Figure S1. Conserved phenotypes exist among several Tn mutant strains of *A. baumannii*. A: Murine, bone marrow-derived macrophages were infected with chemically-killed, mid log-phase *Ab* 17978 VU or one of the Tn mutant strains made in the *Ab* 17978 UN background – Tn5A7, Tn2, or Tn7 – at an MOI of 10. At 18 h.p.i., supernatants were collected from each well, and the concentration of IL-1β was quantified via ELISA. B: Representative electron microscopy images of *Ab* 17978 VU and the Tn mutant strains Tn5A7 and Tn20A11. Numerous appendages (pili) are visible on the cell surface of both Tn mutant strains, but not on the cell surface of *Ab* 17978 VU. C: Quantification of the differences in cell surface appendages between strains in terms of the number of cells with pili and the average number of pili per cell. A and C: Columns depict the mean, and error bars show standard deviation of the mean. All means were compared with the mean of the first column using a one-way ANOVA adjusted for multiple comparisons. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Figure S2. No differences in blood neutrophil or lymphocyte abundance exist between mice infected with *Ab* 17978 VU and mice infected with *Ab* 17978 UN. A-D: Mice were challenged intranasally with 3×10^8 CFU of mid log-phase *Ab* 17978 VU or *Ab* 17978 UN. At 24 h.p.i., mice were euthanized, blood was collected via cardiac puncture, and blood cell counts were determined using an automated hematology analyzer. Circles represent individual animals, columns depict the mean, and error bars show standard deviation of the mean. Means were compared using an unpaired Welch's *t*-test. ns: not significant.

Figure S3. Lung and serum levels of IL-1β do not differ between mice infected with *Ab* 17978 VU and mice infected with *Ab* 17978 UN at 24 h.p.i. Mice were challenged intranasally with 3×10^8 CFU of mid log-phase *Ab* 17978 VU or *Ab* 17978 UN. At 24 h.p.i., mice were euthanized, blood was collected via cardiac puncture, and lungs were harvested. IL-1β in the serum (A) or lung homogenates (B) was quantified using ELISA. A: N=3-5 biological replicates per group, per experiment. Experiments were repeated for a total of at least two times, with graphs depicting representative data. B: N=3-5 biological replicates per group, per experiment. Graphs depict average results from at least two independent experiments. A and B: Columns depict the mean, and error bars show standard deviation of the mean. Means were compared using an unpaired Welch's *t*-test. ns: not significant.

Figure S4. The accessory pilus assembly genes present in *Ab* 17978 UN do not contribute to increased phagocytosis by macrophages, biofilm formation, bacterial surface associated motility, bacterial association with epithelial cells and erythrocytes, or differential production of cytokines by infected macrophages. A: Macrophage-like RAW 264.7 cells were infected with mid log-phase *Ab* 17978 VU, *Ab* 17978 UN, or *Ab* 17978 UN *Δpilus* at an MOI of 15. At 1 h.p.i., and extracellular bacteria were killed with gentamicin at 1 h.p.i. Thirty minutes after the addition of gentamicin, RAW cells were lysed, and intracellular bacterial burdens were determined. B: Stationary-phase cultures of *Ab* 17978 VU, *Ab* 17978 UN, and *Ab* 17978 UN *Δpilus* were diluted 1:10 in PBS, coated onto the wells of a 96-well plate, and incubated at 37 °C without agitation for 24-48 hours. After incubation, biofilm formation was quantified using crystal violet staining, and the ratio of biofilm to biomass was determined by measuring the optical densities at 580 nm and 600 nm, respectively. C: A549 cells were infected with mid log-phase *Ab* 17978 VU, *Ab* 17978 UN, or *Ab* 17978 UN *Δpilus* at an MOI of 100, and incubated at 37 °C for two hours. At 2 h.p.i., non-adherent bacteria were removed by washing with PBS, A549 cells were lysed, and the number of cell-associated bacteria was determined. D: Stationary-phase *Ab* 17978 VU, *Ab* 17978 UN, and *Ab* 17978 UN *Δpilus* were spotted on the surface of motility agar plates at the center and incubated overnight at 37 °C without agitation. After incubation, the maximum motility radius was measured. E: *Ab* 17978 VU, *Ab* 17978 UN, and *Ab* 17978 UN *Δpilus* were grown in static liquid culture (left) or on LBA plates (right), and incubated overnight at 4 °C with an equal volume of 1% human erythrocytes in PBS. Static liquid cultures of *E. coli* UTI89 and *E. coli* UTI89 *ΔfimAH* served as positive and negative controls, respectively. After incubation, hemagglutination titers (i.e. the maximum dilution at which bacteria are able to agglutinate erythrocytes) were determined. F and G: Murine, bone marrow-derived macrophages were infected with mid log-phase cultures of *Ab* 17978 VU, *Ab* 17978 UN, or *Ab* 17978 UN *Δpilus* at an MOI of 10 and incubated at 37°C. At 18 h.p.i., supernatants of infected BMDMs were collected. The concentrations of IL-10 (A) and IL-1β (B) in the supernatants of infected BMDMs were determined by ELISA. A–G: N=3-4 biological

replicates per group, per experiment. Experiments were repeated for a total of at least two times, with graphs depicting representative (A, B, E-G) or average (C and D) data. Columns depict the mean, and error bars show standard deviation of the mean. A-D, F and G: Means were compared to all other means using a one-way ANOVA adjusted for multiple comparisons. E: Means were compared to the mean of the first column using a one-way ANOVA adjusted for multiple comparisons. CFU/mL: colony forming units per milliliter. **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$; ns: not significant.

Figure S5: The AbaAL44 *katX* gene promotes *Ab* 17978 UN resistance to neutrophil-mediated killing. Murine, bone marrow-derived neutrophils were incubated with mid log-phase *Ab* 17978 VU, *Ab* 17978 UN, or *Ab* 17978 UN *ΔkatX* at an MOI of 1. Two hours post-incubation, neutrophils were lysed, and neutrophil-mediated killing of bacteria was assessed by determining the number of viable bacteria in each well. N=3 per group, per experiment. Experiments were repeated for a total of at least two times, with graphs depicting representative data. Columns depict the mean, and error bars show standard deviation of the mean. Means were compared to all other means using a one-way ANOVA adjusted for multiple comparisons. **: $p<0.01$; ****: $p<0.0001$.

Figure S6: Small, punctate colonies are present in supernatants and lysates of macrophage-like RAW 264.7 cells infected with *Ab* 17978 UN *ΔclsC2* or *Ab* 17978 UN *ΔclsC2*/*clsC2*. A-C: Macrophage-like RAW 264.7 cells were infected with *Ab* 17978 UN, *Ab* 17978 *ΔclsC2*, or *Ab* 17978 *ΔclsC2*/*clsC2* at a target MOI of 15 and starting inoculums were serially diluted in PBS and plated on LBA (A). B: At 30 minutes post-infection, supernatants were serially diluted in PBS and plated on LBA. C: The remaining supernatant was aspirated, cells were washed twice with PBS, lysed with 0.01% Triton X-100, and lysates were serially diluted in PBS and plated on LBA.

Table S1. Bacterial strains and plasmids used in this study

Strain/plasmid	Description	Source	Reference
Ab 17978 VU	Variant of *A. baumannii* ATCC 17978 that lacks AbaAL44, stored at Vanderbilt University	Vanderbilt University	ATCC
Ab 17978 UN	Variant of *A. baumannii* ATCC 17978 that harbors AbaAL44, stored at the University of Nebraska	University of Nebraska	ATCC, (1)
Ab 17978 UN *Δpilus*/pAT02	*Ab* 17978 UN with accessory pilus locus (*smf1, yadV, htrE, and mrkD*) replaced by kan^R cassette		This study
Ab 17978 UN *ΔkatX*	*Ab* 17978 UN with accessory *katX* gene (KZA74_09300) replaced by kan^R cassette		This study
Ab 17978 UN *ΔkatX*/EV	*Ab* 17978 UN *ΔkatX* transformed with pWH1266		This study
Ab 17978 UN *ΔkatX*/p*katX*	*Ab* 17978 UN *ΔkatX* transformed with pWH1266(*katX*)		This study
Ab 17978 UN *ΔclsC2*	*Ab* 17978 UN with accessory cardiolipin synthase gene (*clsC2*) replaced by kan^R cassette		This study
Ab 17978 UN *ΔclsC2*/*clsC2*	*Ab* 17978 UN *ΔclsC2* with *clsC2* reintegrated chromosomally under its native promoter		This study

E. coli UTI89	Prototypical UPEC strain	Dr. Maria Hadjifrangiskou (Nashville, TN)	(2)
E. coli UTI89 *ΔfimAH*	Prototypical UPEC strain with *fimA* and *fimH* genes deleted	Dr. Maria Hadjifrangiskou (Nashville, TN)	(3)
E. coli 100D/pTNS2	*E. coli* 100D transformed with helper plasmid encoding the site-specific TnsABCD Tn7 transposition pathway		(4)
E. coli HB101/pRK2013	*E. coli* HB101 transformed with oriT helper-containing plasmid		(4)
E coli DH5α *λpir116*	Strain that permits maintenance of plasmids at high copy numbers	Dr. Eric Skaar (Nashville, TN)	
pCR2.1	Vector to amplify kan^R cassette for insertion		(5)
pAT02	precET-carb confers carb/amp resistance and *A. baumannii* recombinase		(6)
pWH1266	*E. coli – A. baumannii* shuttle vector conferring ampicillin and tetracycline resistance		(7)
pKNOCK-mTn7-amp	Plasmid with R6K origin of replication and mini-Tn7 element		(4, 8)

Table S2. Annotated genes of the accessory cluster present in *Ab* 17978 UN.

Gene	Locus Tag (CP079931)	Annotation	Notes
*3977**	KZA74_09200	phage/plasmid replication protein	Identical to *3978,* only partially in the island
*4059**	KZA74_09205	helix-turn-helix transcriptional regulator	Identical to *4060*
*3965**	KZA74_09210	putative replication initiation protein	Identical to *3966*
*390**	KZA74_09215	hypothetical protein	No putative conserved domains have been detected
*491**	KZA74_09220	putative Zonular occludens toxin (Zot)	
*191**	KZA74_09225	DUF2523 domain-containing protein	
*492**	KZA74_09230	hypothetical protein	No putative conserved domains have been detected. Truncated relative to Aba810CP_9780 in *A. baumannii* strain 810CP
*493**	KZA74_09235	uncharacterized protein	No putative conserved domains have been detected

*4011**	KZA74_09240	hypothetical protein	No putative conserved domains have been detected
*3978**	KZA74_09245	phage/plasmid replication protein	Identical to *3977*
*4060**	KZA74_09250	helix-turn-helix transcriptional regulator	Identical to *4059*
*3966**	KZA74_09255	putative replication initiation protein	Identical to *3965*
*495**	KZA74_09260	hypothetical protein	No putative conserved domains have been detected
smf-1_1	KZA74_09265	Major fimbrial subunit SMF-1	
yadV_1	KZA74_09270	putative fimbrial chaperone YadV	
htrE_1	KZA74_09275	Outer membrane usher protein HtrE	
mrkD_1	KZA74_09280	Fimbria adhesin protein	
*501**	KZA74_09285	DUF 4882 superfamily protein	This small family consists of several uncharacterized proteins around 325 residues in length and is mainly found in various Acinetobacter species.

			The function of this family is unknown.
frmA	KZA74_09290	S-(hydroxymethyl)glutathione dehydrogenase	
*193**	KZA74_09295	phage capsid protein	
*503**	KZA74_09300	catalase	
*504**	KZA74_09305	hypothetical protein	No putative conserved domains have been detected
*505**	KZA74_09310	hypothetical protein	No putative conserved domains have been detected
clsC2	KZA74_09315	Cardiolipin synthase C	
*506**	KZA74_09320	Uracil-DNA glycosylase	
*507**	KZA74_09325	hypothetical protein	No putative conserved domains have been detected
*509**	KZA74_09330	peptidase	
pqqE	KZA74_09335	Pyrroloquinoline quinone biosynthesis protein PqqE	
pqqD	KZA74_09340	Coenzyme PQQ synthesis protein D	
pqqC	KZA74_09345	Pyrroloquinoline-quinone synthase	

pqqB	KZA74_09350	Coenzyme PQQ synthesis protein B	
pqqA	KZA74_09355	pyrroloquinoline quinone precursor peptide PqqA	
ppk_1	KZA74_09360	Polyphosphate kinase	
yhcR	KZA74_09365	Endonuclease YhcR	
*516**	KZA74_09370	Fur family transcriptional regulator	
vibB	KZA74_09375	Vibriobactin-specific isochorismatase	
dhbA	KZA74_09380	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase	
cysA_2	KZA74_09385	Sulfate/thiosulfate import ATP-binding protein CysA	
modB	KZA74_09390	Molybdenum transport system permease protein ModB	
modA	KZA74_09395	Molybdate-binding protein ModA	
mopA	KZA74_09400	Molybdenum-pterin-binding protein MopA	
antA_2	KZA74_09405	Anthranilate 1,2-dioxygenase large subunit	

antB	KZA74_09410	Anthranilate 1,2-dioxygenase small subunit	
antC	KZA74_09415	anthranilate 1,2-dioxygenase electron transfer component	Partially in AbaLA44

*: Indicates name assigned by genome analysis software PROKKA

93 **Table S3: Predicted mutations in *Ab* 17978 UN.**

Nucleotide Position (CP012004 [VU]/ CP079931 [UN])	Allele in VU (residue)	Allele in UN (residue)	Gene	Description
129,758	IS*Aba18*	Δ1,312 bp	*ACX60_00590–ACX60_00595*	transposase, transposase
207,638/206,237	G (A382)	A (T382)	*actP*	acetate permease
524,616/ 523,217	+A	Δ1 bp	*ACX60_02575/KZA74_02575*	Phosphohydrolase/pseudogene
807,273/805,874	G (P439)	A (P439)	*cysG*	sirohydrochlorin ferrochelatase
1,075,443/1,074,044	A (N258)	T (I258)	*ACX60_05080*/*cgtA*	GTPase *obgE*
1,226,257/1,224,858	G (G12)	A (D12)	*ACX60_05695*/*KZA74_05725*	membrane protein DUF817
1,685,154/1,683,755	T (M78)	C (T78)	*ACX60_07925*/*uppS*	UDP diphosphate synthase
2,068,076/2,111,105	T (stop codon)	A (L376)	Intergenic *ACX60_09755-ACX60_09765/KZA74_10025*	SurA N-terminal domain-containing protein
2,109,791/2,152,820	C (A55)	T (T55)	*ACX60_09960*/*KZA74_10230*	hypothetical protein/ putative transporter
2,135,294/2,178,324	+C	$(C)_{5\rightarrow4}$	*ACX60_10130*/*KZA74_10405*	DNA helicase AAA family ATPase
2,172,876/2,215,906	T (F799)	G (V799)	*ACX60_10365*/*KZA74_10630*	LPS biosynthesis protein LptD

2,189,235/2,232,265	A	G	*ACX60_10450 -ACX60_10455/KZA74_10715-putP*	Intergenic (NAD-dependent deacetylase/proline:sodium symporter PutP)
2,236,009/2,279,039	G	A	*ACX60_10685-ACX60_10690/KZA74_10950-KZA74_10955*	Intergenic (ABC transporter substrate-binding protein/glutathione S-transferase)
2,410,784/2,453,814	C (T2)	A (K2)	*ACX60_11495*/*KZA74_11775*	aspartate:proton symporter APC family permease
2,846,783/2,889,821	Δ1,106 bp	IS*Aba11*	*ACX60_17045/KZA74_13815*	transposase
3,384,892/3,429,018	Δ1 bp	+T	*ACX60_15995-ACX60_16000*/*KZA74_16315 - infA*	Intergenic (AraC family transcriptional regulator/translation initiation factor IF-1)
3,384,893/3,429,019	G	A	*ACX60_15995-ACX60_16000*/*KZA74_16315 - infA*	Intergenic (AraC family transcriptional regulator/translation initiation factor IF-1)
3,852,237	$(ATGGTG)_{9\rightarrow8}$	$(ATGGTG)_{9\rightarrow8}$	*ACX60_18165*/*dmeF*	cobalt transporter

REFERENCES (SUPPLEMENT)

1. Jacobs AC, Hood I, Boyd KL, Olson PD, Morrison JM, Carson S, Sayood K, Iwen PC, Skaar EP, Dunman PM. 2010. Inactivation of phospholipase D diminishes *Acinetobacter baumannii* pathogenesis. Infect Immun 78:1952-62.
2. Mulvey MA, Schilling JD, Hultgren SJ. 2001. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. Infect Immun 69:4572-9.
3. Rosen DA, Pinkner JS, Jones JM, Walker JN, Clegg S, Hultgren SJ. 2008. Utilization of an intracellular bacterial community pathway in *Klebsiella pneumoniae* urinary tract infection and the effects of FimK on type 1 pilus expression. Infect Immun 76:3337-45.
4. Kumar A, Dalton C, Cortez-Cordova J, Schweizer HP. 2010. Mini-Tn7 vectors as genetic tools for single copy gene cloning in *Acinetobacter baumannii*. J Microbiol Methods 82:296-300.
5. Spiliotis M. 2012. Inverse fusion PCR cloning. PLoS One 7:e35407.
6. Tucker AT, Nowicki EM, Boll JM, Knauf GA, Burdis NC, Trent MS, Davies BW. 2014. Defining gene-phenotype relationships in *Acinetobacter baumannii* through one-step chromosomal gene inactivation. MBio 5:e01313-14.
7. Hunger M, Schmucker R, Kishan V, Hillen W. 1990. Analysis and nucleotide sequence of an origin of DNA replication in *Acinetobacter calcoaceticus* and its use for *Escherichia coli* shuttle plasmids. Gene 87:45-51.
8. Carruthers MD, Nicholson PA, Tracy EN, Munson RS. 2013. *Acinetobacter baumannii* utilizes a type VI secretion system for bacterial competition. PLoS One 8:e59388.