



Supporting Online Material for

The Dynamic Architecture of *Hox* Gene Clusters

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Materials and Methods

Tissue sampling, animal care and mouse strains

Tissue samples (as illustrated in Figure 1A) were isolated at day E10.5 to E10.75, with day E0.5 being noon on the day of the vaginal plug. Tissue pieces for Affymetrix gene expression analysis and 4C-sequencing were isolated in PBS and subsequently transferred to PBS supplemented with 10% Fetal Calf Serum, incubated for 45 minutes with 1 mg/ml collagenase (Sigma) and made single cell using a cell strainer (BD Falcon). Wild-type material was then divided for further use in a pool for Affymetrix gene expression analysis and a pool for 4C-sequencing. All experiments were performed in agreement with institutional guidelines and Swiss laws on animal protection.

Mutant mice used in this study were previously described: *Del(8-10)/Del(1-13)-d11LacZ* embryos were obtained by crossing heterozygous *Del(1-13)-d11LacZ* mice (18) versus heterozygous *Del(8-10)* mice (19); *Del(i8-10)/Del(1-13)-d11LacZ* embryos were obtained by crossing heterozygous *Del(1-13)-d11LacZ* mice versus heterozygous *Del(i8-10)* mice (20); Heterozygous control *Del(1-13)-d11LacZ* embryos were obtained from the two previous crosses. Genotyping using standard PCR protocols was done on material remaining after isolation of tissue samples.

Affymetrix gene expression analysis

Total RNA from tissue samples, of which a part was also used in the 4C-sequencing analysis, was isolated using Trizol LS reagent (Invitrogen) and RNeasy Mini Kit columns (Qiagen). Generation of biotinylated cRNA, slide hybridization, washing and scanning was done according to the manufacturers' instructions (Affymetrix). For each sample, two technical replicates were performed. For genes represented by multiple probe-sets, the probe-set with highest value in the posterior trunk was used.

4C-sequencing

4C-seq libraries were constructed according to the previously described 3C-on-Chip (21) protocol, with few minor adjustments. Cells were lysed using a buffer containing 50 mM Tris-HCl (pH 8), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1% Triton and Protease Inhibitor Cocktail (Roche). *Nla*III (New England Biolabs) was used as primary restriction enzyme, *Dpn*II (New England Biolabs) was used as secondary restriction enzyme. Wild-type libraries consisted of pooled tissue samples from 12 (anterior trunk and forebrain) or 24 (posterior trunk) E10.5 to E10.75 embryos. Mutant libraries consisted of a minimum of 8 E10.5 to E10.75 embryos. Random 4C-seq libraries were prepared by sequential *Nla*III and *Sau*3AI (New England Biolabs) digestion and ligation of BACs covering the mouse Hox clusters (HoxD: RP23-331E7; HoxC: RP23-430C12; HoxB: RP23-381I12 and RP23-196F5; HoxA: RP24-298M24). For each viewpoint, a total of 1 mg of each 4C-seq library was amplified using 16 individual PCR reactions with inverse primers including Illumina Solexa adapter sequences (primer sequences in Table S6, locations of restriction fragments used as viewpoints in Figure S3). For random 4C-seq libraries, a total of 1 ng BAC template substituted with 1 mg of Salmon Sperm DNA was amplified using 16 individual PCR reactions. Illumina sequencing was done on multiplexed samples, containing PCR amplified material of up to 5 viewpoints, using 76 bp Single end reads on the Illumina

Genome Analyzer system according to the manufacturer's specifications.

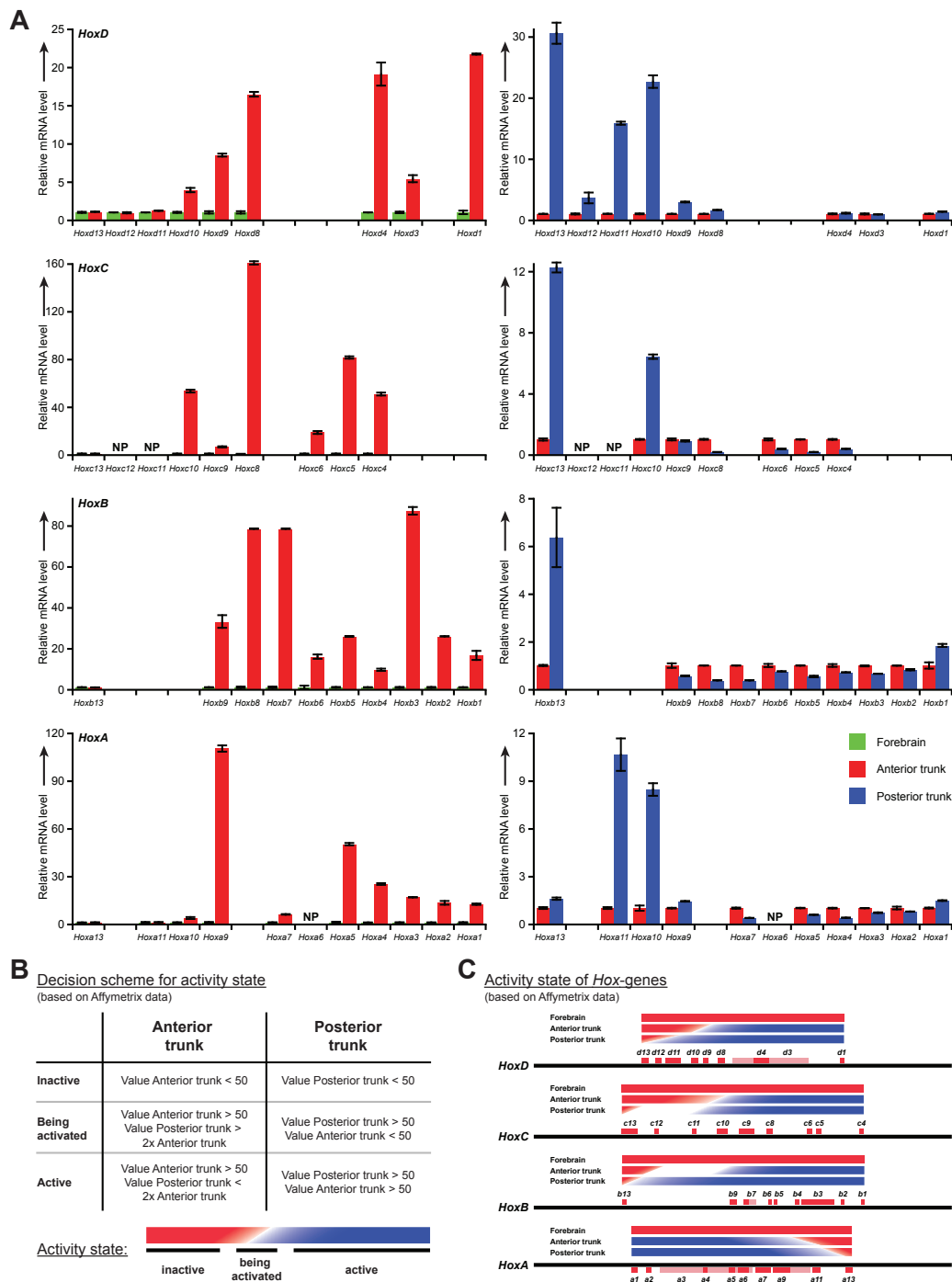
4C-sequencing reads were sorted, aligned, and translated to restriction fragments using a newly developed 4C-seq application, hosted as part of the HTSstation service by the Swiss Institute of Bioinformatics (available, including a detailed documentation, at <http://htsstation.vital-it.ch/> and outlined in Figure S2). Reads in a region directly surrounding the viewpoint were highly enriched and showed considerable experimental variation rather than biological significance, thereby severely influencing overall fragment count. To minimize these effects, the viewpoint itself, the directly neighboring 'undigested' fragment and fragments 2 kb up- and downstream were excluded during the procedure (Table S4A). The fragment counts were then normalized per one million reads. Due to different orders of magnitude in the interactions, the data were analyzed differently depending upon which kind of analysis was performed (long-range interactions with DNA regions located in cis (4 Mb) or local interactions (200-300 kb). For long-range interactions, the identification of interacting regions was done using a running mean approach with window size 29 and thresholding using randomized data (FDR = 0.05). To increase the sensitivity of the method, the domains of very high fragment count covering *Hox* clusters (local saturated domains) were removed from the analysis by fitting the data to a power-law model similar to a previously published model (22). Fragment scores in a one Mb large neighborhood around the viewpoints were fitted to the model, with up- and downstream profiles treated independently to correct for potential asymmetries. Boundaries of the local saturated domains were defined from the fit values as the first position falling below background level, as estimated from a percentile value of all scores, excluding a region 250 kb up- and downstream from each viewpoint. For each *Hox* cluster, percentile values were chosen to best fit overall domain size by visual inspection (percentile values: *HoxD*: 99%; *HoxC*: 99.5%; *HoxB*: 97.5%; *HoxA*: 97.5%; boundaries of local saturated domains in Table S5). Local interactions were studied by first normalizing the value of each restriction fragment to the value of a random digested 4C-seq library, which consisted of BACs covering the respective *Hox* clusters. Quantitative fragment count was then normalized to the total fragment count in the region covered by the random 4C-seq template. Mutant 4C-sequencing patterns were additionally normalized for the amount of deleted fragments. Quantitative log2 ratios were calculated by dividing the quantitative fragment count between tissue samples.

ChIP-sequencing

ChIP was performed according to the Millipore protocol (<http://www.millipore.com>), with three modifications: tissue samples were made single cell by collagenase treatment and applying a cell-strainer cap (BD Biosciences); cells were fixed for 5 minutes in a 2% formaldehyde solution at room temperature and were then lysed in a buffer containing 50 mM Tris HCl pH7.5, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1% Triton X-100 and 1x Complete protease inhibitors. For each ChIP assay, 10 µg of cross-linked chromatin was used. Antibodies used: anti Histone H3K27me3 (#17-622, Millipore); anti H3K4me3 (#17-614, Millipore). 6 to 10 nanograms of immune-precipitated DNA or sheared genomic DNA was prepared for Illumina sequencing according to the manufacturer's instructions. Sequencing was done using 40 bp Single end reads on the Illumina Genome Analyzer system according to the manufacturer's specifications. Reads were mapped to

the mouse genome using bowtie version 0.12.5 (23) allowing 2 mismatches and up to 5 hits per read. Duplicated reads (reads mapping at exactly the same genomic position in the same orientation) were removed as probable PCR artifacts. Genome coverage densities were calculated for each strand separately, then shifted downstream by 70 bp, averaged and normalized by the total number of mapped reads (times 10^{-7}). Enriched regions were detected using MACS version 1.4.0beta (Model-based Analysis of ChIP-seq, (24)) and selected for a p-value < 0.001 and an FDR < 0.05 .

Fig. S1.

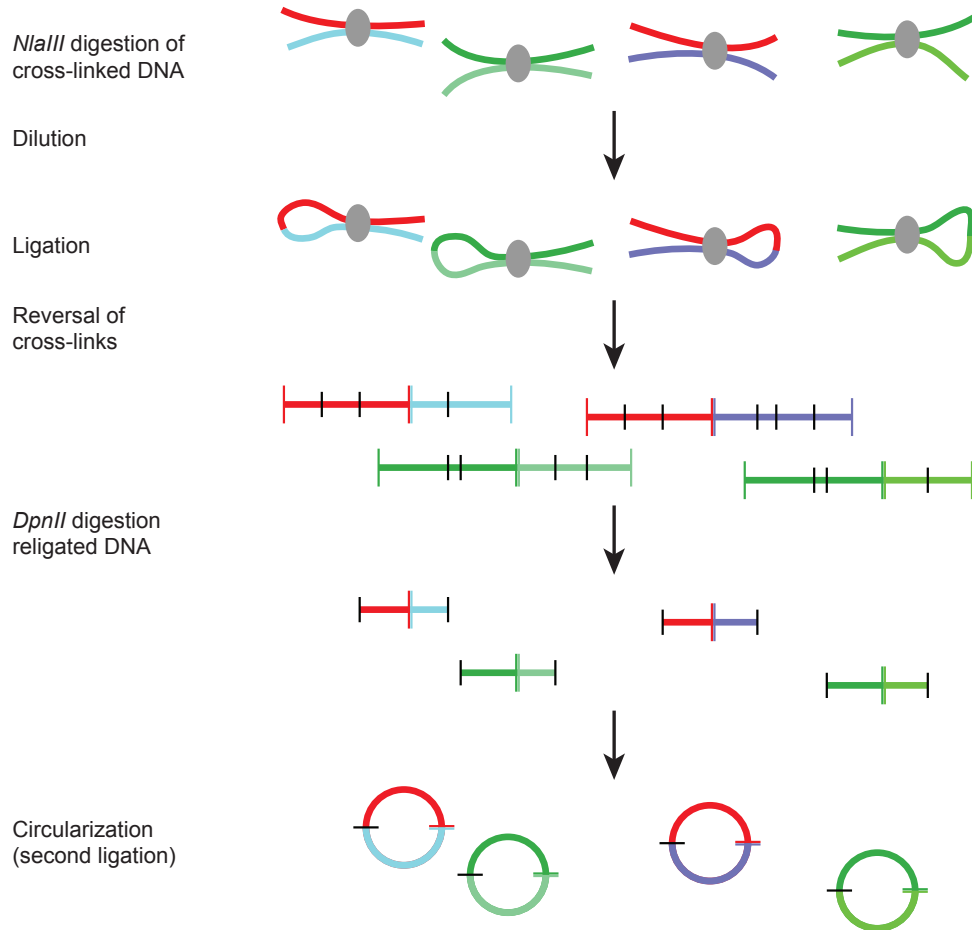


Relative mRNA levels of *Hox* genes in embryonic tissues used in this study. **A.** Affymetrix expression array values for *Hox* genes, normalized to values in forebrain (left) and anterior trunk (right). Error-bars represent standard deviations from two technical replicates. NP: not present on the expression array. **B.** Decision scheme for the determination of activity states of *Hox* genes in tissue samples. Schematic representation of activity states is illustrated below. **C.** Transcriptional activity of *Hox* genes in the various clusters in the different tissue samples.

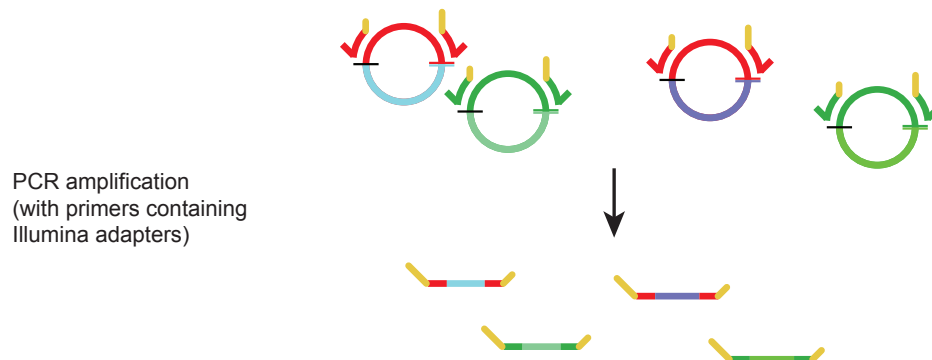
Fig. S2.

Multiplex 4C-seq (Multiplex Circular Chromosome Conformation Capture - sequencing)

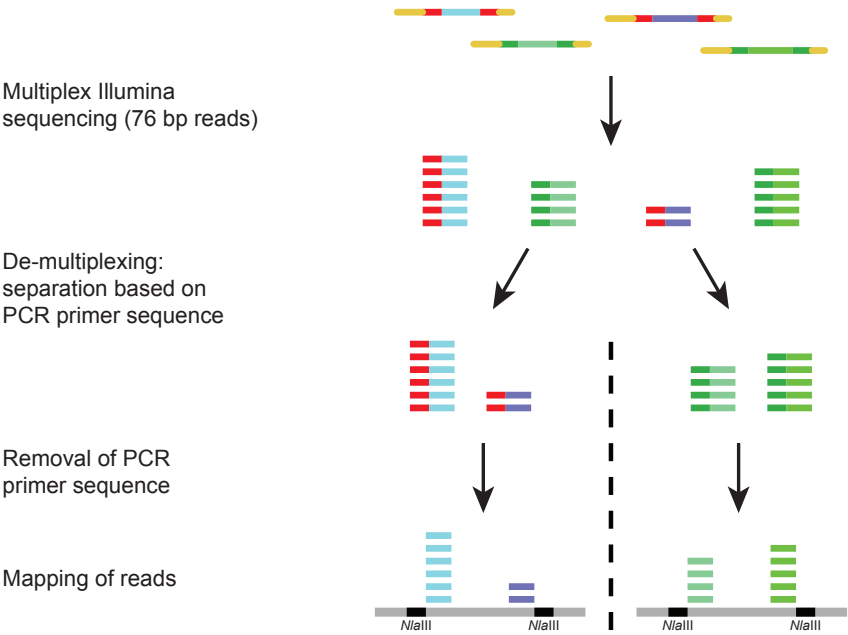
Preparation of 4C library (circular 3C library)



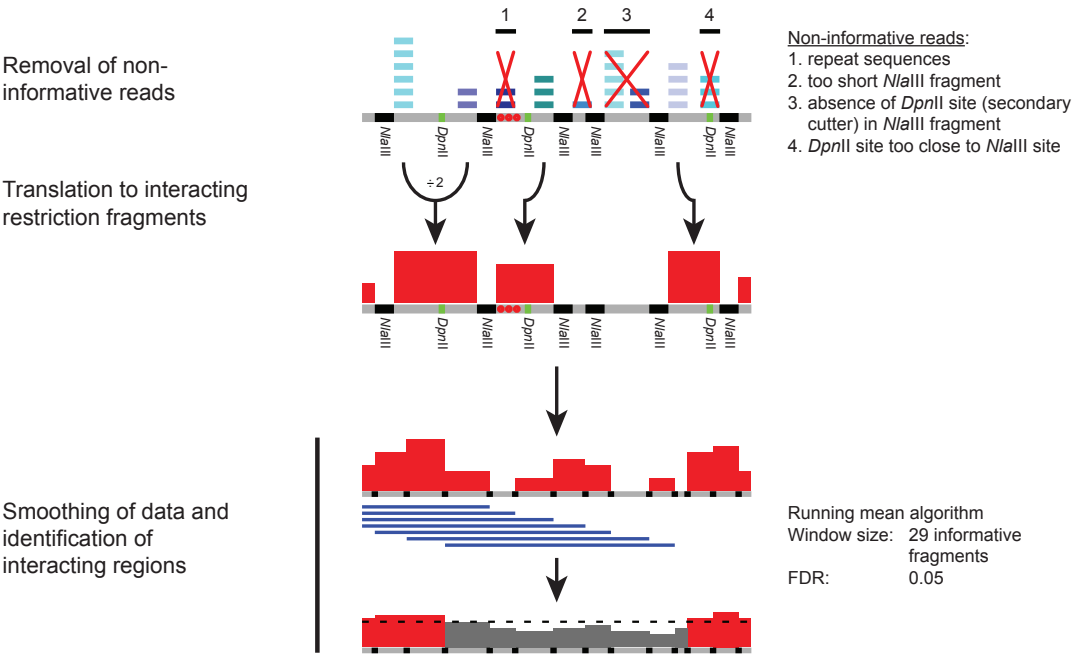
Preparation of PCR-amplified 4C-sequencing material



Mapping of Illumina (Solexa) reads



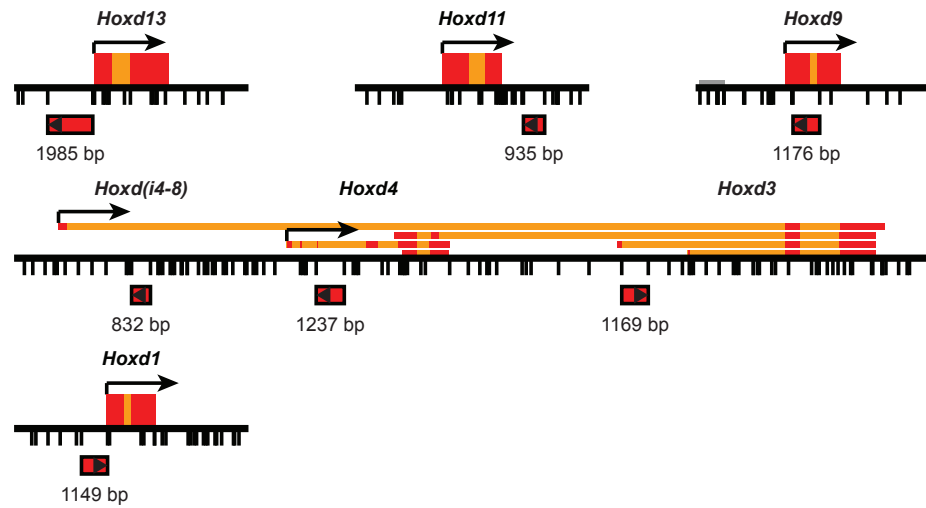
Visualization of interacting fragments and regions



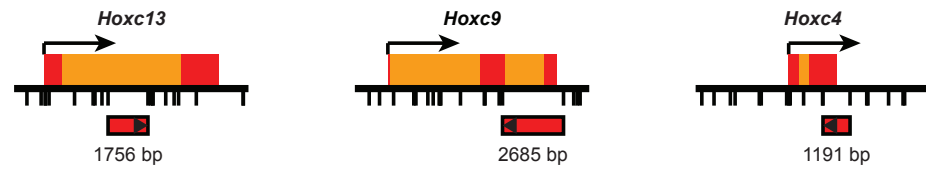
Outline of the Multiplex 4C-seq procedure and data analysis.

Fig. S3

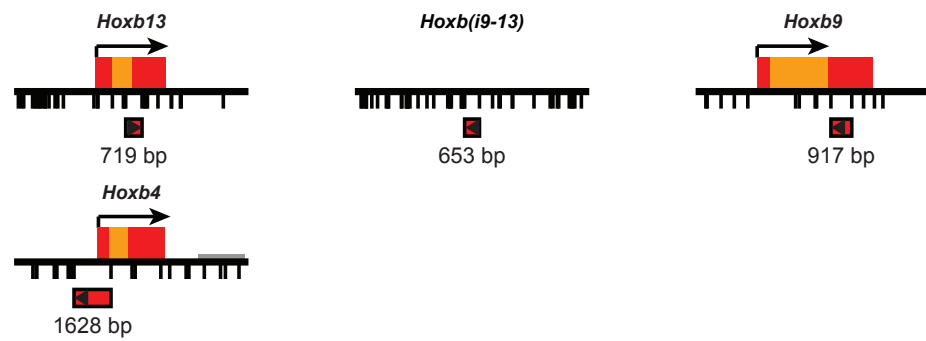
HoxD cluster



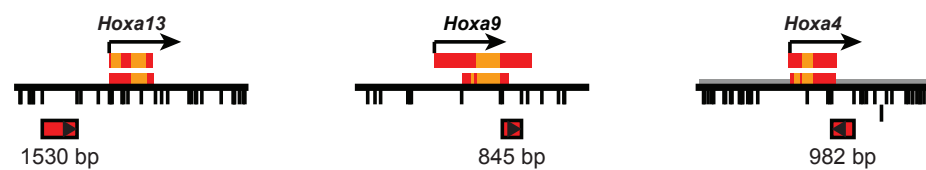
HoxC cluster



HoxB cluster



HoxA cluster

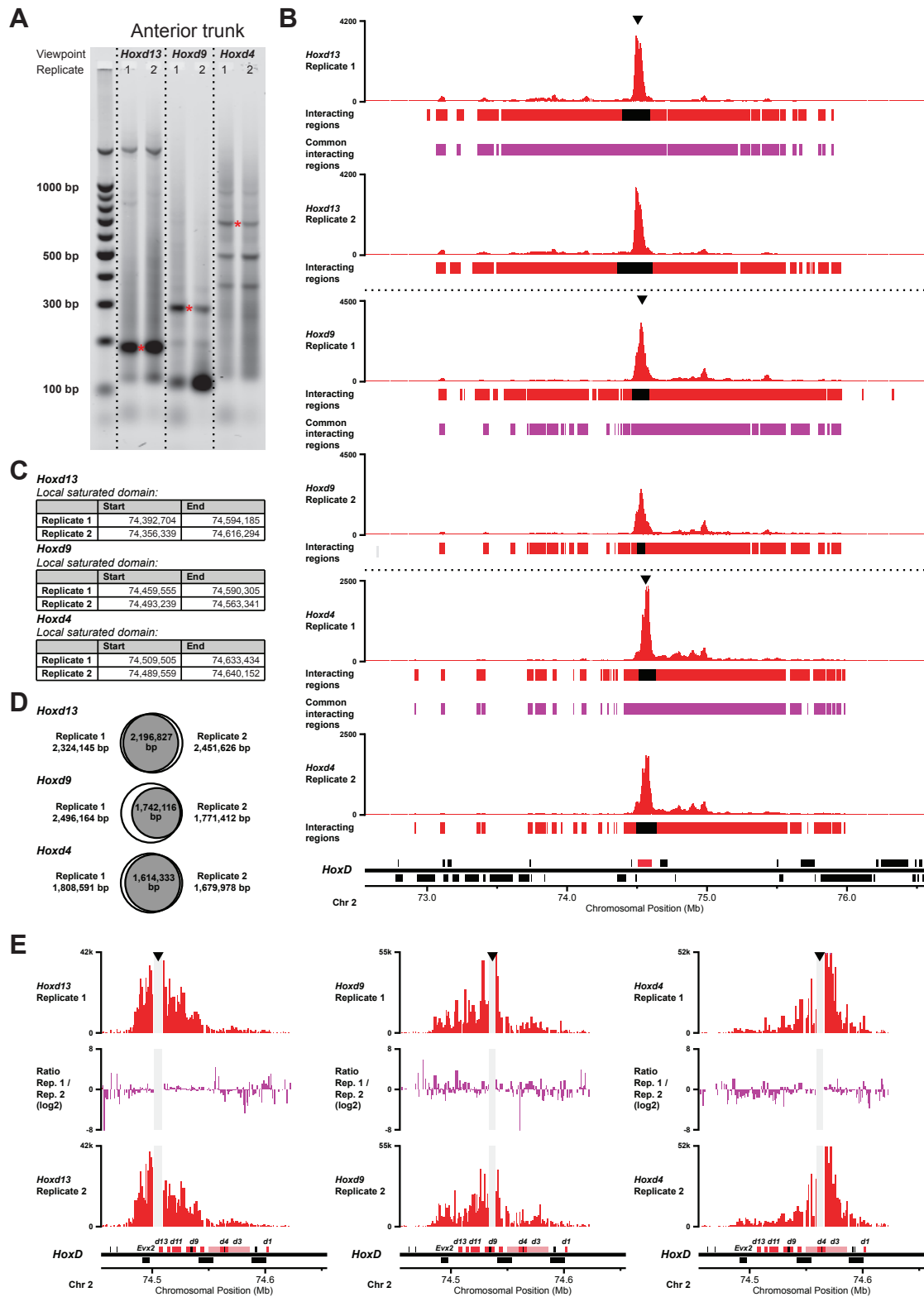


■ Exon ■ Intron ■ Other Hox-gene

5 kb

Location of the *NlaIII* restriction sites (vertical black lines) around the selected viewpoints. Restriction fragments used as viewpoints are depicted by red boxes below each region, with arrowheads indicating the orientation of the inverse forward primer used for amplification. The lengths of the viewpoints are indicated.

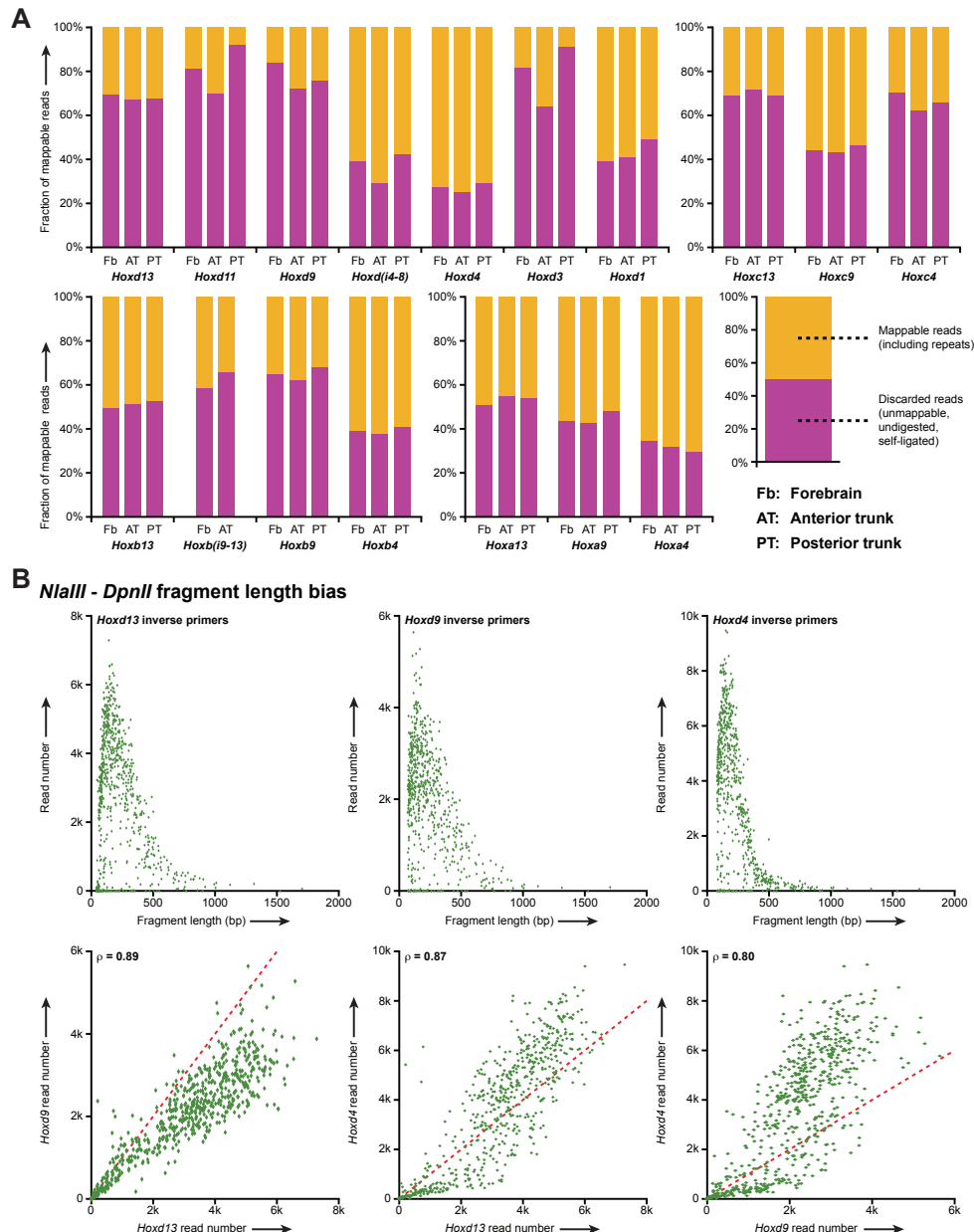
Fig. S4



Reproducibility of multiplex 4C-seq experiments. **A.** Inverse PCR amplification patterns visualized by gel electrophoresis of two independent experimental replicates showing the

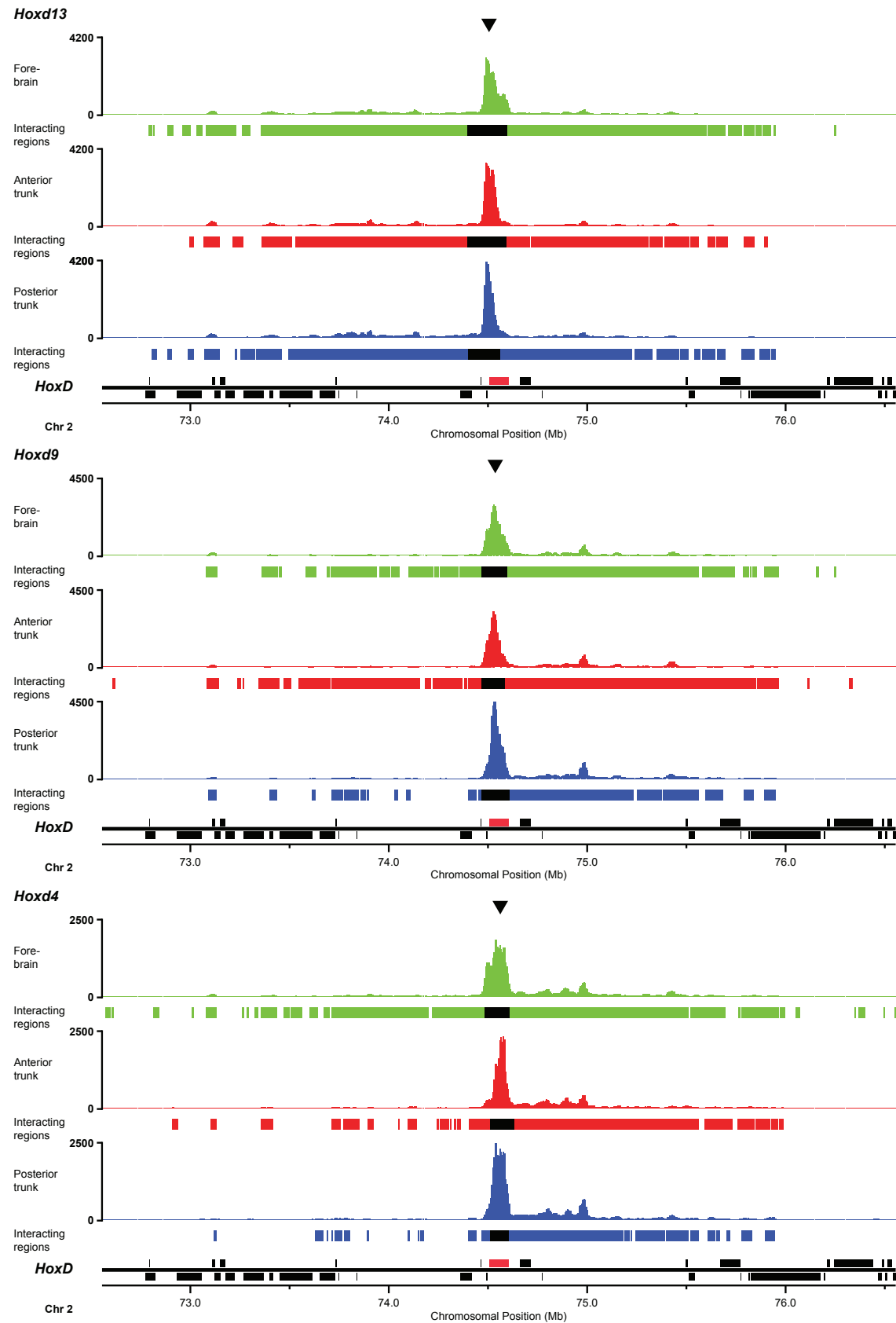
general reproducibility between the 4C-sequencing libraries. Results for three inverse PCR primer sets are given, with the predicted size of the PCR product from undigested fragments indicated by asterisks. **B.** Running-mean 4C-seq interaction patterns of three viewpoints in anterior cells for two independent samples, in a 4 Mb large genomic region surrounding the *HoxD* cluster. Significant interactions are indicated below each pattern (in red, with local saturated regions around the viewpoint depicted in black) and the interacting regions shared between the two replicate samples are shown in between (purple). Genomic coordinates, location of the *HoxD* cluster (red) and other transcriptional units (black) are depicted below. Viewpoints are indicated with arrowheads. **C.** Coordinates of local saturated domains of three viewpoints for two independent samples, as determined by fitting the data to a power-law model. **D.** Venn-diagram showing, for three viewpoints, the large overlap in interacting regions between the two independent samples within the 4 Mb large region surrounding the *HoxD* cluster. **E.** Quantitative local 4C-seq interactions patterns of three viewpoints for two independent samples in anterior trunk cells. Genomic coordinates, location of *Hox* genes (red) and other transcripts (black) are depicted below each panel. Viewpoints are indicated with arrowheads and excluded regions around these viewpoints are indicated with the light grey vertical boxes.

Fig. S5

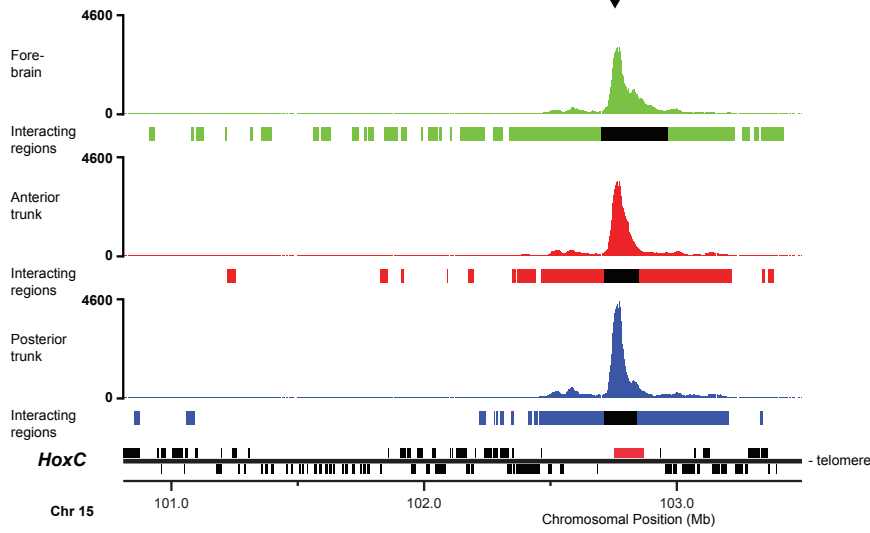


4C-seq quality control. **A.** Fraction of reads that can be mapped for each viewpoint and tissue sample. The numbers of reads are provided in Table S4B. **B.** Fragment length bias in a random template with equimolar presence of ligated fragments. Top: normalized numbers of sequence reads for three random 4C-seq BAC libraries are sorted, based on their predicted size after inverse PCR, consisting of the length of the *NlaIII*-*DpnII* fragment and the genomic sequences of the inverse primers. The numbers of sequence reads are biased depending upon the fragment length, with optimal results obtained when fragments of approximately 150 bp are used. Bottom: comparison of sequence read numbers for individual fragments amplified by different primer sets. A strong correlation is observed between datasets derived from different primer sets, indicating that the normalized sequence read numbers are largely independent from the set of primers used.

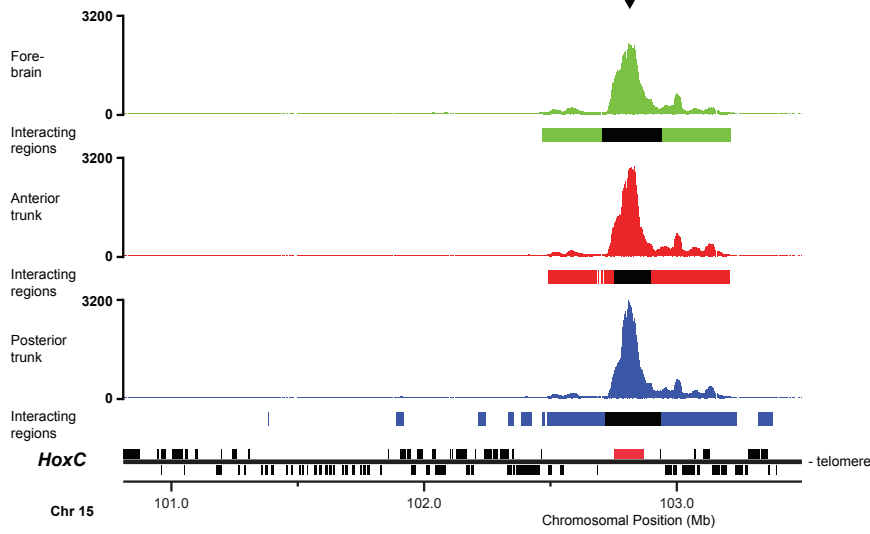
Fig. S6



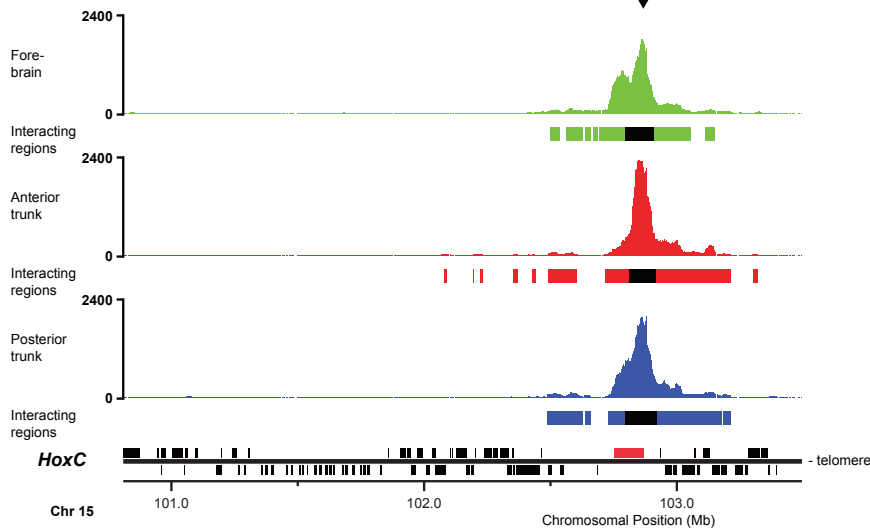
Hoxc13



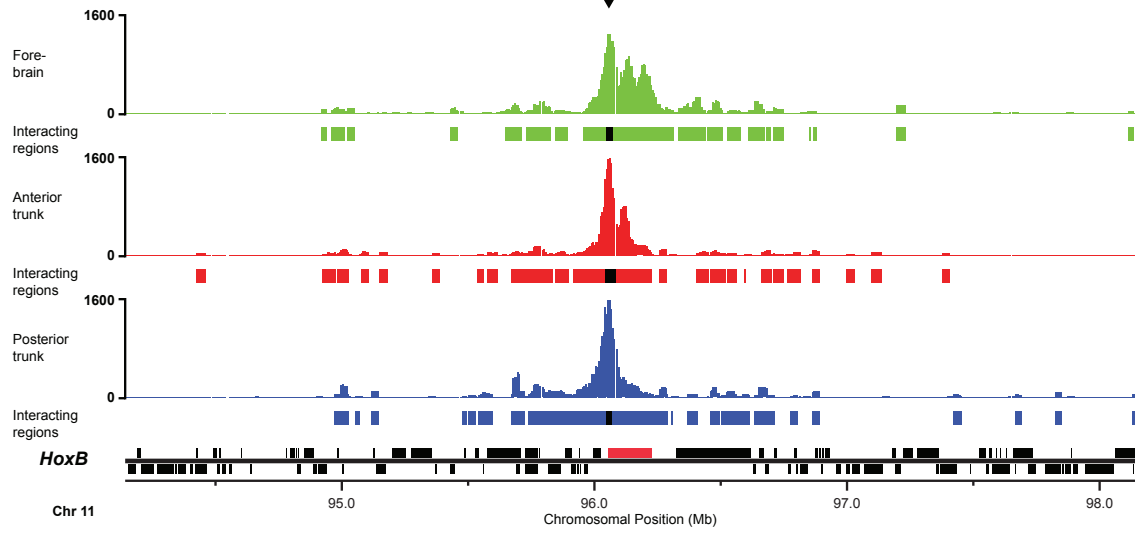
Hoxc9



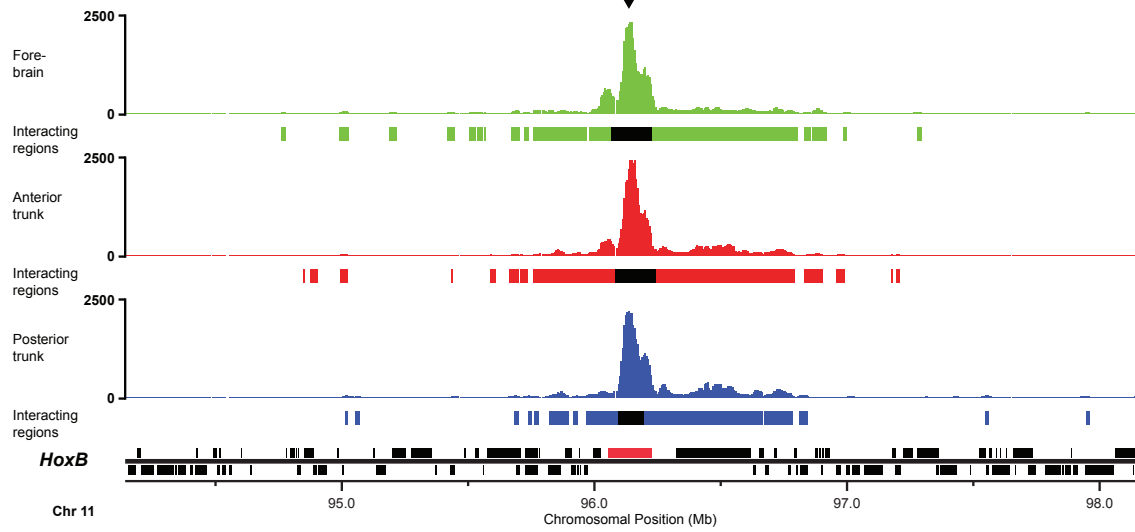
Hoxc4



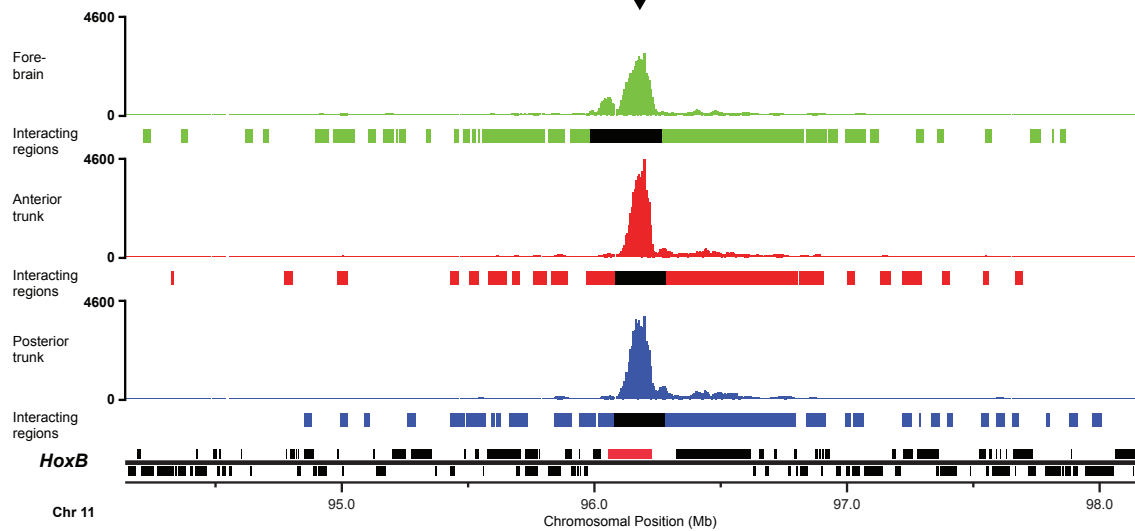
Hoxb13



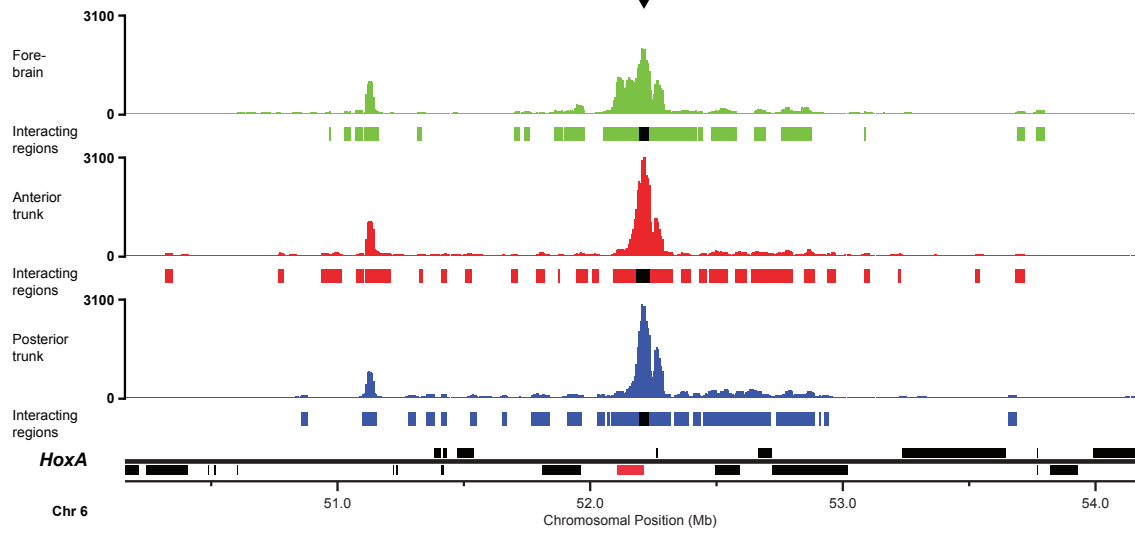
Hoxb9



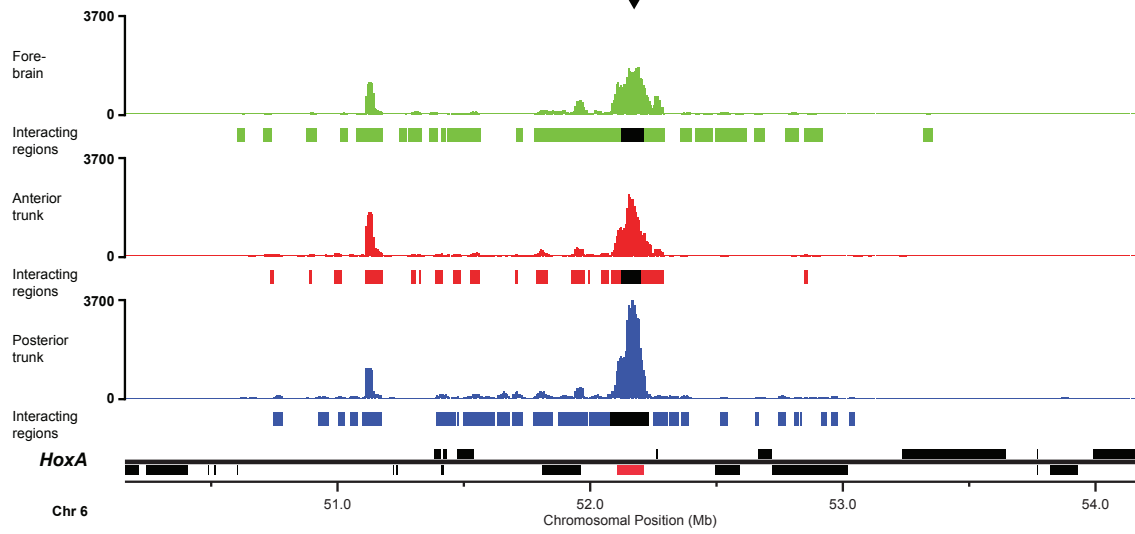
Hoxb4



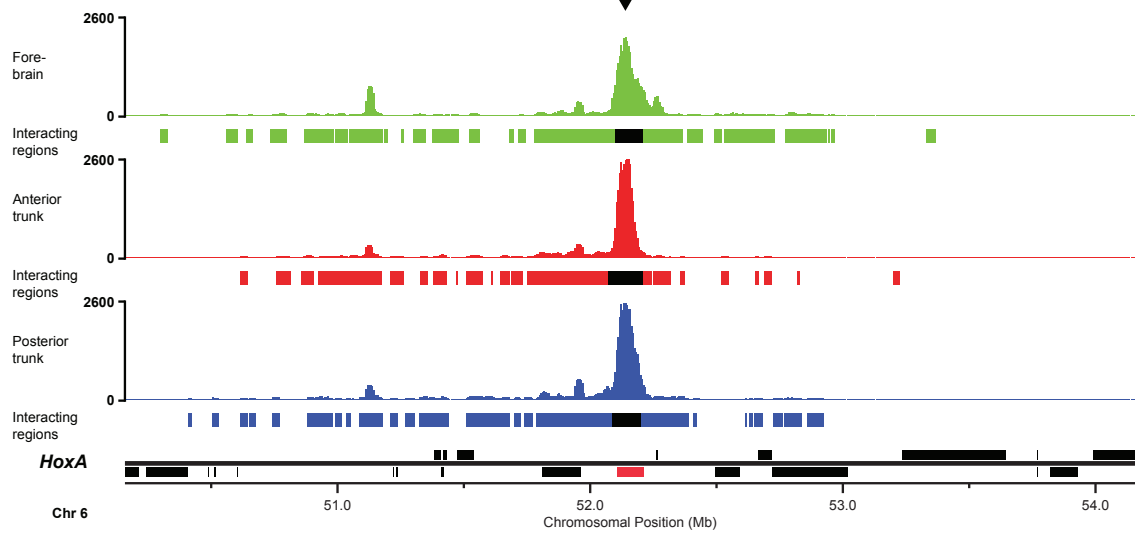
Hoxa13



Hoxa9

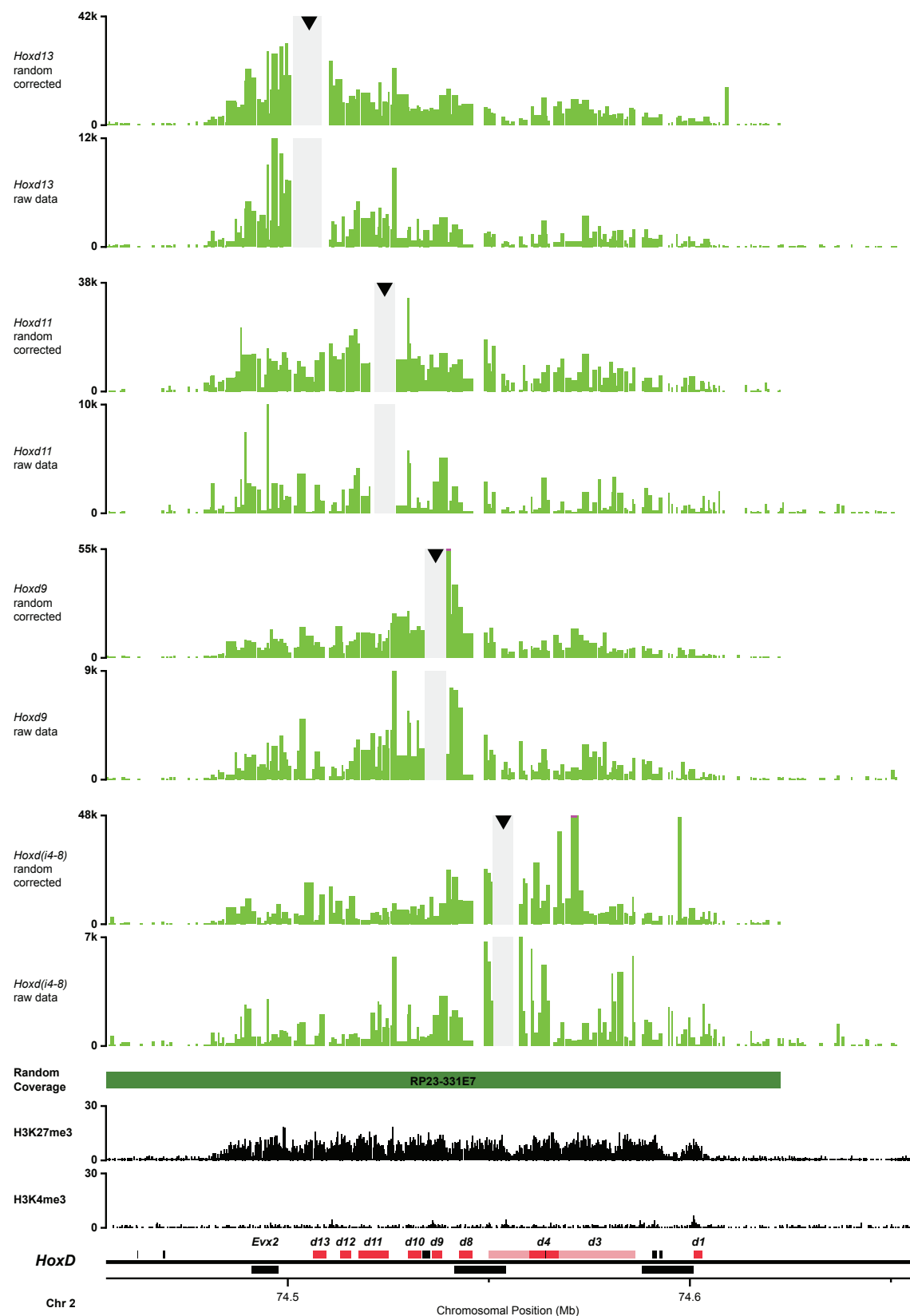


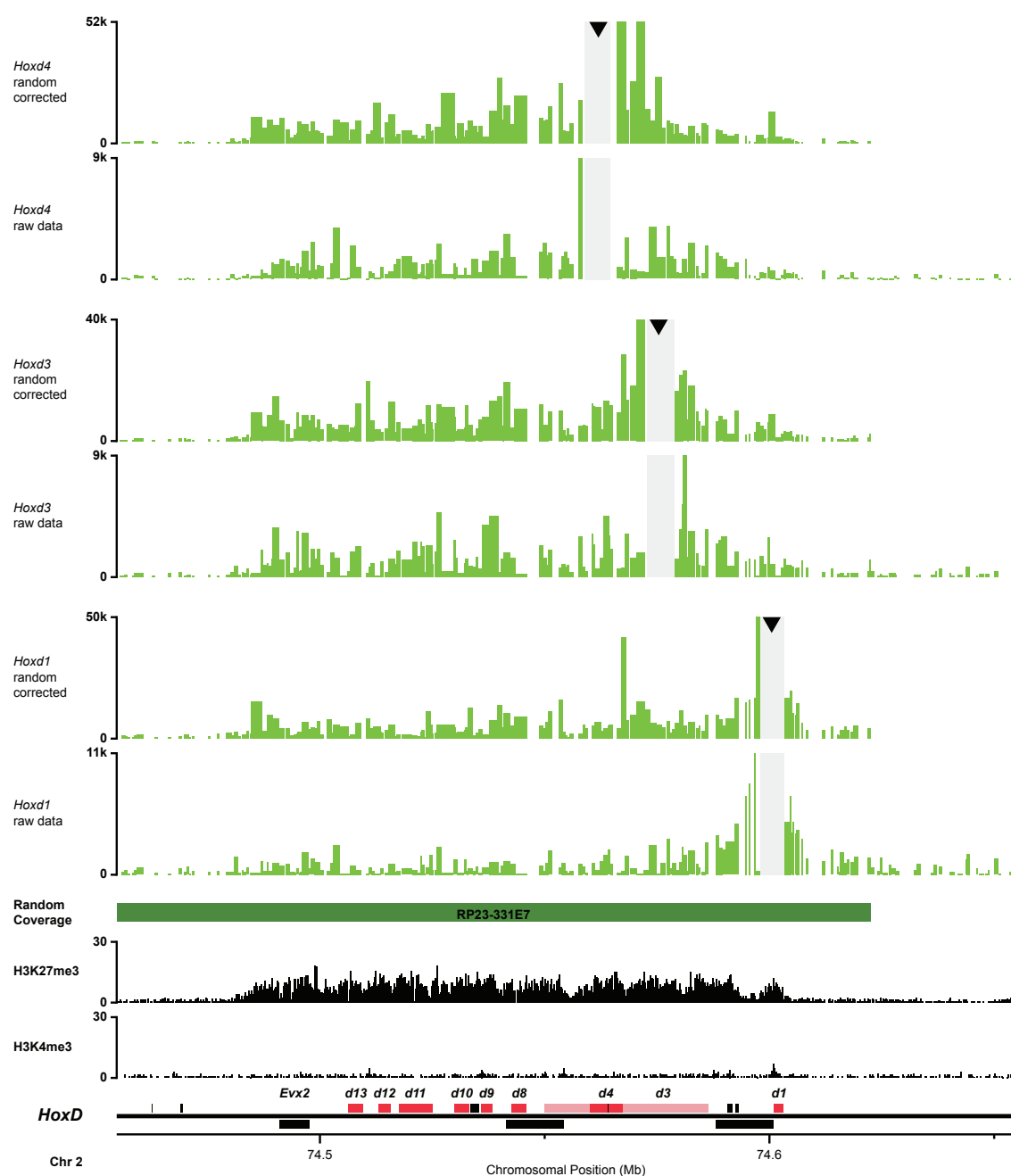
Hoxa4



Long-range contacts of *Hox* genes with DNA regions located in *cis* are primarily observed in the surrounding gene-deserts, rather than with annotated transcription units. Running-mean 4C-seq interaction patterns in 4 Mb genomic regions surrounding the *Hox* clusters, in those tissue samples used in this study. Significant interactions are indicated below each pattern, with local saturated regions around the viewpoint depicted in black. Genomic coordinates, location of *Hox* clusters (red) and other transcriptional units (black) are indicated below each panel. Viewpoints are indicated with arrowheads.

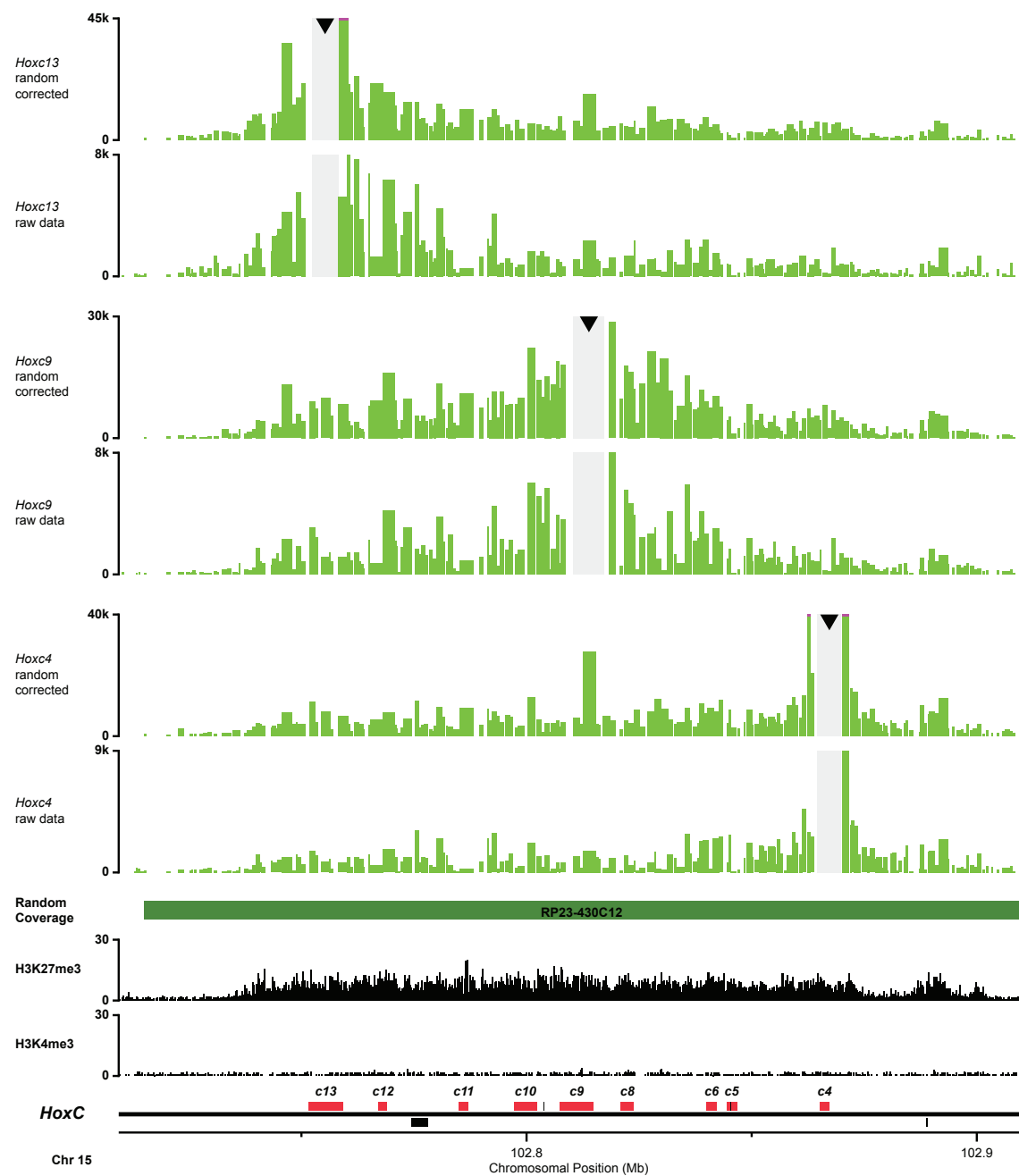
Fig. S7

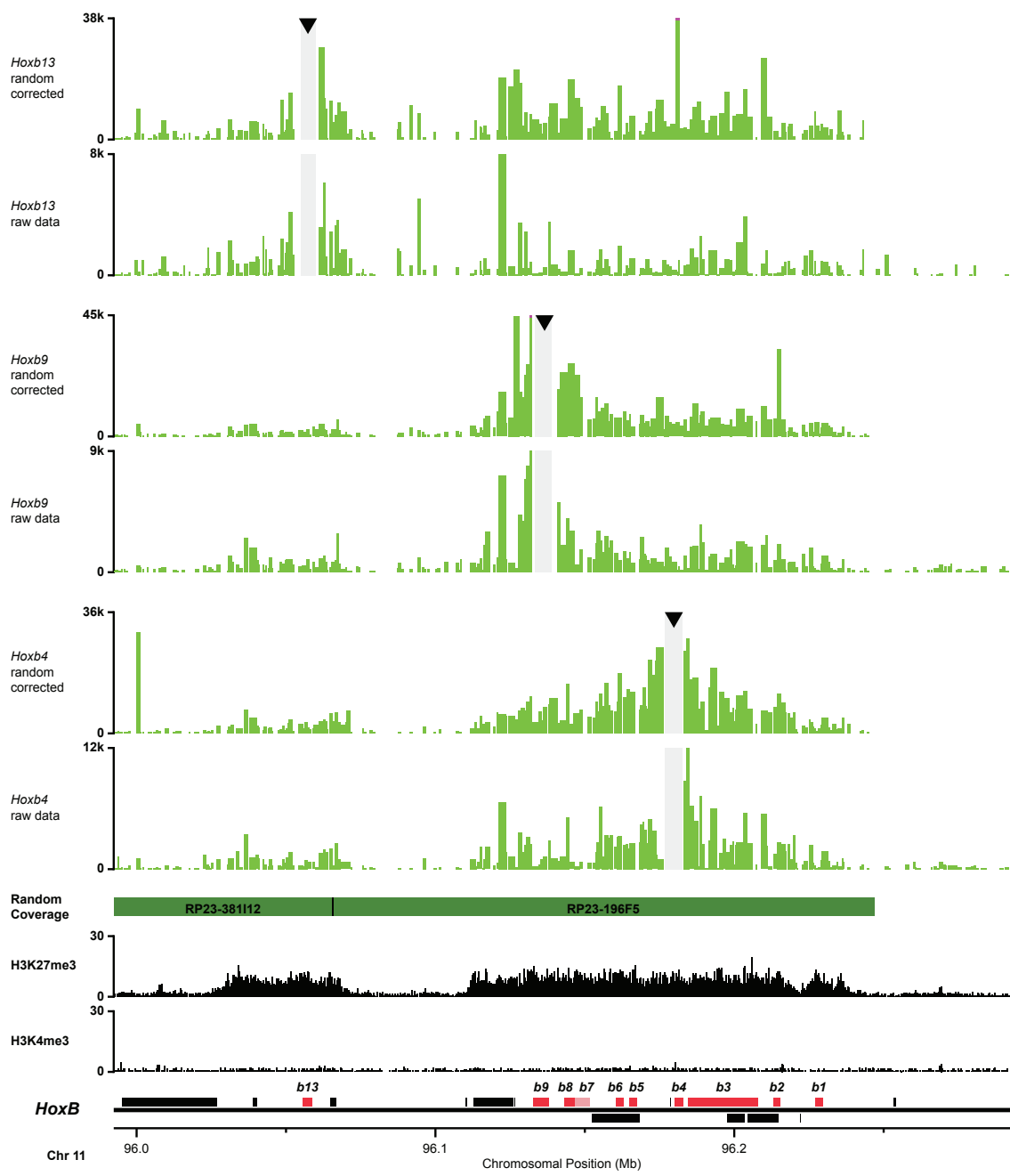


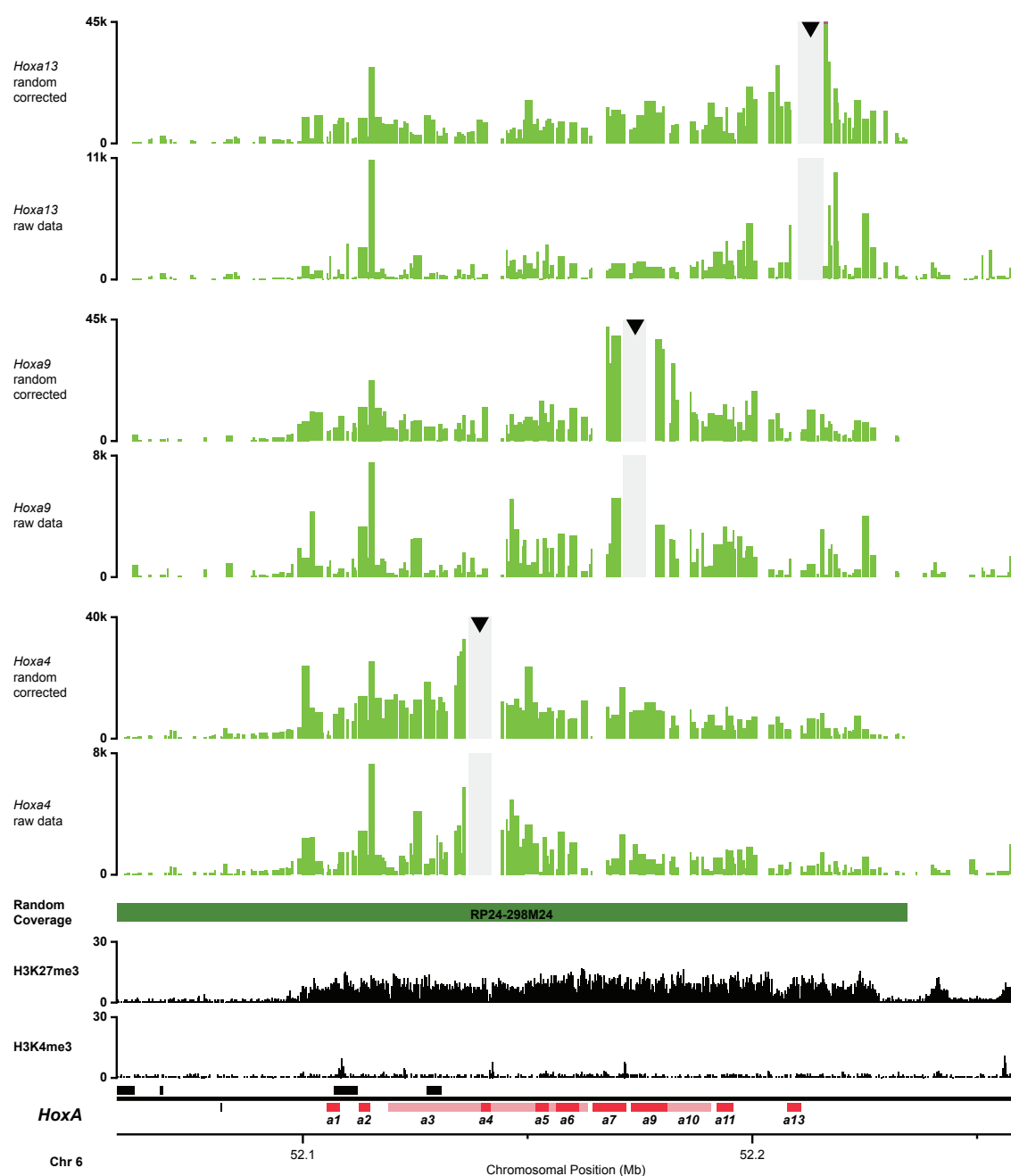


The inactive *HoxD* cluster forms a discrete 3D domain. Quantitative local 4C-seq association patterns (corrected, top; uncorrected, bottom) in forebrain (no *Hoxd* gene expressed), as seen from different viewpoints. Viewpoints are indicated with arrowheads and excluded regions around viewpoints are indicated with vertical light grey boxes. H3K27me3 and H3K4me3 ChIP-seq patterns are depicted below the 4C-seq data. Random 4C-seq template coverage, genomic coordinates, location of *Hoxd* genes (red) and other transcripts (black) are depicted below each panel.

Fig. S8



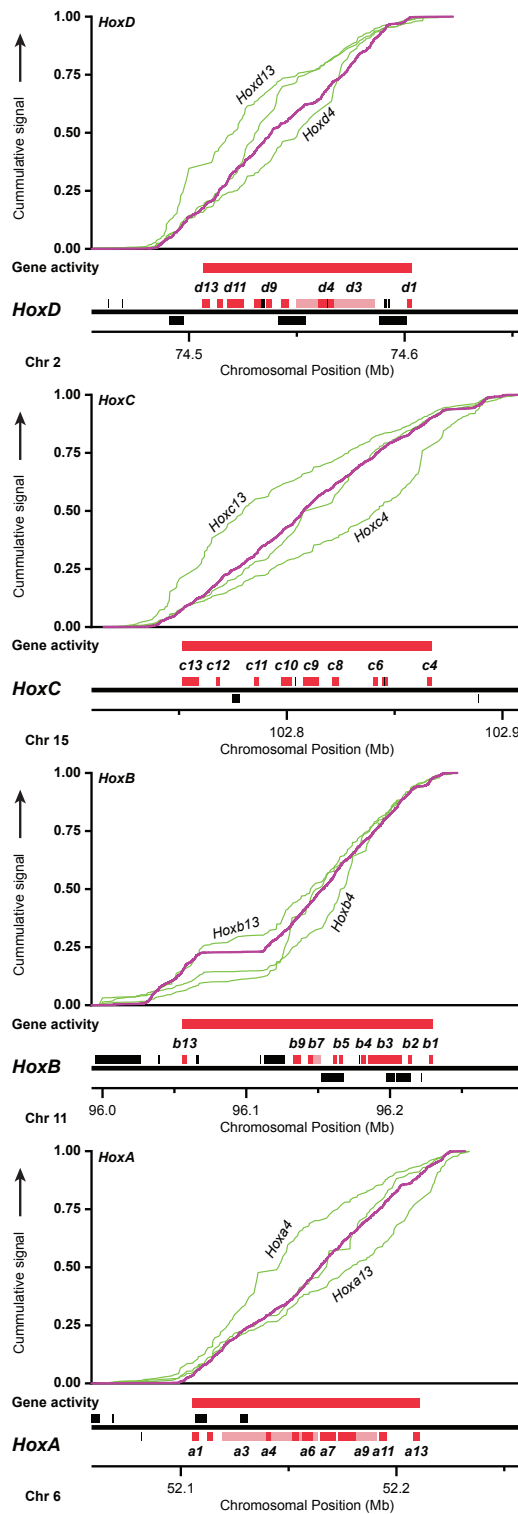




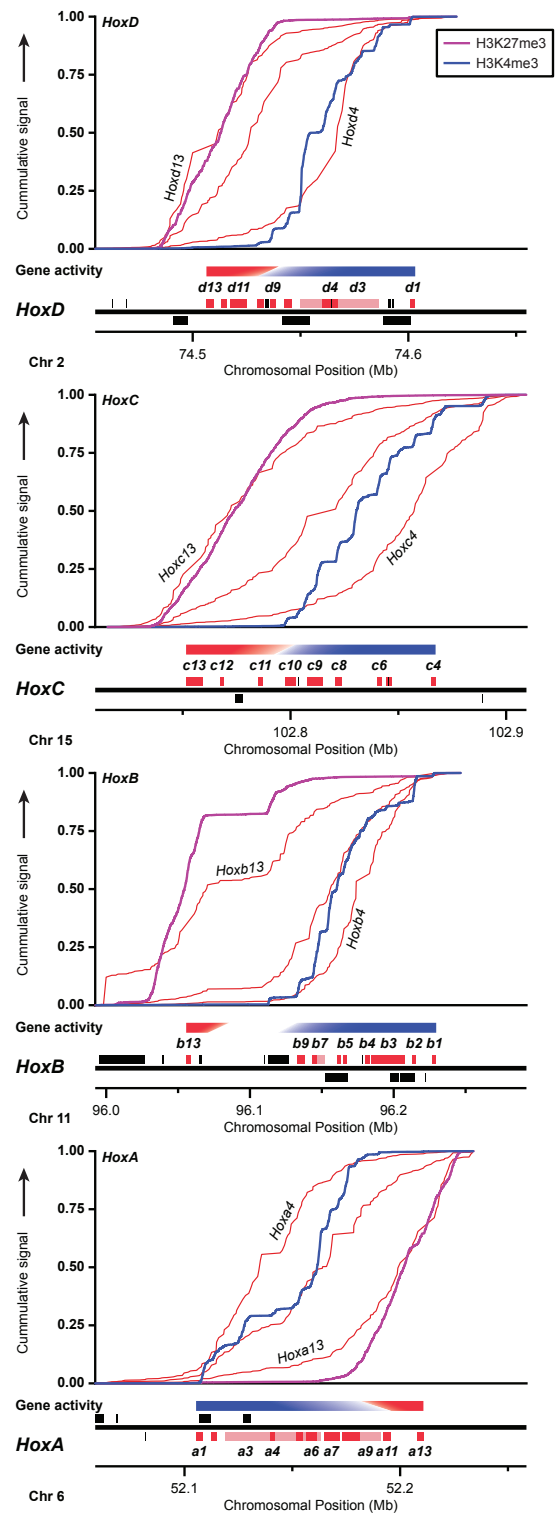
Inactive *Hox* clusters form discrete 3D domains. Quantitative local 4C-seq association patterns (corrected, top; uncorrected, bottom) in forebrain cells, using different viewpoints in the *HoxA*, *B* and *C* clusters. Viewpoints are indicated with arrowheads, and excluded regions around the viewpoints are indicated with vertical light grey boxes. H3K27me3 and H3K4me3 ChIP-seq patterns are depicted below the 4C-seq data. Random 4C-seq template coverage, genomic coordinates, location of *Hox* genes (red) and other transcripts (black) are depicted below each panel.

Fig. S9

Forebrain



Anterior trunk

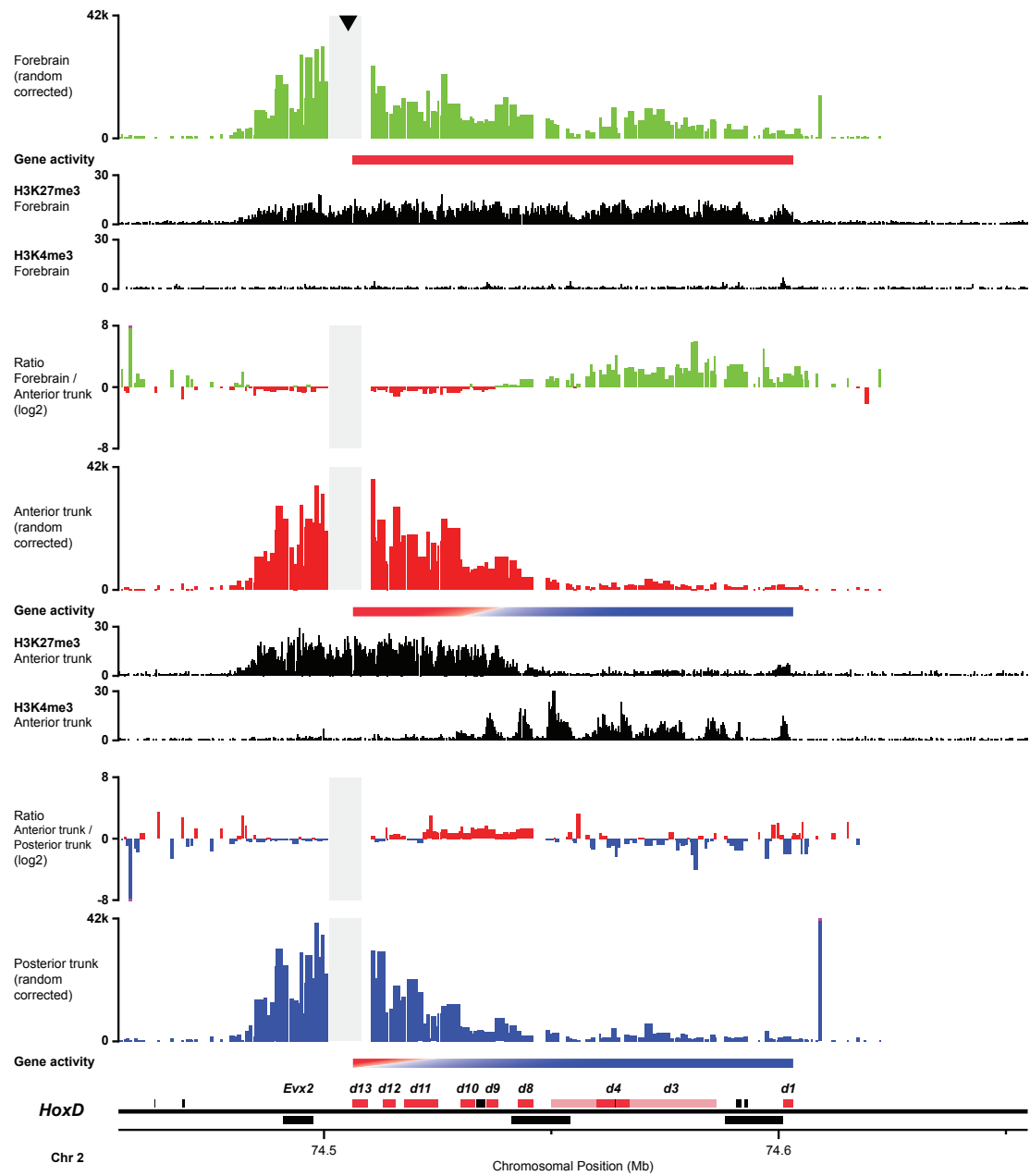


Patterns of local 3D organization coincide with domains of shared chromatin marks. Cumulative local 4C-seq association patterns (green for forebrain, red for anterior trunk) and distribution of chromatin marks (purple is H3K27me3; blue is H3K4me3) in forebrain

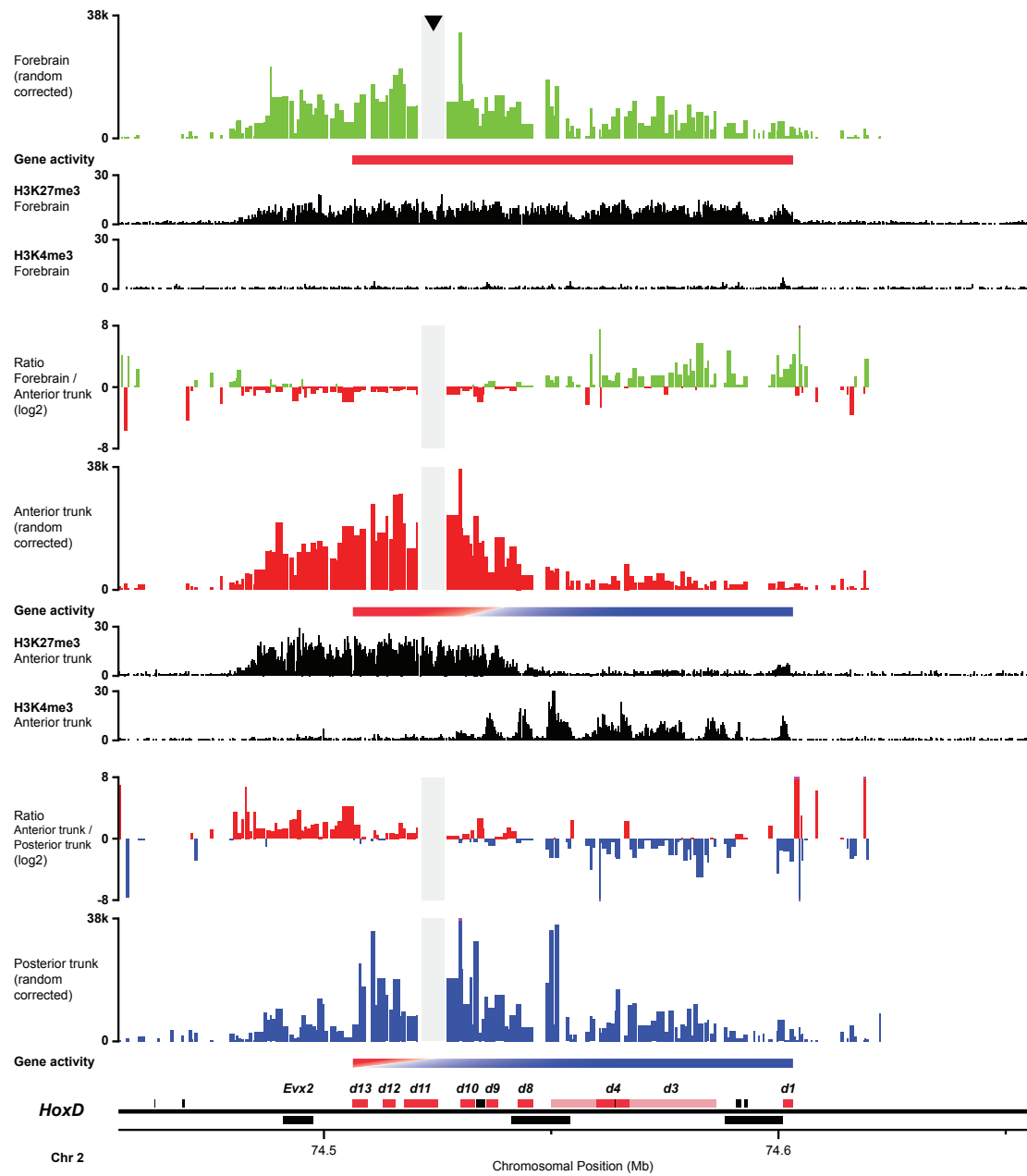
and anterior trunk cells. The expression status of a given gene is depicted below each pattern (in blue, active; in red, inactive). In forebrain, 4C-seq association patterns share similar distributions with the H3K27me3 domains, most of the variation being introduced due to the location of viewpoints. In anterior trunk, the 3D organization around the inactive genes (e.g. group 13 genes) still share similar distributions with the H3K27me3 domains, yet the active genes (e.g. group 4 genes) and to a lesser extent those genes that are shifting from an inactive to an active status (e.g. group 9 genes) adopt a 3D conformation, which now resembles the H3K4me3 domain. Genomic coordinates, location of *Hox* genes (red) and other transcripts (black) are depicted below each panel.

Fig. S10

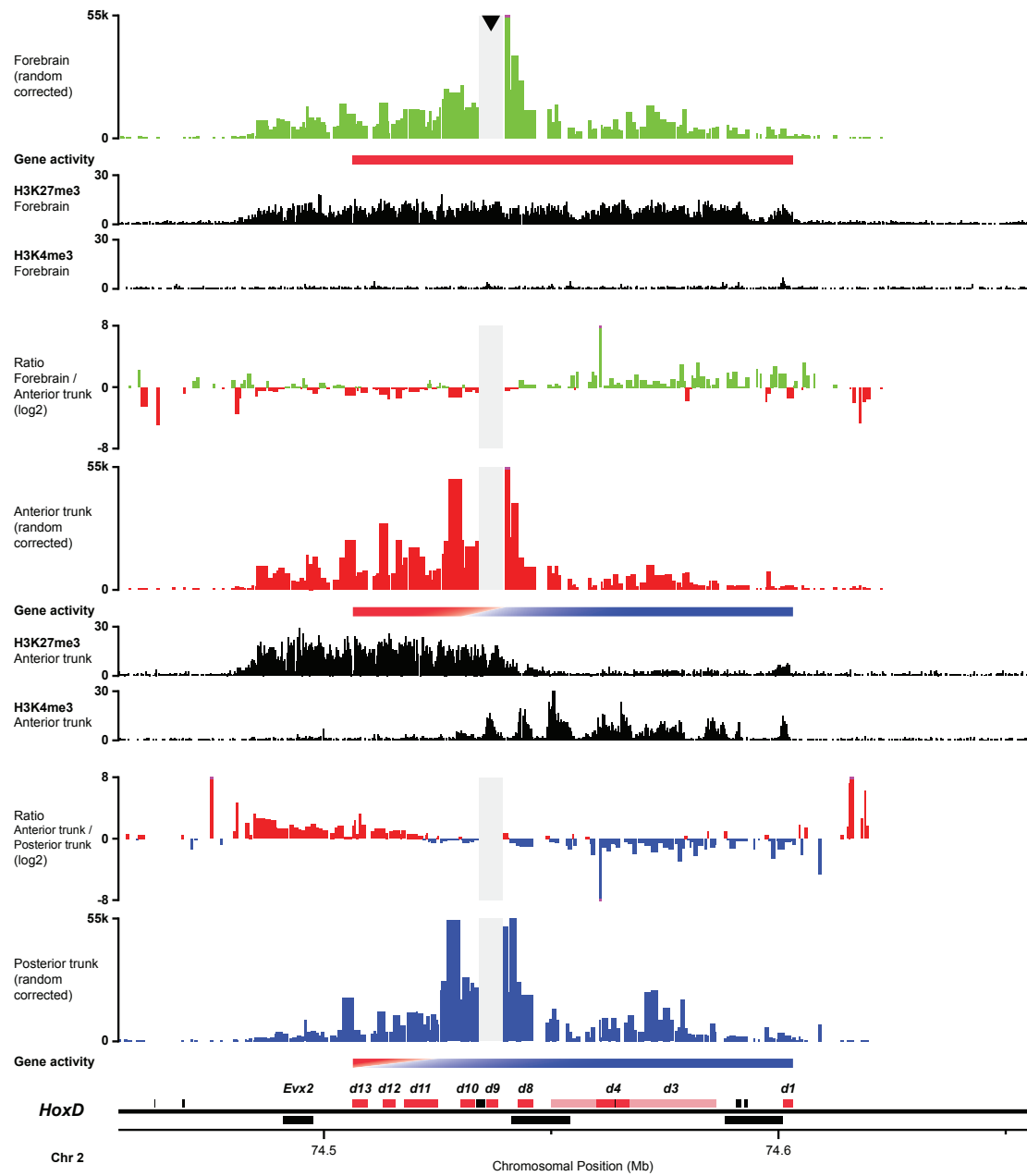
Hoxd13



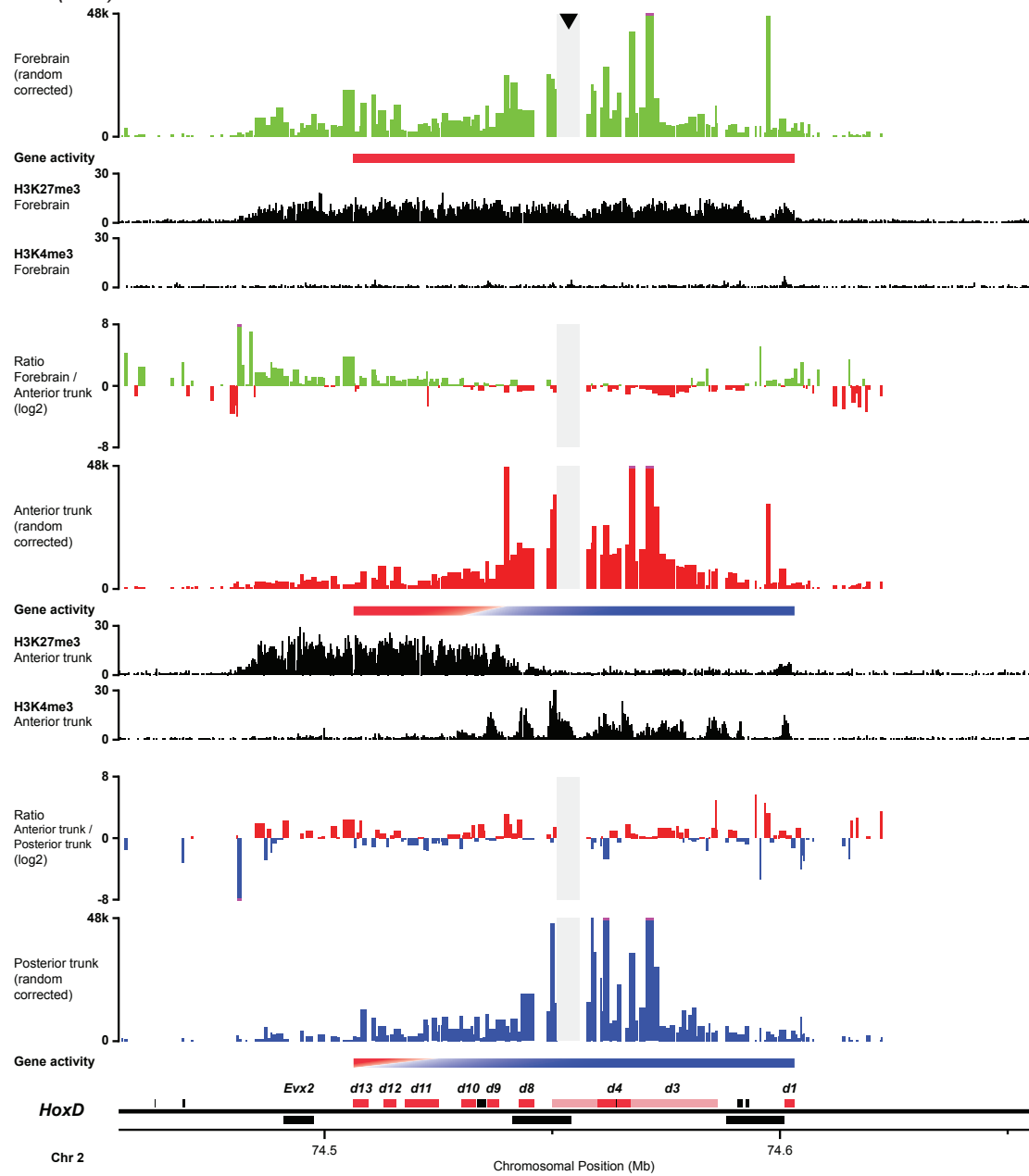
Hoxd11



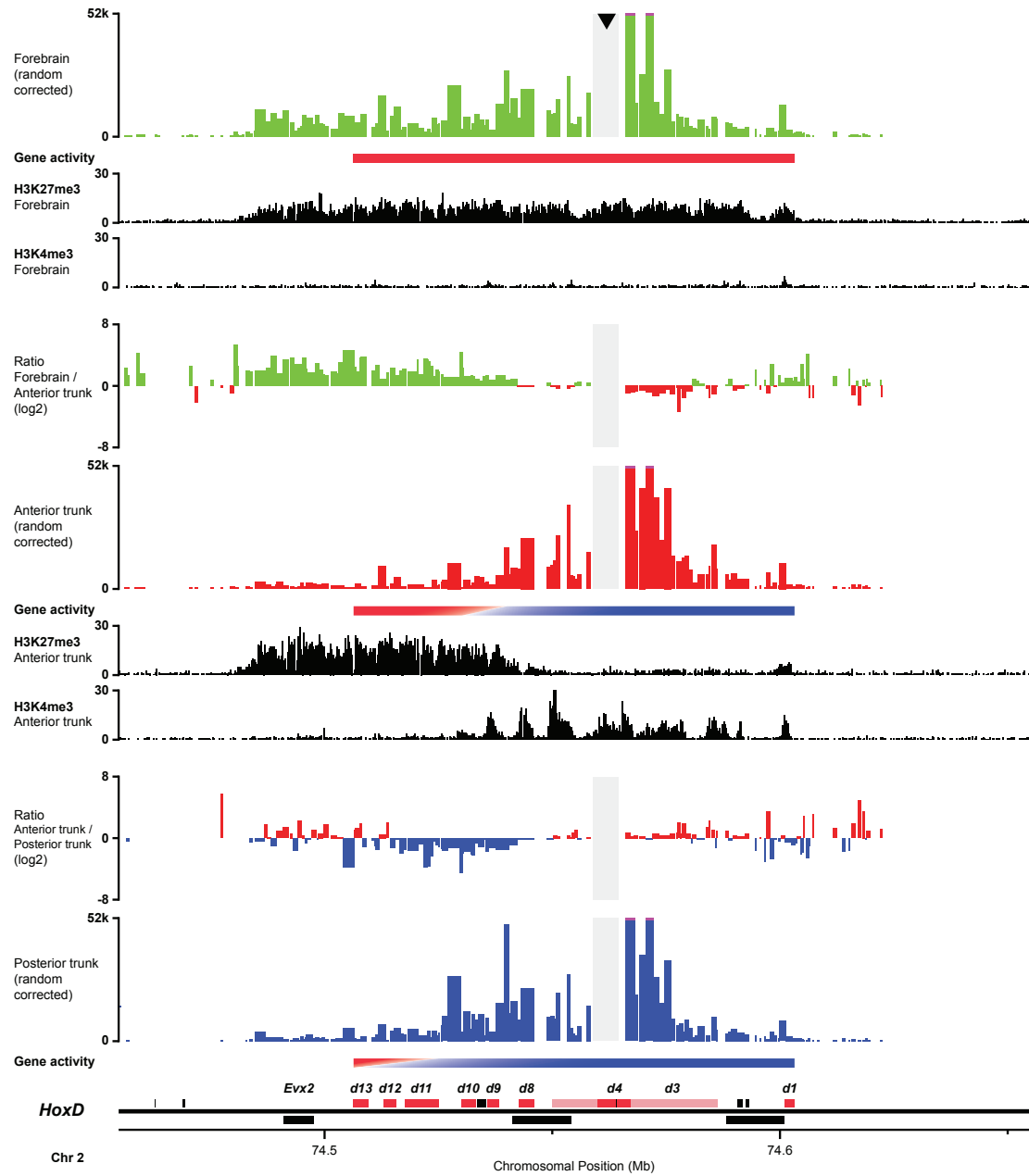
Hoxd9



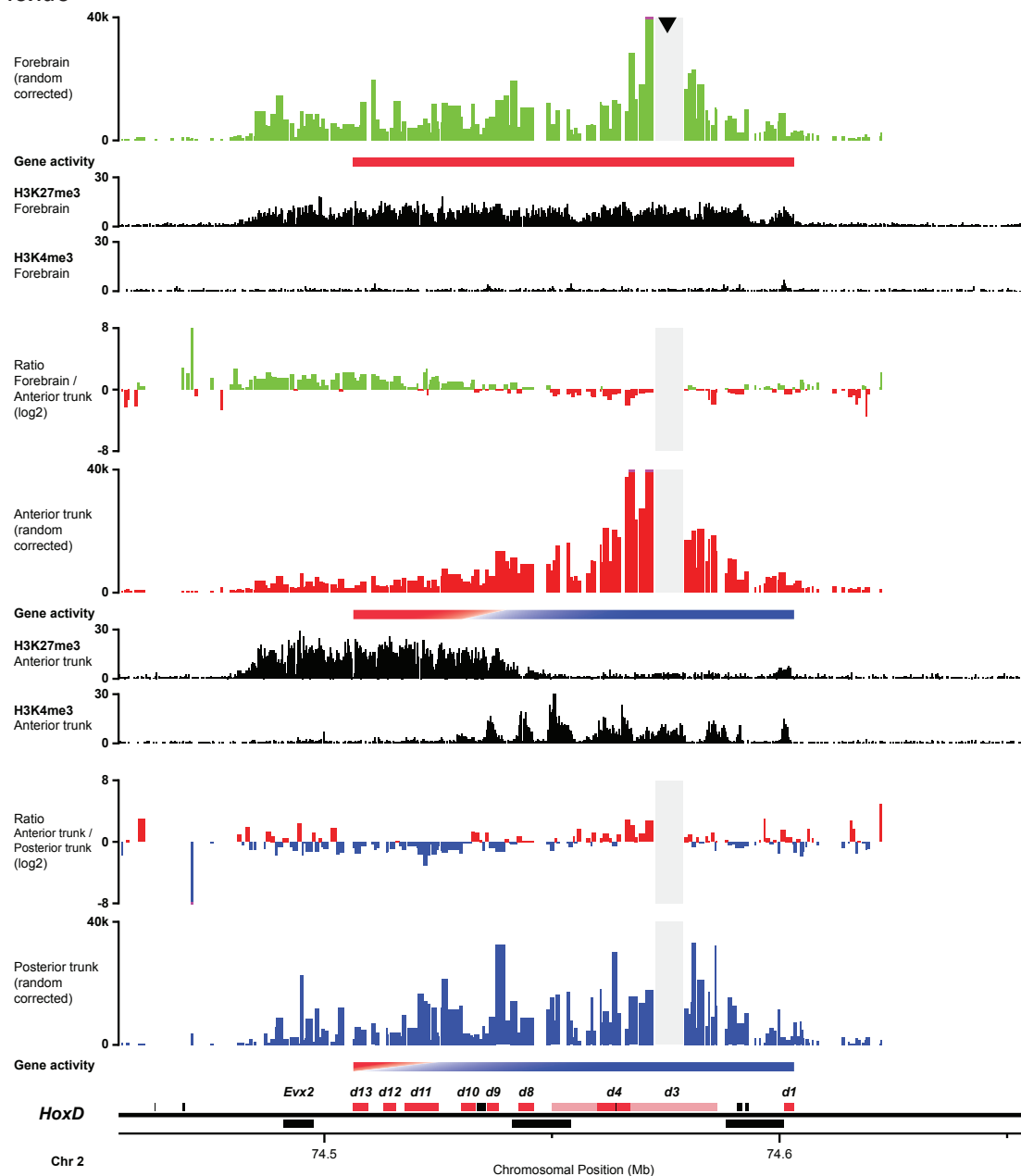
Hoxd(i4-8)

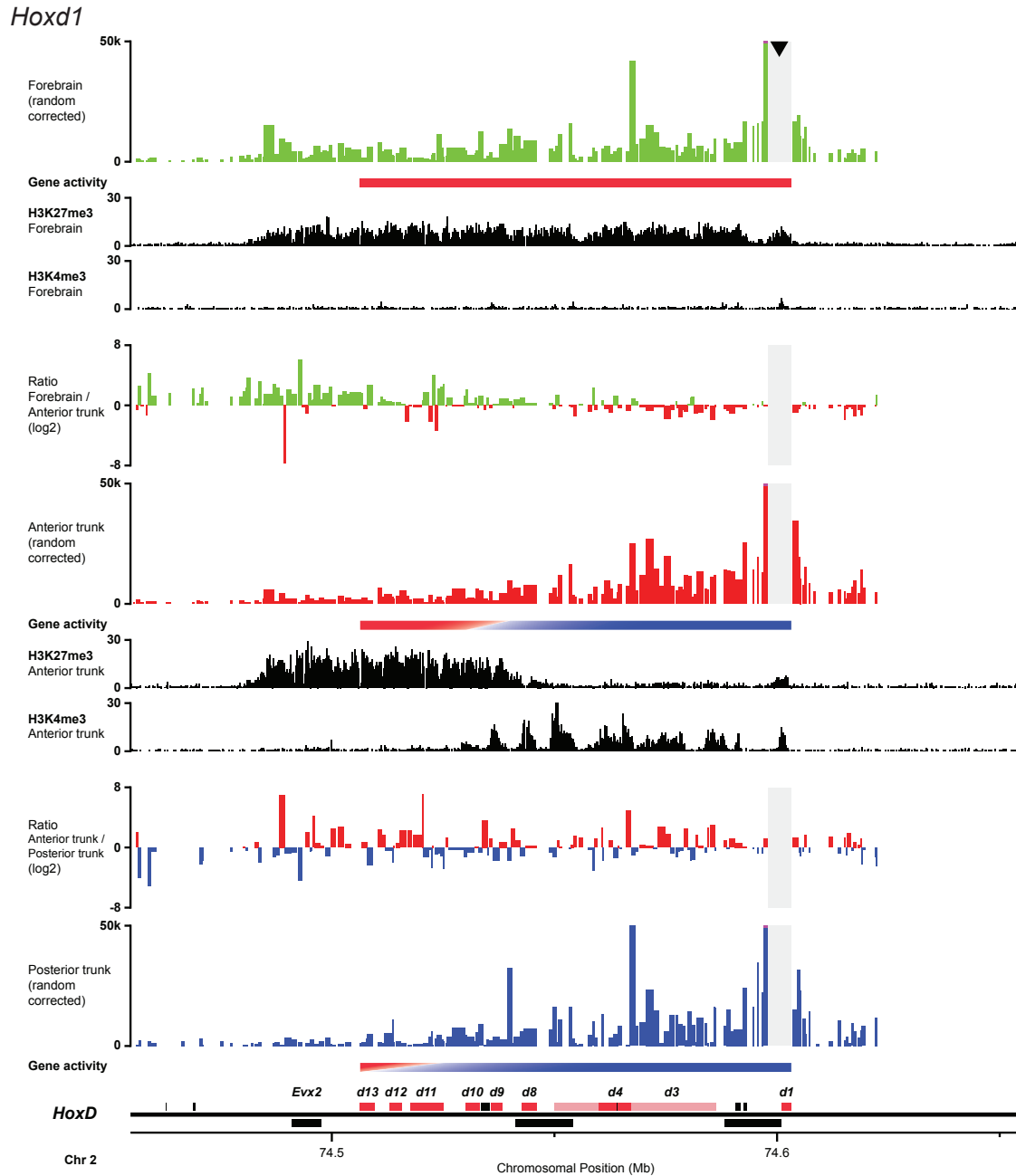


Hoxd4



Hoxd3

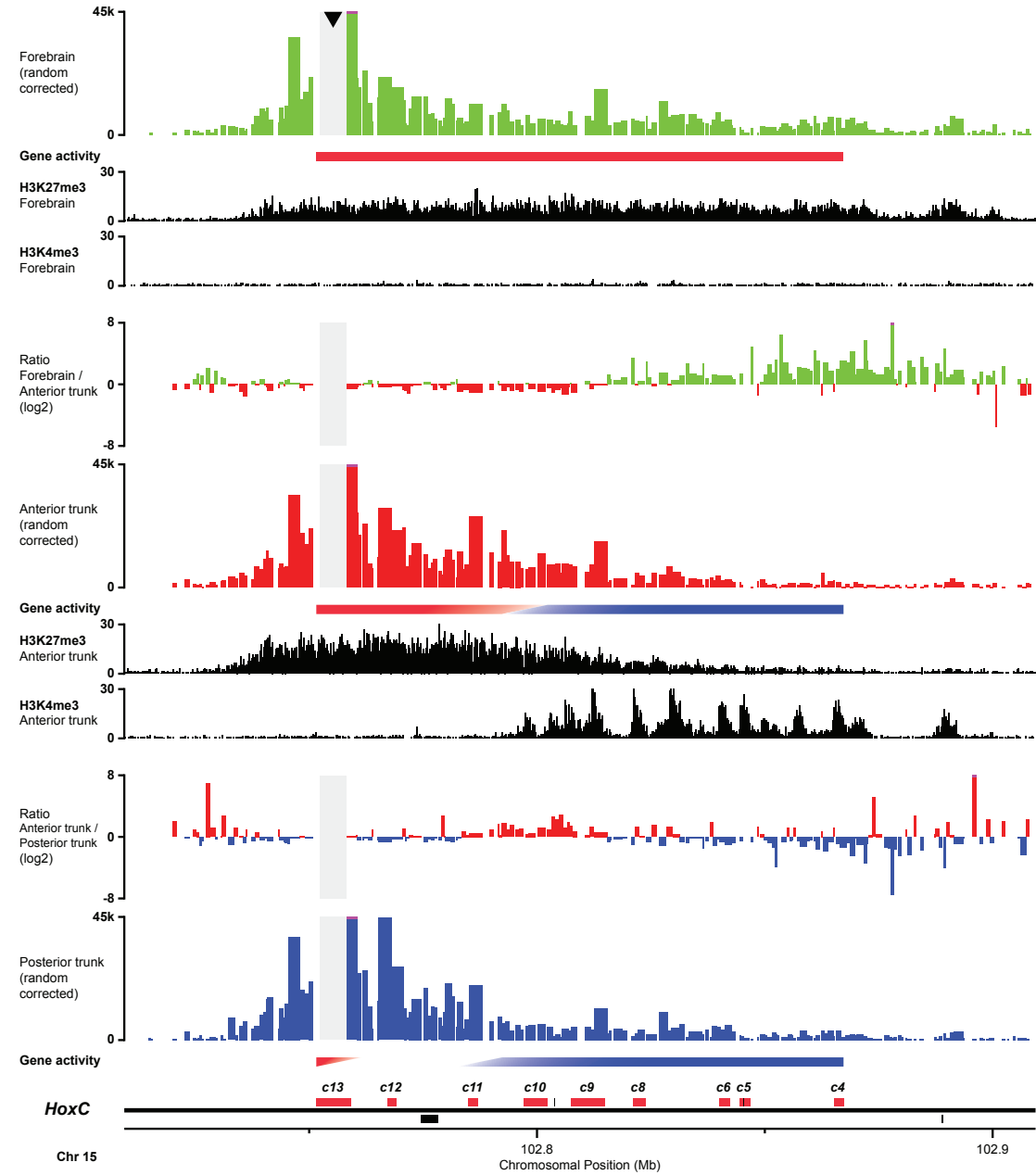




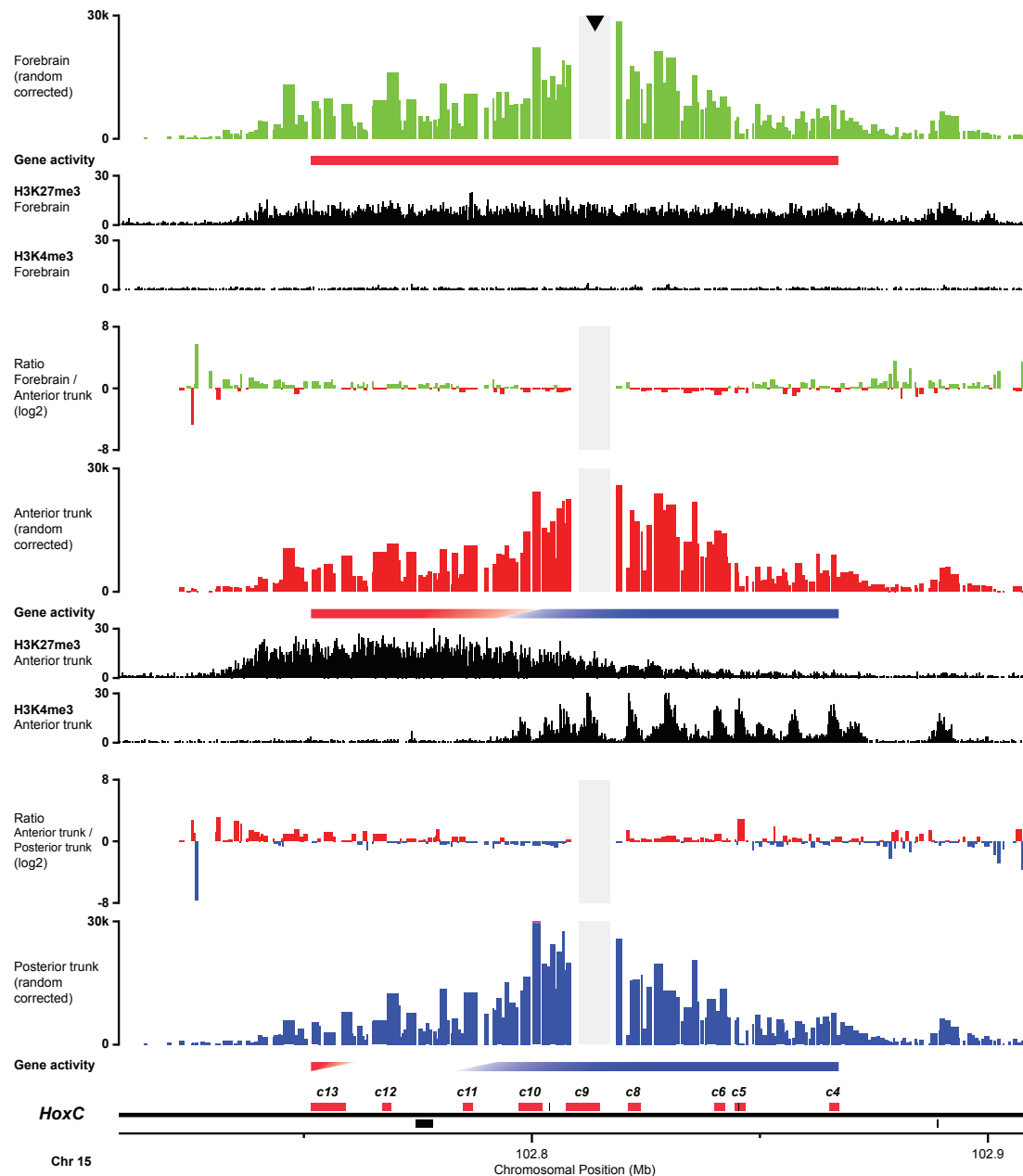
Local association dynamics of *Hoxd* genes. Quantitative local 4C-seq interaction patterns of different *Hoxd* genes, in the tissues used in this study. Quantitative ratios of associations between tissues are indicated in between patterns. Viewpoints are indicated with arrowheads and excluded regions around the viewpoints are indicated with vertical light grey boxes. H3K27me3 and H3K4me3 signals (in both forebrain and anterior trunk) and the collinear expression status of *Hox* genes (blue, active; red, inactive) are depicted below the patterns. Genomic coordinates, location of *Hoxd* genes (red) and other transcripts (black) are indicated.

Fig. S11

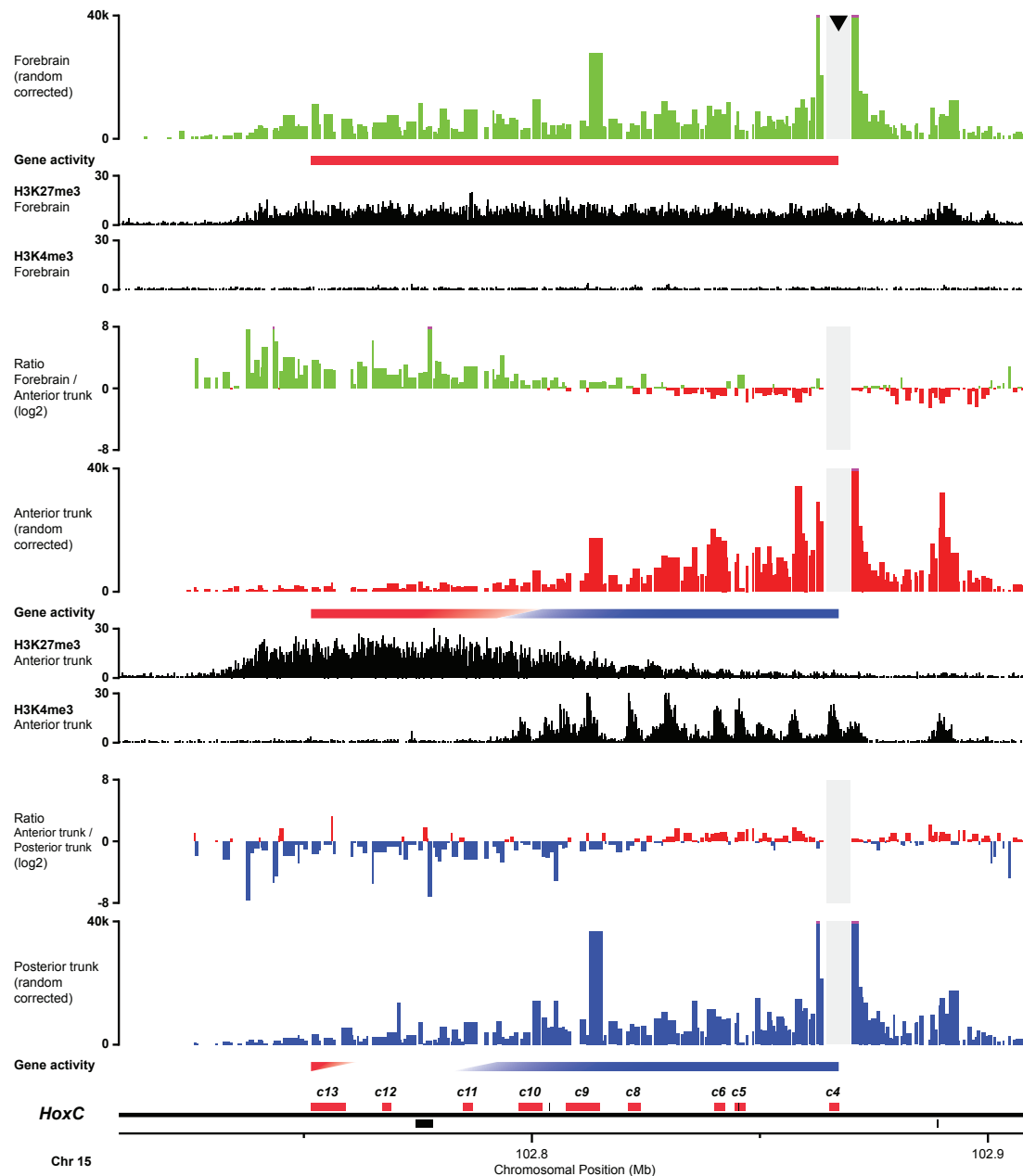
Hoxc13



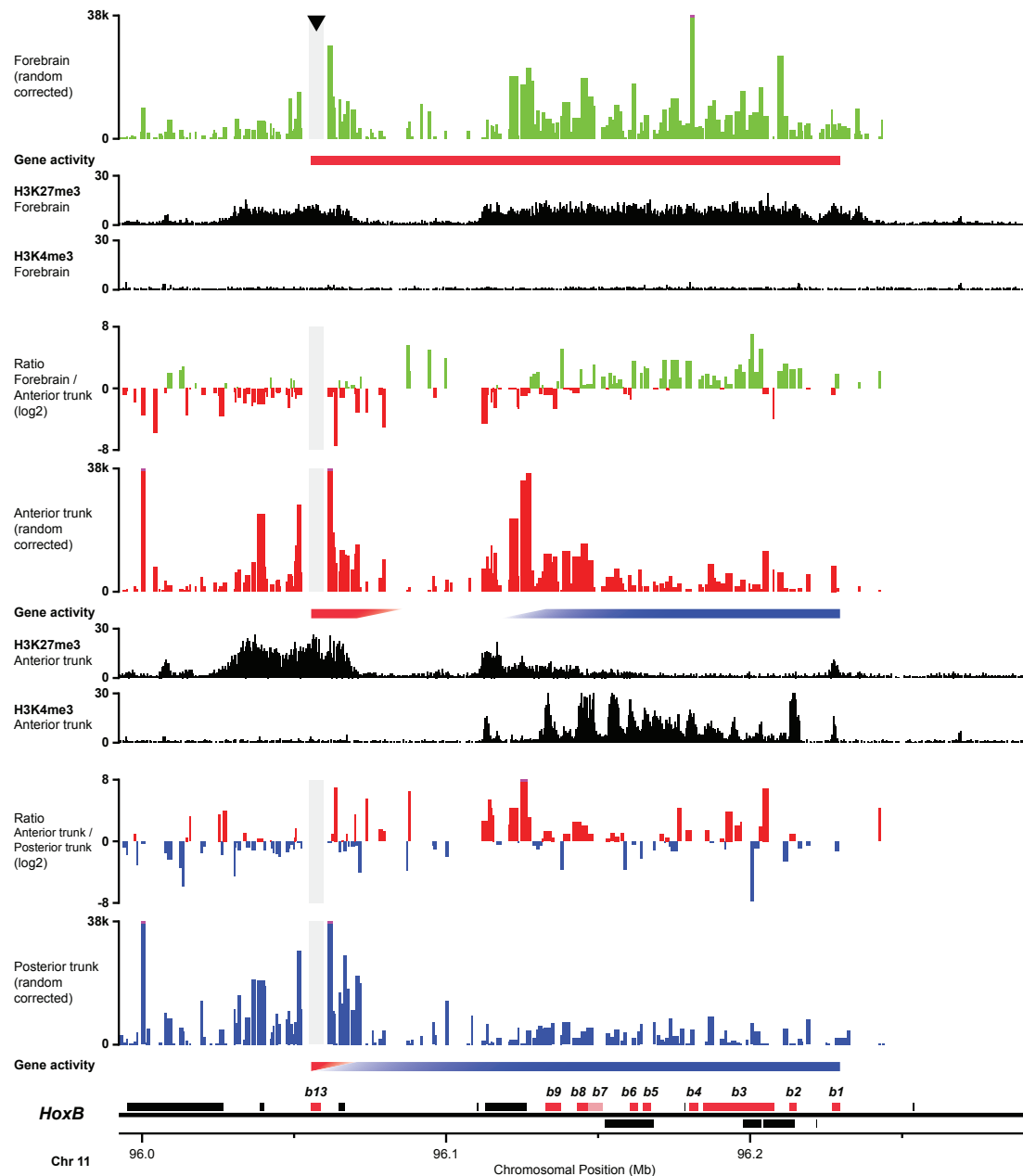
Hoxc9



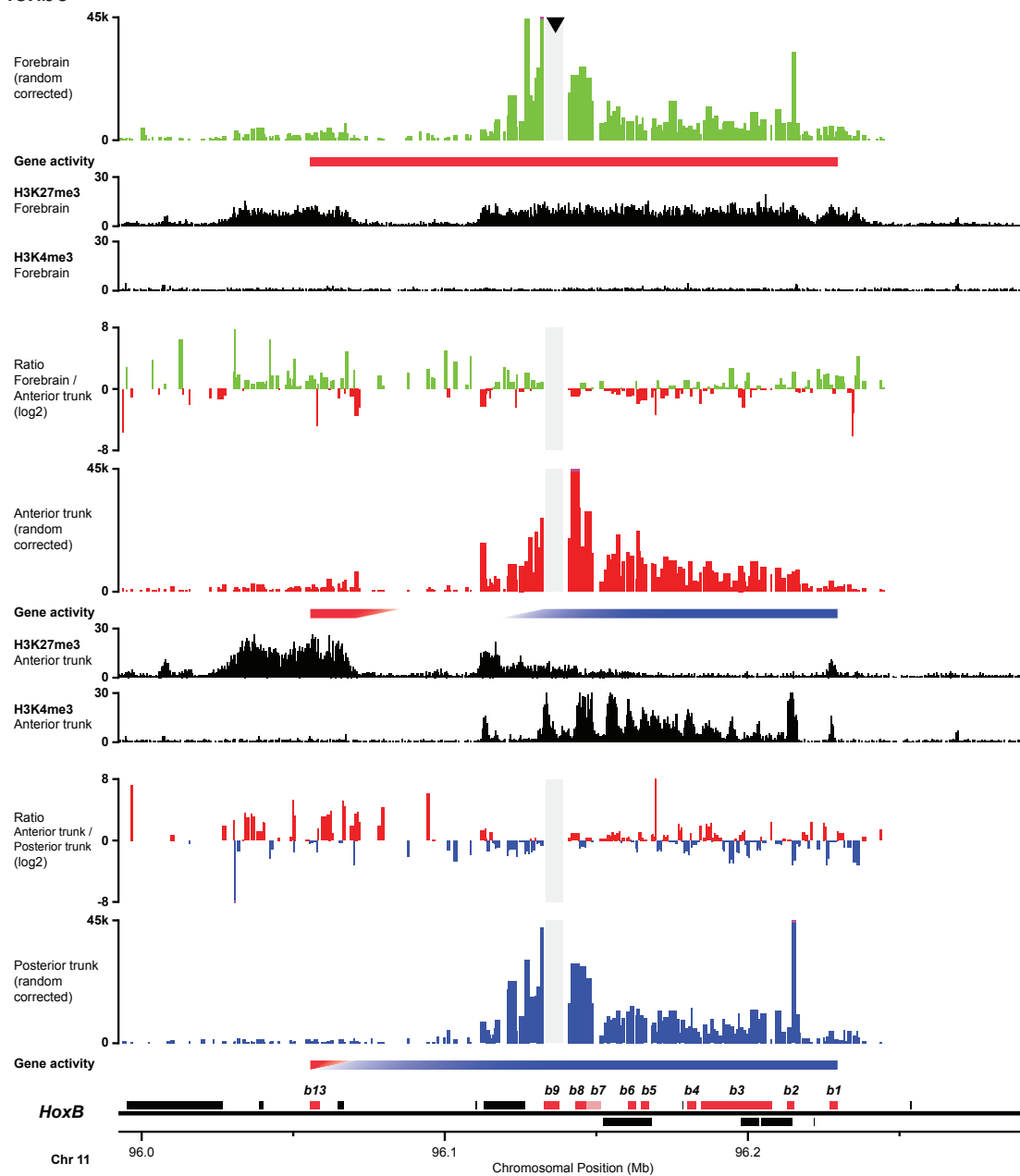
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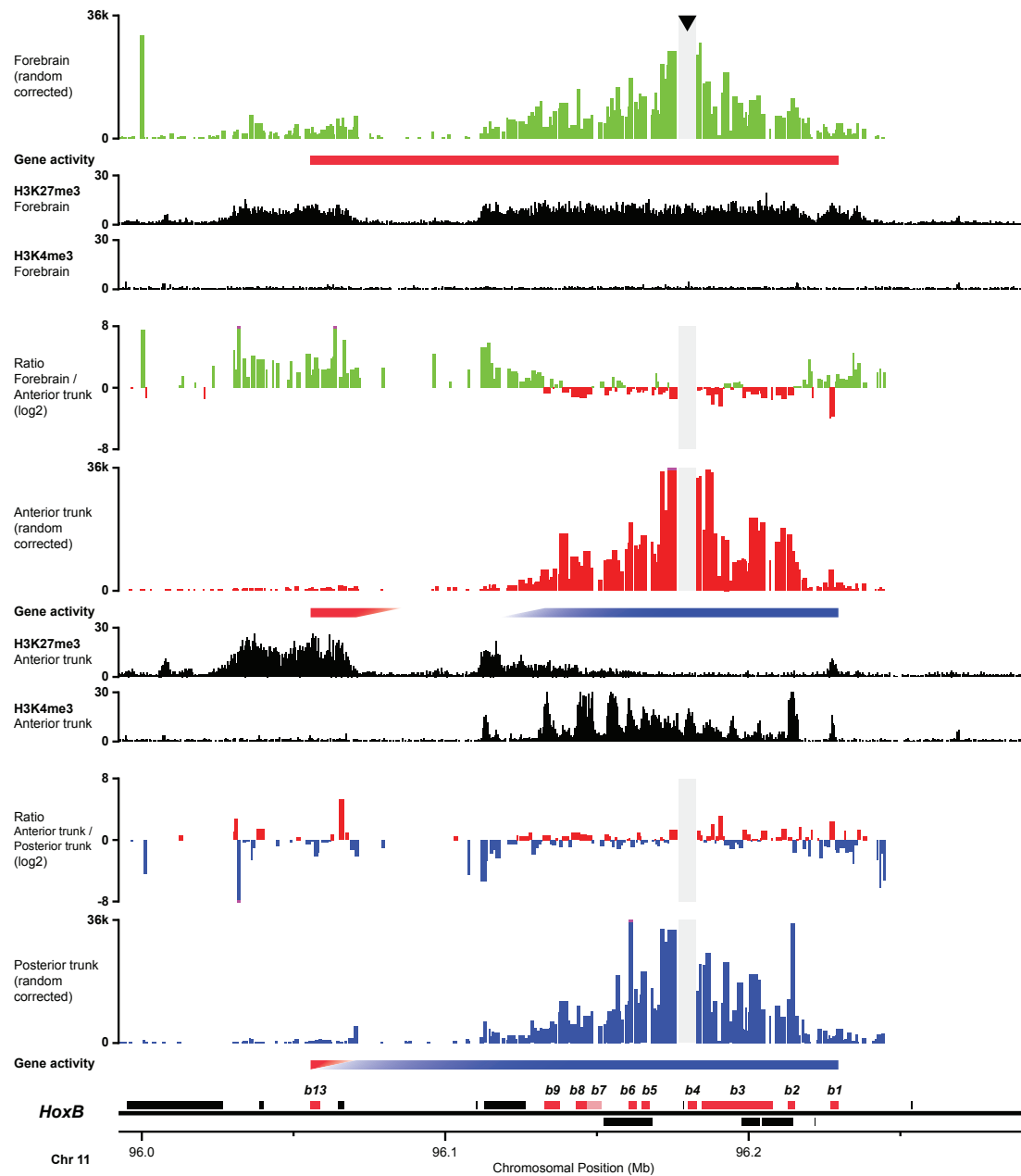
Hoxb13



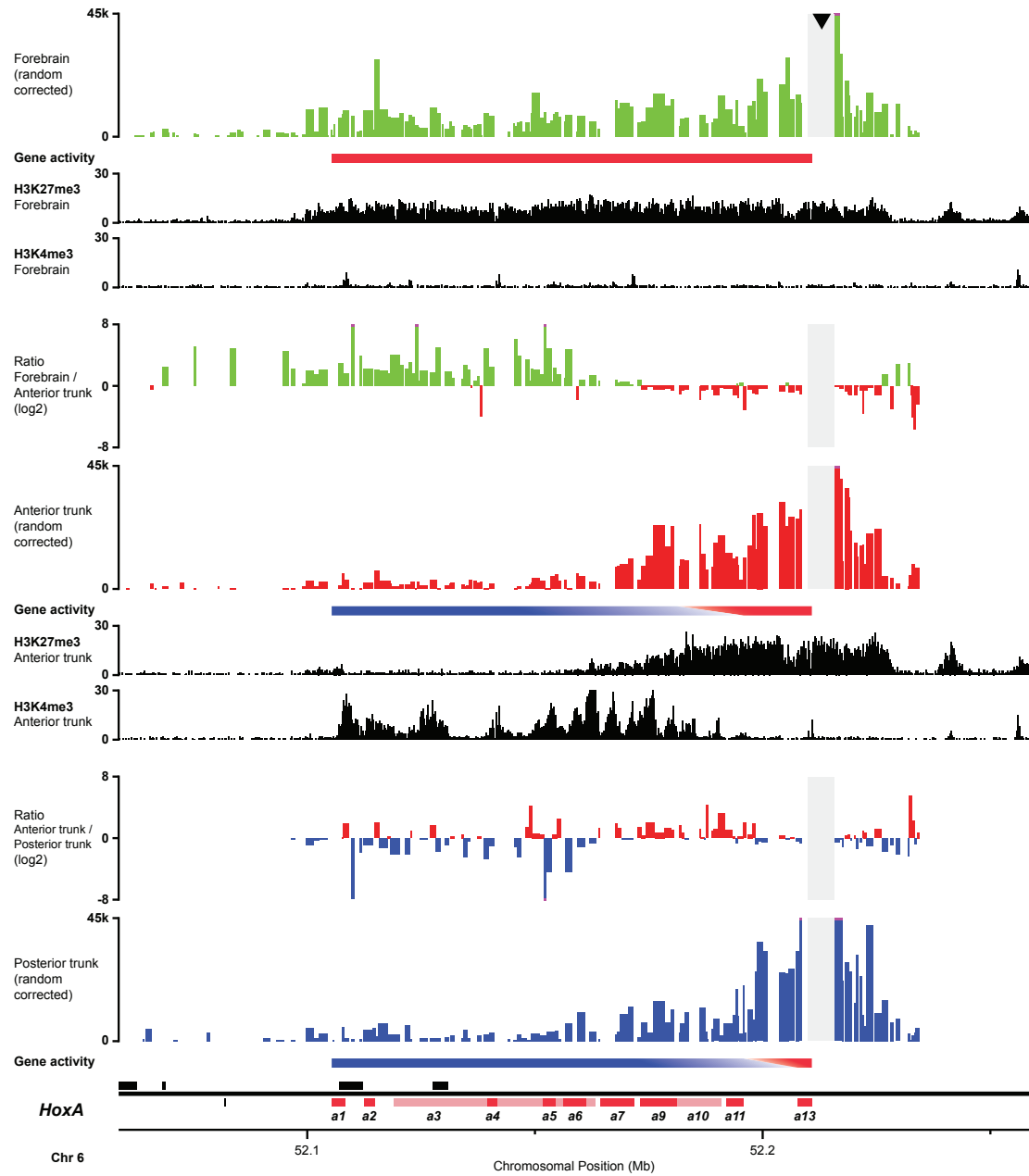
Hoxb9



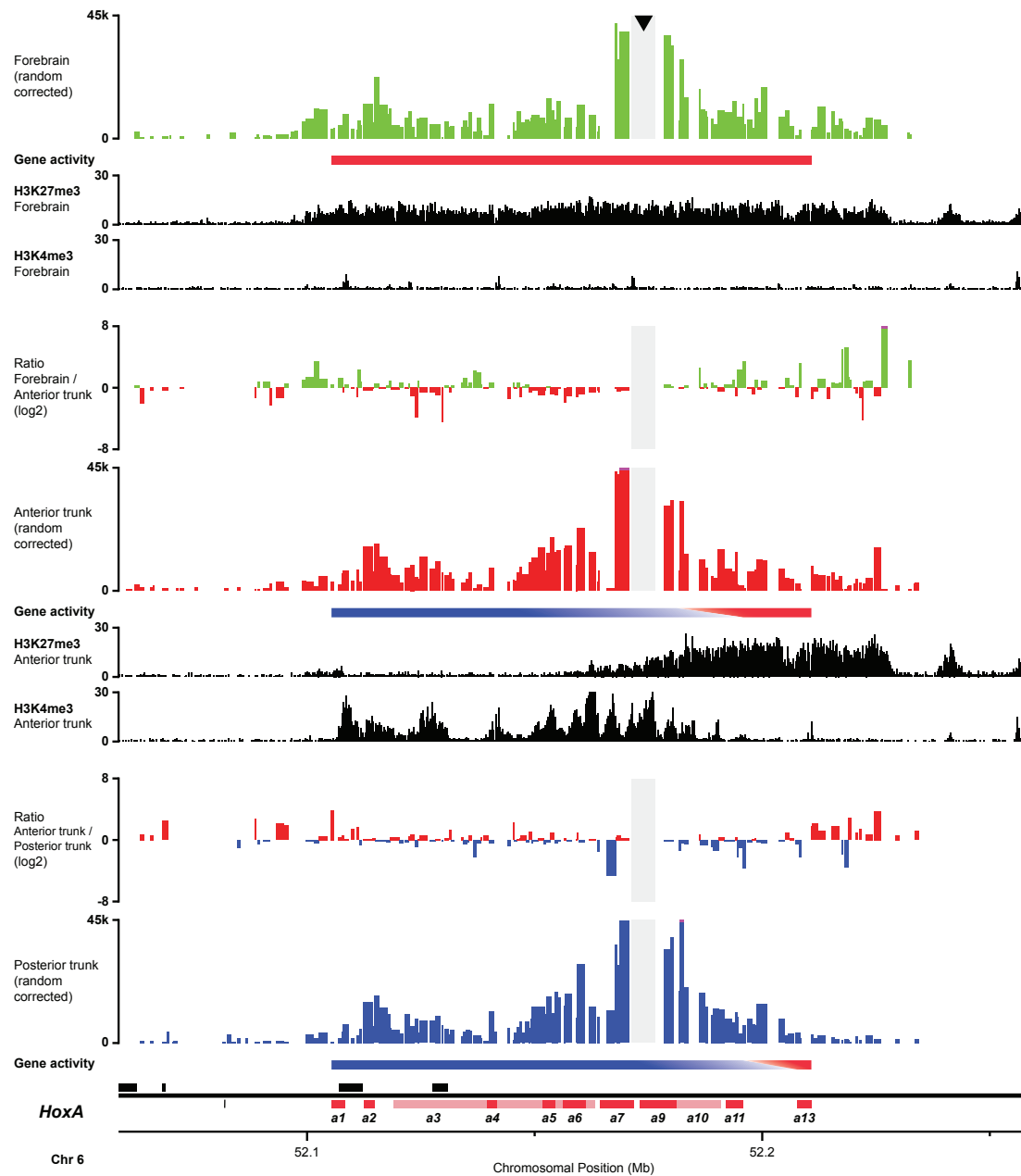
Hoxb4

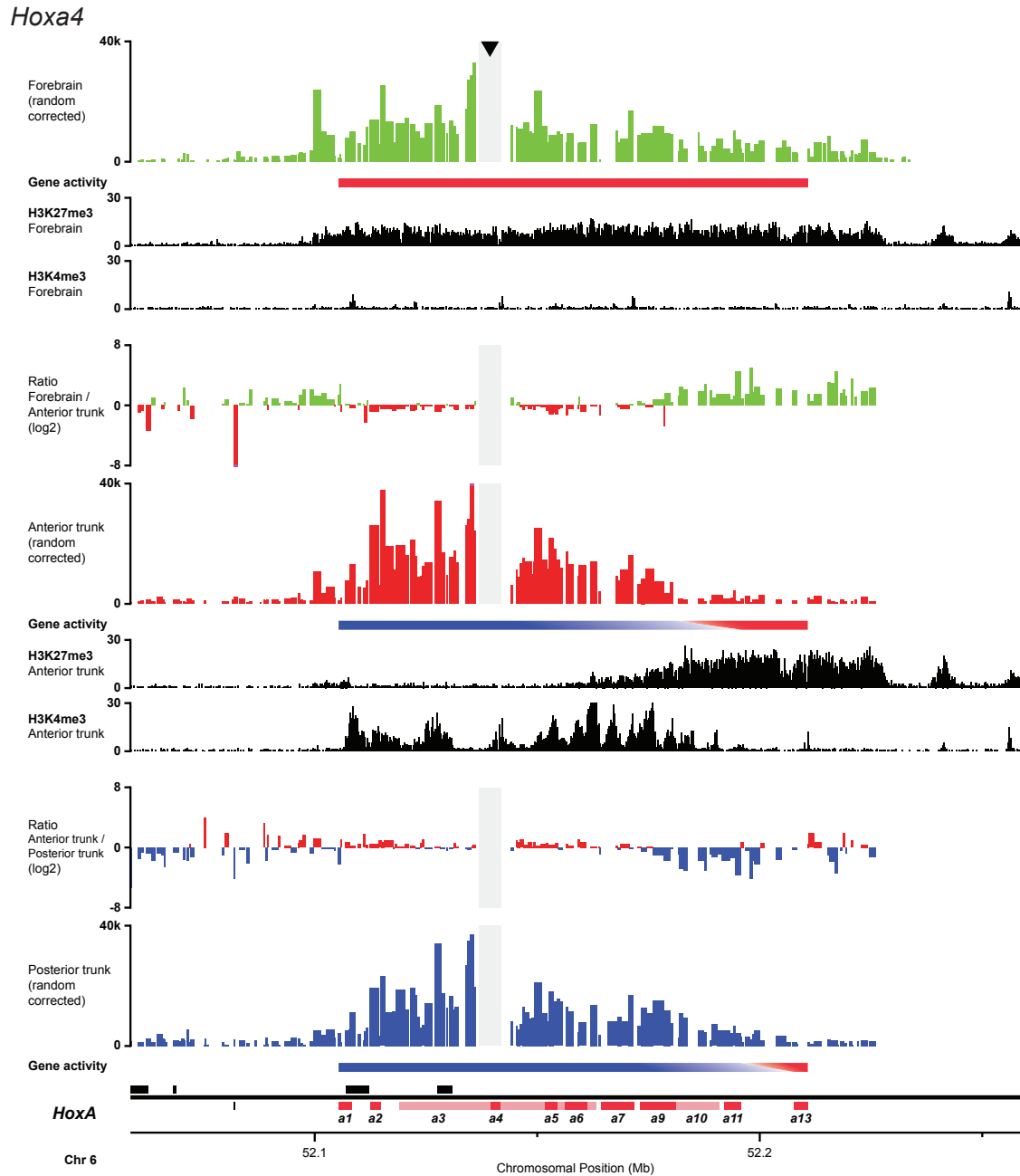


Hoxa13



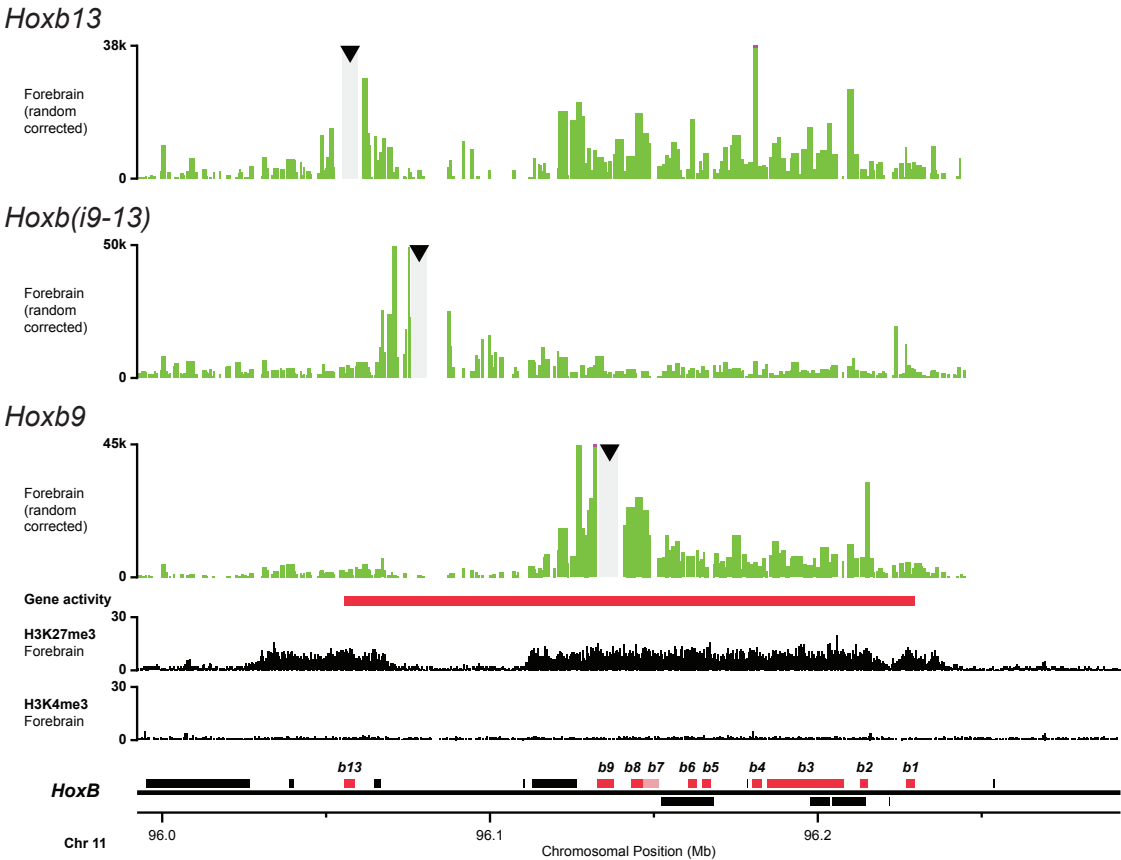
Hoxa9

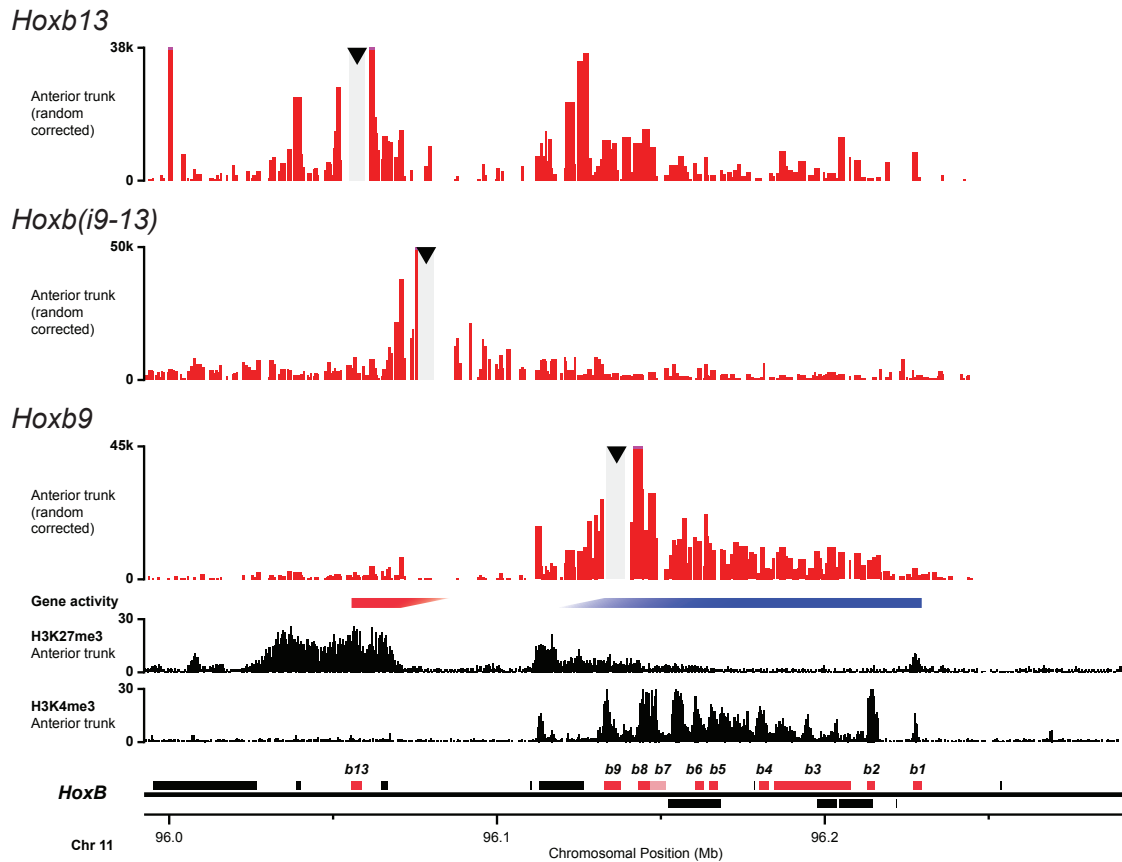




Local association dynamics of *Hox* genes from the *HoxA*, *B* and *C* clusters. Quantitative ratios of associations between the tissues are indicated in between patterns. Viewpoints are indicated with arrowheads and excluded regions around the viewpoints are indicated with vertical light grey boxes. H3K27me3 and H3K4me3 signals (forebrain and anterior trunk) and the collinear expression status of *Hox* genes (blue, active; red) are depicted below the patterns. Genomic coordinates, location of *Hox* genes (red) and other transcripts (black) are indicated.

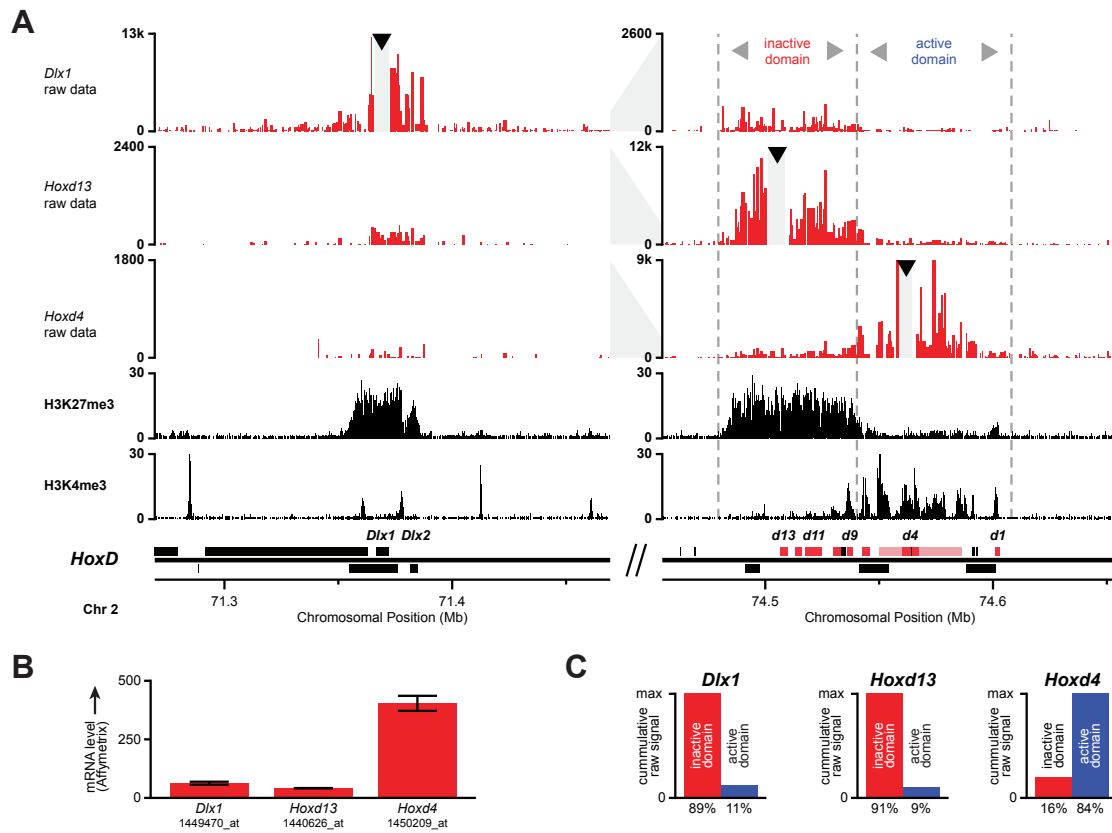
Fig. S12





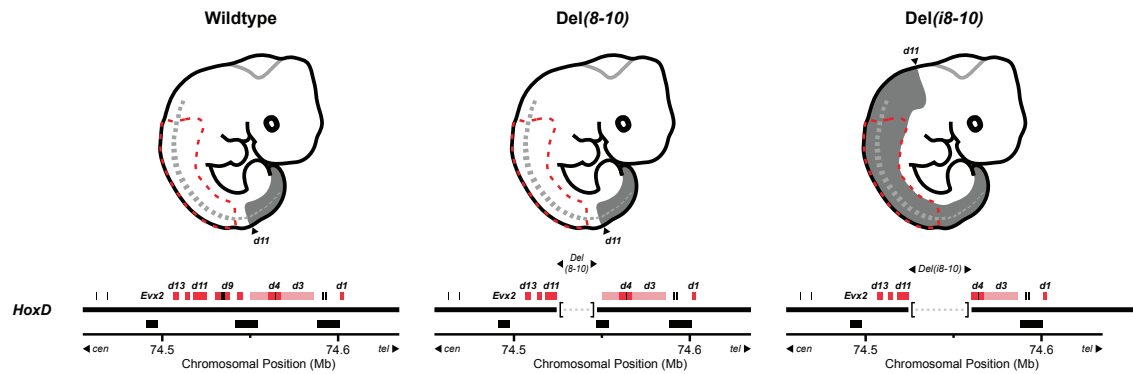
The large intergenic region within the *HoxB* cluster is excluded from the inactive local 3D domain. Quantitative local 4C-seq association patterns, as observed from viewpoints located in the *HoxB* cluster (*Hoxb13* and *Hoxb9*) and from within the intergenic region between *Hoxb13* and *Hoxb9* (*Hoxb(i9-13)*), in forebrain and anterior trunk cells. The intergenic region, which displays very low amounts of H3K27me3 and H3K4me3 signal, does not associate with the surrounding sequences, indicating it's looping out of the *HoxB* 3D domain. Viewpoints are indicated with arrowheads and the excluded region around the viewpoint is shown with vertical light grey boxes. H3K27me3 and H3K4me3 signals and the collinear expression status of a given gene (blue, active; red, inactive) are depicted below each pattern. Genomic coordinates, location of *Hoxb* genes (red) and of other transcripts (black) are indicated.

Fig. S13



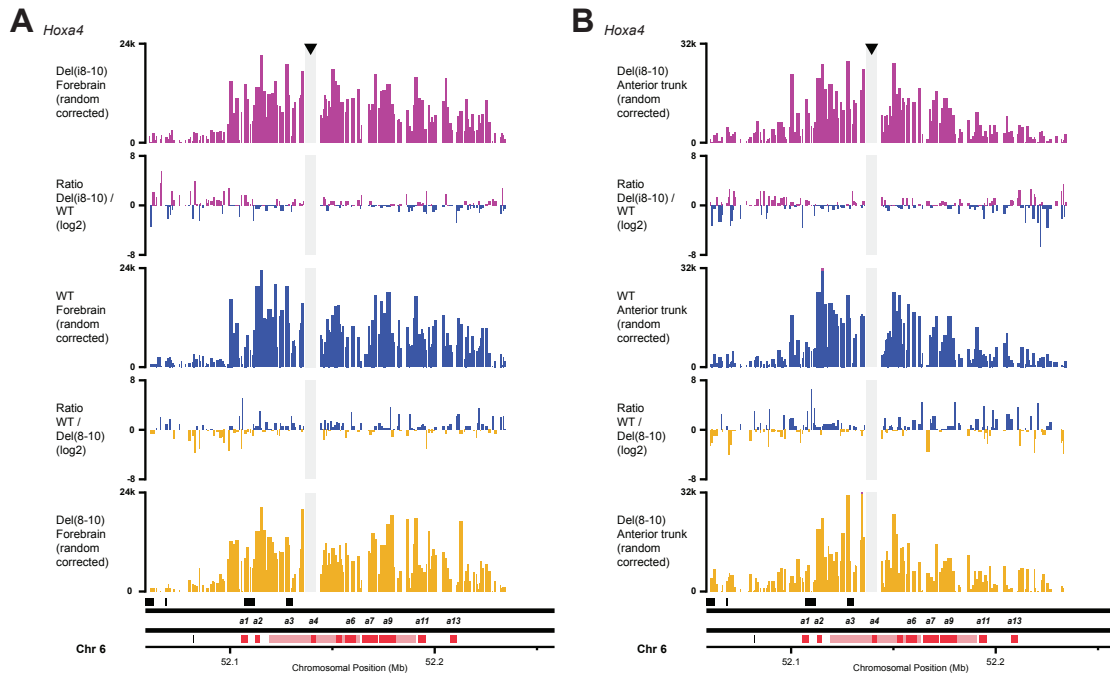
Ultra-long range associations between the *HoxD* cluster and the *Dlx1* locus matches the presence of H3K27me3 domains. **A**. Quantitative local 4C-seq interaction patterns in anterior trunk, between the *Dlx1* gene and *Hoxd* genes, which are separated by ca. 3 Mb. The inactive (see **B**) *Dlx1* locus is organized in a 3D compartment, which again coincides with the extent of its coverage by H3K27me3 marks (left panels). Infrequent, yet significant, interactions between the *Dlx1* locus and the *HoxD* cluster occur (right panels). However, these interactions are observed only between the H3K27me3 domains, both when *Hoxd13* and *Dlx1* are used as viewpoints. Thus, *Dlx1* does not contact the active part of the *HoxD* cluster and, likewise, interactions are lost when *Hoxd4* is used as a viewpoint, showing the high selectivity of these contacts. **B**. Affymetrix expression array values. Error-bars represent standard deviations from two technical replicates. **C**. Total cumulative signal from long-range (*Dlx1*) or local (*Hoxd13* and *Hoxd4*) viewpoints located inside the inactive or active domain at the *HoxD* cluster, in anterior trunk cells. Long-range 3D interaction behavior of the silent *Dlx1* gene is similar to the local interaction behavior of the silent *Hoxd13* gene.

Fig. S14



Hoxd11 embryonic expression patterns in wildtype (left) and the two mutant stocks used in this study. In the Del(8-10) mutant specimen (middle) *Hoxd11* is expressed as in wildtype (domain in grey), i.e. outside the sample dissected as 'anterior trunk' (delimited by a red dashed line). In contrast, in the Del(i8-10) mutant embryo (right), *Hoxd11* is expressed ectopically up to a very anterior level. As a consequence, 'anterior trunk' cells dissected from this mutant stock contain cells expressing *Hoxd11*, unlike in the Del(8-10) mutant sample. The positions and extents of the deletions are shown below. Expression data are taken from (17).

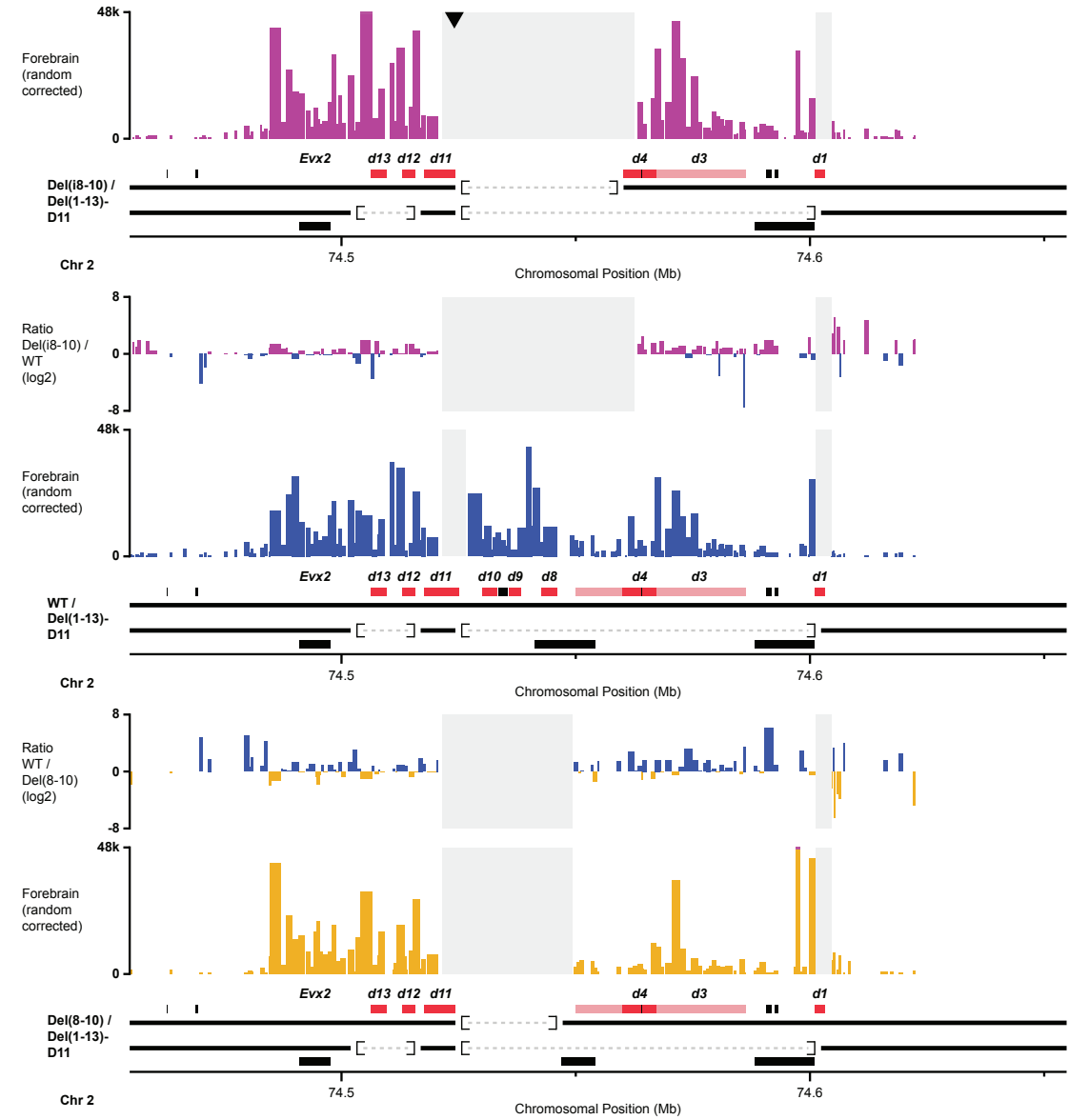
Fig. S15



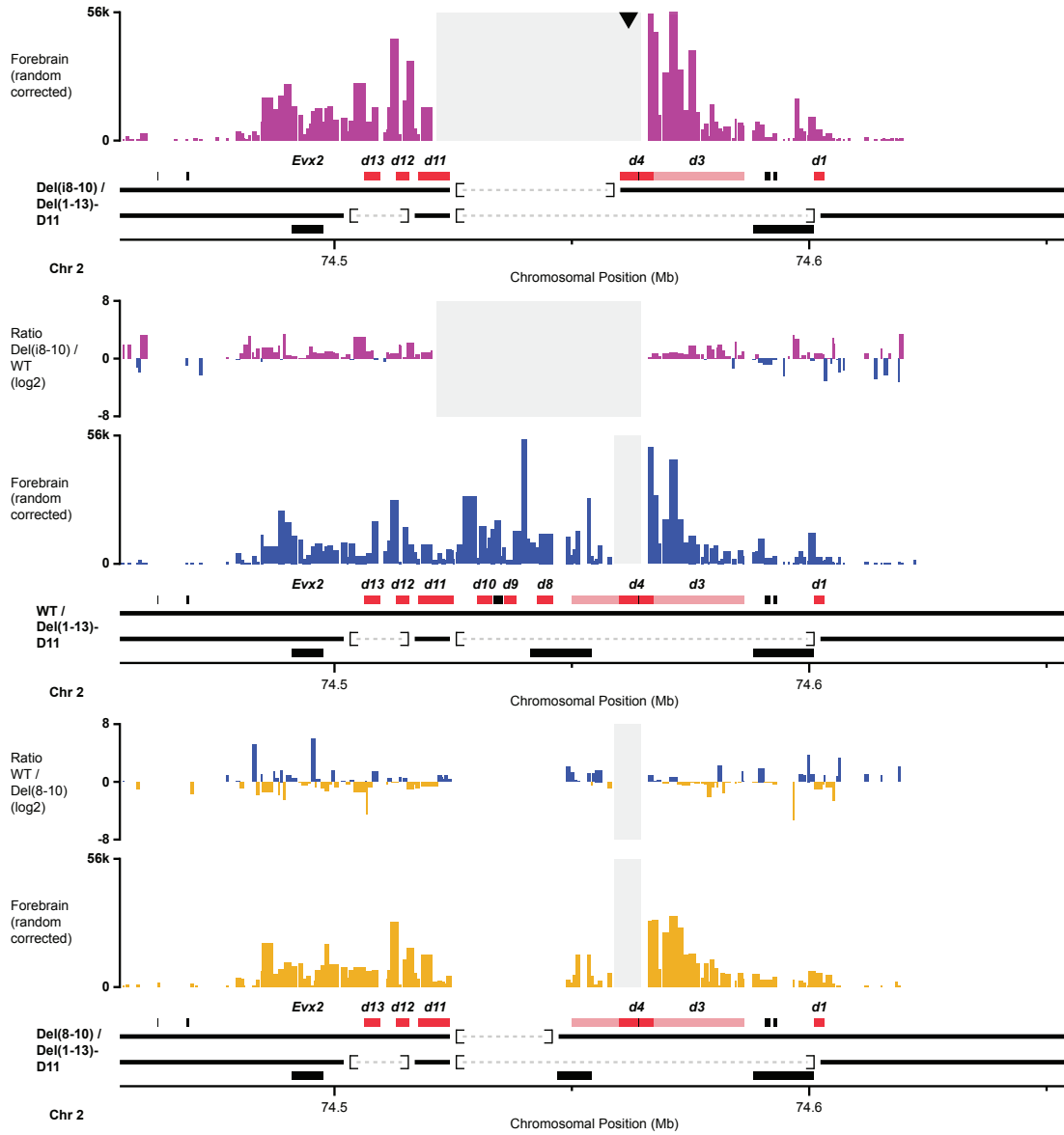
Reproducibility of multiplex 4C-seq experiments on mutant mouse material. Quantitative local 4C-seq association patterns of *Hoxa4* in both forebrain (**A**) and anterior trunk (**B**) cells. Quantitative ratios of interactions between mutants and wild-type controls are indicated in between the profiles. The globally similar 3D organization of the *HoxA* cluster in both *HoxD* mutant and control tissues is used as a quality control. *Hoxa4* viewpoints are indicated with arrowhead and excluded regions around the viewpoints are indicated with vertical light grey boxes. Genomic coordinates, location of *Hoxa* genes (red) and of other transcripts (black) are depicted below each panel.

Fig. S16

Hoxd11



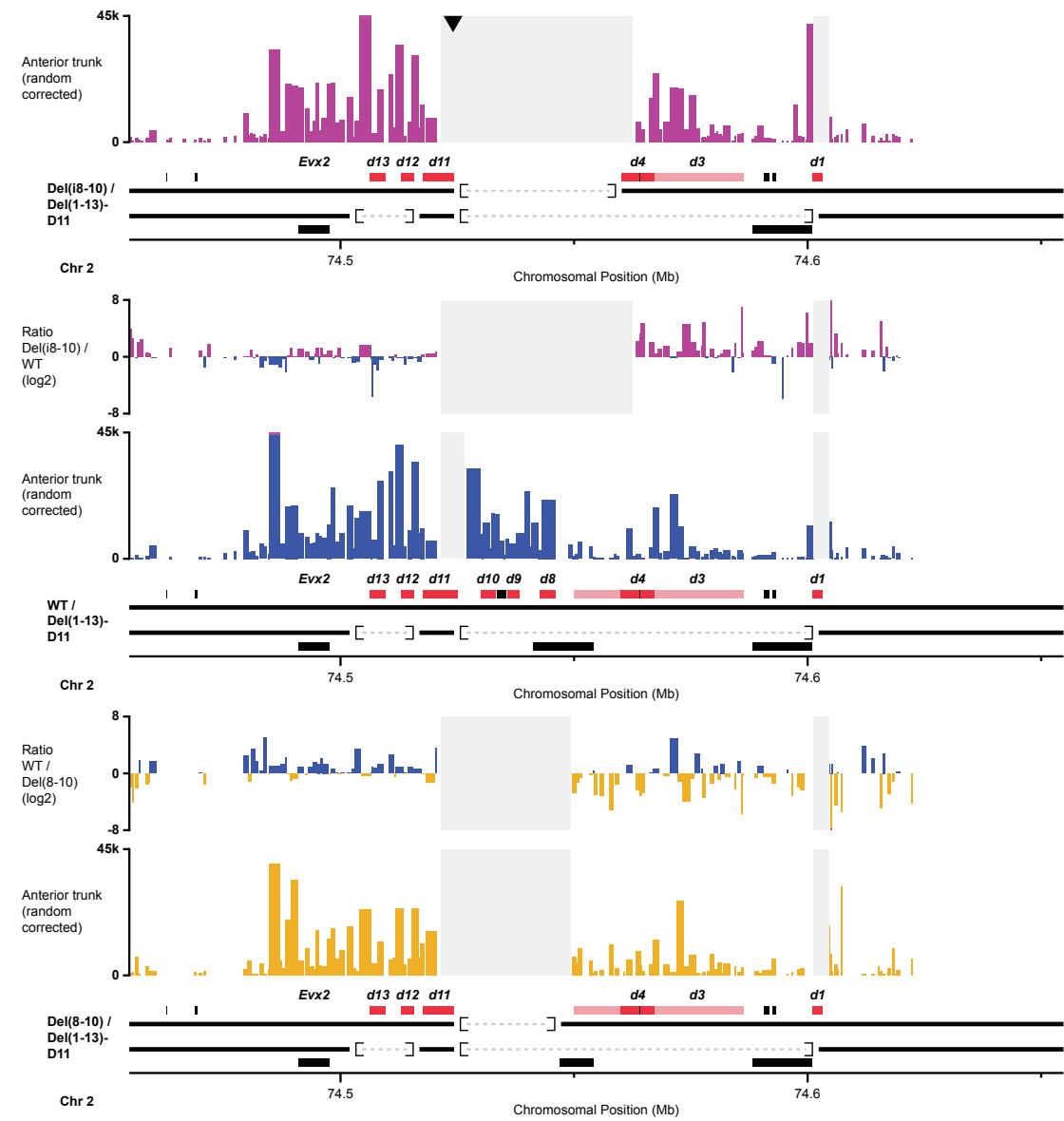
Hoxd4



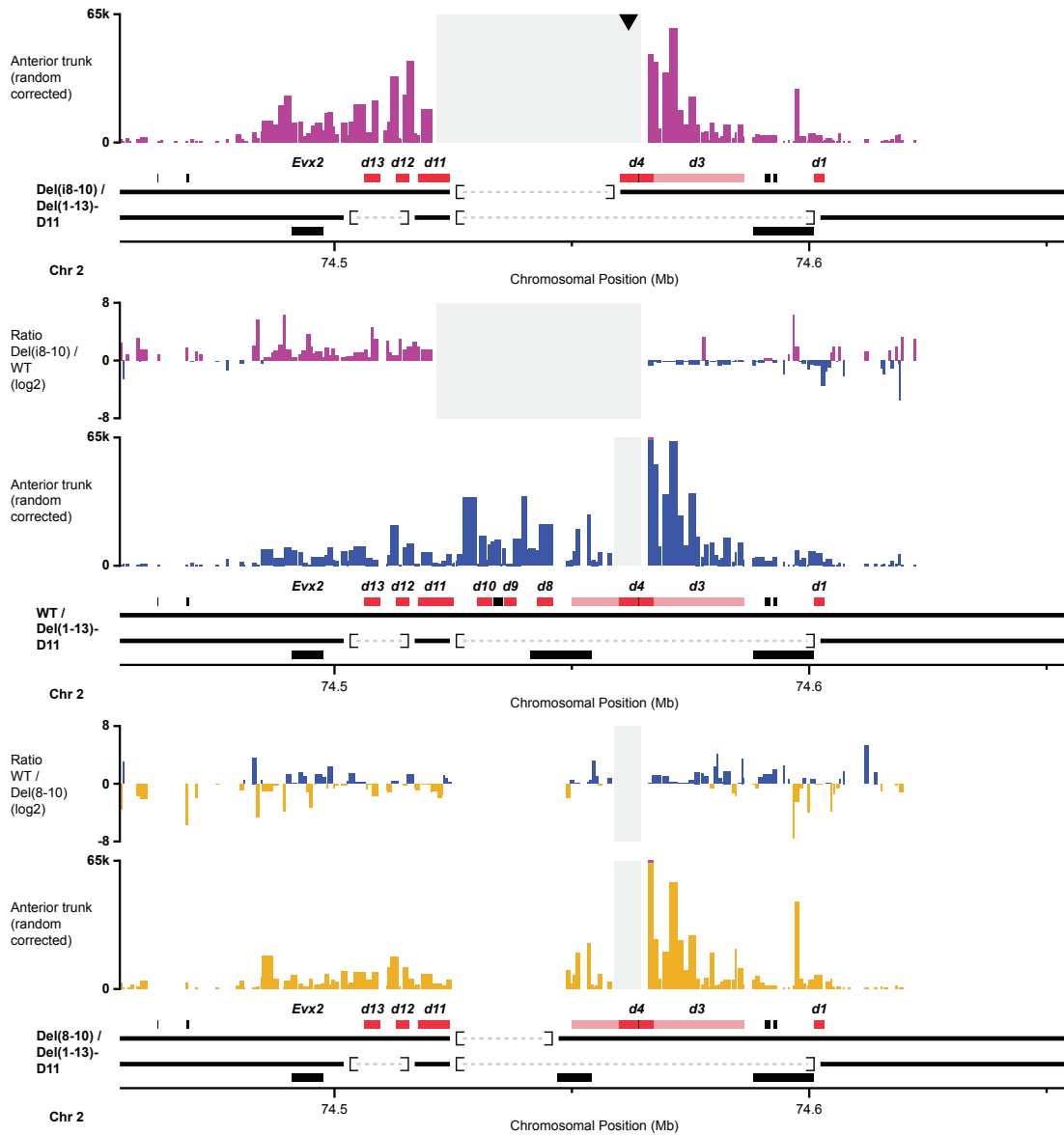
The borders of the 3D domain formed by the inactive *HoxD* cluster in brain cells remain unchanged whenever deletions are introduced within the gene cluster. Quantitative local 4C-seq interactions patterns of *Hoxd* genes in forebrain cells of deletion mutants and wild-type controls animals. Quantitative ratios of interactions between mutants and controls are indicated in between patterns. The viewpoints are indicated with arrowheads and the excluded regions around the viewpoints are indicated with vertical light grey boxes. Below each pattern, the allelic structure, genomic coordinates, locations of *Hoxd* genes (red) and of other transcripts (black) are indicated.

Fig. S17

Hoxd11



Hoxd4



Hoxd11 ectopic expression in anterior trunk cells of *Del(i8-10)* mutant embryos is associated with increased associations of this locus with the telomeric (active) parts of the *HoxD* cluster. Quantitative local 4C-seq interactions patterns of *Hoxd* genes in anterior trunk cells of both mutant and controls embryos. Quantitative ratios of interactions between mutants and controls are indicated in between the profiles. The viewpoints are indicated with arrowheads and the excluded regions around the viewpoints are indicated with vertical light grey boxes. Below each pattern, the allelic structure, genomic coordinates, locations of *Hoxd* genes (red) and of other transcripts (black) are indicated.

Table S1.

Top 20 up-regulated transcripts after a pair-wise comparison between the tissues sampled in this study. Affymetrix expression array values were obtained from two technical replicates on a subset of the cells used for 4C-seq experiments. Genes and transcripts located within the *Hox* clusters are indicated in red.

Anterior Trunk versus Forebrain

Gene / transcript	ENSEMBL ID	Affymetrix probe	Value Forebrain	Value Anterior trunk	Value Posterior trunk	Anterior trunk / Fore-brain
<i>Hoxc8</i>	ENSMUSG0000001657	1452412_at	20.3	3269.7	658.9	161.0
<i>Hoxa9</i>	ENSMUSG00000038227	1455626_at	24.7	2724.6	3892.4	110.3
<i>Hoxb3</i>	ENSMUSG00000048763	1456229_at	25.6	2235.6	1492.9	87.4
<i>Hoxc5</i>	ENSMUSG00000022485	1439885_at	26.2	2136.0	371.0	81.6
<i>Hoxb7 / b8</i>	ENSMUSG00000056648	1452493_s_at	28.3	2224.9	844.9	78.5
<i>C1qtnf3</i>	ENSMUSG00000058914	1422606_at	10.5	796.1	361.1	75.9
<i>Hoxc10</i>	ENSMUSG00000022484	1439798_at	15.4	823.8	5282.3	53.5
<i>Hoxc4</i>	ENSMUSG00000075394	1422870_at	59.0	3008.1	1153.9	51.0
<i>Hoxa5</i>	ENSMUSG00000038253	1448926_at	45.1	2266.4	1310.0	50.2
<i>AK007148</i>	ENSMUSG00000052371	1460056_at	20.4	875.2	1191.8	43.0
<i>Actc1</i>	ENSMUSG00000068614	1415927_at	154.8	5948.9	3625.9	38.4
<i>Myl4</i>	ENSMUSG00000061086	1422580_at	38.1	1278.3	499.1	33.5
<i>Hoxb9</i>	ENSMUSG00000020875	1452317_at	74.7	2477.9	1396.0	33.2
<i>Myh3</i>	ENSMUSG00000020908	1427115_at	46.9	1504.9	478.1	32.1
<i>Myl1</i>	ENSMUSG00000061816	1452651_a_at	66.2	2043.1	683.0	30.9
<i>A1448005</i>	-	1435673_at	19.7	599.7	478.6	30.5
<i>Dmrt2</i>	ENSMUSG00000048138	1426867_at	35.2	972.3	953.5	27.6
<i>Aldh1a2</i>	ENSMUSG00000013584	1422789_at	59.4	1572.3	1738.8	26.5
<i>AK002860</i>	ENSMUSG00000085645	1447886_at	173.5	4538.7	6084.5	26.2
<i>Hoxb2</i>	ENSMUSG00000075588	1449397_at	75.1	1948.2	1607.4	25.9

Posterior Trunk versus Anterior Trunk

Gene / transcript	ENSEMBL ID	Affymetrix probe	Value Forebrain	Value Anterior trunk	Value Posterior trunk	Posterior trunk / Anterior trunk
<i>Hoxd13</i>	ENSMUSG00000001819	1440626_at	38.6	41.5	1269.8	30.6
<i>Hoxd10</i>	ENSMUSG000000050368	1418606_at	40.7	159.8	3616.5	22.6
<i>Hoxd11</i>	ENSMUSG00000042499	1450584_at	27.9	33.9	537.0	15.8
<i>Hoxc13</i>	ENSMUSG00000001655	1425874_at	47.0	46.8	574.6	12.3
<i>Hoxa11</i>	ENSMUSG00000038210	1420414_at	50.8	58.1	617.9	10.6
<i>AK012157</i>	-	1435639_at	21.0	30.7	309.8	10.1
<i>Hoxa10</i>	ENSMUSG00000000938	1431475_a_at	27.8	107.1	905.2	8.5
<i>T</i>	ENSMUSG00000062327	1419304_at	39.0	205.8	1645.8	8.0
<i>Hoxc10</i>	ENSMUSG00000022484	1439798_at	15.4	823.8	5282.3	6.4
<i>Hoxb13</i>	ENSMUSG00000049604	1419576_at	38.5	36.7	233.7	6.4
<i>Hoxa11as</i>	-	1452400_a_at	26.1	25.7	150.3	5.9
<i>Olig1</i>	ENSMUSG00000046160	1416149_at	204.1	32.3	183.4	5.7
<i>Olig2</i>	ENSMUSG00000039830	1416232_at	352.1	46.5	255.2	5.5
<i>Tbx4</i>	ENSMUSG00000000094	1456033_at	37.1	45.1	215.4	4.8
<i>Ifitm1</i>	ENSMUSG00000025491	1424254_at	373.4	582.4	2503.1	4.3
<i>Tbx6</i>	ENSMUSG00000030699	1449868_at	40.1	41.2	176.9	4.3
<i>Tmem30b</i>	ENSMUSG00000034435	1433579_at	334.4	119.3	508.1	4.3
<i>Hoxd12</i>	ENSMUSG00000001823	1450553_at	28.7	27.3	98.6	3.6
<i>Fgf17</i>	ENSMUSG00000022101	1421523_at	383.9	50.0	180.2	3.6
<i>Sall4</i>	ENSMUSG00000027547	1424152_at	1213.2	704.2	2520.4	3.6

Table S2.

mRNA levels of *Hox* genes in those tissues sampled in this study. Affymetrix expression array values were obtained from two technical replicates on a subset of the cells used for 4C-seq experiments.

Gene	ENSEMBL ID	Affymetrix probe	Value Forebrain	Value Anterior Trunk	Value Posterior Trunk
<i>Hoxd13</i>	ENSMUSG00000001819	1440626_at	38.6	41.5	1269.8
<i>Hoxd12</i>	ENSMUSG00000001823	1450553_at	28.7	27.3	98.6
<i>Hoxd11</i>	ENSMUSG000000042499	1450584_at	27.9	33.9	537.0
<i>Hoxd10</i>	ENSMUSG000000050368	1418606_at	40.7	159.8	3616.5
<i>Hoxd9</i>	ENSMUSG000000043342	1419126_at	119.0	1012.3	2981.3
<i>Hoxd8</i>	ENSMUSG000000027102	1431099_at	59.4	978.5	1589.2
<i>Hoxd4</i>	ENSMUSG000000001819	1450209_at	21.1	403.8	451.8
<i>Hoxd3</i>	ENSMUSG000000001823	1421537_at	178.9	968.8	890.7
<i>Hoxd1</i>	ENSMUSG000000042499	1420573_at	17.9	388.3	524.7
<i>Hoxc13</i>	ENSMUSG000000001655	1425874_at	47.0	46.8	574.6
<i>Hoxc12</i>	ENSMUSG000000050328	<i>Not present</i>			
<i>Hoxc11</i>	ENSMUSG000000001656	<i>Not present</i>			
<i>Hoxc10</i>	ENSMUSG000000022484	1439798_at	15.4	823.8	5282.3
<i>Hoxc9</i>	ENSMUSG000000036139	1449867_at	205.3	1387.3	1259.3
<i>Hoxc8</i>	ENSMUSG000000001657	1452412_at	20.3	3269.7	658.9
<i>Hoxc6</i>	ENSMUSG000000001661	1427361_at	85.7	1611.0	607.0
<i>Hoxc5</i>	ENSMUSG000000022485	1439885_at	26.2	2136.0	371.0
<i>Hoxc4</i>	ENSMUSG000000075394	1422870_at	59.0	3008.1	1153.9
<i>Hoxb13</i>	ENSMUSG000000049604	1419576_at	38.5	36.7	233.7
<i>Hoxb9</i>	ENSMUSG000000020875	1452317_at	74.7	2477.9	1396.0
<i>Hoxb7 / b8</i>	ENSMUSG000000056648	1452493_s_at	28.3	2224.9	845.0
<i>Hoxb6</i>	ENSMUSG000000000690	1451660_a_at	97.5	1561.2	1179.6
<i>Hoxb5</i>	ENSMUSG000000038700	1418415_at	25.3	654.6	355.4
<i>Hoxb4</i>	ENSMUSG000000038692	1460379_at	71.1	681.9	486.6
<i>Hoxb3</i>	ENSMUSG000000048763	1456229_at	25.6	2235.6	1492.9
<i>Hoxb2</i>	ENSMUSG000000075588	1449397_at	75.1	1948.2	1607.4
<i>Hoxb1</i>	ENSMUSG000000018973	1453501_at	17.7	294.4	541.4
<i>Hoxa13</i>	ENSMUSG000000038203	1422336_at	42.6	43.1	68.6
<i>Hoxa11</i>	ENSMUSG000000038210	1420414_at	50.8	58.1	617.9
<i>Hoxa10</i>	ENSMUSG000000000938	1431475_a_at	27.8	107.1	905.2
<i>Hoxa9</i>	ENSMUSG000000038227	1455626_at	24.7	2724.6	3892.4
<i>Hoxa7</i>	ENSMUSG000000038236	1449499_at	223.6	1353.0	521.3
<i>Hoxa6</i>	ENSMUSG000000043219	<i>Not present</i>			
<i>Hoxa5</i>	ENSMUSG000000038253	1448926_at	45.1	2266.3	1310.0
<i>Hoxa4</i>	ENSMUSG000000000942	1427354_at	16.4	413.8	163.0
<i>Hoxa3</i>	ENSMUSG000000079560	1427433_s_at	26.0	436.8	310.8
<i>Hoxa2</i>	ENSMUSG000000014704	1419602_at	21.9	292.9	228.3
<i>Hoxa1</i>	ENSMUSG000000029844	1420565_at	29.7	370.7	545.8

Table S3.

Fragment number and genome coverage of the 4C-seq library, with *NlaIII* used as a primary and *DpnII* as a secondary restriction enzymes.

Primary restriction enzyme: *NlaIII*

Secondary restriction enzyme: *DpnII*

	Number of fragments	Fraction of total number (%)	Average length	Genomic coverage	Fraction of total genome (%) [*]
All fragments	12,456,688	100 %	203 bp / frag	2,532,070,302 bp	95 %
Excluded due to restriction site configuration	8,679,038	70 %	129 bp / frag	1,116,024,980 bp	42 %
Excluded due to repeats	1,391,135	11 %	360 bp / frag	500,736,390 bp	19 %
Informative fragments	2,386,515	19 %	382 bp / frag	911,531,282 bp	34 %

* Total genomic length considered: 2,654,911,517 bp

Theoretical average resolution: 1112 bp / fragment

Table S4.

4C-seq quality control. **A.** Regions surrounding the viewpoints that have been excluded. The excluded regions consist of the viewpoint itself, the directly neighboring ‘undigested fragment’ and fragments located within 2 kb on both sides. Signals within these regions were highly enriched and showed considerable experimental variation rather than biological significance. The DNA fragments covered by using these criteria were excluded before normalization of the overall fragment count. **B.** The total reads, reads that can be mapped and reads that are excluded, are shown for different viewpoints and tissue samples.

A. Excluded regions surrounding viewpoints***Wildtype samples***

Viewpoint	Chromosome	Start	End
<i>Hoxd13</i>	2	74,501,237	74,508,317
<i>Hoxd11</i>	2	74,521,560	74,526,558
<i>Hoxd9</i>	2	74,534,116	74,539,344
<i>Hoxd(i4-8)</i>	2	74,551,067	74,556,034
<i>Hoxd4</i>	2	74,558,887	74,564,566
<i>Hoxd3</i>	2	74,572,778	74,578,858
<i>Hoxd1</i>	2	74,597,926	74,603,162
<i>Hoxc13</i>	15	102,752,381	102,758,254
<i>Hoxc9</i>	15	102,810,363	102,817,195
<i>Hoxc4</i>	15	102,864,639	102,869,848
<i>Hoxb13</i>	11	96,054,982	96,059,808
<i>Hoxb(i9-13)</i>	11	96,076,073	96,080,742
<i>Hoxb9</i>	11	96,133,441	96,138,987
<i>Hoxb4</i>	11	96,176,849	96,182,606
<i>Hoxa13</i>	6	52,210,042	52,215,742
<i>Hoxa9</i>	6	52,171,265	52,176,379
<i>Hoxa4</i>	6	52,136,882	52,141,946

Mutant samples**Wildtype/Del(1-13)-D11**

Viewpoint	Chromosome	Start	End
<i>Hoxd11</i>	2	74,521,560	74,526,558
	2	74,601,162	74,604,549
<i>Hoxd4</i>	2	74,558,887	74,564,566
<i>Hoxa4</i>	6	52,136,882	52,141,946

Del(i8-10)/Del(1-13)-D11

Viewpoint	Chromosome	Start	End
<i>Hoxd11</i>	2	74,521,560	74,562,637
	2	74,601,162	74,604,549
<i>Hoxd4</i>	2	74,521,623	74,564,566
<i>Hoxa4</i>	6	52,136,882	52,141,946

Del(8-10)/Del(1-13)-D11

Viewpoint	Chromosome	Start	End
<i>Hoxd11</i>	2	74,521,560	74,549,228
	2	74,601,162	74,604,549
<i>Hoxd4</i>	2	74,558,887	74,564,566
<i>Hoxa4</i>	6	52,136,882	52,141,946

B. Quality of 4C-seq reads

Wildtype samples

HoxD cluster

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxd13</i>	Forebrain	9.41	2.88	6.53
	Anterior trunk	7.38	2.43	4.96
	Posterior trunk	8.22	2.65	5.58
<i>Hoxd11</i>	Forebrain	15.96	3.03	12.93
	Anterior trunk	9.15	2.77	6.38
	Posterior trunk	17.12	1.34	15.77
<i>Hoxd9</i>	Forebrain	20.53	3.31	17.22
	Anterior trunk	15.31	4.29	11.02
	Posterior trunk	16.24	3.96	12.28
<i>Hoxd(i4-8)</i>	Forebrain	4.83	2.93	1.89
	Anterior trunk	6.39	4.54	1.85
	Posterior trunk	3.43	1.98	1.45
<i>Hoxd4</i>	Forebrain	3.84	2.78	1.06
	Anterior trunk	3.36	2.52	0.84
	Posterior trunk	3.58	2.53	1.05
<i>Hoxd3</i>	Forebrain	8.45	1.54	6.91
	Anterior trunk	9.31	3.33	5.97
	Posterior trunk	10.26	0.90	9.36
<i>Hoxd1</i>	Forebrain	14.83	9.01	5.81
	Anterior trunk	13.6	8.06	5.54
	Posterior trunk	12.49	6.35	6.14

HoxC cluster

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxc13</i>	Forebrain	11.05	3.44	7.61
	Anterior trunk	7.89	2.23	4.65
	Posterior trunk	9.26	2.86	6.40
<i>Hoxc9</i>	Forebrain	6.48	3.61	2.87
	Anterior trunk	4.30	2.44	1.86
	Posterior trunk	6.19	3.31	2.88
<i>Hoxc4</i>	Forebrain	8.80	2.60	6.20
	Anterior trunk	6.26	2.35	3.91
	Posterior trunk	9.25	3.16	6.09

HoxB cluster

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxb13</i>	Forebrain	3.35	1.69	1.66
	Anterior trunk	3.52	1.72	1.80
	Posterior trunk	2.97	1.41	1.56
<i>Hoxb(i9-13)</i>	Forebrain	5.30	2.20	3.10
	Anterior trunk	5.66	1.93	3.73
<i>Hoxb9</i>	Forebrain	4.38	1.54	2.83
	Anterior trunk	5.39	2.05	3.34
	Posterior trunk	5.94	1.89	4.05
<i>Hoxb4</i>	Forebrain	1.94	1.19	0.76
	Anterior trunk	4.09	2.55	1.54
	Posterior trunk	2.51	1.48	1.02

HoxA cluster

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxa13</i>	Forebrain	2.84	1.40	1.44
	Anterior trunk	3.45	1.56	1.89
	Posterior trunk	3.07	1.41	1.66
<i>Hoxa9</i>	Forebrain	6.36	3.59	2.77
	Anterior trunk	6.61	3.78	2.83
	Posterior trunk	8.09	4.21	3.89
<i>Hoxa4</i>	Forebrain	4.29	2.81	1.48
	Anterior trunk	4.07	2.77	1.30
	Posterior trunk	3.60	2.53	1.07

Mutant samples

Wildtype/Del(1-13)-D11

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxd11</i>	Forebrain	3.70	2.63	1.06
	Anterior trunk	3.97	2.78	1.19
<i>Hoxd4</i>	Forebrain	3.78	3.04	0.75
	Anterior trunk	4.18	3.13	1.05
<i>Hoxa4</i>	Forebrain	5.93	0.91	5.01
	Anterior trunk	6.38	5.53	0.85

Del(i8-10)/Del(1-13)-D11

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxd11</i>	Forebrain	3.96	2.63	1.33
	Anterior trunk	4.67	3.41	1.26
<i>Hoxd4</i>	Forebrain	4.26	3.41	0.85
	Anterior trunk	5.12	3.91	1.20
<i>Hoxa4</i>	Forebrain	4.81	3.94	0.87
	Anterior trunk	7.16	6.21	0.95

Del(8-10)/Del(1-13)-D11

Viewpoint	Tissue	Total reads (million)	Mappable reads (million)	Excluded reads (million)
<i>Hoxd11</i>	Forebrain	4.82	2.69	2.13
	Anterior trunk	5.02	2.01	3.02
<i>Hoxd4</i>	Forebrain	4.99	3.96	1.03
	Anterior trunk	3.63	2.55	1.08
<i>Hoxa4</i>	Forebrain	5.95	5.08	0.87
	Anterior trunk	6.01	5.10	0.91

Table S5.

4C-seq local saturated domains. Domains of very high fragment counts covering the *Hox* gene clusters that have been removed from the long-range interaction analysis, as determined by fitting the data to a power-law model.

HoxD cluster

Viewpoint	Tissue sample	Chromosome	Start	End
<i>Hoxd13</i>	Forebrain	chr2	74,392,412	74,600,877
	Anterior trunk	chr2	74,392,704	74,594,185
	Posterior trunk	chr2	74,393,976	74,566,497
<i>Hoxd9</i>	Forebrain	chr2	74,461,835	74,602,854
	Anterior trunk	chr2	74,459,555	74,590,305
	Posterior trunk	chr2	74,463,386	74,614,449
<i>Hoxd4</i>	Forebrain	chr2	74,478,036	74,612,146
	Anterior trunk	chr2	74,509,505	74,633,434
	Posterior trunk	chr2	74,507,949	74,607,136

HoxC cluster

Viewpoint	Tissue sample	Chromosome	Start	End
<i>Hoxc13</i>	Forebrain	chr15	102,696,348	102,965,012
	Anterior trunk	chr15	102,706,593	102,852,156
	Posterior trunk	chr15	102,705,389	102,845,832
<i>Hoxc9</i>	Forebrain	chr15	102,700,920	102,944,018
	Anterior trunk	chr15	102,745,519	102,898,398
	Posterior trunk	chr15	102,712,899	102,937,589
<i>Hoxc4</i>	Forebrain	chr15	102,789,560	102,910,946
	Anterior trunk	chr15	102,803,233	102,918,902
	Posterior trunk	chr15	102,788,128	102,922,993

HoxB cluster

Viewpoint	Tissue sample	Chromosome	Start	End
<i>Hoxb13</i>	Forebrain	chr11	96,045,784	96,075,371
	Anterior trunk	chr11	96,035,991	96,084,596
	Posterior trunk	chr11	96,042,044	96,071,005
<i>Hoxb9</i>	Forebrain	chr11	96,060,722	96,230,022
	Anterior trunk	chr11	96,080,379	96,250,175
	Posterior trunk	chr11	96,089,022	96,200,492
<i>Hoxb4</i>	Forebrain	chr11	95,978,849	96,270,670
	Anterior trunk	chr11	96,079,580	96,290,340
	Posterior trunk	chr11	96,074,474	96,282,206

HoxA cluster

Viewpoint	Tissue sample	Chromosome	Start	End
<i>Hoxa13</i>	Forebrain	chr6	52,187,651	52,232,566
	Anterior trunk	chr6	52,177,945	52,243,494
	Posterior trunk	chr6	52,186,582	52,235,158
<i>Hoxa9</i>	Forebrain	chr6	52,120,081	52,218,782
	Anterior trunk	chr6	52,117,390	52,206,173
	Posterior trunk	chr6	52,081,511	52,232,382
<i>Hoxa4</i>	Forebrain	chr6	52,092,252	52,211,219
	Anterior trunk	chr6	52,066,140	52,214,026
	Posterior trunk	chr6	52,083,291	52,203,455

4C – sequencing inverse primers

Analyzed gene / region	Inverse primer	Sequence
<i>Hoxd13</i>	iHoxd13 forward iHoxd13 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAATAAAATCTTAGACCTGGTCATG CAAGCAGAAGACGGGCATACGAGGCCGANTGGTGGCTGTAATAGG
<i>Hoxd11</i>	iHoxd11 forward iHoxd11 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAAGCATACTCTCAGAAGAGGCA CAAGCAGAAGACGGGCATACGACTAGGAAATCTCTAATTTTCAGG
<i>Hoxd9</i>	iHoxd9 forward iHoxd9 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTACGAACACCTCTGCGCCCT CAAGCAGAAGACGGGCATACGACCTCTCAGCTTGCAAGCGAT
<i>Hoxd(14-8)</i>	iHoxd(14-8) forward iHoxd(14-8) reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTACGAGCTGGGCTATTTTCAC CAAGCAGAAGACGGGCATACGACTGGTGGCCGTTCAAACTGA
<i>Hoxd4</i>	iHoxd4 forward iHoxd4 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAAGGACATAAAGCATTCATAGGGG CAAGCAGAAGACGGGCATACGATCCAGTGGAAATTTGGGTGGGAT
<i>Hoxd3</i>	iHoxd3 forward iHoxd3 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTACGACAGCATTTTCATTTGGCCCC CAAGCAGAAGACGGGCATACGAGCTGGCTCACCATTGGCAATTA
<i>Hoxd1</i>	iHoxd1 forward iHoxd1 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAATTTTACCTCGGGCTGGCT CAAGCAGAAGACGGGCATACGACATCTCTGGGGACTGGTCAGA
<i>Hoxc13</i>	iHoxc13 forward iHoxc13 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAGATATTTTCTGTGAGACATTTGTAAC CAAGCAGAAGACGGGCATACGAGCTCAATGTCTCTTCCCTTAACG
<i>Hoxc9</i>	iHoxc9 forward iHoxc9 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAATTCCTCCGGGGAGCCGTACAT CAAGCAGAAGACGGGCATACGAGTGTCCACAGGAGAGAGAGAGT
<i>Hoxc4</i>	iHoxc4 forward iHoxc4 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTACACACAAACCCAGCAGGT CAAGCAGAAGACGGGCATACGATGATGTGAATGGCCCGTGA
<i>Hoxb13</i>	iHoxb13 forward iHoxb13 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAAGGACTGTTCTCGGGGCTAT CAAGCAGAAGACGGGCATACGAMTCTGGCGTTCAGAGAGGCT
<i>Hoxb(19-13)</i>	iHoxb(19-13) forward iHoxb(19-13) reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAATAAAGTGGGGGTTGTAGAGTGT CAAGCAGAAGACGGGCATACGAGCTGAAGACCTCTGGATGGC
<i>Hoxb9</i>	iHoxb9 forward iHoxb9 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAAGATTGAGGAGTCTGGCCACTT CAAGCAGAAGACGGGCATACGATCATCAAAACCAAGCAGGGCA
<i>Hoxb4</i>	iHoxb4 forward iHoxb4 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTATCACACGCACAAGAACACCC CAAGCAGAAGACGGGCATACGACTCCCGAANTAGTGGTGAAT
<i>Hoxa13</i>	iHoxa13 forward iHoxa13 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAACACTTGCACACCAAGAAATGC CAAGCAGAAGACGGGCATACGAGGCGAGGCTCAAGGCTTTTAT
<i>Hoxa9</i>	iHoxa9 forward iHoxa9 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAAGGATGCAATAGATTTCAG CAAGCAGAAGACGGGCATACGAACAGTGTCAAGTATTTTGG
<i>Hoxa4</i>	iHoxa4 forward iHoxa4 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAGCAAGGAGAGGAAACTTAC CAAGCAGAAGACGGGCATACGAGTGTGTATCTGTGCATTC
<i>Dlx1</i>	iDlx1 forward iDlx1 reverse	AATGATACGGCGGACCAACCGAACACTCTTTCCCTACACGAGCGCTCTTCGATCTAATAATTAACGAGACTAATAACGTTCGACA CAAGCAGAAGACGGGCATACGAGTGGCTTTAACGGGCAAGG

Table S6.
Primer Sequences.

References and Notes

1. M. Kmita, D. Duboule, Organizing axes in time and space; 25 years of colinear tinkering. *Science* **301**, 331 (2003).
2. R. Krumlauf, Hox genes in vertebrate development. *Cell* **78**, 191 (1994).
3. N. Soshnikova, D. Duboule, Epigenetic temporal control of mouse Hox genes in vivo. *Science* **324**, 1320 (2009).
4. R. J. Palstra *et al.*, The beta-globin nuclear compartment in development and erythroid differentiation. *Nat. Genet.* **35**, 190 (2003).
5. M. A. Ferraiuolo *et al.*, The three-dimensional architecture of Hox cluster silencing. *Nucleic Acids Res.* **38**, 7472 (2010).
6. J. Fraser *et al.*, Chromatin conformation signatures of cellular differentiation. *Genome Biol.* **10**, R37 (2009).
7. K. C. Wang *et al.*, A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature* **472**, 120 (2011).
8. S. Chambeyron, W. A. Bickmore, Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. *Genes Dev.* **18**, 1119 (2004).
9. C. Morey, N. R. Da Silva, M. Kmita, D. Duboule, W. A. Bickmore, Ectopic nuclear reorganisation driven by a Hoxb1 transgene transposed into Hoxd. *J. Cell Sci.* **121**, 571 (2008).
10. R. Eskeland *et al.*, Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Mol. Cell* **38**, 452 (2010).
11. Material and methods are available as supporting material on Science Online.
12. M. Simonis *et al.*, Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nat. Genet.* **38**, 1348 (2006).
13. A. P. Lee, E. G. Koh, A. Tay, S. Brenner, B. Venkatesh, Highly conserved syntenic blocks at the vertebrate Hox loci and conserved regulatory elements within and outside Hox gene clusters. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6994 (2006).
14. L. Zeltser, C. Desplan, N. Heintz, Hoxb-13: a new Hox gene in a distant region of the HOXB cluster maintains colinearity. *Development* **122**, 2475 (1996).
15. P. Tschopp, B. Tarchini, F. Spitz, J. Zakany, D. Duboule, Uncoupling time and space in the collinear regulation of Hox genes. *PLoS Genet.* **5**, e1000398 (2009).
16. D. M. Wellik, Hox patterning of the vertebrate axial skeleton. *Dev. Dyn.* **236**, 2454 (2007).
17. T. Young *et al.*, Cdx and Hox genes differentially regulate posterior axial growth in mammalian embryos. *Dev. Cell* **17**, 516 (2009).
18. J. Zakany, M. Kmita, P. Alarcon, J. L. de la Pompa, D. Duboule, Localized and transient transcription of Hox genes suggests a link between patterning and the segmentation clock. *Cell* **106**, 207 (2001).

19. B. Tarchini, T. H. Huynh, G. A. Cox, D. Duboule, HoxD cluster scanning deletions identify multiple defects leading to paralysis in the mouse mutant Ironside. *Genes Dev.* **19**, 2862 (2005).
20. B. Tarchini, D. Duboule, Control of Hoxd genes' collinearity during early limb development. *Dev. Cell* **10**, 93 (2006).
21. M. Simonis, J. Kooren, W. de Laat, An evaluation of 3C-based methods to capture DNA interactions. *Nat. Methods* **4**, 895 (2007).
22. E. Lieberman-Aiden *et al.*, Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289 (2009).
23. B. Langmead, C. Trapnell, M. Pop, S. L. Salzberg, Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25 (2009).
24. Y. Zhang *et al.*, Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* **9**, R137 (2008).