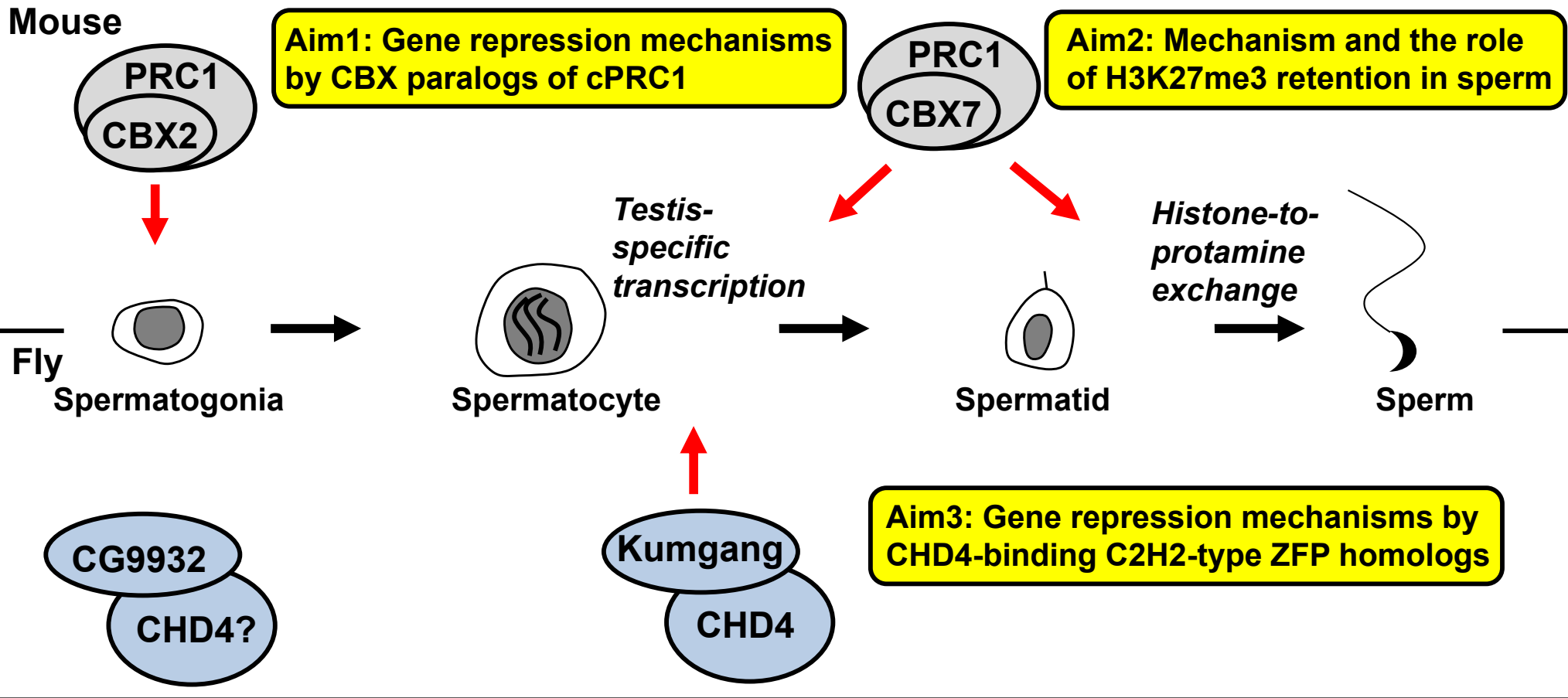
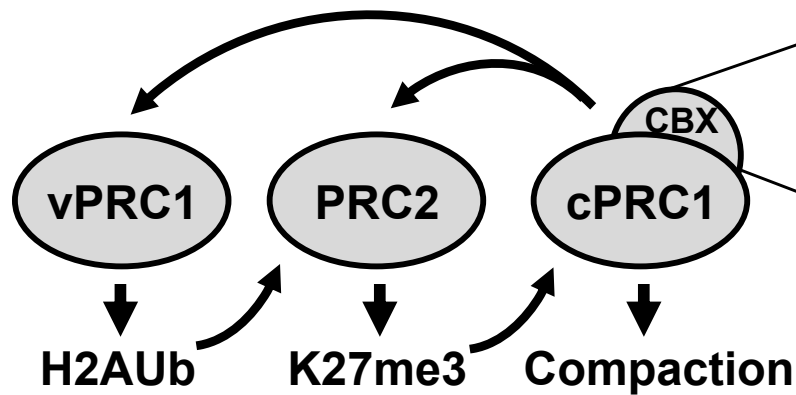


How to block aberrant transcription?

Choosing right promoters
by cell type-specific chromatin modifying complexes



Aim1. Gene repression mechanism by CBX paralogs of cPRC1



Q) Determinants of gene repression?

Q) Why CBX7 but not other CBX?

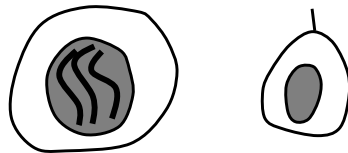
Wild type

Cbx7^{-/-}

vPRC1-cUbMut

Cbx7^{-/-}; rescue-CBX7-dTAG

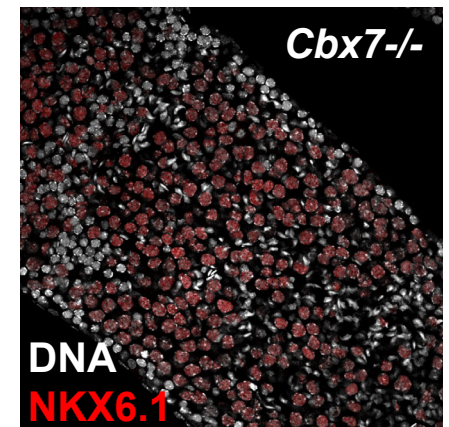
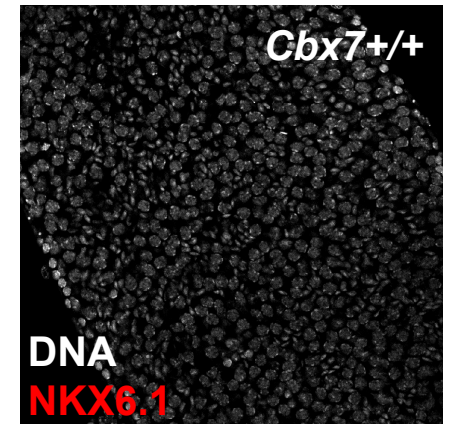
Cbx7^{-/-}; rescue-CBX2-dTAG



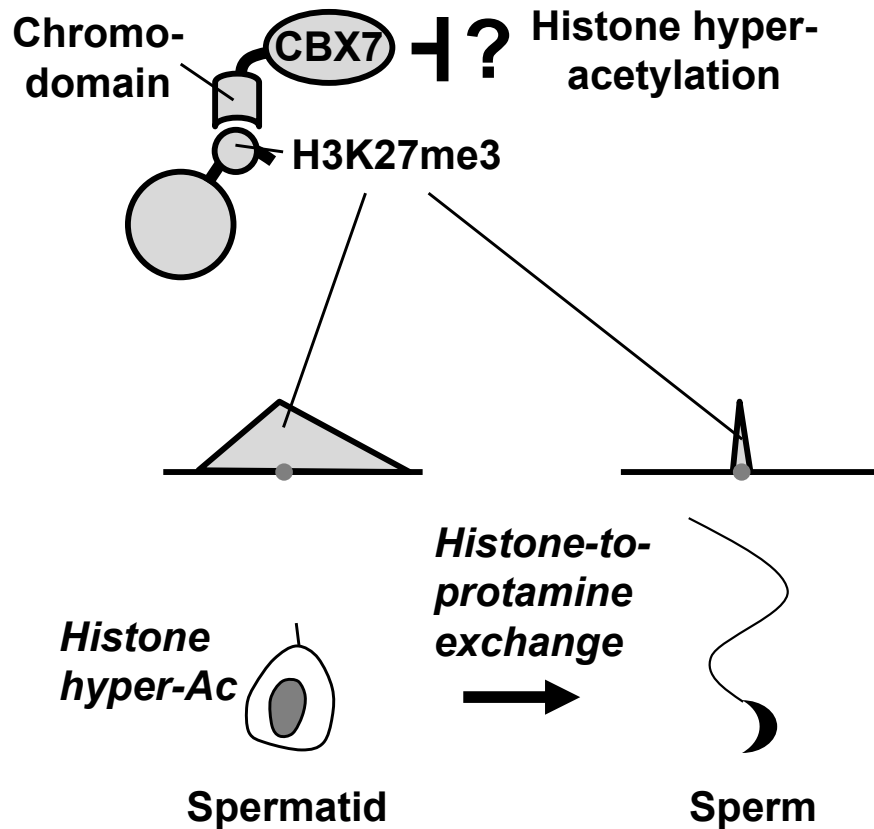
Measure genome-wide:

vPRC1, *PRC2*, *cPRC1*,
H2AUb, H3K27me3,
Compaction

Analyze cellular phenotypes



Aim2. Mechanism and the role of H3K27me3 retention in sperm



CBX7 in H3K27me3 retention in sperm?

Compare WT vs. Cbx7^{-/-} sperm

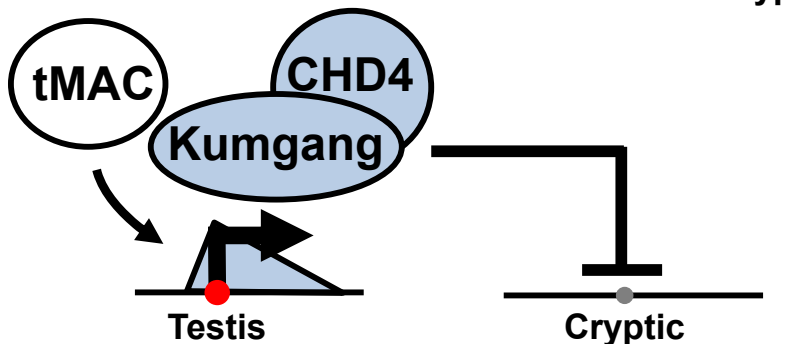
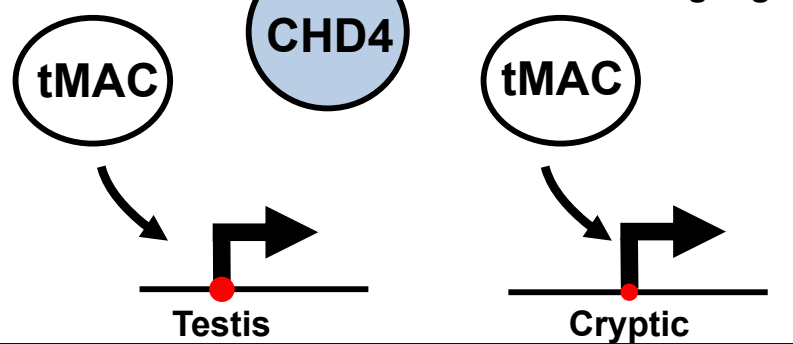
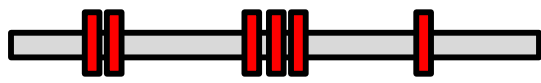




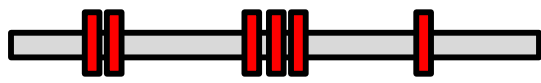




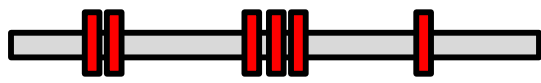




If yes, questions:

- 1) Histone hyper-Ac at normal H3K27me3 loci?
- 2) Sperm chromatin structure?
- 3) Zygotic gene expression & development

If no, H3K27me3 binding protein in spermatids?

H3K27me3 peptide 

Aim3. Gene repression mechanisms by CHD4-binding ZFP homologs

<p>Wild type</p>  <p>tMAC</p> <p>CHD4</p> <p>Kumgang</p> <p>Tetris</p> <p>Cryptic</p>	<p>Hypothesis</p> <p><i>Global disruption of chromatin structure, such as yeast Spt6 mutant (elongation factors)</i></p>															
<p><i>Kumgang^{-/-}</i></p>  <p>tMAC</p> <p>CHD4</p> <p>tMAC</p> <p>Tetris</p> <p>Cryptic</p>	<p>Approach</p> <p><i>Acute-depletion of Kumgang</i></p> <p>DD-DD-Kumgang flies (depletion of Kumgang by TMP withdrawal)</p> <p>Measure time course</p> <ul style="list-style-type: none">- Gene expression- Genome-wide CHD4, tMAC binding- Nucleosome positioning by MNase-seq															
<table><tr><td></td><td>Kumgang</td><td>Fly Scyte</td></tr><tr><td></td><td>CG9932</td><td>Fly general</td></tr><tr><td></td><td>Hunchback</td><td>Fly embryo</td></tr><tr><td></td><td>IKAROS</td><td>Mouse blood</td></tr><tr><td></td><td>ZFP541</td><td>Mouse Scyte</td></tr></table>		Kumgang	Fly Scyte		CG9932	Fly general		Hunchback	Fly embryo		IKAROS	Mouse blood		ZFP541	Mouse Scyte	<p>Q) Role of ZF in Kumgang</p> <p>Individual Zf mutant rescue</p> <p>Q) Role of CHD4-binding ZFPs</p> <p>Kumgang homolog rescue</p>
	Kumgang	Fly Scyte														
	CG9932	Fly general														
	Hunchback	Fly embryo														
	IKAROS	Mouse blood														
	ZFP541	Mouse Scyte														

Future: isolation of dominant suppressor EMS mutants reverting fertility of *kumgang*