

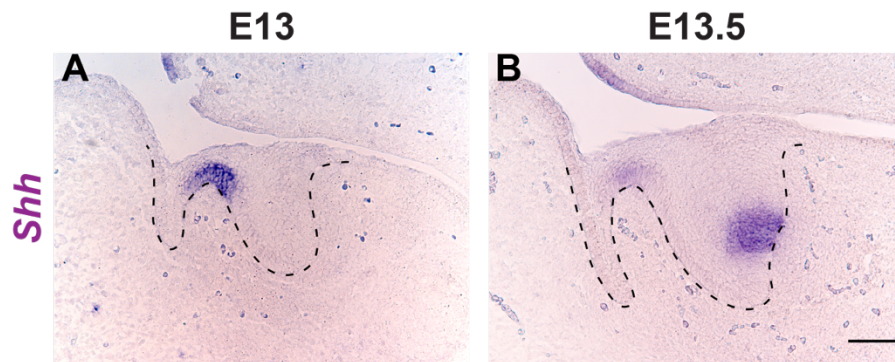
## **Supplemental Data**

Lineage tracing of epithelial cells in developing teeth reveals two strategies for building signaling centers

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Supplemental Figures

Supplemental Figure 1.



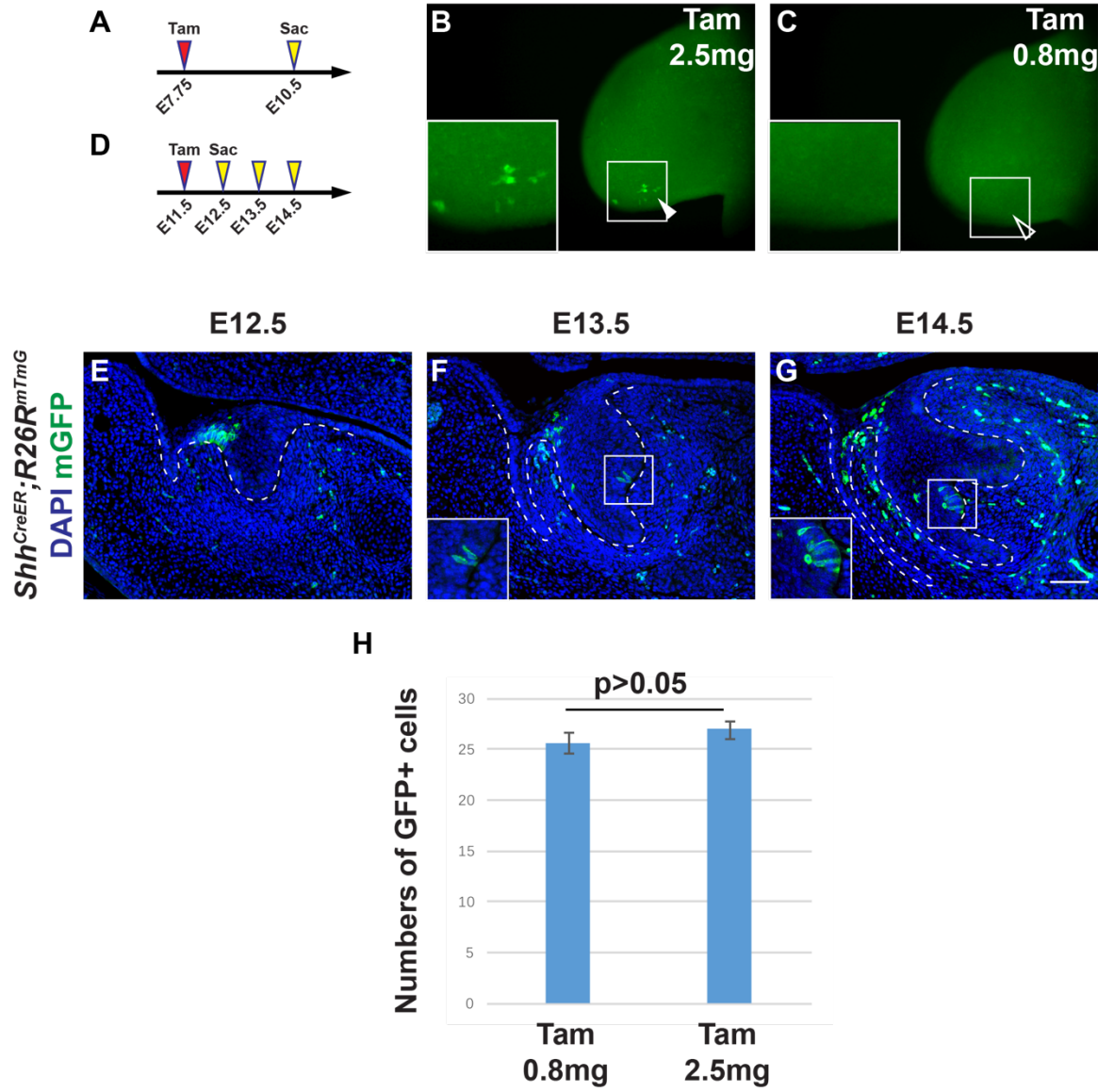
**Figure S1. Expression of *Shh* in incisor tooth germs.**

**A** At E13, expression of *Shh* is only detected in the anterior part of the incisor adjacent to the vestibular lamina.

**B** At early E13.5, a new *Shh* expression domain is observed in the forming EK at the posterior dental epithelium. *Shh* expression in the IK is downregulated.

Scale bar in **(B)** represents 100  $\mu$ m in **(A, B)**

Supplemental Figure 2.



**Figure S2. Dose-dependent response to tamoxifen in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> embryos.**

**A** Timeline depicting the onset of Cre induction (Tamoxifen (Tam) injection, red arrowhead) and sample collections (yellow arrowheads) for **B, C**.

**B, C** In the mouse forelimbs at E10.5, GFP-positive cells (white arrowhead in **B**) are present 48 hours after Cre induction using 2.5 mg/30 g body weight of tamoxifen, but absent (open white arrowhead in **C**) if 0.8 mg/30 g body weight of tamoxifen is given. n=5 embryos for each group.

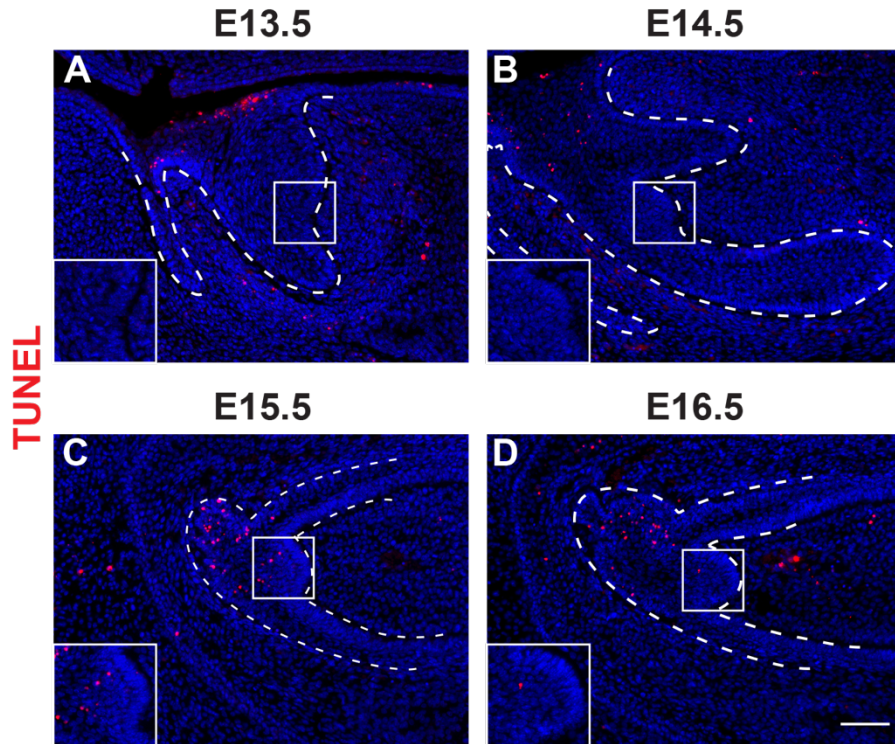
**D** Timeline depicting the onset of Cre induction (Tamoxifen (Tam) injection, red arrowhead) and sample collections (yellow arrowheads) for **E-G**.

**E-G** Lineage tracing analysis of cells expressing *Shh* in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> with 2.5 mg/30 g body weight tam induction. The appearance of GFP-positive cells is observed in the IK region at E12.5- E14.5 as well as in the EK at E13.5 and E14.5. n=5 embryos for each time point.

**H** Number of GFP-positive cells at E12.5 that are induced by 0.8 mg or 2.5 mg/30 g body weight of tamoxifen are quantified (mean  $\pm$  SD,  $p > 0.05$ ). No significant differences are found. The average number of GFP-positive (+) cells per section is calculated using the middle 5 sections of each tooth germ. n = 3 embryos.

Scale bar in (**G**) represents 25  $\mu$ m in (**B, C**), 50  $\mu$ m in (**E-G**)

Supplemental Figure 3.

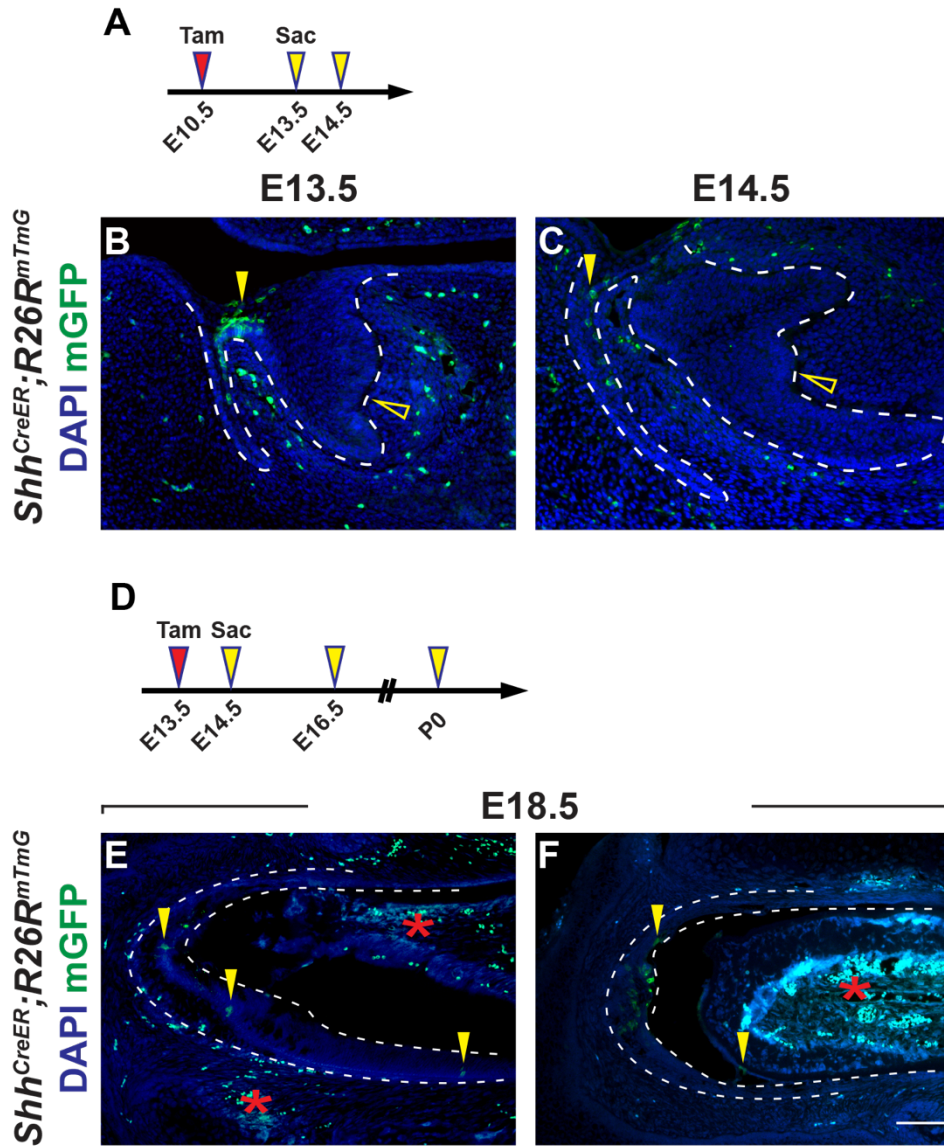


**Figure S3. Apoptosis analyses of incisor EK.**

**A-C** TUNEL staining shows that the EK cells undergoing apoptosis can be detected at E15.5 and E16.5 (**C,D**), but not at E13.5 and E14.5 (**A,B**).

Scale bar in (**D**) represents 50  $\mu$ m in (**A-D**)

Supplemental Figure 4.



**Figure S4. CreER recombination efficiency by tamoxifen induction in the incisor of *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> embryos from E10.5.**

**A** Timeline depicting the onset of Cre induction (Tamoxifen (Tam) injection, red arrowhead) and sample collections (yellow arrowheads) for **B, C**. Tamoxifen concentration is 2.5 mg/30 g body weight.

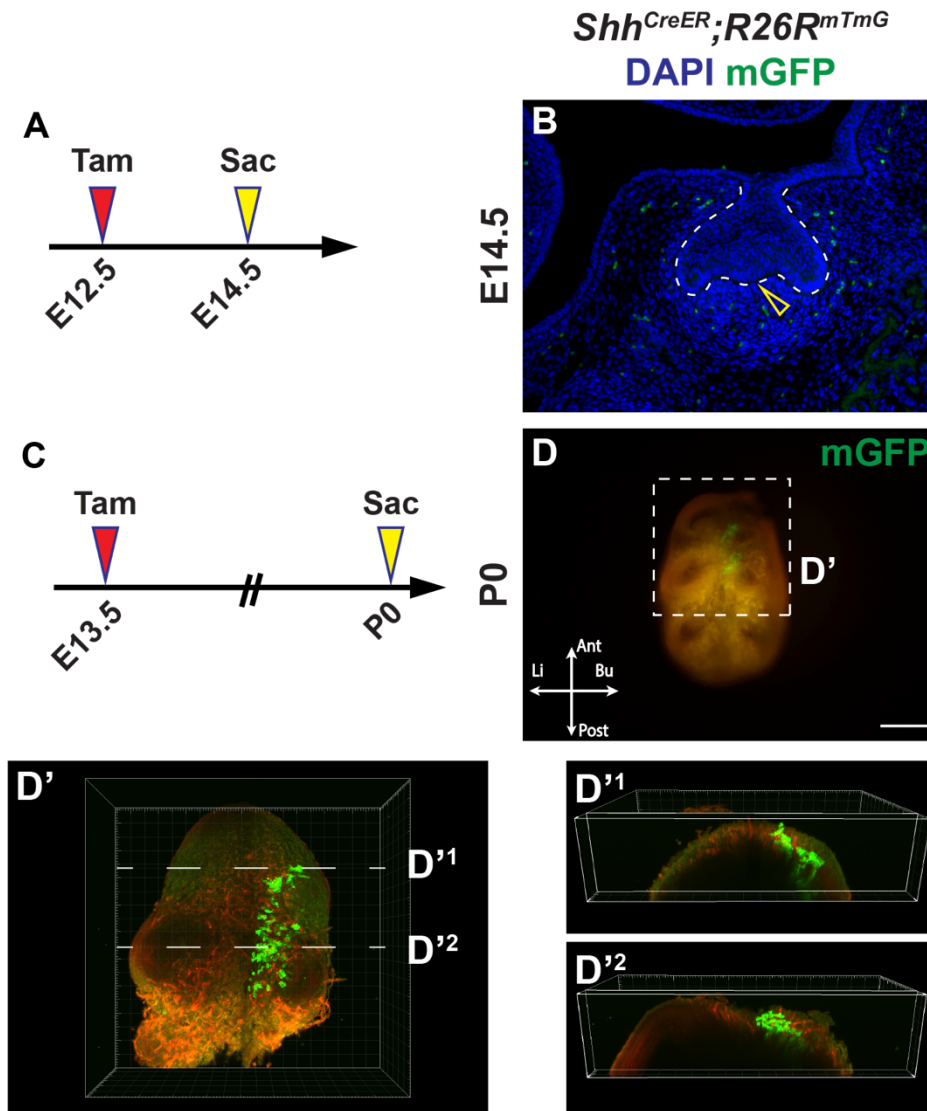
**B, C** Lineage tracing analysis of *Shh* expressing-cells in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> by gavaging 2.5 mg/30 g body weight of tamoxifen at E10.5. GFP-positive cells are only observed in the IK region (yellow arrowheads), absent from the EK (open yellow arrowheads). n=7 embryos for each time point.

**D** Timeline depicting the onset of Cre induction (Tamoxifen (Tam) injection, red arrowhead) and sample collections (yellow arrowheads) for **E and F**. Tamoxifen concentration is 2.5 mg/30 g body weight.

**E, F** Lineage tracing analysis of *Shh* expressing-cells in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> by gavaging 2.5 mg/30 g body weight of tamoxifen induction at E11.5. Some GFP-positive cells are observed in the ameloblast region (yellow arrowheads). Autofluorescent blood cells are marked by the red asterisk. n=9 embryos.

Scale bar in **(F)** represents 50  $\mu$ m in **(B, C, E, F)**

Supplemental Figure 5.





**Figure S5. Lineage tracing of *Shh*-expressing cells in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> embryonic molars.**

**A** Timeline depicting the onset of Cre induction (Tamoxifen (Tam) injection, red arrowhead) and sample collections (yellow arrowheads) for **B**. Tamoxifen concentration: is 2.5 mg/30 g body weight.

**B** Lineage tracing analysis of *Shh* expressing-cells in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> by gavaging 2.5 mg/30 g body weight of tamoxifen at E12.5. GFP-positive cells are absent from the EK (open yellow arrowheads). n=6 embryos.

**C** Timeline depicting the onset of Cre induction (Tamoxifen (Tam) injection, red arrowhead) and sample collections (yellow arrowheads) for **D-D'<sup>2</sup>**. Tamoxifen concentration is 1.25mg/30g body weight.

**D-D'<sup>2</sup>** Whole-mount view (**D**) and 3-dimensional (3D) reconstruction generated by Imaris (**D'**) from lineage traced *Shh* expressing-cells in *Shh*<sup>CreER</sup>; *R26R*<sup>mTmG</sup> at E12.5. The dashed lines mark the positions of the optical sections in (**D'<sup>1</sup>**, **D'<sup>2</sup>**). Li, lingual; Bu, buccal; Ant, anterior; Post, posterior. Scale bar in (**D**) represents 50  $\mu$ m in (**B**, **D**)

## Supplemental Movie Legends

### Movie S1.

3-dimensional (3D) reconstruction generated in Imaris from lineage traced *Shh* expressing-cells in *Shh<sup>CreER</sup>; R26R<sup>mTmG</sup>* at E12.5.

### Movie S2.

3-dimensional (3D) reconstruction generated in Imaris from lineage traced *Shh* expressing-cells in *Shh<sup>CreER</sup>; R26R<sup>mTmG</sup>* at E13.5.

### Movie S3.

3-dimensional (3D) reconstruction generated in Imaris from lineage traced *Shh* expressing-cells in *Shh<sup>CreER</sup>; R26R<sup>mTmG</sup>* at E14.5.