RESEARCH REPORTS
Biological

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TMJ Development and Growth Require Primary Cilia Function

APPENDICES

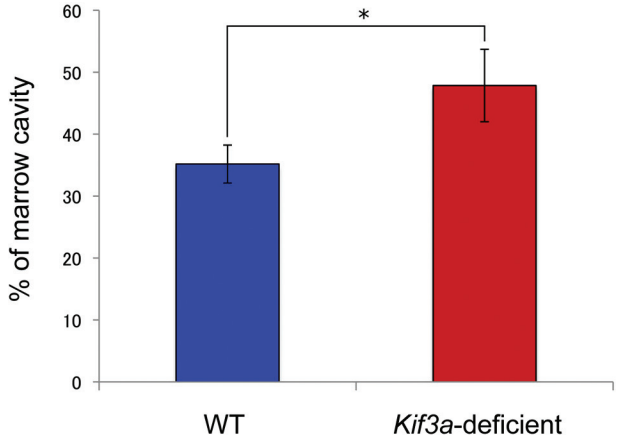
MATERIALS & METHODS

Ciliar Position in Superficial/Polymorphic Fibroblastic Cells

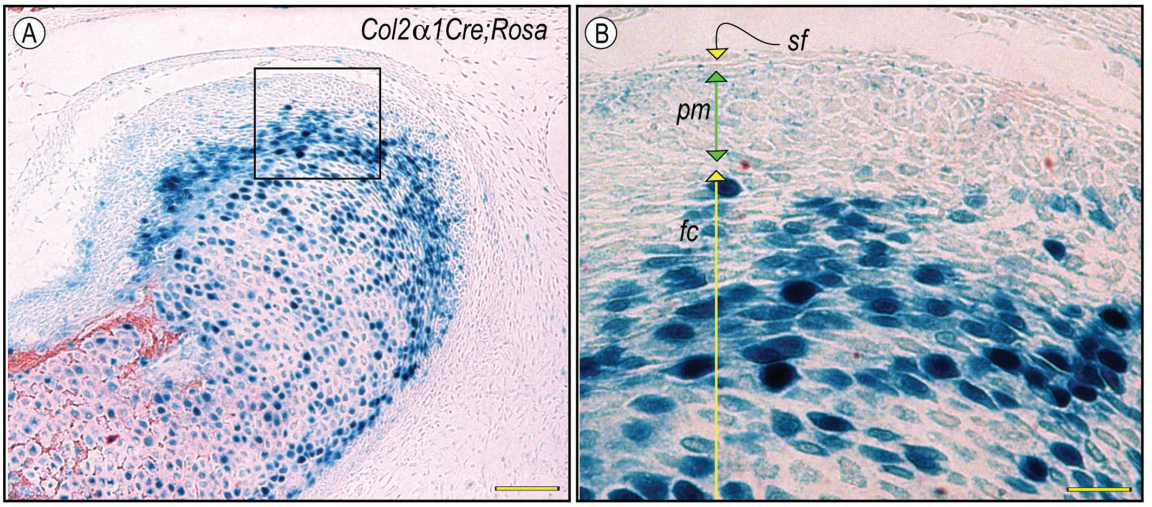
To analyze the position of the cilia in superficial/polymorphic layers, we immunostained para-sagittal and frontal sections from P0, P7, and P15 wild-type and *Kif3a*-deficient condyles with acetylated α-tubulin monoclonal antibody, followed by Dapi-nuclear staining, and photographed the results. Random areas of superficial/polymorphic cell layers were examined (approximately 100 cells/field, n = 6), and the fraction of cells displaying a clear and well-positioned cilium was determined.

Imaging

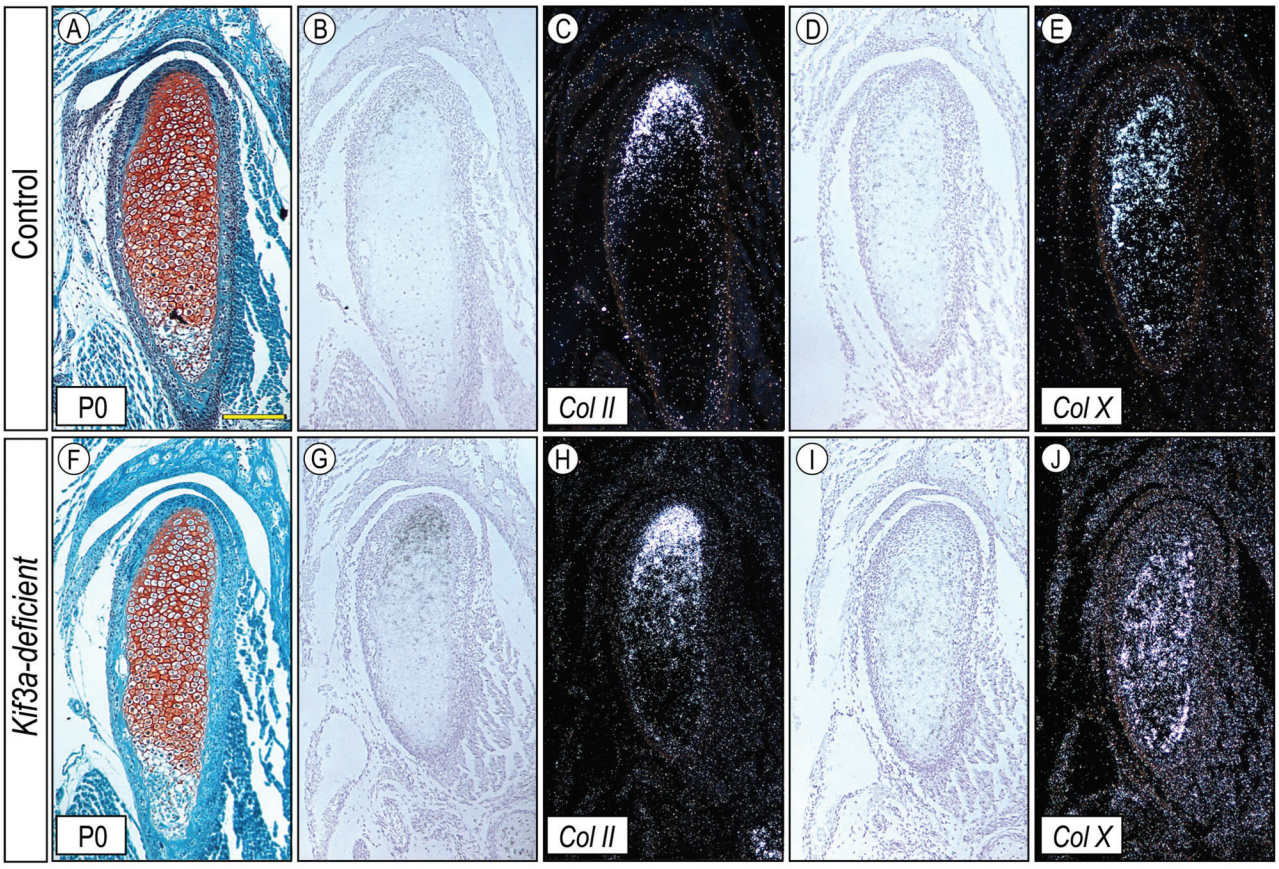
Consecutive sections from P15 control and *Kif3a*-deficient condyles were hybridized with isotope-labeled riboprobes encoding *type II collagen* (*Col II*) and *fibromodulin* (*Fm*), and dark- and bright-field images were captured by means of a digital camera. Dark-field images of *Col-II* transcripts were pseudo-colored with Adobe Photoshop software and overlaid onto dark-field *Fm*-images to facilitate the appreciation of the length of a polymorphic cell layer.



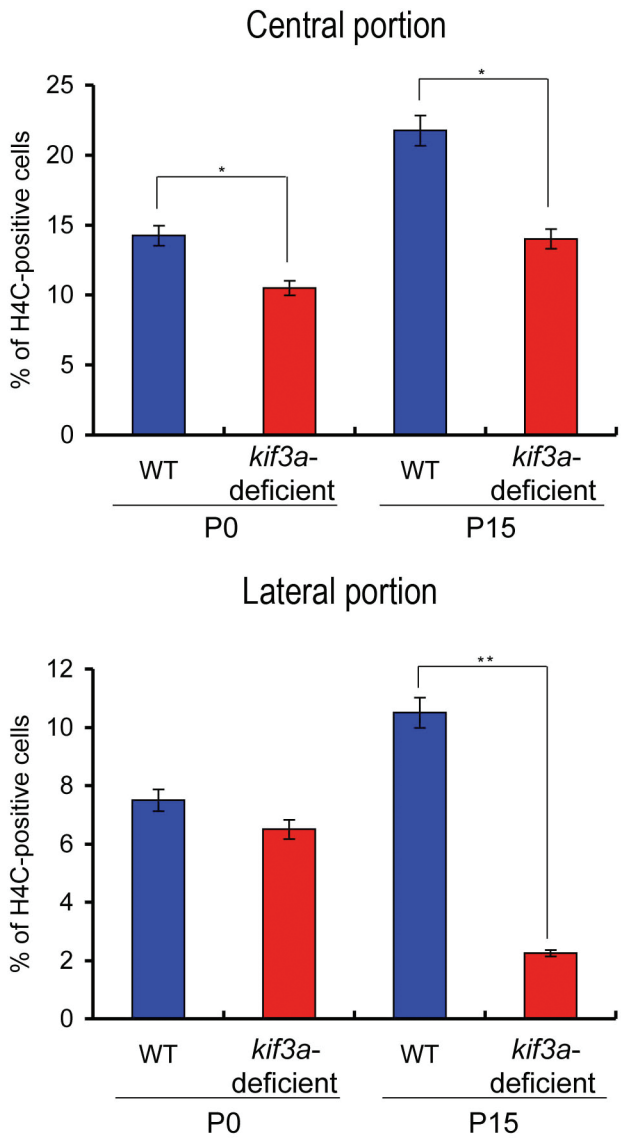
Appendix Figure 1. Defects in subchondral bone formation in *Kif3a*-deficient condyles. For quantification of loss of trabeculae in condylar subchondral bone, serial sections from 3-month-old wild-type and *Kif3a*-deficient mice were processed for histomorphometric analysis. Percentage of marrow cavity space *per* 0.5 x 0.5 mm^2 area was calculated from 8 randomly selected fields/sample. Statistical analysis was carried out by an unpaired Student's *t* test. *p*-values less than 0.02 were considered statistically significant (*$p < 0.02$). All statistical data are presented as means ± SD.



Appendix Figure 2. *Col2α1*-Cre activity in differentiating chondrocytes in mandibular condyles. Parasagittal sections from P0 *Col2α1*-Cre;Rosa mice were processed for β-galactosidase staining. Boxed areas in A are shown at higher magnification in B. Note specific *Col2α1*-Cre activity in differentiating chondrocytes and absence in superficial/polymorphic fibroblastic cells in developing mandibular condyles. *sf*, superficial layer; *pm*, polymorphic layer; *fc*, flattened chondrocyte layer. Scale bars: in A, 165 μm; and in B, 55 μm.



Appendix Figure 3. Chondrocyte maturation and zonal organization in newborn control and *Kif3a*-deficient mandibular condyles. Frontal sections from P0 control (**A-E**) and *Kif3a*-deficient (**F-J**) condyles were processed for safranin-O/fast green staining (A, F) and *in situ* hybridization with isotope-labeled riboprobes encoding Collagen type II (*Col II*) or Collagen type X (*Col X*). *Col II* and *Col X* expression characterizes flattened immature and hypertrophic chondrocytes in condylar cartilage (C, H and E, J, respectively), and *Col II*- and *Col X*-positive chondrocytes in *Kif3a*-deficient mice occupy a wider region of condylar cartilage. Scale bars: 150 μm in A for A-J.



Appendix Figure 4. Decreases in mitotic activity of chondroprogenitor cells in *Kif3a*-deficient condyles. The number of *H4C*-positive proliferating chondroprogenitor cells present in the central and lateral portions of the condyles was counted in hybridized sections from control and *Kif3a*-deficient newborn and P15 condyles. For quantification of proliferating chondroprogenitor cells in the polymorphic layer, *H4C*-positive and -negative cells present in multiple sections from apex (central) and/or lateral portions of condyles were counted microscopically (approximately 100 cells/field, n = 6) and used to calculate ratios of labeled cells/total cells. Statistical analysis was carried out by an unpaired Student's *t* test. *p*-values less than 0.05 were considered as statistically significant (*$p < 0.05$, **$p < 0.001$). All statistical data are presented as means ± SD. Note that the mitotic activity of chondroprogenitor cells is slightly reduced in the central portion of P15 *Kif3a*-deficient condyles (*$p < 0.05$, *Kif3a* mutant *vs.* control), but is significantly reduced in the lateral portion (**$p < 0.01$, *Kif3a* mutant *vs.* control).