# Appendix: A *Klebsiella pneumoniae* antibiotic resistance mechanism that subdues host defences and promotes virulence.

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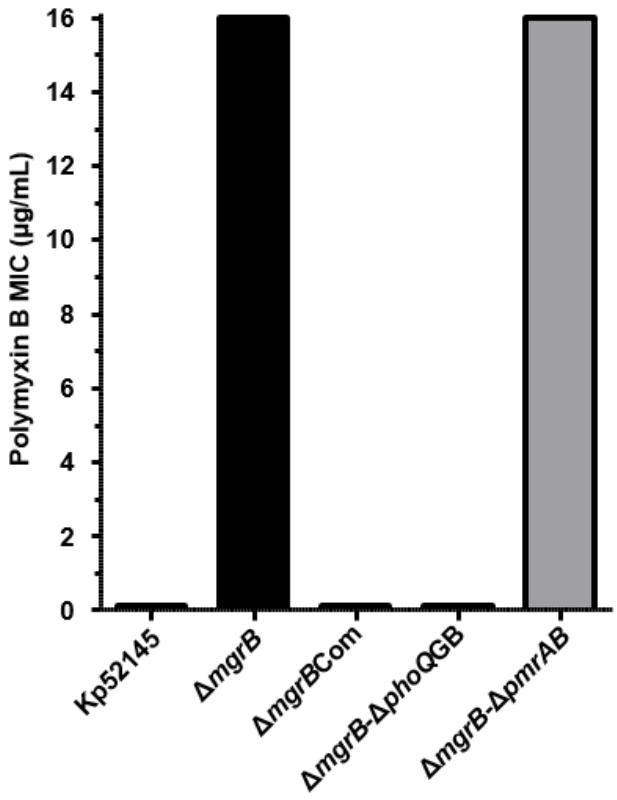



**Appendix Figure S1.**

Etest® minimal inhibitory concentrations (MIC) to polymyxin B of the *Klebsiella pneumoniae* 52145, 52145-Δ*mgrB*, 52145-Δ*mgrB*Com, 52145-Δ*mgrB*-Δ*phoQ*GB and 52145-Δ*mgrB*-Δ*pmrAB* strains.

| Strain | Observed ion (*m/z*) | Acyl substitution | Proposed fatty acid, phosphate and carbohydrate composition[a] |
|---|---|---|---|
| 52145 (wild-type) | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| 52145-Δ*mgrB* | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 1852 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C16:0, 2P |
| | 1866 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0(3-OH), 1x C16:0, 2P |
| | 1948 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 1x PEtN, 2P |
| | 1955 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 1x Ara4N, 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| | 2079 | Hepta-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 1x C16:0, 2P |
| 52145-Δ*mgrB*Com | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| 52145-Δ*mgrB*-Δ*phoQ*GB | 1797 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C12:0, 2P |
| | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| 52145-Δ*mgrB*-Δ*pmrAB* | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 1852 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C16:0, 2P |
| | 1866 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0(3-OH), 1x C16:0, 2P |
| | 1948 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 1x PEtN, 2P |
| | 1955 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 1x Ara4N, 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| | 2079 | Hepta-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 1x C16:0, 2P |
| 52145-Δ*mgrB*-Δ*phoQ*GB-Δ*pmrAB* | 1797 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C12:0, 2P |
| | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| 52145-Δ*mgrB*-Δ*phoQ*GB-*phoPQ*Com | 1824 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 2P |
| | 1840 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 2P |
| | 1852 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C16:0, 2P |
| | 1866 | Hexa-acyl | 4x C14:0(3-OH), 1x C14:0(3-OH), 1x C16:0, 2P |
| | 1948 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 1x PEtN, 2P |
| | 1955 | Hexa-acyl | 4x C14:0(3-OH), 2x C14:0, 1x Ara4N, 2P |
| | 2063 | Hepta-acyl | 4x C14:0(3-OH), 2x C14:0, 1x C16:0, 2P |
| | 2079 | Hepta-acyl | 4x C14:0(3-OH), 1x C14:0, 1x C14:0(3-OH), 1x C16:0, 2P |

[a] C14:0(3-OH), R-3-hydroxymyristoyl acyl chain; C14:0, myristate acyl chain; P, phosphate; C16:0, palmitate; PEtN, phosphoethanolamine; Ara4N, 4-amino-4-deoxy-l-arabinose; C12:0, laureate.

**Appendix Table S1.**

Lipid A species identified by negative-ion MALDI-TOF mass spectrometry analysis among the *Klebsiella pneumoniae* 52145 strains when grown in LB medium at $37^0$C.

| Strain[a] | Origin | Colistin susceptibility | *mgrB* sequence mutation[b] | Additional antimicrobial resistance determinants[c] | Multilocus sequence type |
|---|---|---|---|---|---|
| T1a | Turkey | Sensitive | Wild-type | KPC-2 | ST-258 |
| T1b | Turkey | Resistant | Truncated by IS5-like | KPC-2 | ST-258 |
| C21 | Colombia | Resistant | Truncated by ISKpn13 | CTX-M-2 | ST-1271 |
| C22 | Colombia | Resistant | Truncated by ISKpn14 | CTX-M-15 | ST-101 |
| C2 | Colombia | Resistant | Truncated by IS10R | KPC-2 | ST-258 |
| 15I5 | France | Resistant | Truncated protein (29 aa) | - | ST-70 |

[a] Strain designation and isolates were identical to those reported by Poirel *et al* (Poirel et al, 2015)

[b] IS, insertion sequence, aa, amino acid

[c] KPC, *Klebsiella pneumoniae* carbapenemase 2; CTX-M, CTX-M extended spectrum beta-lactamase

**Appendix Table S2.**

Origins, characteristics and genotypes of the clinical *Klebsiella pneumoniae* strains used in this study.

| Bacterial strain or plasmid | Genotype or comments[a] | Source or reference(s) |
|---|---|---|
| Strains | | |
| *Escherichia coli* | | |
| C600 | *thi thr leuB tonA lacY supE* | |
| GT115 | F$^-$*mcrA*Δ(*mrr-hsdRMS-mcrBC*) ϕ80Δ*lacZ*ΔM15 Δ*lacX*74 *recA1rpsL* (StrA) *endA1*Δ*dcm uidA(*Δ*MluI)::pir-116* Δ*sbcC-sbcD* | InvivoGen |
| SY327 | *λ (lac pro) argE (Am) rif nalA recA56 (λ pir)* | Miller, 1988 |
| β2163 | (F$^-$) RP4-2-Tc::Mu Δ*dapA*::(*erm-pir*) [Km$^R$Em$^R$] | Demarre, 2005 |
| *Klebsiella pneumoniae* | | |
| 52145 | Clinical isolate; serotype O1:K2; sequence type ST66 | Brisse, 2009; Lery, 2014 |
| 52145-Δ*mgrB* | Kp52145, Δ*mgrB*; the *mgrB* gene was inactivated | This study |
| 52145-Δ*mgrB*Com | 52145-Δ*mgrB* complemented with mini-Tn7TKmKp52145*mgrB*; *mgrB* activity was restored | This study |
| 52145-Δ*pmrC* | Kp52145, Δ*pmrC*; the *pmrC* gene was inactivated | This study |
| 52145-Δ*pagP* | Kp52145, Δ*pagP*; the *pagP* gene was inactivated | This study |
| 52145-Δ*manC* | Kp52145, Δ*manC*; the *manC* gene was inactivated; no CPS expression | This study |
| 52145-Δ*mgrB*-Δ*phoQ*GB | Kp52145, Δ*mgrB*, Δ*phoQ*::KM-GenBlock; the *mgrB* and *phoQ* genes were inactivated; Km$^R$ | This study |
| 52145-Δ*mgrB*-Δ*pmrAB* | Kp52145, Δ*mgrB*, Δ*pmrAB*; the *mgrB* and *pmrAB* genes were inactivated | This study |
| 52145-Δ*mgrB*-Δ*phoQ*GB-*phoPQ*Com | 52145-Δ*mgrB*-Δ*phoQ*GB complemented with pGP-Tn7-Cm_KpnPhoPQCom; *phoPQ* activity was restored | This study |
| 52145-Δ*mgrB*-Δ*lpxO* | Kp52145, Δ*mgrB*, Δ*lpxO*; the *mgrB* and *lpxO* genes were inactivated | This study |
| 52145-Δ*pagP*-Δ*mgrB* | Kp52145, Δ*pagP*, Δ*mgrB*; the *pagP* and *mgrB* genes were inactivated | This study |
| 52145-Δ*pmrC*-Δ*mgrB* | Kp52145, Δ*pmrC*, Δ*mgrB*; the *pmrC* and *mgrB* genes were inactivated | This study |
| 52145-Δ*mgrB*-Δ*pmrF* | Kp52145, Δ*mgrB*, Δ*pmrF*; the *mgrB* and *pmrF* genes were inactivated | This study |
| 52145-Δ*mgrB*-Δ*manC* | Kp52145, Δ*mgrB*, Δ*manC*; the *mgrB* and *manC* genes were inactivated; no CPS expression | This study |
| 52145-Δ*pmrC*-Δ*lpxO*-Δ*mgrB* | Kp52145, Δ*pmrC*, Δ*lpxO*, Δ*mgrB*; the *pmrC, lpxO* and *mgrB* genes were inactivated | This study |
| 52145-Δ*mgrB*-Δ*lpxO*-Δ*pmrF* | Kp52145, Δ*mgrB*, Δ*lpxO*, Δ*pmrF*; the *mgrB*, *lpxO* and *pmrF* genes were inactivated | This study |
| 52145-Δ*pmrC*-Δ*lpxO*-Δ*mgrB*-Δ*pmrF* | Kp52145, Δ*pmrC*, Δ*lpxO*, Δ*mgrB*, Δ*pmrF*; the *pmrC*, *lpxO*, *mgrB* and *pmrF* genes were inactivated | This study |
| 52145-Δ*pmrC*-Δ*lpxO*-Δ*mgrB*-Δ*pmrF*-Δ*pagP* | Kp52145, Δ*pmrC*, Δ*lpxO*, Δ*mgrB*, Δ*pagP*; the *pmrC*, *lpxO*, *mgrB*, *pmrF* and *pagP* genes were inactivated | This study |
| 52145-Δ*mgrB*-Δ*lpxO*-*lpxO*Com | 52145-Δ*mgrB*Δ*lpxO* complemented with pGP-Tn7-Cm_KpnLpxOCom; *lpxO* activity was restored | This study |

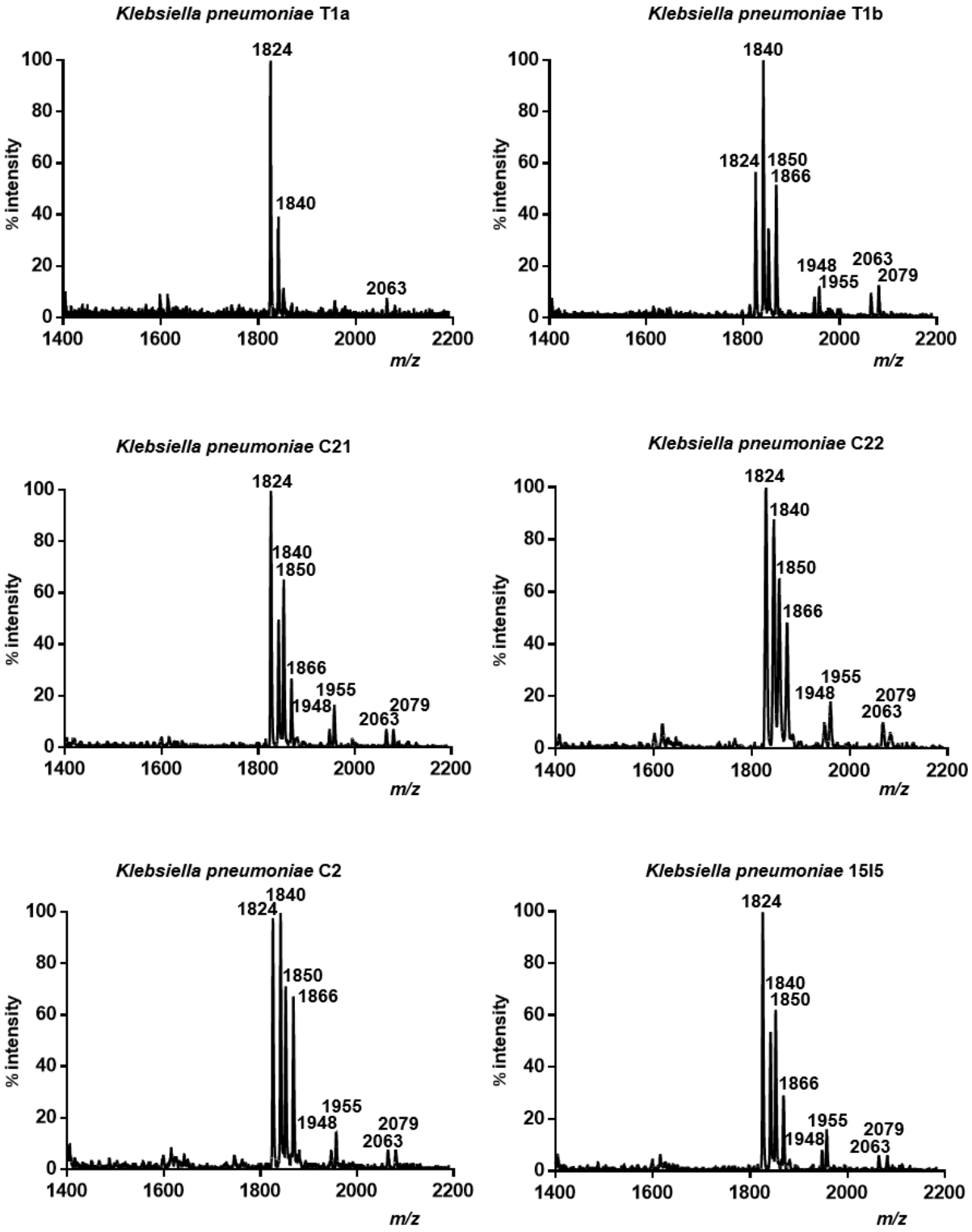
*Continued*

| | | |
|---|---|---|
| Plasmids | | |
| pGEM-T Easy | Cloning plasmid; $Amp^R$ | Promega |
| pGPI-SceI-2 | Suicide vector, R6Kγ origin of replication, $Mob^+$, carries a I-SceI endonuclease site; $Tmp^R$ | Aubert, 2014 |
| pDAI-SceI-SacB | Expresses the I-SceI endonuclease, *sacB* gene; $Tet^R$ | Aubert, 2014 |
| pKD4 | PCR template plasmid for the λ Red recombinase system; $Km^R$ | Datsenko, 2000 |
| pKOBEG-sacB | λ phage *redγβα*, arabinose inducible; $Cm^R$ | Derbise, 2003 |
| pTSNSK-Tp | pTSNSK-Tp containing a transposase for Tn7 insertion; $Km^R$, $Tmp^R$ | Crépin, 2012 |
| pUC18R6KT-mini-Tn7TKm | pUC18R6KT-mini-Tn7T complementation vector; $Amp^R$, $Km^R$ | Choi, 2005 |
| pGP-Tn7-Cm | pGP-Tn7 complementation vector; $Amp^R$, $Cm^R$ | Crépin, 2012 |
| pMAKSACB | Suicide vector, Psc101 replication origin, Mob+, *sacB* gene; $Cm^R$ | Favre, 2000 |
| pFLP2 | Plasmid encoding FLP to remove cassettes between FRT sites, *sacB* gene; $Tmp^R$ | Hoang, 1998 |
| pGPL01 | Firefly luciferase (*lucFF*) transcriptional fusion suicide vector, R6Kγ origin of replication; $Amp^R$ | Gunne, 1996 |
| pGEMΔ*mgrB* | pGEM-T Easy containing Δ*mgrB*; $Amp^R$ | This study |
| pGEMΔ*pmrAB* | pGEM-T Easy containing Δ*pmrAB*; $Amp^R$ | This study |
| pGEMΔ*manC* | pGEM-T Easy containing Δ*manC*; $Amp^R$ | This study |
| pGEMΔ*pmrF* | pGEM-T Easy containing Δ*pmrF*; $Amp^R$ | This study |
| pGEMΔ*pagP* | pGEM-T Easy containing Δ*pagP*; $Amp^R$ | This study |
| pGPI-SceI-2Δ*mgrB* | pGPI-SceI-2 containing Δ*mgrB*; $Tmp^R$ | This study |
| pGPI-SceI-2Δ*pmrAB* | pGPI-SceI-2 containing Δ*pmrAB*; $Tmp^R$ | This study |
| pGPI-SceI-2Δ*manC* | pGPI-SceI-2 containing Δ*manC*; $Tmp^R$ | This study |
| pGPI-SceI-2Δ*pmrF* | pGPI-SceI-2 containing Δ*pmrF*; $Tmp^R$ | This study |
| pGPI-SceI-2Δ*pagP* | pGPI-SceI-2 containing Δ*pagP*; $Tmp^R$ | This study |
| pMAKSACBΔ*lpxO* | pMAKSACB containing Δ*lpxO*; $Km^R$ | This study |
| pUC18R6KT-mini-Tn7TKmKp52145*mgrB* | pUC18R6KT-mini-Tn7TKm containing *mgrB* gene for complementation; $Amp^R$, $Km^R$ | This study |
| pGP-Tn7-Cm_KpnPhoPQCom | pGP-Tn7-Cm containing *phoPQ* operon for complementation; $Amp^R$, $Cm^R$ | Llobet, 2015 |
| pGP-Tn7-Cm_KpnLpxOCom | pGP-Tn7-Cm containing *lpxO* gene for complementation; $Amp^R$, $Cm^R$ | Llobet, 2015 |
| pGPLKpnProLpxO | pGPL01 containing the *lpxO* promoter region; $Amp^R$ | Llobet, 2015 |
| pGPLKpnProPmrH | pGPL01 containing the *pmrH* promoter region; $Amp^R$ | Llobet, 2011 |
| pGPLKpnProPmrC | pGPL01 containing the *pmrC* promoter region; $Amp^R$ | Insua, 2013 |
| pGPLKpnProPagP | pGPL01 containing the *pagP* promoter region; $Amp^R$ | This study |
| pGPLKpnProPhoP | pGPL01 containing the *phoP* promoter region; $Amp^R$ | Llobet, 2011 |
| pGPLKpnProcps | pGPL01 containing the *cps* promoter region; $Amp^R$ | Llobet, 2011 |

[a] $Km^R$, kanamycin resistant; $Em^R$, erythromycin resistant; $Tmp^R$, trimethoprim resistant; $Amp^R$, ampicillin resistant; $Tet^R$, tetracycline resistant; $Cm^R$, chloramphenicol resistant

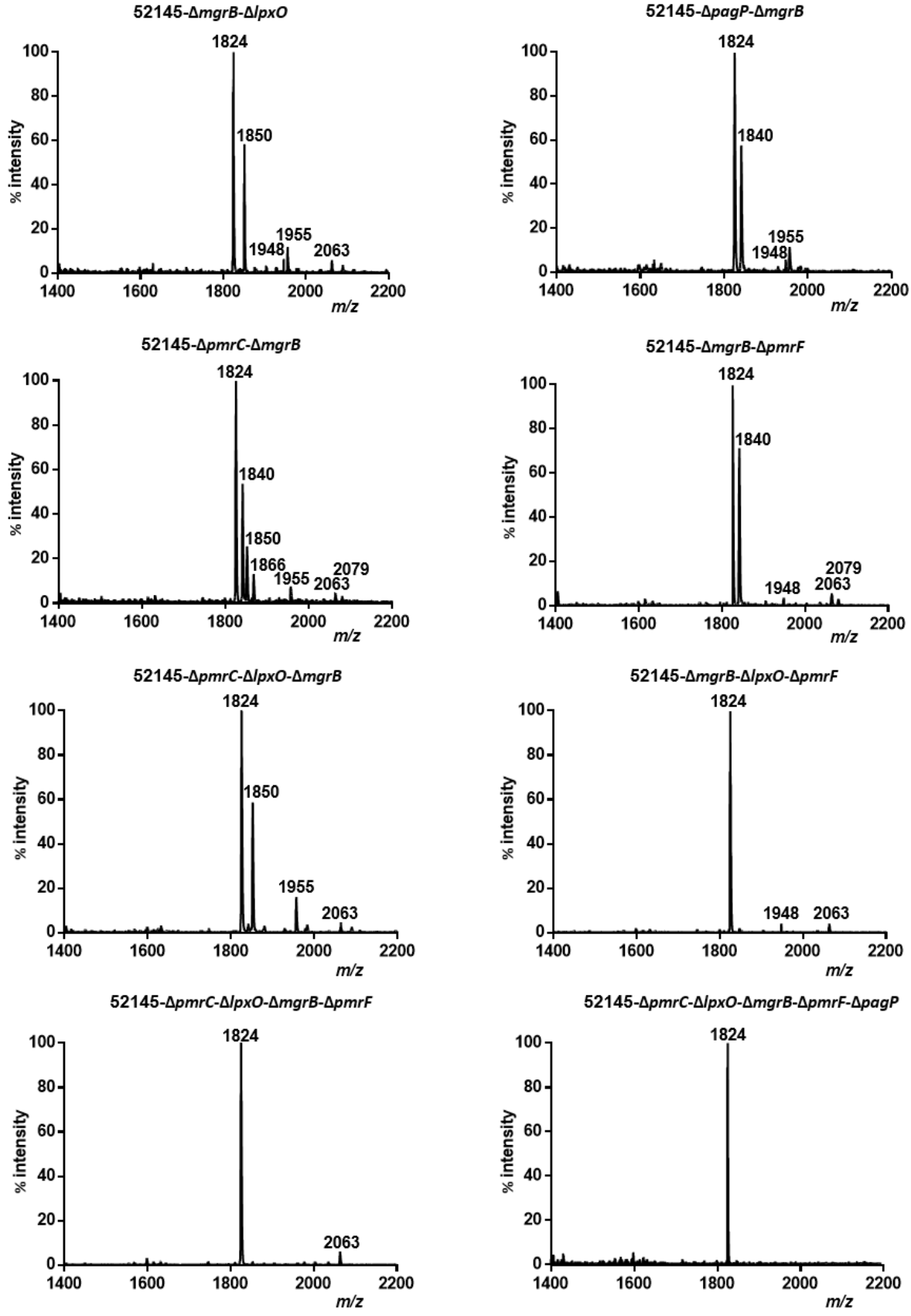
**Appendix Table S3.**

Bacterial strains and plasmids used in this study



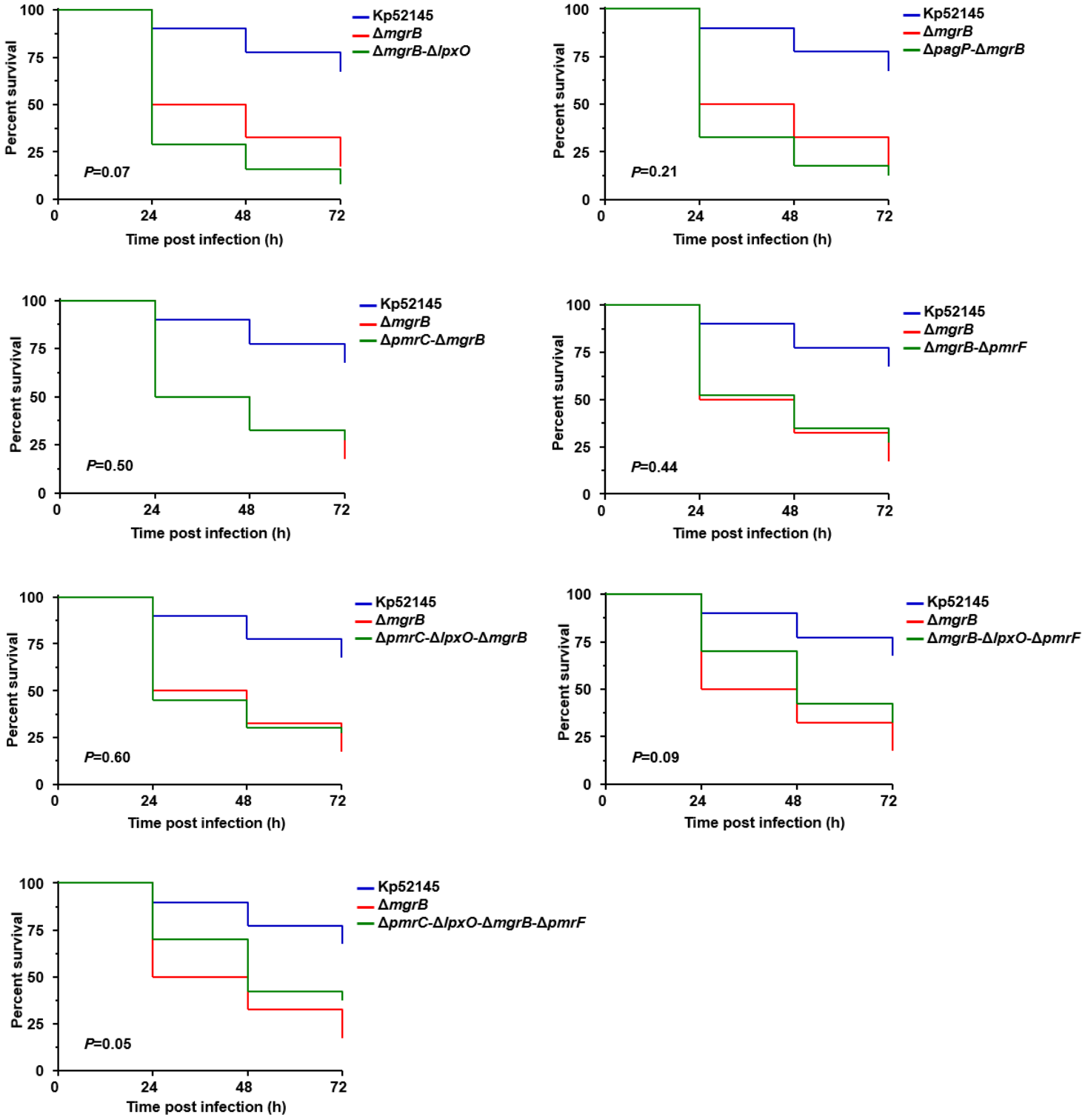

**Appendix Figure S2.**

Negative ion MALDI-TOF mass spectrometry spectra of lipid A isolated from the clinical *Klebsiella pneumoniae* T1a (A), T1b (B), C21 (C), C22 (D), C2 (E) and 15I5 (F) strains. Data represent the mass to charge (*m/z*) ratios of each lipid A species detected and were representative of three independent lipid A extractions.



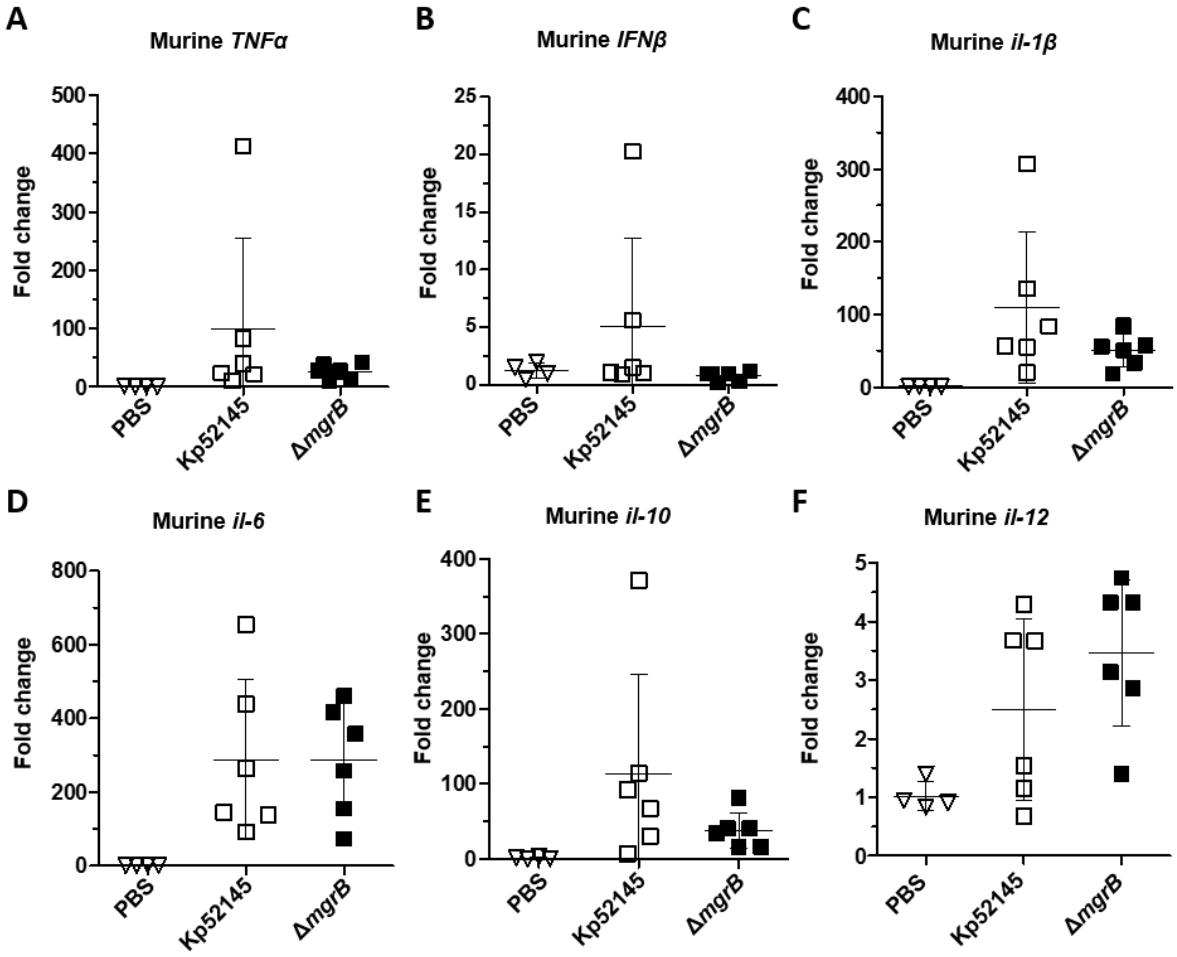

**Appendix Figure S3.**

Negative ion MALDI-TOF mass spectrometry spectra of lipid A isolated from the *Klebsiella pneumoniae* 52145-Δ*mgrB*-Δ*lpxO*, 52145-Δ*pagP*-Δ*mgrB*, 52145-Δ*pmrC*-Δ*mgrB*, 52145-Δ*mgrB*-Δ*pmrF*, 52145-Δ*pmrC*-Δ*lpxO*-Δ*mgrB*, 52145-Δ*mgrB*-Δ*lpxO*-Δ*pmrF*, 52145-Δ*pmrC*-Δ*lpxO*-Δ*mgrB*-Δ*pmrF* and 52145-Δ*pmrC*-Δ*lpxO*-Δ*mgrB*-Δ*pmrF*-Δ*pagP* strains. Data represent the mass to charge (*m/z*) ratios of each lipid A species detected and were representative of three independent lipid A extractions.



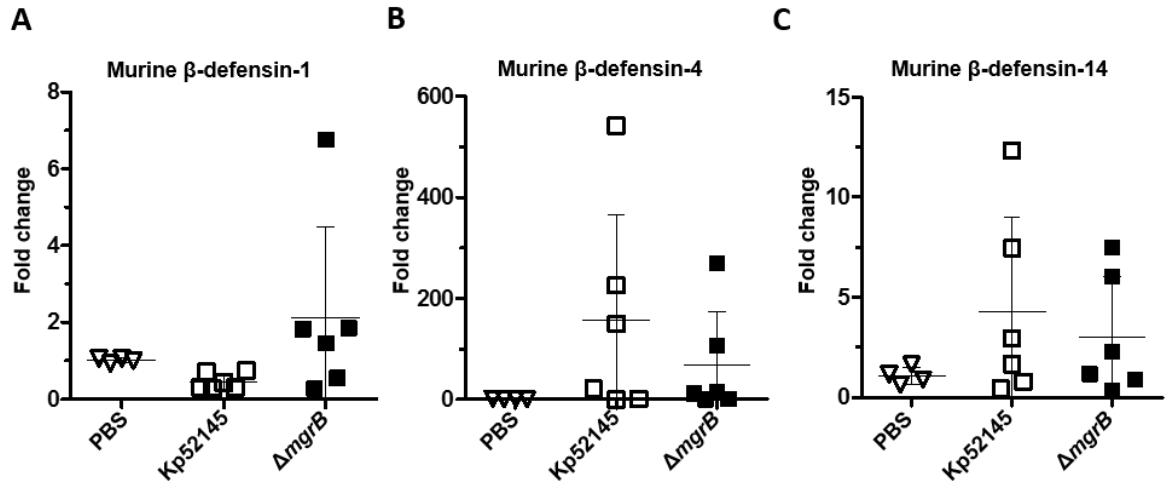

**Appendix Figure S4.**

Kaplan-Meier plots showing the percent survival of *Galleria mellonella* over 72 hours post-infection with $10^5$ organisms of *Klebsiella pneumoniae* 52145 (blue), 52145-Δ*mgrB* (red), and the seven double, triple and quadruple mutant (green) strains. Forty larvae were infected in each group. Level of significance was determined using the log-rank (Mantel-Cox) test with Bonferroni correction (α=0.017). *P*-values presented correspond to the difference between 52145-Δ*mgrB* and the various multiple mutant strains.



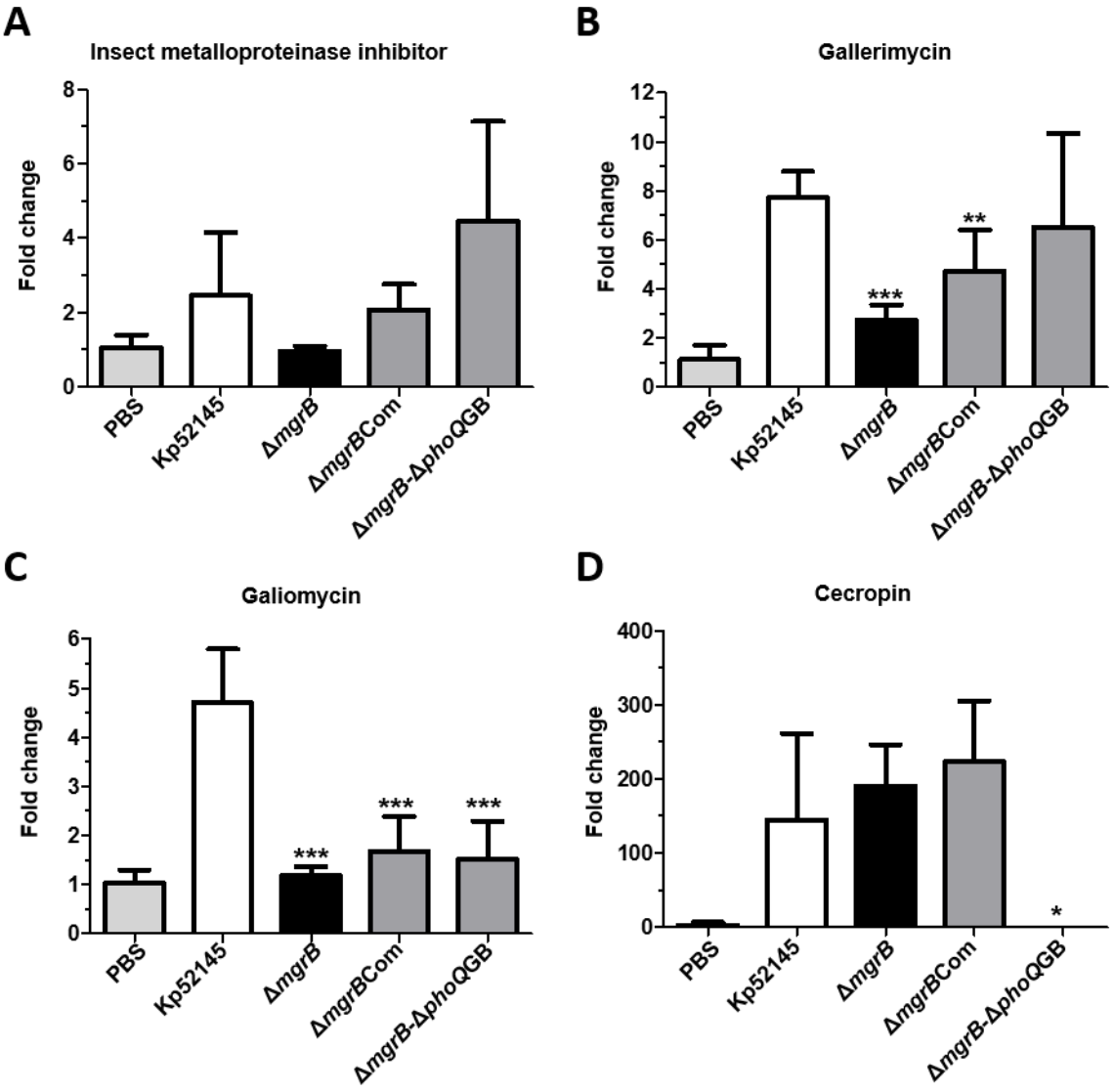

**Appendix Figure S5.**

Expression of murine tissue necrosis factor-alpha (*TNFα*; A), interferon-beta (*IFNβ*; B), interleukin [il] 1-beta (*il-1β*; C), *il-6* (D), *il-10* (E) and *il-12* (F) produced in the lung after 24 hours infection with *Klebsiella pneumoniae* 52145 and 52145-Δ*mgrB* as determined by reverse transcriptase quantitative real-time PCR (n=4 PBS vehicle controls, n=6 infected mice per group). Values are presented as the mean ± SD of two independent cDNA preparations measured in duplicate. Level of significance versus Kp52145 was determined using the two-way Unpaired t-test.



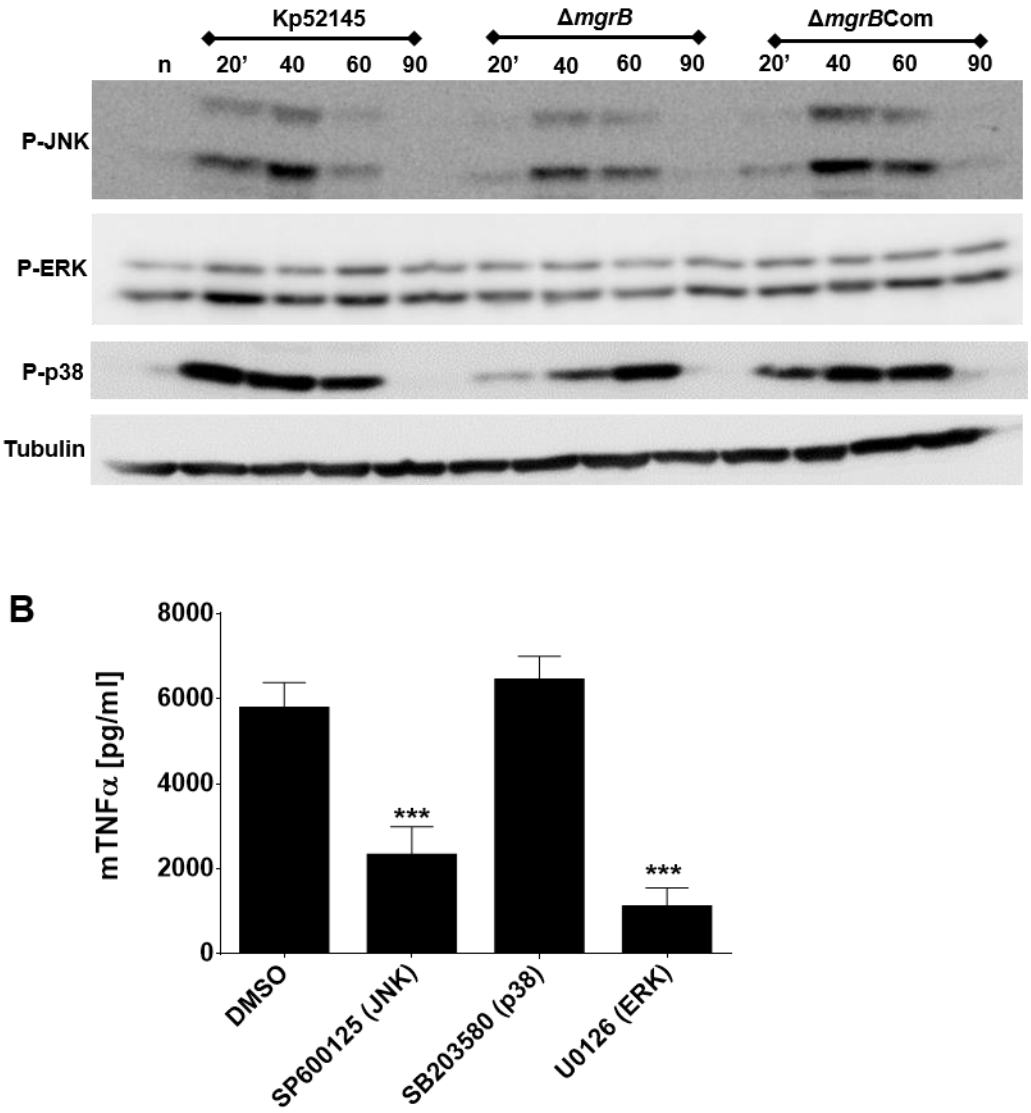

**Appendix Figure S6.**

Expression of the murine antimicrobial peptides beta defensin-1 (A), beta defensin-4 (B) and beta defensin-14 (C) produced in the lung after 24 hours infection with 52145 and 52145-Δ*mgrB* as determined by reverse transcriptase quantitative real-time PCR (n=4 PBS vehicle controls, n=6 infected mice per group). Values are presented as the mean ± SD of two independent cDNA preparations measured in duplicate. Level of significance versus Kp52145 was determined using the two-way Unpaired t-test.




**Appendix Figure S7.**

Expression of insect metalloproteinase inhibitor (A), gallerimycin (B), galiomycin (C) and cecropin (D) produced by *Galleria mellonella* after eight hours infection with *Klebsiella pneumoniae* 52145, 52145-Δ*mgrB*, 52145-Δ*mgrB*Com and 52145-Δ*mgrB*-Δ*phoQ*GB as determined by reverse transcriptase quantitative real-time PCR. Three larvae per group were infected and values are presented as the mean ± SD of two independent cDNA preparations measured in duplicate. *, $P$=0.01; **, $P$=0.004, ***, $P \leq 0.0002$; versus Kp52145 determined using the two-way Unpaired t-test.




**Appendix Figure S8.**

(A) Early inflammatory responses were analysed by immunoblot of phospho-ERK (P-ERK), phospho-p38 (P-p38), phospho-JNK (P-JNK) and tubulin levels in lysates of MH-S cells infected with *K. pneumoniae* 52145, 52145-Δ*mgrB*, 52145-Δ*mgrB*Com for the indicated times. (B) TNFα secretion upon *K. pneumoniae* infection in iBMDM macrophages was analysed in the presence of the MAPK ERK inhibitor (U0126, 10 μM, 2h before infection), JNK inhibitor (SP600125, 10 μM, 2 hours before infection), p38 inhibitor (SB203580, 10 μM, 2 hours before infection) or DMSO (vehicle solution). Macrophages were stimulated for 6 hours with 1 x $10^5$ UV-killed *K. pneumoniae* 52145 and TNFα levels were determined in the supernatant of infected cells. ***, $P<0.0001$; versus DMSO control determined using the two-way Unpaired t-test.

| Primer Name | Sequence (5'-3')[a] |
|---|---|
| *mgrB*_UPFWD | ATA TCG CCC ATT CGT TGC CT |
| *mgrB*_UPRVS | GGA TCC CCG TGG TAA GAC ATT TTT CTG CC |
| *mgrB*_DWNFWD | GGA TCC TTC ACT CCA CTC TCC GCA TC |
| *mgrB*_DWNRVS | GGC TAA GCA TCT CTT TCC CA |
| *pmrC*_FWD | GAT CTC ATT GGT GTC GCA GCT CGG CGT GAA TTT GCG AAA CGT GTA GGC TGG AGC TGC TTC |
| *pmrC*_RVS | CGA AGA TGC CAC GCA GAA AGC GGT GAT GCA TAA CGC TCC CCA TAT GAA TAT CCT CCT TAG |
| *pagP*_FWD | TGT CCG GAA ACG CCA GCG CGT CGT TTT CAT CGA CCC TTA GGT GTA GGC TGG AGC TGC TTC |
| *pagP*_RVS | AAG TAA ACT TAC CGT TAT TGT AGG TGC CGG GAA TAT AGG TCA TAT GAA TAT CCT CCT TAG |
| *pmrC*_checkFWD | CCT TCA CTT TGC ATC GCC AG |
| *pmrC*_checkRVS | CCT GTT CAT TCT GCT CAG C |
| *pagP*_checkFWD | TTA AGC CTC CGA CGA ACA GG |
| *pagP*_checkRVS | GCA CAA GAC CTT CCG CTT AT |
| *lpxO*_UPFWD | CCC AGG CGC AGA TTG CCC AG |
| *lpxO*_UPRVS | GGA TCC CGG ATC CGG ACT CAC TAT AGG GCG GAT ATT GAA CGG CCG ATG |
| *lpxO*_DWNFWD | GGA TCC CGG ATC CGG ACT CAC TAT AGG GGC GGT AAA TGT GGA ATG GTC G |
| *lpxO*_DWNRVS | TCC GTT CAC TGC GTG CCC TG |
| *pmrAB*_UPFWD | CGA TCC TGG ATG CGG ATA AA |
| *pmrAB*_UPRVS | GGA TCC AAC GTC AGC ACC CAT TGA |
| *pmrAB*_DWNFWD | GGA TCC CGC ACA CGT ATC CTT CAC TT |
| *pmrAB*_DWNRVS | CAG AAC GTC ACC GAC TTG AA |
| *pmrF*_UPFWD | ACC CAT TAT AAA GGC CGC CA |
| *pmrF*_UPRVS | GGA TCC GGC AGG CTA TCC TGT TCG TT |
| *pmrF*_DWNFWD | GGA TCC TCG GCC GCA TCT ATA ACG AC |
| *pmrF*_DWNRVS | GTG CAC ATG CCG TAC ACT TC |
| *pagP*_UPFWD | CGG CTT CGA CGC TAA TTT |
| *pagP*_UPRVS | GGA TCC AGG TAT GGT AAC CTT CGC TA |
| *pagP*_DWNFWD | GGA TCC TTC CCG GCA CCT ACA ATA AC |
| *pagP*_DWNRVS | ATC CTG TGC ACC ATG CTA TC |
| *manC*_UPFWD | TCG AAA GTG CTG AGT GGT CC |
| *manC*_UPRVS | GGA TCC TCA AGC GGT GCT CTT CGT TAC AG |
| *manC*_DWNFWD | GGA TCC TAA CTG ACC GAG AAC CAG TCC AC |
| *manC*_DWNRVS | GCC TCG TCA TCG AAC AGG AAG |
| PhoPQ_checkFWD | ATC CTG ATG GCT GAC AAG GC |
| PhoPQ_checkRVS | TCG GGG ATA AAC GGT AGT GG |
| *lpxO*_checkFWD | CTC GGG GTT GTA CCA GAT CG |

*Continued*

| | |
|---|---|
| *lpxO*_checkRVS | TTA ACG GCG GCT TTT TCC TG |
| *pagP*_Pro_FWD | ATC GAA TTC GCT GAT ACC GAC ATT CAC CA |
| *pagP*_Pro_RVS | GGA TCC CTT CGC TAA GGG TCG ATG AAA |
| *lucFF*_checkFWD | GAA GCG AAG GTT GTG GAT CT |
| *lucFF*_checkRVS | AAC CGG GAG GTA GAT GAG AT |
| *pagP*_Pro_checkFWD | GTC GTT GAC ATC GAG CAT CT |
| *phoP*_Pro_checkRVS | CAG CGC CGG TTC GAT ATA AA |
| *lpxO*_Pro_checkFWD | CTG TCC CGT GAA TTC TCC AT |
| *pmrH*_Pro_checkFWD | CGG CTG GAA CTA CGA GAT TTA C |
| *pmrC*_Pro_checkRVS | CGT CGG CGA TGT TTG AGT AT |
| *cps*_Pro_checkRVS | CTA TCT TCC TGT GGC TGC TC |
| *mhPRT*-FWD | GAT CAG TCA ACG GGG GAC AT |
| *mHPRT*-RVS | GGT CCT TTT CAC CAG CAA GC |
| *mTNFα*-FWD | TTC TGT CTA CTG AAC TTC GGG GTG ATC GGT CC |
| *mTNFα*-RVS | GTA TGA GAT AGC AAA TCG GCT GAC GGT GTG GG |
| *mIFNβ*-FWD | ATG GTG GTC CGA GCA GAG AT |
| *mIFNβ*-RVS | CCA CCA CTC ATT CTG AGG |
| *mil-1β*-FWD | AGA TGA AGG GCT GCT TCC AAA |
| *mil-1β*-RVS | AAT GGG AAC GTC ACA GAC CA |
| *mil-6*-FWD | ATG GAT GCT ACC AAA CTG GAT |
| *mil-6*-RVS | TGA AGG ACT CTG GCT TTG TCT |
| *mil-10*-FWD | GGA CTT TAA GGG TTA CTT GGG TTG CC |
| *mil-10*-RVS | CAT GTA TGC TTC TAT GCA GTT GAT GA |
| *mil-12*-FWD | GGA AGC ACG GCA GCA GAA TA |
| *mil-12*-RVS | AAC TTG AGG GAG AAG TAG GAA TGG |
| mβ-defensin-1-FWD | AAC ACG GTA CAC AGG CTT CC |
| mβ-defensin-1-RVS | TCA CAG ATG TCC AAG TCC CA |
| mβ-defensin-4-FWD | CTC CAC TTG CAG CCT TTA CC |
| mβ-defensin-4-RVS | GTG CAT CCC CTA GAA CTG GA |
| mβ-defensin-14-FWD | ATC TTG TTC TTG GTG CCT GC |
| mβ-defensin-14-RVS | CTT CTT TCG GCA GCA TTT TC |

[a] Underlined text denotes a BamHI restriction site incorporated into the primer

**Appendix Table S4.**

Oligonucleotide primers used in this study

**Appendix References.**


Aubert D, Hamad M, Valvano M (2014) A markerless deletion method for genetic manipulation of *Burkholderia cenocepacia* and other multidrug-resistant gram-negative bacteria. In Host-Bacteria Interactions, Vergunst AC, O'Callaghan D (eds) pp 311-327. Springer New York

Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebize R, Diancourt L, Grimont P (2009) Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One 4**:** e4982

Choi KH, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, Schweizer HP (2005) A Tn7-based broad-range bacterial cloning and expression system. Nat Methods 2**:** 443-448

Crépin S, Harel J, Dozois CM (2012) Chromosomal complementation using Tn7 transposon vectors in Enterobacteriaceae. Appl Environ Microbiol 78**:** 6001-6008

Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A 97**:** 6640-6645

Demarre G, Guerout AM, Matsumoto-Mashimo C, Rowe-Magnus DA, Marliere P, Mazel D (2005) A new family of mobilizable suicide plasmids based on broad host range R388 plasmid (IncW) and RP4 plasmid (IncPalpha) conjugative machineries and their cognate *Escherichia coli* host strains. Res Microbiol 156**:** 245-255

Derbise A, Lesic B, Dacheux D, Ghigo JM, Carniel E (2003) A rapid and simple method for inactivating chromosomal genes in Yersinia. FEMS Immunol Med Microbiol 38**:** 113-116

Favre D, Viret JF (2000) Gene replacement in gram-negative bacteria: the pMAKSAC vectors. Biotechniques 28**:** 198-200, 202, 204

Gunn JS, Miller SI (1996) PhoP-PhoQ activates transcription of *pmrAB*, encoding a two-component regulatory system involved in *Salmonella typhimurium* antimicrobial peptide resistance. J Bacteriol 178**:** 6857-6864

Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP (1998) A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. Gene 212**:** 77-86

Insua JL, Llobet E, Moranta D, Perez-Gutierrez C, Tomas A, Garmendia J, Bengoechea JA (2013) Modeling *Klebsiella pneumoniae* pathogenesis by infection of the wax moth *Galleria mellonella*. Infect Immun 81**:** 3552-3565

Lery LM, Frangeul L, Tomas A, Passet V, Almeida AS, Bialek-Davenet S, Barbe V, Bengoechea JA, Sansonetti P, Brisse S, Tournebize R (2014) Comparative analysis of *Klebsiella pneumoniae* genomes identifies a phospholipase D family protein as a novel virulence factor. BMC Biol 12**:** 41

Llobet E, Campos MA, Gimenez P, Moranta D, Bengoechea JA (2011) Analysis of the networks controlling the antimicrobial-peptide-dependent induction of *Klebsiella pneumoniae* virulence factors. Infect Immun 79**:** 3718-3732

Llobet E, Martinez-Moliner V, Moranta D, Dahlstrom KM, Regueiro V, Tomas A, Cano V, Perez-Gutierrez C, Frank CG, Fernandez-Carrasco H, Insua JL, Salminen TA, Garmendia J,

Bengoechea JA (2015) Deciphering tissue-induced *Klebsiella pneumoniae* lipid A structure. Proc Natl Acad Sci U S A 112**:** E6369-6378

Miller VL, Mekalanos JJ (1988) A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J Bacteriol 170**:** 2575-2583

Poirel L, Jayol A, Bontron S, Villegas MV, Ozdamar M, Turkoglu S, Nordmann P (2015) The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. J Antimicrob Chemoth 70: 75-80