

Supporting Information

A highly active and regioselective cannabigerolic acid synthase engineered from a promiscuous prenyltransferase NphB

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27 **Table of Contents**

28

29 **Steady-state concentration of CBGA**

30 **Synthesis of cannabigerol (CBG) and CBGA**

31

32 **Scheme S1.** Synthetic pathway for production of cannabinoids

33 **Figure S1.** Synthesis of CBGA using the wild-type NphB enzyme

34 **Figure S2.** NMR spectra

35 **Figure S3.** Fitting of kinetics data for NphB variants toward OA and GPP

36 **Figure S4.** Time-course of CBGA concentrations for the reactions catalyzed by NphB
37 variants

38 **Figure S5.** Root mean square deviation (RMSD) of backbone atoms during MD simulation
39 for quadruple variant modeled structure without substrates

40 **Figure S6.** RMSD of backbone atoms during MD simulation for quadruple variant
41 complexed with OA and GSPP

42

43 **Table S1.** Synthetic NphB genes used in this study

44 **Table S2.** Plasmids constructed in this study

45 **Table S3.** NMR peak assignments

46 **Table S4.** Kinetic parameters of NphB variants obtained using a three-dimensional
47 Michaelis-Menten equation

48

49 **References**

50

51 **Steady-state concentration of CBGA**

52 The dependence of the steady-state concentration of CBGA on the rate constant k_1 of forming
 53 CBGA from OA and GPP was analyzed as below:

54



55

56 where k_2 is the rate of the CBGA decomposition reaction, k_3 is the rate of the CBGA
 57 degradation, $[OA]_0$ and $[GPP]_0$ are initial concentration of the substrates

58

59 Rate of CBGA synthesis = $k_1[OA][GPP]$

60 Rate of CBGA decomposition = $k_2[CBGA] + k_3[CBGA]$

61

62 At the steady-state,

$$63 k_1[OA][GPP] = k_2[CBGA] + k_3[CBGA]$$

64

65 Replacing $[OA]$ and $[GPP]$ with $([OA]_0 - [CBGA])$ and $([GPP]_0 - [CBGA])$, respectively

$$66 k_1([OA]_0 - [CBGA])([GPP]_0 - [CBGA]) = k_2[CBGA] + k_3[CBGA]$$

$$67 k_1([OA]_0[GPP]_0 - ([OA]_0 + [GPP]_0)[CBGA] + [CBGA]^2) = k_2[CBGA] + k_3[CBGA]$$

68

69 Dividing both sides with k_1 ,

$$70 [OA]_0[GPP]_0 - ([OA]_0 + [GPP]_0)[CBGA] + [CBGA]^2 = (k_2 + k_3) / k_1[CBGA]$$

71

72 Dividing both sides with $[CBGA]$,

$$73 [OA]_0[GPP]_0 / [CBGA] - ([OA]_0 + [GPP]_0) + [CBGA] = (k_2 + k_3) / k_1$$

74

75 Defining the equilibrium constant K as k_2/k_1 ,

$$76 [OA]_0[GPP]_0 / [CBGA] + [CBGA] = K + k_3 / k_1 + [OA]_0 + [GPP]_0$$

77

78 Differentiating with respect to k_1 ,

$$79 \frac{d}{dk_1} \left(\frac{[OA]_0[GPP]_0}{[CBGA]} + [CBGA] \right) = \frac{d}{dk_1} \left(K + \frac{k_3}{k_1} + [OA]_0 + [GPP]_0 \right)$$

$$80 - \frac{[OA]_0[GPP]_0 d[CBGA]}{[CBGA]^2} + \frac{d[CBGA]}{dk_1} = - \frac{k_3}{k_1^2}$$

$$81 \frac{d[CBGA]}{dk_1} \left(1 - \frac{[OA]_0[GPP]_0}{[CBGA]^2} \right) = - \frac{k_3}{k_1^2}$$

$$82 \quad \frac{d[CBGA]}{dk_1} = \frac{\frac{k_3}{k_1^2}}{\frac{[OA]_0[GPP]_0}{[CBGA]^2} - 1}$$

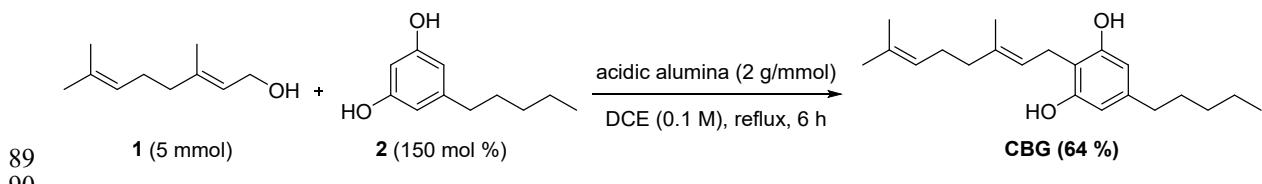
$$83 \quad \frac{d[CBGA]}{dk_1} = \frac{k_3[CBGA]^2}{([OA]_0[GPP]_0 - [CBGA]^2)k_1^2}$$

84 Since $[OA]_0[GPP]_0 - [CBGA]^2$ is positive,

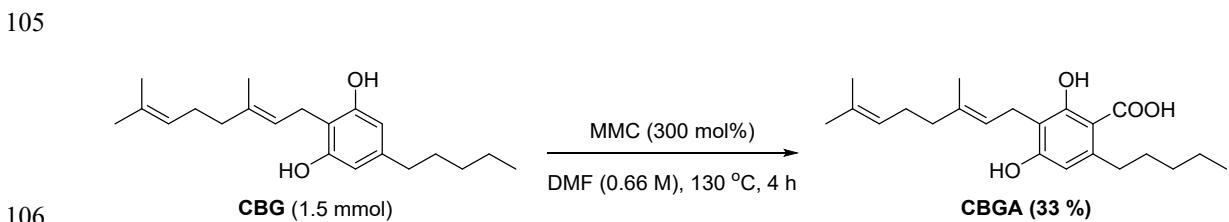
85 $\frac{d[CBGA]}{dk_1} > 0$ which indicates that the steady-state concentration of CBGA increases as k1
86 increases.

87

88 Synthesis of cannabigerol (CBG) and CBGA



A round-bottomed 2-neck flask equipped with a magnetic stirrer bar was charged with geraniol (0.867 mL, 5 mmol, 1.0 equiv.), olivetol (1.351 g, 7.5 mmol, 1.5 equiv.), acidic alumina (10 g, 2 g/mmol) and dichloroethane (50 mL, 0.1 M). The flask was fitted with a reflux condenser and heated to reflux temperature. The reaction was monitored by thin-layer chromatography (TLC) until the limiting reagent was completely consumed. After the reaction was complete, the mixture was cooled to room temperature and filtered through a Celite filter. The filter cake was rinsed with ethyl acetate, and the combined filtrate was concentrated under reduced pressure. The concentrated residue was purified by flash column chromatography (*n*-hexane: ethyl acetate = 20:1) to afford a white to yellow solid (1.156 g, 64 % yield); ¹H Nuclear Magnetic Resonance (NMR) (500 MHz, Chloroform-*d*) δ 6.25 (2H, s), 5.27 (1H, t, *J* = 7.1 Hz), 5.05 (1H, t, *J* = 6.8 Hz), 3.40 (2H, d, *J* = 7.0 Hz), 2.44 (2H, t, *J* = 7.9 Hz), 2.10 (2H, m), 2.06 (2H, m), 1.81 (3H, s), 1.68 (3H, s), 1.59 (3H, s), 1.56 (2H, m), 1.35 – 1.27 (4H, m), 0.89 (3H, t, *J* = 6.8 Hz). ¹³C NMR (126 MHz, Chloroform-*d*) δ 154.9, 142.9, 139.1, 132.2, 123.9, 121.9, 110.7, 108.5, 39.8, 35.6, 31.6, 3.09, 26.5, 25.8, 22.7, 22.4, 17.8, 16.3, 14.1.



107 A round-bottomed 2-neck flask equipped with a magnetic stirrer bar was charged with CBG
108 (542.2 mg, 1.5 mmol, 1.0 equiv.) and a 2.0 M solution of methyl magnesium carbonate (MMC,
109 2.25 mL, 3.0 equiv.) in dimethylformamide. The reaction mixture was heated to 130 °C in an
110 oil bath and stirred for 4 hr. The reaction was monitored by thin-layer chromatography (TLC)
111 until the limiting reagent was completely consumed. After the reaction was complete, the
112 reaction mixture was cooled to room temperature. It was then diluted with a mixture of
113 dichloromethane and methanol (1:1) and carefully quenched with a 10 % hydrochloric acid
114 solution to pH 2. Once the solid was fully dissolved, the reaction mixture was extracted with
115 excess diethyl ether, and the organic layer was evaporated under reduced pressure. The
116 residue was purified using flash column chromatography (*n*-hexane: ethyl acetate = 5:1, with
117 a small amount of formic acid) to afford a yellow solid (178.4 mg, 33 % yield); ¹H NMR (500
118 MHz, Chloroform-*d*) δ : 11.88 (s, 1H), 6.29 (s, 1H), 5.29 (t, 1H, *J* = 7.0 Hz), 5.07 (t, 1H, *J* = 6.5

119 Hz), 3.45 (d, 2H, J = 7.0 Hz), 2.88 (t, 2H, J = 7.5 Hz), 2.09 (m, 4H), 1.83 (s, 3H), 1.69 (s, 3H),
120 1.58 (m, 5H), 1.33 (m, 4H), 0.89 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ : 176.2, 163.7,
121 160.6, 147.6, 139.3, 132.1, 123.8, 121.4, 111.6, 111.4, 103.2, 39.8, 36.6, 32.1, 31.5, 26.5,
122 25.8, 22.6, 22.1, 17.8, 16.3, 14.1. Melting point 81.9–82.5 °C. LRMS (ESI-TOF) m/z [M+H]⁺
123 calculated for $\text{C}_{22}\text{H}_{32}\text{O}_4$ 361.49; found: 361.43.

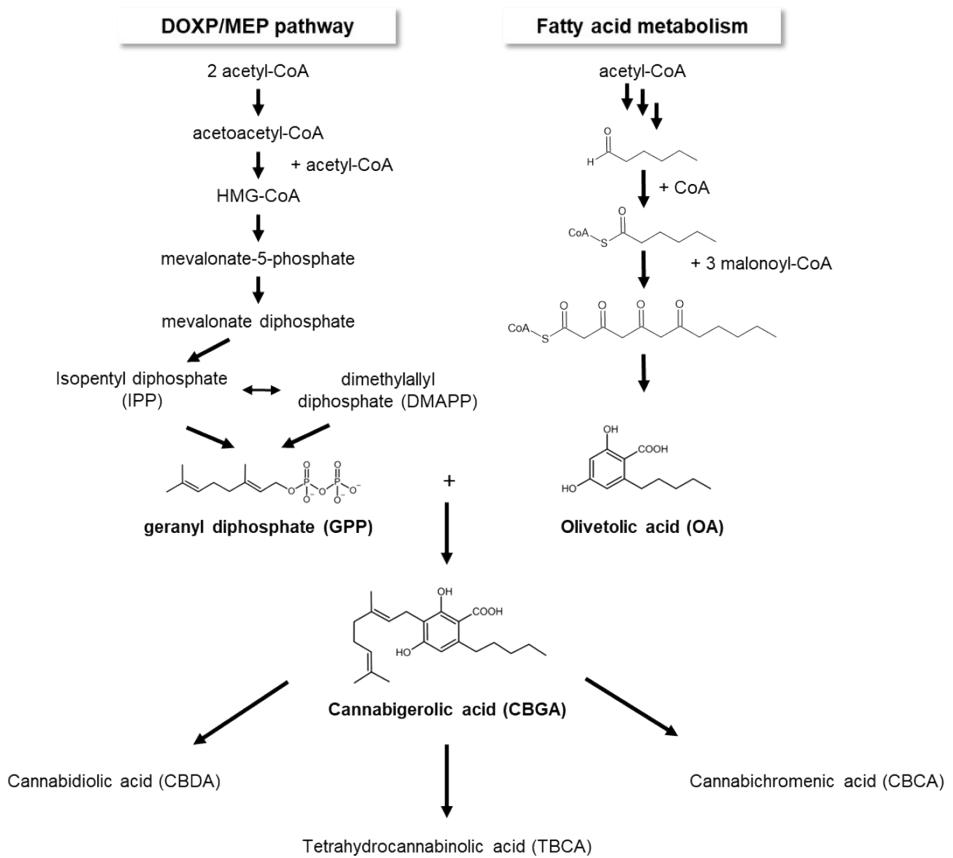
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125 CBG was characterized by ^1H - and ^{13}C NMR. CBGA was characterized by ^1H -, ^{13}C , HSQC,
126 and HMBC NMR. NMR spectra were recorded on a 500 MHz NMR instrument (JEOL, JNM-
127 ECZ500R). Chemical shifts for ^1H NMR are reported in δ units (parts per million, ppm) relative
128 to the residual signal of Chloroform-*d* (7.26 ppm). ^{13}C NMR spectra are reported in ppm
129 relative to Chloroform-*d* (77.16 ppm), with all measurements obtained with ^1H decoupling. ^1H -
130 and ^{13}C NMR Data of the CBGA compound were matched to previously reported paper¹.

131

132 **Scheme S1.** Biological pathways for the production of cannabinoids.

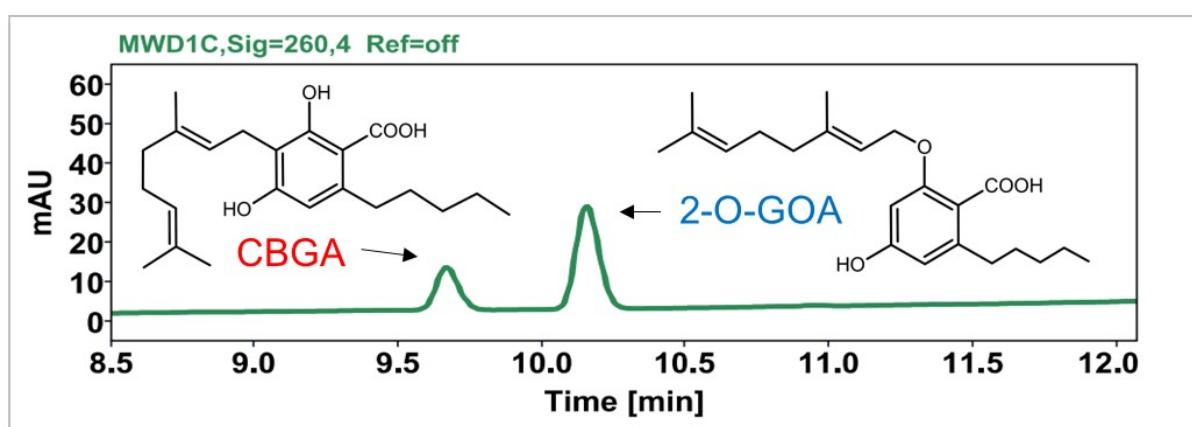
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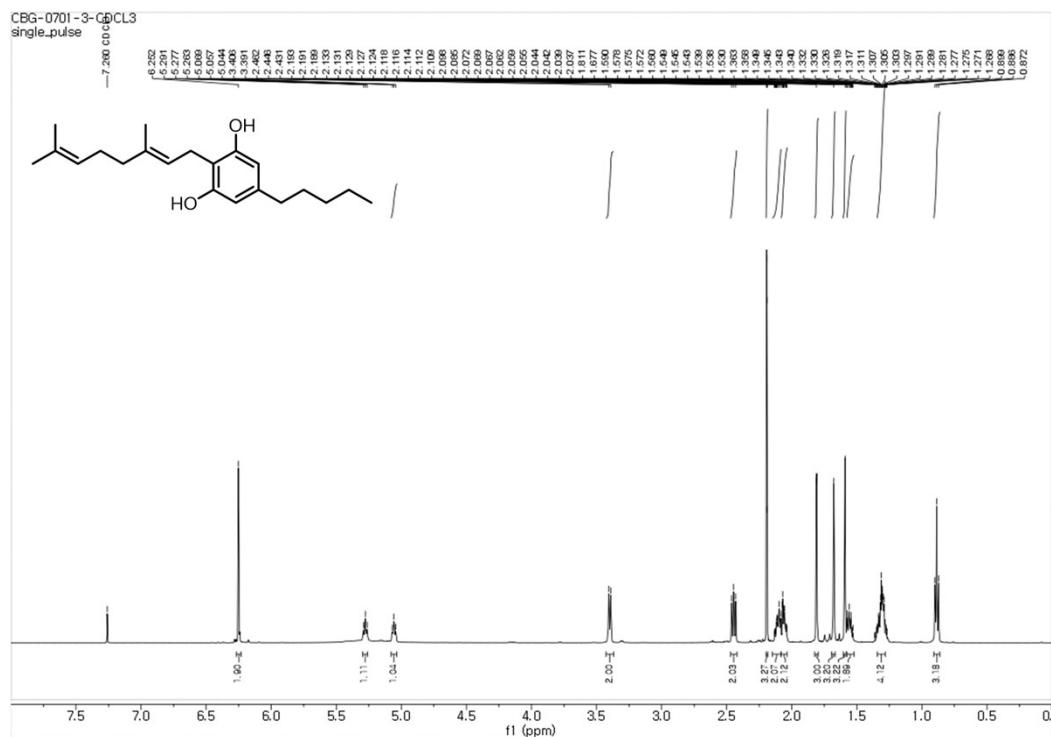
135

136 **Figure S1.** Synthesis of CBGA using the wild-type NphB enzyme. 5 μ M enzyme and 2 mM
137 substrates (OA and GPP) were incubated at 30 °C for 18 h. The peak for CBGA (~9.6 min)
138 was assigned using the chemically synthesized one (Figure S2). The wild-type enzyme
139 produces CBGA and 2-O-GOA, and the other peak (~10.2 min) was assigned to 2-O-GOA.
140



143 Figure S2. NMR spectra

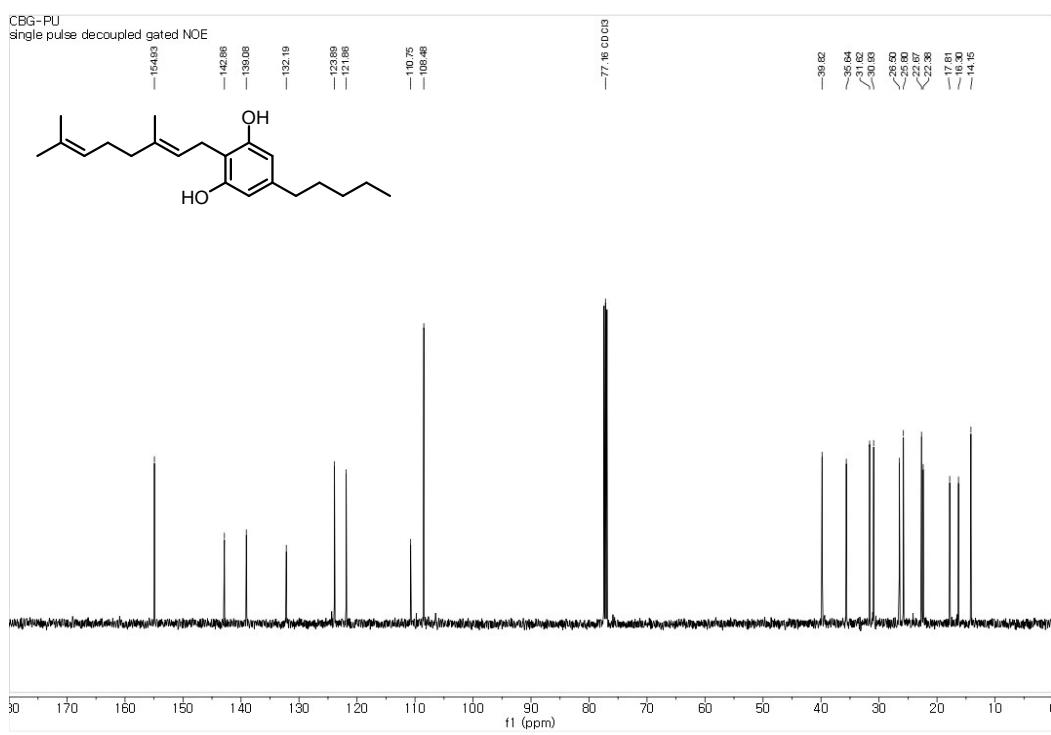
144 (A) ^1H NMR of CBG (500 MHz, Chloroform-*d*)



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147 (B) ^{13}C NMR of CBG (126 MHz, Chloroform-*d*)



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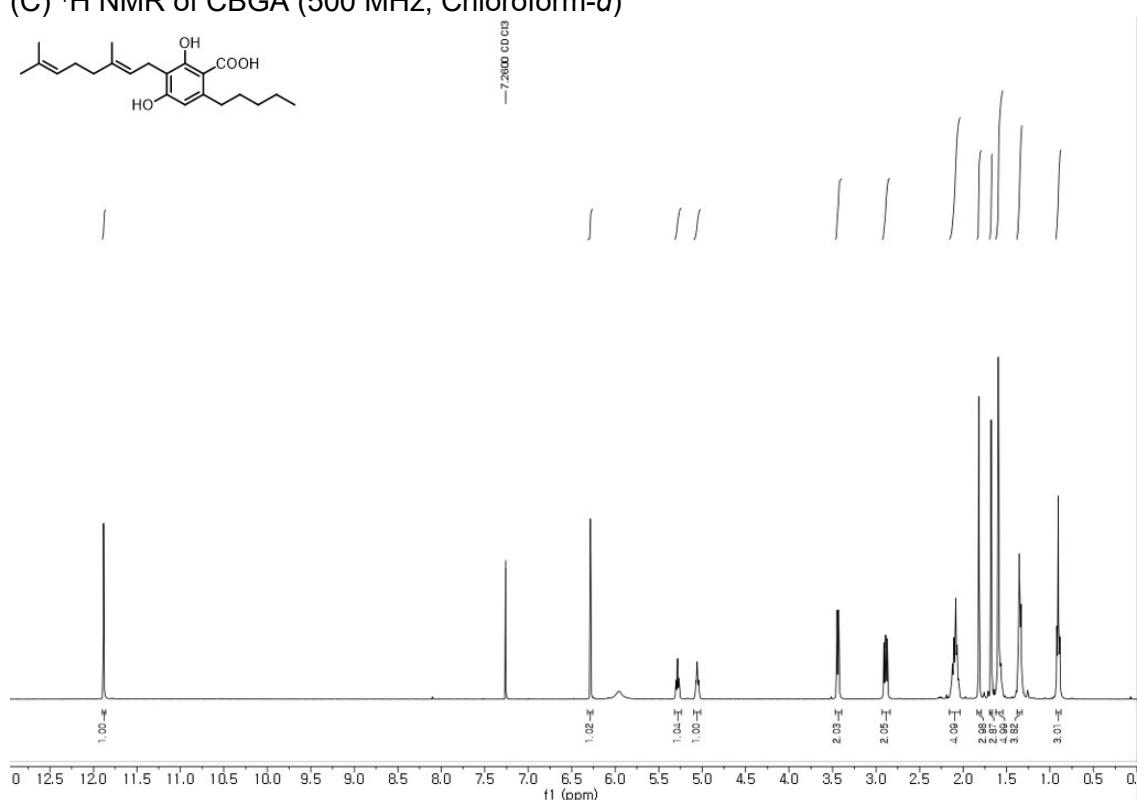
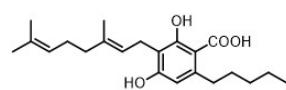
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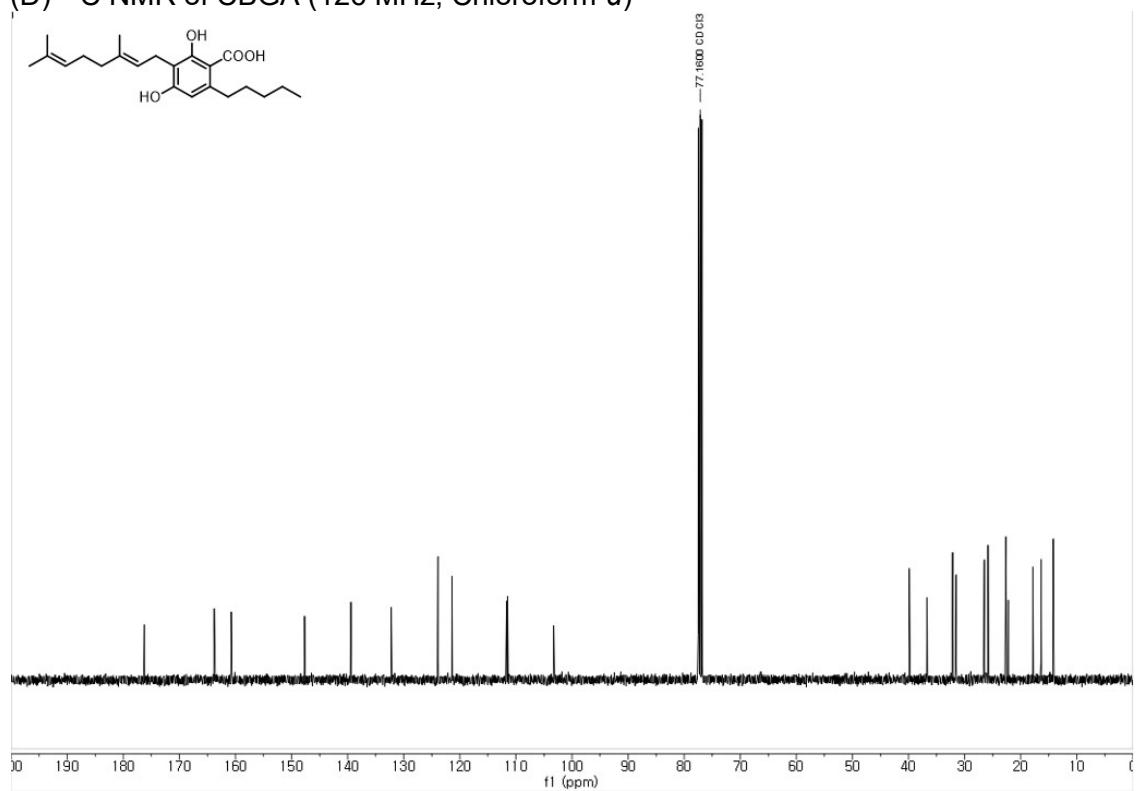
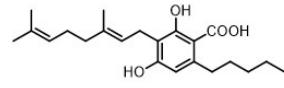
153 (C) ^1H NMR of CBGA (500 MHz, Chloroform-*d*)



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156 (D) ^{13}C NMR of CBGA (126 MHz, Chloroform-*d*)

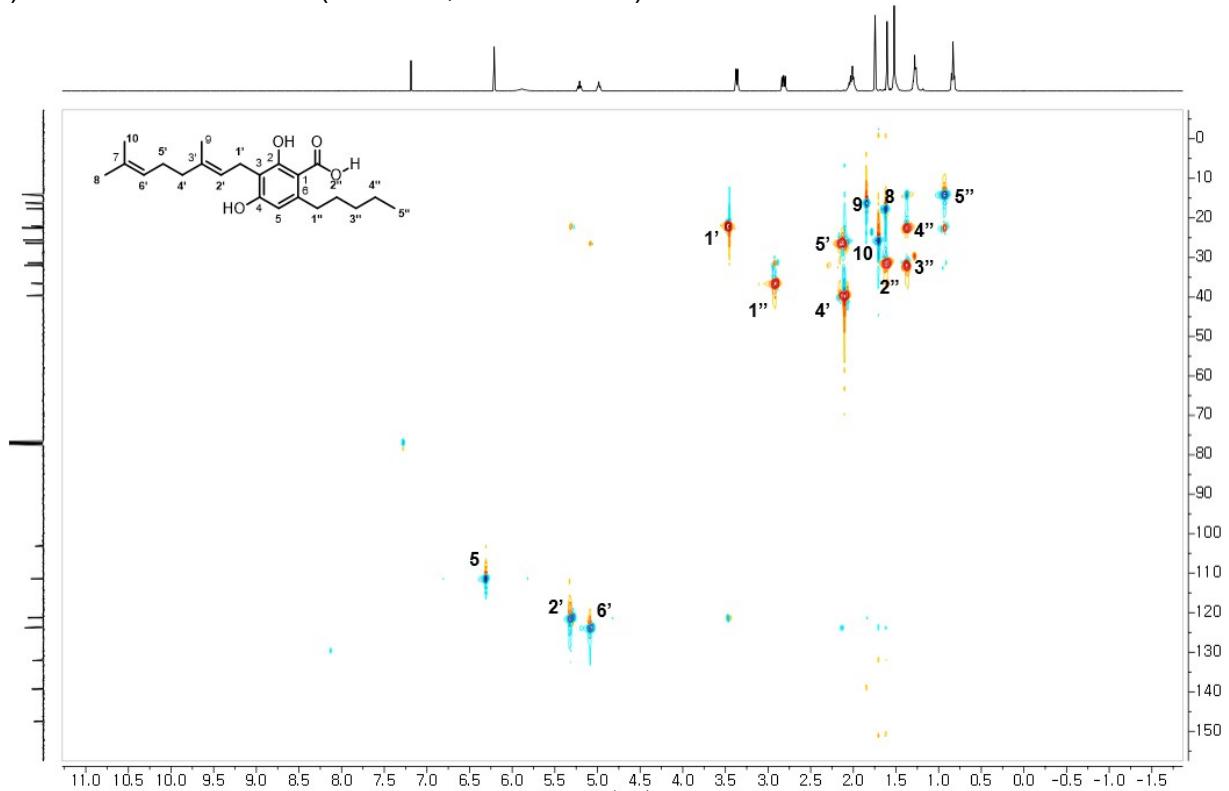


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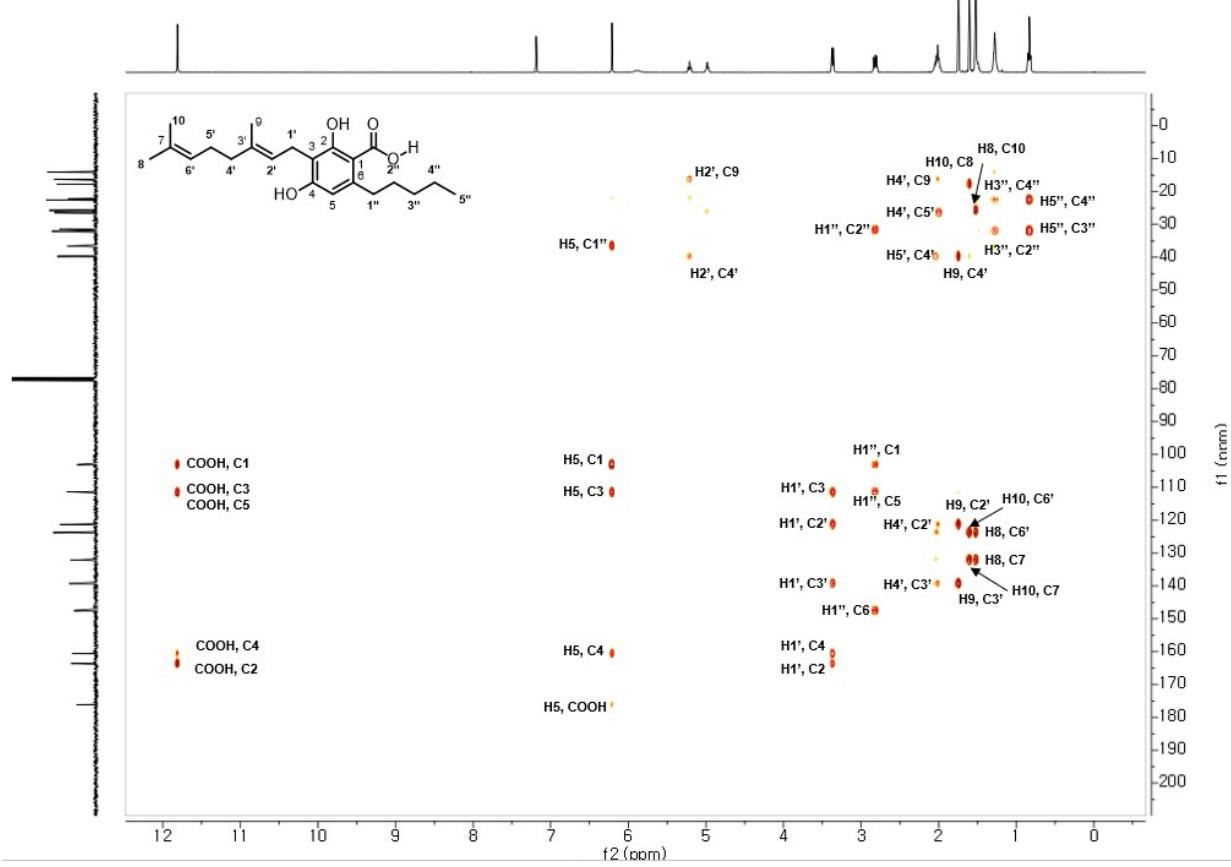
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161 (E) HSQC NMR of CBGA (500 MHz, Chloroform-*d*)

162

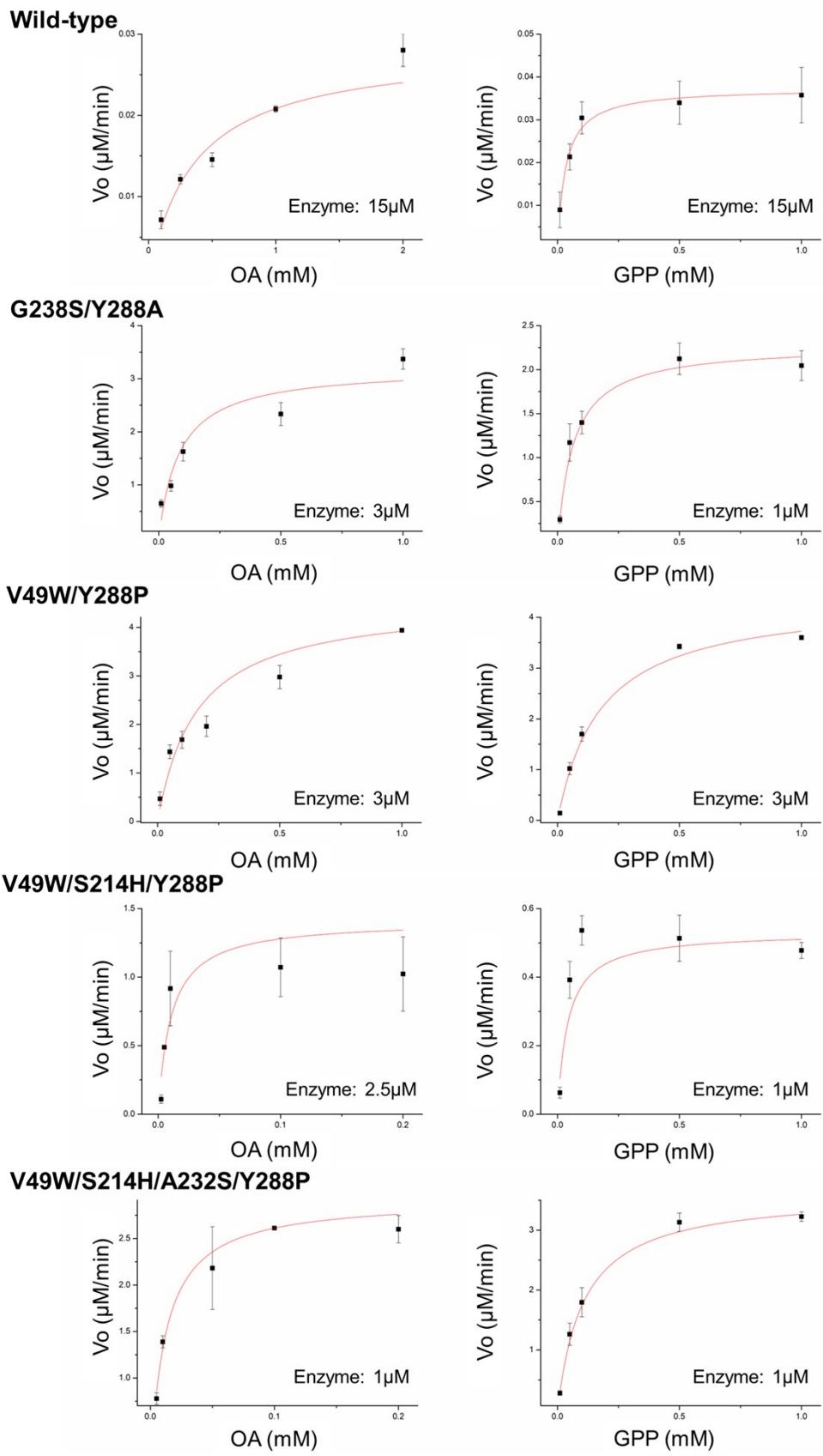
163

164 (F) HMBC NMR of CBGA (500 MHz, Chloroform-*d*)

165

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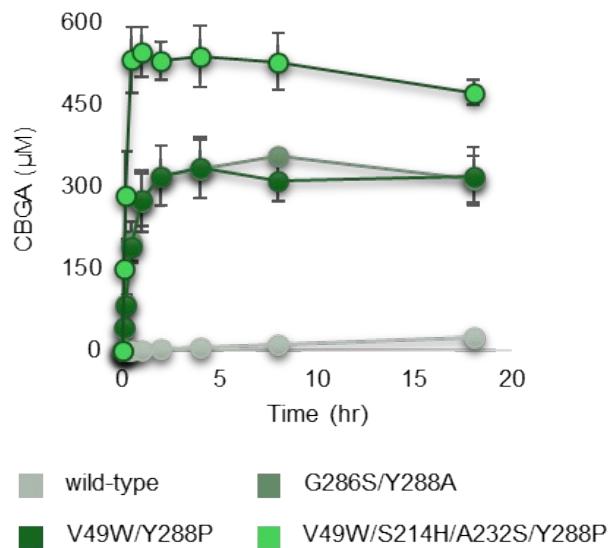
167 **Figure S3.** Fitting of kinetics data for NphB variants toward OA and GPP



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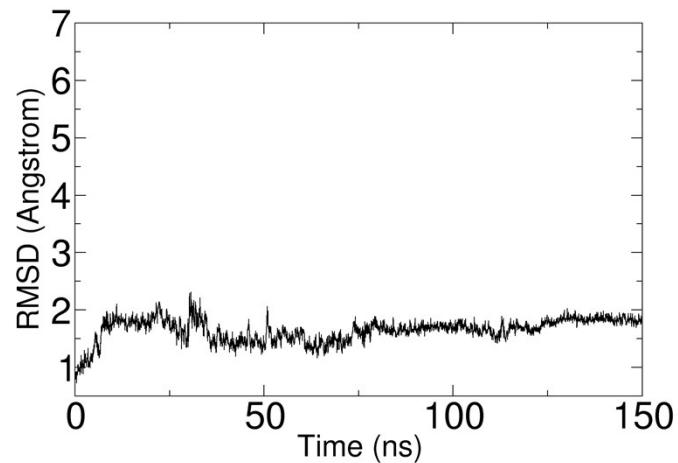
170 **Figure S4.** Time-course of CBGA concentrations for the reactions catalyzed by NphB
171 variants. 5 μ M of each enzyme was incubated with 2 mM OA and GPP at 30°C.



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173
174

175 **Figure S5.** Root mean square deviation (RMSD) of backbone atoms during MD simulation of
176 the quadruple variant without substrates.

177

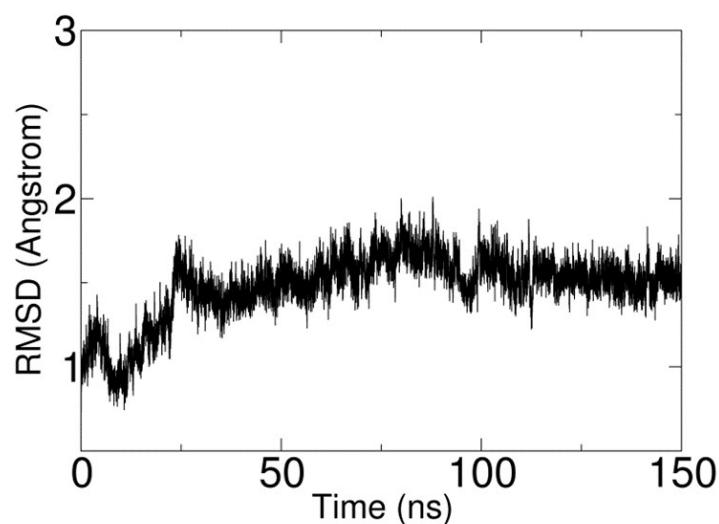


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180 **Figure S6.** RMSD of backbone atoms during MD simulation for the quadruple variant
181 complexed with OA and GSPP.

182



183

184

185 **Table S1.** Synthetic NphB genes used in this study.

186

Gene	Sequence (5'→3')
Wild-type NphB	CATATGAGTGAAGCGCGGATGTGGAACGTGTATCGGCATGGAAAG AAGCGGCTGGCTGCTGGGTGTGGCGTGTGCTCGTATAAAATCTATCC GCTGCTGAGCACCTTCAGGATACGCTGGTTGAAGGCCTCTGTGGTT GTCTCAGCATGGCCTCTGGCCCATAGTACCGAAGTGGATTAGTAT TTCCGTTCCGACGTCCCACGGTACCGTACCGCACCCTGGTTGAAAAA GGCCTTTCCGCCACGGGTACCGGTTGGATGACCTGCTGGCAGATA CCCCAAAACACCTGCCGGTCAGCATGTTGCTATTGACGCCGAAGTGAC CGGCCTTCAAGAAAACCTATGCGTTTCCCACCGATAACATGCCGG GTGTGCCGAACTGTCAGCAATCCCGTCATGCCGCCGGCAGTTGAGA AAATGCTGAAC TGTCGCGCTAACGGCCTGGATAAAGTTCAAGATGACCT CAATGGACTATAAAAACGCCAAGTCAACCTGTACTTAGTGAACGTCC GCCAGACCCCTGGAAAGCAGAACATCGGCTCTGGCTCTGGTGCCTGAAC GCCTGCATGTCCCAGTAAGTAACTGGGTCTGAAATTGCAAACGCTCATT TCGGTGTATCCGACCCCTGAAC TGGGAAACGGGCAAATTGATCGTCTG GTTTCGAGTGTATCCAATGACCCGACGCTGGTCCGAGCTCTGATGAA GGTACATCGAAAAATTCAACATGCAACCAAAGCTCCGTATGCGTA CGTTGCCGAAAAACGTACGCTGGTCTACGGTCTGACCCCTGAGCCCAGA GAAGAATATTACAAACTGGGTGCGTATTACACATTACGGACGTGCAACG CGGTCTGCTGAAAGCATTGACTCTCTGGAAAGATTGACTCGAG
NphB M1	AGTGAAGCGCGGATGTGGAACGTGTATCGGCATGGAAAG CGGCTGGCTGCTGGGTGTGGCGTGTGCTCGTATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTTGAAGGCCTCTGTGGT TTGGTTAGCATGGCCTCTGGCCCATAGTACCGAAGTGGATT GTATTCGTTCCGACGTCCCACGGTACCGTACCGCACCCTGGTT GAAAAAGGCCCTGTTCCGCCACGGGTACCGGTTGGATGACCTGCT GGCAGATAACCAAAACACCTGCCGGTCAGCATGTTGCTATTGACG GCGAAGTGACCCGGCGGTTCAAGAAAACCTATGCGTTTCCCAC GATAACATGCCGGGTGTGGCCGAAC TGTCAGCAATCCCGTCATGCC GCCGGCAGTTGCAGAAAATGCTGAAC TGTCGCGCTAACGGCCTG GATAAGTTCAAGTTCACCTCAATGGACTATAAAACGCCAAGTCAAC CTGTACTTAGTGAAC TGTCGCCCCAGACCCCTGGAAGCAGAAC CTGCTGAAATTGCAAACGCTCATTCTCGGTATCCGACCCCTGAA TGGGAAACGGGCAAATTGATCGTCTGTGTTCGAGCTGATCTCCAAT GACCCGACGCTGGTCCGAGCTGATGAAGGTGACATCGAAAAATT TCACAACATGCAACCAAAGCTCCGTATGCGTACGTTGGGAAAGA GTACGCTGGTCTACGGTCTGACCCCTGAGCCCAGAAGAAGAATATTAC AAACTGGCGCGCGTACCAACATTACGGACGTGCTGCCGGTCTGC TGAAAGCATTGACTCTCTGGAAAGATTGA
NphB M2	AGTGAAGCGCGGATGTGGAACGTGTATCGGCATGGAAAG CGGCTGGCTGCTGGGTGTGGCGTGTGCTCGTATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTTGAAGGCCTCTGTGGT TGTCTCAGCATGGCCTCTGGCCCATAGTACCGAAGTGGATT GTATTCGTTCCGACGTCCCACGGTACCGTACCGCACCCTGGTT GAAAAAGGCCCTGTTCCGCCACGGGTACCGGTTGGATGACCTGCT GGCAGATAACCAAAACACCTGCCGGTCAGCATGTTGCTATTGACG GCGAAGTGACCCGGCGGTTCAAGAAAACCTATGCGTTTCCCAC GATAACATGCCGGGTGTGGCCGAAC TGTCAGCAATCCCGTCATGCC GCCGGCAGTTGCAGAAAATGCTGAAC TGTCGCGCTAACGGCCTG GATAAGTTCAAGTTCACCTCAATGGACTATAAAACGCCAAGTCAAC CTGTACTTAGTGAAC TGTCGCCCCAGACCCCTGGAAGCAGAAC CTGCTGAAATTGCAAACGCTACCGGGTATCCGACCCCTGAA GGTCTGAAATTGCAAACGCTACCGGGTATCCGACCCCTGAA

	CTGGGAAACGGGCAAAATTGATCGTCTGTGTTCGCAGTGATCTCCA ATGACCCGACGCTGGTCCGAGCTCTGATGAAGGTGACATCGAAAAA TTTCACAACATATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAA CGTACGCTGGTCTACGGTCTGACCCCTGAGCCCCAAAAGAAGAATATT CAAACCTGGAAGCGTATTACCACATTACGGACGTGCAACGCGGTCTGC TGAAAGCATTGACTCTCTGGAAGATTGA
NphB M3	AGTGAAGCGGGGGATGTGGAACGTGTATGCGGCAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGT TGTCTTCAGCATGGCCTCTGGCCGCATAGTACCGAAGTGGATTAA GTATTTCCGTTCCGACGTCCCACGGTGACCCGTACCGGACCGTGGT GAAAAGGCCCTGTTCCGCCACGGGTACCGGATGACCTGCTGACCG GGCAGATACCCAAAAAACACCTGCCGGTCAGCATGTTGCTATTGACG GCGAAGTGACCGGCGGTTCAAGAAAACCTATGCGTTTCCCACCG GATAACATGCCGGGTGTGGCCGAACGTCACTGAGCAATCCGTCGATGCC GCCGGCAGTTGCAGAAAATGCTGAACGTTGCGCGTTACGGCCTG GATAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAAC CTGTACTTAGTGAACGTCCGCCACGGCTGGAAAGCAGAACCGT CCTGGCTCTGGTGCCTGAACTGGGCTGCATGTCGTTGACCG GGTCTGAAATTTCGAAACGCTCAATGCGTGTATCCGACCGTGAAC TGGGAAACGGGCAAAATTGATCGTCTGTGTTACCGTGTGATCTCCAAT GACCCGACGCTGGTCCGAGCTGTGATGAAGGTGACATGAAAGAATT TCACAACATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAAC GTACGCTGGTCTACGGTCTGACCCCTGAGCCCCAAAAGAAGAATATTAC AAACTGGCGGCGTATTACCACATTACGGACGTGCAACGCGGTCTGC GAAAGCATTGACTCTCTGGAAGATTGA
NphB M4	AGTGAAGCGGGGGATGTGGAACGTGTATGCGGCAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGT TATTTTCAGCATGGCCTCTGGCCGCATAGTACCGAAGTGGATTAA TATTTCCGTTCCGACGTCCCACGGTGACCCGTACCGGACCGTGGT AAAAGGCCCTGTTCCGCCACGGGTACCGGATGACCTGCTG GCAGATACCCAAAAAACACCTGCCGGTCAGCATGTTGCTATTGACGG CGAAGTGACCGGCGGTTCAAGAAAACCTATGCGTTTCCCACCG ATAACATGCCGGGTGTGGCCGAACGTCACTGAGCAATCCGTCGATGCC CCGGCAGTTGCAGAAAATGCTGAACGTTGCGCGTTACGGCCTGGA TAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAACCT GTACTTAGTGAACGTCCGCCACGGCTGGAAAGCAGAACCGT TGGCTCTGGTGCCTGAACTGGGCTGCATGTCGTTGACATGAACTGGG CTGAAATTTCGAAACGCTCATACGCGGTGTATCCGACCGTGAACG GGAAACGGGCAAAATTGATCGTCTGTGTTCGCAGTGTGATCTCCAATG ACCCGACGCTGGTCCGAGCTGTGATGAAGGTGACATGAAAGAATT CACAACATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACG TACGCTGGTCTACGGTCTGACCCCTGAGCCCCAAAAGAAGAATATTACA AACTGGCGGCGTATTACCACATTACGGACGTGCAACGCGGTCTGC AAAGCATTGACTCTCTGGAAGATTGA
NphB M5	AGTGAAGCGGGGGATGTGGAACGTGTATGCGGCAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTTGAAGGCGGTTCTGTGGT TATTTTCAGCATGGCCTCTGGCCGCATAGTACCGAAGTGGATTAA TATTTCCGTTCCGACGTCCCACGGTGACCCGTACCGGACCGTGGT AAAAGGCCCTGTTCCGCCACGGGTACCGGATGACCTGCTG GCAGATACCCAAAAAACACCTGCCGGTCAGCATGTTGCTATTGACGG CGAAGTGACCGGCGGTTCAAGAAAACCTATGCGTTTCCCACCG ATAACATGCCGGGTGTGGCCGAACGTCACTGAGCAATCCGTCGATGCC CCGGCAGTTGCAGAAAATGCTGAACGTTGCGCGTTACGGCCTGGA

	TAAAGTTCAGTTACCTCAATGGACTATAAAAACGCCAAGTCACCT GTACTTAGTGAACGTCCGCCAGACCTGGAAAGCAGAACCGTCC TGGCTCTGGTGCCTGAACCTGGGCTGCATGTCCCAGAACCTGGT CTGAAATTGCAAACGCTCATTGGCGGTATCGAACCTGAACCTG GGAAACGGGCAAATTGATCGTCTGTGTTCGCAGTGATCTCCAATG ACCCGACGCTGGTCCGAGCTCTGATGAAGGTGACATCGAAAAATT CACAACTATGCAACCAAAGCTCCGTATCGTACGTTGGCGAAAAACG TACGCTGGTCTACGGTCTGACCCCTGAGCCCCGAAAGAAGAATATTACA AACTGTGCGCGTATTACCACATTACGGACGTGCAACGCCGCTGCTG AAAGCATTGACTCTCTGGAAGATTGA
NphB M6	AGTGAAGCGCCGGATGTGGAACGTGTATGCCGCAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGGTGAAGGCGGTTCTGTGGT TGTCTTCAGCATGGCCTCTGGCCGATAGTACCGAACCTGGATTAA GTATTCGTTCCGACGTCCCACGGTGACCCGTACCGAACCGTGGT GAAAAGGCCTGTTCCGCCACGGGTATCCGGTGGATGACCTGCT GGCAGATACCCAAAAACACCTGCCGGTACGCATGTTGCTATTGACG GCGAAGTGACCGGGCGTTCAAGAAAACCTATGCGTTTCCCACC GATAACATGCCGGGTGTGGCCGAACGTCAAGCAATCCGTCGATGCC GCCGGCAGTTGCAGAAAATGCTGAACCTGTCGCGTACGGCCTG GATAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAC CTGTACTTAGTGAACGTCCGCCAGACCCCTGGAAGCAGAACCGT CCTGGCTCTGGTGCCTGAACCTGGGCTGCATGTCCCAGATGAACTG GGTCTGAAATTGCAAACGCTCATATTGGTGTATCCGACCCCTGAA TGGGAAACGGGCAAATTGATCGTCTGTGTTCGCAGTGATCTCCAAT GACCCGACGCTGGTCCGAGCTCTGATGAAGGTGACATCGAAAAATT TCACAACATGCAACCAAAGCTCCGTATCGTACGTTGGCGAAAAAC GTACGCTGGTCTACGGTCTGACCCCTGAGCCCCGAAAGAAGAATATTAC AAACTGGAAGCGTTTACACATTACGGACGTGCAACGCCGCTGCT AAAGCATTGACTCTCTGGAAGATTGA
NphB M7	AGTGAAGCGCCGGATGTGGAACGTGTATGCCGCAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGGTGAAGGCGGTTCTGTGGT TATTTTCAGCATGGCCTCTGGCCGATAGTACCGAACCTGGATTAA TATTTCCGTTCCGACGTCCCACGGTGACCCGTACCGAACCGTGGTT AAAAAGGCCTGTTCCGCCACGGGTATCCGGTGGATGACCTGCTG GCAGATACCCAAAAACACCTGCCGGTACGCATGTTGCTATTGACGG CGAAGTGACCGGGCGTTCAAGAAAACCTATGCGTTTCCCACCG ATAACATGCCGGGTGTGGCCGAACGTCAAGCAATCCGTCGATGCC CCGGCAGTTGCAGAAAATGCTGAACCTGTCGCGCGTACGGCCTGGA TAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAC GTACTTAGTGAACGTCCGCCAGACCCCTGGAAGCAGAACCGTCC TGGCTCTGGTGCCTGAACCTGGGCTGCATGTCCCAGATGAACTGGT CTGAAATTGCAAACGCTCATTGGTGTATCCGACCCCTGAACTGG GAAACGGGCAAATTGATCGTCTGTGTTCGCAGTGATCTCCAATGAC CCGACGCTGGTCCGAGCTCTGATGAAGGTGACATCGAAAAATTCA CAACTATGCAACCAAAGCTCCGTATCGTACGTTGGCGAAAAACGTA CGCTGGTCTACGGTCTGACCCCTGAGCCCCGAAAGAAGAATATTACAA CTGAGCGCGTATTACCACATTACGGACGTGGAACGCCGCTGCTGAA AGCATTGACTCTCTGGAAGATTGA
NphB M8	AGTGAAGCGCCGGATGTGGAACGTGTATGCCGCAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTCGTGTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGGTGAAGGCGGTTCTGTGGT TATTTTCAGCATGGCCTCTGGCCGATAGTACCGAACCTGGATTAA TATTTCCGTTCCGACGTCCCACGGTGACCCGTACCGAACCGTGGTT AAAAAGGCCTGTTCCGCCACGGGTATCCGGTGGATGACCTGCTG

	GCAGATACCCAAAAACACCTGCCGGTCAGCATGTTGCTATTGACGG CGAAGTGACCGGGCGTTCAAGAAAACCTATGCGTTTCCGACCG ATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCCTCGATGCCG CCGGCAGTTGCAGAAAATGCTGAACCTGTCAGCTGCCGGTTACGGCCTGGA TAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAACCT GTACTTAGTGAACCTGTCGCCAGACCCCTGGAAGCAGAACATCGGTCC TGGCTCTGGTGCCTGAACCTGGCCTGCATGTCCTGAATGAACCTGGGT CTGAAATTTGCAAACGCTCATTCCGTGTATCCGACCCCTGAACCTGG GAAACGGGCAAAATTGATCGTCTGTGTTCGCAGTGATCTCCAATGAC CCGACGCTGGTCCGAGCTCTGATGAAGGTGACATGCCAATTTCA CAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTA CGCTGGTCTACGGTCTGACCCTGAGCCGAAAGAAGAATATTACAAA CTGCAGGCGTTTACACATTACGGACGTGCAACGCCGGTCTGCTGAA AGCATTGACTCTCTGGAAGATTGA
NphB M9	AGTGAAGCGGGGATGTGGAACGTGTATGCCGAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTGAAGGCGGTTCTGTGGT TATGTTCACTGGCCTCTGGCCGACAGGTGACCCGTACGCGACCGTGGT GATTTCCGTTCCGACGTCCCACGGTACCGTGGATGACCTGCT GAAAAGGCCTGTTCCGGCACGGGTACCGGTTCCGACGGTGGATGACCTGCT GGCAGATAACCCAAAAACACCTGCCGGTACGATGTTGCTATTGACG GCGAAGTGACCGGGGTTCAAGAAAACCTATGCGTTTCCGACCC GATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCCTCGATGCC GCCGGCAGTTGCAGAAAATGCTGAACCTGTCAGCTGGCGTTACGGCCTG GATAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAAC CTGTACTTAGTGAACCTGTCGCCAGACCCCTGGAAGCAGAACATCGGT CCTGGCTCTGGTGCCTGAACCTGGCCTGCATGTCCTGAATGAACCTG GGTCTGAAATTTGCAAACGCTCATTCCGTGTATCCGACCCCTGAA CTGGGAAACGGGCAAAATTGATCGTCTGTGTTCGCAGTGATCTCCA ATGACCCGACGCTGGTCCGAGCTCTGATGAAGGTGACATGCCA TTTCACAACATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAA CGTACGCTGGTCTACGGTCTGACCCTGAGCCGAAAGAAGAATATT CAAACCTGGCGGGTATTACACATTACGGACGTGCTGCCGGTCTG TGAAAGCATTGACTCTCTGGAAGATTGA
NphB M10	AGTGAAGCGGGGATGTGGAACGTGTATGCCGAATGGAAGAAG CGGCTGGTCTGCTGGGTGTGGCGTGTGCTGATAAAATCTATCCG CTGCTGAGCACCTTCAGGATACGCTGGTGAAGGCGGTTCTGTGGT TATTTCACTGGCCTCTGGCCGACAGGTGACCCGTACGCGACCGTGGT TATTCGTTCCGACGTCCCACGGTACCGTGGATGACCTGCT AAAAAGGCCTGTTCCGGCACGGGTACCGGTTCCGACGGTGGATGACCTGCT GCAGATAACCCAAAAACACCTGCCGGTACGATGTTGCTATTGACGG CGAAGTGACCGGGGTTCAAGAAAACCTATGCGTTTCCGACCC ATAACATGCCGGGTGTGGCCGAACTGTCAGCAATCCCCTCGATGCC CCGGCAGTTGCAGAAAATGCTGAACCTGTCAGCTGGCGTTACGGCCTGGA TAAAGTTCACTTACCTCAATGGACTATAAAAACGCCAAGTCAACCT GTACTTAGTGAACCTGTCGCCAGACCCCTGGAAGCAGAACATCGGTCC TGGCTCTGGTGCCTGAACCTGGCCTGCATGTCCTGAATGAACCTGGGT CTGAAATTTGCAAACGCTCATTCTGGTGTATCCGACCCCTGAACCTG GAAACGGGCAAAATTGATCGTCTGTGTTCGCAGTGATCTCCAATGAC CCGACGCTGGTCCGAGCTCTGATGAAGGTGACATGCCA CAACTATGCAACCAAAGCTCCGTATGCGTACGTTGGCGAAAAACGTA CGCTGGTCTACGGTCTGACCCTGAGCCGAAAGAAGAATATTACAAA CTGGCGGGTATTACACATTACGGACGTGCAACGCCGGTCTGCTGAA AGCATTGACTCTCTGGAAGATTGA

189 **Table S2.** Plasmids constructed in this study. The backbone of the plasmids is pET28a.
190

Plasmids	Information
pSPEL1066	Wild-type NphB
pSPEL1067	NphB-V49W/Y288P
pSPEL1132	NphB-M1
pSPEL1133	NphB-M2
pSPEL1134	NphB-M3
pSPEL1135	NphB-M4
pSPEL1136	NphB-M5
pSPEL1137	NphB-M6
pSPEL1138	NphB-M7
pSPEL1139	NphB-M8
pSPEL1140	NphB-M9
pSPEL1141	NphB-M10
pSPEL1214	NphB-F213M
pSPEL1215	NphB-S214R
pSPEL1216	NphB-A232T
pSPEL1252	NphB-S214G
pSPEL1253	NphB-S214C
pSPEL1254	NphB-S214F
pSPEL1255	NphB-S214K
pSPEL1256	NphB-S214H
pSPEL1257	NphB-S214T
pSPEL1258	NphB-S214N
pSPEL1265	NphB-S214V
pSPEL1266	NphB-S214A
pSPEL1269	NphB-S214D
pSPEL1296	NphB-S214R/V288V
pSPEL1297	NphB-S214R/V288G
pSPEL1299	NphB-S214R/V288A
pSPEL1300	NphB-S214R/V288P
pSPEL1306	NphB-S214H/V288V
pSPEL1307	NphB-S214H/V288G
pSPEL1309	NphB-S214H/V288A
pSPEL1310	NphB-S214H/V288P
pSPEL1318	NphB-G286S/Y288A
pSPEL1337	NphB-V49W/S214H/V288P
pSPEL1352	NphB-S214H/V288P/A232S
pSPEL1353	NphB-S214H/V288P/A232T
pSPEL1355	NphB-S214H/V288P/A232N
pSPEL1358	NphB-V49W/S214H/A232S/V288P

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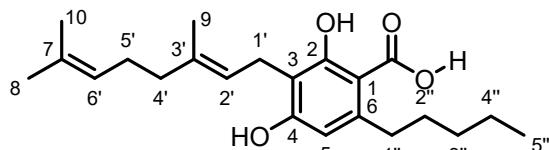
193 **Table S3.** NMR peak assignments

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195 (A) Structure of CBGA with atom numbering and $^1\text{H}/^{13}\text{C}$ NMR assignments

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No.	^{13}C (ppm)	^1H (ppm)
1	103.2	-
2	163.7	-
3	111.4	-
4	160.6	-
5	111.6	6.29 (s, 1H)
6	147.6	-
7	132.1	-
8	17.8	1.58 (m, 2H)
9	16.3	1.83 (s, 3H)
10	25.8	1.69 (s, 3H)
1'	22.1	3.45 (d, 2H, $J = 7.0$ Hz)
2'	121.4	5.29 (t, 1H, $J = 7.0$ Hz)
3'	139.3	-
4'	39.8	2.09 (m, 2H)
5'	26.5	2.09 (m, 2H)
6'	123.8	5.07 (t, 1H, $J = 6.5$ Hz)
1''	36.6	2.88 (t, 2H, $J = 7.5$ Hz)
2''	31.5	1.58 (m, 3H)
3''	32.1	1.33 (m, 4H)
4''	22.6	1.33 (m, 4H)
5''	14.1	0.89 (s, 3H)
COOH	176.2	11.88 (s, 1H)

209
 210 (B) Chemical shifts of 1D NMR signals and 2D NMR correlations of CBGA
 211

Atom	δ (ppm)	HSQC	HMBC
C1	103.2	-	1'', 5, COOH
C2	163.7	-	1', COOH
C3	111.4	-	5, 1'', COOH
C4	160.6	-	5, 1', COOH
C5	111.6	5	1'', COOH
H5	6.29 (s, 1H)	5	1, 3, 4, 1'', COOH
C6	147.6	-	1''
C7	132.1	-	8, 10
C8	17.8	8	10
H8	1.58 (m, 2H)	8	7, 10, 6'
C9	16.3	9	2', 4'
H9	1.83 (s, 3H)	9	2', 3', 4'
C10	25.8	10	8
H10	1.69 (s, 3H)	10	7, 8, 6'
C1'	22.1	1'	-
H1'	3.45 (d, 2H, J = 7.0 Hz)	1'	2, 3, 4, 2', 3',
C2'	121.4	2'	9, 1', 4'
H2'	5.29 (t, 1H, J = 7.0 Hz)	2'	9, 4',
C3'	139.3	-	9, 1', 4'
C4'	39.8	4'	9, 2, 5'
H4'	2.09 (m, 2H)	4'	9, 2', 3', 5'
C5'	26.5	5'	4'
H5'	2.09 (m, 2H)	5'	4'
C6'	123.8	6'	8, 10
H6'	5.07 (t, 1H, J = 6.5 Hz)	6'	-
C1''	36.6	1''	5
H1''	2.88 (t, 2H, J = 7.5 Hz)	1''	1, 5, 6, 2''
C2''	31.5	2''	1'', 3''
H2''	1.58 (m, 3H)	2''	-
C3''	32.1	3''	4''
H3''	1.33 (m, 4H)	3''	2'', 4''
C4''	22.6	4''	3'', 5''
H4''	1.33 (m, 4H)	4''	3''
C5''	14.1	5''	-
H5''	0.89 (s, 3H)	5''	3'', 4''
COOH	176.2	-	5
COOH	11.88 (s, 1H)	-	1, 2, 3, 4, 5,

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242 **Table S4.** Kinetic parameters of NphB variants obtained using a three-dimensional Michaelis-
243 Menten equation

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	k _{cat} (min ⁻¹)	K _m (OA) (mM)	K _m (GPP) (mM)
WT	0.0028 ± 0.00028	0.604 ± 0.1679	0.0208 ± 0.00651
G286S/Y288A	4.39 ± 0.935	2.26 ± 0.804	0.0386 ± 0.00762
V49W/Y288P	1.60 ± 0.112	0.204 ± 0.0425	0.130 ± 0.0294
V49W/S214H/Y288P	0.494 ± 0.0460	0.0336 ± 0.01545	0.0225 ± 0.01085
V49W/S214H/A232S/Y288P	3.04 ± 0.137	0.0129 ± 0.00317	0.0646 ± 0.01490

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246 The three-dimensional Michaelis-Menten equation is shown below:

$$247 v = \frac{k_{cat} \times [Enzyme]}{\left(1 + \frac{K_m(OA)}{[OA]}\right) \times \left(1 + \frac{K_m(GPP)}{[GPP]}\right)}$$

248

249 **References**

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251 1. L. Marchetti, V. Brighenti, M. C. Rossi, J. Sperlea, F. Pellati and D. Bertelli, *Molecules*, 2019, **24**.

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