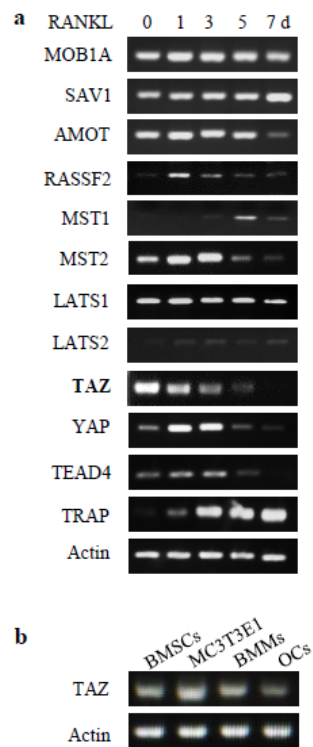
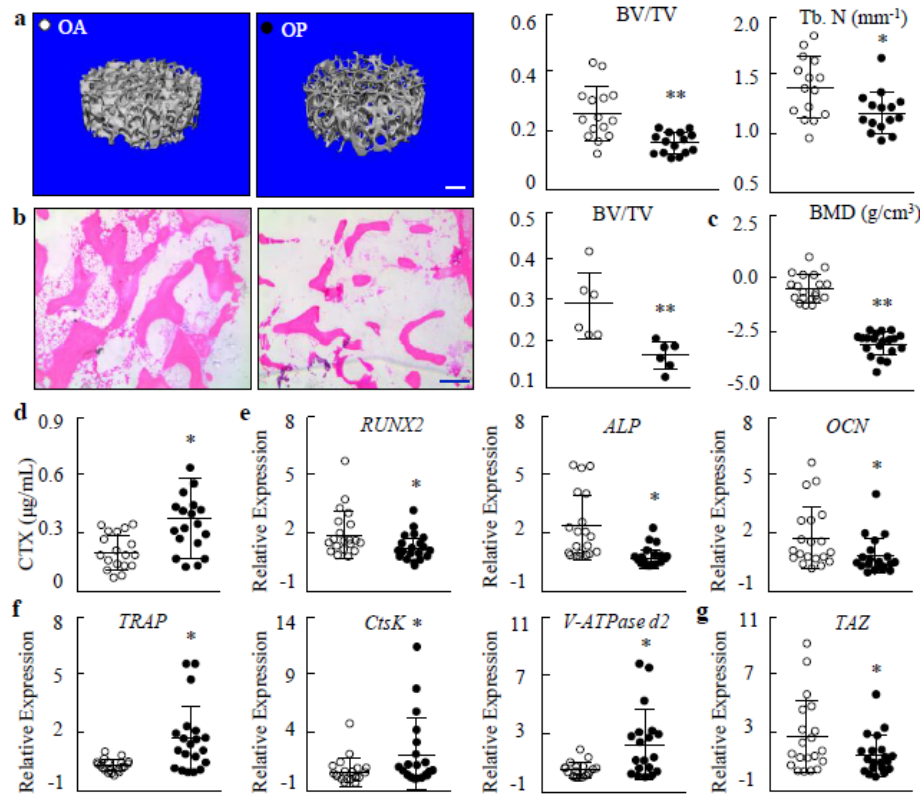


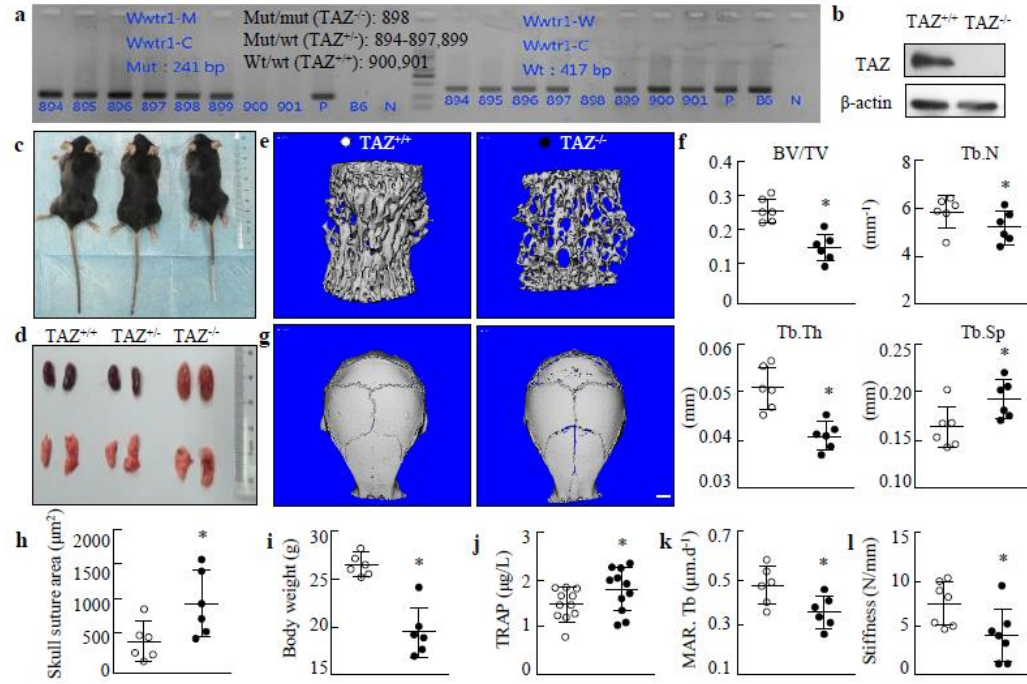
Supplementary Figures and figure legends



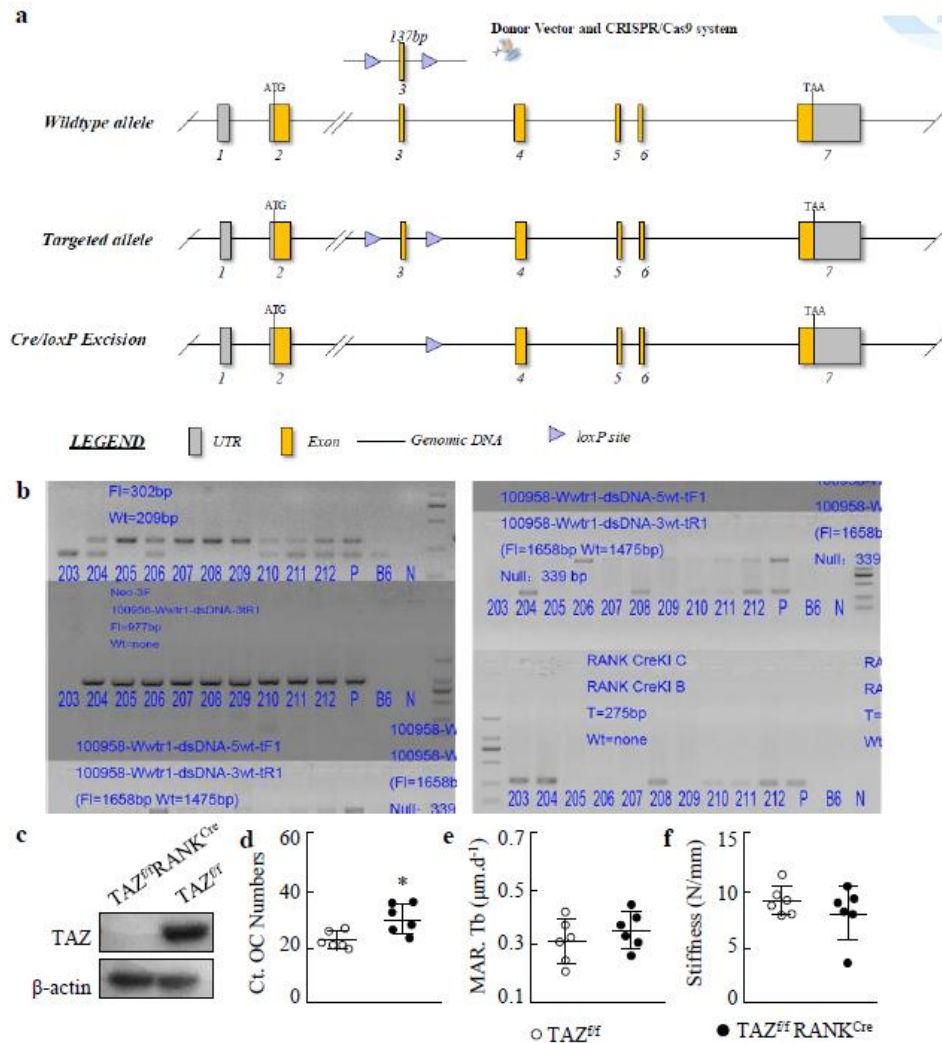
Supplementary Figure 1. (a) Semi-quantitative PCR gene expression profiling of key signaling molecules of the Hippo signaling pathway from BMM-derived osteoclasts stimulated with RANKL for 1, 3, 5, and 7 days. Unstimulated BMMs were used as basal mock control. **(b)** Semi-quantitative PCR gene expression profiling of TAZ from bone marrow stem cells (BMSCs), MC3T3E1 osteoblasts, BMMs and osteoclasts (OCs).



Supplementary Figure 2. Expression of TAZ is downregulated in human osteoporosis. **(a)** Three-dimensional micro-CT reconstructions and quantitative histomorphometric assessment of the femoral head from patients with osteoarthritis (OA, control) or osteoporosis (OP) ; n = 15. Scale bar denotes 1 mm. **(b)** H&E staining and quantitative histomorphometric assessment of femoral head tissue sections from OA and OP patients. Scale bar 1 μ m. (n = 6). **(c)** BMD of the OA and OP femurs by dual-energy x-ray absorptiometry scan. **(d)** ELISA assessment of serum CTX levels in the OA and OP patients. **(e)** and **(f)** Real-time qPCR analysis of the expression of osteoblast marker genes **(e)** and osteoclast marker genes **(f)** from the OA and OP femoral heads, respectively. **(g)** Real-time qPCR analysis of the expression of the TAZ gene from the OA and OP femoral heads. E-G, n = 20. $\bigcirc$ and $\bullet$ represents OA and OP group, respectively. Unpaired student's *t*-test was performed. **p* < 0.05, and ***p* < 0.01.

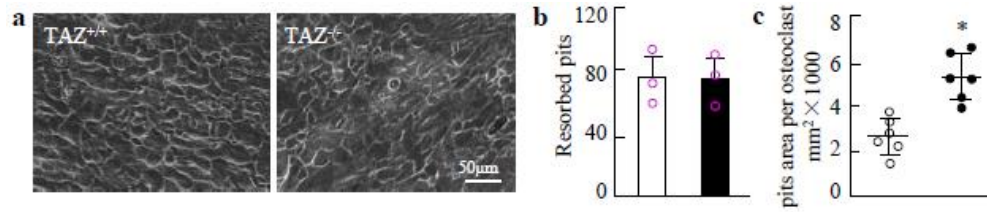


Supplementary Figure 3. TAZ global knockout exhibits osteoporotic phenotype. **(a)** Genotyping of *TAZ*^{+/+} WT, *TAZ*^{+/-} heterozygous, and *TAZ*^{-/-} KO mice. **(b)** Efficiency of TAZ knockout in *TAZ*^{-/-} BMMs. **(c)** Gross appearance of *TAZ*^{+/+}, *TAZ*^{+/-} and *TAZ*^{-/-} mice at 8 weeks of age. **(d)** Gross anatomical appearance of the kidneys and lungs from 8-week-old *TAZ*^{+/+}, *TAZ*^{+/-} and *TAZ*^{-/-} mice. **(e and g)** Representative 3D micro-CT reconstructions of (e) the second lumbar and (g) calvaria from 8-week-old *TAZ*^{+/+} and *TAZ*^{-/-} mice. Scale bar 100 μ m and 1mm respectively. **(f)** Quantitative morphometric measurements of second lumbar trabecular BV/TV, Tb.N, Tb.Th and Tb.Sp. **(h)** Quantitative morphometric measurement of skull suture area. **(i)** Body weight of 8-week-old *TAZ*^{+/+} and *TAZ*^{-/-} mice. **(j)** ELISA assessment of serum TRAP levels in *TAZ*^{+/+} and *TAZ*^{-/-} mice. $n = 6$. **(k)** MAR of trabecular bone by calcein-alizarin red double labeling. **(l)** Mechanical three-point bending tests of stiffness bearing of *TAZ*^{+/+} and *TAZ*^{-/-} tibial bone. $\bigcirc$ and $\bullet$ represents *TAZ*^{+/+} and *TAZ*^{-/-} group, respectively. Unpaired student's *t*-test was performed. $*p < 0.05$.

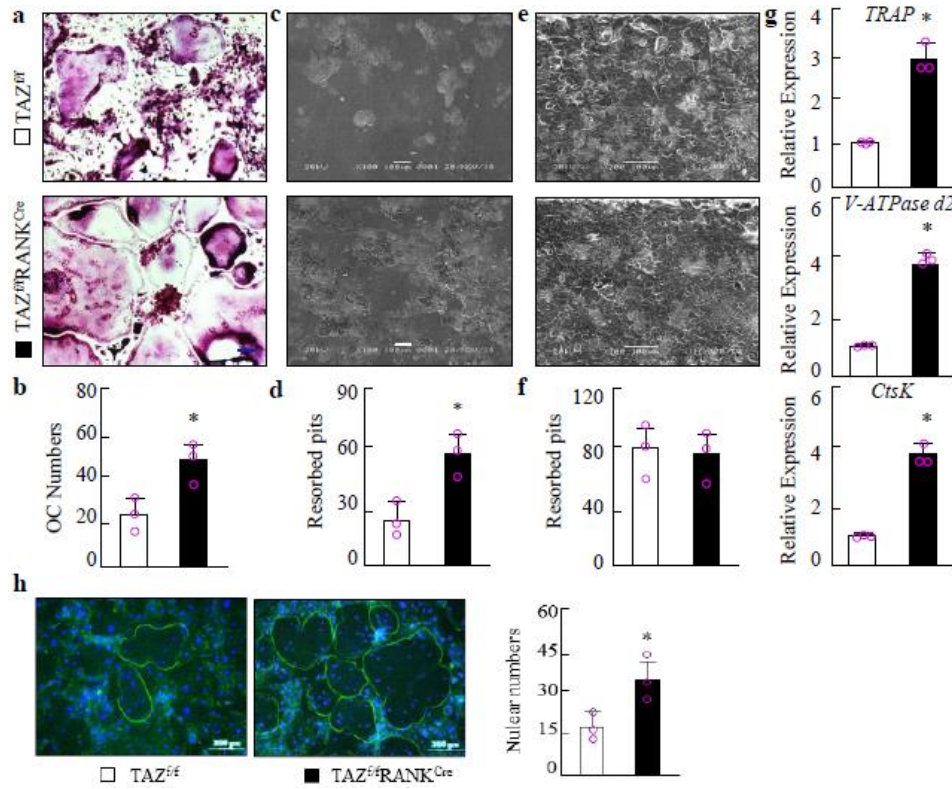


Supplementary Figure 4. Osteoclast-specific deletion of TAZ exhibits osteoporotic phenotype.

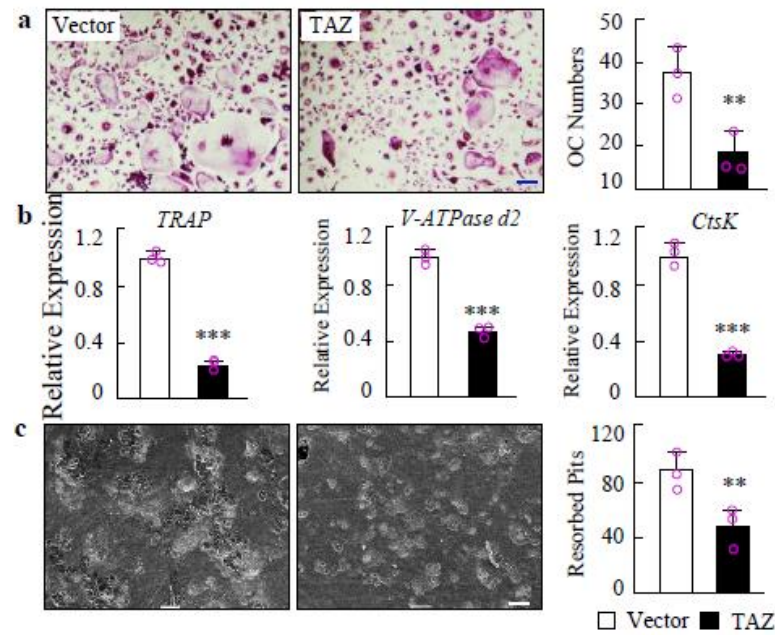
(a) CRISPR/Cas9 strategy for the generation of $TAZ^{f/f}$ mice required for use subsequently to generate the conditional knockout of TAZ in osteoclast. (b) Genotyping of $TAZ^{f/f}$ and $TAZ^{f/f}RANK^{Cre}$ mice, 205, 207 and 209 were genotyped as $TAZ^{f/f}$; 208 were genotyped as $TAZ^{f/f}RANK^{Cre}$. (c) Efficiency of TAZ knockout in $TAZ^{f/f}RANK^{Cre}$ BMMs. (d) osteoclast numbers of cortical bone in $TAZ^{f/f}$ and $TAZ^{f/f}RANK^{Cre}$. (e) MAR of trabecular bone by calcein-alizarin red double labeling. (f) Mechanical three-point bending tests of stiffness bearing of $TAZ^{f/f}$ and $TAZ^{f/f}RANK^{Cre}$ tibial bone. ○ and ● represents $TAZ^{f/f}$ and $TAZ^{f/f}RANK^{Cre}$ group, respectively. Unpaired student's t -test was performed. * $p < 0.05$.



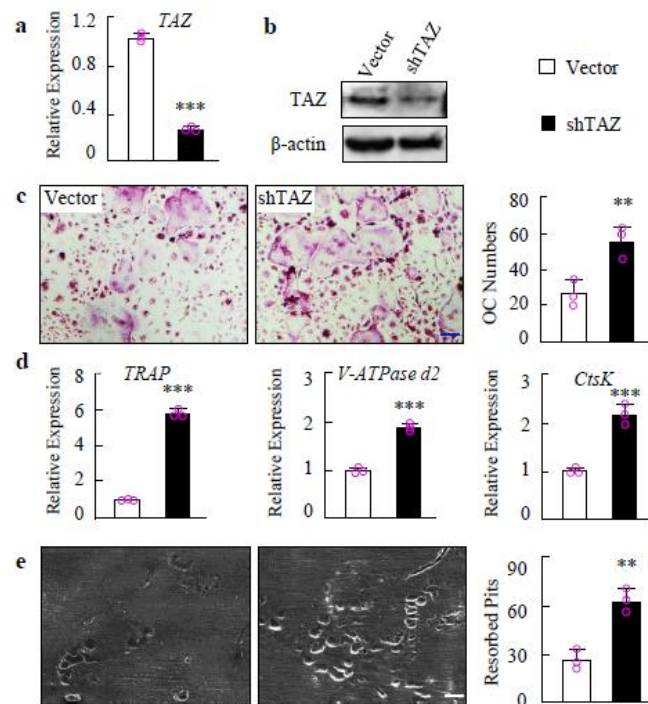
Supplementary Figure 5 Bone resorption for the equal numbers of mature $TAZ^{+/+}$ and $TAZ^{-/-}$ osteoclasts. (a) Scanning electron micrograph of bone resorption pits from osteoclasts derived from equal $TAZ^{+/+}$ and $TAZ^{-/-}$ osteoclasts stimulated with RANKL for 7 days. Scale bar 100 μm . (b) The numbers of bone resorption pits was quantified. (c) Average pit area of bone resorption. $\bigcirc$ and $\bullet$ represents $TAZ^{+/+}$ and $TAZ^{-/-}RANK^{Cre}$ group, respectively. Unpaired student's t -test was performed. $*p < 0.05$.



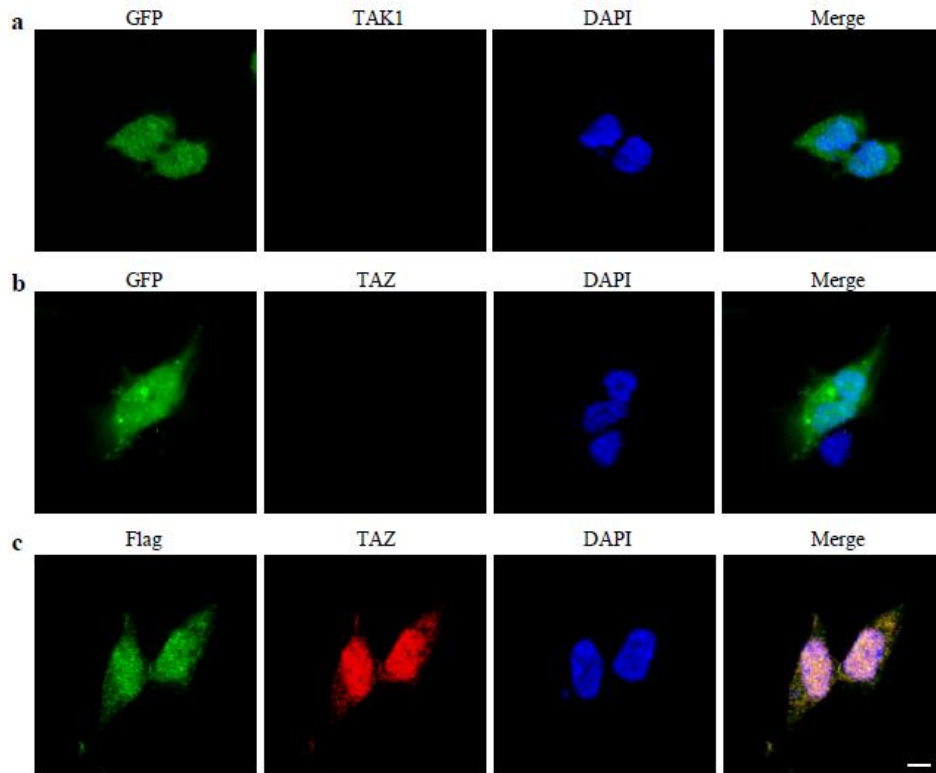
Supplementary Figure 6. Osteoclast-specific deletion of TAZ enhances RANKL-osteoclast formation and bone resorption. **(a)** TRAP stained images of osteoclasts derived from $TAZ^{fl/fl}$ and $TAZ^{fl/fl}RANK^{Cre}$ BMMs stimulated with RANKL for 7 days. Scale bar 10 μm . **(b)** The number of TRAP-positive multinucleated osteoclasts were quantified. **(c)** Scanning electron micrograph of bone resorption pits from osteoclasts derived from $TAZ^{fl/fl}$ and $TAZ^{fl/fl}RANK^{Cre}$ BMMs stimulated with RANKL for 8 days. Scale bar 100 μm . **(d)** The numbers of bone resorption pits was quantified. **(e)** Scanning electron micrograph of bone resorption pits from osteoclasts derived from equal $TAZ^{fl/fl}$ and $TAZ^{fl/fl}RANK^{Cre}$ osteoclasts stimulated with RANKL for 7 days. Scale bar 100 μm . **(f)** The numbers of bone resorption pits was quantified. **(g)** Real-time qPCR analysis of the expression of osteoclast marker genes *TRAP*, *CtsK*, and *V-ATPase d2* from $TAZ^{fl/fl}$ and $TAZ^{fl/fl}RANK^{Cre}$ BMMs stimulated with RANKL for 7 days. **(h)** F-actin/DAPI staining and statistic for the nuclear numbers per osteoclast $TAZ^{fl/fl}$ and $TAZ^{fl/fl}RANK^{Cre}$. $\square$ and $\blacksquare$ represents $TAZ^{fl/fl}$ and $TAZ^{fl/fl}RANK^{Cre}$ group, respectively. $n = 3$ for each group. One-way ANOVA followed by a post-hoc Tukey test were performed. ** $p < 0.01$, and *** $p < 0.001$.



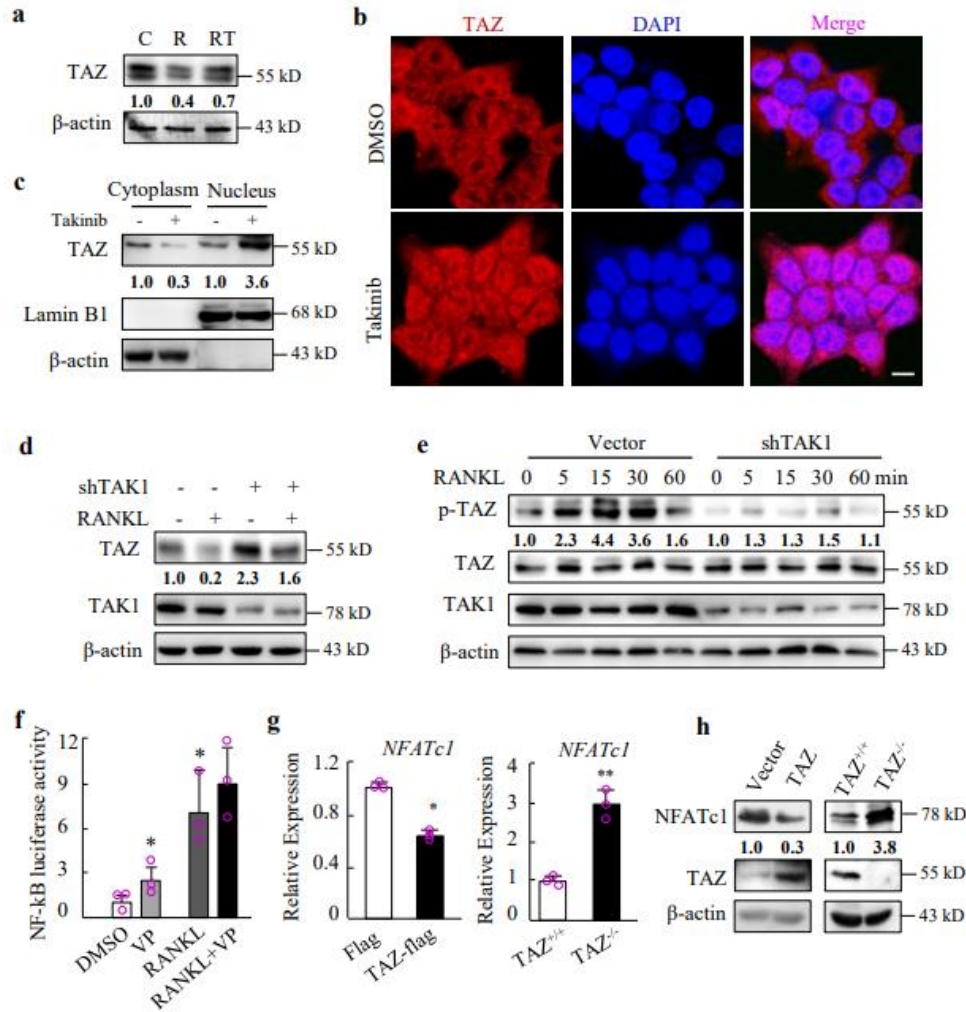
Supplementary Figure 7. Overexpression of TAZ suppressed RANKL-induced osteoclast differentiation and bone resorption. **(a)** TRAP stained and quantitative measurement of the number multinucleated osteoclasts derived from BMMs transduced with empty vector or TAZ overexpression lentiviral particles (TAZ) and then stimulated with 100 ng/mL RANKL for 7 days. Scale bar 10 μ m. **(b)** Real-time qPCR analysis of the expression of osteoclast marker genes. **(c)** Scanning electron micrographs and quantitative measurement of the numbers of bone resorption pits. Scale bar 50 μ m. $n = 3$ for each group. One-way ANOVA followed by a post-hoc Tukey test were performed. ** $p < 0.01$, and *** $p < 0.001$.



Supplementary Figure 8. Silence of TAZ enhanced RANKL-induced osteoclast differentiation and bone resorption. **(a)** silence efficiency of TAZ gene and **(b)** protein. **(c)** TRAP stained images and quantitative measurement of the number of multinucleated osteoclasts derived from BMMs transduced with control vector or TAZ silencing (shTAZ) lentiviral particles and then stimulated with RANKL for 7 days. Scale bar 10 μ m. **(d)** Real-time qPCR analysis of the expression of osteoclast marker genes. **(e)** Scanning electron micrographs and quantitative measurement of the numbers of bone resorption pits. Scale bar 50 μ m. One-way ANOVA followed by a post-hoc Tukey test were performed. ** $p < 0.01$, and *** $p < 0.001$.



Supplementary Figure 9. TAK1 and TAZ antibody test. **(a)** Overexpression of TAK1-GFP in HEK293T and staining with TAK1 primary antibody and red second antibody. **(b)** Overexpression of TAZ-GFP in HEK293T and staining with TAZ primary antibody and red second antibody. **(c)** Overexpression of TAZ-Flag in HEK293T and staining with Flag and TAZ primary antibody and green and red second antibody, respectively. Scale bar = 10 μ m.



Supplementary Figure 10. Reciprocal suppression between TAZ and the NF- κ B pathway regulates osteoclast differentiation. **(a)** Western blot analysis evaluating the effect of takinib on TAZ protein following RANKL stimulation for 48 h in BMMs. C, R, and RT represent control, RANKL, and RANKL + takinib, respectively. **(b)** TAZ immunofluorescence staining of HEK293T treated with DMSO or takinib. Scale bar = 10 μ m. **(c)** Western blot analysis of TAZ localization to nuclear or cytoplasmic fractions in BMMs following stimulation with takinib. β -actin was used as marker for cytoplasmic fraction and Lamin B1 as marker for nuclear fraction. **(d)** Western blot analysis evaluating the effect of TAK1 silence on TAZ protein following RANKL stimulation for 48 h in BMMs. **(e)** Western blot analysis evaluating the effect of TAK1 silence on TAZ phosphorylation during RANKL stimulation in BMMs. **(f)** Luciferase analysis of NF- κ B activity stimulated with verteporfin of TAZ inhibitor and RANKL in RAW264.7 for 6 h. **(g)** Real-time qPCR analysis of NFATc1 expression. **(h)** Western blot analysis of NFATc1 protein expression using total cellular proteins. One-way ANOVA was followed by post-hoc Tukey test. $**p < 0.01$.

Supplementary Table 1. Patient Information

	Osteoarthritis (OA)	Osteoporosis (OP)
Number	20	20
Age (Year)	62.48±1.79	62.53±1.86
BMI	25.74±2.76	22.06±2.59*
Basic Disease		
Hypertension	7	4
Chronic Type-B Hepatitis	3	1
Fatty liver	3	1
Hyperuricemia	3	1
Other diseases	Cataract (1), Cholecystolithiasis (1), Asthma (1), Hypohepatia (1)	Hepatic Cyst (3), Gallbladder Polyps (2),

Unpaired student's *t*-test was performed. * $p < 0.05$.

Supplementary Table 2. Patient Information 2

	Normal bone density (N)	Osteoporosis (OP)
Number	6	6
Age (Year)	65.43±4.58	64.45±6.06
BMI	22.68±2.49	24.02±2.40*
BMD	-0.22±0.88	-3.38±0.71**

Unpaired student's *t*-test was performed. * $p < 0.05$, ** $p < 0.01$.

Supplementary Table 3. TAZ global knockout breed number information

	Wildtype (TAZ ^{+/+})	Heterozygous (TAZ ^{+/-})	Knockout (TAZ ^{-/-})
Female	282	402	3
Male	198	286	18
Total	480	688	21
Embryo Genotype	17	31	16

Supplementary Table 4. Human Primer Pair Sequences

<i>Genes</i>	Forward (5'-3')	Reverse (5'-3')
<i>TAZ</i>	GGCTGGGAGATGACCTTCAC	CTGAGTGGGGTGGTTCTGCT
<i>TRAP</i>	GACTGTGCAGATCCTGGGTG	GGTCAGAGAATACGTCCTCAAAG
<i>CtsK</i>	TCCTTCCAGTTTTACAGCA	GTTTCCCCAGTTTTCTCC
<i>V-ATPase d2</i>	AAGGTGCGGAGCTGTACTTC	TCAGGTCTTCTAGGGTCTCACA
<i>RUNX2</i>	AGTTCCCAAGCATTTTCATC	GGCAGGTAGGTGTGGTAGT
<i>ALP</i>	CCCTTCACTGCCATCCTGTA	GCCTGGTAGTTGTTGTGAGC
<i>OCN</i>	CACACTCCTCGCCCTATT	GGTCTCTTCACTACCTCGCT
<i>β-actin</i>	AGCCATGTACGTAGCCATCC	CTCTCAGCAGTGGTGGTGAA

Supplementary Table 5. Mice Primer Pair Sequences

<i>Genes</i>	Forward (5'-3')	Reverse (5'-3')
<i>TAZ</i>	GGCCCTATCATTCACGGGAG	TTGACGGTCATGGGTGTCTG
<i>TRAP</i>	TCCTGGCTCAAAAAGCAGTT	ACATAGCCCACACCGTTCTC
<i>CtsK</i>	CTTCCAATACGTGCAGCAGA	TCTTCAGGGCTTTCTCGTTC
<i>V-ATPase ϵ</i>	AAGCCTTTGTTTGACGCTGT	TTCGATGCCTCTGTGAGATG
<i>NFATc1</i>	CAGCTGCCGTCGCACTCTGGTC	CCCGGCTGCCTTCCGTCTCAT
<i>ALP</i>	CTTCTTCTTGCTGGTGGA	AGGTGTCTTTCTGGGATGT
<i>OCN</i>	TTCAACGATCTGAGATTTGTGGG	GGATGAGGAATGCGCCCTA
<i>MOB1A</i>	GGGTTTATGCCCATATCTACCAC	CCAACTCACGCCTATCAATCAG
<i>SAV1</i>	ATGCTGTCCCGCAAGAAAAC	AATGAAGGCATGAGATTCCGC
<i>AMOT</i>	CCGCCAGAATACCCTTTCAAG	CTCATCAGTTGCCCCTCTGT
<i>RASSF2</i>	CCCTAATCCCATGCGGACAAG	GGAGTTGCAGGTTTGTGACCTTC
<i>MST1</i>	AGCCCTCACGTAGTCAAGTAT	TCTTGTTCCGTAGCCGAATGATA
<i>MST2</i>	GCAAAACGCAACACTGTAATAGG	AGCCCTCATCGGATGTATATCAG
<i>LATS1</i>	AATTTGGCACACATCATAAAGCC	ACGAGGGTCTTGGTAACTCATT
<i>LATS2</i>	GGACCCCAGGAATGAGCAG	CCCTCGTAGTTTGCACCACC
<i>YAP</i>	TGAGATCCCTGATGATGTACCAC	TGTTGTTGTCTGATCGTTGTGAT
<i>TEAD4</i>	GAGCGACTCGGCAGATAAGC	CCACACGGCGGATAGATAGC
<i>β-actin</i>	AGCCATGTACGTAGCCATCC	CTCTCAGCAGTGGTGGTGAA
<i>GAPDH</i>	AATGGATTTGGACGCATTGGT	TTTGCACTGGTACGTGTTGAT

Supplementary Table 6. Quantitative statistical analysis for western blot

Figure	Mean $\pm$ SD											
F1e	1.00 $\pm$ 0.00	0.86 $\pm$ 0.03	0.83 $\pm$ 0.05	0.84 $\pm$ 0.04	0.79 $\pm$ 0.05	0.67 $\pm$ 0.04	0.67 $\pm$ 0.03	0.56 $\pm$ 0.04	0.54 $\pm$ 0.02	0.44 $\pm$ 0.03	0.19 $\pm$ 0.01	0.25 $\pm$ 0.03
F1f	1.00 $\pm$ 0.00	0.96 $\pm$ 0.03	0.83 $\pm$ 0.05	0.98 $\pm$ 0.06	0.89 $\pm$ 0.06	0.64 $\pm$ 0.01	0.71 $\pm$ 0.03	0.44 $\pm$ 0.01	0.51 $\pm$ 0.02	0.42 $\pm$ 0.00	0.28 $\pm$ 0.01	0.22 $\pm$ 0.02
F5c	1.00 $\pm$ 0.00	1.20 $\pm$ 0.09*	2.92 $\pm$ 0.28*	2.41 $\pm$ 0.22*	1.66 $\pm$ 0.50							
F5d	1.00 $\pm$ 0.00	0.54 $\pm$ 0.08*	0.45 $\pm$ 0.05*	0.24 $\pm$ 0.13*	0.23 $\pm$ 0.09*							
F5e	1.00 $\pm$ 0.00	0.32 $\pm$ 0.14*	0.22 $\pm$ 0.06*									
F6a	1.00 $\pm$ 0.00	2.01 $\pm$ 0.37*	3.82 $\pm$ 0.05*	2.19 $\pm$ 0.37*	1.62 $\pm$ 0.45	1.00 $\pm$ 0.31	1.22 $\pm$ 0.23	1.53 $\pm$ 0.24	1.38 $\pm$ 0.43	1.26 $\pm$ 0.30		
F6b	1.00 $\pm$ 0.00	0.52 $\pm$ 0.14*	2.19 $\pm$ 1.59	2.10 $\pm$ 1.53	1.16 $\pm$ 0.02	1.04 $\pm$ 0.24	1.45 $\pm$ 1.15	1.94 $\pm$ 2.10				
F6d	p-TAK1	1.00 $\pm$ 0.00	4.96 $\pm$ 0.31*	1.59 $\pm$ 0.81								
	TAZ	1.00 $\pm$ 0.00	0.95 $\pm$ 0.15	2.63 $\pm$ 0.49*								
	I- κ B α	1.00 $\pm$ 0.00	0.23 $\pm$ 0.10*	0.6 $\pm$ 0.09*								
	p-p65	1.00 $\pm$ 0.00	4.34 $\pm$ 1.58*	1.07 $\pm$ 0.16								
SF10b	cytoplasm	1.00 $\pm$ 0.00	0.34 $\pm$ 0.02*	nucleus	1.00 $\pm$ 0.00	3.59 $\pm$ 1.32*						
SF10c		1.00 $\pm$ 0.00	0.42 $\pm$ 0.01*	0.73 $\pm$ 0.09*								
SF10d		1.00 $\pm$ 0.00	0.24 $\pm$ 0.05*	2.26 $\pm$ 0.14	1.58 $\pm$ 0.24*							
SF10e		1.00 $\pm$ 0.00	2.34 $\pm$ 0.52*	4.49 $\pm$ 1.19*	3.59 $\pm$ 1.04*	1.64 $\pm$ 0.49	1.04 $\pm$ 0.13	1.37 $\pm$ 0.54				
SF10h		1.00 $\pm$ 0.00	0.28 $\pm$ 0.08*	1.00 $\pm$ 0.00	3.80 $\pm$ 0.37*							

One or two-way ANOVA followed by a post-hoc Tukey test were performed. * $p < 0.05$.