**Cell Reports, Volume *20***

## Supplemental Information

# The Conserved Sonic Hedgehog Limb Enhancer Consists of Discrete Functional Elements that Regulate Precise Spatial Expression

**Laura A. Lettice, Paul Devenney, Carlo De Angelis, and Robert E. Hill**

**Table S1- Relates to Figures 5 and 6**
**Oligonucleotides used.**

| **Px330 oligos** | |
|---|---|
| WMS guideF | CACCGTGGTCATAAAATACAGTACA |
| WMS guideR | AAACTGTACTGTATTTTATGTCCAC |
| EBox guideF | CACCGCACTGAGGGGAAAAGTCATC |
| EBox guideF | AAACGATGACTTTTCCCCTCAGTGC |
| 3' guideF | CACCGAACAATTTATGGATCATCAG |
| 3' guideR | AAACCTGATGATCCATAAATTGTTC |
| | |
| **Hox gene IVT constructs** | |
| HoxD9F | CATGAT**CATATG**TCGTCCAGTGGCACCC |
| HoxD9R | CATGAT**CTCGAG**GTCTCCTTTAGGGCACTTCTC |
| HoxD10F | CATGAT**CATATG**TCCTTTCCCAACAGCTCTC |
| HoxD10R | CATGAT**CTCGAG**AGAAAAGGTGAGGTTGGCGGTC |
| HoxD11F | CATGAT**CATATG**AACGACTTTGACGAGTGCG |
| HoxD11R | CATGAT**CTCGAG**AAATAAGGGGTTTCCAGTGAAATATTG |
| HoxD12F | CATGAT**CATATG**TGTGAGCGCAGTCTCTAC |
| HoxD12R | CATGAT**CTCGAG**ATAGAGGGCCAGTGCTTGCTC |
| HoxD13F | CATGAT**CATATG**AGCCGCTCGGGACTTGG |
| HoxD13R | CATGAT**CTCGAG**GGAGACAGTGTCTTTGAGCTTG |
| | |
| **Hoxsites EMSA oligos** | |
| Hoxsite 1F | TTGTCCTGG**TTTATG**TCGCTTTTG |
| Hoxsite 1R | CAAAAGCGA**CATAAA**CCAGGACAA |
| MutHoxsite 1F | TTGTCCTGG**TTgcgG**TCGCTTTTG |
| MutHoxsite 1R | CAAAAGCGA**CcgcAA**CCAGGACAA |
| Hoxsite 2F | CAAACTTA**CATAAAA**GTGACCTTGT |
| Hoxsite 2R | ACAAGGTCAC**TTTTATG**TAAGTTTG |
| MutHoxsite 2F | CAAACTTA**CATAcgc**GTGACCTTGT |
| MutHoxsite 2R | ACAAGGTCAC**gcgTATG**TAAGTTTG |
| Hoxsite 3F | TGTACTGTAT**TTTATG**ACCAGATGACT |
| Hoxsite 3R | AGTCATCTGGT**CATAAAA**TACAGTACA |
| MutHoxsite 3F | TGTACTGTA**TTTcgcG**ACCAGATGACT |
| MutHoxsite 3R | AGTCATCTGGT**CgcgAAA**TACAGTACA |
| Hoxsite 4F | CTGATGATC**CATAAA**TTGTTGGAA |
| Hoxsite 4R | TTCCAACAA**TTTATG**GATCATCAG |
| MutHoxsite 4F | CTGATGATC**CcgcAA**TTGTTGGAA |
| MutHoxsite 4R | TTCCAACAA**TTgcgG**GATCATCAG |
| | |
| **LacZ Transgenic constructs** | |
| ZRS F | GATCAT**AAGCTT**TACTTTAAGCCATCTTTG |
| ZRS R | GATCAT**AAGCTT**CACATAGAACACTTAGTGAG |
| | |
| Mutate ZRS oligos | |
| Cu F | GACCTTGTACTaTA**TTTTATG**ACCAGATGACTTTTCCCTC |
| Cu R | GAGGGAAAGTCATCTGGT**CATAAAA**TAtAGTACAAGGTC |
| MutHoxsite 1F | CAGTTTGAGATTGTCCTGG**TcgcTG**TCGCTTTTGGCAAAC |
| MutHoxsite 1R | GTTTGCCAAAAGCGA**CAgcgA**CCAGGACAATCTCAAACTG |
| MutHoxsite 2F | GTCGCTTTTGGCAAACTTA**CATAcgc**GTGACCTTGTACTG |
| MutHoxsite 2R | CAGTACAAGGTCAC**gcgTATG**TAAGTTTGCCAAAGCGAC |
| MutHoxsite 3F | GACCTTGTACTGTA**TTTcgcG**ACCAGATGACTTTTCCCTC |
| MutHoxsite 3R | GAGGGAAAGTCATCTGGT**CgcgAAA**TACAGTACAAGGTC |
| MutHoxsite 3F+Cu | GACCTTGTACTaTA**TTTcgcG**ACCAGATGACTTTTCCCTC |
| MutHoxsite 3R+Cu | GAGGGAAAGTCATCTGGT**CgcgAAA**TAtAGTACAAGGTC |
| | |

Figure S1-relates to Figure 1

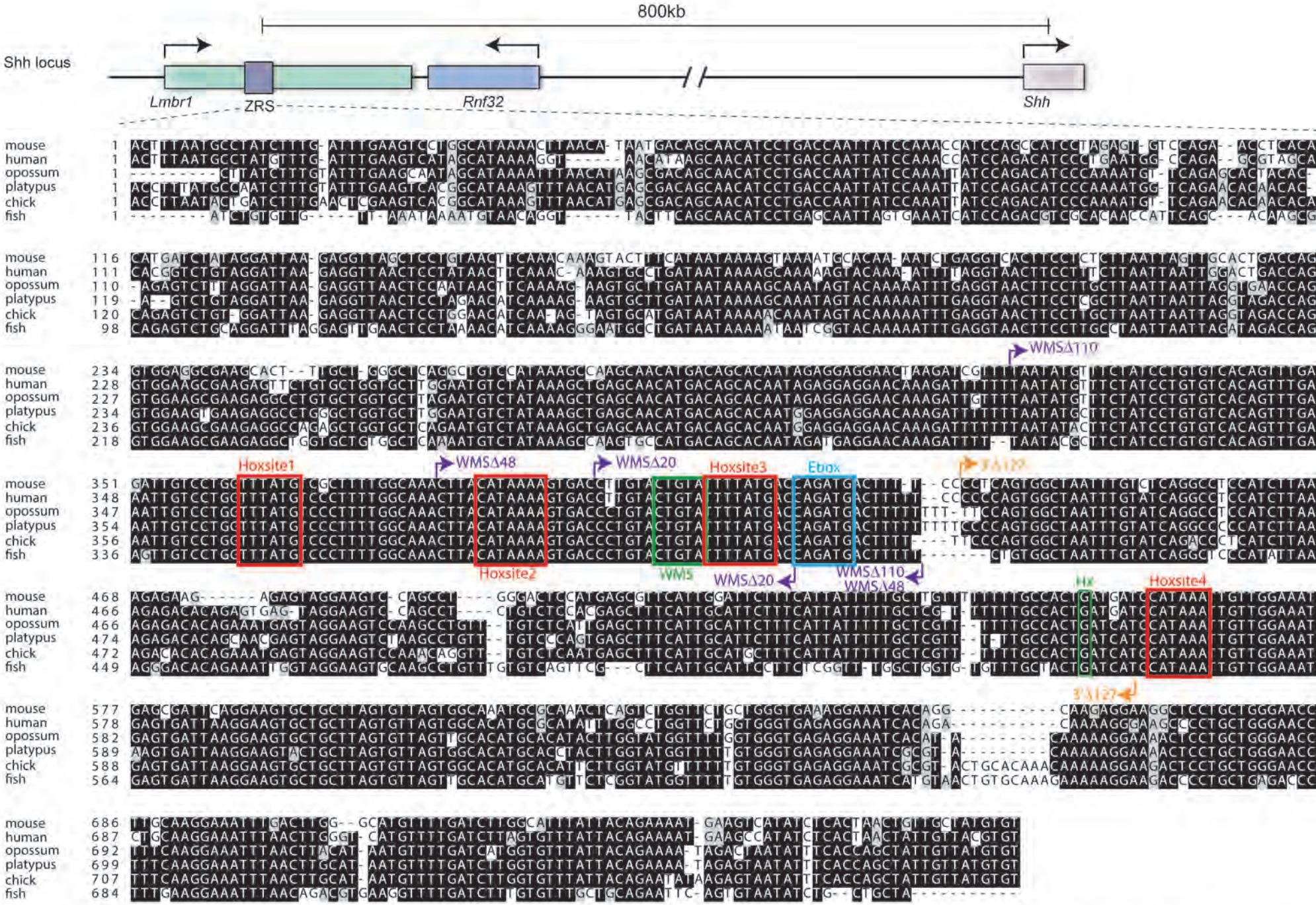
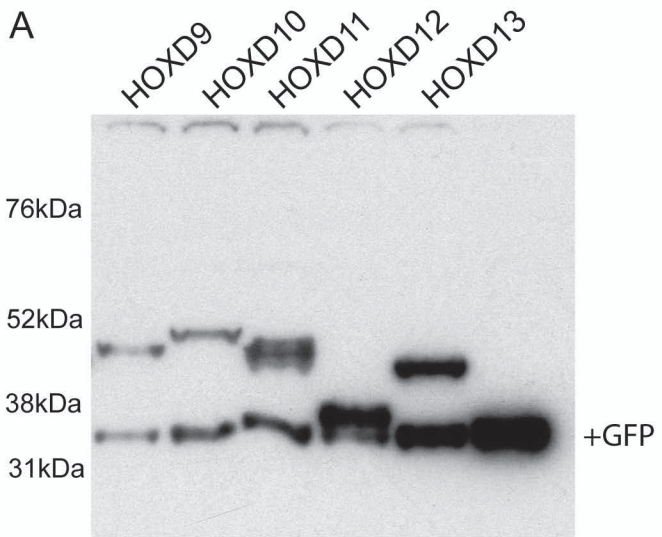
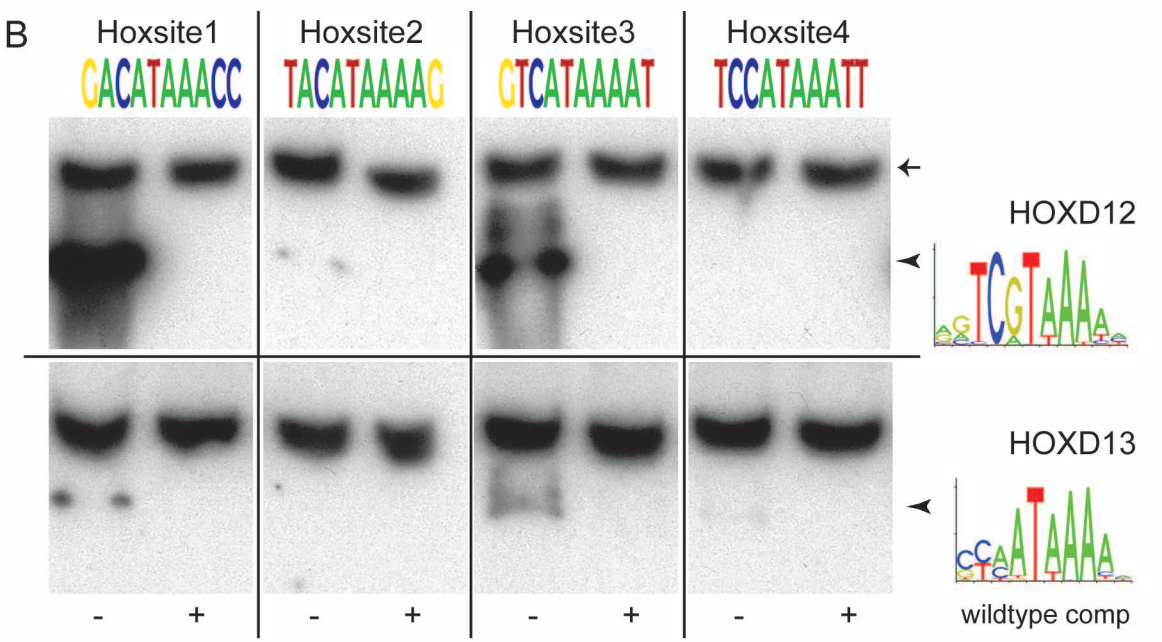



Figure S1. Top line shows a graphical representation of the Lmbr1 to Shh genomic region with the position of ZRS indicated by the grey box. The direction of transcription of the genes is indicated by the arrows. Underneath is the sequence line-up comparing the ZRS in diverse vertebrate species (listed to the left of the sequence) including three different vertebrate classes (mammal, birds and fish) representing >400Myrs of evolution. The sequences for the WMS site and Hx mutation (green) and the Ebox (blue) are boxed. Also boxed in red are the positions of the 4 Hoxsites. The start and end positions of all the large deletions (WMSΔ110, WMSΔ48, WMSΔ20 and 3'Δ127) are indicated.

Figure S2 - relates to Figure 5
Western blots of HOXD proteins and analysis of HOXD12 and D13 binding.




(A) shows a western blot of protein from the IVT reactions probed with an anti HisTag antibody. In addition to the Hox gene containing vector, each reaction included the GFP control vector and a GFP band can be seen in all lanes.
(B) shows the sequence of the Hoxsites1 -4 in the same orientation. The consensus binding sites for the proteins HoxD12 and D13 are shown as position weight matrices under their gene names, adjacent to the relevent EMSAs (D12 in upper panels; D13 in lower panels). For each doublet of EMSAs, the lanes are shown as binding to a labelled Hoxsite oligonucleotide with no competition (lanes marked as - ) and with excess of the wildtype oligo as competitor (lanes marked as +). The specific binding is indicated by the arrowheads, while the non-specific band (arrow) indicates the increased length of exposure time necessary, compare with the equivalent band in Figure 4.