Supplementary Figure Legends

Supplementary Fig. 1 Pearson correlation heatmap among methylomes of merged scRRBS data of 8 single mESC cells, pooled 5-, 10-, 20- mESC cells, and bulk amount of mESCs. Color key from green to red indicates correlation from low to high, respectively.

Supplementary Fig. 2 The number and proportion of CpG sites detected in merged single sperm RRBS dataset overlapped with those from RRBS of bulk amount of sperm.

Supplementary Fig. 3 The methylation profile of several representative loci covered in our scRRBS of 8 individual cells and bulk mESCs (A-C). The upward blue bars indicate the methylated CpG sites, whereas the downward red bars represent unmethylated CpG sites, respectively.

Supplementary Fig. 4 The average methylation levels of different annotated genomic regions across the scRRBS data of 8 individual mESC cells.

Supplementary Fig. 5 The methylation profiles of several representative loci in scRRBS data of 5 single sperm cells and bulk amount of sperm (A-D). The upward blue and downward red bars indicate methylated and unmethylated CpG sites, respectively.

Supplementary Fig. 6 The proportion of fully-methylated (≥90% methylated with reads depth ≥3) and unmethylated (≤10% methylated with reads depth ≥3) CpG sites detected in single mESC cells and bulk amount of mESCs.

Supplementary Fig. 7 The methylation profile of a representative locus in male pronuclei in our scRRBS dataset compared with that using bulk amount of cells from Meissner's lab (Smith et al. 2012).

A. The methylation profile of a representative locus on chromosome 3 in male pronuclei. The upward blue bars in the left panel represent fully-methylated CpG sites, whereas the downward red bars represent unmethylated CpG sites. The

green bar in right panel shows the average methylation levels of the CpG sites covered in this region.

B. The same region as displayed in panel A, which turned to be demethylated from sperm to 2-cell embryos in the published RRBS dataset from Meissner's lab (Smith et al. 2012). The upward blue bars in each track represent methylated CpG sites..

Supplementary Fig. 8 Methylation dynamics of male and female pronuclei in different annotated genomic regions measured by scRRBS analysis.

Supplementary Fig. 9 Isolation of male and female pronuclei and immunostaining of 5mC and 5hmC in the zygotes.

A. Piezo micromanipulator assisted biopsy of male and female pronuclei from a zygote. The arrows and arrow heads indicate female and male pronuclei, respectively. Staining using Hoechst 33342 revealed two separated pronuclei in the zygote. PN, pronucleus.

B. 5mC and 5hmC immunostaining of the zygotes. Mouse zygotes were whole-mount stained using anti-5-methylcytosine and anti-5-hydroxymethylcytosine antibodies. Male and female symbols indicate the male and female pronucleus, respectively. PB: polar body.

Supplementary Fig. 10 The size distribution of the single cell RRBS libraries assessed by Fragment Analyzer. The DNA fragment size in typical RRBS libraries is about 150 - 350 bp, with some visible peaks corresponding to the MspI fragments for some repeat elements, and some primer dimer contaminants (around 120 bp). Represent RRBS libraries of pooled 20 mESC cells (A), a single mESC cell (B), a single metaphase II oocyte (C), and a single male pronucleus (D) were shown.

Supplementary Fig. 11 The averaged mapping efficiencies of scRRBS of the single sperm cells and negative controls (transferring only carryover buffer into the lysate but omitting the single sperm cell).

Supplementary Table 1 Summary of unique covered CpG sites and their mean

coverage depths at 1×, 5× and 10× and the bisulfite conversion rate of the samples of metaphase II oocytes, polar bodies, and female and male pronuclei.

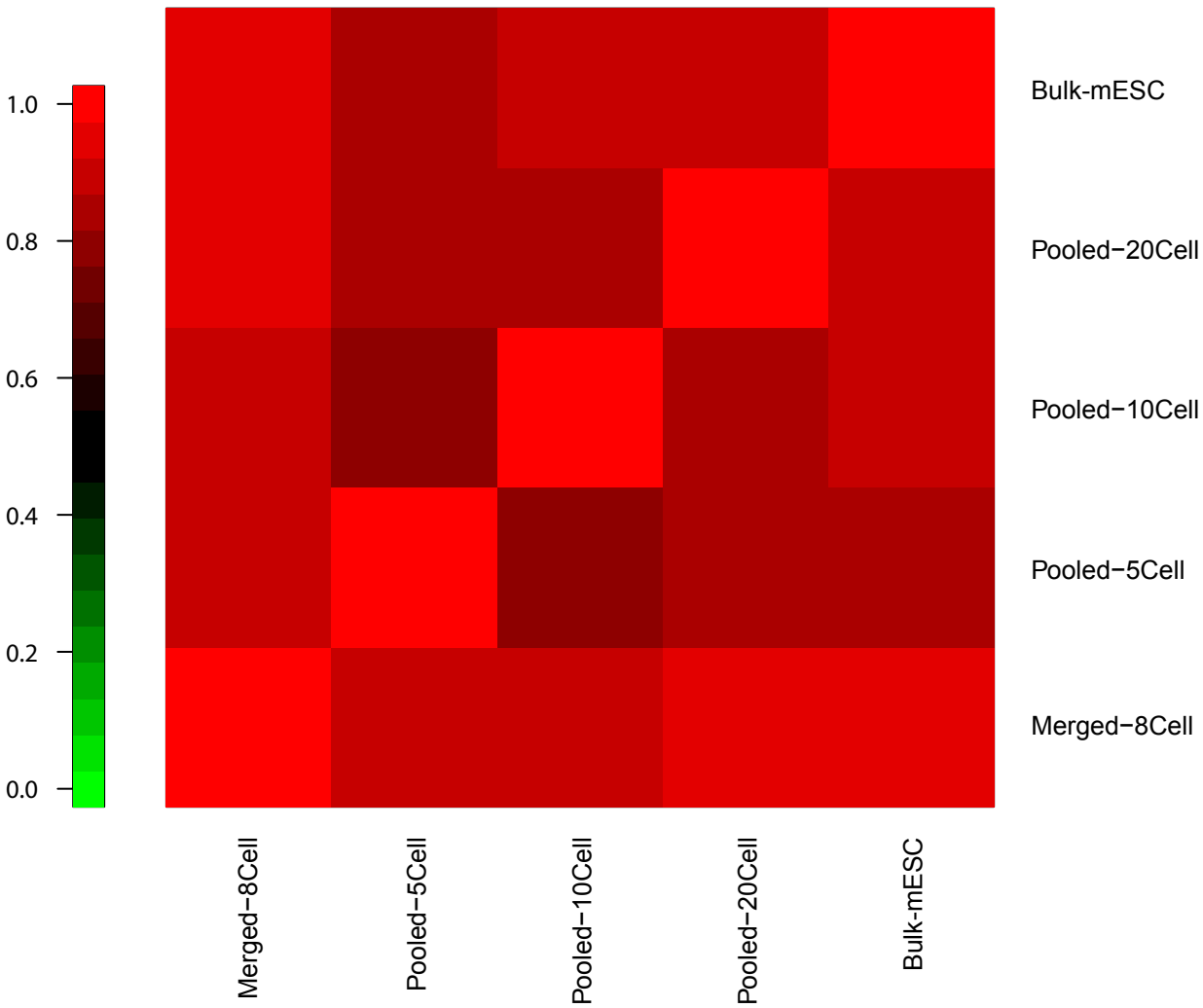
Supplementary Table 2 The number of CpG sites covered simultaneously in any two of the RRBS libraries of mESCs.

Supplementary Table 3 The number of CpG sites overlapped among merged different numbers of single mESC cell RRBS samples, pooled of 5-, 10-, 20-cells and bulk amount of ESCs.

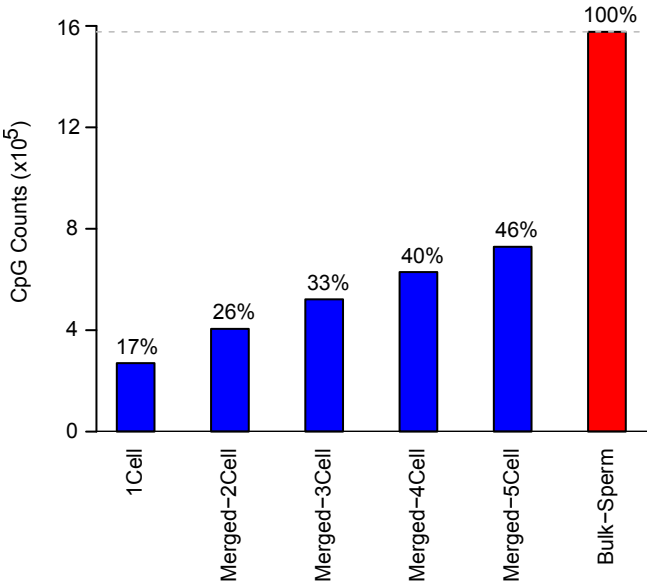
Supplementary Table 4 The DNA methylation levels of male and female pronuclei across different pronucleus stages in different annotated genomic regions.

Supplementary Table 5 The primer sequences for the single cell methylation sensitive restriction digestion coupled with nested PCR.

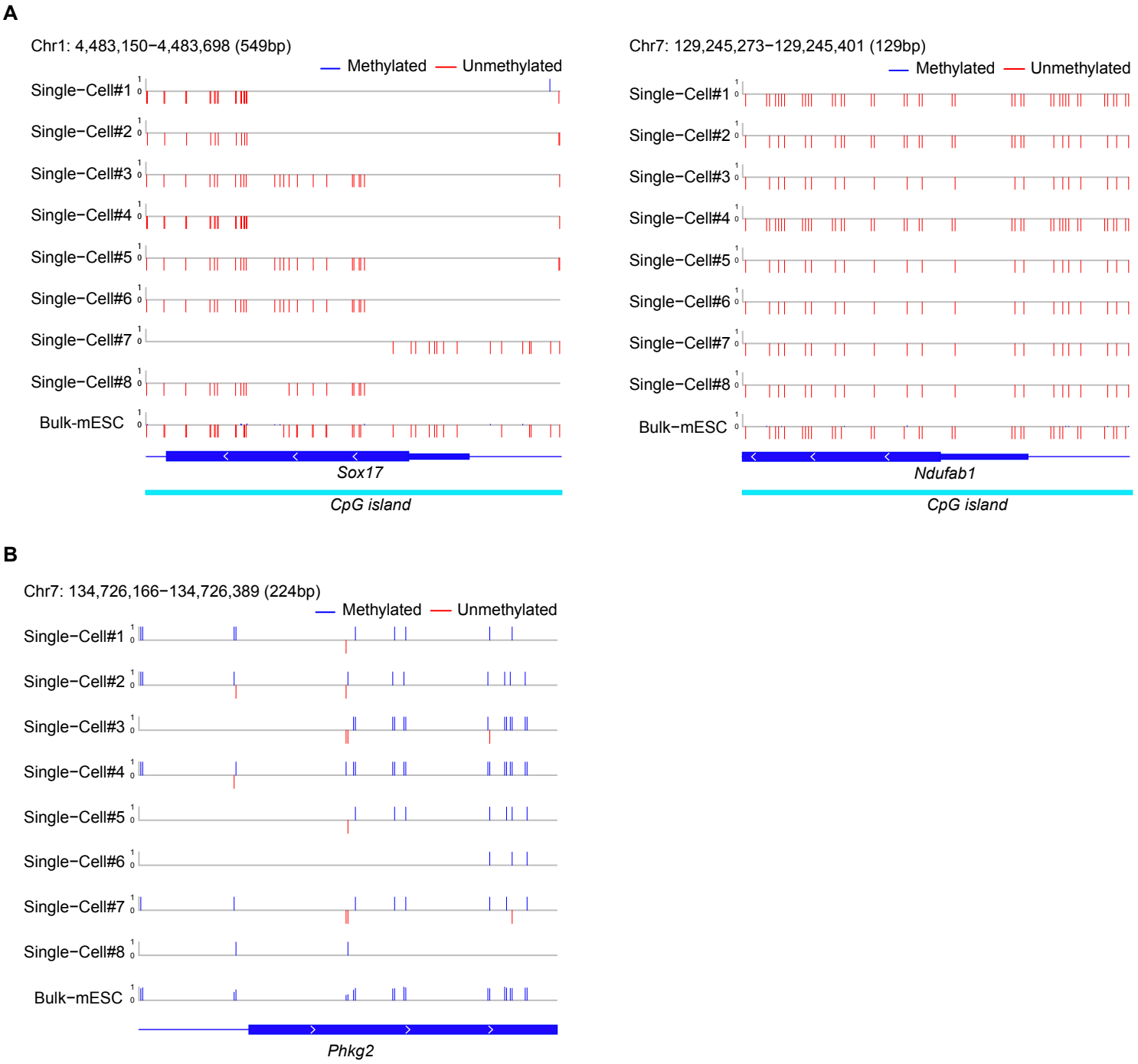
Supplementary Table 6 Summary of the sequencing qualities and reads mapping.



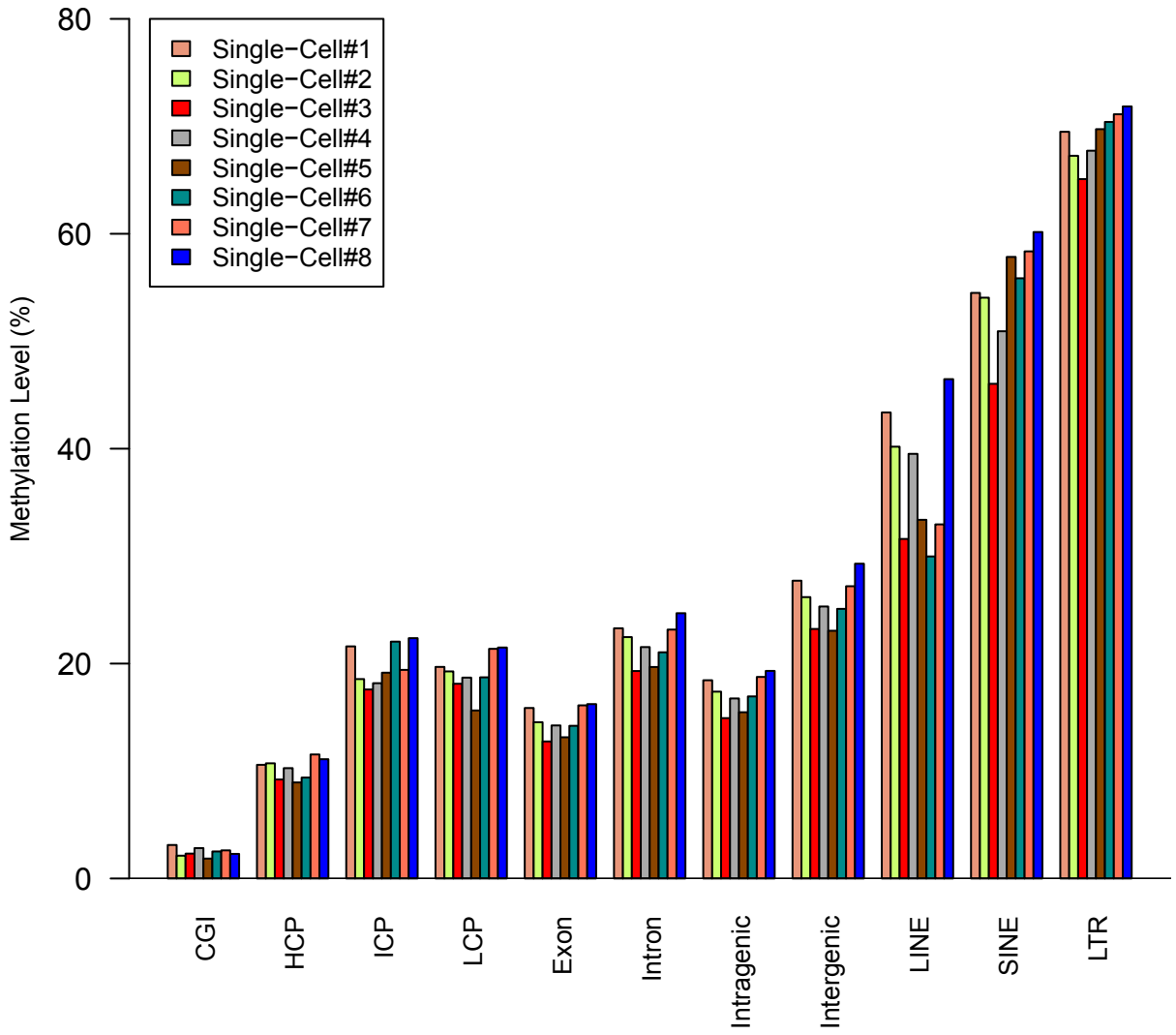
Supplementary Figure 1



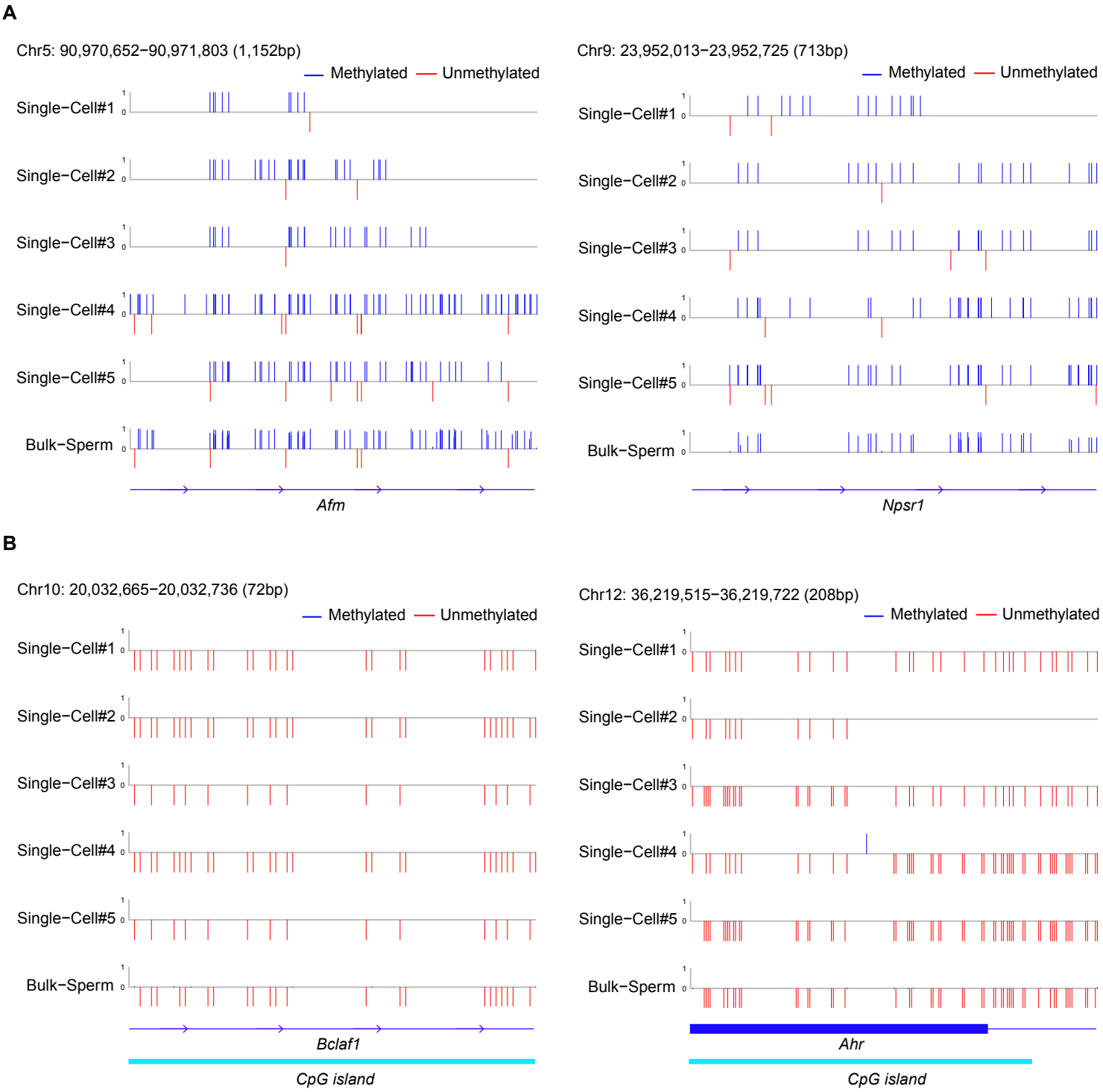
Supplementary Figure 2



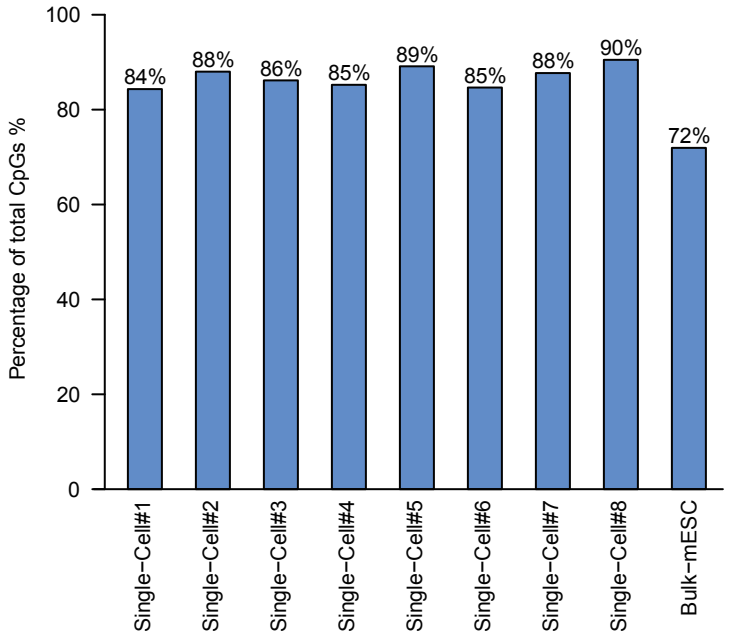
Supplementary Figure 3



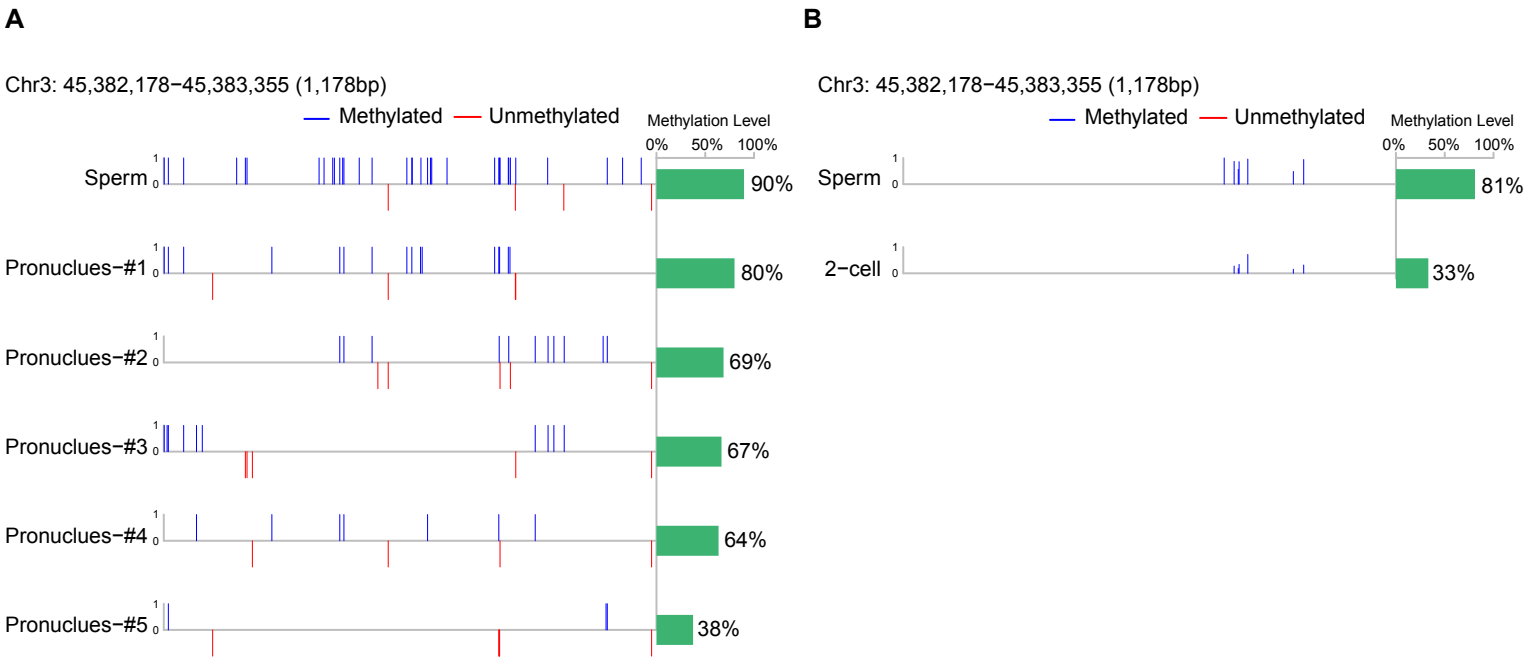
Supplementary Figure 4



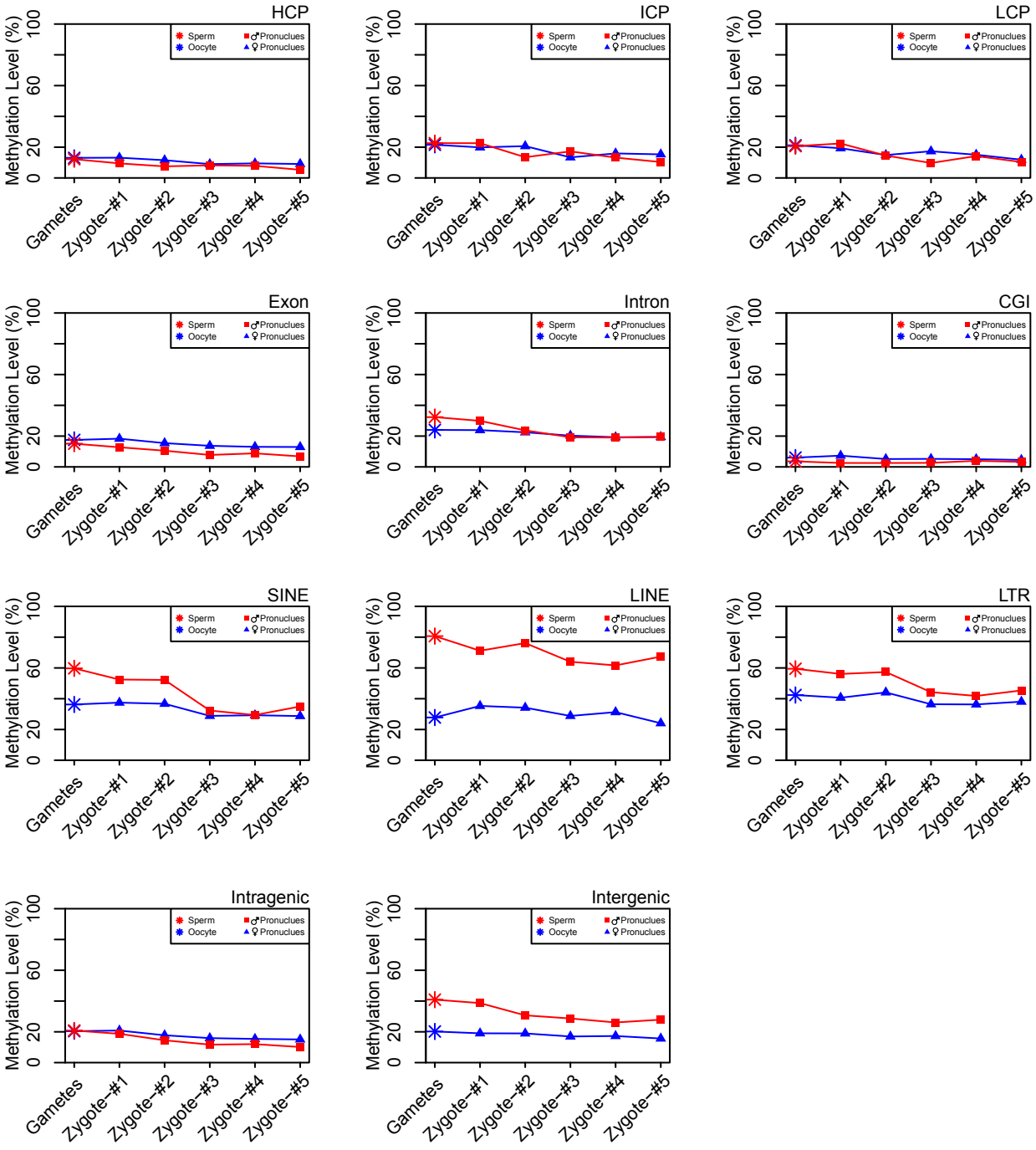
Supplementary Figure 5



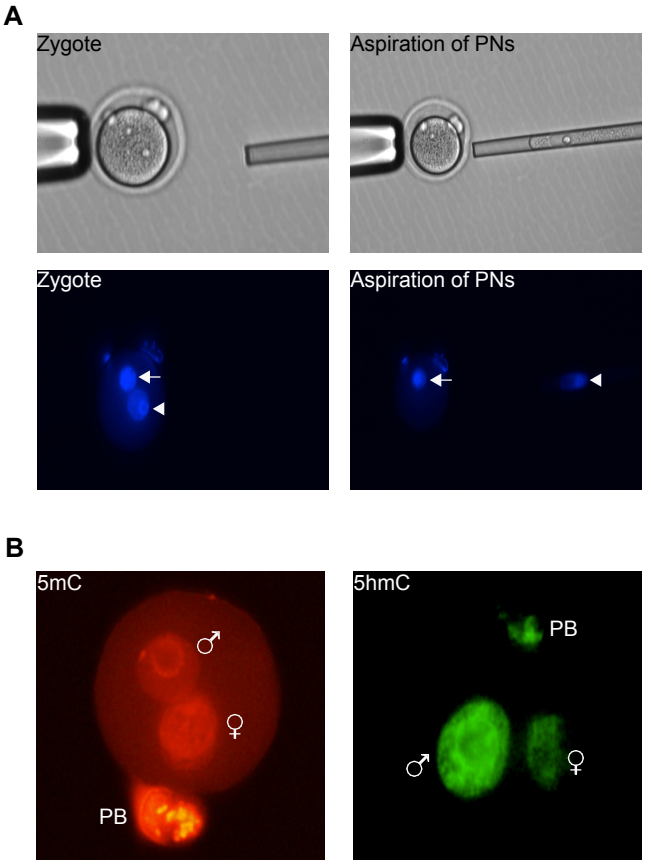
Supplementary Figure 6



Supplementary Figure 7

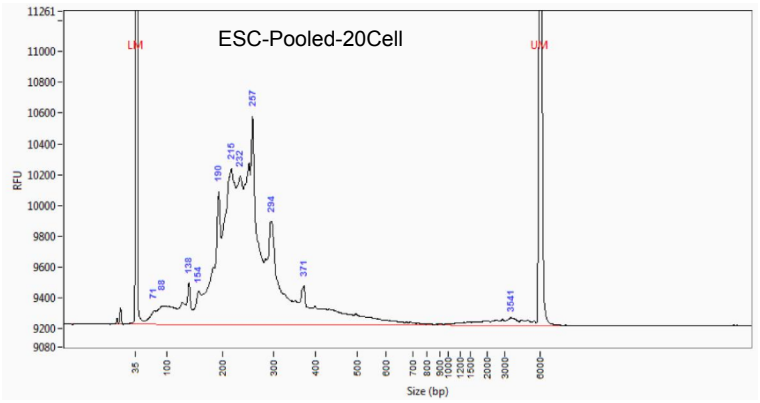


Supplementary Figure 8



Supplementary Figure 9

A



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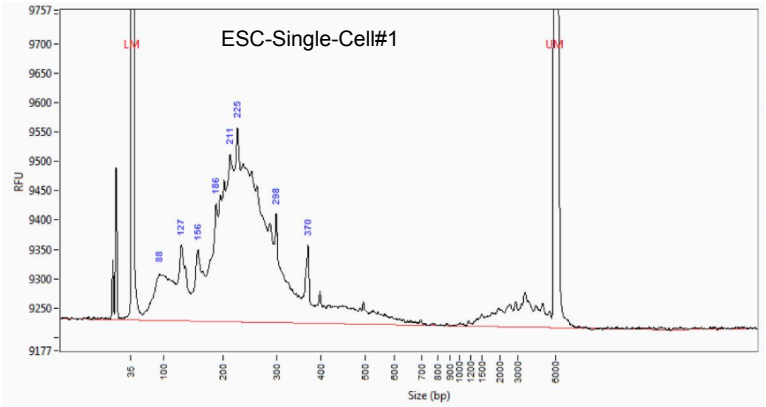

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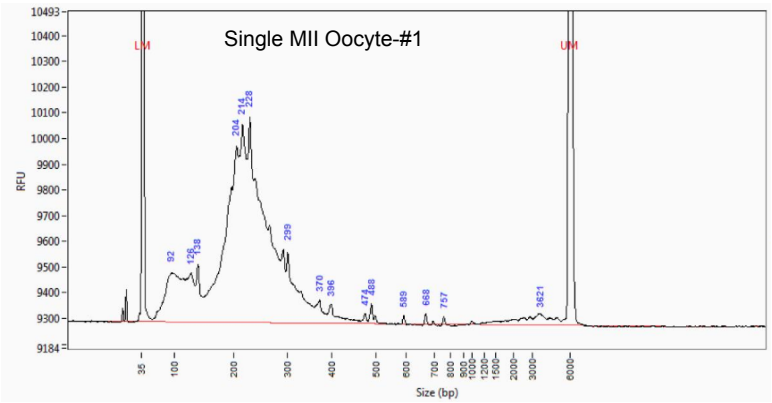

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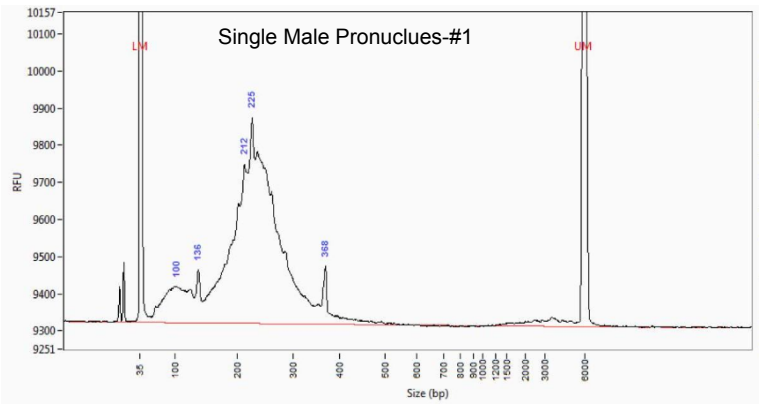
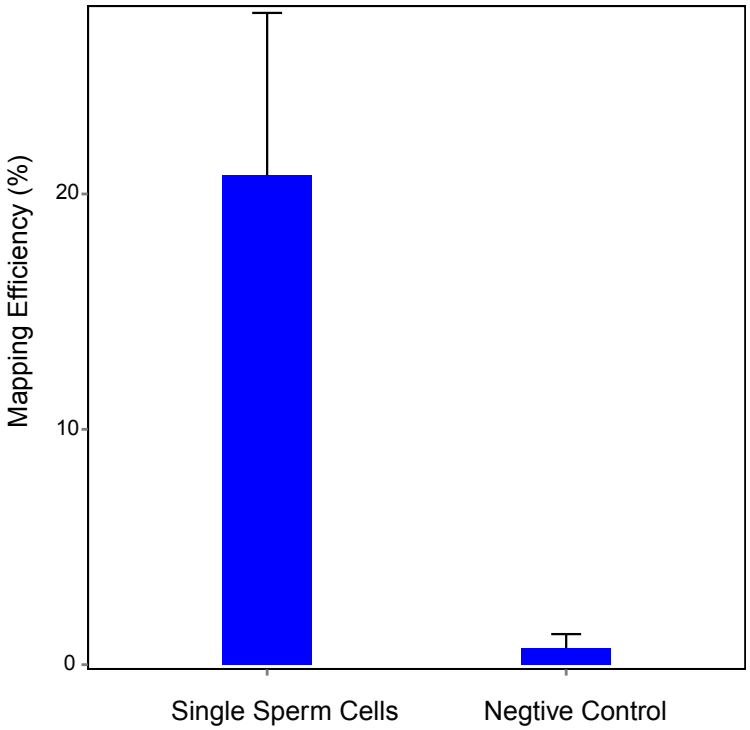


Supplementary Figure 10



Supplementary Figure 11