SUPPLEMENTARY INFORMATION

The long noncoding RNA, Jpx, is a molecular switch for X-chromosome inactivation

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Figure S1: *ΔJpx* has no effects in male cells.

(A) The *Jpx* gene, targeting vector, and products of homologous targeting before and after Cre-mediated excision of the *Neo* positive-selection marker. DT, diphtheria toxin for negative selection. (CpG)n, CpG island. The numbered boxes represent five *Jpx* exons.

(B) Southern analysis of SacI-digested genomic DNA from *Jpx* knockout male ES clones using probe 1. Wildtype (WT), two Neo+ mutants, and the derivative Neo- clones for the *Jpx* knockout are shown. The Neo- male clones, 1C4 and 1D4, were derived from the Neo+ 6B5 and 7F11 clones, respectively, by Cre-mediated excision.

(C) DNA FISH of wildtype and Δ*Jpx*/Y cells. *Xist* probe (pSx9), FITC-labeled (green). The *Jpx* probe (Cy3-labelled, red) is located in the region of deletion.

(D) Brightfield photographs of undifferentiated (d0) and differentiated (d4-d12) *ΔJpx*/Y and WT ES cells. ES cells were differentiated by EB suspension culture for 4 days and plated on gelatin-coated petridishes after d4.

(E) Cell death plots of WT and *ΔJpx*/+ ES cells, with averages and standard errors (SE) from 3 independent differentiation experiments. Trypan-blue exclusion method is based on the fact that dead cells take up the dye, whereas viable cells exclude it. % cell death = ([blue cells] / [blue cells + clear cells]) x 100. Sample size (n) = 150-200 for d0 timepoints, 500-2000 cells for all other timepoints.

(F) RT-PCR analysis of indicated transcripts from d0-d16. EtBr-stained gels are shown. M, 100-bp markers.

(G) Two-color RNA FISH for Xist and Pgk1 expression. Xist probe, FITC-labeled pSx9. Pgk1 probe, Cy3-labeled pCAB17.

(H) Real-time qRT-PCR of Xist and Tsix RNA in WT and mutant male cells. RNA levels were normalized to that of β-actin. Averages and SE for 6 independent differentiation

experiments are shown for each RNA. *P*, calculated by pairwise comparison using the Student *t*-test. Differences were insignificant for all days, shown only for d16.

Figure S2: Immunostaining for Oct4 and Nanog demonstrate appropriate downregulation of stem cell markers during differentiation of *ΔJpx*/+ cells.

WT and *ΔJpx*/+ female ES cells were immunostained for Oct4 and Nanog on d0 and d12 of differentiation. In each case, ES colonies appropriately expressed Oct4 and Nanog in the undifferentiated and the EB derivatives appropriately lost the pluripotency markers by d12 of differentiation.

Figure S3: Strand-specific RNA FISH analysis of Xist and Tsix RNA.

Wildtype and mutant female ES cells were subjected to two-color strand-specific RNA FISH analysis to confirm Xist and Tsix origins of large RNA clouds and pinpoint signals, respectively. Xist probe (green), riboprobe cocktail. Tsix probe (red), pCC3.

Figure S4: *Jpx* does not obviously act as an enhancer for *Xist*.

Luciferase (Luc) assay to test the ability of *Jpx* to enhance expression from the *Xist* promoter.

Supplemental Figure 1

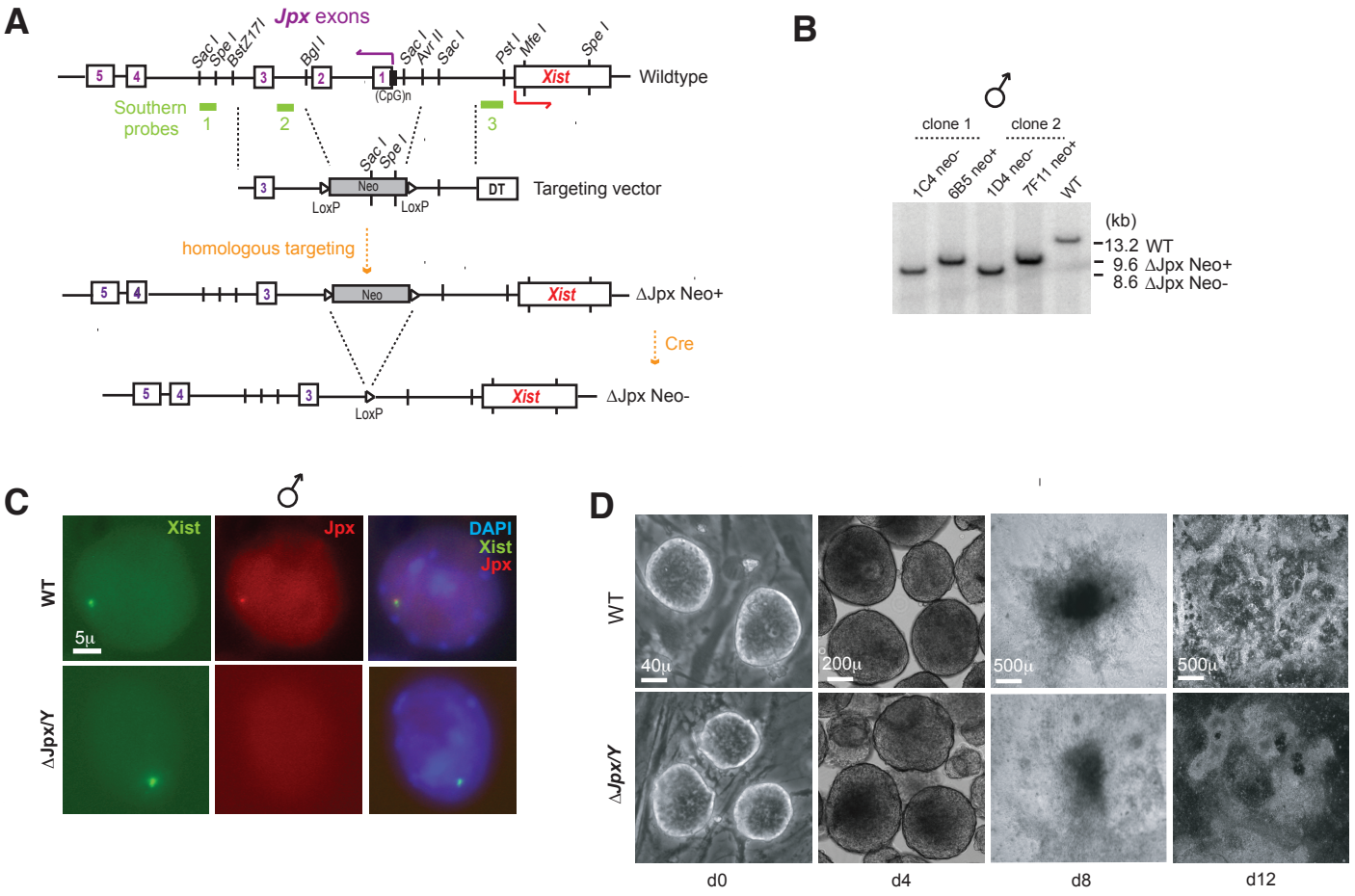

Figure S1

Supplemental Figure 2

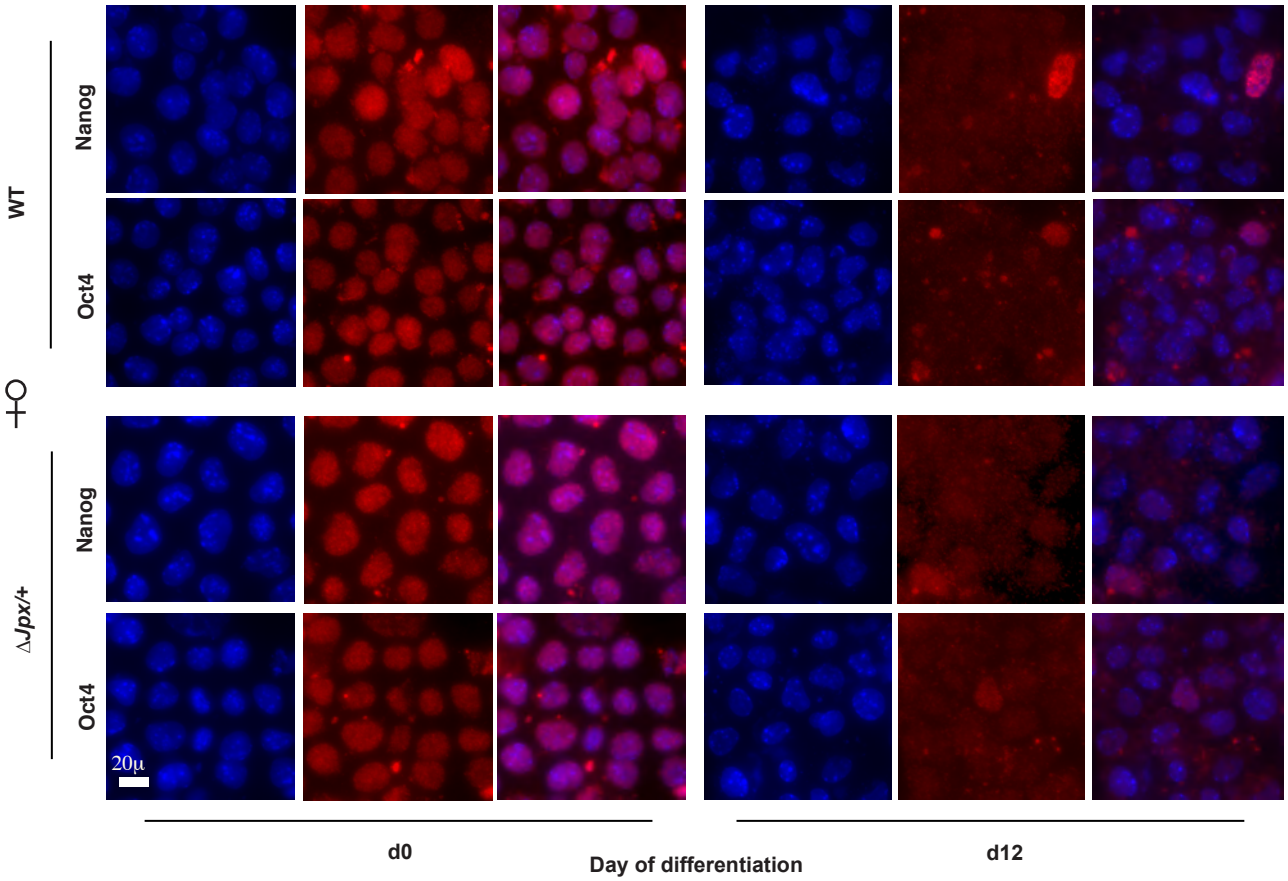

Figure S2

Supplemental Figure 3

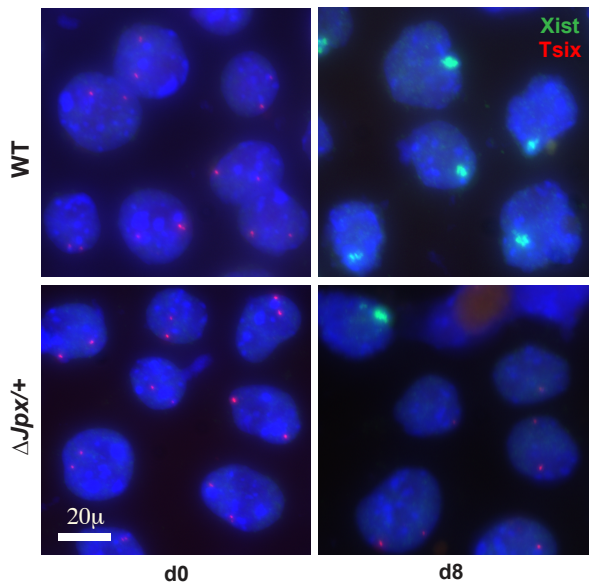

Figure S3

Supplemental Figure 4

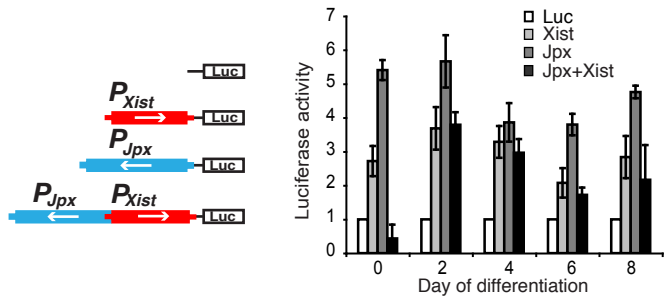

Figure S4