Figure EV1. Lack of both Jmjd2a and Jmjd2c impairs mitotic progression in ESCs.

A–C 2ac and 2abc ESC clones (A) and 2a and 2c ESC clones (B) expressing H2B-Venus were cultured in 2i medium in the absence or presence of OHT and subsequently subjected to time-lapse imaging. Y-axes show the duration of mitosis (from peak chromosome condensation to anaphase onset) for individual cells. Red lines show median values. Data are representative of results obtained in at least two different experiments. (C) Representative images demonstrating proper chromosome alignment after 20 min in mock, but not OHT-treated 2ac ESCs.
Figure EV2. 2ac and 2abc KO ESCs show reproducible and comparable changes in H3K9me3 and H3K36me3 patterns.

A WB analyses of 2ac and 2abc ESC lines. Results are representative of at least three independent experiments.

B Quantification of the histone modification WB presented in (A). Each membrane was reprobed with an antibody detecting H4 for normalization. Data are presented as log2 fold change (OHT versus control treated).

C, D Results of (C) principal component analyses and (D) Pearson’s correlation coefficient analyses of H3K9me3 and H3K36me3 read counts across all TSS regions (± 1 kb).

ChIP-seq data were generated in two different experiments involving a total of four independently derived ESC lines cultured in the absence or presence of OHT.

Data information: All data were obtained using ESCs cultured in 2i medium. Source data are available online for this figure.
Figure EV3. 2ac and 2abc KO ESCs show consistent and comparable changes in H3K9me3 and H3K36me3 distributions.

A Density plots showing average ChIP-seq signals with 95% confidence intervals of the mean indicated in grey. Y-axes show mean tags per million (TPM). Data were obtained using the indicated ESC lines. TSSs were classified as “Bound” if containing binding sites for both Jmjd2a and Jmjd2c within ± 1 kb. Note that H3K4me3 and H3 ChIP-seq data were only obtained for the ESC lines 2ac #5 and 2abc #9.
B Heat map showing H3K36me3 ChIP-seq data for Jmjd2a/c bound TSSs (± 5 kb) sorted according to read number in 2abc #13+OHT.
C ChIP-qPCR validations exemplifying differential effects on histone methylation. Als2, Mfn1, Ska1, Tcf1 and Pim3 are examples of down-regulated target genes, Gadd45g is induced, while the expression levels of Gstz1 and Kif15 are unaltered upon loss of Jmjd2a/c expression. Graphs show mean ± SD for three technical replicates and are representative of results obtained in at least two independent experiments.

Data information: All data were obtained using ESCs cultured in 2i medium.
Figure EV3.
Figure EV4. TSS regions with substantial gain of H3K9me3 show reduced levels of H3K4me3.

A Density plots are presented for the 100 Jmjd2a/c bound TSSs showing the greatest increase in average H3K9me3 levels. Fold change in H3K9me3 read counts was calculated for each ESC line (OHT versus Ctrl) for regions of ± 1 kb of TSSs. Plots show average ChIP-seq signals with 95% confidence intervals of the mean.

B Independent ChIP-qPCR validations. Note that the target genes show impaired expression upon loss of Jmjd2a/c expression. Graphs show mean ± SD for three technical replicates and are representative of results obtained in at least two independent experiments.

Data information: All data were obtained using ESCs cultured in 2i medium.
Figure EV5. Consistent accumulation of H3K9me3 at jmdj2a/c targets with impaired expression.

A, B Density plots showing average ChIP-seq signals with 95% confidence intervals of the mean. Plots are shown for the gene subsets defined for Fig 6C and D, and data were obtained using antibodies specific for (A) H3K9me3 or H3K36me3 or (B) H3K4me3 or H3.

Data information: All data were obtained using ESCs cultured in 2i medium.