

Uchimura_Fig. S1

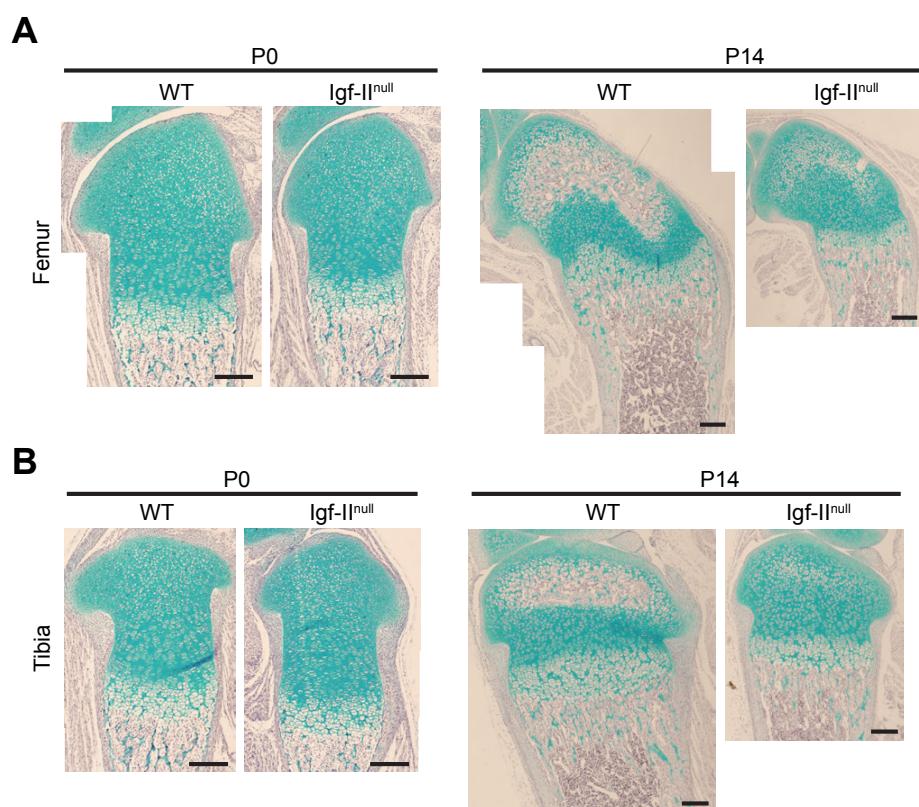
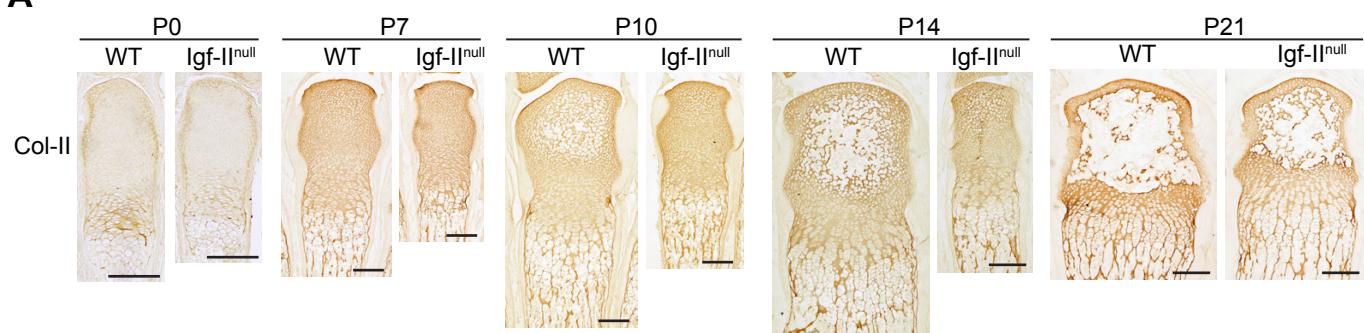


Figure. S1. Histological analysis of distal femur and proximal tibia at postnatal day 0 and 14 showing a delay in long bone development in the Igf-II null. Alcian blue and hematoxylin staining of (A) distal femur and (B) proximal tibia at P0 and P14. Scale bar = 200 μ m.

Uchimura_Fig. S2

A



B

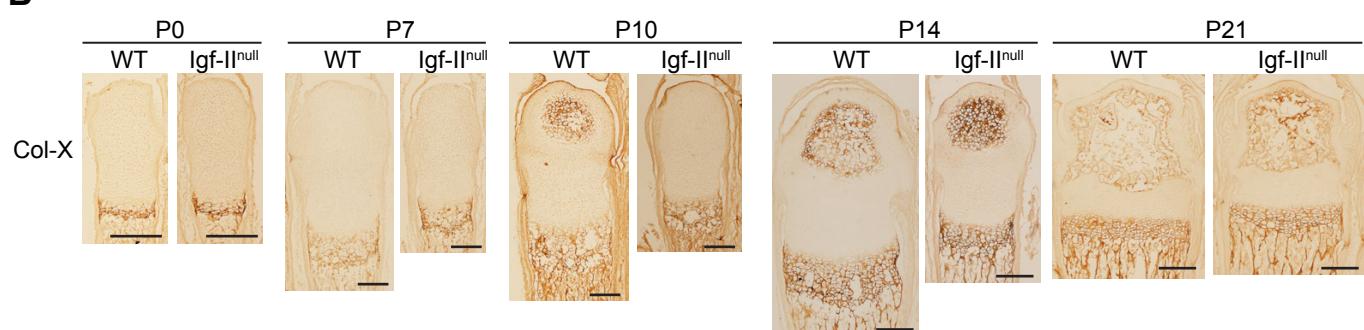


Figure. S2. Immunohistochemistry (IHC) of collagen II and collagen X of the Igf-II null and the WT metatarsal bones from P0 to P21. (A) Collagen II (Col-II) IHC. (B) Collagen X (Col-X) IHC. Scale bar = 200μm.

Uchimura_Fig. S3

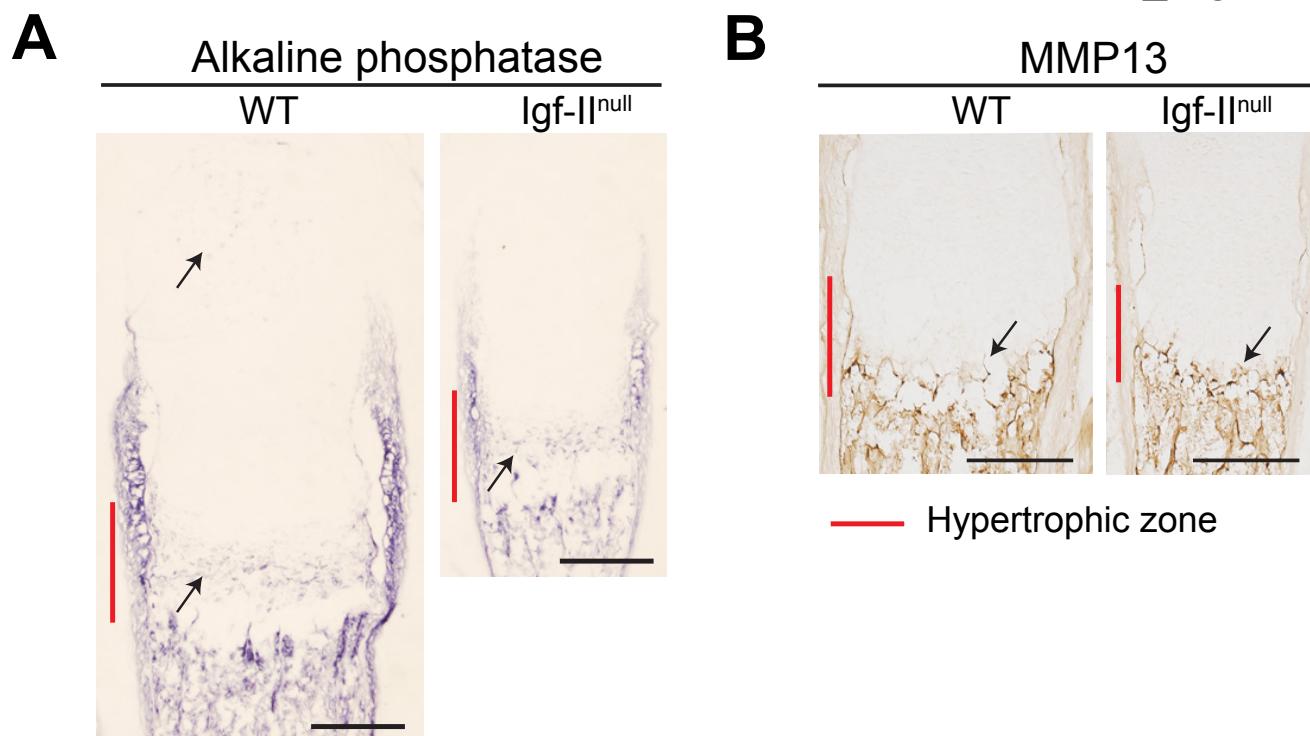


Figure. S3. Histological analysis of alkaline phosphatase activity and MMP13 expression in the growth plate of the WT and Igf-II null metatarsal bones at P7. (A) Alkaline phosphatase (ALP) activity assay. Arrows indicate ALP-positive chondrocytes in the hypertrophic zone in the Igf-II null and the WT bone, as well as the SOC of the WT bone. (B) MMP13 expression. Arrows indicate positive MMP13 in chondrocytes of the late hypertrophic zone. Scale bar = 200μm.

Uchimura_Fig. S4

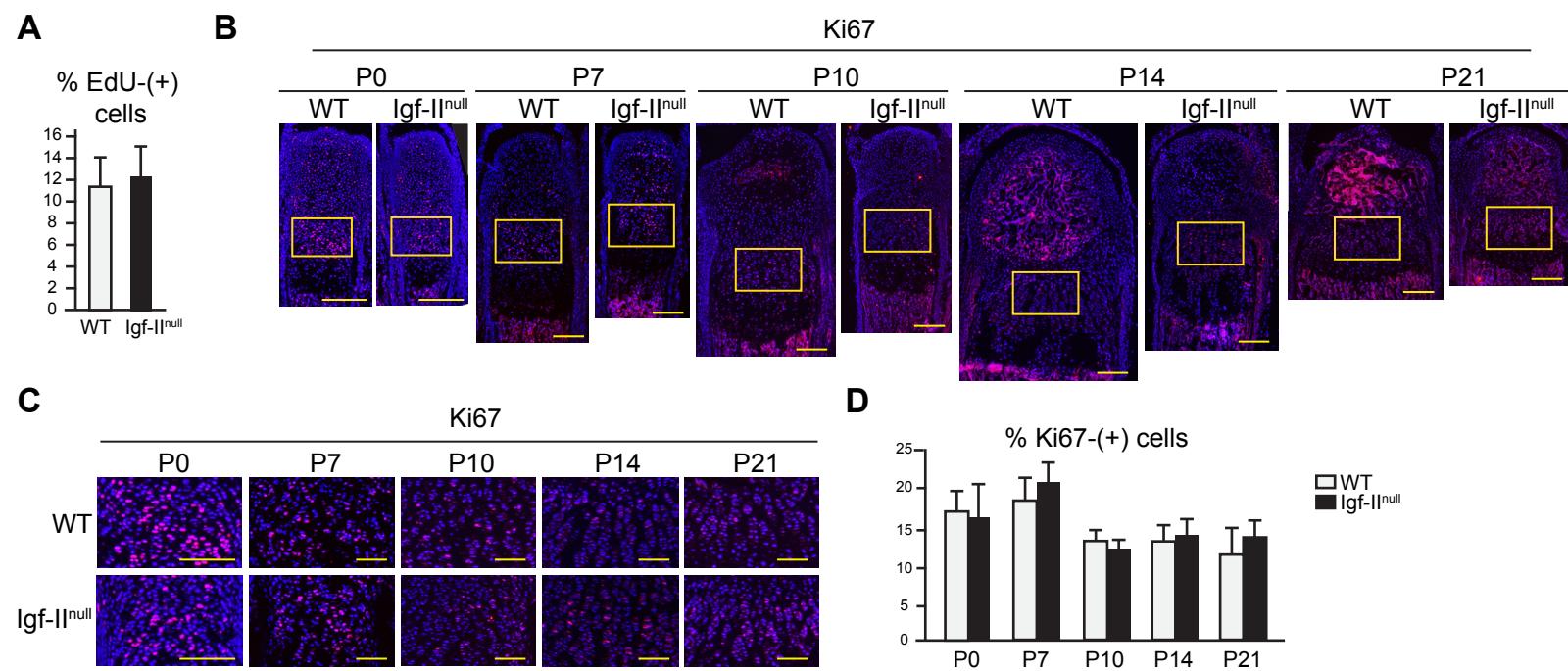


Figure. S4. Quantification of chondrocyte proliferation in the columnar zone of the WT and Igf II-null metatarsal bones. (A) Quantification of EdU incorporation at P7. Data are presented as total number of Ki67-positive cells divided by the total number of cells (DAPI positive) in the columnar zone. (B) IHC analysis of proliferation marker Ki67 expression in the growth plate at postnatal stages P0-P21. Rectangles denote the areas magnified. Scale bar = 200μm. (C) Magnified areas of Ki67 staining. Scale bar = 50μm. (D) Quantification of the percentage of Ki67-positive cells in the columnar zone. Data are presented as total number of Ki67-positive cells divided by the total number of cells (DAPI positive) in the columnar zone. In (A) and (D), mean ± standard deviation are presented.

Uchimura_Fig. S5

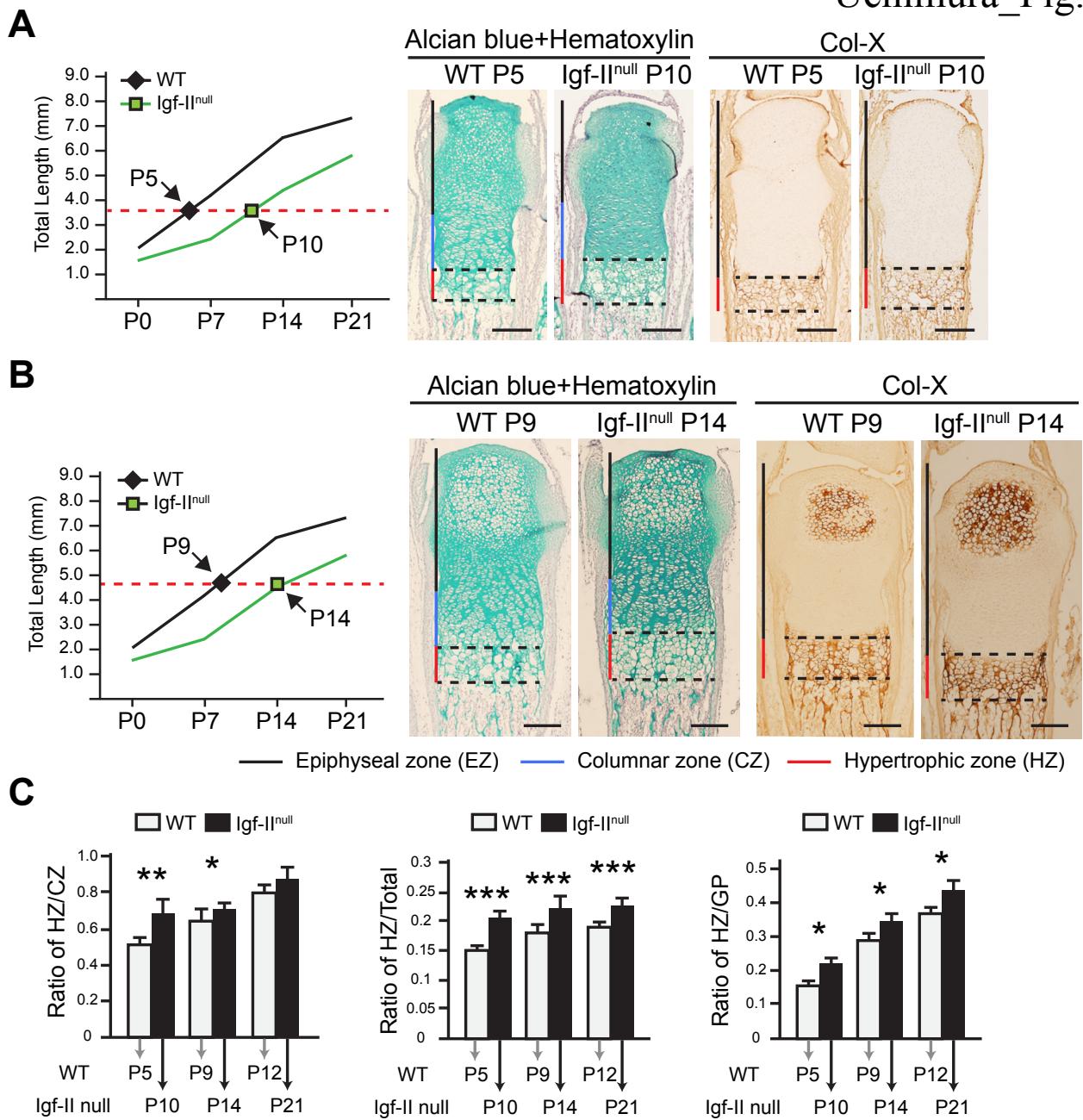


Figure. S5. Histological analyses from additional length-matched bones between the Igf-II null and the WT. Based on the growth curve, two other pairs of length-matched bones were analyzed: (A) P5 WT vs P10 Igf-II null and (B) P9 vs P14 Igf-II null. Alcian blue/hematoxylin and Col-X staining were performed. Scale bar = 200 μ m. (C) Quantification of the ratio of HZ/CZ at three different sets of time point between WT and mutant bones. HZ = hypertrophic zone, CZ = columnar zone. Unpaired *t*-tests were conducted to determine statistical differences between the WT and the Igf-II null. *, *p* < 0.05, **, *p* < 0.01, ***, *p* < 0.001.

Uchimura_Fig. S6

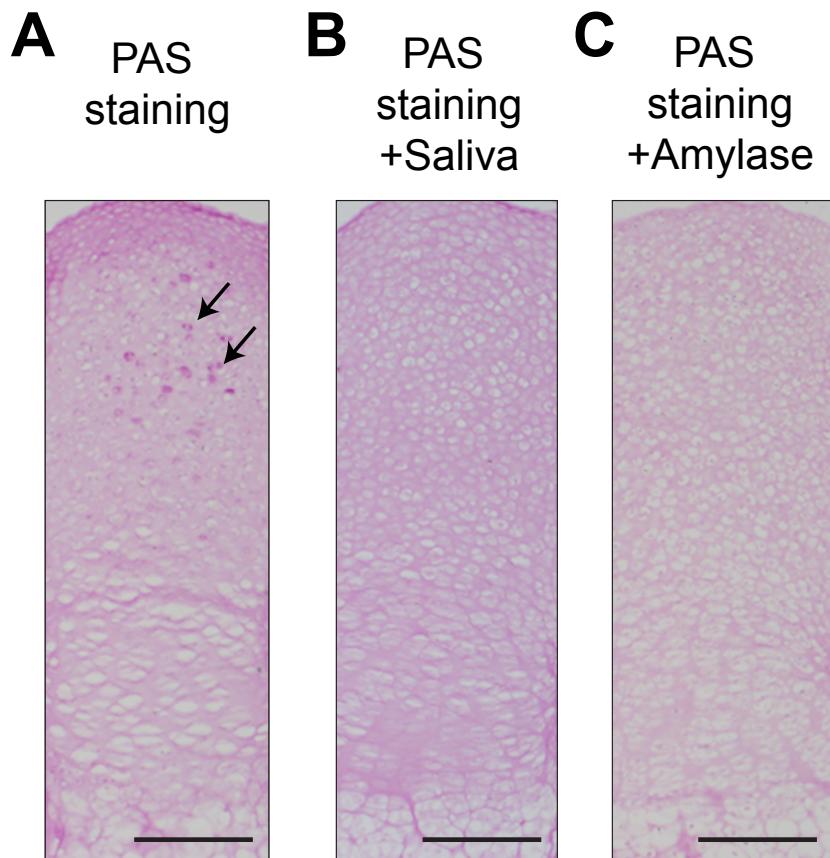


Figure. S6. Validation of Periodic Acid Schiff (PAS) staining for detecting glycogen in growth plate chondrocytes of P7 metatarsal bones in wild type mice. (A) An image of PAS-stained section. Arrows indicate strong punctate staining in the forming secondary ossification center. (B). An image of a section pretreated with 100% saliva before PAS staining. (C) An image of a section pretreated with 10% α -amylase before PAS staining. Since saliva contains α -amylase, which specifically degrades glycogen, the absence of strong punctate staining in saliva and amylase-treated samples indicates that these punctate staining (arrows) represent glycogen deposit. The fainter pink color observed in PAS-stained sections is likely caused by other polysaccharides such as glycosaminoglycans in cartilage matrix. Scale bar = 200 μ m.

Uchimura_Fig. S7

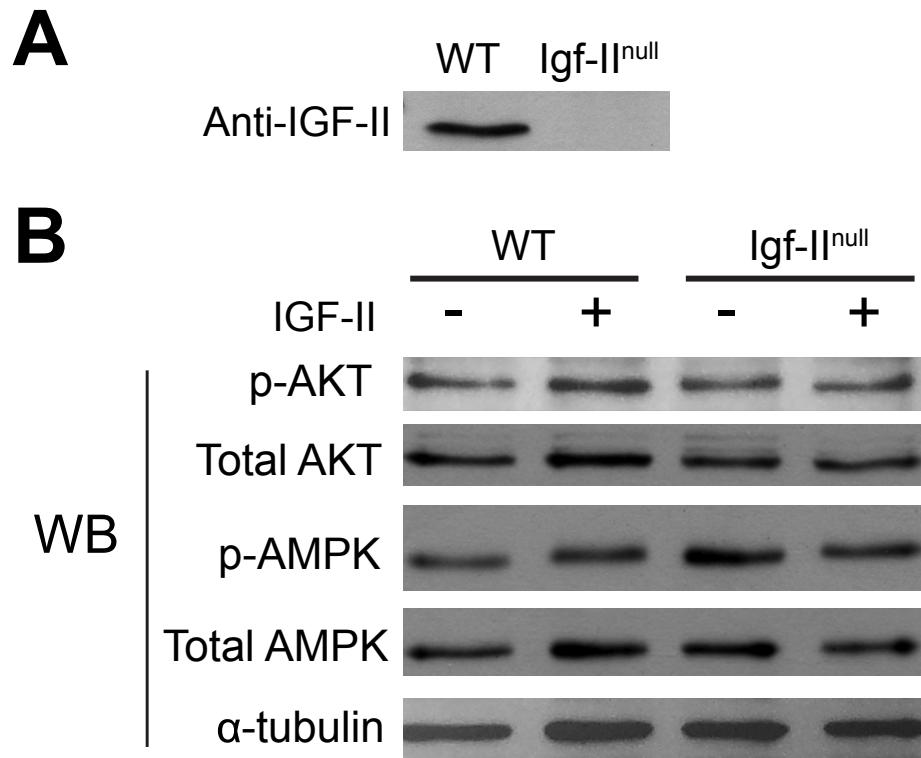


Figure. S7. Western Blot analysis on epiphyseal chondrocytes of the wild type (WT) and Igf-II null upon IGF-II treatment. (A). Western Blot analysis to detect IGF-II protein in epiphyseal chondrocytes. (B). Western Blot analysis to detect phospho-Akt (Ser473), total Akt, phospho-AMPK (Thr172), total AMPK, and α -tubulin.

Uchimura_Supplemental Table 1

Antibody name	Source	Identification number	Dilution Factor	Citations
Goat anti-Ihh	Santa Cruz	C-15	1 to 500	Aguilar, A. et al. 2009. Endocrinology. 150(6): 2732-2739.
Rabbit anti-Ki67	Vector Lab	VP-K451	1 to 1000	Shaaban et al. American Journal of Pathology 160 (2):597.
Rabbit anti-Sox9	Chemicon	AB5535	1 to 850	Carrasco, M., et al. (2012). J. Clin. Invest. 122(10):3504-3515; Sylva, M., et al. (2011). PLoS One. 6(8):e22616).
Rabbit anti-Runx2	Santa Cruz	M-70	1 to 500	Zhang, W. et al. 2014. Journal of bone and mineral research 29: 1232-43.
Mouse anti-collagen II	Thomas Linsenmayer, Tufts Univ.--who deposited into Dev Stud Hyb Banks	II-II6B3	Not diluted	Knight RD et al. 2011. Development. 138(10):2015-24
Mouse anti-collagen X	Thomas Linsenmayer, Tufts Univ.--who deposited into Dev Stud Hyb Banks	X-AC9	Not diluted	Schmid et al. J. Cell Biol. 1985. 100, 598-605.; Bond et al 2011. J. Bone Miner. Res. 26(12), 2911-2922.
Rabbit anti-collagen X	Calbiochem	234196	1:20	Chung, K.S., et al. 1995. <i>Dev. Biol.</i> 170 , 387.
Mouse anti-MMP13	Abcam	VIIIA2	1 to 200	Vikman et al. Vasc Health Risk Manag. 5: 333-41
Mouse anti-HIF1alpha	Brent Cochran, Tufts Univ.-- who purchsed from Novus Biologicals	NB100-122	1 to 50	Wang et al 2007. J Clin. Invest. Jun 1; 117(6): 1616–1626.
Mouse anti-HIF2alpha	Brent Cochran, Tufts Univ.-- who purchsed from Novus Biologicals	NB100-106	1 to 100	Wang et al 2007. J Clin. Invest. Jun 1; 117(6): 1616–1626.
Rat anti-Thy1.2	eBioscience	53-2.1	1 to 100	Nakamura et al. J Histochem Cytochem 58: 455-62. Ledbetter et al. Immunol Rev 47: 63-90.
Rat anti-CD44	Dev Stud Hyb Bank	5D2-27	1 to 10	Huges and August, J. Biol. Chem. 256, 664-671.
Rabbit anti-Akt	Cell Signaling	C73H10	1 to 500	Uchimura et al. J Cell Biochem. 2015. 116: 2858
Rabbit anti-phospho Akt	Cell Signaling	C31E5E	1 to 500	Uchimura et al. J Cell Biochem. 2015. 116: 2858
Rabbit anti-AMPK	Cell Signaling	23A3	1 to 500	Reihill et al 2007. Biochemical and Biophysical Research Communications, 354(4), 1084.
Rabbit anti-phospho AMPK	Cell Signaling	40H9	1 to 500	Reihill et al 2007. Biochemical and Biophysical Research Communications, 354(4), 1084.
Mouse anti-alpha tubulin	Dev Stud Hyb Bank	12G10	1 to 10	Uchimura et al. J Cell Biochem. 2015. 116: 2859
Goat anti-rabbit IgG HRP-conjugate secondary antibody	Millipore/Chemicon	401315	1 to 10,000	Uchimura et al. J Cell Biochem. 2015. 116: 2860
Goat anti-mouse IgG HRP-conjugate secondary antibody	Millipore/Chemicon	401205	1 to 10,000	Uchimura et al. J Cell Biochem. 2015. 116: 2860