

Follistatin regulates tooth root furcation morphogenesis

LIU YANG^{1,#}, JU-KYUNG JEONG^{1,#}, HWAJUNG CHOI¹, TAK-HEUN KIM¹, HYUK-JAE EDWARD KWON², SUNG-WON CHO³, MARTIN MATTHEW MATZUK⁴, EUI-SIC CHO^{*1}

¹Cluster for Craniofacial Development and Regeneration Research, Institute of Oral Bioscience,
Jeonbuk National University School of Dentistry, Jeonju 54896, Republic of Korea

²Department of Oral Biology, School of Dental Medicine, University at Buffalo, The State University of New York, Buffalo, NY 14214, USA

³Division of Anatomy and Developmental Biology, Department of Oral Biology,

BK21 FOUR Project, Yonsei University College of Dentistry, Seoul 03722, Republic of Korea

⁴Department of Pathology & Immunology and Center for Drug Discovery, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Appendix

Appendix Materials and Methods

Generation of *Wnt1-Cre;Fst* cKO mice and X-gal staining

To generate *Wnt1-Cre;Fst^{fl/fl}* (mutant) mice, *Wnt1-Cre;Fst^{fl/+}* (control) mice were crossed with *Fst^{fl/fl}* mice. Mouse offspring were genotyped by polymerase chain reaction analysis using previously described primers (Soriano 1999; Ko *et al.*, 2008; Jorgez *et al.*, 2004). To analyze the level of Cre activity, *Wnt1-Cre* mice were crossed with *R26R* reporter mice and the mandibles of the double transgenic mice were processed for X-gal staining as described previously (Kim *et al.*, 2013).

Immunofluorescence

Tissue sections were permeabilized by incubation in 0.2% Triton X-100 for 10 min at room temperature. After rinsing with phosphate buffered saline (PBS), nonspecific binding sites on the cells were blocked with 5% bovine serum albumin in PBS for 30 min at room temperature. Tissue sections were then incubated

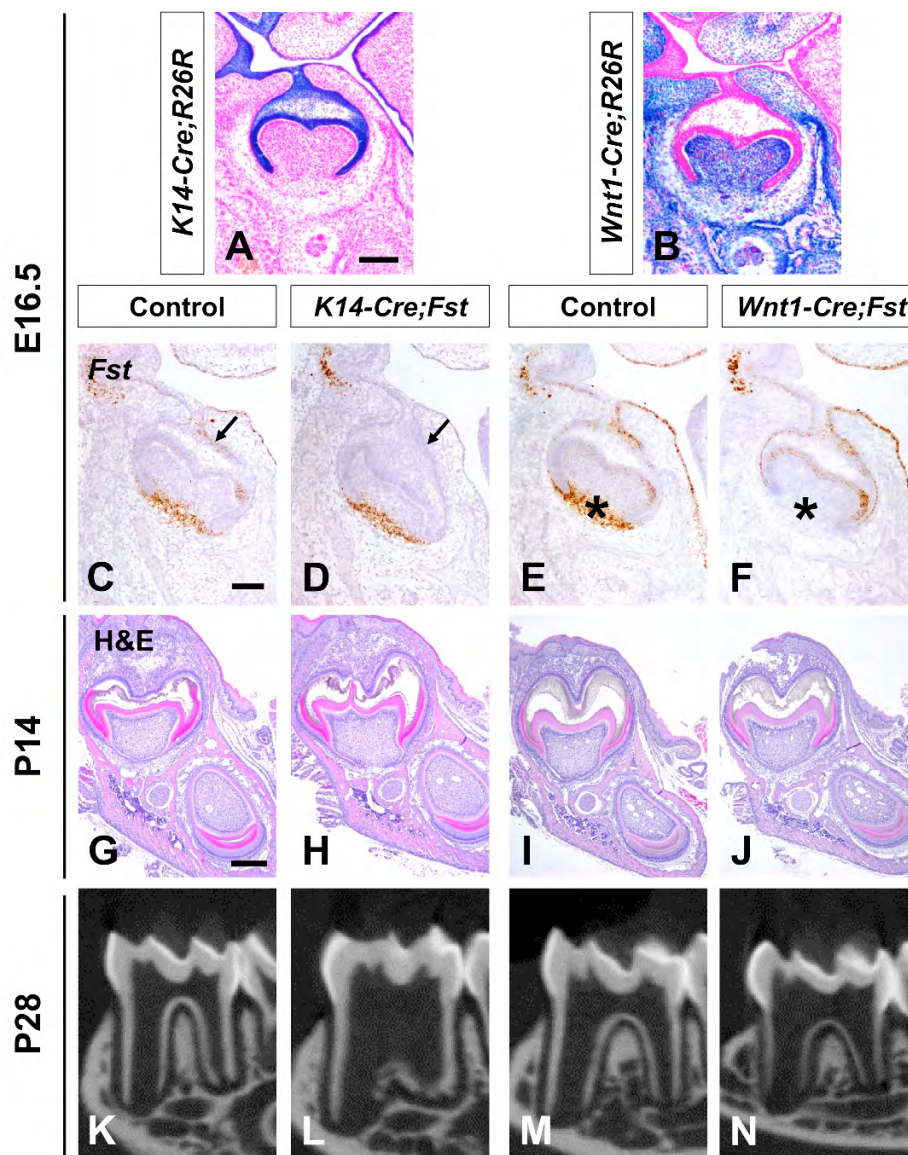
with primary antibodies against Ki67 (NB110-89719; 1:200; Novus Biologicals, Centennial, CO, USA), Nestin (MAB353; 1:200; Millipore, Billerica, MA, USA) and K14 (ab7800; 1:200; Abcam) for 16 h at 4 °C. Normal rabbit or mouse immunoglobulin (Santa Cruz Biotechnology, Dallas, TX, USA) was used as a negative control for the primary antibodies. Alexa Fluor®-conjugated secondary antibodies (Invitrogen, New York, NY, USA) were used for detection. DAPI was used for counterstaining. Cell staining was evaluated using a fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

References

KO SO, CHUNG IH, XU X, OKA S, ZHAO H, CHO ES, DENG C, CHAI Y. (2007). Smad4 is required to regulate the fate of cranial neural crest cells. *Dev Biol.* 312(1):435-447. doi: 10.1016/j.ydbio.2007.09.050

* These authors contributed equally to this work.

Appendix Figure



Appendix Figure. Histological and radiographic analysis of *Fst* conditional knockout molars. (A, B) Cre activity of tooth germ of mandibular molars from *K14-Cre;R26R* (A) and *Wnt1-Cre;R26R* (B) mice at E16.5 were analyzed by X-gal staining. (C-F) *In situ* hybridization of *Fst* with tooth germ of mandibular molars from *K14-Cre;Fst* and *Wnt1-Cre;Fst* with their littermate controls at E16.5. Black arrows and asterisks indicate the difference of *Fst* expression. (G-J) H&E-stained sections of tooth germ of mandibular molars from *K14-Cre;Fst* and *Wnt1-Cre;Fst* with their littermate controls at P14. (K-N) Microradiographs of mandibular molars from *K14-Cre;Fst* and *Wnt1-Cre;Fst* with their littermate controls at P28. Scale bar: 100 μ m (A-F) and 300 μ m (G-J).