



**Supplementary Fig. S2.** TtsA is essential for typhoid toxin secretion. **(a and b)** Detection of typhoid toxin on the bacterial surface and in typhoid toxin carrier intermediates (puncta) after *S. Typhi* infection of cultured epithelial cells. Henle-407 cells were infected with wild-type,  $\Delta ttsA$ , or the complemented  $\Delta ttsA$  mutant strains, all expressing chromosomally encoded FLAG-tagged CdtB. 24 hs post-infection, bacteria were stained with an antibody directed to FLAG-tagged CdtB (green) to visualize typhoid toxin, a rabbit antibody directed to *S. Typhi* LPS (red) (to visualize bacterial cells), and DAPI (blue) (to visualize DNA) **(a)** (scale bar: 5  $\mu$ m). The quantification of typhoid toxin-associated fluorescence on bacterial cells is shown **(b)**. The fluorescence signal intensity of typhoid toxin (green) on bacterial cells was normalized to the LPS signal (red) on the bacteria. Shown are the average ratios green/red and the corresponding standard deviations from 3 independent experiments, in which a total of 30 images were analyzed. **(c)** Western blot analysis of CdtB expression in the different strains used in this analysis. All data in **(a-c)** were derived from at least three independent experiments.