Supporting Information

Engineering a balanced acetyl-CoA metabolism in *Saccharomyces cerevisiae* for lycopene production through rational and evolutionary engineering

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Table S1 Strains used in this study except in Table 1.

Strain/plasmid	Description	Source
Strains		
DH5α	*supE44 ΔlacU169 (φ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Lab stock
BL03-E-1	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE*	This study
BL03-E-2	BL03-D-4, *Δ911b* □ P_{EFT1}-*AtoB*	This study
BL03-E-3	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE, Δ911b* □ P_{EFT1}-*AtoB*	This study
BL03-E-5	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE*-P_{EFT1}-*Erg10*	This study
BL03-E-7	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE-SH3*-P_{EFT1}-*Erg10*	This study
BL03-E-8	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE-sumo-AtoB*	This study
BL03-E-9	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE-R-AtoB*	This study
BL03-E-11	BL03-D-4, *Δ106a* □ P_{HSP104}-*eutE-2A*	This study
BL03-G-1	BL03-E-10, *Δ911b* □ P_{SSA1}-*PDC*	This study
BL03-G-2	BL03-E-10, *Δ911b* □ P_{SSA1}-*ADH2*	This study
BL03-G-3	BL03-E-10, *ΔADH1*	This study
BL03-G-4	BL03-E-10, *Δ911b* □ P_{SSA1}-*mvaS*	This study
BL03-G-5	BL03-D-4, *Δ911b* □ $P_{Cit1\text{-}OhmgR}$	This study
BL03-G-8	BL03-E-10, *Δ911b* □ P_{SSA1}-*IDI*-P_{HSP104}-*Oerg12*	This study
W2-A-1	W2, *Δ911b* □ P_{SSA1}-*Oerg12*	This study
W2-A-2	W2, *Δ911b* □ P_{SSA1}-*IDI*	This study
W2-A-3	W2, *Δ911b* □ P_{SSA1}-*IDI*-P_{HSP104}-*Oerg12*	This study
BL03-2A-1	BL03-E-10, *ΔCHO2* □ *HIS3*	This study
BL03-D-5	BL03-D-4, *ΔCHO2* □ *HIS3*	This study
SC03-D-1	S288C, Δ*Gal80*□ P_{HSP26}-*CrtB*-T_{ADH1}-P_{HSP26}-*CrtI*-T_{GPM1}-P_{HSP26}-*CrtE*-$T_{CYC1,}$ *Δ416d*□ P_{Cit1}-*OhmgR*-T_{Guo}	This study
SC03-D-2	SC03-D-1, *ΔCHO2*	This study

Table S2. Oligonucleotides used in this study. Homology to genome or homologous overhang-nucleotides (underlined); genomic target (red, bold); short synthetic terminator (blue, italics).

Primers	Sequence (5' to 3')
ΔAld6	
gRNA-DOWN-Ald6-F	cttctccgcagtgaaagataaatgatc**gcttctttatgtaagaaggt**gttttagagc
Ald6-UP-F1211	ggacgtgtaaaaagatatgcatccagcttctatatc
Ald6-UP-R1211	gaaatgcaggttggtacataaacaagagaagtaaaagactgaacacttc
Ald6-DOWN-F1211	cttttacttctcttgttttatgtaccaacctgcatttctttccgtcatatacacaaa
Ald6-DOWN-R1211	gttcgaagaaggatgttattatatgatctctgatggc
Ald6-delete-check-F	atgatagaattggattatgtaaaaggtgaagatac
Ald6-delete-check-R	ttacaacttaattctgacagcttttacttcagt
Cit_1- tHMG1	
gRNA-DOWN-416d-F	cttctccgcagtgaaagataaatgatc**tagtgcacttaccccacgtt**gttttagagc
$Cit_1$180109-F-2	tattaaccgcttttactattatcttctacgctgacagtaa
$Cit_1$180109-F	actattatcttctacgctgacagtaactaagaaaaaaggagccatcaaaaaccatt
$Cit_1$180109-R	cttcgtaaatagtattatattgctatatgttttgcc
tHMG1180109-F	catatagcaatataatactatttacgaagatgactgcagaccaattggtgaaaac
tHMG1180109-R	*tttgaaagatactctttatttctagacagttatata*ttaggatttaatgcaggtgacgg
tHMG1180109-R-2	aatgtggtaacaaaggtgttgcctcacttgtcgctttgaaagatactctttatttcta
416-check-F	ggaaaatatacatcgcaggggttgact
416-check-R	agacctagcgataaaatcaggtttgacat
eutE-AtoB/ Erg10	
gRNA-DOWN-106a-F	cttctccgcagtgaaagataaatgatc**atacggtcagggtagcgccc**gttttagagc
106UP0621-F	attcggtcacacttttgtcgcagtgttgc
106UP0621-R	ctagcattgacacacatctcaagtcatctc
HSF04-180621F	cttgagatgtgtgtcaatgctagcgattcaaaggcgttattcagcatcat
HSF04-180621R	atattctgtatattttatggtacgtgtagttga
eutE180621-F	ctacacgtaccataaaatatacagaatatatgaatcaacaggatattgaacaggtg
eutE180621-R	*tttgaaagatactctttatttctagacagttatata*ttaacaatgcgaaacgcatcg
eutE180621-R-2	caatttgtcgacaaccgagcctttgtttgaaagatactctttatttcta
EFT_1-180621-F	tagaaataaagagtatctttcaaaagatagagtatgaaagaaagaagctgacg
EFT_1-180621-R	ttttatctgttattaaaaattcttgggtgc
AtoB20180621-F	gcacccaagaatttttaataacagataaaaatgaaaaattgtgtcatcgtcagtg
AtoB20180621-R	*tttgaaaaaatttatttctagacagttatata*ttaattcaaccgttcaatcaccatc
Erg1020180621-F	gcacccaagaatttttaataacagataaaaatgtctcagaacgtttacattgtatcga
Erg1020180621-R	gtaacagatacagacatcacacgccactgaagagaatgaaggcagccaagacat
AtoB20180621-R-2	gtaacagatacagacatcacacgccatttgaaaaaatttatttctagacagttat
106DOWN0621-F	tggcgtgtgatgtctgtatctgttactta
106DOWN0621-R	atcgttggtggtggtaccagttctgattt
eutE-106-180621-R-2	gtaacagatacagacatcacacgccatttgaaagatactctttatttcta
eutE-SH3-AtoB/ Erg10	
eutE180805-R	caggaggaggaccagaaccagaaccagaaccagaaccaacaatgcgaaacgcatcg
eutE180805-R-2	ttaacgacgacgtttaggaggcagagcaggaggaggaccagaaccaga
eutE180805-R-3	*tttgaaagatactctttatttctagacagttatata*ttaacgacgacgtttaggaggc

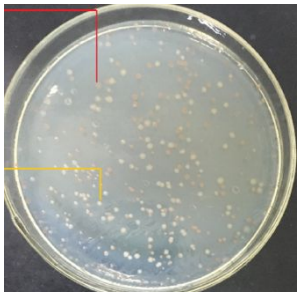
EFT_1-180805-R	aatcaaacaaagctctaacatattcagccattttatctgttattaaaaattcttgggtgc
EFT_1-180805-R-2	aaatggcaaatcttcttcatcattaccattaaaatcaaacaaagctctaacatatt
EFT_1-180805-R-3	ggtttatctctaattctcaaaatatcacctttttaaatggcaaatcttcttcatcat
EFT_1-180805-R-4	agaatcttcagcattccaccattgttcttctggttatctctaattctcaaaatatc
EFT_1-180805-R-5	atatggaactggaatcatacctcttttaccttcagaatcttcagcattccaccat
EFT_1-180805-R-6	agaaccagaaccagaaccatatttttcaacatatggaactggaatcatacctc
EFT_1-180805-R-7	accagaaccagaaccagaaccagaaccata
AtoB20180805-F	ttctggttctggttctggttctggtatgaaaaattgtgtcatcgtcagtg
Erg1020180805-F	ttctggttctggttctggttctggtatgtctcagaacgtttacattgtatcga
SUMO/2A/R	
106UP-HSF04-eutE-F	attcggtcacacttttgtcgcagtgttgc
106UP-HSF04-eutE-Rsumo	accagaaccagaaccagaaccagaaccaacaatgcgaaacgcatcgactaat
sumo-F	
sumo-R	ggttctggttctggttctggttctggttcggactcagaagtcaatcaagaagc
AtoB-106DOWN-F	atacgtagcaccaccaatctgttctc
AtoB-106DOWN-R	aacagattggtggtgctacgtataaaaattgtgtcatcgtcagtgcgg
106UP-HSF04-eutE-R-2A	atcgttggtggtggtaccagttctgattt
AtoB-106DOWN-F-2A	caatttcaacaaagaaaaattagtagcaccaacaatgcgaaacgcatcgactaat
AtoB-106DOWN-F-2A-2	gctggtgatgttgaattgaatccaggtccaaaaaattgtgtcatcgtcagtgcgg
AtoB-R-106DOWN-F	ctaatttttctttgttgaaattggctggtgatgttgaattgaatc
eutE-2A	ggttctggttctggttctggttctggtaaaaattgtgtcatcgtcagtgcgg
eutE-T_{guo}-F	
eutE-R-2A	attcggtcacacttttgtcgcagtgttgc
106DOWN-F-2A	caatttcaacaaagaaaaattagtagcaccaacaatgcgaaacgcatcgactaat
106DOWN-F-2A-2	tgttgaattgaatccaggtccataa*tatataactgtctagaaataaatttttttcaaa*
106DOWN0218-R	ctaatttttctttgttgaaattggctggtgatgttgaattgaatccaggtccatata
EFT_1-AtoB	atcgttggtggtggtaccagttctgattt
gRNA-DOWN-911b-F	cttctccgcagtgaaagataaatgatc**gtaatattgtcttgtttccc**gttttagagc
911UP0621-F	tgtccattgccggcctgcaattttcc
911UP0621-R	acttaaatgatgccgtacgtctttg
$EFT_1$18706-F	caaagacgtacggcatcatttaaagtagatagagtatgaaagaaagaagctgacg
AtoB20180706-R-2	taaagtatgtatcgggaagtctccacctttgaaaaaatttatttctagacagttat
911DOWN0621-F	ggtggagacttcccgatacatacttta
911DOWN0621-R	ttactaacattcgtagaatatttcaagcct
106-check-F	actactacatagtatatgcggcgctacc
106-check-R	acgtacatgatcttgtcgtcacaagcct
911-check-F	acgatcctgaccctgagaccttagaac
911-check-R	aattcatcctcctcgtctgcaagacaga
PDC	
SSA1-181010-F-2	actttccagaaacagatatctatattttataacaaagacgtacggcatcatttaaagt
SSA1-F0311	caaagacgtacggcatcatttaaagtcaaaggctcggttgtcgacaaattgtt
SSA1-R0311	attatctgttatttacttgaatttttgtttcttgtaatac
PDC-F0311	caaaaattcaagtaaataacagataatatgagttatactgtcggtacctatttagcg

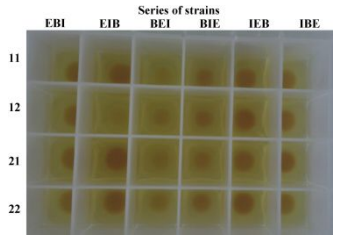
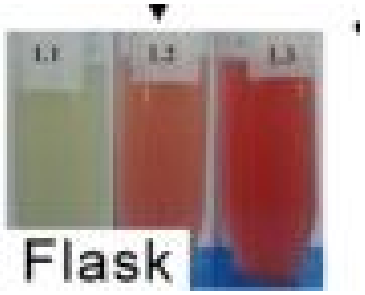
PDC-R0311	*tttgaaaaaatttatttctagacagttatata*ctagaggagcttgttaacaggctta
PDC-R0311-2	taaagtatgtatcgggaagtctccacctttgaaaaaatttatttctagacagttat
PDC20181010-R-3	cagactcagaaaattttatgcaacaacattaaagtatgtatcgggaagtctccacc
mvaS	
mvaS-F0326	caaaaattcaagtaaataacagataatatgactatcggtatcgacaagatctc
mvaS-R0326	*tttgaaaaaatttatttctagacagttatata*ttagtttctgtaagatctaacagt
Oerg12	
Oerg12-F0531	*caaaaattcaagtaaataacagataat*atggctccaggtaactctttgtct
Oerg12-R0531	tttgaaaaaatttatttctagacagttatatattaagaagctcttggaccagcc
IDI	
IDI-F0604	caaaaattcaagtaaataacagataatatgactgccgacaacaatagtatgccc
IDI-R0604	taaagtatgtatcgggaagtctccaccatcagtgggaaacattcaagaggcca
ADH2	
ADH2-F0613	caaaaattcaagtaaataacagataatatgtctattccagaaactcaaaaagcca
ADH2-R0613	taaagtatgtatcgggaagtctccacctgaaattatagggtggacgtcaagacg
OhmgR	
CIT1-OhmgR-F0527	caaagacgtacggcatcatttaaagtctaagaaaaaaggagccatcaaaaaccatt
CIT1-OhmgR-R0527	taaagtatgtatcgggaagtctccacctttgaaagatactctttatttctagacag
mvaS or ADH2 at 720	
720-F0531	gtgtgcgaaaagtactttggatcagcctttccttcacgttcggtccactt
PGK-mvaS-F0619	gcctttccttcacgttcggtccacttttcctccttcttgaattgatgtta
PGK-mvaS-R0619	tgttttatatttgttgtaaaaagtagataattac
mvaS0620-F	tatctacttttttacaacaaatataaaacaatgactatcggtatcgacaagatctc
mvaS0619-R	ctagcttaggctaagaaactccttctttgaaaaaatttatttctagacagttat
ADH2-F0620	tatctacttttttacaacaaatataaaacaatgtctattccagaaactcaaaaagcca
ADH2-R0620	ctagcttaggctaagaaactccttctgaaattatagggtggacgtcaagacg
720-R0531	ggatagataatgggggcgcgcctgcctagcttaggctaagaaactccttc
IDI+Oerg12	
SSA1-181010-F-2	actttccagaaacagatatctatattttataacaaagacgtacggcatcatttaaagt
SSA1-F0531	caaagacgtacggcatcatttaaagtcaaaggctcggttgtcgacaaattgtt
IDI-R0624	atgatgctgaataacgcctttgaatcgatcagtgggaaacattcaagaggcca
HSF04-F	cgattcaaaggcgttattcagcatcat
HSF04-R	atattctgtatattttatggtacgtgtagttga
Oerg12-F0624	cacgtaccataaaatatacagaatatatggctccaggtaactctttgtct
Oerg12-R-2	taaagtatgtatcgggaagtctccacctttgaaaaaatttatttctagacagttat
PDC20181010-R-3	cagactcagaaaattttatgcaacaacattaaagtatgtatcgggaagtctccacc
MAT	
CRISPR/Cpf1-MATalpha-F	atgatcaatttctactaagtgtagat**aaaattaagaacaaagcatc**gctttttttgtt
MAT-F0807	acgataactggttggaaagcgtaa
MAT-R0807	agacttgtggcgaagatgaatagt
MAT-F	agtcacatcaagatcgtttatgg
MAT-A	actccacttcaagtaagagtttg
MAT-alpha	gcacggaatatgggactacttcg
ADH1 deletion	

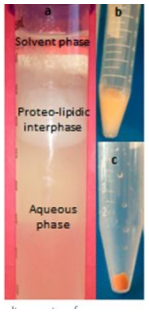
ADH1-UP-F	aaggtgagacgcgcataaccgctaga
ADH1-UP-R	ttccaacttaccgtgggattcgtag
ADH1-DOWN-F	ctacgaatcccacggtaagttggaagacaccagagaagctttggacttctt
ADH1-DOWN-R	cagaatctttgttatcggtaagatgtg
ADH1-Check0508-F	ttgccgaaagaacctgagtgcatttgca
ADH1-Check0508-R	gctcgttcgagagctgtgtgttcttgt
CRISPR/Cpf1-ADH1-F	atgatcaatttctactaagtgtagat**gctgttcaatacgccaaggc**gctttttttgtt
PDC_1-ACC_1	
gRNA-DOWN-ACC_1-F	cttctccgcagtgaaagataaatgatc**agaacaatttgaacttgaat**gttttagagc
ACC_1-UP-F	gctttagcaagccagttcgtacgcagc
ACC_1-UP-R	caacaaaaatatgcgtttagcgggcgggtgattgtgctaggctatactgtgccag
$PDC_1$180716-F	ccgcccgctaaacgcatatttttgttg
$PDC_1$180716-R	tttgattgatttgactgtgttattttgcgt
ACC_1-DOWN-F	caaaataacacagtcaaatcaatcaaaatgagcgaagaaagcttattcgagtct
ACC_1-DOWN-R	gaccagaccggttttctcgtccacgtg
ACC_1-check-F	ctcatttgaatcagcttatggtgatggc
ACC_1-check-R	ctttaccaccaccaccttcggatgcc
***CHO2* deletion in BL03-E-10 or BL03-D-4**	
CHO2-F-2	taattttatacgttagttcaacctaacaatccaggatttcattaacaaga
CHO2-F0507	ctaacaatccaggatttcattaacaagactattactcttggcctcctctagtaca
CHO2-R0507	ctcagacgaaagttcgacgccaattgacggaataccacttgccacctatcacc
CHO2-R-2	gtcaccattgactctcctcatatactcagacgaaagttcgacgccaattg
Construction of SC03-D-1	
BIE-B-ADH1-F	cccgaacgacctcaaaatgtctgctacattcatgtagcttacagtaagccacaattct
BIE-B-ADH1-R	agaattgtggcttactgtaagctacggacttcttcgccagaggtttggtcaagtc
BIE-I-GPM1t-F	gacttgaccaaacctctggcgaagaagtccgtagcttacagtaagccacaattct
BIE-I-GPM1t-R	agaattgtggcttactgtaagctactattcgaactgcccattcagcttttccctt
BIE-E-CYC1-F	aagggaaaagctgaatgggcagttcgaatagtagcttacagtaagccacaattct
BIE-E-CYC1-R	gctctcgattaacctgtgtaatatcagagcatcgcaaattaaagccttcgagcgt
ARS416d-F	tattaaccgcttttactattatcttctacgctgacagtaa
CIT1-F	ttatcttctacgctgacagtaagttcaggtacccgcgttaaggggctgcc
CIT1-R	tagaagtaacagtttcagacatcttcgtaaatagtattatattgctatatgt
OhmgR-F	atgtctgaaactgttacttctagat
OhmgR-R	ttgcctcacttgtcgcttatgatttgaaagatactctttatttcta
ARS416d-R	aatgtggtaacaaaggtgttgcctcacttgtcgcttatga
***CHO2* deletion in SC03-D-1**	
CRISPR-Cpf1-UP-F	gaagctcgtcaaaactggacctctattgaaaacatcaaagaattg
CRISPR-Cpf1-UP-R	atctacacttagtagaaattgatcatttatctttcactgcggagaag
CRISPR/Cpf1-DOWN-R	tagaggtccagttttgacgagcttcaaaacgttccttttccttctta
CRISPR/Cpf1-CHO2-F	atgatcaatttctactaagtgtagat**aatcacccagaagcggtatt**gcttttttgtt
CHO2-UP-F0406	gcggcactaaacttccaacattaaat
CHO2-UP-R0409	acttgaatccagacaaagtggttttacaactggacat

CHO2-DOWN-F0409	taaaaccactttgtctggattcaagttaacattttgaatagaagt
CHO2-DOWN-R0406	atcttacaaataatcctcaggacgac
CHO2-CHECK-R0406	gtaaacctatctcgctaccccaagt

Table S3 Photographs of lycopene-producing *Escherichia coli* and *Saccharomyces cerevisiae* collected from literatures.

Plate	Tube/shake flask	Bioreactor	Yield (mg/g CDW)	Reference
E. coli				
			49.9	Hussain MH et al., 2021; Creative Commons CC-BY-NC-ND license
NP	NP		94	Liu N et al., 2020; Reprinted in part with permission from [1]. Copyright [2020] [Elsevier B.V.] License # 5261070916136

NP		NP	34.5	Wei Y et al., 2018; Reprinted in part with permission from [2]. Copyright [2018] [Elsevier B.V.] License # 5262190729092
NP		NP	148	Xu X et al., 2016; Reprinted in part with permission from [15]. Copyright [2016] [Elsevier B.V.] License # 5264070486223
NP			34.3	Zhu F et al., 2015; Reprinted in part with permission from [3]. Copyright [2015] [Elsevier B.V.] License # 5261090602121

NP	Please see Supplementary Figure 2	NP	88	Jin W et al., 2015; can't get the license
NP		NP	49.7	Gallego-Jara J et al., 2015; Creative Commons CC-BY-NC-ND license
NP	NP	Please see Figure 4	32	Kim Y-S et al., 2011; can't get the license
NP		NP	5.7	Kim S-W et al., 2009; Reprinted in part with permission from [7]. Copyright [2009] [Elsevier B.V.] License #

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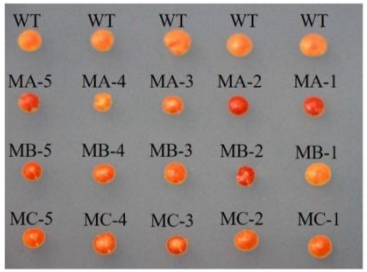
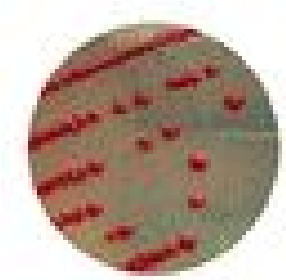
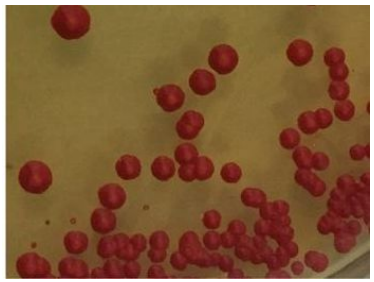
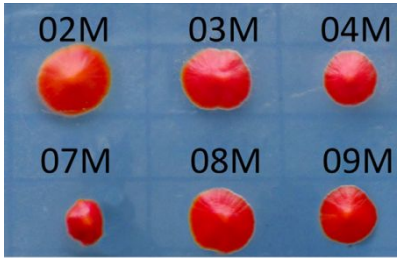
S. cerevisiae				
	NP	NP	NP ~14	Zhou P et al., 2020; Reprinted in part with permission from [9]. Copyright [2020] [Elsevier B.V.] License # 5261110664852
	NP		~73	Shi B et al., 2019; Reprinted in part with permission from [10]. Copyright [2019] [American Chemical Society]
Please see Figure S3	Please see Figure S2	NP	41.8	Hong J et al., 2019; can't get the license

			19.8	Zhou P et al., 2018; Reprinted in part with permission from [12]. Copyright [2018] [John Wiley & Sons, Inc.] License # 5261180959951
NP	NP		55.6	Chen Y et al., 2016; Creative Commons CC-BY-NC-ND license
	NP		24.4	Xie W et al., 2015; Reprinted in part with permission from [14]. Copyright [2015] [Elsevier B.V.] License # 5261111052669

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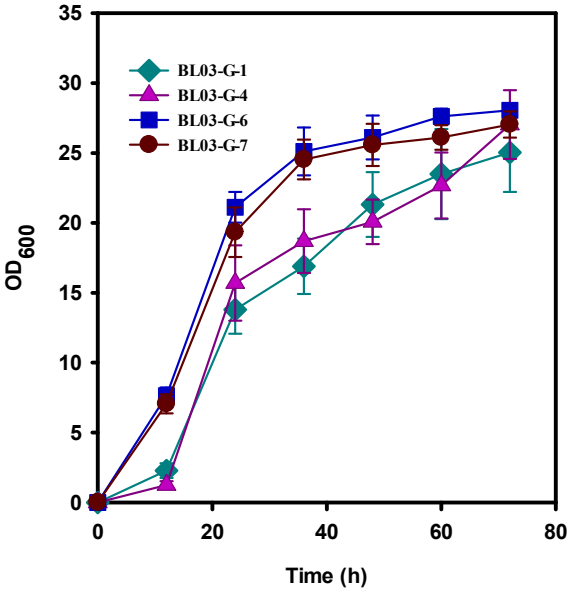

Figure S1 Growth curves of different strains with pathway engineering. Strains were cultured in YPM medium for 72 h. Each value represents the average ± SD of three biological replicates.

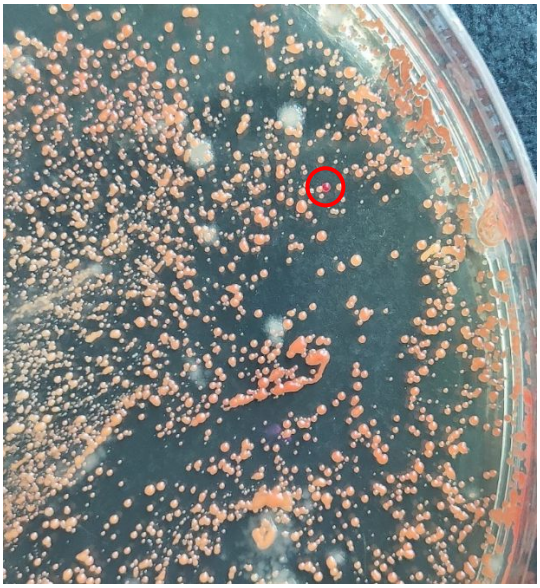

Figure S2 Adaptive laboratory evolution using simulated microgravity. This ALE was successfully applied for improving lycopene productivity in engineered *S. cerevisiae*, and hyper-producer W2 was isolated. Strains were cultured on YPD plates for 48 h.

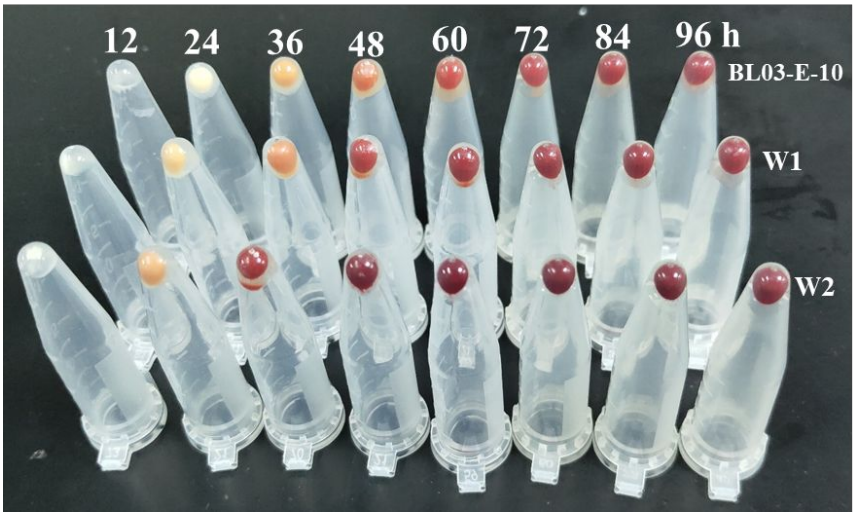

Figure S3 Shake-flask fermentations of BL03-E-10, W1 and W2. Strains were cultured in YPM medium for 96 h, and then collected by centrifugation at intervals.

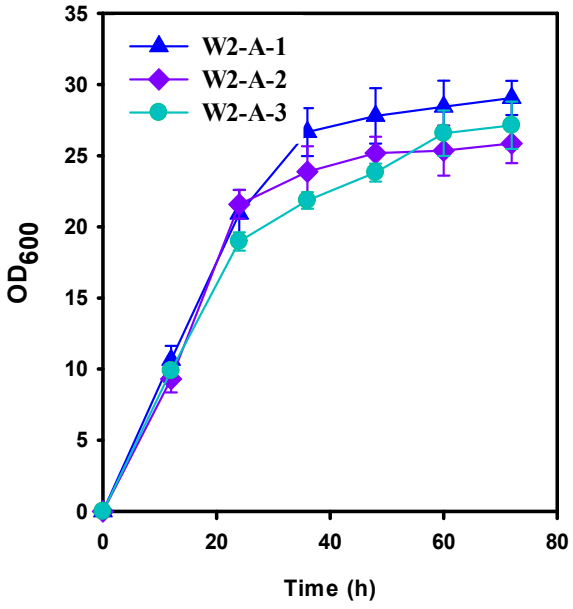

Figure S4 Growth curves of different strains in the shake-flask fermentation. Strain W2 was transformed with *idi* or (and) *oerg12* to determine if coupling overexpression with this evolved strain would further increase lycopene levels. Each value represents the average ± SD of three biological replicates.

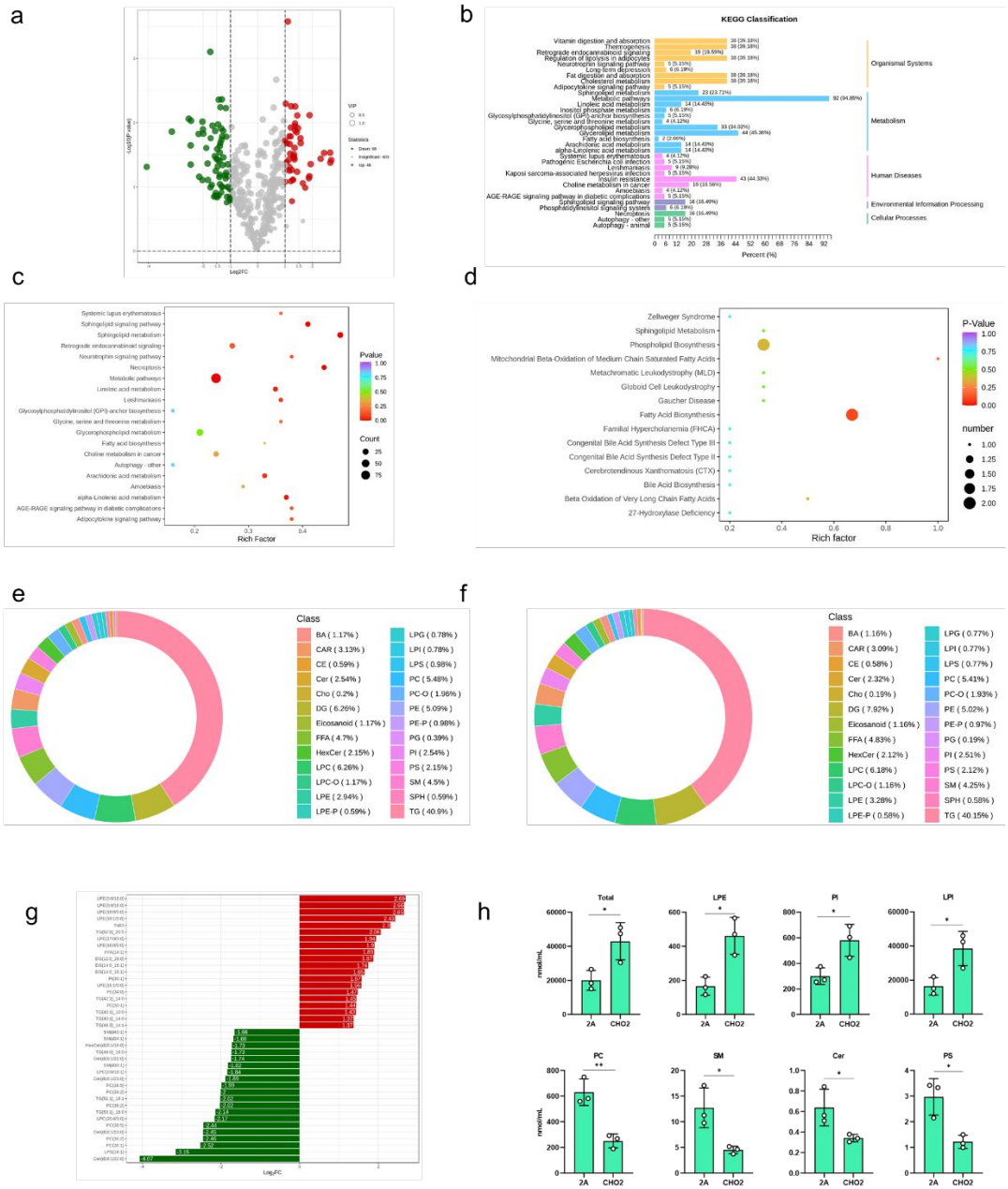

Figure S5 UHPLCQTOF-MS-based quantitative lipidomics. (a) Volcano Plot showing differential metabolites between BL03-E-10 and BL03-2A-1 cultivated in YPM medium for 24 h. (b) KEGG classification of the pathways from differential metabolites. (c, d) Statistics of KEGG and HMDB enrichment. The x axis indicates the rich factor corresponding to each pathway, and the y axis indicates name of the metabolic pathway. The size and color of bubbles represent the number and degree of enrichment of different metabolites, respectively. (e, f) Lipid class counts of BL03-E-10 and BL03-2A-1. (g) Top 20 differential metabolites between BL03-E-10 and BL03-2A-1. (h) Comparison of significant different lipid subclass, the Y-axis indicates the concentration of lipids (nmol/mL). 2A = BL03-E-10; CHO2 = BL03-2A-1. Statistically significant differences are denoted * $p < 0.05$, ** $p < 0.01$ (two-tailed Student's t-test). Total: total lipids; LPE: lysophosphatidylethanolamine; PI: phosphatidylinositol; LPI: Lysophosphatidylinositol; PC: phosphatidylcholine; PG: phosphoglyceride; Cer: ceramide; PS: phosphatidylserine; SM: sphingomyelin; PS: phosphatidylserine.

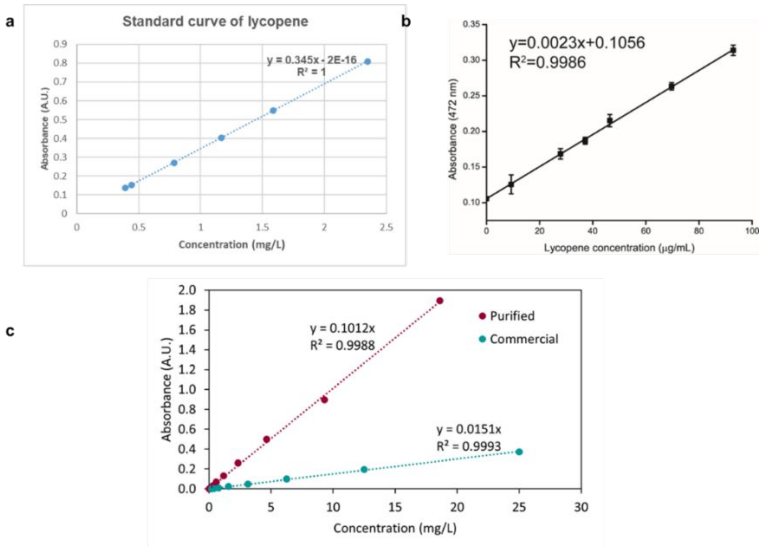

Figure S6 Different standard curves of lycopene. (a) The standard curve of lycopene used in our study redetermined by the spectrometer (Creative Commons CC-BY-NC-ND license). (b) The standard curve of lycopene acquired from the reference of Luo Z, *et al*., 2020 (Reprinted in part with permission from [Luo, Z.; Liu, N.; Lazar, Z.; Chatzivasileiou, A.; Ward, V.; Chen, J.; Zhou, J.; Stephanopoulos, G., Enhancing isoprenoid synthesis in *Yarrowia lipolytica* by expressing the isopentenol utilization pathway and modulating intracellular hydrophobicity. Metab. Eng. 2020, 61, 344-351.]. Copyright [2020] [Elsevier B.V.]. License # 5261941298774). (c) The standard curve of lycopene acquired from the reference of Chatzivasileiou AO, *et al*., 2019 (Creative Commons CC-BY-NC-ND license).

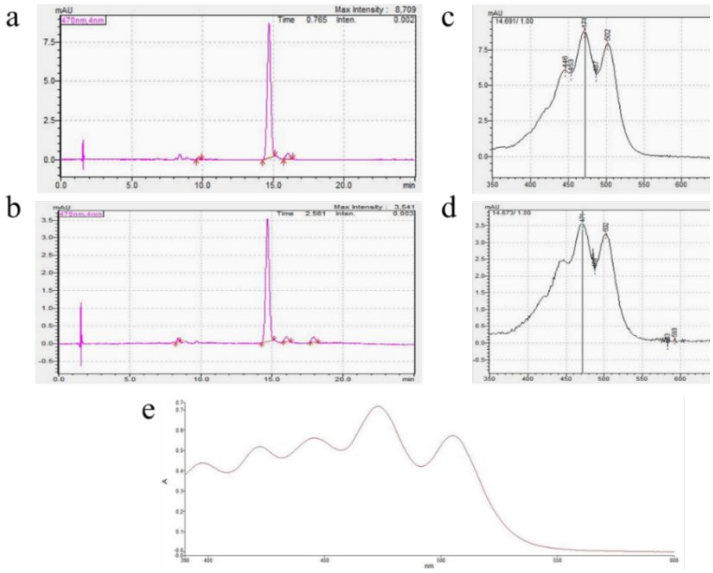

Figure S7 Verification of the method for quantifying lycopene concentration by ultraviolet-visible absorption spectra acquired from our previous study (Su B, *et al*., 2020**)** (Creative Commons CC-BY-NC-ND license)**.** HPLC analysis of the standard (a) and carotenoid extract of strain in this study (b). The spectrum of peak in standard (c) and carotenoid extract of strains in this study (d). (e) Spectral scanning for the carotenoid extract of strains in this study by using UV-spectrophotometer.

Supplementary Methods

Whole-genome resequencing

Strains chosen for whole-genome resequencing were cultivated in 50 mL YPD medium at 30°C in a shaker at 200 rpm for 24 h. Genomic DNA was extracted according to the manufacturer's protocol using the HiPure Yeast DNA Kit (Magen, Guangzhou, China). At least 5 μg of each genomic DNA sample was provided to Shanghai Majorbio Bio-pharm Technology Co. Ltd, for sequencing using the Illumina HiSeq 2000 platform. Paired-end reads of ~250 bp were generated. The average sequencing depths of the samples were 70 to 90. Fastq DNA-seq raw data were deposited in the Genome Sequence Archive (GSA) server at the BIG Data Center (http://bigd.big.ac.cn, GSA accession No. CRA005264).

Transcriptional analysis

The total RNA was extracted from each yeast strain at 6 h, 12 h, 24 h and 48 h cultivation using the HiPure Yeast RNA Kit (Magen, China) according to the manual of application. The RNA (about 500 ng) samples were reversely transcribed using HiScript II Q RT Super-Mix for qPCR (+gDNA wiper) Kit (Vazyme, China). Quantitative PCR was proceeded using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) on a QuantStudio 6 Flex Real-Time PCR System (Life Technologies). To normalize the different samples, the internal control gene *ACT1* was chose and the relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Widely targeted NMR-based metabolomics

Sample preparation and extraction

Samples cultivated in YPM medium for 12 h (adjusting to the same OD_{600}) was thawed on ice, vortex for 10 s and mix well, 300 μL of pure methanol was added to 50 μL of cells, whirl the mixture for 3 min and centrifuge it with 12,000 rpm at 4 ℃ for 10 min. Then collect the supernatant and centrifuge it at 12,000 rpm at 4 ℃ for 5 min. Leave in a refrigerator at -20 ℃ for 30 min, centrifuge at 12000 r/min at 4 ℃ for 3 min, and take 150 μL of supernatant in the liner of the corresponding injection bottle for on-board analysis.

UPLC Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD，https://sciex.com.cn/; MS, QTRAP® System, https://sciex.com/). The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); column temperature, 40℃; flow rate, 0.4 mL/min; injection volume, 2 μL or 5μL; solvent system, water (0.1% formic acid): acetonitrile (0.1% formic acid); gradient program, 95:5 V/V at 0 min, 10:90 V/V at 10.0 min, 10:90 V/V at 11.0 min, 95:5 V/V at 11.1 min, 95:5 V/V at 14.0 min.

QTOF-MS/MS

The Triple TOF mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (TripleTOF 6600, AB SCIEX) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. In each cycle, 12 precursor ions whose intensity greater than 100 were chosen for fragmentation at collision energy (CE) of 30 V (12

MS/MS events with product ion accumulation time of 50 msec each). ESI source conditions were set as following: Ion source gas 1 as 50 Psi, Ion source gas 2 as 50 Psi, Curtain gas as 25 Psi, source temperature 500℃, Ion Spray Voltage Floating (ISVF) 5500 V or -4500 V in positive or negative modes, respectively.

ESI-Q TRAP-MS/MS

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: source temperature 500℃; ion spray voltage (IS) 5500 V (positive), -4500 V (negative); ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 50, 50, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

Differential metabolites selected

Significantly regulated metabolites between groups were determined by VIP >= 1 and absolute Log2FC (fold change) >= 1. VIP values were extracted from OPLS-DA result, which also contain score plots and permutation plots, was generated using R package MetaboAnalystR. The data was log transform (log2) and mean centering before OPLS-DA. In order to avoid overfitting, a permutation test (200 permutations) was performed.

KEGG annotation and enrichment analysis

Identified metabolites were annotated using KEGG Compound database (http://www.kegg.jp/kegg/compound/), annotated metabolites were then mapped to KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html). Significantly enriched pathways are identified with a hypergeometric test's p-value for a given list of metabolites.

Quantitative lipidomics

Sample preparation and extraction

Sample cultivated in YPM medium for 24 h (adjusting to the same OD_{600}) was thawed on ice, whirl around 10 s, and then centrifuge it with 3000 rpm at 4℃ for 5 min. Take 50 μL of one sample and homogenize it with 1mL mixture (include methanol, MTBE and internal standard mixture). Whirl the mixture for 15 min. Then add 200 μL of water and whirl the mixture for 1 min, and centrifuge it with 12,000 r/min at 4℃ for 10 min. Extract 500 μL supernatant and concentrate it. Dissolve powder with 200 μL mobile phase B, then stored in -80℃. Finally take the dissolving solution into the sample bottle for LC-MS/MS analysis.

HPLC Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD， https://sciex.com.cn/ ; MS, QTRAP® 6500+ System, https://sciex.com/). The analytical conditions were as follows, UPLC: column, Thermo Accucore™C30 (2.6 μm, 2.1 mm×100 mm i.d.); solvent system, A: acetonitrile/water (60/40,V/V, 0.1% formic acid, 10 mmol/L ammonium formate), B:

acetonitrile/isopropanol (10/90 VV/V, 0.1% formic acid, 10 mmol/L ammonium formate); gradient program, A/B（80:20, V/V）at 0 min, 70:30 V/V at 2.0 min, 40:60 V/V at 4 min, 15:85 V/V at 9 min, 10:90 V/V at 14 min, 5:95 V/V at 15.5 min, 5:95 V/V at 17.3 min, 80:20 V/V at 17.3 min, 80:20 V/V at 20 min; flow rate, 0.35 ml/min; temperature, 45℃; injection volume: 2 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

ESI-MS/MS Conditions

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® 6500+ LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 500 ℃; ion spray voltage (IS) 5500 V（Positive）,-4500 V(Neagtive); Ion source gas 1 (GS1), gas 2 (GS2), curtain gas (CUR) was set at 45, 55, and 35 psi, respectively. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions was done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

Differential metabolites selected

Significantly regulated metabolites between groups were determined by VIP >= 1

and absolute Log2FC (fold change) >= 1. VIP values were extracted from OPLS-DA result, which also contain score plots and permutation plots, was generated using R package MetaboAnalystR. The data was log transform (log2) and mean centering before OPLS-DA. In order to avoid overfitting, a permutation test (200 permutations) was performed.

KEGG annotation and enrichment analysis

Identified metabolites were annotated using KEGG Compound database (http://www.kegg.jp/kegg/compound/), annotated metabolites were then mapped to KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html). Pathways with significantly regulated metabolites mapped to were then fed into MSEA (metabolite sets enrichment analysis), their significance was determined by hypergeometric test's p-values.