Title:

Loss of the nutrient sensor TAS1R3 leads to reduced bone resorption

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Abstract

The taste receptor type 1 (TAS1R) family of heterotrimeric G protein-coupled receptors participates in monitoring energy and nutrient status. TAS1R member 3 (TAS1R3) is a bi-functional protein that recognizes amino acids such as L-glycine and L-glutamate or sweet molecules such as sucrose and fructose when dimerized with TAS1R member 1 (TAS1R1) or TAS1R member 2 (TAS1R2), respectively. It was recently reported that deletion of TAS1R3 expression in *Tas1R3* mutant mice leads to increased cortical bone mass but the underlying cellular mechanism leading to this phenotype remains unclear. Here, we independently corroborate the increased thickness of cortical bone in femurs of 20-week-old male *Tas1R3* mutant mice and confirm that *Tas1R3* is expressed in the bone environment. *Tas1R3* is expressed in undifferentiated bone marrow stromal cells (BMSCs) *in vitro* and its expression is maintained during BMP2-induced osteogenic differentiation. However, levels of the bone formation marker Procollagen Type I N-terminal Propeptide (PINP) are unchanged in the serum of 20-week-old *Tas1R3* mutant mice as compared to controls. In contrast, levels of the bone resorption marker Collagen Type I C-telopeptide are reduced greater than 60% in *Tas1R3* mutant mice. Consistent with this, *Tas1R3* and its putative signaling partner *Tas1R2* are expressed in primary osteoclasts and their expression levels positively correlate with differentiation status. Collectively, these findings suggest that high bone mass in *Tas1R3* mutant mice is due to uncoupled bone remodeling with reduced osteoclast function and provide rationale for future experiments examining the cell-type-dependent role for TAS1R family members in nutrient sensing in postnatal bone remodeling.

Keywords

Taste receptor, TAS1R3, TAS1R1, TAS1R2, bone, osteoclast, osteoblast

Background

Osteoporosis is a disease of low bone mineral density that is both a significant health problem and a considerable socioeconomic burden, accounting for 1.5 million fractures annually in the United States [18,2]. Osteoporosis is characterized by an imbalance in bone remodeling in which bone resorption by osteoclasts outpaces bone formation by osteoblasts. Current treatments for osteoporosis have significant limitations [18,3,1,7], and a better understanding of the endogenous mechanisms regulating bone remodeling is necessary for developing novel treatment strategies. Recent evidence implicates the taste receptor type 1 (TAS1R) family as a novel mechanism regulating postnatal bone homeostasis [16].

The TAS1R family members TAS1R1, TAS1R2, and TAS1R3 act as nutrient sensors to monitor energy and nutrient status and are expressed in numerous oral and extra-oral tissues including the gastrointestinal tract, brain, bladder, pancreas, male reproductive organs, immune system, adipose tissue, and bone [11]. TAS1R3 is a bi-functional, class C heterotrimeric G protein-coupled receptor that recognizes amino acids such as L-glycine and L-glutamate when dimerized with TAS1R1 or sweet molecules such as sucrose and fructose when dimerized with TAS1R2. Regardless of the ligand, these two responses share a common signaling pathway involving α-gustducin-mediated activation of Phospholipase C-β2 (PLCβ2) [11].

Recently, Simon *et al.* reported that deletion of the gene encoding TAS1R3 in *Tas1R3* mutant mice leads to increased cortical bone mass [16]; however, the underlying cellular mechanism leading to this phenotype was not examined. Here, we corroborate the finding of high bone mass in *Tas1R3* mutant mice and report that levels of the bone resorption marker Collagen Type I C-telopeptide are reduced greater than 60% in these mice while bone formation markers are unchanged. Consistent with this, *Tas1R3* and its putative partner *Tas1R2* are expressed in primary osteoclasts and their expression levels positively correlate with differentiation status. Collectively, these findings suggest that high bone mass in *Tas1R3* mutant mice is due to uncoupled bone remodeling with reduced osteoclast function and provide rationale for future experiments examining the cell type-dependent role for TAS1R family members in nutrient sensing in postnatal bone remodeling.

Materials and Methods

Animals

Tas1R3 mutant mice were described previously [19]. Wild type C57Bl/6 mice were purchased from Charles River Labs. Animals were given humane care with compliance to institutional guidelines and were allowed *ad libitum* access to standard chow and tap water under temperature controlled conditions. Euthanasia was accomplished via exposure to CO_2 followed by cervical dislocation.

Micro computed tomography (µCT) and histomorphometric analyses

For µCT analyses, bones were collected immediately after sacrifice and fixed in 10% neutral buffered formalin (Millipore, Billerica, Massachusetts, USA). Bones were exchanged to 70% ethanol, stored at 4°C, and analyzed at the Harvard School of Dental Medicine µCT Core Facility on a SCANCO µCT 35 scanner. Bone volume and mineral density were determined using the following settings: FOV/Diameter: 7168 µm; voxel size: 7.0 µm; slice increment: 7.0 µm; projections: 500; sample time: 600 ms; µ-scaling: 4096; energy: 55000 V; intensity: 145 µA. The reader is directed to an excellent guide to µCT in rodents [4]. Femora were subsequently embedded in methyl methacrylate without decalcification, stained using hematoxylin and eosin, von Kossa/McNeal, or for TRAP activity counterstained with hematoxylin as previously described [15]; quantification for standard histomorphometric parameters (n=3 per genotype) by a blinded scorer using morphometric software (OsteoMetrics, Atlanta, Georgia, USA). The reader is directed to an excellent guide to bone histomorphometry [5].

Cell culture

MC3T3-E1 and MLO-Y4 cells were obtained from ATCC. Bone marrow stromal cells (BMSCs) were isolated from the long bones of male C57Bl/6 mice (Charles River Labs, Wilmington, Massachusetts, USA) between ten and thirteen weeks of age as previously described [9]. Cells were maintained in DMEM (MC3T3-E1 and MLO-Y4 cells)

or α-MEM (BMSCs) supplemented with 10% FBS (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). BMSC differentiation was accomplished by treatment with 100 ng/ml BMP2 (R&D Systems, Minneapolis, Minnesota, USA) for seven days; RNA was isolated on day 0 and day seven for subsequent RT-PCR analyses.

Primary bone marrow macrophages (BMMs) were flushed from femurs and tibiae of C57Bl/6 mice. Red blood cells were lysed with RBC lysis buffer (150 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA, pH7.4). BMMs were spun down, resuspended in minimal essential media α (Thermo-Fisher Scientific) supplemented with 1% CMG 14-12 cultured supernatant containing M-CSF, and plated on 100 mm tissue culture plates overnight. The following day, the non-adherent population of cells was counted and plated for experiments. Cells were maintained in medium containing M-CSF for two more days, at which time media was replaced with medium containing 1% CMG 14-12 culture supernatant and 30 ng/mL RANKL (R&D Systems) to initiate osteoclast differentiation for up to three days before harvesting.

Gene expression analyses

For gene expression studies, total RNA was isolated from isolated cells or marrow-free humerii as previously described [13]. Reverse transcription was performed using the SuperScript III First-Strand Synthesis SuperMix (Thermo-Fisher Scientific); cDNA from primary murine osteoclasts was generated as described previously [15]. PCR was carried out with GoTaq (Promega, Madison, Wisconsin, USA) or OneTaq (New England Biolabs, Ipswich, Massaschusetts, USA) and the following primer pairs: *Tas1R1*, 5'-CTGCCAAAGGACAGAATCCTC-3' and 5'-GAACCGCATGGCTTGGAAG-3'; *Tas1R2*, 5'- AGCGTGTGGTCTACAGTGTG-3' and 5'-TCTCCCTGAGTAGCTGCCAT-3'; *Tas1R3*, 5'-TGGGGGCCTCTTTGTGTCT-3' and 5'-TGGGTTGTGTTCTCTGGTTGA-3'; *Ctsk*, 5'- AGCGAACAGATTCTCAACAGC-3' and 5'-AGACAGAGCAAAGCTCACCAT-3'; *Osterix/Sp,*, 5'-AGAGATCTGAGCTGGGTAGAGG-3' and 5'-AAGAGAGCCTGGCAAGAGG-3'; *Rankl/Tnfsf11*, 5'-AGCCATTTGCACACCTCAC-3' and 5'-CGTGGTACCAAGAGGACAGAGT-3'; *Trap/Acp5*, 5'-CGTCTCTGCACAGATTGCAT-3' and 5'-AAGCGCAAACGGTAGTAAGG-3'; *Hprt*, 5'-CCTGCTGGATTACATTAAAGCACTG-3' and 5'-

GTCAAGGGCATATCCAACAACAAAC-3'. Negative controls for PCR targets were accomplished using reactions lacking template DNA.

For quantitative real-time PCR experiments, RNA was harvested from BMSCs or osteoclasts using TRIzol Reagent (Thermo-Fisher Scientific) or RNEasy Kit (QIAGEN, Hilden, Germany). cDNA was prepared using the iScript cDNA Synthesis Kit (Biorad, Hercules, California, USA). Reactions were run using LightCycler 480 SYBR Green I Master (Roche, Basel, Switzerland) on the Roche LightCycler 480 using the primer pairs listed above.

Serum immunoassays

Serum samples were obtained as previously described [17] and levels of PINP, CTx, and TRAP were measured using the Mouse PINP ELISA Kit, Mouse CTX-1 ELISA Kit, and Mouse TRAP ELISA Kit by NeoScientific (Cambridge, Massachusetts, USA), respectively, according to the manufacturer's instructions.

Statistical considerations

Data were plotted in GraphPad Prism 6 and statistical tests completed as detailed in the figure or table legends; $p<0.05$ was considered significant.

Results

Increased cortical bone in Tas1R3 mutant mice

We first sought to corroborate the report of increased cortical bone mass in the femora of *Tas1R3* mutant mice. Similar to the report by Simon et al [15], μCT analyses performed on a small cohort of twenty-week-old male mice revealed a modest increase (+13%) in average thickness of the cortical bone at the femoral mid-diaphysis of *Tas1R3* mutant mice as compared to wild type littermates (Figure 1A-B). And, consistent with our previous results [9], RT-PCR analysis on RNA from marrow-free humeral diaphyses of nine-week-old wild type mice confirmed that

Tas1R3 is expressed in the postnatal bone micro-environment along with its putative signaling partners *Tas1R1* and *Tas1R2* (Figure 1C).

TAS1R3 in bone formation

We next sought to determine if osteoblast lineage cells express TAS1R3. These experiments revealed that *Tas1R3* transcript is present in undifferentiated primary murine bone marrow stromal cells (BMSCs) (Figure 2A) and its expression level is maintained during BMP2-induced differentiation of these cells (Figure 2B); increased expression of the osteoblast lineage marker *Osterix/Sp7* (Figure 2C) serves as a positive control for differentiation in this assay. Consistent with this result, *Tas1R3* transcript is present in the osteoblastic cell line MC3T3-E1 and the osteocyte-like cell line MLO-Y4 (Figure 2D); no attempt was made to quantitatively compare expression levels of *Tas1R3* between these cell lines or against primary BMSCs.

These findings raised the possibility that loss of TAS1R3 function in the osteoprogenitor lineage might underlie high bone mass in *Tas1R3* mutant mice. To test this idea, we examined osteoblast parameters by static histomorphometry and osteoblast function by quantifying serum levels of the bone formation marker Procollagen Type I N-terminal Propeptide (PINP). No differences were observed between control and *Tas1R3* mutant mice in these analyses (Table 1 and Figure 2E) suggesting that, although cells of the bone-forming lineage express TAS1R3, its absence does not lead to altered osteoblast differentiation or activity.

TAS1R3 in bone resorption

We next examined a possible role for TAS1R3 in bone resorption and found that serum levels of the bone resorption marker Collagen Type I C-telopeptide (CTx) are reduced by greater than 60% in *Tas1R3* mutant mice (Figure 3A). Consistent with this idea, *Tas1R3* is expressed in primary murine osteoclasts *in vitro* along with its putative signaling partner *Tas1R2* (Figure 3B); *Tas1R1* expression was not observed in these cells (data not shown). Additionally, increased expression of *Tas1R3* and *Tas1R2* correlate with the robust increase in expression of the

mature osteoclast marker *Cathepsin K (Ctsk)* observed during the differentiation period (Figure 3C-E). These data raised the possibility that defective osteoclast differentiation may underlie the reduction in bone resorption observed in *Tas1R3* mutant mice. However, static histomorphometric analyses indicate that osteoclast number is unchanged in the absence of TAS1R3 (Table 1), which is supported by the fact that skeletal expression levels of *Rankl/Tnfsf11* (Figure 3F) and *Trap/Acp5* (Figure 3G) and serum levels of TRAP (Figure 3H) are comparable between control and *Tas1R3* mutant mice. Collectively, our findings suggest that global absence of TAS1R3 function leads to uncoupled bone remodeling due to reduced osteoclast activity.

Discussion

Our study corroborates the finding of elevated cortical bone mass in *Tas1R3* mutant mice recently reported by Simon *et al.* [16] and addresses the open question regarding the cellular mechanism(s) underlying high bone mass in mice. Although *Tas1R3* expression has been previously observed in the skeleton [10], we are the first to detail the specific skeletal cell types that express TAS1R family members *in vitro*. In particular, *Tas1R3* and *Tas1R2* are expressed endogenously in murine osteoclast precursors, and their expression levels parallel the robust increase in the osteoclast marker *Ctsk* during osteoclast differentiation. In contrast, *Tas1R1* expression is undetectable in these assays, suggesting TAS1R3 functions in conjunction with TAS1R2 in osteoclasts. This idea is consistent with the fact that, similar to *Tas1R3* mutant mice, global loss of *Tas1R2* also leads to high bone mass through an undetermined mechanism [16].

The specific role that TAS1R2 and TAS1R3 play in osteoclast cells remains unknown. However, a requirement for TAS1R3 function in bone resorption *in vivo* is confirmed by the fact that *Tas1R3* mutant mice display a profound reduction in serum levels of the bone resorption marker CTx in comparison to wild type mice. In contrast, TAS1R family members are expressed in the osteoblast lineage *in vitro*, yet serum levels of the bone formation marker PINP are unchanged in *Tas1R3* mutant mice. While we are unable to comment on the specific molecule(s) mediating activation of TAS1R2 and TAS1R3 in the osteoclast lineage *in vivo*, glucose is one compelling possibility among the host of putative ligands [14]. For example, reducing glucose availability impairs osteoclast activity *in vitro* [8], and glycolytic inhibitors reduce bone resorption *in vivo* [12,6]. This presents the hypothesis that reduced glucose

sensing by osteoclasts in *Tas1R3* mutant mice leads to defective bone resorption. Additionally, prior work indicates that loss of TAS1R3 function is associated with decreased mammalian target of rapamycin complex 1 (mTORC1) activity and increased autophagy [17]. We must also acknowledge that the global nature of the *Tas1R3* mutation, coupled with the expression of this receptor in numerous extra-skeletal sites [11], leaves open the possibility that impairment of bone resorption in *Tas1R3* mutant mice is secondary to losing TAS1R3 function in another physiological context. Future studies involving cell type-dependent knockout of *Tas1R3* are required to differentiate between these possibilities, but these studies are not yet possible due to the lack of a conditional *Tas1R3* allele.

In conclusion, our findings provide novel insight into the role of TAS1R family members in skeletal biology and postnatal bone remodeling. These results and the stated limitations provide rationale for future experiments examining the cell type-dependent role for TAS1R family members in nutrient sensing by osteoclasts and/or osteoblasts in the postnatal skeleton.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JWL, BJD, and LP conceived of, designed, and supervised the study. MSE, NW, JBN, MP, HEF, JWA, SRS, AB, RS, EMW, JN, and HD generated the data and performed the statistical analyses. All authors interpreted the results, and JWL, BJD, NW, and TDW drafted the manuscript. All authors read and approved the final manuscript.

References

1. Allendorph GP, Vale WW, Choe S (2006) Structure of the ternary signaling complex of a TGF-beta superfamily member. Proc Natl Acad Sci U S A 103:7643-7648. doi:0602558103 [pii]

10.1073/pnas.0602558103
2. Berasi SP, Varadarajan U, Archambault J, Cain M, Souza TA, Abouzeid A, Li J, Brown CT, Dorner AJ, H JS, Jelinsky SA (2011) Divergent activities of osteogenic BMP2, and tenogenic BMP12 and BMP13 independent of receptor binding affinities. Growth Factors. doi:10.3109/08977194.2011.593178
3. Black DM, Bauer DC, Schwartz AV, Cummings SR, Rosen CJ (2012) Continuing Bisphosphonate Treatment for Osteoporosis - For Whom and for How Long? The New England journal of medicine. doi:10.1056/NEJMp1202623
4. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Muller R (2010) Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J Bone Miner Res 25:1468-1486. doi:10.1002/jbmr.141
5. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR, Parfitt AM (2013) Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 28:2-17. doi:10.1002/jbmr.1805
6. El Hajj Dib I, Gallet M, Mentaverri R, Sevenet N, Brazier M, Kamel S (2006) Imatinib mesylate (Gleevec) enhances mature osteoclast apoptosis and suppresses osteoclast bone resorbing activity. European journal of pharmacology 551:27-33. doi:10.1016/j.ejphar.2006.09.007
7. Heinecke K, Seher A, Schmitz W, Mueller TD, Sebald W, Nickel J (2009) Receptor oligomerization and beyond: a case study in bone morphogenetic proteins. BMC Biol 7:59. doi:10.1186/1741-7007-7-59
8. Indo Y, Takeshita S, Ishii KA, Hoshii T, Aburatani H, Hirao A, Ikeda K (2013) Metabolic regulation of osteoclast differentiation and function. J Bone Miner Res 28:2392-2399. doi:10.1002/jbmr.1976
9. Kokabu S, Gamer L, Cox K, Lowery J, Tsuji K, Raz R, Economides A, Katagiri T, Rosen V (2012) BMP3 suppresses osteoblast differentiation of bone marrow stromal cells via interaction with Acvr2b. Mol Endocrinol 26:87-94. doi:10.1210/me.2011-1168
10. Kokabu S, Lowery JW, Toyono T, Seta Y, Hitomi S, Sato T, Enoki Y, Okubo M, Fukushima Y, Yoda T (2015) Muscle regulatory factors regulate T1R3 taste receptor expression. Biochem Biophys Res Commun 468:568-573. doi:10.1016/j.bbrc.2015.10.142
11. Laffitte A, Neiers F, Briand L (2014) Functional roles of the sweet taste receptor in oral and extraoral tissues. Current opinion in clinical nutrition and metabolic care 17:379-385. doi:10.1097/MCO.0000000000000058
12. Li B, Yu S (2003) Genistein prevents bone resorption diseases by inhibiting bone resorption and stimulating bone formation. Biol Pharm Bull 26:780-786
13. Lowery JW, Intini G, Gamer L, Lotinun S, Salazar VS, Ote S, Cox K, Baron R, Rosen V (2015) Loss of BMPR2 leads to high bone mass due to increased osteoblast activity. J Cell Sci 128:1308-1315. doi:10.1242/jcs.156737
14. Morini G, Bassoli A, Temussi PA (2005) From small sweeteners to sweet proteins: anatomy of the binding sites of the human T1R2_T1R3 receptor. Journal of medicinal chemistry 48:5520-5529. doi:10.1021/jm0503345
15. Pacheco-Costa R, Hassan I, Reginato RD, Davis HM, Bruzzaniti A, Allen MR, Plotkin LI (2014) High bone mass in mice lacking Cx37 because of defective osteoclast differentiation. J Biol Chem 289:8508-8520. doi:10.1074/jbc.M113.529735
16. Simon BR, Learman BS, Parlee SD, Scheller EL, Mori H, Cawthorn WP, Ning X, Krishnan V, Ma YL, Tyrberg B, MacDougald OA (2014) Sweet taste receptor deficient mice have decreased adiposity and increased bone mass. PLoS One 9:e86454. doi:10.1371/journal.pone.0086454
17. Wauson EM, Zaganjor E, Lee AY, Guerra ML, Ghosh AB, Bookout AL, Chambers CP, Jivan A, McGlynn K, Hutchison MR, Deberardinis RJ, Cobb MH (2012) The G protein-coupled taste receptor T1R1/T1R3 regulates mTORC1 and autophagy. Mol Cell 47:851-862. doi:10.1016/j.molcel.2012.08.001
18. Whitaker M, Guo J, Kehoe T, Benson G (2012) Bisphosphonates for Osteoporosis - Where Do We Go from Here? The New England journal of medicine. doi:10.1056/NEJMp1202619
19. Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS (2003) The receptors for mammalian sweet and umami taste. Cell 115:255-266

Table 1. Histomorphometric analysis of femora obtained from twenty-week-old wild type and *Tas1R3* knock-out (KO) mice. Data are expressed as mean±SEM and *p* values generated by unpaired t tests. Ob.N: osteoblast number; B.Pm: bone perimeter; Ob.S: osteoblast surface; BS: bone surface; Oc.N: osteoclast number.

	Parameter	Wild Type	*Tas1R3* KO	*p* value
Trabecular	Osteoblast Number (Ob.N/B.Pm)	20.78±3.288	16.07±2.767	0.3344
	Osteoblast Surface (Ob.S/BS)	14.8±3.523	12.88±2.921	0.6956
	Osteoclast Number (Oc.N/B.Pm)	7.621±0.9471	8.358±1.009	0.6226
	Osteoclast Surface (Oc.S/BS)	16.03±2.427	15.88±2.127	0.9639
Cortical	Osteoclast Number (Oc.N/B.Pm)	5.230±1.540	6.219±1.863	0.7035
	Osteoclast Surface (Oc.S/BS)	0.09106±0.02639	0.1183±0.04087	0.6314

Figure Legends

Fig. 1: Evaluation of bone mass in *Tas1R3* mutant mice. **A-B:** A, Quantification of average thickness of the cortical bone (cortical thickness, C.Th) at the femoral mid-diaphysis of twenty-week-old male *Tas1R3* knock-out (KO) as compared to wild types; data are expressed as mean±SEM, n=3 for each genotype and * indicates $p<0.05$ by unpaired t test. B, Images of mid-diaphyseal bone for wild type and *Tas1R3* KO most representative of the genotype mean in A; scale bar is 200 μm. **C:** RT-PCR for TAS1R family members in nine-week-old marrow-free humeral diaphysis from wild type mouse; *Hprt* serves as loading control. Data are representative of five marrow-free humerii.

Fig. 2: TAS1R3 in bone formation. **A:** RT-PCR for *Tas1R3* in undifferentiated wild type primary murine bone marrow stromal cells (BMSC); *Hprt* serves as loading control. **B-C:** Quantification of *Tas1R3* (B) and *Osterix* (C) expression during BMP2-induced differentiation of BMSCs (data are expressed as mean±SEM from four separate isolation/differentiation experiments and * indicates $p<0.05$ by ratio paired t-test). **D:** RT-PCR for *Tas1R3* in osteoblastic MC3T3-E1 cells and osteocyte-like MLO-Y4 cells; *Hprt* serves as loading control. **E:** Quantification of Procollagen Type I N-terminal Propeptide (PINP) levels in serum from twenty-week-old male wild type and *Tas1R3* knock-out (KO) mice; data are expressed as mean±SEM. $n\geq5$ for each group. $p=0.7$ by unpaired t test.

Fig. 3: TAS1R3 in bone resorption. **A:** Quantification of Collagen Type I C-telopeptide (CTx) levels in serum from twenty-week-old male wild type and *Tas1R3* knock-out (KO) mice; data are expressed as mean±SEM. n=3 for each group. * indicates $p<0.04$ by unpaired t test.**B:** RT-PCR for TAS1R family members in differentiated primary murine osteoclasts (OCLs). *Hprt* serves as loading control. Data are representative of four independent differentiated OCL samples. **C-E:** Quantitative RT-PCR for *Tas1R3* (C) and *Tas1R2* (D) expression from non-adherent murine bone marrow cells undergoing osteoclast differentiation *in vitro*. *Cathepsin K (Ctsk)* serves as positive control for osteoclast differentiation (E). Data are expressed as mean±SEM fold change relative to time course day zero from five independent experiments. * indicates $p<0.02$ by ratio paired t test. **F-G:** Quantitative RT-PCR for *Rankl/Tnfsf11* (F) and *Trap/Acp5* (G) using RNA obtained from twenty-week-old humerii; data are expressed as mean±SEM. n=5

for each group. **H:** Quantification of TRAP levels in serum from twenty-week-old male mice; data are expressed as mean±SEM. n=8 for each group.