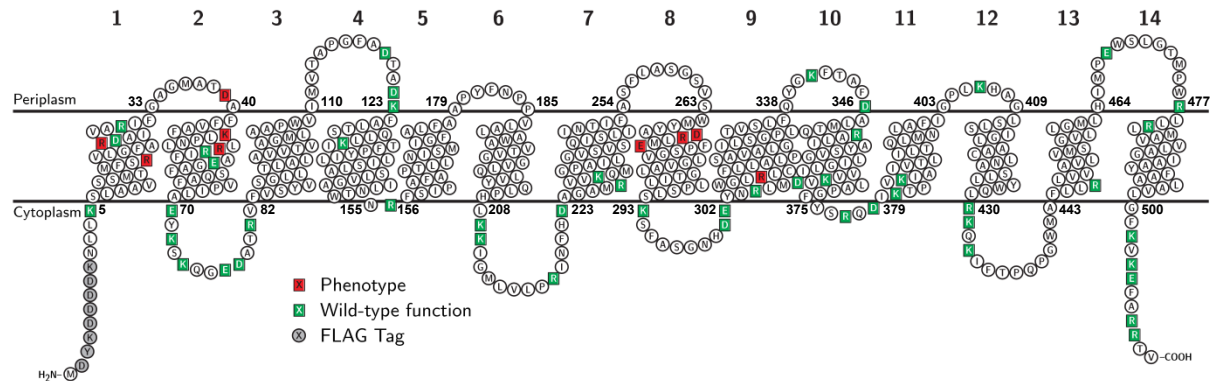


Supplemental material

Charge requirements of lipid II flippase activity in *Escherichia coli*

Emily K. Butler, Wee Boon Tan, Hildy Joseph, Natividad Ruiz

A

■ Phenotype
■ Wild-type function
○ FLAG Tag

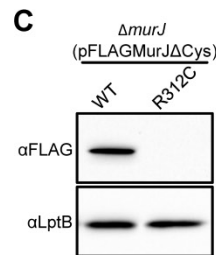
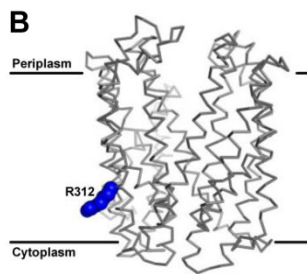


Figure S1. Relevance of charged residues in MurJ. (A) Charged residues whose substitution resulted in partial or total loss of function phenotype (red boxes) and wild-type function (green boxes) are shown in the topological map of MurJ (1). To generate this figure, data were compiled from this work and our previous publication, see Results section (1). N-terminal residues corresponding to the FLAG tag are highlighted in grey. Transmembrane domain (TMD) numbers are indicated at the top of the figure. Figure was prepared using the TEXTopo software package in LaTeX (2). Substitutions that affect function are located in TMDs 1, 2, 8, 9, and the periplasmic loop 1 of MurJ. Numbers indicate the residues bounding TMDs and correspond to the native MurJ sequence. (B) Structural model of MurJ (1) as viewed from the membrane plane with approximate membrane boundaries marked. The side chain of R312 is shown as spheres in blue. (C) Anti-FLAG immunoblot of samples prepared from overnight cultures shows no detectable signal from the functional FLAG-MurJ/R312C variant. Data are representative of at least three independent experiments. Anti-LptB immunoblotting was performed to control for sample loading.

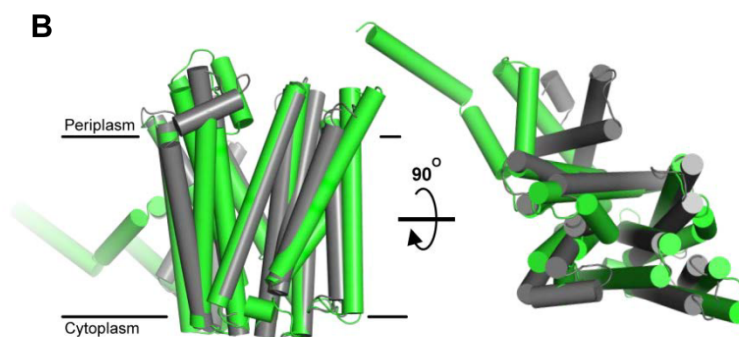
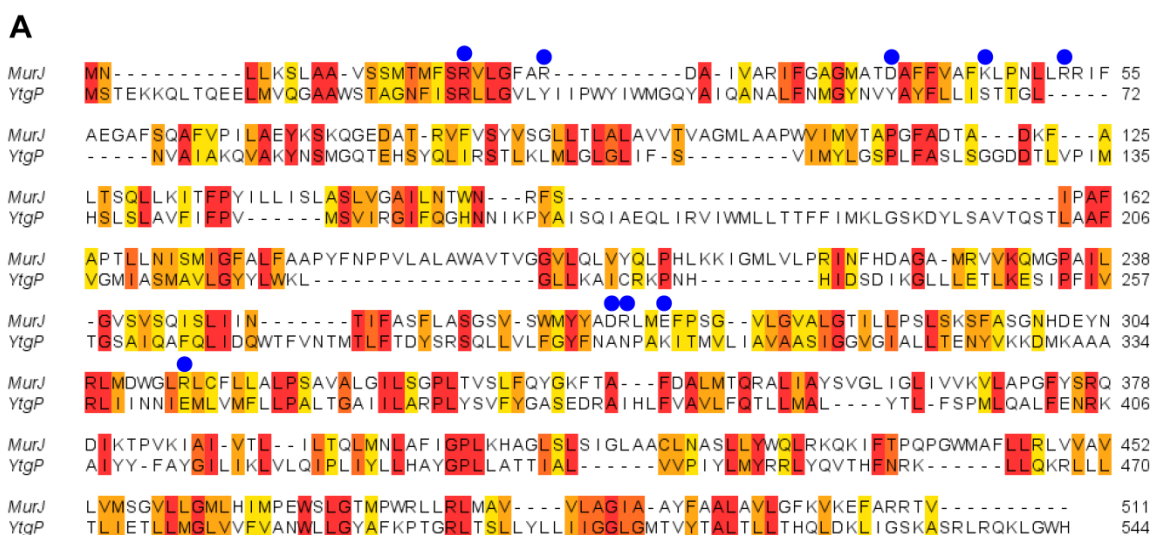


Figure S2. Structural homologs MurJ and YtgP have low conservation at the sequence level. (A) Alignment of the MurJ (NP_415587) and YtgP (YP_002285353.1) sequences using ClustalOmega (3) reveals 21.7% identity. Residues are colored according to JalView (4) conservation scores (1-10), where 10 = red, 9 = orange, and 8 = yellow. The MurJ residues critical for function (R18, R24, D39, K46, R52, D269, R270, and E273) and biogenesis (R312) are marked with blue dots. (B) Alignment of the YtgP (green) and MurJ (grey) structural models using CEalign (5) demonstrates homology between their respective TMD 1-12 regions (RMSD = 1.72 Å). These regions were modeled using the crystal structures of MOP exporters as templates (1). The topology of the remaining C-terminal region in the YtgP model remains to be investigated.

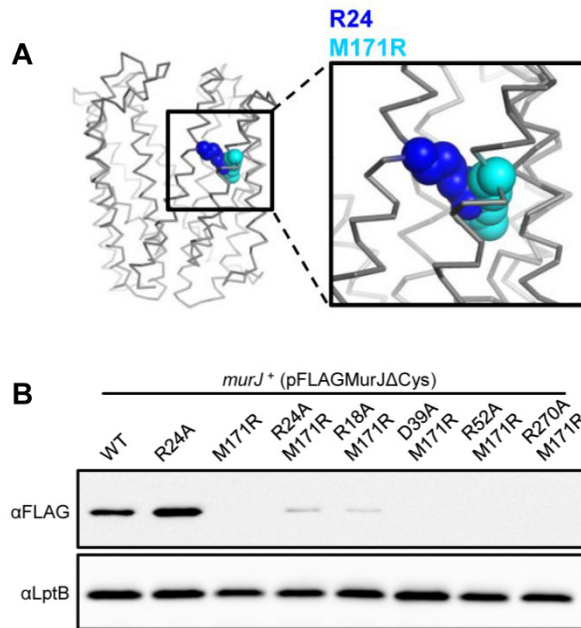


Figure S3. The M171R substitution specifically suppresses the loss of function conferred by R24A and decreases levels of FLAG-MurJ. (A) View of the MurJ structure from the membrane plane showing the predicted proximity of the side chains of R24 (blue spheres) and M171R (cyan spheres). (B) Anti-FLAG immunoblot detection of FLAG-MurJ. Loss-of-function D39A/M171R, R52A/M171R, and R270A/M171R variants are, like the functional M171R variant, present at a level below the limit of detection. Functional R24A/M171R and non-functional R18A/M171R are detected at similar levels, which are decreased relative to the wild-type (WT) FLAG-MurJ. Data are representative of at least three independent experiments. Anti-LptB immunoblotting was performed to control for sample loading.

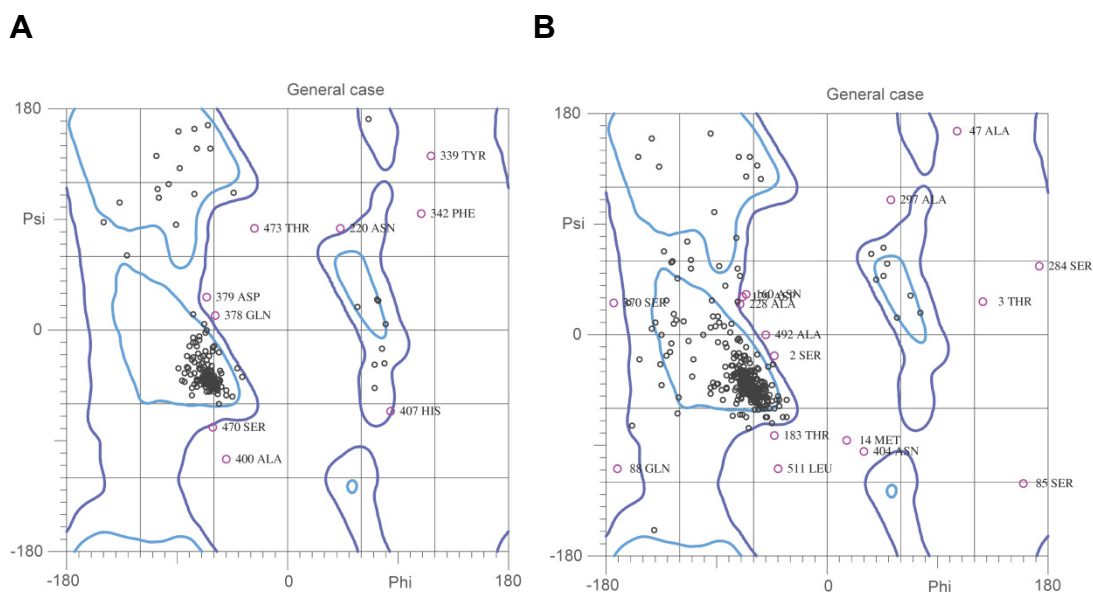


Figure S4. Quality assessment of structural models. Ramachandran plots show that the MurJ and YtgP structural models satisfy conformational restraints of most residues, as 98.2% of MurJ (A) and 97.05% of YtgP (B) residues were found in allowed regions. Plots were generated using the MolProbity web service (6). All charged residues in MurJ and YtgP that are relevant to this study are within allowed regions.

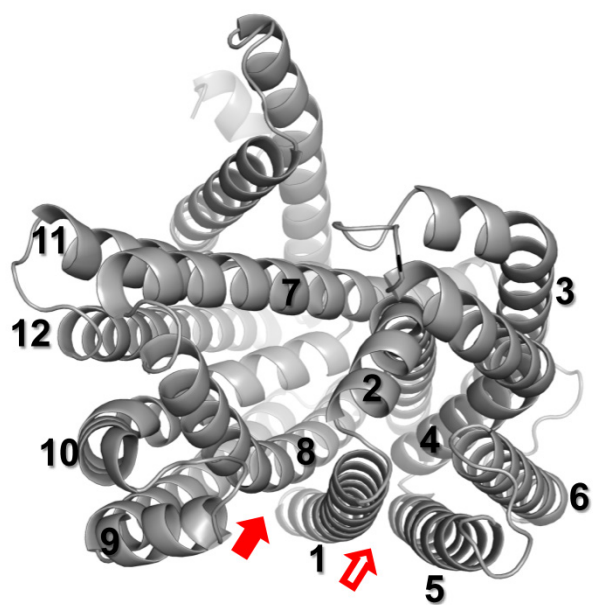


Figure S5. Model of MurJ structure showing “open slots” between transmembrane domains. View of the MurJ structural model from the periplasmic space, tilted to expose the putative slots between transmembrane domains 1 and 8 (red filled arrow) and transmembrane domains 1 and 5 (red open arrow). Numbers indicate transmembrane domains 1 - 12.

Table S1. Charge conservation of specific residues in transmembrane domains 1, 2, and 8 is required for proper function of FLAG-MurJ variants.

Strain	Relevant genotype	TMD ^b	Efficiency of plating ^a	
			LB	YT
NR2131	$\Delta murJ$ (pFLAGMurJ Δ Cys)		1.00	1.0 \pm 0.7
NR2893	$\Delta murJ$ (pFLAGMurJ Δ Cys/R18K)	1	0.4 \pm 0.3	0.4 \pm 0.3
NR2894	$\Delta murJ$ (pFLAGMurJ Δ Cys/R24K)	1	2.2 \pm 1.6	0.7 \pm 0.3
NR2687	$\Delta murJ$ (pFLAGMurJ Δ Cys/R52A)	2	1.1 \pm 0.1	<10 ⁻³
NR2895	$\Delta murJ$ (pFLAGMurJ Δ Cys/R52K)	2	1.5 \pm 0.3	2.1 \pm 1.4
NR2684	$\Delta murJ$ (pFLAGMurJ Δ Cys/D269A)	8	0.4 \pm 0.2	<10 ⁻⁶
NR2896	$\Delta murJ$ (pFLAGMurJ Δ Cys/D269E)	8	0.6 \pm 0.3	0.6 \pm 0.3
NR2897	$\Delta murJ$ (pFLAGMurJ Δ Cys/R270K)	8	0.6 \pm 0.3	0.1 \pm 0.0
NR2712	$\Delta murJ$ (pFLAGMurJ Δ Cys/E273A)	8	2.7 \pm 1.5	<10 ⁻⁴
NR2710	$\Delta murJ$ (pFLAGMurJ Δ Cys/E273D)	8	0.5 \pm 0.1	0.6 \pm 0.3

^a Efficiency of plating values for growth of haploid strains expressing *flag-murJ* alleles carrying indicated substitutions were calculated as described in Materials and Methods. A value = 1 (set by the wild-type allele) indicates full complementation by *flag-murJ* alleles; a value <1 indicates partial complementation.

^b TMD indicates transmembrane domain where the substituted residue is located.

Table S2. Sensitivity to low-osmolarity medium conferred by functional K46 and D39 variants.

Strain	Relevant genotype	Efficiency of plating ^a	
		LB	YT
NR2131	$\Delta murJ$ (pFLAGMurJ Δ Cys)	1.0	0.2 \pm 0.0
NR2683	$\Delta murJ$ (pFLAGMurJ Δ Cys/K46A)	0.0 \pm 0.0	6.7 (\pm 3.2) $\times 10^{-4}$
NR2844	$\Delta murJ$ (pFLAGMurJ Δ Cys/K46E)	2.1 \pm 1.8	3.9 (\pm 2.7) $\times 10^{-3}$
NR3015	$\Delta murJ$ (pFLAGMurJ Δ Cys/D39E)	0.7 \pm 0.3	2.8 (\pm 1.4) $\times 10^{-3}$

^a Efficiency of plating values for growth of haploid strains expressing *flag-murJ* alleles carrying indicated substitutions were calculated as described in Materials and Methods. A value = 1 indicates (set by the wild-type allele) full complementation by *flag-murJ* alleles; a value <1 indicates partial complementation.

Table S3. Strains used in this study.

Strain	Genotype	Reference
DH5α	<i>fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	
MC4100	<i>araD139, Δ(lac)U169, strA, thi</i>	(7)
NR754	<i>araD</i> ⁺ revertant of MC4100	(8)
NR1152	NR754 <i>murJ</i> Ω(-14:: <i>bla araC</i> P _{BAD})	(9)
NR2117	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pRC7MurJ)	(1)
NR2131	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys)	(1)
NR2449	NR754 (pFLAGMurJΔCys)	(1)
NR2988	NR754 (pFLAGMurJΔCys/R18A)	This study
NR2892	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R18E, pRC7MurJ)	This study
NR2935	NR754 (pFLAGMurJΔCys/R18E)	This study
NR2893	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R18K)	This study
NR3139	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R18A/M171R, pRC7MurJ)	This study
NR3143	NR754 (pFLAGMurJΔCys/R18A/M171R)	This study
NR2989	NR754 (pFLAGMurJΔCys/R24A)	This study
NR2845	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R24E, pRC7MurJ)	This study
NR2851	NR754 (pFLAGMurJΔCys/R24E)	This study
NR2894	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R24K)	This study
NR3058	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R24A/M171R)	This study
NR3057	NR754 (pFLAGMurJΔCys/R24A/M171R)	This study
NR3138	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/A37D)	This study
NR3015	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/D39E)	This study
NR2990	NR754 (pFLAGMurJΔCys/D39A)	This study
NR2682	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/D39N, pRC7MurJ)	This study
NR3055	NR754 (pFLAGMurJΔCys/D39N)	This study
NR2846	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/D39K, pRC7MurJ)	This study
NR2852	NR754 (pFLAGMurJΔCys/D39K)	This study
NR3020	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/D39A/A37D, pRC7MurJ)	This study
NR3023	NR754 (pFLAGMurJΔCys/D39A/A37D)	This study
NR3140	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/D39A/M171R, pRC7MurJ)	This study
NR3144	NR754 (pFLAGMurJΔCys/D39A/M171R)	This study
NR2683	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/K46A)	(1)
NR2991	NR754 (pFLAGMurJΔCys/K46A)	This study
NR2844	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/K46E)	This study
NR2996	NR754 (pFLAGMurJΔCys/K46E)	This study
NR2687	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R52A)	(1)
NR2992	NR754 (pFLAGMurJΔCys/R52A)	This study
NR2847	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R52E, pRC7MurJ)	This study
NR2853	NR754 (pFLAGMurJΔCys/R52E)	This study
NR2895	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R52K)	This study
NR3141	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R52A/M171R, pRC7MurJ)	This study
NR3145	NR754 (pFLAGMurJΔCys/R52A/M171R)	This study
NR3170	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/K74C)	This study
NR2614	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/E77C)	This study
NR2609	NR754 <i>ΔmurJ::FRT ΔhsdR::kan</i> (pFLAGMurJΔCys/R81C)	This study

Strain	Genotype	Reference
NR2637	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D122C)	This study
NR2813	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K123C)	This study
NR2815	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K132C)	This study
NR2638	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R156C)	This study
NR3075	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/M171R)	This study
NR3056	NR754 (pFLAGMurJ Δ Cys/M171R)	This study
NR2579	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K210C)	This study
NR2684	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D269A)	(1)
NR2993	NR754 (pFLAGMurJ Δ Cys/D269A)	This study
NR2848	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D269K, pRC7MurJ)	This study
NR2854	NR754 (pFLAGMurJ Δ Cys/D269K)	This study
NR2896	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D269E)	This study
NR2994	NR754 (pFLAGMurJ Δ Cys/R270A)	This study
NR2849	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R270E, pRC7MurJ)	This study
NR2855	NR754 (pFLAGMurJ Δ Cys/R270E)	This study
NR2897	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R270K)	This study
NR3142	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R270A/M171R, pRC7MurJ)	This study
NR3146	NR754 (pFLAGMurJ Δ Cys/R270A/M171R)	This study
NR2712	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/E273A)	(1)
NR2995	NR754 (pFLAGMurJ Δ Cys/E273A)	This study
NR2710	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/E273D)	This study
NR2850	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/E273R, pRC7MurJ)	This study
NR2856	NR754 (pFLAGMurJ Δ Cys/E273R)	This study
NR2466	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D301C)	This study
NR2816	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R305C)	This study
NR2582	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D308C)	This study
NR2468	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R312C)	This study
NR2641	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K341C)	This study
NR2615	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/D346C)	This study
NR2642	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R352C)	This study
NR2652	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K368C)	This study
NR2616	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R377C)	This study
NR2819	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K381C)	This study
NR2613	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K385C)	This study
NR2697	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K406C)	This study
NR2699	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R430C)	This study
NR2700	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K431C)	This study
NR2701	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K433C)	This study
NR2709	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R447C)	This study
NR2931	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/E468C)	This study
NR2702	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R477C)	This study
NR2703	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R480C)	This study
NR2820	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K502C)	This study
NR2704	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/K504C)	This study
NR2705	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/E505C)	This study
NR2706	NR754 $\Delta murJ::FRT \Delta hsdR::kan$ (pFLAGMurJ Δ Cys/R509C)	This study
NR1305	NR1152 (pCA24Not)	(10)

Strain	Genotype	Reference
NR3128	NR754 (pHIS-YtgP)	This study
NR3064	NR1152 (pHIS-YtgP)	This study
NR3129	NR754 (pHIS-YtgP/R29A)	This study
NR3067	NR1152 (pHIS-YtgP/R29A)	This study
NR3130	NR754 (pHIS-YtgP/R29K)	This study
NR3068	NR1152 (pHIS-YtgP/R29K)	This study
NR3131	NR754 (pHIS-YtgP/R152A)	This study
NR3073	NR1152 (pHIS-YtgP/R152A)	This study
NR3132	NR754 (pHIS-YtgP/E171A)	This study
NR3074	NR1152 (pHIS-YtgP/E171A)	This study
NR3133	NR754 (pHIS-YtgP/R175A)	This study
NR3069	NR1152 (pHIS-YtgP/R175A)	This study
NR3134	NR754 (pHIS-YtgP/R175K)	This study
NR3070	NR1152 (pHIS-YtgP/R175K)	This study
NR3135	NR754 (pHIS-YtgP/K301A)	This study
NR3071	NR1152 (pHIS-YtgP/K301A)	This study
NR3136	NR754 (pHIS-YtgP/K301R)	This study
NR3072	NR1152 (pHIS-YtgP/K301R)	This study

Table S4. Primers used in this study.

PRIMER	SEQUENCE 5'-3'	PURPOSE
5MurJR18E 2	CTCGATGACCATGTTTTCGGAAGTGCTTGGCTTCGCACGAG	SDM
3MurJR18E 2	CTCGTGCGAAGCCAAGCACTTCCGAAAACATGGTCATCGAG	SDM
5MurJR18K	CGATGACCATGTTTTCGAAAGTGCTTGGCTTCGCAC	SDM
3MurJR18K	GTGCGAAGCCAAGCACTTTCGAAAACATGGTCATCG	SDM
5MurJR24K	GTGCTTGGCTTCGCAAAAGACGCAATTGTCG	SDM
3MurJR24K	CGACAATTGCGTCTTTTGCGAAGCCAAGCAC	SDM
5MurJR24E	GTGTGCTTGGCTTCGCAGAGGACGCAATTGTCGCCAG	SDM
3MurJR24E	CTGGCGACAATTGCGTCCTCTGCGAAGCCAAGCACAC	SDM
5MurJA37D	CTTTGGCGCAGGGATGGACACCGACGCCTTTTTTCG	SDM
3MurJA37D	CGAAAAAGGCGTCGGTGTCCATCCCTGCGCCAAAAG	SDM
5MurJD39K	GCAGGGATGGCAACCAAGGCCTTTTTCGTCGCT	SDM
3MurJD39K	AGCGACGAAAAAGGCCTTGGTTGCCATCCCTGC	SDM
5MurJD39E	GCAGGGATGGCAACCGAGGCCTTTTTCGTCGCTT	SDM
3MurJD39E	AAGCGACGAAAAAGGCCTCGGTTGCCATCCCTGC	SDM
5MurJD39N	CAGGGATGGCAACCAATGCCTTTTTTCGTCG	SDM
3MurJD39N	CGACGAAAAAGGCATTGGTTGCCATCCCTGC	SDM
5MurJK46E	GACGCCTTTTTCGTCGCTTTTGAGCTTCCTAACTTGTTACGCC	SDM
3MurJK46E	GGCGTAACAAGTTAGGAAGCTCAAAAAGCGACGAAAAAGGCGTC	SDM
5MurJR52K	TTCGTCGCTTTTAAACTTCCTAACTTGTTAAAGCGTATCTTTGCCGAAGG	SDM
3MurJR52K	CCTTCGGCAAAGATACGCTTAAACAAGTTAGGAAGTTTAAAAGCGACGAA	SDM
5MurJR52E	CGTCGCTTTTAAACTTCCTAACTTGTTAGAGCGTATCTTTGCCGAAG	SDM
3MurJR52E	CTTCGGCAAAGATACGCTCTAACAAGTTAGGAAGTTTAAAAGCGACG	SDM
5MurJK74C	GGCGGAATATAAAAAGTTGCCAGGGTGAAGACGCCAC	SDM
3MurJK74C	GTGGCGTCTTCACCTGGCAACTTTTATATTCCGCC	SDM
5MurJE77C	GAATATAAAAGTAAGCAGGGTTGCGACGCCACGCGGGTCTTTGTC	SDM
3MurJE77C	GACAAAGACCCGCGTGGCGTCGCAACCCTGCTTACTTTTATATTC	SDM
5MurJR81C	GGGTGAAGACGCCACGTGCGTCTTTGTCTCTTATGT	SDM
3MurJR81C	ACATAAGAGACAAAGACGCACGTGGCGTCTTCACCC	SDM
5MurJD122C	GGCTTCGCTGACACAGCTTGCAAATTTGCCCTGACCAG	SDM
3MurJD122C	CTGGTCAGGGCAAATTTGCAAGCTGTGTCAGCGAAGCC	SDM
5MurJK123C	TTCGCTGACACAGCTGACTGCTTTGCCCTGACCAGCCAG	SDM
3MurJK123C	CTGGCTGGTCAGGGCAAAGCAGTCAGCTGTGTCAGCGAA	SDM
5MurJK132C	GCCCTGACCAGCCAGCTACTATGCATTACCTTCCCTATATCTTG	SDM
3MurJK132C	CAAGATATAGGGAAAGGTAATGCATAGTAGCTGGCTGGTCAGGGC	SDM
5MurJR156C	GATTCTGAATACGTGGAAGTGTCTCGATTCCGGCGTTTG	SDM
3MurJR156C	CAAACGCCGGAATCGAGAAAACAGTTCCACGTATTCAGAATC	SDM
5MurJM171R	TGCTTAACATCAGCCGATTGGTTTCGCGCT	SDM
3MurJM171R	AGCGCGAAACCAATCCGGCTGATGTAAAGCA	SDM

PRIMER	SEQUENCE 5'-3'	PURPOSE
5MurJK210C	GCTACCGCACCTGAAGTGCATCGGCATGCTGGTCC	SDM
3MurJK210C	GGACCAGCATGCCGATGCACTTCAGGTGCGGTAGC	SDM
5MurJD269E	GATGTATTACGCCGAACGCTTAATGGAGTTTC	SDM
3MurJD269E	GAAACTCCATTAAGCGTTCGGCGTAATACATC	SDM
5MurJD269K	GTCTTGGATGTATTACGCCAAGCGCTTAATGGAGTTCCGT	SDM
3MurJD269K	ACGGAAACTCCATTAAGCGCTTGGCGTAATACATCCAAGAC	SDM
5MurJR270K	GGATGTATTACGCCGACAAGTTAATGGAGTTTCCGTCC	SDM
3MurJR270K	GGACGGAAACTCCATTAACTTGTTCGGCGTAATACATCC	SDM
5MurJR270E	CTTGGATGTATTACGCCGACGAGTTAATGGAGTTTCCGTCCGG	SDM
3MurJR270E	CCGGACGGAAACTCCATTAACTCGTCGGCGTAATACATCCAAG	SDM
5MurJE273D	CGACCGCTTAATGGATTTTCCGTCCGGTGTG	SDM
3MurJE273D	CACACCGGACGGAAAATCCATTAAGCGGTTCG	SDM
5MurJE273R	CGCCGACCGCTTAATGAGGTTTCCGTCCGGTGT	SDM
3MurJE273R	ACACCGGACGGAAACCTCATTAAAGCGGTCCGGCG	SDM
5MurJD301C	GAAAAGTTTGGCCAGTGGCAATCATTGTGAATACAACCGTTTGTATGG	SDM
3MurJD301C	CCATCAAACGGTTGTATTACAATGATTGCCACTGGCAAACTTTTC	SDM
5MurJR305C	CAATCATGATGAATACAACCTGCTTGTGACTGGGGGTTGC	SDM
3MurJR305C	GCAACCCCAAGTCCATCAAGCAGTTGTATTCATCATGATTG	SDM
5MurJD308C	ATGAATACAACCGTTTGTATGTGCTGGGGGTTGCGTCTTAGC	SDM
3MurJD308C	GCTAAGACGCAACCCCAAGCACATCAAACGGTTGTATTCAT	SDM
5MurJR312C	GATGGACTGGGGGTTGTGTCTTAGCTTCCTGTT	SDM
3MurJR312C	AACAGGAAGCTAAGACACAACCCCAAGTCCATC	SDM
5MurJK341C	GTTTCGCTGTTCCAGTACGGTTGCTTTACCGCGTTTGTATGCGCTG	SDM
3MurJK341C	CAGCGCATCAAACGCGGTAAAGCAACCGTACTGGAACAGCGAAAC	SDM
5MurJD346C	CCAGTACGGTAAATTTACCGCGTTTGTGCGCTGATGACC	SDM
3MurJD346C	GGTCATCAGCGCACAAAACGCGGTAAATTTACCGTACTGG	SDM
5MurJR352C	GCGCTGATGACCCAGTGCAGCTTAATTGCCTAC	SDM
3MurJR352C	GTAGGCAATTAACGCGCACTGGGTCATCAGCGC	SDM
5MurJK368C	GTTTGTATTGGCCTGATTGTAGTGTGCGTGTGGCTCCTGGCTTTTATTC	SDM
3MurJK368C	GAATAAAAGCCAGGAGCCAACACGCACACTACAATCAGGCCAATCAAAC	SDM
5MurJR377C	GGCTCCTGGCTTTTATTCCCTGTCAGGACATTA AAAACGCCAG	SDM
3MurJR377C	CTGGCGTTTTAATGTCCTGACAGGAATAAAAAGCCAGGAGCC	SDM
5MurJK381C	CTCCTGGCTTTTATTCCCGCCAGGACATTTGTACGCCAGTGAAAATT	SDM
3MurJK381C	AATTTTCACTGGCGTACAAATGTCCTGGCGGAATAAAAAGCCAGGAG	SDM
5MurJK385C	CCAGGACATTA AAAACGCCAGTGTGCATTGCCATCGTTACGCTGATTT	SDM
3MurJK385C	AAATCAGCGTAACGATGGCAATGCACACTGGCGTTTTAATGTCCTGG	SDM
5MurJK406C	GGCGTTTATTGGTCCGTTGTGCCATGCCGGGCTGTCACTTT	SDM
3MurJK406C	AAAGTGACAGCCCGCATGGCACAACGGACCAATAAACGCC	SDM
5MurJR430C	GCTGCTTTACTGGCAGTTGTGCAAGCAGAAAATCTTTACCC	SDM
3MurJR430C	GGGTAAAGATTTTCTGCTTGCACTGCCAGTAAAGCAGC	SDM
5MurJK431C	CTGCTTTACTGGCAGTTGCGTTGCCAGAAAATCTTTACCCCGCAA	SDM
3MurJK431C	TTGCGGGGTAAAGATTTTCTGGCAACGCAACTGCCAGTAAAGCAG	SDM

PRIMER	SEQUENCE 5'-3'	PURPOSE
5MurJK433C	CTGGCAGTTGCGTAAGCAGTGCATCTTTACCCCGCAACCCG	SDM
3MurJK433C	CGGGTTGCGGGGTAAAGATGCACTGCTTACGCAACTGCCAG	SDM
5MurJR447C	GATGGCGTTTCTGTTGTGCCTGGTGGTGGCGGTAC	SDM
3MurJR447C	GTACCGCCACCACCAGGCACAACAGAAACGCCATC	SDM
5MurJE468C	GGTATGTTACATATCATGCCGTGCTGGTCATTGGGTACCATGCC	SDM
3MurJE468C	GGGCATGGTACCCAATGACCAGCACGGCATGATATGTAACATAACC	SDM
5MurJR477C	TGGGTACCATGCCCTGGTGGTACTGCGTTTAAATGGC	SDM
3MurJR477C	GCCATTAACGCAGTAAGCACCAGGGCATGGTACCCA	SDM
5MurJR480C	GCCCTGGCGTTTACTGTGCTTAAATGGCGGTCTGTC	SDM
3MurJR480C	GCACGACCGCCATTAAGCACAGTAAACGCCAGGGC	SDM
5MurJK502C	ACTGGCGGTACTGGGCTTCTGCGTTAAAGAATTTGCCCGCC	SDM
3MurJK502C	GGCGGGCAAATTCCTTAACGCAGAAGCCCAGTACCGCCAGT	SDM
5MurJK504C	GGTACTGGGCTTCAAAGTTTGCGAATTTGCCCGCCGGACGG	SDM
3MurJK504C	CCGTCCGGCGGGCAAATTCGCAAACCTTTGAAGCCCAGTACC	SDM
5MurJE505C	GGCGGTACTGGGCTTCAAAGTTAAATGCTTTGCCCGCCGGAC	SDM
3MurJE505C	GTCCGGCGGGCAAAGCATTAACTTTGAAGCCCAGTACCGCC	SDM
5MurJR509C	TTAAAGAATTTGCCCGCTGCACGGTGTAAAGGGTTCGAC	SDM
3MurJR509C	GTGCACCCTTACACCGTGCAGCGGGCAAATTCCTTAA	SDM
Sp5ORF0390	GCCTCGACAGAAAAAAAAACAACCTGACACAAGAAGAATTG	pHIS-YtgP construction
Sp3ORF0390	CCATGCCAGCCTAATTTCTGACGAAGGCGACT	pHIS-YtgP construction
5SpyMurJR29A	GCTGGTAATTTTCATTAGCGCACTTTTGGGAGTGTTATAC	SDM
3SpyMurJR29A	GTATAACTCCCAAAGTGCCTAATGAAATTACCAGC	SDM
5SpyMurJR29K	GCTGGTAATTTTCATTAGCAAACCTTTTGGGAGTGTTAT	SDM
3SpyMurJR29K	ATAACTCCCAAAGTTTGCTAATGAAATTACCAGC	SDM
5SpyMurJR152A	CCTGTAATGAGTGTTCATTGCAAGTATCTTTCAAGGCC	SDM
3SpyMurJR152A	GGCCTTGAAAGATACCTGCAATGACTCATTACAGG	SDM
5SpyMurJE171A	GATTAGTCAGATTGCTGCCCAACTCATTGCGGTTATC	SDM
3SpyMurJE171A	GATAACCCGAATGAGTTGGGCAGCAATCTGACTAATC	SDM
5SpyMurJR175A	CTGAGCAACTCATTGCGGTTATCTGGATG	SDM
3SpyMurJR175A	CATCCAGATAACCGCAATGAGTTGCTCAG	SDM
5SpyMurJR175K	GATTGCTGAGCAACTCATTAAGGTTATCTGGATGCTTTTG	SDM
3SpyMurJR175K	CAAAAGCATCCAGATAACCTTAATGAGTTGCTCAGCAATC	SDM
5SpyMurJK301A	CTTTAACGCAAACCCAGCCGCAATTACAATGGTCTTAATTG	SDM
3SpyMurJK301A	CAATTAAGACCATTGTAATTGCGGCTGGGTTTGCCTTAAAG	SDM
5SpyMurJK301R	CTTTAACGCAAACCCAGCCAGGATTACAATGGTCTTAATTG	SDM
3SpyMurJK301R	CAATTAAGACCATTGTAATCCTGGCTGGGTTTGCCTTAAAG	SDM

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