

Figure S1. The structures of Cpd9, Cpd36 and Cpd46.

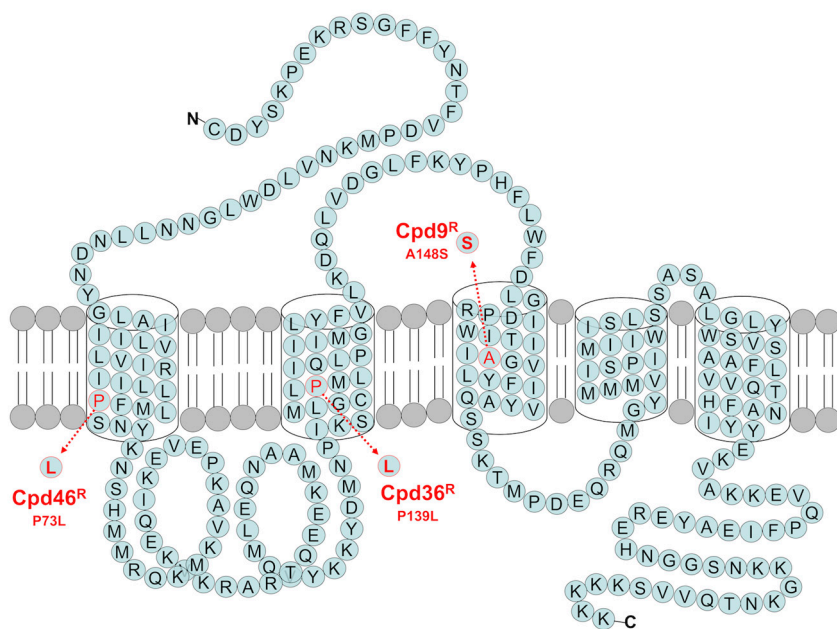


Figure S2. Missense mutations identified in YidC2 of drug-resistant *S. aureus* isolates.

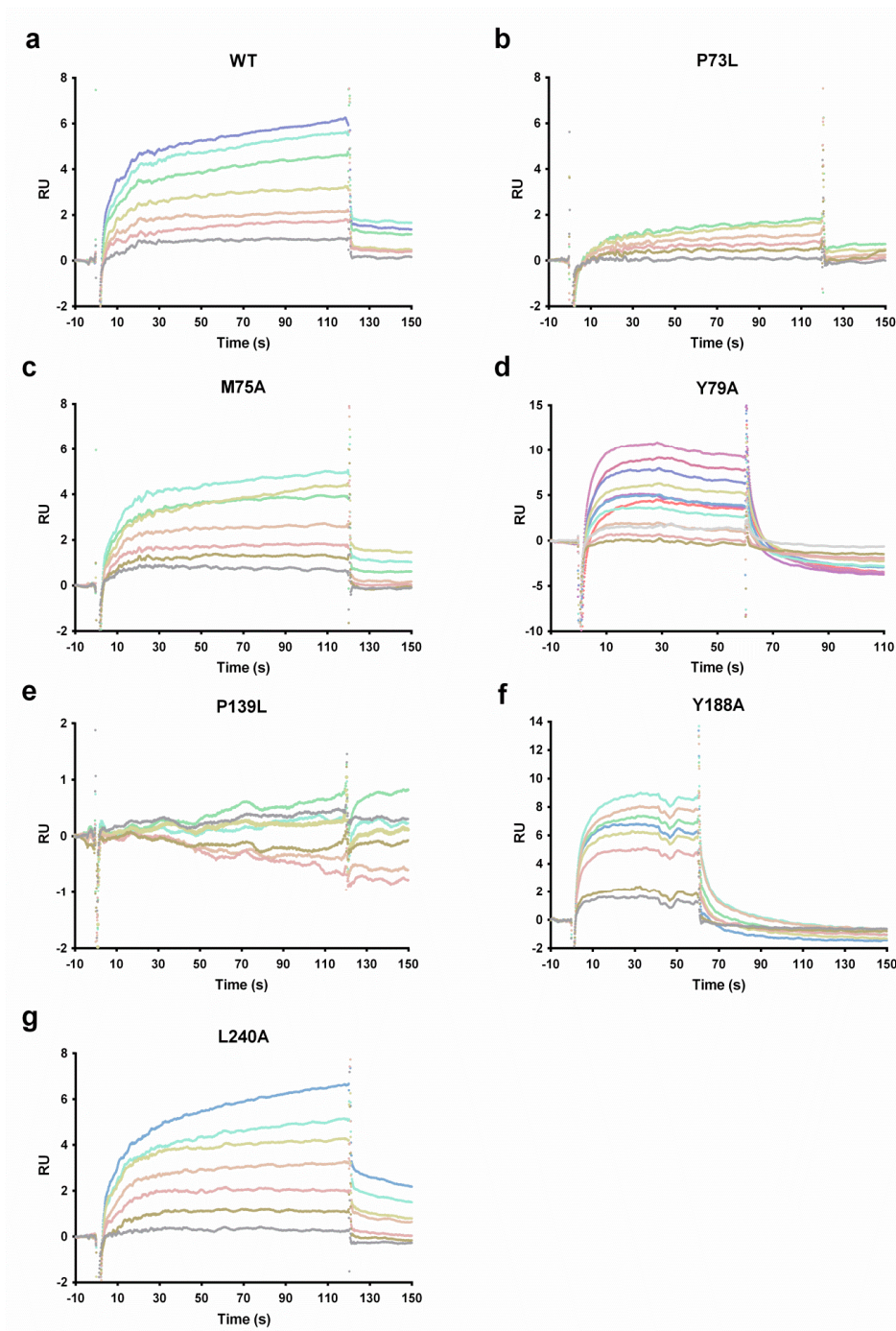


Figure S3. Steady-state analysis of SPR data: affinity values for SaYidC2 variants with Cpd36. Fitting of steady-state responses from SaYidC2-Cpd36 interaction measured by surface plasmon resonance. The steady-state signal reached at the end of the analyte injection (120 seconds at 30 μ l/minute) was plotted against the analyte concentration and the resulting curve fitted with a 1:1 binding model.

Table S1. Bacterial strains used in this study.

Bacterial strain	Source
<i>Acinetobacter baumannii</i> BCRC80276	Bioresource Collection and Research Center (Taiwan)
<i>S. aureus</i> BCRC12552 (a.k.a. NCTC8325)	
<i>Bacillus cereus</i> ATCC11778	American Type Culture Collection (United States)
<i>Enterococcus faecium</i> ATCC35667	
<i>Escherichia coli</i> ATCC25922	
<i>Listeria monocytogenes</i> ATCC19113	
MRSA ATCC33592	
<i>Staphylococcus aureus</i> ATCC12598	
<i>Staphylococcus epidermidis</i> ATCC12228	
<i>Staphylococcus intermedius</i> ATCC29663	
<i>Staphylococcus haemolyticus</i> ATCC29970	
<i>Staphylococcus hominis</i> ATCC27844	
<i>Staphylococcus saprophyticus</i> ATCC15305	
<i>Streptococcus pyogenes</i> ATCC19615	
<i>Streptococcus agalactiae</i> ATCC13813	
<i>Salmonella</i> Typhimurium ATCC14028	
<i>Bacillus subtilis</i> 168	Bacillus Genetic Stock Center (United States)
<i>Staphylococcus lugdunensis</i>	National Taiwan University Hospital
vancomycin-resistant <i>Enterococcus faecium</i>	

Table S2. Primers used in this study.

Name	Sequence (5'→3')
Primers for mutation validation	
CDS789-F	tgttgatcctatcccgaag
CDS789-R	caaacaacccaaacactgcga
CDS792-F	aaagcacaacaaggagtgtg
CDS792-R	actgcagcaaattgcaaaattag
CDS936-F	tgcatgttatactagcgatgc
CDS936-R	cgttcagcaagttcttctgc
CDS2037(YidC2)-F	tccactattgtttcatacgtactc
CDS2037(YidC2)-R	agcgaaaaataatgtgcgcc
CDS2050-F	ggtagtgttagaaatgatctgtcca
CDS2050-R	tcgagtatctatttcttaagagaagc
CDS2053-F	aaactgcagatgaatttaatcgcagcagca
CDS2053-R	aaagaattcttatccagcaaatgtcatgaatg
Primers for plasmid construction	
yidC2-P73L-F	tagtttgataacatgaatagtaacaagataacacgaa
yidC2-P73L-R	attcgtgttatcttgttactattcatgttatcaactat
yidC2-M75A-F	actattttatagtttgataacgcgaatggtaacaagataa
yidC2-M75A-R	gtgttatcttgttaccattcgcgttatcaactataaaaatag
yidC2-Y79A-F	gcatcatatgactattttagcgtttgataacatgaatggta
yidC2-Y79A-R	gttaccattcatgttatcaaacgctaaaaatagtcatatgatg
yidC2-P139L-F	catttgaattaatactagtaaacagcctaatagcactc
yidC2-P139L-R	aagagtgcattaggctgttactagtattaattcaaatgcc
yidC2-Y188A-F	ataccaagcttgataaaggcaagcacaccggcaataat
yidC2-Y188A-R	tattattgccggtgtgcttgcccttatccaagcttgg
yidC2-L240A-F	agtaagtttgacaattgcgaatgccgcactgaca
yidC2-L240A-R	tctgtcagtgccgcatcgcaattgtccaactacttc

Table S3. Mutations identified in the chromosomes of Cpd9-resistant and Cpd36-resistant isolates by whole-genomic resequencing technique.

	Mutation ^a	Locus ^b	Amino acid shift	Location
Cpd9-resistant	A G	292840	No change	hypothetical protein
	T A	337798	<i>Intergenic Region</i>	176 bp at 5' side: possible N-acetyltransferase
	T A	2306559	<i>Intergenic Region</i>	7 bp at 5' side: 50S ribosomal protein L36
	T C	853895	No change	monovalent cation/H ⁺ antiporter subunit A
	A G	906830	<i>Intergenic Region</i>	2 bp at 5' side: regulatory protein Spx
	A C	2158671	A184S	membrane protein YidC2 precursor
	G A	1720642	No change	universal stress family protein
Cpd36-resistant	A G	2158805	P139L	membrane protein YidC2 precursor
	G T	2170476	K409N	F ₀ F ₁ ATP synthase subunit alpha
	A G	2173080	R39C	F ₀ F ₁ ATP synthase subunit c
	C G	28785	G424A	two-component regulator YycH
	C A	849570	L410V	putative monovalent cation/H ⁺ antiporter subunit D
	C T	1013496	No change	putative transcriptional regulator
	A G	1634860	A150T	luciferase family monooxygenase

^aThe presentation of nucleotide is "Novel / Reference"

^bThe position in the whole-genome sequence of *S. aureus* NCTC8325 (NC_007795).

Table S4. Missense mutations identified in the *yidC2* of Cpd9-, Cpd36- and Cpd46-resistant isolates of *S. aureus*.

	Mutation	Codon change	Amino acid shift
Cpd9-resistant isolate	G550T	GCC→TCC	A184S
Cpd36-resistant isolate	C416T	CCA→CTA	P139L
Cpd46-resistant isolate	C218T	CCA→CTA	P73L

Table S5. The identity and similarity of *S. aureus* YidC2 to the YidC2 and YidC of different Gram-positive and Gram-negative bacteria.

	Accession number	<i>S. aureus</i> YidC2	
		Identity (%)	Similarity (%)
Gram-positive YidC2			
<i>Bacillus cereus</i>	WP_000727738.1	31	50
<i>Bacillus subtilis</i>	AIY95428.1	28	51
<i>Enterococcus faecium</i>	WP_002330077.1	32	53
<i>Listeria monocytogenes</i>	WP_003722505.1	36	58
<i>Streptococcus pyogenes</i>	WP_002985918.1	30	47
<i>Streptococcus agalactiae</i>	WP_000751931.1	30	52
Gram-negative YidC			
<i>Acinetobacter baumannii</i>	WP_001188089.1	12	20
<i>Escherichia coli</i>	WP_000378258.1	13	23
<i>Salmonella Typhimurium</i>	WP_000378281.1	13	23

The alignments of the amino acid sequence of were performed using EMBOSS Needle of EMBL-EBL website.

Table S6. Antimicrobial susceptibilities of *E. coli* wild-type strain ATCC25922, efflux pump deficient (ΔcrA) strain JW0452-3, and lipid A deficient (*lpxA2*-ts) strain SM101.

Antibiotics	MIC ($\mu\text{g/ml}$)		
	<i>E. coli</i> ATCC25922	<i>E. coli</i> JW0452-3 ^a	<i>E. coli</i> SM101 ^a
	37°C	37°C	28°C
Cpd36	>64	>64	>64
Cpd46	>64	>64	>64
Erythromycin	64	4	≤ 0.125
Rifampicin	8	8	≤ 0.125
Ampicillin	32	16	8
Gentamicin	1	0.5	0.25

^aThe JW0452-3 and SM101 strains of *E. coli* were obtained from the Coli Genetic Stock Center.