***eLife’s* transparent reporting form**

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. Authors can upload supporting documentation to indicate the use of appropriate reporting guidelines for health-related research (see [EQUATOR Network](http://www.equator-network.org/%20)), life science research (see the [BioSharing Information Resource](https://biosharing.org/" \t "_blank)), or the [ARRIVE guidelines](http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1000412) for reporting work involving animal research. Where applicable, authors should refer to any relevant reporting standards documents in this form.

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**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
* You should state the statistical method of sample size computation and any required assumptions
* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

For quantification of tubules with spermatids, 2 mice were analyzed for each genotype (*Hellsctrl* and *HellscKO*).

For quantification of TUNEL positive tubules, 1 mouse was analyzed for each genotype (*Hellsctrl* and *HellscKO*).

**Replicates**

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

For proteomic analysis, each IP and mass spec analysis was done once, excepted for IP/mass spec with mouse protein extracts treated with benzonase (Mouse testis rep2) which were done in duplicates.

For yeast two hybrid assays, for each combination of constructs, two diploids were tested.

For DMC1 ChIP-seq, experiments were done in duplicate, see Methods and Figure 3- figure supplement 1.

For 5hmC, experiments were done in duplicate, see Figure 4- figure supplement 2.

Proteomic data is available on the Pride Archive (<https://www.ebi.ac.uk/pride/archive> ).

PXD017337.

**Username:** [reviewer86230@ebi.ac.uk](mailto:reviewer86230@ebi.ac.uk)

**Password:** mHSXnRSX

NGS data has been deposited at GEO (GSE145768)

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE145768>

reviewer token:

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**Statistical reporting**

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

Comparison of qPCR data (Figure 3F) was performed by two-tailed Mann Whitney test. The p value is reported in the legend. The raw data is in Figure 3- source data file 1.

Comparisons of PRDM9, H3K4me3 and DMC1 signal presented in Figure 4- figure supplement 2 were performed by Kruskal-Wallis test corrected for multiple comparisons. Results are provided in Supplementary File 4.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

**Group allocation**

* Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
* Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

**Additional data files (“source data”)**

* We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
* Where provided, these should be in the most useful format, and they can be uploaded as “Source data” files linked to a main figure or table
* Include model definition files including the full list of parameters used
* Include code used for data analysis (e.g., R, MatLab)
* Avoid stating that data files are “available upon request”

Please indicate the figures or tables for which source data files have been provided:

**Table 1- source data File 1.** Purification of protein complexes

**Figure 2- source data file 1**. Quantification of spermatid and TUNEL positive sections

**Figure 3- source data file 1**. PRDM9 and H3K4me3 ChIP-qPCR