Supporting Information

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SI Methods

Cell Lines. U87 human glioblastoma epithelium-like cells (kindly provided by Dr. Maciej Lesniak) were cultured in MEM with penicillin/streptomycin and 10% FBS. Normal human astrocytes (NHA) were purchased from Lonza and cultured in provided astrocyte growth media supplemented with rhEGF, insulin, ascorbic acid, GA-1000, L-glutamine and 5% FBS. HeLa cells were grown in DMEM plus penicillin/streptomycin and with 10% FBS.

Western Blot Analysis. NHA and U87 cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 5 μg/μL protease inhibitors). Total protein concentrations of the lysates were determined using a BSA Protein Assay Kit (Thermo). For each sample, 20 μg were loaded on 4–20% Tris-HCl gradient gel for separation by SDS-PAGE electrophoresis. The proteins were transferred to Hybond-C Extra nitrocellulose membrane (Thermo) at 90 Volts for 90 min. The membrane was blocked for 1h in 5% BSA in TBST and incubated overnight at 4 °C with rabbit anti-NK-1R antibody (1:2,000), then incubated with HRP-conjugated anti-rabbit antibody (1:2,000) for 1 h at room temperature. ECL Western Blotting Detection Reagent (Amersham) was used for chemiluminescence signal detection.

NK-1 Receptor Transfection. The plasmid coding for the human NK-1 receptor in the mammalian expression vector pCDNA3(+) was kindly provided by Dr. Lefkowitz (Duke University). Transfections were carried out using Lipofectamine 2000 (Invitrogen) according to manufacturer protocol, with 1 ng of the plasmid for each well containing 10^4 HeLa cells seeded on an 18-mm cover-slip in a 12-well plate.

TIRF Microscopy. Actin was purified from chicken muscle acetone powder as described (26). A total of 10 μM F-actin was polymerized with 10% biotin-G actin (27) and 33% TMR-G actin (28). Glass flow cells (20 μL volume) were washed with 1 mg/mL neutravidin in AB buffer (25 mM KCl, 25 mM imidazole pH 7.5, 1 mM EGTA, 4 mM $MgCl_2$, and 10 mM DTT), followed by blocking with 1 mg/mL BSA in 1× AB buffer. Mg^{2+}-F-actin (200 nM) in AB buffer was added to the flow cells for 2 min. Flow cells were washed with TIRF buffer (AB, 4.3 mg/ml glucose oxidase, 0.7 mg/mL catalase, 4.5 mg/mL glucose, and 0.5% BME) and imaged. sAB-4 (0.5 μM) or sAB-19-Cy5 (0.25 μM) were diluted in TIRF buffer and added to the chamber after the start of the movie. Movies were acquired with a 0.2-s exposure for 200 frames.

Electron Microscopy. Actin filaments (19.2 μM) and sAB-27 (6.4 μM) were incubated together for 5 s, spotted on a holey carbon film (Quantifoil, Cu 400 mesh), washed twice with 0.1 M NaCl, and stained with 1% uranyl acetate. Images were obtained at 75,000×.

Cell-viability Assay. U87 cells were seeded in 96-well plate at density 5000 cells/well in 0.1 mL growth media. Cells were attached over night and incubated in starving media for 3 h. Cells were incubated with 20 nM of the SPv- sAB-27 conjugate for 3, 5, 9, 24, and 48 h, and SPv or sAB-27 alone for 48 h in starving media. Twenty μL of the CellTiter 96 AQ_{ueous} assay (Promega) One-Solution reagent was added to each well. The plate was incubated in a humidified, 5% CO_2 atmosphere until color was developed and the absorbance was recorded at 490 nm.

Internalization Quantification. U87 cells were seeded in a 96-well black bottom plate at 5,000 cells/well in 100 μL full growth media. Cells were allowed to attach overnight and then incubated in starving media for 3 h, followed by stimulation with 20 nM, 50 nM, 100 nM, 200 nM, and 500 nM Cy5-labeled sAB-27-SPv conjugate for 24 h in starving media. The cells were extensively washed with PBS to remove residual conjugate from wells. 100 μLof PBS was added to each well and the remaining fluorescence was measured in triplicates using the following parameters: excitation wavelength 640 nm, emission wavelength 675 nm, with 8 nm excitation bandwidth and 15 nm emission bandwidth. A standard curve was used to calculate the concentration of internalized conjugate based on fluorescence values.

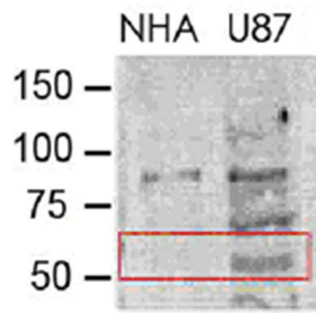


Fig. S1. Western blot analysis of NK1R expression in U87 cells and normal human astrocytes. Box indicates the expected size (≈53 kDa) of the NK1R. Expression is detected in U87 glioblastoma cells, but not in normal human astrocytes.

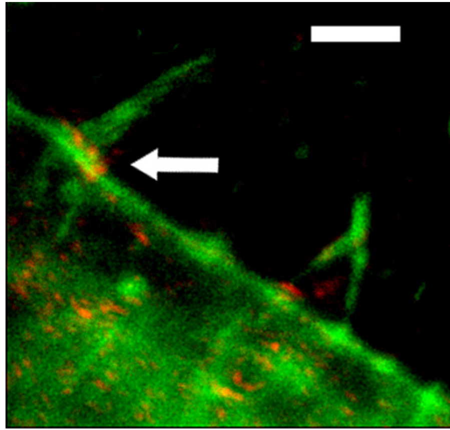
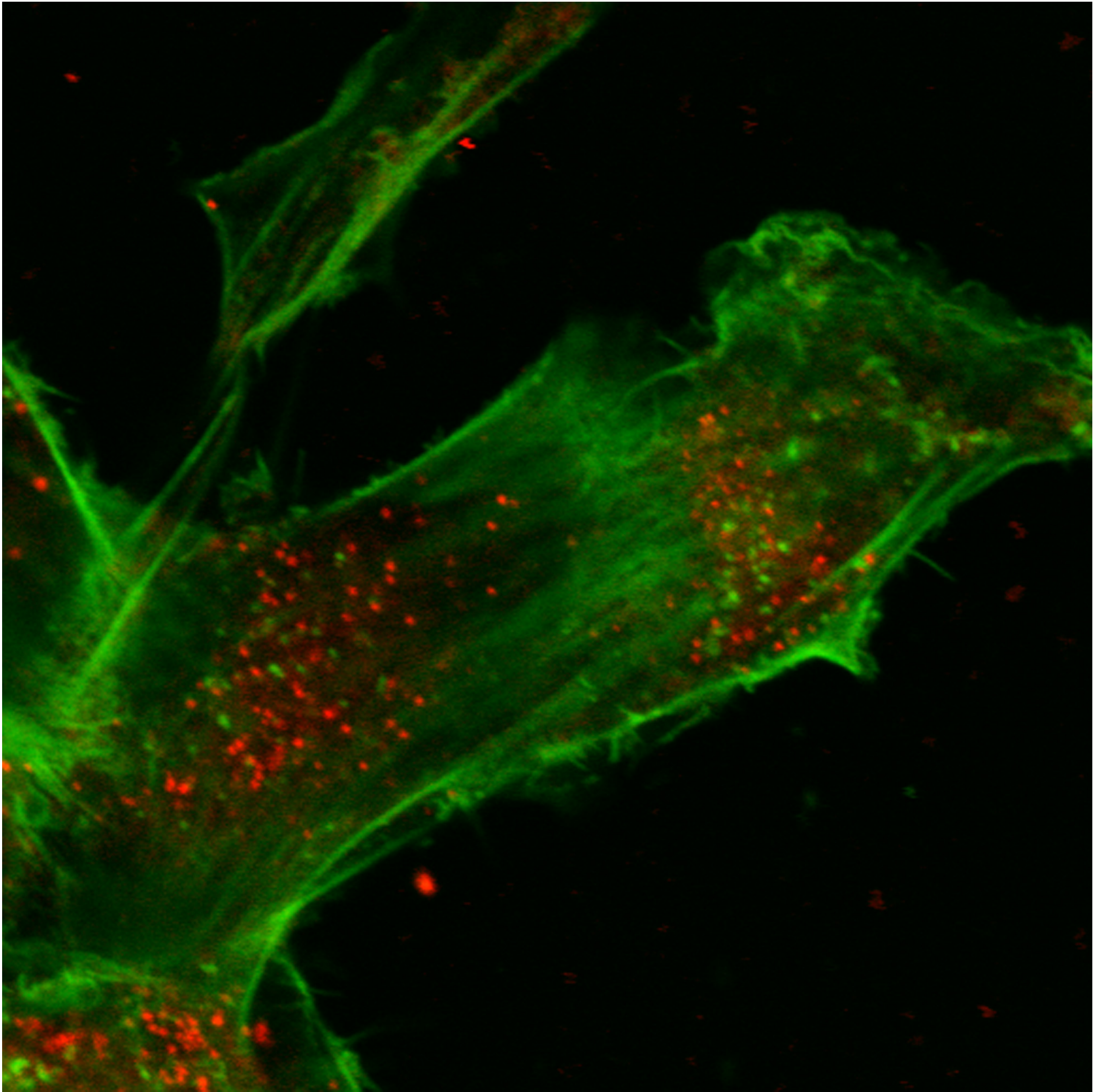


Fig. S2. Immunostaining of fixed cells treated with TMR-phalloidin (green) using Alexa 488-labeled sAB-19 (red). Arrow indicates localization of the sAB to the base of fillopodia. (Scale bar, 2 μm.)



Movie S1. A confocal z-series scan of U87 cell after treatment with 20 nM sAB-4-SPv conjugate (red) followed by fixing and staining with phalloidin (green).

Movie S1