Supporting Information for:

Yarrowia lipolytica as a metabolic engineering platform for the production of very long-chain wax esters

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Supplementary Figures

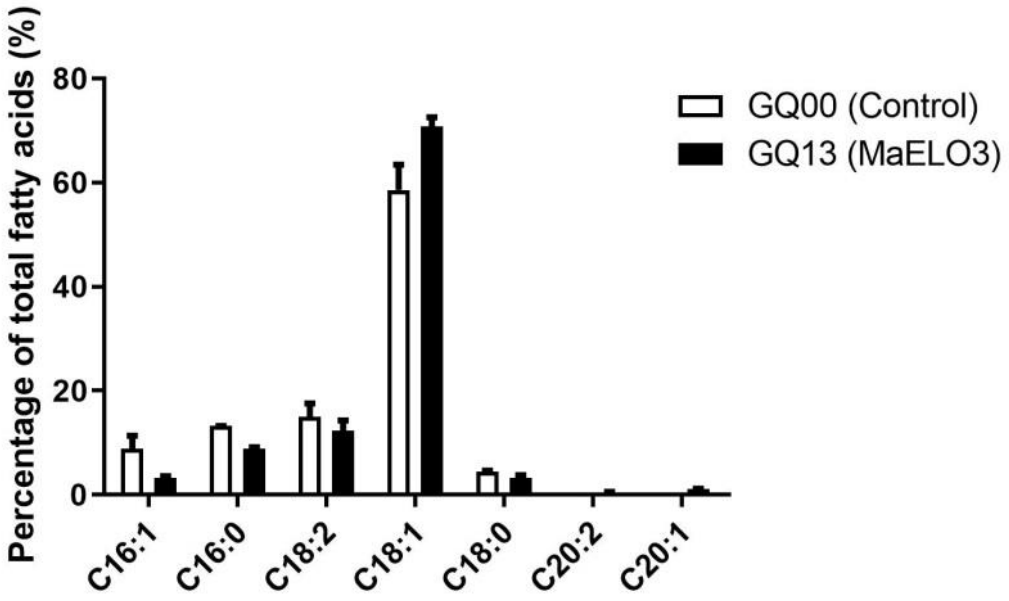


Figure S1. FAMES analysis of fatty acids produced by strains GQ00 and GQ13. Empty vector (GQ00) and vector overexpressed C16/18-elongase gene *MaELO3* (GQ13) were chromosomally integrated into *Y. lipolytica* strain Po1f and VLCFAs production by the resulting strains was compared. Cultures were grown in YPD medium for three days. The bars represent the mean, and the error bars represent the standard deviation of biological triplicates.

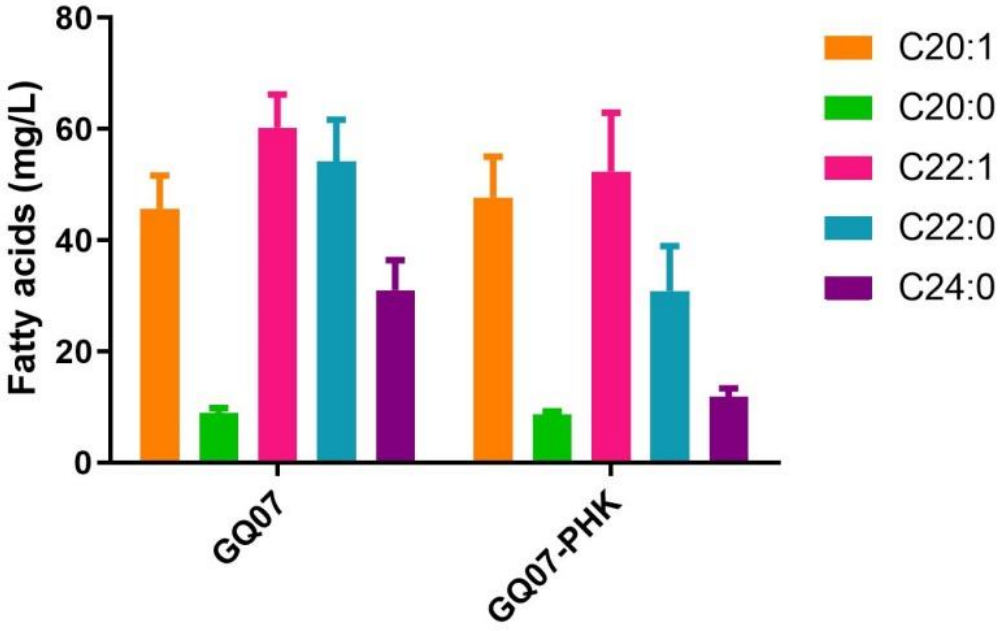


Figure S2. Production of VLCFAs in the engineered *Y. lipolytica* strains GQ07 and GQ07-PHK. VLCFAs accumulation of strain GQ07-PHK including codon-optimized *AnPK* from *Aspergillus nidulans* and *BsuPta* from *Bacillus subtilis* compared to control strain GQ07 in shake flask experiments. The bars represent the mean, and the error bars represent the standard deviation of biological triplicates.

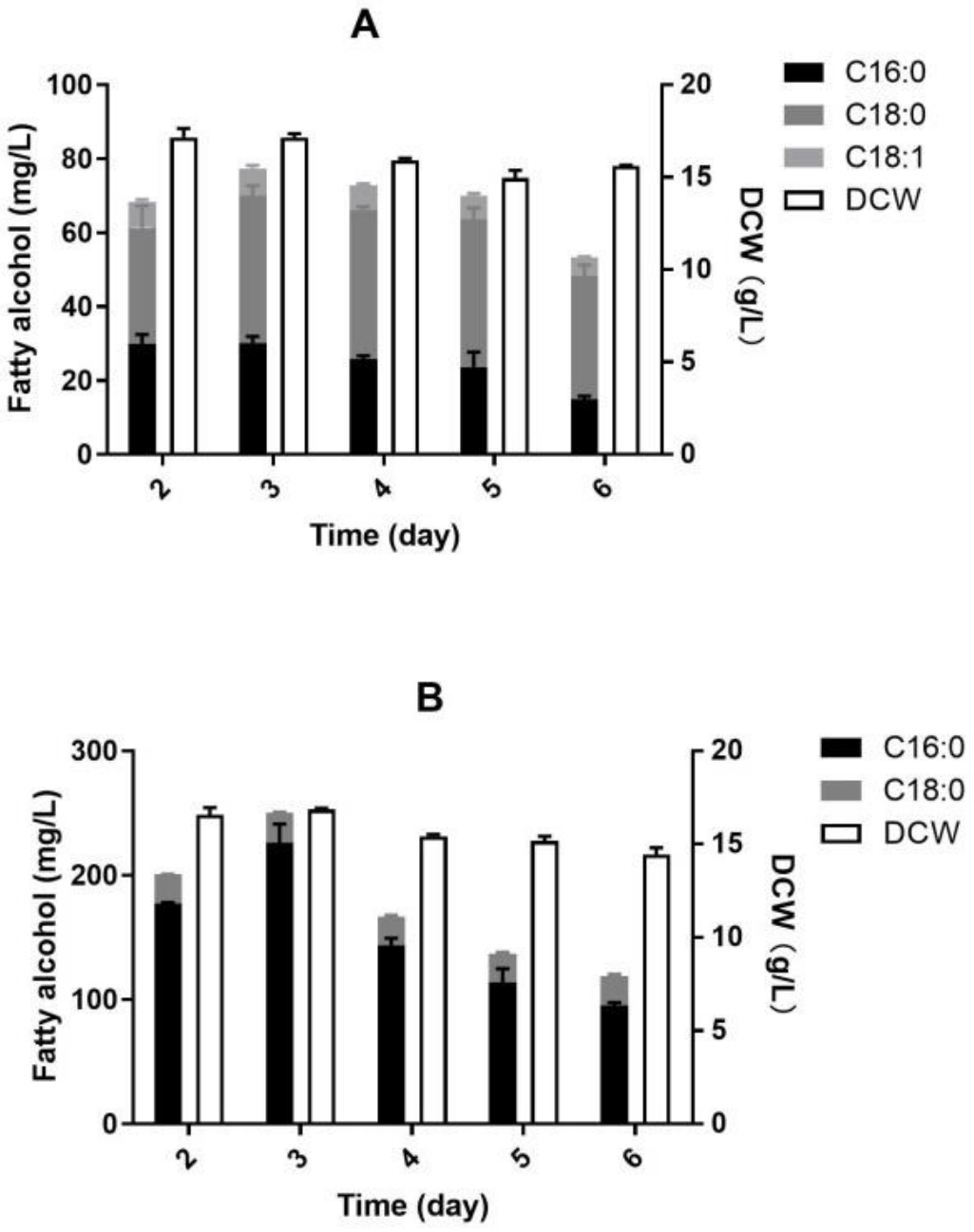


Figure S3. Time courses of fatty alcohols production and cell growth (dry cell weight) of *Y. lipolytica* strains with *MaFAR* (A) or *TaFAR* (B). The bars represent the mean, and the error bars represent the standard deviation of biological triplicates.

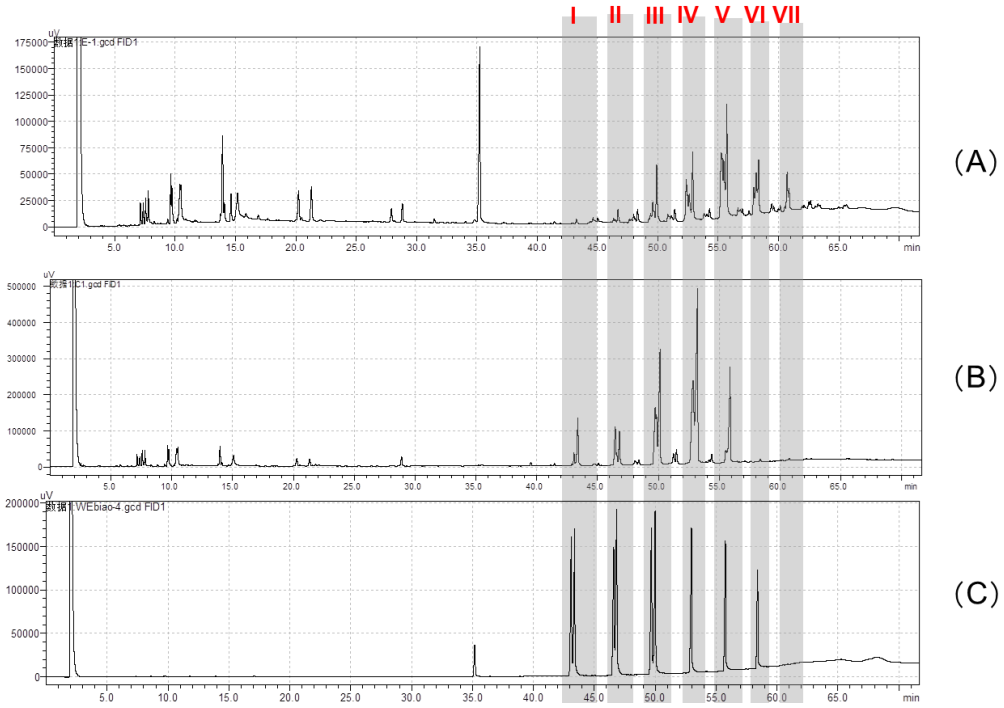
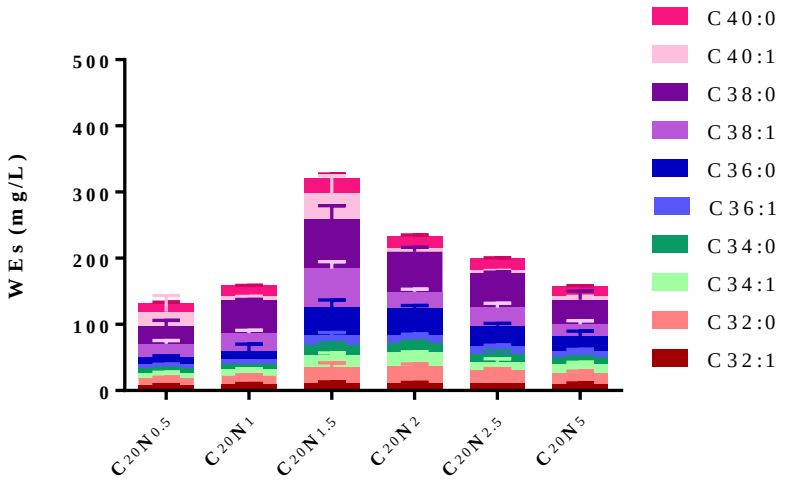
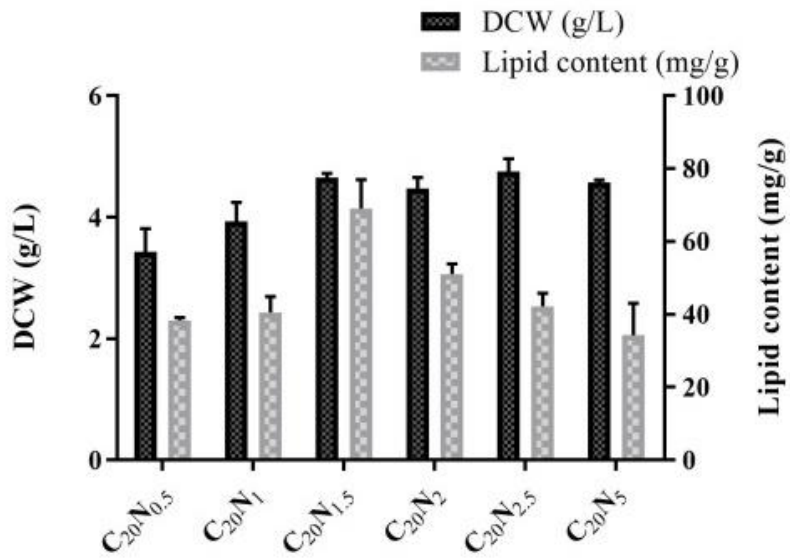


Figure S4. GC chromatograms of WEs isolated from recombinant strains carrying different *FAR* and *WS* genes. (A) WE03 (*MhWS* and *MaFAR*); (B) WE01 (*MhWS* and *TaFAR*); (C) A mixture of WEs standards. The peaks labeled with gray bars (I-VII) were identified as (I), C32:0/C32:1; (II), C34:0/C34:1; (III), C36:0/C36:1; (IV), C38:0/C38:1; (V), C40:0/C40:1; (VI), C42:0/C42:1; (VII), C44:0/C44:1 based on the standards used in this study.

A


C 40:0
C 40:1
C 38:0
C 38:1
C 36:0
C 36:1
C 34:0
C 34:1
C 32:0
C 32:1
WEs (mg/L)
500
400
300
200
100
0
C20N0.5
C20N1
C20N1.5
C20N2
C20N2.5
C20N5

B


DCW (g/L)
Lipid content (mg/g)
DCW (g/L)
Lipid content (mg/g)
6
4
2
0
100
80
60
40
20
0
C20N0.5
C20N1
C20N1.5
C20N2
C20N2.5
C20N5

C

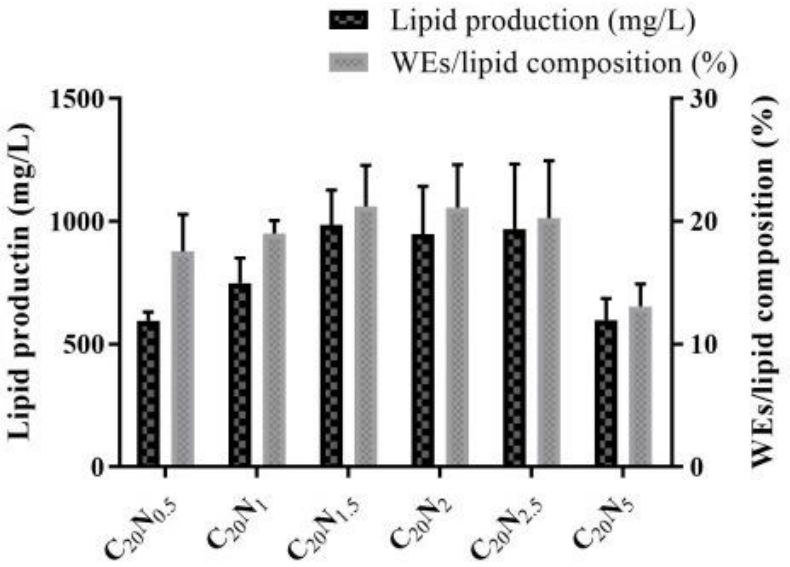
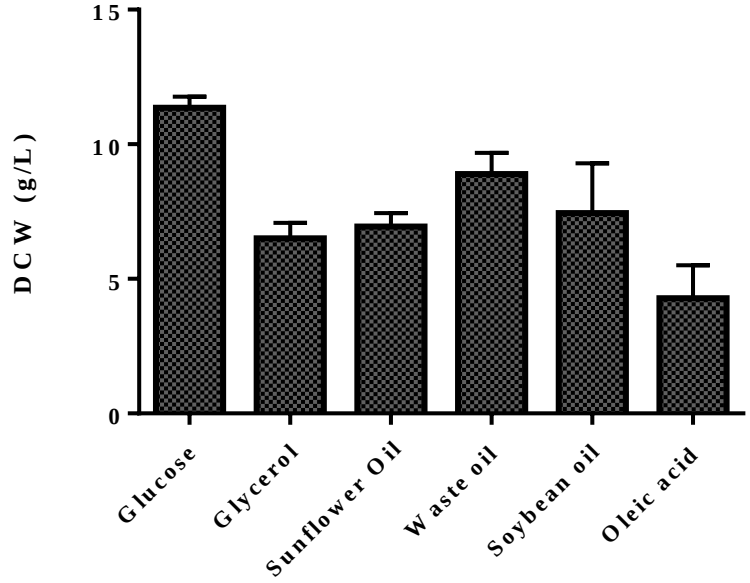
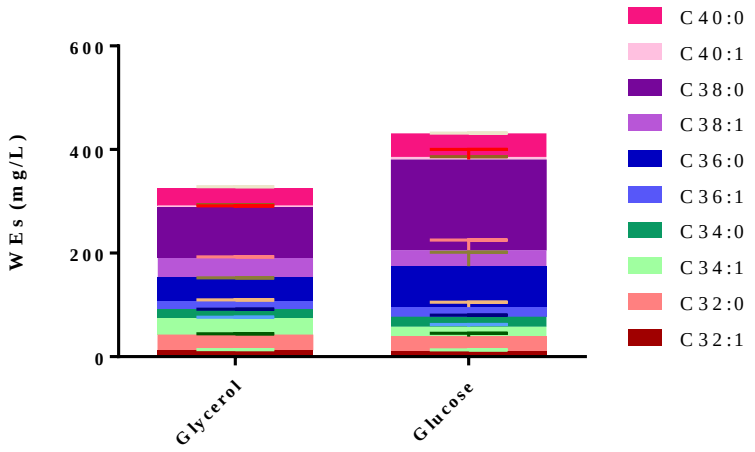

Lipid production (mg/L)
WEs/lipid composition (%)
Lipid productin (mg/L)
WEs/lipid composition (%)
1500
1000
500
0
30
20
10
0
C20N0.5
C20N1
C20N1.5
C20N2
C20N2.5
C20N5

Figure S5. Effects of carbon:nitrogen ratio on WEs production. (A) C:N optimization for WEs production in the WE01 strain, where C_i and N_j denote the concentrations of glucose (*i* g/L) and ammonium (*j* g/L), respectively . (B) Cell growth and lipid content of WE01 strain in different C:N ratio. (C) Lipid production and WEs/lipid composition of WE01 strain in different C:N ratio. Error bars represent the standard deviation of biological triplicates.

A


15
10
5
0
DCW (g/L)
Glucose
Glycerol
Sunflower Oil
Waste oil
Soybean oil
Oleic acid

B


600
400
200
0
WEs (mg/L)
Glycerol
Glucose
C40:0
C40:1
C38:0
C38:1
C36:0
C36:1
C34:0
C34:1
C32:0
C32:1

C

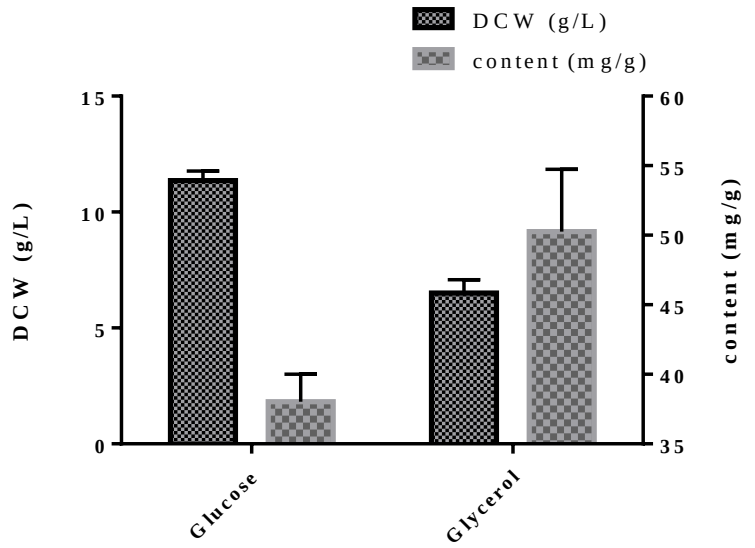

DCW (g/L)
content (mg/g)
15
10
5
0
DCW (g/L)
60
55
50
45
40
35
content (mg/g)
Glucose
Glycerol

Figure S6. Effects of different carbon sources on WEs production in shake flask culture. (A) Comparison of DCW in YP medium with different carbon sources. (B) Comparison of WEs titers in glucose or glycerol medium. (C) Comparison of DCW and WEs contents in glucose or glycerol medium. Three repeats were conducted for each strain, and error bars represent standard deviations.

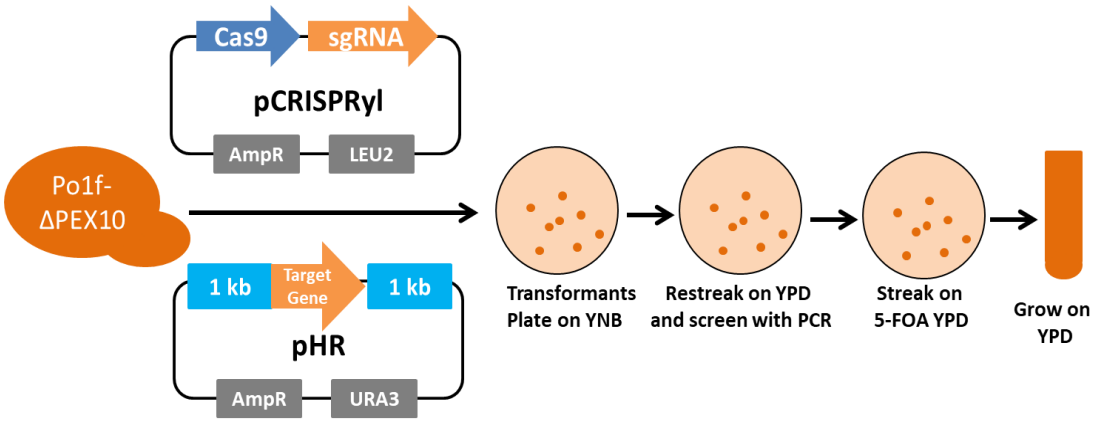


Figure S7 Schematic diagram of gene integration with CRISPR/Cas9. Transformation and outgrowth in selective media, followed by plating on rich medium and screening. Successful colonies were then plated on rich medium containing 5-FOA, which used for URA3 marker plasmid removal, and grown in rich medium, which used to cure the LEU2 marker plasmid.

Supplementary Tables

Table S1. Plasmids used in this study

Plasmids	Description	Reference
pINA1269	*Y. lipolytica* integrative plasmid, hp4d promoter, *XPR2* terminator, *LEU2* selection marker, Amp^r	[1]
pINA1312	*Y. lipolytica* integrative plasmid, hp4d promoter, *XPR2* terminator, *ura3d1* selection marker, Km^r	[1]
p32UT	pINA1312 php4d::UAS4B+TEF	[2]
p32AtKCS	pINA1312 vector containing codon-optimized *AtKCS* from *Arabidopsis thaliana*	This study
p32UTAtKCS	p32UT vector containing codon-optimized *AtKCS* from *Arabidopsis thaliana*	This study
p32UTCraKCS	p32UT vector containing codon-optimized *CraKCS* from *Crambe abyssinica*	This study
p32UTAtKCS_UTCraKCS	p32UTAtKCS plasmid containing *CraKCS* from *Crambe abyssinica* with UAS4B+TEF promoter	This study
p32UTMaFAR	p32UT vector containing codon-optimized *MaFAR* from *Marinobacter aquaeolei* VT8 Maqu_2220	This study
p32UTMaFAR-UTMaFAR	PINA1312 vector containing two copies of MaFAR with UAS4B-TEF promoter	This study
p32UTTaFAR	p32UT vector containing codon-optimized *TaFAR* from *Tyto alba*	This study
p32UTMaELO3	p32UT vector containing codon-optimized *MaELO3* from *Mortierella alpina*	This study
p32UTAbWS	p32UT vector containing codon-optimized *AbWS* from *Acinetobacter baylyi* ADP1	This study
p32UTMhWS	p32UT vector containing codon-optimized *MhWS* from *Marinobacter hydrocarbonoclasticus* DSM 8798	This study
p32MaFAR	pINA1312 vector containing codon-optimized *MaFAR* from *Marinobacter aquaeolei* VT8 Maqu_2220	This study
p32TaFAR	pINA1312 vector containing codon-optimized *TaFAR* from *Tyto alba*	This study
p32AmFAR	pINA1312 vector containing codon-optimized *AmFAR* from *Apis mellifera*	This study
p32MmFAR	pINA1312 vector containing codon-optimized *MmFAR* from *Mus musculus*	This study
p32ScFAR	pINA1312 vector containing codon-optimized *ScFAR* from *Simmondsia chinensis*	This study
p32EgFAR	pINA1312 vector containing codon-optimized *EgFAR* from *Euglena gracilis*	This study
p69UTMaFAR	p69UT vector containing codon-optimized *MaFAR* from *Marinobacter aquaeolei* VT8 Maqu_2220	This study
p69UTTaFAR	P69UT vector containing codon-optimized *TaFAR* from *Tyto alba*	This study
p69UTMhWS_UTMaFAR	p69UTMaFAR vector containing codon-optimized *MhWS* from	This study

	Marinobacter hydrocarbonoclasticus DSM 8798 with UT promoter	
p69UTAbWS_UTMaFAR	p69UTMaFAR vector containing codon-optimized *AbWS* from *Acinetobacter baylyi* ADP1 with UT promoter	This study
p69UTMhWS_UTTaFAR	p69UTTaFAR vector containing codon-optimized *MhWS* from *Marinobacter hydrocarbonoclasticus* DSM 8798 with UT promoter	This study
p69UTAbWS_UTTaFAR	p69UTTaFAR vector containing codon-optimized *AbWS* from *Acinetobacter baylyi* ADP1 with UT promoter	This study
p69UTMaELO3	pINA1269 vector containing codon-optimized MaELO3 from *Mortierella alpina* php4d::UAS4B+TEF	This study
P69UTAMPD	pINA1269 vector containing *AMPD* gene from *Y. lipolytica* php4d::UAS4B+TEF	This study
p69ACL1	pINA1269 vector containing *ACL1* gene from *Y. lipolytica*	[2]
p69ACC1	pINA1269 vector containing *ACC1* gene without intronic sequences from *Y. lipolytica*	[2]
p69ACC1-ACL1	pINA1269 vector containing *ACC1* gene without intronic sequences and *ACL1* gene from *Y. lipolytica*	This study
p69DGA1	pINA1269 vector containing *DGA1* gene from *Y. lipolytica*	[2]
p69EcAldh	pINA1269 vector containing *Aldh* gene from *E. coli*	This study
pCRISPRyl_F1	pCRISPRyl with F1 targeting sgRNA	Unpublished
pCRISPRyl_D17	pCRISPRyl with D17 targeting sgRNA	[3]
pCRISPRyl_XPR2	pCRISPRyl with XPR2 targeting sgRNA	[3]
pHR_F1_hrGFP	1kb_F1_up-UAS8B-TEF-hrGFP-CYC-1kb_F1_down, CEN1 URA3 AmpR ColE1	[4]
pHR_F1_MaELO3	1kb_F1_up-UAS4B-TEF-MaELO3-CYC-1kb_F1_down, CEN1 URA3 AmpR ColE1	This study
pHR_D17_hrGFP	1kb_D17_up-UAS8B-TEF-hrGFP-CYC-1kb_D17_down, CEN1 URA3 AmpR ColE1	[3]
pHR_D17_AnPK	1kb_D17_up-UAS8B-TEF-AnPK-CYC-1kb_D17_down, CEN1 URA3 AmpR ColE1	This study
pHR_XPR2_hrGFP	1kb_XPR2_up-UAS8B-TEF-hrGFP-CYC-1kb_XPR2_down, CEN1 URA3 AmpR ColE1	[3]
pHR_XPR2_BsuPta	1kb_XPR2_up-UAS8B-TEF-BsuPta-CYC-1kb_XPR2_down, CEN1 URA3 AmpR ColE1	This study

Table S2. Primers used in this study

Primer Names	Sequences
Primers used to build plasmids	
32AtKCS-f	caaccacacacatccacgtgatgacctccgtgaacgtcaagct
32AtKCS-r	cgtccagaacggccgatcctaaggatccggtacctccatggcc
32UTAtKCS-f	gagtataagaatcattcaaacacgtgatgacctccgtgaacgt
32UTAtKCS-r	tggggacaggccatggaggtaccggatccttaggatcggccgt
32UTCraKCS-f	gtataagaatcattcaaacacgtgatgacctctatcaacgtgaagct
32UTCraKCS-r	caggccatggaggtaccggatccttaagatcggccgttctgggct
32UTAtKCS_UTCraKCS-f	tagtctgcagcccaagctagctt atcgat aagctagcttatcgatacgcg
32UTAtKCS_UTCraKCS-r	acacctcagcatgcacgcgtatcgat ggacacgggcatctcacttgcgt
32UTMaFAR-f	tataagaatcattcaaa cacgtg atggccatccagcaggtgc
32UTMaFAR-r	caggccatggaggtacc ggatcc ttaggcggccttctttcg
32UTTaFAR-f	tataagaatcattcaaa cacgtg atggtctccatccccgagt
32UTTaFAR-r	caggccatggaggtacc ggatcc ttagtatcgcatggtggagg
32UTMaELO3-f	gtataagaatcattcaaacacgtgatggagtctggccccatgcccgc
32UTMaELO3-r	aggccatggaggtacc ggatcc ttactgggccttcttctgggc
32UTAbWS-f	gtataagaatcattcaaacacgtgatgcgacccctgcaccccatt
32UTAbWS-r	gggacaggccatggaggtaccggatccttagttggcggtcttaatgt
32MaFAR-f	atacaaccacacacatc cacgtg atggccatccagcaggtgc
32MaFAR-r	caggccatggaggtacc ggatcc ttaggcggccttctttcg
32TaFAR-f	atacaaccacacacatc cacgtg atggtctccatccccgagt
32TaFAR-r	caggccatggaggtacc ggatcc ttagtatcgcatggtggagg
32AmFAR-f	acaaccacacacatc cacgtg atgtccaccatctccg
32AmFAR-r	caggccatggaggtacc ggatcc ttagacaatcattcggc
32MmFAR-f	acaaccacacacatc cacgtg atggtttcaatcccagaatact
32MmFAR-r	caggccatggaggtacc ggatcc tcagtatctcatagtgctggat
32ScFAR-f	acaaccacacacatc cacgtg atggaggagatgggttc
32ScFAR-r	caggccatggaggtac cggatcc ttagttcaggacgtgctc
32EgFAR-f	acaaccacacacatc cacgtg atgaacgacttctacg
32EgFAR-r	caggccatggaggtac cggatcc ttacagcatggctcg
69UTMaFAR-f	ggctctcaagggcatcggtcgacaagctttagtctgcagcccaagctagc
69UTMaFAR-r	cttagtttcgggttcc cacgtg ttaggcggccttctttcgct
69UTTaFAR-f	ggctctcaagggcatcggtcgacaagctttagtctgcagcccaagctagc
69UTTaFAR-r	cttagtttcgggttcccacgtgttagtatcgcatggtggaggaggct
69UTFAR_UTWS-f	tgaaggctctcaagggcatcggtcgac aagctt aagctagcttatcgatacgcgtgc
69UTFAR_UTWS-r	taagctagcttgggctgcagactaaagctt catctcacttgcgtatg
69UTMaELO3-f	ggctctcaagggcatcggtcgacaagctttagtctgcagcccaagctagc
69UTMaELO3-r	cttagtttcgggttcc cacgtg ttactgggccttcttctgggc
69EcAldh-f	atacaaccacacacatc cacgtg atgaattttcatcatctggc
69EcAldh-r	atccttagtttcgggttcc cacgtg tcaggcctccaggcttatcc
F1_MaELO3-f	gagaatacaacgcctgccat actagt aagctagcttatcgatacgcgt
F1_MaELO3-r	caagcctgtggaggacttcaag cctagg ggacacgggcatctcact

Table S3. Codon optimized sequences of genes used in this study.

Name	Codon optimized sequence (5' → 3')
AtKCS	atgacctccgtgaacgtcaagctgctgtaccgatacgtcctgaccaacttcttcaacctgtgcctgttccccctgaccgccttcctggccggcaagg cttcccgactgaccatcaacgacctgcacaacttcctgtcctacctccagcacaacctcatcaccgtcaccctcctgttcgccttcaccgtcttcggcc tcgtcctctacatcgtcacccgacccaaccccgtctaccttgtcgactactcctgctacctgccccccccccacctcaaggtctccgtctccaaggtc atggacatcttctaccagatccgaaaggccgacacctcctcccgaaacgtcgcctgcgacgacccctcctccctggacttcctccgaaagatccag gagcgatccggcctcggcgatgagacctactcccccgagggcctgatccacgttcccccccgaaagaccttcgccgcctcccgagaggagacc gagaaggtcatcatcggagccctcgagaacctgtttgagaacaccaaggtcaaccccccgagagatcggcatcctcgtcgtcaactcctccatgttc aaccccacccctccctgtccgctatggtcgtcaacacttttaagctgcgatccaacatcaagtccttcaacctcggcggcatgggctgttccgccg gcgttatcgccatcgacctcgccaaggacctcctccacgtccacaagaacacctacgccctggtcgtctccaccgagaacatcacccagggcatc tacgctggcgagaaccgatccatgatggtctccaactgcctctttcgagttggcggcgccgccatcctgctgtccaacaagtccggcgaccgacg acgaagcaagtacaagctggtccacactgtgcgaacccacaccggcgccgatgacaagtccttccgatgcgtccagcaggaggatgacgagtc cggcaagatcggcgtgtgcctgagcaaggacatcaccaacgtcgccggcaccactctgaccaagaacatcgccacccctgggccccttattctg cctctctccgagaagtttctgttcttcgccacctttgtcgccaagaagctgctgaaggacaagatcaagcattactacgtccccgacttcaagcttgcc gtcgatcacttctgcatccacgccggcggccgagccgtcatcgacgagcttgagaagaacctgggactctcccccatcgacgtcgaggcctccc gatccaccctgcaccgattcggcaacacctcttcctcctccatctggtacgagcttgcctacatcgaggccaagggacgaatgaagaagggcaac aaggcctggcagatcgccctcggctccggcttcaagtgcaactcggccgtctgggtcgccctgcgaaacgtcaaggcctccgccaactccccct ggcagcactgcatcgaccgataccccgttaagatcgactccgacctgtccaagtccaagacccacgtccagaacggccgatcctaa
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