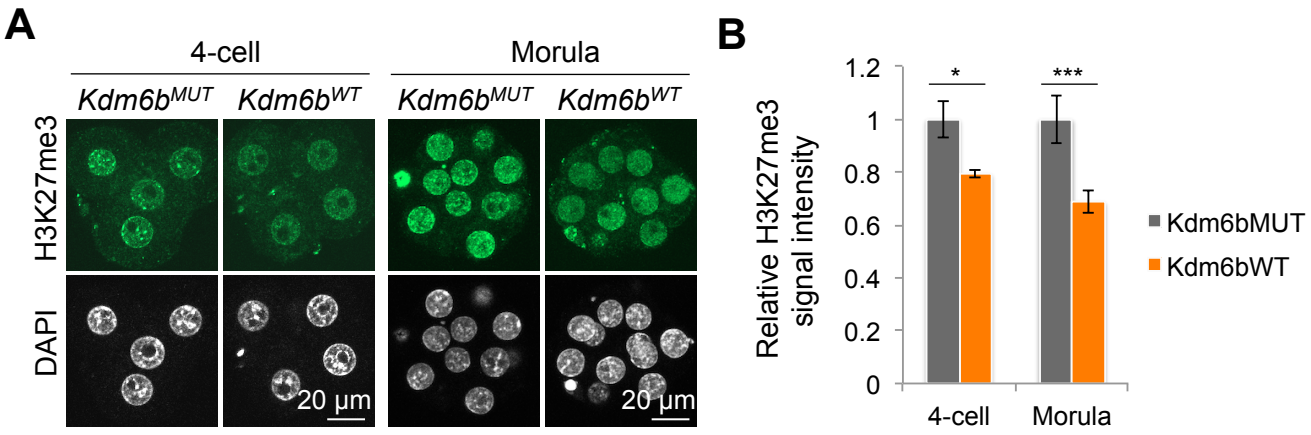
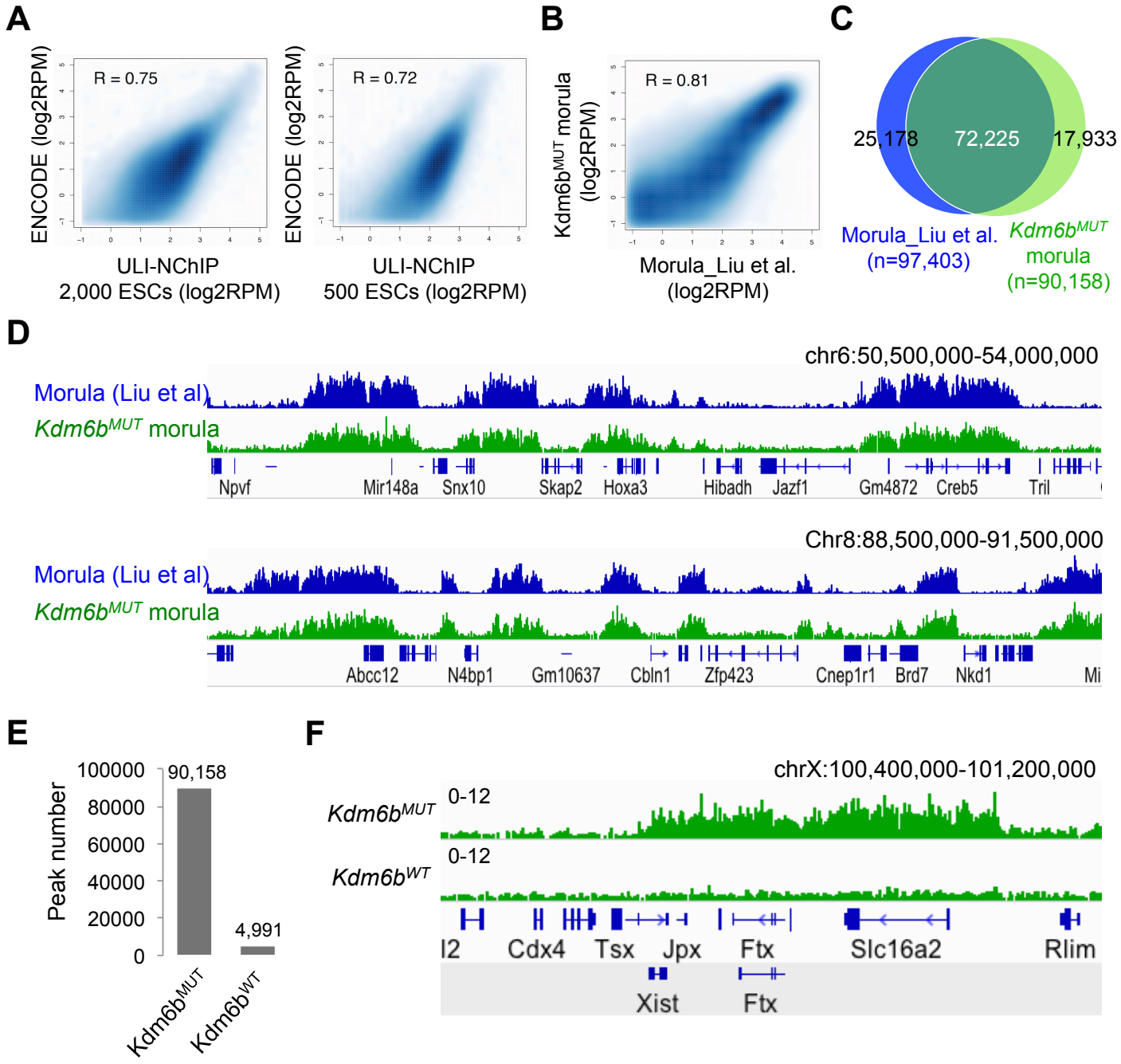
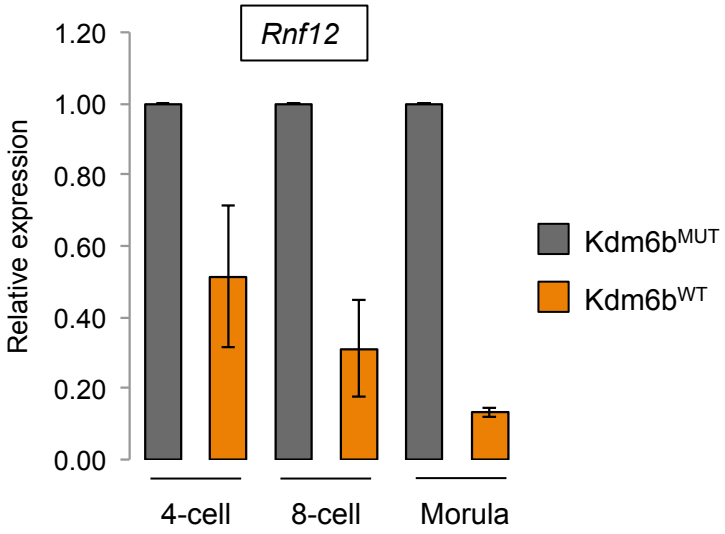
Inoue_Supplemental Figure S1



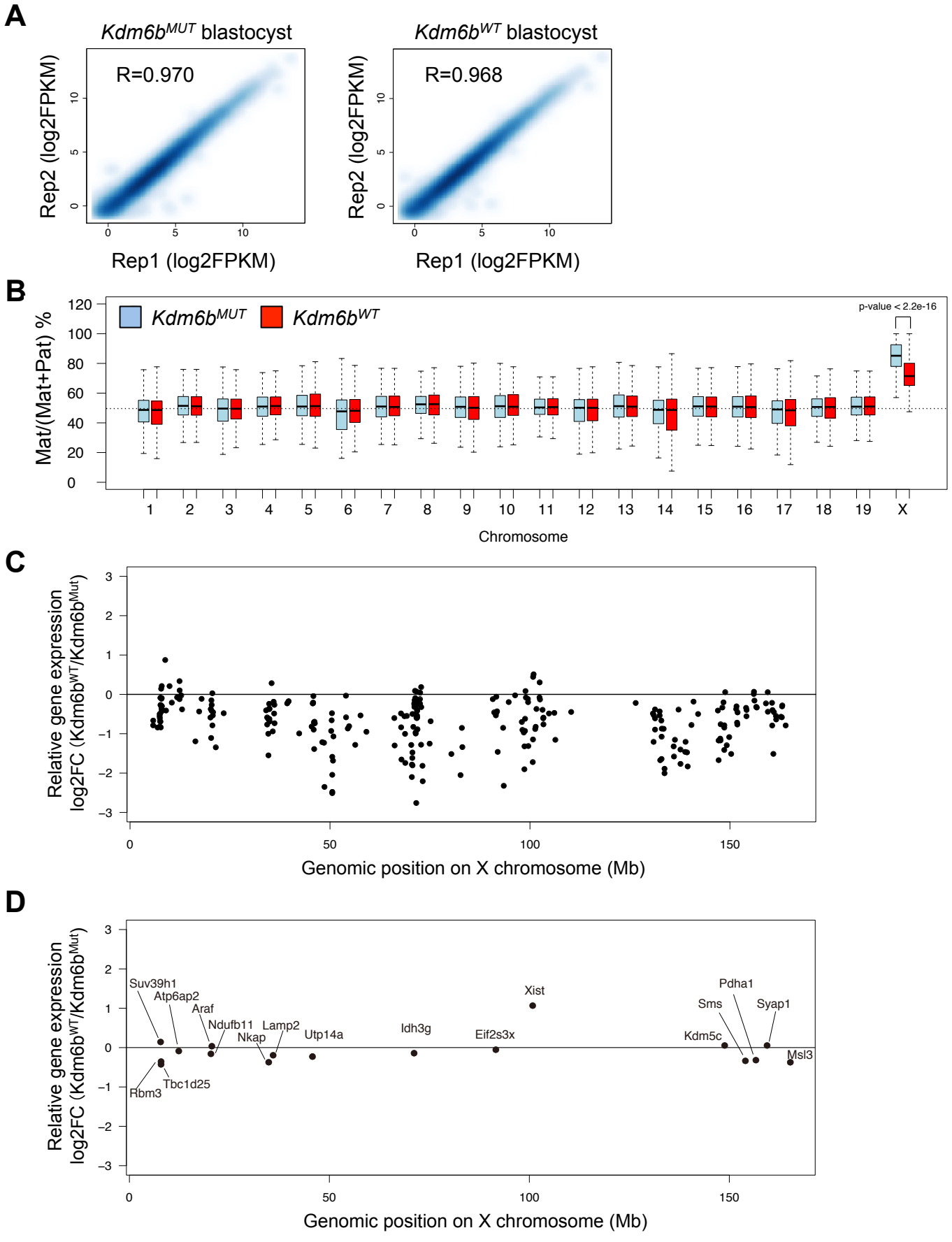
# Inoue_Supplemental Figure S2



Inoue_Supplemental Figure S3



# Inoue_Supplemental Figure S4



**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 *Rnf12* in *Kdm6b*-injected embryos.** The data were normalized to *18S,* and then the values of *Kdm6b*$^{MUT}$ 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 *Rnf12* is downregulated rather than upregulated in *Kdm6b*$^{WT}$-injected embryos. We speculate that this is likely due to maternal XCI occurring as early as the 4-cell stage in *Kdm6b*$^{WT}$-injected embryos, given that *Rnf12* is a non-escapee X-linked gene (Borensztein et al. 2017).

**Figure S4. Maternal X chromosome inactivation in *Kdm6b*$^{WT}$-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 *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 *Kdm6b*$^{WT}$- and *Kdm6b*$^{MUT}$-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 *Kdm6b*$^{WT}$- and *Kdm6b*$^{MUT}$-injected blastocyst embryos

**Table S2**. Summary of datasets generated in this study