Supporting Information

Regenerative Bone-Targeted Nanoparticles Modulate Osteoclast Function

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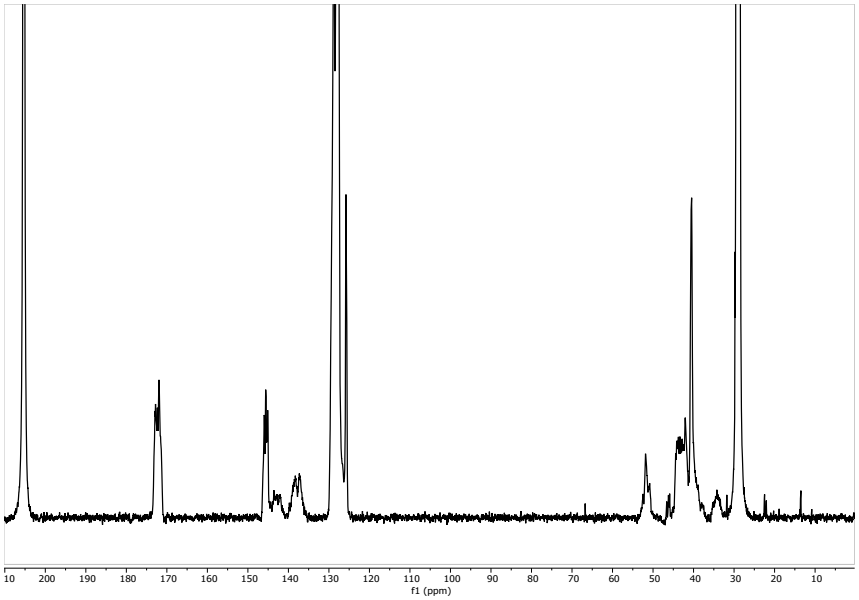
Table S1. The molecular weights and the PDI of the polymers were obtained by gel permeation chromatography (GPC). The size of the SCP-NP was measured using dynamic light scattering (DLS). NP concentration was calculated using ZetaView (Particle Metrix) that was used to determine the peptide/NP calculation

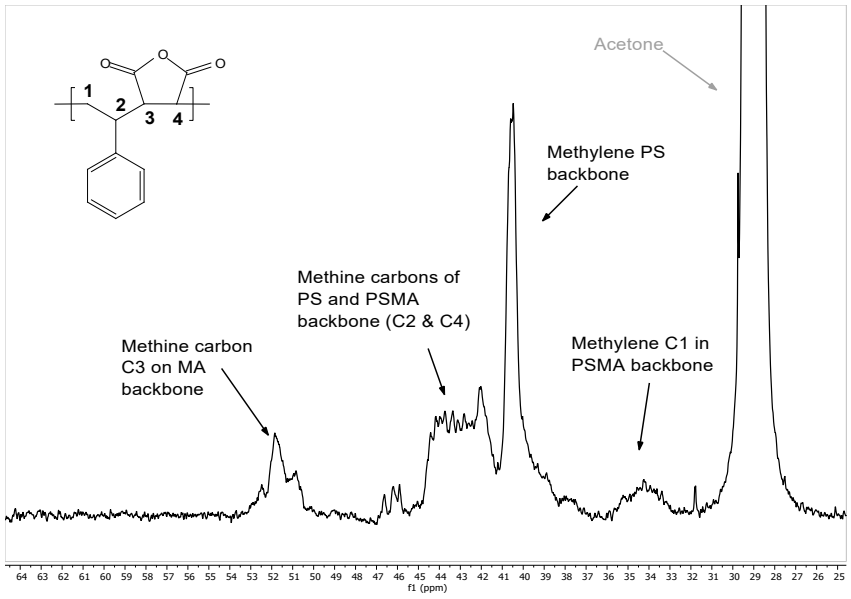
NP	Block 1 (PSMA)	Block 2 (PS)	Diblock Copolymer		SCP-diblock Copolymer		Size	Zeta	PDI	Loading capacity	Loading efficiency
	Mn [kDa]	Mn [kDa]	Mn [kDa]	PDI	Mn [kDa]	PDI	(nm)	(mV)		(LC%)	(LE%)
$SCP_{27,000}$-NP	20	25	45	1.0	57	1.1	56 ± 7	-51 ± 6	0.2	-	-

Peptides/polymer chain- 7 SCP/polymer chain.

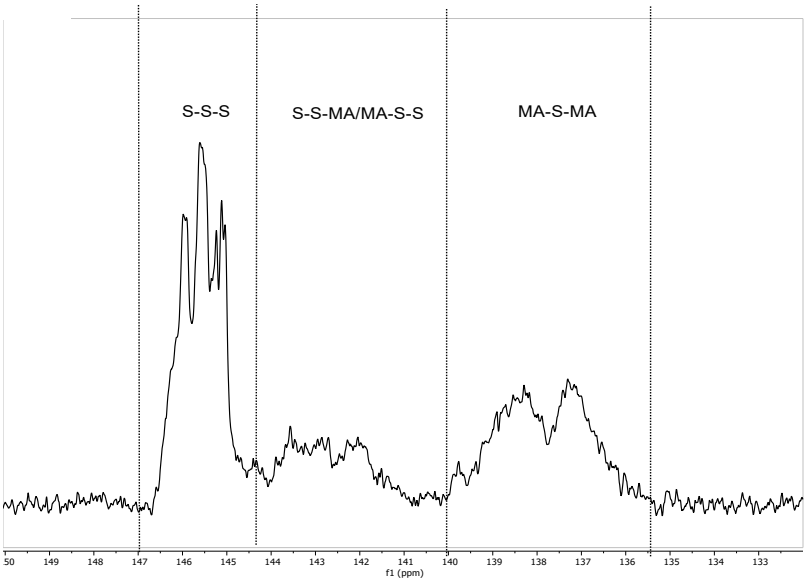
A



B


1
2
3
4
Acetone
Methylene PS backbone
Methine carbons of PS and PSMA backbone (C2 & C4)
Methine carbon C3 on MA backbone
Methylene C1 in PSMA backbone
f1 (ppm)

C


S-S-S
S-S-MA/MA-S-S
MA-S-MA
f1 (ppm)

D

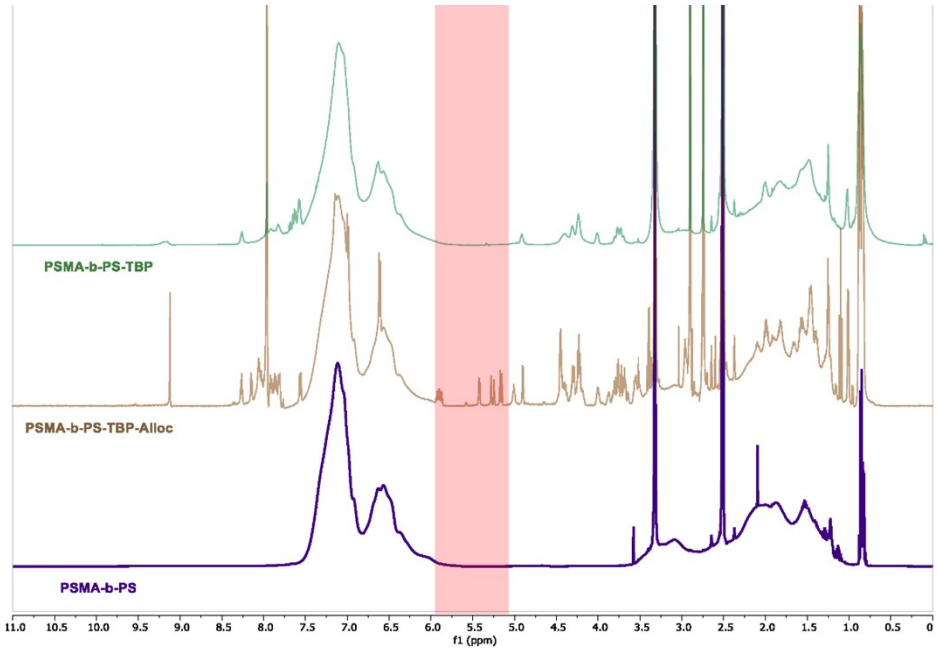


Figure S1. (A) ^{13}C NMR of PSMA-b-PS in acetone-D6 at 50 mg/mL was obtained using a Bruker 300 MHz NMR with 8000 scans, a delay time of 6 seconds, and inverse gating to mitigate the Nuclear Overhauser Effect. (B) The peaks depicting the PSMA backbone and (C) the peaks identifying alternating MSM, MSS/SSM, and SSS triads. **(**D**)** NMR spectra of PSMA-b-PS, PSMA-b-PS-TBPalloc, and PSMA-b-PS-TBP. Peaks highlighted in red belong to vinylic protons on the alloc protecting group.

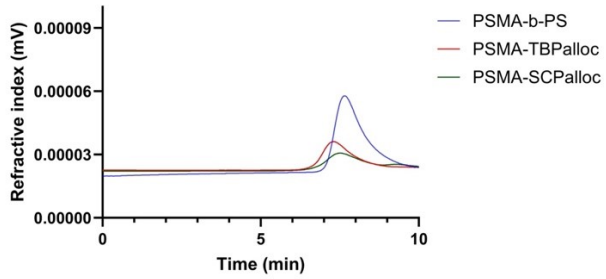


Figure S2. Gel permeation chromatography spectra of PSMA-b-PS, PSMA-b-PS-TBPalloc, and PSMA-b-PS-SCPalloc.

Table S2. Hydrophobic residues shown in the TBP and SCP peptides, showing the differences in the distribution of hydrophobic amino acids that could affect how the peptides fold when interacting with polymers.

TBP	SCP
TPLSYLKGLVTVG	VPVGTLSYLKLTG
Green: hydrophobic uncharged residues (F, I, L, M, V, W, A, and P)	
Black: other residues (G, S, T, C, N, Q, and P)	

Table S3. Amino acids with their protecting groups used for the synthesis of TBP and SCP.

Amino Acid	**Source Company**
Fmoc-Thr(tBu)-OH	aapptec
Fmoc-Pro-OH	aapptec
Fmoc-Leu-OH	aapptec
Fmoc-Ser(tBu)-OH	aapptec
Fmoc-Tyr(tBu)-OH	aapptec
Fmoc-Lys(Alloc)-OH	Sigma Aldrich
Fmoc-Gly-OH	aapptec
Fmoc-Val-OH	aapptec

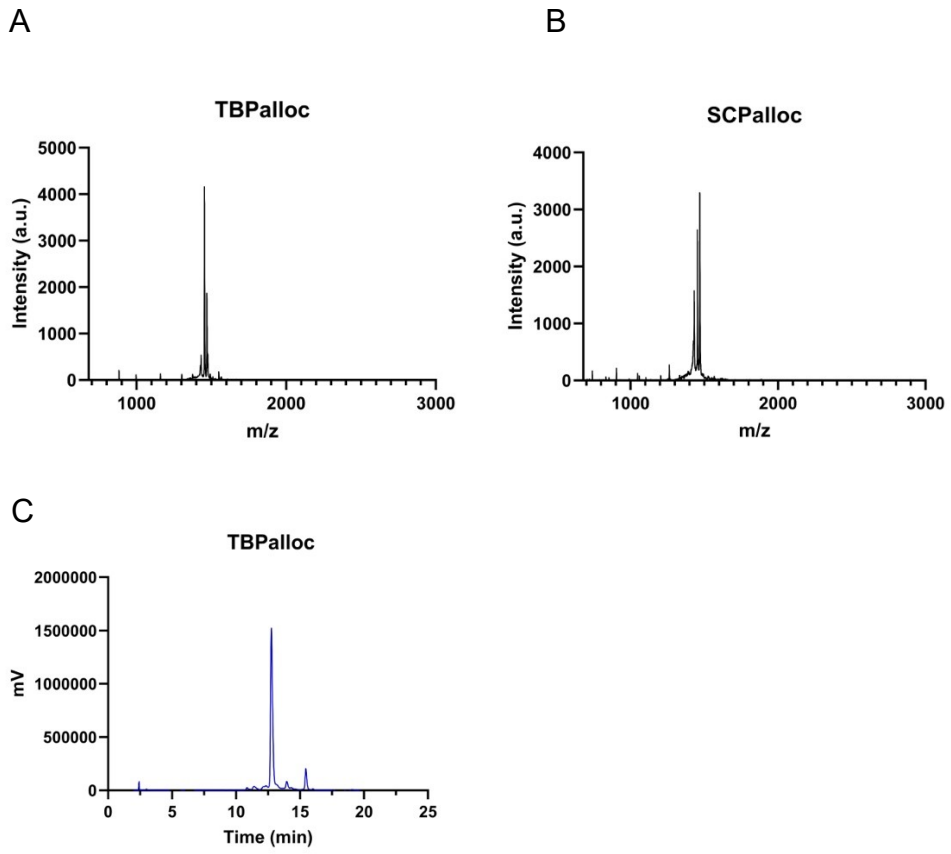


Figure S3. A) MALDI spectra of TBPalloc and B) SCPalloc. The purity of these peptides was determined by HPLC. C) The chromatogram of TBPalloc.

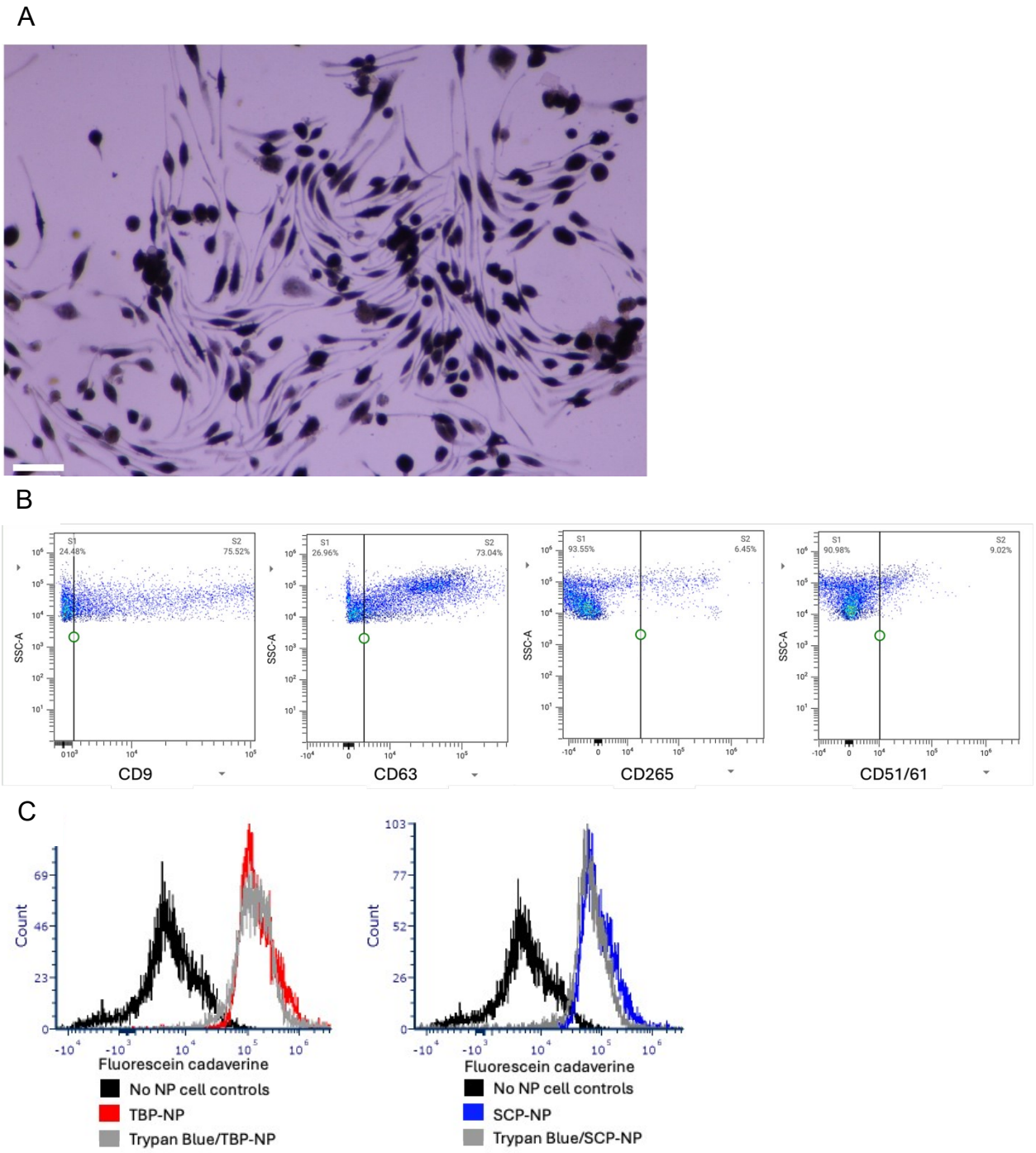


Figure S4: A) Morphological changes observed in differentiated osteoclasts after treatment with SCP-NP. (B) Flow cytometry of the osteoclasts with osteoclast-specific markers- CD9, CD63,

CD265, and CD51/61. (C) Histograms of osteoclasts treated with TBP-NPs and SCP-NP conjugated with fluorescein cadaverine for 1 hr. and quenching with trypan blue.

Table S4. Primer sequences obtained from Integrated DNA Technologies (IDT).

Primer name	Primer sequence
NFATc1- forward	TCCACCCACTTCTGACTTCC
NFATc1- reverse	CTTCGCCCACTGATACGAG
cFOS - forward	GTTCGTGAAACACACCAGGC
cFOS- reverse	GGCCTTGACTCACATGCTCT
CTSK- forward	TCCGCAATCCTTACCGAATA
CTSK- reverse	AACTTGAACACCCACATCCTG
ATP6v0d2- forward	CAAAGCCAGCCTCCTAACTC
ATP6v0d2- reverse	GTTGCCATAGTCCGTGGTC
GAPDH- forward	AGGTCGGTGTGAACGGATTTG
GAPDH- reverse	TGTAGACCATGTAGTTGAGGT

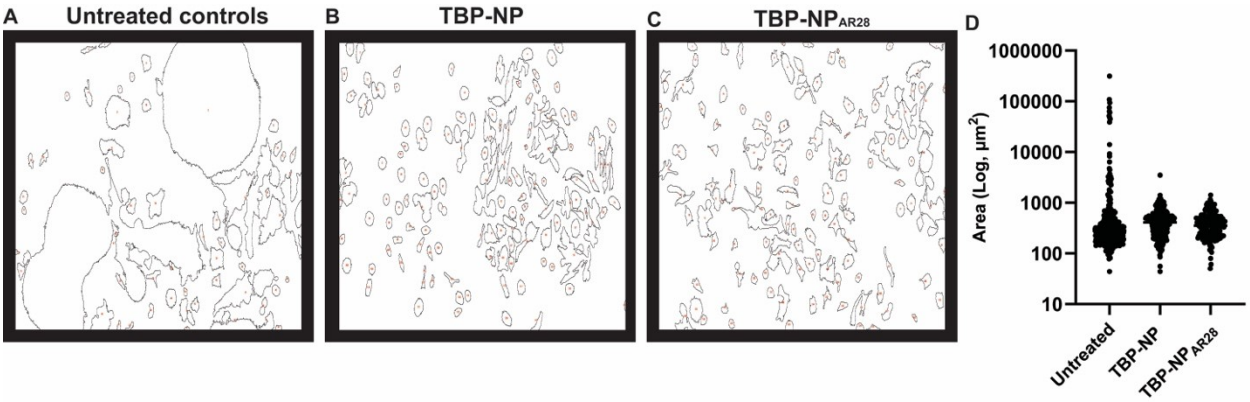


Figure S5. Quantification of cell area analyzed through ImageJ. Untreated osteoclasts (A) and osteoclasts treated with TBP-NP (B) and TBP-NPAR28 (C). Graphical representation of cell area with and without treatment of NPs (D).

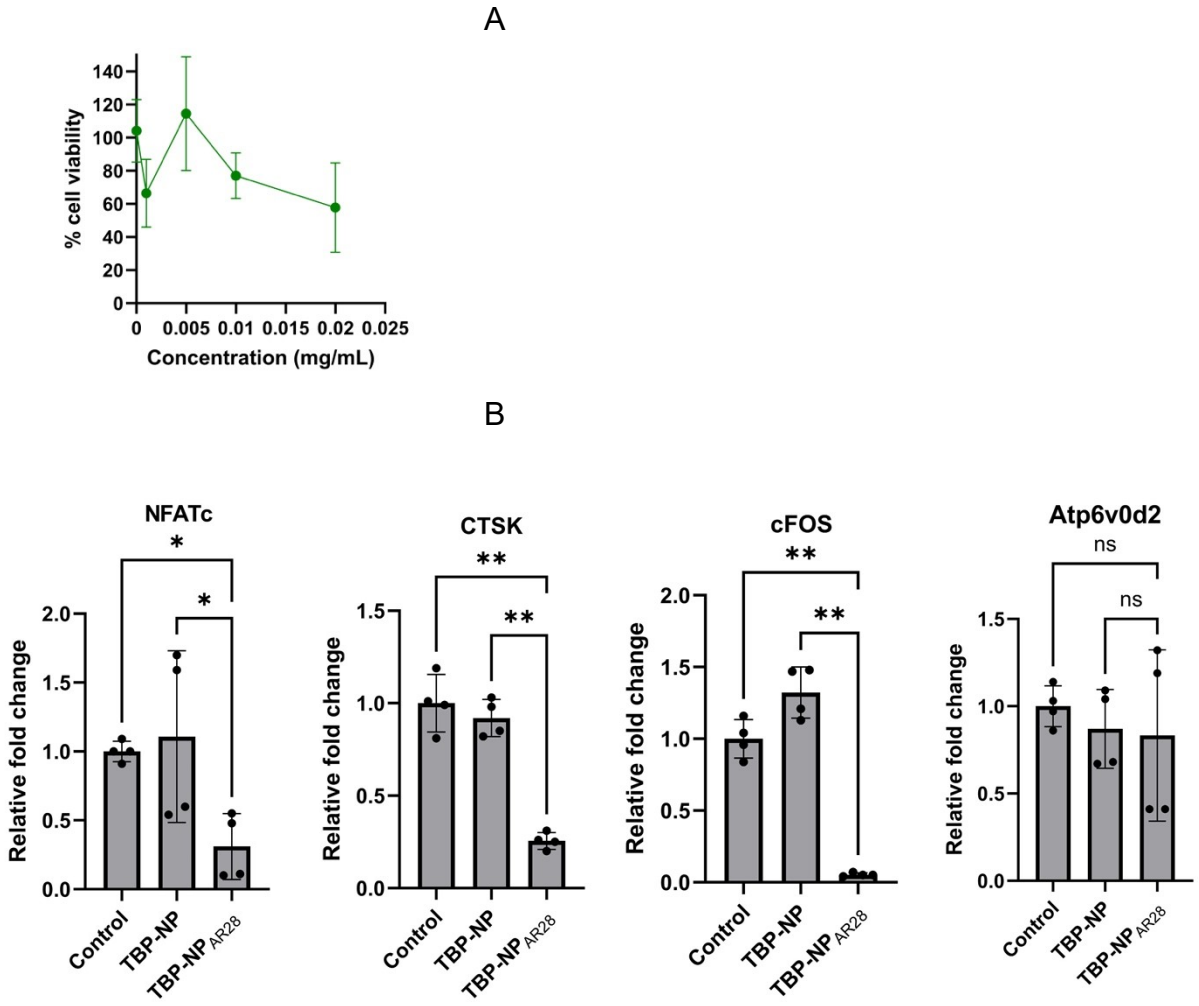


Figure S6. A) Cytocompatibility of TBP-NP_{AR28} was assessed through Alamar blue assay. The concentration represents the polymer concentration used from 0- 0.025 mg/mL which corresponds to 0, 0.4, 2, 4, and 8 μM of AR28 (calculated based on loading capacity). (n=3) **B)** Gene expression of genes involved in osteoclastogenesis and osteoclast function analyzed through qRT-PCR after 3 days of treatment with TBP-NP and TBP-NP_{AR28}. Control= untreated cells. Data represented as

Mean ± Standard deviation. Statistical significance determined by 2way ANOVA and Dunnett's multiple comparisons test. *p<0.05, **p<0.01.

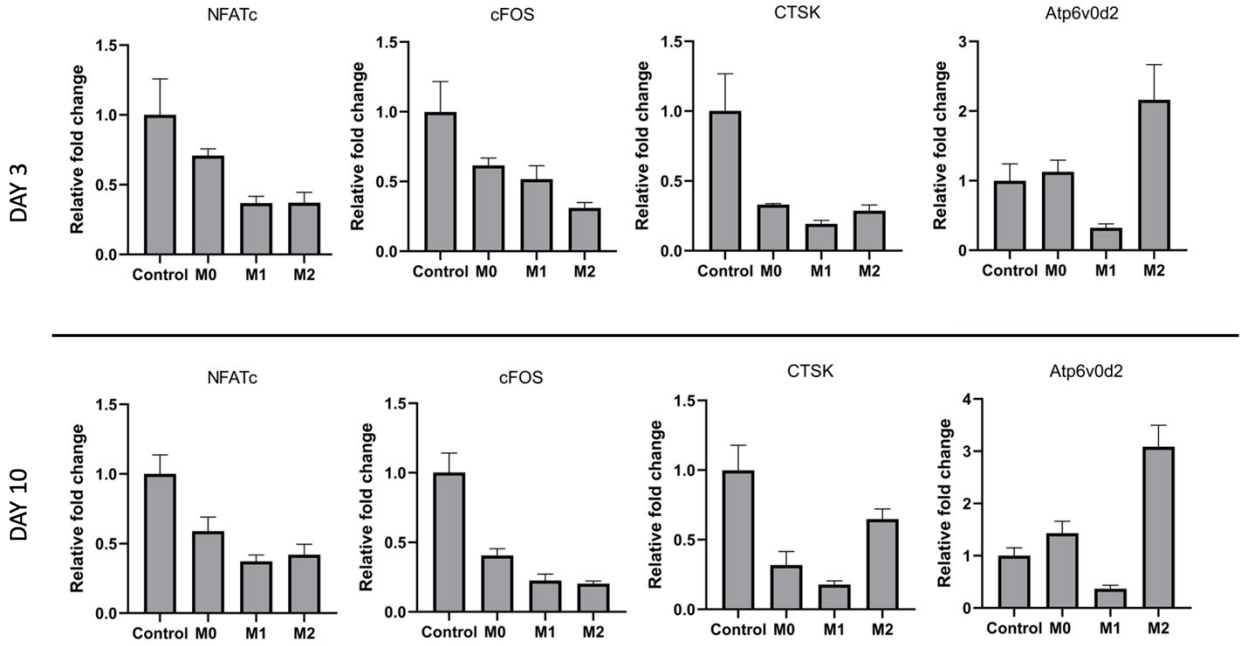


Figure S7. Osteoclasts were treated with conditioned media of TBP-NP-treated M0, M1, and M2 macrophages. RNA was extracted from osteoclasts 3- and 10-days post-treatment with conditioned media, and qRT-PCR was performed to analyze the expression of NFATc, cFOS, CTSK, and Atpv0d2.