



Supplementary Materials

Novel Fatty Acid Chain-Shortening by Fungal Peroxygenases Yielding 2C-Shorter Dicarboxylic Acids

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These Supplementary Materials include the GC-MS analyses of the reactions by rCciUPO with myristic, myristoleic, palmitic and palmitoleic acids (Figure S1); AaeUPO with stearic, oleic, linoleic and α -linolenic acids (Figure S2) and AaeUPO with myristic, myristoleic, palmitic and palmitoleic acids (Figure S3); and several doses of H₂O₂ (without enzyme) on the products of the reaction of rCciUPO with stearic acid (Figure S4).

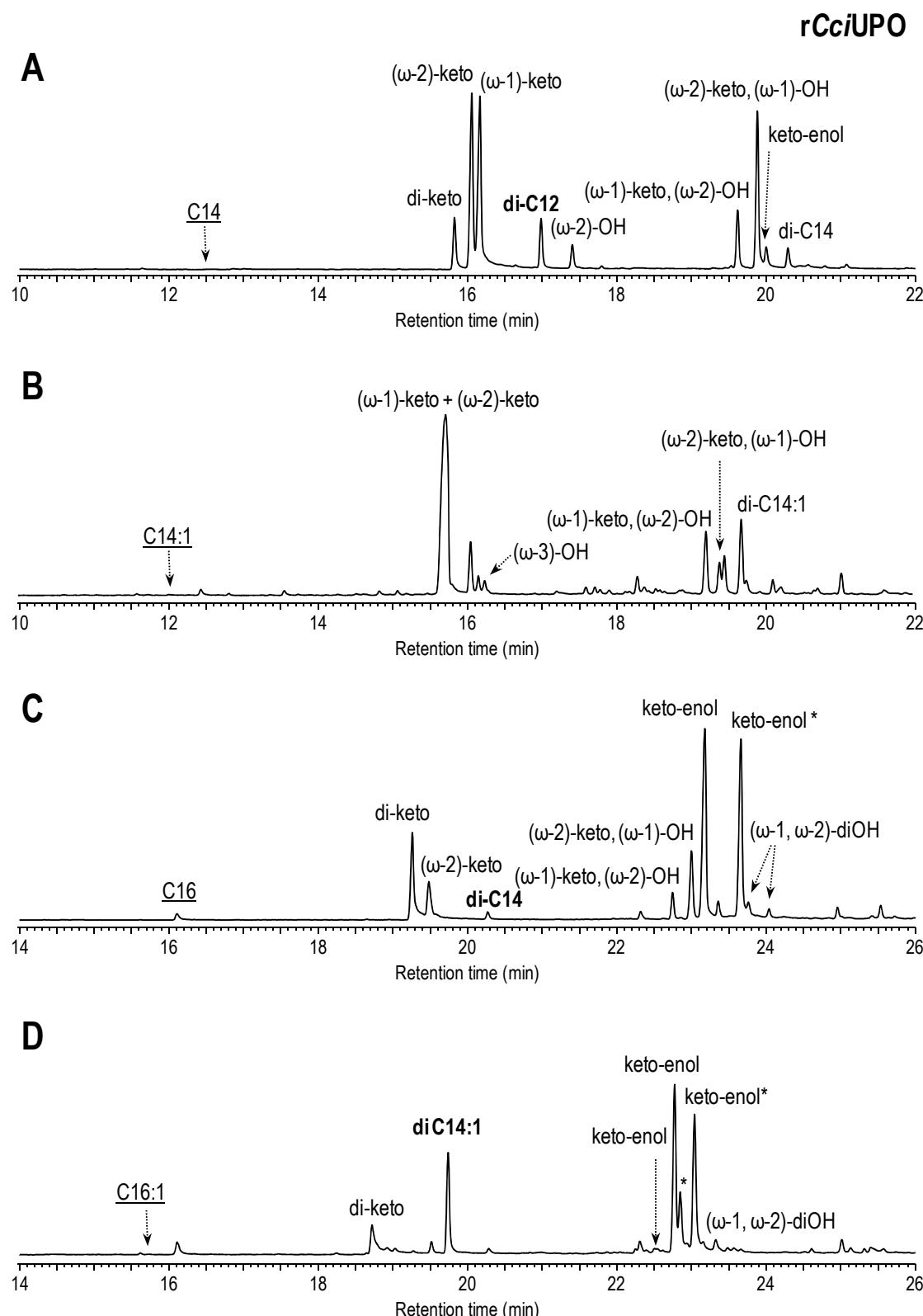


Figure S1. GC-MS analysis of rCciUPO reactions with myristic (**A**), myristoleic (**B**), palmitic (**C**) and palmitoleic (**D**) acids (underlined) showing the shortened dicarboxylic acids (in bold) together with the hydroxy, keto, and enol derivatives of the substrate. Reaction conditions: A (0.1 mM substrate, 0.2 μ M enzyme, 2.5 mM H₂O₂, 0.5 h, 20% acetone); B (0.5 mM substrate, 1 μ M enzyme, 20 mM H₂O₂, 24 h, 20% acetone); C (0.1 mM substrate, 0.8 μ M enzyme, 15 mM H₂O₂, 3 h, 40% acetone) and D (0.1 mM substrate, 0.8 μ M enzyme, 15 mM H₂O₂, 3 h, 20% acetone). Peaks with asterisk were tentatively identified as keto-enol isomers.

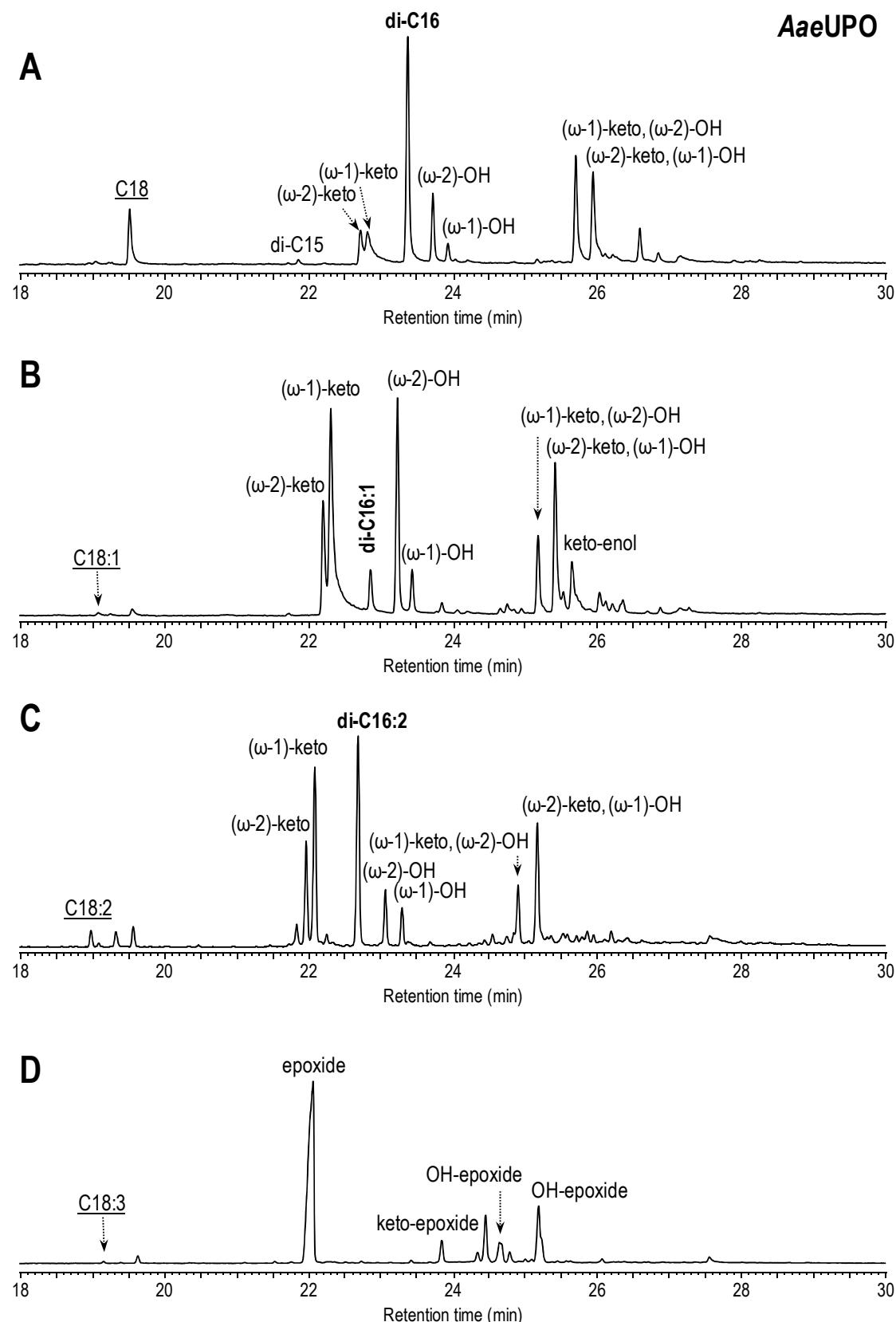


Figure S2. GC-MS analysis of *AaeUPO* reactions with 0.1 mM stearic (**A**), oleic (**B**), linoleic (**C**) and linolenic (**D**) acids (underlined) showing the dicarboxylic acids (in bold) together with the hydroxy, keto, enol and epoxide derivatives. Reaction conditions: A (1 μM enzyme, 20 mM H₂O₂, 25 h); B (0.4 μM enzyme, 5 mM H₂O₂, 1 h) and C, D (0.2 μM enzyme, 2.5 mM H₂O₂, 0.5 h).

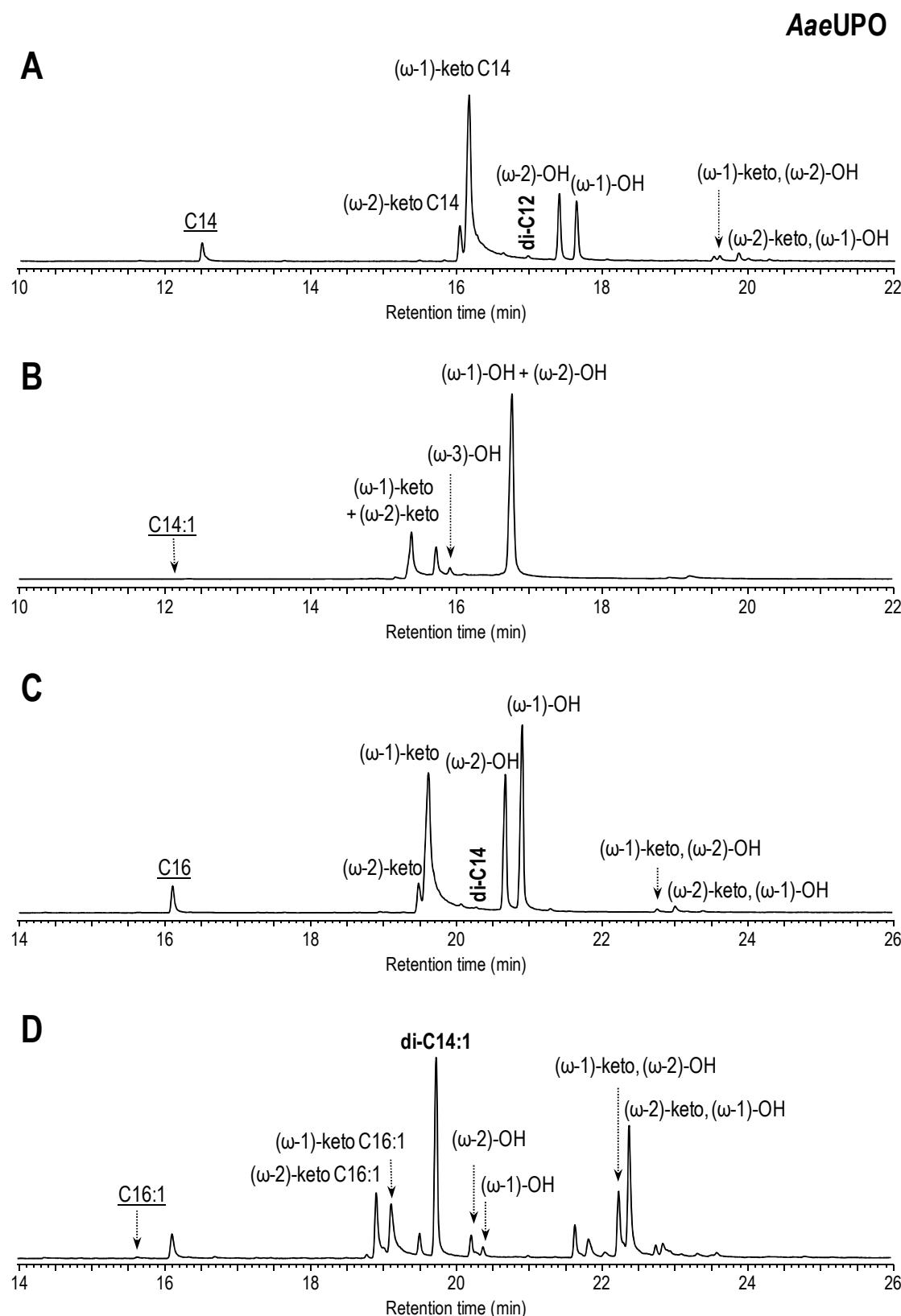


Figure S3. GC-MS analysis of *AaeUPO* reactions with myristic (A), myristoleic (B), palmitic (C) and palmitoleic (D) acids (underlined) showing the shortened dicarboxylic acids (in bold) together with the hydroxy, keto, and enol derivatives of the substrate. Reaction conditions: A (0.1 mM substrate, 0.4 μ M enzyme, 5 mM H₂O₂, 1 h, 20% acetone); B (0.1 mM substrate, 0.2 μ M enzyme, 2.5 mM H₂O₂, 0.5 h, 20% acetone); C (0.1 mM substrate, 0.8 μ M enzyme, 15 mM H₂O₂, 3 h, 40% acetone) and D (0.1 mM substrate, 0.8 μ M enzyme, 15 mM H₂O₂, 3 h, 20% acetone).

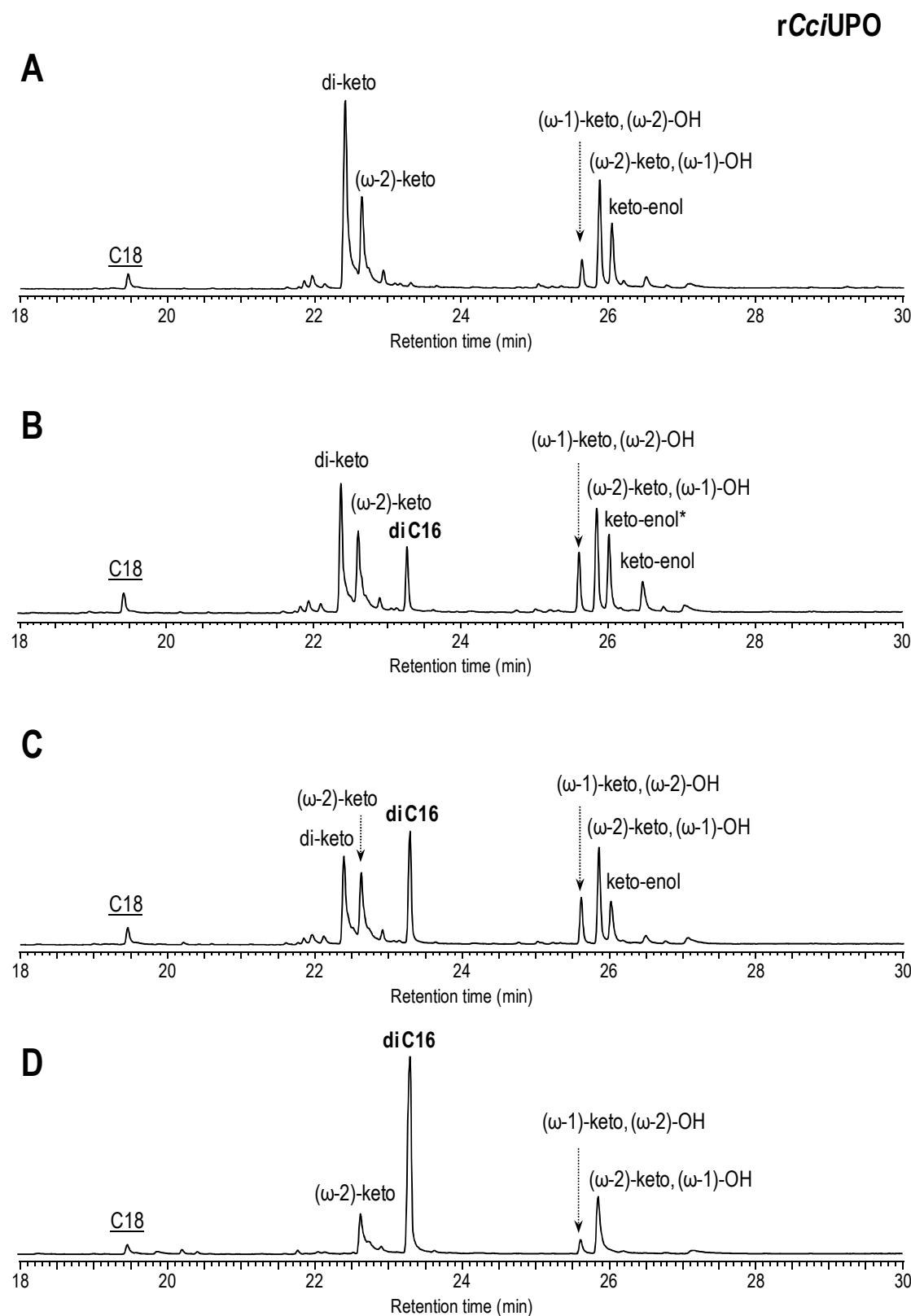


Figure S4. GC-MS of the reaction of $1.2 \mu\text{M}$ rCcIUPO with 0.1 mM stearic acid (underlined), after 3 h and 10 mM H_2O_2 (**A**). GC-MS of the products of the reaction A after addition of 1 mM (**B**), 3 mM (**C**) and 5 mM (**D**) H_2O_2 during 2 h in the absence of any enzyme. Higher doses of H_2O_2 (up to 15 mM) did not show differences respect to D. Solvent (acetone) was added in a 40% of the total volume in all cases. The 2C shorter dicarboxylic acid (in bold) is shown together with the hydroxy, keto and enol derivatives. Peak with asterisk was tentatively identified as keto-enol isomer.