Supporting information

The phospholipid-repair system LplT/Aas in Gram-negative bacteria protects the bacterial membrane envelope from host phospholipase A_2 attack

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Content:

I. Tables

Table S1. Primer sequences used in this study

Primers	Sequences (5' – 3')*
lplT-knockout-forward	GTGAGTCAGTGCACACTAACACTTCGTTGTGGTCGAAGGGGATGAAAGCGATGGGAATTAGCCATGGTCC
lplT-knockout-reverse	GCCAATGCCAATGGGCACGACCGGGATGCCTATCATTACCGCCAGCGAGTGTAGGCTGGAGCTGCTTCG
aas-knockout-forward	AATCTCCCTCCATTCGCTTTTACTGAATCAGAGCAAAGGGAGTTGGAATGATGGGAATTAGCCATGGTCC
aas-knockout-reverse	GCATTTCGCCAGCGATTTTGCGACGGTCACGGGCACGCGGCGCATCCGGTGTAGGCTGGAGCTGCTTCG
lplT-Kn-ccdb-forward	GATGAGTGAGTCAGTGCACACTAACACTTCGTTGTGGTCGAAGGGGATGATAGGAACTTCAAGATCC
lplT-Kn-ccdb-reverse	CGGCGCTGCCAGATCCACAGCGCCGTTATTGCCAGCGCAAACAGCGCACCTTATATTCCCCAGAACATCAGG
aas-Kn-ccdb-forward	AATCTCCCTCCATTCGCTTTTACTGAATCAGAGCAAAGGGAGTTGGAATGATAGGAACTTCAAGATCC
aas-Kn-ccdb-reverse	CAGCCCTTTTACCCCGGCAGTGTAGTTCATCATTGCGGGCATGCGGCGACTTATATTCCCCAGAACATCAGG
aas-H36A-forward	CGTTCTAATTACGCCTAAT**GC**CGTCTCTTTTATTGATGG
aas-H36A-reverse	CCATCAATAAAAGAGACG**GC**ATTAGGCGTAATTAGAACG
lplT-D30A-forward	CTGCGTTTGGCG**C**TAATGCCCTACTG
lplT-D30A-reverse	CAGTAGGGCATTA**G**CGCCAAACGCAG
lplT-knockin-forward	ATGAGTGAGTCAGTGCACACTAACACTTCG
lplT-knockin-reverse	TTAATGACGGCGCTGCCAGATCCACAGCG
aas-knockin-forward	ATGCTTTTTAGCTTTTTTCGAAATTTG
aas-knockin-reverse	CATCGTGTTGTTCCGCTTCGTCTACC

* Bold red letters show the nucleotides mutated. Underlined letters represent the homologue arms

Table S2. PL compositions in psPLA$_2$-treated *E. coli* W3110 WT, Δ*lplT*, Δ*aas* and Δ*aas/lplT* cells*

Strain	Treatment	Time (min)	CL	PE	PG	MCL	LPE	LPG
W3110	+ AF	0	7.54	66.25	18.62	3.58	2.2	1.81
		10	7.55	66.99	18.51	3.43	1.9	1.69
		20	7.53	67.08	18.66	3.33	1.79	1.6
		30	7.85	67.52	18.12	3.29	1.68	1.54
		60	7.66	67.51	18.4	3.2	1.67	1.57
		120	7.82	67.56	18.37	3.07	1.61	1.48
W3110	+ AF + psPLA$_2$	0	7.94	65.25	19.39	3.55	2.18	3.87
		10	6.95	67.85	18.21	3.28	1.34	3.71
		20	7.21	67.12	18.38	3.41	1.58	3.88
		30	7.39	67.49	19.56	3.32	1.95	2.24
		60	6.96	67.96	18.28	3.21	1.38	3.59
		120	7.32	67.49	18.99	3.24	1.36	2.96
Δ*lplT*	+ AF	0	8.96	57.88	19.66	4.76	3.58	5.17
		10	9.3	57.71	19.33	4.91	3.43	5.31
		20	9.22	58.18	19.32	4.66	3.38	5.24
		30	9.03	58.16	19.36	4.82	3.44	5.2
		60	8.93	58.17	19.4	4.65	3.4	4.86
		120	8.74	59.05	19.36	4.79	3.3	4.75
Δ*lplT**	+ AF + psPLA$_2$	0	7.61	64.58	18.82	3.36	3.07	2.56
		10	12.46	52.14	9.51	3.76	16.23	5.9
		20	14.77	45.63	4.31	6.94	22.3	6.03
		30	13.48	44	4.11	10.88	21.65	5.88
		60	12.54	41.77	4.5	14.43	18.23	8.52
		120	11.02	38.26	6.21	20.68	12.51	11.32
Δ*aas*	+ AF	0	7.7	60.06	21.98	3.53	2.81	3.92
		10	5.29	62.2	24.21	2.9	2.56	2.84
		20	4.99	63.55	25.02	2.5	1.97	1.99
		30	5.2	65.71	23.82	2.03	1.71	1.53
		60	5.63	65.3	24.12	1.97	1.48	1.5
		120	6.28	64.62	23.74	2.17	1.53	1.66
Δ*aas*	+ AF + psPLA$_2$	0	10.76	50.74	16.52	7.46	7.28	7.24
		10	11.58	48.11	10.21	6.79	15.64	7.67
		20	13.11	42.21	6.62	9.48	20.27	8.32
		30	12.41	39.62	7.1	12.51	19.23	9.13
		60	12.36	36.53	8.31	15.02	16.97	10.82
		120	12.21	32.7	9.85	18.26	13.52	13.46

Δ***aas/lplT***	+ AF	0	8.91	58.36	22.55	3.78	2.8	3.61
		10	10	59.67	21.66	3.3	2.39	2.98
		20	8.28	59.11	25.08	2.89	2.07	2.58
		30	8.53	59.19	25.39	2.49	2.08	2.32
		60	10.56	55.8	23.51	3.57	3.26	3.29
		120	11.48	56.87	22.41	3.1	2.95	3.18
Δ***aas/lplT***	+ AF + $psPLA_2$	0	10.76	50.74	16.52	2.46	2.28	2.24
		10	11.58	48.11	10.21	6.79	15.64	7.67
		20	13.11	42.21	6.62	9.48	20.27	8.32
		30	12.41	39.62	7.1	12.51	19.23	9.13
		60	12.36	36.53	8.31	15.02	16.97	10.82
		120	12.21	32.7	9.85	18.26	13.52	13.46

Note:
*Data were obtained from scanned TLC images shown at Fig. 4a and Fig. S6 and expressed as mol% of the total PL in each sample.
**: A minor band appearing above the origin in Figure 4a was considered as a contaminant of lipid extraction and was not included in the table since it was not found in the Δ*lplT* control (+AF only) (Fig. S6).

II. Figures

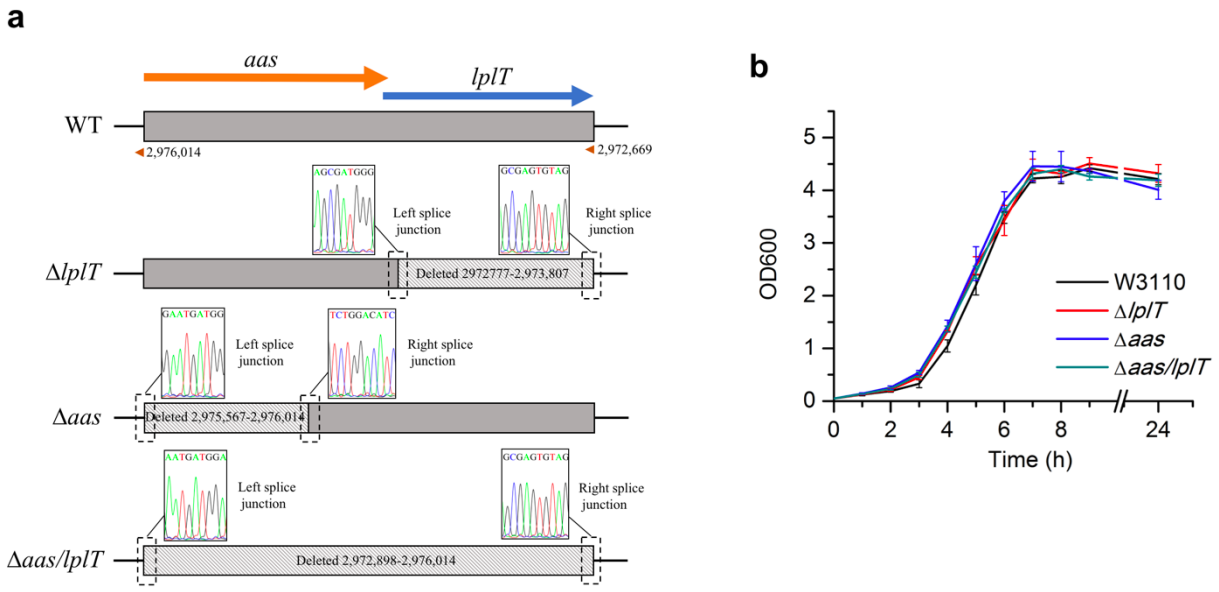

Fig. S1. Generation of *lplT* or/and *aas* gene knockout strains of *E. coli*. a) scheme of the *aas-lplT* operon in *E. coli*. The genes of *aas* and *lplT* were under control of the same promoter and have an overlapping sequence of five terminal nucleotides. The deletion region in Δ*lplT,* Δ*aas* or Δ*aas/lplT* strain was highlighted with wide downward diagonal. The DNA splice junctions were confirmed by DNA sequencing as shown in the chromatography traces.; b) growth curve of *E. coli* W3110 WT, Δ*lplT,* Δ*aas* and Δ*aas/lplT* strains. The four *E. coli* strains were incubated in LB broth at 37 ^{o}C with shaking and absorbance was measured at OD_{600} at indicated times.

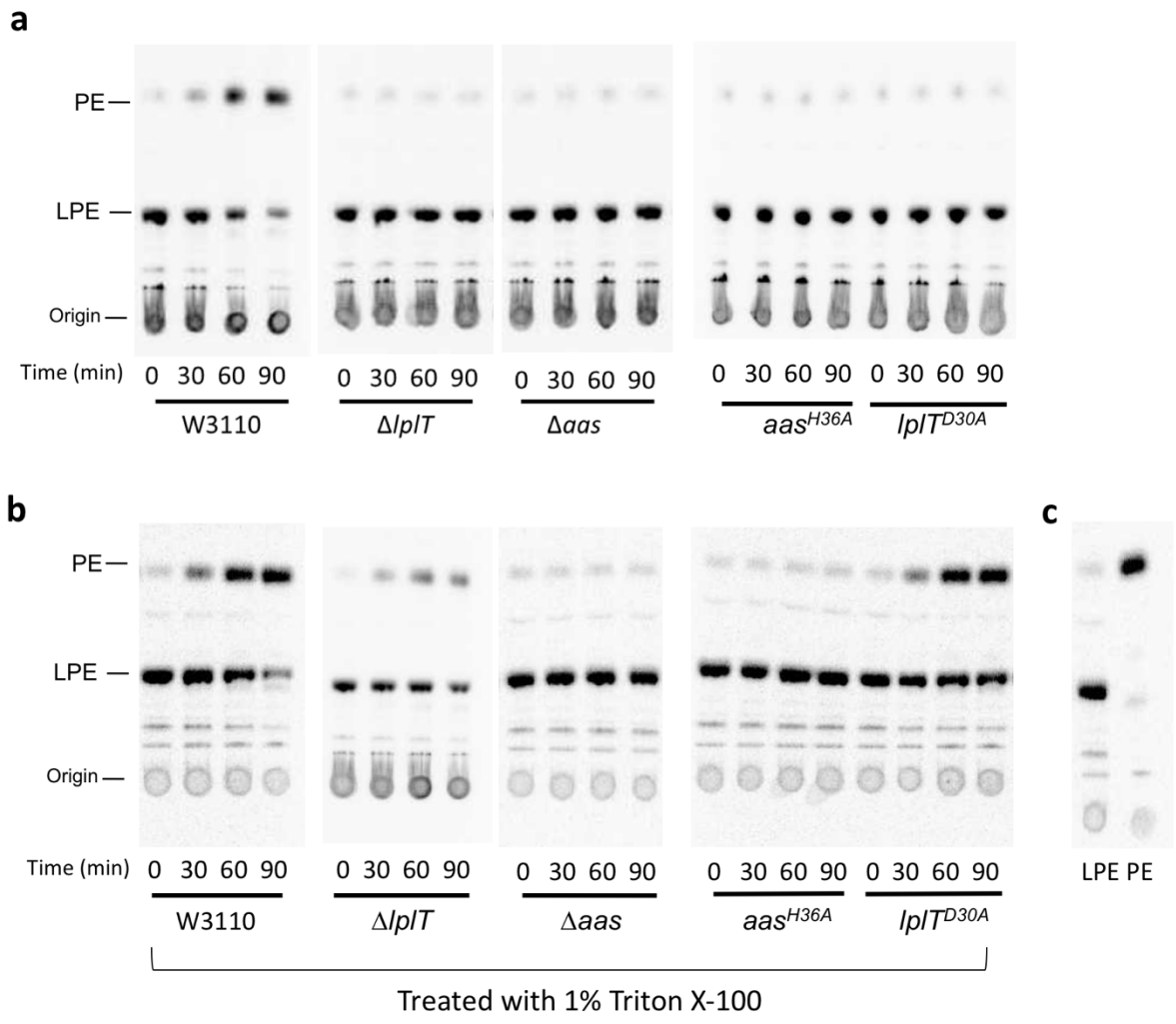

Fig. S2. LPE acylation assays. a) TLC images of [^{32}P] LPE acylation reactions using spheroplasts generated from *E. coli* WT, Δ*lplT,* Δ*aas, aas*^{H36A} *and lplT*^{D30A} cells. The reactions were sampled at indicated times for lipid extraction and TLC analysis. b) TLC analysis of the reactions performed using spheroplasts solubilized by 1% Triton X-100. **c)** [^{32}P] LPE substrate used in the assays and [^{32}P] PE extracted from *E. coli* UE54 strain.

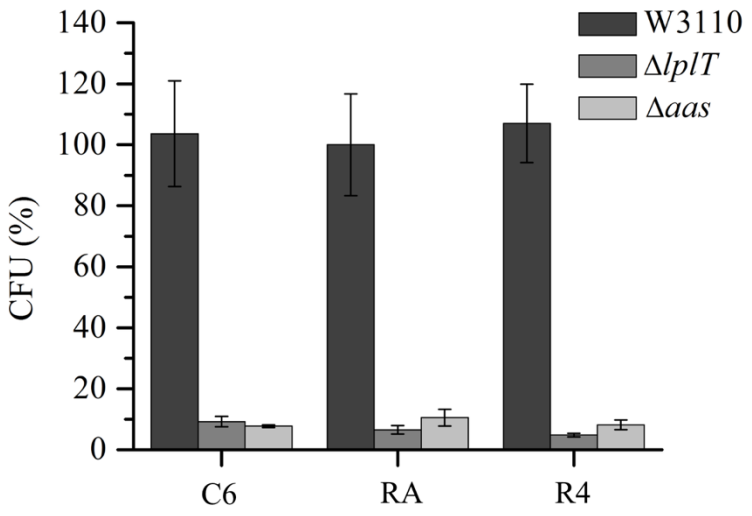

Fig. S3. Viability tests of *E. coli* WT, Δ*lplT* and Δ*aas* with three different AF. ~10^6 of *E. coli* cells harvested at log phase were treated with 12 units $psPLA_2$ and 20 µL of each AF: C6, RA or R4 for 90 min. Data are expressed as percentage of CFU compared to that of W3110 WT (- AF, - $psPLA_2$). Values are means ± S.D. of 3 or more repeated experiments.

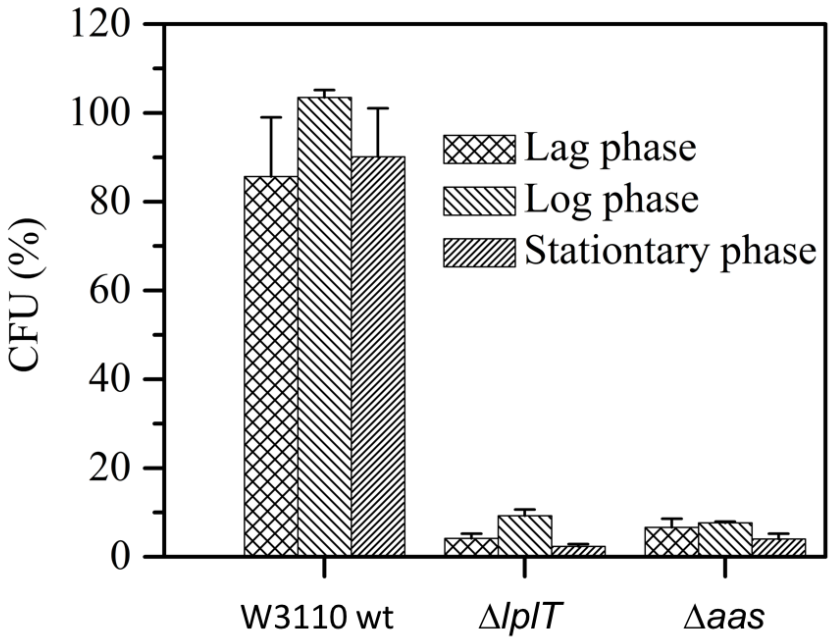

Fig. S4. Viability tests of *E. coli* WT, Δ*lplT* and Δ*aas* at different cell growth stages. ~10^6 of *E. coli* cells harvested at lag, log or stationary phase were treated with 12 units $psPLA_2$ and 20 µl of AF for 90 min. Data are expressed as percentage of CFU compared to that of W3110 WT (- AF, - $psPLA_2$). Values are means ± S.D. of 3 or more repeated experiments.

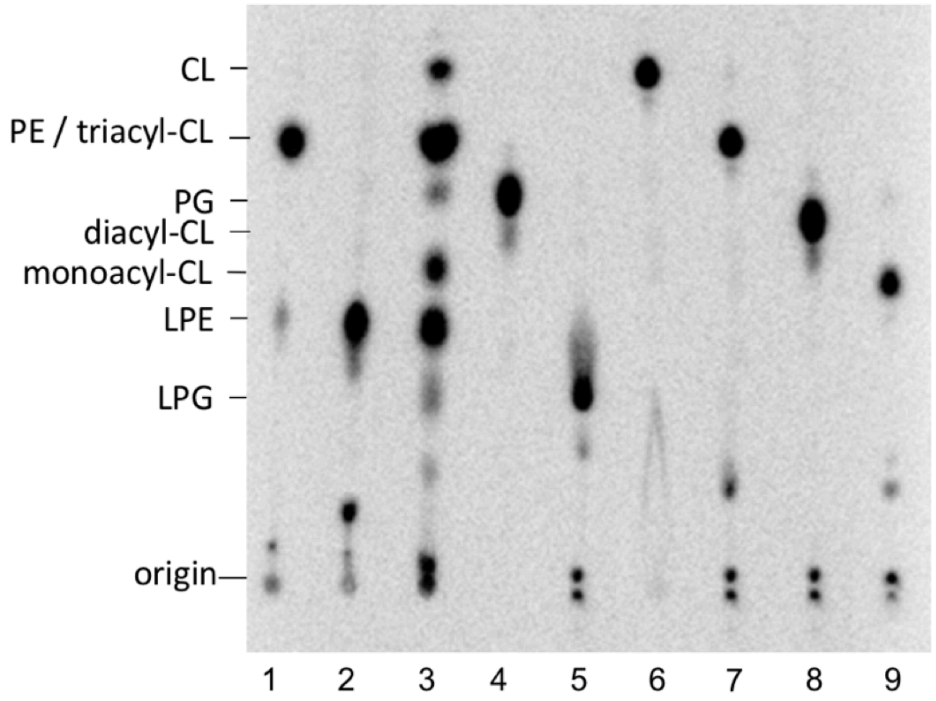

Fig. S5. Determination of PL species in sPLA$_2$-mediated bactericidal reaction by TLC. 1) [^{32}P] PE extracted from *E. coli* strain UE54; 2) [^{32}P] LPE generated by digestion of the sample 1 using venom PLA$_2$; 3) total lipid extract from ~10^6 Δ*plT* cells treated with 12 units of psPLA$_2$ and 20 µl AF for 30 min at 37 ℃; 4) [^{32}P] PG purified from *E. coli* AL95 strain; 5) [^{32}P] LPG generated by digestion of the sample 4 using venom PLA$_2$; 6) [^{32}P] CL purified from *E. coli* AL95 strain; the samples 7-9 are CL lyso-derivatives generated by venom PLA$_2$ from the sample 6 and separated by TLC. The bands corresponding to triacyl-CL (7), diacyl-CL (8), or monoacyl-CL (9) were scraped from the TLC plate, extracted using chloroform.

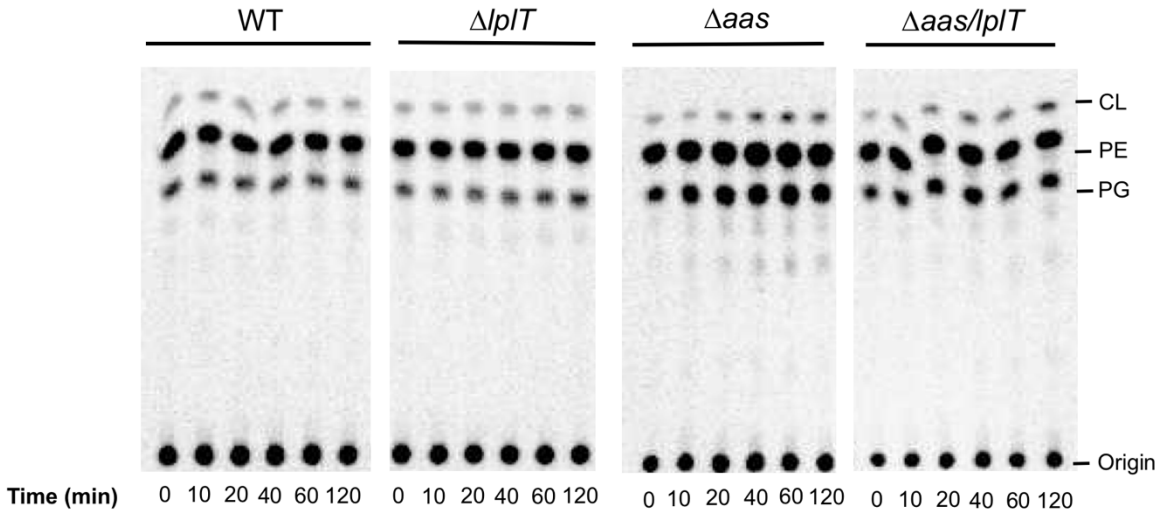

Fig. S6. TLC images of PL extracted from [^{32}P] labeled *E. coli* cells of W3110 *E. coli* WT, Δ*lplT,* Δ*aas* and Δ*aas/lplT* strains in the absence of psPLA$_2$. The cells were treated with 20 μl of AF for indicated times prior to lipid extraction as described in Experimental Approaches. Individual PL content is expressed as mol% of total PL in each sample.

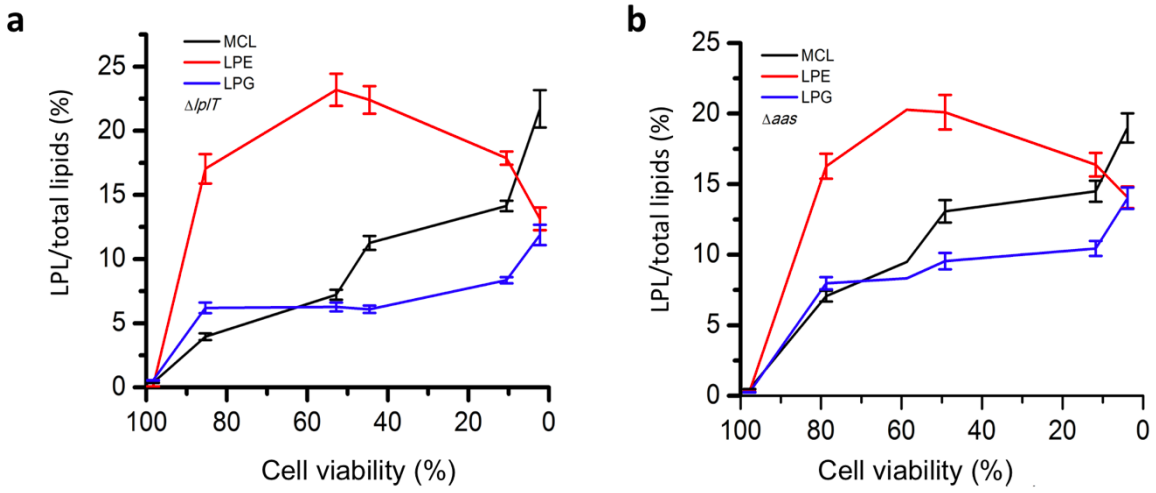

Fig. S7. Cell viability vs degradation of individual PL in the psPLA2-mediatd bactericidal reactions. a) Δ*lplT* strain; b)Δ*aas* strain**.** Each LPL (LPE, LPG or monoacyl-CL (MCL)) was expressed as mol% in the total radiolabeled PL pool. The data were obtained from Table S2. The viability (%) was calculated by comparing CFU of W3110 WT control (- AF, - $psPLA_2$).

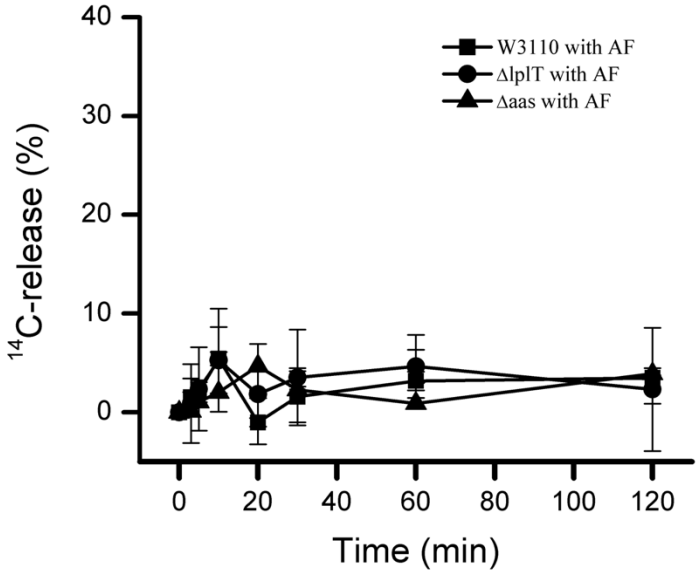

Fig. S8. No detectable free fatty acid released from *E. coli* WT, Δ*lplT or* Δ*aas* strain in the absence of sPLA$_2$. Free fatty acid release assay of *E. coli* W3110 WT, Δ*lplT and* Δ*aas* cells. ~10^6 *E. coli* cells labelled with [^{14}C] oleic acid were incubated with 20 µL AF for indicated times. The radioactivity in the supernatant fraction was determined by scintillation counting. PL degradation is expressed as the % of total [^{14}C] radioactivity.

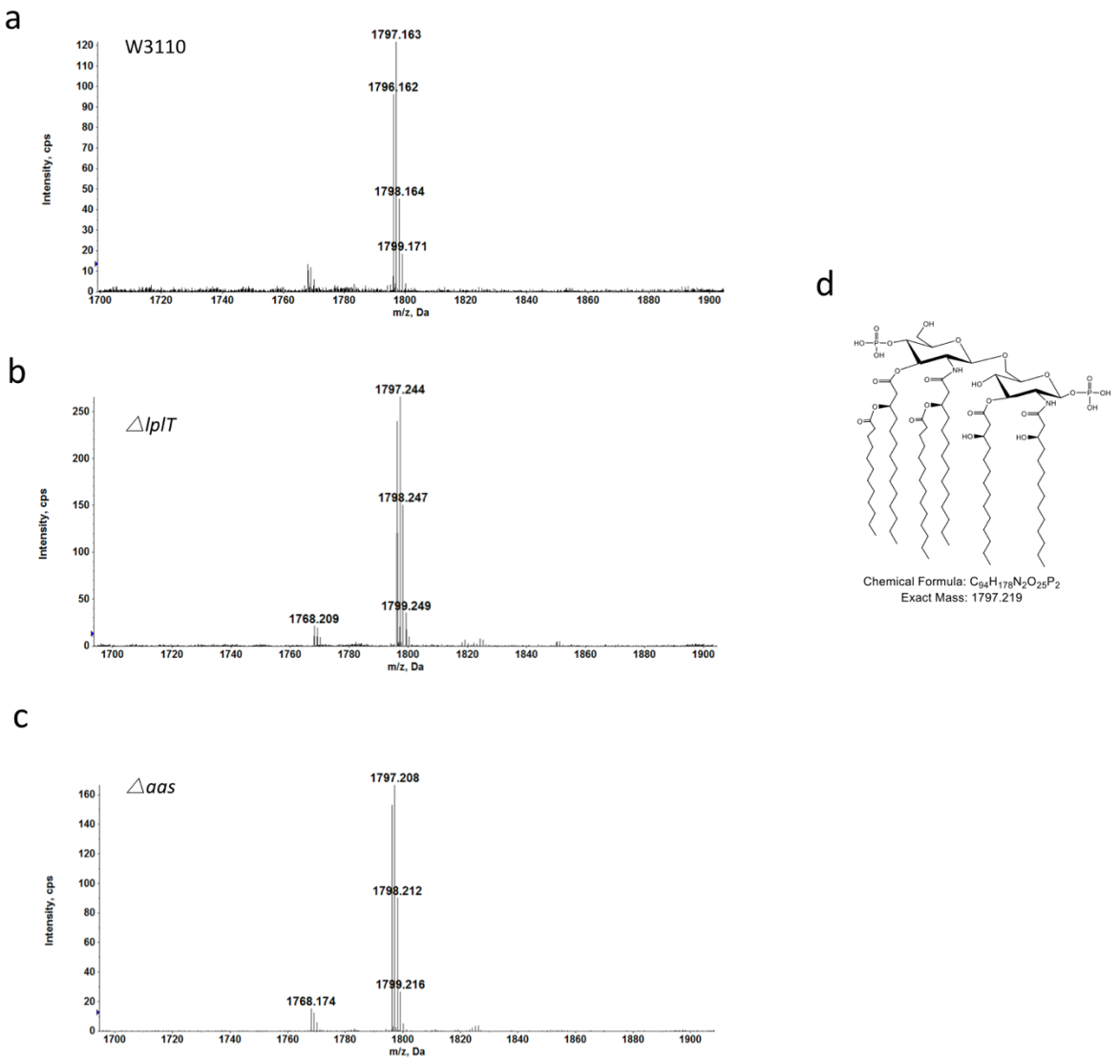

Fig. S9. LC-ESI mass spec analysis of lipid A isolated from *E. coli.* *a)* W3110 WT; b) Δ*lplT; c)* Δ*aas* strain. d) the chemical structure of lipid A based on mass spec results.