SUPPLEMENTAL MATERIALS

Biochemical Characterization of CYP505D6, a Self-Sufficient Cytochrome P450 from the White-Rot Fungus *Phanerochaete chrysosporium*

Kiyota Sakai,[a] Fumiko Matsuzaki,[b,c] Lisa Wise,[a] Yu Sakai,[a] Sadanari Jindou,[d] Hirofumi Ichinose,[b] Naoki Takaya,[e] Masashi Kato,[a] Hiroyuki Wariishi,[f] Motoyuki Shimizu[a]#

[a] *Department of Applied Biological Chemistry, Faculty of Agriculture, Meijo University, Nagoya, Japan.*

[b] *Faculty of Agriculture, Kyushu University, Fukuoka, Japan.*

[c] *Research Center for Transomics Medicine, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.*

[d] *Faculty of Science and Technology, Meijo University, Nagoya, Japan.*

[e] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.*

[f] *Faculty of Art and Science, Kyushu University, Fukuoka, Japan.*

Running Head: Diverse hydroxylation of fatty compounds by CYP505D6

#Address correspondence to Motoyuki Shimizu, moshimi@meijo-u.ac.jp.

TABLE S1 Reaction products of saturated fatty alcohol (C9–18) and fatty acid (C9–18) conversions by CYP505D6

Substrate		Ratio of reaction product (%)						
		ω-1	ω-2	ω-3	ω-4	ω-5	ω-6	ω-7
Fatty alcohol								
C9	nonanol	11	19	46	12	8	4	-[a]
C10	decanol	10	16	42	19	8	5	-
C11	undecanol	13	20	22	25	12	8	-
C12	dodecanol	13	36	29	6	5	8	3
C13	tridecanol	16	28	21	18	12	15	-
C14	tetradecanol	21	23	26	11	11	8	-
C15	pentadecanol	24	34	21	12	6	3	-
C16	hexadecanol	43	29	17	11	-	-	-
C17	heptadecanol	54	24	15	7	-	-	-
C18	octadecanol	14	40	28	18	-	-	-
Fatty acid								
C9	nonanoic acid	9	38	20	18	11	4	-
C10	decanoic acid	17	15	45	13	6	4	-
C11	undecanoic acid	10	34	28	15	8	5	-
C12	dodecanoic acid	14	25	32	12	10	7	-
C13	tridecanoic acid	8	32	25	21	7	7	-

C14	tetradecanoic acid	17	16	26	13	14	14	-
C15	pentadecanoic acid	7	42	35	6	5	5	-
C16	hexadecanoic acid	56	36	5	3	-	-	-
C17	heptadecanoic acid	64	22	9	5	-	-	-
C18	octadecanoic acid	13	48	25	14	-	-	-

[a] Not detected. Data are presented as mean values of three independent experiments. The standard errors were <17%.

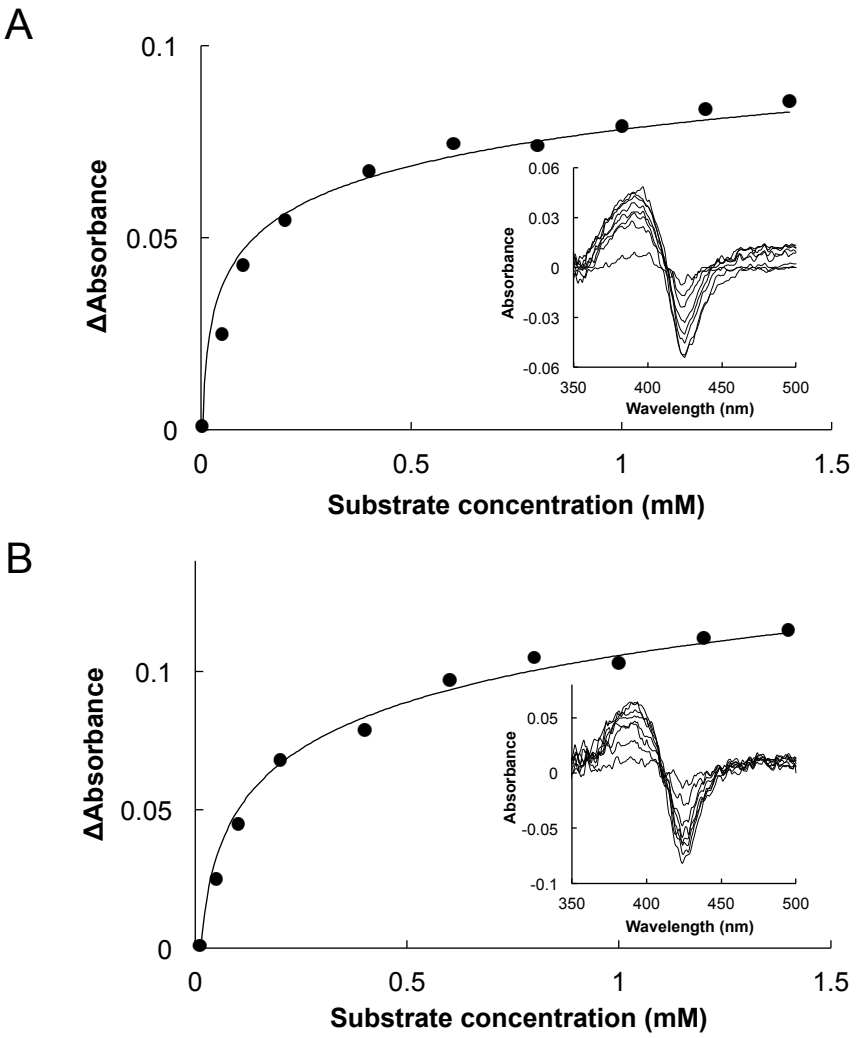

FIG S1 Absorbance spectral changes upon binding of 1-dodecanol (A) and dodecanoic acid (B) to CYP505D6. CYP505D6 in 50 mM sodium phosphate buffer (pH 7.5) was titrated with a freshly prepared solution of 1-dodecanol and dodecanoic acid, and the absorbance changes were recorded following each addition (1-dodecanol; 0–1.4 mM, dodecanoic acid; 0–1.4 mM, final concentration). Insets show the plots of substrate-induced absorption changes from difference spectra generated by subtraction of the starting spectrum from subsequent spectra collected at each point in the titration. In both cases, A_{390} minus A_{420} data from the difference spectra are plotted versus each relevant substrate. A fit of each data set to a rectangular hyperbola produces K_d values of 81.5 μM against 1-dodecanol and 155.8 μM against dodecanoic acid, respectively, for the binding to CYP505D6.

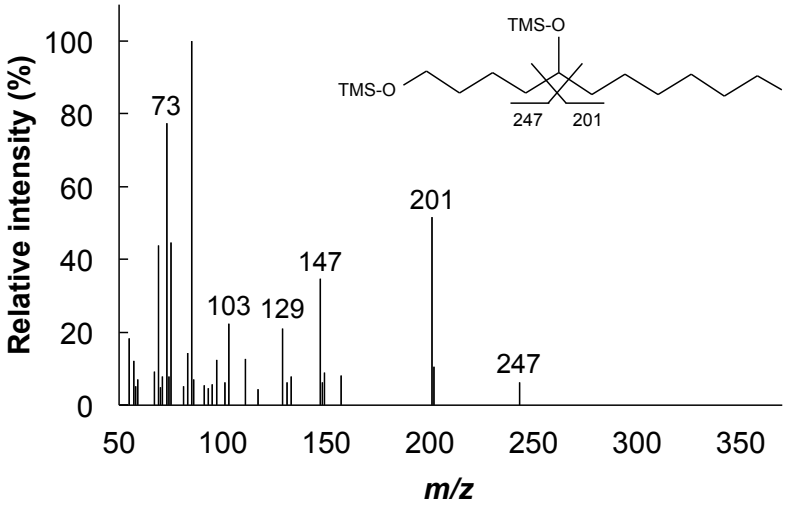

FIG S2 Mass spectrum of 1,5-dodecanediol generated from 1-dodecanol in a CYP505D6-catalyzed reaction. The mass spectrum was obtained from the GC peak at the retention time (35.11 min) shown in Fig. 4A.

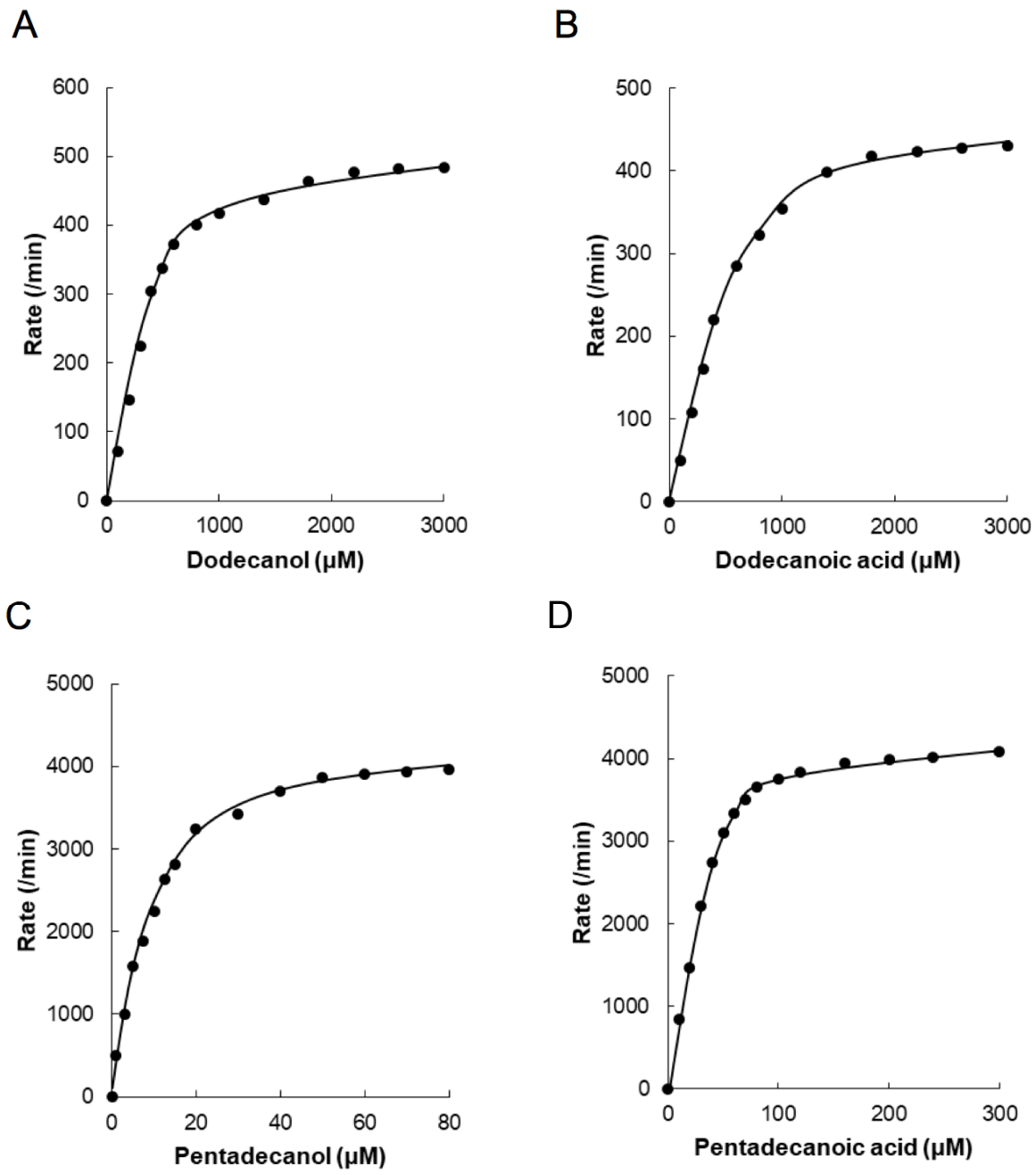

FIG S3 Steady-state kinetics of 1-dodecanol (A), dodecanoic acid (B), 1-pentadecanol (C), and pentadecanoic acid (D) reacted with CYP505D6. Rate of NADPH oxidation catalyzed by CYP505D6 in the presence of various concentrations of fatty alcohols and fatty acids (C12 and C15). K_m and k_{cat} values are described in Table 1.

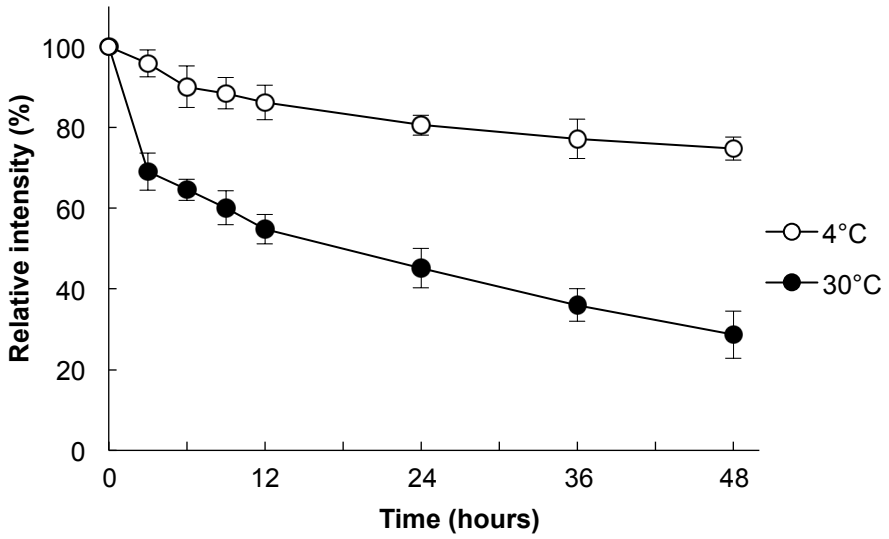

FIG S4 Thermostabilities of CYP505D6 at 4°C (open circles) and 30°C (closed circles). The thermostability of CYP505D6 was determined using dodecanoic acid as the substrate. Purified CYP505D6 was preincubated at 4°C or 30°C for 3, 6, 9, 12, 24, 36, or 48 h. Residual CYP505D6 activity was assayed in reaction mixtures (0.5 ml) containing 125 μM dodecanoic acid, 125 μM NADPH, 1 μM FMN, 1 μM FAD, 10% (v/v) glycerol, and purified enzyme in 50 mM sodium phosphate (pH 7.5). The activity of CYP505D6 without preincubation was set to 100%. Data are presented as mean values ± standard deviation (error bars) of three independent experiments.

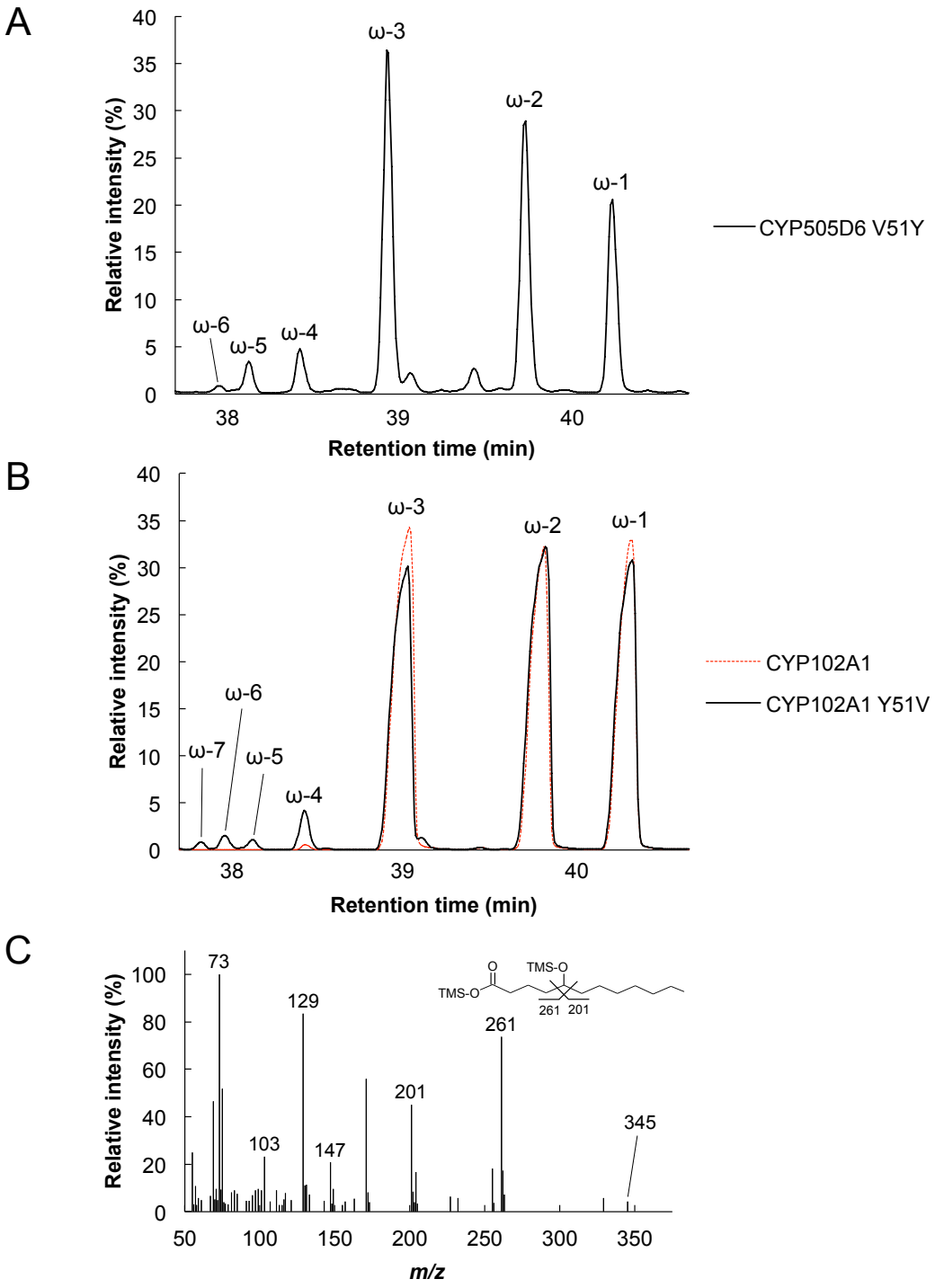

FIG S5 Total ion chromatograms and mass spectrum of the products of reactions catalyzed by CYP505D6 V51Y, CYP102A1, and CYP102A1 Y51V with dodecanoic acid as the substrate. The TMS derivation reaction products from reactions catalyzed by CYP505D6 V51Y (A), and CYP102A1 and CYP102A1 Y51V (B) were analyzed using GC-MS. The mass spectrum of 5-hydroxydodecanoic acid was obtained from the GC peak at the retention time (37.77 min) shown in Fig. S5B (C).