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Supplemental Information

Timed Collinear Activation of Hox Genes during Gastrulation Controls the Avian Forelimb Position

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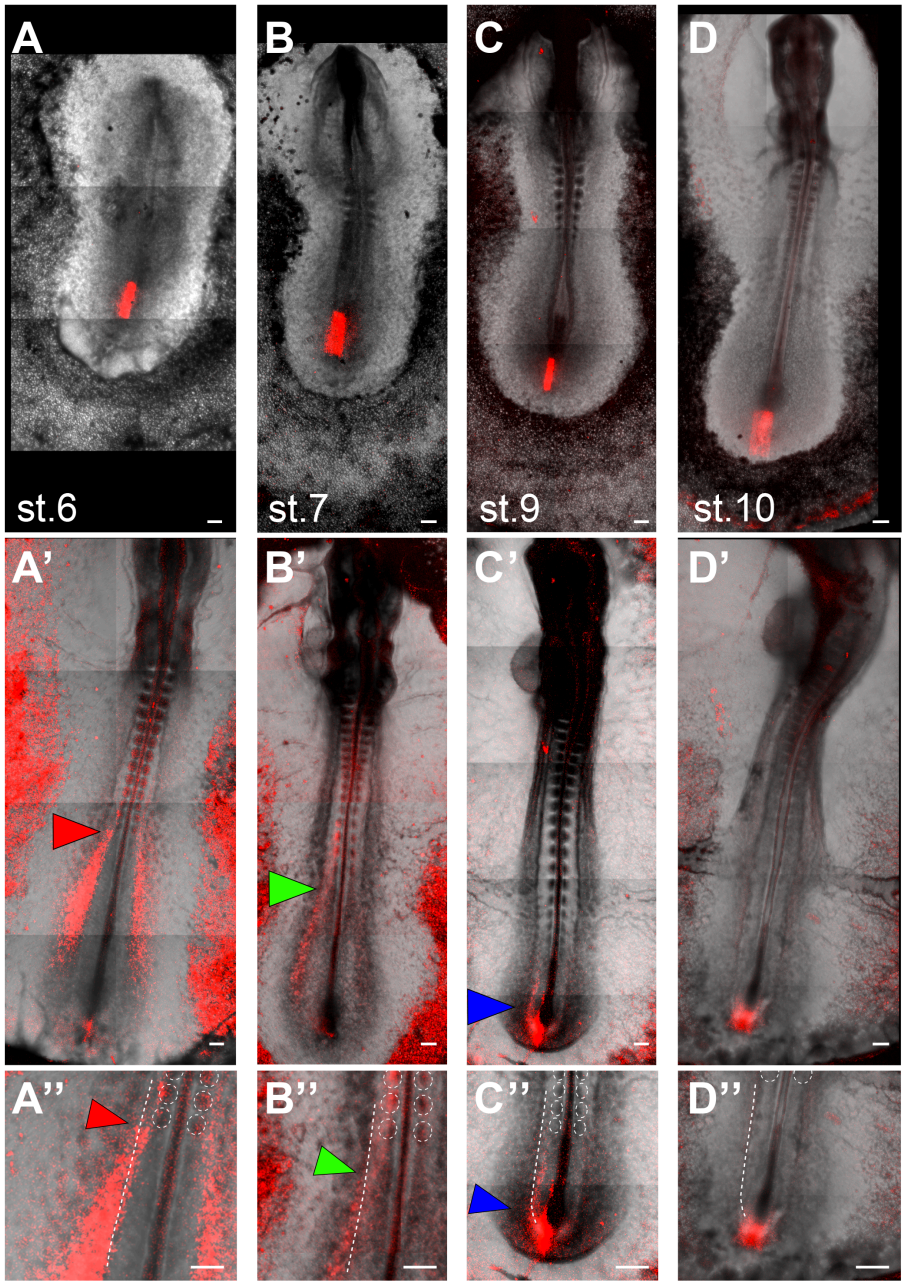

Figure S1. Progressive formation of the LPM during gastrulation. Related to Figure 2.
(A-D) Photoconversion (red) of the presumptive LPM in the PS of *hUbC:mEOS2FP* transgenic quail embryos at stage 6 (A), 7 (B), 9 (C) and 10 (D). (A'-D') Distribution of red-labeled cells 24h after photoconversion. Cells photoconverted at stage 6 (A) are found in the forelimb and interlimb domains (A', red arrow head). Cells photoconverted at stage 7 (B) are found more posteriorly, in the interlimb and hindlimb domains (B', green arrowhead). Cells photoconverted at stage 9 (C) are only found in the hindlimb domain (C', blue arrowhead). Photoconversion of the remaining PS at stage 10 (D) only labels the tail bud tissue (D'). Note the gradual posterior distribution, as cells are photoconverted at later stages. (A"-D") Higher magnification of (A'-D') showing the anterior limit of photoconverted cells (arrowheads). Dashed white lines outline the border between lateral plate and somitic mesoderm; dashed white circles outline somites. n=16 photoconverted embryos. Scale bar is 100µm. See also Video S3.

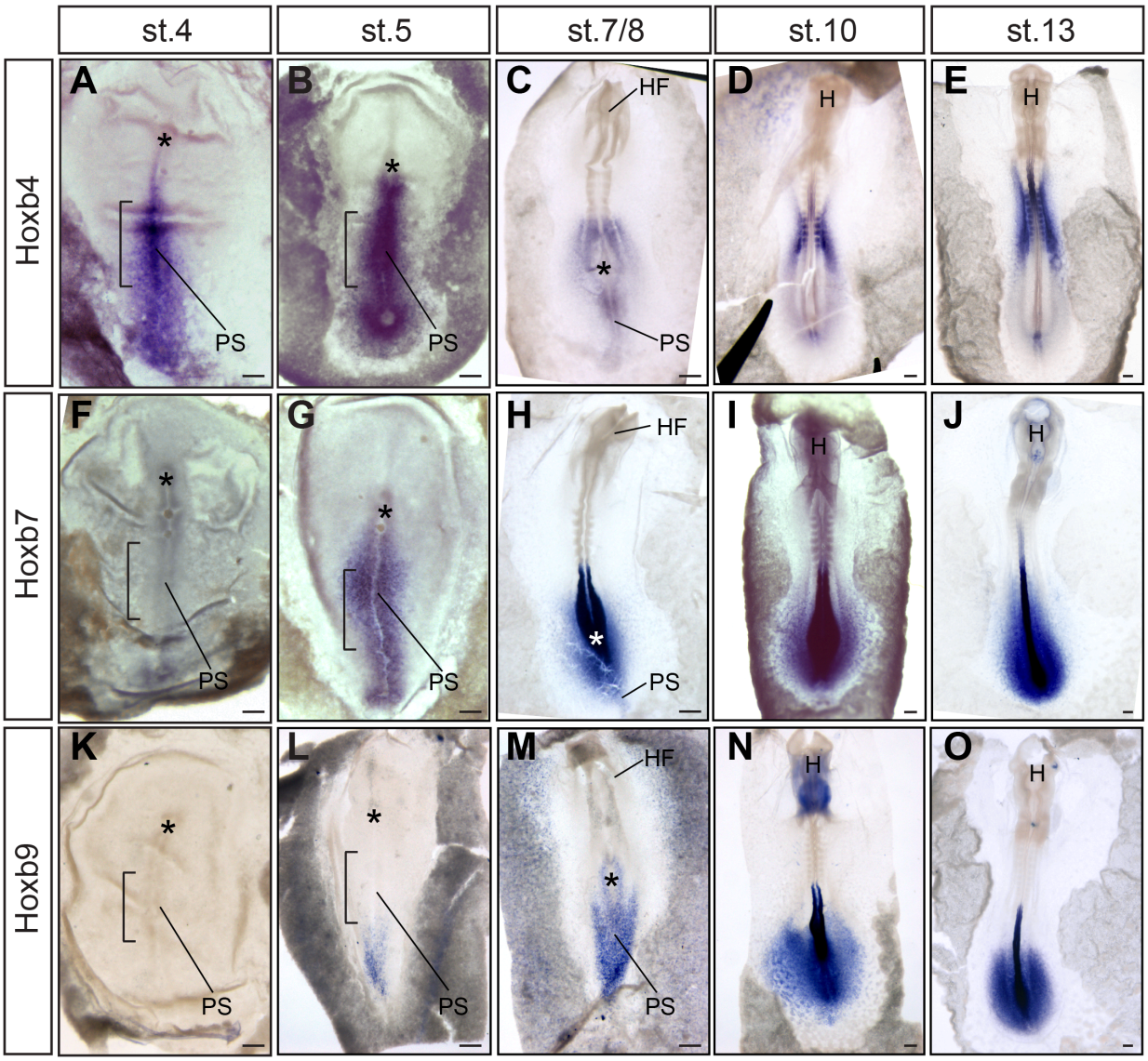

Figure S2. Characterization of Hoxb temporal and spatial collinearity with respect to the LPM. Related to Figure 2.

(A-O) Hoxb4 (A-E), Hoxb7 (F-J) and Hoxb9 (K-O) expression patterns. Asterisks represent the Hensen's node; black brackets outline the presumptive LPM; PS, primitive streak; HF, head folds; H, head. Scale bar is 100μm.

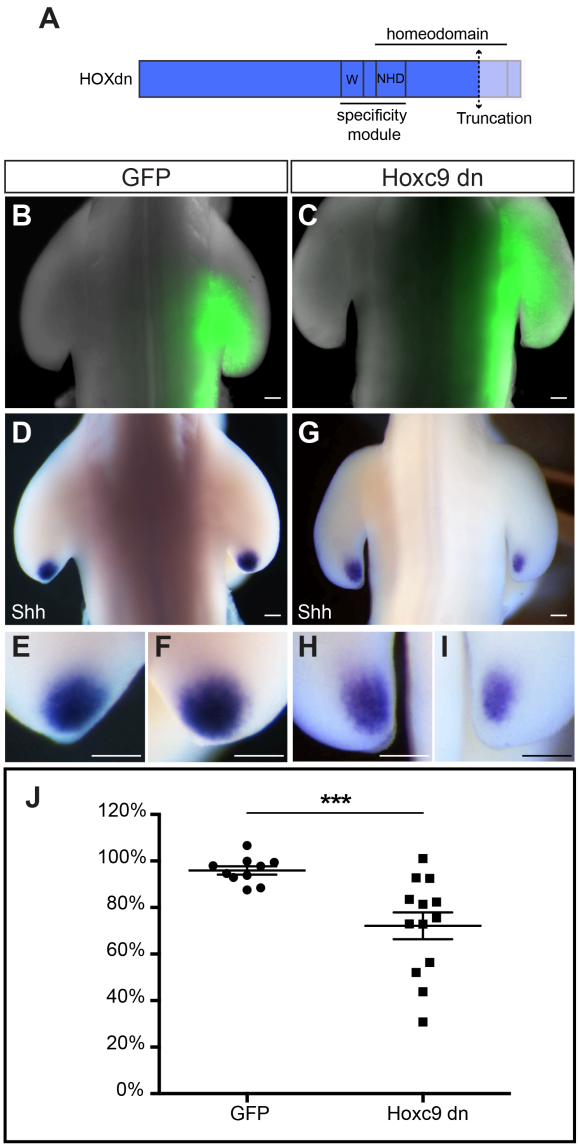

Figure S3. Hoxc9dn downregulates Sonic Hedgehog (Shh) expression in the posterior limb mesenchyme. Related to Figures 2 and 3.

(A) Schematic representation of truncated HOX (HOXdn) proteins. The truncated C-terminal part of the protein (including the C-terminal part of the Homeodomain) is highlighted by the dashed double arrow and the light blue shaded area. The specificity module includes the hexapeptide motif (W) and N-terminal residues of the Homeodomain (NHD) as previously characterized [S1].

(B-C) Embryos electroporated with GFP alone (B, n=10 embryos) or in combination with Hoxc9dn (C, n=13 embryos) in the forelimb domain at stage 15 and re-incubated for 48h.

(D-F) Shh expression (D) in a GFP electroporated embryo (B). (E) and (F) are higher magnification of the posterior limb mesenchyme on the contralateral (E) and GFP electroporated (F) sides.

(G-I) Shh expression (G) in a GFP/Hoxc9dn electroporated embryo (C). (H) and (I) are higher magnification of the posterior limb mesenchyme on the contralateral (H) and GFP/Hoxc9dn electroporated (I) sides. Note the significant decrease in Shh expression in the electroporated limb mesenchyme despite the mosaicism in expression due to the electroporation technique

(J) Measurements of the area of Shh expression domain of GFP- (n=10 embryos) and GFP/Hoxc9dn- (n=13 embryos) electroporated limbs, normalized to the contralateral side. Error bars represent mean±SEM. Mann-Whitney statistical test, *** p<0,001. Scale bar is 100µm.

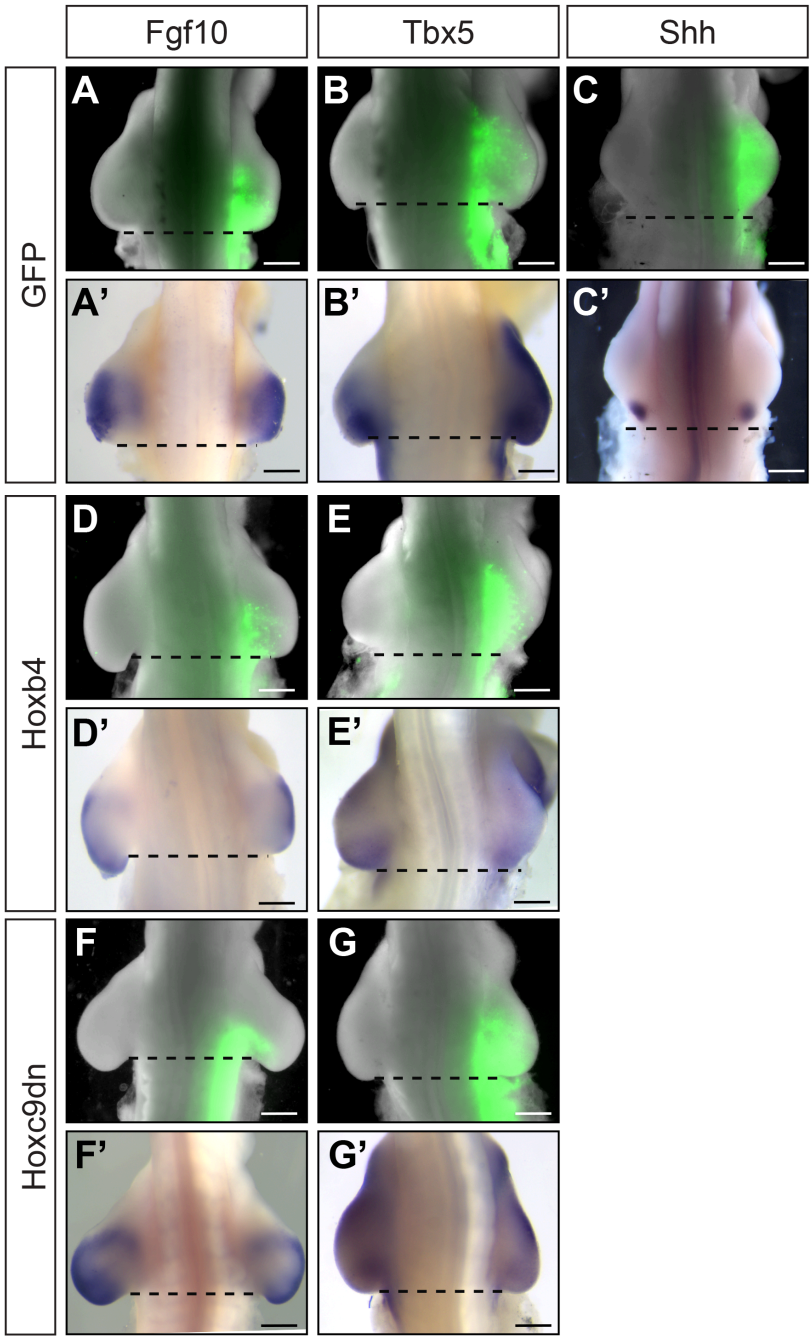

Figure S4. Electroporation of GFP, Hoxb4 and Hoxc9dn alone do not perturb the forelimb position. Related to Figure 4.

(A-C) Embryos electroporated with GFP, in the interlimb domain at stage 14 and re-incubated for 48h (n=15 embryos). (A'-C') shows Fgf10 (A'), Tbx5 (B') and Shh (C') expression in corresponding embryos.

(D-E) Embryos electroporated with Hoxb4/GFP in the interlimb domain at stage 14 and re-incubated for 48h (n=18 embryos). (D'-E') shows Fgf10 (D') and Tbx5 (E') expression in corresponding embryos.

(F-G) Embryos electroporated with Hoxc9dn/GFP in the interlimb domain at stage 14 and re-incubated for 48h (n=14 embryos). (F'-G') shows Fgf10 (F') and Tbx5 (G') expression in corresponding embryos.

Black dashed line, posterior border of the forelimb buds. Scale bar is 200μm.

Supplemental references

S1. Nishimoto, S., Minguillon, C., Wood, S., and Logan, M.P.O. (2014). A combination of activation and repression by a colinear Hox code controls forelimb-restricted expression of Tbx5 and reveals Hox protein specificity. PLoS Genet. *10*, e1004245.