SUPPLEMENTARY MATERIAL

Supplementary Figure 1: Analysis of STxB-saporin conjugates.

(A) SDS-PAGE was performed on a Tris-Tricine gel, and proteins stained with Coomasie Blue. Lane 1, non-cleavable *STxB-saporin*; Lane 2, cleavable *STxB-ss-saporin*; Lane 3, STxB-Cys; Lane 4 and Lane 6, cleavable *STxB-ss-saporin* +DTT; Lane 5, non-cleavable *STxB-saporin* +DTT; Lane 7, unconjugated saporin. **(B)** N-glycosidase activity of unconjugated saporin versus *STxB-ss-saporin*. **(C)** N-glycosidase activity of non-cleavable *STxB-saporin* versus cleavable *STxB-ss-saporin*. Percent depurination was calculated by relating the aniline fragment (arrow head) to the 5.8s rRNA. A non-aniline, toxin-treated control is shown.

Supplementary Figure 2: Retrograde transport inhibition of *STxB-ss-saporin* in HeLa cells.

(A) HeLa cells stably expressing GFP-tagged endoA2 incubated with 0.2 µM STxB-Cy3 or 0.1 µM STxB-ss-saporin and analyzed by total internal reflection microscopy. Scale bar: 5 µm. **(B)** 50 nM STxB-Cy3 and 50 nM Cy3-coupled STxB-ss-saporin were bound to HeLa cells on ice, which were subsequently incubated for 5 minutes at 37°C. Labelings were then analyzed by confocal microscopy. Note the substantial colocalization between both makers. Pearson's correlation coefficient is reported. Scale bar: 10 µm. (**C**) 0.5 µg/ml *STxB-ss-saporin* was incubated for 45 minutes at 37°C with HeLa cells in the absence or presence of 25 µM Retro-2. After washing, immunofluoresence was perfomed using anti-STxB (red) and anti-giantin (green) antibodies, and cells were analyzed by confocal microscopy. Scale bars: 10 µm. **(D)** Immunofluoresence experiments were also perfomed using anti-STxB (red) and goat anti-saporin (green) antibodies in control or Retro-2 treated cells, as described in (**C**). Scale bars: 10 µm.

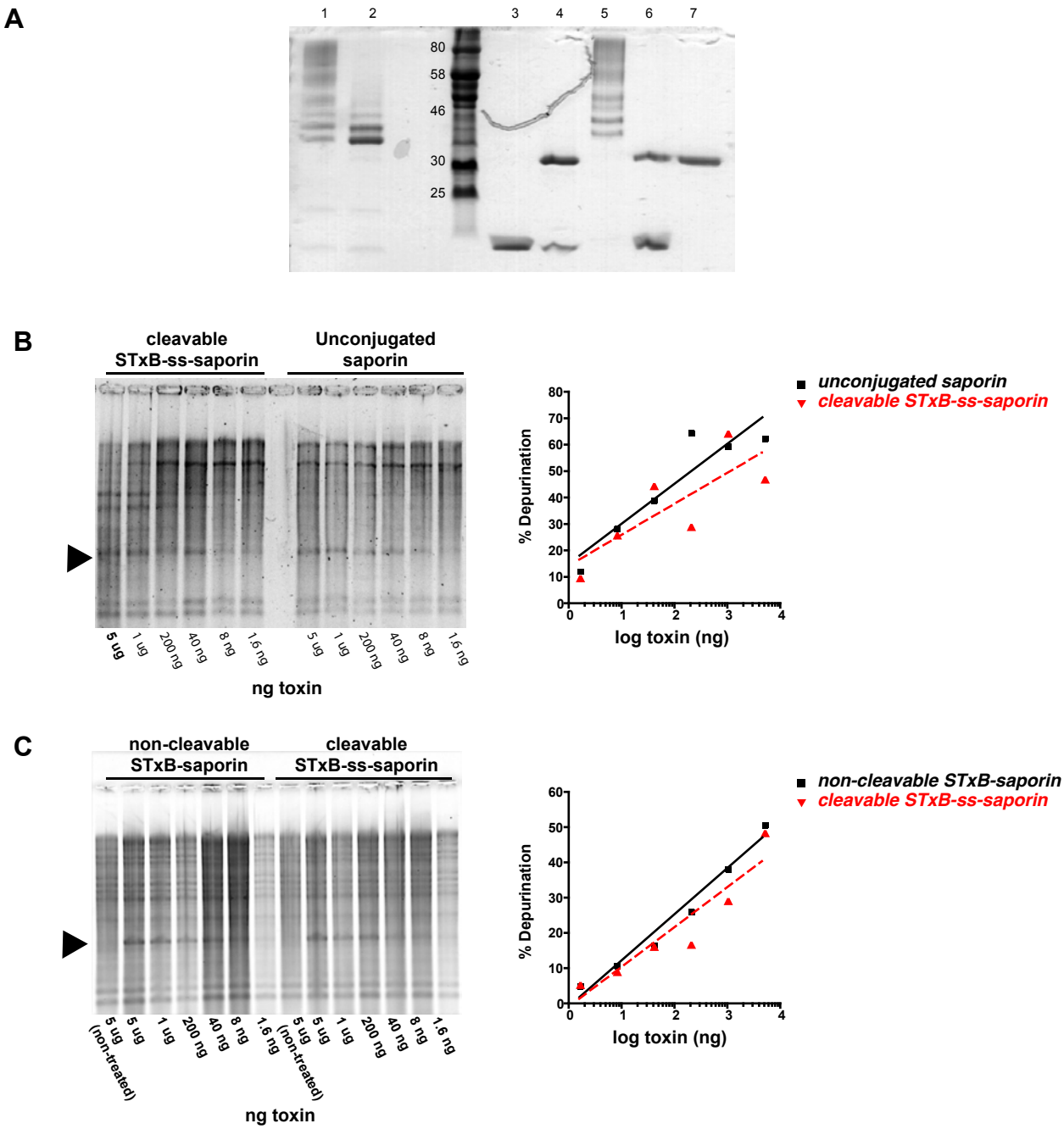
Supplementary Figure 3: Effect of temperature and endosomal acidification on *STxB-ss-saporin* cytotoxicity.

(A) Representative intoxication assay after a 4 hour exposure to STx-1 or *STxB-ss-saporin* in control conditions (black curves) or at 19.5°C (red curves). **(B)** Similarly, intoxication assays were perfomed after bafilomycin A1 treatment. A representative experiment is shown.

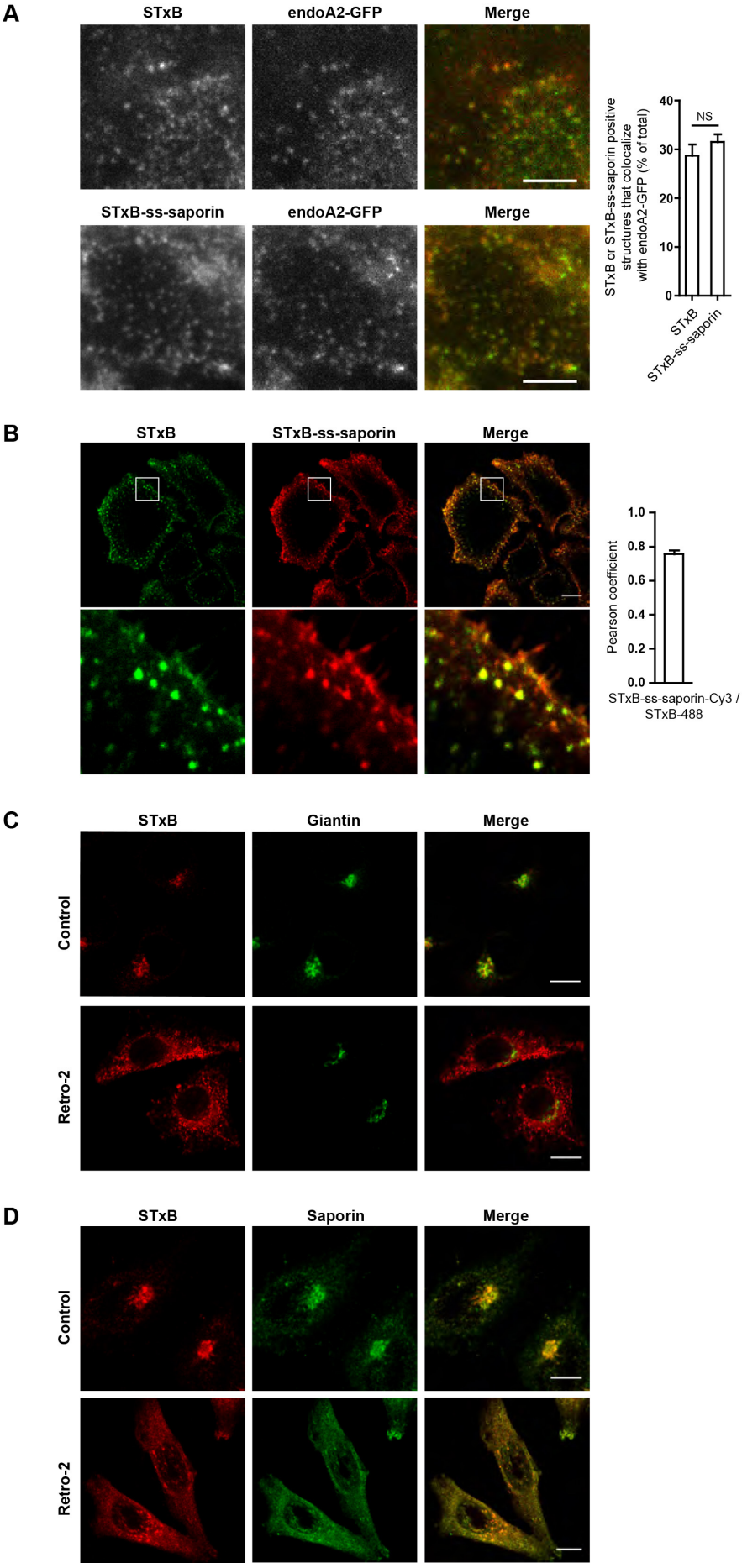
Supplementary Figure 4: Western Blot analysis of Sec22B, Rab6a', Rab5, or Rab7 depletion.

Western blot analysis of siRNA-mediated depletion of **(A)** Sec22B, **(B)** Rab6a', **(C)** Rab5, or **(D)** Rab7 versus control (scrambled) siRNA using antibodies against each of these proteins and CHC (clathrin heavy chain) as a loading control.

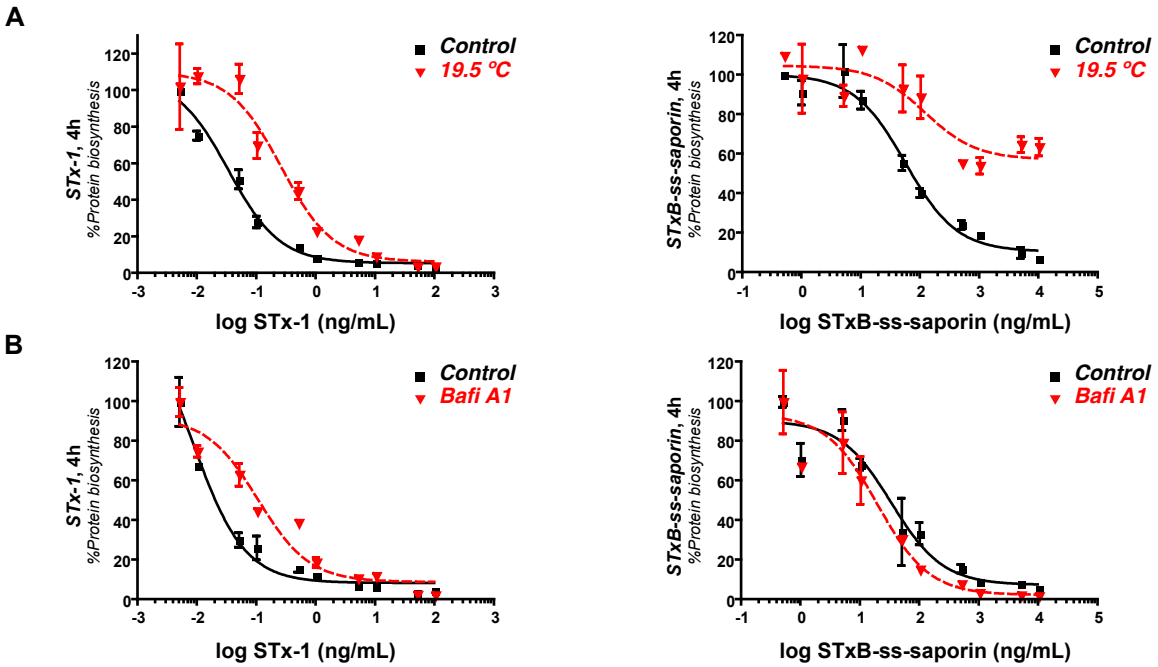
Supplementary Figure 1. Garcia-Castillo *et al*.



Supplementary Figure 2. Garcia-Castillo *et al*.



Supplementary Figure 3. Garcia-Castillo *et al.*



Supplementary Figure 4. Garcia-Castillo *et al.*