Supplementary material

Figure S1.

(A) The susceptibility of AB5075-UW was determined using a minimum inhibitory concentration (MIC) broth microdilution method. The MIC was determined to be 125 µg/ml. (B) The susceptibility of AB5075-UW (WT), and Tn insertion mutants ΔadeI (AB02300), ΔadeJ (AB02296), ΔadeK (AB02291) and ΔadeN (AB04557) were tested using the broth microdilution method. Each strain was subjected to either no ciprofloxacin (growth control - GC) or different concentrations of ciprofloxacin. The optical density was measured after 24h. All mutants (except ΔadeN) were more susceptible to ciprofloxacin when compared WT. Data bars represent geometric mean ± SE (n = 8) of two independent experiments. (C) The susceptibility of ACICU was determined using a minimum inhibitory concentration (MIC) broth microdilution method. The MIC was determined to be ~62.5 µg/ml. (D) The susceptibility of AYE was determined using a minimum inhibitory concentration (MIC) broth microdilution method.
The MIC was determined to be 125 µg/ml. (E) The susceptibility of D1279779 was determined using a minimum inhibitory concentration (MIC) broth microdilution method. The MIC was determined to be 0.39 µg/ml. (F) The susceptibility of ATCC 19606 was determined using a minimum inhibitory concentration (MIC) broth microdilution method. The MIC was determined to be 1.56 µg/ml.
Figure S2.

(A) ABUW_0098 gene-specific primers were used to confirm insertional inactivation of ABUW_0098 gene. The sizes of the molecular weight standards are shown on the left (lane 1). Lane 2 corresponds to AB5075-UW (Wildtype) strain. Lane 3 corresponds to strain AB00272 (ABUW_0098 gene disrupted by the T26 transposon). (B) M13 universal primers were used to confirm insertion of ABUW_0098 gene with a 405bp upstream region. The sizes of the molecular weight standards are shown on the left (lane 1). Primers used for the reaction correspond to AB5075-UW (Lane 2), AB00272 (Lane3), AB5075-UW expressing empty vector - pVRL1Z (Lane 4), AB00272 expressing empty vector - pVRL1Z (Lane 5) and AB00272 expressing ABUW_0098 gene with its presumably endogenous promoter (405bp upstream region of ABUW_0098) on a pVRL1Z plasmid (Lane 6). (C) ABUW_0099 gene-specific primers were used to confirm insertional inactivation of ABUW_0099 gene. The sizes of the molecular weight standards are shown on the left (lane 1) Lanes 2 and 3 correspond to AB00275; lanes 4 and 5 correspond to AB00274 (ABUW_0099 gene disrupted by the T26 transposon) and lane 6 corresponds to AB5075-UW (Wildtype) strain.
Figure S3.

Cell morphology of AB5075-UW expressing empty pVRL1Z plasmid, AB00272 mutant strain expressing empty pVRL1Z plasmid and AB00272 mutant strain complemented with pVRL1Z\textsubscript{ABUW\_0098} grown without antibiotics to mid-log phase (no treatment) followed by exposure to sub-MIC ciprofloxacin (31.25µg/ml) at 1h, 2h and 4h. Scale bar, 5µm. Inset scale bar, 2µm.
Figure S4.

Cell morphology of AB00272 mutant, AB00272 mutant complemented with pVRL1Z_{ABUW_0098}, as well as controls including AB5075-UW parental strain, AB5075-UW with empty pVRL1Z plasmid and AB00272 mutant with empty pVRL1Z plasmid. Cells were grown to mid-log phase without antibiotics (no treatment) followed by exposure to sub-MIC ciprofloxacin (31.25\,\mu g/ml). Cells were harvested for microscopy before adding antibiotics (no treatment) and at 1h and 2h post-treatment. Scale bar, 20\,\mu m.
Figure S5.

(A) Graph shows 6.25h growth rate in Mueller-Hinton broth of AB5075-UW (parental strain, blue line), AB00274 (green) and AB00275 (light green) mutant strains (ABUW_0099 gene insertionally-inactivated). (B) All strains were shocked with 31.25µg/ml of ciprofloxacin after 2h 6min of growth. (C) All strains were shocked with 46.87µg/ml of ciprofloxacin after 2h 6min of growth. (D) All strains were shocked with 62.5µg/ml of ciprofloxacin after 2h 6min of growth (OD_{600nm} ~0.5). There was no significant difference in the growth of the ΔABUW_0099 mutant strain and AB5075-UW with and without ciprofloxacin. Error bars show the standard error of three biological replicates each.
Cell morphology of AB0027 and AB00275 mutant strains (ABUW_0099 gene insertionally-inactivated) and AB5075-UW complemented with pVRL1ZABUW_0098, as well as control AB5075-UW with empty pVRL1Z plasmid. Cells were grown to mid-log phase without antibiotics (no treatment) followed by exposure to sub-MIC ciprofloxacin (31.25µg/ml). Cells were harvested for microscopy before adding antibiotics (no treatment) and at 1h, 2h and 4h post-treatment. Scale bar, 2µm.
### Table S1.

Primers used in this study.

| Primer     | Sequence                      | Description                                               |
|------------|-------------------------------|-----------------------------------------------------------|
| M13F       | TGTAAAACGACGGCCAGT            | To confirm insertion of the construct in pVRL1Z plasmid   |
| M13R       | CAGAAACAGCTATGAC              | pVRL1Z plasmid                                            |
| ABUW_0098F | TCGCATCGACAAATAACA            | To confirm the disruption of ABUW_0098 gene              |
| ABUW_0098R | TGGTTTCGTGCATCGACT            | ABUW_0098 gene                                           |
| ABUW_0099F | CACGAAACAGGCAATGGTGA          | To confirm the disruption of ABUW_0099 gene              |
| ABUW_0099R | TGCTCGGGACATGAAAGTTC          | ABUW_0099 gene                                           |
| aciT_F     | CCGTCGTTGCTTGGTTTTTA         | To investigate the induction of aciT in *A. bau mannii* strains via RT-qPCR |
| aciT_R     | TATGGGCCATTGGCACAAGC          | *baumannii* strains via RT-qPCR                           |
| rpoB_F     | CCACGTTTTCGATTACACA           | Housekeeping gene *rpoB* used as a control               |
| rpoB_R     | GCATATGGACGTCTTCTGCA          | in the RT-qPCR experiment.                               |
Table S2.
gBlocks (Intergrated DNA Technologies) Gene fragment used in this study

| Description                      | Sequence                                                                 |
|----------------------------------|--------------------------------------------------------------------------|
| ABUW_0098 gene with a 405bp       | ATAAAGCTTGTATCGTGAAAATAATCATTAAACCGCTGCTCAAGGAGTAGCGCTTTTTTTTTT   |
| upstream region                   | TATGAGAATTAGCTATAAAAAACAAAGCAGTTATTGACAAAAATTATTGCAAGAAGAAAAA   |
|                                  | AGGCCAAAAAAATAGGAAAAACATTTATTTTGTGAGGCAAAAAACAGCATAGTT     |
|                                  | TTTTGATAGCAACGCCAGAAATGGATGCTGAGATAGAAGATGAACTTTTTAAAAAT  |
|                                  | TTACAAAAATTGTAATGACAACAAAAAAGATGTTGCGACAAAAGATATGTGCACAGCAAAA |
|                                  | TAAACAGGGTTTTTTTTCTAAAAACATCCATAATGCTTTCTAAAGCTCAACA       |
|                                  | CAATTCATGAAATTAAAGTGGTTTTTGGAATGAGTCTGTTTGTTCAAGACGAATGT  |
|                                  | TTTTAGAGCTTTTTTATTAAGACGGAGGGTTCTATGTAACACGAATTTTGCGCG     |
|                                  | TATCGCCGGACTCAGTTTAATTGCTGTCGCGTTTGCTGTGTTCTTTTTTCTCT    |
|                                  | TACCCGTGTTGGCGTTGTTTTATGCTTGCGAGGATGTTTTTTTTGCGGCTATTGG  |
|                                  | AAGTGCTCGTTTTGAGGTCAAGTCACAAAAAGTCCAGTCACATATAGTTA        |
|                                  | TTAAACTGCACTAATAGCTAGGAGGTTATGTTTACATTTTTTTGTTACATGTAAG   |
|                                  | GATAAAACAGCGACAGAAAGCTTTTGCCAATATAGAACAACACGCCACAGACAGCAAA |
|                                  | TATATGAGATGATCGACGACATGTTCTAGCCGATAACGGCCGCCACGCCG        |

Data S1.
Transcriptomics and Proteomics fold-change data