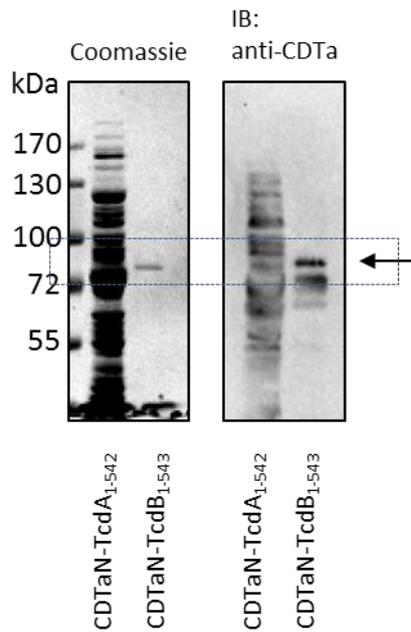


Supplementary Materials: The Binary Toxin CDT of *Clostridium difficile* as a Tool for Intracellular Delivery of Bacterial Glucosyltransferase Domains

Lara-Antonia Beer, Helma Tatge, Carmen Schneider, Maximilian Ruschig, Michael Hust, Jessica Barton, Stefan Thiemann, Viola Fühner, Giulio Russo and Ralf Gerhard

A



B

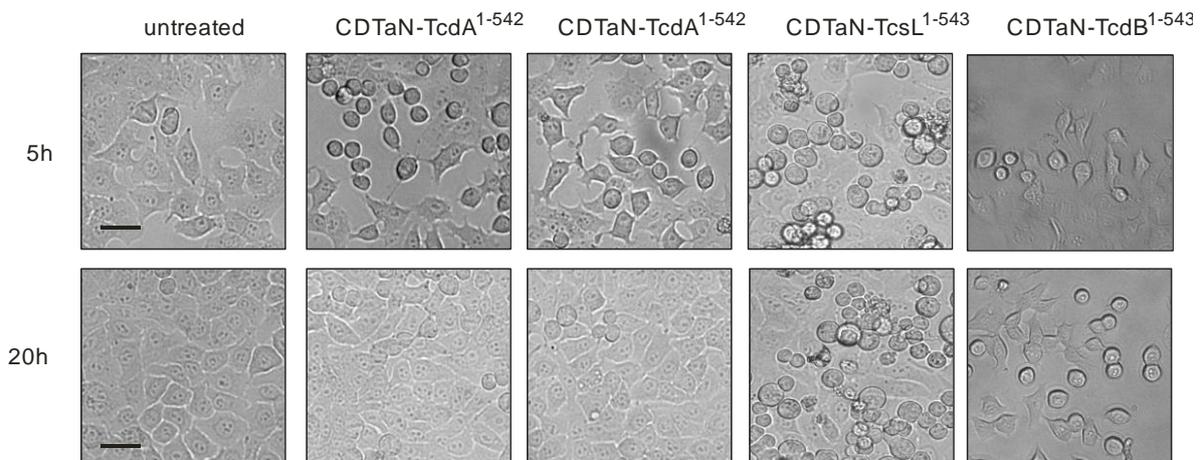


Figure S1. Unstable CDTaN-TcdA¹⁻⁵⁴² fusion protein. A) Eluate from Ni²⁺-affinity purification of 6xHis tagged CDTaN-TcdA¹⁻⁵⁴² (91 kDa) by Ni-Ida columns (Macherey-Nagel) showed enrichment of unspecific proteins in Coomassie-stained 7.5% SDS-PAGE (left panel). For comparison, purified 6xHis tagged CDTaN-TcdB¹⁻⁵⁴³ (91 kDa, arrow) is shown. Half of the SDS-gel was blotted onto nitrocellulose and specific detection of the same CDTaN-fusion proteins was performed by immunoblot analysis

with anti-CDTa polyclonal rabbit serum (right panel). CDTaN-TcdB¹⁻⁵⁴³ along with few degradation products, that were not visible in Coomassie gel, were specifically detected. On the contrary, no specific band accounting for CDTaN-TcdA¹⁻⁵⁴² was observed. Thus, 6xHis tagged CDTaN-TcdA¹⁻⁵⁴² is below detection limit in SDS-PAGE and Immunoblot analysis. **B)** For a sensitive proof of functional CDTaN-TcdA¹⁻⁵⁴² a cell rounding assay with HEp-2 cells was performed. Cells were seeded in a 24-well plate at a density of approximately 5×10^5 cells/well in Minimum Essential Medium Eagle supplemented with 10% fetal bovine serum, 100 μ M penicillin and 100 μ g/ml streptomycin and were let grown over night. 1000 ng/ml activated CDTb in combination with 500 ng CDTaN-fusion protein (as indicated) were applied. In case of CDTaN-TcdA¹⁻⁵⁴² with unknown protein concentration, 2 μ l of protein solution was added to 1 ml cell culture medium containing 1000 μ g activated CDTb. Morphological changes of cells were monitored by phase contrast microscopy 5 h and 20 h after addition of toxins. Transient morphological changes were induced by CDTaN-TcdA¹⁻⁵⁴² in some cells (shown are two replicates) after 5 h. After 20 h of incubation, no morphological changes of cells were observed anymore. In contrast, HEp-2 cells were affected by CDTaN-TcdB¹⁻⁵⁴³ or CDTaN-TcdL¹⁻⁵⁴³ after 5 h, and the effect was observed even after 20 h of incubation. Scale bar in untreated controls represent 25 μ m.