

## **Inhibition of Sirtuin 3 prevents titanium particle-induced bone resorption and osteoclastogenesis via suppressing ERK and JNK signaling**

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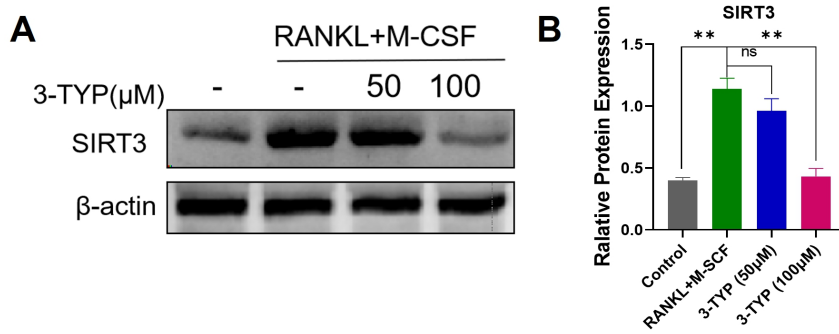
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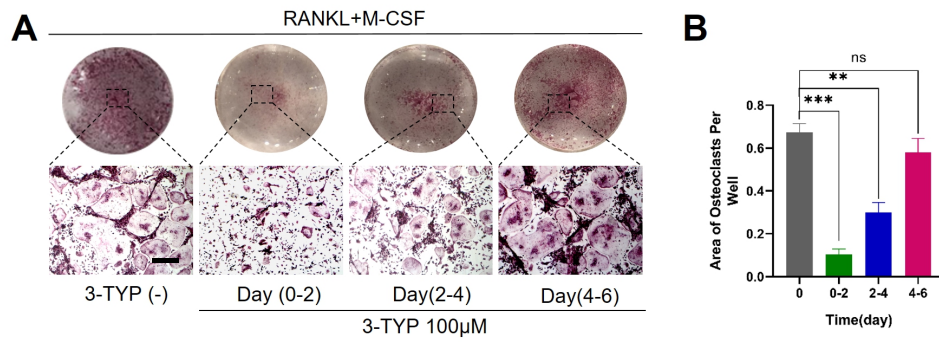
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Genes	Forward (5'-3')	Reverse(5'-3')
CTSK	GGGAGAAAAACCTGAAGC	CGAGACTCAGGGGTCTTA
NFATc1	CCGTTGCTTCCAGAAAATAACA	TGTGGGATGTGAACTCGGAA
TRAP	TGTGGCCATCTTTATGCT	TTCGGGGTTTCTTTACTG
MMP9	CTGGACAGCCAGACACTAAAG	CTCGCGGCAAGTCTTCAGAG
Atp6v0d2	GTGAGACCTTGAAGACCTGAA	TCGGGGACTCGTGTAAGAG
DC-STAMP	AAAACCCTTGGGCTGTTCTT	AATCATGGACGACTCCTTGG
IL-1 $\beta$	ACTCATTGTGGCTGTGGAGA	TGTCCGAGGCTCTACTTGTT
IL-6	TCGTGGAAATGAGAAAAGAGTG	ACATACTTGTTGCTACTACGTGA
TNF- $\alpha$	CTGAGGTCAATCTGCCAAGTAC	GAAACCTCAGTAACGAGACACTTC
GAPDH	AGCCATGTACGTAGCCATCC	CTCTCAGCAGTGGTGGTGAA

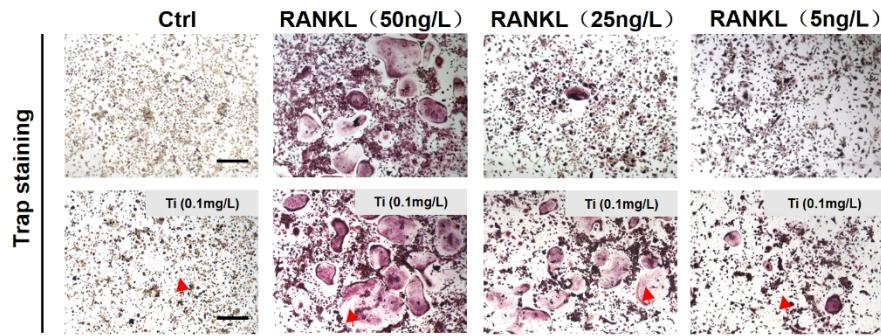
**Table S1:** Sequences of the primers used for RT-PCR. CTSK : cathepsin K ; NFATc1:nuclear factor of activated T - cell cytoplasmic 1 ; TRAP: Tartrate resistant acid phosphatase ; MMP9 : matrix metalloproteinase 9 ; Atp6v0d2 : ATPase H<sup>+</sup> Transporting V0 Subunit D2 ; DC-STAMP : dendritic cell-specific transmembrane protein ; IL-1 $\beta$ : interleukin-1 $\beta$ , IL-6: interleukin-6 ; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.



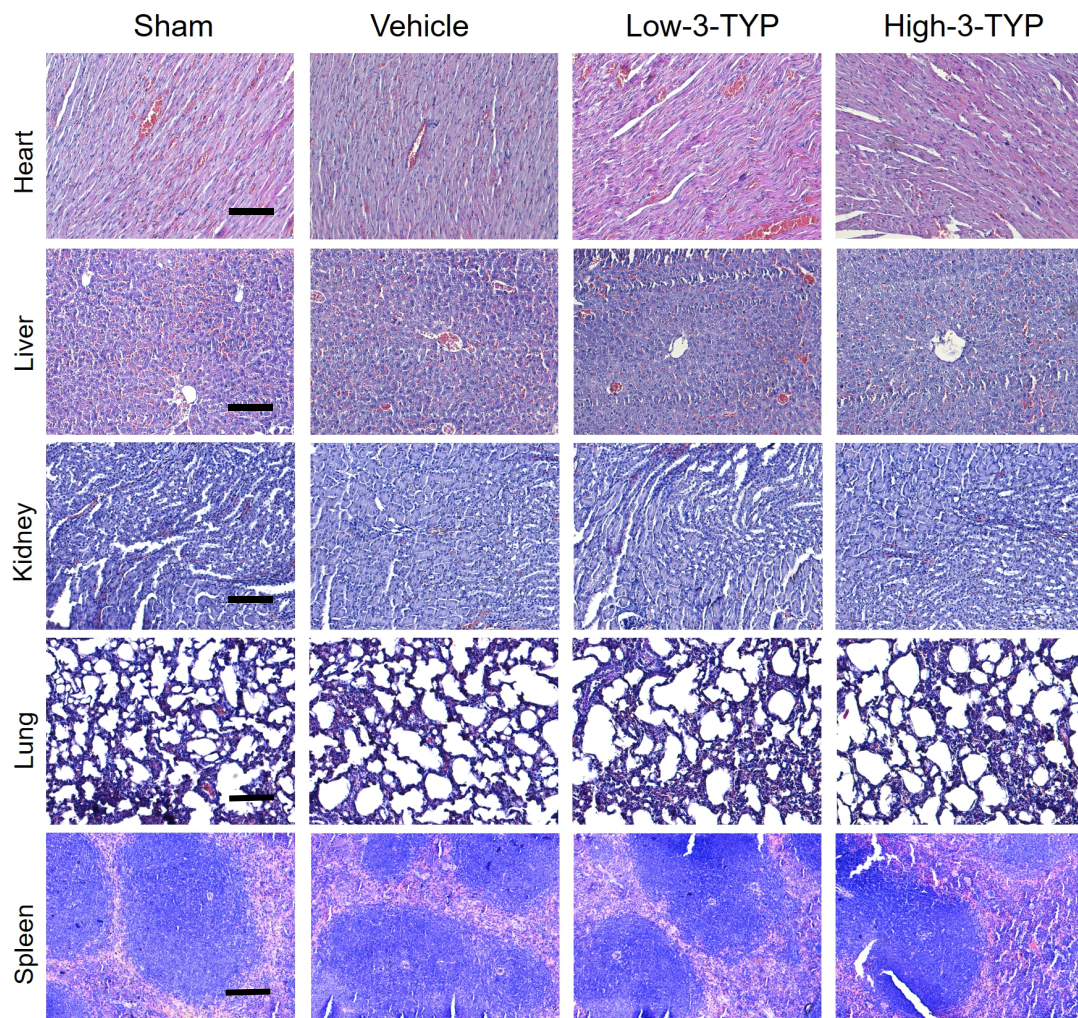
**Figures S1.** 3-TYP inhibits the increase of SIRT3 expression in the development of RANKL-induced osteoclasts. (A) SIRT3 expression was assessed via Western blotting at the day 5. (B) Results of western blotting are quantified. Data are means  $\pm$  SD. (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ ,  $n=3$ ).



**Figures S2.** 3-TYP inhibits the RANKL-induced osteoclasts. (A) BMMs were cultured in media containing 100µM 3-TYP, RANKL (50ng/mL), and M-CSF (30ng/mL) for the indicated periods of time, revealing that 3-TYP suppresses osteoclasts differentiation during early time points. (B) Quantification of TRAP-positive multinucleated cells and osteoclasts per well (nuclei  $\geq 3$ ). Scale bar: 100µm. Data are means  $\pm$  SD. (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ ,  $n=3$ ).



**Figure S3.** Titanium particles promoted the differentiation of BMMs into mature osteoclasts in the presence of RANKL (Red arrow: titanium particles). Scale bar: 100 $\mu$ m.



**Figure S4.** Toxicities of 3-TYP on heart, liver, kidney, lung and spleen. H&E staining of the organ tissue sections. Scale bar: 50 $\mu$ m.