Supplementary Materials and Methods

Tartrate-resistant acid phosphatase staining

The osteoclasts in tissue samples were detected by Tartrate-Resistant Acid Phosphatase (TRAP) staining. The bone tumors were harvested and decalcified in 10% ethylenediaminetetraacetic acid (EDTA)/ PBS for 14 days. The decalcifying solution was changed every other day. The decalcified bone tissues were processed, paraffin embedded, and cut into sections. The bone tissue sections were deparaffinized and stained with a TRAP staining kit purchased from Sigma (St. Louis, MO) according to the manufacturer's instructions, followed by Orange G staining.

Chromatin immunoprecipitation (ChIP) assay

ChIP was performed by a ChIP-IT kit (Active Motif, Inc., Carlsbad, CA). LN^{Neo} and LN^{RANKL} cells were serum-starved overnight and treated with 200 μg/ml of RANKL, 200 μg/ml of RANKL plus 200 μg/ml of OPG, for LN^{Neo}cells or 1 μg/ml of OPG for LN^{RANKL} cells for an additional 48h. The cells were formaldehyde-fixed and sheared into 200-1500 bp of DNA fragments by enzymatic shearing mixture for 10 min at 37 °C. A portion of the cross-linked protein-DNA complexes was reserved as input DNA, and the rest of the protein-DNA complexes were subjected to immunoprecipitation with anti-cMyc antibody (Cell Signaling Technology, Danvers, MA) and anti-rabbit IgG as a negative control. The precipitated DNA was extracted and purified for both semi-quantitative and quantitative real-time PCR analyses. The PCR primers 5'-CCTGGTCACTCTTCCTCAAC-3' (forward) and 5'-CTCTAGGCAAGATTTGTGAC-3' (reverse) for RANKL promoter and 5'-GCCATGCCGTATCAGGAAATC-3' (forward) and 5'-CTATGTGACTCTCCTGGGAAC-3'(reverse) for c-Met promoter were used to amplify cMyc DNA from the input and anti-cMyc-

and anti-rabbit IgG-precipitated DNA. The amplified PCR products were detected and analyzed on a 3% agarose gel. Quantitative real-time PCR was also conducted to further quantify the PCR products from the ChIP analysis. The purified DNA was mixed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and the cMyc primer pair and amplified using the ABI 7500 Fast detection system (Applied Biosystems). The relative expression of each sample was then normalized with that of the input to get the fold change.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts of LNCaP, RANKL-treated LNCaP, $LN^{Neo}$and LN^{RANKL}cells were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, Waltham, MA). EMSA was performed using the non-invasive LightShift Chemiluminescent EMSA kit according to the manufacturer's instructions. The double-stranded oligonucleotide of the cMyc/Max binding motif (underlined) within the RANKL promoter is 5'-CAATTTAGAACACATGCTTTAATAAC-3', which was used for biotin-labeled and unlabeled probes (and later was used as a competitor at 400x) in the binding reactions. For the supershift assay, 2 µg of thecMyc (sc-764X) and Max (sc-197X) antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were pre-mixed with the nuclear extracts for 30 min on ice before adding the binding reaction mixture, further incubated at RT for 20 min. The samples were subjected to electrophoresis on a 5% TBE gel in 0.5x TBE buffer for 1h and transferred onto a nylon membrane in 0.5x TBE buffer for 1h. The nylon membrane was UV cross-liked at 120 mJ/cm^2 for 1 min, blocked, and incubated with streptavidin-HRP solution at RT for 15 min. The membrane was washed for four times for 5 min each before adding the substrate. The membrane was then exposed to X-ray film to detect the biotin-labeled DNA by chemiluminescence.

Semi-quantitative PCR

One μl of cDNA was subjected to PCR analyses, which involves an initial denaturation at 94°C for 10 min, followed by 36 cycles of 94°C,1 min; 55 °C,30 sec for RANKL, RANK, and AR, 48°C, 1 min for OPG; 72°C, 1 min and a final extension at 72°C for 10 min. For E-cadherin, N-cadherin, and c-Met gene amplification, the PCR reactions ran for a total of 32 cycles with annealing temperatures of 55°C and 47°C, respectively for 30 sec. For snail, vimentin, and GAPDH amplification, the PCR reactions ran for a total of 28 cycles with annealing temperatures at 48°C for 30 sec. The amplified PCR products were detected and analyzed on 1% agarose gel. PCR primer sequences are listed below.

Genes	PCR Primer Sequence
RANKL	F: 5'-TGG ATC ACA GCA CAT CAG AGC AG-3'
	R: 5'-TGG GGC TCA ATC TAT ATC TCG AAC-3'
E-cadherin	F: 5'-GCC AAG CAG CAG TAC ATT CTA CAC G-3'
	R: 5'-GCT GTT CTT CAC GTG CTC AAA ATC C-3'
AR	F: 5'-AGCTACTCCGGACCTTACG-3'
	R: 5'-AGGTGCCATGGGAGGGTTAG-3'
N-cadherin	F: 5'-GAT GTT GAG GTA CAG AAT CGT-3'
	R: 5'-GGT CGG TCT GGA TGG CGA-3'
Snail	F: 5'-CAG ACC CAC TCA GAT GTC AA-3'
	R: 5'-CAT AGT TAG TCA CAC CTC GT-3'
Vimentin	F: 5'-GGA CTC GGT GGA CTT CTC-3'
	R: 5'-CGC ATC TCC TCC TCG TAG-3'
c-Met	F: 5'-TGGGAATCTGCCTGCGAA-3'
	R: 5'-CCAGAGGACGACGCCAAA-3'

Soft agar colony formation

Six-well plates were first coated with 1 ml of 0.5% agarose mixed in RPMI1640 supplemented with 10% FBS and placed at room temperature until they solidified. One thousand prostate cancer cells were then mixed in 1 ml of 0.3% agarose in RPMI1640 supplemented with 10%

FBS and laid over the pre-coated 0.5% agarose layer, and 1 ml feeding media (RPMI1640 containing 10% FBS) were added into the well after the top agarose layer solidified. Media were changed every three days. Once the colonies were formed, the plates were stained with 0.5% crystal violet for 30 min at room temperature and washed with distilled water until the crystal violet in the agarose became clear and only the colonies were stained blue. The colonies in triplicate wells (>100 um) were then counted and images of each well were taken by camera.

***In vitro* osteoclastogenesis assay**

Osteoclast precursor cells (RAW 264.7 cells) and prostate cancer cells were co-cultured in 48-well plates containing 500 μl of α-MEM media supplemented with 10% FBS, and were set up in quadruplicates. Osteoclast precursor cells were seeded with prostate cancer cells in an 8:1 ratio (15,000 RAW 264.7 cells and 1,875 prostate cancer cells) per well. Recombinant mouse RANKL (100 ng/ml, provided by Dr. Xu Feng {McHugh, 2000 #27; Zhang, 2003 #103}) was added to the assay as the positive control. Additionally, recombinant OPG (1 μg/ml) (R&D Systems, Minneapolis, MN) was added to designated wells to block RANKL-mediated increased osteoclastogenesis. The cells were checked under a microscope daily from day 4 to day 7. Cells were fixed and subjected to tartrate-resistant acid phosphatase (TRAP) staining (Sigma-Aldrich, Inc., St. Louis, MO). $TRAP^+$ multinucleated cells (≥3 nuclei) in the entire well were counted as mature osteoclasts and images were taken under light microscopy.

***In vitro* migration and invasion assays**

Migration and invasion by cancer cells were examined in 24-well plates using collagen I and growth factor reduced Matrigel (BD Biosciences, Bedford, MA) coated transwells (8 μm pore size), respectively, as previously described {Odero-Marah, 2008 #30}, and were set up in

triplicates. Cells were serum-starved in RPMI 1640 or T-medium (Invitrogen, Carlsbad, CA) overnight and dissociated in 10 mM EDTA/HEPES. The lifted cells were washed and counted, and $5x10^4$ cells were seeded in 100 μl of serum-free RPMI 1640 or T-medium in the upper chamber of the transwell in the presence or absence of 1 μg/ml OPG, and 400 μl of RPMI 1640 or T-medium supplemented with 5% FBS was placed in the lower chamber of the transwell. After 24h (migration) and 48h (invasion) of culturing at 37 °C, the migrated and invaded cells were fixed with 10% formaldehyde, stained with 0.5% crystal violet, and the cells on the upper side of the membrane of the chamber were removed and quantified.