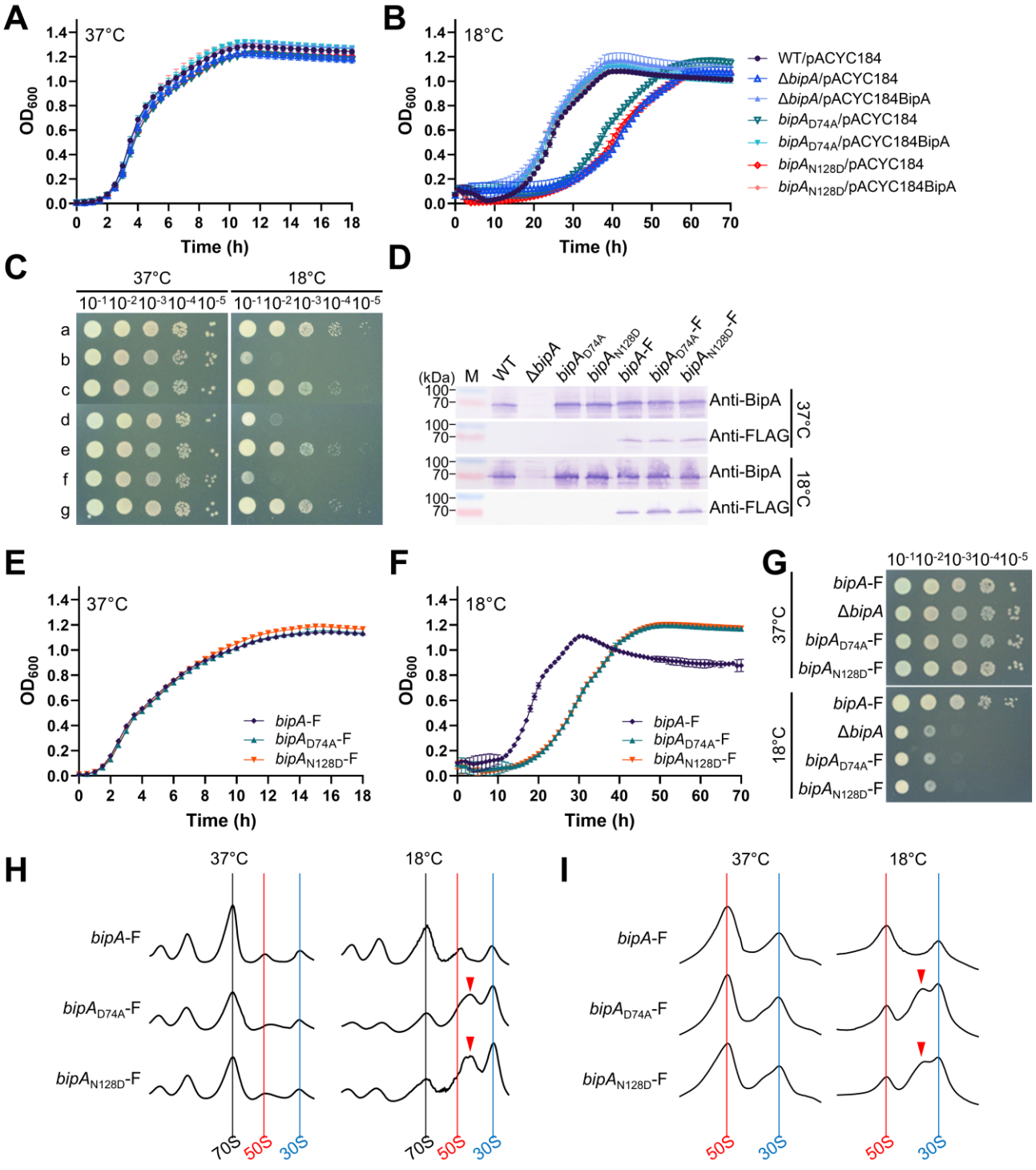


Fig. S1. Complementation and functional characterization of chromosomal *bipA* mutant strains. (A and B) Growth curves of chromosomal *bipA* GTPase-defective mutant strains transformed with either the empty vector pACYC184 or pACYC184BipA were measured at 37°C (A) and 18°C (B). The same symbols are used in both panels. The data presented are the mean ± SD of three independent experiments. (C) The

indicated strains carrying pACYC184 or pACYC184BipA were serially diluted, spotted onto LB agar plates supplemented with chloramphenicol, and incubated at the indicated temperatures. Labels a–g correspond to the following strain/plasmid combinations: (a) WT/pACYC184, (b) Δ*bipA*/pACYC184, (c) Δ*bipA*/pACYC184BipA, (d) $bipA_{D74A}$/pACYC184, (e) $bipA_{D74A}$/pACYC184BipA, (f) $bipA_{N128D}$/pACYC184, and (g) $bipA_{N128D}$/pACYC184BipA. (D) Western blotting analysis was performed to confirm the expression of BipA and FLAG-tagged BipA in the WT strain and chromosomal *bipA* GTPase-defective mutant strains generated using the CRISPR/Cas9 system. Whole-cell samples were analyzed using SDS–PAGE followed by immunoblotting with anti-BipA or anti-FLAG antibodies. M, molecular weight marker. (E and F) Growth curves of strains expressing chromosomally encoded BipA-F, $BipA_{D74A}$-F, or $BipA_{N128D}$-F were measured at 37°C (E) and 18°C (F). The data are presented as the mean ± SD of three independent experiments. The same symbols are used in both panels. (G) Overnight cultures were serially diluted (10^{-1}–10^{-5}), spotted onto LB agar plates, and incubated at the indicated temperatures. (H and I) Polysome (H) and ribosomal subunit (I) profiles of the indicated strains were analyzed using sucrose gradient sedimentation, as described in the Materials and Methods. Black, red, and blue vertical lines indicate the positions of the 70S ribosome, 50S subunit, and 30S subunit, respectively. Red arrowheads indicate abnormal 44S ribosomal particles.