Supplementary Methods

Quantitative PCR for Sox9 and Gata6 promoters

The Taqman probes and primers for both of the promoters were designed within the previously described quantitative PCR amplicons (Boyer *et al*, 2006) by using *PrimerExpress* v1.5 software (Applied Biosystems) and are shown in Supplementary Table IV.

ChIP assay for UbH2A

The UbH2A ChIP was done as described in materials and methods. The optimal amount of chromatin and antibody for ChIP reaction was determined beforehand at the *HoxA7* promoter. We used 2.5 μl of anti-UbH2A antibody (clone E6C5, #05-678, Upstate) for 8 x 10^4 nuclei. Affinity-purified rabbit anti-mouse IgM antibody (#12-488, Upstate) was subsequently used as a bridging secondary antibody. Quantitative PCR for the *HoxA7* promoter was done by Brilliant II SYBR Green QPCR Master Mix (600828, Stratagene) using the primer pair reported previously (Stock *et al*, 2007). ChIP at the *Xist* promoter and *Tsix* 3'-end were quantified by Taqman PCR, and the probes and primers were described in Supplementary Table IV. The quantitative PCR probe and primers for *Tsix* 3'-end are the same to those for *Tsix* (4) in qRT-PCR.

References

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441:** 349-353

Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M, Koseki H, Brockdorff N, Fisher AG, Pombo A (2007) Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat Cell Biol* **9**: 1428-1435