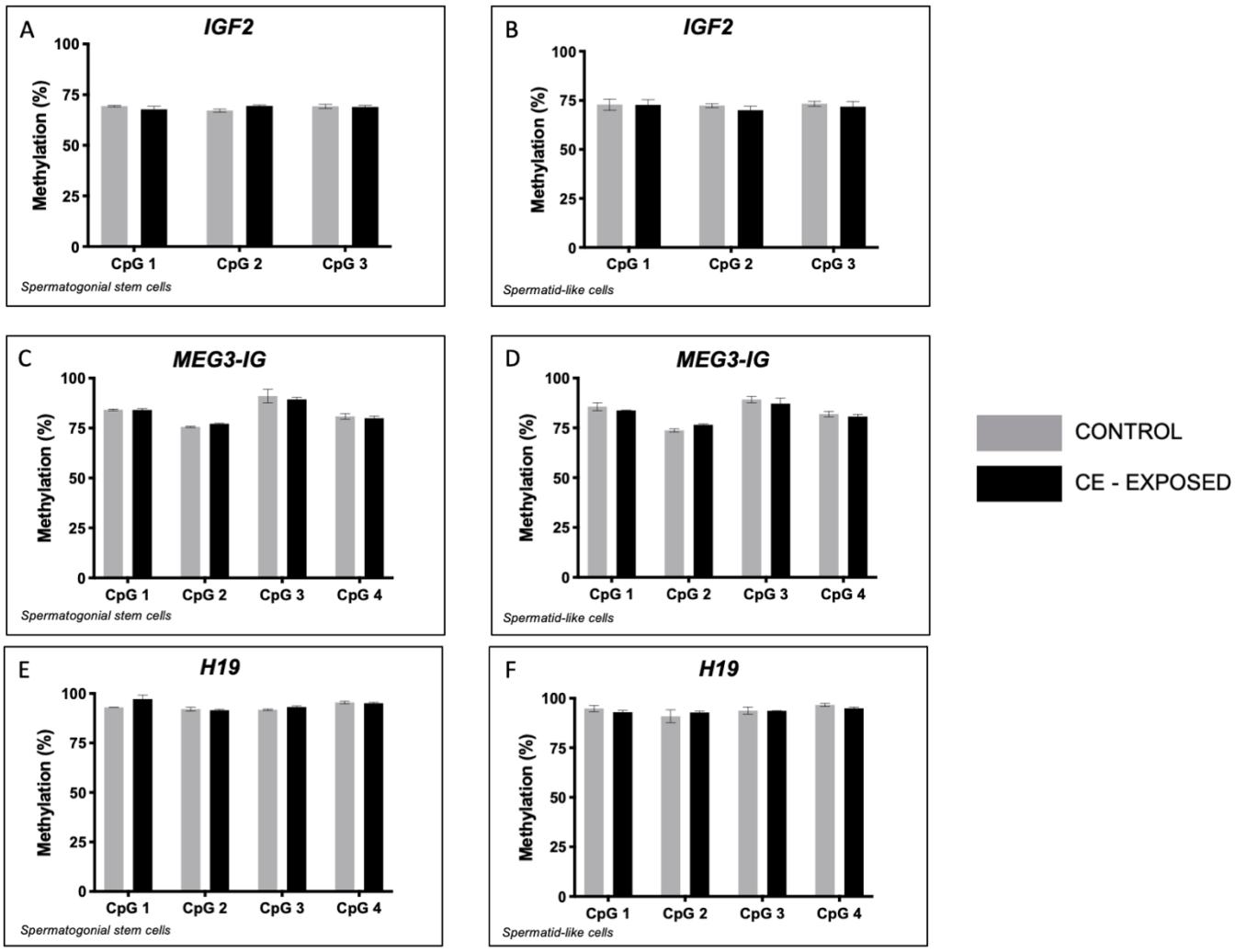
SUPPLEMENTAL INFORMATION

Cannabis alters DNA methylation at maternally imprinted and autism candidate genes in spermatogenic cells

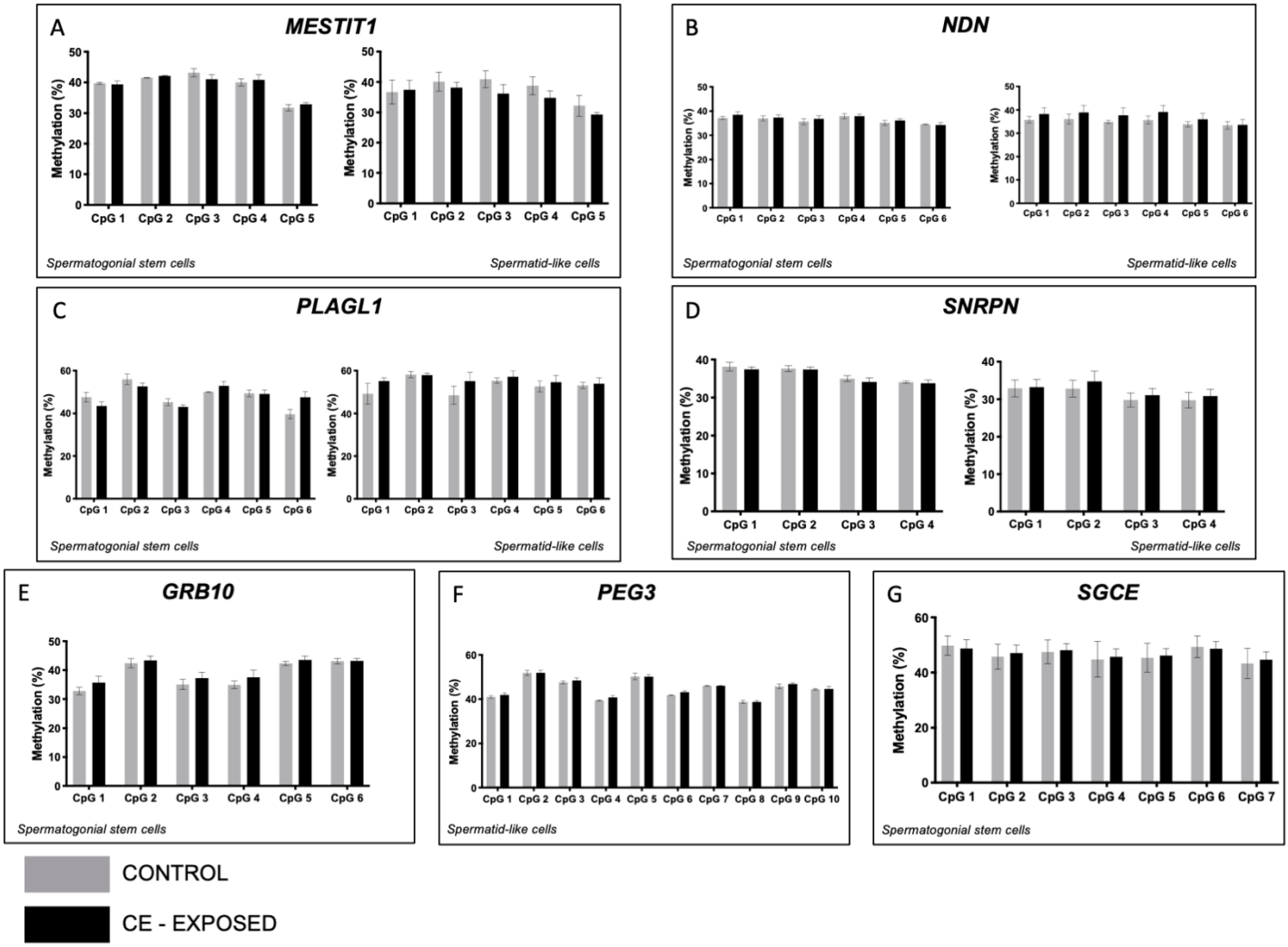
Rose Schrott[a,b,c,†], Katherine W. Greeson[d,e,†], Dillon King[a,b], Krista M. Symosko Crow[d,e], Charles A. Easley IV[d,e] and Susan K. Murphy[a,b]

[a] *Division of Reproductive Sciences, Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, 27701, USA;* [b] *Integrated Toxicology and Environmental Health Program, Nicholas School of the Environment, Duke University, Durham, NC, 27701, USA;* [c] *Department of Environmental Health Science, College of Public Health, University of Georgia, Athens, GA, 30602, USA;* [d] *Regenerative Bioscience Center, University of Georgia, Athens, GA, 30602, USA*

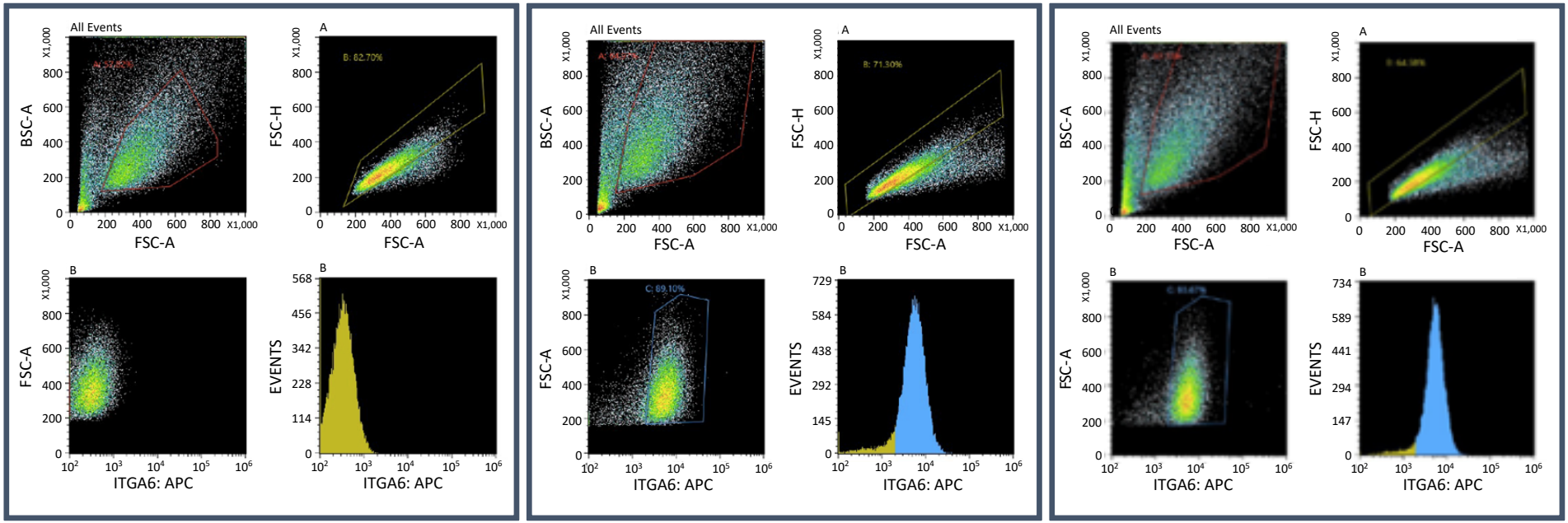
† These authors contributed equally



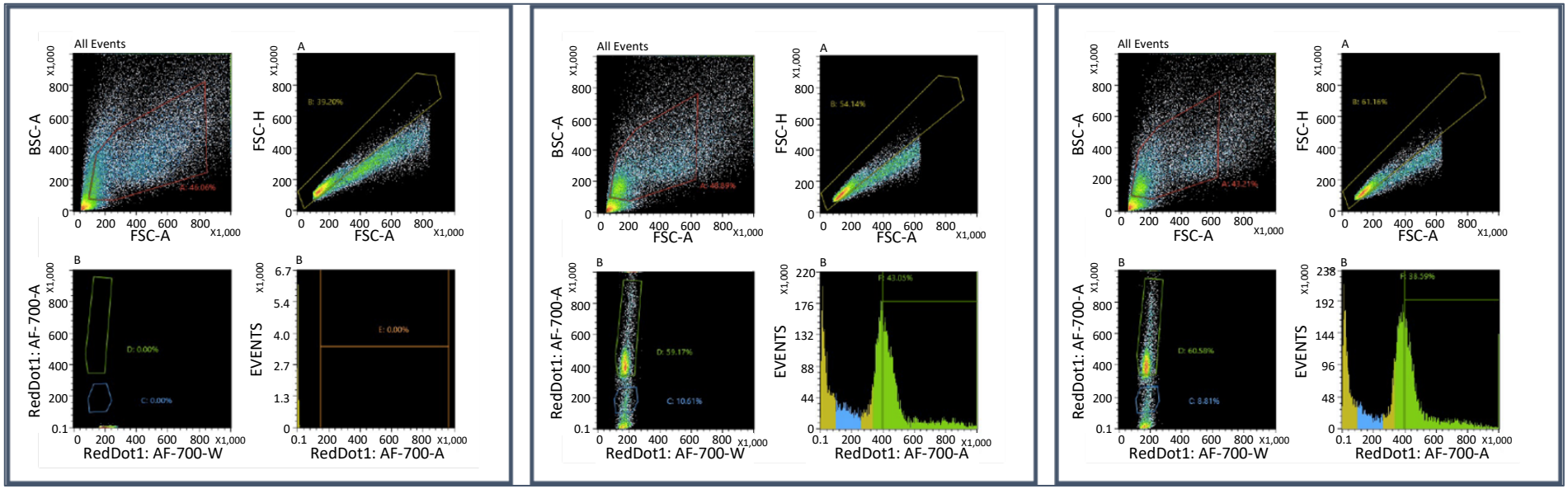
Supplemental Figure 1. CE does not significantly impact DNA methylation at paternally imprinted genes in SSC-like cells (**A, C, E**) or haploid spermatid-like cells (**B, D, F**). Two-factor ANOVA of bisulfite pyrosequencing data for **A, B:** *IGF2,* **C, D:** *MEG3-IG,* and **E, F:** *H19* demonstrates no significant effect of CE exposure on DNA methylation at these genes in either spermatogenic cell type. Grey=controls, black=CE-exposed.



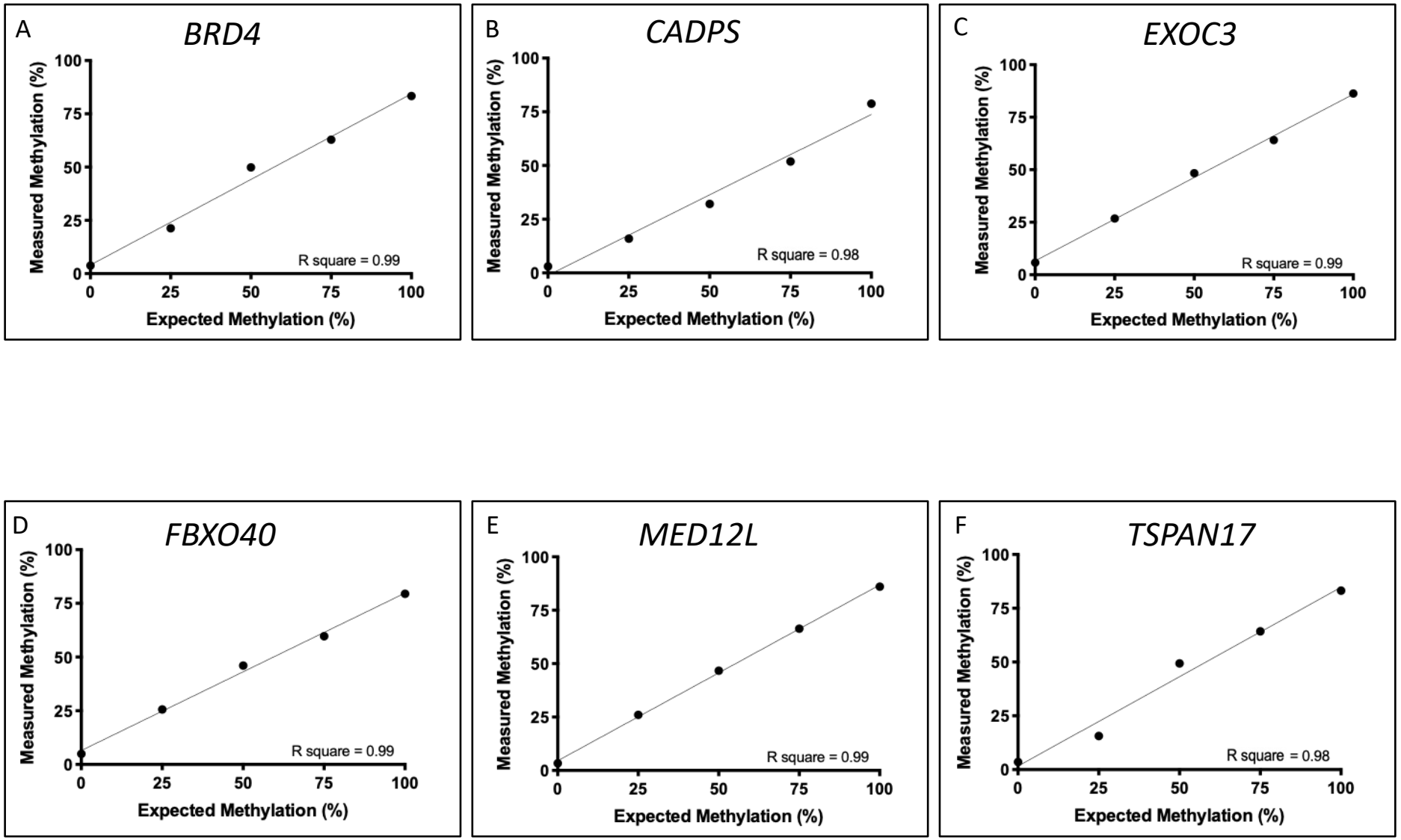
Supplemental Figure 2. CE does not significantly impact DNA methylation at several maternally imprinted genes in SSC-like cells or haploid spermatid-like cells. In panels **A-D**, left graph shows methylation levels at individual CpG sites in SSC-like cells and right graph shows levels in spermatid-like cells. Panels **E** and **F** show methylation in SSC-like cells and panel **G** shows methylation in haploid spermatid-like cells. Two-factor ANOVA of bisulfite pyrosequencing data for **A:** *MEST*, **B:** *NDN*, **C:** *PLAGL1*, **D:** *SNRPN*, **E:** *GRB10*, **F:** *PEG3*, and **G:** *SGCE*.



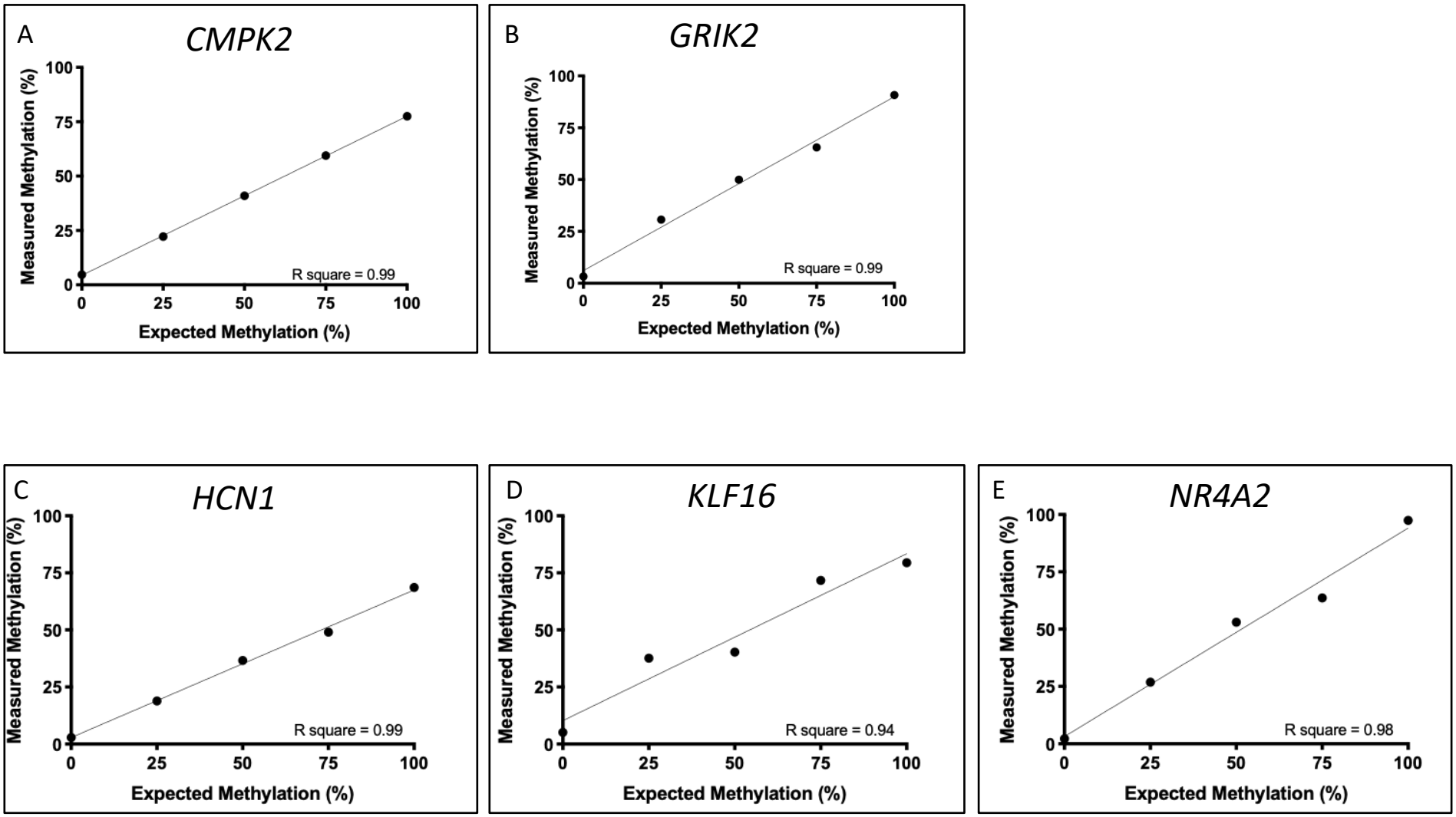
Supplemental Figure 3. Flow cytometry sort report for the isolation of spermatogonial stem cell-like cells. (Left) Unstained control cells. "All events" show the forward scatter versus the back scatter to visualize all cells. Panel "A" shows isolation of the single cells, leaving doublets. Panels labeled "B" show the population of unstained cells. (Center) Cells exposed to vehicle control. "All events" show all cells and gates the main cell population. Panel "A" shows isolation of single cells. Panels labeled "B" show isolation of the ITGA6+ stained cells. (Right) Cells exposed to CSE. "All events" show all cells and gates the main cell population. Panel "A" shows isolation of single cells. Panels labeled "B" show isolation of the ITGA6+ stained cells.



Supplemental Figure 4. Flow cytometry sort report for the isolation of haploid spermatid-like cells. (Left) Unstained control cells. “All events” show the forward scatter versus the back scatter to visualize all cells. Panel “A” shows isolation of the single cells, leaving doublets. Panels labeled “B” show the population of unstained cells. (Center) Cells exposed to vehicle control. “All events” show all cells and gates the main cell population. Panel “A” shows isolation of single cells. Panels labeled “B” show the populations of cells present based on DNA content. B-left shows the distinct cell populations captured. B-right shows the cell cycle profile for these stained cells, based on DNA content. The G1 peak is at 400 on the x-axis for diploid cells, and haploid cells are centered at 200. (Right) Cells exposed to CSE. “All events” show all cells and gates the main cell population. Panel “A” shows isolation of single cells. Panels labeled “B” show the populations of cells present based on DNA content. B-left shows the distinct cell populations captured. B-right shows the cell cycle profile for these stained cells, based on DNA content. The G1 peak is at 400 on the x-axis for diploid cells, and haploid cells are centered at 200.



Supplemental Figure 5. Measured methylation (y axes) by bisulfite pyrosequencing of samples with increasing amounts of methylated human DNA (x axes) for assays designed to query genes from the SFARI-only gene list. Bisulfite pyrosequencing validation curves for **A:** *BRD4;* **B:** *CADPS;* **C:** *EXOC3;* **D:** *FBXO40;* **E:** *MED12L;* and **F:** *TSPAN17.* R^2 values are indicated.



Supplemental Figure 6. Measured methylation (y axes) by bisulfite pyrosequencing of samples with increasing amounts of methylated human DNA (x axes) for assays designed to query genes possessing bivalent chromatin from the SFARI gene list. Bisulfite pyrosequencing validation curves for **A:** *CMPK2;* **B:** *GRIK2;* **C:** *HCN1;* **D:** *KLF16;* and **E:** *NR4A2.* R^2 values are indicated.