Supporting Information

Olivares-Navarrete *et al.* 10.1073/pnas.0805420105

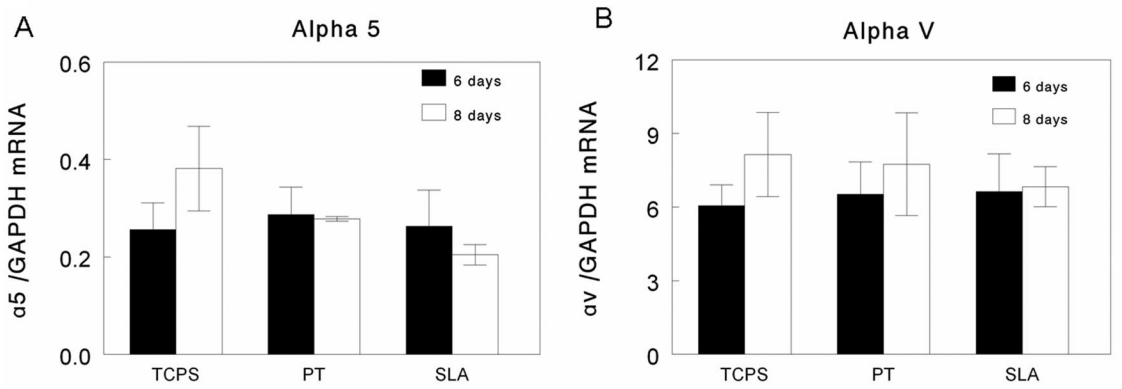

Fig. S1. Expression of α5 and αv integrin subunits is not substrate sensitive in confluent and postconfluent cultures of MG63 osteoblast-like cells. MG63 cells were cultured on TCPS, PT Ti disks ($R_a < 0.02$ μm) or PT disks that were grit-blasted and acid-etched to produce a complex micron-scale and submicron scale topography (SLA; $R_a = 3.2$ μm). At 6 days (confluence) and 8 days (postconfluence) after seeding, cells were harvested and expression of α5 and αv mRNAs was determined by real-time PCR as a function of GAPDH mRNA levels. Data are expressed as means $\pm$ SEM. Each value represents six different samples, where each sample is the combined mRNA from three separate cultures. No differences in expression were observed.

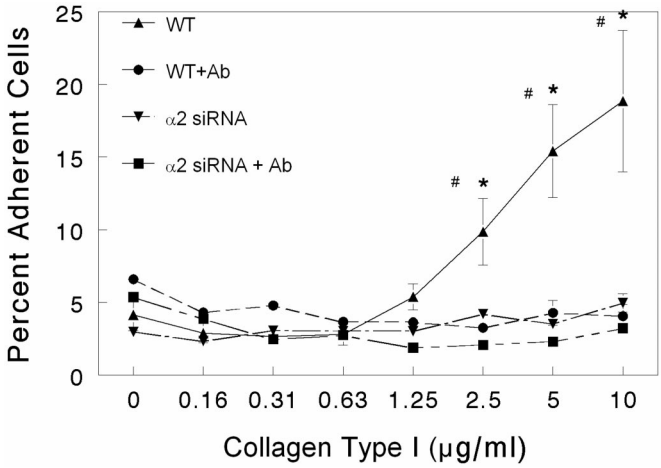

Fig. S2. $\alpha 2$ integrin subunits are required for MG63 cell adhesion to type I collagen. WT and $\alpha 2$ knockdown cells were seeded onto type I collagen-coated TCPS in the presence and absence of antibodies to $\alpha 2$. Briefly, 96-well culture plates were coated for 1 h at room temperature with 50 μl of PBS containing 0–10 ng/ml bovine type I collagen (Vitrogen-100; Cohesion). All wells were then blocked in 1% BSA for 1 h to prevent nonspecific protein adsorption. MG63 or $\alpha 2$-silenced cells were released from their tissue culture flasks by 0.25% trypsin and resuspended in PBS containing 2 mM dextrose and 2 μg/ml calcein-AM (Molecular Probes). These cells were then seeded onto the 96-well plate (15,000 cells per 100 μl/well) and allowed to attach for 3 h at room temperature in the presence and absence of 1 μg/ml anti-human $\alpha 2$ antibody (MAB1950; Chemicon). After the incubation, the initial fluorescence (485-nm excitation, 535-nm emission) was measured to determine the original cell density. The plate was then filled with PBS, covered with sealing tape, and centrifuged upside-down at 250 $\times$ *g* for 5 min to detach the cells. The liquid containing any detached cells was discarded and the wells were refilled with 100 μl of PBS/dextrose for the postspin fluorescence reading to determine the remaining cells. The postspin fluorescence value was normalized by the original fluorescence value to calculate the fraction of adherent cells. Data are means $\pm$ SEM for six separate cultures per variable and are from one of two independent experiments, both with comparable results. Statistical significance was determined by ANOVA followed by Bonferroni's modification of Student's *t* test. *, $P < 0.05$, WT vs. $\alpha 2$ siRNA; #, $P < 0.05$, $\alpha 2$ siRNA alone vs. siRNA plus antibody.

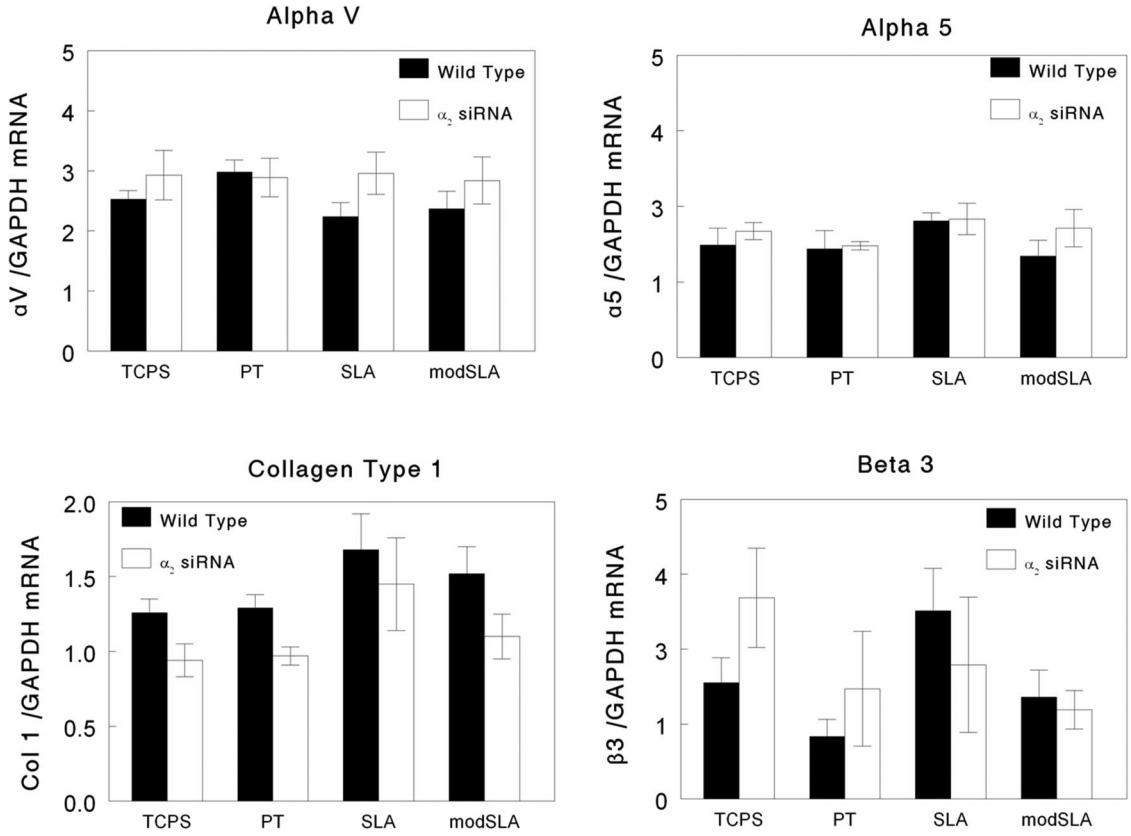

Fig. S3. Silencing α2 integrin levels does not affect expression of αv, α5, or β3 integrins or collagen type 1, regardless of substrate microstructure or surface energy. WT and α2-silenced MG63 cells were cultured on tissue TCPS, PT Ti disks ($R_a < 0.02$ μm) or PT disks that were grit-blasted and acid-etched to produce a complex micron-scale and submicron scale topography (SLA; $R_a = 3.2$ μm). In addition, SLA disks were modified to have high surface energy (modSLA). At 7 days after seeding, cells were harvested, and expression of αv, α5, β3, and type 1 collagen mRNAs was determined by real-time PCR as a function of GAPDH mRNA levels. Data are expressed as means $\pm$ SEM. Each value represents six different samples, where each sample is the combined mRNA from three separate cultures. Data were analyzed by ANOVA, and statistical differences between groups were determined by using Bonferroni's modification of Student's *t* test. No differences in expression were observed.