Supplementary Figure 1 Generation of ppkl-knockout and complemented strains from A. baumannii ATCC 17978. (A) Nucleic acid electrophoresis map of marker (Line M) and apramycin resistance gene linked with the homologous recombination upstream and downstream DNA fragment of ppkl (Line 1). (B) The correct single exchanged Line 1, Line 3, Line 8, Line 13, Line 19, and Line 20 clone was screened by PCR using primers ppkl-out forward/reverse and Apr-seq forward/reverse and marker (Line M). (C) Nucleic acid electrophoresis maps of the apramycin resistance gene using primers ppkl-out F/ppkl-out R in Δppkl::Apr (Line 1), the negative control (Line 2), and the marker (Line M). (D) Nucleic acid electrophoresis maps of ppkl in Δppkl::Apr (Line 1) using primers ppkl-in F/ppkl-in R, the positive control (Line 2), and the negative control (Line 3). (E) Nucleic acid electrophoresis map of ppkl in A. baumannii ATCC 17978 using primers ppkl-BamHI-F/ ppkl-SalI-R (Line 1) and the marker (Line M).
**Supplementary Figure 2** Western blot analysis of PPK1 in logarithmic phase Δppk1::Apr/PJL02-ppk1 under induction with different concentrations of IPTG (0 mM, 0.25 mM, 0.5 mM, 1 mM and 2 mM).

**Supplementary Table 1 Bacterial strains and plasmids used in this study.**

| Strains or plasmids | Relevant features | Source |
|---------------------|------------------|--------|
| A. baumannii ATCC 17978 | Type strains | ATCC |
| Δppk1::Apr/PJL02-ppk1 | Complementation strains of ppk1 | This study |
| Δppk1::Apr | ppk1 deficient strains | This study |
| pUC57-Apr | Source for apr gene | This study |
| pCVD442 | A suicide plasmid for construction Δppk1::Apr | This study |
| E. coli β2155 | E. coli strains for conjugation | This study |
| PJL02 | Complementation plasmid of ppk1 | (1) |
| E. coli WM3064 | A donor strain for complementation of ppk1 | This study |
Supplementary Table 2 Primers used in this study

| Primers name | Oligonucleotide (5’-3’) |
|--------------|-------------------------|
| ppk1-up-F    | CTCAAGTTTGGTTTGGTGCGTAC |
| ppk1-up-R    | GTCTAACCTAATCTTCTTTCCAACC |
| ppk1-down-F  | CAGGCGAAAAATTGCATAATGCTCAAG |
| ppk1-down-R  | GCTGGTAAACAACTTTATAGCCTTTTAGCC |
| ppk1-Apr-F   | AGAGATTTTGGTTGAAAGAGATTAGGTTAGAC |
| ppk1-Apr-R   | CTTTGAGCATTATGCAATTTTCGCTGGGAATA |
| ppk1-out-F   | GAAAAGCCTTTATTGTTTCG |
| ppk1-out-R   | TGTGCAAATTGCATAGTCATTGGC |
| ppk1-in-F    | GTAAAGAACATCATGTCATCTTTTGCTGC |
| ppk1-in-R    | CCAAATCTTCAACGTCTTTCAAGT |
| Apr-seq-F    | CAGAGCAGATCATCTCTGATCCATTG |
| Apr-seq-R    | CAATGGATCAGAGATGACTCTGCTCTG |
| PJL02-out-F2 | GGAATTTGAGCGGATAACAATTCAC |
| PJL02-out-R2 | GTGCTGCAAGGCGATTAAGTTG |
| ppk1-BamHI-F | GACGACAAAGGATCCATGATACAGCGATTAC |
| ppk1-SalI-R  | CTCGTTACCGTCTGACTTTTTAAAGTTTCTAATAAT |

Reference

1. Jie J, Chu X, Li D, Luo ZA-O. 2021. A set of shuttle plasmids for gene expression in Acinetobacter baumannii. PLoS One 16:e0246918.