

Supplementary Materials and Methods

Internalization and Recycling Assay

Internalization of membrane receptors was induced by cooling cells on ice for 30 minutes, followed by stimulation at 37°C for 30 minutes. Internalization was then stopped by placing cells back on ice for 30 minutes, and recycling to the plasma membrane was induced by re-incubation at 37°C for 15 minutes. Cells were fixed at the various steps of internalization/recycling and examined by confocal microscopy. Co-localization of Trop-2 and integrin subunits in trafficking vesicles was quantified using the “Pearson–Spearman correlation co-localization” ImageJ plugin. The Pearson’s correlation coefficient (PCC) was calculated for each image, and then means \pm SEM were calculated for each experimental group in order to score the extent of co-localization. In order to avoid overestimation, signals from membrane edges containing the Trop-2/ β ₁ complex were excluded by drawing a mask surrounding only the cytoplasmic area.