SUPPORTING INFORMATION

The UDP-GalNacA biosynthesis genes *gna-gne2* are required to maintain cell envelope integrity and *in vivo* fitness in multi-drug resistant *Acinetobacter baumannii*

Short title: Contribution of UDP-GalNacA to pathogenesis of *A. baumannii*

Sébastien Crépin, Elizabeth N. Ottosen, Courtney E. Chandler, Anna Sintsova, Robert K. Ernst and Harry L.T. Mobley

CONTENTS

Table S1. *A. baumannii* strain AB0057 Gna and Gne2 closest homologs.

Table. S2. Genes belonging to the KL4 locus and their associated.

Table S3. Strains and plasmids used in this study.

Table S4. Primers and oligonucleotides.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Colonization of the bloodstream by the Δ*gna* and Δ*gne2* mutants.

Colonization of the spleen, liver and kidneys was determined by inoculating CBA/J mice via tail vein injection with 10^7 CFU of either the WT strain (57), the Δ*gna* mutant **(A)** or the Δ*gne2* mutant **(B)**. At 24 hpi, mice were sacrificed, organs were harvested, and the bacterial burden was determined by CFU enumeration on LB agar (57, Δ*gna* and Δ*gne2*) and LB-Km agar (57 eV, Δ*gna* eV, Δ*gna* compl., Δ*gne2* eV and Δ*gne2* compl.). Bacterial numbers are presented as the Log_{10} CFU g^{-1} of tissue. Each data point represents a sample from an individual mouse, and horizontal bars indicate the median values. Statistical significance was calculated by the Mann-Whitney test (*, P<0.05; ****, P<0.0001; NS, not significant). Abbreviations: 57: WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gna* and pABBR_Km-*gne2*).

Fig. S2. Growth curves in LB and M9 minimal media.

(A) Growth of the WT (57) and its derivative strains in LB broth. **(B)** Growth of the strains in M9 minimal medium supplemented with 0.4% glucose and 0.2% casamino acids. All results are the mean values and standard deviations of three independent experiments. For ease of reading, standard deviations were removed from graphs. The quantification of growth, or growth potential, and comparison between strains was performed by calculating the area under the curve of each

growth curve. Abbreviation: 57: WT; compl.: complemented (pABBR_Km-*gna*, pABBR_Km-*gne2* and pABBR_Km-*a/e2*).

Fig. S3. Resistance to normal human serum.

Survival in 90% normal human serum (NHS) and growth in 90% heat-inactivated human serum (HI). A total of 10^7 CFU ml^{-1} of the WT (57) and either the Δ*gna* **(A)** or the Δ*gne2* **(B)** strains was incubated in either 90% NHS and HI, and the number of CFUs was quantified by CFU enumeration on LB agar every hour. Since no CFUs were recovered from strains Δ*gna,* Δ*gna* eV, Δ*gne2* and Δ*gne2* eV incubated with 90% NHS, no statistical tests were performed for these groups. The dashed line corresponds to the limit of detection. All results are the mean values and standard deviations of three independent experiments. Statistical significance was calculated by the Student's *t*-test (B-C, HI) (NS, not significant). Abbreviations: 57: WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gna* and pABBR_Km-*gne2*).

Fig. S4. Capsule production depends on the *gna-gne2* (*a/e2*) locus

(A) Microscopic analysis of capsule production. Maneval's staining of bacteria cultured for 24 h on LB agar plates. Images are representative of three independen experiments. **(B)** Analysis of the polysaccharides from lysates of bacteria cultured for 24 h on LB agar plates by SDS-PAGE and stained with 0.1% Alcian blue (section of the gel corresponding to the capsular polysaccharides). The strain AB5075 Δ*wzc* was used as a negative control. **(C)** Pellet of the strains following centrifugation of the overnight cultures normalized to an OD_{600} of 1.0. The 57, AB5075 and the complemented strains present a thick and loose pellet, suggesting capsule production, while all the mutant strains show a tight and sticky pellet, which is indicative of non-capsulated strains. **(D)** Colony morphology of the strains cultured on BHI Congo red plates for 48 h at 37°C. **(E-F)** Autoaggregation assay. Cultures of WT (57) and *gna* (E) and *gne2* (F) were standardized in 10 ml of LB to an OD_{600} of 2.0 in a culture tube and incubated statically at 37°C for 4 h. A 200-µl sample was taken 1 cm below the surface at time 0 and 4 h post-inoculation for

OD_{600} measurement. The percent autoaggregation was determined by dividing the OD_{600} value of the aggregated cells by the OD_{600} value of a control growth tube. The MDR strain AB5075 and its isogenic *wzc* mutant were used as controls (B-D). All results are the mean values and standard deviations of three independent experiments. Images are representative of three independent experiments (A-D). Statistical significance was calculated by the one-way ANOVA with Tukey's multiple comparisons test (D and E) (**, P <0.01; ***, P <0.005; NS, Not significant). Abbreviations: 57: WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gna*, pABBR_Km-*gne2* and pABBR_Km-*a/e2*).

Fig. S5. The *gna-gne2* (*a/e2*) locus is important for twitching motility

(A) Twitching motility of the bacterial strains cultured to an OD_{600} 2.0 and an aliquot was stabbed to the bottom of a 1% EIKEN agar plate, then incubated at 37°C for 18 h. Twitching motility images are representative of three independent experiments. The red bars mark the twitching motility diameter. The AB5075 Δ*pilT* mutant was used as negative control. Images are representative of three independent experiments. **(B, C, D)** Quantification of the twitching diameter (in mm) from three independent experiments. The MDR strain AB5075 and its isogenic *wzc* and *pilT* mutants were used as controls (A-D). All results are the mean values and standard deviations of three independent experiments. Statistical significance was calculated by the one-way ANOVA with Tukey's multiple comparisons test (B, C, D) (*, P <0.05; ***, P <0.005; ****, P <0.001; NS, Not significant). Abbreviations: 57: WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gna* and pABBR_Km-*gne2*).

Fig. S6. The *gna-gne2* (*a/e2*) locus is important maintain the cell envelope integrity

(A, B) Minimal inhibitory concentration (MIC) of vancomycin on the WT (57) and its derivative strains. MIC values were determined by E-test. **(C)** Growth of the strains on MacConkey plates. Strains were cultured to an OD_{600} of 0.6 and normalized to 10^7 CFU ml^{-1} and serial dilutions were spotted on MacConkey plates. Images are representative of three independent experiments. **(D)** Colony morphology of the strains cultured on LB agar supplemented with BCIP-Toluidine (XP), a substrate of the periplasmic alkaline phosphatase PhoA. Images are representative of three

independent experiments. **(E, F)** Survival in the presence of polymyxin B (1 µg ml^{-1}). The number of surviving CFUs was quantified by CFU enumeration of LB agar at 15-, 30- and 60 mins p.i. The MDR strain AB5075 and its isogenic *wzc* mutant were used as controls (C,D). All results are the mean values and standard deviations of three independent experiments. Statistical significance was calculated by the one-way ANOVA with Tukey's multiple comparisons test (A) (*, *P* <0.05; **, *P* <0.01; ***, *P* <0.005; NS, Not significant). Abbreviations: 57: WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gna* and pABBR_Km-*gne2*).

Fig. S7. Lipooligosaccharide biosynthesis is influence by the *gna-gne2* (*a/e2*) locus.
Analysis of LOS extracted from of bacterial cultures cultured to mid-log phase and separated by SDS-PAGE, and stained with Pro-Q emerald 300 Lipopolysaccharide gel stain kit. The *E. coli* MG1655 (rough LPS) was used as a positive control. Images are representative of three independent experiments. Abbreviations: 57: WT; compl.: complemented (pABBR_Km-*a/e2*).

Fig. S8. Cell envelope hydrophobicity and the envelope stress response (ESR) are controlled by the *gna-gne2* (*a/e2*) locus.
(A-C) Bacterial adherence to hydrocarbons. Approximately 10^7 CFU ml^{-1} were incubated with either 25% *n-octane* (A; Δ*a/e2* mutant) or hexadecane (B; Δ*gna* and C; Δ*gne2*) and the number of CFUs from the aqueous phase were recovered at 15 mins post-inoculation. An increase in the cell envelope hydrophobicity is represented by a lower number of CFUs recovered from the aqueous phase. **(D-E)** Expression of genes involved in the envelope stress response (ESR, *dsbA*, *degP*, *baeR* and *rstA*) between WT, Δ*gna* (F), Δ*gne2* (G), and their respective complemented strain. Gene expression was evaluated by qRT-PCR and compared between the WT, the mutants and the complemented strain. The dashed line corresponds to the cutoff for a significant difference in expression. All results are the mean values and standard deviations of three independent experiments. Statistical significance was calculated by the one-way ANOVA with Tukey's multiple comparisons test (A-C) and by the Student's *t*-test (D,E) (*, *P*<0.05; **, *P*<0.01; ****, *P*<0.0001; NS, not significant). Abbreviations: 57, WT; eV, empty vector (pABBR_Km);

compl.: complemented (pABBR_Km-*a/e2*, pABBR_Km-*gna* and pABBR_Km-*gne2*).

Fig. S9. Resistance to antibiotics is influenced by the *gna*.

(A) Minimal inhibitory concentration (MIC) of amoxicillin, gentamicin and imipenem, on WT (57) and its derivative strains. MIC values were determined by the E-test method. **(B)** Percent survival in the presence of a defined concentration of antibiotic. A total of 10^7 CFU ml^{-1} were incubated in the presence of different antibiotics and the percent survival was determined by dividing the number of CFUs recovered from the antibiotic challenge by the number of CFUs recovered from the corresponding untreated sample. All results are the mean values and standard deviations of three independent experiments. Statistical significance was calculated by the one-way ANOVA with Tukey's multiple comparisons test (*, P<0.05; **, P<0.01; ***, P<0.005; ****, P<0.0001; NS, not significant). Abbreviations: 57, WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gna*).

Fig. S10. Resistance to antibiotics is influenced by the *gne2*.

(A) Minimal inhibitory concentration (MIC) of amoxicillin, gentamicin and imipenem, on WT (57) and its derivative strains. MIC values were determined by the E-test method. **(B)** Percent survival in the presence of a defined concentration of antibiotic. A total of 10^7 CFU ml^{-1} were incubated in the presence of different antibiotics and the percent survival was determined by dividing the number of CFUs recovered from the antibiotic challenge by the number of CFUs recovered from the corresponding untreated sample. All results are the mean values and standard deviations of three independent experiments. Statistical significance was calculated by the one-way ANOVA with Tukey's multiple comparisons test (*, P<0.05; **, P<0.01; ***, P<0.005; ****, P<0.0001; NS, not significant). Abbreviations: 57, WT; eV: empty vector (pABBR_Km); compl.: complemented (pABBR_Km-*gne2*).

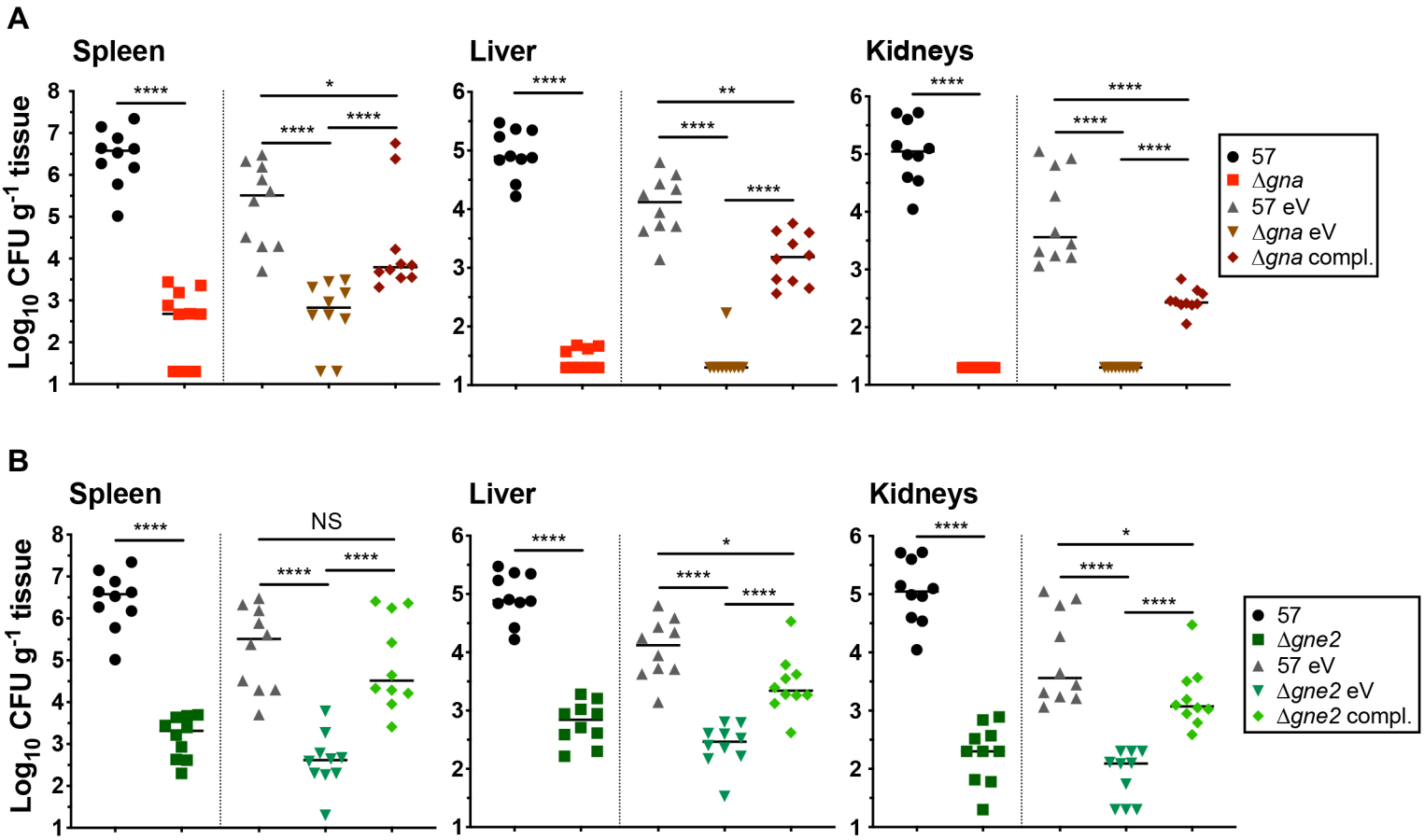
A
Spleen
Liver
Kidneys
Log10 CFU g-1 tissue
57
Δgna
57 eV
Δgna eV
Δgna compl.
B
Spleen
Liver
Kidneys
NS
Log10 CFU g-1 tissue
57
Δgne2
57 eV
Δgne2 eV
Δgne2 compl.

Fig. S1.

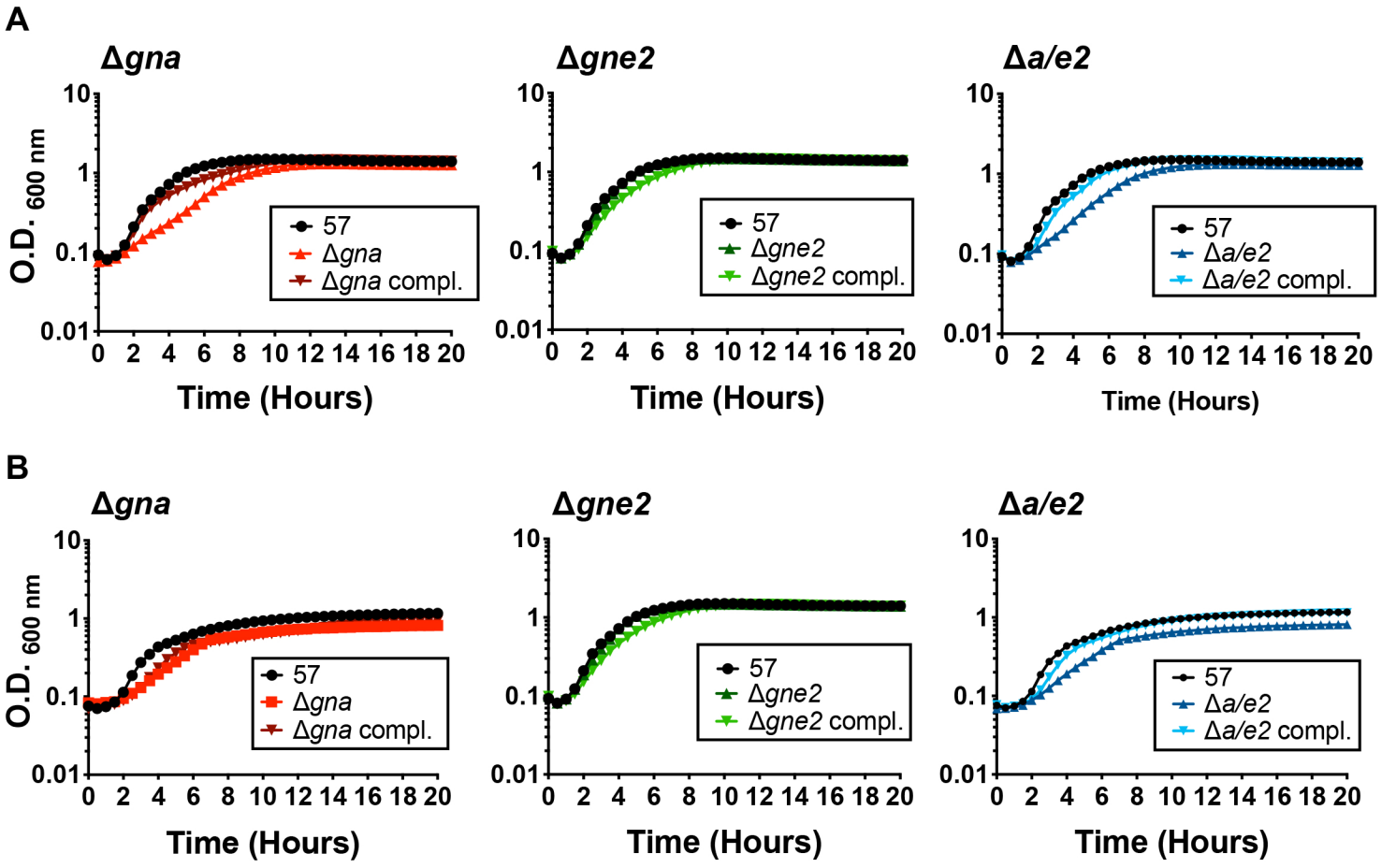
A
Δgna
Δgne2
Δa/e2
O.D. 600 nm
10
1
0.1
0.01
0 2 4 6 8 10 12 14 16 18 20
Time (Hours)
57
Δgna
Δgna compl.
Δgne2
Δgne2 compl.
Δa/e2
Δa/e2 compl.
B
Δgna
Δgne2
Δa/e2
O.D. 600 nm
Time (Hours)
57
Δgna
Δgna compl.
Δgne2
Δgne2 compl.
Δa/e2
Δa/e2 compl.

Fig. S2.

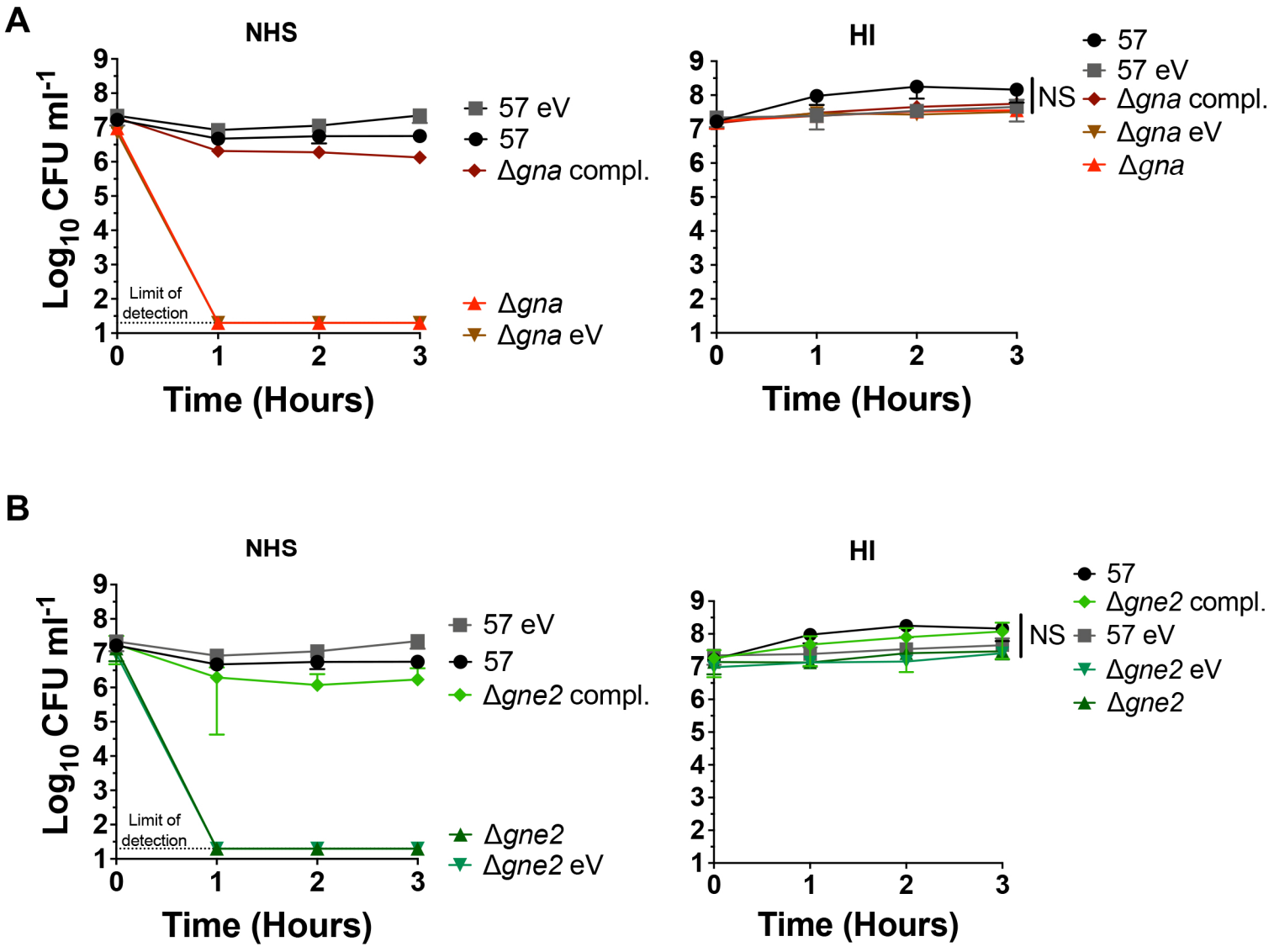
A
NHS
HI
Log10 CFU ml-1
Time (Hours)
Limit of detection
57 eV
57
Δgna compl.
Δgna
Δgna eV
NS
B
Δgne2 compl.
Δgne2
Δgne2 eV

Fig. S3.

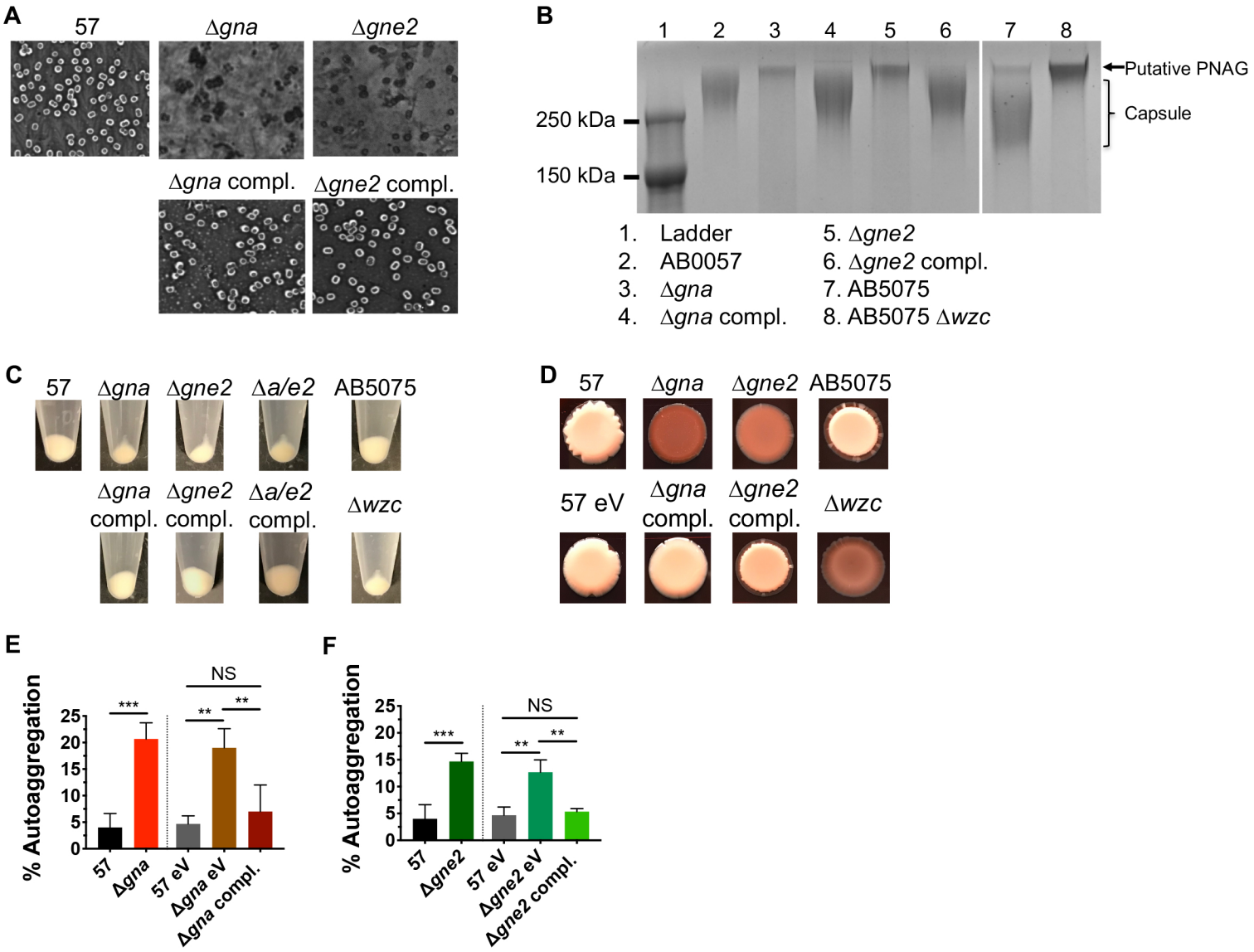
A
57
Δgna
Δgne2
Δgna compl.
Δgne2 compl.
B
1 2 3 4 5 6 7 8
250 kDa
150 kDa
Putative PNAG
Capsule
1. Ladder
2. AB0057
3. Δgna
4. Δgna compl.
5. Δgne2
6. Δgne2 compl.
7. AB5075
8. AB5075 Δwzc
C
57
Δgna
Δgne2
Δa/e2
AB5075
Δgna compl.
Δgne2 compl.
Δa/e2 compl.
Δwzc
D
57
Δgna
Δgne2
AB5075
57 eV
Δgna compl.
Δgne2 compl.
Δwzc
E
% Autoaggregation
NS
***
**
**
57
Δgna
57 eV
Δgna eV
Δgna compl.
F
% Autoaggregation
NS
***
**
**
57
Δgne2
57 eV
Δgne2 eV
Δgne2 compl.

Fig. S4.

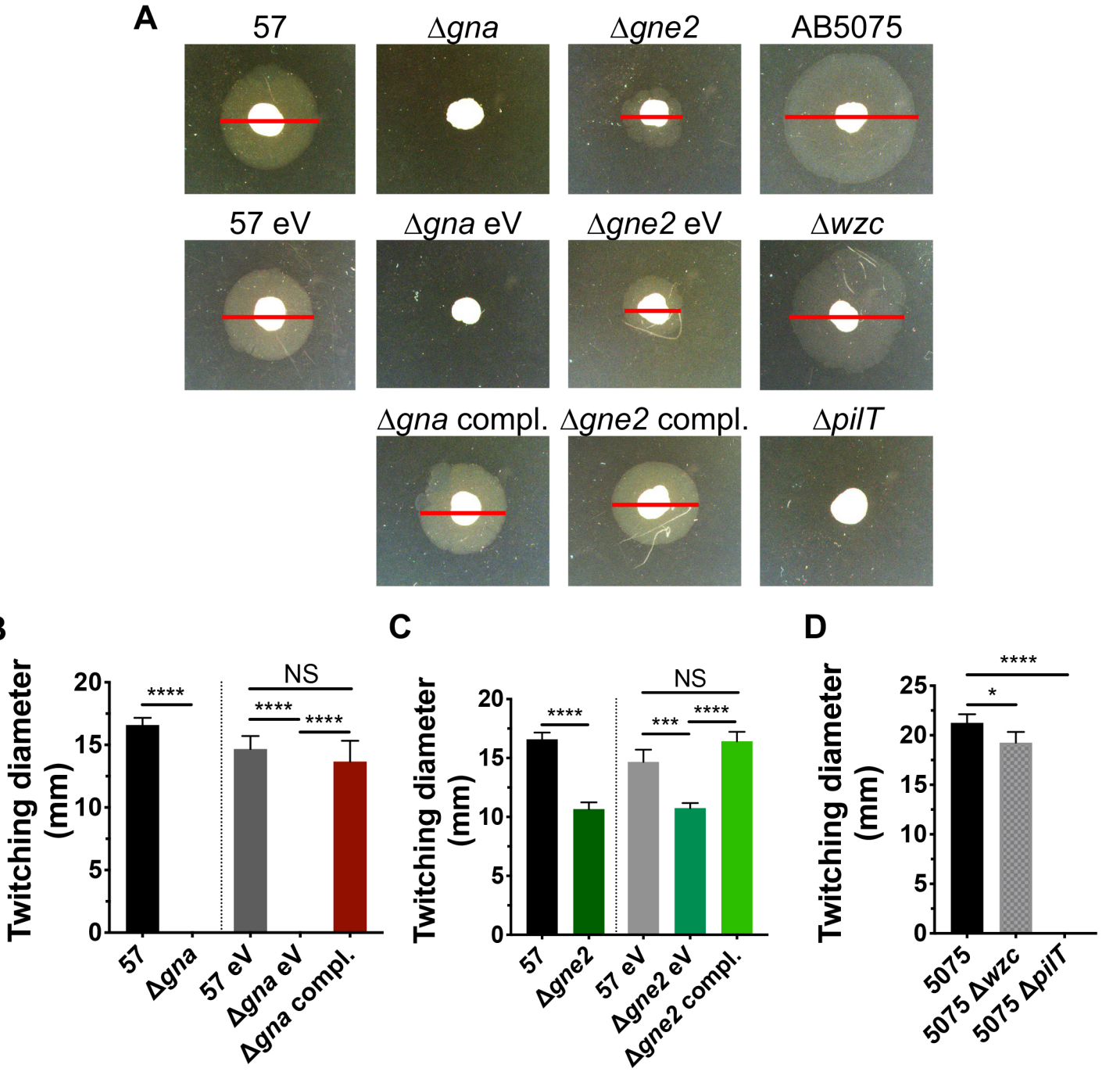
A
57
Δgna
Δgne2
AB5075
57 eV
Δgna eV
Δgne2 eV
Δwzc
Δgna compl.
Δgne2 compl.
ΔpilT
B
Twitching diameter (mm)
20
15
10
5
0
****
NS
****
****
57
Δgna
57 eV
Δgna eV
Δgna compl.
C
Twitching diameter (mm)
20
15
10
5
0
****
NS
***
****
57
Δgne2
57 eV
Δgne2 eV
Δgne2 compl.
D
Twitching diameter (mm)
25
20
15
10
5
0
****
*
5075
5075 Δwzc
5075 ΔpilT

Fig. S5.

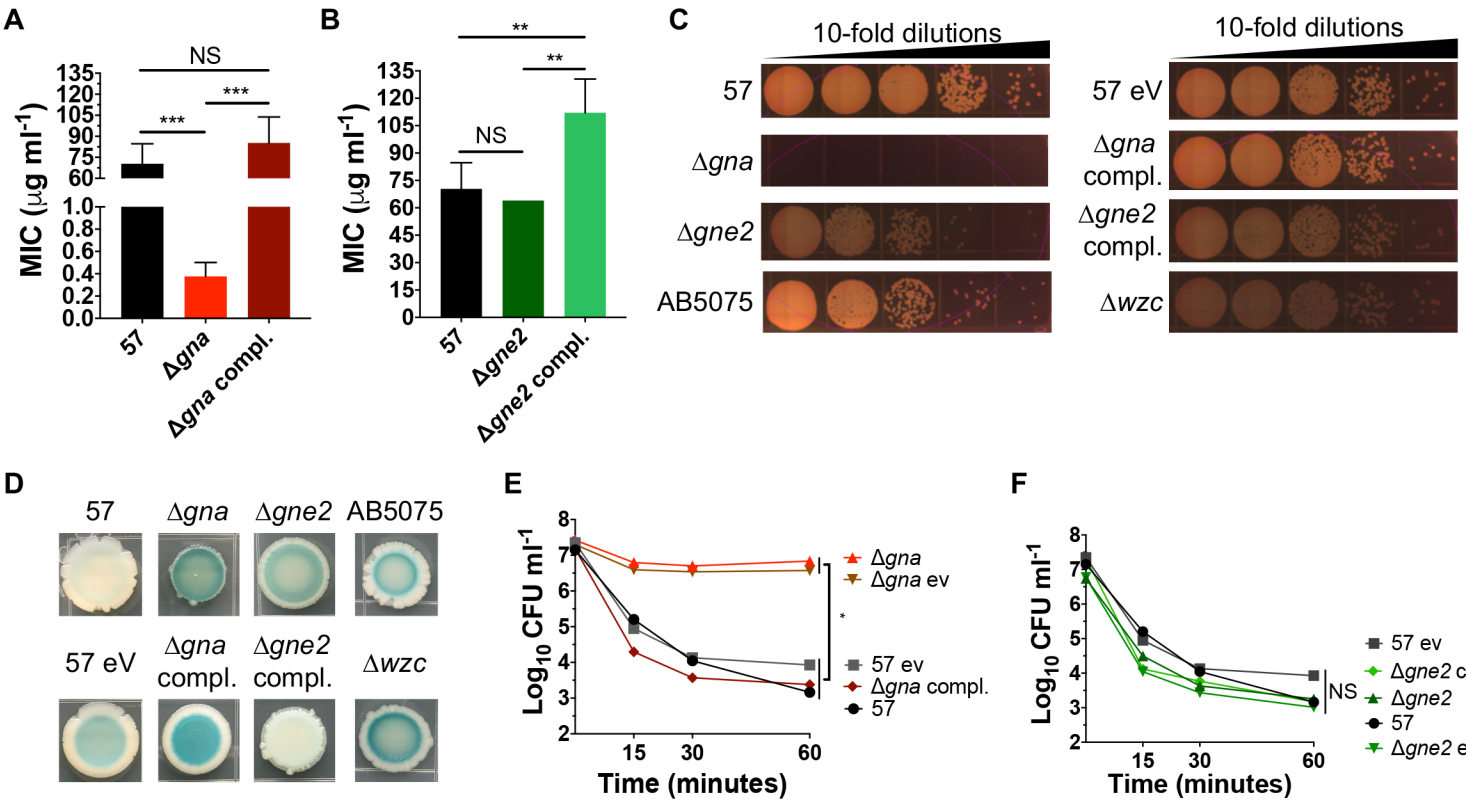
A
NS
***
***
MIC (μg ml-1)
135
120
105
90
75
60
1.0
0.8
0.6
0.4
0.2
0.0
57
Δgna
Δgna compl.
B
**
**
NS
MIC (μg ml-1)
135
120
105
90
75
60
45
30
15
0
57
Δgne2
Δgne2 compl.
C
10-fold dilutions
57
Δgna
Δgne2
AB5075
10-fold dilutions
57 eV
Δgna compl.
Δgne2 compl.
Δwzc
D
57
Δgna
Δgne2
AB5075
57 eV
Δgna compl.
Δgne2 compl.
Δwzc
E
Log10 CFU ml-1
8
7
6
5
4
3
2
15
30
60
Time (minutes)
Δgna
Δgna ev
*
57 ev
Δgna compl.
57
F
Log10 CFU ml-1
8
7
6
5
4
3
2
15
30
60
Time (minutes)
NS
57 ev
Δgne2 compl.
Δgne2
57
Δgne2 eV

Fig. S6.

1. *E. coli* MG1655
2. AB0057
3. Δ*a/e2*
4. Δ*a/e2* compl.

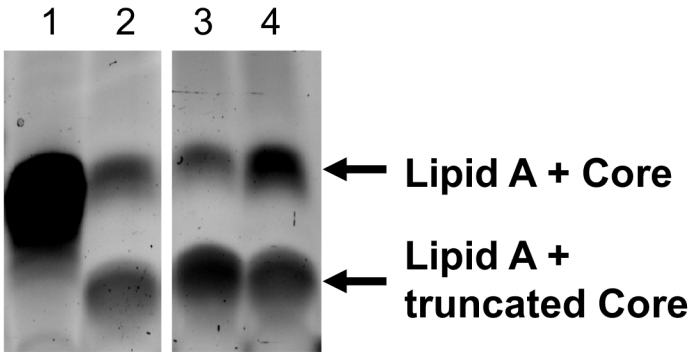

Fig. S7.

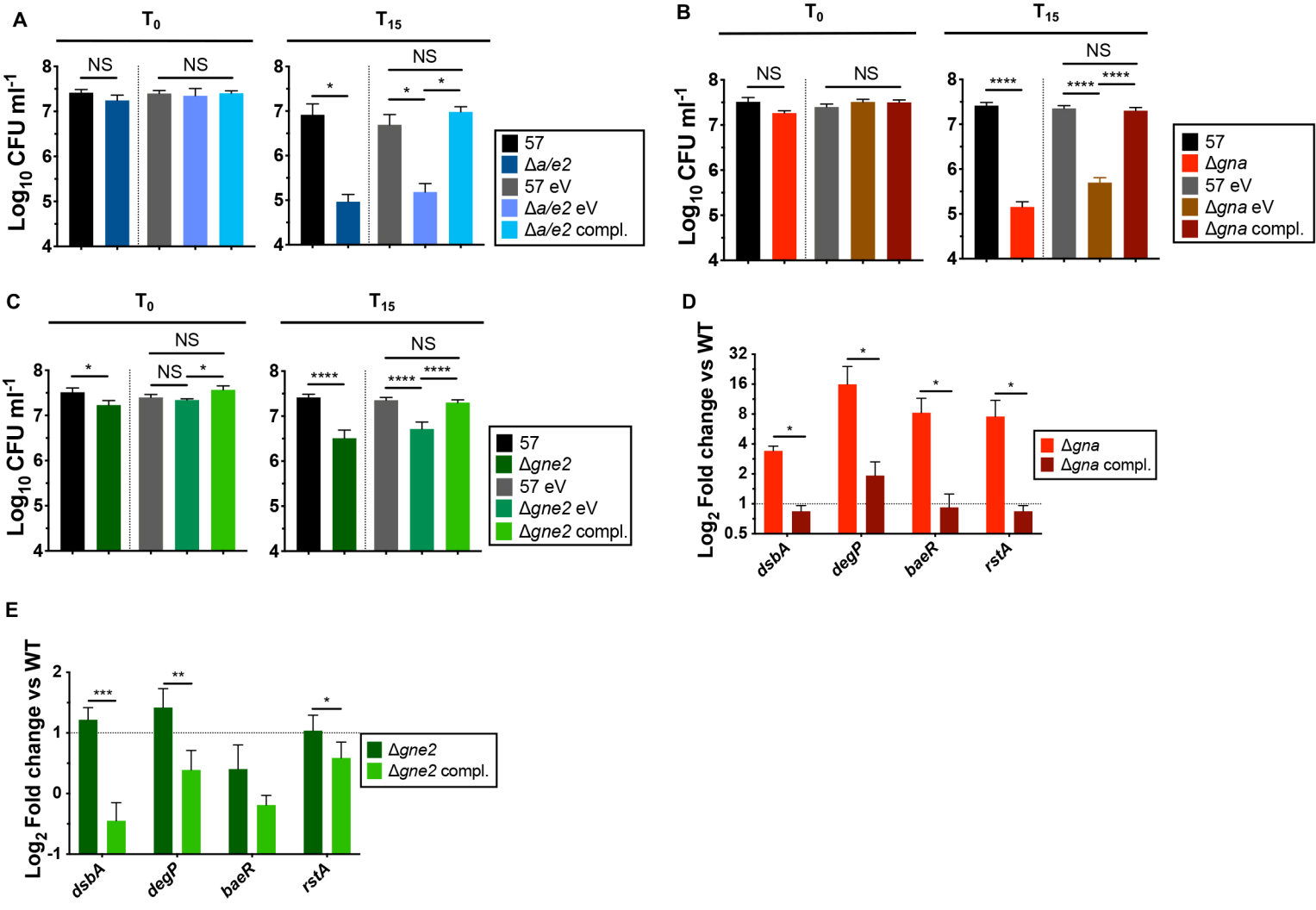
A
T0
T15
Log10 CFU ml-1
NS
*
57
Δa/e2
57 eV
Δa/e2 eV
Δa/e2 compl.
B
****
Δgna
Δgna eV
Δgna compl.
C
Δgne2
Δgne2 eV
Δgne2 compl.
D
Log2 Fold change vs WT
Δgna
Δgna compl.
dsbA
degP
baeR
rstA
E
***
**
Δgne2
Δgne2 compl.

Fig. S8.

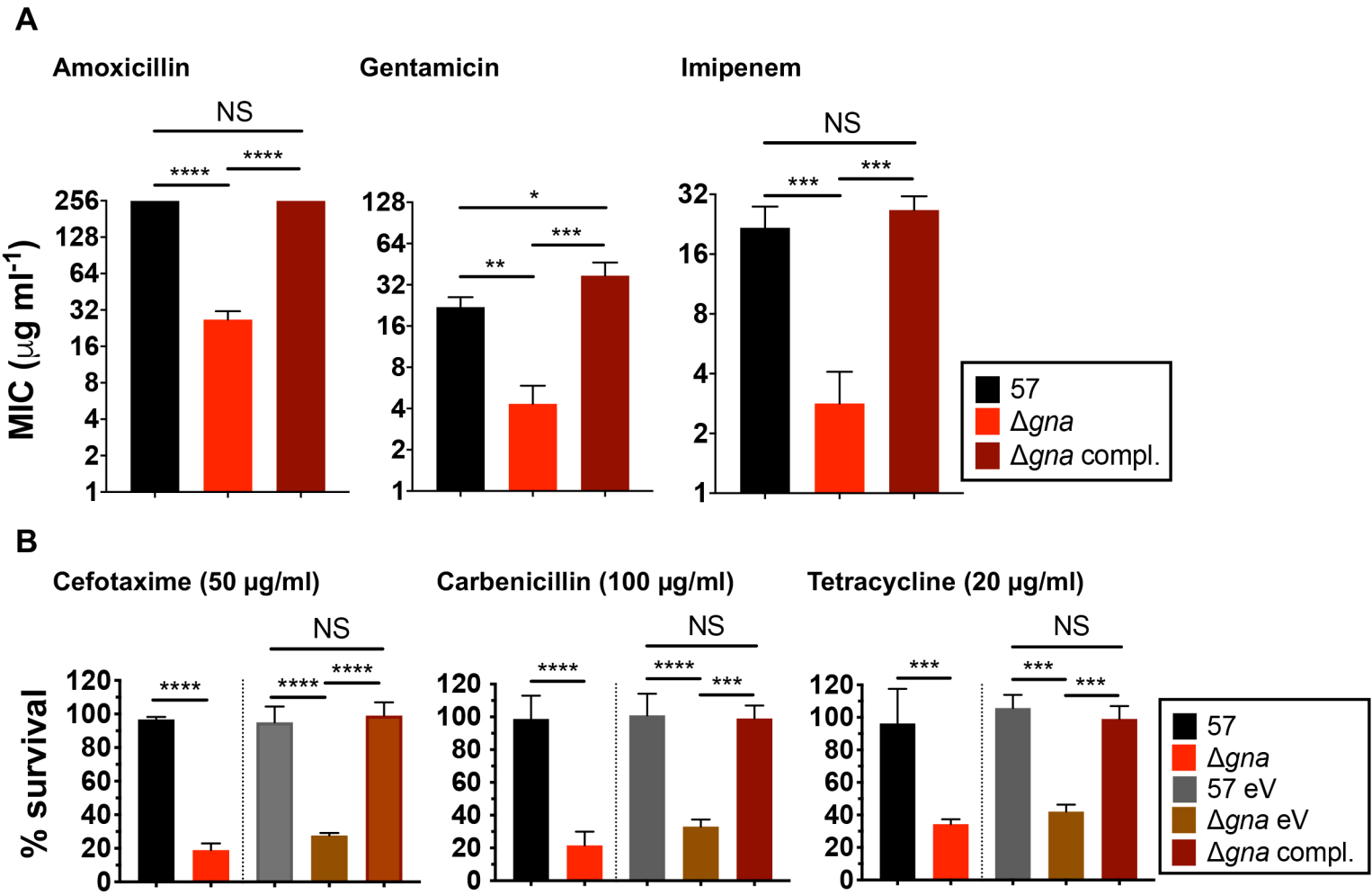
A
Amoxicillin
Gentamicin
Imipenem
NS
****
****
*
**
***
***
***
MIC (μg ml-1)
256
128
64
32
16
8
4
2
1
128
64
32
16
8
4
2
1
32
16
8
4
2
1
57
Δgna
Δgna compl.
B
Cefotaxime (50 μg/ml)
Carbenicillin (100 μg/ml)
Tetracycline (20 μg/ml)
NS
****
****
***
% survival
120
100
80
60
40
20
0
57
Δgna
57 eV
Δgna eV
Δgna compl.

Fig. S9.

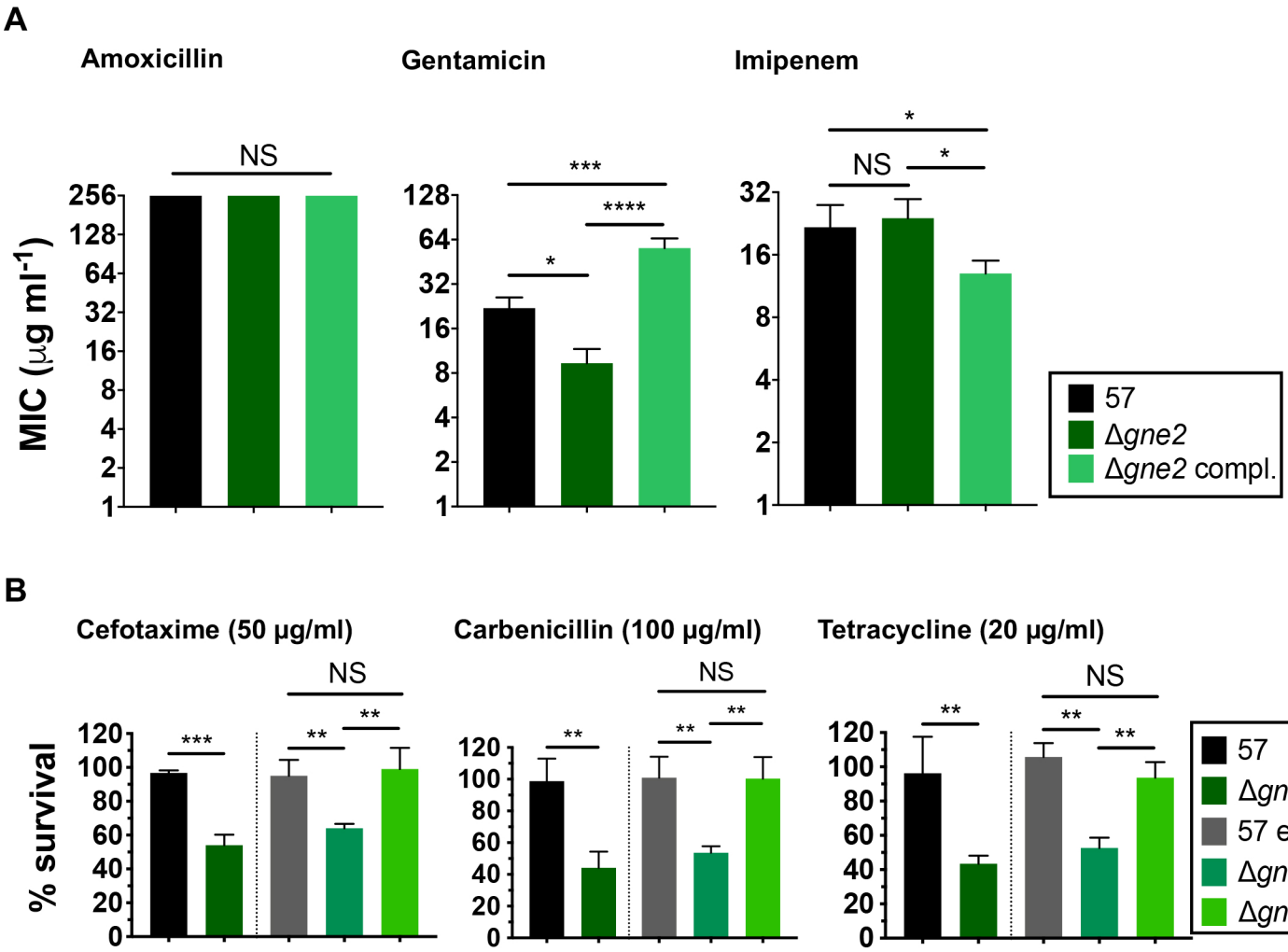
A
Amoxicillin
Gentamicin
Imipenem
MIC (μg ml-1)
NS
***
****
*
NS
*
*
256
128
64
32
16
8
4
2
1
57
Δgne2
Δgne2 compl.
B
Cefotaxime (50 μg/ml)
Carbenicillin (100 μg/ml)
Tetracycline (20 μg/ml)
% survival
NS
**
***
120
100
80
60
40
20
0
57
Δgne2
57 eV
Δgne2 eV
Δgne2 compl.

Fig. S10.

S1 Table. *A. baumannii* strain AB0057 Gna and Gne2 closest homologs.

Proteins	Predicted function	Domain	Homolog (species)	% coverage / identity / positive
Gna (TviB)	UDP-*N*-acetylglucosamine C-6 dehydrogenase	Dehydrogenase superfamily, UDP-glucose/GDP-mannose dehydrogenase family	WpbP (*P. aeruginosa)*	99 / 75 / 87
			TviB (*S.* Typhi)	99 / 69 / 84
Gne2 (TviC)	UDP-*N*-acetylglucosaminuronic acid C-4 epimerase	Epimerase superfamily, NAD dependent epimerase/dehydratase family	WpbO (*P. aeruginosa*)	98 / 73 / 86
			TviC (*S*. Typhi)	99 / 66 / 81

S2 Table. Genes belonging to the KL4 locus and their associated.

Gene	Function	Fitness defect[a] Log_2	*P* value
wzc	Protein tyrosine kinase; involved in capsule export	-6.2	0.002
wzb	Low molecular weight protein tyrosine phosphatase; involved in capsule export	-8.3	0.06
wza	Outer membrane protein; involved in capsule export	-8.2	0.04
gna (*tviB*)	UDP-*N*-acetylglucosamine C-6 dehydrogenase	-6.9	0.053
gne2 (*tviC*)	UDP-*N*-acetylglucosaminuronic acid C-4 epimerase	-8.8	0.002
wzx	Putative oligosaccharide-unit translocase	NA[b]	
ptr1	Predicted pyruvyltransferase	NA	
gtr10	Predicted glycosyltransferase	-1.7	0.007
wzy	Predicted oligosaccharide-unit polymerase	-8.2	0.1
gtr11	Predicted glycosyltransferase	-1.2	0.45
gtr12	Predicted glycosyltransferase	-7.3	0.009
qnr	UDP-N-acetyl-D-quinovosamine biosynthesis protein	-4.1	6.8e-5
itrB1	Predicted initiating transferase for oligosaccharide synthesis	-6.7	0.04
atr3	Predicted acetyl- or acyl- transferase	-7.3	0.005
gdr	UDP-N-acetyl-glucosamine 4,6-dehydratase	-5.0	3.6e-6
gne3	Predicted UDP-glucosamine-4-epimerase	-3.9	0.0004
atr4	Predicted acetyl- or acyl- transferase	-3.5	0.06
atr5	Predicted acetyl- or acyl- transferase	-6.9	0.004
galU	UTP-glucose-1-phosphate uridylyltransferase	-5.3	0.0006
ugd	UDP-glucose 6-dehydrogenase	-3.8	0.005
gpi	Glucose-6-phosphate isomerase	-5.0	7.3e-5
gne1	Predicted UDP-glucosamine-4-epimerase	-5.8	0.005
pgm	Phosphoglucomutase/phosphomannomutase	-1.6	0.0008

[a] Calculated fitness defect in the spleen according to Crepin *et al*. 2018 (Crépin *et al.*, 2018)
[b] Genes were not recovered from the output pools, Crepin *et al*. 2018 (Crépin *et al.*, 2018)

S3 Table. Strains and plasmids used in this study.

Strains	Characteristic(s)[a]	Source or reference
E. coli		
MGN-617	*hi thr leu tonA lacY glnV supE* Δ*asdA4 recA*::RP4 2-Tc::Mu [pir]; Km^r	(Dozois *et al.*, 2000)
MG1655	F^- lambda$^-$ *ilvG rfb-50 rph-1*	(Blattner, 1997)
DH5 α λ pir	sup E44, Δl*acU169* (ΦlacZΔM15), *recA1*, *endA1*, *hsdR17*, *thi-1*, *gyrA96*, *relA1*, λpir phage lysogen	Laboratory collection
S17-1	λ*pir* lysogen of S17.1 (Tp^r Sm^r *thi pro hsdR$^-$ M$^+$ recA* RP4::2-Tc::Mu-*km*::Tn*7*)	(Simon *et al.*, 1983)
A. baumannii		
57; WT; $AB0057^{Km}$;	AB0057 Δ*km*::FRT; Km susceptible	(Crépin *et al.*, 2018)
57 eV	$AB0057^{Km}$ + pABBR_Km; Km^R	(Crépin *et al.*, 2018)
Δ*gna*	$AB0057^{Km}$ Δ*gna*	This study
Δ*gna* eV	$AB0057^{Km}$ Δ*gna* + pABBR_Km; Km^R	This study
Δ*gna* compl.	$AB0057^{Km}$ Δ*gna* + pABBR_Km-*gna*; Km^R	This study
Δ*gne2*	$AB0057^{Km}$ Δ*gne2*	This study
Δ*gne2* eV	$AB0057^{Km}$ Δ*gne2* + pABBR_Km; Km^R	This study
Δ*gne2* compl.	$AB0057^{Km}$ Δ*gne2* + pABBR_Km-*gne2*; Km^R	This study
Δ*gna-gne2* (Δ*a/e2*)	$AB0057^{Km}$ Δ*gna-gne2* (Δ*a/e2*)	(Crépin *et al.*, 2018)
Δ*gna-gne2* (Δ*a/e2*) eV	$AB0057^{Km}$ Δ*gna-gne2* (Δ*a/e2*) + pABBR_Km; Km^R	This study
Δ*gna-gne2* (Δ*a/e2*) compl.	$AB0057^{Km}$ Δ*gna-gne2* (Δ*a/e2*) + pABBR_Km-*gna-gne2* (*a/e2*); Km^R	This study
AB5075	MDR Tibia/osteomyelitis isolate	(Jacobs *et al.*, 2014)
AB5075 Δ*wzc*	AB5075 Δ*wzc*	(Tipton *et al.*, 2018)
AB5075 Δ*pilT*	ABUW Δ*3031*::T26 tnab1_kr121205p08q137	(Gallagher *et al.*, 2015)
Plasmids		
pCVD442_MCS	pCVD442 + MCS; The IS*1* element was swapped by a MCS; Ap^r, R6K, *sacB*	(Crépin *et al.*, 2018)
pCVD_Km	pCVD442_MCS_Amk; Amk^r, R6K, *sacB*	This study

pCVD_AmK_Δ*gna-gne2* (*a/e2*)	Δ*gna-gne2* (Δ*a/e2*) in pCVD_AmK; Amk^r, R6K, *sacB*	(Crépin *et al.*, 2018)
pCVD_Km_Δ*gna*	Δ*gna* in pCVD_Km; Kmk^r, R6K, *sacB*	This study
pCVD_Km_Δ*gne2*	Δ*gne2* in pCVD_Km; Kmk^r, R6K, *sacB*	This study
pABBR_Km	pABBR_MCS_Km; Km^r	(Crépin *et al.*, 2018)
pABBR_Km-*gna-gne2* (*a/e2*)	Genes *gna-gne2* cloned into pABBR_Km; Km^r	This study
pABBR_Km-*gna*	Gene *gna* cloned into pABBR_Km; Km^r	This study
pABBR_Km-*gne2*	Gene *gne2* cloned into pABBR_Km; Km^r	This study

S4 Table. Primers and oligonucleotides.

Name	Sequence 5' → 3'	Purpose
Mutant		
GA_pCVD_Km_F	GGCAGGTATATGTGATGGGTTAAAAAGGA	Inverted PCR of pCVD_Km to clone, by Gibson assembly, the gene of interest (*goi*)
GA_pCVD_Km_R	GGCAACTTTATGCCCATGCAACA	
GA_gna_5'frag_F	TAGTTTC**TGTTGCATGGGCATAAAGTTGCC**GCCTAAAGCATCCGTTAAAGTCAT	Amplification of ~ 1 Kb upstream of *gna*
GA_gna_5'frag_R	**GTCGAATATCTGACTCTGCTTGAGAACCTATAATCGCTATTCTTAAATCG**GCAAGTTG	
GA_gna_3'frag_F	**CGATTTAAGAATAGCGATTATAGGTTCTCAAGCAGAGTCAGATATTCGAC**TATAAATC	Amplification of ~ 1 Kb dowstream of *gna*
GA_gna_3'frag_R	**CCTTTTTAACCCATCACATATACCTGCC**GCCTGTGAATGCCTTACATCTCC	
delta_gna_screen	ATAAGCTTACTGTCCCAAACGGTCTA	Screening to confirm the *gna* mutation; used with GA_*gna*_3'frag_R
GA_gne2_5'frag_F	TCTGTTGCATGGGCATAAAGTTGCCGCCATTGGCAGTTGAGTTTGGA	Amplification of ~ 1 Kb upstream of *gne2*
GA_gne2_5'frag_R	TTTTAGTACTATACCAGTTGATTGCTTGCTCACATATTGTTTGATATTGGCTCATTTT	
GA_gne2_3'frag_F	CCAATATCAAACAATATGTGAGCAAGCAATCAACTGGTATAGTACTAAAATTTAGTCTTTG	Amplification of ~ 1 Kb upstream of *gne2*
GA_gne2_3'frag_R	CCTTTTTAACCCATCACATATACCTGCCGCCTGTGAATGCCTTACATCTCC	
delta_gne2_screen	TTTACTGGAGGCGTAATTTCAGGGTATG	Screening to confirm the *gne2* mutation; used with GA_gne2_3'frag_R
pCVD_Screen_F	GATTTGCAGACTACGGGCCTAAAG	Screening to confirm the cloning of the Δ*goi* into pCVD_Km
pCVD_Screen_R	CGAACTAAACCCTCATGGCTAACG	
Complementation		
AB0057_ *gna-gne2*_F_NruI	ACAAGT**TCGCGA**TAAACGCAGGCTGAACAGATTCTAGC	Cloning of the *gna-gne2* locus into pABBR_Km
AB0057_ *gna-gne2*_R_SalI	AAATC**GTCGAC**GCAACCCCAACTCTATTGAGAGGTATTTTCA	

GA_pABBR_Km_R	GCCTTCCCCATTATGATTCTTCTCG	Inverted PCR of pABBR_Km to clone, by Gibson assembly, the *goi*
GA_pABBR_Km_F	ACGATTCCGAAGCCCAACCTTT	
GA_AB0057_gna_F_pABBR	**CGAGAAGAATCATAATGGGGAAGGC**TAAACGCAGGCTGAACAGATTCTAG	Cloning, by Gibson assembly, of *gna*, with its native promoter, into pABBR_Km
GA_AB0057_gna_R_pABBR	**ATGAAAGGTTGGGCTTCGGAATCGT**GATCAGCAATGGAACGAGGTACT	
GA_AB0057_gne2prom_F_pABBR	**TCATAATGGGGAAGGC**CCCTTTGTGTATTTATCTTTAATTTGATATTGTCCAC	Cloning, by Gibson assembly, of *gne2*, with the native promoter found upstream of *gna*, into pABBR_Km
GA_AB0057_gne2prom_R_gne2	**TTTGATATTGGCTCAT**AAAAGTTAGCCTTGTTCCTTACAAATTGTAT	
GA_AB0057_gne2_F_gne2prom	**AACAAGGCTAACTTTT**ATGAGCCAATATCAAACAATATGTGAGCA	
GA_AB0057_gne2_R_pABBR	**TGGGCTTCGGAATCGT**GCAACCCCAACTCTATTGAGAGGTATTTTCA	
pABBR_Km_Km_Screen	TGATATTGCTGAAGAGCTTGGCG	Screening to confirm the cloning of the *goi* into pABBR_Km
qRT-PCR		
ESR		
degP_F_qPCR	GTAATCAGAGCACCTTCCGGTTTAG	Amplification of a portion of *degP*
degP_R_qPCR	TGACTCGTTCATACCTAGGCGTTAT	
baeR_F_qPCR	TGTTCACGTACCTTACGGCAAATC	Amplification of a portion of *baeR*
baeR_R_qPCR	GGTCAGGATGCTTACACGAACTTT	
rstA_F_qPCR	CGCCCACACTATCATCAACCAA	Amplification of a portion of *rstA*
rstA_R_qPCR	GCACCCATTTCCAGACCAAGTA	
dsbA_F_qPCR	GGGTAGTGTAATGGCAGCAGATTT	Amplification of a portion of *dsbA*
dsbA_R_qPCR	GGTACTTCCACTTTGCCTGGATTG	

Bolded nucleotides denote restriction site.
Bolded and underlined nucleotides denote the region of homology for the Gibson assembly.

REFERENCES

Blattner, F.R. (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453-1462.

Crépin, S., Ottosen, E.N., Peters, K., Smith, S.N., Himpsl, S.D., Vollmer, W., and Mobley, H.L.T. (2018) The lytic transglycosylase MltB connects membrane homeostasis and *in vivo* fitness of *Acinetobacter baumannii*. *Mol Microbiol* **109**(6):745-762

Dozois, C.M., Dho-Moulin, M., Bree, A., Fairbrother, J.M., Desautels, C., and Curtiss, R. (2000) Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the *tsh* genetic region. *Infect Immun* **68**: 4145-4154.

Gallagher, L.A., Ramage, E., Weiss, E.J., Radey, M., Hayden, H.S., Held, K.G., Huse, H.K., Zurawski, D.V., Brittnacher, M.J., and Manoil, C. (2015) Resources for genetic and genomic analysis of emerging pathogen *Acinetobacter baumannii*. *J Bacteriol* **197**: 2027-2035.

Jacobs, A.C., Thompson, M.G., Black, C.C., Kessler, J.L., Clark, L.P., McQueary, *et al*. (2014) AB5075, a highly virulent isolate of *Acinetobacter baumannii*, as a Model Strain for the evaluation of pathogenesis and antimicrobial treatments. *MBio* **5**: e01076-01014.

Simon, R., Priefer, U., and Pühler, A. (1983) A broad host range mbilization system for *in vivo* genetic engineering: Transposon mutagenesis in Gram negative bacteria. *Bio/Technology* **1**: 784-791.

Tipton, K.A., Chin, C.-Y., Farokhyfar, M., Weiss, D.S., and Rather, P.N. (2018) Role of capsule in resistance to disinfectants, host antimicrobials, and desiccation in *Acinetobacter baumannii*. *Antimicrob Agents and Chemother* **62**(12):e01188-18.