Figure S7

(A) COS-1 cells were treated with EHD1 siRNA1 for 72 h. Cells were pulsed with Alexa 594-CTXB for 5 min, washed, and chased for 15 or 60 min. Cells were then fixed and stained for GM130. The images of control cells in Fig 1E are shown as a reference. Scale bars, 10 µm. (B) COS-1 cells were treated with EHD1 siRNA1 or siRNA2 for 72 h. Cells were then fixed and stained for TfnR. The graph shows percentages of cells showing aberrant TfnR-positive tubules as mean ± SD of three independent experiments. At least 20 cells were counted per experiment. (C) COS-1 cells were transfected with EHD1 siRNA1 and TfnR-GFP. Seventy-two hours after transfection, cells were imaged with an epifluorescent microscopy. Selected stills from Movies S2 are shown. The tubule indicated by red asterisks lingered over 10 sec. Scale bars, 5 µm.