SUPPLEMENTARY MATERIALS

Fig. S1. CTCF consensus motifs underlie chromatin boundary elements.

A. Schematic depiction of differentiation protocol to generate cervical motor neurons from ESCs. Terminally differentiated motor neurons (MN) form after 4 days of RA/SAG treatment. RA: retinoic acid, SAG: Smoothened agonist.

B,C. PhyloP conservation and sequence tracks for the indicated species. CTCF 15bp core motif underlying C5|6 **(B)** and C6|7 **(C)** is highlighted in red. Positive PhyloP scores signify conservation at base-pair resolution.

Fig. S2. CTCF demarcates a chromatin boundary at the *HoxC* and *HoxD* clusters.

A,B. Normalized ChIP-seq read densities for the indicated proteins/modifications across the *HoxD* **(A)** and *HoxC* **(B)** clusters in ESCs and in MNs from two biological replicates. Dashed line highlights the boundary. CTCF motifs are highlighted in red.

Fig. S3. Δ5|6 ESCs appropriately differentiate into motor neurons.

Stainings of MNs three days after exposure to RA/SAG. Hb9, Lhx3 and Isl1/2 are markers of spinal motor neurons. Class III b-tubulin is a marker of neurons.

Fig. S4. Dynamics of *HoxA* gene expression during differentiation.

RT-qPCR signal is normalized against GAPDH expression levels. Fold change in expression at the indicated time-points after RA/SAG treatment is calculated relative to

baseline expression in ESCs. Error bars represent the standard deviation across two technical replicates.

Fig. S5. CTCF plays a boundary function within the *HoxC* cluster

A. Normalized ChIP-seq read densities across the *HoxC* cluster for the indicated proteins/modifications in ESCs and MNs in WT and $\Delta 5|6_{HoxC}$ motor neurons. **B.** Sequencing chromatogram of $\Delta 5|6_{HoxC}$ line depicts a 13bp deletion overlapping the CTCF core motif. **C.** RT-qPCR signal is normalized against GAPDH expression levels. Fold change in expression versus WT MNs is depicted. Error bars represent the standard deviation across two biological replicates.

Fig. S6. Chromatin boundary is disrupted in Δ5|6 mutant.

A,B. WT vs. Δ5|6 ratio of normalized ChIP-seq signal mapping to 1kb windows tiled across the indicated portion of the *HoxA* cluster for H3K27me3 **(A)** and H3K4me3 **(B)**

C,D. ChIP-qPCR at the loci diagrammed in **A,B** for H3K27me3 **(C)** and H3K4me3 **(D)**. Error bars represent the standard deviation across two biological replicates. The "HoxD" primer probes a region within the repressed segment of the *HoxD* cluster in MNs. This region is immediately caudal to the CTCF-demarcated chromatin boundary.

E. Normalized ChIP-seq tracks for the indicated protein/modifications along the *HoxC* cluster in MNs (4 days after RA/SAG).

Fig. S7. Topological organization of the *HoxA* cluster in ESCs

A,B. Normalized ChIP-seq read densities for CTCF and 4C contact profiles in WT, Δ5|6, and Δ5|6:7|9 ESCs using a viewpoint (red) in the rostral **(A**, 4C.Hoxa5-A**)** and caudal **(B**, 4C.Hoxa10**)** segment of the cluster. The median and 20th/80th percentile of sliding 5kb windows determine the main trendline. Color scale represents enrichment relative to the maximum attainable 12-kb median value.

Fig. S8. Loss of CTCF alters topological structure of the *HoxA* locus

A,B,C. Normalized ChIP-seq read densities for CTCF and 4C contact profiles in WT, Δ5|6, and Δ5|6:7|9 MNs using the 4C.HOXA5-A **(A)**, 4C.HOXA10 **(B)**, or 4C.HOXA5-B **(C)** viewpoints. Normalized interaction frequency of individual biological replicates across 2.5kb tiled windows is depicted on the left. Mean and standard deviation of replicates are plotted on the right.

Fig. S9. Generation of Δ5|6:7|9 mutant via CRISPR genome-editing

Guide-RNA targeting the CTCF core motif, C7|9 (orange) is depicted below the WT sequence. Sequence and chromatogram of mutant alleles in the Δ5|6:7|9 line are presented above and below. One allele (deletion allele) contains a 21bp deletion overlapping the CTCF core motif. The other allele ("insertion allele") contains a 20bp insertion that disrupts the CTCF core motif.

Fig. S10. Mutation of an adjacent YY1 binding site does not phenocopy CTCF ablation

A. Normalized ChIP-seq read densities across the HoxA cluster for the indicated proteins/modifications in MNs from two merged biological replicates. YY1 ChIP-seq was performed in ESCs, and taken from GSE31786. Genes that are activated in Δ5|6 cells during differentiation are annotated in green; repressed in red. Genetic mutants of the YY1, CTCF, or both binding sites are depicted. **B.** Sequencing chromatogram of $YY1_{\Delta 5|6:7|9}$ and $YY1\text{-}CTCF_{\Delta 5|6:7|9}$ lines depicts a 13bp and 49bp deletion overlapping the highlighted CTCF and YY1 binding motifs. The $CTCF_{\Delta 5|6:7|9}$ chromatogram is depicted in Fig. S9. **C.** RT-qPCR signal is normalized against GAPDH expression levels. Fold change in expression versus WT MNs is depicted. Standard deviations are calculated across two technical replicates.

MATERIALS AND METHODS

Cell culture and motor neuron differentiation

ESCs were trypsinized and seeded at 2.5×10^4 cells/ml in ANDFK medium (Advanced DMEM/F12 : Neurobasal (1:1) Medium, 15% Knockout Serum Replacement, Pen/Strep, 2 mM L-Glutamine, and 0.1 mM 2-mercaptoethanol) to initiate formation of embryoid bodies (Day 0). Medium was exchanged on Day 2 of differentiation. Patterning of embryoid bodies was induced by supplementing the media on Day 2 with 1 μM all-*trans*-Retinoic acid (RA, Sigma) and 0.5 μM smoothened agonist (SAG, Calbiochem). For ChIP experiments, the same conditions were used but scaled up to seed 3×10^7 cells on Day 0. Biological replicates represent differentiations performed on different days.

CRISPR genome editing

An optimal gRNA target sequence closest to the genomic target site was chosen using the http://crispr.mit.edu/ design tool. The gRNA (Table S3) was cloned into the SpCas9-2A-GFP vector (Addgene: PX458) via BbsI digestion and insertion (18). ES cells were seeded into 12-well plates at 100,000 cells per well, and transfected with 0.5ug of the appropriate guide RNA (Table S3) and Cas9 endonuclease using Lipofectamine 2000 (Life Technologies). The transfection was performed according to the manufacturer's recommended protocol, using a 3:1 ratio of Lipofectamine:DNA. After transfection, GFP positive cells were sorted using the Sony SY3200 cell sorter, and 20,000 cells were plated on a 15cm dish. 7-10 days later, single ESC clones were picked, trypsinized in 0.25% Trypsin-EDTA for 5', and plated into individual wells of a 96-well plate for genotyping. Genomic DNA was harvested via QuickExtract (Epicentre) DNA extraction, and genotyping PCRs were performed using primers surrounding the target site (Table S3). The resulting PCR products were purified and sequenced to determine the presence of an insertion or deletion event. When necessary, the PCR product was subject to TOPO cloning and sequencing in order to distinguish the amplified products of distinct alleles. Sequencing chromatograms were aligned in Benchling.

Expression analysis

RNA was purified from cells with TRIzol (Invitrogen), and RT-qPCRs were performed in replicate on 5ng of total RNA using the QuantiTect SYBR Green RT-PCR kit.

Immunocytochemistry

Embryoid bodies were fixed with 4% paraformaldehyde in PBS, embedded in OCT (Tissue-Tek) and sectioned for staining: 16 hours at 4C for primary antibodies and 4 hours at RT for secondary antibodies. After staining, samples were mounted with Fluoroshield with DAPI (Sigma-Aldrich). Images were acquired with a Leica SP5 confocal microscope. Antibodies used in this study include: mouse anti-Isl1, mouse anti-Lhx3, mouse anti-Hb9 (Developmental Studies Hybridoma Bank, 1:100), anti-β-III-tubulin (Sigma T2200, 1:4,000,). Conjugated secondary antibodies from Invitrogen used 1:2,000; Alexa 488 (A11029, A11034), Alexa 568 (A11036, A11031); Alexa 647 (A21245, A21236).

Chromatin immunoprecipitation

EBs were trypsinized and fixed in 10 mM Hepes, pH 7.6, 1% formaldehyde, 15 mM NaCl, 0.15 mM EDTA and 0.075 mM EGTA, for 15 min at room temperature. The reaction was quenched with 0.125M glycine at room temperature. After washing with PBS, cells were collected, and the pellet was lysed in 50 mM Hepes, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% Igepal CA630, and 0.25% Triton X-100. After centrifugation, isolated nuclei were washed once in 10 mM Tris pH 8.0, 200 mM NaCl, 1 mM EDTA and 0.5 mM EGTA, then resuspended in 10 mM Tris pH 8.0, 1 mM EDTA, 0.5 mM EGTA and 0.5% N-Lauryl Sarcosine, and sonicated using a Diagenode Bioruptor to an average chromatin size of 200 bp. Chromatin was diluted with 2X IP buffer (2% NP-40, 200mM NaCl in 10mM Tris-HCl pH8, 1mM EDTA), to which antibodies were added to each IP (Table S3). IPs were performed overnight, after which Dynabeads (Life Technologies) were incubated for an additional 2 hours. The beads were

washed 5 times with 1mL of 25 mM Hepes pH 7.6, 1 mM EDTA, 0.1% N-Lauryl Sarcosine, 1% NP-40, and 0.5 M LiCl. After a final wash with 750 µl of 10 mM Tris pH 8.0, 1 mM EDTA and 50 mM NaCl, the beads were resuspended in 100 mM sodium bicarbonate, 200 mM NaCl, and 1% SDS. The chromatin was incubated 15 min at room temperature and the cross-linking was reversed at 65°C for 16 hr. After reversal, a Proteinase K digest was performed for 2hrs at 55°C, and DNA was extracted using a PCR purification kit (Qiagen). 10% of the input was treated in parallel.

Preparation of 4C template

Cells were processed for 4C-seq as described previously by van de Werken et al (28). Cells were trypsinized and counted. 1 x 10^7 cells were crosslinked with 2% formaldehyde and 10% FBS in PBS for 10 min at room temperature. The reaction was quenched with glycine. Cells were lysed on ice with 1ml lysis buffer (50mM Tris pH 7.3, 150mM NaCl, 5mM EDTA, 0.5% NP-40, 1% Triton X-100) for 15 min. Nuclei were spun down and resuspended in 360ul H2O (or frozen). 60ul of 10X DpnII restriction buffer was added along with 15ul 10% SDS, and left shaking for 1hr at 37C. 150ul of 10% Triton X-100 was added, and nuclei were again incubated for 1hr at 37C. 5ul undigested control was stored, and nuclei were incubated overnight with 200U of DpnII (NEB, R0543M) restriction enzyme. A fresh 200U of DpnII was added the following morning for 6hrs. DpnII was inactivated with 80ul 10% SDS, and a proximity ligation reaction was performed in a 7ml volume using 4000U T4 DNA Ligase (Roche M0202M). Crosslinks were reversed at 65C O/N after adding 300ug Proteinase K. Samples were then treated with 300ug RNAse A for 45 min at 37C, and DNA was

ethanol precipitated. A 2nd restriction digest was performed overnight in a 500ul reaction with 50U Csp6l (Fermentas, ER0211). The enzyme was inactivated at 65C for 25 min, and a proximity ligation reaction was performed in a 14ml volume with 6000U T4 DNA Ligase. Sample DNA was ethanol precipitated, and purified using the QIAquick PCR purification kit.

Library construction

Libraries for ChIP-seq were prepared according to manufacturer's instructions (Illumina). Briefly, IP'ed DNA (~5 ng) was end-repaired using End-It Repair Kit (Epicenter), tailed with deoxyadenine using Klenow exo− (New England Biolabs), and ligated to custom adapters with T4 Rapid DNA Ligase (Enzymatics). Fragments of 200-400bp were size-selected using Agencourt AMPure XP beads, and subjected to PCR amplification using Q5 DNA polymerase (New England Biolabs). Libraries were quantified by qPCR using primers annealing to the adapter sequence and sequenced at a concentration of 12 pM on an Illumina HiSeq. Barcodes were utilized for multiplexing. For RNA-seq libraries, polyA+ RNA was isolated using Dynabeads Oligo(dT)25 (Invitrogen) and constructed into strand-specific libraries using the dUTP method *(29)*. Once dUTP-marked double-stranded cDNA was obtained, the remaining library construction steps followed the same protocol as described above for ChIP-seq libraries.

Libraries for 4C-seq were constructed by adding barcoded illumina adapters to the 5' end of each primer (Table S3). PCR reactions were performed using the Expand Long Template PCR System (Roche), and 100-700bp DNA was gel purified and quantified before sequencing.

Data analysis

Sequenced reads from experiments were mapped with Bowtie using parameters –v2 –m4 (ChIP-seq), or –v2 –m40 (RNA-seq). Normalized ChIP-seq read densities were visualized on the UCSC genome browser (http://genome.ucsc.edu). RNA-seq reads were assigned to genes using DEGseq (R package) and the ENSEMBL annotation. Normalized differential gene expression was calculated with DEseq (R package), which bases differential expression calls on an underlying negative binomial distribution of read counts. Relevant expression and p-values are listed in Table S1. Heatmaps were generated in R. 4C-seq data was analyzed either using the 4cseq_pipeline (28), or manually by mapping reads to a reduced genome composed of windows surrounding DpnII sites. Reads mapping to individual restriction sites were counted and normalized by the total number of reads mapping to chr6. UCSC tracks represent read density across a 2.5kb sliding window run across the *HoxA* cluster.

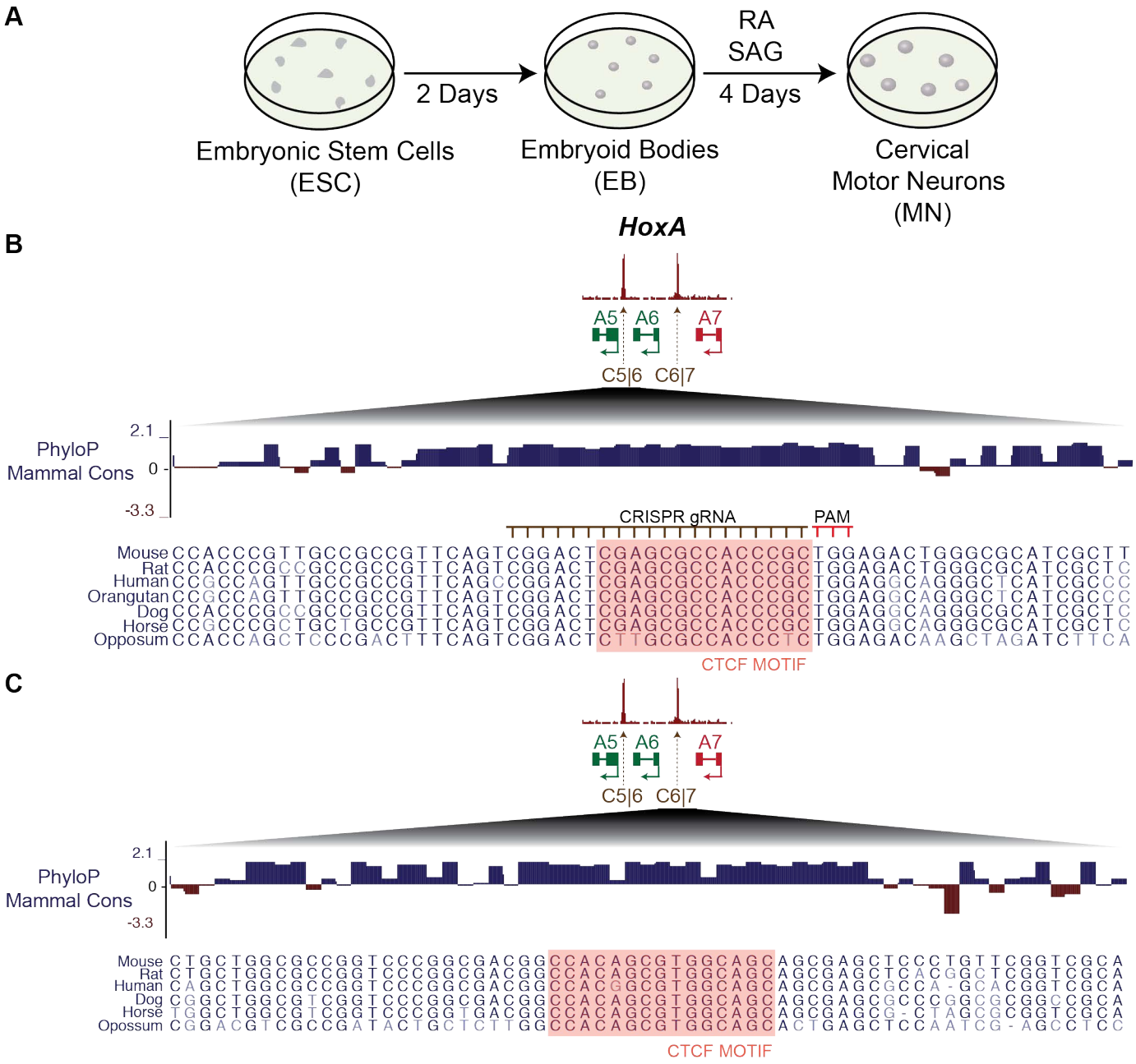

Figure S1 | CTCF consensus motifs underlie chromatin boundary elements

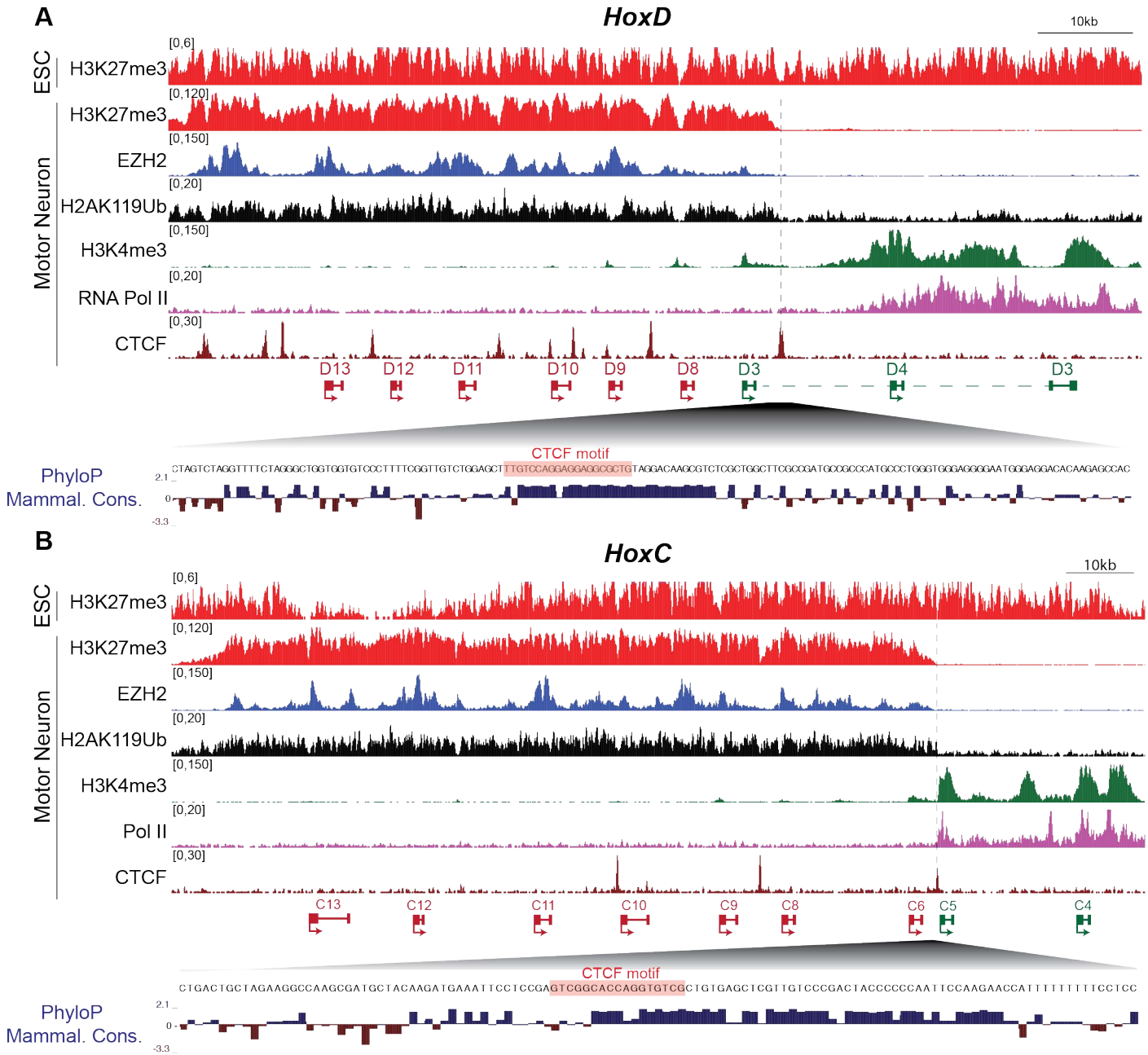

Figure S2 | CTCF demarcates a chromatin boundary at the HoxC and HoxD clusters

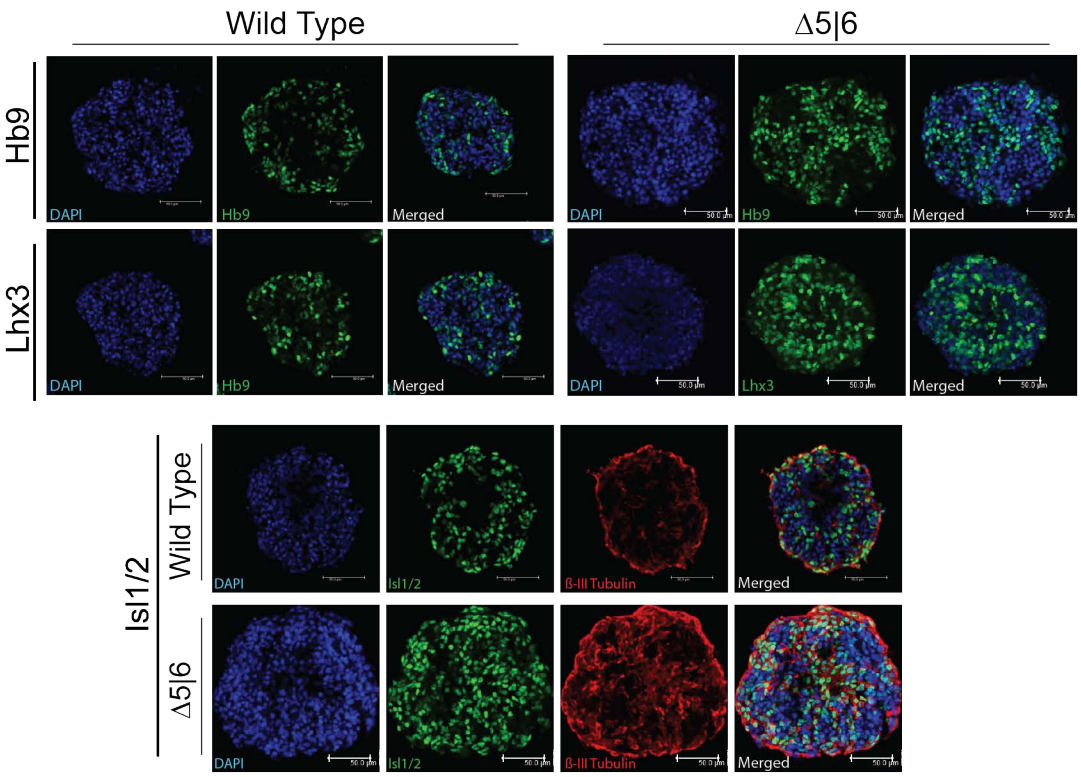

Figure S3 | Δ5|6 ESCs appropriately differentiate into motor neurons

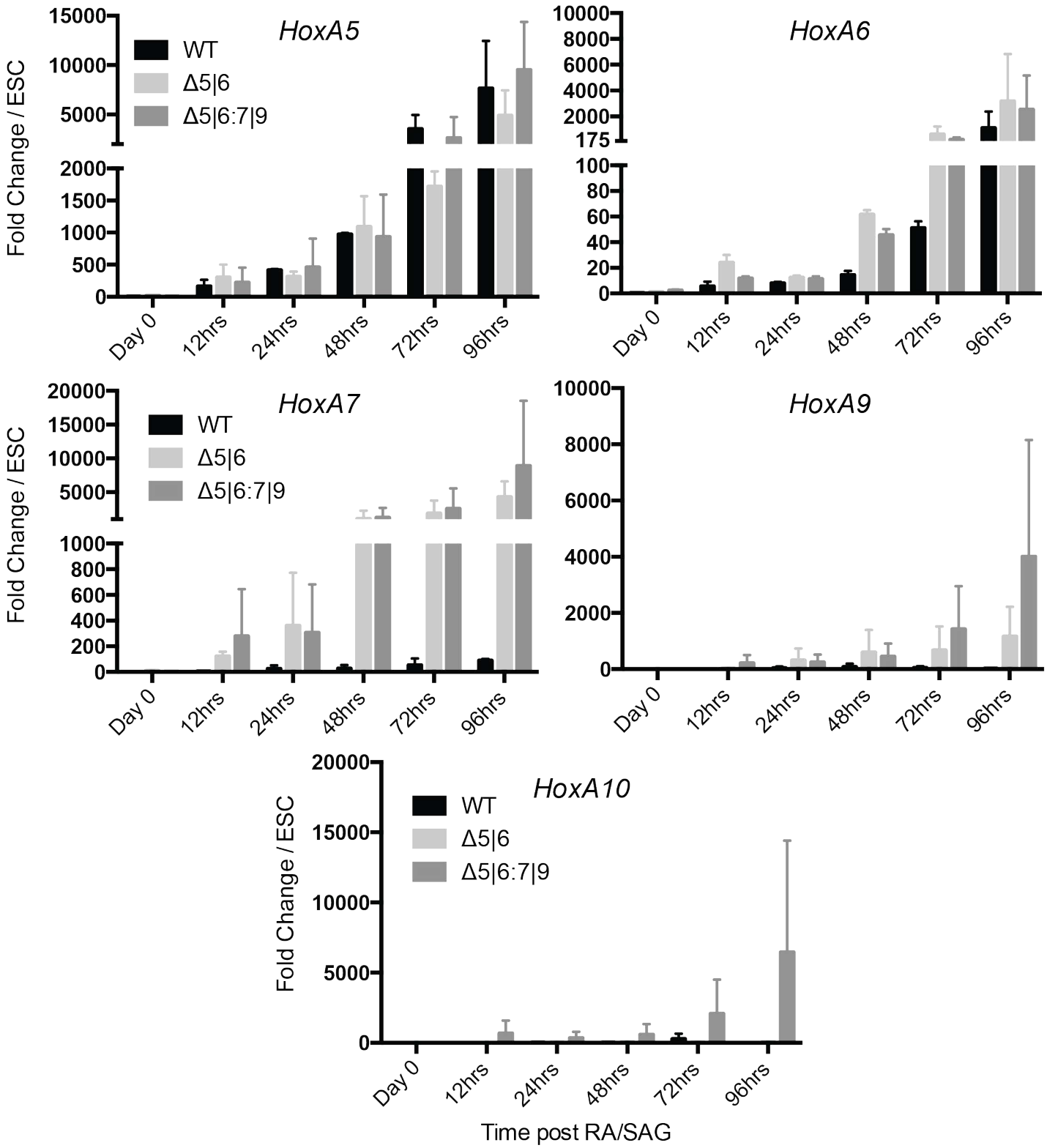

Figure S4 | Dynamics of HoxA gene expression during differentiation

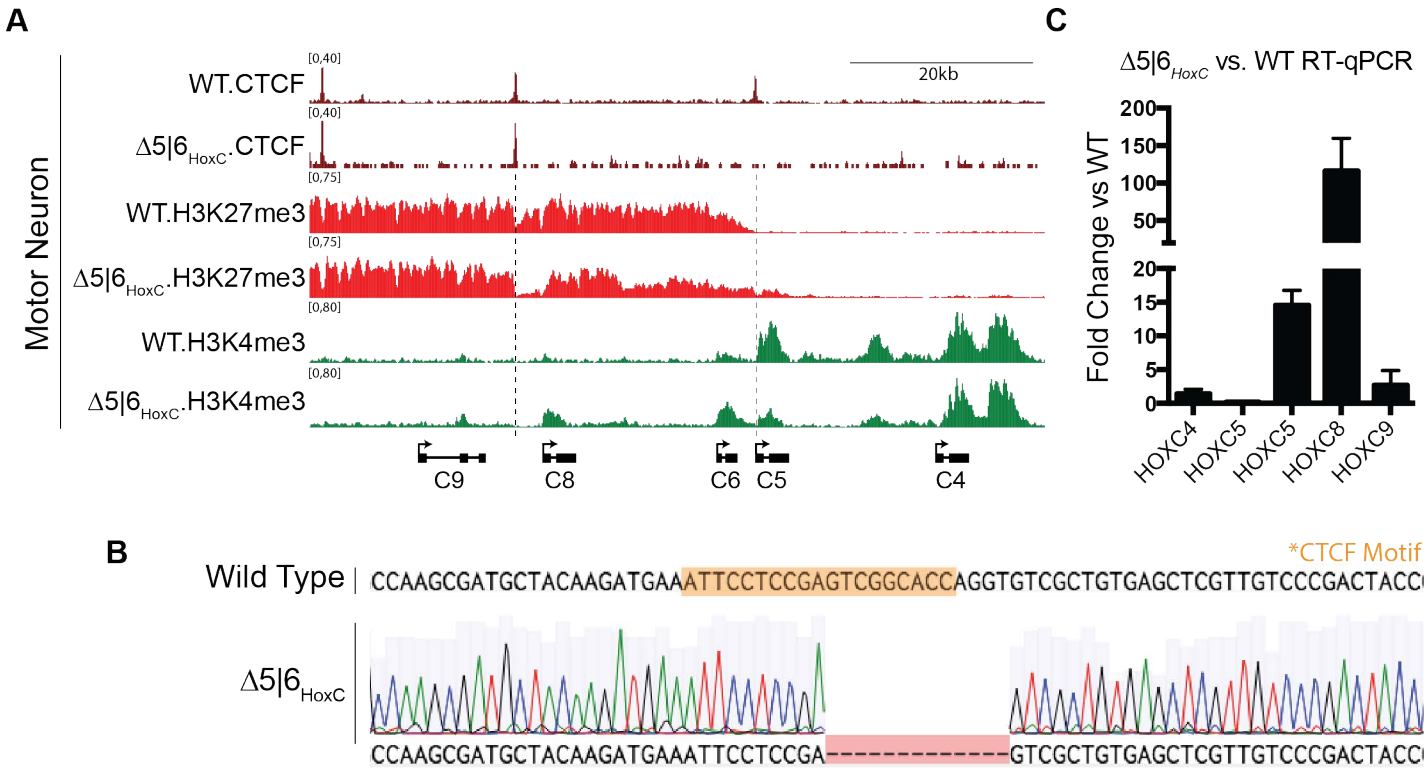

Figure S5 | CTCF plays a boundary function within the *HoxC* cluster

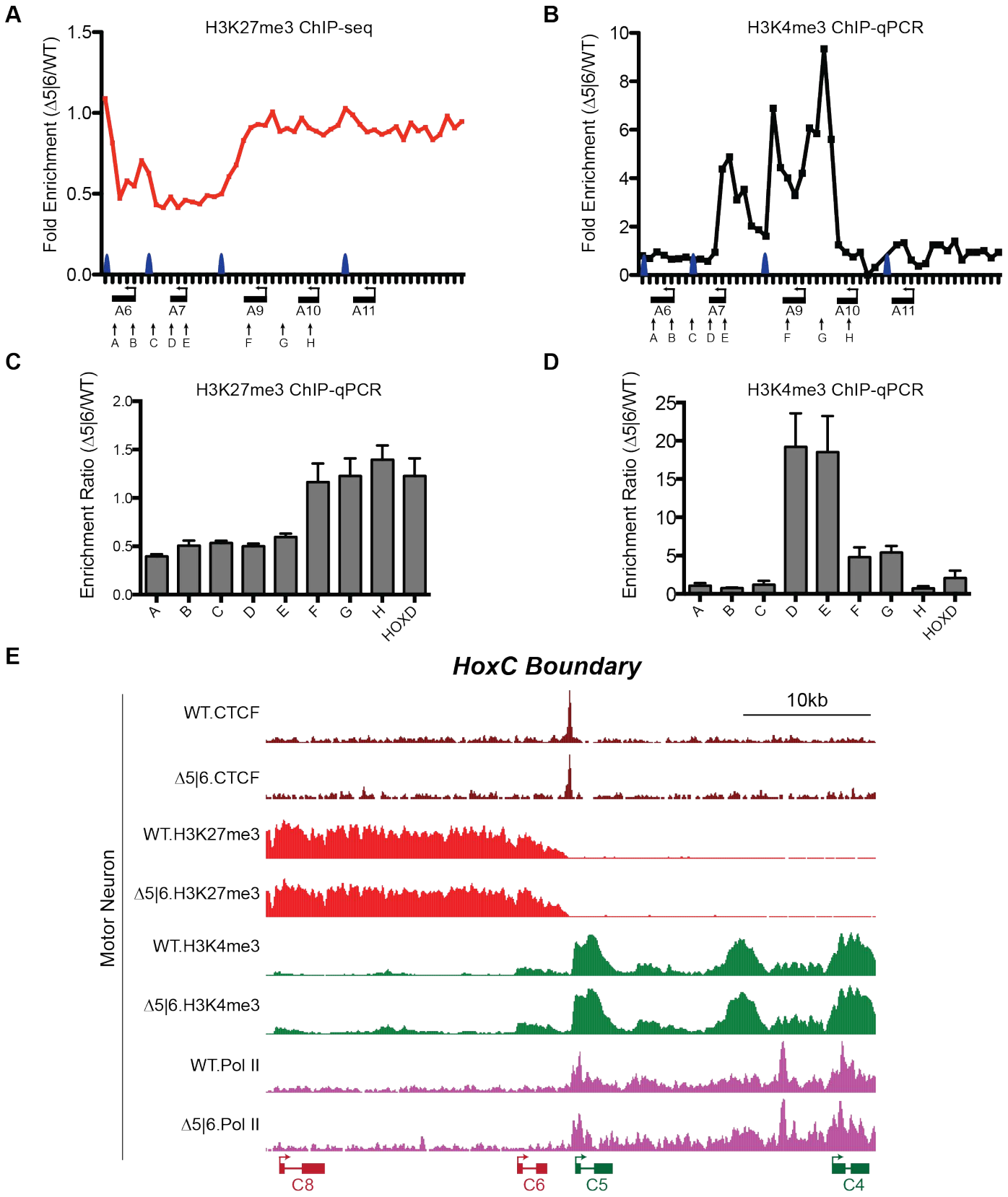

Figure S6 | Chromatin boundary is disrupted in Δ5|6 mutant

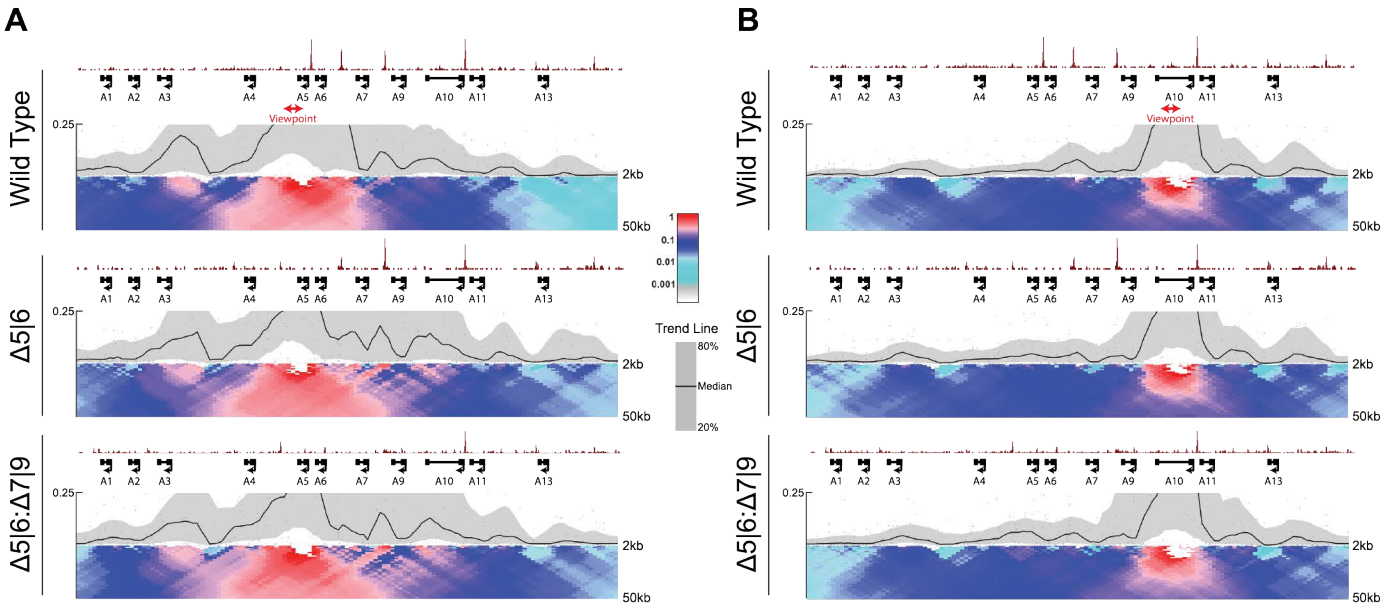

Figure S7 | Topological organization of the *HoxA* cluster in ESCs

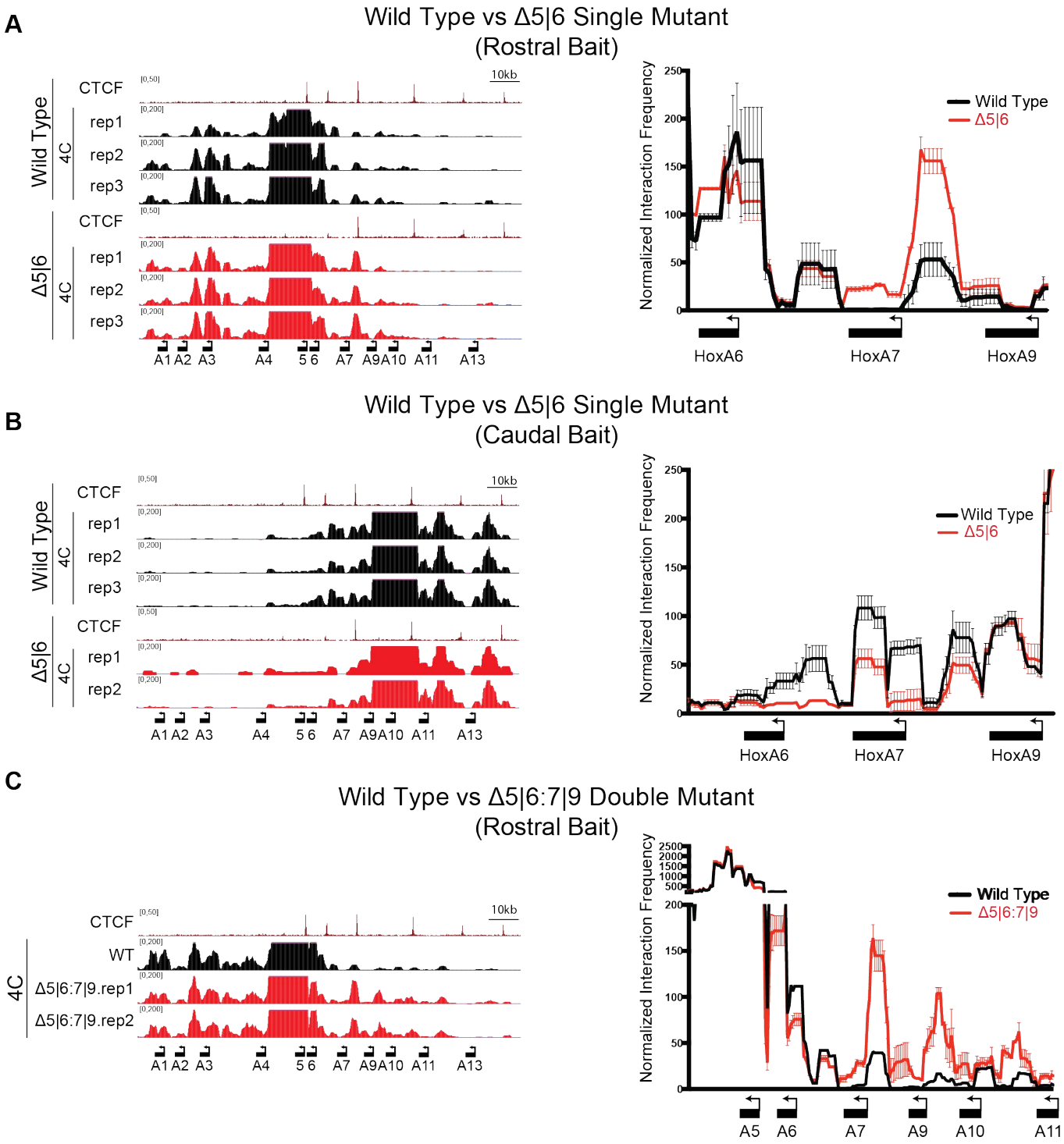

Figure S8 | Loss of CTCF alters topological structure of the HoxA locus

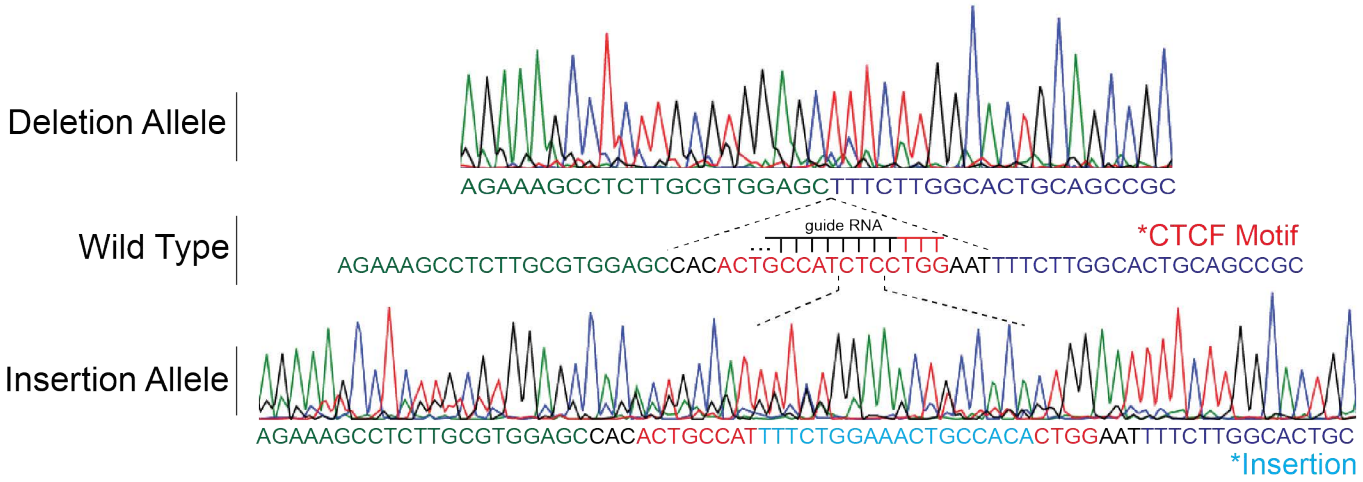

Figure S9 | Generation of Δ5|6:7|9 double mutant via CRISPR genome-editing

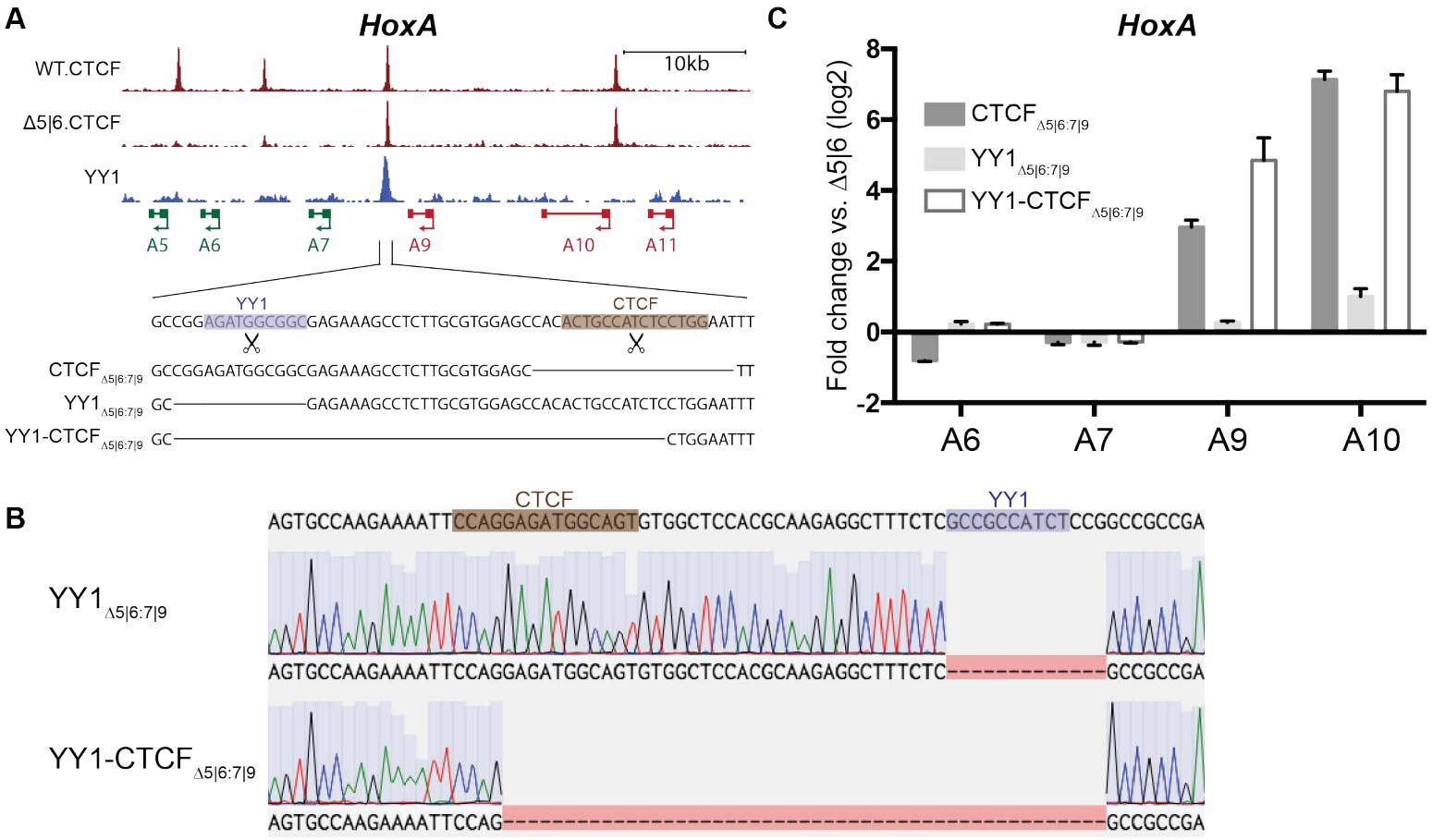

Figure S10 | Mutation of an adjacent YY1 binding site does not phenocopy CTCF ablation

TABLE S1

				Mean Norm. Counts			
Sample A	**Sample B**	**Time Point**	**Gene**	**Counts A**	**Counts B**	**Fold Change**	**P-Value**
WT	Δ5|6	ESC	HoxA7	28.26	46.93	1.66	0.72915
WT	Δ5|6	ESC	HoxA9	5.43	10.15	1.87	0.75422
WT	Δ5|6	ESC	HoxA10	10.87	20.30	1.87	0.70507
WT	Δ5|6	Day 4	HoxA7	35.09	898	25.59	0.00046
WT	Δ5|6	Day 4	HoxA9	4.38	23.64	5.40	0.08749
WT	Δ5|6	Day 4	HoxA10	1.00	2.18	2.18	0.63613
WT	Δ5|6:7|9	Day 4	HoxA7	42.05	389.02	9.25	0.13785
WT	Δ5|6:7|9	Day 4	HoxA9	9.23	634.93	68.79	0.01268
WT	Δ5|6:7|9	Day 4	HoxA10	1.00	278.45	278.20	0.00235

Line	**Time Point A**	**Time Point B**	**Gene**	**Counts A**	**Counts B**	**Fold Change**	**P-Value**
WT	ESC	Day 4	HoxA1	29.35	1023.46	34.87	0.023486545
WT	ESC	Day 4	HoxA2	4.35	577.36	132.79	0.005333454
WT	ESC	Day 4	HoxA3	11.96	919.88	76.93	0.008930133
WT	ESC	Day 4	HoxA4	4.35	930.14	213.92	0.002794114
WT	ESC	Day 4	HoxA5	10.87	5659.81	520.68	0.000669904
WT	ESC	Day 4	HoxA6	4.35	77.94	17.93	0.078501127
WT	ESC	Day 4	HoxA7	28.26	42.05	1.49	0.788431222
WT	ESC	Day 4	HoxA9	5.43	9.23	1.70	0.79814746
WT	ESC	Day 4	HoxA10	11.87	1.00	0.08	0.124951383
WT	ESC	Day 4	HoxA11	17.30	1.00	0.06	0.125101718
WT	ESC	Day 4	HoxA13	3.17	1.00	0.32	0.81619525

TABLE S2

sequence	score	mismatches	UCSC gene	Name
GGGACTCCAGCTCCACCCGCCGG	**1.4**	**3MMs [1:8:12]**	**NM_001081373**	**off-target.1**
CGGCCCTGAGCGCCACCCGCTGG	**1**	**3MMs [4:6:7]**	**NM_153075**	**off-target.2**
GGAACTCTAGCGCCACCCTCAGG	**0.5**	**4MMs [1:3:8:19]**		**off-target.3**
AAGACTTGAGCACCACCCGCAGG	0.5	4MMs [1:2:7:12]		
CTGACTCGGGCGCCACCAGCTGG	**0.4**	**3MMs [2:9:18]**	**NM_021293**	**off-target.4**
GGGACTAGATCGCCACCCACGAG	0.3	4MMs [1:7:10:19]		
TGGACTCCAGAGCCACCCTCCAG	0.3	4MMs [1:8:11:19]		
CGGTCTGCAGCGCCTCCCGCCGG	0.3	4MMs [4:7:8:15]		
CAGGCTCCAGCGCCAGCCGCAGG	0.3	4MMs [2:4:8:16]		
CACAGTCGAGCGCCAACCGCTAG	0.3	4MMs [2:3:5:16]	NM_172832	
CGCACACCAGCGCCCCCCGCAGG	0.2	4MMs [3:6:8:15]		
CCGACTCGGTCGCCTCCCGCTGG	**0.2**	**4MMs [2:9:10:15]**	**NM_172903**	**off-target.5**
GGGACTGCAGCGCCACCTGCTGG	0.2	4MMs [1:7:8:18]		
CCGACTGGAGGGCCACCCTCAAG	0.2	4MMs [2:7:11:19]	NM_023196	
CAGGCTCGGGCGCCACCTGCTGG	0.2	4MMs [2:4:9:18]	NM_018771	
CGGACTCCTGCCCCACCCGAAGG	0.2	4MMs [8:9:12:20]		
TGGACTCTAGTGCCACCTGCAGG	0.2	4MMs [1:8:11:18]		
TGGACTCCAGCGTCTCCCGCGGG	0.2	4MMs [1:8:13:15]		
AGGACTCTAGCTCCACCTGCTAG	0.2	4MMs [1:8:12:18]		
CGGACCACAGCGCCACCTGCTGG	0.1	4MMs [6:7:8:18]		
CGGACGGGAGCGTCACCCGACAG	0.1	4MMs [6:7:13:20]	NM_018858	
CCGACTCGGGCACCACCAGCTGG	0.1	4MMs [2:9:12:18]		
GGGAGTCGAGCGCCAACCTCGGG	0.1	4MMs [1:5:16:19]	NM_001025426	
CGGCCTGGAGCGACAGCCGCAGG	**0.1**	**4MMs [4:7:13:16]**	**NM_011488**	**off-target.6**

***gRNA used to target C5|6 had a score of 92**

***Green off-target candidates were tested. None showed mutation by PCR based genotyping.**

TABLE S3

Oligos, Antibodies and Plasmids

RT-qPCR oligos (Figure S5)

Gene Name	Forward Sequence	Reverse Sequence
HoxA1	ACCAAGAAGCCTGTCGTTCC	TAGCCGTACTCTCCAACTTTCC
HoxA2	CCTGGATGAAGGAGAAGAAGG	GTTGGTGTACGCGGTTCTCA
HoxA3	TCAAGGCAGAACACTAAGCAGA	ATAGGTAGCGGTTGAAGTGGAA
HoxA4	TGTACCCCTGGATGAAGAAGAT	AAGACTTGCTGCCGGGTATAG
HoxA5	TGTACGTGGAAGTGTTCCTGTC	GTCACAGTTTTCGTCACAGAGC
HoxA6	ACCGACCGGAAGTACACAAG	AGGTAGCGGTTGAAGTGGAAT
HoxA7	GAAGCCAGTTTCCGCATCTAC	CTTCTCCAGTTCCAGCGTCT
HoxA9	TCCCTGACTGACTATGCTTGTG	ATCGCTTCTTCCGAGTGGAG
HoxA10	GAAGAAACGCTGCCCTTACAC	TTTCACTTGTCTGTCCGTGAG
HoxA11	CGAGAGTTCTTCTTCAGCGTCT	TGGAGCCTTAGAGAAGTGGATT
HoxA13	GCGGTGTCCATGTACTTGTC	GCTGCCCTACGGCTACTTC
HoxC4	AGCAAGCAACCCATAGTCTACC	GCGGTTGTAATGAAACTCTTTCTC
HoxC5	CACAGATTTACCCGTGGATGAC	CTTTCTCGAGTTCCAGGGTCT
HoxC6	TAGTTCTGAGCAGGGCAGGA	CGAGTTAGGTAGCGGTTGAAGT
HoxC8	GTAAATCCTCCGCCAACACTAA	CGCTTTCTGGTCAAATAAGGAT
HoxC9	GCAAGCACAAAGAGGAGAAGG	CGTCTGGTACTTGGTGTAGGG

ChIP-qPCR oligos (Figure S7)

Primer-pair Name	Forward Sequence	Reverse Sequence
A	CTCCAGCTTCTCACATCACAAC	GGCCATGCTATTTCTTCAAGTC
B	TGTGATCCACCTGAAAACACTC	GGTTTTCAACGATCTCACACAG
C	GTACTTAGGTGGCTTGGGACTG	GGAATTCTGTGTTGGAGGAGAG
D	CAAGACCTTCCTGTGACTTTCC	CAAAGGCAAGGAGAATTGAGAC
E	AGGACAGAAAAGAGGGGGATAG	CCATTTGCCTTGGAATTTTTAC
F	TCTTCTTCTTCCTCCCACAGTC	GGGAAGAGAGGGAAAGAGAGAG
G	CTTCCCTTATGCACACCTTAGC	CTTCCATTCCAAATCGTTCTTC
H	ATTGTGGTGTGCTTTTCTTGTG	TGGCTAAAATCCTTCATTCACC
HoxD	CTCTGGAGTGGGTTTTTGTTTC	CAAGGAGCTTGTTTTTCCAAAG

CRISPR gRNA oligos

gRNA Target	Forward Sequence	Reverse Sequence
C5|6	CACCGGGACTCGAGCGCCACCCGC	AAACGCGGGTGGCGCTCGAGTCCC
C7|9	CACCGGAGCCACACTGCCATCTCC	AAACGGAGATGGCAGTGTGGCTCC
YY1	CACCGTCTCGGCGGCCGGAGATGG	AAACCCATCTCCGGCCGCCGAGAC
HOXC	CACCGATTCCTCCGAGTCGGCACC	AAACGGTGCCGACTCGGAGGAATC
	*G for U6 promoter transcription initiation	
	*BbsI Overhang	*BbsI Overhang

4C-seq viewpoint oligos

gRNA Target	Forward Sequence (DpnII)	Reverse Sequence (Csp6I)
4C.HOXA5-A	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNAGTATCTGTGAATGCAGATC	CAAGCAGAAGACGGCATACGAAGGCGGGATTCTGAAGTT
4C.HOXA5-B	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNTGTTTAAGCAAACTGTGATC	CAAGCAGAAGACGGCATACGATCAAGTCACCTCTACAACAGC
4C.HOXA10	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGAGCCGTCCCTGTCTGGATC	CAAGCAGAAGACGGCATACGAGAACACAGGAGTTCCACCTA
	*Illimuna adapters	
	*Barcode	

Genotyping primers

Name	Forward Sequence (DpnII)	Reverse Sequence (Csp6I)
HoxA.Δ5|6.geno	CACCCTTGCACAATTTATGATGA	GGATACAAAGCCGGGGAAATAA
HoxA.Δ7|9.geno	AGGACTGTGACCCTGAAATGG	GCCTCCGATAAAGAGACTGC
HoxC.Δ5|6.geno	GGCACTATTCGTTTCAAAAACC	TCTTTATCCTATGCGCTCTTCC
off_target.1	CCTATGAAATGCAGCAGAAATG	TTCAGTGGCTCTAGCTTCCAAC
off_target.2	GGCTGGACAAAGAATACAGACC	CAGCTGGTAACTGCCGATTG
off_target.3	AGCCGTAGGTAGTTGGTCTTTC	CGGGCAAGTTAAGTTTATCTCC
off_target.4	TTCCTGTGTCATTTTTCTGTGC	ACCTTCCTTCCCCTTTTACTTG
off_target.5	CAGTTGCGCAGGCTAAGAG	ACCTAGAGGGAGGCACTATTCC
off_target.6	CCTAGTGGTCGGTCTTGTAAGC	CTCCGAGTGGAGTGTGATAATG

Antibodies for ChIP

Antibody	Company	Catalog #	Application
H3K27me3	Abcam	ab6002	ChIP
H3K4me3	Abcam	ab8580	ChIP
Pol II	Santa Cruz Biotech	sc-899	ChIP
CTCF	Millipore	07-729	ChIP
H2AK119Ub1	Cell Signaling	8240	ChIP
EZH2	Reinberg Lab, NYU	N/A	ChIP
Isl1	Developmental Studies Hybridoma Bank	N/A	Immunocytochemistry
Lhx3	Developmental Studies Hybridoma Bank	N/A	Immunocytochemistry
Hb9	Developmental Studies Hybridoma Bank	N/A	Immunocytochemistry
B-III Tubulin	Sigma	T2200	Immunocytochemistry

CRISPR gRNA-Cas9 plasmid

SpCas9-2A-GFP vector (Addgene: PX458)