

Supplemental Material

Figure legends

Figure S1. Stx transport to the TGN is not sensitive to p38 β inhibition.

(A) HeLa cells were transfected with 100 nM of the indicated siRNA for two days prior to fixation and permeabilisation. siRNA1 and 2 are targeted against two different sequences of p38 α , while siRNA b1 and b2 are targeted against different parts of the p38 β mRNA. The cells were stained with antibodies against p38 α (upper panels) or p38 β (lower panels). Bar, 10 μ m.

(B) Cells were transfected with 100 nM of the indicated siRNA for two days. StxB was then added, and the incubation continued for 45 minutes. StxB was immunoprecipitated from the lysates, and the precipitate separated by SDS-PAGE before autoradiography. The intensity of the bands was quantified and the average plotted with error bars showing deviations. This experiment was repeated twice.

Figure S2. StxB inhibits cytosolic Ca²⁺ oscillations.

Cytosolic Ca²⁺ was measured in HeLa cells loaded with the calcium probe Fura-2AM at 37°C. 1 μ g/ml Stx was added to the medium as indicated, and the incubation continued for 25 minutes followed by stimulation with 4 μ M ionomycin (A) or 1 mM ATP (B, right trace). The left trace in (B) represents cells without toxin.

(C) Cytosolic Ca²⁺ was measured in HeLa cells loaded with the calcium probe Fura-2AM at 37°C. 1 μ g/ml StxB was added to the medium three minutes after the Ca²⁺ measurement

started. This continued for 25 minutes followed by stimulation with 4 μM ionomycin (left), 1 mM ATP (middle) or 1 μM histamine (right). Traces show the Fura-2 fluorescent ratio (340nm/380nm). Time scale bar is represented for each experiment as indicated in the figure. Each trace is a representative Ca^{2+} response for 15 to 30 individual cells per experimental condition.

Figure S3. p38 inhibition and TMB-8 reduce StxB transport to the TGN in HEp2 cells.

(A) HEp2 cells were transfected with 100 nM p38 α siRNA1 for 48 hours before StxB-Sulf₂ was added. The experiment was performed as described for Fig. S1B. Inset: 1/100 of the StxB-IP supernatant was analyzed by Western immunoblot with the indicated antibodies. (B) HEp2 cells were incubated with the indicated inhibitors (30 μM SB203580 and 100 μM TMB-8, respectively) or the carrier (DMSO, 0.1% final concentration) for 30 minutes prior to incubation with StxB for 45 minutes. These experiments were repeated twice with duplicates, error bars show deviations.

Figure S4. p38 colocalizes with Stx on EEA1 positive endosomes.

Hela cells were incubated with 2 $\mu\text{g/ml}$ Stx for 20 minutes prior to fixation and permeabilisation. The cells were stained with the indicated antibodies. Bar, 20 μm .