

Chondrocytes Directly Transform into Bone Cells in Mandibular Condyle Growth

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Appendix

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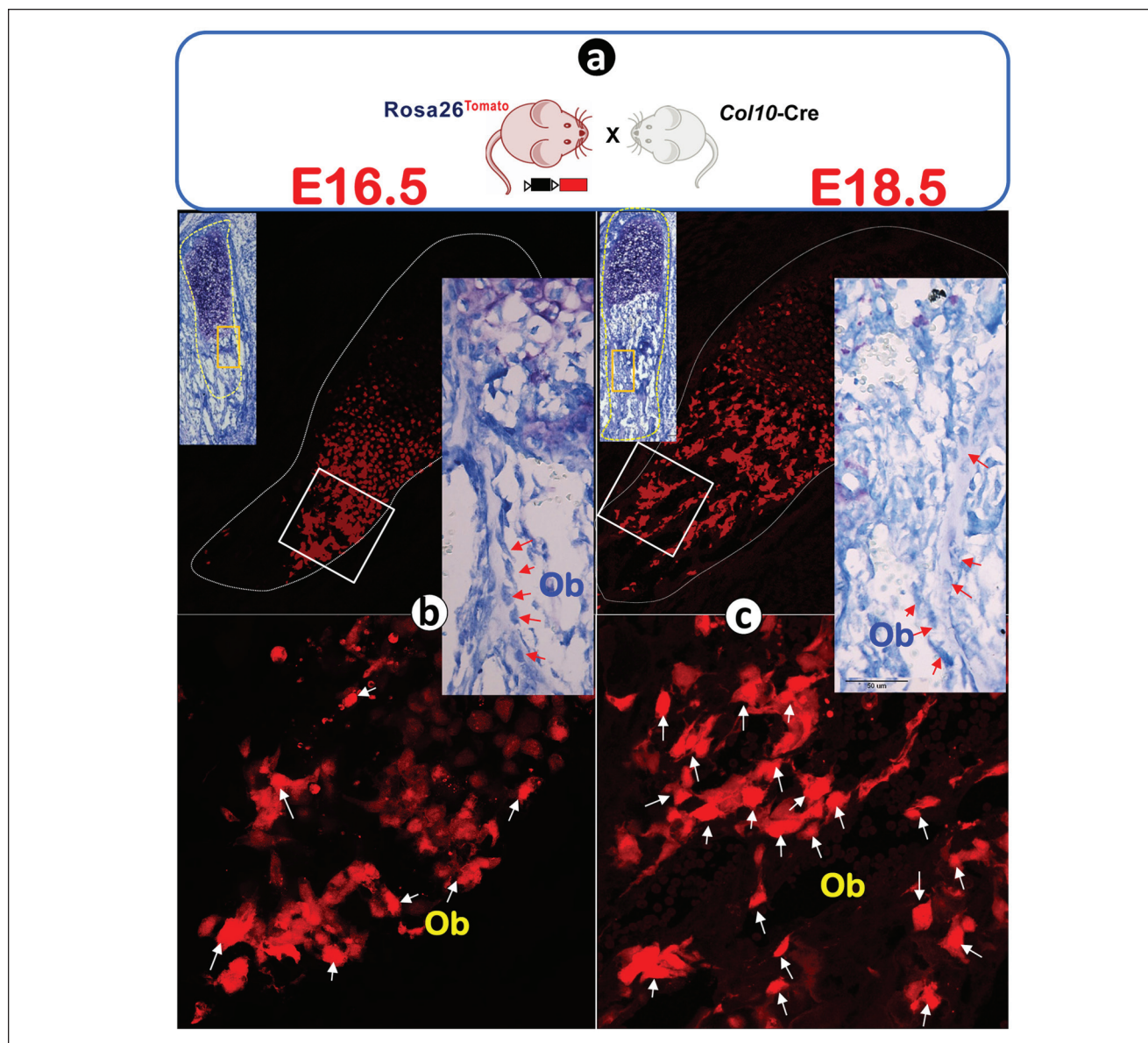
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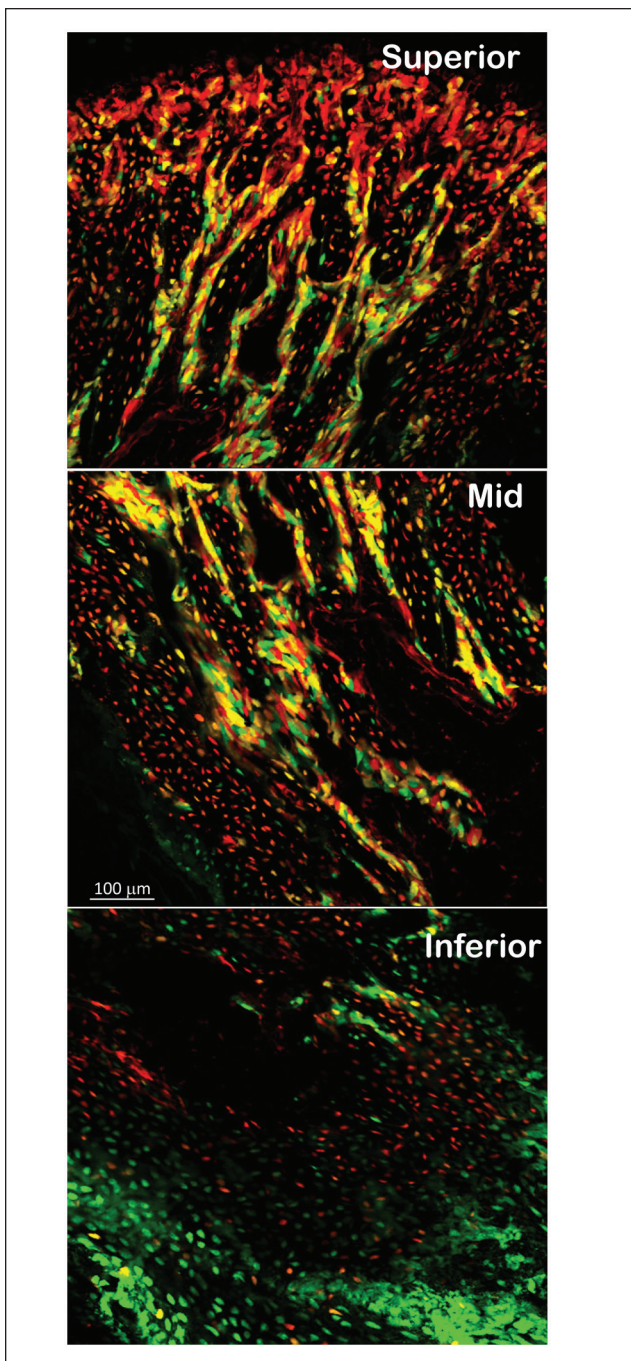
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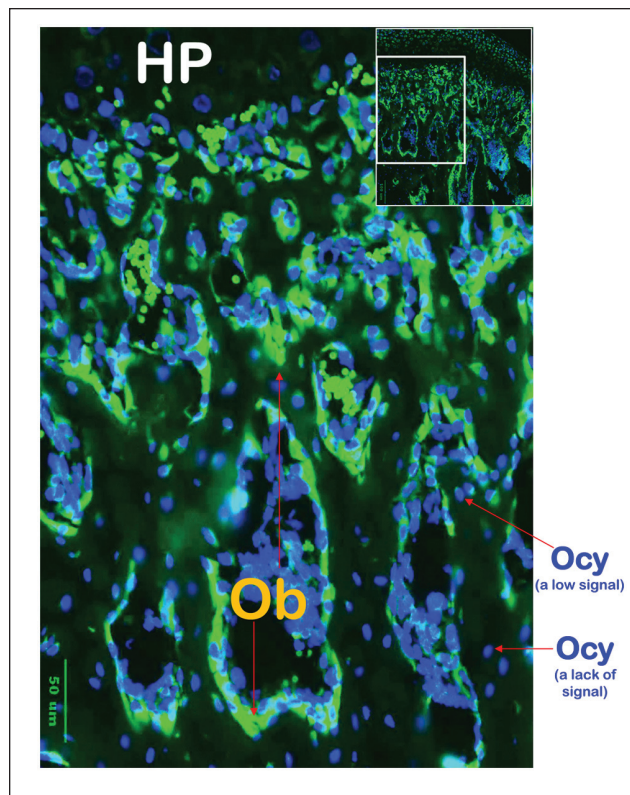
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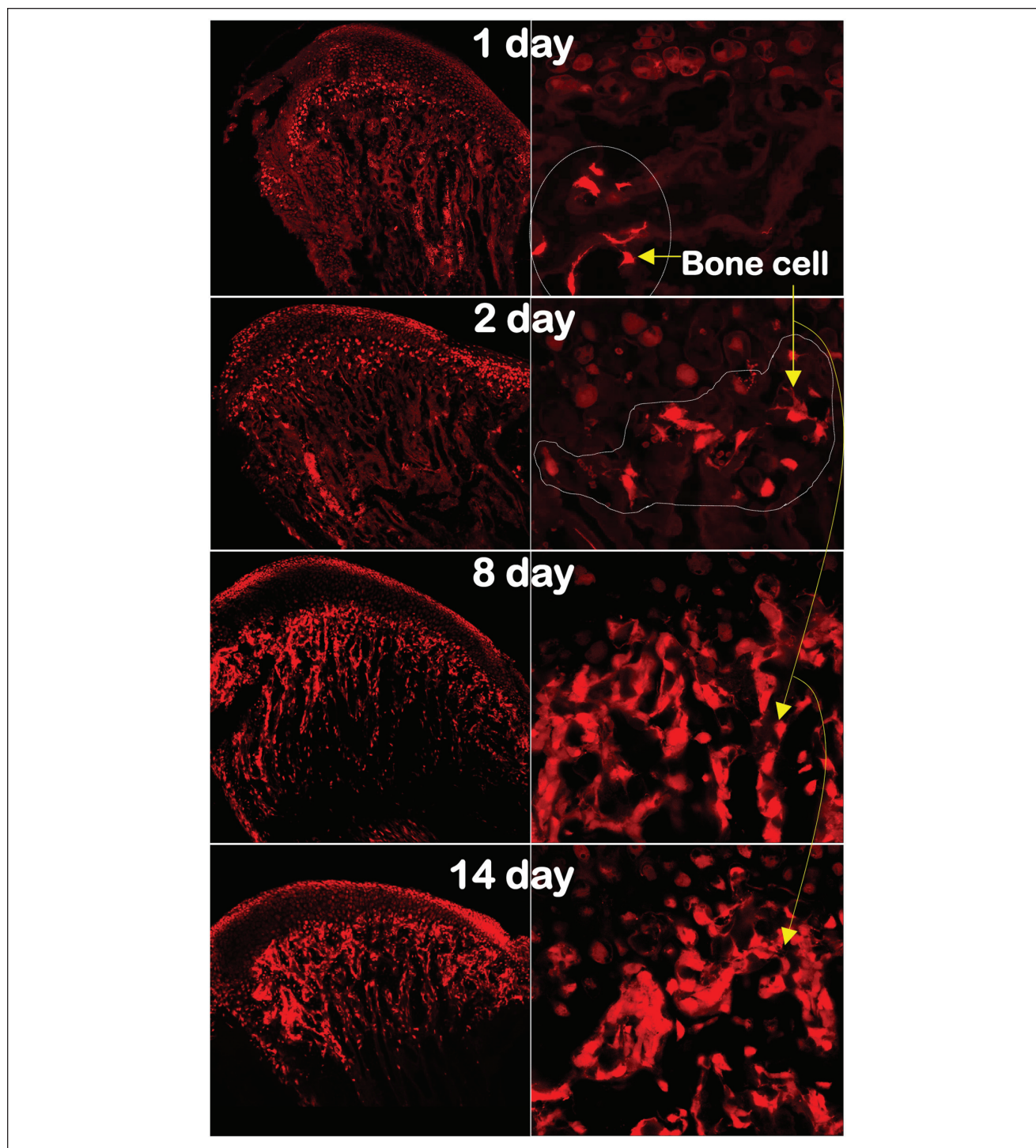
Appendix Figure 1. Ossification is initiated from the inferior region of condylar ramus. (a) The cartoon shows condyles from mice with the cross of *Rosa26^{-tdtomato}* and *Col10-Cre*. (b) The E16.5 confocal image reveals red chondrocytes on top and a few red bone cells in the inferior area (low magnification, upper panel; high magnification of inset, lower panel, red arrows) and (c) an expansion of red bone collar in E18.5 (red arrows). Toluidine blue–stained images from adjacent sections are included to assist in visualizing the confocal images. Ob, osteoblasts.



Appendix Figure 2. In the superior, middle, and inferior regions of the bone underlying the mandibular condylar cartilage, the number of red, yellow, and green cells were manually counted to quantitate the percentage of chondrocytes transformed into bone cells in the condylar ramus. Counts were made from 4 condyle samples (see Fig. 4 in the main article for detail).



Appendix Figure 3. The *Col1* in situ hybridization revealed positive signal in all osteoblasts (Ob) and some osteocytes (Ocy) in a 3-wk-old mouse condyle sample. Mouse antisense RNAs for *Col1* were used for in situ hybridization as described previously (Lu et al. 2011). An in situ hybridization with fluorescein labeling approach (Roche, Indianapolis, IN, USA) was used to label *Col1* mRNAs in subchondral bone, in which all Ob and some Ocy cells were in green color. HP, hypertrophic chondrocytes.



Appendix Figure 4. A time series illustrates the gradual transformation of chondrocytes into bone cells in subchondral bone. The compound mice were collected at days 1, 2, 8, and 14 after activation of $\text{Agr-Cre}^{\text{ERT2}}$ by tamoxifen injection at 2 wk of age. The confocal images showed a gradual increase in red bone cells in subchondral bone, with few in days 1 and 2 but a sharp increase in days 8 and 14. These images further support the concept of the direct transformation of chondrocytes into bone cells.

Appendix Reference

Lu Y, Yuan B, Qin C, Cao Z, Xie Y, Dallas SL, McKee MD, Drezner MK, Bonewald LF, Feng JQ. 2011. The biological function of DMP-1 in osteocyte maturation is mediated by its 57-kDa C-terminal fragment. *J Bone Miner Res.* 26(2):331–340.