

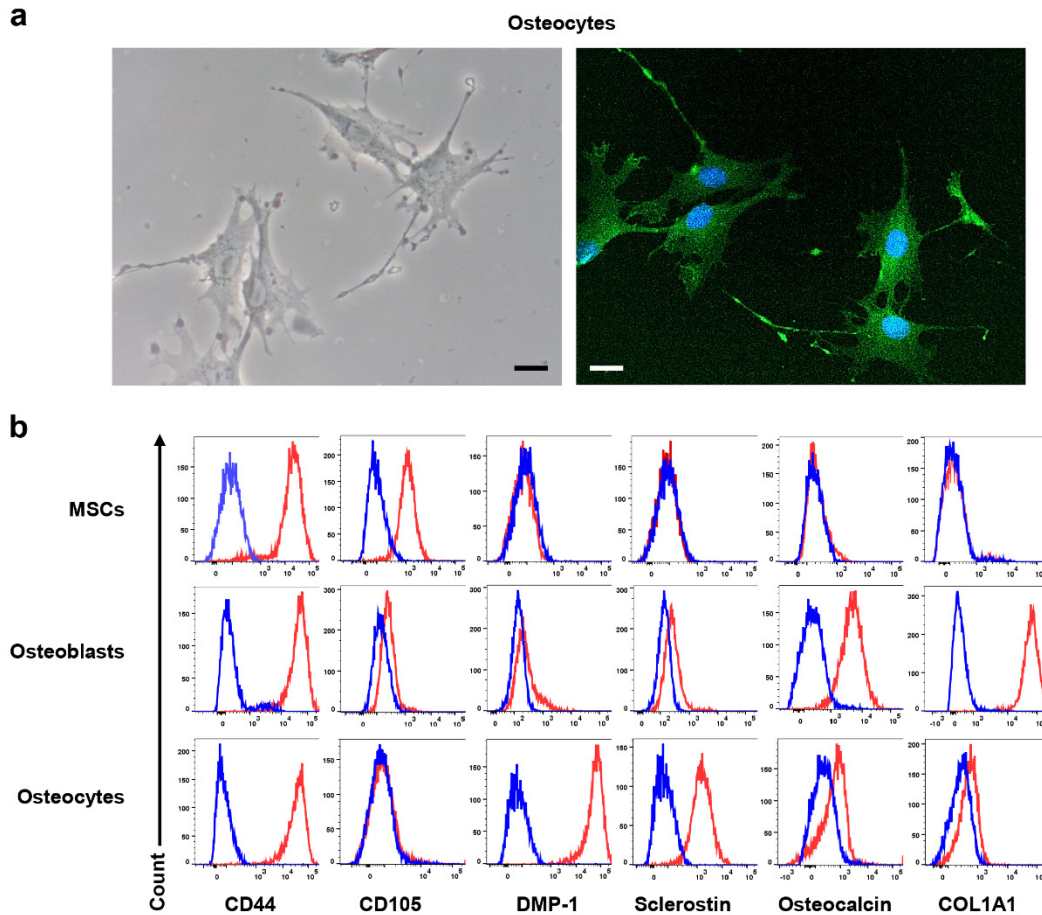
SUPPLEMENTAL INFORMATION

Osteocyte CIITA Aggravates Osteolytic Bone Lesions in Myeloma

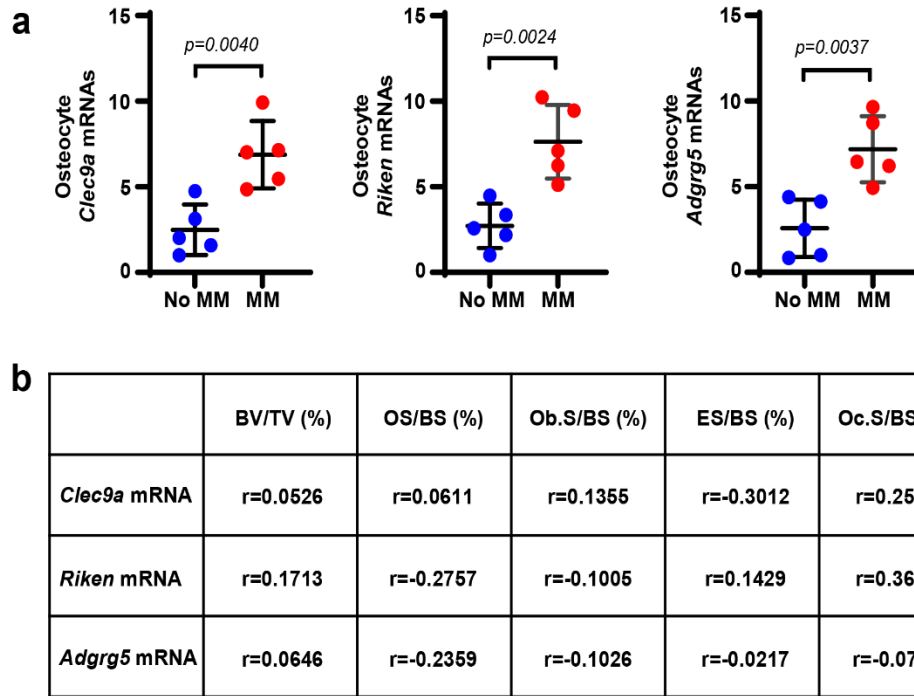
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Supplementary Figures

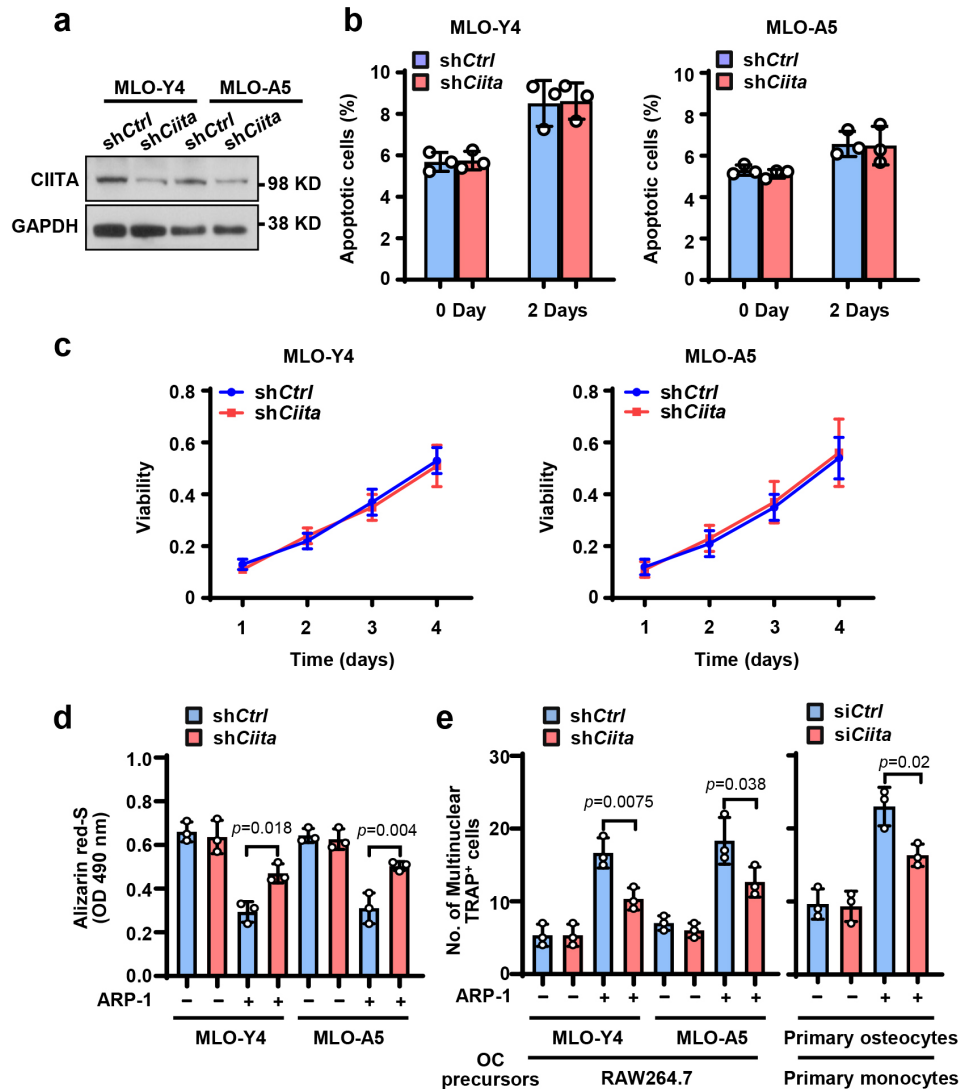
Supplementary Tables



Supplementary Fig. 1. Characterization of osteocytes. Cells were isolated from the femurs of C57BL/6 mice. **a** Representative images show the morphology of primary osteocytes after 7 days of culturing under light microscopy (left) or staining for E11/GP38 under fluorescent microscope (right). Scale bar: 20 μ m. Images shown are representative of three independent experiments. **b** Identification of MSCs, osteoblasts, and osteocytes using flow cytometry. Shown are representative histograms of the surface markers for MSCs (CD44 and CD105), osteocytes (DMP-1 and sclerostin), and osteoblasts (osteocalcin and COL1A1). Blue lines indicate isotype controls. Data shown are representative of two independent experiments.

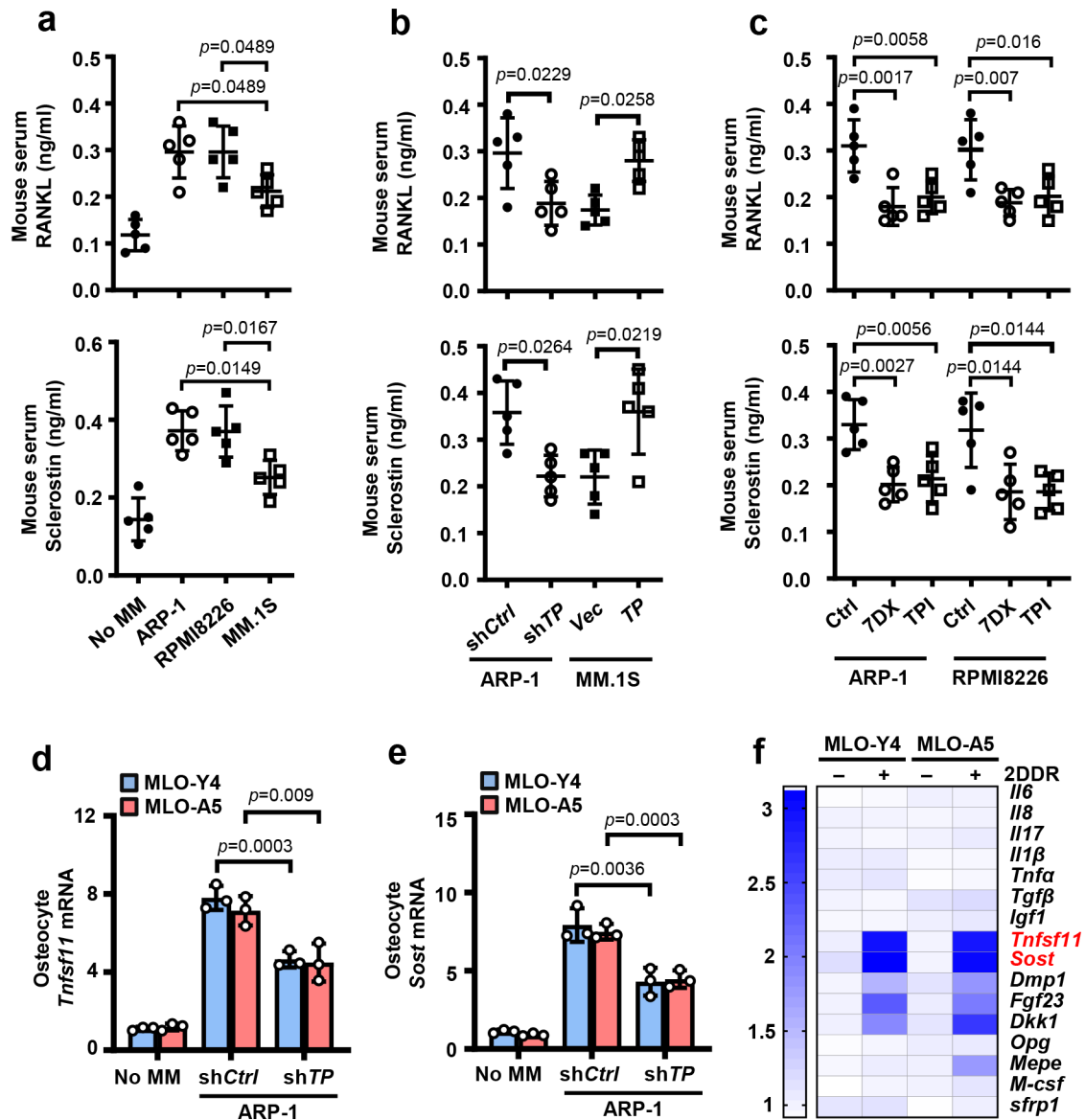


Supplementary Fig. 2. The correlation between the expression of candidate genes expressed in osteocytes and the status of bone lesions in myeloma-bearing mice. **a** qPCR analysis shows the levels of *Clec9a*, *Riken*, and *Adgrg5* mRNAs in the osteocytes isolated from myeloma-bearing or control mice. The data are mean \pm SD, n=5 mice/group. *P* value was determined using unpaired two-tailed *t*-test. **b** Correlation coefficients between the levels of *Clec9a*, *Riken*, or *Adgrg5* mRNAs in osteocytes and the percentages of BV/TV, OS/BS, Ob.S/BS, ES/BS, and Oc.S/BS in myeloma-bearing mice ($n = 10$) and control mice ($n = 10$). The correlations were evaluated using the Pearson coefficient with two-tailed *P* value. *r*, correlation coefficient.



Supplementary Fig. 3. Knockdown of CIITA in osteocytes reduces myeloma-induced enhancement of osteoclastogenesis and inhibition of osteoblastogenesis. **a** Western blots show CIITA expression in MLO-Y4 or MLO-A5 osteocytes transfected with non-targeted shRNA (*shCtrl*) or *Ciita* shRNA (*shCiita*). GAPDH levels served as protein loading controls. Data shown are representative of two independent experiments. **b-c** Percentages of apoptosis in 0- or 48-h cultures (**b**) and the viability of *shCtrl* or *shCiita* MLO-Y4 or MLO-A5 cells in 4-day cultures (**c**). Data shown are mean \pm SD, $n=3$ biological replicates. **d-e** The *shCtrl* or *shCiita* MLO-Y4 or MLO-A5 cells or *siCtrl* or *siCiita* primary osteocytes were co-cultured with or

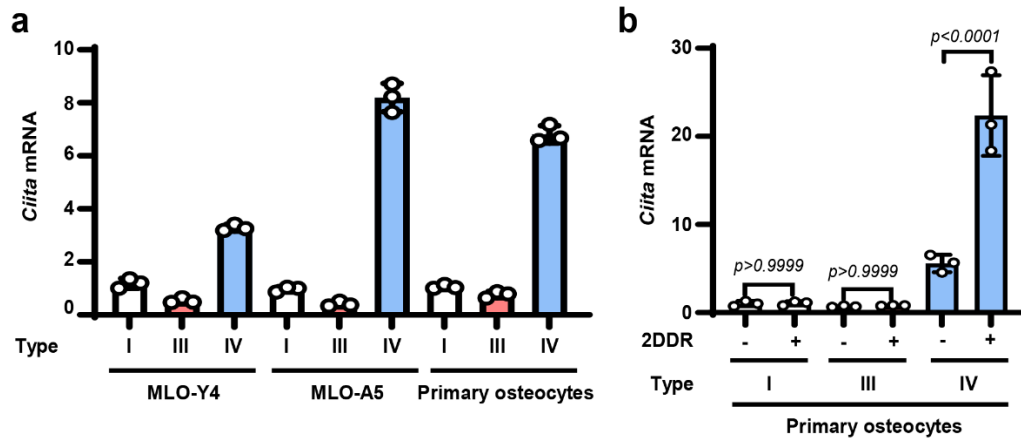
without myeloma ARP-1 cells for 2 days, and then provided with fresh medium for another 2 days for collecting osteocyte CM. The osteoblast precursor MC3T3-E1 and the osteoclast precursor Raw264.7 or primary monocytes were cultured with the osteocyte CM. After culturing, the cells were subjected to Alizarin red-S staining for osteoblast differentiation or to TRAP staining for osteoclast differentiation. Shown are summarized data from Alizarin red-S staining (**d**) and numbers of multinuclear (≥ 3) TRAP⁺ cells (**e**). Data are mean \pm SD, n = 3 biological replicates. *P* values were determined using one-way ANOVA with Tukey's multiple comparisons test.



Supplementary Fig. 4. Myeloma TP/2DDR increases RANKL and SOST expression in

osteocytes. **a** The high TP-expressing human myeloma cell lines ARP-1 and RPMI8226 and the low TP-expressing human myeloma cell line MM.1S (5×10^5 cell/mouse) were injected into the femurs of SCID mice. **b** Shown are the levels of RANKL (top) and sclerostin (bottom) in the serum of SCID mice injected with ARP-1 cells expressing non-targeted control shRNAs (*shCtrl*) or TP shRNAs (*shTP*) or with MM.1S cells expressing empty control vector (*Vec*) or TP cDNAs

(*TP*). **c** ARP-1 or RPMI8226 cells were injected into the femurs of SCID mice. After 3 weeks, mice were treated with PBS as vehicle controls (Ctrl) or the TP inhibitor 7DX (200 $\mu\text{g}/\text{kg}$) or TPI (300 $\mu\text{g}/\text{kg}$). After treatment, mouse sera were collected for ELISA analysis. Shown are the levels of RANKL (top) or sclerostin (bottom) in mouse serum. Data are mean \pm SD, n = 5 mice/group. **d-e** Osteocyte cell lines MLO-Y4 and MLO-A5 were co-cultured with sh*Ctrl* or sh*TP* ARP-1 cells for 24 h. qPCR analysis showed the relative expression of *Tnfsf11* (**d**) or *Sost* (**e**) in osteocytes. Data are mean \pm SD, n = 3 biological replicates. **f** MLO-Y4 or MLO-A5 osteocytes were cultured with 1 mM 2-deoxy-D-ribose (2DDR) for 24 h. Quantitative PCR analysis showed the relative expression of cytokine genes in osteocytes. *P* values were determined using one-way ANOVA with Tukey's multiple comparisons test.



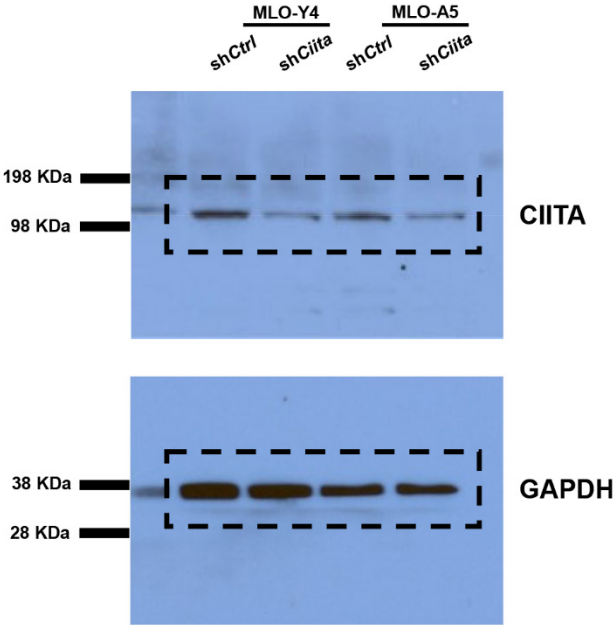
Supplementary Fig. 5. 2DDR enhances the expression of *Ciita* type IV in osteocytes. a

Quantitative PCR shows the relative expression of various isoforms of *Ciita* in MLO-Y4, MLO-A5, and primary murine osteocytes. Data are mean \pm SD, n = 3 biological replicates. **b**

Quantitative PCR shows the relative expression of *Ciita* type IV in primary murine osteocytes that were cultured with or without 1 mM 2DDR for 2 days. Data are mean \pm SD, n = 3 biological replicates. *P* values were determined using unpaired two-tailed *t*-test.

Uncropped Figures

Supplementary Fig. 3a



Supplementary Tables

table S1. Identification of AP2 α that binds to the CIITA in osteocytes using mass spectrometry.

Gene name	Peptide
<i>CIITA</i>	K.LAWELGR.R
	K.FTIEPFK.A
	K.LLQAAEEK.F
	K.GLVQHPPR.A
	R.GLLAGLFQK.K
	K.SYWAGAVSR.A
	K.SLKDVEDLGK.L
	R.ELATPDWAER.Q
	R.FLAGLIFQPPAR.C
	K.WPEPVEQFYR.S
	K.FTAAGAQQLAASLR.R
	K.RPFPEELPADLK.H
	R.SSSEDTAGELPAVR.D

R.AALDSPPGALAEAK.L

AP2 α

K.VTVAEVQR.R

K.IGLNLPAGR.R

K.AVAEFLNR.Q

R.LSLLSSTSK.Y

K.EFTDLLAQDR.S

R.QHSDPNEQVTR.K

Mass spectrum analysis of the upper band.

Protein	Unique peptide	Total peptides	% peptide ratio
CIITA	31	55	56.36
KRT1	16	21	76.19
KRT2	15	18	83.33
LRPPRC	9	10	90.00
DHX9	6	6	100.00
SMC2	8	8	100.00

IARS	7	7	100.00
CAND1	5	5	100.00

Mass spectrum analysis of the lower band.

Protein	Unique peptide	Total peptides	% peptide ratio
ACTB	11	20	55.00
TFAP2A	8	9	88.89
GOT2	6	6	100.00
LRPAP1	2	2	100.00

table S2. Primers for real-time reverse transcription PCR analysis.

Gene	Forward	Reverse
<i>Gapdh</i>	CCTCCTGCAGACAGACGTAA	AGCATCGACCAGTGCTACAG
<i>Ciita</i>	TGGGATCTTCCAGCGGAAGC	ACAACAGGGCTGTGACTATAGC
<i>Alp</i>	CCAACTCTTTTGTGCCAGAGA	GGCTACATTGGTGTTGAGCTTTT
<i>Coll1a1</i>	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
<i>Bglap</i>	CTCTGACCTCACAGATGCCA	TTATTGCCCTCCTGCTTGGA
<i>Trap</i>	CACTCCCACCCTGAGATTTGT	CATCGTCTGCACGGTTCTG
<i>Calcr</i>	GAGGTTCTTCTCGTGAACAG	AGTCAGTGAGATTGGTAGGAGC
<i>Ctsk</i>	GAAGAAGACTCACCAGAAGCAG	TCCAGGTTATGGGCAGAGATT
<i>Il6</i>	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>Il8</i>	CAAGGCTGGTCCATGCTCC	TGCTATCACTTCCTTTCTGTTGC
<i>Il17</i>	TTTAACTCCCTTGGCGCAAAA	CTTCCCTCCGCATTGACAC
<i>Il1β</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
<i>Tnfa</i>	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
<i>Tgfβ</i>	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
<i>Igf1</i>	CTGGACCAGAGACCCTTTGC	GGACGGGGACTTCTGAGTCTT
<i>Tnfsf11</i>	CAGCATCGCTCTGTTCCCTGTA	CTGCGTTTTTCATGGAGTCTCA
<i>Sost</i>	AGCCTTCAGGAATGATGCCAC	CTTTGGCGTCATAGGGATGGT
<i>Dmp1</i>	CATTCTCCTTGTGTTCCCTTGGG	TGTGGTCACTATTTGCCTGTG
<i>Fgf23</i>	ATGCTAGGGACCTGCCTTAGA	AGCCAAGCAATGGGGAAGTG
<i>Dkk1</i>	CTCATCAATTCCAACGCGATCA	GCCCTCATAGAGAACTCCCG
<i>Opg</i>	ACCCAGAAACTGGTCATCAGC	CTGCAATACACACTCATCACT
<i>Mepe</i>	GTCTGTTGGACTGCTCCTCTT	CACCGTGGGATCAGGATACA

<i>M-csf</i>	ATGAGCAGGAGTATTGCCAAGG	TCCATTCCCAATCATGTGGCTA
<i>Sfrp1</i>	TCTAAGCCCCAAGGTACAACC	GCTTGCACAGAGATGTTCAATG
<i>Clec9a</i>	GAAGTGCCAATCCCCTAGCAA	CAGTCACTACCTGAATGGAGAGA
<i>Riken</i>	CTCCAGTTGCTGTGGGAATG	CTGAATAGCCAGCCAAGCAC
<i>Adgrg5</i>	GATTCCACCACACGTATCCAC	CAGTGCGTAGTGTAGGACAGC
<i>Ciita</i> <i>Type I</i>	CAGGGACCATGGAGACCATAGT	CAGGTAGCTGCCCTCTGGAG
<i>Ciita</i> <i>Type III</i>	GGTTCCTGGCCCTTCTGG	ATCCATGGTGGCACACAGACT
<i>Ciita</i> <i>Type IV</i>	CAGCACTCAGAAGCACGGG	ATCATGGTGGCACACAGACT
<i>TP</i>	ACAGGAGGCACCTTGGATAA	GCTCACTCTGACCCACGATA
<i>RANKL</i>	ATCGTTGGATCACAGCACAT	TTATGGGAACCAGATGGGAT
<i>SOST</i>	ACACAGCCTTCCGTGTAGTG	GGTTCATGGTCTTGTTGTTCTCC
<i>GAPDH</i>	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG

table S3. Primers for constructing vector constructs in luciferase assay.

Name	Forward	Reverse
<i>Luc-Tnfsf11</i>	CATCTCGAG GGAGGCAGAGAGGCAGAGACA	CAGAAGCTT CTCCCTTCCTCGGCCACCCA
<i>Luc-Tnfsf11-Δ1</i>	CATCTCGAG GCCTTCACCAGCTGAGCCGTC	CAGAAGCTT CTCCCTTCCTCGGCCACCCA
<i>Luc-Tnfsf11-mut</i>	CAGAAGAATCCTCATCCTTT TTATCCATCATGTGCGCAAG CACGCACAC	GTGTGCGTGCTTGCGCACAT GATGGATAAAAAGGATGAGG ATTCTTCTG
<i>Luc-Sost</i>	CATCTCGAG AGGCGGACCACAGCCTCCTT	CAGAAGCTT CCCTCTGCCTCATGCCAGCC
<i>Luc-Sost-Δ1</i>	CATCTCGAG GCCTGGTGGTTGCCTCTCCC	CAGAAGCTT CCCTCTGCCTCATGCCAGCC
<i>Luc-Sost-Δ2</i>	CATCTCGAG TGCCCGTGGGAGGTAAGTGGG	CAGAAGCTT CCCTCTGCCTCATGCCAGCC
<i>Luc-Sost-mut1</i>	GGCCACAGTTTGTGTTTGGAG AATGATTGATAGGCCAGGG AGCCAG	CTGGCTCCCTGGGCCTATCAA TCATTCTCAAACAAACTGTG GGCC
<i>Luc-Sost-mut2</i>	CTTGGCTTTTAATTGTCTGG CTTACTGATCCCCTCAGGCA TTCTCAAACA	TGTTTTGAGAATGCCTGAGG GGATCAGTAAGCCAGACAAT TAAAAGCCAAG

table S4. Primers for ChIP-PCR.

Genes	Forward	Reverse
<i>Irf1</i>	TGCAGAAAGAGGGGGACGGT	GCGGCGAAGGGGAAGTACAA
<i>Ciita</i>	GCTGGAGCTCACCATGTCCC	TGGGCCAAATTGGGTGACCA
<i>Tnfrsf11</i>	CCAACCACTGGACCCAACCC	AGATGGCAGGGTACCCCAGG
<i>Sost</i>	AAACCACAGCCTGACTGCC	GTGTGCTTAGCGTCAGCCCT