

**The type VII secretion system protects *Staphylococcus aureus* against antimicrobial host fatty acids**

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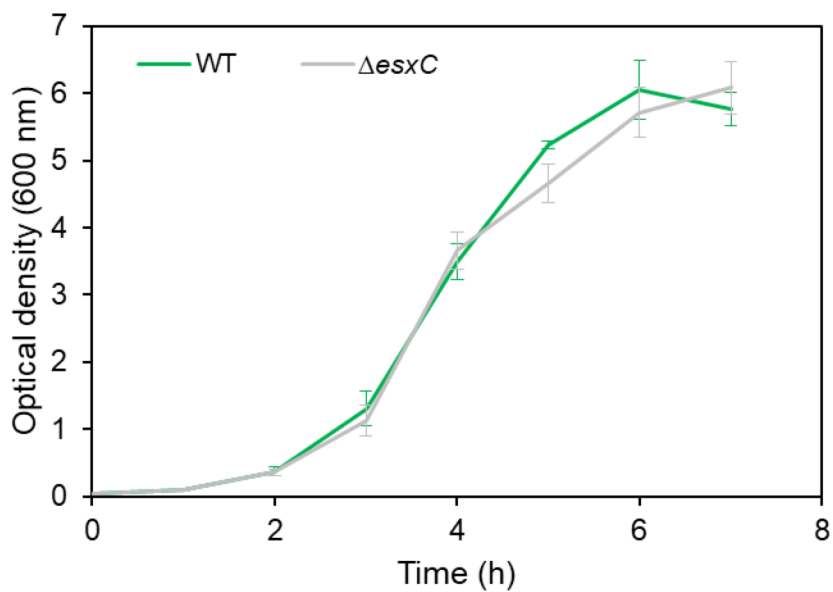
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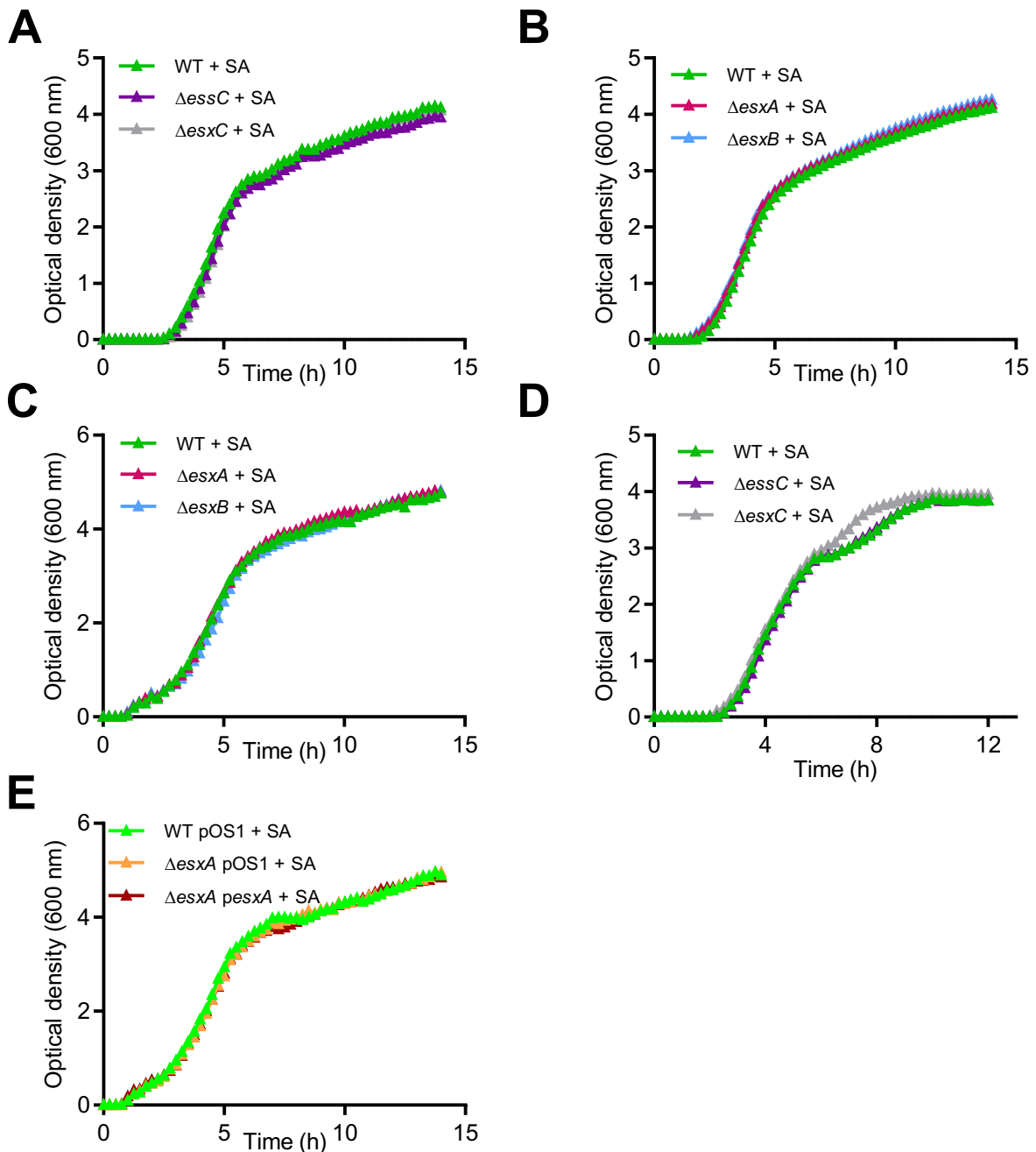
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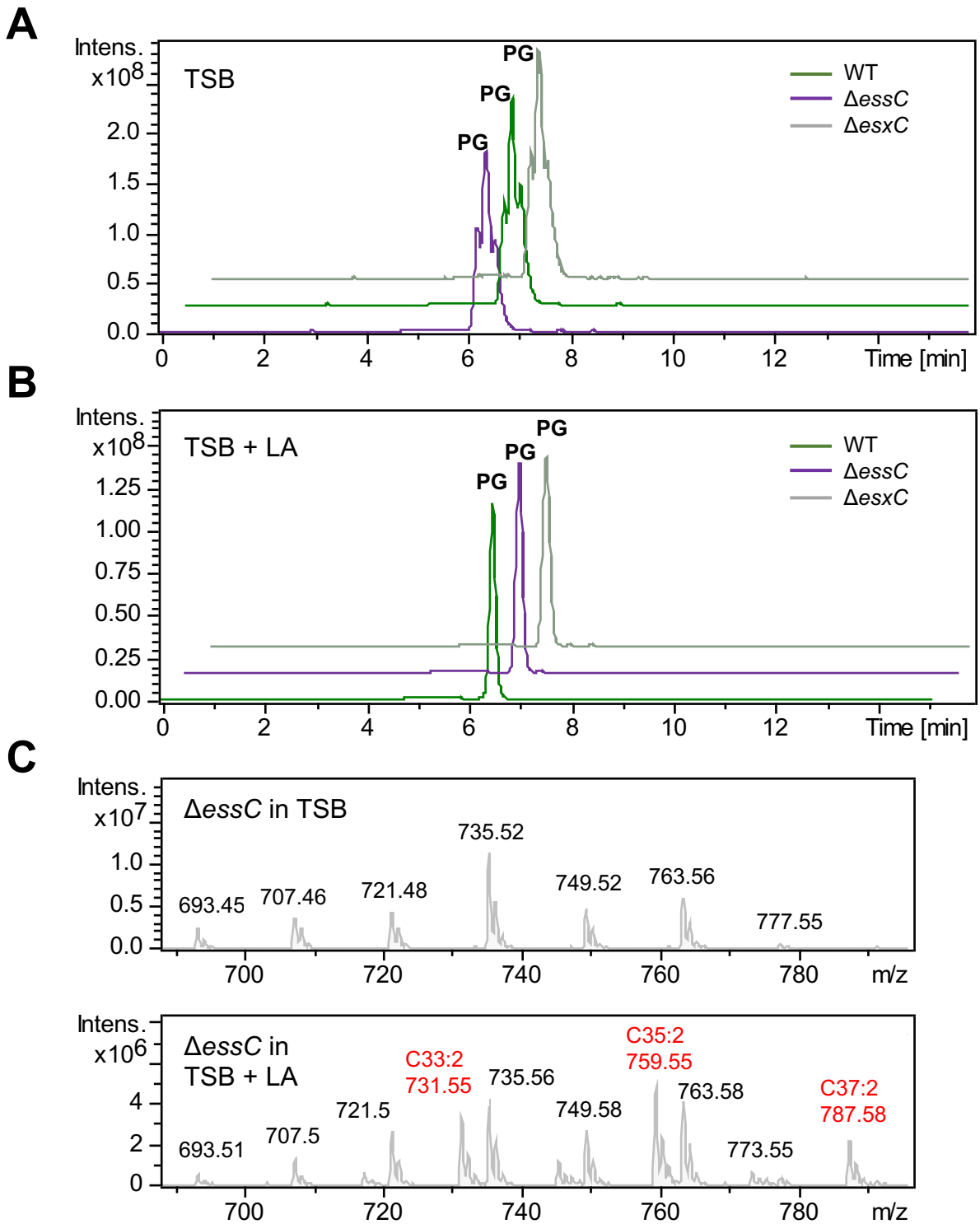
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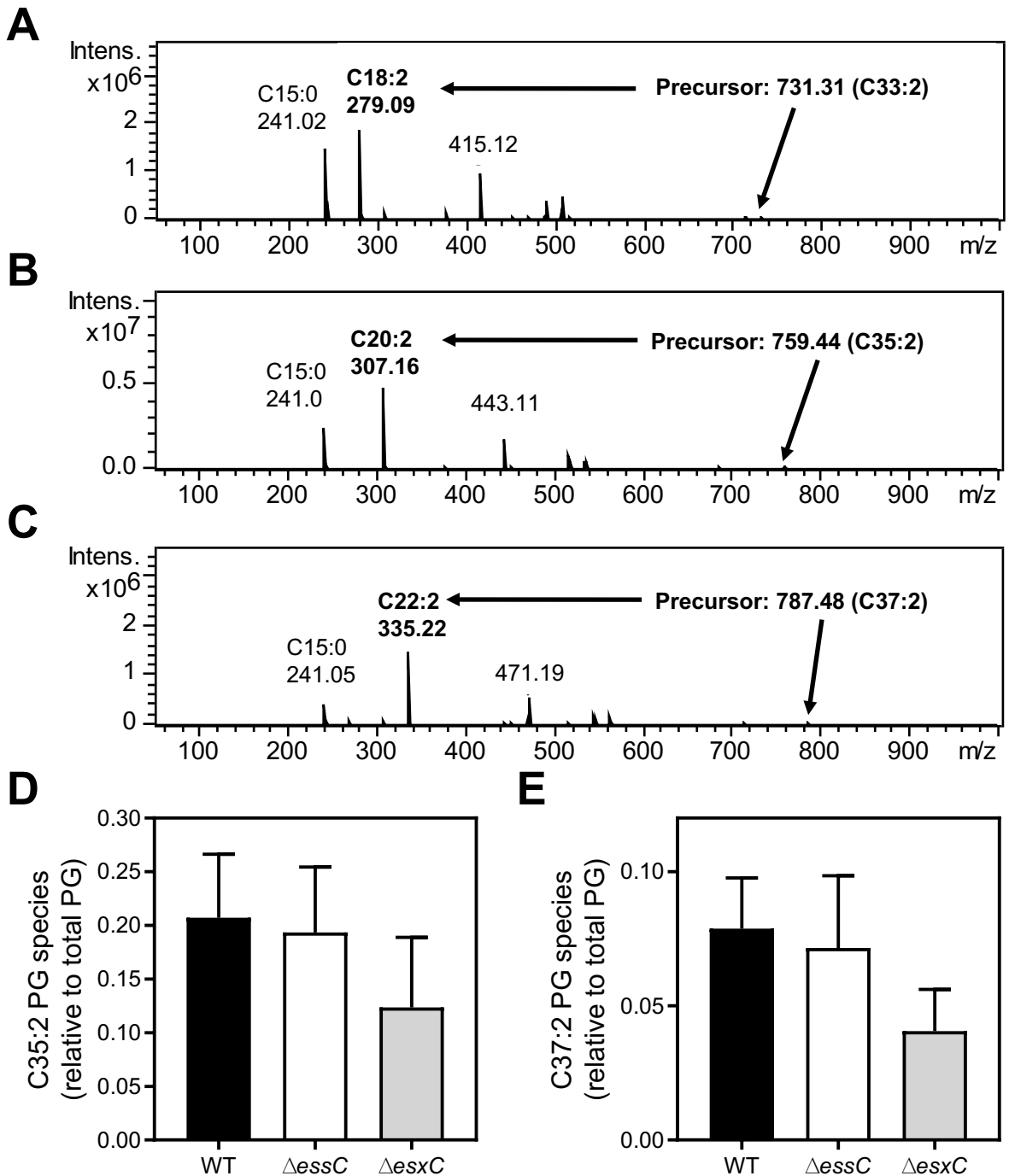
**Fig. S1. USA300 JE2 WT and  $\Delta$ esxC strains display similar growth rates.** WT and  $\Delta$ esxC were grown in TSB, and OD<sub>600</sub> monitored with a Novaspec Pro spectrophotometer. Data shown are means of three independent experiments, and the error bars indicate the standard errors of the mean.



**Fig. S2. T7SS mutants are not inhibited by stearic acid.** The following *S. aureus* T7SS mutants and their respective WT strains were grown in TSB or TSB supplemented with stearic acid (SA): (A) USA300 JE2 WT,  $\Delta\text{essC}$ , and  $\Delta\text{esxC}$ ; (B) USA300 LAC WT,  $\Delta\text{esxA}$ , and  $\Delta\text{esxB}$ ; (C) Newman WT,  $\Delta\text{esxA}$ , and  $\Delta\text{esxB}$ ; (D) RN6390 WT,  $\Delta\text{essC}$ , and  $\Delta\text{esxC}$ ; (E) Newman WT with the empty pOS1 plasmid (WT pOS1) and Newman *esxA* mutant with either pOS1 ( $\Delta\text{esxA}$  pOS1) or pOS1-*esxA* ( $\Delta\text{esxA pesxA}$ ). Data shown are representative of at least three independent experiments.



**Fig. S3. USA300 JE2 WT and T7SS mutants display similar lipids.** Representative HPLC chromatograms of the indicated bacteria grown in TSB (A) or in TSB supplemented with LA (B), in negative ionisation mode. Phosphatidylglycerol (PG) is highlighted. C. Representative HPLC chromatograms of native PG species of  $\Delta$ essC grown in TSB (top panel) or in TSB supplemented with LA (bottom panel), in negative ionisation mode.



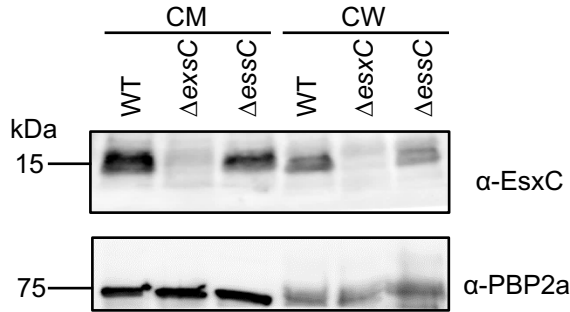
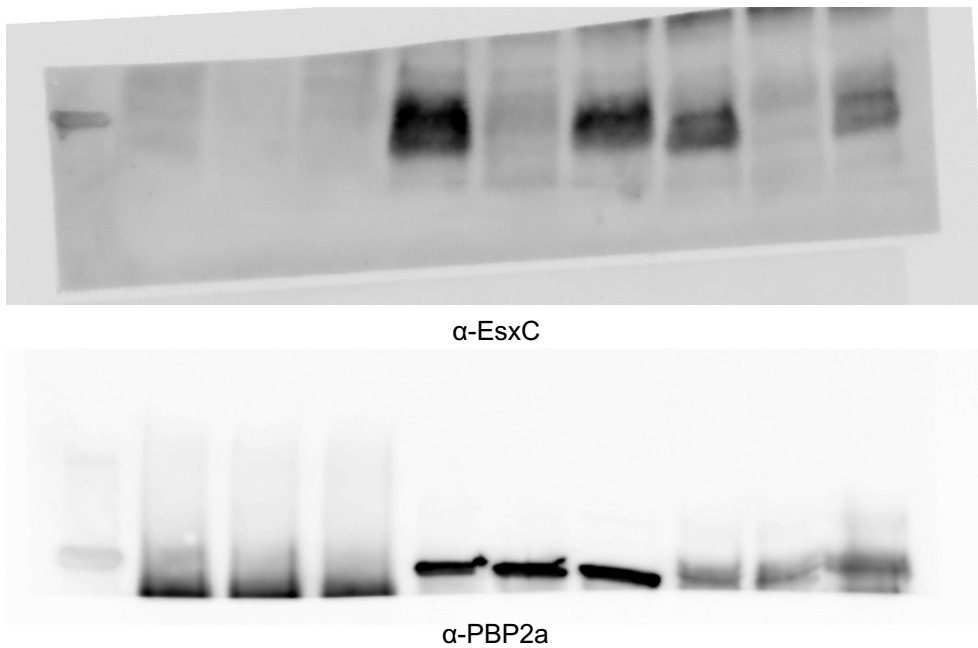
**Fig. S4. LA (C18:2) is elongated and incorporated into *S. aureus* phosphatidylglycerol (PG) species.** A-C. Representative mass spectrometry fragmentation spectra for PG species containing unsaturated fatty acids, in negative ionisation mode.

A) PG species with mass 731 m/z, containing C18:2 fatty acid (279 m/z).

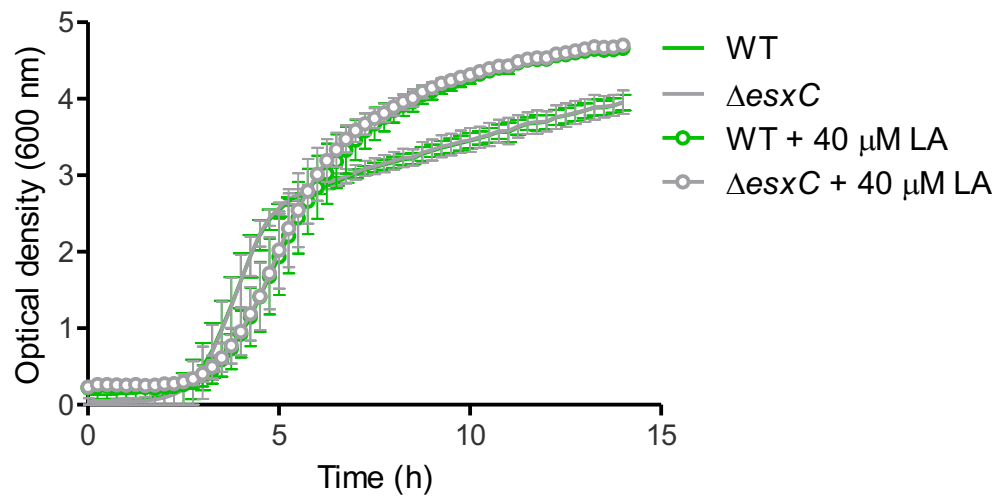
B) PG species 759 m/z, containing C20:2 fatty acid (307 m/z)

C) PG species 787 m/z, containing C22:2 fatty acid (335 m/z).

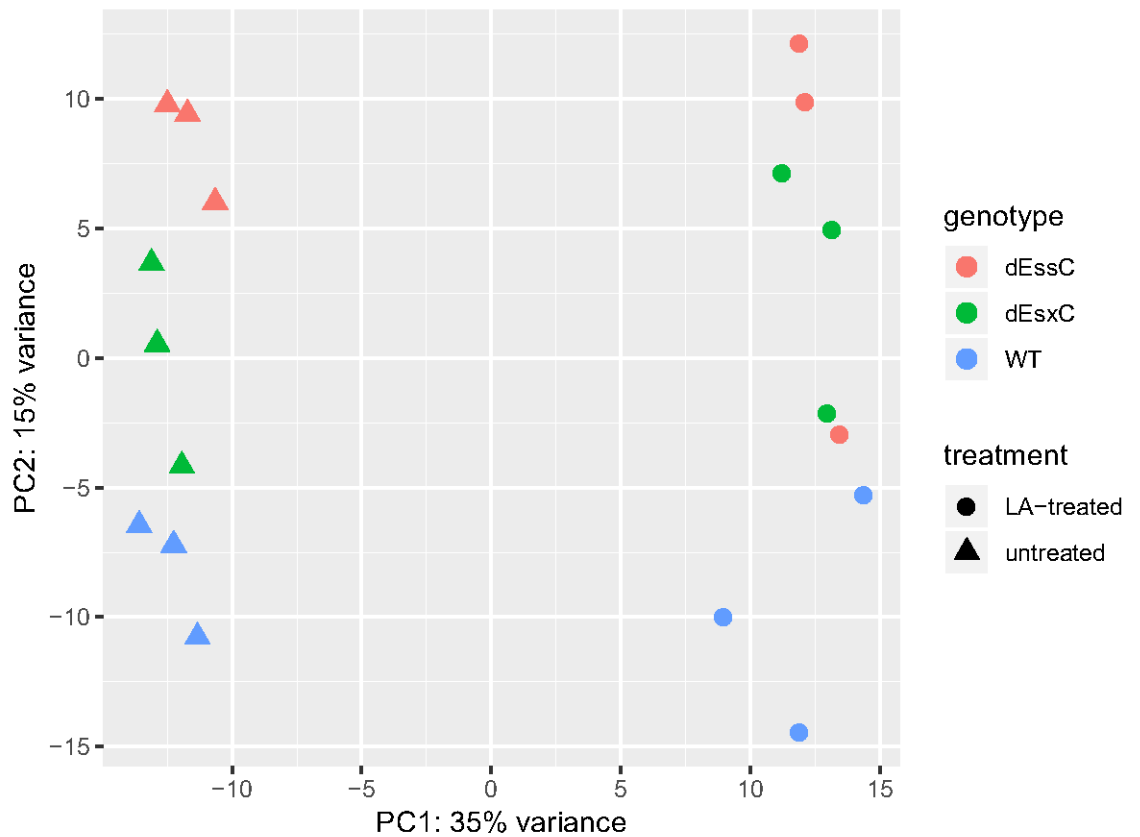
D) and E. Relative quantification of the indicated PG species containing an unsaturated fatty acid in WT,  $\Delta$ essC and  $\Delta$ esxC. C20:2- (D) and C22:2-containing PG species (E) are presented as ratios of total PG species. Data shown are the means and error bars represent SD of three independent experiments.

**A****B**

**Fig. S5. Localisation of EsxC in *S. aureus* WT and  $\Delta essC$  mutants.** A) Immunoblot analysis of cell membrane (CM) or cell wall (CW) fractions of WT (USA300),  $\Delta essC$ , and  $\Delta esxC$  with anti-EsxC sera or anti-PBP2a antibodies (loading control). The images have been cropped for clarity. After sample transfer, the blots were cut and probed in parallel with anti-EsxC and anti-PBP2a antibodies (loading control). B) Images of unprocessed blots shown in A.

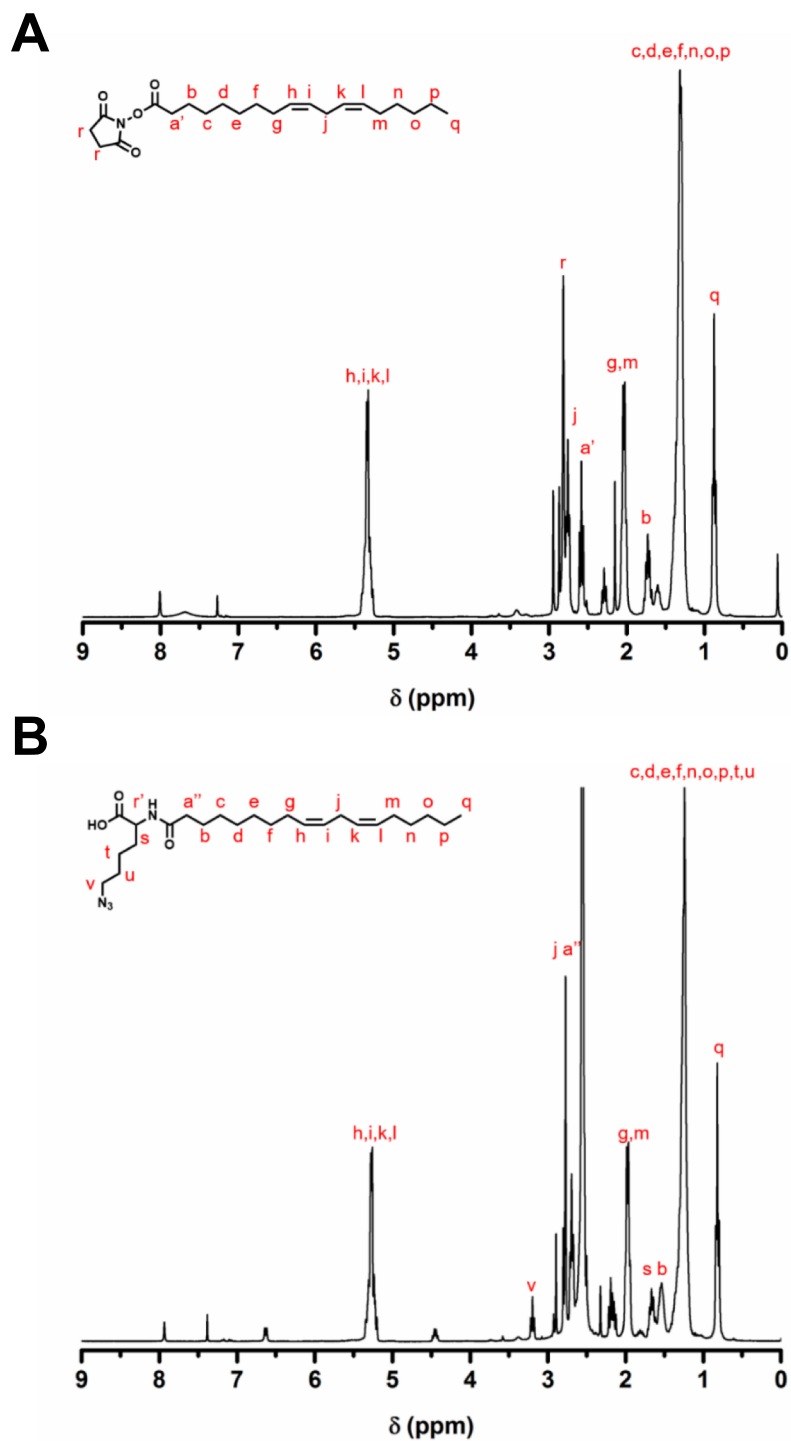


**Fig. S6. USA300 JE2 WT and  $\Delta esxC$  growth similarly in presence of sub-inhibitory levels of linoleic acid.** *S. aureus* WT USA300 and  $\Delta esxC$  were grown in TSB or TSB supplemented with 40  $\mu\text{M}$  linoleic acid (LA). Means  $\pm$  standard error of the mean (SEM) are shown,  $n = 3$



**Fig. S7. Principal component analysis (PCA) of the *S. aureus* cellular proteomic profiles.** PCA was performed on all the identified proteins of USA300 JE2 WT and T7SS mutants grown in TSB (untreated) or TSB + LA (LA-treated). Each dot represents a biological replicate.





**Fig. S8.**  $^1\text{H}$  NMR spectra of NHS-LA (A) and azide-LA (B) in  $\text{CDCl}_3$ . Both spectra were recorded on a Bruker Advance 300 spectrometer (300 MHz) at 27  $^\circ\text{C}$ . The letters indicate the chemical shift  $\delta$  (in parts per million, ppm) of the protons in each molecule.

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

Strain or plasmid	Description	Source or reference
<b><i>Staphylococcus aureus</i></b>		
USA300 LAC	Community-acquired MRSA (CA-MRSA)	Olaf Schneewind
LAC $\Delta$ esxA	<i>S. aureus</i> USA300 LAC defective for EsxA	(1)
LAC $\Delta$ esxB	<i>S. aureus</i> USA300 LAC defective for EsxB	(1)
USA300 LAC JE2	Plasmid-cured USA300 LAC	BEI Resources (NARSA) (2)
JE2 $\Delta$ esxC	<i>S. aureus</i> USA300 LAC JE2 defective for EsxC	This study
JE2 $\Delta$ essC	<i>S. aureus</i> USA300 LAC JE2 defective for EssC	This study
Newman	Methicillin-sensitive <i>Staphylococcus aureus</i>	Olaf Schneewind
Newman $\Delta$ esxA	<i>S. aureus</i> Newman defective for EsxA	(1)
Newman $\Delta$ esxB	<i>S. aureus</i> Newman defective for EsxB	(1)
RN6390	NCTC8325 derivative, $\Delta$ rbsU, $\Delta$ tcaR, cured of $\phi$ 11, $\phi$ 12, and $\phi$ 13	Tracy Palmer (3)
RN6390 $\Delta$ esxC	<i>S. aureus</i> RN6390 defective for EsxC	Tracy Palmer (3)
RN6390 $\Delta$ essC	<i>S. aureus</i> RN6390 defective for EssC	Tracy Palmer (3)
RN4220	<i>S. aureus</i> restriction negative, cloning tool	BEI Resources (NARSA)
<b>Plasmids</b>		
pKORI	Temperature-sensitive allelic exchange vector	Olaf Schneewind (4)
pKORI $\Delta$ essC	pKORI used to generate essC mutant	This study
pKORI $\Delta$ esxC	pKORI used to generate esxC mutant	This study
pOS1	Insertless vector for genetic complementation	Olaf Schneewind
pOS1CK	pOS1 with P1 constitutive promoter of sarA	(1)
pOS1-esxA	esxA complementation vector	(1)
pOS1-esxC	esxC complementation vector	This study

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

<b>Primer</b>	<b>Sequence (5' – 3')</b>	<b>Product</b>
AttB1-esxC Up-Fwrd	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAGCTAACGCTATGAAAACACC	AttB1-
esxC-up-Rev-soeing	ACCCATATCTTCACCTCAATAAACATACCTCCCTCCTATTT	$\Delta$ esxC-
esxC-down-Fwd	TATTGAGGTGAAGATATGGGTGG	AttB2
esxC-down-Rev-attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCATTACTCCTCTGCTTTA	
AttB1-essC-up-fwd	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCTACACATTTGTGTTGGCACC	AttB1-
essC-upstream-rev	TGTCTTTGCCTCAGTCCTATAC	$\Delta$ essC-
essC-dpwn-soeing-fwd	GTATAGGACTGAGGCAAAGACACAATGAATTAATAGGAGGGAGG	AttB2
esxC-down-Rev-attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCATTACTCCTCTGCTTTA	
esxC-RBS-PstI-fwd	GCGCTGCAGTTGAGAGGAGAGAAAATGAATTTAATGATATTGAAAC	RBS-
esxC-SmaI-rev	GCGGCGCCCGGGTTAATTCATTGCTTTATTA	esxC

## Supplemental references

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