

Figure S1 Allele-specific Expression Assays to Measure the X Inactivation Ratio. (A) Location of genes on the X chromosome with assays for allele-specific expression. (B) Pctk1 analysis using previously described Light Cycler Assay (Percec et al. 2002). The left panel shows the amplification curve of two control progeny samples: CastXm-3 is heterozygous for entire X chromosome; 246-1 is homozygous for 129S1 for the entire X chromosome except for the proximal end of the paternal X chromosome, which is Cast. The right panel

depicts the corresponding melting curves: peak at 60°C corresponds to the Cast allele product; peak at 65°C corresponds to 129S1 allele product. Peak heights were used to calculate the X inactivation ratio (129S1 /(Cast + 129S1)). The ratio for CastXm-3 = 0.23; the ratio for 246-1= 0.50. **(C)** *Mecp2* and *Xist* assays using RFLPs. Lanes shown are pBR322 DNA-*MspI* Digest (M), uncut PCR product (U) and cut PCR product (using *Tsp*509I for *Mecp2* and *SmII* for *Xist*) for control and RX2 progeny samples. *Mecp2 Tsp*509I 129S1 digested fragment is 217 bp and Cast digested fragments are 155 bp and 62 bp. *Xist SmII* 129S1 digested fragments are 279 bp, 82 bp and 24 bp, and the Cast digested fragments are 361 bp and 24 bp. Progeny tested in lanes 1-6 are CastXm-1, CastXm-7, 6443-1, 6443-3, 3695-1 and 3695-2, respectively. The ratio as measured by *Mecp2* for corresponding lanes are 0.31 (1), 0.22 (2), 0.40 (3), 0.28 (4), 0.58 (5) and 0.62 (6), and as measured by *Xist* for corresponding lanes are 0.31 (1), 0.21 (2), 0.46 (3) and 0.33 (4). Progeny tested in (B) and lanes 1-6 in (C) were from control or RX2-derived male mated with 129S1 female.