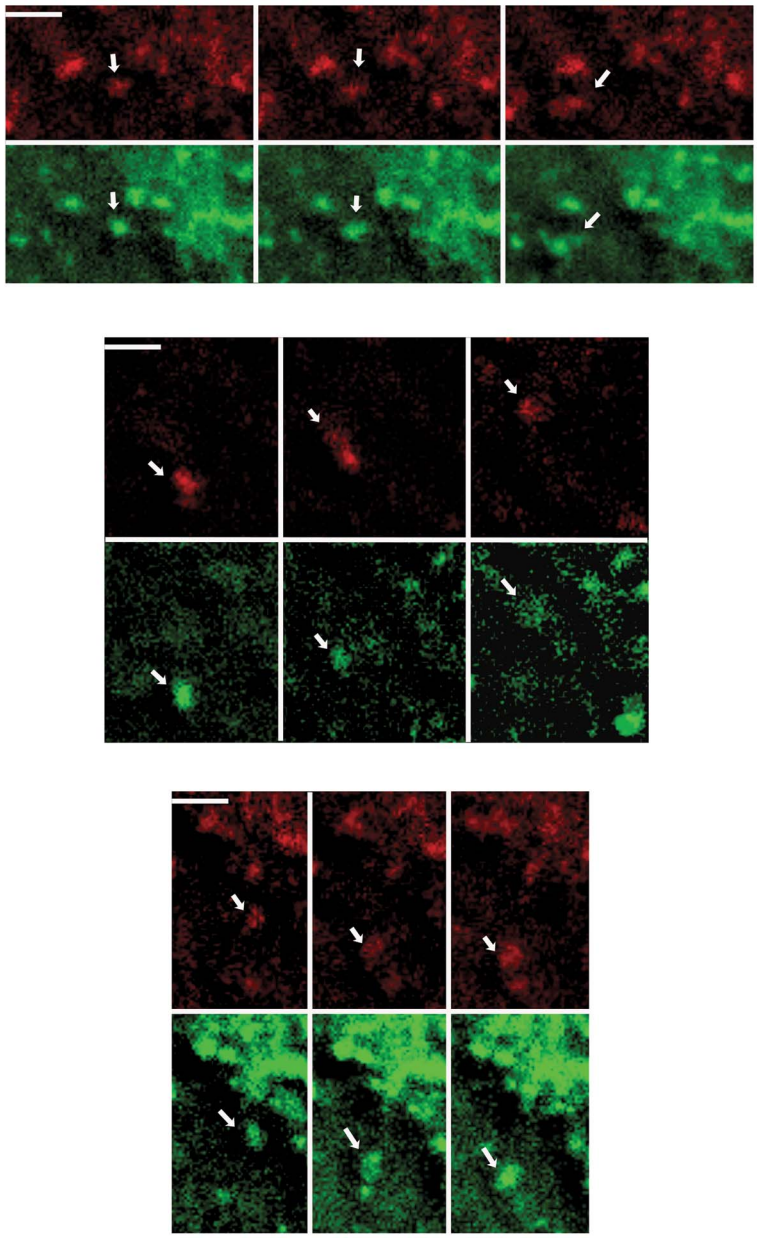

Supplementary Figure 1. Internalization of cholera toxin via caveolae. Alexa Fluor 647-conjugated cholera toxin subunit β (Molecular Probes) was incubated with cells expressing Caveolin-1-EGFP at 4ºC for 15 min. After removing excess cholera toxin, the temperature was raised to 37ºC to initiate endocyosis. Cholera toxin was found to localize into discrete spots (red images), all of which colocalize with caveolin-1-EGFP structures (green images). Three examples of these cholera toxin-containing structures are indicated by arrows. For each example, three consecutive images, each 10 s apart in time, are shown to illustrate the movement of these structures. Treating cells with nocodazole (60 μM) completely inhibited this movement, indicating that these EGFP-labeled caveolin structures containing cholera toxin were internalized and moving in a microtubule-dependent manner. Such structures were not observed in cells treated with

filipin (5 μg/ml), an inhibitor of caveolin-dependent endocytosis, suggesting that the internalization of cholera toxin here is via caveolae. During the observation window (50–100 s), 54% of the cholera toxin-containing structures showed microtubule-dependent movement in Caveolin-1-EGFP expressing cells; and this fraction is similar in untransfected cells (52%). In both transfected and untransfected cells, we started to observe such mobile structures within 5 minutes of the initiation of endocytosis. These results indicate that the expression of Caveolin-1-EGFP did not significantly perturb caveolin-mediated endocytosis. Scale bars: 3 μm.