Supplementary Information (SI) for Green Chemistry.

Supplementary Materials

Cytoplasm-peroxisomal spatial combination engineering in *Candida tropicalis* for enhanced terpenoid production

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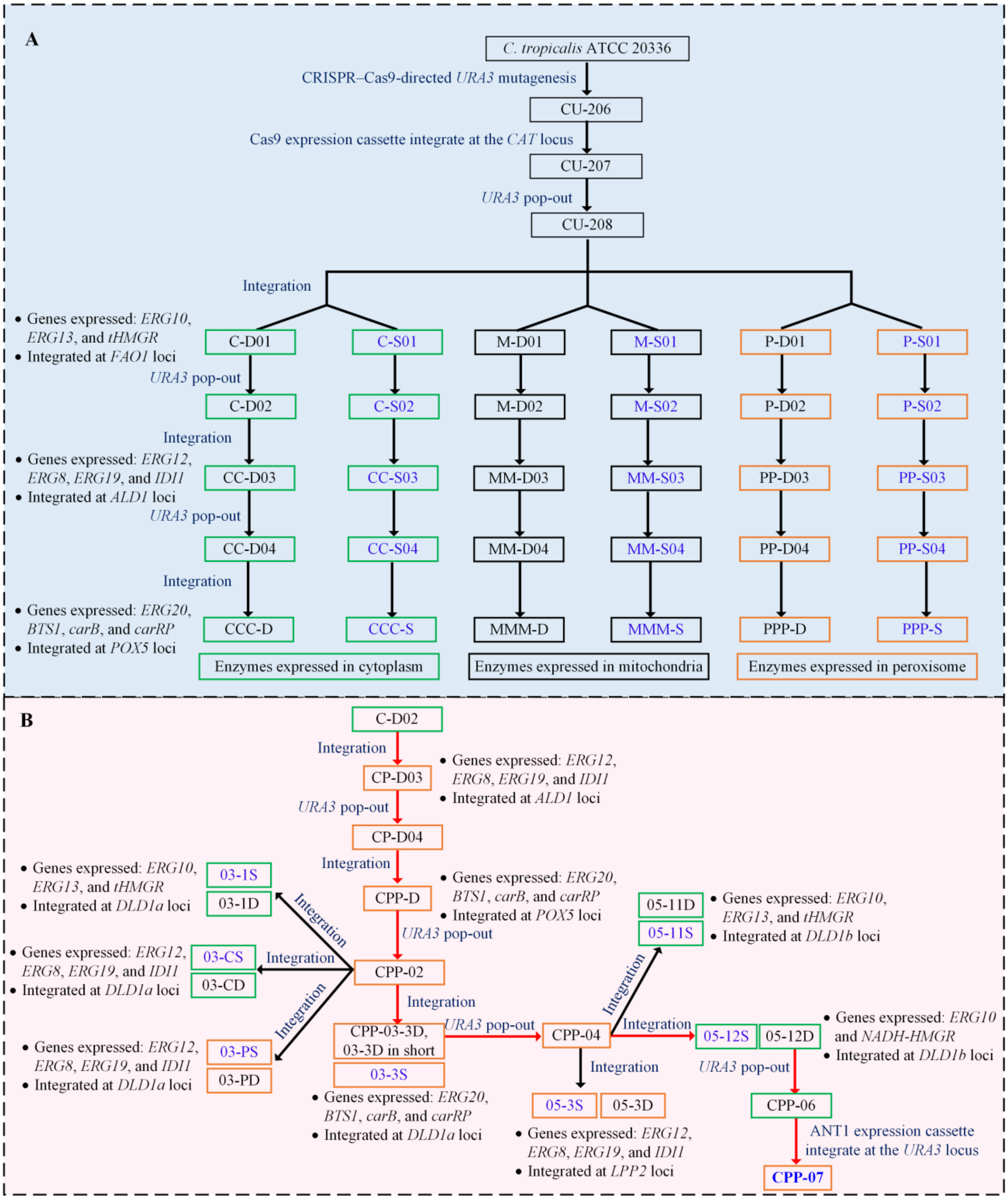


Fig. S1. The workflow for the construction of yeast strains for β-carotene production. (A) Flowchart for the construction of β-carotene synthetic pathway in different subcellular compartments. (B) Flowchart for the construction of strain CPP-07. Green box represents the transformant obtained by expressing genes in the cytoplasm of the former strain. Black box represents the transformant obtained by expressing genes in mitochondria of the former strain. Orange box represents the transformant obtained by expressing genes in peroxisome of the former strain. The blue text in box represents expressed one copy of the cassette. The black text in box represents expressed double copies of the cassette.

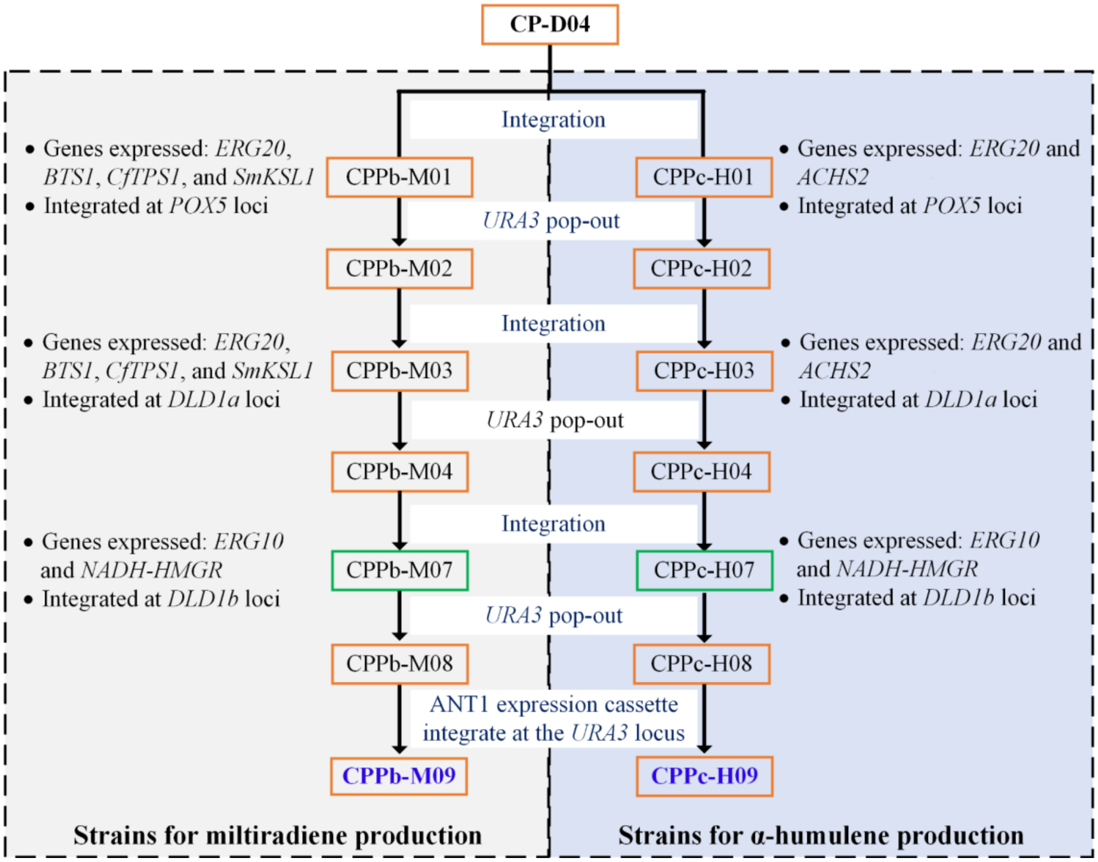


Fig. S2. The workflow for the construction of the major yeast strains for miltiradiene and α-humulene production. Green box represents the transformant obtained by expressing genes in the cytoplasm of the former strain. Orange box represents the transformant obtained by expressing genes in peroxisome of the former strain. The blue text in box represents expressed one copy of the cassette. The black text in box represents expressed double copies of the cassette.

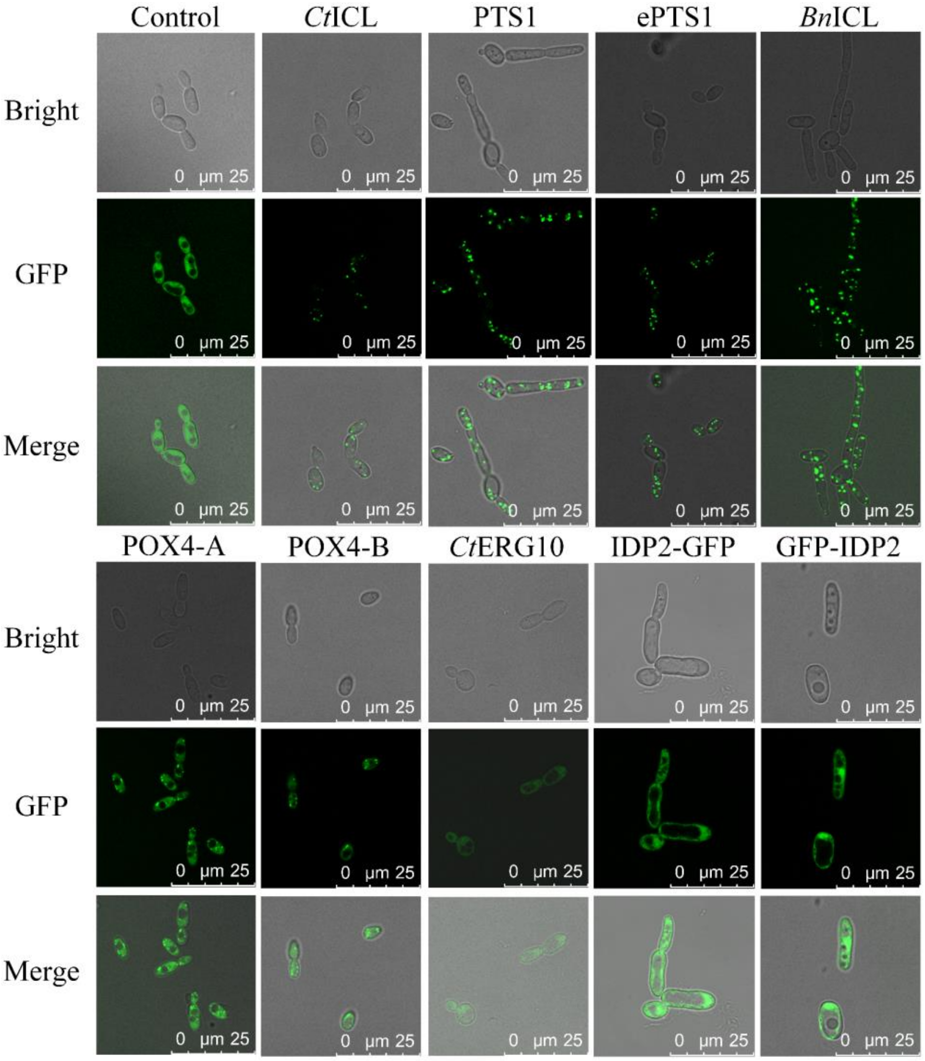


Fig. S3. Confirmation of different peroxisomal targeting signals in *C. tropicalis* with green fluorescence protein as a reporter. Control represents a transformant expressed the yeGFP3 without target signal (*C. tropicalis* 05-3 [1]). *Ct*ICL, PTS1, ePTS1, *Bn*ICL, POX4-A, POX4-B, and *Ct*ERG10 represent transformants expressed yeGFP3-*Ct*ICL, yeGFP3-PTS1, yeGFP3-ePTS1, yeGFP3-*Bn*ICL, POX4-A-yeGFP3, POX4-B-yeGFP3 fusion protein, respectively. IDP2-yeGFP3 and yeGFP3-IDP2 represent transformants expressed *Ct*IDP2 and yeGFP3 in different order. All the gene was expressed under the control of the *GAP1* promoter and terminator, and the expression cassette was integrated into the *CAT* locus as described previously [2]. PTS1, the typical peroxisomal targeting signal type 1 which consists of Ser-Lys-Leu (SKL); ePTS1, enhanced PTS1 (LGRGRR-SKL) [3]; *Ct*ICL, the C-terminal region of *C. tropicalis* isocitrate lyase; *Ct*ERG10, the C-terminal region of *C. tropicalis* acetoacetyl-CoA thiolase; *Bn*ICL, the C-terminal region of *Brassica napus* isocitrate lyase [4]; *Ct*IDP2, *C. tropicalis* isocitrate dehydrogenase [5]; POX4-A and POX4-B, amino acids 1-118 and 309-427 from *C. tropicalis* acyl-CoA oxidase respectively [6]. The pictures were observed using a Confocal Laser Scanning Microscope Leica TCS SP8 (Leica Microsystems, Germany) (10×63).

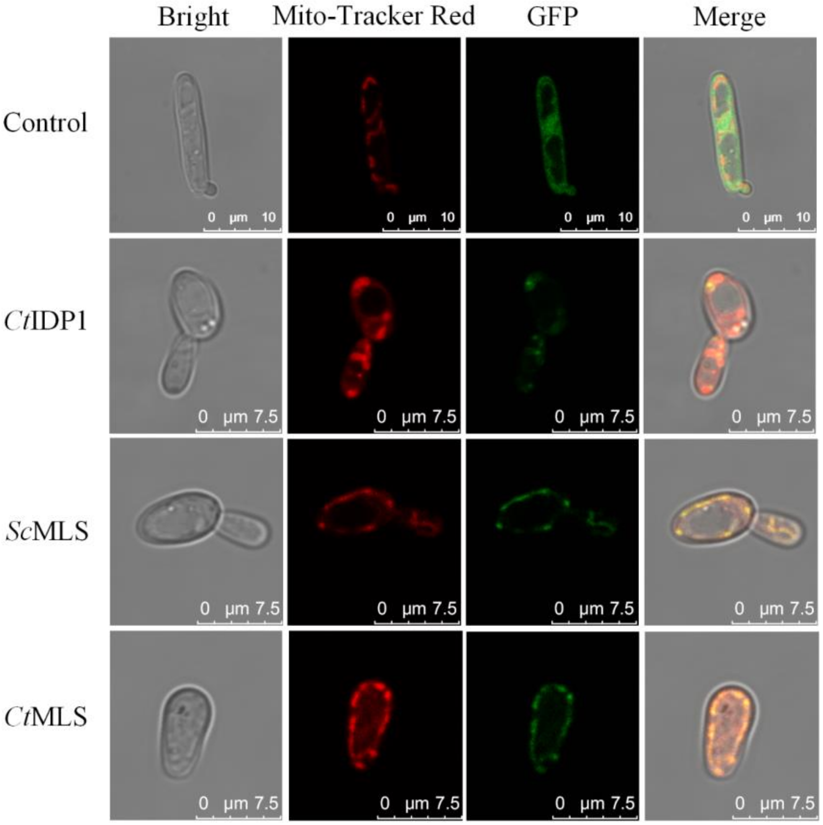


Fig. S4. Confirmation of mitochondrial targeting signals in *C. tropicalis* with yeGFP3 as a reporter. Control represents a transformant expressed the yeGFP3 without target signal (*C. tropicalis* 05-3). *Ct*IDP1, *Sc*COX4, and *Ct*COX4 represent transformants expressed *Ct*IDP1-yeGFP3, *Sc*COX4-yeGFP3, and *Ct*COX4-yeGFP3 fusion protein, respectively. Mitochondria (red) were counterstained with Mito-Tracker Red CMXRos (Beyotime, China). The pictures were observed using a Confocal Laser Scanning Microscope Leica TCS SP8 (10×63).

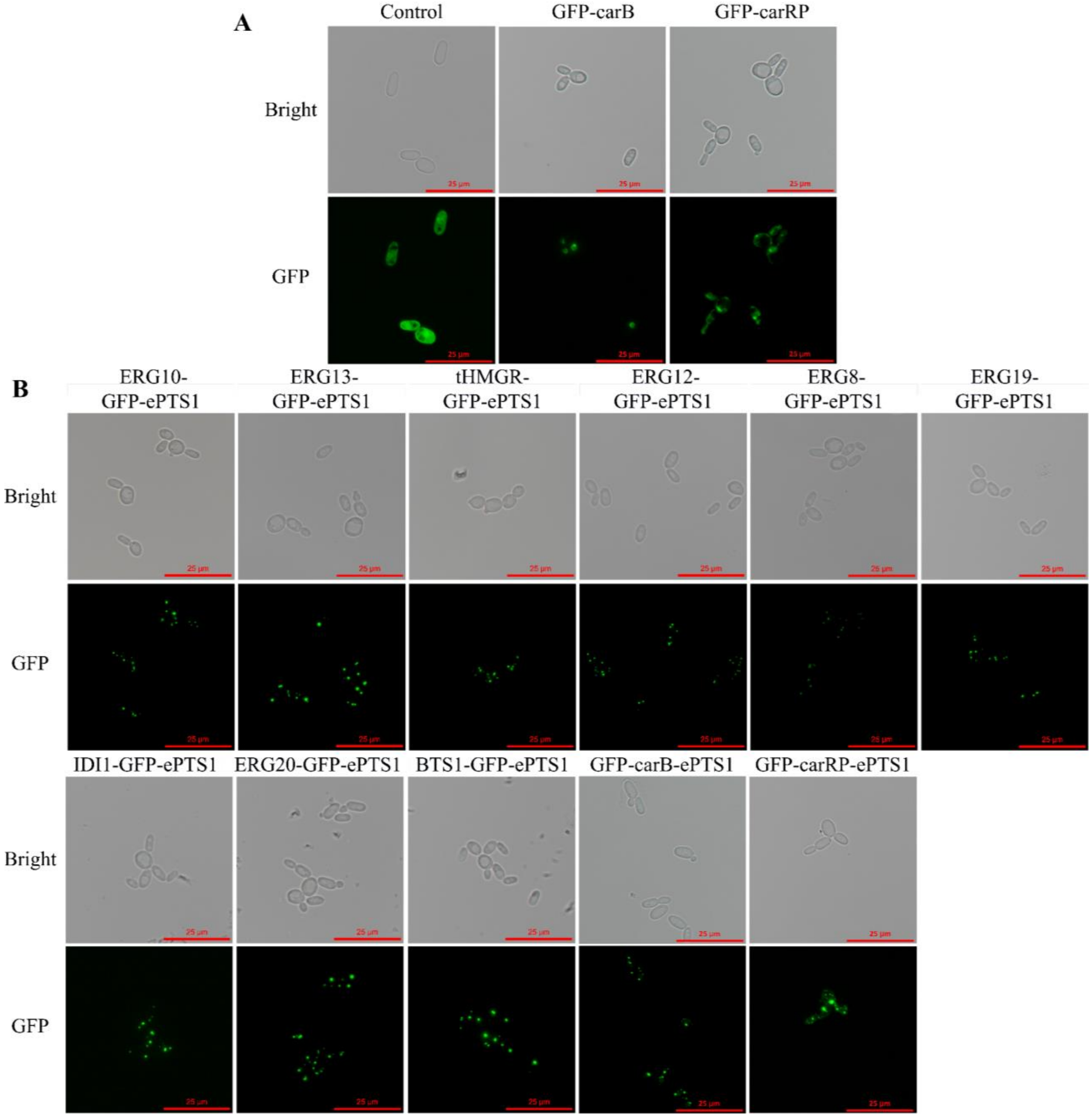


Fig. S5. Investigation of the subcellular localization of enzymes of the β-carotene pathway. (A) Investigation of the subcellular localization of fusion proteins GFP-CarB and GFP-CarRP in *C. tropicalis*. (B) Investigation of the subcellular localization of fusion proteins ERG10-GFP-ePTS1, ERG13-GFP-ePTS1, tHMGR-GFP-ePTS1, ERG12-GFP-ePTS1, ERG8-GFP-ePTS1, ERG19-GFP-ePTS1, IDI1-GFP-ePTS1, ERG20-GFP-ePTS1, BTS1-GFP-ePTS1, GFP-CarB-ePTS1, and GFP-CarRP-ePTS1 in *C. tropicalis*. Control represents *C. tropicalis* 05-3. The pictures were observed using a Nikon Eclipse 80i microscope (10×40).

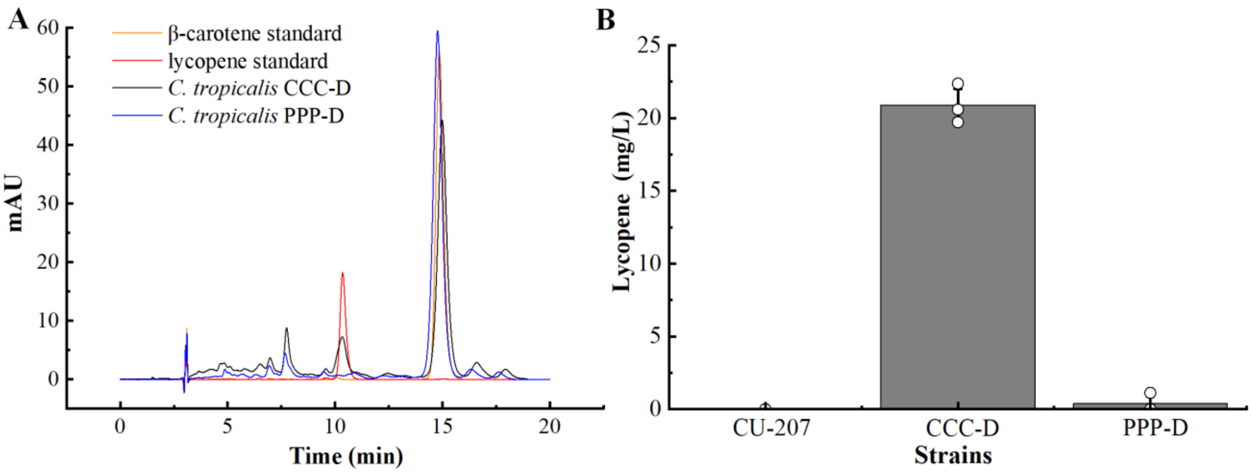


Fig. S6. Compartmentalization of β-carotene pathway into peroxisome results in a lower titer of lycopene. (A) HPLC spectra of lycopene, β-carotene, and their standards. (B) The lycopene titer difference in the strain CCC-D, PPP-D and CU-207. Data are means values ± standard deviations of biological triplicates.

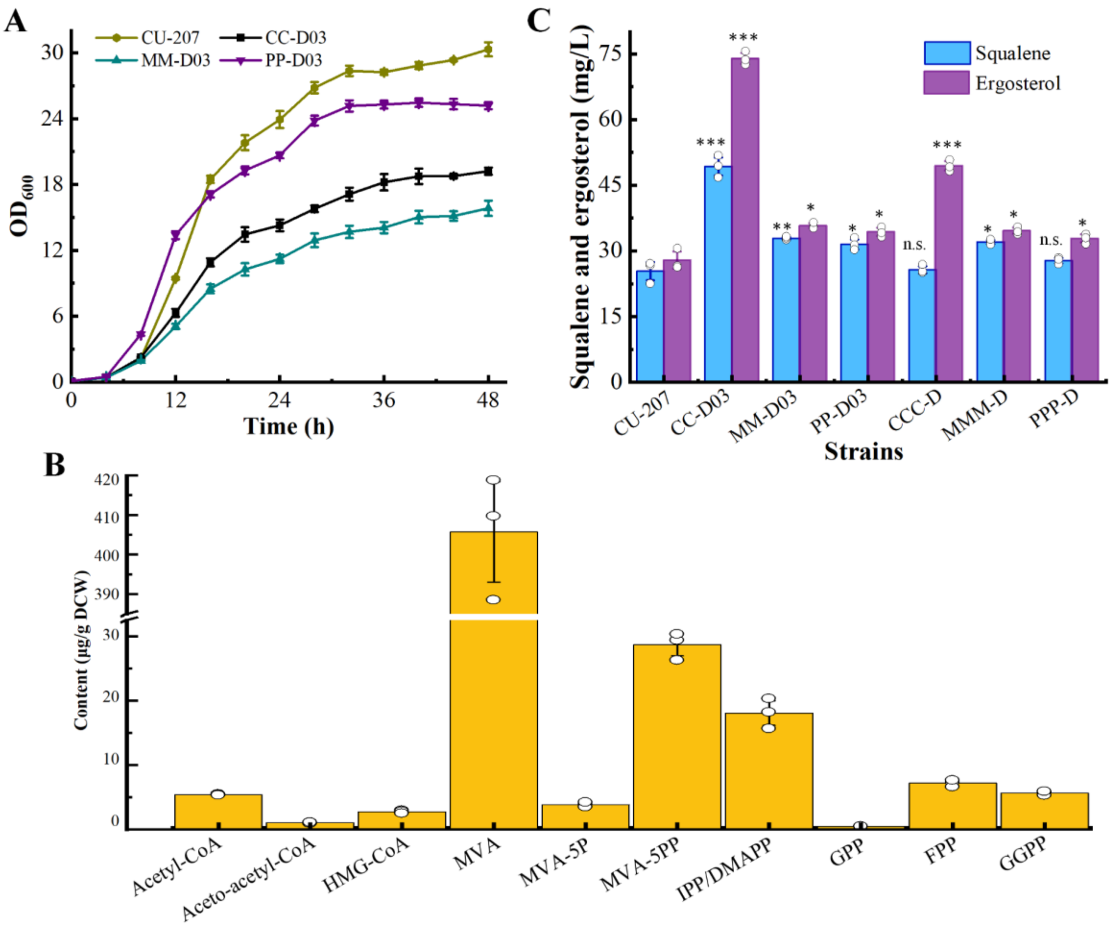


Fig. S7. The effect of MI and MII genes overexpression on cell growth, metabolic intermediates change, squalene and ergosterol production. (A) Growth curve of *C. tropicalis* CC-D03, MM-D03, PP-D03, and the control strain CU-207. (B) Analysis of the metabolic intermediates levels in engineered strains CC-D03. (C) Squalene and ergosterol production of engineered strains CC-D03, MM-D03, PP-D03, CCC-D, MMM-D, PPP-D, and the control strain CU-207. Data are means values ± standard deviations of biological triplicates. n.s., no significance, * for $p<0.05$, *** for $p<0.01$, *** for $p<0.001$.

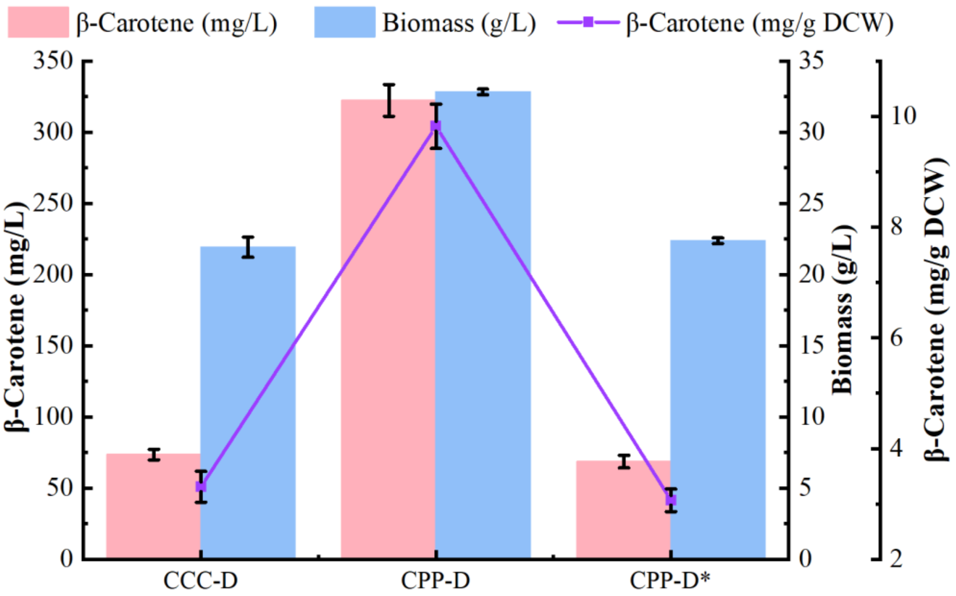


Fig. S8. Effect of deletion of *PEX5* gene on β-carotene production. Strains were cultivated in a 250 mL shake flask containing 15 mL YPD60 medium for 3 d.

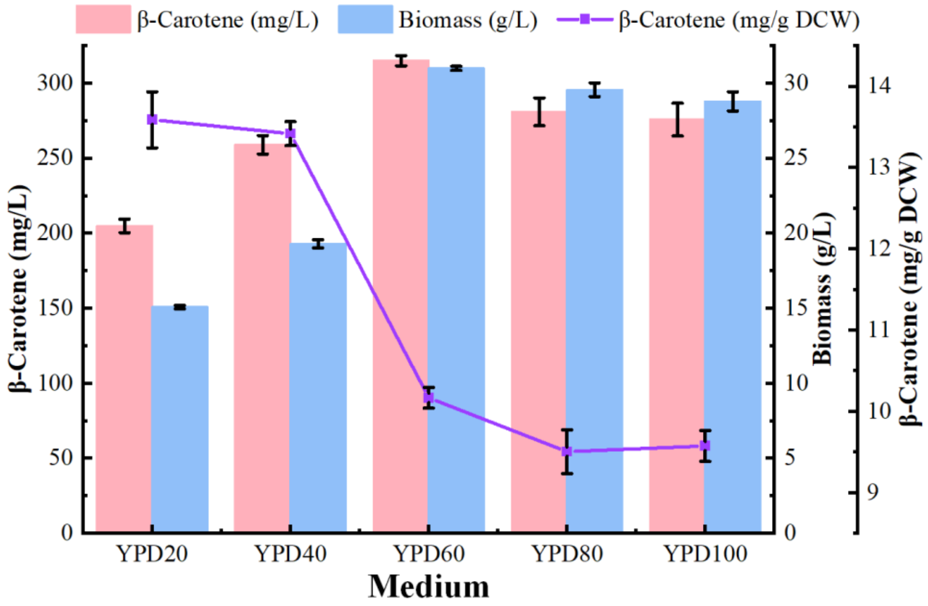


Fig. S9. β-Carotene titer and content depended on the glucose concentration in the medium. YPD20, YPD40, YPD60, YPD80, and YPD100 represent modified YPD medium with 20, 40, 60, 80, and 100 g/L of glucose, respectively. Data are means values ± standard deviations of biological triplicates.

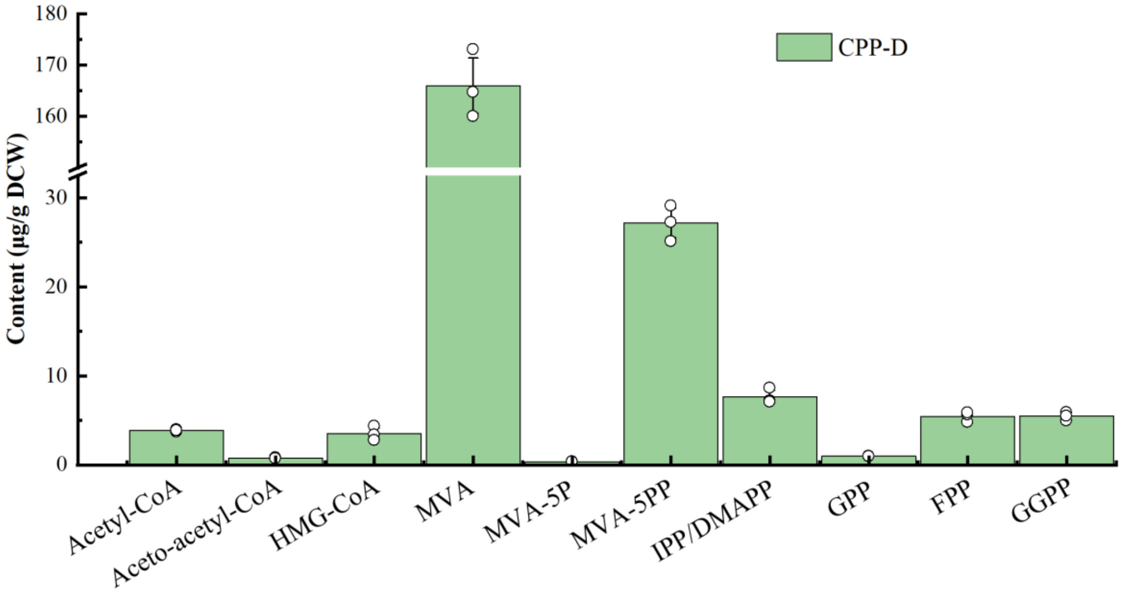


Fig. S10. Analysis of the metabolic intermediate levels in engineered strains CPP-D. Data are mean values ± standard deviations of biological triplicates**.**

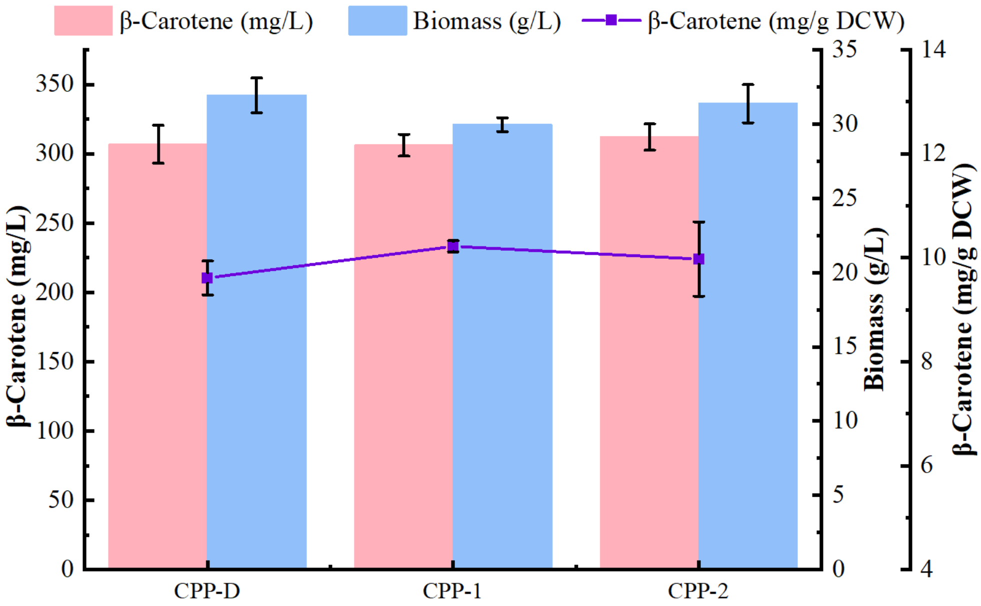


Fig. S11. The effect of expressing genes *carB* and *carRP*, *ERG20* and *BTS1* on β-carotene production in the strain CPP-D. CPP-1 represents a transformant expressed double copies of *ERG20* and *BTS1* in the peroxisome of CPP-D, CPP-2 represents a transformant expressed double copies of *carB* and *carRP* in the peroxisome of CPP-D. Data are means values ± standard deviations of biological triplicates.

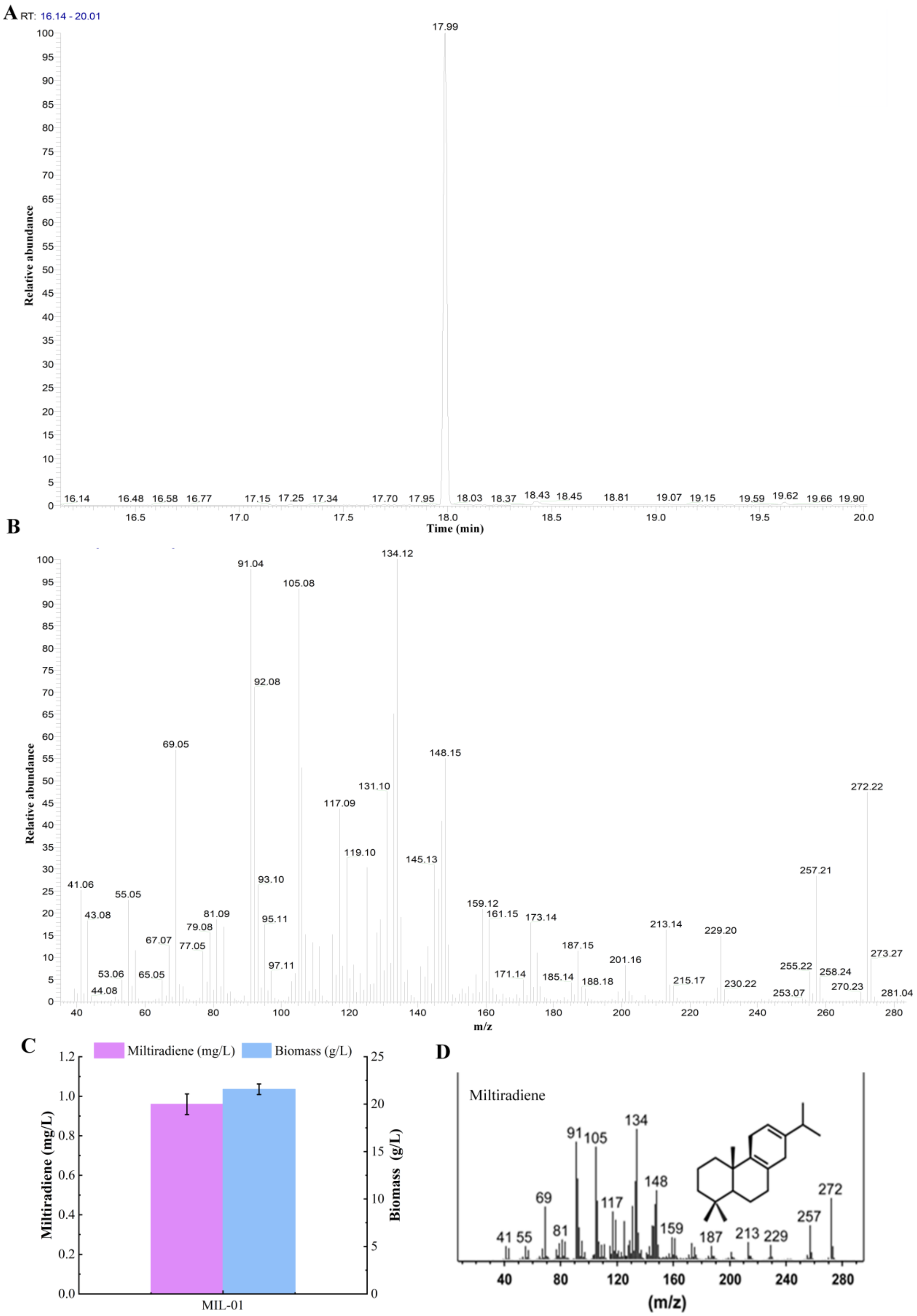

A
RT: 16.14 - 20.01
Relative abundance
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
17.99
16.14
16.48
16.58
16.77
17.15
17.25
17.34
17.70
17.95
18.03
18.37
18.43
18.45
18.81
19.07
19.15
19.59
19.62
19.66
19.90
16.5
17.0
17.5
18.0
18.5
19.0
19.5
20.0
Time (min)
B
Relative abundance
134.12
91.04
105.08
92.08
69.05
148.15
131.10
117.09
272.22
119.10
145.13
257.21
41.06
55.05
93.10
159.12
161.15
173.14
43.08
81.09
95.11
213.14
79.08
229.20
67.07
77.05
187.15
201.16
273.27
97.11
255.22
53.06
65.05
171.14
258.24
185.14
188.18
215.17
230.22
270.23
44.08
253.07
281.04
40
60
80
100
120
140
160
180
200
220
240
260
280
m/z
C
Miltiradiene (mg/L)
Biomass (g/L)
Miltiradiene (mg/L)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Biomass (g/L)
0
5
10
15
20
25
MIL-01
D
Miltiradiene
41
55
69
81
91
105
117
134
148
159
187
213
229
257
272
40
80
120
160
200
240
280
(m/z)

Fig. S12. GC-MS analysis of miltiradiene in *C. tropicalis*. (A) Gas chromatography profile of miltiradiene produced by strain MIL-01 (retention time 17.99 min). (B) Mass spectrum of miltiradiene produced by strain MIL-01 (MW = 272). (C) Miltiradiene production by *C. tropicalis* expressing genes *CfTPS1* and *SmKSL1* in the cytoplasm. (D) Mass spectrum of miltiradiene standard (MW = 272) [7].

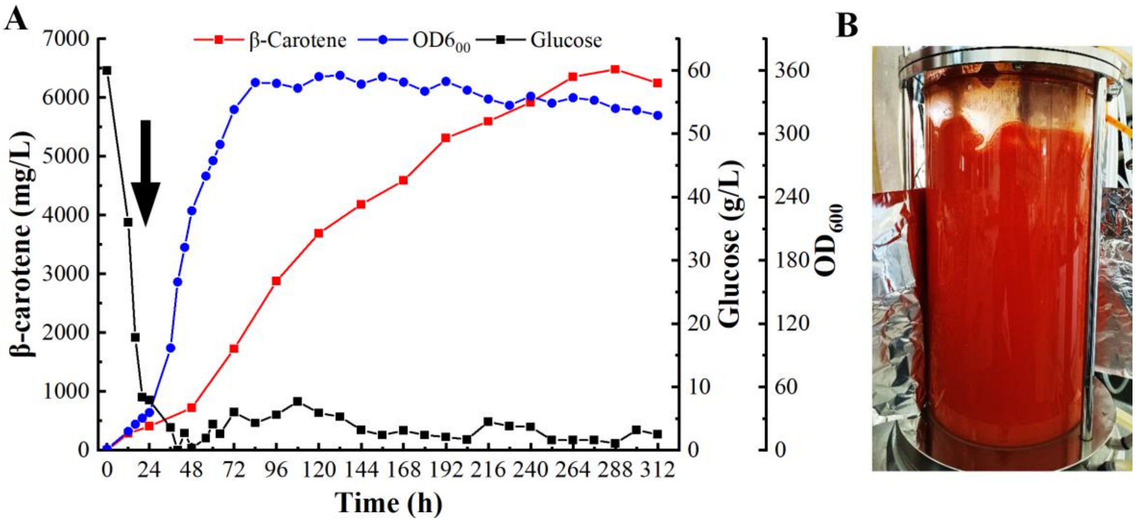
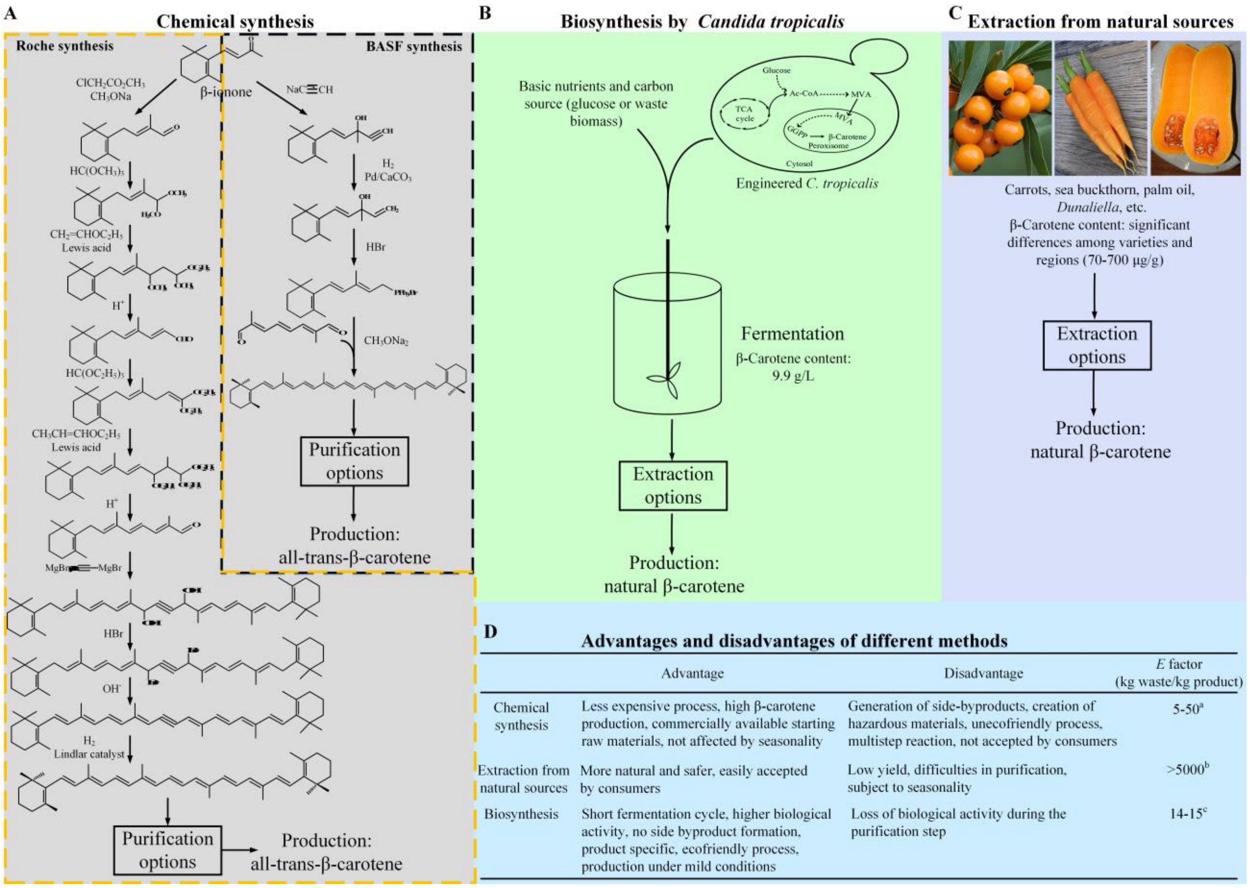


Fig. S13. Fed-batch fermentation of strain CPP-07 to produce β-carotene. (A) Fermentation profiles of the strain CPP-07 for β-carotene. The black arrow indicates the start of glucose concentration control. (B) Appearance of the fermentation broth for β-carotene (the ninth day).



Advantages and disadvantages of different methods

	Advantage	Disadvantage	*E* factor (kg waste/kg product)
Chemical synthesis	Less expensive process, high β-carotene production, commercially available starting raw materials, not affected by seasonality	Generation of side-byproducts, creation of hazardous materials, unecofriendly process, multistep reaction, not accepted by consumers	5-50[a]
Extraction from natural sources	More natural and safer, easily accepted by consumers	Low yield, difficulties in purification, subject to seasonality	>5000[b]
Biosynthesis	Short fermentation cycle, higher biological activity, no side byproduct formation, product specific, ecofriendly process, production under mild conditions	Loss of biological activity during the purification step	14-15[c]

Fig. S14. Production of β-carotene. (A) Chemical synthesis of β-carotene (Roche synthesis, and BASF synthesis) [8, 9]. (B) Process scheme of β-carotene production by *C. tropicalis*. (C) Extraction of β-carotene from bio-sources. (D) Advantages and disadvantages of different methods to produce β-carotene [10]. [a]*E*-factor for the chemical synthesis of fine chemicals [11], [b]*E*-factor associated with the production of β-carotene via the plant extraction method from carrot, estimated based on a β-carotene content of 190 μg/g of dry weight, [c]*E*-factor for β-carotene production utilizing *C. tropicalis* CPP-07, estimated from data obtained from a 5-L fermenter.

Table S1. Primers used in this study

Primer name	Sequence (5' to 3')
SKL-F	GTATGGATGAATTGTACAAATCCAAGTTGTAACTATCCAACAAACTCTAGG
SKL-R	CCTAGAGTTTGTTGGATAGTTACAACTTGGATTTGTACAATTCATCCATAC
CtICL-F	GTATGGATGAATTGTACAAAACTGAAGACCAATTCAAGGATAACAAAGCTAAGGTTTAACTATCCAACAAACTCTAGG
CtICL-R	CCTAGAGTTTGTTGGATAGTTAAACCTTAGCTTTGTTATCCTTGAATTGGTCTTCAGTTTTGTACAATTCATCCATAC
ePTS-F	GTATGGATGAATTGTACAAATTGGGTAGAGGTAGAAGATCCAAGTTGTAACTATCCAACAAACTCTAGG
ePTS-R	CCTAGAGTTTGTTGGATAGTTACAACTTGGATCTTCTACCTCTACCCAATTTGTACAATTCATCCATAC
POX4A-F	CAATTAAAGAATTTAAACAATGACTTTTACAAAGAAAAACGTTAGT
POX4A-R	AATTCTTCACCTTTAGACATAGAACCACCACCACCGACACCAATTCTGGTACCG
POX4B-F	CAATTAAAGAATTTAAACAATGAGAATGTTGGCTAGAATGTCC
POX4B-R	AATTCTTCACCTTTAGACATAGAACCACCACCACCACATTGGTCAATGGCTTCAGC
PERG10-F	TGGATGAATTGTACAAAGGTGGTGGTGGTTCTATGGCTCTCCCACCAGTCTACAT
PERG10-F	CTAGAGTTTGTTGGATAGTTACAACTTGGCGTCGGCGTC
IDP2-CF	GTATGGATGAATTGTACAAAGGTGGTGGTGGTTCTATGGGCGAAATTCAGAAAAT
IDP2-CR	CCTAGAGTTT GTTGGATAGT CTAGTAGCCCAAGTTTTTGTTC
IDP2-NF	CAATTAAAGAATTTAAACAATGGGCGAAATTCAGAAAATA
IDP2-NR	AATTCTTCACCTTTAGACATAGAACCACCACCACCGTAGCCCAAGTTTTTGTTC
YURA3-F	ACGCGTGATTGTTTCCTTTGTTGATTG
YURA3-R	ACGCGTAAGTTCGGGTTTAATAAGTT
rURA3-F	AACTGCAGGTTGTACCCGTCGTTAGAGTA
rURA3-R	GCTCTAGACTTCTGCACTTTTGAAATCGGGT
ANT1-F	TCAATTAAAGAATTTAAACAATGTCACTTTCACCAATAGAA
ANT1-R	TACCTCTTTGCAGAAAACTCACTTTAACGACTTTATAGACTT
SmKSL1-F	ATCTACAAATCATCCAACAGCCACCATGTCCTTGGCTTTCAACCCT
SmKSL1-R	TAGAATAAATATTTCTGATCTACTTACCTCTAACGTTGTTAGCAACCAATG
CfTPS1-F	TCAATTAAAGAATTTAAACAGCCACCATGGGTTCCTTGTC
CfTPS1-R	TACCTCTTTGCAGAAAACTTAAGCAACAGGTTCGAACAAA
ACHS2-F	ATCTACAAATCATCCAACAATGTCCCCAGCTCAAGCTC
ACHS2-R	CCACCAATTTCAGTACACCTAAATGGTGAATGGGTGGACCAACAA

Table S2. Plasmids used in this study

Plasmid name	**Characteristics**	**Source**
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	Lab stock
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-PTS1-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-PTS1-$\mathrm{T_{GAP1}}$-CAT1	Lab stock
Ts-ALD1-gda-URA3-$\mathrm{P_{GAP1}}$-PEX3-mScarlet-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette ALD1-gda-URA3-$\mathrm{P_{GAP1}}$-PEX3-mScarlet-$\mathrm{T_{GAP1}}$-ALD1	Lab stock
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-ePTS1-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-ePTS1-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-CtICL-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-CtICL-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-BnICL-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-BnICL-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-POX4-A-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-POX4-A-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-POX4-B-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-POX4-B-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-ERG10-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-CtERG10-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-CtIDP2-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-yeGFP3-CtIDP2-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-CtIDP2-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-CtIDP2-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-CtIDP1-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-CtIDP1-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-ScMLS-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-ScMLS-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-CtMLS-yeGFP3-$\mathrm{T_{GAP1}}$	The plasmid harboring cassette CAT1-gda324-URA3-$\mathrm{P_{GAP1}}$-CtMLS-yeGFP3-$\mathrm{T_{GAP1}}$-CAT1	This study
Ts-POX5-gda324-URA3-$\mathrm{T_{PGK1}}$-carB-$\mathrm{P_{FBA1}}$-$\mathrm{P_{GAP1}}$-ERG20-$\mathrm{T_{syn7}}$-	The plasmid harboring cassette POX5-gda324-URA3-$\mathrm{T_{PGK1}}$-carB-$\mathrm{P_{FBA1}}$-$\mathrm{P_{GAP1}}$-	This study

T_{ADH2}-BTS1-P_{FBA1}-P_{GAP1}-carRP-T_{ENO1}	ERG20-T_{syn7}-T_{ADH2}-BTS1-P_{FBA1}-P_{GAP1}-carRP-T_{ENO1}-POX5	
Ts-FAO1-gda324-URA3-T_{PGK1}-ERG13-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-tHMGR-T_{syn7}-P_{GAP1}-ScMLS-ERG10-T_{ENO1}	The plasmid harboring cassette FAO1-gda324-URA3-T_{PGK1}-ERG13-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-tHMGR-T_{syn7}-P_{GAP1}-ScMLS-ERG10-T_{ENO1}-FAO1	This study
Ts-ALD1-gda324-URA3-T_{PGK1}-ERG12-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-ERG8-T_{syn7}-T_{ADH2}-ERG19-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-IDI1-T_{ENO1}	The plasmid harboring cassette ALD1-gda324-URA3-T_{PGK1}-ERG12-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-ERG8-T_{syn7}-T_{ADH2}-ERG19-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-IDI1-T_{ENO1}-ALD1	This study
Ts-POX5-gda324-URA3-T_{PGK1}-carB-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-ERG20-T7syn-T_{ADH2}-BTS1-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-carRP-T_{ENO1}	The plasmid harboring cassette POX5-gda324-URA3-T_{PGK1}-carB-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-ERG20-T_{syn7}-T_{ADH2}-BTS1-ScMLS-P_{FBA1}-P_{GAP1}-ScMLS-carRP-T_{ENO1}-POX5	This study
Ts-FAO1-gda324-URA3-T_{PGK1}-ePTS1-ERG13-P_{FBA1}-P_{GAP1}-tHMGR-ePTS1-T_{syn7}-P_{GAP1}-ERG10-ePTS1-T_{ENO1}	The plasmid harboring cassette FAO1-gda324-URA3-T_{PGK1}-ePTS1-ERG13-P_{FBA1}-P_{GAP1}-tHMGR-ePTS1-T_{syn7}-P_{GAP1}-ERG10-ePTS1-T_{ENO1}-FAO1	This study
Ts-ALD1-gda324-URA3-T_{PGK1}-ePTS1-ERG12-P_{FBA1}-P_{GAP1}-ERG8-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ERG19-P_{FBA1}-P_{GAP1}-IDI1-ePTS1-T_{ENO1}	The plasmid harboring cassette ALD1-gda324-URA3-T_{PGK1}-ePTS1-ERG12-P_{FBA1}-P_{GAP1}-ERG8-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ERG19-P_{FBA1}-P_{GAP1}-IDI1-ePTS1-T_{ENO1}-ALD1	This study
Ts-POX5-gda324-URA3-T_{PGK1}-ePTS1-carB-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-carRP-ePTS1-T_{ENO1}	The plasmid harboring cassette POX5-gda324-URA3-T_{PGK1}-ePTS1-carB-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-carRP-ePTS1-T_{ENO1}-POX5	This study
Ts-DLD1a-gda324-URA3-T_{PGK1}-ERG13-P_{FBA1}-P_{GAP1}-tHMGR-T_{syn7}-P_{GAP1}-ERG10-T_{ENO1}	The plasmid harboring cassette DLD1a-gda324-URA3-T_{PGK1}-ERG13-P_{FBA1}-P_{GAP1}-tHMGR-T_{syn7}-P_{GAP1}-ERG10-T_{ENO1}-DLD1a	Lab stock
Ts-DLD1a-gda-URA3-T_{PGK1}-ERG12-P_{FBA1}-P_{GAP1}-ERG8-T_{syn7}-T_{ADH2}-ERG19-P_{FBA1}-P_{GAP1}-IDI1-T_{ENO1}	The plasmid harboring cassette DLD1a-gda-URA3-T_{PGK1}-ERG12-P_{FBA1}-P_{GAP1}-ERG8-T_{syn7}-T_{ADH2}-ERG19-P_{FBA1}-P_{GAP1}-IDI1-T_{ENO1}-DLD1a	Lab stock
Ts-DLD1a-gda-URA3-T_{PGK1}-ePTS1-ERG12-P_{FBA1}-P_{GAP1}-ERG8-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ERG19-P_{FBA1}-P_{GAP1}-IDI1-ePTS1-T_{ENO1}	The plasmid harboring cassette DLD1a-gda-URA3-T_{PGK1}-ePTS1-ERG12-P_{FBA1}-P_{GAP1}-ERG8-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ERG19-P_{FBA1}-P_{GAP1}-IDI1-ePTS1-T_{ENO1}-DLD1a	This study
Ts-DLD1a-gda324-URA3-T_{PGK1}-ePTS1-carB-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-carRP-ePTS1-T_{ENO1}	The plasmid harboring cassette DLD1a-gda324-URA3-T_{PGK1}-ePTS1-carB-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-carRP-ePTS1-T_{ENO1}-DLD1a	This study

Ts-DLD1b-gda324-URA3-T_{PGK1}-ERG13-P_{FBA1}-P_{GAP1}-tHMGR-T_{syn7}-P_{GAP1}-ERG10-T_{ENO1}	The plasmid harboring cassette DLD1b-gda324-URA3-T_{PGK1}-ERG13-P_{FBA1}-P_{GAP1}-tHMGR-T_{syn7}-P_{GAP1}-ERG10-T_{ENO1}-DLD1b	This study
Ts-DLD1b-gda324-URA3-T_{PGK1}-NADH-HMGR-P_{FBA1}-P_{GAP1}-ERG10-T_{ENO1}	The plasmid harboring cassette DLD1b-gda324-URA3-T_{PGK1}-NADH-HMGR-P_{FBA1}-P_{GAP1}-ERG10-T_{ENO1}-DLD1b	This study
Ts-LPP2-gda324-URA3-T_{PGK1}-ERG12-P_{FBA1}-P_{GAP1}-ERG8-T_{syn7}-T_{ADH2}-ERG19-P_{FBA1}-P_{GAP1}-IDI1-T_{ENO1}	The plasmid harboring cassette LPP2-gda324-URA3-T_{PGK1}-ERG12-P_{FBA1}-P_{GAP1}-ERG8-T_{syn7}-T_{ADH2}-ERG19-P_{FBA1}-P_{GAP1}-IDI1-T_{ENO1}-LPP2	This study
Ts-LPP2-gda324-URA3-T_{PGK1}-ePTS1-ERG12-P_{FBA1}-P_{GAP1}-ERG8-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ERG19-P_{FBA1}-P_{GAP1}-IDI1-ePTS1-T_{ENO1}	The plasmid harboring cassette LPP2-gda324-URA3-T_{PGK1}-ePTS1-ERG12-P_{FBA1}-P_{GAP1}-ERG8-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ERG19-P_{FBA1}-P_{GAP1}-IDI1-ePTS1-T_{ENO1}-LPP2	This study
Ts-YURA3-gda324-URA3-P_{GAP1}-ANT1-T_{ENO1}	The plasmid harboring cassette YURA3-gda324-URA3-P_{GAP1}-ANT1-T_{ENO1}-YURA3	This study
Ts-POX5-gda324-URA3-T_{PGK1}-SmKSL1-P_{FBA1}-P_{GAP1}-ERG20-T_{syn7}-T_{ADH2}-BTS1-P_{FBA1}-P_{GAP1}-CfTPS1-T_{ENO1}	The plasmid harboring cassette POX5-gda324-URA3-T_{PGK1}-SmKSL1-P_{FBA1}-P_{GAP1}-ERG20-T_{syn7}-T_{ADH2}-BTS1-P_{FBA1}-P_{GAP1}-CfTPS1-T_{ENO1}-POX5	This study
Ts-POX5-gda324-URA3-T_{PGK1}-ePTS1-SmKSL1-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-CfTPS1-ePTS1-T_{ENO1}	The plasmid harboring cassette POX5-gda324-URA3-T_{PGK1}-ePTS1-SmKSL1-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-CfTPS1-ePTS1-T_{ENO1}-POX5	This study
Ts-DLD1a-gda324-URA3-T_{PGK1}-ePTS1-SmKSL1-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-CfTPS1-ePTS1-T_{ENO1}	The plasmid harboring cassette DLD1a-gda324-URA3-T_{PGK1}-ePTS1-SmKSL1-P_{FBA1}-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-BTS1-P_{FBA1}-P_{GAP1}-CfTPS1-ePTS1-T_{ENO1}-DLD1a	This study
Ts-POX5-gda324-URA3-P_{GAP1}-ERG20-T_{syn7}-T_{ADH2}-ACHS2-P_{FBA1}	The plasmid harboring cassette POX5-gda324-URA3-P_{GAP1}-ERG20-T_{syn7}-T_{ADH2}-ACHS2-P_{FBA1}-POX5	This study
Ts-POX5-gda324-URA3-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ACHS2-P_{FBA1}	The plasmid harboring cassette POX5-gda324-URA3-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ACHS2-P_{FBA1}-POX5	This study
Ts-DLD1a-gda324-URA3-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ACHS2-P_{FBA1}	The plasmid harboring cassette DLD1a-gda324-URA3-P_{GAP1}-ERG20-ePTS1-T_{syn7}-T_{ADH2}-ePTS1-ACHS2-P_{FBA1}-DLD1a	This study

Table S3. Codon-optimized gene sequences, peroxisomal targeting signal sequences, and mitochondrial targeting signals sequences used in this study

Name	Sequences (5'-3')	Source
SmKSL1	ACTAGTGCCACCATGTCCTTGGCTTTCAACCCTGCTGCTACCGCTTTCTCCGGTAACGGTGCTAGATCTAGAAGAGAAAACTTCCCTGTTAAGCACGTTACCGTTAGAGGTTTCCCAATGATTACCAACAAGTCCTCCTTCGCTGTTAAGTGTAACTTGACCACTACCGATTTGATGGGTAAGATTGCTGAAAAGTTCAAGGGTGAAGATTCCAACTTCCCTGCTGCCGCTGCTGTTCAACCTGCTGCTGATATGCCATCCAACTTATGTATTATCGATACCTTGCAAAGATTGGGTGTTGATAGATACTTCAGATCCGAAATTGATACCATTTTGGAAGATACCTACAGATTGTGGCAGAGAAAGGAAAGAGCCATTTTCTCCGATACCGCTATTCACGCTATGGCTTTCAGATTGTTGAGAGTTAAGGGTTACGAAGTTTCCTCCGAAGAATTGGCTCCATACGCTGATCAAGAACACGTTGATTTGCAAACCATTGAAGTTGCTACCGTTATTGAATTGTACAGAGCTGCTCAAGAAAGAACCGGTGAAGATGAATCCTCCTTGAAGAAGTTGCACGCTTGGACCACTACCTTCTTGAAGCAAAAGTTGTTGACCAACTCCATTCCTGATAAGAAATTGCACAAGTTGGTTGAATACTACTTGAAGAACTACCACGGTATTTTGGATAGAATGGGTGTTAGACAAAACTTGGATTTGTACGATATTTCCTACTACAGAACCTCCAAGGCTGCTAACAGATTCTCTAACTTGTGTTCCGAAGATTTCTTGGCTTTCGCTAGACAAGATTTCAACATTTGTCAAGCTCAACACCAAAAGGAATTGCAACAATTGCAAAGATGGTACGCTGATTGTAAGTTGGATACCTTGAAGTACGGTAGAGATGTTGTTAGAGTTGCTAACTTCTTGACCTCCGCTATTATTGGTGATCCTGAATTGTCCGATGTTAGAATTGTTTTCGCTCAACACATTGTTTTGGTTACTAGAATTGATGATTTCTTCGATCACAGAGGTTCTAGAGAAGAATCCTACAAGATTTTGGAATTGATTAAGGAATGGAAGGAAAAGCCTGCTGCTGAATACGGTTCCGAAGAAGTTGAAATTTTGTTCACCGCTGTTTACAACACCGTTAACGAATTGGCTGAAAGAGCTCACGCTGAACAAGGTAGATCCGTTAAGGATTTCTTGATTAAGTTGTGGGTTCAAATTTTGTCCATTTTCAAGAGAGAATTGGATACCTGGTCCGATGATACCGCTTTGACCTTGGATGATTACTTGTCCGCTTCCTGGGTTTCCATTGGTTGTAGAATTTGTATTTTGATGTCCATGCAATTCATTGGTATTAAGTTGTCCGATGAAATGTTGTTGTCCGAAGAATGTATTGATTTGTGTAGACACGTTTCCATGGTTGATAGATTGTTGAACGATGTTCAAACCTTCGAAAAGGAAAGAAAAGAAAACACCGGTAACTCCGTTACCTTGTTATTGGCTGCTAACAAGGATGATTCCTCCTTCACCGAAGAGGAAGCTATTAGAATTGCTAAGGAAATGGCTGAATGTAACCGTCGTCAATTGATGCAAATTGTTTACAAGACCGGTACCATTTTCCCAAGACAATGTAAGGATATGTTCTTGAAGGTTTGTAGAATTGGTTGTTACTTGTACGCTTCCGGTGATGAATTCACCTCCCCACAACAAATGATGGAAGATATGAAGTCCTTGGTTTACGAACCATTGACCATTCACCCATTGGTTGCTAACAACGT	*Salvia miltiorrhiza* (NCBI Accession Number: EF635966.2)

	TAGAGGTAAG**TTGGGTAGAGGTAGAAGATCCAAGTTG**TAGCTCGAG	
CfTPS1	GTCGACGCCACCATGGGTTCCTTGTCCACCATGAACTTGAACCACTCCCCAATGTCCTACTCCGGTATTTTGCCATCCTCTTCCGCTAAGGCTAAGTTGTTATTGCCTGGTTGTTTCTCCATTTCCGCTTGGATGAACAACGGTAAGAACTTGAACTGTCAATTGACCCACAAGAAGATTTCCAAGGTTGCTGAAATTAGAGTTGCTACCGTTAACGCTCCACCTGTTCACGATCAAGATGATTCCACCGAAAACCAATGTCACGATGCTGTTAACAACATTGAAGATCCAATTGAATACATTAGAACCTTGTTGAGAACCACCGGTGATGGTAGAATTTCCGTTTCCCCATACGATACCGCTTGGGTTGCTTTGATTAAGGATTTGCAAGGTAGAGATGCTCCTGAATTCCCATCCTCCTTGGAATGGATTATTCAAAACCAATTGGCTGATGGTTCCTGGGGTGATGCTAAGTTCTTCTGTGTTTACGATAGATTGGTTAACACCATTGCTTGTGTTGTTGCTTTGAGATCCTGGGATGTTCACGCTGAAAAGGTTGAAAGAGGTGTTAGATACATTAACGAAAACGTTGAAAAGTTGAGAGATGGTAACGAAGAACACATGACCTGTGGTTTCGAAGTTGTTTTCCCTGCTTTGTTGCAAAGAGCTAAGTCCTTGGGTATTCAAGATTTGCCATACGATGCTCCTGTTATTCAAGAAATTTACCACTCTAGAGAACAAAAGTCCAAGAGAATTCCATTGGAAATGATGCACAAGGTTCCAACTTCCTTGTTGTTCTCCTTGGAAGGTTTGGAAAACTTGGAATGGGATAAGTTGTTGAAGTTGCAATCCGCCGATGGTTCCTTCTTAACCTCCCCATCCTCCACCGCTTTCGCTTTCATGCAAACTAGAGATCCAAAGTGTTACCAATTCATTAAGAACACCATTCAAACCTTCAACGGTGGTGCTCCACACACCTACCCTGTTGATGTTTTCGGTAGATTGTGGGCTATTGATAGATTACAAAGATTGGGTATTTCTAGATTCTTCGAATCCGAAATTGCTGATTGTATTGCTCACATTCACAGATTCTGGACCGAAAAGGGTGTTTTCTCCGGTAGAGAATCCGAATTCTGTGATATTGATGATACCTCCATGGGTGTTAGATTGATGAGAATGCACGGTTACGATGTTGATCCAAACGTTTTGAAGAACTTCAAGAAGGATGATAAGTTCTCCTGTTACGGTGGTCAAATGATTGAATCCCCATCCCCAATTTACAACTTGTACAGAGCTTCCCAATTGAGATTCCCTGGTGAACAAATTTTGGAAGATGCTAACAAGTTCGCTTACGATTTCTTGCAAGAAAAGTTGGCTCACAATCAAATTTTGGATAAGTGGGTTATTTCCAAGCACTTGCCTGATGAAATTAAGTTGGGTTTGGAAATGCCATGGTACGCTACCTTGCCAAGAGTTGAAGCTAGATACTACATTCAATACTACGCTGGTTCCGGTGATGTTTGGATTGGTAAGACCTTGTACAGAATGCCTGAAATTTCCAACGATACCTACCACGAATTGGCTAAGACCGATTTCAAGAGATGTCAAGCTCAACACCAATTCGAATGGATTTACATGCAAGAATGGTACGAATCCTGTAACATGGAAGAATTCGGTATTTCTAGAAAAGAGTTGTTAGTTGCTTATTTCTTGGCTACCGCTTCCATTTTCGAATTGGAAAGAGCTAACGAAAGAATTGCTTGGGCTAAGTCCCAAATTATTTCCACCATTATTGCTTCCTTCTTCAACAACCAAAACACCTCCCCTGAAGATAAGTTGGCTTTCTTGACCGATTTCAAGAACGGCAACTCCACCAACATGGCTTTGGTTACCTTGACCCAATTCTTGGAAGGCTTCGAT	*Plectranthus barbatus* (NCBI Accession Number: KF444506.1)

	AGATACACCTCCCACCAATTGAAGAACGCTTGGTCCGTTTGGTTGAGAAAGTTGCAACAAGGTGAAGGTAACGGTGGCGCTGATGCTGAATTGTTAGTTAACACCTTAAACATTTGTGCTGGTCACATTGCTTTCAGAGAAGAAATTTTGGCTCACAACGATTACAAGACCTTGTCCAACTTGACCTCCAAGATTTGTAGACAATTGTCCCAAATTCAAAACGAAAAGGAGTTGGAAACCGAAGGTCAAAAGACCTCCATTAAGAACAAGGAATTAGAGGAAGACATGCAAAGATTGGTTAAGTTGGTTTTGGAAAAGTCTAGAGTTGGTATTAACAGAGATATGAAGAAGACCTTCTTGGCTGTTGTTAAGACCTACTATTACAAGGCTTACCACTCCGCTCAAGCTATTGATAACCACATGTTCAAGGTTTTGTTCGAACCTGTTGCT**TTGGGTAGAGGTAGAAGATCCAAGTTG**TAGGCTAGC	
ACHS2	GAATTCATGTCCCCAGCTCAAGCTCCACAAGTTTCTGCTCCAACTCAAAAGGCTGCTGATGAAGAAGCTAACAGAAGATCCGCTGGTTACCACCCATCCTTTTGGGGTGAATTTTTCTTGACTCACTCCTCTGGTTACACCAAGTCCGACACTAAGATTCAACAAAAGCACGAAGAATTGAAGCAACAAGTTAGAGGTATGATCTTGGACGCTGCTGCTGATACTTCTCAAAAGTTGGAATTGATCGACGCTGCTTTGAGATTGGGTGTTGGTTACCACTTCGAAGCTGAAATTCAATCCCAATTACAAAAGATCCACGGTCAAGGTTCCTTCCACTCTGACTTATATACTGCTTGTATCTGGTTCAGAGTCTTGAGAGGTCAAGGTTTCACCGTTTCCGCTGATGTTTTCAACATTATGAAGAACAAGGACGGTGGTTTCGAAGCTAGAGATGCTAGAACTTTGTTGTGTTTGTACGAAACTACCCACTTGAGAATCCAAGGTGAACAAGTTTTGGAAGAAGCTCTTGAATTTTCCAGAAAGCAATTAGGTGACTTGTTGGCTGAATTGTCTTCCCCATTGGCTGAATACGTTAACAACTCTTTGGAATTGCCATACCACAAGGGTATGCAAAGATTGGAAGCTAGACAATACATCCCAATTTACGAATCTTACGCTAACAAGAACGACACCTTGTTGCAATTTGCTAAGTTGGATTTCAACTTGTTGCAAGCTCTTCACCAATCCGAAATTAGAGAAATTACCAGATGGTGGAAGGACTTGGACTTTAAGGCTAGATTGCCATACGCTAGAGATAGATTGGTTGAATGTTACTTCTGGATCTTGGGTGTTCAATACGAACCACAATACTCCATTTCTCGTGTTTTCTTGACTAAGGTTATCTCCTTGGCTTCCGTTTTCGATGACACCTACGACATTTACGGTACTTTCGATGAATTGAAGTTGTTGACTGACGCTGTTGAAAGATGGGAACCAGAAGCTACCGATTCTTTGCCAGGTTACATGCAAATTTTGTACGGTGCTTTGTTGAAGGTTTTCGAAGAATACAAGGACGAATTGATCAACGCTGGTGGTAGAGATTACTGTTTGTACTACGCTAAGGAAGCTATGAAGGGTTTGGTTAGATCCTACCACACCGAAGCTGTTTCTTTCCACACTGGTTACGTTCAAAACTTCGAAGAATATTTGGACAACTCCGCTGTTTCTTCTGGTTACCCAATGTTGACTGTTGAAGCTCTTATTGGTATGGGTGCTCCATACGCTACTAGAGAATCTTTGGACTGGGCTTTGAAGGTTCCAAAGATTATTAAGGCTTCCTCTGACATTTGTAGATTGGTTGACGACTTGAGAACCTACAAGGTTGAAGAAGAAAGAGGTGATGCTCCATCCGGTGTTCACTGTTACATGAGAGACTACAACGTTTCTGAAGAAGAAGCCTGTACCAAGATTGAAGAAATGATTGAT	*Aquilaria crassna* (NCBI Accession Number: AMQ67166.1)

	TTGGCCTGGAAGGCTATTAACGAAGAAATTCAAAAGCCAAACCACTTGCCATTGCCAATTTTGTTGCCAGCTTTGAACTTTGCTAGAATGATGGAAGTTTTGTACCAAAACATCGACGGTTACACCAACTCTGGTGGTAGAACTAAGGAAAGAATTTCCTCTTTGTTGGTCCACCCATTCACCATT**TTGGGTAGAGGTAGAAGATCCAAGTTG**TAGGAGCTC	
PTS1	TCCAAGTTG	Typical peroxisomal targeting signal type 1, amino acid sequence: SKL
ePTS1	TTGGGTAGAGGTAGAAGATCCAAGTTG	Enhanced PTS1, amino acid sequence: LGRGRRSKL[3]
*Ct*ICL	ACTGAAGACCAATTCAAGGATAACAAAGCTAAGGTT	The C-terminal region of *C. tropicalis* isocitrate lyase, amino acid sequence: TEDQFKDNKAKV (NCBI Accession Number: RCK55682.1）
*Ct*ERG10	GTCGTTATTGAAAAGATTGACGCCGACGCCAAGTTG	The C-terminal region of *C. tropicalis* acetoacetyl-CoA thiolase A, amino acid sequence: VVIEKIDADAKL(NCBI Accession Number: D13470.1）
*Bn*ICL	TCCAAGGCTTTGGGTAAGGGTGTCACTGAAGAACAATTCAAGGAAACTTGGACTAGACCAGGTGCTGCCGGTATGGGTGCCGGTACTTCCTTGGTCGTTGCTAAGTCCAGAATG	The C-terminal region of *Brassica napus* isocitrate lyase (NCBI

		Accession Number: NP_001302775.1）
*Ct*IDP2	ATGGGCGAAATTCAGAAAATAACAGTCAAGAACCCAATCGTCGAAATGGACGGTGACGAAATGACCCGTATCATCTGGCAATTCATCAAAGACAAGTTGATCTTGCCTTACTTGAATGTCGACTTGAAATACTACGACTTGGGCATTGAGTACAGAGACAAGACCGACGACAAGGTCACCACCGATGCTGCTGAAGCCATCTTGCAATATGGTGTCGGTGTCAAGTGTGCCACCATCACCCCAGATGAAGCCAGAGTTAAAGAATTCAACTTGAAGAAGATGTGGCTCTCTCCAAACGGTACATTGAGAAACGTCATTGGCGGTACTGTCTTTAGAGAACCAATTGTCATTGACAACATCCCAAGAATTGTGCCTAGCTGGGAAAAACCAATCATCATTGGTAGACACGCTTTCGGCGACCAATACAAGGCCACCGATGTGGTTATCCCAGCTGCCGGTGACTTGAAATTGGTGTTCAAGCCAAAAGACGGCGGCGAAGTACAAGAATTCCCAGTCTACCAGTTCGACGGTCCAGGTGTCGCCTTGAGCATGTACAACACCGACGCTTCAATTACTGATTTCGCTGAAAGCTCCTTCCAATTAGCCATTGAGCGTAAATTGAACTTGTTTTCTTCCACCAAAAACACCATCTTGAAGAAATACGACGGGAAATTCAAAGACATATTCGAAGGCTTGTACGCCAGCAAATACAAGACCAAGATGGACGAATTGGGCATCTGGTACGAGCACAGATTGATTGACGATATGGTTGCGCAGATGTTGAAGTCTAAAGGTGGTTACATCATCGCCATGAAAAACTACGATGGTGATGTCCAATCCGACATTGTCGCACAAGGTTTCGGTTCCTTGGGCTTGATGACCTCTGTTTTAGTTACCCCAGACGGCAAGGCTTTTGAGTCCGAAGCTGCCCACGGTACTGTCACTAGACATTATAGACAACACCAACAAGGCAAAGAGACCTCCACCAACTCCATTGCCTCCATCTACGCCTGGACCAGAGGGTTGATCCAAAGAGGTAAGTTGGATGACACTCCGGAAGTTGTCAAGTTTGCTGAAGAGTTGGAAAAGGCTGTCATCGAGACTGTCTCCAAGGACAACATCATGACTAAAGACTTGGCCTTGACTCAGGGTAAGACCGACAGATCTTCGTATGTCACGACTGAAGAATTCATTGATGGTGTTGCTAATAGATTGAACAAAAACTTGGGCTACTAG	*C. tropicalis* (NCBI Accession Number: O13294.3）
POX4-A	ATGACTTTTACAAAGAAAAACGTTAGTGTATCACAAGGTCCTGACCCTAGATCATCCATCCAAAAGGAAAGAGACAGCTCCAAATGGAACCCTCAACAAATGAACTACTTCTTGGAAGGCTCCGTCGAAAGAAGTGAGTTGATGAAGGCTTTGGCCCAACAAATGGAAAGAGACCCAATCTTGTTCACAGACGGCTCCTACTACGACTTGACCAAGGACCAACAAAGAGAATTGACCGCCGTCAAGATCAACAGAATCGCCAGATACAGAGAACAAGAATCCATCGACACTTTCAACAAGAGATTGTCCTTGATTGGTATCTTTGACCCACAGGTCGGTACCAGAATTGGTGTC	The N-terminal region (amino acids 1-118) of *C. tropicalis* acyl-CoA oxidase (NCBI Accession Number: M12160.1）
POX4-B	AGAATGTTGGCTAGAATGTCCACCATTGCCTTGAGATACGCCATTGGTAGAAGACAATTCAAGGGTGACAATGTCGATCCAAAAGATCCAAACGCTTTGGAAACCCAATTGATAGATTACCCATTGCACCAAAAGAGATTGTTCCCATACTTGGCTGCTGCCTACGTCATCTCC	The middle region (amino acids 309-427) of *C. tropicalis*

	GCTGGTGCCCTCAAGGTTGAAGACACCATCCATAACACCTTGGCTGAATTGGACGCTGCCGTTGAAAAGAACGACACCAAGGCTATCTTTAAGTCTATTGACGACATGAAGTCATTGTTTGTTGACTCTGGTTCCTTGAAGTCCACTGCCACTTGGTTGGGTGCTGAAGCCATTGACCAATGT	acyl-CoA oxidase (NCBI Accession Number: M12160.1）
*Sc*MLS	ATGTTATCCTTAAGACAATCTATTAGATTCTTTAAGCCAGCTACTAGAACCTTATGTTCTTCCAGATACTTGTTGCAA	The N-terminal region (amino acids 1-26) of *S. cerevisiae* cytochrome c oxidase (NCBI Accession Number: NP_011328.1）
*Ct*MLS	ATGCTTTCCCGTACCACTTTAAGAGTTGCCAGACAACAAACCAGATTATTGTCTACTTCCAGAATCTTGTTCAACAGCAAGACCGACCAA	The N-terminal region (amino acids 1-30) of *C. tropicalis* cytochrome c oxidase (NCBI Accession Number: RCK67380.1）
*Ct*IDP1	ATGATCAGAGCTAGTGCTATCCAACGTACCGCAATGTTGCTGAGACAATTGCGCGGGTTTTCGACCAGTGCTACCTTGGCC	The N-terminal region (amino acids 1-27) of *C. tropicalis* isocitrate dehydrogenase (NCBI Accession Number: AB004556.1）

Note: The chemical base bold represents ePTS1.

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