

**Supplementary Table I** Cell count result in *Xist* RNA-FISH in day3 EB

Genotype	Strong <i>Xist</i> signal	Weak <i>Xist</i> signal	No <i>Xist</i> signal	Total No. of nuclei counted
X <sup>Δ</sup> X	120 (66.3%)	21 (11.6%)	40 (22.0%)	181 (100%)
XY <i>Eed</i> <sup>-/-</sup>	0 (0%)	0 (0%)	83 (100%)	83 (100%)
X <sup>Δ</sup> Y <i>Eed</i> <sup>-/-</sup> 1 <sup>*1</sup>	146 (59.8%)	23 (9.4%)	75 (30.7%)	244 (100%)
X <sup>Δ</sup> Y <i>Eed</i> <sup>-/-</sup> 2 <sup>*2</sup>	119 (63.6%)	16 (8.6%)	52 (27.8%)	187 (100%)

\*1 X<sup>Δ</sup>Y *Eed*<sup>-/-</sup> cell clone 1

\*2 X<sup>Δ</sup>Y *Eed*<sup>-/-</sup> cell clone 2

**Supplementary Table II** Relative amount of *Xist* RNA to undifferentiated female ES cells

Genotype	Condition	Relative <i>Xist</i> expression (Mean $\pm$ SD)	p-value <sup>*1</sup> vs. XX <sup>*2</sup>	p-value vs. XY <i>Eed</i> <sup>-/-</sup> <sup>*3</sup>
XX	undifferentiated	1.0 $\pm$ 0.4	NA <sup>*4</sup>	< 0.005
XY <i>Eed</i> -TG	undifferentiated	1.4 $\pm$ 0.3	not significant <sup>*5</sup>	< 0.005
XY <i>Eed</i> <sup>-/-</sup>	undifferentiated	6.5 $\pm$ 1.1	< 0.005	NA
X <sup><math>\Delta</math></sup> Y <i>Eed</i> <sup>-/-</sup> 1 <sup>*6</sup>	undifferentiated	23.3 $\pm$ 2.6	< 0.0005	< 0.0005
X <sup><math>\Delta</math></sup> Y <i>Eed</i> <sup>-/-</sup> 2 <sup>*7</sup>	undifferentiated	31.2 $\pm$ 11.1	< 0.01	< 0.05
XX	EB day 4	34.1 $\pm$ 1.1	NA	< 0.005
XY <i>Eed</i> <sup>-/-</sup>	EB day 4	9.8 $\pm$ 6.8	< 0.005	NA
X <sup><math>\Delta</math></sup> Y <i>Eed</i> <sup>-/-</sup>	EB day 4	163.3 $\pm$ 35.6	< 0.005	< 0.005
XX	EB day 12	19.4 $\pm$ 2.3	NA	< 0.005
XY <i>Eed</i> <sup>-/-</sup>	EB day 12	7.5 $\pm$ 2.4	< 0.005	NA
X <sup><math>\Delta</math></sup> Y <i>Eed</i> <sup>-/-</sup>	EB day 12	219.8 $\pm$ 13.8	< 0.00005	< 0.00005

<sup>\*1</sup> p-values were determined by student's t-test

<sup>\*2</sup> p-values vs. XX cells in the same condition

<sup>\*3</sup> p-values vs. XY *Eed*<sup>-/-</sup> cells in the same condition

<sup>\*4</sup> Not applicable

<sup>\*5</sup> p>0.05

<sup>\*6</sup> X <sup>$\Delta$</sup> Y *Eed*<sup>-/-</sup> cell clone 1

<sup>\*7</sup> X <sup>$\Delta$</sup> Y *Eed*<sup>-/-</sup> cell clone 2

**Supplementary Table III** The number of nuclei showing co-localization of H4K20m1 and *Xist* RNA at the onset of XCI

Genotype	EB differentiation	H4K20m1			No. of <i>Xist</i> -positive nuclei examined
		++	+ ~ ±	-	
X <sup>Δ</sup> X	2 days	41 (25.5%)	76 (47.2%)	44 (27.3%)	161 (100%)
X <sup>Δ</sup> Y <i>Eed</i> <sup>-/-</sup>	2 days	1 (0.8%)	4 (3.3%)	116 (95.9%)	121 (100%)
X <sup>Δ</sup> X	4 days	94 (39.7%)	99 (41.8%)	44 (18.6%)	237 (100%)
X <sup>Δ</sup> Y <i>Eed</i> <sup>-/-</sup>	4 days	0 (0.0%)	18 (10.7%)	150 (89.3%)	168 (100%)

**Supplementary Table IV** Primers and probes used for real-time PCR

Amplicon	PCR primers	Reference (Genbank Accession#) <sup>*1</sup>	TaqMan probe	Reference (Genbank Accession#) <sup>*1</sup>
<i>Xist</i> (RT-PCR)	gga gtg aag agt gct gga gag a gct gct ggc agt cct tga	L04961.1@9544-9565 (Fw) <sup>*2</sup> L04961.1@9615-9598 (RC) <sup>*3</sup>	5'-FAM- ccc aaa ggg aca aac aa -MGB-3'	L04961.1@9567-9583 (Fw)
<i>Tsix(4)</i> (RT-PCR, ChIP)	ctg tga att att tgt cag cgt gaa tca aga gat cag aca ccc tgg gta tta g	AJ421479.1@105507-105533 (Fw) AJ421479.1@105584-105560 (RC)	5'-FAM- acg aga caa caa tat cc -MGB-3'	AJ421479.1@105534-105550 (Fw)
<i>Xist-P</i> (ChIP)	ttt gtg gcc act cct ctt ctg cgt tgc acg cct tta act gat c	AJ421479.1@106232-106252 (Fw) AJ421479.1@106297-106276 (RC)	5'-FAM- tct ceg cct tca geg cc -MGB-3'	AJ421479.1@106256-106272 (Fw)
<i>Xist-GB1</i> (ChIP)	tgc ata tgg acc cct ggc gcc att tta tag act tct gag cag c	L04961.1@1178-1195 (Fw) L04961.1@1242-1218 (RC)	5'-VIC- ccc gtg gct tta ag -MGB-3'	L04961.1@1203-1216 (Fw)
<i>Xist-GB2</i> (ChIP)	cag cgg gat ggc aag atg ggg tcc tgg aga tca aag tga gat	L04961.1@9304-9287 (RC) L04961.1@9224-9247 (Fw)	5'-VIC- ttg ctg cca agc ctg -MGB-3'	L04961.1@9265-9251 (RC)
<i>5'-Tsix</i> (ChIP)	gca gac tta gtt act cgt tct tgg t ggg cac aga ttc aga aaa gtg gaa	X99946.1@80060-80036 (Fw) X99946.1@79988-80011 (RC)	5'-FAM- aca cgc tac cag taa ttt -MGB-3'	X99946.1@80029-80012 (RC)
<i>Sox9-P</i> (ChIP)	agc cca gct ccg ctt tg tgg cgt tta cag caa tgg aa	NT_165773.2@24184076-24184092 (Fw) NT_165773.2@24184130-24184111 (RC)	5'-VIC- cga gca gct gtt gca -MGB-3'	NT_165773.2@24184094-24184108 (Fw)
<i>Gata6-P</i> (ChIP)	ccc aga gcg ttg aat tcc a gac cct gtt tgg gga tgc t	NT_039674.7@8051865-8051883 (Fw) NT_039674.7@8051919-8051901 (RC)	5'-FAM- agg acc cca cga cct -MGB-3'	NT_039674.7@8051885-8051899 (Fw)

<sup>\*1</sup> The reference is shown as a nucleotide position in the indicated Genbank sequence

<sup>\*2</sup> Fw: Forward orientation to the reference sequence

<sup>\*3</sup> RC: Reverse complement to the reference sequence