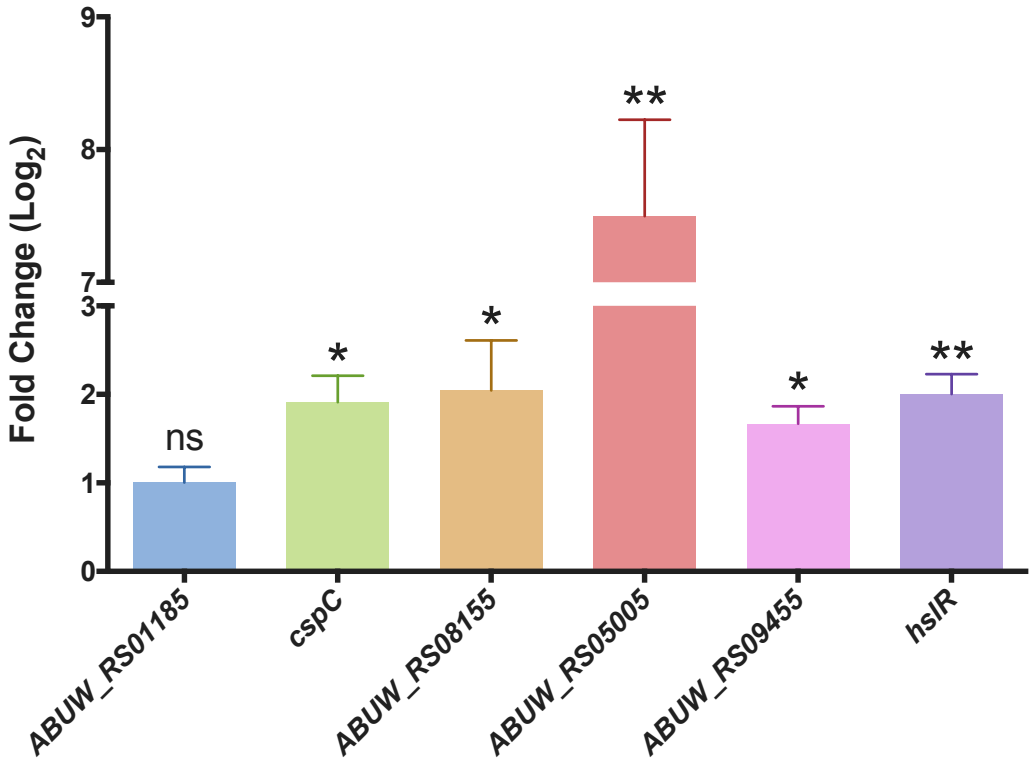


Figure S1: RT-qPCR validation of RNA-seq findings. RT-qPCR was performed in triplicate using gene specific primers for six selected genes. The 16S rRNA gene was used as an internal control. Fold change of expression within biofilm relative to planktonic samples was determined using the $2^{-\Delta\Delta CT}$ method. Error bars ±SD are shown. Student's *t* test was used to determine statistical significance relative to wildtype expression levels. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

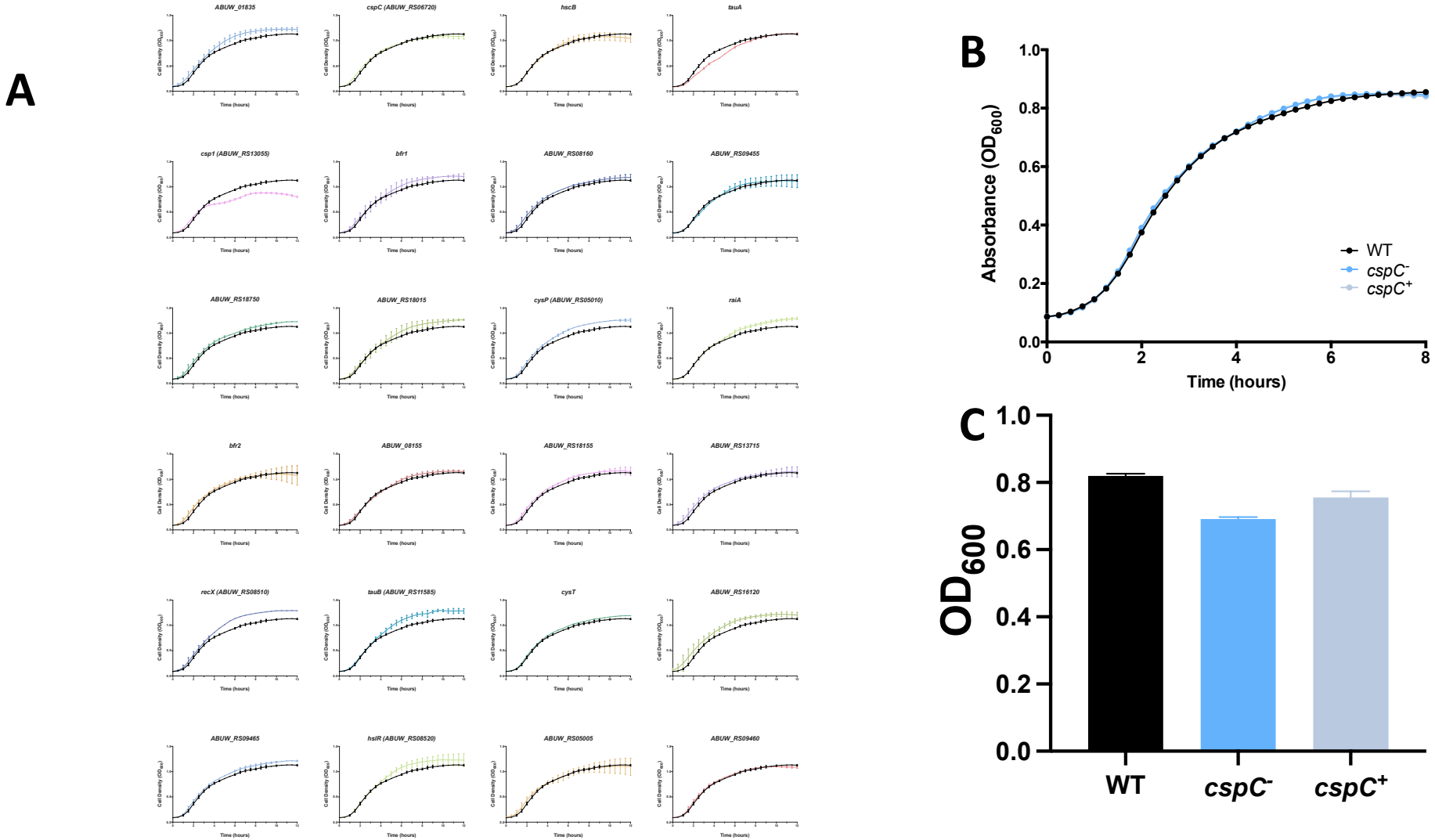


Figure S2. The majority of transposon mutants tested exhibit wild type growth. Growth of the prioritized transposon mutants was profiled in a 96-well plate in LB media at 37°C with shaking, and assessed by measuring optical density (OD_{600}) until stationary phase (**A**). Same as A, but with the *cspC* transposon mutant and wild-type strain, both bearing an empty pMQ557 vector, alongside a *cspC* mutant complementing strain, in LB supplemented with hygromycin (**B**). Same as B, but without shaking. Data presented is from 24h growth, as with our biofilm assays (**C**). n=3 for **A** and **B**, n= 10 for **C**. Error bars represent ±SEM.

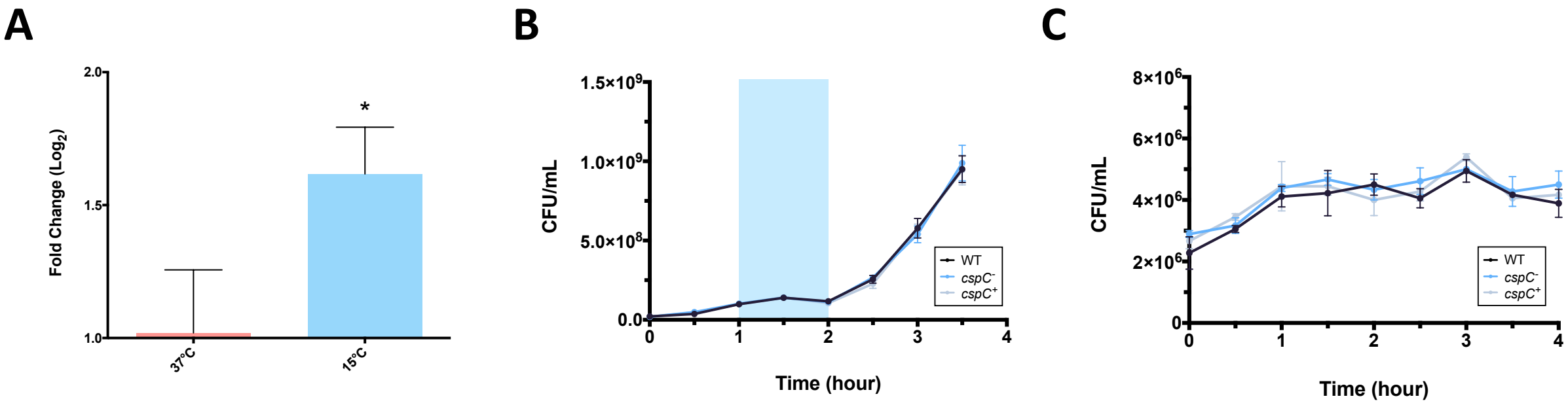


Figure S3. *cspC* transcription is induced during cold stress but its disruption has no effect on cold-shock recovery or survival. RT-qPCR was used to measure *cspC* transcript abundance after 15 minutes of cold-stress (**A**). The 16S rRNA gene was used as an internal control. Fold change of expression under cold-stress relative to 37°C was determined using the $2^{-\Delta\Delta CT}$ method. Cell viability was assessed after one hour of induced cold-shock (blue shading) (**B**) and sustained cold-stress conditions (**C**). Assays were performed in biological triplicate with 3 technical replicates each. Error bars represent ±SEM. Student's *t* test was used to determine statistical significance. *, P<0.05.

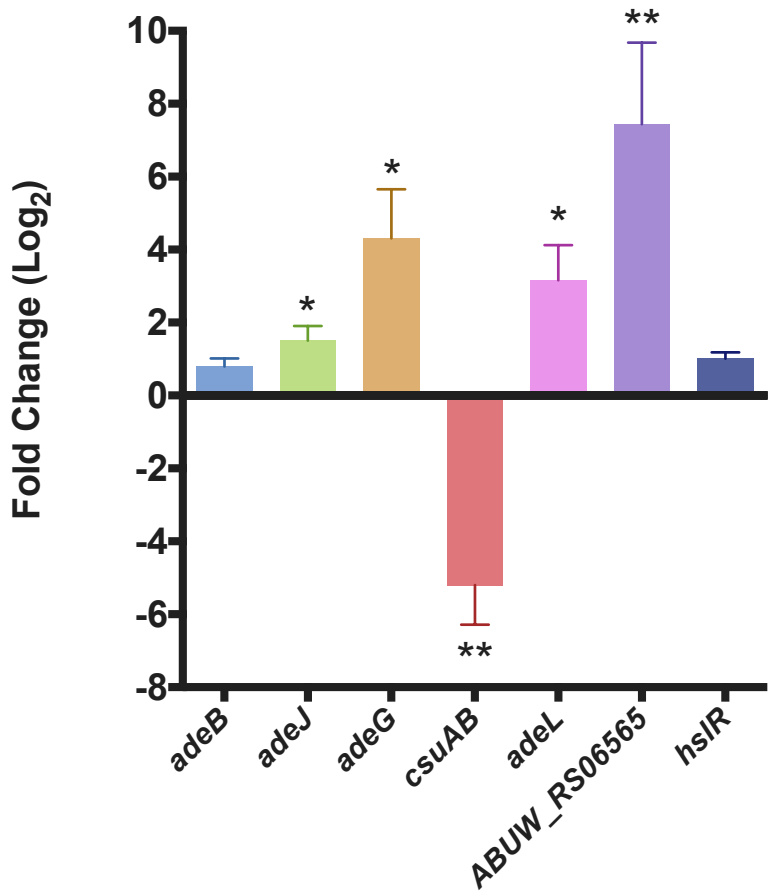
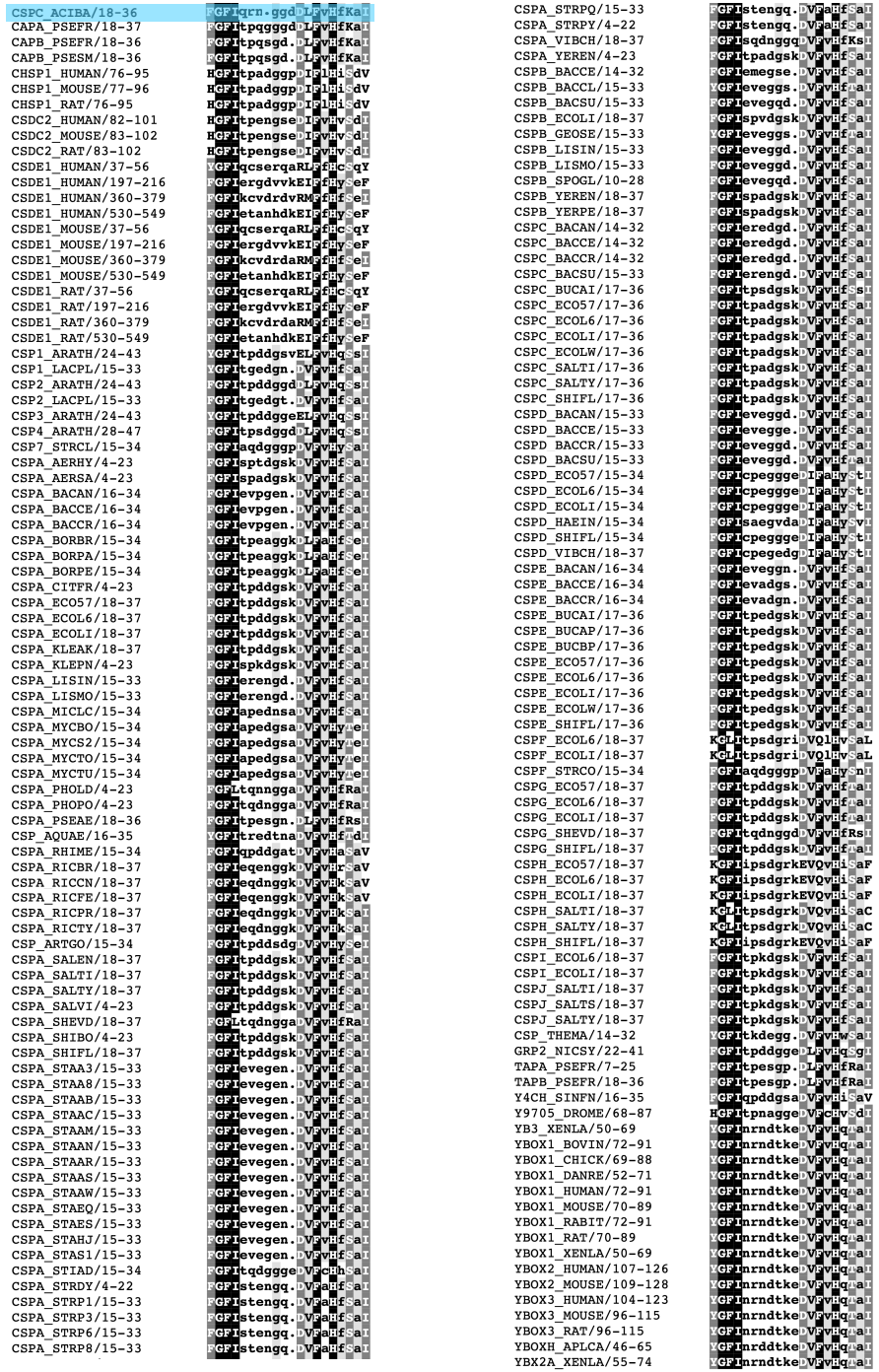


Figure S4: RT-qPCR validation of RNA-seq findings for the *cspC* mutant. RT-qPCR was performed in triplicate on genes selected from our RNA-seq dataset. The 16S rRNA gene was used as an internal control. Shown is fold change of expression in the *cspC* mutant relative to wildtype; determined using the $2^{-\Delta\Delta CT}$ method. Error bars are ±SD. Student's *t* test was used to determine statistical significance. *, $P < 0.05$; **, $P < 0.01$.

Figure S5. Alignment of conserved cold-shock protein nucleic acid binding motifs. A Clustal alignment of the conserved cold shock domain signature (Prosite entry PS00352) found in CspC of *A. baumannii* strain AB5075. Included in the alignment are the 174 sequences in UniProtKB/Swiss-Prot identified as belonging to the same class as CspC, with CspC highlighted in blue.



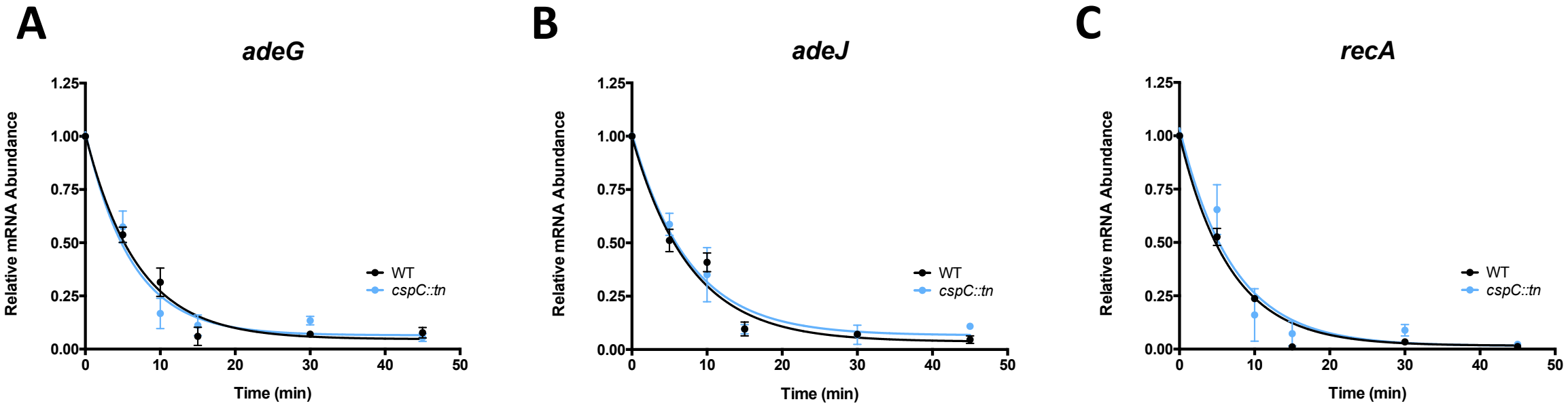


Figure S6. CspC does not influence mRNA half life of efflux pump components *adeG* and *adeJ*. Exponentially growing *A. baumannii* wildtype and *cspC* mutant strains were treated with 250 µg/mL rifampin to arrest transcription, and changes in transcript abundance were measured for *adeG* (A) and *adeJ* (B) using RT-qPCR. The mRNA decay of *recA* (C) was also included as a control, as the half-life for this mRNA has previously been determined as 4.5 min (confirmed herein). Values represent mean fold change in transcript abundance relative to that immediately prior to rifampin treatment (t=0). Data is from triplicate cultures, ±SD. Lines shown are the exponential, one phase decay curve, represented as $R(t) = R_0e^{-kt}$, which were used to calculate mRNA half lives.

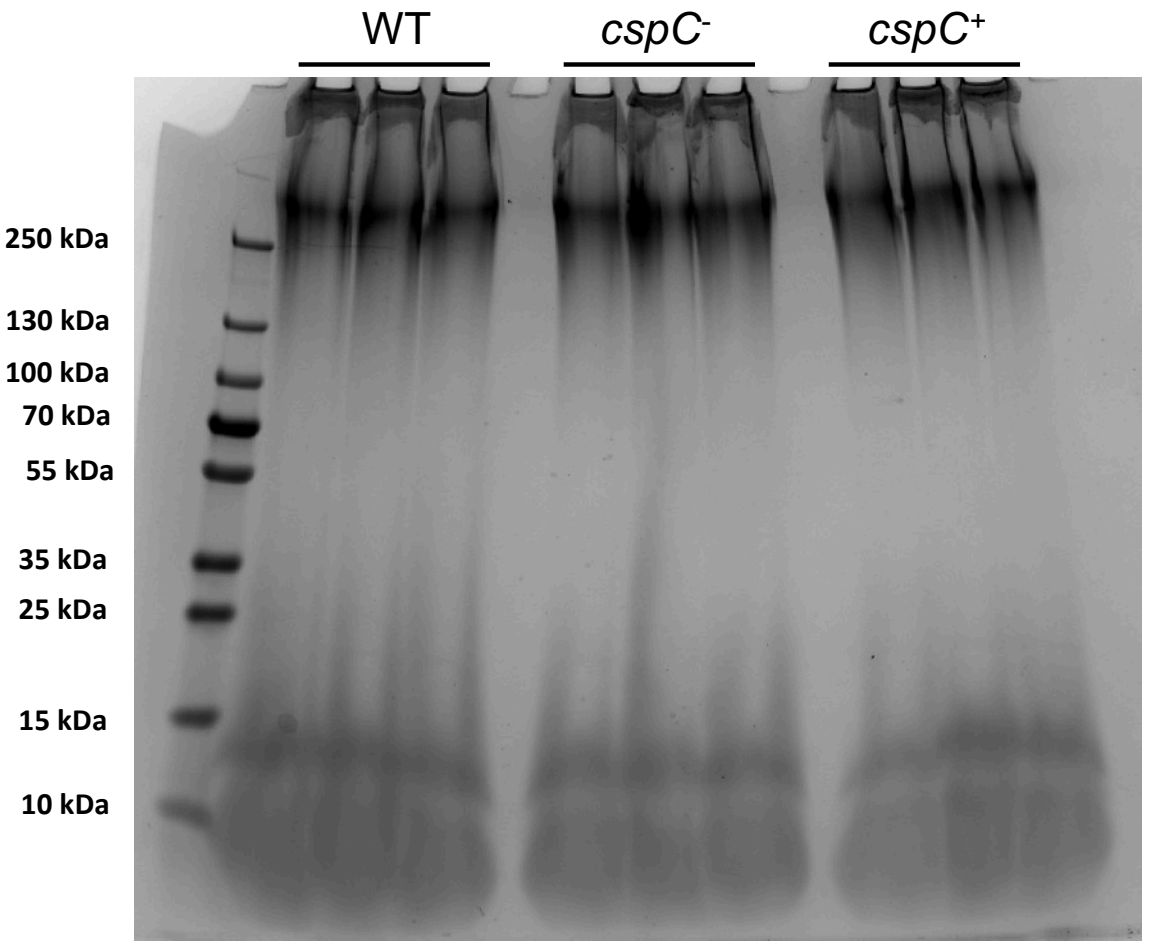


Figure S7. Capsular exopolysaccharide abundance is not altered in an *A. baumannii cspC* mutant. Polysaccharides were extracted and analyzed as described by Geisinger and Isberg [1]. The OD_{600} of WT, *cspC*$^{-}$ and *cspC*$^{+}$ cultures grown for 15h was adjusted to ensure equal amounts of samples were processed. Samples were loaded onto a 4-20% TGX Tris-glycine gel. Gels were stained with 0.1% Alcian Blue 8GX.

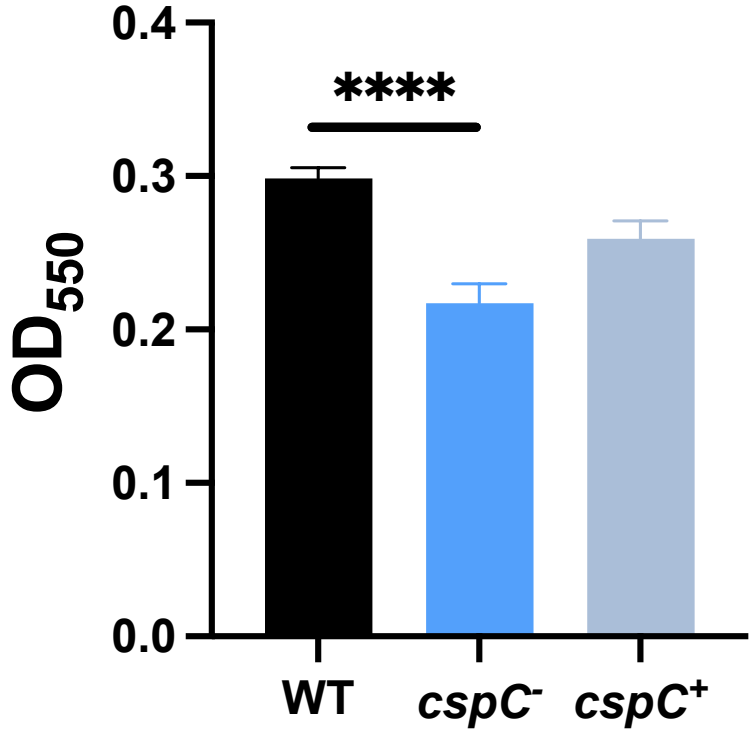


Figure S8. *A. baumannii cspC* mutants have an impaired ability to adhere to plastic surfaces. Adhesion of the strains noted was assessed using the method of Tomaras and coworkers [2] with modifications. Overnight cultures (24h) were standardized to the same OD_{600} and added to the wells of a 96-well plate. Plates were incubated for 45 mins, before wells were thrice washed with PBS and the remaining cellular biomass assessed using a crystal violet assay. Error bars from 10 replicates each are shown ±SEM. Student's *t* test was used to determine statistical significance. ****, $P < 0.0001$.

Table S1. Primers used in this study.

Primer	Sequence*	Description†	Source
OL5087	ATGCCCGGGACCCTATAACAAATGGATGTAACC GG	*cspC* F for *tn* screening	This study
OL5088	ATGCTCGAGGCGCTCTAACAAACAATCTCTCG	*cspC* R for pMQ557 and *tn* screening	This study
OL5214	ATGCCCGGGGTGCTCATTCATCATCAAAACAACT	*cspC* F for pMQ557	This study
OL4163	ATCTTCTCTCATCCGCCAAA	pMQ557 F MCS screening	This study
OL4164	CTGTTTCTCCATACCCGTAG	pMQ557 R MCS screening	This study
OL398	TCCTACGGGAGGCAGCAGT	*16s* F to confirm DNA removal	[3]
OL399	GGACTACCAGGGTATCTA ATCCTGTT	*16s* R to confirm DNA removal	[3]
OL4498	CCTAGAGATAGTGGACGTTACTCG	*16s* F for qPCR normalization	[4]
OL4499	CCAGTATCGAATGCAATTCCCAAG	*16s* F for qPCR normalization	[4]
OL5216	ACAGCTCGTGAACAAGGC	*cspC* F qPCR	This study
OL5218	CGCCGTTACGTTGAATAAAACC	*cspC* R qPCR	This study
OL5369	ATTGGCTGTGCACTGGAATATCTG	*ABUW_RS01185* F qPCR	This study
OL5370	AAGCCTTACGGGTTTGAGATGC	*ABUW_RS01185* R qPCR	This study
OL5375	CTTGCTCCTGAAACTCCTGCTTAC	*ABUW_RS08155* F qPCR	This study
OL5376	GTTGTATCTACGCCACCTGCAATAC	*ABUW_RS08155* R qPCR	This study
OL5373	CCTTAGCAGAGCATCCAGAGTTACA	*ABUW_RS05005* F qPCR	This study
OL5374	AGTCAAACTATGGCGGGCAAATC	*ABUW_RS05005* R qPCR	This study
OL5379	TATCTGTACCAGCGACCGAACA	*ABUW_RS09455* F qPCR	This study
OL5380	GCTGACAACACCAGACGGATAAAG	*ABUW_RS09455* R qPCR	This study
OL5406	TGTCGGTATGGAGCTCGTAGTT	*hslR* F qPCR	This study

OL5407	GGAAGCAATCAACTCCCGTCTT	*hslR* R qPCR	This study
OL6278	AACGGACGACCATCTTTGAG	*adeB* F qPCR	[5]
OL6279	CAGTTGTTCCATTTCACGCA	*adeB* R qPCR	[5]
OL6065	TTCAACTGTTTCAGCCGATG	*adeJ* F qPCR	This study
OL6066	GCGGGTATTCTTACGCTAAC	*adeJ* R qPCR	This study
OL6067	TAGCCTAAGGCACCTCGG	*adeG* F qPCR	This study
OL6068	GGTGCGCTCAACCAGAAATATTC	*adeG* R qPCR	This study
OL6061	GGTGGTAGTCAAACTGAAGGA	*csuAB* F qPCR	[6]
OL6062	GCAACTACATCAGCAGAAGC	*csuAB* R qPCR	[6]
OL6294	ACACGTCCACGTACTGACACACTT	*adeL* F qPCR	[7]
OL6295	GCAACCGCTGCTTCACCGATTTAT	*adeL* R qPCR	[7]
OL6280	GTCCACAAGCGGGATTTATTT	*ABUW_RS06565* F qPCR	This study
OL6281	CATCCATGGCCTGATGAAAC	*ABUW_RS06565* R qPCR	This study

*Underline indicates restriction enzyme site

†F = Forward; R = Reverse; MCS = Multiple Cloning Site

Table S4. RNAseq Fold Changes for Components of the K Locus. Genes were identified by BlastP analysis and the work of Wyers et al. [8]

Locus Tag	Gene	K Locus Region	Function	RNAseq Fold Change*
ABUW_RS 18570	*pgm*	Region 3	phosphomannomutase/phosphoglucomutase	-1.13
ABUW_RS 18575	*gne1*	Region 3	UDP-glucose 4-epimerase GalE	-1.28
ABUW_RS 18580	*gpi*	Region 3	glucose-6-phosphate isomerase	-1.15
ABUW_RS 18585	*ugd*	Region 3	UDP-glucose/GDP-mannose dehydrogenase family protein	-1.23
ABUW_RS 18590	*galU*	Region 3	UTP--glucose-1-phosphate uridylyltransferase GalU	1
ABUW_RS 18595		Region 2	polysaccharide biosynthesis protein	1.14
ABUW_RS 18600		Region 2	DegT/DnrJ/EryC1/StrS aminotransferase family protein	1.17
ABUW_RS 18605		Region 2	acetyltransferase	1.1
ABUW_RS 18610		Region 2	sugar transferase	1.27
ABUW_RS 18615		Region 2	glycosyltransferase family 4 protein	1.34
ABUW_RS 18620		Region 2	oligosaccharide repeat unit polymerase	-1.1
ABUW_RS 18625		Region 2	hypothetical protein	1.02
ABUW_RS 18630		Region 2	oligosaccharide flippase family protein	-1.36
ABUW_RS 18635	*wecC*	Region 2	UDP-N-acetyl-D-mannosamine dehydrogenase	1.27
ABUW_RS 18640	*gne2*	Region 2	UDP-N-acetylglucosamine 2-epimerase (non-hydrolyzing)	1.14
ABUW_RS 18645	*gna*	Region 2	Vi polysaccharide biosynthesis UDP-N-acetylglucosamine C-6 dehydrogenase TviB	-1.1
ABUW_RS 18650	*wza*	Region 1	polysaccharide biosynthesis/export family protein	1.26
ABUW_RS 18655	*wzb*	Region 1	low molecular weight phosphotyrosine protein phosphatase	1.07
ABUW_RS 18660	*wzc*	Region 1	polysaccharide biosynthesis tyrosine autokinase	1.07

*Data is presented as mutant/wild-type.

Supplemental Information References

1. Geisinger, E. and R.R. Isberg, *Antibiotic modulation of capsular exopolysaccharide and virulence in Acinetobacter baumannii.* PLoS Pathog, 2015. **11**(2): p. e1004691.

2. Tomaras, A.P., et al., *Attachment to and biofilm formation on abiotic surfaces by Acinetobacter baumannii: involvement of a novel chaperone-usher pili assembly system.* Microbiology (Reading), 2003. **149**(Pt 12): p. 3473-3484.

3. Nadkarni, M.A., et al., *Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set.* Microbiology (Reading), 2002. **148**(Pt 1): p. 257-266.

4. Fiester, S.E., et al., *Iron-Regulated Phospholipase C Activity Contributes to the Cytolytic Activity and Virulence of Acinetobacter baumannii.* PLoS One, 2016. **11**(11): p. e0167068.

5. Abdi, S.N., et al., *AdeB efflux pump gene knockdown by mRNA mediated peptide nucleic acid in multidrug resistance Acinetobacter baumannii.* Microb Pathog, 2020. **139**: p. 103825.

6. Moon, K.H., B.S. Weber, and M.F. Feldman, *Subinhibitory Concentrations of Trimethoprim and Sulfamethoxazole Prevent Biofilm Formation by Acinetobacter baumannii through Inhibition of Csu Pilus Expression.* Antimicrob Agents Chemother, 2017. **61**(9).

7. Amin, I.M., et al., *A method for generating marker-less gene deletions in multidrug-resistant Acinetobacter baumannii.* BMC Microbiol, 2013. **13**: p. 158.

8. Wyres, K.L., et al., *Identification of Acinetobacter baumannii loci for capsular polysaccharide (KL) and lipooligosaccharide outer core (OCL) synthesis in genome assemblies using curated reference databases compatible with Kaptive.* Microb Genom, 2020. **6**(3).