Electronic Supplementary Information

Biosynthesis of retinyl esters in *Yarrowia lipolytica* through metabolic engineering and fermentation condition optimization

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A1 Plasmids and strains construction details

For *Y. lipolytica* transformants selection, the selection marker *LEU2* in plasmid pKi-1 was replaced by *TRP1* and *HYG* to construct pKi1-TRP and pKi1-HYG, and the selection marker *URA3* in plasmid 113-GPD-TEF was replaced by *LEU2* to construct 113-LEU-GPD-TEF. To screen β-carotene 15,15'-dioxygenase (BCO) and retinol dehydrogenase (RDH) with high activity in *Y. lipolytica*, enzymes from different organisms found in the BRENDA enzyme database were considered. *BCO* genes derived from the uncultured marine bacterium 66A03 (*BLH*, GenBank: AAY68319.1), *Danio rerio* (*DrBCO1*, GenBank: AAH49331.1), *Gallus gallus* (*GgBCO1*, GenBank: CAB90825.1), *Homo sapiens* (*HsBCO1*, GenBank: AAG15380.1) and *Rattus norvegicus* (*RnBCO1*, GenBank: BAB60807.1) were codon-optimized and synthesized (Tsingke, Beijing, China). *RDH* genes derived from *E. coli* K12 (*YBBO*, GenBank: NP_415026.1) and *Homo sapiens* (*ADH1B*, GenBank: NP_000659.2; *DHRS3*, GenBank: NP_004744.2; *RDH8*, GenBank: NP_056540.3; *RDH12* GenBank: NP_689656.2) were also codon-optimized and synthesized (Tsingke, Beijing, China). These genes were ligated with the expression plasmid pKi1-TRP under the control of the *ut8* promoter and the *CYC1* terminator. Linear plasmids were transformed into the T3 strain,[1] which overexpresses all genes in the biosynthetic pathway from acetyl-CoA to β-carotene (*AtoB*, *HMGS*, *HMGR*, *ERG12*, *ERG8*, *ERG19*, *IDI1*, *ERG20*, *GGS1*, *CarRP*, and *CarB*), and integrated into the genome randomly. Transformants were selected on SC-TRP plates and verified by clone PCR to generate strains named VA1 to VA9. Twenty transformants from each transformant plate were selected randomly and fermented in shake flasks to detect retinoid production.

To verify the ability of different β-carotene-producing strains to produce retinol, we used four strains. Of genes in the β-carotene synthesis pathway, the T1 strain[2] expresses *CarRP* and *CarB*; the T1GI12VC strain[2] overexpresses *ERG12*, *ERG8*, *ERG19*, *IDI1*, *GGS1*, *GPS*, *CarRP*, and *VHb*; and the T3 strain[1] overexpresses all genes in the biosynthetic pathway from acetyl-CoA to β-carotene including *AtoB*, *HMGS*, *HMGR*, *ERG12*, *ERG8*, *ERG19*, *IDI1*, *ERG20*, *GGS1*, *CarRP*, and *CarB*. The T3GI12VC-ΔCLA4/MHY1 strain[2] overexpresses all genes of the mevalonate and carotenoid pathways, and has the endogenous *CLA4* and *MHY1* genes, which control filament formation, deleted. We expressed *BLH* from the uncultured marine bacterium 66A03 and *RDH12* from *Homo sapiens* in these strains and obtained the VAT1, VATIGI12VC, VAT3 and VAT3GI12VC-ΔCLA4/MHY1 strains, respectively. To obtain higher expression of *BLH* and *RDH12*, we transformed *BLH*- and *RDH12*-containing DNA fragments into the T3GI12VC-ΔCLA4/MHY1 strain via non-homologous end joining (NHEJ), and screened out multiple transformants with different retinoid production including the VA1-1, VA1-2 and VA1-3 strain.

To strengthen the mevalonate pathway, the p-car-M1 plasmid[1] containing acetyl-CoA acetyltransferase gene (*AtoB*, GenBank: b2224), HMG-CoA synthase gene (*HMGS*, Genbank: YALI0_F30481g), HMG-CoA reductase gene (*HMGR*, GenBank: Bpet3342), mevalonate kinase gene (*ERG12*, GenBank: YALI0_B16038g) was transformed into the VA1-3 strain to obtain the VA10 strain. To strengthen the carotenoid pathway, the geranylgeranyl diphosphate synthase gene from *Haematococcus pluvialis* (*HpGGPPs*, Genbank: KP759940.1) under the control of the *TEF1* promoter and the *CYC1* terminator was codon-optimized and synthesized (Tsingke, Beijing, China). The

gene for the bifunctional enzyme phytoene synthase/lycopene cyclase (*CarRP*; GenBank: AJ250827.1), under the control of the *GPD1* promoter and the *XPR2* terminator was amplified from the p-car-M3 plasmid.[1] 113-LEU-HpGGPPs-CarRP was transformed into the VA10 strain to obtain the VA11 strain.

To improve the production of retinyl esters, the endogenous diacylglycerol acyltransferase 1/2 genes (*DGA1*, Genbank: YALI0_E32769g; *DGA2*, Genbank: YALI0_D07986g) were amplified from the *Y. lipolytica* genome. Lecithin retinol acyltransferase genes derived from *Homo sapiens* (*HsLRAT*, Genbank: AF071510) and *Rattus norvegicus* (*RnLRAT*, Genbank: AF255060) and retinol-binding protein 1 gene derived from *Homo sapiens* (*RBP1*, Genbank: M11433) were codon-optimized and synthesized (Tsingke, Beijing, China). These genes were constructed into the plasmid pKi1-HYG under the control of the *ut8* promoter and the *CYC1* terminator. HsLRAT was fused with RBP1 via a GGGGS amino acid linker and ligated into the plasmid pKi1-HYG to obtain the plasmid pKi1-HYG-HsLRATRBP1. These plasmids were transformed into the VA11 strain to obtain the VA12 to VA17 strain.

A2 Shake-flask cultivations details

For shake-flask cultivations, single colonies were inoculated in 24-well plates containing 1.5 mL YPD medium. Next, 1 mL of the culture was inoculated in a 300-mL shake flask containing 50 mL medium and cultured for 96 h at 30 °C and 200 rpm. 10% dodecane was added to collect retinoids. YP (20 g/L tryptone, 10 g/L yeast extract) was used as the basic component of the fermentation medium, and different carbon sources, including glucose, oleic acid, palmitic acid, palm oil, peanut oil and olive oil (50 g/L), were added to YP when necessary. Tween 80 (0.1%) was added to the medium as an emulsifier when lipid carbon sources were used. For retinol fermentation, different fermentation conditions were optimized in shake flasks, including the addition of different metal ions, antioxidants and cultivation temperatures. Metal ions, including Zn^{2+} and Fe^{2+}, were added to the medium in the form of sulfates with final concentration of 1.5, 3 and 6 mM; the doses of the antioxidant butylated hydroxytoluene (BHT) tested were 0.1%, 0.5%, 1.0% and 2.0%; and the cultivation temperatures tested were 26 °C, 28 °C, 30 °C and 32 °C. For retinyl ester fermentation, different fermentation conditions were optimized in shake flasks, including the proportion of dodecane and cultivation temperatures. The proportions of dodecane tested were 10%, 20% and 30%; the cultivation temperatures tested were 26 °C, 28 °C, 30 °C and 32 °C for 96 h, as well as 30 °C for 48 h and 35 °C for another 48 h.

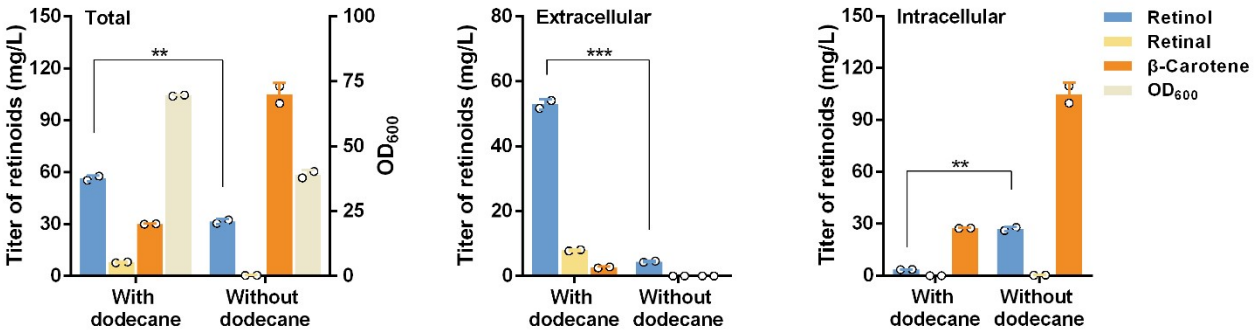

Fig. S1 The effect of adding dodecane on the production of retinoids and OD_{600}. Data represent the mean ± SD of two biological replicates. Statistical analysis was performed by using Student's *t*-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

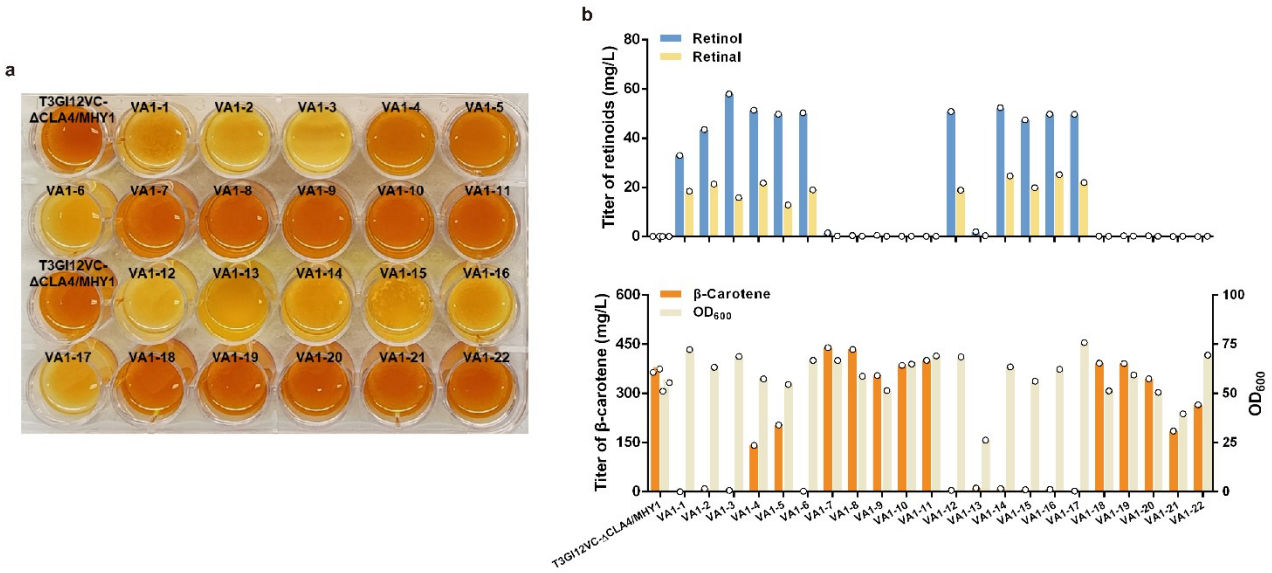

Fig. S2 Selection of transformants transformed *BLH* and *RDH12* via NHEJ in T3GI12VC-ΔCLA4/MHY1. (a) Appearance of the 24-well plate fermentation of different transformants. (b) The retinoid production and OD_{600} of different transformants. Data of control represent the mean ± SD of two biological replicates.

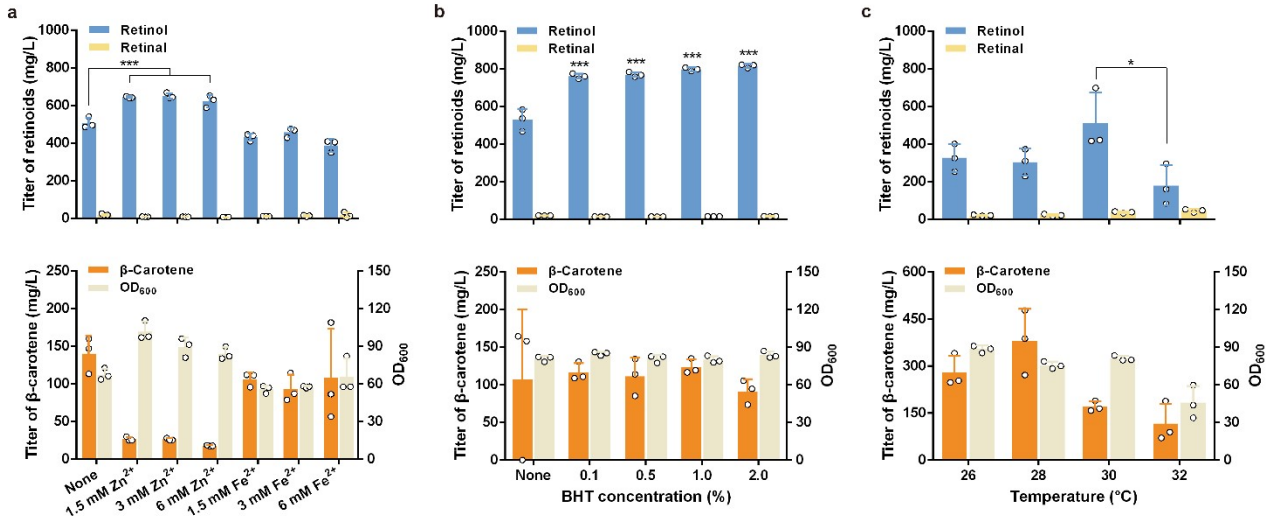

Fig. S3 Optimization of the cultivation conditions for retinol production in VA11 using glucose as the carbon source in shake flasks. (a) The retinoid production and OD_{600} with adding different concentrations of $Zn^{2+}$and Fe^{2+}. (b) The retinoid production and OD_{600} with adding different concentrations of BHT. (c) The retinoid production and OD_{600} under different culture temperatures. Data represent the mean ± SD of three biological replicates. Statistical analysis was performed by using one-way ANOVA (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

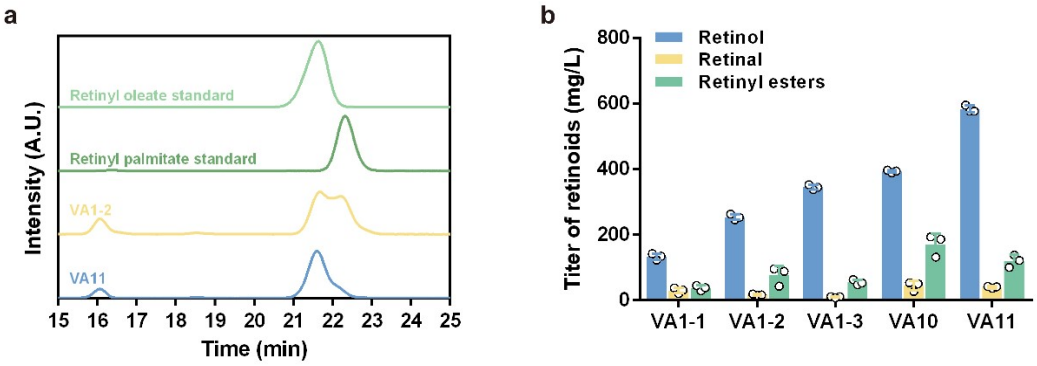

Fig. S4 Accumulation of retinyl esters in retinol-producing strains. (a) HPLC analysis of retinyl ester standards and the fermented product of VA1-2 and VA11 strains. (b) The retinoid production of retinol-producing strains. Data represent the mean ± SD of three biological replicates.

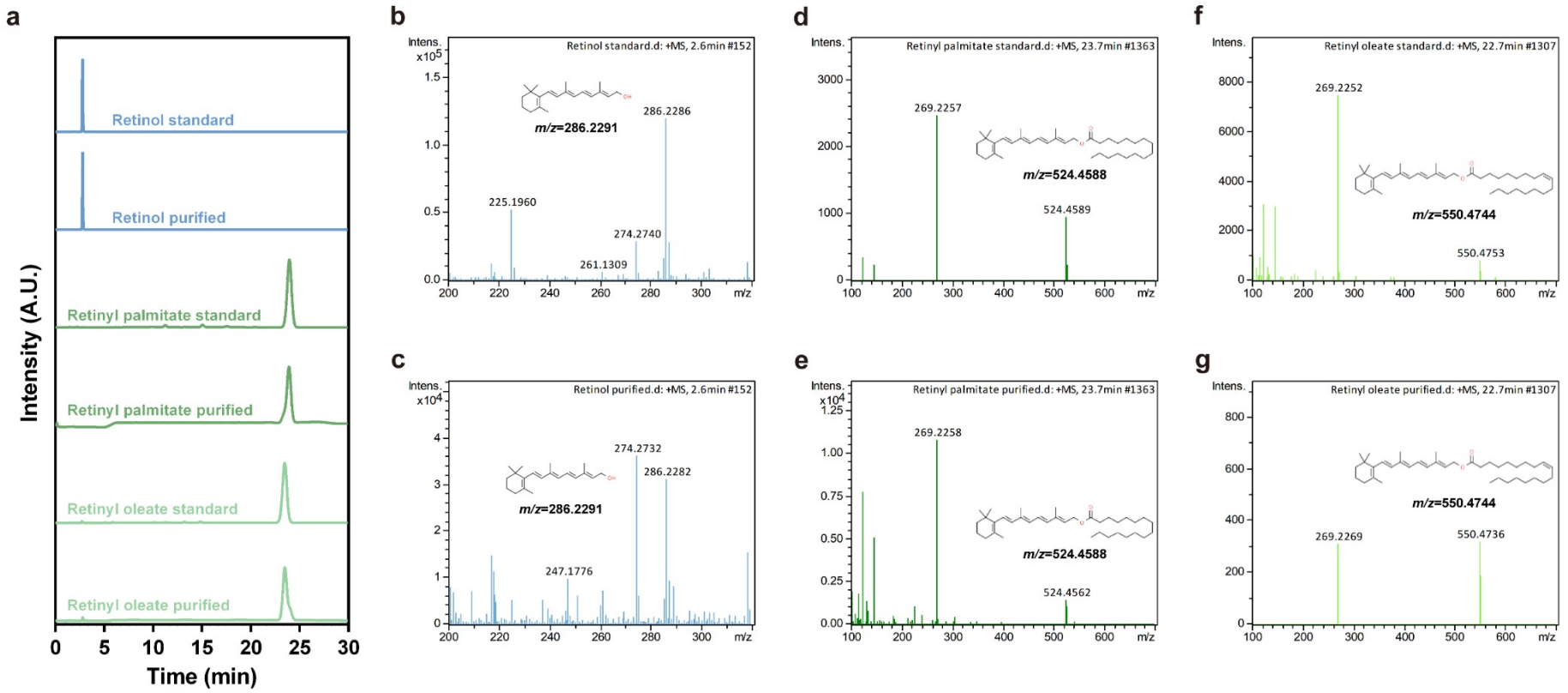

Fig. S5 The LC-MS spectra of standard and purified retinoids. (a) HPLC spectra of standard and purified retinoids. (b) MS spectra of retinol standard. (c) MS spectra of purified retinol. (d) MS spectra of retinyl palmitate standard. (e) MS spectra of purified retinyl palmitate. (f) MS spectra of retinyl oleate standard. (g) MS spectra of purified retinyl oleate.

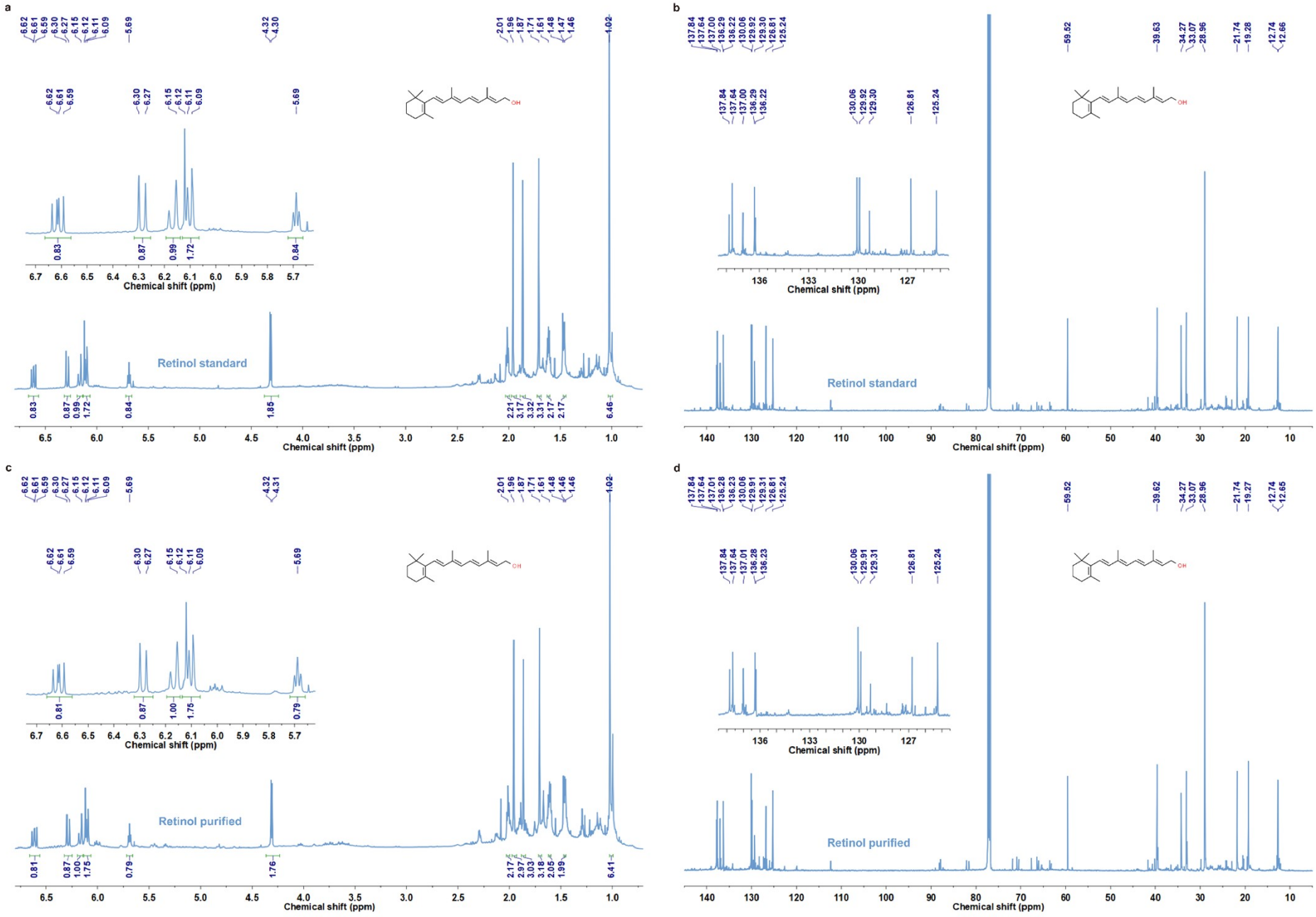
a
Retinol standard
Chemical shift (ppm)
b
Retinol standard
Chemical shift (ppm)
c
Retinol purified
Chemical shift (ppm)
d
Retinol purified
Chemical shift (ppm)

Fig. S6 The NMR spectra of standard and purified retinol. (a) The ^{1}H NMR spectra of retinol standard. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 – 6.54 (m, 1H), 6.29 (d, J = 15.2 Hz, 1H), 6.15 (s, 1H), 6.13 – 6.04 (m, 2H), 5.69 (s, 1H), 4.31 (d, J = 7.0 Hz, 2H), 2.01 (s, 2H), 1.96 (s, 3H), 1.87 (s, 3H), 1.71 (s, 3H), 1.61 (s, 2H), 1.46 (d, J = 5.8 Hz, 2H), 1.02 (s, 6H). (b) The ^{13}C NMR spectra of retinol standard. ^{13}C NMR (400 MHz, $CDCl_3$) δ 137.84 (s), 137.64 (s), 137.00 (s), 136.29 (s), 136.22 (s), 130.06 (s), 129.92 (s), 129.30 (s), 126.81 (s), 125.24 (s), 59.52 (s), 39.63 (s), 34.27 (s), 33.07 (s), 28.96 (s), 21.74 (s), 19.28 (s), 12.74 (s), 12.66 (s). (c) The ^{1}H NMR spectra of purified retinol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 – 6.56 (m, 1H), 6.29 (d, J = 15.2 Hz, 1H), 6.15 (s, 1H), 6.13 – 6.05 (m, 2H), 5.69 (s, 1H), 4.31 (d, J = 7.0 Hz, 2H), 2.01 (s, 2H), 1.96 (s, 3H), 1.87 (s, 3H), 1.71 (s, 3H), 1.61 (s, 2H), 1.48 – 1.45 (m, 2H), 1.02 (s, 6H). (d) The ^{13}C NMR spectra of purified retinol. ^{13}C NMR (400 MHz, $CDCl_3$) δ 137.84 (s), 137.64 (s), 137.01 (s), 136.28 (s), 136.23 (s), 130.06 (s), 129.91 (s), 129.31 (s), 126.81 (s), 125.24 (s), 59.52 (s), 39.62 (s), 34.27 (s), 33.07 (s), 28.96 (s), 21.74 (s), 19.27 (s), 12.74 (s), 12.65 (s).

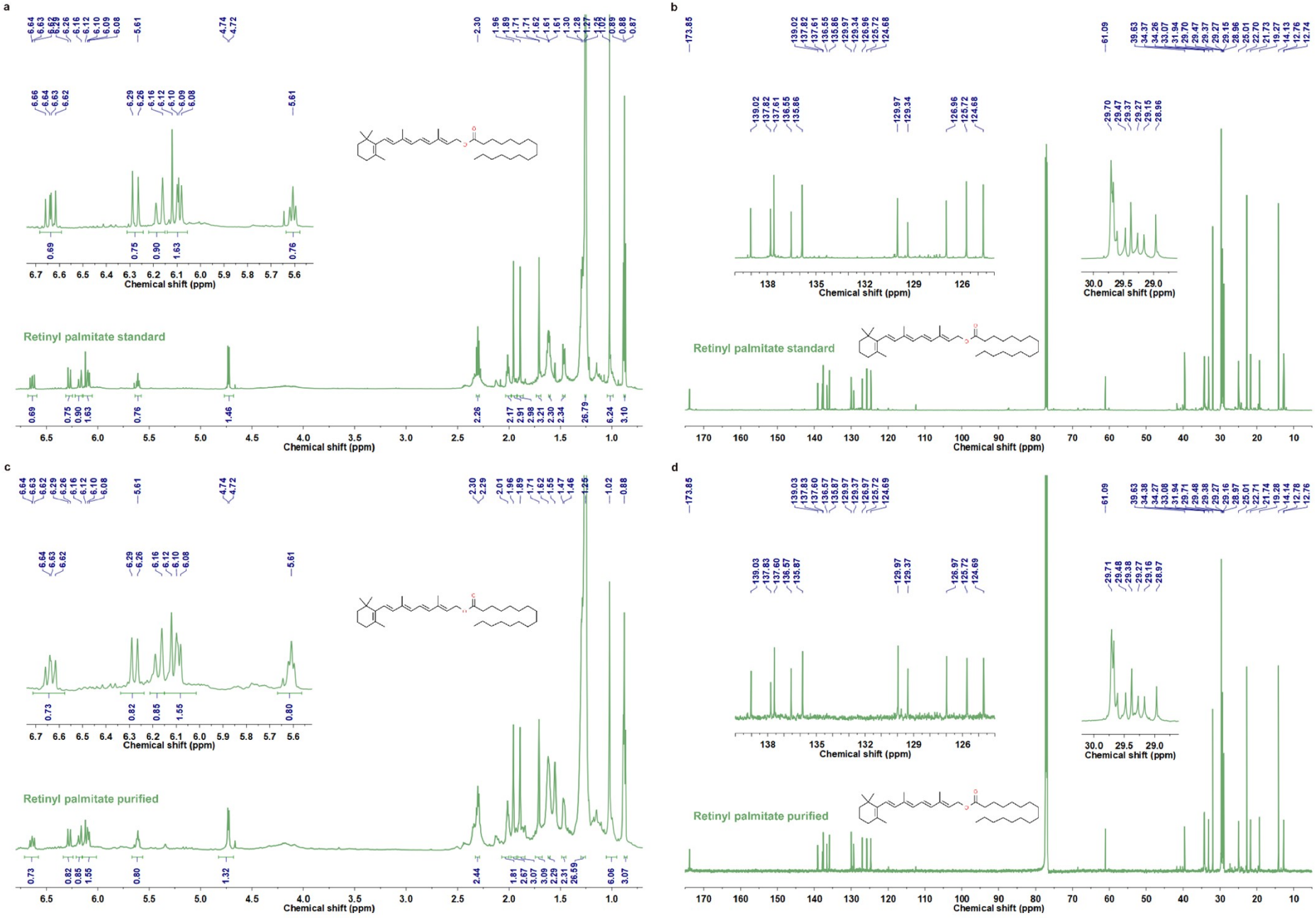
a
b
c
d
Retinyl palmitate standard
Retinyl palmitate purified
Chemical shift (ppm)

Fig. S7 The NMR spectra of standard and purified retinyl palmitate. (a) The ^{1}H NMR spectra of retinyl palmitate standard. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (dd, J = 15.1, 11.3 Hz, 1H), 6.28 (d, J = 15.2 Hz, 1H), 6.16 (s, 1H), 6.10 (dd, J = 13.6, 10.0 Hz, 2H), 5.61 (s, 1H), 4.73 (d, J = 7.2 Hz, 2H), 2.30 (s, 2H), 2.01 (s, 2H), 1.96 (s, 3H), 1.89 (s, 3H), 1.73 – 1.69 (m, 3H), 1.61 (d, J = 3.4 Hz, 2H), 1.48 – 1.45 (m, 2H), 1.25 (s, 26H), 1.02 (s, 6H), 0.88 (s, 3H). (b) The ^{13}C NMR spectra of retinyl palmitate standard. ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.85 (s), 139.02 (s), 137.82 (s), 137.61 (s), 136.55 (s), 135.86 (s), 129.97 (s), 129.34 (s), 126.96 (s), 125.72 (s), 124.68 (s), 61.09 (s), 39.63 (s), 34.37 (s), 34.26 (s), 33.07 (s), 31.94 (s), 29.70 (s), 29.47 (s), 29.37 (s), 29.27 (s), 29.15 (s), 28.96 (s), 25.01 (s), 22.70 (s), 21.73 (s), 19.27 (s), 14.13 (s), 12.75 (d, J = 3.2 Hz). (c) The ^{1}H NMR spectra of purified retinyl palmitate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 – 6.58 (m, 1H), 6.28 (d, J = 15.2 Hz, 1H), 6.16 (s, 1H), 6.15 – 6.01 (m, 2H), 5.61 (s, 1H), 4.73 (d, J = 7.2 Hz, 2H), 2.30 (d, J = 7.1 Hz, 2H), 2.01 (s, 2H), 1.96 (s, 3H), 1.89 (s, 3H), 1.71 (s, 3H), 1.62 (s, 2H), 1.47 (d, J = 5.5 Hz, 2H), 1.30 – 1.25 (m, 26H), 1.02 (s, 6H), 0.88 (s, 3H). (d) The ^{13}C NMR spectra of purified retinyl palmitate. ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.85 (s), 139.03 (s), 137.83 (s), 137.60 (s), 136.57 (s), 135.87 (s), 129.97 (s), 129.37 (s), 126.97 (s), 125.72 (s), 124.69 (s), 61.09 (s), 39.63 (s), 34.38 (s), 34.27 (s), 33.08 (s), 31.94 (s), 29.71 (s), 29.48 (s), 29.38 (s), 29.27 (s), 29.16 (s), 28.97 (s), 25.01 (s), 22.71 (s), 21.74 (s), 19.28 (s), 14.14 (s), 12.77 (d, J = 3.1 Hz).

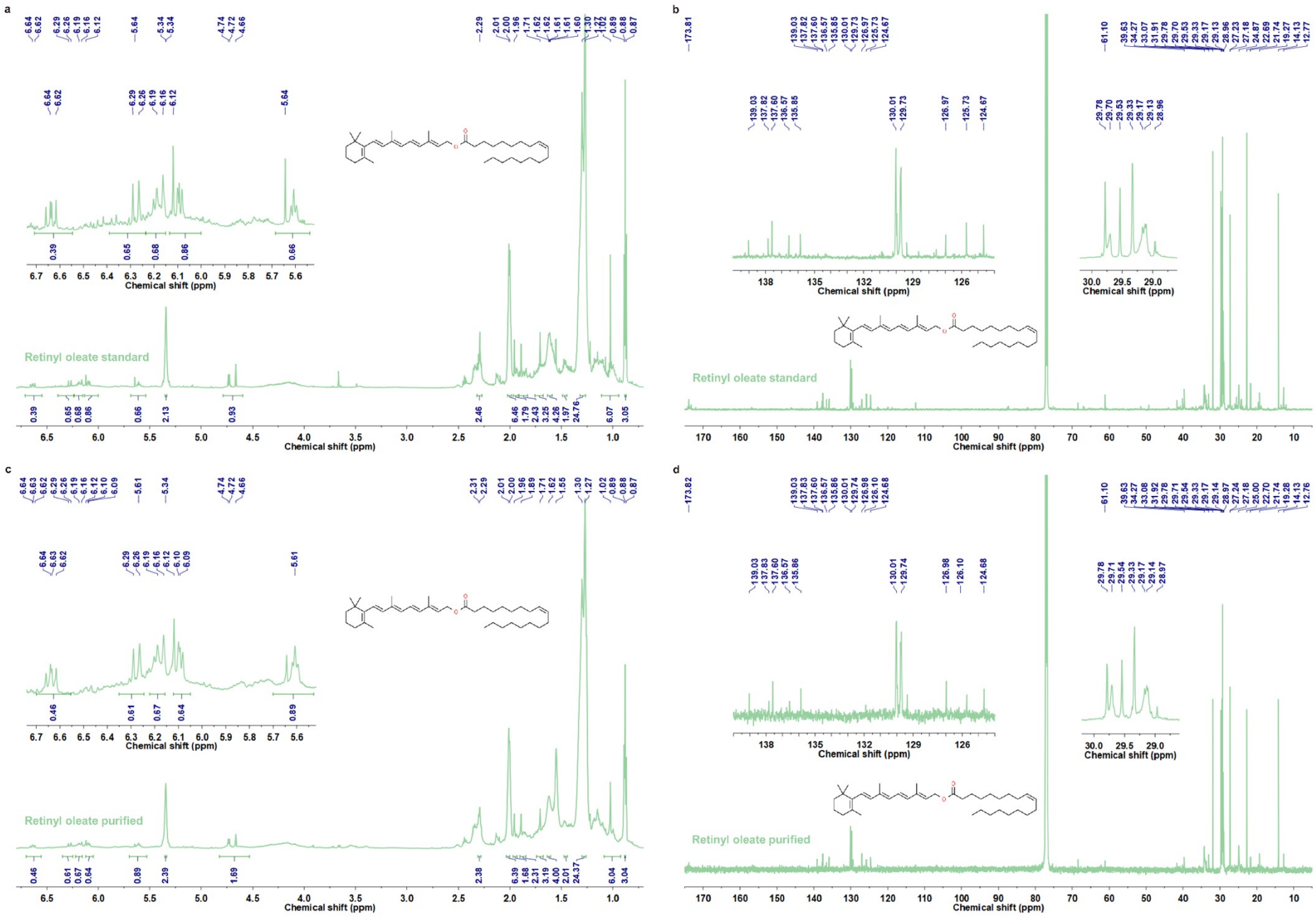
a
Retinyl oleate standard
Chemical shift (ppm)
b
Retinyl oleate standard
Chemical shift (ppm)
c
Retinyl oleate purified
Chemical shift (ppm)
d
Retinyl oleate purified
Chemical shift (ppm)

Fig. S8 The NMR spectra of standard and purified retinyl oleate. (a) The ^{1}H NMR spectra of retinyl oleate standard. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.63 (d, J = 15.1 Hz, 1H), 6.28 (d, J = 15.3 Hz, 1H), 6.18 (d, J = 16.1 Hz, 1H), 6.12 (s, 1H), 5.64 (s, 1H), 5.34 (d, J = 1.8 Hz, 2H), 4.79 – 4.60 (m, 1H), 2.29 (s, 2H), 2.01 (d, J = 6.1 Hz, 6H), 1.96 (s, 2H), 1.89 (s, 2H), 1.71 (s, 3H), 1.63 – 1.59 (m, 4H), 1.49 – 1.44 (m, 2H), 1.28 (d, J = 16.9 Hz, 24H), 1.02 (s, 6H), 0.87 (d, J = 7.1 Hz, 3H). (b) The ^{13}C NMR spectra of retinyl oleate standard. ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.81 (s), 139.03 (s), 137.82 (s), 137.60 (s), 136.57 (s), 135.85 (s), 130.01 (s), 129.73 (s), 126.97 (s), 125.73 (s), 124.67 (s), 61.10 (s), 39.63 (s), 34.27 (s), 33.07 (s), 31.91 (s), 29.78 (s), 29.70 (s), 29.53 (s), 29.33 (s), 29.13 (s), 28.96 (s), 27.23 (s), 24.96 (d, J = 9.4 Hz), 22.69 (s), 21.74 (s), 19.27 (s), 14.13 (s), 12.77 (s). (c) The ^{1}H NMR spectra of purified retinyl oleate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 – 6.55 (m, 1H), 6.28 (d, J = 15.3 Hz, 1H), 6.18 (d, J = 15.8 Hz, 1H), 6.12 – 6.05 (m, 1H), 5.61 (s, 1H), 5.34 (s, 2H), 4.82 – 4.53 (m, 2H), 2.30 (d, J = 7.0 Hz, 2H), 2.01 (d, J = 5.6 Hz, 6H), 1.96 (s, 2H), 1.89 (s, 2H), 1.71 (s, 3H), 1.62 (s, 4H), 1.46 (d, J = 6.7 Hz, 2H), 1.28 (d, J = 17.1 Hz, 24H), 1.02 (s, 6H), 0.88 (s, 3H). (d) The ^{13}C NMR spectra of purified retinyl oleate. ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.82 (s), 139.03 (s), 137.83 (s), 137.60 (s), 136.57 (s), 135.86 (s), 130.01 (s), 129.74 (s), 126.98 (s), 126.29 – 125.19 (m), 124.68 (s), 61.10 (s), 39.63 (s), 34.27 (s), 33.08 (s), 31.92 (s), 29.78 (s), 29.71 (s), 29.54 (s), 29.33 (s), 29.14 (s), 28.97 (s), 27.24 (s), 24.89 – 24.68 (m), 22.70 (s), 21.74 (s), 19.28 (s), 14.13 (s), 12.76 (s).

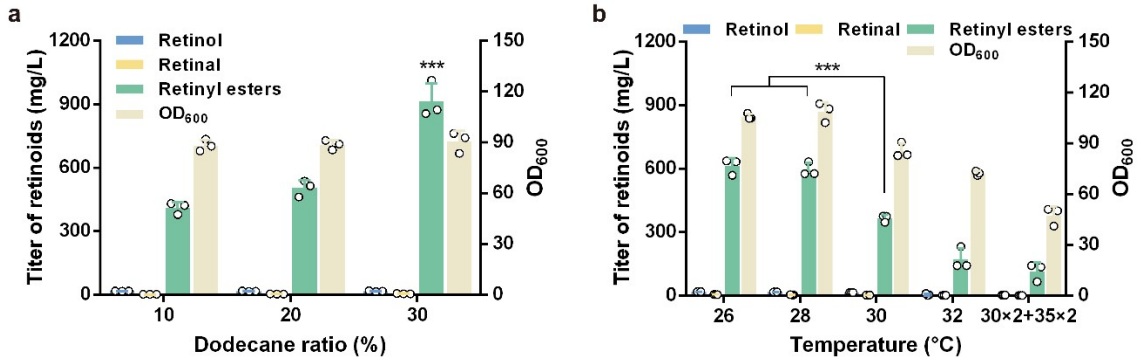

Fig. S9 Optimization of the cultivation conditions for retinyl ester production in VA11 using palmitic acid as the carbon source in shake flasks. (a) The retinoid production and OD_{600} with adding different ratios of dodecane. (b) The retinoid production and OD_{600} under different culture temperatures. Data represent the mean ± SD of three biological replicates. Statistical analysis was performed by using one-way ANOVA (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

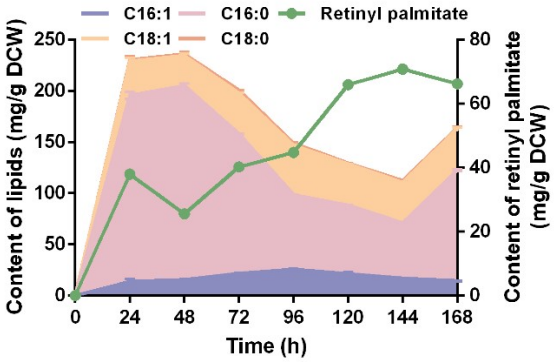

Fig. S10 The content of intracellular lipids and retinyl palmitate of fed-batch cultivation for retinyl palmitate production. Data represent the mean ± SD of two biological replicates.

Table S1 Characteristics of retinoids in different strains of the main reports.

Main product	Organism	Metabolic engineering strategies	Titer (g/L)	Content (mg/g DCW)	Composition of retinoids	Reference
Retinal	*Saccharomyces cerevisiae*	Inactivated alcohol dehydrogenases (Adh6, Adh7, Sfa1 and Gre2) and aldehyde dehydrogenase (Hfd1), and optimized β-carotene synthesis.	0.07	-	0.07 g/L retinal	[3]
Retinoic acid	*Saccharomyces cerevisiae*	Mined endogenous aldehyde dehydrogenase (Hfd1) and overexpressed in multiple copies.	0.65	10	0.55 g/L retinoic acid, 0.1 g/L retinol and retinal	[4]
Retinol	*Saccharomyces cerevisiae*	Identification and combinational expression of two isoenzymes Mbblh and Ssbco, multi-copy integration and engineering precursor supply.	5.21	-	4.12 g/L retinol and 1.09 g/L retinal	[5]
	Yarrowia lipolytica	Multi-copy integration of *Mb.blh*, optimized metabolic pathway, and increased retinol stability using antioxidant and detergent extraction.	5.12	42	4.86 g/L retinol and 0.26 g/L retinal	[6]
	Yarrowia lipolytica	Improved the metabolic flux of β-carotene, and optimized the expression level of *Mb.blh* and *RDH12* and the fermentation process.	5.4	-	5.4 g/L retinol	[7]
	Yarrowia lipolytica	Screened BCOs and RDHs, adjusted copy numbers of the key genes, strengthened metabolic flux of the precursor, and optimized the cultivation conditions.	6.69	66	5.89 g/L retinol, 0.08 g/L retinal and 0.72 g/L retinyl esters	This study
Retinyl esters	*Escherichia coli*	Expressing of *CrtEBIY*, *BLH*, *LRAT* and *CRBP*, reconstructed heterologous retinyl palmitate biosynthetic pathway.	0.08	-	0.07 g/L retinyl palmitate, 0.01 g/L retinyl acetate, retinol and retinal	[8]
	Yarrowia	Changed the cultivation conditions from nitrogen-	9.11	109	8.18 g/L retinyl esters, 0.9 g/L	This study

	lipolytica	rich to nitrogen-limited condition using glucose as the carbon source.			retinol and 0.03 g/L retinal	
	Yarrowia lipolytica	Adjusted carbon source and introduced retinol-binding protein.	4.68	68	4.53 g/L retinyl palmitate and 0.15 g/L retinol	This study

Table S2 The yeast strains used in this study.

Name	Description	Reference
T1	Heterologous expression *CarB* and *CarRP* genes in Po1f at *TRP1* site	[2]
T1GI12VC	T1 with integrated linearized plasmids JMP-HYG-GPS-IDI1-ERG12, 114-VHb-ERG8-ERG19 and 113-URA3-CarRP-GGS1 then removed *URA3*, *LEU2* and *HYG* markers using the Cre-loxP system	[2]
T3	Po1f with integrated linearized plasmids p-car-M1, p-car-M2 and p-car-M3 then removed *URA3*, *LEU2* and *TRP1* markers using the Cre-loxP system	[1]
T3GI12VC-ΔCLA4/MHY1	T3 with integrated linearized plasmids JMP-HYG-GPS-IDI1-ERG12, 114-VHb-ERG8-ERG19 and 113-URA3-CarRP-GGS1 then knocked out *CLA4* and *MHY1* genes	[2]
VA1	T3 with integrated linearized plasmid pKi1-TRP-BLH-RDH12	This stduy
VA2	T3 with integrated linearized plasmid pKi1-TRP-DrBCO1-RDH12	This study
VA3	T3 with integrated linearized plasmid pKi1-TRP-GgBCO1-RDH12	This study
VA4	T3 with integrated linearized plasmid pKi1-TRP-HsBCO1-RDH12	This study
VA5	T3 with integrated linearized plasmid pKi1-TRP-RnBCO1-RDH12	This study
VA6	T3 with integrated linearized plasmid pKi1-TRP-BLH-YBBO	This study
VA7	T3 with integrated linearized plasmid pKi1-TRP-BLH-ADH1B	This study
VA8	T3 with integrated linearized plasmid pKi1-TRP-BLH-DHRS3	This study
VA9	T3 with integrated linearized plasmid pKi1-TRP-BLH-RDH8	This study
VAT1	T1 with integrated linearized plasmid pKi1-TRP-BLH-RDH12	This stduy
VAT1GI12VC	T1GI12VC with integrated linearized plasmid pKi1-TRP-BLH-RDH12	This study
VAT3	T3 with integrated linearized plasmid pKi1-TRP-BLH-RDH12	This study
VAT3GI12VC-ΔCLA4/MHY1	T3GI12VC-ΔCLA4/MHY1 with integrated linearized plasmid pKi1-TRP-BLH-RDH12	This study
VA1-1	T3GI12VC-ΔCLA4/MHY1 with integrated linearized plasmid pKi1-TRP-BLH-RDH12, transformant 1	This study
VA1-2	T3GI12VC-ΔCLA4/MHY1 with integrated linearized plasmid pKi1-TRP-BLH-RDH12, transformant 2	This study
VA1-3	T3GI12VC-ΔCLA4/MHY1 with integrated linearized plasmid pKi1-TRP-BLH-RDH12, transformant 3	This study
VA10	VA1-3 with integrated linearized plasmid p-car-M1	This study
VA11	VA10 with integrated linearized plasmid 113-LEU-HpGGPPs-CarRP	This study
VA12	VA11 with integrated linearized plasmid pKi1-HYG-DGA1	This study

VA13	VA11 with integrated linearized plasmid pKi1-HYG-DGA2	This study
VA14	VA11 with integrated linearized plasmid pKi1-HYG-HsLRAT	This study
VA15	VA11 with integrated linearized plasmid pKi1-HYG-RBP1	This study
VA16	VA11 with integrated linearized plasmid pKi1-HYG-HsLRATRBP1	This study
VA17	VA11 with integrated linearized plasmid pKi1-HYG-RnLRAT	This study

Table S3 The primers used in this study.

Name	Sequence (5'-3')	Description
Trp-F	ATGGACTTTCTCTACTCTTCGACATGT CTAC	To amplified *TRP1* from *Y. lipolytica* genome
Trp-R	TTACCCCCTGGCGTTTTTGACAAACAG C	To amplified *TRP1* from *Y. lipolytica* genome
pKi1-Trp-F	GTTTGTCAAAAACGCCAGGGGGTAAG TCGTTTCTACGACGCATTGATG	To amplified vector of pKi1-TRP
pKi1-Trp-R	ATGTCGAAGAGTAGAGAAAGTCCATT GTGGATGTGTGTGGTTGTATG	To amplified vector of pKi1-TRP
HYG-F	ATGAAAAAGCCTGAACTCACCGC	To amplified *HYG* from *Y. lipolytica* genome
HYG-R	CTATTCCTTTGCCCTCGGACG	To amplified *HYG* from *Y. lipolytica* genome
pKi1-HYG-F	CACTCGTCCGAGGGCAAAGGAATAGG TCGTTTCTACGACGCATTGATG	To amplified vector of pKi1-HYG
pKi1-HYG-R	TCGCGGTGAGTTCAGGCTTTTTCATTG TGGATGTGTGTGGTTGTATG	To amplified vector of pKi1-HYG
LEU-113-F	TTTCAGTCTCCTCTTCACCACCAAAAT GCAGATCTTTGTTAAGACTTTGACCG	To amplified *LEU2* from *Y. lipolytica* genome
LEU-113-R	AAATTACATATCCATAGTCTAACCTTT ACTCCTTCTTGAGCAGCTCCTTG	To amplified *LEU2* from *Y. lipolytica* genome
113-F	AGGTTAGACTATGGATATGTAATTTAA CT	To amplified vector of 113-LEU-GPD-TEF
113-R	TTTGGTGGTGAAGAGGAGACTG	To amplified vector of 113-LEU-GPD-TEF
UT8-pKi1-F	TCTAGAACTAGTGGATCCTAGTCATAT GGGTACCAAGGAAGCATGCGGT	To amplified BCOs and RDHs cassettes from synthesized plasmids for construction of pKi1-TRP-BCOs-RDHs
CYC1-pKi1-R	ACCGCATGCTTCCTTGGTACCTTCACG CGCAAATTAAAGCCTTCGAG	To amplified BCOs and RDHs cassettes from synthesized plasmids for construction of pKi1-TRP-BCOs-RDHs
HpGGPPs-TEF-F	ACTTTTTGCAGTACTAACCGCAGATTT ATGATCCGAGCGATGCACAAC	To amplified *HpGGPPs* from synthesized plasmids
HpGGPPs-TEF-R	TGACATAACTAATTACATGAATTTTCA GTTCTTCCGGTAGCCAATCAG	To amplified *HpGGPPs* from synthesized plasmids
CarRP-GPD-F	ATTAAACACACATCAACAGATGCTGCT GACCTACATGGA	To amplified *CarRP* from p-car-M3
CarRP-GPD-R	GACAGGCCATGGAGGTACGTTAGATG GTGTTCAGGTTTC	To amplified *CarRP* from p-car-M3
DGA1-ut8-F	TTCTGAGTATAAGAATCATTCAAAGAT GACTATCGACTCACAATACTACAAG	To amplified *DGA1* from *Y. lipolytica* genome
DGA1-CYC1-R	GACATAACTAATTACATGAATCGATTT ACTCAATCATTCGGAACTCTGG	To amplified *DGA1* from *Y. lipolytica* genome

DGA2-ut8-F	GAGTATAAGAATCATTCAAAGATTTATGGAAGTCCGACGACGAAAAATCG	To amplified *DGA2* from *Y. lipolytica* genome
DGA2-CYC1-R	GTGACATAACTAATTACATGAATTTCTACTGGTTCTGCTTGTAGTTGTAAG	To amplified *DGA2* from *Y. lipolytica* genome
HsLRAT-ut8-F	TTCTGAGTATAAGAATCATTCAAAGATGAAGAACCCTATGCTGGAGGTC	To amplified *HsLRAT* from synthesized plasmids for fused with *RBP1*
HsLRAT(GS)RBP1-R	GGCATGGAGCCACCACCGCCGCCAGCCATCCACAGAAAG	To amplified *HsLRAT* from synthesized plasmids for fused with *RBP1*
RBP1(GS)HsLRAT-F	CTGGCGGCGGTGGTGGCTCCATGCCTGTCGATTTCACCGGAT	To amplified *RBP1* from synthesized plasmids for fused with *HsLRAT*
RBP1-CYC1-R	GACATAACTAATTACATGAATCGATTTACTGGACCTTCTTGAACACCTGC	To amplified *RBP1* from synthesized plasmids for fused with *HsLRAT*
RT-GAPDH-F	CGGACGAATCGGACGAATTGTGA	The reference gene for detection of the copy number
RT-GAPDH-R	CAGCGTACTCGGTGTCGATGAAG	The reference gene for detection of the copy number
RT-BLH-F	CATCGCCTTCGAGCTCATCG	To detected the copy number and relative expression of *BLH*
RT-BLH-R	CCTCGGAAGCGATCATCTTGG	To detected the copy number of *BLH*
RT-BLH-R1	ACGAAGGAGAAGTGTCGTCG	To detected the relative expression of *BLH*
RT-RDH12-F	CCAACGTCCTCTTCACCCGAG	To detected the copy number and relative expression of *RDH12*
RT-RDH12-R	CGCTTGCAGTCGGAGAAGTAC	To detected the copy number of *RDH12*
RT-RDH12-R1	AGGGAGGAGTGTCGGACAAG	To detected the relative expression of *RDH12*
RT-Actin-F	CTACGAGCTTCCCGATGGCC	The reference gene for detection of the relative expression
RT-Actin-R	CGTGGATACCAGCAGCCTCA	The reference gene for detection of the relative expression
RT-DGA1-F	TCGCGGGAATCCGATATGCC	To detected the relative expression of *DGA1*
RT-DGA1-R	GTGGAATTGCGCAGCATAGC	To detected the relative expression of *DGA1*
RT-DGA2-F	TCTCGACAATCGTCGCAGCC	To detected the relative expression of *DGA2*
RT-DGA2-R	CCCGCAGGTTTCTTCTGGAC	To detected the relative expression of *DGA2*
RT-ARE1-F	CGGAGAAGAGCTCAATGTGCC	To detected the relative expression of *ARE1*
RT-ARE1-R	TTCCTTCTCGTCGTCCGAGTC	To detected the relative expression of *ARE1*
RT-LRO1-F	CAGCAGTGGAGGTGGCTGAC	To detected the relative expression of *LRO1*
RT-LRO1-R	TGAGAATCTTGCCCTTGACGG	To detected the relative expression of

		LRO1
RT-SCT1-F	CCCGAAAATCAGTGGACACGG	To detected the relative expression of *SCT1*
RT-SCT1-R	CACGTGGCCGGATTTCTCTG	To detected the relative expression of *SCT1*
RT-GPAT-like-F	AGGGTCTCGCTGAGGAATCC	To detected the relative expression of *GPAT-like*
RT-GPAT-like-R	TCGAGCATGTCGGCCTAGATC	To detected the relative expression of *GPAT-like*
RT-LAC1-F	TCACCGAGTTTGCCACCGTC	To detected the relative expression of *LAC1*
RT-LAC1-R	CTGCAGAGCCGCCAGAAGAC	To detected the relative expression of *LAC1*
RT-LAG1-F	GCGCGATGAAGATGGCCAGG	To detected the relative expression of *LAG1*
RT-LAG1-R	CTCGCACAATCATGTACAGCC	To detected the relative expression of *LAG1*

Table S4 The plasmids used in this study.

Name	Description	Reference
pKi-1	*Y. lipolytica* integrative vector, *ut8* promoter with *CYC1* terminator, *LEU2* selection marker	[1]
pKi1-TRP	*Y. lipolytica* integrative vector, *ut8* promoter with *CYC1* terminator, *TRP1* selection marker	This stduy
pKi1-HYG	*Y. lipolytica* integrative vector, *ut8* promoter with *CYC1* terminator, *HYG* selection marker	This stduy
113-GPD-TEF	*Y. lipolytica* integrative vector, *GPD* promoter with *XPR2* terminator, *TEF* promoter with *CYC1* terminator, *URA3* selection marker	[1]
113-LEU-GPD-TEF	*Y. lipolytica* integrative vector, *GPD* promoter with *XPR2* terminator, *TEF* promoter with *CYC1* terminator, *LEU2* selection marker	This stduy
pKi1-TRP-BLH-RDH12	pKi1-TRP vector containing *ut8p-BLH-CYC1t* and *ut8p-RDH12-CYC1t*	This stduy
pKi1-TRP-DrBCO1-RDH12	pKi1-TRP vector containing *ut8p-DrBCO1-CYC1t* and *ut8p-RDH12-CYC1t*	This study
pKi1-TRP-GgBCO1-RDH12	pKi1-TRP vector containing *ut8p-GgBCO1-CYC1t* and *ut8p-RDH12-CYC1t*	This study
pKi1-TRP-HsBCO1-RDH12	pKi1-TRP vector containing *ut8p-HsBCO1-CYC1t* and *ut8p-RDH12-CYC1t*	This study
pKi1-TRP-RnBCO1-RDH12	pKi1-TRP vector containing *ut8p-RnBCO1-CYC1t* and *ut8p-RDH12-CYC1t*	This study
pKi1-TRP-BLH-YBBO	pKi1-TRP vector containing *ut8p-BLH-CYC1t* and *ut8p-YBBO-CYC1t*	This stduy
pKi1-TRP-BLH-ADH1B	pKi1-TRP vector containing *ut8p-BLH-CYC1t* and *ut8p-ADH1B-CYC1t*	This study
pKi1-TRP-BLH-DHRS3	pKi1-TRP vector containing *ut8p-BLH-CYC1t* and *ut8p-DHRS3-CYC1t*	This study
pKi1-TRP-BLH-RDH8	pKi1-TRP vector containing *ut8p-BLH-CYC1t* and *ut8p-RDH8-CYC1t*	This study
YLEP-BleoR-Cre	Episomal overexpression for Cre recombinase	Lab stock
p-car-M1	Integrative vector containing the Module 1 of β-carotene biosynthetic pathway	[1]
113-LEU-HpGGPPs-	113-LEU-GPD-TEF vector containing *TEFp-HpGGPPs-CYC1t* and	This study

CarRP	*GPDp*-CarRP-*XPR2t*	
pKi1-HYG-DGA1	pKi1-HYG vector containing *ut8p-DGA1-CYC1t*	This study
pKi1-HYG-DGA2	pKi1-HYG vector containing *ut8p-DGA2-CYC1t*	This study
pKi1-HYG-HsLRAT	pKi1-HYG vector containing *ut8p-HsLRAT-CYC1t*	This study
pKi1-HYG-RBP1	pKi1-HYG vector containing *ut8p-RBP1-CYC1t*	This study
pKi1-HYG-HsLRATRBP1	pKi1-HYG vector containing *ut8p-HsLRATRBP1-CYC1t* (fusion *HsLRAT* and *RBP1* with GGGGS linker)	This study
pKi1-HYG-RnLRAT	pKi1-HYG vector containing *ut8p-RnLRAT-CYC1t*	This study

Table S5 The bioproduction cost evaluation of retinoids.

Product	**Titer (mg/L)**	**Yield (g/g carbon source)**	**Feedstock price (dollar/ton)**	**Estimated feedstock cost (dollar/kg)**	**Market price (dollar/kg)**
Retinol	5.89	0.013	478	37	13-38
Retinyl oleate	8.18	0.015	478	32	-
Retinyl palmitate	4.53	0.018	1011	56	17-45

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