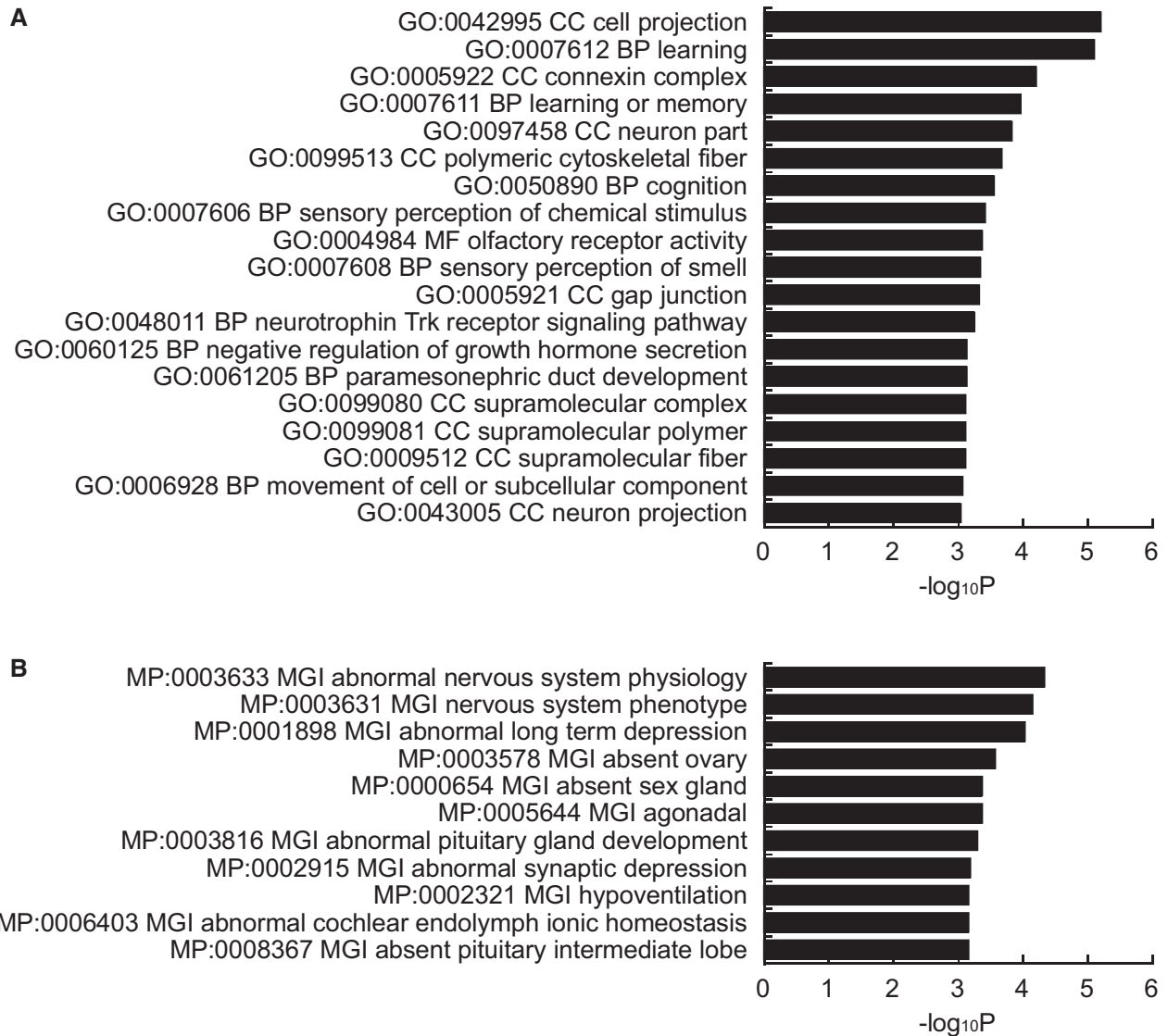


## Expanded View Figures

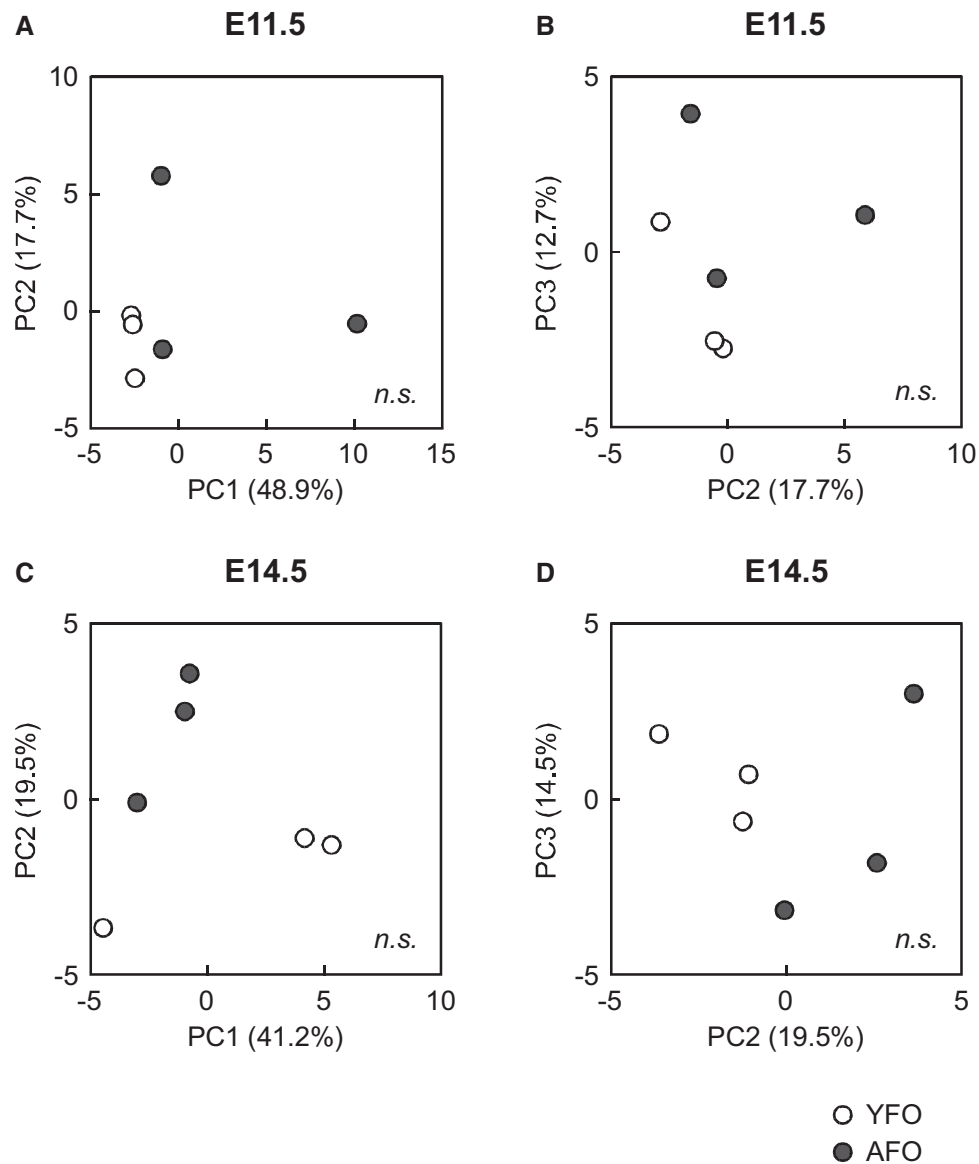


**Figure EV1. Gene ontology analysis of hypo-methylated DMRs.**

A Top 19 GO terms ( $P < 0.001$ ) associated with the biological process (BP), cellular component (CC), and molecular function (MF).

B Top 11 MGI phenotypes ( $P < 0.001$ ) of genes near hypo-methylated DMRs in sperm from aged mice. Both indicate that genes associated with neuronal function are enriched.

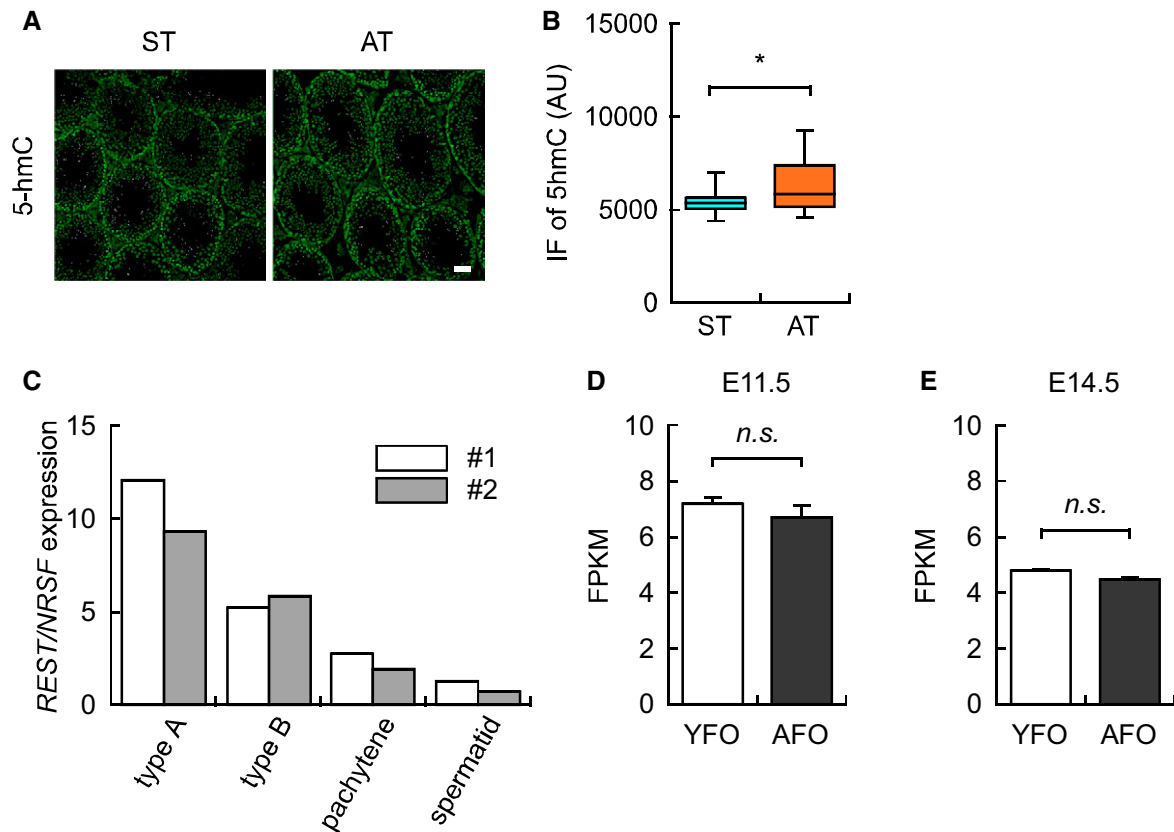
Data information: Data are presented in the bar graph format. Statistical significance was determined using Fisher's exact test.



**Figure EV2. PCA of the comprehensive transcriptome of offspring derived from young and aged fathers.**

- A A two-dimensional scatter plot of the PCA analysis of gene expression at E11.5. PC1 and PC2 accounted for 48.9 and 17.7% of the contribution of the variance, respectively.
- B A two-dimensional scatter plot of the PCA analysis of gene expression at E11.5. PC2 and PC3 accounted for 17.7 and 12.7% of the contribution of the variance, respectively.
- C A two-dimensional scatter plot of the PCA analysis of gene expression at E14.5. PC1 and PC2 accounted for 41.2 and 19.5% of the contribution of the variance, respectively.
- D A two-dimensional scatter plot of the PCA analysis of gene expression at E14.5. PC2 and PC3 accounted for 19.5 and 14.5% of the contribution of the variance, respectively.

Data information: Data are presented in the scatter plot format. The open circle indicates an individual YFO pattern, and a dark circle denotes an individual AFO pattern. PC1, principal component 1. PC2, principal component 2. PC3, principal component 3. Statistical significance was determined using the prcomp R package.



**Figure EV3. 5-Aza treatment globally increased 5-hydroxymethylcytosine levels in the testes.**

A Representative images of 5-hydroxymethylcytosine (5-hmC) in the testis of saline- and 5-Aza-treated mice (ST and AT, respectively). Scale bar: 50  $\mu$ m.

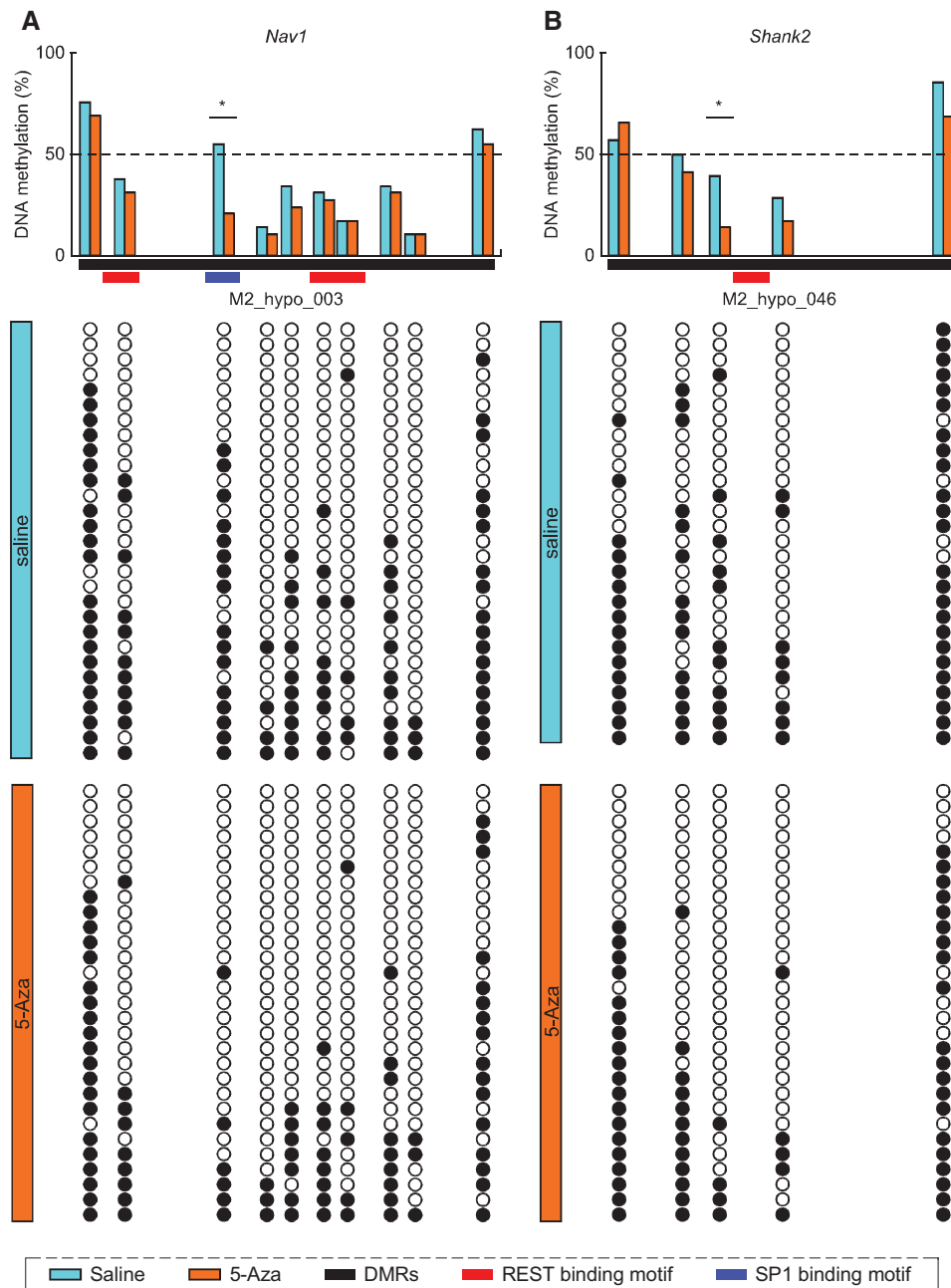
B Quantification of 5-hmC immunofluorescence in the testis of ST ( $n = 798$  spermatogonia from 4 male mice) and AT ( $n = 1,031$  spermatogonia from 4 male mice).

C Reanalysis of *REST/NRSF* expression in the germline cells (i.e., type A spermatogonia, type B spermatogonia, pachytene spermatocytes, and spermatid) in a public database of the testis ( $n = 2$ ) (Namekawa *et al*, 2006). The open and light bars indicate samples #1 and #2, respectively.

D *REST/NRSF* expression in E11.5 brains from YFO ( $n = 3$ , from different mothers) and AFO ( $n = 3$ , from different mothers) based on RNA-seq data.

E *REST/NRSF* expression in the E14.5 brains from YFO ( $n = 3$ , from different mothers) and AFO ( $n = 3$ , from different mothers) based on RNA-seq data.

Data information: Data are presented in the box plot (the central band in each box represents the median, boxes indicate the middle quartiles, and whiskers extended to the minimum and maximum values) or bar graph (mean  $\pm$  SEM) format. \* $P < 0.05$ , determined using Student's *t*-test.



**Figure EV4. 5-Aza treatment significantly induced DNA hypo-methylation in sperm genomic loci.**

- A Percentages of DNA methylation in DMRs (M2\_hypo\_003) near the *Nav1* gene (38.0% vs. 29.0% in Saline vs. 5-Aza, respectively;  $P < 0.05$ ) in sperm from young father with Saline (blue,  $n = 29$  from different 3 male) and 5-Aza treatment (orange,  $n = 29$  from different 3 male).
- B Percentage of DNA methylation of DMRs (M2\_hypo\_046) near *Shank2* gene (49.7% vs. 42.8% in Saline vs. 5-Aza, respectively;  $P < 0.05$ ) in sperm from young father with Saline (blue,  $n = 28$  from different 3 male) and 5-Aza treatment (orange,  $n = 29$  from different 3 male).

Data information: Horizontal red and blue bars indicate REST/NRSF and Sp1 binding motifs, respectively (for more detail, see Discussion). Black and open circles indicate methylated and unmethylated CpG sites, respectively. Statistical significance was determined by Fisher's exact test.

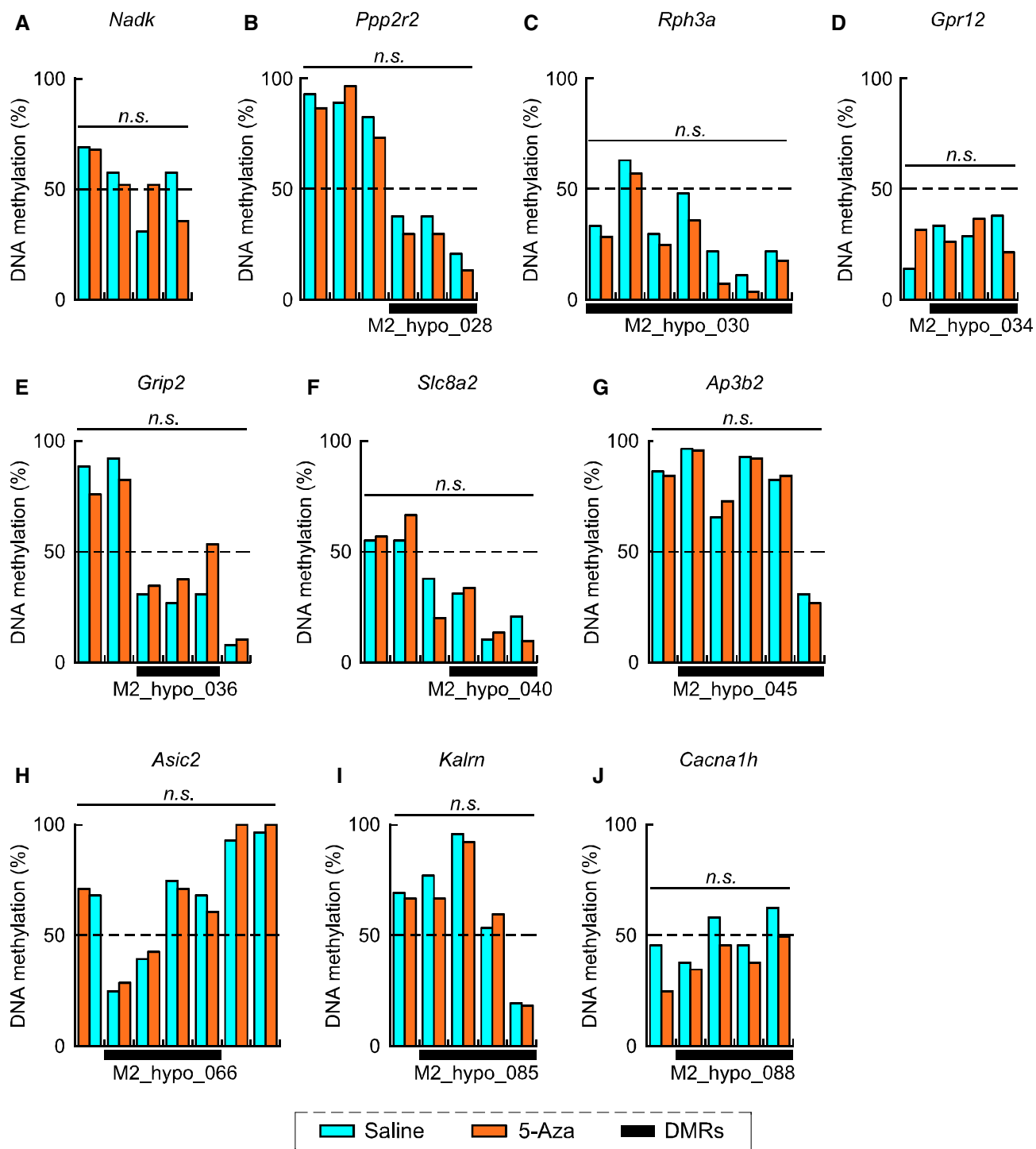


Figure EV5.

**Figure EV5. 5-Aza treatment-induced DNA hypo-methylation in sperm genomic loci at non-significant levels.**

- A Percentages of DNA methylation of CpG sites near *Nadk* (a control) (53.3% vs. 50.8% in saline vs. 5-Aza, respectively;  $P = 0.744$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- B Percentages of DNA methylation of DMRs (M2\_hypo\_028) near *Ppp2r2* (33.3% vs. 22.6% in saline vs. 5-Aza, respectively;  $P = 0.275$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- C Percentages of DNA methylation of DMRs (M2\_hypo\_030) near *Rph3a* (29.6% vs. 21.9% in saline vs. 5-Aza, respectively;  $P = 0.334$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- D Percentages of DNA methylation of DMRs (M2\_hypo\_034) near *Gpr12* (30.0% vs. 23.0% in saline vs. 5-Aza, respectively;  $P = 0.869$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- E Percentages of DNA methylation of DMRs (M2\_hypo\_036) near *Grip2* (29.6% vs. 40.7% in saline vs. 5-Aza, respectively;  $P = 0.654$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- F Percentages of DNA methylation of DMRs (M2\_hypo\_040) near *Slc8a2* (53.0% vs. 50.6% in saline vs. 5-Aza, respectively;  $P = 0.882$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- G Percentages of DNA methylation of DMRs (M2\_hypo\_045) near *Ap3b2* (69.1% vs. 69.6% in saline vs. 5-Aza, respectively;  $P = 0.578$ ) in sperm from young fathers with saline (blue;  $n = 30$ , from different 3 males) or 5-Aza treatment (orange;  $n = 29$ , from different 3 males).
- H Percentages of DNA methylation of DMRs (M2\_hypo\_066) near *Asic2* (51.8% vs. 49.2% in saline vs. 5-Aza, respectively;  $P = 0.812$ ) in sperm from young fathers with saline (blue;  $n = 28$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- I Percentages of DNA methylation of DMRs (M2\_hypo\_085) near *Kalrn* (55.6% vs. 56.3% in saline vs. 5-Aza, respectively;  $P = 0.821$ ) in sperm from young fathers with saline (blue;  $n = 29$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).
- J Percentages of DNA methylation of DMRs (M2\_hypo\_088) near *Cacna1h* (52.5% vs. 39.5% in saline vs. 5-Aza, respectively;  $P = 0.075$ ) in sperm from young fathers with saline (blue;  $n = 29$ , from different 3 males) or 5-Aza treatment (orange;  $n = 30$ , from different 3 males).

Data information: Data are presented in the bar graph format. Statistical significance was determined using Fisher's exact test.