



**Figure 4. Hfq and bS21-2 influence production of T6SS proteins via different pathways. (A)** Diagram of the Francisella Pathogenicity Island (FPI) genes, which encode T6SS proteins. **(B)** Cells lacking bS21-2 have more Hfq. Bottom: Immunoblots probed with anti-VSV-G antibody. Whole cell lysates from bacteria containing Hfq-VSV-G and either with (WT) or without ( $\Delta rpsU2$ ) bS21-2, in biological triplicate. Top: Quantification of immunoblots. Band intensities for each protein were normalized to total protein per lane on the membrane. **(C)** Loss of bS21-2 leads to more *hfq* translation. Relative fluorescence for translational fusion reporters containing the 5' UTR of either *hfq* or *tul4* fused to *gfp* in cells with (WT) or without ( $\Delta rpsU2$ ) bS21-2, in biological triplicate. Values relative to WT for each 5' UTR are shown. **(D)** Only some of the T6SS proteins are influenced by loss of Hfq. Bottom: Immunoblots probed with antibodies to indicated T6SS proteins in lysates of WT cells, cells lacking bS21-2 ( $\Delta rpsU2$ ), or cells lacking Hfq ( $\Delta hfq$ ). Top: Quantification of immunoblots. Band intensities for each protein were normalized to total protein per lane on the membrane. **(E)** Hfq does not influence translation of the T6SS protein PdpA. Relative fluorescence for translational fusion reporters containing the 5' UTR of either *pdpA* or *tul4* fused to *gfp* in WT cells, cells lacking bS21-2 ( $\Delta rpsU2$ ), or cells lacking Hfq ( $\Delta hfq$ ), in biological triplicate. **(F)** Hfq is a negative regulator of T6SS gene transcript abundance. Quantitative real-time PCR was used to determine the relative transcript abundance for indicated FPI-encoded genes in WT cells, cells lacking bS21-2 ( $\Delta rpsU2$ ), or cells lacking Hfq ( $\Delta hfq$ ), normalized to the *tul4* gene. The *rpoA1* and *bfr* genes are included as additional negative controls, as their expression is not meaningfully influenced by bS21-2. (A-E) Error bars represent 1 SD. Experiments were repeated at least twice and data from a representative experiment are shown. (A-D) \*  $p < 0.05$  after Bonferroni correction. (E) \*  $p < 0.005$  after Bonferroni correction.