SUPPLEMENTAL FIGURES

SUPPLEMENTAL FIGURE 1. **Partial alignment of the two catalytic domains from PpoA.** A. Partial alignment of heme peroxidase domain from PpoA with *G. graminis* 7,8-LDS **(**Q9UUS2.1), sheep PGHS-1 (P05979.2) and sheep PGHS-2 **(**P79208.1). The catalytic tyrosine as well as the proximal and distal histidines, which are conserved in the four sequences and have been identified before (54), are shaded black. Mutated amino acids are labeled with an arrow. B. Partial alignment of P450 domain from PpoA with known P450 enzymes [CYP450_119 (1F4T:B), CYP450_cin (1T2B:B), CYP450_130 (2UUQ:A), CYP450_nor (1JFB:A), CYP4503A4 (1TQN:A), CYP4502C9 (1R9O:A)] and predicted CYP450 domains from different putative fungal Ppo-like enzymes [*A. niger* 1 (XP_001395220), *A. niger* 2 (XP_001401954) *A. fumigatus* 1 (EDP50447), *A. fumigatus* 2 (ABV21632.1), *A. fumigatus* 3 (EDP47075.1), *F. graminearum* 1 (FGSG_02668)[A], *F. graminearum* 2 (FGSG_10960)[A], *F. oxysporum* 1 (FOXG_12909)[A]]. Amino acids matching the P450 consensus sequence according to the PROSITE database (http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00081) are shaded grey. Additionally, the cysteine responsible for the ligation of heme iron is shaded black. The mutated histidine and cysteine are labeled with an arrow. The alignment was carried out using CLUSTALW.
[A]Annotation code in accordance to the *Fusarium* database: (http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/)

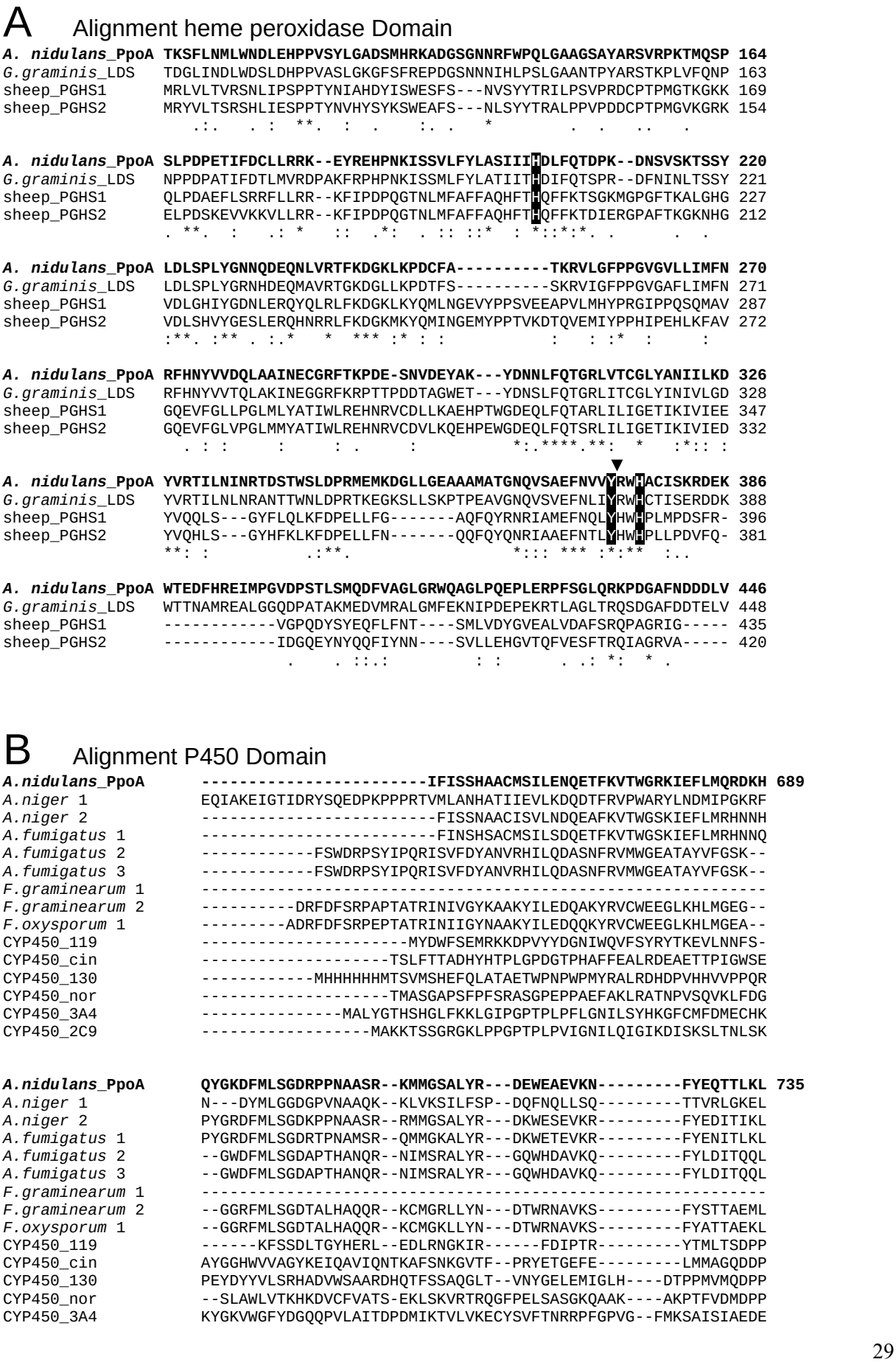
SUPPLEMENTAL FIGURE 2. **Mass spectrometric analysis of products formed by PpoA from 18:2$^{\Delta 9Z,12Z}$.** A) Electron impact mass spectrum of the Me_3Si ether methyl ester derivative of product 2 (5,8-DiHODE). B) Electron impact mass spectrum of the Me_3Si ether methyl ester derivative of product 1.

SUPPLEMENTAL FIGURE 3. **Kinetic analysis of PpoA.** A) Michaelis Menten plot, the initial reaction rate of O_2-consumption for the reaction of 15 µg PpoA with different linoleic acid concentrations is plotted in dependence of the substrate concentration. Reactions were performed in 50 mM phosphate buffer (pH = 7.2). B) The method of Hanes and Woolf was used for the linearization of the Michaelis Menten plot. Kinetic parameters were obtained using this method.

SUPPLEMENTAL FIGURE 4. **Peroxidase activity assay.** 15 µg PpoA were incubated with 100 µM 18:2$^{\Delta 9Z,12Z}$ and 100 µM N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) in 50 mM HEPES pH = 7.4 and the increase of the absorbance at 611 nm (due to oxidized TMPD) was recorded.

SUPPLEMENTAL FIGURE 5. **Kinetic analysis of PpoA_H1004A.** A) Michaelis Menten plot, the initial reaction rate of O_2-consumption for the reaction of 15 µg PpoA_H1004A with different linoleic acid concentrations is plotted in dependence of the substrate concentration. Reactions were performed in 50 mM phosphate buffer (pH = 7.2). B) The method of Hanes and Woolf was used for the linearization of the Michaelis Menten plot. Kinetic parameters were obtained using this method.

Supplemental Figure 1



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A.niger 1           LELNALQLSKDLNQVD--IIRDVAIPLNARIMADLFC-LDMKTPENESGSMNAATVYKHL
A.niger 2           LRQHSYQLGG-VNQVD--IVRDVANYAQVHFCANVFS-LPLKTESNPRGIFAESELYEIL
A.fumigatus 1       LHRYSYKLAG-VNQVD--VVRDIANLAQVHFCASVFS-LPLKTESNPRGIFTESELYQIM
A.fumigatus 2       LTEKSCRIGN-VNQVD--ISRDVGNLAHVHFASNVFS-LPLKSREHPHGIITAHEMFEAM
A.fumigatus 3       LTEKSCRIGN-VNQVD--ISRDVGNLAHVHFASNVFS-LPLKSREHPHGIITAHEMFEAM
F.graminearum 1     ------------DQID--IIRDVAIPLNAQLLSDMFY-FDMRTDENPDGELGAAELYRSL
F.graminearum 2     LNEKSYTLAG-KKQVD--VVRDVGNVAHTHFVARMFN-LPLKTKQNPKGVFSEQELYMIL
F.oxysporum 1       LAEKSYRLAG-KMQVD--VVRDVGNVAHTHFVARMFN-LPLKTSENPKGVFSEQELYMIL
CYP450_119          LHDELRSMSADIFSPQ--KLQTLETFIRETTRSLLDS-IDPREDD------IVKKLAVPL
CYP450_cin          VHKKYRQLVAKPFSPE--ATDLFTEQLRQSTNDLIDARIELGEGD------AATWLANEI
CYP450_ 130         VHTEFRKLVSRGFTPR--QVETVEPTVRKFVVERLEKLRANGGGD------IVTELFKPL
CYP450_nor          EHMHQRSMVEPTFTPE--AVKNLQPYIQRTVDDLLEQMKQKGCANGP--VDLVKEFALPV
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A.fumigatus 1       AVVFTSIFYDADIGKSFELNQAARAVTQQLG----QLTLANVELIA--------------
A.fumigatus 2       AVIFTAIFFDAEPVKSFELRHKAREAANKLG----RLVELNVKAIK--------------
A.fumigatus 3       AVIFTAIFFDAEPVKSFELRHKAREAANKLG----RLVELNVKAIK--------------
F.graminearum 1     LDIRVWGVNNNDPGQAWNRRRRAQEGVKRIY----DSTKKLVSEVEVARPRG--------
F.graminearum 2     AVIFVCIFFDIDPAKSFPLRQAAREVAQQLG----KIVEMNVKLAT--------------
F.oxysporum 1       AVIFVCIFFDIDPAKSFPLRQGAREVAQKLG----GIIEMNVKLAN--------------
CYP450_119          PIIVISKILGLPIEDKEKFKEWSDLVAFRLG----KPGEIFELGKK--------------
CYP450_cin          PARLTAILLGLPPEDGDTYRRWVWAITHVENP--EEGAEIFAELVAH-------------
CYP450_130          PSMVVAHYLGVPEEDWTQFDGWTQAIVAANAV--DGATTGALDAVG--------------
CYP450_nor          PSYIIYTLLGVPFNDLEYLTQQNAIRTNGSST--AREASAANQELLD-------------
CYP450_3A4          MDVITSTSFGVNIDSLNNPQDPFVENTKKLLR--FDFLDPFFLSITVFPFLIPILEVLNI
CYP450_2C9          CSIIFHKRFDYKDQQFLNLMEKLNENIKILSSPWIQICNNFSPIIDYFPGTHN-----KL
                              .

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A.niger 2           -------KAGFIANLVSSLHR-HDVLSEYGEHMIQRLLHS----------NVPPAEIVWT
A.fumigatus 1       -------KTGFIANLVNSLHR-HDVLSEYGVHMIQRLLDS----------GMPAPEIVWT
A.fumigatus 2       -------SSGLIATLLGNMPANRNALFEYGVHMVERLLQS----------GLDPEQVTWS
A.fumigatus 3       -------SSGLIATLLGNMPANRNALFEYGVHMVERLLQS----------GLDPEQVTWS
F.graminearum 1     ----IGLISAVSNRIGAKSYLKKDSLRSCGLKLVEELLAQ----------GSSIDHVTDN
F.graminearum 2     -------SVGIKGLFTSKPNKNDDPLAAYGENMAKGLKKA----------GLSIDDIVWS
F.oxysporum 1       -------SIGVKGLFTSKPDKNDDPLARYGENMAKGLKKA----------GLSTEDIVWS
CYP450_119          -------YLELIGYVKDHLNS--------GTEVVSRVVNS----------NLSDIEKLGY
CYP450cin           -------ARTLIAERRTNPGN-----DIMSRVIMSKIDGE----------SLSEDDLIGF
CYP450_130          -----SMMAYFTGLIERRRTEPADDAISHLVAAGVGADGD----------TAGTLSILAF
CYP450_nor          ---------YLAILVEQRLVEPKD--DIISKLCTEQVKPG----------NIDKSDAVQI
CYP450_3A4          CVFPREVTNFLRKSVKRMKESRLEDTQKHRVDFLQLMIDSQNSKETESHKALSDLELVAQ
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A.niger 2           HLLPTAGGMVANQAQLFSQCLDYYLSDEGSIHLPDIKRLAKENT-------SEADALLLR
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A.fumigatus 2       QVLPTAVAMVPNQAQVFTQIIDYYLSDKGRKHLPDIKRFAKEDS-------PASDEVLLR
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F.oxysporum 1       QILPTAGAMVPNQAQVFAQTLDWYLSPAGEKYRPELARIAALETG------DETDALLLG
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CYP450_130          TFTMVTGGNDTVTGMLGGSMPLLHRRPDQRRLLLDDP-----------------EGIPD
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A.niger 1           YVLEAQRLTS--MECTVRVCKAHRTIN-----DQEFKPGDVVITLLGPACRDPTSI--PD
A.niger 2           YFMEGARLRS--SVGLPRLVAKPTVVD-DGGSKYTLKPGQSVLCNLVSASMDPRSF--PE
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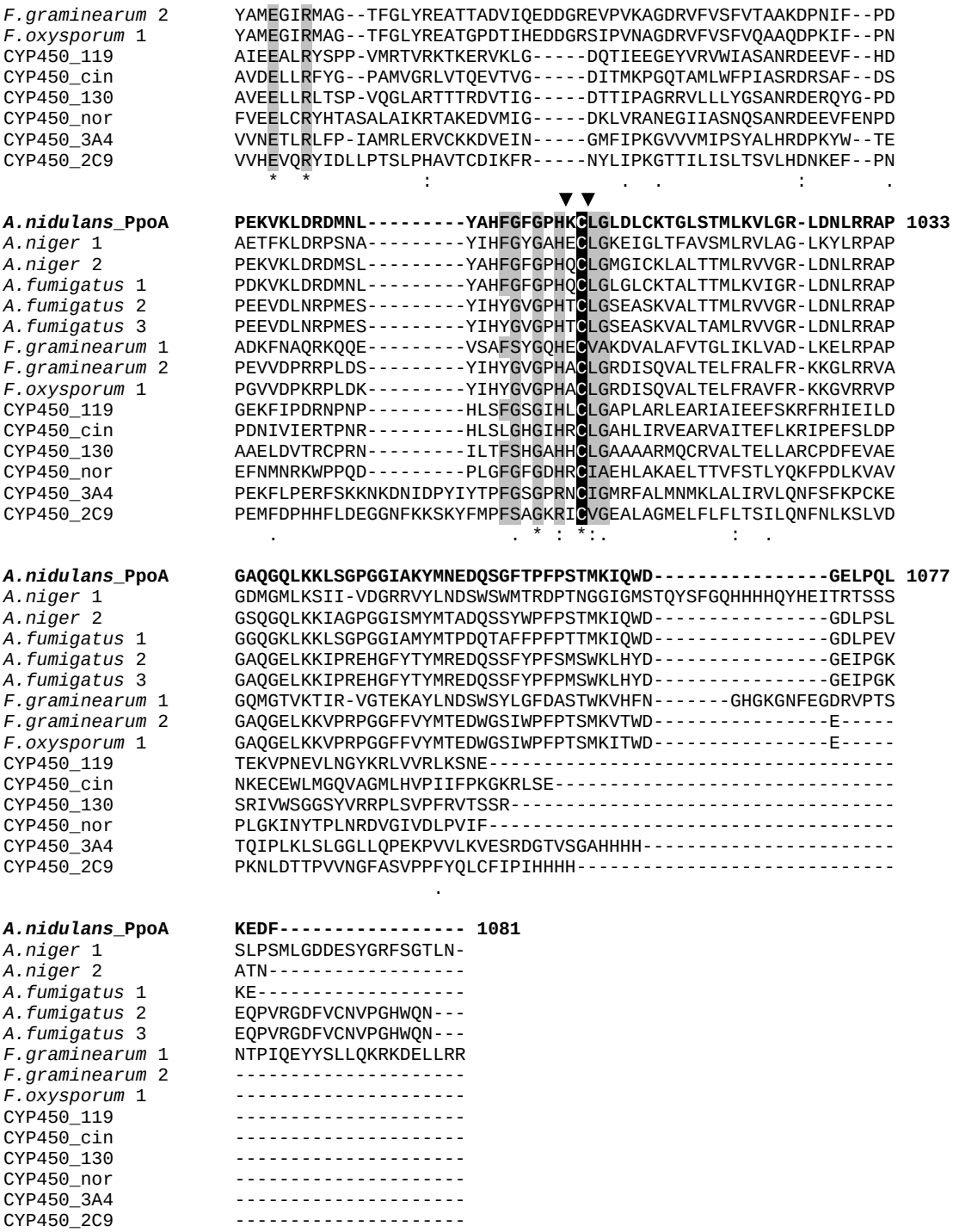
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F.graminearum 1 NTPIQEYYSLLQKRKDELLRR
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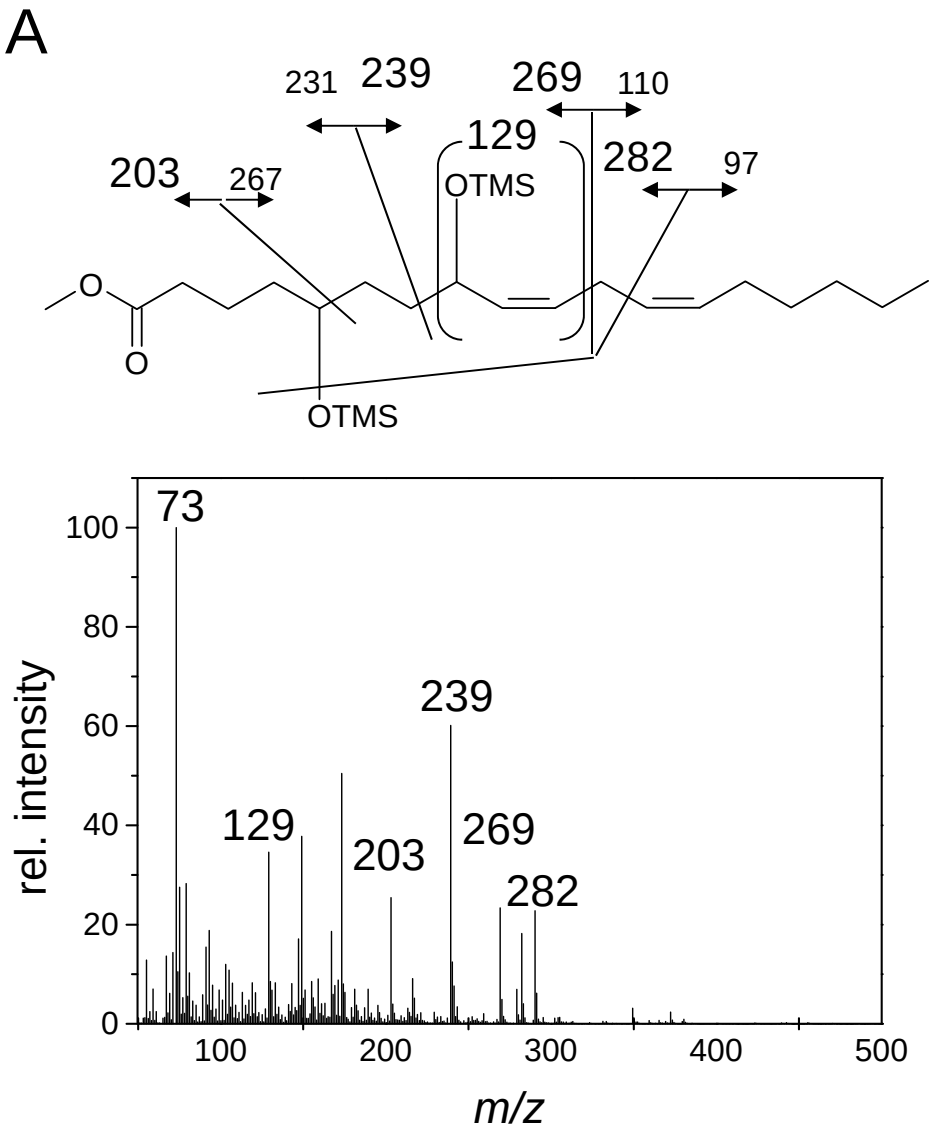
Supplemental Figure 2



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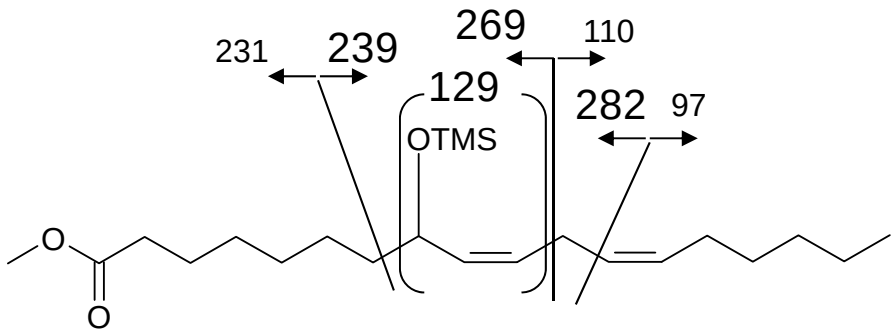
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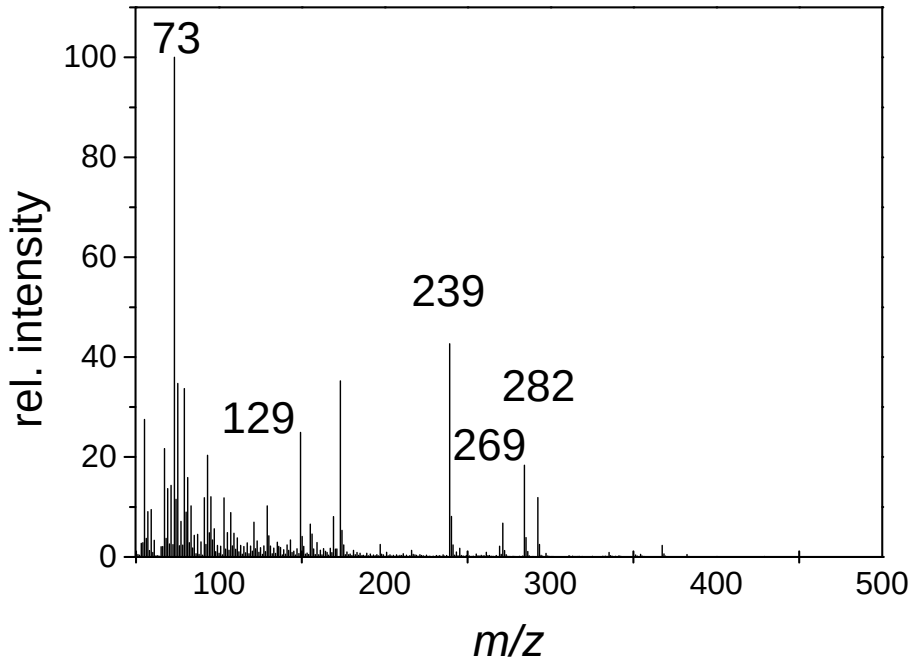
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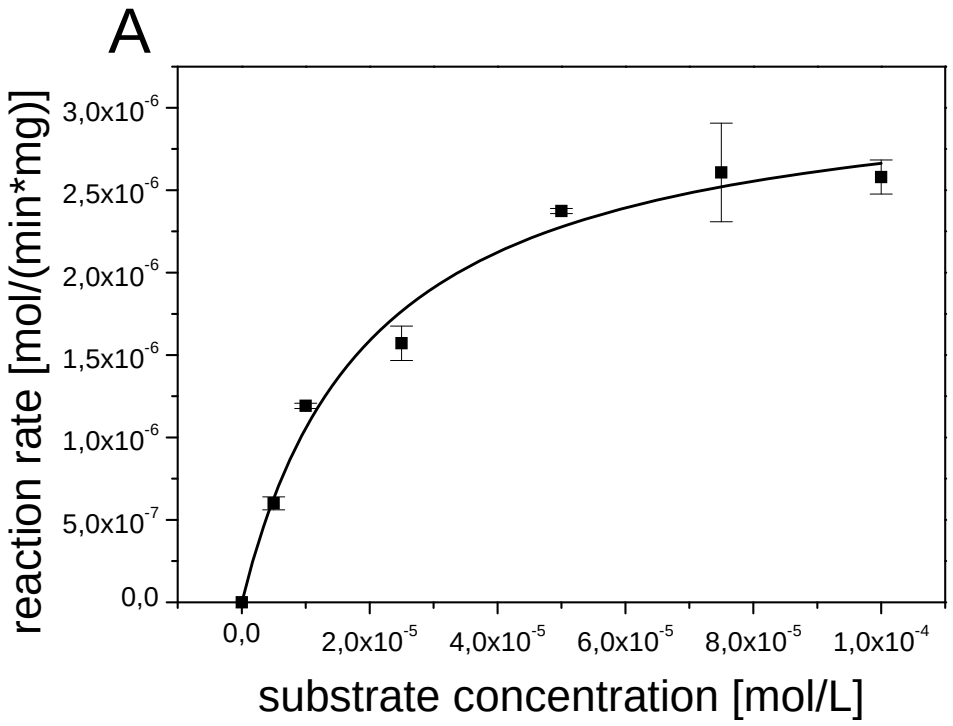
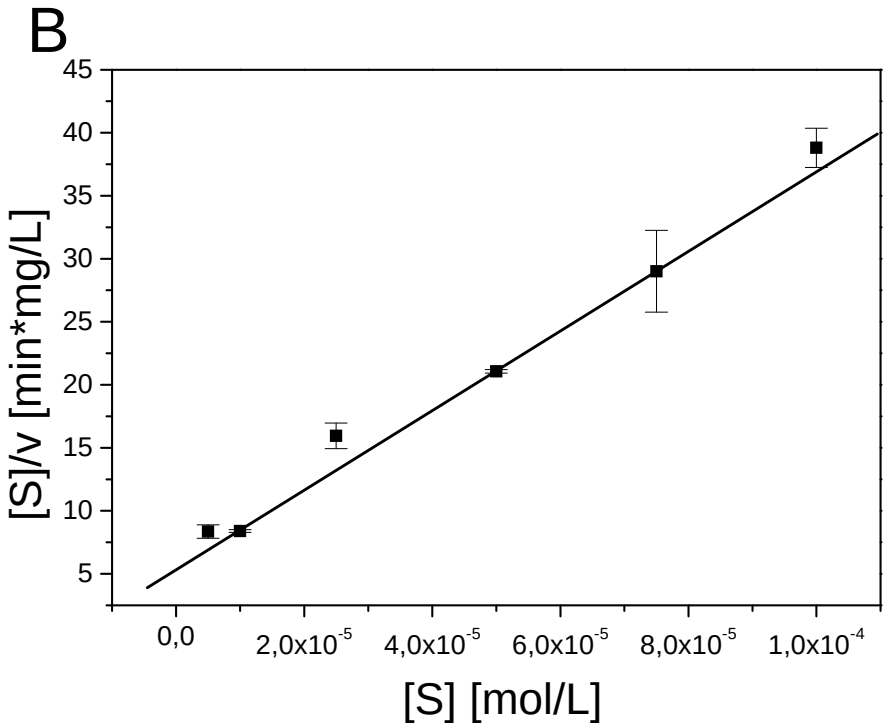
Supplemental Figure 3





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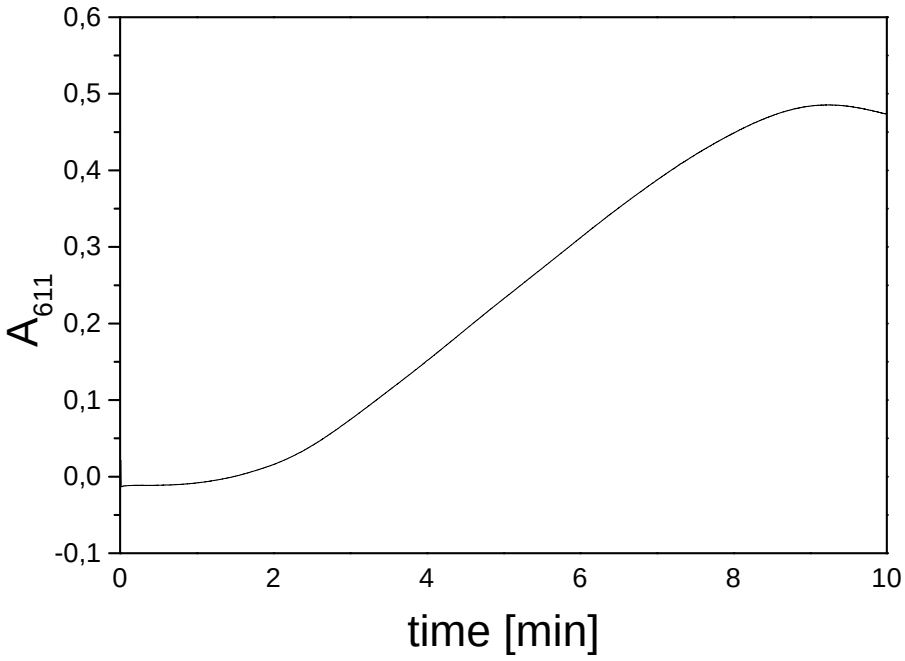

Supplemental Figure 5