

Supplementary Information

Improved selectivity of an engineered multi-product terpene synthase

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Figure S1 Comparison of the reactions of FPP (a) and **S1** (b) catalyzed by Cop2.

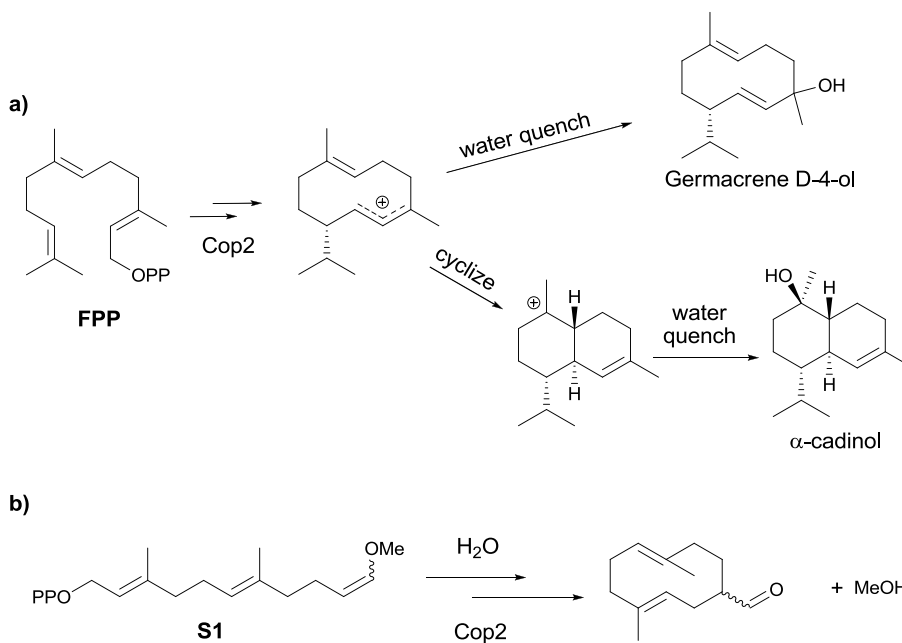


Figure S2 Product distributions in % with the enzymes indicated and 300 μM of FPP including error bars.

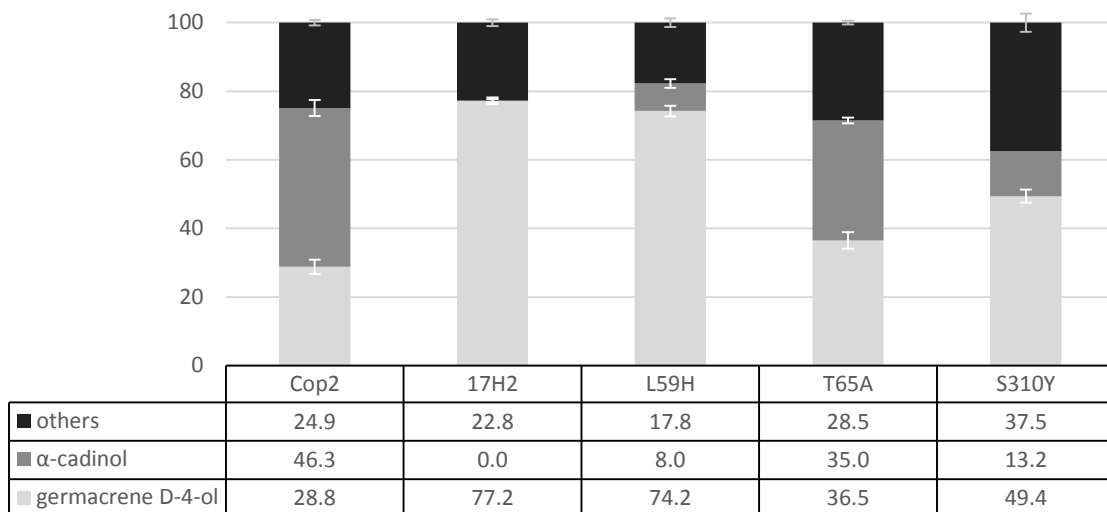


Figure S3 Product formation of Cop2 with FPP, using Michaelis-Menten kinetics for curve fitting with the software Prism.

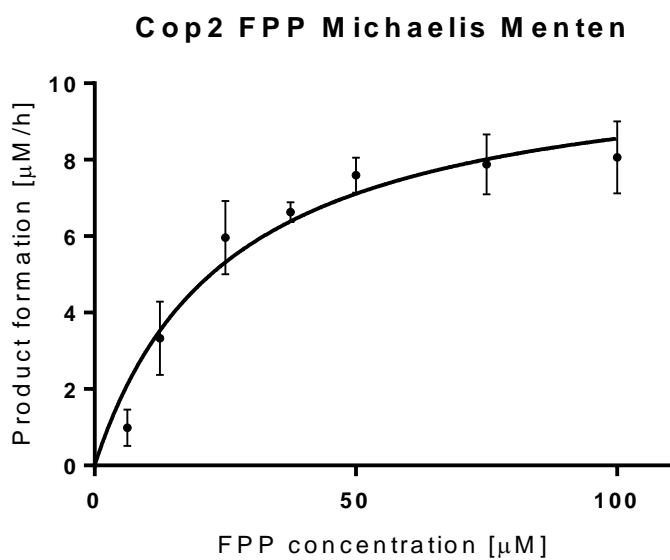
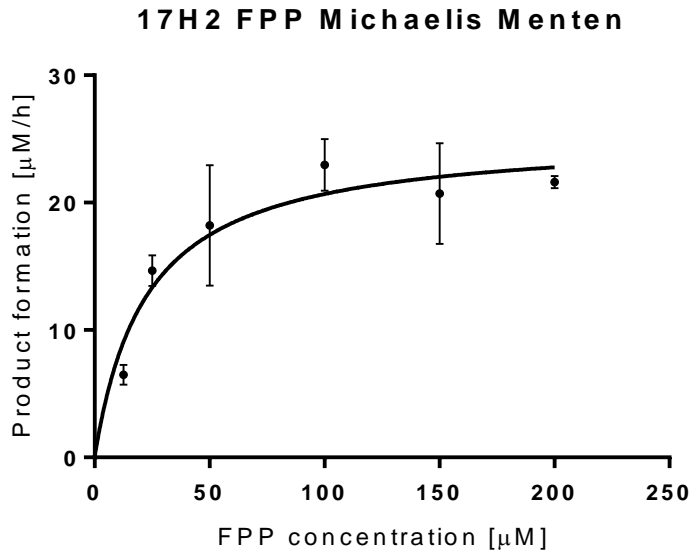


Figure S4 Product formation of 17H2 with FPP, using Michaelis-Menten kinetics for curve fitting with the software Prism.



Nucleotide and amino acid sequences (base pair mutations highlighted in yellow, amino acid mutations highlighted in green)

Figure S5 Nucleotide sequence of full-length, wild-type Cop2

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ATGCCAAGTCCCGCGGGAGCGCTTCCCAAGTCCTTCATACTCCCGACCTGGTCAACGACTGCCCATTCCTTCGAGTCAACC
CACTCTGCGACGAGGTTGGGAGACTATCCGAGCAATGGTTCCTCCGCTATGCAAATTATTCGCCCCCTCGGGCAGTGGCGTTTA
TGGCACTCAAAGCGGGCGAGCTCACTGCTGCATGCTACCCAGATGCCGATGCCTTCCATCTCCGAGTCAGCGACGACTTCATG
AACTTCTCTTCAATGCCGACGATTGGCTAGACGACTTCGACATTGAGGACACGTACGGGCTGGCCAATTGTACTGTTTCGAGCC
CTGCGCGACCCAGTCAACTTCATTACTGATAAACGCGCAGGACTAATGACGAAATCATATTTTTCTCGATTCTGAAGACCGCT
GGGCTCGCTGTACAGAGCGCTTTATCCAAACGTTGGCGCTCTACTTTGAATCAGTAGTAACGAAAAGCAAGCTCGTAACAA
TGGCACTTTGCCGATTTAGAGAGCTACATCACCATTGAAAGGAATAACAGCGGCTGTAAACCTTGTATGCACTCATCGAGTT
CTGTGCTGGCATTGATTTGCCGGACGAGGTTATCAATCACCAATTATTCAATCGCTCGAAGATGCTTGAATGACCTTATTGCT
TGGTGAACGATATCTTCTTTCAACAGGGAGCAATCTCGCCATGACAGCTTCAACATGGTTTCCATCGTTATGCACCAAGAG
GGCTTTGCGCTTCAGGAAGCTGTCAACTTTGTTGGAGAGCTTTGTAAGGCGATGGAACGTTTTCAGGCGGATAAGAGGA
ATCTCCCTTCATGGGGTCCAGAGATTGACGGGGAGGTCGCAATGTATGTTGATGGGCTGCAGAATTGGATAGTCGGGTCTCTA
AATTGGTCCATCGACGGGACAGAGCGCTATTTCCGCAAAGACGGGCTGGAATAAAGAAGCATAGAATAGTCAAGCTATTCC
CCAAGCGCCATTGAAAACACCTGCTGTGCGGGTTTTGCCTAA
    
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Figure S6 Amino acid sequence of full-length, wild-type Cop2

MHHHHHHPSPAGALPKSFILPDLVNDCPFPLRVNPLCDEVGRLSEQWFLRYANYSPRAVAF
MALKAGELTAACYPDADAFHLRVSDDFMNFLFNADDWLDDFDIEDTYGLANCTVRALRDPVNF
ITDKRAGLMTKSYFSRFLKTAGPRCTERFIQTLALYFESVVTQKQARNNGTLPDLESYITIRRNN
SGCKPCYALIEFCAGIDLDPDEVINHPIIQSLEDASNDLIAWSNDIFSFNREQSRHDSFNMVSIVMH
QKGFALQEAVNFVVGELCKKAMERFQADKRNLP SWGPEIDGEVAMYVDGLQNWIVGSLNWSID
GTERYFGKDGPGIKKHRIVKLFPRPLKTPAVRVLA-

Figure S7 Nucleotide sequence of full-length 17H2

ATGCCAAGTCCCGCGGGAGCGCTTCCAAGTCCTCATACTCCCGACCTGGTCAACGACTGCCATTCCCCCTTCGAGTCAACC
CACTCTGCGACGAGGTTGGGAGACTATCCGAGCAATGGTTCCTCCGCTATGCAAATTATTCGCCCCCTCGGGCAGTGGCGTTTA
TGGCACAAAGCGGGCGAGCTCTGCTGCTGCTACCCAGATGCCGATGCCTTCCATCTCCGAGTCAGCGACGACTTCATG
AATTCCTCTTCAATGCCGACGATTGGCTAGACGACTTCGACATTGAGGACACGTACGGGCTGGCCAATTGACTGTTTCGAGCC
CTGCGCGACCCAGTCAACTTCATTACTGATAAAGCGCGAGGACTAATGACGAAATCATATTTTTCTCGATTCTGAAGACCGCT
GGGCCTCGCTGTACAGAGCGCTTTATCCAACGTTGGCGCTCTACTTTGAATCAGTAGTAACGCAAAGCAAGCTCGTAACAA
TGGCACTTTGCCGATTTAGAGAGCTACATCACCATTGGAAGGAATAACAGCGGCTGTAAACCTTGTATGCACTCATCGAGTT
CTGTGCTGGCATTGATTTGCCGACGAGGTTATCAATCACCCAATTATTCAATCGCTCGAAGATGCTCGAATGACCTTATTGCT
TGGTGAACGATATCTTCTTTCAACAGGGAGCAATCTCGCCATGACAGCTTCAACATGGTTTCCATCGTTATGCACCAGAAG
GGCTTTGCGCTTCAGGAAGCTGTCAACTTTGTTGGAGAGCTTTGTAAGGCGATGGAACGTTTTTCAGGCGGATAAGAGGA
ATCTCCCTTCATGGGGTCCAGAGATTGACGGGGAGGTCGCAATGTATGTTGATGGGCTGCAGAATTGGATAGTGGGTCTCTA
AATTGGTACATCGACGGGACAGAGCGCTATTTCCGCAAAGACGGGCTGGAATAAAGAAGCATAGAATAGTCAAGCTATTC
CCAAGCGCCATTGAAAACACCTGCTGTGCGGGTTTTGGCCTAA

Figure S8 Amino acid sequence of full-length 17H2

MHHHHHHPSPAGALPKSFILPDLVNDCPFPLRVNPLCDEVGRLSEQWFLRYANYSPRAVAF
MAH KAGELAAACYPDADAFHLRVSDDFMNFLFNADDWLDDFDIEDTYGLANCTVRALRDPVN
FITDKRAGLMTKSYFSRFLKTAGPRCTERFIQTLALYFESVVTQKQARNNGTLPDLESYITIRRNN
NSGCKPCYALIEFCAGIDLDPDEVINHPIIQSLEDASNDLIAWSNDIFSFNREQSRHDSFNMVSIVMH
HQKGFALQEAVNFVVGELCKKAMERFQADKRNLP SWGPEIDGEVAMYVDGLQNWIVGSLNWI
DGTERYFGKDGPGIKKHRIVKLFPRPLKTPAVRVLA-

Figure S9 Nucleotide sequence of full-length L59H

ATGCCAAGTCCCGCGGGAGCGCTTCCAAGTCCTCATACTCCCGACCTGGTCAACGACTGCCATTCCCCCTTCGAGTCAACC
CACTCTGCGACGAGGTTGGGAGACTATCCGAGCAATGGTTCCTCCGCTATGCAAATTATTCGCCCCCTCGGGCAGTGGCGTTTA
TGGCACAAAGCGGGCGAGCTCACTGCTGCTGCTACCCAGATGCCGATGCCTTCCATCTCCGAGTCAGCGACGACTTCATG
AATTCCTCTTCAATGCCGACGATTGGCTAGACGACTTCGACATTGAGGACACGTACGGGCTGGCCAATTGACTGTTTCGAGCC
CTGCGCGACCCAGTCAACTTCATTACTGATAAAGCGCGAGGACTAATGACGAAATCATATTTTTCTCGATTCTGAAGACCGCT
GGGCCTCGCTGTACAGAGCGCTTTATCCAACGTTGGCGCTCTACTTTGAATCAGTAGTAACGCAAAGCAAGCTCGTAACAA
TGGCACTTTGCCGATTTAGAGAGCTACATCACCATTGGAAGGAATAACAGCGGCTGTAAACCTTGTATGCACTCATCGAGTT
CTGTGCTGGCATTGATTTGCCGACGAGGTTATCAATCACCCAATTATTCAATCGCTCGAAGATGCTCGAATGACCTTATTGCT
TGGTGAACGATATCTTCTTTCAACAGGGAGCAATCTCGCCATGACAGCTTCAACATGGTTTCCATCGTTATGCACCAGAAG
GGCTTTGCGCTTCAGGAAGCTGTCAACTTTGTTGGAGAGCTTTGTAAGGCGATGGAACGTTTTTCAGGCGGATAAGAGGA
ATCTCCCTTCATGGGGTCCAGAGATTGACGGGGAGGTCGCAATGTATGTTGATGGGCTGCAGAATTGGATAGTCGGGTCTCTA

AATTGGTCCATCGACGGGACAGAGCGCTATTTGGCAAAGACGGGCCTGGAATAAAGAAGCATAGAATAGTCAAGCTATTCC
CCAAGCGCCCATTGAAAACACCTGCTGTGCGGGTTTTGCCTAA

Figure S10 Amino acid sequence of full-length L59H

MHHHHHHPSPAGALPKSFILPDLVNDCPFPLRVNPLCDEVGRLSEQWFLRYANYSPPRAVAF
MALKAGELTAAACYPDADAFHLRVSDDFMNFLFNADDWLDDFDIEDTYGLANCTVRALRDPVNF
ITDKRAGLMTKSYFSRFLKTAGPRCTERFIQTLALYFESVVTQKQARNNGTLPDLESYITIRRNN
SGCKPCYALIEFCAGIDLPEVINHPHIIQSLEDASNDLIAWSNDIFSFNREQSRHDSFNMVSIVMH
QKGFALQEAVNFVGEELCKKAMERFQADKRNLPSWGPEIDGEVAMYVDGLQNWIVGSLNWSID
GTERYFGKDGPGIKKHRIVKLFPKRPLKTPAVRVLA-

Figure S11 Nucleotide sequence of full-length T65A

ATGCCAAGTCCCGCGGGAGCGCTTCCAAGTCCTCATACTCCCGACCTGGTCAACGACTGCCATTCCCCCTCGAGTCAACC
CACTCTGCGACGAGGTTGGGAGACTATCCGAGCAATGGTTCCTCCGCTATGCAAATTATTCGCCCCCTCGGGCAGTGGCGTTTA
TGGCACTCAAAGCGGGCAGACTCGCTGCTGCATGCTACCCAGATGCCGATGCCTTCCATCTCCGAGTCAGCGACGACTTCATG
AACTTCTCTTCAATGCCGACGATTGGCTAGACGACTTCGACATTGAGGACACGTACGGGCTGGCCAATTGTACTGTTGAGCC
CTGCGCGACCCAGTCAACTTCATTACTGATAAACGCGCAGGACTAATGACGAAATCATATTTTTCTCGATTCTGAAGACCGCT
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TGGCACTTTGCCGATTTAGAGAGCTACATCACCATTGAAAGGAATAACAGCGGCTGTAAACCTGTTATGCACTCATCGAGTT
CTGTGCTGGCATTGATTTGCCGGACGAGGTTATCAATCACCCAATTATTCAATCGCTCGAAGATGCTTGAATGACCTTATTGCT
TGGTGAACGATATCTTCTTTCAACAGGGAGCAATCTCGCCATGACAGCTTCAACATGGTTTTCCATCGTTATGCACCAGAAG
GGCTTTGCGCTTCAGGAAGCTGTCAACTTTGTTGGAGAGCTTTGTAAAAAGCGATGGAACGTTTTTCAGGCGGATAAGAGGA
ATCTCCCTTCATGGGGTCCAGAGATTGACGGGGAGGTCGCAATGTATGTTGATGGGCTGCAGAATTGGATAGTCGGGTCTTA
AATTGGTCCATCGACGGGACAGAGCGCTATTTGGCAAAGACGGGCCTGGAATAAAGAAGCATAGAATAGTCAAGCTATTCC
CCAAGCGCCCATTGAAAACACCTGCTGTGCGGGTTTTGCCTAA

Figure S12 Amino acid sequence of full-length T65A

MHHHHHHPSPAGALPKSFILPDLVNDCPFPLRVNPLCDEVGRLSEQWFLRYANYSPPRAVAF
MALKAGELTAAACYPDADAFHLRVSDDFMNFLFNADDWLDDFDIEDTYGLANCTVRALRDPVNF
ITDKRAGLMTKSYFSRFLKTAGPRCTERFIQTLALYFESVVTQKQARNNGTLPDLESYITIRRNN
SGCKPCYALIEFCAGIDLPEVINHPHIIQSLEDASNDLIAWSNDIFSFNREQSRHDSFNMVSIVMH
QKGFALQEAVNFVGEELCKKAMERFQADKRNLPSWGPEIDGEVAMYVDGLQNWIVGSLNWSID
GTERYFGKDGPGIKKHRIVKLFPKRPLKTPAVRVLA-

Figure S13 Nucleotide sequence of full-length S310Y

ATGCCAAGTCCCGCGGGAGCGCTTCCAAGTCCTCATACTCCCGACCTGGTCAACGACTGCCATTCCCCCTCGAGTCAACC
CACTCTGCGACGAGGTTGGGAGACTATCCGAGCAATGGTTCCTCCGCTATGCAAATTATTCGCCCCCTCGGGCAGTGGCGTTTA
TGGCACTCAAAGCGGGCAGACTACTGCTGCATGCTACCCAGATGCCGATGCCTTCCATCTCCGAGTCAGCGACGACTTCATG
AACTTCTCTTCAATGCCGACGATTGGCTAGACGACTTCGACATTGAGGACACGTACGGGCTGGCCAATTGTACTGTTGAGCC
CTGCGCGACCCAGTCAACTTCATTACTGATAAACGCGCAGGACTAATGACGAAATCATATTTTTCTCGATTCTGAAGACCGCT

GGGCCTCGCTGTACAGAGCGCTTTATCCAAACGTTGGCGCTCTACTTTGAATCAGTAGTAACGCAAAGCAAGCTCGTAACAA
TGGCACTTTGCCGATTTAGAGAGCTACATCACCATTGGAAGGAATAACAGCGGCTGTAAACCTTGTTATGCACTCATCGAGTT
CTGTGCTGGCATTGATTTGCCGGACGAGGTTATCAATCACCCAATTATTCAATCGCTCGAAGATGCTTGAATGACCTTATTGCT
TGGTCGAACGATATCTTCTTTCAACAGGGAGCAATCTCGCCATGACAGCTTCAACATGGTTTCCATCGTTATGCACCAGAAG
GGCTTTGCGCTTCAGGAAGCTGTCAACTTTGTTGGAGAGCTTTGAAAAAGGCGATGGAACGTTTTTCAGGCGGATAAGAGGA
ATCTCCCTTCATGGGGTCCAGAGATTGACGGGGAGGTCGCAATGTATGTTGATGGGCTGCAGAATTGGATAGTCGGGTCTCTA
AATTGGTACATCGACGGGACAGAGCGCTATTTGGCAAAGACGGGCCTGGAATAAAGAAGCATAGAATAGTCAAGCTATTCC
CCAAGCGCCATTGAAAACACCTGCTGTGCGGGTTTTGGCCTAA

Figure S14 Amino acid sequence of full-length S310Y

MHHHHHHPSPAGALPKSFILPDLVNDCPFPLRVNPLCDEVGRLSEQWFLRYANYSPPRAVAF
MALKAGELTAACYPDADAFHLRVSDDFMNFLFNADDWLDDFDIEDTYGLANCTVRALRDPVNF
ITDKRAGLMTKSYFSRFLKTAGPRCTERFIQTLALYFESVVTQKQARNNGTLPDLESYITIRRN
SGCKPCYALIEFCAGIDLPDEVINHPIIQSLEDASNDLIAWSNDIFSFNREQRSRHDSFNMVSIVMH
QKGFALQEAVNFVVELCKKAMERFQADKRNLP SWGPEIDGEVAMYVDGLQNWIVGSLNWYID
GTERYFGKDGPGIKKHRIVKLFPKRPLKTPAVRVLA-