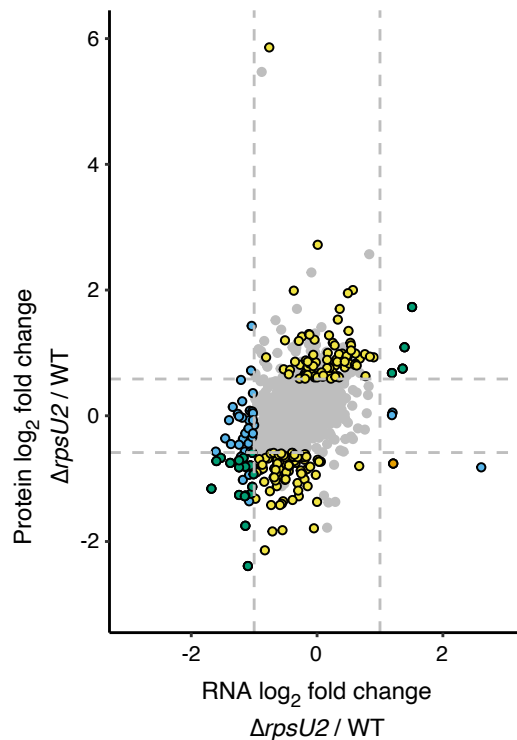
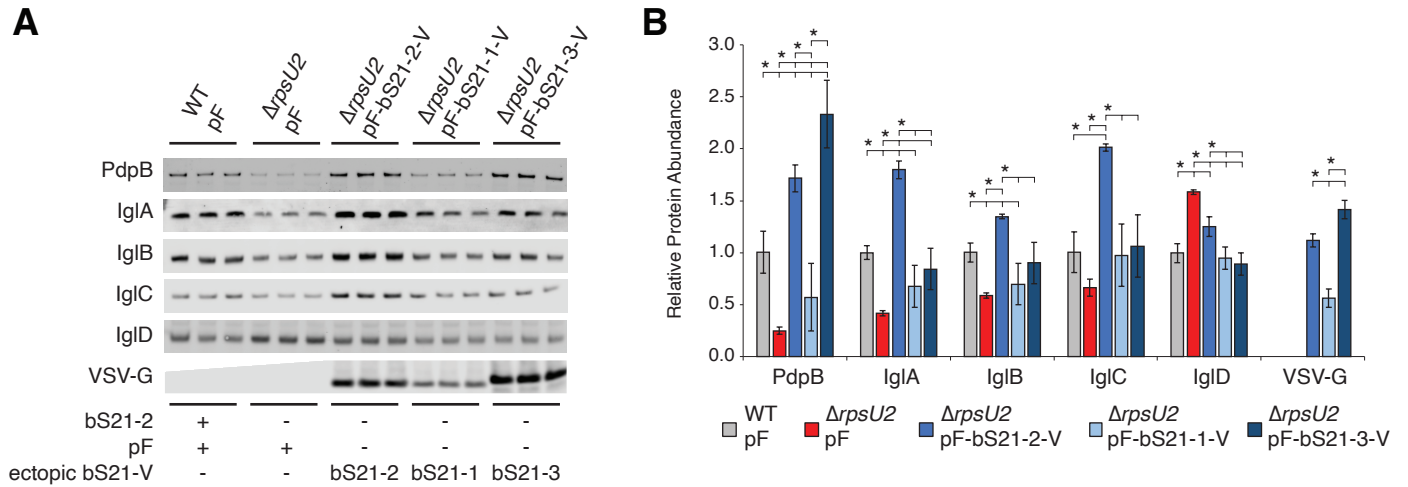


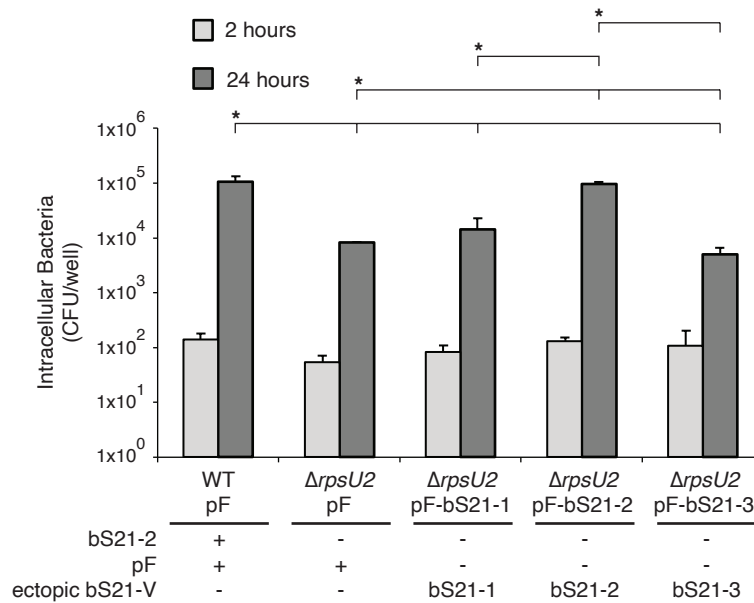
**Figure 1. *F. tularensis* ribosomes are heterogenous with respect to bS21.** **A.** Chart demonstrating purity of wild-type ribosomes. Categories represent classification of proteins identified by mass spectrometry of ribosomes purified from wild-type *F. tularensis* LVS cells. Numbers represent the percentage of spectral counts corresponding to proteins in each category, combined from quadruplicate samples. **B.** Wild-type *F. tularensis* LVS ribosomes contain more than one bS21 homolog. Table detailing the number of spectral counts corresponding to bS21 homologs identified from individual ribosome purifications (A – D) from wild-type cells. Spectral counts corresponding to bS21-1 and/or bS21-3 cannot be unambiguously assigned due to complete sequence identity of detected peptides. ND: not detected. **C.** Each bS21 homolog can be incorporated into ribosomes. Top: Sucrose gradient sedimentation profile from actively-translating wild-type cells containing an empty vector. 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. Bottom: Immunoblot analysis of fractions from sucrose gradient sedimentation performed on actively-translating cells ectopically expressing indicated bS21 homolog with VSV-G epitope tag. Wells correspond to fractions 1 – 21 from profile above.



**Figure 2. Loss of bS21-2 leads to changes in protein abundance that cannot be explained by changes in transcript abundance.** Cells with (WT, wild-type) and without bS21-2 ( $\Delta rpsU2$ ) were analyzed using RNA-Seq (x-axis) and DIA whole cell mass spectrometry (y-axis). Genes are represented by dots. Most genes with changes in protein (161 yellow dots) do not have corresponding changes in transcript abundance. One gene (orange dot) has discordant changes in transcript and protein abundance. Green dots (23) represent genes with concordant changes in transcript and protein abundance. Blue dots (60) indicate genes with altered transcript abundance only. Horizontal dashed lines indicate +/- 1.5-fold cutoff for differential protein abundance; vertical dashes indicate +/- 2-fold cutoff for differential transcript abundance. Colored dots with black outlines represent genes with significant changes in protein (+/- 1.5-fold change, adjusted p-value <0.05) and/or transcript (+/- 2-fold change, adjusted p-value <0.05) abundance as indicated above, while grey dots without outline represent genes with changes that did not meet the statistical thresholds. Three grey dots are located outside the bounds of the axis as represented.



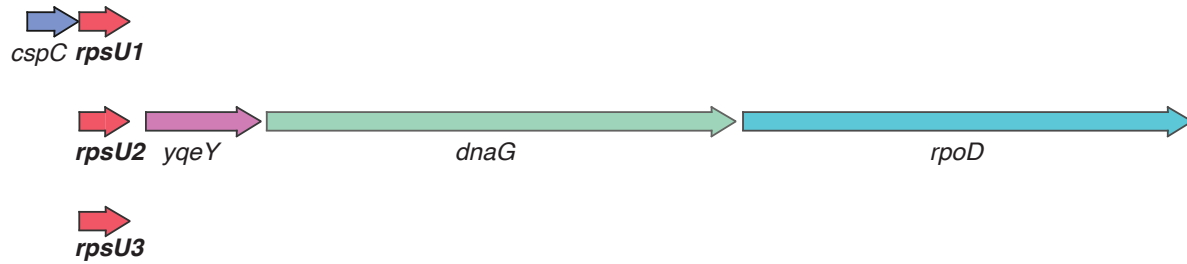
**Figure 3. bS21-2 is a regulator of T6SS protein abundance. A.** Immunoblot analysis of indicated T6SS protein abundance. As indicated, cells either contained (wild-type) or lacked ( $\Delta rpsU2$ ) bS21-2 and either an empty vector control (pF) or a vector ectopically expressing VSV-G-tagged bS21-2 (pF-bS21-2-V). Immunoblot against VSV-G was included to demonstrate production of VSV-G-tagged bS21 homologs. **B.** Quantification of immunoblots from (A). Band intensities for each protein were normalized to total protein per well on the membrane. Error bars represent 1 SD. Experiments were repeated at least twice and data from a representative experiment are shown. Lines above bars indicate statistical comparison among groups by t-test. Asterisk indicates group to which all other groups are compared, if horizontal line connects to line above group, \* $p < 0.05$  using Benjamini-Hochberg correction.



**Figure 4. Cells without bS21-2 have an intramacrophage growth defect, which can be complemented by ectopic expression of bS21-2.** Growth and survival of *F. tularensis* LVS cells within J774A.1 cells. Murine macrophage-like J774A.1 cells were infected with indicated bacterial cells at a multiplicity of infection of 5 – 10. J774A.1 cells were lysed and bacteria were plated for enumeration (colony forming units [CFU]) at 2 and 24 hours post-infection. Error bars represent 1 SD. Error bars for the LVS  $\Delta rpsU2$  cells at the 24 hour time point are too small to be illustrated. Experiments were repeated at least twice and data from a representative experiment are shown. Lines above bars indicate statistical comparison among groups by t-test. Asterisk indicates group to which all other groups are compared, if horizontal line connects to line above group, \*p < 0.05 using Benjamini-Hochberg correction.



*Francisella tularensis*



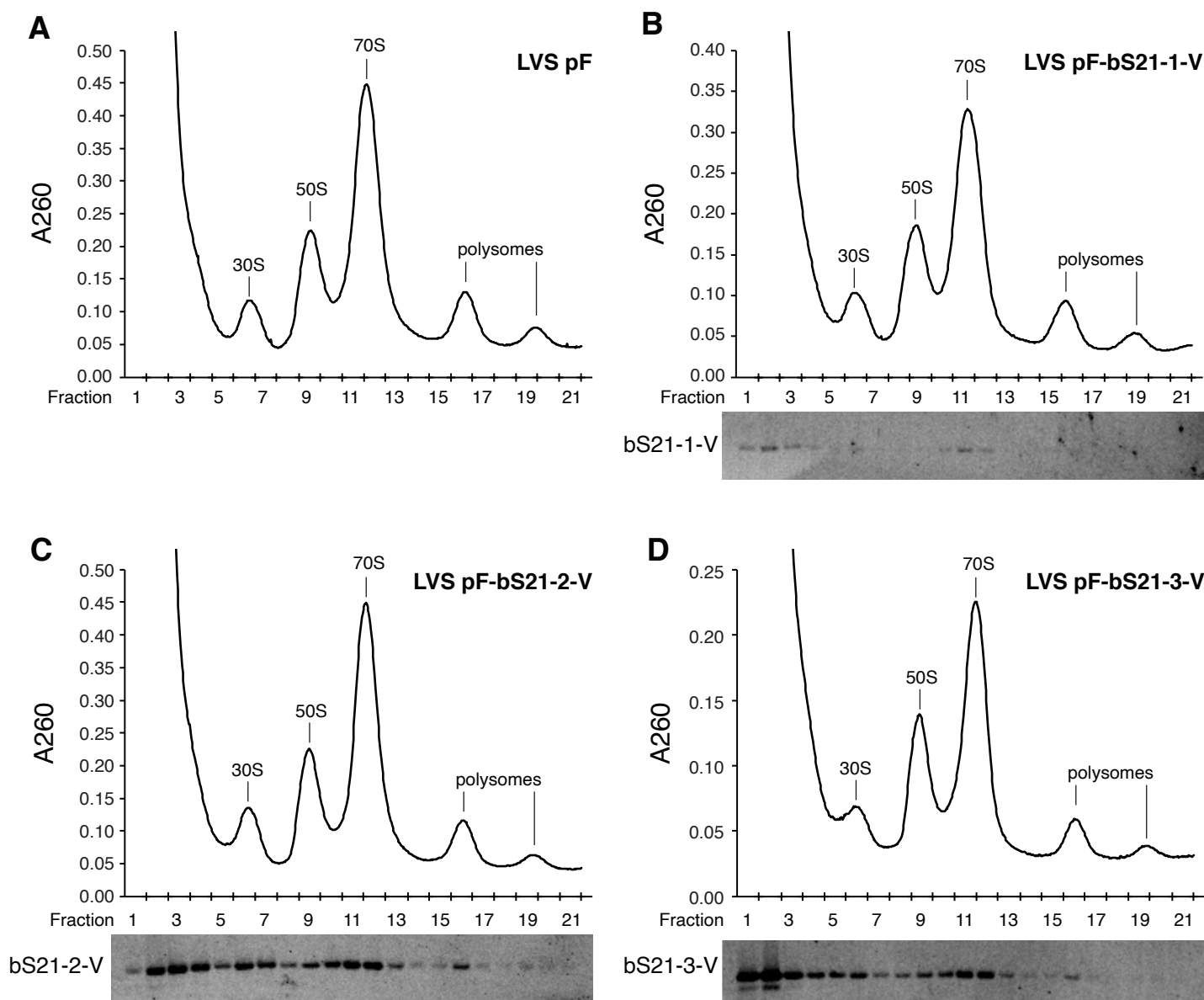
*Escherichia coli*



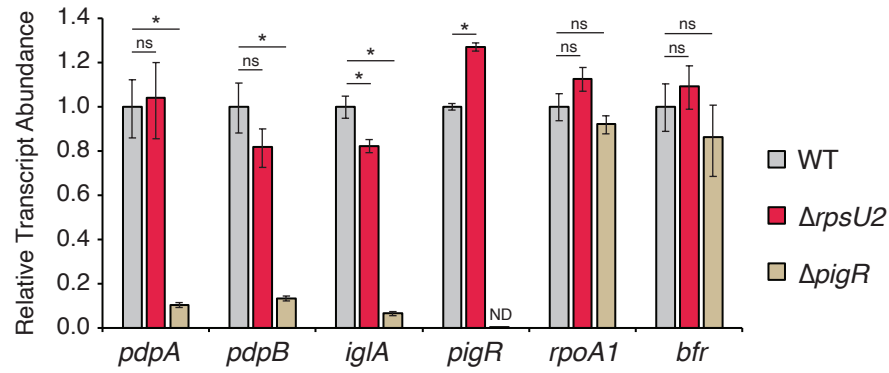
**Figure S1. *F. tularensis* encodes three *rpsU* genes.** *F. tularensis* *rpsU2*, which encodes bS21-2, is syntenic with the only *rpsU* in *E. coli*, which is located in the macromolecular synthesis operon (1). This operon in *E. coli* includes *rpsU* (encoding bS21), *dnaG* (encoding DNA primase), and *rpoD* (encoding RNA polymerase  $\sigma^{70}$ ). In *F. tularensis*, this operon also includes *yqeY*, the product of which may be involved in tRNA aminoacylation. *rpsU1*, encoding bS21-1, is located immediately downstream of *cspC* (encoding cold-shock protein CspC), while *rpsU3*, 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*.

	<i>F. tularensis</i> subsp <i>holarctica</i> LVS bS21-1	<i>F. tularensis</i> subsp <i>holarctica</i> LVS bS21-3	<i>F. tularensis</i> subsp <i>holarctica</i> LVS bS21-2	<i>E. coli</i> bS21
<i>F. tularensis</i> subsp <i>holarctica</i> LVS bS21-1	100.0	72.3	54.0	50.8
<i>F. tularensis</i> subsp <i>holarctica</i> LVS bS21-3		100.0	47.6	48.5
<i>F. tularensis</i> subsp <i>holarctica</i> LVS bS21-2			100.0	60.0
<i>E. coli</i> bS21				100.0

**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.

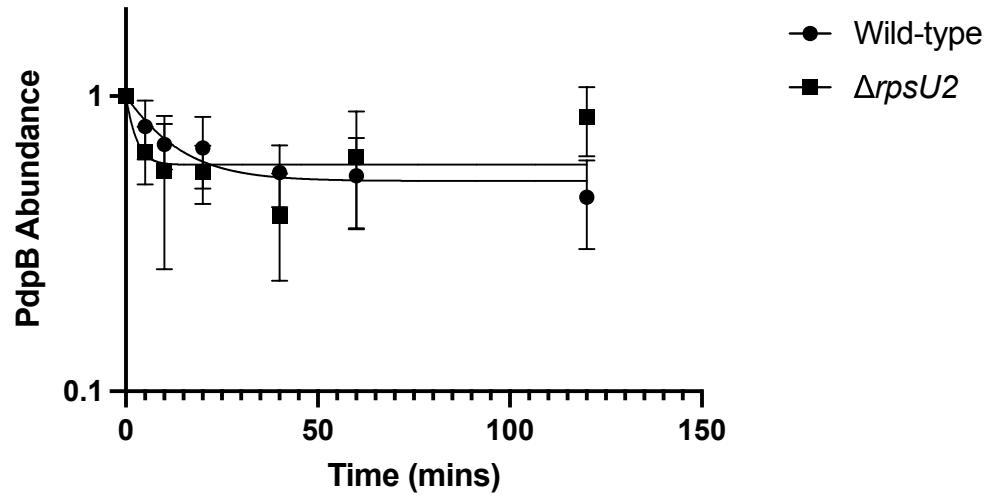


**Figure S3. Each bS21 homolog can be detected in translationally-active ribosomes.** For **A – D**, top: Sucrose gradient sedimentation profile from actively-translating wild-type *F. tularensis* cells with either empty vector or ectopic expression of indicated bS21 homolog. Nucleic acid content was 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 ( $\Delta rpsU2$ ), or cells lacking the transcription factor PigR ( $\Delta pigR$ ). 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 ( $\Delta rpsU2$ ). 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.