Supporting Information

Title

Combinatorial Surface Roughness Effects on Osteoclastogenesis and Osteogenesis

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Table S1. qPCR primer sequences of target genes for osteoclast differentiation and osteogenic differentiation.

Gene name	Forward primer sequence	Reversed primer sequence
mGAPDH	TGACCACAGTCCATGCCATC	GACGGACACATTGGGGGTAG
mRANK	GCAGCTCAACAAGGATACGG	GGTGCAGTTGGTCCAAGGTT
mMMP-9	GGGCGTGTCTGGAGATTCG	CACCTGGTTCACCTCATGGTC
mCTSK	CCAGTTTTACAGCAGAGGTGTG	CTTGCTTCCCTTCTGGGTG
mTRAP	CACTCCCACCCTGAGATTTGT	CATCGTCTGCACGGTTCTG
rGAPDH	CTTCACCACCATGGAGAAGGC	GGCATGGACTGTGGTCATGAG
rRunx2	GAGCACAAACATGGCTGAGA	TGGAGATGTTGCTCTGTTCG
rCollagen I	GAGCGATTACTACTGGATTGACCC	CAAGGAATGGCAGGCGAGAT
rALP	GGGACTGGTACTCGGATAACGA	CTGATATGCGATGTCCTTGCA
rOCN	CGACTCTGAGTCTGACAAA	GCCGGAGTCTATTCACCACCTT

Table S2. qPCR primer sequences of clastokines.

Gene name	Forward primer sequence	Reversed primer sequence
mGAPDH	TGACCACAGTCCATGCCATC	GACGGACACATTGGGGGTAG
mWint10b	TCGATACCCACAACCGCAACT	AAGAGGCGGCTGGTCTTGTT
mBMP-6	AAGGCTACGCTGCCAACTACT	AGCATGGTTTGGGGACGTACT
mSPHK1	CGAACGGAAGAACCATGCCAG	GGAGGCTACACAGGGGTTTCT
mCTHRC1	TGCGAGTTCTGTTCAGTGGCT	ATGGCTTCGATGGGAAGAGGT
mSclerotin	CACTACACCCGCTTCCTGACA	TCCGGGATGCAGCGGAAATC
mSemaphorin4D	TATGCGGTCTTCACCCCACAG	TGTCGATACACGCTCCAGGTC

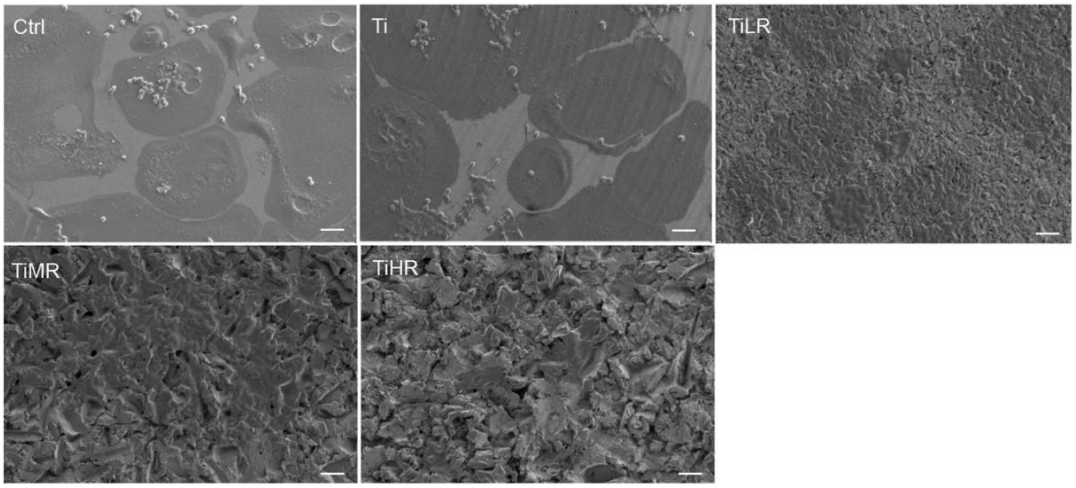

Figure S1. Morphologies of different rough surfaces and osteoclasts induced on these different surfaces. RAW264.7 macrophages were grown on glass control and different rough titanium surfaces and induced with RANKL for 4 days and their morphology imaged by SEM. Scale bar is 10 μm in all panels.

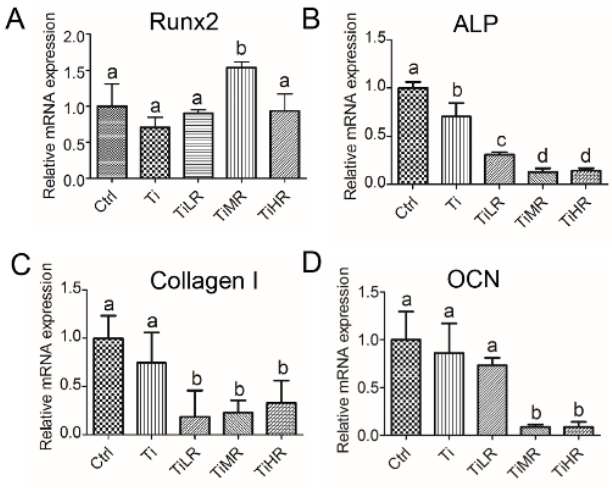

Figure S2. Gene expression of osteogenic markers in mouse osteoprogenitor cells cultured in conditioned medium of different types of osteoclasts. Mouse osteoprogenitor cells (MC3T3) were cultured in the conditioned medium of RAW264.7 derived osteoclasts on glass control and different titanium rough surfaces for 7 days. Gene expression of osteogenic markers including **(A)** Runx2 (n = 3), **(B)** ALP (n = 3), **(C)** Collagen I (n = 3) and **(D)** OCN (n = 3) were analyzed. A significant difference was indicated by a, b, c d. Groups with different letters mean significant difference and groups sharing the same letter are not significantly different.

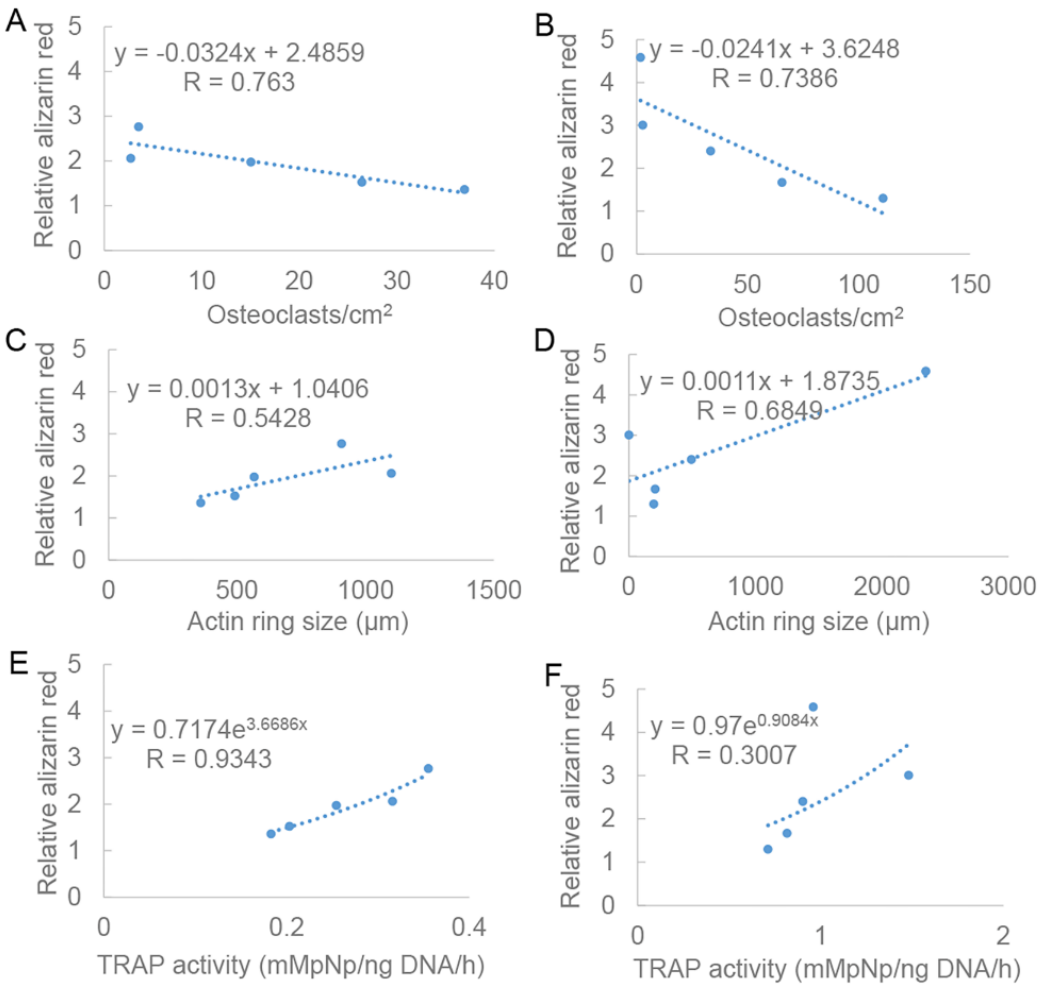

Figure S3. The coupling between osteoclast phenotype and its anabolic effects. Number of osteoclast per cm^2 of **(A)** RAW264.7 derived osteoclasts and **(B)** primary osteoclasts, actin ring size of **(C)** RAW264.7 derived osteoclasts and **(D)** primary osteoclasts, and TRAP activity of **(E)** RAW264.7 derived osteoclasts and **(F)** primary osteoclasts induced by different surface roughness was coupled with their anabolic effects on osteoblastic cells.