

Figure S1: Overview of LPS and LOS structure. (A) A cartoon representation of the LOS and LPS structure of Gram-negative bacteria. The lipid A anchor, the core-oligosaccharide, and the O-antigen domains are indicated. Rough-LPS or R-LPS are LPS molecules lacking the O-antigen, as produced by *E. coli* K-12 strains. Organisms that synthesize LOS, such as *A. baumannii*, lack the machinery to produce the O-antigen polysaccharide. (B) The chemical structure of the lipid A moiety from either *E. coli* K-12 or *A. baumannii*. Isolates that are polymyxin resistant can modify their lipid A with additional moieties (not shown).

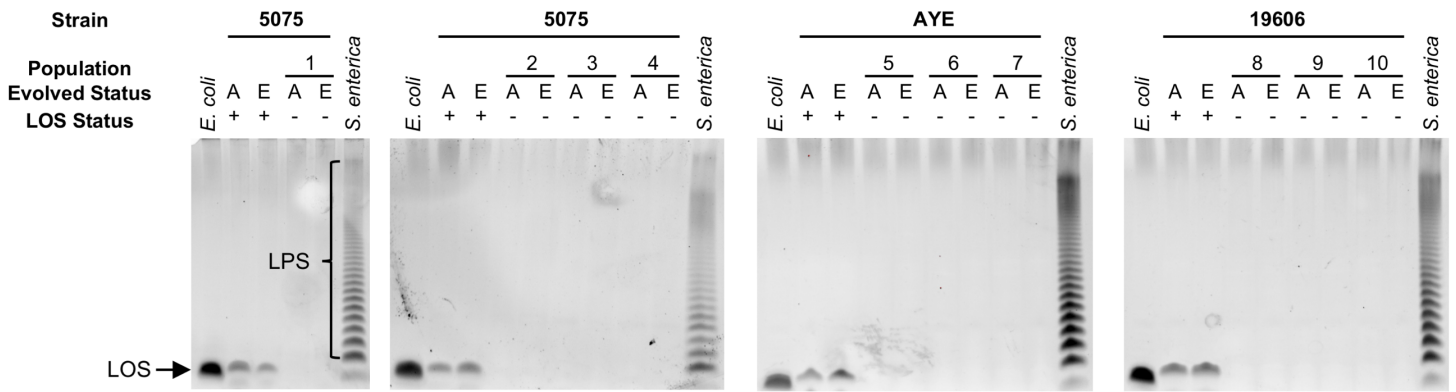


Figure S2: Populations remain LOS-deficient after evolution experiment. SDS-PAGE gels of proteinase K-treated cell lysates were stained for LPS using the ProQ-emerald 300 LPS staining kit. *E. coli* K-12 produces a LPS variant that is exclusively lipid A plus core sugars. *S. enterica* produces a full-length O-antigen, resulting in the characteristic banding pattern. *A. baumannii* produce LOS, which has a few additional sugars relative to *E. coli* K-12, causing it to run slightly higher (indicated by black arrow). For evolved status: A, ancestor and E, evolved.

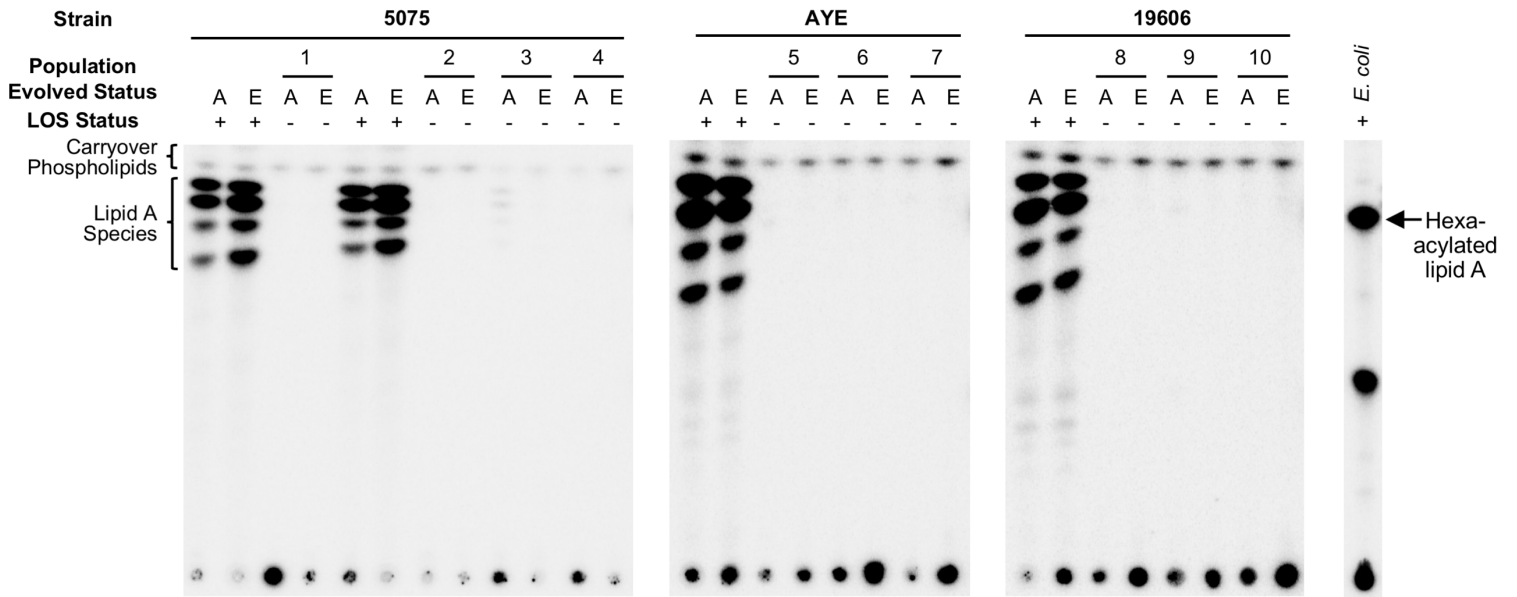


Figure S3: Populations do not produce lipid A after evolution experiment. TLC analysis of lipid A extracted from populations before and after evolution experiment. In each case, LOS-deficient populations do not produce any detectable radiolabeled lipid A. The four spots present in *A. baumannii* preps that do produce lipid A represent the four main lipid A species that are produced in a wild-type cell (43). The arrow indicates the major lipid A species for *E. coli* K-12. *E. coli* K-12 controls were run on the same TLC plate as *A. baumannii* samples.

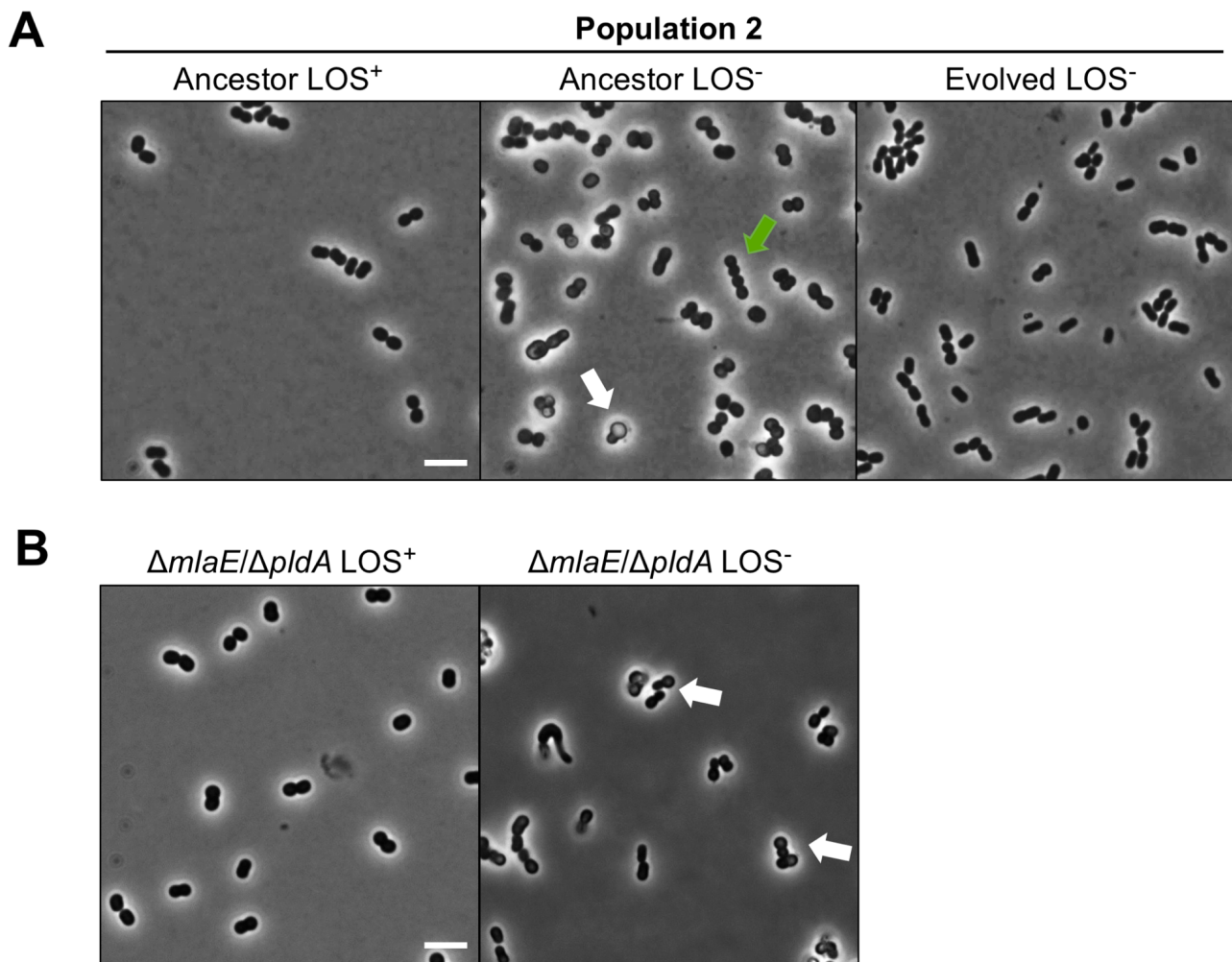


Figure S4: Phase microscopy shows that altered morphologies are independent of *mIa* and *pldA*. (A) Phase microscopy of wild-type, population 2 LOS-deficient ancestor, and population 2 LOS-deficient evolved show that while the LOS-deficient ancestor exhibits a wide array of morphologies that drastically differ from wild-type, the evolved population 2 LOS-deficient strain exhibits a relatively uniform morphology. (B) Phase microscopy of the 19606 $\Delta mlaE/\Delta pldA$ double mutant LOS-deficient strain exhibits clear morphological defects relative to its isogenic, LOS⁺ parent. Scale bars are 5 μm . Representative images of biological replicates are shown. We noticed cells with both atypical morphology (white arrow) and irregular division (green arrow).

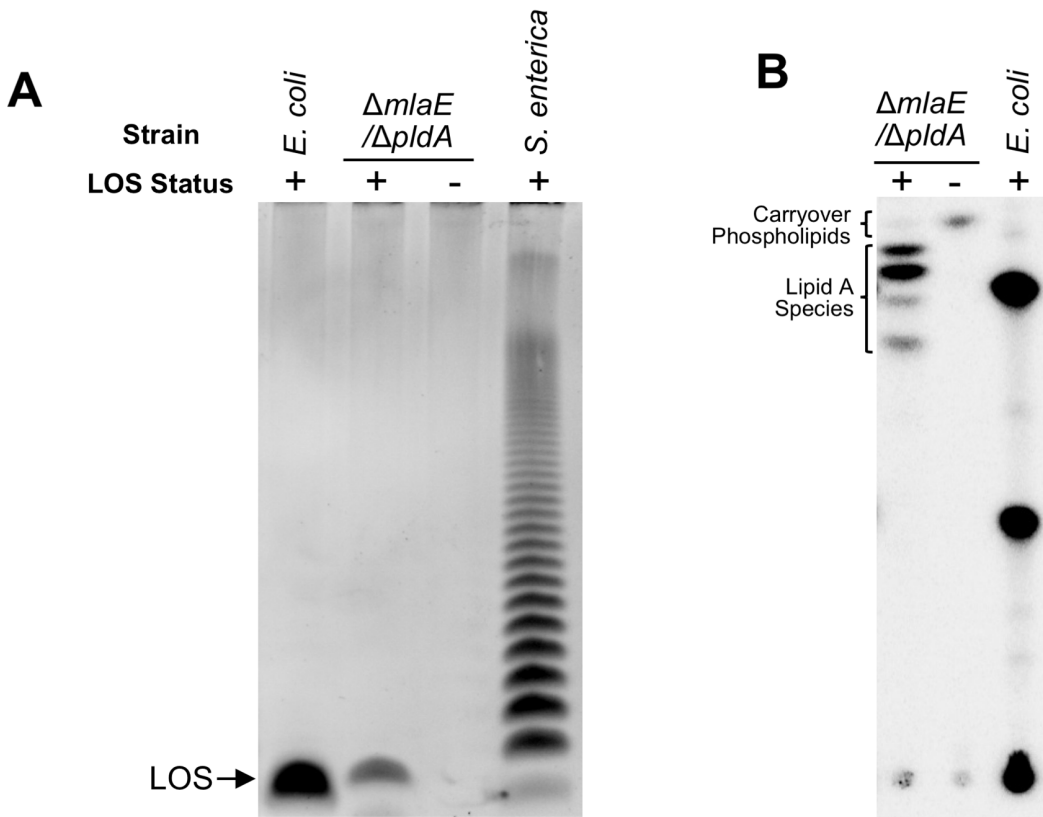


Figure S5: The $\Delta mlaE/\Delta pldA$ LOS-deficient strain lacks LOS and lipid A. (A) SDS-PAGE separation and staining of proteinase-K treated lysate reveals that the LOS-deficient double mutant does not produce LOS. (B) TLC analysis of radiolabeled lipid A extractions confirm that the LOS-deficient, 19606 $\Delta mlaE/\Delta pldA$ double mutant does not produce lipid A.

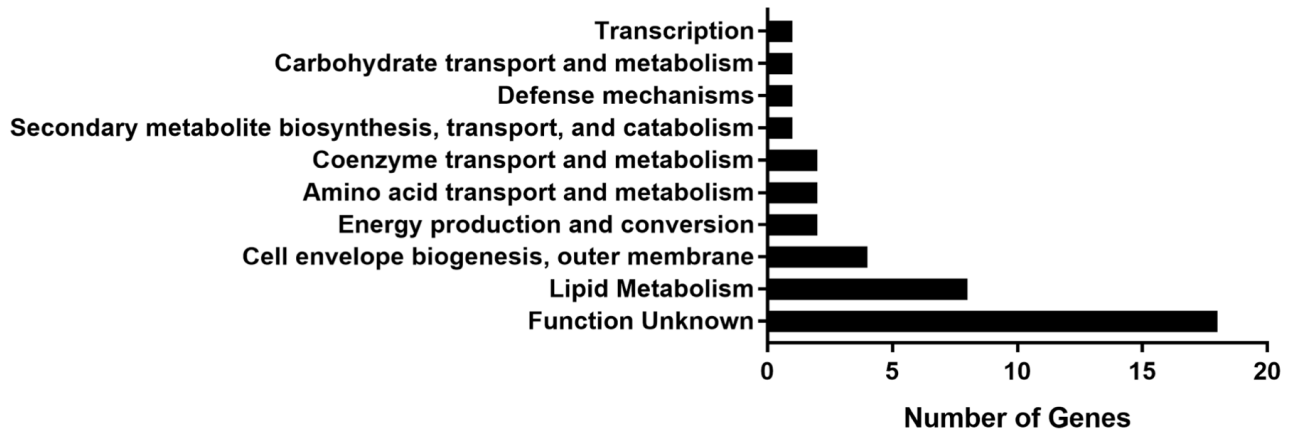
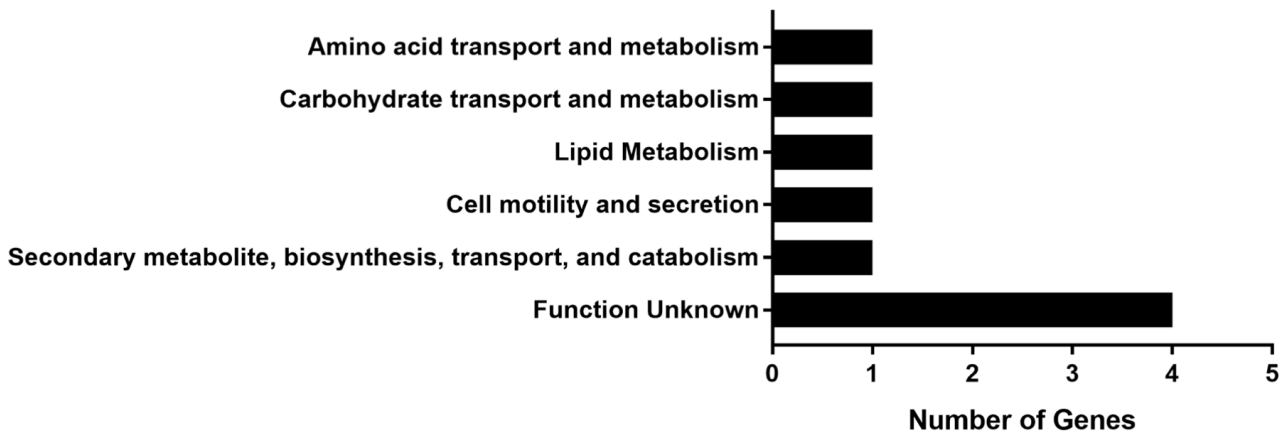
A**B**

Figure S6: Differentially regulated genes largely encode for proteins of unknown function. Upregulated (A) and downregulated (B) genes in the evolved LOS⁻ versus LOS⁺ distributed by COG category. A large number of both up- and down-regulated genes have no predicted function. The two COG categories with a higher frequency of genes assigned to it are lipid metabolism and cell envelope biogenesis.

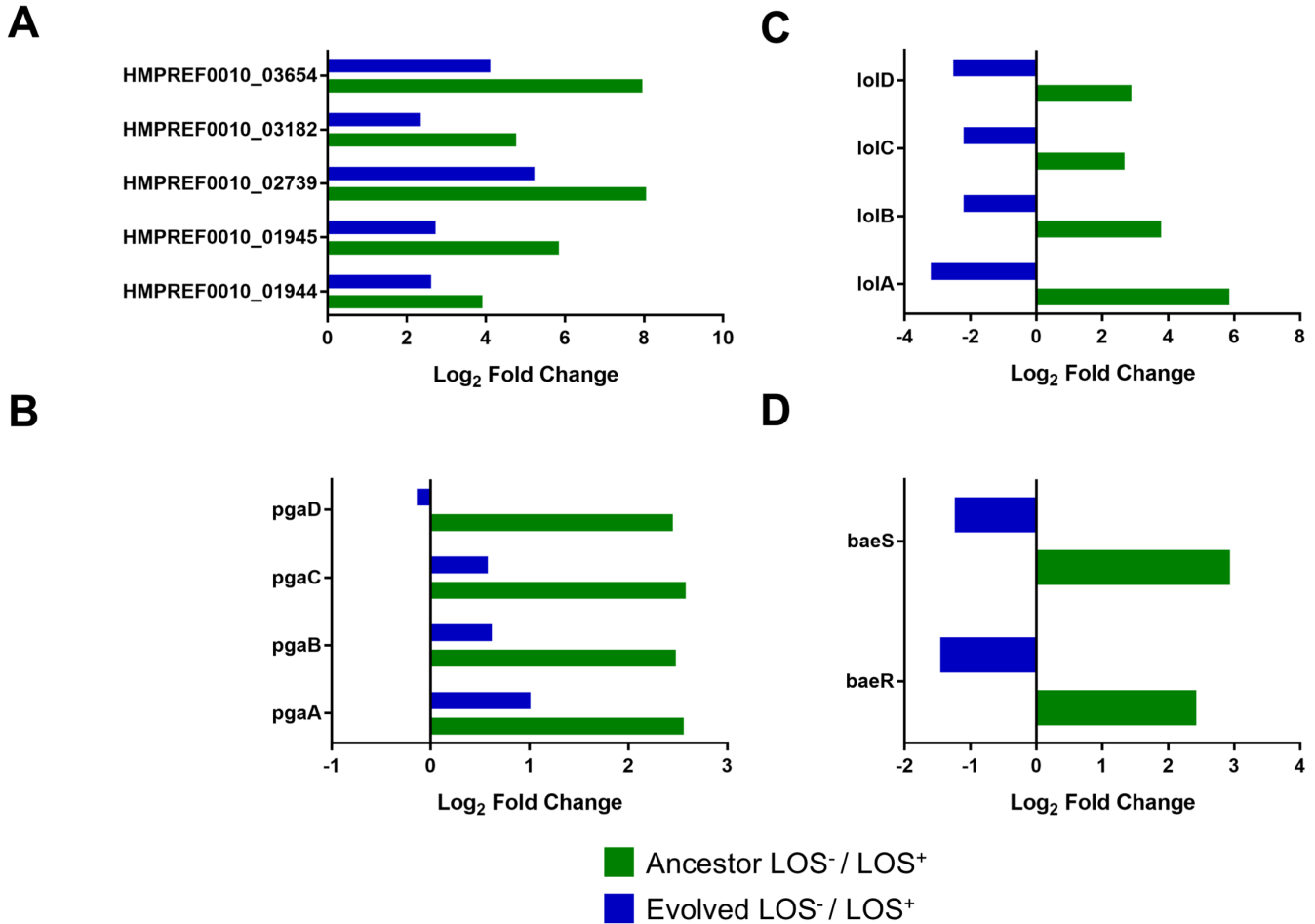


Figure S7: Gene expression for lipoproteins, lipoprotein transport, PNAG biosynthesis, and *baeRS* is decreased after evolution. (A) The expression of five lipoproteins which have been shown to be a conserved response to LOS-deficiency in *A. baumannii*. After evolution, the evolved LOS⁻ bacteria show a decrease in overall expression relative to LOS⁺ cells; however, these lipoproteins are still expressed. HMPREF0010_01944, HMPREF0010_01945, and HMPREF0010_02739 have been shown to be cell surface exposed (26). (B) The expression of genes involved in PNAG biosynthesis. After the evolution experiment, the evolved LOS⁻ no longer upregulate any *pga* genes. Similar trends were observed for the lipoprotein transport pathway (C) and *baeRS* two-component system (D). In (A-D), the x-axis is log₂ fold change. Green bars indicate expression for ancestor LOS⁻ / ancestor LOS⁺ and blue bars indicate expression for evolved LOS⁻ / evolved LOS⁺.

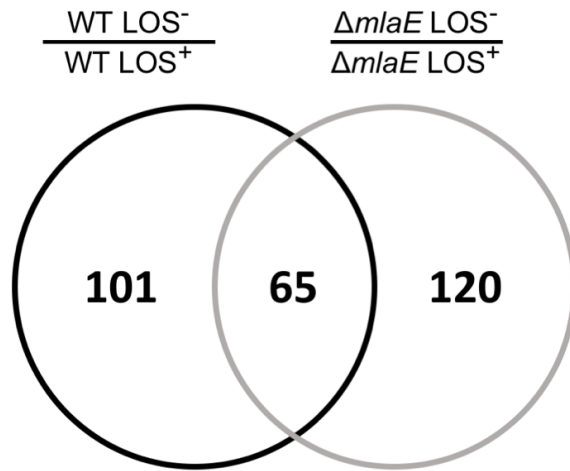


Figure S8: A 19606 $\Delta mlaE$ LOS^- has a unique set of genes that are differentially regulated. A Venn-Diagram showing unique or conserved responses to LOS -deficiency between wild-type and a $\Delta mlaE$ mutant. All genes in the Venn-Diagram had fold-change values \log_2 of ± 2 .

Table S1. Antibiotic resistance profiles for additional populations

Population	Strain	Evolution Status	Polymyxin B*	Vancomycin*	Daptomycin*	Bacitracin*
1	5075	Ancestor LOS-	32	0.25	2	0.38
		Evolved LOS-	1024	3	96	6
		Fold Change	32	12	48	15
5	AYE	Ancestor LOS-	128	0.25	3	0.75
		Evolved LOS-	512	4	24	3
		Fold Change	4	16.0	8	4
8	19606	Ancestor LOS-	256	0.38	3	0.5
		Evolved LOS-	1024	4	96	6
		Fold Change	4	10.5	32	12

* All units are in µg/mL

Table S2: All mutations accumulated in evolved LOS⁻ populations

Evolution/LOS Status	Strain	Population	Locus	Gene	Mutation	Sequence	Impact*	Frequency†
Evolved LOS-	5075	Population 1	ABUW_0289	<i>PBP1A</i>	Insertion	CTTGGTGA	Val349fs	97.89
			ABUW_3259	<i>mIaA</i>	Insertion	CCAGCTAATT	Gln136fs	98.91
			ABUW_1745	<i>lpxA</i>	SNP	C>T	Gln255stop	100
		Population 2	ABUW_0384	<i>mIaE</i>	Deletion	C519	Leu175fs	99.93
			ABUW_1199	<i>smr/mutS2</i>	SNP	A>T	Gln162Leu	99.89
			ABUW_1664 <> ABUW_1666	--	Insertion	GTTCGAG	--	99.42
			ABUW_1803	<i>pIaA</i>	Replacement	TT>AAAG	Leu22fs	90.34
			ABUW_0152	<i>lpxC</i>	Large Insertion Confirmed by Sanger Sequencing			
		Population 3	ABUW_0385	<i>mIaD</i>	Insertion	T	Ile161fs	100
			ABUW_1199	<i>smr/mutS2</i>	SNP	A>T	Gln162Leu	100
			ABUW_1803	<i>pIaA</i>	Deletion	T84	Tyr28fs	95.65
			ABUW_3566	<i>pgsA</i>	SNP	C>T	Ala56Val	87.69
			ABUW_0152	<i>lpxC</i>	Insertion	ATTT	Gly108fs	90
		Population 4	ABUW_0289	<i>PBP1A</i>	Deletion	T2007	Asn669fs	100
			ABUW_1803	<i>pIaA</i>	Deletion	731T	Val246fs	100
	ABUW_3566		<i>pgsA</i>	SNP	G>T	Ala108Asp	100	
	ABUW_0152		<i>lpxC</i>	Large Insertion Confirmed by Sanger Sequencing				
	AYE	Population 5	ABAYE0388	<i>mIaC</i>	Deletion	363T	Tyr122fs	94.3
			ABAYE1587	<i>lpxA</i>	Large Insertion Confirmed by Sanger Sequencing			
		Population 6	ABAYE0388	<i>mIaC</i>	Deletion	363T	Tyr122fs	100
			ABAYE1587	<i>lpxA</i>	Large Insertion Confirmed by Sanger Sequencing			
		Population 7	ABAYE0388	<i>mIaC</i>	Deletion	272C	Thr91fs	100
			ABAYE3503	<i>H-NS</i>	SNP	G>A	Arg91His	100
	ABAYE1587	<i>lpxA</i>	Large Insertion Confirmed by Sanger Sequencing					
	19606	Population 8	HMPREF0010_00357	<i>lpxA</i>	SNP	C>T	Gln72stop	99.94
			HMPREF0010_00206 <>	--	Insertion	T	--	100
			HMPREF0010_00207					
		Population 9	HMPREF0010_02485	<i>ompR</i>	SNP	C>T	Arg31Cys	100
			HMPREF0010_02484	<i>ompR</i>	SNP	C>A	Pro10Tyr	100
			HMPREF0010_00357	<i>lpxA</i>	SNP	C>T	Gln72stop	98.39
Population 10		HMPREF0010_02484	<i>envZ</i>	SNP	G>T	Met264Ile	100	
		HMPREF0010_02484	<i>envZ</i>	SNP	C>A	synonymous	98.53	
HMPREF0010_02607	<i>mIaC</i>	Deletion	363-364 TT	Tyr122fs	100			

Evolution/LOS Status	Strain	Population	Locus	Gene	Mutation	Sequence	Impact*	Frequency†
Evolved LOS-	19606	Population 10	HMPREF0010_03503	<i>lpxC</i>	Large Insertion Confirmed by Sanger Sequencing			

*fs refers to frame shift

† Frequency refers to the number of times the SNP was identified relative to the total number of reads available for that sequence.

Table S3: All mutations accumulated in ancestor and evolved populations

Evolution/LOS Status	Strain	Population	Locus	Gene	Mutation	Sequence	Impact*	Frequency†	
Ancestor LOS-	5075	Population 1	ABUW_1745	<i>lpxA</i>	SNP	C>T	Gln255stop	100	
			ABUW_3827	<i>hypothetical</i>	Insertion	462A	Gln155fs	88.64	
		Population 2	ABUW_0384	<i>mIaE</i>	Deletion	519C	Leu175fs	99.82	
			ABUW_1199	<i>smr/mutS2</i>	SNP	A>T	Gln162Leu	99.71	
			ABUW_1664 <> ABUW_1666	--	Insertion	GTTCGAG	--	99.72	
		Population 3	ABUW_0152	<i>lpxC</i>	Large Insertion Confirmed by Sanger Sequencing				
			ABUW_1199	<i>smr/mutS2</i>	SNP	A>T	Gln162Leu	100	
		Population 4	ABUW_0152	<i>lpxC</i>	Insertion	ATTT	Gly108fs	90	
			ABUW_1199	<i>smr/mutS2</i>	SNP	A>T	Gln162Leu	100	
			ABUW_1664 <> ABUW_1666	--	Insertion	GTTCGAG	--	100	
	AYE	Population 5	ABUW_0152	<i>lpxC</i>	Large Insertion Confirmed by Sanger Sequencing				
			ABAYE0388	<i>mIaC</i>	Deletion	363T	Tyr122fs	94.3	
		Population 6	ABAYE1587	<i>lpxA</i>	Large Insertion Confirmed by Sanger Sequencing				
			ABAYE0388	<i>mIaC</i>	Deletion	363T	Tyr122fs	91.18	
		Population 7	ABAYE1587	<i>lpxA</i>	Large Insertion Confirmed by Sanger Sequencing				
			ABAYE0388	<i>mIaC</i>	Deletion	272C	Thr91fs	100	
	19606	Population 8	ABAYE1587	<i>lpxA</i>	Large Insertion Confirmed by Sanger Sequencing				
			HMPREF0010_00357	<i>lpxA</i>	SNP	C>T	Gln72stop	99.94	
		Population 9	HMPREF0010_00357	<i>lpxA</i>	SNP	C>T	Gln72stop	100	
	Ancestor LOS+	5075	Population 10	HMPREF0010_003502	<i>lpxC</i>	Large Insertion Confirmed by Sanger Sequencing			
Population 1			--	--	--	--	--	--	
		Population 2	ABUW_2146	<i>rnd</i>	Insertion	493TA	Lys165fs	96.99	

Evolution/LOS Status	Strain	Population	Locus	Gene	Mutation	Sequence	Impact*	Frequency†	
	5075	Population 3	ABUW_2146	<i>rnd</i>	Insertion	493TA	Lys165fs	96.99	
		Population 4	ABUW_2146	<i>rnd</i>	Insertion	493TA	Lys165fs	96.99	
	AYE	Population 5	--	--	--	--	--	--	
		Population 6	--	--	--	--	--	--	
		Population 7	--	--	--	--	--	--	
	19606	Population 8	--	--	--	--	--	--	
		Population 9	--	--	--	--	--	--	
		Population 10	--	--	--	--	--	--	
	Evolved LOS+	5075	Population 1	ABUW_1199	<i>smr/mutS2</i>	SNP	A>T	Gln162Leu	99.17
				ABUW_1664 <> ABUW_1666	--	Insertion	GAGTTTA	--	100
ABUW_2471				<i>dcaF</i>	SNP	C>T	Pro44Leu	99.52	
Population 2			ABUW_0913	<i>pckG</i>	SNP	G>A	Arg157His	99.71	
			ABUW_1199	<i>smr/mutS2</i>	SNP	C>T	Gln162Leu	99.82	
Population 3			ABUW_1664 <> ABUW_1666	--	Insertion	GTTCGAG	--	99.5	
			ABUW_0913	<i>pckG</i>	SNP	G>A	Arg157His	99.71	
			ABUW_1199	<i>smr/mutS2</i>	SNP	C>T	Gln162Leu	99.82	
Population 4			ABUW_1664 <> ABUW_1666	--	Insertion	GTTCGAG	--	99.5	
			ABUW_0913	<i>pckG</i>	SNP	G>A	Arg157His	99.71	
			ABUW_1199	<i>smr/mutS2</i>	SNP	C>T	Gln162Leu	99.82	
Population 4			ABUW_1664 <> ABUW_1666	--	Insertion	GTTCGAG	--	99.5	

Evolution/LOS Status	Strain	Population	Locus	Gene	Mutation	Sequence	Impact*	Frequency†
Evolved LOS+	AYE	Population 5	--	--	--	--	--	--
	AYE	Population 6	--	--	--	--	--	--
	AYE	Population 7	--	--	--	--	--	--
	19606	Population 8	HMPREF0010_00206 <> HMPREF0010_00207	--	Insertion	T	--	100
	19606	Population 9	HMPREF0010_00206 <> HMPREF0010_00207	--	Insertion	T	--	100
	19606	Population 10	HMPREF0010_00206 <> HMPREF0010_00207	--	Insertion	T	--	100

* fs refers to frameshift

† Frequency refers to the number of times the SNP was identified relative to the total number of reads available for that sequence.

Table S4: Transcriptomic fold changes for the Mla-independent gene set

Mla-Independent Fold Changes		
Locus*	WT LOS ⁻ / LOS ⁺	<i>mlaE</i> LOS ⁻ / LOS ⁺
03356	10.42	9.64
02733	9.51	9.13
00185	7.94	7.84
02739	8.49	7.8
03654	8.58	7.06
00186	8.15	6.98
104195	5.52	6.04
01945	6.77	5.45
<i>macA</i>	5.95	5.1
<i>lolA</i>	5.84	5.01
01944	4.83	4.95
03355	5.14	4.92
00247	3.25	4.68
<i>macB</i>	5.68	4.47
02727	4.16	4.4
02568	5.39	4.36
02579	5.02	4.05
03182	4.46	4.05
<i>macC</i>	5.45	3.93
<i>ompW</i>	3.3	3.88
02249	3.88	3.85
00385	2.63	3.71
00069	4.15	3.69
<i>lolB</i>	4.32	3.66
02797	3.23	3.51
02675	3.76	3.47
00266	3.28	3.42
<i>mIaB</i>	3.98	3.1
<i>argA</i>	3.4	3.1
02025	3.34	3.07
<i>kynU</i>	3.67	2.83
00694	3.36	2.83
<i>baeS</i>	3.37	2.81
<i>mIaC</i>	3.4	2.7
02248	2.42	2.67
<i>dsbA</i>	3.95	2.66

02071	3.35	2.64
03516	2.87	2.64
02462	3.41	2.63
00184	2.78	2.55
01616	2.25	2.55
03111	2.04	2.43
03519	2.09	2.38
03113	2.03	2.27
03145	3.08	2.26
01939	2.49	2.25
02269	2.97	2.22
<i>proY</i>	2.53	2.2
00792	3.17	2.13
03727	2.27	2.1
00990	2.69	2.07
<i>csgG</i>	-2.53	-2.14
00606	-4.31	-2.16
03013	-2.68	-2.21
03661	-2.08	-2.31
<i>rhtB</i>	-3.17	-2.37
104360	-3.62	-2.42
<i>pgpB</i>	-2.48	-2.43
03531	-3.47	-2.68
03347	-3.19	-2.82
00516	-3.05	-2.88
00599	-2.41	-3.42
03348	-4.47	-4.96
00597	-2.74	-5.08
00598	-3.49	-5.38

*Locus is preceded by HMPREF0010_ for unannotated genes

Table S5: Transcriptomic fold changes for the Mla-independent gene set

Mla-dependent Fold Changes		
Locus*	WT LOS ⁻ /LOS ⁺	<i>mlaE</i> LOS ⁻ /LOS ⁺
00466	-0.68	7.67
00467	-1.49	7.1
00468	-0.93	6.73
00469	-1.63	6.51
00470	-0.62	5.7
00471	-0.24	5.16
00472	0.31	4.66
01245	-0.15	4.46
00194	-1.32	4.24
<i>acoA</i>	-1.17	4.11
00473	-0.11	4.1
104260	1.57	4.1
00463	-0.45	4.09
<i>benA</i>	1.39	4.02
<i>acoB</i>	-1.39	3.94
<i>aroP3</i>	-1.71	3.94
<i>benB</i>	0.78	3.92
00193	-2.16	3.77
00192	-2.53	3.76
<i>atoE</i>	-1.47	3.73
<i>acoC</i>	-1.34	3.71
00474	0.49	3.7
<i>lipA2</i>	-1.22	3.69
104270	-1.69	3.5
02254	-1.64	3.48
<i>lpdA2</i>	-1.15	3.46
104275	-1.88	3.45
00637	-1.81	3.4
104765	0	3.35
<i>mmsA</i>	-1.16	3.33
00396	1.89	3.26
<i>budC</i>	-0.57	3.25
<i>mmsB</i>	-0.97	3.18
<i>paaF</i>	-2.14	3.15
<i>paaJ</i>	-2.17	3.12

caiD	-1.63	3.03
bdhA	-1.57	3
00673	-0.25	2.98
00680	0.24	2.94
00646	-1.15	2.88
paaE	-1.98	2.83
<i>paal</i>	-2.17	2.81
benC	0.13	2.77
01003	-1.84	2.75
01101	-0.51	2.75
<i>paaX</i>	-2.15	2.73
03117	1.42	2.7
paaB	-1.98	2.69
<i>paaK</i>	-2.09	2.69
03057	1.17	2.68
03231	-0.22	2.68
paaC	-2.13	2.65
<i>paal</i>	-2.36	2.64
03725	1.14	2.62
vanK	0.68	2.58
paaH	-1.88	2.55
pobA	-0.11	2.47
02778	-0.44	2.45
03058	1.28	2.43
03115	1.58	2.42
03131	1.54	2.42
01007	-1.84	2.41
02800	-0.83	2.4
paaY	-1.9	2.36
01142	1.91	2.36
mgh	-2.12	2.31
02412	0.7	2.29
vanA	0.52	2.29
acsA	0.72	2.29
02936	1.9	2.28
01005	-1.92	2.27
01008	-2.03	2.27
00005	0.15	2.26
03119	1.83	2.26

03121	1.66	2.25
03533	0.49	2.25
01010	-2.25	2.24
03112	1.95	2.23
<i>pcaT</i>	-0.08	2.22
01011	-2.1	2.22
23485	0.88	2.21
<i>paaZ</i>	-1.58	2.2
03724	1.11	2.2
01271	1.05	2.18
00635	-0.27	2.16
01006	-0.74	2.16
02419	0.66	2.16
03110	1.65	2.16
02411	0.85	2.14
00275	1.73	2.12
<i>benP1</i>	0.56	2.12
<i>actP</i>	-0.13	2.12
00272	1.35	2.09
01002	-0.8	2.08
02329	1.11	2.08
03118	1.82	2.08
03120	1.58	2.08
00267	1.13	2.07
02252	-0.92	2.06
02409	0.54	2.03
00013	0.32	2.02
00985	0.58	2.01
<i>clpV</i>	-1.6	-2
<i>gltD</i>	-0.78	-2
<i>01112</i>	-1.45	-2.01
01115	-1.04	-2.04
01111	-1.51	-2.07
01116	-1.2	-2.09
<i>icmF</i>	-1.53	-2.09
00650	-1.88	-2.19
<i>01117</i>	-1.59	-2.19
104310	-1.51	-2.26
00579	-0.68	-2.3

00600	-1.74	-2.31
00722	-1.9	-2.39
00231	-0.47	-2.42
<i>gabT</i>	-0.68	-2.48
00649	-1.87	-2.61
<i>gltB</i>	-1.67	-2.7
104800	1.88	-2.84

*Locus is preceded by HMPREF0010_ for unannotated genes

Table S6: Strains and Plasmids

Strain	Genotype	Reference
<i>E. coli</i> W3110	Wild type, F- λ -, <i>rph-1</i> IN(<i>rrnD</i> , <i>rrnE</i>)1	<i>E. coli</i> Genetic Stock Center (Yale)
<i>A. baumannii</i> ATCC19606	Wild type	ATCC
<i>A. baumannii</i> 5075	Wild type	ATCC
<i>A. baumannii</i> AYE	Wild type	ATCC
<i>A. baumannii</i> ATCC19606	$\Delta mlaE$, $\Delta pldA$	This Study
<i>A. baumannii</i> ATCC19606, LOS-	$\Delta mlaE$, $\Delta pldA$	This Study
<i>A. baumannii</i> ATCC19606, LOS+	$\Delta mlaE$	This Study
<i>A. baumannii</i> ATCC19606, LOS-	$\Delta mlaE$	This Study
<i>A. baumannii</i> ATCC19606, LOS+	$\Delta mlaE$, $\Delta pldA$, pMMB67EHKn- <i>m</i> <i>laE</i>	This Study
<i>A. baumannii</i> ATCC19606, LOS-	$\Delta mlaE$, $\Delta pldA$, pMMB67EHKn- <i>m</i> <i>laE</i>	This Study
<i>A. baumannii</i> 5075 LOS- Ancestor Pop 1	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS- Ancestor Pop 2	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS- Ancestor Pop 3	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS- Ancestor Pop 4	See Table S3	This Study
<i>A. baumannii</i> AYE LOS- Ancestor Pop 5	See Table S3	This Study
<i>A. baumannii</i> AYE LOS- Ancestor Pop 6	See Table S3	This Study
<i>A. baumannii</i> AYE LOS- Ancestor Pop 7	See Table S3	This Study
<i>A. baumannii</i> ATCC19606 LOS- Ancestor Pop 8	See Table S3	This Study
<i>A. baumannii</i> ATCC19606 LOS- Ancestor Pop 9	See Table S3	This Study
<i>A. baumannii</i> ATCC19606 LOS- Ancestor Pop 10	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS+ Evolved Pop 1	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS+ Evolved Pop 2	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS+ Evolved Pop 3	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS+ Evolved Pop 4	See Table S3	This Study
<i>A. baumannii</i> AYE LOS+ Evolved Pop 5	See Table S3	This Study
<i>A. baumannii</i> AYE LOS+ Evolved Pop 6	See Table S3	This Study

<i>A. baumannii</i> AYE LOS+ Evolved Pop 7	See Table S3	This Study
<i>A. baumannii</i> ATCC19606 LOS+ Evolved Pop 8	See Table S3	This Study
<i>A. baumannii</i> ATCC19606 LOS+ Evolved Pop 9	See Table S3	This Study
<i>A. baumannii</i> ATCC19606 LOS+ Evolved Pop 10	See Table S3	This Study
<i>A. baumannii</i> 5075 LOS- Evolved Pop 1	See Table S2	This Study
<i>A. baumannii</i> 5075 LOS- Evolved Pop 2	See Table S2	This Study
<i>A. baumannii</i> 5075 LOS- Evolved Pop 3	See Table S2	This Study
<i>A. baumannii</i> 5075 LOS- Evolved Pop 4	See Table S2	This Study
<i>A. baumannii</i> AYE LOS- Evolved Pop 5	See Table S2	This Study
<i>A. baumannii</i> AYE LOS- Evolved Pop 6	See Table S2	This Study
<i>A. baumannii</i> AYE LOS- Evolved Pop 7	See Table S2	This Study
<i>A. baumannii</i> ATCC19606 LOS- Evolved Pop 8	See Table S2	This Study
<i>A. baumannii</i> ATCC19606 LOS- Evolved Pop 9	See Table S2	This Study
<i>A. baumannii</i> ATCC19606 LOS- Evolved Pop 10	See Table S2	This Study
Plasmid	Description	Reference
pMMB67EHKn	pMMB67EH with Kan ^R	30
pMMB67EHKn- <i>mIaE</i>	pMMB67EH with <i>mIaE</i>	This Study
pRecABtet	pMMB67EH with REC _{AB} system, tet ^R	44
pFLPtet	pMMB67EH with FLP recombinase, tet ^R	44
pKD4	FRT-flanked Kan ^R	44

Table S7: Primers

Primer	Sequence
Recombineering_F_ <i>pldA</i>	5'- TAAGTTTGACCTATAAAAATGCCGCTTTTACAGCGGCTTTT TTATTAACCAATTTGAATATTGGCTTGAGGGTGTTTGAGAC GGCTTTTTTTAGGAGTAGCAATTAATAGGCGAGTGTTAA GGGCCTAACGGCCTGCAAACATAAGTATATCCTCCTTA GTTCCATTCCG
Recombineering_R_ <i>pldA</i>	5'- TTTAGAAGATTTAAAACGTGTTGCACGTCAATATTTAATTG AACAAACACCTGTAAAAGCTGTCGTTGCACCTTTTGCAAAA CGTGATGAATTGCAACAACACTGGGCTTTACGATCAAACAAG TTAATTAATAAAAATTGGAGATGAACAGCGATTGTGTAG GCTGGAGCTGCTTCG
Recombineering_F_ <i>mlaE</i>	5'- GGTACACCTGAGCAACTCAAGGCACATGCTTCTCCATTTG TAAAACAGTTCTTAACCGTTTCGGTAGAAGGTCCGGTTGA ATATCAGTTTAGCCACCAAGCTTATTTAGATAACGAGGTTT GTCCTATATCCTCCTTAGTTCCATTCCG
Recombineering_R_ <i>mlaE</i>	5'- GGTGTAGCCATCGCTCAAGTTCGTGCCCACTAAACCACTC ACTTTCATTGCTAAAAAGAATAAAGCGATACCGAAGATAAT GACAAAGATACCTACGGCCAGCTCACTAGTACGTGATTTT ACAGCGATTGTGTAGGCTGGAGCTGCTTCG
Promoter_ <i>mlaA</i> _F	5'- CAAATAAACTTCGAGTAATTTAAAC
Coding Region_ <i>mlaA</i> _R	5'- TTTTTCGGTTTTATCAGTGTTATC
pMMB67EHKn- <i>mlaE</i> _F	5'- CCGGAATTCATGAATACGATTGCCTGG*
pMMB67EHKn- <i>mlaE</i> _R	5'- CGCGGATCCTTAAATCCCTCCGAACATG*