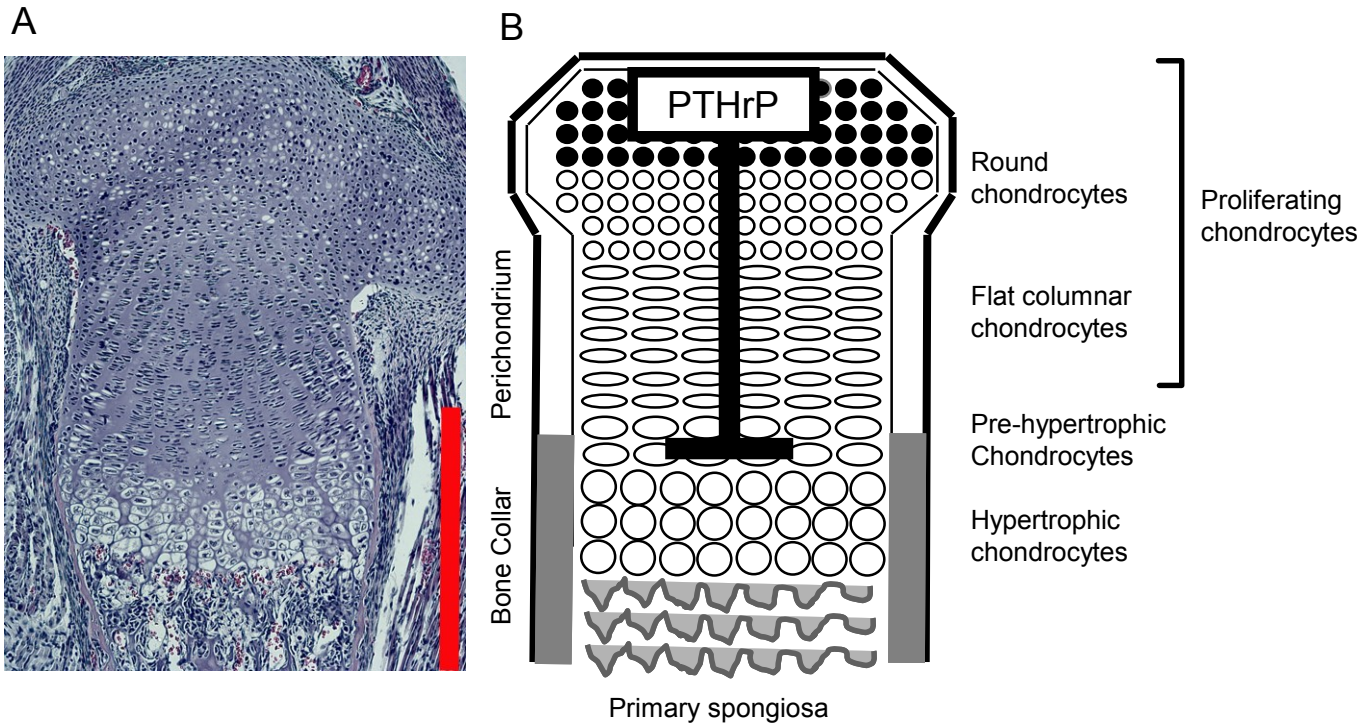
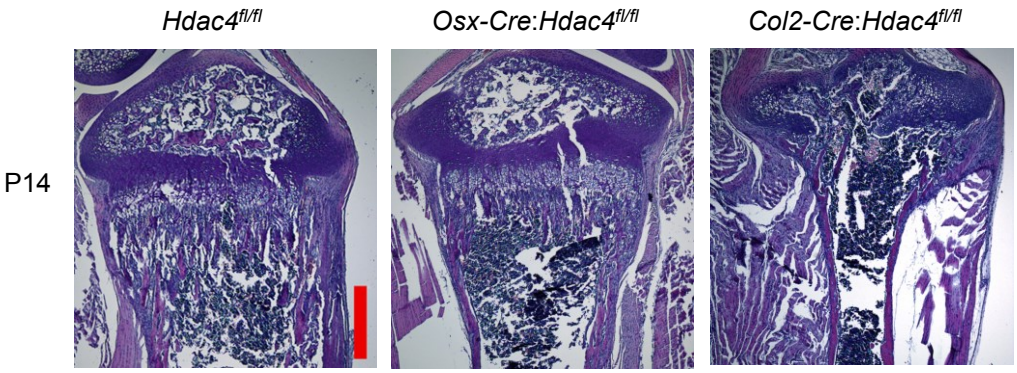


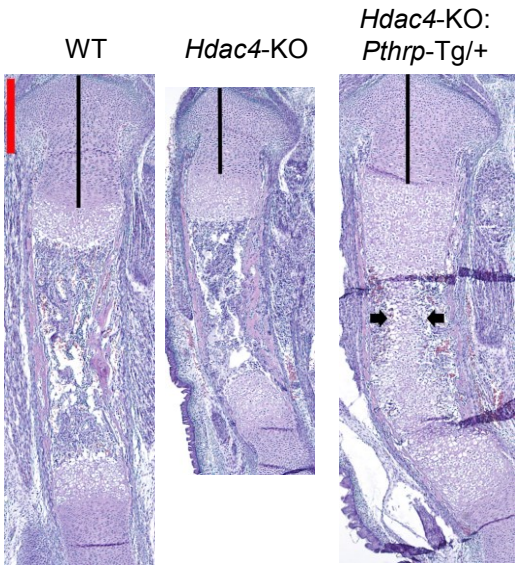
Supplemental Figure 1. Schematic view of the developing growth plate. PTHrP suppresses chondrocyte hypertrophy.

(A) Proximal tibial growth plate of WT mouse at birth (X100). Same figure as in Figure 2A. (B) Schematic view of the developing growth plate before the formation of the secondary ossification center. (C) H&E stain of the whole tibia from the PTHrP-Tg/+ mouse at birth, P8, and P14 (X40). Black arrows indicate hypertrophic chondrocytes at P8. The reproducibility of the phenotype was confirmed by two independent animals for each genotype (C). Scale bars (red lines): 500 μm (A, C).



Supplemental Figure 2. The bone-specific conditional *Hdac4* knockout mouse by *Osx-Cre* exhibits a normal phenotype.

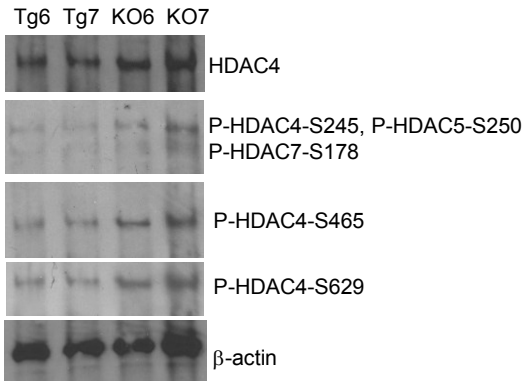
H&E stain of the proximal tibial growth plates at P14 (X40). The *Hdac4*$^{fl/fl}$ mouse and the *Osx-Cre*:*Hdac4*$^{fl/fl}$ mouse are littermates. The reproducibility of the phenotype was confirmed by three independent animals for each genotype. Scale bar (red line): 500 μm.



Supplemental Figure 3. Possible existence of other mediators of PTHrP signaling in the *Hdac4*-KO mouse.

H&E stain of the whole tibia at E17.5 (X40). The mice shown are littermates. Black lines indicate the length of proliferating chondrocyte region. Black arrows indicate early bone formation. The reproducibility of the phenotype was confirmed by two independent animals for each genotype. Scale bar (red line): 500 μm.

A



B

Pthrp-Tg8-area 1 (Total cell number in the confocal image: 77)

Sum of HDAC4 intensities in the HDAC4 and DAPI double positive areas: 6.67×10^7
Sum of HDAC4 intensities in the HDAC4 and whole cell stain double positive areas: 1.06×10^8
Nucleus / Whole Cell ratio of HDAC4: $6.67 \times 10^7 / 1.06 \times 10^8 = 62.9$ %

Nucleus / Whole Cell ratio of HDAC4 in other areas

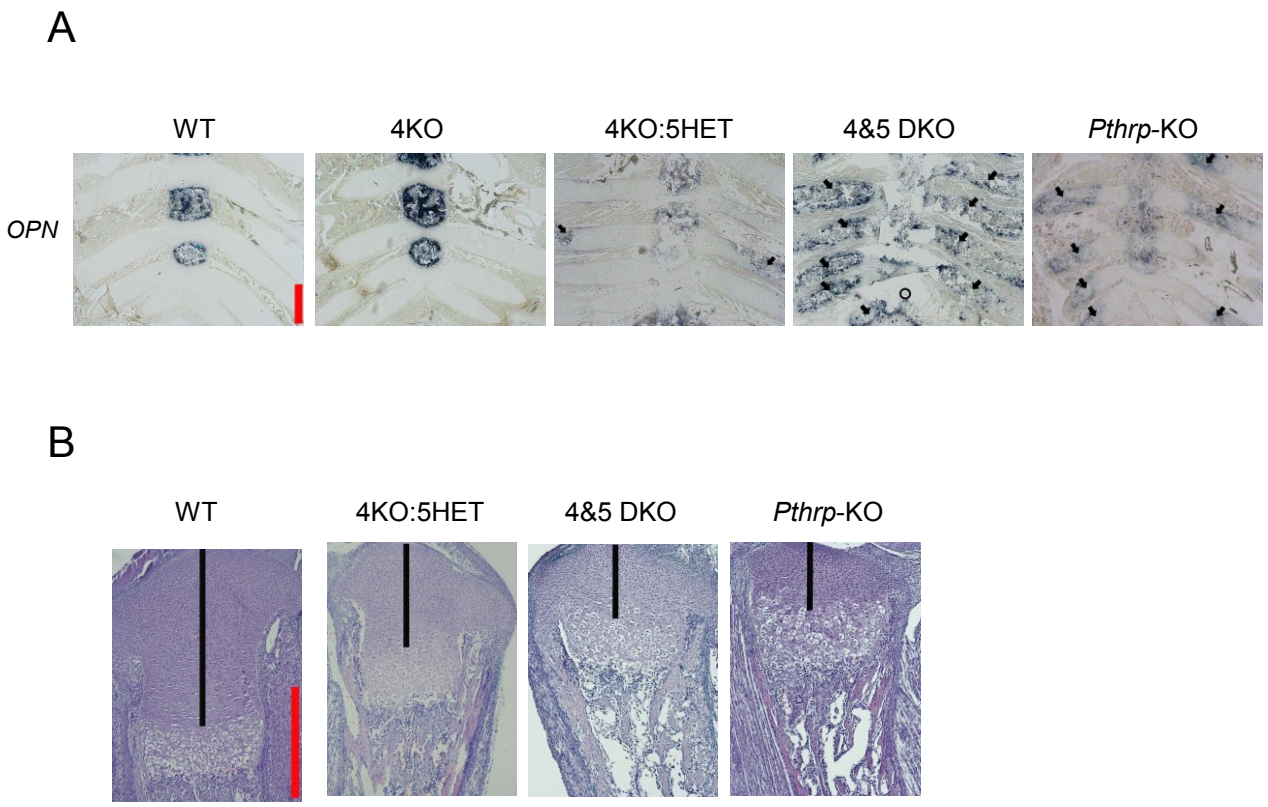
Tg8-area 2: 48.3 % (Cell number: 78)
Tg8-area 3: 53.9 % (Cell number: 73)
Tg8-area 4: 56.3 % (Cell number: 86)
Tg8-area 5: 63.8 % (Cell number: 81)
Tg8-area 6: 54.2 % (Cell number: 88)
Tg8-area 7: 56.6 % (Cell number: 68)
Tg8-area 8: 55.8 % (Cell number: 73)

Average ratio ± SEM (area 1 to 8): 56.5 ± 1.8 % (Total cell number: 624)

Supplemental Figure 4. Supplemental information for the graphs in Figure 3.

(A) Western blots at the all 14-3-3 binding sites using microdissected proliferating chondrocyte regions in the proximal tibial growth plates at birth (n=2, biological replicates). The full, uncut gels are available in the published online Supplemental Material. Different sets of animals from Figure 3C (Tg4, Tg5, KO4, KO5). To calculate the average band intensities for the graph in Figure 3D, we analyzed these 8 animals (Tg4–Tg7 and KO4–KO7: n = 4, biological replicate).

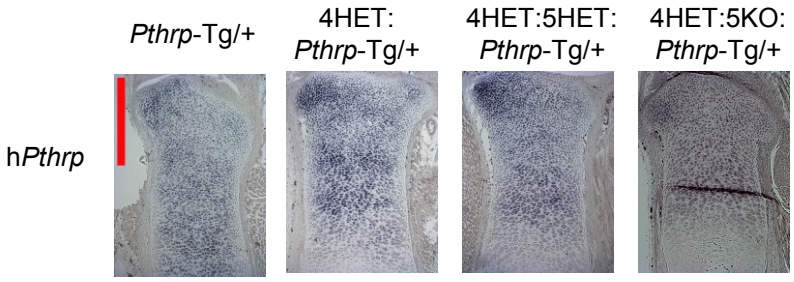
(B) We performed 3-color IHC (HDAC4, DAPI, Whole cell stain) for 5 sets of Tg (Tg8–Tg12) and KO (KO8–KO12) at birth. For example, a calculation for Tg8 is shown here. We captured confocal microscopy images of round chondrocytes (X620) from 8 different areas in the same proximal tibial growth plates. We analyzed each whole image using a software, PerkinElmer Volocity that calculates the sum of HDAC4 intensities in the HDAC4 and DAPI double positive areas (HDAC4 signals in nuclei) and the sum of HDAC4 intensities in the HDAC4 and whole cell stain double positive areas (HDAC4 signals in the whole cell) (See Method). For the dot plots in the graph (Figure 3F), we calculate the average ratios (nuclei vs. whole cell) from area 1 to 8. The data for all the animals are shown in Supplemental Table 1.



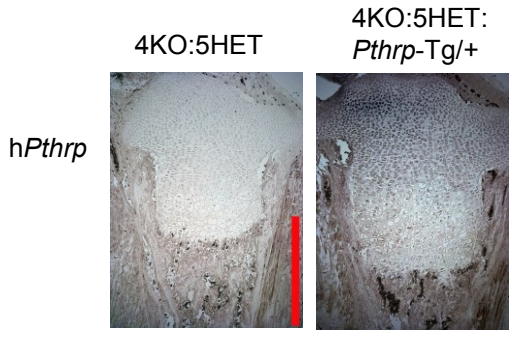
Supplemental Figure 5. HDAC5 mediates PTHrP signaling in chondrocytes.

(A) *in situ* hybridization for *Osteopontin (OPN)* mRNA in the anterior rib cage at birth (X40). Black arrows indicate representative *OPN* expression areas in the rib cartilage. Same mice as shown in Figure 5B. (B) H&E stain of the proximal tibial growth plates at E18.5 at high magnification (X100). The mice shown are littermates, except the *Pthrp*-KO mouse. Black lines indicate the length of proliferating chondrocyte region. The reproducibility of the phenotype was confirmed by two independent animals for each genotype. Scale bars (red lines): 500 μm (A, B).

A



B



Supplemental Figure 6. The genetic deletion of *Hdac4* and/or *Hdac5* does not reduce the *Pthrp* overexpression in the *Pthrp*-Tg/+ mouse.

(A, B) *in situ* hybridization for human *Pthrp* mRNA on the proximal tibia (A) or the proximal tibial growth plate (B) at birth (X100). Same mice as shown in Figure 6A, 6B, and 6C. The mice shown were born from the same parents (A) or are littermates (B). Scale bars (red lines): 500 μm (A, B).

Animals	Number of the analyzed areas	Total cell number in the analyzed areas	HDAC4 ratio nuclei to whole cell (%) (Average ± SEM)
Tg8	8	624	56.5 ± 1.8
Tg9	9	981	45.9 ± 2.8
Tg10	7	781	45.0 ± 1.9
Tg11	12	1,237	54.9 ± 2.5
Tg12	17	1,845	54.2 ± 1.9
KO8	6	643	37.1 ± 1.6
KO9	6	489	43.0 ± 2.4
KO10	10	1,034	34.2 ± 2.4
KO11	14	1,318	45.7 ± 1.5
KO12	14	1,208	36.5 ± 1.6

Supplemental Table 1. Supplemental information for the graph in Figure 3F

The raw data for Figure 3F are shown here. The detailed calculation for the ratios is shown in Supplemental Figure 3B and Method. We captured multiple images from different areas in the round chondrocyte regions in the same proximal tibial growth plate. Each image has 80 to 120 cells that were scanned at random levels by the confocal plane. We obtained similar average ratios of HDAC4 in nuclei vs. whole cells by analyzing 5, 10, and 15 images from the same animal. Thus, we could get representative average ratios by analyzing at least 5 images (including 400 to 500 cells).

A. Genotyping primers

(1) PTHrP-KO mouse
PTHrP-WT Forward (FWD) (on PTHrP Exon 3): 5'-AGATCCACACAGCCGAAATC-3'
PTHrP-KO FWD (on NEO cassette): 5'-CATCGCCTTCTATCGCCTTCTTGAC-3'
Common Reverse (REV) (on PTHrP intron): 5'-CTTATAATCCCAGCATCTGAGAGGC-3'

(2) Mouse Col2 promoter: human PTHrP-Tg mouse
PTHrP-Tg-FWD (on human GH sequence): 5'-GGACCTAGAGGAAGGCATCCAAAC-3'
PTHrP-Tg-REV (on human GH sequence): 5'-TTGCTGTAGGTCTGCTTGAAGATC-3'

(3) HDAC4-KO mouse
Common FWD (on HDAC4 intron): 5' ATCTGCCCACCAGAGTATGTG-3'
HDAC4-WT REV (on HDAC4 Exon 5): 5'-CTTGTTGAGAACAAACTCCTGCAGCT-3'
HDAC4-KO REV (on LacZ cassette): 5'-GATTGACCGTAATGGGATAGGTTACG-3'

(4) HDAC4-Floxed mouse
Same primers sets with HDAC4 WT allele

(5) Col2-Cre mouse
Col2-Cre FWD (on Col2 intron): 5'-TTTTGCCACTGCTTTTGAGA-3'
Col2-Cre REV (on IRES cassette): 5'-GGAAAGACCCCTAGGAATGC-3'

(6) Osx-Cre-GFP mouse
GFP-FWD: 5'-TCATCTGCACCACCGGCAAGC-3'
GFP-REV: 5'-AGCAGGACCATGTGATCGCGC-3'

(7) HDAC5-KO mouse
Common FWD (on HDAC5 intron): 5'-GGTGTGTTCCTGTGCAGTTT-3'
HDAC5-WT REV (on HDAC5 Exon 4): 5'-CTTGAGGACCAGGAGCTCC-3'
HDAC5-KO REV (on LacZ cassette): 5'-GTTTGAGGGGACGACGACAG-3'

(8) Runx2-KO mouse
Runx2-KO FWD (on Neo cassette): 5'-TCTGGATTCATCGACTGTGG-3'
Runx2-KO REV (on Runx2 intron): 5'-AGGCTGGAGTCTTGGAGGA-3'

B. Target regions for *in situ* hybridization

(1) Ihh (Mouse, NM_010544.3: 2,476 bp) : 230 to 2,030 (1,801 bp)
(2) Col10a1 (Mouse, NM_009925.4: 3,139 bp): 644 to 1,535 (892 bp)
(3) PTHrP (Human, NM_198965.1: 1,331 bp) : 357 to 712 (356 bp)
(4) Col2a1 (Rat, K02904, 521 bp): 1 to 521 (521 bp)
(5) Osteopontin (Mouse, NM_001204201: 1,475 bp): 455 to 1,269 (815 bp)

Supplemental Table 2. Genotyping primers and the target regions for *in situ* hybridization

(A) Genotyping primers
(B) Target regions of *in situ* hybridization probes