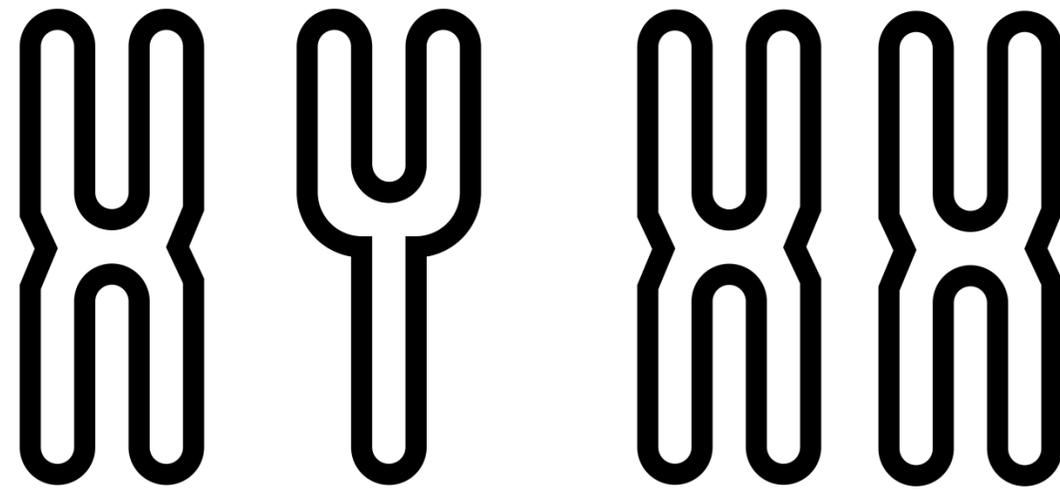# Xist spatially amplifies SHARP/SPEN recruitment to balance chromosome-wide silencing and specificity to the X chromosome

Joanna W. Jachowicz, Mackenzie Strehle, Abhik K. Banerjee, Mario R. Blanco, Jasmine Thai and Mitchell Guttman
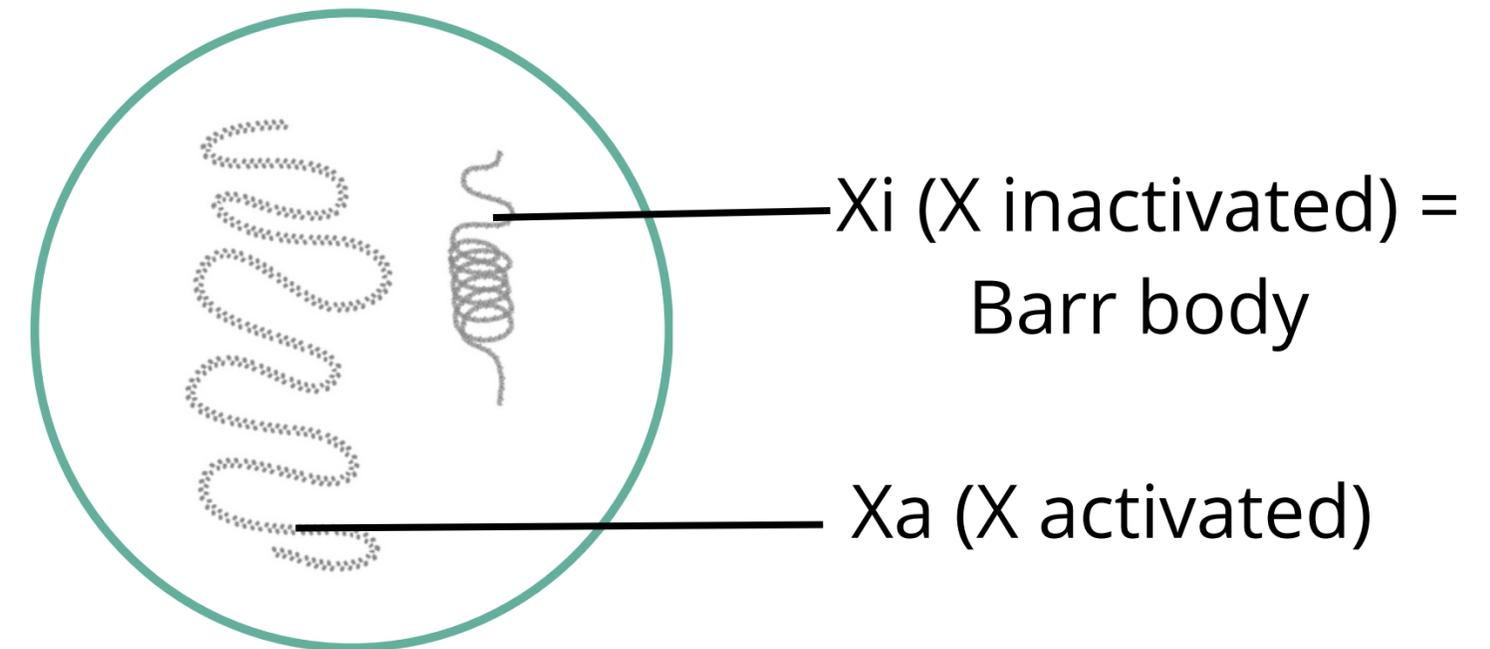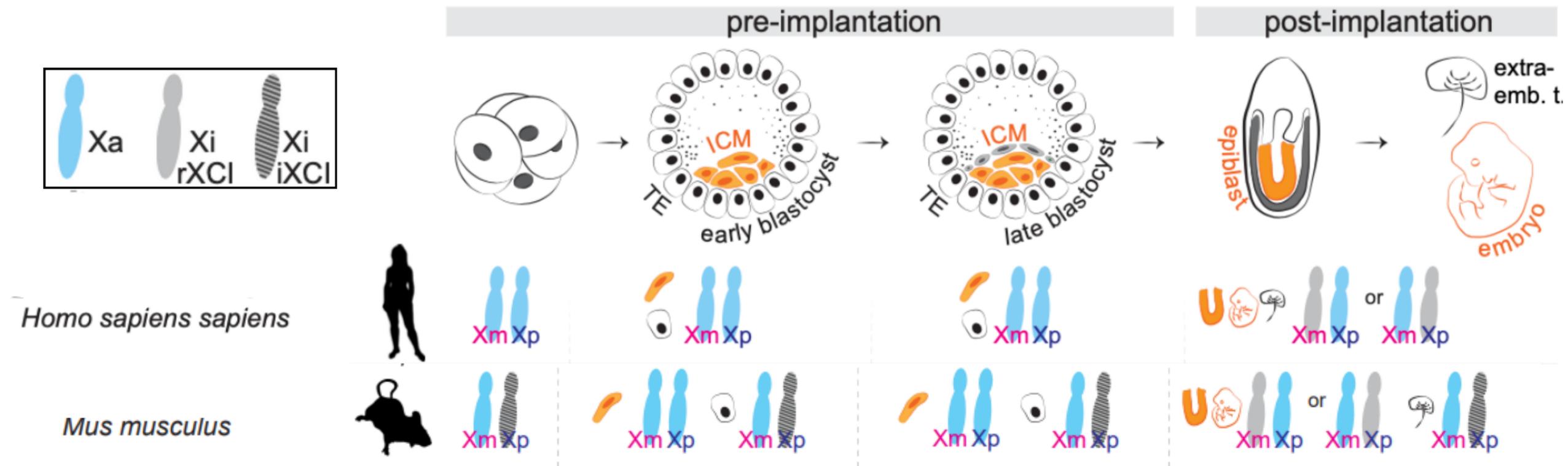
nature structural & molecular biology

UNIVERSITÀ DEGLI STUDI DI TRIESTE

Collyn HAAR & Diego MORICE

X inactivation

Double dosing of X gene is incompatible with early development

One of the X chromosome is **silenced** bi heterochromatisation

Xi (X inactivated) = Barr body

Xa (X activated)

The X inactivation phenomena

- Choice of X

Furlan, G.; Galupa, R. Mechanisms of Choice in X-Chromosome Inactivation. Cells
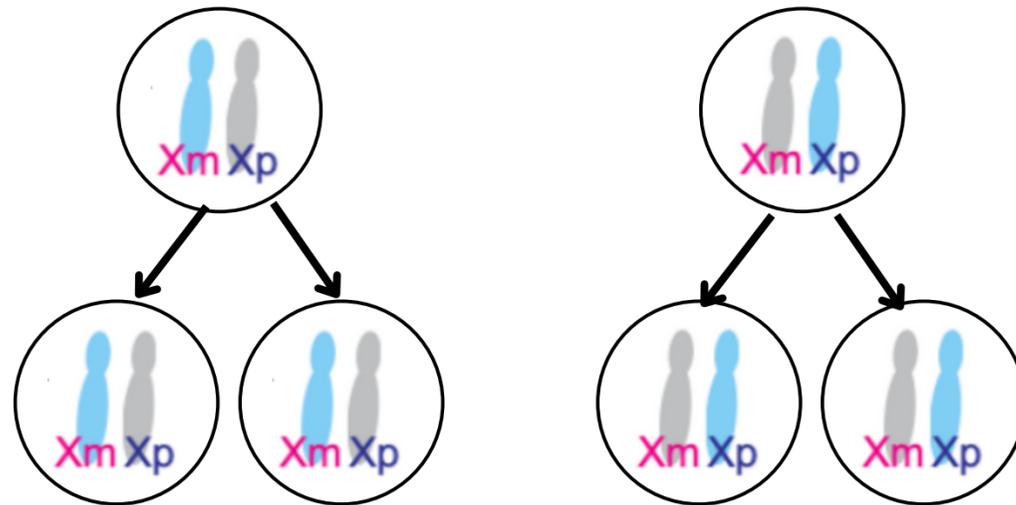
The X inactivation phenomena

- ## THE X CHOICE MECANISM

  → Random

  → Conserved in the cell's progeny

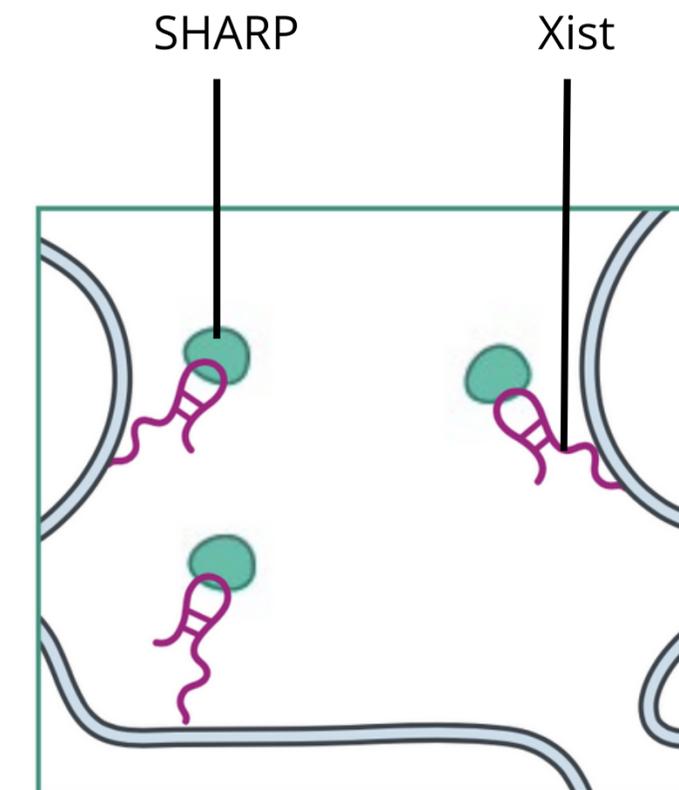- ## MOSAICISM PHENOMENA

  → Visual example of X choice

Mechanism of X silencing

Xist :
- Coded by X chromosome
- Non coding RNA 15000 nt
- Located all over the X chromosome

SHARP :
- Protein 400 kDA - 3664 AA
- 4 RNA reconizion motif
- Domain C-terminal

Jachowicz et al., Nat Struct Mol Biol, 2022

SHARP   Xist

SHARP : RRM   IDR   SPOC
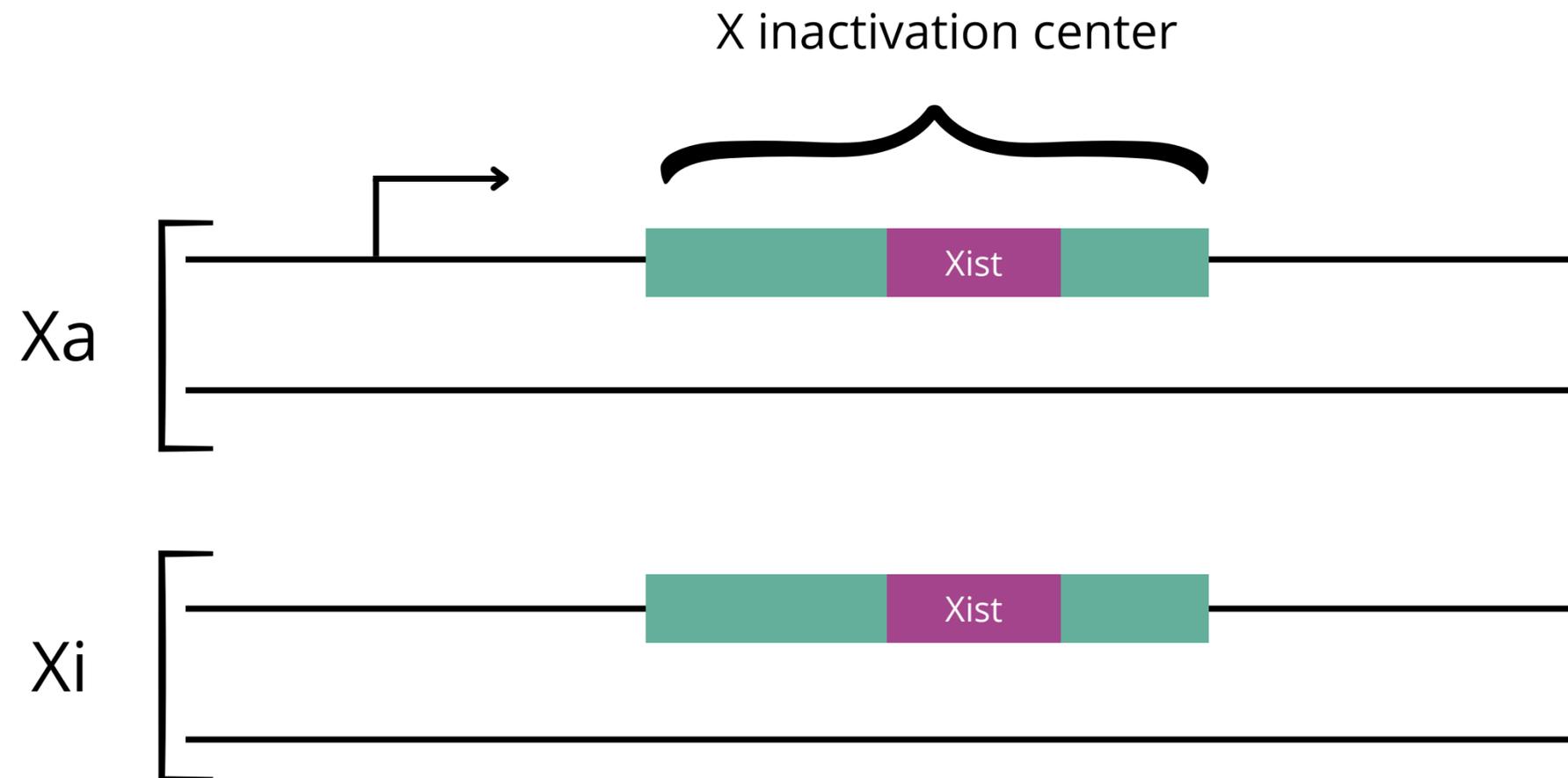
RNA recognition motif
Intransicaly disordered region
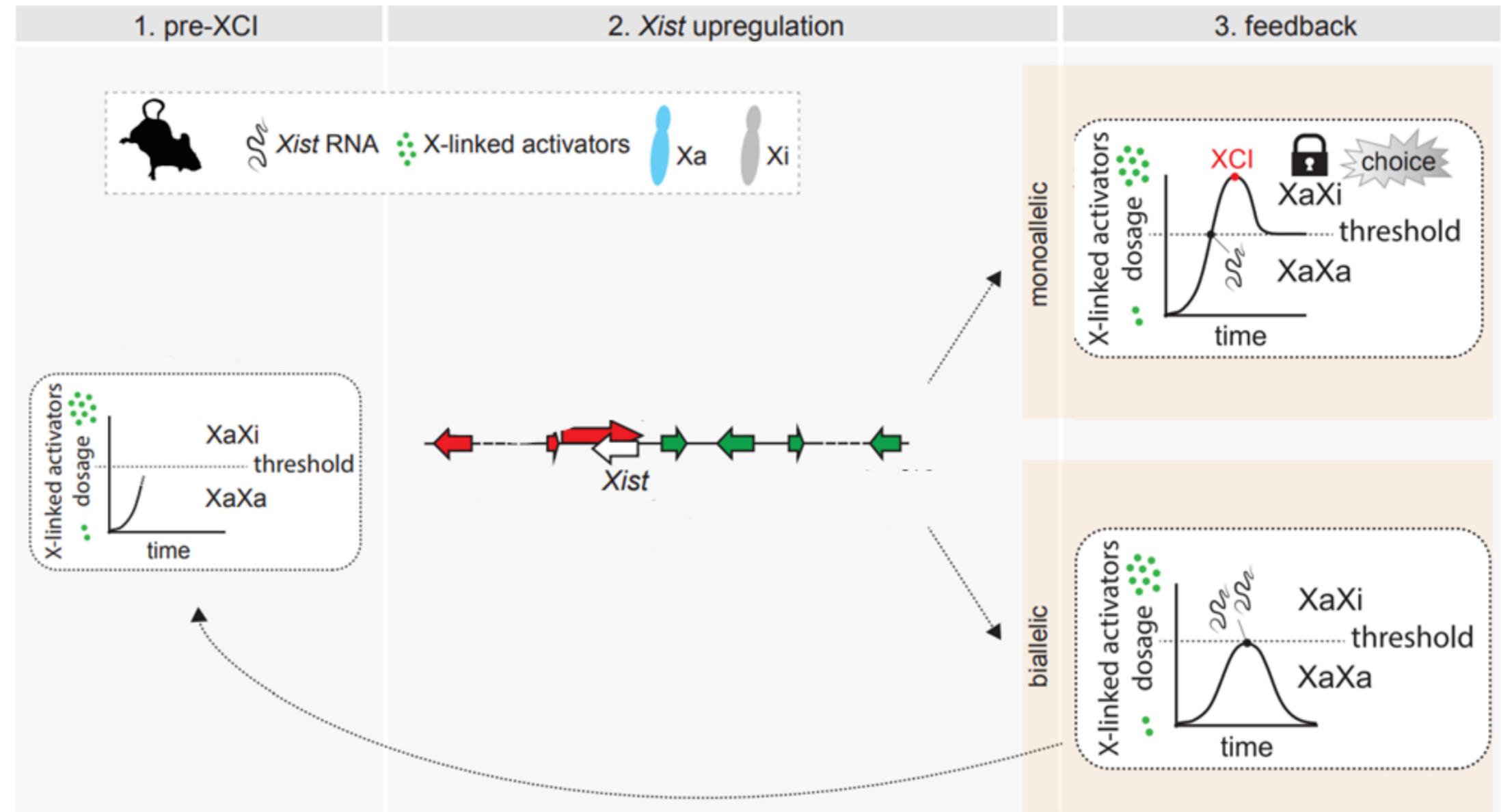SPEN paralog and ortholog C-terminal

Mechanism of X silencing
1. Initiation

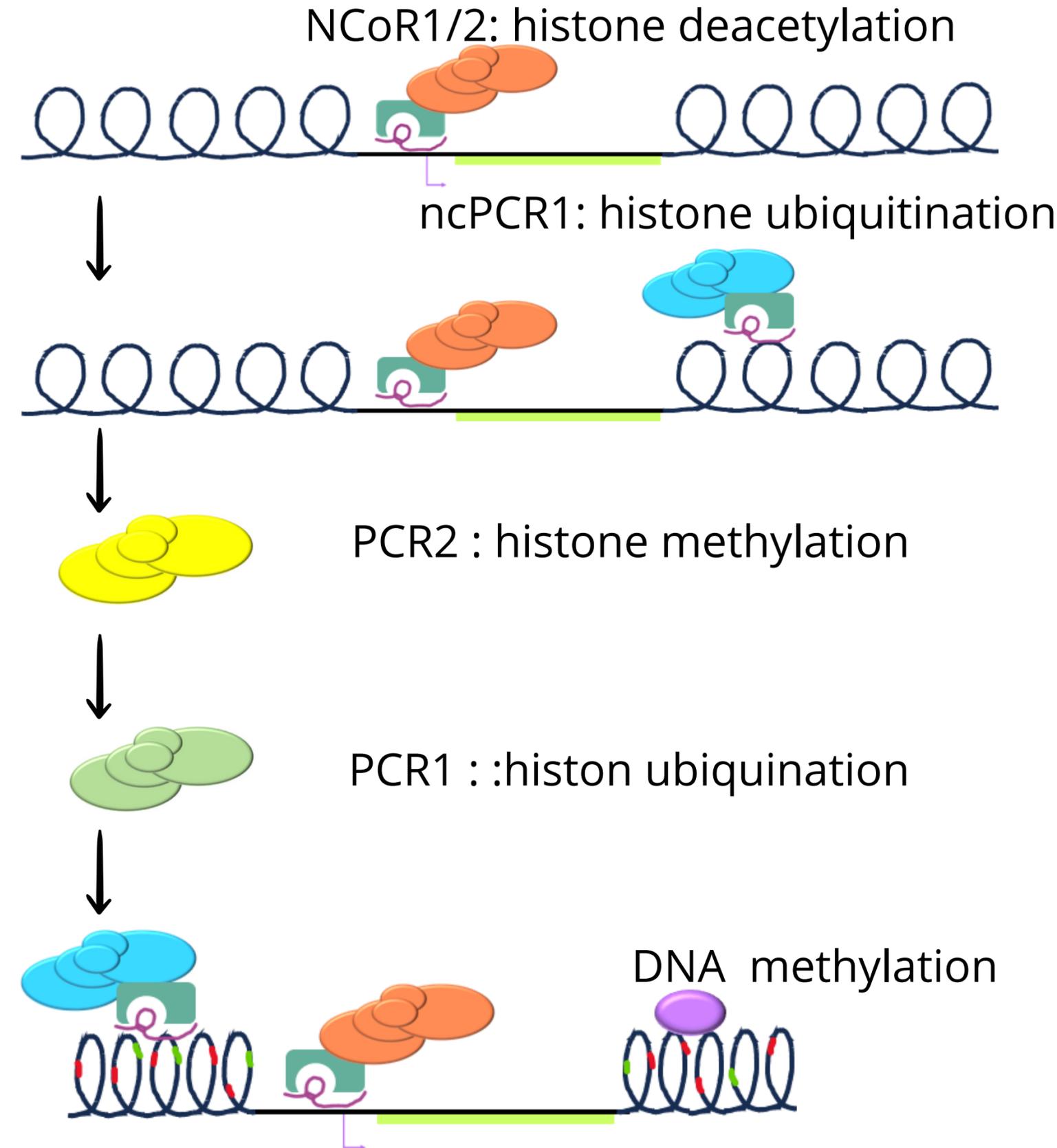- X inactivation center

Mechanism of X silencing

1. Initiation

- Race to inactivation
- Concentration of X linked activation factor

Furlan, G.; Galupa, R. Mechanisms of Choice in X-Chromosome Inactivation. Cells

Mechanism of X silencing

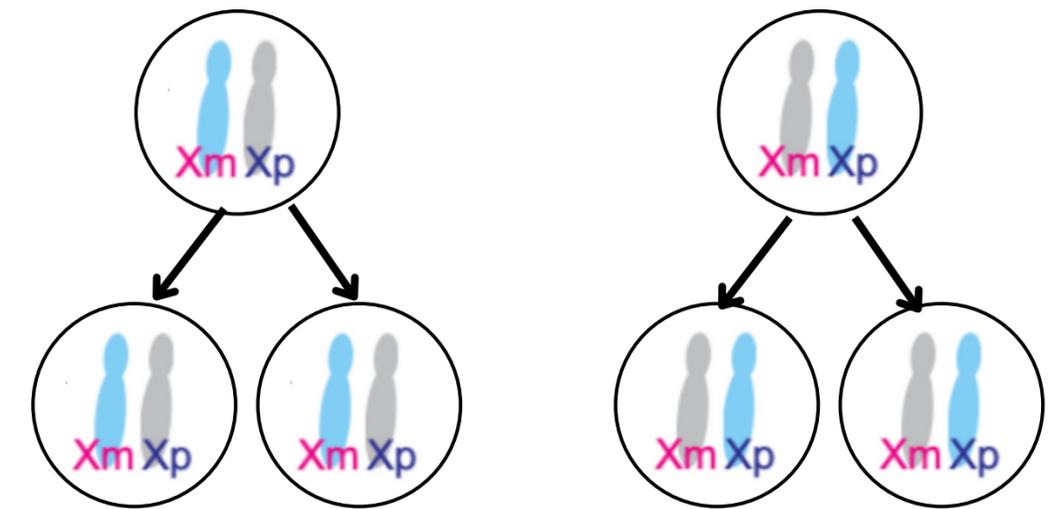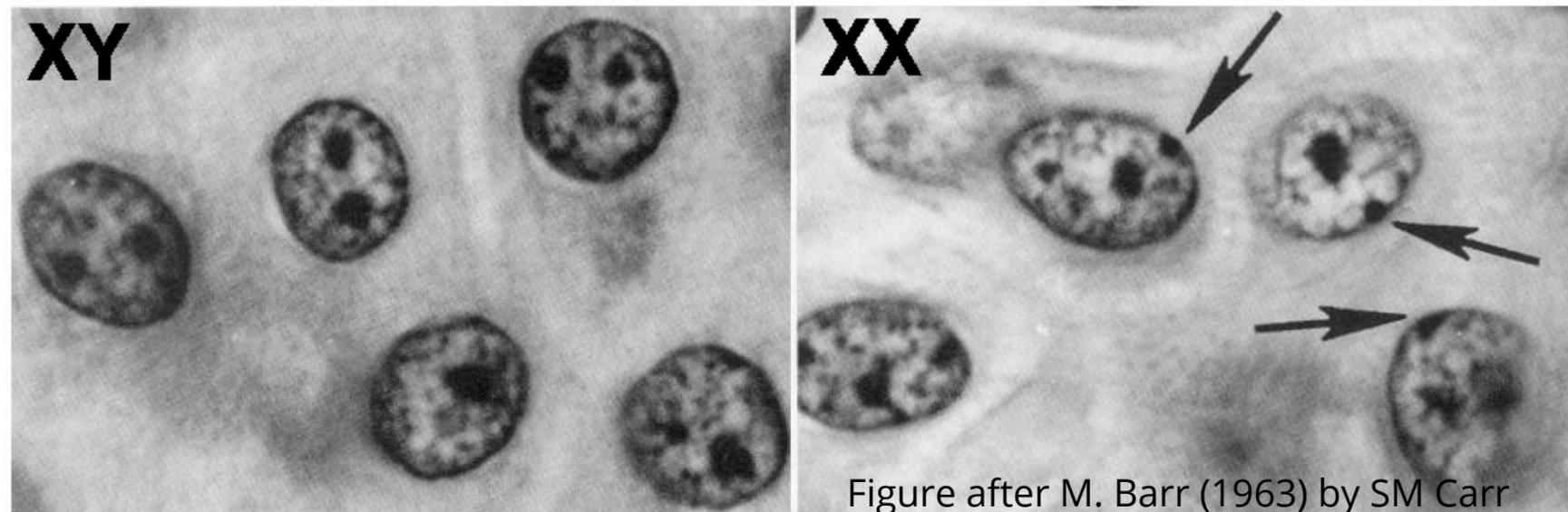## 2. Establishment

- Xist transcription in X inactivation center
- Sharp recuitment by Xist
- Sharp recruit proteins complexes

NCoR1/2: histone deacetylation

ncPCR1: histone ubiquitination

PCR2 : histone methylation

PCR1 : :histon ubiquination

DNA methylation

Mechanism of X silencing

3. Maintenance

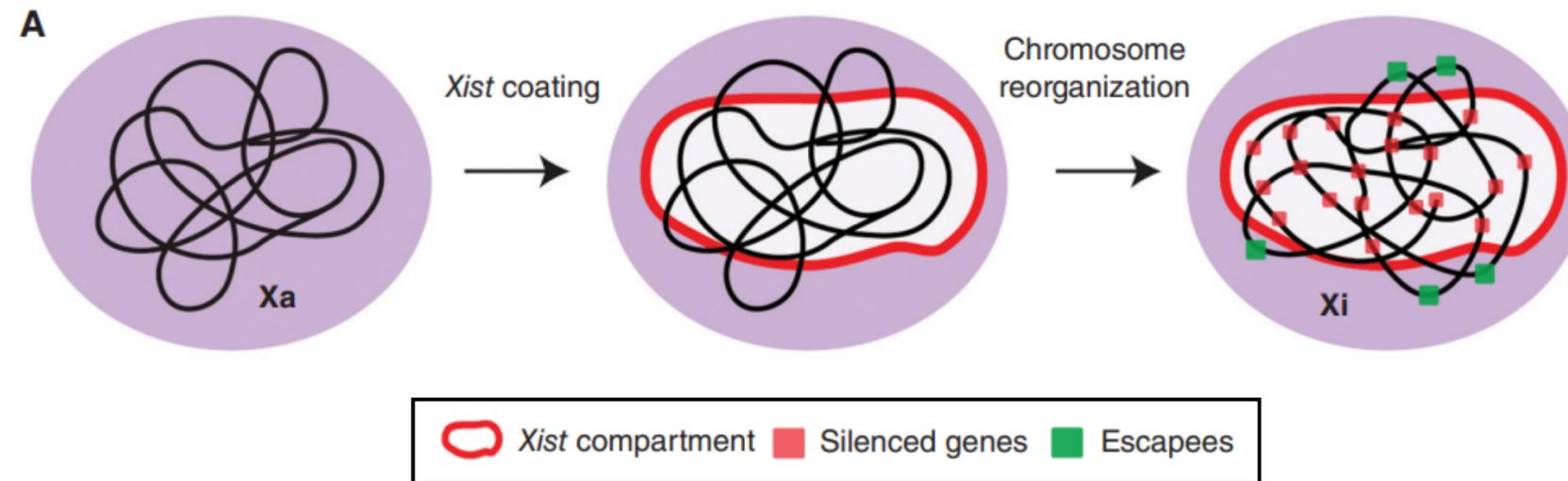Figure after M. Barr (1963) by SM Carr

- DNA methylation
- Xist continuous expression

→ Silences the chromosome with **continued expression of Xist**

→ Xi **maintains its configuration** and **propagates** by cloning in all his divisions
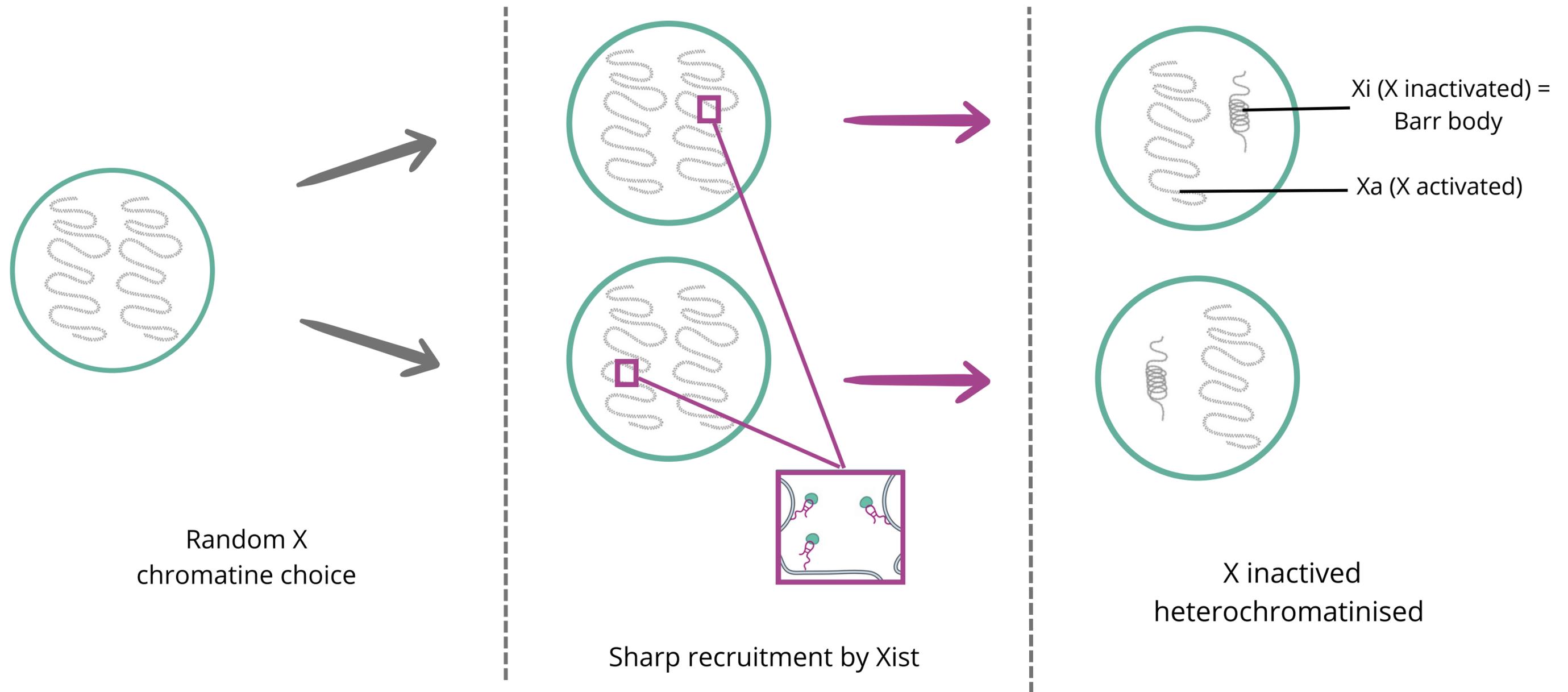
Some genes escape the silencing

From : The Molecular and Nuclear Dynamics of X-Chromosome Inactivation

⟶ 15 to 30% escape inactivation

⟶ Xist coating responsible

The Daily

Sunday, August 30, 2006

The X chx not inactivated !

Or at least not complaetly, some not at all, how is it some gene are only possible? partialy inactvated and

Global inactivation

Random X
chromatine choice

Sharp recruitment by Xist

Xi (X inactivated) =
Barr body

Xa (X activated)

X inactived
heterochromatinised

And if it's go wrong ?

If it didn't have X inactivation → Abortion

But some **diseases** can be in link with an **abnormal inactivation**
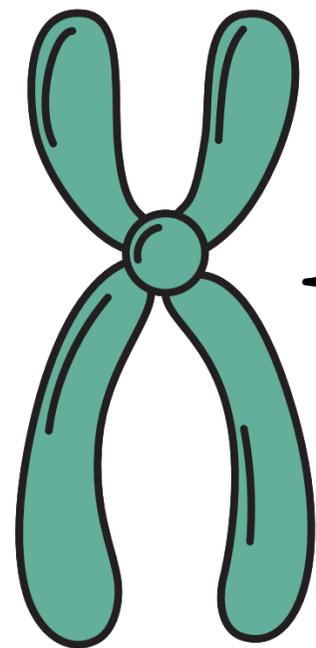
Code for 80% of immunity gene
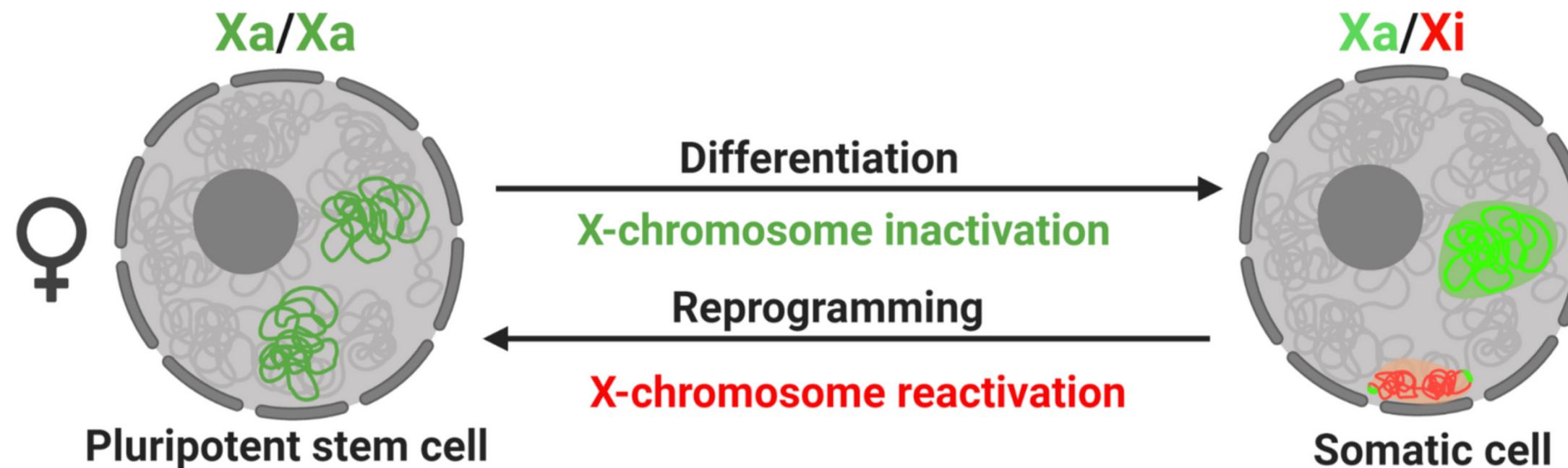
Many autoimmune disorders have a female bias

Current studies on Xist

→ Influence of mosaiscim

→ Reactivating the other x in case of x linked desease

From : Amitesh Panda et al, Cell, 2020,

PROBLEMATIQUE

A greater understanding of those phenomenon at the molecular level is needed.

All the complexes necessary to x silencing are recruited by Xist.

But you have only 1 Xist mol for every megabase of DNA that need silencing

How does x silencing hapend in those condition ?

Check for updates

Xist spatially amplifies SHARP/SPEN recruitment to balance chromosome-wide silencing and specificity to the X chromosome

Joanna W. Jachowicz [1,3], Mackenzie Strehle [1,3], Abhik K. Banerjee[1,2], Mario R. Blanco[1], Jasmine Thai[1] and Mitchell Guttman [1]✉

To better understand how Xist works, the article address five main questions :

1. **How do Xist recruit sharp ?**
2. **How do SHARP assemblies form ?**
3. **How does SHARP recruitment hapen?**
4. **Does SHARP foci play a role in X silencing?**
5. **Why is this strategy necessary?**

Methods

Experimental model
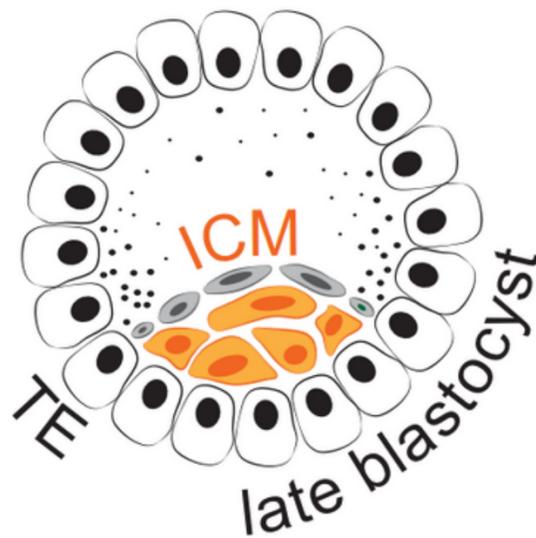
Femal F1 hybrids (Bl6xCast)
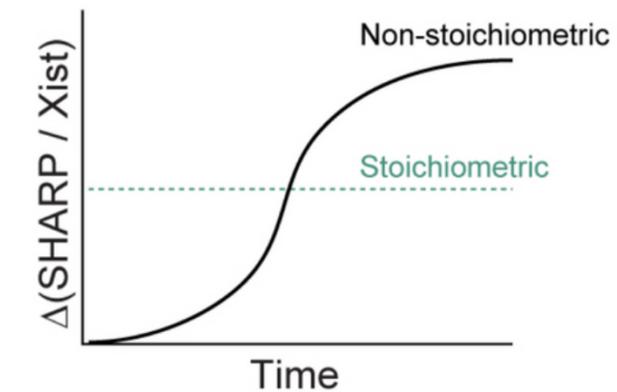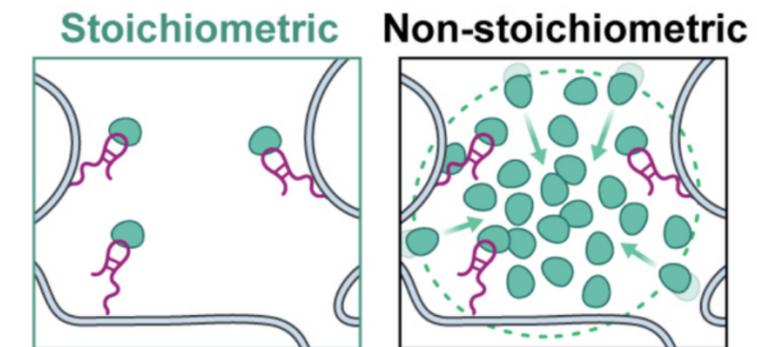Mouse embryonic stem cells

Induction of Xist by dox

Furlan, G.; Galupa, R. Mechanisms of Choice in X-Chromosome Inactivation. Cells
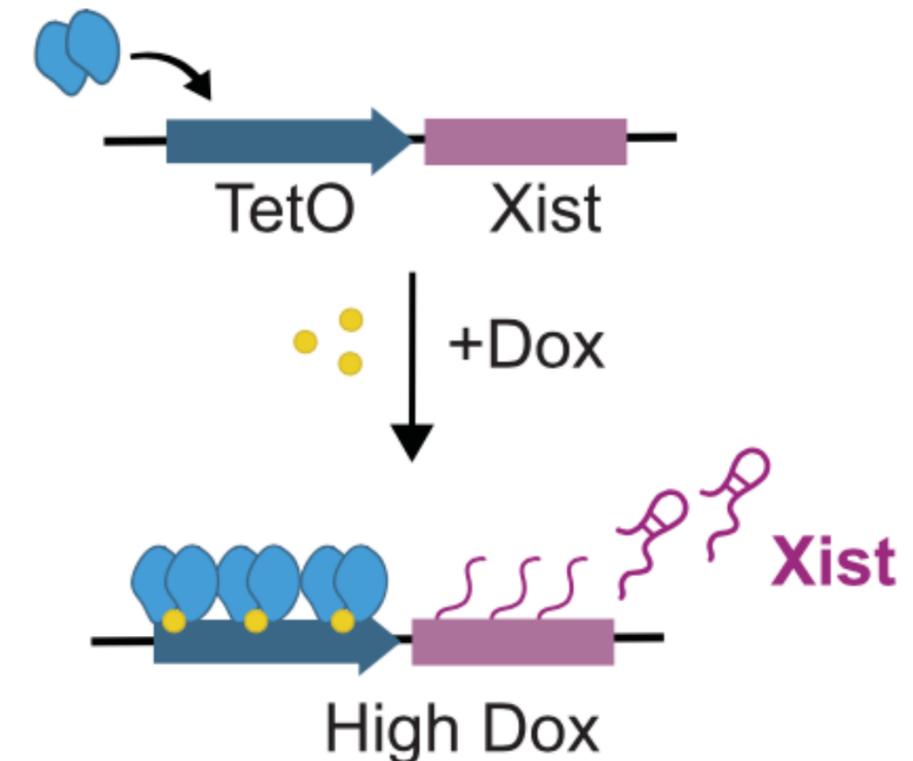
1. How do Xist recruit sharp ?

Methods

HaloTag construction

Promotor | SHARP | HaloTag

Antibody against HaloTag

SHARP → Fluorescence

No SHARP → No fluorescence

Stoichiometric | Non-stoichiometric

Non-stoichiometric

Δ(SHARP / Xist)

Stoichiometric

Time
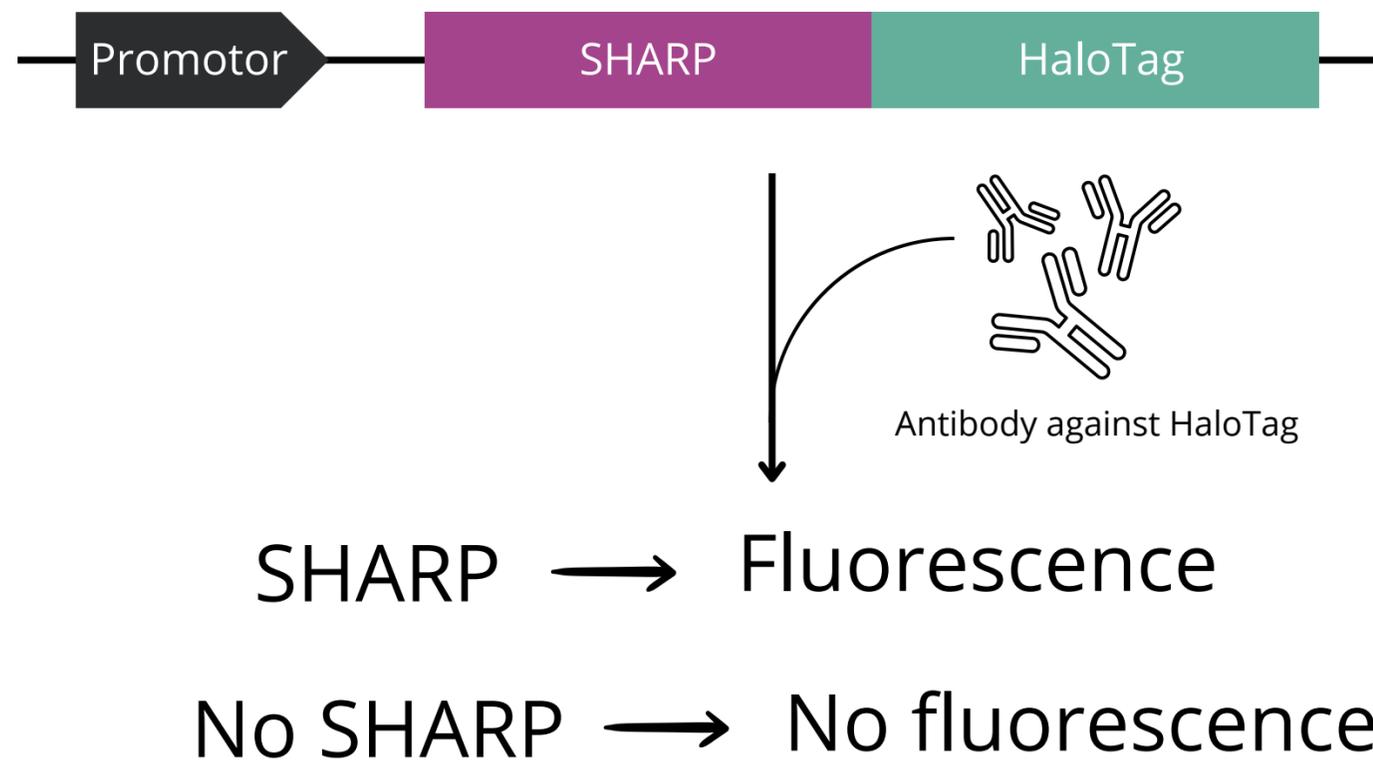
SHARP/Xist = 1 (constant)

↓

Recruitment is stoichiometric

1. How do Xist recruit sharp ?

Result

RNA-fish on mouse mES
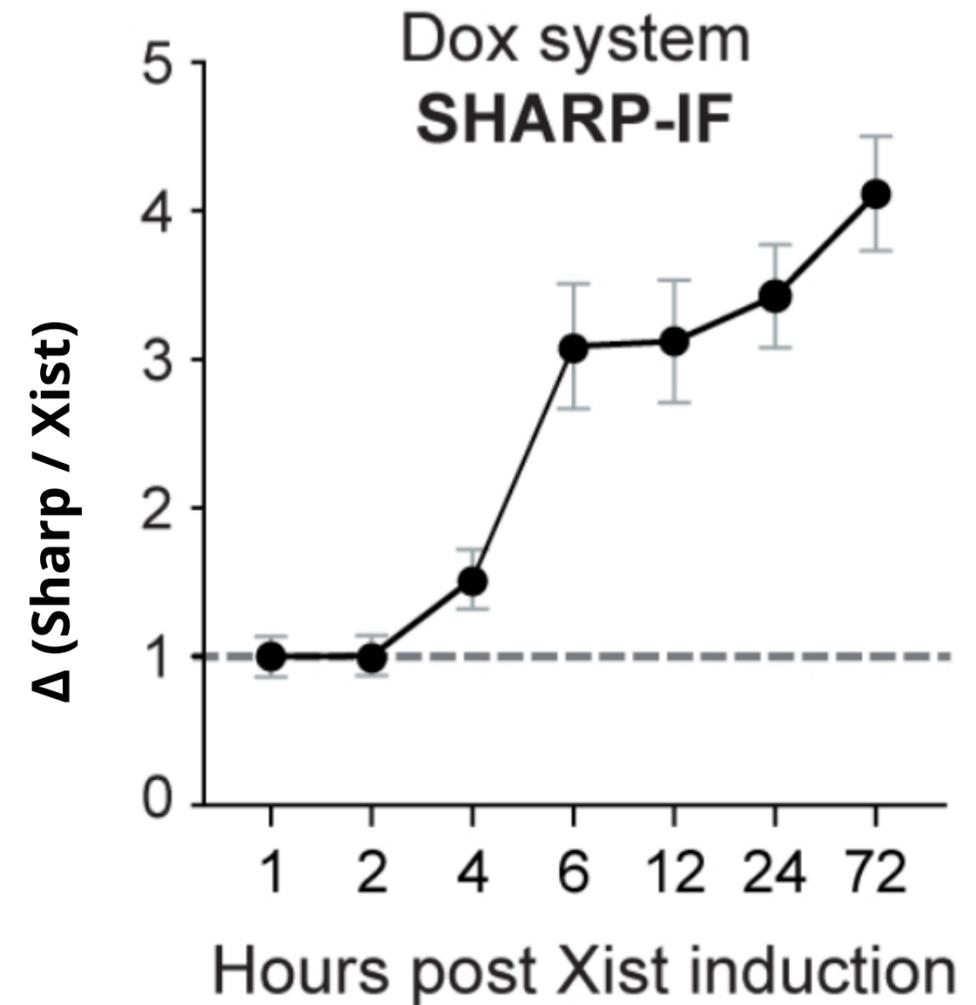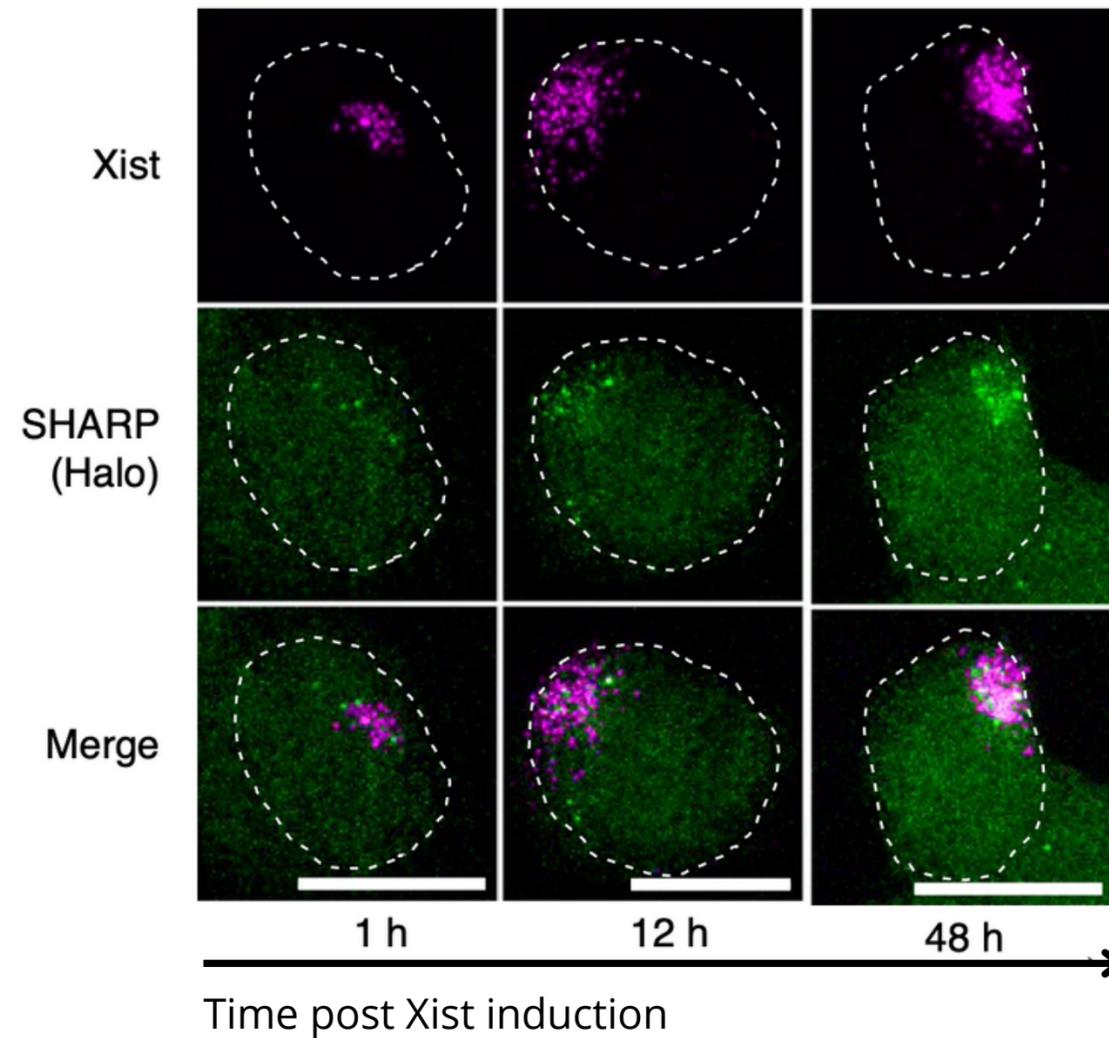
Stocheometric excess

2. How do sharp assemblies form ?
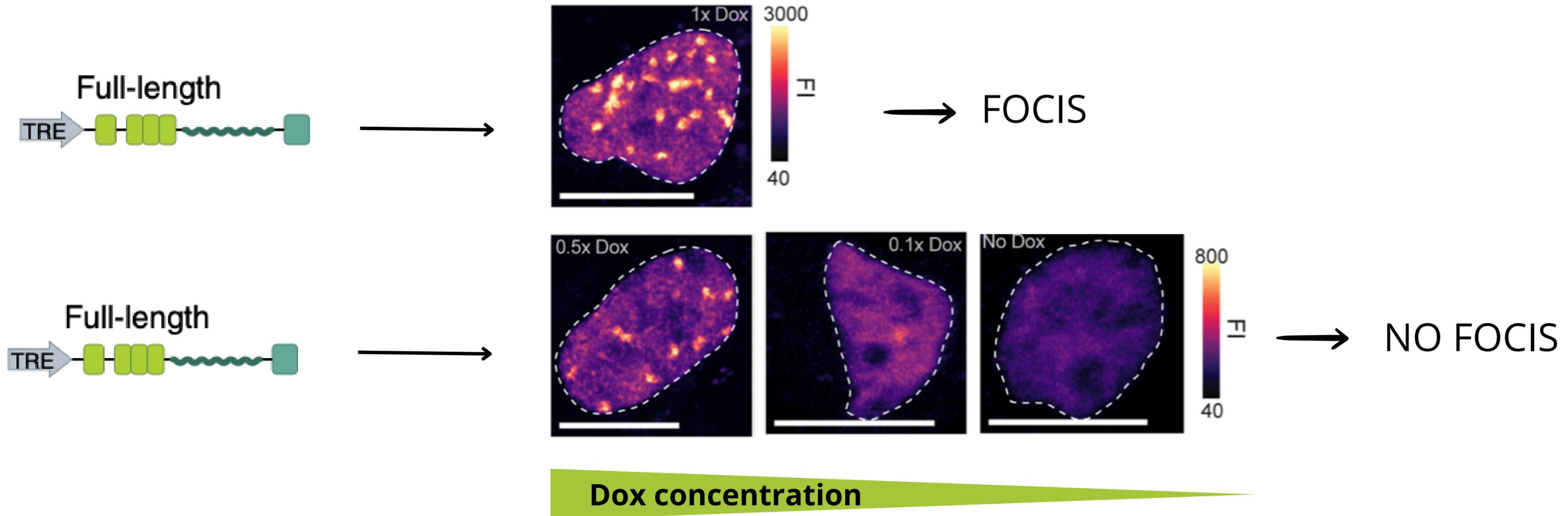
Sharp form concentration dependant assemblie in the nuclues

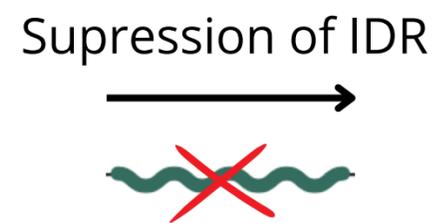# 2. How do sharp assemblies form ?

Sharp deletion analysis :

FUS = Form multivalent homotypics interactions
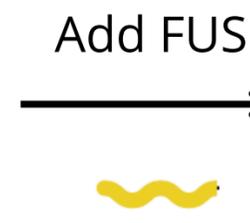
Methods

Deletion   Rescue

Differents models

Full-length
RRM   IDR   SPOC

Supression of IDR

ΔIDR

Add FUS

FUS-ΔIDR
FUS

Fluorescence technique

FL-SHARP
TRE GFP   RRM   IDR   SPOC

ΔIDR-SHARP
TRE GFP   RRM   SPOC

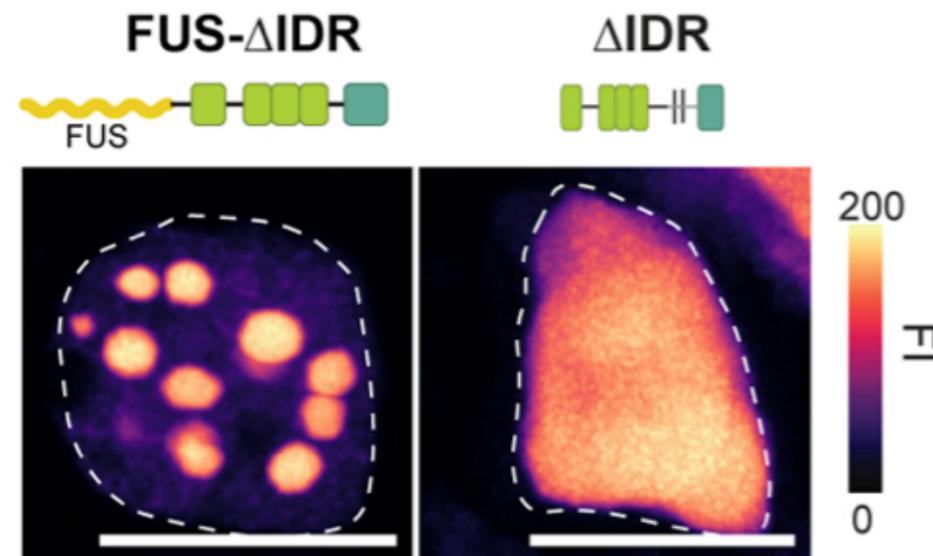FUS-ΔIDR-SHARP
TRE FUS mCh   RRM   SPOC

2. How do sharp assemblies form ?
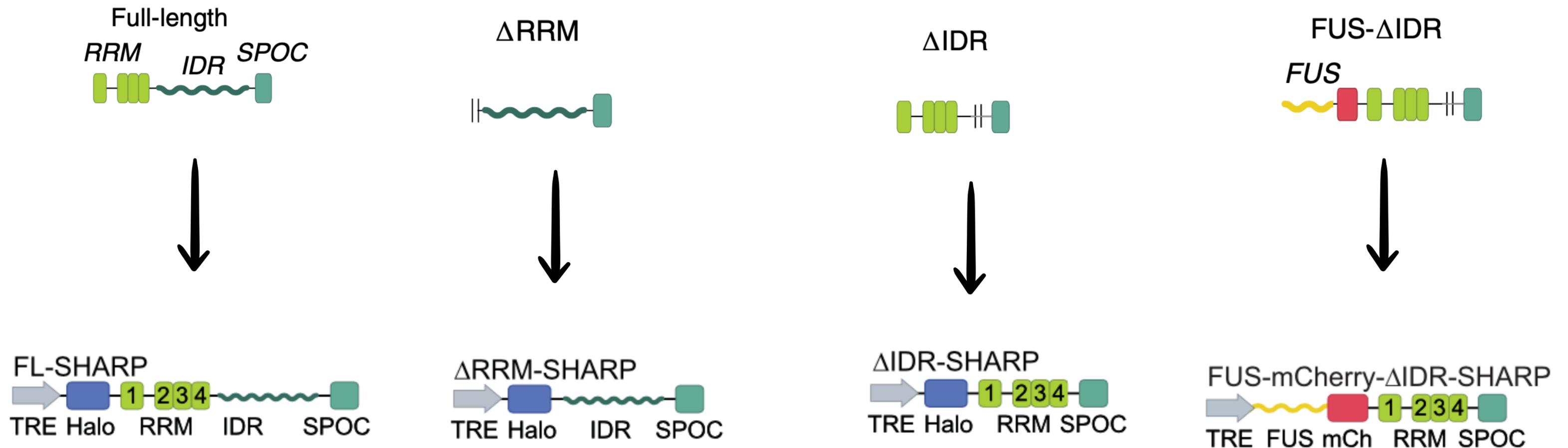
Result

IDR deletion
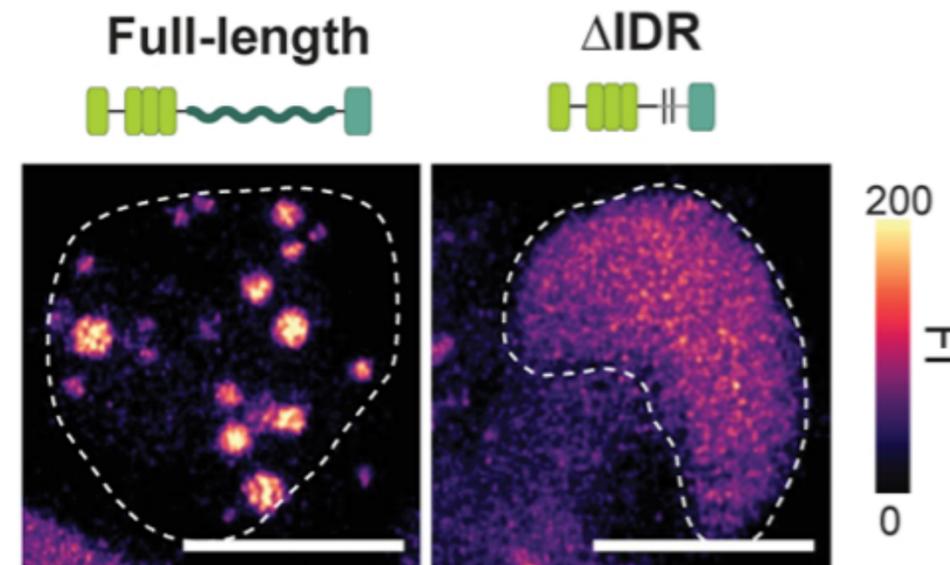
Rescue (FUS)

Foci formation depend on homotypic multivalent interaction between IDRs

3. How does Sharp recruitment hapen?

Methods
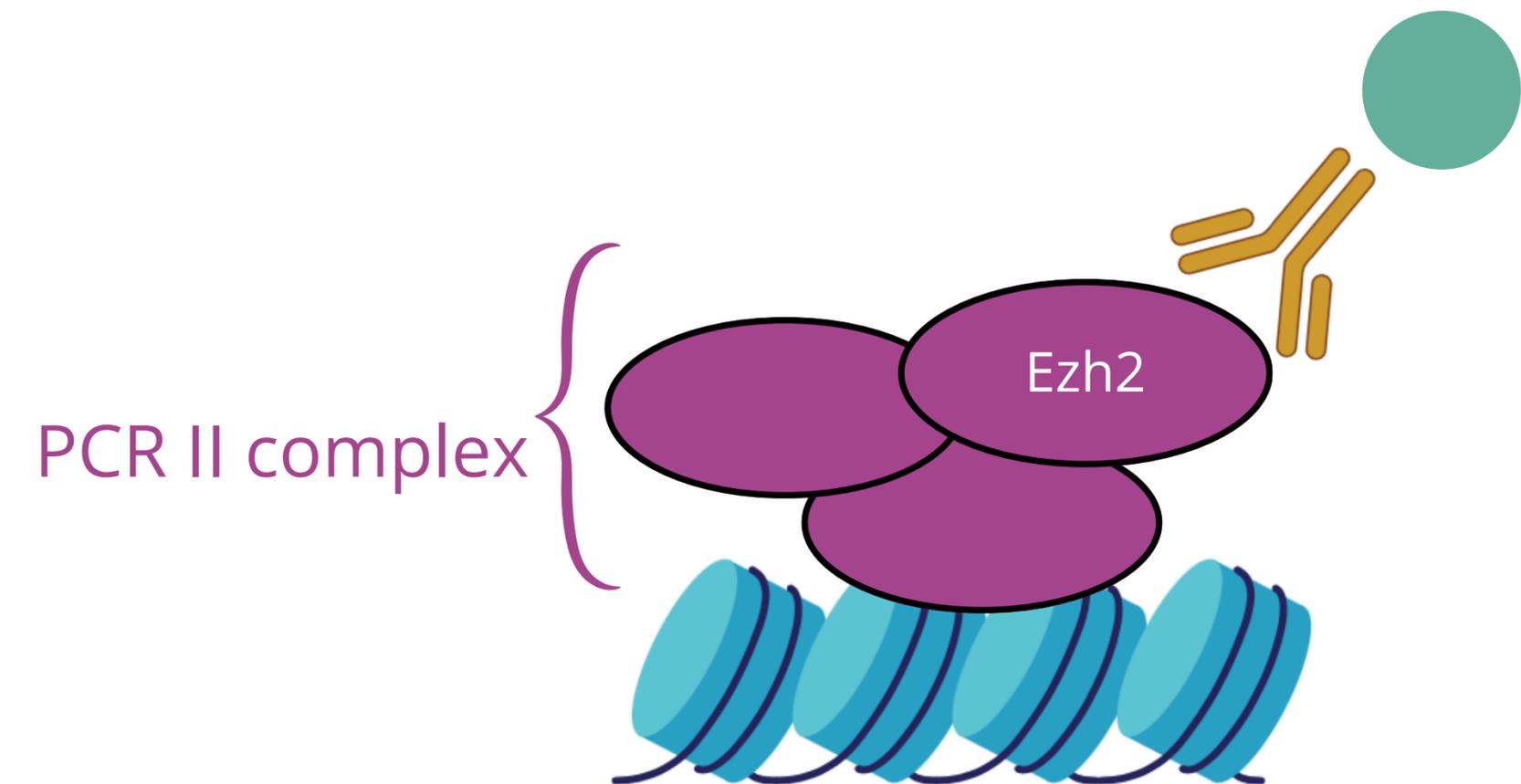

Visualisation by RNA Fish   **Direct visualisation**
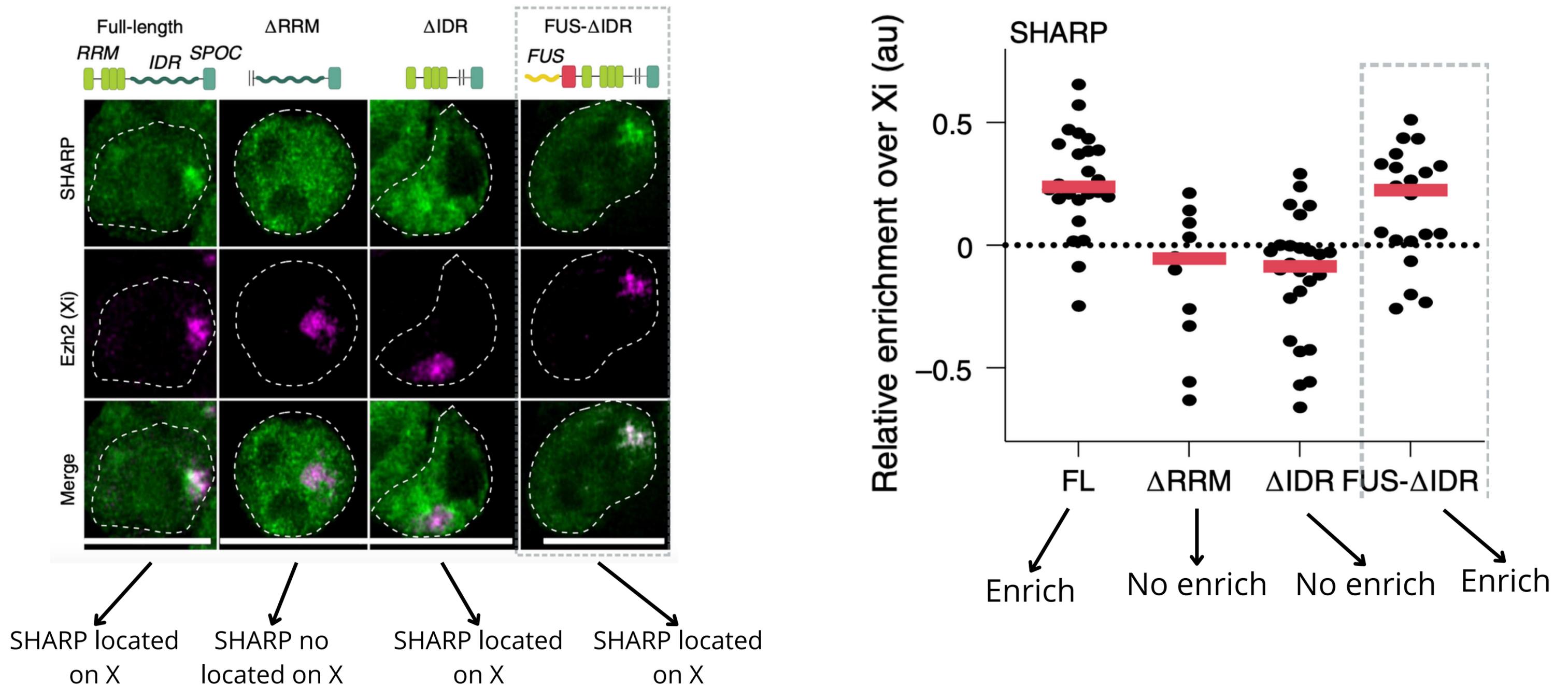
3. How does Sharp recruitment hapen?

Methods

⟶ Ezh2 use to mark Xi

⟶ Immunomarquage antobody against Ezh2

PCR II complex

Ezh2

3. How does Sharp recruitment hapen?

Result

SHARP located on X   SHARP no located on X   SHARP located on X   SHARP located on X

Enrich   No enrich   No enrich   Enrich

3. How does Sharp recruitment hapen?

Result

	Full-length RRM IDR SPOC	ΔRRM	ΔIDR	FUS-ΔIDR FUS
Sharp located on Xi ?	+++	-	+++	+++
Sharp can enrich Xi ?	+++	-	-	+++

→ RRMs of SHARP are essential for localisation / link to Xi
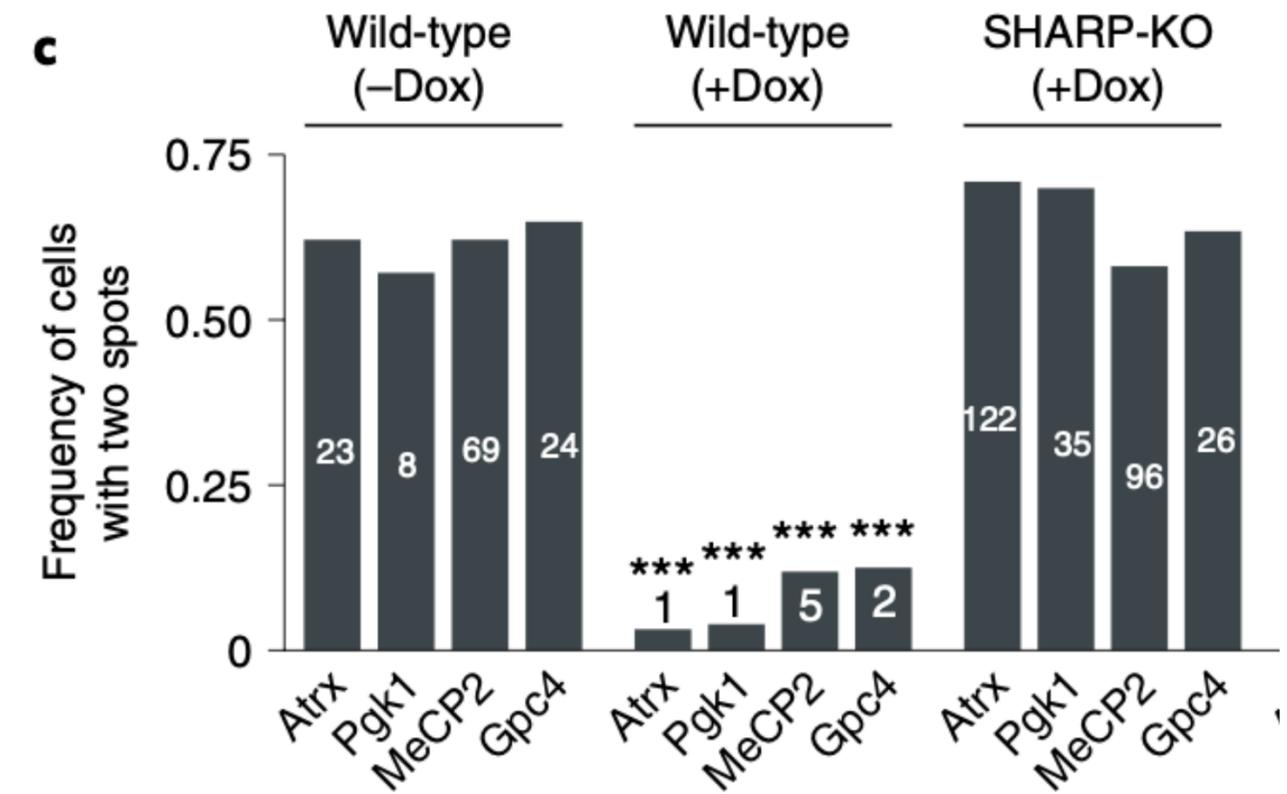
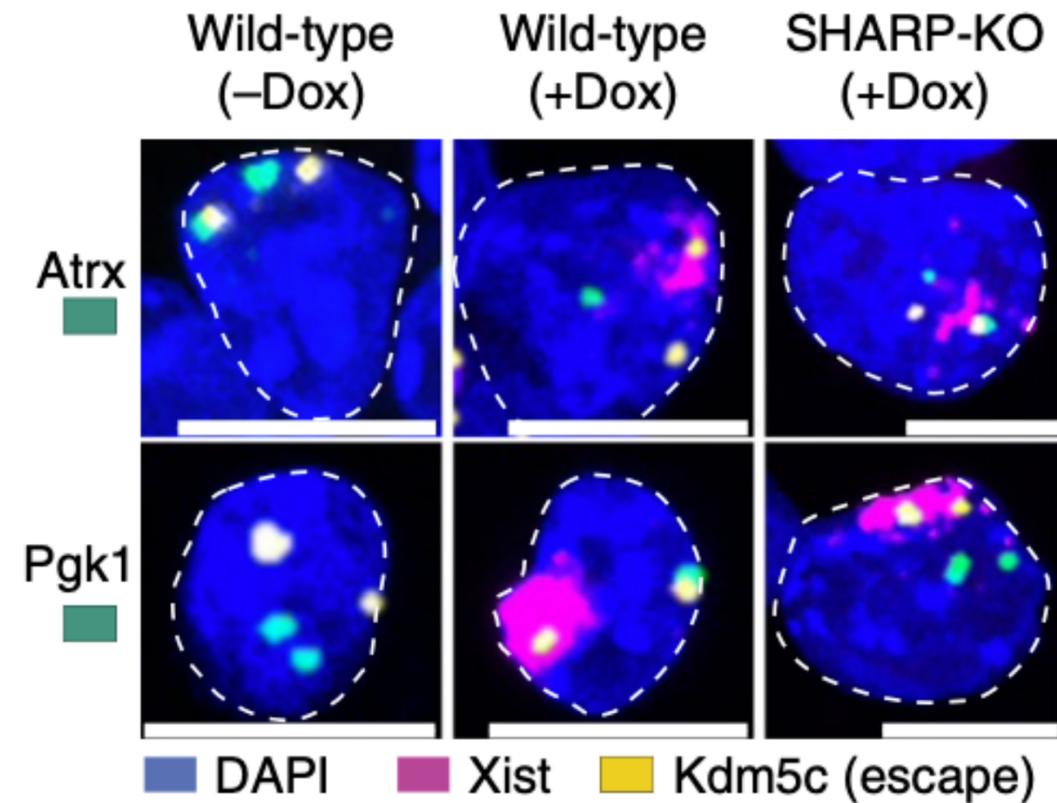→ IDRs of SHARP are essential for enrichment on the Xi

4. Does SHARP foci play a role in X silencing?

Methods

Silencing **No silencing**

Silenced gene during X inactivation

DAPI   Xist   Kdm5c (escape)

Xa   Xi   Xa   Xa

Gene "escape"

Gene silenced during X inactivation

1 bp —

Kdm6a ⟶ **Gene escape**

50,000,000 bp — Gpc4

MeCP2

Gene silenced during X inactivation

Xist

100,000,000 bp — Atrx
Pgk1

150,000,000 bp — Kdm5c ⟶ **Gene escape**

167,000,000 bp —

Chromosome X

⟶ Visualisation by RNA-FISH
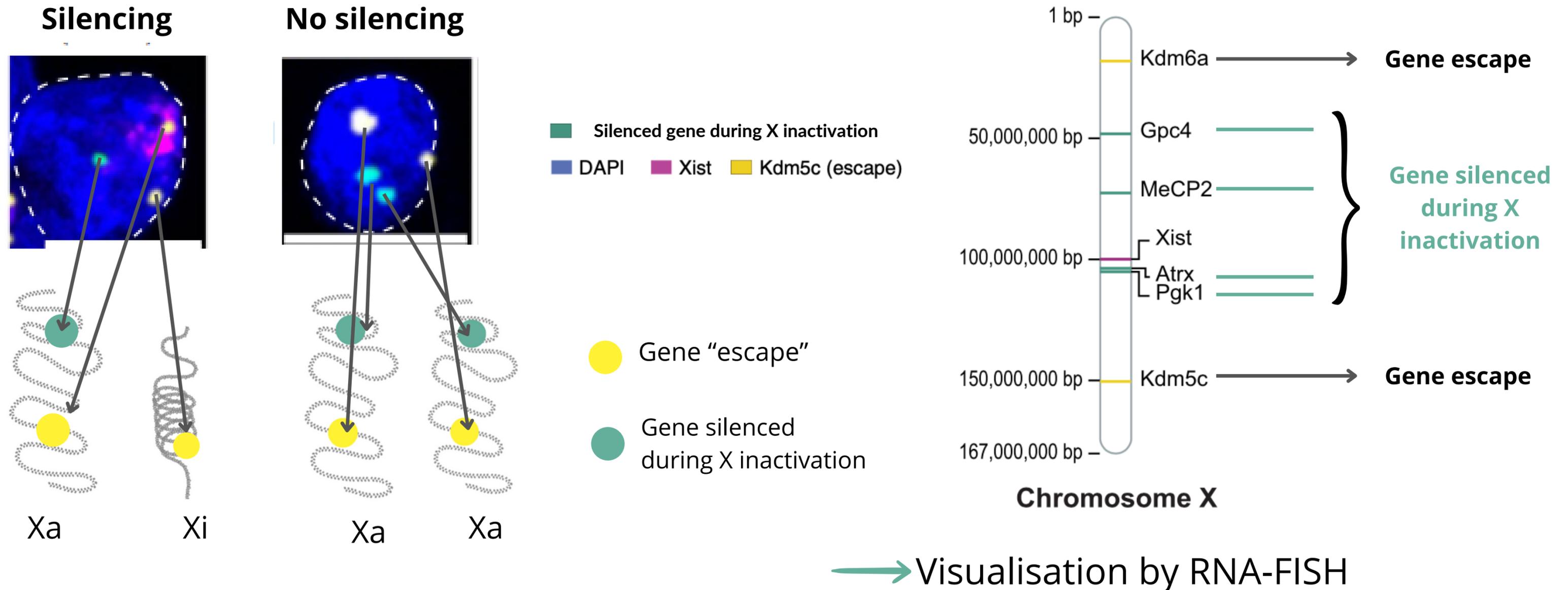
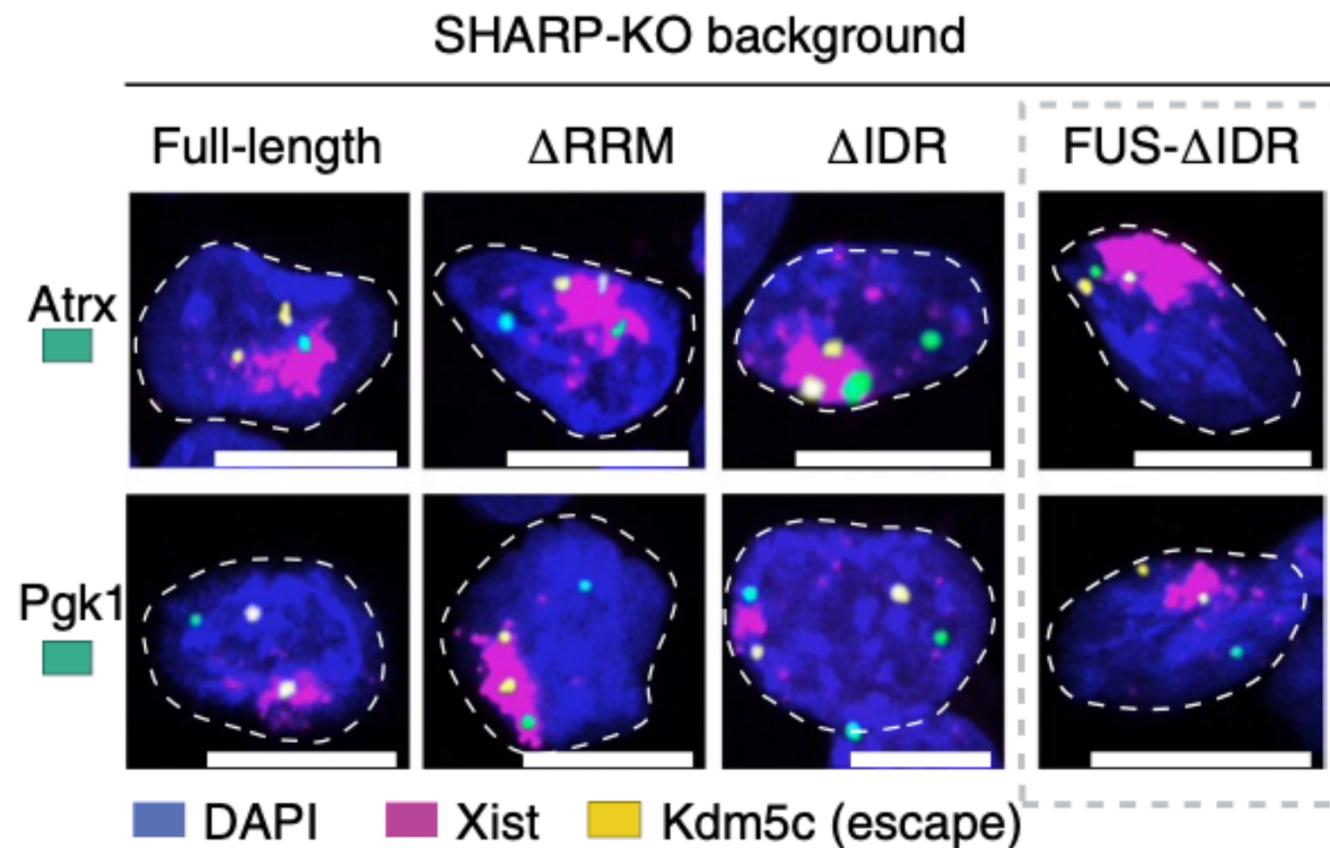# 4. Does SHARP foci play a role in X silencing?

Result

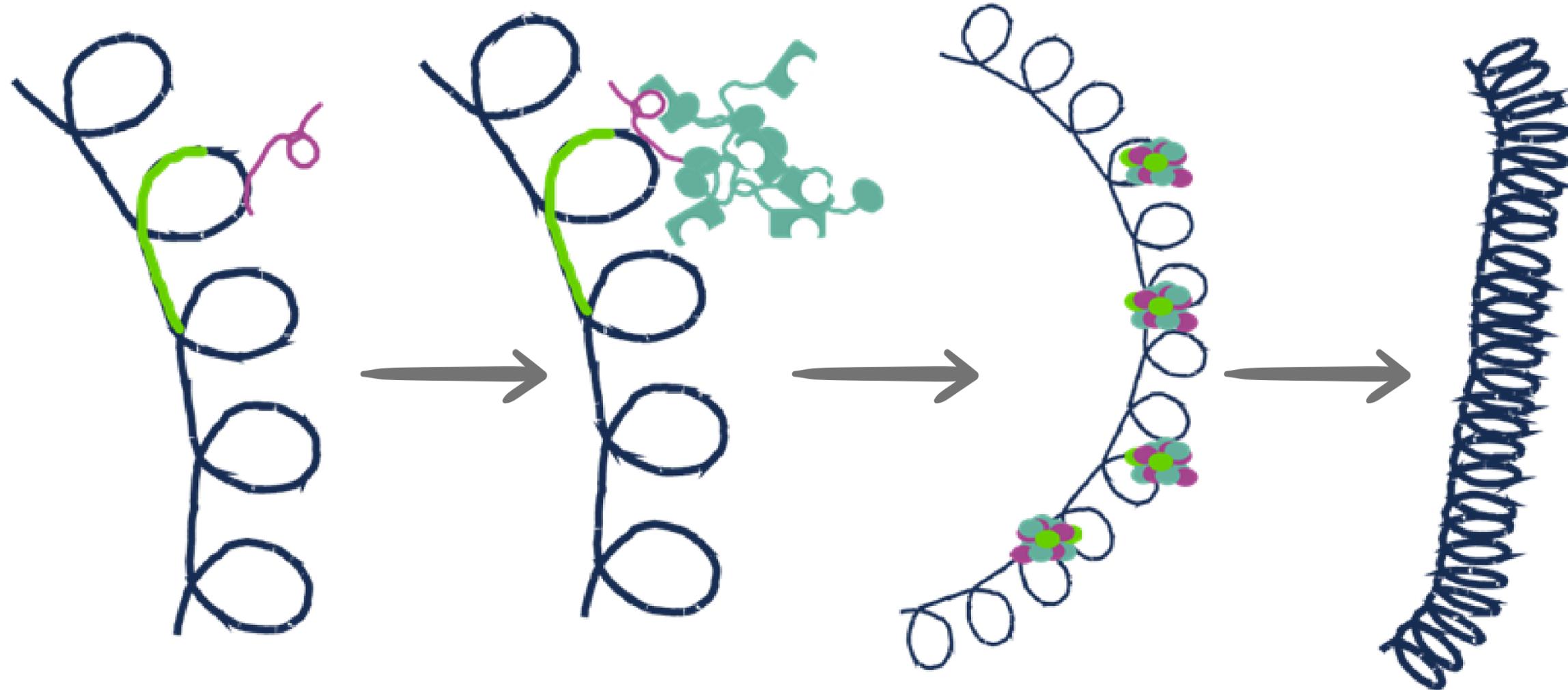No inactivation of Xist without SHARP

4. Does SHARP foci play a role in X silencing?

Result
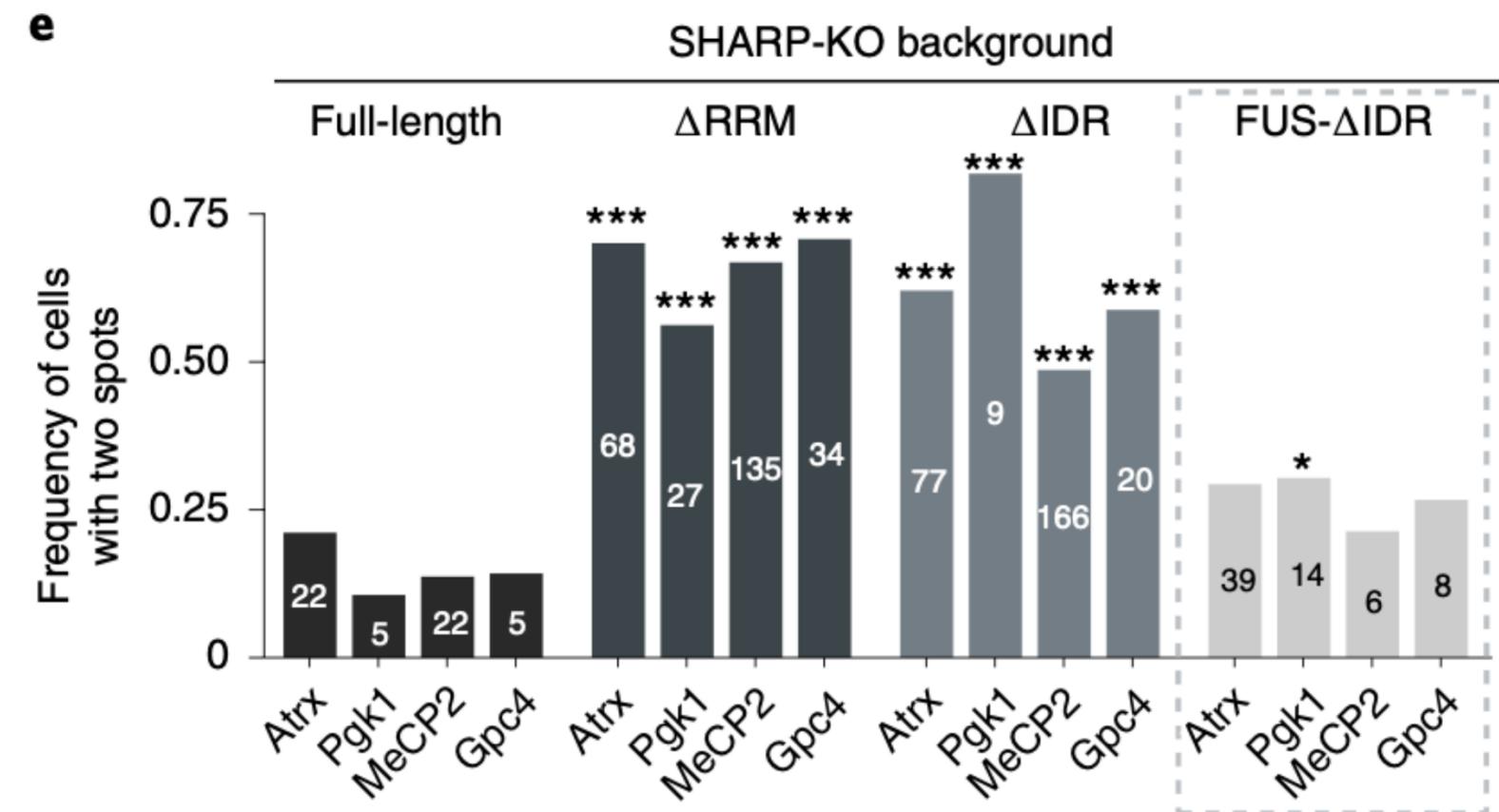
No inactivation of Xist without RRM & IDR domain of sharp

Conclusion diagram of Sharp recruitment on Xist

Sharp recruitment by Xist

Excess stochiometric recruitment

- RRMs allow Sharp linking on Xist
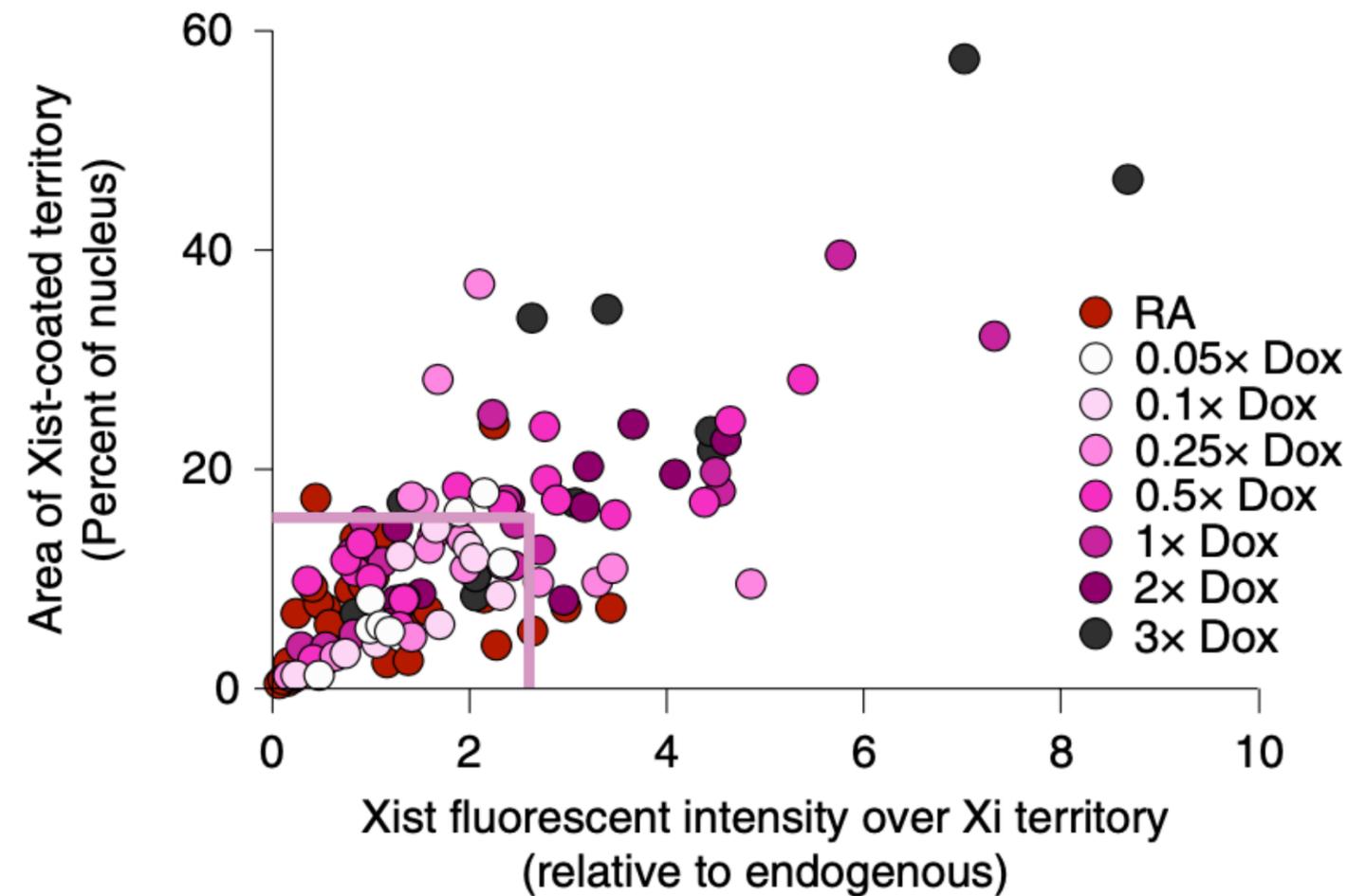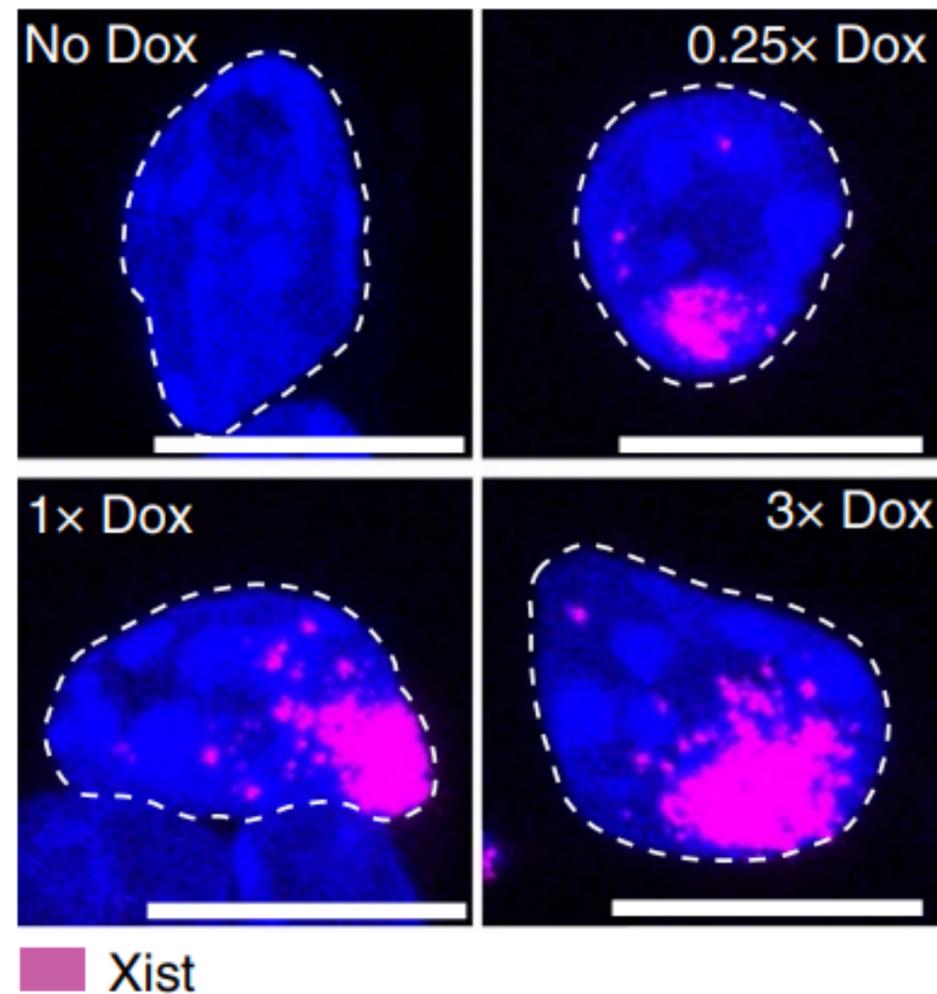- Interaction between IDRs allow SHARP non stoichiometric recruitment

Responsible of X silencing

5. Why is this strategy necessary?

→ **Low number of Xist** mol is critical mechanical by which cells limit Xist
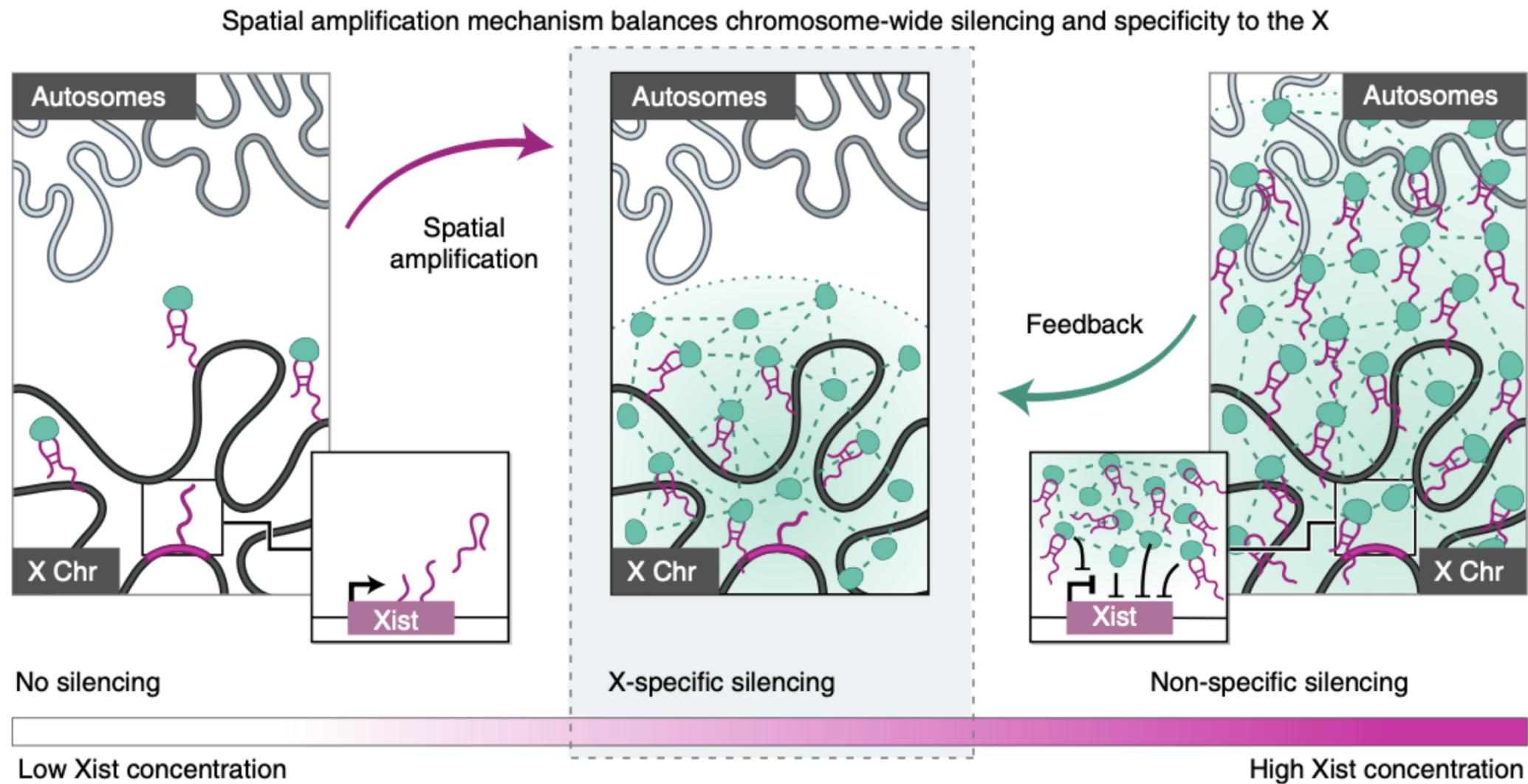**spreading to autosomal region** ?

Methods : **RNA - Fish quantification**

5. Why is this strategy necessary?

Xist

RA
0.05× Dox
0.1× Dox
0.25× Dox
0.5× Dox
1× Dox
2× Dox
3× Dox

→ Xist quantity is correlate with Xist territory

→ Xist is in low quantity so as not to fix the autosomes
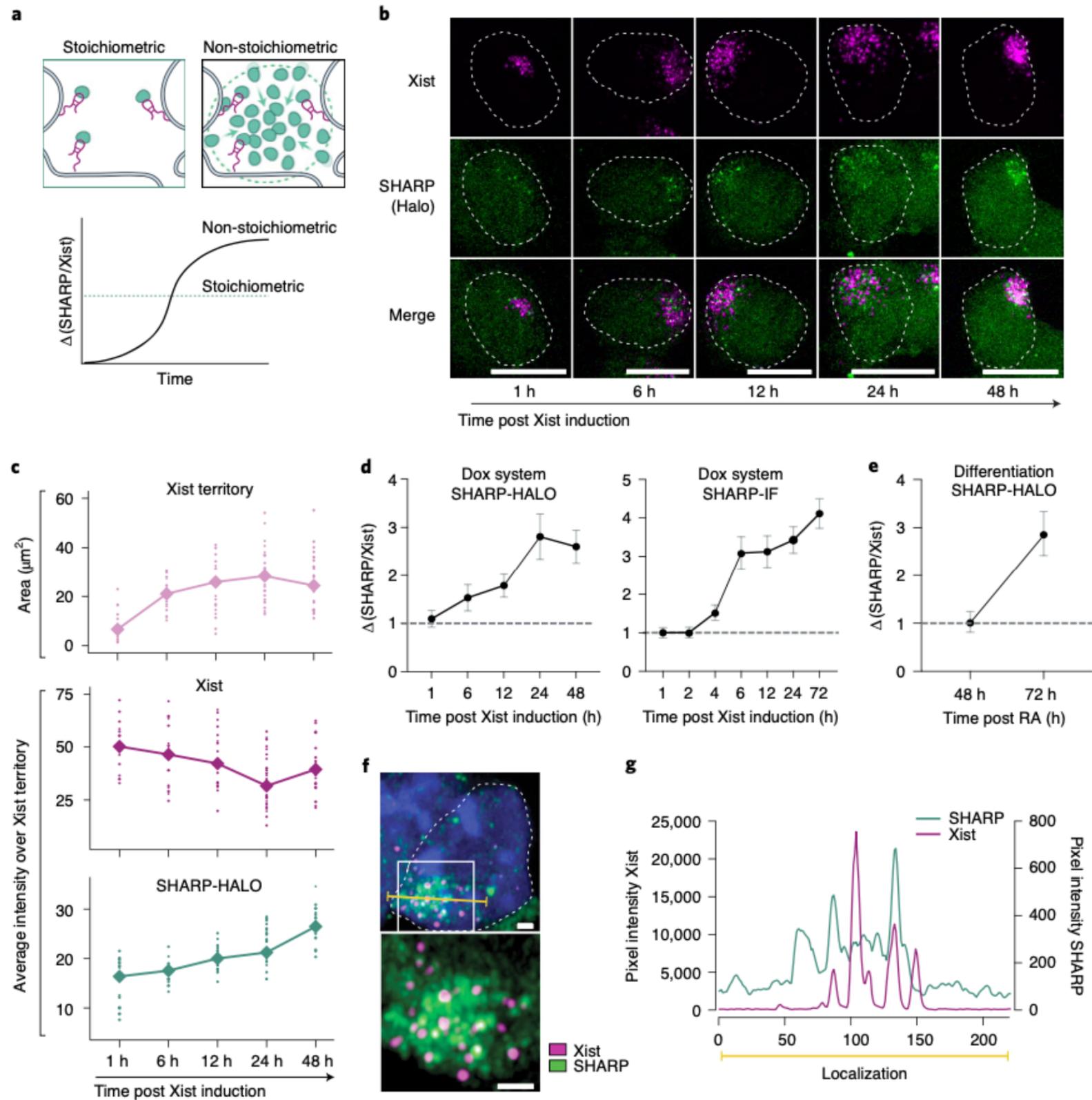
5. Why is this strategy necessary?

Spatial amplification mechanism balances chromosome-wide silencing and specificity to the X

Perspectives ?

Discussion/conclusion

Xist in small quantity → SHARP's RRMs link Xist → SHARP's IDRs interaction allow SHARP recruitment

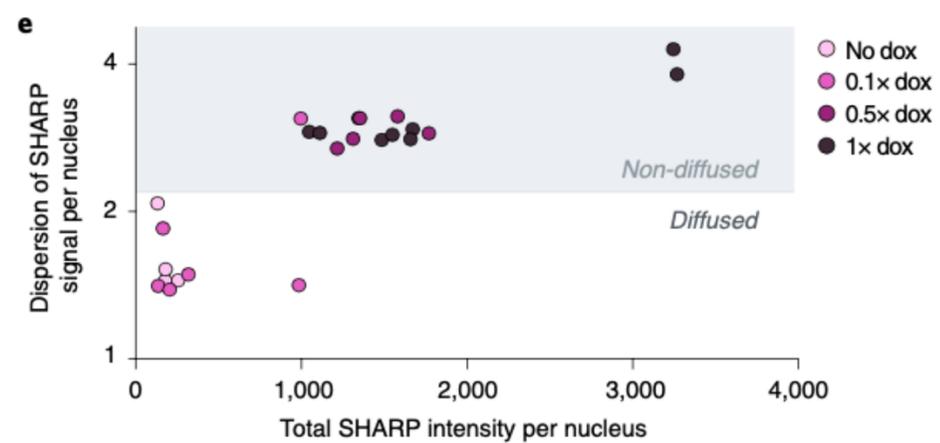SHARP recruit transcription factor responsible of heterochromatisation

No Inactivation of autosomaux
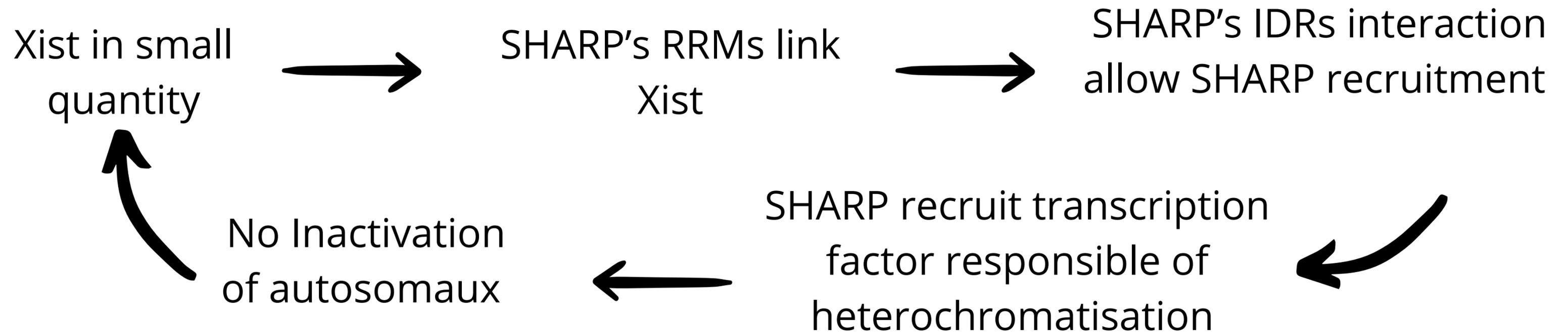
Do you have any question ?

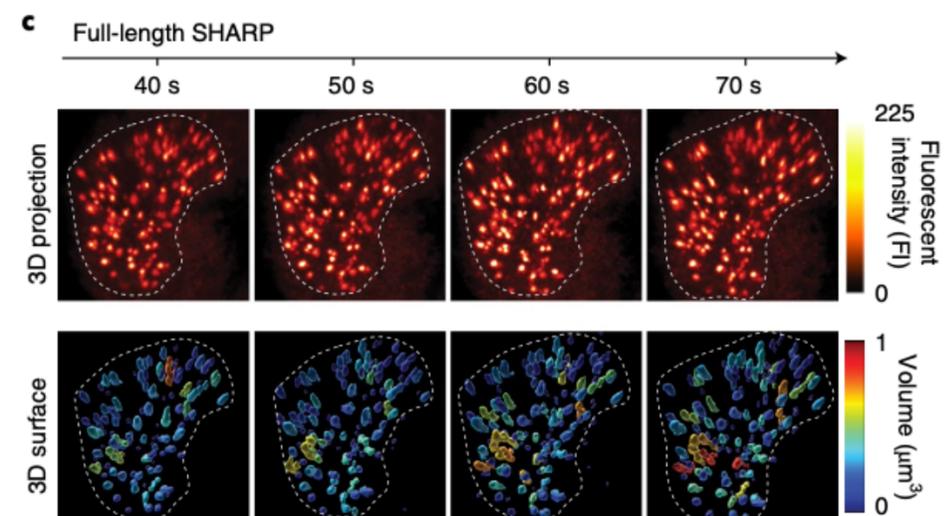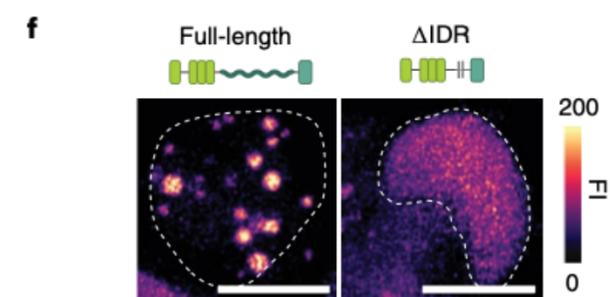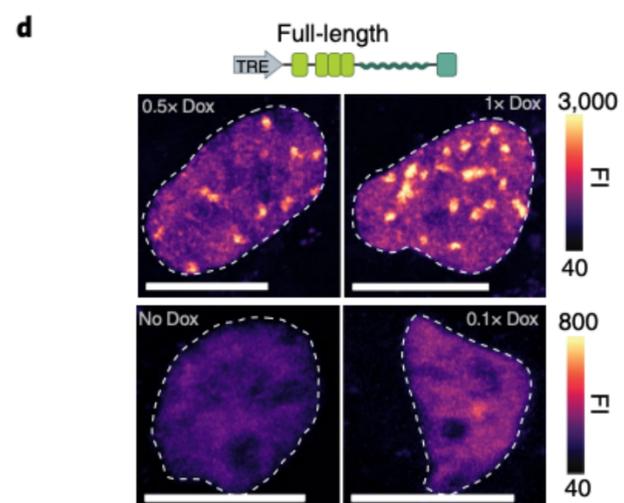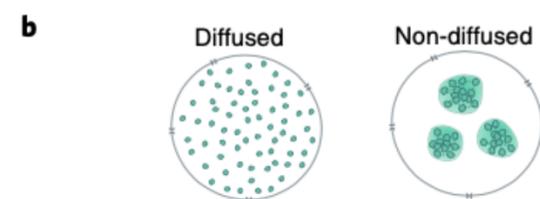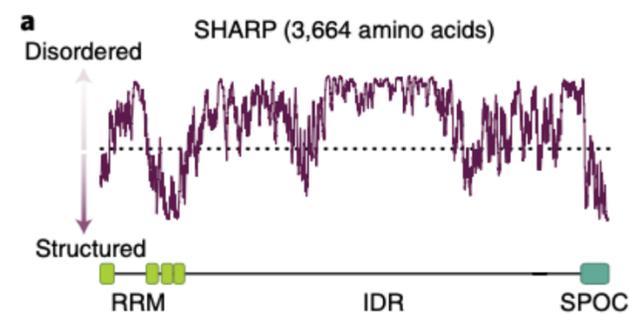# Bibliography

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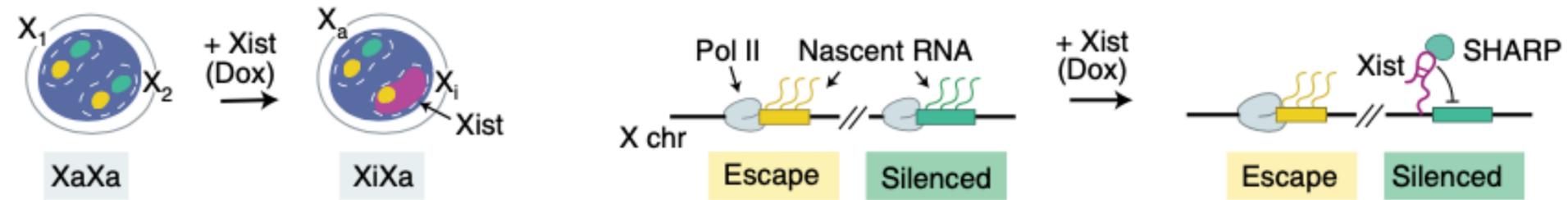
Annexes :

Annexes :

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