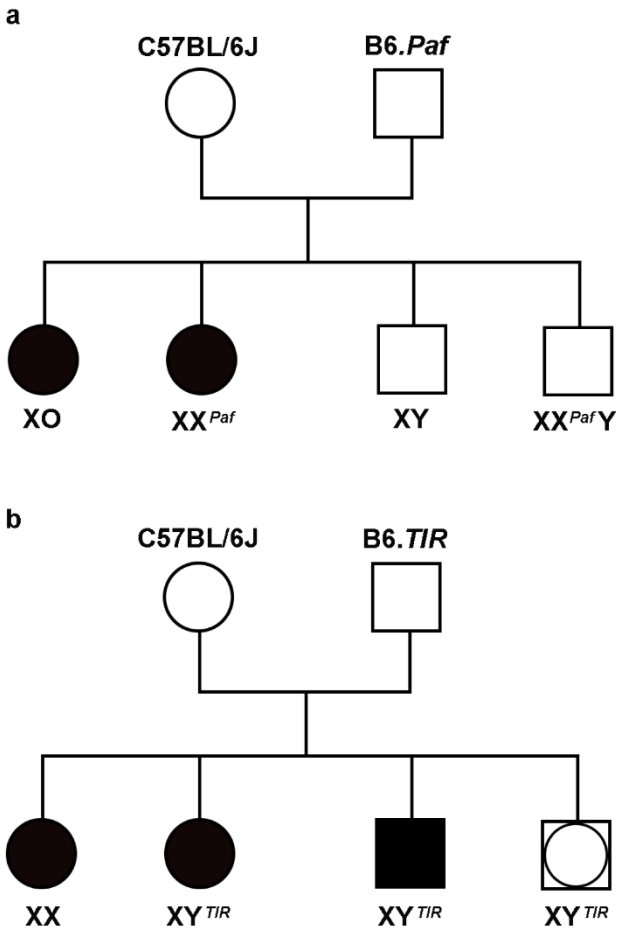

Figure S1. Mouse crosses used in the study.

a. The *Paf* cross; **b.** The TIR cross. Circles represent females, squares represent males, combined circle and square represents hermaphrodites. Filled shapes represent groups used in our study.

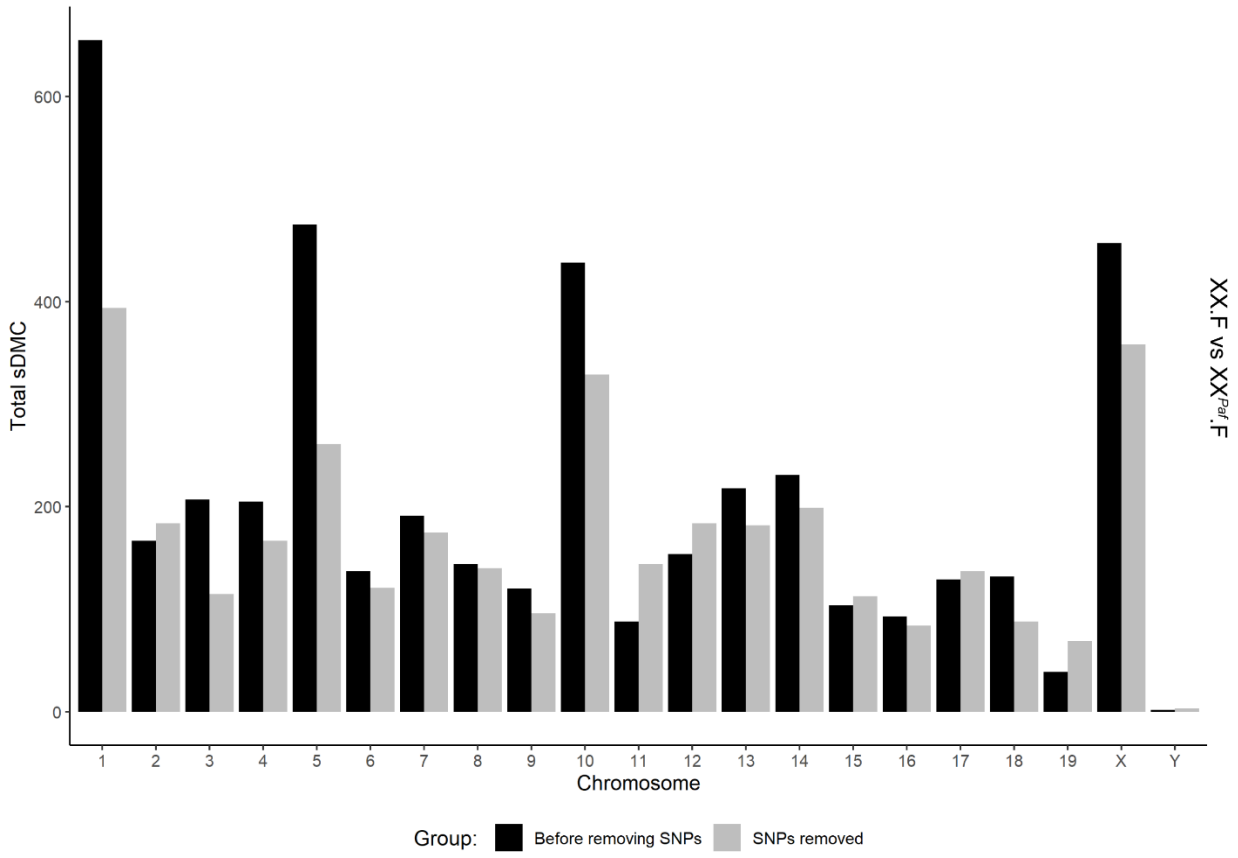

Figure S2. Methylation heterogeneity associated with SNPs was reduced after SNP removal in the comparison between the two groups of XX females from *Paf* and TIR crosses.

The number of differentially methylated CpG sites (DMC) between sample groups XX.F (TIR cross**)** and XX^{Paf}.F (*Paf* cross). Removal of SNPs resulted in altered DMC profiles, reflecting the contribution of SNPs to methylation differences across samples. Removing SNPs showed effects of reducing both false positive DMC (e.g. on chromosomes 1, 5, 10, and X) and false negative (e.g. on chromosomes 2, 11, 12, 15, 17, and 19). DMC identified on the Y chromosome came from pseudoautosomal regions.

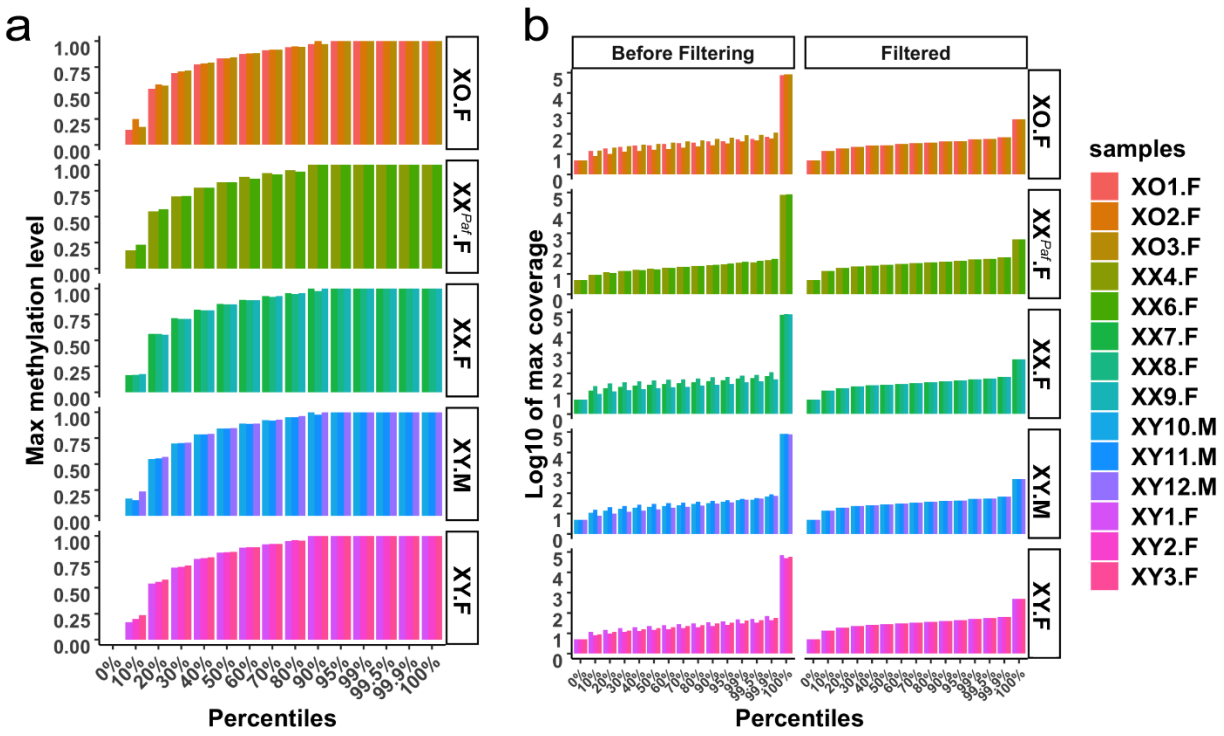

Figure S3. Comparable distributions of methylation levels and removal of CpG sites with extremely high coverage across all 14 samples.

X-axis represents the percentile of all CpG sites in each sample. Y-axis represents the **(a)** maximum methylation level and **(b)** maximum coverage (log10 value) of CpG site in each percentile. **(a)** Samples showed comparable distribution of methylation levels. **(b)** CpG sites with coverage > 500X were filtered to account for PCR bias.

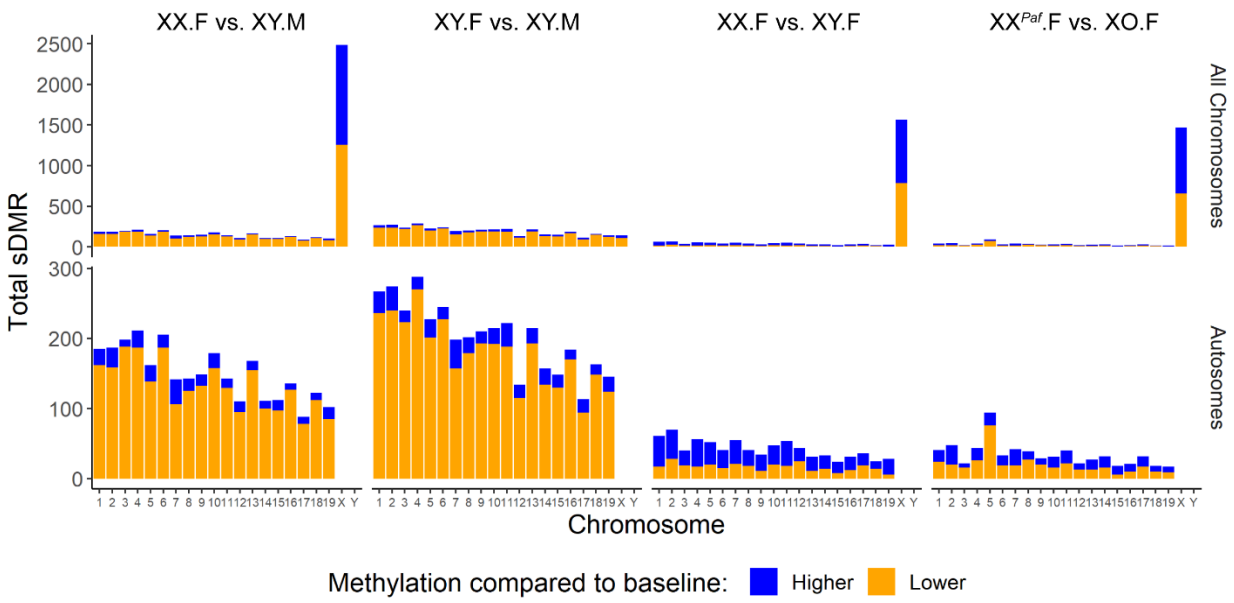

Figure S4. Differential distributions of sDMRs related to sex phenotype and sex chromosome complement.

Counts of sDMRs per chromosome in each comparison. Top row shows the distribution of sDMR across all chromosomes. Bottom row shows the autosomal sDMRs. The x-axis represents chromosome ID, and the y-axis represents the number of sDMRs. Orange portion of the bar corresponds to sDMRs with lower methylation compared to baseline. Blue corresponds to sDMRs with higher methylation level compared to baseline. Baseline samples are XX.F (XX.F vs XY.M), XY.F (XY.F vs XY.M), XX.F (XX.F vs XY.F), and XX^{Paf}.F (XX.^{Paf}F vs XO.F).

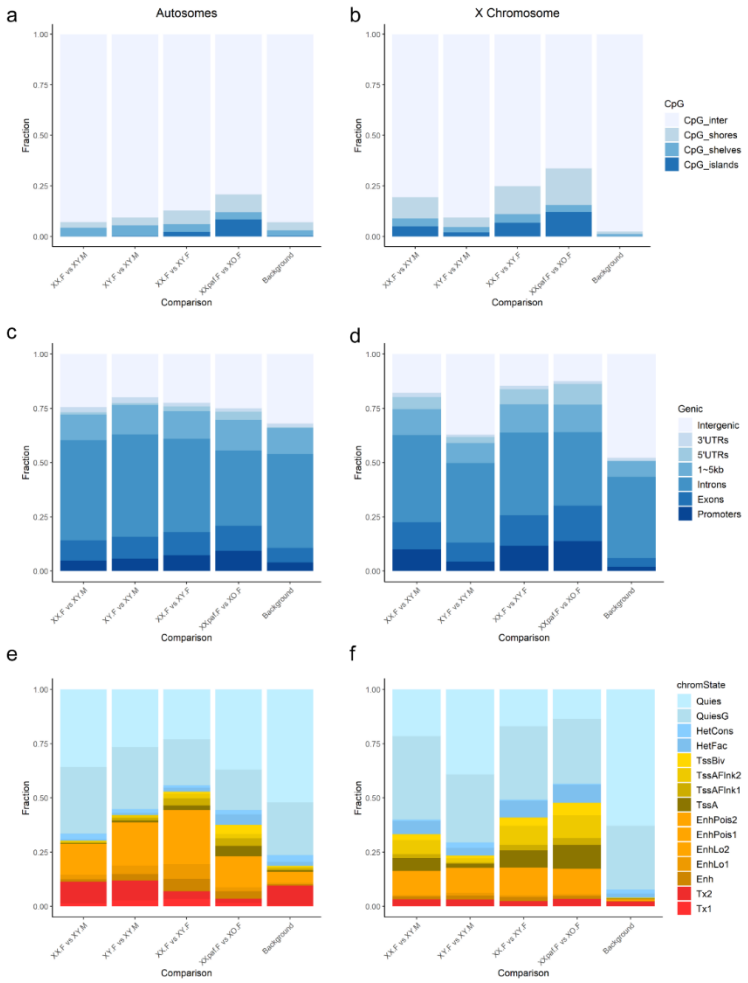

Figure S5. Distributions of sDMRs differ between autosomes and the X chromosome in CpG island annotation, genic annotation, and estimation of chromatin states.

Basic annotations of sDMRs in each of the four comparisons and a background group spanning all CpG sites on autosomes (left) and X chromosome (right). Each bar represents either sDMR from one comparison or an expected distribution estimated from all CpG sites. The y-axis represents the fraction (relative frequency) of sDMRs or CpG sites overlapped with different annotated regions. **a-b**. CpG island (CGI) annotations. CpG shores are defined as 2Kb upstream/downstream from the end of CpG islands and CpG shelves are defined as another 2Kb upstream/downstream from the farthest limit of CpG shores. **c-d.** Genic annotations included 1-5Kb upstream of the TSS (1~5kb), the promoter (< 1Kb upstream of the TSS), 5'UTR, exons, introns, and 3'UTR. In all four comparisons, sDMR overlapped with promoters and exons more frequently than the background. **e-f** Annotations of chromatin states estimated with chromHMM.

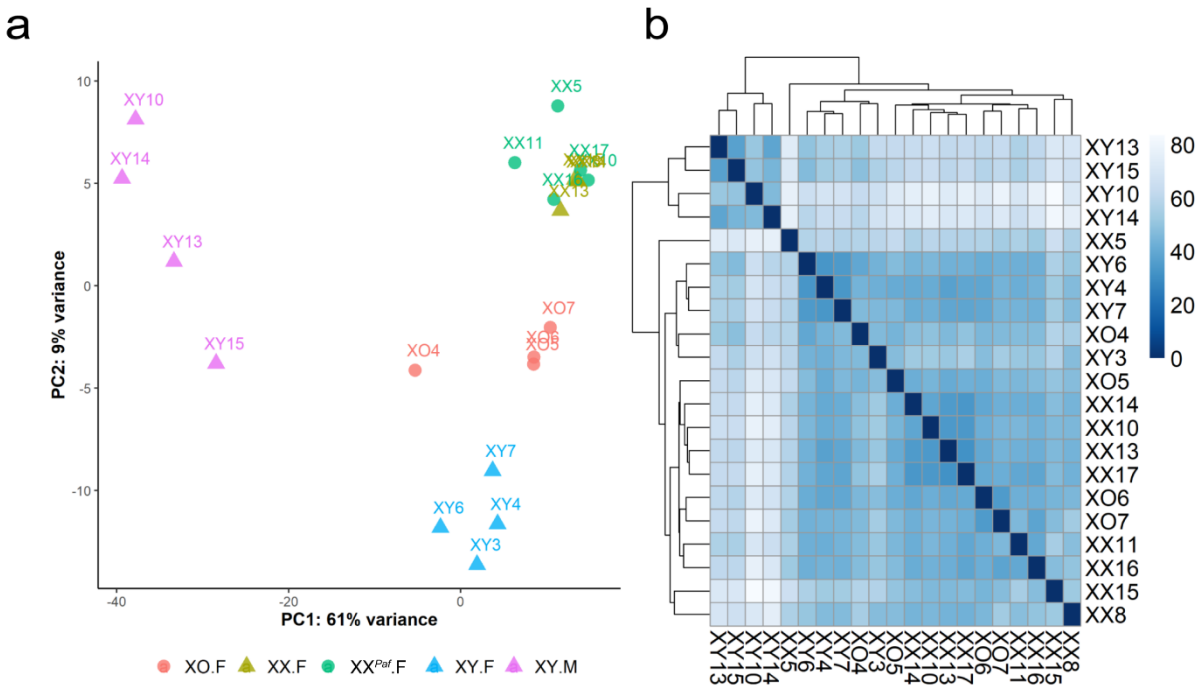

Figure S6. Quality assessment of RNA-seq data for samples illustrates sex-biased gene expression associated with phenotypic sex and sex-chromosome complement.

a. Plot of the first two Principal Components (PCs) in a principal component analysis of the RNA-seq data. Shapes represent strains and each color represents one sample group. A total of 70% of the variance is explained by the first two PCs in this plot. **b**. Heatmap shows the Euclidean distance between each sample, as well as their hierarchical clustering. Higher color intensity represented lower Euclidean distance, and vice versa.

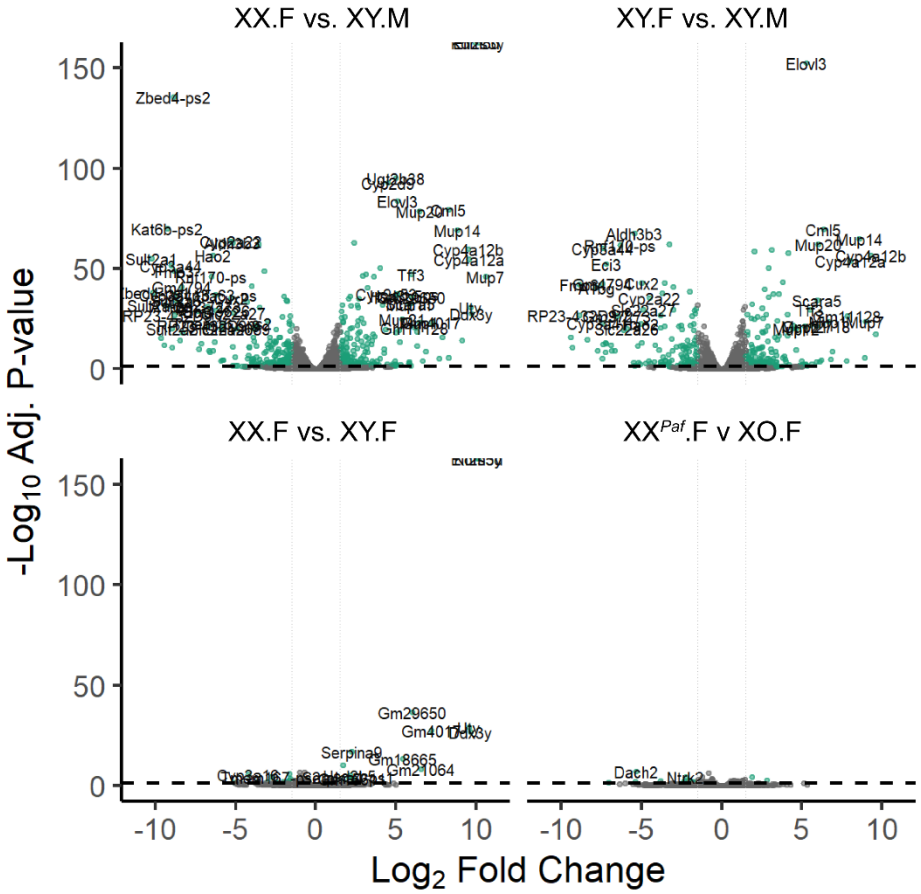

Figure S7: Differential gene expression analysis with EdgeR shows sex-associated DEG, especially in the comparisons XX.F vs XY.M and XY.F vs XY.M.

Volcano plots for differential gene expression (DGE) analysis using EdgeR. DEG (absolute $\log_2$ fold change > 1.5; adjusted P-value < 0.05) are shown with green color. The dotted line marks the point where the adjusted p-value is equal to 0.05. The comparisons XX.F vs XY.M and XY.F vs XY.M show a larger list of DEG than the comparisons XX.F vs XY.F and XX^{Paf}.F vs XO.F, which is consistent with the number of sDMR identified in respective comparisons.

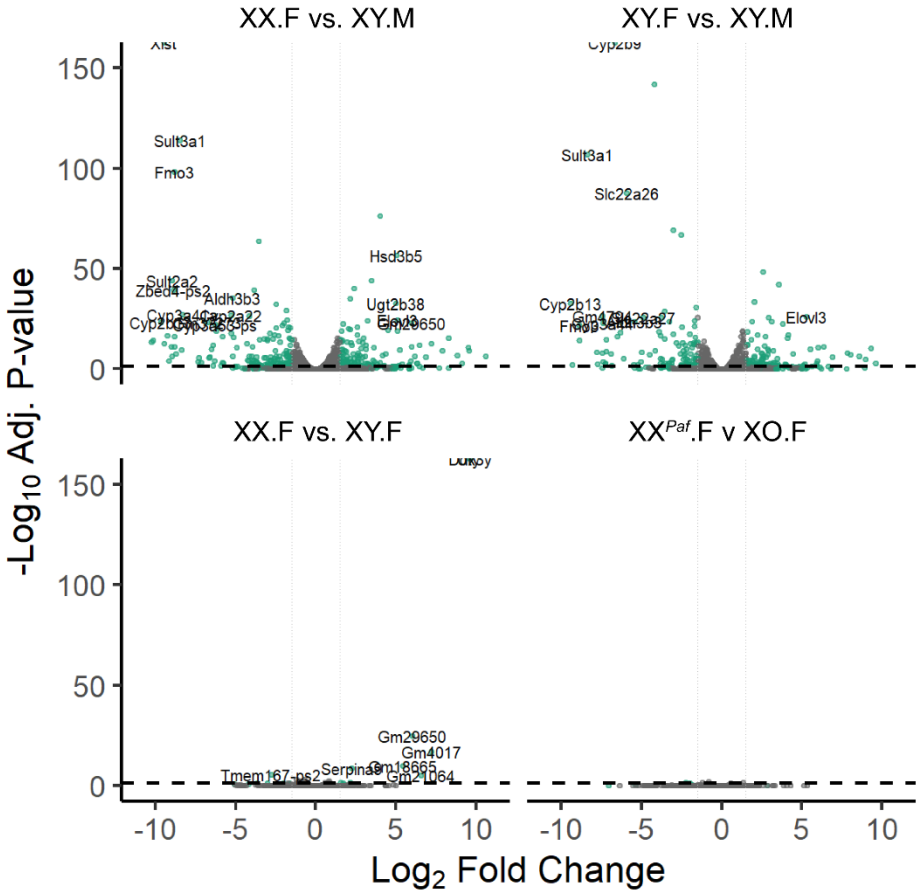

Figure S8. Differential gene expression analysis with DESeq confirms the result from EdgeR that more differentially expressed genes are found in the comparisons XX.F vs XY.M and XY.F vs XY.M.

Volcano plots for the differential gene expression (DGE) analysis using DESeq. Differentially expressed genes (DEG) (absolute $\log_2$ fold change > 1.5; adjusted P-value < 0.05) are shown with green color. The dotted line marks the point where the adjusted p-value is equal to 0.05. The comparisons XX.F vs XY.M and XY.F vs XY.M show more DEG than the comparisons XX.F vs XY.F and XX^{Paf}.F vs XO.F, which is consistent with the number of sDMR identified in respective comparisons.

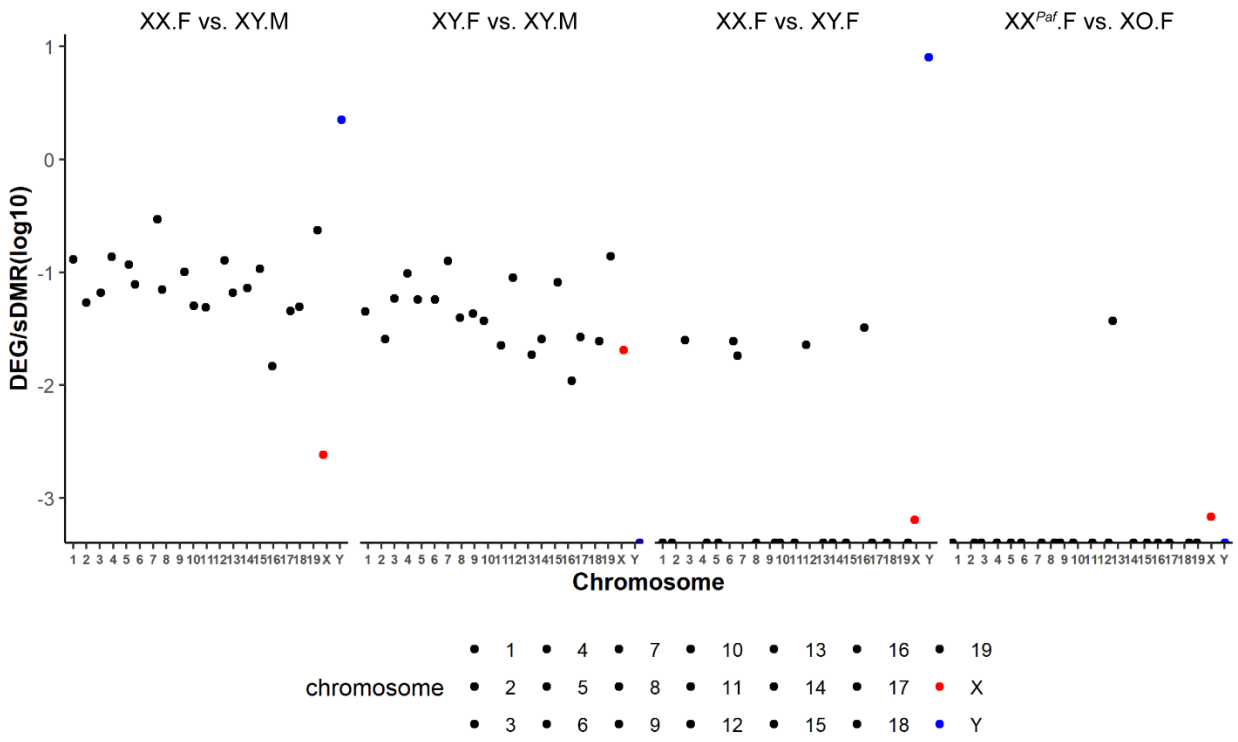

Figure S9. Ratio of numbers of DEG over the number of sDMRs across chromosomes**.**
The x-axis represents the chromosome index and the y-axis represents the ratio of DEG/sDMR (in log10) on each chromosome. In comparisons between groups with different X-chromosome dosage (XX.F vs XY.M, XX.F vs XY.F, and XX^{Paf}.F vs XO.F), the ratios of DEG/sDMR(log10) on the X chromosome were smaller than that on autosomes. Black dots correspond to autosomes, red dots to the X chromosome, blue dots- the Y chromosome.

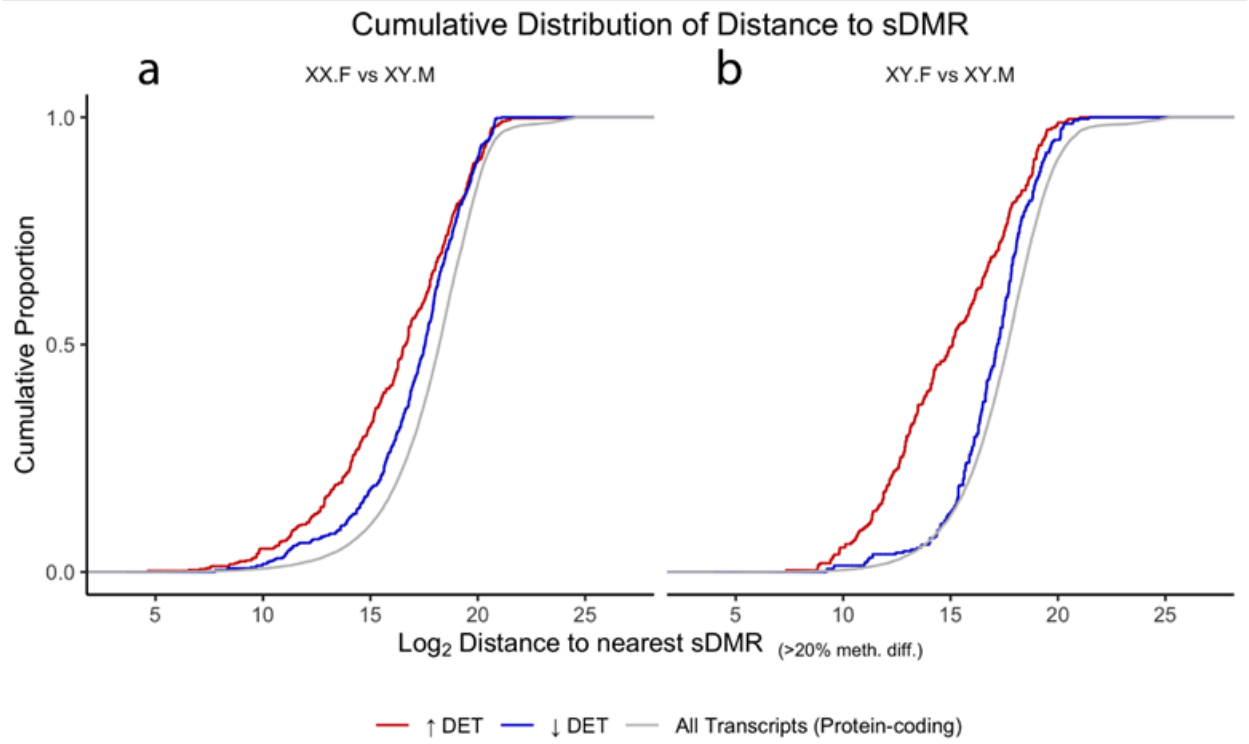

Figure S10. sDMRs are enriched around the TSS of transcripts that are significantly over-expressed in phenotypic males (XY.M).

a. Cumulative distribution of differentially expressed transcripts (DET) and sDMRs detected when comparing XX.F and XY.M. **b**. Cumulative distribution of DETs and sDMRs detected when comparing XY.F and XY.M. Cumulative distribution plot of the $\log_2$ distance (in bp) from the transcription start site (TSS) of a DET to the nearest sDMR (>20% methylation difference). Only protein-coding transcripts are included. The red line represents upregulated transcripts (higher abundance in phenotypic males), whereas the blue line represents downregulated transcripts (lower abundance in phenotypic males).