

Figure S1. Inhibition of cell proliferation in mandibular explant culture (associated with Figure 2). (A) BrdU incorporation in vehicle (DMSO)-treated or proliferation-inhibited (aphidicolin (APH) + hydroxyurea (HU)) mandibles. Blue: DAPI. Green: BrdU. Red: β -catenin. Scale bars: 50 μ m. (B) Shh in situ hybridisation in vehicle or APH+HU treated mandible cultures. Arrowheads: molars. Arrows: incisors. Scale bar: 500 μ m.

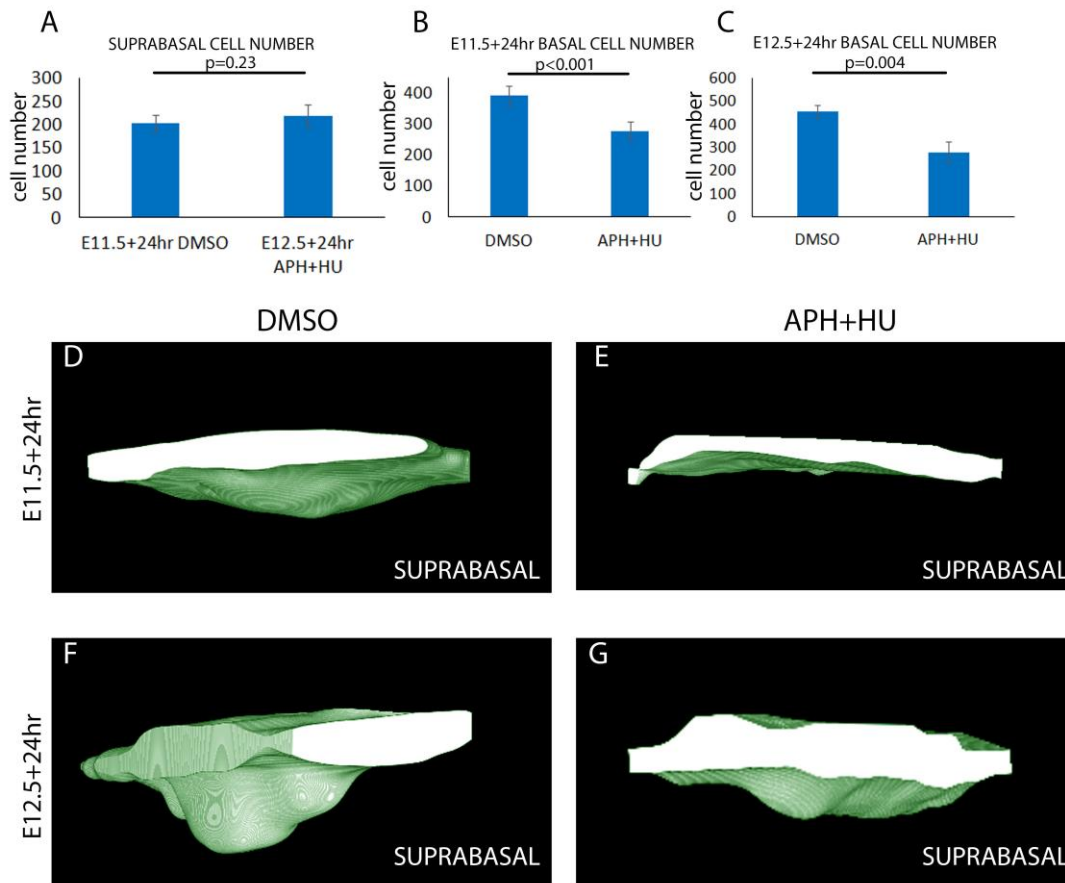


Figure S2. Basal and suprabasal cell quantification and 3D rendering of suprabasal volume (associated with Figure 3). (A) Comparison of suprabasal cell numbers between E11.5+24hr control placodes and E12.5+24hr proliferation inhibited placodes. n = 9. (B-C) Quantification of basal cell numbers in E11.5+24hr (B) and E12.5+24hr (C) placodes. n = 9. (D-E) Mandibular molars cultured with vehicle (D) or proliferation inhibitors (E) from E11.5 for 24 hrs. (F-G) Mandibular molars cultured with vehicle (F) or proliferation inhibitors (G) from E12.5 for 24 hrs

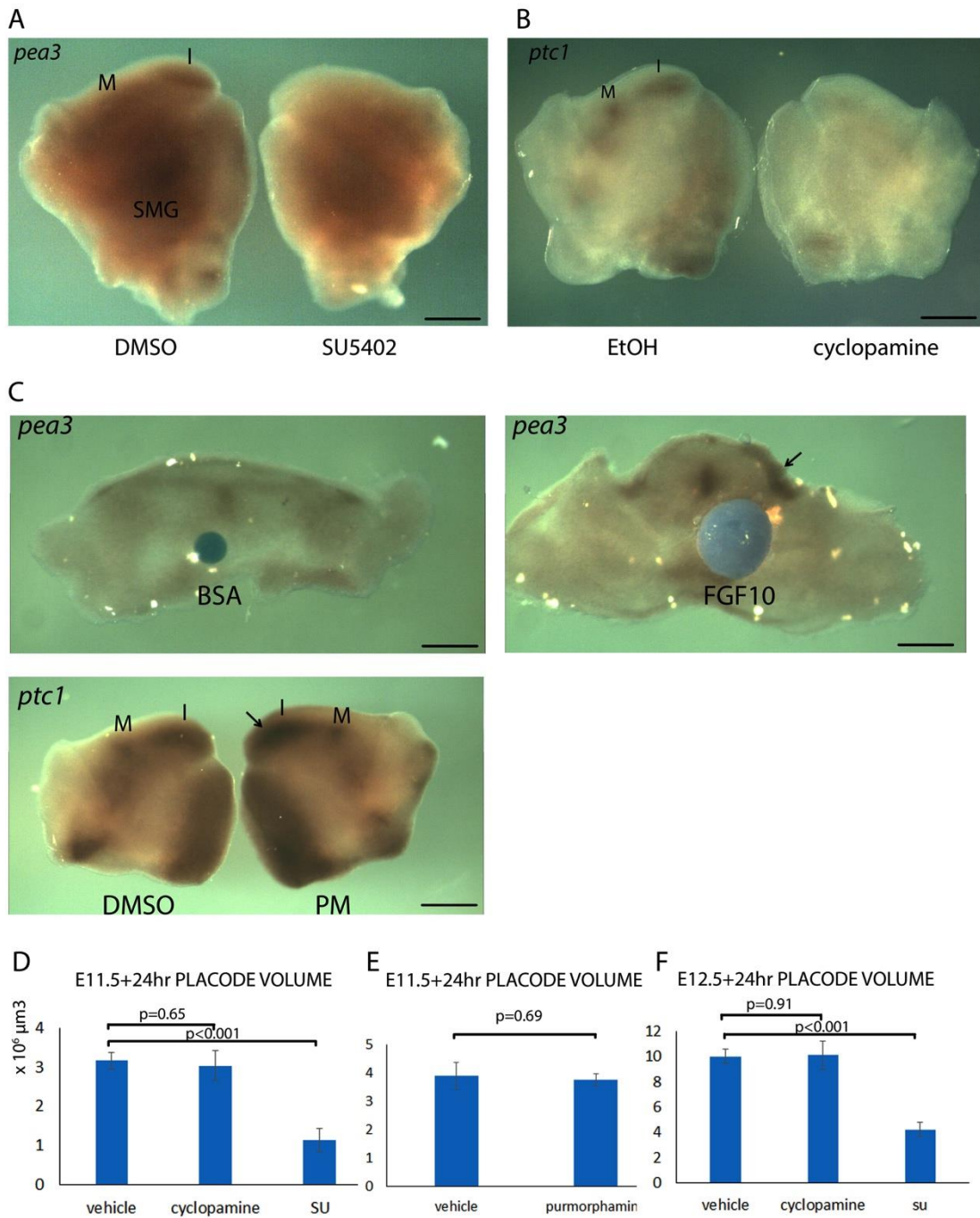


Figure S3. In situ hybridisation showing gene expression under manipulations of FGF and Shh pathways (associated with Figures 4 and 5). (A) *pea3* expression in cultured E11.5 mandible was inhibited by FGF inhibitor (SU5402). (B) *ptc1* expression was inhibited by Smoothened inhibitor (cyclopamine). (C) Top panels: *pea3* expression in cultured frontal slices of E11.5 mandible was induced by FGF10 beads. Arrow: induced expression of *pea3* in the tongue epithelium near the bead. Bottom panel: *ptc1* expression was enhanced by purmorphamine treatment in the tooth regions of cultured E11.5 mandibles. Arrow: enhanced *ptc1* expression. M: molar. I: incisor. SMG: submandibular gland. Scale bars: 500 μm. (D-F) Quantification of placode volumes in control, cyclopamine, purmorphamine and SU treated placodes, cultured from E11.5 and E12.5. n = 9.

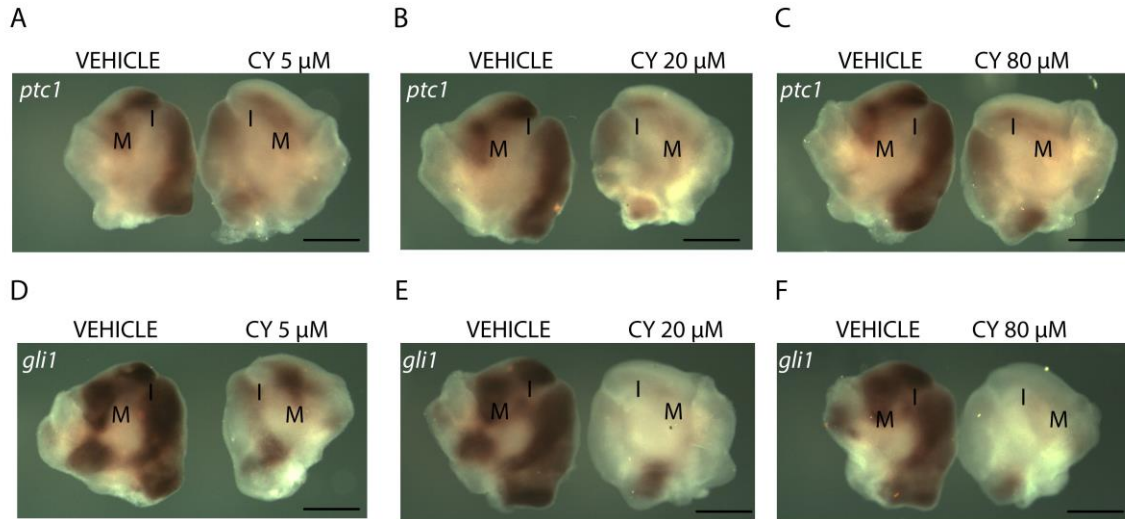


Figure S4. The efficacy of different concentrations of cyclopamine tested by in situ hybridisation (associated with Figures 6). (A-C) *ptc1* in situ hybridisation with 5 μ M (A), 20 μ M (B) and 80 μ M (C) cyclopamine or vehicle (EtOH). (D-F) *gli1* in situ hybridisation with 5 μ M (D), 20 μ M (E) and 80 μ M (F) cyclopamine or vehicle (EtOH). I: incisor. M: molar. CY: cyclopamine. Scale bars: 500 μ m.

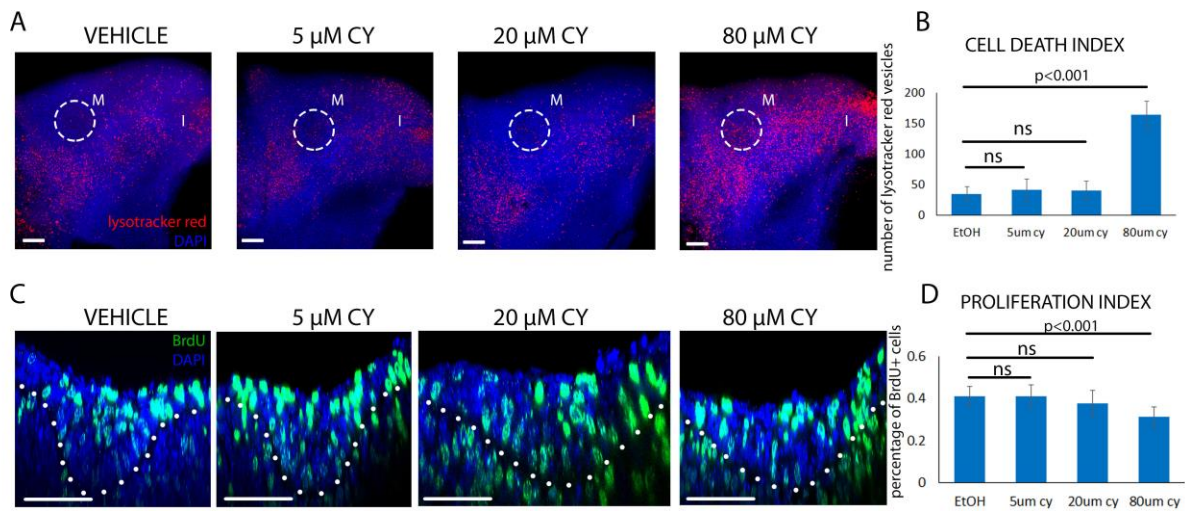


Figure. S5. Lysotracker red and BrdU labelling under different concentrations of cyclopamine treatments (associated with Figure 6). (A) Lysotracker red labelling of cultured mandibles. 3D image stacks were taken to determine the invaginated molar regions (circles). Red: Lysotracker red. Blue: DAPI. M: molar. I: incisor. (B) Quantification of Lysotracker red puncta in the molar region (circle). $n = 9$. (C) BrdU labelling after different concentrations of cyclopamine treatments. Green: BrdU. Blue: DAPI. Scale Bars: 50 μ m. White dots: outline of the epithelium. (D) Quantification of the percentage of BrdU positive cells. In the placodes. $n = 9$.

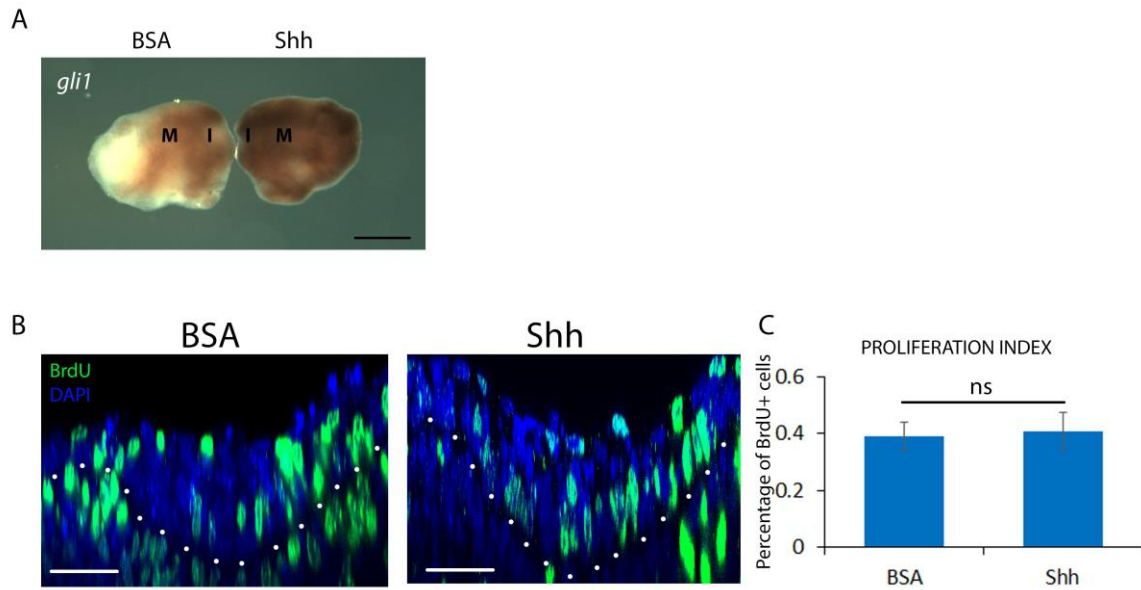


Figure S6. Shh protein treatment does not affect proliferation (associated with Figure 6). (A) *gli1* in situ hybridisation showing the efficacy of the Shh protein used in culture. M: molar. I: incisor. Scale bar: 500 μ m. (B) BrdU labelling in placodes treated with BSA (left) or Shh protein (right). Green: BrdU. Blue: DAPI. White dots: outline of the epithelium. Scale bars: 50 μ m. (C) Quantification of the percentage of BrdU positive cells in the placode. n = 9.

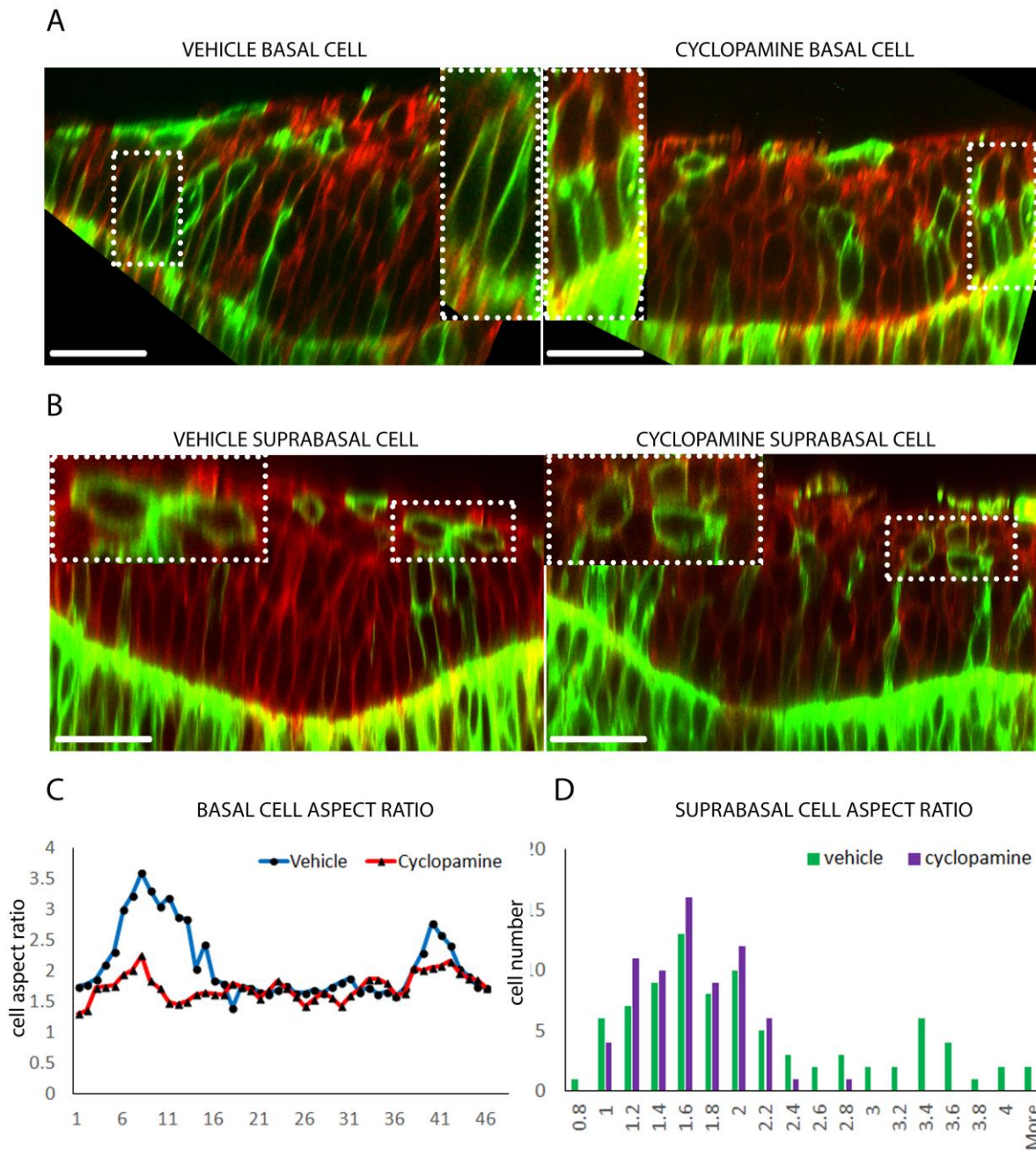


Figure S7. Cell elongation measurement using mTmG mosaic labelling (associated with Figure 7). (A) mTmG tooth placode treated with vehicle (EtOH) (left) and cyclopamine (right), showing the elongation of basal cells. Red: mTomato cells. Green: mGFP cells. Dotted rectangles: basal cells on the converging region of the placode. (B) mTmG tooth placode treated with vehicle (EtOH) (left) and cyclopamine (right), showing the elongation of suprabasal cells. Dotted rectangles: suprabasal cells near the surface (but not in the peridermal layer) of the placode. (C) Basal cell aspect ratio across a placode, showing the loss of elongated cells on the two constriction sides under cyclopamine treatment. (D) Suprabasal cell aspect ratio showing the loss of elongated cell population under cyclopamine treatment. $n = 6$.