

SUPPORTING INFORMATION

The role of zinc efflux during *Acinetobacter baumannii* infection

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Table S1. Strains included in the study.

Name	Details	Origin
AB5075_UW	Wild-type	Manoil Lab
<i>czcI</i> ::T26	tnab1_kr130913p10q193	Manoil Lab
<i>czcA</i> ::T26	tnab1_kr130913p05q135	Manoil Lab
<i>czcD</i> ::T26	tnab1_kr130917p09q147	Manoil Lab
<i>czcE</i> ::T26	tnab1_kr121128p05q140	Manoil Lab
<i>czcF</i> ::T26	tnab1_kr121204p05q155	Manoil Lab
ATCC 17978	Wild-type	ATCC
ATCC 17978 + pAT04	IPTG-inducible recombinase plasmid	Davies Lab
17978Δ <i>czcA</i>	Erythromycin ^R	This study

Table S2. Oligonucleotides included in the study.

Name	Forward (5' -> 3')	Reverse (5' -> 3')
qABUW_0265 (<i>czcI</i>)	AATATAGCAGCGGCTTTGC	ATGGTCTTGTAAAGTTAAAGGT
qABUW_0266 (<i>czcC</i>)	AATGCAGTTGCAAGATGTGC	GTTGGAAAAGTTGAGCTTG
qABUW_0267 (<i>czcB</i>)	GAATTCCCGCGAAACAAGAT	TTATTACTCACGATAACCGC
qABUW_0268 (<i>czcA</i>)	GCTGCAACTTACAACGGTGA	TTTGTTTACTAAGCTCGTCC
qABUW_0269 (<i>czcD</i>)	TGCTTGGTTGCCATACAGA	ACACTTGAAATTTCAGGTGG
mABUW_0265 (<i>czcI</i> ::T26)	GTAGCTCGCCTGCTATAGTTG	CAGGCTTGAGATAAGTTACAACC
mABUW_0268 (<i>czcA</i> ::T26)	CTGCTTGAACAATCGCAAGG	CCTTTAACTCTTACAAACAC
mABUW_0269 (<i>czcD</i> ::T26)	GGTGGACATCATGGTCATGATCA TAG	ATGCTGATGTGAATGCGTCTTGT C
mABUW_2851-2 (<i>czcE</i> ::T26)	GGAGTATTGCAATTCACTTCTC	CCAGATGAACATAGCGATCG
mABUW_3664-5 (<i>czcF</i> ::T26)	TACGGTGACAGATGTTGCC	CGGCATCTCATCGGATTATC ctatcaacacactttaagTTAATGTCCATG CTCAGCCTC
17978Δ _{czcA} _UFR	GCCAGATTAGTCGATCAGCA	CGCTAAATACCAGCAAGCTTT
17978Δ _{czcA} _DFR	attnaacgggaggaaataaGCGACCCAAAT GGGTAGTGAA	CCGCCATATTCCAACCAAT TTATTCCTCCGTTAAAT
17978Δ _{czcA} _NOL Ery	TATGGTCTCAAGGAATTCCGC CTTAAGAGTGTGTTGATAG	

Table S3. Zinc-responsive genes (>4-fold).

Locus-tag	Gene Product	Description	Fold change	Fold change (\log_2)	P value (adjusted)
Up-regulated					
ABUW_0010	adeT	RND type efflux pump	4.7	2.2	0.181
ABUW_0073		CobW/P47K family protein	4.2	2.1	0.123
ABUW_0077	hutU	urocanate hydratase	7.0	2.8	0.067
ABUW_0079		proline-specific permease ProY	4.2	2.1	0.118
ABUW_0080	hutI	imidazolonepropionase	4.7	2.2	0.152
ABUW_0183		hypothetical protein	4.0	2.0	0.027
ABUW_0184		Na+/solute symporter	8.9	3.2	0.019
ABUW_0199		hypothetical protein	4.9	2.3	0.207
ABUW_0226		hypothetical protein	4.0	2.0	0.183
ABUW_0255		succinyl-CoA:coenzyme A transferase	4.6	2.2	0.018
ABUW_0265		cation efflux system protein	22.4	4.5	0.000
ABUW_0266	czcC	cation efflux system protein	18.2	4.2	0.000
ABUW_0267	czcB	cation efflux system protein (EsvF1)	9.5	3.2	0.000
ABUW_0268	czcA	cation efflux system protein	11.3	3.5	0.000
ABUW_0299	gltD	glutamate synthase, small subunit	4.3	2.1	0.143
ABUW_0827	pmrC	lipid A phosphoethanolamine transferase	4.8	2.3	0.176
ABUW_0830		hypothetical protein	6.2	2.6	0.217
ABUW_0932		non-ribosomal peptide synthetase	19.3	4.3	0.125
ABUW_0963		glycosyltransferase	4.6	2.2	0.145
ABUW_0964		hypothetical protein	5.3	2.4	0.149
ABUW_1018	cysW	sulfate ABC transporter, permease protein CysW	7.9	3.0	0.145
ABUW_1019	cysT	sulfate ABC transporter, permease protein CysT	11.8	3.6	0.183
ABUW_1020		alpha/beta hydrolase fold protein	13.0	3.7	0.145
ABUW_1021	cysP	thiosulfate-binding protein	8.2	3.0	0.201
ABUW_1095		hypothetical protein	5.1	2.4	0.112
ABUW_1112		transcriptional regulator, AsnC family	5.3	2.4	0.092
ABUW_1113		indole-3-pyruvate decarboxylase	6.6	2.7	0.533
ABUW_1335		efflux transporter, RND family	4.7	2.2	0.115
ABUW_1352		hypothetical protein	5.2	2.4	0.180
ABUW_1418		hypothetical protein	7.5	2.9	0.232
ABUW_1424		hypothetical protein	5.0	2.3	0.236

ABUW_1480		aldo/keto reductase oxidoreductase, short chain dehydrogenase/reductase family	23.8	4.6	0.124
ABUW_1481			11.6	3.5	0.172
ABUW_1593	acnB	aconitate hydratase 2	4.8	2.3	0.066
ABUW_1616		hypothetical protein	5.8	2.5	0.199
ABUW_1624		alcohol dehydrogenase, iron-containing	6.1	2.6	0.148
ABUW_1831	pcaI1	3-oxoadipate CoA-transferase subunit A	6.7	2.7	0.160
ABUW_2084		transcriptional regulator, AraC family	5.3	2.4	0.149
ABUW_2091		transcriptional regulator, AraC family	4.1	2.0	0.146
ABUW_2106	pcaT	metabolite:H ⁺ symporter	9.6	3.3	0.155
ABUW_2113		succinate dehydrogenase	4.8	2.3	0.123
ABUW_2114		transcriptional regulator, GntR family	5.5	2.5	0.176
ABUW_2130	acoB	acetoin:2,6-dichlorophenolindophenol oxidoreductase subunit beta	5.1	2.3	0.128
ABUW_2131	acoA	acetoin:2,6-dichlorophenolindophenol oxidoreductase alpha subunit	5.0	2.3	0.020
ABUW_2201	iscR	iscRSUA operon repressor	4.3	2.1	0.361
ABUW_2308		hypothetical protein	37.0	5.2	0.091
ABUW_2309		hypothetical protein	82.6	6.4	0.042
ABUW_2336		putative Acyl-CoA dehydrogenase	4.9	2.3	0.088
ABUW_2345		hypothetical protein	5.3	2.4	0.175
ABUW_2365	ahpF1	alkyl hydroperoxide reductase, F subunit	10.3	3.4	0.181
ABUW_2367		chromate transporter	4.0	2.0	0.149
ABUW_2370	arsR1	transcriptional regulator, ArsR family	15.8	4.0	0.141
ABUW_2371	arsC1	arsenate reductase	4.4	2.1	0.232
ABUW_2378	hyu	Asp/Glu racemase	5.6	2.5	0.125
ABUW_2379	tauD	taurine dioxygenase	5.4	2.4	0.168
ABUW_2380	tauC	taurine transport system permease protein	6.1	2.6	0.172
ABUW_2381	tauB	taurine import ATP-binding protein	7.6	2.9	0.132
ABUW_2382	tauA	taurine ABC transporter, periplasmic binding protein	5.8	2.5	0.107
ABUW_2400	mdcC	malonate decarboxylase acyl carrier protein	13.7	3.8	0.293
ABUW_2415	srpH	serine acetyltransferase	4.5	2.2	0.341
ABUW_2416		hypothetical protein	4.0	2.0	0.360

		glutamate/aspartate ABC transporter, ATP-binding protein	8.1	3.0	0.174
ABUW_2422		ArtM protein	15.5	4.0	0.149
ABUW_2423		ABC-type amino acid transport system	4.3	2.1	0.125
ABUW_2424		hypothetical protein	4.4	2.1	0.037
ABUW_2448		oxidoreductase	6.4	2.7	0.003
ABUW_2458		paaB	6.4	2.7	0.005
ABUW_2529	paaB	enoyl-CoA hydratase	6.4	2.7	0.005
ABUW_2530	cauD	enoyl-coA hydratase phenylacetate-CoA oxygenase/reductase, PaaK subunit	6.9	2.8	0.005
ABUW_2531	paaK	phenylacetate-CoA oxygenase, PaaJ subunit	5.0	2.3	0.020
ABUW_2532	paaJ	phenylacetate-CoA oxygenase, PaaI subunit	4.8	2.2	0.024
ABUW_2533	paaI2	phenylacetate-CoA oxygenase, PaaH subunit	4.1	2.0	0.364
ABUW_2534	paaH	phenylacetate-CoA oxygenase, PaaN phenylacetic acid degradation protein PaaN	4.3	2.1	0.281
ABUW_2536	paaN	hypothetical protein	4.6	2.2	0.019
ABUW_2588		putative membrane protein	5.6	2.5	0.135
ABUW_2744		feruloyl-CoA synthetase	5.5	2.5	0.024
ABUW_2780	hcaC	long-chain specific acyl-coa dehydrogenase	4.2	2.1	0.027
ABUW_2781	hcaD	porin	5.8	2.5	0.115
ABUW_2782	hcaE	hypothetical protein	8.9	3.2	0.144
ABUW_2783		hypothetical protein	5.0	2.3	0.113
ABUW_2784		arginine N-succinyltransferase	6.3	2.7	0.149
ABUW_2799	astA2	succinylornithine transaminase	5.3	2.4	0.149
ABUW_2800		Co/Zn/Cd efflux system	4.6	2.2	0.157
ABUW_2851		citN	50.0	5.6	0.067
ABUW_2892		citrate transporter	5.6	2.5	0.149
ABUW_2944		oxidoreductase, short chain dehydrogenase/reductase family	4.1	2.0	0.042
ABUW_2951		rieske (2Fe-2S) protein	4.9	2.3	0.144
ABUW_2952		hypothetical protein	13.5	3.8	0.159
ABUW_2953		rieske (2Fe-2S) protein	23.3	4.5	0.125
ABUW_2971	betB	betaaine aldehyde dehydrogenase	4.2	2.1	0.145
ABUW_2972	betA	choline dehydrogenase	4.6	2.2	0.162
ABUW_3176	nuoB	NADH dehydrogenase I chain B	4.3	2.1	0.151
ABUW_3397	fumA	fumarate hydratase	7.4	2.9	0.107
ABUW_3477		hypothetical protein	4.4	2.1	0.098
ABUW_3478	rne	ribonuclease E	4.2	2.1	0.221
ABUW_3661		ISPpu12 transposase	14.5	3.9	0.149

ABUW_3663	lspA1	ISPPu12 lipoprotein signal peptidase	25.9	4.7	0.131
ABUW_3664	cadA	heavy metal detoxification protein	19.6	4.3	0.144
ABUW_3665	cadR	transcriptional regulator-like protein	6.7	2.7	0.159
ABUW_3667	arsC2	arsenate reductase	21.3	4.4	0.138
ABUW_3668	arsR2	arsenical resistance operon repressor	8.5	3.1	0.240
ABUW_3669	arsC	arsenate reductase	5.2	2.4	0.321
ABUW_3785	cycA1	D-serine/D-alanine/glycine transporter	6.0	2.6	0.203
ABUW_3786	cycA2	D-serine/D-alanine/glycine transporter	11.1	3.5	0.126
ABUW_3788	dadX	alanine racemase	6.5	2.7	0.135
ABUW_3790	lrp	leucine-responsive regulatory protein	4.4	2.1	0.266
ABUW_3797		hypothetical protein	11.3	3.5	0.048
ABUW_3806	acnD	2-methylisocitrate dehydratase, Fe/S-dependent	7.9	3.0	0.201
ABUW_3807	prpC	2-methylcitrate synthase	5.2	2.4	0.261
ABUW_3808	prpB	methylisocitrate lyase	5.5	2.5	0.272
ABUW_3809		transcriptional regulator, GntR family	9.4	3.2	0.215
ABUW_3812	lldD	L-lactate dehydrogenase (cytochrome)	4.5	2.2	0.175
ABUW_3813	lldR	L-lactate utilization transcriptional repressor	4.3	2.1	0.232
ABUW_3814	lldP	L-lactate permease	8.1	3.0	0.176
ABUW_3853	ssuA1	aliphatic sulfonates-binding protein	7.1	2.8	0.149
ABUW_3854	ssuA2	aliphatic sulfonates-binding protein	8.9	3.2	0.149
ABUW_3855	ssuD	alkanesulfonate monooxygenase	7.8	3.0	0.201
ABUW_3856	ssuC	aliphatic sulfonates transport permease protein SsuC	6.8	2.8	0.280

Down-regulated

ABUW_0143		TonB-dependent receptor protein	-4.88	-2.3	0.145
ABUW_0263		hypothetical protein	-4.47	-2.2	0.311
ABUW_0304		type IV pilin structural subunit	-4.48	-2.2	0.255
ABUW_0563		beta-lactamase OXA-23	-5.22	-2.4	0.088
ABUW_0786		hypothetical protein	-5.50	-2.5	0.149
ABUW_1076		hypothetical protein	-5.18	-2.4	0.043
ABUW_1168	bauF	siderophore-interacting protein	-6.89	-2.8	0.006
ABUW_1169	basA	non-ribosomal peptide synthetase	-7.78	-3.0	0.032

ABUW_1170	basB	non-ribosomal peptide synthetase	-4.15	-2.1	0.061
ABUW_1171		hypothetical protein	-8.31	-3.1	0.020
ABUW_1172		hypothetical protein	-6.82	-2.8	0.003
ABUW_1173	bauD	ferric acinetobactin transport system permease	-7.50	-2.9	0.018
ABUW_1174	bauC	ferric acinetobactin transport system permease	-4.19	-2.1	0.073
ABUW_1175	bauE	ferric acinetobactin transport system ATP-binding protein	-4.62	-2.2	0.018
ABUW_1176	bauB	ferric acinetobactin transport system periplasmic binding protein	-6.18	-2.6	0.013
ABUW_1177	bauA	ferric acinetobactin receptor	-7.02	-2.8	0.014
ABUW_1178	basC	nonribosomal peptide synthetase BasC	-6.00	-2.6	0.019
ABUW_1179	basD	nonribosomal peptide synthetase BasD	-5.54	-2.5	0.027
ABUW_1180	basE	2,3-dihydroxybenzoate-AMP ligase	-8.78	-3.1	0.019
ABUW_1181	basF	2,3 dihydro-2,3 dihydroxybenzoate synthase	-9.69	-3.3	0.018
ABUW_1182	basG	histidine decarboxylase	-7.55	-2.9	0.013
ABUW_1183		hypothetical protein	-10.75	-3.4	0.022
ABUW_1184		ABC transporter, ATP-binding protein	-8.88	-3.2	0.032
ABUW_1185		ABC transporter	-6.50	-2.7	0.035
ABUW_1186	basH	thioesterase	-6.51	-2.7	0.005
ABUW_1187	basI	phosphopantetheinyl transferase	-4.37	-2.1	0.013
ABUW_1188	basJ	isochorismate synthetase	-10.21	-3.4	0.013
ABUW_1397		hypothetical protein	-4.86	-2.3	0.062
ABUW_1597		hypothetical protein	-5.29	-2.4	0.221
ABUW_1598		hypothetical protein	-15.08	-3.9	0.035
ABUW_1655		TonB-dependent siderophore receptor	-6.91	-2.8	0.207
ABUW_1816	aro1	3-deoxy-7-phosphoheptulonate synthase	-4.28	-2.1	0.024
ABUW_1844		hypothetical protein	-4.43	-2.1	0.017
ABUW_1919		hypothetical protein	-11.65	-3.5	0.092
ABUW_1920		hypothetical protein	-4.55	-2.2	0.145
ABUW_2074		transcriptional regulator, fur family	-4.03	-2.0	0.027
ABUW_2075		isochorismatase	-6.96	-2.8	0.012
ABUW_2076		2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase	-11.68	-3.5	0.024
ABUW_2154		hypothetical protein	-5.61	-2.5	0.202
ABUW_2155		hypothetical protein	-7.67	-2.9	0.138

		outer-membrane receptor for Fe(III)-coprogen,Fe(III)-ferrioxamine B and Fe(III)-rhodotulic acid	-4.53	-2.2	0.599
ABUW_2165		hypothetical protein	-5.67	-2.5	0.611
ABUW_2166		hypothetical protein	-5.62	-2.5	0.460
ABUW_2168		rhizobactin siderophore biosynthesis protein RhbD	-12.71	-3.7	0.029
ABUW_2178		hypothetical protein	-4.73	-2.2	0.148
ABUW_2181		TonB-dependent receptor	-4.80	-2.3	0.138
ABUW_2182		dimethylmenaquinone methyltransferase	-4.07	-2.0	0.146
ABUW_2183		hypothetical protein	-4.49	-2.2	0.018
ABUW_2349		hypothetical protein	-7.97	-3.0	0.251
ABUW_2627		hypothetical protein	-4.55	-2.2	0.067
ABUW_2868		TonB dependent outer membrane siderophore receptor protein	-8.81	-3.1	0.092
ABUW_2916	pfeA	heme oxygenase	-6.28	-2.7	0.521
ABUW_2981		TonB family protein	-6.02	-2.6	0.570
ABUW_2982		hypothetical protein	-7.08	-2.8	0.529
ABUW_2983		hypothetical protein	-6.70	-2.7	0.410
ABUW_2984		hypothetical protein	-4.90	-2.3	0.644
ABUW_2985		putative transmembrane sensor protein FecR	-4.20	-2.1	0.256
ABUW_2986		RNA polymerase sigma factor FecI	-7.73	-2.9	0.191
ABUW_2987		NAD(P) transhydrogenase subunit beta	-26.78	-4.7	0.113
ABUW_3312	pntB	NAD(P) transhydrogenase subunit alpha	-23.73	-4.6	0.107
ABUW_3313	pntA2	NAD(P) transhydrogenase subunit alpha	-26.33	-4.7	0.115
ABUW_3314	pntA1	putative transcriptional regulator	-4.51	-2.2	0.293
ABUW_3352		TonB protein	-4.44	-2.1	0.113
ABUW_3426		thiamine biosynthesis protein ThiC	-5.12	-2.4	0.115
ABUW_3624	thiC	hypothetical protein	-4.36	-2.1	0.308
ABUW_3684					

Table S4. Murine metal concentrations.

		Manganese		Iron		Copper	
		Mean (μ M) ^{a,b}	S.E.M. (μ M)	Mean (μ M)	S.E.M. (μ M)	Mean (μ M) ^a	S.E.M. (μ M)
Serum	Zinc replete	b.d.		25.82	4.08	18.32	0.80
	Zinc deficient	b.d.		70.12	14.54	17.18	0.78
Lung	Zinc replete	0.50	0.01	135.70	17.49	b.d.	
	Zinc deficient	0.40	0.03	134.20	17.16	b.d.	
Nasopharynx	Zinc replete	0.57	0.07	79.58	5.35	b.d.	
	Zinc deficient	0.45	0.03	83.95	2.13	b.d.	
Spleen	Zinc replete	0.39	0.03	259.50	14.17	b.d.	
	Zinc deficient	0.25	0.02	255.00	16.95	b.d.	
BAL	Zinc replete	0.03	0.00	6.43	0.98	0.61	0.09
	Zinc deficient	0.03	0.00	13.45	2.04	0.89	0.07

^a b.d. = below detection.

^b Numbers in bold are statistically significantly different between zinc deficient and zinc replete (Student *t*-test; P < 0.05).

LPRSAIFMSVLFC_{LL}IFQSLWNIAAAFCA**H**ENQE_{KAL}**HHFGHHA**
ALNVYQTSS**H**THVE**H**TETLDAAH**K**APLN_LQD**HH**D**H**LPTCF**H**VVM
TEVAKQAQTPV**L****H**V**H**ELSQIY**H**WSNSYQSP**H**LNALKPPPVL**T**PL

Figure S1. Bioinformatic analysis of *A. baumannii* CzcI. The primary sequence of CzcI (ABUW_0265) includes a putative signal peptide for periplasmic translocation (in dark green; determined using SignalP-5.0) and 17 histidine residues (in red) with a putative role in zinc binding.

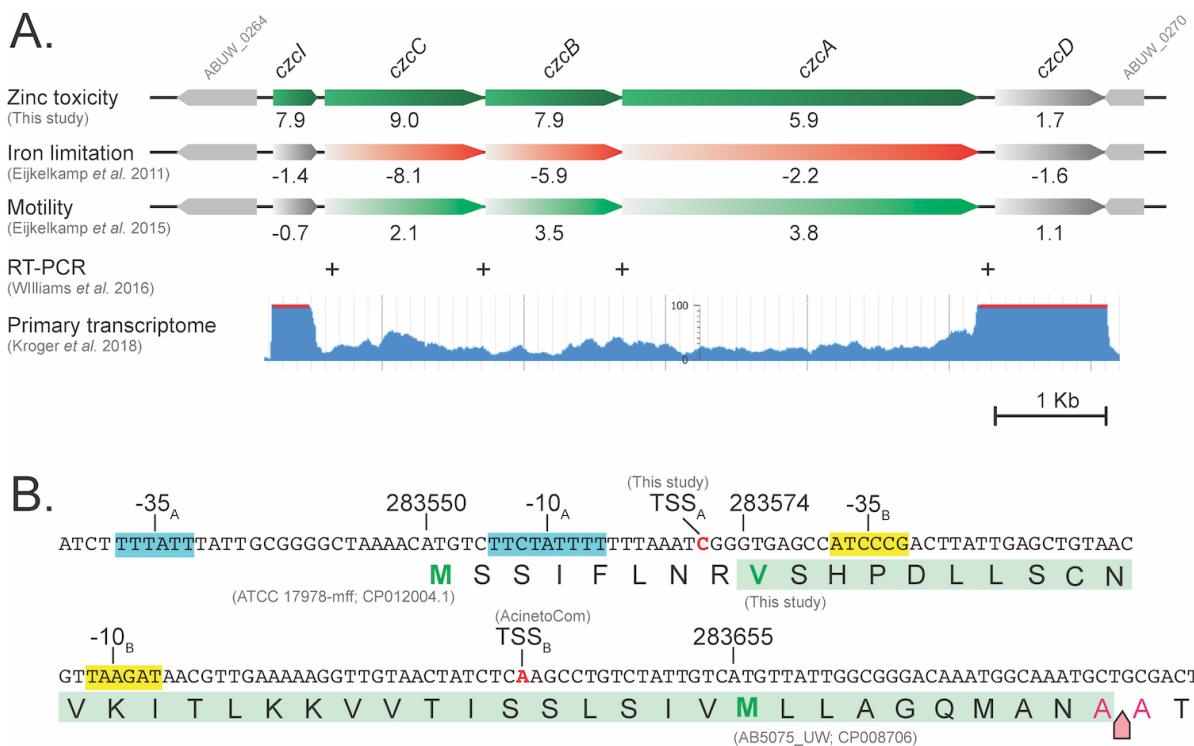


Figure S2. Genetic analysis of the *czcICBAD* cluster. (A) The transcriptional properties of the *czcICBAD* cluster indicates the presence of multiple promotor. Previous transcriptional responses to iron starvation ³ and transcription of motile vs non-motile cells ⁴ were included, with the times-fold difference per genes indicated below the relevant ORF. The transcriptional links between ORFs were previously determined by Williams *et al.* are indicated by “+” underneath the results from the motility transcriptome ⁵. The relevant section of the primary transcriptome of *A. baumannii* strain ATCC 17978-mff has been included (AcinetoCom) ⁶. **(B)** Genetic elements the *czcC* regulatory region. The 5'-end of *czcC* and its regulatory region from *A. baumannii* strain AB5075_UW (CP008706) are displayed. Two distinct -10/-35 sites are highlighted, A (in teal) and B (in yellow), with the conserved nucleotides across the *A. baumannii* transcriptome ⁶ underlined. Two distinct transcription start sites (TSS_A and TSS_B) were identified (in red). The derived amino acid sequence harbours three alternative start sites; methionine (M) as predicted in ATCC 17978-mff, methionine (M) as predicted in AB5075_UW and valine (V) as predicted in this study, based on the -10_A/−35_A/TSS_A sequences

and the signal peptide prediction (in green shading). The predicted cleavage site is indicated by the pink arrow in between two alanine (A) residues.

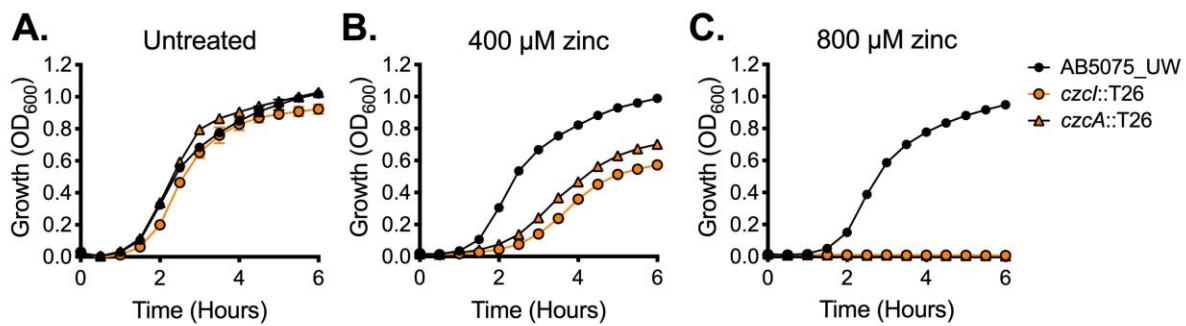


Figure S3. *czcI* mutation leads to hyper susceptibility to zinc stress. Growth of *A. baumannii* AB5075_UW and *czcI*::T26 mutant cells as determined by measuring the optical density at 600 nm (OD₆₀₀) under (A) untreated conditions or following supplementation with (B) 400 μ M zinc or (C) 800 μ M zinc. For all panels, the data represent the mean of at least biological triplicates (\pm SEM). The visibility of error bars may be occluded by the symbols.

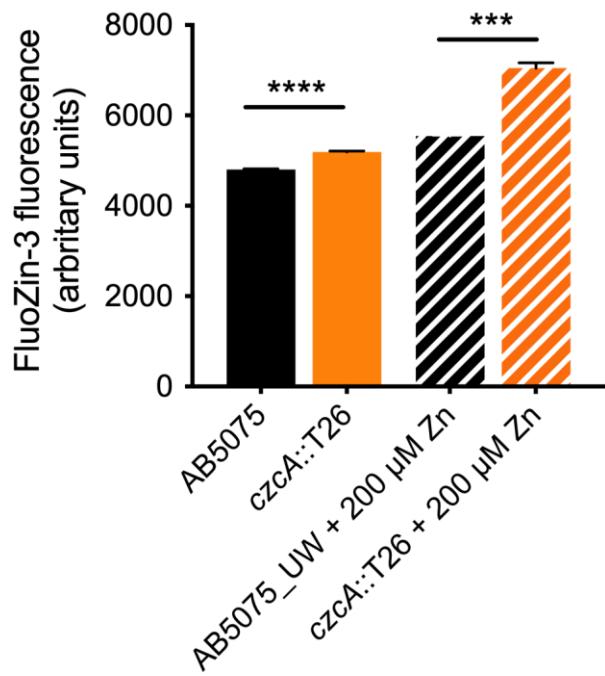


Figure S4. Cellular zinc analysis using FluoZin-3 AM labelling. The accumulation of zinc in the AB5075_UW wild-type or *czcA*::T26 mutant cells with or without 200 μ M zinc supplementation, as determined by FluoZin-3 AM labelling and analysis by flow cytometry.