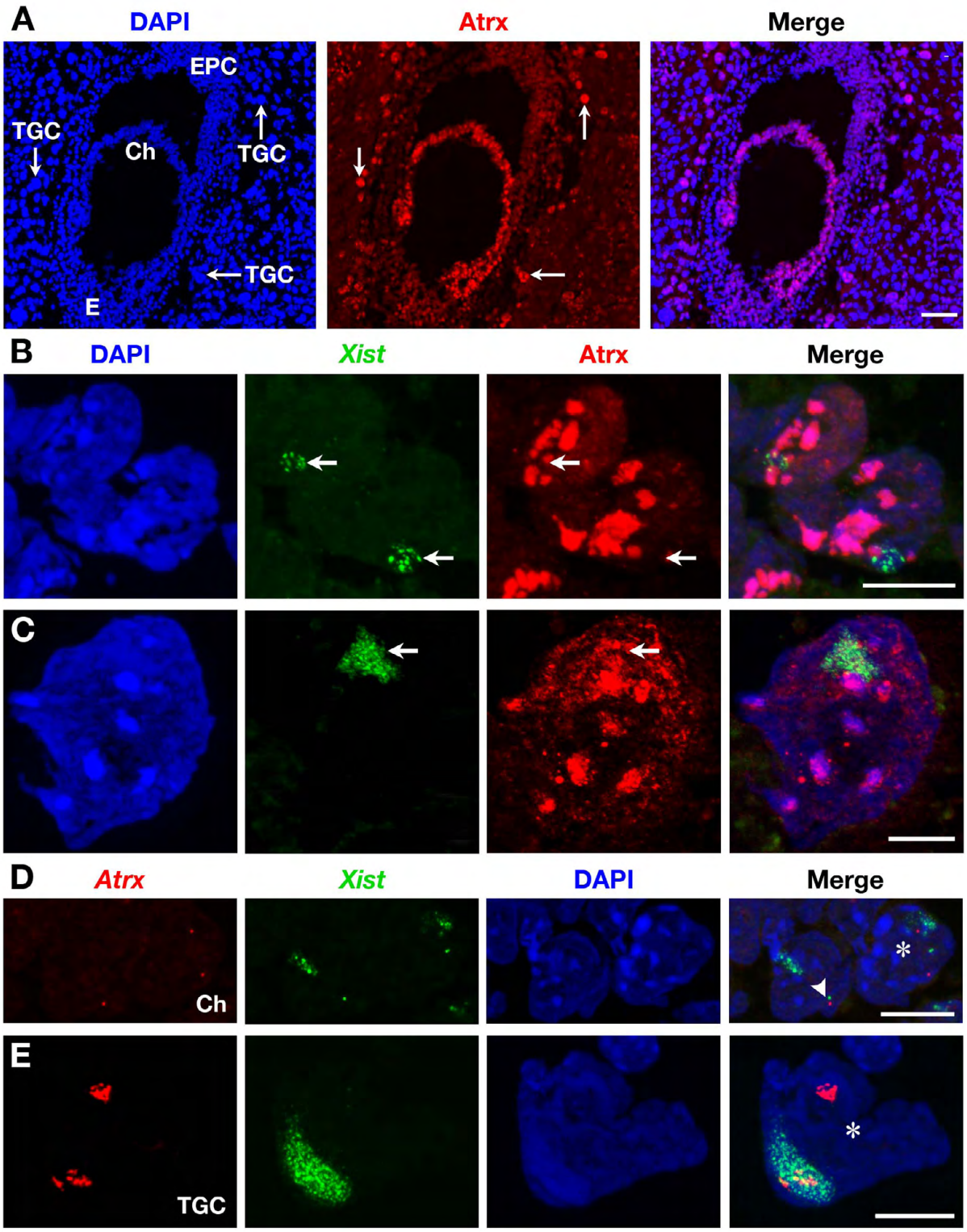

Fig. S1. Atrx protein and *Atrx* RNA expression during imprinted X inactivation of extra-embryonic lineages at the postimplantation stage. (**A**) Longitudinal section of E7 female embryo immunostained with an antibody against Atrx. Scale bar: 100 µm. (**B**,**C**) RNA FISH for *Xist* (green) and immunofluorescence for Atrx (red). (B) Two nuclei from the chorion showing no overlap of the *Xist* domain with the Atrx protein. Only 5.8% (5/86) of nuclei from this extra-embryonic tissue exhibit partial overlap between *Xist* and Atrx. Scale bar: 10 µm. (C) One representative TGC showing *Xist* that partially overlaps with Atrx. They represent 25% (6/24) of TGCs. Scale bar: 100 µm. (**D**,**E**) RNA FISH to detect *Xist* RNA (green) and *Atrx* primary transcripts (red). (D) Two nuclei from E7 female embryonic chorion showing monoallelic (arrowhead) and biallelic (asterisk) *Atrx* expression. Scale bar: 10 µm. (E) One representative TGC showing biallelic *Atrx* expression (asterisk). Scale bar: 100 µm. Ch, chorion; E, embryo; EPC, ectoplacental cone; TGC, trophoblast giant cell.

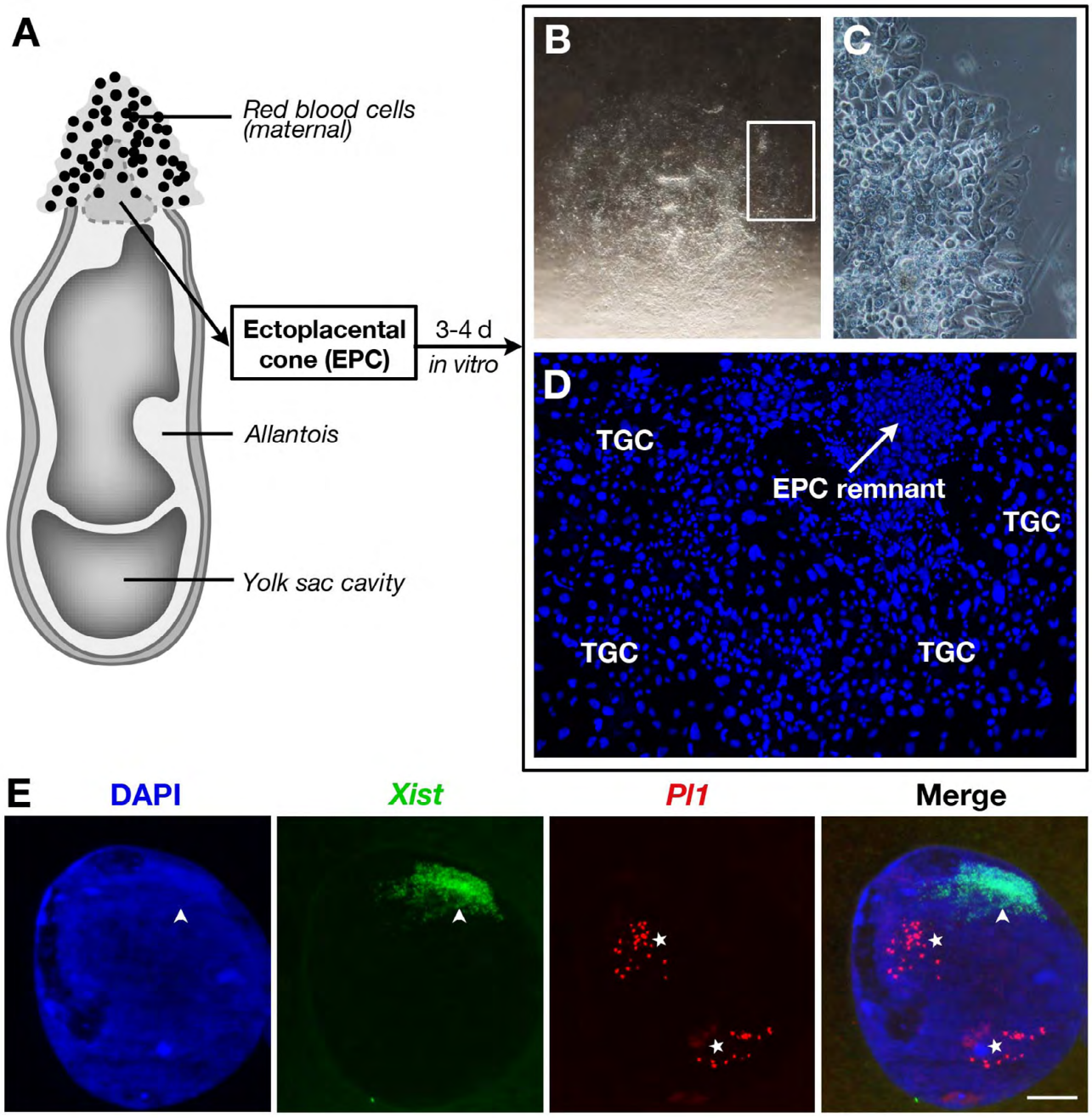

Fig. S2. Secondary TGC differentiation from E7 ectoplacental cone. (**A**) Drawing of an E7 embryo prior to EPC dissection. The EPC was separated from the embryo and yolk sac. It was also deprived of surrounding red blood cells prior to being deposited on a glass coverslip in culture medium. (**B-D**) TGCs developed from an E7 EPC after 3-4 days of culture. Individual explant forms an outgrowth (B) that spreads as a monolayer of flattened TGCs (C). TGC with giant nuclei can be detected, spread around the EPC remnant (arrow), as shown by DAPI staining (D). (**E**) Transcriptional activity of *Pl1* assayed by RNA FISH in secondary female TGC derived from E7 EPC. *Pl1* RNA (red, indicated by a star), a specific marker for TGCs, is detected on chromosome 13, whereas *Xist* RNA (green) accumulates on the X chromosome. Dense DAPI intensity and the *Xist* domain colocalize as indicated by the arrowhead. Scale bar: 10 µm.

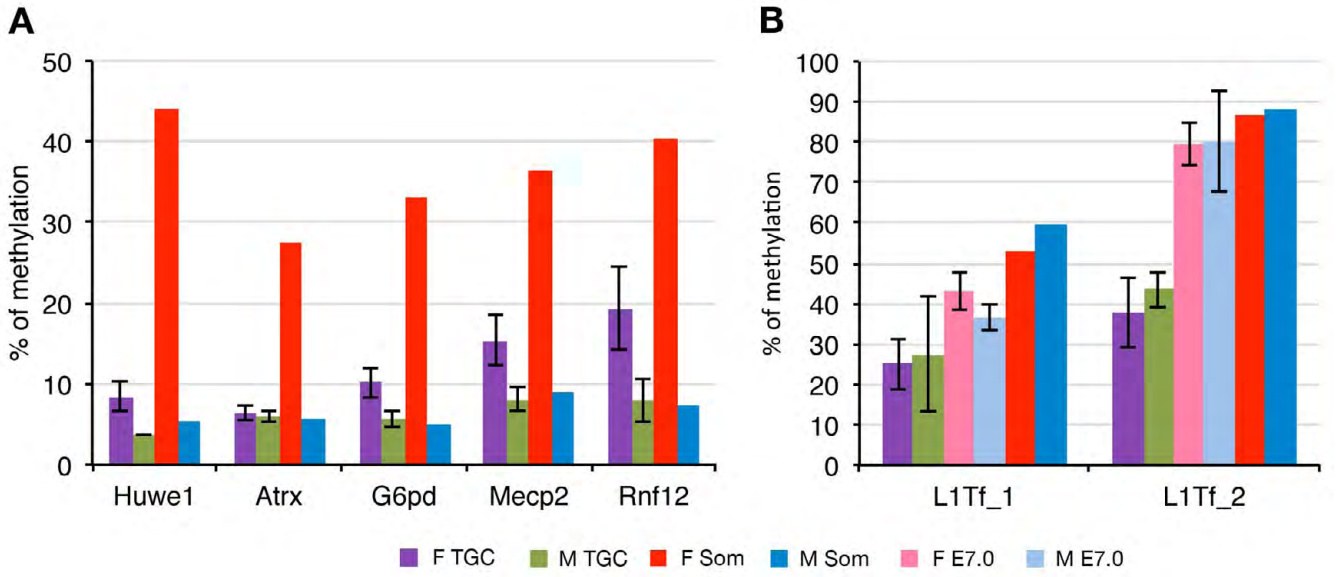

Fig. S3. DNA methylation analysis by bisulfite sequencing of X-linked genes and LINE-1 (Tf) promoters in secondary TGCs. Percentage of methylation in female (F) and male (M) is shown. (**A**) X-linked promoter (*Huwe1*, *Atrx*, *G6pd*, *Mecp2* and *Rnf12*) methylation in secondary TGCs generated from single E7 EPCs cultured for 3 days and somatic tissues (som, adult liver cells). (**B**) Methylation of L1-Tf elements located immediately upstream (1) and downstream (2) of the *Huwe1* gene as described (Chow et al., 2010) in secondary TGCs and somatic tissues.

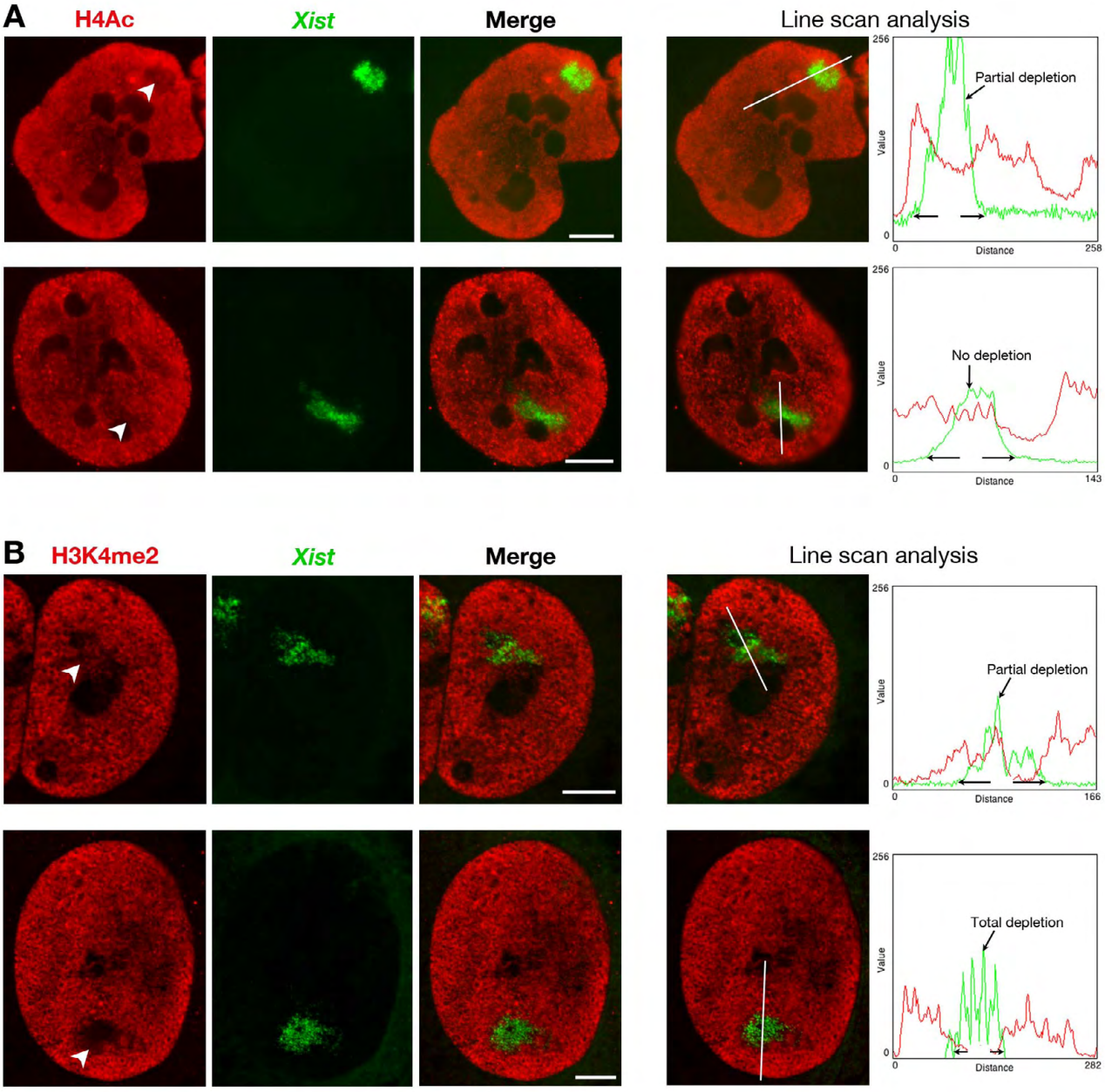

Fig. S4. 3D images and line scan analysis of H4 acetylation and H3K4 dimethylation of secondary TGCs. (**A**) H4 acetylation at the Xi. Upper panel, partial depletion; lower panel, no depletion. (**B**) H3K4 dimethylation at the Xi. Upper panel, partial depletion; lower panel, total depletion.

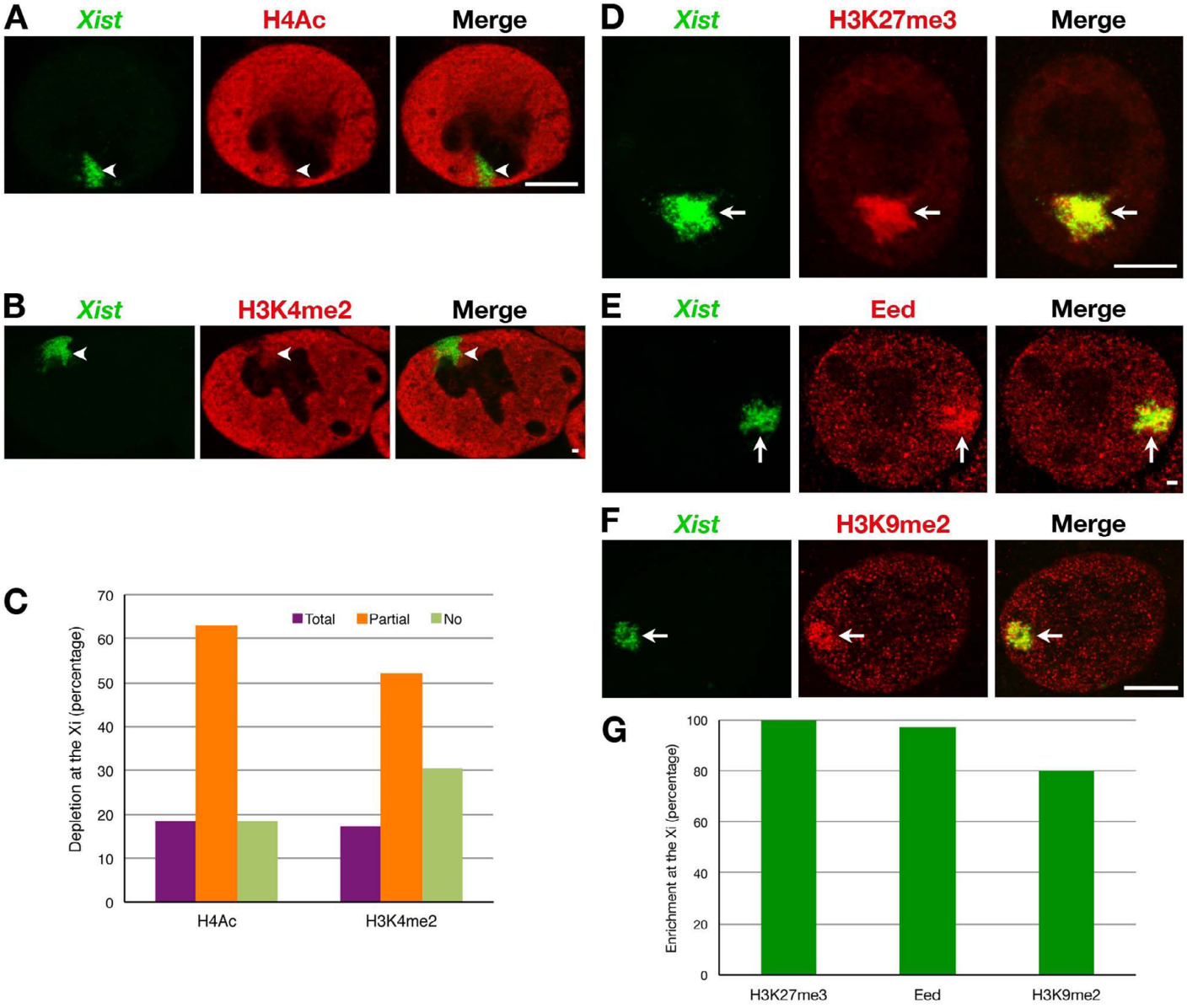

Fig. S5. 3D analysis of active marks (H4 acetylation, H3K4 dimethylation) and repressive marks (H3K27me3 and Eed) of primary female TGCs derived from E3 blastocyst. See Fig 3. (**A**) H4 acetylation at the Xi, showing partial depletion. (**B**) H3K4 dimethylation at the Xi, showing partial depletion. (**C**) Quantification of active marks: H4 acetylation (*n*=38 from four embryos) and H3K4me2 (*n*=46 from five embryos). (**D**) H3K27me3 at the Xi, showing colocalization. (**E**) Eed at the Xi, showing colocalization. (**F**) H3K9me2 at the Xi, showing colocalization. (**G**) Quantification of repressive marks: H3K27me3 (*n*=41 from four embryos), Eed (*n*=61 from four embryos) and H3K9me2 (*n*=20 from one embryo).

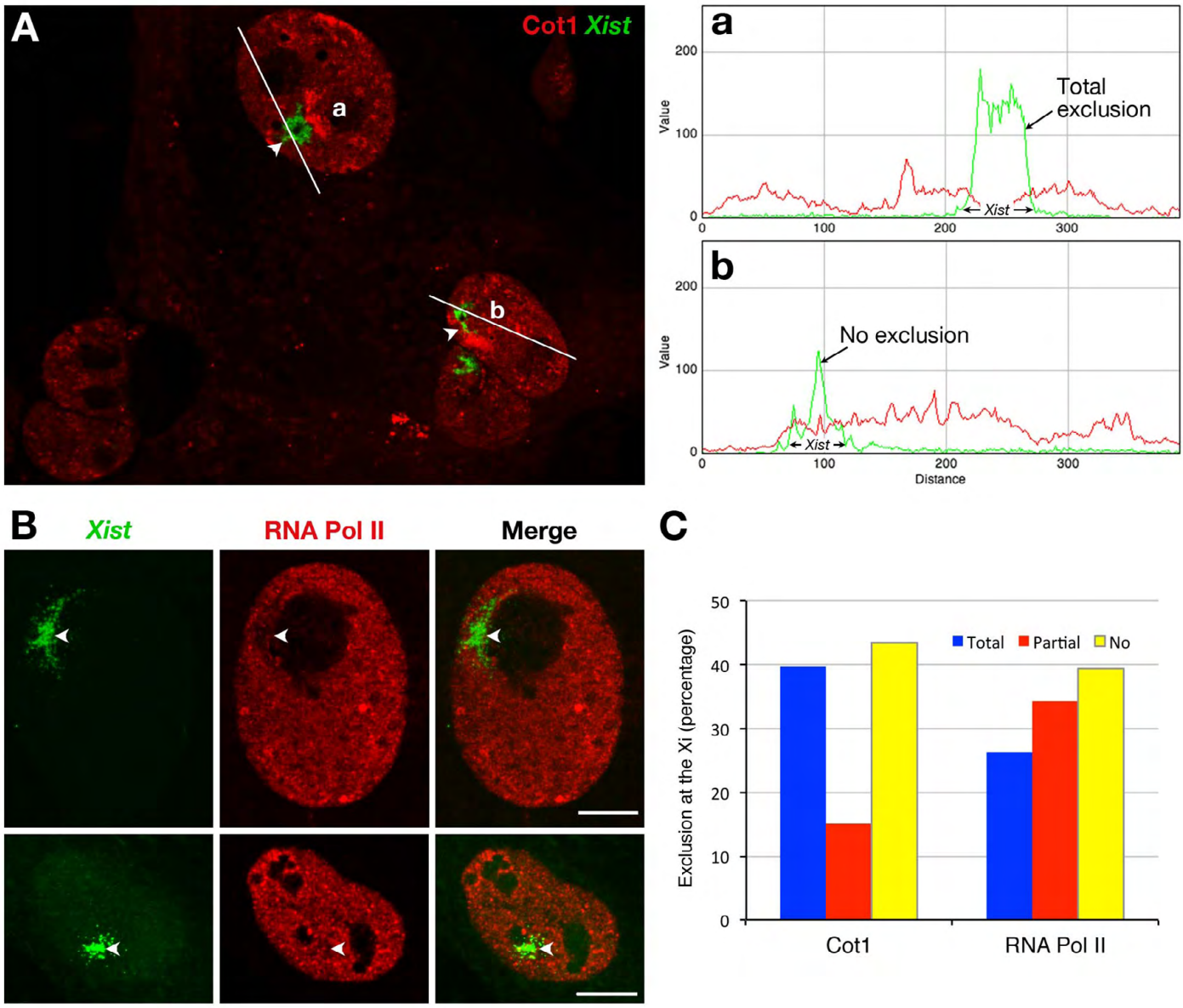

Fig. S6. No silent *Xist* RNA nuclear compartment on the paternal X chromosome in primary TGCs. (**A**) *Cot1* and *Xist* RNA FISH. (**a**,**b**) Representative examples of 3D analysis and line scans showing the total (a) and no (b) exclusion of *Cot1* RNA in the *Xist* RNA domain. (**B**) RNA Pol II immunofluorescence and *Xist* RNA FISH. Exclusion of RNA Pol II (red) in the *Xist* RNA domain (green); upper panel, partial exclusion; lower panel, no exclusion. (**C**) Quantification of primary TGCs exhibiting total, partial or no exclusion of *Cot1* RNA and RNA Pol II. For *Cot1* RNA FISH, three blastocysts were cultured and 12, 12 and 29 primary TGCs were analyzed, respectively (n=53). For RNA Pol II immunofluorescence, five blastocysts were cultured and 1, 5, 14, 20 and 21 primary TGCs were analyzed, respectively (n=61). The results indicate the mean (percentage). Scale bars: 10 μm.

Table S1. Efficiency of RNA primary transcripts for different genes in different cell types in male embryos

Gene	Tissue/cell	Total no. of cells	Expression (%)
Kif4	E7 embryo	90	90
	E7.5 embryo	50	100
	E7 VE	49	79.6
	E7.5 VE	27	96.3
	E7 EPC+Ch	65	87.7
	E7.5 EPC+Ch	9	77.8
	E7 TGC	4	100
Huwe1	E7 embryo	89	97.7
	E7.5 embryo	59	89.8
	E7 VE	74	77.0
	E7.5 VE	65	86.1
	E7 EPC+Ch	73	95.9
	E7.5 EPC+Ch	39	82.1
	E7 TGC	12	100
	E7.5 TGC	8	87.5
	Secondary TGC*	38	100
Atrx	Secondary TGC*	64	95.3
Rnf12	Secondary TGC*	71	98.6
G6pd	Secondary TGC*	37	89.2

VE, visceral endoderm; EPC, ectoplacental cone; Ch, chorion; TGC, trophoblast giant cell.
*Secondary TGC obtained upon 3-4 days of E7 EPC culture.

Table S2. Biallelic expression of X-linked genes in TGCs analyzed on embryo sections at different postimplantation stages

	X-linked genes				
	% of *in vivo* TGCs showing escape (*n*=number of analyzed TGCs/embryo)				
Embryonic stage	*Kif4*	*Rnf12*	*Mecp2*	*Huwe1*	*Atrx*
E6.5	3.1 (*n*=32)	14.3 (*n*=7) 0 (*n*=11)	0 (*n*=2) 17.2 (*n*=29)	14.8 (*n*=27) 0 (*n*=4)	42.8 (*n*=7) 33.3 (*n*=3)
E7.0	0 (*n*=2) 0 (*n*=17) 0 (*n*=16)	10.0 (*n*=10) 5.5 (*n*=18) 25.0 (*n*=4)	25.0 (*n*=8) 25.0 (*n*=12) 0 (*n*=18)	42.3 (*n*=52) 14.3 (*n*=7) 20.0 (*n*=10)	33.3 (*n*=15) 22.2 (*n*=9) 37.5 (*n*=8) 33.3 (*n*=15)
E7.5	21.4 (*n*=14) 16.7 (*n*=12)	0 (*n*=14) 20 (*n*=25) 16.6 (*n*=18)	27.8 (*n*=18) 23.5 (*n*=17)	15.4 (*n*=13) 28.6 (*n*=28)	24.0 (*n*=25) 42.3 (*n*=26)
E8.0	14.3 (*n*=21) 35.7 (*n*=14)	25.9 (*n*=27) 40.0 (*n*=10)	11.1 (*n*=18) 45.4 (*n*=11)	27.3 (*n*=11) 20.0 (*n*=10)	52.6 (*n*=19) 28.6 (*n*=7) 55.2 (*n*=29)

Table S3. Primer information for Sequenom epityper analysis [sequence, annealing temperature (Ta) and product size]

ID	Primer	Sequence	Ta (°C)	Size (bp)
Huwe1	Huwe1_F	GAGATTTTATGTTTTTTAAAGG	60	329
	Huwe1_R	ACTCCTACACTCAATAACCAACCTC		
Atrx	Atrx_F	TTAAGTTTTAGTTGGGGTTTTTTAT	60	313
	Atrx_R	ATAACTACTAAAATCCCAATACTTTTC		
G6pdx	G6pdx_F	GGAAAGTTAGGTTATATATAATGGTTGG	58	490
	G6pdx_R	AATCTAATCCTCATAAACCCAATAC		
Mecp2	Mecp2_F	GAGGAGGAGGGAGTAAAATTTAGAG	60	296
	Mecp2_R	CATCCTAAACCCCAACTATACAAAC		
Rnf12	Rnf12_F	TTTTATTTTATTTTTTTAAATTTTAGTT	60	459
	Rnf12_R	AACCCAATTAAATTCCCTCCTAAC		
Tf-1	Tf-1_F	AGGTTTTTGAAGTTTATTGAGAGTT	58	342
	Tf-1_R	TATTCAAACTAATTTCCTAAATCCC		
Tf-2	Tf-2_F	AGTAGAGGTTTTAAAAATTTAATTTTTAGT	55	313
	Tf-2_R	CAAAAAATCCTAAAACCAAAATAAC		
	10bp 5'tag	AGGAAGAGAG		
	T7 3'tag	CAGTAATACGACTCACTATAGGGAGAAGGCT		