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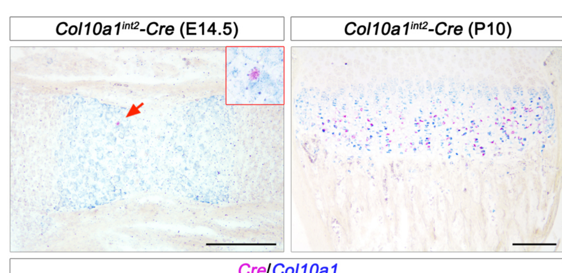

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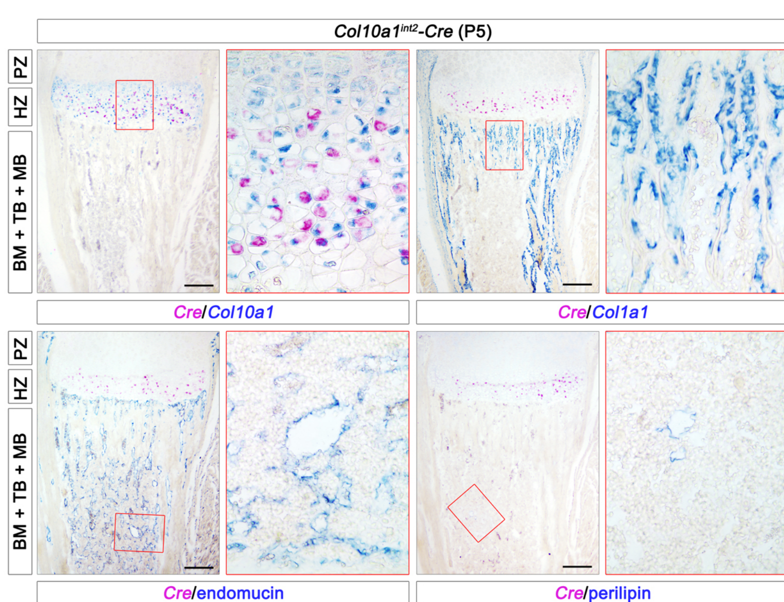

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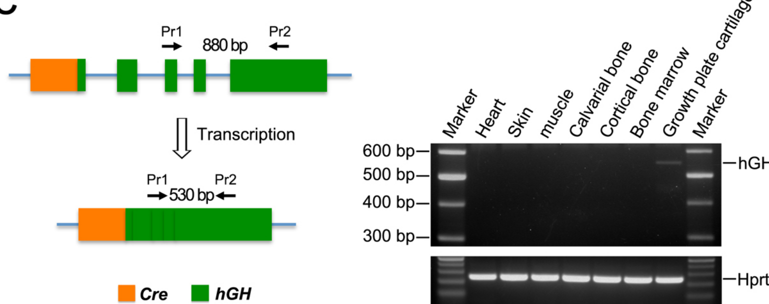

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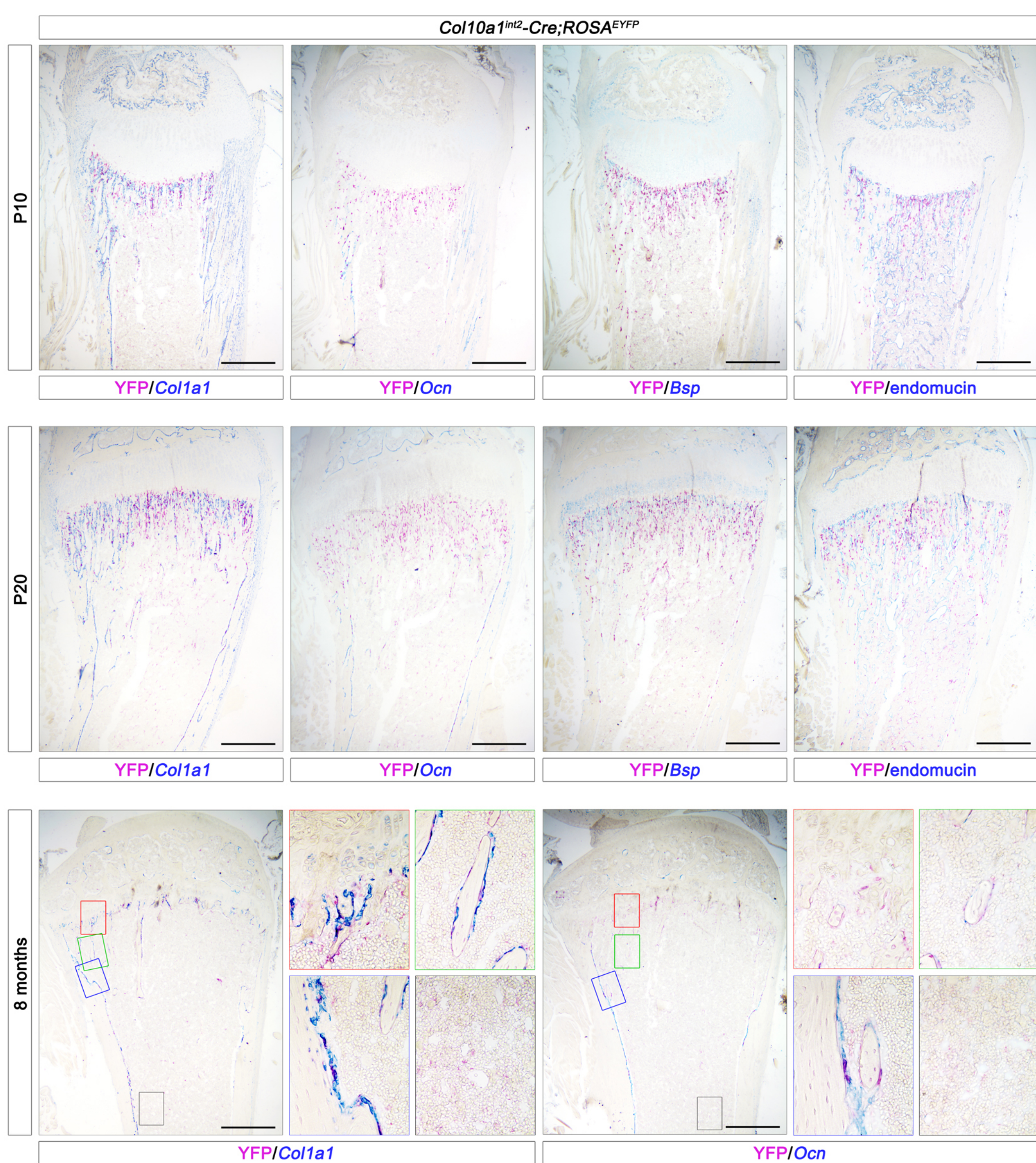

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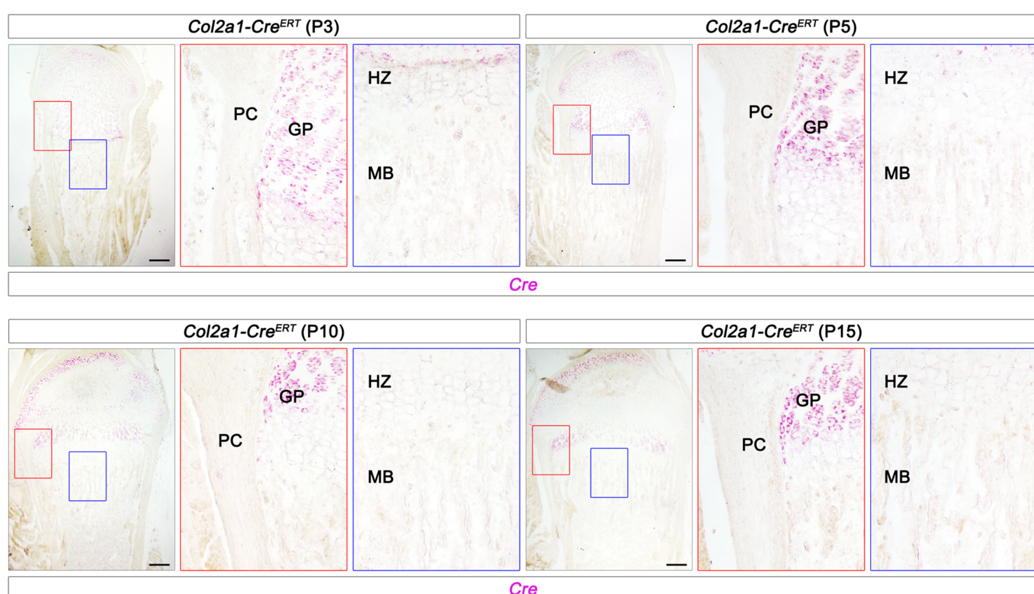

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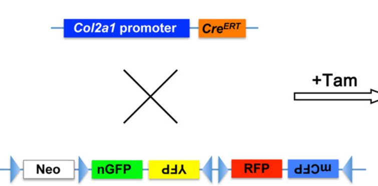

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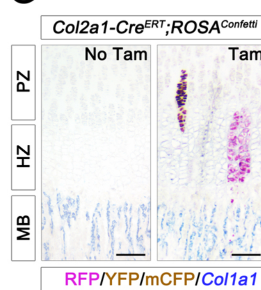

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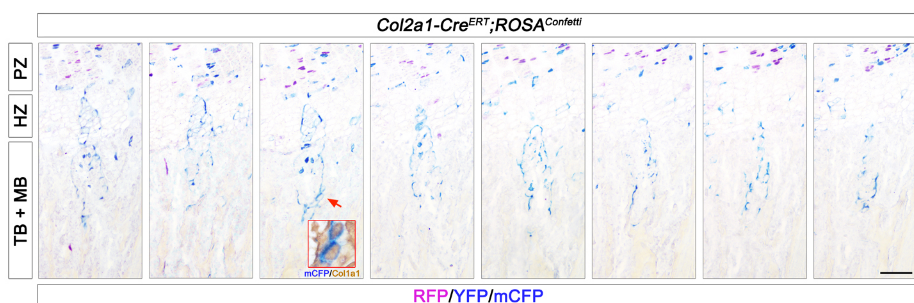

Figure S1 Osteogenic fate of hypertrophic chondrocyte. **(A)** At E14.5 and P10, *in situ* hybridization analyses showed that *Cre* (fuchsia) co-localized with *Col10a1* (blue; a marker for hypertrophic chondrocytes) in *Col10a1*^{int2}*-Cre* tibia. The insert showed the high-magnification image indicated by red arrow. **(B)** At P5, *in situ* hybridization analyses showed that *Cre* (fuchsia) co-localized with *Col10a1* (blue; a marker for hypertrophic chondrocytes). Double staining of *Cre* (fuchsia) by *in situ* hybridization and endomucin (a marker for endothelial cells) or perilipin (a marker for adipocytes) by IHC revealed that *Cre*$^{+}$ cells (fuchsia) did not express endomucin or perilipin. The right showed the high-magnification image boxed in red on the left. PZ: proliferation zone; HZ: hypertrophic zone; MB: metaphyseal bone; TB: trabecular bone; BM: bone marrow. **(C)** Reverse transcriptase-PCR analysis using the primers for *hGH* mRNA splicing showed the restricted expression of *Cre-hGH* mRNA in growth plate cartilage from *Col10a1*^{int2}*-Cre* transgenic tibia. The PCR primers (Pr1 and Pr2) are located in the 3rd and 5th exon of *hGH*, respectively. A 530 bp product represents the spliced *Cre-hGH* mRNA. **(D)** Double staining of YFP (fuchsia) by IHC and the transcripts (blue) of *Col1a1*, *Ocn* and *Bsp* (markers for osteoblastic differentiation) by *in situ* hybridization analyses in *Col10a1*^{int2}*-Cre;ROSA*^{EYFP} tibia at the age of 10 days (upper), 20 days (middle) and 8 months (lower). Considerable YFP^{+} cells were obviously localized throughout the metaphysis beneath the growth plate, and some YFP^{+} cells co-expressed *Col1a1*, *Ocn* and *Bsp* mRNA. Double staining of YFP (fuchsia) and endomucin (blue, marker for endothelial cell) by IHC showed that some YFP^{+} cells were adjacent to the endothelial cells. **(E)** *In situ* hybridization analyses showed that *Cre* (fuchsia) expressed in the growth plate cartilage, but not in perichondrial or bone marrow stromal cells at P3, P5, P10 and P15. The right showed the high-magnification image boxed in red and blue on the left. PC: perichondrium; GP: growth plate; HZ: hypertrophic zone; MB: metaphyseal bone. **(F)** *ROSA*^{Confetti} locus contained the coding genes of four fluorescent proteins. A neomycin resistance roadblock cassette floxed by *Lox*P was inserted upstream. After the neomycin roadblock is removed upon Cre activation, *ROSA*^{Confetti} locus results in a random fashion to four possible outcomes. nGFP is nuclear, mCFP is membrane associated, and the other two are cytoplasmic. **(G)** After a single dose of tamoxifen exposure (50 mg/kg) at P5, 10-day-old *Col2a1-Cre*^{ERT}*;ROSA*^{Confetti} tibia was stained by RFP (red) antibody and GFP (brown) antibody that recognizes mCFP (specific with membrane staining) and YFP (specific with cytoplasmic staining). Two clonal columns of YFP^{+} (brown) and RFP^{+} (red) were detected in *Col2a1-Cre*^{ERT}*;ROSA*^{Confetti} tibia with tamoxifen treatment, but not in that without tamoxifen administration. PZ: proliferation zone; HZ: hypertrophic zone; MB: metaphyseal bone. **(H)** Serial sections from *Col2a1-Cre*^{ERT}*;ROSA*^{Confetti} mice upon a single dose of tamoxifen induction (100 mg/kg) displayed a clonal column (blue; mCFP membrane staining) extending from hypertrophic chondrocyte zone to the metaphysis. Double labeled mCFP^{+}Col1a1^{+} cells

were detected on metaphyseal bones underneath the growth plate cartilage (inserted with red frame). PZ: proliferation zone; HZ: hypertrophic zone; MB: metaphyseal bone; TB: trabecular bone. Scale bar is 200 μm (A, B, E), 500 μm (D), 100 μm (H, G).

Material and methods

Mice

ROSA^{EYFP} [3], *Col2a1-Cre*^{ERT} [4] and *ROSA*^{Confetti} [5] mice have been previously reported. Mice were bred and maintained in animal facility according to institutional guidelines. All experimental protocols were designed according to the recommendation of the Beijing Experimental Animal Regulation Board (SYXK/JING/2005/0031).

Generation of *Col10a1*^{int2}*-Cre* transgenic line

The DNA fragments containing the 8.2 kb murine *Col10a1* promoter (from −8039 bp to +199 bp), 1.2 kb Cre cDNA, 2.1 kb *hGH* polyadenylation signal and 3.2 kb 2nd intron of murine *Col10a1* were injected into FVB/N zygotes to generate *Col10a1*^{int2}*-Cre* transgenic mice. Three founders were obtained, each expressing Cre in the hypertrophic chondrocytes, and 1 was selected for subsequent studies. No differences in the specificity of transgene expression were observed between the founders.

Induction of lineage tracing

Lineage tracing of *Col2a1-Cre*^{ERT}*;ROSA*^{EYFP} and *Col2a1-Cre*^{ERT}*;ROSA*^{Confetti} was induced at P5 by intraperitoneal injection of a single pulse of tamoxifen (Sigma Aldrich, T5648-1G, 50 or 100mg/kg). Control mice for labeling studies included *Col2a1-Cre*^{ERT}*;ROSA*^{EYFP} and *Col2a1-Cre*^{ERT}*;ROSA*^{Confetti} mice without administration of tamoxifen.

BrdU labeling

Mice were intraperitoneally injected with BrdU (Sigma Aldrich, 35002-5G, 100mg/kg body weight) 3 hours before sacrifice.

Von Kossa staining

For detection of mineralization, the undecalcified tibiae were embedded in methyl methacrylate, and 6 μm sections were cut and stained with von Kossa stainmethod (2% silver

nitrate). After digestion with 20 μg/ml proteinase K, detection of YFP was then performed using monoclonal rabbit anti-GFP antibody (Cell Signaling Technology, 2956, 1:500, 37 ℃, 2 hours) and visualized with HighDef red IHC (AP) (Enzo Life Sciences, ADI-950-140-0030).

Isotopic *in situ* hybridization

RNA probe for *Cre* were labeled with ^{35}S-dUTP (Perkin-Elmer, NEG039H001MC) using the MAXIscript *in vitro* transcription kit (Ambion, T3/AM1316, T7/AM1312). *In situ* hybridization on 6 μm paraffin sections was performed with standard procedures. Slides were dipped in emulsion (Ilford, K5, 02746-100) and exposed for 5–30 days before developing.

Multicolor chromogenic *in situ* hybridization and immunohistochemistry

DNP-11-UTP (perkinelmer, NEL555001EA) or digoxigenin-11-UTP (Roche Applied Science, 11277073910) was employed to label RNA probes for *Col1a1*, *Ocn*, *Bsp* and *Cre* using the MAXIscript *in vitro* transcription kit (Ambion, T3/AM1316, T7/AM1312). *In situ* hybridization on 6 μm paraffin sections was performed with standard procedures. Sections were then incubated in 1:2000 AP-conjugated, polyclonal sheep anti-digoxigenin antibody (Roche Applied Science, 11093274910) or in 1:200 HRP conjugate, monoclonal rat anti-DNP antibody (perkinelmer, FP1129) at 4 ℃ for 10 hours. Immunoreactive cells were visualized with HighDef blue (HRP) (Enzo Life Sciences, ADI-950-151-0006), HighDef red IHC (AP) (Enzo Life Sciences, ADI-950-140-0030) or BCIP/NBT (AP) (Roche Applied Science, 11681451001). Inactivation of HRP or AP was achieved by washing slides with 3% hydrogen peroxide for 40 minutes at RT or incubating slides in PBS for 20 minutes at 65 ℃, respectively. The primary antibodies used for immunohistochemistry following *in situ* hybridization were monoclonal rabbit anti-GFP (Cell Signaling Technology, 2956, 1:500,37 ℃, 2 hours), polyclonal goat anti-GFP (abcam, ab6662, 1:200, 37 ℃, 2 hours), polyclonal rabbit anti-RFP (Rockland, 600-401-379, 1:500, 37 ℃, 2 hours), monoclonal rat anti-endomucin (Santa Cruz Biotechnology, sc-65495, 1:200, 37 ℃, 2 hours), Polyclonal guinea pig anti-perilipin (Fitzgerald, 20R-PP004, 1:400, 37 ℃, 2 hours), polyclonal rabbit anti-Col1a1 (Millipore, AB765P,1:200, 37 ℃, 2 hours). HRP-conjugated secondary antibodies (used following AP-conjugated anti-digoxigenin antibody) or AP-conjugated secondary antibodies (used following HRP-conjugated anti-DNP antibody) were then applied (Zhongshan Biotech, DS-0001, PV-6001). Immunoreactive cells were visualized with

HighDef blue (HRP) (Enzo Life Sciences, ADI-950-151-0006), HighDef red IHC (AP) (Enzo Life Sciences, ADI-950-140-0030) or DAB (HRP) (Zhongshan Biotech, ZLI-9019).

Immunofluorescence

Antigen retrieval of 6 μm paraffin sections was performed in citrate buffer (pH 6.0) using a pressure cooker (Biocare Medical, DC2008). The primary antibodies (monoclonal rabbit anti-PDGFR-β, Cell Signaling Technology, 3169, 1:200; polyclonal goat anti-GFP, abcam, ab6662, 1:200) were mixed and incubated at 4 ℃ overnight. Immunoreactive cells were visualized with fluorochrome-conjugated secondary antibodies (Zhongshan Biotech, ZF-0511, ZF-0317), stained with DAPI and observed under confocal microscopy (Zeiss, LSM 510).

Reverse transcriptase-PCR

Total RNA was isolated from growth plate cartilage, bone marrow and cortical bone of tibia, as well as calvarial bone, skin, muscle and heart tissue. cDNA synthesis was carried out using ReverTra Ace qPCR RT Master Mix (FSQ-201), followed by PCR with primers for *Cre-hGH* mRNA splicing: Pr1: 5'-GAAGCCTATATCCCAA GGAA-3' and Pr2: 5'-ACTGGAGTGGCAACTTCCAG-3'.