Cell Reports, Volume *32*

Supplemental Information

Modulating Isoprenoid Biosynthesis Increases Lipooligosaccharides and Restores *Acinetobacter baumannii* Resistance to Host and Antibiotic Stress

Lauren D. Palmer, Keaton E. Minor, Joshua A. Mettlach, Emilio S. Rivera, Kelli L. Boyd, Richard M. Caprioli, Jeffrey M. Spraggins, Zachary D. Dalebroux, and Eric P. Skaar

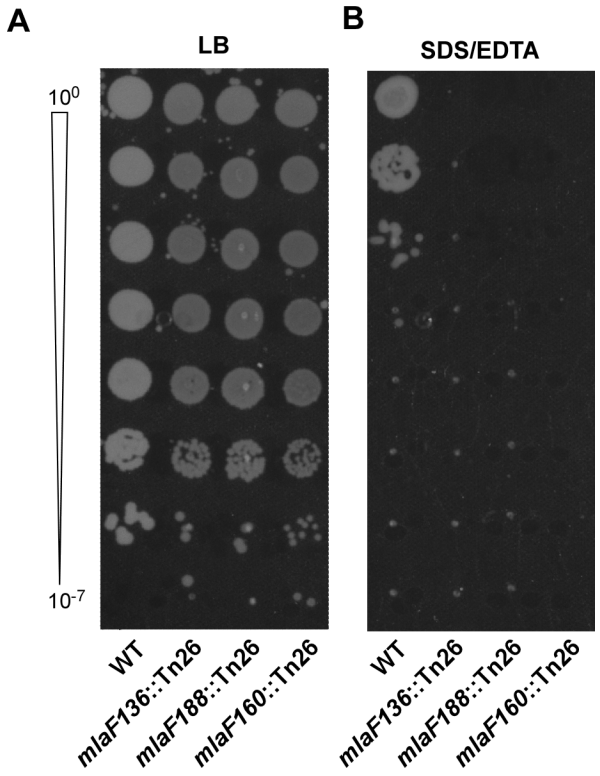

Figure S1: *A. baumannii* AB5075 *mlaF* mutants are defective at colony formation on SDS/EDTA. Related to Figure 1. (A-B) Wild-type (WT) and *mlaF* strains were diluted and spotted to lysogeny broth (LB) plates without (A) and with (B) 0.01% SDS and 0.15% EDTA.

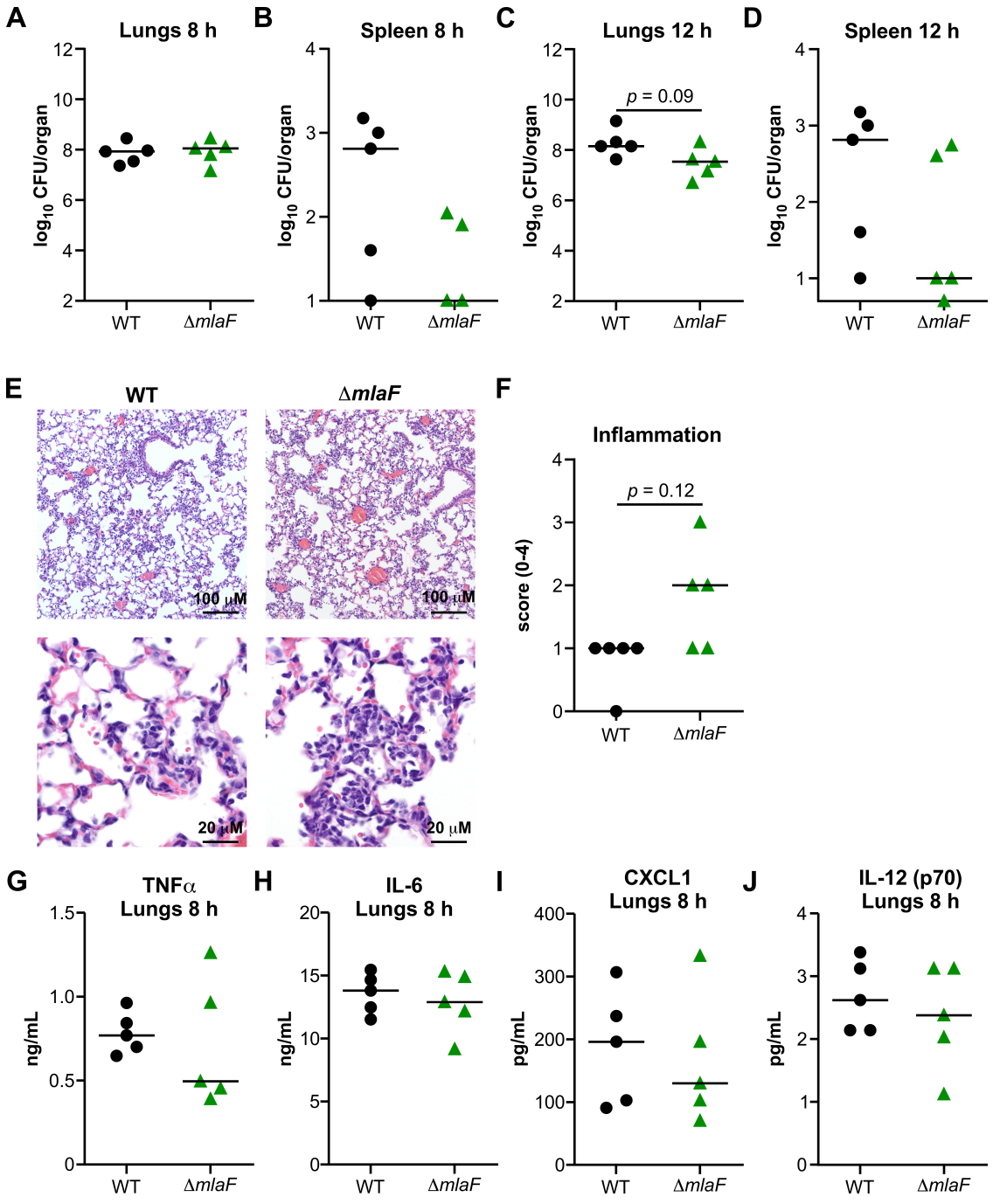

Figure S2: Infection at 8 h, 12 h, and histology at 36 h. Related to Figure 2. (A-B) At 8 h post infection, bacteria were enumerated from the lungs and spleens. (C-D) At 12 h post infection, bacteria were enumerated from the lungs and spleen. (E) At 8 h post infection, lungs were inflated and fixed prior to hematoxylin and eosin staining. (F) Inflammation was scored by a blinded pathologist. n=5; *p* value is by Mann-Whitney test. (G-J) At 8 h post infection, cytokines and chemokines in lung homogenates were quantified by Luminex. Medians are shown. No significance by Mann-Whitney test.

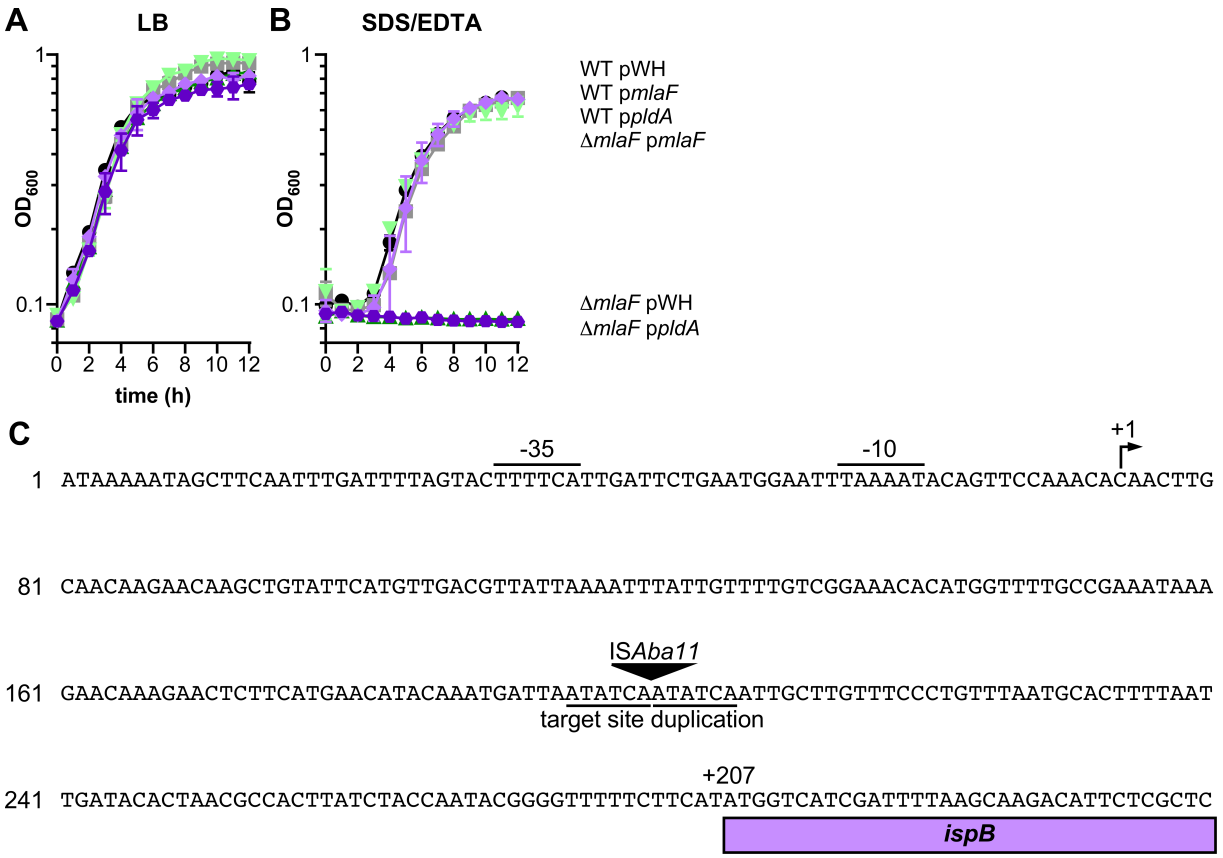

Figure S3: Constitutive expression of *pldA* and *mlaF*, and the predicted *ispB* promoter. Related to Figure 3. (A-B) Strains constitutively expressing *pldA* or *mlaF in trans* were grown in lysogeny broth (LB) without (A) and with (B) 0.01% SDS and 0.15 mM EDTA (n=3). Data are mean ± SEM. (C) Predicted *ispB* promoter region containing the IS*Aba11* insertion.

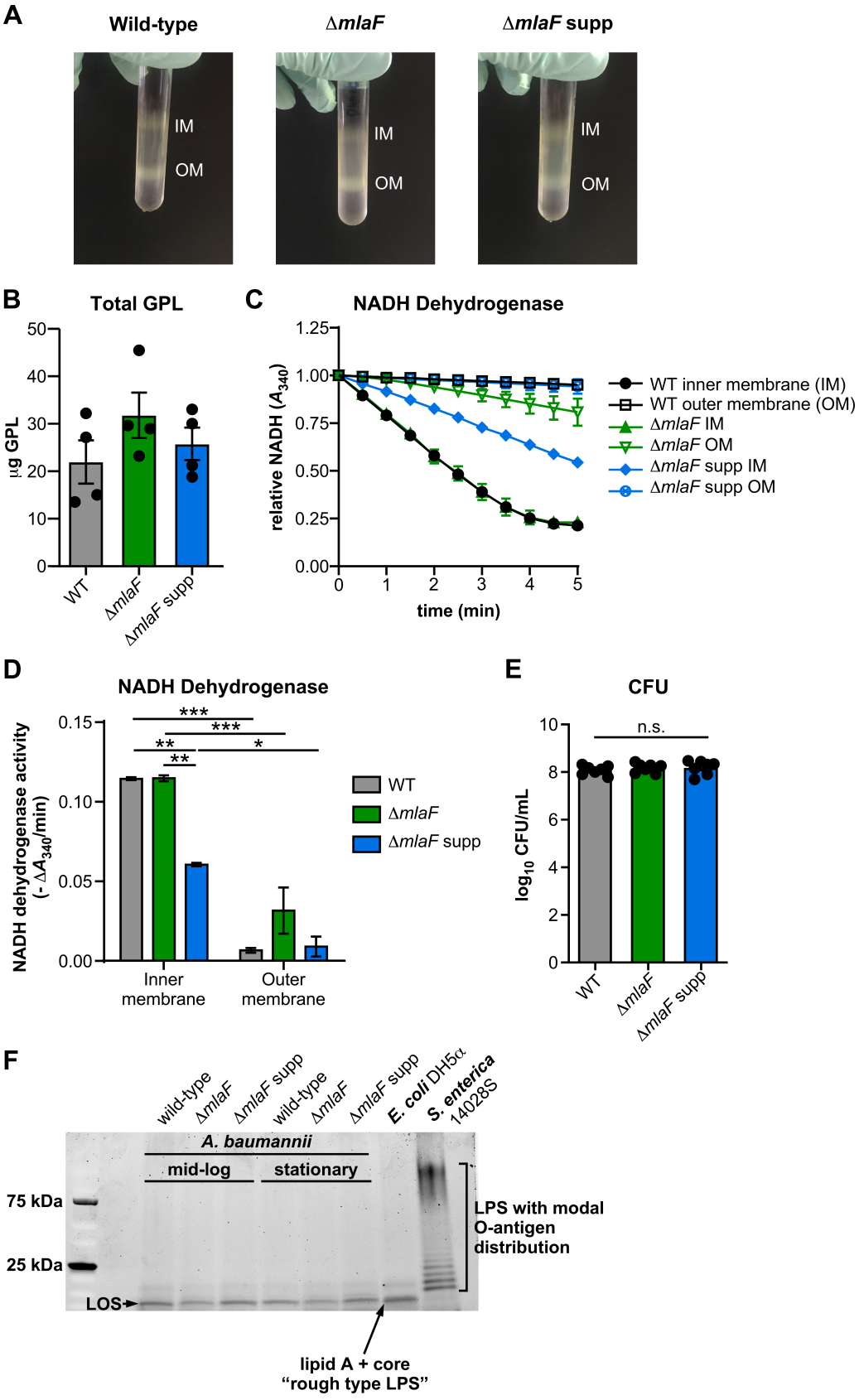
A
Wild-type
ΔmlaF
ΔmlaF supp
IM
OM
B
Total GPL
µg GPL
WT
ΔmlaF
ΔmlaF supp
C
NADH Dehydrogenase
relative NADH (A340)
time (min)
WT inner membrane (IM)
WT outer membrane (OM)
ΔmlaF IM
ΔmlaF OM
ΔmlaF supp IM
ΔmlaF supp OM
D
NADH Dehydrogenase
NADH dehydrogenase activity (- ΔA340/min)
Inner membrane
Outer membrane
WT
ΔmlaF
ΔmlaF supp
E
CFU
log10 CFU/mL
n.s.
F
wild-type
ΔmlaF
ΔmlaF supp
E. coli DH5α
S. enterica 14028S
A. baumannii
mid-log
stationary
75 kDa
25 kDa
LOS
LPS with modal O-antigen distribution
lipid A + core
"rough type LPS"

Figure S4: Supplemental information for membrane fractionation and LOS quantification. Related to Figure 4. (A) Membrane separation for *A. baumannii*. (B) Total glycerophospholipids (GPL) (n=4; significance is by one-way ANOVA with Tukey's multiple comparisons). (C) NADH dehydrogenase assay for inner membrane (IM; filled symbols) and outer membrane (OM; open symbols) fractions measured decrease in NADH by absorbance at 340 nm using 50 µg total protein and normalized to time = 0 s (n=2). (D) NADH dehydrogenase activity was determined by initial rate (n=2; significance is determined by two-way ANOVA with Tukey's multiple comparisons). (E) CFU quantification of samples used for LOS analysis (n=7; no significance by one-way ANOVA). (F) Representative gel showing LOS and LPS from *A. baumannii* strains, *E. coli* DH5α, and *S. enterica* serovar Typhimurium 14028S. Data are mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

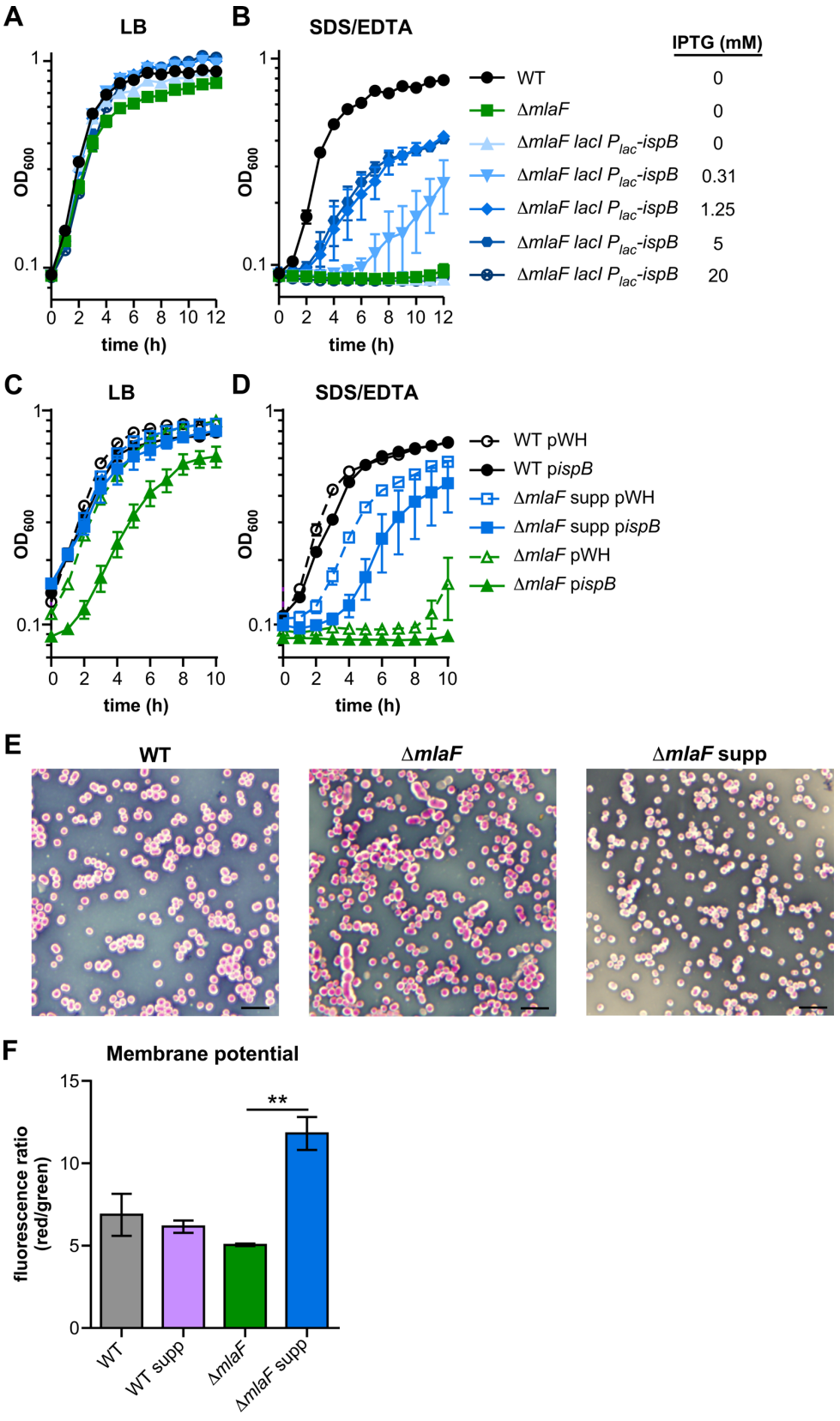
A
LB
B
SDS/EDTA
IPTG (mM)
WT 0
ΔmlaF 0
ΔmlaF lacI Plac-ispB 0
ΔmlaF lacI Plac-ispB 0.31
ΔmlaF lacI Plac-ispB 1.25
ΔmlaF lacI Plac-ispB 5
ΔmlaF lacI Plac-ispB 20
OD600
time (h)
C
LB
D
SDS/EDTA
WT pWH
WT pispB
ΔmlaF supp pWH
ΔmlaF supp pispB
ΔmlaF pWH
ΔmlaF pispB
E
WT
ΔmlaF
ΔmlaF supp
F
Membrane potential
fluorescence ratio (red/green)
**
WT
WT supp
ΔmlaF
ΔmlaF supp

Figure S5: Additional characterization of Δ*mlaF* suppressor strain phenotypes. Related to Figure 5. (A-B) Representative growth curves of the inducible *ispB* Δ*mlaF* strain in LB without (A) and with (B) 0.01% SDS and 0.175 mM EDTA (n=2). (C-D) Representative growth curves of strains constitutively expressing *ispB* in trans (filled symbols) in LB without (C) and with (D) 0.01% SDS and 0.1 mM EDTA (n=3). (E) Congo red exclusion to visualize capsule. Scale bar is 5 μm. (F) Membrane potential was assessed in mid-logarithmic growth in LB (n=2-3, significance is by one-way ANOVA with Tukey's multiple comparisons). Data are mean ± SEM. ** $p < 0.01$.

Table S1: Glycerophospholipids in the total membranes. Related to Figure 4. Data represent four biological replicates and significance was determined by 2-way ANOVA with Dunnett's multiple comparisons test. Data are mean ± standard deviation in ng GPL/extraction (normalized to 1.5 mg protein). Significance is denoted compared to wild-type as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; there were no statistically significant differences between Δ*mlaF* and Δ*mlaF* suppressor (supp) by 2-way ANOVA with Tukey's multiple comparisons test. GPL: glycerophospholipid; PGL: phosphatidylglycerol; aPGL: acyl-phosphatidylglycerol; PE: phosphatidylethanolamine; CL: cardiolipin.

	Total membranes		
GPL species	**Wild-type**	**Δ*mlaF***	**Δ*mlaF* supp**
PGL *m/z* 719 > 253	1317 ± 492	5256 ± 1642***	4276 ± 1227**
PGL *m/z* 747 > 281	4861 ± 2016	5983 ± 1575	5668 ± 1473
PGL *m/z* 773 > 281	2062 ± 1407	2116 ± 1286	1603 ± 871
aPGL *m/z* 955 > 253	2 ± 2	19 ± 14	10 ± 3
aPGL *m/z* 958 > 255	10 ± 4	91 ± 36	78 ± 10
aPGL *m/z* 986 > 255	172 ± 88	384 ± 124	427 ± 153
aPGL *m/z* 1012 > 255	36 ± 25	82 ± 52	55 ± 22
PE *m/z* 688 > 253	88 ± 43	486 ± 39	324 ± 120
PE *m/z* 716 > 281	10419 ± 4055	13238 ± 3007**	11104 ± 2912
PE *m/z* 742 > 281	1760 ± 1264	2980 ± 2588	1568 ± 905
CL *m/z* 1402 > 255	1244 ± 881	1179 ± 475	657 ± 504

Table S2: Glycerophospholipids in the outer membrane fraction. Related to Figure 4. Data represent three biological replicates and significance was determined by 2-way ANOVA with Dunnett's multiple comparisons test. Data are mean ± standard deviation in ng GPL/extraction (normalized to 0.5 mg protein). Significance is denoted compared to wild-type as * $p < 0.05$; there were no statistically significant differences between Δ*mlaF* and Δ*mlaF* suppressor (supp) by 2-way ANOVA with Tukey's multiple comparisons test. GPL: glycerophospholipid; PGL: phosphatidylglycerol; aPGL: acyl-phosphatidylglycerol; PE: phosphatidylethanolamine; CL: cardiolipin.

	Total membranes		
GPL species	**Wild-type**	**Δ*mlaF***	**Δ*mlaF* supp**
PGL *m/z* 719 > 253	82.1 ± 35.5	295.7 ± 61.7*	254.6 ± 83.3*
PGL *m/z* 747 > 281	613.4 ± 246.4	659.9 ± 134.3	565.5 ± 158.2
PGL *m/z* 773 > 281	139.9 ± 10.5	130.7 ± 86.8	63.7 ± 34.4
aPGL *m/z* 955 > 253	0.2 ± 0.1	1.2 ± 0.5	0.7 ± 0.1
aPGL *m/z* 958 > 255	1.8 ± 0.2	10.4 ± 3.2	8 ± 3.8
aPGL *m/z* 986 > 255	15.3 ± 3.6	30.1 ± 5.1	29.4 ± 6.9
aPGL *m/z* 1012 > 255	4.3 ± 1.2	7.6 ± 2.3	5.4 ± 1.4
PE *m/z* 688 > 253	1.9 ± 1.2	11.7 ± 0.8	9.2 ± 6
PE *m/z* 716 > 281	335.3 ± 165.7	451.6 ± 158.4	400.7 ± 161.8
PE *m/z* 742 > 281	62.3 ± 23.3	66 ± 25	58.1 ± 9.1
CL *m/z* 1402 > 255	450.8 ± 182.1	377.1 ± 151.1	328.5 ± 150.2

Table S3: Oligonucleotides used in this study. Related to STAR Methods section.

Sequence	Source	Identifier
CCCGGGTCTGAAATTGAAGGC	IDT (Coralville, IA)	BM17
CCTAGTTAGTCACATATGATTATTCATAATGGCAATCA	IDT (Coralville, IA)	BM18
GAATAATGACATATGCGTCCATGAATACGATTG	IDT (Coralville, IA)	BM19
CCCGGGATCAGTTGCTGTTC	IDT (Coralville, IA)	BM20
TGATTGCCATTATGAATAATCATATGTGACTAACTAGGAGGA	IDT (Coralville, IA)	BM21
CAATCGTATTCATGGACGCATATGTCATTATTCCCTCC	IDT (Coralville, IA)	BM22
CGAGGGGAACGCGTCATTT	IDT (Coralville, IA)	LP63
GCTCCACTCTGGAACAGCATTC	IDT (Coralville, IA)	LP64
GGTCGTACAGGTCGTGC	IDT (Coralville, IA)	LP69
AGACCACCACCCGGTAC	IDT (Coralville, IA)	LP70
AAAGGATCCTTTAAGAGATTTAAAAAAAGGCC	IDT (Coralville, IA)	LP71
AAAGTCGACTCATGGACGAACCTCG	IDT (Coralville, IA)	LP72
CACACCCGTCCTGTGGATCCGCGTGACAAAAGAGATATG	IDT (Coralville, IA)	LP127
TGAACGCCATCTCGAGGATATACCTGATAAAGTCAGCG	IDT (Coralville, IA)	LP143
CAGGTATATCCTCGAGATGGCGTTCAGACAATTTG	IDT (Coralville, IA)	LP144
AAGGCTCTCAAGGGCATCGGTCGACGGTACCTTAGTACCAGTTCATGAGTG	IDT (Coralville, IA)	LP145
GTATATCCTCGAGATATGGTACCGTCGACC	IDT (Coralville, IA)	LP148
ATACTCGAGATGAATAATAAAACTCCTCTCTCG	IDT (Coralville, IA)	LP146
AAAGGTACCTCATGGACGAACCTCG	IDT (Coralville, IA)	LP147
ACCGCTGACTTTATCAGGTATATCCTCGAGATGGTCATCGATTTTAAGCAAG	IDT (Coralville, IA)	LP246
TCTCAAGGGCATCGGTCGACGGTACCCTACTGTATTCGATGTAATGCAAG	IDT (Coralville, IA)	LP247
GGCGCAAGGGCTGCTAAAGGCGTCAGGCTGTCCATGCC	IDT (Coralville, IA)	LP258
TTCGAACCCCAGAGTCCCGCTCACAATGAAGTTGCGAATGG	IDT (Coralville, IA)	LP259
GTTAAAAAGGATCGATCCTCTAGAGGATCCAAATTGACCTTTCACTTC	IDT (Coralville, IA)	LP270
ACTTCGAAGCAGCTCCAGCCTACACATGAAGAAAAACCCCGTATTG	IDT (Coralville, IA)	LP271
AGGAACTAAGGAGGATATTCATATGATATTATTTTGCCTATGCATATAAATTTTTC	IDT (Coralville, IA)	LP272
ATGATTACGAATTCGAGCTCGGTACCAATAAGCTCACTGGTCGTG	IDT (Coralville, IA)	LP273
TATACATGCCCCAAAAGCC	IDT (Coralville, IA)	LP275
CATGCATGAGCTCACTAGTGGATCCTCACTGCCCGCTTTCCAG	IDT (Coralville, IA)	LP342

AGGTACCGGGCCCAAGCTTCTCGAGCTACTGTATTCGATGTAATGCAAG	IDT (Coralville, IA)	LP345
GCGCTTTTGAAGCTAATTCG	IDT (Coralville, IA)	LP354
ATTTCACTTATCTGGTTGGCC	IDT (Coralville, IA)	LP355
CGATGACCATGAGCTCGAATTCTGTTTCCTG	IDT (Coralville, IA)	LP356
ATTCGAGCTCATGGTCATCGATTTTAAGC	IDT (Coralville, IA)	LP357
CAGTTTCAACTTTTACGTAGCGAC	IDT (Coralville, IA)	LP358