Supplemental Figure Legends

FIGURE S1. Evaluation of the assay system. (A) Structure of the construct used to produce a doxycycline-inducible expression system. Left, reverse tetracycline transactivator (rtTA) expression vector; right, expression vector for test fragments. Test fragments were expressed under the control of pTight doxycycline-inducible promoter. A puromycin resistance gene expression cassette was also sandwiched between PBIRs, which represent piggyBac transposon inverted repeats. (B, C) Expression of EGFP mRNA and Xist RNA induced by the addition of dox was detected by RNA-FISH. (D,E) Distribution of each of EGFP mRNA and Xist RNA was quantified in comparison with Malat 1 by qRT-PCR.

FIGURE S2. Abundance of each test RNA fragment in the nucleus was compared relative to 6-kb TSS. Error bars, standard deviation (SD).

FIGURE S3. Quantitative RT-PCR comparing absolute copy number of 950-nt mut and 950-nt as transcripts between the cytoplasmic and nuclear fractions.

FIGURE S4. CRISPR-Cas9 mediated disruption of the Xist locus in J1rtTA/N ES cells (A) Tetracycline transactivator in conjunction with a nuclear localization signal (nls-rtTA) introduced at the ROSA26 locus in J1rtTA/N ES cells (Wutz et al, 2000). (B) Schematic for a deletion of the Xist 5′ region containing the 950-nt sequence in J1rtTA/N ES cells. (C) Correct editing was confirmed by genotyping PCR using primers shown in (B).