Vital Roles of β-catenin in Trans-differentiation of Chondrocytes to Bone Cells

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Figure S1

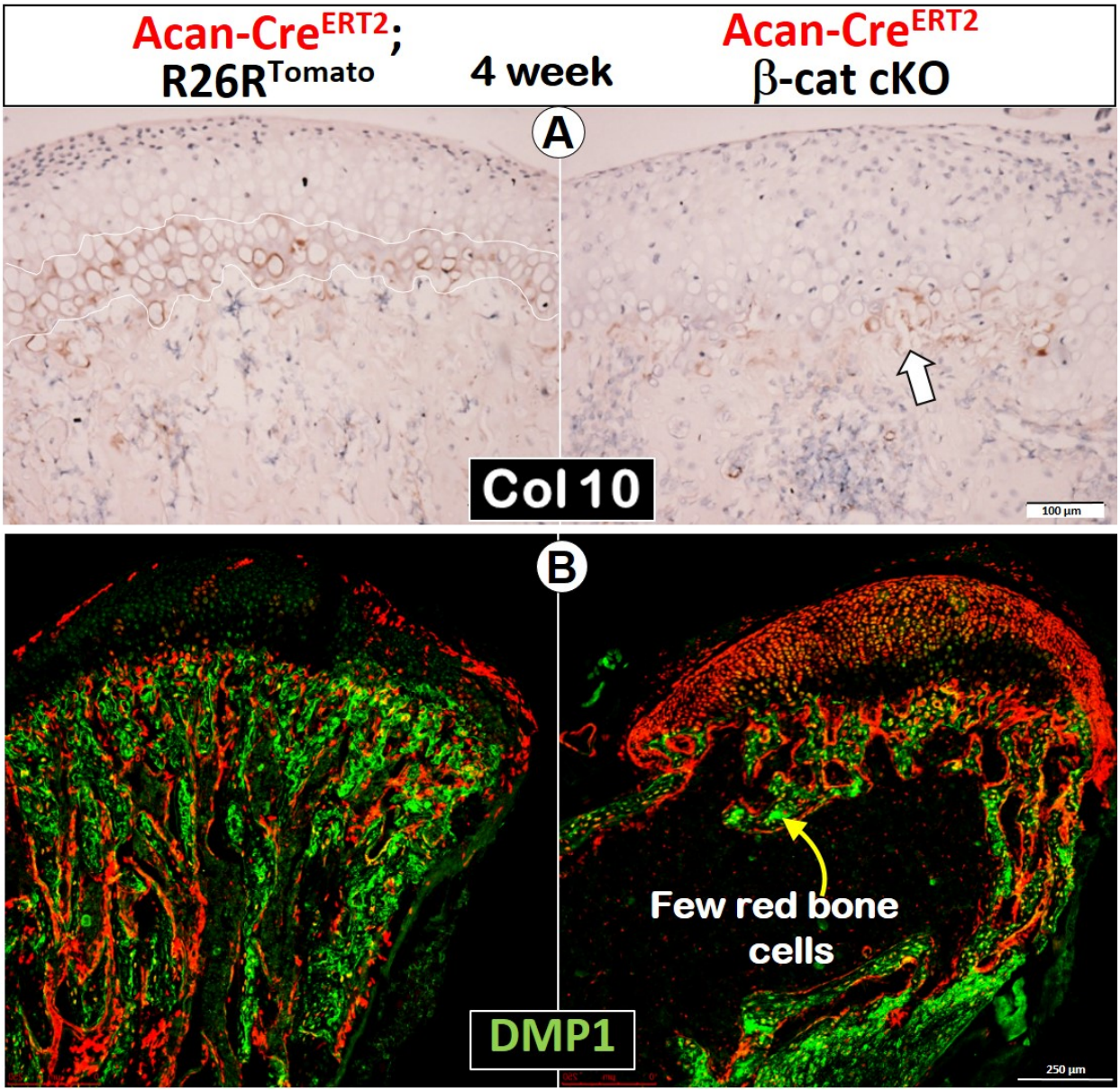

Figure S1. Deletion of *β-catenin* in chondrocytes by using *Acan*-Cre^{ERT2} (Cre was activated at P3) led to defective chondrocyte maturation, and less subchondral bone formation. (A) Col 10 expression was largely undetectable in 4-week old cKO mice (arrow). **(B)** DMP1 IHC combined with cell lineage tracing revealed much less subchondral bone volume with few transformed red bone cells in cKO mice.

Figure S2

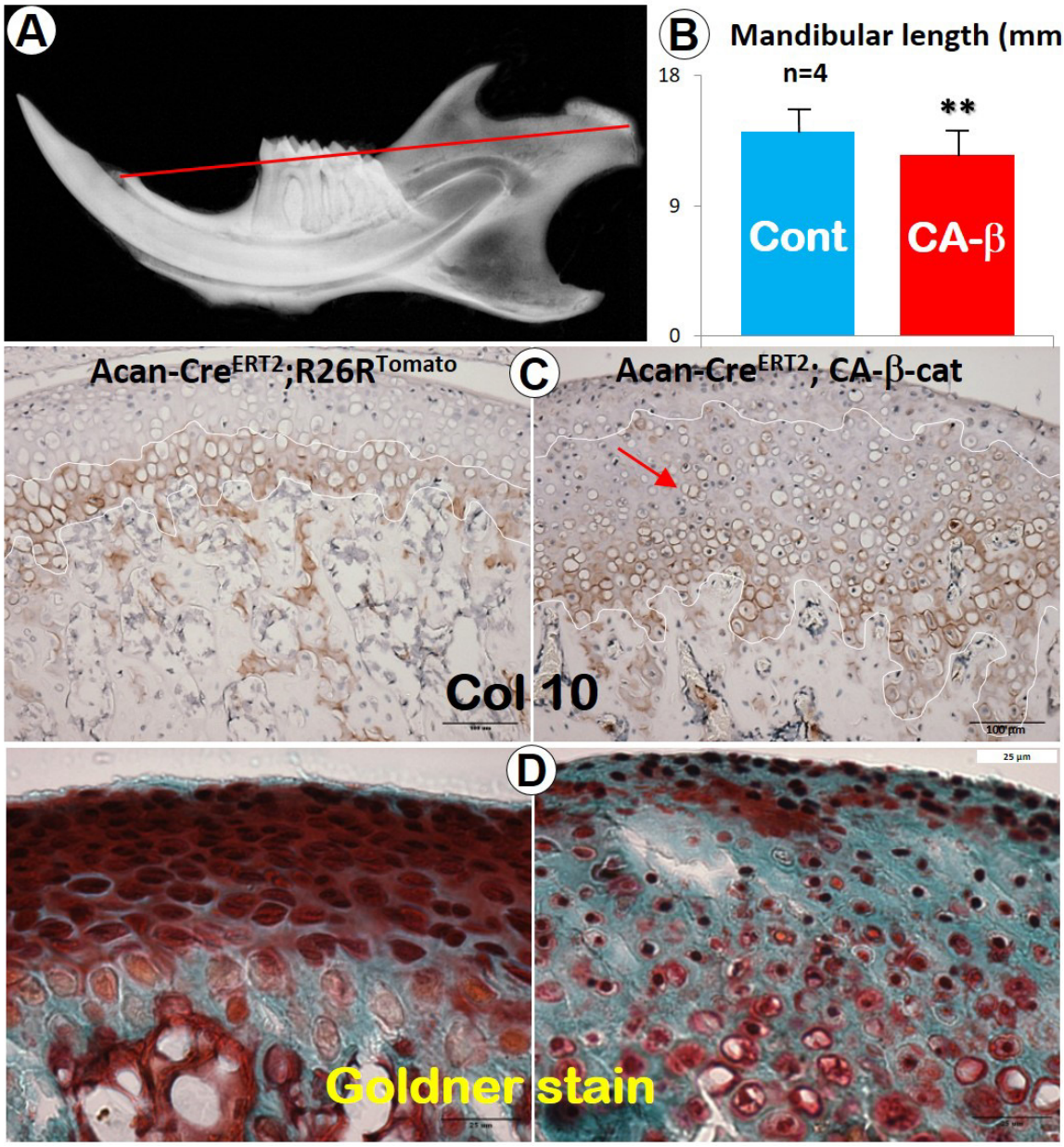

Figure S2. Constitutive activation of *β-catenin* in MCC (*Acan*-Cre^{ERT2} was activated at P14) accelerated chondrogenesis with widespread calcification in the cartilage. (A) Mandibular length was measured from the middle of the condylar head curvature to the most anterior point on the incisal alveolar process, and quantified by Image J software. **(B)** The mandible length of 4-week old CA-β-cat mice was significantly shorter than controls (n=4). **(C)** Col 10 IHC in the CA-β-cat MCC displayed an enlarged area of hypertrophic chondrocytes (arrow). **(D)** Goldner staining reflected a widely mineralized region (indicated by green color), indicating cartilage calcification in CA-β-cat MCC.

Figure S3

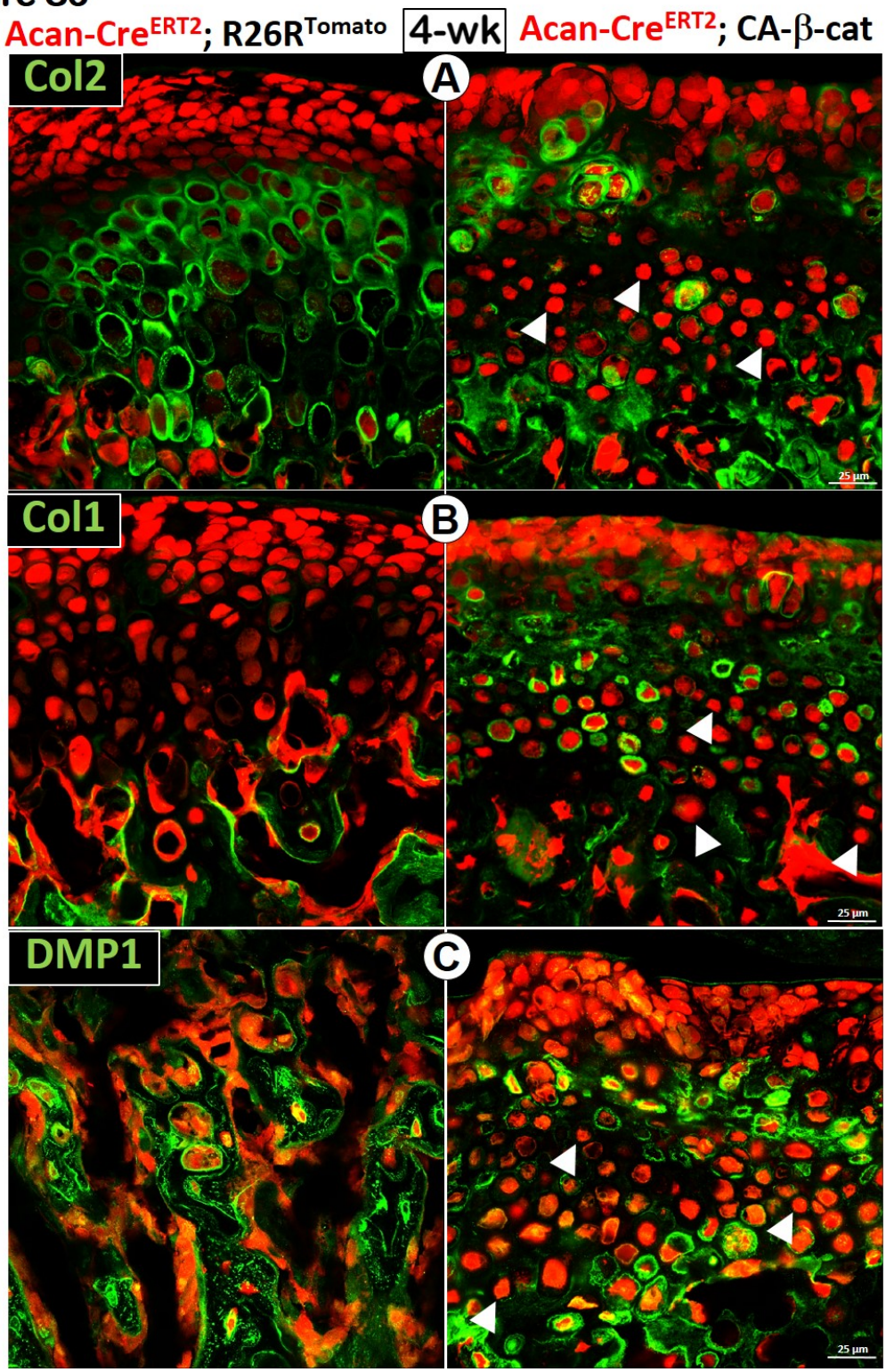

Figure S3. Variable gene expression profiles in the CA-β-cat cells. (A-C) In CA-β-cat MCC, there were many round and relatively small red cells (arrowheads), which were clustered and did not express Col 2 **(A)**, Col 1 **(B)**, or DMP1 **(C)**.

Figure S4

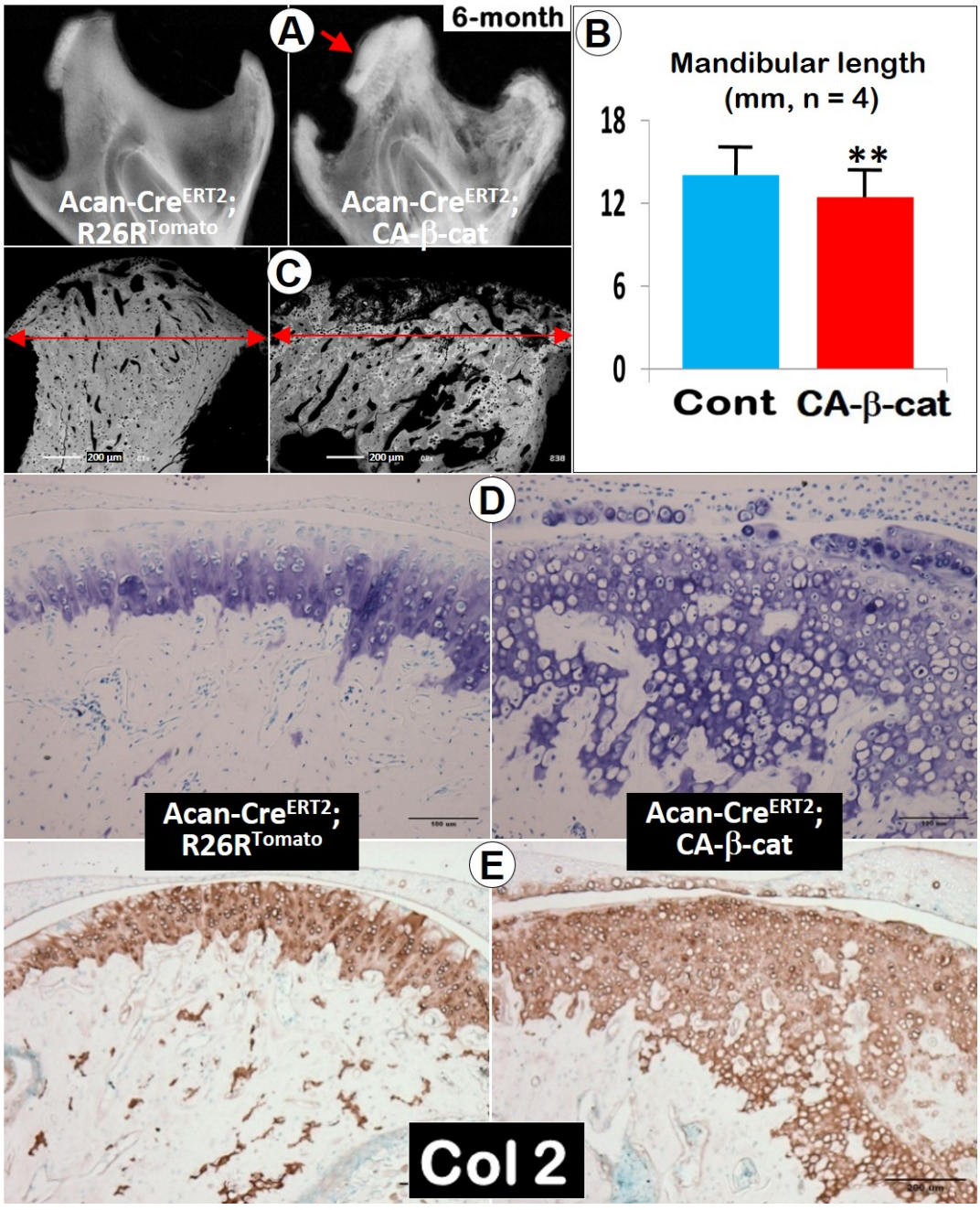

Figure S4. Stabilization of *β-catenin* in adulthood displayed a more severe phenotype. (A) X-ray showed short but hypermineralized mandibular condylar process in CA-β-cat mice **(B)** Quantitative data demonstrated that the length of the mandible was significantly reduced in CA-β-cat mice (n=4). **(C)** The back-scattered SEM revealed a wider condyle with uneven mineralization in the CA-β-cat condylar neck. **(D)** Toluidine blue staining showed wider staining area with more hypertrophic chondrocytes, and larger calcified region within the CA-β-cat MCC. **(E)** Col 2 IHC confirmed the increased chondrogenesis and expanded hypertrophic chondrocyte layer in CA-β-cat mice.

Figure S5

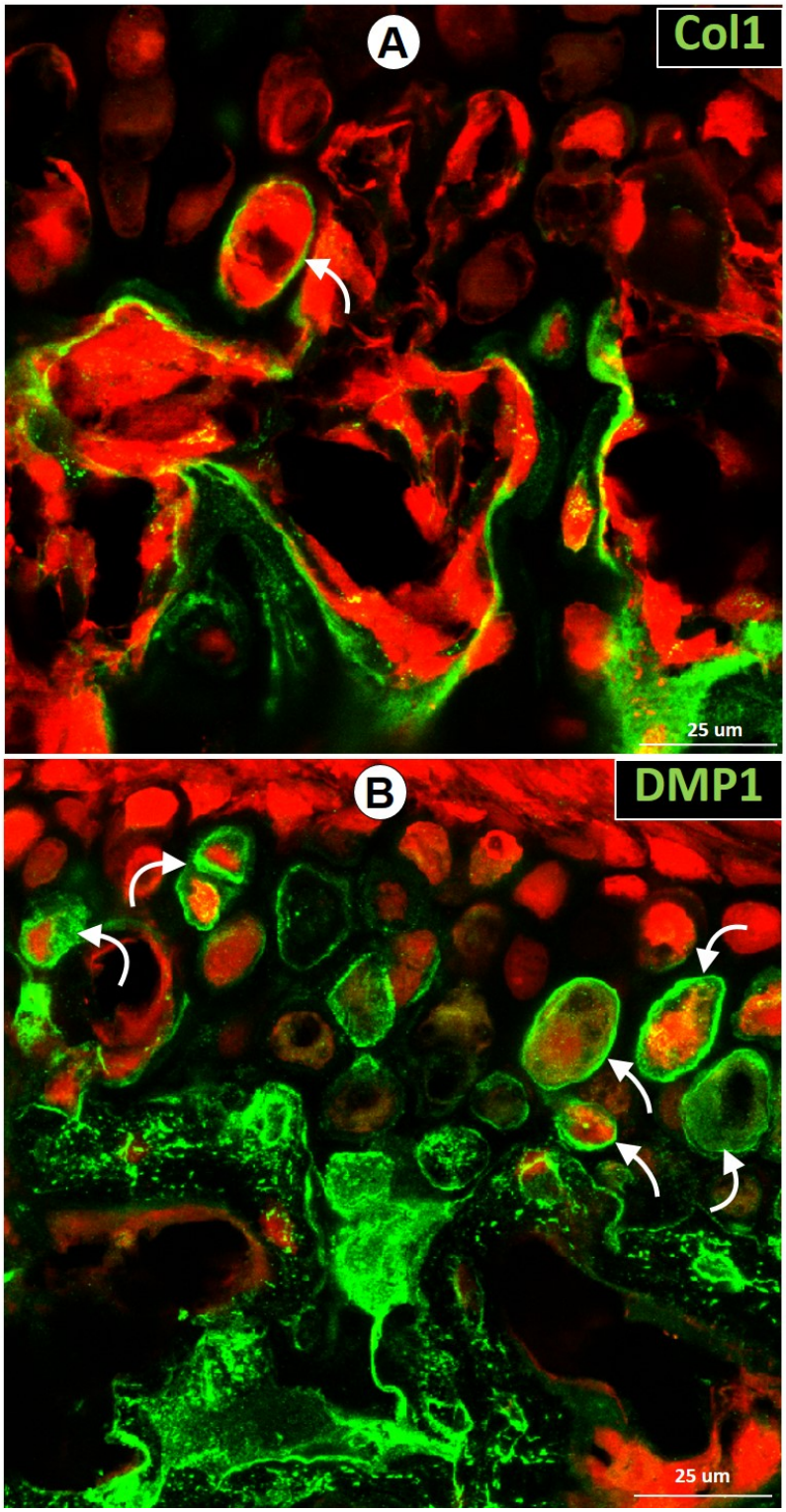

Figure S5. Normal hypertrophic chondrocytes have the ability to express osteogenic markers. **(A)** By using *Acan*-Cre^{ERT2}; R26R^{Tomato} mice, Col 1 (early osteogenic marker) was detected in hypertrophic chondrocytes (arrow). **(B)** Some hypertrophic chondrocytes expressed DMP1 (late osteogenic marker, arrows).