Supplementary Figure S1: Effect of SKF10047 treatment on co-immunoprecipitation of β1 integrin by σ1 receptor. Lanes 4 and 5 total cell extracts, Lanes 1 and 2 immunoprecipitation for control and SKF10047 treated cells respectively, Lanes 7 and 8 (control bead reactions). Western blotting antibody shown below blot.

Supplementary Figure S2: Localization of the σ1 receptor in lipid raft sub cellular fractions following treatment with 2% cyclodextrin. Fractions (1-10) were slot blotted to nitrocellulose and subsequently probed with antibodies to the σ1 receptor. Graph shows densitrometric analysis of slot blotted lipid raft sub cellular fractions.

Supplementary Figure S3. Controls for σ1 receptor RNAi. A σ1 receptor gene silencing vector was constructed using the pSilencer RNAi vector (Ambion) as described previously (7) which effects both σ1 receptor mRNA and protein levels without effecting basal gene expression levels. As a control a σ1 receptor RNAi randomized sequence was constructed in pSilencer. Transfection was accomplished using Lipofectamine 2000 (Invitrogen) and Optimem (Gibco) according to the manufacturers' instructions. Transfection efficiency was verified by co-transfecting a GFP expressing plasmid. *A,* Silencing efficiency was initially quantified by protein extraction from cultures four days post-transfection. σ1 receptor protein levels in the cell lines were compared by separating equal amounts of protein extracted. The proteins were blotted to PVDF membrane and even protein blotting across the membrane was confirmed by staining with Ponceau Red (data not shown). σ1 receptor protein levels were detected by immunoblotting using the σ1 receptor antibody. The blots were quantified by densitrometric analysis of three replicates. *B,* Silencing efficiency was also quantified by determining the level of σ1 receptor mRNA expression by real time PCR (n=3). *C,* Subsequently for all experiments σ1 receptor silencing was quantified by slot blotting in duplicate 5 μg of total cell protein onto nitrocellulose followed by detection using a σ-1 receptor specific antibody, and an anti-actinin antibody (as controls for non-specific effects on gene expression). Results for three replicate experiments from Figure 4A are shown. *D,* Densitrometric quantification of above slot blots.

Supplementary Information

Commercial antibodies used in this study

Mouse anti-human β1−integrin (Chemicon); Rabbit anti-human β1−integrin (Chemicon); β1−integrin blocking antibody (Coulter); Rabbit anti-human Na^+/K^+-ATPase (Chemicon); Rabbit anti-human Flottillin (Chemicon); Mouse anti-human Actin (Sigma); Mouse anti c-myc (Invitrogen); Swine anti-rabbit IgG HRP (Dako); Goat anti-mouse IgG HRP (Dako); Swine anti-rabbit IgG FITC. Mouse anti-actinin antibody (Sigma).

Buffers

PBS: 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, 150 mM NaCl, pH 7.4. PBST: as above with 0.1% Tween 20. TBS: TBST: Tris-HCl (pH 8.0), 125 mM NaCl. TBST: as above with 0.1% Tween 20.

Immunostaining of cells for β1-integrin (detailed protocol)

Sterile 13 mm diameter glass coverslips in 24 well plates were coated with poly-L-Lysine (0.25 mg/ml) for 30 mins at 37^{o}C and washed twice with PBS. Cells were seeded at a density of 2 x 10^4 cells/ml and grown for 48 hours. Cells were washed once in PBS and then fixed in 2 % paraformaldehyde for 7 mins at room temperature. The cells were washed twice in PBS and blocked with 8 % BSA in PBS for 30 mins. Cells were again washed twice with PBS, excess liquid was removed and primary antibody was added diluted with 1 % BSA in PBS for 1 hour. Following a further two washes in PBS the cells were incubated with a FITC conjugated anti-IgG antibody diluted in PBS with 1 % BSA for 30 mins at room temperature in the dark. After three final PBS washes coverslips were mounted in Vectashield (Vector Laboratories). Controls to assess autofluorescence or non-specific labeling were also undertaken and consisted of treatment with 1 % BSA without either primary antibody, secondary antibody or both. Cells were visualized on a Leica DMIRB confocal with a 100 x objective and analysis was performed with Leica confocal software.

Transmembrane protein prediction and hydropathy analysis

Transmembrane protein prediction and hydropathy analysis was performed by a Kyle and Doolittle method (41) and TMpred (42). σ1 receptor peptides $\sigma1_{161\text{-}180}$ and $\sigma1_{191\text{-}210}$ were modeled using ArgusLab software (43) ($\phi = -135$ deg, $\psi = -45$ deg, and $\omega = 180$ deg). Energy was minimalized by Universal Force Field prediction. The docked location of cholesterol ($C_{29}H_{50}O$) to each peptide was predicted by using simulated annealing as implemented using ArgusDock.