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**Supplemental Information**

# Stage-Specific Roles for Tet1 and Tet2 in DNA Demethylation in Primordial Germ Cells

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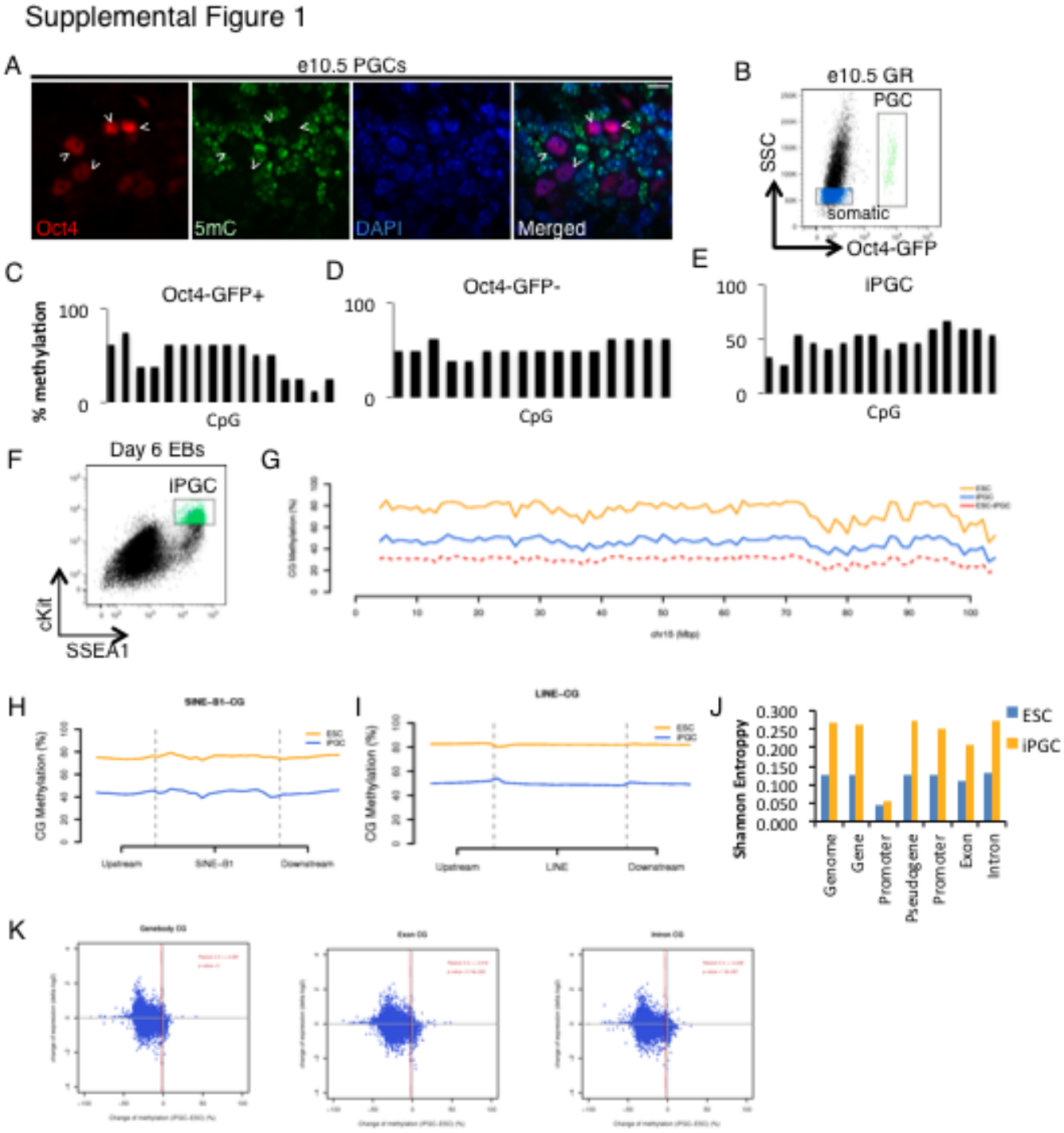


**Figure S1. DNA Methylation in PGCs and iPGCs, Related to Figures 1 and 2.**

A: Immunofluorescence of e10.5 genital ridges for Oct4 (red) and 5mC (green).

Arrowheads denote Oct4+ PGCs.

B: Flow cytometry of e10.5 *Oct4-gfp* genital ridges (GR). GFP+ cells (green) are PGCs. GFP- somatic cells (blue).

C,D,E: Percent methylation at *Snrpn* ICC in GFP+ PGCs (C), GFP- somatic cells from e10.5 embryos (D) and iPGCs (E). The x-axis denotes individual CpG dinucleotides, and the percent methylation at each CpG is graphed on the y-axis.

F: Flow cytometry of V6.5 EBs at Day 6 of differentiation, showing gating strategy for SSEA1+/cKit$^{bright}$ iPGCs (green).

G: Metaplot of methylation across murine chromosome 15. Methylation percentage (y-axis) is graphed along the coordinate distance of the chromosome in megabases (x-axis).

H,I: Methylation of SINE B1 (H) and LINE (I) transposable elements.

J: Shannon Entropy analysis of ESCs and iPGCs at genomic regions indicated.

K: Pearson correlations of change of methylation between iPGCs and ESCs against gene expression or repression. Correlations are performed on CG methylation found in gene body, exon, and intronic regions. Scale bar=5um.

Supplemental Figure 2

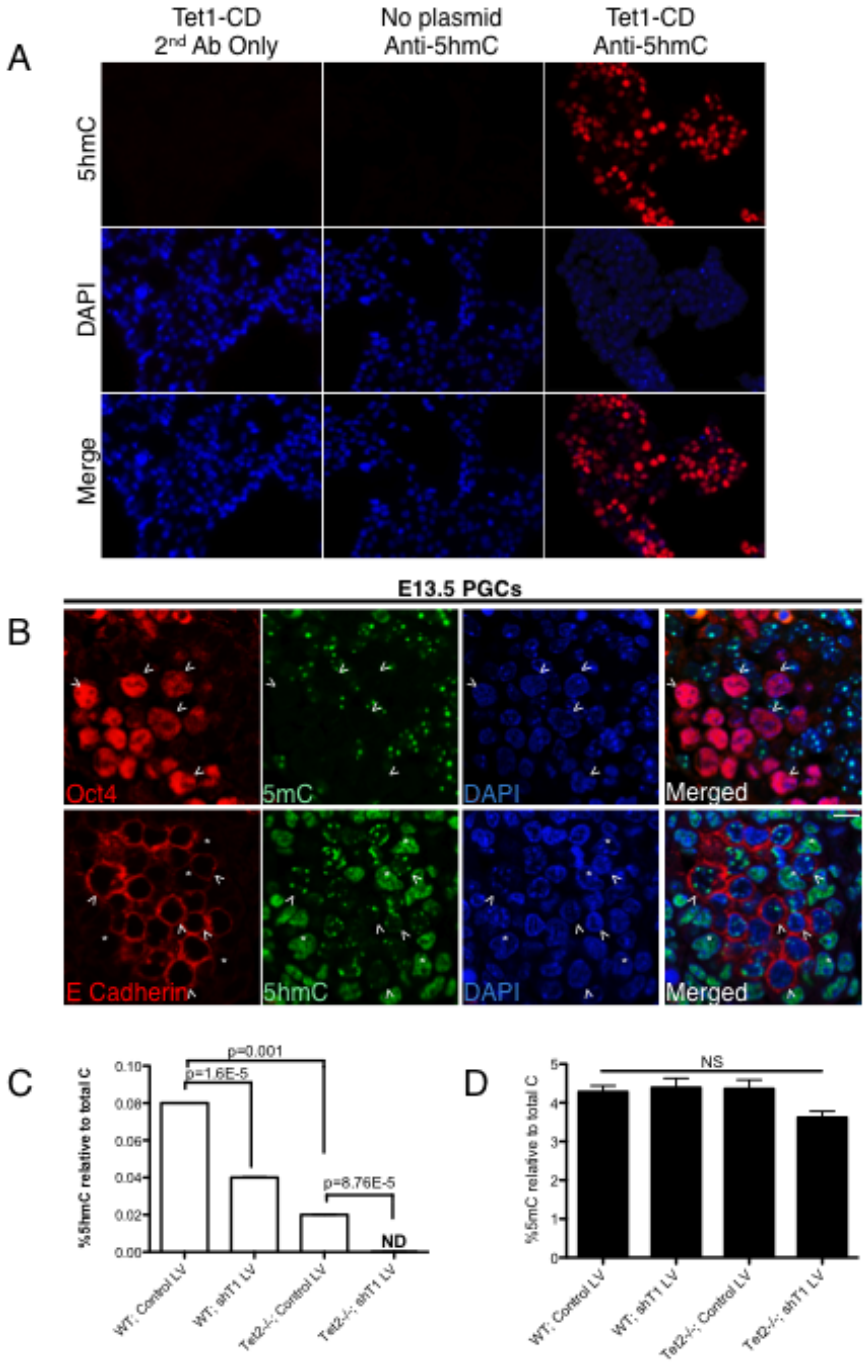

**Figure S2. 5hmC Analysis of PGCs and ESCs, Related to Figures 2 and 3**

A: HEK293 cells, which do not have detectable 5hmC, were transfected with Tet1-CD overexpression construct. Staining for 5hmC (red) was performed as indicated. 5hmC signal was only detectable by fluorescent microscopy with transfection of Tet1-CD.

B: 5hmC (green) staining of Oct4 and E-Cadherin (red) positive germ cells shows a punctate expression at e13.5. Scale bar=5um.

C,D: 5hmC (C) and 5mC (D) in undifferentiated ESCs by mass spectrometry. Measurements are displayed as mean $\pm$ SD n=3. Significance is shown on graph.

**Table S1. BS-Seq of Undifferentiated ESCs and iPGCs, Related to Figure 1**

| Sample | Library | CG | CHG | CHH | CA | CC | CT |
|---|---|---|---|---|---|---|---|
| ES | mBS47 | 74.97% | 1.43% | 0.97% | 2.15% | 0.19% | 0.49% |
| iPGC | mBS44 | 52.19% | 0.70% | 0.47% | 0.95% | 0.16% | 0.27% |
| ES | mBS49 | 73.39% | 0.77% | 0.52% | 1.16% | 0.14% | 0.28% |
| iPGC | mBS48 | 44.74% | 0.56% | 0.40% | 0.76% | 0.17% | 0.27% |
| ES | mBS57 | 75.92% | 0.77% | 0.55% | 1.12% | 0.16% | 0.31% |
| iPGC | mBS56 | 45.16% | 0.42% | 0.31% | 0.55% | 0.15% | 0.22% |

BS-Seq of undifferentiated ESCs and iPGCs. All experiments were performed pairwise so that the ESC and iPGC pair in each of the three experiments were sequenced in neighboring lanes. Methylation was calculated in various sequence contexts as indicated. Black: Differentiations performed with R26-GFP ESCs. Blue: Experiments using V6.5 ESCs and iPGCs. Deep sequencing to >6X was performed on libraries mBS48 and mBS49. See also Figure 1.