Supplementary Legends

Fig. S1 Expression analysis of *esx* deletion mutants and growth curves.

A. Expression analysis of *esxA* and *esxB* genes in USA300 WT strain and in different *esx* mutants by quantitative real-time PCR (qRT-PCR). Relative quantification was performed using 16S rRNA as internal control. **B.** Growth curve in TSB medium does not reveal any growth defects.

Fig. S2 *esxA* mutant induces more actin polymerization.

A. Confocal microscopy of A549 cells infected by the WT and the *esx* mutant strains Δ*esxA* and Δ*esxB* 4h *p.i.* Vancomycin-Bodipy-FL stains *S. aureus* wall, while phalloidin stains F-actin. Polymerized actin (condensed form) can be observed as brightly stained cell membranes on rounded cells (white arrows). **B.** The graph shows quantitation of rounded infected cells with cell membranes bearing polymerized actin for the WT and *esx* mutant-infected cells. Data are representative of 3 independent experiments. * indicates significant differences, one way ANOVA, Tukey's multiple comparisons test $P<0.05$.

Fig. S3 Flow cytometry analysis of A549 cells transfected with truncated *esxA*

24h post transfection cells were dissociated and then stained with Aqua Live-Dead and Annexin V-APC. The percentage of apoptotic cells (early and late apoptotic cells) was measured in the subpopulation bearing EYFP, *i.e.* transfected cells. Data are shown as a percentage of cells transfected with the control vector. Data are from one representative experiment.

Fig S4. FACS analysis of apoptosis after staurosporine treatment

24h post transfection, A549 cells transfected with the plasmid p*esxA*-EYFP were treated with 2.5 mM staurosporine for 30 min, followed by AquaLive-Dead and Annexin V-APC staining. The percentage of apoptotic cells (early and late apoptotic cells) was measured in the subpopulation bearing EYFP, *i.e.* transfected cells. Representative flow cytometry scatter plots depicting the early apoptotic (bottom right quadrant), late apoptotic (top right quadrant) and dead cells (top left quadrant).

Fig. S5 Δe*sxAB* mutant induces more apoptosis in epithelial cells compared to WT.

Quantitation of apoptotic cells using flow cytometry for A549 cells infected by WT and Δe*sxAB* strain at 6h *p.i*. Cells were stained with Aqua Live-Dead (1:500), and with Annexin V-FITC. Data are the mean of 3 independent experiments +/- s.d. * indicates significant differences using a one tailed Mann Whitney test ($P< 0.05$).

Fig S6. Bacterial counts from supernatants from continuous infection assays

Bacterial counts from cell culture supernatants of cells infected with WT or Δ*esxAB* at 7.5h *p.i.* in the absence of lysostaphin, as described in Methods.

Fig. S7. Hemolysin expression in *esx* deletion mutants

A. After overnight culture of WT and *esx* mutants, 5 μl of culture were spotted on the blood agar plates and incubated for 24h. Equal zones of hemolysis were observed for the WT and mutant strains. **B.** Immunoblotting analysis of bacterial supernatants. Proteins were precipitated with TCA, separated on SDS-PAGE, and detected by immunoblotting with anti-α-hemolysin.