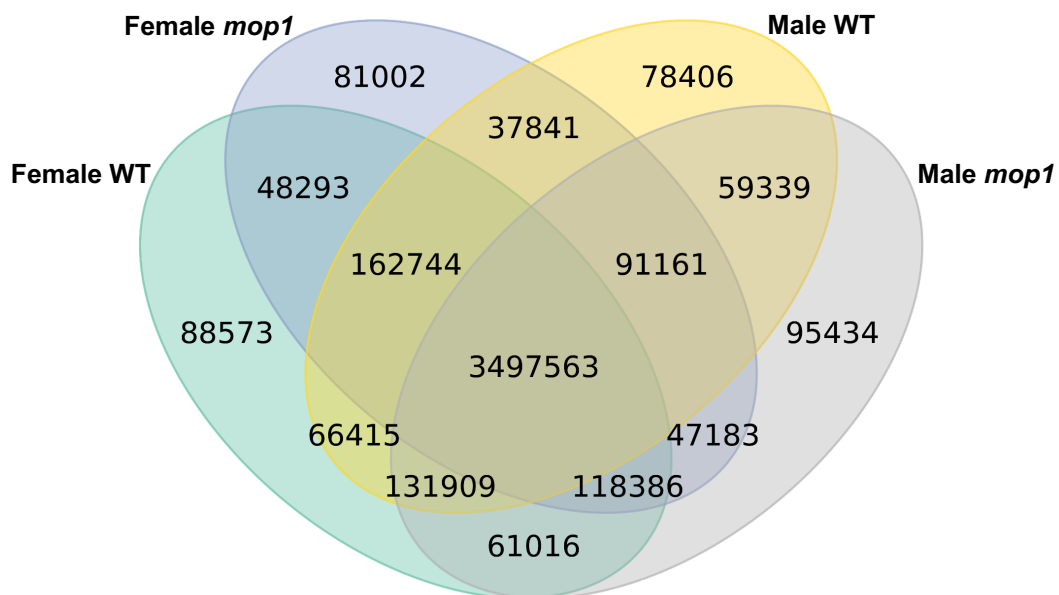


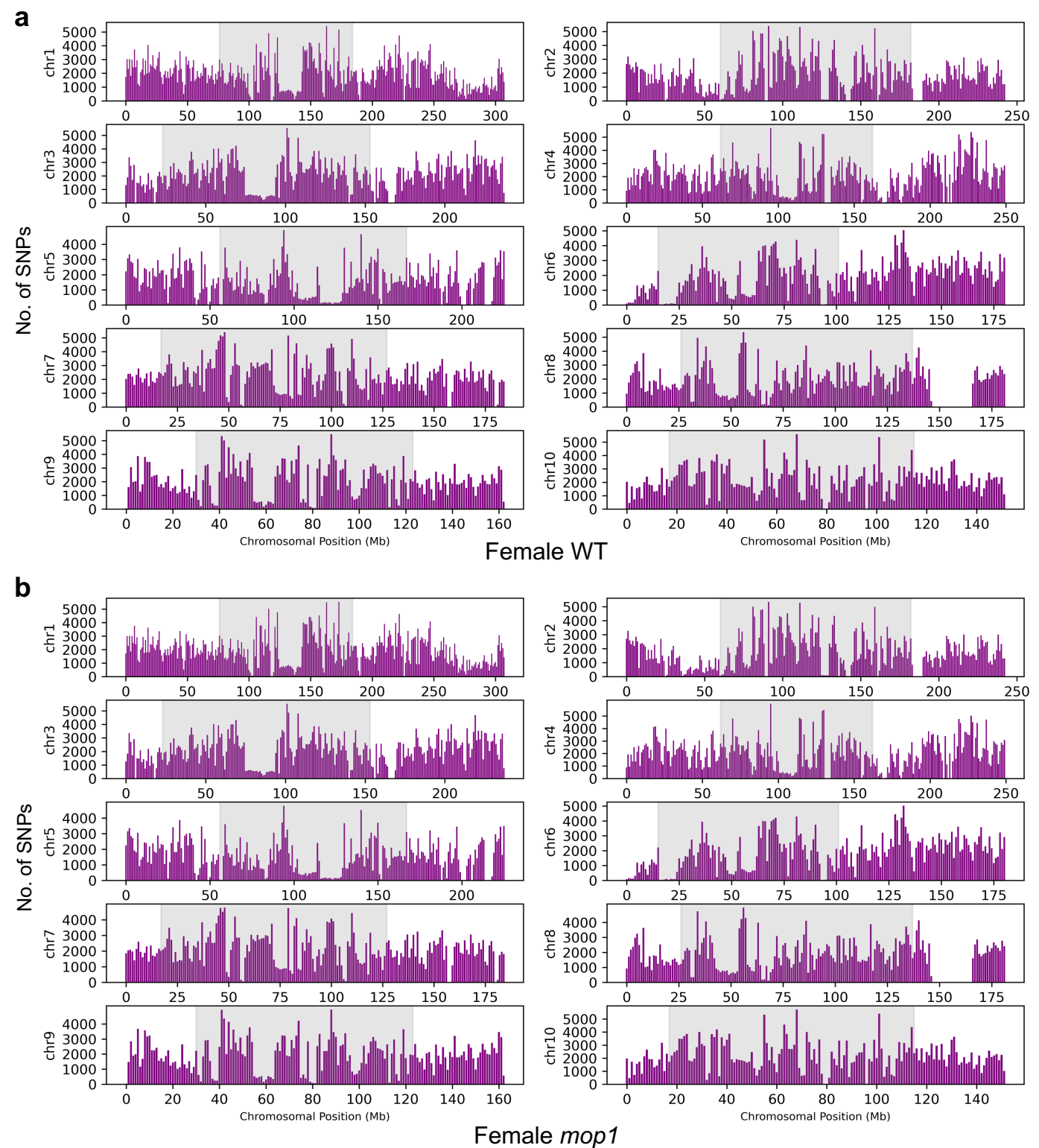
***mop1* affects maize recombination landscapes by modulating methylation of MITEs near genes in open chromatin**

Hasan *et al.*

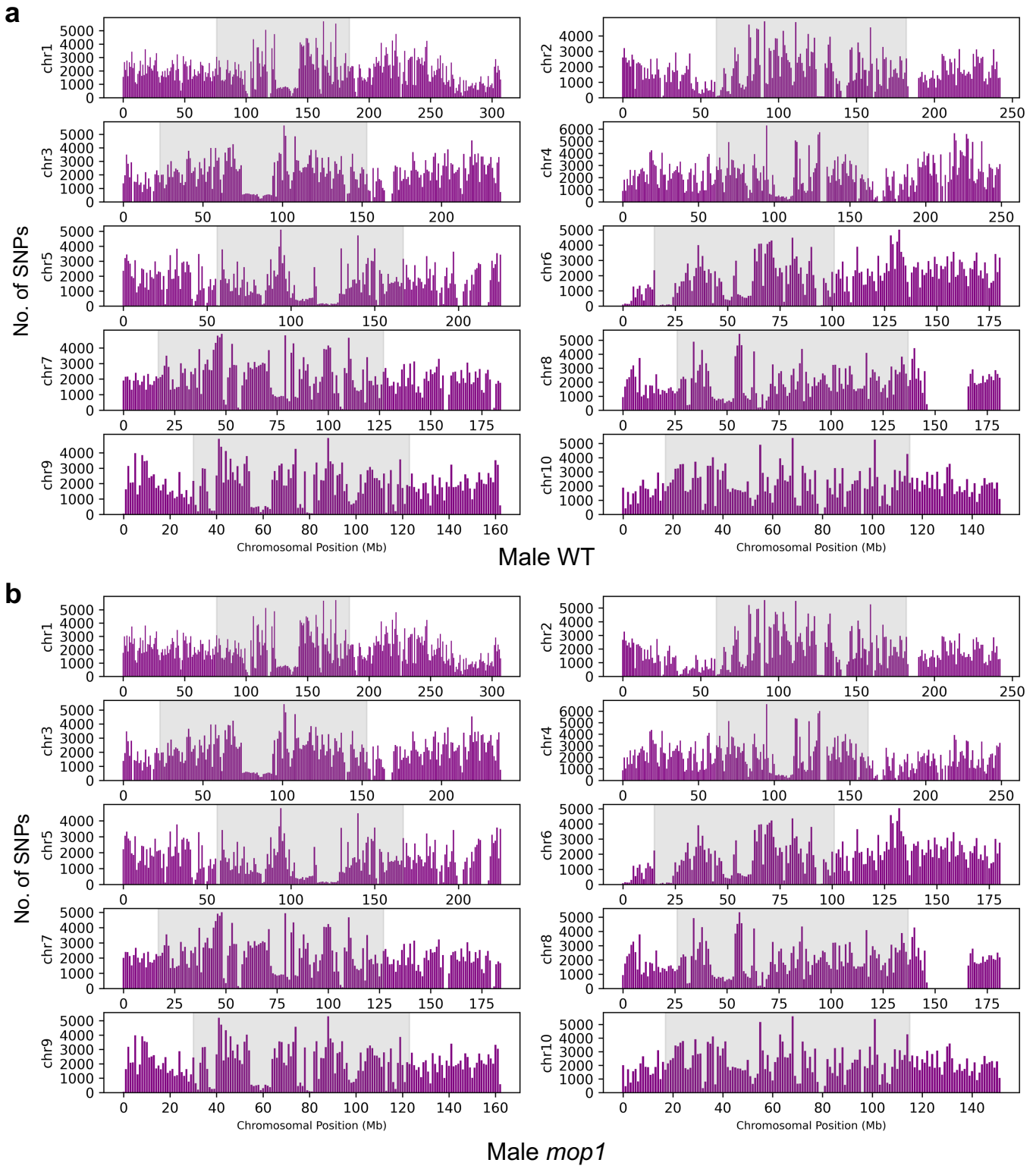


Supplementary Fig. 1. SNPs are predominantly shared across the four BC₁ populations.

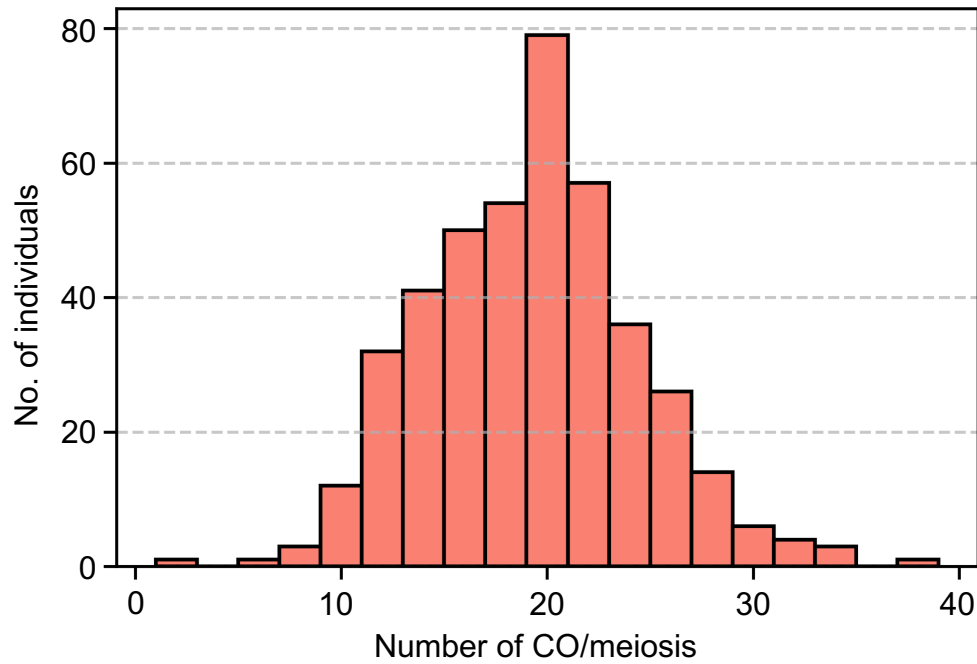
SNPs were identified between B73 and Mo17 in each population.



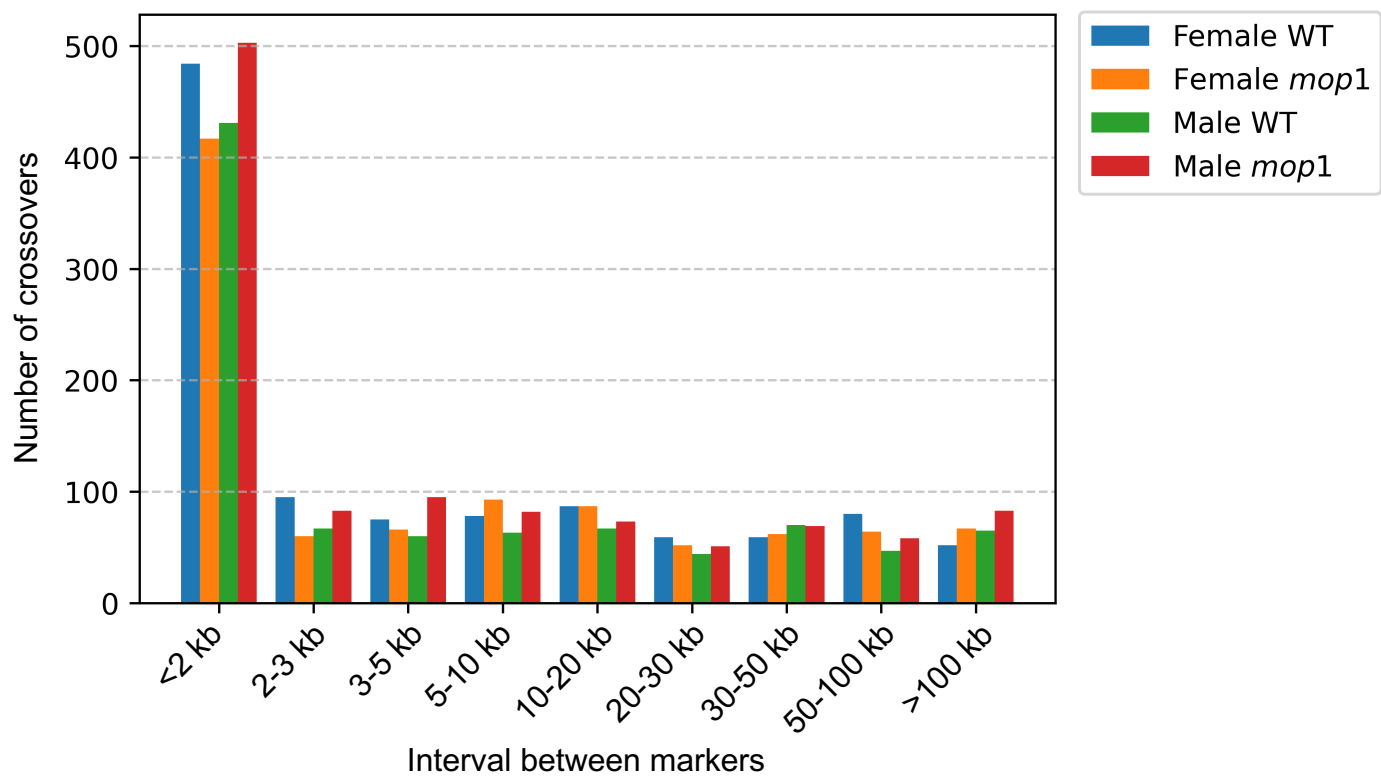
Supplementary Fig. 2. SNPs span most of the genomes in female WT and female *mop1*. **a**, Female WT. **b**, Female *mop1*. The number of SNPs was calculated in 1 Mb non-overlapping windows along each chromosome. Gray-shaded regions indicate pericentromeric regions.



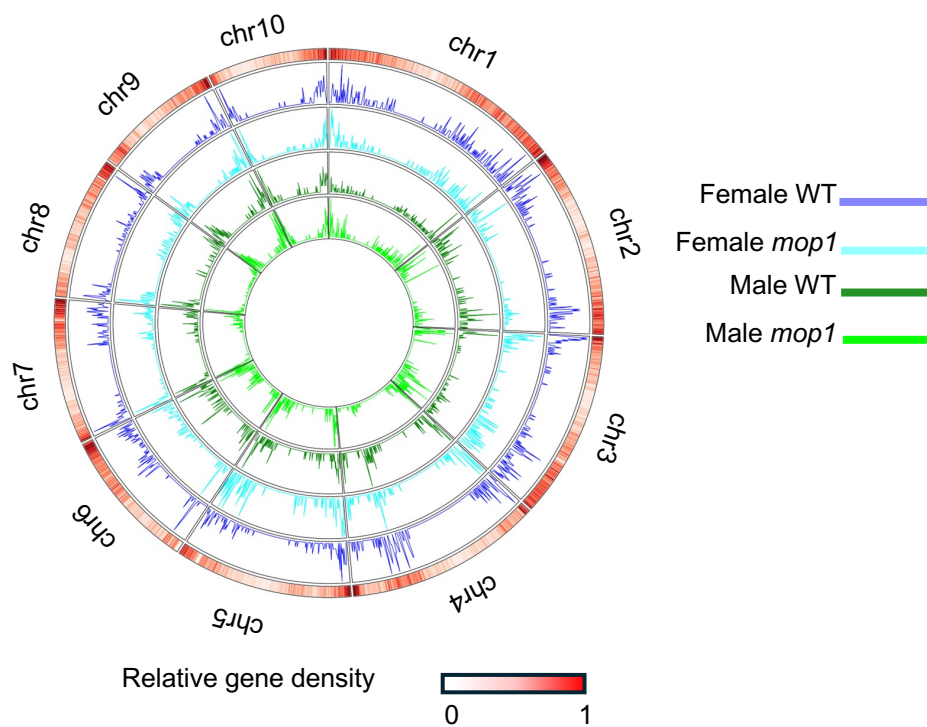
Supplementary Fig. 3. SNPs span most of the genomes in male WT and male *mop1*.
a, Male WT. **b**, Male *mop1*. The number of SNPs was calculated in 1 Mb non-overlapping windows along each chromosome. Gray-shaded regions indicate pericentromeric regions.



Supplementary Fig. 4. Distribution of crossover numbers per individual. The y-axis indicates the number of individuals, and the x-axis represents the number of crossovers detected.

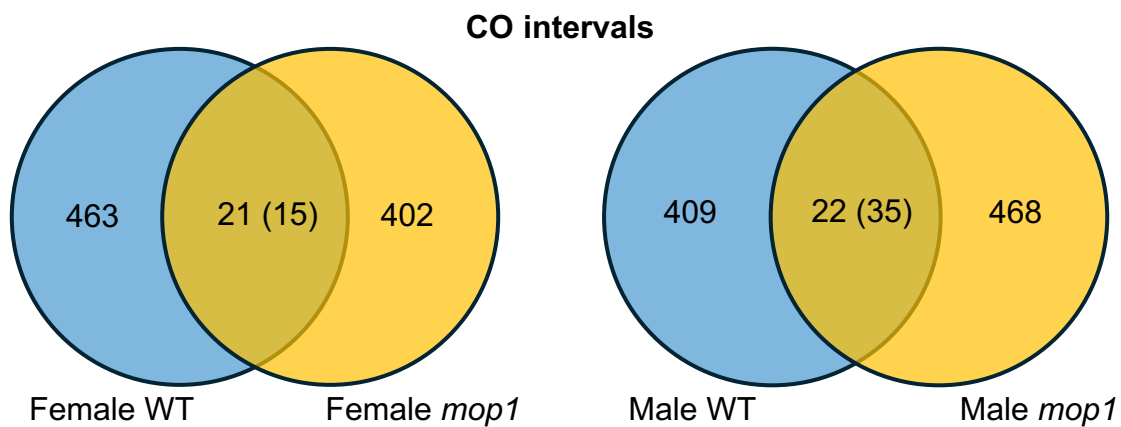


Supplementary Fig. 5. Frequency of crossovers detected across different intervals.

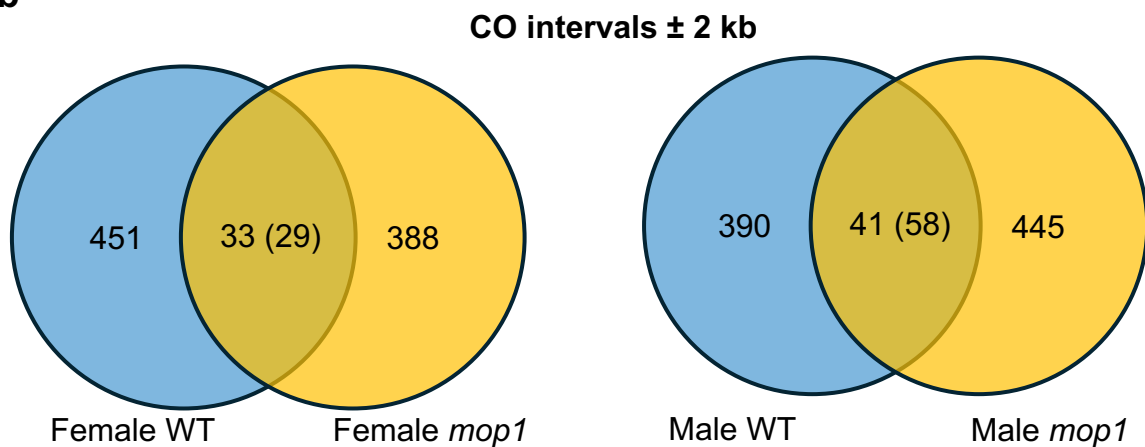


Supplementary Fig. 6. Distribution of crossovers and relative gene density across the 10 chromosomes of maize. Source data are provided as a Source Data file.

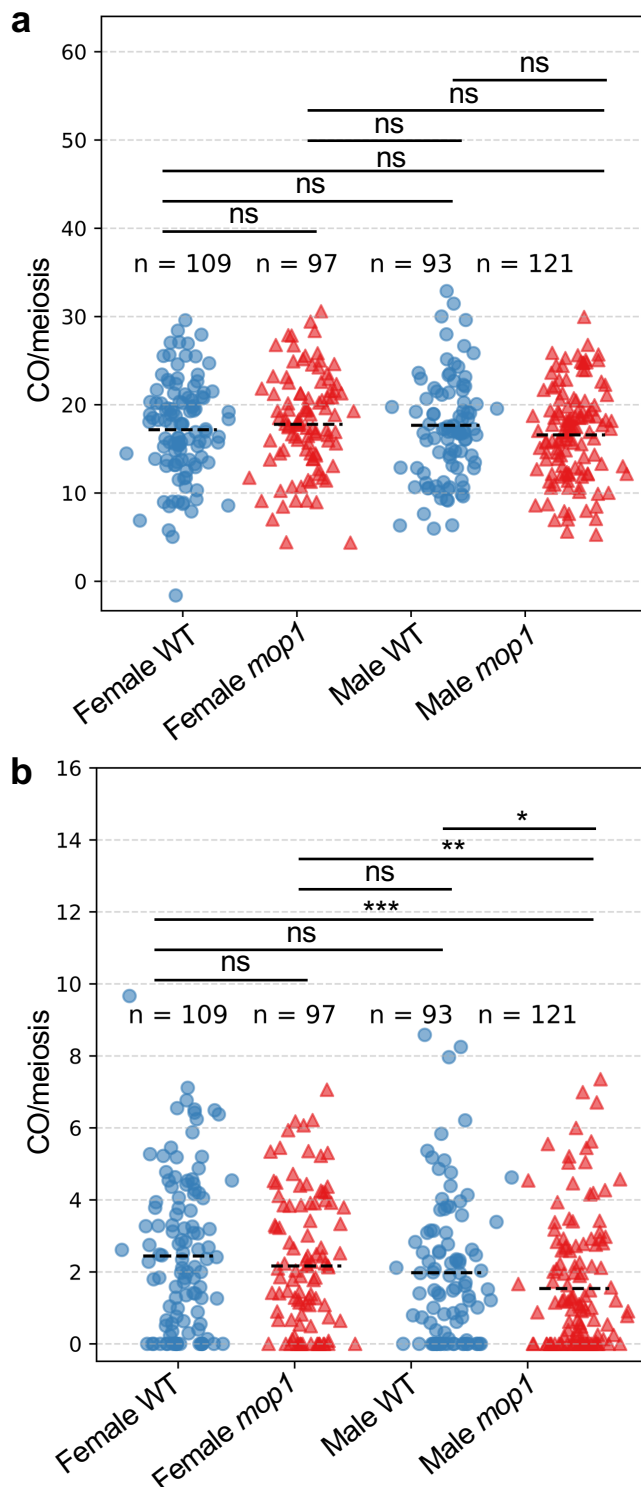
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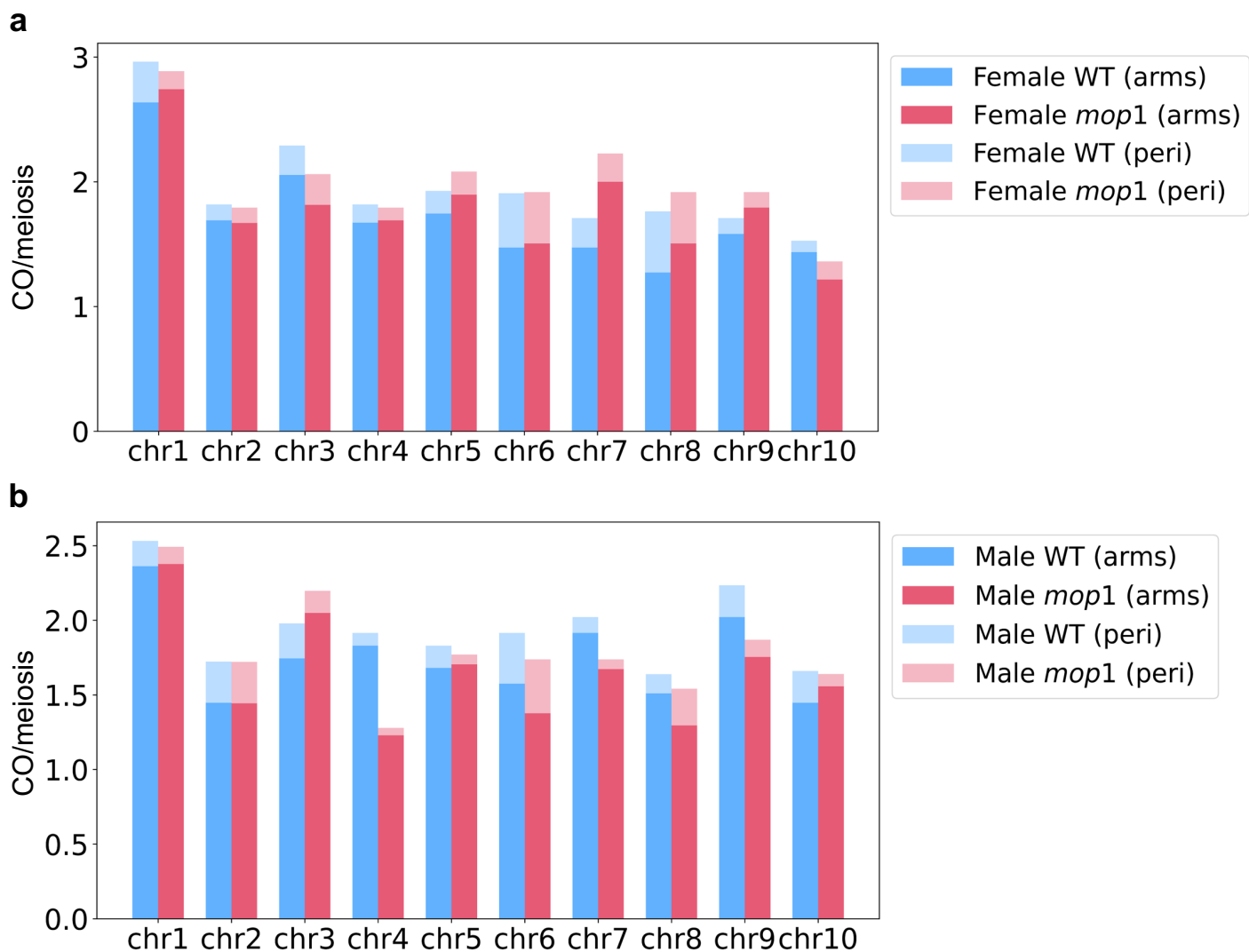
b



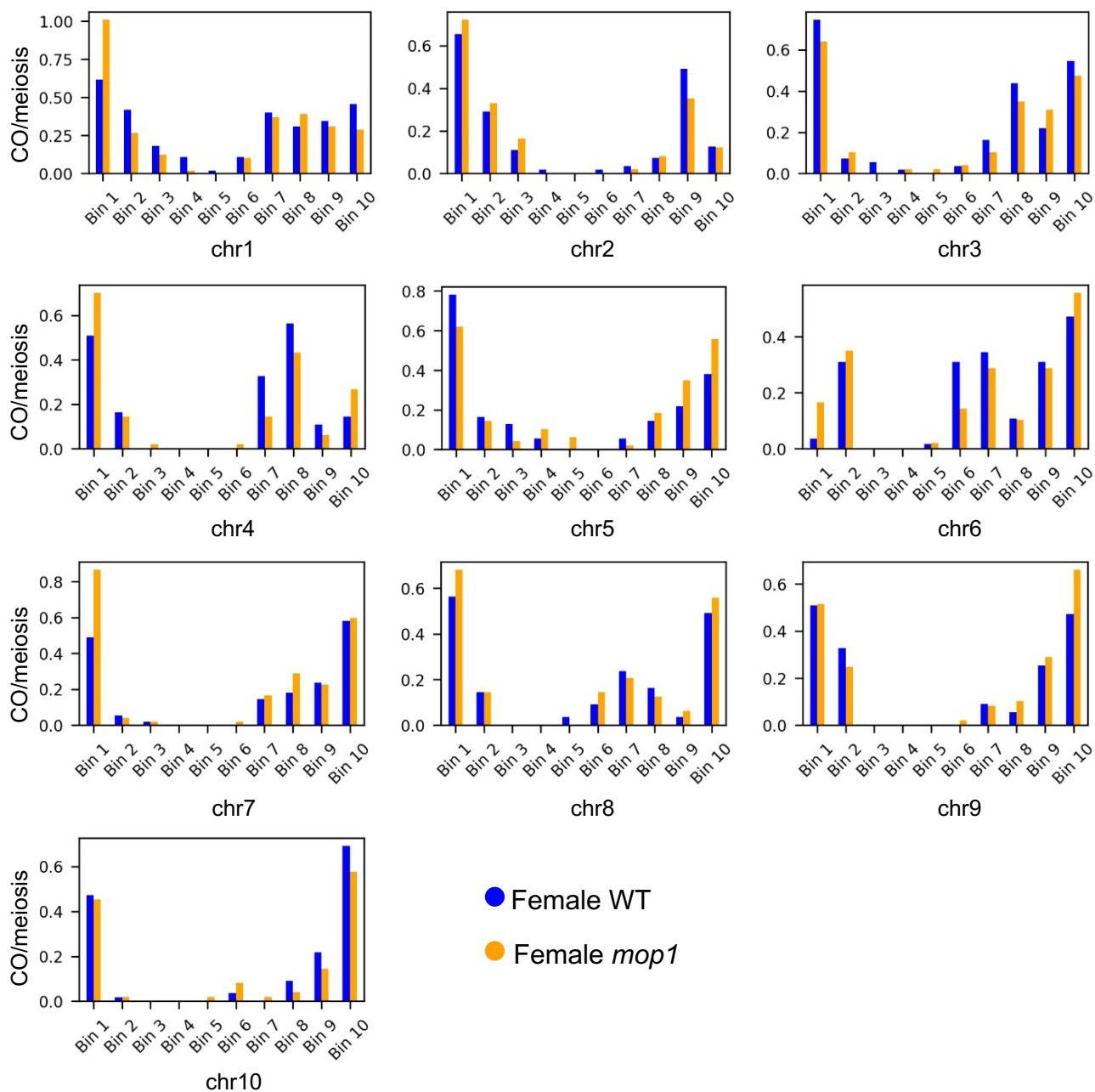
Supplementary Fig. 7. Overlap of crossover (CO) intervals between WT and *mop1*. **a**, CO intervals. **b**, CO intervals and their flanking 2 kb regions.



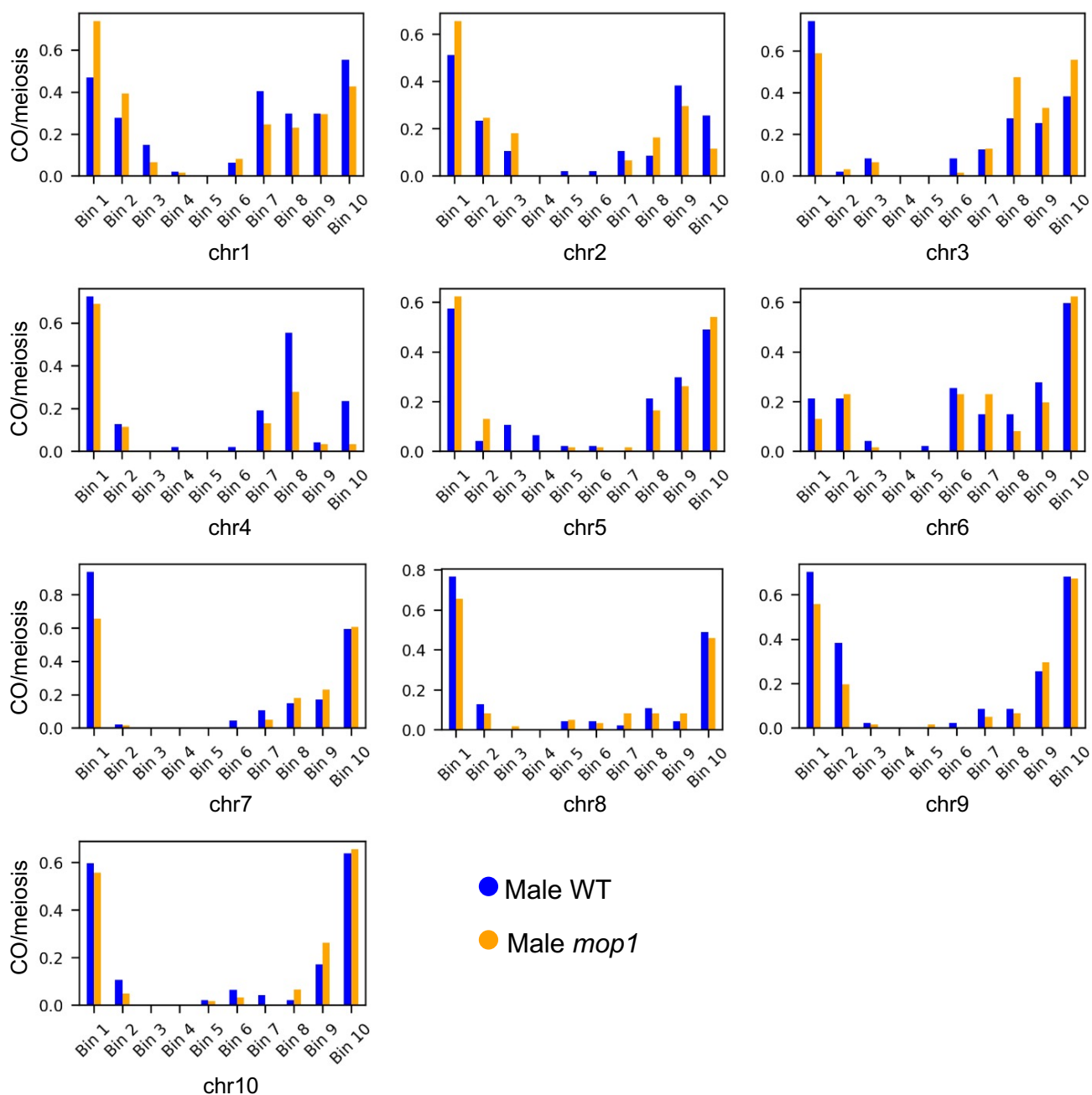
Supplementary Fig. 8. Average crossovers (COs) per meiosis in chromosomal arms and pericentromeric regions across four populations. a, Chromosomal arms. **b**, Pericentromeric regions. Individual points represent data values, and black dashed lines indicate the mean. Statistical significance was assessed using a two-tailed Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction. *, $P < 0.05$; ***, $P < 0.001$; ns, not significant. Source data are provided as a Source Data file.



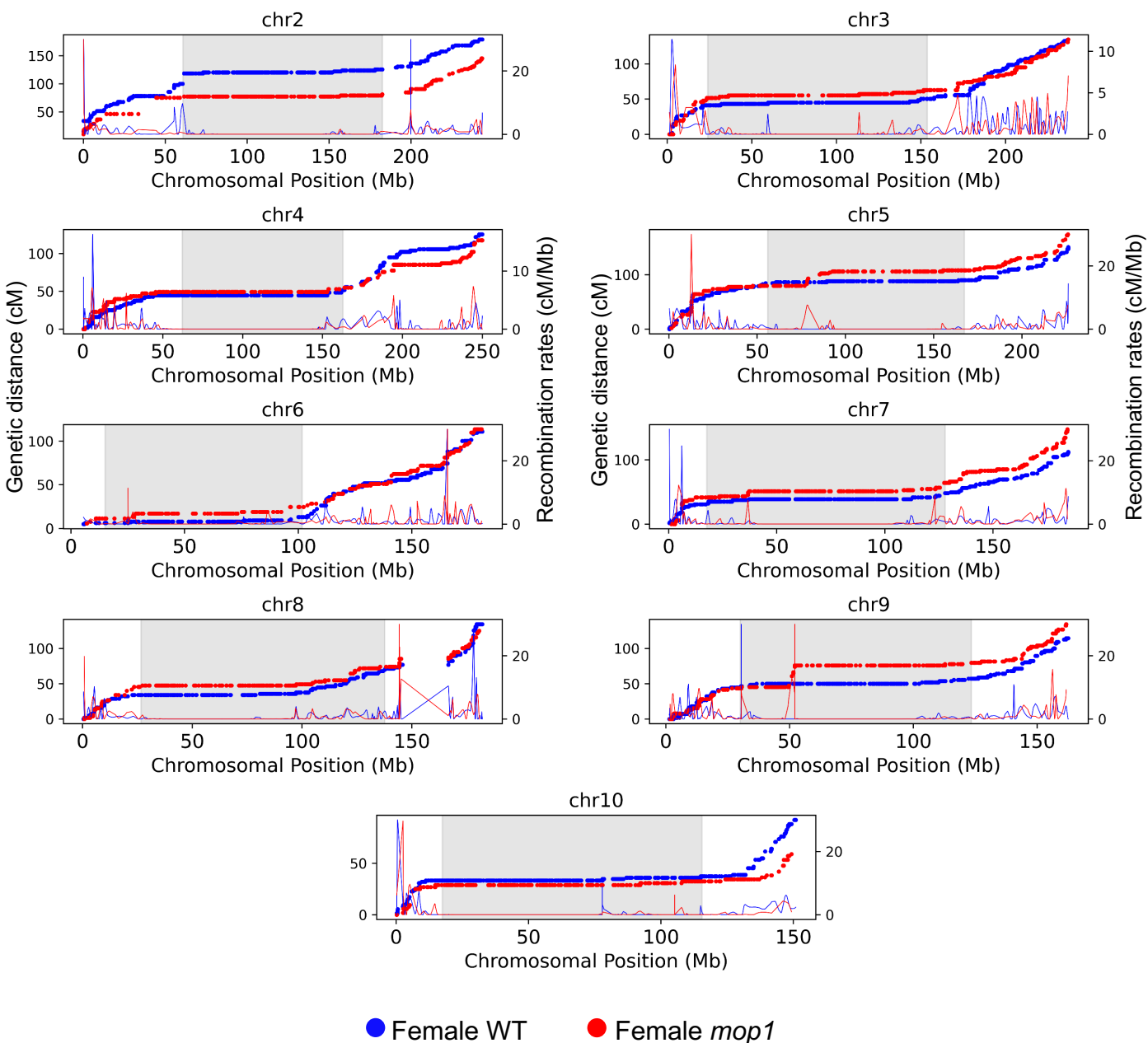
Supplementary Fig. 9. Crossover (CO) rates increase in chromosomal arms and decrease in pericentromeric regions in *mop1* mutants for certain maize chromosomes, with more pronounced effects in females. a, Females. b, Males. Arms, chromosomal arms; Peri, pericentromeric regions. Source data are provided as a Source Data file.



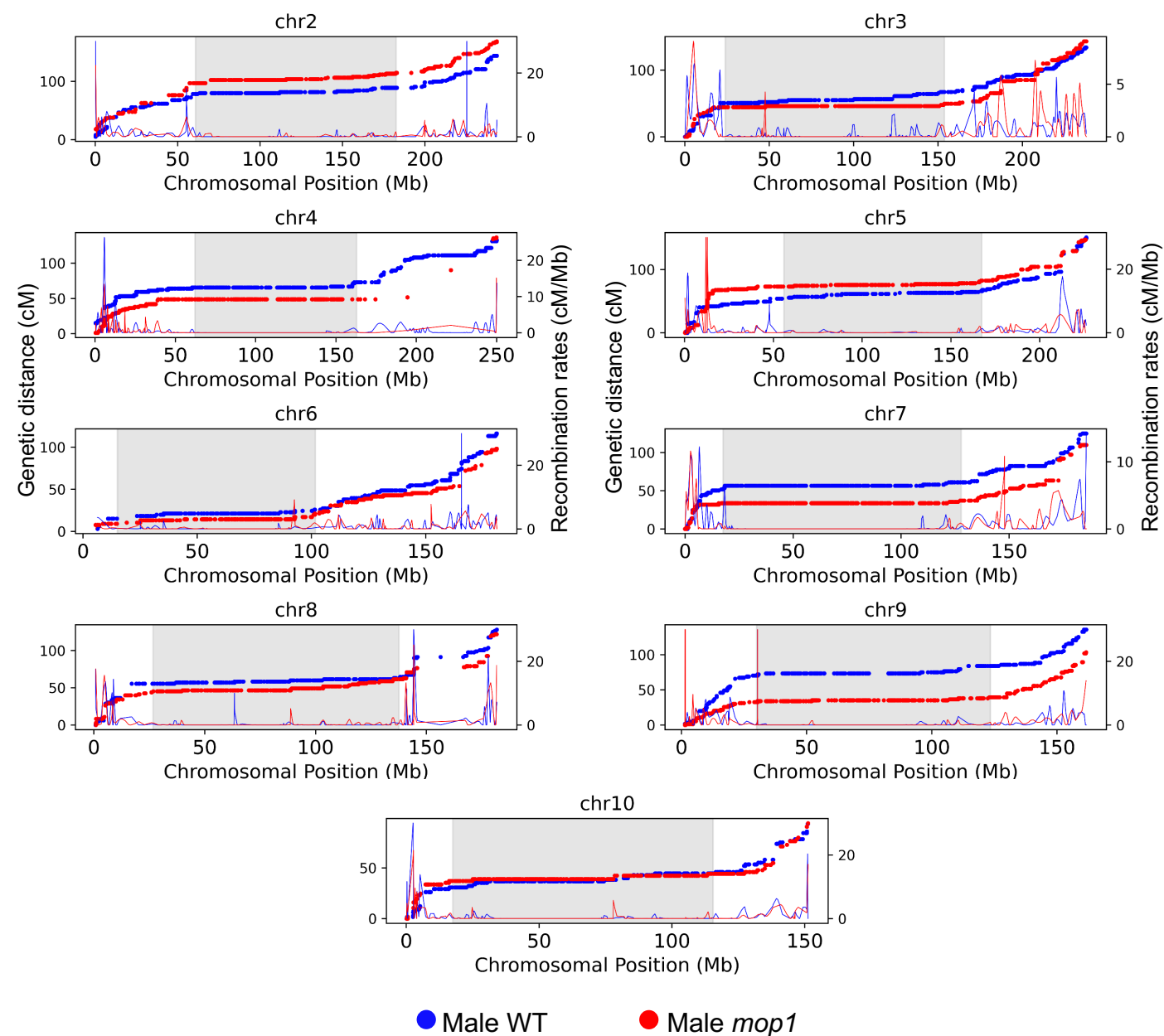
Supplementary Fig. 10. Increased crossovers (COs) are observed at the ends of specific chromosomes in female *mop1* mutants compared with female WT. Source data are provided as a Source Data file.



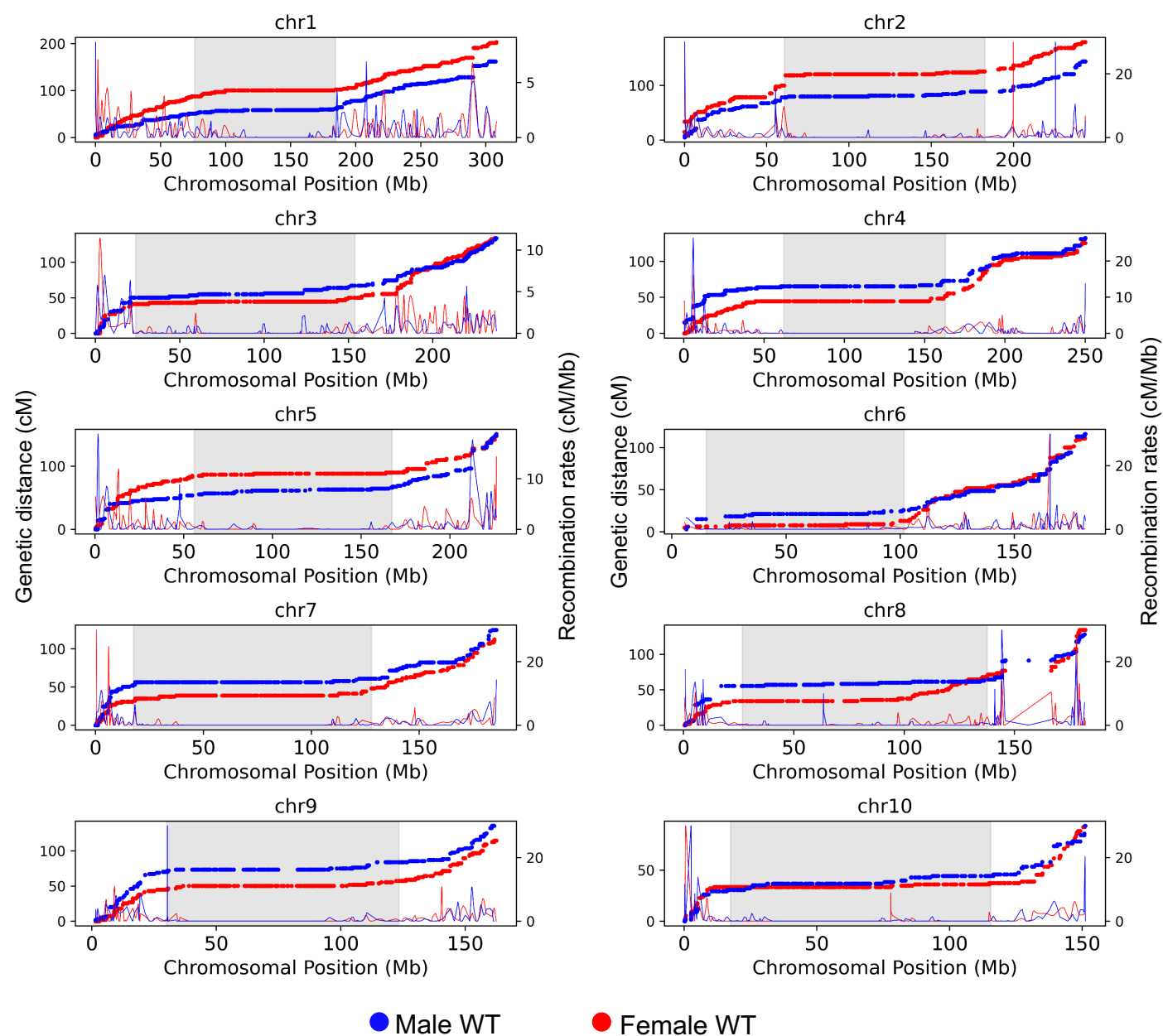
Supplementary Fig. 11. Increased crossovers (COs) are observed at the ends of specific chromosomes in male *mop1* mutants compared with male WT. Source data are provided as a Source Data file.



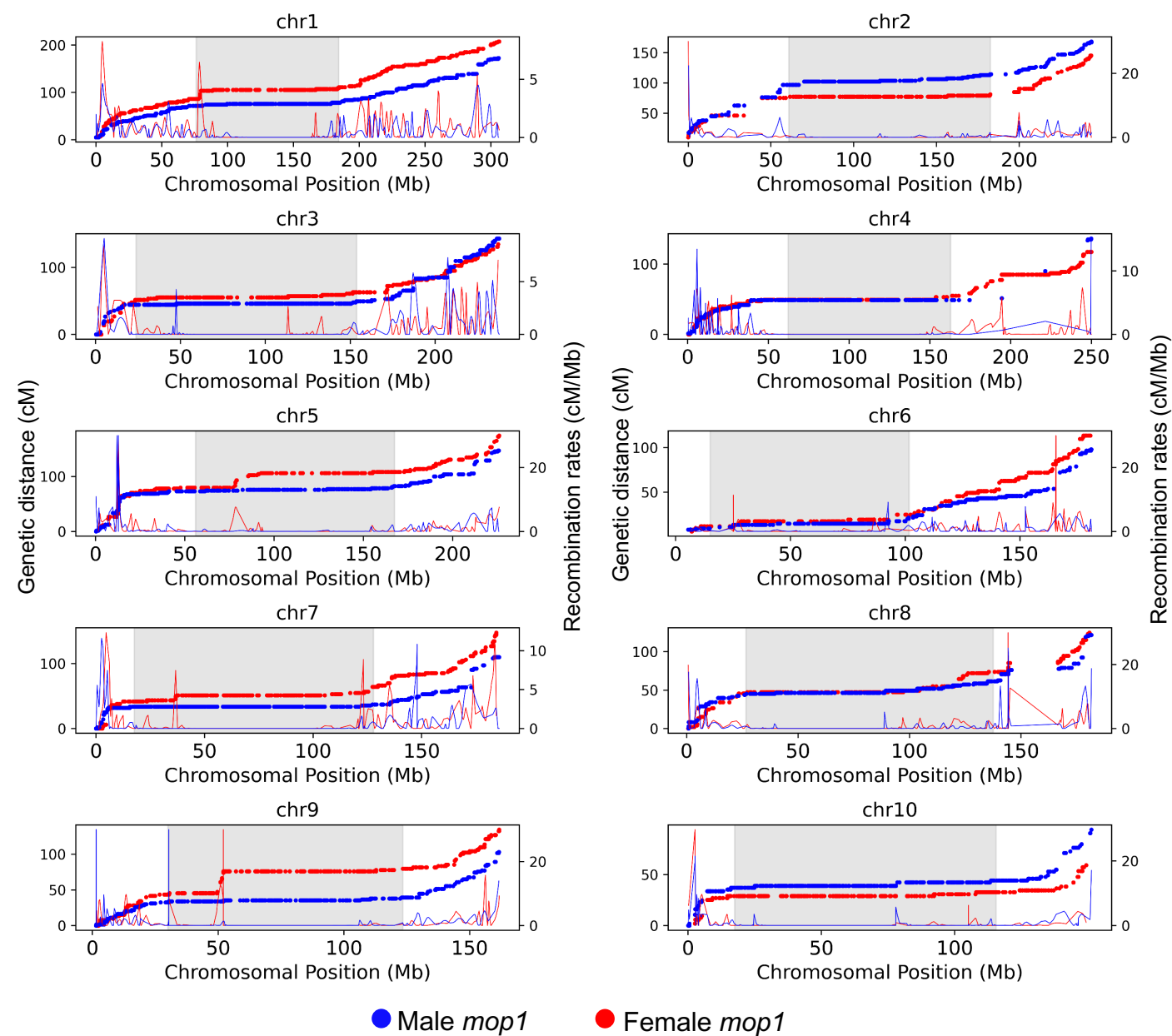
Supplementary Fig. 12. Cumulative genetic distances and recombination rates between female WT and female *mop1* across maize chromosomes. Gray-shaded areas represent pericentromeric regions. Source data are provided as a Source Data file.



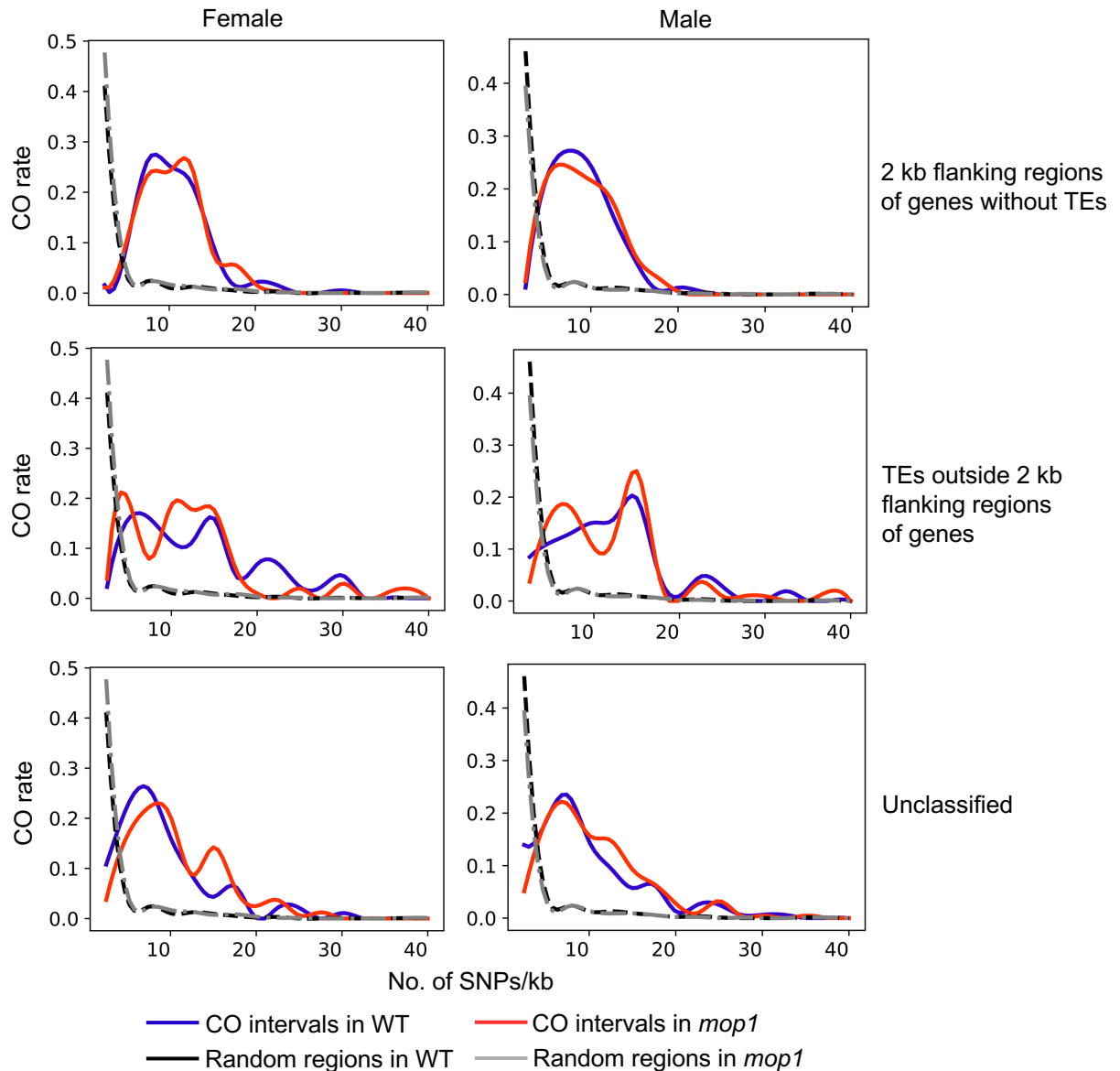
Supplementary Fig. 13. Cumulative genetic distances and recombination rates between male WT and male *mop1* across maize chromosomes. Gray-shaded areas represent pericentromeric regions. Source data are provided as a Source Data file.



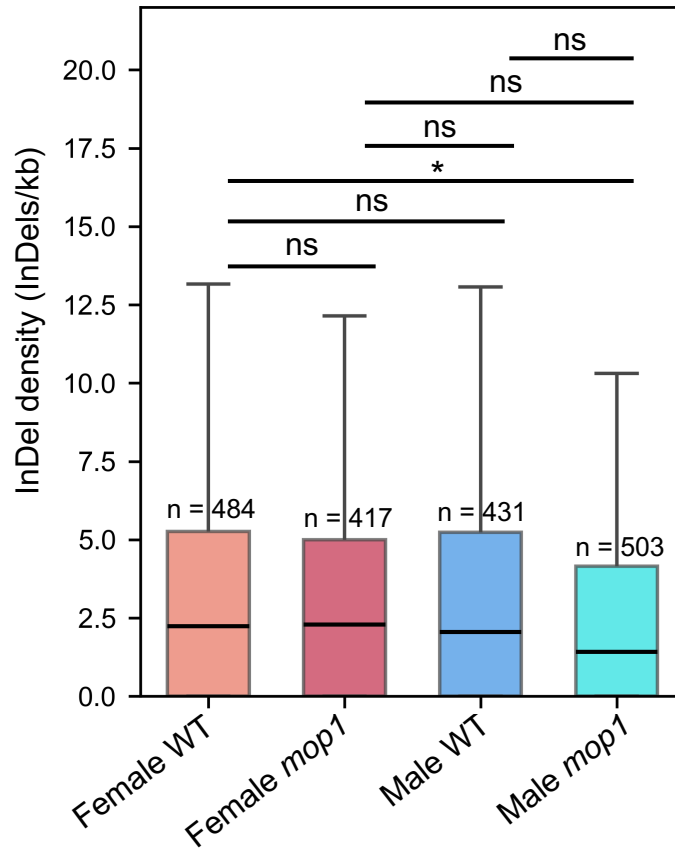
Supplementary Fig. 14. Cumulative genetic distances and recombination rates between male WT and female WT across maize chromosomes. Gray-shaded areas represent pericentromeric regions. Source data are provided as a Source Data file.



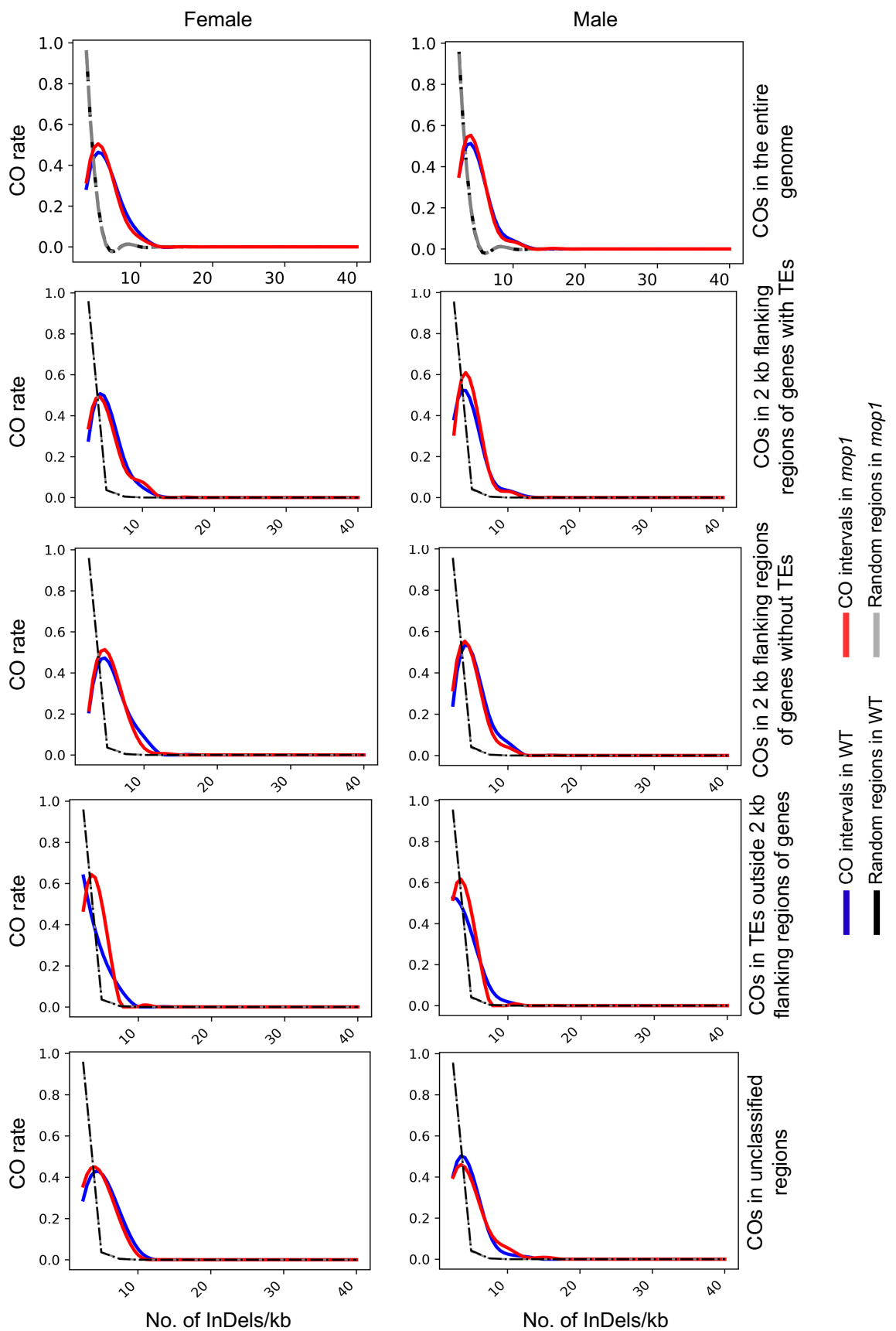
Supplementary Fig. 15. Cumulative genetic distances and recombination rates between male *mop1* and female *mop1* across maize chromosomes. Gray-shaded areas represent pericentromeric regions. Source data are provided as a Source Data file.



Supplementary Fig. 16. Relationship between crossover (CO) rates and single nucleotide polymorphism (SNP) diversity in female and male meiosis across different genomic contexts. Source data are provided as a Source Data file.

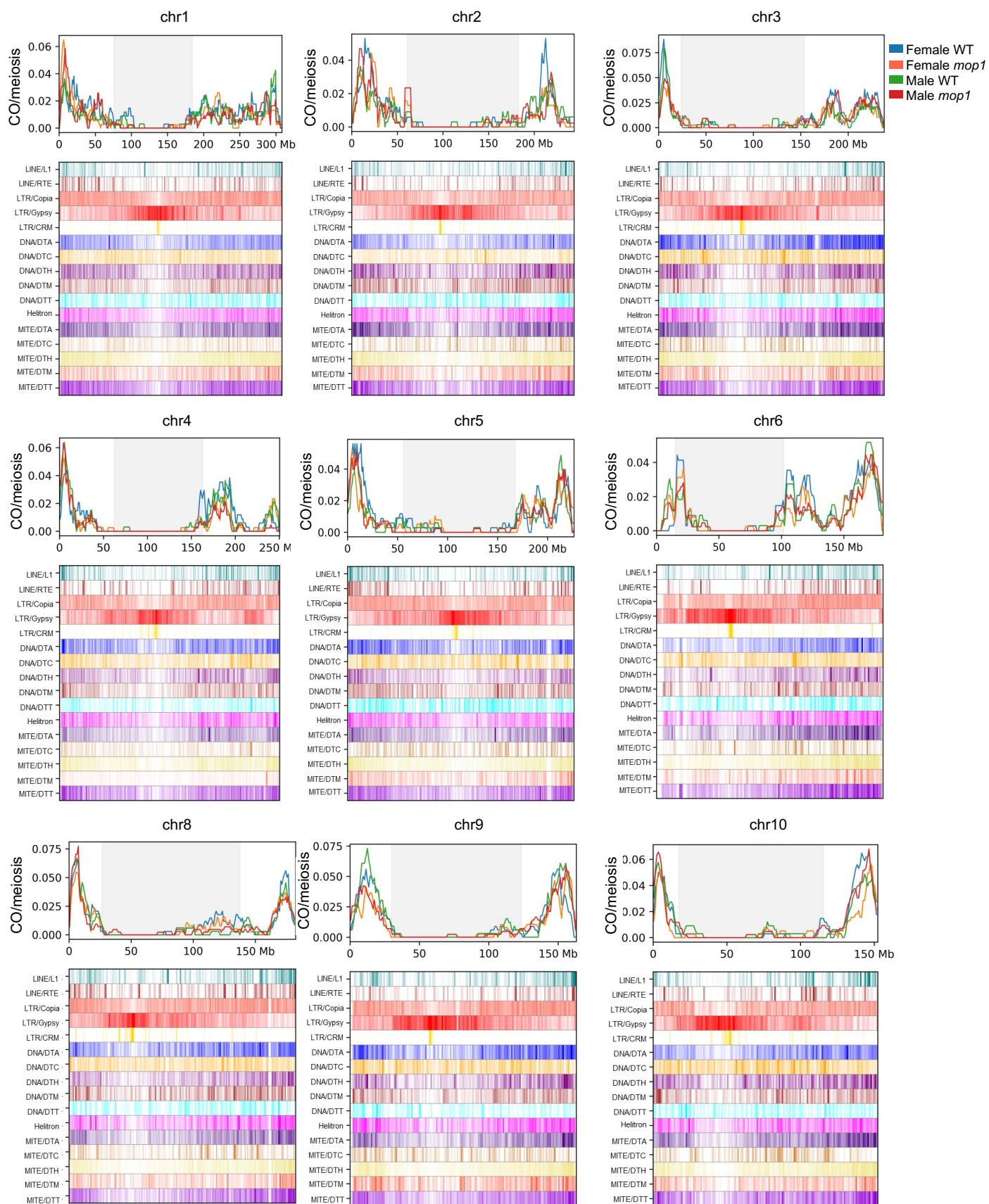


Supplementary Fig. 17. Average InDel (insertion + deletion) density within crossover intervals. Boxes represent the interquartile range (IQR) between the first (Q1) and third (Q3) quartiles, with the horizontal line inside each box indicating the median (Q2). Whiskers extend to the smallest and largest data points within $1.5 \times \text{IQR}$. Statistical significance was assessed using a two-tailed Kruskal-Wallis test, followed by Dunn's post hoc test with Bonferroni correction. *, $P < 0.05$; ns, not significant. Exact P values are provided in the source data associated with this paper. Source data are provided as a Source Data file.

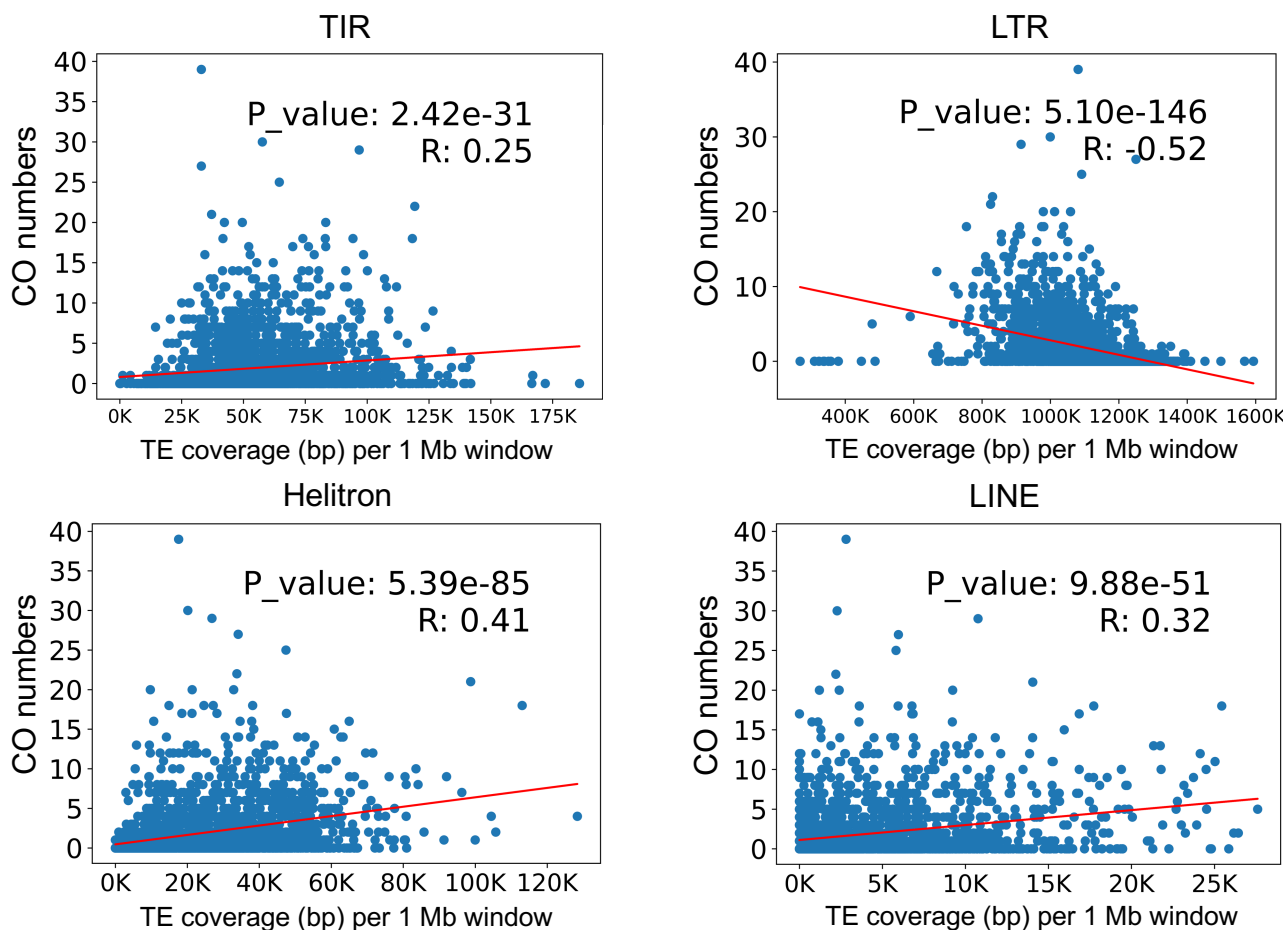


Supplementary Fig. 18. Relationship between crossovers (CO) rates and InDel (insertion + deletion) diversity in female and male meiosis across different genomic contexts.

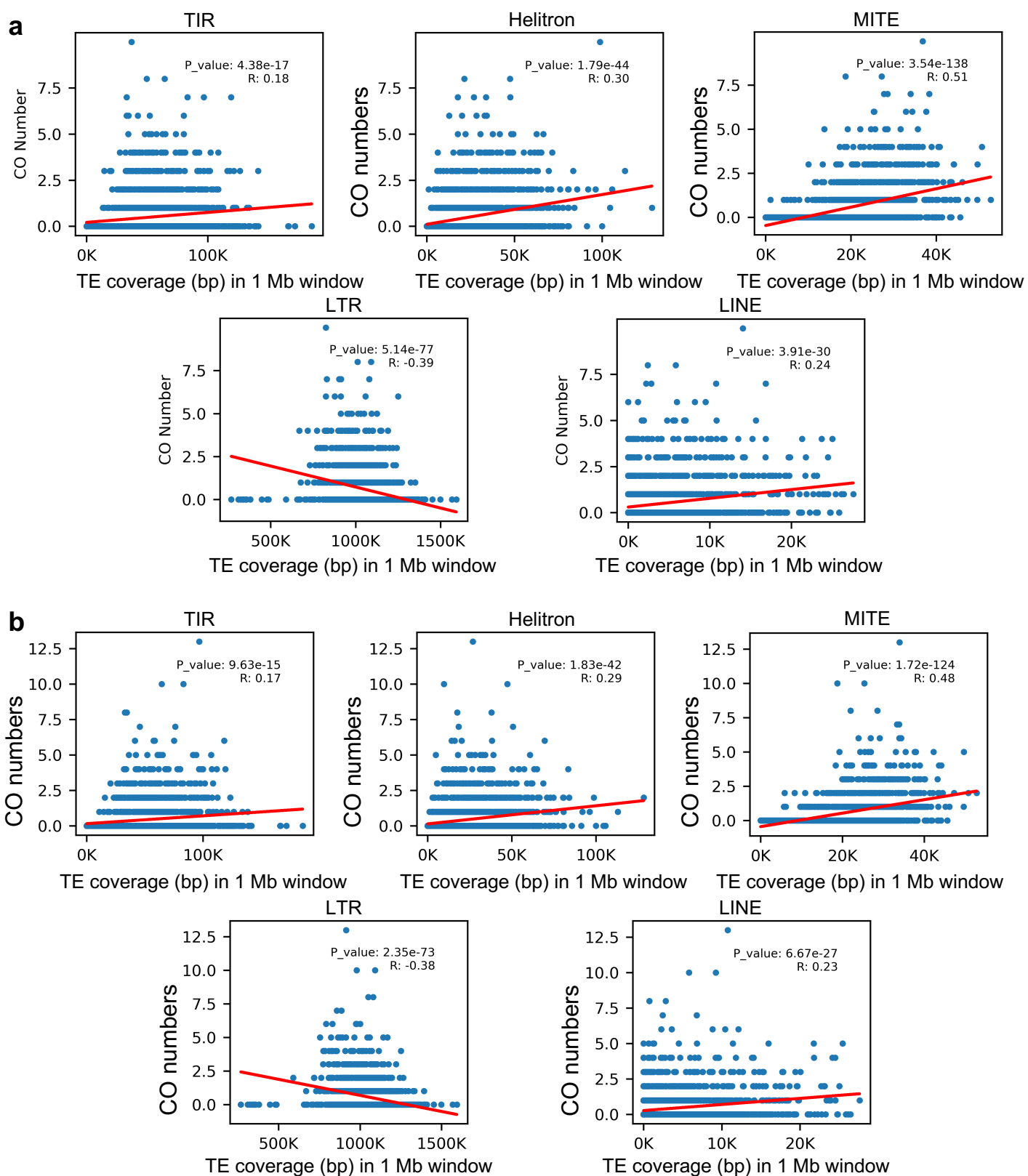
Source data are provided as a Source Data file.



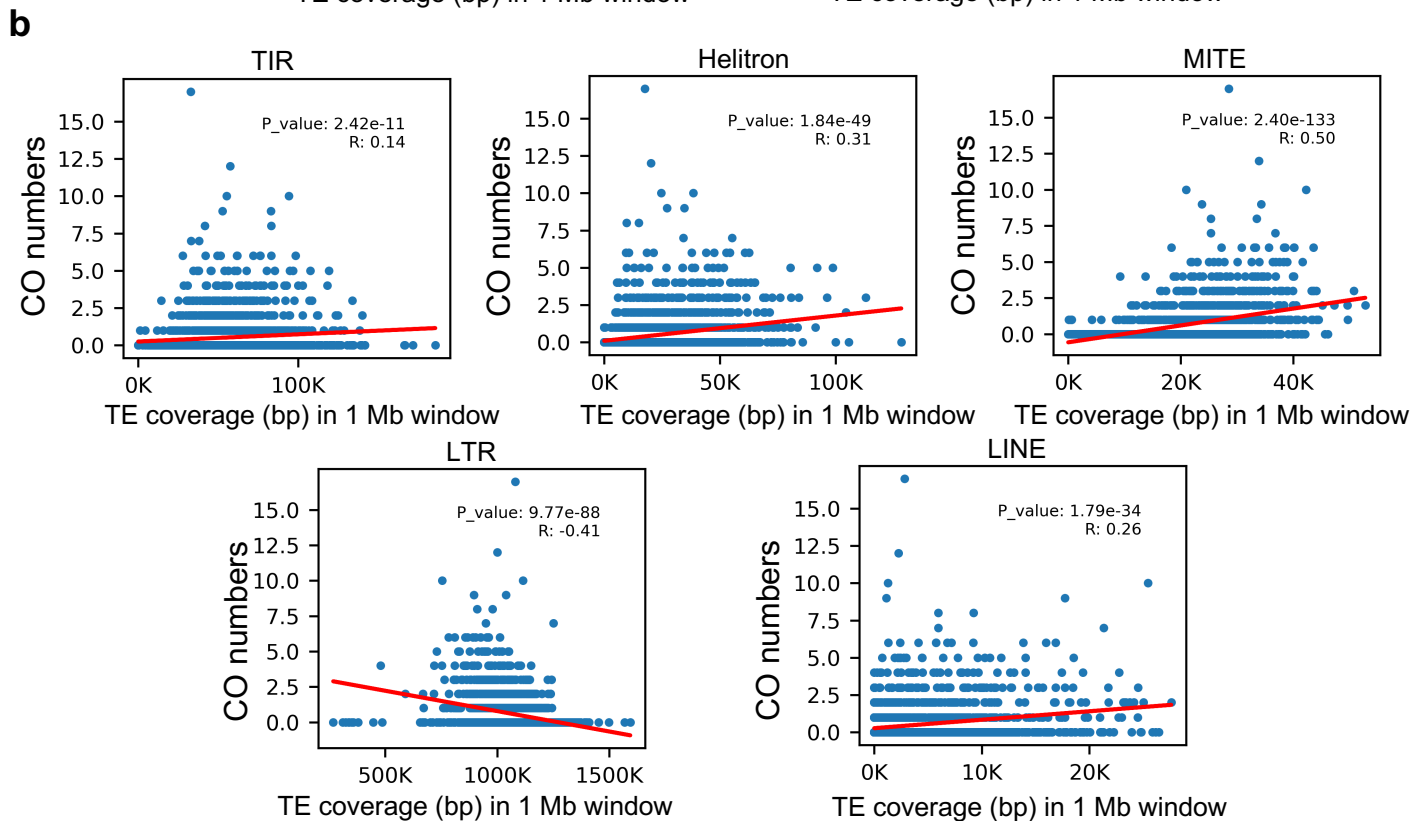
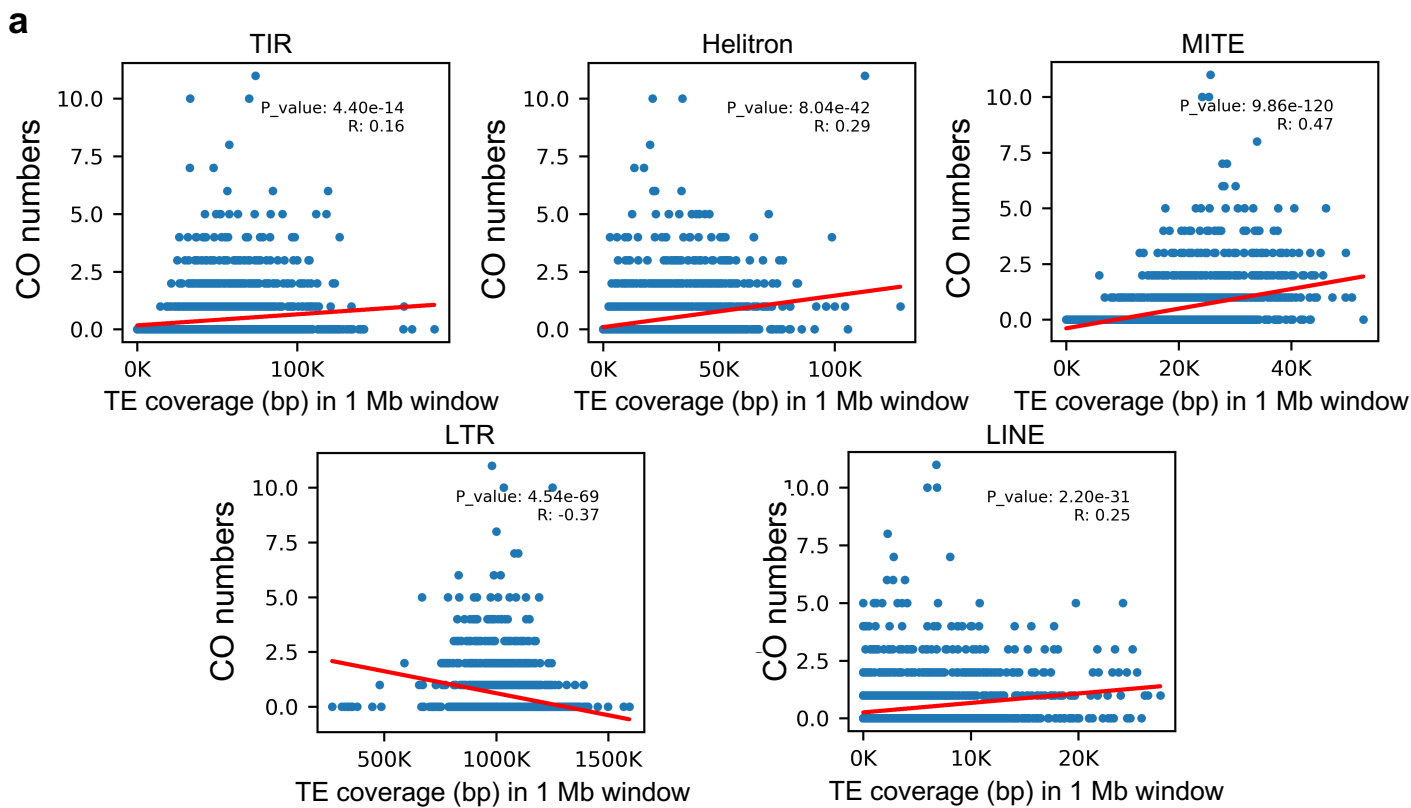
Supplementary Fig. 19. Crossovers (COs) per meiosis in all four populations and relative TE density across chromosomes. Gray-shaded areas represent pericentromeric regions. Source data are provided as a Source Data file.



Supplementary Fig. 20. Spearman's rank correlation between crossover (CO) numbers (from all four populations) and different types of transposable elements. Each dot represents a genomic window. Red lines represent the local regression fit. TIR, terminal inverted repeat DNA transposons; LTR, long terminal repeat retrotransposons; LINEs, long interspersed nuclear elements. Source data are provided as a Source Data file.

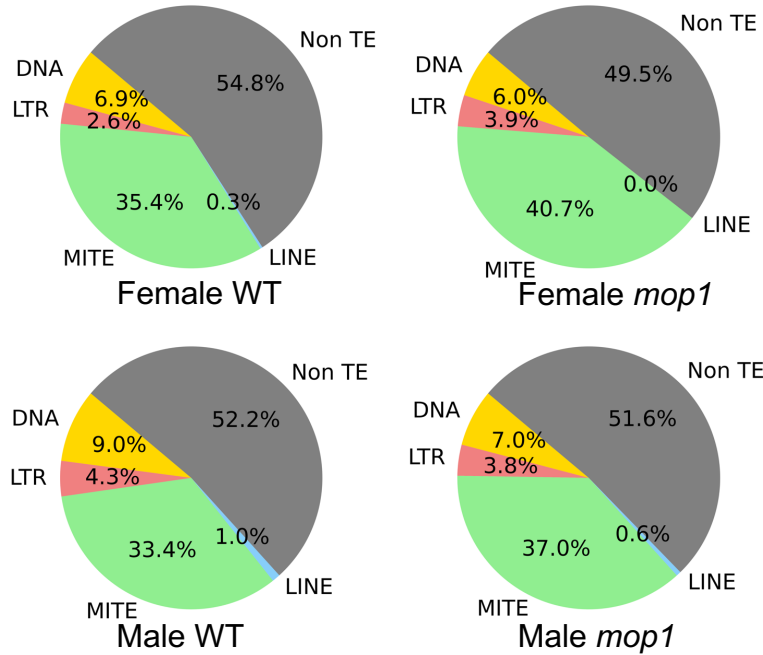


Supplementary Fig. 21. Spearman's rank correlation between crossover (CO) numbers and different types of transposable elements in female WT and female *mop1*. **a**, Female WT. **b**, Female *mop1*. Each dot represents a genomic window. Red lines represent the local regression fit. TIR, terminal inverted repeat DNA transposons; LTR, long terminal repeat retrotransposons; LINEs, long interspersed nuclear elements; MITEs, miniature inverted-repeat transposable elements. Source data are provided as a Source Data file.

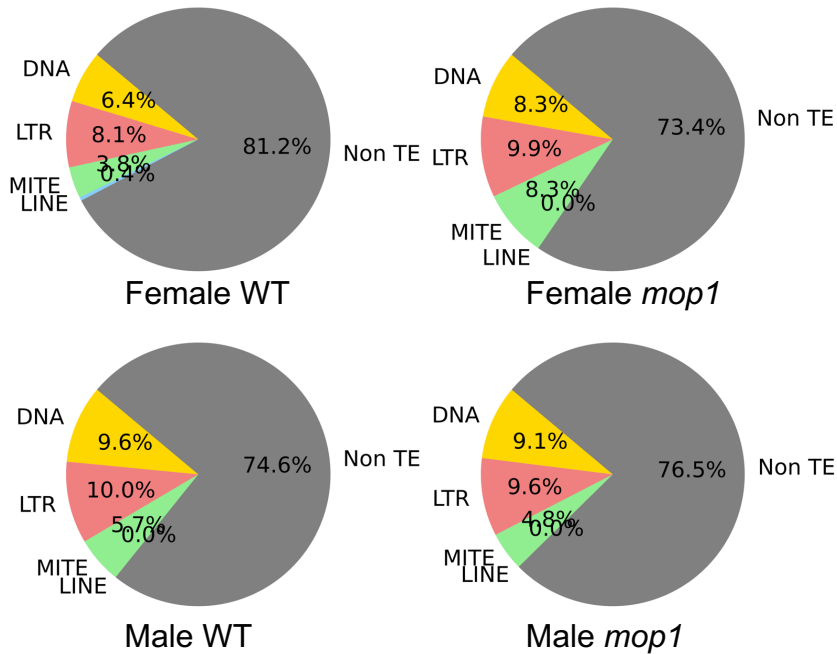


Supplementary Fig. 22. Spearman's rank correlation between crossover (CO) numbers and different types of transposable elements in male WT and male *mop1*. **a**, Male WT. **b**, Male *mop1*. Each dot represents a genomic window. Red lines represent the local regression fit. TIR, terminal inverted repeat DNA transposons; LTR, long terminal repeat retrotransposons; LINEs, long interspersed nuclear elements; MITEs, miniature inverted-repeat transposable elements. Source data are provided as a Source Data file. 23

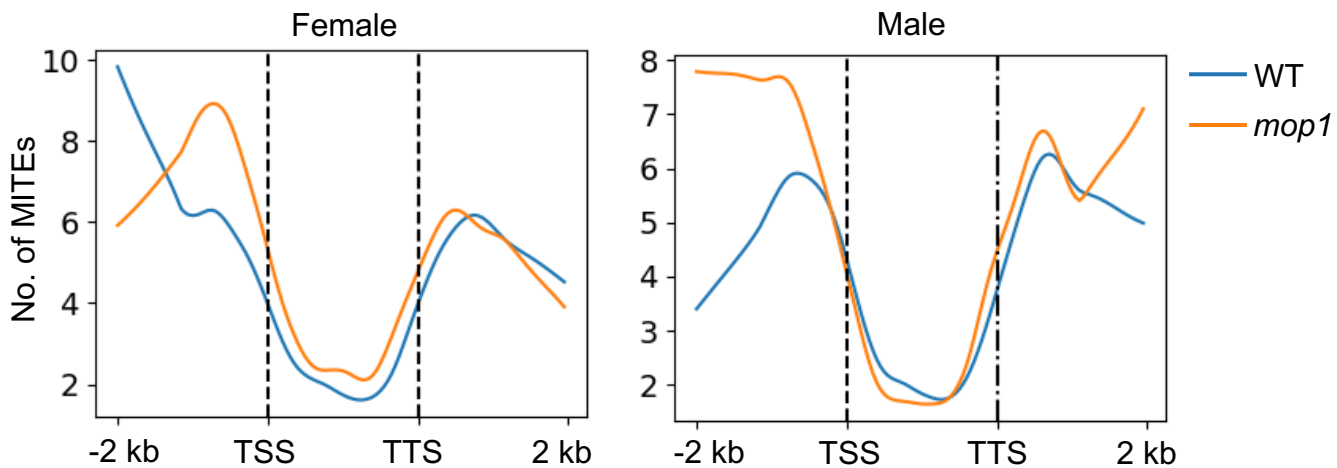
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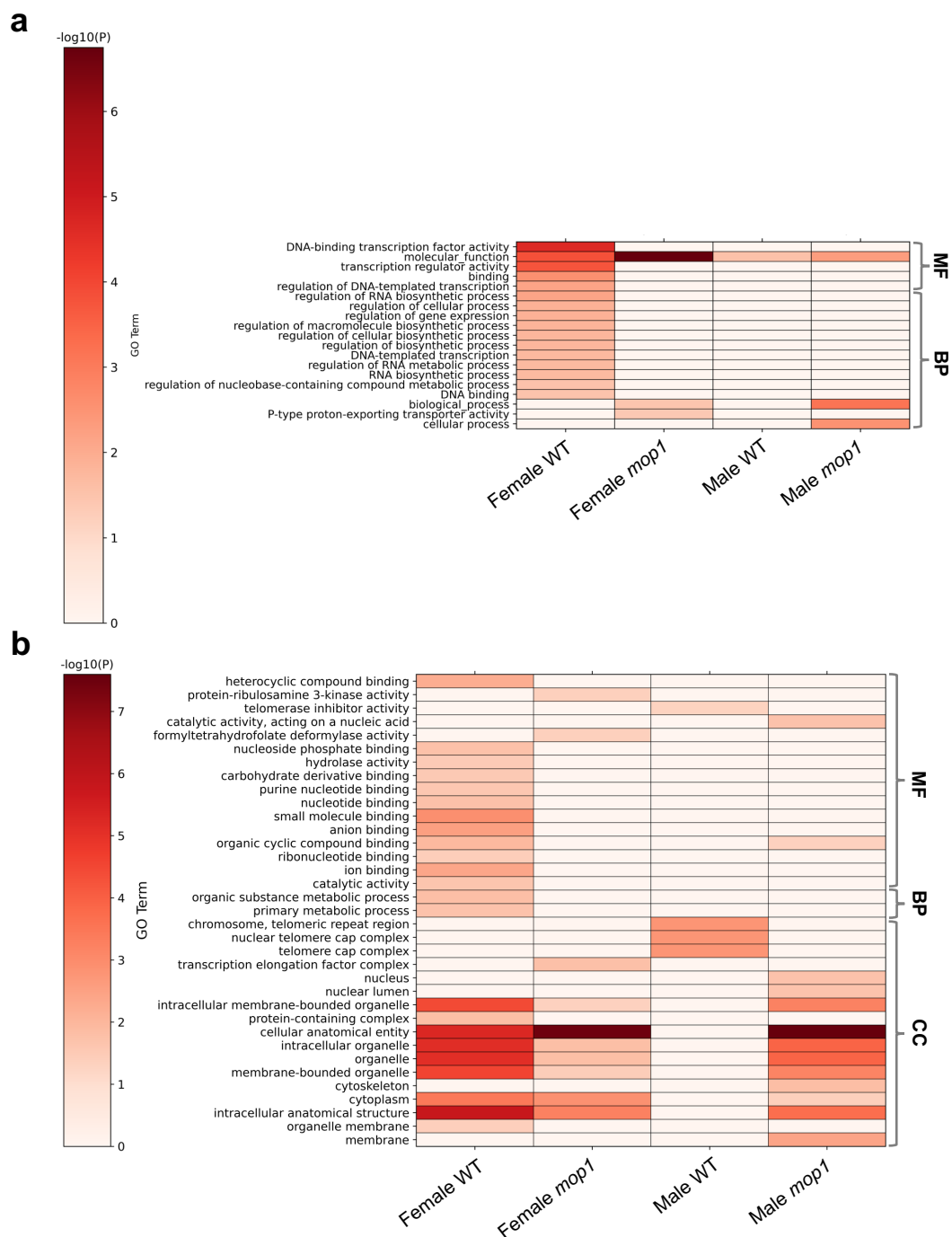
b



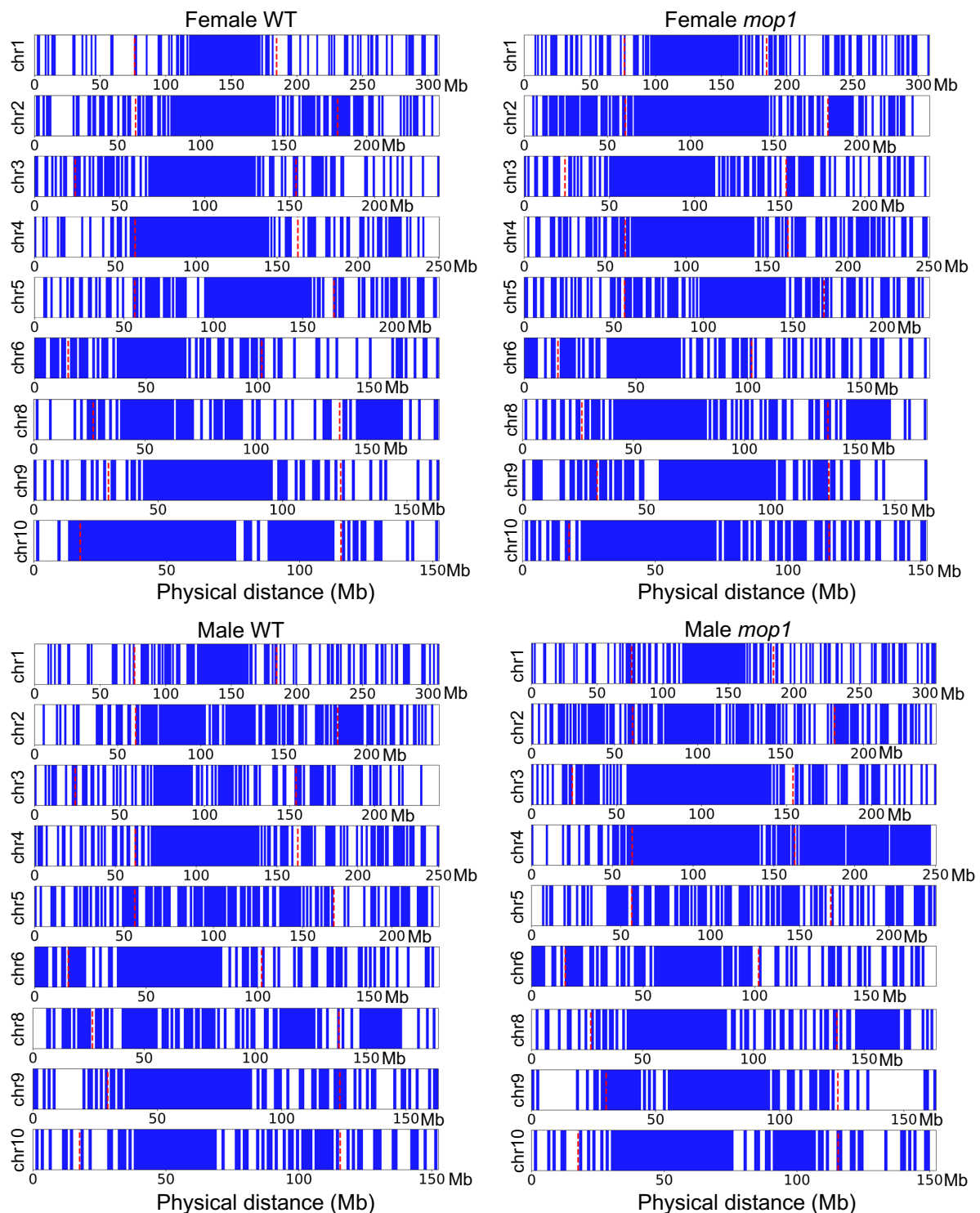
Supplementary Fig. 23. Crossover (CO) locations in relation to transposable elements (TEs). a, COs within 2 kb flanking regions of genes. b, COs outside 2 kb flanking regions of genes. Source data are provided as a Source Data file.



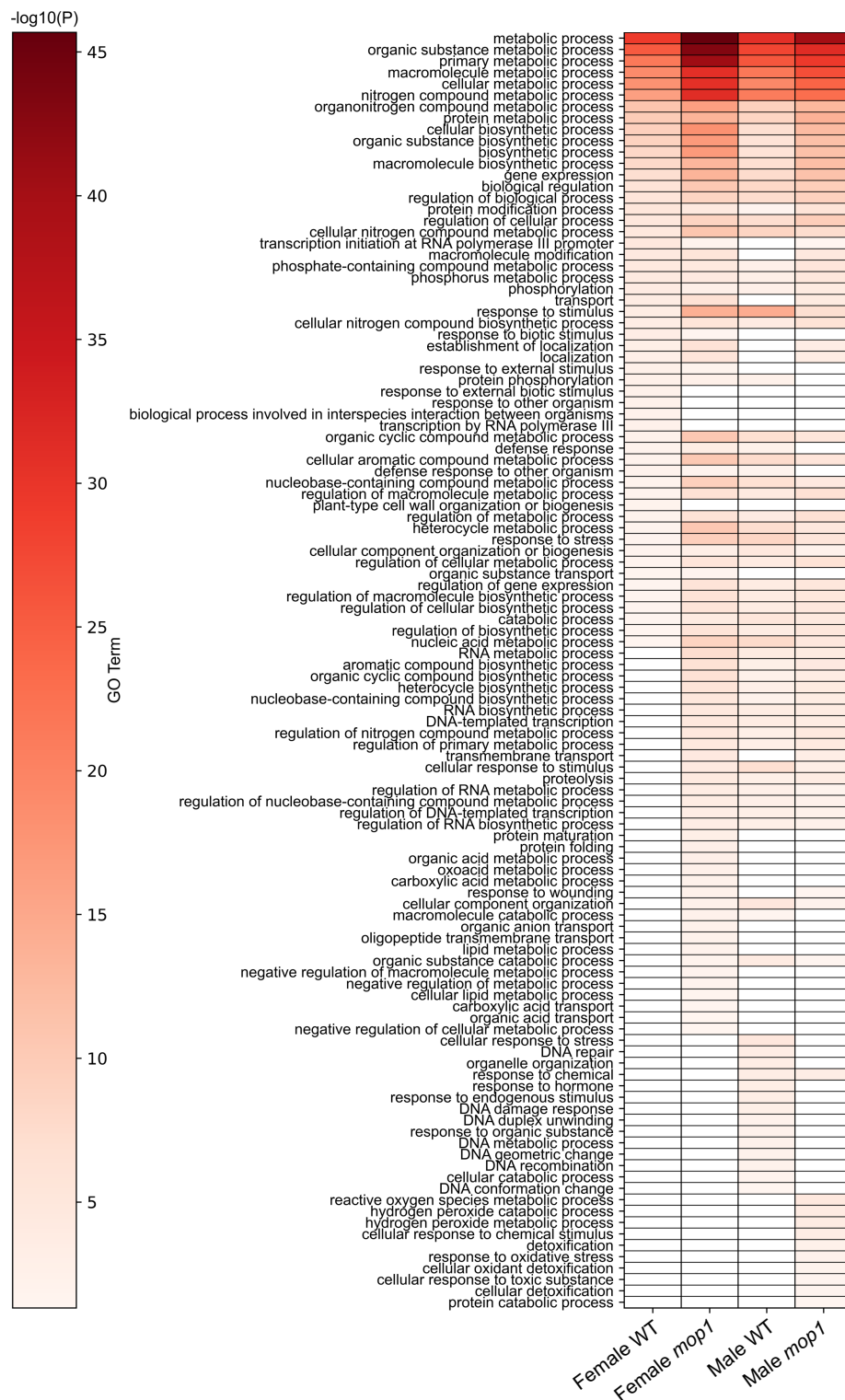
Supplementary Fig. 24. Distribution of MITEs overlapping with crossovers within gene bodies and their 2 kb flanking regions. MITEs, miniature inverted-repeat transposable elements; TSS, transcription start sites; TTS, transcription termination sites. Source data are provided as a Source Data file.



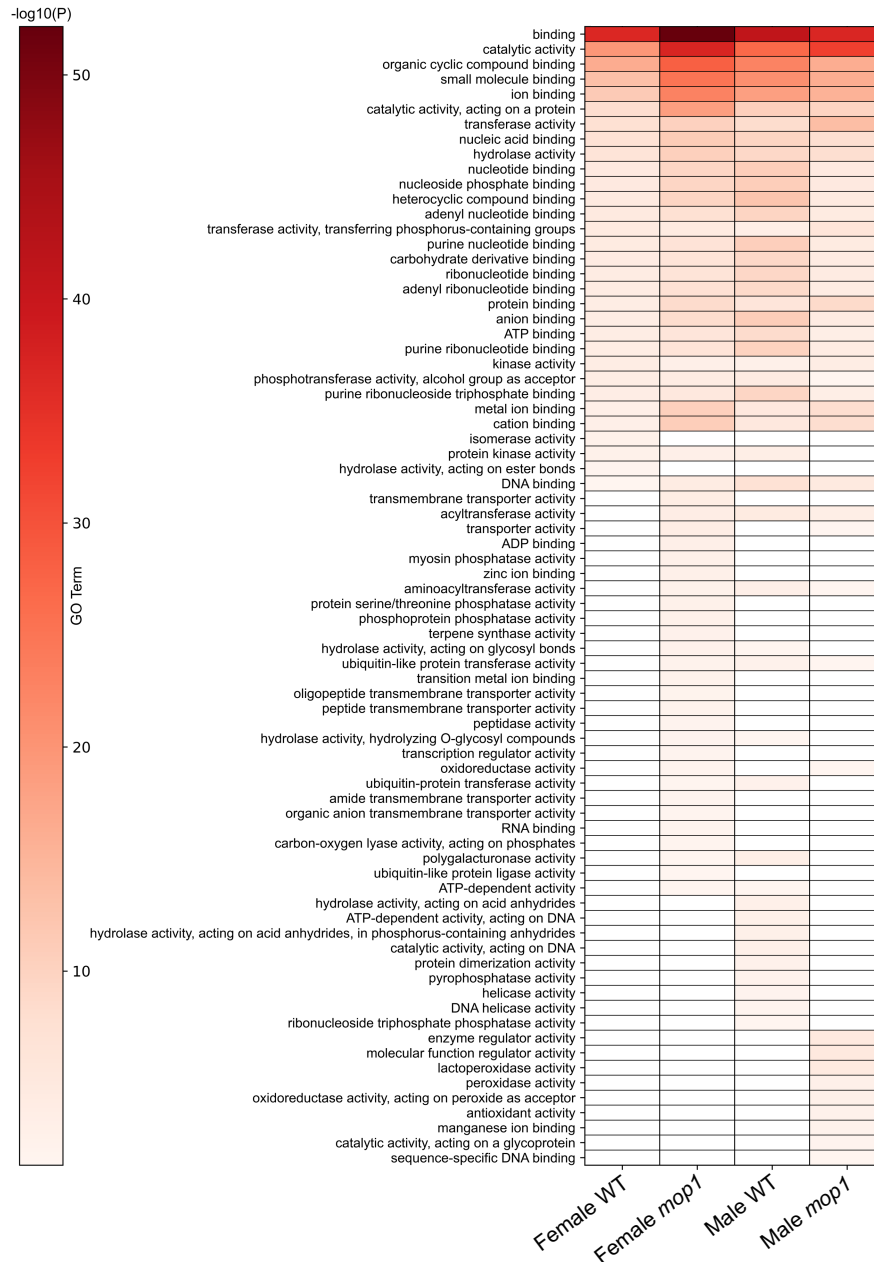
Supplementary Fig. 25. Gene ontology (GO) analysis of genes within crossover (CO) intervals mapped to 2 kb regions and hotspot regions. a, CO intervals mapped to 2 kb regions. b, CO hotspot regions. MF, Molecular Function; BP, Biological Process; CC, Cellular Component. Only significant GO terms ($P < 0.05$) are shown. Source data are provided as a Source Data file.



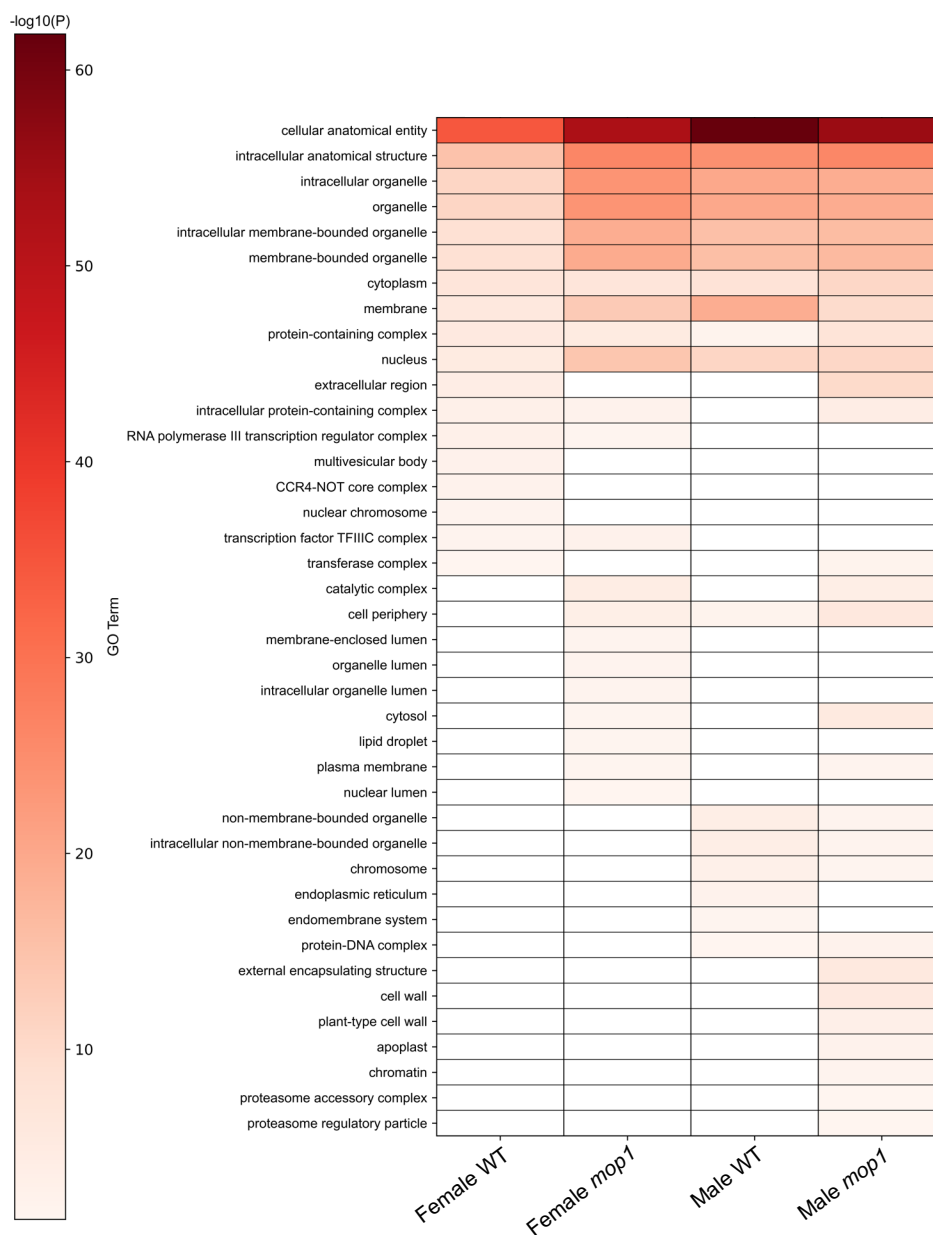
Supplementary Fig. 26. Coldspots are primarily located in pericentromeric regions. Blue regions indicate coldspots on the chromosome, and red vertical dotted lines mark the boundaries between chromosomal arms and pericentromeric regions. Coldspots are defined as regions of at least 1 Mb without crossovers. Source data are provided as a Source Data file.



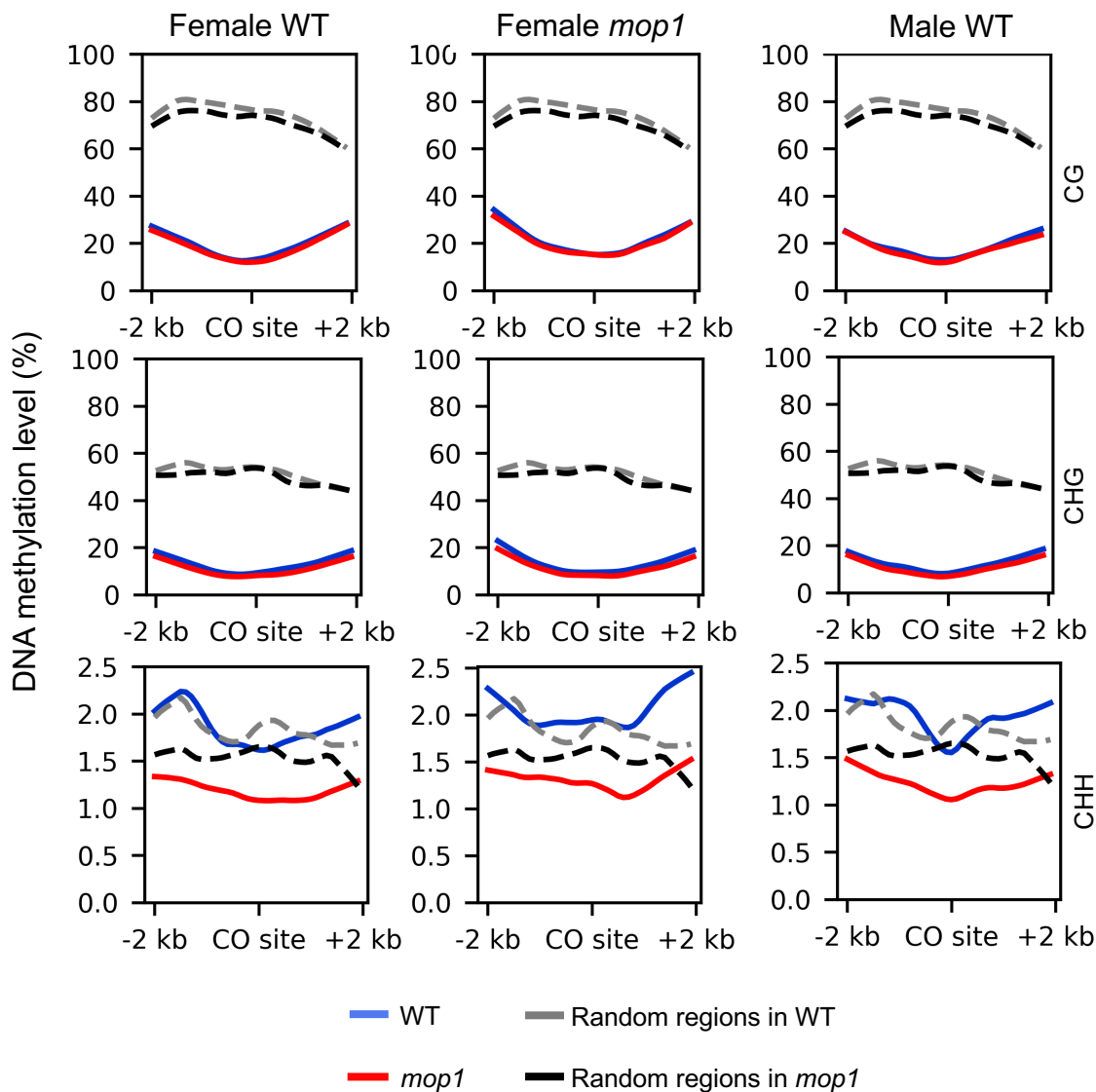
Supplementary Fig. 27. Gene ontology (GO) analysis of genes within coldspot regions for biological process (BP). Only significant GO terms ($P < 0.05$) are shown. Source data are provided as a Source Data file.



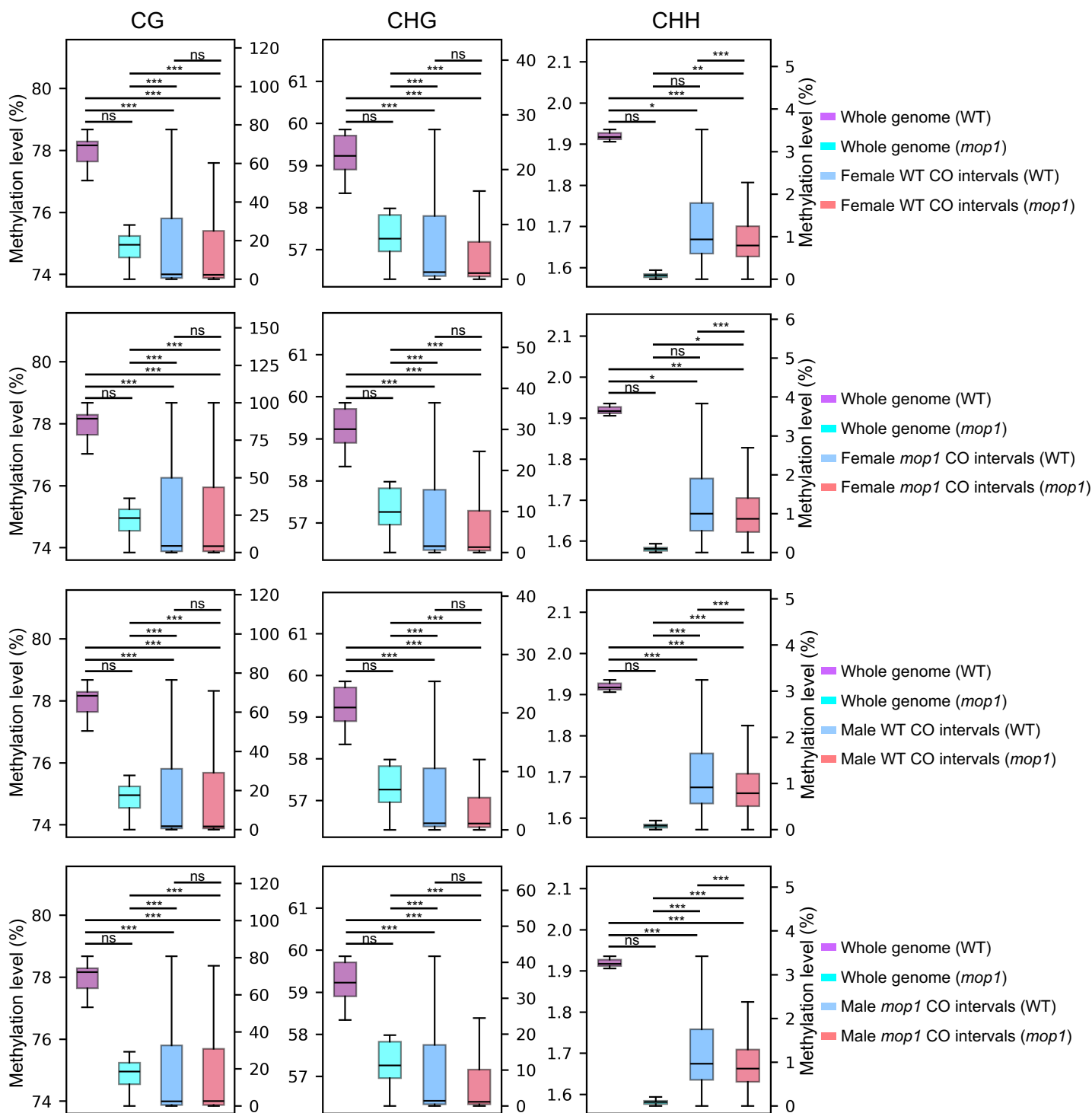
Supplementary Fig. 28. Gene ontology (GO) analysis of genes within coldspot regions for molecular function (MF). Only significant GO terms ($P < 0.05$) are shown. Source data are provided as a Source Data file.



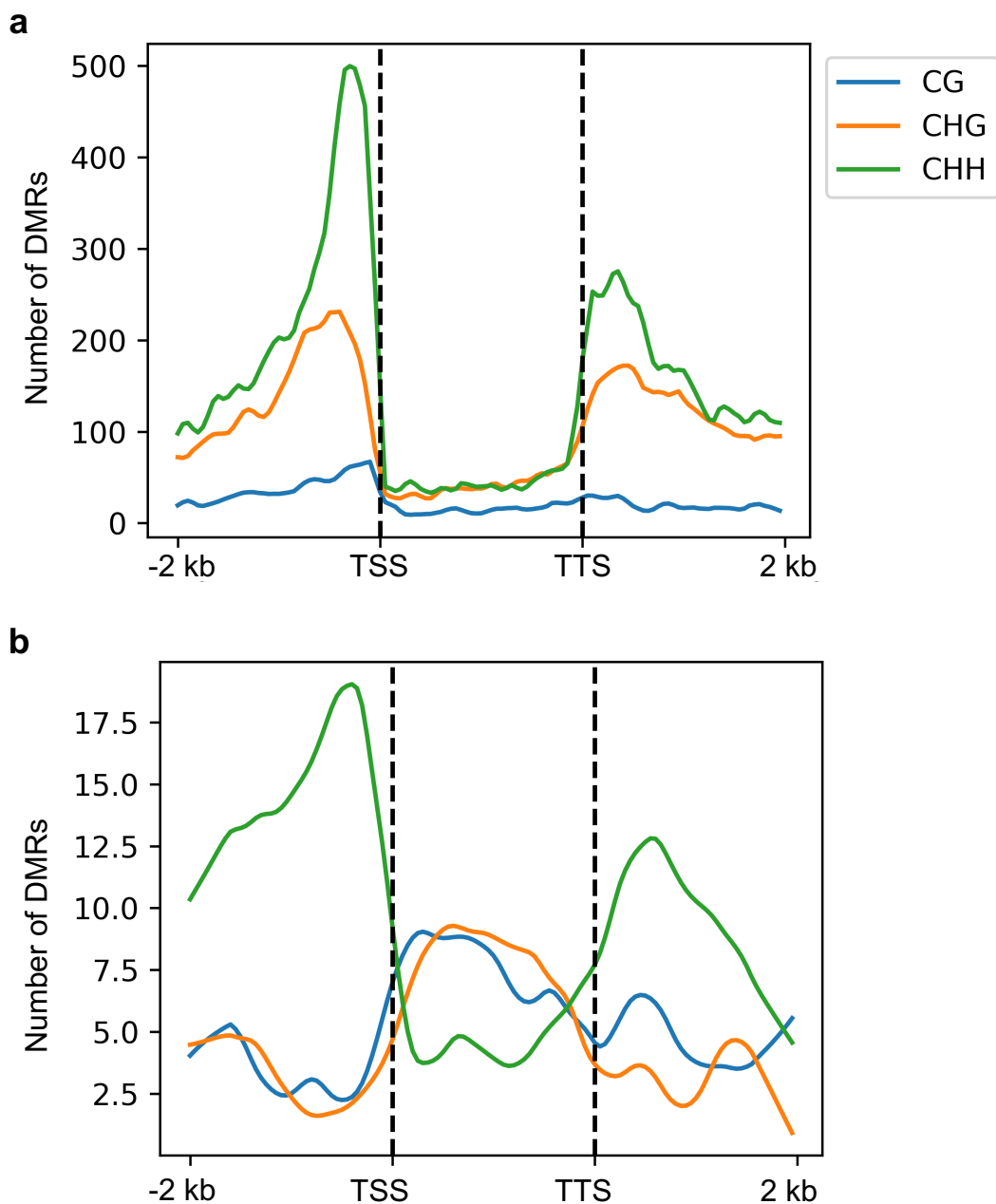
Supplementary Fig. 29. Gene ontology (GO) analysis of genes within coldspot regions for cellular components (CC). Only significant GO terms ($P < 0.05$) are shown. Source data are provided as a Source Data file.



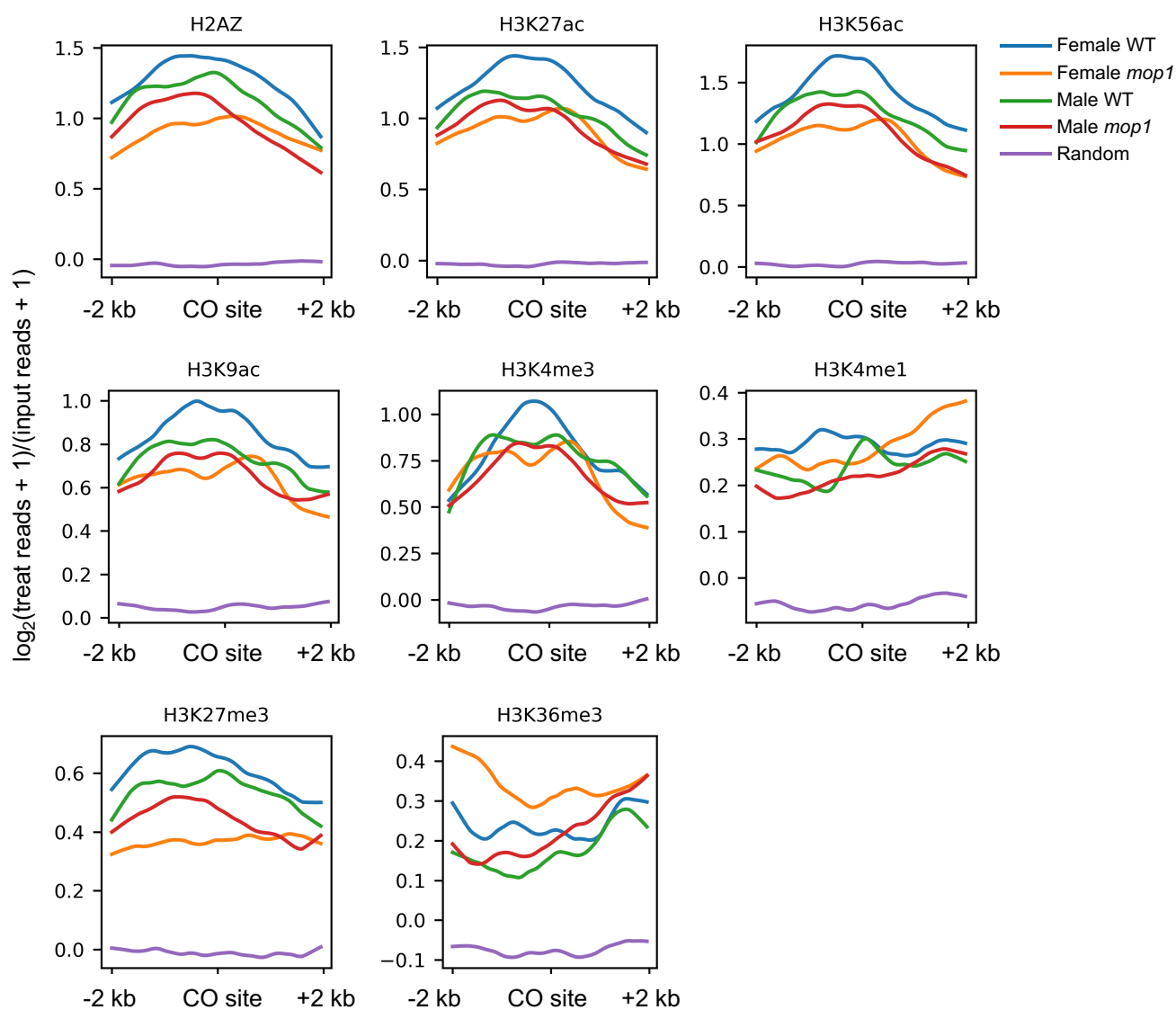
Supplementary Fig. 30. *mop1* exhibits reduced CHH (H = A, T, or C) DNA methylation levels at crossover (CO) sites. Only COs mapped within 2 kb intervals were included in the analysis. Methylation data were obtained from immature anthers¹. Source data are provided as a Source Data file.



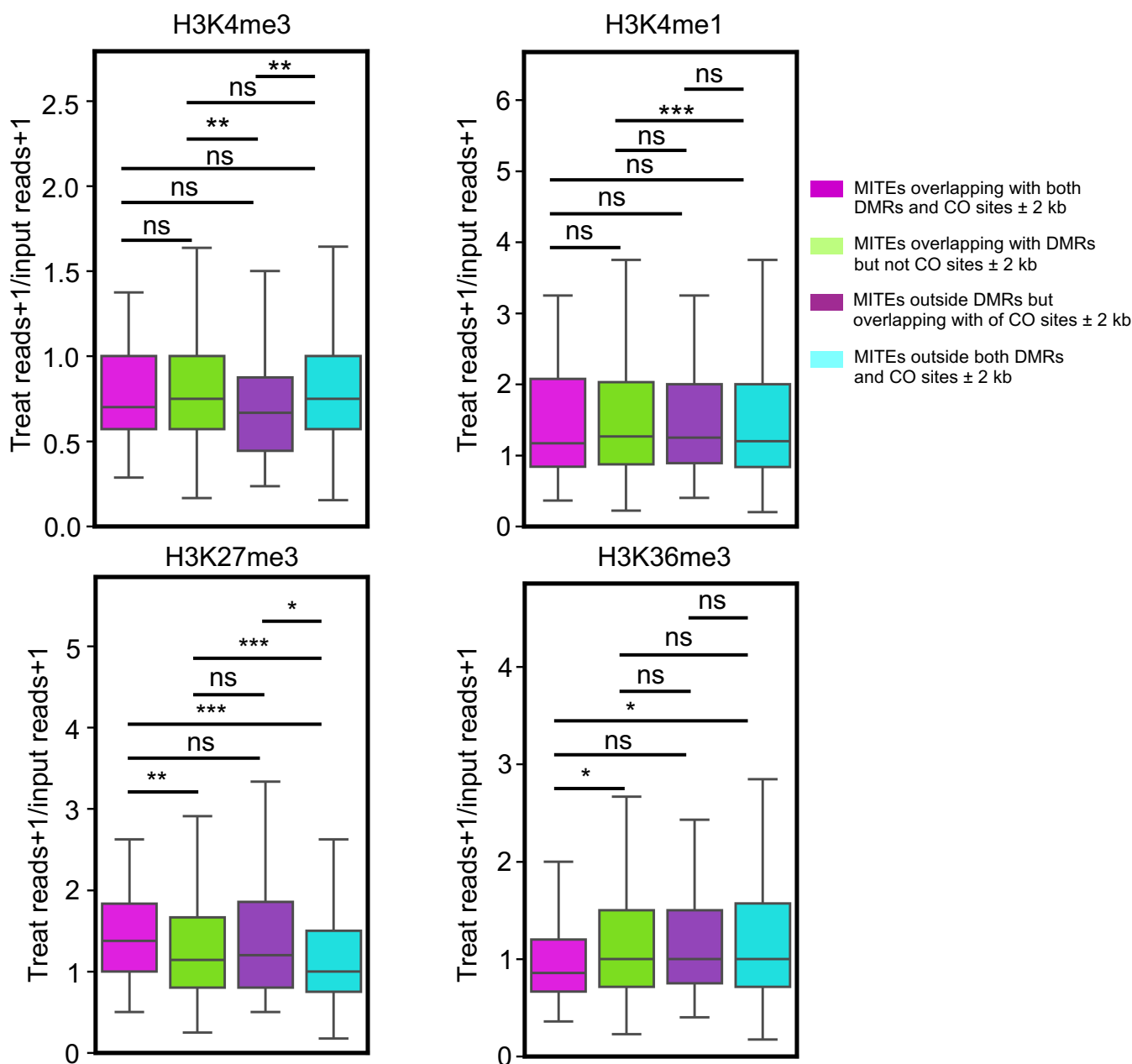
Supplementary Fig. 31. Comparison of DNA methylation levels at crossover (CO) intervals between WT and *mop1*. Boxes represent the interquartile range (IQR) between the first (Q1) and third (Q3) quartiles, with the horizontal line inside each box indicating the median (Q2). Whiskers extend to the smallest and largest data points within $1.5 \times \text{IQR}$. Statistical significance was assessed using a two-tailed paired Student's *t*-test for whole-genome data and a two-tailed Wilcoxon signed-rank test for CO interval data in each population. The Y-axis on the left side shows the methylation levels of the whole genome, and the right side shows the methylation levels of the CO intervals. Only COs mapped within 2 kb intervals were included. Methylation data were obtained from immature anthers¹. Source data are provided as a Source Data file.



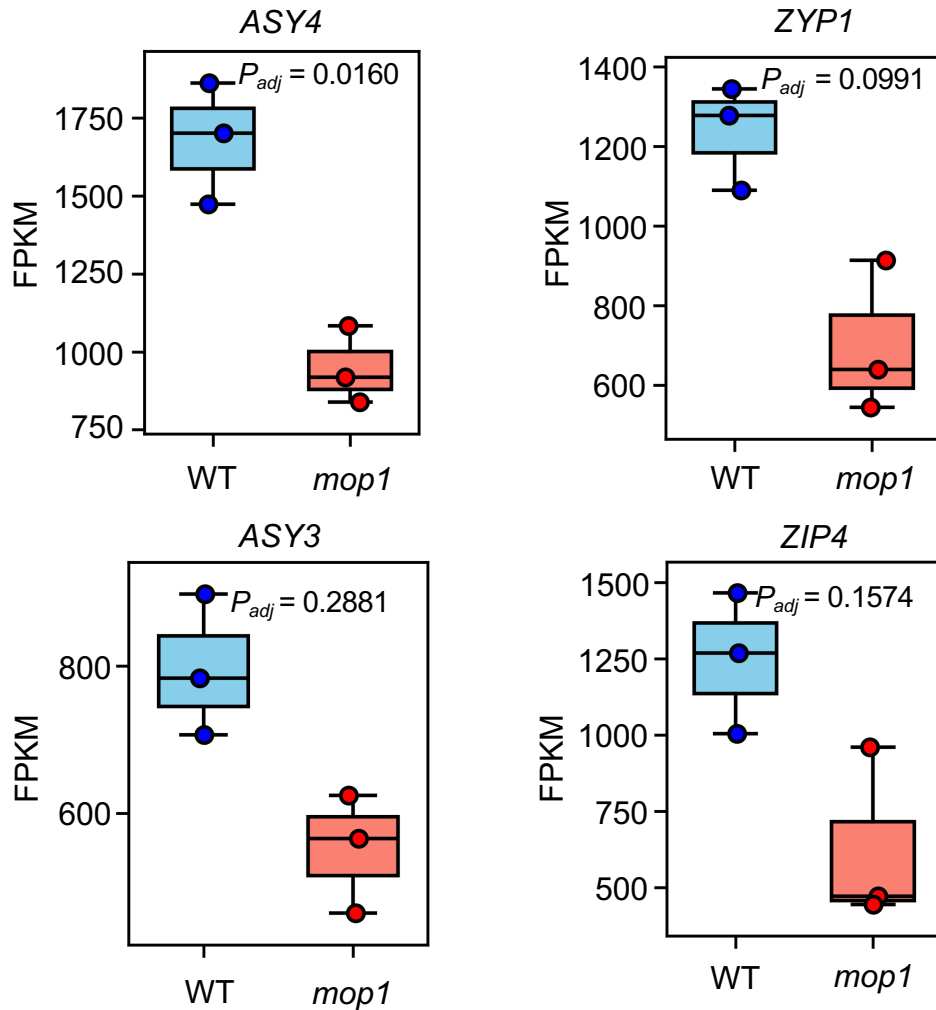
Supplementary Fig. 32. Distribution of differentially methylated regions (DMRs) within gene bodies and their 2 kb flanking regions. a, hypomethylated DMRs. b, hypermethylated DMRs. Source data are provided as a Source Data file.



Supplementary Fig. 33. Crossover (CO) regions are enriched for higher levels of active histone marks. Only COs mapped within 2 kb intervals were included. Histone data were obtained from wild type leaves². Source data are provided as a Source Data file.

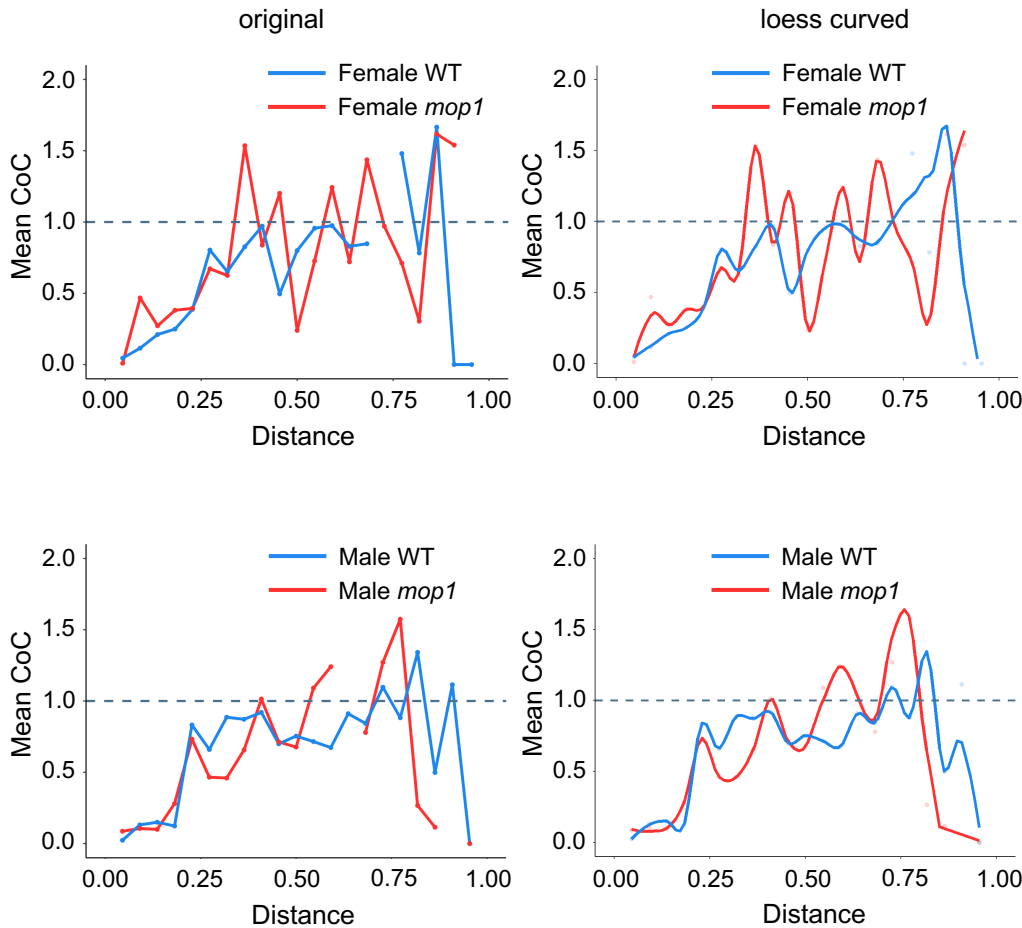
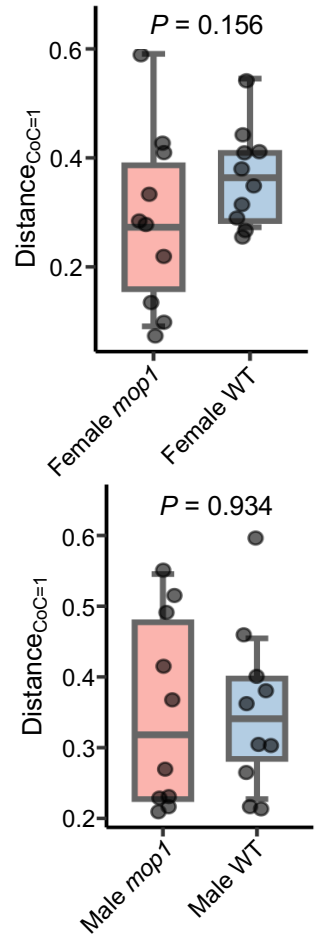


Supplementary Fig. 34. Comparison of histone modification levels on miniature inverted-repeat transposable elements (MITEs) across four categories. Boxes represent the interquartile range (IQR) between the first (Q1) and third (Q3) quartiles, with the horizontal line inside each box indicating the median (Q2). Whiskers extend to the smallest and largest data points within $1.5 \times$ IQR. Error bars represent the standard error of the mean (SEM). Statistical significance was assessed using a two-tailed Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Histone data were obtained from wild type leaves². Source data are provided as a Source Data file.

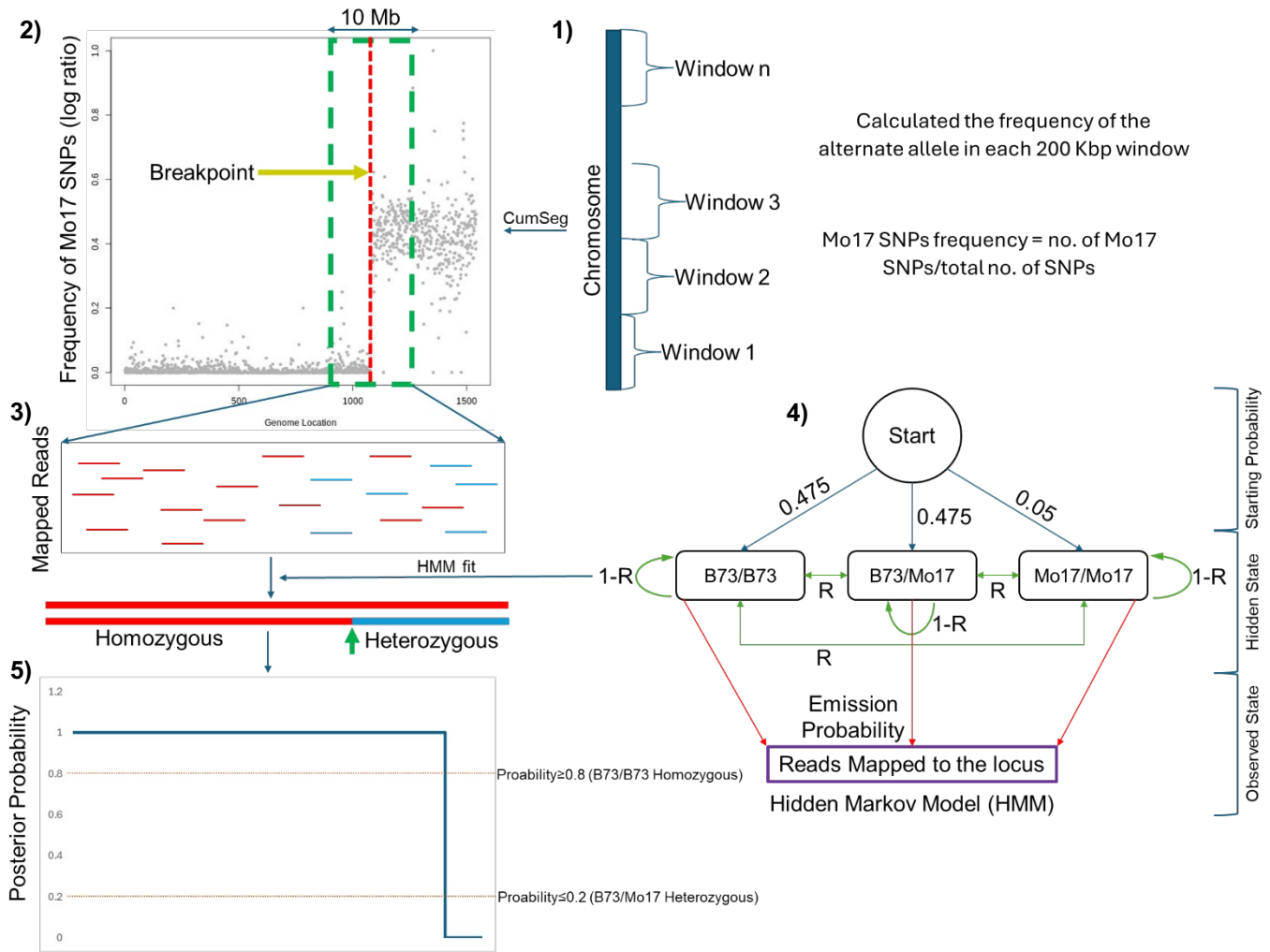


Supplementary Fig. 35. Normalized expression levels of *ASY4*, *ASY3*, *ZYP1*, and *ZIP4* in immature anthers of WT and *mop1*.

Statistical analysis was conducted by DESeq2. Boxes represent the interquartile range (IQR) between the first (Q1) and third (Q3) quartiles, with the horizontal line inside each box indicating the median (Q2). Whiskers extend to the smallest and largest data points within $1.5 \times \text{IQR}$. Sample size ($n = 3$) indicates three biological replicates. Source data are provided as a Source Data file.

a**b**

Supplementary Fig. 36. No significant changes in crossover interference in *mop1* mutants. **a**, Mean CoC values for female WT versus female *mop1* and male WT versus male *mop1*. **b**, Mean inter-interval distance at a CoC value of 1. Error bars represent the standard error of the mean (SEM). Statistical significance was assessed using two-tailed Student's *t*-test. Boxes represent the interquartile range (IQR) between the first (Q1) and third (Q3) quartiles, with the horizontal line inside each box indicating the median (Q2). Whiskers extend to the smallest and largest data points within 1.5 × IQR., and dots outside the whiskers represent outliers. Sample size (n = 10) indicates ten maize chromosomes. Source data are provided as a Source Data file.



Supplementary Fig. 37. Bioinformatics pipeline for detecting crossover intervals in the populations. 1) Each chromosome was divided into 200 kb windows and the frequency of Mo17 SNPs in each window was calculated. 2) Potential breakpoints were detected using CumSeg, an R package, and SNPs within 5 Mb upstream and downstream of each breakpoint were extracted. 3) A representative 10 Mb region around a breakpoint, showing 150 bp Illumina reads aligned to the B73 reference genome. 4) A Hidden Markov Model (HMM) was applied, where the number of reads overlapping SNP loci (observed state) was used to calculate the probability of the genotype (hidden state). The initial probabilities were set at 0.475 for B73/B73 homozygous, 0.475 for B73/Mo17 heterozygous, and 0.05 for Mo17/Mo17 homozygous. Although Mo17/Mo17 homozygous is not expected in the BC₁ population, a 5% error tolerance was incorporated into the model. 5) The posterior probability of SNPs belonging to the B73/B73 homozygous genotype was plotted on the Y-axis against genomic position on the X-axis. A posterior probability ≥ 0.8 indicates a B73/B73 homozygous SNP, while ≤ 0.2 indicates a B73/Mo17 heterozygous SNP.

Supplementary Table 1. Number of final SNPs identified in each chromosome in each population.

Chromosome	Female WT	Female <i>mop1</i>	Male WT	Male <i>mop1</i>
1	601,292	601,044	604,722	620,864
2	446,403	420,436	416,297	433,229
3	494,225	496,318	496,194	483,280
4	505,106	512,916	537,039	492,851
5	359,307	350,133	361,949	344,274
6	349,153	343,896	350,949	336,901
7	419,998	390,183	392,158	397,141
8	331,168	316,730	329,894	326,593
9	339,789	317,620	320,180	338,820
10	328,457	334,896	315,995	328,037
Total	4,174,898	4,084,172	4,125,377	4,101,990

Supplementary Table 2. Overall crossover (CO) numbers and average COs per meiosis in different populations.

Population	Total COs	Samples	Average CO/meiosis
Female WT	1,069	109	19.43
Female <i>mop1</i>	968	98	19.75
Male WT	914	94	19.44
Male <i>mop1</i>	1,097	122	17.98

Supplementary Table 3. Comparison of genetic distances of the 10 chromosomes in the four populations used in this study

Chromosome	Female WT	Female <i>mop1</i>	Male WT	Male <i>mop1</i>	<i>mop1</i> to WT ratio (female)	<i>mop1</i> to WT ratio (male)	Female to male ratio (WT)	Female to male ratio (<i>mop1</i>)
chr1	203.1	207.34	161.63	172.51	1.02	1.07	1.26*	1.20
chr2	179.29	145.42	143.81	168.57	0.81	1.17	1.25*	0.86
chr3	134.17	134.61	133.78	143.11	1.00	1.07	1.00	0.94
chr4	125.31	117.32	132.36	136.41	0.94	1.03	0.95	0.86
chr5	150.87	174.44	150.58	146.93	1.16	0.98	1.00	1.19
chr6	110.93	113.62	116.25	98.08	1.02	0.84	0.95	1.16
chr7	112.26	147.45	124.71	109.65	1.31*	0.88	0.90	1.34*
chr8	134.32	124.94	128.06	121.86	0.93	0.95	1.05	1.03
chr9	114.52	134.56	135.6	103.12	1.17	0.76*	0.84	1.30
chr10	92.22	59.05	93.4	93.93	0.64*	1.01	0.99	0.63*
Total	1356.99	1358.75	1320.18	1294.17	1.00	0.98	1.03	1.05

*, $P < 0.05$, one-tailed chi-square test

Supplementary Table 4. Statistical analysis of motif enrichment between WT and *mop1*.

Motif ID	Sex	WT_%	mop1_%	WT count	WT total	mop1 count	mop1 total	P value	Q value	Significant FDR0.05
1-ACGACGACGACGACGWGGVCS	Female	28.3	28.1	113	399	88	313	1.0000	1.0000	FALSE
1-ACGACGACGACGACGWGGVCS	Male	36.5	31.2	124	340	119	382	0.0334	0.1668	FALSE
2-GVGRGRRRGAGRGRGRGRRRGRVGRVG	Female	43.1	44.1	172	399	138	313	0.7323	0.9154	FALSE
2-GVGRGRRRGAGRGRGRGRRRGRVGRVG	Male	43.2	37.7	147	340	144	382	0.0300	0.1668	FALSE
3-CAGCAGCAGCAGCAGCAGCNSS	Female	33.3	31.0	133	399	97	313	0.4015	0.7652	FALSE
3-CAGCAGCAGCAGCAGCAGCNSS	Male	35.9	29.1	122	340	111	382	0.0055	0.1096	FALSE
4-TATATATATWTWWWWWWAWWWWWAWTTA	Female	30.6	37.1	122	399	116	313	0.0141	0.1409	FALSE
4-TATATATATWTWWWWWWAWWWWWAWTTA	Male	29.7	29.3	101	340	112	382	0.9109	0.9588	FALSE
5-AAAWAAAAARAARAARARWAHWWRW	Female	37.6	43.1	150	399	135	313	0.0471	0.1852	FALSE
5-AAAWAAAAARAARAARARWAHWWRW	Male	41.2	42.7	140	340	163	382	0.5676	0.8219	FALSE

Note: Statistical significance was assessed using two-tailed binomial tests with Benjamini–Hochberg correction, implemented in `scipy.stats` (Python).

Supplementary references

1. Zhao M, *et al.* The *mop1* mutation affects the recombination landscape in maize. *Proc Natl Acad Sci U S A* **118**, (2021).
2. Ricci WA, *et al.* Widespread long-range cis-regulatory elements in the maize genome. *Nat Plants* **5**, 1237-1249 (2019).