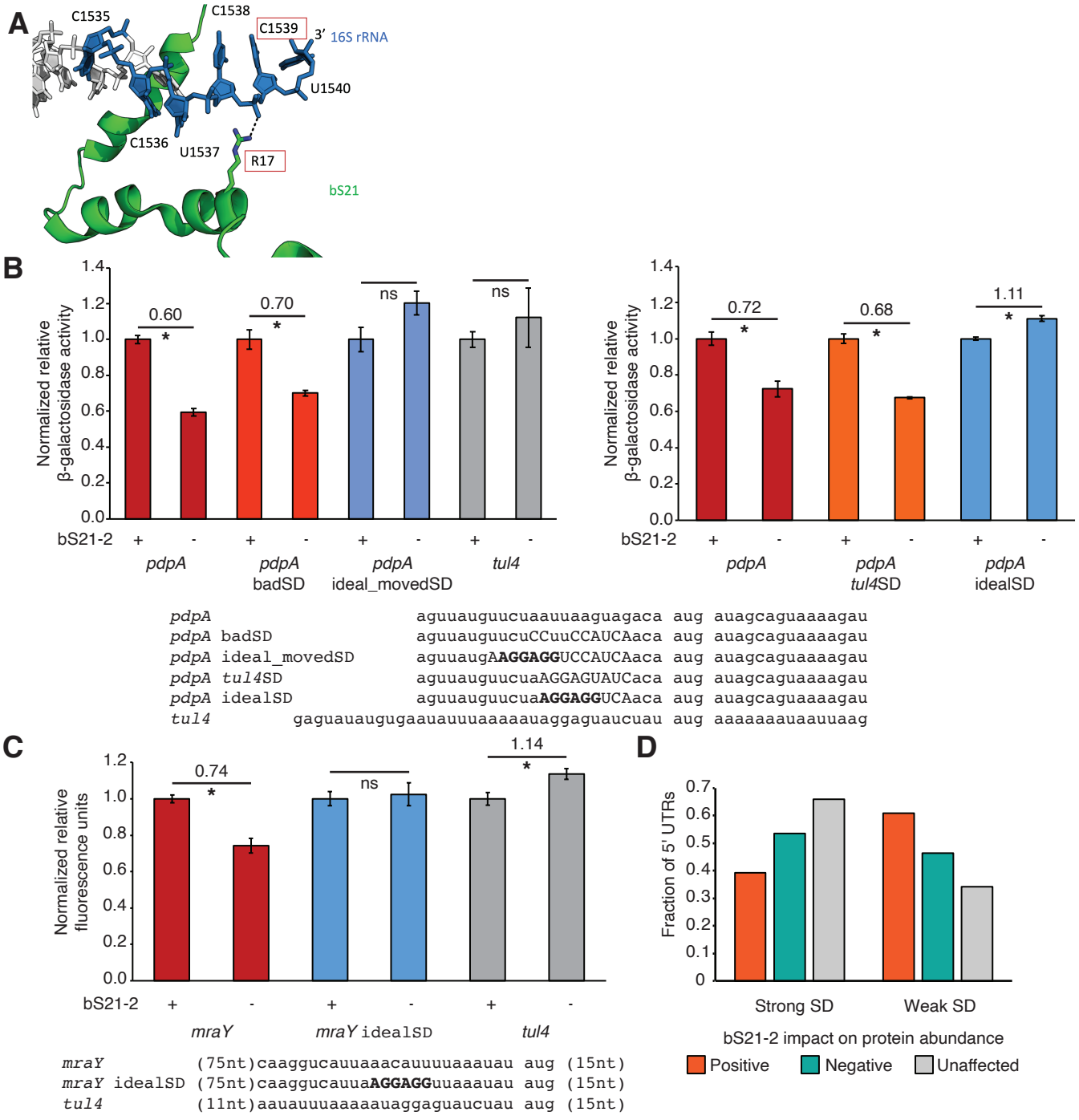

Figure 3. Genes with ideal Shine-Dalgarno (SD) sequences are not responsive to bS21-2. (A) bS21 interacts with the anti-Shine Dalgarno (ASD) sequence. In *E. coli*, amino acid R17 of the sole bS21 protein (green) directly interacts with C1539 of 16S, which is part of the ASD (blue). Measured distance is 2.7Å (PDB 6o7k; Kaledhonkar et al. 2019). **(B)** Introduction of an ideal SD in the *pdpA* leader leads to loss of bS21-2 responsiveness. Top: Relative β-galactosidase activity from modified or wild-type *pdpA* 5′ UTRs in cells with (+; WT) or without (-; Δ*rpsU2*) bS21-2, in biological triplicate. Bottom: Alignment of modifications to *pdpA* 5′ UTR. **(C)** Introduction of an ideal SD in the *mraY* leader leads to loss of bS21-2 responsiveness. Top: Relative fluorescence is reported for indicated translational fusion reporters in cells with (+; WT) or without (-; Δ*rpsU2*) bS21-2, in biological triplicate. Bottom: Alignment of indicated 5′ UTRs. (B-C) Value in parenthesis indicates the number of identical nts omitted from 5 ′ end of the UTRs. Capital letters: altered from WT; bold: ideal SD sequences. Error bars represent 1 SD. *p<0.05 by t-test. ns = not significant. Experiments were repeated at least twice and data from a representative experiment are shown. **(D)** The absence of strong SD-ASD interactions is correlated with bS21-2 influencing translation. Fraction of genes that are positively-impacted (n=74), negatively-impacted (n=84), or unaffected (n=82) by bS21-2, categorized by strength of SD. “Strong” SD: 4 or more nts complementary to ASD; “weak” SD: 3 or fewer complementary nts.