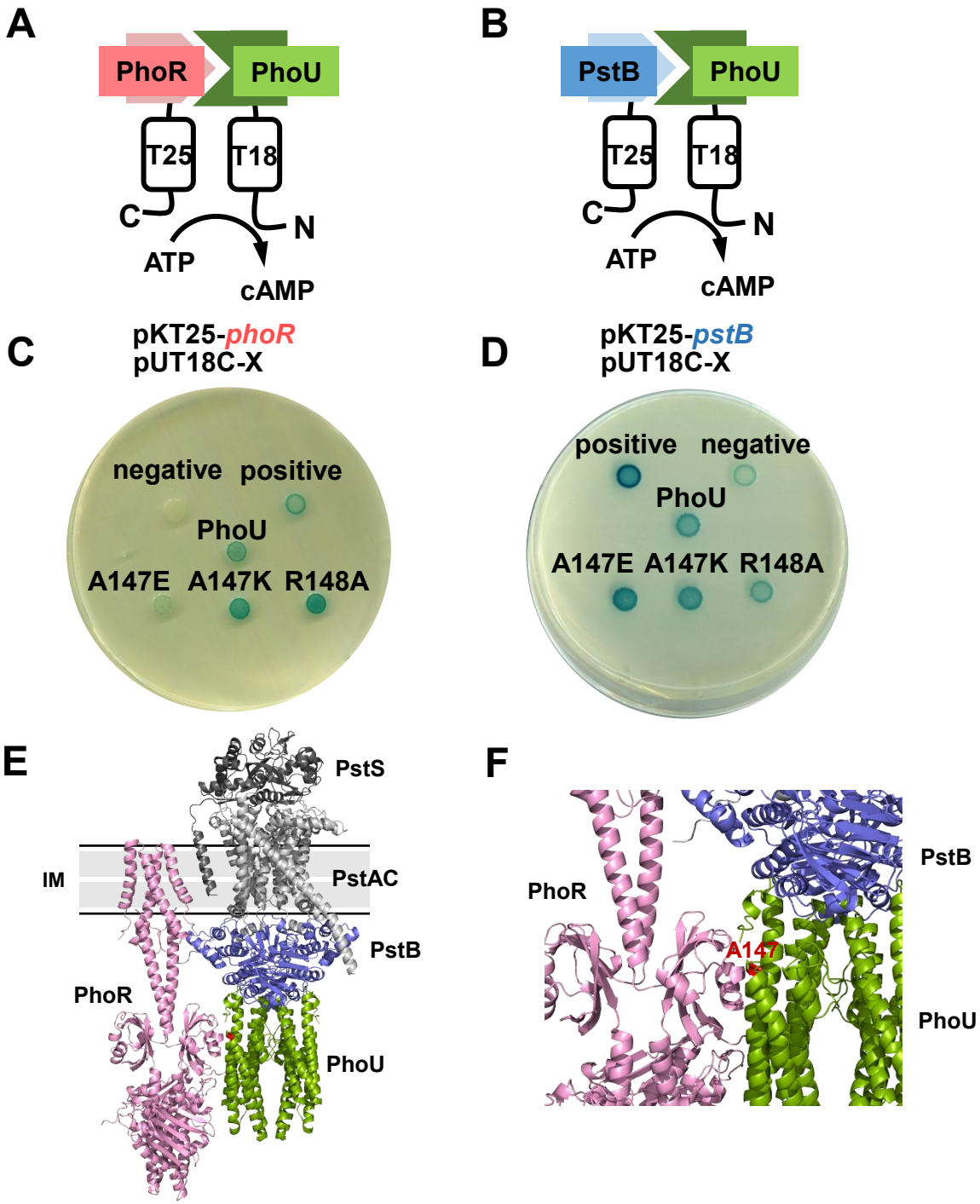


**Fig. S1.** PhoU Ala147 is not required for interaction with PstB. (A–B) Schematic representation of the bacterial two-hybrid assay used to assess interactions between PhoR or PstB and PhoU or its variants. (C–D) *Escherichia coli* BTH101 strains were co-transformed with plasmid pairs: pKT25 derivatives expressing a C-terminal fusion of the *cyaA* T25 fragment to *phoR* (C) or *pstB* (D), and pUT18 or pUT18C derivatives expressing N-terminal fusions of the *cyaA* T18 fragment to wild-type *phoU* or C-terminal fusions to *phoU*$^{A147E}$, *phoU*$^{A147K}$, or *phoU*$^{R148A}$. Cells containing pUT18C served as a negative control. Cells co-expressing pUT18-*mgtC* and pKT25-*mgtR* served as a positive control. Cultures were spotted onto LB agar plates containing 80 µM X-Gal and 0.5 mM IPTG and incubated at 30°C for 48 h. Blue colonies indicate positive interactions. (E–F) Alphafold-assisted modeling of PhoR-PhoU-Pst complex. PhoU (UniProt: A0A0F6B922) and its interacting partners PhoR (A0A0F6AXL9) and PstB (A0A0F6B923) in *Salmonella* Typhimurium 14028s, were used to generate complex prediction. PhoU is shown in green, with Ala147 highlighted in red. PhoR and PstB are shown in pink and sky blue, respectively.