FGF8 Signaling Alters the Osteogenic Cell Fate in the Hard Palate

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Appendix

Supplemental Materials

Animal

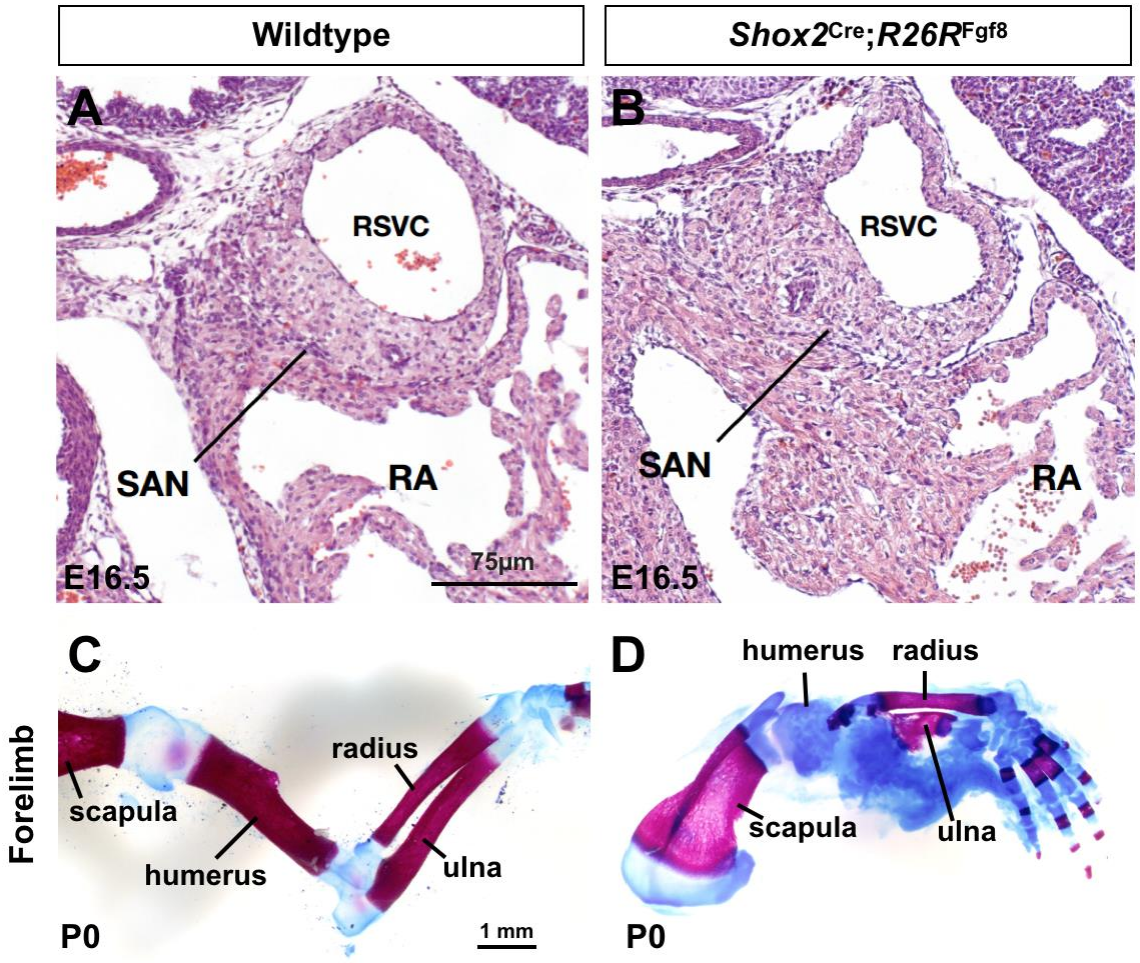
The generation and genotyping protocols of *Dmp1*^{Cre} mice have been described previously (Lu et al. 2007).

Antibody information

Primary antibodies used in the study were: anti-Runx2 (1:300, Santa Cruz, sc-390351), anti-Sp7/Osterix (1:500, Abcam, ab22552), anti-Ki67 (1:500, Abcam, ab15580), anti-Col II (1:500, Abcam, ab34712), anti-GFP (1:500, Abcam, ab13970), anti-Sox9 (1:300, Abcam, ab3696), anti-PI3K (1:50, abcam, ab86714), anti-p-ERK1/2 (1:50, Cell Signaling, 4370S), anti-PLCγ (1:50, Cell Signaling, 14008). Secondary antibodies, all from Life Technologies and used at 1:1000 dilution, were: goat-anti rabbit (A11008, A11037), donkey-anti mouse (A21202, A31571), goat-anti mouse (A11005), goat-anti chicken (A11039).

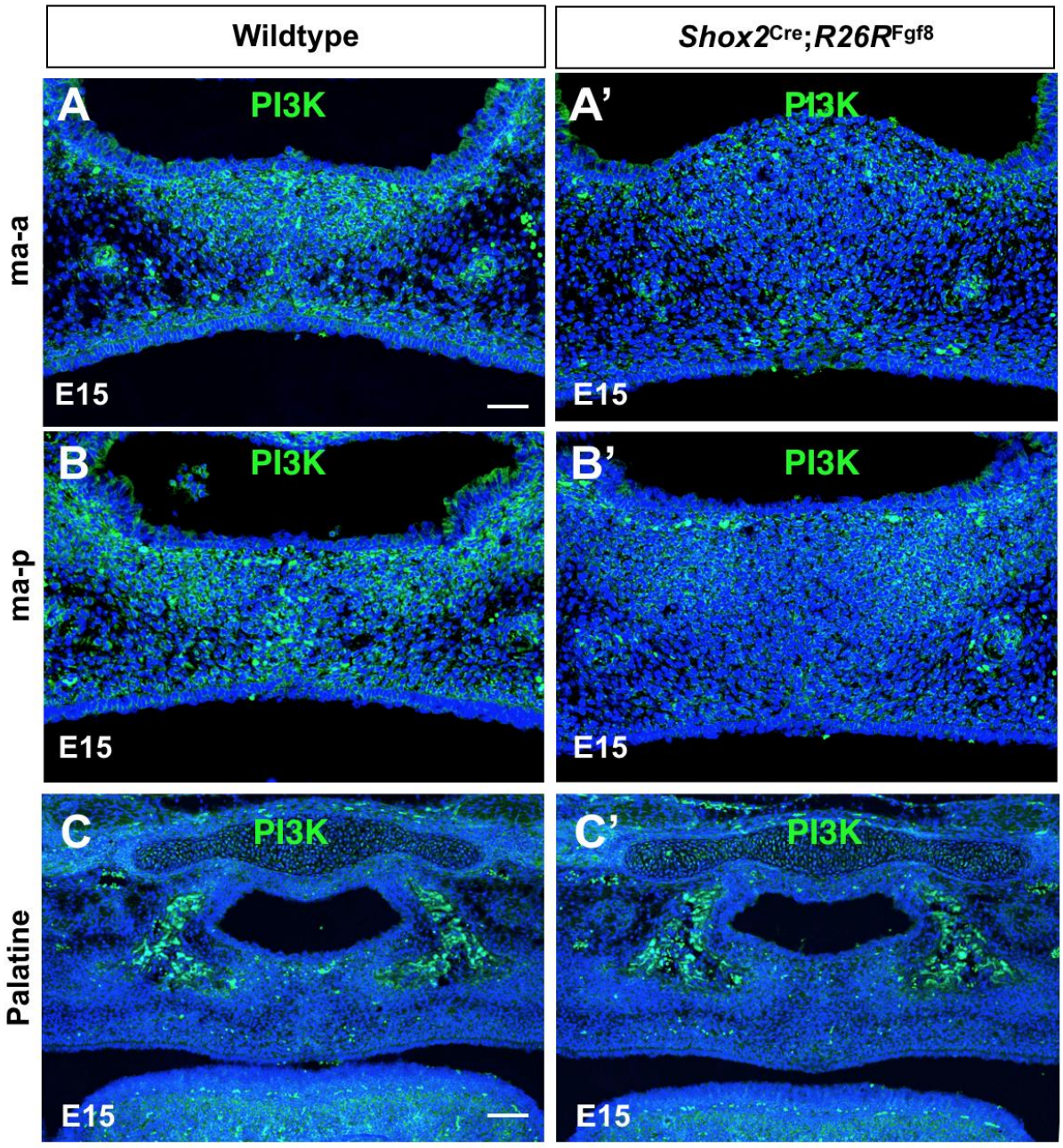
Reference

Lu Y, Xie Y, Zhang S, Dusevich V, Bonewald LF, Feng JQ. 2007. DMP1-targeted Cre expression in odontoblasts and osteocytes. J Dent Res. 86(4):320-5.



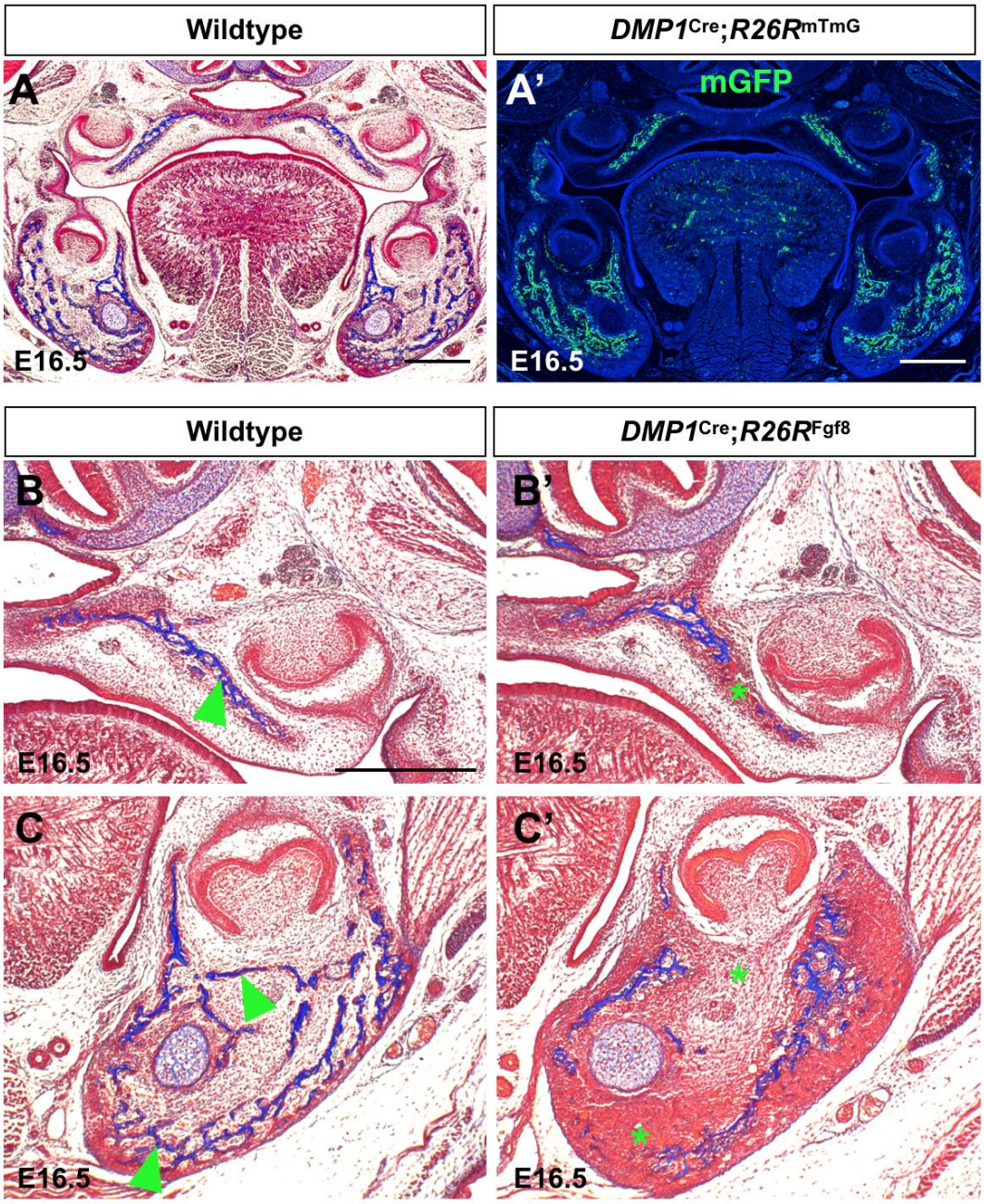
Appendix Figure 1. Ectopic *Fgf8* activation in *Shox2*$^{+}$ cells leads to cardiac and limb defects

(A, B) H&E staining on sections of E16.5 control *Shox2*^{Cre};*R26R*^{Fgf8} embryonic hearts reveals hyperplastic right atrial wall immediately adjacent to the sinoatrial node in the mutant. (C, D) Alcian Blue/Alizarin Red skeletal staining shows a virtual loss of humerus and severely truncated radius and ulna in a P0 *Shox2*^{Cre};*R26R*^{Fgf8} mouse forelimb (D), as compared to the control (C). RA, right atrium; SAN, sinoatrial node, RSVC, right superior vena cava. Scale bars = 75-μm (A, B); = 1-mm (C, D).



Appendix Figure 2. Enhanced FGF8 signaling reduces PI3K levels in the future palatine process of the maxilla but not in the future palatine domain

Immunostaining staining shows reduced PI3K levels in the future palatine process of the maxilla region (A' B') but not the future palatine domain (C') of an E15 *Shox2*^{Cre};*R26R*^{Fgf8} palate, as compared to control (A, B, C). Scale bars = 50-µm (A-B'), = 100-µm (C, C').



Appendix Figure 3. *Fgf8* overexpression in *Dmp1*$^{+}$ cells leads to osteopenia in both the maxilla and mandible. (A, A') Azon red/Aniline blue staining on section of E16.5 wild type craniofacial region reveals match of the forming maxilla-mandibular bones (A) with *Dmp1*$^{+}$ cells (marked by mGFP staining) in E16.5 *Dmp1*^{Cre};*R26R*^{mTmG} mice (A'). (B-C') Azon red/Aniline blue staining shows osteopenia (marked by green asterisks) in both the maxillary bone and mandibular bone in E16.5 *Dmp1*^{Cre};*R26R*^{Fgf8} mice (B' C'), as compared to controls (B, C). Scale bars = 400-μm.

Appendix Table. Primer sequences for qPCR.

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
Fgf8	ATCGTGCTGGAGAACAACTAC	ACCCAACAGCAAACAATATGC
Runx2	AGGGACTATGGCGTCAAACA	GGCTCACGTCGCTCATCTT
Sp7	CGCTTTGTGCCTTTGAAAT	CCGTCAACGACGTTATGC
Bglap	CAGACACCATGAGGACCATC	GGACTGAGGCTCTGTGAGGT
Sox9	CGGAGGAAGTCGGTGAAGA	GTCGGTTTTGGGAGTGGTG
Col2a	TGGCTTCCACTTCAGCTATG	AGGTAGGCGATGCTGTTCTT
Myc	GTGACCAGATCCCTGAATTG	CTCGTCGTTTCCTCAATAAGT
Fgfr1	GGTGAACGGGAGTAAGATCGG	CCCCGCATCCTCAAAGGAG
Fgfr2	CCTCGATGTCGTTGAACGGTC	CAGCATCCATCTCCGTCACA
Fgfr3	GCCTGCGTGCTAGTGTTCT	TACCATCCTTAGCCCAGACCG
Fgfr4	GCTCGGAGGTAGAGGTCTTGT	CCACGCTGACTGGTAGGAA