

Review Article



Supplementary Figure 1: Bone marrow and RAW264.7 cell viability determination. MTT assay was performed on 3 days treated BMCs with M-CSF (20 ng/ml) and RANKL (40 ng/ml) (A) or 3 days treated RAW264.7 cells with RANKL (40 ng/ml) (B) in the presence or absence of RSV, SRT2183, and SRT1720 at specified concentrations. Three independent experiments were performed with \geq 3 wells per condition. Note: * p<0.05, compared with vehicle control. Data expressed as 20 mean ± SD.

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Supplementary Figure 2: SR12183 and SR11720 inhibit osteoclast and actin belt formation in RAW264.7 cells, while RSV does not. 3.4 days treated (5 μ M RSV, 5 μ M SRT2183, or 0.6 μ M SRT1720) RAW264.7 cells in the presence of 40 ng/ml RANKL were fixed and stained with TRAP (A) to visualize and count osteoclasts (B) or with FITC-phalloidin (C) to visualize actin belts. Osteoclasts with 3 or more nuclei were counted (D). Three independent experiments were performed with \geq 3 wells per condition. Note: * p \leq 0.05, compared with vehicle control. Data expressed as mean \pm SD.



Supplementary Figure 3: SRT2183 and SRT1720 disrupt actin belts of mature osteoclasts in RAW264.7 cells, while RSV does not. The mature osteoclasts in the tissue culture plate were treated at different time points (3 h, 6 h, 24 h, and 30 h) with 5 μ M RSV, 5 μ M SRT2183, or 0.6 μ M SRT1720, then fixed at mentioned time points and stained with FITC-Phalloidin to visualize actin belts. Three independent experiments were performed with \geq 3 wells per condition. Note: * p<0.05, compared with vehicle control. Data expressed as mean \pm SD.



Supplementary Figure 4: RSV activates AMPK α in RANKL induced BMMs and RAW264.7 cells. Western blot analysis of AMPK α (AMP-activated protein kinase-alpha) and phosphorylated AMPK α in BMMs (A) and RAW 264.7 cells (B) treated with or without 5 μ M RSV in the presence of RANKL for 24 hours. Three independent experiments were performed. Note: * p<0.05, compared with vehicle control. Data expressed as mean ± SD.