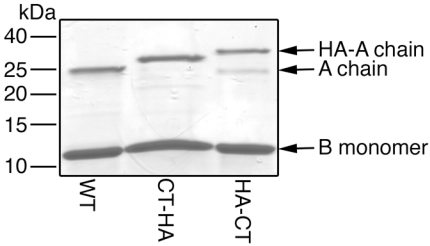**SUPPLEMENTAL FIGURE LEGENDS**

Supp. Fig. 1. Purification and Analysis of Fusion Proteins. WT (28 kDa) and HA-tagged (30 kDa) CT were purified from *e.coli* and analyzed by SDS-PAGE and Coomassie blue staining.
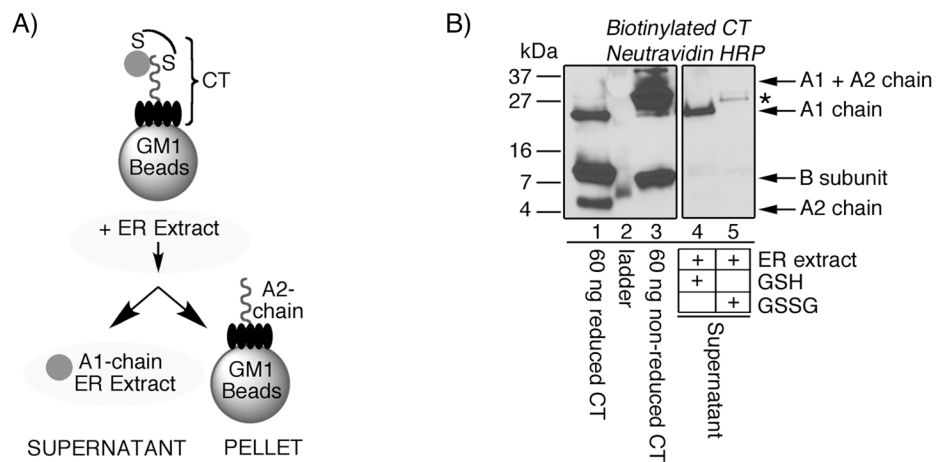
Supp. Fig. 2. The A1-chain is unfolded by PDI. (A) A schematic of the release assay for CT is shown. This diagram was made with BioDraw (Cambridgesoft, Cambridge, MA). Briefly, CT was bound to GM1 beads and incubated with ER extract in the presence of reducing agent, GSH, or oxidizing agent, GSSG. The beads, bound to CT, were pelleted; the ER extract and any toxin that was released appeared in the supernatant. (B) Biotinylated CT A1-chain is released from the A2-chain and B-subunit by ER extract in the presence of GSH (lane 4) but not GSSG (lane 5). The A2-chain remains in the pellet (not shown). Lanes 1 and 2 are controls. All samples were analyzed by SDS-PAGE and probed with neutravidin HRP. * indicates an unidentified band.

Supp Fig. 3. Modification of a refolding assay. (A) A schematic of the refolding assay illustrates the protocol. This diagram was made with BioDraw (Cambridgesoft, Cambridge, MA). Briefly, we incubated CT with GM1-coupled beads and ER lumenal proteins in reducing conditions. The ER lumenal chaperone protein, PDI, binds, unfolds, and releases the A1-chain into the supernatant from the B-subunit-GM1-bead complex (this complex, the pellet, also contains any unfolded A-subunit). The supernatant was left untreated or treated with GSSG (for varying lengths of time, as indicated) to mediate release from PDI and subsequent refolding of the A1-chain; this was followed by trypsin treatment to degrade any unfolded or poorly folded substrate. All samples were analyzed by SDS-PAGE under non-reducing conditions, except the 4 ng control, which was reduced to separate the A1-chain. (B) The unfolding and refolding of WT CT was analyzed. Samples were immunoblotted with a toxin antibody recognizing the A- and B-subunits.
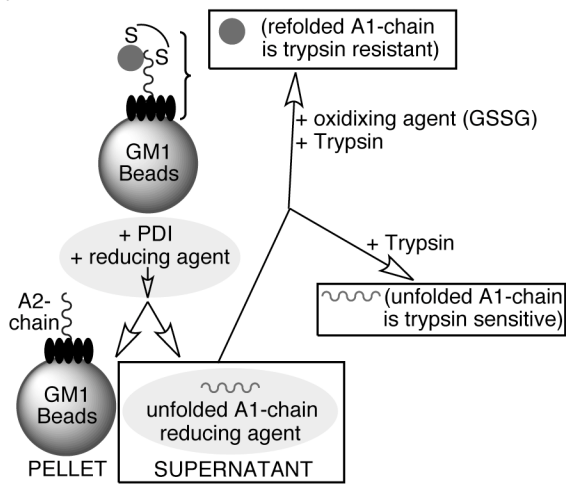
Supplemental Figure 1

Supplemental Figure 2

A)

B)

Biotinylated CT
Neutravidin HRP

Supplemental Figure 3

A)

B)