Supporting Information

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SI Materials and Methods

LNA Nucleofection in Mouse ES Cells. A total of 16.7 female ES cells were grown in DMEM supplemented with 15% calf serum (hyclone) and LIF. Cells were differentiated in medium without LIF for 6 d before nucleofection. For mucleofection, 2×10^6 cells were suspended in 100 μL of mES nucleofector solution (Lonza). Cy-3 labeled LNAs were added to a final concentration of 2 μM. Cells were transferred to a nucleofection cuvettete and processed using the A-30 program. Nucleofected cells were resuspended in 2 mL of culture medium. A total of 100 μL of this suspension was cytospun onto slides at each time point using a Shandon cytospin3 centrifuge. Xist RNA FISH was performed as described in *Materials and Methods*.

Nascent RNA FISH for PGK1 and HPRT. FISH probes were prepared by nick translation of BACs for PGK1 and HPRT. A total of 10 μg Cot1 DNA and 10 μg yeast tRNA was vacuum dried and resuspended in 16 μL of hybridization buffer containing 50% formamide, 10% dextran, 2× SSC, and 0.1% BSA. A total of 100 ng probe in 2 μL of hybridization solution was added to the above mix and denatured for 10 min at 80 °C and for 10 min at 37 °C. A total of 2 μL of RNase inhibitors was added to the probe mix to a final volume of 20 μL. A total of 10 μL of probe was used per slide. Slides were incubated overnight at 37 °C. After hybridization, slides were washed twice in buffer containing 50% formamide in 2× SSC at 37 °C and twice in 2× SSC buffer at 37 °C. Slides were then mounted with Vectashield containing DAPI and visualized immediately.

Nucleofection of siRNAs and shRNAs. siRNA and shRNA duplexes identical to the LNA-C1 sequence were synthesized and 2 μM were nucleofected in parallel with LNA-C1 using the T-20 program as described in *Materials and Methods*. Slides were processed for RNA FISH as described.

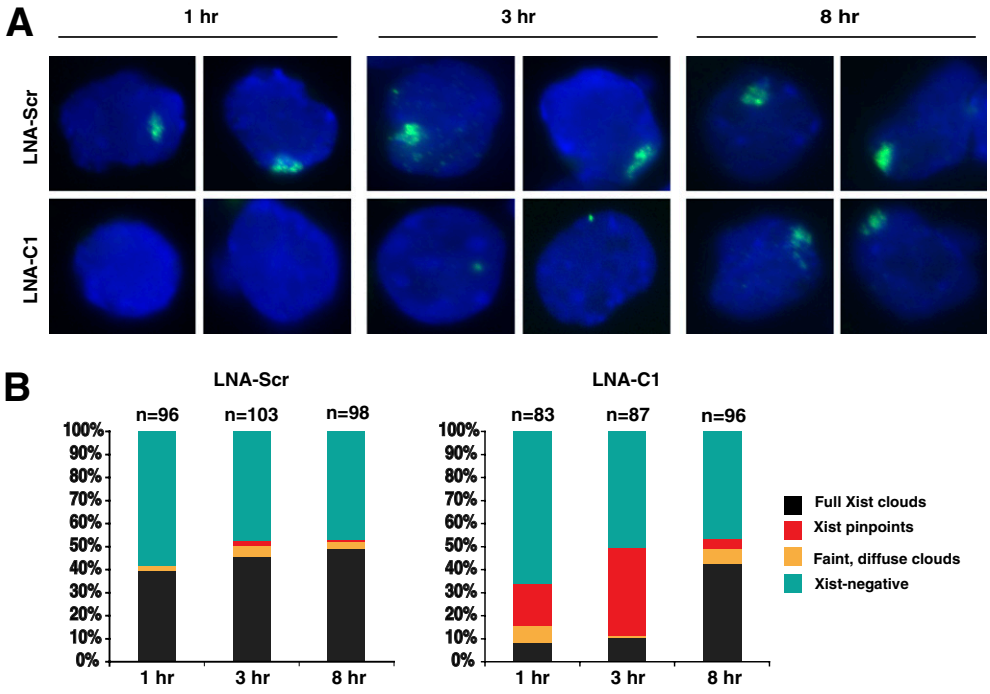

Fig. S1. Nucleofection of LNA-C1 into female mouse ES cells results in loss of Xist RNA from the Xi by 1 h and recovery by 8 h. Xist RNA FISH (green) at indicated time points after LNA-C1 nucleofection in mESCs. Two representative images are shown for each time point. Quantitation of results shown in *A*.

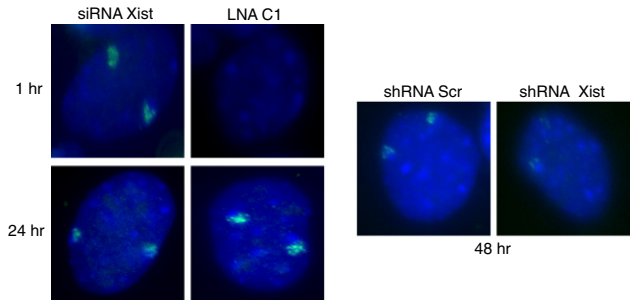

Fig. S2. Comparative kinetics of LNA versus siRNAs and shRNAs. siRNAs toward Xist repeat C do not result in displacement of Xist at the 1-h time point, whereas LNAs do. Treatment with shRNAs for 48 h is required for mild Xist knockdown. Xist RNA, green.

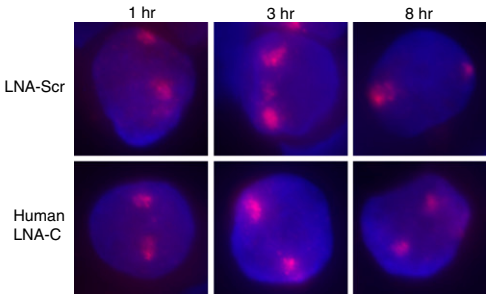

Fig. S3. Human XIST repeat C is not required for localization of RNA to Xi. XIST RNA FISH (red) in 293 cells at indicated time points after LNA-Scr or LNA-C1 nucleofection.

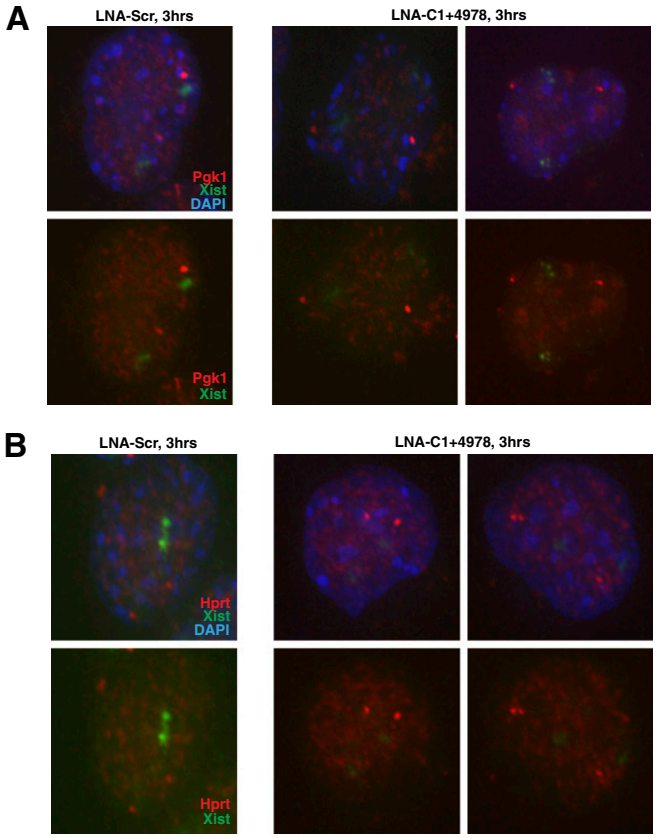

Fig. S4. Genes that are silenced on the Xi are not reactivated upon displacement of Xist by LNAs. Two-color RNA FISH detecting Xist RNA and either Pgk1 (*A*) or Hprt (*B*) nascent RNAs at 3 h after MEFs were nucleofected with LNA-Scr or repeat C LNAs (LNA-C1 and LNA-4978).