## Inoue_Supplemental Figure S1

### A

|          | 4-cell          | Morula          |
|----------|-----------------|-----------------|
| Kdm6b\textsuperscript{MUT} | ![Image](image1) | ![Image](image2) |
| Kdm6b\textsuperscript{WT}   | ![Image](image3) | ![Image](image4) |

|          | 4-cell          | Morula          |
|----------|-----------------|-----------------|
| H3K27me3 | ![Image](image5) | ![Image](image6) |
| DAPI     | ![Image](image7) | ![Image](image8) |

20 µm

### B

**Relative H3K27me3 signal intensity**

|          | Kdm6b\textsuperscript{MUT} | Kdm6b\textsuperscript{WT} |
|----------|-----------------------------|-----------------------------|
| 4-cell   | ![Bar graph](image9)        | ![Bar graph](image10)      |
| Morula   | ![Bar graph](image11)       | ![Bar graph](image12)      |

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Inoue_Supplemental Figure S3

![Graph showing relative expression of Rnf12 in 4-cell, 8-cell, and morula stages for Kdm6b^WT and Kdm6b^MUT conditions.](image-url)
SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Ectopic $Kdm6b^{WT}$ mRNA injection results in reduction of H3K27me3 in preimplantation embryos, related to Figure 3

(A) Representative images of $Kdm6b$-injected 4-cell and morula embryos immunostained with H3K27me3 antibody. $Kdm6b$ mRNA was injected into zygotes. The embryos were fixed at 46 (4-cell) and 78 (morula) hrs after fertilization.

(B) Relative H3K27me3 signal intensity. The signal intensities of multiple blastomeres were measured and averaged to obtain the value of a single embryo. The average signals of $Kdm6b^{MUT}$ embryos were set as 1.0. The total numbers of embryos examined were 9 ($Kdm6b^{MUT}$) and 9 ($Kdm6b^{WT}$) for 4-cell and 19 ($Kdm6b^{MUT}$) and 22 ($Kdm6b^{WT}$) for morulae. Error bars indicate SE. ***, $p<0.001$, * $p<0.05$ (two-tailed Student t-test).

Figure S2. Validation of H3K27me3 ULI-NChIP in embryonic stem cells (ESCs) and $Kdm6b$-injected morula embryos, related to Figure 3

(A) Scatterplot showing a correlation between H3K27me3 peaks detected in mouse ESCs in the ENCODE project and in our hands using 500 or 2,000 ESCs.

(B, C) Scatterplot (B) and Venn diagram (C) showing a correlation between H3K27me3 peaks of a published dataset (Liu et al. 2016) and $Kdm6b^{MUT}$-injected morula embryos.

(D) Genome browser views of representative loci showing almost identical H3K27me3 enrichment in the public dataset and $Kdm6b^{MUT}$-injected morula embryos.

(E) The number of H3K27me3 peaks detected in $Kdm6b^{MUT}$- and $Kdm6b^{WT}$-injected morula embryos.

(F) Genome browser view of the $Xist$ locus showing loss of H3K27me3 domain in $Kdm6b^{WT}$-injected embryos. The parental alleles were not distinguished in these tracks.
Figure S3. RT-qPCR analysis of \textit{Rnf12} in \textit{Kdm6b}-injected embryos. The data were normalized to \textit{18S}, and then the values of \textit{Kdm6b}\textsuperscript{MUT} embryos were set as 1.0. Error bars indicate SE of three biological replicates. Each experiment used a pool of 18-24 embryos per group. Note that \textit{Rnf12} is downregulated rather than upregulated in \textit{Kdm6b}\textsuperscript{WT}-injected embryos. We speculate that this is likely due to maternal XCI occurring as early as the 4-cell stage in \textit{Kdm6b}\textsuperscript{WT}-injected embryos, given that \textit{Rnf12} is a non-escapee X-linked gene (Borensztein et al. 2017).

Figure S4. Maternal X chromosome inactivation in \textit{Kdm6b}\textsuperscript{WT}-injected blastocyst embryos, related to Figure 4

(A) Scatter plot showing the correlation between biological duplicate of RNA-seq samples.

(B) Box plot showing the maternal allelic expression ratios [Mat/(Mat+Pat)] of individual chromosomes in \textit{Kdm6b}-injected blastocysts. Middle lines in the boxes represent the medians. Box edges and whiskers indicate the 25th/75th and 2.5th/97.5th percentiles, respectively.

(C, D) The relative expression levels of X-linked genes between \textit{Kdm6b}\textsuperscript{WT} and \textit{Kdm6b}\textsuperscript{MUT}-injected blastocyst embryos. The expression levels of the maternal allele were analyzed. Each dot represents an individual gene showing enough SNP reads (RPM>0.5). Panel D shows known escapees, and panel C shows the rest of genes.

SUPPLEMENTAL TABLES

Table S1. Allelic gene expression in \textit{Kdm6b}\textsuperscript{WT} and \textit{Kdm6b}\textsuperscript{MUT}-injected blastocyst embryos

Table S2. Summary of datasets generated in this study