

## Supplemental Figures

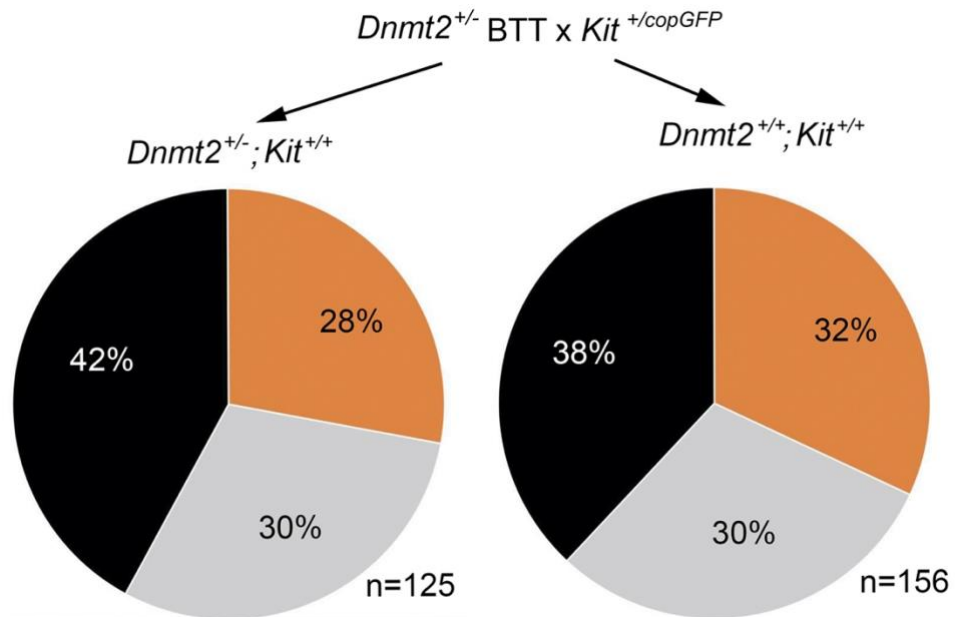
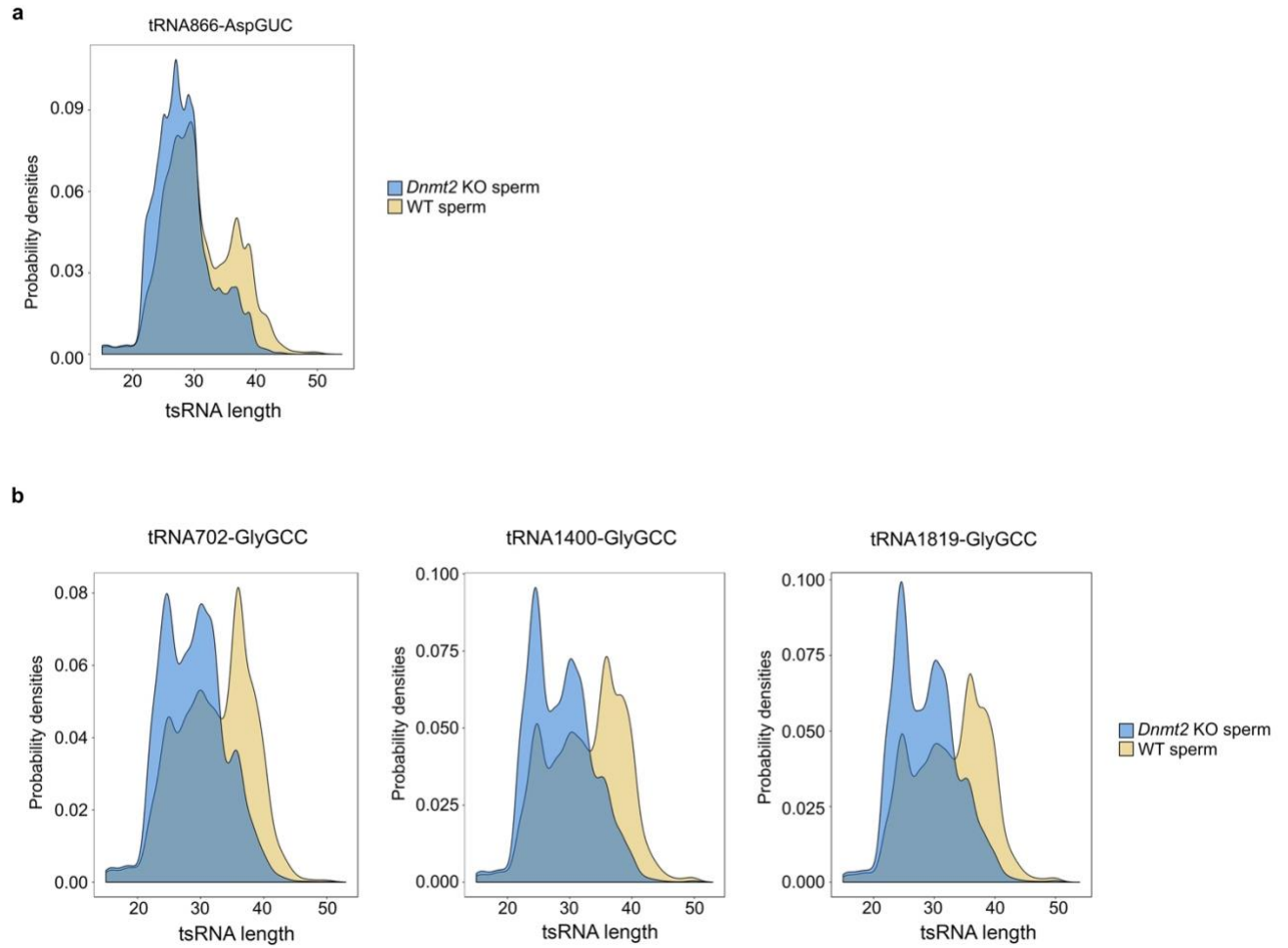
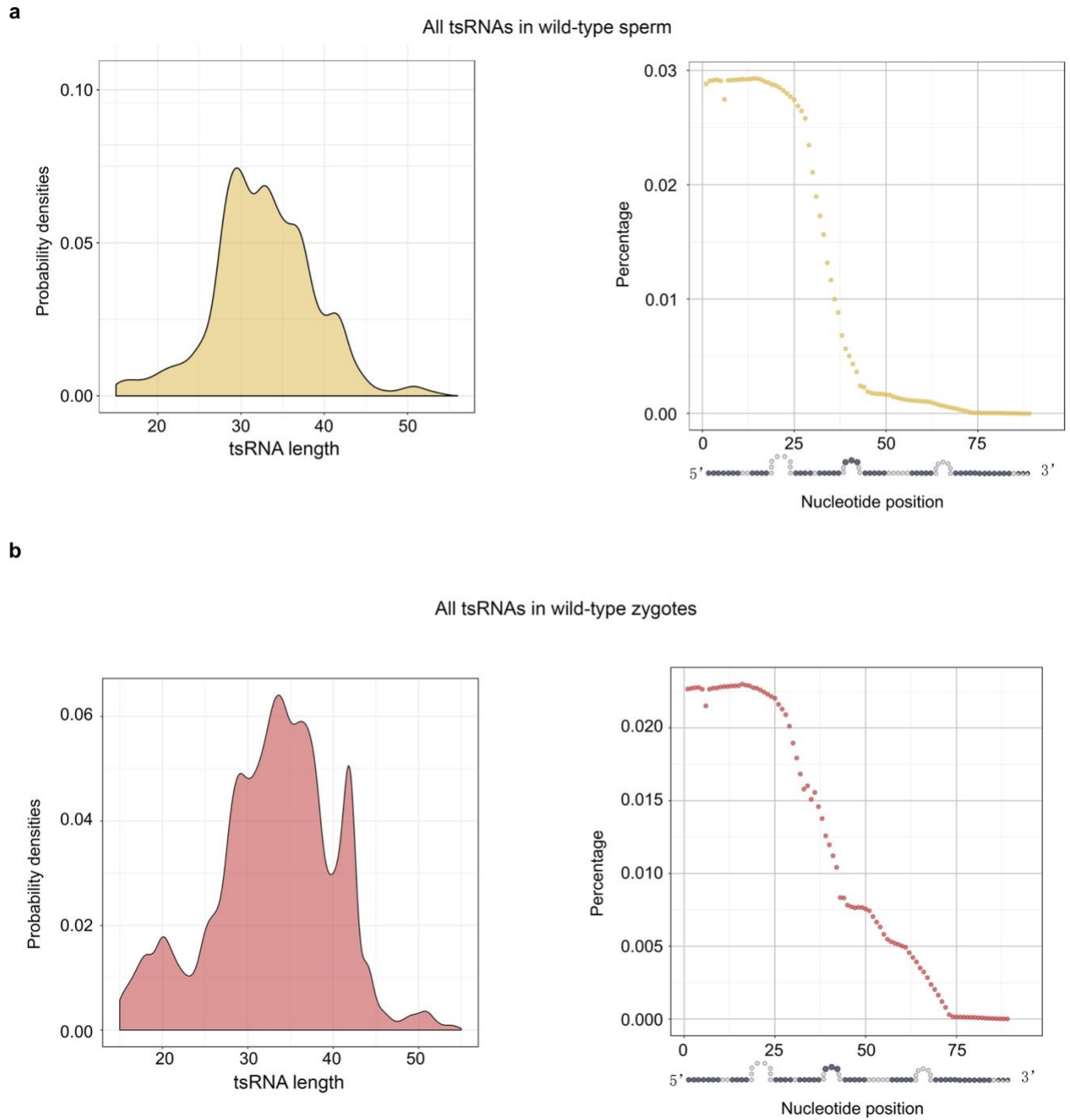


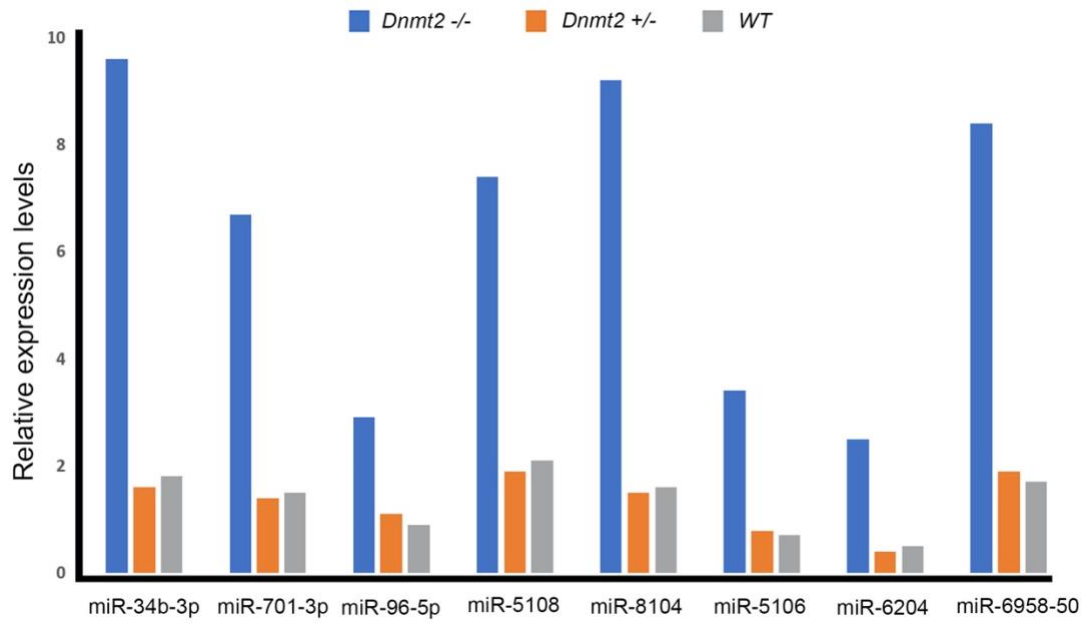
Figure S1. The penetrance of the WTT phenotype in  $Dnmt2^{+/-}; Kit^{+/+}$  and  $Dnmt2^{+/-}; Kit^{+/+}$  offspring derived from breeding pairs of  $Dnmt2^{+/-}$  males and  $Kit^{+/copGFP}$  females.



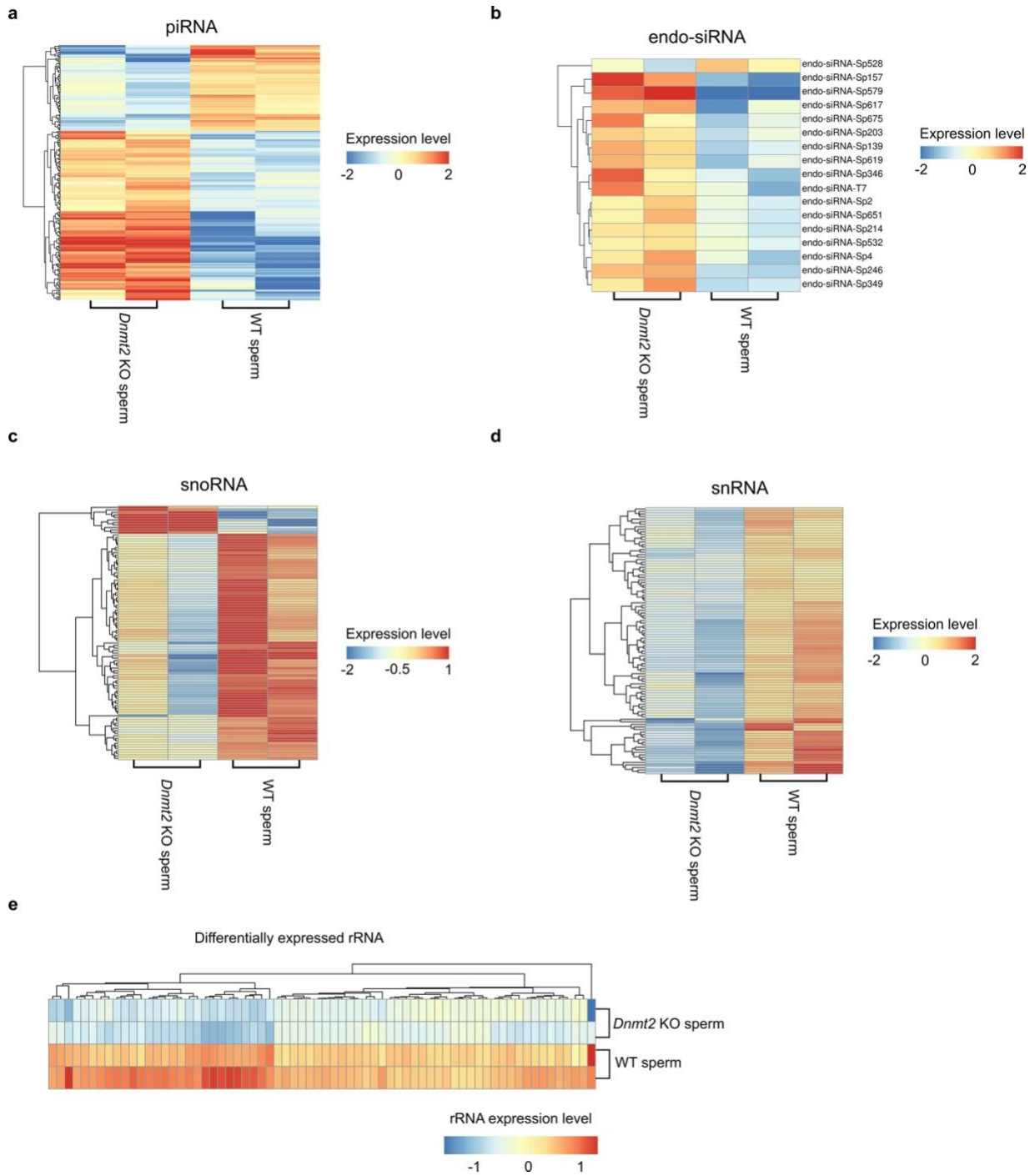
**Figure S2. Length distribution of four tsRNAs derived from tRNA-Asp<sub>GUC</sub> (a) and tRNA-Gly<sub>GCC</sub> (b) in wild-type (WT) and *Dnmt2* knockout (KO) sperm.**



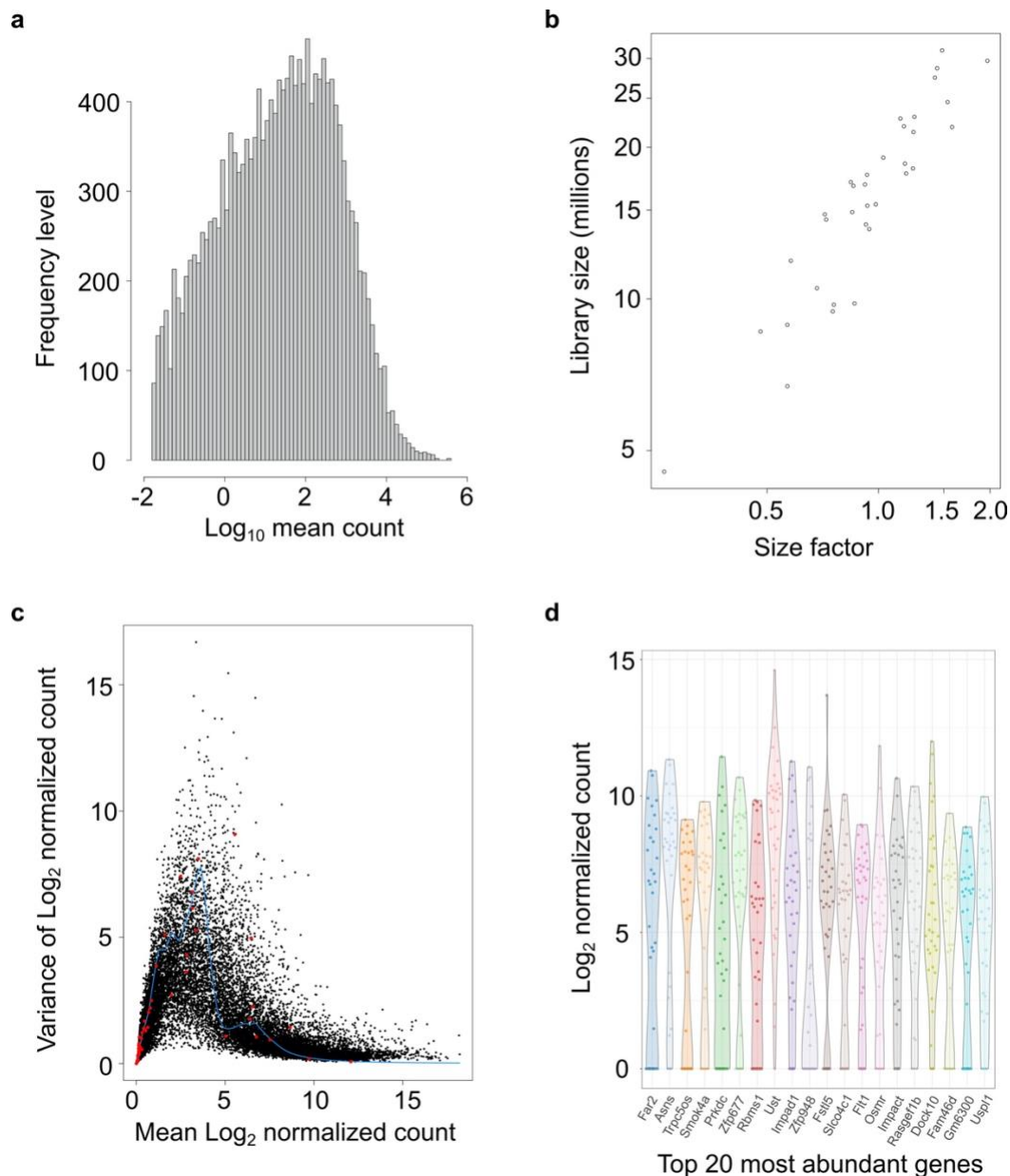
**Figure S3. Length distribution and nucleotide position percentage of all tRNAs from wild-type sperm (a) and zygote (b).** The datasets used were from a report by Yang, et. al., (Science Advancement, 2016, 2(6); doi: 10.1126/sciadv.1501482).



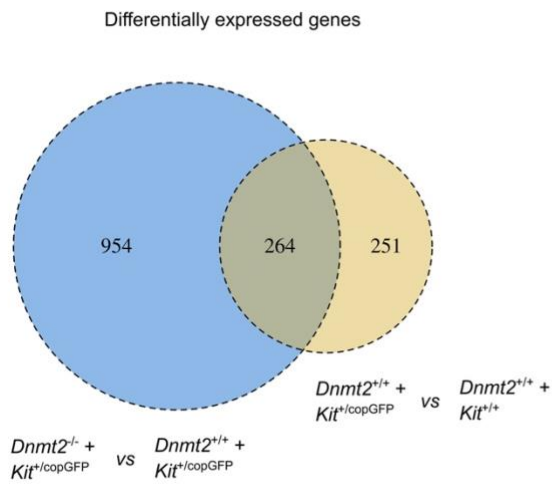
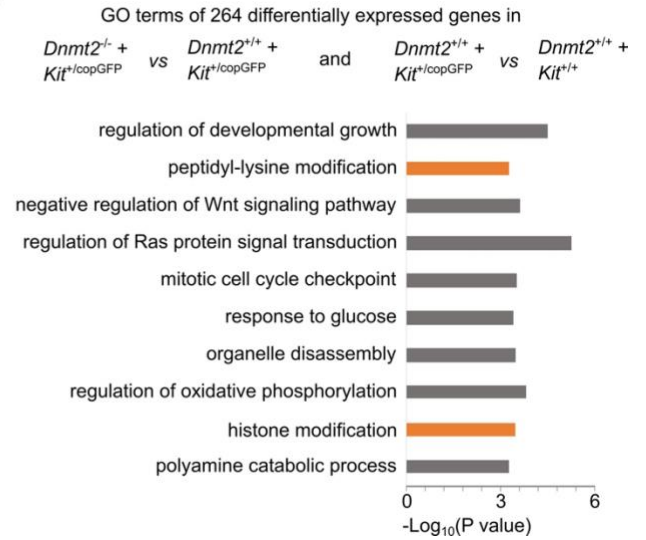
**Figure S4. qPCR analyses of the levels of eight miRNAs in wild-type (WT), *Dnmt2*<sup>+/-</sup> and *Dnmt2*<sup>-/-</sup> sperm.**



**Figure S5. Dysregulated piRNAs (a), endo-siRNAs (b), snoRNAs (c), snRNAs (d), and rRNAs (e) in *Dnmt2* KO sperm compared to WT sperm. For detailed lists of these sncRNAs, please see Table S2.**



**Figure S6. Quality control data of single zygote RNA-seq analyses.** (a) Histograms showing the  $\log_{10}$  values of mean counts of the filtered gene dataset. (b) Scatter plots with the size factor from deconvolution plotted against the library size for all cells in the dataset. (c) Scatter plots with variance of the filtered and normalized  $\log_2$  counts plotted against the mean  $\log_2$  normalized counts. The blue line indicates the mean-dependent trend fitted to the variances of the endogenous genes. The red dots represent the variance estimates for the spike-in transcripts. (d) Violin plots of normalized  $\log$ -expression for the top 20 highly variable genes in filtered and normalized gene datasets.

**a****b**

**Figure S7. Differentially expressed genes between zygotes derived from *Kit<sup>+/copGFP</sup>* oocytes injected with *Dnmt2*-null or WT sperm overlap with those between zygotes from *Kit<sup>+/copGFP</sup>* or WT oocytes injected with WT sperm (a) and GO term enrichment analyses of the 264 overlapping genes (b).**