Supporting Information

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SI Text

Antibodies used for Histological Analysis. Primary antibodies used were a chicken anti-β-gal antibody (1:1,000 dilution), a rabbit anti-pankeratin antibody (1:200), and a polyclonal anti histone H3 phosphorylated on serine 10 (1:100), all from Abcam. Secondary antibodies included a goat anti-rabbit-IgG linked to Alexa Fluor 488 (1:100, Jackson ImmunoResearch) and an anti-chicken-IgG linked to cyanin 3 (1:200, Abcam).

Staining of Cartilage and Bone with Alizarin Red/Alcian Blue. Newborn mice were eviscerated and incubated in water at 65° for 1 min to facilitate complete removal of the skin and even penetration of the dyes. Mice were then fixed in 100% ethanol overnight and then stained with 0.015% alcian blue (Sigma) and 0.005% alizarin red (Sigma) in 5% acetic acid and 70% ethanol for 24 h. Mice were cleared with KOH, then incubated in increasing concentrations of glycerol according to standard protocols.

Analysis of Apoptosis by TUNEL. In situ analysis of DNA fragmentation was performed using the ApopTag Fluorescein In Situ Apoptosis Detection Kit (Chemicon International), according to the manufacturer's recommendations.

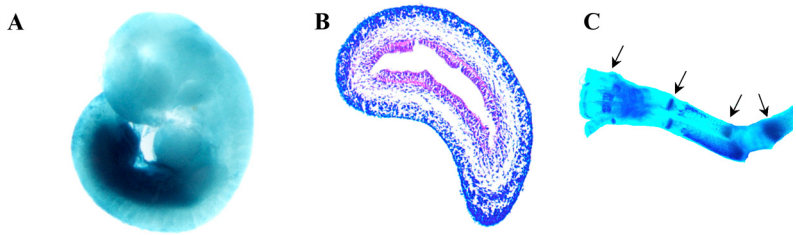

Fig. S1. Expression of basonuclin 2 gene around the gut and joints. (*A*) Whole-mount staining of *bnc2*$^{+/-}$ embryo at E9.5 showing intense bnc2-driven lacZ expression in the gut. (*B*) Paraffin section of the gut. X-gal staining in outer mesoderm. (*C*) X-gal staining of the anterior limb at E16.5. Staining around the joints (arrows) resembling staining observed in adult *Drosophila* (see figure 1*F* in Bishop S, Klein T, Arias A, Couso J (1999) *Development* 126:2993–3003.].

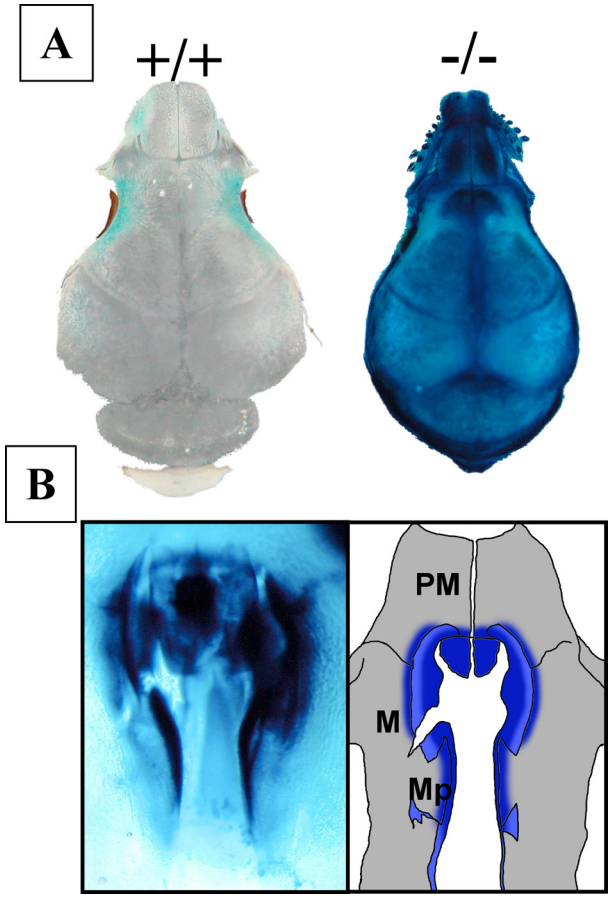

Fig. S2. Expression of *bnc2*^{lacZ} in the cranial vault and around the palatal cleft.(*A*) Superior view of newborn skull after X-gal staining showing high β-gal activity in the *bnc2*$^{-/-}$ mouse, particularly in the sutures and the posterior fontanel. The wt is virtually unstained. (*B*) Inferior view of the head after removal of mandible demonstrating strong staining around the palatal cleft of a *bnc2*$^{-/-}$ mouse (*Left*). Schematized in *Right*. Bones with high enzyme activity are those strongly affected by lack of bnc2. PM: premaxillary bone, M: maxillary, Mp: maxillary process. X-gal staining of whole mount embryos was carried out according to standard procedures [Pereira F (2001) *Curr Protoc Mol Biol* Chapter 14:Unit 14.14.].