Supplementary Data to

Two novel allene oxides and synthases of human and plant pathogenic fungi, *Coccidioides immitis* and *Zymoseptoria tritici*

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Oxidation of arachidonic acid by 8*R*-DOX-AOS (EAS28473) of *C. immitis*

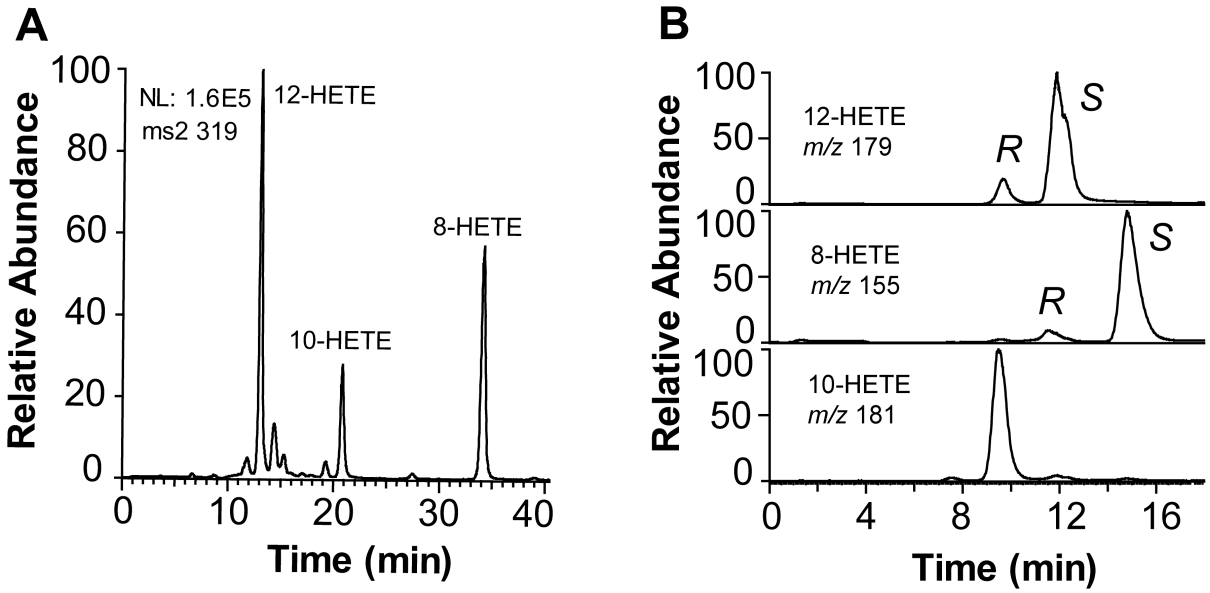

Fig. S1. LC-MS analysis of the oxidation of 20:4*n*-6 to HPETE by 8*R*-DOX-AOS (EAS28473). A. NP-HPLC-MS/MS analysis of HETE after reduction of HPETE to HETE with TPP. The MS/MS spectra of 8-, 10- and 12-HETE were as reported (1). B. CP-HPLC-MS/MS analysis of HETE. The elution order of the *R* and *S* stereoisomer of 8- and 12-HETE is marked (column: Reprosil Chiral-AM)(cf. Ref. (2)). NL, intensity normalized to 100%.

Catalytic properties of recombinant 9*R*-DOX-AOS (EGP87976) of *Z. tritici*

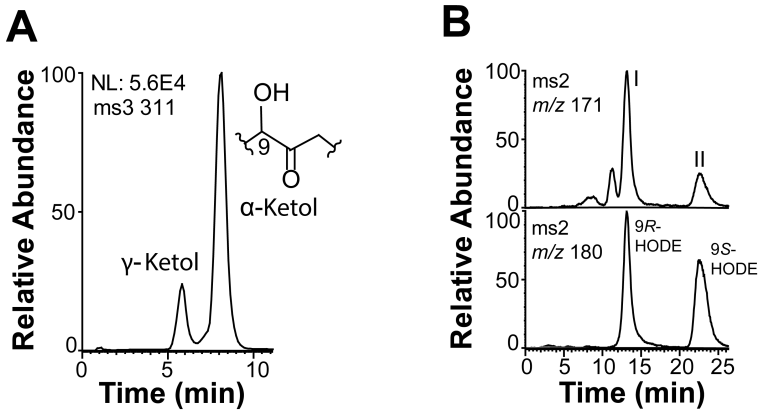

Fig. S2. LC-MS analysis of products formed from 9*R*-HPODE and 18:2*n*-6 by 9*R*-DOX-AOS (EGP87976). A. RP-HPLC-MS/MS analysis of polar products (γ-ketol; α-ketol) formed from 9*R*-HPODE by recombinant 9*R*-DOX-AOS (EGP87976). B. Steric analysis of 9-HPODE formed from 18:2*n*-6 by CP-HPLC-MS/MS analysis after reduction to an alcohol (Reprosil Chiral-AM). Top chromatogram shows analysis of 9-HODE (*m/z* 171) and the bottom chromatogram analysis of added rac [$^{13}C_{18}$]9-HODE (*m/z* 180). Part of 9*R*-HPODE was transformed by the AOS activity, but it remained the main stereoisomer.

Transformation of hydroperoxides by 8*R*- and 8*S*-DOX-AOS

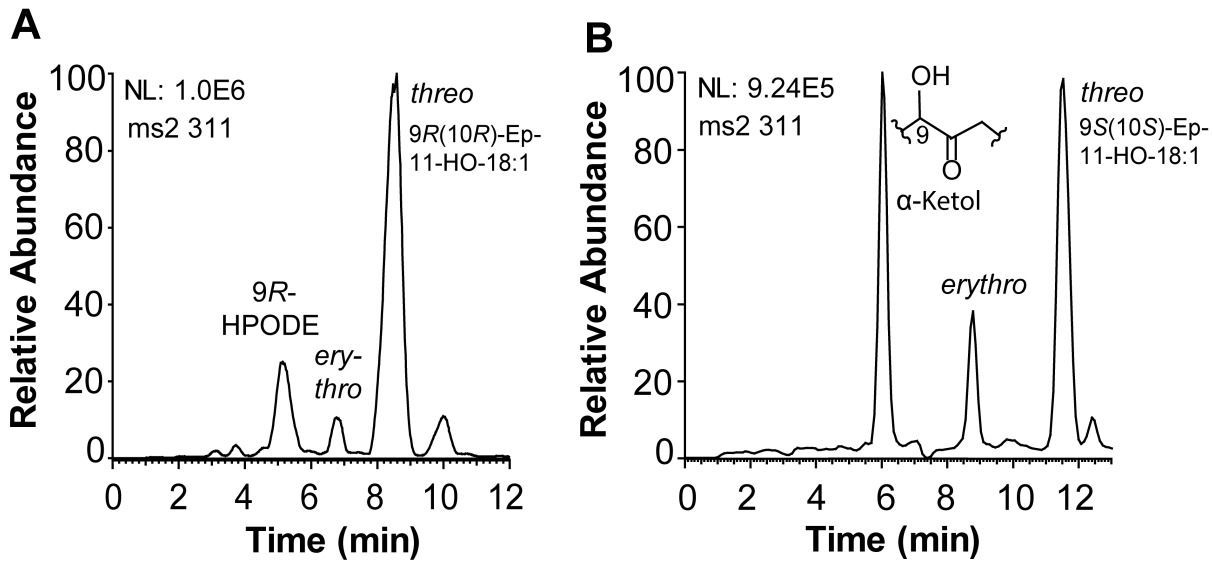

Fig. S3. NP-HPLC-MS/MS analysis of the products formed by transformation of 9*R*- and 9*S*-HPODE by recombinant 8*R*- and 8*S*-DOX-AOS. A. Products formed from 9*R*-HPODE by 8*R*-DOX-AOS (EAS28473) consisted mainly of *threo* 9*R*(10*R*)-epoxy-11-hydroxy-12*Z*-octadecenoic acid. B. 9*S*-HPODE was transformed by 8*S*-DOX-AOS (EGP83657) to an α-ketol and to epoxy alcohols (*erythro* and *threo* 9*S*(10*S*)-epoxy-11-hydroxy-12*Z*-octadecenoic acids (cf. Ref. (3)).

References

1. Bylund, J., Ericsson, J., and Oliw, E. H. (1998) Analysis of cytochrome P450 metabolites of arachidonic and linoleic acids by liquid chromatography-mass spectrometry with ion trap MS. *Anal. Biochem.* **265**, 55-68
2. Schneider, C., Yu, Z., Boeglin, W. E., Zheng, Y., and Brash, A. R. (2007) Enantiomeric separation of hydroxy and hydroperoxy eicosanoids by chiral column chromatography. *Methods Enzymol.* **433**, 145-157
3. Oliw, E. H., Garscha, U., Nilsson, T., and Cristea, M. (2006) Payne rearrangement during analysis of epoxyalcohols of linoleic and alpha-linolenic acids by normal phase liquid chromatography with tandem mass spectrometry. *Anal. Biochem.* **354**, 111-126