Supplementary Information

Biocatalytic synthesis of non-vicinal aliphatic diols

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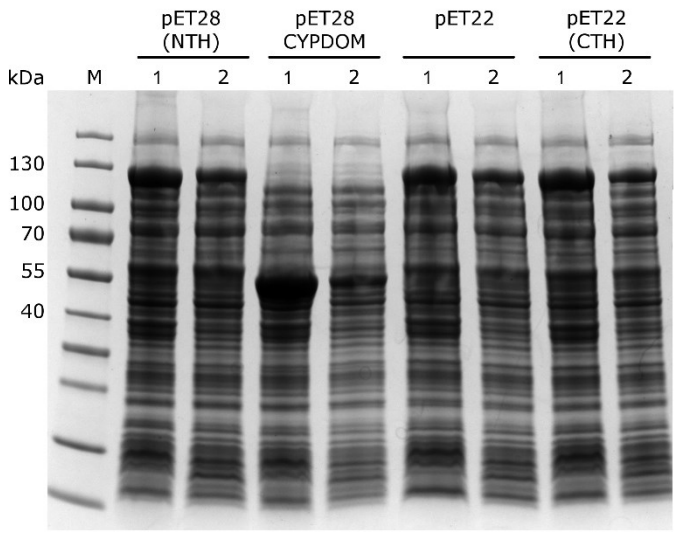


Figure S1. SDS-PAGE analysis of heterologous expression of CYP505A30 constructs. Lane M: molecular weight marker, lanes 1: total protein fraction, lanes 2: soluble protein fractions.

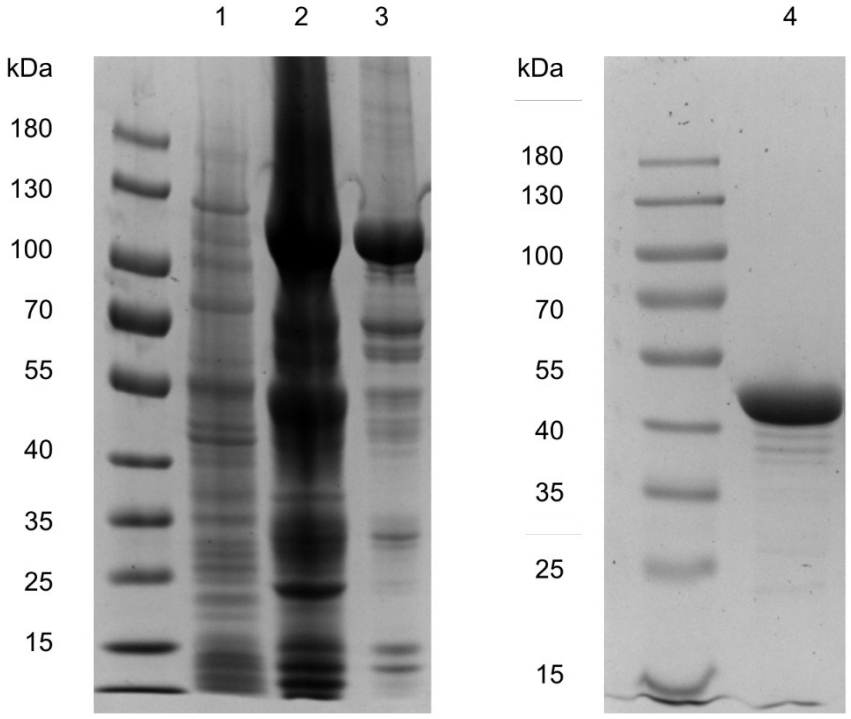


Figure S2. SDS-PAGE analysis of purified CYP505A30 and CYP505A30HD. Lane 1: crude extract containing CYP505A30, lane 2: pooled IMAC fractions of CYP505A30, lane 3: purified CYP505A30 after anion exchange chromatography, lane 4: purified heme domain.

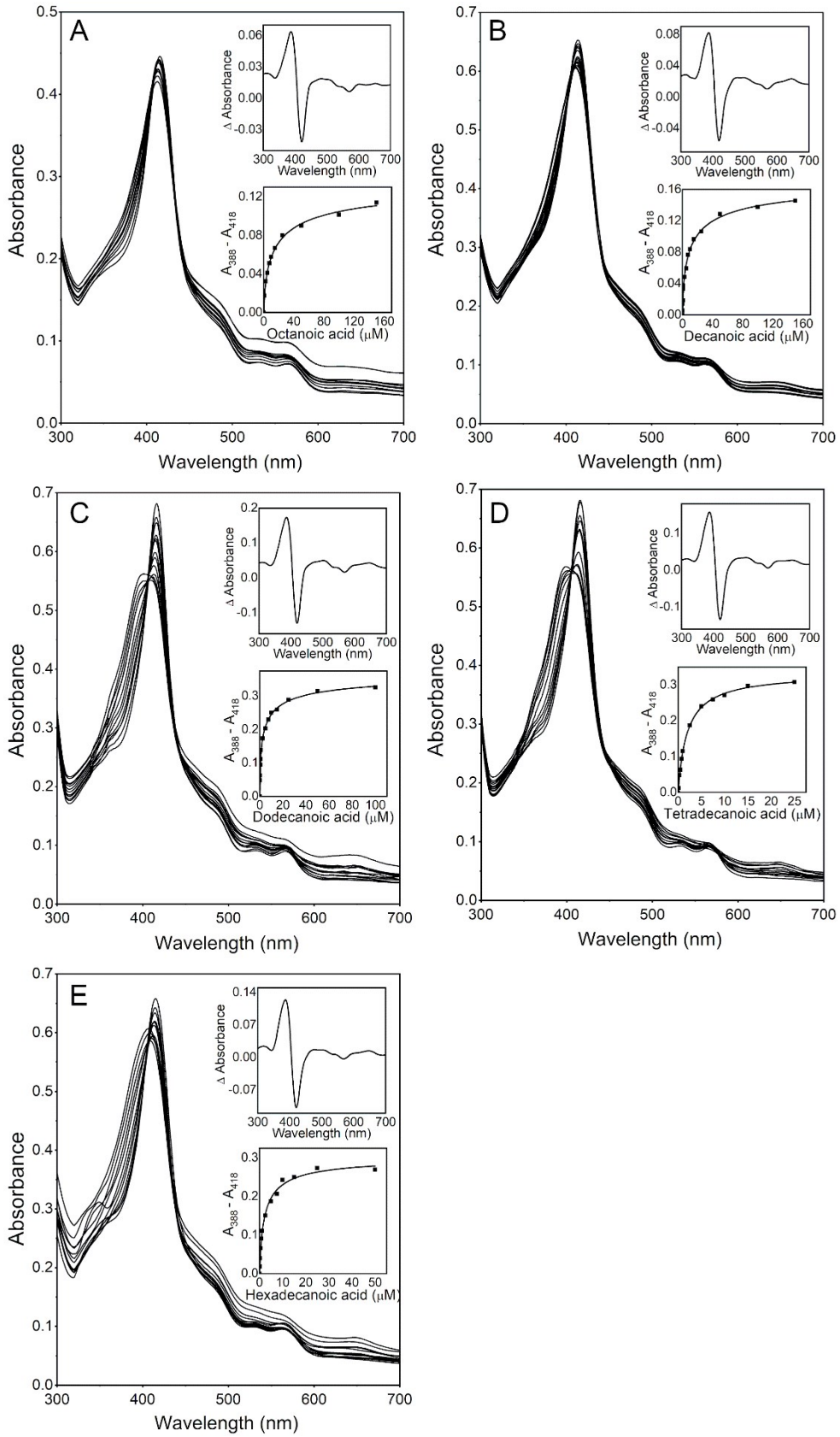


Figure S3. UV-Vis spectra of CYP505A30 titrated with fatty acids. A) octanoic acid B) decanoic acid C) dodecanoic acid D) tetradecanoic acid E) hexadecanoic acid. Inset top: Difference spectra. Inset bottom: Dissociation constant (K_D) analysis.

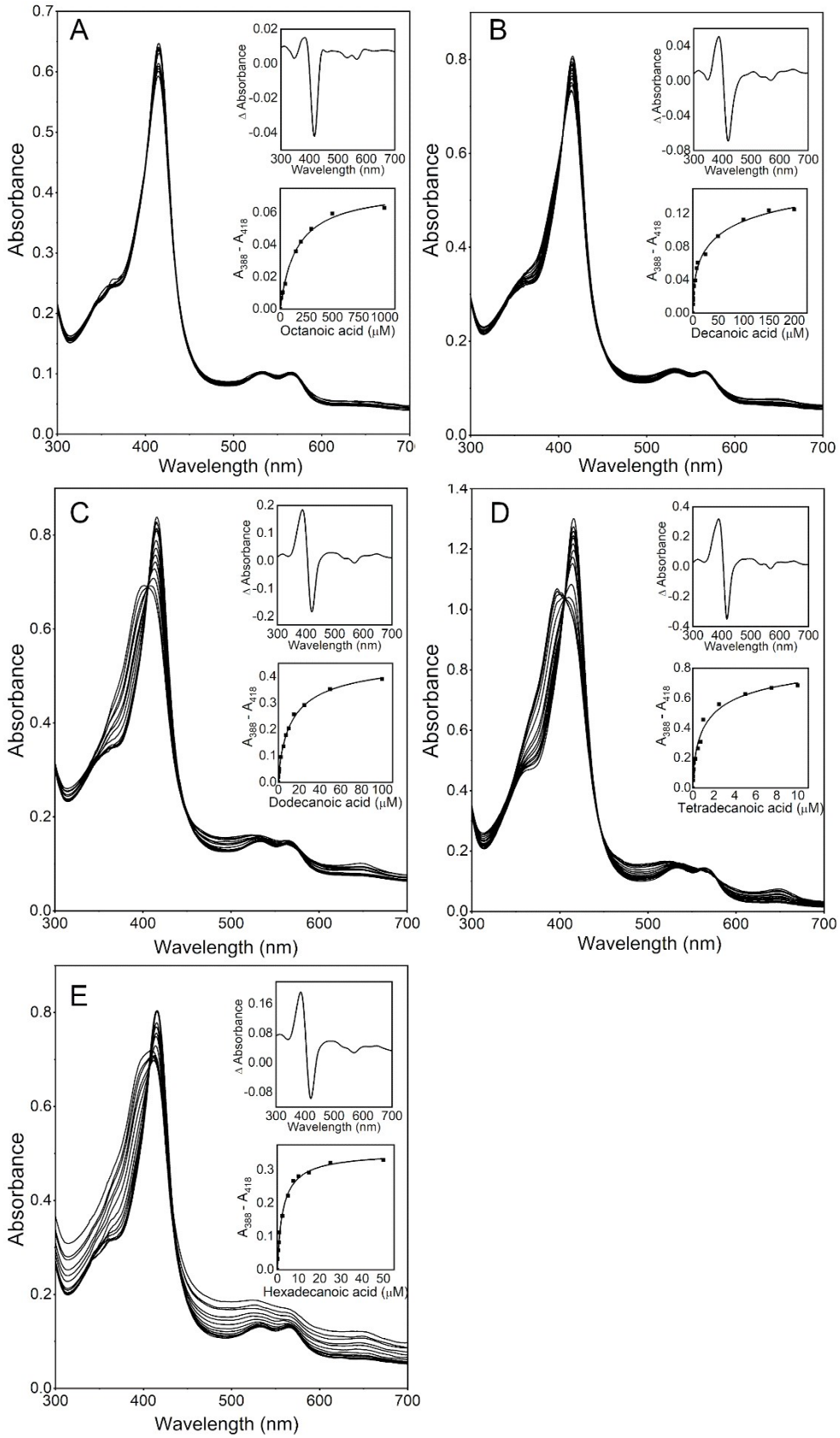


Figure S4. UV-Vis spectra of CYP505A30HD titrated with fatty acids. A) octanoic acid B) decanoic acid C) dodecanoic acid D) tetradecanoic acid E) hexadecanoic acid. Inset top: Difference spectra. Inset bottom: Dissociation constant (K_D) analysis.

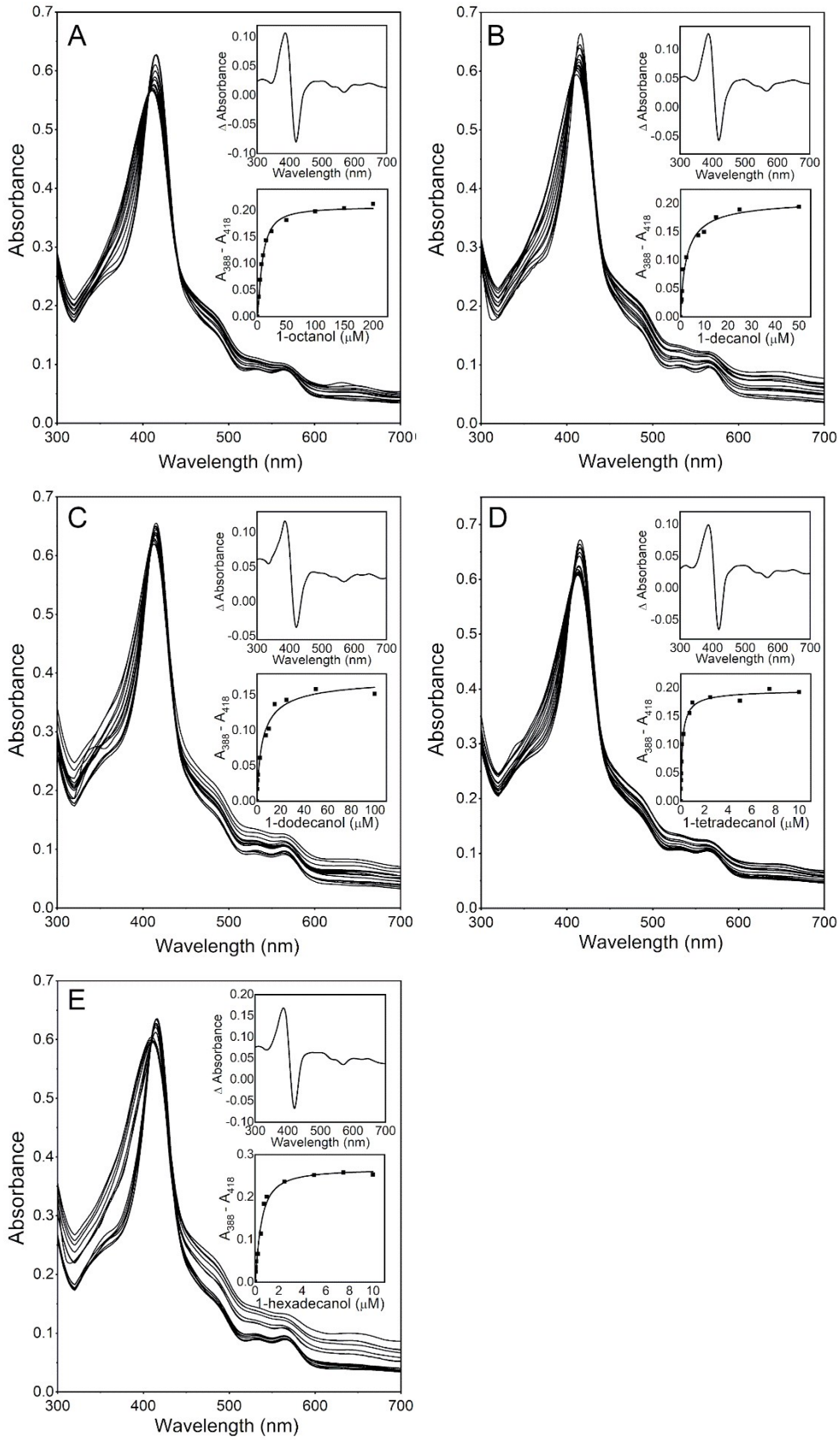


Figure S5. UV-Vis spectra of CYP505A30 titrated with fatty alcohols. A) 1-octanol B) 1-decanol C) 1-dodecanol D) 1-tetradecanol E) 1-hexadecanol. Inset top: Difference spectra. Inset bottom: Dissociation constant (K_D) analysis.

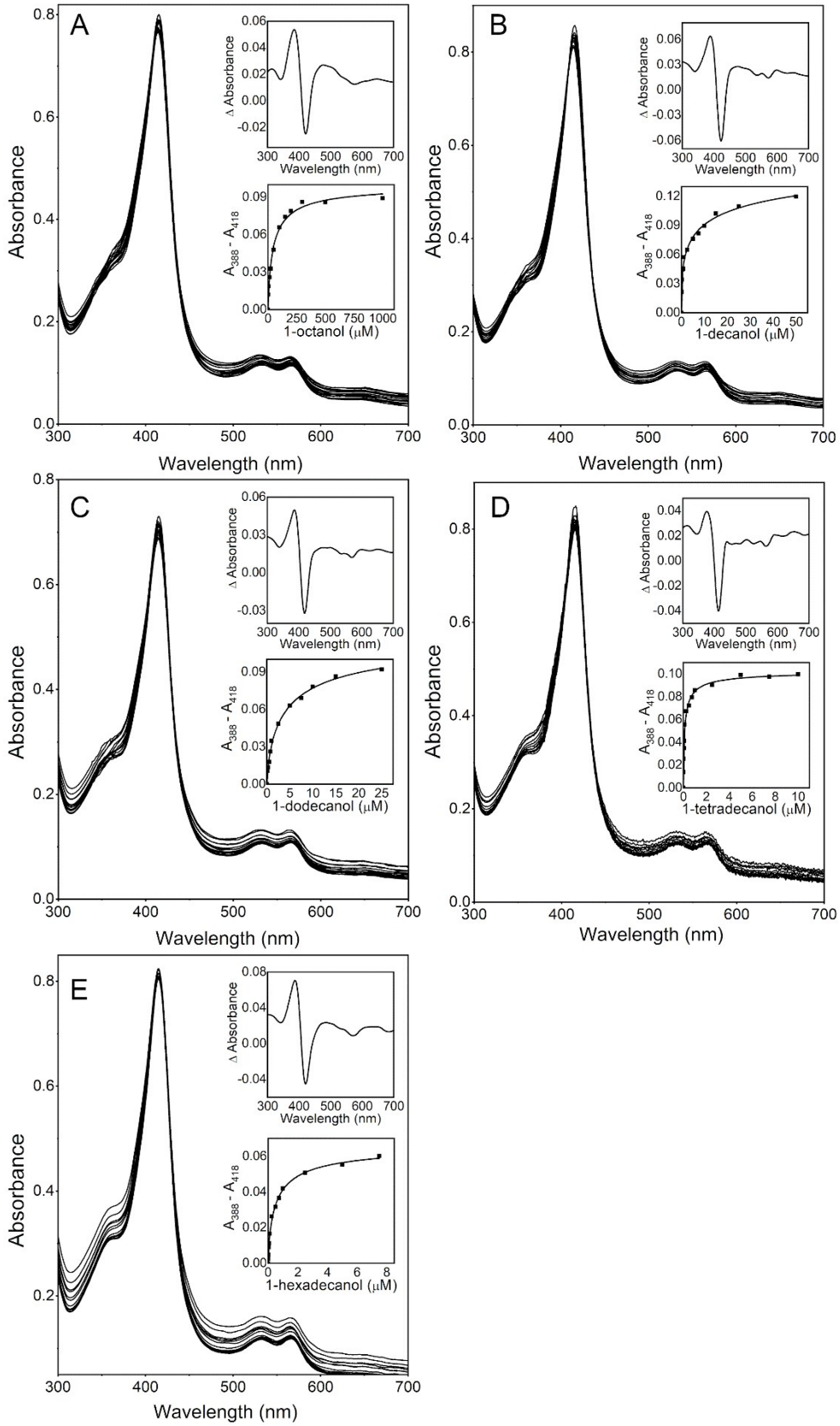


Figure S6. UV-Vis spectra of CYP505A30HD titrated with fatty alcohols. A) 1-octanol B) 1-decanol C) 1-dodecanol D) 1-tetradecanol E) 1-hexadecanol. Inset top: Difference spectra. Inset bottom: Dissociation constant (K_D) analysis.

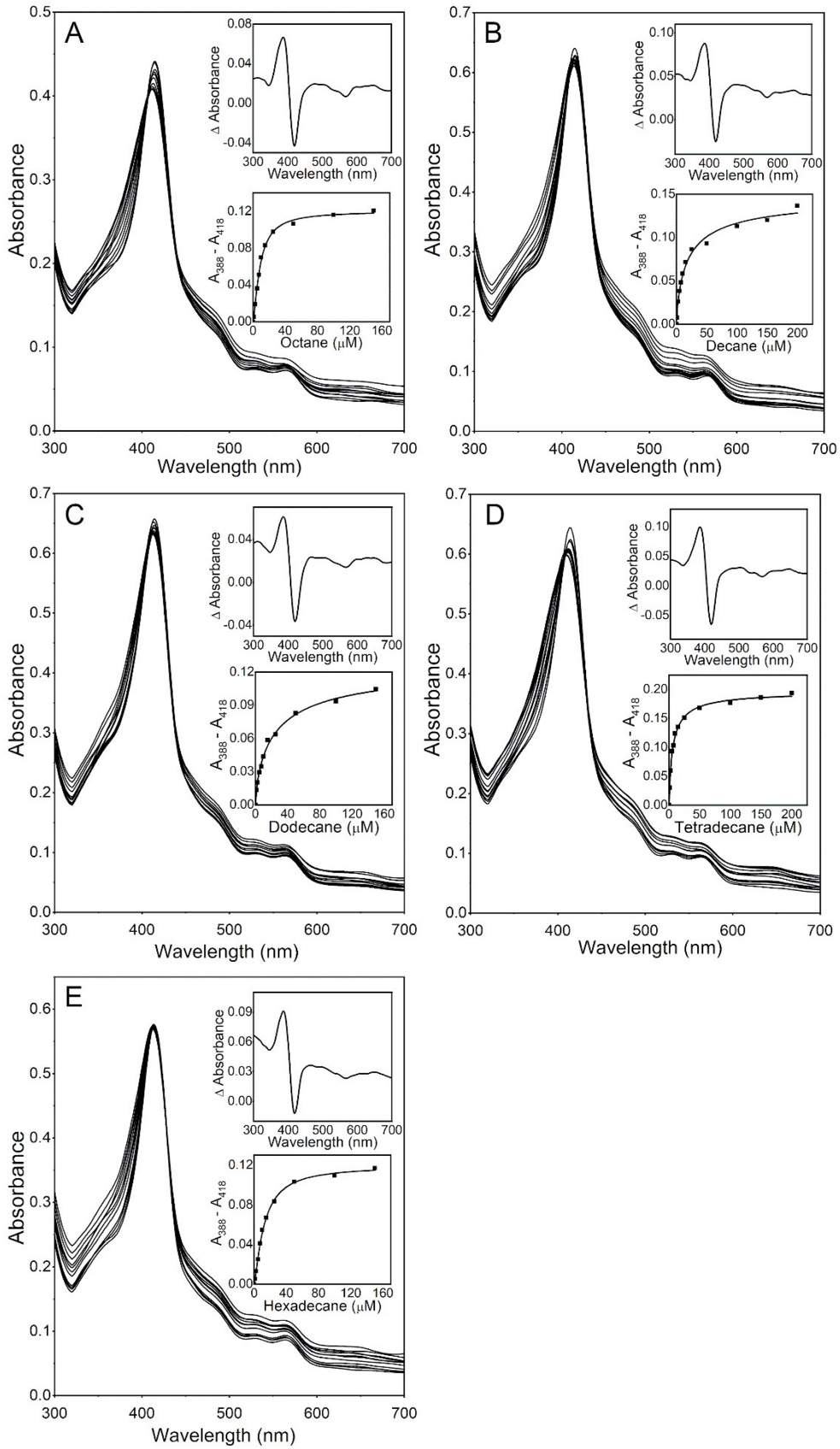


Figure S7. UV-Vis spectra of CYP505A30 titrated with *n*-alkanes. A) *n*-octane B) *n*-decane C) *n*-dodecane D) *n*-tetradecane E) *n*-hexadecane. Inset top: Difference spectra. Inset bottom: Dissociation constant (K_D) analysis.

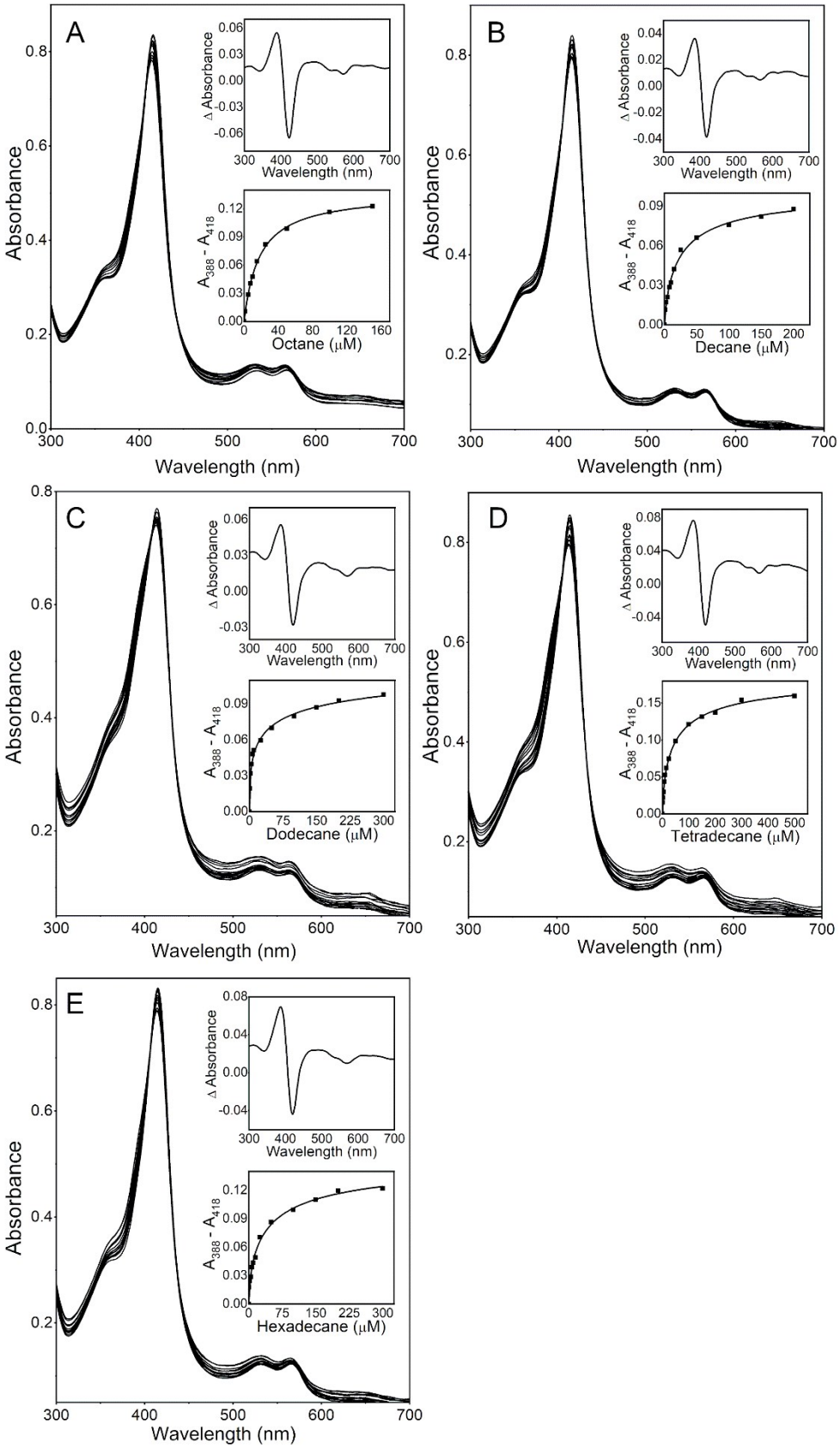


Figure S8. UV-Vis spectra of CYP505A30HD titrated with *n*-alkanes. A) *n*-octane B) *n*-decane C) *n*-dodecane D) *n*-tetradecane E) *n*-hexadecane. Inset top: Difference spectra. Inset bottom: Dissociation constant (K_D) analysis.

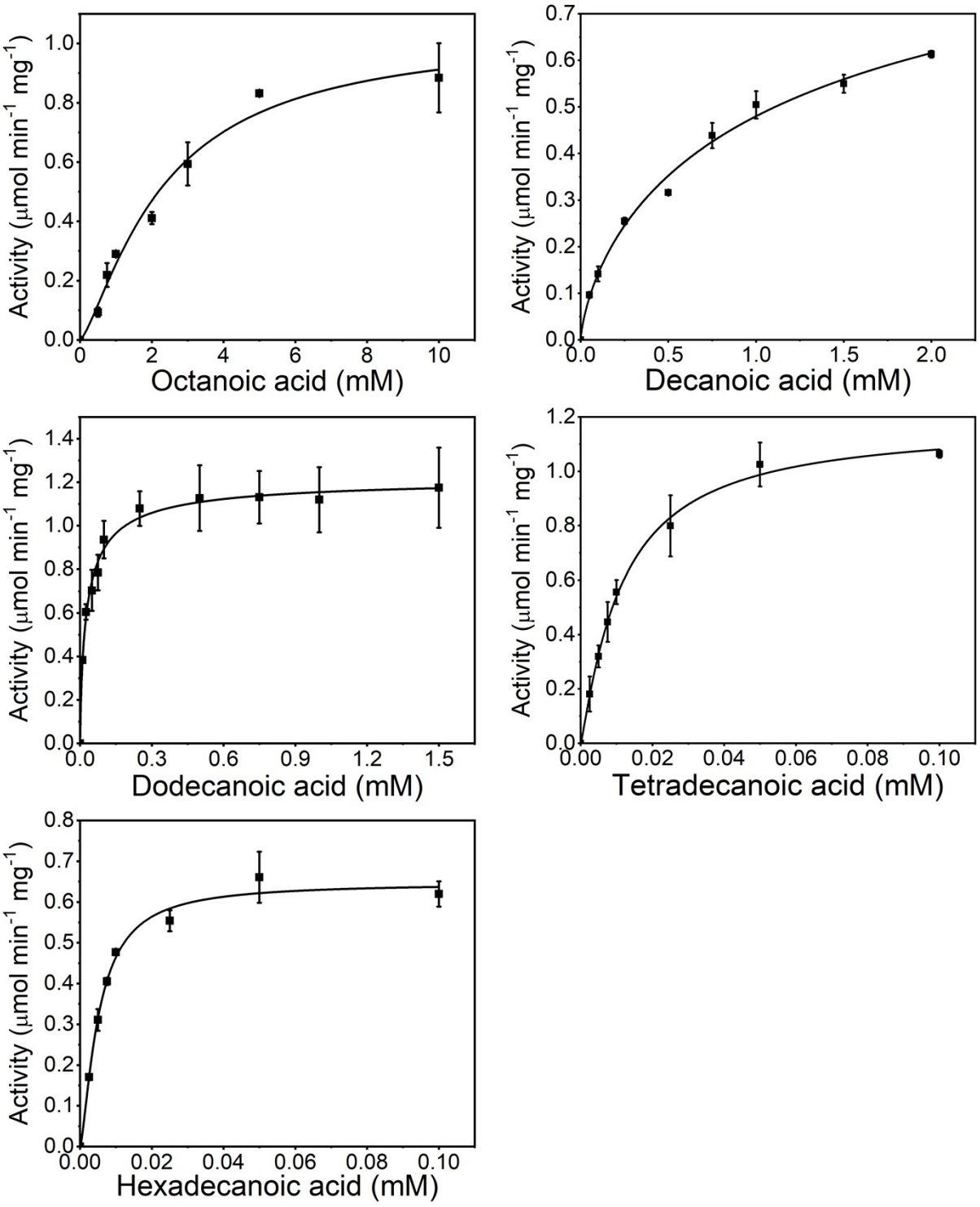


Figure S9. Kinetic characterization of CYP505A30 with fatty acids

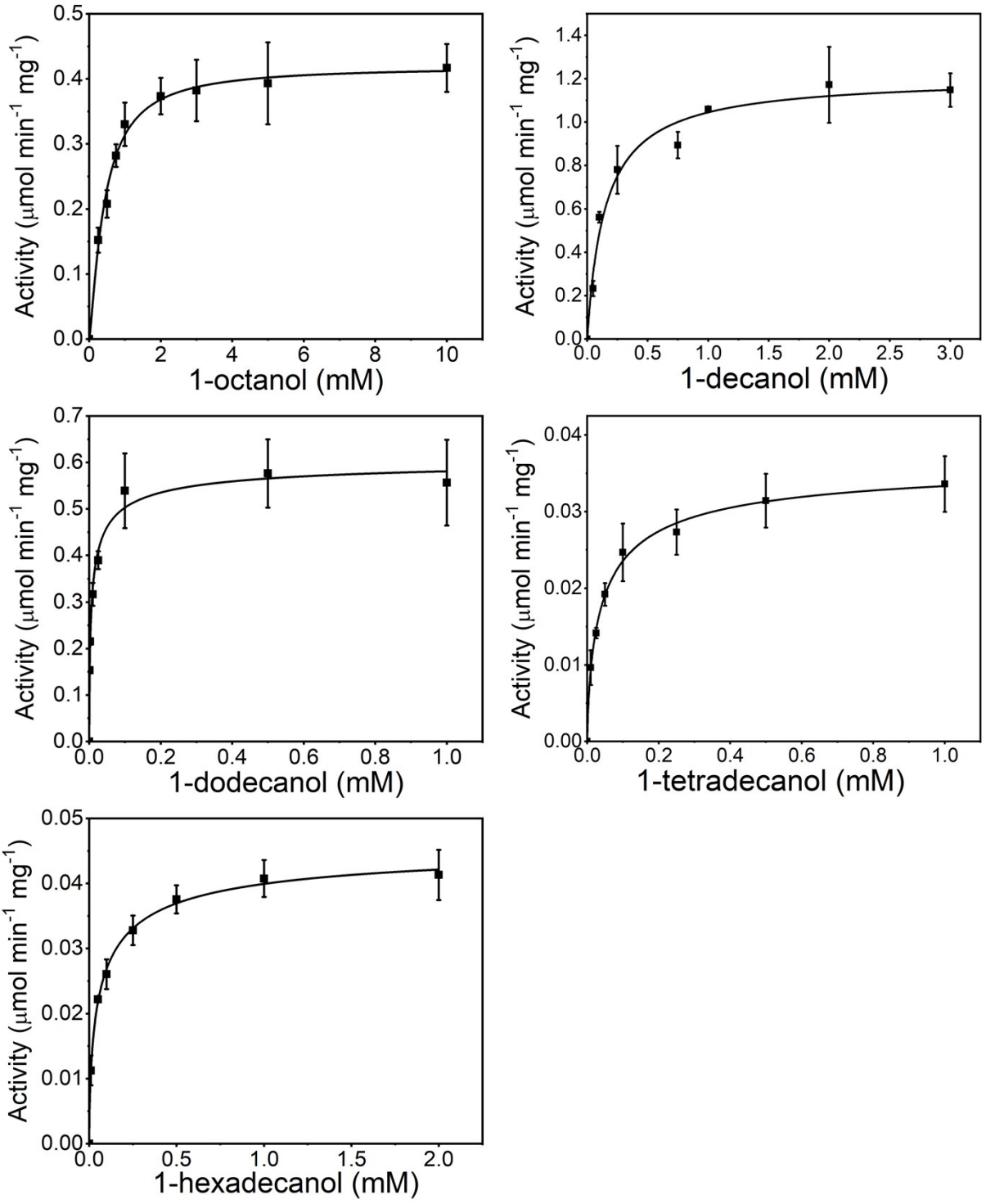


Figure S10. Kinetic characterization of CYP505A30 with primary fatty alcohols

Table S1. Spectral and kinetic parameters of CYP505A30

Substrate	K_D (μM)	% HS	K_D HD (μM)	% HS HD	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (μM^{-1} s^{-1})	H_2O_2 Uncoupling (%)[c]
Fatty acids								
C8 (octanoic acid)	16 ± 1	35	161 ± 12	9	3550 ± 340	2.5 ± 0.1	<0.001	3.3 ± 0.3
C10 (decanoic acid)	12 ± 1 (21.1 ± 2.0)	45	66 ± 7	18	550 ± 60	1.5 ± 0.1	0.003	3.5 ± 0.4
C12 (dodecanoic acid)	3.3 ± 0.1 (6.1 ± 0.4)	98	14 ± 1 (9.0 ± 0.9)[a]	57	26 ± 3 (21.2 ± 2.4)	2.3 ± 0.2	0.09 (0.23)	1.5 ± 0.1
C14 (tetradecanoic acid)	1.9 ± 0.2 (7.4 ± 0.2)	100	1.5 ± 0.2 (3.9 ± 0.3)	100	13 ± 1 (7.7 ± 1.9)	2.5 ± 0.1	0.19 (0.16)	
C16 (hexadecanoic acid)	2.3 ± 0.1 10.5 ± 0.2)	83	2.7 ± 0.1 (1.0 ± 0.4)	48	6 ± 1	1.2 ± 0.1	0.2	
Fatty alcohols								
C8 (1-octanol)	8.5 ± 0.7	65	48 ± 3	13	450 ± 50	0.85 ± 0.01	0.002	16.3 ± 0.5
C10 (1-decanol)	2.5 ± 0.2	60	13 ± 2	17	150 ± 12	2.3 ± 0.2	0.015	9.5 ± 0.3
C12 (1-dodecanol)	4.9 ± 0.2	46	4.40 ± 0.30	9	4.0 ± 0.2	1.2 ± 0.1	0.3	2.8 ± 0.2
C14 (1-tetradecanol)	0.13 ± 0.02	59	0.10 ± 0.02	14	33 ± 2	0.07 ± 0.01	0.002	
C16 (1-hexadecanol)	0.46 ± 0.03	78	0.50 ± 0.03	9	45 ± 3	0.08 ± 0.01	0.002	
Alkanes								
C8 (*n*-octane)	12 ± 1	37	18 ± 2	18		0.04 ± 0.01[b]		3.4 ± 0.5
C10 (*n*-decane)	19 ± 2	42	24 ± 1	13		0.05 ± 0.01		7.8 ± 0.1
C12 (*n*-dodecane)	27 ± 3	32	44 ± 3	14		0.08 ± 0.01		
C14 (*n*-tetradecane)	20 ± 1	60	47 ± 4	23		0.07 ± 0.01		
C16 (*n*-hexadecane)	12 ± 1	36	35 ± 4	18		0.14 ± 0.01		

[a] Values in parenthesis are as reported by Baker and co-workers. [b] Values are for k_{obs} at 10 mM. [c] H_2O_2 uncoupling was measured as the percentage of NADPH that is used to produce hydrogen peroxide during the reaction.

Table S2. Turnover frequency (TOF) and total turnover number (TTN) for CFE reactions with different substrates

Substrate	TOF [min^{-1}][a]	Conversion (after 2 h)	TTN[b]
Octane	73.8 ± 4.5		10718 ± 550
Decane	8.8 ± 0.4		3125 ± 280
1-octanol	42.6 ± 0.1	>99 %	2500
2-octanol	57.5 ± 6.3	>99 %	2500
3-octanol	31.1 ± 1.2	83 %	2500
4-octanol	18.4 ± 0.9	71 %	2500
1-decanol	23.6 ± 3.1	>99 %	5000
2-decanol	50.2 ± 4.7	>99 %	3325 ± 106
1-dodecanol	59.2 ± 5.2	>99 %	5000
2-dodecanol	30.4 ± 6.8	90 %	5000
octanoic acid	33.3 ± 0.6	90 %	2500
decanoic acid	36.1 ± 5.3	>99 %	4200 ± 106
dodecanoic acid	57.3 ± 0.9	85 %	4850 ± 212

[a]TOF calculated for 30 min reactions. For octane reactions, product concentrations were calculated using standards of 2- and 3-octanol for simple alcohols formed, and 1,8-octanediol as standard for diols. For decane reactions, product concentrations were calculated using 2-decanol for *n*-decanol formed, and 1,10-decanediol for diols. For fatty acids and fatty alcohols, the remaining substrate concentration was calculated using their corresponding standards.

[b]TTN achieved over 24 h reactions. For fatty acids and fatty alcohols 10 mM substrate was consumed over 24 h. Alkane TTNs were calculated from the total concentration of product formed after 24 h. [CYP505A30] = 4 µM.

Table S3. GC-FID and GC-MS methods

Analysis	Substrates	Derivatization	Temperature program
GC-MS **Column A: FactorFour CF-5ms (60 m x 0.25 mm x 0.25 µm)**			
Alkanes Fatty alcohols Fatty acids	C8 – C10 C8 – C12 C8 – C12	BSTFA	100 °C hold 0 min → 300 °C (10 min^{-1}) hold 10 min
GC-FID **Column B: BPX90 (30 m x 0.25 mm x 0.25 µm)**			
Alkanes Fatty alcohols Fatty acids	C8 – C10 C8 – C12 C8 – C12	None None TMSH	80 °C hold 2 min → 280 °C (15 min^{-1}) hold 4.66 min

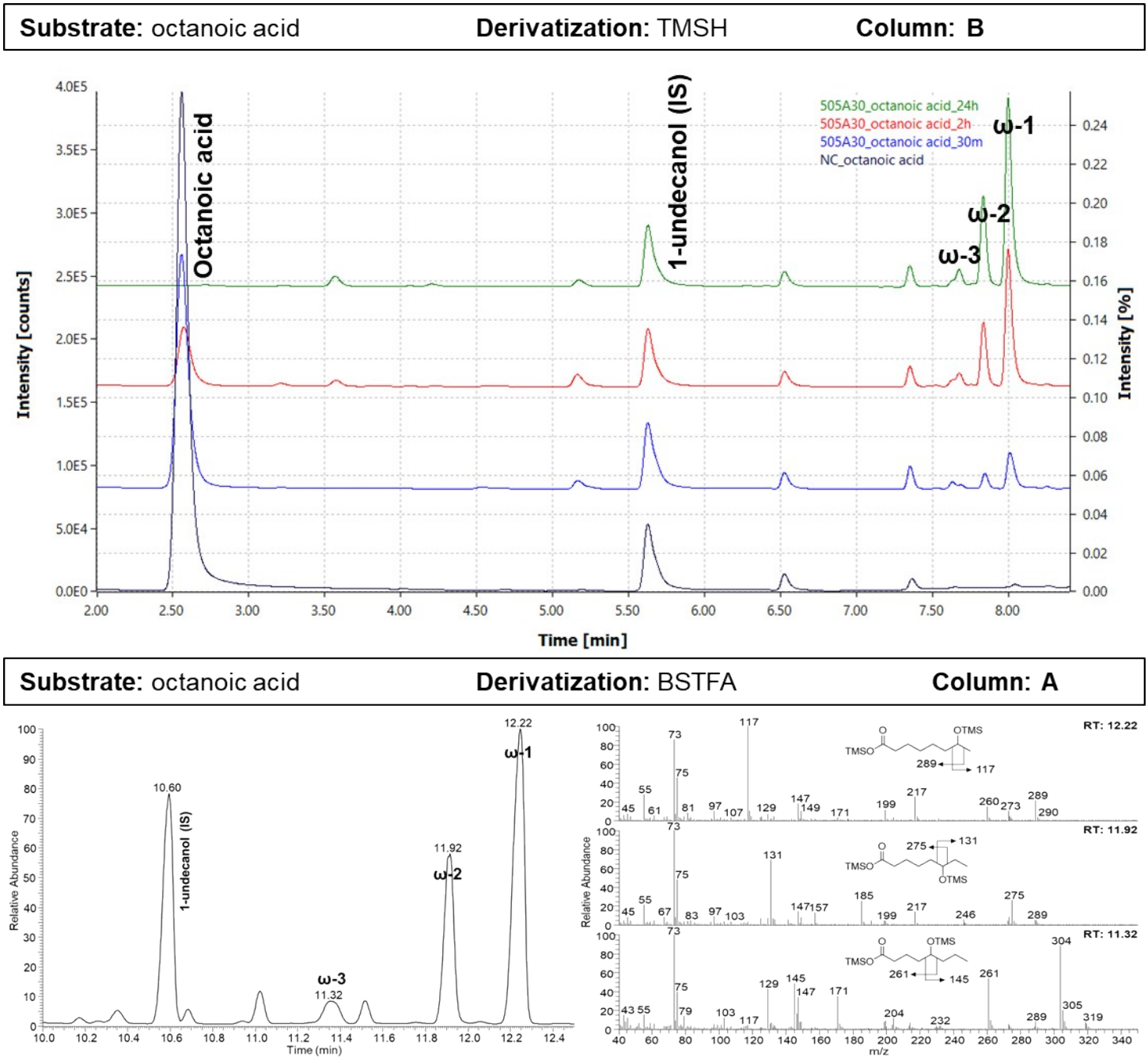
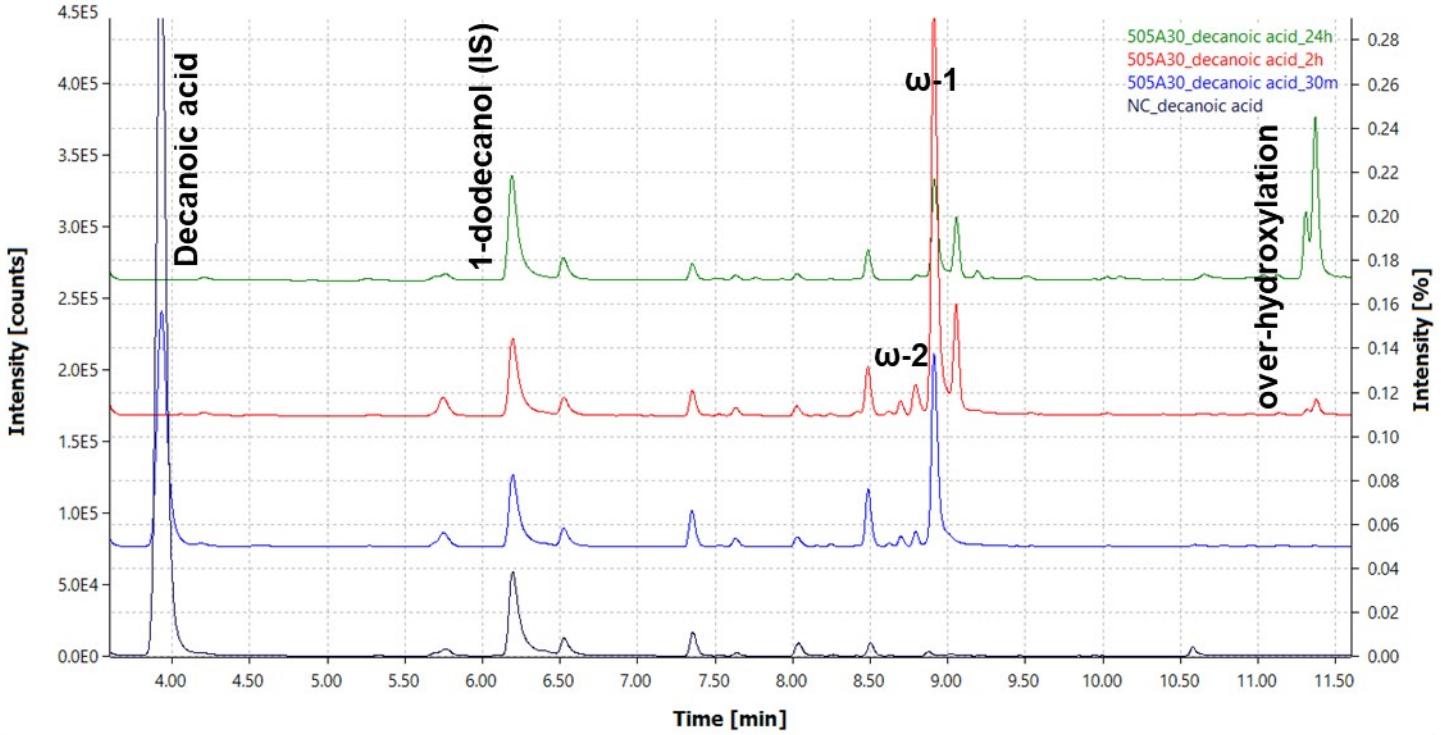


Figure S11. GC chromatograms for reactions with fatty acids (C8 - C12) after 30 min, 2 h, and 24 h of CFE biotransformations. Negative control (NC) are 24 h biotransformations with CFE carrying the empty plasmid. GC/MS chromatogram and spectra of the hydroxy-fatty acids formed after 24 h reactions with CFE, unless otherwise is stated.

Substrate: decanoic acid	**Derivatization:** TMSH	**Column: B**



Substrate: decanoic acid	**Derivatization:** BSTFA	**Column: A**

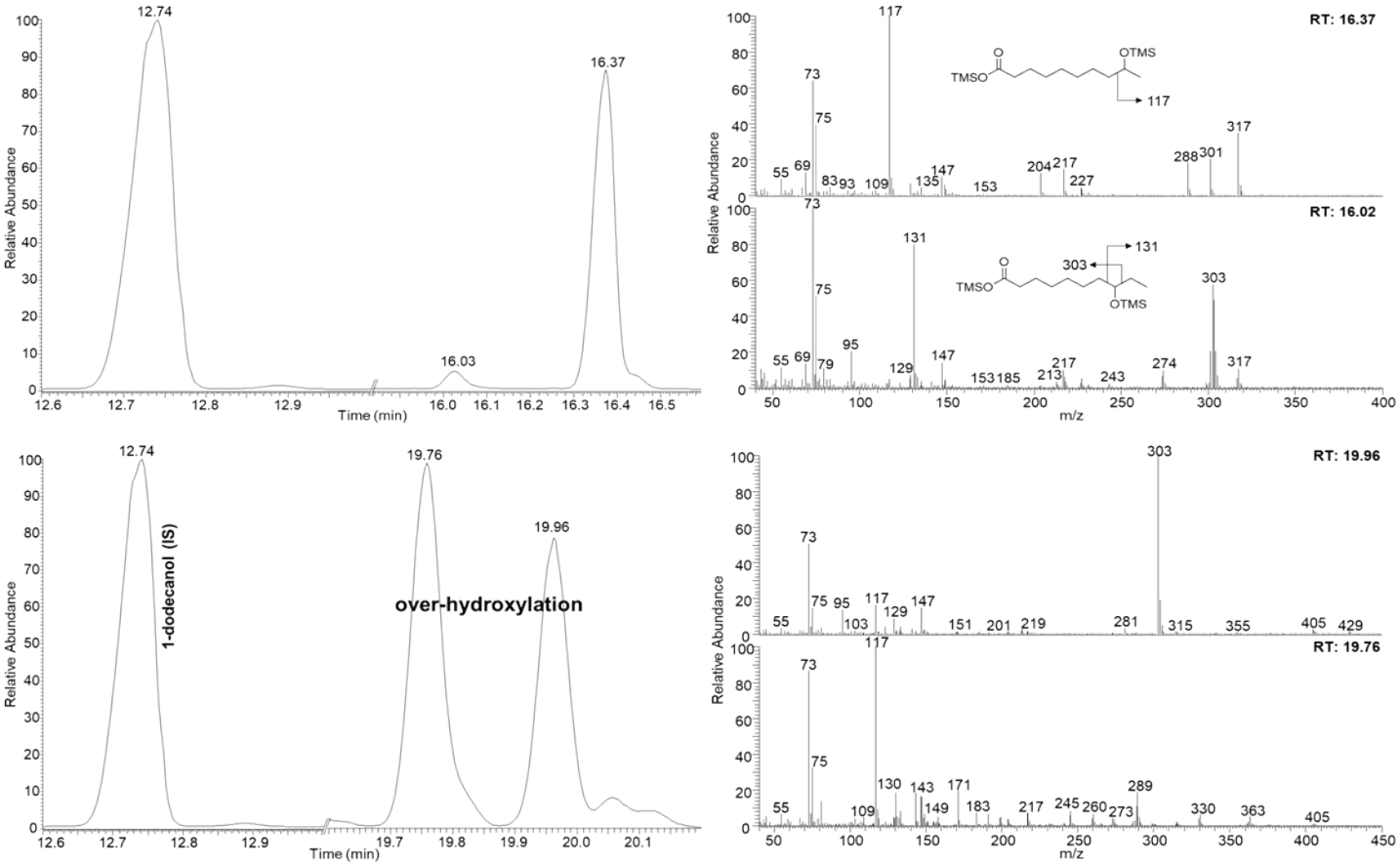
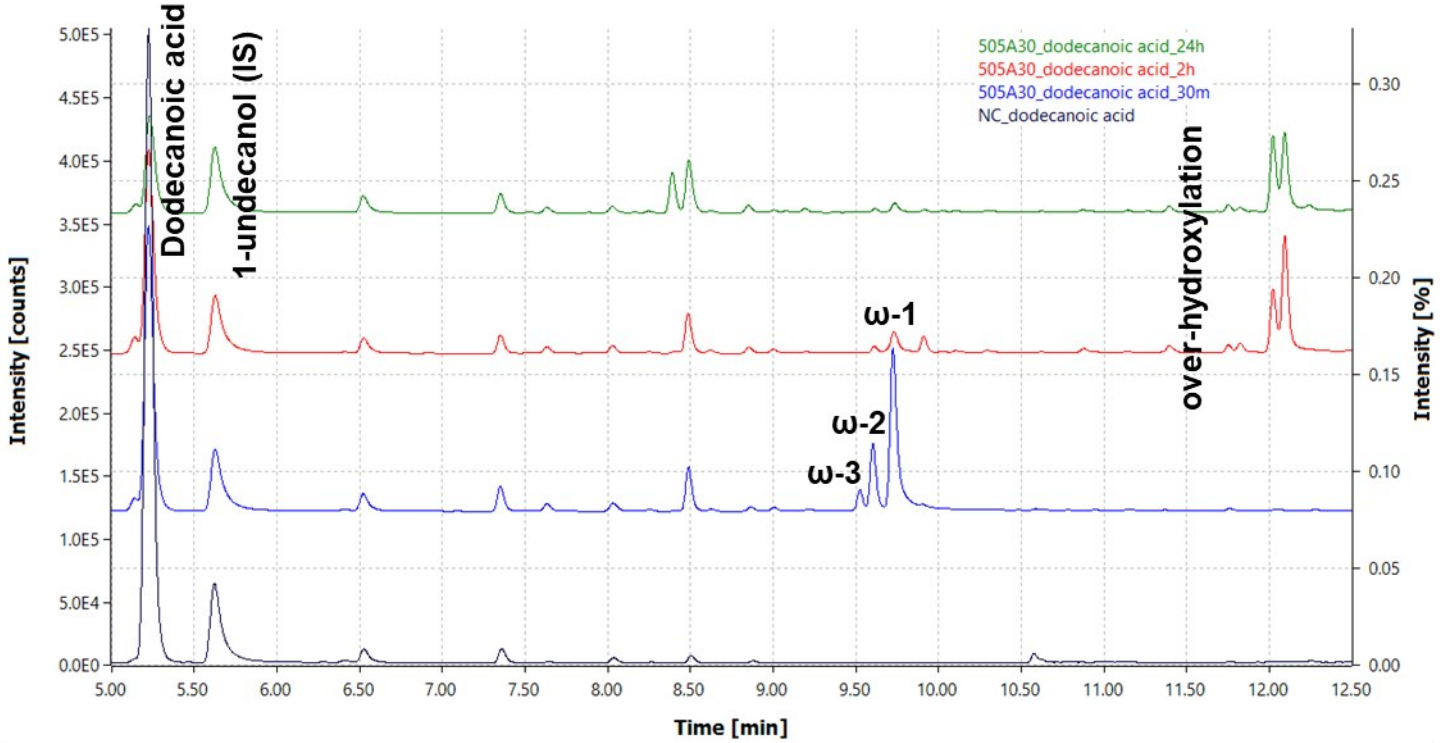


Figure S11. Cont.

Substrate: dodecanoic acid **Derivatization:** TMSH **Column: B**



Substrate: dodecanoic acid **Derivatization:** BSTFA **Column: A**

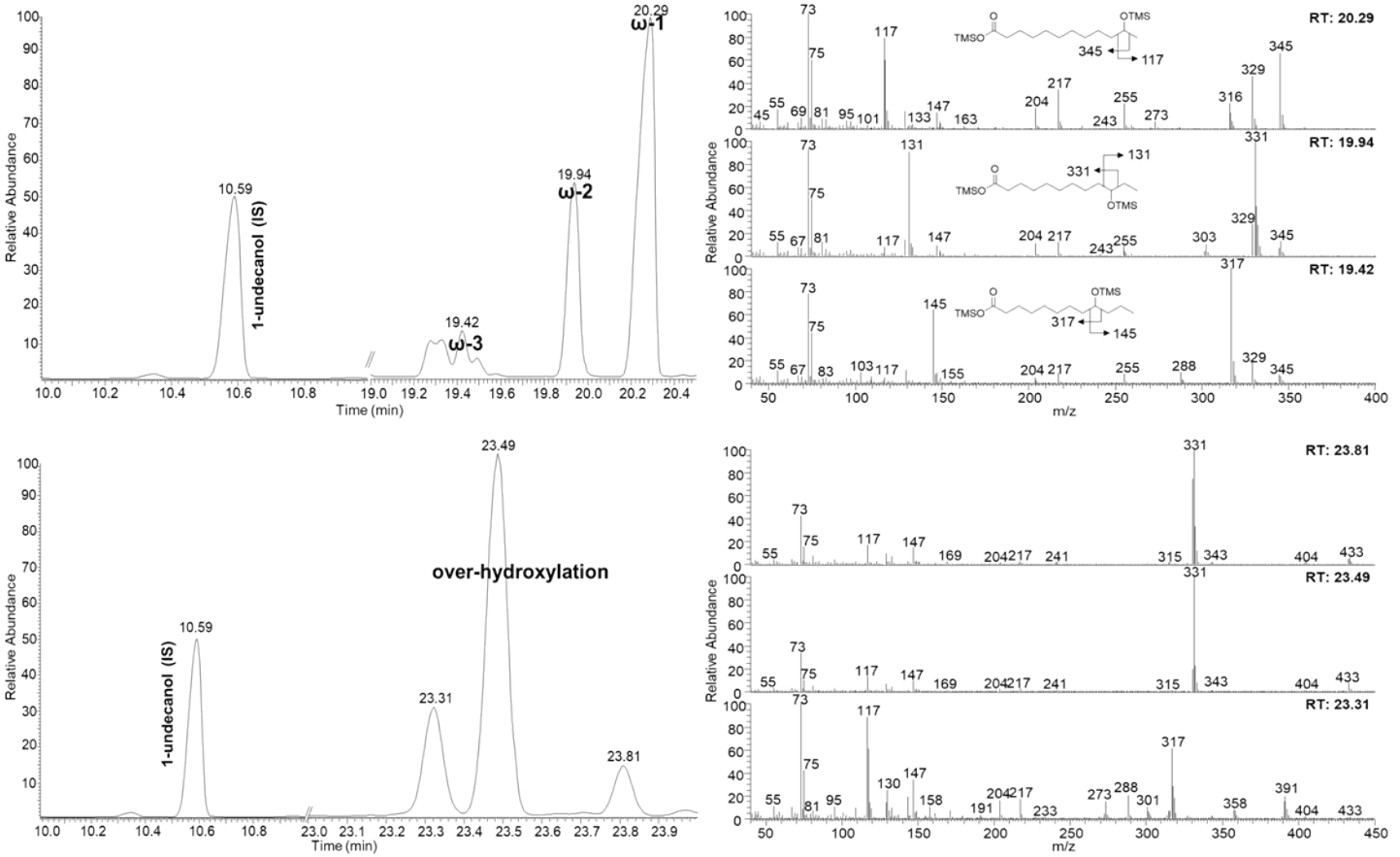
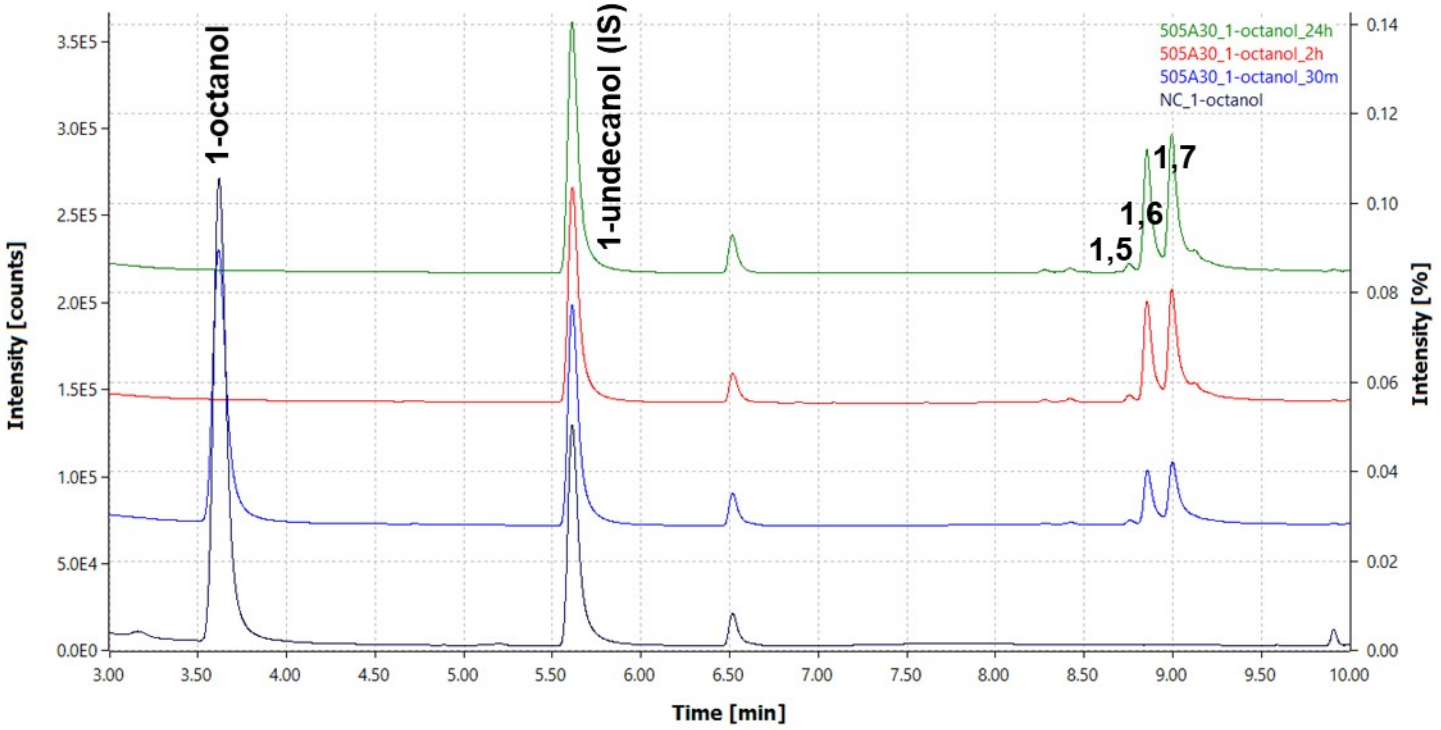


Figure S11. Cont. GC/MS chromatogram and spectra of production of hydroxy dodecanoic acid (ω-1, ω-2, and ω-3) after 30 min reactions with CFE. GC/MS chromatogram and spectra of over-hydroxylation of the substrate after 24 h reaction with CFE.

Substrate: 1-octanol **Derivatization:** none **Column: B**



Substrate: 1-octanol **Derivatization:** BSTFA **Column: A**

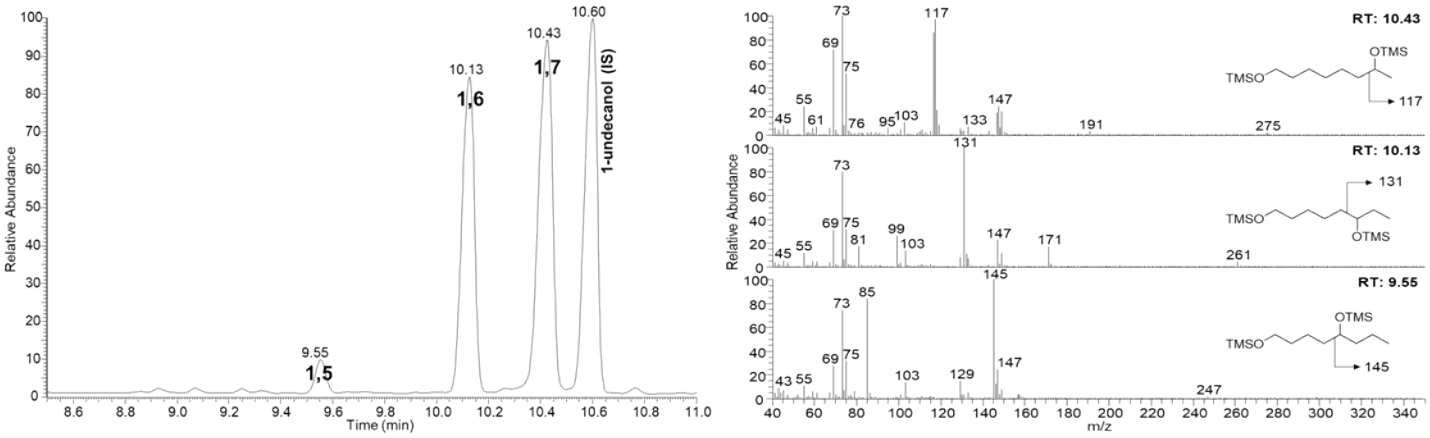
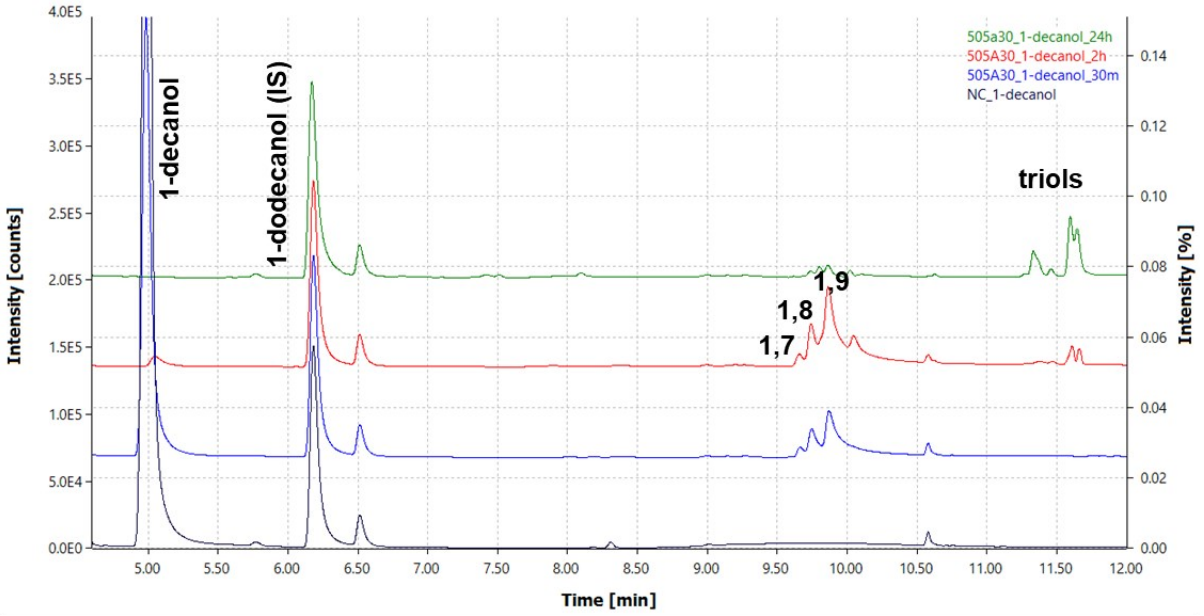


Figure S12. GC chromatograms for reactions with primary fatty alcohols (C8 - C12) after 30 min, 2 h, and 24 h of CFE biotransformations. Negative control (NC) are 24 h biotransformations with CFE carrying the empty plasmid. GC/MS chromatogram and spectra of the diols (and triols) formed after 24 h reactions with CFE, unless otherwise is stated.

Substrate: 1-decanol	**Derivatization:** none	**Column: B**



Substrate: 1-decanol	**Derivatization:** BSTFA	**Column: A**

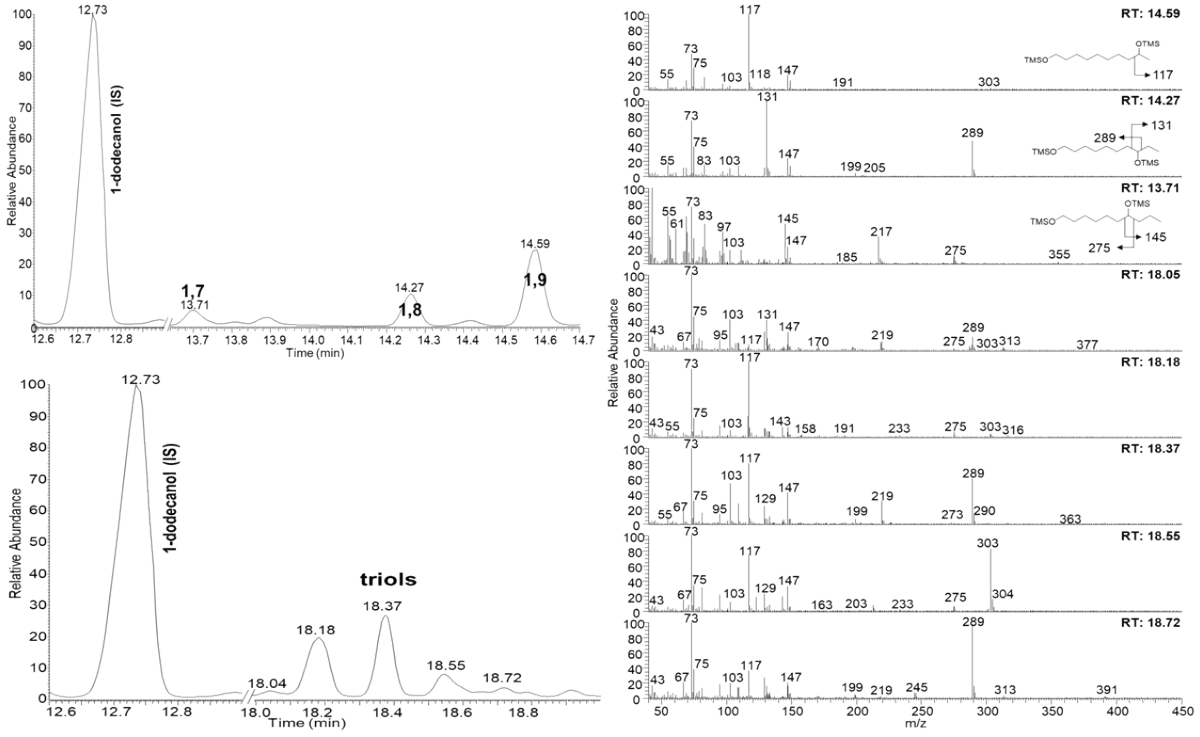


Figure S12. Cont.

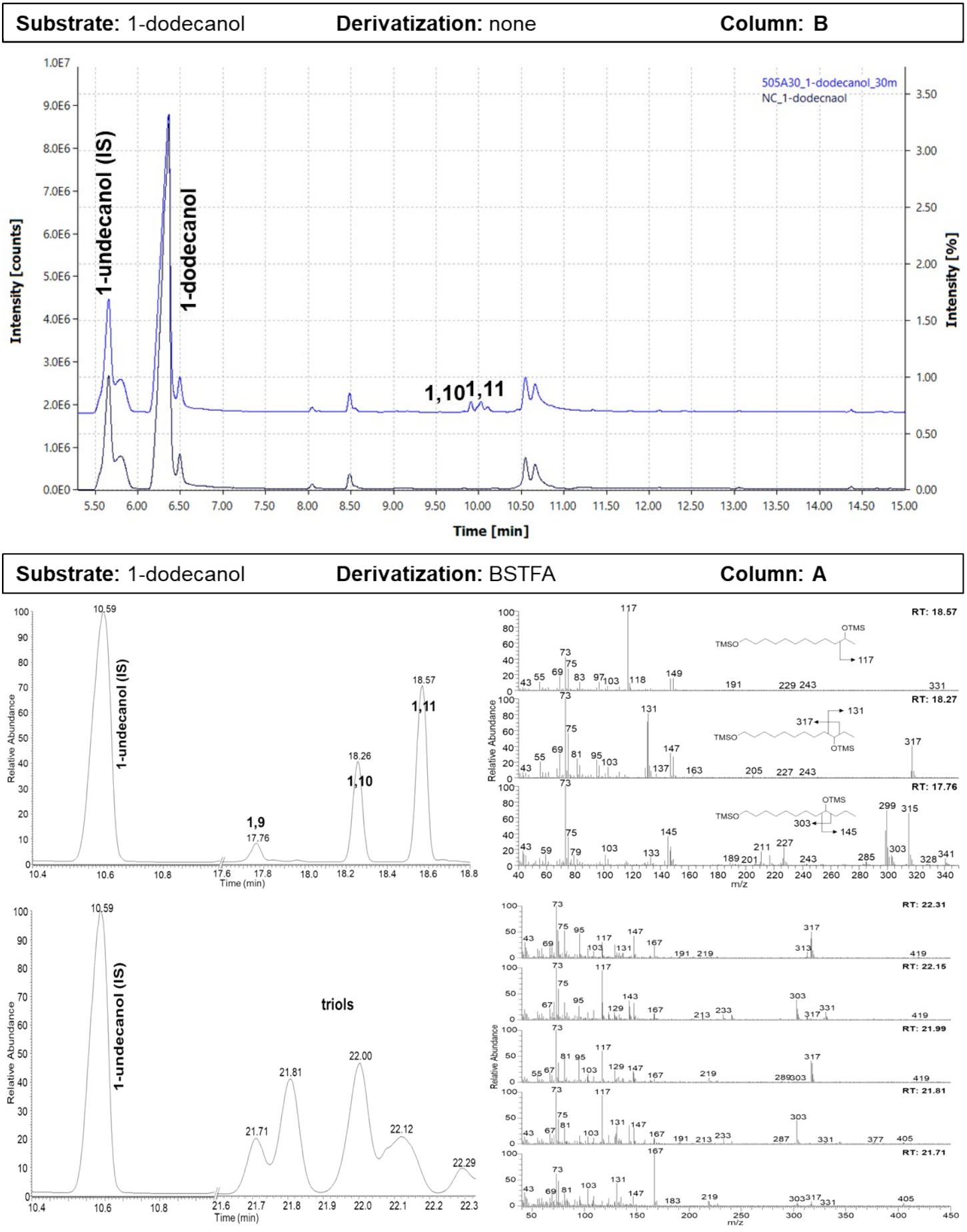
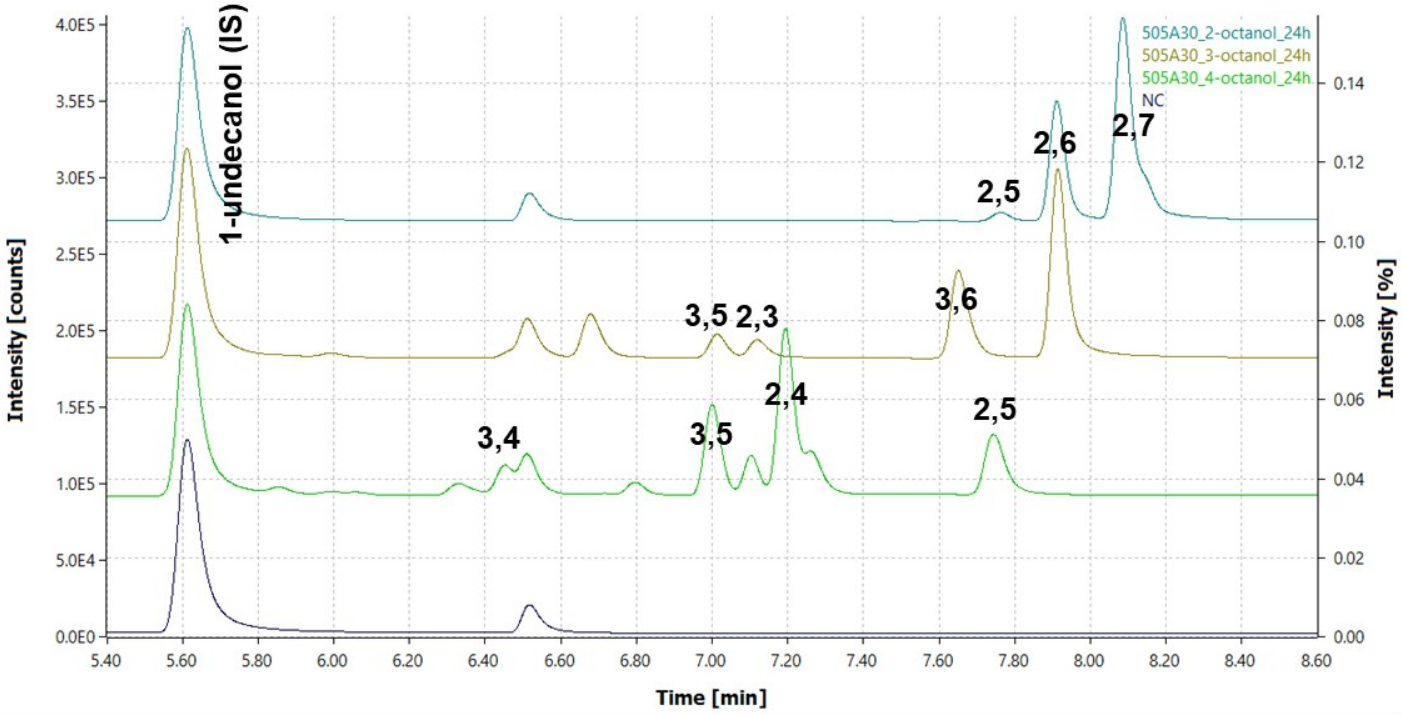


Figure S12. Cont. GC chromatograms for reactions with 1-dodecanol after 30 min CFE biotransformations. Negative control (NC) 24 h biotransformations with CFE carrying the empty

plasmid. GC/MS chromatogram and spectra of the diols (and triols) formed after 2 h reactions with CFE.

Substrate: *n*-octanol **Derivatization:** none **Column: B**



Substrate: *n*-octanol **Derivatization:** BSTFA **Column: A**

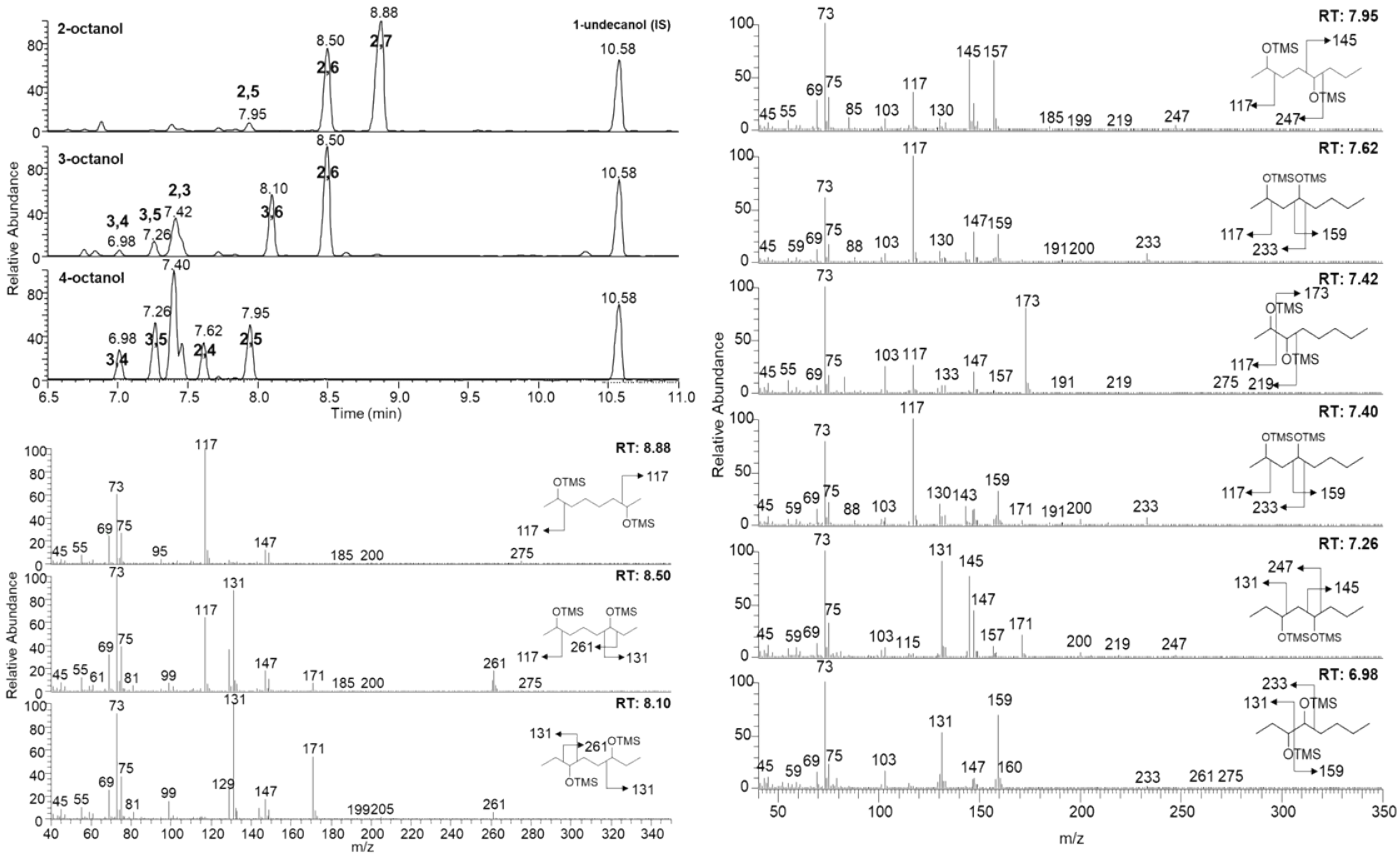
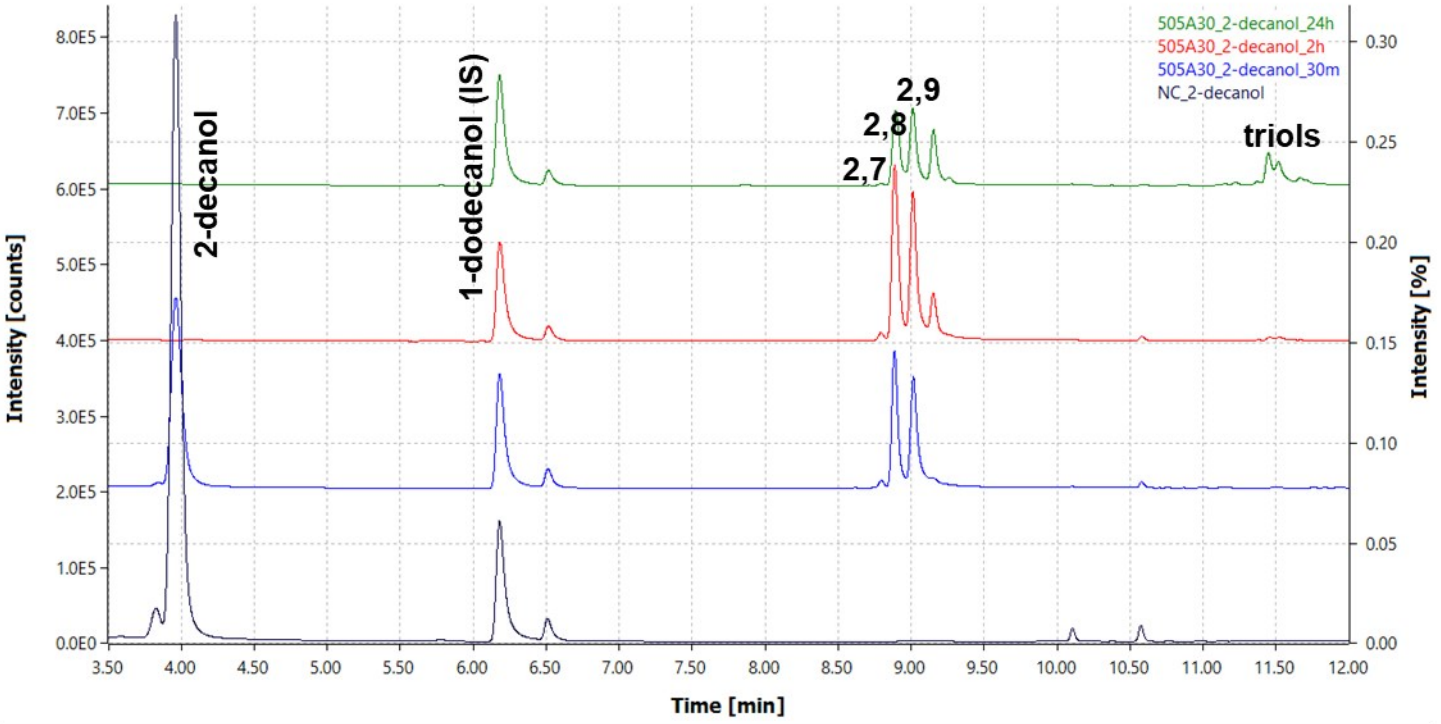


Figure S13. GC chromatograms for reactions with 2-, 3-, and 4-octanol after 24 h of CFE biotransformations. Negative control (NC) are 24 h biotransformations with CFE carrying the empty plasmid. GC/MS chromatogram and spectra of the diols formed after 24 h reactions with CFE.

Substrate: 2-decanol **Derivatization:** none **Column: B**



Substrate: 2-decanol **Derivatization:** BSTFA **Column: A**

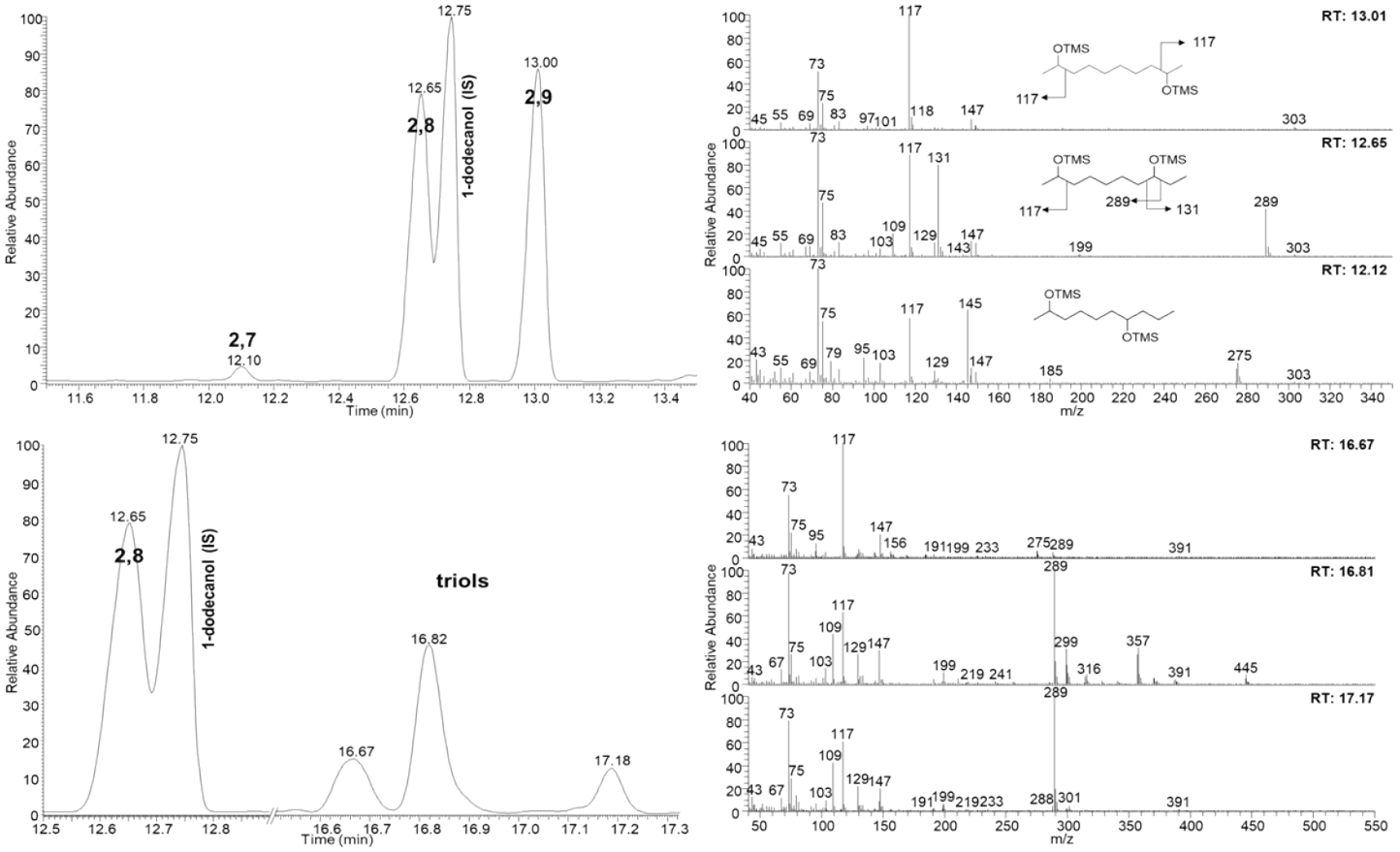
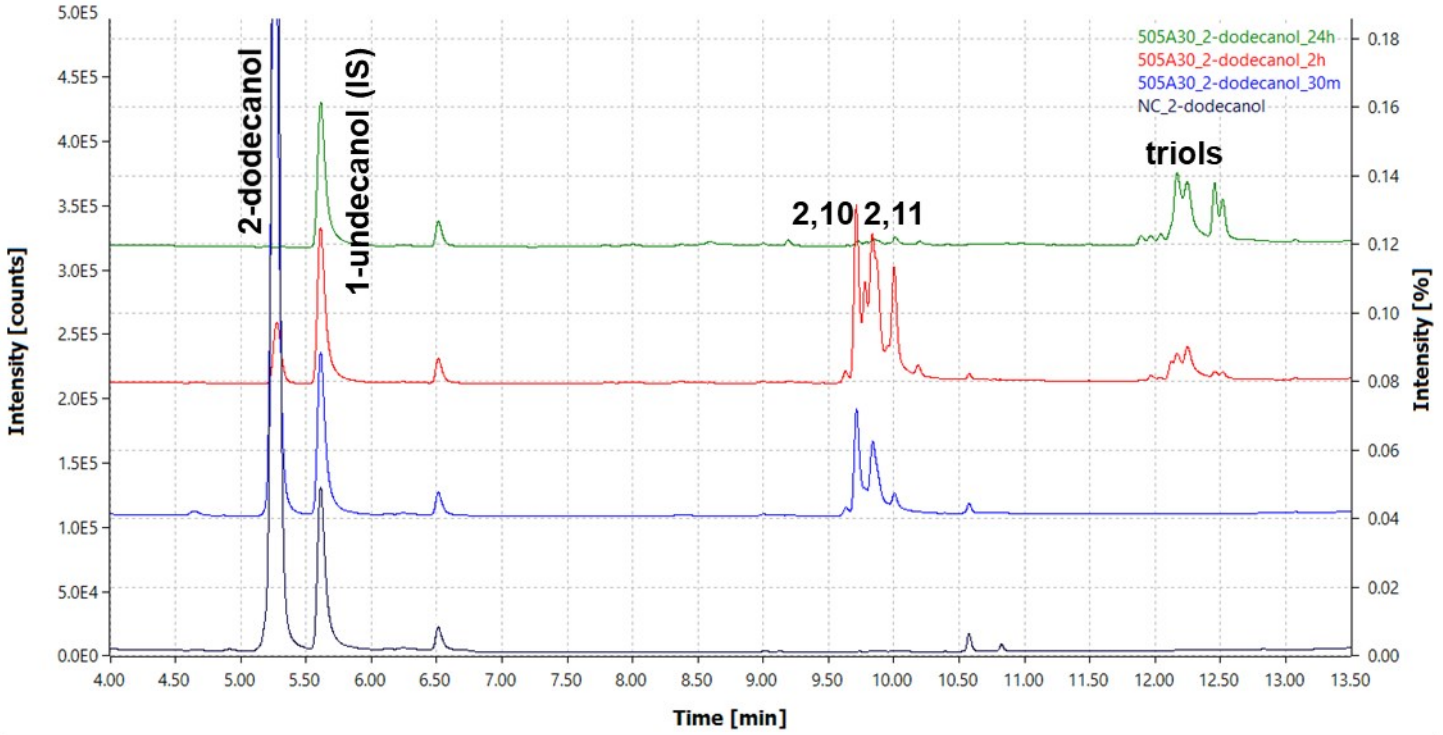


Figure S14. GC chromatograms for reactions with secondary fatty alcohols (C10 and C12) after 30 min, 2 h, and 24 h of CFE biotransformations. Negative control (NC) are 24 h biotransformations with CFE carrying the empty plasmid. GC/MS chromatogram and spectra of the diols (and triols) formed after 24 h reactions with CFE.

Substrate: 2-dodecanol **Derivatization:** none **Column: B**



Substrate: 2-dodecanol **Derivatization:** BSTFA **Column: A**

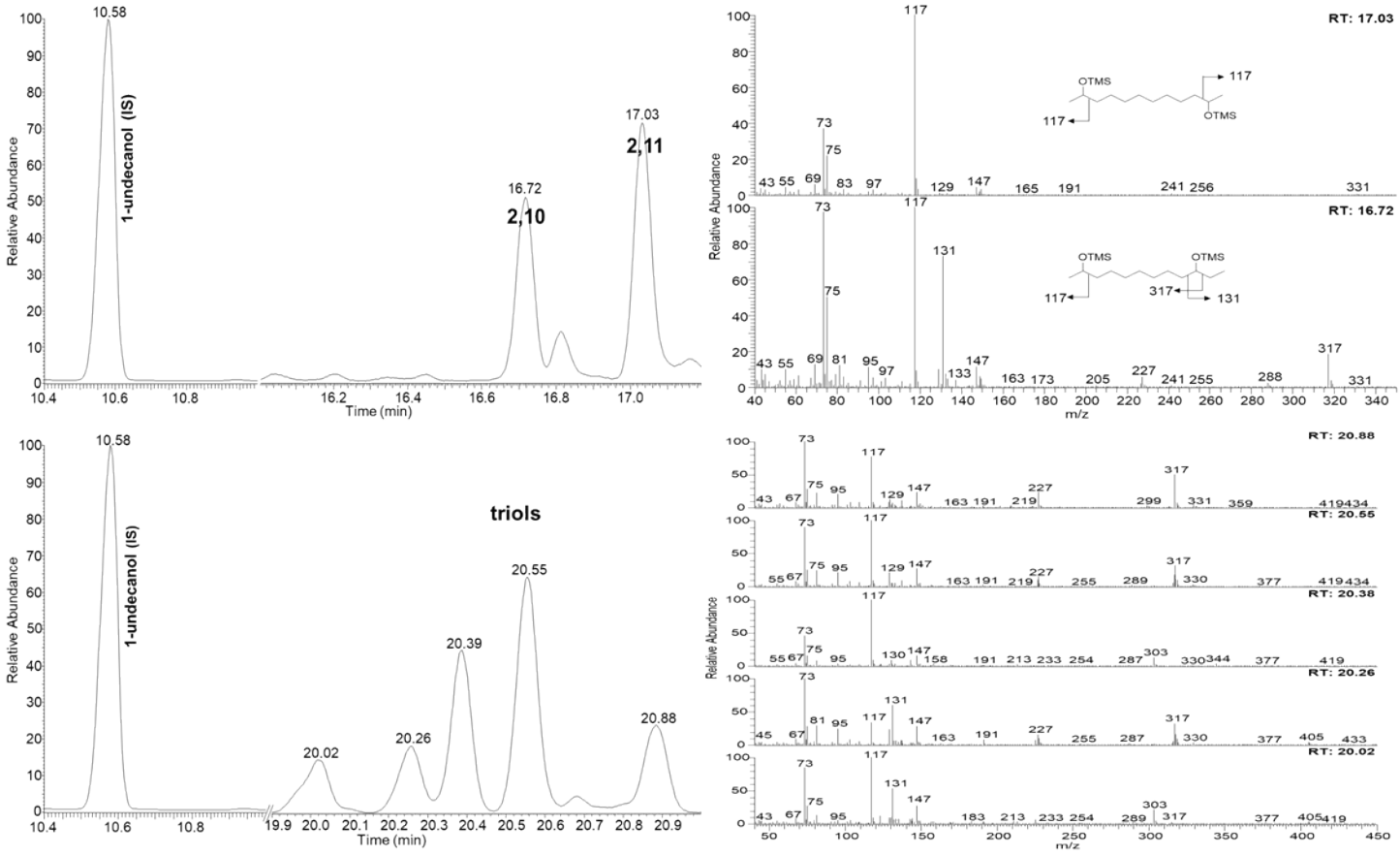
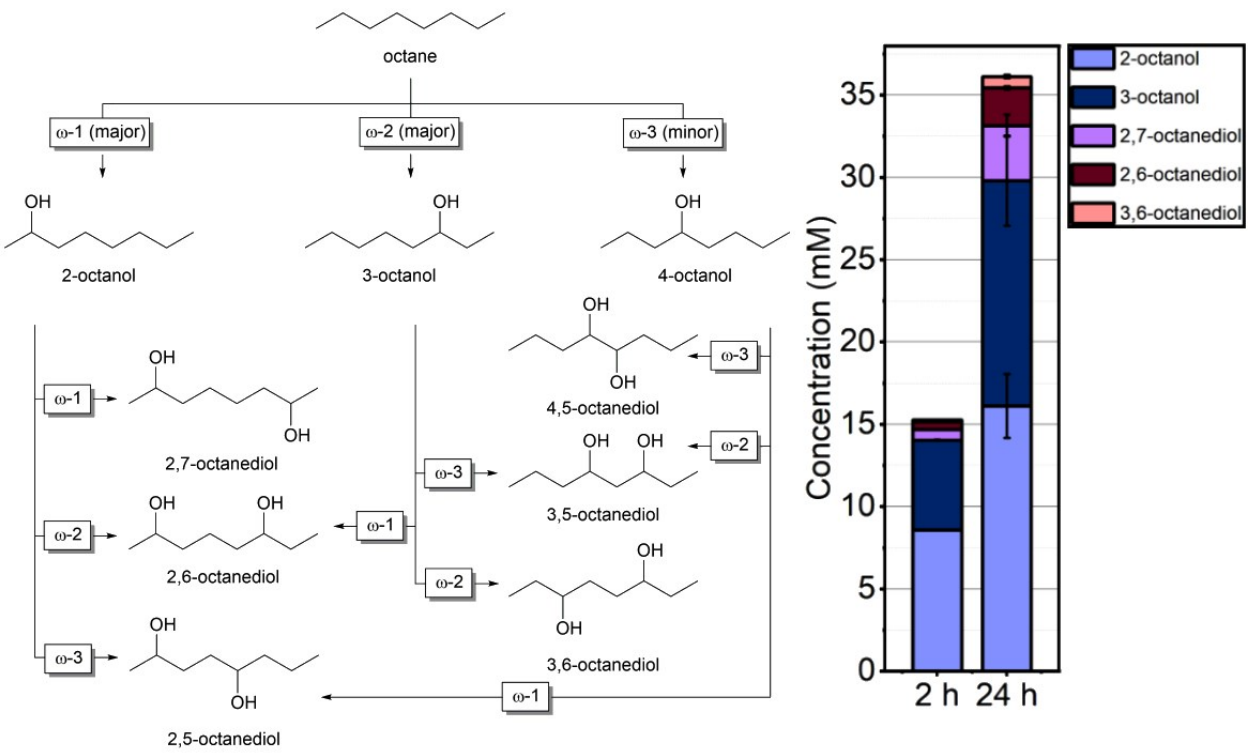
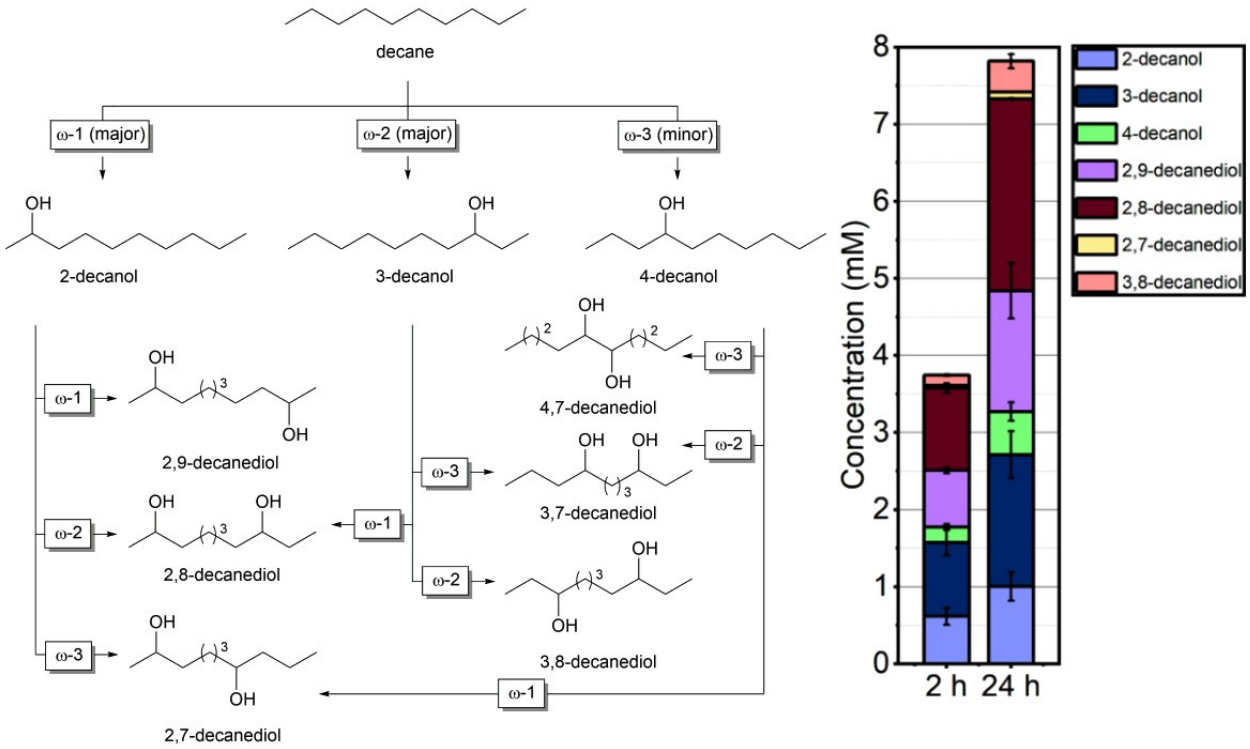


Figure S14. Cont. GC/MS chromatogram and spectra of diols and triols after 2 h reactions with CFE.



Scheme S1. Sequential regioselective oxyfunctionalization of *n*-octane by CYP505A30 in the production of diols. Total products formed (mM) after 2 h and 24 h reactions with CFE. The concentration of *n*-octanols were calculated using their respective standards. For octanediols, concentrations were calculated using 1,8-octanediol as standard.



Scheme S2. Sequential regioselective oxyfunctionalization of *n*-decane by CYP505A30 in the production of diols. Total products formed (mM) after 2 h and 24 h reactions with CFE. The concentration of *n*-decanols were calculated using 2-decanol as standards and 1,10-decanediol as standard for decanediols. [CYP505A30] = 4 µM.

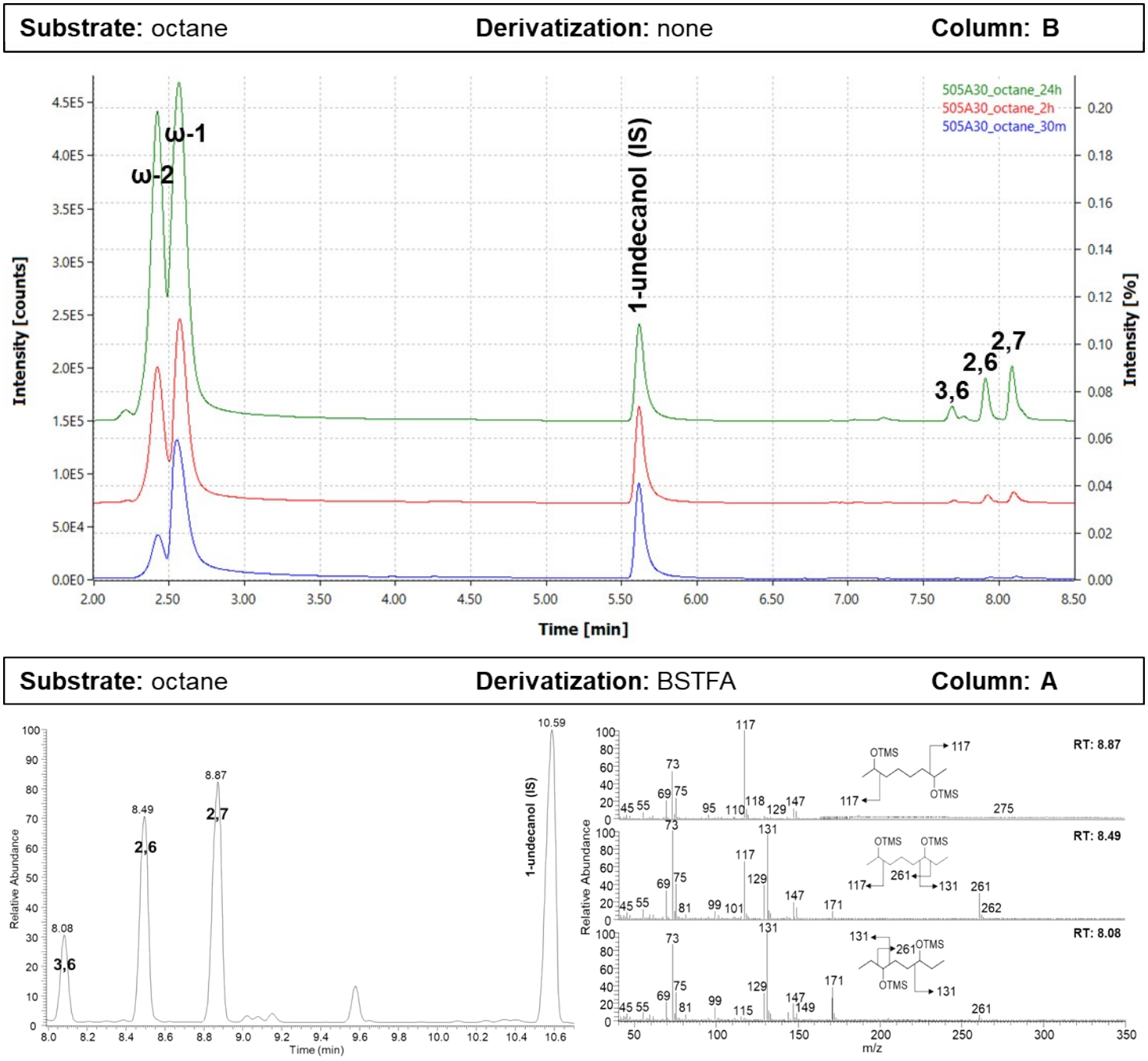
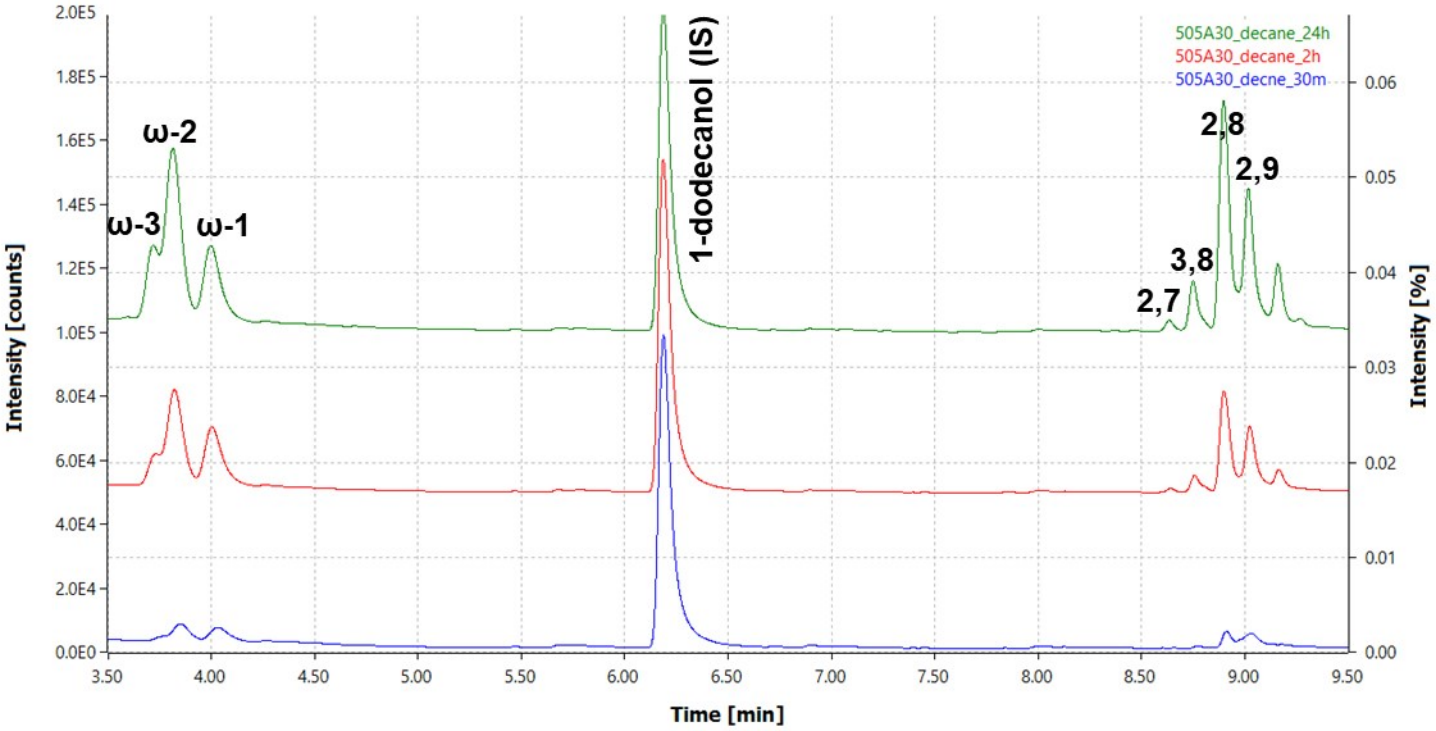


Figure S15. GC chromatograms for reactions with *n*-alkanes (C8 and C10) at 30 min, 2 h, and 24 h of CFE biotransformations. GC/MS chromatogram and spectra of the diols formed after 24 h CFE biotransformations.

Substrate: decane **Derivatization:** none **Column: B**



Substrate: decane **Derivatization:** BSTFA **Column: A**

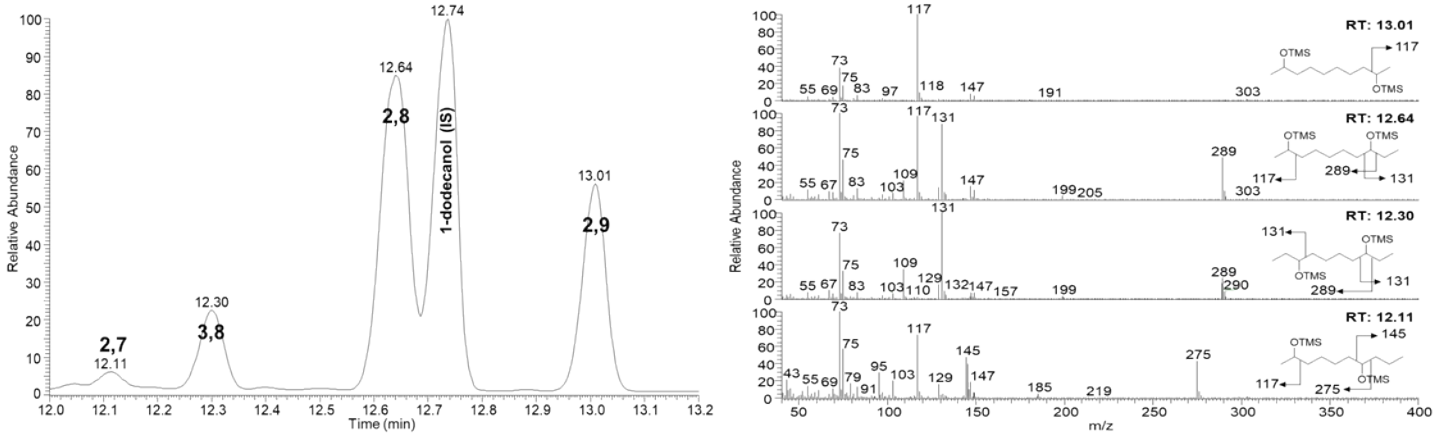


Figure S15. Cont.