

Supplemental Material

to

Discovery of a Linoleate 9S-Dioxygenase and an Allene Oxide Synthase in a Fusion Protein of *Fusarium oxysporum*

by

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Table S1. Oligonucleotides used for site-directed mutagenesis of FOXB_01332.

Oligonucleotides used for site-directed mutagenesis of 9S-DOX-AOS		
Replacement	5'- Forward primer	5'- Reverse primer
F416W	gagttcaatcttctaccgctggcactcttgcactccaagc	gcttgagatgcaagagtgccagcggtagagaagattgaactc
E946V	gttctgtcactgctttctactgtggtctgtcttcttcttc	gaaggaagaacgacagaaccacgtagaaagcagtgacaggaac
S949A	ctgctttctacgaggttctgcttcttcttctgctcggag	ctccggacgaaggaagaacgccagaacctgtagaaagcag
N921V	gttgcttgcgcagggaatgctggtgatcaggtcactgataac	gttatcagtgacctgatcaacgacattccctgctgcaagcaac

Fig. S1.

ATEG_02036	1	MSSVIVALAVLVLSLLYLTLFRNDLTHLIIIEKLQSFRTGSGWELSPRSRLPRATKAALS
FOXB_01332	1	-----
ATEG_02036	61	SITGTGVGIWSRLYARIFHSDELAEEEDDEKYQAGEAYGDPKVIATSLIKDLRAIGVKGR
FOXB_01332	1	-----MSFNEKFQACEISYGDSEKEDPSSLNNPEKIVADLMKDFAGVRSQAS
ATEG_02036	121	RSDLRRTLEEMKKNKGKPMDDQMHMEKIIATVAMLPRTSKARQRLTGVLIDQLWRSLOHP
FOXB_01332	47	PAQLLGLVKELEKQGPDDKKGTTPELLIGIITALPATSKARTALTINKLIDTLWGNLOHP
ATEG_02036	181	PLSYFG-----NKYQYRTPD
FOXB_01332	107	PLSYMGGDVKYDVVNSDKPAHKHNCELYDTIEFKVPGTDVLLREQVPQAPDGLHQYRMPD
ATEG_02036	196	GSYNNPLEPNLGRAGSPYARSIPRIKTMHGVRPDPGLLFDLLMARDSTFKENPAGISSM
FOXB_01332	167	GSFNNILEPNLGRAGIPYARSVKSEKRLHGVRPDPGLLFDLLMARDETTFOENPAGISSM
		HD
ATEG_02036	256	LFYHASIIIHDI FR TNRRDPNLSDTSSYL DLAPLYGSSLEDQLKVRTMEKGLKLPDTFHE
FOXB_01332	227	LFYHAAIIIHDI FR TNRTDMNKSDTSSYL DLAPLYGSSLEKQHEVRTMEKGLKLPDTFHE
ATEG_02036	316	KRLLGQPAGVNVILVMYSRFHNYVADMLLKINENGRFTL--PPTSSEEARKKALAKQDED
FOXB_01332	287	KRLLGQPAGVNVILVLYSRFHNYVADMLLKINENGRFSLSVPPNA SEEDKAKALAKQDHD
ATEG_02036	374	LFQVARLVNGLYVNISLHDYLRGLTNTTHSASDWTLDPRIA VGRITFDPDGVPRGIGNQI
FOXB_01332	347	LFNVARLVTCGLYINICLHDYLRATNTTHSASDWTLDPRIAIDKQFDGQGVPRGIGNQV

YRFH

ATEG_02036 434 SAEFNLLYRFHVSISRDEKWTNEFLKSLFP-DLNKPLDQLTPQEFMMGLRVEQSIDKD
 FOXB_01332 407 SVEFNLLYRFHSCISKRDEKWTNEFLKLFPCRKAEDLQDVSWTELGOALLIEQNTPKD

F

ATEG_02036 493 PSKREFGGLKRSFDGKFNDA DLVQILKDSMEDPAGLFGPRNVPKALRMIEIAGIWSARKW
 FOXB_01332 467 PSVRITFDGLERQADGTFKDEDLVRILKDAMEDPAGTFGARVMPKALKVVEVLGI IQGRKW

ATEG_02036 553 DLGSLNEMRDFFKLKRHATFEDINPDPEIADLIRKLYDHPDMVEMYPGMFLEDAKPRLDP
 FOXB_01332 527 QCASLNEMRDFFGGLKRYDSFSEINSNPDIANLEKLYTDDPMVEIYPGLMIEDIKPQRNP

ATEG_02036 613 CGGGCPPTVGRAVFSDAVTLVRSDFRLTIDYTA SNLTNWGTRREVQODYDILGGSMFHKL
 FOXB_01332 587 GSGIMPTY SVGRAVLSDAVTLVRSDFRNTIDYTVSNLTAWGNREVQODYKTGGSMLYKL

ATEG_02036 673 IQRAIPGWFPNNSLHATQPMFTKMKNEQIAREIGTIDHYSIADPAPPPRKIVLTDYATNI
 FOXB_01332 647 IQRGVPNWFPNNSIAVMQPMFTKKANEQIAREIGTFDQYILDDPKAPPKVAVLTSGPAIK

ATEG_02036 733 KVLKDQASRVPWARYLNDVFPG-KTYNDMLCGDDPANAAQKLVHSILFSPDQFLDLL
 FOXB_01332 707 QILSNTKQVVPWLKPLNTEFPGKKDFGWEMLAGDQFQNYTHANFSKAMSKIPNMHNAV

ATEG_02036 792 SETTTKLGSELKANTLWLTKDLHQVDIIRDVAIPLNARIADLFLCDMKTPENFTGSMN
 FOXB_01332 767 HAFIEREGKLINKETFTLKKGLDQIDIIRDVAIPLNTQLIADLFYFDLRTENEDGKLG

ATEG_02036 852 AATVYRHLNVRIWGFNNNDPALMLQRRKWAIESAEALIEETTKIVNEQAQPAQSCVILKN
 FOXB_01332 827 VAELYRSLDRIWGVNNNDPAQAWNRRRRAQEGAKRMIETTKTIVAEADAGRPRCIGLV

N

ATEG_02036 912 LMTRRQATG-----TLRWYGNNAKEMMEMGMSAEVADICWLTAIGGVGTFSGVVAN
 FOXB_01332 887 SAVANRIGRSYLKKDSLRSCLKIVEELLAQGNNDQVTDNLWLTAFGGIGVETVAFYE

NVDQ **E**

VLQ **EAxR**

ATEG_02036 965 VLQYYFRYENLCHWEETQKVTQPDTPAADRTLRQYVLEARNLTSMECTVRVCAREVTVVD
 FOXB_01332 947 VLSFFLRPENELIWAEVQAIAQKGD---DAILHAYVAEAQRNLTSSQRNVRVATAPGEVQ

VLS

YGxHECxxKxxAxxF

ATEG_02036 1025 GHDFKPGEVIVNHLGLACRDPENIPDADKFRLLDRPASAYIQVGYGAHECLGKETAITFAV
 FOXB_01332 1003 GQAIQPGTAVVLMLEAGRNPKVEVPDAGKFNPPQRKKEDVSAFSGQHECIAKDVAFVFT

ATEG_02036 1085 SMIRILAGLKYLRPAPGEMGVLSVSMADGRQAFNLNDSWSNLTDPTSKSNMHGKASAVD-
 FOXB_01332 1063 GILKLVADLKELRPAPGQMGTVKTIQVGTEKAFNLNDSWSYLGFDASTWKVHFNGHGKGF

ATEG_02036 -----
 FOXB_01332 1123 EGERVPTKSTPIQEYYYYLLQKRKDEILGN

Fig. S1. Alignment of ATEG_02036 and FOXB_01332 by ClustalW algorithm, marked by Boxshade. Some conserved elements are marked in red above the alignment. The positions which were subject to site-directed mutagenesis are marked in blue letters below the alignment.

Fig. S2.

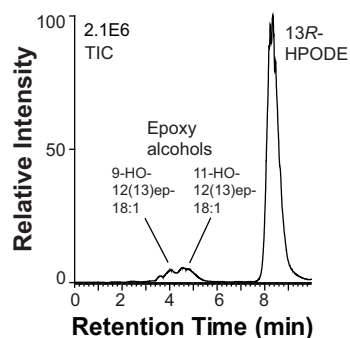


Fig. S2. RP-HPLC-MS/MS analysis of products formed by incubation of 13R-HPODE with 9S-DOX-AOS. The main products are two epoxyalcohols, which were formed in equal amounts as judged from the ion intensities of m/z 193 (9-hydroxy-12(13)epoxy-(10E)-octadecenoic acid) and m/z 197 (11-hydroxy-12(13)epoxy-(9Z)-octadecenoic acid) in the MS/MS analysis (m/z 311 \rightarrow full scan).

Fig. S3.

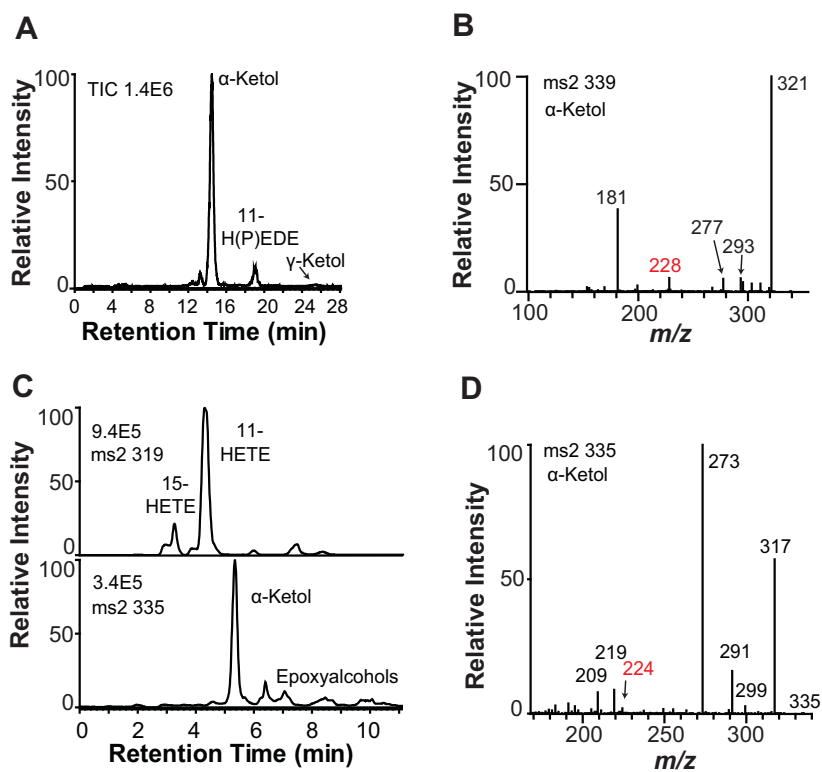


Fig. S3. Oxidation of 20:2n-6 and 20:4n-6 by 9S-DOX-AOS. A, NP-HPLC-MS/MS analysis of products formed from 20:2n-6. B, MS/MS spectrum of the α -ketol formed from 20:2n-6. C, NP-HPLC-MS/MS analysis of products formed from 20:4n-6. D, MS/MS spectrum of the α -ketol formed from 20:4n-6.