Supporting Information

Prochazka et al. 10.1073/pnas.1002784107

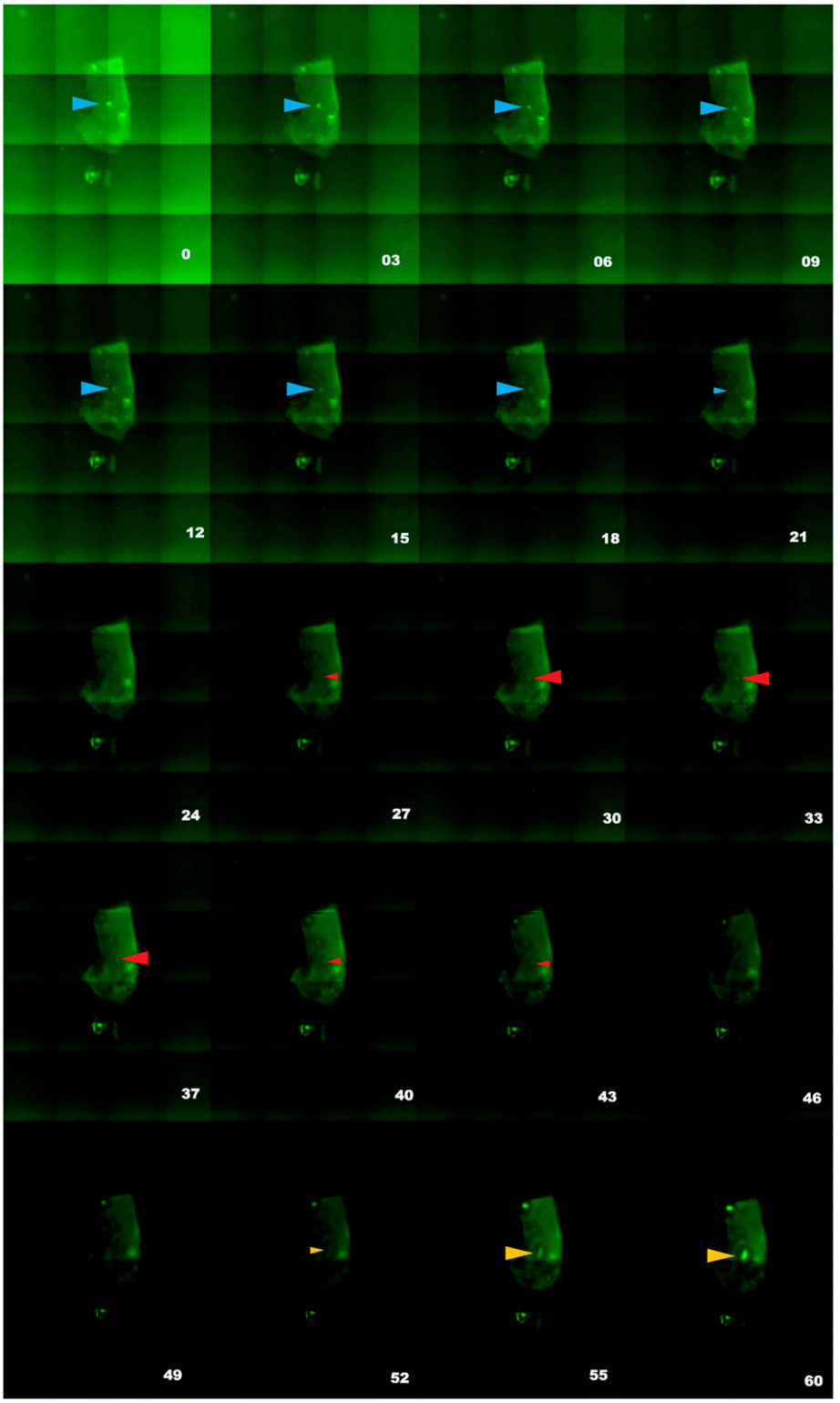


Fig. S1. Series of photos of the Shh-EGFP mandible of mouse embryo from ED 12.7. The mandible of ED 12.7 mouse embryo (body weight, 90 mg) was explanted and cultured under a time-lapse microscope. Hours of culture are indicated by white numbers. The sequence shows three distinct *Shh* signaling centers: MS (blue arrowhead), R2 (red arrowhead), and the primary enamel knot (pEK) of the first molar (M1pEK) (yellow arrowhead). Weak/vanishing fluorescence signal is indicated by small arrowheads.

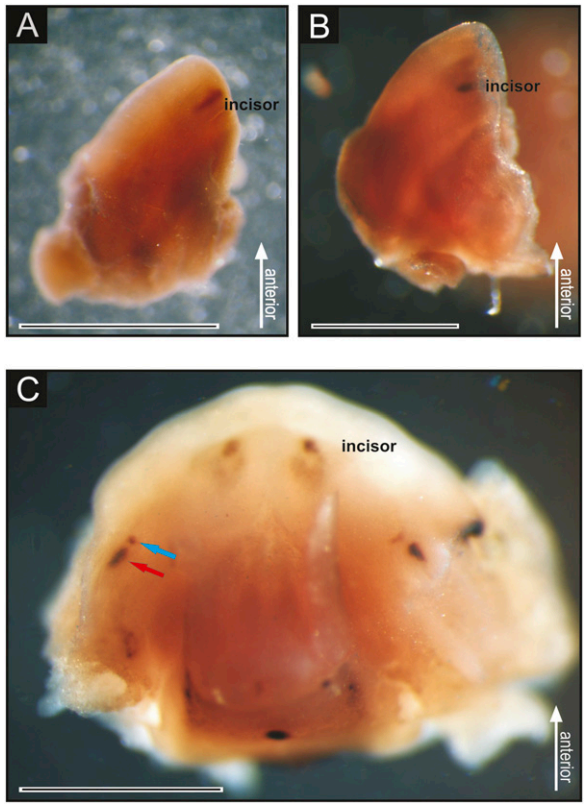


Fig. S2. Shh whole-mount in situ hybridization of mouse embryonic mandibles. (*A*) The mandible of a mouse embryo at ED 13.3 (body weight, 114 mg) documents the *Shh*-negative period I. (*B*) The mandible of a mouse embryo at ED 13.3 (body weight, 158 mg) documents the *Shh* negative period II. During the negative periods, no distinct *Shh* signal patch is present in the cheek teeth region (compare with Fig. 2*A*). (*C*) A rare case of coexistence of two *Shh* expression patches found at ED 13.5. Based on the present data, the anterior patch corresponds to MS residuum (blue arrow), the posterior patch to the R2 signaling center (red arrow). The M1 pEK is not yet present. Similar *Shh* pattern has been published in WT mice and K14-EDA mice, and the anterior small patch has been interpreted as a weak expression of an extra (diastemal) signaling center, the posterior patch as the signaling of the M1 pEK (1, 2). (Scale bars, 100 μm.)

1. Mustonen T, et al. (2004) Ectodysplasin A1 promotes placodal cell fate during early morphogenesis of ectodermal appendages. *Development* 131:4907–4919.
2. Kangas AT, Evans AR, Thesleff I, Jernvall J (2004) Nonindependence of mammalian dental characters. *Nature* 432:211–214.

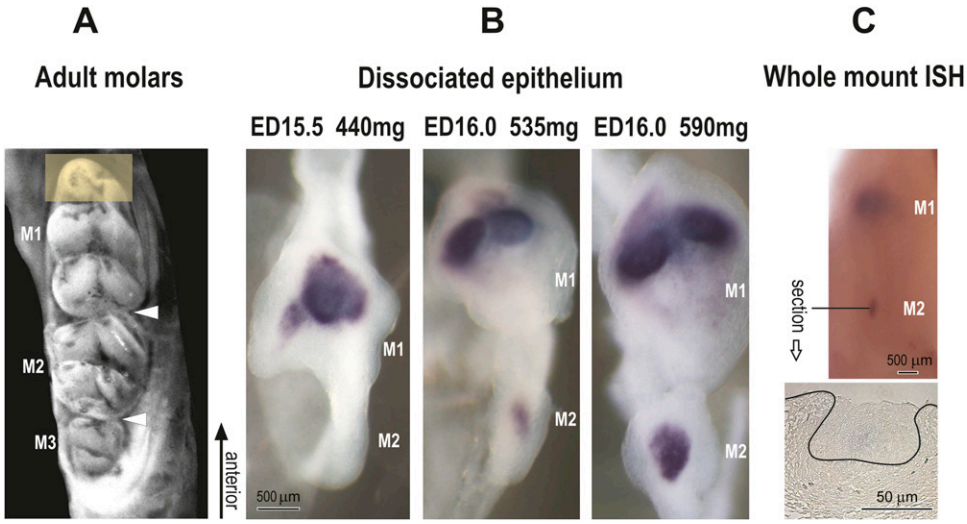
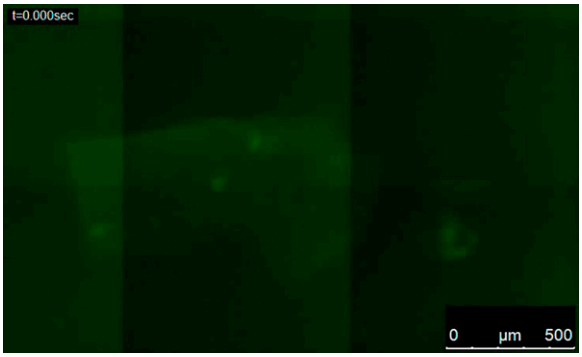
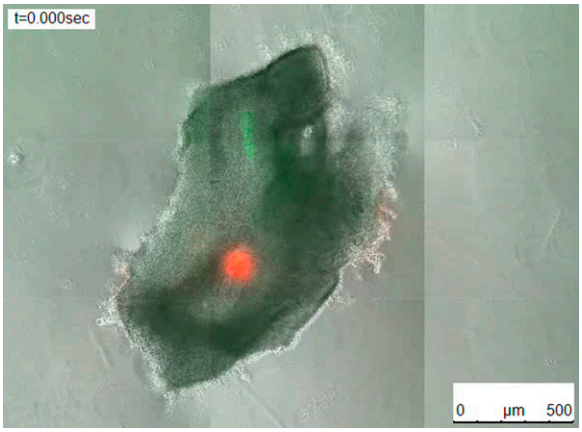


Fig. S3. Lower molars in the mouse. (*A*) Adult mouse molars. The first molar (M1) differs from the second (M2) or third (M3) molar by comprising the long anterior extension called anteroconid (yellow). Note the similarity between the M2 and the M1 without the anteroconid. Arrowheads indicate tooth boundaries. (*B*) Dissociated epithelia from mandibles of different age/weight mouse embryos were hybridized with *Shh* antisens probe. *Shh* expression in the M2 starts at ED 16.0 (weight class 530–550 mg). (*C*) A section of a whole-mount *Shh* hybridized mandible of the same weight class shows that the expression starts in the M2 at the bud-cap transition.



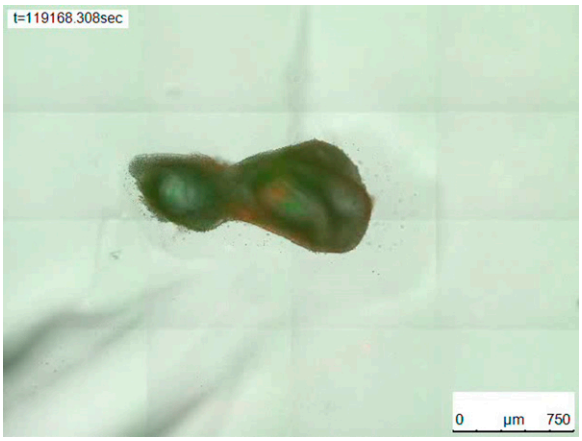
Movie S1. Time-lapse movie of the Shh-EGFP mandible of a mouse embryo from ED 12.7. The mandible of ED 12.7 mouse embryo (body weight, 90 mg) was explanted and cultured under a time-lapse microscope. This records the same experiment as shown in Fig. S1. Note the appearance and disappearance of distinct green fluorescence signals. Time of culture is shown in seconds.

Movie S1



Movie S2. Time-laps movie of the DiI labeled Shh-EGFP mouse embryonic mandible from ED 12.7. The mandible of ED 12.7 mouse embryo (body weight, 90 mg) was explanted and cultured under a time-lapse microscope after DiI microinjection in the MS signaling center. The culture time is shown in seconds.

Movie S2



Movie S3. Time-laps movie of the DiI labeled Shh-EGFP mouse embryonic mandible from ED 13.3. The mandible of ED 13.3 mouse embryo (body weight, 145 mg) was explanted and cultured under a time-lapse microscope after DiI microinjection in the R2 signaling center. The culture time is shown in seconds.

Movie S3