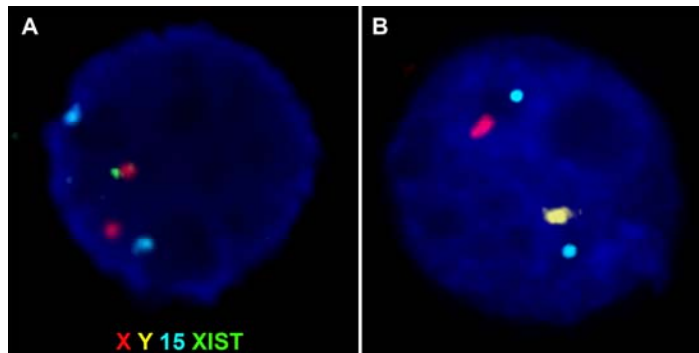


Supplemental Data

## X Chromosome Inactivation Is Initiated in Human Preimplantation Embryos

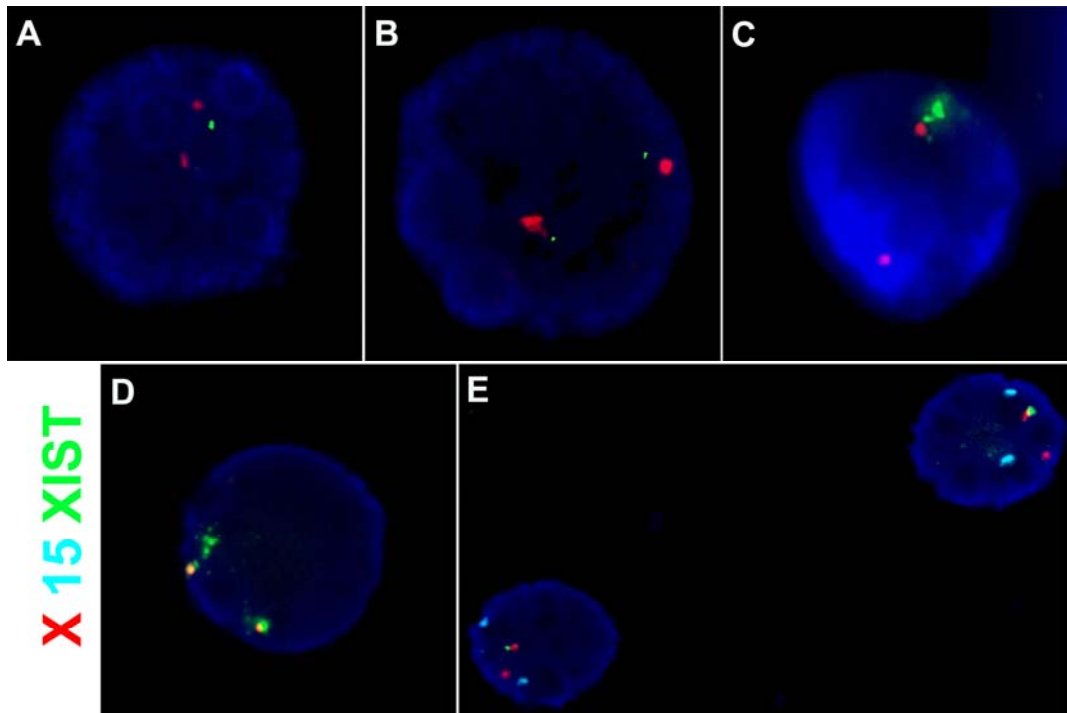
Ilse M. van den Berg, Joop S.E. Laven, Mary Stevens, Iris Jonkers, Robert-Jan Galjaard, Joost Gribnau,  
and J. Hikke van Doorninck

Figure S1. Single Cell RNA/DNA FISH Analysis of Human Embryos



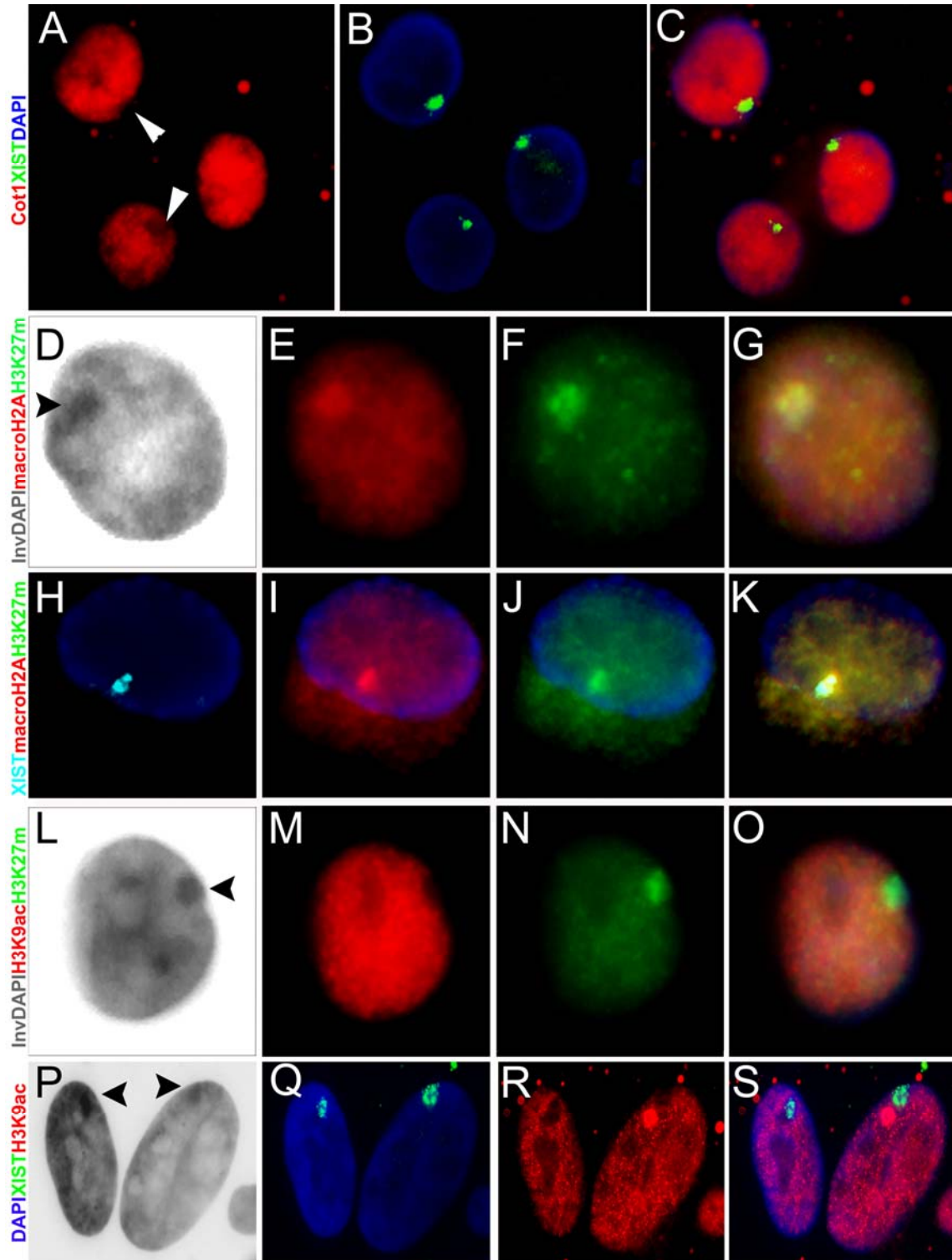
(A) Diploid blastomere from a female 8-cell embryo displaying two X centromeres (red), two chromosome 15 centromeres (aqua), and an *XIST* pinpoint (green). (B) Diploid male blastomere from a 12-cell male embryo with an X chromosome (red), a Y chromosome (yellow) and two chromosomes 15 (aqua). *XIST* RNA was absent.

**Figure S2. Examples of Different Patterns of *XIST* RNA Signals in Female Embryos**



(A) Single pinpoint of *XIST* (green) near the X centromere (red) at the 8-cell stage. (B) Two pinpoint signals of *XIST* at morula stage. (C) Single cloud signal in blastocyst. (D) Double cloud signals of *XIST* in late morula embryo. (E) Two cells from a morula, one with a pinpoint *XIST* signal and the other with an intermediate cloud of *XIST* RNA

Figure S3. Epigenetic Changes on the Inactive X Chromosome in Human Control Cells



RNA FISH and immunocytochemistry of human female cumulus cells (A-G, L-O) and female amniocytes (H-K, P-S) with probes and antibodies commonly used to characterize the inactive X chromosome. *Cot1* RNA FISH staining of cumulus cells (red in A) shows an excluded area (indicated with arrowheads), that overlaps with *XIST* RNA staining (green in B) as shown in the overlay in (C). The third nucleus is not in focus and the *Cot1* depleted region can thus not be seen. Inverted DAPI staining reveals the position of the inactive X/Barr body (arrowhead in D) in cumulus cells that overlaps with macroH2A staining (red in E) and H3K27Me3 (green in F), merged in G. (H-K) In female amniocytes, *XIST* RNA (H) shows a complete overlap with the accumulated nuclear domains of macroH2A (red in I) and H3K27me3 (green in J) as shown in the overlay (merged in K). (L) Cumulus cell with Barr body that is indicated with an arrowhead in the inverted DAPI image. H3K9 acetylation staining shows exclusion of the Barr body area (red in M) and H3K27Me3 gives a strong localized signal staining (green in N) that overlaps exactly with the exclusion of H3K9ac and the position of the Barr body (merged in O). In female amniocytes the Barr body (arrowheads in P) overlaps the *XIST* cloud (green signal in Q) and at this area H3K9ac is depleted (red in R) as shown in the overlay (merged in S). The bright red spot in R and S is aspecific background staining.

**Table S1. XIST Patterns in Sex Euploid and Aneuploid Human Embryonic Cells**

Embryos	Genotype	# Cells	"Expected" XIST pattern <sup>a</sup>	(%)	"Unexpected" XIST pattern	Unexpected patterns: more (+) or less (-) XIST spots
<b>XY cleavage stage</b>						
euploid (N=3)	XY	17	16	(94)	1	+
mosaic (N=3)	XY	13	12	(92)	1	+
	XX	1	1	(100)		
	XO	3	3	(100)		
	XYY	1	1	(100)		
<b>XY morulas</b>						
euploid (N=2)	XY	24	24	(100)		
mosaic (N=7)	XY	67	54	(81)	13	+
	XX	3	0	(0)	3	+
	XO	12	7	(58)	5	+
<b>XY blastocysts</b>						
euploid (N=3)	XY	52	52	(100)		
mosaic (N=2)	XY	46	39	(85)	7	+
	XX	1	1	(100)		
	XO,YO	2	2	(100)		
<b>XX cleavage stage</b>						
euploid (N=2)	XX	11	8	(73)	3	+
mosaic (N=3)	XX	15	10	(67)	5	2+ and 3-
	XO	1	1	(100)		
	XXXX	1		(0)	1	-
<b>XX morulas</b>						
euploid (N=4)	XX	30	20	(67)	10	4+ and 6-
mosaic (N=9)	XX	59	41	(69)	18	7+ and 11-
	XO	11	9	(82)	2	+
	XXX	5	3	(60)	2	-
<b>XX blastocysts</b>						
euploid (N=4)	XX	110	110	(100)		
mosaic (N=2)	XX	34	32	(88)	2	+
	XO	1	1	(100)		
	XXXX	1		(0)	1	-

Data include only cells with an analysable X/Y, 15 and XIST. <sup>a</sup> An "Expected" pattern is an XIST signal for every n-1 X chromosome.