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## Supplemental information

# Oxidative stress is intrinsic to staphylococcal adaptation to fatty acid synthesis antibiotics

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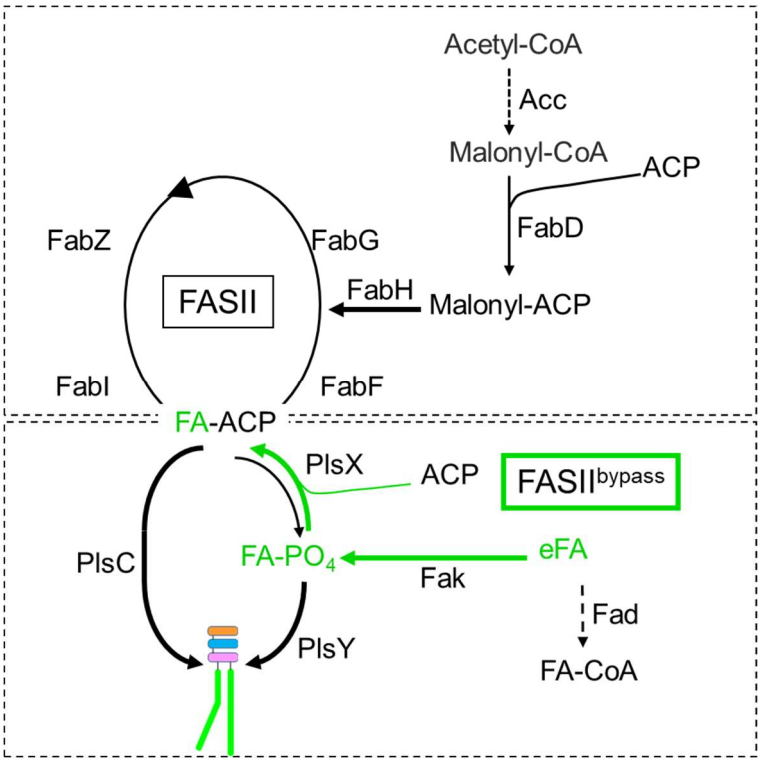


**Fig S1. Schematic model of FASII and FASII bypass in *S. aureus*.** Related to Fig. 1. **Upper:** *S. aureus* synthesizes fatty acids (FAs) *via* the fatty acid synthesis pathway FASII (boxed in black), comprising 3 dedicated initiation enzymes (upper right), and 4 enzymes that constitute the recursive FASII cycle (for review, [1]). The final product, FA-ACP (ACP, acyl carrier protein), provides FAs for transfer to a glycerol phosphate backbone for phospholipid synthesis. **Lower:** FASII bypass (boxed in green) can take over when FASII is inhibited. Environmental FAs are phosphorylated by fatty acid kinase (Fak; [2]). Both FASII and FASII bypass products lead to phospholipid synthesis: Starting from FA-ACP (the FASII product), PlsX and PlsY catalyze FA attachment to position 1 of the glycerolphosphate backbone. PlsC then joins a second FA to position 2 of the mono. Starting from eFAs, the resulting phosphorylated FA (FA-$PO_4$) is used by PlsY to join a FA to position 1. Reverse activity of PlsX generates FA-ACP, which is the substrate for PlsC to join the FA to position 2 on the lysophosphatidic acid. eFAs are also substrates for Fad (fatty acid degradation) enzymes, dotted arrow, which are encoded by *S. aureus*, whose activity was recently demonstrated [3].

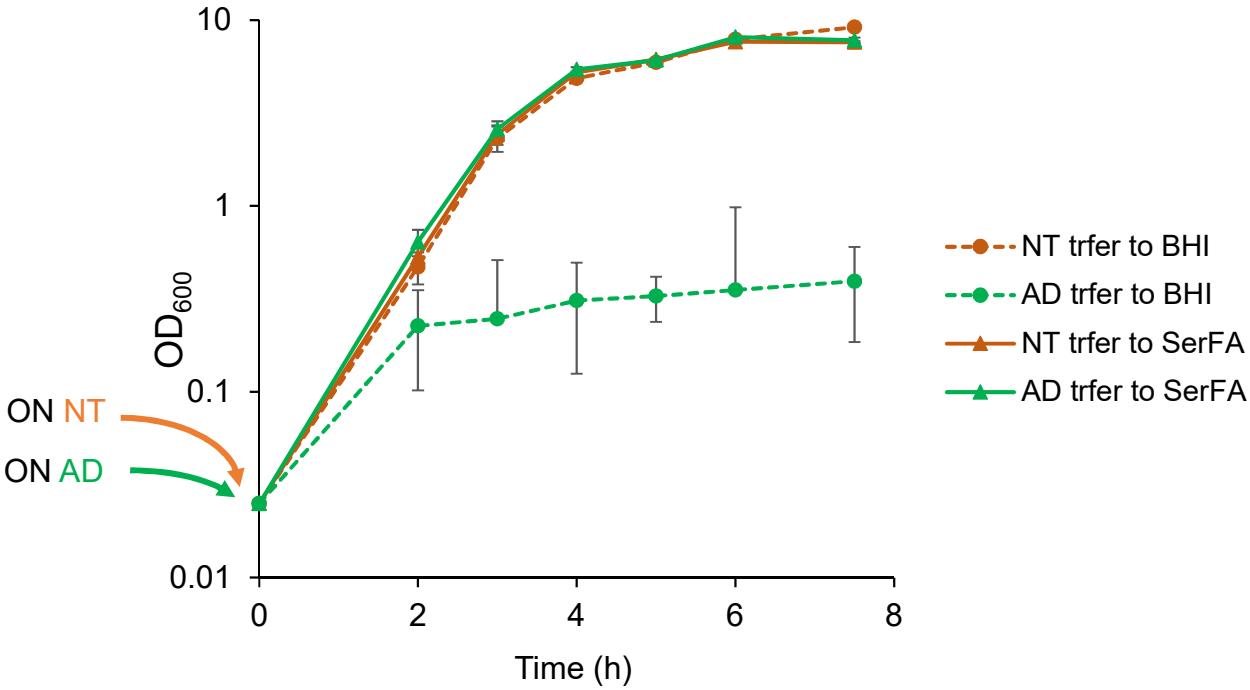


**Fig. S2. Regrowth of non-treated and anti-FASII-adapted *S. aureus* USA300 cultures varies according to medium.** Related to Chapter "Anti-FASII treatment leads to long-term S. aureus adaptation without detectable chromosomal rearrangements". Overnight (ON) non-treated (NT) and anti-FASII adapted (AD) *S. aureus* USA300 cultures were prepared in SerFA containing or not anti-FASII AFN-1252. They were then diluted to $OD_{600}$ 0.025, and transferred to ('trfer to') SerFA or BHI medium without selection. Growth of cultures was monitored. Samples are as indicated above. Shown are the mean and standard deviation of 3 biological replicates.

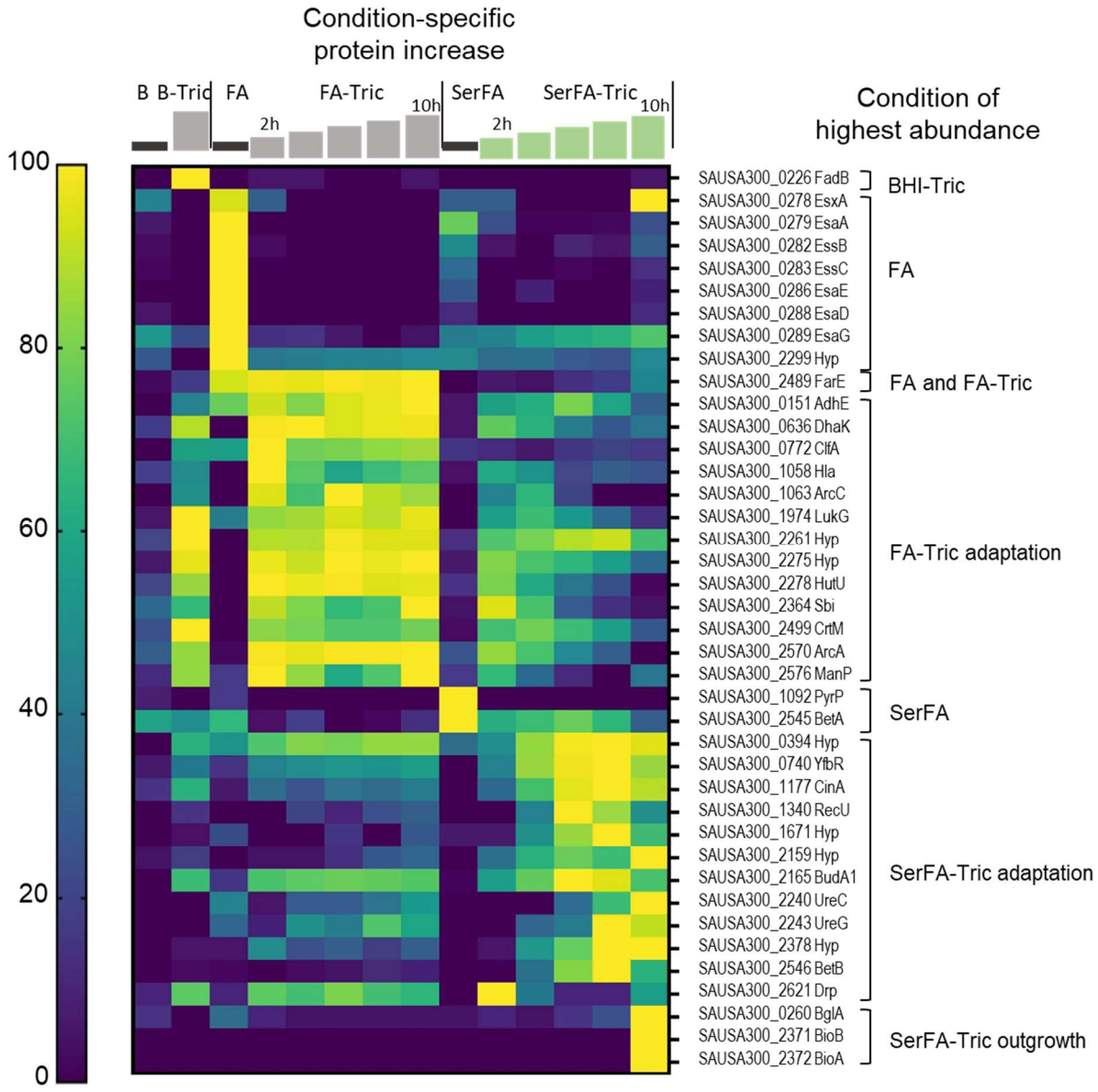


**Fig. S3. Heat map of condition-specific highly expressed proteins.** Related to Fig. 1. Gene loci and encoded proteins at right show the highest differential expression in conditions indicated after each bracket. FA-Tric and SerFA-Tric kinetics samples were each analyzed as a group regardless of the time of increased expression. Hyp, hypothetical protein. The heat map scale (at left) is determined relative to weighted value for each protein (navy, down-represented; yellow, up-represented; see Star Methods for analyses). Sampling times (h) above steps correspond to 2, 4, 6, 8, and 10 h for FA-Tric and SerFA-Tric. B-Tric (BHI with triclosan without added FAs) corresponds uniquely to a 6 h time point. Green steps corresponds to the adaptation condition.

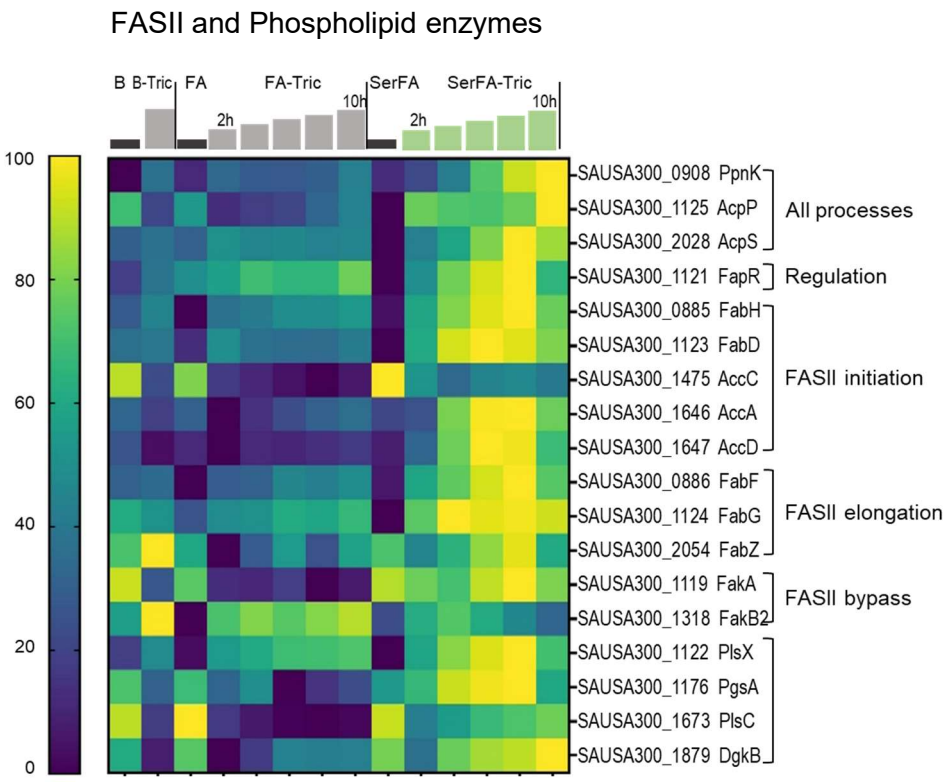


**Fig. S4. Heat map of FASII and phospholipid synthesis and recycling functions whose expression is affected in SerFA-Tric growth.** Related to Fig. 1. Proteins were compiled based on confirmed FASII and phospholipid synthesis and recycling proteins. Proteins unaffected or nondetected in any condition are not included. Results are limited to SerFA (control) and SerFA-Tric adaptation conditions. Gene names and functional categories are at right. Incremental time points for SerFA-Tric 2, 4, 6, 8, and 10 h samples are represented by steps. Correspondence between color and expression is determined relative to weighted value for each protein, as on scale at left (navy, down-represented; yellow, up-represented; see Star Methods for analyses).

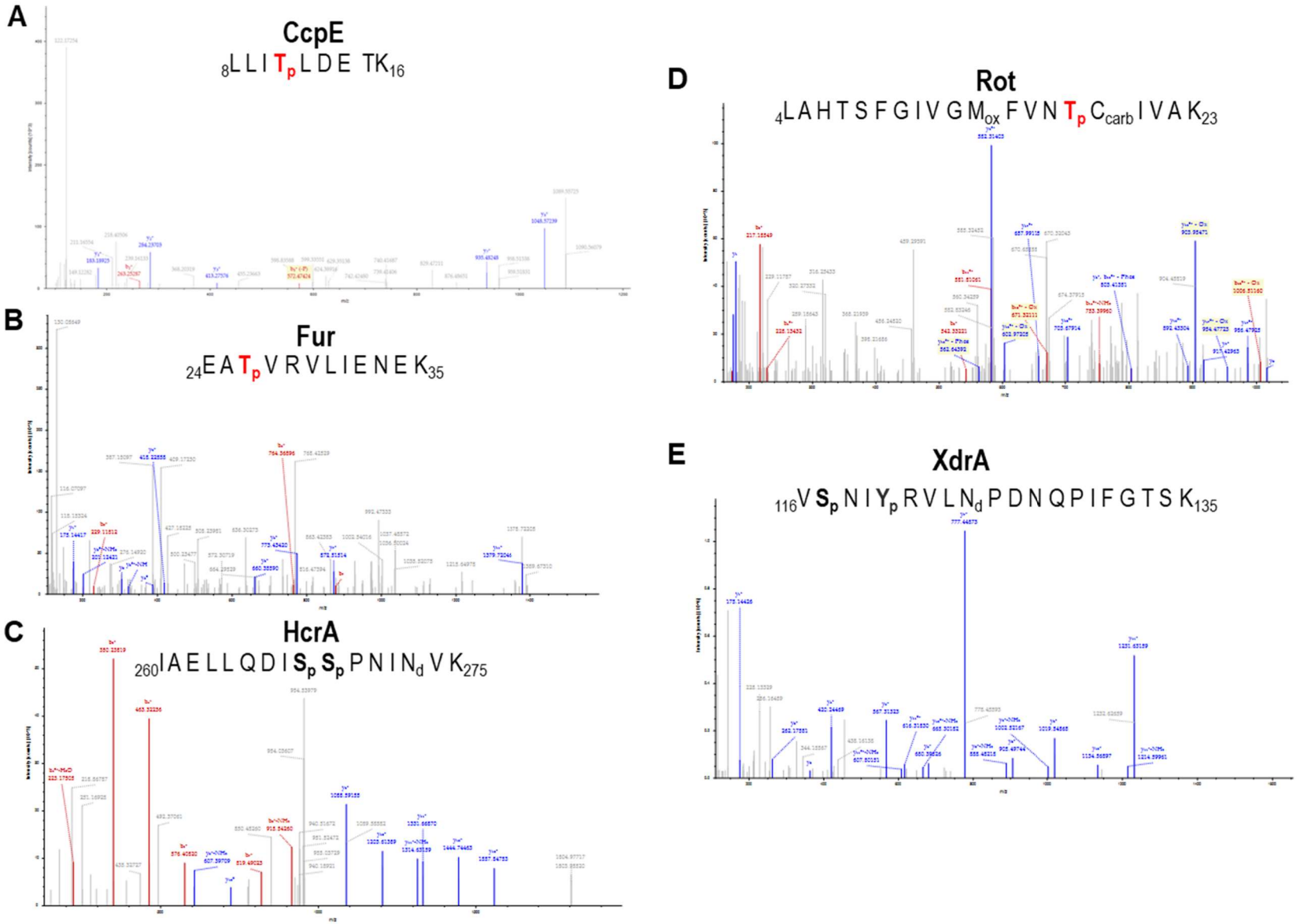


**Fig. S5. Tandem Mass spectra of identifying differentially expressed phosphorylation sites of CcpE (A), Fur (B), HcrA (C), Rot (D) and XdrA (E) proteins.** Related to Fig. 2. The identified phosphorylation sites are indicated in bold red when localization is validated and in bold black when 2 phosphorylation sites are possible. d: deamidation, carb: carbamidomethylation, ox: oxidation, p: phosphorylation. All peptides are labeled by dimethyl tag on peptide N-term and K.

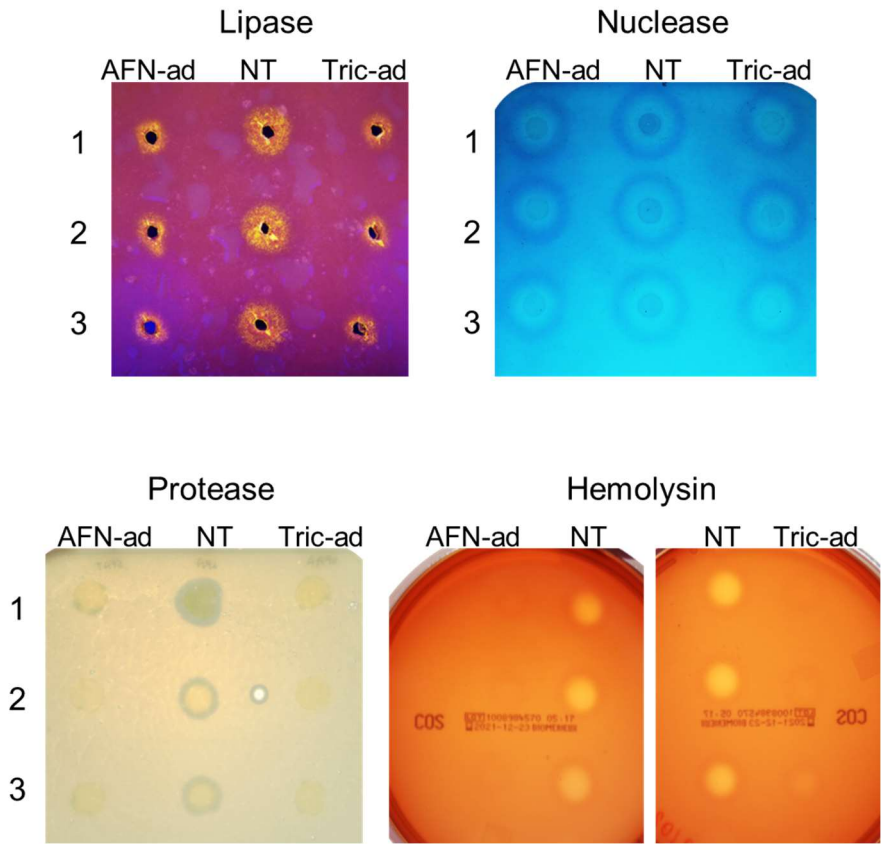


**Fig. S6. Secreted virulence factor activities.** Related to Fig. 3.Non-treated (NT) and anti-FASII-adapted cultures treated with triclosan (Tric-ad) or AFN-1252 (AFN-ad) were grown overnight. Cultures (for protease detection) and culture supernatants (lipase, nuclease, and hemolysin detection) were spotted on appropriate detection medium. Three independent cultures were prepared. See Star Methods section for protocol.

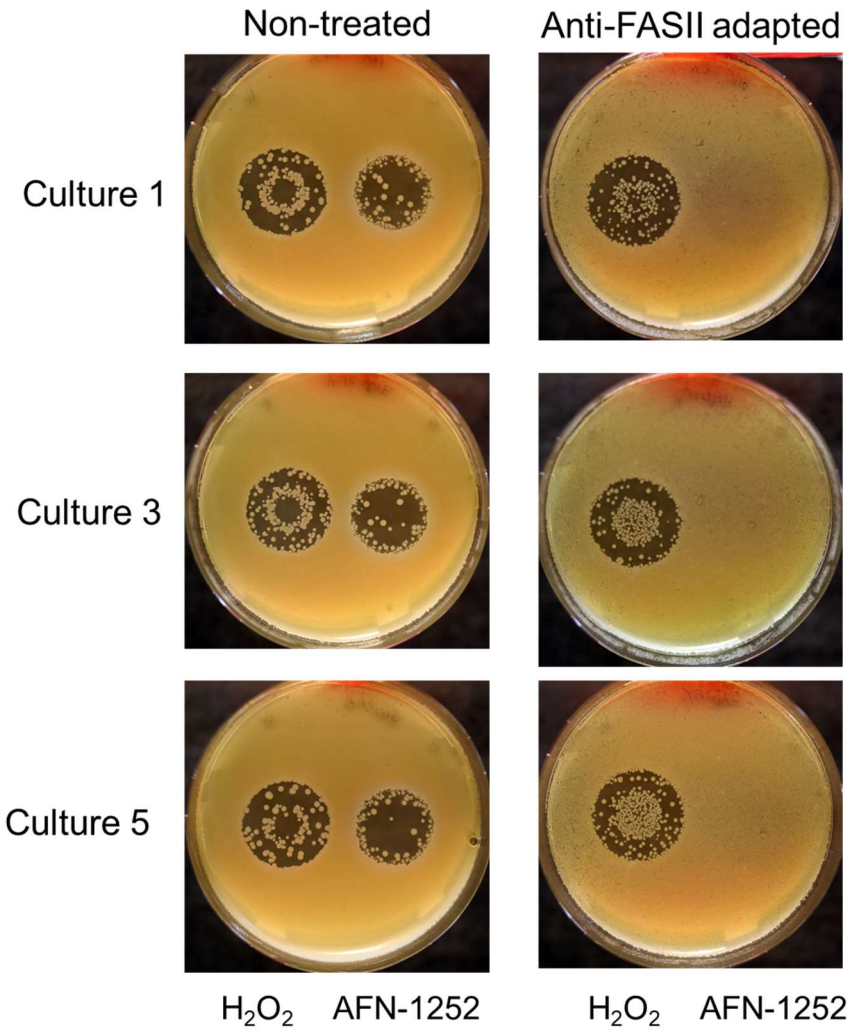


**Fig. S7. *S. aureus* anti-FASII adaptation confers increased $H_2O_2$ resistance.** Related to Fig. 4. USA300 non-treated and AFN-1252-adapted overnight cultures were prepared, and lawns (100 µl of dilutions adjusted to $OD_{600}$ = 0.1) were spread on SerFA solid medium. They were spotted with 1.5 mm $H_2O_2$ and 4 nm (1.5 µg) AFN-1252, and photographed after 48 h incubation at 37°C. See Star Methods section for protocol.

**Supplementary references**

[S1]. Zhang, Y.M., and Rock, C.O. (2008). Membrane lipid homeostasis in bacteria. Nat Rev Microbiol 6, 222-233. 10.1038/nrmicro1839.
[S2]. Parsons, J.B., Broussard, T.C., Bose, J.L., Rosch, J.W., Jackson, P., Subramanian, C., and Rock, C.O. (2014). Identification of a two-component fatty acid kinase responsible for host fatty acid incorporation by *Staphylococcus aureus*. Proc Natl Acad Sci U S A 111, 10532-10537. 10.1073/pnas.1408797111.
[S3]. Kuiack, R.C., Tuffs, S.W., Dufresne, K., Flick, R., McCormick, J.K., and McGavin, M.J. (2023). The *fadXDEBA* locus of *Staphylococcus aureus* is required for metabolism of exogenous palmitic acid and in vivo growth. Mol Microbiol 120, 425-438. 10.1111/mmi.15131.