

ORIGINAL RESEARCH ARTICLE

Jawbone periosteal cell spheroids exhibit early osteogenic responses through F-actin/NF- κ B-mediated mechanotransduction

Supplementary Files

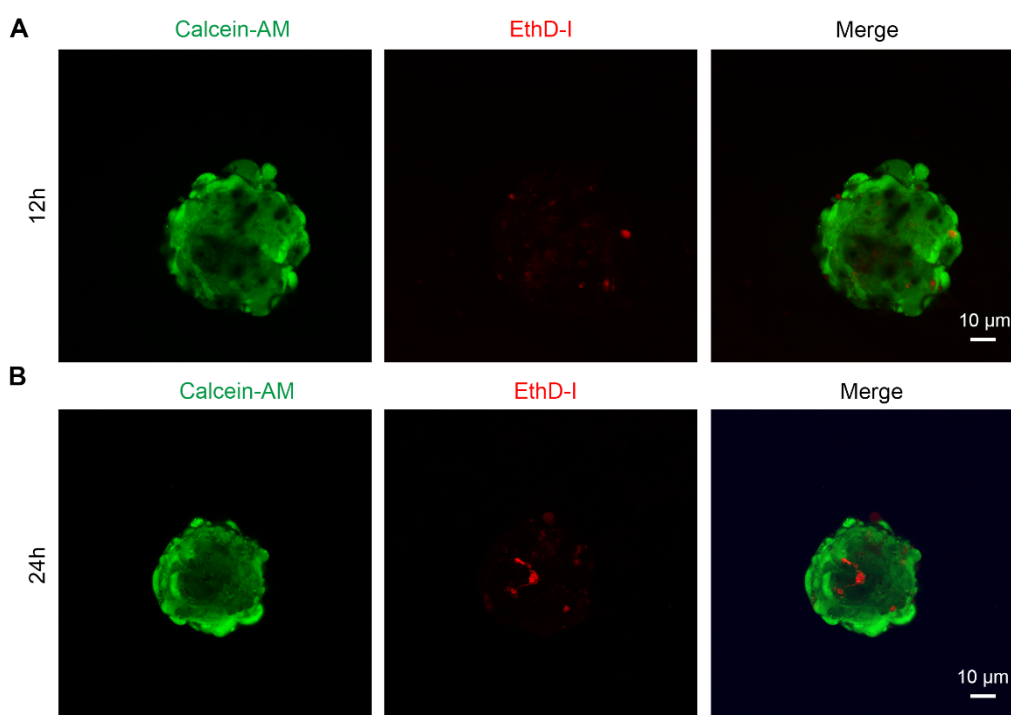


Figure S1. Representative live/dead staining images of jawbone periosteum-derived cell spheroids at (A) 12 h and (B) 24 h of culture. Live cells are shown in green (Calcein AM), whereas dead cells are shown in red (ethidium homodimer-1 [EthD-I]) (scale bar = 10 μ m, magnification = 40 $\times$).

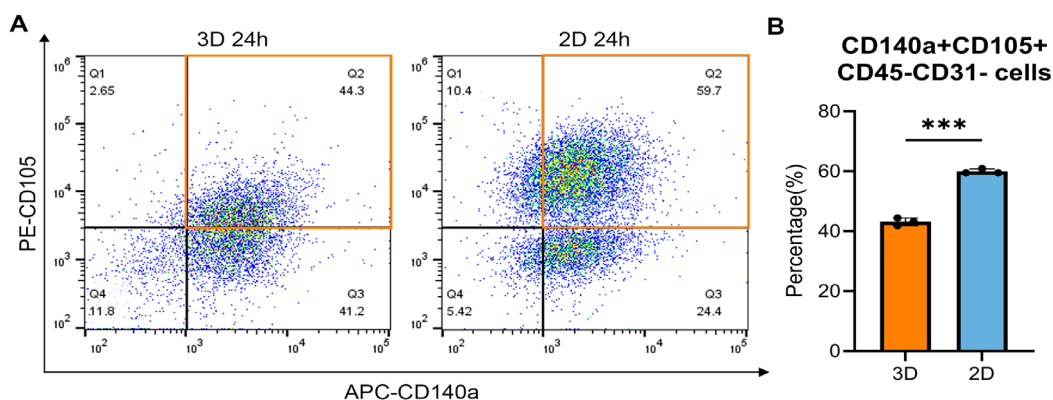


Figure S2. Flow cytometric analysis of the skeletal stem cell population in 3D jawbone periosteum-derived cell spheroids and 2D monolayer cultures at 24 h. (A) Representative flow cytometry plots showing the skeletal stem cell population in 3D jawbone periosteum-derived cell spheroids and 2D monolayer cultures after 24 h of *in vitro* culture. (B) Flow cytometric quantification of the proportion of the skeletal stem cell population (CD140a⁺CD105⁺CD45⁻CD31⁻) in 3D spheroids and 2D monolayer cultures after 24 h of *in vitro* culture ($n = 3$). Statistical comparisons between two groups were performed using Student's *t*-test. *** $p < 0.001$ indicates statistical significance. Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional.

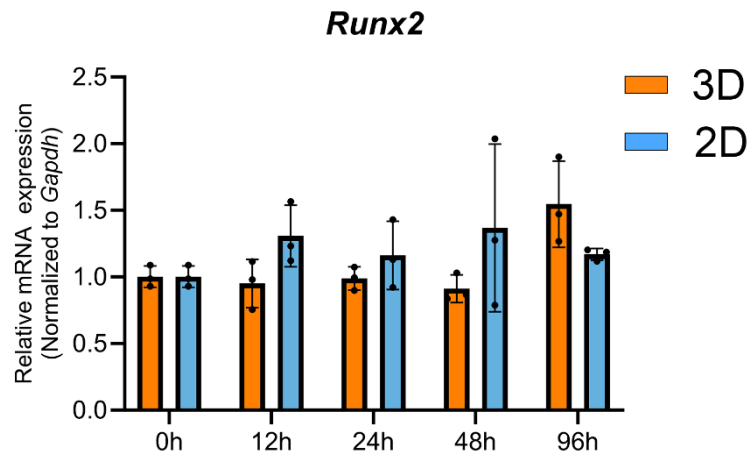


Figure S3. Relative *Runx2* mRNA expression in 3D jawbone periosteum-derived cell spheroids versus 2D monolayers over 96 h of culture ($n = 3$). Statistical comparisons between two groups were performed using Student's *t*-test.

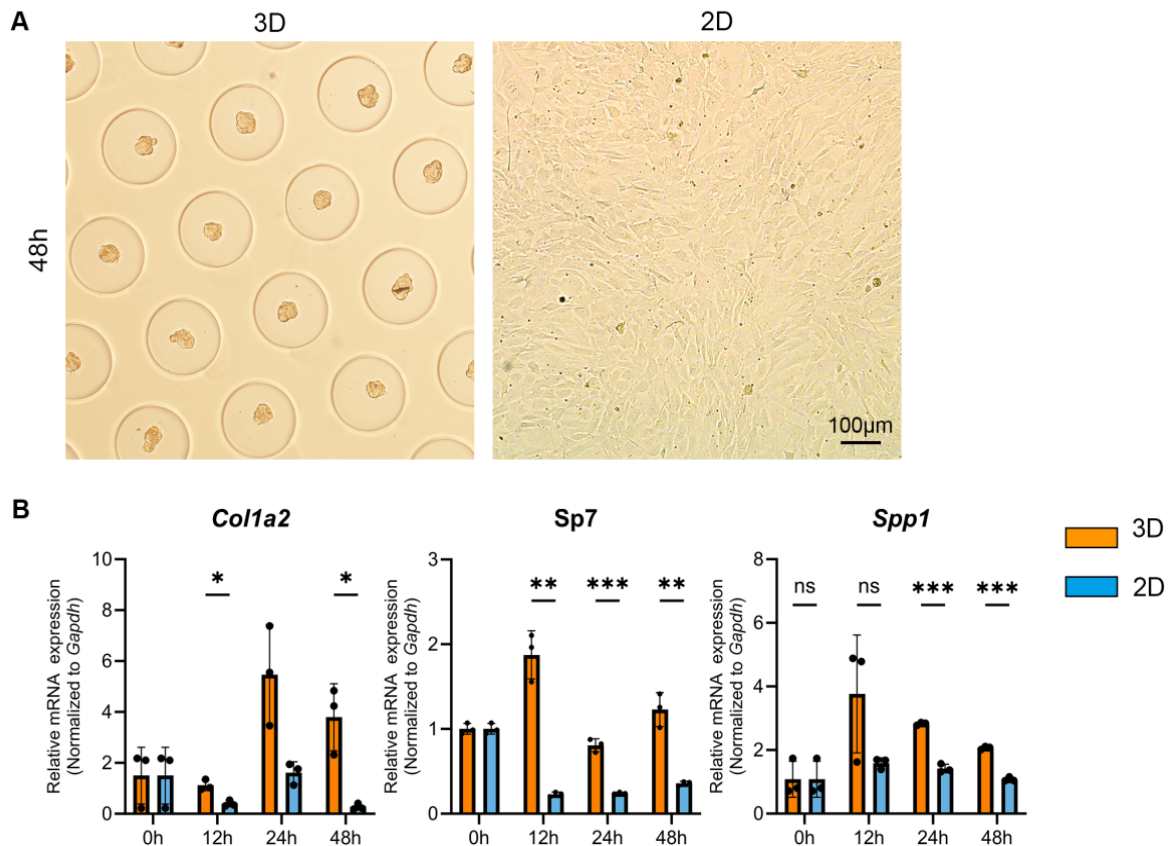


Figure S4. Osteogenic gene expression in 3D spheroids versus 2D monolayers under matched cell density. (A) Bright-field images of 3D spheroids and 2D monolayers under matched cell density at 48 h after seeding (scale bar = 100 μ m, magnification = 10 $\times$). (B) Relative mRNA expression levels of *Col1a2*, *Sp7*, and *Spp1* in jawbone periosteum-derived cells cultured as 3D spheroids and 2D monolayers under matched cell density ($n = 3$). Statistical comparisons between two groups were performed using Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate statistical significance, whereas “ns” indicates no statistical significance.

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional.

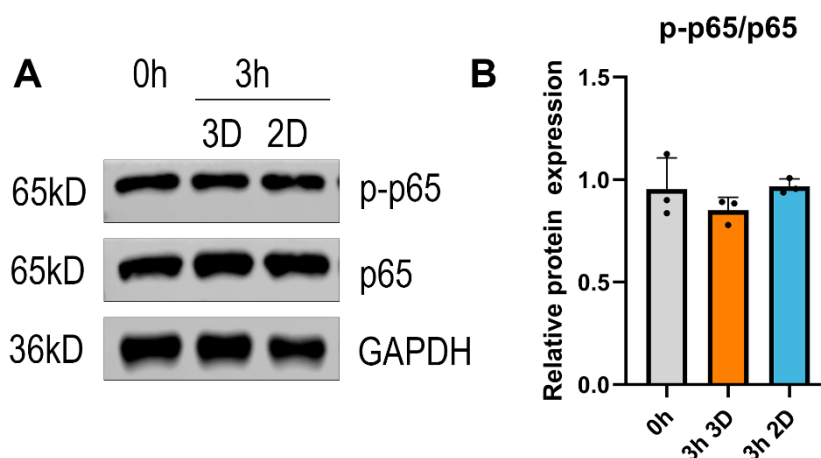


Figure S5. Phosphorylation of p65 in 3D spheroids versus 2D monolayer cultures at 3 h after seeding. (A) Western blot analysis of p-p65 and total p65 in 2D monolayers (0 h) and 3D spheroids (3 h after seeding). (B) Densitometric quantification of the p-p65/p65 ratio ($n = 3$). Statistical analysis was performed using one-way analysis of variance.

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; p-p65: Phosphorylated p65.

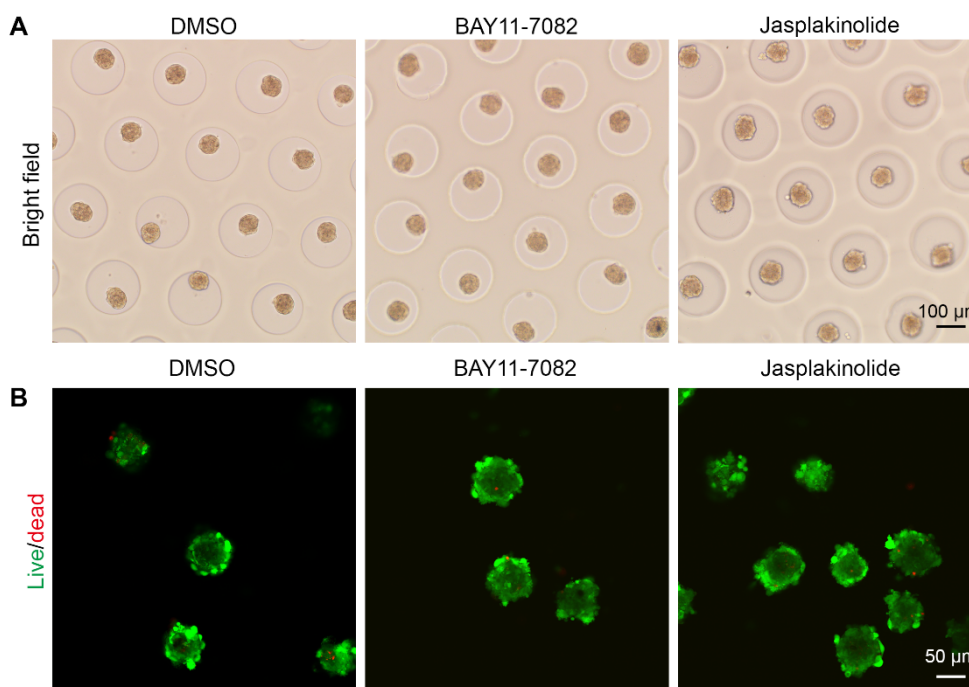


Figure S6. Effects of pharmacological treatments on spheroid formation and cell viability. Representative (A) bright-field images and (B) live/dead staining images of jawbone periosteum-derived cell spheroids after 48 h of culture with dimethyl sulfoxide (DMSO) vehicle control, BAY11-7082 (1 μ M), or jasplakinolide (50 nM). Live cells were stained with Calcein AM (green), whereas dead cells were stained with ethidium homodimer-1 (red) (scale bar = 100 μ m, magnification = 10 $\times$; scale bar = 50 μ m, magnification = 20 $\times$). None of the treatments significantly affected spheroid morphology, formation efficiency, or cell viability compared to the DMSO control.

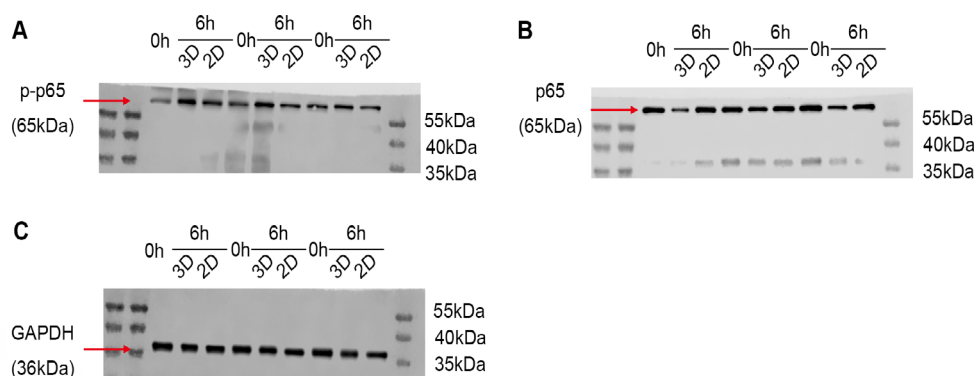


Figure S7. Original images for Western blotting for phosphorylated p65, total p65 and GAPDH in Figure 3G. (A) Western blotting for p-p65 (65 kDa), (B) Western blotting for p65 (65 kDa) and (C) Western blotting for GAPDH (36 kDa).
Abbreviations: 2D: Two-dimensional; 3D: Tree-dimensional; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; p-p65: Phosphorylated p65.

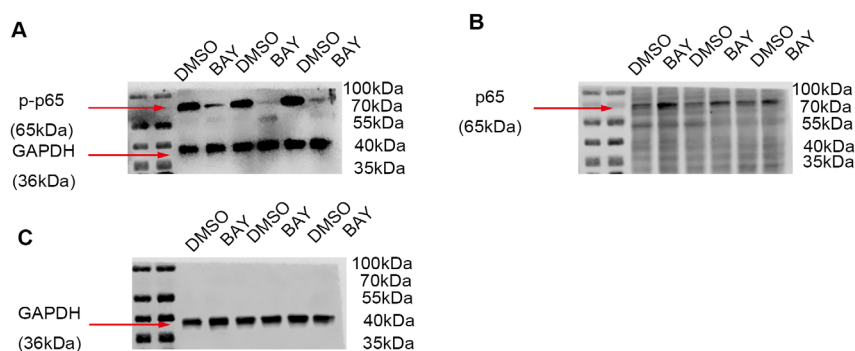


Figure S8. Original images for Western blotting for phosphorylated p65, total p65 and GAPDH in Figure 4C. (A) Western blotting for p-p65 (65 kDa), (B) Western blotting for p65 (65 kDa) and (C) Western blotting for GAPDH (36 kDa).
Abbreviations: DMSO: Dimethyl sulfoxide; BAY: BAY11-7082; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; p-p65: Phosphorylated p65.

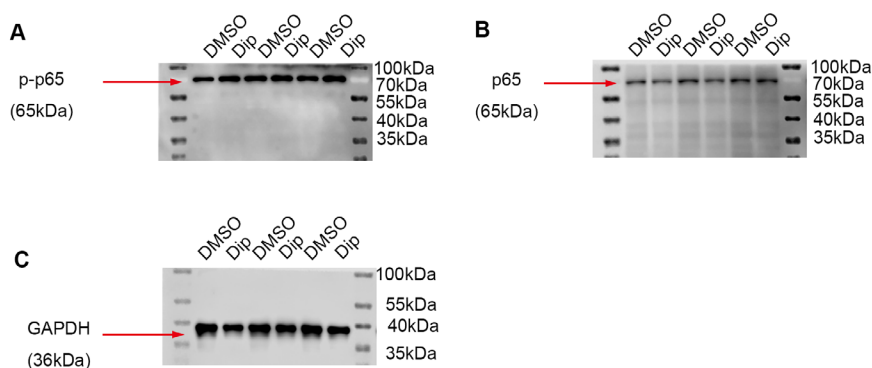


Figure S9. Original images for Western blotting for phosphorylated p65, total p65 and GAPDH in Figure 4G. (A) Western blotting for p-p65 (65 kDa), (B) Western blotting for p65 (65 kDa) and (C) Western blotting for GAPDH (36 kDa).
Abbreviations: DMSO: Dimethyl sulfoxide; Dip: Diprovocim; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; p-p65: Phosphorylated p65.

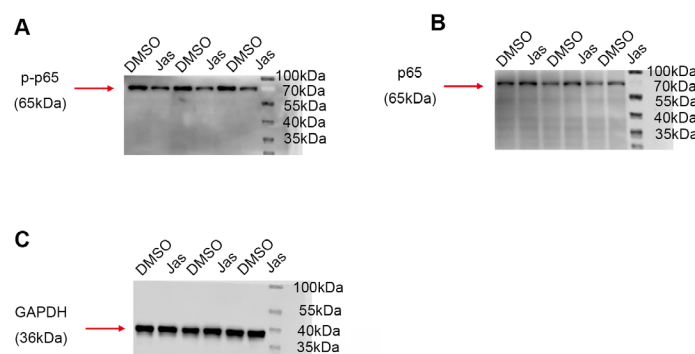


Figure S10. Original images for Western blotting for phosphorylated p65, total p65 and GAPDH in Figure 5B. (A) Western blotting for p-p65 (65 kDa), (B) Western blotting for p65 (65 kDa) and (C) Western blotting for GAPDH (36 kDa).
Abbreviations: DMSO: Dimethyl sulfoxide; Jas: Jasplakinolide; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; p-p65: Phosphorylated p65.

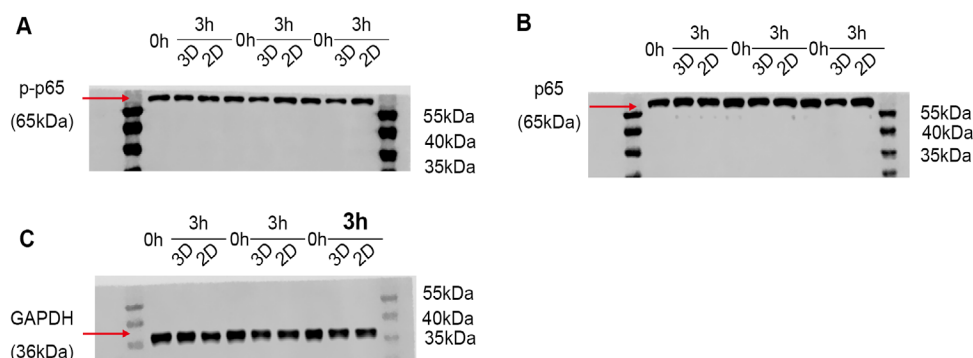


Figure S11. Original images for Western blotting for phosphorylated p65 and total p65 in Figure S5A. (A) Western blotting for p-p65 (65 kDa), (B) Western blotting for p65 (65 kDa) and (C) Western blotting for GAPDH (62 kDa).
Abbreviations: 2D: Two-dimensional; 3D: Tree-dimensional; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; p-p65: Phosphorylated p65.

Table S1. Quality control summary of RNA sequencing

Sample name	aggregate_P_1	aggregate_P_2	aggregate_P_3	monolayer_P_1	monolayer_P_2	monolayer_P_3
Total raw reads (M)	23.92	23.92	23.92	23.92	23.92	23.92
Clean reads (M)	23.73	23.76	23.75	23.76	23.76	23.75
Clean bases (Gb)	1.19	1.19	1.19	1.19	1.19	1.19
Clean reads Q30 (%)	93.57	93.88	94.17	93.67	93.92	93.98
Clean reads ratio (%)	99.21	99.3	99.3	99.3	99.33	99.29
Genome mapping rate (%)	95.82	96.11	96.13	96.03	96.1	96.17
Unique genome mapping (%)	82.54	82.77	82.84	81.02	81.02	81.46
Gene mapping rate (%)	91.43	91.56	91.65	91.95	92.11	92.06
Unique gene mapping (%)	86.68	86.81	86.94	86.51	86.65	86.73
RIN	Passed QC (RIN > 8.0)	Passed QC (RIN > 8.0)	Passed QC (RIN > 8.0)	Passed QC (RIN > 8.0)	Passed QC (RIN > 8.0)	Passed QC (RIN > 8.0)
Batch	Batch 1	Batch 1	Batch 1	Batch 1	Batch 1	Batch 1

Abbreviations: QC: Quality control; RIN: RNA integrity number.