Supporting Information

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SI Materials and Methods

ChIP was carried out according to a previously described protocol (1). Embryonic day (E) 10.5 or E11.5 mouse forelimbs were collected, homogenized through 18G needles, and fixed for 15 min in 1% formaldehyde solution at room temperature. Crosslinked samples were sonicated to yield 200- to 500-bp fragments. IgG was used as a control in these assays. The PCR primers were designed as described previously (1–3). The following antibodies were used for the ChIP experiments: anti-Hoxa5 (sc-13199; Santa Cruz Biotechnology), anti-Hand2 (sc-9409; Santa Cruz Biotechnology), and anti-Plzf (sc-22839; Santa Cruz Biotechnology). The primers used to generate protein expression constructs and reporter vectors were as follows:

Gene name	Forward primer	Reverse primer
HoxA5	5′ agtgtcctcgagatga gctcttattttgtaaactc 3′	5′ agtgtctctagatcagggg cggaaagcccccc 3′
HoxB5	agtgtcaggcctatgagc tcgtactttgta	agtgtctctagatcaaggttgg aaggcgctg
HoxC5	agtgtcaggcctatgagc tcctacgtagcc	agtgtctctagactaaagagc ttctttgctc
PLZF	agtgtcgaattcaatggat ctgacaaaaatgggc	agtgtctctagatcacacatag cacaggtagag
ZRS	agtgtcggtacctactttaa tgcctatctttgatttg	agtgtcgagctccacatagcaa cagttagtgag

1. Capellini TD, et al. (2006) Pbx1/Pbx2 requirement for distal limb patterning is mediated by the hierarchical control of Hox gene spatial distribution and Shh expression. *Development* 133(11):2263–2273.
2. Barna M, et al. (2002) Plzf mediates transcriptional repression of HoxD gene expression through chromatin remodeling. *Dev Cell* 3(4):499–510.
3. Galli A, et al. (2010) Distinct roles of Hand2 in initiating polarity and posterior *Shh* expression during the onset of mouse limb bud development. *PLoS Genet* 6(4): e1000901.

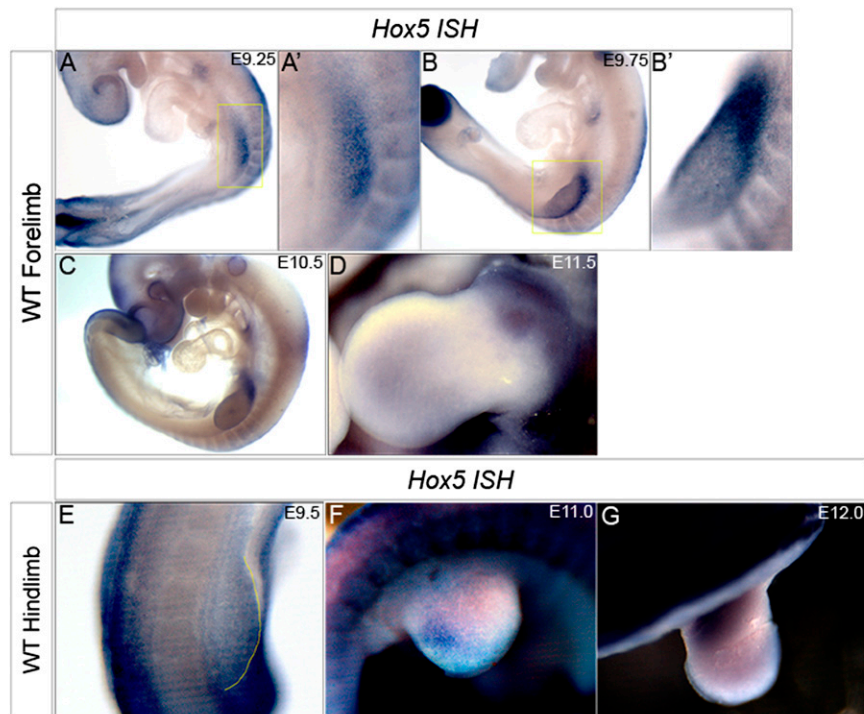

Fig. S1. *Hox5* expression patterns in WT forelimbs and hindlimbs at different stages. (*A*–*D*) *Hox5* genes exhibit dynamic expression during early forelimb development. *Hox5* expression appears uniform across the entire forelimb bud at E9.25 (*A* and *A*′), but becomes localized to anterior regions of forelimb buds at later stages (*B*–*D*). *A*′ and *B*′ represent magnified regions of the forelimbs outlined in yellow boxes in *A* and *B*, respectively. (*E*) At E9.5, *Hox5* is broadly expressed throughout the WT hindlimb. (*F*) At E11.0, *Hox5* expression is localized in the posterior region of the hindlimb. (*G*) *Hox5* expression at E12.0 is restricted to the proximal hindlimb. In situ hydridization was performed using combined probes for *Hoxa5, Hoxb5*, and *Hoxc5*.

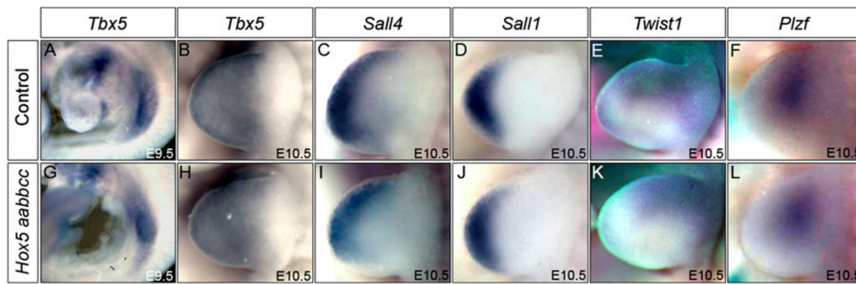

Fig. S2. Expression of anterior patterning regulators, including *Tbx5*, *Sall4/1*, *Twist1*, and *promyelocytic leukemia zinc finger* (*Plzf*), is unaltered in *Hox5* mutant forelimbs. *Tbx5* (*A* and *G*, *B* and *H*), *Sall4* (*C* and *I*), *Sall1* (*D* and *J*), *Twist1* (*E* and *K*), and *Plzf* (*F* and *L*) expression are unchanged at E10.5 in *Hox5* mutant limb buds compared with controls.

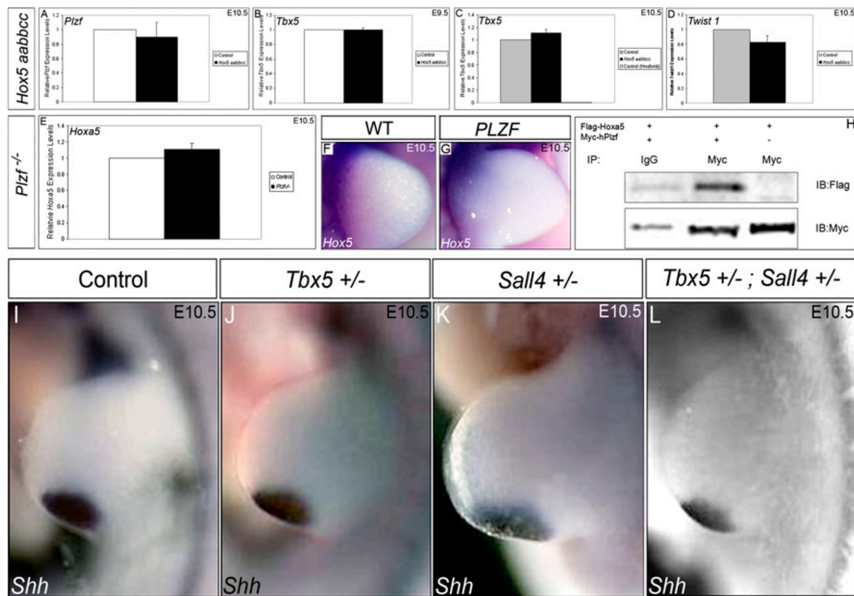

Fig. S3. *Hox5* and *Plzf* expression are independent. (*A*) *Plzf* expression levels are not altered in *Hox5* mutant forelimbs compared with controls. (*B* and *C*) Quantitative RT-PCR (qRT-PCR) data reveal that the *Tbx5* expression levels are unchanged in *Hox5* mutants at E9.5 (*B*) and E10.5 (*C*). (*D*) qRT-PCR data show that *Twist1* expression levels are unchanged in *Hox5* mutants at E10.5. (*E*) qRT-PCR data reveal that *Hoxa5* expression is unchanged in E10.5 *Plzf* mutants. (*F* and *G*) Whole-mount *Hox5* in situ hybridization demonstrating that *Hox5* expression is unaffected in $PLZF^{-/-}$ mutant forelimbs at E10.5. (*H*) Coimmunoprecipitation (IP) of Myc-tagged Plzf with Hoxa5 was observed from immunoprecipitation with anti-Myc (Plzf) antibodies and immunoblotting (IB) for anti-Flag (Hoxa5). IgG was used as a negative control. (*I–L*) *Shh* expression is unaltered in $Tbx5^{+/-}$, $Sall4^{+/-}$, and $Tbx5^{+/-}$;$Sall4^{+/-}$ forelimbs at E10.5.

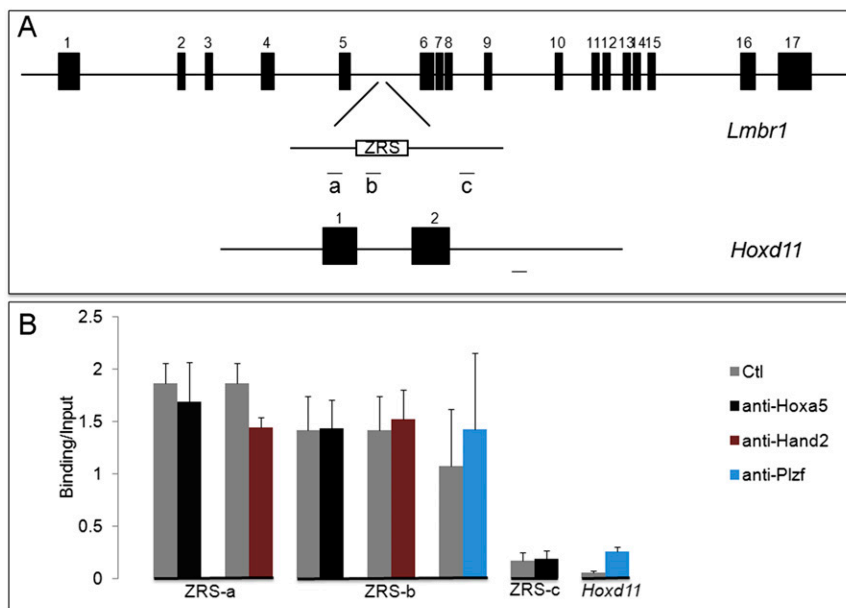

Fig. S4. ChIP results at the zone of polarizing activity regulatory sequence (ZRS). (*A*) Neither *Hoxb5* nor *Plzf* shows enriched binding at the ZRS in vivo owing to very high background binding at ZRS and perhaps the small number of responding cells. (*B*) Very high background levels (with no antibody or nonspecific antibody) are observed at regions a and b in the ZRS compared with region c and other regions outside the ZRS (e.g., *Hoxd11*). ChIP results at the region downstream of *Hoxd11* demonstrate that the Plzf antibody can be used successfully in these experiments at other sites, as reported previously (2).