Supporting Information

Identification of multiple serine hydrolases involved in virulence and cell envelope integrity of *Klebsiella pneumoniae*

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Supplementary tables

Table S1. List of bacterial strains used in this study.

Strain	Description	Reference/Source
K. pneumoniae MP103	KPNIH1 derivative with the KPC-3 carbapenemase-encoding gene deleted and Parent strain of Moinul Transposon Mutant Library	[1]
degP:Tn	Transposon insertion mutant in MKP103 KPNIH1_04540	
ychK:Tn	Transposon insertion mutant in MKP103 KPNIH1_15760	
ybfF:Tn	Transposon insertion mutant in MKP103 KPNIH1_07725	
catD:Tn	Transposon insertion mutant in MKP103 KPNIH1_12245	
pldB:Tn	Transposon insertion mutant in MKP103 KPNIH1_00895	
degQ:Tn	Transposon insertion mutant in MKP103 KPNIH1_23755	
yjfP:Tn	Transposon insertion mutant in MKP103 KPNIH1_02230	

Table S2. List of serine hydrolases identified in *K. pneumoniae* by MS-ABPP

Gene	Previous annotation	MW [kDa]	Strain KPNIH1 locus tag
degP	Serine endoprotease	49,5	KPNIH1_04540
ychK	Patatin-like phospholipase	33,3	KPNIH1_15760
ybfF	Acyl-CoA esterase	28,5	KPNIH1_07725
catD	3-oxoadipate enol-lactonase	27,3	KPNIH1_12245
pldB	Lysophospholipase L2	38,2	KPNIH1_00895
degQ	Serine endoprotease	47,2	KPNIH1_23755
YqiA	Esterase	21,5	KPNIH1_22780
YcfP	Esterase	21,1	KPNIH1_09915
bioH	Pimeloyl-ACP methyl ester esterase	28,3	KPNIH1_24570
yjfP	Esterase	26,5	KPNIH1_02230

Table S3. The homology percentage data used for Figure S1.

All BLASTp results for a homolog of the K. pneumoniae SHs are provided in a separate Excel file

	DegP	YchK	YbfF	CatD	PldB	DegQ	YqiA	YcfP	BioH	YjfP
Bifidobacterium adolescentis ATCC 15703	0	0	0	0	0	0	0	0	0	28.4
Bifidobacterium longum NCC2705	0	0	26.6	24.3	0	0	0	0	0	27.4
Collinsella aerofaciens ATCC 25986	0	0	0	0	0	0	0	0	0	24.8
Bacteroides caccae ATCC 43185	0	0	0	0	0	0	0	0	0	
Bacteroides fragilis ATCC 25285	0	0	0	0	0	0	0	0	0	25.7
Bacteroides ovatus ATCC 8483	0	0	0	0	0	0	0	0	20.0	24.4
Parabacteroides distasonis ATCC 8503	0	0	0	0	0	0	0	0	0	
Prevotella copri DSM 18205	0	0	0	0	0	0	0	0	0	41.3
Clostridium sporogenes ATCC 15579	0	0	0	0	0	0	0	0	0	0
Enterococcus faecalis V583	0	0	26.6	0	0	0	0	0	23.6	0
Enterococcus faecium ATCC BAA-472	0	0	0	0	0	0	0	0	29.3	0
Lactobacillus ruminis ATCC 25644	0	0	0	0	0	0	0	0	0	0
Ruminococcus gnavus ATCC 29149	0	0	0	0	0	0	0	0	0	0
Fusobacterium nucleatum subsp. nucleatum ATCC 23726	0	0	0	0	0	0	0	0	0	0
Edwardsiella tarda ATCC 23685	77.9	0	0	0	0	62.0	0		0	0
Enterobacter cancerogenus ATCC 35316	91.9	0	0	0	84.9	82.0	0	90.0	0	0
Escherichia coli K12 MG1655	90.2	81.1	0	24.7	79.7	0	0	0	0	0
Klebsiella pneumoniae subsp. pneumoniae KPNIH1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Akkermansia muciniphila ATCC BAA-835	0	0	0	0	0	0	0	0	0	0
Homo sapiens	0	0	0	0	25.2				30.2	

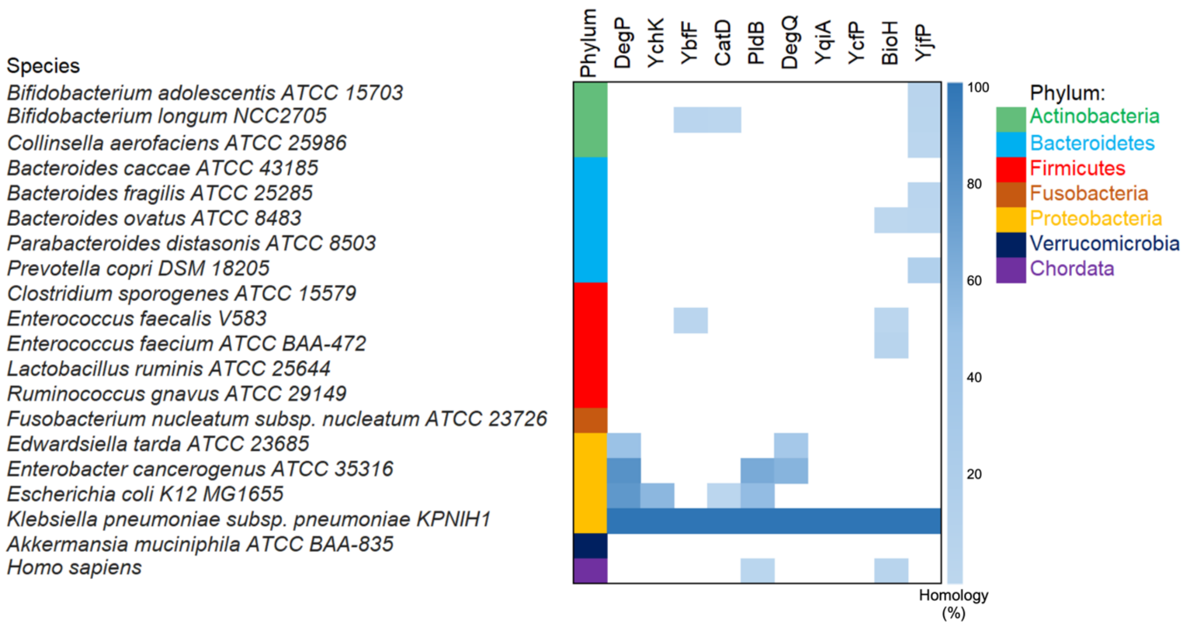
Table S4. Collection statistics of x ray crystallography datasets used to determine the structures of YjfP and YqiA. Values in parentheses refer to the high-resolution shell.

	YjfP	**YqiA**
Beamline	Australian Synchrotron MX2	Australian Synchrotron MX2
Wavelength (Å / keV)	0.954 / 13.00	0.954 / 13.00
Detector	DECTRIS EIGER X 16M	DECTRIS EIGER X 16M
Space group	*C* 2	$P\,3_2\,2$
a, b, c (Å)	102.71 68.20 65.36	62.46 62.46 80.36
α, β, γ (°)	90.00 77.16 90.00	90.00 90.00 120
Rotation range (°)	250	310
Resolution (Å)	45.11-1.30 (1.32-1.30)	44.87-1.50 (1.53-1.50)
Total Reflections	513,218 (23,779)	507,705 (23,786)
Unique Reflections	106,974 (5,160)	29,609 (1,434)
Multiplicity	4.8 (4.6)	17.1 (16.60)
Completeness (%)	99.2 (97.4)	100.00 (100.00)
I/σ(I)	9.6 (1.7)	13.8 (1.7)
$CC_{1/2}$	0.998 (0.369)	0.999 (0.568)
R_{merge}	0.115 (2.702)	0.121 (5.951)
R_{pim}	0.088 (2.083)	0.043 (2.148)

Table S5. Refinement and validation statistics of YjfP and YqiA crystal structures.

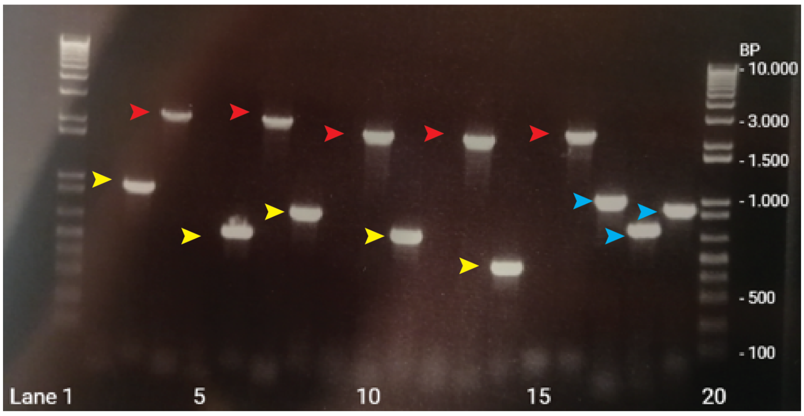
	YjfP	**YqiA**
PDB ID	9BD4	9BI7
Resolution Range (Å)	45.11-1.30	44.87-1.50
Reflections, working	101,407	28,024
Reflections, free	5,336	1,528
R_{work} (%)	16.13	17.46
R_{free} (%)	17.48	21.31
Number of residues	479	192
Number of waters	305	94
Ligand		2 Ca, 1 Cl
Average *B* factors (Å^2)	16.22	30.84
Ligands		23.88
Waters	25.01	33.35
RMSD		
Bonds (Å)	0.006	0.009
Angles (°)	0.89	1.00
Ramachandran		
Favoured (%)	97.89	98.42
Outliers (%)	0	0
Rotamer outliers (%)	0.25	0
Molprobity score	0.88	1.08

Supplementary figures



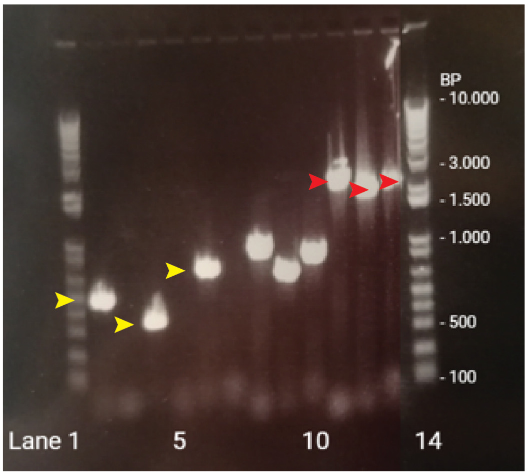
Supplementary figure S1. Homology analysis of *K. pneumoniae* serine hydrolases. Heatmap of homologs of *K. pneumoniae* serine hydrolases across 20 representative gut commensal bacterial species and in humans (Chordata), as sourced from the Human Microbiome Project Reference Genomes for the Gastrointestinal Tract database using BLAST-P. In the heatmap, each filled cell indicates that the species has a homolog of the *K. pneumoniae* SHs, as determined by a threshold e-value of 1×10^{-10}; white background denotes the absence of a homolog. The underlying percentage homology data is listed in table S3.

A



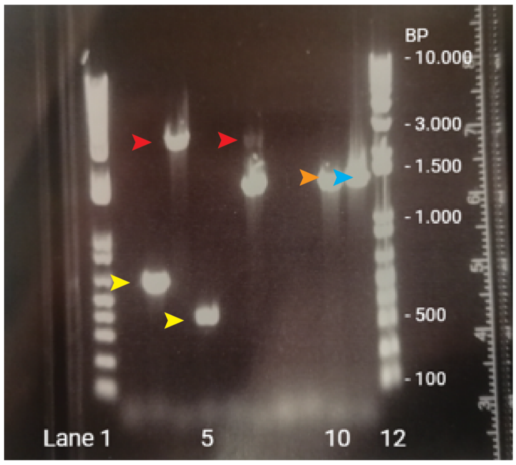
1. 1 Kb ladder
2. *pld*B forward + PMC-140 (*pld*B1 lysate)
3. *pld*B reverse + PMC-140 (*pld*B1 lysate)
4. *pld*B forward + *pld*B reverse (*pld*B1 lysate)
5. *pld*B forward + PMC-140 (*pld*B3 lysate)
6. *pld*B reverse + PMC-140 (*pld*B3 lysate)
7. *pld*B forward + *pld*B reverse (*pld*B3 lysate)
8. *yfj*P forward + PMC-140 (*yfj*P1 lysate)
9. *yfj*P reverse + PMC-140 (*yfj*P1 lysate)
10. *yfj*P forward + *yfj*P reverse (*yfj*P1 lysate)
11. *yfj*P forward + PMC-140 (*yfj*P3 lysate)
12. *yfj*P reverse + PMC-140 (*yfj*P3 lysate)
13. *yfj*P forward + *yfj*P reverse (*yfj*P3 lysate)
14. *ych*K forward + PMC-140 (*ych*K1 lysate)
15. *ych*K reverse + PMC-140 (*ych*K1 lysate)
16. *ych*K forward + *ych*K reverse (*ych*K1 lysate)
17. *pld*B forward + *pld*B reverse (MKP-103 lysate)
18. *yfj*P forward + *yfj*P reverse (MKP-103 lysate)
19. *ych*K forward + *ych*K reverse (MKP-103 lysate)
20. 1 Kb ladder

B



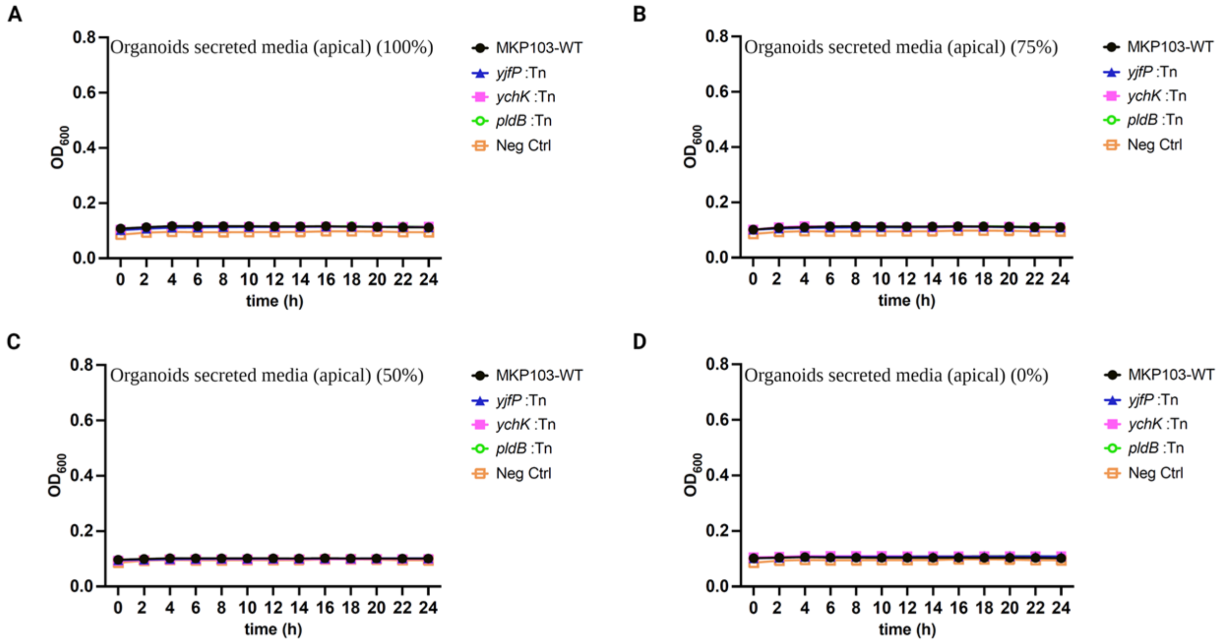
1. 1 Kb ladder
2. *pld*B forward + PMC-140 (*pld*B2 lysate)
3. *pld*B reverse + PMC-140 (*pld*B2 lysate)
4. *yfj*P forward + PMC-140 (*yfj*P2 lysate)
5. *yfj*P reverse + PMC-140 (*yfj*P2 lysate)
6. *ych*K forward + PMC-140 (*ych*K2 lysate)
7. *ych*K reverse + PMC-140 (*ych*K2 lysate)
8. *pld*B forward + *pld*B reverse (MKP-103 lysate)
9. *yfj*P forward + *yfj*P reverse (MKP-103 lysate)
10. *ych*K forward + *ych*K reverse (MKP-103 lysate)
11. *pld*B forward + *pld*B reverse (*pld*B2 lysate)
12. *yfj*P forward + *yfj*P reverse (*yjf*P2 lysate)
13. *ych*K forward + *ych*K reverse (*ych*K2 lysate)
14. 1 Kb ladder

C

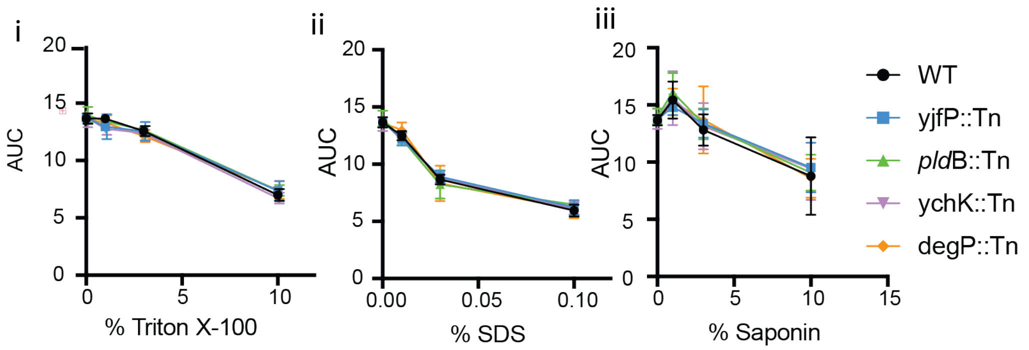


1. 1 Kb ladder
2. *deg*P forward + PMC-140 (*deg*P1 lysate)
3. *deg*P reverse + PMC-140 (*deg*P1 lysate)
4. *deg*P forward + *deg*P reverse (*deg*P1 lysate)
5. *deg*P forward + PMC-140 (*deg*P2 lysate)
6. *deg*P reverse + PMC-140 (*deg*P2 lysate)
7. *deg*P forward + *deg*P reverse (*deg*P2 lysate)
8. *deg*P forward + PMC-140 (*deg*P3 lysate)
9. *deg*P reverse + PMC-140 (*deg*P3 lysate)
10. *deg*P forward + *deg*P reverse (*deg*P3 lysate)
11. *deg*P forward + *deg*P reverse (MKP-103 lysate)
12. 1Kb ladder

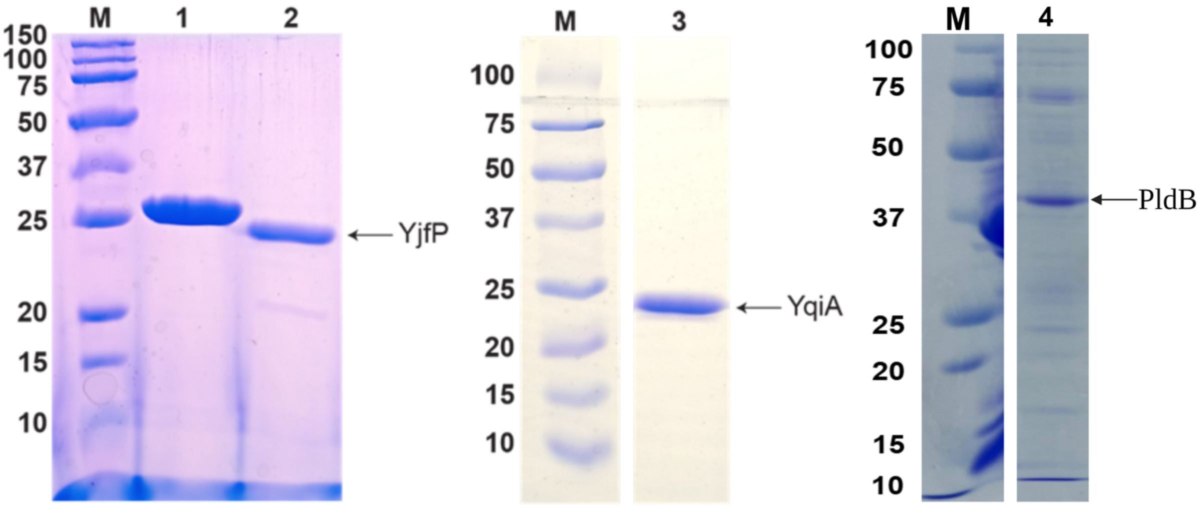
Supplementary figure S2. PCR validation of transposon mutants. PCR products were analysed by agarose gel electrophoresis and stained with GelRed Nucleic acid stain and visualized by UV-light. The correct insertion of the transposon into the pldB, yjfP, was investigated by three PCR reactions using DNA within cell lysates as a template : i) using combination a combination of a gene-specific forward or ii) reverse primer each in combination with a transposon-specific primer (PMC-140) that should give a band only if the transposon is correctly inserted into the gene (as indicated by yellow arrowhead) iii) A gene-specific PCR with gene-specific forward and reverse primers that in the case of transposon insertion should lead to an increase in amplicon length of approx 1.3 kbp (correct length indicated by red arrowheads). Amplicon size for intact genes are: *pld*B: 993 bp, *yjf*P: 717 bp; *ych*K: 903 bp; *deg*P: 1581 bp (blue arrowheads). PCR products were analysed by agarose gel electrophoresis and stained with GelRed Nucleic acid stain and visualized by UV-light. A) Strains *pld*B::Tn1, *pld*B::Tn3, *yjf*P::Tn1, *yjf*P::Tn3, *ych*K::Tn1, and WT; B) *pld*B::Tn2, *yjf*P::Tn2, *ych*K::Tn2, and WT; C) *deg*P::Tn1; *deg*P::Tn2; *deg*P::Tn3 and WT. For strain *deg*P::Tn3, neither PCR reaction with a combination of transposon and gene-specific primers (lane 8,9) yielded a product, whereas amplification of the *deg*P gene yielded a product of the length as for the WT amplicon (orange arrowhead, lane 10) suggesting that the *deg*P gene is intact in this strain.



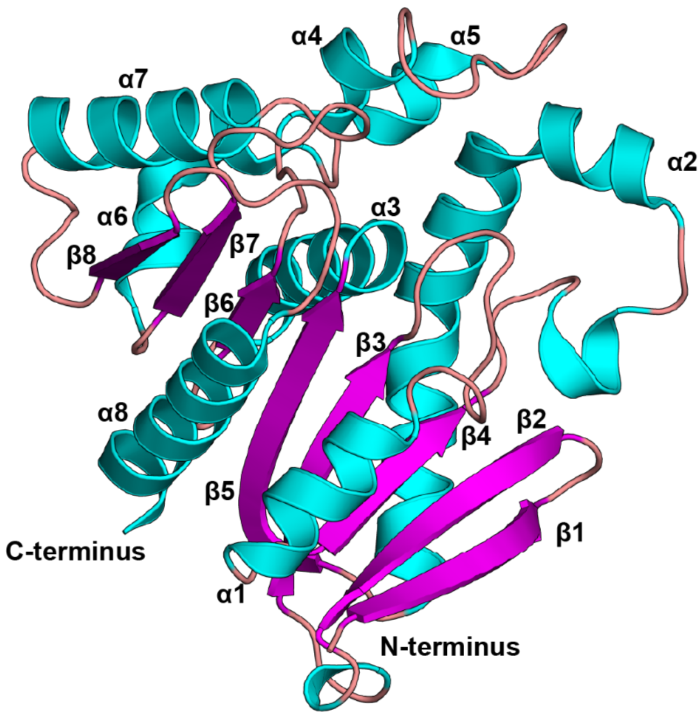
Supplementary figure S3. Growth curves of *K. pneumoniae* MKP103 and isogenic SH-deficient transposon mutant on organoids secreted substances. The growth curves of *K. pneumoniae* and SH-deficient mutants were measured over 24 hours in: **A**) media containing 100% secretions from organoid monolayers (collected from the apical side), **B**) media with 75% organoid secretions, **C**) media with 50% organoid secretions, and **D**) media without organoid secretions (consisting only of differentiated organoid media). Growth curves in A-D show means ± standard deviation of n=3 independent biological culture replicates



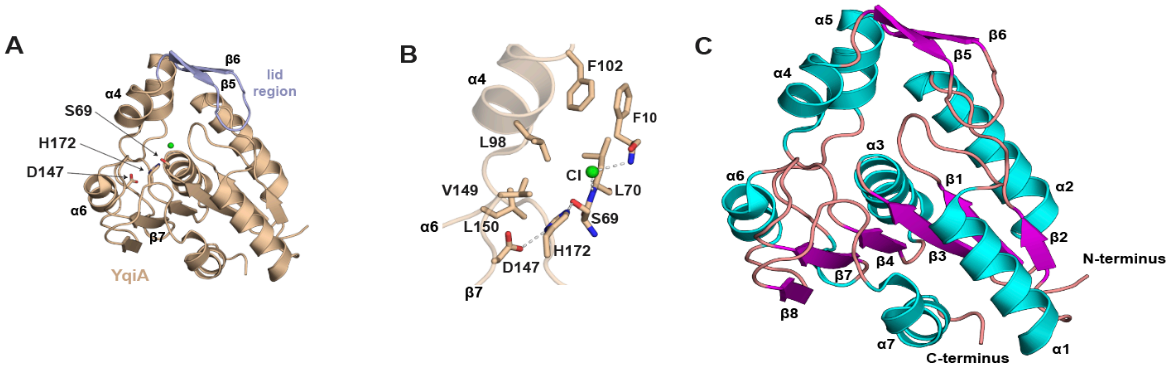
Supplementary figure S4. Detergent susceptibility of SH-deficient transposon mutants. WT or indicated transposon mutant strains were cultivated in microplates in the presence of different concentrations of detergent (i) Triton X-100, ii) SDS, iii) saponin) and growth was monitored by OD600 measurements over 24h. Area under the curve analysis was performd to quantify detergent-induced growth impairment. The graph shows means ± standard deviation of n=3 independent biological replicate cultures (recorded with three technical replicates each.)



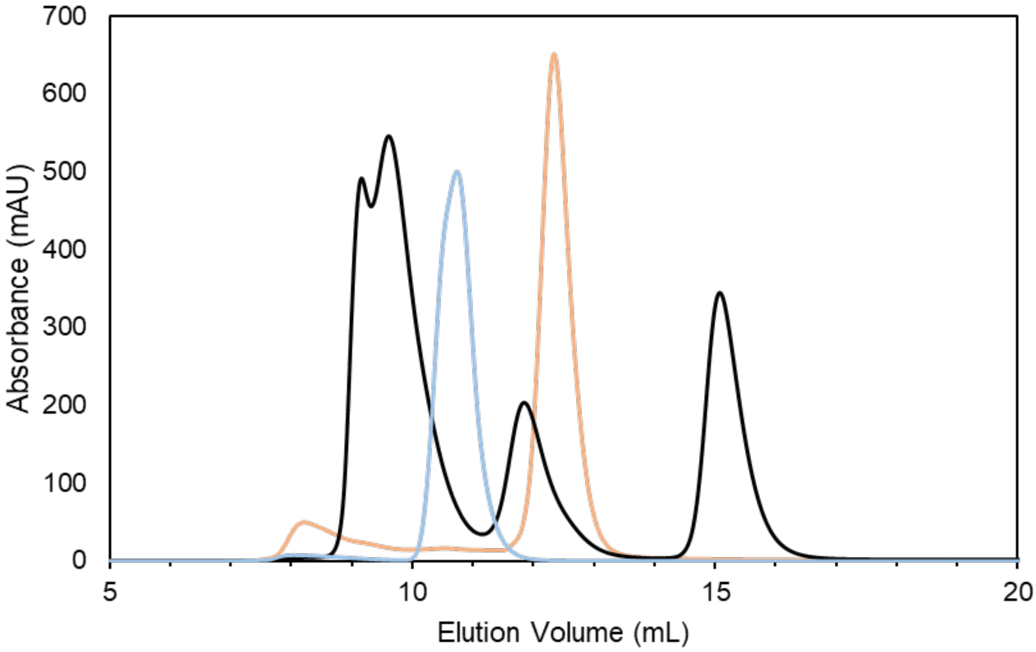
Supplementary figure S5. SDS PAGE of purified YjfP, YqiA, and His6-PldB. YjfP, YqiA, and PldB purity were assessed by loading onto SDS PAGE gels of 12 % polyacrylamide. Molecular weight markers are in lanes labelled M and the sizes of the bands (in kDa) indicated. His_6 YjfP was loaded into lane 1, YjfP after 3C cleavage loaded into lane 2, YqiA loaded into lane 3, and His_6-PldB loaded into lane 4. The predicted masses after cleavage of YjfP, YqiA, and PldB are 27 kDa, 22 kDa, and 38 kDa, respectively.



Supplementary figure S6. **Secondary structures of YjfP**. Secondary structure elements are displayed as follows: α helices (cyan), β sheets (magenta), and loops (beige).



Supplementary figure S7. **Structural analysis of YqiA. A**) YqiA (beige) crystallised as a monomer, where β strands 4 and 5 form a lid region. The catalytic triad (Ser69, Asp147, His172) is more expose than in the YjfP structure. **B)** Interactions between side chains of the catalytic triad are shown. A chlorine atom (green) is modelled in the oxyanion hole coordinated by backbone amides of Leu70 and Phe10. Several hydrophobic side residues on α helix 4 and a loop between β strand 7 and α helix 6 are likely important for substrate recognition. **C**) Secondary structure elements are displayed as follows: α helices (cyan), β sheets (magenta), and loops (beige).



Supplementary figure S8. **Gel-filtration chromatogram of YjfP and YqiA**. Gel filtration of YjfP (cyan), YqiA (orange), and protein standards (black). The protein standards run include thyroglobulin (670 kDa), bovine gamma globulin (158 kDa), chicken ovalbumin (44 kDa), and equine myoglobin (17 kDa). YjfP elutes after bovine gamma globulin and before chicken ovalbumin, consistent with a 54 kDa homodimer. YqiA elutes after chicken ovalbumin consistent with a 22 kDa monomer.

Supplementary references

1. Ramage B, *et al.* Comprehensive Arrayed Transposon Mutant Library of Klebsiella pneumoniae Outbreak Strain KPNIH1. *J Bacteriol* **199**, (2017).