Supplementary Materials

Biosynthesis of Medium- to Long-chain α,ω-Diols from Free Fatty Acids Using CYP153A Monooxygenase, Carboxylic Acid Reductase and *E. coli* Endogenous Aldehyde Reductases

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1. Codon optimized nucleotide sequence of sfp

5′_ATGAAGATCTACGGTATCTACATGGACCGCCCTCTGTCTCAAGAGGAGAACGAGCGTTTCATG
ACTTTCATCTCCCCAGAGAAGCGTGACCGTTGTCGTCGTTTCTACCATAAGGAAGACGCACATCG
TACTCTGCTGGGTGATGTACTGGTACGTTCTGTTATCAGCCGTCAATACCAGCTGGACAAAAGCG
ACATCCGTTTCTCTACCCAGGAATACGGTAAACCGTGTATCCCTGACCTGCCAGATGCACACTTC
AATATCTCCCACTCTGGTCGCTGGGTTATCGGTCGCTGCGATTCTCAGCCGATTGGCATTGATATC
GAAAAAACCAAACCGATCTCTCTGGAAATCGCTAAACGCTTCTTCTCCAAAACCGAATACAGCG
ACCTGCTGGCTAAAGACAAAGACGAACAGACCGACTACTTCTATCACCTGTGGAGCATGAAAGA
AAGCTTTATTAAACAGGAAGGCAAAGGCCTGTCCCTGCCGCTGGATTCCTTTAGCGTGCGCCTGC
ACCAGGATGGCCAGGTCTCTATTGAACTGCCGGATTCTCACTCCCCGTGCTATATTAAAACCTAT
GAAGTGGACCCGGGCTACAAAATGGCGGTTTGCGCCGCGCACCCGGATTTTCCGGAAGATATTA
CGATGGTTTCCTATGAAGAACTGCTG_3′

2. Codon optimized nucleotide sequence of PPK2

5′_ATGCCGATGGTTGCTGCAGTTGAATTTGCTAAATCTCCGGCTGAAGTTCTGCGTGTTGGTTCAG
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GTAAATTCGGCAACCCGAAACGTCTGCTGCTGATTCTGCAGGCGATGGATACTGCGGGTAAAGG
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CGTATTGGGATGATTACATGGATGCTTACTCTGTAGCATTTGAAAAAACCAGCACCGAAATCGCT
CCGTGGCATGTAGTTCCGGCTAATAAAAAATGGTACGCACGCATCGCTGTTCAGCAGCTGCTGCT
GGATGCCCTGGGTGGTCTGCAGCTGGATTGGCCGAAAGCGGATTTTGATGTTGCTGCTGAACGTG
CTCTGGTTGTTGAATCT_3′

Table S1. Retention time of the substrates and products by Gas Chromatography (nonpolar capillary column-5% phenyl methyl siloxane capillary, HP-5) as described in Materials and Methods

Chain length / Substrate / Product	Retention time [min]				
	C_8	C_{10}	C_{12}	C_{14}	C_{16}
*Fatty acid	10.91	13.50	15.90	18.05	20.03
*ω-Hydroxy fatty acid	15.52	17.60	19.64	21.40	22.70
**Fatty alcohol	8.05	11.03	13.75	16.15	18.35
**Fatty aldehyde	-	-	12.75	-	-
**α,ω-diols	12.09	14.70	17.06	19.23	21.20
**Benzoic acid	10.70				
**Benzyl alcohol	7.48				
**Benzaldehyde	6.34				

-Not detected
*Derivatized with BSTFA
**Not derivatized with BSTFA

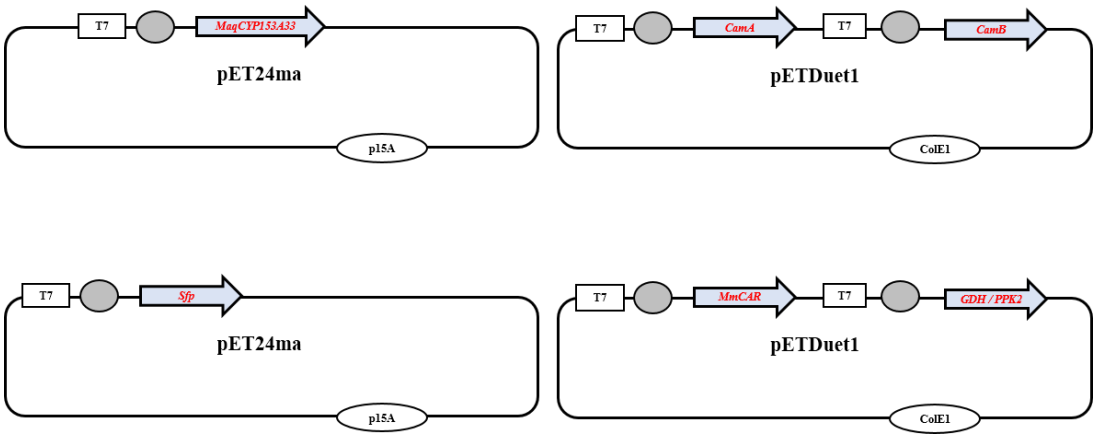

Figure S1. Schematic representation of plasmid system used in this study.

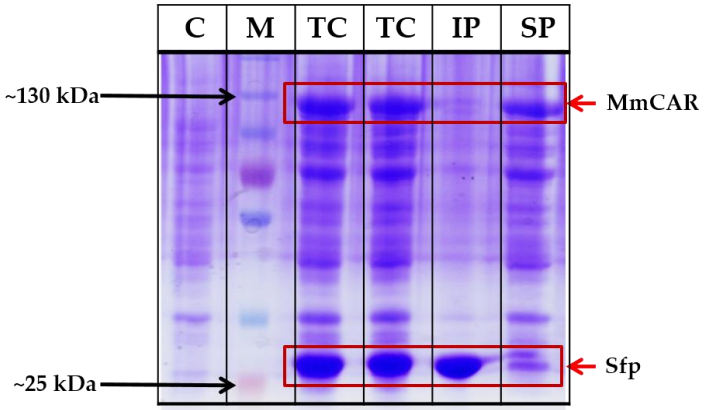

Figure S2. SDS PAGE analysis of recombinant *E. coli* expressing MmCAR (~128 kDa) and Sfp (~27.3 kDa). C, control; M, Marker; TC, total cells; IP, insoluble proteins; SP, soluble proteins. Protein expression was carried out using 0.01 mM IPTG at 20 °C and 200 rpm.

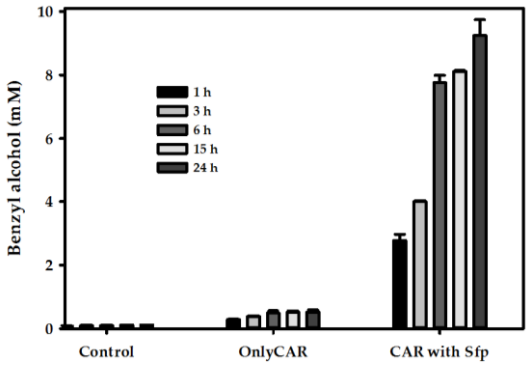

Figure S3. Effect of Sfp to make active MmCAR. Reaction conditions: *E. coli* BW25113 (ΔfadD, DE3) cells expressing MmCAR, with/without Sfp. Substrate, 10 mM benzoic acid; Volume, 10 mL in 100 mL flask; Temp, 30°C; Buffer, 100 mM potassium phosphate buffer (pH: 7.5) with 1% glucose (w/v) and 10 mM $MgCl_2$; Cell OD_{600}, 30. Control, cells carrying 'empty' pET24ma and pETDuet-1 plasmids (without CAR and sfp genes).

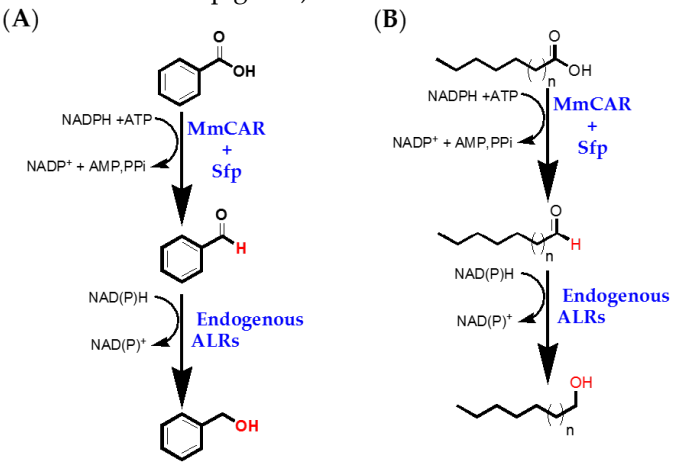

Figure S4. Proposed reaction scheme for the biosynthesis of benzyl alcohol and fatty alcohols (C_8-C_{16}).

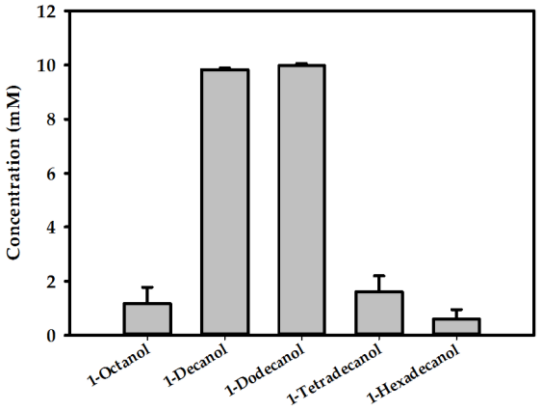

Figure S5. *In vivo* production of fatty alcohol using *E. coli* BW25113 (ΔfadD, DE3) cells expressing MmCAR, and Sfp. Reaction conditions: Substrate, 10 mM benzoic acid and FFAs (C_8-C_{16}); Volume, 10 mL in 100 mL flask; Temp, 30 °C; Buffer, 100 mM potassium phosphate buffer (pH: 7.5) with 1% glucose (w/v) and 10 mM $MgCl_2$; Cell OD_{600}, 30.

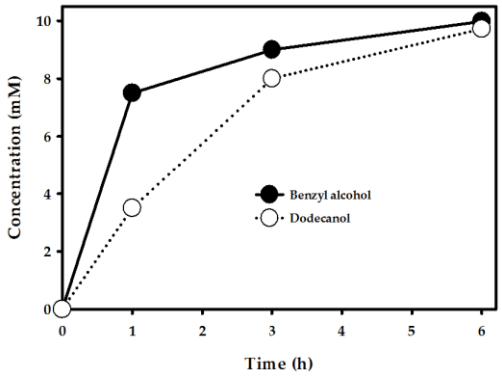

Figure S6. Production of alcohols by *E. coli* endogenous ALRs using *E. coli* BW25113 (ΔfadD, DE3). Reaction conditions: Substrate, 10 mM benzaldehyde and lauric aldehyde; Volume, 10 mL in 100 mL flask; Temp, 30 °C; Buffer, 100 mM potassium phosphate buffer (pH: 7.5) with 1% glucose (w/v); and Cell OD_{600}, 30.

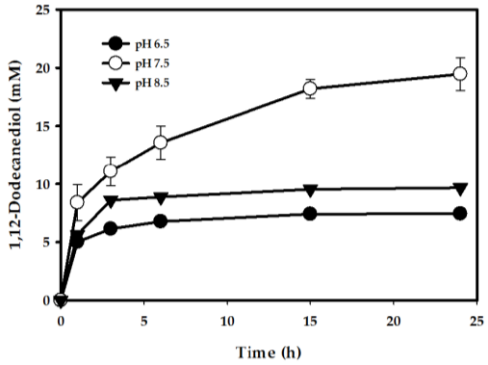

Figure S7. Effect of pH for the optimum activity of CAR using *E. coli* BW25113 (ΔfadD, DE3) cells expressing MmCAR, Sfp, and endogenous ALRs. Reaction conditions: Substrate, 30 mM ω-

ODDA; Volume, 10 mL in 100 mL flask; Temp, 30 °C; Buffer, 100 mM potassium phosphate buffer (pH: 6.5-8.5) with 1% glucose (w/v) and 10 mM $MgCl_2$; Cell OD_{600}, 30.

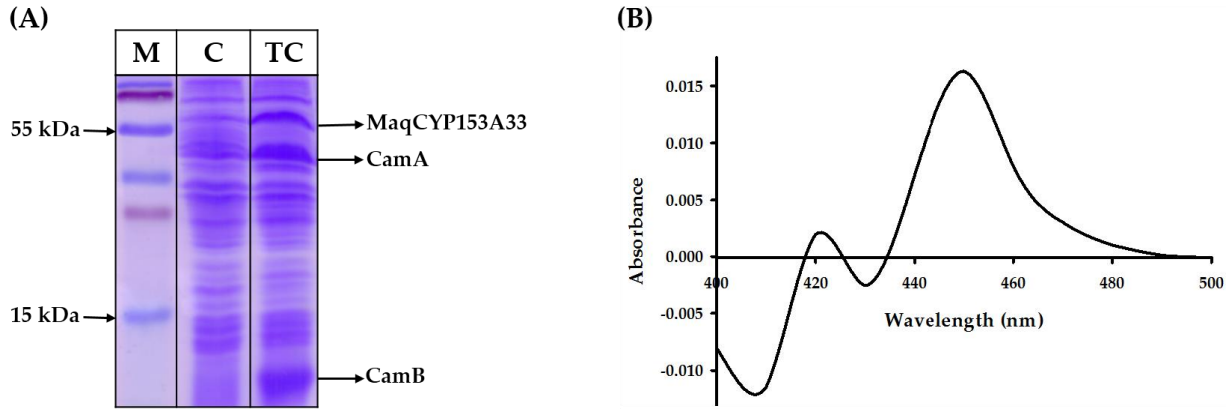

Figure S8. (A) SDS PAGE analysis of recombinant *E. coli* expressing MaqCYP153A33 (~55.2 kDa), CamA (~47.8 kDa), and CamB (11.6 kDa). C, control; M, Marker; TC, total cells. Protein expression was carried out using 0.01 mM IPTG, 0.5 mM 5-ALA, and 0.1 mM $FeSO_4$ at 30 °C and 200 rpm. **(B)** CO binding spectrum of active MaqCYP153A33.