

Supplementary Materials

Figure S1. Overview of the knock-in strategy at the *cyp52m1* locus. 1. Genomic organization of wild type *Starmerella bombicola*. 2. Integration cassette with the P450 of interest. 3. Genomic organization of the engineered *S. bombicola*.

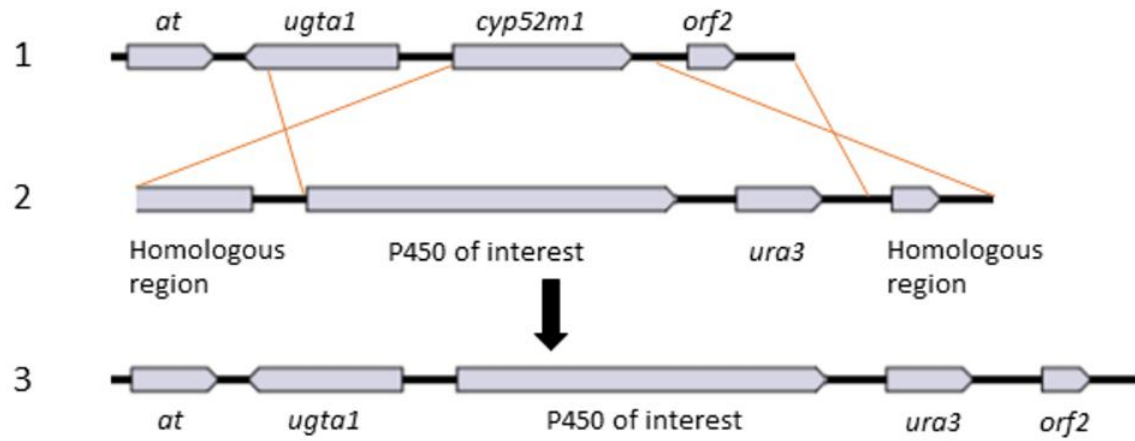


Figure S2. Ordered coding sequence of the *Ustilago maydis cyp1* gene.

ATGTCGCTCAAAGTGCAGCTGCCGCACTCTTCCTTATGCAGGATCGGCCAAAGGACAACGGGCGCTCAGGGTG
GACTTAGCTCAGACAAGGCCGCTCAGAAGGAAAAGGTAGGTCCCCTTAAAAGTGGCCTGCTTTTCTCGTACAT
GCAGAACTTCTGCGCTCTTCAAACCCGCTTGCGCAGACCTTCGAGGACTACTGAGCTCGATACAATGGACTTCA
AGCCTTTCCTCACAACACTTCAAGGCCGAGGGCTTTGCCGGGGATGTGTTGGCACCAGGCGCTTCCTAC
AACCAGACATGGAACACTATGGCGTCCAAGTTCAATGGTAGAGGTGGCAACAGAGTCGAAACTGGAAAGGTG
TTGGAAGCAGCAAGCGAATCGCTCAAGGAGACTGTACCACTTCTGCAGCTTGTGTACGAGCTCGGCACCATC
CACTACTCGTTTTCTTGTGCGACTTTTCTGGGAACTATCTACTTGCTATATCGCTACTGGGACTGCGCAGTCG
GTTGCGAGAGGAGACCGGACCTCAAAGGGCCTAAAGGTCTCCCCCTTGATCGGCAACCTTATGTGGGCGCTAA
AGAACCGCGATCCGCTCAGCTACCAAGTTTATGCCAGCAGAAGTACGGATACGGTAACACCCATACGCTCCC
CGGACTCGGACGTCTGATCGACATTTACGTCCTGATTGGATCGAGCACGTTCAAGATCAAGTTCAGCAAC
TACGTCAAAGGTGAGCAGTTCCACGATCAGATGCGTGATGTGCTTGGTGATGGAATCTTTACCTCGGACGGCG
AGCGTTGGAAGATGCAGAGAAAGGTAGCTTCGCGTATCTTTACTGTCAGCTCATTCAAGGCGATCATCACGCA
GACGATTTCGAGAGGATTGTGCTCTCGTCAACAACATCATCGAGACGTATGCAAGGCAAGGCACCGTTTTCAAC
CTGCAAGAACTCTACTTCAAGTTCACGCTCAGCTCGTTTGTCAAGATCGCATTCAAGACATCAAATCTCTT
TCGGAACCGGACCGCCCTGATACCTTGGCGATGCGTTCAACTATGCTCAGAAGGTGCTCGACATGCGCTTCGT
ACAGCCGTGGTGGAAGATCGCCGAGCGATTCAACGAGACCGGAAGAAAGATGCGAGCTGCACGAAAGATTG
TCGAAGAATTCACCACCAACATTGTCGAAGCAAGAAGAAAGGAGAGCGAGGCGATGGGAGAAAAATCCAAG
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ACCAACAGCTCAAGGATACGATTCTGAACCTGATGATTGCAGGACGCGATACCACTGCAGAAGCACTTTCGTG
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ATTTGGGGACCCGACGATGCGAATTCAAGCCAAGCCGCTGGACCGATCAAGAGACTGGTCTTCCATCAAGT
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GGTGGTGCAGCTCATACATCACATCTTGGCAAGTTCGACCTGGAATTGATCGACCTTGGACCCGGCAGGTGCG
GCTGGCTTTGGAAGGTCCCTGATTACCTCAACTCTTAACTCACCTATGAAGCGACCACTCATGGTCAAGGC
TACCCTTCGATGTTGCAAGGAAGGCACTAGA

Figure S3. Ordered mutated *B. megaterium cyp102a1* reductase sequence (BMR). Mutated codons are highlighted in bold face.

TGCTGATGAAGTAAAAGGCGTTTCGCTACTCCGTATTT**GATT**GCGGCGATAAAAACTGGGCTACTACGTATCAA
AAAGTGCCTGCTTTTATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCA
GATGCAAGCGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTAC
TTTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACCTCAATTTGTCGACAGCGCCGCGGA
TATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGAACGTCGTAGCAAGCAAAGAACTTCAACAGCCAGGC
AGTGACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAGCTTCTTATCAAGAAGGAGATCATTTAG
GTGTTATTCTCGCAACTATGAAGGAATAGTAAACCGTGTAACAGCAAGGTTTCGGCCTAGATGCATCACAGCA
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CTGCAATACGTGGAGCTTCAAGATCCTGTTACGCGCACGCGAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCC
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AAAAGAACAAGGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGTTACCTCATGAAGACTATCTGT
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CAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGACGGCAAGAAATTGATTGAACTTCTTGATCAAGGAG
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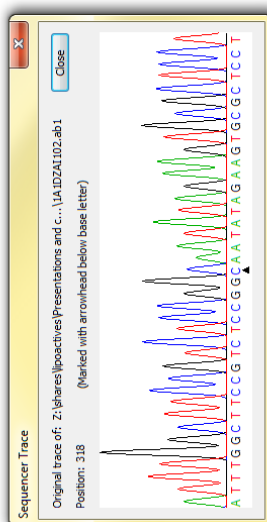
[illegible]

Figure S5. Effect of ethyl palmitate on colony forming units. Effect of different concentrations of ethyl palmitate on colony forming units four days after addition. 100 % corresponds to 3.1×10^9 colonies per mL. Error bars represent standard deviation (SD; $n=3$).

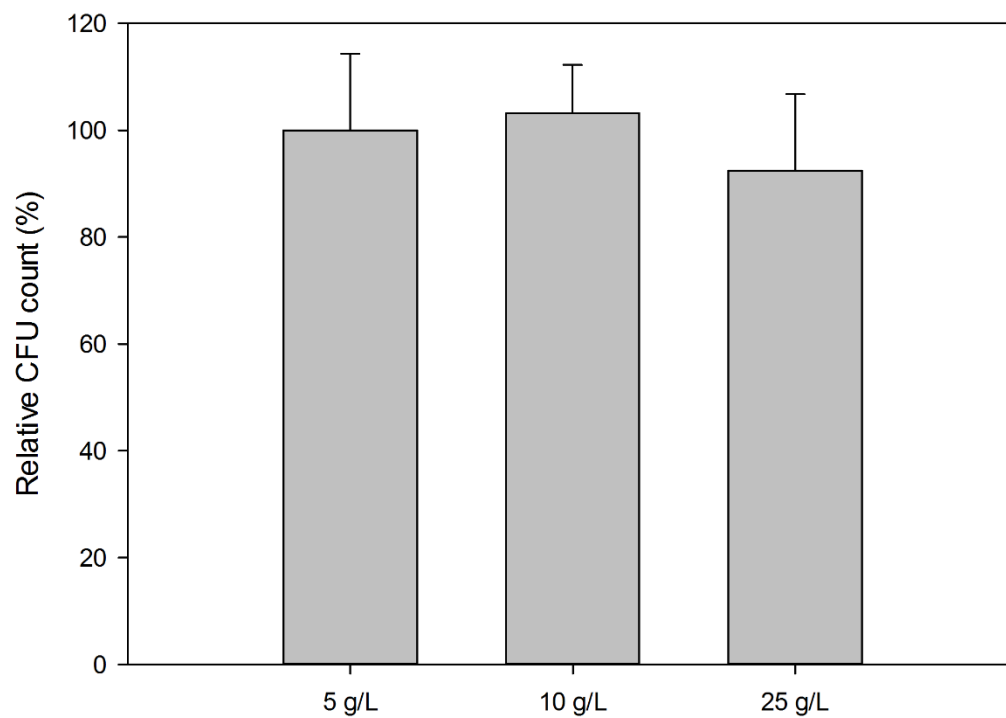
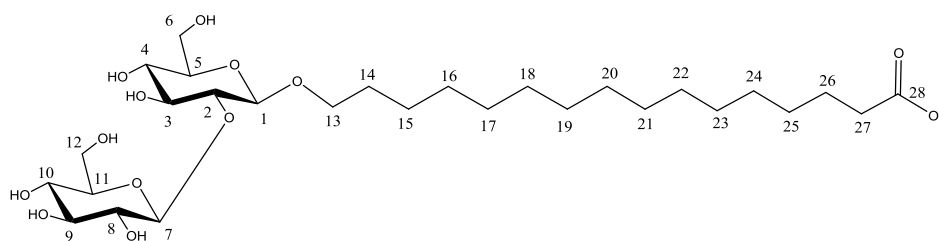


Figure S6. ^1H and ^{13}C NMR spectra of C16:0 terminal hydroxylated sophorolipids.

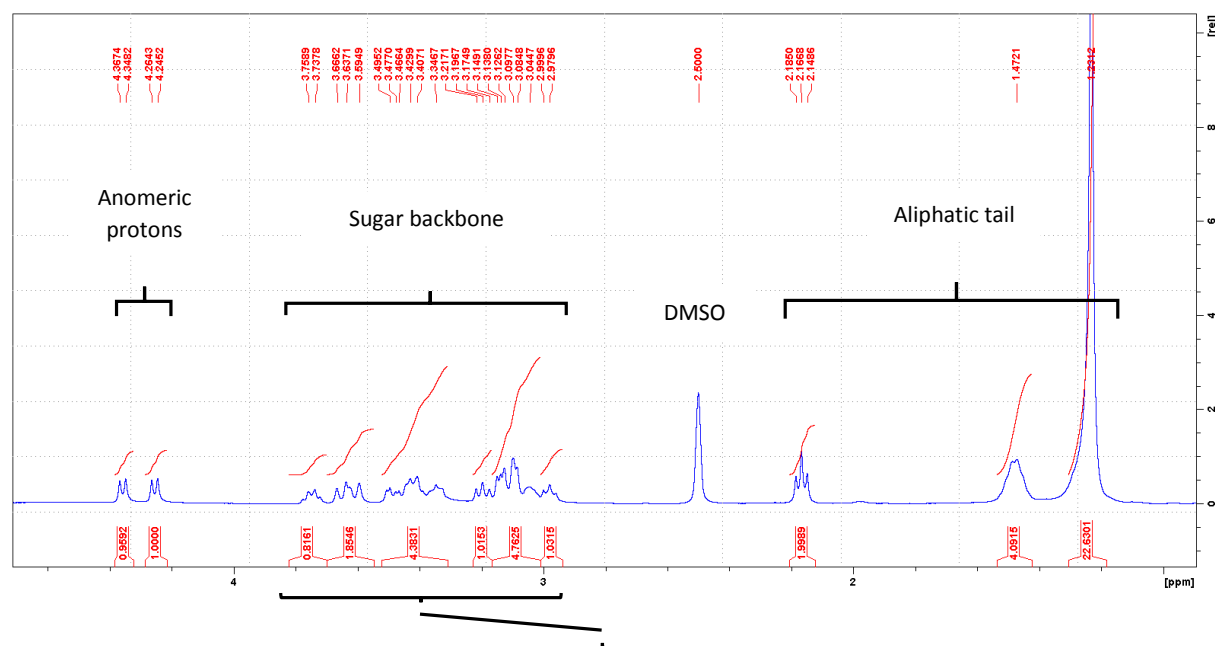


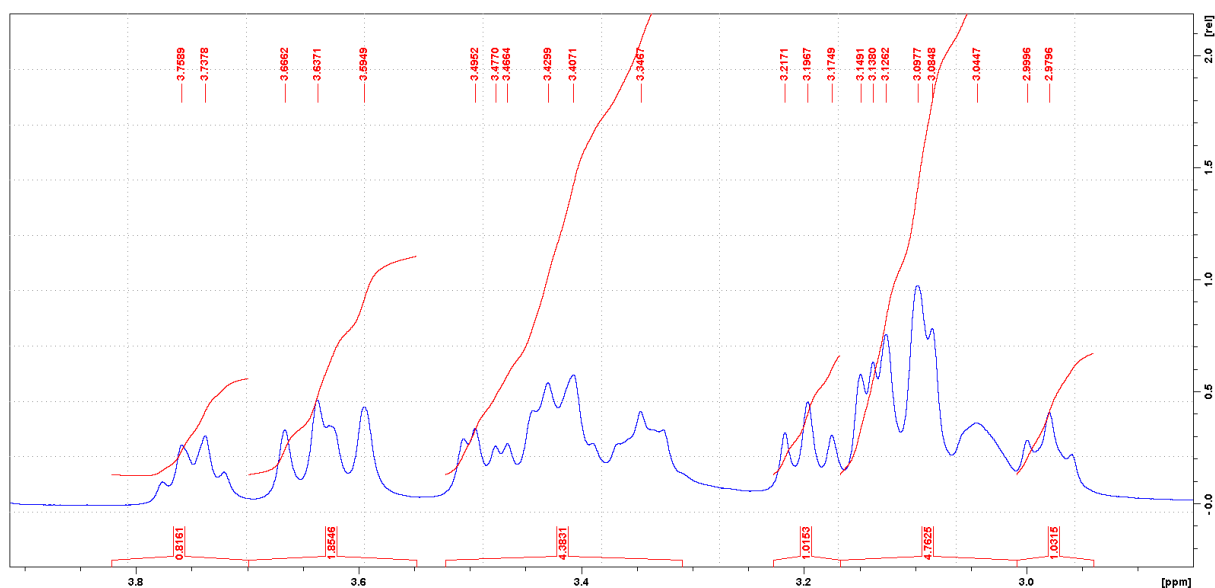
Structure of the synthesized glycolipid with numbering

Overview assignments ^1H chemical shifts

$\delta\ ^1\text{H}$ (ppm)	Multiplicity	Integral	# Protons	Annotation
1.18 – 1.31	m	22.63	22	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
1.42 – 1.64	m	4.09	4	14, 26
2.16	t	2.00	2	27
2.94 – 3.01	m	1.03	1	8
3.01 – 3.17	m	4.76	5	4, 5, 9, 10, 11
3.17 – 3.23	m	1.01	1	2
3.31 – 3.52	m	4.38	4 + H_2O	3, 6A, 12A, 13A
3.55 – 3.70	m	1.85	2	6B, 12B
3.70 – 3.82	m	0.81	1	13B
4.25	d	1	1	1
4.36	d	0.96	1	7
Total:			44	

^1H spectrum with zoom





Overview assignments ^{13}C chemical shifts

$\delta^{13}\text{C}$ (ppm)	Type of Carbon	# Carbons	Annotation
24.59	CH_2	1	26
25.56	CH_2	1	15
28.62 – 29.15	CH_2	10	16, 17, 18, 19, 20, 21, 22, 23, 24, 25
29.31	CH_2	1	14
33.82	CH_2	1	27
60.80	CH_2	1	12
60.90	CH_2	1	6
68.71	CH_2	1	13
69.69	CH	1	10
69.80	CH	1	4
74.94	CH	1	8
76.01	CH	1	3
76.09	CH	1	9
76.63	CH	1	5
77.07	CH	1	11
82.35	CH	1	2
101.35	CH (anomeric)	1	1
104.17	CH (anomeric)	1	7
174.66	COOH	1	28
Total:		28	

¹³C spectrum

