

## Supplemental Material

**FIG S1 MALDI-TOF mass spectrometry of negative control strain W3110 *pmrA*<sup>-</sup>.** This strain serves as a negative control for the W3110 *pmrD*<sup>-</sup> strain. **(A,B)** W3110 *pmrA*<sup>-</sup> produces hexa-acylated lipid A in both 10  $\mu$ M (low) and 10 mM (high)  $Mg^{2+}$  growth conditions, generating major peaks at  $m/z$  1797.0 and  $m/z$  1796.4, respectively. Minor peaks at  $m/z$  1717.1 (low)/1717.5 (high) and  $m/z$  1769.1 (low)/1768.4 (high) correspond to lipid A species lacking a phosphate group or bearing a shorter acyl chain, respectively.

**FIG S2 *pmrD* impacts transcription of *pmrA* and its downstream genes.** Relative gene expression of *pmrA*, *arnT*, *eptA*, *pmrD* and *rstA* when *E. coli* W3110 wild-type and *pmrD* mutant were grown in N-minimal medium with 10  $\mu$ M  $Mg^{2+}$  (low). Results are representative of 3 technical replicates.

Table S1 – Docx  
Strains and plasmids used in this study.

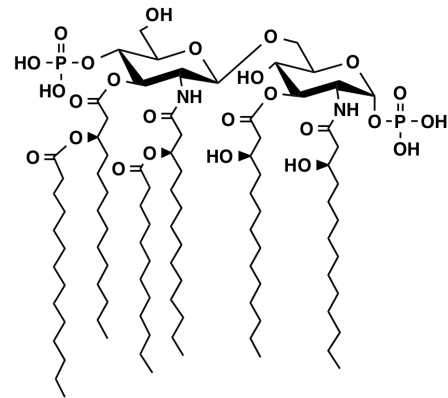
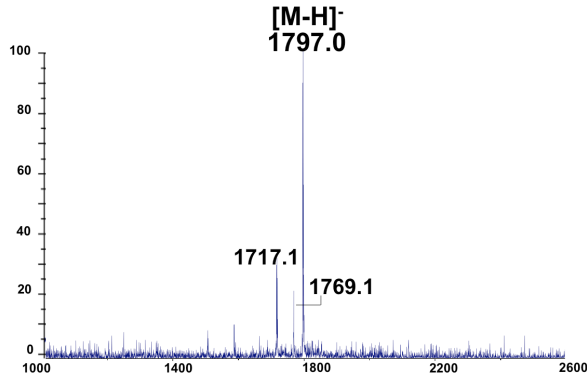
Table S2 – Docx  
Oligonucleotides used in this study.

Table S3 – Docx  
Densitometry analysis values for Figure 4.

Table S4 – Excel  
RNAseq data High vs. Low  $Mg^{2+}$  Replicates 1 and 2, wild-type

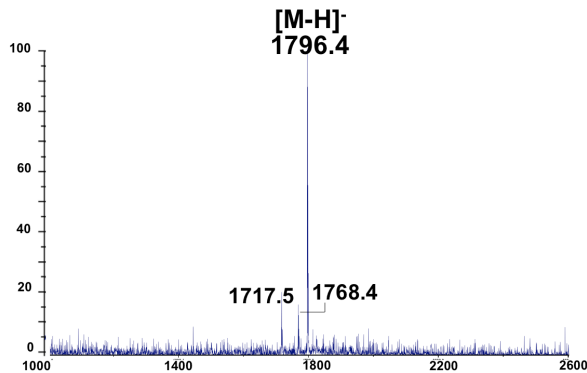
Table S5 – Excel  
RNAseq data High vs. Low  $Mg^{2+}$  Replicates 1 and 2, *pmrD*<sup>-</sup>

**A. W3110 *pmrA*<sup>-</sup> Low Mg<sup>2+</sup>**

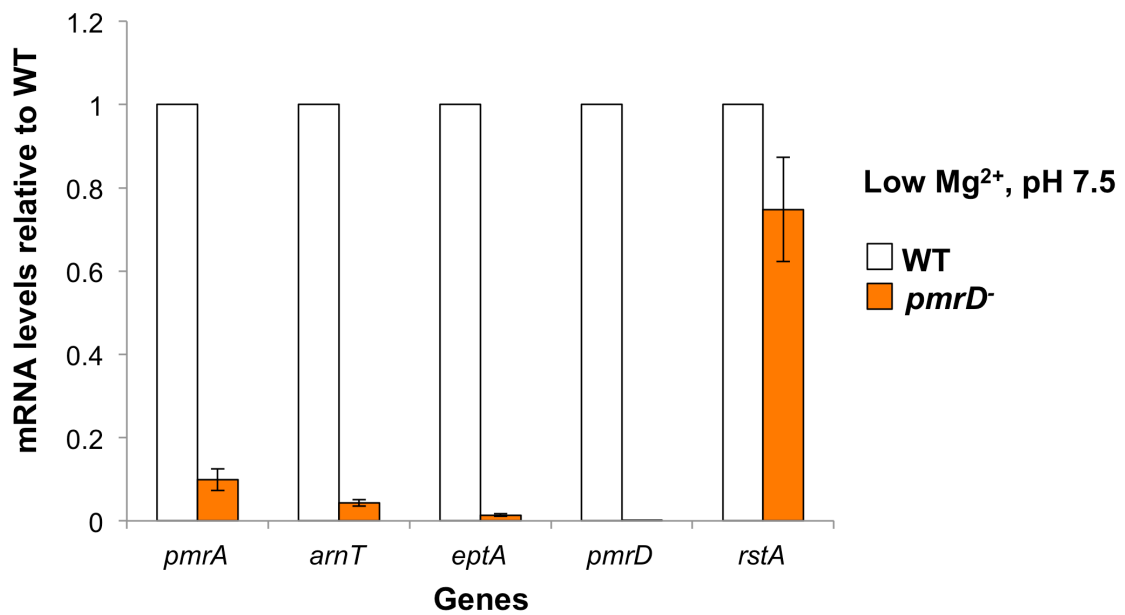


Exact mass:  
1797.2

**B. W3110 *pmrA*<sup>-</sup> High Mg<sup>2+</sup>**



**FIG S1 MALDI-TOF mass spectrometry of negative control strain W3110 *pmrA*<sup>-</sup>.** This strain serves as a negative control for the W3110 *pmrD*<sup>-</sup> strain. **(A,B)** W3110 *pmrA*<sup>-</sup> produces hexa-acylated lipid A in both 10 $\mu$ M (low) and 10mM (high) Mg<sup>2+</sup> growth conditions, generating major peaks at *m/z* 1797.0 and *m/z* 1796.4, respectively. The peaks at *m/z* 1717.1 (low Mg<sup>2+</sup>) and *m/z* 1717.5 (high Mg<sup>2+</sup>) correspond to 1-dephosphorylated hexa-acylated lipid A, and *m/z* 1769.1 (low Mg<sup>2+</sup>) and *m/z* 1768.4 (high Mg<sup>2+</sup>) to loss of an ethylene group.



**FIG S2 *pmrD* impacts transcription of *pmrA* and its downstream genes.** Relative gene expression of *pmrA*, *arnT*, *eptA*, *pmrD* and *rstA* when *E. coli* W3110 wild-type and *pmrD* mutant were grown in N-minimal medium with 10 $\mu$ M Mg<sup>2+</sup> (low). Results are representative of 3 technical replicates.

**Table S1.** Strains and plasmids used in this study.

Strains	Genotype or Description	Source or Reference
W3110	Wild type, F <sup>-</sup> rph-1 INV( <i>rrmD</i> , <i>rrnE</i> ) 1 <i>rph</i> -1	<i>E. coli</i> genetic stock center (Yale)
W3110 <i>pmrD</i> :: <i>kan</i>	W3110 containing kanamycin cassette at <i>pmrD</i>	This work
W3110 <i>pmrD</i> :: <i>kan</i> + <i>pmrD</i>	W3110 <i>pmrD</i> - complemented with pWSK29 <i>pmrD</i>	This work
W3110 <i>phoPQ</i> :: <i>cam</i>	W3110 containing chloramphenicol cassette at <i>phoPQ</i>	This work
W3110 <i>phoPQ</i> :: <i>cam</i> , <i>pmrD</i> :: <i>kan</i>	W3110 <i>phoPQ</i> - containing kanamycin cassette at <i>pmrD</i>	This work
W3110 <i>phoPQ</i> :: <i>cam</i> , <i>pmrD</i> :: <i>kan</i> + <i>pmrD</i>	W3110 <i>phoPQpmrD</i> - expressing pWSK29 <i>pmrD</i>	This work
W3110 $\Delta$ <i>pmrA</i>	W3110 with <i>pmrA</i> deleted, no marker	This work
W3110 $\Delta$ <i>pmrA</i> + <i>pmrD</i>	W3110 <i>pmrA</i> - expressing pWSK29 <i>pmrD</i>	This work
MG1655	F <sup>-</sup> , lambda <sup>-</sup> , <i>rph</i> -1	Gift from S. Payne
MG1655 <i>pmrD</i> :: <i>kan</i>	MG1655 containing kanamycin cassette at <i>pmrD</i>	This work
MG1655 <i>pmrD</i> :: <i>kan</i> + <i>pmrD</i>	MG1655 <i>pmrD</i> - complemented with pWSK29 <i>pmrD</i>	This work
WD101	W3110 constitutive <i>pmrA</i> mutant, polymyxin B resistant	(1)
EHEC EDL 933	Serotype O157:H7	ATCC
ETEC H10407	Serotype O78:H11	ATCC
XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lacIqZ</i> $\Delta$ <i>M15</i> ::Tn10 (Tetr)]	Stratagene
Plasmids		
pET21a	Vector containing a T7 promoter; Amp resistance	Novagen
pET21a <i>pmrD</i>	pET21a containing W3110 <i>pmrD</i> coding sequence	This work
pWSK29	Low copy vector containing T7 and T3 RNA polymerase promoters; Amp resistance	(2)
pWSK29 <i>pmrD</i>	pWSK29 containing W3110 <i>pmrD</i> coding sequence plus ribosome binding site	This work
pWSK29EHEC <i>pmrD</i>	pWSK29 containing EHEC <i>pmrD</i> coding sequence plus ribosome binding site	This work
pWSK29ETEC <i>pmrD</i>	pWSK29 containing ETEC <i>pmrD</i> coding sequence plus ribosome binding site	This work

1. **Trent MS, Ribeiro AA, Lin S, Cotter RJ, Raetz CR.** 2001. An inner membrane enzyme in *Salmonella* and *Escherichia coli* that transfers 4-amino-4-deoxy-L-arabinose to lipid A: induction on polymyxin-resistant mutants and role of a novel lipid-linked donor. *J. Biol. Chem.* **276**:43122–43131.
2. **Wang RF, Kushner SR.** 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* **100**:195–199.

**Table S2.** Oligonucleotides used in this study.

Primer	Sequence	Restriction Site
1 ECpmrDF_Ndel	GCGCGCCATATGGAATGGCTGGTCAAAAAA	Ndel
2 ECpmrDR_BamHI	GCGCGCGGATCCTTACTGAGTTTTCCCTGC	BamHI
3 pmrDkeiocheck_R	GAGTGGGTGCAACGTCAGCAA	-
4 pmrAkeiocheck_R	GCTGCGGATGATATTCTGCAA	-
5 pmrBkeiocheck_R	TTTGGCTATATGCTGGTCGCG	-
6 k1 (1)	CAGTCATAGCCGAATAGCCT	-
7 GyrBF	ACGCTGCTGTTGACCTTCTT	-
8 GyrBR	TCCTGCTTGCCTTTCTTCAC	-
9 PmrDF	ATGGAATGGCTGGTCAAAAA	-
10 PmrDR	CATTCTGCAAAGGCGAGAGT	-
11 EptAF	CAGCGACTGGCAAATCT	-
12 EptAR	TAGTTTCACGCGGGTAGC	-
13 PmrAF	GGGCGGTGAAGAGTTGATT	-
14 PmrAR	TTGGTCGAGGGTTCATTGTC	-
15 ArnTF	TCAGCCAAGCCGCTATATTC	-
16 ArnTR	ATCACCGCTGACAAATCTCC	-
17 RstAF	GTGGAAGATGATGCGGAAGT	-
18 RstAR	CCTGGTAGCATGATGTGCGAGT	-
19 PhoP1	ATAATCGCGTTACACTATTTTAATAATTAAGAC AGGGAGAAATAAAAATGTGTAGGCTGGAGCT GCTTCG	-
20 PhoQP2	TTAACGTAATGCGTGAAGTATGGGCATATTTA TTCATCTTTTCGGCGCAGAATGGGAATTAGCCA TGGTCC	-

1. **Datsenko KA, Wanner BL.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **97**:6640–6645.

**Table S3.** Densitometry analysis values for Figure 4.

	<b>% Modified<sup>a</sup></b>	<b>% Unmodified<sup>b</sup></b>
<b>WD101</b>	98.52	1.48
<b>WT</b>	89.42	10.58
<b>phoPQ<sup>-</sup></b>	40.06	59.94
<b>phoPQD<sup>-</sup></b>	5.49	94.51
<b>phoPQpmrD<sup>-</sup> + pmrD</b>	90.56	9.44
<b>pmrA<sup>-</sup></b>	1.28	98.72
<b>pmrA<sup>-</sup> + pmrD</b>	1.08	98.92

<sup>a</sup> “% modified” refers to percentage of radiolabeled lipid A species in each strain profile containing pETN or L-Ara4N additions

<sup>b</sup> “% unmodified” refers to percentage of radiolabeled lipid A species in each strain profile that are unmodified hexa-acylated *bis*-phosphorylated lipid A