Online Supplemental Information for:

The pluripotency factor, Oct4, interacts with Ctcf and also controls X-chromosome pairing and counting

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Figure S1: Expression kinetics of XCI and pluripotency factors suggest that XCI and cell differentiation are tightly coupled.

Quantitative RT-PCR analysis of indicated gene products in wildtype female ES cells from d0 to d9. ES cells were differentiated by suspension culture into embryoid bodies and RNA prepared at indicated timepoints using Trizol (Invitrogen) for qRT-PCR analysis. cDNA was generated using random hexamers or appropriate strand-specific primers, after which 200 ng cDNA was amplified in the presence of SYBR Green on a BioRad iCycler iQ real-time detection system. Primers for *Oct4, Rex1, Nanog*, and *c-Myc* have been described by Toyooka et al., *Development* 135, pp. 909-918 (2008). *Xite*, *Tsix*, and *Xist* primers have described in the Materials and Methods. Error bars represent one standard deviation from the mean.

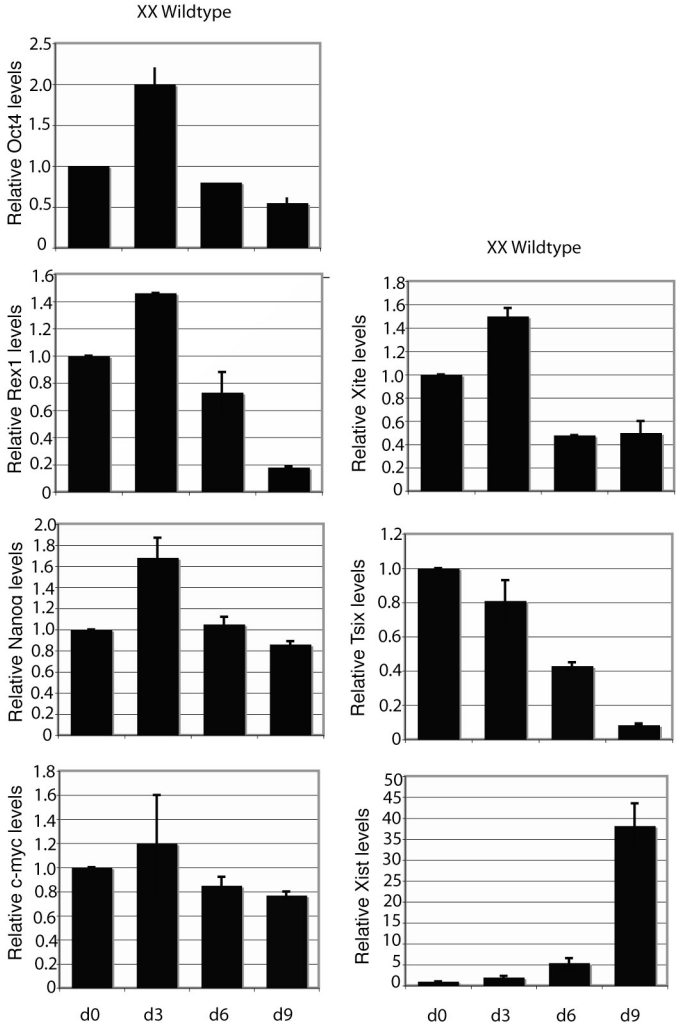

Figure S2: qRT-PCR of Oct4, Rex1, Foxd3, and Nanog transcripts demonstrates that d4 Oct4-knockdown cells are differentiating in culture.

Female ES cells were knocked down for indicated factors on d2 and harvested 46-48 hours later. RNA was prepared and reverse-transcribed prior to qRT-PCR using primers for Oct4, Rex1, Foxd3, and Nanog. Primer sequences have been published in Toyooka et al., *Development* 135, pp. 909-918 (2008).

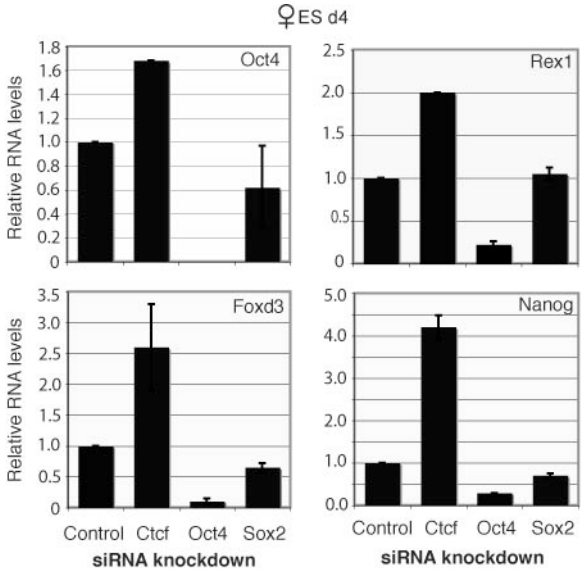

Figure S3: Analysis of Oct4-deficient female ES cells on d1 of differentiation.

a, Western blotting confirms knockdown of indicated proteins in wildtype female ES cells nucleofected with siRNAs on d0 and analyzed on d1.
b, Distribution of X-X distances in knockdown cells as indicated. n, nuclei counted. ND= Xic-Xic distance/d, where d = 2 x (nuclear area/π)$^{0.5}$. The significance of the difference (*P*) between samples and control siRNA was calculated using the Kolmogorov-Smirnov (KS) test, a non-parametric test to determine whether two data-sets have a similar distribution (SPSS 13.0 software).
c, Cumulative frequency curves of the analysis carried out in panel **b**. ND 0.0-0.2 are shown.
d, Percentage of cells displaying indicated number of RNA clusters on d1.
e, Representative example of the weak and diffuse Xist clouds (arrow) in Xist^{+} cells scored in **d.**

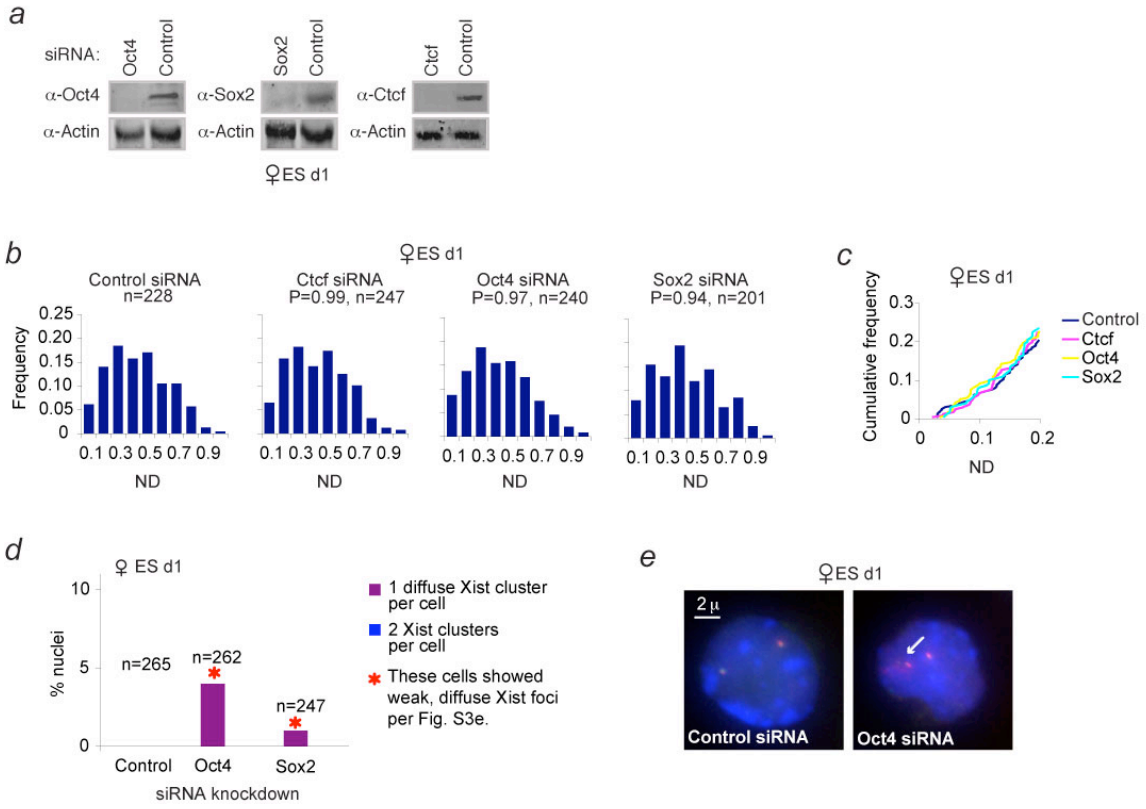

Figure S4: Quantitative RT-PCR of Xist, Tsix, and Xite expression in d1 and d4 Oct4-knockdown female cells.

Total RNA was isolated using Trizol from d1 and d4 female ES cells that were either knocked down with control siRNAs (Ctl) or Oct4 siRNAs. RNAs were then treated with RNase-free DNAse, RNA reverse transcribed using appropriate strand-specific primers, and 400 ng of cDNA was amplified in SYBER Green using the BioRad iCycler iQ or the Applied Biosystems 7500 Real-Time PCR detection system. All samples were normalized to a β-actin internal control. *Xist*, *Tsix*, *Xite*, and *β-actin* PCR primers are as described in the Methods. Error bars represent one standard deviation from the mean.