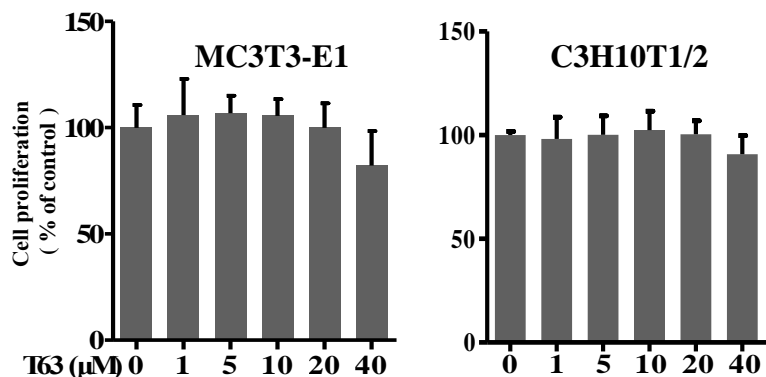


# Small molecule T63 suppresses osteoporosis by modulating osteoblast differentiation via BMP and WNT signaling pathways

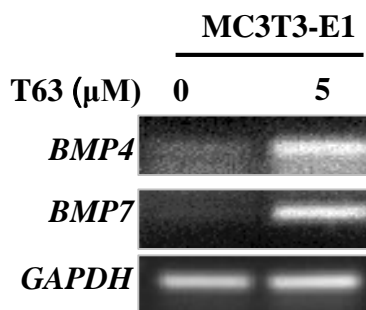
Xiao-li Zhao, Jin-jing Chen, Guo-ning Zhang, Yu-cheng Wang, Shu-yi Si, Lin-Feng Chen\*, Zhen Wang\*

**S1**

a

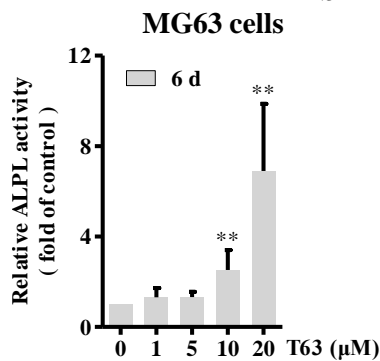


b

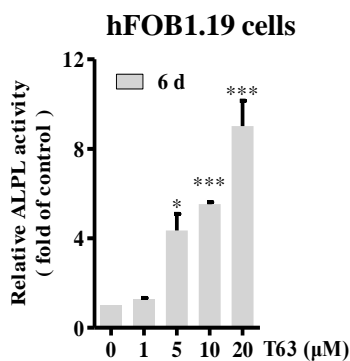


**S2**

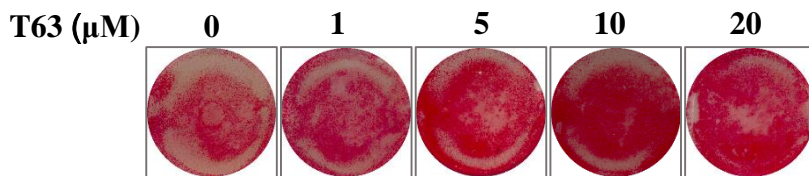
a

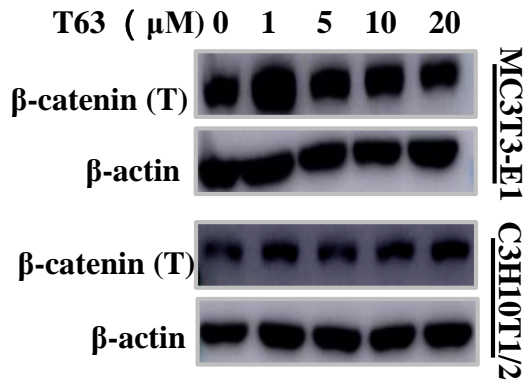
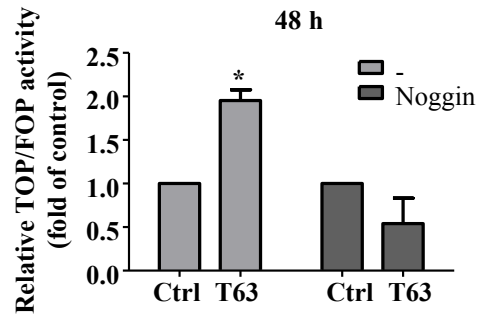
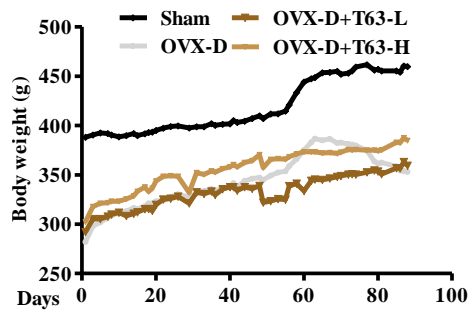
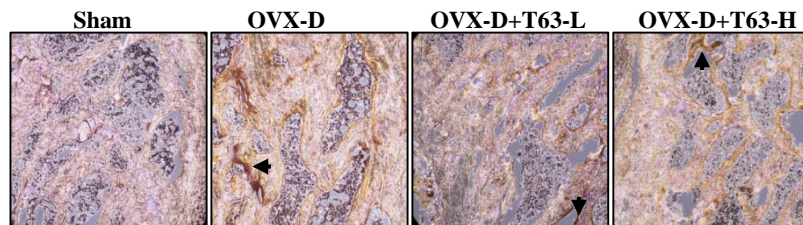
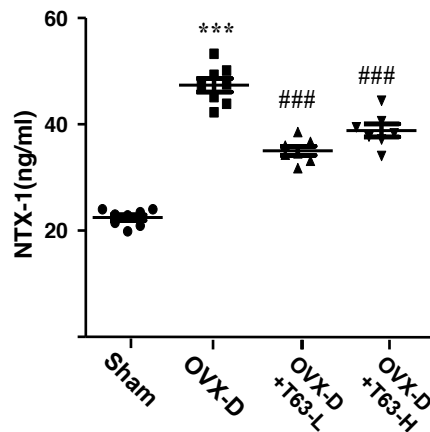


b



c



**S3****a****b****S4****a****b****c**

**Supplementary Figure S1.** Effect of T63 on the proliferation and osteoblastic differentiation of C3H10T1/2 and MC3T3-E1 cells. (a) C3H10T1/2 or MC3T3-E1 cells were seeded in 96-well plate for 24 h, and then treated with different concentrations of T63 for another 48 h. Cell proliferation was determined by MTT assay. (b) Expression of BMP4 and BMP7 genes in T63-treated MC3T3-E1 cells. The cells were treated with 5  $\mu$ M T63 for 12 days in OS medium and mRNA expressions were determined by semi-quantitative PCR.

**Supplementary Figure S2.** Effect of T63 on osteoblastic differentiation of human cells. (a)& (b) The levels of ALPL activity. The cells were seeded in 6-well plates ( $2 \times 10^5$  cells /well) for 24 h and treated with different concentrations of T63 in OS medium for 6 d.  $**p < 0.01$ ,  $***p < 0.001$ . (c) Osteoblast mineralization was observed by Alizarin Red S staining in MG63 cells for 18 d.

**Supplementary Figure S3.** BMP and canonical WNT/ $\beta$ -catenin pathways are involved in the regulation of T63-induced RUNX2 expression and osteoblast differentiation. (a) T63 activates canonical WNT/ $\beta$ -catenin signaling. MC3T3-E1 cells were seeded in 6-well plates ( $3 \times 10^5$  cells /well) for 24 h and treated with different concentrations of T63 for another 48 h. The levels of indicated proteins were determined by western blot. (b) Noggin (200 ng/ml) suppresses T63 increased- TCF/LEF reporter activity at 48 h in MC3T3-E1 cells,  $*p < 0.05$  (n=3).

**Supplementary Figure S4.** T63 attenuates OVX-D-induced bone loss. (a) Body weight of each rat was measured every one or two days. (b) TRAP staining was performed on femur sections. (c) Serum NTX-1 level.  $***p < 0.001$  versus Sham group.  $####p < 0.001$  versus OVX-D group.

## S5. Uncropped western blots for the blots used Figures

Fig. S5a: full Western blot of Fig. 4a.

Fig. S5b: full Western blot of Fig. 4c.

Fig. S5c: full Western blot of Fig. 4d.

Fig. S5d: full Western blot of Fig. 5b.

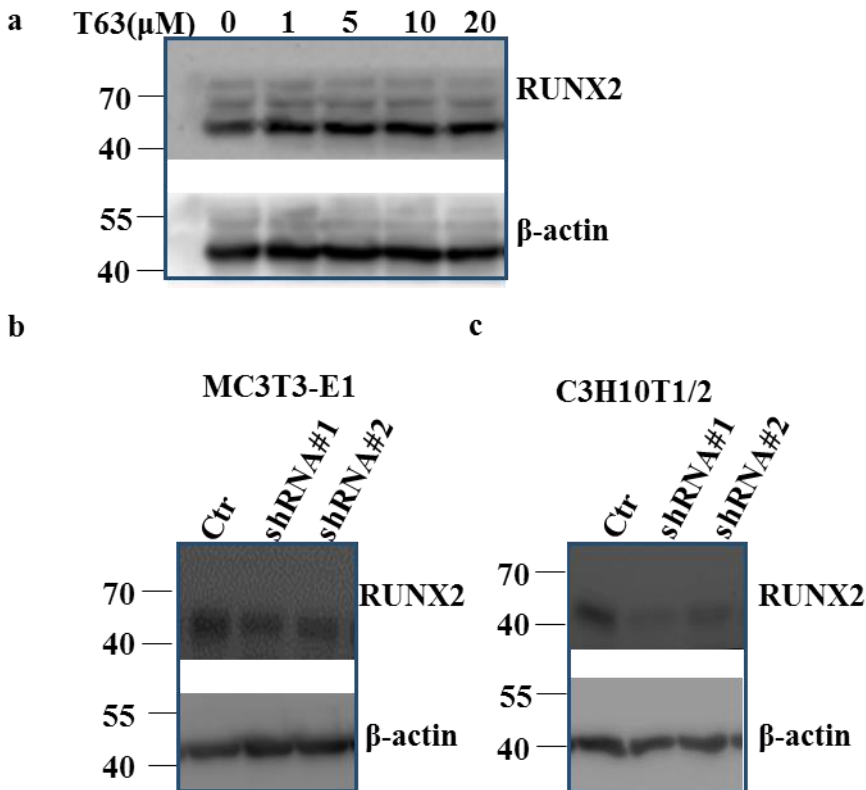
Fig. S5e: full Western blot of Fig. 5c.

Fig. S5f: full Western blot of Fig. 6b.

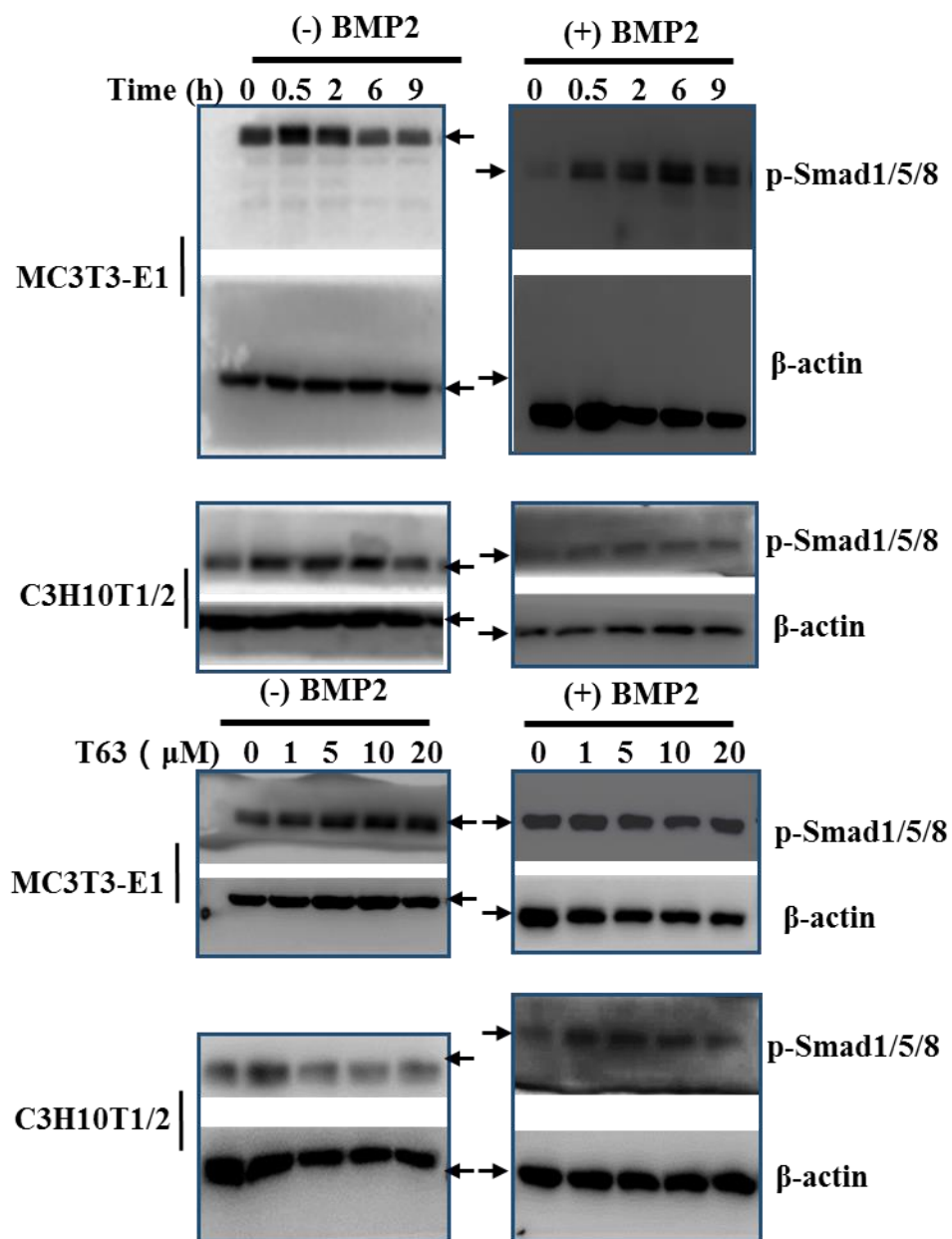
Fig. S5g: full Western blot of Fig. 6c.

Fig. S5h: full Western blot of Fig. 6d.

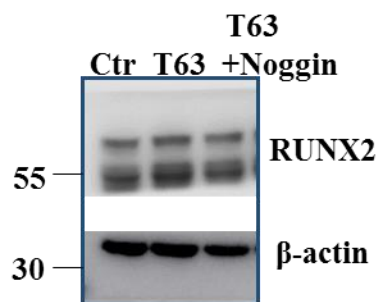
Fig. S5i: full Western blot of supplementary Fig. S3a.



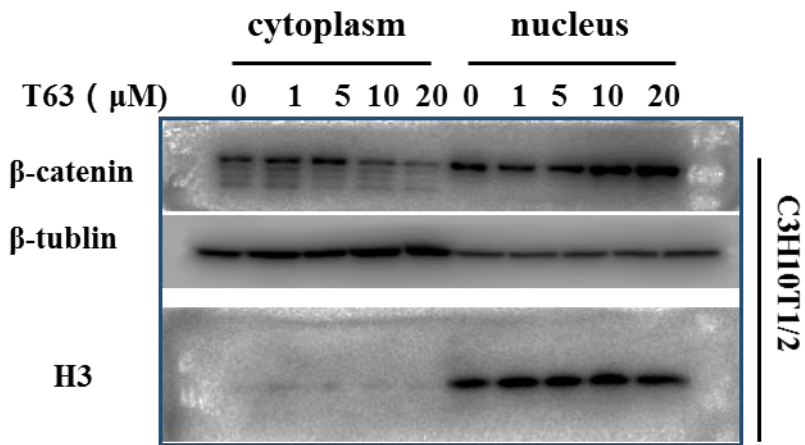
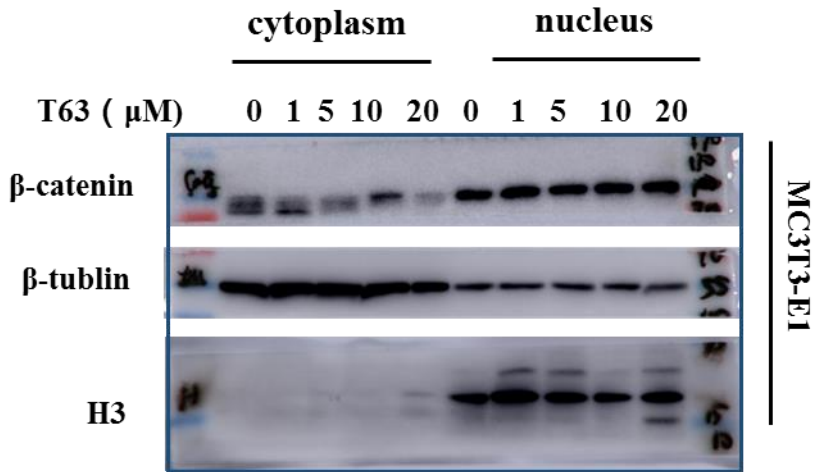
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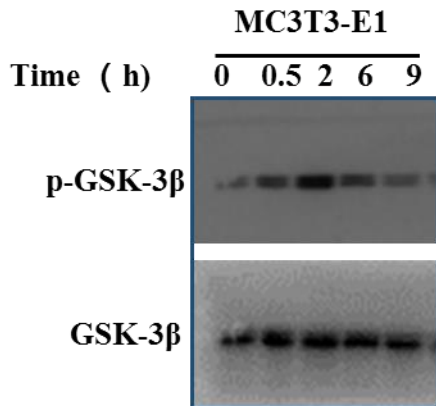
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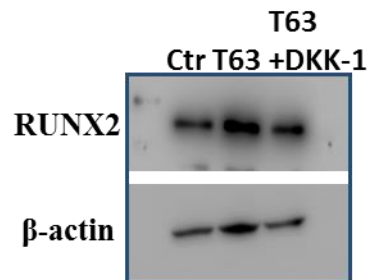
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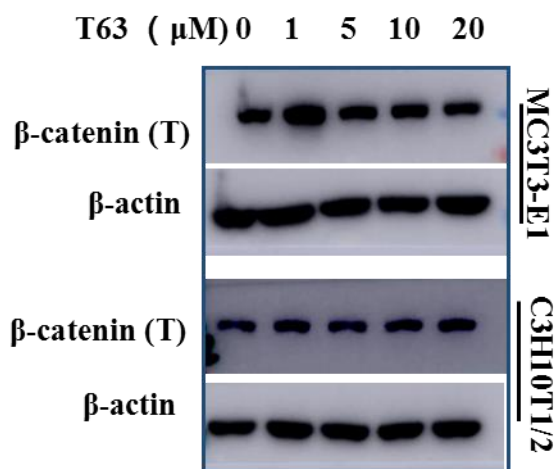
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h

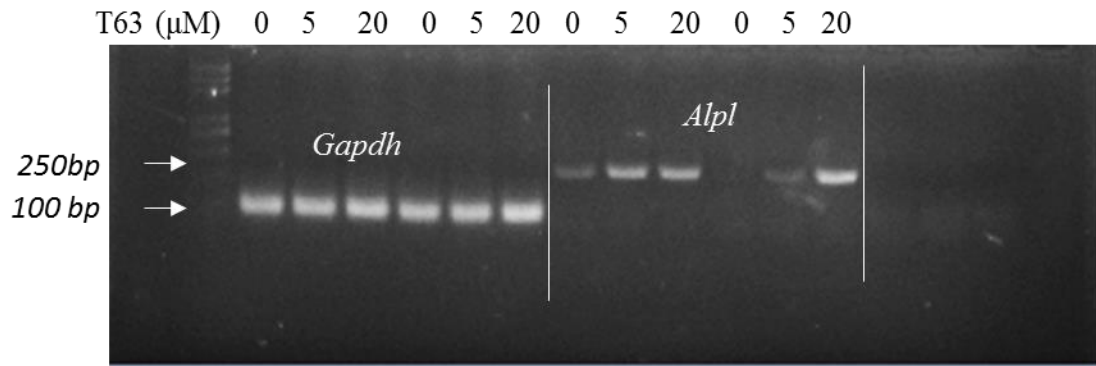


**i**

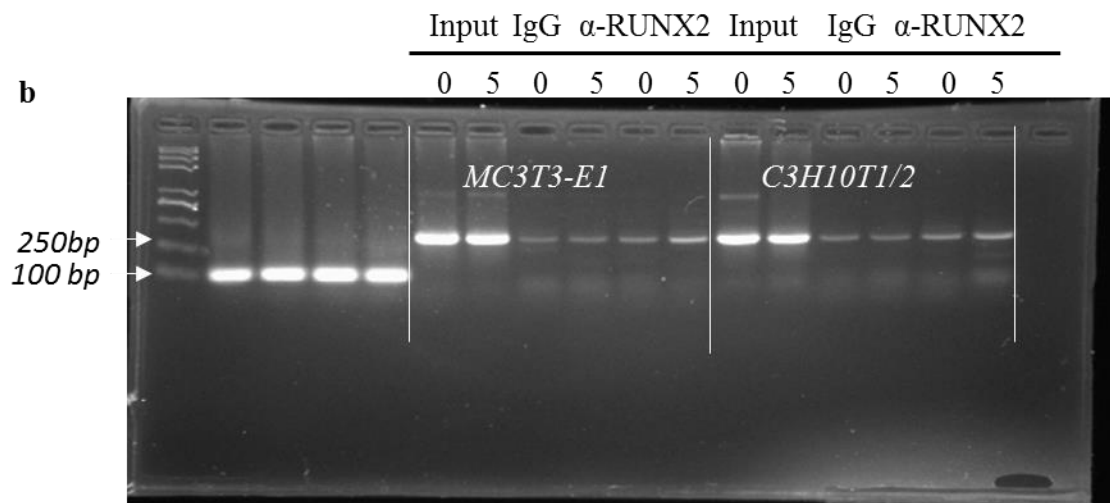


## S6. Uncropped gel for Figures

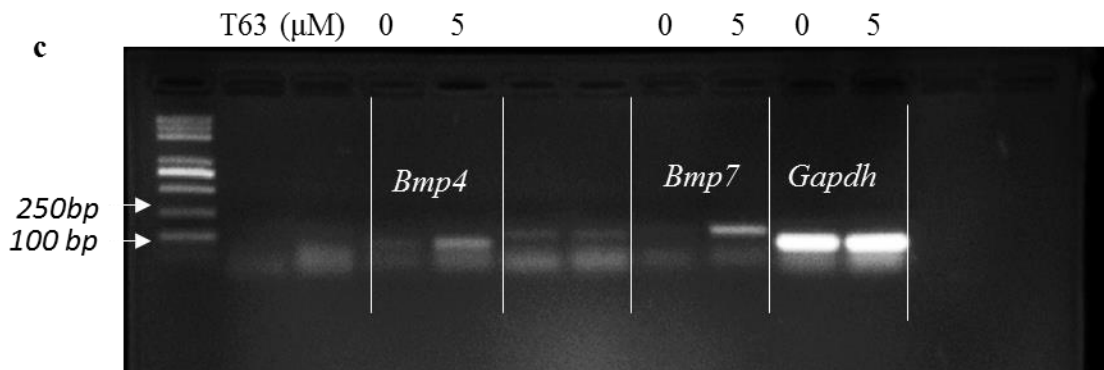
**a**



**b**



**c**



**Fig. S6a:** full gel of Fig.2b.

**Fig. S6b:** full gel of Fig. 4b.

**Fig. S6c:** full gel of supplementary Fig. S1b.