

Supplemental Figure Legends

Supplemental Figure 1: SK-CO15 cells were transfected with siRNA targeted to the clathrin heavy chain, or control scramble siRNA, wounded, and subjected to Alexa 546 - labeled human transferrin (red, arrowheads) internalization assay. Cells were fixed and immunolabeled for clathrin heavy chain (blue) to monitor efficiency of clathrin heavy chain knock down. The dashed lines outline leading edge of the migrating cells. Scale bar, 10 μ m.

Supplemental Figure 2: Validation of the ELISA-based protocol for determination of beta1 integrin internalization.

A) Surface-biotinylated proteins were pulled down from cell lysates using avidin beads and analyzed by immunoblotting using P5D2 monoclonal anti-beta1 integrin antibody. Note the antibody recognizes a single band that corresponds to beta1 integrin. **B)** Determination of the linearity range in the ELISA assay of beta1 integrin internalization. Internalization of surface biotinylated proteins was performed during 0, 5, 10, 20, 30 and 40 minutes. Internalized biotinylated proteins were captured on streptavidine plates and the amount of captured (internalized) beta1 integrin was determined by ELISA using β 1 integrin antibody followed by HRP secondary antibody.

Supplemental Figure 3: Beta1 integrin co-fractionates with epithelial lipid rafts.

Lipid rafts from T84 epithelial cells were isolated by floatation in continuous 5-30% sucrose gradients. Fractions were subjected to SDS-PAGE and immunoblotted for β 1 integrin and the membrane raft protein, caveolin-1. A representative immunoblot demonstrates that β 1 integrin co-fractionates with caveolin-1 in light density fractions which are characterized by increased light scattering at 600 nm.

Supplemental Figure 4: DMA and EIPA do not influence internalization of β 1 integrin but efficiently block macropinocytosis.

Co-internalization of β 1 integrin antibody (red) and a macropinocytosis marker, FITC-labeled dextran 3000 (green) was performed in wounded SK-CO15 cells in the presence DMA, EIPA or vehicle. Nuclei are highlighted with ToPro3 (blue). Scale bar, 20 μ m.