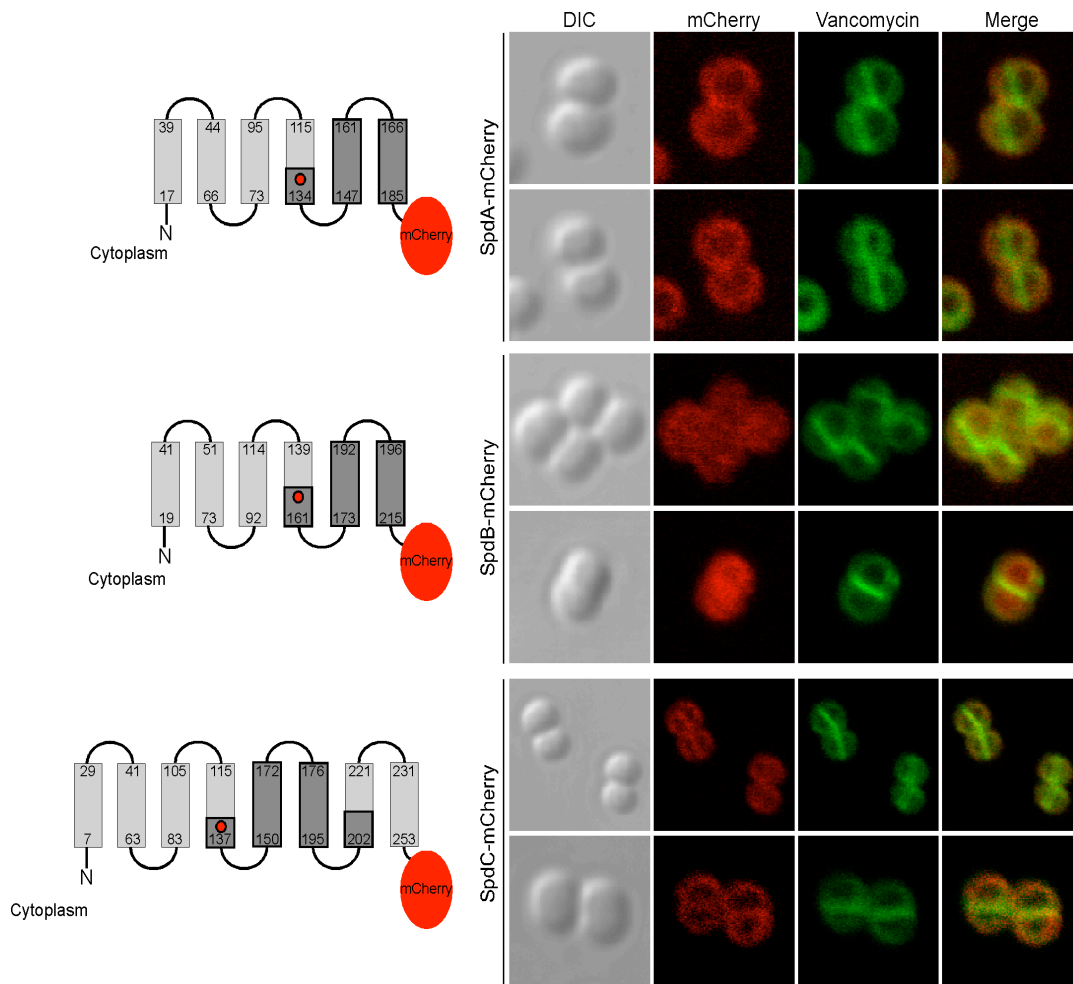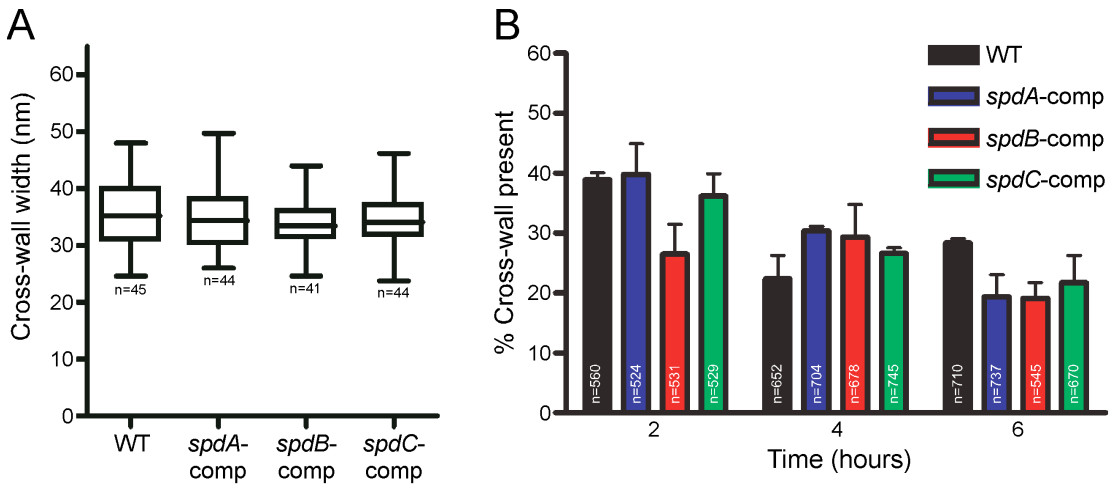**Figure S1. *spd* mutants do not display a growth defect.**
Staphylococcus aureus strain Newman (WT, wild-type) and isogenic variants carrying *bursa aureus* insertions in *spdA*, *spdB* or *spdC* were inoculated into tryptic soy broth (TSB) and staphylococcal growth at 37°C with rotation was monitored as an increase in optical densitiy (OD_{600}) measured over time.

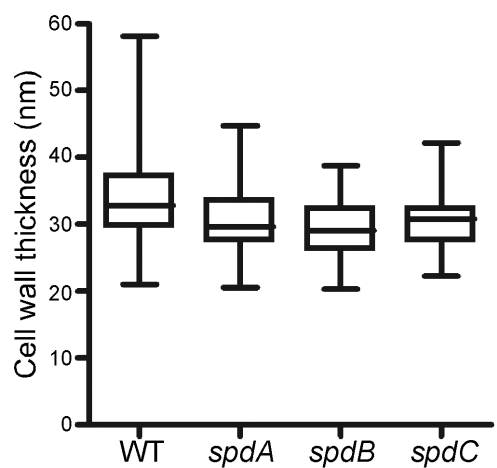**Figure S2. Cellular distribution of Spd-mCherry hybrids.**
Left panel - The drawing illustrates translational hybrids that were derived from coding sequences for SpdA, SpdB, SpdC and mCherry to generate fusions that position the red fluorescent protein in the bacterial cytoplasm. *S. aureus* Newman harboring plasmids expressing the indicated Spd-mCherry hybrids was grown to mid-logarithmic phase, fixed, and incubated with BODIPY-vancomycin to stain the staphylococcal cross-wall and outer peptidoglycan layers. Representative micrographs are displayed with the DIC images in the left column, followed by fluorescence images for the Spd-mCherry hybrids, BODIPY-vancomycin, and a merge of the two fluorescent images in the right column.

Figure S3. Cell division and cross wall diameter phenotype of *spd* mutants with complementing plasmids.

A) The width of the cross-walls from wild-type (WT) and complemented spd mutants was determined from the indicated number of bacteria (n) of each strain from images derived by electron microscopic analysis. The non-parametric Anova (Kruskal-Wallis) test and the Dunn's multiple comparison test were used to assess significance of these observations. There was no significant difference between WT and complemented mutant strains.

B) Overnight cultures of wild-type and complemented *spd* mutant staphylococci were diluted into fresh media and aliquots removed after 2, 4, and 6 hours of incubation. Cells were fixed and stained with BODIPY-vancomycin. The percent amount of cross-walls was quantified and compared to the total number of cells per field of view. The number of cells counted is listed in each bar (n). Error bars represent the SEM.

Figure S4. Cell wall envelope features of *spd* mutants
Wild-type (WT) and *spd* mutant staphylococci were fixed and
subjected to transmission electron microscopy as shown in
Fig. 7. The diameter of the outer peptidoglycan layer was
determined for 50 cells each.