Figure S1. Transcriptional analysis of transposon insertion mutants. (A) Locus map and insertion sites of 18E8 and 26F2 with primer sets in colored boxes used to determine transcript expression. Primers were designed to amplify upstream and downstream of the insertion site in each gene. (B) Expression of target sites in WT GN050, 18E8 (RTX::Tn) or 26F2 (tolC::Tn) was normalized to expression of the housekeeping gene gyrB. Data bars are the mean of 2-3 biological replicates performed in technical triplicate and the error is ± 1 SD.
**Figure S2.** Determined domain architecture of ArtA in 33 *Ax* genomes with respect to the GN050 RTX adhesin. Using an anvi’o pipeline, 8 genomes were found to encode a complete ArtA while 25 other sequences in the gene cluster encode the highly conserved CT including vWA, RTX repeats and T1SS signal sequence. In genomes containing an ArtA CT but lacking further annotation, manual determination of the VCBS repeating units were determined and denoted as “determined.” R; retention module. VCBS; repeating units. vWA; von Willebrand type A. RTX; Ca^{2+} binding RTX repeats. T1S; T1SS signal sequence.
Figure S3. Alignment of CT aa from ArtA in 33 Ax genomes. Sequences obtained from the anvi’o analysis and aligned by Clustal Omega. CT domains are annotated.
Figure S4. Pangenomic analysis of 88 Ax genomes with respect to GN050 synteny. Genome layers are ordered by the maximum likelihood relationship of 77 single-copy core genes. Displayed is the presence or absence of gene clusters of homologous genes based on amino acid sequence identity. By this method, genomes that contained the artA gene cluster are shown in pink and genomes that lacked the artA gene cluster are shown in black. Minimum requirement for presence of artA in a genome included annotation of a vWA domain, RTX-repeats and a T1SS signal sequence (Figure S3).
Figure S5. CF serum antibodies that recognize ArtA also recognize a HMW product produced by other Ax CF isolates. (A) Precipitated supernatants from overnight cultures of GN050::26F2, WT GN050, or Ax CF isolates Ax3, Ax4, Ax12, GN008, Ax5, AX13, AX14. (B) Western blot using CF serum ID: 9429 that was previously found to have GN050 ArtA reactivity. Reactivity of the serum against HMW bands was found in CF isolates Ax3, Ax4, Ax12, and GN008. Detection of a HMW similar to ArtA is denoted by red arrows.
Table S1. Bacterial strains and plasmids used in this study with relevant descriptions.

| Strain/Genotype | Description                  | Reference |
|-----------------|-------------------------------|-----------|
| A. xylosoxidans |                               |           |
| GN008           | CF isolate                    | 1         |
| GN050           | Ear infection isolate         | 1         |
| 18E8            | GN050 artA::himar1            | This study|
| 26F2            | GN050 tolC::himar1            | This study|
| Ax3             | CF isolate                    | 1         |
| Ax4             | CF isolate                    | 1         |
| Ax5             | CF isolate                    | 1         |
| Ax12            | CF isolate                    | 1         |
| Ax13            | CF isolate                    | 1         |
| Ax14            | CF isolate                    | 1         |

| Plasmid         | Description                                                            | Reference |
|-----------------|------------------------------------------------------------------------|-----------|
| pDBD1           | Derivative of pCM62 (2)                                                | This study|
| pDBD2sfGFP      | pDBD1 with GN050 30S ribosomal protein S21 promoter region and sfGFP    | This study|
| Modified himar1 | 30S ribosomal protein S21 promoter region driving CmΩ open reading frame added at Nhel site of himar1 | 3         |
| Mutant   | %WT Toxicity | Location    | Gene Locus Tag                          | Function                                                                 | Source                        |
|----------|--------------|-------------|-----------------------------------------|---------------------------------------------------------------------------|-------------------------------|
| 59D12    | 44.9         | 445,064     | HPS44_01820 / HPS44_01825               | Intergenic                                                               |                               |
| 156B3    | 2.9          | 476,296     | HPS44_01970                            | tyrB; aromatic-amino-acid transaminase                                   | KeggGhostKoala               |
| 150G10   | 4.3          | 477,901     | HPS44_01975 / HPS44_01980              | Intergenic                                                               |                               |
| 149H1    | 9.9          | 480,415     | HPS44_01980                            | lon; ATP-dependent Lon protease                                          | KeggGhostKoala               |
| 85E3     | 69.7         | 480,814     | HPS44_01985                            | clpX, CLPX; ATP-dependent Clp protease ATP-binding subunit ClpX           | KeggGhostKoala               |
| 61F6     | 37.1         | 481,968     | HPS44_01985 / HPS44_01990              | Intergenic                                                               |                               |
| 28D5     | 63.6         | 495,682     | HPS44_02085                            | squalene synthase HpnC                                                   | NCBI_PGAP                    |
| 169H10   | 67.8         | 547,106     | HPS44_02295                            | putative transposase                                                     | KeggGhostKoala               |
| 162C10   | 43.7         | 583,825     | HPS44_02455 / HPS44_02460              | Intergenic                                                               |                               |
| 180B7    | 38.5         | 635,186     | HPS44_02670                            | hpaI, hpcH; 4-hydroxy-2-oxoheptanedioate aldolase                        | KeggGhostKoala               |
| 41A7     | 59.0         | 639,490     | HPS44_02690                            | squalene synthase HpnC                                                   | NCBI_PGAP                    |
| 116G8    | 66.4         | 702,028     | HPS44_03015 / HPS44_03020              | Intergenic                                                               |                               |
| 24B11    | 58.2         | 1,031,493   | HPS44_04410                            | tktA, tktB; transketolase                                                | KeggGhostKoala               |
| 116E9    | 2.9          | 1,065,563   | HPS44_04550                            | hypothetical protein                                                      | NCBI_PGAP                    |
| 50C9     | 5.1          | 1,068,113   | HPS44_04570                            | tadC; tight adherence protein                                            | KeggGhostKoala               |
| 25B5     | 3.3          | 1,070,374   | HPS44_04575 / HPS44_04575              | cpAF, tadA; pilus assembly protein CpaF                                    | KeggGhostKoala               |
| 46A7     | 5.7          | 1,079,434   | HPS44_04620 / HPS44_04625              | Intergenic                                                               |                               |
| 125F10   | 2.8          | 1,105,359   | HPS44_04720                            | elk-wzc; tyrosine-protein kinase Etk/Wzc                                  | KeggGhostKoala               |
| 162D8    | 9.4          | 1,145,736   | HPS44_04875                            | vcaM; ATP-binding cassette, subfamily B, multidrug efflux pump          | KeggGhostKoala               |
| 113D2    | 44.3         | 1,159,820   | HPS44_04955                            | short chain dehydrogenase                                                | NCBI_PGAP                    |
| 29H7     | 64.1         | 1,264,383   | HPS44_05495 / HPS44_05500              | Intergenic                                                               |                               |
| 115E2    | 61.3         | 1,276,873   | HPS44_05570                            | dapA; 4-hydroxy-tetrahydrodipicolinate synthase                          | KeggGhostKoala               |
| 12H7     | 24.0         | 1,417,632   | HPS44_06255 / HPS44_06260              | Intergenic                                                               |                               |
| 13E2     | 25.0         | 1,417,632   | HPS44_06255 / HPS44_06260              | Intergenic                                                               |                               |
| 154E8    | 7.8          | 1,504,306   | HPS44_06660                            | aldolase                                                                  | NCBI_PGAP                    |
| 155E10   | 19.7         | 1,514,265   | HPS44_06705                            | aarC, cat1; succinyl-CoA:acetate CoA-transferase                          | KeggGhostKoala               |
| 30A6     | 64.7         | 1,531,250   | HPS44_06795                            | CBS domain-containing protein                                            | NCBI_PGAP                    |
| 57F6     | 11.9         | 1,546,665   | HPS44_06875                            | hypothetical protein                                                      | KeggGhostKoala               |
| 30C12    | 5.0          | 1,557,551   | HPS44_06935                            | pstS; phosphate transport system substrate-binding protein               | KeggGhostKoala               |
| 196E1    | 4.6          | 1,655,395   | HPS44_07355                            | purK; 5-(carboxyamino)imidazole ribonucleotide synthase                   | KeggGhostKoala               |
| 106F7    | 4.1          | 1,657,324   | HPS44_07375                            | purC; phosphoribosylaminomidazole-succinocarboxamide synthase             | KeggGhostKoala               |
| Gene  | Score | Start     | End       | Function                                                                 | Database     |
|-------|-------|-----------|-----------|--------------------------------------------------------------------------|--------------|
| 101F2 | 49.2  | 1,717,670 | ubiD; 4-hydroxy-3-polypropenylbenzoate decarboxylase                     | KeggGhostKoala |
| 162B10| 10.5  | 1,735,318 | /HPS44_07780 | Intergenic                                                                 | NCBI_PGAP    |
| 81G4  | 56.3  | 1,738,305 | HPS44_07785 | /HPS44_07790                                                               | NCBI_PGAP    |
| 29E9  | 2.4   | 1,738,366 | type II toxin-antitoxin system RelE/ParE family toxin onypeptide           | NCBI_PGAP    |
| 150G8 | 33.6  | 1,875,291 | hypothetical protein                                                      | NCBI_PGAP    |
| 167E8 | 64.6  | 1,930,755 | porin                                                                 | KeggGhostKoala |
| 161D4 | 5.6   | 2,062,402 | cheB; two-component system, chemotaxis family, protein-glutamate methyltransferase/glutaminase | KeggGhostKoala |
| 104H9 | 66.8  | 2,114,136 | rsmD; 16S rRNA (guanine966-N2)-methyltransferase                         | KeggGhostKoala |
| 110C2 | 7.6   | 2,118,500 | asmA; AsmA protein                                                       | KeggGhostKoala |
| 177E7 | 64.3  | 2,126,718 | fhuE, fpvA, fptA; outer-membrane receptor for ferric coprogen and ferric-rhodotorulic acid | KeggGhostKoala |
| 8D2   | 44.2  | 2,128,870 | ychF; ribosome-binding ATPase                                            | KeggGhostKoala |
| 111C3 | 11.2  | 2,129,309 | ychF; ribosome-binding ATPase                                            | KeggGhostKoala |
| 158C4 | 36.4  | 2,135,718 | HAMP domain-containing protein                                           | NCBI_PGAP    |
| 82H1  | 6.4   | 2,166,340 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 76H9  | 6.5   | 2,166,466 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 143G6 | 4.3   | 2,166,466 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 14B2  | 3.9   | 2,171,051 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 110C4 | 3.6   | 2,173,666 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 47B12 | 20.9  | 2,173,750 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 9E10  | 8.0   | 2,174,159 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 17C7  | 10.2  | 2,174,525 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 82G8  | 12.4  | 2,174,764 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 15F4  | 16.9  | 2,175,262 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 36A3  | 39.1  | 2,175,262 | wbpP; UDP-N-acetylglucosamine/UDP-N-acetylgalactosamine 4-epimerase       | KeggGhostKoala |
| 70E8  | 33.9  | 2,175,998 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 3H9   | 22.0  | 2,176,097 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 161B6 | 3.5   | 2,176,097 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 85E8  | 7.4   | 2,176,277 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 137H4 | 8.3   | 2,176,277 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 70D2  | 65.4  | 2,201,817 | glycosyltransferase family 4 protein                                     | NCBI_PGAP    |
| 160D8 | 20.8  | 2,216,823 | dcd; dCTP deaminase                                                      | KeggGhostKoala |
| 158C9 | 39.8  | 2,328,780 | heme acquisition protein HasAp                                           | NCBI_PGAP    |
| 60F2  | 26.7  | 2,392,034 | helix-turn-helix transcriptional regulator                               | NCBI_PGAP    |
| 159E5 | 4.6   | 2,509,162 | hemK, prmC, HEMK; release factor glutamine methyltransferase             | KeggGhostKoala |
| Chromosome | Start | End   | Description                                                                 |
|------------|-------|-------|-----------------------------------------------------------------------------|
| 163A10     | 2     | 2.2   | Preprotein translocase subunit SecD (SecD) (PDB:3AQP) COG20_FUNCTION         |
| 165G7      | 3.2   | 2.546,686 | Preprotein translocase subunit SecD (SecD) (PDB:3AQP) COG20_FUNCTION     |
| 198D10     | 4.6   | 2.546,901 | Preprotein translocase subunit SecD (SecD) (PDB:3AQP) COG20_FUNCTION     |
| 28H4*      | 62.7  | 2,599,180 | Intergenic                                                                  |
| 28H9*      | 44.9  | 2,599,180 | Intergenic                                                                  |
| 81B9       | 54.5  | 2,680,581 | hypothetical protein NCBI_PGAP                                             |
| 5H7        | 6.2   | 2,700,177 | circularly permuted type 2 ATP-grasp protein NCBI_PGAP                     |
| 163B6      | 16.7  | 2,700,443 | circularly permuted type 2 ATP-grasp protein NCBI_PGAP                     |
| 27E11      | 8.2   | 2,706,718 | ahcY; adenosylhomocysteinase KeggGhostKoala                              |
| 103E10     | 64.6  | 2,729,433 | dapF; diaminopimelate epimerase KeggGhostKoala                            |
| 46E6       | 62.1  | 2,737,467 | Intergenic                                                                  |
| 141B2*     | 2.9   | 2,745,704 | lipB; lipoyl(octanoyl) transference KeggGhostKoala                         |
| 141H3*     | 3.3   | 2,745,794 | lipB; lipoyl(octanoyl) transference KeggGhostKoala                         |
| 9A2        | 6.8   | 2,758,668 | wbPD, wbHB; UDP-2-acetamido-3-amino-2,3-dideoxy-glucuronate N-acetyltransferase KeggGhostKoala |
| 6A2        | 5.1   | 2,759,073 | wbPD, wbHB; UDP-2-acetamido-3-amino-2,3-dideoxy-glucuronate N-acetyltransferase KeggGhostKoala |
| 28E2       | 2.9   | 2,759,073 | wbPD, wbHB; UDP-2-acetamido-3-amino-2,3-dideoxy-glucuronate N-acetyltransferase KeggGhostKoala |
| 65F2       | 21.0  | 2,759,640 | wbPD, wbHB; UDP-2-acetamido-3-amino-2,3-dideoxy-glucuronate N-acetyltransferase KeggGhostKoala |
| 118C6      | 2.6   | 2,759,073 | wbPD, wbHB; UDP-2-acetamido-3-amino-2,3-dideoxy-glucuronate N-acetyltransferase KeggGhostKoala |
| 8A6        | 14.3  | 2,759,250 | wbpE, wbHC; UDP-2-acetamido-2-deoxy-ribo-hexuluronate aminotransferase wbpE, wbHC; UDP-2-acetamido-2-deoxy-ribo-hexuluronate aminotransferase wbpE, wbHC; UDP-2-acetamido-2-deoxy-ribo-hexuluronate aminotransferase KeggGhostKoala |
| 57A12      | 5.5   | 2,759,604 | wbpE, wbHC; UDP-2-acetamido-2-deoxy-ribo-hexuluronate aminotransferase KeggGhostKoala |
| 48H12      | 15.6  | 2,760,072 | wbpE, wbHC; UDP-2-acetamido-2-deoxy-ribo-hexuluronate aminotransferase KeggGhostKoala |
| 45D1       | 23.7  | 2,760,549 | glycosyltransferase NCBI_PGAP                                             |
| 144C11     | 3.3   | 2,760,549 | glycosyltransferase NCBI_PGAP                                             |
| 28D1       | 3.1   | 2,760,640 | glycosyltransferase NCBI_PGAP                                             |
| 31F2       | 3.0   | 2,760,694 | glycosyltransferase NCBI_PGAP                                             |
| 42F4       | 32.5  | 2,760,694 | glycosyltransferase NCBI_PGAP                                             |
| 190D11     | 5.3   | 2,760,790 | glycosyltransferase NCBI_PGAP                                             |
| 106A6      | 4.3   | 2,761,091 | glycosyltransferase NCBI_PGAP                                             |
| 87D12      | 3.7   | 2,761,207 | glycosyltransferase NCBI_PGAP                                             |
| 33F6       | 2.7   | 2,761,270 | glycosyltransferase NCBI_PGAP                                             |
| 101A5      | 3.7   | 2,761,270 | glycosyltransferase NCBI_PGAP                                             |
| 105F5      | 4.0   | 2,761,270 | glycosyltransferase NCBI_PGAP                                             |
| 161D7      | 3.9   | 2,761,596 | DegT/DnrJ/EryC1/StrS family aminotransferase NCBI_PGAP                   |
| Accession | Score | Start | End | Description |
|-----------|-------|-------|-----|-------------|
| 162A5     | 1.9   | 2,761,847 | 2,761,847 | DegT/DnrJ/EryC1/StrS family aminotransferase |
| 51G4      | 8.4   | 2,762,483 | 2,762,483 | DegT/DnrJ/EryC1/StrS family aminotransferase |
| 187H3     | 4.9   | 2,762,483 | 2,762,483 | DegT/DnrJ/EryC1/StrS family aminotransferase |
| 133D7     | 20.9  | 2,762,487 | 2,762,487 | DegT/DnrJ/EryC1/StrS family aminotransferase |
| 32D4      | 9.6   | 2,762,597 | 2,762,597 | DegT/DnrJ/EryC1/StrS family aminotransferase |
| 35A7      | 59.7  | 2,764,918 | 2,764,918 | DUF2837 family protein |
| 28E10     | 35.0  | 2,770,051 | 2,770,051 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 47D8      | 45.6  | 2,770,186 | 2,770,186 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 7D7       | 14.9  | 2,770,337 | 2,770,337 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 3C6       | 19.1  | 2,770,569 | 2,770,569 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 36D12     | 38.0  | 2,770,655 | 2,770,655 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 101F4*    | 5.4   | 2,770,853 | 2,770,853 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 101G6*    | 5.2   | 2,770,853 | 2,770,853 | wbpl, wibD; UDP-GlcNAc3NAcA epimerase |
| 30D2      | 12.7  | 2,771,317 | 2,771,317 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 39H11     | 48.0  | 2,771,317 | 2,771,317 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 117A11    | 4.5   | 2,771,317 | 2,771,317 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 92A4      | 3.1   | 2,771,360 | 2,771,360 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 160C12    | 3.1   | 2,771,360 | 2,771,360 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 164B6     | 4.6   | 2,771,557 | 2,771,557 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 142F5     | 3.8   | 2,771,784 | 2,771,784 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 162H9     | 3.3   | 2,772,019 | 2,772,019 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 122F7     | 3.9   | 2,772,075 | 2,772,075 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 34D3      | 9.0   | 2,772,151 | 2,772,151 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 44F12     | 47.0  | 2,772,194 | 2,772,194 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 176E6     | 16.3  | 2,772,194 | 2,772,194 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 137E6     | 5.5   | 2,772,273 | 2,772,273 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 17D10     | 8.1   | 2,772,286 | 2,772,286 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 50H12     | 13.0  | 2,772,286 | 2,772,286 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 178G7*    | 20.8  | 2,772,443 | 2,772,443 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| 178H7*    | 20.4  | 2,772,443 | 2,772,443 | asnB, ASNS; asparagine synthase (glutamine-hydrolysing) |
| Accession | Expression | Score | Description                                                                 | Database       |
|-----------|------------|-------|-----------------------------------------------------------------------------|----------------|
| HPS44_12610 | 2.772,522   | 2.9   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala |
| HPS44_12610 | 2.772,615   | 3.6   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala |
| HPS44_12610 | 2.772,784   | 11.0  | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala |
| HPS44_12610 | 2.772,852   | 4.3   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala |
| HPS44_12615 | 2.773,083   | 7.7   | glycosyltransferase                                                          | NCBI_PGAP     |
| HPS44_12615 | 2.773,083   | 30.9  | glycosyltransferase                                                          | NCBI_PGAP     |
| HPS44_12615 | 2.773,383   | 9.1   | glycosyltransferase                                                          | NCBI_PGAP     |
| HPS44_12615 | 2.773,534   | 28.4  | glycosyltransferase                                                          | NCBI_PGAP     |
| HPS44_12630 | 2.776,773   | 6.6   | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12630 | 2.776,885   | 6.6   | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12630 | 2.777,086   | 16.8  | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12630 | 2.777,322   | 35.4  | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12630 | 2.777,548   | 17.8  | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12630 | 2.777,690   | 24.2  | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12635 | 2.778,266   | 28.2  | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12635 | 2.779,021   | 6.0   | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12640 | 2.779,189   | 8.3   | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12640 | 2.779,210   | 14.4  | galE, GALE; UDP-glucose 4-epimerase                                          | KeggGhostKoala|
| HPS44_12645 | 2.779,734   | 7.3   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12645 | 2.779,734   | 5.3   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12645 | 2.780,168   | 4.3   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12645 | 2.780,394   | 7.9   | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12645 | 2.780,753   | 38.7  | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12645 | 2.781,126   | 32.1  | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12645 | 2.781,128   | 30.0  | asnB, ASNS; asparagine synthase (glutamine-hydrolysing)                      | KeggGhostKoala|
| HPS44_12650 | 2.782,909   | 7.3   | hypothetical protein                                                          | NCBI_PGAP     |
| HPS44_12650 | 2.783,115   | 24.7  | hypothetical protein                                                          | NCBI_PGAP     |
| HPS44_12650 | 2.783,692   | 8.4   | hypothetical protein                                                          | NCBI_PGAP     |
| HPS44_12650 | 2.784,100   | 44.0  | hypothetical protein                                                          | NCBI_PGAP     |
| HPS44_12655 | 2.784,941   | 27.6  | wzm, rfbA; lipopolysaccharide transport system permease protein               | KeggGhostKoala|
| HPS44_12660 | 2.785,158   | 16.1  | wzt, rfbB; lipopolysaccharide transport system ATP-binding protein            | KeggGhostKoala|
| HPS44_12660 | 2.785,289   | 2.8   | wzt, rfbB; lipopolysaccharide transport system ATP-binding protein            | KeggGhostKoala|
| HPS44_12660 | 2.785,613   | 6.7   | wzt, rfbB; lipopolysaccharide transport system ATP-binding protein            | KeggGhostKoala|
| HPS44_12660 | 2.785,709   | 5.3   | wzt, rfbB; lipopolysaccharide transport system ATP-binding protein            | KeggGhostKoala|
| Gene    | Coverage | Identity | Description                                                                 | Database       |
|---------|----------|----------|-----------------------------------------------------------------------------|----------------|
| HPS44_12660 | 3.9      | 2,785,709| wzt, rbfB; lipopolysaccharide transport system ATP-binding protein           | KeggGhostKoala|
| HPS44_12660 | 7.3      | 2,786,151| wzt, rbfB; lipopolysaccharide transport system ATP-binding protein           | KeggGhostKoala|
| HPS44_12665 | 44.5     | 2,786,529| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 12.6     | 2,786,640| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 6.6      | 2,786,744| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 31.4     | 2,786,884| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 29.5     | 2,786,884| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 14.8     | 2,786,911| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 14.3     | 2,787,105| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 30.2     | 2,787,124| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12665 | 13.8     | 2,787,327| WbqC family protein                                                         | NCBI_PGAP      |
| HPS44_12670 | 10.2     | 2,788,422| fmt; methionyl-rrnA formyltransferase                                         | KeggGhostKoala|
| HPS44_12680 | 4.7      | 2,789,706| fmt; methionyl-rrnA formyltransferase                                         | KeggGhostKoala|
| HPS44_12700 | 11.7     | 2,792,949| methyltransferase domain-containing protein                                  | NCBI_PGAP      |
| HPS44_12705 | 9.9      | 2,794,061| carB, CPA2; carbamoyl-phosphate synthase large subunit                       | KeggGhostKoala|
| HPS44_12715 | 18.6     | 2,796,173| NAD-dependent epimerase/dehydratase family protein                           | NCBI_PGAP      |
| HPS44_12725 | 9.7      | 2,798,597| aminotransferase class I/II-pyridoxal phosphate-dependent enzyme             | NCBI_PGAP      |
| HPS44_12725 | 1.7      | 2,798,993| aminotransferase class I/II-pyridoxal phosphate-dependent enzyme             | NCBI_PGAP      |
| HPS44_12730 | 19.4     | 2,800,024| galE; UDP-glucose 4-epimerase                                                | KeggGhostKoala|
| HPS44_12730 | 11.5     | 2,800,177| galE; UDP-glucose 4-epimerase                                                | KeggGhostKoala|
| HPS44_12730 | 2.8      | 2,800,147| galE; UDP-glucose 4-epimerase                                                | KeggGhostKoala|
| HPS44_12730 | 2.8      | 2,800,321| galE; UDP-glucose 4-epimerase                                                | KeggGhostKoala|
| HPS44_12730 | 55.9     | 2,800,683| galE; UDP-glucose 4-epimerase                                                | KeggGhostKoala|
| HPS44_12730 | 13.8     | 2,800,863| galE; UDP-glucose 4-epimerase                                                | KeggGhostKoala|
| HPS44_12730 | 36.1     | 2,804,097| rfbC, mIC; dTDP-4-dehydrohamnose 3,5-epimerase                              | KeggGhostKoala|
| HPS44_12730 | 15.3     | 2,804,100| rfbC, mIC; dTDP-4-dehydrohamnose 3,5-epimerase                              | KeggGhostKoala|
| HPS44_12780 | 56.8     | 2,810,883| DUF502 domain-containing protein                                             | NCBI_PGAP      |
| HPS44_12925 | 6.7      | 2,842,256| mrcA; penicillin-binding protein 1A                                           | KeggGhostKoala|
| HPS44_12925 | 31.4     | 2,843,285| mrcA; penicillin-binding protein 1A                                           | KeggGhostKoala|
| HPS44_12975 | 3.9      | 2,854,132| dsbD, dipZ; thioredoxin:protein disulfide reductase                         | KeggGhostKoala|
| HPS44_12975 | 18.7     | 2,854,216| dsbD, dipZ; thioredoxin:protein disulfide reductase                         | KeggGhostKoala|
| HPS44_12975 | 9.2      | 2,854,981| dsbD, dipZ; thioredoxin:protein disulfide reductase                         | KeggGhostKoala|
| HPS44_12975 | 15.1     | 2,854,981| dsbD, dipZ; thioredoxin:protein disulfide reductase                         | KeggGhostKoala|
| HPS44_13035 | 22.2     | 2,861,605| rpmD; large subunit ribosomal protein L30                                    | KeggGhostKoala|
| HPS44_13195 | 10.4     | 2,884,109| tuf; elongation factor Tu                                                    | KeggGhostKoala|
| HPS44_13220 | 6.1      | 2,889,219| response regulator transcription factor                                      | NCBI_PGAP      |
| Gene ID | Predicted Function |
|--------|-------------------|
| HPS44_13250 | rpoB; DNA-directed RNA polymerase subunit beta |
| HPS44_13390 | parA, soj; chromosome partitioning protein |
| HPS44_13440 | tctD; two-component system, OmpR family, response regulator TctD |
| HPS44_13505 | katE, CAT, catB, srpA; catalase |
| HPS44_13975 | sporadically distributed protein |
| HPS44_13990 | DUF3893 domain-containing protein |
| HPS44_15235 | DNA-binding beta-propeller fold protein |
| HPS44_15800 | TIGR04141 family |
| HPS44_15975 | rubredoxin |
| HPS44_16505 | SDR family oxidoreductase |
| HPS44_16575 | lytic transglycosylase domain-containing protein |
| HPS44_16595 | hypothetical protein |
| HPS44_16755 | hypothetical protein |
| HPS44_16970 | YbhB/YbcL family Raf kinase inhibitor-like protein |
| HPS44_16505 | SDR family oxidoreductase |
| HPS44_16575 | lytic transglycosylase domain-containing protein |
| HPS44_16595 | rubredoxin |
| HPS44_17070 | hypothetical protein |
| HPS44_17145 | hypothetical protein |
| HPS44_17215 | hypothetical protein |
| HPS44_17220 | hypothetical protein |
| HPS44_17235 | hypothetical protein |
| HPS44_17240 / HPS44_17245 | Intergenic |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| HPS44_17255 | hypothetical protein |
| GeneID | Percent | StartPosition | GeneID | Percent | StartPosition |
|--------|---------|---------------|--------|---------|---------------|
| 55D7   | 3.8     | 3,786,085     | RsbU/P | phosphoserine phosphatase RsbU/P | KeggGhostKoala |
| 7D11   | 6.5     | 3,786,133     | RsbU/P | phosphoserine phosphatase RsbU/P | KeggGhostKoala |
| 90B9   | 3.2     | 3,786,748     | RsbU/P | phosphoserine phosphatase RsbU/P | KeggGhostKoala |
| 97D8   | 2.7     | 3,787,979     | Intergenic |         |               |
| 1F11   | 5.8     | 3,787,983     | Intergenic |         |               |
| 113F3  | 4.6     | 3,791,898     | Intergenic |         |               |
| 187B10 | 13.7    | 3,792,455     | STAS domain-containing protein | NCBI_PGAP |
| 140H4  | 1.7     | 3,792,609     | STAS domain-containing protein | NCBI_PGAP |
| 178F7  | 25.6    | 3,792,834     | Hypothetical protein | NCBI_PGAP |
| 39G5   | 6.1     | 3,793,106     | Intergenic |         |               |
| 15E9   | 9.9     | 3,806,668     | dnaQ: DNA polymerase III subunit epsilon | KeggGhostKoala |
| 113C8  | 17.6    | 3,806,668     | dnaQ: DNA polymerase III subunit epsilon | KeggGhostKoala |
| 174H6  | 60.0    | 3,812,798     | gtrB, csbB: polyisoprenyl-phosphate glycosyltransferase | KeggGhostKoala |
| 33E6   | 50.0    | 3,818,962     | livM: branched-chain amino acid transport system permease protein | KeggGhostKoala |
| 20H8   | 14.2    | 3,843,779     | tolQ: biopolymer transport protein TolQ | KeggGhostKoala |
| 177C11 | 58.2    | 3,843,892     | ybgC: acyl-CoA thioster hydrolase | KeggGhostKoala |
| 187A10 | 34.2    | 3,844,032     | ybgC: acyl-CoA thioster hydrolase | KeggGhostKoala |
| 155H9  | 6.2     | 3,856,544     | Intergenic |         |               |
| 64B5   | 40.9    | 3,861,199     | lgt, umpA: phosphatidylglycerol—prolipoprotein diacylglycerol transferase [EC:2.5.1.145] | KeggGhostKoala |
| 150D4  | 28.5    | 3,873,364     | Hypothetical protein | NCBI_PGAP |
| 24E5   | 4.9     | 3,951,422     | LysR family transcriptional regulator | NCBI_PGAP |
| 151D3  | 3.5     | 4,054,860     | Maleylacetate reductase | KeggGhostKoala |
| 81A6   | 67.2    | 4,068,280     | Hypothetical protein | NCBI_PGAP |
| 27H6   | 43.4    | 4,122,320     | Helix-turn-helix domain-containing protein | NCBI_PGAP |
| 58B4   | 14.9    | 4,125,992     | Etta: energy-dependent translational throttle protein Etta | KeggGhostKoala |
| 73E11  | 68.8    | 4,130,387     | MinC: septum site-determining protein MinC | KeggGhostKoala |
| 123F4  | 5.1     | 4,190,681     | DapB: 4-hydroxy-tetrahydrodipicolinate reductase | KeggGhostKoala |
| 191C6  | 5.5     | 4,190,681     | DapB: 4-hydroxy-tetrahydrodipicolinate reductase | KeggGhostKoala |
| 54D11  | 17.7    | 4,191,375     | DapB: 4-hydroxy-tetrahydrodipicolinate reductase | KeggGhostKoala |
| 141B7* | 11.5    | 4,191,381     | DapB: 4-hydroxy-tetrahydrodipicolinate reductase | KeggGhostKoala |
| 141G7* | 27.2    | 4,191,381     | DapB: 4-hydroxy-tetrahydrodipicolinate reductase | KeggGhostKoala |
| 161H4  | 3.1     | 4,194,332     | PnpK, NADK; NAD+ kinase | KeggGhostKoala |
| Accession | % Identity | Start | End | Description | Database |
|-----------|------------|-------|-----|-------------|----------|
| HPS44_19510 | 59.2 | 4,279,105 | 4,279,105 | hypothetical protein | NCBI_PGAP |
| HPS44_19520 | 9.8 | 4,282,155 | 4,282,155 | pnp, PNPT1; polyribonucleotide nucleotidyltransferase | KeggGhostKoala |
| HPS44_19580 | 7.0 | 4,293,653 | 4,293,653 | secG; preprotein translocase subunit SecG | KeggGhostKoala |
| HPS44_19580 | 12.1 | 4,293,932 | 4,293,932 | secG; preprotein translocase subunit SecG | KeggGhostKoala |
| HPS44_20275 | 13.9 | 4,466,735 | 4,466,735 | Intergenic | |
| HPS44_20295 | 50.0 | 4,470,327 | 4,470,327 | msrQ; methionine sulfoxide reductase heme-binding subunit | KeggGhostKoala |
| HPS44_20295 | 7.1 | 4,479,708 | 4,479,708 | hypothetical protein | NCBI_PGAP |
| HPS44_20295 | 3.8 | 4,479,708 | 4,479,708 | hypothetical protein | NCBI_PGAP |
| HPS44_20315 | 3.7 | 4,480,596 | 4,480,596 | rseA; sigma-E factor negative regulatory protein RseA | KeggGhostKoala |
| HPS44_20315 | 3.3 | 4,480,596 | 4,480,596 | rseA; sigma-E factor negative regulatory protein RseA | KeggGhostKoala |
| HPS44_20335 | 4.9 | 4,480,887 | 4,480,887 | rseA; sigma-E factor negative regulatory protein RseA | KeggGhostKoala |
| HPS44_20335 | 3.4 | 4,481,174 | 4,481,174 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 3.6 | 4,481,174 | 4,481,174 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 4.2 | 4,481,174 | 4,481,174 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 2.6 | 4,481,174 | 4,481,174 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 6.6 | 4,481,192 | 4,481,192 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 3.2 | 4,481,192 | 4,481,192 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 3.0 | 4,481,192 | 4,481,192 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 4.0 | 4,481,192 | 4,481,192 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 2.0 | 4,481,420 | 4,481,420 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 4.5 | 4,481,420 | 4,481,420 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 2.9 | 4,481,849 | 4,481,849 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 2.2 | 4,481,912 | 4,481,912 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 8.4 | 4,481,999 | 4,481,999 | rseB; sigma-E factor negative regulatory protein RseB | KeggGhostKoala |
| HPS44_20335 | 38.2 | 4,482,080 | 4,482,080 | degP, htrA; serine protease Do | KeggGhostKoala |
| HPS44_20335 | 61.2 | 4,483,265 | 4,483,265 | degP, htrA; serine protease Do | KeggGhostKoala |
| HPS44_20380 | 9.8 | 4,484,006 | 4,484,006 | lepA; GTP-binding protein LepA | KeggGhostKoala |
| HPS44_20380 | 35.2 | 4,484,402 | 4,484,402 | lepA; GTP-binding protein LepA | KeggGhostKoala |
| HPS44_20380 | 4.3 | 4,485,203 | 4,485,203 | lepA; GTP-binding protein LepA | KeggGhostKoala |
| HPS44_20480 | 63.7 | 4,505,053 | 4,505,053 | DUF2325 domain-containing protein | NCBI_PGAP |
| HPS44_20565 | 11.5 | 4,523,683 | 4,523,683 | elA; ElaA protein | KeggGhostKoala |
| Gene ID | %G | Map Location | Description | Database |
|--------|---|--------------|-------------|---------|
| 46B2   | 61.5 | 4,555,988 | mdh; malate dehydrogenase | KeggGhostKoala |
| 97E12  | 22.0 | 4,577,672 | Intergenic | |
| 48H11  | 35.7 | 4,582,176 | DLD, Lpd, pmdH; dihydrodipicolinate dehydrogenase | KeggGhostKoala |
| 114B7  | 66.0 | 4,604,190 | phbB; acetoacetyl-CoA reductase | KeggGhostKoala |
| 119E12 | 62.3 | 4,739,421 | instB, MTIF2; translation initiation factor IF-2 | KeggGhostKoala |
| 66A10  | 5.0  | 4,740,145 | Intergenic | |
| 48H11  | 35.7 | 4,742,940 | DLD, lpd, pdhD; dihydrolipoamide dehydrogenase | KeggGhostKoala |
| 115G12 | 16.6 | 4,824,207 | hemY; HemY protein | KeggGhostKoala |
| 114C11 | 31.9 | 4,876,328 | hypothetical protein | NCBI_PGAP |
| 159G12 | 10.5 | 4,915,830 | exbB; biopolymer transport protein | KeggGhostKoala |
| 110F2  | 6.0  | 4,980,301 | Intergenic | |
| 66F8   | 28.6 | 4,915,630 | exbB; biopolymer transport protein | KeggGhostKoala |
| 24A2   | 9.0  | 4,950,407 | phoU; phosphate transport system protein | KeggGhostKoala |
| 26G2   | 6.5  | 4,950,407 | phoU; phosphate transport system protein | KeggGhostKoala |
| 83F2   | 15.0 | 4,951,026 | phoU; phosphate transport system protein | KeggGhostKoala |
| 10B11  | 11.5 | 4,969,335 | glycosyltransferase family 2 protein | NCBI_PGAP |
| 186E12 | 68.6 | 5,029,107 | parE; topoisomerase IV subunit B | KeggGhostKoala |
| 111D11 | 15.6 | 5,074,021 | hflK; modulator of FtsH protease HflK | KeggGhostKoala |
| 35H3   | 7.0  | 5,082,422 | Intergenic | |
| 110G9  | 22.7 | 5,097,897 | tadB; tight adherence protein B | KeggGhostKoala |
| 136F2  | 53.2 | 5,109,233 | EAL domain-containing protein | NCBI_PGAP |
| 40H11  | 45.5 | 5,109,605 | EAL domain-containing protein | NCBI_PGAP |
| 134E1  | 42.5 | 5,110,255 | Intergenic | |
| 73F9   | 54.8 | 5,110,278 | Intergenic | |
| 18E8   | 32.4 | 5,122,901 | Ca2+ binding protein, RTX toxin-related (PDB: 1AF0) | COG20_FUNCTION |
| 26F2   | 14.0 | 5,132,467 | lapE; outer membrane protein, adhesin transport system | KeggGhostKoala |
| 185E1  | 59.9 | 5,276,139 | K00666; fatty-acyl-CoA synthase | KeggGhostKoala |
| 69F4   | 33.8 | 5,514,006 | K00666; fatty-acyl-CoA synthase | KeggGhostKoala |
| 50B2   | 29.1 | 5,526,029 | PAS domain S-box protein | NCBI_PGAP |
| 90E7   | 46.9 | 5,526,483 | PAS domain S-box protein | NCBI_PGAP |
| 46D1   | 28.5 | 5,526,687 | PAS domain S-box protein | NCBI_PGAP |
| 141G11 | 2.5  | 5,527,009 | PAS domain S-box protein | NCBI_PGAP |
| 155D4  | 38.4 | 5,607,906 | fliS; flagellar secretion chaperone FlIS | KeggGhostKoala |
| 156D10 | 7.5  | 5,607,906 | fliS; flagellar secretion chaperone FlIS | KeggGhostKoala |
| 112C4  | 55.0 | 5,608,083 | fliS; flagellar secretion chaperone FlIS | KeggGhostKoala |
| 69B3   | 7.6  | 5,617,962 | purF, PPAT; amidophosphoribosyltransferase | KeggGhostKoala |
| ID     | Similarity | Position     | Gene         | Description                                      | Database       |
|--------|------------|--------------|--------------|--------------------------------------------------|----------------|
| 150F6  | 27.8       | 5,625,515    | HPS44_25640  | hypothetical protein                              |                |
| 165E9  | 19.6       | 5,652,274    | HPS44_25785  | type II toxin-antitoxin system RatA family toxin | NCBI_PGAP      |
| 70D11  | 46.6       | 5,735,646    | HPS44_26230  | AAA family ATPase                                 | NCBI_PGAP      |
| 67C2   | 15.9       | 5,750,917    | HPS44_26300  | ldcA; muramoyltetrapeptide carboxypeptidase       | KeggGhostKoala |
| 53E5   | 12.6       | 5,751,046    | HPS44_26300  | ldcA; muramoyltetrapeptide carboxypeptidase       | KeggGhostKoala |
| 14D1   | 11.3       | 5,751,241    | HPS44_26300  | ldcA; muramoyltetrapeptide carboxypeptidase       | KeggGhostKoala |
| 62A7   | 5.4        | 5,751,241    | HPS44_26300  | ldcA; muramoyltetrapeptide carboxypeptidase       | KeggGhostKoala |
| 156E9  | 33.3       | 5,971,841    | HPS44_27310  | parS; two-component system, OmpR family, sensor kinase ParS | KeggGhostKoala |
| 33A11  | 67.1       | 6,211,977    | HPS44_28430  | hypothetical protein                              | NCBI_PGAP      |
| 10F2   | 60.7       | 6,390,695    | HPS44_29175  | transposase                                       | NCBI_PGAP      |

* Indicates possible strain duplication due to cross-contamination during processing of the same plate. We considered insertions that mapped to the same nucleotide but isolated from separate transformations as independent events.
**Movie S1.** Live imaging of J774a.1 cells infected with GN050 pDBD2sfGFP at an MOI:5 for 30 min followed by treatment with antibiotics to select for intracellular bacteria. After 30 min of treatment, the infection was replaced with antibiotic-free medium containing 0.36 µl/ml PI and imaged every 15 min for 20 h. Chloramphenicol (10 µg/ml) was supplemented into the infection to promote plasmid maintenance and sfGFP expression. Scale = 25 µm.

**Movie S2.** FITC and dsRED channels extracted from supplemental movie 1 to emphasize bacterial proliferation in relation to cytotoxicity. Scale = 25 µm.

**Movie S3.** Live imaging of J774a.1 cells infected with GN050 pDBD2sfGFP at an MOI:5 for 30 min followed by treatment with antibiotics to select for intracellular bacteria. After 30 min of treatment, the infection medium was replaced with medium containing 50 µg/ml gentamicin, 10 µg/ml polymyxin B, 0.36 µl/ml PI and imaged every 15 m for 20 h. Chloramphenicol (10 µg/ml) was supplemented into the infection to promote plasmid maintenance and sfGFP expression. Scale = 25 µm.

**Movie S4.** FITC and dsRED channels extracted from supplemental movie 2 to emphasize bacterial proliferation in relation to cytotoxicity. Scale = 25 µm.
SUPPLEMENTAL METHODS

Construction of pDBD2sfGFP and Modification of himar1

Antibiotic resistance is problematic in Achromobacter species. Initial characterization of antibiograms suggested that chloramphenicol could be useful at high concentrations (100-200 µg/ml) for selection in several clinical isolates from the Medical College campus, including GN050. As IncP plasmids appear to replicate in Achromobacter (4,5), we constructed pDBD2 utilizing the broad host range vector pCM62 (2). The CmΩ (6) interposon was cloned into the EcoRI site of pCM62 and chloramphenicol resistant clones were selected. To reduce the size of the plasmid the genes encoding tetracycline resistance (tetA and tetR) and terminator sequences were removed using the Q5 mutagenesis kit (New England Biolabs). This plasmid is called pDBD1. Plasmid DBD2 was constructed from pDBD1 by cloning the 492 bp 30S ribosomal protein S21 promoter region into the KpnI/SacI sites of pDBD1. sfGFP was amplified from pBADsfGFP (gift from the Mekalanos laboratory) and cloned into the EcoRI site. All cloning steps utilized the Gibson assembly Master Mix (New England Biolabs) and primers purchased from Integrated DNA Technologies (IDT). himar1 was modified to express chloramphenicol resistance as a single copy gene after transposition to the genome of GN050. Briefly, the 30S ribosomal protein S21 promoter region and CmΩ open reading frame were constructed as a cassette in a potential allelic replacement vector, pDBD4. The cassette was amplified from pDBD4 and inserted into the NheI site of himar1. All clones and plasmids were verified by double strand DNA sequence analysis.

Transposon mutagenesis screen

To identify GN050 himar1 insertion mutants that were unable to induce cytotoxicity in J774a.1 macrophages, a crystal violet-based screen was optimized using a 96-well plate format. J774a.1 macrophages were seeded at a density of 2.9x10⁴ cells per well. GN050 himar1 insertion mutants that were recovered from LB plates supplemented with 200 µg/ml chloramphenicol were picked into
individual wells of a 96-well plate containing 200 µl of DMEM. After suspension of the colony, 5 µl of bacterial culture was added to an individual well of seeded J774a.1 macrophages (MOI of 50-200) and subject to a light centrifugation of 600 x g for 5 min to synchronize the infection. Each well of the bacterial suspensions were replica plated using a 96-prong tamper onto LB agar containing 100 µg/ml chloramphenicol. These would be used for isolating downstream mutants of interest. After 4 h, wells were washed with HBSS (Gibco) and stained with 3% crystal violet in 5% methanol 5% ethanol 5% isopropanol for 5 min at room temperature (RT). Each well was then washed with deionized water to remove excess crystal violet followed by an image capture to document the staining pattern of each well.

Wells that retained a majority of crystal violet staining were thought to be infected with a GN050 mutant that was non-toxic to J774a.1 cells. These selected wells were paired with mirrored replica plate mutants that grew on medium containing 100 µg/ml chloramphenicol, confirming the presence of the selectable marker. Each selected GN050 mutant was struck on LB for further analysis. Mutants that grew in a comparable manner to WT GN050 on LB were processed in a secondary screen with a standardized MOI. Here, J774a.1 cells seeded at 1.75 x 10^5 cells per well in 24-well plates were infected with WT GN050 and mutant derivatives at an MOI of 10:1 for 6-8 h after a centrifugation of 600 x g for 5 min. To quantify cytotoxicity, adenylate kinase (AK) release assays were carried out as previously described (1). Genome location of himar1 insertions were determined for GN050 mutants that were at 70% or below of WT toxicity in the secondary screen.

**Sequencing and functional annotation of himar1 insertion mutants**

Clones, possessing similar growth properties to GN050 but defective for cytotoxicity in primary and secondary screens, were processed for genomic DNA isolation using the DNeasy ultraclean microbial kit according to manufacturers’ instructions (Qiagen). One microgram of genomic DNA was digested with NotI in a 20 µl reaction. Restriction endonuclease reactions were heated to 65°C for 20 min to inactivate NotI and 50 ng of DNA was ligated to rescue plasmid containing the R6Kori from
himar1. Ligations were transformed into DH5α λpir and selected on medium with 30 µg/ml chloramphenicol. The resulting colonies were screened for kanamycin resistance. Plasmid DNA was isolated from colonies expressing both chloramphenicol and kanamycin resistance and subjected to DNA sequence analysis utilizing primers that bind to each end of himar1.

**Quantitative reverse transcription-PCR (RT-qPCR)**

GN050 and derived strains were grown in LB to an OD_{600} of 0.8-1.0 and 3 mls of culture were harvested by centrifugation at 16,100 x g for 5 min at RT. The supernatant was removed and the resulting pellet was suspended in 500 μl of TRIzol reagent (Ambion). For every 500 μl of TRIzol sample, 100 μl was mixed in a phasemaker tube (Invitrogen) and subject to centrifugation at 16,100 x g for 30 min at 4ºC. Total RNA was extracted using the RNeasy plus universal minikit (Qiagen) and quantified using a Qubit fluorometer. Using 1 μg of total RNA, DNase and reverse transcriptase reactions were performed using SuperScript IV Vilo (Thermo Scientific) per the manufacturers’ instructions. To detect any contaminating DNA, reactions excluding reverse transcriptase were performed for each group. Approximately 75 ng of cDNA was used in each qPCR experiment performed in technical triplicates using primers (0.5 µM) generated by the IDT PrimerQuest tool. Primers and template were mixed with nuclease-free water and 10 µl of 2X SsoAdvanced Universal SYBR green Supermix (Bio-Rad). qPCR was performed on a CFX Connect real-time system (Bio-Rad) and analyzed using CFX Maestro software (v4.1).

**Functional annotation of insertion mutants**

Transposon insertions that mapped to an ORF were assigned functional annotation identifiers. Ax GN050 genome containing ORF identifiers (FASTAS) were exported from the NCBI genome database (CP053617) and converted into an anvi’o (v7, “Hope”)‐useable contig database file (.db). Functional annotations were identified against COG and PFAM databases. Protein sequences
were exported from anvi’o and imported into GhostKOALA (Kanehisa Laboratories) for KEGG annotation.

Pangenomic and phylogenomic analyses

Pangenomic analysis of Ax strains were performed using the anvi’o suite (v7, “Hope”). Ax genomes for 88 isolates were obtained from NCBI RefSeq databases (FASTAS) and were used to generate the anvi’o-readable contigs database file (.db; ‘anvi-generate-contigs-database’). A single storage database was generated for the 88 genomes by using ‘anvi-gen-genomes-storage’ with the command ‘—gene-caller NCBI_PGAP’. The pangenome analysis was performed using ‘anvi-pan-genome’ under default settings (Use DIAMOND [7], ‘—minbit = 0.5’ and ‘—mcl-inflation = 2’) and resulting protein clusters were visualized using ‘anvi-display-pan’. The figure was exported (.svg) and manipulated for aesthetics using inkscape (v1.0).

To determine the phylogenetic relationship of the 88 Ax isolates, an anvi’o (v7, “Hope”) workflow for phylogenomics was used. A new bin collection in ‘anvi-display-pan’ was generated to identify core single-copy genes (SCG’s) across Ax genomes. Because the strains were highly similar, custom homogeneity indices (Min number of genomes gene cluster occurs = 88, Max number of genes from each genome = 1, Max functional homogeneity index = 0.95, Min geometric homogeneity index = 0.9) were applied to identify 77 SCG’s. Alignments from the pangenomic analysis described above and using the bin collection with 77 SCG’s were exported (.fa) using ‘anvi-get-sequences-for-gene-clusters’ with the command ‘—concatenate-gene-clusters’ and ‘—max-num-genes-from-each-genome = 1’. A phylogenetic tree (maximum-likelihood method) was generated using ‘anvi-gen-phylogenomic-tree’ and visualized in ‘anvi-interactive’. The tree was exported into a Newick file (.nwk) then rooted by midpoint and visualized in FigTree (v1.4.4). The figure was exported (.svg) and manipulated for aesthetics using inkscape (v1.0).
**Microscopy and image processing**

Microscopy images were generated using a Nikon Eclipse Ti inverted microscope system equipped with a CoolSNAP ES2 charge-coupled-device (CCD) camera (Photometrics) with NIS-Elements AR imaging software (v5.2). For binding assays, images were captured using a Nikon 10X (Plan Apo 0.3 NA) or 100X (Plan Apo 1.4 NA oil) lens. For live imaging, images were captured in intervals using a Nikon 10X (Plan Fluor 0.3 NA) or 60X (Plan Apo 1.4 NA oil) lens. Infections were carried out on a motorized stage inside a Tokei Hit environmental chamber temperature-controlled at 37°C and 5% Clinical Blood Gas (Airgas). In both cases image intensity of propidium iodide (cytotoxicity assays), GFP signal (bacterial proliferation assays), or Alexa Fluor-488 signal (binding assays) was quantified using CellProfiler (v3.1.5) using the ‘Measure-Image-Intensity’ module.

**Transmission electron microscopy (TEM)**

Cells in culture dishes were fixed in mixture of 2.5% glutaraldehyde + 2% paraformaldehyde in 100mM sodium cacodylate buffer pH 7.4 (8) for 1 h at RT. Following fixation the cells were washed 3x5 min in cacodylate buffer then post fixed in reduced 1% osmium tetroxide (9) for 2 h on ice. Cells were scraped and the suspension was centrifuged at 8,000 x g for 10 min. The cell pellet was kept intact during washing (3 x 5 min) with distilled water then dehydrated through a graded methanol series followed by 2 x 10 min washes in acetonitrile before infiltrating with EMBed 812 epoxy resin and polymerization overnight at 70°C. Ultrathin sections (70 nm) were cut, stained with uranyl acetate and lead citrate, and examined in a JEOL 1400 transmission electron microscope. Images were recorded using an AMT “nanosprint 12” digital camera.

**Western blotting analyses**

To prepare bacterial supernatants, GN050 and derived strains were grown in Tryptic Soy Broth (TSB) dialysate medium overnight at 37°C shaking at 250 rotations per minute (rpm). Cultures were subject to centrifugation at 3,823 x g for 10 min at RT. Supernatants were harvested and filter
sterilized through a 0.2 µm filter. Approximately 9.6 ml of supernatant was mixed with 12 ml of saturated ammonium sulfate solution (Thermo Scientific) and placed on ice for 3 h. Precipitated material was collected by centrifugation at 31,209 x g for 30 min at 4ºC followed by another brief centrifugation (31,209 x g for 5 min at 4ºC) to remove as much supernatant as possible. Pellets were suspended in 240 µl 1x SDS loading buffer (0.1 M dithiothreitol) and boiled for 5 min before loading on 8% acrylamide, SDS gels.

For artA expression analyses, *E. coli* BL21 (DE3) pLysS pET15bartA was grown in 25 ml Terrific Broth (TB) shaking at 250 rpm to an OD$_{600}$ of 0.5-0.7. Induction of gene expression was initiated by addition of isopropyl β - d-1 thiogalactopyranoside (IPTG; 0.5 mM final concentration) or mock buffer (uninduced) and incubated for an additional 3 h. Aliquots of 40 µl from growing cultures were mixed with 10 µl of 5x SDS loading buffer (0.1 M dithiothreitol).

Antigen preparations described above were analyzed by SDS-PAGE (8% acrylamide gel) and probed with CF patient serum (1:500, overnight, 4ºC [Cystic Fibrosis Foundation]), anti-His antibody (1:10,000, overnight, 4ºC [GenScript]), and either horse radish peroxidase (HRP)-conjugated anti-human IgG (1:20,000, 2 h, RT [Thermo Scientific]) or HRP-conjugated anti-mouse IgG (1:10,000, 2 h, RT [Invitrogen]). Coomassie stained gels and western blots were imaged using a ChemiDoc Touch Gel imaging System (Bio-Rad).

**Figures and statistical analyses**

Figures were generated and statistical analyses were performed using python (v3.8.5) in a JupyterLab notebook environment and figures were modified using Inkscape software (v1.1). When applicable, one-way or two-way ANOVA statistical tests were performed in combination with Tukey’s post hoc analysis. Generally, cutoff for significance was at least p<.001 unless otherwise specified. For each method, the statistical analysis used is indicated in the figure legend.
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