SUPPLEMENTARY MATERIAL

The macrosatellite DXZ4 mediates CTCF-dependent long-range intrachromosomal interactions on the human inactive X chromosome

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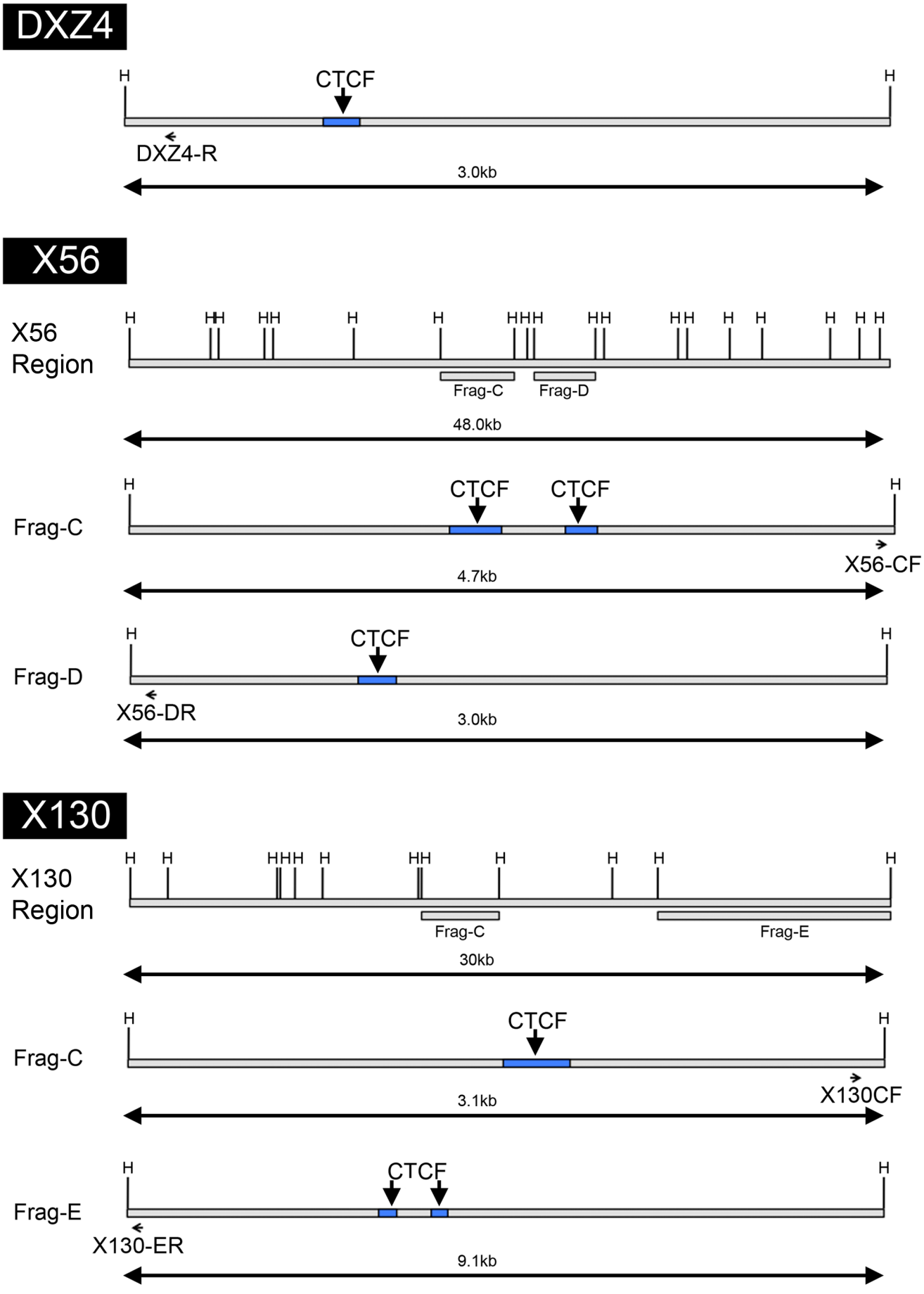
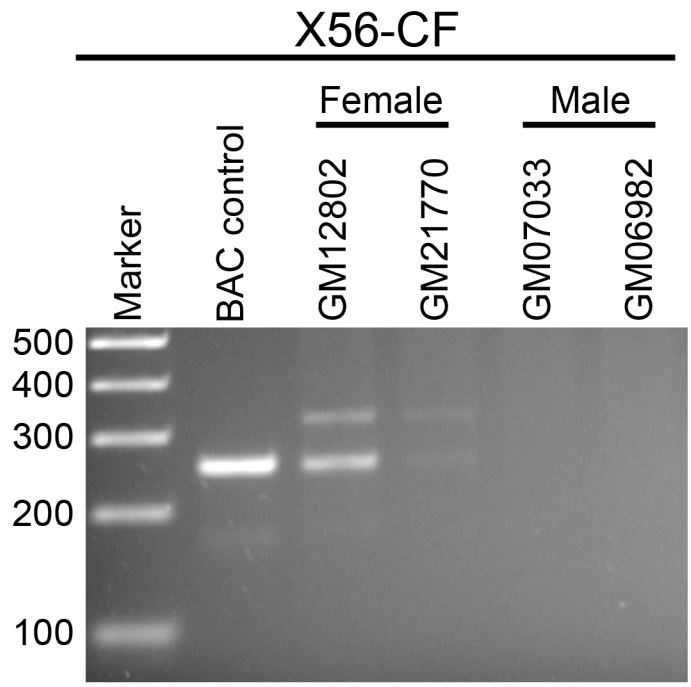
SUPPLEMENTARY FIGURES

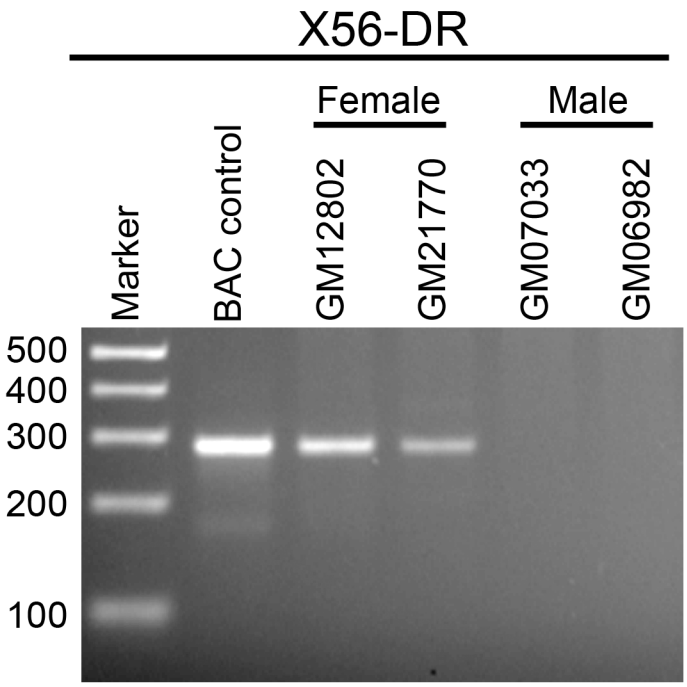
Supplementary Figure 1 Physical maps of regions assessed by chromosome conformation capture (3C). For the three regions (DXZ4, X56, and X130) schematic *Hin*dIII restriction maps are shown. The locations of *Hin*dIII sites are indicated (H) above each map. The size of each schematic is indicated in kilobases (kb) above the double-headed arrow below each map. The locations of putative CTCF binding sites are highlighted in blue and indicated by the annotated downward facing arrows. For X56 and X130, a regional overview map is shown at the top indicating the fragments (Frag-C and Frag-D for X56; Frag-C and Frag-E for X130) that were assessed in conjunction with DXZ4 by 3C. Immediately below each of these regional maps is a schematic map of the corresponding fragments. The locations of primers used in PCR reactions to assess the 3C library are indicated below each fragment map by left- or right-facing labeled arrows. Primer sequences are given in Supplementary Table 3.


DXZ4
H
CTCF
H
DXZ4-R
3.0kb
X56
X56
Region
H
Frag-C
Frag-D
48.0kb
Frag-C
CTCF
CTCF
H
X56-CF
4.7kb
Frag-D
H
CTCF
H
X56-DR
3.0kb
X130
X130
Region
H
Frag-C
Frag-E
30kb
Frag-C
CTCF
H
X130CF
3.1kb
Frag-E
H
CTCF
H
X130-ER
9.1kb

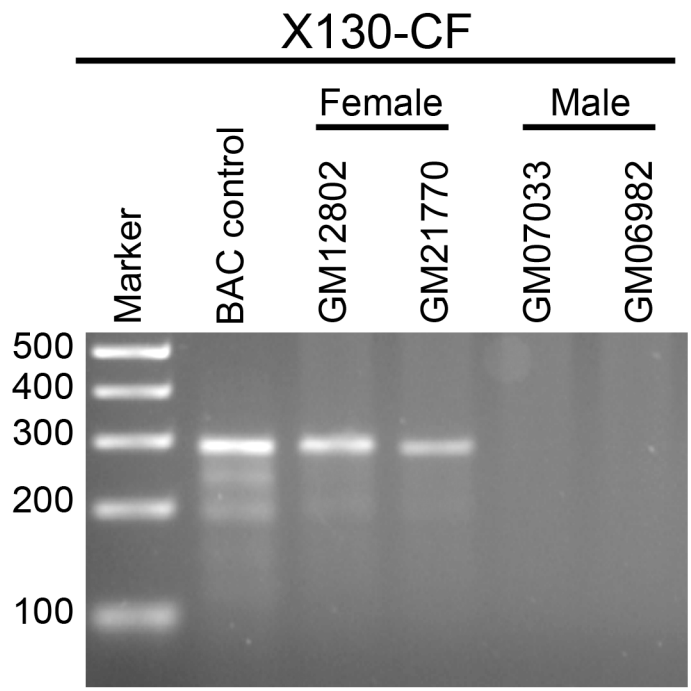
Supplementary Figure 2 Ethidium-bromide-stained agarose gels showing examples of PCR analysis of *Hin*dIII 3C libraries. PCR was performed for two sites within the X56 locus to DXZ4 (top) and two sites within the X130 locus to DXZ4 (bottom). Samples shown are for two independent male and female normal diploid cell lines as well as a BAC control. The molecular-weight marker is indicated, and the sizes given in base pairs to the left of each gel.

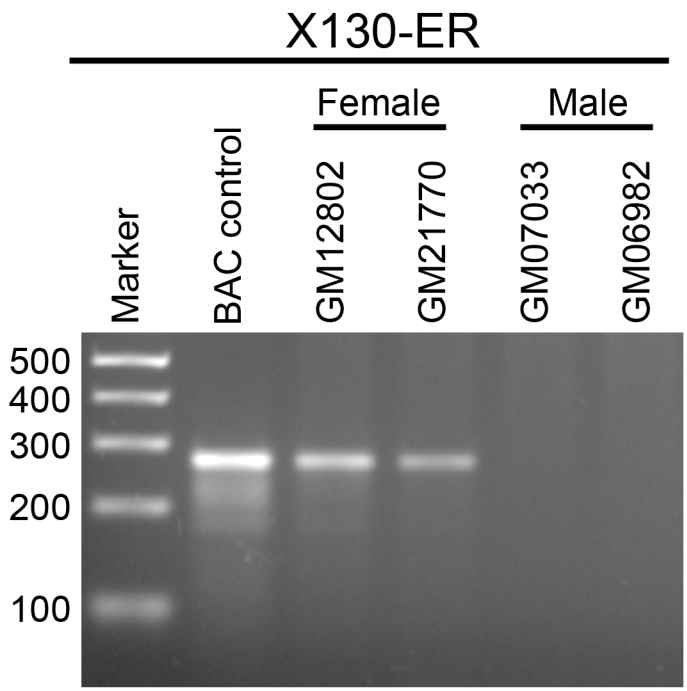
X56-DXZ4



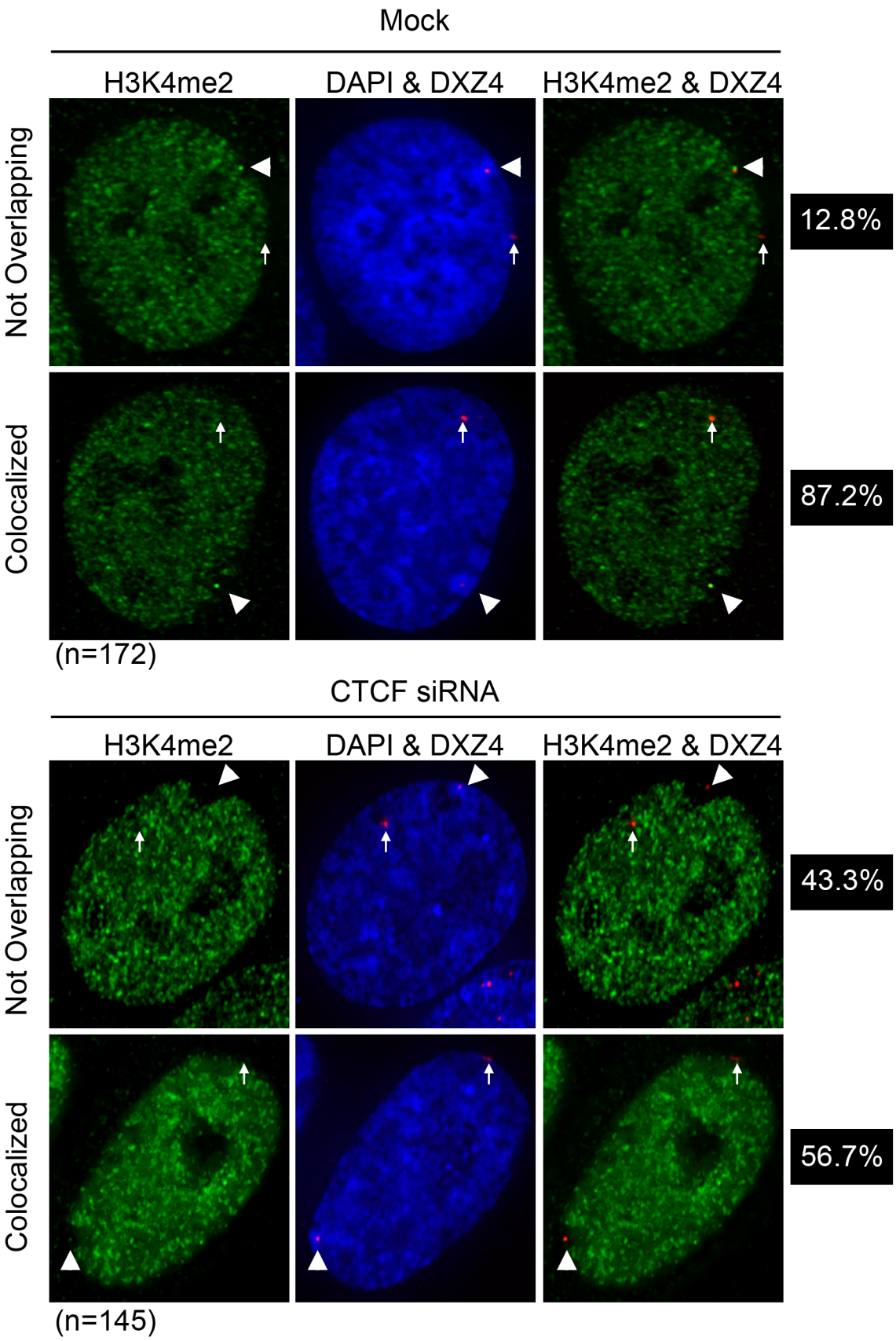


X130-DXZ4



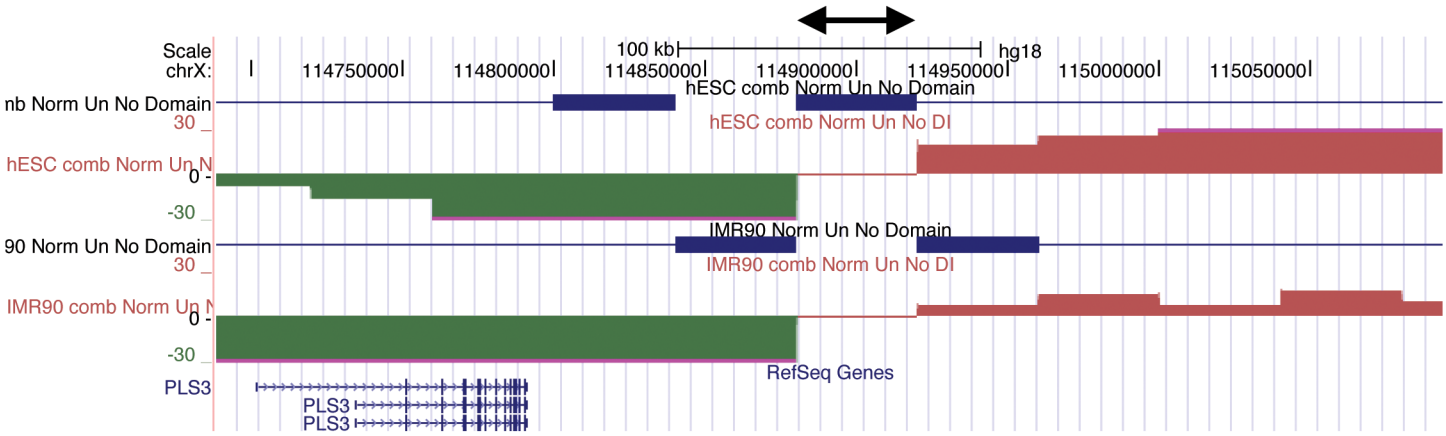


Supplementary Figure 3 Anti-H3K4me2 indirect immunofluorescence coupled with DXZ4 FISH in Mock treated (Top panels) and CTCF siRNA treated (Bottom panels) hTERT-RPE1 cells. Each panel set shows the distribution of H3K4me2 (Green, left), DXZ4 FISH merged with DAPI (Red and Blue, middle) and H3K4me2 merged with DXZ4 FISH (Green and red, right). The top row for each group shows an example of non-overlapping DXZ4 with H3K4me2 at the Xi and the percentage of cells in that class are indicated to the far right and the number of nuclei examined indicated at the bottom of each group. The bottom row shows an example of colocaized signals. A white arrowhead indicates the location of the Xi, whereas the white arrow indicates the location of the Xa.

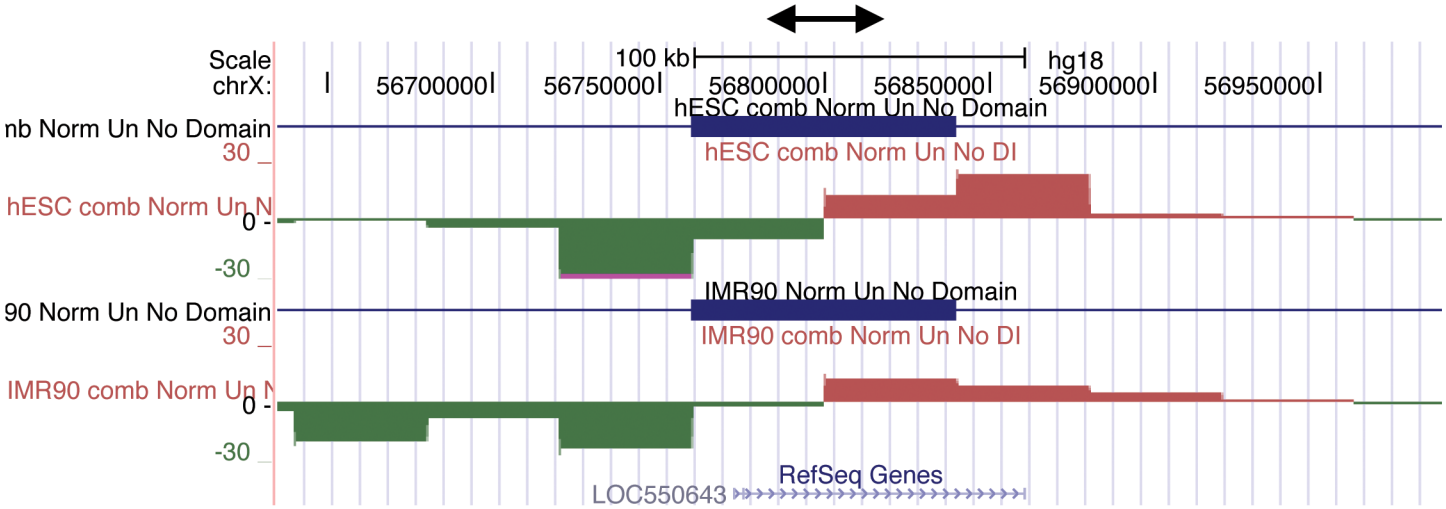

Mock
H3K4me2
DAPI & DXZ4
H3K4me2 & DXZ4
Not Overlapping
Colocalized
12.8%
87.2%
(n=172)
CTCF siRNA
H3K4me2
DAPI & DXZ4
H3K4me2 & DXZ4
Not Overlapping
Colocalized
43.3%
56.7%
(n=145)

Supplementary Figure 4 Location of the DXZ4, X56 and X130 tandem arrays at the border of topological domains. Images show a snapshot of the UCSC Genome Browser (http://genome.ucsc.edu) with the Topological Domains annotation activated (30). The DXZ4, X56 and X130 browser shots are indicated to the left and the black double-headed arrow above the browser image indicates the approximate location of the corresponding tandem repeat.

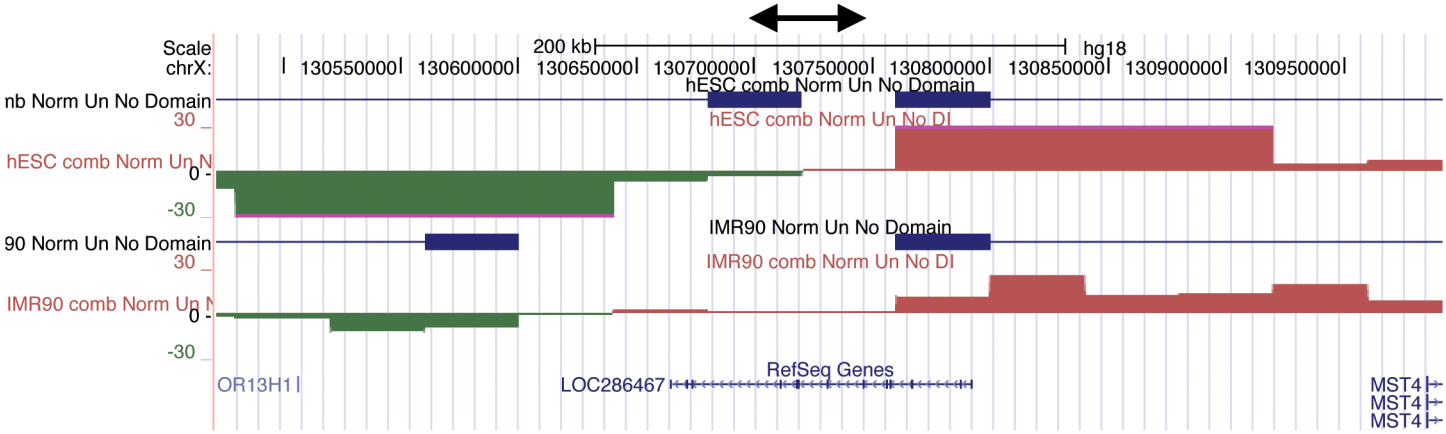
DXZ4



X56



X130



SUPPLEMENTARY TABLES

Supplementary Table 1 Oligonucleotides used in this study.

Oligonucleotide	Sequence	Application
DXZ4-R	CTTGCCACCCTGTCTTGTTG	3C
X56-CF	CGTCTGAACTATATCCTGTCT	3C
X56-DR	CTGTTCACCATGACATTTCAC	3C
X130-CF	CTACTCTTACTCACTCAGCAT	3C
X130-ER	ATATGGAGTAGAACTGCTAGG	3C
DXZ4-F23	GGACAGTCCCAAGCCACTC	CTCF ChIP analysis
DXZ4-R26	AGATGCTGATCCGCCATGTG	CTCF ChIP analysis
X56-XXC1-F	GTACTTGGCATTTGTGGTGC	CTCF & H3K4me2 ChIP
X56-XXC1-R	AGCGAGGAGTTCCTCTCTCG	CTCF & H3K4me2 ChIP
X130-CTCF-3-F	TGTTTGGTGGCGCCTTTGTG	CTCF & H3K4me2 ChIP
X130-CTCF-3-R	TCCACCTCTTACCAGACCAG	CTCF & H3K4me2 ChIP
DXZ4-F19	GAGATGCCCATGAACTCAAG	H3K4me2 ChIP
DXZ4-R19	GCCAGGGGGATAGGTGTG	H3K4me2 ChIP
X56-EST-F1	TCTCAGGTCTCTTGGCTTTG	RT-PCR
X56-EST-R1	GGGCCTTAATGGATTTATAGG	RT-PCR
X130-EST-F2	CCGAGTGAAGAGGATTGAAG	RT-PCR
X130-EST-R2	CATTTTCAGGGACCTCACAGG	RT-PCR
X56-Prom-F1	TGTACGTCTCAGGATACAGG	Luciferase construct
X56-Prom-R1	CTTGGCTTCCCGTGCGTTTC	Luciferase construct
X130-Prom-F1	TCTCTTCAAGGTCCTCCTAG	Luciferase construct
X130-Prom-R1	CGTGGTGCCTTCAGGCTCTG	Luciferase construct

Supplementary Table 2 Number of nuclei scored for DXZ4-X56 and DXZ4-X130 interactions by FISH. The number given in column 1 corresponds to the number indicated for males and females in the interaction-frequency graphs in Figure 4A.

Number	Cells	Sex	DXZ4 v X56	DXZ4 v X130
1	GM12802	Female	177	171
2	WI38	Female	59	60
3	IMR90	Female	43	43
4	hTERT-RPE1	Female	46	51
5	GM07059	Female	110	104
6	GM21770	Female	101	123
7	GM07011	Female	114	108
8	GM08728	Female	116	120
1	hTERT-BJ1	Male	58	48
2	GM06982	Male	63	69
3	GM07033	Male	105	115
4	GM07026	Male	103	118
5	GM07030	Male	101	112
6	GM08729	Male	107	94
7	HepG2	Male	121	117