SUPPLEMENTAL FIGURES AND LEGENDS

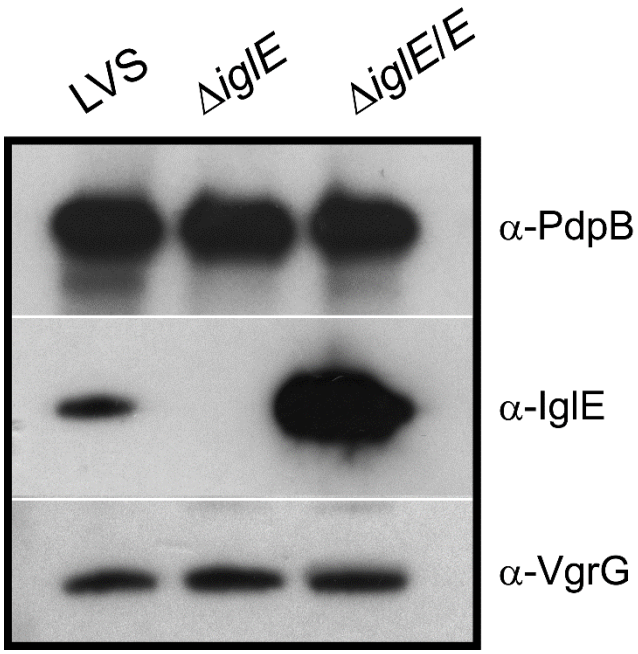

Figure S1. IglE expression in *F. tularensis* LVS. Analysis of IglE protein synthesis from LVS, Δ*iglE* or Δ*iglE* expressing IglE in *trans*. Proteins contained in the pellet fraction were separated by SDS-PAGE and identified by immunoblot analysis using an anti-IglE antiserum. Analysis of PdpB and VgrG was performed to exclude polar effects of the *iglE* deletion. The experiment was repeated three times and a representative example is shown.

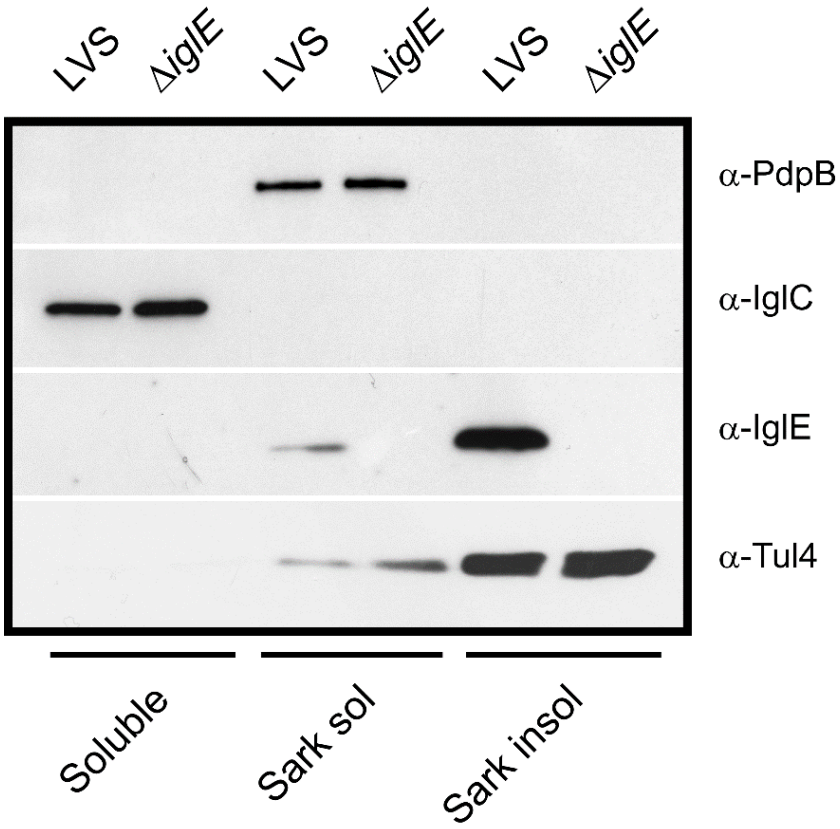

Figure S2. IglE localizes to the outer membrane. Subcellular localization of IglE in *F. tularensis*. LVS and Δ*iglE* were fractioned into soluble and membrane-associated fractions, and Sarkosyl solubilization was used to further separate inner (Sarkosyl-soluble) and outer (Sarkosyl-insoluble) membranes. Protein fractions were separated by SDS-PAGE and analyzed using standard Western blot techniques and appropriate antiserum. To detect IglE, anti-IglE antibodies were used, while antibodies recognizing IglC, PdpB, or Tul4 were used as markers for soluble, inner membrane and outer membrane fractions, respectively. The experiment was repeated three times and a representative example is shown.

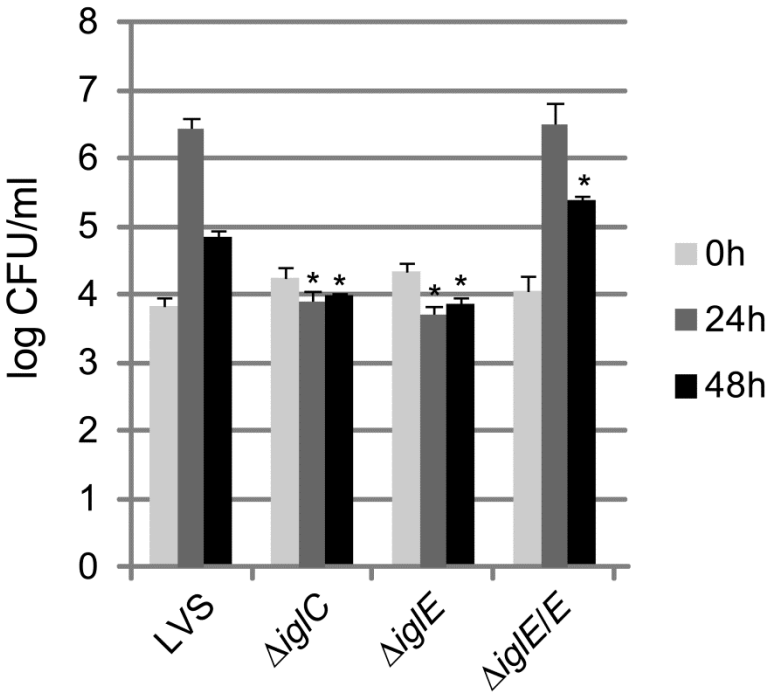

Figure S3. Intracellular growth of *F. tularensis* strains. J774 cells were infected by various strains of *F. tularensis* at an MOI of 200 for 2 h. Upon gentamicin treatment, cells were allowed to recover for 30 min after which they were lysed immediately (corresponds to 0 h) or after 24 h or 48 h with PBS-buffered 0.1 % sodium deoxycholate solution and plated to determine the number of viable bacteria ($\log_{10}$). All infections were repeated two times and a representative experiment is shown. Each bar represents the mean values and the error bar indicates the SD from triplicate data sets. The asterisks indicate that the $\log_{10}$ number of CFU was significantly different from the parental LVS strain as determined by a 2-sided *t*-test with equal variance, including the Bonferroni correction for multiple pair-wise comparisons (*, $P \leq 0.05$; **, $P \leq 0.01$).

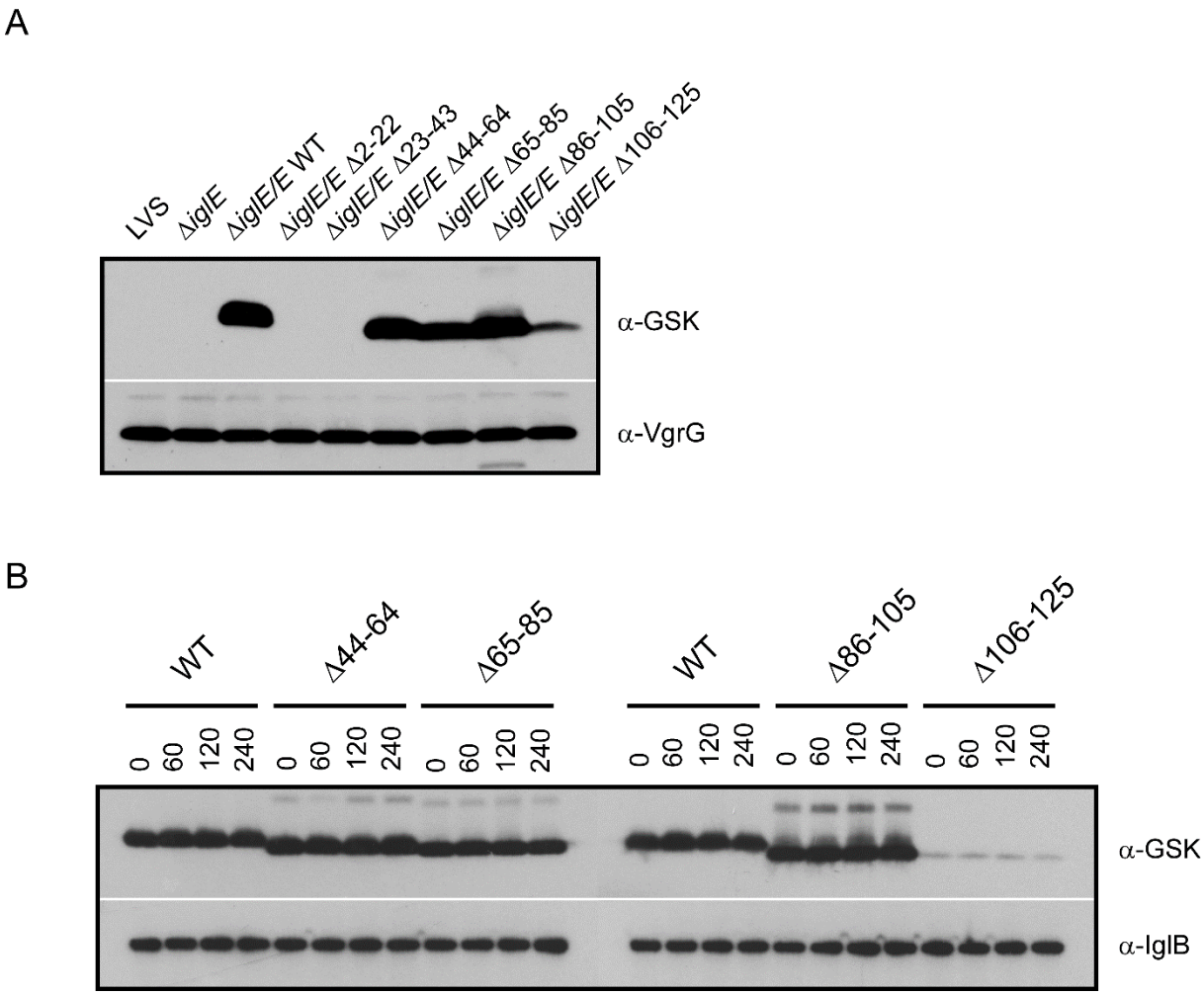

Figure S4. Expression (A) and stability (B) of mutant forms of IglE expressed in LVS Δ*iglE*. Proteins contained in the pellet fraction were separated by SDS-PAGE and identified by immunoblot analysis using an anti-GSK antiserum. Antibodies specific for VgrG was used as a loading control (A). The stability of mutant forms of IglE was analyzed over time using anti-GSK antiserum after stopping *de novo* synthesis. Antibodies specific for IglB was used as a loading control (B). The experiments were repeated two times and a representative example is shown.

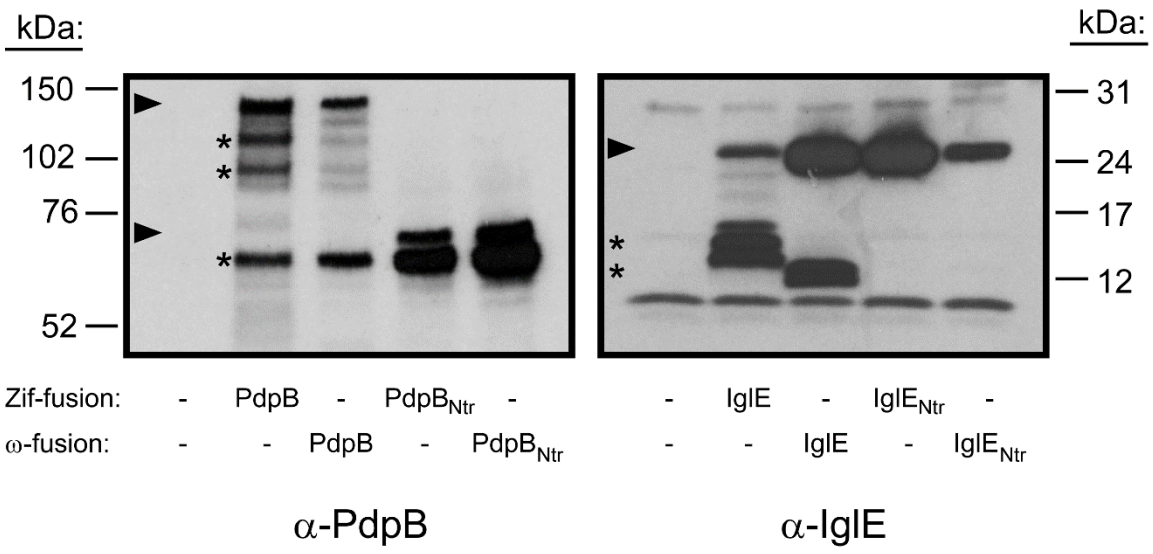

Figure S5. Expression of IglE and PdpB in the B2H reporter strain KDZif1ΔZ. Full-length or N-terminally truncated IglE (aa 23-125) or PdpB (aa 590-1093) variants were fused to either Zif- or ω of the *E. coli* RNA polymerase and expressed in the *E. coli* B2H reporter strain. The negative control (-/-) refers to *E. coli* transformed with empty vectors. Upon IPTG induction, equivalent amounts of bacterial lysates were analyzed for the presence of fusion protein using antisera recognizing PdpB or IglE. Left panel; full-length and truncated forms of PdpB are indicated with black arrows. Right panel; full-length and truncated forms of IglE migrate similarly and are indicated with a black arrow. Note that full-length and truncated versions of PdpB, similar to full-length versions of IglE, result in degradation products (indicated with asterisks).

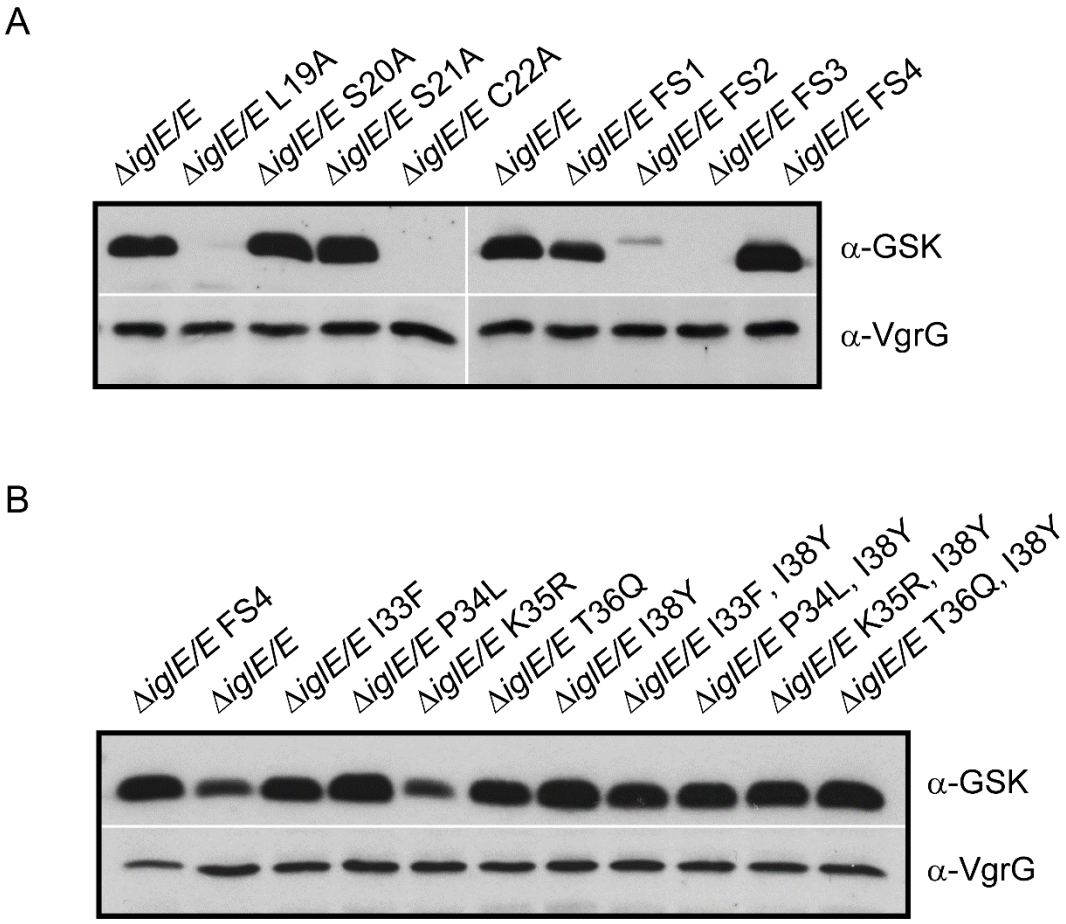

Figure S6. Analysis of IgE protein synthesis by *F. tularensis* strains. GSK-tagged IglE present in the pellet fraction of the indicated IglE mutant strains were separated by SDS-PAGE and identified by immunoblot using antiserum specific for GSK. Antibodies directed against VgrG were used as a loading control. The experiment was repeated at least three times and a representative example is shown.

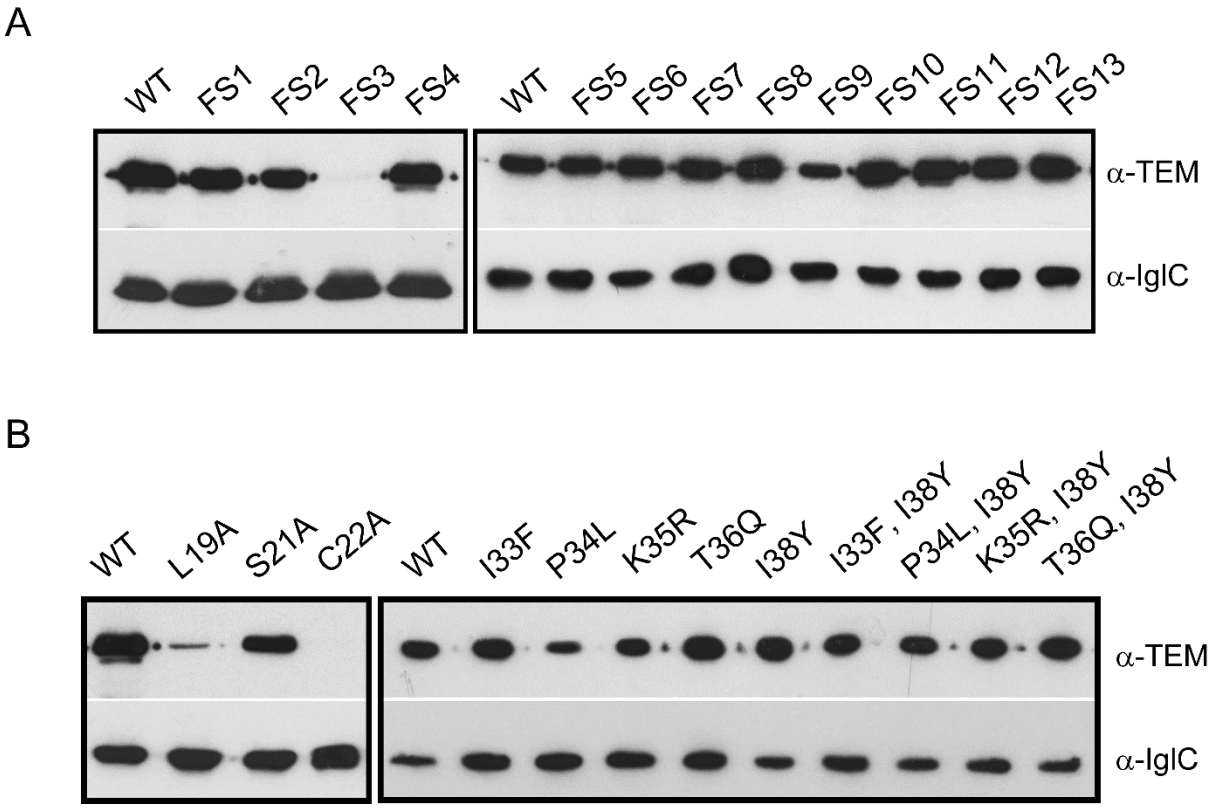

Figure S7. Production of IglE frameshift mutants fused to TEM β-lactamase. Total cell lysates of *Francisella* LVS harboring various IglE-TEM fusions were prepared and examined by Western-blot analysis using an antibody against TEM β-lactamase or IglC. The latter was used as a loading control. The experiment was repeated at least three times and a representative example is shown.