Eunice Kennedy Shriver National Institute of Child Health and Human Development

THE HUMAN PLACENTA PROJECT
http://www.nichd.nih.gov/hpp

How we shuffle our genes in the germ-line and why it might matter for human fertility

50% of his genes

By Mohamed Mahgoub

Todd Macfarlan, PhD

Meiotic Recombination

Meiotic Recombination is essential for:

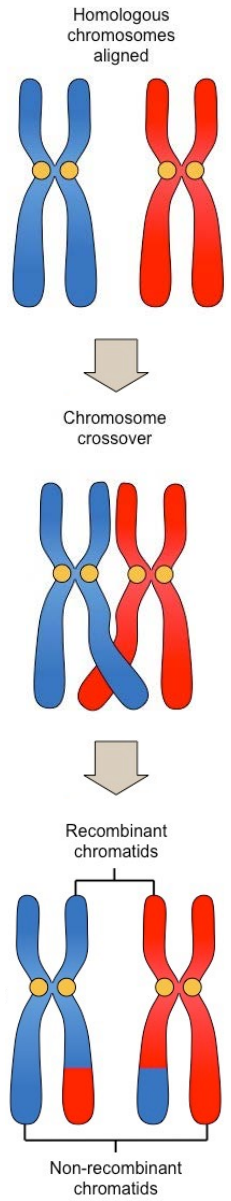
- Mixing alleles and generating genetic diversity
- Alignment and segregation of chromosomes

Meiotic Recombination involves:

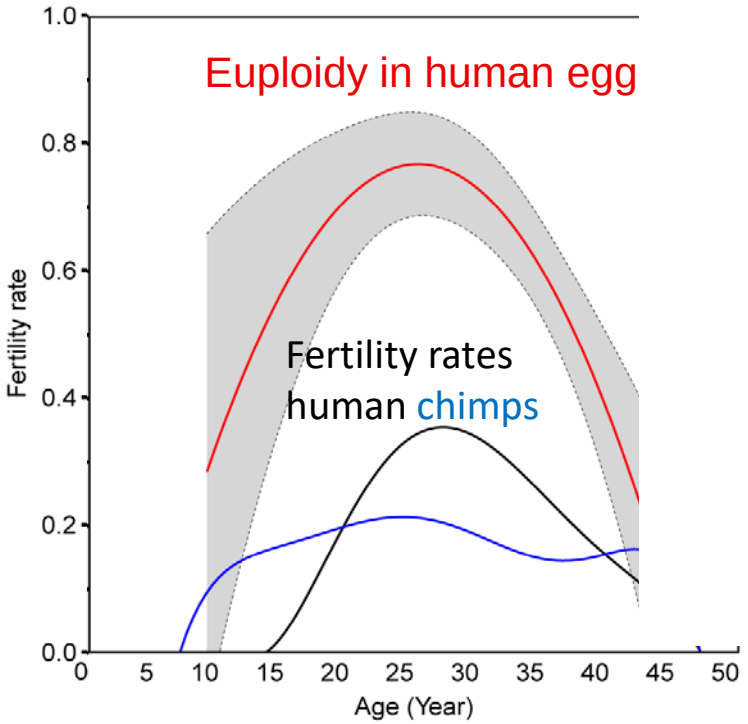
- A programmed double strand break (DSB) in one chromosome
- Homology directed repair of the DSB

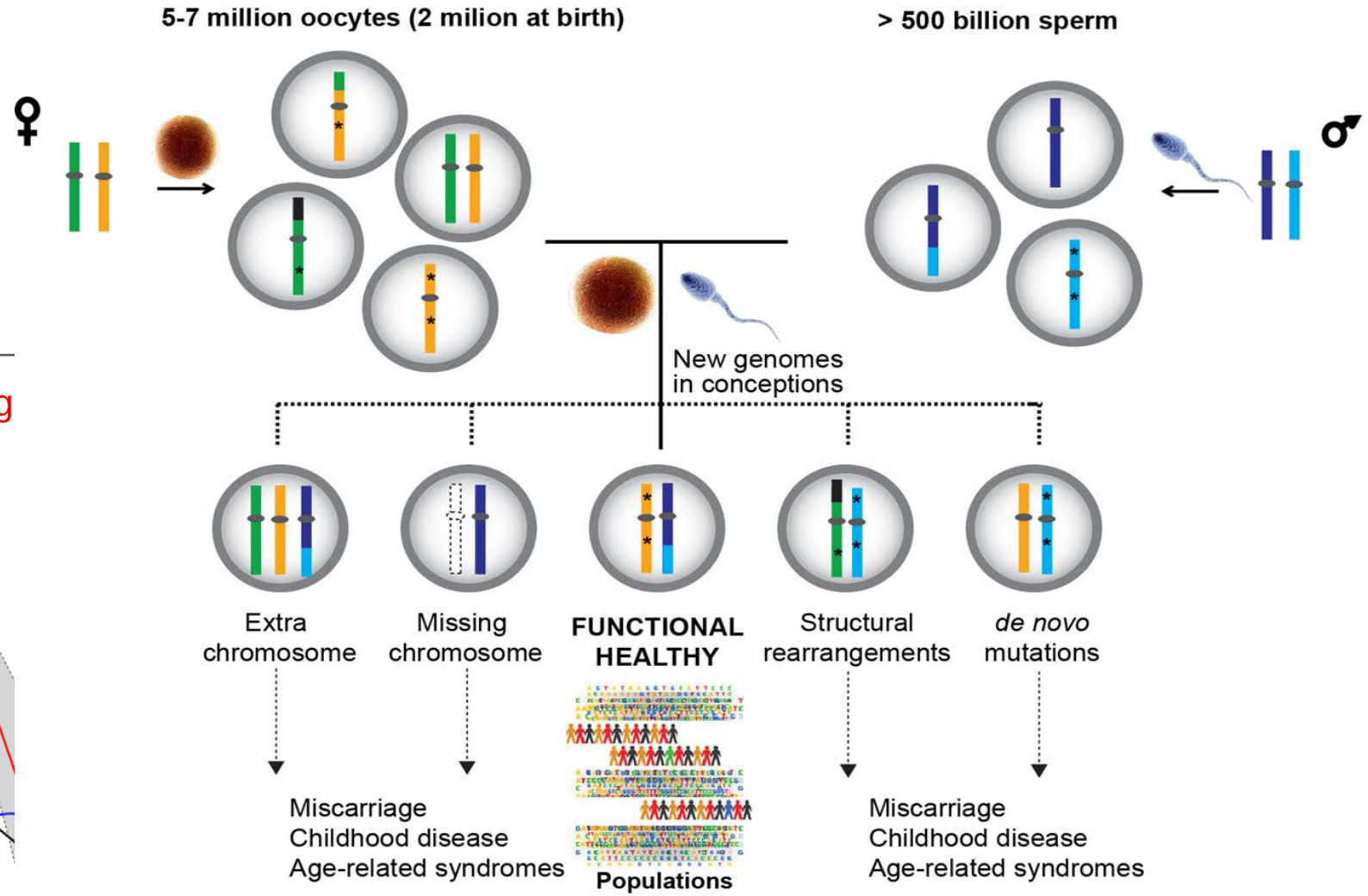
Meiotic Recombination is not a random process

- Typically 2-3 crossovers per chromosome
- Crossovers occur in hotspots
- Crossovers (or lack of them) determine which alleles stay linked



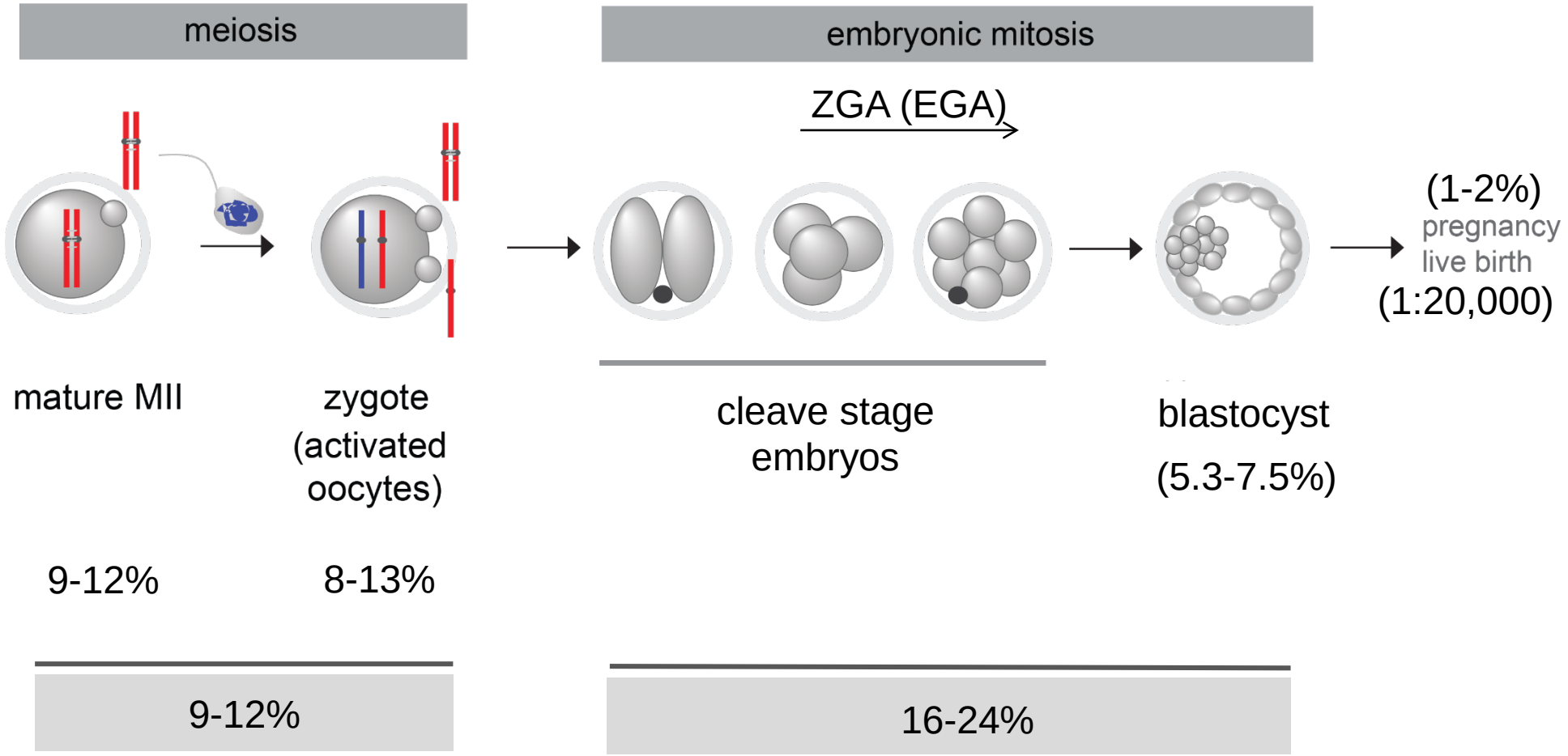
Consequences of Meiotic Recombination Errors

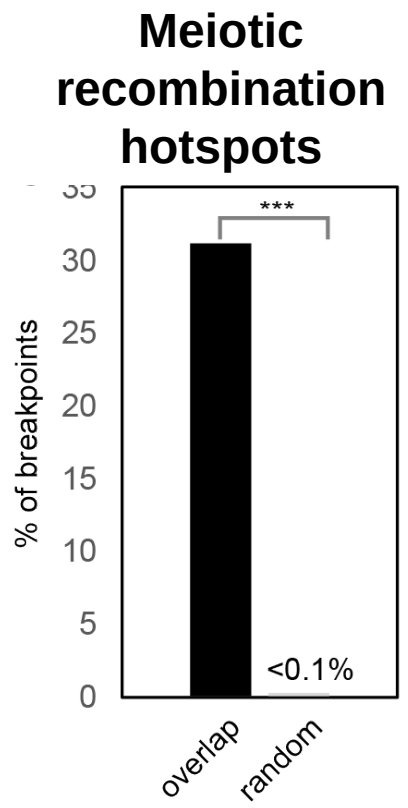




From Eva Hoffman

Gross chromosome rearrangements (GCRs) overlap hotspots

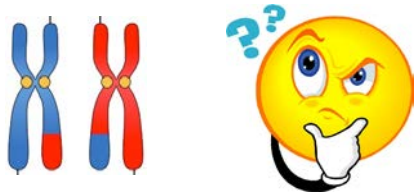




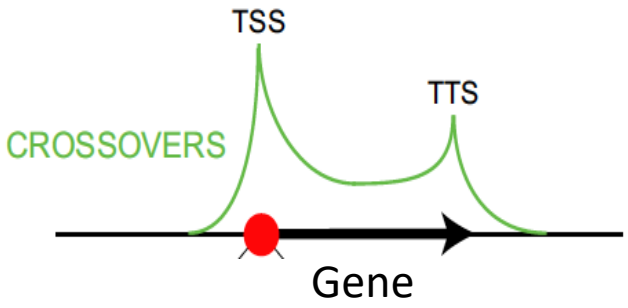
Percentage GCRs at each stage (from WGS)

From Eva Hoffman Lab

Where are hotspots distributed?

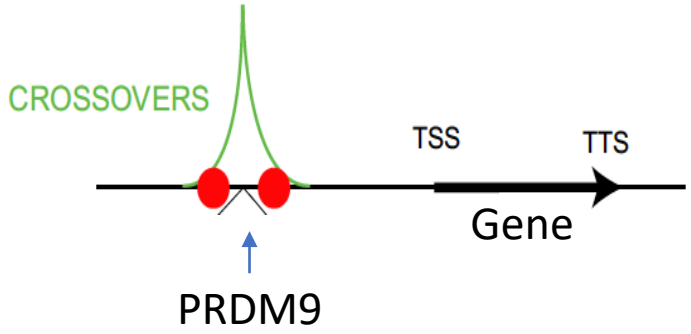


"Ancestral State"
Within genes
(Plants, Yeast, Birds)



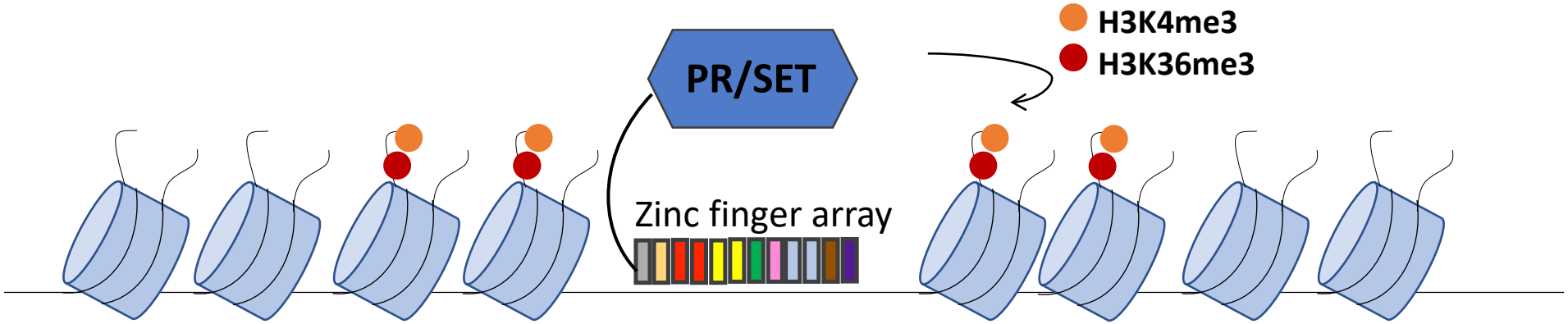
- Hotspots are fixed

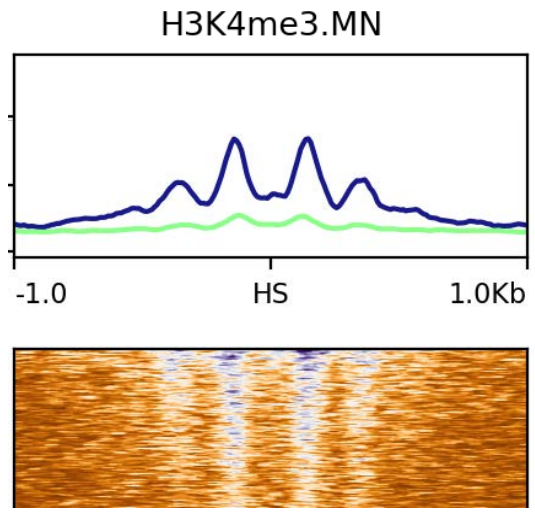
"Evolved State"
Away from genes
(Many Vertebrates, Most Mammals)



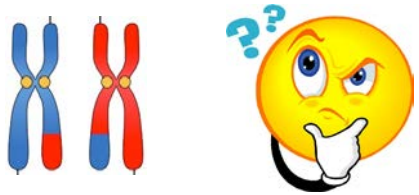
- PRDM9 allele diversity generates unique hotspots
- Hotspots change over evolutionary time scales

PRDM9 binds DNA and places a chemical signature that determines where DSBs will occur

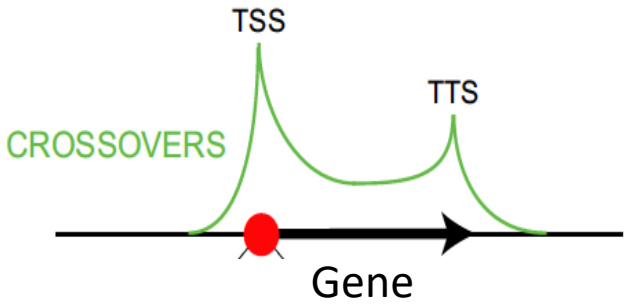




In *Prdm9* KOs, hotspots revert to the ancestral state

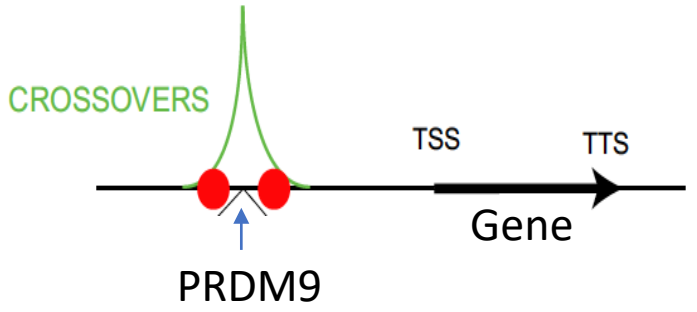


"Ancestral State"
Within genes
(Plants, and Yeast)



H3K4me3

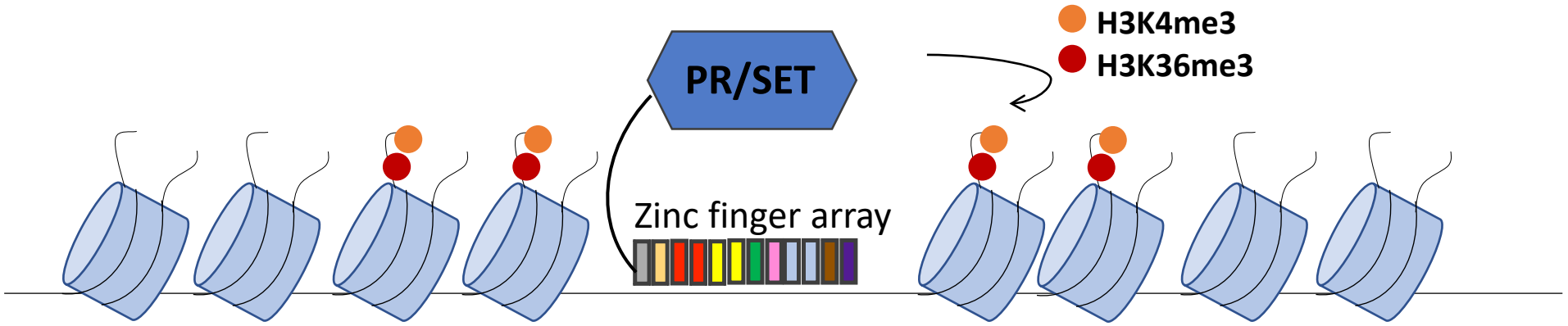
"Evolved State"
Away from genes
(Most Mammals)



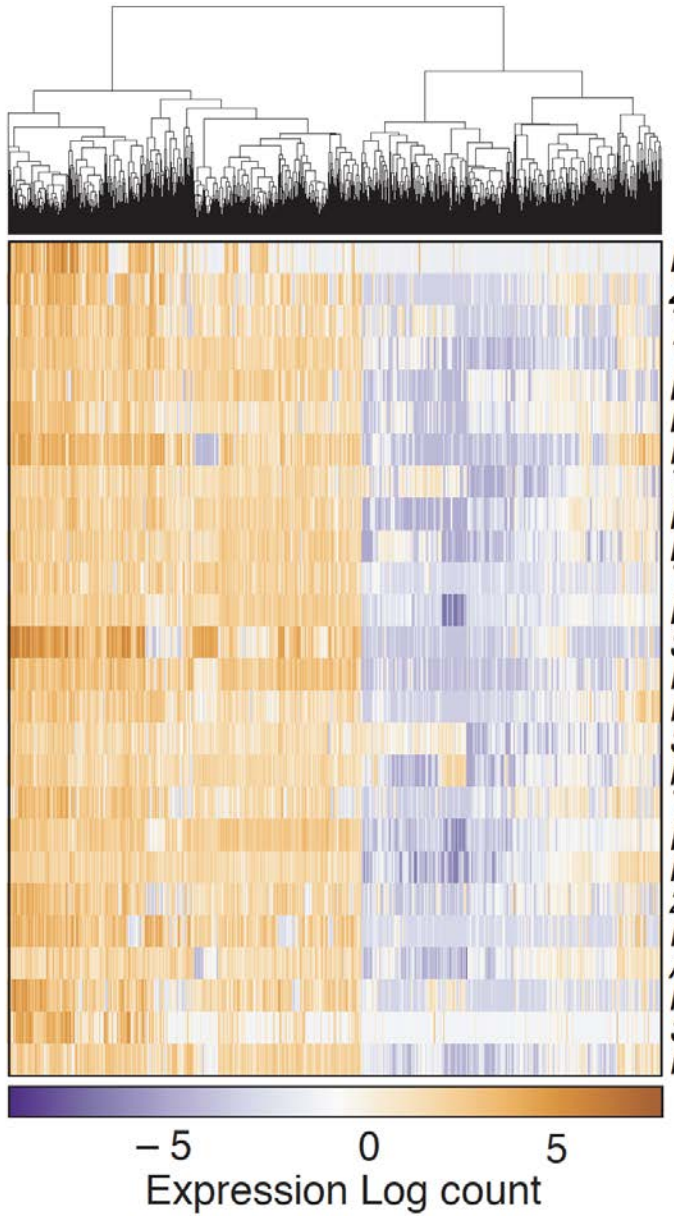
H3K4me3 + H3K36me3

*Dogs also have hotspots at promoters because they lost the Prdm9 gene!!!

Can we identify factors that may recognize the dual mark?



Identification of genes co-expressed with Prdm9



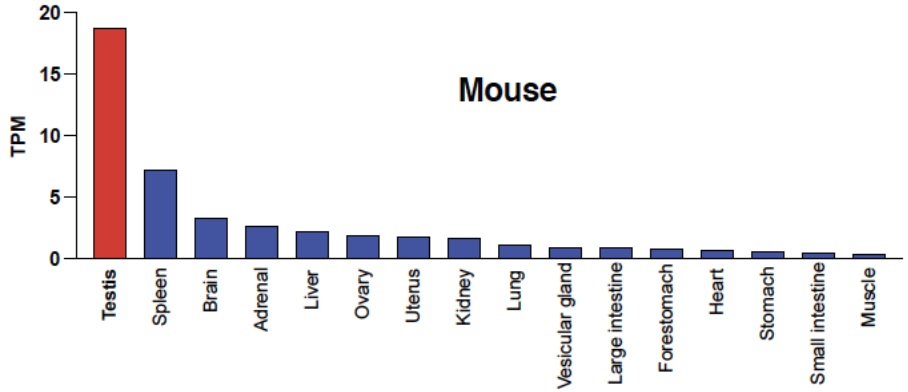
Zcwpw1

Unknown factor ???

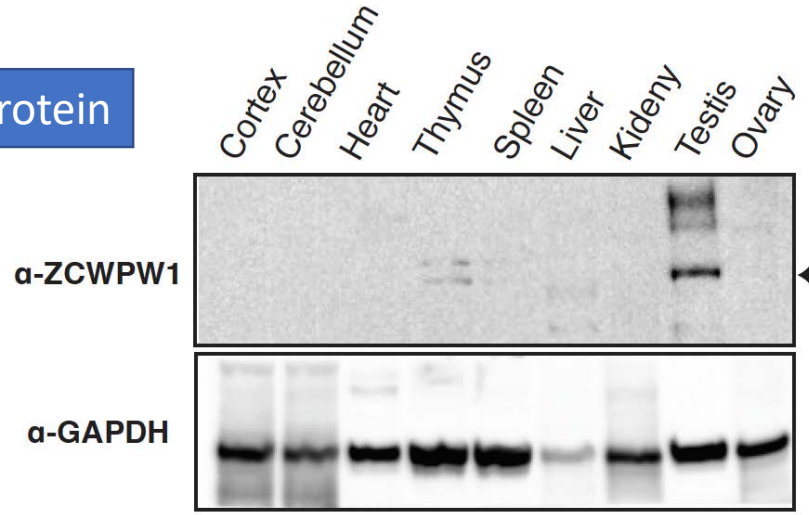
Known meiosis related factors

Zcwpw1 mainly expressed in testis

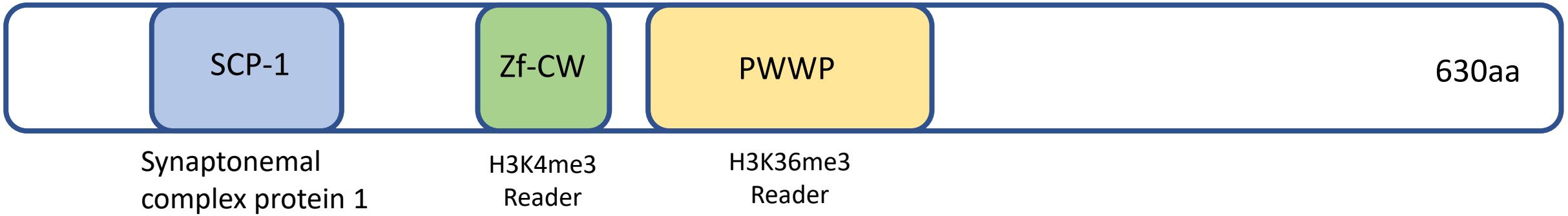
RNA

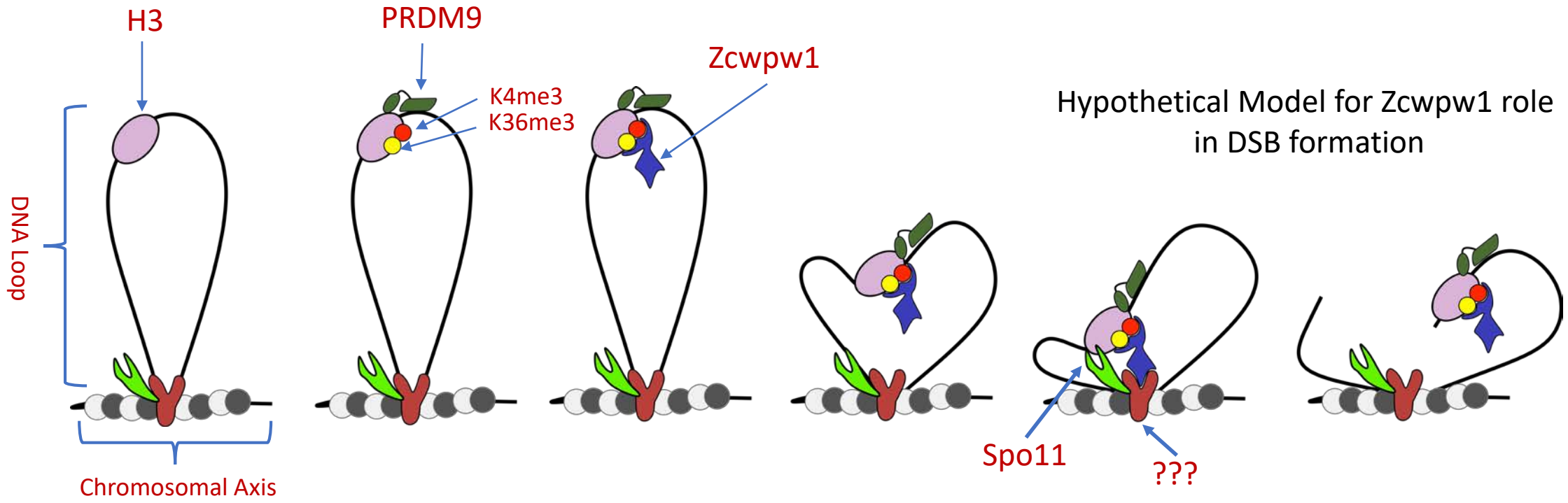


Protein



Zcwpw1 domains suggest it is a histone methyl reader





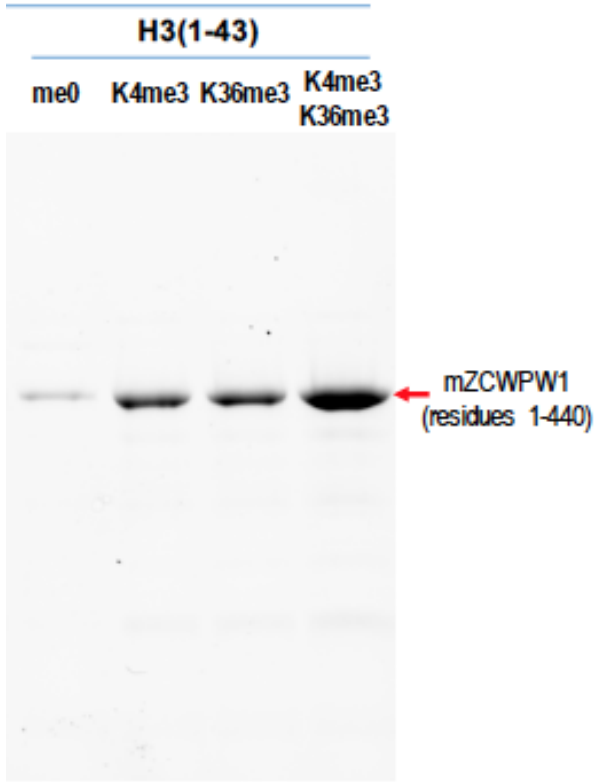
ZCWPW1 binds to the dual H3K4me3/H3K36me3 mark *in vitro*

Histone Peptide Pull down Assay

Mix biotinylated H3 peptides with Zcwpw1

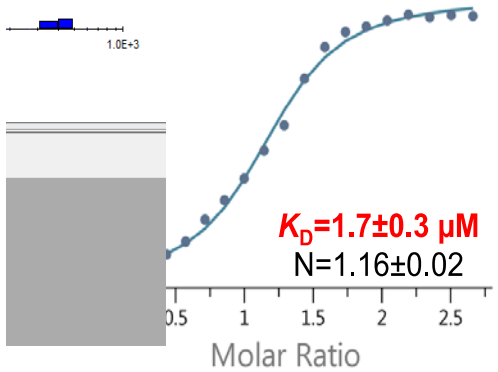
↓

Streptavidin pull down

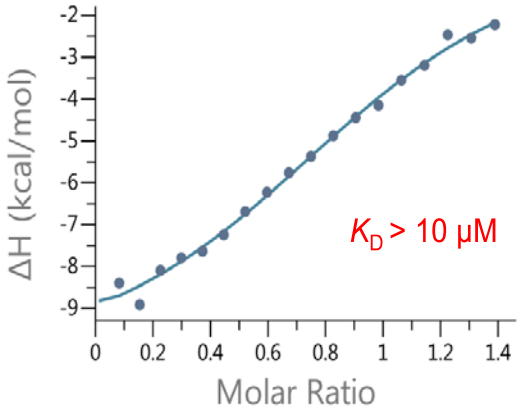


Isothermal titration calorimetry

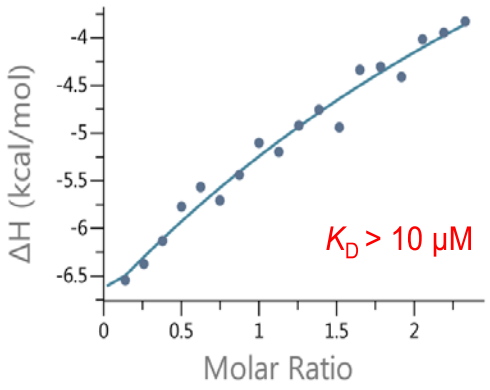
H3 (1-44)
K4me3K36me3



H3 (1-18)
K4me3

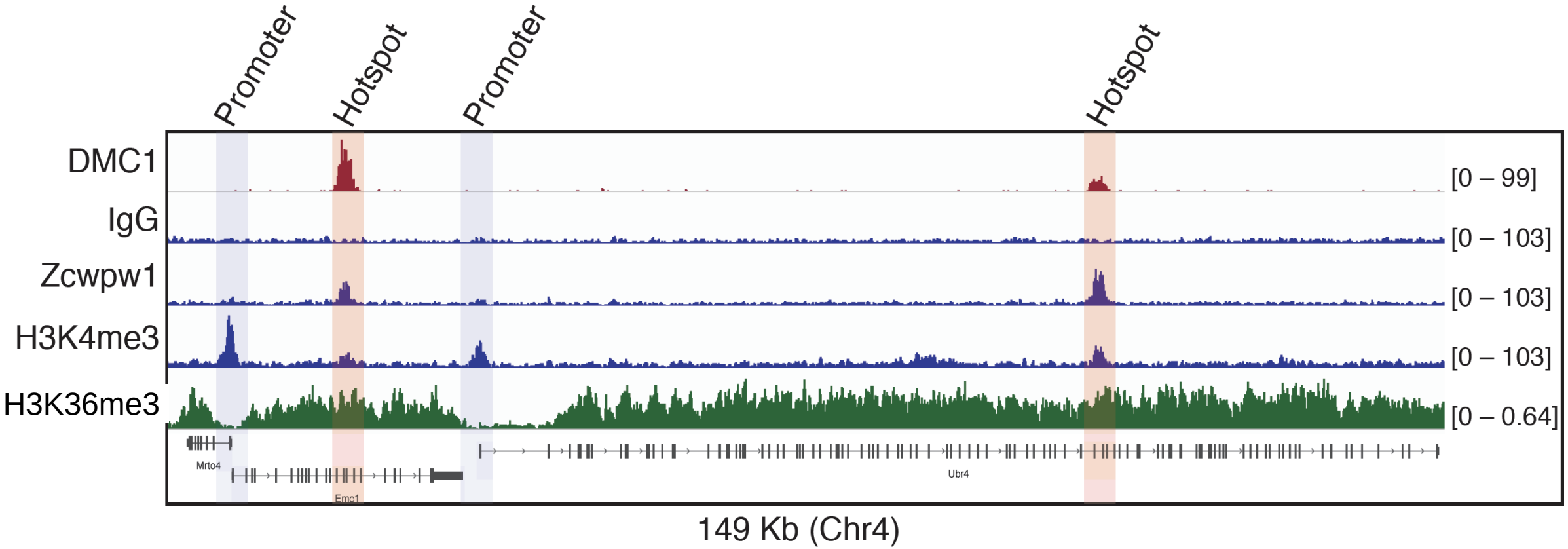


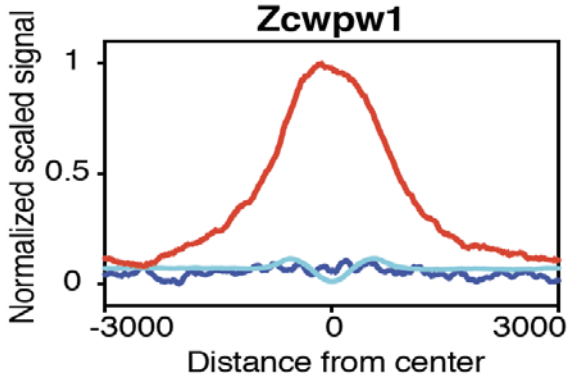
H3 (21-44)
K36me3

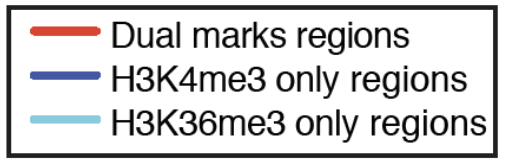


KD = Equilibrium dissociation constant, measures binding affinity

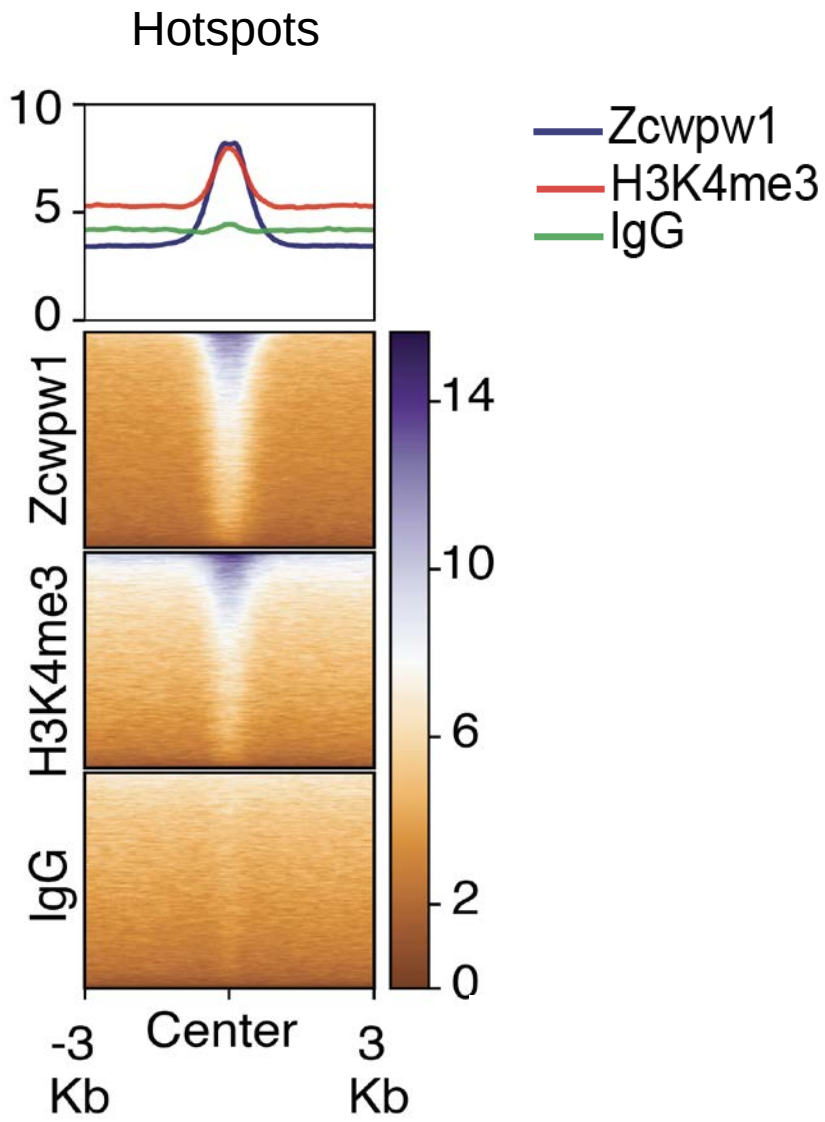
ZCWPW1 binds only to dual marked sites *in vivo*



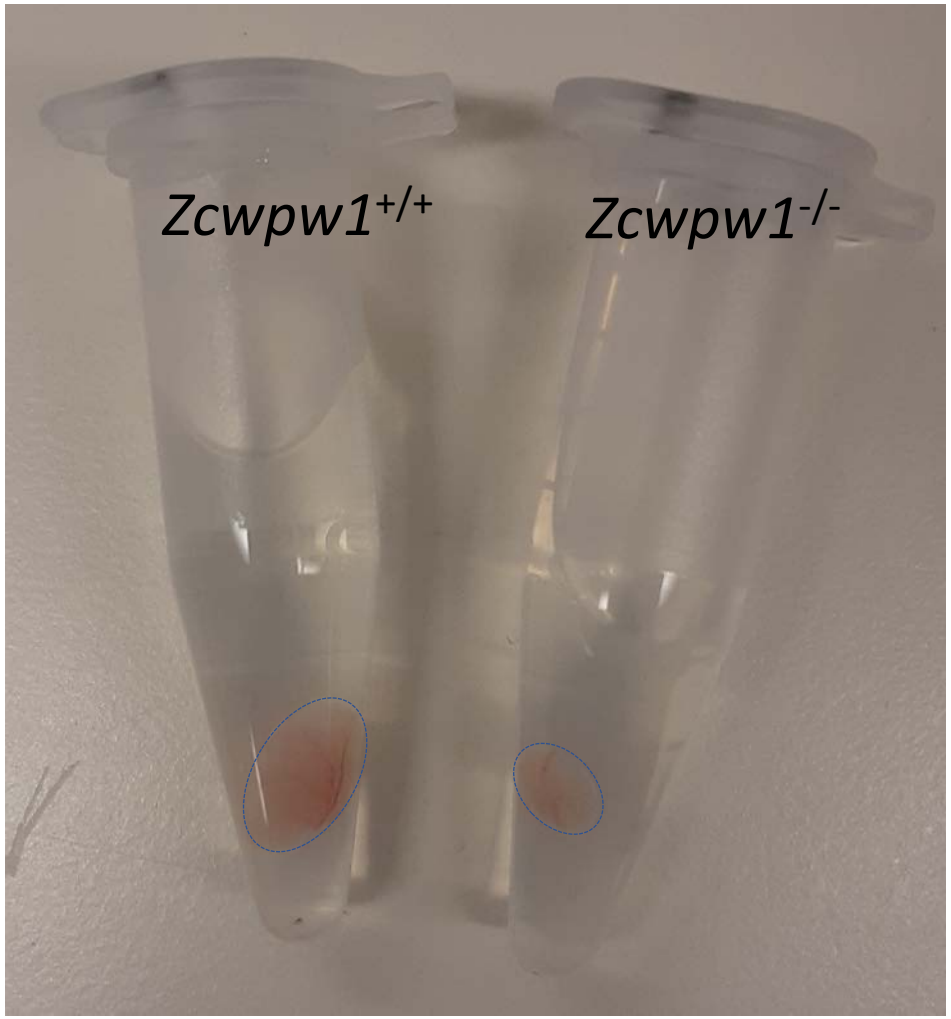


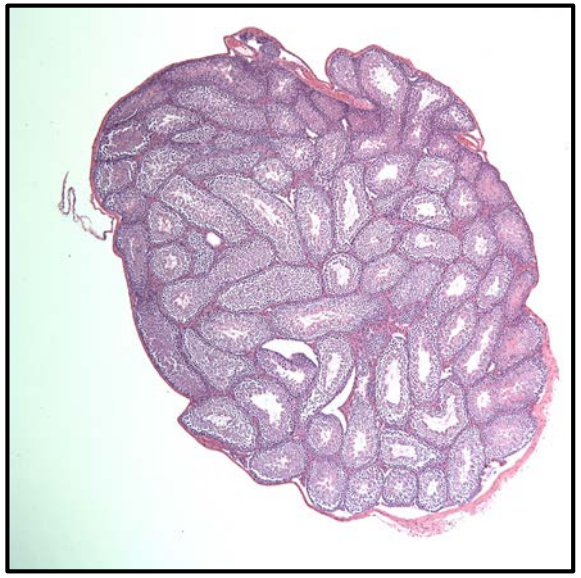
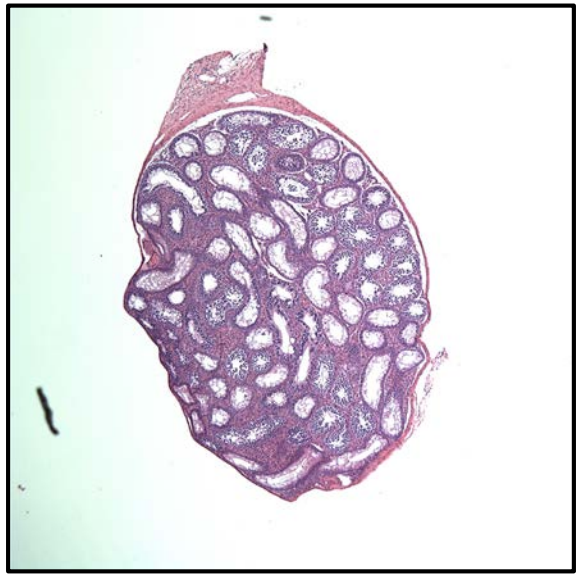
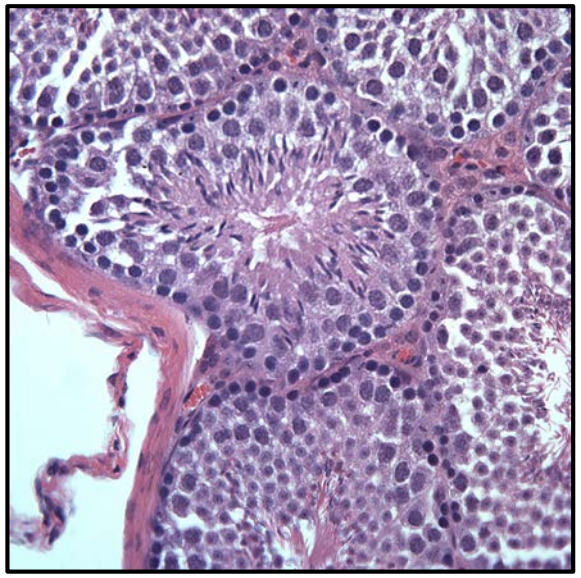
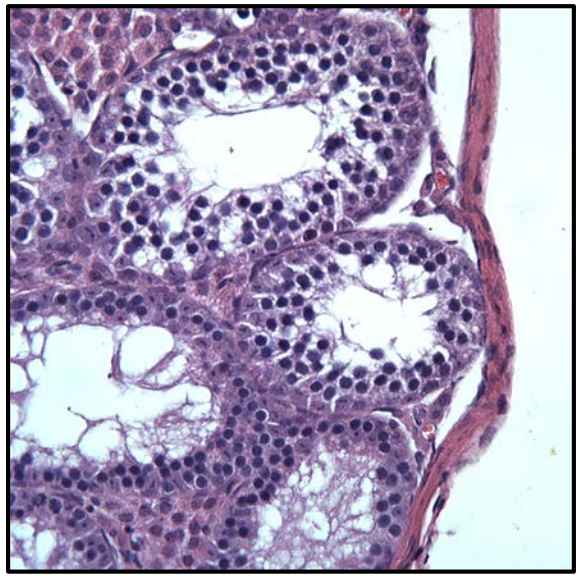


ZCWPW1 binds to PRDM9 determined hotspots *in vivo*

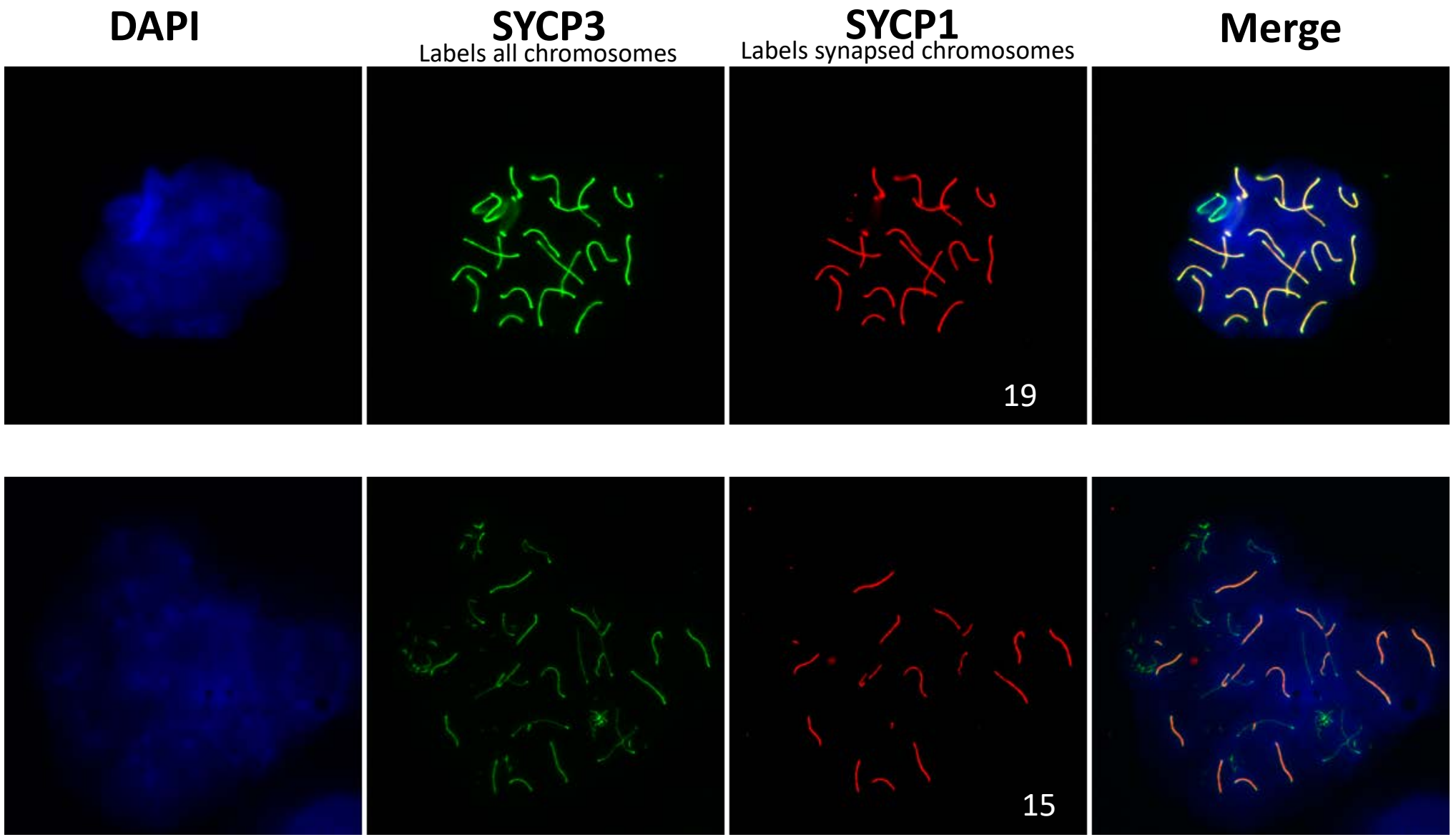


Zcwpw1$^{-/-}$ mice are azoospermic



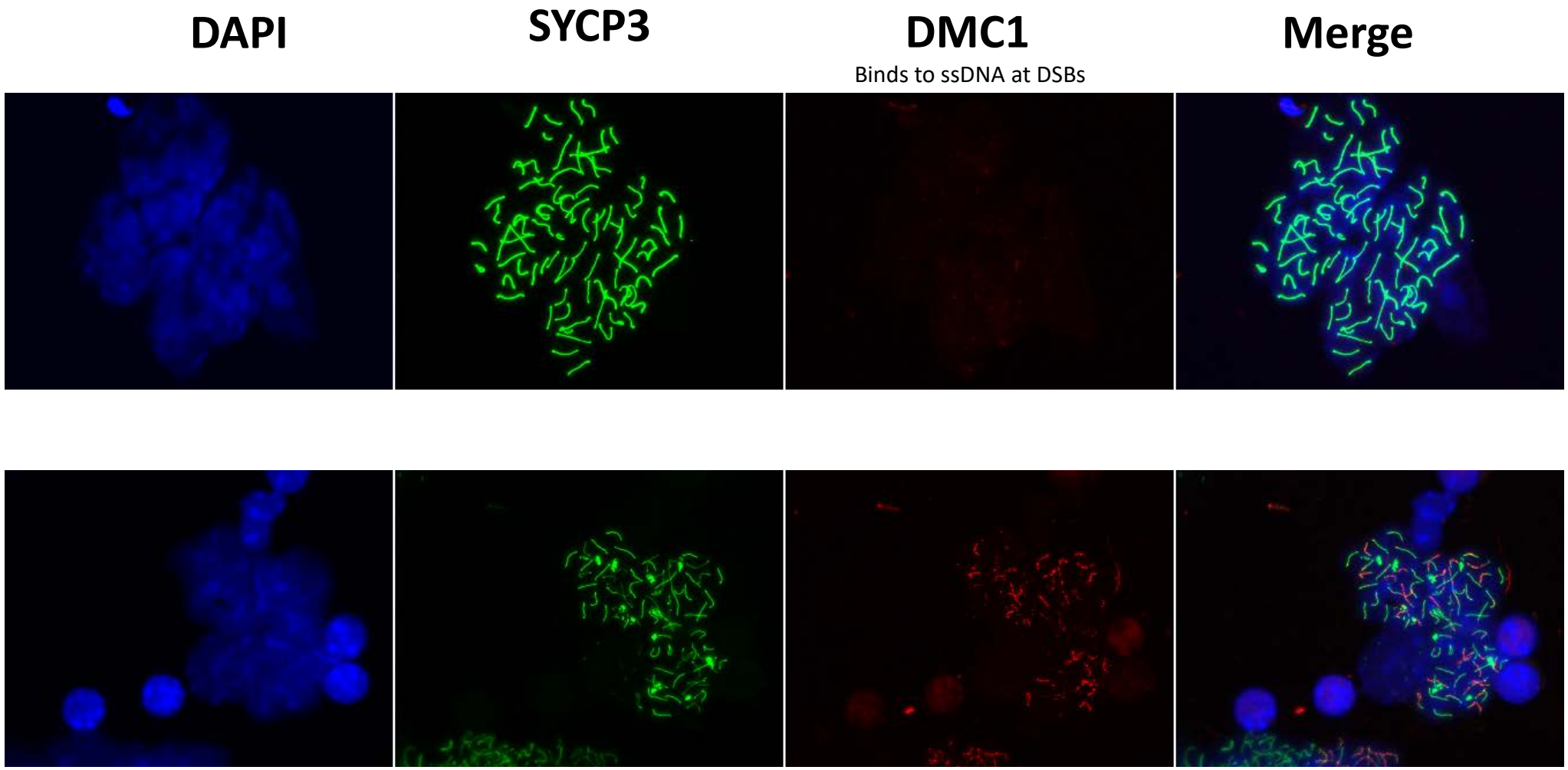
	Zcwpw1$^{+/+}$	*Zcwpw1*$^{-/-}$
4X		
40X		

Partial asynapsis in *Zcwpw1*$^{-/-}$ *spermatocytes*



chromosomal Spreads 40X

DSB repair failure in *Zcwpw1*$^{-/-}$ *spermatocytes*

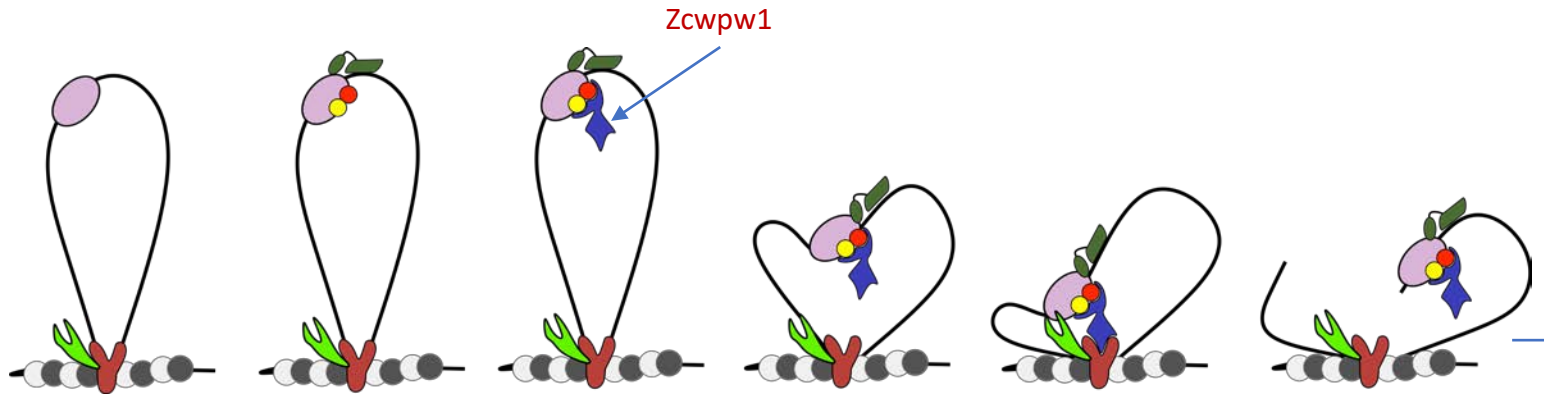


chromosomal Spreads
40X

Alternative models for ZCWPW1 function

Model (1)

Zcwpw1 is essential for PRDM9-dependent DSBs

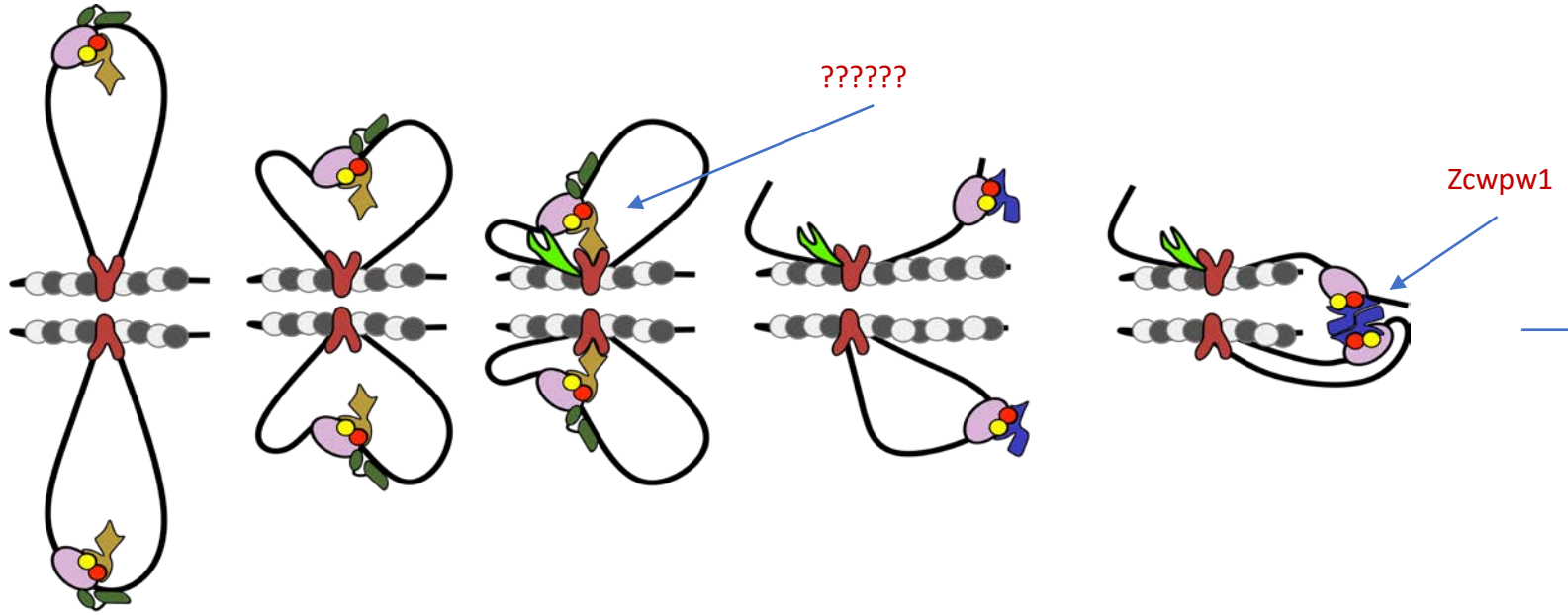


Expected DSBs mapping in *Zcwpw1*$^{-/-}$

DSBs at promoters (Mimic PRDM9 KO)

Model (2)

Zcwpw1 is dispensable for PRDM9 dependent DSBs, but is essential for repair of DSBs



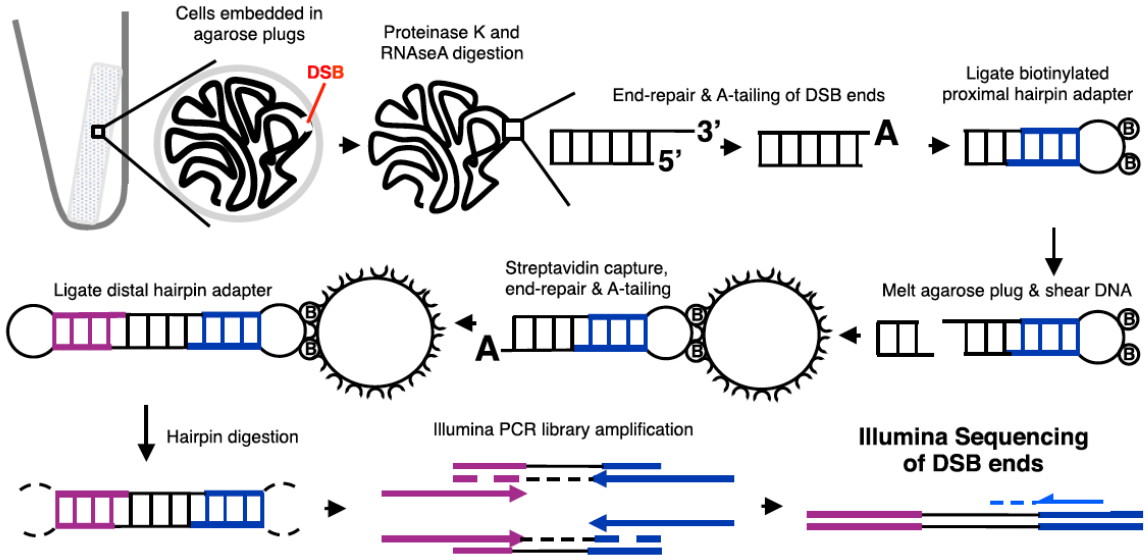
DSBs at Prdm9 binding sites

END-Seq directly maps DSBs

Molecular Cell

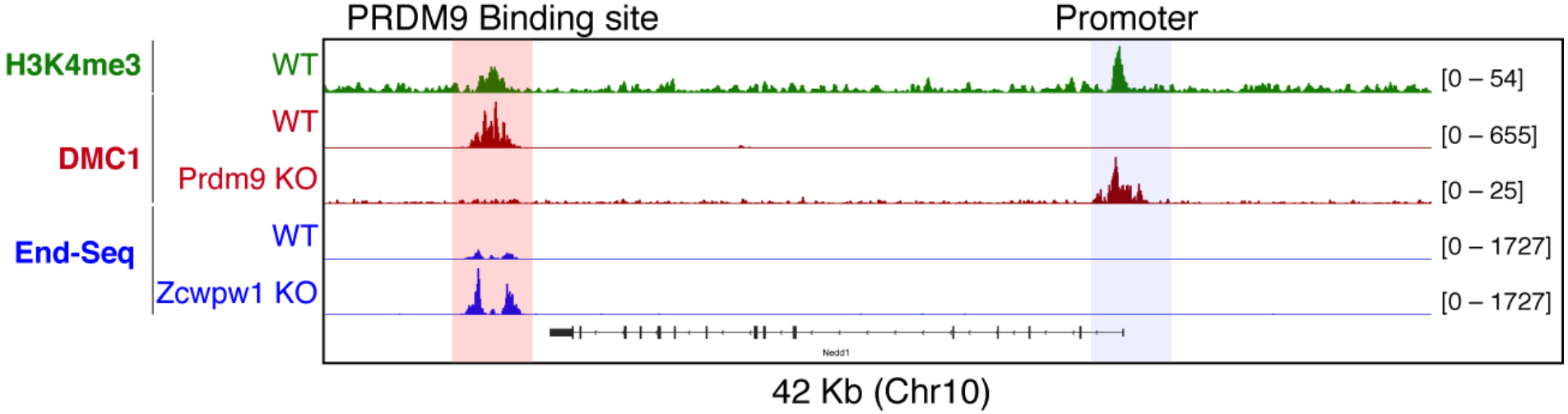
DNA Breaks and End Resection Measured Genome-wide by End Sequencing

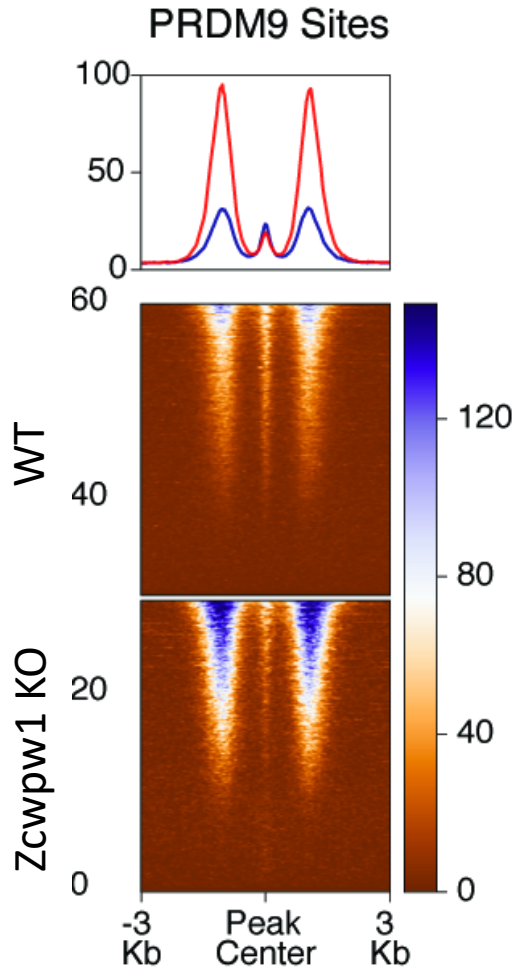
Andres Canela,[1] Sriram Sridharan,[1] Nicholas Sciascia,[1] Anthony Tubbs,[1] Paul Meltzer,[2] Barry P. Sleckman,[3] and André Nussenzweig[1,*]



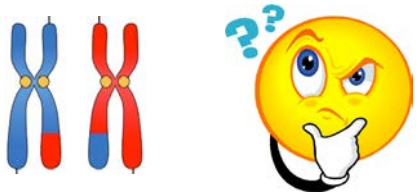
- Gene editing
- V(D)J recombination
- Class switch recombination
- Fragile sites (ERFS, CFS)
- End-resection *in vivo*
- Chemotherapeutic agents

DSBs don’t re-locate in *Zcwp1*$^{-/-}$ *testes*

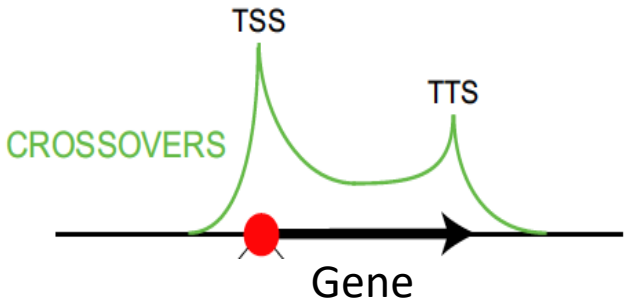




Co-emergence of PRDM9/ZCWPW1 re-engineered the landscape of recombination hotspots

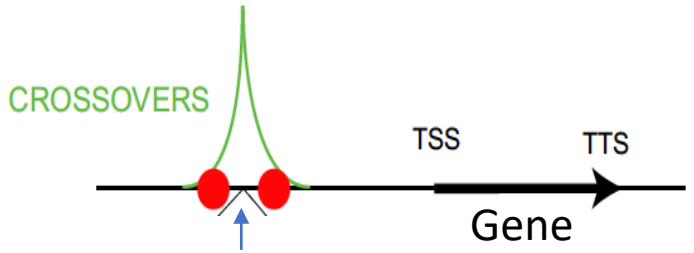


"Ancestral State"
Within genes
(Plants, and Yeast)



H3K4me3

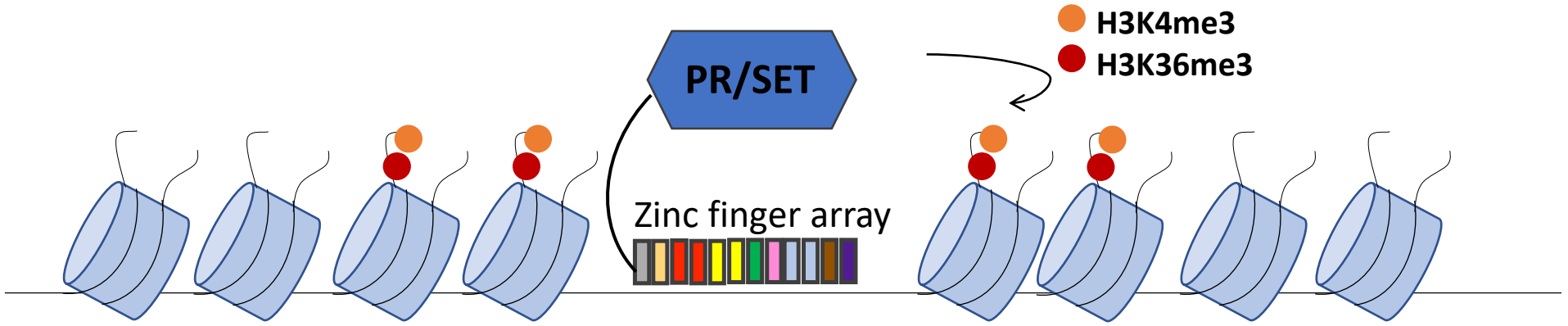
"Evolved State"
Away from genes
(Most Mammals)



PRDM9- Writer-- initiate breaks
ZCWPW1- Reader--facilitate repair

H3K4me3 + H3K36me3

Could defects in the *PRDM9* system contribute to infertility in humans?

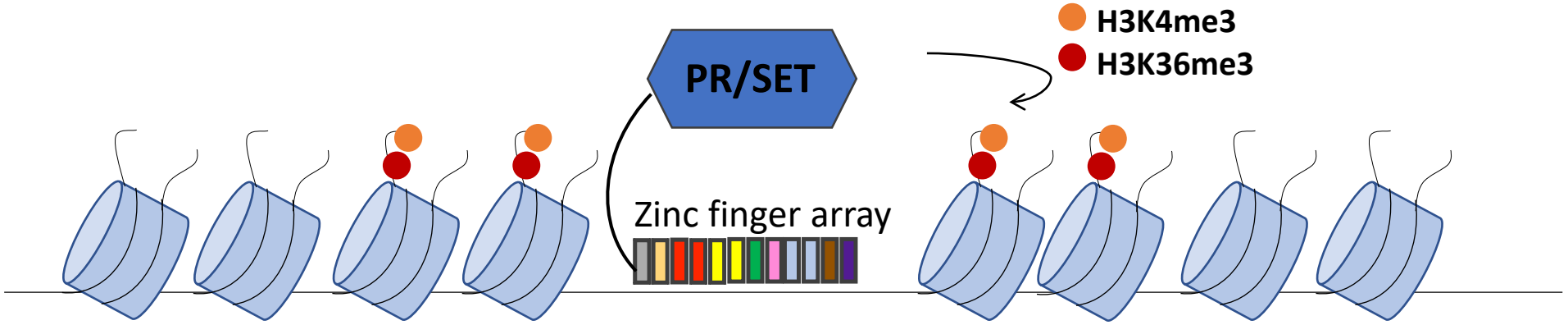


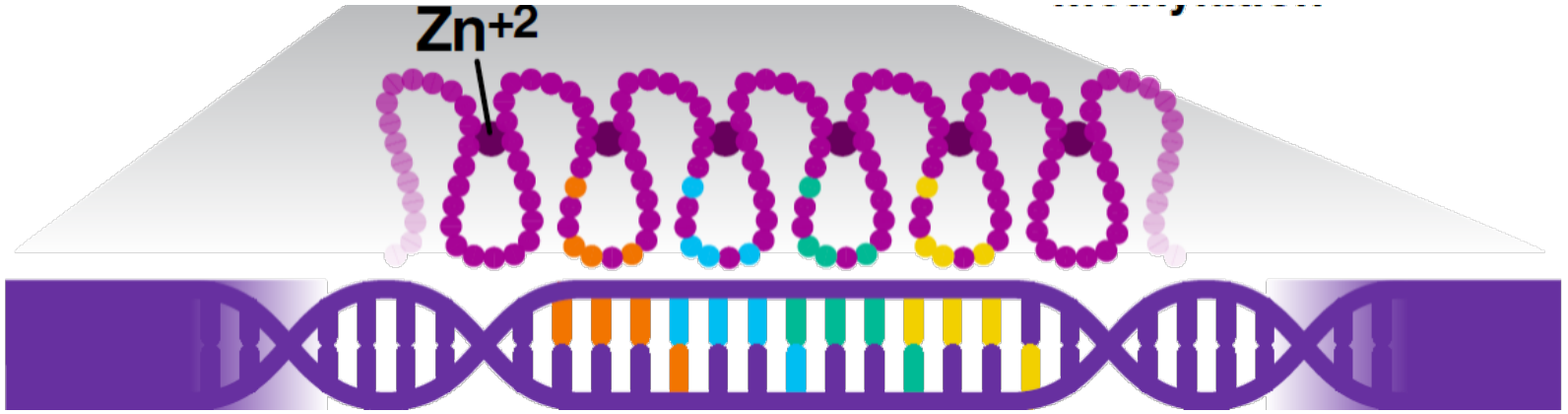
Rationale

- *Prdm9* and *Zcwpw1* knockout in mice lead to sterility in males (azoospermia)
- Two small studies in Japan found SNPs in *PRDM9* in cases of azoospermia
- *PRDM9* is a rapidly evolving gene that includes a coding mini-satellite sequence

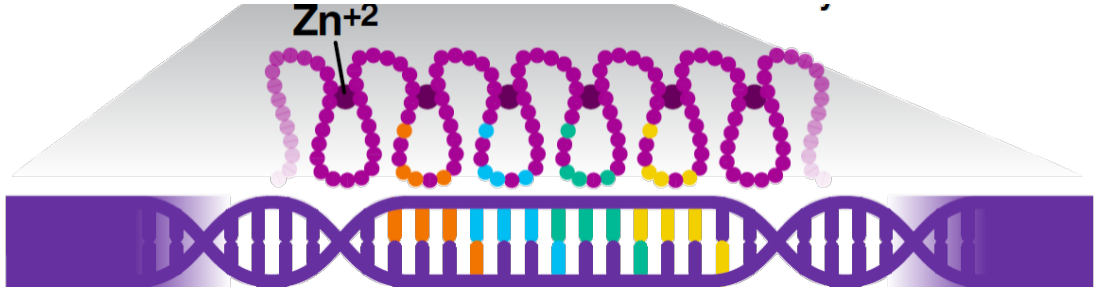
The *PRDM9* zinc finger array is a mini-satellite

PRDM9 is a DNA binding histone methyltransferase

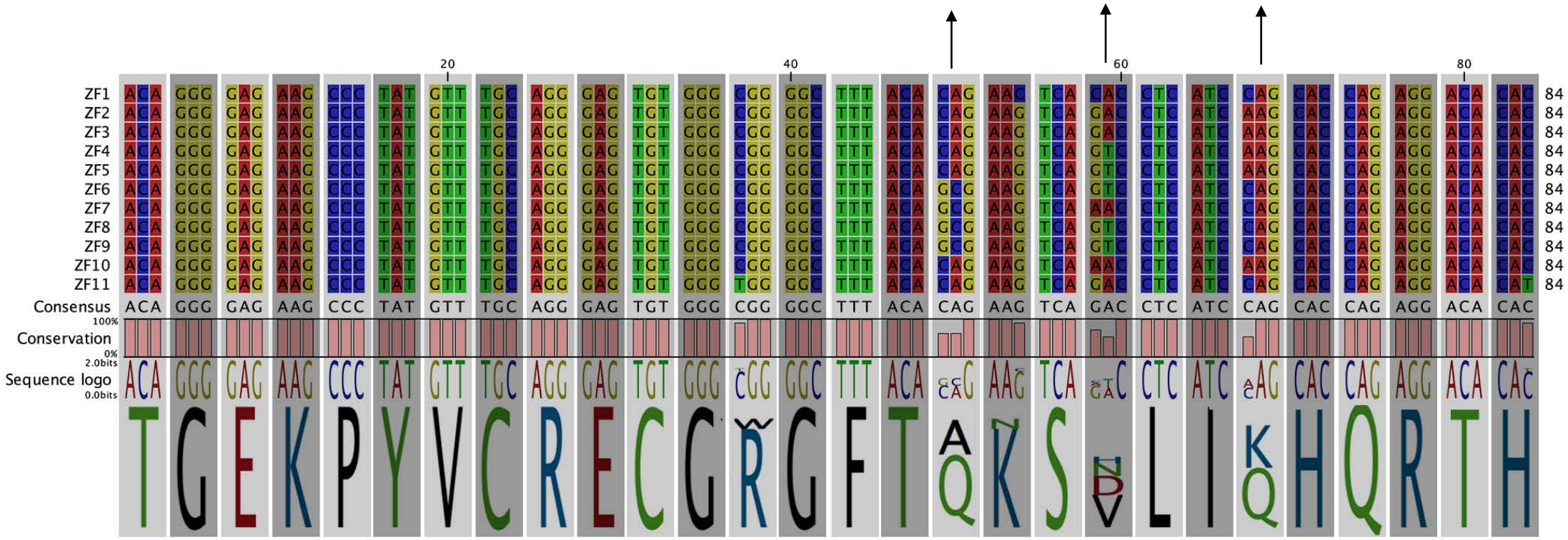




The *PRDM9* gene contains a mini-satellite that encodes its zinc fingers

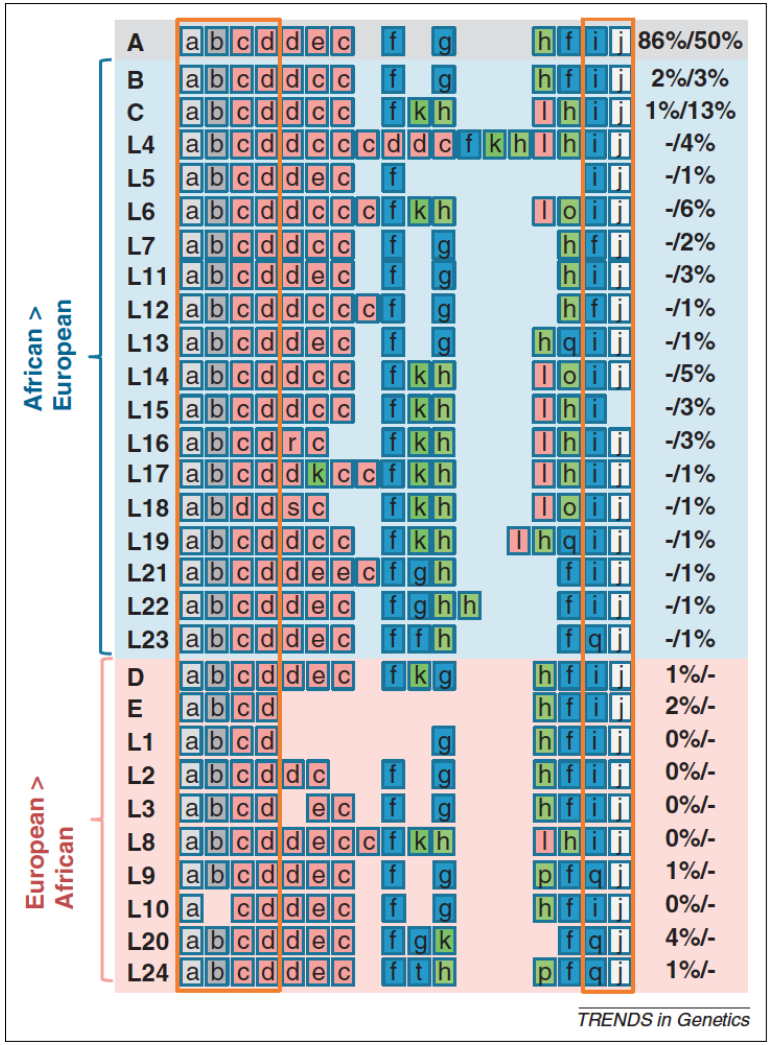


Positions – 1, 3, and 6 Fingerprint amino acids: these control DNA binding



PRDM9 allele frequencies in human populations

European/African

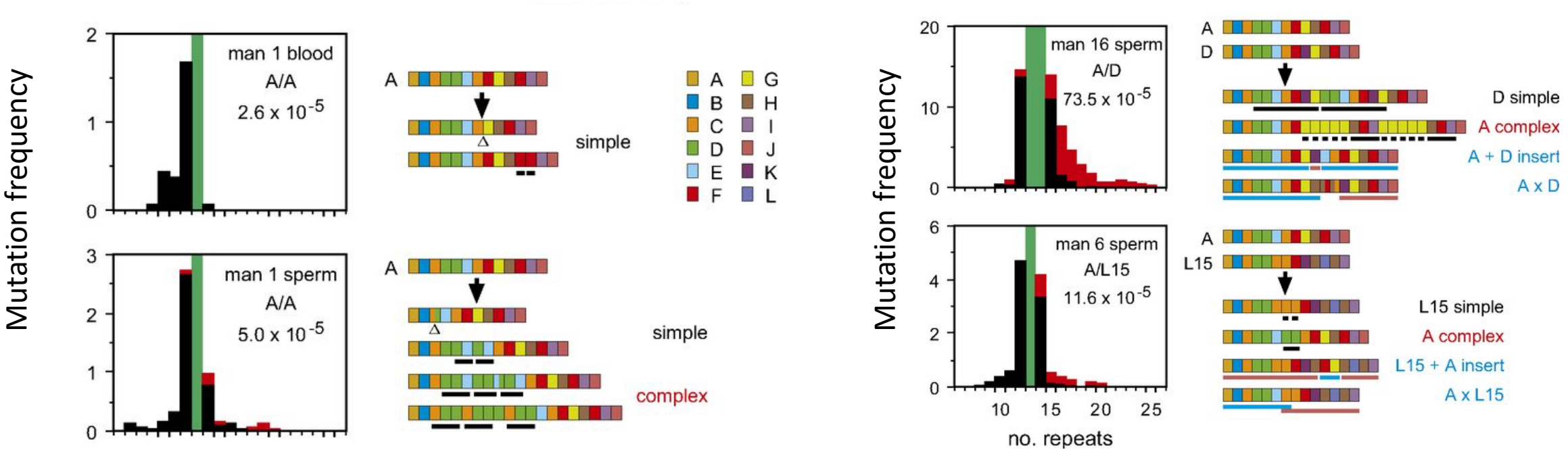


Each *Prdm9* allele will encode a unique protein that will bind to different DNA sequences and therefore specify unique hotspots!

Ponting CP. Trends Genet. 2011;27(5):165-71

New *PRDM9* alleles are produced by recombination of zinc fingers

*Prdm9 requires specialized genotyping because it is a mini-satellite

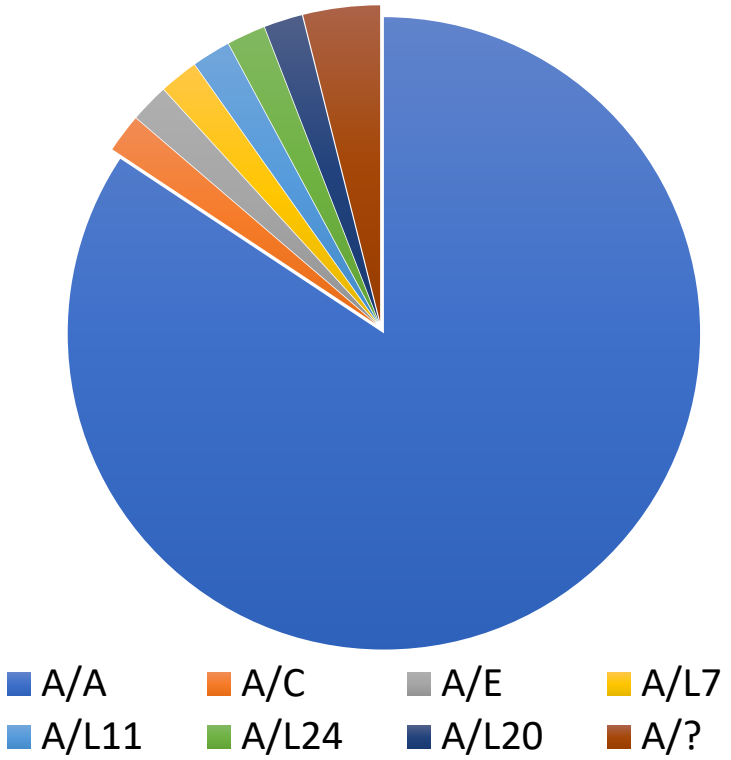


New alleles arise due to simple and complex recombination events

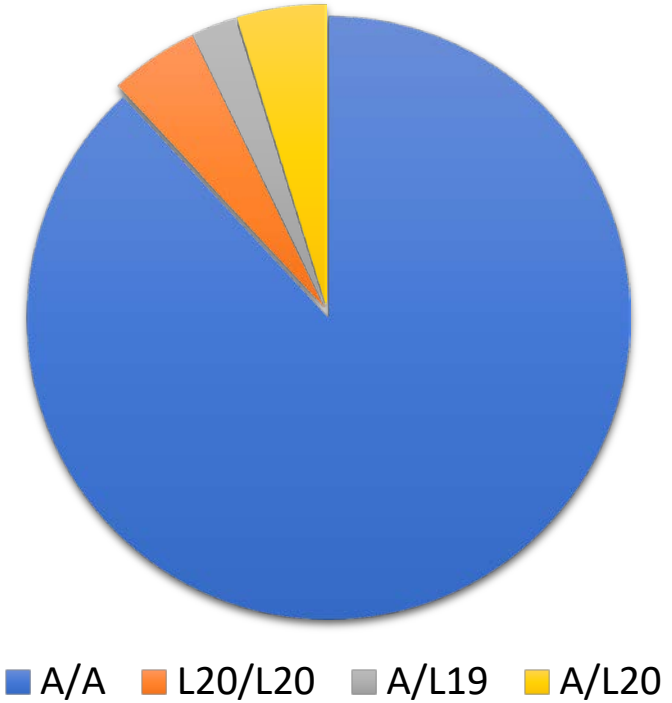
Alec J. Jeffreys et al. PNAS 2013;110:2:600-605

PAC-BIO Genotyping of *PRDM9* identifies two novel PRDM9 alleles in azoospermic males

Azoospermia PRDM9 Genotypes (n=51)



Normospermia PRDM9 Control Genotypes (n=42)

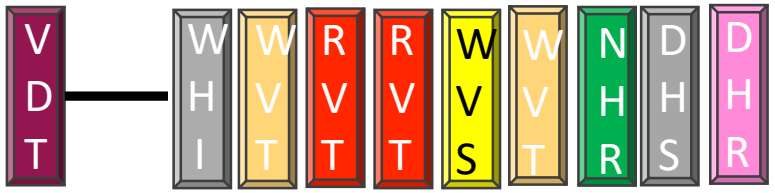


Novel alleles are likely derived variants of rarer alleles

PRDM9 C allele

Novel allele – patient C11

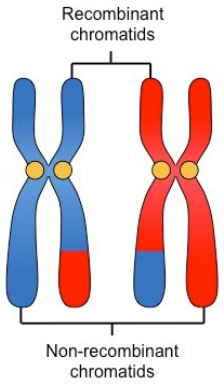
PRDM9 D allele

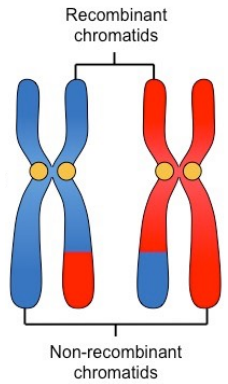


Novel allele – patient A7

Summary and Perspectives

- A small case study identifies two novel *PRDM9* alleles from azoospermic patients
- Are these non- or neo-functional dominant alleles? Are they causative?
- Could removal of such alleles could restore fertility?

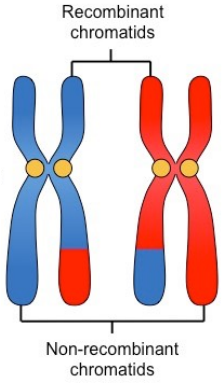


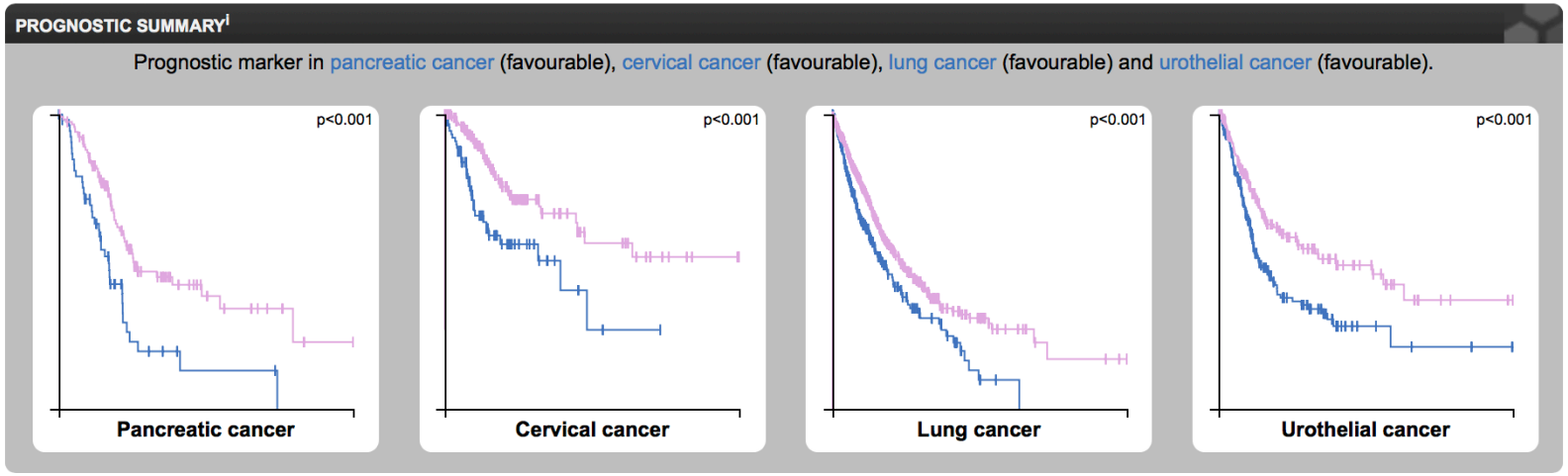


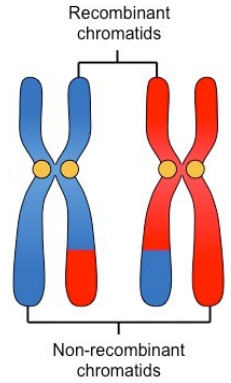
Summary and Perspectives

Could PRDM9/ZCWPW1 contribute to other human diseases/cancer?

- Rare PRDM9 alleles are associated with childhood B-ALL (Hussin et al Genome Research 2013)
- PRDM9 reactivation occurs in ~10% of cancers, GCRs accumulate at PRDM9 binding sites (Houle et al, Geneome Research 2018)
- ZCWPW1 expression correlates with better survival in several cancer types







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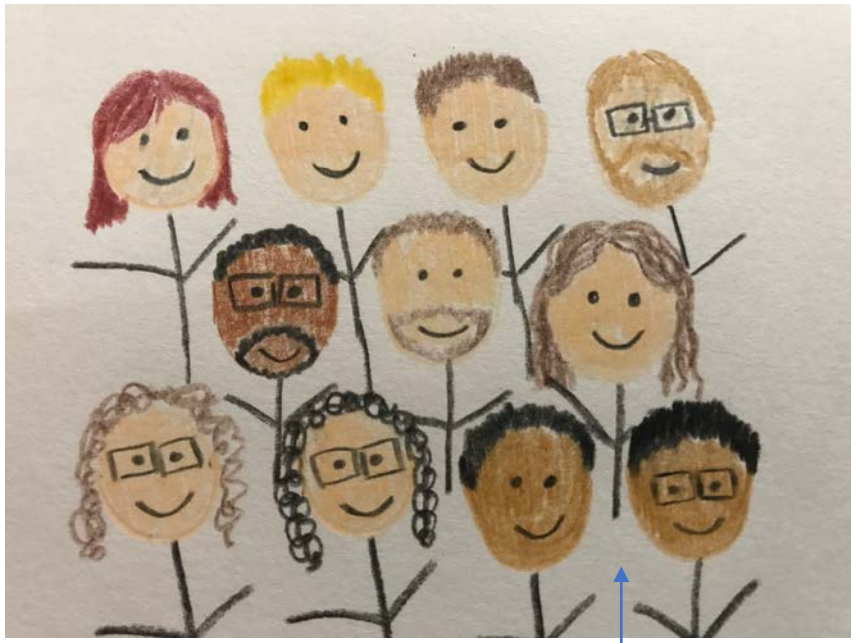

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