Supplemental Results and Discussion

Francisella species are extremely resistant to polymyxin in comparison to other bacteria. We therefore set out to identify genes necessary for *F. novicida* polymyxin resistance. We initiated a screen of 470 transposon mutants from a library [\(1\)](#page-11-0) representing 229 genes that have previously been identified as necessary for virulence *in vivo* and/or intracellular replication in mammalian cells. Each mutant was grown overnight in the presence of polymyxin B, and compared to wild-type. Mutants that failed to reach at least 75% of wild-type growth were deemed to have mutations in genes required for complete fitness in the presence of polymyxin (Supplemental Tables 1 and 2).

We identified 120 genes as playing roles in *F. novicida* resistance to polymyxin. The majority of these are categorized as having Unknown Function by COG analysis, while the remainder are primarily grouped into pathways necessary for the generation of envelope structures or within metabolic pathways that can act upstream of envelope biogenesis and modification (Supplemental Figure 2). Resistance to polymyxin is often mediated by alterations to the structure of lipid A and O-antigen, components of lipopolysaccharide (LPS) in the bacterial outer membrane. Notably, we identified *FTN_0544*, *FTN_0545*, and *lpxE* which have roles in lipid A modification and have previously been implicated in polymyxin resistance [\(2-4\)](#page-11-1), providing validation for the results of the screen (Supplemental Table 1). Additionally, *wbtD*, *wbtF*, and *wbtH*, which are part of the O-antigen biosynthetic machinery were also identified (Supplemental Table 1)[\(5\)](#page-11-2). To further confirm the results of the screen, we generated deletion mutants in two genes encoding proteins of unknown function, *FTN_0109* and *FTN_1254*. These deletion mutants demonstrated a significant decrease in polymyxin resistance compared to wild-type, providing additional support for screen's validity (Supplemental Figure 3).

In addition to a number of genes known to be involved in the biogenesis or modification of envelope structures, and numerous genes encoding hypothetical proteins, the screen also implicated numerous potential metabolic pathways in mediating polymyxin resistance (Supplemental Table 1 and

Supplemental Figure 2). These pathways may be involved in creating necessary precursors for envelope structures, and/or increasing metabolic output, allowing sufficient energy to resist and repair damage induced by polymyxin. In either case, the results suggest an important interplay between the metabolic status of the bacterial cell and its ability to resist the action of polymyxin. It is important to note that this screen does not differentiate between mutant strains which failed to grow in the presence of polymyxin, mutant strains which can replicate in polymyxin but subsequently are killed, and those mutant strains which are actively killed more effectively by polymyxin. However, this broad screen of 229 genes already implicated in *Francisella* virulence allows the foundation for future studies to determine their precise contribution to polymyxin resistance and virulence.

Supplemental Methods

Screen for polymyxin resistance determinants

Four hundred and seventy transposon mutants, representing 229 genes were obtained from the *Francisella* two-allele transposon mutant library [\(1,](#page-11-0) [6\)](#page-11-3). Each transposon mutant was grown overnight in a well of a 96 well plate containing cation-adjusted Mueller Hinton broth (MH/C-A) with 0.2% L-cysteine (BD Biosciences). Subsequently, each mutant was diluted to an OD_{600} of 0.03 in MH/C-A containing 100µg/mL of polymyxin B (USB Corporation, Cleveland, OH). Following overnight growth at 37°C with aeration, the OD_{600} was measured and used to calculate the percent growth compared to wild-type bacteria. Strains that grew to an OD_{600} of less than 75% than that of wild-type were deemed to have increased sensitivity.

Bacterial growth kinetics.

The indicated strains were grown overnight and subsequently diluted to an OD_{600} of 0.03 in TSB with 0.2% L-cysteine or Chamberlain's Defined Media (CDM). Subcultures were placed at 37°C with aeration in a Biotek Synergy Mx plate reader and OD_{600} was measured each hour for 15 hours.

Lipid A isolation and analysis.

Total lipid A was isolated from the indicated strains as described previously [\(2\)](#page-11-1). Lipid A was analyzed by LC/MS as described previously [\(2\)](#page-11-1). Briefly, LC/MS of lipids was performed using a Shimadzu LC system coupled to a QSTAR XL quadrupole time-of-flight tandem mass spectrometer.

Surface charge analysis by zeta potential

Zeta electrokinetic potentials of the indicated strains were calculated as described previously [\(2\)](#page-11-1). Briefly, bacteria were subcultured and grown to $OD_{600} = 1.0$, and subsequently washed and resuspended at a 5× concentration in 20 mM potassium chloride. Twenty microlitres of the concentrated bacteria were added to 3.2 ml of 20 mM potassium chloride in the zeta potential electrokinetic cuvette from Brookhaven

Instruments Corporation (BIC, Holtsville, NY). The bacterial cell sizes and zeta electrokinetic potentials were measured using the 90Plus size and zeta potential analyser (BIC). Data were analysed using BIC Zeta Potential Analyser Software Version 5.20, which corrects for bacterial cell size.

Antimicrobial resistance.

The indicated strains were grown overnight and subsequently diluted to an OD_{600} of 0.03 in MH/C-A with 0.2% L-cysteine containing the specified doses of Triton X (Fisher Scientific, Pittsburgh, PA), hydrogen peroxide (Fisher Scientific, Pittsburgh, PA), kanamycin (Teknova, Hollister, CA), and streptomycin (Teknova). Following overnight growth at 37° C with aeration, OD₆₀₀ was measured and used to calculate the percent growth compared to the growth of the strain in media alone.

Intracellular survival.

Murine bone marrow-derived macrophages were infected as described in the Experimental Procedures with the indicated bacterial strains. At 4 hours post infection, macrophages were lysed with 1% saponin. Lysates were serially diluted in PBS and plated onto TSA containing 0.1% cysteine to enumerate colony forming units.

Campylobacter manipulations and experiments

The GB11 (wild-type) and its cognate Δ*cas9* deletion mutant have been described previously [\(7\)](#page-11-4). Strains were routinely grown on Columbia blood agar plates containing 7% sheep blood (Becton Dickinson, Breda, The Netherlands), supplemented with vancomycin (Sigma-Aldrich, Zwijndrecht, The Netherlands) and chloramphenicol (Sigma-Aldrich) under micro-aerophilic conditions at 37°C using anaerobic jars and an Anoxomat (Mart Microbiology B.V., Drachten, The Netherlands). To measure bacterial permeability, plate-grown bacteria were recovered, washed in PBS, and diluted to a concentration of 7.5×10^7 cfu/mL in PBS containing 30μ g/mL ethidium bromide (Sigma-Aldrich). Fluorescence was measured immediately in a Fluostar Optima plate reader (BMG Labtech) using an excitation of 250nm

and an emission of 605nm. Erythromycin susceptibility was determined using an Epsilometer-test (bioMérieux, Zaltbommel, The Netherlands). Bacteria were diluted to a 1 MacFarland suspension, swabbed onto Columbia blood agar plates, and an E-test deposited. Plates were incubated overnight, as described above, and at 24 hours the MIC was determined.

Supplemental Figure Legends

Supplemental Figure 1. Model of the Cas9/dual RNA complex mediating *FTN_1103* **repression.**

Cas9 associates with two small RNAs, tracrRNA and scaRNA. This complex is then targeted to the *FTN_1103* transcript, encoding a bacterial lipoprotein (BLP), and ultimately mediates the repression of BLP production by altering the stability of its mRNA. Since BLP can be recognized by TLR2, leading to a proinflammatory innate immune response, the ability of Cas9 to act as a regulatory element against this transcript is critical for *Francisella* evasion of the innate immune response.

Supplemental Figure 2. COG categories of genes identified as being involved in polymyxin B

resistance. COG categories were assigned to each locus identified within the screen as defined by the *Francisella novicida* U112 genome database through NCBI (Accession #: NC_008601.1). Quantities of each COG category were plotted as percent of all categories identified within the screen.

Supplemental Figure 3. *FTN_1254* **and** *FTN_0109* **contribute to** *F. novicida* **polymyxin resistance.** Wild-type (WT), *FTN_0544*, *FTN_1254,* or *FTN_0109* deletion mutants were grown overnight in TSB with or without polymyxin B (200 ug/mL). Percent growth compared to untreated cultures is plotted (n=3). **; *p* ≤0.005, ***; *p* ≤0.001.

Supplemental Figure 4. Growth kinetics of the *cas9* **deletion mutant in the presence of polymyxin B.** Overnight cultures of wild-type (WT; circles) or the *cas9* deletion mutant (squares) were diluted to an $OD₆₀₀$ of $~0.03$ into the media containing indicated doses of polymyxin B in a 96-well plate. Cultures were incubated at 37 \degree C with aeration in a Biotek Synergy Mx plate reader and OD₆₀₀ was measured each hour for 18 hours (n=3).

Supplemental Figure 5. Complementation of the *cas9* **deletion mutant restores polymyxin resistance.** Wild-type (WT), *cas9* deletion mutant, or a *cas9*:complement strain were grown overnight with or without polymyxin B (200 ug/mL). Percent growth compared to untreated cultures is plotted $(n=3)$. **; $p \le 0.005$.

Supplemental Figure 6. Other *cas* **genes are not involved in polymyxin resistance.** Wild-type (WT) or deletion mutants in the indicated *cas* genes were grown overnight with or without polymyxin B (400 ug/mL). Percent growth compared to untreated cultures is plotted (n=3). **; $p \le 0.005$

Supplemental Figure 7. Cas9 is not involved in modification of lipid A. Total lipid A was analyzed by LC/MS from wild-type (WT), the *cas9* deletion mutant, or the *cas9*/1103 double deletion strain.

Supplemental Figure 8. Cas9 is not involved in alteration of cell surface charge. Cultures of wildtype (WT), the *cas9* deletion mutant, or the *cas9*/*1103* double deletion strain were subjected to zeta electrokinetic potential analysis. Data presented for each strain is pooled from 3 independent cultures, with multiple technical replicates.

Supplemental Figure 9. *FTN_1103* **regulation by tracrRNA and scaRNA is necessary for polymyxin resistance. (**A) Wild-type (WT), tracrRNA or tracrRNA*/1103* deletion mutants, or (B) WT, scaRNA or scaRNA*/1103* deletion mutants, were grown overnight in TSB containing the indicated concentrations of polymyxin B. Percent growth compared to untreated cultures is plotted (n=3). Data presented was generated during the same experiment as Figures 1a, b, utilizing the same controls, and plotted separately for clarity. **; $p \leq 0.005$.

Supplemental Figure 10. Cas9 regulatory axis provides resistance to Triton X. (A) Wild-type (WT), *cas9* and *cas9/1103* deletion mutants, (B) tracrRNA and tracrRNA/*1103* deletion mutants, or (C) scaRNA and scaRNA/*1103* deletion mutants were grown overnight in TSB, in the presence or absence of the nonionic detergent Triton X (0.0125%). Percent growth compared to untreated cultures is plotted (n=3). Data

presented was generated during the same experiment, utilizing the same controls, and plotted separately for clarity. \ast ; $p \leq 0.05$.

Supplemental Figure 11. Cas9 regulatory axis is not required for resistance to hydrogen peroxide. (A) Wild-type (WT), *cas9* and *cas9/1103* deletion mutants, (B) WT, tracrRNA and tracrRNA/*1103* deletion mutants, or (C) WT, scaRNA and scaRNA/*1103* deletion mutants were grown overnight in TSB containing the indicated concentrations of hydrogen peroxide. Percent growth compared to untreated cultures is plotted $(n=3)$. Data presented was generated during the same experiment, utilizing the same controls, and plotted separately for clarity. $\sp{p} > 0.05$.

Supplemental Figure 12. Cas9 regulatory axis is required for resistance to kanamycin. Wild-type (WT), *cas9*, *cas9/1103*, tracrRNA, tracrRNA/*1103*, scaRNA, and scaRNA/*1103* deletion mutants were grown overnight in TSB containing the indicated concentrations of kanamycin. Percent growth compared to untreated cultures is plotted (n=3). * $p > 0.05$, ***; $p \le 0.001$.

Supplemental Figure 13. Cas9 regulatory axis is required for resistance to streptomycin. Wild-type (WT), *cas9*, *cas9/1103*, tracrRNA, tracrRNA/*1103*, scaRNA, and scaRNA/*1103* deletion mutants were grown overnight in TSB containing the indicated concentrations of streptomycin. Percent growth compared to untreated cultures is plotted $(n=3)$. * $p > 0.05$.

Supplemental Figure 14. *cas9* **mutant does not have altered viability during growth in broth.** Prior to propidium iodide staining (Figure 1c), wild-type (WT), *cas9*, or *cas9*/*1103* deletion mutants were grown to mid-log phase in TSB, washed and plated to enumerate colony forming units $(n=3)$.

Supplemental Figure 15. Cas9 regulatory axis mutants exhibit wild-type growth kinetics in rich or synthetic media. Wild-type (WT), *cas9*, tracrRNA, scaRNA, *cas9/1103*, tracrRNA/*1103* and

scaRNA/*1103* deletion mutants were grown in (A) TSB or (B) Chamberlain's defined media (CHB) for 15 hours, and OD_{600} was measured every hour (n=3).

Supplemental Figure 16. *FTN_1103* **regulation by tracrRNA and scaRNA is necessary for enhanced envelope integrity.** Wild-type (WT), tracrRNA, tracrRNA/*1103*, scaRNA, or scaRNA/*1103* deletion mutants were grown to mid-log phase, washed, and stained with ethidium bromide. Fluorescence was measured at excitation 250nm and emission 605nm (n=3). **; $p \le 0.005$, ***; $p \le 0.001$.

Supplemental Figure 17. *FTN_1103* **regulation by tracrRNA and scaRNA is necessary for enhanced envelope integrity during intracellular infection. (**A) Bone marrow-derived macrophages were infected with wild-type (WT), tracrRNA, tracrRNA/*1103*, scaRNA*,* or scaRNA*/1103* deletion mutants at a multiplicity of infection (MOI) of 20:1 (bacteria per macrophage). At 4 hours post infection, macrophages were permeabilized with saponin and lysates were plated to enumerate intracellular bacterial levels (n=3). (B) Macrophages were infected as above, and at 4 hours post infection, macrophages were permeabilized with saponin and stained with anti-*Francisella* antibody (green), and propidium iodide (nucleic acids; red). Co-localization was quantified as no less than 50% PI overlap with *Francisella*, and 1,000 bacteria were counted for each strain. (C) Representative fluorescence micrographs of WT, tracrRNA, tracrRNA/*1103*, scaRNA*,* or scaRNA*/1103* deletion mutants. DAPI (DNA; blue), anti-*Francisella* antibody (green), and propidium iodide (nucleic acids; red). Arrows indicate representative PI and anti-*Francisella* co-localization. **; *p* ≤0.005, ***; *p* ≤0.001.

Supplemental Figure 18. *FTN_1103* **regulation by tracrRNA and scaRNA promotes evasion of the inflammasome.** Wild-type (WT), ASC^{\perp} , and $TLR2^{\perp}$, knockout macrophages were infected with WT, tracrRNA, tracrRNA/*1103*, scaRNA, or scaRNA/*1103* deletion mutants at a multiplicity of infection (MOI) of 20:1. At 5.5 hours post infection, cells were assayed for cytotoxicity through LDH release (n=3). *; *p*≤0.05, **; *p* ≤0.005, ***; *p* ≤0.001.

Supplemental Figure 19. Other CRISPR/Cas components are not required for evasion of the inflammasome. Wild-type bone marrow-derived macrophages were infected with wild-type (WT), *cas9*, *cas1*, *cas2*, *cas4*, tracrRNA, crRNA, or scaRNA deletion mutants at a multiplicity of infection (MOI) of 20:1. At 5.5 hours post infection, cells were assayed for cytotoxicity through LDH release (n=3). ***; *p* ≤ 0.001 .

Supplemental Figure 20. Sublethal treatment with polymyxin induces increased permeability without loss of bacterial viability. Wild-type bacteria were treated with 40µg/mL polymyxin B for 30min, at 37°C with aeration. (A) Treated and untreated cultures were plated and colony forming units enumerated. (B) Treated and untreated cultures were grown to mid-log phase, washed, and stained with ethidium bromide and fluorescence measured (n=3). ***; $p \le 0.001$.

Supplemental Figure 21. Virulence of the *cas9* **mutant is not restored in ASC-/- or TLR2-/- mice.** (A) Wild-type (WT), ASC^{-/-}, TLR2^{-/-}, or ASC/TLR2-deficient mice were inoculated subcutaneously with 10^5 cfu of the *cas9* deletion strain. Forty-eight hours post infection, spleens were harvested and plated to quantify bacterial levels (n=5). (B, C) Groups of 5 (B) WT or TLR2^{-/-} and (C) WT or ASC^{-/-} mice were inoculated subcutaneously with 10⁸ cfu of WT or *cas*9 deletion strains. Mice were monitored for survival over 20 days. **; *p* ≤0.005.

Supplemental Figure 22. ASC and TLR2 control inflammasome activation and cytokine response against cas9 deletion mutants. (A) Wild-type (WT), TLR2^{-/-}, ASC^{-/-} and ASC/TLR2-deficient bone marrow-derived macrophages were infected with wild-type (WT), *cas9*, or *cas9/1103* deletion mutants at a multiplicity of infection (MOI) of 20:1 (bacteria per macrophage). At 5.5 hours post infection, cells were assayed for cytotoxicity using the LDH release assay $(n=3)$. (B) Identical infections as above were

performed and at 4 hours post infection, infection supernatants collected, and ELISA performed for the pro-inflammatory cytokine IL-6 (n=3). *; *p*≤0.05, **; *p* ≤0.005.

Supplemental Figure 23. *Campylobacter jejuni* **Cas9 controls envelope permeability and**

erythromycin resistance. (A) Cultures of wild-type GB11 (WT) and the *cas9* deletion mutant were washed, stained with ethidium bromide, and fluorescence measured (n=4). (B) MIC breakpoints as determined by erythromycin E-test for WT or the *cas*9 deletion mutant. ***; $p \le 0.001$.

Supplemental References

- 1. Llewellyn AC, Jones CL, Napier BA, Bina JE, & Weiss DS (2011) Macrophage replication screen identifies a novel Francisella hydroperoxide resistance protein involved in virulence. (Translated from eng) *PLoS One* 6(9):e24201 (in eng).
- 2. Llewellyn AC*, et al.* (2012) NaxD is a deacetylase required for lipid A modification and Francisella pathogenesis. (Translated from eng) *Mol Microbiol* 86(3):611-627 (in eng).
- 3. Kanistanon D*, et al.* (2008) A Francisella mutant in lipid A carbohydrate modification elicits protective immunity. (Translated from eng) *PLoS Pathog* 4(2):e24 (in eng).
- 4. Wang X, Karbarz MJ, McGrath SC, Cotter RJ, & Raetz CR (2004) MsbA transporter-dependent lipid A 1-dephosphorylation on the periplasmic surface of the inner membrane: topography of francisella novicida LpxE expressed in Escherichia coli. (Translated from eng) *J Biol Chem* 279(47):49470-49478 (in eng).
- 5. Raynaud C*, et al.* (2007) Role of the wbt locus of Francisella tularensis in lipopolysaccharide Oantigen biogenesis and pathogenicity. (Translated from eng) *Infect Immun* 75(1):536-541 (in eng).
- 6. Gallagher LA*, et al.* (2007) A comprehensive transposon mutant library of Francisella novicida, a bioweapon surrogate. (Translated from eng) *Proc Natl Acad Sci U S A* 104(3):1009-1014 (in eng).
- 7. Louwen R*, et al.* (2013) A novel link between Campylobacter jejuni bacteriophage defence, virulence and Guillain-Barre syndrome. (Translated from Eng) *Eur J Clin Microbiol Infect Dis* 32(2):207-226 (in Eng).

Supplemental Figure 2.

Supplemental Figure 3.

Supplemental Figure 4.

Supplemental Figure 5.

Supplemental Figure 6.

Supplemental Figure 7.

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Supplemental Figure 11.

Supplemental Figure 12.

Supplemental Figure 13.

Supplemental Figure 14.

Supplemental Figure 15.

Supplemental Figure 16.

Supplemental Figure 17.

Supplemental Figure 18.

Supplemental Figure 19.

Supplemental Figure 20.

A BArbitrary Fluorescence Units (10³) 10^{10} 20 *** $10⁹$ $10^{8} 15$ cfu/mL $10⁷$ $10 10⁶$ $10⁵$ $\overline{5}$ $10⁴$ P dymnyting o Traded Polymphia B Treated $10³$ O₃ H H H H O3

Supplemental Figure 21.

Supplemental Figure 22.

Supplemental Figure 23.

Locus	Name	CO _G	Description	% WT Growth in Polymyxin
FTN_0019	pyrB	COG0540F	aspartate carbamoyltransferase	68.9
FTN 0021	carA	COG0505EF	carbamoyl phosphate synthase small subunit	62.4
FTN_0036	pyrD	COG0167F	dihydroorotate oxidase	68.7
FTN_0097		COG0814E	hydroxy/aromatic amino acid permease (HAAAP) family protein	73.0
FTN_0098	gidB	COG0357M	16S rRNA methyltransferase GidB	69.0
FTN_0109			hypothetical protein FTN 0109	0.2
FTN_0113	ribC	COG0307H	riboflavin synthase subunit alpha	0.0
FTN 0132			hypothetical protein FTN_0132	63.9
FTN_0177	purH	COG0138F	bifunctional	70.1
			phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	
FTN_0178	purA	COG0104F	adenylosuccinate synthetase	57.0
FTN 0196	cyoB	COG0843C	cytochrome bo terminal oxidase subunit I	73.4
FTN 0197	cycC	COG1845C	cytochrome bo terminal oxidase subunit III	65.7
FTN_0202	pdxY	COG2240H	pyridoxal kinase	68.1
FTN_0296	IysP	COG0833E	lysine:H+ symporter	28.5
FTN_0298	glpX	COG1494G	fructose 1,6-bisphosphatase II	68.5
FTN_0300	$\overline{}$	COG1215M	glycosyl transferase	63.5
FTN 0325	$\overline{}$	COG2854Q	membrane protein	71.9
FTN_0330	minD	COG2894D	septum site-determining protein MinD	71.0
FTN 0331	minC	COG0850D	septum site-determining protein MinC	64.9

Supplemental Table 1. Transposon mutants with increased sensitivity to polymyxin B

Locus Tag	Gene name	COG	Gene product	% WT growth in polymyxin	Library Plate	Well
FTN 0113	ribC	COG0307H	riboflavin synthase subunit alpha	$\mathbf{0}$	NR-8043	E03
FTN_0109	$\overline{}$		hypothetical protein FTN 0109	0.2	NR-8048	G06
FTN 0546	$\qquad \qquad \blacksquare$	COG1807M	dolichyl-phosphate-mannose-protein mannosyltransferase family protein	1.0	NR-8060	A06
FTN 1470	i sp A	COG0142H	geranyl diphosphate synthase/farnesyl diphosphate synthase	2.0	NR-8058	F05
FTN 0113	ribC	COG0307H	riboflavin synthase subunit alph	4.0	NR-8060	C12
FTN 0545	\blacksquare	COG0463M	glycosyl transferase	4.3	NR-8040	D ₀₉
FTN 1056	clpX	COG1219O	ATP-dependent protease ATP-binding subunit ClpX	5.1	NR-8061	H ₀₉
FTN_0545	\blacksquare	COG0463M	glycosyl transferase	5.9	NR-8055	G11
FTN_0546	$\qquad \qquad \blacksquare$	COG1807M	dolichyl-phosphate-mannose-protein mannosyltransferase family protein	11.9	NR-8050	D ₀₉
FTN_0296	lysP	COG0833E	lysine: H+ symporter	28.5	NR-8058	A05
FTN 0417	folD	COG0190H	bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/ 5,10- methylene-tetrahydrofolate cyclohyd	29.6	NR-8047	G04
FTN 0544	naxD	COG3394S	N-acetylhexosamine deacteylase	36.5	NR-8055	F02
FTN 0544	maxD	COG3394S	N-acetylhexosamine deacteylase	40.6	NR-8052	G01
FTN 0444	\blacksquare		membrane protein	48.1	NR-8061	C10
FTN_0945	rsuA	COG1187J	16S rRNA pseudouridine synthase	50.1	NR-8064	E06
FTN 1240	\blacksquare	COG0271T	hypothetical protein FTN 1240	50.7	NR-8058	C11
FTN_0444	$\overline{}$		membrane protein	51.4	NR-8047	CO ₃
FTN_1313			hypothetical protein FTN 1313	52.5	NR-8046	F07
FTN 0416	lpxE	COG0671I	lipid A 1-phosphatase	52.6	NR-8043	E11
FTN 0561	apaH	COG0639T	diadenosine tetraphosphatase	53.7	NR-8056	H07
FTN 0599	\blacksquare		hypothetical protein FTN 0599	55.6	NR-8052	G11
FTN 0515	glgC	COG0448G	glucose-1-phosphate adenylyltransferase	55.6	NR-8046	F05
FTN 1597	prfC	COG4108J	peptide chain release factor 3	57.0	NR-8063	F ₀₆
FTN 0178	purA	COG0104F	adenylosuccinate synthetase	57.0	NR-8037	A05

Supplemental Table 2. Percent wild-type growth of each transposon mutant in polymyxin B

Supplemental Table 3. Primers used in this study

