Supplementary Material

Intrinsic Surface Effects of Tantalum and Titanium on Integrin α5β1/ERK1/2 Pathway-Mediated Osteogenic Differentiation in Rat Bone Mesenchymal Stromal Cells

Mengmeng Lu[a] Xiaohua Zhuang[b] Kaiwei Tang[c] Peishi Wu[c]
Xiaojing Guo[a] Linling Yin[a] Huiliang Cao[c] Derong Zou[a]

[a]Department of Stomatology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, [b]Department of Stomatology, Gongli Hospital, Second Military Medical University, Shanghai, [c]State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai, China

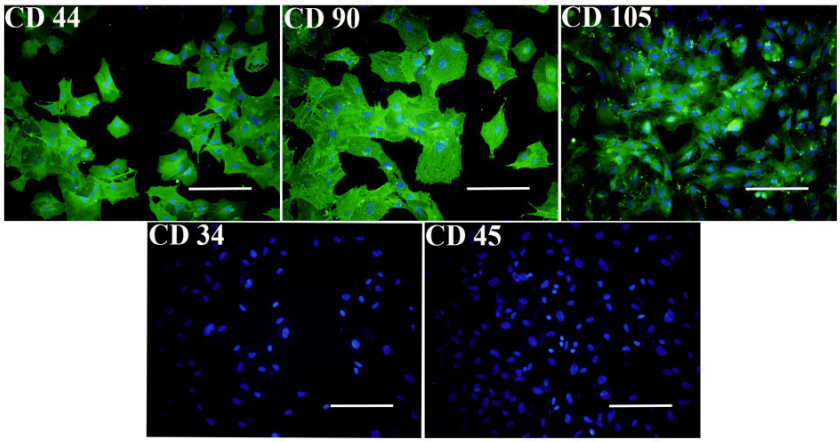

Figure S1. Immunofluorescence staining for rBMSC identification: cells were positively stained with CD44, CD90, and CD105 (green), while negatively stained with CD34 and CD45; cell nuclei were stained with DAPI (blue). Scale bar: 200 μm.

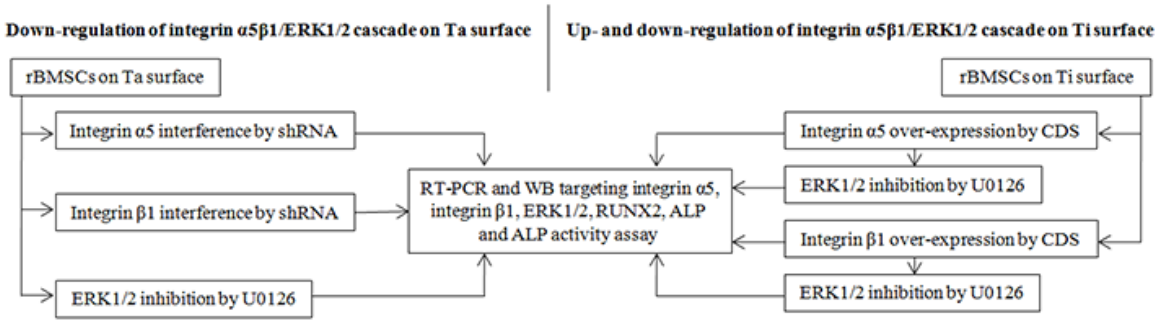

Figure S2. Technical strategy applied for integrin α5β1/ERK1/2 pathway verification on the Ta and Ti surfaces.

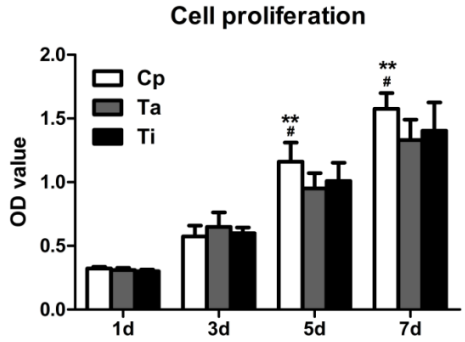

Figure S3. rBMSC proliferation on Ta, Ti, and Cp surfaces. Cell proliferation measured by the CCK8 assay at 1, 7, and 14 days; comparison was conducted between substrates at the same time point. $^{**}p < 0.01$ compared with Ta; $^{\#}p < 0.05$ compared with Ti.

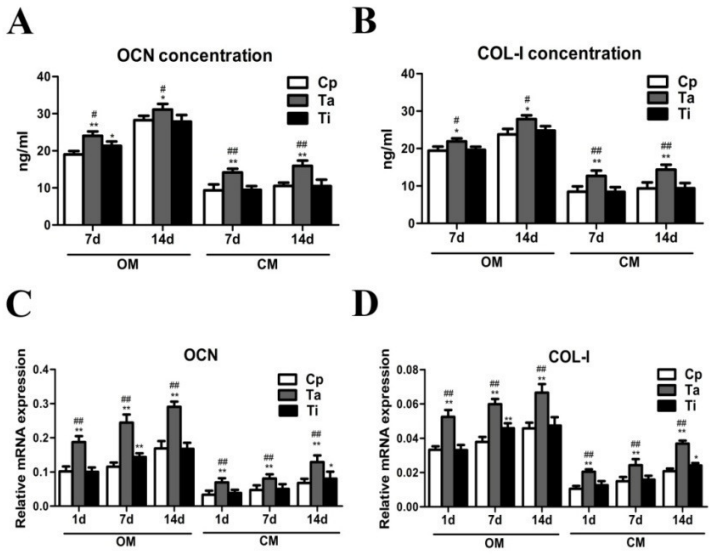

Figure S4. Expression of OCN and COL-I in rBMSCs on Ta, Ti, and Cp substrates. Concentration of (A) OCN and (B) COL-I in the supernatants of OM and CM at day 7 and 14. mRNA expression levels of (C) *Ocn* and (D) *Col-I* in OM and CM at day 1, 7, and 14; the expression level of *Gapdh* was used to normalize that of the target genes, and comparisons were conducted between samples in the same medium at the same time point. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with Cp; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with Ti.

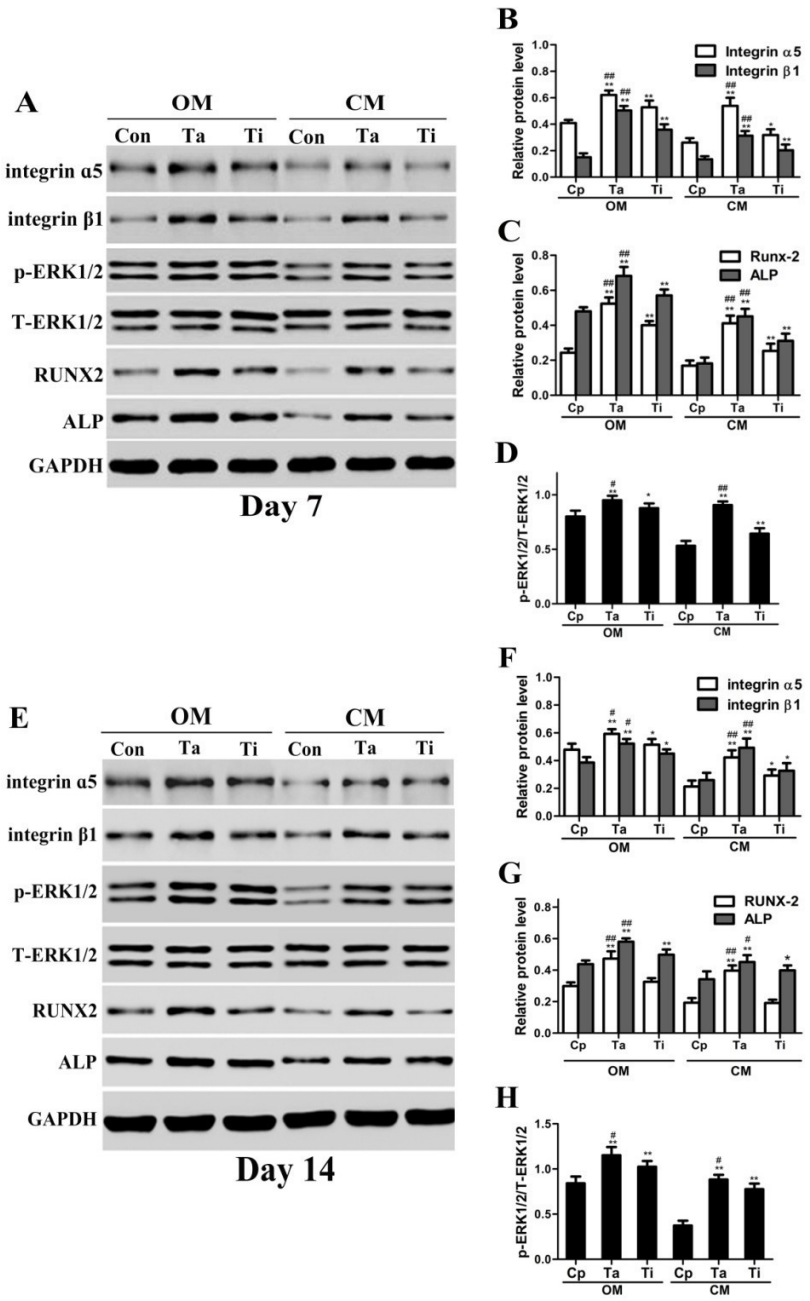

Figure S5. Osteogenic phenotype of rBMSCs on Ta, Ti, and Cp substrates. (A) Osteogenic protein expression in OM and CM at day 7, and (E) at day 14. Band gray value of (B) integrin α5, integrin β1, (C) RUNX2, ALP, and (D) p-ERK1/2 at day 7 and (F–H) at day 14. The gray value of integrin α5, integrin β1, RUNX2, and ALP was normalized to that of GAPDH, and the grey value of p-ERK was normalized to that of T-ERK1/2; comparisons were conducted between samples in the same medium. *$p < 0.05$, **$p < 0.01$ compared with Cp; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with Ti.

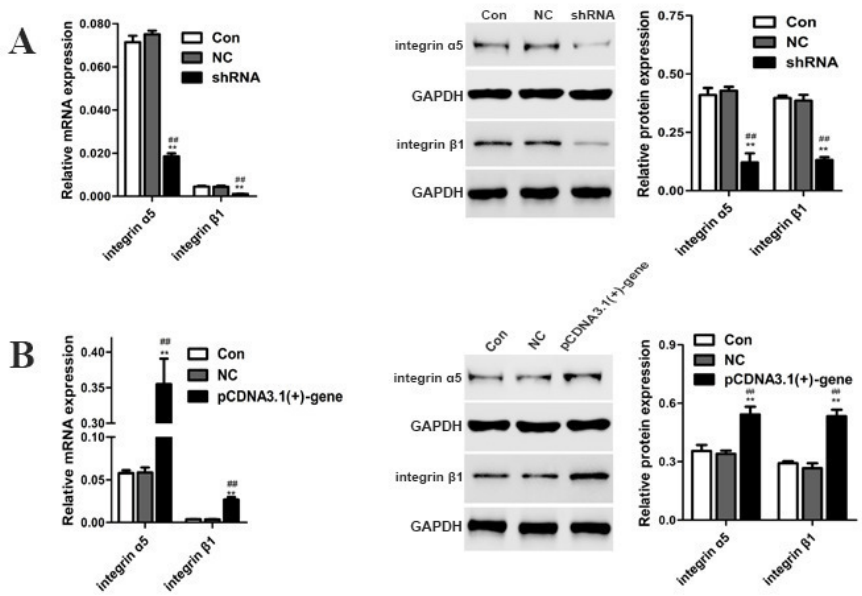

Figure S6. Transfection efficiency of rBMSCs. mRNA and protein expression levels of (A) cells transfected with the blank lentiviral vector (NC), integrin α5-shRNA or integrin β1-shRNA, and of (B) those treated with blank pCDNA3.1(+) vector (NC), integrin α5-coding sequence or integrin β1-coding sequence. $^{**}p < 0.01$ compared with NC; $^{\#\#}p < 0.01$ compared with the control (wild-type).

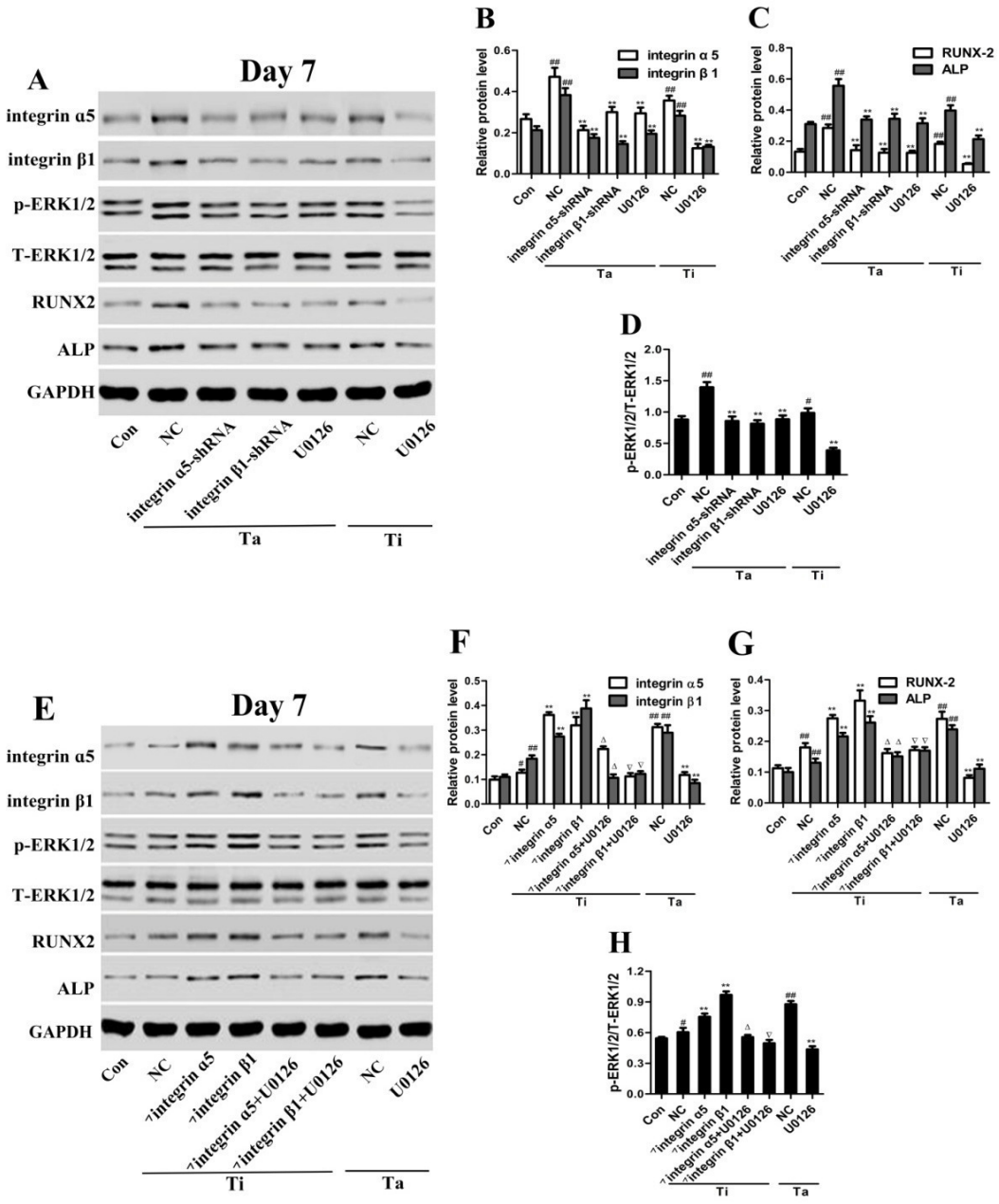

Figure S7. Osteogenic phenotype of rBMSCs following intervention of the integrin α5β1/ERK1/2 pathway on Ta and Ti surfaces. (A) Protein expression of cells treated with the blank vector (NC), integrin α5-shRNA, integrin β1-shRNA, or ERK1/2 inhibitor (U0126) on the Ta surface and those transfected with NC or treated with the ERK1/2 inhibitor on the Ti surface. (E) Protein expression of cells treated with NC, integrin α5-coding sequence (^integrin α5), and integrin β1-coding sequence (^integrin β1) in the presence or absence of the ERK1/2 inhibitor on the Ti surface, and

those transfected with NC or treated with the ERK1/2 inhibitor on the Ta surface in CM at day 7. The band gray value of (B, F) integrin α5, integrin β1, (C, G) RUNX2, ALP was normalized to that of GAPDH, and the grey value of p-ERK (D, H) was normalized to that of T-ERK1/2. $^{**}p < 0.01$ compared with NC on the same substrate; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the control (wild-type on the Cp substrate); $^{\Delta}p < 0.01$ compared with ^integrin α5; $^{\nabla}p < 0.01$ compared with ^integrin β1.