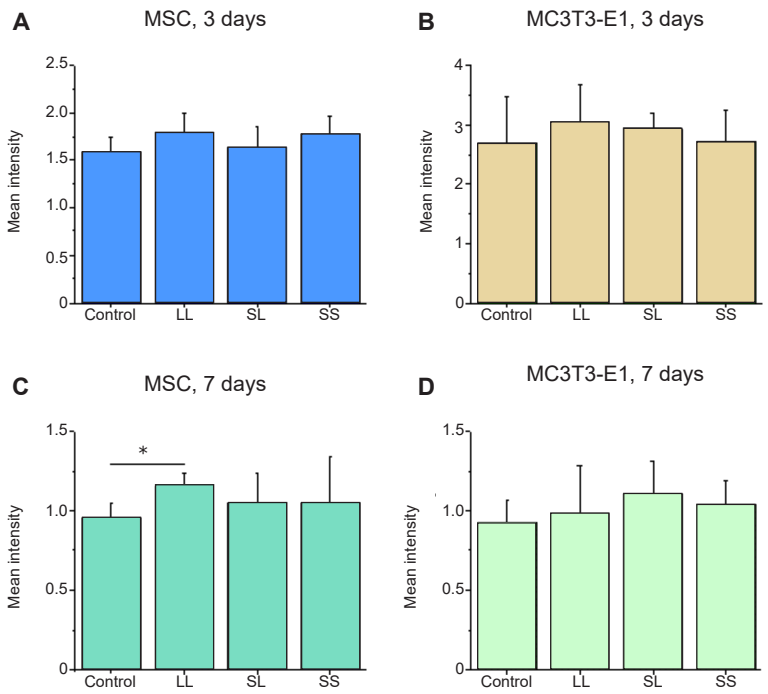
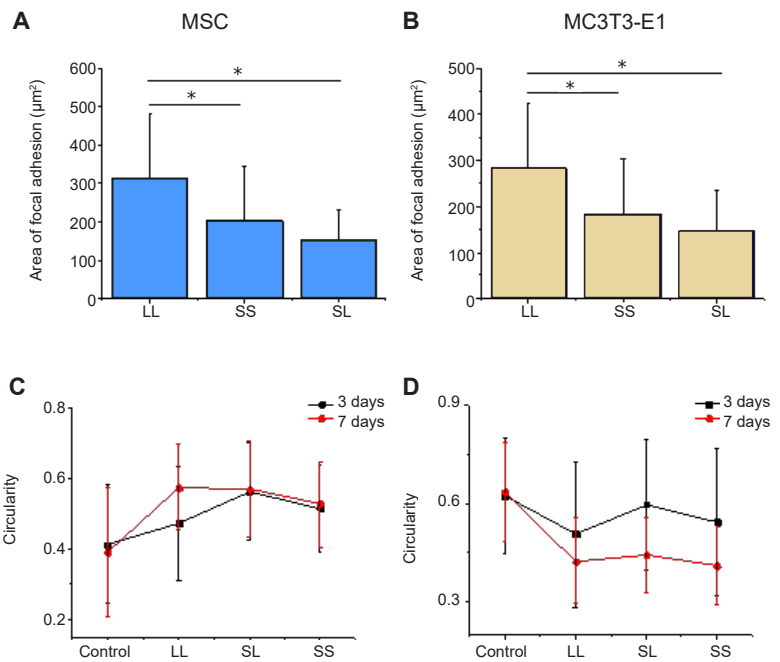
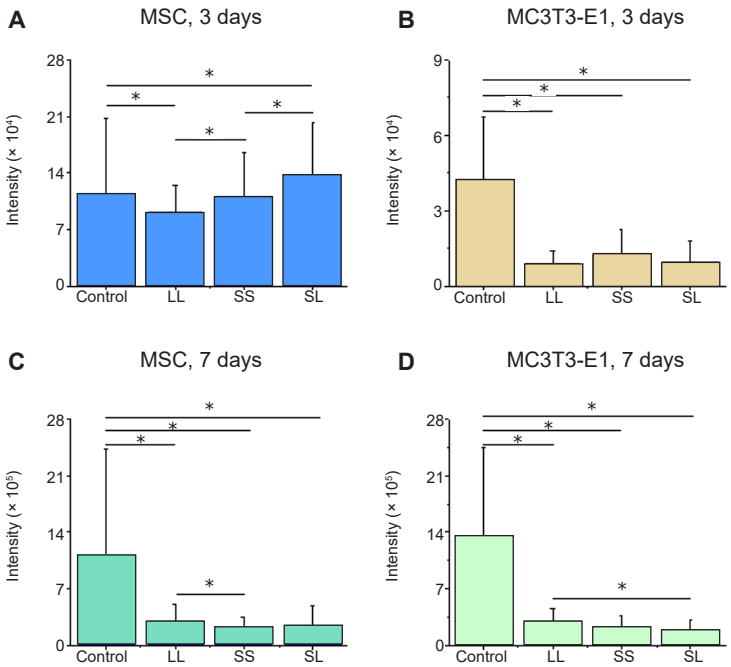


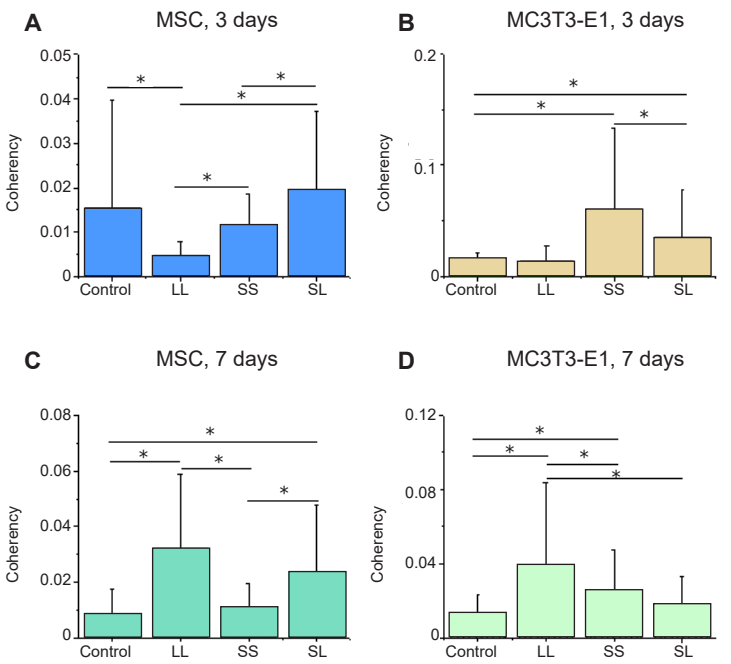
Additional Figure 1. Statistical analysis of the fluorescence intensity of TUNEL-stained cells cultured on micropatterned substrates. (A–D) MSCs and MC3T3-E1 cells cultured for 3 or 7 days. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling.



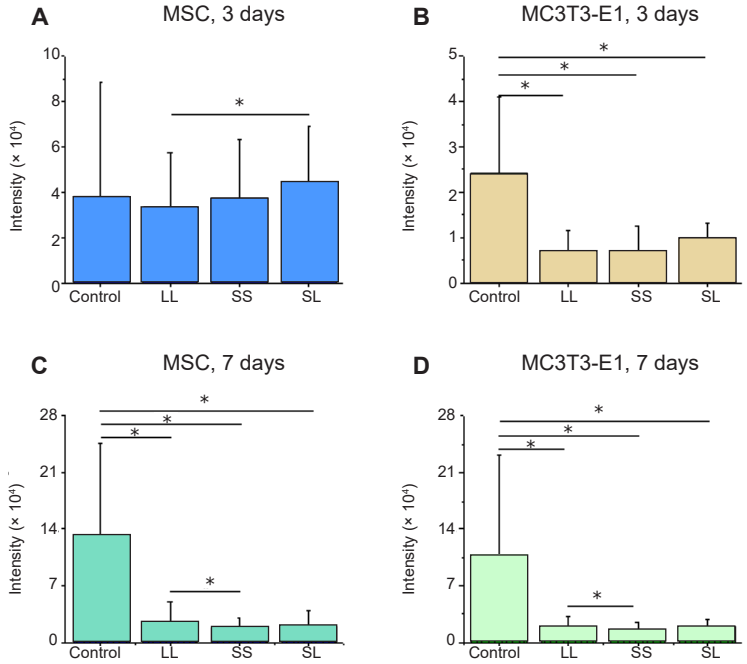
Additional Figure 2. Statistical analysis of the area and circularity of focal adhesions. (A) Area of focal adhesion of MSCs. (B) Area of focal adhesion of MC3T3-E1 cells. (C) Circularity of MSCs. (D) Circularity of MC3T3-E1 cells. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). CON: control; LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing.



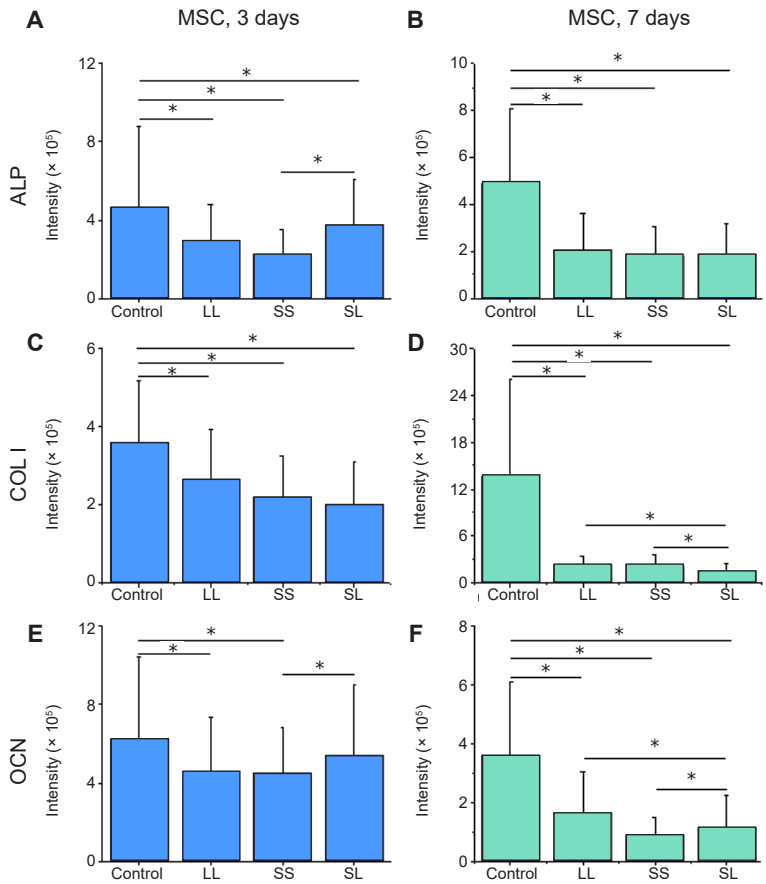
Additional Figure 3. Statistical results of fluorescence intensity of F-actin. (A–D) MSCs and MC3T3-E1 cells cultured for 3 or 7 days. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing.



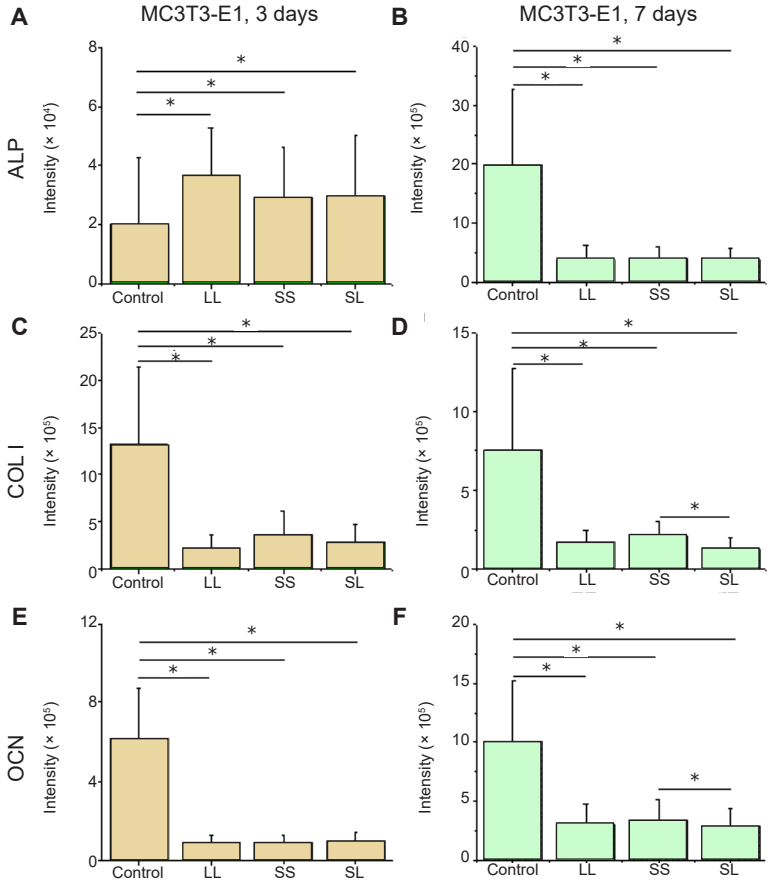
Additional Figure 4. Statistical results of F-actin coherency. (A–D) MSCs and MC3T3-E1 cells cultured for 3 or 7 days. The larger the value, the more ordered the actin, and the more consistent the direction of stress fibres. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing.



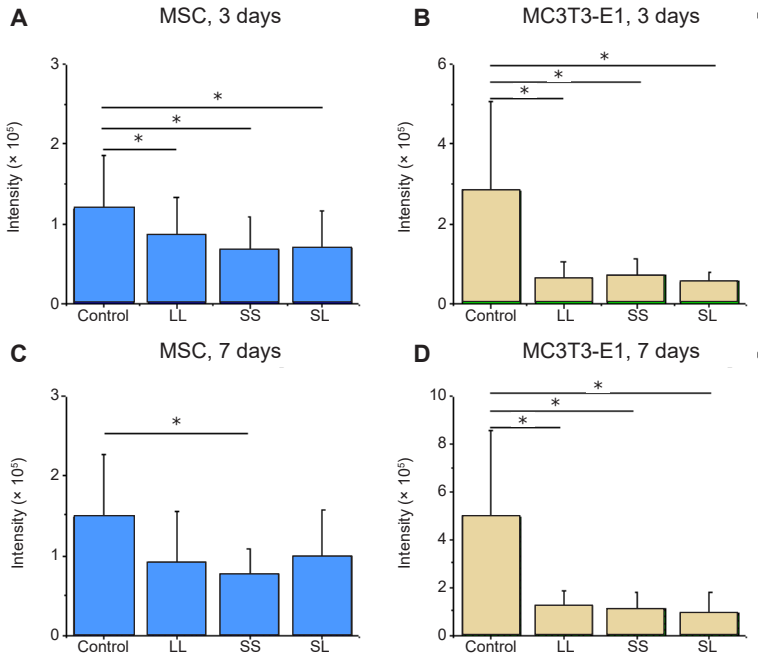
Additional Figure 5. Statistical results of fluorescence intensity of P-MLC2. (A–D) MSCs and MC3T3-E1 cells cultured for 3 or 7 days. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). LL: large circles with large spacing; MSC: mesenchymal stem cell; P-MLC2: phosphorylated myosin light chain 2; SL: small circles with large spacing; SS: small circles with small spacing.



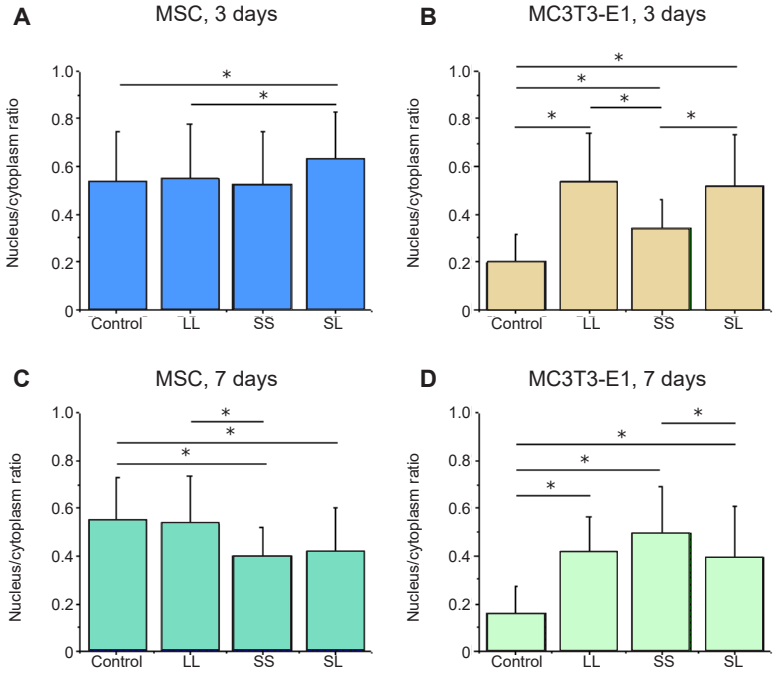
Additional Figure 6. Fluorescence intensity of osteogenic differentiation markers in MSCs cultured on micropatterned substrates. (A–F) Statistical results of fluorescent intensity of ALP, COL I, and OCN, respectively, after culture for 3 days (A, C, E) or 7 days (B, D, F). Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). ALP: alkaline phosphatase; COL I: type I collagen; LL: large circles with large spacing; MSC: mesenchymal stem cell; OCN: osteocalcin; SL: small circles with large spacing; SS: small circles with small spacing.



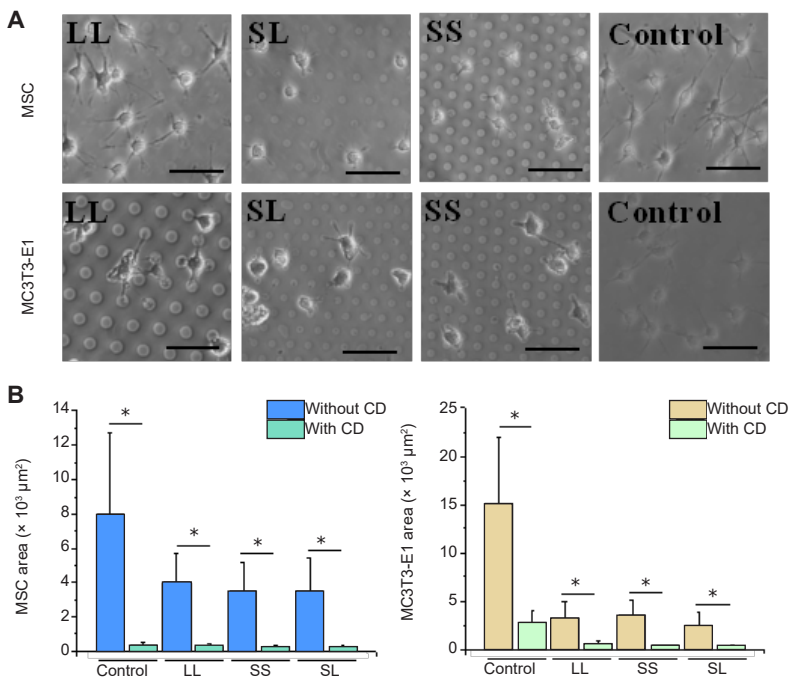
Additional Figure 7. Fluorescence intensity of osteogenic differentiation markers in MC3T3-E1 cells cultured on micropatterned substrates. (A–F) Statistical results of fluorescent intensity of ALP, COL I, and OCN in cells cultured for 3 days (A, C, E) or 7 days (B, D, F), respectively. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). ALP: alkaline phosphatase; COL I: type I collagen; LL: large circles with large spacing; OCN: osteocalcin; SL: small circles with large spacing; SS: small circles with small spacing.



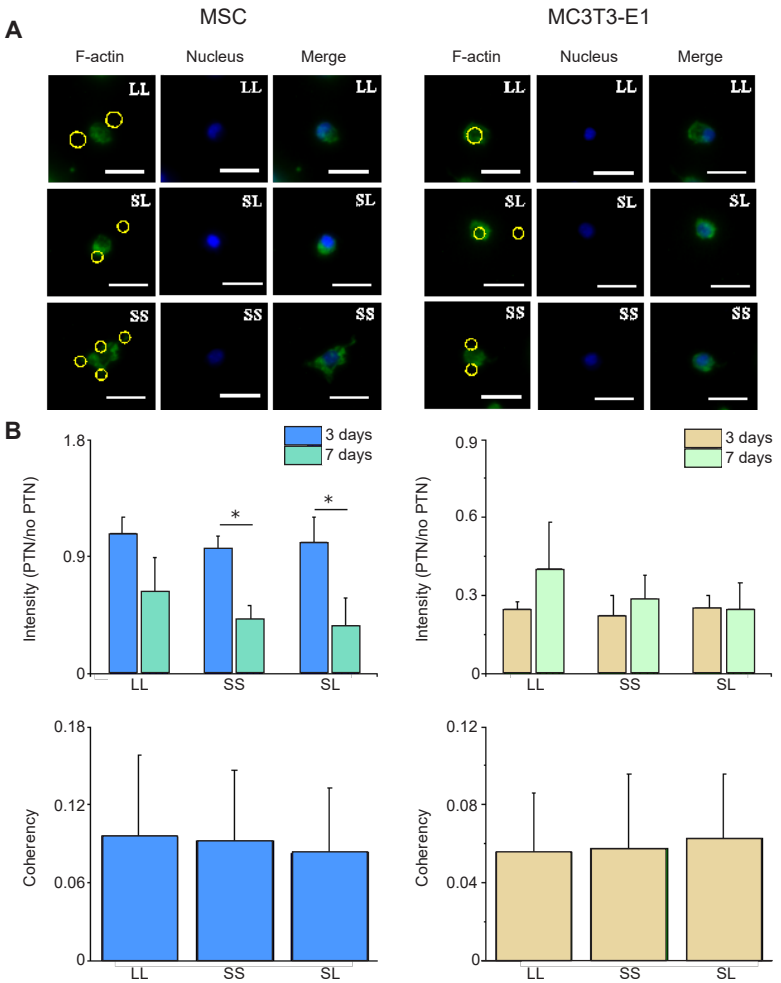
Additional Figure 8. Statistical results of fluorescence intensity of YAP. (A–D) MSCs and MC3T3-E1 cells cultured for 3 or 7 days. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing; YAP: yes-associated proteins.



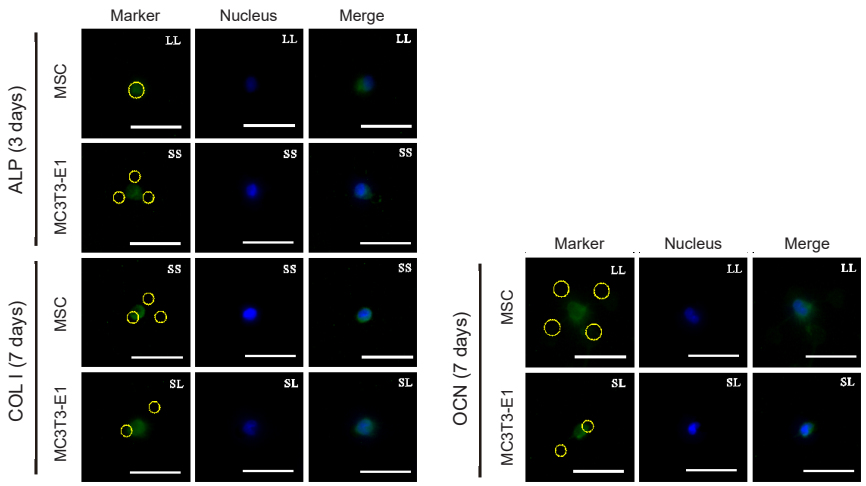
Additional Figure 9. Statistical analyses of the nuclear/cytoplasmic ratio of YAP. (A–D) MSCs and MC3T3-E1 cells cultured for 3 or 7 days. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing; YAP: yes-associated proteins.



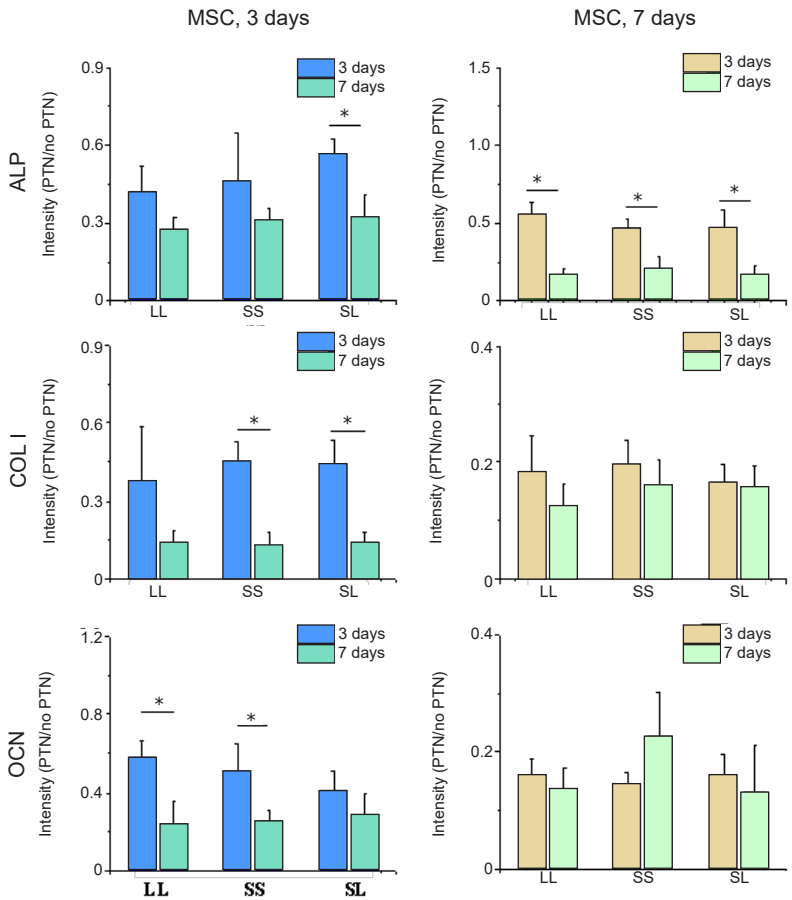
Additional Figure 10. Cell phenotypes and area after CD treatment for 24 hours. (A) Bright-field images of the cell on the micropatterned substrates. Cells were shrunk obviously. Scale bars: 50 μm. (B) Statistical analysis of the spreading area of the cells. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). CD: cytochalasin D; LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing.



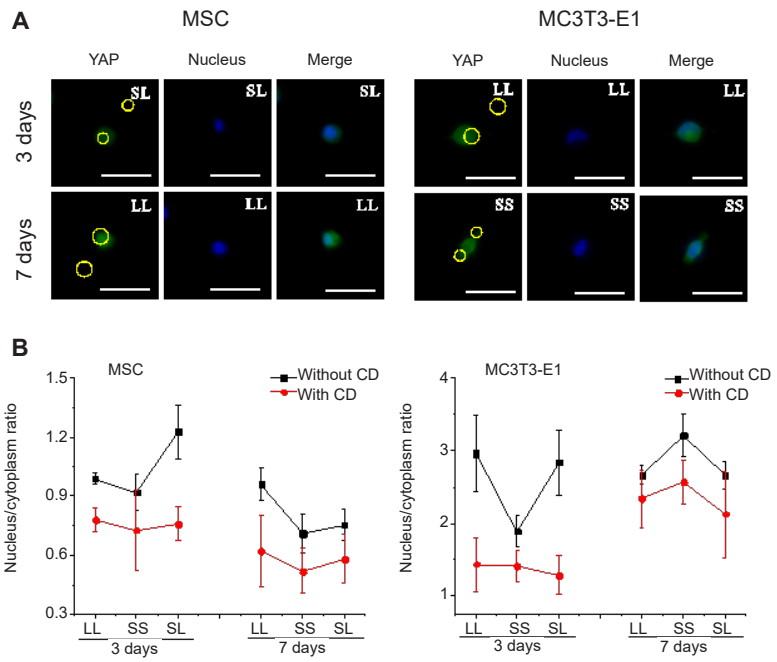
Additional Figure 11. Statistical results of F-actin staining after CD treatment for 24 hours. (A) Fluorescent images of F-actin in shrunk cells. Scale bars: 50 μm. (B) Statistical results of fluorescence intensity of F-actin. (C) Statistical results of F-actin coherency. The larger the value, the better the order of actin, and the more consistent the direction of stress fibre. Data are presented as mean ± SD (n = 3). *P < 0.05 (one-way analysis of variance followed by Tukey's *post hoc* analysis). CD: cytochalasin D; LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing.



Additional Figure 12. Fluorescent images of osteogenic differentiation markers (green, FITC-labeled) in individual cells treated with CD and cultured on the micropatterned substrates for 3 (ALP) or 7 days (COL I, OCN). The yellow circles represent the location of micropatterned islands. Scale bars: 50 μm. ALP: alkaline phosphatase; CD: cytochalasin D; COL I: type I collagen; FITC: fluorescein isothiocyanate; MSC: mesenchymal stem cell; OCN: osteocalcin.



Additional Figure 13. Fluorescence intensity of osteogenic markers in cells cultured on micropatterned substrates after CD treatment. Data are presented as mean ± SD ($n = 3$). *$P < 0.05$ (one-way analysis of variance followed by Tukey's *post hoc* analysis). ALP: alkaline phosphatase; CD: cytochalasin D; COL I: type I collagen; LL: large circles with large spacing; MSC: mesenchymal stem cell; OCN: osteocalcin; PTN: pattern; SL: small circles with large spacing; SS: small circles with small spacing.



Additional Figure 14. Statistical results of YAP localisation after CD treatment. (A) Fluorescent images of YAP. Nuclear transfer of YAP could not be observed. The yellow circles represent the location of micropatterned islands. Scale bars: 50 μm. (B) Statistical analyses of the nuclear/cytoplasmic ratio of YAP in MSCs and MC3T3-E1 cells. Data are presented as mean ± SD ($n = 3$), and were analysed by one-way analysis of variance followed by Tukey's *post hoc* analysis. CD: cytochalasin D; LL: large circles with large spacing; MSC: mesenchymal stem cell; SL: small circles with large spacing; SS: small circles with small spacing; YAP: yes-associated proteins.