Supplementary information

Calculation of wax ester stoichiometric theoretical yields - As most glucose is utilized via the ED pathway by PD630,[1] and this pathway is highly upregulated in RHA1 during growth on glucose under lipid accumulating conditions,[2] the production of a 32-carbon WE can be defined by the following stoichiometric reaction:

$$8\ Glucose + 6\ ATP + 8\ NADPH \rightarrow C32\ WE + 10\ \mathrm{NADH} \quad (1)$$

Assuming that the remaining ATP requirements are fulfilled by oxidative phosphorylation and NADPH is generated through the pentose phosphate pathway (~5% of glucose flux in PD630[1]):

$$1\ NADH \rightarrow \sim 2.5\ ATP \quad (2)$$

$$1\ Glucose + 1\ ATP \rightarrow 12\ \mathrm{NADPH} + 6\mathrm{CO_2} \quad (3)$$

WE production in *Rhodococcus* can be simplified to:

$$8.66\ Glucose \rightarrow C32\ WE + 7.6\ \mathrm{NADH} \quad (4)$$

Based on these considerations, the theoretical yield of C32 WEs is ~0.32 g per g glucose.

Supplemental tables

Table S1. Candidate genes of screened constitutive promoters.

Name[a]	Locus[b]	Predicted gene product
P_1	RS04990	Quinolinate synthetase
P_2	RS05290	Division cluster transcriptional repressor
P_3	ro01178	Hypothetical protein
P_4	RS15810	MerR-family transcriptional regulator
P_5	RS19555	Bacterioferritin
P_6	RS19805	Membrane protein
P_7	RS20995	Transcriptional regulator
P_8	RS21170	Cold-shock protein
P_9	RS30940	Ribosomal subunit interface protein
P_{10}	RS36130	MerR-family transcriptional regulator
P_{11}	RS44810	Bacterial RNase P
P_{12}	RS11020	Hypothetical protein
P_{13}	RS30980	Stearoyl-CoA 9-desaturase
P_{14}	RS05505	Hypothetical protein
P_{15}	RS21725	CarD-family transcriptional regulator
P_{16}	RS30245	Co-chaperone GroES
P_{17}	RS10475	Molecular chaperone GroEL
P_{18}	RS09620	50S ribosomal protein L10
P_{19}	RS09350	Elongation factor Tu
P_{20}	RS19475	Superoxide dismutase

[a]Name of promoter

[b]Gene predicted to be under control of the promoter

Table S2. Synthesized DNA[a]

Gene / Fragment
ws1 of *Marinobacter hydrocarbonoclasticus* DSM 8798
ATGACCCCGCTCAACCCTACTGATCAATTATTTTTATGGCTGGAGAAGCGCCAGCAGCCCATGCACGTCGGTGGCCTCCAGCTGTTCTCGTTCCCGGAGGGCGCA CCCGACGACTACGTCGCGCAGCTCGCCGACCAGCTCCGCCAGAAGACGGAGGTCACCGCGCCGTTCAATCAGCGGCTTTCGTATCGACTAGGCCAGCCGGTGTG GGTCGAGGACGAGCACCTCGACCTCGAGCACCACTTCCGTTTCGAGGCCCTGCCCACCCCCGGACGTATCCGCGAATTGCTGTCGTTCGTCTCTGCTGAACACTC GCACCTCATGGACCGGGAACGCCCGATGTGGGAGGTGCACCTCATCGAGGGACTAAAGGACCGCCAGTTCGCCCTGTACACGAAGGTCCACCATTCGCTCGTCG ACGGCGTAAGTGCGATGCGCATGGCGACCCGGATGCTGTCCGAGAACCCCGACGAGCATGGGATGCCGCCGATCTGGGACCTGCCGTGCCTGTCACGGGACCG AGGAGAGTCAGATGGGCACAGTCTGTGGCGCTCGGTGACGCACCTGCTGGGACTGTCCGACCGTCAGCTCGGCACGATCCCTACAGTAGCAAAAGAACTCCTGA AGACCATCAACCAGGCCCGCAAGGACCCGGCGTACGACTCCATCTTCCACGCCCCAAGATGCATGCTCAACCAGAAGATCACCGGCTCCCGCCGCTTCGCGGCCC AGTCGTGGTGCCTGAAGCGCATTCGCGCGGTTTGCGAGGCCTACGGTACGACGGTCAACGACGTGGTGACAGCTATGTGCGCGGCCGCCCTACGCACGTACCTT ATGAACCAAGACGCCTTACCGGAAAAGCCCCTGGTCGCATTCGTGCCTGTGTCGTTGCGACGGGACGATAGCTCGGGCGGCAACCAGGTGGGTGTCATATTAGC GTCCTTGCACACCGACGTGCAGGACGCGGGTGAGCGACTGCTCAAGATCCACCACGGGATGGAGGAAGCGAAGCAGCGTTACCGGCACATGTCGCCCGAGGA GATCGTGAACTACACGGCCCTAACTTTGGCCCCCGCGGCGTTCCACCTGCTCACCGGTCTGGCTCCGAAGTGGCAGACGTTCAACGTCGTCATCTCCAACGTGCC AGGACCATCGCGGCCTCTGTACTGGAACGGTGCCAAGTTAGAAGGCATGTACCCGGTCTCGATCGACATGGACAGGCTCGCTCTGAACATGACATTAACCAGCT ACAACGACCAGGTCGAGTTCGGCCTCATCGGCTGCCGGCGGACGTTGCCGTCCCTGCAACGCATGCTCGACTACCTGGAGCAGGGCCTGGCCGAACTGGAACTG AACGCCGGCTTA
ws2 of *Marinobacter hydrocarbonoclasticus* DSM 8798
ATGAAACGACTTGGAACACTTGATGCATCTTGGCTGGCTGTCGAGTCCGAGGATACCCCGATGCACGTCGGCACTTTGCAAATCTTCAGCCTTCCTGAAGGCGCG CCCGAAACGTTCCTGCGCGACATGGTCACCCGCATGAAGGAGGCGGGTGACGTGGCGCCCCCCTGGGGCTACAAGCTCGCCTGGTCGGGCTTCCTCGGGCGGG TGATCGCACCTGCGTGGAAGGTCGACAAGGACATCGACCTTGACTACCACGTGCGGCACAGTGCGCTTCCCCGACCCGGTGGCGAGCGCGAGCTCGGGATCTTA GTATCAAGACTTCACAGCAACCCGCTGGACTTCTCGAGGCCGTTATGGGAGTGCCACGTCATCGAGGGGCTCGAGAACAACCGCTTCGCCCTGTACACCAAGAT GCACCACTCGATGATCGACGGCATCAGTGGGGTGCGTCTGATGCAGCGCGTCCTCACCACGGACCCCGAGCGTTGCAACATGCCGCCGCCGTGGACTGTTCGTC CGCACCAGCGGCGTGGTGCCAAGACCGATAAAGAGGCCAGCGTCCCGGCCGCCGTGTCGCAGGCCATGGACGCGCTCAAGCTACAGGCTGACATGGCCCCGAG ACTGTGGCAGGCCGGGAATCGACTTGTGCACTCCGTCCGTCACCCGGAGGACGGCCTCACGGCCCCCTTCACGGGGCCGGTGTCAGTCCTCAACCATCGCGTGA CAGCCCAGCGTCGGTTCGCGACCCAGCACTACCAGCTCGATAGGCTTAAGAATCTCGCGCACGCCAGCGGCGGGAGTCTCAACGACATCGTCCTGTACCTGTGC GGCACCGCGCTGAGACGGTTCCTAGCCGAGCAGAACAACCTGCCTGACACGCCGTTAACGGCGGGAATCCCCGTCAACATCAGGCCGGCGGACGACGAGGGAA CGGGCACCCAGATCAGCTTCATGATCGCGTCCTTGGCAACAGACGAGGCAGATCCGCTCAACCGGCTCCAGCAGATCAAGACTTCTACGAGACGTGCCAAGGAA CACCTGCAGAAGCTTCCGAAGAGCGCTTTGACACAGTACACGATGCTCCTGATGTCCCCGTACATCCTGCAATTAATGAGCGGACTGGGTGGCCGGATGCGACC AGTGTTCAACGTCACGATCTCGAACGTGCCGGGCCCTGAGGGTACGCTCTACTACGAGGGAGCACGTCTCGAAGCGATGTACCCGGTCTCGTTGATCGCCCACG GCGGGGCGCTGAACATCACGTGCCTGTCCTACGCAGGATCGTTGAATTTCGGCTTCACAGGTTGCCGGGACACCTTGCCCTCCATGCAGAAGCTGGCGGTCTACA CCGGTGAGGCCCTCGACGAACTGGAGTCGCTTATCTTACCTCCCAAGAAGCGCGCGAGGACACGCAAG
atfA of *Acinetobacter baylyi* ADP1
ATGAGACCGCTGCACCCGATCGACTTCATCTTCCTCTCCCTCGAGAAGCGGCAGCAGCCGATGCACGTGGGCGGCCTGTTCCTGTTCCAGATCCCCGACAACGCG CCCGACACGTTCATCCAGGACCTCGTCAACGACATCCGCATCTCCAAGTCCATCCCGGTCCCGCCGTTCAACAACAAGCTGAACGGTCTGTTCTGGGACGAGGAC GAGGAGTTCGACCTGGACCATCACTTCCGGCACATCGCGCTGCCGCACCCCGGCCGGATCCGTGAGCTCCTCATCTACATCTCGCAGGAGCACTCGACGCTGCTC GACCGCGCCAAGCCGCTGTGGACCTGCAACATCATCGAAGGCATCGAGGGCAACCGGTTCGCCATGTACTTCAAGATCCACCACGCGATGGTCGACGGCGTCGC CGGGATGCGCCTGATCGAGAAGTCGCTGTCGCACGACGTCACCGAGAAGTCGATCGTCCCCCCGTGGTGCGTCGAGGGCAAGCGCGCGAAGCGTCTGCGCGAG CCGAAGACGGGCAAGATCAAGAAGATCATGTCCGGCATCAAGTCCCAGCTGCAGGCGACCCCGACCGTCATCCAGGAGCTGAGCCAGACCGTGTTCAAGGACA TCGGCCGCAACCCGGACCACGTCAGCAGCTTCCAGGCCCCGTGCAGCATCCTGAACCAGCGCGTCTCCTCGTCGCGCCGCTTCGCGGCGCAGTCGTTCGACTTGG ACCGGTTCCGCAACATCGCCAAGAGCCTCAACGTCACGATCAACGACGTCGTCCTCGCCGTCTGCTCGGGCGCCCTGCGGGCGTACCTGATGAGCCACAACTCCC TGCCCAGCAAGCCCCTGATCGCGATGGTGCCCGCGTCCATCCGCAACGACGACTCCGACGTGTCGAACCGCATCACGATGATCCTCGCGAACCTGGCCACCCACA AGGACGACCCGCTCCAGCGTCTCGAGATCATCCGGCGGTCGGTGCAGAACTCGAAGCAGCGGTTCAAGCGGATGACCAGCGACCAGATCCTCAACTACTCGGC GGTGGTCTACGGCCCCGCCGGCCTCAACATCATCAGCGGCATGATGCCCAAGCGCCAGGCGTTCAACCTCGTGATCTCCAACGTGCCGGGCCCGCGGGAGCCCC TCTACTGGAACGGCGCGAAGCTCGACGCCCTGTACCCGGCGTCGATCGTGCTCGACGGTCAGGCCCTGAACATCACCATGACGTCGTACCTCGACAAGCTGGAG GTCGGCCTGATCGCCTGCCGGAACGCCCTCCCGCGCATGCAGAACCTGCTGACGCACCTCGAAGAGGAGATCCAGCTCTTCGAGGGTGTCATCGCCAAGCAGGA AGACATCAAGACCGCCAAC
atfA2 of *Alcanivorax borkumensis* ATCC 700651
ATGGCAAGGAAATTAAGTATCATGGACTCAGGTTGGCTCATGATGGAGACCCGCGAGACGCCGATGCACGTCGGTGGACTCGCACTGTTCGCGATCCCGGAGG GTGCCCCCGAGGACTACGTCGAGTCGATCTACCGCTACCTCGTGGACGTGGACTCCATCTGCCGGCCGTTCAACCAGAAGATCCAGAGCCATCTGCCGCTGTACC TGGACGCCACGTGGGTGGAAGACAAGAACTTCGACATCGACTACCACGTTAGGCACTCGGCTTTGCCACGCCCCGGTCGTGTACGGGAGCTGTTAGCGCTTGTA AGCAGGCTGCACGCCCAGCGCCTCGACCCGAGCCGTCCCCTGTGGGAGTCCTACCTGATTGAAGGCCTGGAAGGTAATAGGTTTGCGCTATATACCAAGATGCA CCACTCCATGGTGGACGGTGTCGCAGGCATGCACTTGATGCAGTCGCGACTCGCCACATGCGCCGAGGACCGGCTCCCGGCACCGTGGAGCGGGGAGTGGGAC GCGGAGAAGAAGCCGCGGAAATCGCGCGGCGCCGCCGCCGCGAACGCGGGCATGAAGGGCACCATGAACAACCTGCGCCGCGGTGGCGGGCAGTTGGTTGA CCTGCTAAGGCAGCCGAAGGACGGCAACGTCAAGACCATCTACCGGGCTCCGAAAACCCAGCTCAACCGCAGGGTCACCGGTGCCCGGAGGTTTGCTGCGCAG TCCTGGAGCCTCAGCCGGATCAAGGCGGCGGGTAAGCAGCACGGCGGTACTGTGAACGACATCTTCCTGGCGATGTGCGGAGGCGCATTGCGACGTTATCTACT GAGCCAGGACGCTCTCTCCGACCAGCCATTGGTGGCGCAGGTCCCGGTGGCCCTGCGTTCGGCCGACCAGGCCGGCGAGGGCGGCAACGCCATCACGACAGTT CAAGTCAGCCTAGGCACCCACATCGCCCAGCCTCTCAACCGGCTTGCCGCGATCCAGGACTCGATGAAGGCAGTCAAATCACGGCTGGGCGACATGCAGAAGTC GGAGATCGACGTCTACACCGTGCTCACCAACATGCCGCTGTCGCTCGGCCAGGTGACCGGCTTGTCGGGTCGGGTCTCTCCGATGTTCAACCTGGTCATCTCGAA

	CGTGCCCGGCCCGAAGGAGACCCTACACCTAAACGGCGCTGAGATGCTGGCCACCTACCCGGTCTCCCTGGTCCTGCATGGCTACGCTCTGAACATCACCGTCGT CTCGTACAAGAACTCGTTAGAGTTCGGGGTTATCGGCTGCCGCGACACCTTGCCGCACATCCAGCGTTTCCTTGTGTACCTCGAGGAGTCACTGGTGGAACTCGA GCCC
pLB[b]	
	GGCTGCAGGTCGACTCTAGACATTACTCGCATCCATTCTCAGGCTGTCTCGTCTCGTCTCGGCGCGCCATCGATCATTTTAATTAACCAGGATACATAGATTACCAC AACTCCGAGCCCTTCCACCATTAATGAATCGGCCAACGC

[a]Fragment/gene name followed by nucleotide sequence. Genes encoding WSs were codon-optimized for *Rhodococcus*.

[b]Used for universal linkers, linearization sites and Gibson overlaps.

Table S3. Bacterial strains used in this study.

Strains	Use	Reference
<u>*Escherichia coli* strains</u>		
E. coli DH5α	Propagation of DNA	n/a
E. coli NEB® 10-beta	Cloning pMiniT plasmids	(New England Biolabs)
<u>*Rhodococcus* strains</u>		
R. jostii RHA1	Wild-type, protein expression, WE accumulation	[3]
RHA1::T1/M6[a]	RHA1 strains carrying *fcrA* and *ws2* co-expression cassettes driven by different strength promoters.	This study
RHA1::T1/T1		
RHA1::T1/T2		
RHA1::T2/M6		
RHA1::T2/T1		
RHA1::T2/T2		

[a]Named according to promoters driving *fcrA* and *ws2* expression, respectively (e.g., T1/T2 designates the strain in which: *fcrA* and *ws2* expression are driven by P_{T1} and P_{T2}, respectively)

Table S4. Plasmids used in this study

Plasmid	Description / Use	Reference
pTipQC2	*Rhodococcus* expression vector	[4]
pmCherry	Source of *mCherry*	(Takarabio)
pTip-*mCherry*	Cloning intermediate in the production of pSYN-*mCherry*	This study
pSET152	Integrative vector	[5, 6]
pSYN-*mCherry*	Modular integrative vector. Promotor-less backbone carrying *mCherry*. Promoter characterization	This study
pSYN-P_X-mCherry	pSYN carrying *mCherry* under control of P_X. Used to characterize promoter	This study
pSYN-P_X-*fcrA*	pSYN carrying *fcrA* under control of P_X. WE accumulation	This study
pUC57-*ws1* pUC57-*ws2* pUC57-*atfA* pUC57-*aftA2*	Synthesized WSs. Cloning intermediates	(GENEWIZ)
pTip-*ws1* pTip-*ws2* pTip-*atfA* pTip-*aftA2*	Overproduction of WSs in RHA1 to promote WE accumulation	This study
pSYN-P_X-*ws2*	pSYN carrying *ws2* under control of P_X. Cloning intermediates for cassette assembly	This study
pLB	Integrative recipient vector for *fcrA* and *ws2* co-expression cassettes	This study
pMiniT	Cloning of TSS junction amplicons	(NEB)

31 Table S5. Primers used in this study

Name	Sequence (5’ to 3’)
Cloning Primers used to amplify indicated gene or fragment	
mCherry_fwd	CTTTAAGAAGGAGATATACATATGGTGAGCAAGGGCGAG
mCherry_rev	CACGGGTGCCGGTGGGTCGACTAGTCACTTGTACAGCTCGTCCATG
MOD-MCS_fwd	GGCTGCAGGTCGACTCTAGAGCGGCCGCATCGATCATTGATATCGTCTAGAAATA ATTTTGTTTAACTTTAAG
MOD-MCS_rev	GCGTTGGCCGATTCATTAATACTAGAGTCCCGCTGAGG
UL1-frcA-UL2_fwd	CATTACTCGCATCCATTCTCAGGCTGTCTCGTCTCGTCTCGGCGCGCCGTTTTCCCA GTCACGACGTT
UL1-frcA-UL2_rev	GCTTGGATTCTGCGTTTGTTTCCGTCTACGAACTCCCAGCGAGTCAGTGAGCGAGG AAGC
UL2-ws2-ULX_fwd	GCTGGGAGTTCGTAGACGGAAACAAACGCAGAATCCAAGCGTTTTCCCAGTCACG ACGTT
UL2-ws2-ULX_rev	GGTGGAAGGGCTCGGAGTTGTGGTAATCTATGTATCCTGGTTAATTAAGAGTCAG TGAGCGAGGAAGC
Promoter amplification primers	
Pnit_fwd	GGTCGACTCTAGAGCGGCCGCTCACTCTTCTGCTCGGCC
Pnit_rev	AACAAAATTATTTCTAGACGATATCGCCGTCCATTATACCTCCTCACGTGACGTGA G
PT1_fwd	GGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PT1_rev	AACAAAATTATTTCTAGACGATATCCTTGCGACGAAAGGAACTC
PT2_fwd	GGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PT2_rev	AACAAAATTATTTCTAGACGATATCGAAAGGAACTCTACAACAGCGAC
P1_fwd	GGTCGACTCTAGAGCGGCCGCGAGTCCGAGGAAGGTCGAC
P1_rev	AACAAAATTATTTCTAGACGATATCTTCGCTCGCCTCGACGC
P2_fwd	GGTCGACTCTAGAGCGGCCGCGTAGGCGGGGGTCGATCC
P2_rev	AACAAAATTATTTCTAGACGATATCTGGTGACCATCAACCCTCC
P3_fwd	GGTCGACTCTAGAGCGGCCGCTACGTGCGGGCGTTCC
P3_rev	AACAAAATTATTTCTAGACGATATCGCTTCGGCGCCCGGGGA
P4_fwd	GGTCGACTCTAGAGCGGCCGCATGCCGATACTCCCTGAATATCGATCC
P4_rev	AACAAAATTATTTCTAGACGATATCGGGCCGGAGCGGGTTTCA
P5_fwd	GGTCGACTCTAGAGCGGCCGCCCGTCCGAGTTCGCGTA
P5_rev	AACAAAATTATTTCTAGACGATATCGTTGCGAATCTGGTCGTG
P6_fwd	GGTCGACTCTAGAGCGGCCGCACCAGATAGCCGGTCCC
P6_rev	AACAAAATTATTTCTAGACGATATCTGCCATTGCCCATGACCA

P7_fwd	GGTCGACTCTAGAGCGGCCGCTTCCTTCGACCAGTTCGG
P7_rev	AACAAAATTATTTCTAGACGATATCGATCGGGATCGACTTCCC
P8_fwd	GGTCGACTCTAGAGCGGCCGCACTGATGTCTCCGGGCT
P8_rev	AACAAAATTATTTCTAGACGATATCTCGGAGTAGTGAACGAAG
P9_fwd	GGTCGACTCTAGAGCGGCCGCGAGCGCAGATCAACGTCG
P9_rev	AACAAAATTATTTCTAGACGATATCGTCCCTTGACGACGATCTC
P10_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
P10_rev	CAAAATTATTTCTAGACGATATCCGGAGCGGGTTTCACTAC
P11_fwd	GGTCGACTCTAGAGCGGCCGCGTGTGTCCAGGACGTCGG
P11_rev	AACAAAATTATTTCTAGACGATATCGTTAACAACCGCCCTGCTC
P12_fwd	GGTCGACTCTAGAGCGGCCGCTTCGGGCAGGGCCGTGCG
P12_rev	AACAAAATTATTTCTAGACGATATCGTCGAGCCGCAGATCCCATG
P13_fwd	GGTCGACTCTAGAGCGGCCGCGGCGCTGTGCGAGAAGTAC
P13_rev	AACAAAATTATTTCTAGACGATATCACTCTTCGATATCGCGCC
P14_fwd	GGTCGACTCTAGAGCGGCCGCCTCGACCAGCAGTCGC
P14_rev	AACAAAATTATTTCTAGACGATATCGTCGTCACGACCTTCCTGATC
P15_fwd	GGTCGACTCTAGAGCGGCCGCTTGCCGTTGACCGCGGCG
P15_rev	AACAAAATTATTTCTAGACGATATCATTCCTTCTGCTCACCCTTGATGGTGC
P16_fwd	GGTCGACTCTAGAGCGGCCGCGAGCTGCCGAGGGGGACG
P16_rev	AACAAAATTATTTCTAGACGATATCCGTGTCGGGAATGACCAG
P17_fwd	GGTCGACTCTAGAGCGGCCGCTCGCGCTCGGCGGTGGC
P17_rev	AACAAAATTATTTCTAGACGATATCCTTGGGGCCCAACGTCACC
P18_fwd	GGTCGACTCTAGAGCGGCCGCTGATGAACCACACCCCCG
P18_rev	AACAAAATTATTTCTAGACGATATCCACCGTCAGACCACGGTATTC
P19_fwd	GGTCGACTCTAGAGCGGCCGCAAGCCGCCCGCAAGGCCG
P19_rev	AACAAAATTATTTCTAGACGATATCTGGTGATGGCCGCGGTCAG
P20_fwd	GGTCGACTCTAGAGCGGCCGCTTCCGAGGTCGTCGATCAG
P20_rev	AACAAAATTATTTCTAGACGATATCGTGATGCTTGTCGTGATG
PM1_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PM1_rev	AACAAAATTATTTCTAGACGATATCTCGTCTTGCCTGCCCGG
PM2_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PM2_rev	AACAAAATTATTTCTAGACGATATCACATCAACCCTTGCCC
PM3_fwd	GGTCGACTCTAGAGCGGCCGCTGACACGTCCCCATCGTG
PM3_rev	CAAAATTATTTCTAGACGATATCCGGAGCGGGTTTCACTAC
PM4_fwd	GGTCGACTCTAGAGCGGCCGCAACCCACTCGCCCCTGCC
PM4_rev	CAAAATTATTTCTAGACGATATCCGGAGCGGGTTTCACTAC
PM5_fwd	GGTCGACTCTAGAGCGGCCGCTTTCGTCGCAAGGAAAGAG

PM5_rev	CAAAATTATTTCTAGACGATATCCGGAGCGGGTTTCACTAC
PM6_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PM6_rev	AACAAAATTATTTCTAGACGATATCGGCAGTTCATCCTCTCCC
PM7_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PM7_rev	AACAAAATTATTTCTAGACGATATCGAACTCTACAACAGCGACACCTC
PM8_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PM8_rev	AACAAAATTATTTCTAGACGATATCTGGAGTCGGTTGACCAG
PM9_fwd	CTGCAGGTCGACTCTAGAGCGGCCGCACCGCTCTGGTCAGCGAC
PM9_rev	AACAAAATTATTTCTAGACGATATCCGTCGACGTCGCCGGGT
ARF-TSS primers	
mCherry_RT	Phosph-CGCCCTCGATCTCGAACT
mCherry_Inv1	ATGGAGGGCTCCGTGAAC
mCherry_Inv2	GCAAAGGCTGGGACGTTAGTG
PCR screening / Sequencing primers	
pSYN_fwd	GTTTTCCCAGTCACGACGTT
pSYN_rev	GAGTCAGTGAGCGAGGAAGC
pLB_fwd	GGCGATTAAGTTGGGTAACG
pLB_rev	AACCGTATTACCGCCTTTGA
pTip_fwd	GCAGCGTGGACGGCG
pTip_rev	CACCGGCACCCGCAGCGA
pMiniT_fwd	ACCTGCCAACCAAAGCGAGAAC
pMiniT_rev	TCAGGGTTATTGTCTCATGAGCG
pSYN_flank_fwd	CGGTGATGTCCTGGTATCG
pSYN_flank_rev	GTGACGGTCGACAACATCCT

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Supplemental figures

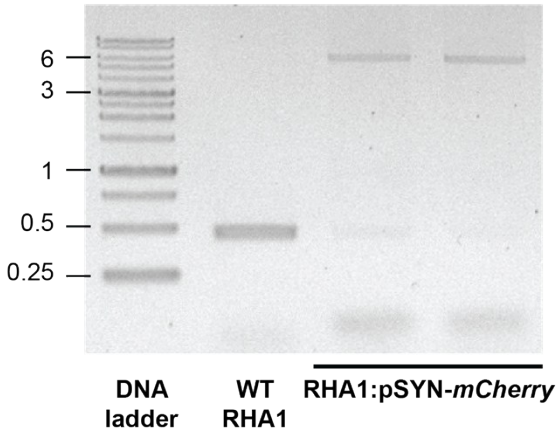

Figure S1. PCR analysis of the integration of pSYN-*mCherry* into the RHA1 genome.

Amplicons resulting from PCR amplification of the φC31 *attB* integration site in *RS20555* using a flanking primer pair (pSYN_flank; Table S5) in the indicated RHA1 strains. In the WT strain, the primers are separated by 0.45 kb. pSYN-mCherry is 6.5 kb. PCR was performed on colonies grown on solid LB media. From left to right: Lane 1, 1 kb DNA ladder, major bands are indicated (kb); Lane 2, WT RHA1; Lanes 3 and 4, biological replicates of RHA1:pSYN-*mCherry*.

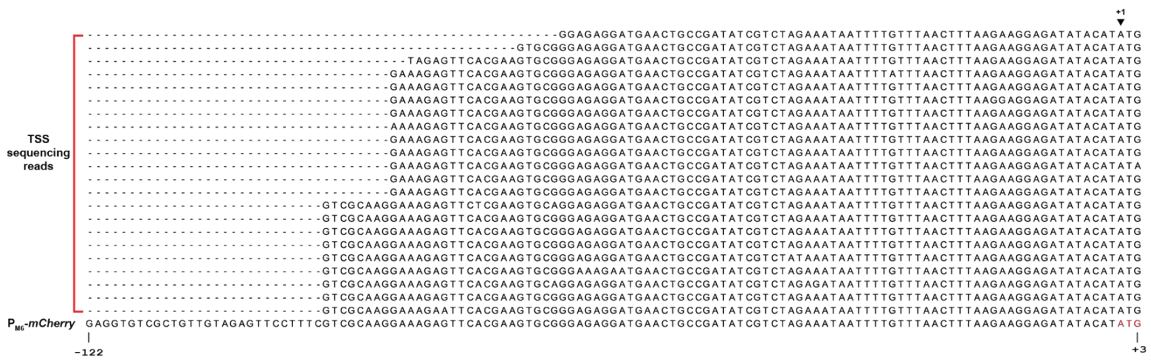

Figure S2. ARF-TSS results for P_{M6}.

Sequence reads obtained from ARF-TSS analysis mapped using RHA1::pSYN-P_{M6}-*mCherry*.

Resulting sequencing reads were trimmed and aligned against the sequence of P_{M6}-*mCherry*.

▼+1 represents the first A in the ATG start codon of P_{M6}-*mCherry*.

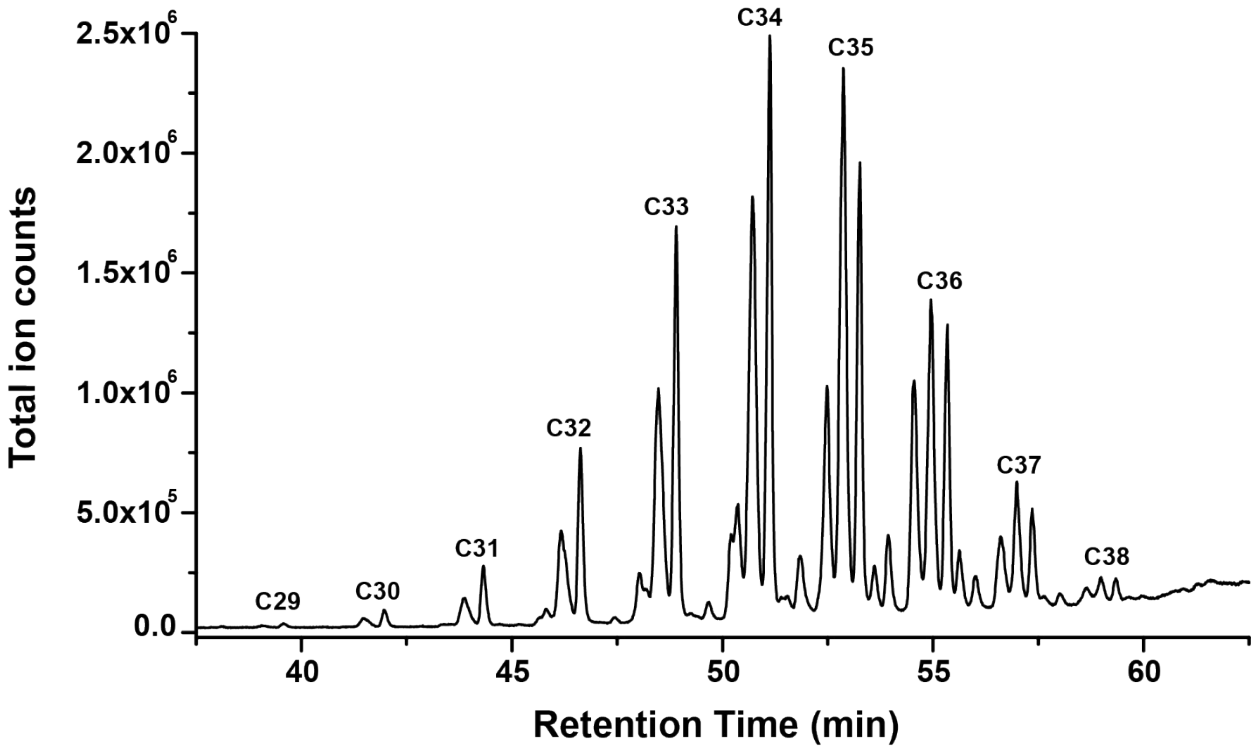

Figure S3. WE profile of *R. jostii* RHA1 constitutively expressing a single-copy of *fcrA*.

GC/MS analysis of WEs isolated from RHA1::pSYN-P_{M6}-*fcrA*. Cells were grown in N^- medium (4 g/L glucose, 0.05 g/L ammonium chloride). Cells were harvested in stationary phase.

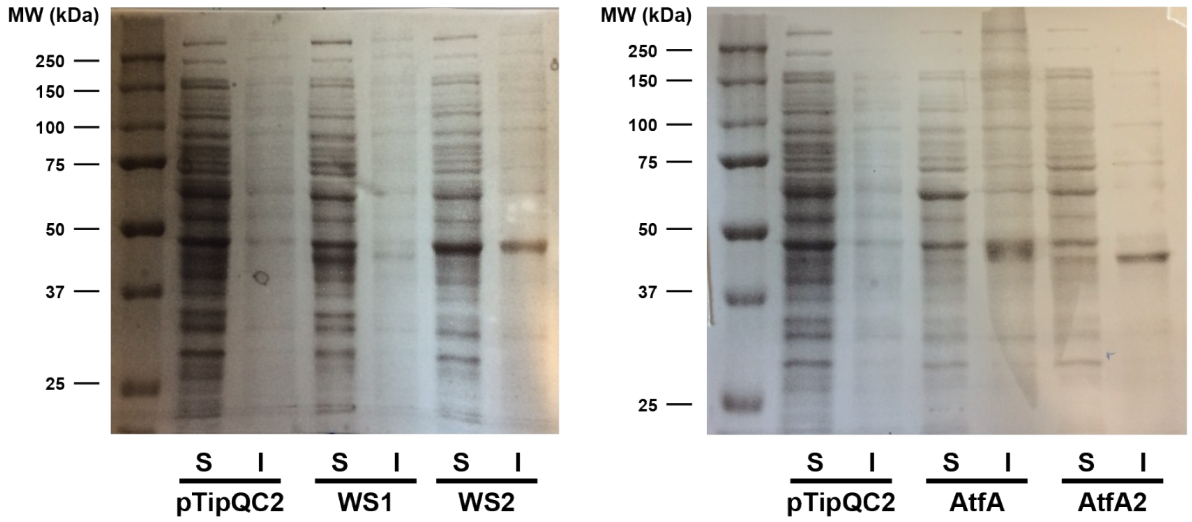

Figure S4. SDS-PAGE analysis of heterologous production of wax synthases in RHA1.

Lanes were loaded with soluble (S) and insoluble (I) fractions of RHA1 containing: WS1, pTip-*ws1*; WS2, pTip-*ws2*; AtfA, pTip-*atfA*; AtfA2, pTip-*atfA2*; pTipQC2, empty vector, or molecular weight markers. Expected molecular weights of WSs: WS1, 51.5 kDa; WS2, 52.5 kDa; AtfA, 51.8 kDa; AtfA2, 49.8 kDa.

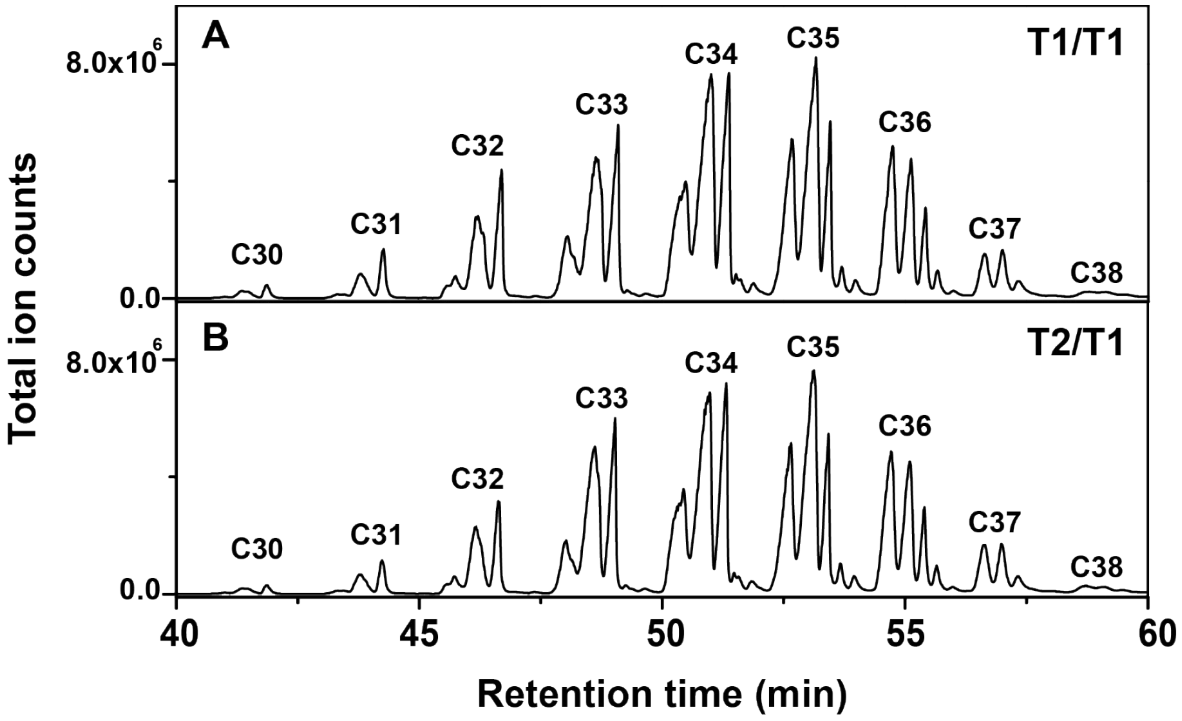

Figure S5. WE profile of *R. jostii* RHA1 strains containing chromosomal *fcrA* and *ws2*.

GC/MS analysis of WEs isolated from (A) RHA1::pSYN-P_{T1}-*fcrA*-P_{T1}-*ws2* and (B) RHA1::pSYN-P_{T2}-*fcrA*-P_{T1}-*ws2*. Cells were grown in N^- medium (4 g/L glucose, 0.05 g/L ammonium chloride) and harvested in stationary phase.

References

1. W. D. Hollinshead, W. R. Henson, M. Abernathy, T. S. Moon and Y. J. Tang, *Biotechnol Bioeng*, 2016, **113**, 91-100.
2. A. Juarez, J. A. Villa, V. F. Lanza, B. Lázaro, F. de la Cruz, H. M. Alvarez and G. Moncalián, *microb Cell Fact*, 2017, **16**, 35.
3. M. P. McLeod, R. L. Warren, W. W. L. Hsiao, N. Araki, M. Myhre, C. Fernandes, D. Miyazawa, W. Wong, A. L. Lillquist, D. Wang, M. Dosanjh, H. Hara, A. Petrescu, R. D. Morin, G. Yang, J. M. Stott, J. E. Schein, H. Shin, D. Smailus, A. S. Siddiqui, M. A. Marra, S. J. M. Jones, R. Holt, F. S. L. Brinkman, K. Miyauchi, M. Fukuda, J. E. Davies, W. W. Mohn and L. D. Eltis, *Proc Natl Acad Sci USA* 2006, **103**, 15582-15587.
4. N. Nakashima and T. Tamura, *Appl Environ Microbiol*, 2004, **70**, 5557-5568.
5. Y. Hong and M. K. Hondalus, *FEMS microbiology letters*, 2008, **287**, 63-68.
6. M. Bierman, R. Logan, K. O'Brien, E. T. Seno, R. Nagaraja Rao and B. E. Schoner, *Gene*, 1992, **116**, 43-49.