

Supplemental Data

Supplementary Table 1 Details of FISH probes

Genome co-ordinates are given using the mm9 assembly of the mouse genome

Locus	Whitehead Name	Coordinates (mm9)	Size (bp)
<i>Hoxd3</i>	WI1-121N10	Chr2: 74,566,983 – 74,605,438	38,455
<i>Hoxd13</i>	WI1-469P2	Chr2: 74,474,157 -74,513,003	38,846
<i>GCR</i>	WI1-2157A11	Chr2: 74,242,615 -74,282,044	39,429
<i>Lnp</i>	WI1-482L15	Chr2: 74,329,582 -74,372,986	43,404
<i>Hoxb1</i>	WI1-2671L18	Chr11: 96,201,164- 96,242,956	41,793
<i>Hoxb13</i>	WI1-1356F15	Chr11: 96,060,900-96,099,631	38,732
<i>Hoxc4</i>	WI1-0991J24	Chr15: 103,018,285 -103,057,123	38,838
<i>Hoxc13</i>	WI1-1176M4	Chr15: 102,910,943 - 102,949,345	38,403
<i>En2</i>	WI1-2728F4	Chr5: 28,477,913 – 28,517,563	39,650
<i>Shh</i>	WI1-574O18	Chr5: 28,754,458 – 28,795,879	41,421
<i>Mnx1</i>	WI1-1204B6	Chr5: 29,791,124 – 29,827,491	36,367

Supplementary Table 2. Median inter-probe distances for each FISH probe set in all cell lines and conditions. Data from replicate experiments are indicated

Cell line/condition	Probe pair	Median interprobe distance (µm)
Figure 1 & Figure S1		
WT (clone36) Serum	<i>Hoxd3-Hoxd13</i>	Rep1: 0.276, Rep2: 0.317
WT (clone36) 2i	<i>Hoxd3-Hoxd13</i>	Rep1: 0.424, Rep2: 0.423
WT (clone36) Serum	<i>GCR-Lnp</i>	Rep1: 0.334, Rep2: 0.36
WT (clone36) 2i	<i>GCR-Lnp</i>	Rep1: 0.36, Rep2: 0.422
<i>Ring1B</i> ^{-/-} Serum	<i>Hoxd3-Hoxd13</i>	Rep1: 0.379, Rep2: 0.443
<i>Ring1B</i> ^{-/-} 2i	<i>Hoxd3-Hoxd13</i>	Rep1: 0.483, Rep2: 0.459
<i>Ring1B</i> ^{-/-} Serum	<i>GCR-Lnp</i>	Rep1: 0.3, Rep2: 0.36
<i>Ring1B</i> ^{-/-} 2i	<i>GCR-Lnp</i>	Rep1: 0.3, Rep2: 0.334
<i>Eed</i> ^{-/-} Serum	<i>Hoxd3-Hoxd13</i>	Rep1: 0.483, Rep2: 0.481
<i>Eed</i> ^{-/-} 2i	<i>Hoxd3-Hoxd13</i>	Rep1: 0.39, Rep2: 0.469
<i>Eed</i> ^{-/-} Serum	<i>GCR-Lnp</i>	Rep1: 0.334, Rep2: 0.334
<i>Eed</i> ^{-/-} 2i	<i>GCR-Lnp</i>	Rep1: 0.36, Rep2: 0.334
E14 Serum	<i>Hoxb1-Hoxb13</i>	0.276
E14 2i	<i>Hoxb1-Hoxb13</i>	0.347
E14 Serum	<i>Hoxc4-Hoxc13</i>	0.3
E14 2i	<i>Hoxc4-Hoxc13</i>	0.36
Figure 2		
Blastocysts	<i>Hoxd3-Hoxd13</i>	0.422
Blastocysts	<i>GCR-Lnp</i>	0.334
Figure 5 & S4		
WT J1 Serum	<i>Hoxd3-Hoxd13</i>	0.3

WT J1 2i	<i>Hoxd3-Hoxd13</i>	0.443
WT J1 Serum	<i>GCR-Lnp</i>	0.36
WT J1 2i	<i>GCR-Lnp</i>	0.334
3B3L Serum	<i>Hoxd3-Hoxd13</i>	Rep1: 0.3, Rep2: 0.3
3B3L 2i	<i>Hoxd3-Hoxd13</i>	Rep1: 0.334, Rep2: 0.334
3B3L Serum	<i>GCR-Lnp</i>	0.3
3B3L 2i	<i>GCR-Lnp</i>	0.334
3A3L Serum	<i>Hoxd3-Hoxd13</i>	0.3
3A3L 2i	<i>Hoxd3-Hoxd13</i>	0.36
WT J1 Serum	<i>En2-Shh</i>	0.324
WT J1 2i	<i>En2-Shh</i>	0.385
WT J1 Serum	<i>Shh-Mnx1</i>	0.48
WT J1 2i	<i>Shh-Mnx1</i>	0.608
WT J1 Serum	<i>En2-Mnx1</i>	0.478
WT J1 2i	<i>En2-Mnx1</i>	0.59
3B3L Serum	<i>En2-Shh</i>	0.329
3B3L 2i	<i>En2-Shh</i>	0.247
3B3L Serum	<i>Shh-Mnx1</i>	0.44
3B3L 2i	<i>Shh-Mnx1</i>	0.44
3B3L Serum	<i>En2-Mnx1</i>	0.471
3B3L 2i	<i>En2-Mnx1</i>	0.466

Supplementary Table 3 Probability values calculated by Mann-Whitney U tests comparing inter-probe distances between two populations. Inter-probe distances of *HoxD*, *Lnp-GCR* (Ctrl), *HoxC*, and *HoxB* probes in different cell types and conditions are shown

Genotype/ Condition/ Probes Sample 1	Genotype/ Condition/ Probes Sample 2	P-value (Mann-Whitney U Test)
Figure 1 & Figure S1		
WT (clone36) Serum <i>HoxD</i>	WT (clone36) 2i <i>HoxD</i>	Rep1: <0.0001, Rep2: 0.0003
WT (clone36) Serum <i>HoxD</i>	Ring1B ^{-/-} Serum <i>HoxD</i>	Rep1: <0.0001, Rep2: <0.0001
WT (clone36) Serum <i>HoxD</i>	Eed ^{-/-} Serum <i>HoxD</i>	Rep1: <0.0001, Rep2: <0.0001
WT (clone36) Serum <i>HoxD</i>	Ring1B ^{-/-} 2i <i>HoxD</i>	Rep1: <0.0001, Rep2: <0.0001
WT (clone36) Serum <i>HoxD</i>	Eed ^{-/-} 2i <i>HoxD</i>	Rep1: <0.0001, Rep2: <0.0001
WT (clone36) Serum Ctrl	WT (clone36) 2i Ctrl	Rep1: 0.4215, Rep2: 0.2564
WT (clone36) Serum Ctrl	Ring1B ^{-/-} Serum Ctrl	Rep1: 0.1352, Rep2: 0.5583
WT (clone36) Serum Ctrl	Eed ^{-/-} Serum Ctrl	Rep1: 0.7539, Rep2: 0.4776
WT (clone36) Serum Ctrl	Ring1B ^{-/-} 2i Ctrl	Rep1: 0.0797, Rep2: 0.1836
WT (clone36) Serum Ctrl	Eed ^{-/-} 2i Ctrl	Rep1: 0.5062, Rep2: 0.0865
E14 Serum <i>HoxB</i>	E14 2i <i>HoxB</i>	0.0334
E14 Serum <i>HoxC</i>	E14 2i <i>HoxC</i>	0.0024
Figure 2		
WT serum <i>HoxD</i>	Blastocysts <i>HoxD</i> (all)	<0.0001
WT serum <i>HoxD</i>	Blastocyst 1 <i>HoxD</i>	0.0050
WT serum <i>HoxD</i>	Blastocyst 2 <i>HoxD</i>	0.0170
WT serum <i>HoxD</i>	Blastocyst 3 <i>HoxD</i>	0.0168
WT serum <i>HoxD</i>	Blastocyst 4 <i>HoxD</i>	0.0043
WT serum <i>HoxD</i>	Blastocyst 5 <i>HoxD</i>	<0.0001
WT serum <i>HoxD</i>	Blastocyst 6 <i>HoxD</i>	<0.0001

WT serum <i>HoxD</i>	Blastocyst 7 <i>HoxD</i>	0.0068
WT serum <i>HoxD</i>	Blastocyst 8 <i>HoxD</i>	0.0002
WT serum <i>HoxD</i>	Blastocyst 9 <i>HoxD</i>	0.0019
WT serum <i>HoxD</i>	Blastocyst 10 <i>HoxD</i>	0.1389
WT serum <i>HoxD</i>	Blastocyst 11 <i>HoxD</i>	0.0537
WT serum <i>HoxD</i>	Blastocyst 12 <i>HoxD</i>	0.0047
WT serum <i>HoxD</i>	Blastocyst 13 <i>HoxD</i>	0.0237
WT 2i <i>HoxD</i>	Blastocysts <i>HoxD</i> (all)	0.0879
WT Serum Ctrl	Blastocysts Ctrl	0.6855
WT 2i Ctrl	Blastocysts Ctrl	0.2017
Figure 5 and Figure S4		
WT J1 Serum <i>HoxD</i>	WT J1 2i <i>HoxD</i>	Rep1: <0.0001, Rep2:
WT J1 Serum <i>HoxD</i>	3B3L Serum <i>HoxD</i>	Rep1: 0.2027, Rep2:
WT J1 Serum <i>HoxD</i>	3B3L 2i <i>HoxD</i>	Rep1: 0.2790, Rep2:
3B3L Serum <i>HoxD</i>	3B3L 2i <i>HoxD</i>	Rep1: 0.8658 Rep2:
WT J1 Serum <i>HoxD</i>	3A3L Serum <i>HoxD</i>	0.7219
WT J1 Serum <i>HoxD</i>	3A3L 2i <i>HoxD</i>	0.1128
3A3L Serum <i>HoxD</i>	3A3L 2i <i>HoxD</i>	0.2779
WT J1 Serum Ctrl	WT J1 2i Ctrl	0.2195
WT J1 Serum Ctrl	3B3L Serum Ctrl	0.0513
WT J1 Serum Ctrl	3B3L 2i Ctrl	0.1445
3B3L Serum Ctrl	3B3L 2i Ctrl	0.5587
WT J1 Serum <i>Shh-Mnx1</i>	WT J1 2i <i>Shh-Mnx1</i>	0.0001
WT J1 Serum <i>Shh-Mnx1</i>	3B3L Serum <i>Shh-Mnx1</i>	0.2274
WT J1 2i <i>Shh-Mnx1</i>	3B3L 2i <i>Shh-Mnx1</i>	<0.0001
3B3L Serum <i>Shh-Mnx1</i>	3B3L 2i <i>Shh-Mnx1</i>	0.3214
WT J1 Serum <i>En2-Shh</i>	WT J1 2i <i>En2-Shh</i>	0.0228
WT J1 Serum <i>En2-Shh</i>	3B3L Serum <i>En2-Shh</i>	0.4785
WT J1 2i <i>En2-Shh</i>	3B3L 2i <i>En2-Shh</i>	<0.0001
3B3L Serum <i>En2-Shh</i>	3B3L 2i <i>En2-Shh</i>	0.0028
WT J1 Serum <i>En2-Mnx1</i>	WT J1 2i <i>En2-Mnx1</i>	0.0101
WT J1 Serum <i>En2-Mnx1</i>	3B3L Serum <i>En2-Mnx1</i>	0.3550
WT J1 2i <i>En2-Mnx1</i>	3B3L 2i <i>En2-Mnx1</i>	0.0001
3B3L Serum <i>En2-Mnx1</i>	3B3L 2i <i>En2-Mnx1</i>	0.3190

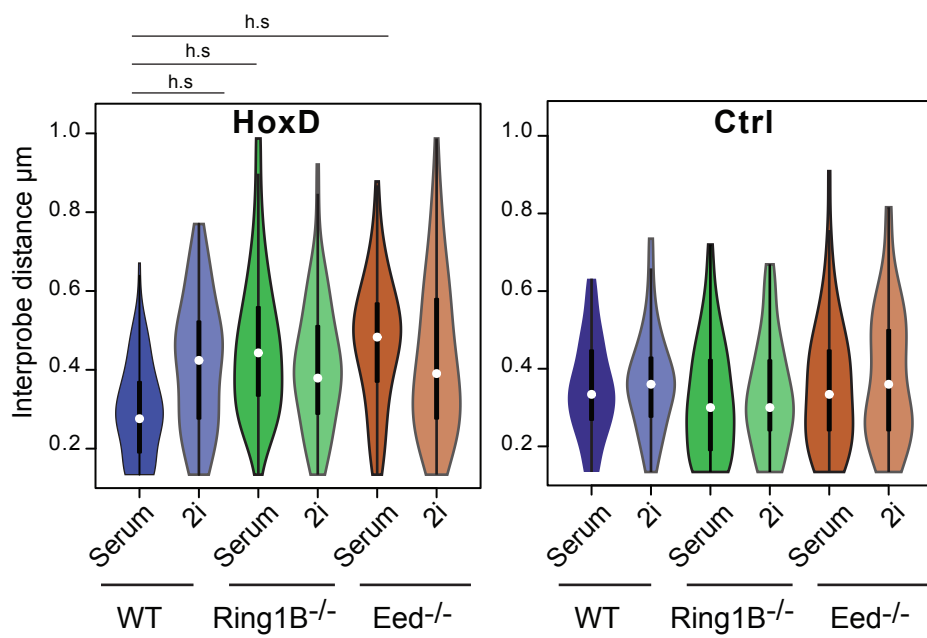
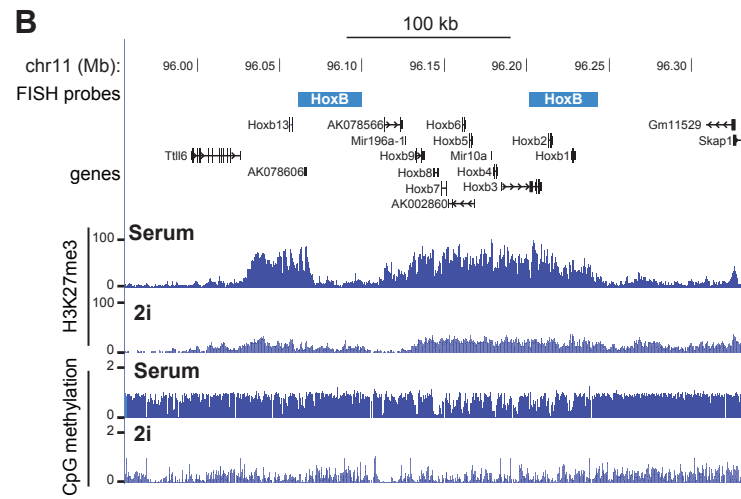
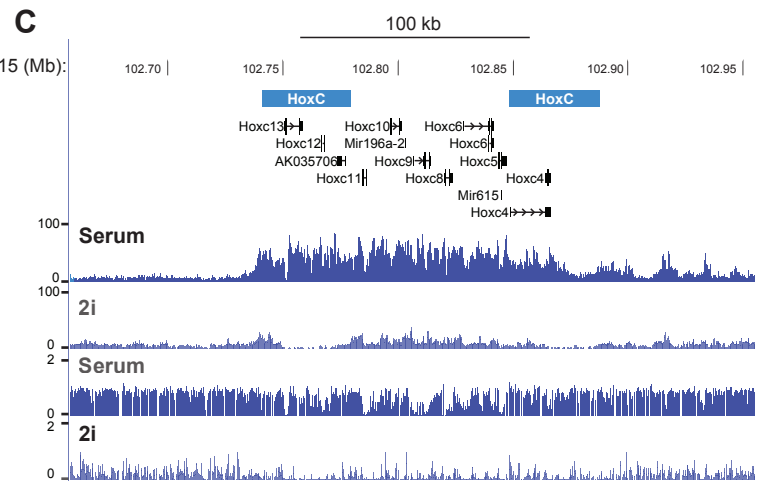
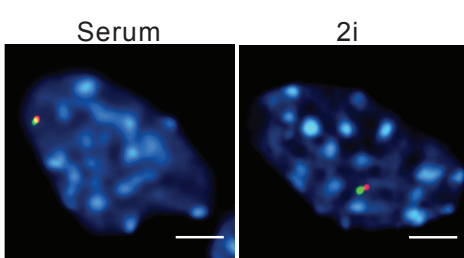
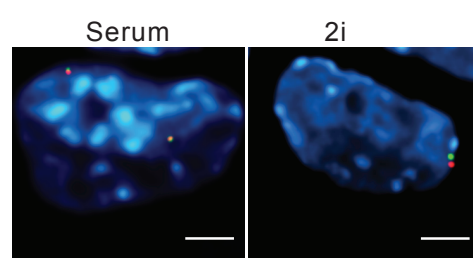
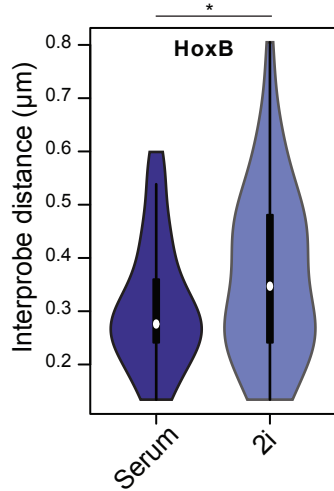
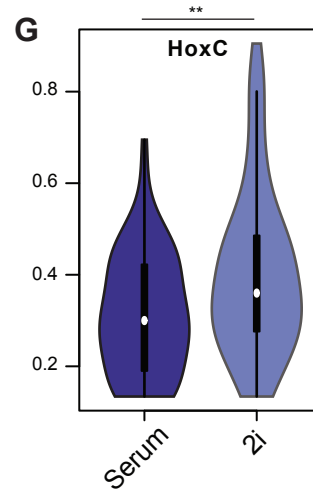
A**B****C****D****E****F****G**

Figure S1

Supplementary Figure 1

A. Violin plots showing the distribution of inter-probe distances for HoxD and control (Ctrl) loci (from Figure 1A) in WT, *Ring1B*^{-/-} and *Eed*^{-/-} cells grown in serum or 2i. Data are biological replicate for the data in Figure 1. h.s = p<0.0001. Details of statistical analysis are given in Tables S2 and S3.

B. UCSC genome browser tracks (mm9 assembly of the mouse genome) showing the location on chromosome 11 of FISH probes used to measure compaction across the *HoxB* locus. Probe co-ordinates are given in Supplementary Table 1. Below are shown the H3K27me3 (Marks et al., 2012) and DNA methylation (Habibi et al., 2013) profiles for this region of the mouse genome in mESCs grown in serum or 2i.

C. As in (B) but for the *HoxC* locus on chromosome 15.

D. Representative images of *HoxB* probe hybridisation signals (red and green) in WT E14 mESCs grown in serum or 2i. Scale bars represent 10 µm.

E. As in (D) but for *HoxC*.

F. Violin plots showing the distribution of inter-probe distances for the *HoxB* locus in mESCs cells grown in serum or 2i. The vertical line and spot within each plot indicate the interquartile range and median, respectively. *p<0.05, **p<0.01.

G. As in (F) but for *HoxC*.

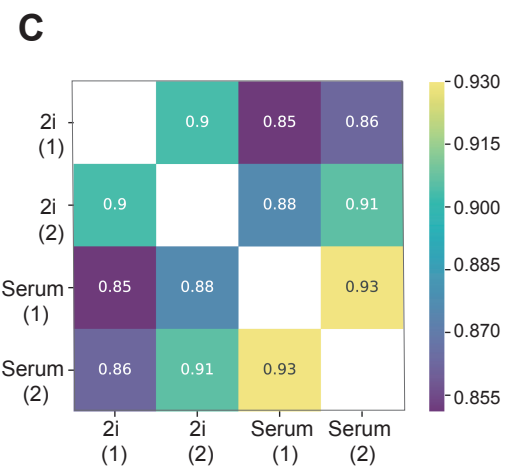
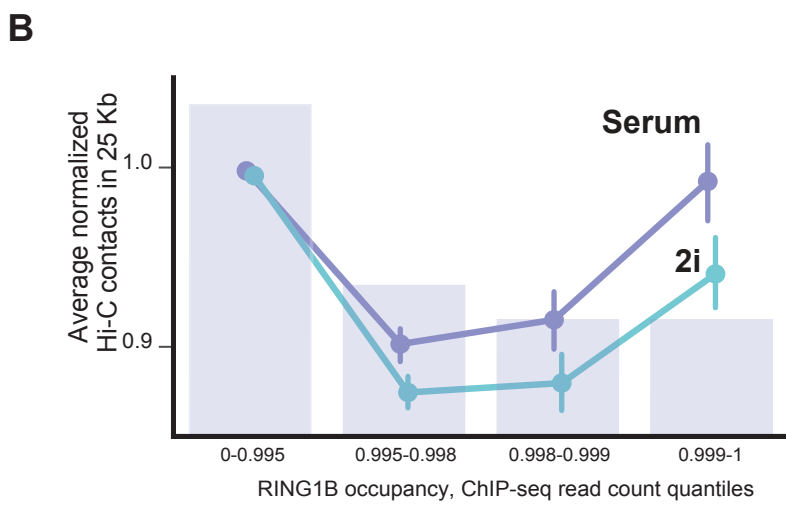
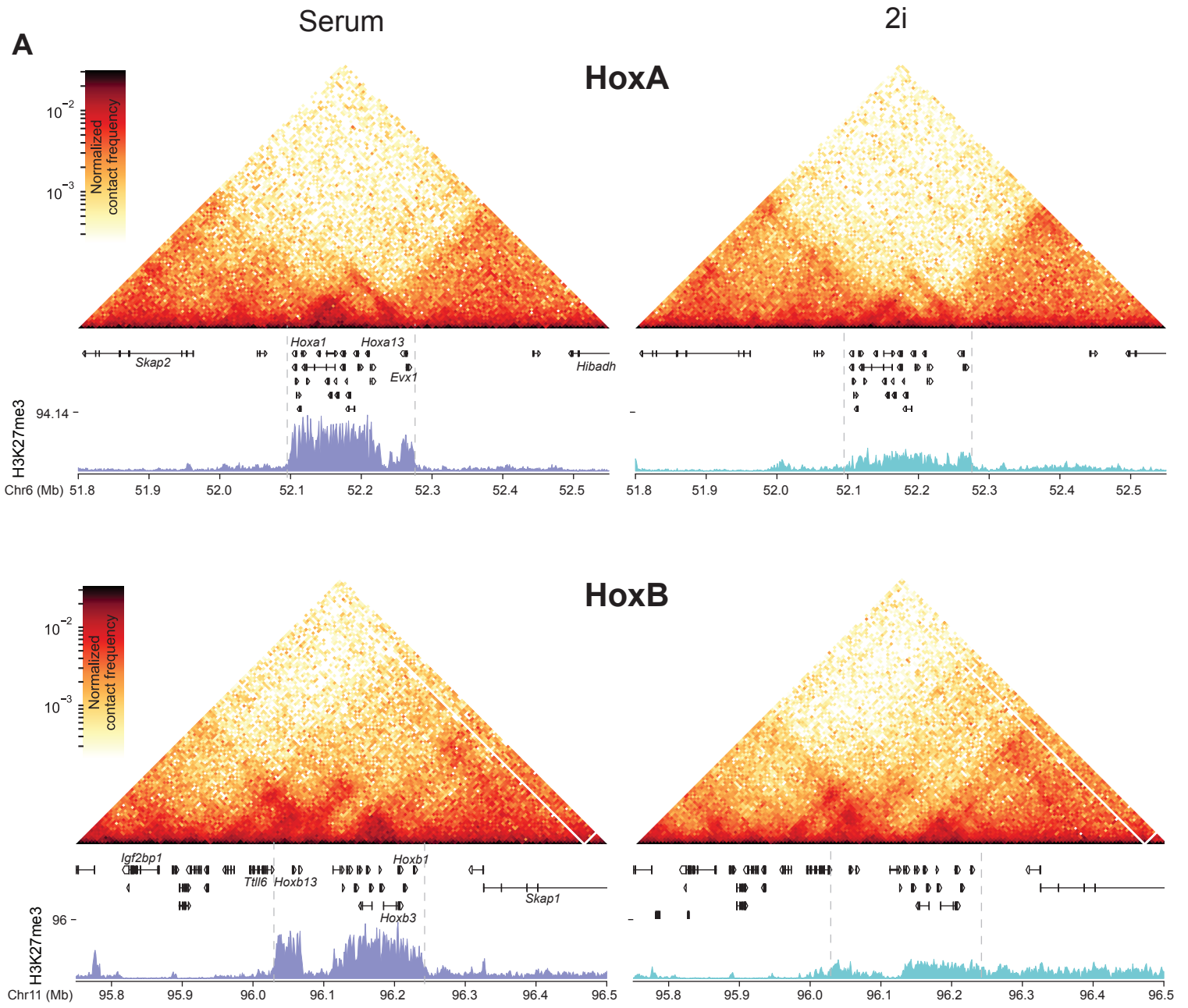


Figure S2

Supplementary Figure 2

- A. Hi-C heatmaps (normalised contact frequencies) for cells grown in serum and 2i media for the *HoxA* (top) and *HoxB* (bottom) clusters along with gene annotations and H3K27me3 ChIP-seq profiles (Marks et al., 2012). Boundaries of the *Hox* clusters are marked with dashed lines.
- B. Same as Fig. 3B, but with RING1B ChIP-seq quantification instead of H3K27me3 (both Hi-C data compared to RING1B data from serum-grown cells).
- C. Correlation of \log_2 of insulation score profiles (100 kb window) across serum and 2i Hi-C replicates.

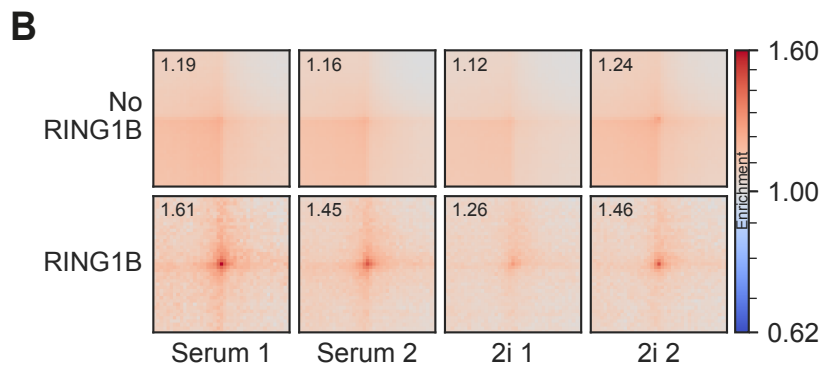
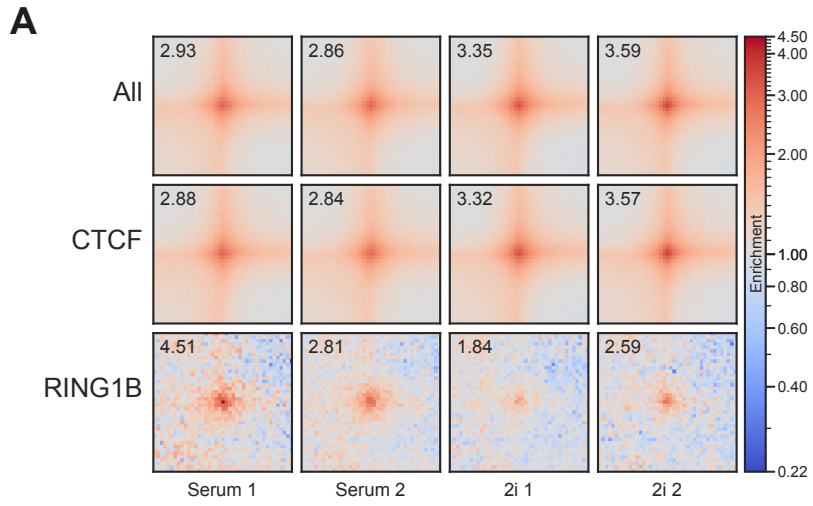


Figure S3

Supplementary Figure 3

- A. As for main Figure 4C, but for individual replicates.
- B. As for main Figure 4D, but for individual replicates of our data.

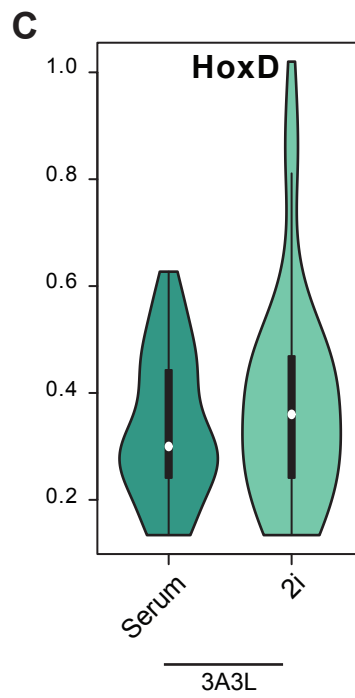
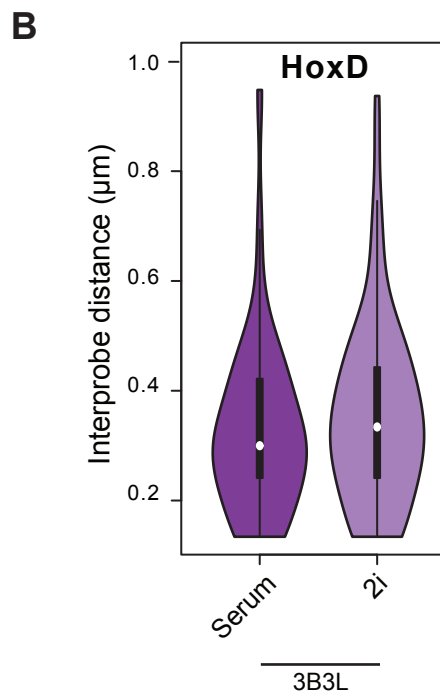
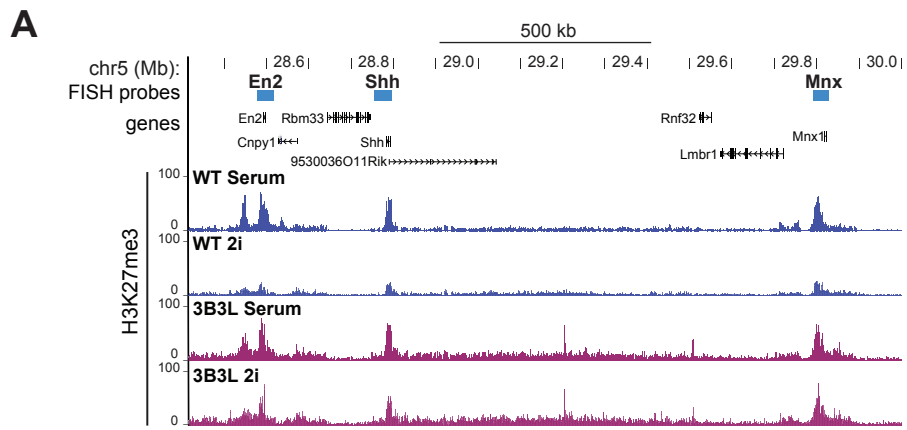


Figure S4

Supplementary Figure 4

A. UCSC genome browser tracks (mm9 assembly) showing the location on chromosome 5 of FISH probes used to measure distal interactions across the *Shh* locus. Probe co-ordinates are given in Supplementary Table 1. Below are shown the H3K27me3 profiles for this region of the mouse genome in WT (Marks et al., 2012) and 3B3L mESCs grown in serum or 2i.

B. Violin plots showing distribution of inter-probe distances at the *HoxD* locus 3B3L cells cultured in serum/LIF and 2i/LIF. This is a biological replicate for the data in Figure 5E.

C. As for (A) but for 3A3L cells.