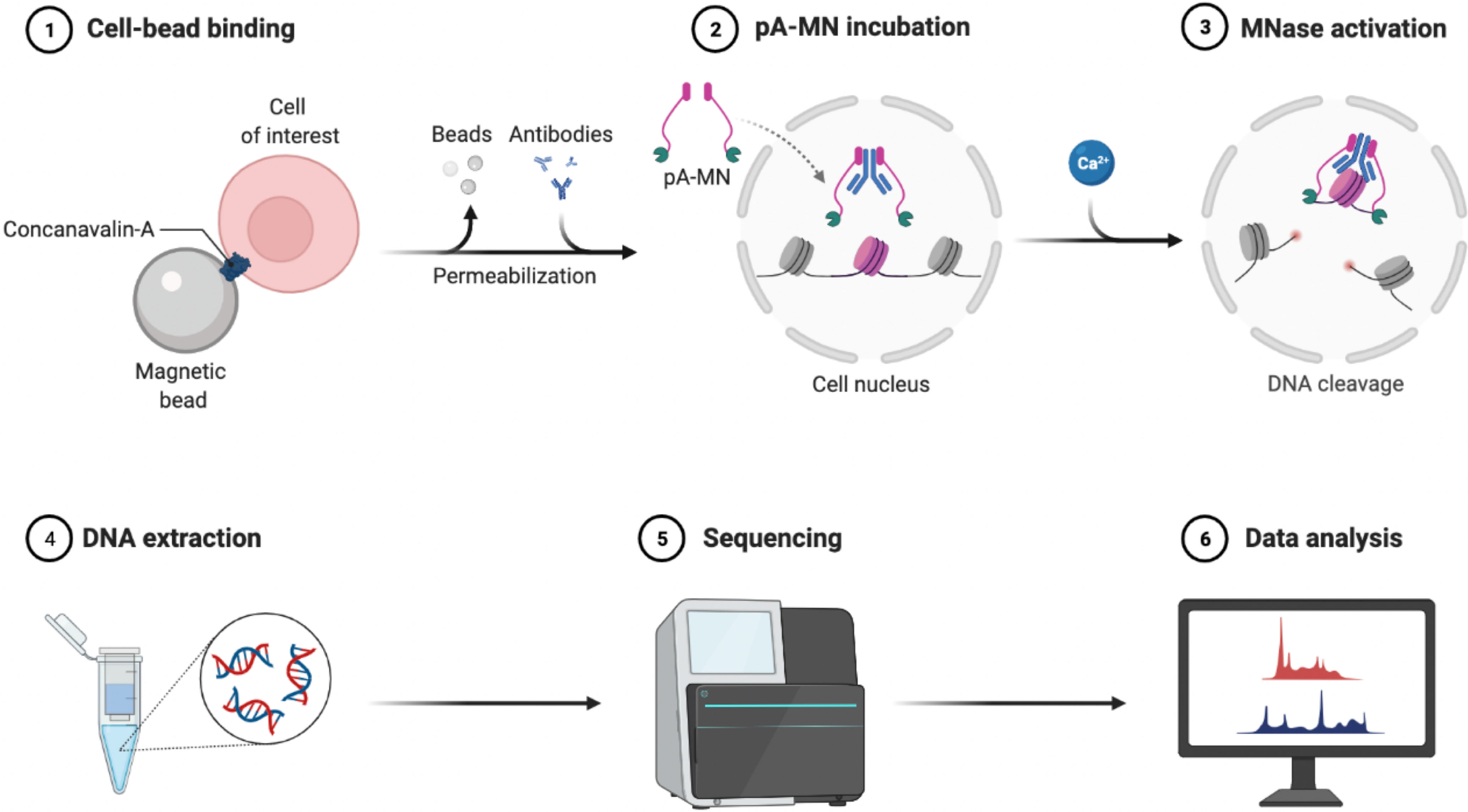
H3K27me3 enrichment on the Xi and loss of H3K27me3 around the TSS



Demonstrate a dramatic loss of H3K27me3 around the transcription start site (TSS) and throughout the body of X-gene linked in mutant cells

Cleavage Under Target & Release Using Nuclease (CUT & RUN)

→ CUT & RUN isolates protein-DNA complexes on native chromatin



Is there a link between known epigenetic marks and gene dysregulation due to *Firre* RNA?

Metagene plot of H3K27me3 for cells with Firre deletion

→ Small increase of H3K27me3 in **downregulated genes**

→Most dysregulated genes (90%) had no increase in H3K27me3.

→No modifications observed for other active epigenetic marks H3K36me3 and H3K4me3 (CUT&RUN)