Supplementary Data

Progressive accumulation of epigenetic heterogeneity during human ES cell culture

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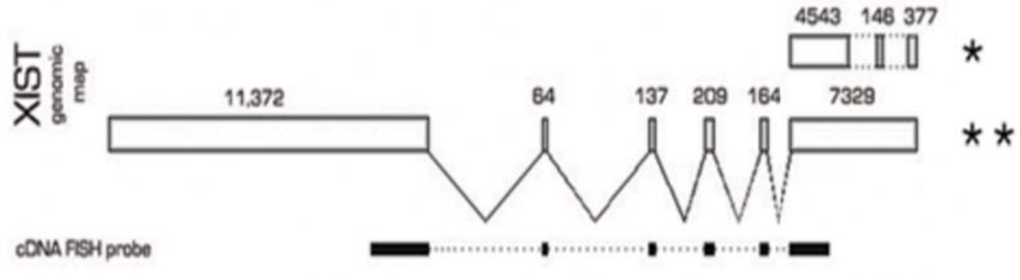

Figure S1. Structure of the XIST gene on Xq13.2 with exon sizes shown from 5' end on the left. cDNA sequence used for preparation of the RNA FISH probe is shown in closed boxes. The 3' end of XIST has been reported to contain either three small exons (*) (15) or a single 3' terminal exon (**) (Ensembl human genome database).

Table S1. Primer sequences used in the study

Gene Symbol		Primer sequence (5' - 3')
XIST	**F**	TCTCGGAGAAGGATGTCAAAAGA
	R	GGACACATGCAGCGTGGTAT
POU5F1	**F**	CCCCTGGTGCCGTGAAG
	R	CTCGAGTTCTTTCTGCAGAGCTT
ACTB	**F**	GGCACCCAGCACAATGAAG
	R	GCCGATCCACACGGAGTACT
GAPDH	**F**	GAGTCAACGGATTTGGTCGT
	R	TTGATTTTGGAGGGATCTCG
GATA2	**F**	CCCACCTACCCCTCCTATGT
	R	TGCCCATTCATCTTGTGGTA
GCM1	**F**	CGCAATACCAACAACCACAA
	R	CAAGGGGATGAGCTTCAGAGG
DFc*		GTGGATCGAATTCTGCCAGT
DFg*		GCTAAACTTGCCTTACTGCTTTTT
DRcg*		TCTTTCTCTCTGGCCTGCAC
Dseq*		AAGGCCACAAAGTCTGCATC
WFcg*		GCAGTGGAACTGATGAAGACC
WRg*		GAGGTTTCCCGGAGAAGAAA
WRc*		GCAAAGGCATTCTCTTCGAG
Wseq*		TGAAAGATCAAGGAGATCAGCA

F-forward; **R**-Reverse; * see text for details

Table S2. Genotyping and expression analysis of polymorphic SNPs from two X-linked genes in H9 hESCs

Sample name	Gene name (SNP ID)	
	DMD (rs228406)	WDR44 (rs10521584)
gDNA		
H9 p47	biallellic	biallellic
H9 p48 (4% O_2)	biallellic	n/a
H9 p48 (20% O_2)	biallellic	biallellic
H9 p54 (4% O_2)	biallellic	biallellic
H9 p54 (20% O_2)	biallellic	n/a
cDNA		
H9 p32 d1	biallellic	biallellic
H9 p32 d3	biallellic	biallellic
H9 p32 d5	biallellic	biallellic
H9 p33 d1	biallellic	n/a
H9 p33 d3	biallellic	biallellic
H9 p33 d5	biallellic	biallellic
H9 p33 d7	biallellic	biallellic
H9 p47 col1	n/a	biallellic
H9 p47 col2	n/a	biallellic
H9 p47 col3	biallellic	biallellic
H9 p47 col4	n/a	biallellic
H9 p47 col5	biallellic	biallellic
H9 p47 col6	biallellic	biallellic
H9 p47 col7	biallellic	biallellic
H9 p47 col8	n/a	biallellic
H9 p47 col9	biallellic	biallellic

Table S3. Antibodies used for NU-ELISA

Epitope	Host[a]	Type[b]	Source	Catalog #	Working Concentration
H2A	Rb	P	IMGENEX	IMG-358	1:1,000
H3K4me3	Rb	M	Upstate	05-745	1:1,000
H3K9me1	Rb	P	Upstate	07-450	1:1,000
H3K9me3	Rb	P	Upstate	07-442	1:1,000
H3K36me3	Rb	P	Abcam	Ab9050	1:1,000
H3K79me2	Rb	M	Upstate	05-835	1:1,000
H4K20me3	Rb	P	Upstate	07-463	1:1,000
MACROH2A1	Rb	P	Rasmussen Lab	-	1:1,000
HRP Gt anti-Rb	Gt	P	Pierce	31460	1:5,000

[a] Rb=Rabbit, Gt=Goat. [b] P=Polyclonal IgG, M=Monoclonal IgG.

Table S4. H9 hESCs low passage (p45) and high passage(p113) relative PTM values

PTM	[a] Mean of H9 P45 PTM	[b] SD of H9 P45 PTM	[a] Mean of H9 P113 PTM	[b] SD of H9 P113 PTM
H3K4me3	0.774	0.085	0.845	0.016
H3K9me1	0.838	0.124	0.799	0.098
H3K9me3	0.485	0.049	0.553	0.058
H3K36me3	3.289	0.955	3.278	0.358
H3K79me2	1.743.	0.526	1.777	0.389
H4K20me3	1.162	0.182	1.041	0.065
MACROH2A1	0.648	0.073	0.829	0.151

[a] Mean of 4 biological replicates on ELISA plates. The relative PTM values of each cell passage were calculated as the ELISA signal (after background subtraction) divided by the H2A content from the same wells and rows (after background subtraction).

[b] Standard deviations (SD) of each cell passage PTM