Ras Signaling Regulates Stem Cells and Amelogenesis in the Mouse Incisor

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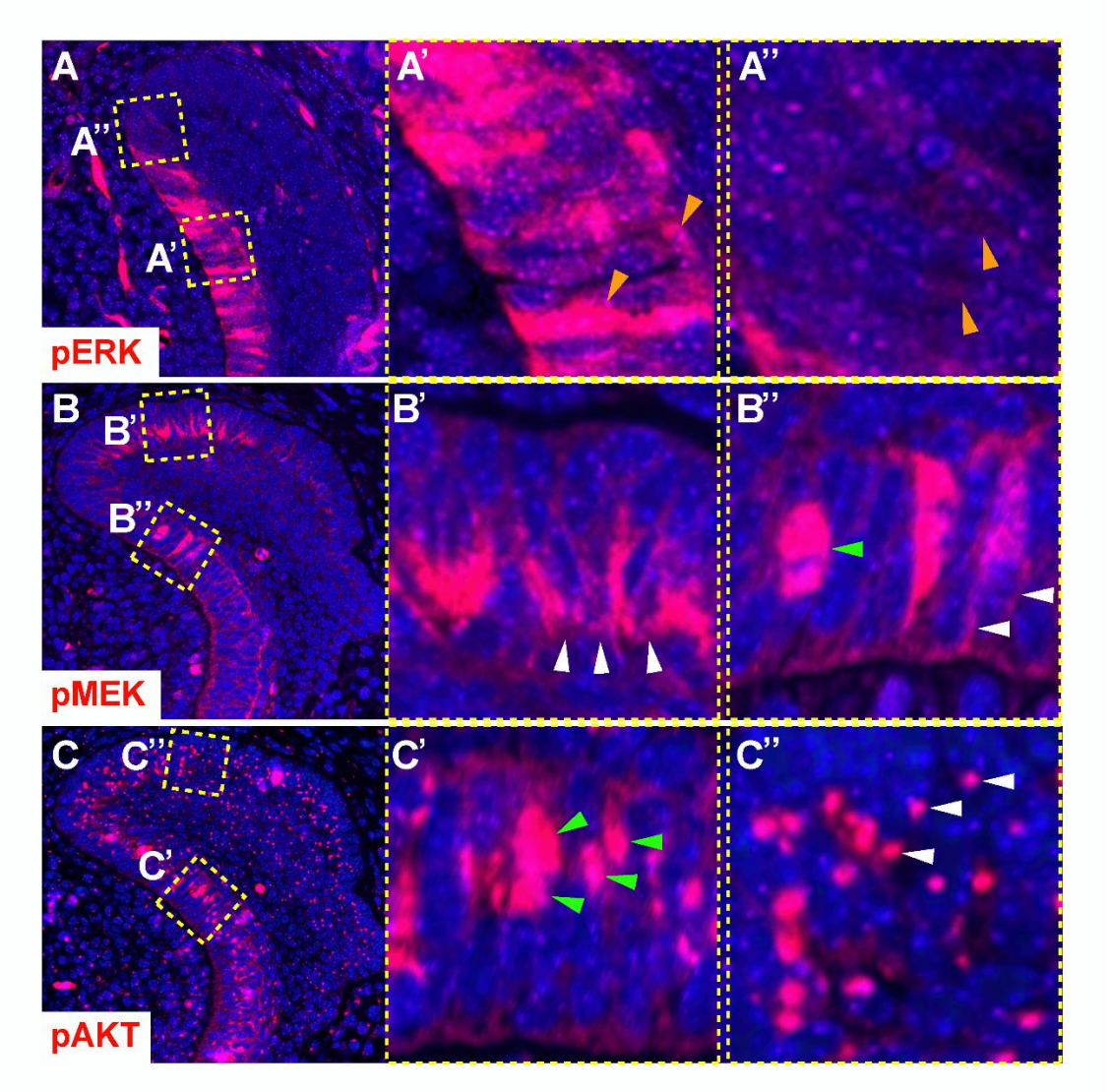
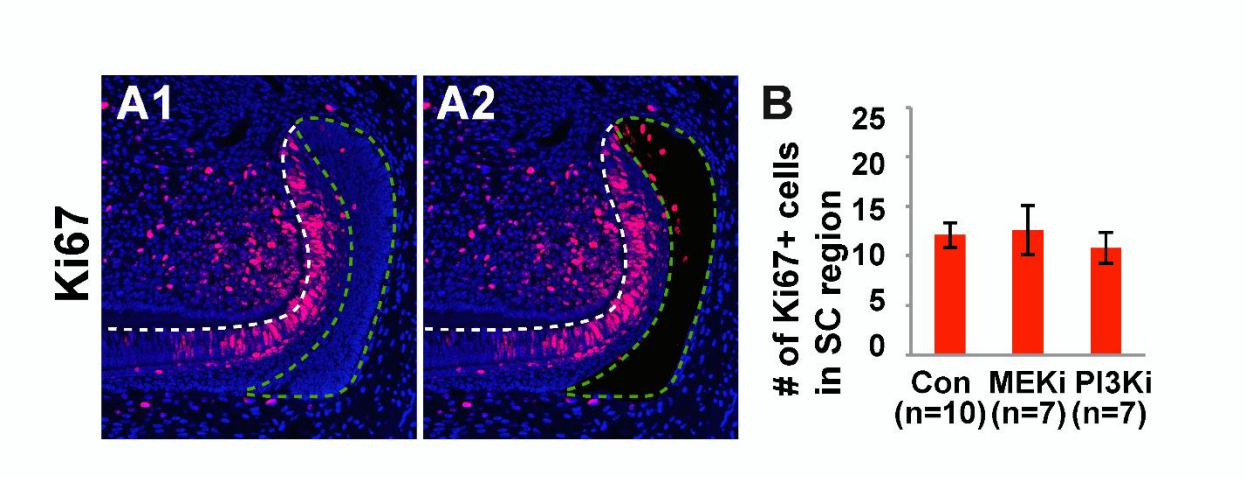
Appendix

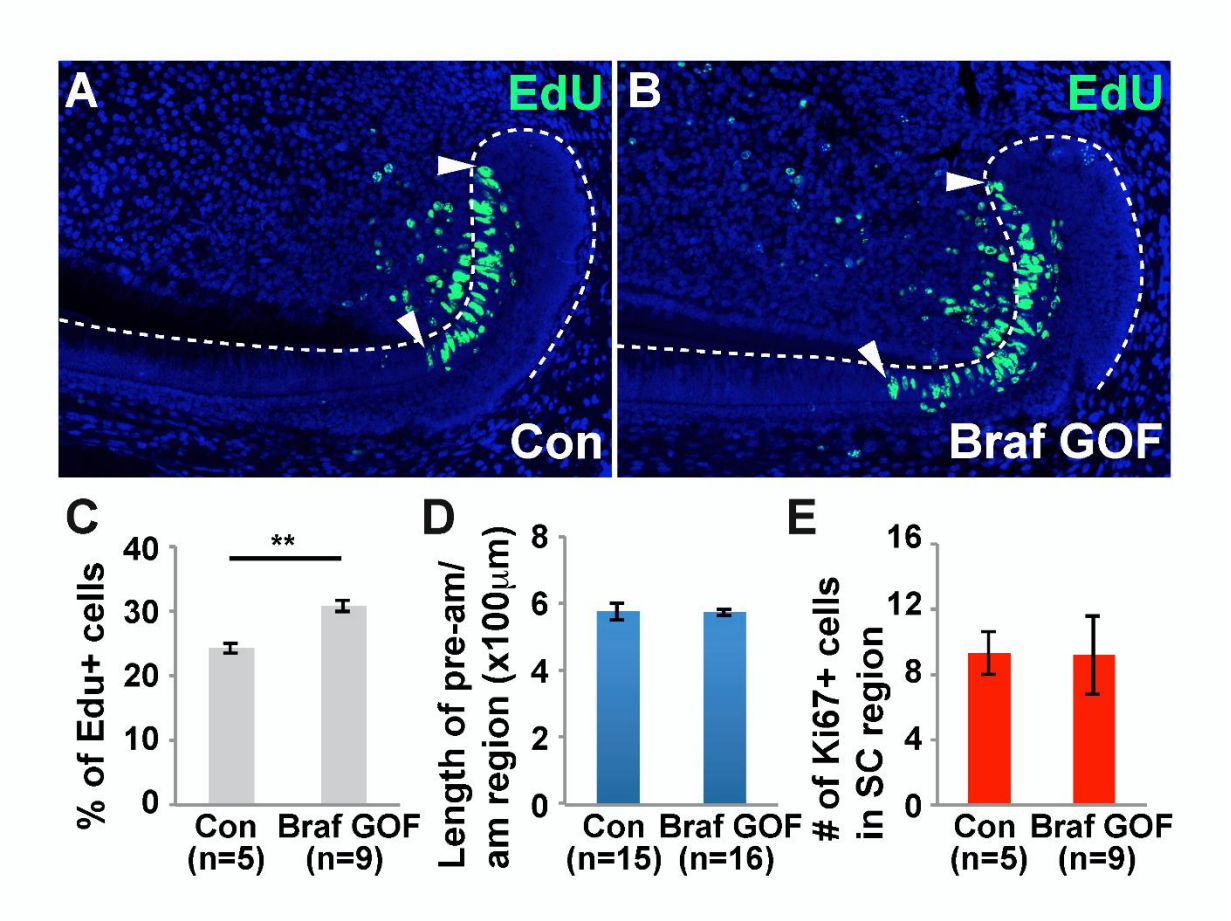


Appendix Figure 1. Cellular localization of pERK, pMEK, and pAKT in the mouse laCL. (A) Immunofluorescence with an antibody against pERK in the mouse laCL. Magnified boxed regions show high intensity (A') and low intensity (A") cytoplasmic pERK staining (orange arrows). (B) pMEK staining in the laCL with magnified views of

polarized membrane staining in the OEE (B’; white arrows) and membrane (B”; white arrows) and nuclear staining (B”; green arrow) in the T-A. (C) pAKT staining in the laCL was nuclear (C’; green arrows) or punctate in the cytoplasm (C”; white arrows). laCL, labial cervical loop; OEE, outer enamel epithelium; T-A, transit-amplifying.



Appendix Figure 2. Neither MEKi nor PI3Ki treatment affected DESC proliferation. (A1, A2) Immunofluorescence with antibody against Ki67 labeled proliferating cells in the DESC region of the proximal laCL (green outline). Dapi staining was removed in the green outlined SC region by turning off the 405 nm wavelength light to better visualize the Ki67 staining (A2). (B) MEKi and PI3Ki treatment did not affect DESC proliferation as shown by quantification of the number of Ki67+ cells in the green outlined DESC region. DESC, dental epithelial stem cell; laCL, labial cervical loop. Error bars represent SEM.



Appendix Figure 3. Effect of increased ERK phosphorylation in the *Braf*^{L597V} gain-of-function (Braf GOF) mouse. (A-C) An increased percentage of EdU+ cells was observed in the T-A region (delimited by white arrows) of Braf GOF mice compared to control. (D) No difference in length of the pre-am/am region was observed in Braf GOF mutants compared to control. (E) No difference in number of Ki67+, proliferating cells in the proximal laCL was observed. T-A, transit-amplifying; pre-am, preameloblast; am, ameloblast. Error bars represent SEM; ** $p<0.01$.