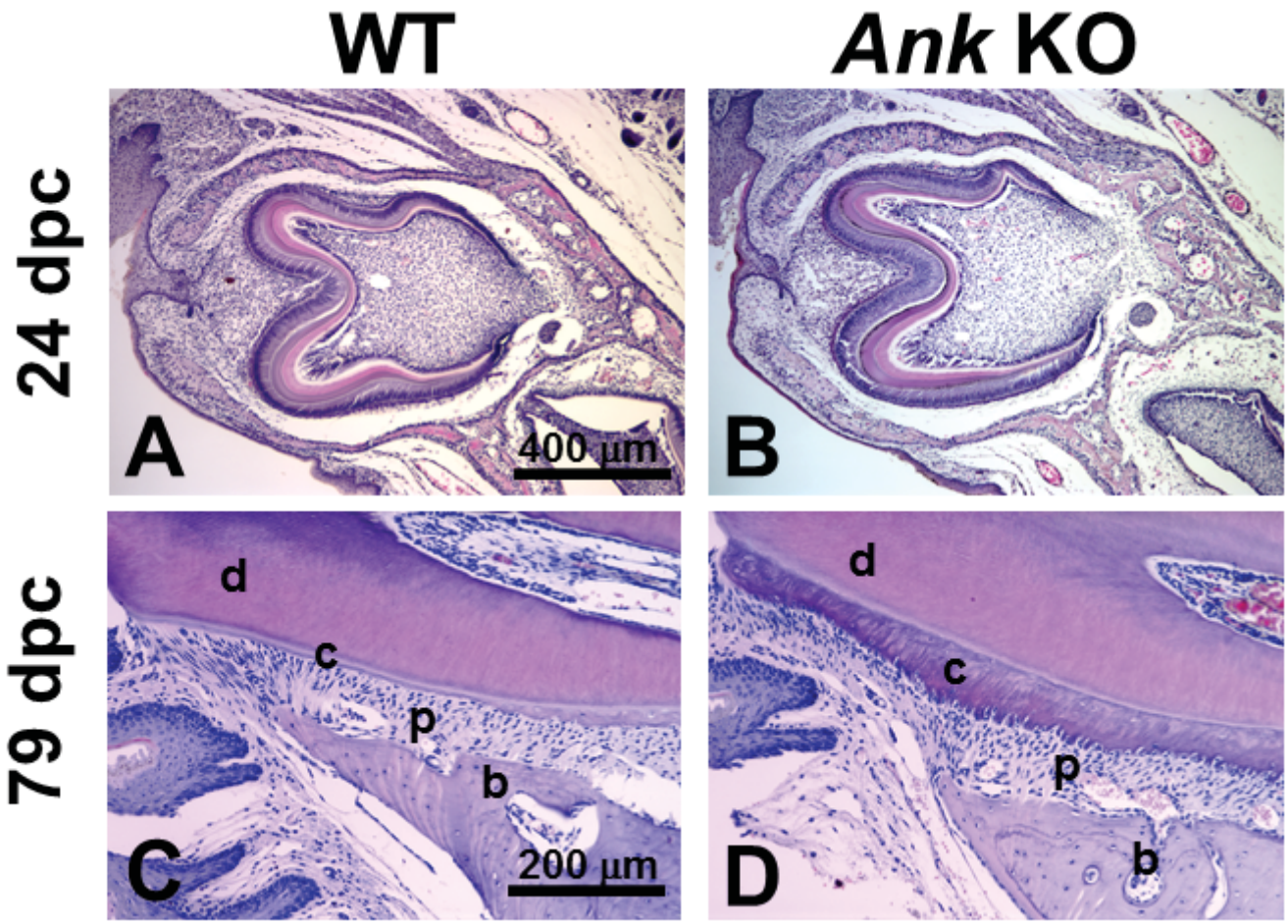
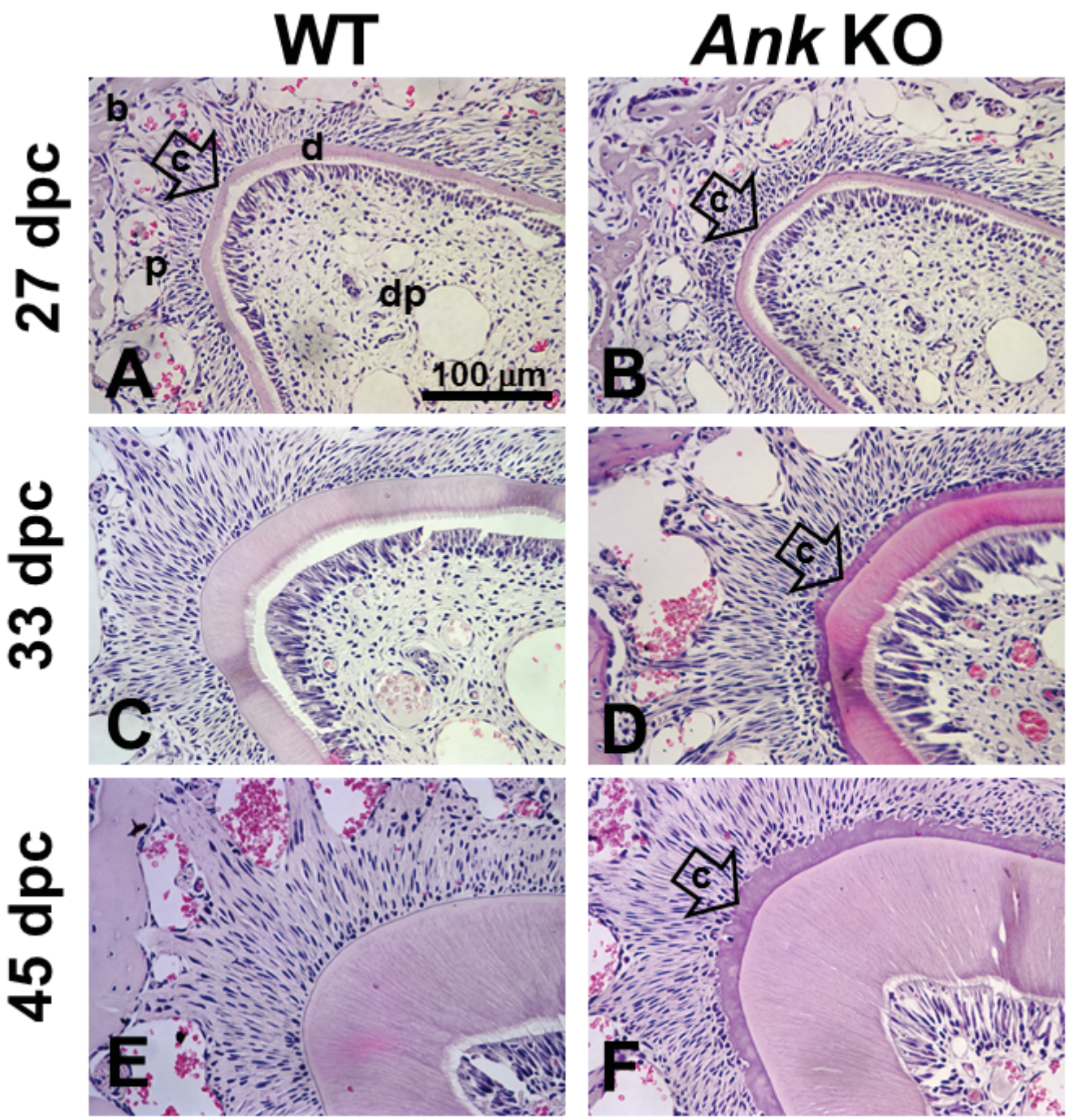
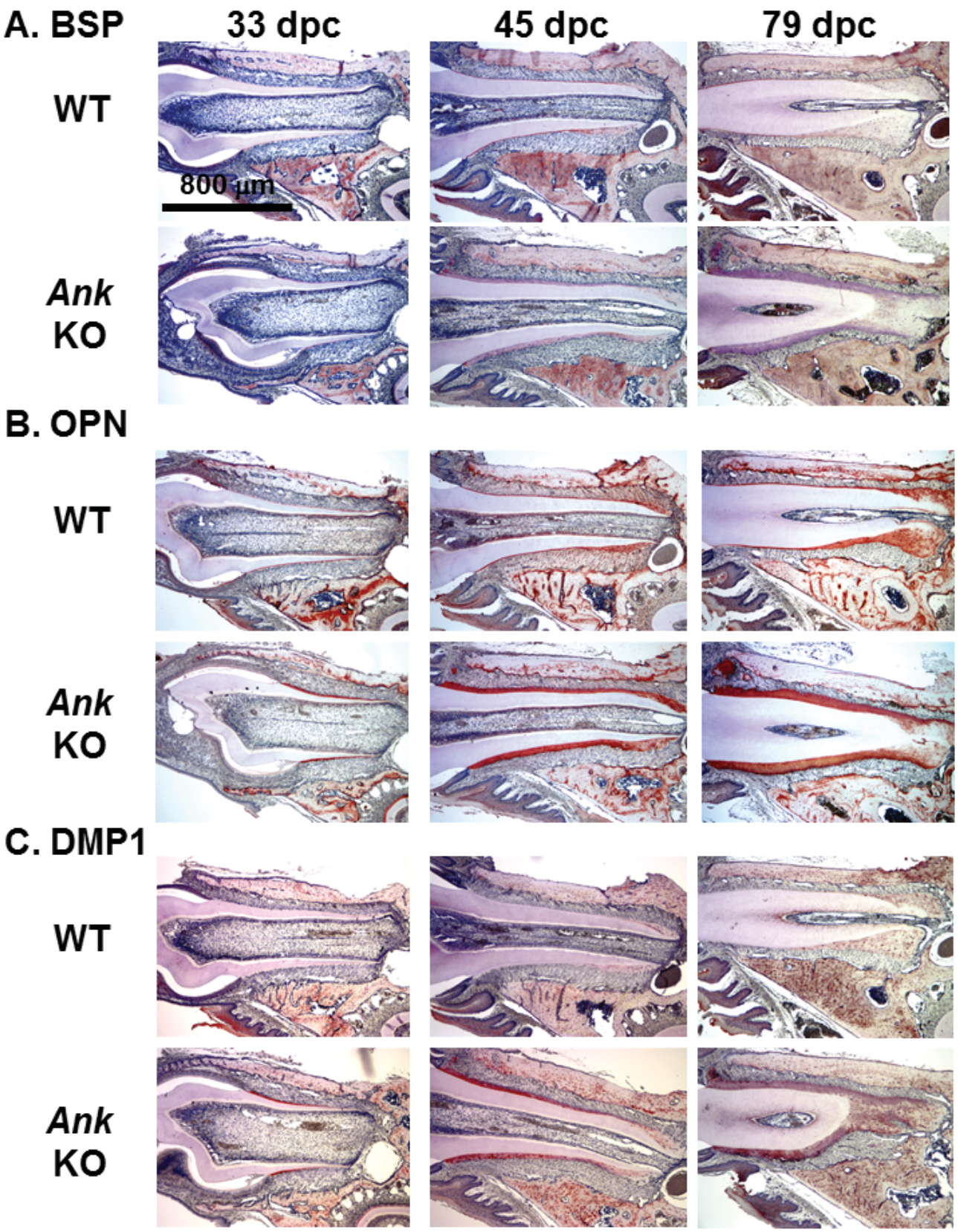
Foster et al – Supplemental Figure 1



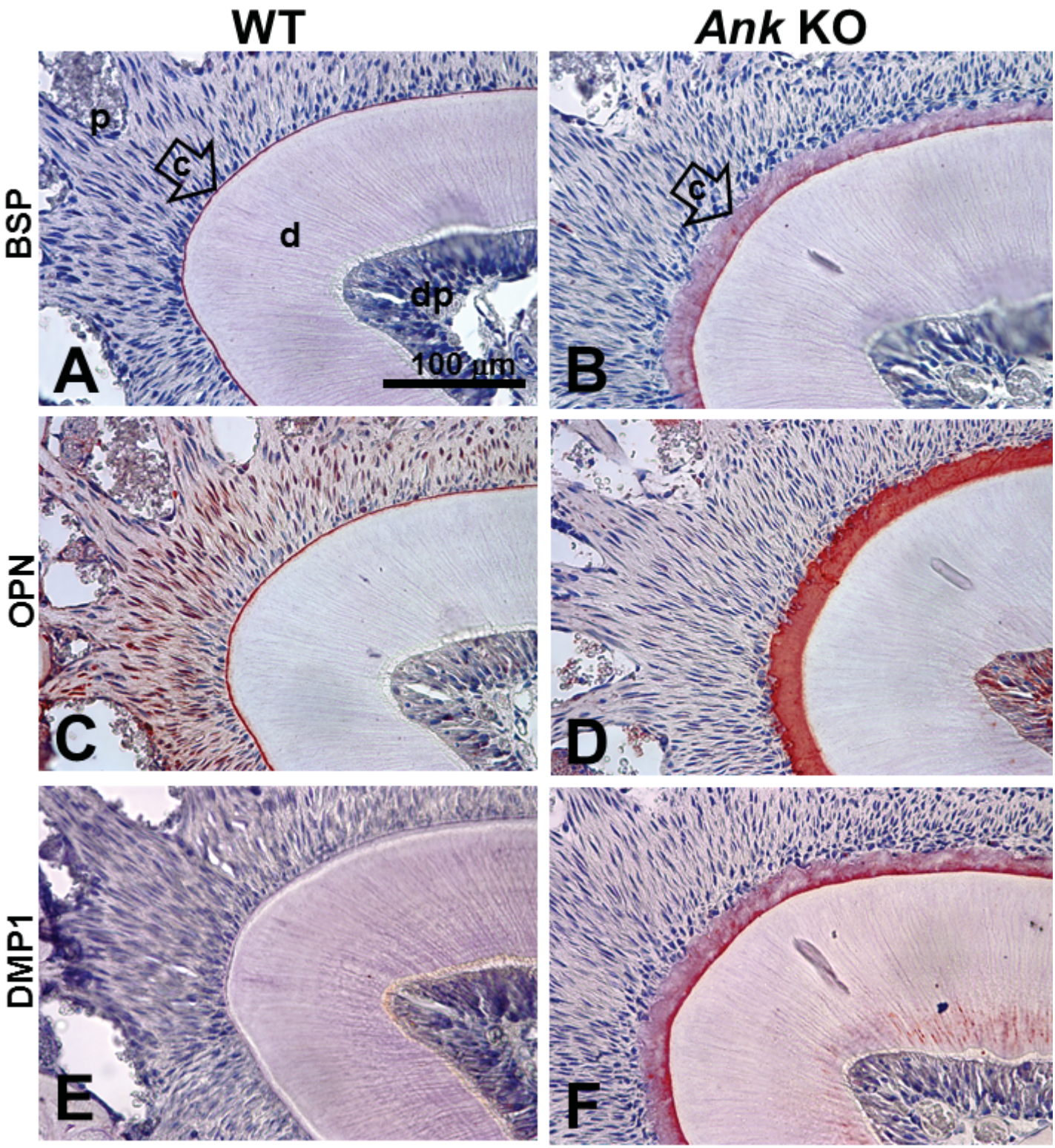
Foster et al – Supplemental Figure 2



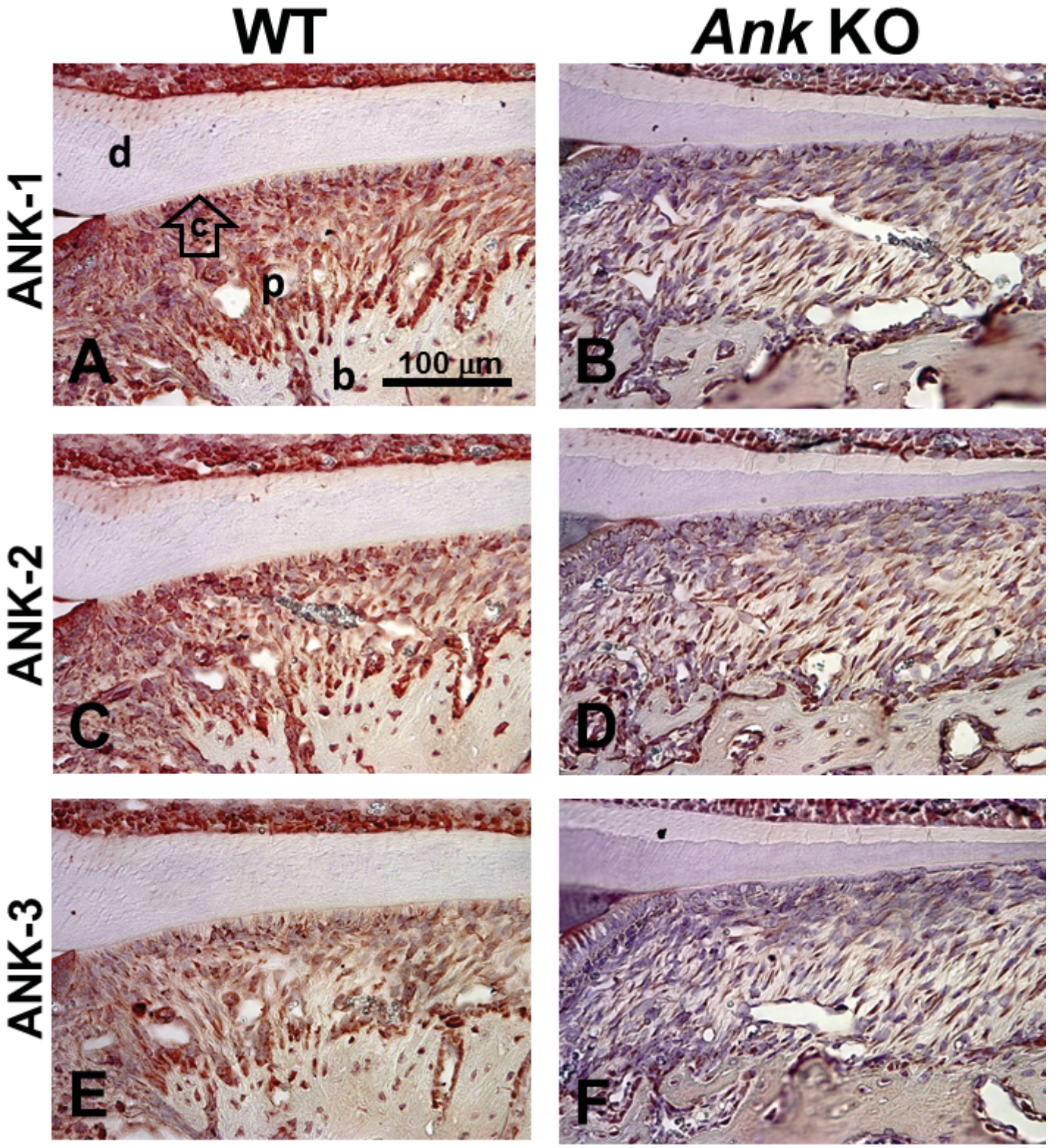
Supplemental Figure 3



Foster et al – Supplemental Figure 4



Foster et al – Supplemental Figure 5



Supplemental Figure 1. Loss of ANK affects targets molar root cementum. 24 dpc: At the early age of 24 dpc, where molars were in crown stage of development, no differences were noted between **(A)** WT and **(B)** *Ank* KO teeth. **79 dpc:** By the advanced age of 79 dpc, **(C)** WT cementum remained thin while **(D)** *Ank* KO cervical cementum hypercementosis progressed, though PDL space was well maintained. In 79 dpc sections, the lingual aspect of the first mandibular molar is featured. Abbreviations: d=dentin, c=cementum, p=periodontal ligament, b=alveolar bone. Panels A and B at original magnification 100X, panels C and D at original magnification 200X.

Supplemental Figure 2. Loss of ANK causes cementum hyperplasia in incisor. The continuously erupting mouse incisor features acellular cementum on the lingual "root analogue" aspect. Cementum remains thin in WT at ages **(A)** 27, **(C)** 33, and **(E)** 45 dpc.
Like the cervical cementum of the molar, the *Ank* KO incisor exhibited hypercementosis by **(B)** 27 dpc and increased at ages **(D)** 33 dpc and **(F)** 45 dpc. As with the molar tooth, the dentin, pulp chamber, and PDL of the incisor did not appear different from WT. Abbreviations: dp=dental pulp, d=dentin, c=cementum, p=periodontal ligament, b=alveolar bone. Original magnification 200X for panels A-F.

Supplemental Figure 3. Distribution of extracellular matrix proteins in the dentoalveolar complex of *Ank* KO vs. WT. Histological sections from 33, 45, and 79 dpc mice were used for IHC. **(A) <u>BSP</u>:** In WT, BSP staining was strong in bone and defined the entire acellular cementum at all time points. *Ank* KO cervical cementum showed diffuse and limited BSP staining at all time points, while bone BSP was no different. **(B) <u>OPN</u>:** In WT, OPN was

observed at bone cement lines, in PDL, odontoblasts, and strongly labeled acellular and cellular cementum. OPN localization in KO was similar to WT, notable for strong and even OPN labeling of cervical cementum over times points from 33 to 79 dpc. **(C) DMP1:** DMP1 in WT was localized to bone around osteocytes, and cervical cementum, but not acellular cementum. In *Ank* KO, DMP1 was strongly localized to cervical cementum as early as 33 dpc, particularly to perilacunar spaces around cells and cells in the process of being embedded. Increased DMP1 was noted at 45 and 79 dpc, also. Abbreviations: d=dentin, p=periodontal ligament, b=alveolar bone. Original magnification 100X.

Supplemental Figure 4. Alterations in *Ank* KO incisor cementum extracellular matrix proteins parallel those observed in molar acellular cementum. Histological sections from 45 dpc mice were used for IHC. **BSP: (A)** In WT incisor, BSP protein staining defined the entire acellular cementum. **(B)** *Ank* KO incisor cementum showed limited BSP staining. **OPN: (C)** WT incisor cementum exhibited strong staining for OPN. **(D)** *Ank* KO incisor cementum was strongly labeled for OPN protein. **DMP1: (E)** DMP1 was not labeled in WT incisor cementum. **(F)** In *Ank* KO, DMP1 was strongly increased in cementum of incisor tooth, particularly the initial cementum formed. Abbreviations: d=dentin, c=cementum, p=periodontal ligament. Original magnification 400X.

Supplemental Figure 5. Multiple antibodies confirmed widespread ANK expression in the developing dentoalveolar complex. ANK localization in developing tooth was confirmed by multiple antibodies against different ANK epitopes. In 33 dpc WT mouse molars, **(A)** ANK-1

and **(C)** ANK-2 antibodies indicate widespread ANK localization as described for ANK-3 antibody in **(E)** and shown in Figure 6. ANK immunostaining, however, was not seen in 33 dpc *Ank* KO mouse molars using **(B)** ANK-1, **(D)** ANK-2, and **(F)** ANK-3 antibodies. Abbreviations: d=dentin, c=cementum, p=periodontal ligament, b=alveolar bone. Original magnification 400X.