Figure S1

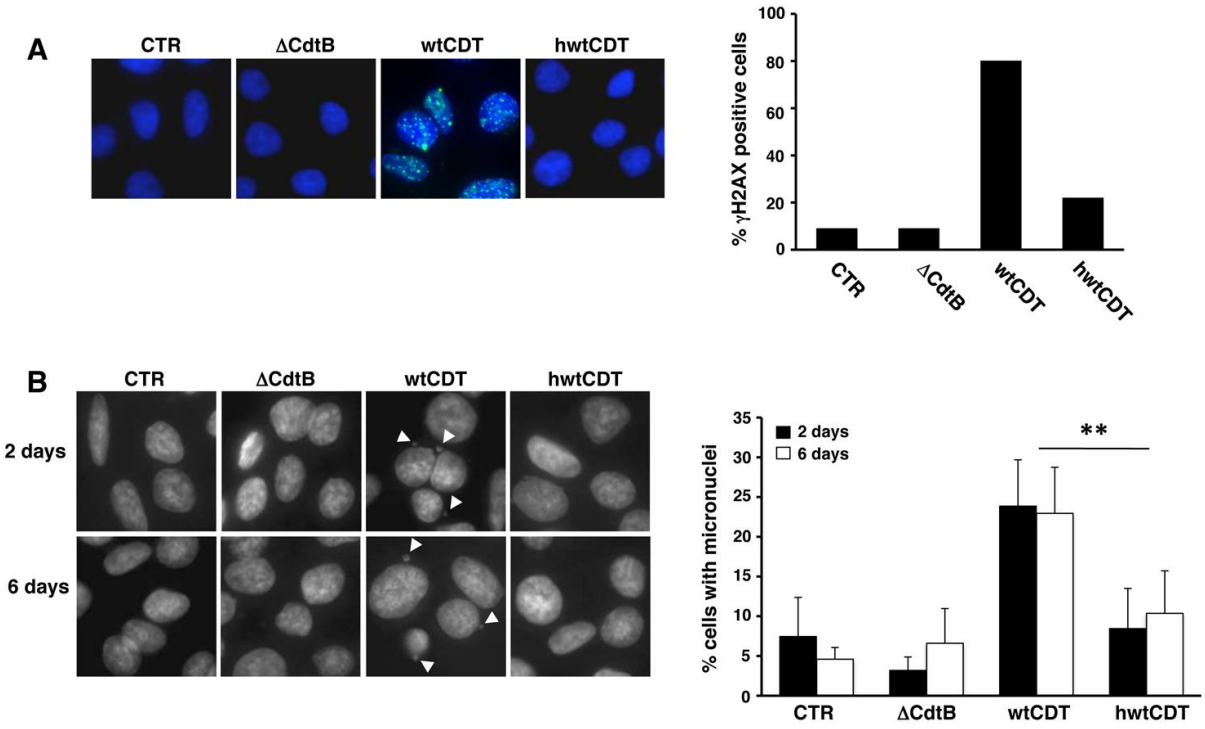

Fig. S1. Heat inactivation abrogates the DNA-damaging activity of CDT.

A. Big Blue fibroblasts were left untreated (CTR) or exposed to the lysates containing the inactive (ΔCdtB), wild-type (wtCDT) toxin or the wild-type toxin heat inactivated at 90°C for 10 min (hwtCDT) at a 1:1000 dilution for 8 h in complete medium. Phosphorylated H2AX (γH2AX) was detected by indirect immunofluorescence (green), and nuclei were counterstained with DAPI (blue). Magnification 63×. The quantification of cells positive for γH2AX is shown in the right panel.

B. Big Blue fibroblasts were left untreated (CTR) or exposed to the lysates containing the inactive (ΔCdtB), wild-type (wtCDT) toxin or the wild-type toxin heat inactivated (hwtCDT) at a 1:20 000 dilution in complete medium, and incubated for the indicated periods of time. Nuclei were stained with DAPI, and visualized by fluorescence microscopy. Magnification 63×. Micronuclei are indicated with head arrows. The right panel shows the quantification of cells carrying micronuclei (mean ± SD of 50 cells randomly chosen per condition). Statistical analysis was performed using the Student's *t*-test.

Figure S2

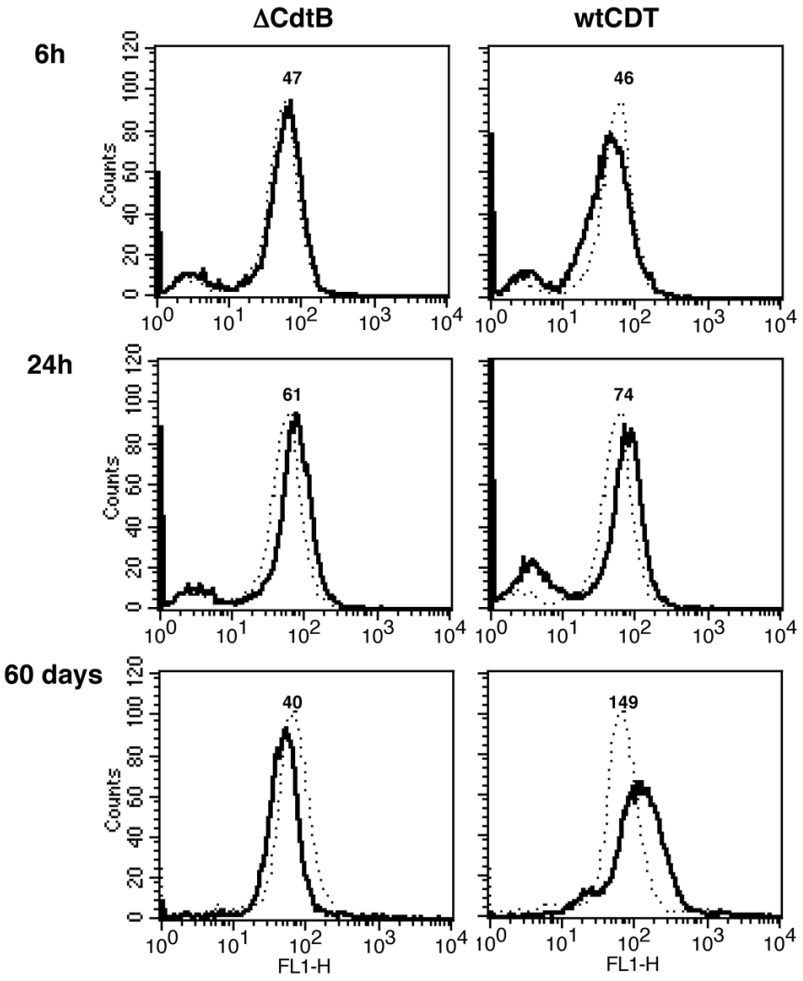

Fig. S2. Chronic exposure to CDT promotes ROS production. Big Blue fibroblasts were left untreated (CTR) or exposed to bacterial lysates containing the inactive (ΔCdtB) or the wild-type (wtCDT) toxin at a 1:20 000 dilution in complete medium for the indicated periods of time. Levels of intracellular ROS were assessed by FACS analysis. The dotted line represents the mean fluorescence intensity (MFI) in untreated cells. The numbers in the histograms represent the MFI of ΔCdtB- or wtCDT-treated cells.