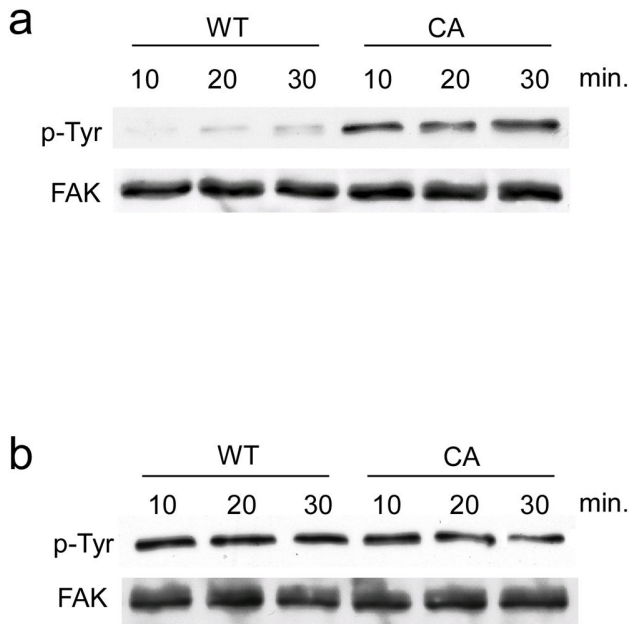
Supplemental Figure 1



Legend: C4-2 cells expressing constitutively active αvβ3 (CA, D723R) readily bind ligand and signal without the need for stimulation (inside-out signaling). C4-2 cells re-expressing β3 WT or CA were plated in Vn coated dishes (a) or pretreated with VEGF (10ng/mL) followed by plating in Vn coated dishes (b). Cell lysates from select time points were separated by SDS-PAGE, transferred to nitrocellulose and analyzed by immunoblotting with anti- phospho FAK Y379 (Chemicon) and FAK antibodies (Cell Signaling Technology). a) C4-2 CA αvβ3 cells bind Vn and signal through αvβ3 resulting in FAK Y379 phosphorylation at high levels as early as 10 minutes. C4-2 αvβ3 WT cells required at least 20 minutes for minimal FAK Y379 phosphorylation. b) Incubation of αvβ3 WT cells with VEGF prior to plating on Vn provides αvβ3 WT cells with an activating stimulus that promotes rapid signal internalization following ligand binding. FAK Y397 phosphorylation is similar for both C4-2 cell lines at all time points tested.

Supplemental Table 1. Analysis of C4-2 expression of integrin αvβ3 expression and state of activation

	β3 (–)	β3 WT	β3 D723R	β3 S752P
MFI	75.1	323.95	314.56	301.99
Activation Index	1.0	2.9	10.2	n.d.

NOTE: Expression levels of αvβ3 following retroviral infection for β3 expression was determined by FACS analysis (FACSCalibur/CellQuest software package; Becton Dickinson, San Jose, CA). Cells were incubated with mouse monoclonal anti- αvβ3 (LM609; Chemicon, Temecula, CA) or with control mouse IgG (Invitrogen, Carlsbad, CA) for 1h at 25°C. Cells were washed 2x in PBS then incubated with Alexa-Flour 488 conjugated anti-mouse IgG (Invitrogen) and subjected to FACS analysis. The functional activity of the β3 integrin was assessed on the basis of fibrinogen binding by suspending cells in RPMI with 0.2% BSA, followed by treatment with or without cRGDfv (50μM) and Fluorescein isothiocyanate (FITC)-labeled fibrinogen at a final concentration of 1μM for 30 min. Cells were washed and the numbers of FITC-Fibrinogen bound cells were determined by FACS analysis. The percentages of activation index of cell types were calculated as follows:

$$\% \text{ Activation Index} = \frac{\text{MFI(FITC-Fgn)} - \text{MFI(FITC-Fgn + cRGDfv)}}{\text{MFI of } \beta3 \text{ Expression}} \text{ X } 100$$

Resulting values were normalized to the activation index of C4-2 β3(–) cells, which were assigned a value of 1.

Supplemental Table 2. Adhesion and migration of C4-2 cells to bone matrix proteins and extract

Parameter		C4-2 cell line			
		αvβ3 (–)	αvβ3 WT	αvβ3 CA	αvβ3 inactive
Adhesion to Vn (cells x1000)	no Mn^{2+}	6 ± 1	7 ± 1	14 ± 1	4 ± 1
	Mn^{2+}	5 ± 2	14 ± 1	14 ± 1	5 ± 1
Migration to Vn (cells per field)	Alone	28 ± 3	124 ± 4	179 ± 7	64 ± 5
	+ anti-αvβ3	24 ± 3	40 ± 5	33 ± 3	32 ± 2
Migration to BSP (cells per field)	no Mn^{2+}	2 ± 1	22 ± 4	73 ± 6	4 ± 1
	Mn^{2+}	2 ± 1	46 ± 3	100 ± 9	8 ± 2
Migration to SPARC (cells per field)	alone	3 ± 1	33 ± 3	50 ± 3	17 ± 3
	+ anti-αvβ3	1 ± 0	15 ± 3	11 ± 1	14 ± 2
% Adhesion to Bone Extract	no Mn^{2+}	5 ± 1	6 ± 2	20 ± 3	4 ± 1
	Mn^{2+}	8 ± 1	19 ± 0	27 ± 3	6 ± 1
Migration to Bone Extract (cells per field)		15 ± 1	38 ± 2	64 ± 3	21 ± 2

Adhesion and migration experiments were performed in triplicate (anti-αvβ3= 20ug/ml; Mn^{2+}=1mM; Vn, BSP, SPARC and bone extracts were a 10ug/ml). Cell counts were determined by counting the number of adherent or migrated cells from 10 fields of each experiment. β3 (–) cells lack αvβ3 heterodimer expression; β3 WT cells express resting, yet activatable αvβ3; β3 CA cells express constitutively active αvβ3; β3 inactive cells express resting, non-activatable αvβ3.