

A polyketide-based biosynthetic platform for diols, amino alcohols and hydroxy acids

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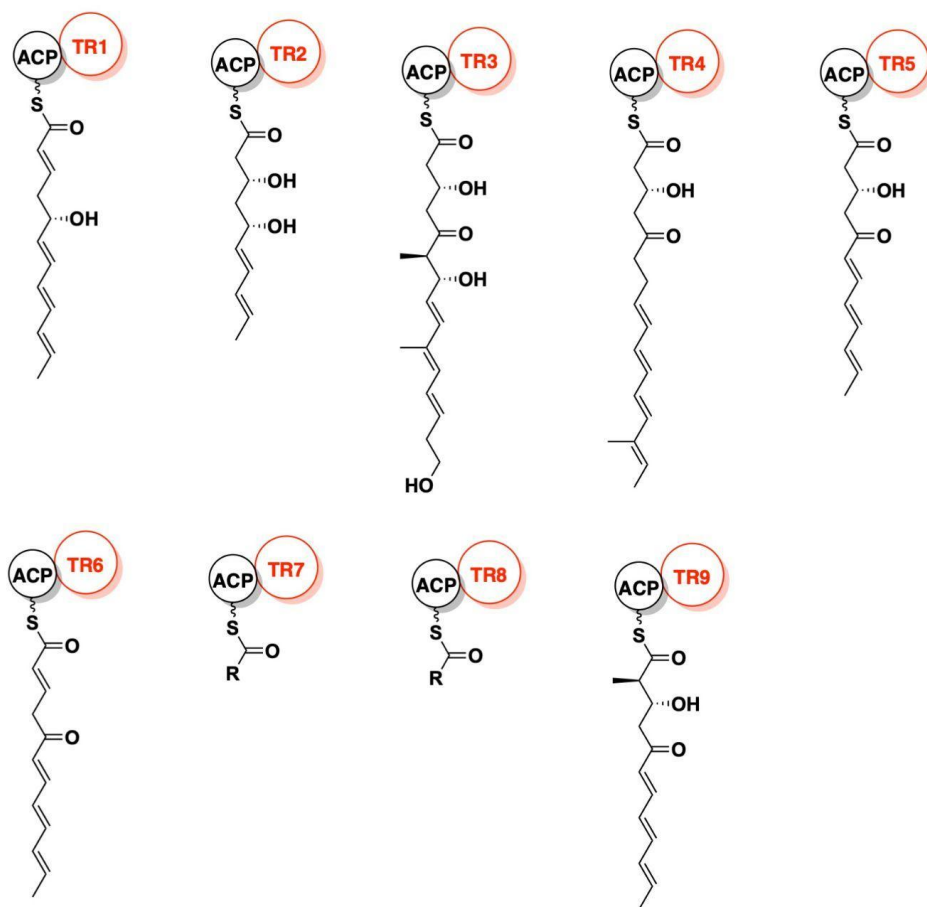
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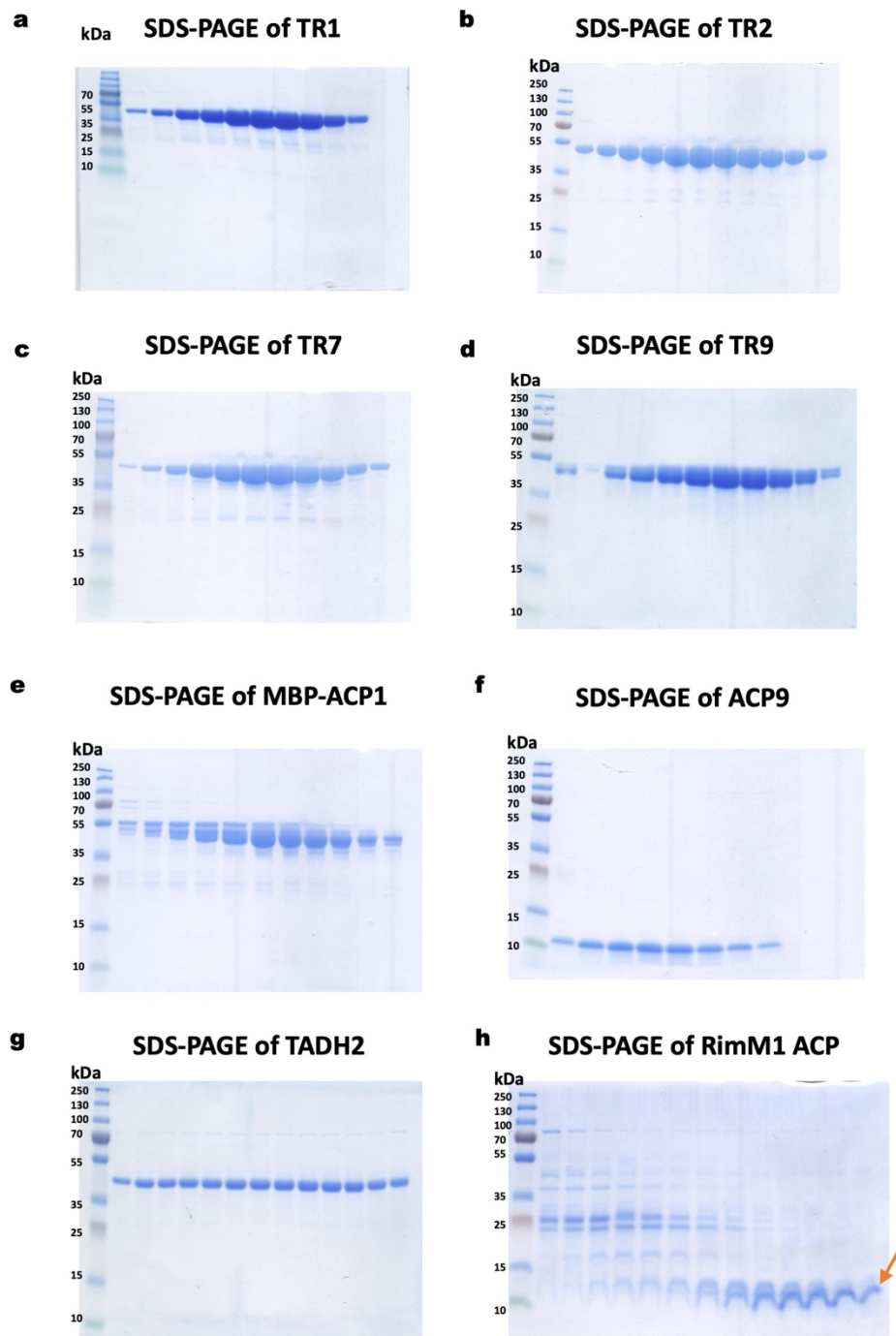
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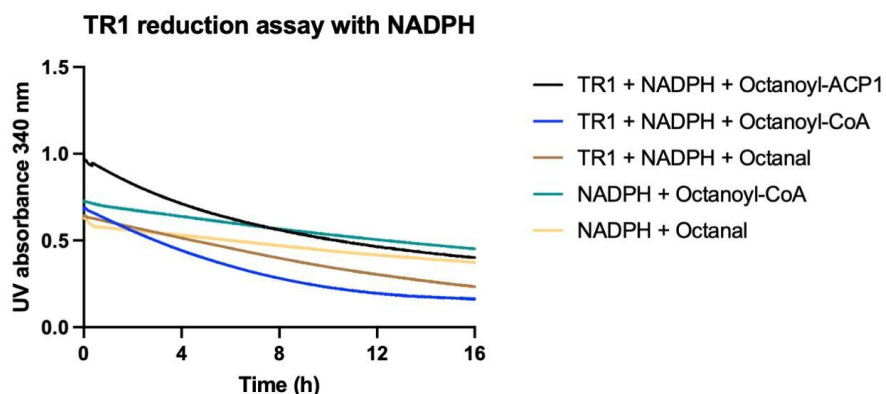
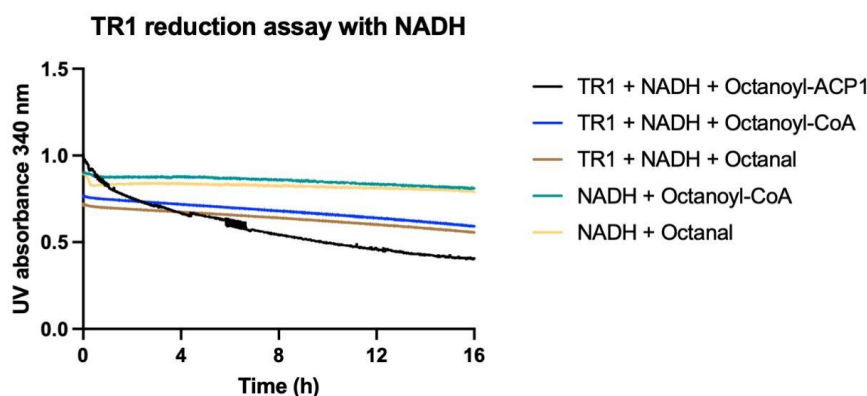
Supplementary Figure 1. Phylogenetic analysis of PKS TRs. TR1 is from coelimycin pathway¹, TR2 is from B24891 pathway¹, TR3 is from cyclizidine pathway², TR4 is from iminimycin pathway³, TR5 is from argimycin pathway⁴, TR6 is from streptazone pathway⁵, TR7 and TR8 are from unknown pathways, and TR9 is from venediol pathway⁶.



Supplementary Figure 2. Natural substrates of PKS TRs. TR1 is from coelimycin pathway¹, TR2 is from B24891 pathway¹, TR3 is from cyclizidine pathway², TR4 is from iminimycin pathway³, TR5 is from argimycin pathway⁴, TR6 is from streptazone pathway⁵, TR7 and TR8 are from unknown pathways with unknown products, and TR9 is from venediol pathway⁶. All natural products are in the range of medium-chain or long-chain.



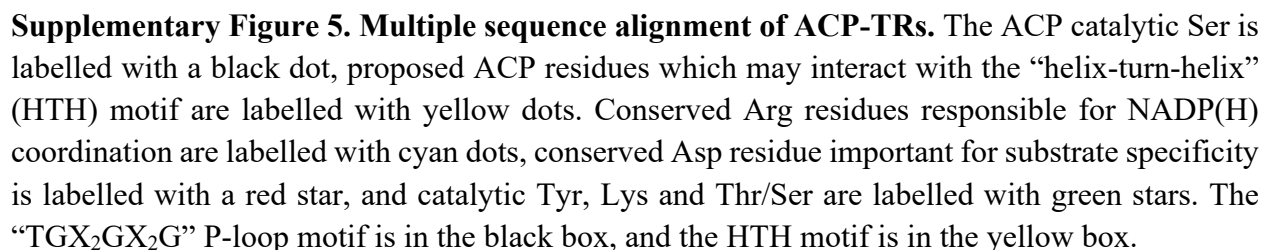
Supplementary Figure 3. SDS-PAGE analysis of purified TR1 (a), TR2 (b), TR7 (c), TR9 (d), maltose binding protein (MBP)-ACP1 (e), ACP9 (f), TADH2 (g)⁷, and RimM1 ACP (h). The protein ladders are PageRule Plus prestained protein ladders, 10 to 250 kDa. All samples were directly pooled from size-exclusion chromatography (SEC) purification.

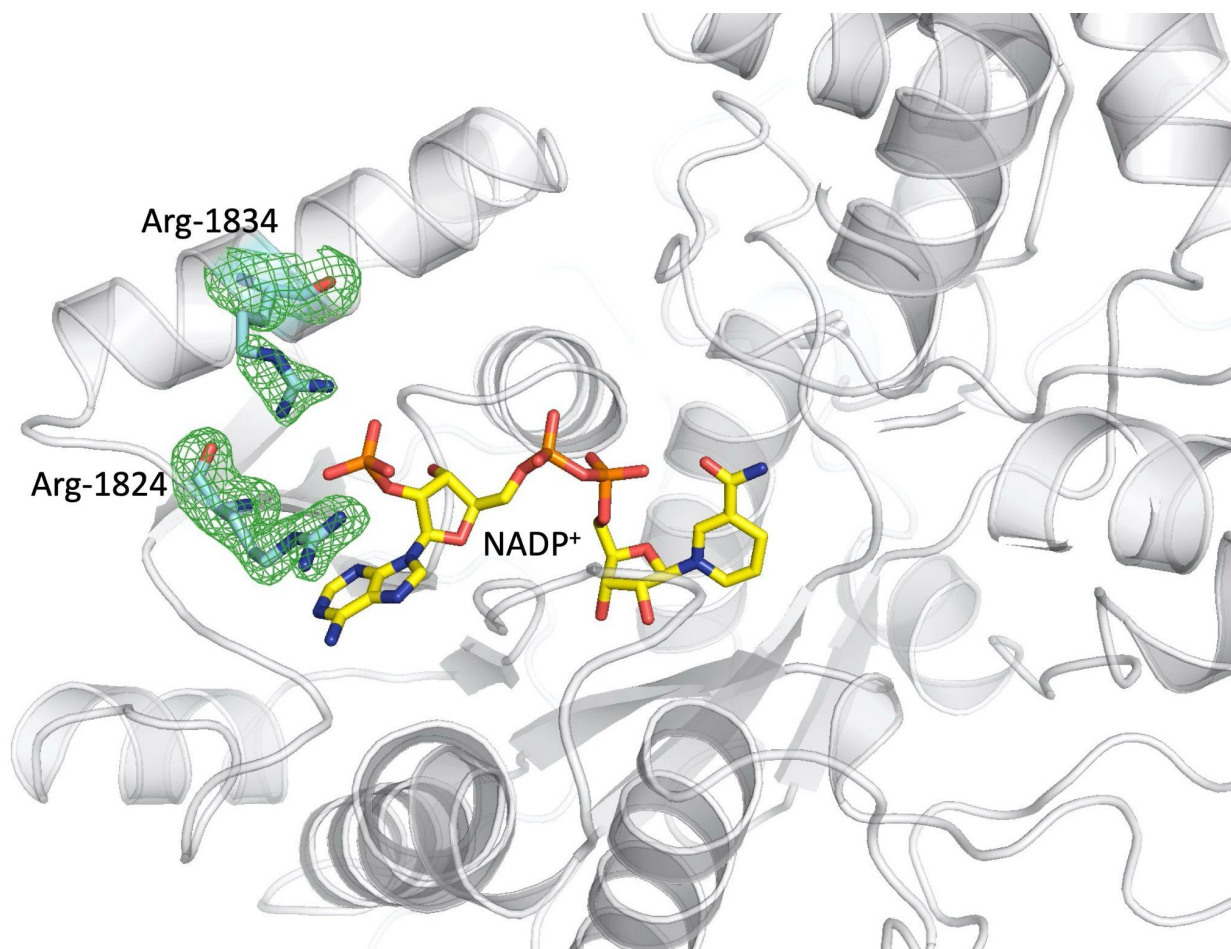
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Cofactor	Substrate	TR1-catalysed reaction		TR9-catalysed reaction	
		Initial reaction rate ($\times 10^{-3} \Delta UV_{340nm}/min$)	R ²	Initial reaction rate ($\times 10^{-3} \Delta UV_{340nm}/min$)	R ²
NADPH	Octanoyl-ACP	2.7	0.986	26.3	0.992
	Octanoyl-CoA	2.1	0.997	9.4	0.998
	Octanal	0.2	0.965	2.1	0.998
NADH	Octanoyl-ACP	3.2	0.763	1.3	0.658
	Octanoyl-CoA	0.1	0.971	0.5	0.838
	Octanal	1.1	0.988	0.4	0.956

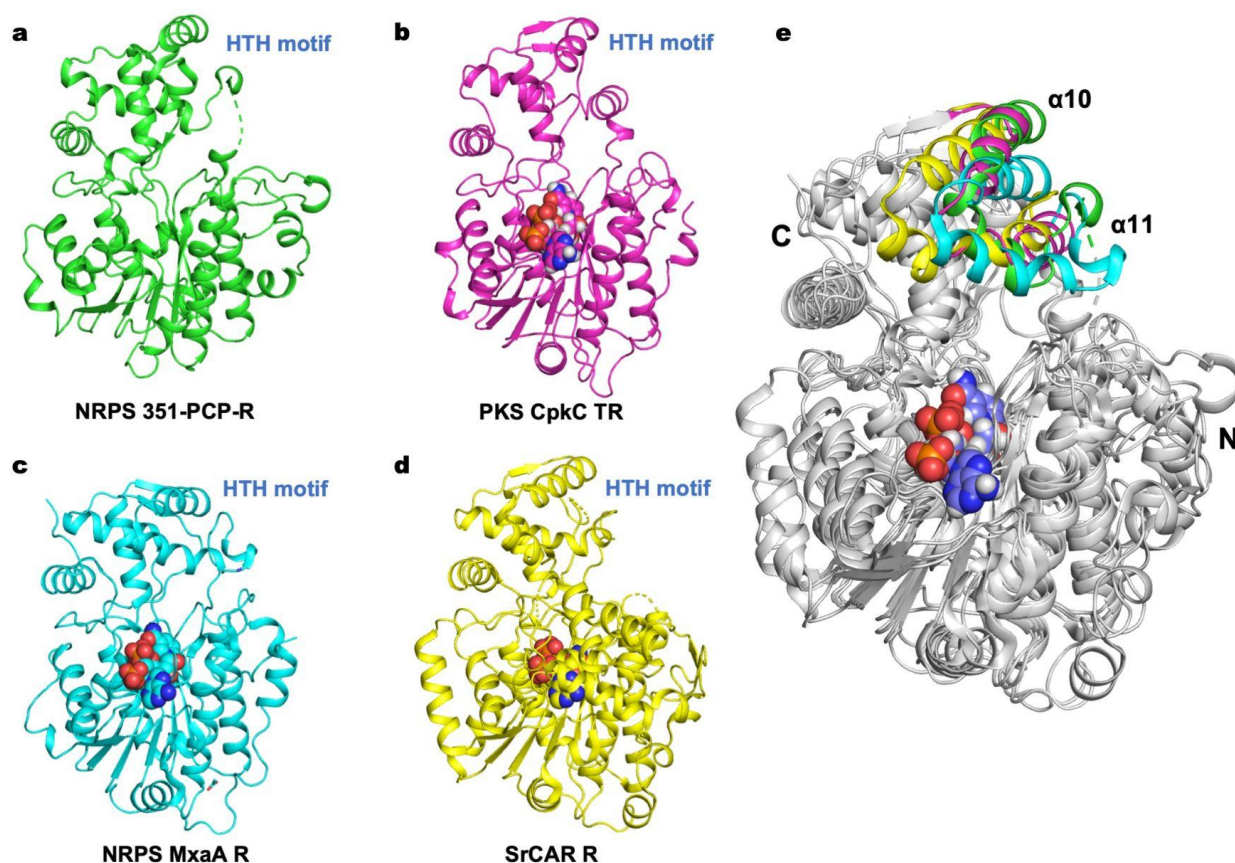
Supplementary Figure 4. TR substrate scope analysis and initial reaction rate measurement.

a-b, TR1 substrate scope analysis, with NADPH (a) or NADH (b) as the cofactor. Black: TR1 + NAD(P)H + octanoyl-MBP-ACP1; Blue: TR1 + NAD(P)H + octanoyl-CoA; Brown: TR1 + NAD(P)H + octanal; Green: NAD(P)H + octanoyl-CoA; Yellow: NAD(P)H + octanal. Each experiment was repeated in duplicate. c, Initial reaction rates of TR1- and TR9-catalysed reactions. R²: the coefficient of determination in linear regression. Octanoyl-ACP is octanoyl-ACP1 for the TR1 reaction and octanoyl-ACP9 for the TR9 reaction.

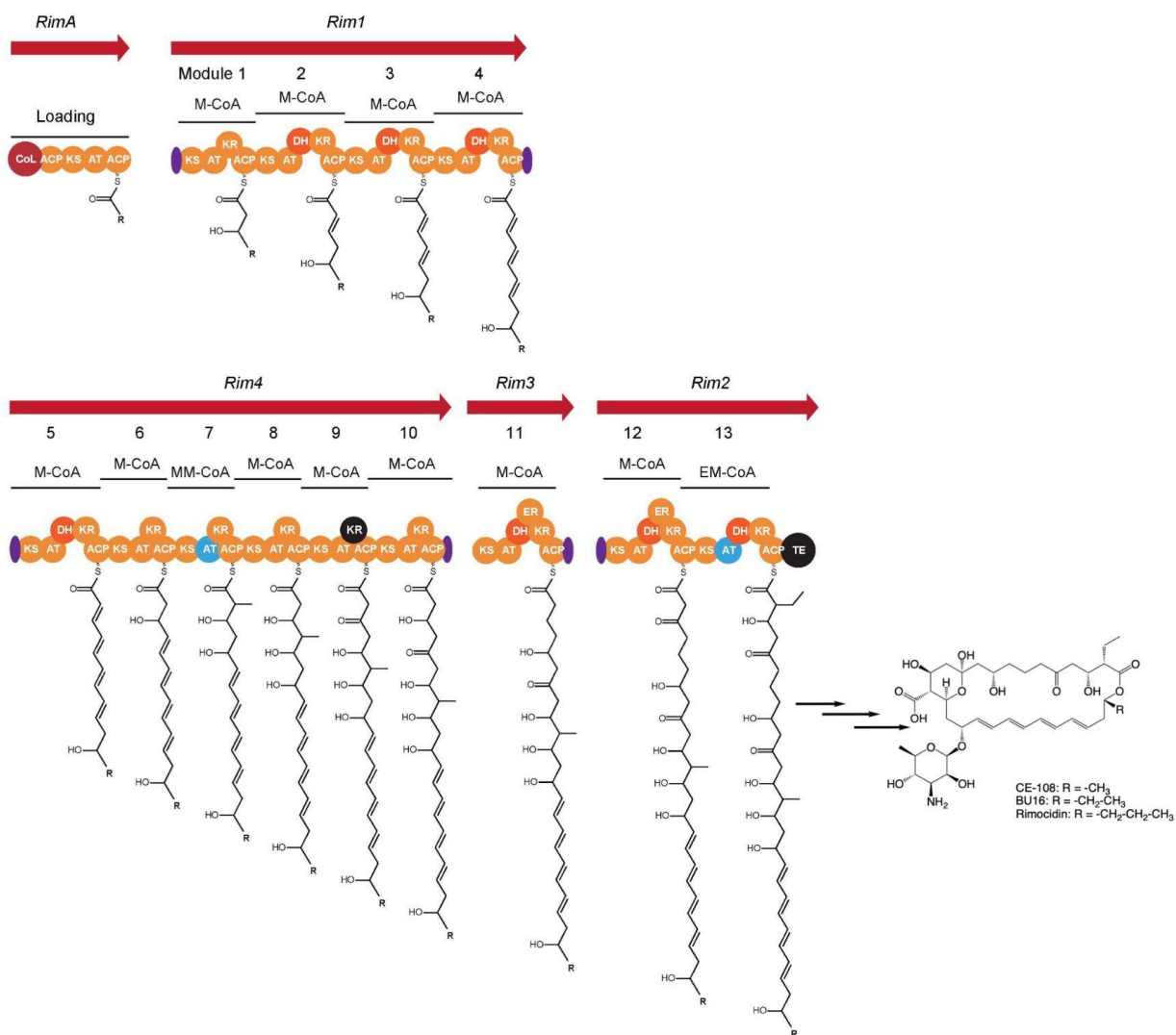




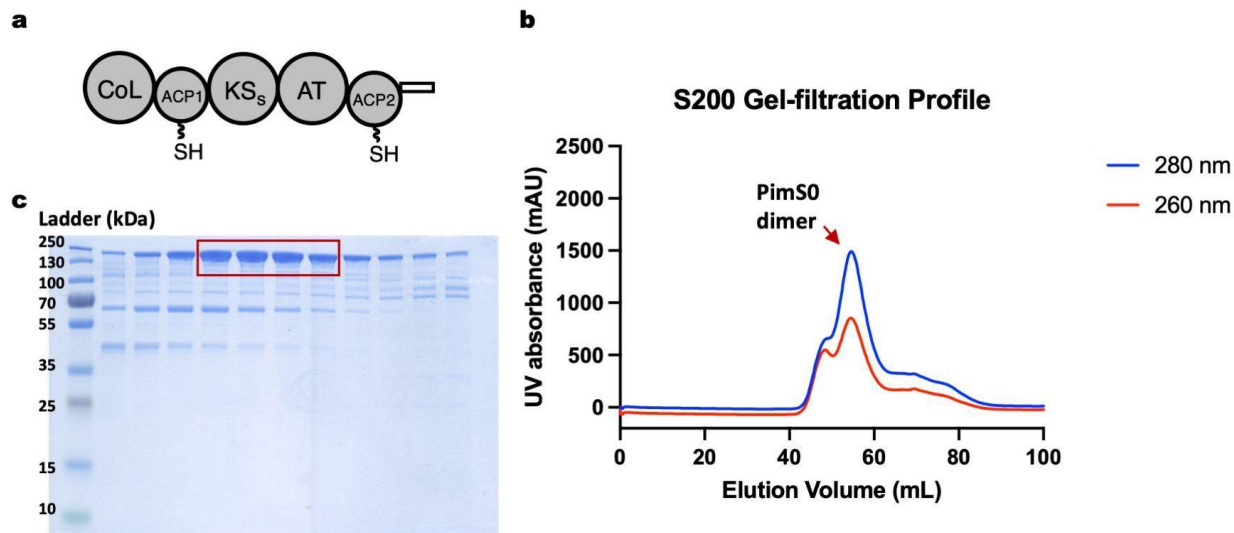
Supplementary Figure 6. the mFo-DFc omit map of Arg-1824 and Arg-1834 contoured 3.0 sigma level coloured in green.



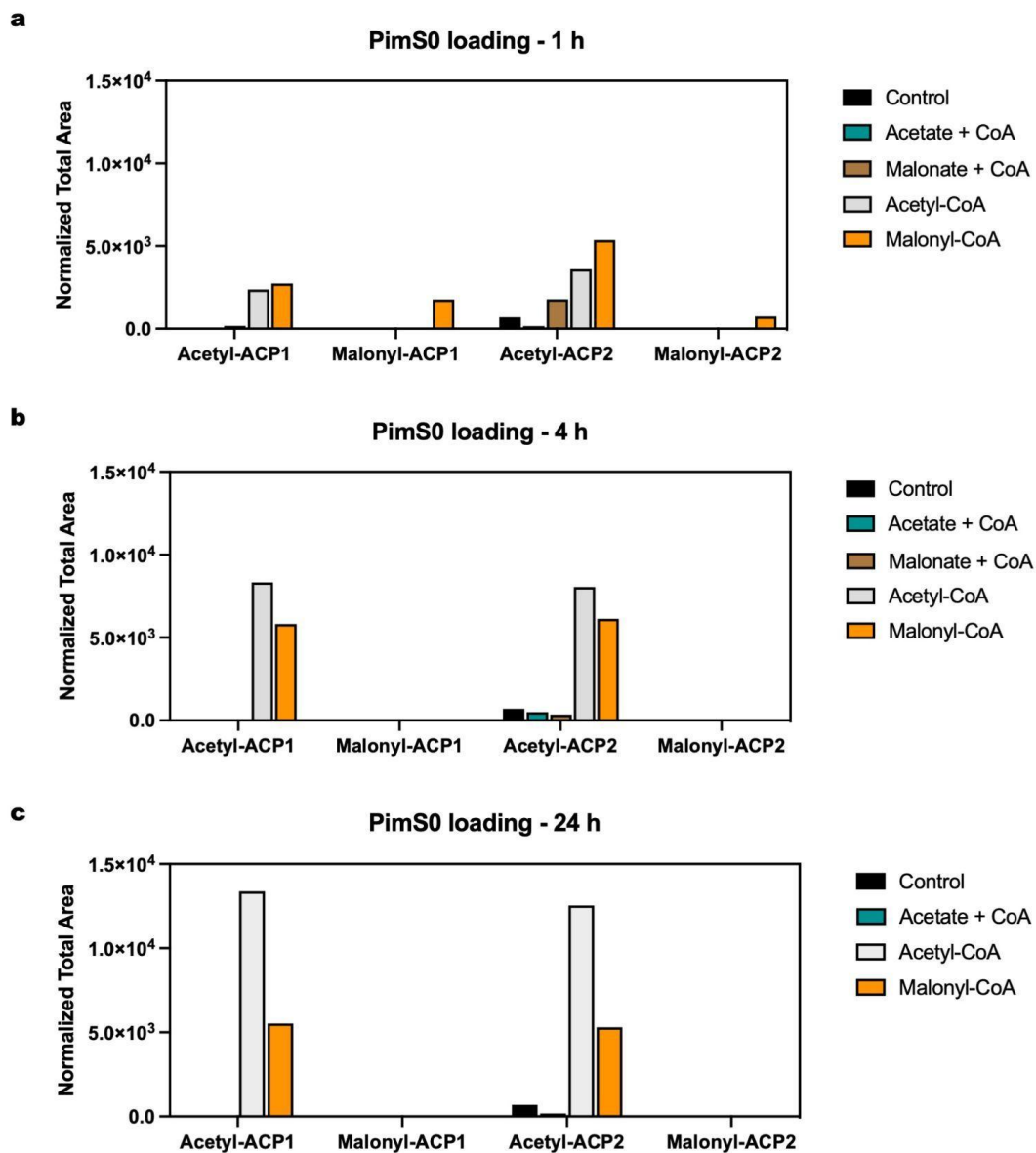
Supplementary Figure 7. Terminal thioreductase structures in carrier protein-containing multi-domain complexes. The “helix-turn-helix” (HTH) motif composed of $\alpha 10$ and $\alpha 11$ is labelled in NRPS 351-PCP-R (a, green, PDB code: 6VTJ), PKS CpkC TR or TR1 (b, magenta, PDB code: 8V1X), NRPS MxaA R (c, cyan, PDB code: 4U7W), and SrCAR R (d, yellow, PDB code: 5MSR). e, Overall alignment of the four crystal structures (N = N-terminus, C = C-terminus). Cofactors are shown in spheres.



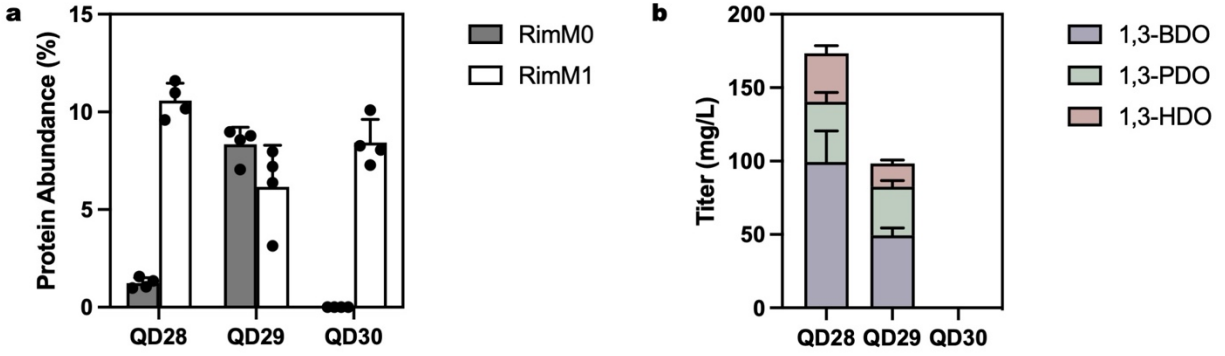
Supplementary Figure 8. Scheme of the rimocidin biosynthetic gene cluster. Rimocidin loading module (RimA or RimM0) is predicted to promiscuously initiate with either acetyl-, propionyl-, or butyryl- starter units, producing CE-108, BU16, and rimocidin^{8,9} as the pathway products.



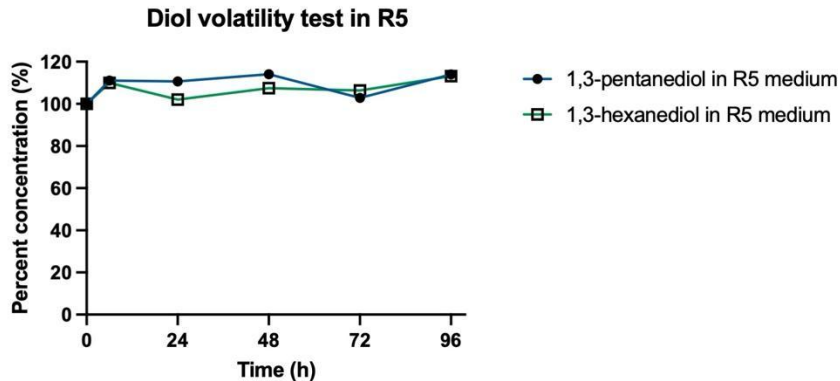
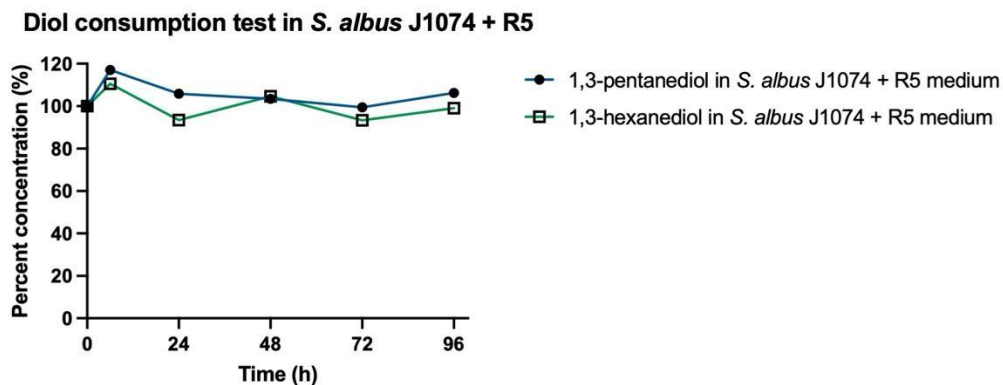
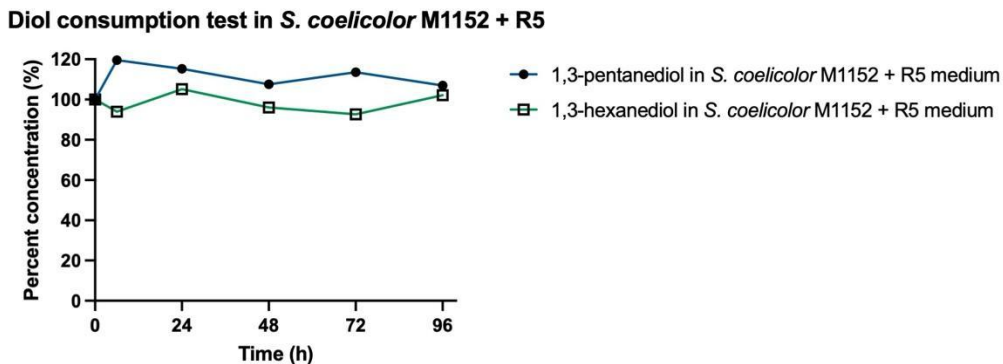
Supplementary Figure 9. Purification of *holo*-PimS0 (pPant-attached PimS0) loading module in *E. coli* BAP1 cells. a, Domain organisation of PimS0 loading module¹⁰. b, Gel-filtration elution profile of PimS0 purification, with X-axis being elution volume, and Y-axis being UV 280 nm absorbance for the blue line and UV 260 nm absorbance for the red line. PimS0 dimer eluted at 54 min. c, SDS-PAGE analysis of PimS0 from gel-filtration, fractions in the red box were collected for subsequent assays. Furthermore, with coexpression of GroEL-GroES and DnaJ-DnaK-GrpE chaperones in *E. coli* BL21(DE3), we successfully purified the *apo*-PimS0 dimer to 80% homogeneity. Next we tested whether PimS0 can load acetate, malonate, acetyl-CoA, or malonyl-CoA *in vitro* (Supplementary Figure 10). *Holo*-PimS0 loaded two of the tested substrates, acetyl-CoA and malonyl-CoA. In comparison, neither acetate nor malonate was accepted by *apo*-PimS0 despite the presence of a putative CoA-ligase (CoL) domain. Both PimS0 ACP1 and ACP2 were functional, as demonstrated by detection of acetyl-ACP1 and acetyl-ACP2. Moreover, malonyl-ACP1 and malonyl-ACP2 were detected after 1 hour of malonyl-CoA incubation, but absent after 4 hours, suggesting that KSs may be capable of malonyl-ACP decarboxylation to generate acetyl-ACPs. The fact that PimS0 readily accepted acetyl-CoA and malonyl-CoA as substrates suggested a similar initiation mode for RimM0 with a putatively altered substrate pool, which may include acetyl-CoA, propionyl-CoA, and butyryl-CoA starter units.



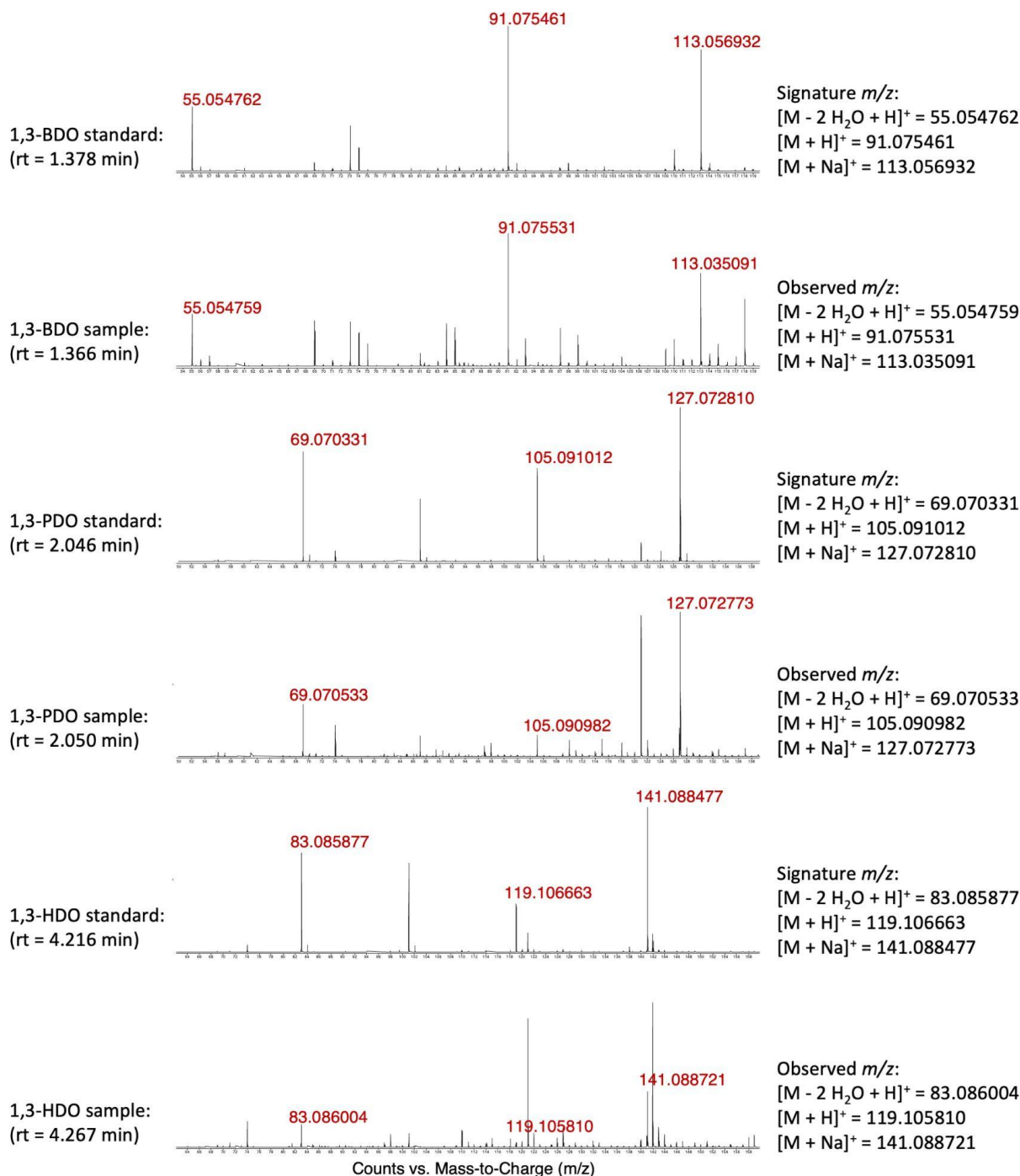
Supplementary Figure 10. Substrate scope analysis of PimS0 loading module at 1 h (a), 4 h (b), and 24 h (c). Black: no substrate control; Cyan: acetate + CoA; Brown: malonate + CoA; White: acetyl-CoA; Orange: malonyl-CoA.



Supplementary Figure 11. Promoter screening results of *rimM0* in *S. albus* RimM0M1-TR2 (QD28-QD30). QD28: *S. albus* *PrpsL(RO)-rimM0* + *Pgapdh(EL)-rimM1-TR2*; QD29: *S. albus* *Pgapdh(EL)-rimM0* + *Pgapdh(EL)-rimM1-TR2*; QD30: *S. albus* *kasOP*-rimM0* + *Pgapdh(EL)-rimM1-TR2*. a, Protein abundance of RimM0 and RimM1 in engineered *S. albus* when using *PrpsL(RO)*, *Pgapdh(EL)*, or *kasOP** promoter to drive *rimM0* transcription. The *rimM1-TR2* promoter is *Pgapdh(EL)*. Grey: RimM0 protein abundance; White: RimM1 protein abundance. b, 1,3-Diol production titers after 7 d cultivation in R5 medium. Purple: 1,3-BDO; Green: 1,3-PDO; Red: 1,3-HDO. All data are represented as mean value and error bars indicate standard deviation (s.d.) of four biological replicates (n = 4).

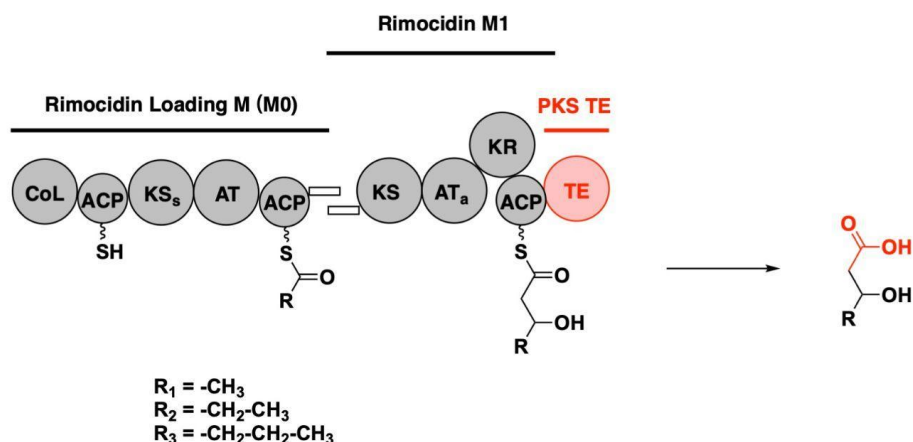
a**b****c**

Supplementary Figure 12. 1,3-Diol volatility and consumption test. a, Volatility test of 1,3-pentanol (blue) and 1,3-hexanediol (green) in R5 medium. b, *S. albus* J1074 consumption test of 1,3-pentanol (blue) and 1,3-hexanediol (green) in R5 medium. c, *S. coelicolor* M1152 consumption test of 1,3-pentanol (blue) and 1,3-hexanediol (green) in R5 medium.

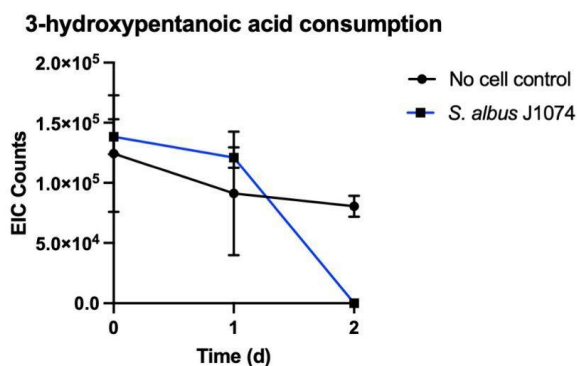


Supplementary Figure 13. MS spectra of 1,3-diols produced in *S. albus* RimM0M1-TR1 (QD27). From top to bottom are MS spectra of 1,3-BDO standard at rt = 1.378 min, QD27 at rt = 1.366 min, 1,3-PDO standard at rt = 2.046 min, QD27 at rt = 2.050 min, 1,3-HDO standard at rt = 4.216 min, QD27 at rt = 4.267 min. All observed m/z match well with expected signature m/z (dehydrated m/z^{11} , $[M + H]^+ m/z$, and $[M + Na]^+ m/z$).

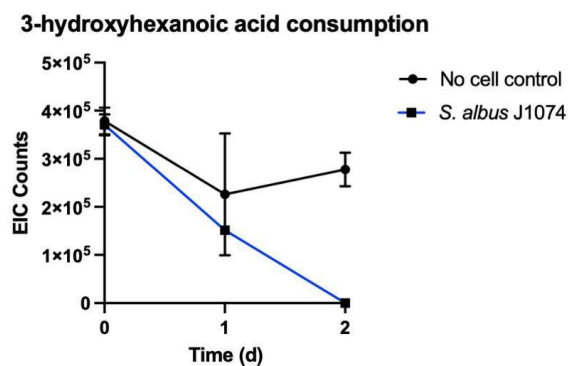
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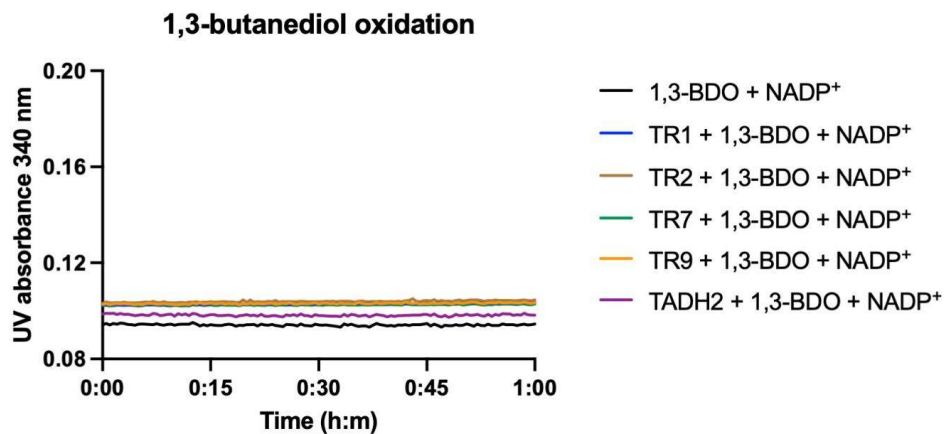
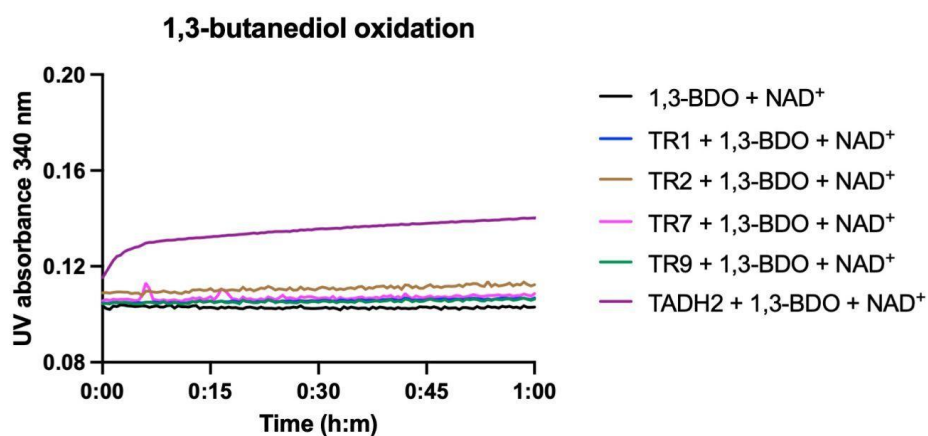
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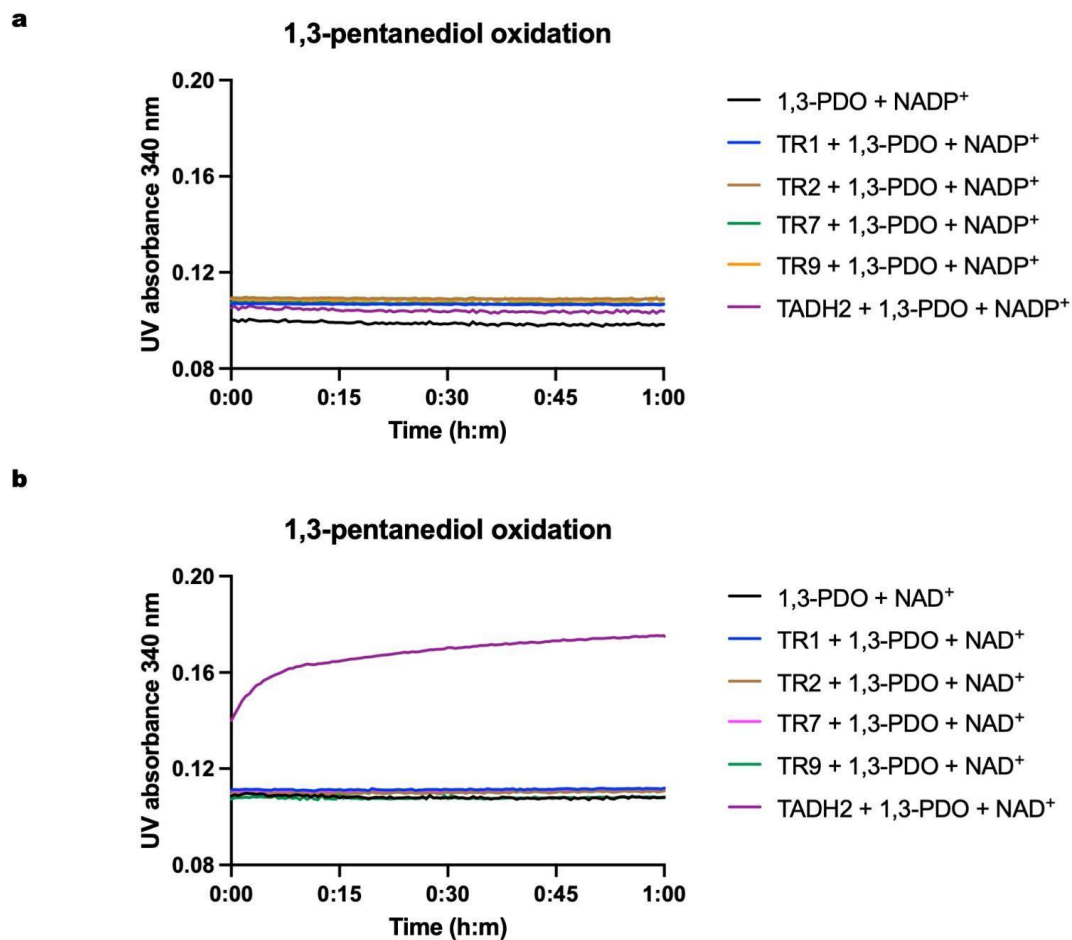
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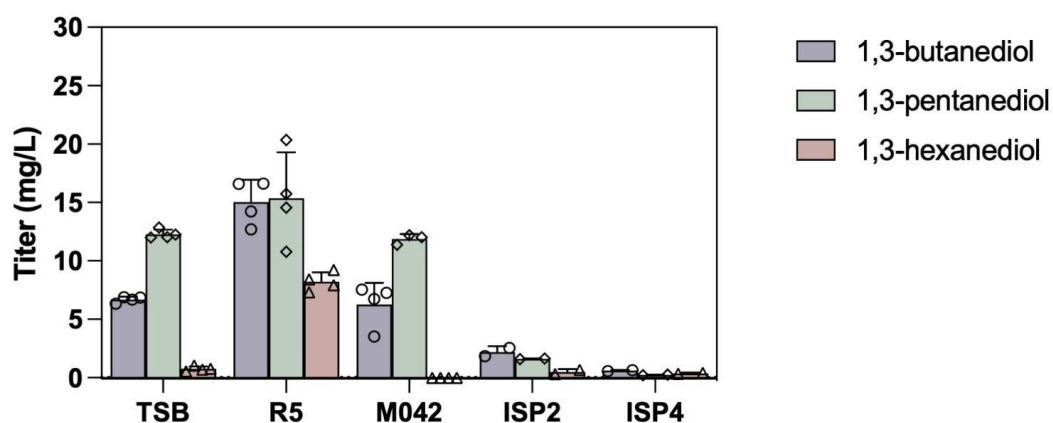
Supplementary Figure 14. *S. albus* RimM0M1-DEBS TE (QD18) production test. a, Reaction scheme of RimM0M1-DEBS TE for 3-hydroxy acid production in *Streptomyces*. No 3-hydroxy acid was detected. b, Consumption test of 3-hydroxypentanoic acid in R5 (black) or R5 + *S. albus* (blue). c, Consumption test of 3-hydroxyhexanoic acid in R5 (black) or R5 + *S. albus* (blue). All cultivation data are represented as mean value and error bars indicate standard deviation (s.d.) of three biological replicates (n = 3).

a**b**

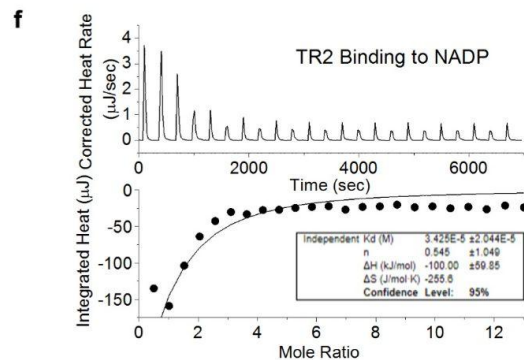
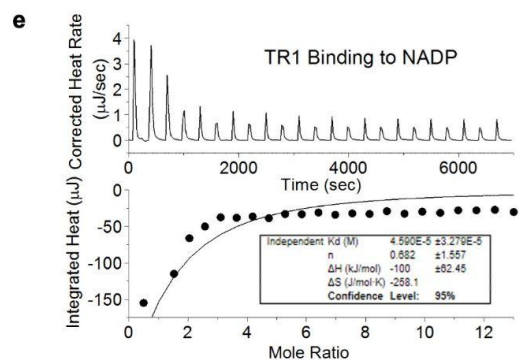
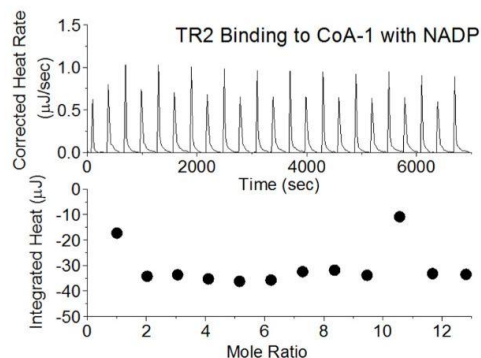
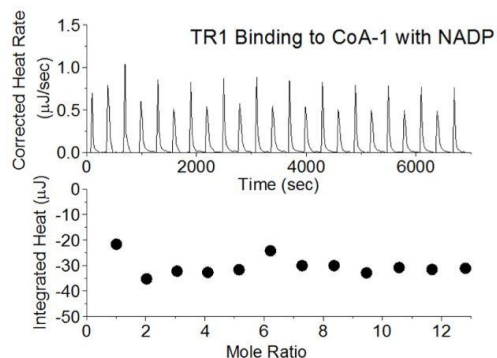
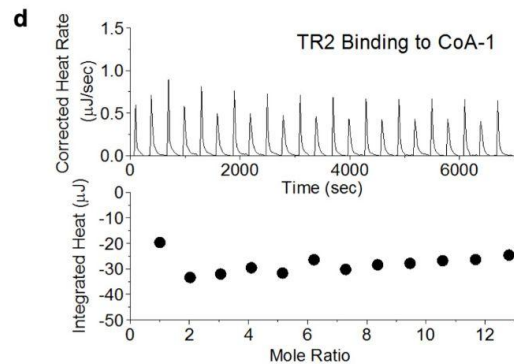
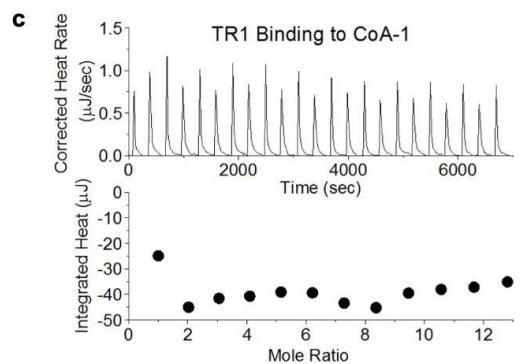
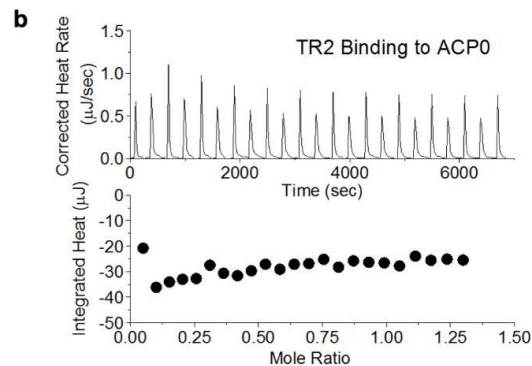
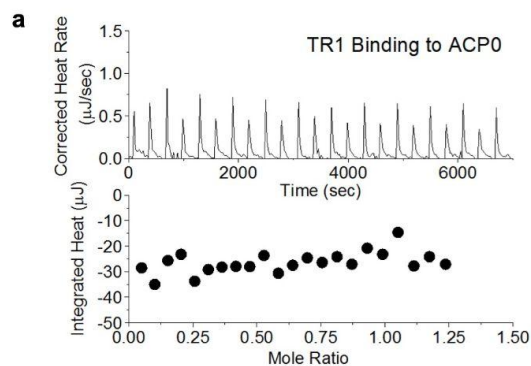
Supplementary Figure 15. TR substrate scope analysis with 1,3-butanediol, using NADP⁺ (a) or NAD⁺ (b) as the cofactor. Black: no enzyme control; Blue: TR1; Brown: TR2; Magenta: TR7; Green: TR9; Purple: TADH2 positive control. Each experiment was repeated in triplicate.



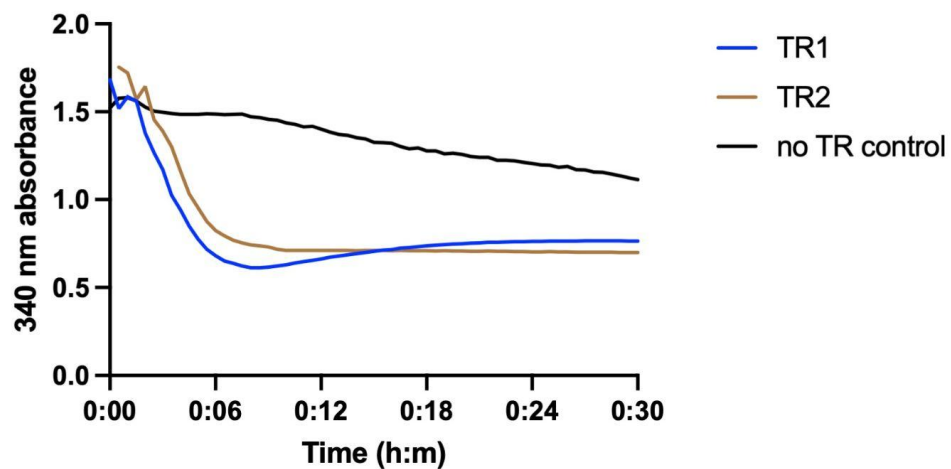
Supplementary Figure 16. TR substrate scope analysis with 1,3-pentenediol, using NADP⁺ (a) or NAD⁺ (b) as the cofactor. Black: no enzyme control; Blue: TR1; Brown: TR2; Magenta: TR7; Green: TR9; Purple: TADH2 positive control. Each experiment was repeated in triplicate.



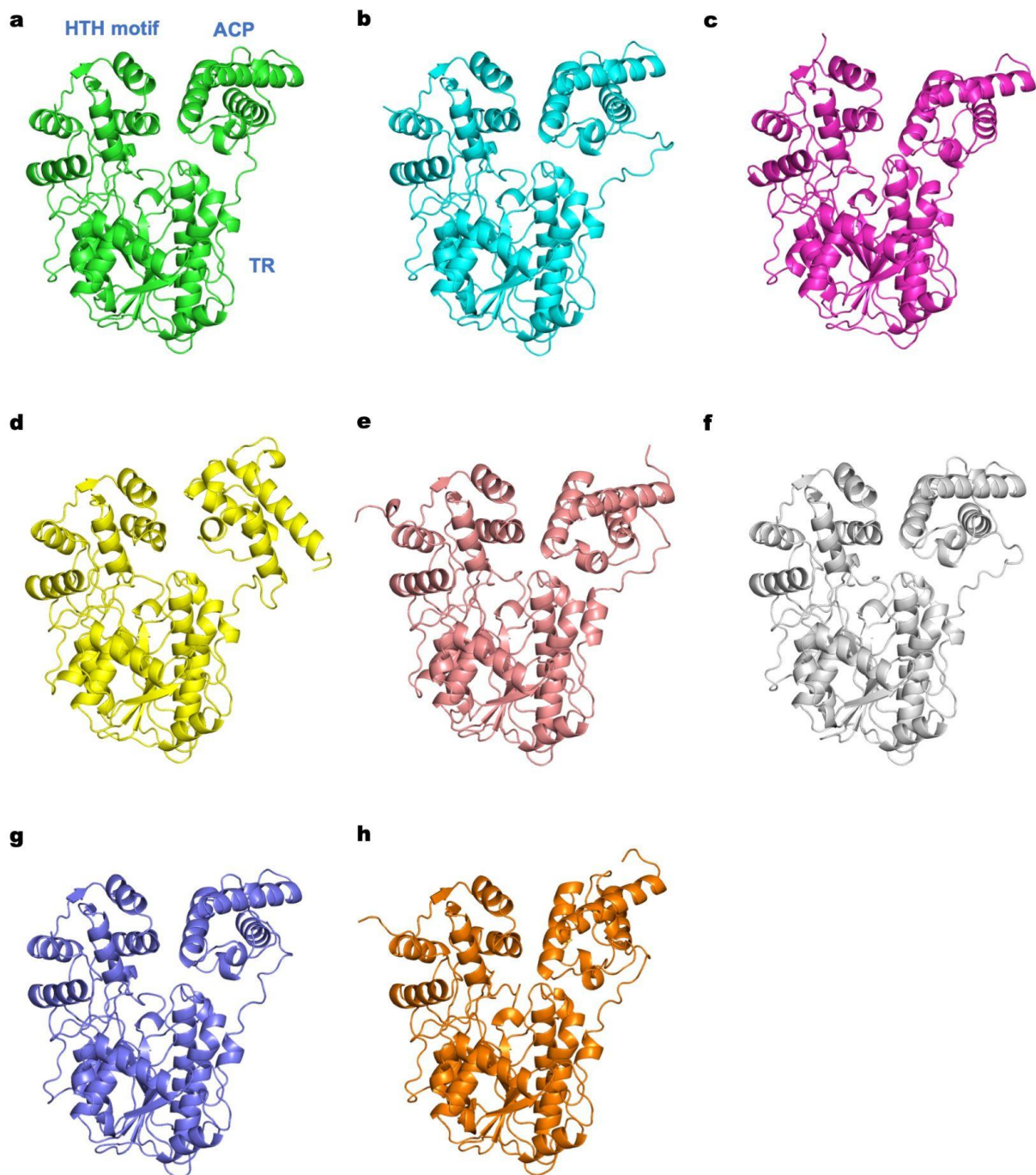
Supplementary Figure 17. Medium screening for *S. albus* RimM0M1-TR1 (QD27). 3 d RimM0M1-TR1 cultivation results in TSB, R5, M042, and 5 d cultivation results in ISP2, ISP4 were presented here. 15.0 mg/L 1,3-BDO, 15.4 mg/L 1,3-PDO, and 8.2 mg/L 1,3-HDO were produced in R5 after 3 d. All cultivation data are represented as mean value and error bars indicate standard deviation (s.d.) of at least two biological replicates. For TSB, R5 and M042 groups, n = 4; for ISP2 and ISP4 groups, n = 2.



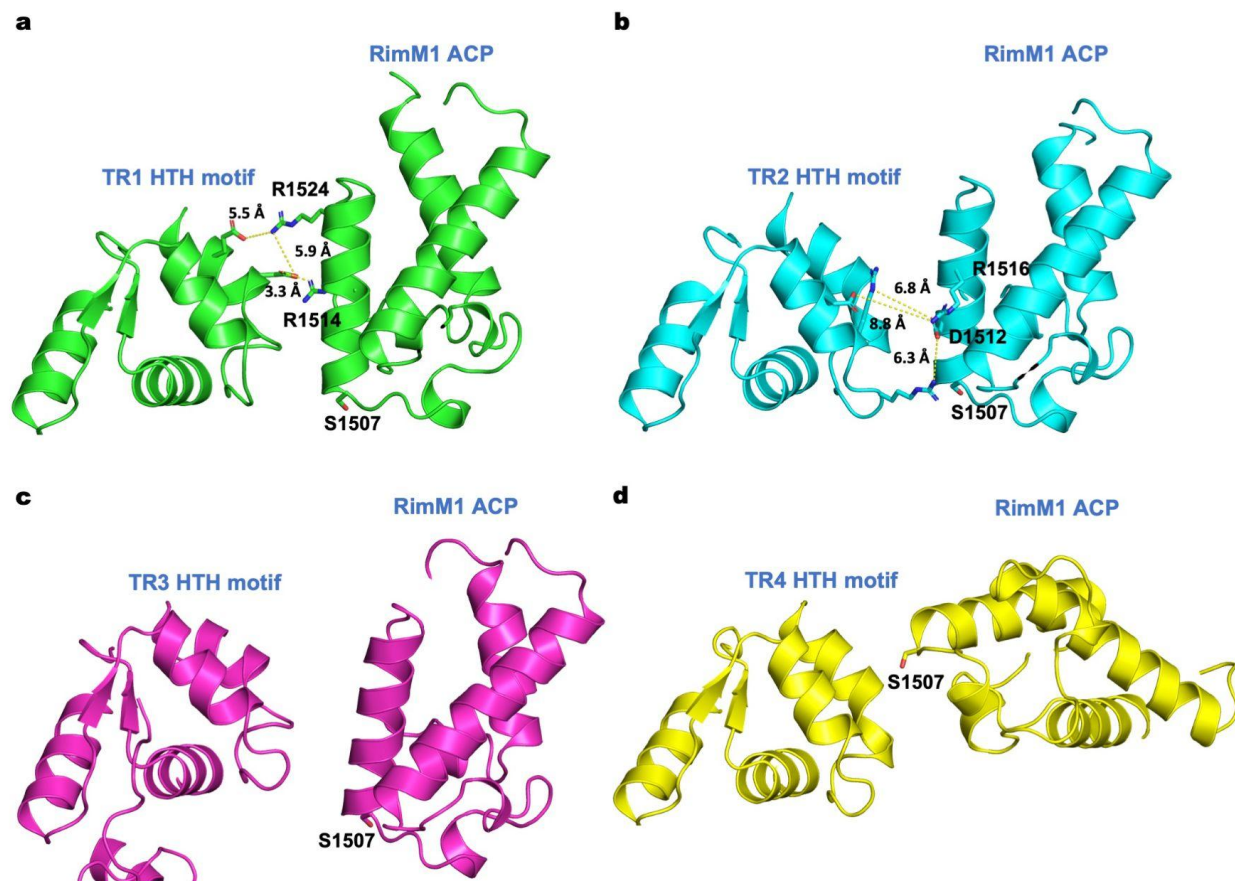
Supplementary Figure 18. TR1 and TR2 binding to ACP and enzyme substrates. ITC experiments measure the binding of TR1 or TR2 to either RimM1 ACP (ACP0) protein, 3-hydroxybutyryl-CoA (CoA-1), or NADP⁺. Each panel shows the experiment injections as a function of time above and the integrated heat of each injection as a function of the molar ratio of components below. a-b, TR1 or TR2 (10 μ M) show little or no binding to RimM1 ACP protein (50 μ M in the syringe and 0.5 μ M injections). c-d, neither TR1 nor TR2 (10 μ M) binds 3-hydroxybutyryl-CoA (1 mM in the syringe, 10 μ M injections) alone or in the presence of 1 mM NADP⁺ in both the syringe and cell. e-f, TR1 or TR2 (20 μ M) binds NADP⁺ (1 mM in the syringe, 10 μ M injections). In the bottom panel, the data points (circles) are fit to an independent binding curve (solid line) with the following fit parameters: $K_{d, \text{TR1_NADP}} = 4.6 \pm 3.3 \times 10^{-5}$ M, $n = 0.7 \pm 1.5$, $\Delta H = 100 \pm 60$ kJ/mol; $K_{d, \text{TR2_NADP}} = 3.4 \pm 2.0 \times 10^{-5}$ M, $n = 0.6 \pm 1.0$, $\Delta H = 100 \pm 60$ kJ/mol.



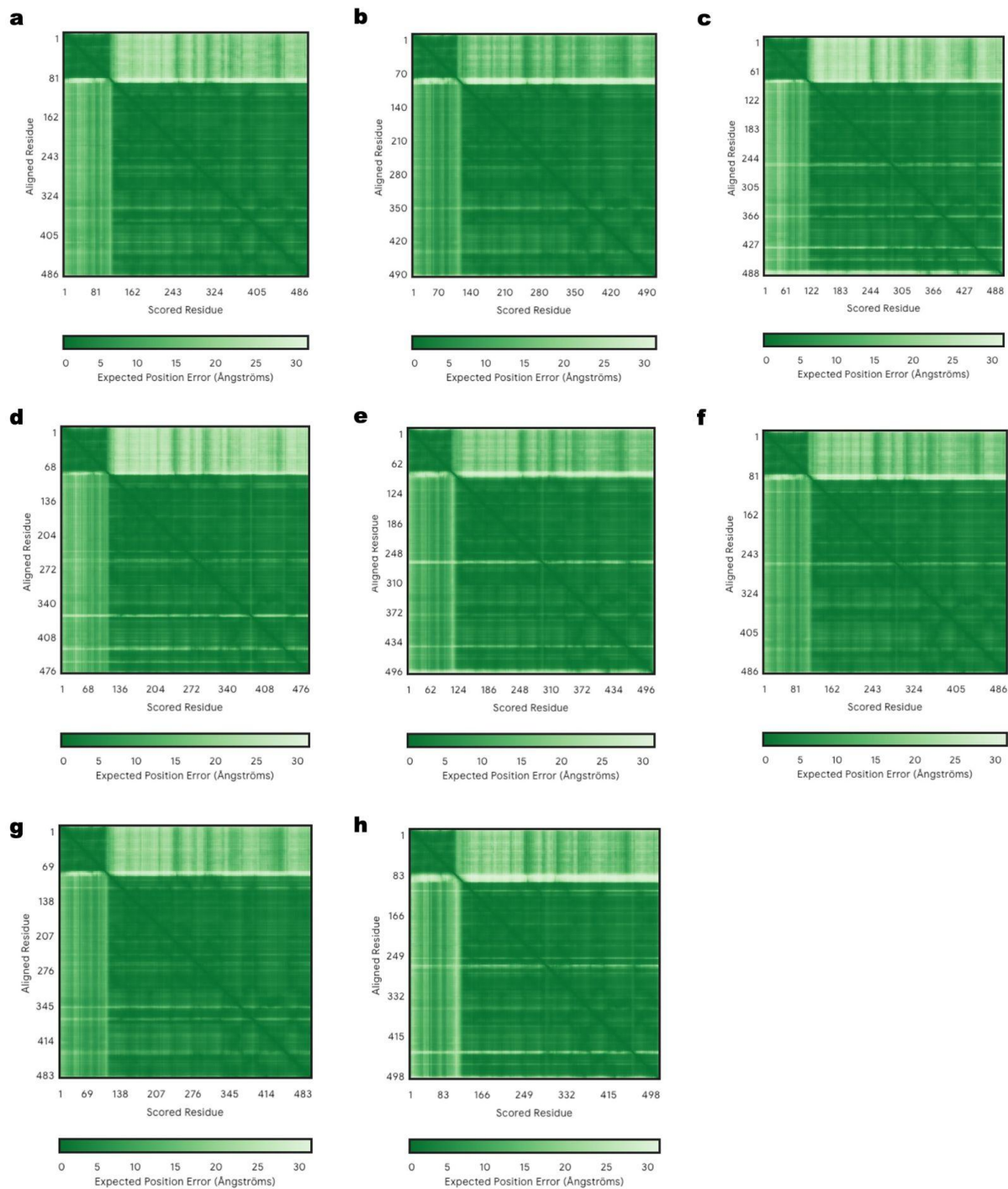
Supplementary Figure 19. TR-catalysed reactions on 3-hydroxybutyryl-CoA. NADPH is the cofactor, and NADPH consumption was detected here. Black: no TR control; Blue: TR1 reaction; Brown: TR2 reaction. TR1 and TR2 have similar reaction rates. Each experiment was repeated in triplicate.



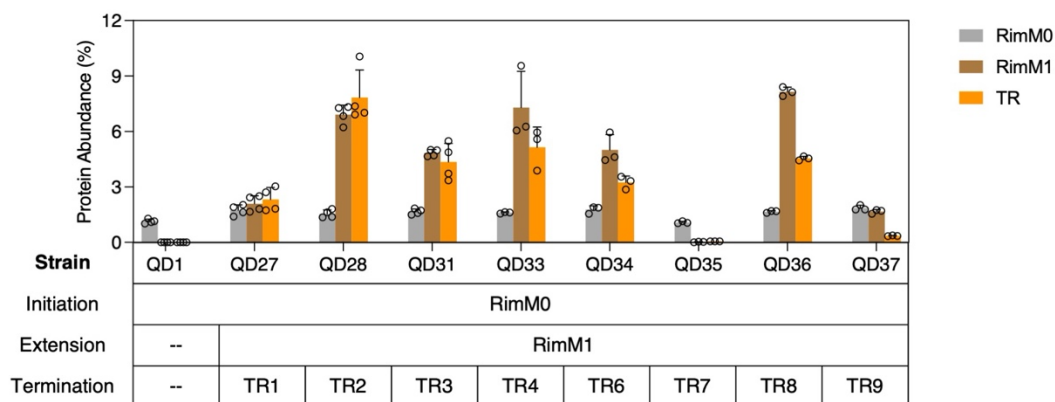
Supplementary Figure 20. AlphaFold3 models of chimeric RimM1 ACP-TR complexes. a-h, RimM1 ACP-TR1/TR2/TR3/TR4/TR6/TR7/TR8/TR9.



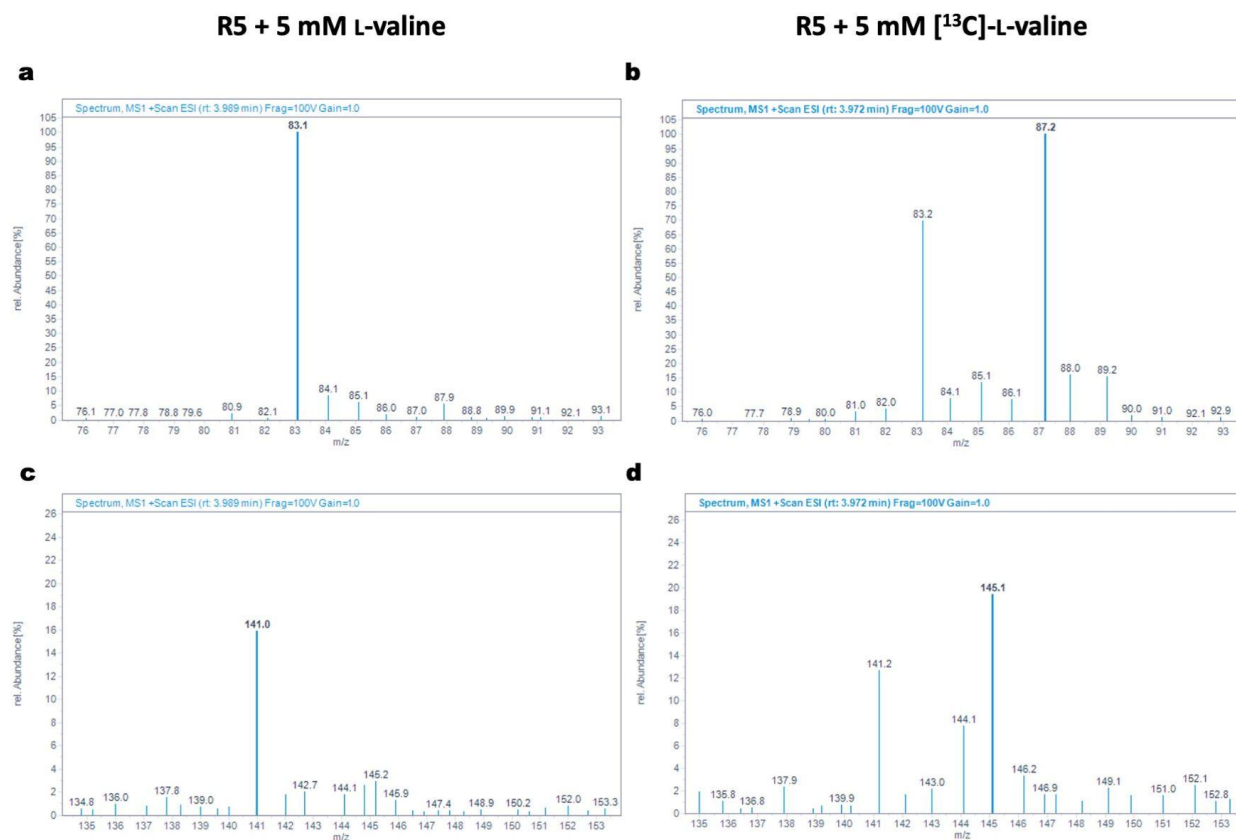
Supplementary Figure 21. Predicted RimM1 ACP-TR domain-domain interactions are dominantly charge-charge interactions. a, Predicted charge-charge interactions between RimM1 ACP and TR1 HTH motif. The three bond distances are 3.3 Å, 5.5 Å, and 5.9 Å. b, Predicted charge-charge interactions between RimM1 ACP and TR2 HTH motif. The three bond distances are 6.3 Å, 6.8 Å, and 8.8 Å. c, No charge-charge interaction between RimM1 ACP and TR3 HTH motif was predicted by AlphaFold 3. d, No charge-charge interaction between RimM1 ACP and TR4 HTH motif was predicted by AlphaFold 3. Moreover, RimM1 ACP in the ACP-TR4 model positions its catalytic serine (S1507, RimM1 amino acid numbering) away from TR active site. Notably, all of the RimM1 ACP residues (D1512, R1514, R1516, R1524) identified here are mostly conserved in TR-cognate ACPs, suggesting potentially conserved charge-charge interaction-driven ACP-TR recognition in their native PKSs.



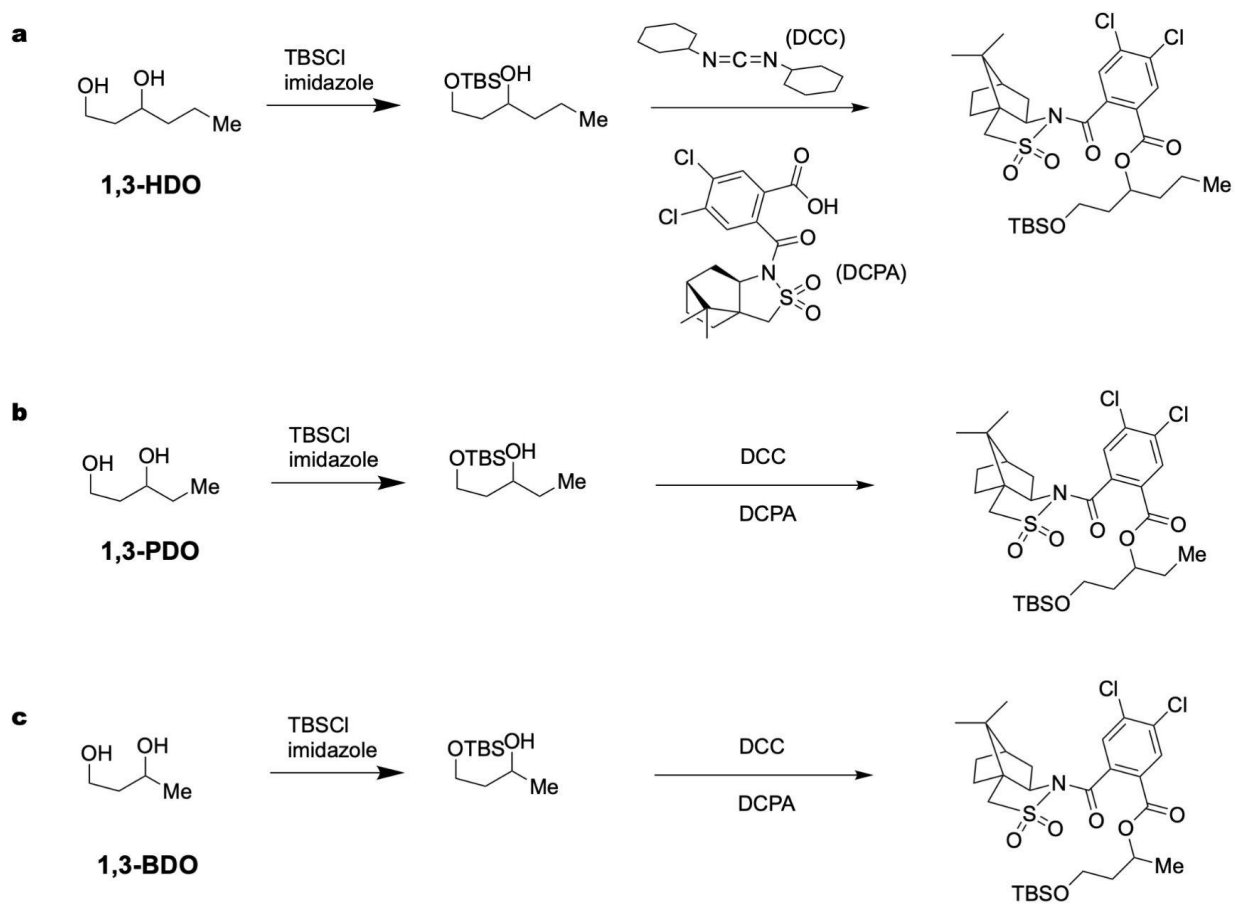
Supplementary Figure 22. Expected position error maps of RimM1 ACP-TR models. a-h, RimM1 ACP-TR1/TR2/TR3/TR4/TR6/TR7/TR8/TR9.



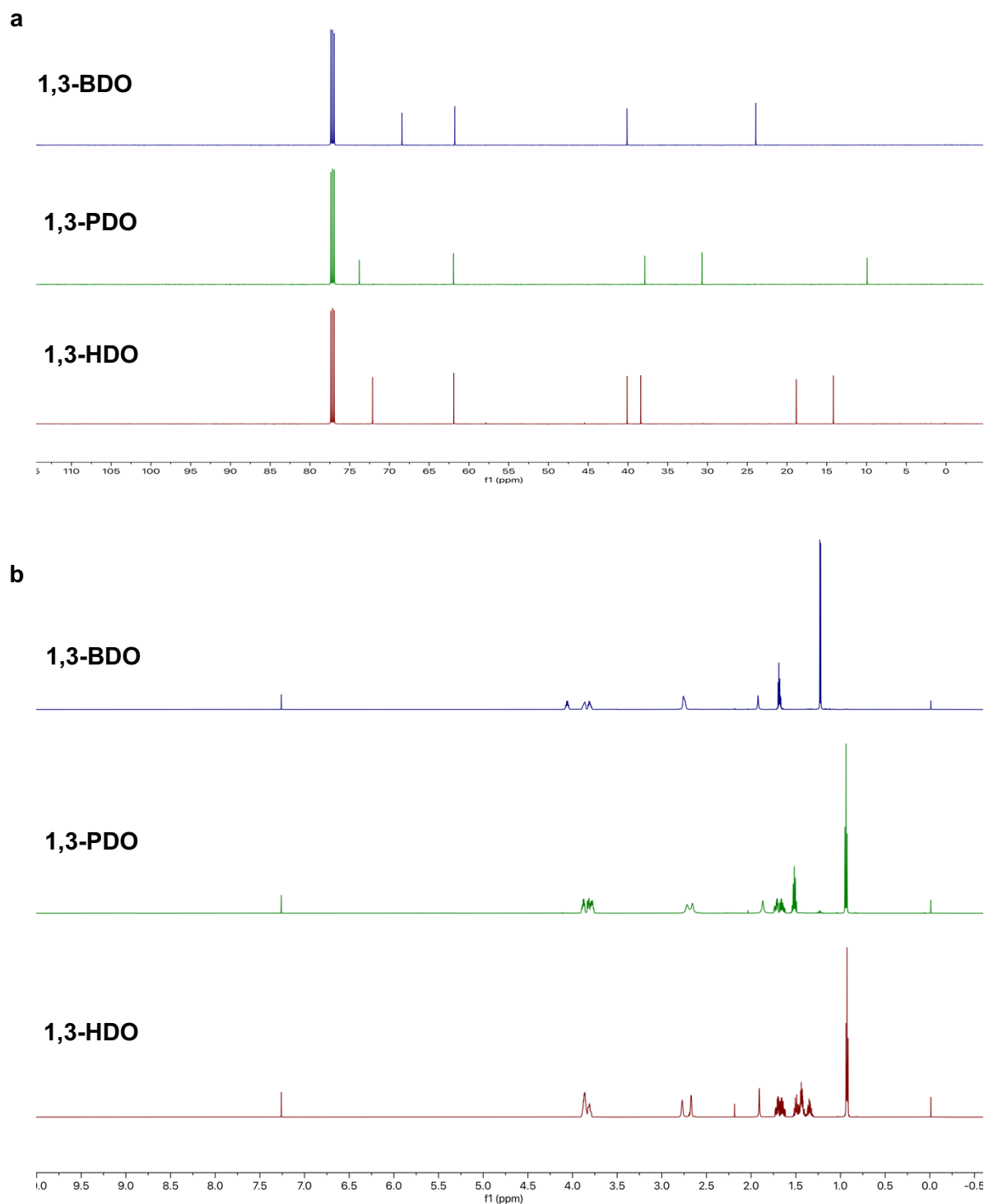
Supplementary Figure 23. Protein abundance of RimM1-TR chimaeras in engineered *S. albus* RimM0M1-TR after 3 d cultivation. Grey: RimM0; Brown: RimM1; Orange: TR. RimM0 abundances are similar in all strains. In comparison, RimM1-TR2/3/4/6/8 abundance is optimal, followed by RimM1-TR1. RimM1-TR7 was not detected in QD35, in agreement with no diol production. RimM1-TR9 abundance is quite low which explains the relatively low diol titer in QD37. All cultivation data are represented as mean value and error bars indicate standard deviation (s.d.) of at least three biological replicates. For QD1, QD27, QD28, QD31 and QD33 groups, n = 4; for QD34 – QD37 groups, n = 3.



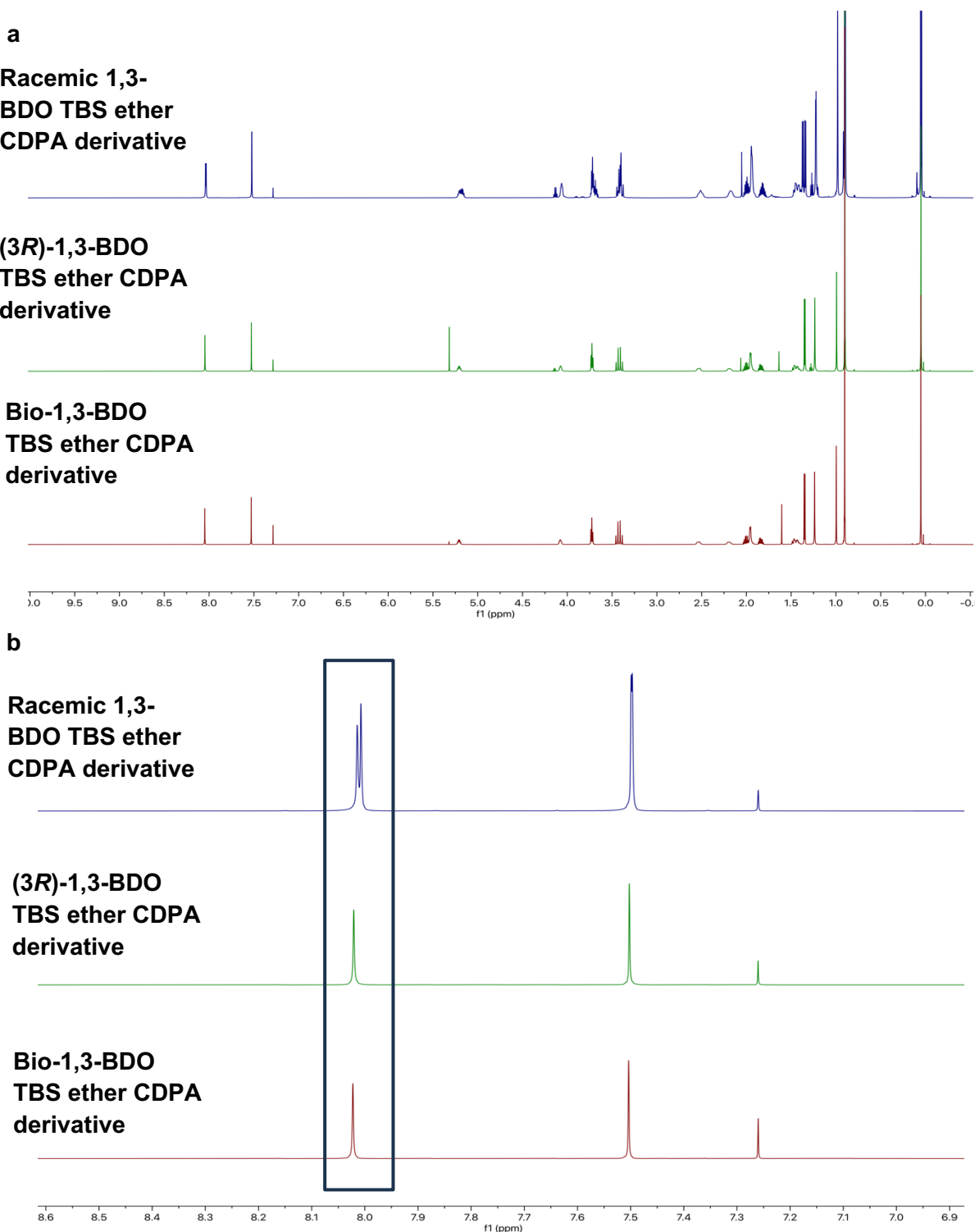
Supplementary Figure 24. [¹³C]labelled L-valine feeding experiment on *S. albus* RimM0M1-TR2 (QD28). R5 medium + 5 mM L-valine or [¹³C]labelled L-valine was selected for comparison. a, 1,3-hexanediol MS spectrum in L-valine addition group (rt = 3.99 min, expected dehydrated 83.1 *m/z*, observed 83.1 *m/z*). b, 1,3-hexanediol MS spectrum in [¹³C]labelled L-valine addition group (rt = 3.97 min, expected dehydrated 87.1 *m/z*, observed 87.2 *m/z*). c, 1,3-hexanediol MS spectrum in L-valine addition group (rt = 3.99 min, expected [M + Na]⁺ 141.1 *m/z*, observed 141.0 *m/z*). d, 1,3-hexanediol MS spectrum in [¹³C]labelled L-valine addition group (rt = 3.97 min, expected [M + Na]⁺ 145.1 *m/z*, observed 145.1 *m/z*).



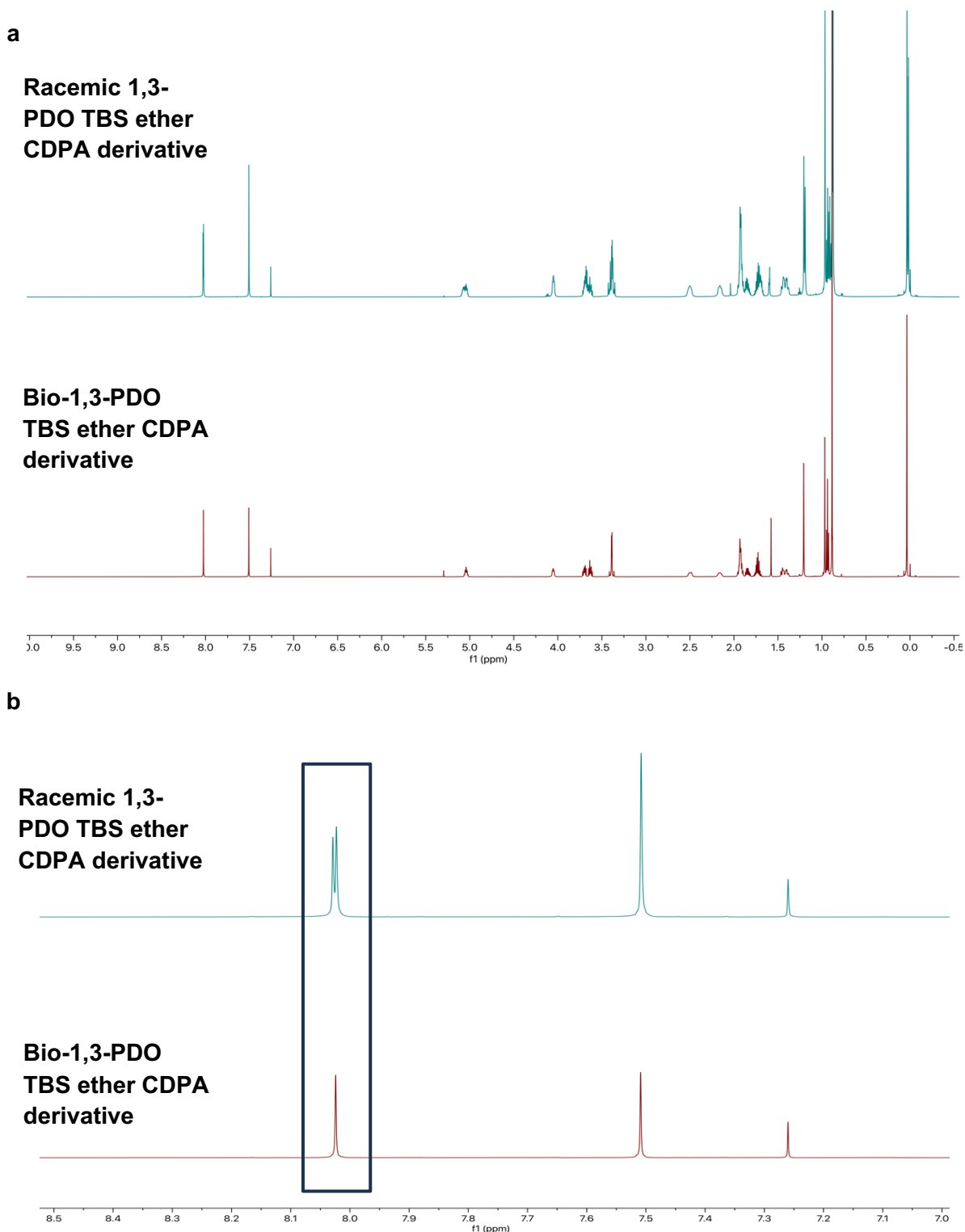
Supplementary Figure 25. The strategy of chiral CDPA coupling to derivatize 1,3-diols to form diastereomers. a, 1,3-HDO. b, 1,3-PDO. c, 1,3-BDO.



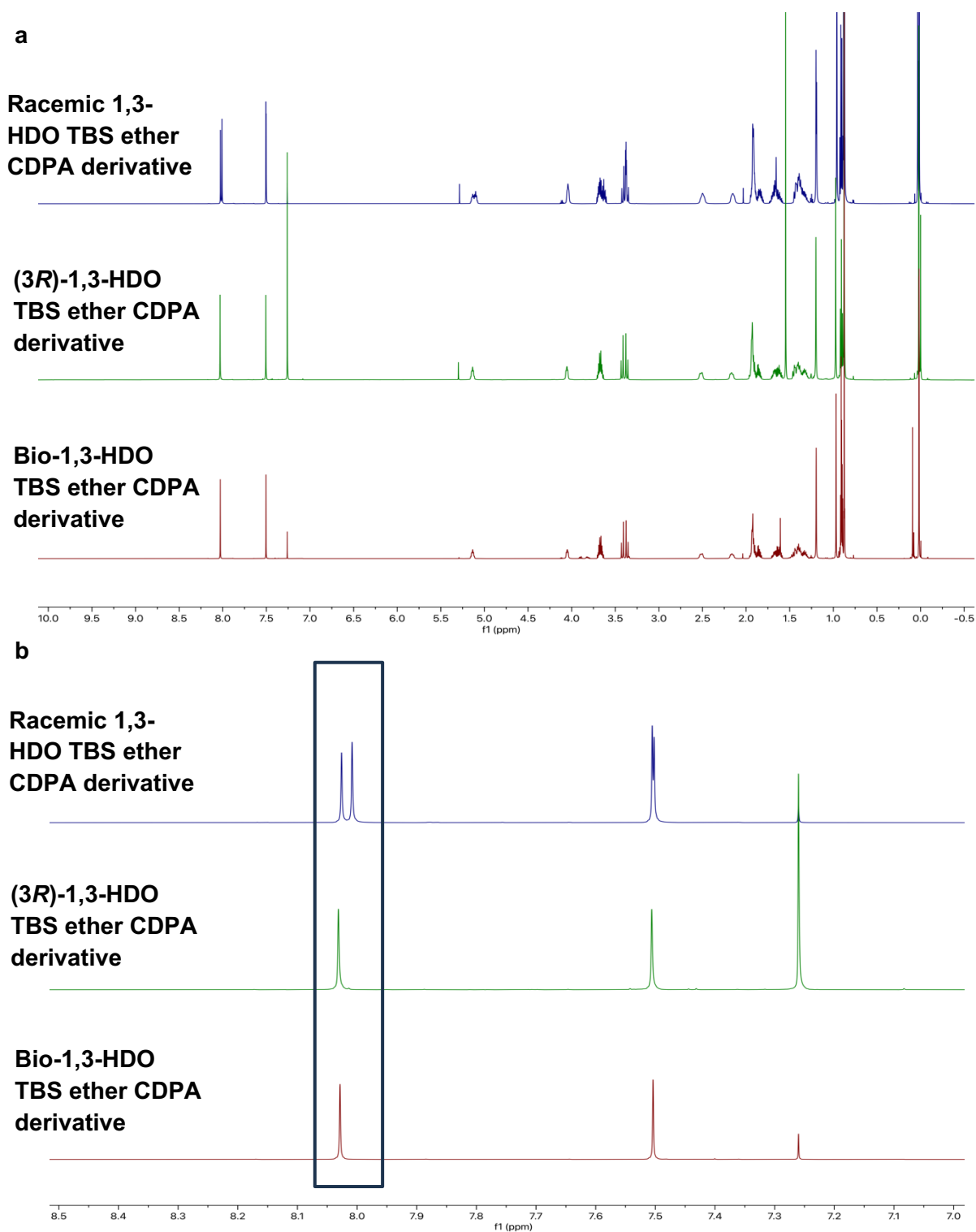
Supplementary Figure 26. ^{13}C NMR spectra (a) and ^1H NMR spectra (b) of purified bio-1,3-diols from *S. albus* RimM0M1-TR2 + FkbS (QD76) culture.



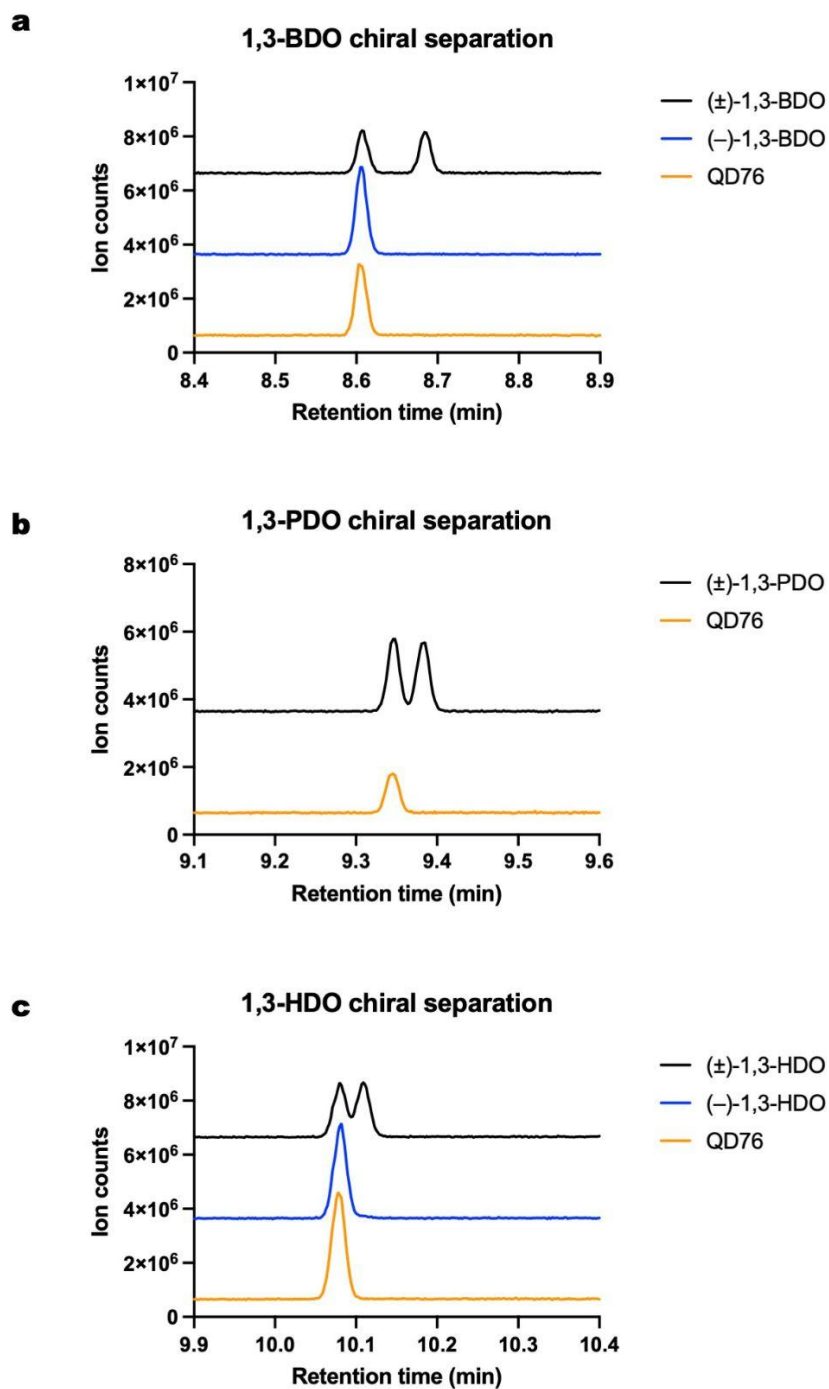
Supplementary Figure 27. ^1H NMR spectra of bio-1,3-BDO derivative from *S. albus* RimM0M1-TR2 + FkbS (QD76) culture. a, full ^1H NMR spectra of TBS ether CDPA derivatives of racemic 1,3-BDO standard, (3R)-BDO standard, and purified bio-1,3-BDO sample. b, The aromatic regions of the ^1H NMR spectra. Comparison of the highlighted regions clearly show that bio-1,3-BDO from the cell culture has 100% “R” chirality at the C3 position.



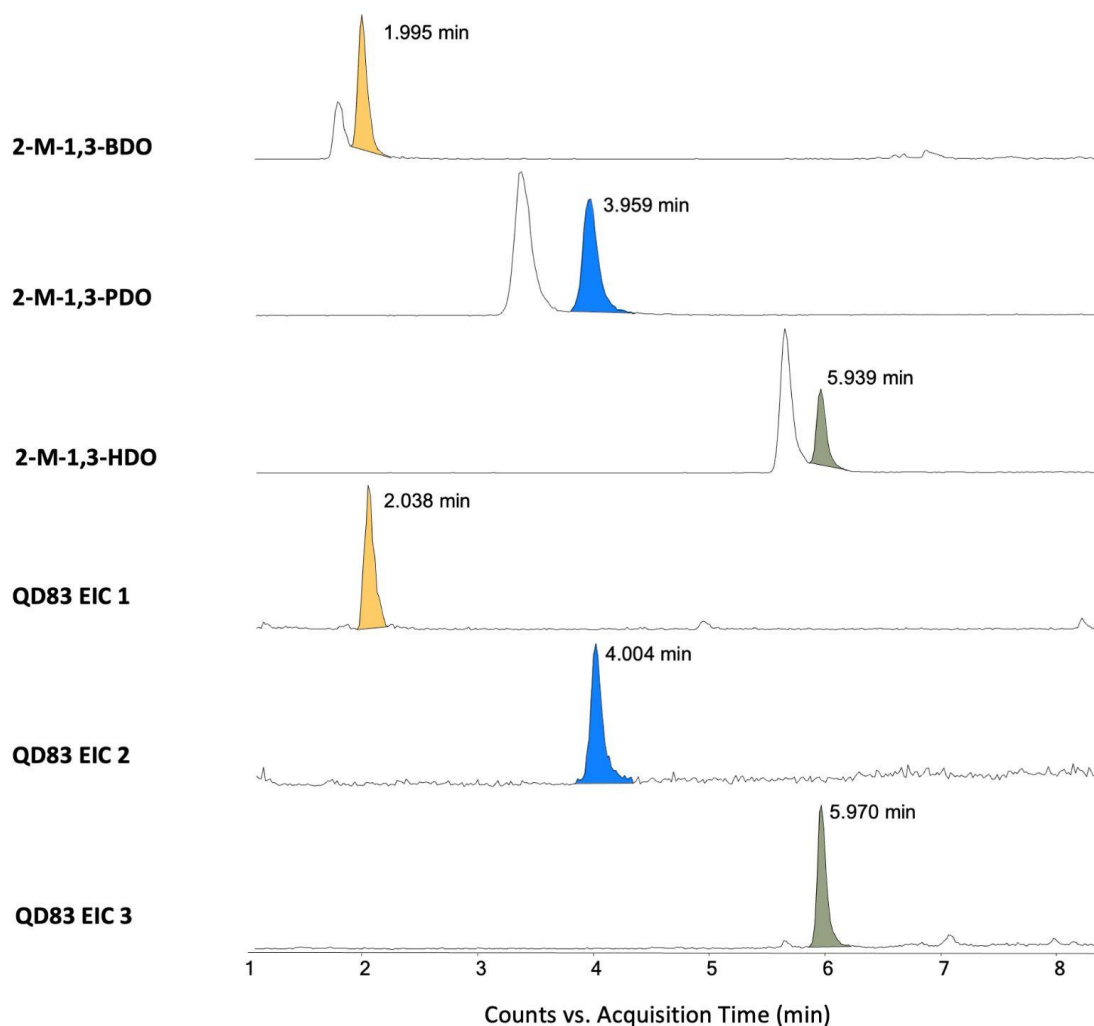
Supplementary Figure 28. ^1H NMR spectra of bio-1,3-PDO derivative from *S. albus* RimM0M1-TR2 + FkbS (QD76) culture. a, full ^1H NMR spectra of TBS ether CDPA derivatives of racemic 1,3-PDO standard and purified bio-1,3-PDO sample. b, The aromatic regions of the ^1H NMR spectra.



Supplementary Figure 29. ^1H NMR spectra of bio-1,3-HDO derivative from *S. albus* RimM0M1-TR2 + FkbS (QD76) culture. a, full ^1H NMR spectra of TBS ether CDPA derivatives of racemic 1,3-HDO standard, (3*R*)-HDO standard, and purified bio-1,3-HDO sample. b, The aromatic regions of the ^1H NMR spectra. Comparison of the highlighted regions clearly show that bio-1,3-HDO from the cell culture has 100% “R” chirality at the C3 position.

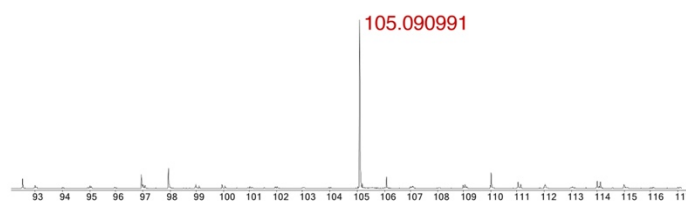


Supplementary Figure 30. Chiral separation of *S. albus* RimM0M1-TR2 + FkbS (QD76) products by GC-MS analysis. All produced 1,3-diols are in (3*R*)- configuration. a, 1,3-BDO. b, 1,3-PDO. c, 1,3-HDO.

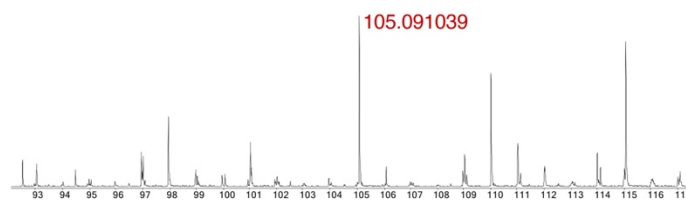


Supplementary Figure 31. LC-MS detection of 2-methyl-1,3-diols produced in *S. albus* RimM0M1(RimM7 AT)-TR2 + FkbS (QD83). From top to bottom: 2-M-1,3-BDO standard EIC ($[M + H]^+ = 105.091006 \text{ } m/z$); 2-M-1,3-PDO standard EIC ($[M + Na]^+ = 141.088601 \text{ } m/z$); 2-M-1,3-HDO standard EIC ($[M + Na]^+ = 155.104251 \text{ } m/z$); QD83 EIC 1 = $105.091006 \text{ } m/z$; EIC 2 = $141.088601 \text{ } m/z$; EIC 3 = $155.104251 \text{ } m/z$. Rim M1 KR is predicted to be B1-type, suggesting no (2*S*)-methyl epimerization occurrence at the C2 position. The (3*R*)- and (3*S*)- (2*S*)-methyl-1,3-diol diastereomer standards were separated to allow product diastereomeric ratio (dr) assessment, and our observation of high 2-methyl-1,3-diol product dr is indicative of strict Rim M1 KR stereospecificity.

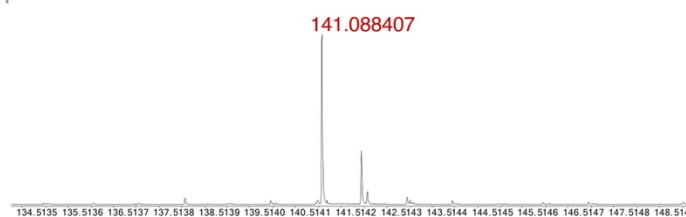
2-M-1,3-BDO standard:
(rt = 1.976 min)
 $m/z = 105.090991$



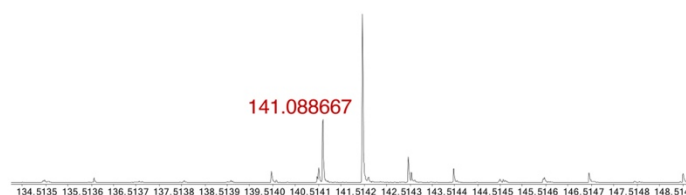
2-M-1,3-BDO sample:
(rt = 2.038 min)
 $m/z = 105.091039$



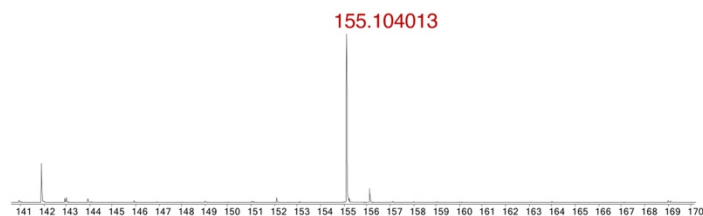
2-M-1,3-PDO standard:
(rt = 3.940 min)
 $m/z = 141.088407$



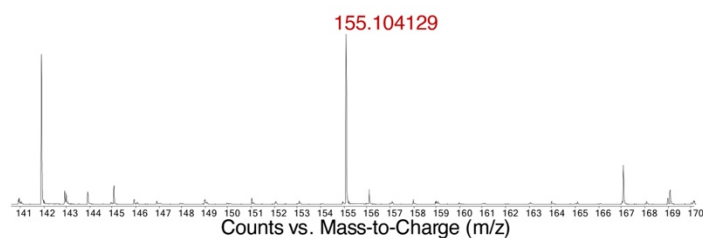
2-M-1,3-PDO sample:
(rt = 4.004 min)
 $m/z = 141.088667$



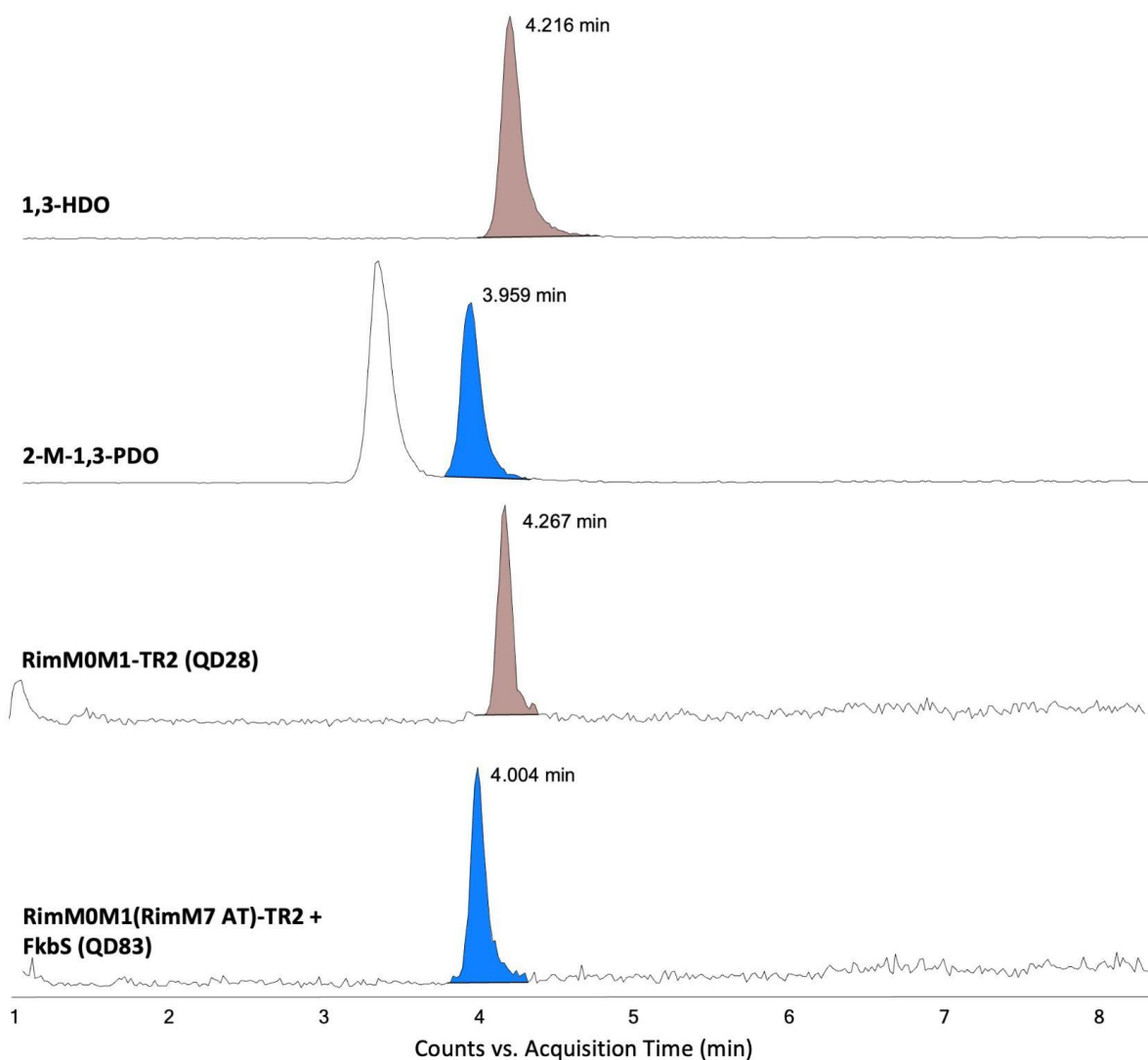
2-M-1,3-HDO standard:
(rt = 5.939 min)
 $m/z = 155.104013$



2-M-1,3-HDO sample:
(rt = 5.970 min)
 $m/z = 155.104129$

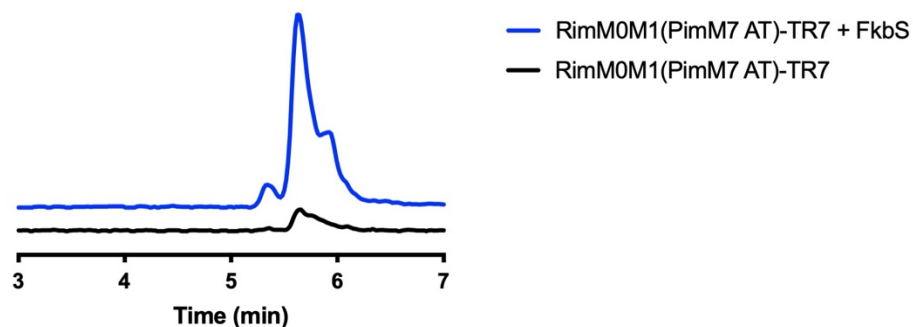


Supplementary Figure 32. MS spectra of 2-methyl-1,3-diols produced in *S. albus* RimM0M1(M7 AT)-TR2 + FkbS (QD83). $[M + H]^+$ m/z were presented for 2-M-1,3-BDO standard and sample, and $[M + Na]^+$ m/z were shown here for 2-M-1,3-PDO and 2-M-1,3-HDO standards and samples.

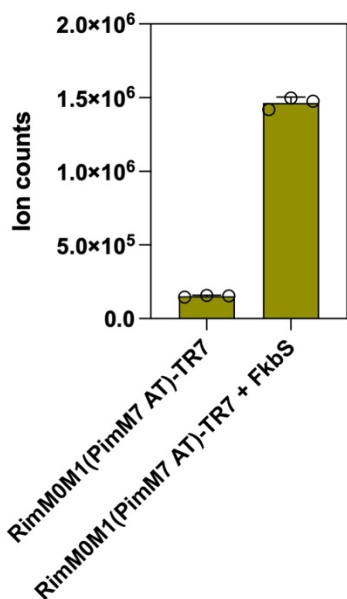


Supplementary Figure 33. Comparative analysis of 1,3-diols produced in *S. albus* QD28 and QD83. From top to bottom are EIC traces of 1,3-HDO standard, 2-M-1,3-PDO standard, *S. albus* RimM0M1-TR2 (QD28) sample, and *S. albus* RimM0M1(RimM7 AT)-TR2 + FkbS (QD83) sample. 1,3-HDO and 2-M-1,3-PDO are isomers ($[M + Na]^+ = 141.088601\ m/z$). 1,3-HDO was produced only in QD28, whereas 2-M-1,3-PDO was produced only in QD83, confirming precision of PKS-TR engineering.

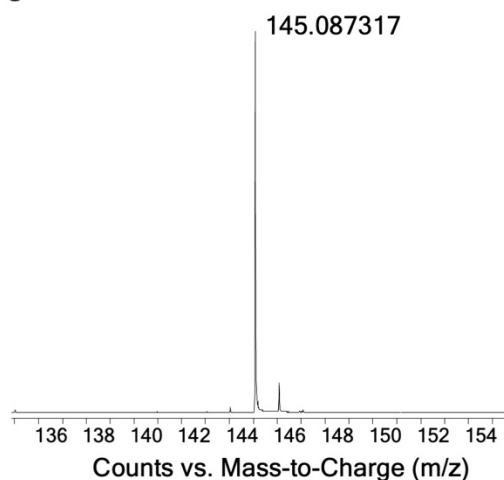
a EIC trace: 2-methyl-3-hydroxyhexanoic acid



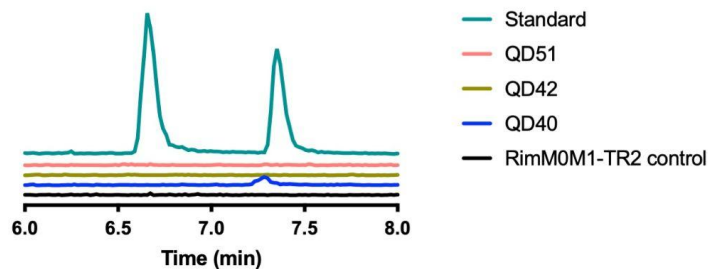
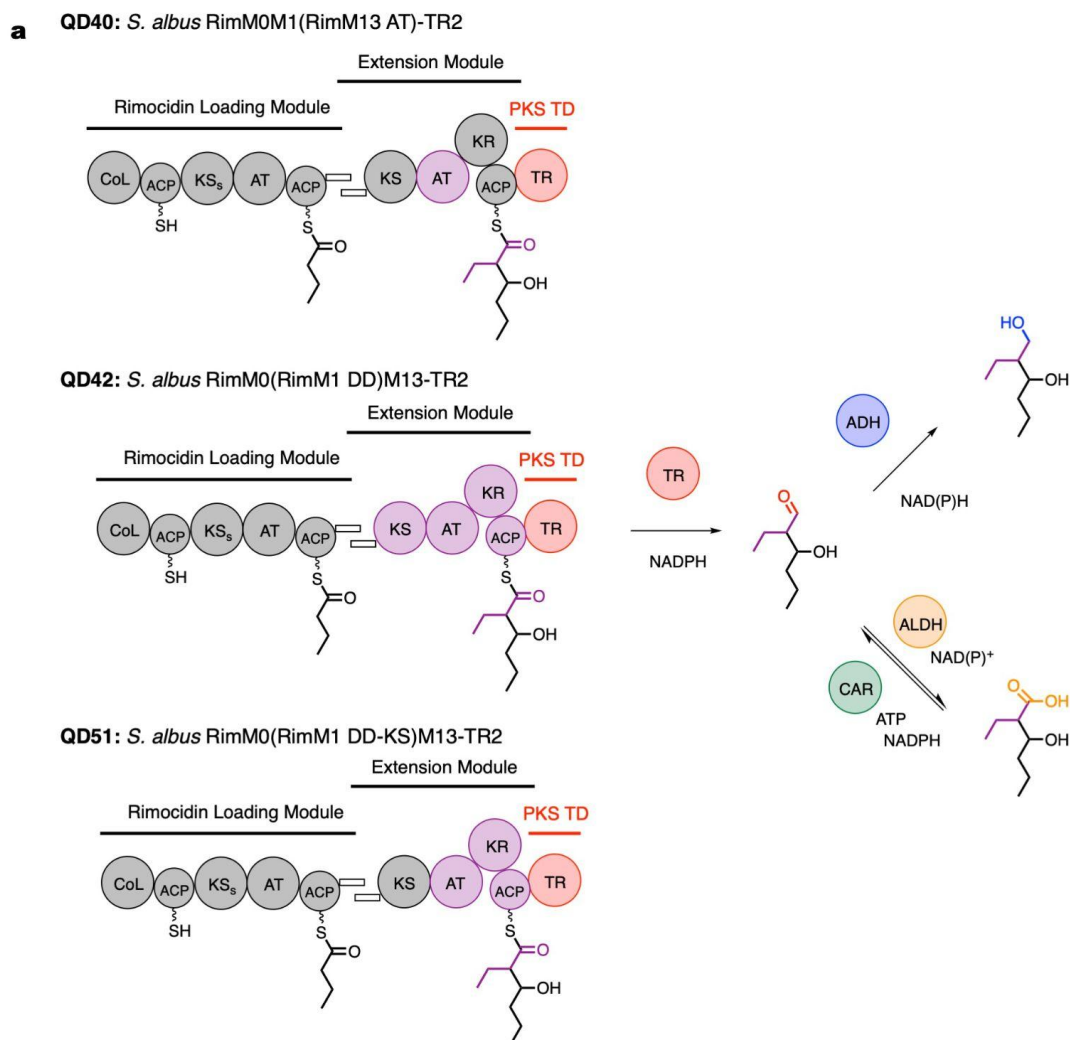
b



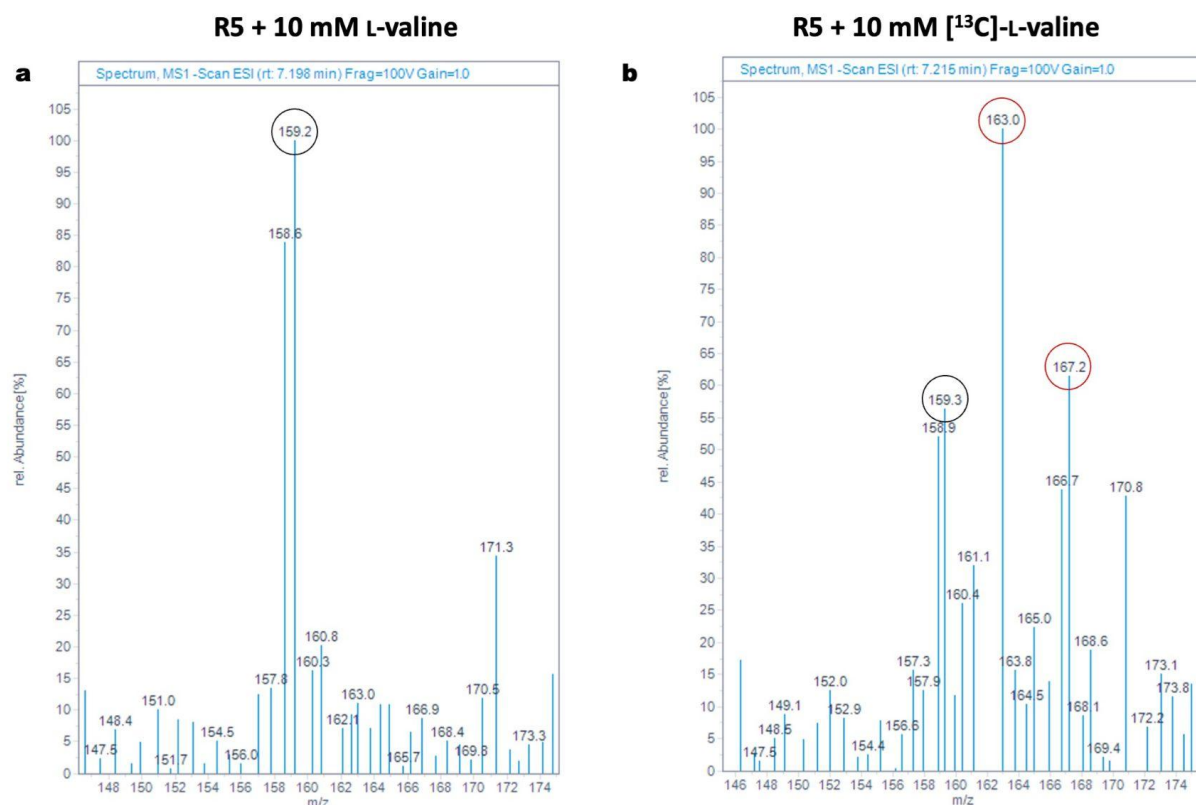
c



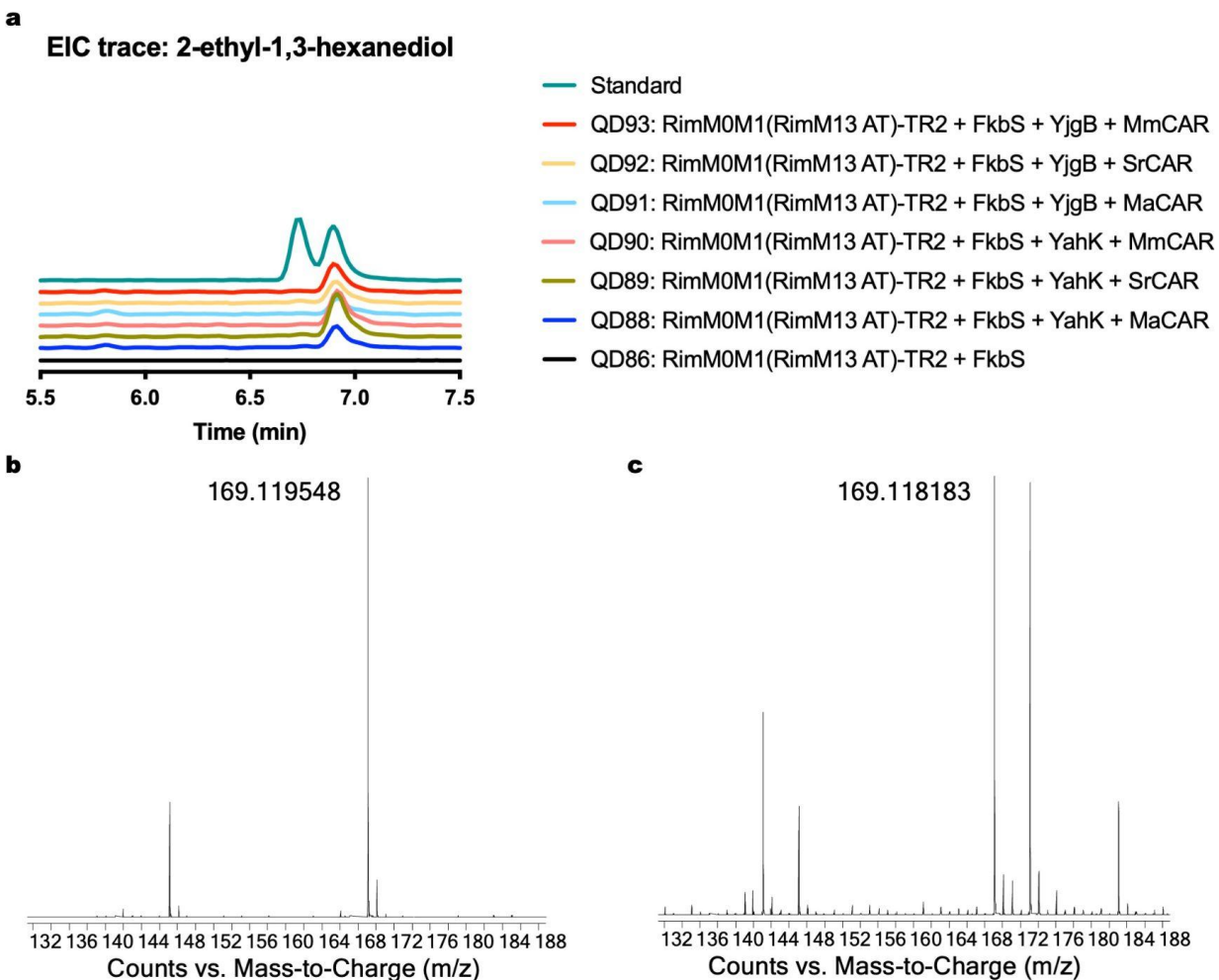
Supplementary Figure 34. Detection of 2-methyl-3-hydroxyhexanoic acid produced in *S. albus*. a, EIC profiles of 2-methyl-3-hydroxyhexanoic acid: 7 d cultivation of RimM0M1(PiM7 AT)-TR7 (QD69) in R5 + 2% Glucose is shown in black, and 7 d cultivation of RimM0M1(PiM7 AT)-TR7 + FkbS (QD85) in R5 + 2% Glucose + 15 mM L-valine is shown in blue. b, Production profiles of 2-methyl-3-hydroxyhexanoic acid. c, Observed $[M - H]^-$ of 2-methyl-3-hydroxyhexanoic acid in the QD85 sample is 145.087317 m/z (theoretical $[M - H]^-$ 145.087018 m/z , mass error 2.06 ppm). All cultivation data are represented as mean value and error bars indicate standard deviation (s.d.) of three biological replicates ($n = 3$).



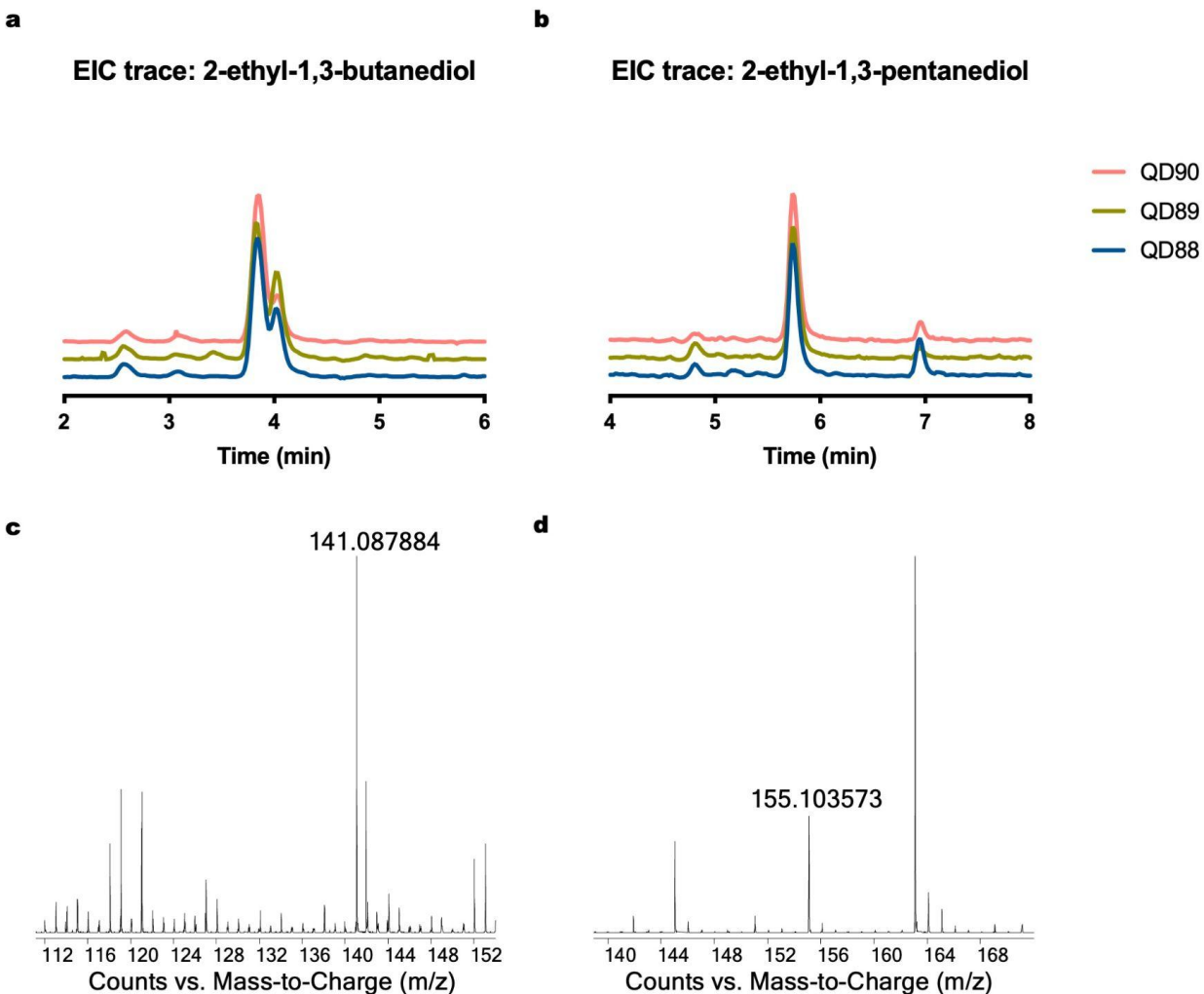
Supplementary Figure 35. Detection of 2-ethyl-3-hydroxyhexanoic acid produced in *S. albus*. a, three PKS pathway designs for 2-ethyl-3-hydroxyhexanoic acid production were tested in *S. albus*. AT: acyltransferase; DD: docking domain; KS: ketosynthase. b, EIC profiles of 2-ethyl-3-hydroxyhexanoic acid in QD28 control (black), QD40 (blue), QD42 (brown), QD51 (pink) after 3 d cultivation in R5 + 2% Glucose + 5 mM L-valine, and 2-ethyl-3-hydroxyhexanoic acid standard (cyan).



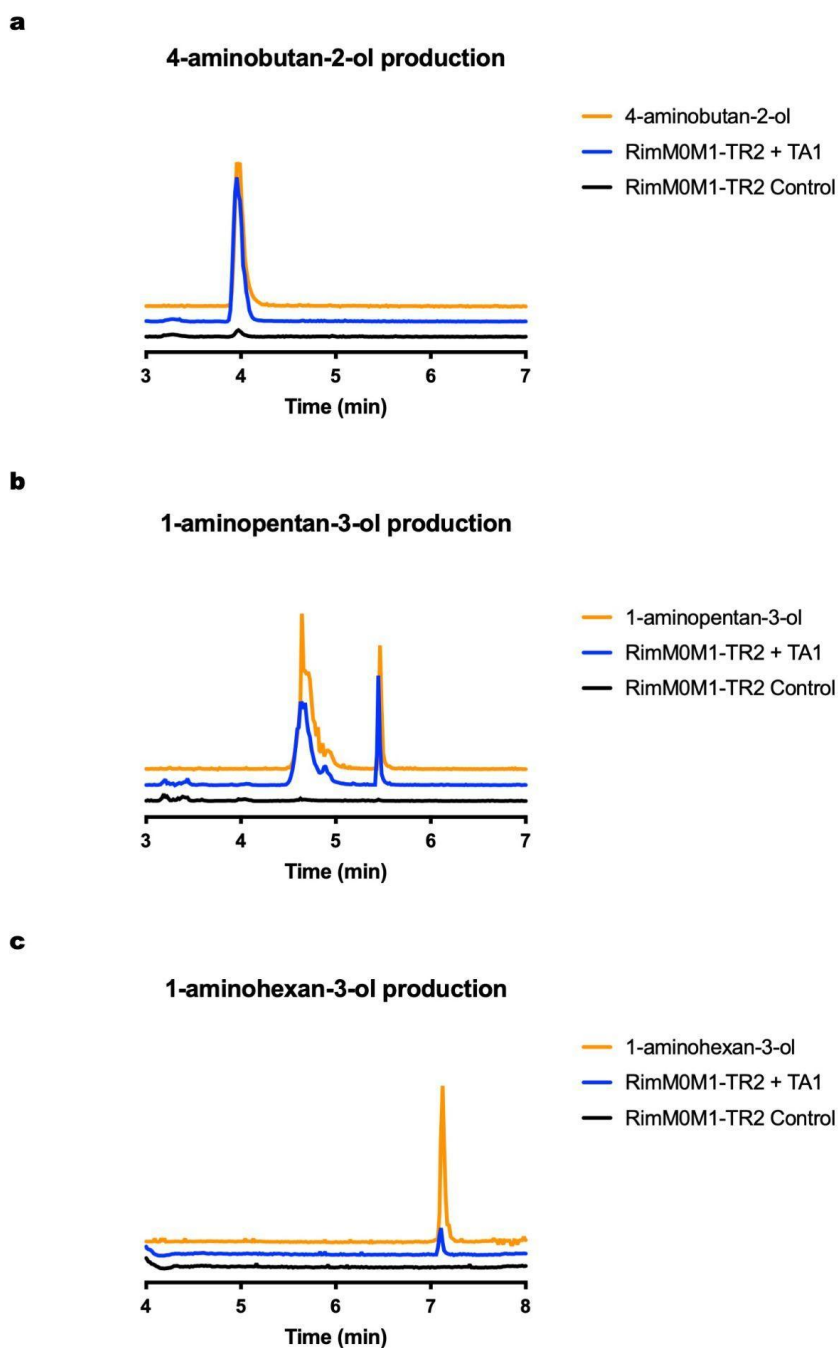
Supplementary Figure 36. [¹³C]labelled L-valine feeding experiment on *S. albus* RimM0M1(RimM13 AT)-TR2 (QD40). R5 medium + 10 mM L-valine or [¹³C]labelled L-valine was selected for comparison. a, 2-ethyl-3-hydroxyhexanoic acid MS spectrum in the L-valine addition group (rt = 7.20 min, expected [M - H]⁻ 159.2 m/z, observed 159.2 m/z in the black circle). b, 2-ethyl-3-hydroxyhexanoic acid MS spectrum in [¹³C]labelled L-valine addition group (rt = 7.21 min, expected [M - H]⁻ 163.2 m/z for a single [¹³C]labelled L-valine incorporation and 167.2 m/z for double [¹³C]labelled L-valine incorporation, observed 163.0 m/z and 167.2 m/z in red circles).



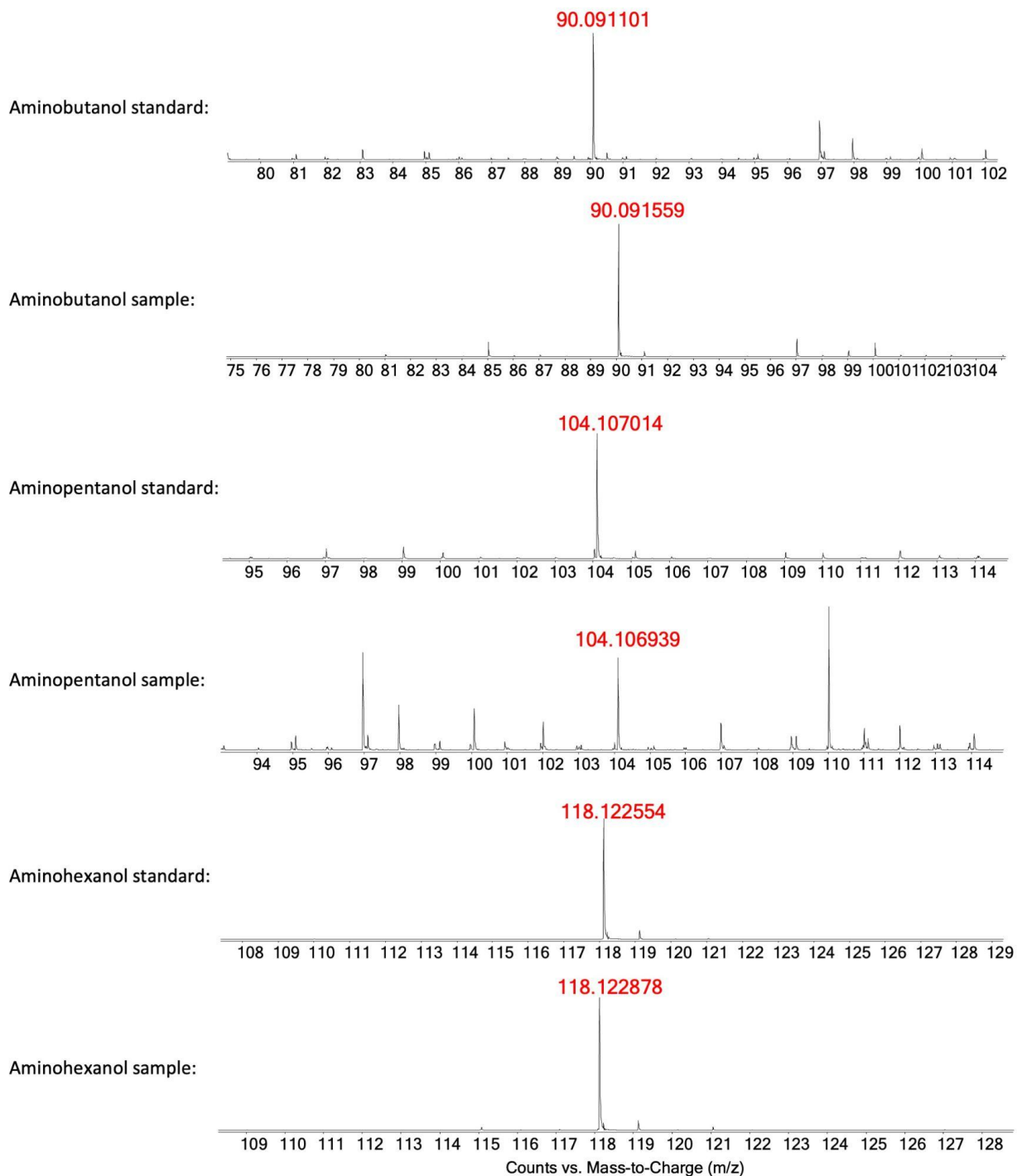
Supplementary Figure 37. Detection of 2-ethyl-1,3-hexanediol produced in *S. albus*. a, EIC profiles of 2-E-1,3-HDO: 7 d cultivation of QD86, QD88 - QD93 in R5 + 2% Glucose + 15 mM L-valine. b, Observed $[M + Na]^+$ m/z of 2-E-1,3-HDO standard is 169.119548. c, Observed $[M + Na]^+$ m/z of 2-E-1,3-HDO in QD89 sample is 169.118183.



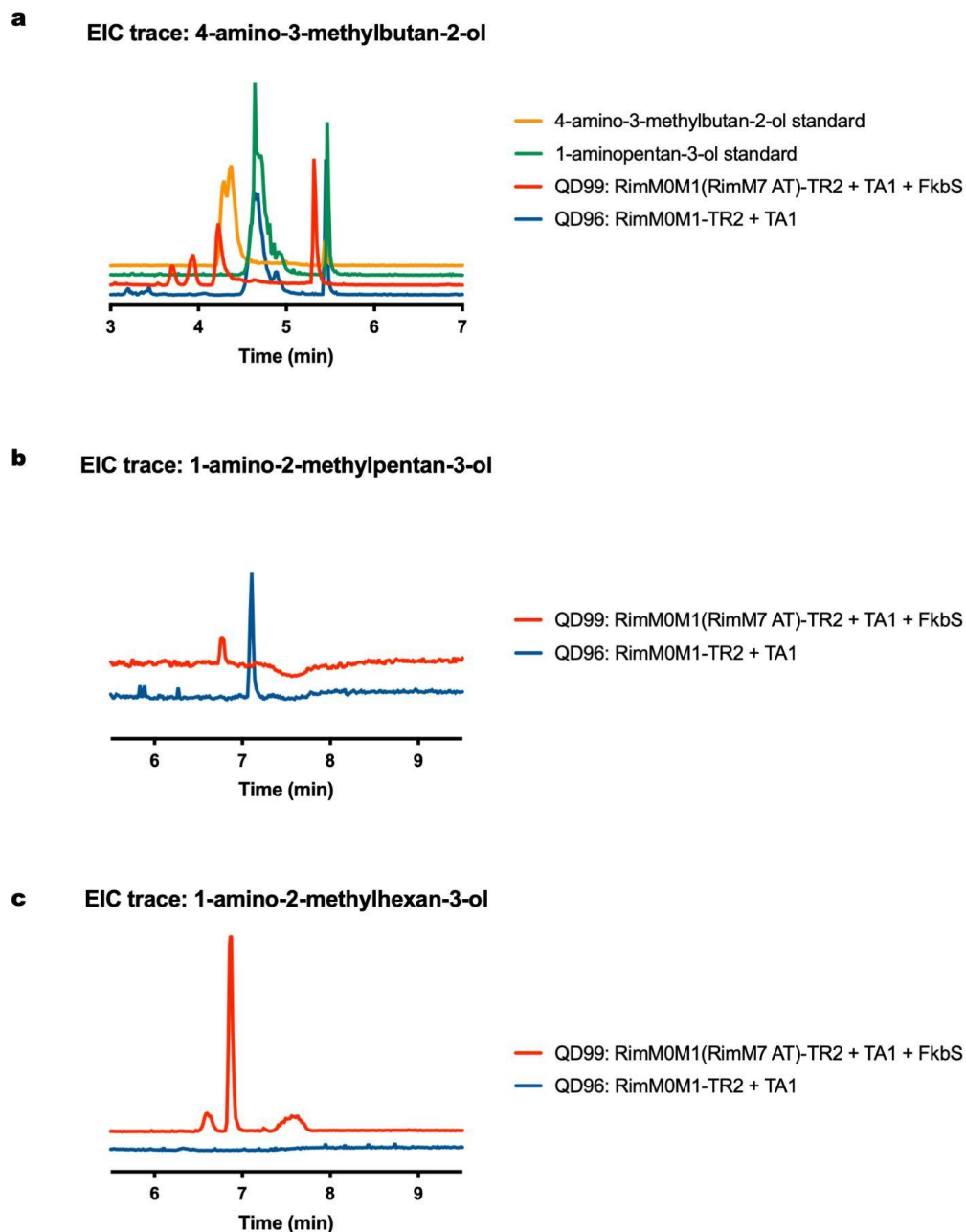
Supplementary Figure 38. Detection of 2-E-1,3-BDO and 2-E-1,3-PDO produced in *S. albus*. a, EIC profile of 2-E-1,3-BDO after 7 d cultivation of QD88 - QD90 in R5 + 2% Glucose + 15 mM L-valine. b, EIC profile of 2-E-1,3-PDO after 7 d cultivation of QD88 - QD90 in R5 + 2% Glucose + 15 mM L-valine. c, Observed $[M + Na]^+$ m/z of 2-E-1,3-BDO in QD89 sample is 141.087884 (theoretical $[M + Na]^+$ 141.088601 m/z , mass error -5.08 ppm). d, Observed $[M + Na]^+$ m/z of 2-E-1,3-PDO in QD89 sample is 155.103573 (theoretical $[M + Na]^+$ 155.104251 m/z , mass error -4.37 ppm).



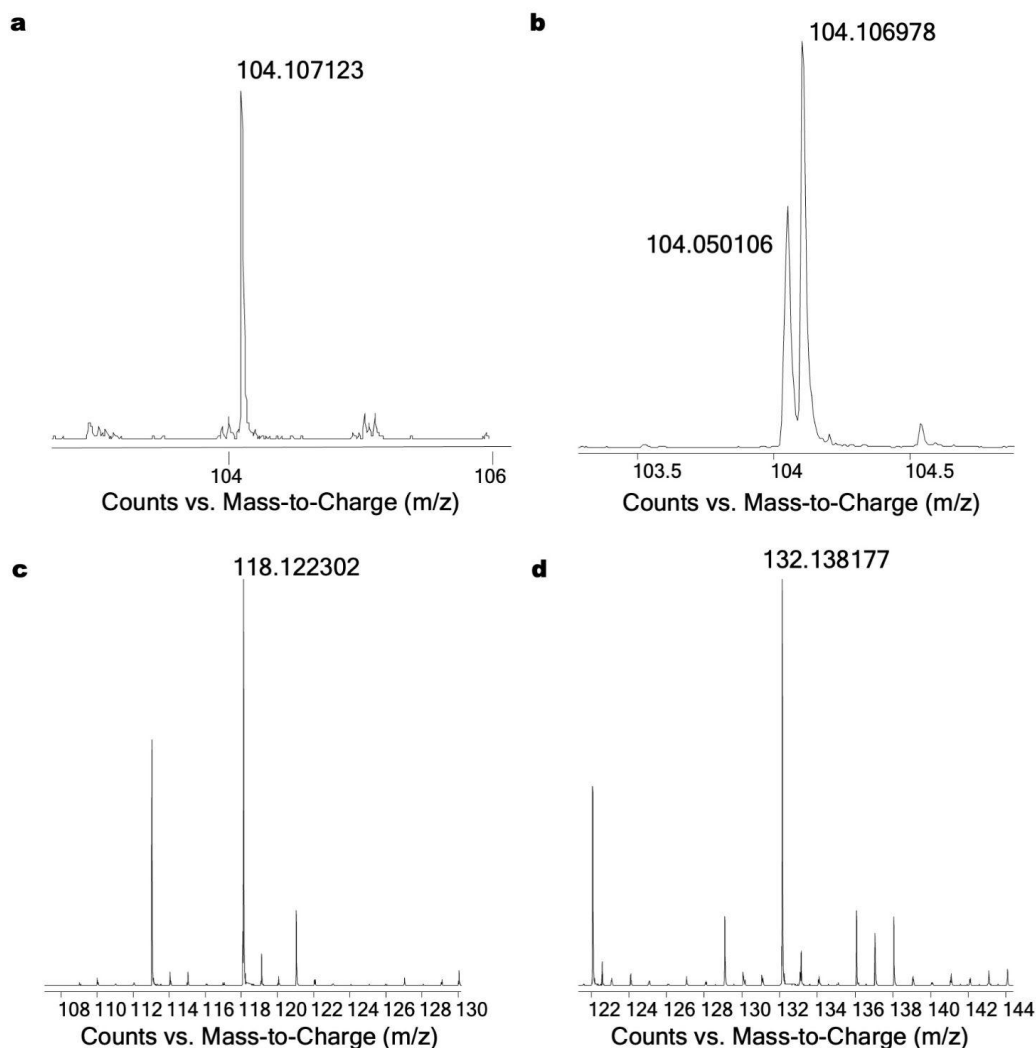
Supplementary Figure 39. LC-MS detection of amino alcohols produced in *S. albus* RimM0M1-TR2 + TA1 (QD96). EIC traces: 4-aminobutan-2-ol (a), 1-aminopentan-3-ol (b), and 1-aminohexan-3-ol (c). RimM0M1-TR2 (QD28) control is coloured in black, RimM0M1-TR2 + TA1 (QD96) is coloured in blue, and the standards are coloured in orange. See Supplementary Figure 40 for detailed mass-to-charge (m/z) detection.



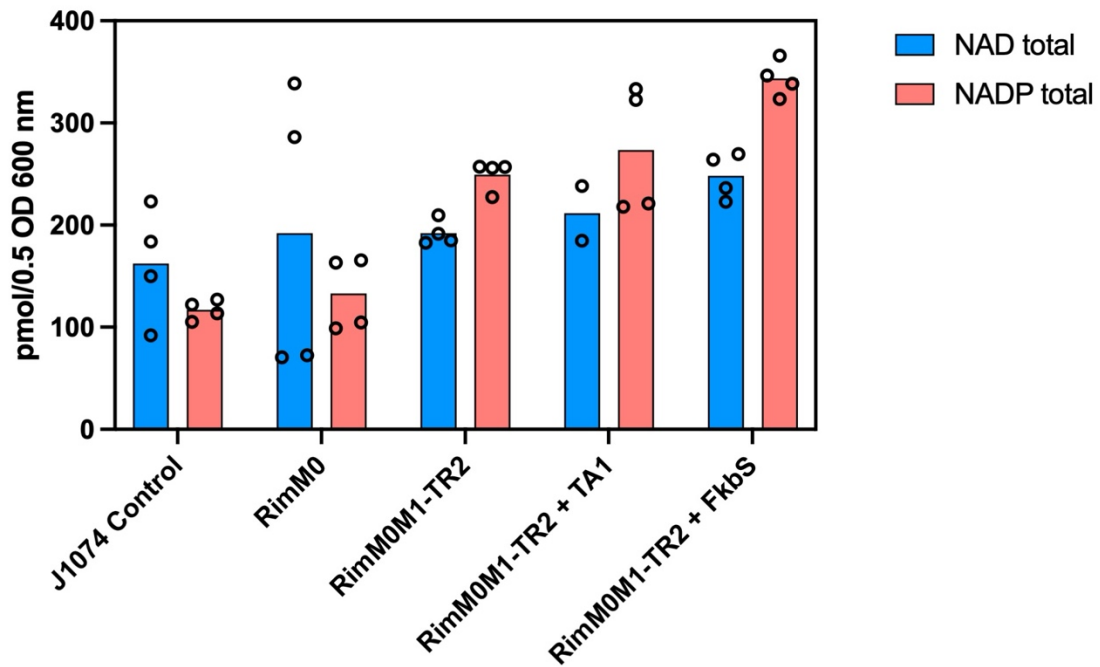
Supplementary Figure 40. MS spectra of amino alcohols produced in *S. albus* RimM0M1-TR2 + TA1 (QD96). $[M + H]^+$ m/z were presented for standards and samples of 4-aminobutan-2-ol, 1-aminopentan-3-ol, and 1-aminohexan-3-ol.



Supplementary Figure 41. LC-MS detection of methyl amino alcohols produced in *S. albus* RimM0M1(RimM7 AT)-TR2 + TA1 + FkbS (QD99). *S. albus* RimM0M1-TR2 + TA1 (QD96) that produced 4-aminobutan-2-ol, 1-aminopentan-3-ol, and 1-aminohexan-3-ol serves as control. EIC traces: 4-amino-3-methyl-butan-2-ol (a), 1-amino-2-methylpentan-3-ol (b), and 1-amino-2-methylhexan-3-ol (c). QD96 control is coloured in blue, RimM0M1(RimM7 AT)-TR2 + TA1 + FkbS (QD99) is coloured in red. See Supplementary Figure 42 for detailed mass-to-charge (m/z) detection.



Supplementary Figure 42. MS spectra of amino alcohols produced in *S. albus* RimM0M1(RimM7 AT)-TR2 + TA1 + FkbS (QD99). $[M + H]^+$ m/z were presented for 4-amino-3-methyl-butan-2-ol standard (a, observed $[M + H]^+$ 104.107123 m/z), and samples of 4-amino-3-methyl-butan-2-ol (b, observed $[M + H]^+$ 104.106978 m/z), 1-amino-2-methylpentan-3-ol (c, theoretical $[M + H]^+$ 118.122641 m/z , observed $[M + H]^+$ 118.122302 m/z , mass error -2.87 ppm), and 1-amino-2-methylhexan-3-ol (d, theoretical $[M + H]^+$ 132.138291 m/z , observed $[M + H]^+$ 132.138177 m/z , mass error -0.86 ppm).



Supplementary Figure 43. Intracellular NAD(P) level measurement in *S. albus*. Each experiment was repeated in quadruplicate except for RimM0M1-TR2 + TA1 which was repeated in duplicate.

Supplementary Table 1. Plasmids and strains used in this study

Strains and Plasmids	Description	Source or Reference	JPUB ID
Plasmids			
pHIS	<i>E. coli</i> expression plasmid with N-terminal 6×HIS-TEV tag, kanamycin resistance	Addgene plasmid 29653	
pMBP	<i>E. coli</i> expression plasmid with N-terminal 6×HIS-MBP-TEV tag, kanamycin resistance	Addgene plasmid 29656	
pG-KJE8	<i>E. coli</i> chaperone coexpression plasmid, chloramphenicol resistance	Takara	
pHIS_rimM1 ACP	pHIS plasmid carrying <i>rimM1 ACP</i> gene	This work	JPUB_024305
pHIS_rimA	pHIS plasmid carrying <i>rimM0</i> gene	This work	JPUB_024231
pHIS_pimS0	pHIS plasmid carrying <i>pimS0</i> gene	This work	JPUB_024233
pMBP_ACP1	pMBP plasmid carrying <i>ACP1</i> gene	This work	JPUB_024235
pHIS_ACP9	pHIS plasmid carrying <i>ACP9</i> gene	This work	JPUB_024237
pHIS_TR1	pHIS plasmid carrying <i>TR1</i> gene	This work	JPUB_024221
pHIS_TR2	pHIS plasmid carrying <i>TR2</i> gene	This work	JPUB_024223
pHIS_TR7	pHIS plasmid carrying <i>TR7</i> gene	This work	JPUB_024225
pHIS_TR9	pHIS plasmid carrying <i>TR9</i> gene	This work	JPUB_024227
pHIS_TADH2	pHIS plasmid carrying <i>TADH2</i> gene	This work	JPUB_024229
pSC	PhiC31-based <i>Streptomyces</i> integration plasmid with spectinomycin resistance	Cruz-Morales <i>et al.</i> ¹²	
p41	VWB-based <i>Streptomyces</i> integration plasmid with apramycin resistance	Cruz-Morales <i>et al.</i> ¹²	
pOSV807	pSAM2-based <i>Streptomyces</i> integration plasmid with hygromycin resistance	Addgene plasmid 126600	
pOSV809	PhiBT1-based <i>Streptomyces</i> integration plasmid with kanamycin resistance	Addgene plasmid 126602	
pSC_rimA0	pSC plasmid carrying <i>PrpsL(RO)-rimM0</i>	This work	JPUB_024239
pSC_rimA6	pSC plasmid carrying <i>Pgapdh(EL)-rimM0</i>	This work	JPUB_024169
pSC_rimA7	pSC plasmid carrying <i>kasOP*-rimM0</i>	This work	JPUB_024241
p41_rimB18	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-DEBS TE</i>	This work	JPUB_024263
p41_rimB27	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR1</i>	This work	JPUB_024242
p41_rimB28	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR2</i>	This work	JPUB_024243
p41_rimB31	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR3</i>	This work	JPUB_024244
p41_rimB33	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR4</i>	This work	JPUB_024245
p41_rimB34	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR6</i>	This work	JPUB_024246
p41_rimB35	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR7</i>	This work	JPUB_024247
p41_rimB36	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR8</i>	This work	JPUB_024248
p41_rimB37	p41 plasmid carrying <i>Pgapdh(EL)-rimM1-TR9</i>	This work	JPUB_024249

p41_rimB40	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i>	This work	JPUB_024293
p41_rimB42	p41 plasmid carrying <i>Pgapdh(EL)-rimM13(rimM1 docking domain)-TR2</i>	This work	JPUB_024294
p41_rimB51	p41 plasmid carrying <i>Pgapdh(EL)-rimM13(rimM1 docking domain-KS)-TR2</i>	This work	JPUB_024296
p41_rimB66	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR2</i>	This work	JPUB_024250
p41_rimB67	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR7</i>	This work	JPUB_024251
p41_rimB69	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(pimM7 AT)-TR7</i>	This work	JPUB_024252
p41_rimB73	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(rimM13 AT)-DEBS TE</i>	This work	JPUB_024290
p41_rimB74	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(rimM13 AT)-Pik TE</i>	This work	JPUB_024291
p41_rimB75	p41 plasmid carrying <i>Pgapdh(EL)-rimM1(rimM13 AT)-Rim TE</i>	This work	JPUB_024292
pOSV807_rimJ	pOSV807 plasmid carrying <i>Pgapdh(EL)-rimJ</i>	This work	JPUB_024254
pOSV807_fkbS	pOSV807 plasmid carrying <i>Pgapdh(EL)-fkbS</i>	This work	JPUB_024255
pOSV809_TA1	pOSV809 plasmid carrying <i>Pgapdh(EL)-TA1</i>	This work	JPUB_024256
pOSV809_TA2	pOSV809 plasmid carrying <i>Pgapdh(EL)-TA2</i>	This work	JPUB_024257
pOSV809_yqhD	pOSV809 plasmid carrying <i>Pgapdh(EL)-yqhD</i>	This work	JPUB_024258
pOSV809_chnD	pOSV809 plasmid carrying <i>Pgapdh(EL)-chnD</i>	This work	JPUB_024259
pOSV809_yahK	pOSV809 plasmid carrying <i>Pgapdh(EL)-yahK</i>	This work	JPUB_024260
pOSV809_yjgB	pOSV809 plasmid carrying <i>Pgapdh(EL)-yjgB</i>	This work	JPUB_024261
pOSV809_ScADH2	pOSV809 plasmid carrying <i>Pgapdh(EL)-ScADH2</i>	This work	JPUB_024262
pOSV809_MaCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-MaCAR</i>	This work	JPUB_024298
pOSV809_yahK_MaCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-yahK</i> and <i>Pgapdh(EL)-MaCAR</i>	This work	JPUB_024299
pOSV809_yahK_SrCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-yahK</i> and <i>Pgapdh(EL)-SrCAR</i>	This work	JPUB_024300
pOSV809_yahK_MmCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-yahK</i> and <i>Pgapdh(EL)-MmCAR</i>	This work	JPUB_024301
pOSV809_yjgB_MaCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-yjgB</i> and <i>Pgapdh(EL)-MaCAR</i>	This work	JPUB_024302
pOSV809_yjgB_SrCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-yjgB</i> and <i>Pgapdh(EL)-SrCAR</i>	This work	JPUB_024303
pOSV809_yjgB_MmCAR	pOSV809 plasmid carrying <i>Pgapdh(EL)-yjgB</i> and <i>Pgapdh(EL)-MmCAR</i>	This work	JPUB_024304
Strains			
<i>E. coli</i> BL21(DE3)		NEB	
<i>E. coli</i> BAP1		Pfeifer <i>et al.</i> ¹³	
<i>S. albus</i> J1074		ATCC	
<i>S. coelicolor</i> M1152		ATCC	
<i>S. rimosus</i> ATCC 10970		ATCC	
<i>S. tsukubaensis</i> NRRL 18488		NRRL	
<i>S. natalensis</i> ATCC 27448		ATCC	

QD1	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i>	This work	JPUB_024167
QD2	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>Pgapdh(EL)-rimM0</i>	This work	JPUB_024169
QD3	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>kasOP*-rimM0</i>	This work	JPUB_024171
QD4	<i>S. coelicolor</i> M1152 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i>	This work	JPUB_024191
QD5	<i>S. coelicolor</i> M1152 strain with phiC31-based integration of <i>Pgapdh(EL)-rimM0</i>	This work	JPUB_024192
QD6	<i>S. coelicolor</i> M1152 strain with phiC31-based integration of <i>kasOP*-rimM0</i>	This work	JPUB_024193
QD18	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-DEBS TE</i>	This work	JPUB_024307
QD27	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR1</i>	This work	JPUB_024173
QD28	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i>	This work	JPUB_024175
QD29	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>Pgapdh(EL)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i>	This work	JPUB_024189
QD30	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>kasOP*-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i>	This work	JPUB_024190
QD31	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR3</i>	This work	JPUB_024177
QD33	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR4</i>	This work	JPUB_024179
QD34	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR6</i>	This work	JPUB_024181
QD35	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR7</i>	This work	JPUB_024183
QD36	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR8</i>	This work	JPUB_024185
QD37	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1-TR9</i>	This work	JPUB_024187
QD40	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i>	This work	JPUB_024267
QD42	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM13(rimM1 docking domain)-TR2</i>	This work	JPUB_024309
QD51	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM13(rimM1 docking domain-KS)-TR2</i>	This work	JPUB_024310
QD66	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR2</i>	This work	JPUB_024194
QD67	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR7</i>	This work	JPUB_024196
QD69	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(pimM7 AT)-TR7</i>	This work	JPUB_024265
QD73	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-DEBS TE</i>	This work	JPUB_024272
QD74	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-Pik TE</i>	This work	JPUB_024274

QD75	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> and VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-Rim TE</i>	This work	JPUB_024276
QD76	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , and pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i>	This work	JPUB_024203
QD77	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , and pSAM2-based integration of <i>Pgapdh(EL)-rimJ</i>	This work	JPUB_024201
QD78	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yqhD</i>	This work	JPUB_024208
QD79	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-chnD</i>	This work	JPUB_024210
QD80	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yahK</i>	This work	JPUB_024212
QD81	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yjbB</i>	This work	JPUB_024214
QD82	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-ScADH2</i>	This work	JPUB_024216
QD83	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR2</i> , and pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i>	This work	JPUB_024198
QD84	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR7</i> , and pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i>	This work	JPUB_024200
QD85	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(pimM7 AT)-TR7</i> , and pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i>	This work	JPUB_024266
QD86	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , and pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i>	This work	JPUB_024269
QD87	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , and phiBT1-based integration of <i>Pgapdh(EL)-MaCAR</i>	This work	JPUB_024270
QD88	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yahK</i> and <i>Pgapdh(EL)-MaCAR</i>	This work	JPUB_024278
QD89	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yahK</i> and <i>Pgapdh(EL)-SrCAR</i>	This work	JPUB_024280
QD90	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yahK</i> and <i>Pgapdh(EL)-MmCAR</i>	This work	JPUB_024282
QD91	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13</i>	This work	JPUB_024284

	<i>AT</i>)- <i>TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yjbB</i> and <i>Pgapdh(EL)-MaCAR</i>		
QD92	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yjbB</i> and <i>Pgapdh(EL)-SrCAR</i>	This work	JPUB_024286
QD93	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM13 AT)-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-yjbB</i> and <i>Pgapdh(EL)-MmCAR</i>	This work	JPUB_024288
QD94	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR1</i> , and phiBT1-based integration of <i>Pgapdh(EL)-TA1</i>	This work	JPUB_024204
QD95	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR1</i> , and phiBT1-based integration of <i>Pgapdh(EL)-TA2</i>	This work	JPUB_024206
QD96	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , and phiBT1-based integration of <i>Pgapdh(EL)-TA1</i>	This work	JPUB_024218
QD97	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , and phiBT1-based integration of <i>Pgapdh(EL)-TA2</i>	This work	JPUB_024219
QD98	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-TA1</i>	This work	JPUB_024220
QD99	<i>S. albus</i> J1074 strain with phiC31-based integration of <i>PrpsL(RO)-rimM0</i> , VWB-based integration of <i>Pgapdh(EL)-rimM1(rimM7 AT)-TR2</i> , pSAM2-based integration of <i>Pgapdh(EL)-fkbS</i> , and phiBT1-based integration of <i>Pgapdh(EL)-TA1</i>	This work	JPUB_024308

Supplementary Table 2. Primers used in this study

Primer name	Sequence (5' to 3')
pHIS_rimA-F	TACTTCCAATCCAATGCAatggtccccgtgcatactg
pHIS_rimA-R	TTATCCACTTCCAATGTTATCAATGTTCTGCATCCATAAGACGG
pHIS_pimS0-F	TACTTCCAATCCAATGCAatggtgccccgtccacacagatgactacgc
pHIS_pimS0-R	TTATCCACTTCCAATGTTAtcagctctccgctccatcagccg
pHIS_rimM1 ACP-F	TACTTCCAATCCAATGCAgcccgcctgcccgc
pHIS_rimM1 ACP-R	TTATCCACTTCCAATGTTAgacctggagcgcagatacgcgg
pMBP_ACP1-F	TACTTCCAATCCAATGCAgctgggctaggagatgcag
pMBP_ACP1-R	TTATCCACTTCCAATGTTAtacttcaccatgcaggtactccg
pHIS_ACP9-F	TACTTCCAATCCAATGCAggaggcatgaccggcgcg
pHIS_ACP9-R	TTATCCACTTCCAATGTTActccagcaggaccgtccggagg
pHIS_TR1-F	TACTTCCAATCCAATGCAcgggcggaactgtcggcgg
pHIS_TR1-R	TTATCCACTTCCAATGTTAtcaccggcgccgggg
pHIS_TR2-F	TACTTCCAATCCAATGCAaccggggccagcggct
pHIS_TR2-R	TTATCCACTTCCAATGTTActagcgggcccggggccg
pHIS_TR7-F	TACTTCCAATCCAATGCAggcggacggacgcagggc
pHIS_TR7-R	TTATCCACTTCCAATGTTAtcagcctgccgcggggaag
pHIS_TR9-F	TACTTCCAATCCAATGCAggcaccacgggcagcggc
pHIS_TR9-R	TTATCCACTTCCAATGTTAtccttcggtcatggggcgccgc
pHIS_TADH2-F	TACTTCCAATCCAATGCAatgtctactacagtcggccaag
pHIS_TADH2-R	TTATCCACTTCCAATGTTAtcagtcagccatagtaatgatacatctc
pSC_rimA0-0F	gacgcggagcactgataactagcataacccttggggc
pSC_rimA0-0R	gtggacgggcaccatttaccggctttctctgtgtgaac
pSC_rimA0-1F	atggtgccccgtccacacagc
pSC_rimA0-1R	tcagtgtccgcgtccatcag
p41_rimB27-0F	cgcgatatcgaattcctcgag
p41_rimB27-0R	cttctcctcgttcgacatatggcgatcccccttcag
p41_rimB27-1F	atgtcgaacgaggagaagctgcg
p41_rimB27-1R	gacctcggagcgcagatacg
p41_rimB27-2F	tgcgctccgaggtccggcggaactgtcg
p41_rimB27-2R	cgaggaattcgatatcgcttcttccgattaccggcg
p41_rimB28-2F	tgcgctccgaggtcaccggggccagcggct
p41_rimB28-2R	cgaggaattcgatatcgcgctagcggggccggggcc
p41_rimB31-2F	tgcgctccgaggtcgggtccgccgacccccg
p41_rimB31-2R	cgaggaattcgatatcgcggttacgcctcgacgtgaccgcg
p41_rimB33-2F	ctgcgctccgaggtcggcgacccggcgcg
p41_rimB33-2R	gctagtactcaggaattcgtcacggggcgggcaggtaac
p41_rimB34-2F	ctgcgctccgaggtcggcgggcgccggacggc

p41_rimB34-2R	gctagtactcgaggaattcgtcaggcagcgggcccgcg
p41_rimB35-2F	ctgcgctccgaggtcggcggacgcagggc
p41_rimB35-2R	gctagtactcgaggaattcgtcagcctgccgcggggaagt
p41_rimB36-2F	CTGCGCTCCGAGGTCGCGCCCCAGGACGACACCGGG
p41_rimB36-2R	GCTAGTTACTCGAGGAATTCGTCACGGGGCGGGCGGGAAGTG
p41_rimB37-2F	ctgcgctccgaggtcggcaccacgggcagcggc
p41_rimB37-2R	gctagtactcgaggaattcgtcatccttcgggtcatggggggccgc
p41_RimB40-0F	cgtcgacctgccacgtacgcc
p41_RimB40-0R	ggcgctgtccaggatgacgtgg
p41_RimB40-1F	cctggagcaggcaccggcggacgcc
p41_RimB40-1R	gtgggcaggtcgacgcggcg
p41_RimB42-0F	ctgcgcagcgagttaccggggccagcggctcc
p41_RimB42-0R	ggctcgcgctgcttcgcctcg
p41_RimB42-1F	aagcagcgcgagccggctgcatcgctggcatggc
p41_RimB42-1R	gaactcgtcgcgcagccgggc
p41_RimB51-0F	aaaggccaggaaccgtaaaaaggccgc
p41_RimB51-0R	cttctcctcgttcgacatatggcgatatcccc
p41_RimB51-1F	atgtcgaacgaggagaagctcgcga
p41_RimB51-1R	ggcgctgtccaggatgacgtgg
p41_RimB51-2F	cctggagcaggcaccggcggacgcc
p41_RimB51-2R	cgggtcctggccttttctgccttt
p41_RimB69-0F	cgtgcccacgtacgccttcagcg
p41_RimB69-0R	tcagccaatcgactggcgagcggcat
p41_RimB69-1F	ccagtcgattggctgagctcatgagcgg
p41_RimB69-1R	gcggggggcctcctccaggatgacgtgggcgttggtg
p41_RimB69-2F	gaggcccccccccgcacga
p41_RimB69-2R	gtacgtgggcagcggcaccgg
p41_RimB73-0F	tagcgcgatatcgaattcctcagtaactagcataacccc
p41_RimB73-0R	gacctcggagcgcagatacgcggc
p41_RimB73-1F	tgcgctccgaggtcgactccgtacgcctgcac
p41_RimB73-1R	gaattcgatatcgcgctatcagctgttgcggccac
p41_RimB74-0F	tagcgcgatatcgaattcctcagtaactagcataacccc
p41_RimB74-0R	gacctcggagcgcagatacgcggc
p41_RimB74-1F	tgcgctccgaggtcggcagcggaaactgggccc
p41_RimB74-1R	gaattcgatatcgcgctacttggccggccctcgatgc
p41_RimB75-0F	tagcgcgatatcgaattcctcagtaactagcataacccc
p41_RimB75-0R	gggctgtcggcgacctcggagcgcagatacgcggc
p41_RimB75-1F	gccgcacagcccgccct
p41_RimB75-1R	gaattcgatatcgcgctatcatgcctggccgaggggtggcc
pSC_RimA6-0F	ctagcataacccttggggcc

pSC_RimA6-0R	gaccgaaggagcagcagtactctgctgcaacgtgtg
pSC_RimA6-1F	gctgctccttcggtcgga
pSC_RimA6-1R	atggcgtatcccccttcagatact
pSC_RimA6-2F	gaaaggggatacgcacataggtgcccgtccacaca
pSC_RimA6-2R	cccaaggggttatgctagtatcagt
pSC_RimA7-0F	ctagcataacccttggggcc
pSC_RimA7-0R	gttcgaatgtgaacaagtactagtactctgctgcaacgtgtg
pSC_RimA7-1 (direct use)	agtacttggtcacattcgaacgggtctctgctttgacaacatgctgtcggtgtgtgtaaagtcgtggccaggagaatacga cagcgtgcaggactgggggagttcat
pSC_RimA7-2F	ggactgggggagttcatatggtccccgtccacaca
pSC_RimA7-2R	cccaaggggttatgctagtatcagt
p41_RimB66-0F	ccggcgaggagctggccgccctggccgccg
p41_RimB66-0R	aacacaccgggtctcggtgggg
p41_RimB66-1F	gcagaccggtgtgtcaaggaagacgacg
p41_RimB66-1R	cagccagtaccactggcgctggaagg
p41_RimB66-2F	ccagtgttactggctgcaggaaactccccacgcgccacca
p41_RimB66-2R	cagtcctcgccggacagcgg
p41_RimB67-0F	gccgccctgccgccg
p41_RimB67-0R	aacacaccgggtctcggtgggg
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p41_RimB67-2R	cggcagggcgcccgggcgccagtcggc
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pOSV807_rimJ-1F	ATGAAGGAAATACTGAACGCTATTTCAGGC
pOSV807_rimJ-1R	TCAGTCCTCCAGCGTCCC
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pOSV809_ADH_CAR-1R	aagtatcttcctggcatcttcag
pOSV809_ADH_CAR-2F	cctctcacttcctgtgtgctccttcggtcgg
pOSV809_ADH_CAR-2R	gatgccaggaagatacttcaaaaaacccctcaagaccgtt

Supplementary Table 3. Genes used in this study

Gene name	Sequence (5' to 3')
<i>rimA</i> (<i>rimM0</i>)	atggtgccccgtccacacagctgactacgtgatccagccgcccaccgacgcggcgagggtacggggcgccacacgctgccgagggtgttcgagaccgctgagggcgccccgacgcg gtggccatcgtggacggggacgcttgcggacgtgggcgagtgggcgggcgacgtccgcgcgtggcccggtggctccaggaaatcgggcgctggggcccggtgacgtggtggcggtgcgg ctggccgaacagctgggagttccagaccctgcacctggccgtcgcggccgctggcgccgtactgtctcccgtccacgaggcgaccccgaccgcggaggtccacgcgctgctgaccgcgcgg aaccgctactctctgctgctgctccgggagcgaggcgacgggcaacggcccgctcgcctctggagagcgctggcagcgtgcgcggcggtgctgctggccggggcgctcgagggcgagtg cgaggagccggggatcgggcgctggacgggttgcgggcagcctggacgggcagcgagccccggcggtgacactcaccgccacatgccgtcgcctgatccccctgctcggcaccac ctcggcgcgcccaagctgctgctgcacaccacggcgccctgctggaacaccgcggccgtgctggccgatgccgcgacgcttcggcgacaggtcctgaccgcgtgcccgatgacc cacctgttcgggctccagtccctgcacacggcgctgttcgcccggagcgaggtctgtcaccggctgggacgtggaccgttcctgagctggcggggagcacaacccccgctgctgt tcggctaccgcccgaactgcgggacatcgtcgcgactcgcagggcgggcgagccggcggtggcgccgtggcgccgacccaggtgctgacggcgggcgcccgccctgcaccgcgctcgccg 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<i>YahK</i>	atgaagatcaaggcggctggcgccctactggccaagcagccctcgaaccgatggacatcacggcgaggagccggccgaacgacgtcaagatcgagatcgctactggcggtgtgtca ctccgacctgcaccaggtgctcctccgagtgggcgggcaccgtctacccgtgctgcccgggtcacgagatcgctggccgggtcgtcgggtaggcgaccaggtcgagaagtacccccggag acctgtgggggtgggctgcatcgtcactcctgcaagcactgccaagagtgccgagggacggcctggagaactactcggaccacatgaccggcacctataactccccgacggcgagcaaccc ggacacacctggcgggctacagccagcagatcgtggtgcacgagcgtctacgtcctgcgcatccgccaccggcaggagcagctcgccggcggtggcgccgtgctgtgctggcggtcatcaca cgtactcgccgtcggcactggcagggcgcccgccaagaagggtggcgctcgtgggatcggtggcctcgccacatgggcatcaagctcgcgacggccatggcgcgcatgtcgtggc cttcacgaccagcgaggcgaaacgcgaggcgccaaggccctggggcgcgaggtggtcaacagccgcaacggcgacgaatgggtgcccacctgaagtcttcgacttcatctgaaca cggtcggcgcccccacaacctggagacttaccacccctcctcaagcgtgacgggacatgacgctggtgggtgccccggccactccgcacaagtcggcgagggtcttcaacctgatcatgaa gcggagggggatcgccgggtcgatgacgggggaatccccgagaccaggagatgctcgacttctcgccgagcacggcatcgtggccgacatcgagatgacggggcgatcagatcaacg aggcctacgagcggatgctgctggcgacgtcaagtaccgtctgctacgacaaccgaccttgaccgactaa
<i>YjgB</i>	atgtccatgatcaagtcgtacgccggaaggagggcgaggcgagctggaggtctacgagtagacccccggggaactcgggcgagggacgtcgaggtcgaggtcgattactcggggatctg ccactccgacctgtcgatgatcgacaacgagtggggcttctcgcagtaccgctggtcgcgggcgacgaggtcattggcggggtgtggccctcggcagcgcgccccagacaaggcgctcc agggtggccagcgggctggcatcggctggagggccgctcgtgtggacactgcgacgcttgcactcggcaaccagatcaactgcgagcagggcgcggtgcccagacgatcatgaaccggcg gggttcggcgagaagctccgtcggaactggcagtggtgatccccctgcccagagaacatcgacatcgagagcgcggggtccgtgctgtgctggcgcatcaggtcttcaagccgctcctcatgc accacatcaccgacgactcccgctgtggcgctcatcggtcggggggctggggccacatcgcatcaagctcctgcacgctggtggctgcgaggtgaccgcttctgtccaaccggccaagg agcaggagggtcctcgccatgggtgcccagaaagtgtgtaactccgggacccgcaagccctgaagcgctggtggcgccagtctgacctcatcaaacaccgtgaacgtctcctggactggc agccgtacttcgaggcactacacacggcggaacttccacaccgtcgcgccgctctgacccccctgtccgtccccggttaccctgatcgccggcgaccggagcgtgagcggtgagtgccac cggcacccccctacgaattgcgcaagctgatgcgttcgcgccagggtcgaagtgcgccacgacgagctgttccgatgagcaagatcaacgacggccatccagcacgtacgcgacggcaa ggccccgtaccgctcgtgctcaaggccgacttctaa
<i>ScADH</i> 2	atgtcgtacccccgagaccagaaggccatcatftttctacgaatcgaacggcaagctggagcacaaggacatccgggtgccgaagcccaaacgaacgaactgctgatcaacgtcaagtagcgcg gctcgtgccacaccgacctgcacgctggcacgggaltggccccctgccgacgaagctcccgtggtgggtgggcacgagggcgccggcggtggtggtgcatgggtgagaatgtgaagggc tggaagatcggcgactacggcgcatcaagtggctgaacgggagctgcatggcgtgtgagtactgcgagctcggaacgagtcgaactgcccgcacggcggacctgtccgggtacaccacga cggctccttcaggagtagcgcgaccggcgacgctccaggccgacacatcccgagggcacagacctcgccgaggtggcgccgatcctgtgcccgggacacgtctacaaggcgctca agtcggccaacctcgggcgccgactggcgcccatcagcgcgcggtgctcggaaacctgcccgtgcatgacccaaggccatgggctaccgctcctgggcatcgacgggg ggccgggaaaggaggagctgttcacgtcgtcggcgcgagggtgttcatcgacttcaccaaggagaaggacatcgtgctggcggtgtgaaggccaccaacggcgcgccccatggcatc aacgtctcgtcagcgaaggcggaatcagggcctccacgcttactgcccggcgaaacggcaccgtggtcctcgtcggcctcccgcggcgcggaagtgtcctccgacgtcttcaaccacgtcg tcaagtccatctcgatcgtcggtcctatgtgggcaaccgcccacacccgcgaggccctggacttcttcggcgggggtcgtggaagagtcccatcaaggtcgtaggtctgagctcgtcgcc gagatctacgagaagatggagaaggccagatcgccggcgctacgtcgtcgacacgtccaagtaa

Supplementary Table 4. Statistics for data collection and refinement of CpkC TR

	CpkC TR
<i>Data collection</i>	
Wavelength (Å)	0.97741
Resolution range (Å)	44.76 - 1.84 (1.88 - 1.84)
Detector Distance (mm)	220
Φ (deg.) collected / ΔΦ (deg.)	180/0.25
Exposure time (seconds)	0.3
Temperature of collect (Kelvin)	100
<i>Data statistics</i>	
Space group	P 21 21 21
Unit-Cell parameters (Å)	a=49.63 b=103.55 c=148.32
Total reflections	445567 (44265)
Unique reflections	67220 (4891)
Multiplicity	6.6 (6.7)
Data completeness (%)	100 (99.7)
<I/σ(I)>	12.5 (0.9)
R _{merge} (%)	0.095 (1.90)
R _{pim} (%)	0.059 (1.20)
CC _{1/2}	0.999 (0.358)
<i>Structure Refinement</i>	
Reflections used in refinement	67220 (4891)
Reflections used for R _{free}	1828 (151)
R _{factor}	0.183 (0.338)
R _{free}	0.218 (0.352)
RMS from ideal geometry	
Bond lengths (Å)	0.008
Bond angles (°)	0.77
Average B-factor	35.9
Macromolecules	35.2
Ligands	37.0
Solvent	38.6
Ramachandran Plot	
Favoured region (%)	97.5
Outliers region (%)	0
Rotamer outliers (%)	0.1
Number of TLS groups	16

Supplementary Table 5. Thermodynamic analysis of different synthesis pathways of 2-ethyl-1,3-hexanediol and 2-ethyl-3-hydroxyhexanoic acid

Product	Synthesis pathway	Stoichiometric of synthesis pathway	$\Delta_r G'^{\circ}$ (kcal/mol)
2-ethyl-1,3-hexanediol	PKS KS catalysed decarboxylative Claisen condensation-based pathway	Butyryl-CoA + Ethylmalonyl-CoA + 4 H ⁺ + 3 NAD(P)H → 2-ethyl-1,3-hexanediol + 2 CoA + CO ₂ + 3 NAD(P) ⁺	-2.78
	Thiolase catalysed non-decarboxylative Claisen condensation-based pathway	2 Butyryl-CoA + 3 H ⁺ + 3 NAD(P)H → 2-ethyl-1,3-hexanediol + 2 CoA + 3 NAD(P) ⁺	0.31
2-ethyl-3-hydroxyhexanoic acid	PKS KS catalysed decarboxylative Claisen condensation-based pathway	Butyryl-CoA + Ethylmalonyl-CoA + H ⁺ + NAD(P)H + H ₂ O → 2-ethyl-3-hydroxyhexanoic acid + 2 CoA + CO ₂ + NAD(P) ⁺	-7.36
	Thiolase catalysed non-decarboxylative Claisen condensation-based pathway	2 Butyryl-CoA + NAD(P)H + H ₂ O → 2-ethyl-3-hydroxyhexanoic acid + 2 CoA + NAD(P) ⁺	-4.27

a. Calculated through stoichiometric summations of standard formation Gibbs energies ($\Delta_r G'^{\circ}$) of each substrate and products of the pathways. $\Delta_r G'^{\circ}$ were estimated through the group contribution method under standard conditions (298K, pH 7, zero ionic strength)¹⁴.

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