MATERIALS AND METHODS

Generation of *pMes-Pitx2c* transgenic mice

To generate *pMes-Pitx2c* conditional transgenic mice, the coding sequence of the mouse *Pitx2c* cDNA was cloned into *pMES-IRES-Egfp* vector (Xiong et al. 2009) in front of the *IRES-Egfp* sequence under the control of the chick *β-actin* promoter. A *STOP* cassette flanked by *LoxP* sequences was inserted between the *β-actin* promoter and the *Pitx2c* sequence. Pronuclear injection was conducted at Tulane University Uptown Transgenic/Knockout Facility.

Vector construction and Mutagenesis

The construction of *Myc*-tagged mouse *Wnts* expression vectors have been reported previously (Zhu et al. 2013). *Nog-FLAG* expression vector was generated by cloning full-length mouse *Noggin* into the C-terminus of *pFLAG-CMV* vector (Sigma). *pc-Wnt3* and *pc-Nog* were generated by cloning *Wnt3* full-length cDNAs with a *FLAG* tag or *Nog* full-length cDNA with a *HA* tag into *pcDNA3.1* vector, respectively. To generate *Nog*(Δ*CRD*), PCR was performed with *pc-Nog* plasmid as template with the following primers: forward: 5′-aaaccatggATGGAGCGC TGCCCCAGCCTGGGGGTCAC-3′, reverse: 5′-aaactcgagCTAAGCGTAATCTGGAACATCGT ATGGGTAGCTGCCCACCTTCACGTAGCG-3′. To generate *Nog*(Δ*clip*), overlap PCR was used with the following primers: forward: same as that used for *Nog(ΔCRD)*, reverse: 5′-aaactcgagCT AAGCGTAATCTGGAACATCGTATGGGTAGCAGGAACACTTACACTCGGA-3′, and two reverse complementary sequences, S1: 5′-GGCATGGCCCCCGACGGCCGCTGCCGCAGC AGCGAGCGCAGCAGCGTCTCGTTCAGATC-3′, S2: 5′-GATCTGAACGAGACGCTGCTGC GCTCGCTGCTGCGGCAGCGGCCGTCGGGGGCCATGCC-3′. The forward primer contains a

5′-aaaccatgg-*NcoI* overhang, and the reverse primers contain a 5′-aaactcgag-*XhoI* overhang and a *HA* sequence (marked with underline) followed by a stop codon. All the PCR products were gel-purified, digested with *NcoI* and *XhoI,* and subcloned into *pcDNA3.1* vector.

RT-PCR and Quantitative real time RT-PCR (qRT-PCR)

Molar germs isolated from timed embryos were subjected to RNA extraction with RNase Mini Kit (Qiagene) and reversely transcribed using SuperScript III First Strand Synthesis System (Invitrogen). The expressions of *Noggin*, *Wnt-4*, *-5a*, *-6*, *-7b*, *-10a*, *-10b*, *Tgfb1*, and *Tgfb2* in *K14Cre;pNog* and wild type controls were detected by qRT-PCR or RT-PCR. The primers were showed in supplemental Table 1.

REFERENCES

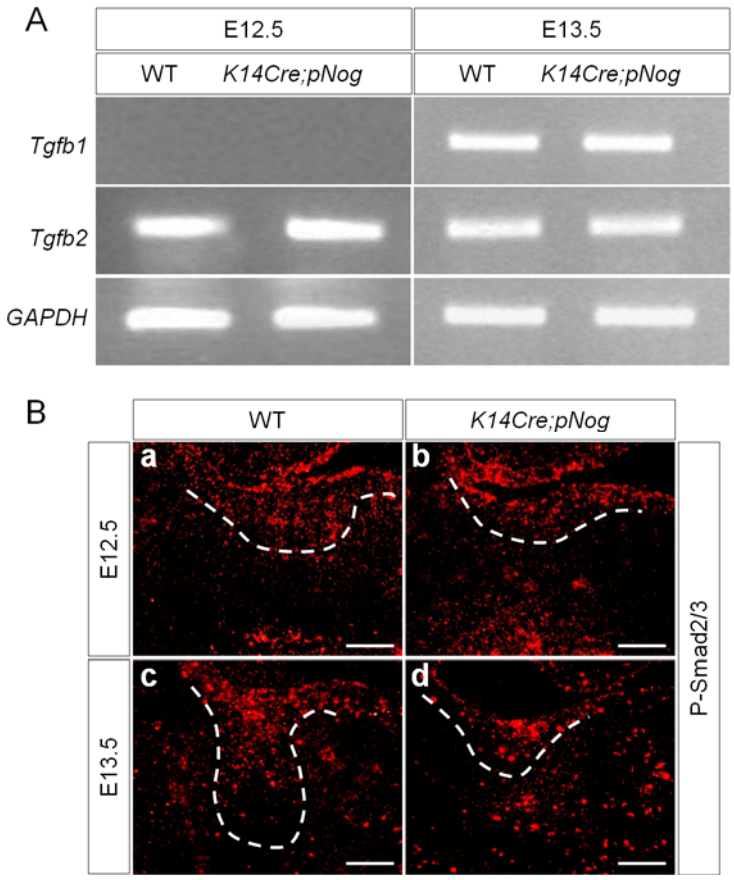
Xiong, W., He, F., Morikawa, Y., Yu, X., Zhang, Z., Lan, Y., Jiang, R., Cserjesi, P., and Chen, Y. (2009). Hand2 is required in the epithelium for palatogenesis in mice. *Dev. Biol.* **330,** 131-41.

Zhu, X., Zhao, P., Liu, Y., Zhang, X., Fu, J., Ivy Yu, H.M., Qiu, M., Chen, Y., Hsu, W., and Zhang, Z. (2013). Intra-epithelial requirement of canonical Wnt signaling for tooth development. *J. Biol. Chem.* **288,** 12080-12089.

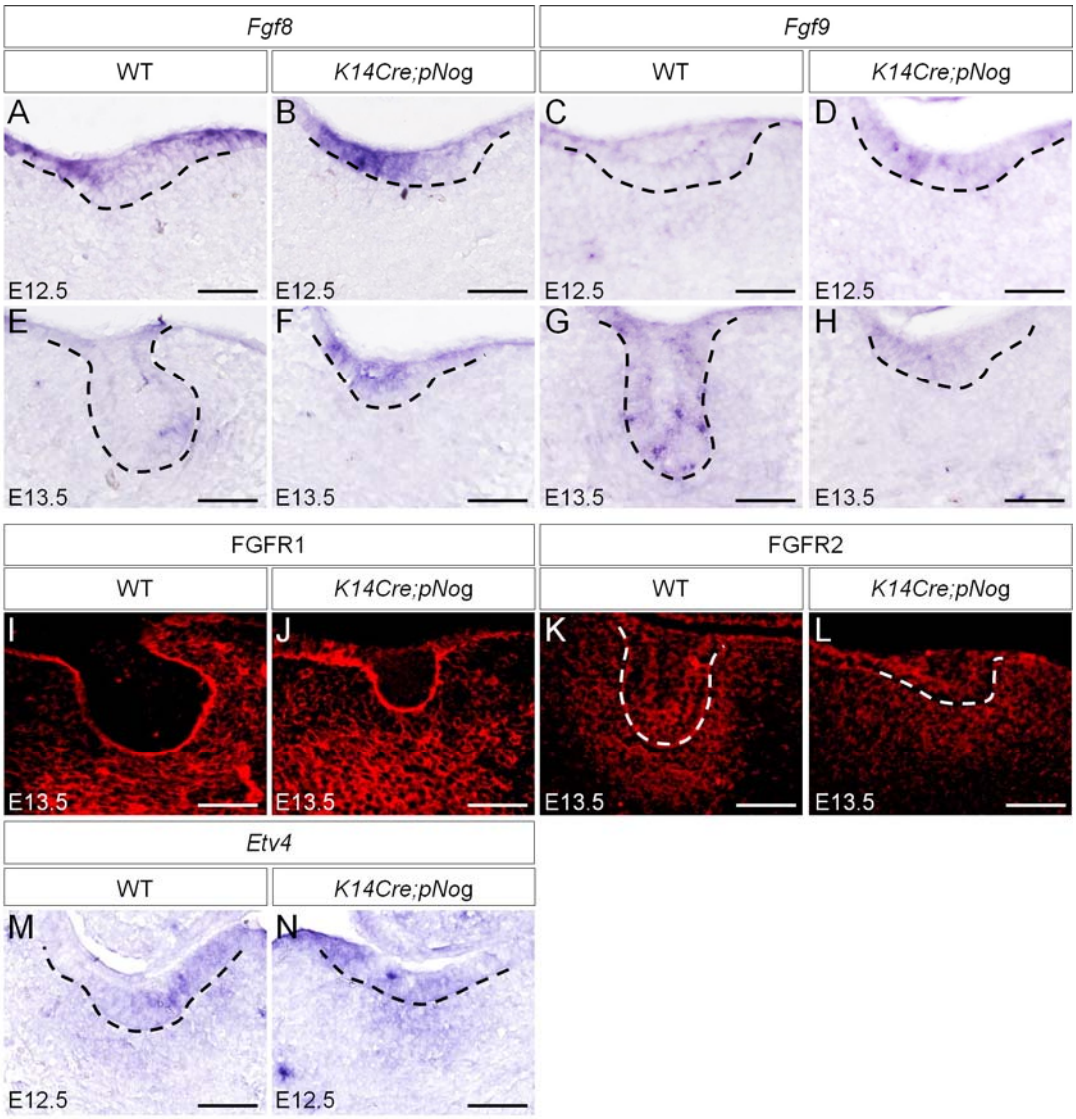
Supplemental Table 1. Oligonucleotide primer sequences used in qRT-PCR or RT-PCR

Gene	Sequence (5′-3′)
Nog	F: CCAGCACTATCTACACATCCG
	R: GATAGGGTCTGGATGTTCGATG
Wnt4	F: GACTCCTCGTCTTCGCCGTGTTCTCG
	R: GTTTGCACATCTGCACCTGCCTCTGG
Wnt5a	F: TGCCATGTCTTCCAAGTTCTTCCTAATG
	R: TGATGCCTGTCTTCGCACCTTCTCCA
Wnt6	F: GGGAGGCTGCGGAGACGATGTGGACTT
	R: TCGGGTGAATCGGCTGCGTAGAGGAG
Wnt7b	F: CTTCGGGCAAGAACTCCGAGTAGGGA
	R: CCAGCCTTCCGCCTGGTTGTAGTAGC
Wnt10a	F: TCGCCATCCATGAGTGCCAGCATCAG
	R: ACCGCA AGCCTTCAGTTTACCCAGA
Wnt10b	F: GGGGTGGCTGTAACCACGACAT GGAC
	R: CCCAGAGTAGGGTCTCGCTCGCAGAA
Tgfb1	F: TGGCTGAACCAAGGAGACGGAATACA
	R: CAGGGTCCCAGACGAAGTTGGCATG
Tgfb2	F: TCCCGCCCACTTTCTACAGACCCTAC
	R: CCATCAATACCTGCAAATCTCGCCTC
GAPDH	F: CGCCTGGAGAAACCTGCCAAGTATGA
	R: TGGAAGAGTGGGAGTTGCTGTTGAAGT

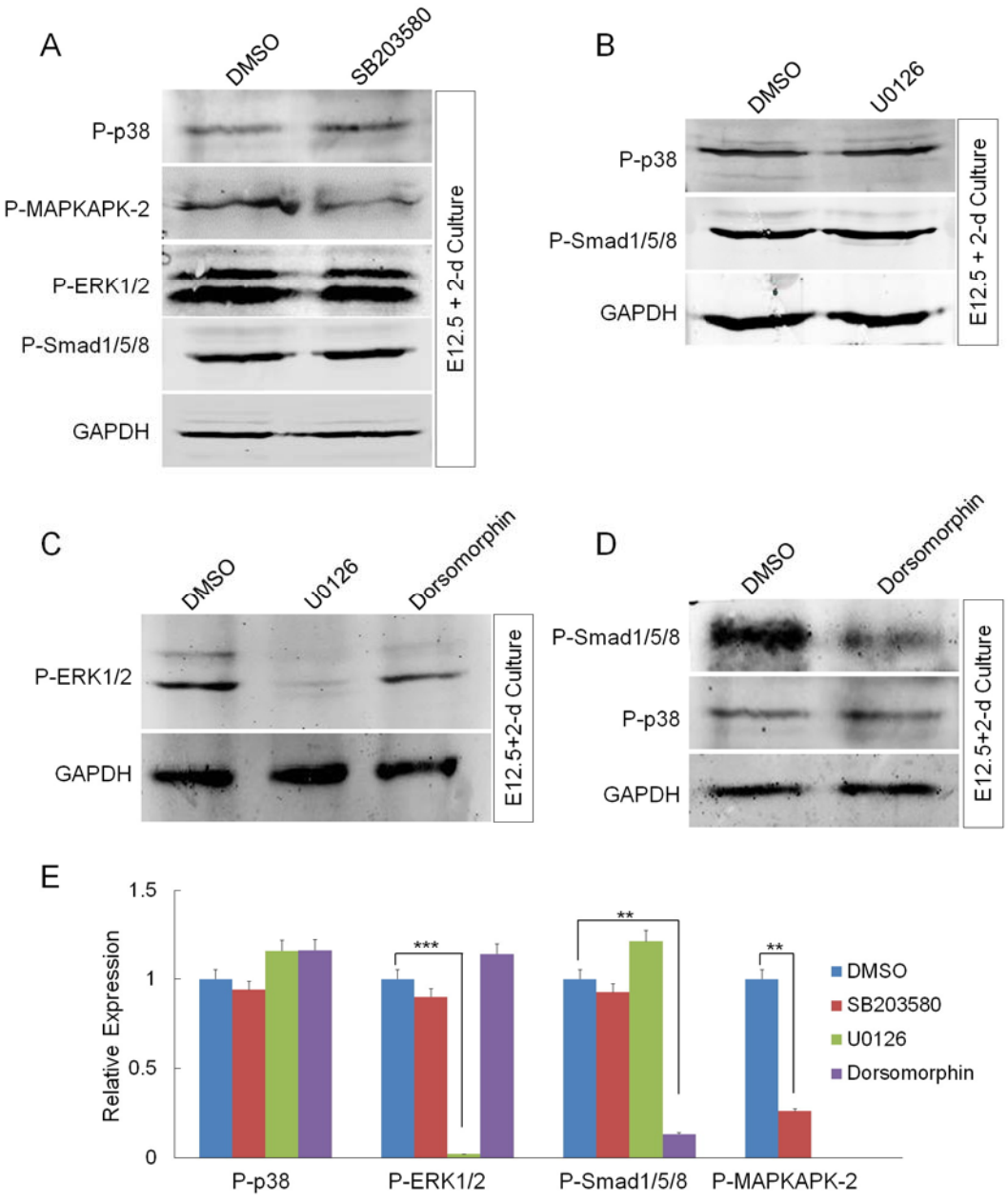
FIGURE LEGENDS



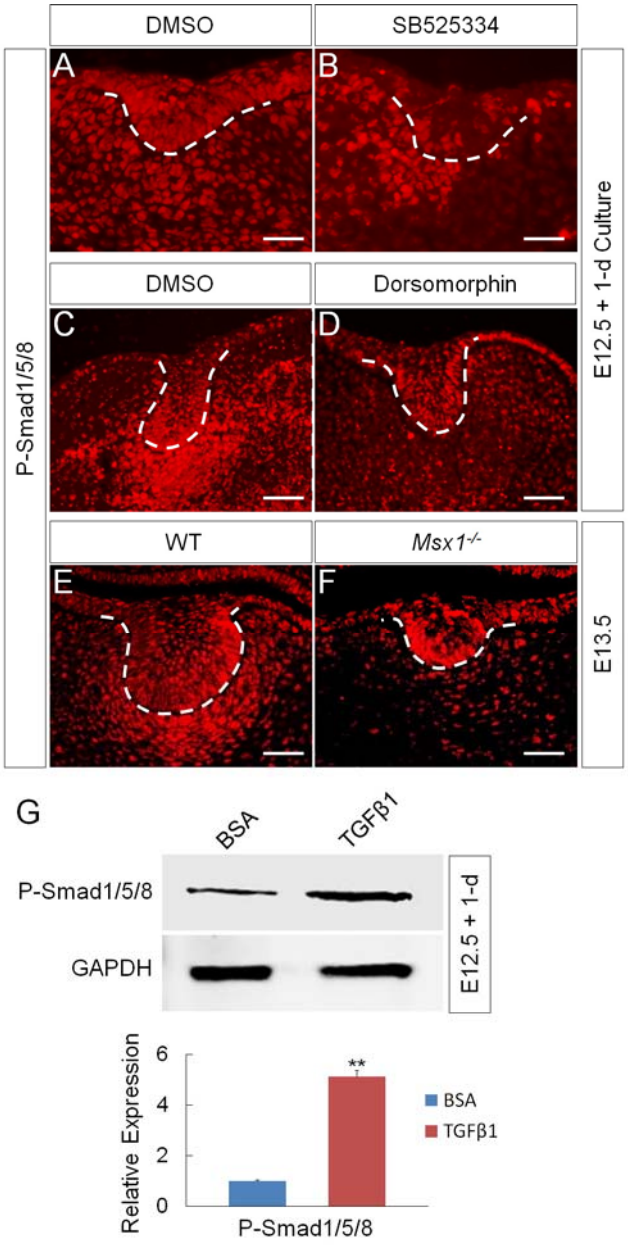
Supplemental Fig. 1. TGFβ signaling is not altered in *K14Cre;pNog* molar germs. (A) RT-PCR shows mRNA expression of *Tgfb1* and *Tgfb2* in controls and *K14Cre;pNog* tooth germs at E12.5 and E13.5. *GAPDH* is included as internal control. **(B)** Immunofluorescence of P-Smad2/3 in controls (a, c) and *K14Cre;pNog* (b, d) tooth germs at E12.5 (a, b) and E13.5 (c, d). Bar = 50 μm.



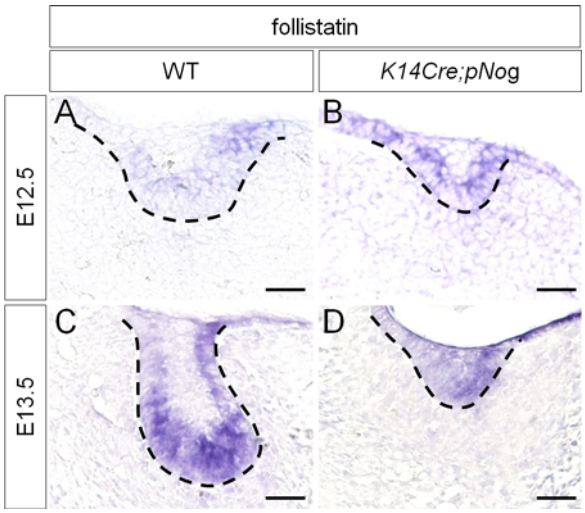
Supplemental Fig. 2. FGF signaling is not affected in *K14Cre;pNog* molar germs. (A-H) In situ hybridization assays on *Fgf8* (A, B, E, F) and *Fgf9* (C, D, G, H) in controls (A, C, E, G) and *K14Cre;pNog* (B, D, F, H) tooth germs at E12.5 (A-D) and E13.5 (E-H). **(I-L)** Immunofluorescence of FGFR1 (I, J) and FGFR2 (K, L) in controls (I, K) and *K14Cre;pNog* (J, L) tooth germs at E13.5. **(M, N)** In situ hybridization of *Etv4* in control (M) and *K14Cre;pNog* (N) tooth germs at E12.5. Bar = 50 μm in all panels.



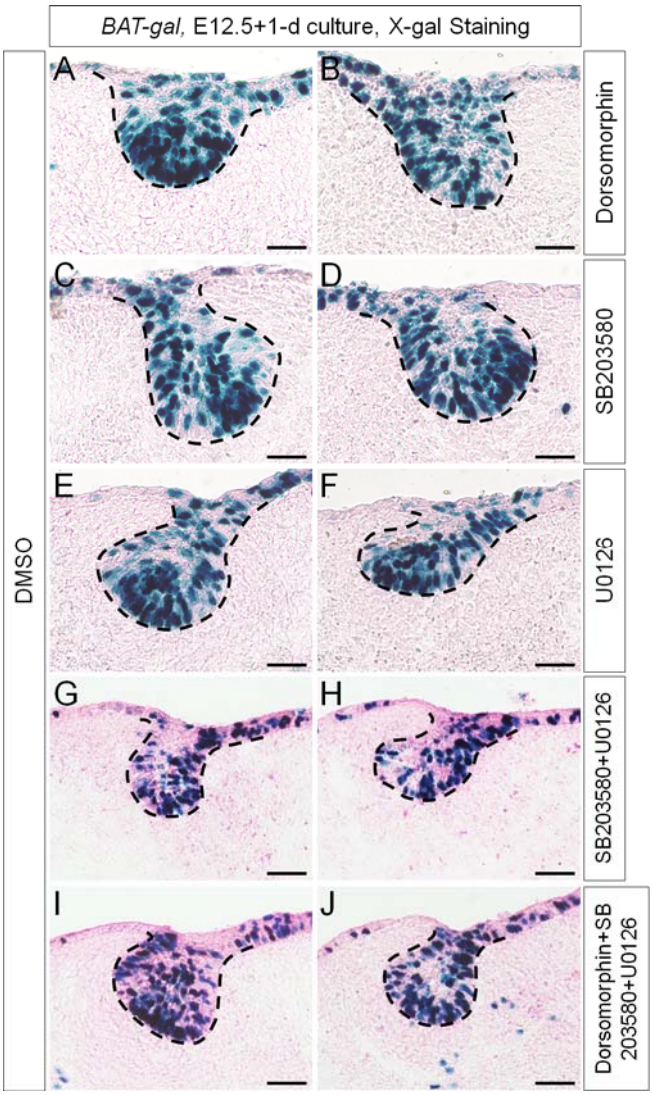
Supplemental Fig. 3. The specificity and efficiency of small inhibitor molecules. (A-D) Representative Western blots show expression of P-p38, P-MAPKAPK-2, P-ERK1/2, and P-Smad1/5/8 in E12.5 tooth germs after 2-day organ culture in the presence of 20 μM of each inhibitor or DMSO as indicated. GAPDH was used as internal control. **(E)** Band quantification of A-D from three individual experiments. The protein levels in DMSO-treated group were set as one unit. **: $P < 0.01$; ***: $P < 0.001$.



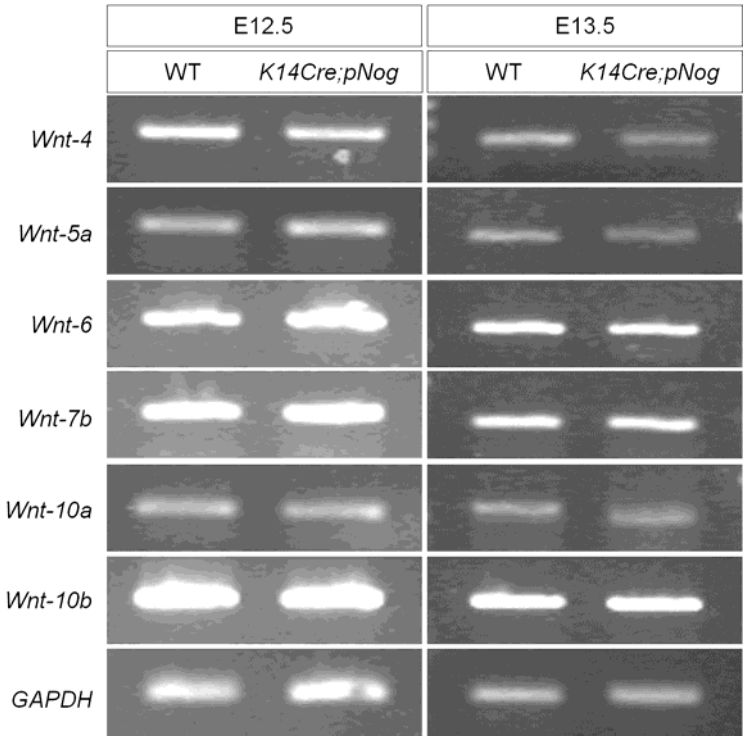
Supplemental Fig. 4. TGFβ signaling is responsible for Smad1/5/8 activation in the dental epithelium. (A-D) Immunofluorescence of P-Smad1/5/8 in E12.5 tooth germ after 1-day organ culture in the presence of SB525334 (B), Dorsomorphin (D) or DMSO (A, C). **(E-F)** Immunofluorescence of P-Smad1/5/8 in control (E) or *Msx1*$^{-/-}$ (F) tooth germs at E13.5. Bar = 50 μm in all panels. (G) Western blot shows P-Smad1/5/8 levels in dental epithelia that were separated from E12.5 tooth germs after 1-day organ culture with or without Tgfb1.



Supplemental Fig. 5. Activin activity is not affected in *K14Cre;pNog* molar germs. In situ hybridization of follistatin in controls (A, C) and *K14Cre;pNog* (B, D) tooth germs at E12.5 (A, B) and E13.5 (C, D). Bar = 50 µm.



Supplemental Fig. 6. Inhibition of BMP-mediated signaling pathways does not attenuate Wnt/β-catenin signaling activity. X-gal staining of E12.5 *BAT-gal* tooth germs after 1 day organ culture in the presence of DMSO (A, C, E, G, I), dorsomorphin (B), SB203580 (D), U0126 (F), SB203580 and U0126 (H), dorsomorphin, SB203580 and U0126 (J). Bar = 50 μm.



Supplemental Fig. 7. Expression of Wnt ligands is not changed in *K14Cre;pNog* molar germs.

RT-PCR shows mRNA expression of *Wnt-4, Wnt-5a, Wnt-6, Wnt-7b, Wnt-10a,* and *Wnt-10b* in controls and *K14Cre;pNog* tooth germs at E12.5 and E13.5. *GAPDH* is used as internal control.