Dynamic 3D chromatin architecture contributes to enhancer specificity and limb morphogenesis

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Supplementary Figure 1

Probing the regulatory sequences and activities of the Pitx1 locus.

a, Table listing putative enhancer regions tested by lacZ reporter assay. Above is the lacZ reporter activity in E11.5 embryos. Numbers represent the number of embryos displaying the staining shown in the table. b, Table listing the genomic location of lacZ sensors 1 and 2 at the Pitx1 locus (mm9) and lacZ reporter staining in E11.5 embryos.
Supplementary Figure 2
Quantification of Pitx1 hindlimb transcription in several deletion mice.

In all analyses, we used a one-sided t test to evaluate the significance of the decrease in Pitx1 expression and n represents the number of wild-type and mutant hindlimb pairs assayed. The s.d. is displayed as error bars and the measure of the center corresponds to the average. a, qRT–PCR mRNA quantification of E11.5 Pitx1<sup>del1/del1</sup> hindlimbs (P = 0.0002, n = 4). b, qRT–PCR mRNA quantification of E11.5 Pitx1<sup>del2/del2</sup> hindlimbs (P = 0.025, n = 4). c, qRT–PCR mRNA quantification of E11.5 Pitx1<sup>del3/del3</sup> hindlimbs (P = 0.019, n = 3). d, qRT–PCR mRNA quantification of E11.5 Pitx1<sup>Pen/Pen</sup> hindlimbs (P = 0.012, n = 4).
Supplementary Figure 3

cHi-C of the extended Pitx1 locus in wild-type forelimb, hindlimb and midbrain tissues.
a, cHi-C interaction map in E11.5 forelimb (blue) and hindlimb (red) tissues over a 3-Mb captured region. Bottom, subtraction of hindlimb and forelimb cHi-C whereby blue indicates a higher chromatin interaction frequency in forelimb and red a higher interaction frequency in hindlimb as compared to each other. Note that only the Pitx1 locus displays clear changes in chromatin interactions within the entire captured region. b, cHi-C interaction map in E11.5 forelimb (blue) and E10.5 midbrain (red) tissues over a 3-Mb captured region. Bottom, subtraction of midbrain and forelimb cHi-C whereby blue indicates a higher chromatin interaction frequency in forelimb and red a higher interaction frequency in midbrain as compared to each other. Note the absence of chromatin interaction changes at the Pitx1 locus, in contrast to the neighboring telomeric domain (see blue domain in subtraction map), which include Cxcl14, a gene transcriptionally repressed in midbrain and active in forelimb.
Supplementary Figure 4

Modeling of the Pitx1 locus 3D architecture in wild-type forelimb and hindlimb.

a,b, Histograms displaying the position and abundance of 14 different types of binding sites (Methods) along the genome, in forelimbs (top) and hindlimbs (bottom) as derived from the E11.5 chi-C data. Each binding site is displayed with a different color. c,d, Contact maps derived from chi-C (above) and SBS model (below) display high similarity. The Pearson correlation, \( r \), and the genomic-distance-corrected Pearson correlation, \( r' \), between the chi-C and SBS matrices (105 bins × 105 bins = 11,025) are \( r = 0.98 \) and \( r' = 0.84 \) in forelimb and \( r = 0.98 \) and \( r' = 0.82 \) in hindlimb. e,f, Subtraction matrices between chi-C and SBS model in wild-type forelimbs (top) and hindlimbs (bottom). Differences above random background are shown in red and blue. g,h, A representative 3D structure of the locus in forelimb (top) and hindlimb (bottom), selected from the ensemble of 'single-cell' model-derived conformations (Methods). In Fig. 4d,e, the corresponding coarse-grained versions are shown to highlight the position of genes and regulators.
Supplementary Figure 5

Subtraction matrix between the SBS models of wild-type forelimb and hindlimb.
The corresponding cHi-C data are shown in Fig. 4c.
Supplementary Figure 6

Relative changes in physical distances between wild-type forelimb and hindlimb 3D structure.

Heat map showing relative changes in physical distances between forelimb and hindlimb 3D structure as measured by the polymer model.
**Supplementary Figure 7**

**Effect of Pitx1 and HoxC loss of function on the 3D structure of the Pitx1 locus in hindlimbs.**

**a.** cHi-C subtraction between wild-type and Pitx1<sup>fs/fs</sup> mutant hindlimb tissue at E11.5. Chromatin interactions more prevalent in mutant or wild-type hindlimb tissues are shown in red and blue, respectively. Significant changes in interactions are highlighted in black boxes (FDR = 0.05). Interactions significantly reduced between regulatory anchors are indicated with a blue arrow (Pitx1–RA3 interaction). Derived viewpoint from cHi-C map, vC, using the Pitx1 viewpoint is shown in red. Below is the subtraction track between wild-type and mutant hindlimb tissues using the respective viewpoint.

**b.** cHi-C subtraction between wild-type and HoxC<sup>del/del</sup> mutant hindlimb tissues at E11.5. Chromatin interactions more prevalent in mutant or wild-type hindlimb tissues are shown in red and blue, respectively. Significant changes are highlighted in black boxes (FDR = 0.05). Interactions significantly reduced between regulatory anchors are indicated with blue arrows (Pitx1–RA3 and Pitx1–Pen). qRT–PCR quantification of Pitx1 in HoxC<sup>del/del</sup> mutant hindlimb tissues at E11.5 showed an average 36% reduction. (We used a one-sided t test to evaluate the significance of decrease in Pitx1 expression and found \( P = 0.02; n = 4 \) wild-type and mutant hindlimb pairs; s.d. is displayed as error bars; the measure of the center is the average of the data points.)
Deletions of the Pitx1 or Neurog1 H3K27me3 domains are not sufficient to perturb the hindlimb-restricted regulatory activity of the locus.

a, cHi-C subtraction between wild-type and Neurog1\textsuperscript{del/del} mutant forelimb tissue at E11.5. Chromatin interactions more prevalent in mutant or wild-type forelimb tissues are shown in red and blue, respectively. Significant changes are highlighted in black boxes (FDR =
0.05). Right, Neurog1^{del/del} embryos do not show changes in Pitx1 expression in E11.5 forelimbs as seen in WISH (photo) and quantified by qRT–PCR. (We used a one-sided t test to evaluate the significance of increased Pitx1 expression and found $P = 0.38; n = 3$ wild-type and mutant limb pairs; the center is the average and the s.d. is displayed by the error bars.) Below, derived vC from the Pitx1 viewpoint in wild-type and Neurog1^{del/del} forelimbs are shown in blue and red, respectively. Below is the subtraction track between wild-type and mutant forelimb tissue using the respective viewpoint. b. Whole chromosome 13 view of vC from the Pitx1 viewpoint. Note that these profiles display the genomic region enriched in cHi-C as well as the non-enriched part of the chromosome. c. Staining of embryos with a lacZ sensor integrated in the RA3 region. Wild-type (top) and Pitx1^{del/del} (bottom) staining display no obvious difference between fore- and hindlimb. Eighteen of 18 embryos displayed the same staining in the wild-type background, and 28 of 28 displayed the same staining in the Pitx1^{del/del} background.
Supplementary Figure 9

Modeling of the Pitx1 locus 3D architecture in Pitx1<sup>inv1/inv1</sup> forelimbs.

**a.** Histograms displaying the position and abundance of 14 different types of binding sites (Methods) along the genome, in Pitx1<sup>inv1/inv1</sup> forelimbs at E11.5. As in Supplementary Fig. 4a,b, each binding site is displayed with a different color. **b.** Contact maps derived from cHi-C (above) and SBS model (below) display high similarity. The Pearson correlation, r, and the genomic-distance-corrected Pearson correlation, r', between the cHi-C and SBS matrices (105 bins * 105 bins = 11,025) are r = 0.97 and r' = 0.74. **c.** Subtraction matrix between cHi-C and SBS model in Pitx1<sup>inv1/inv1</sup> forelimbs. Differences above random background are shown in red and blue. **d.** A representative 3D structure of the locus in Pitx1<sup>inv1/inv1</sup> forelimbs, selected from the ensemble of 'single-cell' model-derived conformations (Methods). In Fig. 6e, the corresponding coarse-grained version is shown to highlight the position of genes and regulators.
Supplementary Figure 10
Quantification of Pitx1 forelimb transcription in several deletion and inversion mice.
WISH and qRT–PCR mRNA quantification of deletions and inversion at the *Pitx1* locus. In all analyses, we used a one-sided *t* test to evaluate the significance of increased *Pitx1* expression and *n* represents the number of wild-type and mutant forelimb pairs assayed. The s.d. is displayed as error bars and the measure of the center corresponds to the average. 

Supplementary Figure 11

Schematic representation of the Pitx1 locus in several species.

Schematic representation of the Pitx1 extended locus in several species, demonstrating the conserved synteny of the region. The species from top to bottom are human (Homo sapiens), mouse (Mus musculus), chicken (Gallus gallus), lizard (Anolis carolinensis), frog (Xenopus (Silurana) tropicalis), zebrafish (Danio rerio), stickleback (Gasterosteus aculeatus), spotted gar (Lepisosteus oculatus), and elephant shark (Callorhinchus milii). Pen (green) is found in tetrapods but not in fish. The previously characterized pelvic enhancer Pel (red) is displayed in red in stickleback.
Conservation of sequences between vertebrates along the Pitx1 regulatory landscape.

Conservation of sequences between vertebrates along the Pitx1 regulatory landscape. A zoomed-in view of the Pen region shows that the element is conserved with human (Homo sapiens), chicken (Gallus gallus), and frog (Xenopus (Silurana) tropicalis), but not with bony or cartilaginous fishes (here stickleback (Gasterosteus aculeatus), spotted gar (Lepisosteus oculatus), and elephant shark (Callorhinchus milii).