

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).