Supplementary information, Figure S4 Quantification of the triple colocalization of TβRI, caveolin-1 and EEA1. (A) HeLa cells expressing Myc-TβRI were labeled and internalized for 30 min in the presence of TGF-β1. Then the cells were immunostained with antibodies against caveolin-1 and EEA1 and imaged by confocal microscopy. (B) The fluorescence intensities of the three channels along the white line of the merged image were shown. (C) The triple colocalization (white signal) was identified by the Blobprob plugin (ImageJ). The Mander’s overlay coefficients were calculated basing on the triple colocalization identified. (D) The fluorescence intensities of the three channels along the white line on the merged image (Figure 2B) were shown. (E) Quantifying the distribution of Myc-TβRI in EEA1-positive vesicles (EEA1), caveolin-1-positive vesicles (Caveolin-1), EEA1 and caveolin-1 double-positive vesicles (EEA1+Caveolin-1), EEA1 and caveolin-1 double-negative vesicles (Alone) after 30 min internalization in the absence or presence of TGF-β1 by visual inspection as described in Methods (n = 4 independent experiments).