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# Supplementary Materials

## (*S*)-2-(Cyclobutylamino)-*N*-(3-(3,4-dihydroisoquinolin-2(1*H*)-yl)-2-hydroxypropyl)isonicotinamide attenuates RANKL-induced osteoclast differentiation by inhibiting NF-κB nuclear translocation

**Supplementary results**

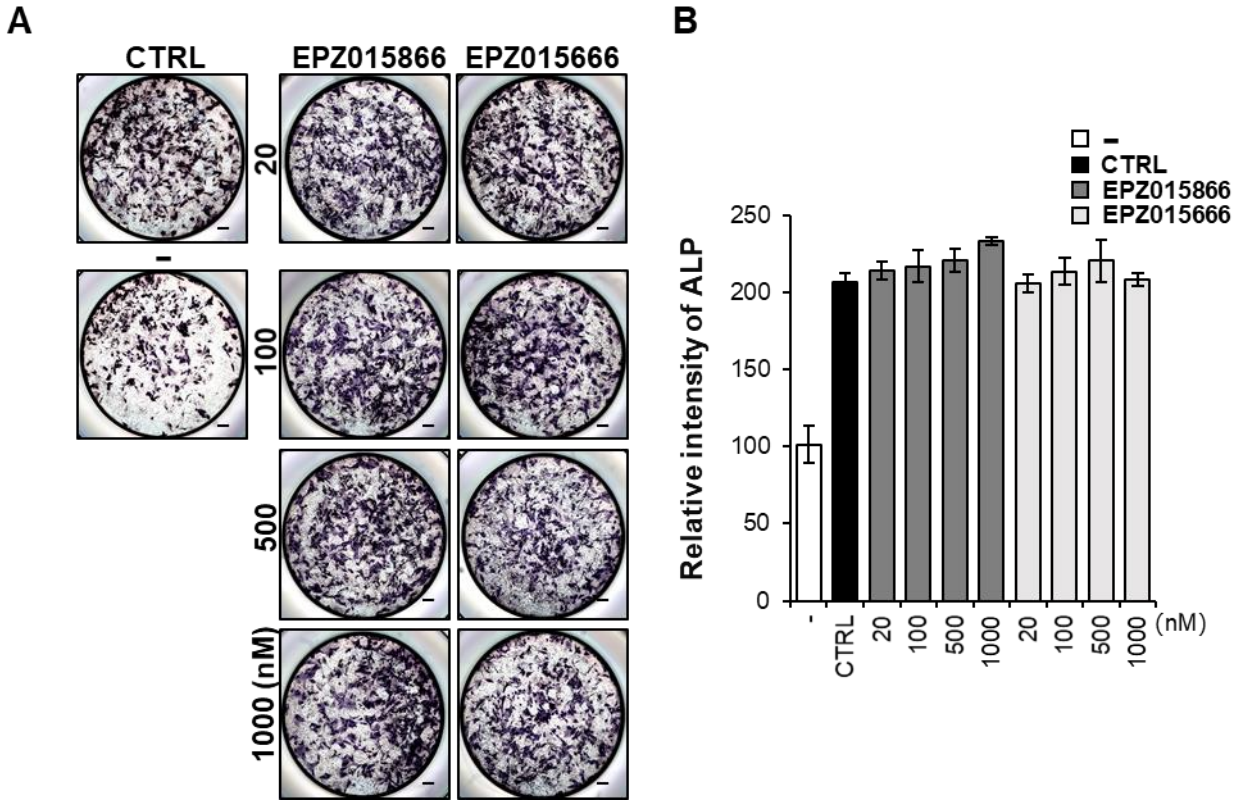


**Figure S1.** EPZ compounds did not affect the osteoblast formation. (**A**) Representative images of ALP staining showed mouse primary pre-osteoblast cells were cultured with BMP2 (100 ng/mL) in the presence or absence of different doses of EPZ compounds (20, 50, 100, 500, and 1000 nM) for 7 days. Scale bar represents 400 μm. (**B**) Quantified analysis of ALP staining intensity. "-" indicates cells were not treated with BMP2 and "CTRL" indicates the control group (cells were treated with BMP2).

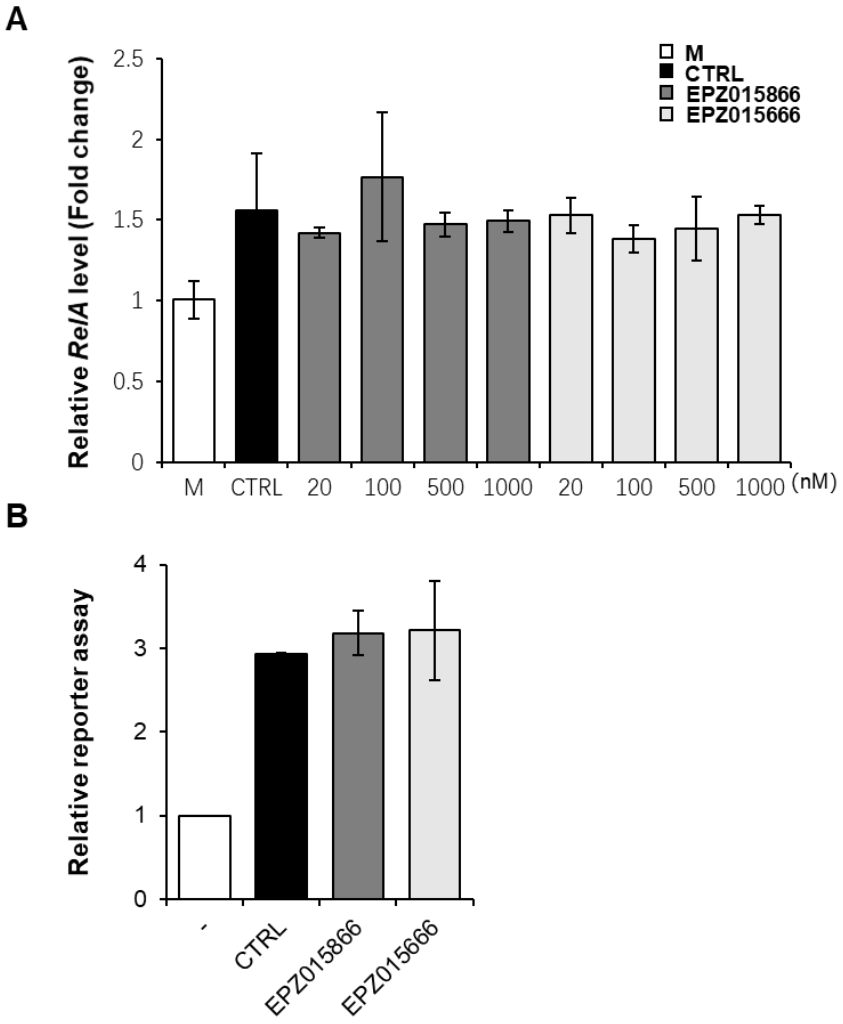


**Figure S2.** NF-κB transcriptional activity was not altered by EPZ compounds. (**A**) The mRNA levels of RelA (p65) in BMMs were determined by qRT-PCR after RANKL or indicated concentrations of EPZ compounds for 4 days. (**B**) The NF-κB reporter HEK293 cell line were incubated with or without 1000 nM of EPZ compounds for 24 hours and then stimulated with RANKL for 6 hours. NF-κB luciferase assay was performed. The data presented are the mean ± SD.

**Table S1.** Primer sequences used for real-time PCR analysis.

| Gene (mouse) | Primer Sequence (5'-3') |
|---|---|
| *Ctsk* | F: ACTTCCGCAATCCTTACCGA<br>R: TTCGCTAGGCTCTTTTCGGA |
| *Dc-stamp* | F: CGCACGATGCTTCATTCTTC<br>R: CAGTGCCAGCCGCAATC |
| *Oc-stamp* | F: CAGAGTGACCACCTGAACAAACA<br>R: TGCCTGAGGTCCCTGTGACT |
| *Acp5* | F: TTTATGCTGGACACAGTGATGCT<br>R: CCCAGGTCTCGAGGCATT |
| *Mmp9* | F: CTGGACAGCCAGACACTAAAG<br>R: CTCGCGGCAAGTCTTCAGAG |
| *Atp6v0d2* | F: GTGAGACCTTGGAAGACCTGAAA<br>R: TCCTCATCTCCGTGTCAATTTTG |
| *Prmt5* | F: CTGAATTGCGTCCCCGAAATA<br>R: AGGTTCCTGAATGAACTCCCT |

| | |
|---|---|
| *Gapdh* | F: TGTGTCCGTCGTGGATCTGA<br>R: GATGCCTGCTTCACCACCTT |

F: forward; R: reverse, Ctsk: cathepsin K.

**Supplementary Method**

*NF-κB luciferase assay*

The NF-κB reporter HEK293 cell lines were seeded into 12-well plates at a density of $1.0 \times 10^5$ per well. After 1 day, cells were incubated with or without 1000 nM of EPZ compounds for 24 hours, and then stimulated with RANKL for 6 hours. Harvest cells for NF-κB luciferase assay. NF-κB Luciferase activity was measured by a luciferase reporter assay system (Promega) and a luminometer (SpectraMax i3x, Molecular Devices) following the manufacturer's manual.