Supplemental Information: Lytic transglycosylases RlpA and MltC assist in *Vibrio cholerae* daughter cell separation

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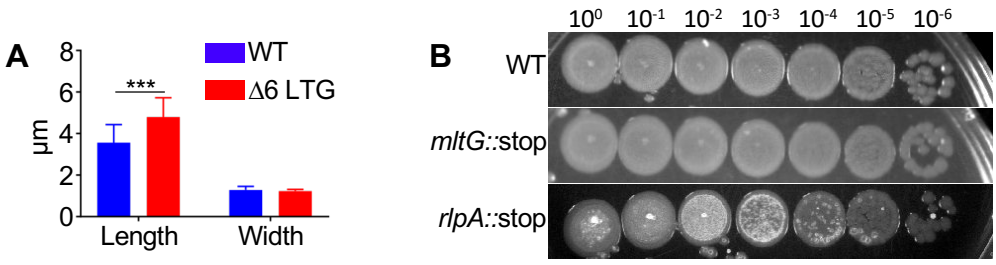


Fig S1. ∆6 LTG exhibits a mild morphological defect and *mltG*⁻, *rlpA*⁻ exhibit no plating defects.

A) Cultures of WT and ∆6 LTG were grown in LB at 37 °C for 5 hrs and imaged on agarose pads. Dimensions of >800 cells were compared within length and within width with a Student's t-test where *** indicates p< 0.0001. **B)** Overnight cultures of WT, *rlpA::*stop, and *mltG::*stop grown in LB at 37°C were spot-plated in serial 10-fold dilutions onto LB and incubated ~18 hrs at 30°C. All data are representative of at least two biological replicates.

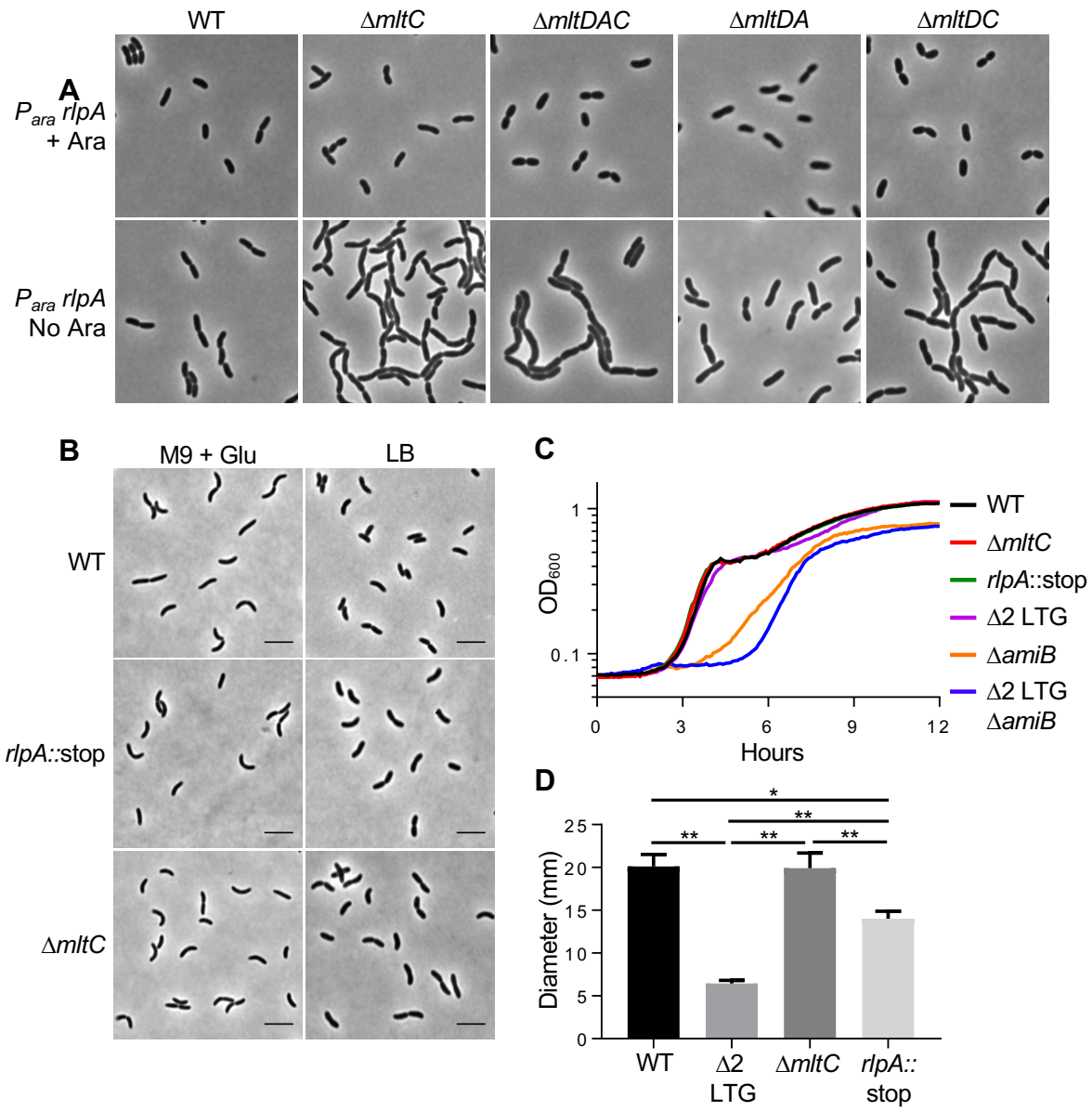


Fig S2. ***rlpA::*stop Δ*mltC* mutant exhibits motility defect but no growth defect.**
A) RlpA was depleted in WT, Δ*mltC*, Δ*mltDAC*, Δ*mltDA*, and Δ*mltDC* backgrounds by placing its native promoter under control of arabinose induction and growing in the absence or presence of arabinose. Cells were imaged on an agarose pad. **B)** WT, *rlpA::*stop, and Δ*mltC* were grown to ~OD_{600}0.5 in M9+0.2%Glu or LB at 37°C and imaged on agarose pads. **C)** Autolysin mutants were grown in 200 µL LB, monitoring OD_{600}. **D)** Overnight cultures of autolysin mutants grown in LB were stabbed into 0.3% agarose LB plates and incubated at 30°C for 16 hrs before measuring the diameter of growth. Diameters of mutant strains were compared to WT by a one-way ANOVA and Tukey's test where * indicates a $p<0.05$ and ** a $p<0.01$. Error bars represent SEM of 3 independent replicates. Scale bars = 5 µm.

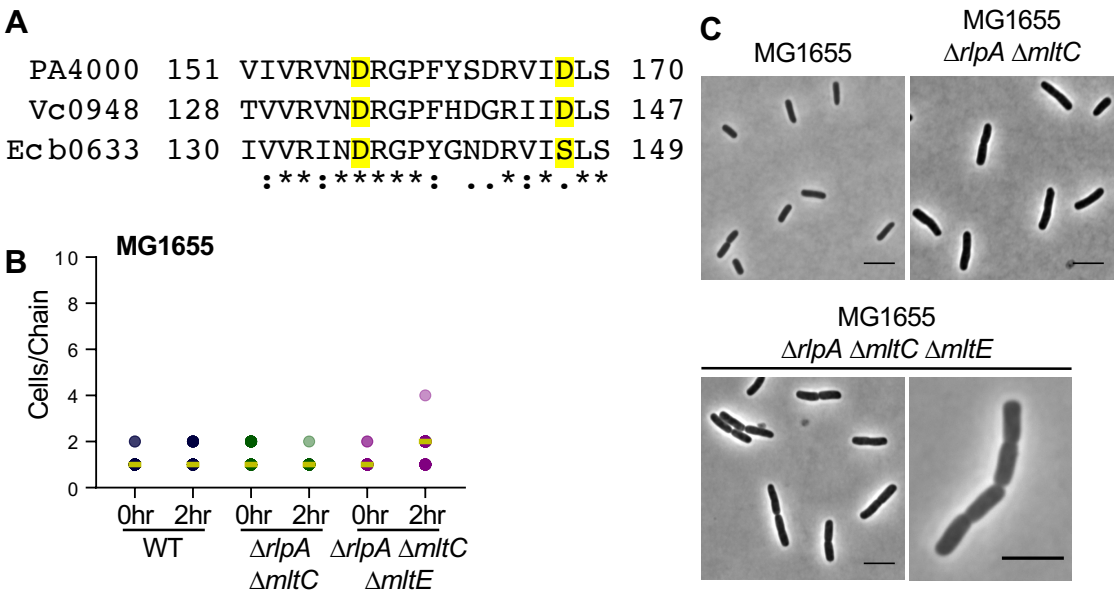


Fig S3. *E. coli* Δ*rlpA* Δ*mltC* Δ*mltE* mutant exhibits slight separation defect.
A) Clustal-W alignment of RlpA homologs PA4000, Vc0948, and Ec b0063 protein sequences. Predicted catalytic residues based on an MltA alignment are highlighted in yellow. In the alignment, * = conserved, : = highly similar, and . = somewhat similar residues. **B)** Cells per chain where manually counted (n >100). Gold bar = median. **C)** WT, Δ*rlpA* Δ*mltC*, and Δ*rlpA* Δ*mltC* Δ*mltE* were grown at 37°C to ~OD_{600} 0.6 and imaged on agarose pads. Scale bars = 5 μm.

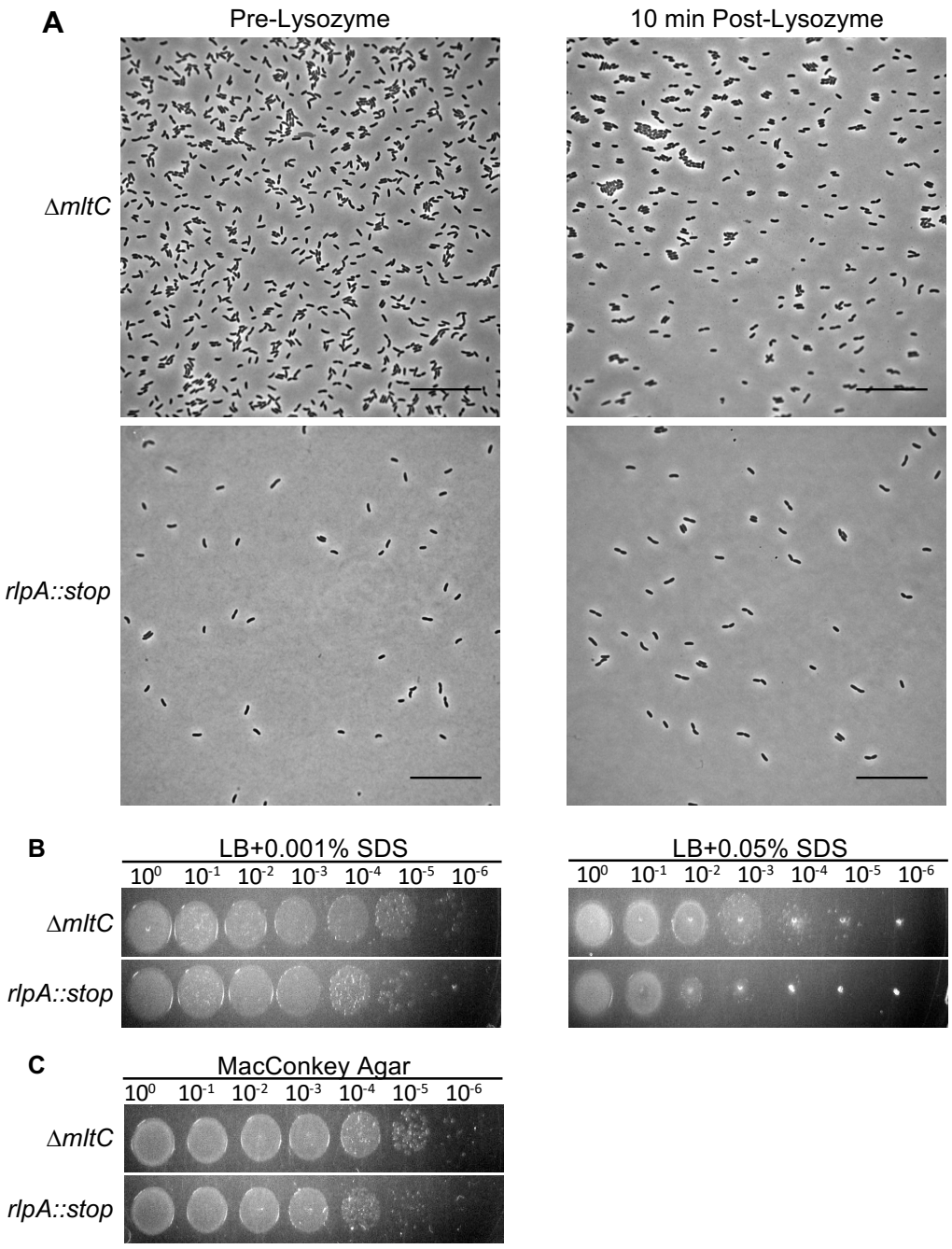


Fig S4. Single mutants of *rlpA::stop* and Δ*mltC* exhibit a mild outer membrane defect

Exponential phase cultures of *rlpA::stop* and Δ*mltC* were exposed to 5 mg mL^{-1} lysozyme for 10 min. Scale bars = 25 μm. Overnight cultures of *rlpA::stop* and Δ*mltC* grown in LB were diluted in LB and spot-plated in 10-fold serial dilutions on **B)** LB containing SDS and on **C)** MacConkey agar and incubated at 18hrs at 30°C. All data are representative of at least two biological replicates.

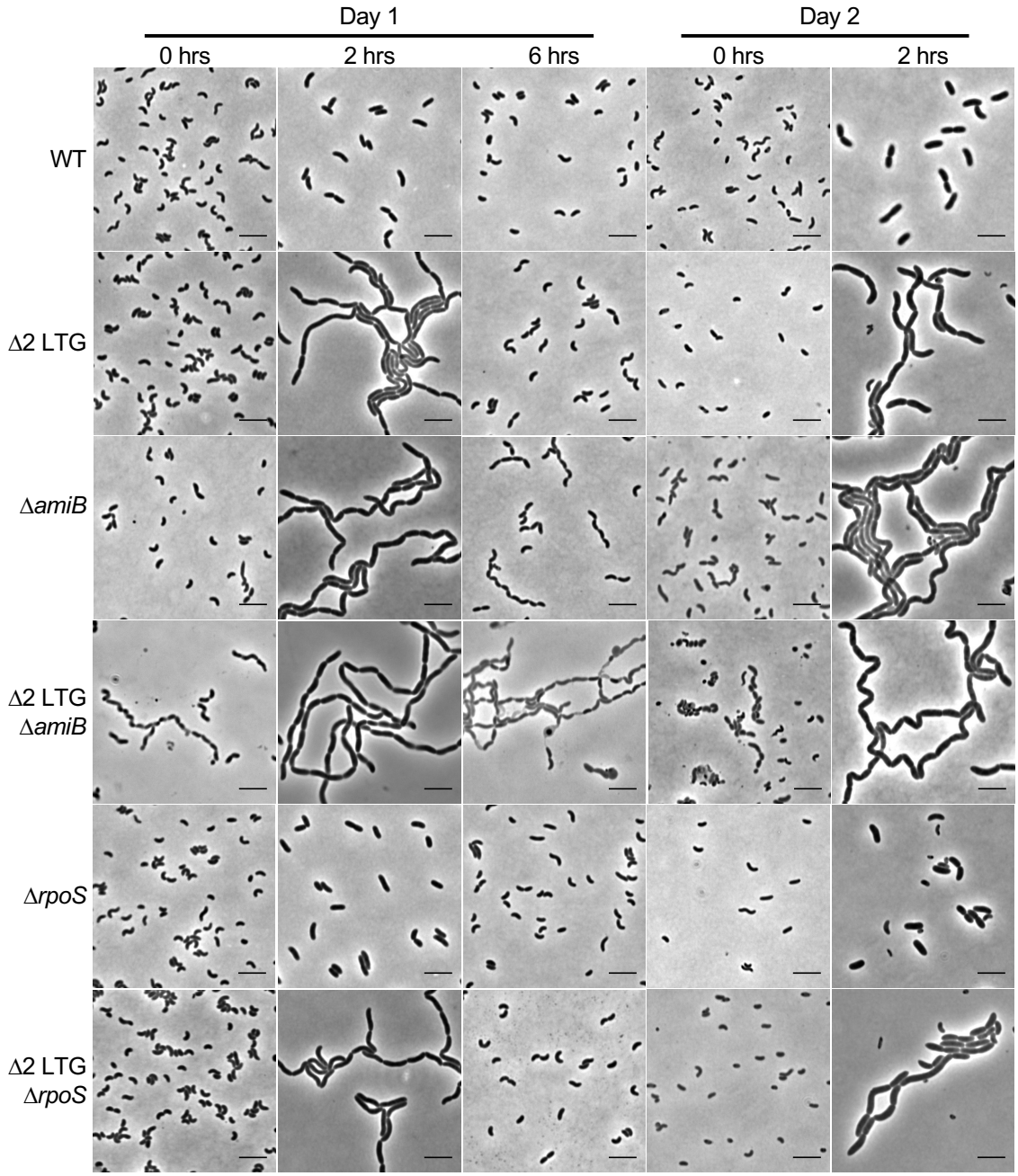
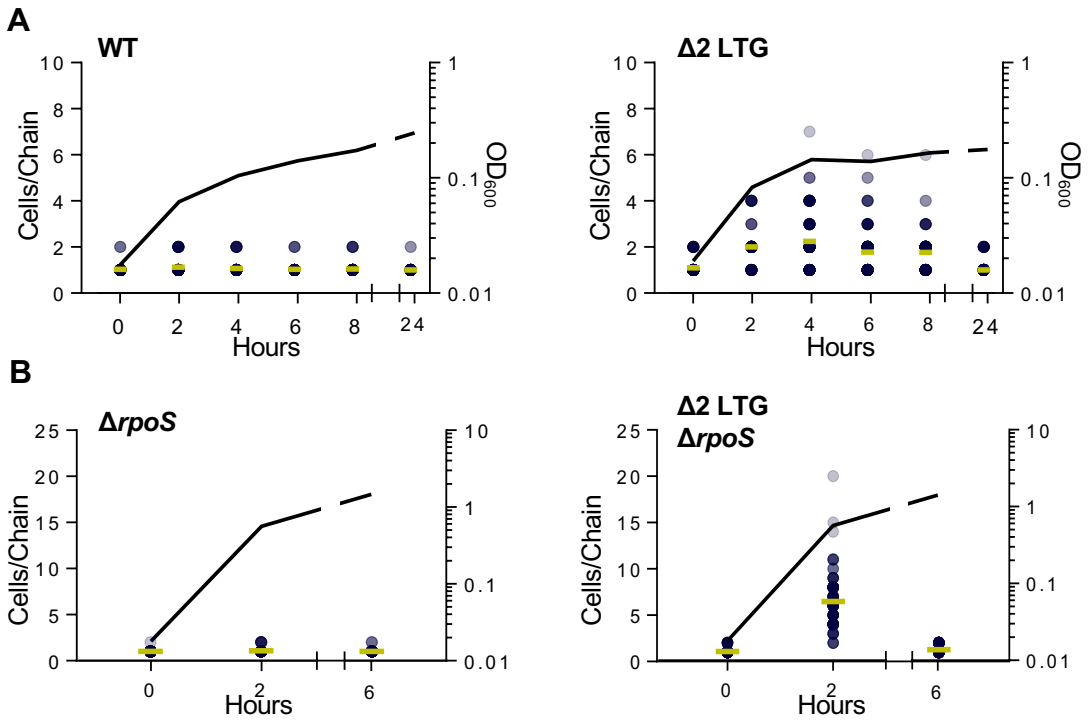


Fig S5. **Resolution of septal autolysin mutant chains is not due to spontaneous suppressors**.

Septal autolysin and Δ*rpoS* mutants were grown in LB at 37°C for 24hrs, then back-diluted into LB at 37°C. Cultures were imaged on agarose pads. Scale bars = 5 μm.



FigS6. Factors secreted during stationary phase do not affect Δ2 LTG chaining defect. **A)** WT and Δ2 LTG were grown in the supernatant of an overnight WT LB culture at 37°C and imaged on agarose pads. Cells per chain where manually counted (n >100). Gold bar = median. **B)** Δ*rpoS* and Δ2 LTG Δ*rpoS* were grown in LB at 37°C and imaged on agarose pads and analyzed as in Fig S5A.

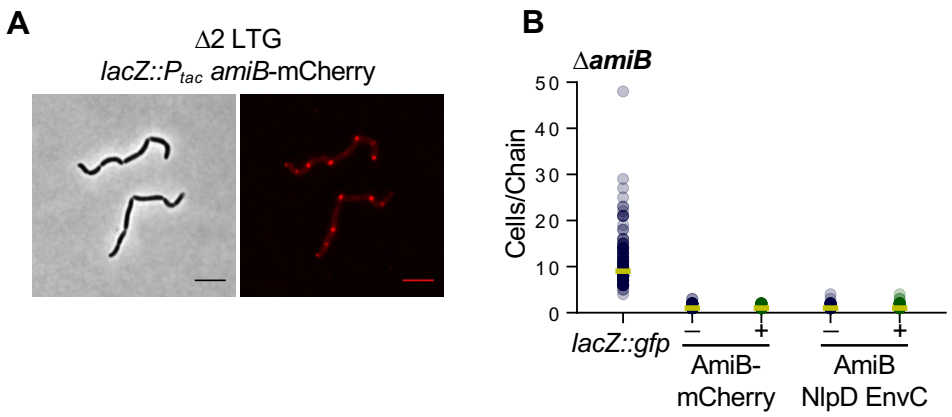


Fig S7. Functional AmiB-mCherry localizes in Δ2 LTG.

A) Expression of P_{tac}: *amiB-mCherry* was induced with 1 mM IPTG in a Δ*amiB* background, grown in LB at 37°C to ~OD_{600} 0.6, and imaged on agarose pads. Cells per chain where manually counted (n >100). Gold bar = median. Gold bars = median. **B)** Expression of P_{tac}: *amiB-mCherry* was induced with 1 mM IPTG in a Δ2 LTG background, grown in LB at 37°C to ~OD_{600} 0.6, and imaged on agarose pads.

Scale bars = 5 μm

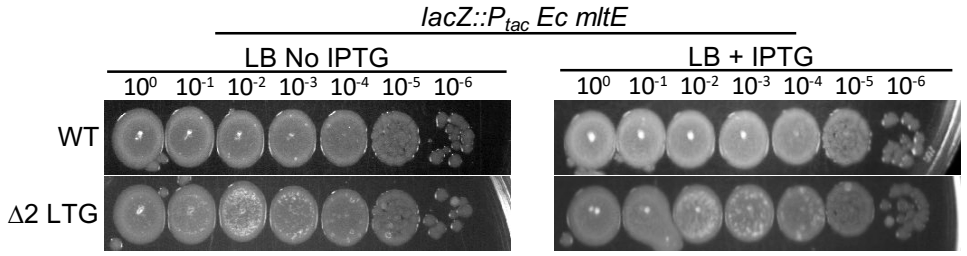


Fig S8. $MltE_{E.\ coli}$ is not toxic to WT or Δ2 LTG *V. cholerae.*

A) Overnight cultures of P_{tac}: $mltE_{E.\ coli}$ in WT and Δ2 LTG backgrounds were spot-plated in serial 10-fold dilutions onto LB +/- 1 mM IPTG and incubated overnight at 30°C.

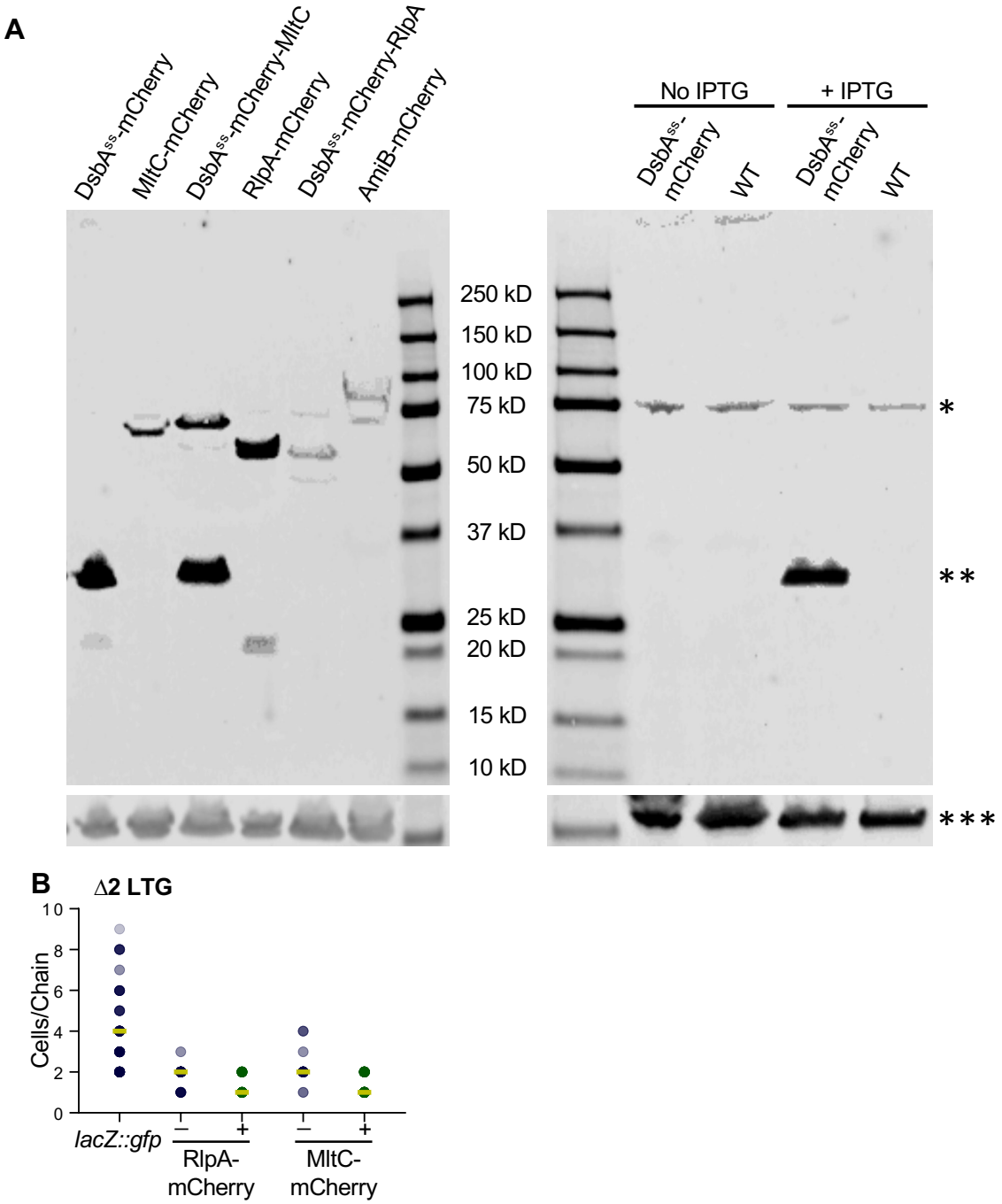


Fig S9. Autolysin-mCherry fusions are functional.

A) With the exception of AmiB-mCherry expressed from chromosomal P_{tac}, mCherry fusions were expressed from IPTG-inducible vector pHL100mob. Fusion protein expression was induced with 0.2% arabinose or 1 mM IPTG in a WT background grown in LB at 37°C and detected with Genetex polyclonal mCherry antibody. Loading control band detected with BioLegend monoclonal RpoA antibody. *=non-specific crossreaction with mCherry antibody, **=soluble mCherry, ***=RpoA.

B) Expression of chromosomal P_{tac}: *rlpA-mCherry* or *mltC-mCherry* was induced with 1 mM IPTG in a Δ2 LTG background, grown in LB at 37°C to ~OD_{600} 0.6, and imaged on agarose pads.

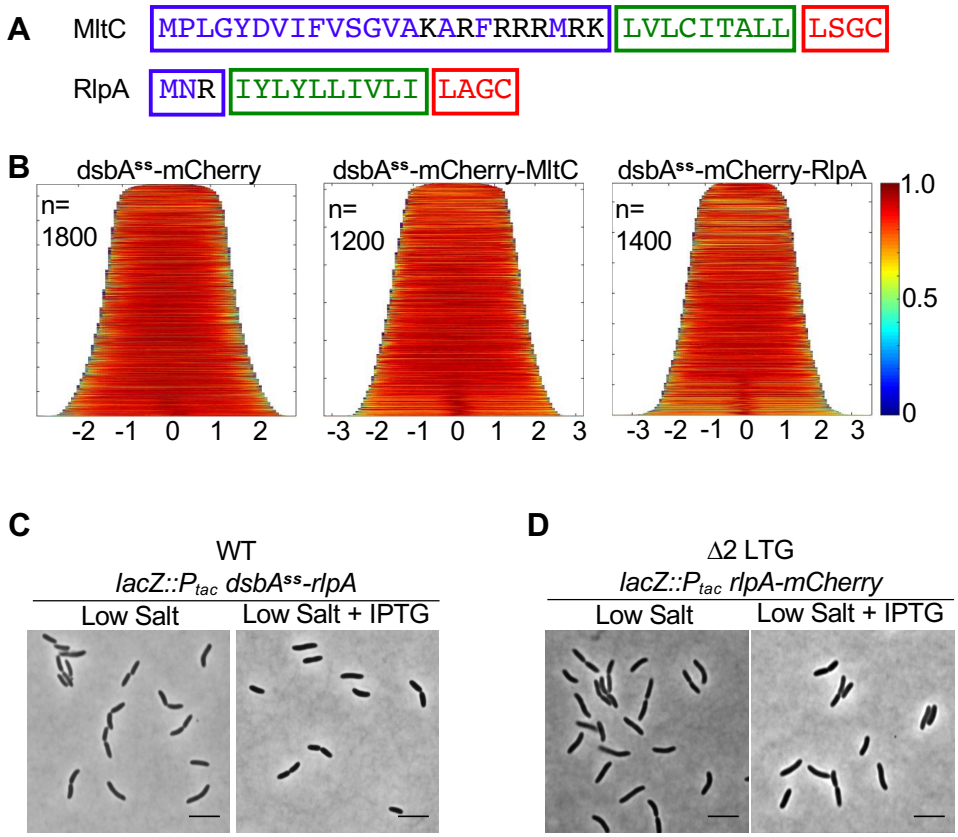


Fig S10. RlpA and MltC can localize without lipidation.

A) Outer membrane signal sequences and lipoboxes in LTG amino acid sequences were predicted by the DOLOP algorithm. **B)** WT carrying pHL100 *dsbA*^{ss}*-mCherry-LTGΔss* was grown in M9 + 0.2% glucose/ kan50 at 30 °C, induced with 1mM IPTG after 2hrs, and imaged on agarose pads at OD_{600}~0.15. Demographs of *mCherry* fusion localization generated with Oufti. **C)** Expression of P_{tac}: *dsbA*^{ss}*-rlpA*$_{[18\text{-}263]}$ was induced in a WT background with 1 mM IPTG, grown in low salt LB for 4 hrs, and imaged on agarose pads. **D)** Expression of P_{tac}: *rlpA-mCherry* was induced in a Δ 2 LTG background, grown in low salt LB for 4 hrs, and imaged on agarose pads.

Table S1. V. cholerae strains

Strain	Description	Source or reference
N16961	Wild-type V. cholerae El Tor strain	Heidelberg, et. al., 2000
AW388	pHL100 mltC-mCherry, pBAD33 yfp-ftsZ	This study
AW390	ΔmltC, rlpA::stop (denoted as Δ2 LTG)	This study
AW414	ΔmltC	This study
AW431	rlpA::stop	This study
AW452	ΔmltA, ΔmltB, ΔmltC, ΔmltD, ΔmltF, Δslt70 (denoted as Δ6 LTG)	This study
AW460	pHL100 rlpA-mCherry	This study
AW465	pHL100 rlpA-mCherry, pBAD33 yfp-ftsZ	This study
AW482	ΔamiB	This study
AW487	*lacZ::GFP*	This study
AW488	*ΔmltC, rlpA::stop, lacZ::GFP*	This study
AW581	*ΔrpoS<>kan*	This study
AW582	*ΔmltC, rlpA::stop, ΔrpoS<>kan*	This study
AW597	*lacZ::P_{tac}yfp-ftsN, pHL100 rlpA-mCherry*	This study
AW598	*lacZ::P_{tac}yfp-ftsN, pHL100 mltC-mCherry*	This study
AW607	*ΔmltC, rlpA::stop, lacZ::P_{tac}shyC*	This study
AW615	*ΔmltC, rlpA::stop, lacZ::P_{tac}shyA*	This study
AW634	*mltG::stop*	This study
AW636	*ΔmltC, rlpA::stop, lacZ::P_{tac} rlpA-mCherry*	This study
AW639	*ΔmltC, rlpA::stop, lacZ::P_{tac} mltC-mCherry*	This study
AW664	*lacZ::P_{tac} amIB-mCherry*	This study
AW665	*ΔmltC, rlpA::stop, lacZ::P_{tac} amiB-mCherry*	This study
AW666	*ΔamiB, lacZ::P_{tac} amiB-mCherry*	This study
AW679	*ΔmltC, rlpA::stop, lacZ::P_{tac} dsbA^{ss}-mCherry*	This study
AW683	*lacZ::P_{tac} Ec mltE*	This study
AW684	*ΔmltC, rlpA::stop, lacZ::P_{tac} Ec mltE*	This study
AW708	ΔmltA, ΔmltB, ΔmltC, ΔmltD, ΔmltF, Δslt70, P_{ara} rlpA	This study
AW717	*ΔmltC, rlpA::stop, lacZ::P_{tac} amiB nlpD envC*	This study
AW719	ΔmltC, rlpA::stop, ΔamiB	This study
AW722	*ΔamiB, lacZ::P_{tac} amiB nlpD envC*	This study
AW733	*pHL100mob dsbA^{ss}-mCherry*	This study
AW734	*pHL100mob dsbA^{ss}-mCherry-mltC*	This study
AW735	*pHL100mob dsbA^{ss}-mCherry-rlpA*	This study
AW736	*pHL100 mltC-mCherry*	This study
AW756	*ΔmltC, rlpA::stop, lacZ::P_{tac} dsbA^{ss}-mltC*	This study
AW757	*lacZ::P_{tac} dsbA^{ss}-rlpA*	This study
AW758	*ΔmltC, rlpA::stop, lacZ::P_{tac} dsbA^{ss}-rlpA*	This study
AW771	*ΔmltD ΔmltA*	This study
AW772	*ΔmltD ΔmltA ΔmltC*	This study
AW773	ΔmltC ΔmltD	This study

Table S2. E. coli strains

Strain	Description	Source or reference
MG1655	*E. coli K-12 strain*	Blattner, et. al., 1997
AW650	ΔrlpA, ΔmltC	This study
AW656	ΔrlpA, ΔmltC, ΔmltE<>FRT-cat-FRT	This study

Table S3. Plasmids

Plasmid	Description	Source or reference	Relevant Primers
	pBAD33 yfp-ftsZ	Gift from Waldor lab	
	pBAD33 yfp-ftsN	Gift from Waldor lab	
pAW32	pHL100 mltC-mCherry	This study	TDP206/TDP208, TDP765/TDP766
pAW33	pHL100 rlpA-mCherry	This study	TDP1164/TDP1167, TDP765/TDP766
pJLZ111	pCVD442 lacZ::gfp	Ritchie, et. al., 2010	
pAW34	pTD101 yfp-ftsN	This study	AIW306/pHL revB
pAW35	pTD101 shyC	This study	AIW329/AIW330
pAW36	pTD101 shyA	This study	AIW327/AIW328
pAM321	pDS132 ΔamiB	Möll, et. al., 2014	
pAW37	pCVD442 ΔmltA	This study	TDP1199/TDP1200, TDP1201/TDP1202
pAW38	pCVD442 ΔmltB	This study	TDP1203/TDP1204, TDP1205/TDP1206
pAW39	pCVD442 ΔmltC	This study	DLP42/DLP43, DLP137/DLP138
pAW40	pCVD442 ΔmltD	This study	TDP1211/TDP1212, TDP1213/TDP1214
pAW41	pCVD442 ΔmltF	This study	TDP1258/TDP1257, TDP1255/TDP1256
pAW42	pCVD442 Δslt70	This study	TDP1215/TDP1216, TDP1217/TDP1218
pAW43	pCVD442 rlpA::stop	This study	DLP48/TDP210, TDP211/DLP51
pAW44	pCVD442 mltG::stop	This study	TDP1251/TDP826, TDP827/TDP1254
pAW45	pAM299 rlpA	This study	DLP186/DLP187
pAW46	pTD101 rlpA-mCherry	This study	AIW356/pHLrevB
pAW47	pTD101 mltC-mCherry	This study	AIW264/pHLrevB
pAW48	pTD101 amiB-mCherry	This study	AIW265/AIW250, AIW251/AIW252
pAW49	*pTD101 dsbA^{ss}-mCherry*	This study	AIWGB4, AIW251/AIW346
pAW50	pTD101 Ec mltE	This study	BR02/AIW380
pAW51	pTD101 amiB nlpD envC	This study	AIW265/AIW257, AIW255/256, AIW258/259
pAW52	*pHL100mob dsbA^{ss}-mCherry*	This study	AIWGB4, AIW251/AIW346
pAW53	*pHL100mob dsbA^{ss}-mCherry-mltC*	This study	AIWGB4, AIW343/AIW344
pAW54	*pHL100mob dsbA^{ss}-mCherry-rlpA*	This study	AIWGB4, AIW347/AIW348
pAW55	*pTD101 dsbA^{ss}-mltC*	This study	AIW343/AIW389
pAW56	*pTD101 dsbA^{ss}-rlpA*	This study	AIW347/AIW391

Table S3. Plasmids (continued)

Plasmid	Description	Source or reference	Relevant Primers
pKM208	P_{tac} λ Red, ampR, Rep^{ts}	Murphy and Campellone, 2003	
pKD3	*FRT-cat-FRT*	Datsenko and Wanner, 2000	
pKD4	*FRT-kan-FRT*	Datsenko and Wanner, 2000	
pCP20	FLP+, λ cI857+, λP_R , Rep^{ts}, ampR, cmR	Cherepanov and Wackernagel, 1995	
pJZ111	*lacZ::gfp*	Ritchie, et. al., 2010	