Supporting Information

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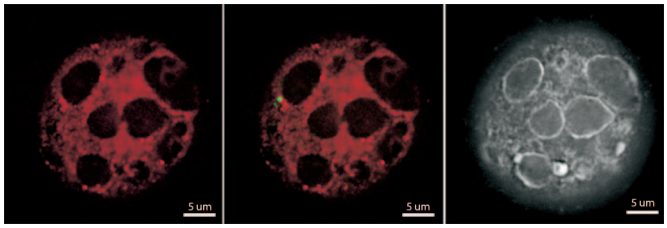

Fig. S1. Expression of the B1 family of SINE repetitive elements at the 2-cell stage in female embryos. A representative example of repeat-probe RNA FISH on a 2-cell embryo. A single blastomere of a 2-cell embryo is shown, with clear B1 SINE expression (*red*) in regions overlapping with and surrounding the paternal *Xist* RNA signal (*green*). DAPI staining of DNA is shown on the *Right* (black and white). It should be noted that SINE transcripts are excluded from adjacent nucleoli (depleted of DAPI) but not from the DAPI-stained DNA surrounding the paternal Xist signal.

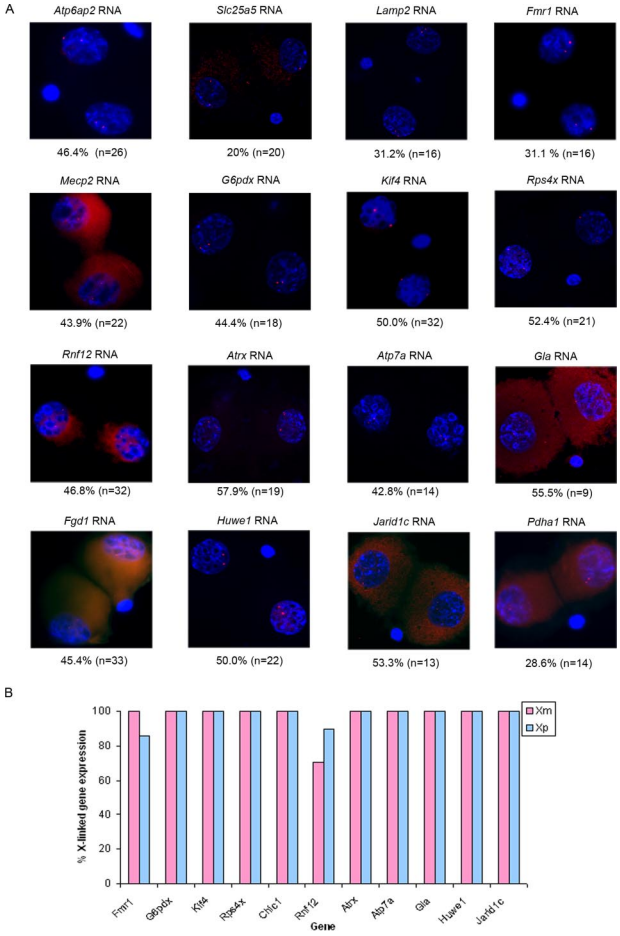

Fig. S2. Transcriptional activity of the paternal and maternal X chromosomes assayed by gene RNA FISH for 16 genes in 2-cell embryos. (*A*) Two primary transcript signals derived from the maternal and paternal X chromosomes (*red*) could be detected for every gene tested. On the bottom of each panel is shown the percentage of biallelic expression observed at the 2-cell stage for a given gene, when considering the totality of embryos, including male and female, as well as the number of embryos tested. (*B*) Percentage X-linked gene expression originating from the maternal (Xm) or paternal (Xp) allele at the 2-cell stage in female embryos. Genes were considered only in cases in which it was possible to formally conclude that the signal was coming from the paternal or maternal allele.

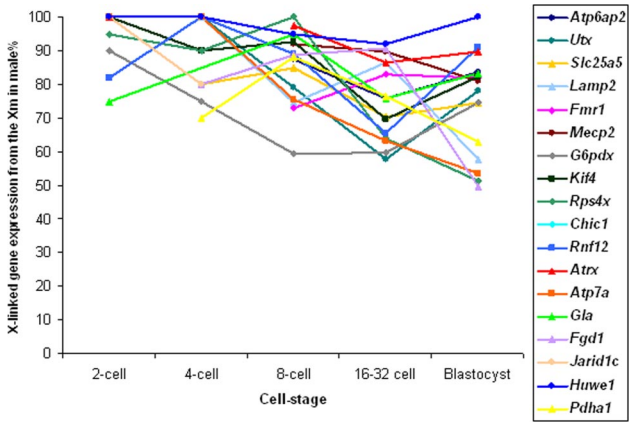

Fig. S3. Kinetics of Xm expression of the 18 different genes during male preimplantation development. Each curve represents the kinetics of one gene. Five different cell stages have been studied (2-cell, 4-cell, 8-cell, 16–32-cell, and trophectoderm cells of blastocyst). For all of the genes, a minimum of 5 embryos was studied for each cell stage.

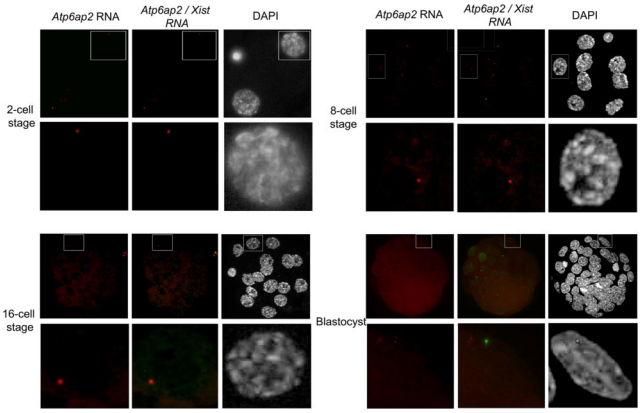

Fig. S4. An example of the transcriptional activity of an X-linked gene, *Atp6ap2* during male embryo preimplantation development. One *Atp6ap2* RNA FISH signal (*red*) could be detected at every stage examined (2-cell, 8-cell, 16-cell, and blastocyst stages). *Xist* (green) is expressed from the 8-cell stage in males.

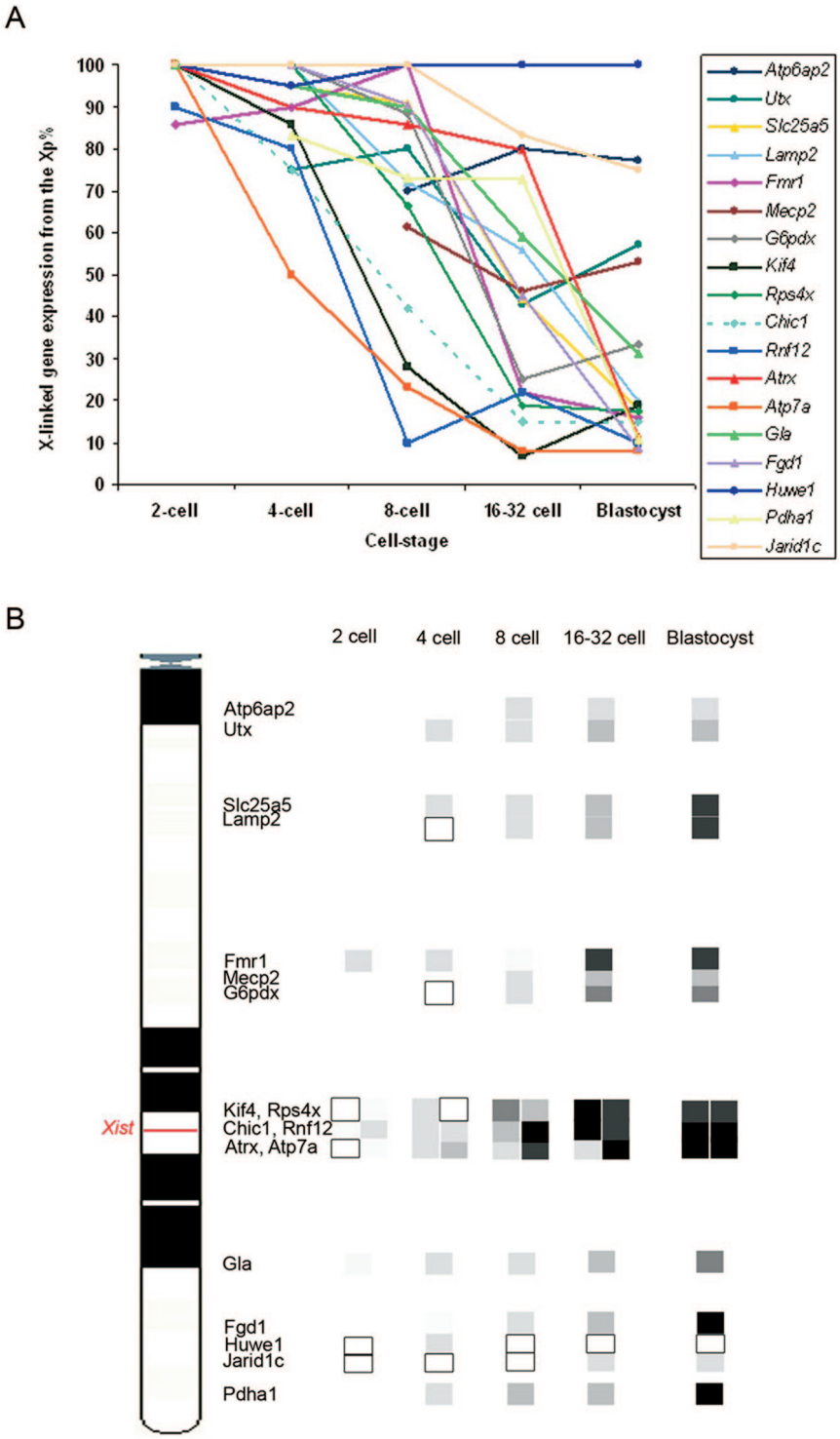

Fig. S5. Kinetics of Xp expression of the 18 different genes during female preimplantation development. For every X-linked gene, gene expression from the paternal allele in female embryos was normalized to the expression from the maternal allele observed in male embryos at the same cell stage. Five different cell stages were studied (2-cell, 4-cell, 8-cell, 16–32-cell, and trophectoderm cells of blastocyst). For all of the genes, a minimum of 5 embryos was studied for each cell stage. (*A*) Each curve represents the kinetics of 1 gene. (*B*) Color coding reflects relative Xp expression level as indicated (white: 100%; light grey: 70–99%; medium-light grey: 50–69%; medium-dark grey: 30–49%; dark grey: 14–29%; black: ≤15%).

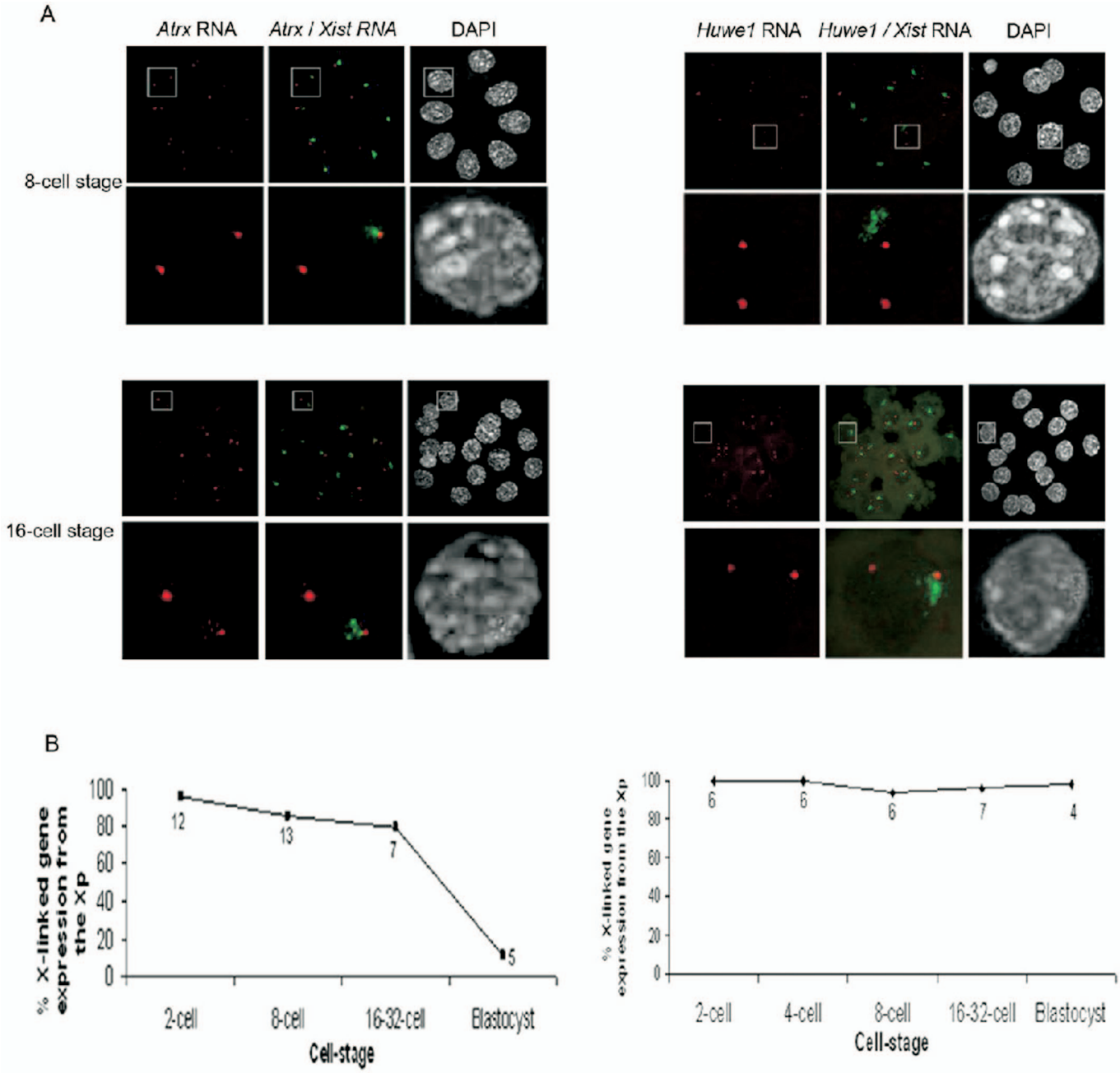

Fig. S6. Specific profile of *Atrx* and *Huwe1* transcriptional activity during X-imprinted inactivation assayed by RNA FISH in preimplantation stages. (*A*) *Atrx* primary transcript signals (red) evidenced by specific RNA FISH at 8-cell (*Upper Left*) and 16-cell (*Bottom Left*) stages. Two signals coexisted, with 1 within the *Xist* domain (green) at the 8-cell and 16-cell stages. *Huwe1* primary transcript signals (red) evidenced by specific RNA FISH at 8-cell (*Upper Right*) and 16-cell (*Bottom Right*) stages. Two signals coexisted, with 1 within the *Xist* domain (green) at the 8-cell and 16-cell stages. (*B*) Kinetics of expression of *Atrx* (*Left*) and *Huwe1* (*Right*) during preimplantation development. Xp-*Atrx* and Xp-*Huwe1* expressions observed in female embryos were normalized as a function of the expression seen in male embryos for the same cell stage and the same gene. Four different preimplantation cell stages were studied (2-cell, 8-cell, 16–32-cell, and trophectoderm cells of blastocyst). The numbers represent the number of studied embryos.

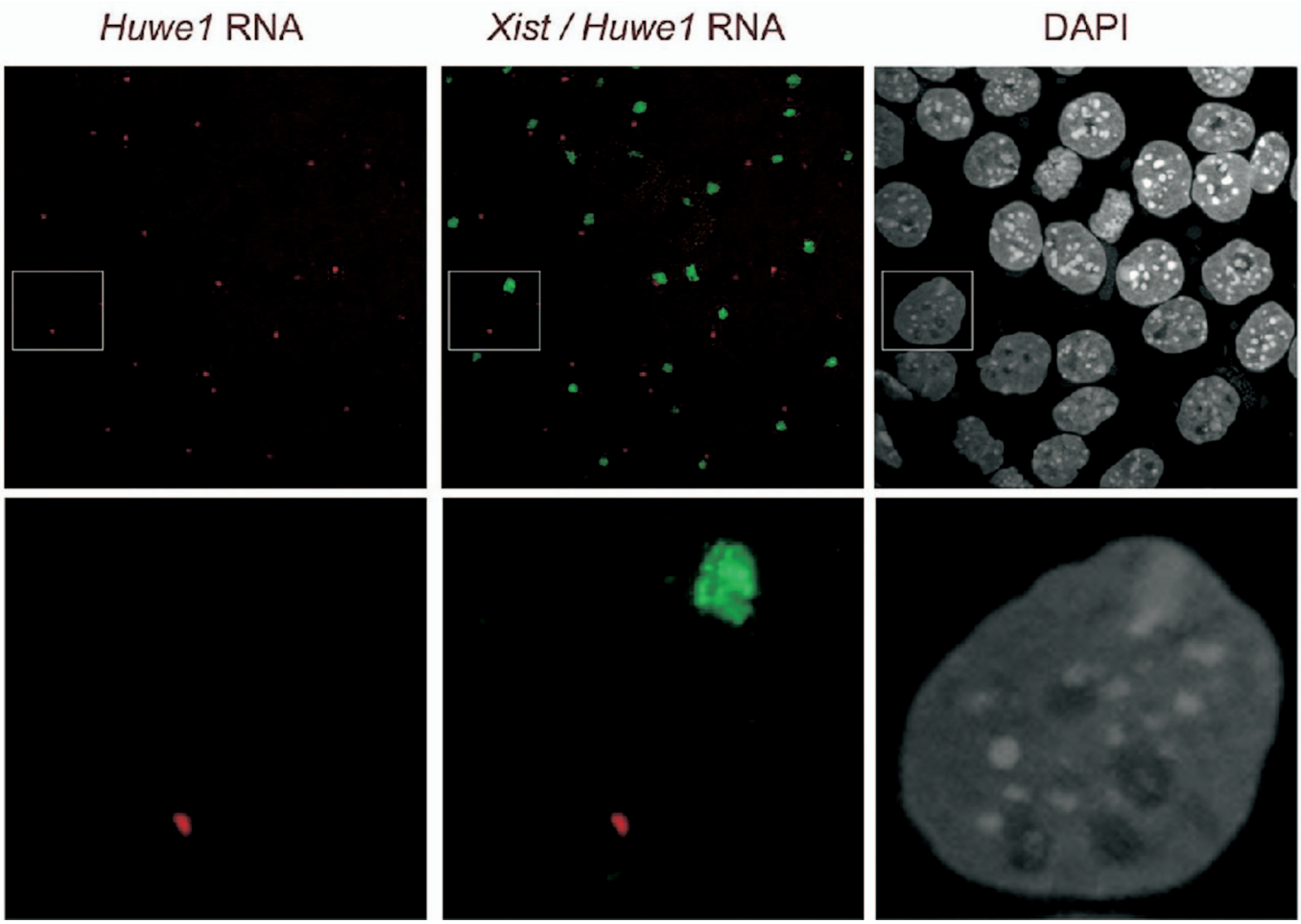

Fig. S7. *Huwe1* expression in female TS cells at Day 5 of differentiation by RNA FISH. RNA FISH analysis shows that only 1 *Huwe1* primary transcript signal (red), distinct from the Xist RNA domain (green), could be seen on the Xp allele.

Table S1. *Atrx* and *Huwe1* expression in 4 different tissues isolated from female embryos at 6.5 dpc

Tissue	Gene	Total no. of cells (no. of embryos)	Cells with monoallelic expression (%)	Cells with biallelic expression (%)
Proximal endoderm	*Atrx*	200 (4)	100	0
	Huwe1	150 (3)	100	0
Extraembryonic ectoderm	*Atrx*	190 (4)	7	93
	Huwe1	126 (3)	84	16
Distal endoderm	*Atrx*	94 (4)	94	6
	Huwe1	104 (3)	98	2
Embryonic ectoderm	*Atrx*	346 (4)	83	17
	Huwe1	202 (3)	94	6

Table S2. List of genomic BAC probes

Clone no.	Gene	Gene title
RP23-43D10	*Atp6ap2*	ATPase
RP23-174N2	*Utx*	Ubiquitously transcribed tetratricopep, repeat gene, X chr.
RP23-286I5	*Slc25a5*	Solute carrier family 25, isoform 5
RP24-173A8	*Lamp2*	Lysosomal membrane glycoprotein 2
RP24-183G11	*Fmr1*	Fragile X mental retardation syndrome 1 homologue
RP23-77L16	*Mecp2*	Methyl CpG binding protein 2
RP23-13D21	*G6pdx*	Glucose-6-phosphate dehydrogenase X-linked
RP24-248G16	*Kif4*	Kinesin family member 4
RP23-331B22	*Rps4x*	Ribosomal protein S4
RP24-240J16	*Rnf12*	Ring finger protein 12
RP23-260I15	*Atrx*	Alpha thalassemia/mental retardation syndrome X-linked homologue
RP23-186F4	*Atp7a*	ATPase, Cu++ transporting, α polypeptide
RP23-26D22	*Gla*	Galactosidase, α
RP23-84O5	*Fgd1*	FYVE, RhoGEF, and PH domain containing 1
RP24-157H12	*Huwe1*	HECT, UBA, and WWE domain containing 1
RP24-148H21	*Jarid1c*	Jumonji, AT rich interactive domain 1C
RP24-211E22	*Pdha1*	Pyruvate dehydrogenase E1 α 1