



**Figure 1. Hfq and bS21-2 influence production of T6SS proteins via different pathways.** (A) 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. (B) Loss of bS21-2 leads to more *hfq* translation. Relative fluorescence is reported 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. (C) 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. (D) 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. (E) 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.