

RESEARCH ARTICLE

Osteocytic PGE₂ receptors EP2/4 signaling create a physiological osteogenic microenvironment in polycaprolactone 3D modules

Supplementary File

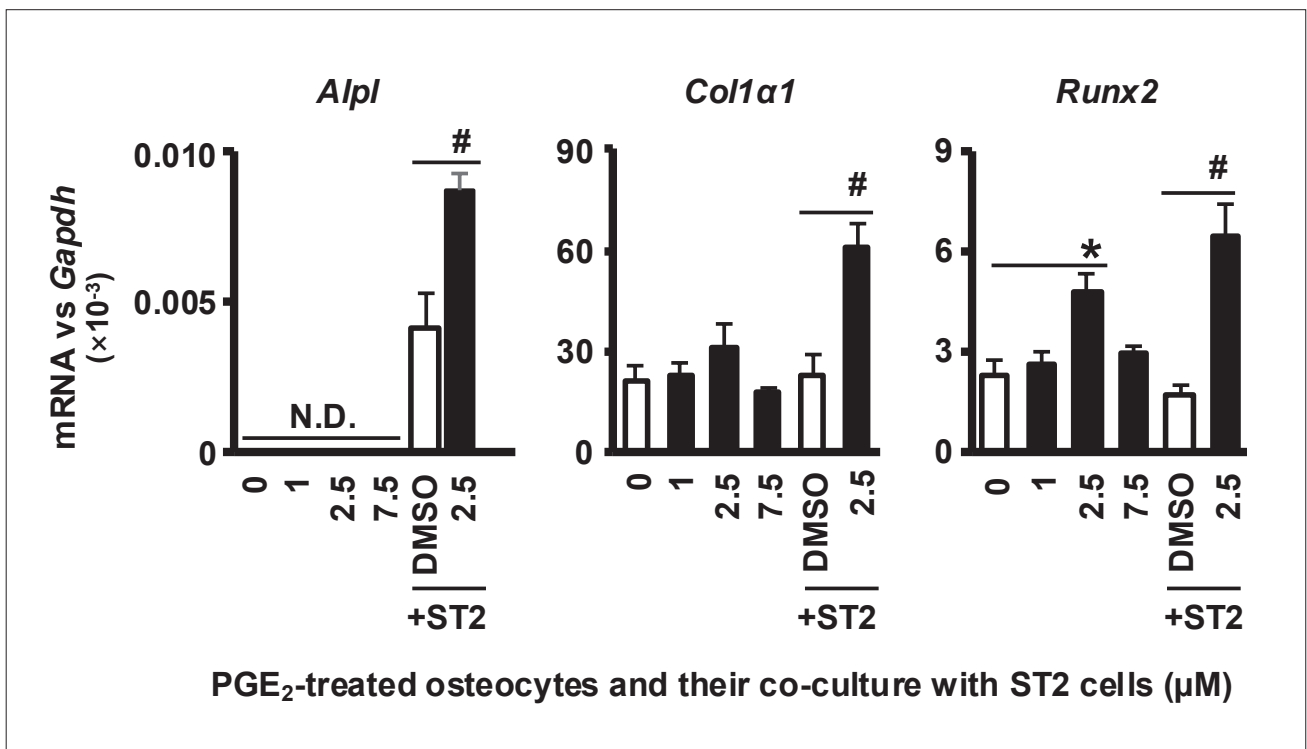


Figure S1. The expression of osteoblast marker genes in PGE₂-treated osteocytic MLO-Y4 cells was examined by qPCR. The MLO-Y4 cells were treated with PGE₂ at the indicated concentrations for one day. The three-day-co-culture of PGE₂-treated MLO-Y4 cells and ST2 cells (1:4) were used as the positive control. **p* < 0.05 vs. DMSO-treated MLO-Y4 cells; #*p* < 0.05 vs. the co-culture of DMSO-treated MLO-Y4 cells and ST2 cells by Student's *t*-test, *n* = 3. Abbreviations: DMSO: Dimethyl sulfoxide; qPCR: Quantitative polymerase chain reaction; N.D.: Not detected.

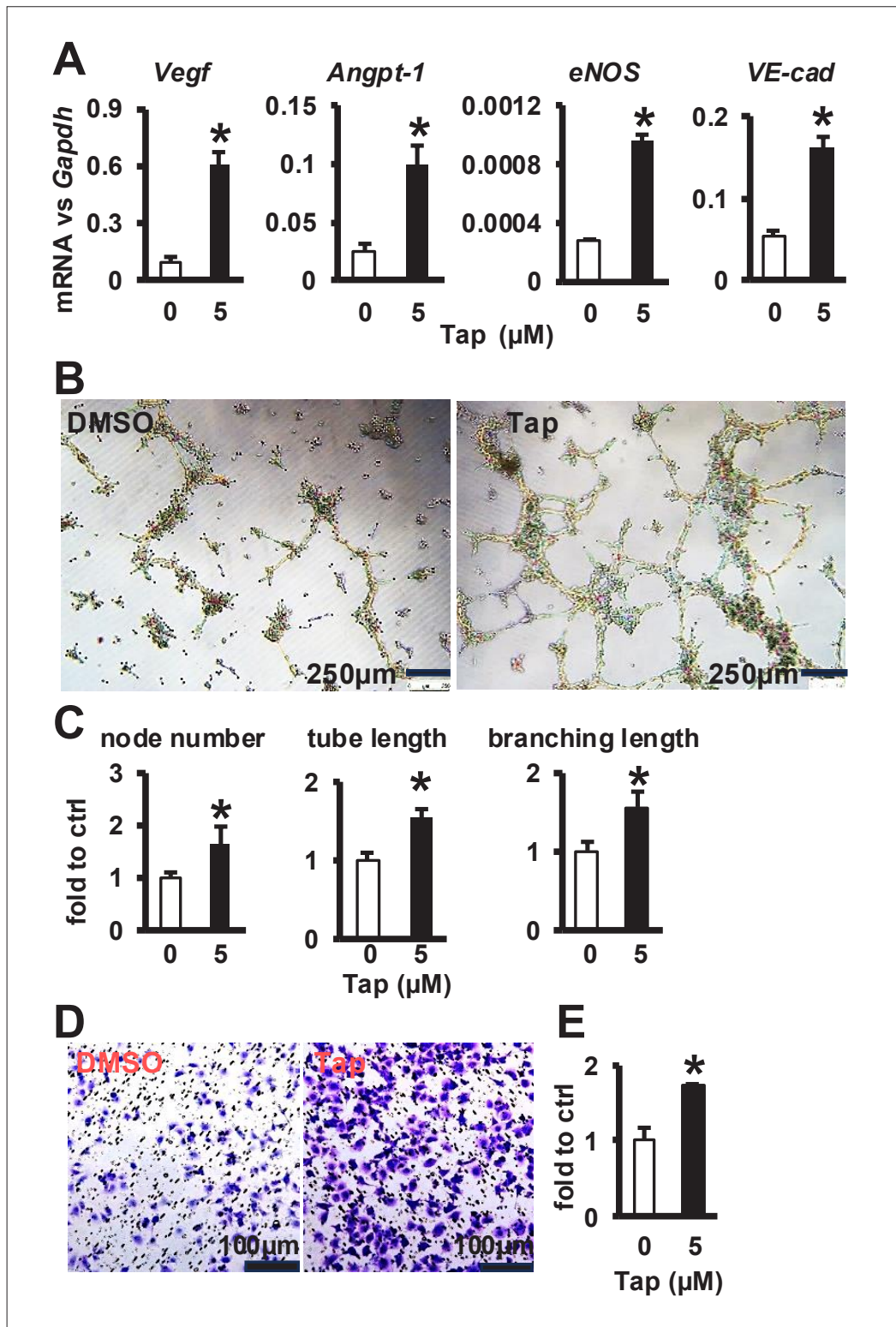


Figure S2. The effect of osteocytes treated with EP2 agonist on angiogenesis in HUVECs. (A) Angiogenic-related gene expression in the co-culture of 5 μM Tap-treated MLO-Y4 cells and HUVECs for three days. (B and C) Images (B) and calculation (C) of vascular tubule formation in the above co-culture for 4 h. (D) Migration of HUVECs cultured in 30% CM for one day. Scale bars: 100 μm. **p* < 0.05 by Student's *t*-test, *n* = 3. Abbreviations: CM: Conditioned medium of 5μM Tap-treated MLO-Y4 cells; HUVECs: Human umbilical vein endothelial cells; DMSO: Dimethyl sulfoxide; ctrl: Control.

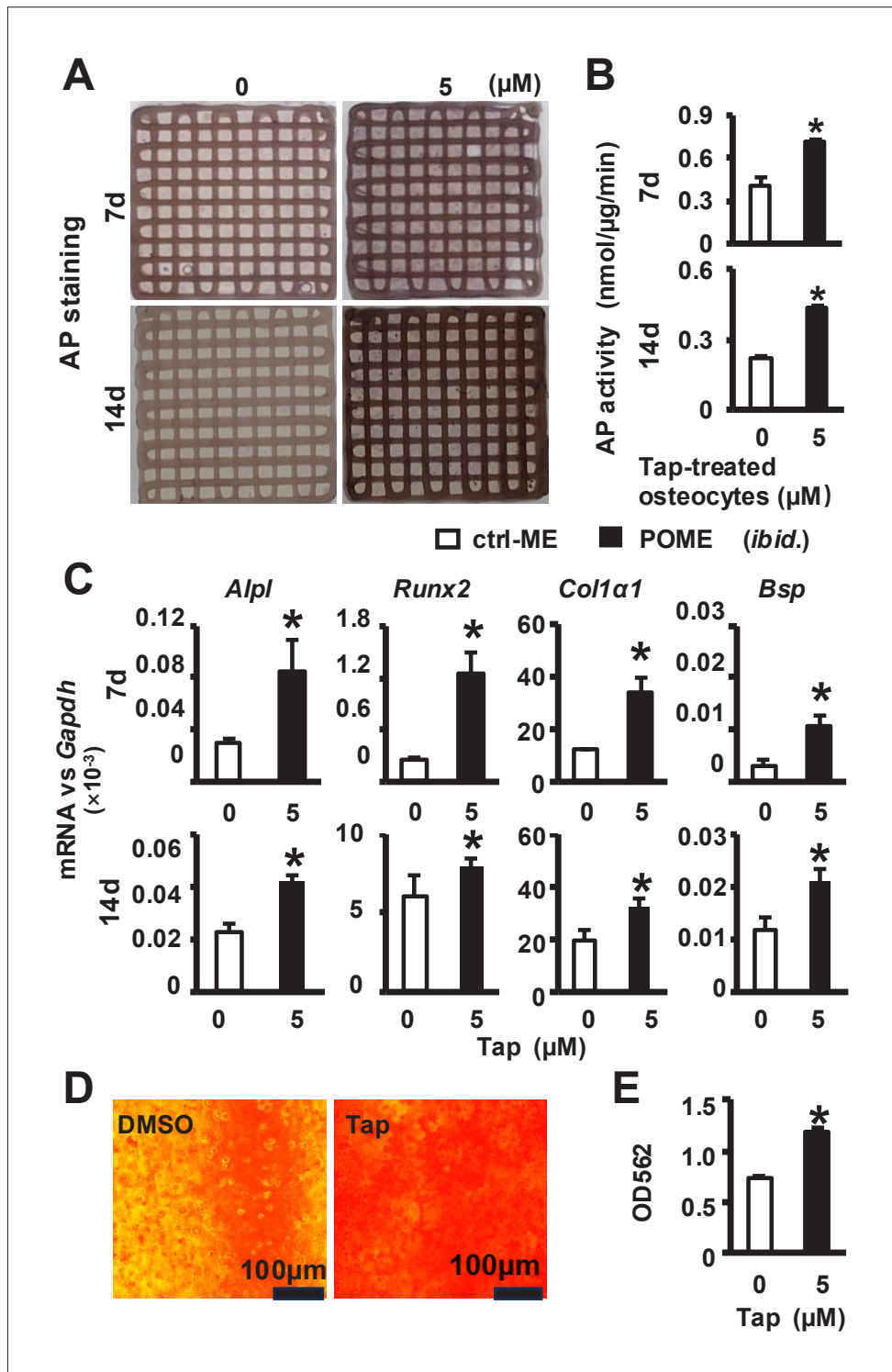


Figure S3. Effects of EP2 agonist-treated osteocytes in PCI3D modules on osteogenesis. Tap-treated MLO-Y4 cells promoted osteogenesis of ST2 cells in PCI3D modules. (A–C) After being cultured for 7 and 14 days, the cells were subjected to AP staining (A), its biochemical activity assay (B), and qPCR for the detection of gene expression of osteoblast marker (C). (D–E) Mineralization assay of bone nozzle formation by Alizarin Red S staining in the PCI3D modules. The PCI3D modules were cultured in a growth medium for seven days and an osteogenic medium for another 21 days. Scale bars: 100 μm . * $p < 0.05$ by Student’s t -test, $n = 3$. Abbreviations: PCI3D: Polycaprolactone- and cell-integrated 3D printing system; POME: Physiological osteogenic microenvironment of the MLO-Y4 cells treated by EP2 agonist Tap for one day; ctrl-ME: Control microenvironment of the MLO-Y4 cells treated by DMSO for one day; AP: Alkaline phosphatase; qPCR: Quantitative polymerase chain reaction; OD: Optical density.