Ahtiainen et al., http://www.jcb.org/cgi/content/full/jcb.201512074/DC1

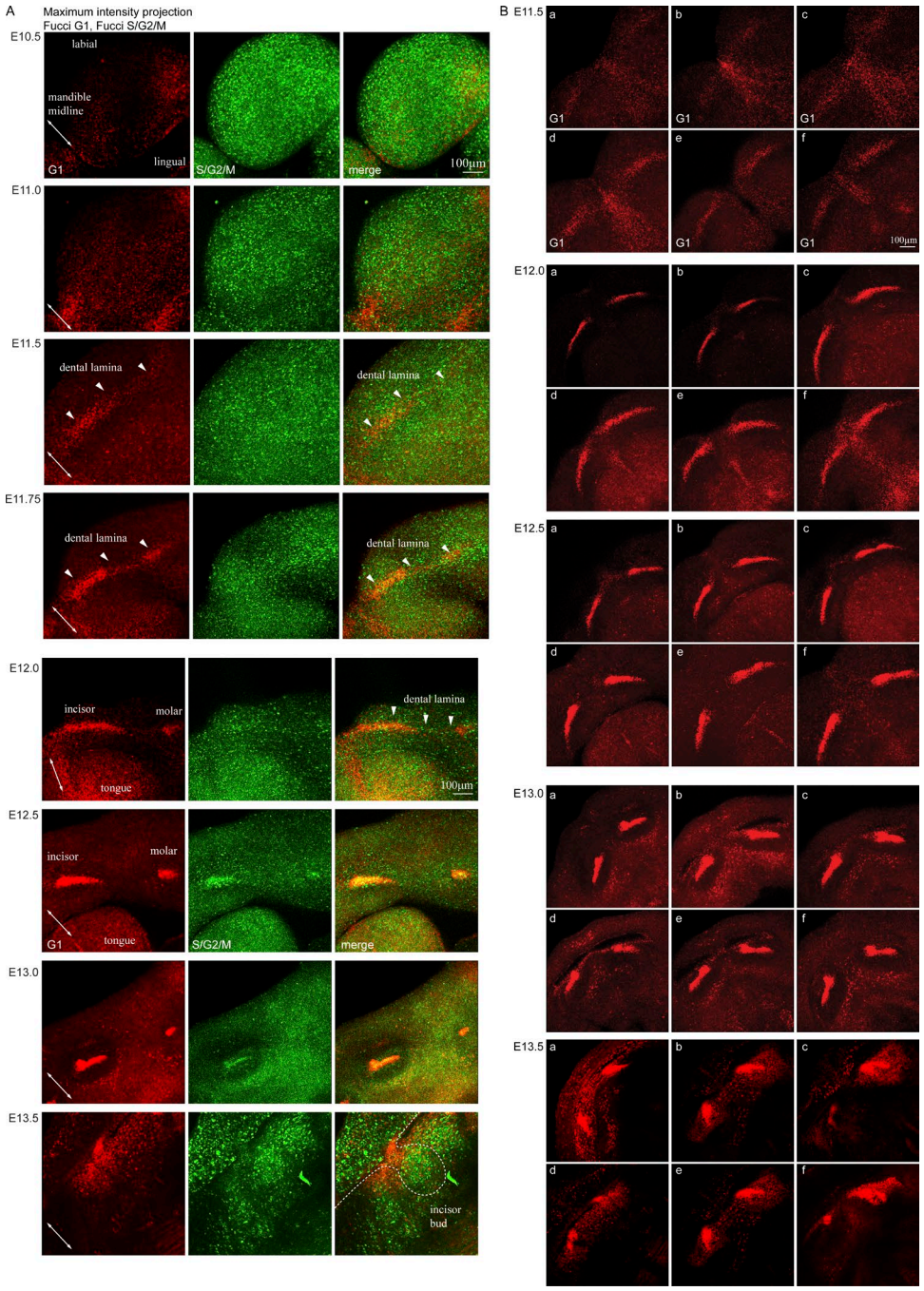


Figure S1. **Distinct distribution of different cell cycle phases in the developing mouse mandible corresponding to prospective tooth areas.** (A) Maximum-intensity projections of confocal fluorescence microscopy *z*-stacks of Fucci cell cycle reporter embryonic mouse mandible whole mounts at different stages of embryonic development. Red, G1 cell cycle phase; green, S/G2/M phase. In the early stages of mandible development, at E10.5 and 11.0, there was an even distribution of both Fucci G1 and S/G2/M cells throughout the tissue area. At E11.5, during the initiation of the dental lamina, G1 cells were seen to enrich in the thickening area. By E12.0, a uniform stripe of G1 cells marked the dental lamina (arrowheads), and this even stripe resolved into separate domains for the molar and incisor regions by E12.5; G1 cells remained in this area up to E13.5. (B) Maximum-intensity projections of confocal fluorescence microscopy *z*-stacks of Fucci G1 cell cycle reporter embryonic mouse whole mount mandibles (individual representative embryos marked a–f). Clustering of G1 cells to distinct areas in the incisor tooth forming regions was observed. Bars, 100 μm.

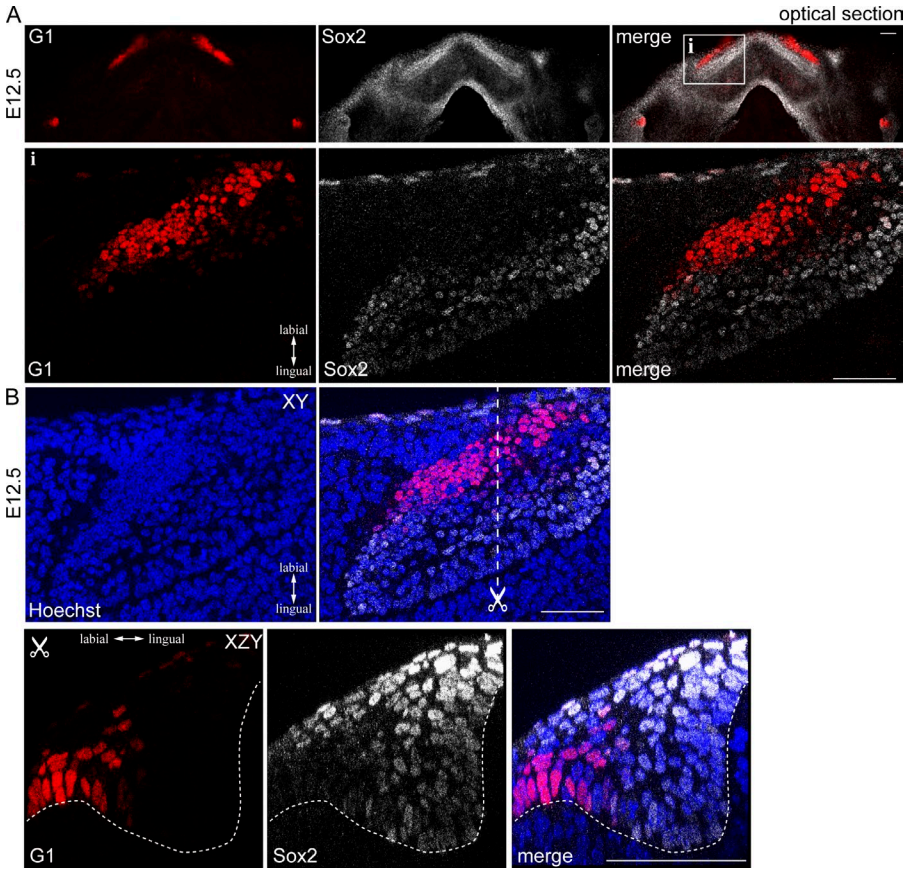


Figure S2. **Immunofluorescence staining of Sox2-positive cells in the incisor shows localization on the lingual side of the early signaling center.** High-resolution imaging of E12.5 Fucci G1 whole-mount samples immunostained for Sox2. Epithelium is delineated with a dotted line, Hoechst-stained nuclei (blue). (A) Top view of an optical *z*-section shows Sox2-positive epithelial cells (white) distinct from the Fucci G1 cell population (red). (B) Representative *xzy* optical section shows that Sox2 positive cells resided on the lingual side of the early signaling center. These data are in agreement with the whole-mount in situ hybridization data and confirm that Sox2-positive cells are distinct from the early signaling center G1 cells. Bars, 100 µm.

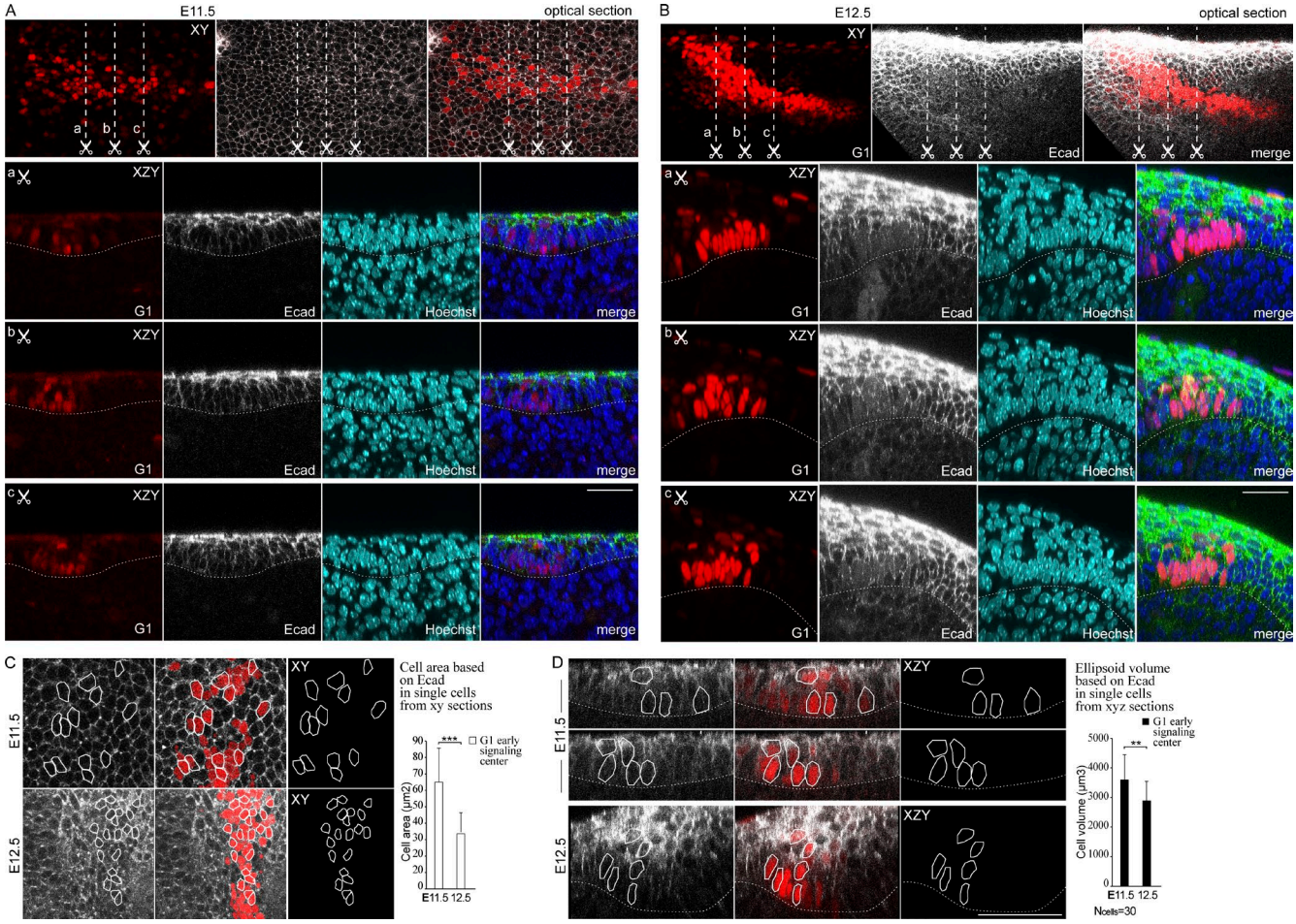


Figure S3. **Reduction in G1 cell size in the maturing early signaling center.** Individual cell areas were measured in E-cadherin (white) immunofluorescence-stained samples for visualization of cell perimeters together with nuclear Hoechst stain (cyan/blue) and Fucci G1 fluorescence (red). Measurements were done in 3D by inspecting *xyz* and *xzy* optical stacks and measuring individual cell area or cell volume based on E-cadherin (Ecad) staining in G1-positive cells. Three representative sections (a–c) are shown from E11.5 (A) and 12.5 (B) samples. (C) Cell area was defined from 152 cells in four samples for each time point and showed a similar reduction as that seen with the averaging from total volume. (D) Similarly, volume based on reducing single cell shapes to ellipsoids (measuring height, width, and depth; n_{cells} = 30 and $n_{samples}$ = 4) showed reduction in cell volume. However, reduction in cell volume was less pronounced than with the averaging method (Fig. 3 E), likely because of variations in cell shape from a true ellipsoid in the earlier time point. The G1 early signaling center cells become more elongated as the cells condense. ***, P < 0.001; **, P < 0.01. Error bars represent SD. Bars, 50 µm.

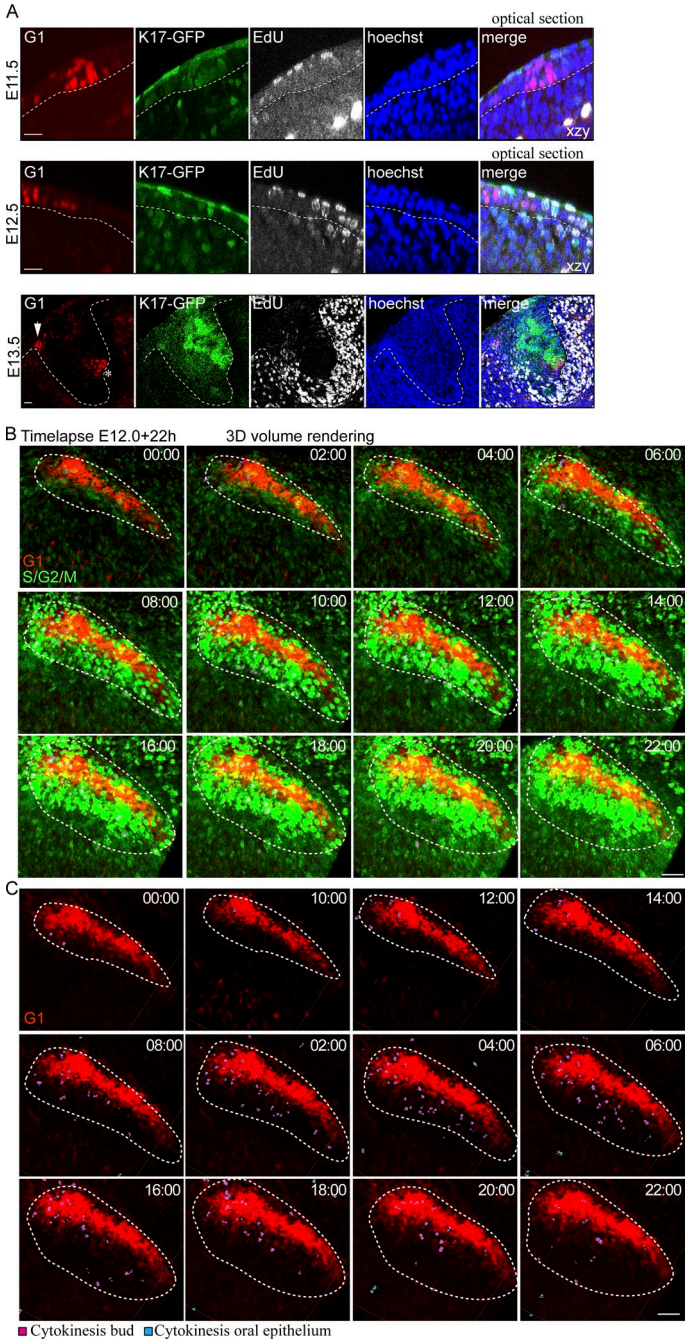


Figure S4. **Early signaling center cells remain in G1, whereas budding morphogenesis takes place through local cell proliferation in the adjacent lingual cell population.** (A) Cell proliferation was assessed using in vivo injections of EdU into pregnant dams. K17-GFP and Fucci G1 embryos were dissected, and EdU was detected from whole mounts (E11.5 and E12.5) or frontal paraffin sections (E13.5). Green, K17-GFP–positive epithelium; red, G1; white, EdU; blue, Hoechst nuclear stain; dotted line, epithelium; arrowhead, early signaling center; asterisk, emerging enamel knot. At E11.5, there was uniform cell proliferation, indicated by EdU incorporation, throughout the tissue. In agreement with the Fucci data, proliferation was seen in the cell population adjacent to the early signaling center G1 cells at E12.5 and E13.5. Cell proliferation in the incisor visualized by live confocal fluorescence imaging of a Fucci whole-mount explant. (B) Still images from time lapse of E12.0 + 22 h. Red, G1 early signaling center cells; green, S/G2/M cells; magenta, mitoses (cytokinesis) in the tooth epithelium; cyan, mitoses in the oral epithelium. There was a wave of cell divisions starting at 6 h in the cells directly adjacent to the G1 area on the lingual side. (C) The G1 population remained persistently localized to the anterior part of the forming bud. Bars, 50 µm.

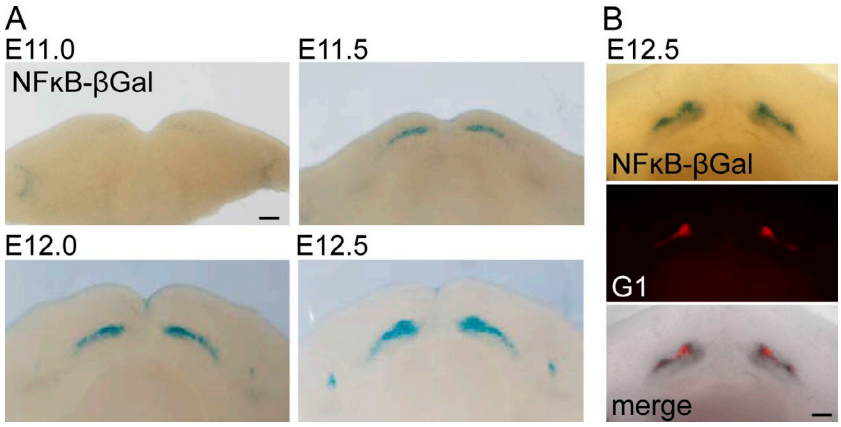
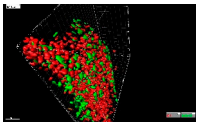
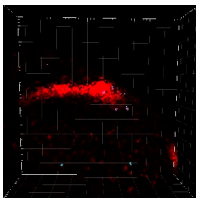


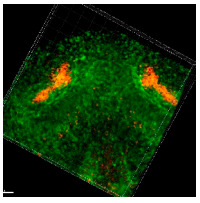
Figure S5. **NF-κB reporter expression in the prospective incisor region.** (A) NF-κB reporter marks Eda/NFκB activity in the prospective incisor region in X-gal–stained mandible whole mounts at developmental stages E11.0, 11.5, 12.0, and 12.5. (B) X-gal–stained NF-κB reporter shows colocalization with Fucci G1 in E12.5 mandible. Bars, 50 µm.



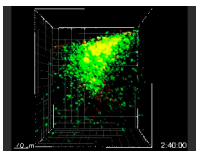
Video 1. **Contribution of different cell cycle phase cells to the forming incisor at different stages of development.** Fixed tissues were imaged with laser scanning confocal microscopy. Video presents 3D surface renderings made from fluorescence confocal *z*-stacks of whole-mount mandibles at various stages of incisor formation. The contribution of G1 and S/G2/M cells at stages E11.5, 12.0, 12.5, and 13.5. Red, G1; green, S/G2/M. Bar, 50 µm.



Video 2. **S/G2/M switch is initiated at E12.0 + 22 h in the cell population directly adjacent to the early signaling center.** A laser scanning confocal time-lapse video of an E12.0 Fucci G1, S/G2/M transgenic mouse mandible. Red, G1; green, S/G2/M. The time lapse is 22 h of a *z*-stack through the tissue acquired every 20 min with a display rate of 5 frames/s. The video shows a 3D volume rendering of the developing incisor. Individual S/G2/M cells that go into cytokinesis are shown in magenta in the cell population contributing to the tooth bud. Cell divisions in oral epithelial cells are shown in cyan. Bar, 50 µm.



Video 3. **Cell proliferation in the cell population adjacent to the G1 early signaling center forms the incisor tooth bud.** Laser scanning confocal time-lapse video of an E12.5 Fucci G1, S/G2/M mouse mandible. Red, G1; green, S/G2/M. The time lapse is 10 h of a *z*-stack through the tissue acquired every 20 min at a display rate of 5 frames/s. The video first shows in 3D volume rendering an overview of the developing mandible from E12.5 up to 10 h. This is followed by a closeup of the left incisor displayed as single channels. Individual cells in the S/G2/M cell population were followed, and cell divisions of representative cells are shown as surface rendering of the nuclei in light yellow. The G1 early signaling center cells stayed localized to the labial part of the developing bud, whereas the cell population on the lingual side proliferated, contributing to rapid bud growth. Bar, 50 µm.



Video 4. **Visualization of the early signaling center and enamel knot by Fucci G1 and TCF/Lef:H2B-GFP reporter.** Laser scanning confocal time-lapse video (associated with Fig. 7 A) of an E13.0 TCF/Lef:H2B-GFP, Fucci G1 transgenic mouse mandible. The time lapse is 14 h of a *z*-stack through the tissue acquired every 20 min at a display rate of 5 frames/s. First shown is a 3D volume rendering of the tissue with cells expressing GFP (green) and cells in G1 phase (red). This is followed by a 3D surface rendering with early signaling center/dental lamina GFP-positive cells in green and G1 cells in red. The enamel knot GFP-positive cells are shown in light yellow, and G1 cells in magenta. The cells in the early signaling center are both G1 and GFP positive and remain in the labial part of the developing bud. At 4–6 h, the first G1 nuclei appear in the enamel knot area, and cells become GFP positive in a wider area in the lingual part of the enamel knot. The two signaling centers remain separate throughout bud morphogenesis. Bar, 50 µm.