

RESEARCH ARTICLE

3D-bioprinted osteocytes expressing Wnt7b protect osteoblast differentiation from microgravity

Supplementary file

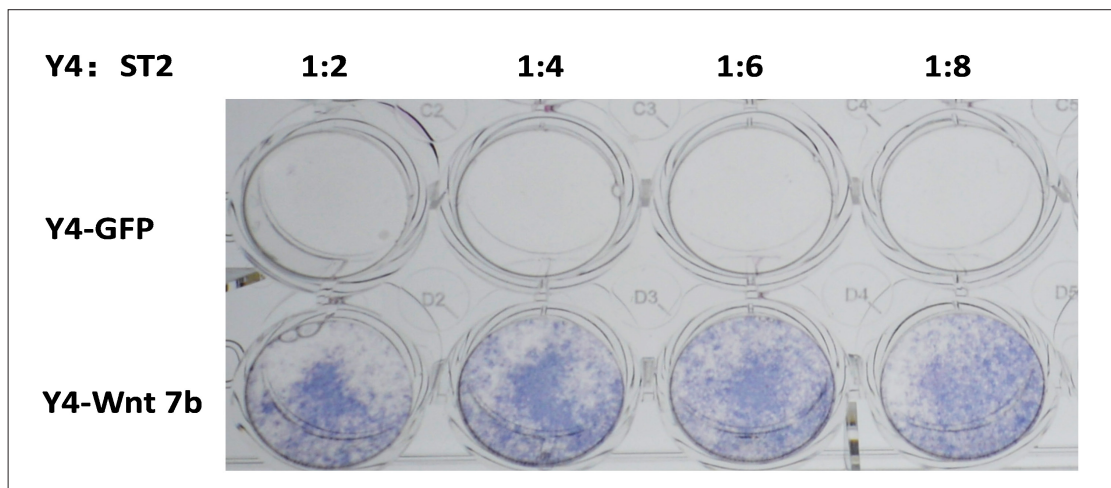


Figure S1. Alkaline phosphatase (AP) staining of MLO-Y4 and ST2 co-cultures inoculated at ratios of 1:2, 1:4, 1:6, and 1:8 for 3 days.

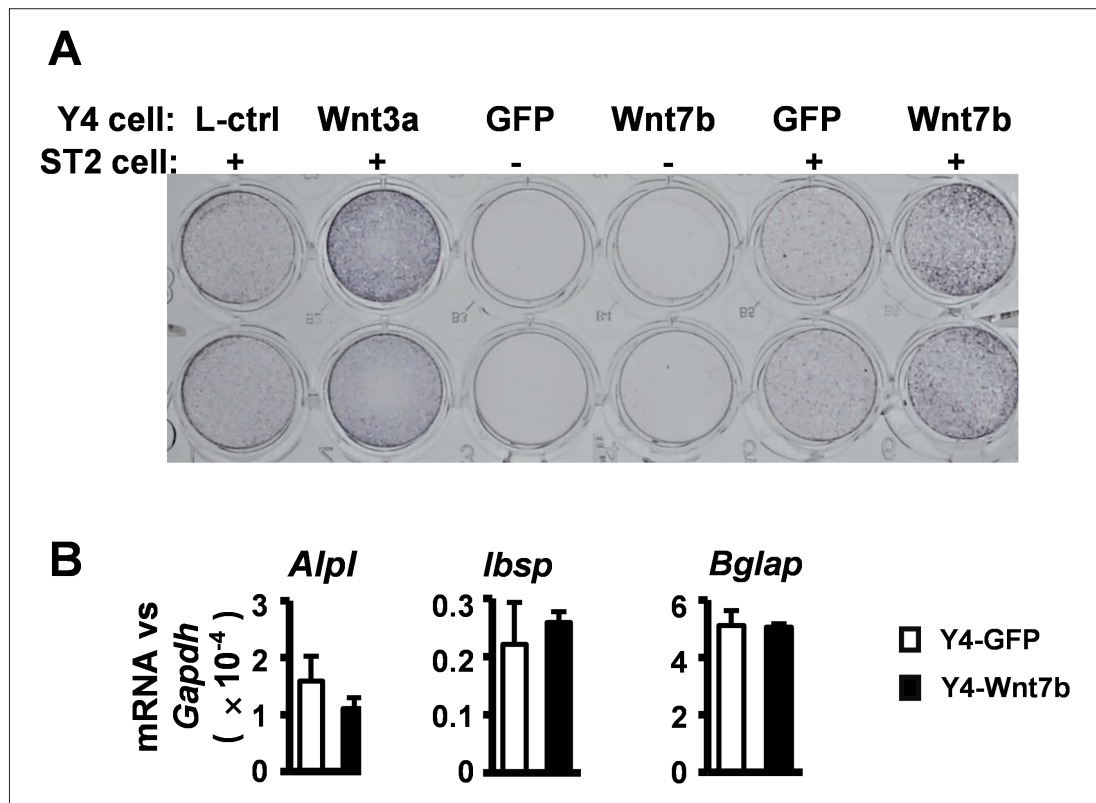


Figure S2. Effect of Wnt7b on inducing osteocytes dedifferentiation into osteoblasts. (A) Alkaline phosphatase (AP) staining of cultured Y4-Wnt7b and its control Y4-GFP cells. The L-ctrl+ST2 and Wnt3a+ST2 groups were used as negative and positive controls, respectively. (B) Reverse transcription quantitative polymerase chain reaction (RT-qPCR) results of osteoblastic marker genes in Y4-Wnt7b ($n = 3$).

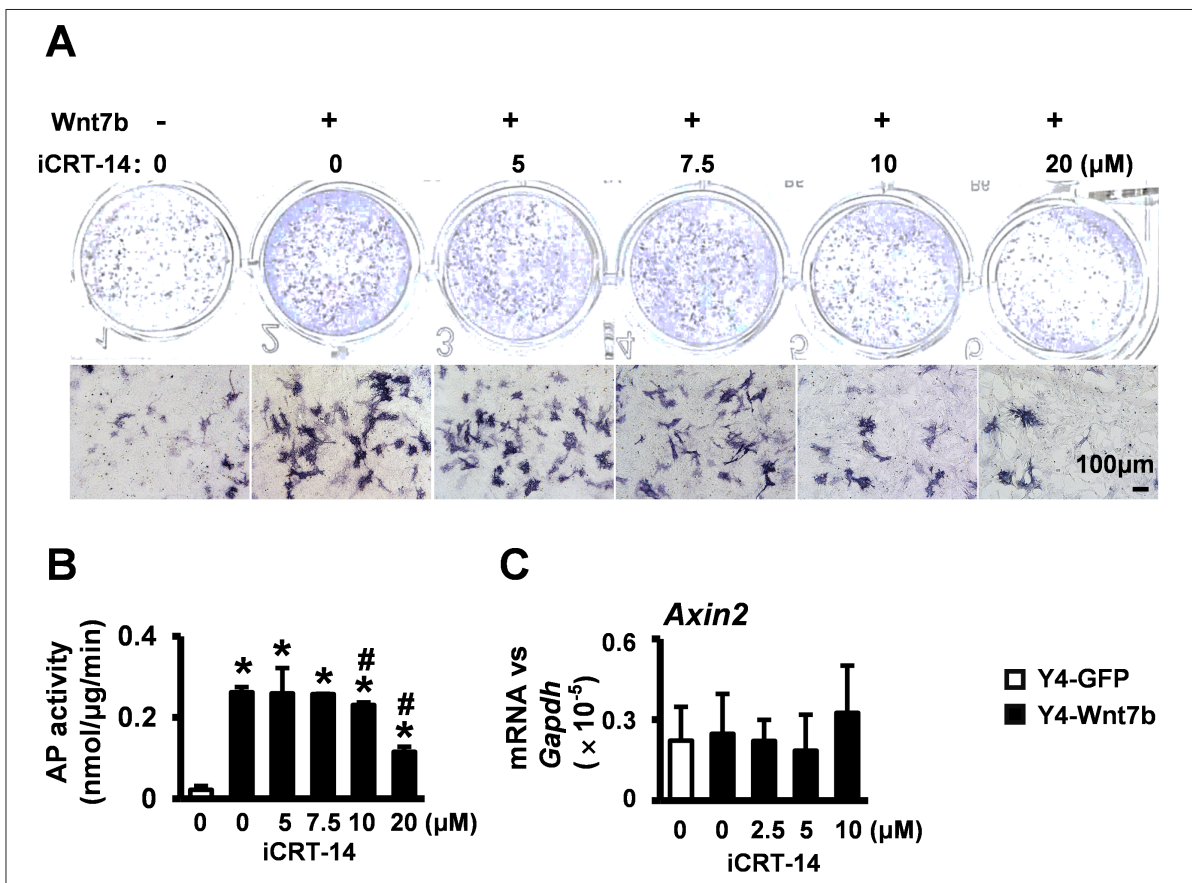


Figure S3. Effect of Wnt canonical signaling antagonist on Wnt7b-induced osteoblast differentiation. The co-culture of Y4-Wnt7b and ST2 cells was treated with iCRT-14 for 3 days and tested for osteoblast differentiation. (A) Alkaline phosphatase (AP) staining. The lower images are enlarged from the 24-well plate. Scale bar: 100 μm. (B) AP biochemical activity assay. (C) Quantitative polymerase chain reaction (qPCR) of Wnt canonical signaling target gene *Axin2* in MLO-Y4 cells. **p* < 0.05 versus Y4-GFP, #*p* < 0.05 versus DMSO-treated Y4-Wnt7b; *n* = 3.

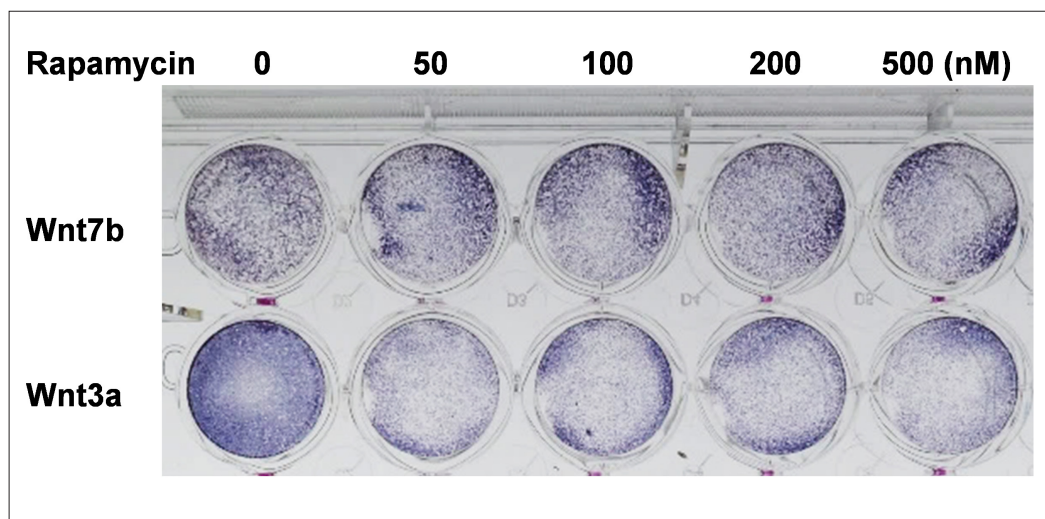


Figure S4. Effect of mTORC1 signaling on Wnt7b-induced osteoblast differentiation. The co-culture of Y4-Wnt7b and ST2 cells was treated with mTOR signaling inhibitor rapamycin for 3 days and tested for osteoblast differentiation by alkaline phosphatase (AP) staining. Rapamycin partially inhibited osteoblast differentiation of ST2 cells induced by Wnt3a-conditioned medium from stimulated MLO-Y4 cells, which served as the positive control.

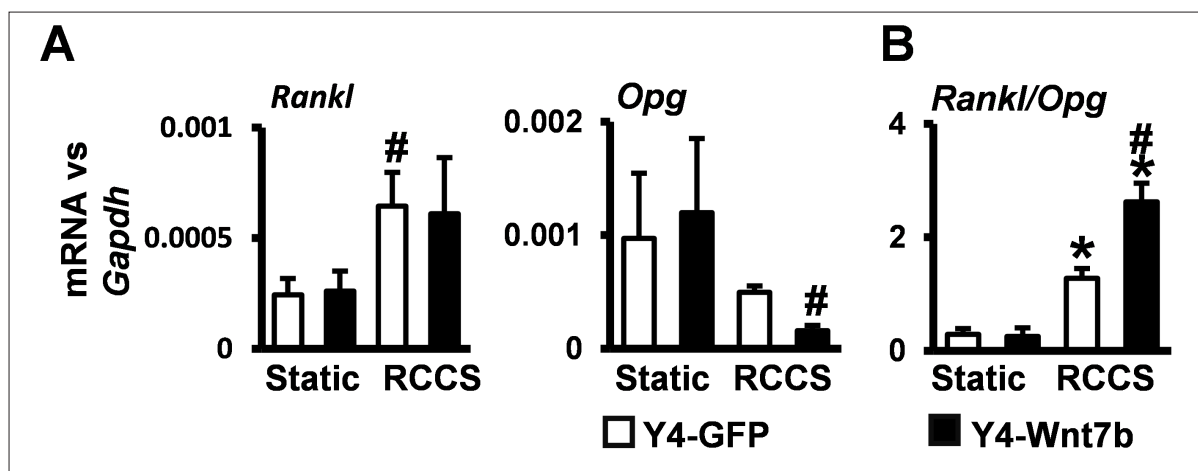


Figure S5. Potential effects of osteocytic Wnt7b on osteoclast differentiation under microgravity conditions. Quantitative polymerase chain reaction (qPCR) results of pro- and anti-osteoclastogenic cytokines in Y4-Wnt7b PCI3D modules cultured in static and microgravity conditions for 7 days ($n = 3$); $*p < 0.05$ versus Y4-GFP group, $\#p < 0.05$ versus static condition.