



Saos $\alpha 2$ WT cells were treated with EV1 for 15 min @ 37° C. Paraformaldehyde fixed (4 %) and TritonX-100 (0.2 %) permeabilized cells were stained with EV1 specific antibody (red) together with a conformation-sensitive $\beta 1$ antibody (A; green; 9EG7, BD Biosciences) or an antibody recognizing total $\beta 1$ (B; green; sc-9970, Santa Cruz). While active $\beta 1$ integrins characterically localized at the cell edges, the EV1 bound to integrins located in cell apices. Blow-ups show a typical EV1 clustering on the top of the cell and its colocalization with total $\beta 1$ (B), but not with active $\beta 1$ (A). When the colocalization was quantified further by using BIOMAGEXD software, it appeared that on average 67% of the total intensity of the EV1 stain coincides with the total $\beta 1$ integrin stain, but only 40% with the active $\beta 1$ integrin stain, when observing meaningful intensity levels of both channels. Scale bar, 15 μ M.