PNAS

Supplementary Information for

Methanol biotransformation toward high-level production of fatty acid derivatives by engineering the industrial yeast *Pichia pastoris*.

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Supplementary Figures

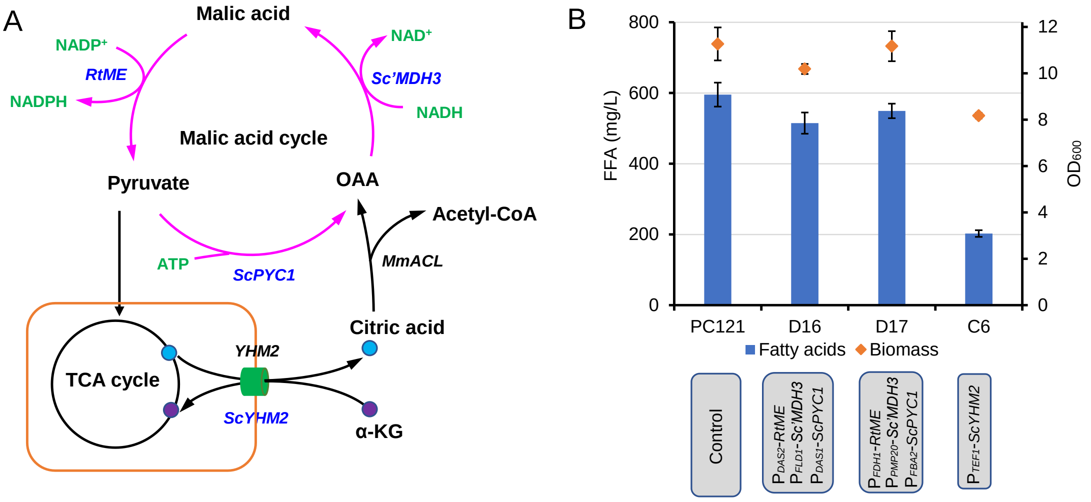


Figure S1. Construction of malate cycle (transhydrogenase cycle) in cytoplasm. (*A*) Schematic diagram of malate cycle. Overexpressed genes are marked in light blue. (*B*) FFA production in engineered strains. Error bars correspond to the SD of the mean (n = 3, corresponding to three biological replicates)

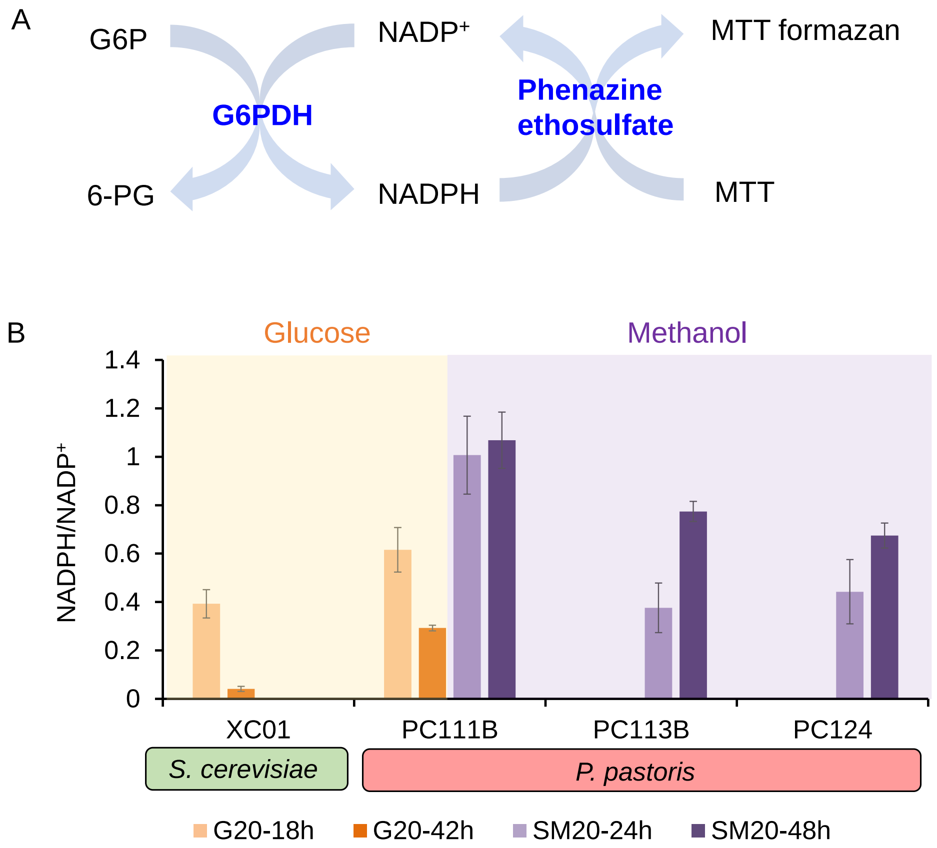


Figure S2. Quantification of NADPH/NADP$^+$ in *S. cerevisiae* and *P. pastoris*. (*A*) Schematic diagram of cofactor recycling assay. (*B*) NADPH/NADP$^+$ of *S. cerevisiae* and *P. pastoris* (PC111B) in glucose medium and methanol medium. PC111B, wild type; PC113B, FFA-producing strain with blocked FFA activation; PC124, FFA over-producing strain with engineered central metabolism. *Due to the slow growth of PC113B, the sampling time was delayed by 24 h. Error bars correspond to the SD of the mean (n = 3, corresponding to three biological replicates).

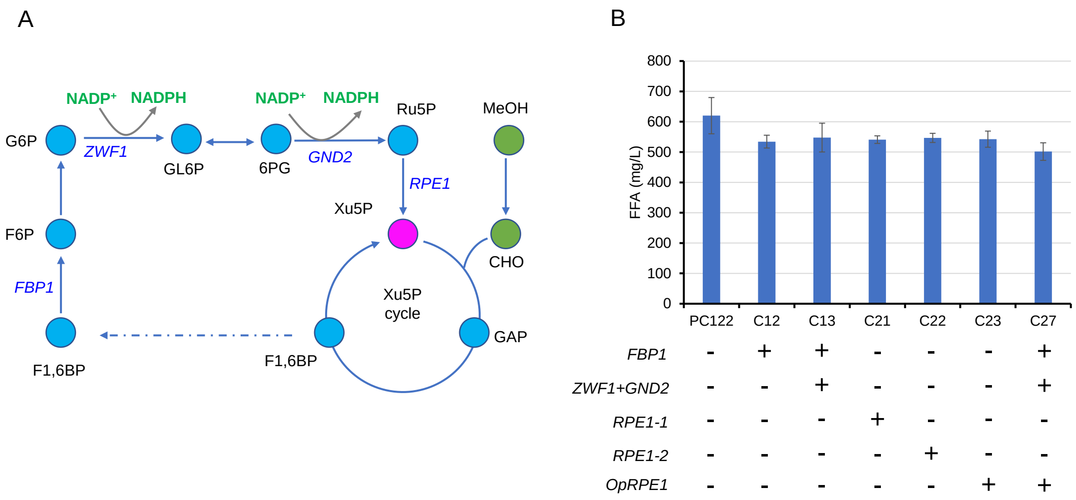


Figure S3. Enhancing gluconeogenesis pathway to increase Xu5p supply. (*A*) Schematic diagram of gluconeogenesis pathway engineering. Overexpressed genes are marked in light blue. (*B*) FFA production in engineered strains with rewired gluconeogenesis pathway. Error bars correspond to the SD of the mean (n = 3, corresponding to three biological replicates).

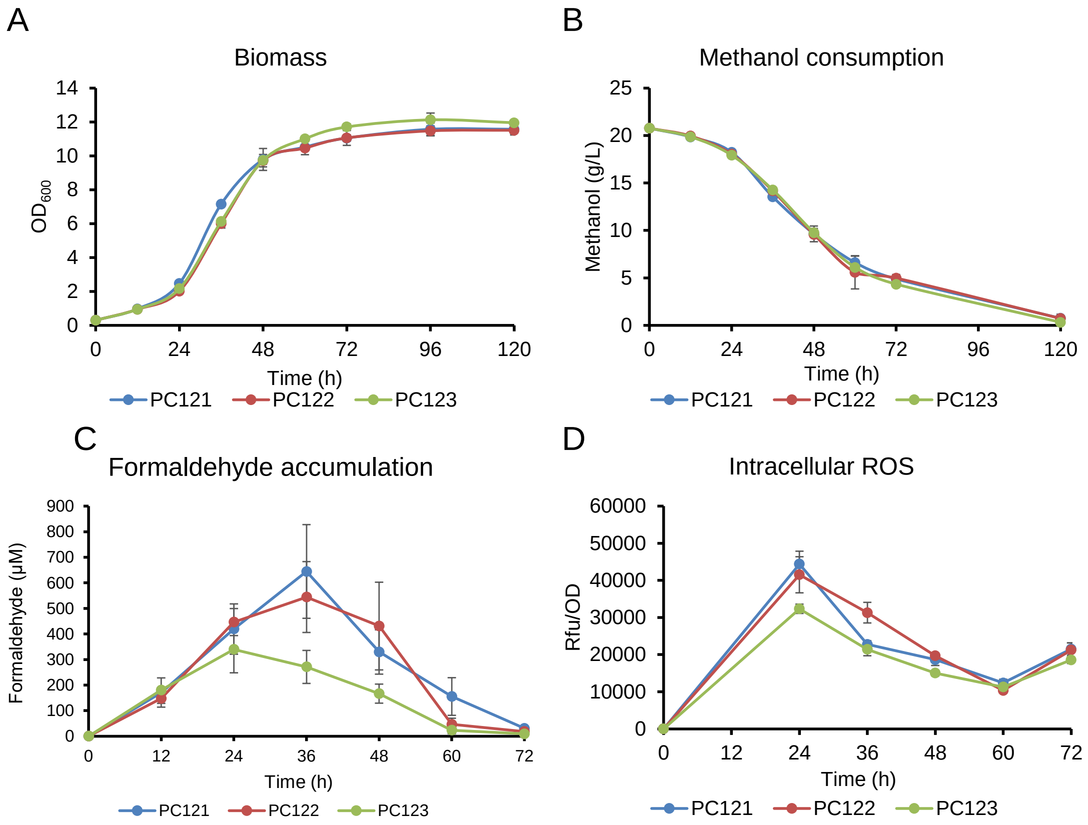
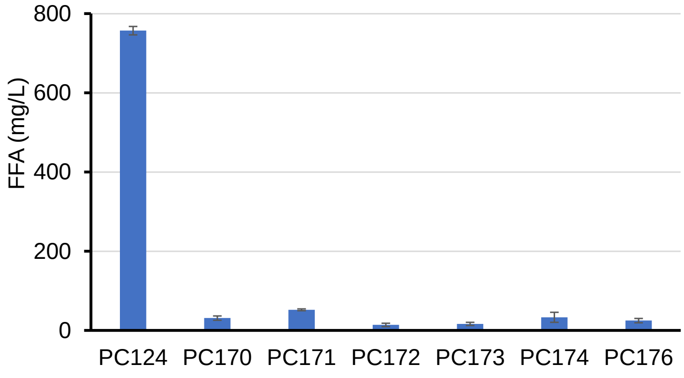


Figure S4. Rewiring central metabolism for improving FFA production from methanol. (*A-D*) The growth curves, methanol consumption, formaldehyde accumulation and intracellular ROS level. Error bars represent SD of triplicate samples.



	PC124	PC170	PC171	PC172	PC173	PC174	PC176
FAA1t::FaCoAR	-	+	-	-	-	-	-
faa1Δ::FAA1	-	-	+	-	-	-	-
faa1Δ::FAA1+FaCoAR	-	-	-	+	+	+	+
hfd1Δ	-	-	-	-	+	+	+
hfd1Δ::FaCoAR	-	-	-	-	-	+	+
faa2Δ::FAA2+FaCoAR	-	-	-	-	-	-	+

Figure S5. FFA accumulation of the fatty alcohols producing strains. Error bars correspond to the SD of the mean (n = 3, corresponding to three biological replicates)

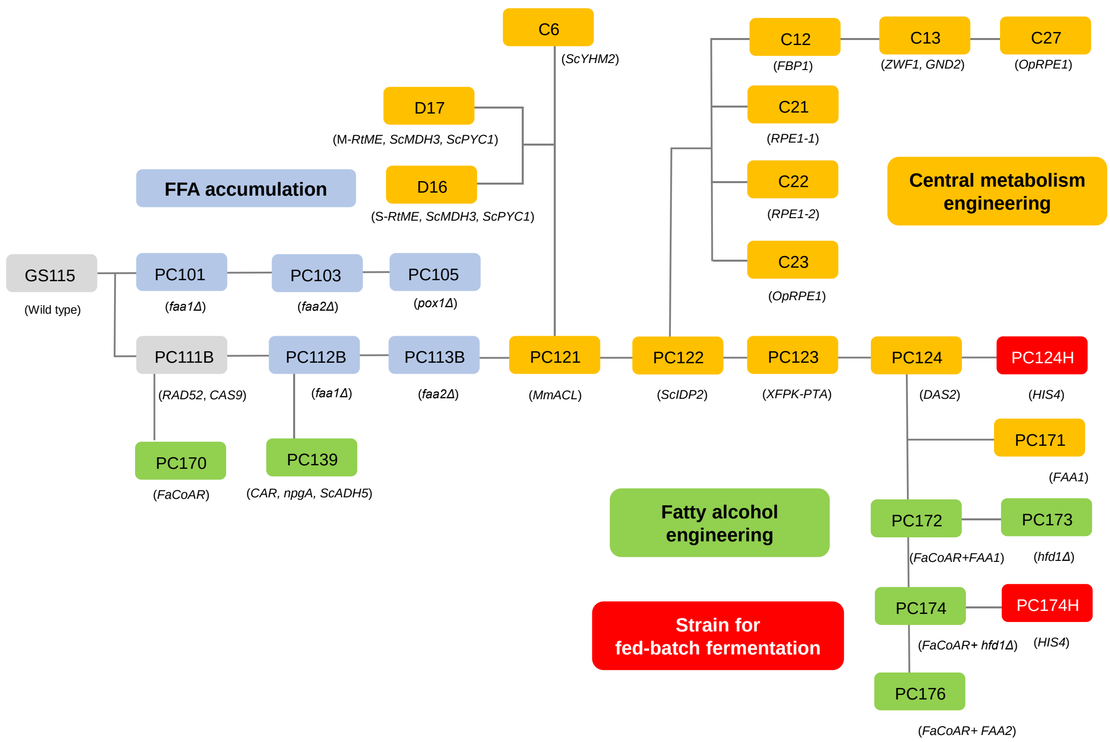


Figure S6. Flowchart of yeast strain construction in this study.

Supplementary Tables

Table S1.Plasmids used in this study.

Plasmid name	Genotype or characteristic	Resource or Reference
pPICZ-Cas9-gFAA1	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gFAA1-T_{AOX1}	(1)
pPICZ-Cas9-gFAA2	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gFAA2-T_{AOX1}	(1)
pPICZ-Cas9-gPOX1	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPOX1-T_{AOX1}	This study
pPICZ-Cas9-gHFD1	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gHFD1-T_{AOX1}	(1)
pPICZ-Cas9-gHIS4	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gHIS4-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI2	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI2-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI3	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI3-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI4	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI4-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI5	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI5-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI6	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI6-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI8	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI8-T_{AOX1}	(1)
pPICZ-Cas9-gPNSI10	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI10-T_{AOX1}	(1)
pPICZ-Cas9-	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI12-	(1)

gPNSI12	T_{AOX1}	
pPICZ-Cas9-gPNSI13	panARS, *Ble*^{R}, P_{HTX1}-Cas9-T_{DAS1}, P_{HTX1}-gPNSI13-T_{AOX1}	(1)
pCAI-gPNSII-4	panARS, *KanMX*, P_{HTX1}-gPNSII-4-T_{AOX1}	(1)
pCAI-gPNSIII-5	panARS, *KanMX*, P_{HTX1}-gPNSIII-5-T_{AOX1}	(1)
pCAI-gFAA1t	panARS, *KanMX*, P_{HTX1}-gFAA1t-T_{AOX1}	This study
pCAI-gFAA2t	panARS, *KanMX*, P_{HTX1}-gFAA2t-T_{AOX1}	This study

Table S2. Strains used in this study

Strain name	Genotype	Resource or Reference
GS115	Mut+, *his4*$^-$, *AOX1*, *AOX2*	From Pro Cai
XC01	*MATa; MAL2-8c; SUC2; his3Δ1; ura3-52; XI-5::*P_{TEF1}*-Cas9-*T_{CYC1}	Lab reserved
PC110	GS115, *HIS4::*P_{GAP}-*PpRAD52*-T_{AOX1}	This study
PC111B	PC110, *PNSI-2::*P_{GAP}-*hCas9*-T_{DAS1}	This study
Engineered strains for FFA production		
PC113B	Mut$^+$, *his*$^-$, *AOX1*, *AOX2*, *his4Δ*::P_{GAP}-*PpRAD52*-T_{AOX1}, *PNSI-3::*P_{GAP}-*hCas9*-T_{DAS1}, *faa1*Δ,*faa2*Δ =[PC111B]+(*faa1Δ,faa2Δ*)	This study
PC121	Mut$^+$, *his*$^-$, *AOX1*, *AOX2*, *his4Δ*::P_{GAP}-*PpRAD52*-T_{AOX1}, *PNSI-3::*P_{GAP}-*hCas9*-T_{DAS1}, *faa1*Δ,*faa2*Δ, *PNSI2*::P_{AOX1}-*MmACL*-KpOpt1-T_{FAA1} =[PC131B]+(*PNSI2::*P_{AOX1}-*MmACL*-KpOpt1-T_{FAA1})	This study
PC122	Mut$^+$, *his*$^-$, *AOX1*, *AOX2*, *his4Δ*::P_{GAP}-*PpRAD52*-T_{AOX1}, *PNSI-3::*P_{GAP}-*hCas9*-T_{DAS1}, *faa1*Δ,*faa2*Δ, *PNSI-2*::P_{AOX1}-*MmACL*-KpOpt1-T_{FAA1}, *PNSI-4::*P_{FLD1}-*ScIDP2o*-T_{DAS2} =[PC121]+(*PNSI-4::*P_{FLD1}-*ScIDP2o*-T_{DAS2})	This study
PC123	Mut$^+$, *his*$^-$, *AOX1*, *AOX2*, *his4Δ*::P_{GAP}-*PpRAD52*-T_{AOX1}, *PNSI-3::*P_{GAP}-*hCas9*-T_{DAS1}, *faa1*Δ,*faa2*Δ, *PNSI-2*::P_{AOX1}-*MmACL*-KpOpt1-T_{FAA1}, *PNSI-4::*P_{FLD1}-*ScIDP2o*-T_{DAS2},*PNSIII-5*::*BbXFPK*-P_{HTX1}-*CkPTA* =[PC122]+(*PNSIII-5::BbXFPK*-P_{HTX1}-*CkPTA*)	This study

PC124	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *faa1*Δ,*faa2*Δ, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$ =[PC123]+(*PNSII-4::*P$_{DAS2}$-*DAS2*-T$_{GAP}$)	This study
PC124H	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *faa1*Δ,*faa2*Δ, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$,PNSIII-5::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *his4Δ*::*HIS4* =[PC124]+(*his4Δ::HIS4*)	This study
Engineered strains for fatty alcohol production		
PC170	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *FAA1t*::P$_{TEF1}$-FaCoAR-KpOpt1-T$_{FBP1t}$ =[PC111B]+(*FAA1t::*P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$)	This study
PC171	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *faa2*Δ, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ*::*FAA1* =[PC124]+(*faa1Δ::FAA1*)	This study
PC172	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *faa2*Δ, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ*::*FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$ =[PC124]+(*faa1Δ::FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$)	This study
PC173	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *faa2*Δ, *PNSI-2*::P$_{AOX1}$-	This study

	MmACL-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ*::*FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *hfd1Δ* =[PC172]+(*hfd1Δ*)	
PC174	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *faa2*Δ, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ*::*FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *hfd1Δ::*P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$ =[PC173]+(*hfd1Δ::*P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$)	This study
PC175	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ*::*FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *hfd1Δ::*P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *faa2Δ::FAA2* =[PC174]+(*faa2Δ::FAA2*)	This study
PC176	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ::*P$_{GAP}$-*PpRAD52*-T$_{AOX1}$, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5::BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ::FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *hfd1Δ::*P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *faa2Δ::FAA2*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$ =[PC175]+(*faa2Δ::FAA2*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$)	This study
PC174H	Mut^{+}, *his*$^{-}$, *AOX1*, *AOX2*, *his4Δ*::*HIS4*, *PNSI-3::*P$_{GAP}$-*hCas9*-T$_{DAS1}$, *PNSI-2*::P$_{AOX1}$-*MmACL*-KpOpt1-T$_{FAA1}$, *PNSI-4::*P$_{FLD1}$-*ScIDP2o*-T$_{DAS2}$, *PNSIII-5*::*BbXFPK*-P$_{HTX1}$-*CkPTA*, *PNSII-4*::P$_{DAS2}$-*DAS2*-T$_{GAP}$, *faa1Δ*::*FAA1*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *hfd1Δ::*P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$, *faa2Δ::FAA2*+P$_{TEF1}$-*FaCoAR-KpOpt1*-T$_{FBP1}$ =[PC174]+(*his4Δ::HIS4*)	This study

Table S3.Primers used in this study.

Primer No.	Name	Sequence (5' - 3')
Primers for gRNA plasmid construction		
P1	AOX1t-Kpn-ARS-R	AAACGTCAAATCATAATCAGCACTAGGTACCGCACAAACGAACGTCTCACTTAATCTTC
P2	HTX1-Cas9-F	GGAGTACTTCTTGTCCATCGTTTCGACTAGTTGTTGTAGTTTTAATATAGTTTGAGTATGAGATGGAACTC
P3	gPOX1-F	GATTTTCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCAAAATCTTGGGACTTTCCGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCT
P4	gPOX1-R	GTTTCGTCCTCACGGACTCATCAGAAAATCTTTGATTTGTTTAGGTAACTTGAACTGGATGTATTAGTTTGG
P5	gFAA1t-F	GAAGCTATGAATGAAAAGCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAG
P6	gFAA1t-R	TGCTTTTCATTCATAGCTTCGACGAGCTTACTCGTTTCGTCC
P7	gFAA2t-F	AAAAATGAAATAAAAAACAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAG
P8	gFAA2t-R	CTGTTTTTTATTTCATTTTTGACGAGCTTACTCGTTTCGTCC
Primers for seamless gene deletion of POX1		

P9	POX1-HRUP-F	GTCAAGTTGTATAGCATAGAACAAGAATGGAAG
P10	POX1-HRUP-R1	CGTAATCAATACACCGTTGTGATTTTCAGTTGTTAGACTGGTTAAAAAAG
P11	POX1-HRDN-F1	ACTGAAAATCACAACGGTGTATTGATTACGTAGTAATGCTATCACAAAGTA
P12	POX1-HRDN-R	GAAGCTTCTTCGTAGCTCTTCATCTTTC
Primers for engineering FFA production		
P13	MmACL-KpOpt1-AOX1p-F	AGATCAAAAAACAACTAATTATTCGAAACGATGTCTGCCAAGGCCATCAG
P14	MmACL-KpOpt1-FAA1t-R	GTCCGTAGAAAACTTCAATCGGCTGCTCGCTCACATACTCATATGTTCTGGAAGGACAT
P15	ScIDP2o-FLD1p-F	TGCTTGTTCATACAATTCTTGATATTCACAATGACAAAGATAAAGGTTGCAAACCCTA
P16	ScIDP2o-DAS2t-R	AACTACTAACCCGTTAGTGGCCAAATCTACTCAAAGTGCTGCTGCTTCGAAC
P17	ScYHM2o-TEF1p-F	TCACTACATACATTTTAGTTATTCGCCAACATGCCATCTACTACTAATACCGCCG
P18	ScYHM2o-AOX1t-R	CAGGCAAATGGCATTCTGACATCCTCTTGATCAATGTTTGGCGACTGGAGTCTC
P19	RtME-DAS2p-F	TCACTCTTATCAAACTATCAAACATCAAAAATGCCTGCTCATTTTGCCCC
P20	RtME-AOX1t-R	CAGGCAAATGGCATTCTGACATCCTCTTGATCATTGTGCTTGTTGTTCTGCTTCTAATA

P21	RtME-FDH1p-F	CAAATACCTCCAACATCACCCACTTAAACAATGCCTGCTCATTTTGCCCC
P22	ScMDH3-FLD1p-F	TGCTTGTTCATACAATTCTTGATATTCACAATGGTCAAAGTCGCAATTCTTGGC
P23	ScMDH3-DAS1t-R	CTCCTAACTAAAACTGTAAAGACTTCCCGTTCAAGAGTCTAGGATGAAACTCTTGCCT
P24	ScMDH3-PMP20p-F	AATCCCACCAAGCAAAAAAAAAAATCTAAGATGGTCAAAGTCGCAATTCTTGGC
P25	ScPYC1-DAS1p-F	TTGATTTTATCTGGAGAATAATCGAACAAAATGTCGCAAAGAAAATTCGCCG
P26	ScPYC1-AOX1t-R	CAGGCAAATGGCATTCTGACATCCTCTTGATCATGCCTTAGTTTCAACAGGAACTTGG
P27	ScPYC1-FBA2p-F	TTGATAAGGTAATTGATTAATTTCATAAATATGTCGCAAAGAAAATTCGCCGG
P28	KpZWF1-DAK1p-F	ACAGGAAACAAAGGAATTTATACACTTTAAATGACCGATACGAAAGCCGTAGAA
P29	KpZWF1t-PI10DN-R	AAGCAAAAGTCCGAAGAAATCTCGAAAACACAGAAAATCAGGAGTAGAGTTGTGAAAAG
P30	KpGND2-PEX5p-F	ATTCTCAACCCAACCATCTAACTAATCGTAATGGTTGAAGCAACAGGAGATATTGG
P31	KpRPE1-2-R	TTTTTCGAATAGCTAGGTGATATGAAGGAAAGGTA
P32	KpGND2t-RPE2t-R	CTTTCCTTCATATCACCTAGCTATTCGAAAAACAACACAGCAAAATATATGGCTGTGC
P33	KpRPE1-2-F	ATGGTTAAAACAATTATTGCTCCTTCAATCCTG
P34	KpRPE1-1-R	CAAGGCAACGGCCCTAGTGA
P35	KpRPE1-1-FLD1p-F	CTTTGCTTGTTCATACAATTCTTGATATTCACAATGGTCAAACCTGTTATCGCTCC

P36	OpRPE-ADH2t-R	CATTACATAAGACGTATACAAACTATTCGGCTCACTCAAGAAGTCCGCGGG
P37	OpRPE-FLD1p-F	TGCTTGTTCATACAATTCTTGATATTCACAATGGTGAAACCAATTATTGCTCCCTC
P38	BbXFPKo-FDH1t-R	TTACTTAATATCAAATTAAATACATTTCAATTATTCATTATCACCAGCAGTAGCAGCAG
P39	BbXFPKo-HTX1p-F	TCATACTCAAACTATATTAAAACTACAACAATGACTTCTCCTGTTATTGGTACTCCATG
P40	CkPTAo-HTX1p-F	ATCCAGTTCAAGTTACCTAAACAAATCAAAATGAAATTGATGGAAAATATTTTTGGTTTGGC
P41	CkPTAo-FBP1t-R	AAATCTCGGAAACAGTGCCAATCGAACGCATTAACCTTGAGCTTGAGCTTGAACAGC
P42	DAS2-GAPt-R	CGAATTTCAGCTATTTCACATACAAATCGATTTACAACTTGTCATGCTTTGGTTTTCCC
P43	DAS2p-F	ATTACTGTTTTGGGCAATCCTGTTGATAAG
Primers for constructing pathways for fatty alcohol production		
P44	FAA1-CAT1t-R	AATCTTTAATTAATAATAAATATAGTTAGCTCAACTGTTTTGCCTATATACTTCATCGACAC
P45	CAT1t-F	GCTAACTATATTTATTATTAATTAAAGATTCTTTAACTTCGG
P46	CAT1t-FBP1t-R	ATAGGGATAATAGAAAAGTAAGGTTCCGCGGTTTAGTTTTCTGATGATGTTTCGATCATCG
P47	FaCoAR-KpO-FBP1t-R	CAAATCTCGGAAACAGTGCCAATCGAACGCATCACCAGTAAATTCCTCTCATGATGGC
P48	FaCoAR-R1-TEF1p-F	CTCACTACATACATTTTAGTTATTCGCCAACATGAACTACTTCCTTACCGGAGGTA
P49	FAA1DN-TEF1p-F	AGTGCGGCAGATAAAAGAGGCGACAGTTATCAACGTACTACGTAGTTCAATTGTACTTTTTC

P50	HFD1UP-TEF1p-R	AGTGCGGCAGATAAAAGAGGCGACAGTTATCAATGAAAAAGATGAATTGCCCAAATAGAAGAC
P51	HFD1DN-FBP1t-F	ATAGGGATAATAGAAAAGTAAGGTTCCGCGACTTTTATTGTTTCGTCTGTATCATCTGTTAAGAG
P52	FAA2-GAPt-R	GAATTTCAGCTATTTCACATACAAATCGATCTACATCTTAGTCTCCCTCAGAAGACTC
P53	FBP1t-GAPt-F	CTTTCCGATCAAATTGGAATGGAAAATTGCCGCGGAACCTTACTTTTCTATTATCCCTA
P54	FAA2DN-TEF1p-F	AGTGCGGCAGATAAAAGAGGCGACAGTTATGGTATAGCTCAATGTGTCATTATCACAGG

SI References

1. P. Cai et al., Recombination machinery engineering facilitates metabolic engineering of the industrial yeast Pichia pastoris. *Nucleic Acids Res* **49**, 7791-7805 (2021).