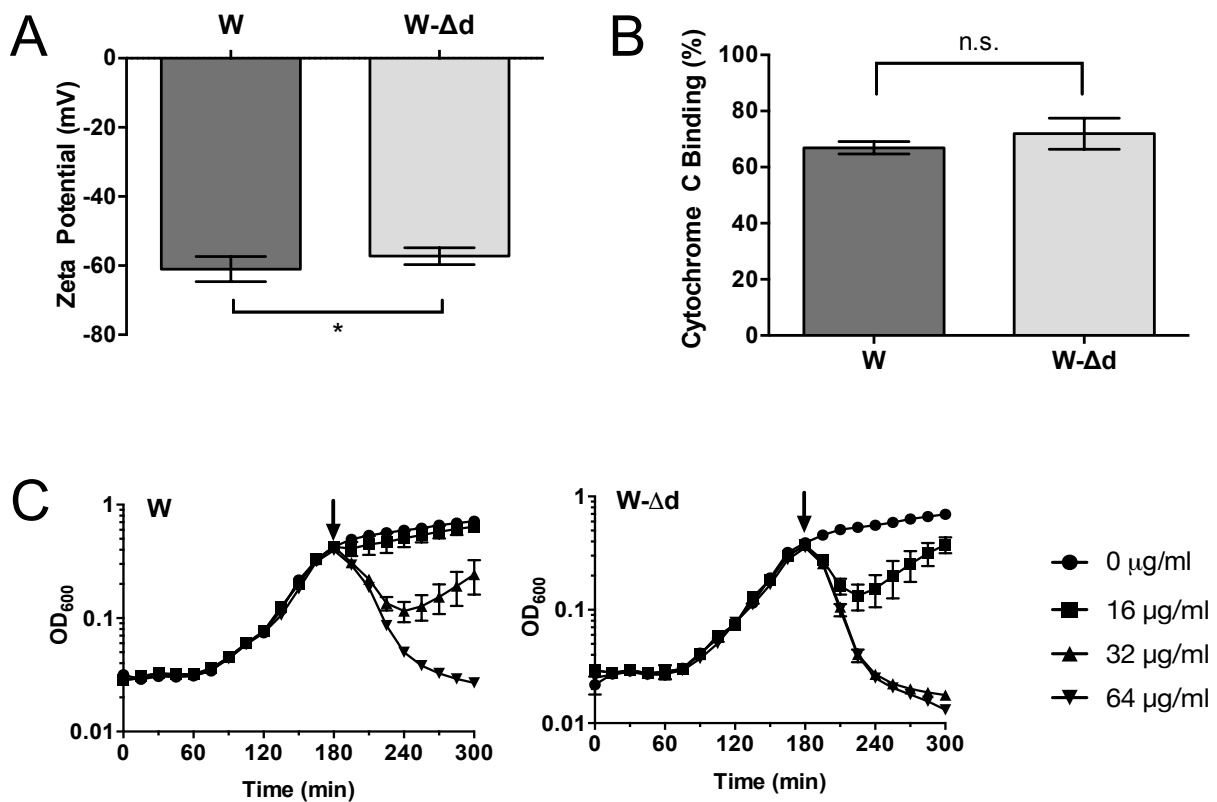




Additional file 4. Effects of *dtlABCDE* deletion on the cell surface charge of *B. subtilis*. Cells of wild-type *B. subtilis* W168 (W) or an isogenic *dtlABCDE* deletion strain were subjected to surface charge analyses. **(A)** Zeta potential measurements. Cells were grown to exponential phase ($OD_{600} = 0.5-1$), harvested, washed and resuspended in dH₂O to $OD_{600} = 0.5$, and the zeta potential measured on a Zetasizer Nano ZS at 25 °C. Data are shown as mean $\pm$ standard deviation of three biological repeats, each measured in technical triplicates. n.s. shows $p > 0.05$ and * shows $p \leq 0.05$ from an un-paired t-test analysis. **(B)** Cytochrome C binding assay. Overnight cultures were resuspended in MOPS/NaOH [pH7] to $OD_{600} = 2.5$ and incubated with 250 $\mu\text{g/ml}$ cytochrome C for 10 minutes at room temperature. Percentage binding was calculated as absorbance difference of the supernatant relative to samples without bacteria. Data are shown as mean $\pm$ standard deviation of three to four biological repeats; n.s. $p > 0.05$ in an unpaired t-test analysis. **(C)** Nisin-dependent killing assay. Cells were grown in LB medium in a Tecan microplate reader to $OD_{600} = 0.4-0.5$. At the time point shown by the arrows, nisin was added at the indicated concentrations and OD_{600} monitored over time. Data are shown as mean and standard deviation of three technical repeats and are representative of three biological repeats.