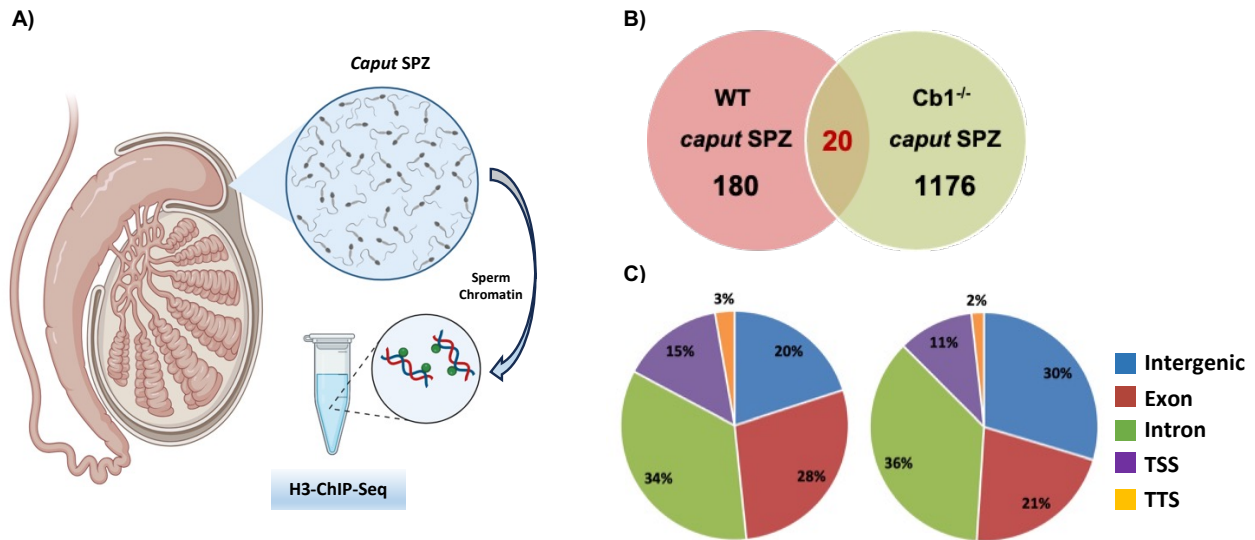


## *Supplementary Material*

### **Supplementary Data: the impairment of histone displacement leads to H3-enriched sperm chromatin**

Chromatin immunoprecipitation sequencing (ChIP-seq) experiments using the anti-H3 antibody were performed in WT and Cb1<sup>-/-</sup> SPZ collected from *caput* epididymis to corroborate the abnormal histone retention characterized in Cb1<sup>-/-</sup> mice. The *caput* SPZ were chosen as accurately reflects the outcomes of histone displacement events occurring in the testis (Fig. S 3A). We excluded Cb1<sup>+/-</sup> SPZ from this analysis since histone displacement efficiently occurs in Cb1<sup>+/-</sup> mice.

Chip-Seq analysis revealed significant differences in the number of H3-marked chromatin regions between WT and Cb1<sup>-/-</sup> *caput* SPZ. In particular, the analysis of H3 signals showed a total of 1335 H3-associated peaks consisting in 180 and 1176 peaks, respectively detected in WT and Cb1<sup>-/-</sup> *caput* SPZ, and 20 peaks relatively to gene loci shared by both genotypes (Fig. S 3B). Such difference in the peak number appeared consistent with the increased H3 amount previously characterized in Cb1<sup>-/-</sup> *caput* SPZ (Chioccarelli et al., 2020b), demonstrating that the defective histone displacement in Cb1<sup>-/-</sup> mice promoted the production of SPZ with abnormally retained chromatin H3-binding sites. Moreover, the accurate description of H3 signals detected in WT and Cb1<sup>-/-</sup> *caput* SPZ revealed that the identified peaks localized in different genomic regions with differences in genic (exons, introns and TSS) and in intergenic genomic location. Nearly 80% of H3-associated peaks were localized in the genic regions in WT *caput* SPZ, while approximately 70% of them were localized in the genic regions of Cb1<sup>-/-</sup> counterpart. Conversely, a reduced population of peaks was localized in intergenic regions of WT *caput* SPZ in comparison to that found in Cb1<sup>-/-</sup> counterpart (Fig. S 3C).



**Figure S2. (A)** Schematic representation of ChIP-seq analysis in WT and *Cb1*<sup>-/-</sup> *caput* SPZ. **(B)** Venn Diagram showing 180 chromatin peaks associated with H3 for WT *caput* SPZ, 1176 peaks for *Cb1*<sup>-/-</sup> *caput* SPZ and 20 peaks shared by both genotypes. **(C)** Pie chart analysis of separate samples showing the distribution of H3 peaks detected in WT and *Cb1*<sup>-/-</sup> *caput* SPZ among various genomic regions.