SUPPLEMENTARY INFORMATION FOR:

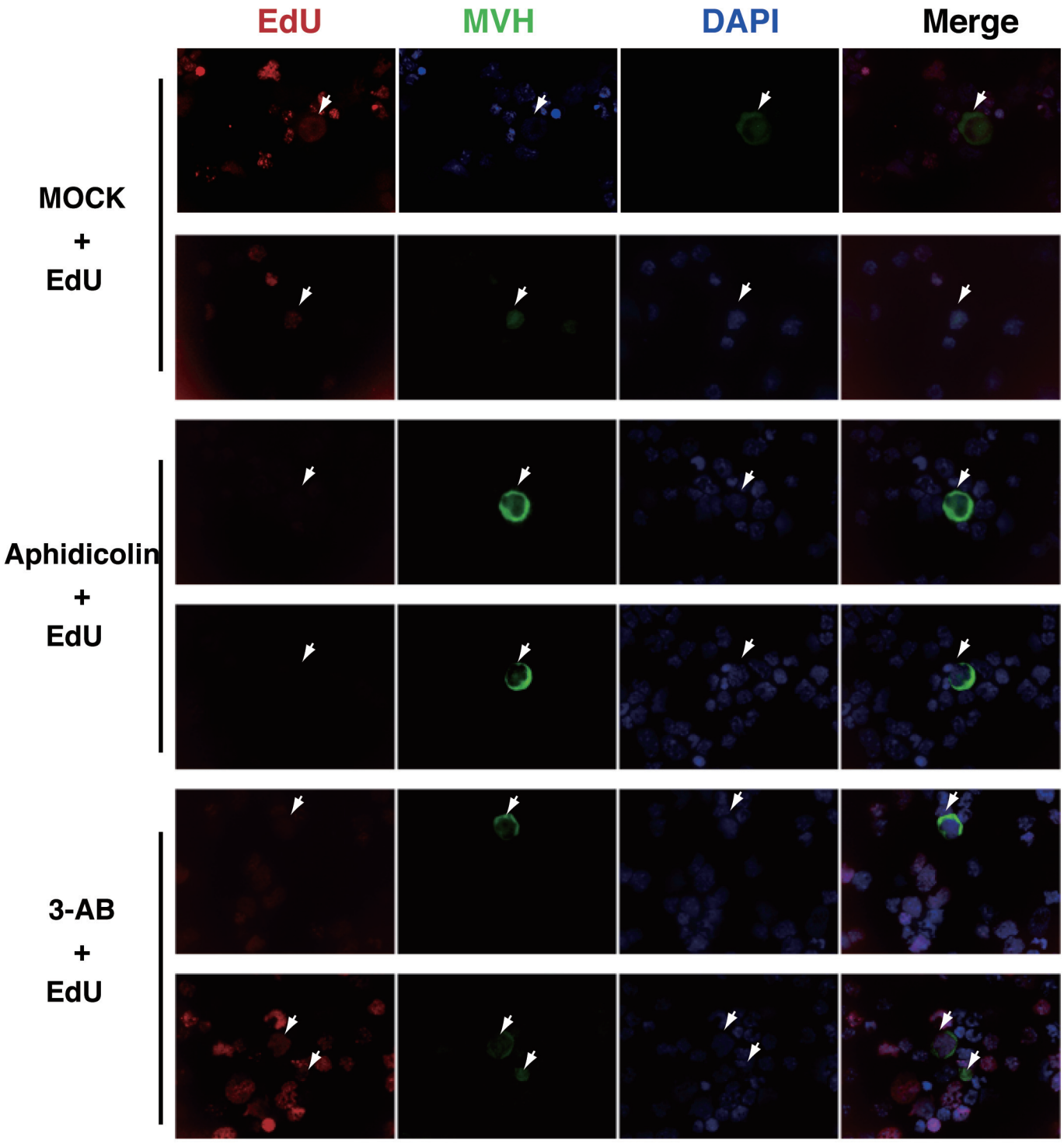
Active DNA demethylation is required for complete imprint erasure in primordial germ cells.

Yuki Kawasaki[1,2,*], Jiyoung Lee[1,2,3,*], Ayumi Matsuzawa[1], Takashi Kohda[1], Tomoko Kaneko-Ishino[4] & Fumitoshi Ishino[1,2]

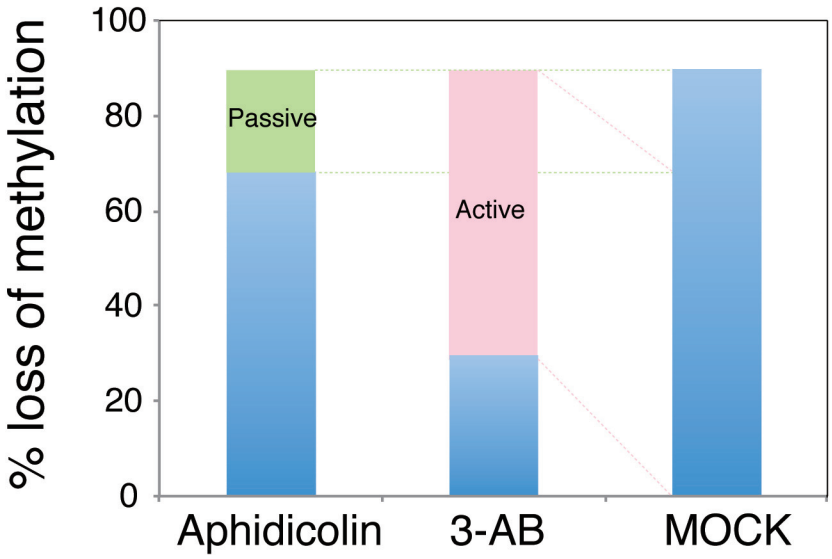
[1] Department of Epigenetics, Medical Research Institute and [2]Global Center of Excellence Program for International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8510, Japan. [3]Japan Science and Technology Agency, PRESTO, Japan. [4]School of Health Sciences, Tokai University, Bohseidai, Isehara, Kanagawa 259-1193, Japan.

Correspondence and requests for materials should be addressed to F.I (email: fishino.epgn@mri.tmd.ac.jp).

*These authors contributed equally to this work.



Supplementary Figure S1. Small molecule inhibitor assay in the fetus during embryonic development. For the detection of DNA replication, EdU was also injected. EdU detection both of PGCs and somatic cells in the genital ridges from inhibitor-treated fetus. The incorporated EdU (red) was detected with Alexa fluor 594 conjugated azide in PGCs and somatic cells. DAPI was used for nuclear staining (blue). For the identification of PGCs, gonad cells were stained with Mvh (green; arrows indicate PGCs). EdU was incorporated in the 3-AB-treated or MOCK fetal genomic DNA, but not in the aphidicolin-treated fetal genomic DNA.



Supplementary Figure S2. Percentage loss of methylation trough the DNA methylation analysis of *H19*-DMRs in inhibitor-treated PGCs in vivo, as shown in Fig. 3c. Pink and light green area suggests active and passive demethylation, respectively.