

Legends for Supplementary Figures

Supplementary Figure S1

ChIP for H3K27m3 in the X^ΔY *Eed-TG* ES cells at known H3K27m3-marked sites

The *Eed* transgene sufficiently rescued the PRC2 activity in the X^ΔY *Eed-TG* ES cells at the *Sox9* and *Gata6* promoters. Assays were done using the X^ΔY *Eed-TG* lines at a passage number of less than 6 since their derivation. Average from three independent samples are shown. Error bars indicate SD. P, promoter.

Supplementary Figure S2

EB development in ES cells with *Eed* and/or *Tsix* mutations

(A) Microscopic view of EB during differentiation. EB were cultured in suspension for 3 days and then replated on gelatinized dishes. (B) Gross morphology of Giemsa-stained EB at day 12.

Supplementary Figure S3

Immuno-FISH for H4K20m1 and *Xist* RNA in the X^ΔY *Eed*^{-/-} EB

A few X^ΔY *Eed*^{-/-} nuclei showed weak H4K20m1 deposition co-localizing with *Xist* RNA during the early stages of differentiation, whereas the X^ΔX nuclei displayed a much clearer signal. Photos were from EB differentiated for 7 days. Insets indicate enlarged view of the areas enclosed by dotted squares. DAPI, 4',6-Diamidino-2-phenylindole (for nuclear staining).

Supplementary Figure S4

A schematic representation of X-chromosome status in the X^ΔY *Eed*^{-/-} cells during differentiation

Upon differentiation augmented *Xist* transcription in the X^ΔY *Eed*^{-/-} cells induces partial silencing of X-linked genes presumably by recruiting PRC1. This results in growth retardation of EB and/or cell death at the onset of differentiation. In a late stage after the critical time window of silencing by *Xist* RNA, the X^ΔY *Eed*^{-/-} cells restore growth and the expression of X-linked genes. Blue flat ovals in the figure represent *Xist* RNA.

Supplementary Figure S5

ChIP for UbH2A in ES cells with *Eed* and/or *Tsix* mutations at the *Xist* promoter and 3'-end of *Tsix*

There was no statistically significant difference in UbH2A modification at both sites

between the wild-type and *Tsix*-deficient ES cells of *Eed*^{+/+} and *-/-* background. Colored columns represent results obtained by using a specific antibody and gray columns represent those from control IgM. The *HoxA7* promoter is a previously reported UbH2A-marked site in ES cells and was examined as a positive control for the ChIP assay. Average from three independent samples are shown. Error bars indicate SD.