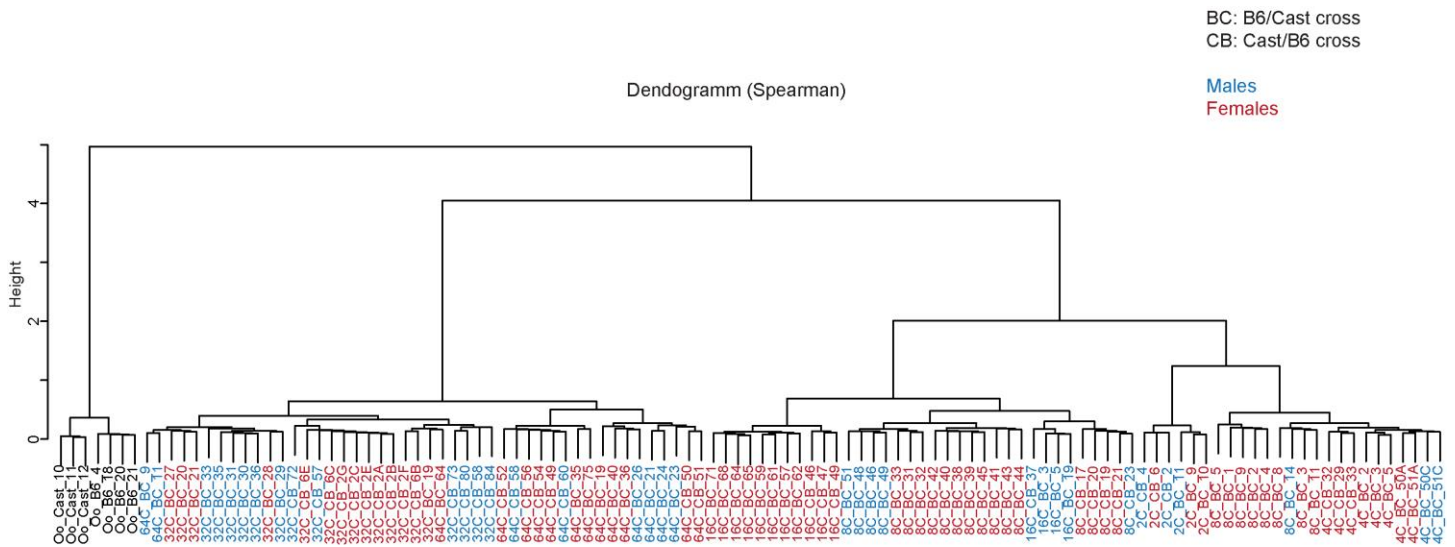


Hierarchical clustering of the single cell transcriptomes

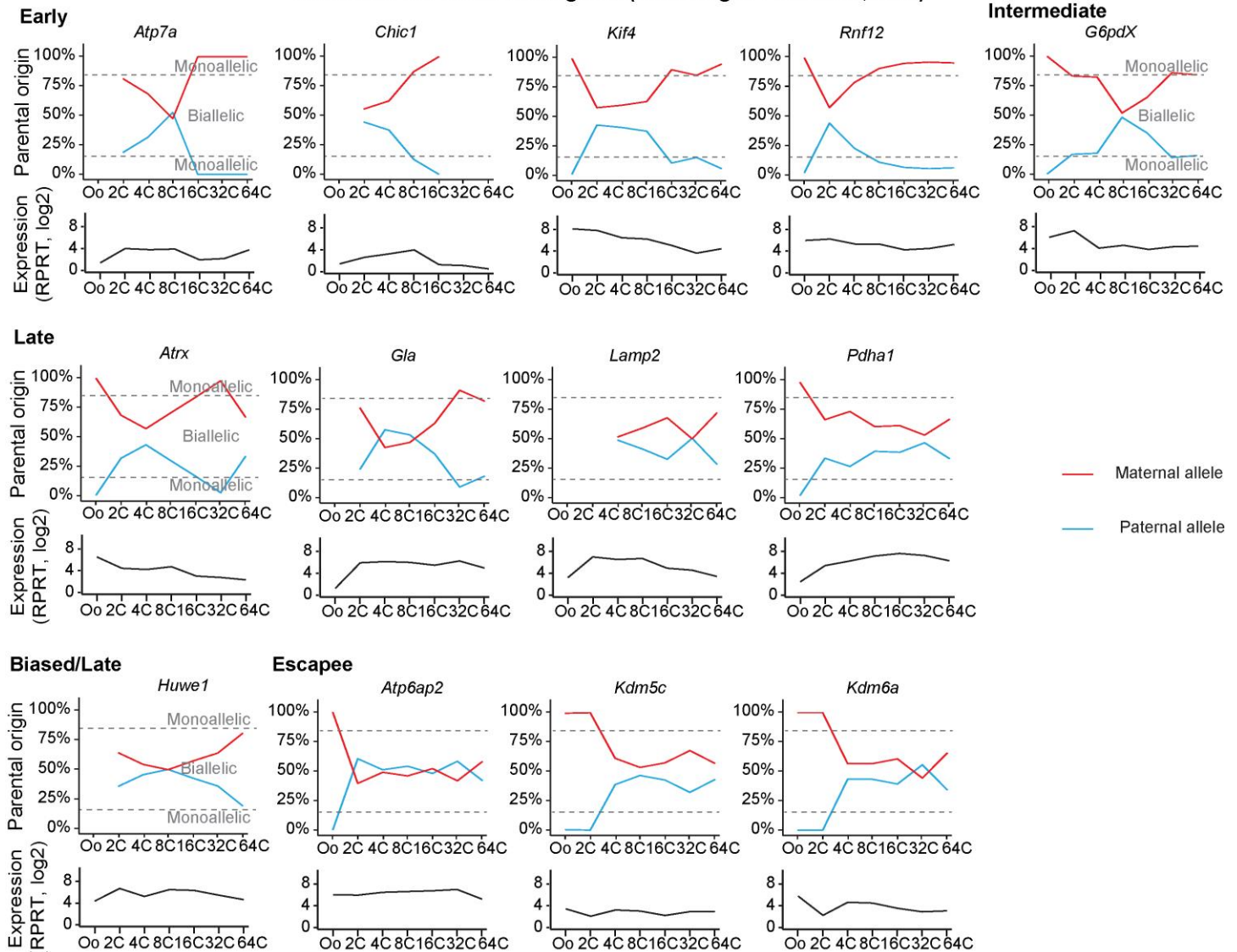


Supplementary Figure 1

Hierarchical clustering of scRNA-seq.

Hierarchical clustering of single cell transcriptomes, based on Spearman's correlation. Cells were clustered by developmental stage, then by cross (BC or CB), and finally according to their sex. ScRNAseq samples called 64C are related to the early blastocyst stage. n=184 single cell samples

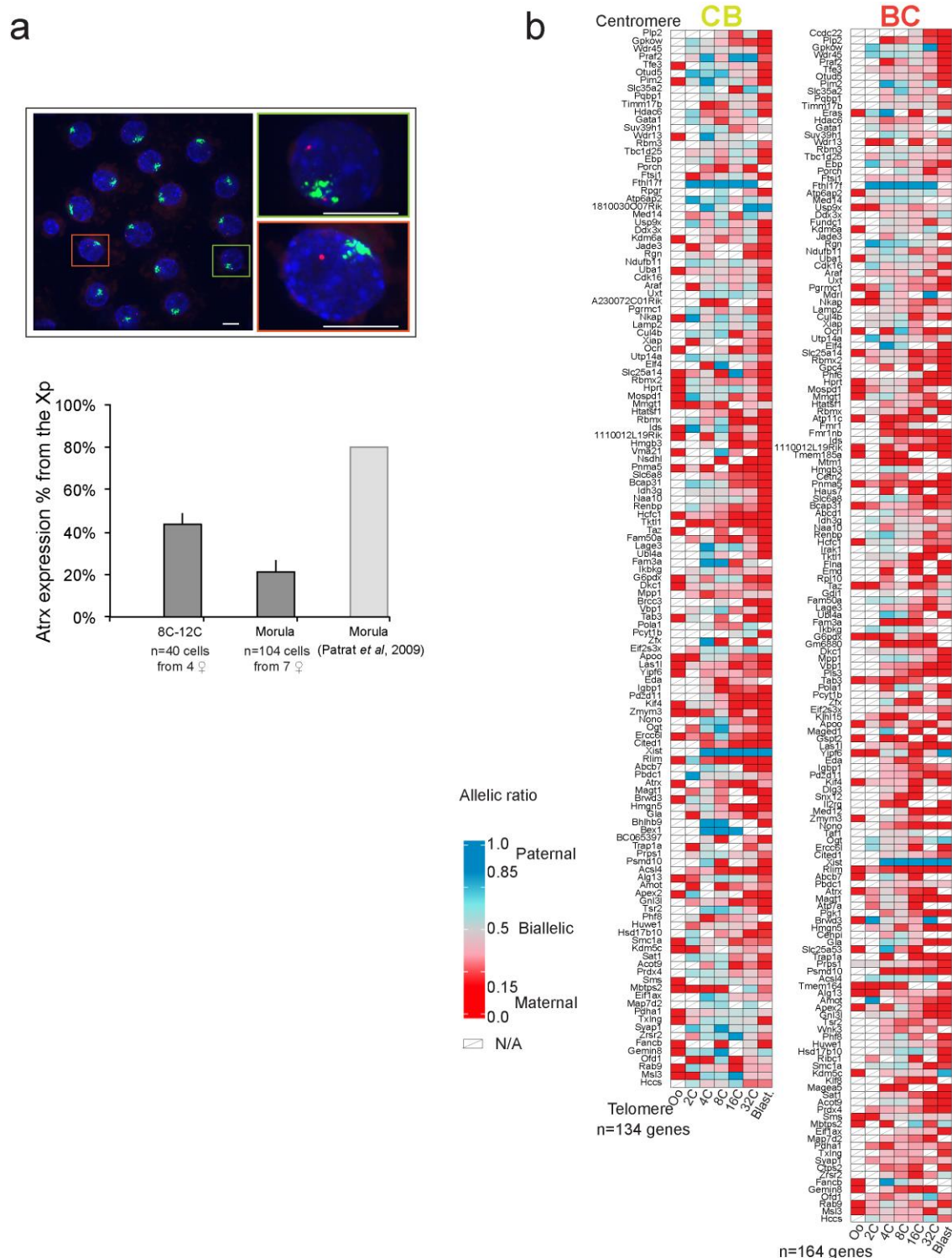
Kinetics of XCI of candidate genes (according to Patrat *et al*, 2009)



Supplementary Figure 2

Single-cell RNA-sequencing data corroborate RNA-FISH-based silencing kinetics.

Candidate X-linked gene silencing kinetics (scRNA-seq) is compared to previous kinetics studied by RNA-FISH in Patrat *et al*, 2009¹⁴. Mean of maternal and paternal reads are respectively represented as red or blue lines. Percentage of parental origin transcript (top panel) and level of expression in RPRT (bottom panel) of each gene are represented between oocytes and blastocysts.



Supplementary Figure 3

Gene validation by RNA-FISH and chromosome-wide representation of parental expression ratio.

(a) Nascent RNA FISH using probes recognizing *Xist* (signal in green) and *Atrx* (signal in red) RNAs on a 16C female embryo. DAPI is

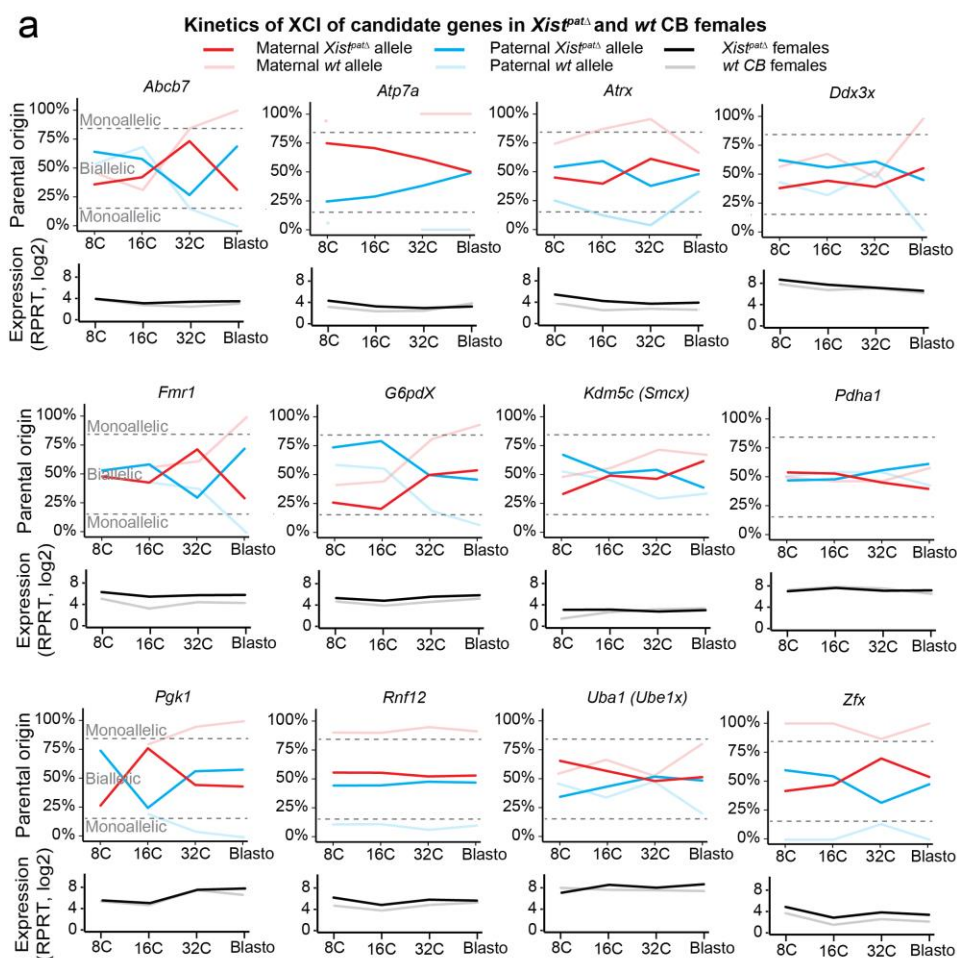
in blue. Right pictures are enhanced pictures of two individual blastomeres. Percentage of nuclei showing pinpoints of nascent transcripts by RNA FISH from Xp and Xm has been assessed and summarized as the median + s.e.m. under the picture. Normalization of the primary transcript detection frequency obtained for the paternal (Xist RNA-associated) allele in female embryos was achieved thanks to the detection frequency obtained for the maternal allele in male embryos at the same stage. Number of embryos and single cell processed are indicated under each genotype. Scale bars represent 10 μ m.

(b) Heatmaps are shown representing the mean of allele-specific expression of X-linked genes, from Oocytes to blastocysts in CB and BC crosses. Strictly maternally expressed genes (allelic ratio ≤ 0.15) are represented in red and strictly paternally expressed genes (allelic ratio ≥ 0.85) in blue. Color gradients are used in between and genes have been ordered by genomic position. Oocytes and 2C stage data, as well as strain-specific gene expression data, have been included, in addition to the heatmaps for the stages shown in Figure 2.

Paternal Biallelic Maternal N/A

Complete single-cell information of X-linked gene expression in preimplantation development.

Heatmap representing the full allele-specific expression data set of informative and well-expressed X-linked genes in each single cell, from oocytes to blastocysts, in male and female embryos derived from BC and CB crosses. Strictly maternally expressed genes (allelic ratio ≤ 0.15) are represented in red and strictly paternally expressed genes (allelic ratio ≥ 0.85) in blue. Color gradients are used in between and genes have been ordered by genomic position. Genes expressed in one or both crosses have been included to the single cell heatmap. n=173 genes.



b Total differentially expressed genes in *Xist^{patΔ}* CB females

Developmental stages	DE genes from the X chromosome				DE genes from the autosomes			
	Total Upregulated	Total Downregulated	Total Upregulated	Total Downregulated	Total Upregulated	Total Downregulated	Total Upregulated	Total Downregulated
	%	n	%	n	%	n	%	n
8-cell	5.0%	27	1.9%	14	95.0%	514	98.1%	541
16-cell	11.6%	15	1.0%	1	88.4%	114	99.0%	129
32-cell	4.7%	29	2.3%	12	95.3%	591	97.7%	620
Blastocyst	29.6%	16	3.6%	2	70.4	38	96.4%	54

Supplementary Figure 5

Lack of X-chromosome inactivation in the absence of *Xist*.

(a) Each plot represents a candidate X-linked gene that was previously studied in Kalantry *et al*, 2009⁶. Allele-specific expression ratio of each gene represents the number of reads mapped on paternal genome divided by the total number of paternal and maternal reads. Mean of maternal and paternal reads are respectively represented as a red or a blue line. Percentage of parental origin transcript (top panel) and level of expression in RPRT (bottom panel) of each gene are represented between 8-cell stage and blastocysts.

(b) A table summarizing the total differentially expressed (DE) genes between CB wt and CB *Xist^{patΔ}* female embryos during early development and their localization on X chromosomes or autosomes. The percentages shown correspond to the distribution of total DE genes between autosomes and X chromosomes. The blastocyst stage is highlighted in red and candidate DE genes from this stage have been analyzed in Figure 4. List of DE genes is available in Supplementary Data Set 2.

Supplementary Table 1: Silencing classes for informative and well-expressed X-linked genes in BC and CB crosses.

Silencing classes for the 173 informative and well-expressed X-linked genes in BC and/or CB crosses.

All genes			All genes			Consistent genes			
Genes	BC	CB	Genes	BC	CB	Genes	BC	CB	All
1110012L19Rik	Early	Early	Mdr1	Late	NA	1110012L19Rik	Early	Early	Early
1810030O07Rik	NA	Esc	Med12	Early	NA	Acsl4	Late	Inter	Late
A230072C01Rik	NA	Late	Med14	Late	Esc	Alg13	Bias	Bias	Bias
Abcb7	Early	Late	Mmg1	Late	Esc	Amot	Late	Bias	Late
Abcd1	Esc	NA	Mospd1	Late	Late	Apex2	Inter	Late	Inter
Acof9	Late	Early	Mpp1	Late	Esc	Apoo	Inter	Late	Inter
Acs14	Late	Inter	Msl3	Esc	Esc	Araf	Esc	Bias	Esc
Alg13	Bias	Bias	Mtm1	Early	NA	Atp6ap2	Esc	Esc	Esc
Amot	Late	Bias	Naa10	Bias	Bias	Atrx	Inter	Early	Inter
Apex2	Inter	Late	Ndufb11	Bias	Esc	Bcap31	Inter	Late	Bias
Apoo	Inter	Late	Nkap	Esc	Bias	Brwd3	Late	Late	Late
Araf	Esc	Bias	Nono	Inter	Late	Cdk16	Late	Late	Late
Atp11c	Inter	NA	Nsdhl	NA	Inter	Cul4b	Late	Bias	Bias
Atp6ap2	Esc	Esc	Ocr1	Inter	Early	Dkc1	Bias	Bias	Bias
Atp7a	Early	NA	Odf1	Bias	Early	Ebp	Late	Bias	Late
Atrx	Inter	Early	Ogt	Esc	Bias	Eda	Late	Late	Late
BC065397	NA	Late	Otd5	Bias	Late	Elf2s3x	Bias	Esc	Esc
Bcap31	Inter	Late	Pbdc1	Esc	Esc	Elf4	Late	Late	Late
Bex1	NA	Late	Pcyt1b	Late	Late	Fancb	Late	Late	Late
Bhlhb9	NA	Late	Pdha1	Bias	Esc	Fthl17f	Late	Late	Late
Brc3	NA	Inter	Pdzd11	Inter	Inter	G6pdx	Inter	Late	Inter
Brwd3	Late	Late	Pgk1	Inter	NA	Gata1	Late	Late	Late
Ccdc22	Late	NA	Pgrmc1	Late	Late	Gla	Inter	Inter	Inter
Cdk16	Late	Late	Phf6	Late	NA	Gnl3l	Inter	Early	Late
Cenpi	Late	NA	Phf8	Late	Late	Gpkow	Late	Late	Late
Cetn2	Late	NA	Pim2	Late	Late	Hccs	Bias	Bias	Bias
Cited1	Bias	Inter	Plp2	Late	Late	Hdac6	Late	Late	Late
Ctps2	Bias	NA	Pls3	Inter	NA	Hmg5	Inter	Early	Inter
Cul4b	Late	Bias	Pnma5	Early	Early	Hsd17b10	Late	Inter	Late
Ddx3x	Esc	Late	Pola1	Late	Late	Htatsf1	Late	Late	Late
Dkc1	Bias	Bias	Porcn	Inter	Late	Huwe1	Bias	Late	Bias
Dlg3	Early	NA	Pqbp1	Bias	Late	Idh3g	Esc	Bias	Bias
Ebp	Late	Bias	Praf2	Late	Bias	Ids	Late	Late	Late
Eda	Late	Late	Prdx4	Inter	Esc	Jade3	Late	Late	Late
Elf1ax	Late	Esc	Prps1	Bias	Bias	Kdm5c	Esc	Esc	Esc
Elf2s3x	Bias	Esc	Psm10	Early	Late	Kdm6a	Late	Bias	Bias
Elf4	Late	Late	Rab9	Bias	Early	Kif4	Early	Early	Early
Emd	Early	NA	Rbm3	Esc	Bias	Lage3	Inter	Late	Bias
Eras	Early	NA	Rbm3	Early	Early	Lamp2	Esc	Bias	Bias
Ercc6l	Early	Late	Rbm32	Late	Late	Las1l	Inter	Early	Early
Fam3a	Early	Late	Renbp	Esc	Late	Magt1	Early	Inter	Early
Fam50a	Esc	Early	Rgn	Late	Late	Map7d2	Inter	Late	Late
Fancb	Late	Late	Ribc1	Early	NA	Mbtps2	Esc	Bias	Esc
Flna	Early	NA	Rlim	Early	Early	Mospd1	Late	Late	Late
Fmr1	Late	NA	Rpgr	NA	Late	Msl3	Esc	Esc	Esc
Fmr1nb	Early	NA	Rpl10	Inter	NA	Naa10	Bias	Bias	Bias
Fthl17f	Late	Late	Sat1	Inter	Bias	Ndufb11	Bias	Esc	Bias
Ftsj1	Esc	Late	Slc25a14	Early	Late	Nkap	Esc	Bias	Bias
Fundc1	Esc	NA	Slc25a53	Early	NA	Nono	Inter	Late	Inter
G6pdx	Inter	Late	Slc35a2	Late	Early	Ocr1	Inter	Early	Late
Gata1	Late	Late	Slc6a8	Inter	Late	Ogt	Esc	Bias	Esc
Gdi1	Esc	NA	Smc1a	Inter	Bias	Otd5	Bias	Late	Late
Gemin8	Early	Esc	Sms	Esc	Esc	Pbdc1	Esc	Esc	Esc
Gla	Inter	Inter	Snx12	Early	NA	Pcyt1b	Late	Late	Late
Gm6880	Early	NA	Suv39h1	Esc	Esc	Pdha1	Bias	Esc	Esc
Gnl3l	Inter	Early	Syap1	Esc	Esc	Pdzd11	Inter	Inter	Inter
Gpc4	Early	NA	Tab3	Late	Inter	Pgrmc1	Late	Late	Late
Gpkow	Late	Late	Taf1	Late	NA	Phf8	Late	Late	Late
Gsp12	Inter	NA	Taz	Early	Late	Pim2	Late	Late	Late
Haus7	Early	NA	Tbc1d25	Esc	Bias	Plp2	Late	Late	Late
Hccs	Bias	Bias	Tfe3	Late	Bias	Pnma5	Early	Early	Early
Hcfc1	Bias	Early	Timm17b	Bias	Bias	Pola1	Late	Late	Late
Hdac6	Late	Late	Tkt1l	Early	Early	Porcn	Inter	Late	Esc
Hmgb3	Late	Early	Tmem164	Late	NA	Pqbp1	Bias	Late	Late
Hmg5	Inter	Early	Tmem185a	Late	NA	Praf2	Late	Bias	Late
Hprt	Inter	Bias	Trap1a	Early	Esc	Prps1	Bias	Bias	Bias
Hsd17b10	Late	Inter	Tsr2	Bias	Late	Rbm3	Esc	Bias	Bias
Htatsf1	Late	Late	Txlng	Bias	Late	Rbm3	Early	Early	Early
Huwe1	Bias	Late	Uba1	Esc	Bias	Rbm32	Late	Late	Late
Idh3g	Esc	Bias	Ubl4a	Late	Late	Rgn	Late	Late	Late
Ids	Late	Late	Usp9x	Esc	Late	Rlim	Early	Early	Early
Igbbp1	Esc	Late	Utp14a	Esc	Bias	Slc6a8	Inter	Late	Late
Ikbkg	Late	Esc	Uxt	Late	Esc	Sms	Esc	Esc	Esc
Il2rg	Late	NA	Vbp1	Inter	Bias	Suv39h1	Esc	Esc	Esc
Irak1	Inter	NA	Vma21	NA	Late	Syap1	Esc	Esc	Esc
Jade3	Late	Late	Wdr13	Bias	Late	Tab3	Late	Inter	Inter
Kdm5c	Esc	Esc	Wdr45	Late	Bias	Tbc1d25	Esc	Bias	Bias
Kdm6a	Late	Bias	Wnk3	Late	NA	Tfe3	Late	Bias	Late
Kif4	Early	Early	Xiap	Late	Bias	Timm17b	Bias	Bias	Bias
Kif8	Inter	NA	Xist	Esc	Esc	Tkt1l	Early	Early	Early
Klh15	Late	NA	Yipf6	Early	Bias	Tsr2	Bias	Late	Bias
Lage3	Late	Late	Zfx	Early	Late	Txlng	Bias	Late	Late
Lamp2	Esc	Bias	Zmym3	Late	Early	Uba1	Esc	Bias	Esc
Las1l	Inter	Early	Zrsr2	Early	Bias	Ubl4a	Late	Late	Late
Magea5	Late	NA				Utp14a	Esc	Bias	Esc
Maged1	Early	NA				Wdr13	Bias	Late	Bias
Magt1	Early	Inter				Wdr45	Late	Bias	Bias
Map7d2	Inter	Late				Xiap	Late	Bias	Bias
Mbtps2	Esc	Bias				Xist	Esc	Esc	Esc

Supplementary Table 2: Summary of escapees and their status in other studies

Information for escapees is provided for CB and BC crosses. Status of each escapee gene has been evaluated in other studies using hybrid cell lines or tissues (Calabrese *et al*, *Cell*, 2012; Berletch *et al*, *PLOS Genet*, 2015; Marks *et al*, *Genome Biol*, 2015; Gendrel *et al*, *Dev Cell*, 2014)

Gene Symbol	BC embryos	CB embryos	TSC (Calabrese et al)	Brain (Berletch et al)	Spleen (Berletch et al)	Ovary (Berletch et al)	Patski (Berletch et al)	NPC* (Marks et al)	NPC** (Gendrel et al)
<i>1810030O07Rik</i>	-	yes	no			yes	yes	yes (1/3)	yes (4/4)
<i>Abcd1</i>	yes	no	no					-	yes (2/4)
<i>Alg13</i>	biased	biased	-			yes		no	yes (1/4)
<i>Amot</i>	no	biased	no				yes	no	no
<i>Araf</i>	yes	biased	no					yes (1/3)	yes (4/4)
<i>Atp6ap2</i>	yes	yes	-					no	yes (2/4)
<i>Cited</i>	biased	no	no					-	no
<i>Ctsp2</i>	biased	-	no				yes	no	yes (3/4)
<i>Cul4b</i>	no	biased	no					-	-
<i>Ddx3x</i>	yes	no	no	yes	yes	yes	yes	yes (3/3)	yes (4/4)
<i>Dkc1</i>	biased	biased	no					yes	no
<i>Ebp</i>	no	biased	no				yes	yes (1/3)	yes (2/4)
<i>Eif1ax</i>	no	yes	-					no	yes (2/4)
<i>Eif2s3x</i>	biased	yes	yes	yes	yes	yes	yes	-	yes (4/4)
<i>Fam50a</i>	yes	no	-				yes	-	yes (2/4)
<i>Ftsj1</i>	yes	no	no					yes (2/3)	yes (4/4)
<i>Fundc1</i>	yes	-	no					no	yes (2/4)
<i>Gdi1</i>	yes	-	no	yes				no	yes (2/4)
<i>Gemin8</i>	no	yes	no					-	yes (4/4)
<i>Hccs</i>	biased	biased	no					no	yes (3/4)
<i>Hcfc1</i>	biased	no	no					yes (2/3)	yes (4/4)
<i>Hprt</i>	no	biased	no					no	no
<i>Huwe1</i>	biased	no	no			yes		no	yes (3/4)
<i>Idh3g</i>	yes	biased	no			yes	yes	no	yes (2/4)
<i>Igfbp1</i>	yes	no	no					no	yes (3/4)
<i>Ikbkg</i>	no	yes	no				yes	no	yes (1/4)
<i>Kdm5c</i>	yes	yes	yes	yes	yes	yes	yes	yes (3/3)	yes (4/4)
<i>Kdm6a</i>	no	biased	yes	yes	yes	yes	yes	yes (3/3)	yes (4/4)
<i>Lamp2</i>	yes	biased	no			yes	yes	no	yes (1/4)
<i>Mbtps2</i>	yes	biased	no					no	yes (1/4)
<i>Med14</i>	no	yes	no					yes (2/3)	yes (4/4)
<i>Mmgt1</i>	no	yes	-			yes		-	yes (2/4)
<i>Mpp1</i>	no	yes	no					no	no
<i>Msl3</i>	yes	yes	no					no	yes (2/4)
<i>Naa10</i>	biased	biased	-					-	yes (2/4)
<i>Ndufb11</i>	biased	yes	no					yes (1/3)	yes (4/4)
<i>Nkap</i>	yes	biased	yes CB only					yes (2/3)***	yes (3/4)
<i>Ofd1</i>	biased	no	no				yes	yes (2/3)	yes (3/4)
<i>Ogt</i>	yes	biased	yes					no	yes (4/4)
<i>Pbdc1</i>	yes	yes	-		yes	yes	yes	-	yes (4/4)
<i>Pdha1</i>	biased	yes	no			yes		no	no
<i>Pqbp1</i>	biased	no	yes BC only					no	yes (3/4)
<i>Praf2</i>	no	biased	-					-	yes (3/4)
<i>Prdx4</i>	no	yes	no					no	no
<i>Prps1</i>	biased	biased	no					no	no
<i>Rab9</i>	biased	no	no					no	yes (3/4)
<i>Rbm3</i>	yes	biased	no					yes (1/3)	yes (2/4)
<i>Renbp</i>	yes	no	no					no	yes (2/4)
<i>Sat1</i>	no	biased	no					no	no
<i>Smc1a</i>	no	biased	no					no	yes (3/4)
<i>Sms</i>	yes	yes	no					no	no
<i>Suv39h1</i>	yes	yes	yes BC only					no	yes (4/4)
<i>Syp1</i>	yes	yes	yes CB only					no	yes (3/4)
<i>Tbc1d25</i>	yes	biased	no					yes (1/3)	yes (2/4)
<i>Tfe3</i>	no	biased	no					-	-
<i>Timm17b</i>	biased	biased	no					yes (2/3)	yes (3/4)
<i>Trap1a</i>	no	yes	no					-	-
<i>Tsr2</i>	biased	no	no					no	no
<i>Txlng</i>	biased	no	no					yes (1/3)	yes (3/4)
<i>Uba1a</i>	yes	biased	-			yes		yes (2/3)	yes (4/4)
<i>Usp9x</i>	yes	no	no			yes		yes (2/3)	yes (3/4)
<i>Utp14a</i>	yes	biased	yes		yes	yes		yes (3/3)	yes (4/4)
<i>Uxt</i>	no	yes	no					-	yes (1/4)
<i>Vbp1</i>	no	biased	no				yes	yes (2/3)	yes (2/4)
<i>Wdr13</i>	biased	no	no				yes	yes (1/3)	yes (3/4)
<i>Wdr45</i>	no	biased	no					yes (1/3)	yes (3/4)
<i>Xiap</i>	no	biased	-					no	no
<i>Xist</i>	yes	yes	yes	yes	yes	yes	yes	yes (3/3)	yes (4/4)
<i>Yipf6</i>	no	yes	yes				yes	no	yes (1/4)
<i>Zrsr2</i>	no	biased	yes					yes (1/3)	yes (3/4)

* NPC data are derived from 3 independent clones. Information of escape for each clone is provided in brackets.

** NPC data are derived from 4 independent clones. Information of escape for each clone is provided in brackets.

*** *Nkap* escapes from Cast Xi and from 129 Xi.