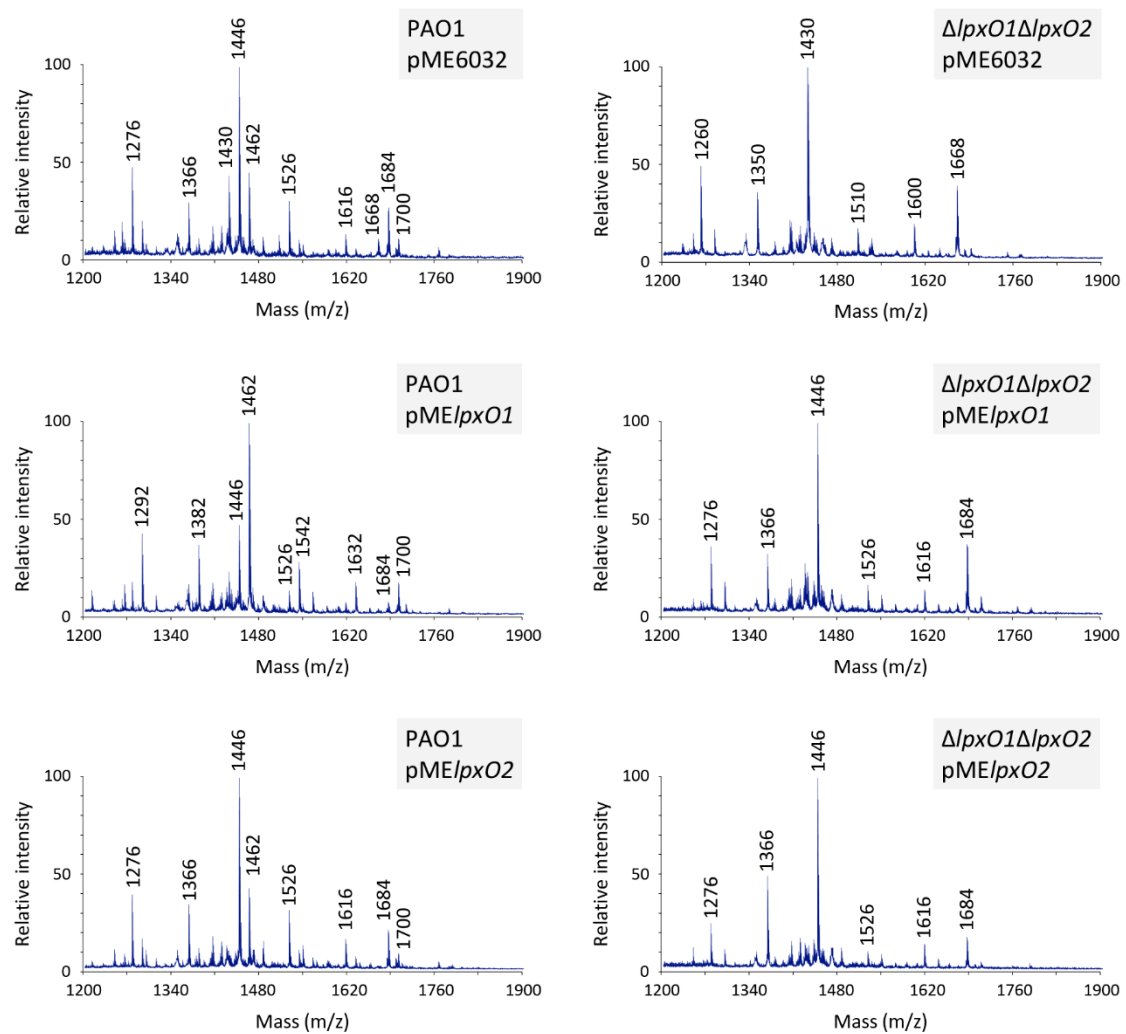
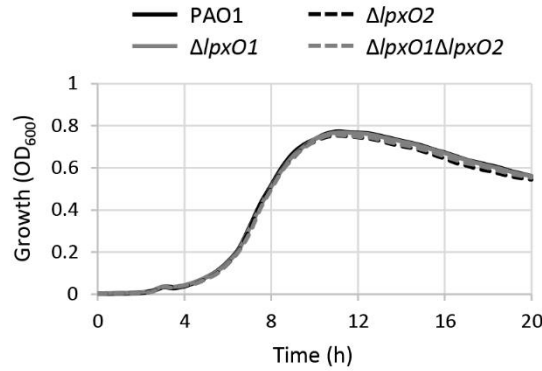


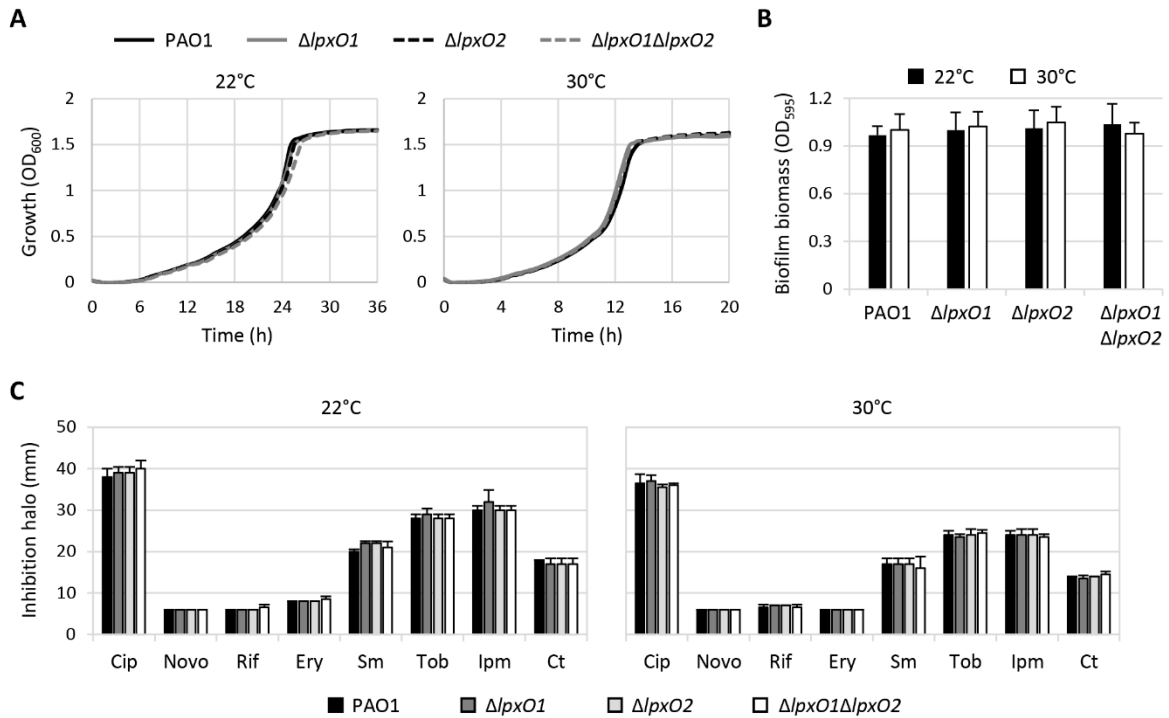
**Figure S1.** MALDI-TOF analysis of lipid A extracted from the *P. aeruginosa*  $\Delta lpxO1$  and  $\Delta lpxO2$  mutants carrying the complementing plasmid pME/lpxO1 or pME/lpxO2 or the empty vector pME6032 as control. Strains were cultured at 37°C in MH supplemented with 0.5 mM IPTG to induce *lpxO1* or *lpxO2* expression. Spectra are representative of two biological replicates.



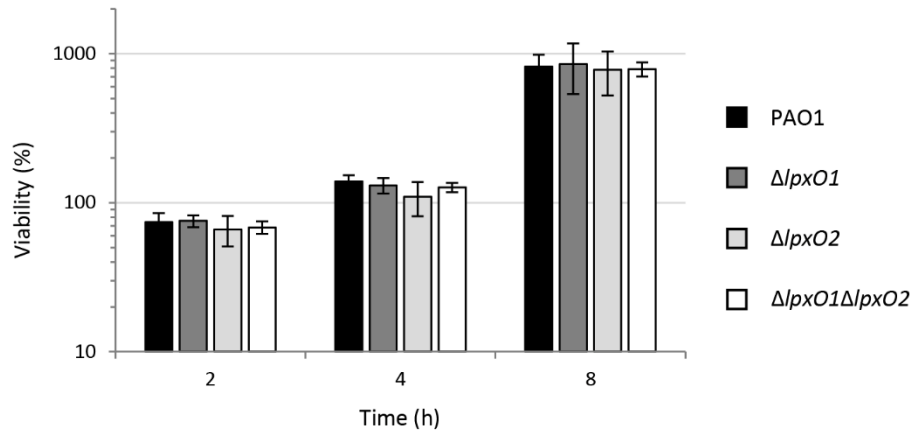
**Figure S2.** MALDI-TOF analysis of lipid A extracted from the wild type strain PAO1 and the  $\Delta lpxO1\Delta lpxO2$  double mutant carrying the complementing plasmid pME/lpxO1 or pME/lpxO2 or the empty vector pME6032 as control. Strains were cultured at 37°C in MH supplemented with 0.5 mM IPTG to overexpress *lpxO1* or *lpxO2*. Spectra are representative of two biological replicates.



**Figure S3.** Growth curves in minimal medium. Planktonic growth ( $OD_{600}$ ) of the wild type PAO1 and  $\Delta lpxO1$ ,  $\Delta lpxO2$  and  $\Delta lpxO1\Delta lpxO2$  deletion mutants at 37°C in M9 supplemented with 50  $\mu$ M  $FeCl_3$  and succinate as carbon source. Growth curves are representative of three independent experiments performed in triplicate.



**Figure S4.** Effect of lipid A hydroxylation on planktonic and biofilm growth and on the antibiotic sensitivity profile of *P. aeruginosa* at 22 and 30°C. (A) Planktonic growth ( $OD_{600}$ ) of the wild type PAO1 and *lpxO* mutants in MH at 22 or 30°C. Growth curves are representative of three independent experiments performed in triplicate. (B) Biofilm formation in 96-well polystyrene microtiter plates of PAO1 and *lpxO* mutants after 24 h at 22 or 30°C under static conditions. Values are the mean ( $\pm$ SD) from four biological replicates. (C) Antibiotic susceptibility of PAO1 and *lpxO* mutants at 22 or 30°C, determined by the Kirby-Bauer disk diffusion assay. Values are the mean ( $\pm$ SD) of three independent assays. Antibiotic abbreviations: Cip, ciprofloxacin; Novo, novobiocin; Rif, rifampicin; Ery, erythromycin; Sm, streptomycin; Tob, tobramycin; Ipm, imipenem; Ct, colistin.



**Figure S5.** Viability over time of the wild type PAO1 and  $\Delta lpxO1$ ,  $\Delta lpxO2$  and  $\Delta lpxO1\Delta lpxO2$  deletion mutants at 30°C in *G. mellonella* hemolymph *ex vivo*. Values are expressed as percentage with respect to time 0 (100%), and represent the mean ( $\pm$ SD) of three independent experiments. In each experiment, the hemolymph was collected from ten larvae and pooled.