*Supplementary Material*

# Free fatty acids interfere with the DNA binding activity of the virulence regulator PrfA of *Listeria monocytogenes*

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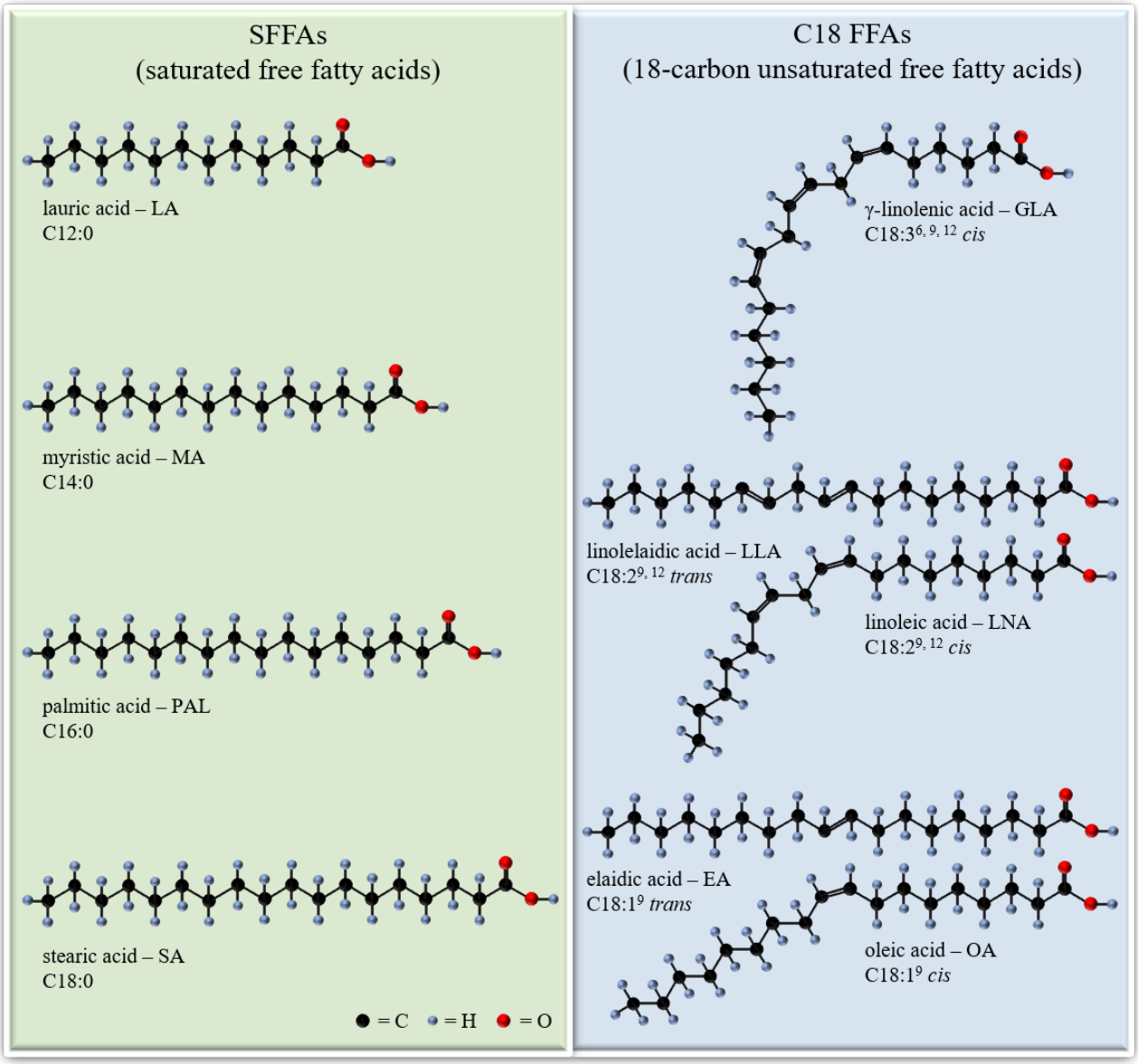
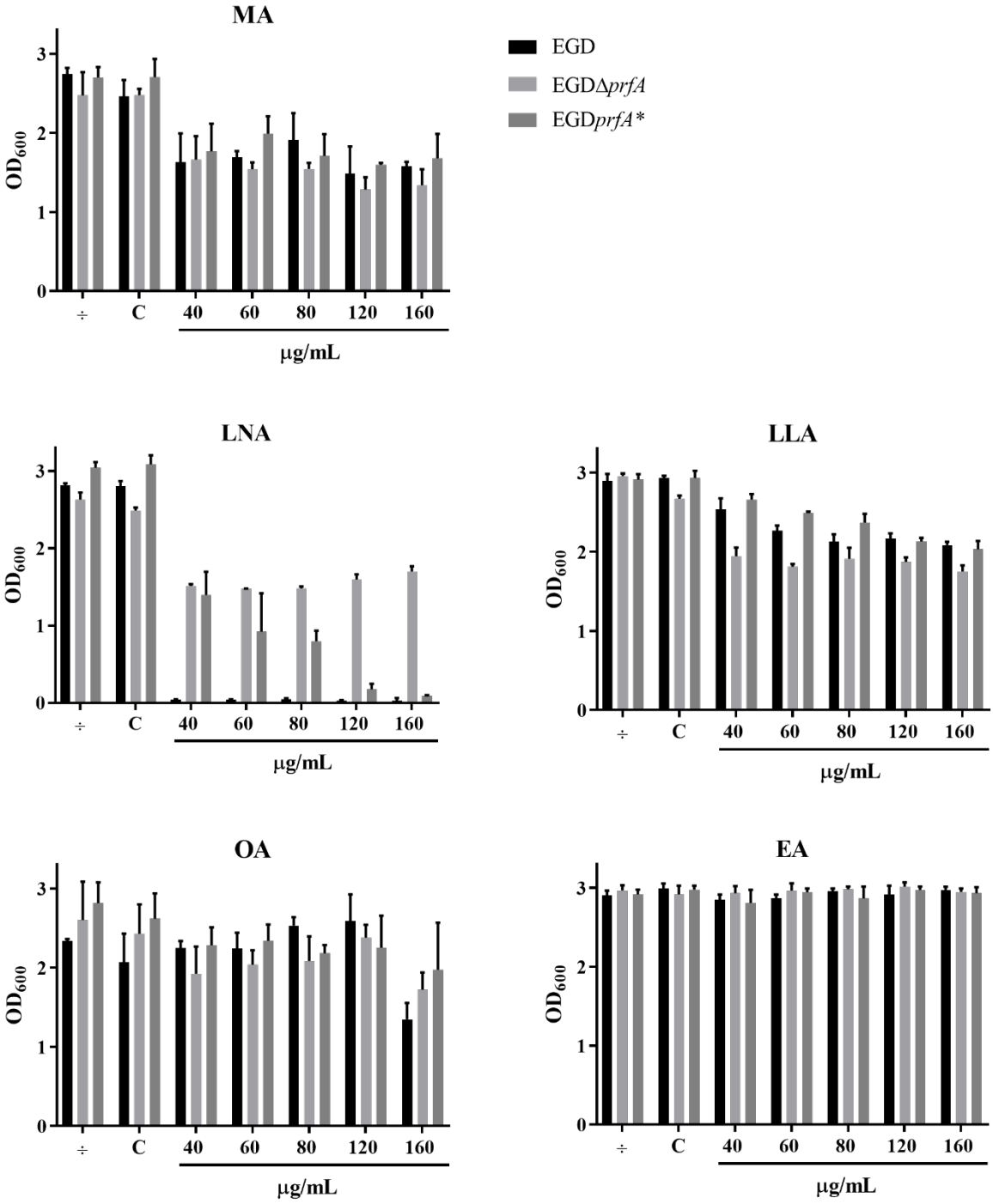
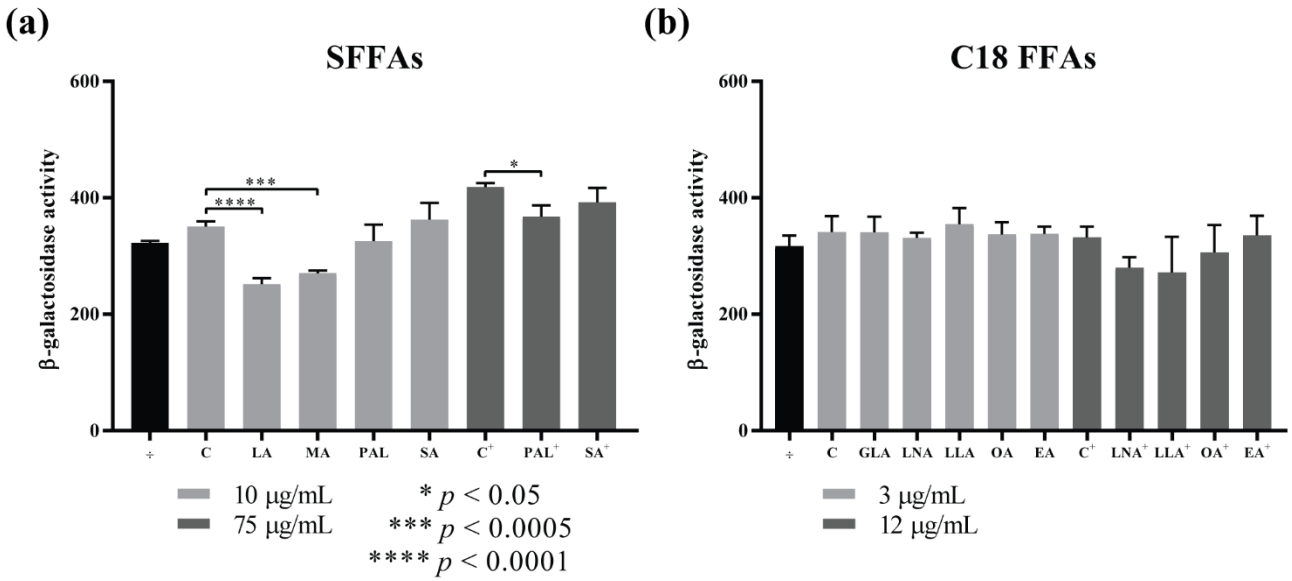


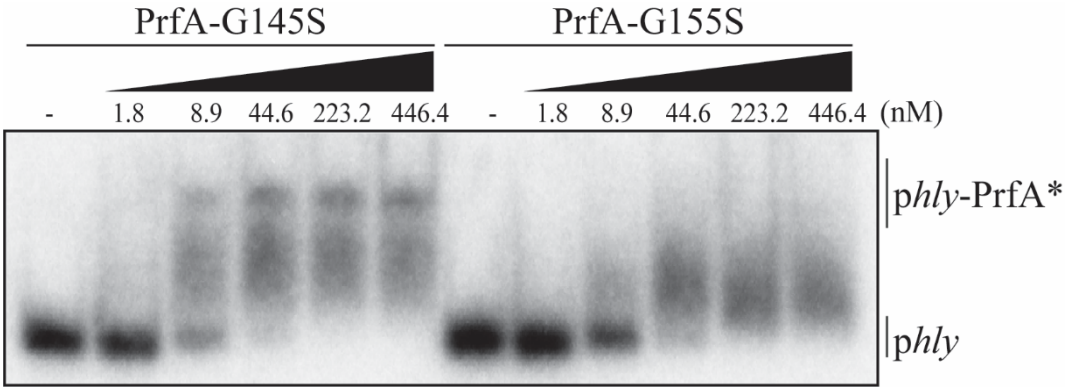

**Supplementary Figure S1:** FFA structures. 2D representation of the saturated FFAs (left) and C18 unsaturated FFAs (right) used in this study.




**Supplementary Figure S2:** Growth of EGD wild-type, EGDΔ*prfA* and EGD*prfA** (*prfA*-G155S) in the presence of increasing concentrations of MA, LNA, LLA, OA and EA. $OD_{600}$ measurements were taken after 20 h of FFA exposure. As controls, the cultures were left untreated (÷) or vehicle was added corresponding to the highest concentration used (C). Results are the average of three biological replicates.




**Supplementary Figure S3:** Expression of p*lhrA36-lacZ* in response to FFA exposure. The promoter region of the PrfA-independent *lhrA* gene cloned into vector pTCV-lac was transformed into EGD*prfA**. The resulting strain was grown to $OD_{600}$ = 0.3 and exposed to (**A**) SFFAs in a concentration of 10 µg/mL (50 µM LA, 44 µM MA, 39 µM PAL, 35 µM SA) or 75 µg/mL (293 µM $PAL^+$, 264 µM $SA^+$), or to (**B**) C18 FFAs in a concentration of 3 µg/mL (corresponding to 11 µM FFA) or 12 µg/mL (corresponding to 43 µM $FFA^+$). As controls, the cultures were left untreated (÷) or vehicle was added corresponding to the concentration present in the FFA-treated cultures (C or $C^+$). Samples for the β-galactosidase assays were withdrawn after 20 h. Results are the average of three biological replicates, each carried out in technical duplicates.




**Supplementary Figure S4:** *In vitro* PrfA*-p*hly* complex formation. EMSA of the interaction between labeled p*hly* and increasing concentrations of purified His-tagged PrfA* variants PrfA-G145S (left) or PrfA-G155S (right). For PrfA-G145S-p*hly*, the smear observed underneath the shifted band most likely reflects the instability of the protein-DNA complex. The PrfA-G155S-p*hly* complex appeared to be highly unstable; in this case, a specific shifted band could not be observed.

**Supplementary Table S1:** Primers used in this study

| Name | Sequence (5'→ 3') | Further information |
|---|---|---|
| | **Cloning** | |
| **His-tag Fw PrfA** | GGGGCCATGGCG**CACCATCACCATCACCAT**AA CGCTCAAGCAGAAGAATTC | Forward primer for 6 × His-tag coding sequence inserted between codon 2 and 3 of *prfA*. NcoI restriction enzyme site is underlined. Nucleotide sequence for 6 × His-tag is in bold. |
| **G145S Rev PrfA** | CATAGGTCAGGATTAAAAGTTGACTGCAAATAG AGCCAAGCTTCCCG | Reverse primer to introduce G145S substitution on *prfA*. Codon 145 where the nucleotide substitution GGT → AGT was introduced is highlighted. |
| **G145S Fw PrfA** | CGGGAAGCTTGGCTCTATTTGCAGTCAACTTTTA ATCCTGACCTATG | Forward primer to introduce G145S substitution on *prfA*. Codon 145 where the nucleotide substitution GGT → AGT was introduced is highlighted. |
| **His-tag Rev PrfA** | CCCCTCTAGATTAATTTAATTTTCCCCAAGTAGC | Reverse primer for constructing 6 × His-tagged PrfA. XbaI restriction enzyme site is underlined. |
| | **NB probes** | |
| **ActA R** | GCTATTAGGTCTGCTTTGTTC | Single stranded probe for *actA* mRNA. |
| **Hly R** | CCATCTTTGTAACCTTTTCTTGG | Single stranded probe for *hly* mRNA. |
| **InlA R** | ATTTGCGGAAGGTGGTGTAG | Single stranded probe for *inlA* mRNA. |
| **PrfA R** | GCTAGACTGTATGAAACTTG | Single stranded probe for *prfA* mRNA. |
| **16S rRNA** | GGCCATTACCCTACCAACTAGCTAATGCAC | Single stranded probe for 16S rRNA. |
| | **EMSAs** | |
| **Phly short Fw** | GTGACTTTTATGTTGAGGCA | Forward primer to amplify a 54bp region containing the *hly* promoter. |
| **Phly short Rev** | CTGCTGTCCCTTTATCG | Reverse primer to amplify a 54bp region containing the *hly* promoter. |