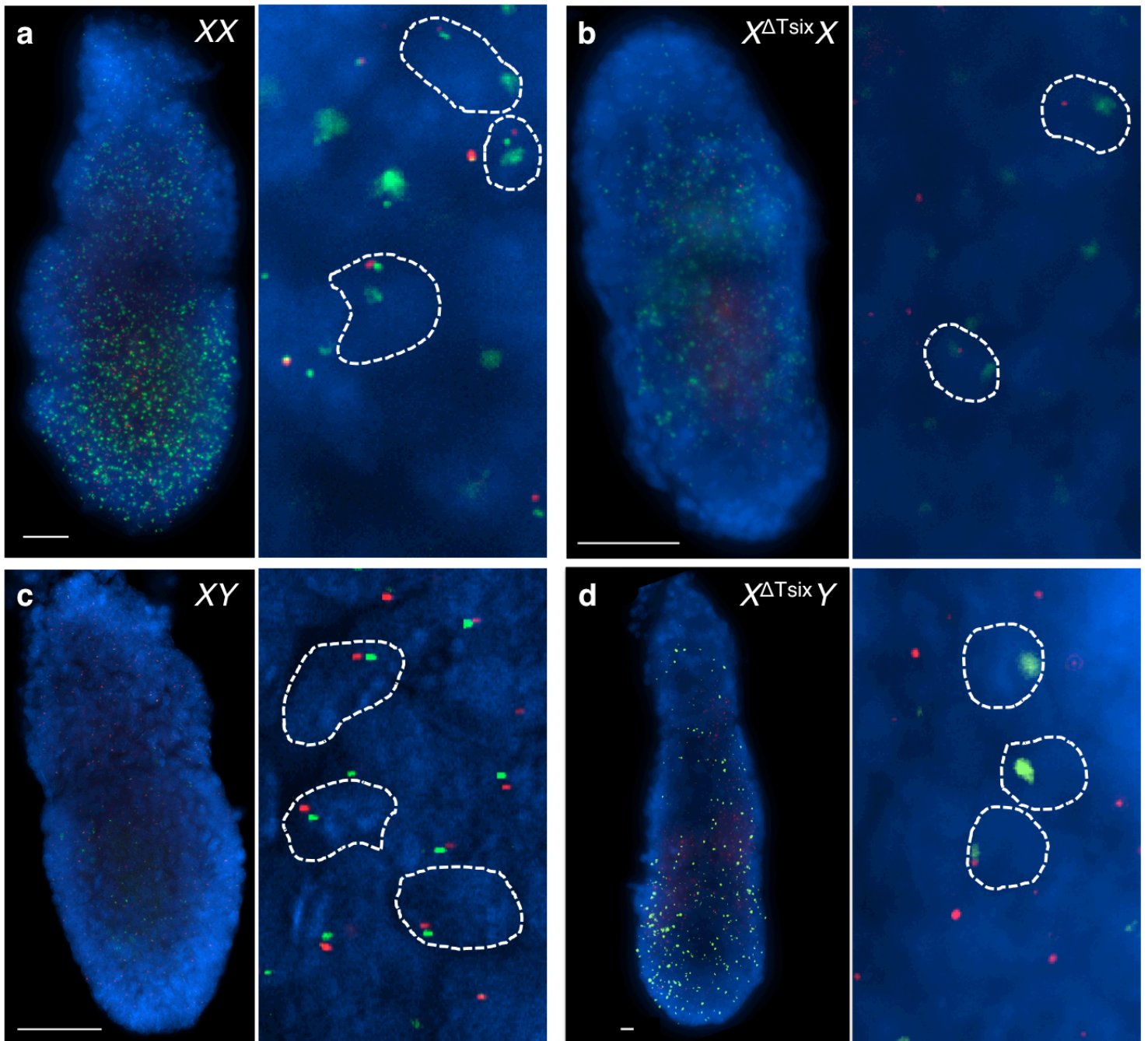
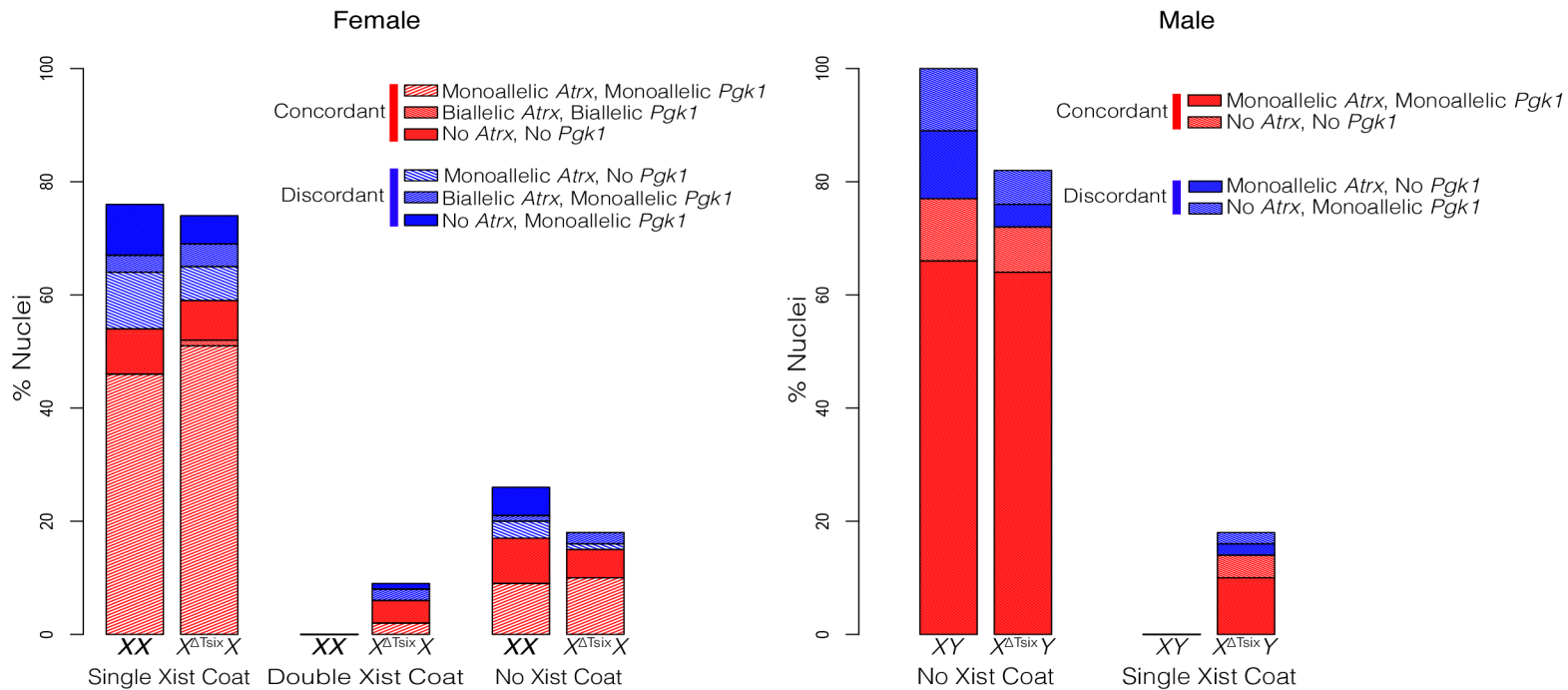
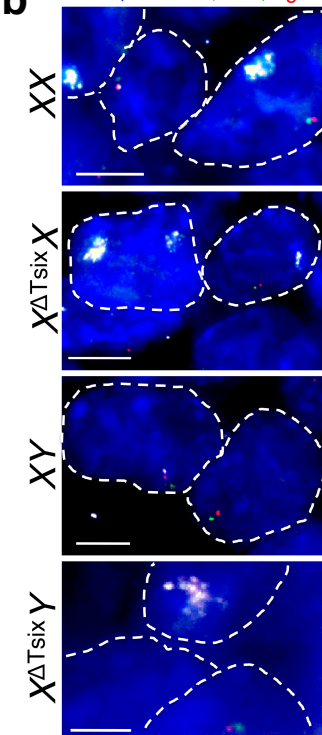
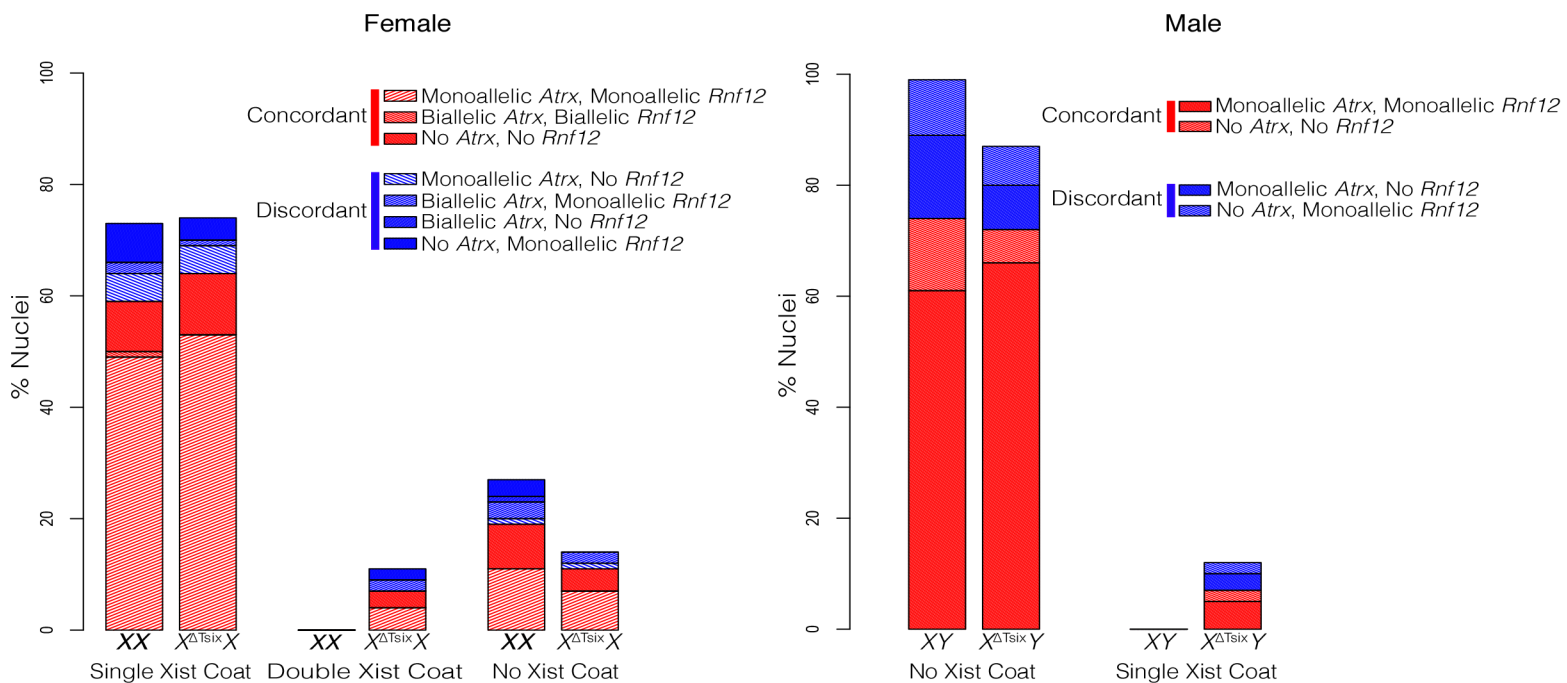
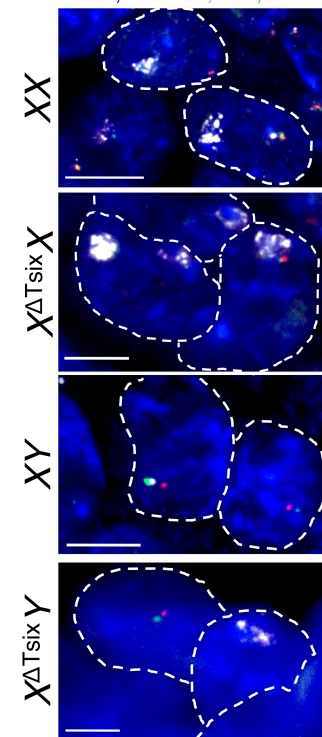


**Supplementary Figure 1. Limited Xist induction from the  $X^{\Delta\text{Tsix}}$  maternal X-chromosome in embryonic day (E) 4.0 embryos.** RNA FISH staining of whole E4.0 XX,  $X^{\Delta\text{Tsix}}X$ , XY, and  $X^{\Delta\text{Tsix}}Y$  embryos. Three embryos were examined per genotype. Xist RNA coating and Tsix RNA are simultaneously detected in green using a double-stranded probe. RNA expressed from the X-linked gene *Atrx* is shown in red. Nuclei are stained blue with DAPI. Insets show representative nuclei. Faint Xist induction from the  $X^{\Delta\text{Tsix}}$  can be seen in fewer than three percent of the  $X^{\Delta\text{Tsix}}X$  and  $X^{\Delta\text{Tsix}}Y$  nuclei (right panels, see inset nuclei). Scale bar, 20  $\mu\text{m}$ .



**Supplemental Figure 2. RNA FISH analysis of X-linked gene expression in intact wild-type (WT) and maternal  $X^{\Delta Tsix}$  mutant E6.5 embryos.** Maximum intensity projections (left) and representative extra-embryonic nuclei (right) from embryos depicted in movies S1-S4. (a) XX, (b)  $X^{\Delta Tsix}X$ , (c) XY and (d)  $X^{\Delta Tsix}Y$ . Xist RNA coating and Tsix RNA pinpoints are detected in green with a common double-stranded probe. RNA expressed from the X-linked gene *Pgk1* is detected in red. Nuclei stained blue with DAPI. Scale bar, 50  $\mu$ m.



**a*****Pgk1* and *Atrx* Allelic Expression in E6.5 Extra-Embryonic Cells****b**DAPI; Xist/Tsix; *Atrx*; *Pgk1***c*****Rnf12* and *Atrx* Allelic Expression in E6.5 Extra-Embryonic Cells****d**DAPI; Xist/Tsix; *Atrx*; *Rnf12*

**Supplemental Figure 3. X-linked genes show concordance of allelic expression in  $X^{\Delta\text{Tsix}}X$  and  $X^{\Delta\text{Tsix}}Y$  E6.5 extra-embryonic tissues.** (a) Bar plots quantifying allelic expression of the X-linked genes *Atrx* and *Pgk1* by RNA FISH in individual nuclei of *XX*,  $X^{\Delta\text{Tsix}}X$ , *XY*, and  $X^{\Delta\text{Tsix}}Y$  E6.5 extra-embryonic cells. Nuclei are subdivided into classes based on observed Xist RNA expression and coating. Concordant expression of genes within a single nucleus is plotted in red. Discordant expression is plotted in blue. In females (left), expression of *Atrx* and *Pgk1* is 78% concordant in *XX* embryos and 79% concordant in  $X^{\Delta\text{Tsix}}X$  embryos (100 total nuclei analyzed per genotype from  $n = 3$  embryos). In males (right), expression of *Atrx* and *Pgk1* is 77% concordant in *XY* embryos and 87% concordant in  $X^{\Delta\text{Tsix}}Y$  embryos (100 total nuclei analyzed per genotype from  $n = 3$  embryos). (b) Representative RNA FISH-stained nuclei. Xist and Tsix RNAs are shown in white, *Atrx* RNA in green, and *Pgk1* RNA in red. Nuclei are stained blue with DAPI. Scale bar, 5  $\mu\text{m}$  (c) Bar plots quantifying allelic expression of the X-linked genes *Atrx* and *Rnf12* in individual nuclei of E6.5 extra-embryonic cells. Analysis was carried out as described in (a). In females (left), *Atrx* and *Rnf12* expression is 78% concordant in *XX* embryos and 82% concordant in  $X^{\Delta\text{Tsix}}X$  embryos (100 total nuclei analyzed per genotype from  $n = 3$  embryos). In males (right), *Atrx* and *Rnf12* expression are 74% concordant in *XY* embryos and 79% concordant in  $X^{\Delta\text{Tsix}}Y$  embryos (100 total nuclei analyzed per genotype from  $n = 3$  embryos). (d) Representative RNA FISH-stained nuclei. Xist and Tsix RNAs are shown in white, *Atrx* RNA in green, and *Rnf12* RNA in red. Nuclei are stained blue with DAPI. Scale bar, 5  $\mu\text{m}$ .