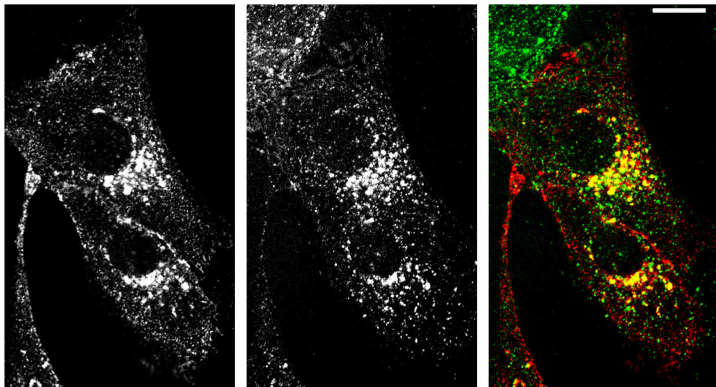
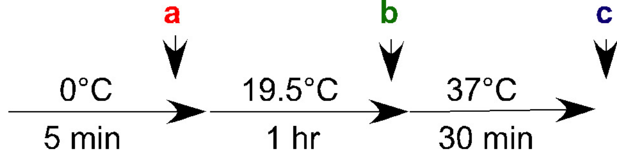


Supplemental Figure S1. Shiga toxin colocalizes with early endosomes following a 19.5°C block. Shown are confocal micrographs of Vero cells allowed to internalize STxB (red) for one hour at 19.5°C. Cells were fixed and decorated with an anti-body against early endosomes EEA1 (green). The merged images indicate overlap between STxB and EEA1 (yellow). Bar is 10 μm.



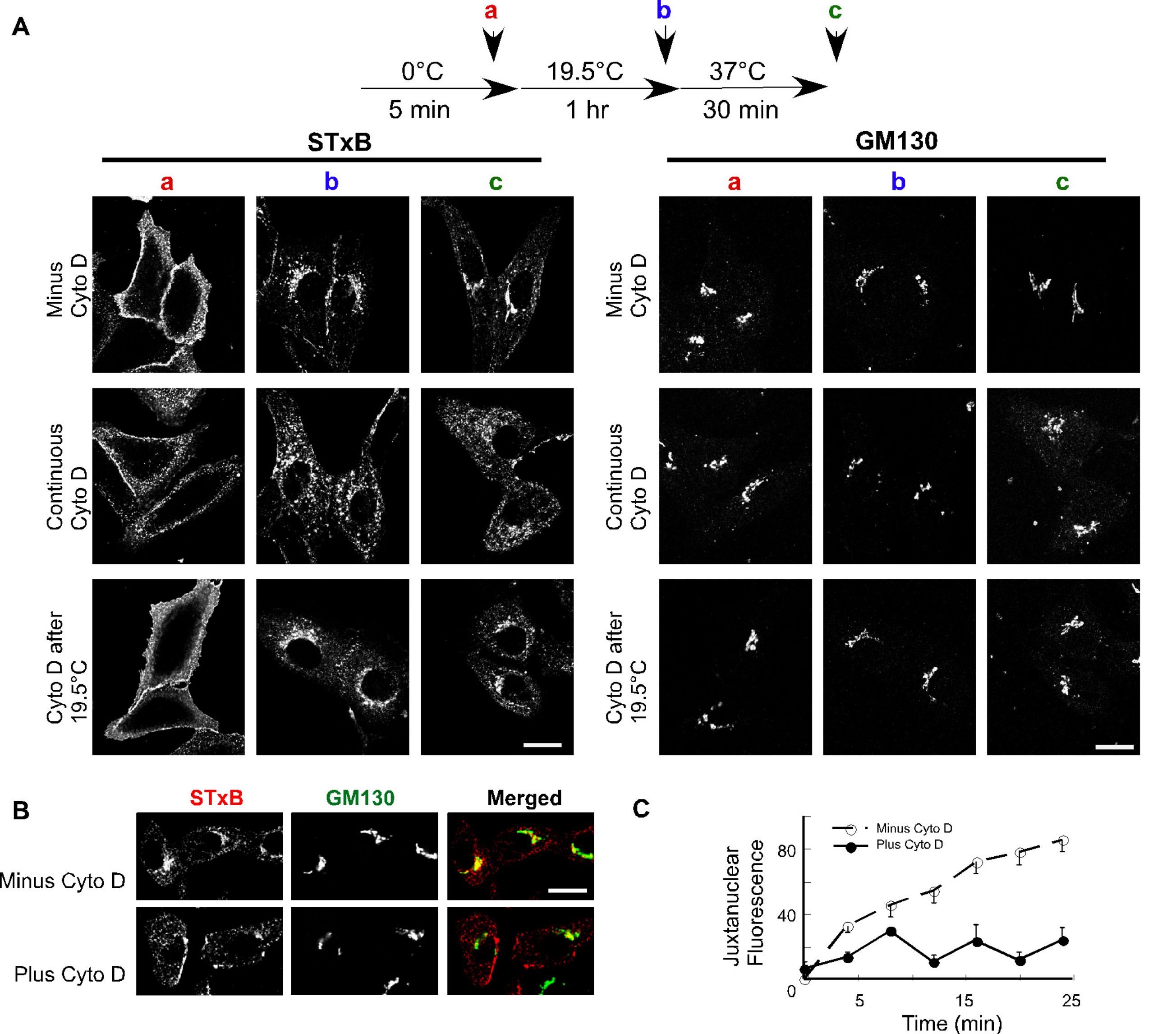
a b c

Minus Noc

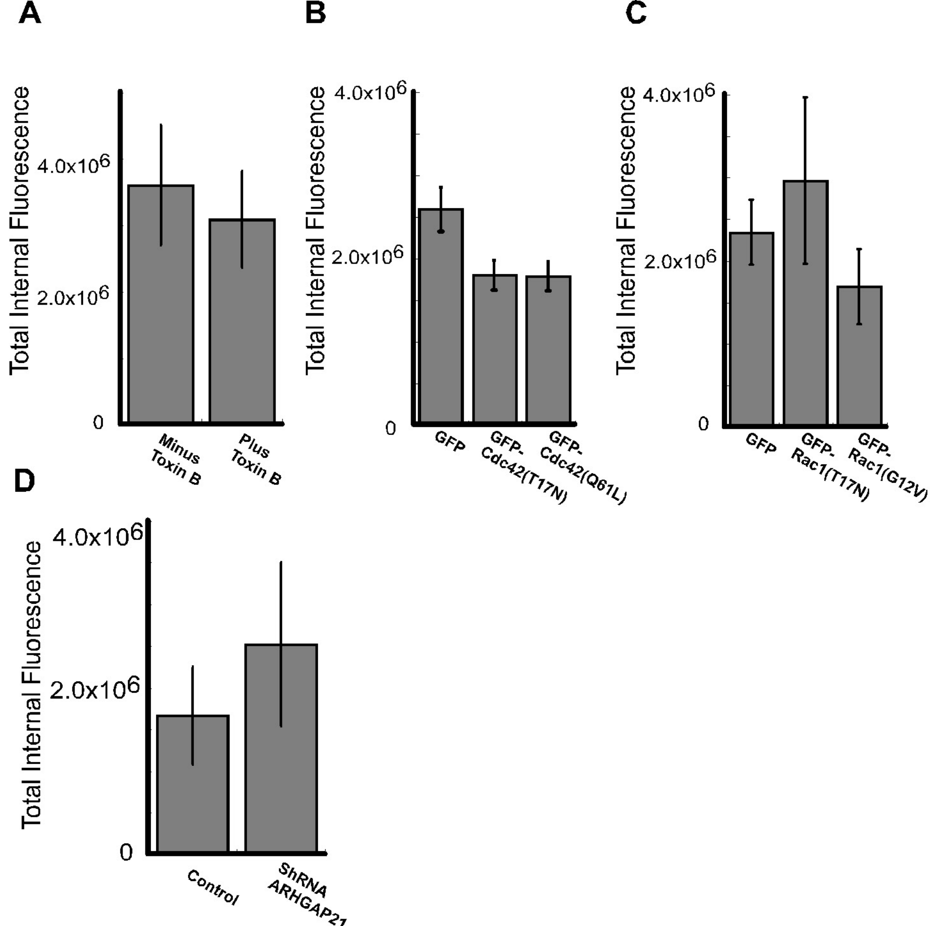
Continuous Noc

Noc after 19.5°C

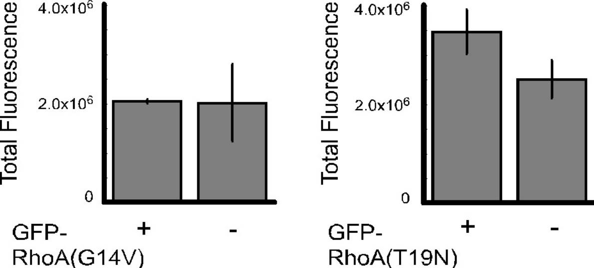
Supplemental Figure S2. The Golgi apparatus remains compact and juxtanuclear during a 19.5°C incubation. Shown are the cells from Figure 1A decorated with an antibody against the Golgi marker GM130. Bar is 20 μm.



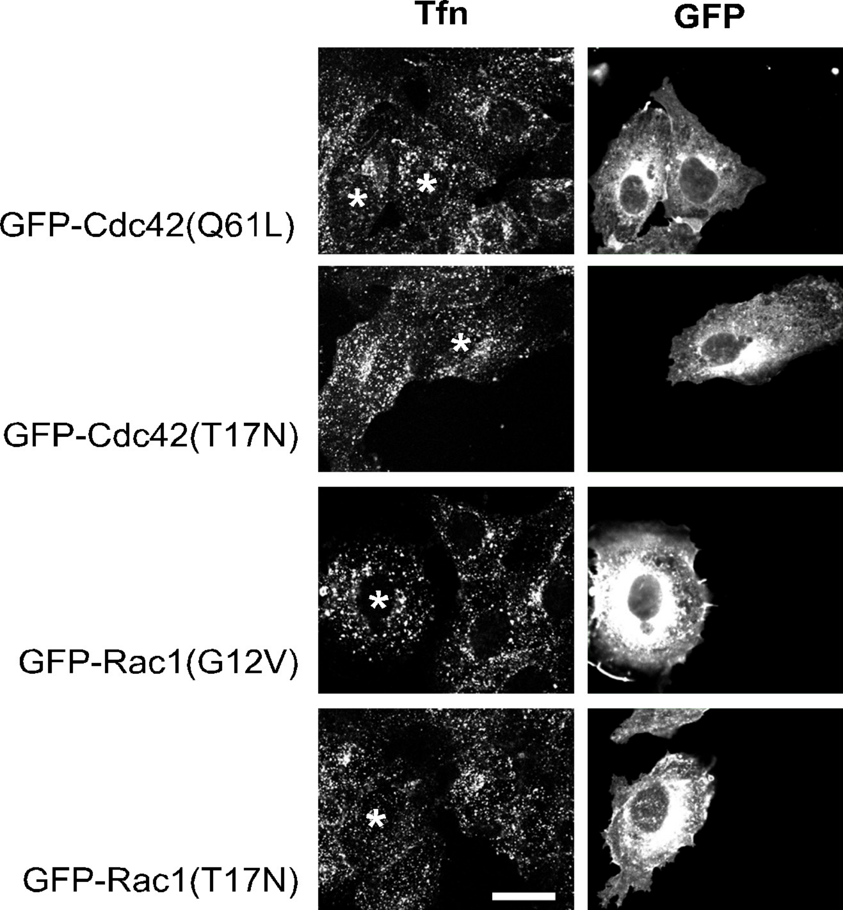
Supplemental Figure S3. STxB transport to the Golgi apparatus requires actin. (A) 0.25 μg/ml cytochalasin D (cyto D) was added to cells during the 0°C incubation with STxB or after the 19.5°C incubation as indicated. Cells were fixed after the 0°C (a in diagram), 19.5°C (b in diagram), or the 37°C (c in diagram) incubation. STxB (left panels) and anti-GM130 (right panels) are shown for the same cells. The bar is 10 μm. (B) Shown are confocal micrographs of Vero cells incubated with Cy3.5-labeled STxB (red) at 0°C, washed, and then incubated for 30 minutes at 37°C. Cyto D was included or omitted as indicated. The cells were fixed, permeabilized, and decorated with an antibody against the Golgi marker GM130 (green). The merge image indicates the overlap between STxB and GM130 (yellow). The bar is 10 μm. (C) Shown are the average levels of fluorescent STxB present in a circular ROI placed at the NBD C6-ceramide-labeled Golgi complexes as a function of time. The cells were treated with cyto D (closed circles) or mock treated (open circles). The ROI was the same size for each cell imaged. The mean intensity within the ROI was determined and plotted as a function of time for three independent experiments. The bars represent SE.



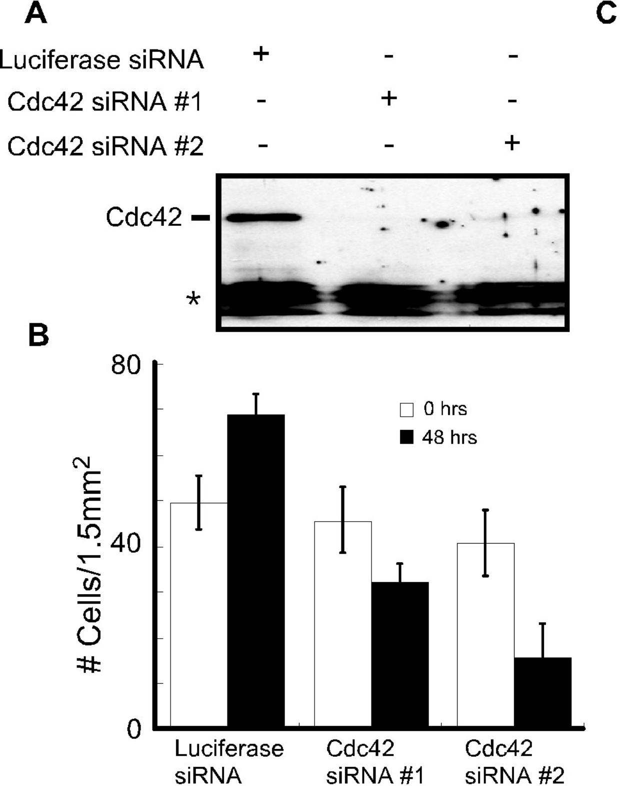
Supplemental Figure S4. Cdc42, Rac1, and toxin B had only minor effects on STxB endocytosis. (A) The total intracellular fluorescence of STxB was quantified in cells treated with and without toxin B. The bars represent SE, n=3 experiments. The effects of toxin B are not significant, p=0.68. (B) The total intracellular fluorescence of STxB was quantified in cells expressing GFP, GFP-Cdc42(T17N) and GFP-Cdc42(Q61L). The bars represent SE, n=4 experiments. The effects of GFP-Cdc42(T17N) and GFP-Cdc42(Q61L) are significant compared to GFP alone, p<0.05. There is in significant difference between GFP-Cdc42(T17N) and GFP-Cdc42(Q61L), p=0.97. (C) The total intracellular fluorescence of STxB was quantified in cells with GFP, GFP-Rac1(T17N), or GFP-Rac1(G12V). When compared to GFP alone, the effects of GFP-Rac1(T17N)(p=0.59) and GFP-Rac1(G12V) are not significantly different (p=0.33). There is no significant difference between GFP-Rac1(T17N) and GFP-Rac1(G12V) (p=0.31). The bars represent SE, n=3. (D) The total intracellular fluorescence of STxB was quantified in cells with and without an shRNA to ARHGAP21. The bars represent SE, n=3 experiments. The effects of ARHGAP21 are not significant (p=0.50).

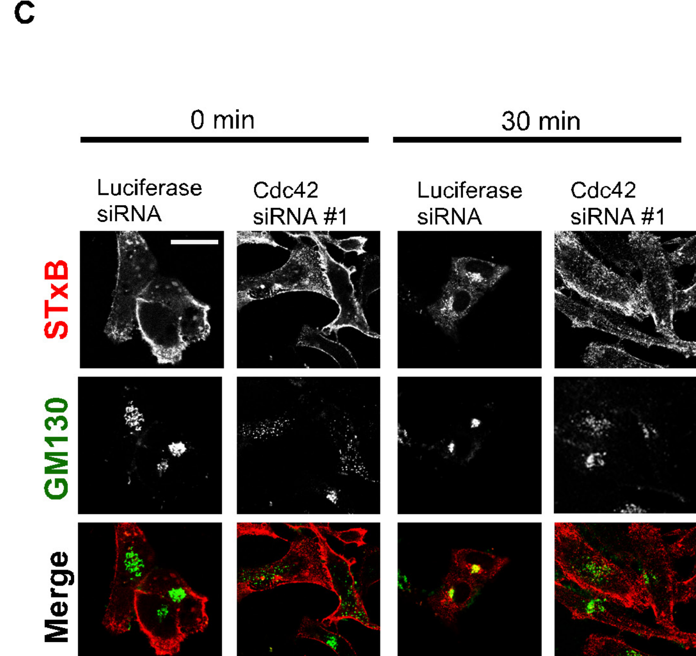


Supplemental Figure S5. RhoA did not affect the binding of STxB to the cell surface. Vero cells were incubated with STxB at 0°C for 2 minutes. The cells were washed three times at 0°C and immediately fixed. The total STxB fluorescence was quantified in control cells and cells expressing RhoA(G14V) or RhoA(T19N) as indicated. There was no significant difference between RhoA(G14V) ($p=0.9733$) or RhoA(T19N) ($p=0.1734$) and control. The bars represent SE, n=3.



Supplemental Figure S6. Cdc42 or Rac1 does not affect transferrin endocytosis. Shown are confocal micrographs of Vero cells expressing GFP-Cdc42(Q61L), GFP-Cdc42(T17N), GFP-Rac1(G12V), or GFP-Rac1(T17N). The transfected cells depicted with astericks. The cells were incubated with transferrin Alexa Fluor 555 conjugate (left panels). After 20 minutes, the cells were fixed and mounted for microscopy. Bar is 20 μm.





Supplemental Figure S7. Cdc42 siRNA inhibits STxB transport to the juxtanuclear Golgi region. Hela cells were transfected with the two previously characterized siRNAs targetting Cdc42 (Deroanne *et al.*, 2005) and a control siRNA targetting luciferase. (A) We confirmed the effectiveness of the knockdown by lysing the cells after 48 hours and loading equal amounts of total lysate on SDS-PAGE. The gels were electroblotted and probed with antibodies against Cdc42. The asterisk indicates a cross reacting band that demonstrates equal loading among the lanes. (B) We noted that the cell number decreased following Cdc42 siRNA treatment suggesting that they failed to remain attached to the coverslip. This was quantified by counting all cells in randomly selected fields after 48 hours of treatment. The number of cells per 1.5 mm^2 was calculated and plotted. We observed that the cell number was reduced by 35% with Cdc42 siRNA #1 and 63% with Cdc42 siRNA #2. The bars represent SE, n=3. (C) We used Cy3.5-labeled STxB (red) to determine whether knocking down Cdc42 expression affected trafficking. Shown are confocal micrographs of Hela cells transfected with siRNA #1 targeting Cdc42 or the control siRNA (luciferase). After 48 hours, the cells were treated with STxB, washed, and incubated for an additional 30 minutes at 37°C. The cells were fixed, permeabilized, and labeled with an antibody against the Golgi marker GM130 (green). In cells treated with Cdc42 siRNA the Golgi apparatus appears dispersed when compared to cells treated with the luciferase siRNA. STxB remains dispersed in cells transfected with Cdc42#1 siRNA, whereas STxB was localized at the Golgi apparatus in cells transfected with the luciferase siRNA. The bar is 20 μm.