

Fig. S1. Expression of *Cux2* and depletion of *Cux2* by *Cux2*-siRNA. (A-E) Expression of *Cux2* in developing chick embryos at HH 13 (A), 14 (B), 17 (C), 19 (D) and 26 (E). (F, G) pCMV-*Cux2*-EGFP and either *Cux2*-siRNA (F) or control-siRNA (G) were co-transfected into COS7 cells. (H) EGFP-positive cells were counted at 24 hours after transfection. (I, J) Expression of *Cux2* in chick forelimb buds after the electroporation of either *Cux2*-siRNA (I) or control-siRNA (J) into the presumptive right forelimb buds. Black dotted lines indicate the width of the left (control side) forelimb bud. Notably, *Cux2* expression was downregulated in the forelimb bud at the somite 20 level at the *Cux2*-siRNA-injected site (I). (I', J') pCAGGS-EGFP was used to assay efficiency of electroporation. White dotted lines indicate the outlines of the right forelimb bud. Numbers in (B) and (I) indicate somite levels. Scale bars, 500 μ m.

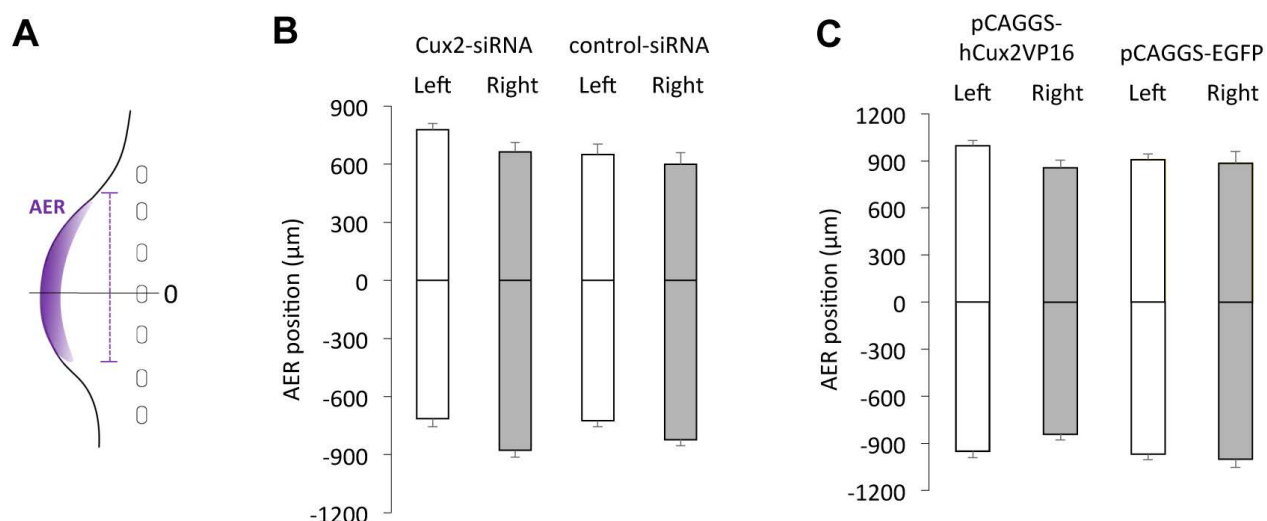


Fig. S2. Analysis of the position of the forelimb buds electroporated with *Cux2-siRNA*, *control-siRNA*, or *pCAGGS-hCux2-VP16*, together with *pCAGGS-EGFP*, or *pCAGGS-EGFP* alone. (A) The position of the AER was measured in embryos stained with *fgf8* probes. The length of the region between seven somites including the forelimb was measured, and its center ("zero") was used as a reference for the AER position along the anterior-posterior axis. (B) Forelimb buds electroporated with *Cux2-siRNA* are shifted posteriorly. (C) Forelimb buds electroporated with *pCAGGS-hCux2-VP16* are reduced and an anterior shift of the posterior limb boundary is observed. Mean \pm s.e.m.

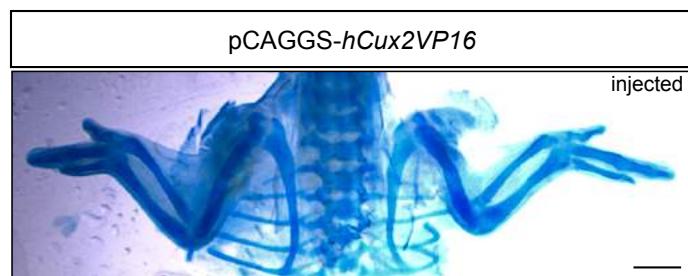


Fig. S3. Cartilage patterns of forelimbs electroporated with pCAGGS-hCux2-VP16. Cartilage patterns of forelimbs electroporated with pCAGGS-hCux2-VP16. Scale bars, 2 mm.

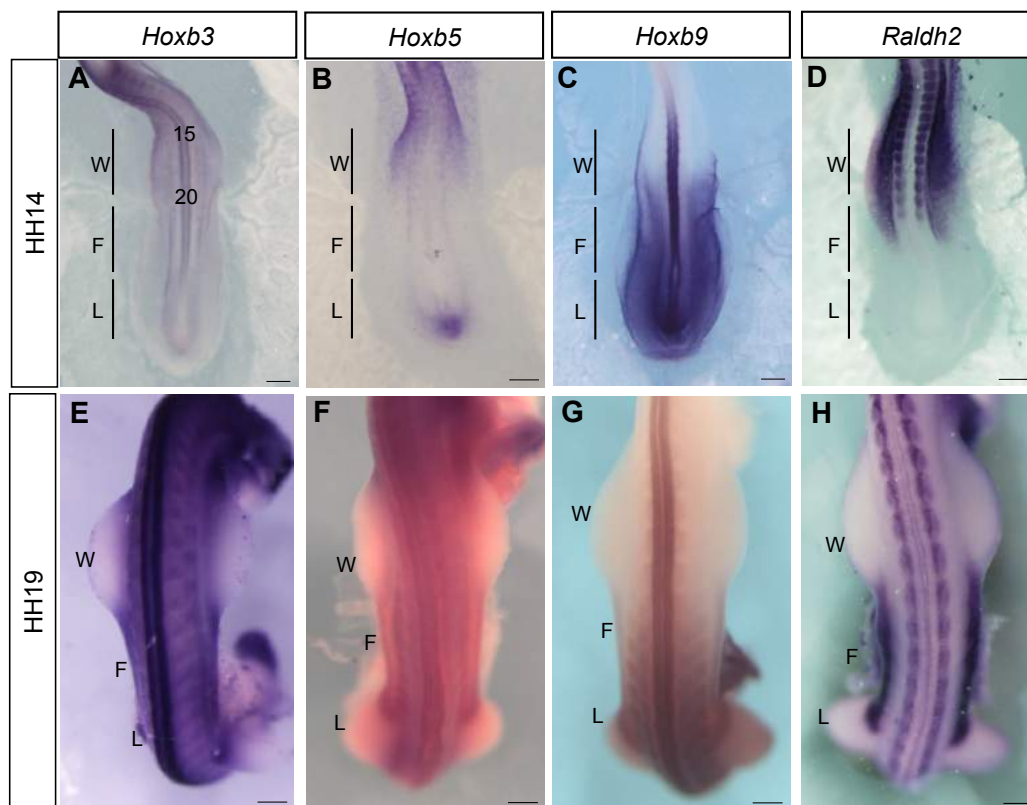


Fig. S4. Expression of *Hoxb3*, *b5*, *b9* and *Raldh2*. (A-H) Expression of *Hoxb3* (A, E), *b5* (B, F), *b9* (C, G) and *Raldh2* (D, H) in developing chick embryos at HH 14 (A-D) and 19 (E-H). Scale bars, 500 μ m.

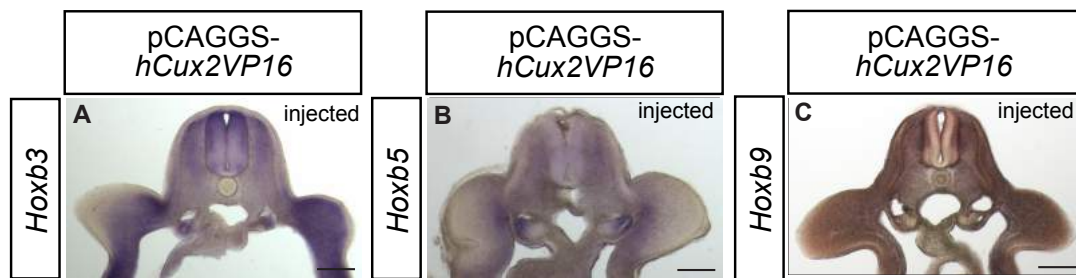


Fig. S5. *Cux2* regulates the expression of *Hoxb* genes. (A-C) Transverse sections of the forelimb buds hybridized with *Hoxb3* (A), *Hoxb5* (B), and *Hoxb9* (C), respectively, after electroporation with pCAGGS-*hCux2-VP16*. Scale bars, 200 μ m.