

Multiomic data identifies skewed X-inactivation and its role in masking Mendelian inheritance patterns

Long-range phasing of whole-genome sequencing data with marked open chromatin regions identifies haplotype-specific expression of disease-related variants in related individuals with differing levels of skewed X-inactivation

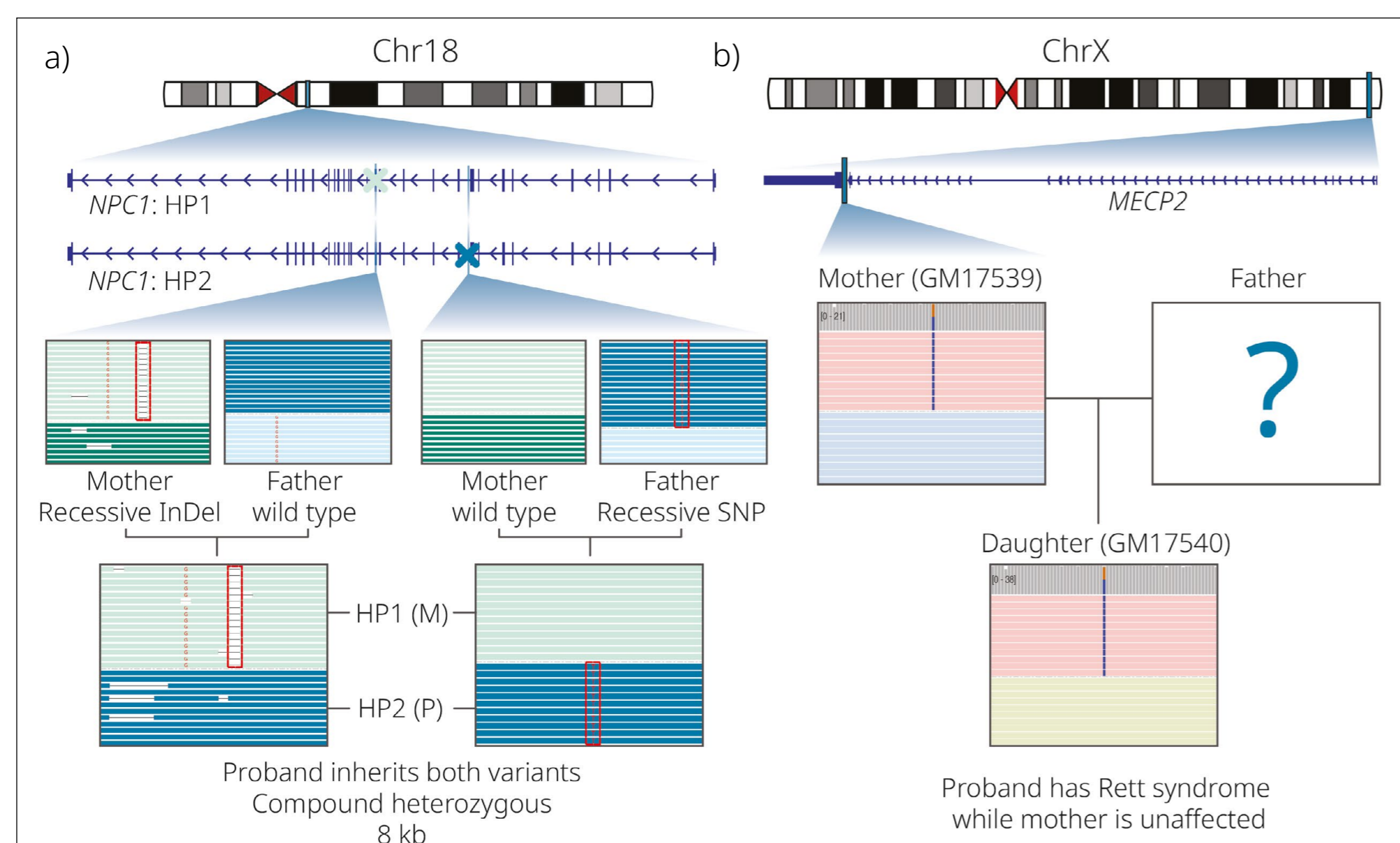


Fig. 1 a) Inherited compound heterozygous mutations b) a causative variant of Rett syndrome.

Local analysis of disease variants does not always explain phenotypic differences in related individuals

Inheritance of rare diseases are often described using traditional Mendelian models. One haplotype is inherited from each parent and one or both may contain a genetic variant that disrupts gene function. For example, inheritance of two autosomal recessive mutations in the *NPC1* gene results in the proband having two non-functional genes due to compound heterozygosity (Fig. 1a). However, in some cases both the parent and offspring can have the same underlying aetiological mutation but have different phenotypes. Here we examine a mother and daughter duo whereby the daughter has Rett syndrome caused by disruption of the *MECP2* gene. The mother is healthy, although they both have the same underlying local genotype (Fig. 1b).

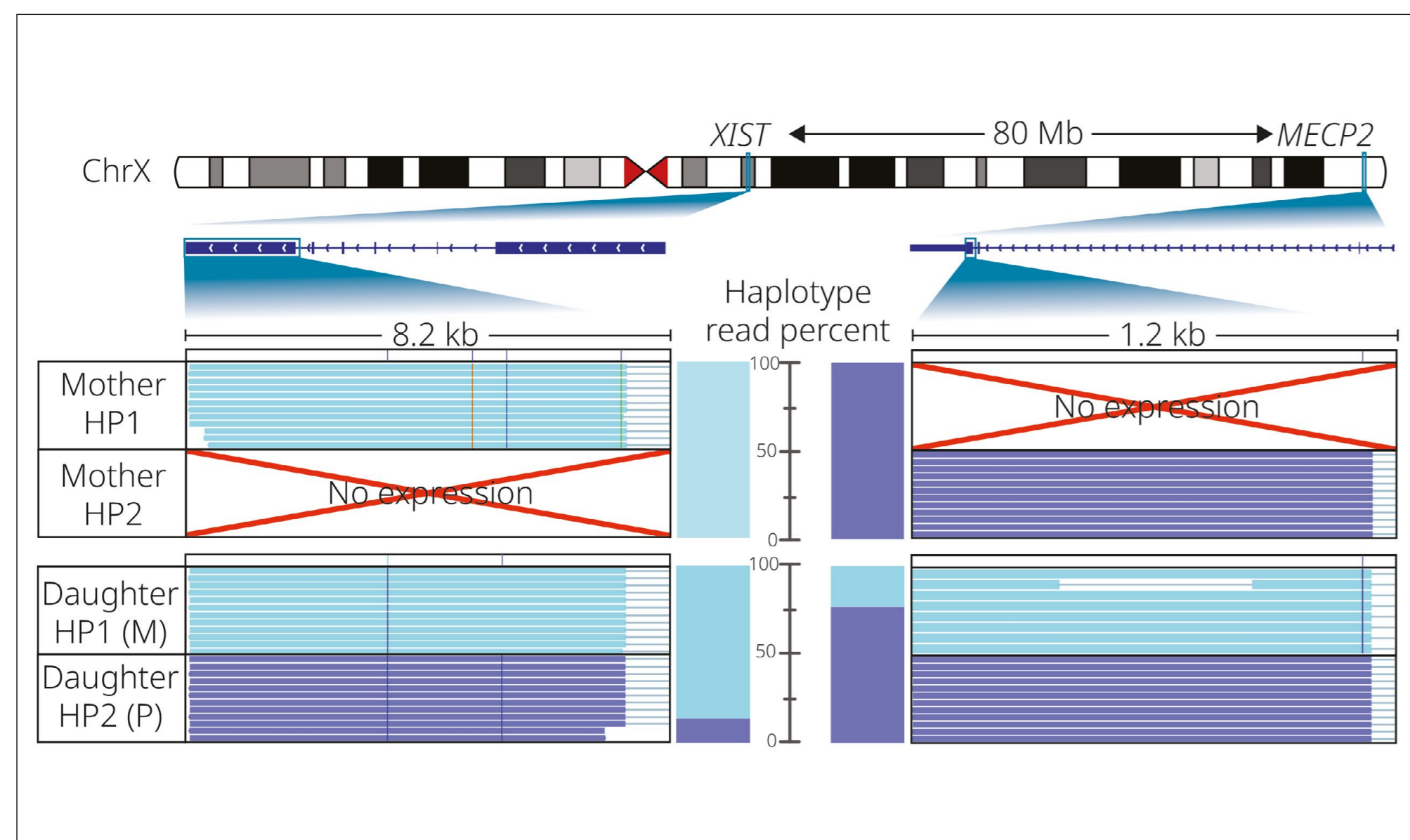


Fig. 3 Haplotype-specific expression of *XIST* and *MECP2* in mother and daughter duo.

Haplotype-specific expression of *XIST* and *MECP2* shows skewed X-inactivation explains phenotypic differences

In an individual with two X chromosomes, the long non-coding RNA *XIST* silences the haplotype from which it is expressed. Haplotype-specific expression of *XIST* in the mother shows that it is only expressed from haplotype 1, suggesting a 100% skewing rate. As the genomic data is phased across the entire chromosome, haplotype-specific expression of the healthy *MECP2* allele is only observed from haplotype 2. In the daughter, both *XIST* and *MECP2* are expressed from both haplotypes, showing the expression of the disease-related variant. Skewing of X-inactivation is still present; however, to a lesser degree.

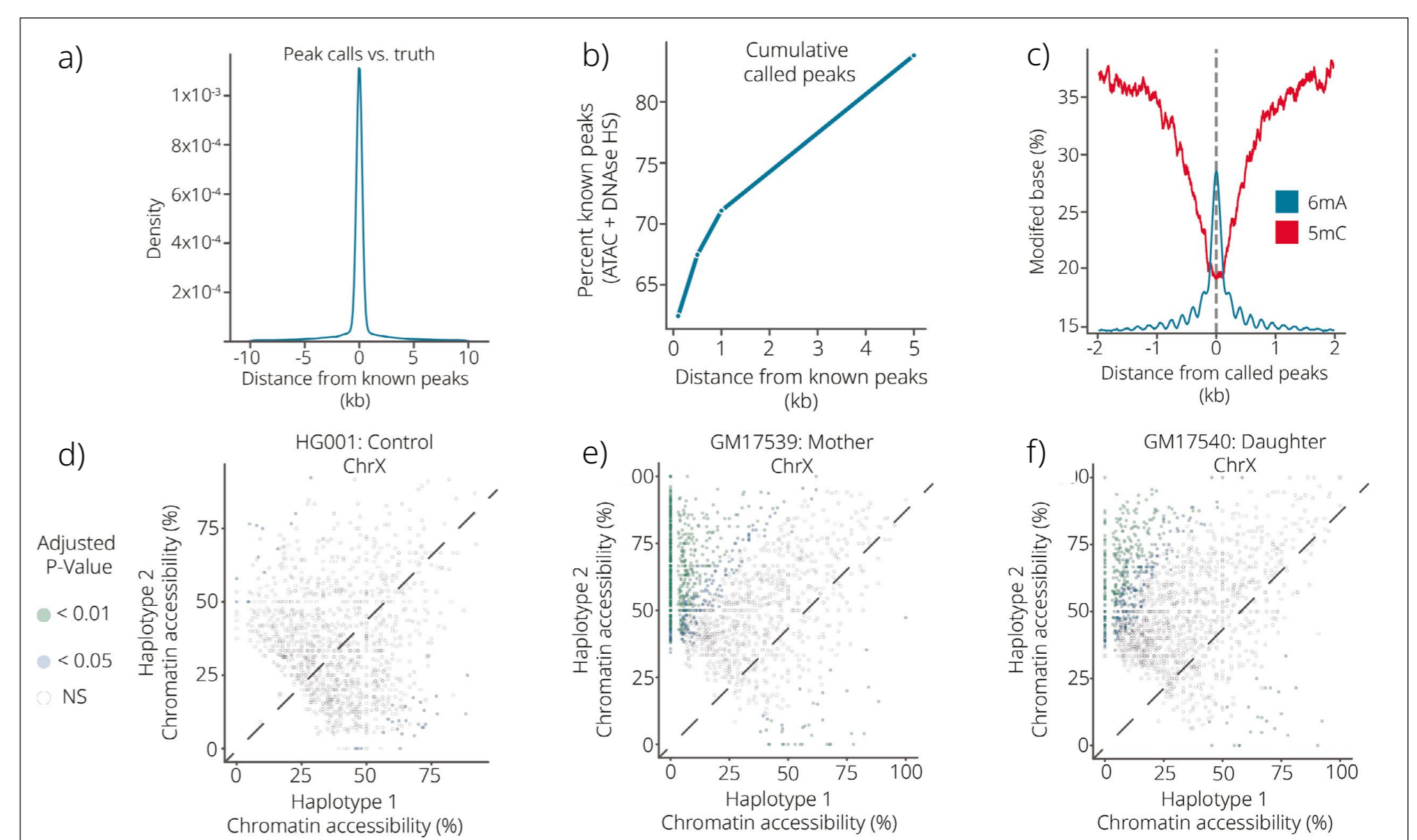


Fig. 2 a-c) Benchmarking open chromatin calling d-e) differential chromatin accessibility.

Long-range phasing of marked open chromatin in whole-genome data identifies skewed X-inactivation

Identification of open chromatin regions provides information about transcriptional activity. Methods such as Fiber-seq¹ achieve this by marking regions of open chromatin with 6mA using a methyltransferase. Areas of *de novo* open chromatin detected in HG001 correlate well with publicly available ATAC-seq peaks and DNase I hypersensitive regions (Fig. 2a-c). Fibertools² was used to identify significantly different regions of chromatin accessibility on Pore-C-phased 6mA reads mapping to the X chromosome. Few regions were differentially accessible in the HG001 control data (Fig. 2d), while many regions were significantly differentially accessible on one haplotype in the mother-daughter duo, suggesting highly skewed X-inactivation (Fig. 2e-f).

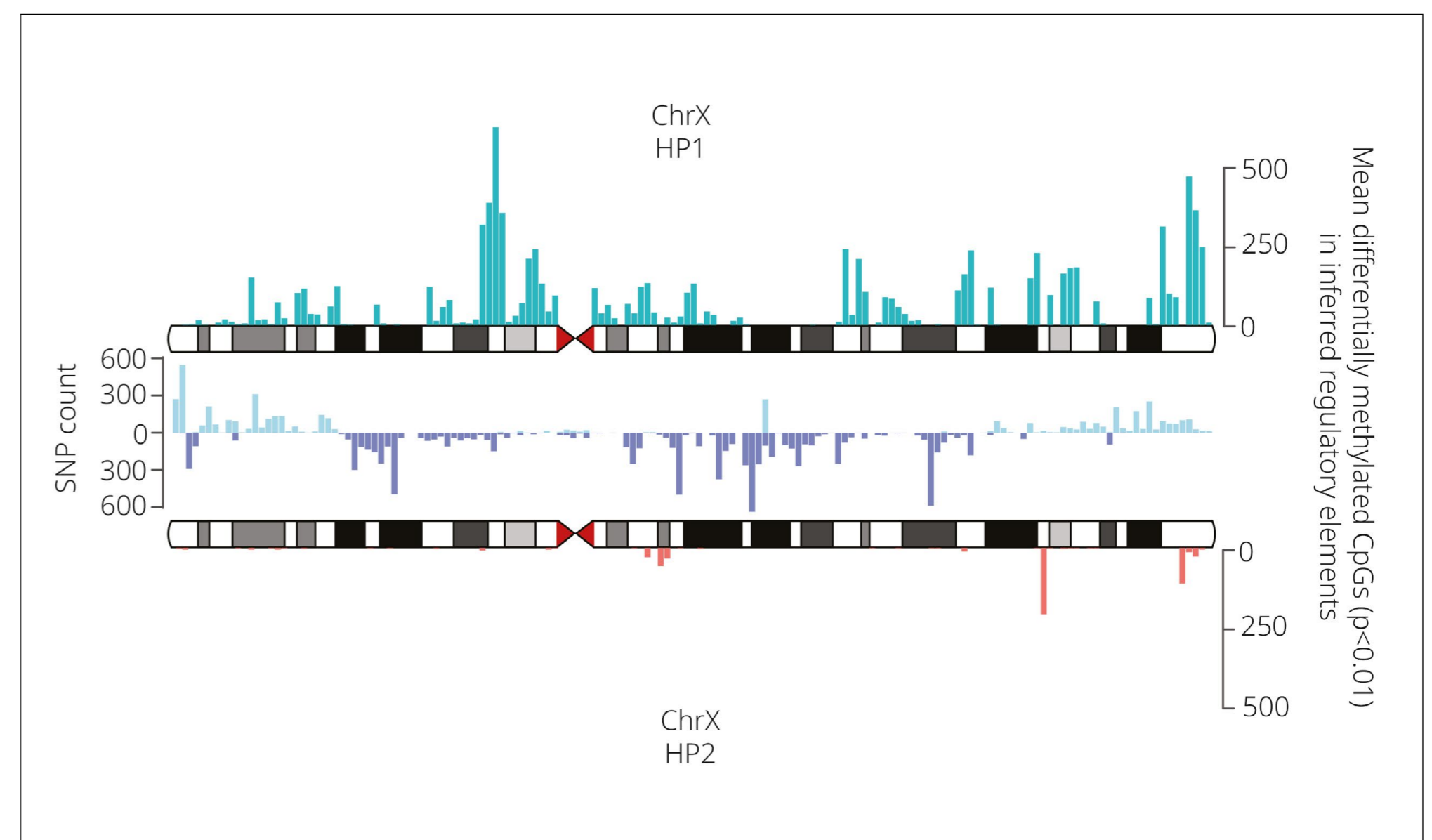


Fig. 4 Inheritance patterns of SNPs and differential methylation calling in open chromatin regions.

SNP inheritance and differential methylation calling suggest recombination affects X-inactivation rates

Homozygous variants in the daughter were matched to heterozygous variants in the mother along the X chromosome to determine which haplotype was inherited at any given location. SNPs identifying haplotype 1 were detected towards the ends of the X chromosome, whereas SNPs from haplotype 2 were detected towards the centre. This suggests recombination between the two X chromosomes in the mother had occurred during meiosis and could explain the differences in X-inactivation skewing rates between mother and daughter. Differential methylation calling was performed at CpG sites within *de novo* open chromatin regions, showing haplotype 1 was predominantly silenced by methylation in the daughter.

¹ Stergachis, A.B. et al. Single-molecule regulatory architectures captured by chromatin fiber sequencing. *Science* 368(6498):1449-1454 (2020). DOI: <https://doi.org/10.1126/science.aaz1646>

² Jha, A. and Bohaczk, S.C. et al. DNA-m6A calling and integrated long-read epigenetic and genetic analysis with fibertools. *Genome Res.* 34(11):1976-1986 (2024). DOI: <https://doi.org/10.1101/gr.279095.124>

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