

**CrrAB regulates PagP-mediated glycerophosphoglycerol palmitoylation in the outer membrane of  
*Klebsiella pneumoniae***

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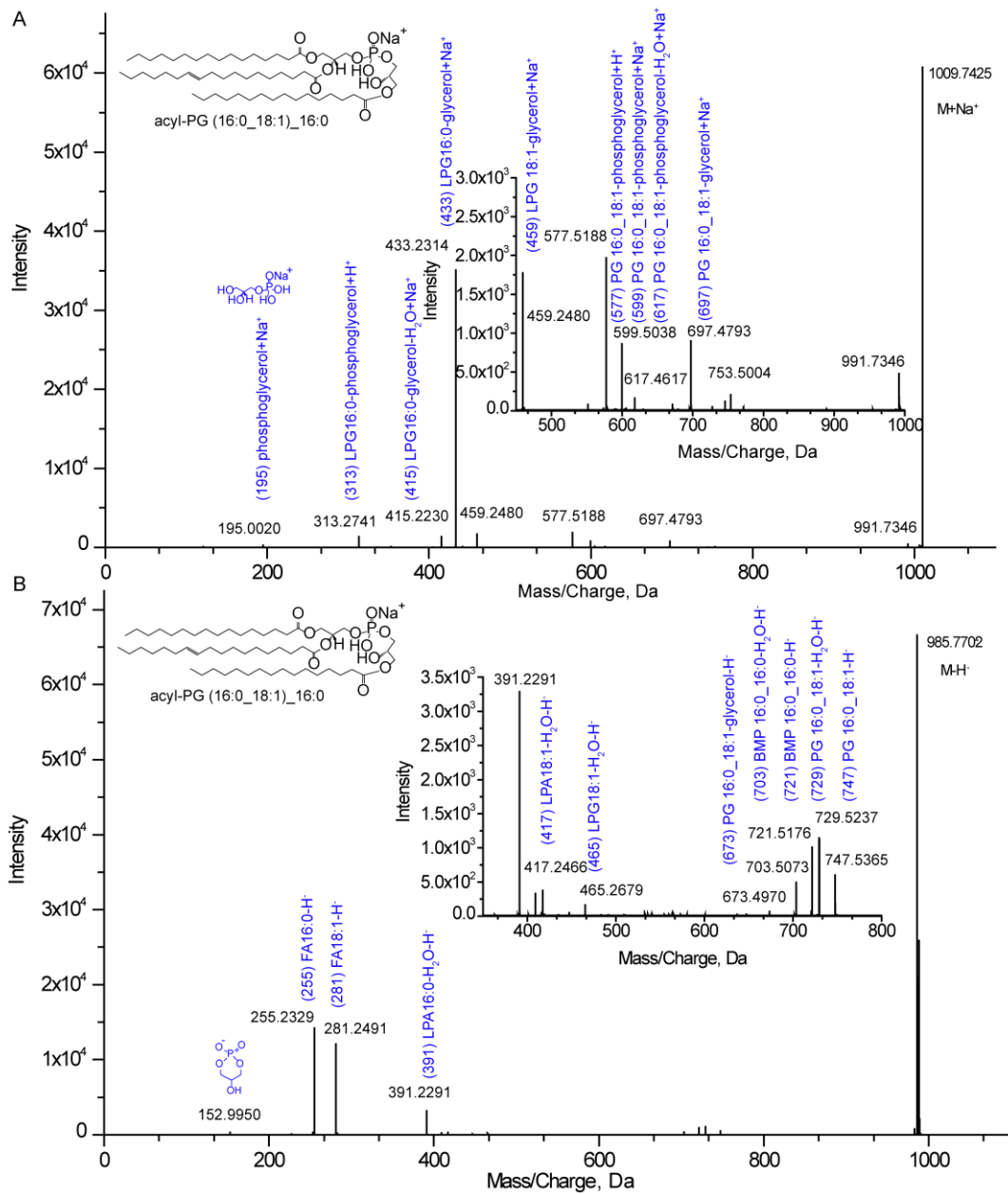


Fig. S1. The representative annotation of acyl-PG based on MS2 spectra in both positive (A) and negative (B) modes.

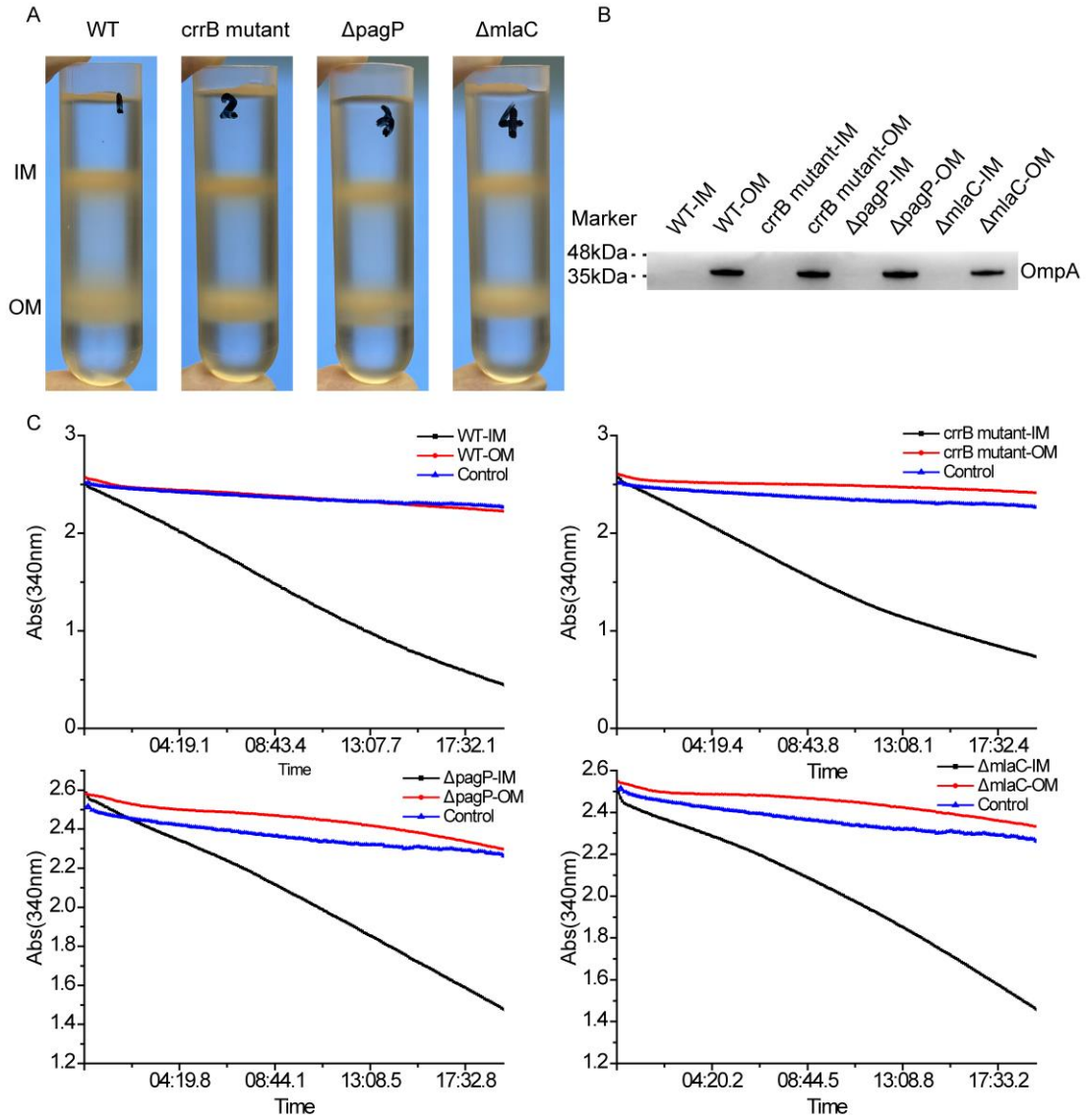


Fig. S2. The purity of IM and OM separation. (A) Representative results for the separation of IM and OM. (B) Western blot for the OM-localized OmpA protein indicated the purity of IM. (C) NADH dehydrogenase assay revealed the purity of OM.

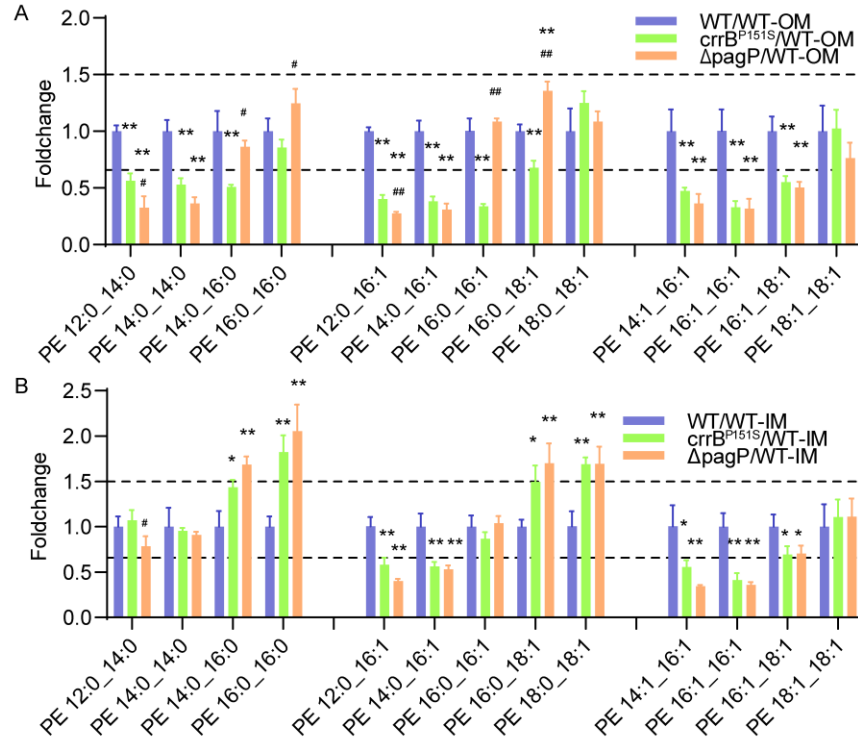


Fig. S3. The alterations of phosphatidylethanolamine contents within the outer and inner membrane. (A) Decreased OM PE contents with a palmitate chain in the *crrB* mutant were increased towards the level of the wild type, while that without a palmitate chain showed a negligible alteration after *pagP* knockout. (B) PagP inactivation had a minor impact on the PE content with the inner membrane in the *crrB* mutant. The experiment was repeated three times with four technical replicates each and the data was in Supplementary File 4. \*(p-value<0.05) or \*\*(p-value<0.01) indicates a significance of *crrB*<sup>P151S</sup> or  $\Delta$ *pagP* compared to WT. #(p-value<0.05) or ##(p-value<0.01) shows a significance of  $\Delta$ *pagP* in comparison to *crrB*<sup>P151S</sup>.

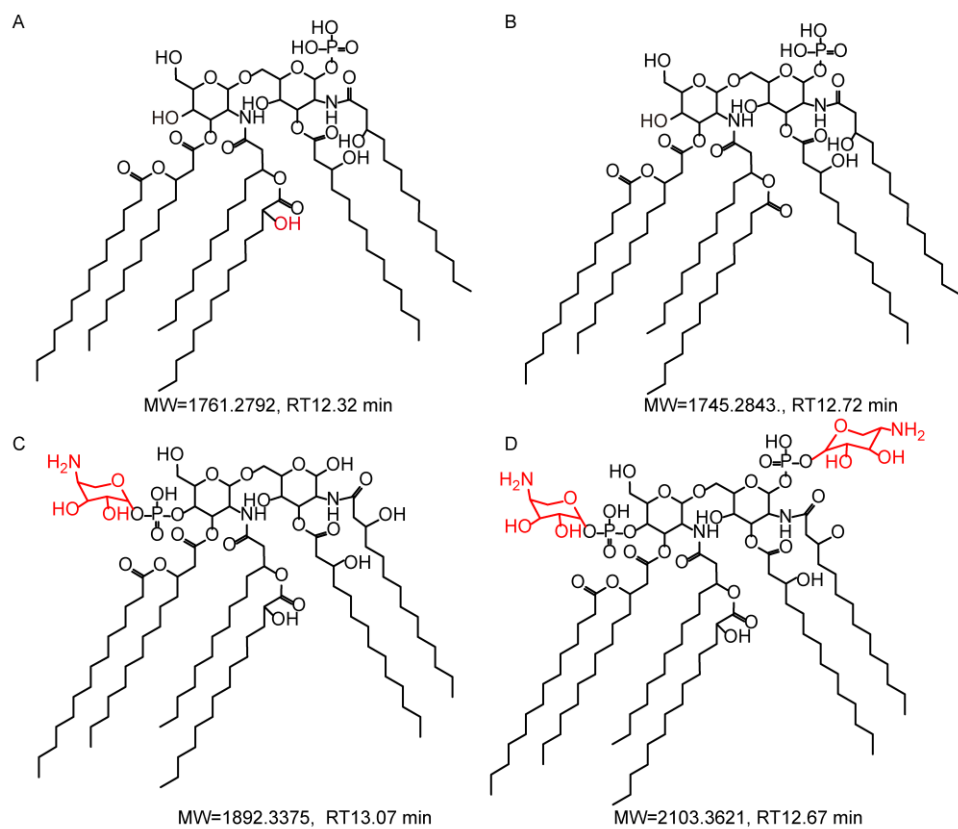


Fig. S4. The representative structures of lipid A correspond to the peaks at the retention time of 12.32 (A), 12.72 (B), 13.07 (C) and 12.67 min (D).

Table S1. Primers for gene knockout used in the study

Primer	Sequence (5'to 3')	nucleotide
pagP-spacer-F2	tagtTTTAATAAGTGGGAGCCCAT	pagP spacer for gene deletion
pagP-spacer-R2	aaacATGGGCTCCCACTTATTA	pagP spacer for gene deletion
pagP-LF	ACCGACATTCACCATTACC	amplification of <i>pagP</i> for validation
pagP-LR	TGACCTTCAGCCAGAGTT	amplification of <i>pagP</i> for validation
pagP-template	CACAAGACCTTCCGCTTATCTCATTATTGC TAAGACTTAGCGAACAACTTTTCTATTAC CACTTTGATTTAAAAGCCACCTAAAATA	90 nt <i>pagP</i> donor template for gene deletion
m1aC-spacer-F2	tagtCGGTACGTCTTGACTTCCAG	<i>m1aC</i> spacer for gene deletion
m1aC-spacer-R2	aaacCTGGAAGTCAAGACGTACCG	<i>m1aC</i> spacer for gene deletion
m1aC-LF	CTGGTGAAGTGCCTGATTA	amplification of <i>m1aC</i> for validation
m1aC-LR	GTAGATGCTCTGCTGCTT	amplification of <i>m1aC</i> for validation
m1aC-template	ACGCCGGCAGCCGGCACGACGCATTAATTT CAGGAGAAATACGATTGAGCGGGCAGCTG AGCTGGACTAGCGAGGGCGAGACGCTGGC GC	90 nt <i>m1aC</i> donor template for gene deletion

Table S2. The fragmentation patterns of the identified lipids based on MRM on negative mode

ID	Q1	Q3	CE	ID	Q1	Q3	CE
acyl-PG 42:0	875.638	227.202	-50	PG 34:1	747.518	281.249	-40
acyl-PG 44:0	903.67	227.202	-50	PG 36:1	775.549	281.249	-40
acyl-PG 46:0	931.701	255.233	-50	PG 30:2	689.44	253.217	-40
acyl-PG 48:0	959.732	255.233	-50	PG 32:2	717.471	253.217	-40
acyl-PG 50:0	987.763	255.233	-50	PG 34:2	745.503	253.217	-40
acyl-PG 42:1	873.623	253.217	-50	PG 36:2	773.534	281.249	-40
acyl-PG 44:1	901.654	253.217	-50	LPE 14:0	424.247	227.202	-40
acyl-PG 46:1	929.685	253.217	-50	LPE 14:1	422.231	225.186	-40
acyl-PG 48:1	957.717	255.233	-50	LPE 16:0	452.278	255.233	-40
acyl-PG 50:1	985.748	255.233	-50	LPE 16:1	450.263	253.217	-40
acyl-PG 52:1	1013.779	281.249	-50	LPE 18:0	480.31	283.264	-40
acyl-PG 44:2	899.638	253.217	-50	LPE 18:1	478.294	281.249	-40
acyl-PG 46:2	927.67	253.217	-50	PE 26:0	606.414	227.202	-40
acyl-PG 48:2	955.701	253.217	-50	PE 28:0	634.445	227.202	-40
acyl-PG 50:2	983.732	253.217	-50	PE 30:0	662.477	227.202	-40
acyl-PG 52:2	1011.763	281.249	-50	PE 32:0	690.508	255.233	-40
acyl-PG 54:2	1039.795	281.249	-50	PE 28:1	632.43	253.217	-40
acyl-PG 46:3	925.654	253.217	-50	PE 30:1	660.461	253.217	-40
acyl-PG 48:3	953.685	253.217	-50	PE 32:1	688.492	253.217	-40
acyl-PG 50:3	981.717	253.217	-50	PE 34:1	716.524	281.249	-40
acyl-PG 52:3	1009.748	281.249	-50	PE 36:1	744.555	281.249	-40
acyl-PG 54:3	1037.779	281.249	-50	PE 30:2	658.445	253.217	-40
LPG 16:0	483.273	255.233	-50	PE 32:2	686.477	253.217	-40

ID	Q1	Q3	CE	ID	Q1	Q3	CE
LPG 16:1	481.257	253.217	-40	PE 34:2	714.508	253.217	-40
LPG 18:1	509.288	281.249	-40	PE 36:2	742.539	281.249	-40
PG 30:0	693.471	227.202	-40	16:0-d31- 18:1 PE	747.718	281.249	-40
PG 32:0	721.503	255.233	-40	16:0-d31- 18:1 PG	778.713	281.249	-40
PG 28:1	663.424	253.217	-40	Palmitic acid- d31	286.593	286.593	-40
PG 30:1	691.456	253.217	-40	16:0 LPE-d9	461.335	264.289	-40
PG 32:1	719.487	253.217	-40				