Ch2 - L2.4

tech discussion Research Paper 2

Research Paper 2

Molecular Cell
Article

CellPress

Capturing the Onset of PRC2-Mediated Repressive Domain Formation

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Reminder

Research Papers (1 each Ch.) are essential parts of your study.

Deep understanding required.

At the Exam, one question will always be proposed concerning one of the Research Papers; not «general» questions, but going deep into one of the experiments presented in the paper, including methodology.

Summary

Polycomb repressive complex 2 (PRC2) maintains gene silencing by catalyzing methylation of histone H3 at lysine 27 (H3K27me2/3) within chromatin. By designing a system whereby PRC2-mediated repressive domains were collapsed and then reconstructed in an inducible fashion *in vivo*, a two-step mechanism of H3K27me2/3 domain formation became evident. First, PRC2 is stably recruited by the actions of JARID2 and MTF2 to a limited number of spatially interacting "nucleation sites," creating H3K27me3-forming Polycomb foci within the nucleus. Second, PRC2 is allosterically activated via its binding to H3K27me3 and rapidly spreads H3K27me2/3 both in *cis* and in far-*cis* via long-range contacts. As PRC2 proceeds further from the nucleation sites, its stability on chromatin decreases such that domains of H3K27me3 remain proximal, and those of H3K27me2 distal, to the nucleation sites. This study demonstrates the principles of *de novo* establishment of PRC2-mediated repressive domains across the genome.

Summary

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essential background information

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main question and strategy

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essential results summary

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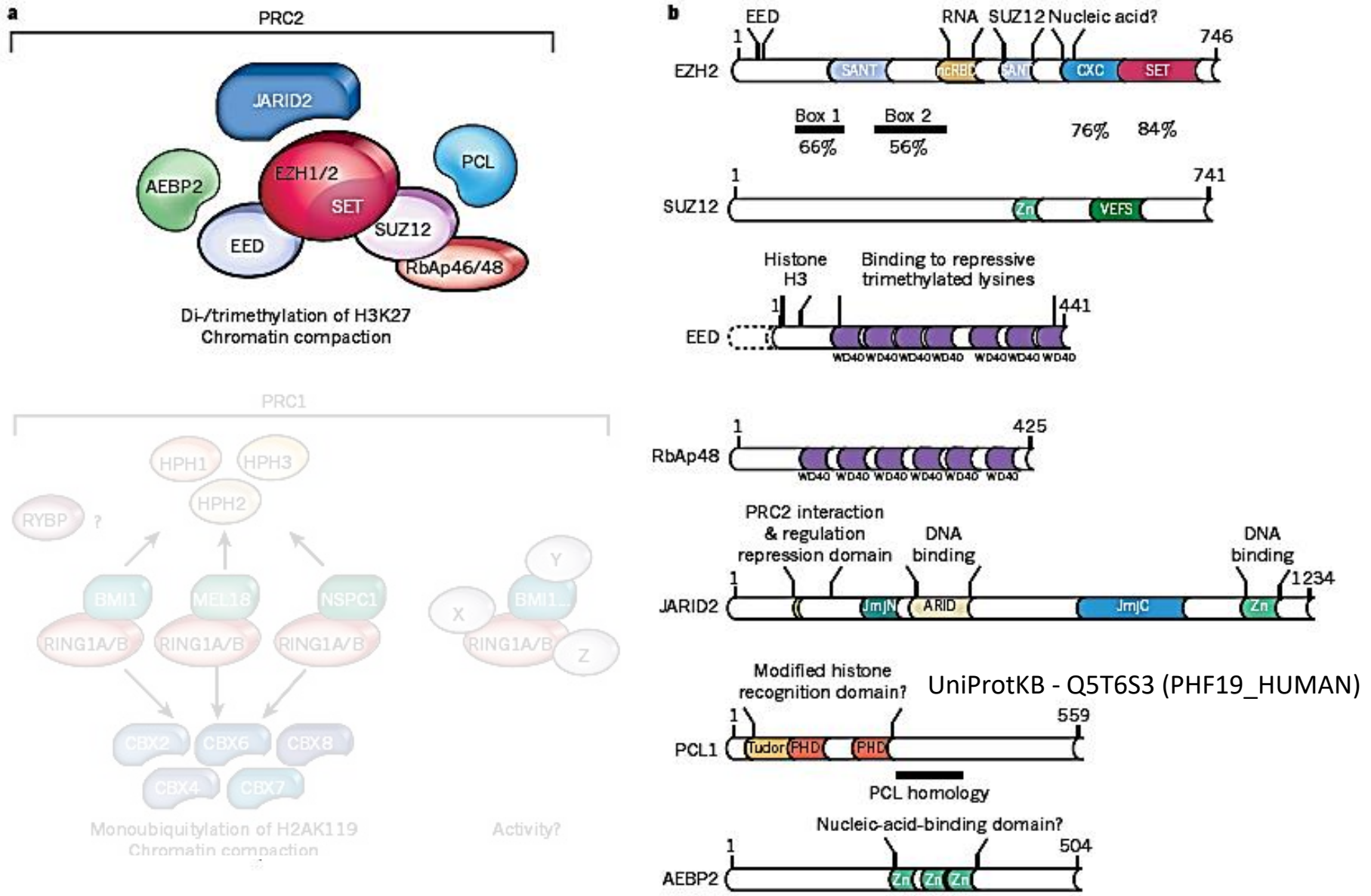
essential novel information obained

PRC2 proceeds further from the nucleation sites, its stability on chromatin decreases such that domains of H3K27me3 remain proximal, and those of H3K27me2 distal, to the nucleation sites. **This study demonstrates the principles of *de novo* establishment of PRC2-mediated repressive domains across the genome.**



UniProtKB - Q5T6S3 (PHF19_HUMAN)

from Margueron & Reinberg, Nature 469:343-349 (2011)

Intro

find the scientific question addressed !

... the mechanism(s) by which PRC2 is initially recruited to specific genomic loci and how PRC2 sets up repressive domains comprising H3K27me2 or H3K27me3 remain unclear.

PRC2 targeting facilitated by JARID2, MTF2 (and other proteins?) for CGI (*CpG islands*)

Thus, we devised a genetic system in mESCs by which we could inducibly reconstruct these domains from scratch. Employing this system, we defined the exact genomic coordinates of PRC2 nucleation and spreading and identified the key factors for its initial recruitment and stability on chromatin

then obtain te necessary background information !

example:

Aa say that << PRC2 targeting is facilitated by JARID2, MTF2 >>

What JARID2, MTF2 are ?

you may use any official database you know
or .....
just google it out, but take care of choosing a good website

then obtain te necessary background information !

example:

Aa say that << PRC2 targeting is facilitated by JARID2, MTF2 >>

What JARID2, MTF2 are ?

NCBI Gene: https://www.ncbi.nlm.nih.gov/gene/3720
https://www.ncbi.nlm.nih.gov/gene/22823

GeneCards: https://www.genecards.org/ (Weinzmann Institute of Science)

UniProt: https://www.uniprot.org/

http://www.rcsb.org/3d-view/3IIW

RESULTS

Disruption of EED-H3K27me3 Interaction Reduced H3K27me3 Levels in mESCs

The crystal structure of EED shows that H3K27me3 is located within an aromatic cage formed by the WD40 repeats of EED and that three amino acids, Phe97, Tyr148, and Tyr365, directly contact the tri-methylated lysine residue (Margueron et al., 2009). To test whether this interaction was necessary for the maintenance of repressive chromatin domains *in vivo*, we generated EED cage mutants having Phe97 or Tyr365 substituted with alanine (F97A or Y365A) in mESCs using the CRISPR/Cas9 system. As a control, we mutated Tyr358 (Y358A), which does not contact the tri-methylated lysine. A western blot (WB) of whole-cell extract preparations from these cage-mutant lines showed a significant reduction in H3K27me3 levels (Figure 1A), while those of the control Y358A mutant were unaffected.

EED is the PRC2 component that binds to H3K27me2/3

The EED domain specifically involved in di-/tri-methylated Lysine binding is called «aromatic Cage» and is formed by WD40 repeats

Where can we get information on this protein ?

NCBI Gene

NCBI Protein

RCSB Protein Data Bank see 3JZG

SwissProt

but also Wikipedia

WD40 repeat

From Wikipedia, the free encyclopedia

The **WD40 repeat** (also known as the **WD** or **beta-transducin repeat**) is a short structural motif of approximately 40 amino acids, often terminating in a tryptophan-aspartic acid (W-D) dipeptide.[2] Tandem copies of these repeats typically fold together to form a type of circular solenoid protein domain called the **WD40 domain**.

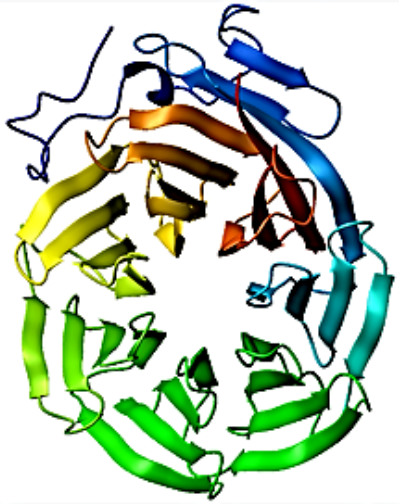
Contents [hide]

1 Structure
2 Function
3 Examples
4 See also
5 References
6 External links

Structure [edit]

WD40 domain-containing proteins have 4 to 16 repeating units, all of which are thought to form a circularised beta-propeller structure (see figure to the right).[3][4] The WD40 domain is composed of several repeats, a variable region of around 20 residues at the beginning followed by a more common repeated set of residues. These repeats typically form a four stranded anti-parallel beta sheet or blade. These blades come together to form a propeller with the most common being a 7 bladed beta propeller. The blades interlock so that the last beta strand of one repeat forms with the first three of the next repeat to form the 3D blade structure.

WD domain, G-beta repeat



Ribbon diagram of the C-terminal WD40 domain of Tup1 (a transcriptional corepressor in yeast), which adopts a 7-bladed beta-propeller fold. Ribbon is colored from blue (N-terminus) to red (C-terminus).[1]

Identifiers	
Symbol	WD40

https://en.wikipedia.org/wiki/WD40_repeat

EED Aromatic cage mutants: F97A or Y365A

control mutant: Y358A

Engineered in mESCs using CRISPR/Cas9 system

- cage muts did not disrupt PRC2 (EED KO does)
- cage-muts mESCs have WT phenotype

CRISPR/Cas9 system
Aa replaced endogenous copies of EED gene with the mutated versions.

Genome Editing

gene substitution using **CRISPR-cas9**

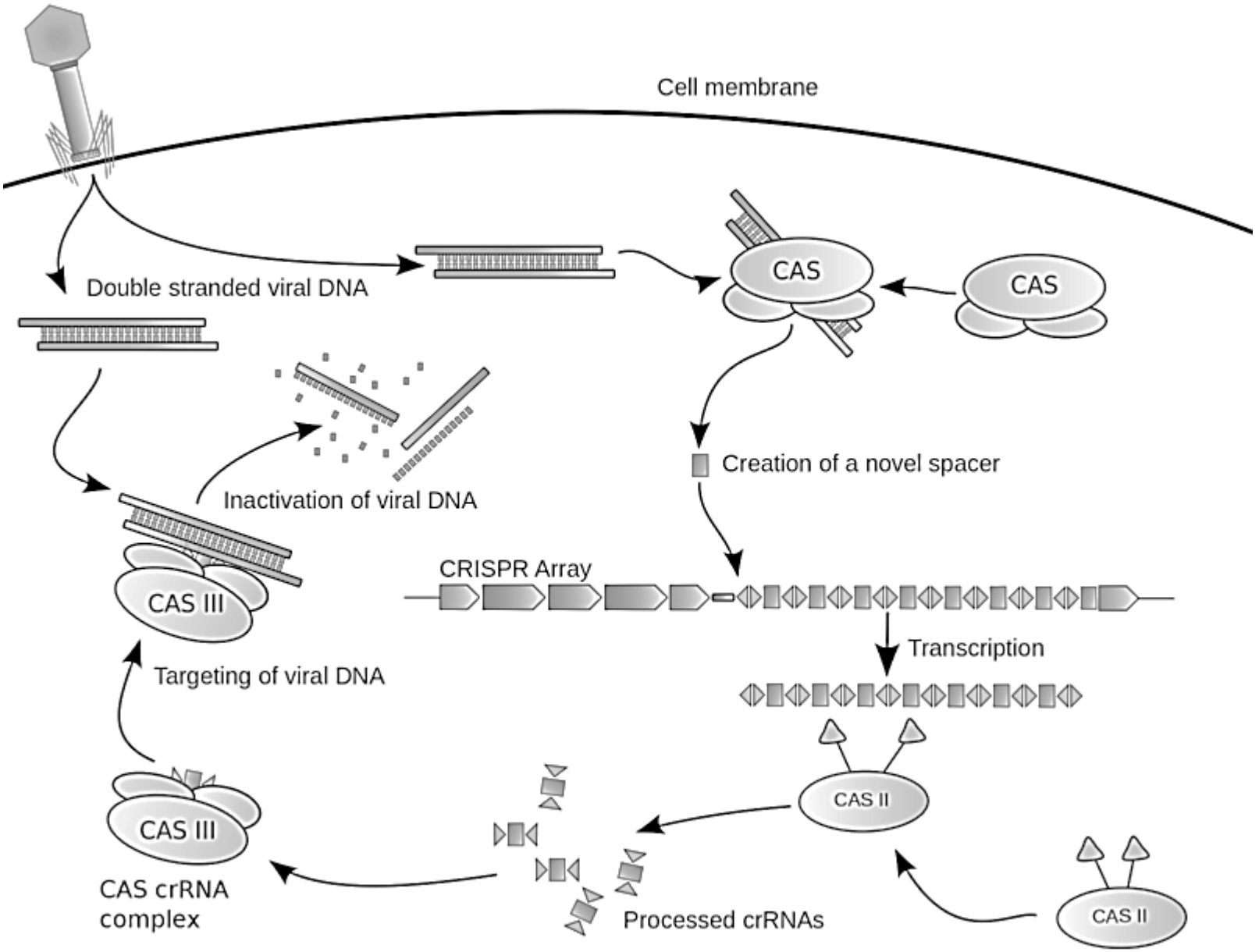
with homologous recombination-dependent repair

CRISPR (clustered regularly interspaced short palindromic repeats) is a family of DNA sequences found within the genomes of prokaryotic organisms such as bacteria and archaea. These sequences are derived from DNA fragments from viruses that have previously infected the prokaryote and are used to detect and destroy DNA from similar viruses during subsequent infections. Hence these sequences play a key role in the antiviral defense system of prokaryotes.

Cas 9 (or "CRISPR-associated protein 9") is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR-Cas9 that can be used to edit genes within organisms.

The CRISPR-Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phages that provides a form of acquired immunity. RNAs harboring the spacer sequence help Cas proteins recognizing and cutting foreign pathogenic DNA. *Other RNA-guided Cas proteins cut foreign RNA*

https://en.wikipedia.org/wiki/CRISPR



https://en.wikipedia.org/wiki/CRISPR

PROTOCOL

Genome engineering using the CRISPR-Cas9 system

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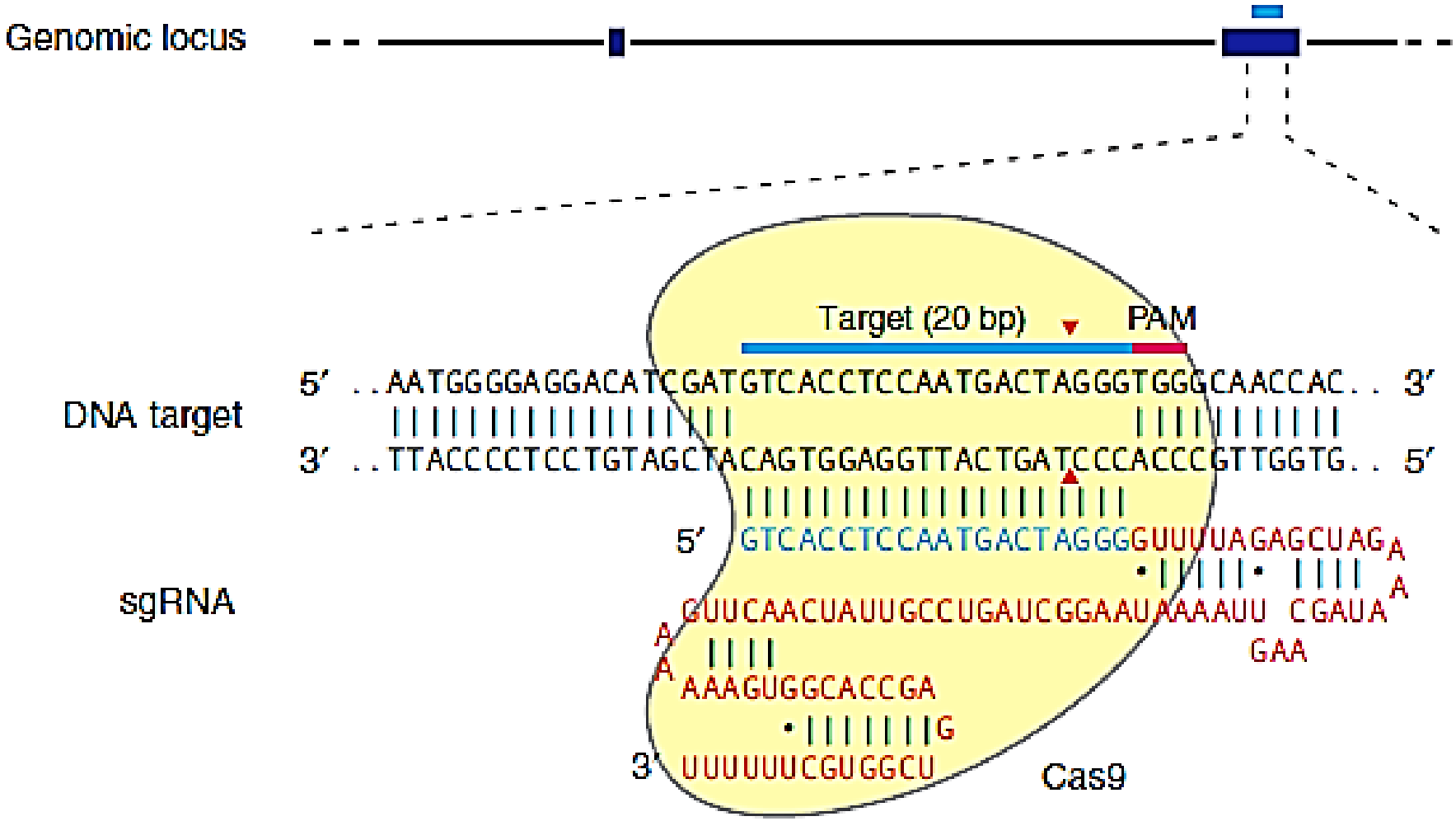
Targeted nucleases are powerful tools for mediating genome alteration with high precision. The RNA-guided Cas9 nuclease from the microbial clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system can be used to facilitate efficient genome engineering in eukaryotic cells by simply specifying a 20-nt targeting sequence within its guide RNA. Here we describe a set of tools for Cas9-mediated genome editing via nonhomologous end joining (NHEJ) or homology-directed repair (HDR) in mammalian cells, as well as generation of modified cell lines for downstream functional studies. To minimize off-target cleavage, we further describe a double-nicking strategy using the Cas9 nickase mutant with paired guide RNAs. This protocol provides experimentally derived guidelines for the selection of target sites, evaluation of cleavage efficiency and analysis of off-target activity. Beginning with target design, gene modifications can be achieved within as little as 1–2 weeks, and modified clonal cell lines can be derived within 2–3 weeks.



Yellow: Cas9 nuclease from *S. pyogenes*

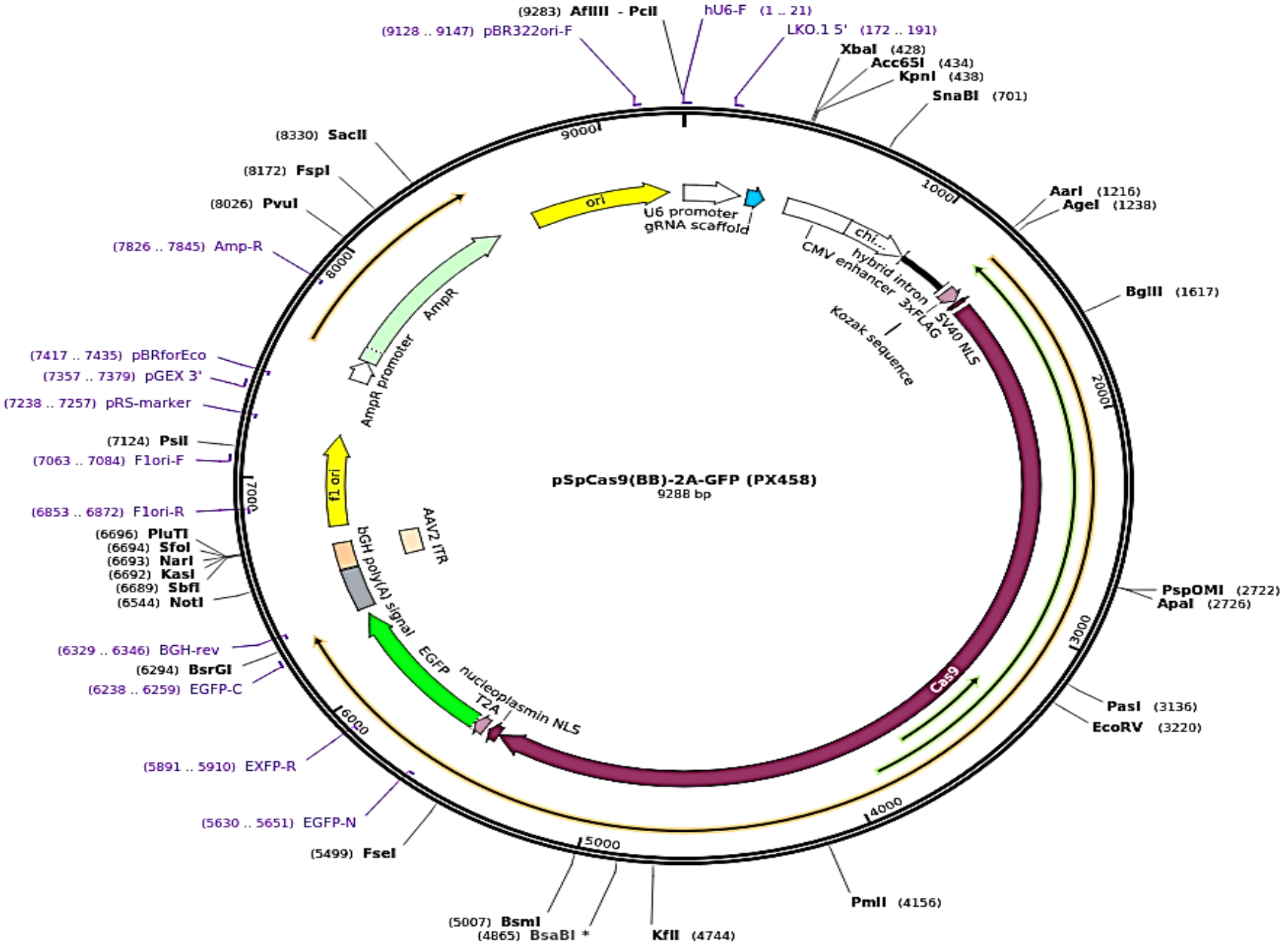
sgRNA : a 20-nt guide sequence (blue) and a scaffold (red)

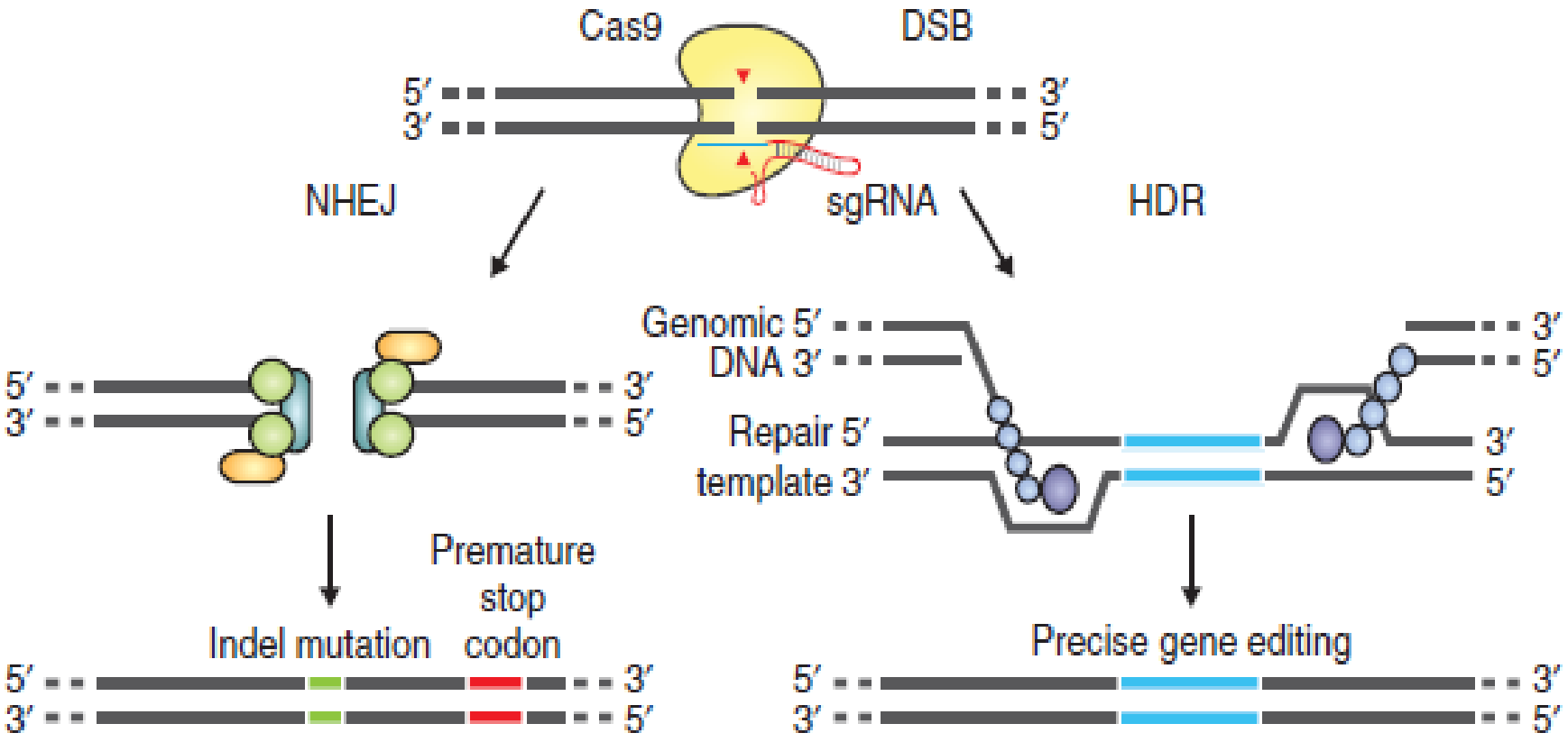
PAM: a requisite 5′-NGG adjacent motif

Cas9 makes a DSB (double strand break) ~3 bp upstream of the PAM (red triangle).

The RNA-guided nuclease function of CRISPR-Cas is reconstituted in mammalian cells through the heterologous expression of human codon–optimized Cas9 and the requisite RNA components.

Structural RNA + the guide RNA can be fused together → sg RNA
(single guide RNA)





Cells will repair the DSB using two possile pathways:

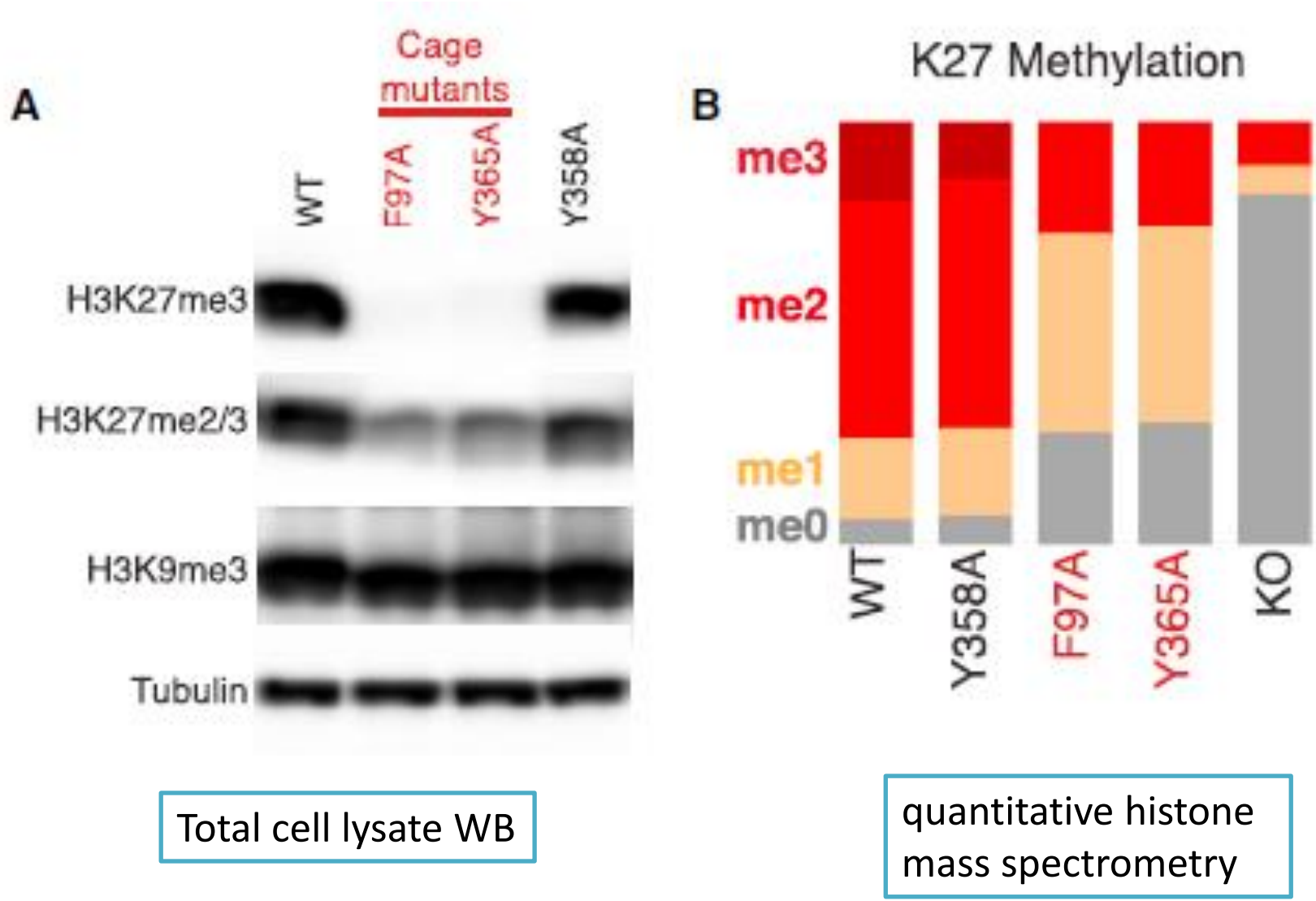
1. NHEJ – nonhomologous end-joining – introduces errors like small indels
2. HDR - homologous recombination dependent repair
   takes information from homologous DNA, whch can be in cis, i.e. one can transfect cells with the mutated version of the gene /locus to be substituted.

An aspartate-to-alanine (D10A) mutation in the RuvC catalytic domain of Cas9 allows transformation of wt Cas9 DNA cleaving activity into a nickase: this will yield single-stranded breaks, and the subsequent preferential repair through HDR.

For this kind of gene substitution, Aa used Cas9-nickase

The homologous DNA for recombination was given in *trans* (plasmid) and contained the require mutant EED sequences.

Oksuz Fig. 1

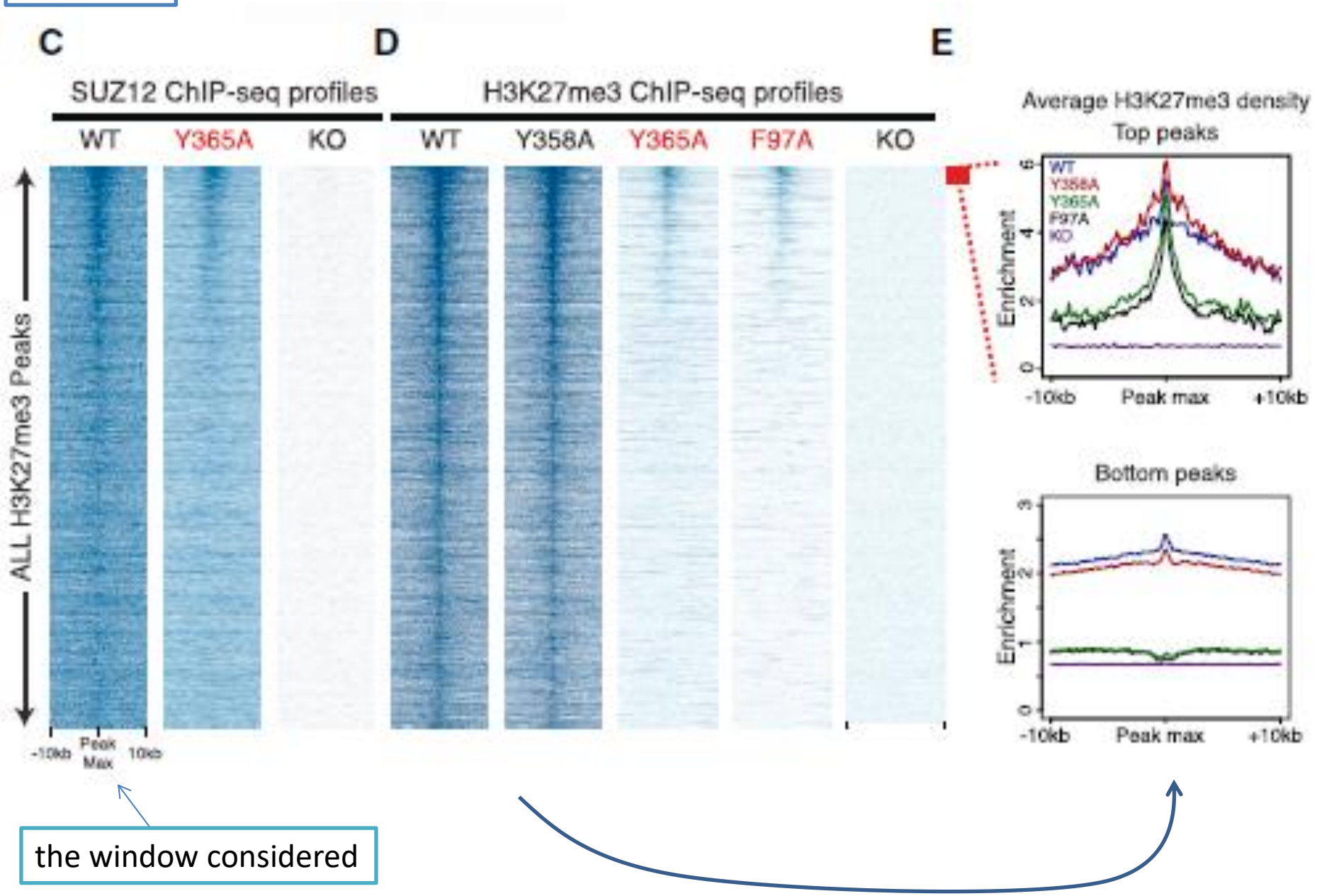


Total cell lysate WB

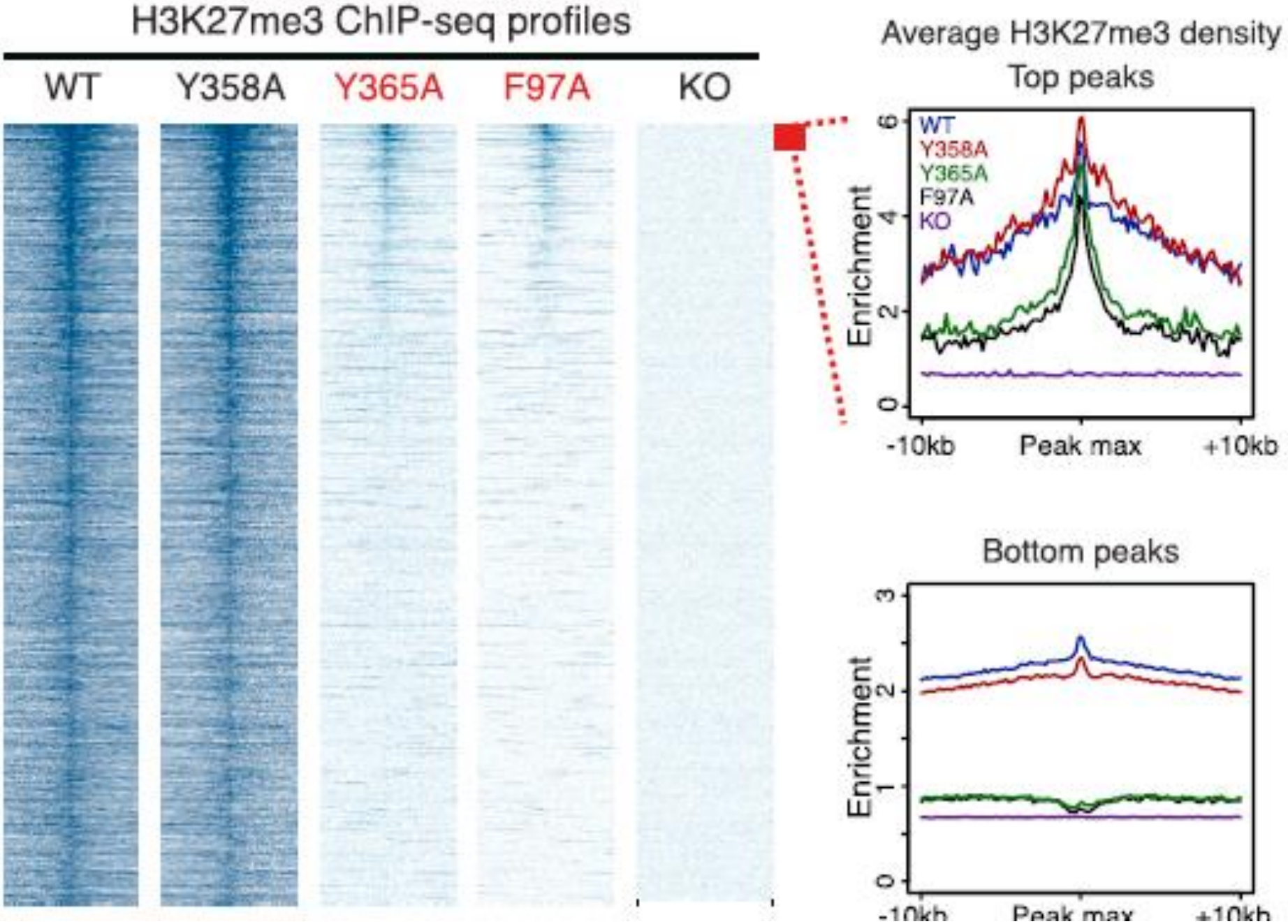
quantitative histone mass spectrometry

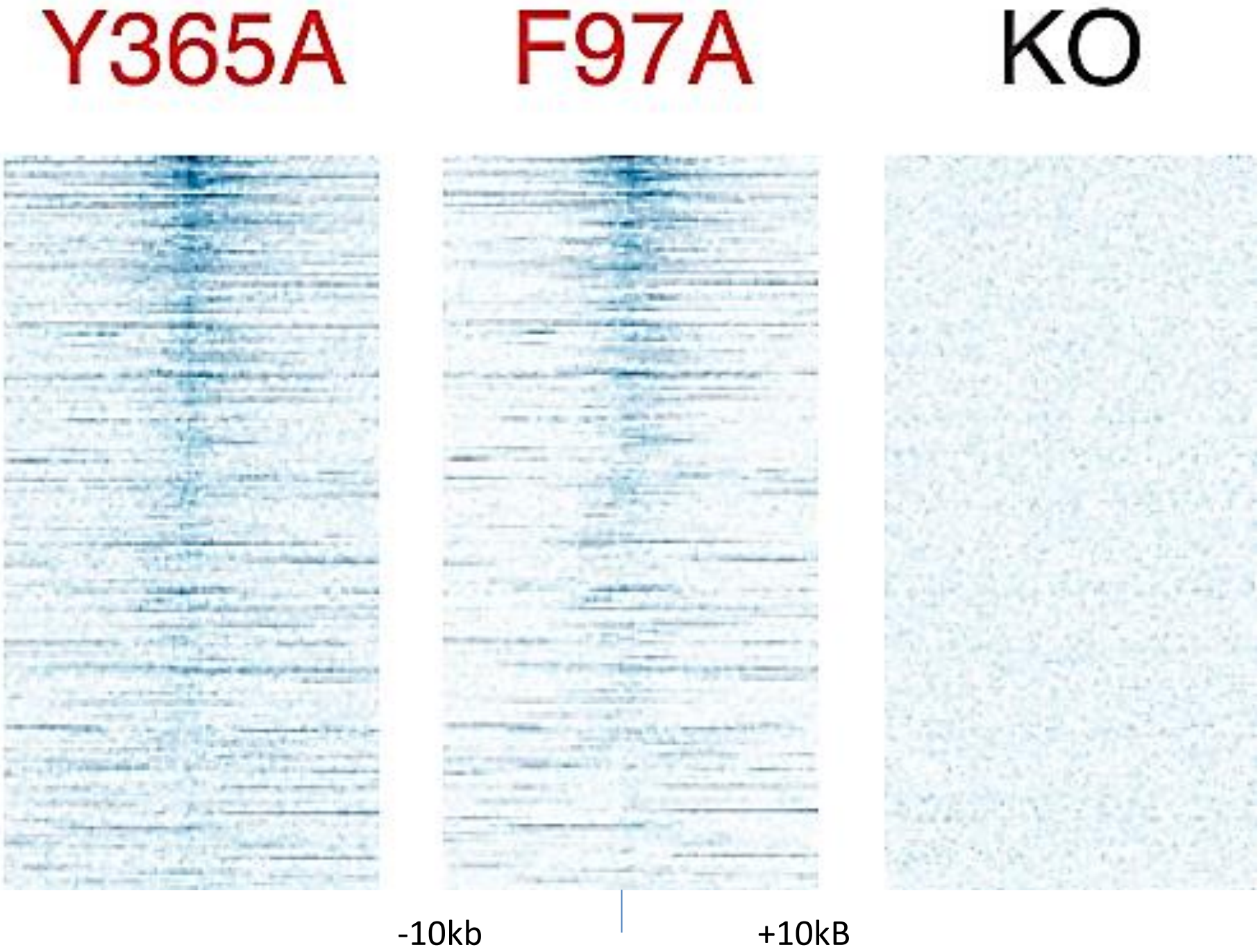
Heatmap

quantitative ChIP-seq

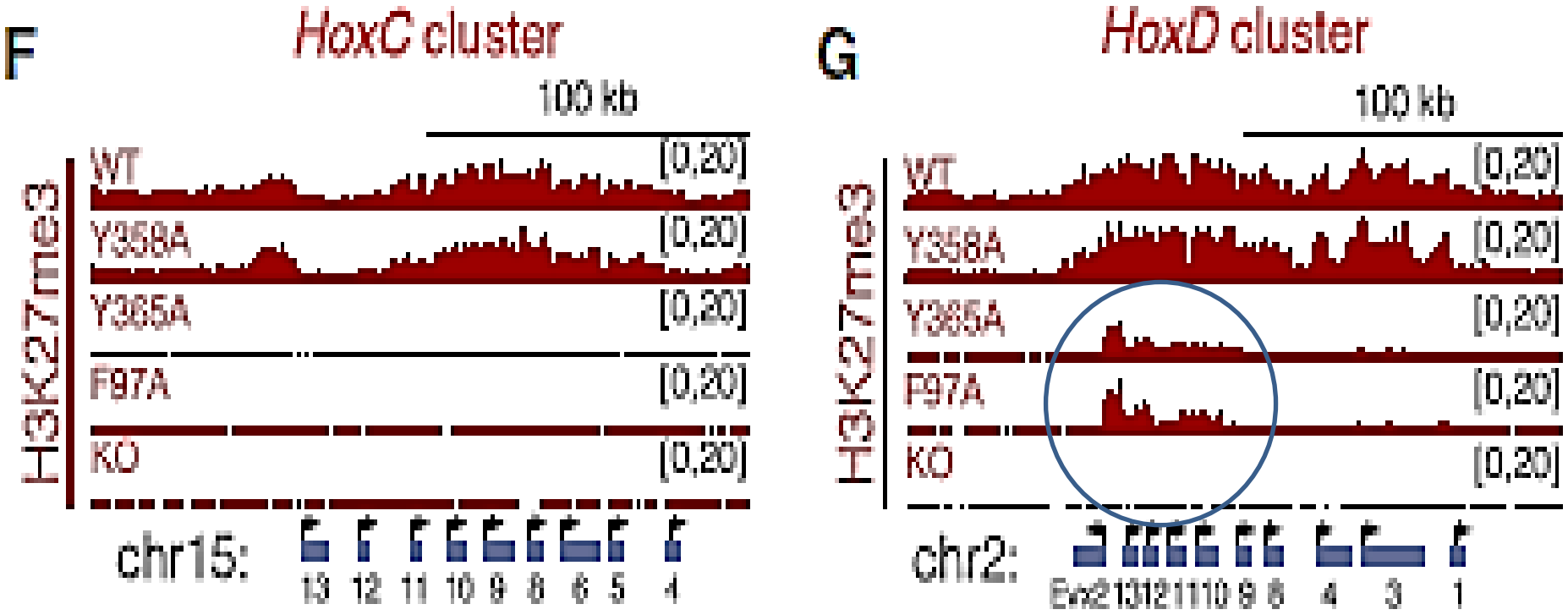


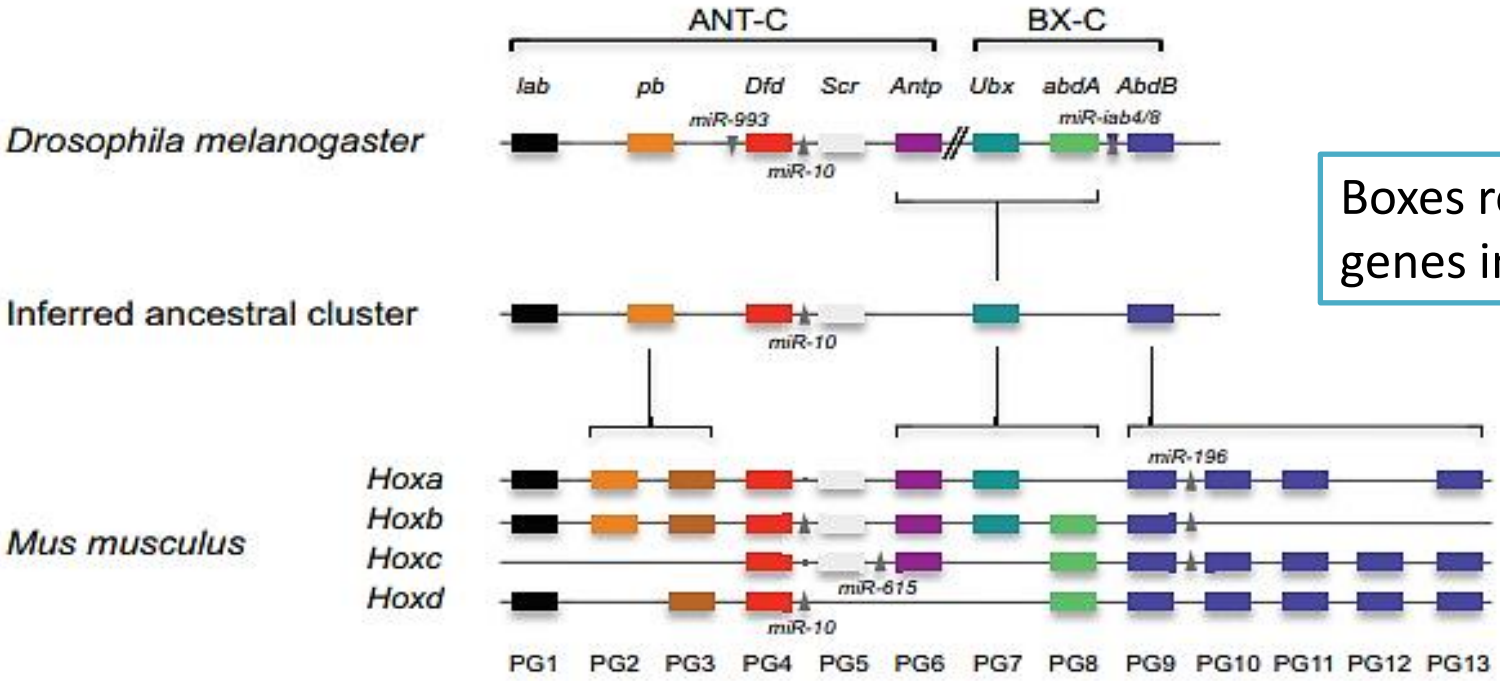
the window considered


H3K27me3 ChIP-seq profiles
WT
Y358A
Y365A
F97A
KO
Average H3K27me3 density
Top peaks
WT
Y358A
Y365A
F97A
KO
Enrichment
0
2
4
6
-10kb
Peak max
+10kb
Bottom peaks
Enrichment
0
1
2
3
-10kb
Peak max
+10kb

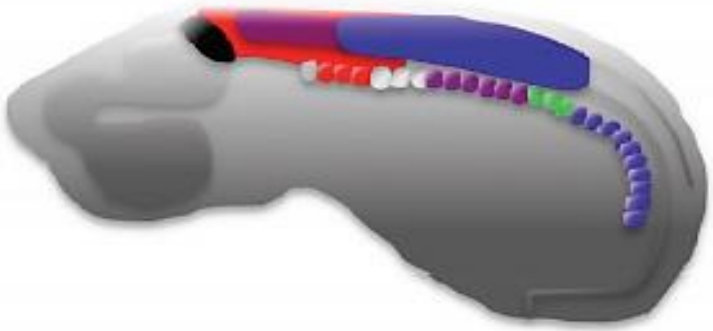

Y365A
F97A
KO
-10kb
+10kB

Details for 2 loci containing the HoxC and the HoxD clusters





Boxes represent single genes in the cluster



...approximate WT levels of H3K27me3 were maintained in cage-mutant mESCs at a limited number of discrete regions within larger Polycomb domains in contrast to other regions in the genome, with a progressive decrease distal to these sites (Figures 1E, cf. top and bottom plots)

At these regions, apparently, Lysine 27 me2/3 binding by EED is not required for PRC2 carrying on methylation

Initial Deposition and Spreading Mechanism of H3K27me2/3 Domains

To test whether regions of active PRC2 recruitment identified above might serve as nucleation sites for the initial establishment of Polycomb chromatin domains, we devised a Cre-lox-based system in which new domains could be established ‘‘de novo’’ in an inducible fashion in vivo, and their formation was tracked over time.

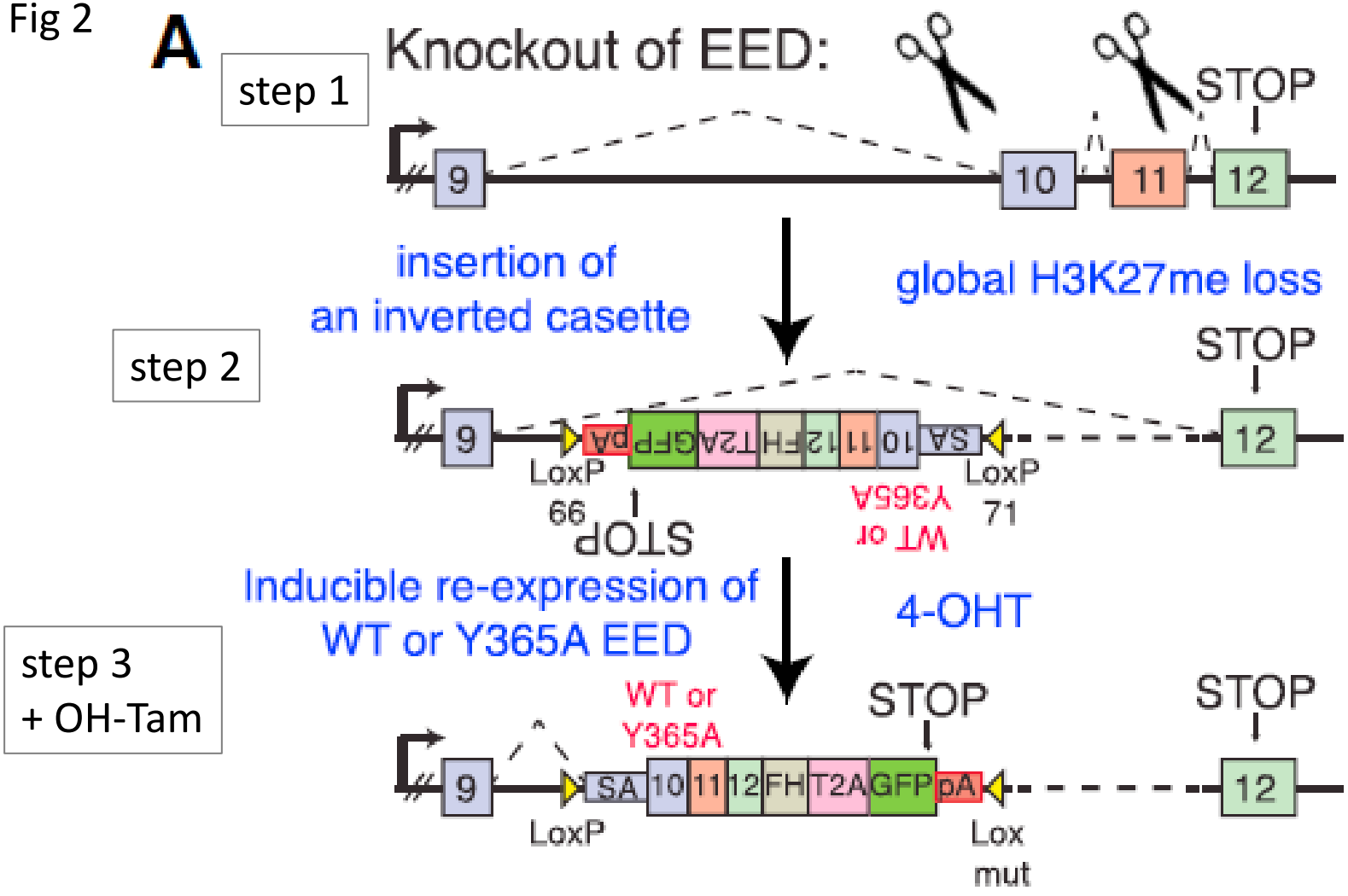
To this end, we deleted the endogenous copy of EED in mESCs possessing a CreERT2 transgene (Figure 2A; see STAR Methods for details).

The cells were propagated until H3K27me2/3 was completely depleted (Figures S3A, see 0 hr, and S3E).

We then expressed FLAG-HA-tagged versions of WT or cage-mutant EED (Y365A) from its endogenous locus in response to 4-Hydroxytamoxifen (4-OHT) to follow de novo H3K27me2/3 deposition.

Targeting scheme to conditionally rescue EED KO mESCs with EED, either WT or Y365A.

Fig 2



Aa. used (C57BL/6) mESCs that contain CreERT2. It encodes a Cre recombinase (Cre) fused to a mutant estrogen ligand-binding domain (ERT2) that requires the presence of tamoxifen for activity.

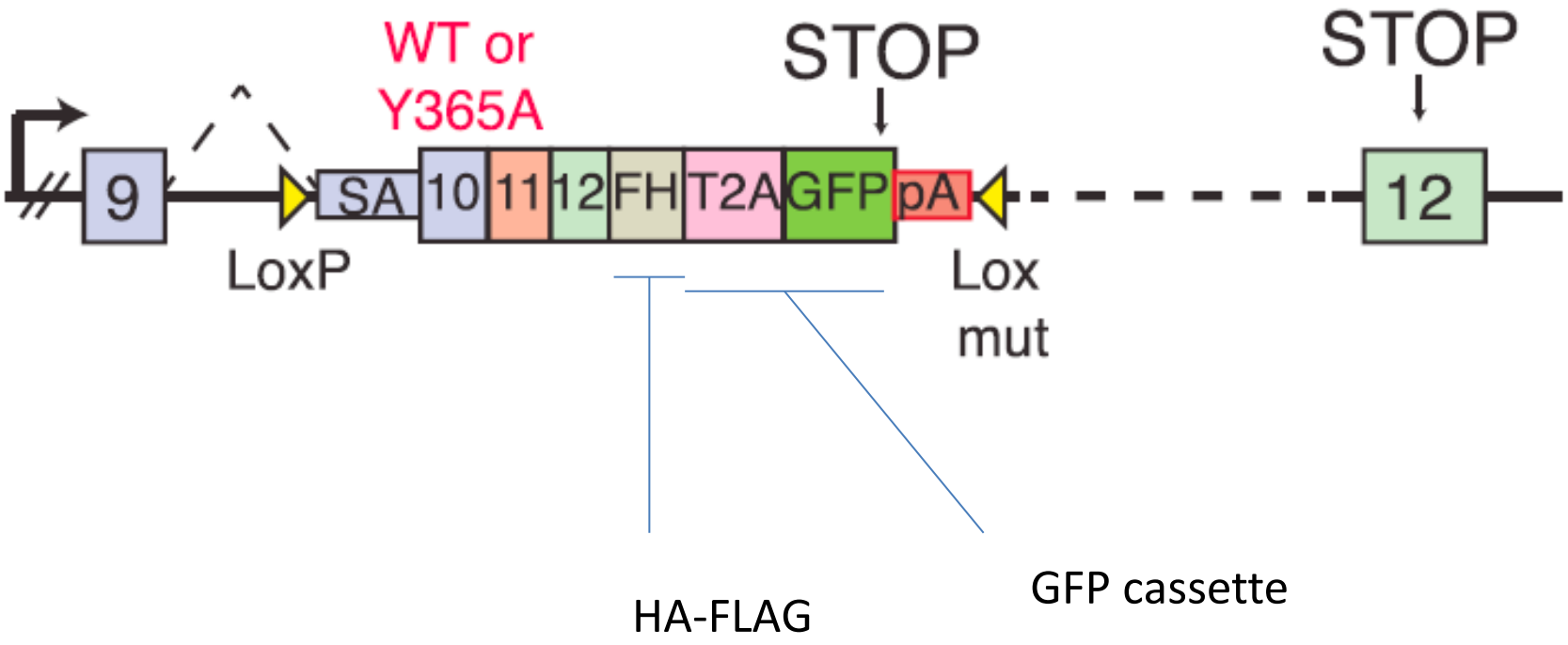
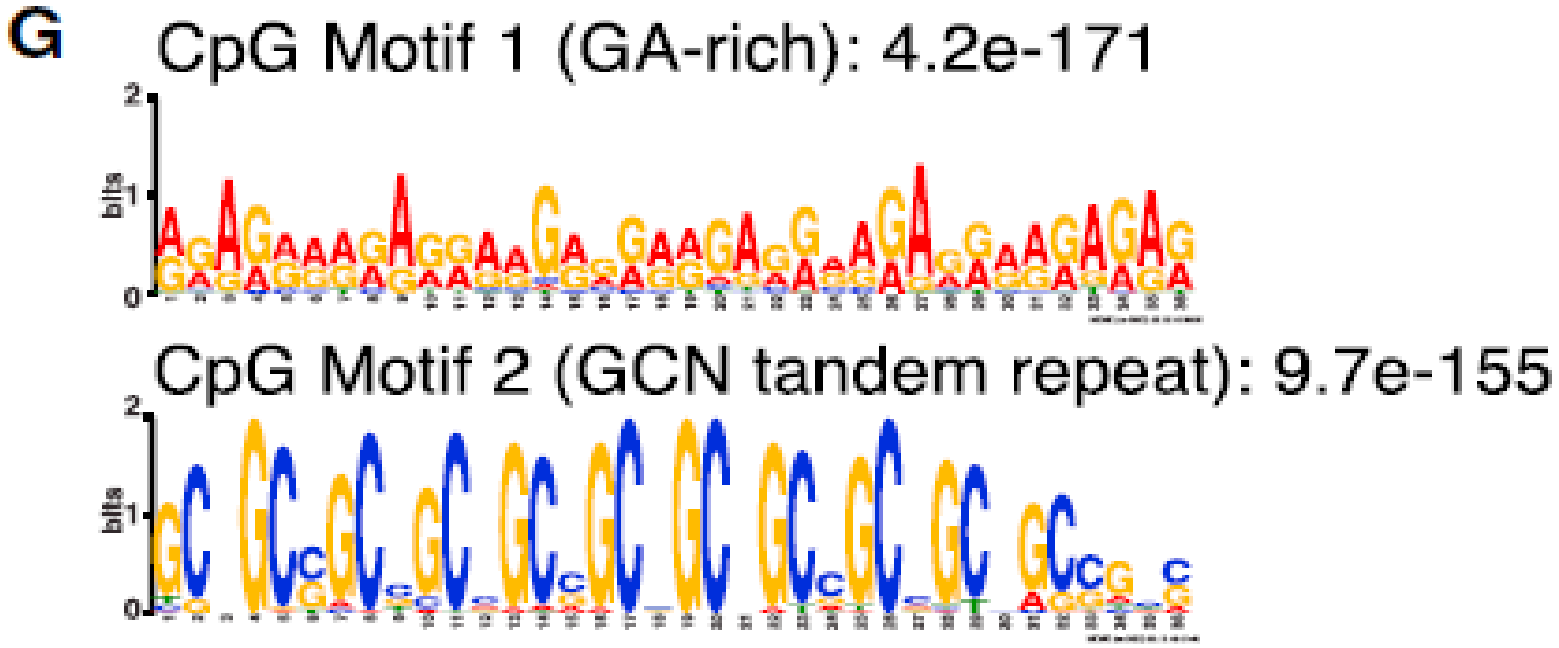

WT or
Y365A
STOP
STOP
9
SA
10
11
12
FH
T2A
GFP
pA
12
LoxP
Lox
mut
HA-FLAG
GFP cassette

Fig. 2


G
CpG Motif 1 (GA-rich): 4.2e-171
bits
2
1
0
CpG Motif 2 (GCN tandem repeat): 9.7e-155
bits
2
1
0

S-Logos