Figure S1. *F. tularensis* encodes three *rpsU* genes. *F. tularensis* *rpsU*2, which encodes bS21-2, is syntenic with the only *rpsU* in *E. coli*, which is located in the macromolecular synthesis operon (1). This operon in *E. coli* includes *rpsU* (encoding bS21), *dnaG* (encoding DNA primase), and *rpoD* (encoding RNA polymerase σ^70^). In *F. tularensis*, this operon also includes *yqeY*, the product of which may be involved in tRNA aminoacylation. *rpsU*1, encoding bS21-1, is located immediately downstream of *cspC* (encoding cold-shock protein CspC), while *rpsU*3, encoding bS21-3, is not apparently in an operon with other genes. Genomic locations of *rpsU* genes were determined using RefSeq NC_007880 for *F. tularensis* and NC_000913 for *E. coli*. 
Figure S2. The three bS21 homologs in *F. tularensis* are distinct. Percent identities of amino acid sequences for *F. tularensis* LVS bS21-1, bS21-2, bS21-3, and *E. coli* bS21 were calculated using the multiple sequence alignment tool ClustalOmega (2). The bS21 homologs in *F. tularensis* are similar to each other, particularly bS21-1 and bS21-3 which are 72% identical at the amino acid level. bS21-2, encoded by the rpsU homolog gene syntenic to the single *E. coli* rpsU gene, is also the most similar to *E. coli* bS21, with 60% amino acid identity.
Figure S3. Each bS21 homolog can be detected in translationally-active ribosomes. For A – D, top: Sucrose gradient sedimentation profile from actively-translating wild-type *F. tularensis* cells with either empty vector or ectopic expression of indicated bS21 homolog. Nucleic acid content was monitored by A260 (y-axis). Peaks corresponding to the 30S, 50S, 70S, and polysomes are indicated. Fractions collected are indicated on the x-axis. For A – D, bottom: Immunoblot analysis of fractions from sucrose gradient sedimentation (above), probing for VSV-G. Wells correspond to fractions 1 – 21 from profile above. A. Cells from wild-type *F. tularensis* LVS with empty vector (LVS pF). B. Cells from wild-type *F. tularensis* LVS with ectopic expression of bS21-1 (LVS pF-bS21-1-V). C. Cells from wild-type *F. tularensis* LVS with ectopic expression of bS21-2 (LVS pF-bS21-2-V). D. Cells from wild-type *F. tularensis* LVS with ectopic expression of bS21-3 (LVS pF-bS21-3-V).
Figure S4. Loss of bS21-2 does not affect transcript abundance of FPI-encoded genes.

Quantitative real-time PCR was used to determine the relative transcript abundance for indicated FPI genes in wild-type cells, cells lacking bS21-2 (ΔrpsU2), or cells lacking the transcription factor PigR (ΔpigR). Cells lacking PigR serve as a positive control, as PigR positively regulates its own transcription and the transcription of pdpA, pdpB, and iglA. The rpoA1 and bfr genes are included as negative controls, as their expression is not influenced by bS21-2 or PigR. Transcript abundances are normalized to tul4, whose expression is not influenced by bS21-2 or PigR. Error bars represent 1 SD from the value (calculated using the mean threshold cycle). ns: not significant. ND: not detected. *adjusted p < 0.05 by t-test.
Figure S5. Loss of bS21-2 does not affect protein degradation of PdpB. One-phase decay of PdpB from antibiotic-chase experiment from wild-type cells and cells lacking bS21-2 (ΔrpsU2). Neither strain showed significant degradation of PdpB through the time points assessed; the calculated half-life for both was greater than 120 minutes. Y-axis is logarithmic and error bars represent 1 SD from the mean.
Supplemental References

1. Lupski JR, Godson GN. 1984. The rpsU-dnaG-rpoD macromolecular synthesis operon of E. coli. Cell 39:251–252.

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