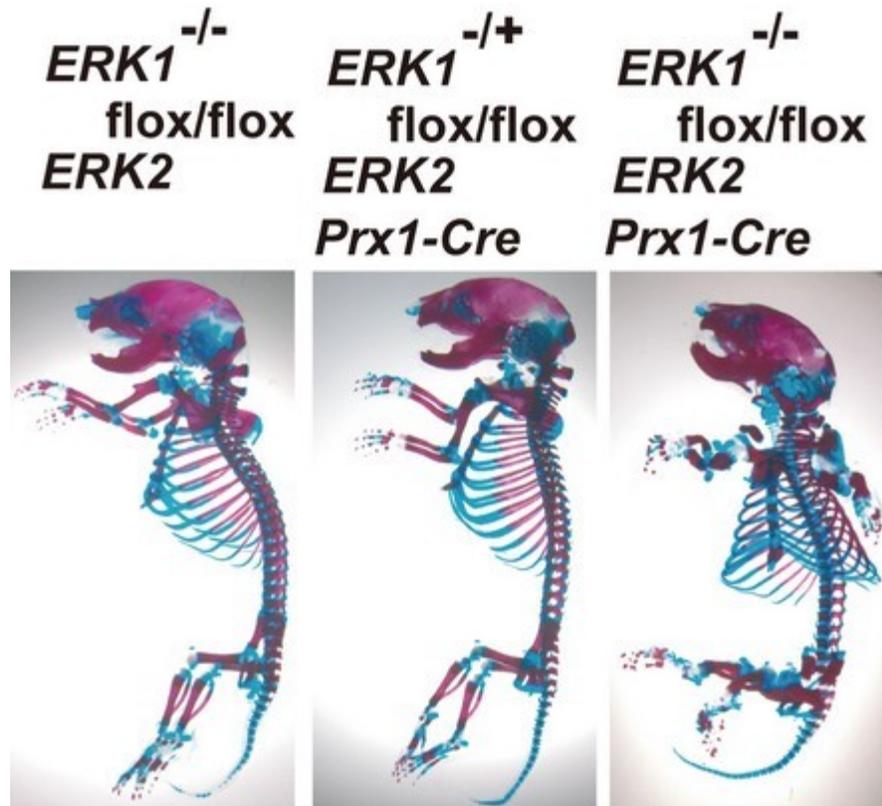


Supplemental Figures

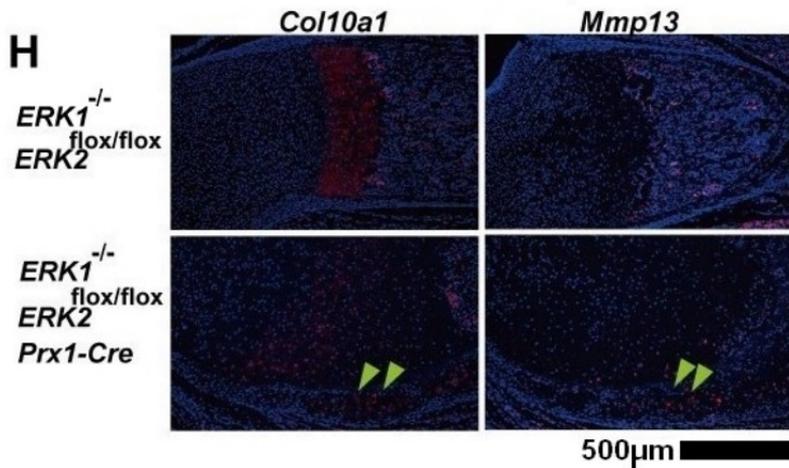
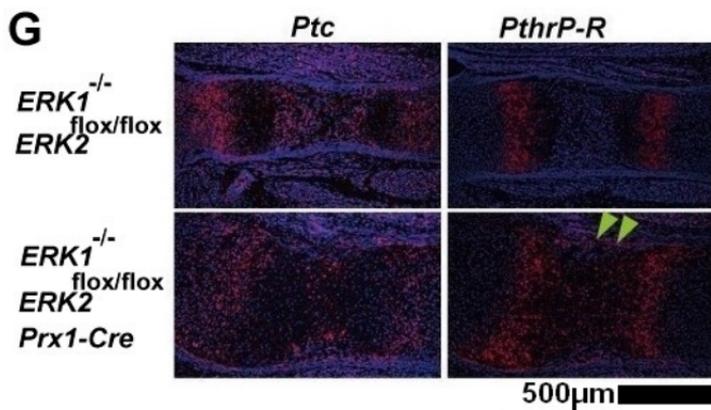
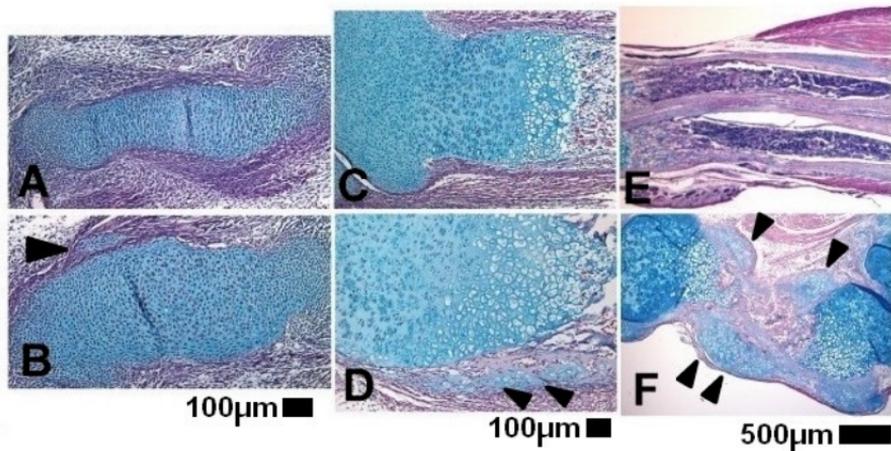
Supplemental Figure 1

Skeletal preparation of the whole body after alizarin red and alcian blue staining at P1.



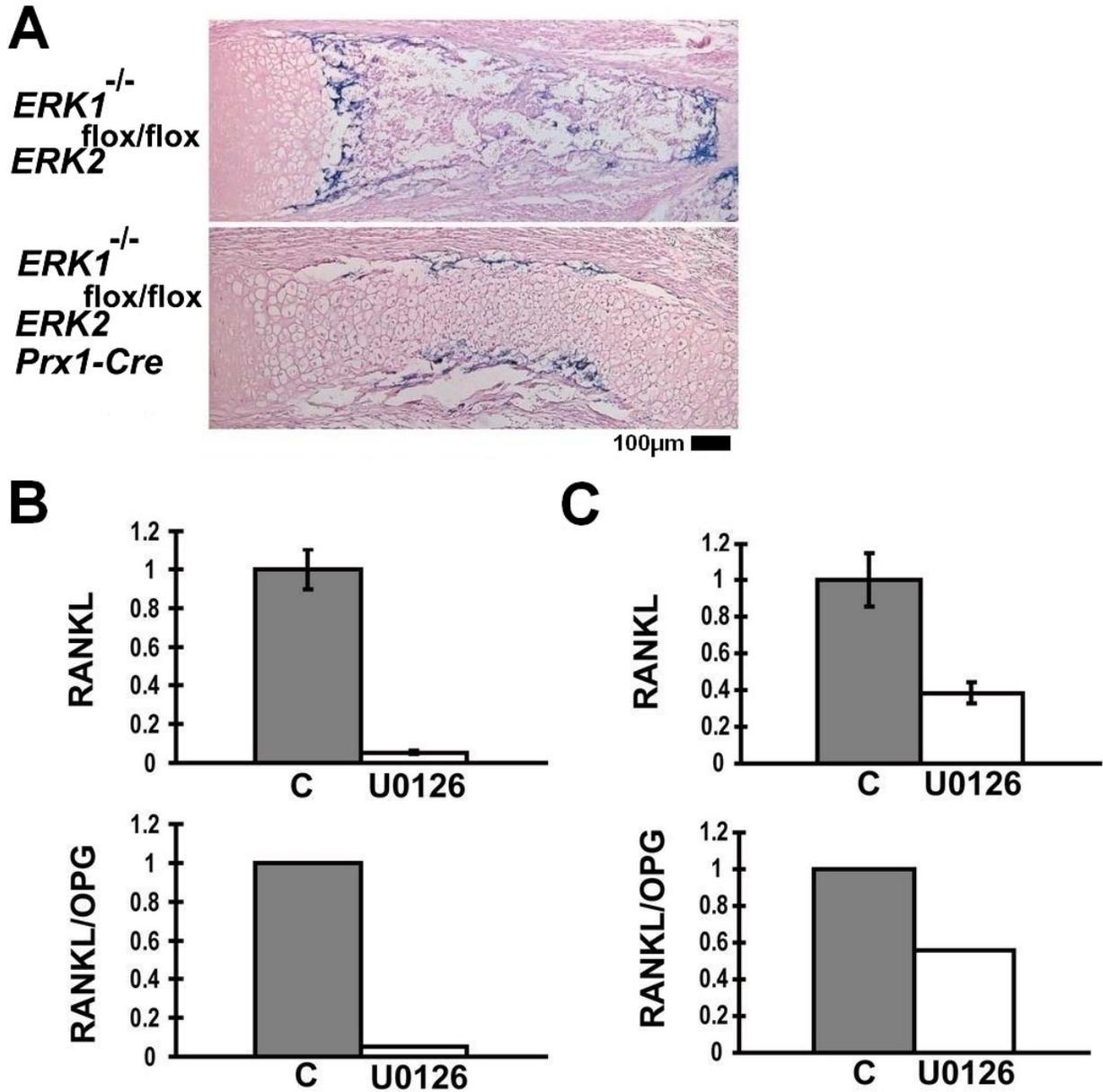
Supplemental Figure 2

Ectopic cartilage formation was observed in the perichondrium of *ERK1*^{-/-}; *ERK2*^{flox/flox}; *Prx1-Cre* embryos and mice (B,D,F, arrowheads), while no alcian blue positive cartilaginous matrix was observed in *ERK1*^{-/-}; *ERK2*^{flox/flox} embryos and mice (A,C,E). (A,B) E13.5 humerus, (C,D) E18.5 femur, (E,F) P5 ulna. (G) In situ hybridization of the femur showing the expression of *patched* (*Ptc*) and *PTH/PTHrP receptor* (*PthrP-R*) at E15.5. Cells in the ectopic cartilage express *patched* and *PTH/PTHrP receptor* (arrowheads). (H) In situ hybridization of the femur showing the expression of *Col10a1* and *Mmp13* in the perichondrium of *ERK1*^{-/-}; *ERK2*^{flox/flox}; *Prx1-Cre* mice at P0 (arrowheads).



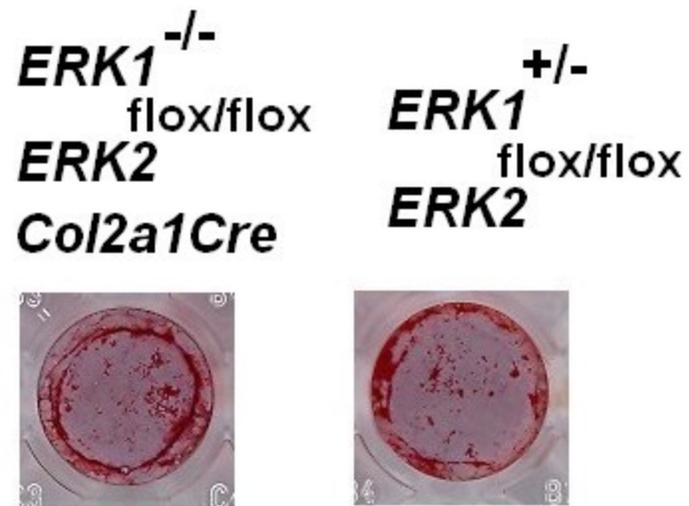
Supplemental Figure 3

(A) Immunohistochemistry for MMP9 showing reduced staining in the tibia of an *ERK1*^{-/-}; *ERK2*^{flox/flox}; *Prx1-Cre* embryo at E15.5. (B,C) 3 h U0126 treatment at 20 μM strongly inhibited *RANKL* expression both in primary calvaria osteoblasts (B) and rib chondrocytes (C) isolated from wild type mice, resulting in a decrease in *RANKL*/*OPG* ratio. *OPG* expression remained unaffected. C: control vehicle DMSO.



Supplemental Figure 4

Calvaria osteoblasts were isolated from $ERK1^{+/-}; ERK2^{\text{flox/flox}}$ and $ERK1^{-/-}; ERK2^{\text{flox/flox}}$; $Col2a1-Cre$ embryos at E18.5. After reaching confluence, the cells were maintained in differentiation medium (alpha MEM, 10% FCS, 5 mM β -glycerophosphate, 100 $\mu\text{g/ml}$ ascorbic acid) for 21 days. The cells were stained with alizarin red to detect mineralization.



Supplemental Figure 5

(A) In situ hybridization of the tibia showing accelerated *osteocalcin* expression in the perichondrium of *Prx1-MEK1* transgenic mice compared with wild type mice at E16.5. (B) Immunohistochemical detection of the FLAG-tagged MEK1(S218/222E, Δ 32-51) using anti-M5 FLAG antibody in a serial section of Figure 10C. Cartilage develops within the transgene-expressing mesenchyme, which stains brown for the FLAG-tagged MEK1. (C) Skeletal preparation of the forelimbs of E14.5 embryos showing a delay in the formation of cartilage anlagen in *Prx1-MEK1* transgenic mice.

