Supplementary information

Rewiring methanol assimilation and reductive glycine pathways in *Saccharomyces cerevisiae* to increase one-carbon recovery

Mingming Qi[a], Chao Zhu[a], Chi Cheng[a, b *], Wei Kang[a, b *], Chuang Xue[a, b *]

[a] MOE Key Laboratory of Bio-Intelligent Manufacturing, State Key Laboratory of Fine Chemicals, Frontiers Science Centre for Smart Materials Oriented Chemical Engineering, School of Bioengineering, Dalian University of Technology, Dalian 116024, China

[b] Ningbo Institute of Dalian University of Technology, Ningbo 315016, China

*** Corresponding author:** Cheng Chi@dlut.edu.cn, Kang Wei@dlut.edu.cn, Xue.1@dlut.edu.cn.

Supplementary Methods:

Whole-genome resequencing and data analysis

The control strain M01 and three evolved strains of M29 were chosen for genome sequencing. The whole genome was sequenced using Illumina HiSeq/Novaseq/MGI2000 at Genewiz Biotech (Suzhou, China). Data analysis was used by fastp (V0.23.0) and annotation for SNV/InDel was performed by Annovar (V21 Apr 2018).

^{13}C Metabolite tracer analysis

For ^{13}C metabolite tracer analysis, strain M45 was cultivated in YPD overnight and washed twice with PBS (pH=7.2) before being transferred to YNB medium with 0.1% yeast extract and 0.4% ^{13}C-methanol (Sigma-Aldrich), initial OD_{600} was around 0.3, then cultured for 144 h. The yeast (about 10^7 cells) was taken, mixed with 1000 μL of extraction solution consisting of methanol, acetonitrile, and water in a ratio of 2:2:1 (v/v). The mixture was vortexed for 30 s and then incubated in liquid nitrogen for 1 min. Subsequently, the samples were allowed to thaw at room temperature and vortexed for 30 s. This freeze-thaw cycle was repeated three times. Following that, the samples were sonicated for 10 min in a 4°C water bath and incubated for 1 h at -40°C to precipitate proteins. The samples were centrifuged at 12000 rpm for 15 min at 4°C. The supernatant was collected and evaporated to dryness by a vacuum concentrator. The dried extracts were then reconstituted in 100 μL of a mixture of acetonitrile and water in a 1:1 ratio (v/v), followed by sonication for 10 min at 50 Hz and 4°C, and centrifugation for 15 min at 16,200 g and 4°C to eliminate insoluble material. The supernatants were transferred to glass vials for subsequent analysis.

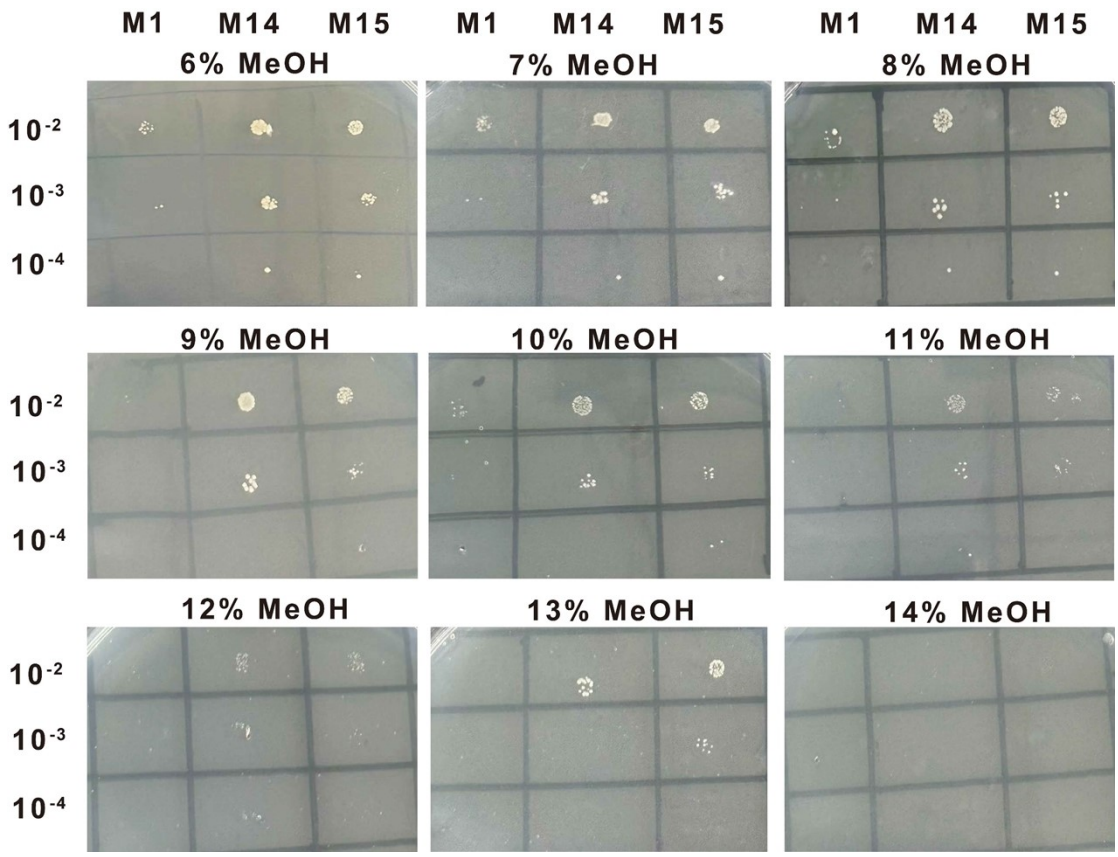
For the analysis of polar metabolites, LC-MS/MS was conducted using an UHPLC system (Vanquish, Thermo Fisher Scientific) coupled with a Waters ACQUITY UPLC BEH Amide column (2.1 mm×100 mm, 1.7 μm) and an Orbitrap Exploris 120 mass

spectrometer (Orbitrap MS, Thermo Fisher Scientific). The mobile phase comprised 25 mmol/L of ammonium acetate and 25 mmol/L of ammonia hydroxide in water (pH=9.75) (A) and acetonitrile (B). The auto-sampler temperature was 4°C, and the injection volume was 2 μL. For the analysis of non-polar metabolites, LC-MS/MS was conducted using an UHPLC system (Vanquish, Thermo Fisher Scientific) coupled with a Phenomenex Kinetex C18 column (2.1 mm×100 mm, 2.6 μm) and an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo Fisher Scientific). The mobile phase consisted of 0.01% acetic acid in water (A) and a mixture of isopropanol and acetonitrile (1:1, v/v) (B). The auto-sampler temperature was 4°C, and the injection volume was 2 μL. The ESI source conditions were set as follows: sheath gas flow rate at 50 Arb, auxiliary gas flow rate at 15 Arb, capillary temperature at 320°C, full MS resolution at 60,000, MS/MS resolution at 15,000, collision energy set to 20/30/40 eV in NCE mode, and spray voltage at 3.8 kV (positive) or -3.4 kV (negative), respectively.

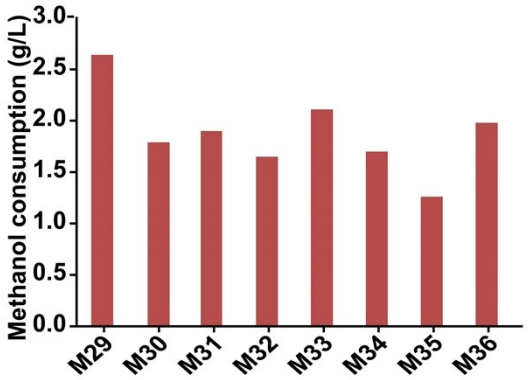
GC-MS was used for the detection of metabolites in the reductive glycine pathway. Firstly, strain M47 was cultivated in YPD overnight and washed twice with PBS (pH = 7.2) before being transferred to YNB medium with 0.1% yeast extract, 2% methanol, 250 mM sodium formate, 100 mM $(NH_4)_2SO_4$, and 35 mM $NaH^{13}CO_3$ (Sigma-Aldrich), initial OD_{600} was around 0.2, then cultured for 144 h. The metabolites were subsequently derivatized for GC-MS analysis following a previously published protocol [1]. The cells were ground in liquid nitrogen, re-suspended in 1 mL of cold (-40°C) 50% aqueous methanol, and placed in dry ice for 30 min before thawing the samples on ice. Then, 0.4 mL of chloroform was added, and the mixture was vortexed for 30 s, and centrifuged for 15 min at 14,000 rpm (4°C). The supernatant was then transferred to new 1.5 mL tubes for evaporation and storage at -80°C prior to analysis.

Metabolites were derivatized for GC-MS analysis as follows: First, 70 μL of O-Isobutylhydroxylamine hydrochloride was added to the dried pellet and incubated for 20 min at 85°C. After cooling, 30 μL of *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) was added and samples were re-incubated for 60 min at 85°C before centrifugation for 15 min at 12,000 rpm (4°C). The supernatant was transferred to an autosampler vial for GC-MS analysis.

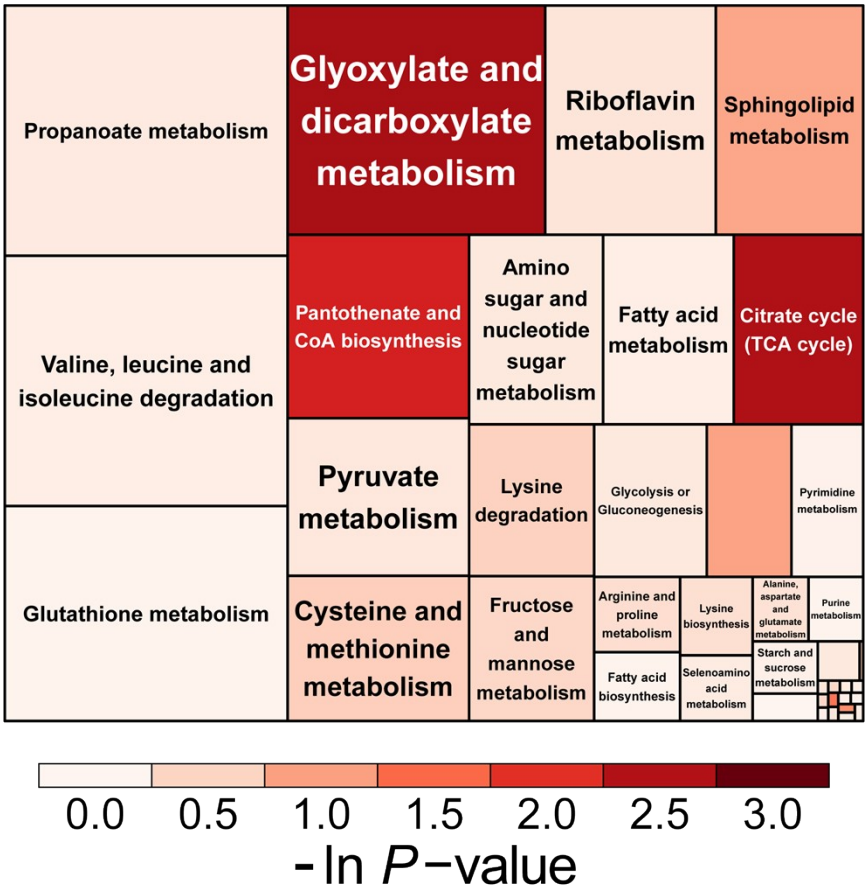
A Shimadzu QP-2020 GC-MS was programmed with an injection temperature of 250°C. The GC column used was a 30 m×0.25 mm×0.25 mm DB-5ms. GC-MS interface temperature was 300°C and ion source temperature was set at 200°C, with 70 V ionization voltage. The mass spectrometer was set to scan m/z range 50-700, with 1 kV detector. GC flow rate with helium carrier gas was 0.92 ml/min. The gradient elution of GC oven temperature was list as follows: 120°C for 3 min, followed by a ramp of 4 °C min^{-1} to 170°C, 0 min hold; 2°C min^{-1} to 182°C, 0 min hold; 20°C min^{-1} to 195°C, 0 min hold; 3°C min^{-1} to 230°C, 0 min hold; 10°C min^{-1} to 265°C, 0 min hold; 3°C min^{-1} to 280°C, 5 min hold, and the injection volume was 5 μL.



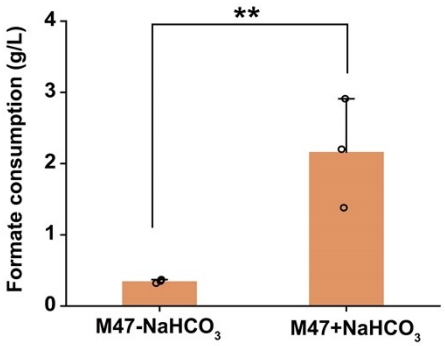
Supplementary Fig. S1 Growth in different concentrations of methanol to evaluate their tolerance to methanol. Growth on solid YNB medium with 0.1% yeast extract, and different concentrations of methanol, methanol (MeOH). Images were taken after incubating at 30°C for 6 days.



Supplementary Fig. S2 Fermentation validation of 8 single clones from the evolved strains G2-80 plate. Strains were cultured in YNB medium with 2% methanol, 0.1% yeast extract at 30°C, 220 rpm, and the initial OD_{600} was around 0.15.

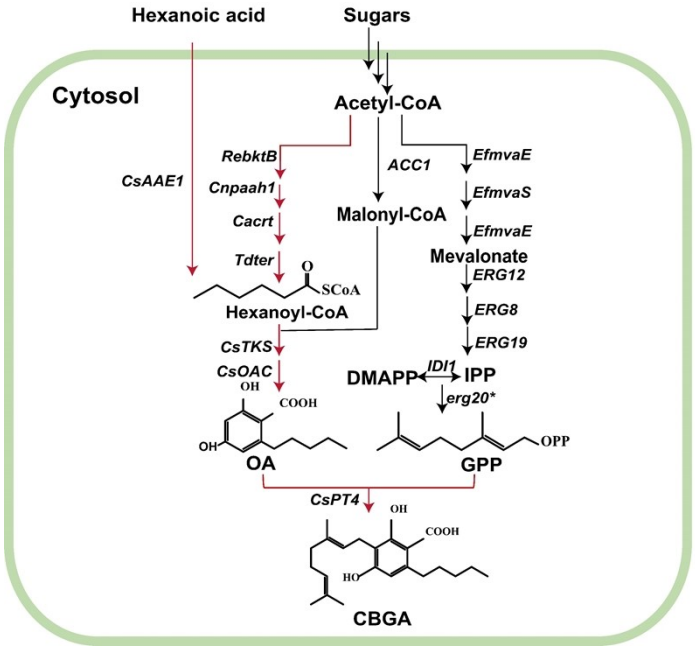


Supplementary Fig. S3 Pathway analysis of ^{13}C-labelled metabolites in strain M045. The labelled metabolites are mainly involved in glyoxylate metabolism and TCA cycle, suggesting that we have successfully rewired the central carbon metabolism of *S. cerevisiae* through modularization and ALE strategies, engineering it into a methylotrophic yeast.



Supplementary Fig. S4 Comparison of formate consumption in strain M47 in media with and without $NaHCO_3$. The consumption of formate in the medium with $NaHCO_3$

was significantly higher than that without $NaHCO_3$, proving the activity of RGP. Strains were cultured in YNB medium with 0.1% yeast extract, 2% methanol, 250 mM sodium formate, 100 mM $(NH_4)_2SO_4$, and 100 mM $NaHCO_3$ at 30°C, 220 rpm.



Supplementary Fig. S5 Biosynthetic pathway of cannabigerolic acid (CBGA) in *S. cerevisiae*. Biosynthetic pathway of CBGA contains heterologous pathway of CBGA (red arrows) and the native pathway of *S. cerevisiae* (black arrows). CsAAE1, acyl activating enzyme from *Cannabis sativa*; ACC1,acetyl-CoA carboxylase; RebktB, β-ketothiolase from *Ralstonia eutropha*; RepaaH1, 3-hydroxybutyryl coenzyme A dehydrogenase from *Ralstonia eutropha*; Cacrt, crotonase from *Clostridium acetobutylicum*; Tdter, trans-2-enoyl-CoA reductase from *Treponema denticola*; CsTKS, polyketide synthase from *C. sativa*; CsOAC, olivetolic acid cyclase from *C. sativa*; CsPT4, geranyl transferase from *C. sativa*; EfmvaE, acetoacetyl-CoA thiolase from *Enterococcus faecalis*; EfmvaS, HMG-CoA synthase from *E. faecalis*; ERG12, mevalonate kinase; ERG8, phosphomevalonate kinase; ERG19, mevalonate pyrophosphate decarboxylase; IDI1, isopentenyl-pyrophosphate delta isomerase; erg20∗, farnesyl pyrophosphate synthetase with F96W/N127W mutantion. IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranylgeranyl pyrophosphate, OA, olivetolic acid, CBGA, cannabigerolic acid.

Supplementary Table 1 Thermodynamic analysis of different glycine biosynthetic pathways

Pathway	Reactions	$\Delta_rG'^m$ (kJ/mol)
RGP	Formate+THF+ATP<=>10-formyl-THF+ADP+Orthophosphate	-2.1
	10-formyl-THF<=>5,10-methenyl-THF+H_2O	5.4
	5,10-methenyl-THF+NADPH<=>5,10-methylene-THF+$NADP^+$	-6.5
	5,10-methylene-THF+NH_4+CO_2+NADH<=>Glycine+THF+NAD^+	-4.9
Methanol-RGP	Methanol+O_2<=>Formaldehyde+H_2O_2	-98.9
	Formaldehyde+THF<=>5,10-methylene-THF+H_2O	-5.2
	5,10-methylene-THF+NH_4+CO_2+NADH<=>Glycine+THF+NAD^+	-4.9

Supplementary Table 2 Plasmids used in this study

Plasmids	Description	Source
p423-SpSgH	2μ; *HIS3*; *AmpR*; *pSNR52-BsaI-BsaI-SpSgRNA-tSUP4*	[2]
p426-SpSgH	2μ; *URA3*; *AmpR*; *pSNR52-BsaI-BsaI-SpSgRNA-tSUP4*	[3]
p423*-ccdB	2μ; *HIS3*; *AmpR*; *pSNR52-BsaI-ccdB-BsaI-tSUP4*	[2]
p426*-ccdB	2μ; *URA3*; *AmpR*; *pSNR52-BsaI-ccdB-BsaI-tSUP4*	[3]
p41K-iCas9	2μ; *KanMX*; *AmpR*; *pTEF1-iCas9-tADH1*	[4]
pESC-URA	2μ; *URA3*; *AmpR*; *pGAL1-MCS1-tCYC1*; *pGAL10-MCS2-tADH1*	[2]
pH23	2μ; *LEU2*; *AmpR*; *tADH1-pGPD1-eGFP-tCYC1-pENO2*	[2]
pH5	2μ; *LEU2*; *AmpR*; *pTEF1-eGFP-tTEF1*	[2]
pH66	2μ; *LEU2*; *AmpR*; *tTEF1-pPGK1-eGFP-tHXT7*	[2]
pH5-PFK1	2μ; *LEU2*; *AmpR*; *pTEF1-PFK1-tTEF1*	this study
pH5-PFK2	2μ; *LEU2*; *AmpR*; *pTEF1-PFK2-tTEF1*	this study
pH5-FBA1	2μ; *LEU2*; *AmpR*; *pTEF1-FBA1-tTEF1*	this study
pH7-RPE1	2μ; *LEU2*; *AmpR*; *pTPI1-RPE1-tTEF1*	this study
pH7-Bmplgx	2μ; *LEU2*; *AmpR*; *pTPI1-Bmplgx-tTEF1*	this study
pH10-BsMDH	2μ; *URA3*; *AmpR*; *pGPM1-BsMDH-tTEF1*	this study
pH9-ADK1	2μ; *LEU2*; *AmpR*; *pTEF2-ADK1-tHXT7*	this study
pH9-FLS	2μ; *LEU2*; *AmpR*; *pTEF2-FLS-tTEF1*	this study
pH5-HPS	2μ; *LEU2*; *AmpR*; *pTEF1-HPS-tTEF1*	this study
pH5-PHI	2μ; *LEU2*; *AmpR*; *pTEF1-PHI-tTEF1*	this study
pH10-AOX1	2μ; *LEU2*; *AmpR*; *pGPM1-AOX1-tTEF1*	this study
pH10-DAK1	2μ; *LEU2*; *AmpR*; *pGPM1-DAK1-tTEF1*	this study
pH11-DAS2	2μ; *LEU2*; *AmpR*; *pENO2-DAS2-tTEF1*	this study
pH11-CAT1	2μ; *LEU2*; *AmpR*; *pENO2-CAT1-tTEF1*	this study
pH7-TKL1	2μ; *LEU2*; *AmpR*; *pTPI1-TKL1-tTEF1*	this study
pH23-FBP1	2μ; *LEU2*; *AmpR*; *pGPD1-FBP1-tCYC1*	this study
pH23-ZWF1	2μ; *LEU2*; *AmpR*; *pGPD1-ZWF1-tCYC1*	this study
pH12-GCV1	2μ; *LEU2*; *AmpR*; *pFBA1-GCV1-tTEF1*	this study
pH12-GCV2	2μ; *LEU2*; *AmpR*; *pFBA1-GCV2-tTEF1*	this study
pH13-GCV3	2μ; *LEU2*; *AmpR*; *pPDC1-GCV3-tCYC1*	this study
pH13-MIS1	2μ; *LEU2*; *AmpR*; *pPDC1-MIS1-tCYC1*	this study
pH23-$SHM2^{A194C/S248T}$	2μ; *LEU2*; *AmpR*; *pGPD1-*$SHM2^{A194C/S248T}$*-tCYC1*	this study
		this study

Supplementary Table 3 Strains used in this study

Strains	Description	Source
INVSc1	*MATa/MATα his 3Δ1 leu2 trp 1-289 ura3-52*	Lian's lab
M01	INVSc1[*YPRCδ15c*::*pPGK1-Tdter-tHXT7*; *SAP155b*::*pTEF1-RebktB-tTEF1;*1414a::*pGAL1-EfmvaS-tCYC1/pGAL10-EfmvaE-tADH1*; *1114a*::*pGAL1-ERG12-tCYC1/pGAL10-IDI-tADH1*;*SAP155c*::*pGAL1-RePaaH1-tCYC1/pGAL10-CaCrt-tADH1*;*1014a*::*pGAL1-ERG19-tCYC1/pGAL10-ERG8-tADH1*; *1622b*:: *pGAL1-CsOAC-tCYC1/pGAL10-CsTKS-tADH1*;*911b*:: *pGPD1-CsAAE1-tCYC1*; *1021b*::*pGAL1-tHMG1-tCYC1/pGAL10-erg20*-tADH1*; *416d*::*pGAL1-CsPT4-tCYC1*; *308a*::*pGAL1-CBCAS-tCYC1*; *X4*:: *pGAL1-CsOAC-tCYC1/pGAL10-CsTKS-tADH1*;*XI3*:: *pGAL1-CsOAC-tCYC1/pGAL10-CsTKS-tADH1*;*XII5*:: *pGAL1-CsOAC-tCYC1/pGAL10-CsTKS-tADH1*;*208a*:: *pGPD1-CsAAE1-tCYC1*;*106a*:: *pGPD1-CsAAE1-tCYC1*; *CAN1y*::*pGAL1-CsPT4-tCYC1*;*YOLCd1b*::*pGAL1-CsPT4-tCYC1*; *1021b*:: *pGAL10-erg20*-tADH1*; *XI1*::*pTEF1-CHK-tTEF1*;*XI2*::*pTEF1-AtIPK-tTEF1*; *XII4*::*pPGK1-FAA2-tHXT7*; *X2*::*pGAL1-IDI1-tCYC1*; *X3*:: *pTEF1-ERO1-tTEF1*; *NS7*:: *pPGK1-IRE1*-tHXT7*; *NS8*:: *pPGK1-PDI1-tHXT7*; *NS9*:: *pPGK1-CNE1-tHXT7*; *NS2*:: *pTEF1-INO2-tTEF1*; *NS3*::*pPGK1-KAR2-tHXT7*; *NS14*:: *pPGK1-HAC1s*-tHXT7*; *YGLCτ3*:: *pTEF1-ERO1-tTEF1*; *Gal80Δ*]	This study
M02	M01[*NS18*:: *pTEF1-HPS-tTEF1*; *NS19*:: *pTEF1-PHI-tTEF1*]	This study
M06	M02[*NS22*:: *pTEF1-PFK2-tTEF1*; *NS23*:: *pTEF1-FBA1-tTEF1*;*YORWΔ22*:: *pTEF1-PFK1-tTEF1*;*NS24*:: *pTPI1-RPE1-tTEF1*; *NS25*:: *pTPI1-Bmglpx-tTEF1; NS10*:: *pGPM1-BsMDH-tTEF1*; *NS11*:: *pGPM1-BsMDH-tTEF1*]	This study
M07	M06[*NS5*:: *pTEF2-ADK1-tTEF1*; *NS6*:: *pTEF2-FLS-tTEF1*]	This study
M09	M06[*XI7*:: *pGPM1-AOX1-tTEF1*;*XI8*:: *pGPM1-DAK1-tTEF1*;*YCRWδ12*:: *pENO2-DAS2-tTEF1*;*YNRCΔ9*::*pENO2-CAT1-tTEF1*]	This study
M14	M09[*V1*::*pGPD1-FBP1-tCYC1*;*V3*::*pGPD1-ZWF1-tCYC1*; *NS17*:: *pGPD1-TKL1-tCYC1*; *YGR067C*:: *tYGR067C*]	This study
M15	M14[*LPL1Δ;IZH3Δ*]	This study
M27	M15-G80-2	This study
M29	M15-G80-2-1	This study
M45	M29[*4-OH*:: *pGPD1-SHM2*$^{A194C/S248T}$*-tCYC1*]	This study
M47	M45[*NS20*::*pFBA1-GCV1-tTEF1*;*NS21*::*pFBA1-GCV2-tTEF1*; *XII3*:: *pPDC1-GCV3-tCYC1*; *XI5*::*pPDC1-MIS1-tCYC1*]	This study

Supplementary Table 4 List of primers used in this study

primers	sequences (5'-3')
for plasmids construction	
IDP2-F	ACACCAGAACTTAGTTTCGACGGATGGATCCATGACAAAGATTAAGGTAGCTAACC
IDP2-R	GCGTGACATAACTAATTACATGATCTCGAGTTACAATGCAGCTGCCTCGAA
PFK1-F	CTAATCTAAGTTTTAATTACAAAGGATCCATGCAATCTCAAGATTCATGCT
PFK1-R	TATGCAACTAGAAAAGTCTTATCAATCTCCCTCGAGTCATTTGTTTTCAGCGGCTAAAG
PFK2-F	TCTAATCTAAGTTTTAATTACAAAGGATCCATGACTGTTACTACTCCTTTTGTGAATG
PFK2-R	ACTAGAAAAGTCTTATCAATCTCCCTCGAGTTAATCAACTCTCTTTCTTCCAACCAA
FBA1-F	CTAATCTAAGTTTTAATTACAAAGGATCCATGGGTGTTGAACAAATCTTAA
FBA1-R	AGAAAACGTCTTATCAATCTCCCTCGAGTTATAAAGTGTTAGTGGTACGGAAAG
GCV1-F	AACCAAGTAATACATATTCAAAAGGATCCATGTCTATAATCAAAAAAATTGTGTTTAAG
GCV1-R	AGAAAAGTCTTATCAATCTCCCTCGAGTTACTGCTTGTAGTAATGTGTGG
GCV2-F	AACCAAGTAATACATATTCAAAAGGATCCATGCTTAGGACAAGAGTGACT
GCV2-R	AAAGTCTTATCAATCTCCCTCGAGTCATTCAGTTTCGTTCGCAATTTC
GCV3-F	AATCTAAGTTTTAATTACAAAGGATCCATGTTACGCACTACTAGACTATG
GCV3-R	AAAAGTCTTATCAATCTCCCTCGAGTCAGTCATCATGAACCAGTGTCT
MIS1-F	ACACAGTCAAATCAATCAAAGGATCCATGTTGTCGAGACTATCTTTATTGAGT
MIS1-R	TGACATAACTAATTACATGATCTCGAGTTAAAATAGACCTTCAATTTCACC
for genome integration	
HPS-HOMO-F	GCAAAATAGTATACCATCTTTGGTTCCTGGACCACATTTTCGCGCGTAATACGACTCAC
HPS-HOMO-R	AAAGGGTTCGCTCAATGGCAGATGCTGCCATACGAGGACAATAGCGCCGATCAAAGTAT
PHI-HOMO-F	AACTCCGTCCACAGTACCCGATCAGCTGGCTCATCGTTATGCGCGCGTAATACGACTCA
PHI-HOMO-R	CTTGCAACGCTAGTAACGCCGTATCCACAGAGAAACCGGGATAGCGCCGATCAAAGTAT
PFK2-HOMO-F	AAGGTTGGCCCGCCAAAATCGTAAGTCTCGGTGCTCTGTTGCGCGCGTAATACGACTCA
PFK2-HOMO-R	TTACTGGAGTAATGCTTATATTATTCGCGTCCCCCCACGCATAGCGCCGATCAAAGTAT
FBA1-HOMO-F	TGTGGTGAATGCACCTGGTGCACGCTGCGAAGGTGACGTAGAGCGCGCGTAATACGACT
FBA1-HOMO-R	TGGCTTTTAACCTTATTACCCCAAGAGAGTGTCTACAGAAATAGCGCCGATCAAAGTAT
PFK1-HOMO-F	TCTTTTAAATACTTATTAACGTACTCAAACAACTACACTTTGAGCGCGCGTAATACGAC
PFK1-HOMO-R	GGTCCCTATTCCGATAATCTTAGCAGAGTGAATAGTAATAATAGCGCCGATCAAAGTAT
RPE1-HOMO-F	CATTAGATAGAGAGGGGCAGATGTTCAAGCTATACCCATTCTACTTATTCCCTTCGAGA
RPE1-HOMO-R	ACTGAAATGAAAATTTCATATTTACTTTTTTATTGTTACTGACCATGATTACGCCAAGC
Bmglpx-HOMO-F	CCTAATTAGTAGGAAGCGGAAAATAATAATATAAGAAAGTCTACTTATTCCCTTCGAGA
Bmglpx-HOMO-R	TTGCGCGCTCAAGCGGCCCTATACTGCACACCTATTACTTGACCATGATTACGCCAAGC
BsMDH-HOMO-F	ACTTTTTACCATCCTTTAGCTTTACCTAATATAATGAAATTAGTCGTGCAATGTATGAC
BsMDH-HOMO-R	TTCGAGAAATAGTTGGTATAAATAACTATAAATAACGTTTATAGCGCCGATCAAAGTAT
BsMDH-HOMO-	AACTAACCTAAGAATTTTGAAATCACAACAAAAATAATTAGTCGTGCAATGTATG

F	AC
BsMDH-HOMO-R	ACAAAAAAGTAGTAATAAATAGGTCCAAATCTTCTTTATTATAGCGCCGATCAAAGTAT
ADK1-HOMO-F	CATTTGAATTTTAGTAGTAACTAATATAAGATTCCCATCCGGGCGCCATAACCAAGGTA
ADK1-HOMO-R	TCATCTATAAAGACTGTACGCATATTTGGAACTAACTGCTATAGCGCCGATCAAAGTAT
FLS-HOMO-F	ACATTTCAAGTGGTTTCTCAAGGGAGAATCATAGTTTAGCGGGCGCCATAACCAAGGTA
FLS-HOMO-R	CTTCGTATACTTATTACGCAGGTAGGAGTGCAATAGTTGAATAGCGCCGATCAAAGTAT
AOX1-HOMO-F	CTTGACATGTAACGTAAGAAAAGAAAAAAGAGATGGCAGATAGTCGTGCAATGTATGAC
AOX1-HOMO-R	AATCATTGCTATCCCCACAAAAAACAGTGCATGTACTTTGATAGCGCCGATCAAAGTAT
DAK1-HOMO-R	TTTTTGCCATACATTTAGCGCTCCACACTCTAAATAAAGTGTGTCGACGCTGCGGGTAT
DAK1-HOMO-F	TTTATAATATGCAAAAGGCGGTAACGATATGCCGCGCAAAATAGCGCCGATCAAAGTAT
DAS2-HOMO-F	GCCGATGAAATAAAATCCTGATATCATCTATATAGTAGTGTGTCGACGCTGCGGGTAT
DAS2-HOMO-R	TGAAGATATATGAATCTACAAGAGAGAGTCAATATTTCTAATAGCGCCGATCAAAGTAT
CAT1-HOMO-F	AGACATTTTTTGGGATTAATTGTTTATAAAAGCTATGAACGTGTCGACGCTGCGGGTAT
CAT1-HOMO-R	GACATAATTGATGGGAAACAGTTATCAAAGTTATTGGATGATAGCGCCGATCAAAGTAT
TKL1-HOMO-R	GATCTGTGAAGGTTTTGAGAGAATGAGGCGAAAACACAGGCTACTTATTCCCTTCGAGA
TKL1-HOMO-F	AAGCGAGTGATTCATCATGAAAAGAATTTATCACTTCGAAATAGCGCCGATCAAAGTAT
YGR067C-HOMO-F	TTTTATTTAAAACCTTCTCTCGGGAGAAGACAATTGTTGAATGGCTGCGGGTCAAAAAA
YGR067C-HOMO-R	GCTCTTGCGGATAAGTCTTCTTTATTATAAAATACACTTACGCGGAATCTGCCAAAATA
FBP1-HOMO-F	GTGGAAAATGACCATAATGATAATTATCAATAGATAAAATCCACACCTCTACCGGCATG
FBP1-HOMO-R	CTAAAATGGTAGGCATGAGTGTTCTCCTTCTTGTGTGTCTGCAAATTAAAGCCTTCGAG
ZWF1-HOMO-F	TGAAGACTACTGAACCATGGCAGCTAATACAATCACGCCCCCACACCTCTACCGGCATG
ZWF1-HOMO-R	AAAAAGGCTAGGTACTTTAAAAATTTCCTACAAATATAGAGCAAATTAAAGCCTTCGAG
GCV1-HOMO-F	ATAAGCCCTAGAAACCTTACACCCTAATTTGCACAAGAAATAACAATACTGACAGTACTAA
GCV1-HOMO-R	CATCTGGAATATAATTCCCCCCTCCTGAAGCAAATTTTTCATAGCGCCGATCAAAGTAT
GCV2-HOMO-F	CATCATATAGGGACATACCTCTCAAGTTATTGTCTTGGATAACAATACTGACAGTACTAAAT
GCV2-HOMO-R	TCCAGGCAGGACGACACGAGAATAGACGGGCTGATCCCGTATAGCGCCGATCAAAGTAT
GCV3-HOMO-F	GACACATCTCTAAGCTGAAACTGAGAATACTGTTGTAAAACATGCGACTGGGTGAGCAT
GCV3-HOMO-R	AAGTCCATTACCCTTAAGGTTGTTGTCACAACCCACGGAGGCAAATTAAAGCCTTCGAG
MIS1-HOMO-F	AAGCTTCGAAGAATATGTAAATATAGTAGTATGAATCTAACATGCGACTGGGTGAGCAT
MIS1-HOMO-R	GAGTGTCATATATCCCTCCTTTAAATTTTTTTACACTTACGCAAATTAAAGCCTTCGAG
SHM2$^{A194C/S248T}$-HOMO-F	TACATACAACTTTTTAAACTAATATACACATTTTAGCAGACCACACCTCTACCGGCATG
SHM2$^{A194C/S248T}$-HOMO-R	ATCCTCATAAGCAGCAATCAATTCTATCTATACTTTAAAAGCAAATTAAAGCCTTCGAG
LPL1-UP-F	CCTCAACATTTATCAAATAGTTTAGCAATG
LPL1-UP-R	TCGAATTTACGGCCCAATTACTAGC

LPL1-DOWN-F	CTAGTAATTGGGCCGTAAATTCGATGAAGCGCTGTATAATATATATGGTTG
LPL1-DOWN-R	TTGAAGCAAGATATTGGAAAGACAAGGG
IZH3-UP-F	ACTACTGCATGAAATCACTTAAGTTCG
IZH3-UP-R	GGTATGACTGACTACCTTTACTTACAA
IZH3-DOWN-F	TAAGTAAAGGTAGTCAGTCATACCTACTTAATTTGATTGTCTGTTGGGA
IZH3-DOWN-R	CTACGAAATTAGCGTATCCGAAAA

Supplementary Table 5 Mutations in different evolved strains

Protein	M29-1	M29-2	M29-3	Annotation
AAD4	C23F		C23F	Enables aryl-alcohol dehydrogenase (NADP+) activity. Predicted to be involved in cellular aldehyde metabolic process.
AAD10	F33Y		F33Y	Enables aryl-alcohol dehydrogenase (NADP+) activity. Predicted to be involved in cellular aldehyde metabolic process.
DAN4	P216S		P216S	Predicted to be involved in fungal-type cell wall organization.
GDA1	V208I		V208I	Enables GDP phosphatase activity and UDP phosphatase activity. Involved in protein glycosylation.
ADP1	K26I		K26I	Predicted to enable ATPase-coupled transmembrane transporter activity.
RPL30	I92T		I92T	Involved in negative regulation of mRNA splicing, via spliceosome and rRNA processing.
TGL3	A12D		A12D	Involved in cell budding and triglyceride catabolic process.
COS2		R311K	R311K	Predicted to be involved in protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway.
YHR219W	S251P	S251P		Predicted to be active in cytoplasm.
YMR317W	E323A	E323A		No found.
COS6	V89F			Predicted to be involved in protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway.
PAU4	I24L			Predicted to be involved in fungal-type cell wall organization.
PFF1		V372F		Predicted to enable metalloexopeptidase activity and zinc ion binding activity. Predicted to be involved in proteolysis.
YPS5		I39T		Predicted to enable aspartic-type endopeptidase activity.
KEX1		A200V		Involved in apoptotic process.
LSG1		G494W		Involved in ribosomal large subunit assembly and ribosomal subunit export from nucleus.

NAM8	A363G	Enables mRNA binding activity. Involved in mRNA splice site recognition and positive regulation of mRNA splicing, via spliceosome. Located in cytoplasm and nucleus.
INA1	D42N	Predicted to be involved in fungal-type cell wall organization.
RAS2	K124T	Involved in several processes, including cytoplasm to vacuole targeting by the Cvt pathway; positive regulation of transcription by galactose; and protein localization to bud neck.

Supplementary Table 6 Integration site used in this study

site	20 bp spacer (5'-3')
NS5	GAGAGAATGACATTGCTCAG
NS6	TAGTTAATATCATCTAGATG
NS10	ATATAAAAAAATTCTTACTA
NS11	TATTCTTAGGAAAATCAACA
NS17	CTTGCGAAAAGAAAAGGGCA
NS18	AACTGCTCAGGGCGGATAAC
NS22	ATCTTAAATGAAAGACAGAG
NS24	ATTATGAAAGTTTTCAACTA
NS25	CACCATTCAAGTTACCGAGA
YORWΔ22	CGTTTGGGTAGTTCAGCTAA
YCRWδ12	TTTAATGGAAACGAAATGCA
YNRCΔ9	GCACGGAACTTCGGACCTAG
XI7	GTCAGTAACAGTGATTGCTG
XI8	GATGAAATAGCCTCAGTTAC
V1	CGTTTATAGACGGCACTGTC
V3	GTGCTCTTAAGTCGTAATGA
X1	GTGCCTGCTGCTATGCTCAA
III3	AGACATATGATAATCGAGCA
4-OH	GTTCGTGAAGCATTCTTAGC

Supplementary Table 7 Heterologous genes used in this study

genes	gene sequence (5'-3')
BsMDH	ATGAAAGCTGCTGTTGTTAATGAATTTAAGAAAGCTTTGGAAATTAAAGAAGTTGAAA GACCAAAATTGGAAGAAGGTGAAGTTTTGGTTAAAATTGAAGCTTGTGGTGTTTGTCA TACTGATTTGCATGCTGCTCATGGTGATTGGCCAATTAAACCAAAATTGCCATTGATTC CTGGTCATGAAGGTGTTGGTATTGTTGTTGAAGTTGCTAAAGGTGTTAAATCTATTAAA GTTGGTGATAGAGTTGGTATTCCATGGTTGTATTCTGCTTGTGGTGAATGTGAATATTG TTTGACTGGTCAAGAAACTTTGTGTCCACATCAATTGAATGGTGGTTATTCTGTTGATG GTGGTTATGCTGAATATTGTAAAGCTCCTGCTGATTATGTTGCTAAAATTCCTGATAAT TTAGATCCTGTTGAGGTCGCTCCTATTTTGTGTGCTGGTGTTACTACTTATAAAGCTTTG AAAGTTTCTGGTGCTAGACCTGGTGAATGGGTTGCTATTTATGGTATTGGTGGTTTGGG TCATATTGCTTTGCAATATGCTAAAGCTATGGGTTTGAATGTTGTTGCTGTTGATATTTC TGATGAAAAATCTAAATTGGCTAAAGATTTGGGTGCTGATATTGCTATTAATGGTTTGA AAGAAGATCCTGTTAAAGCTATTCATGATCAAGTTGGTGGTGTTCATGCTGCTATTTCT GTTGCTGTTAATAAAAAGGCTTTTGAACAAGCTTATCAATCTGTTAAAAGAGGTGGTA

	CTTTGGTTGTCGTTGGTTTGCCAAATGCTGATTTGCCAATTCCAATTTTTGATACTGTTT TGAATGGTGTTTCTGTTAAAGGTTCTATTGTTGGTACTAGAAAAGATATGCAAGAAGCT TTGGATTTTGCTGCTAGAGGTAAAGTTAGACCAATTGTTGAAACTGCTGAGTTGGAAG AAATTAATGAAGTTTTTGAGAGAATGGAGAAAGGTAAAATTAATGGTAGAATTGTTTT GAAATTGAAAGAAGACTAA
BmHPS	ATGGAATTGCAATTGGCTTTGGATTTGGTTAATATTGAAGAAGCAAAACAAGTTGTTG CTGAAGTTCAAGAATATGTTGATATTGTTGAAATTGGTACTCCTGTTATTAAAATTTGG GGTTTGCAAGCTGTTAAGGCTGTTAAAGATGCTTTTCCACATTTGCAAGTTTTGGCTGA TATGAAAACTATGGATGCTGCAGCTTATGAAGTTGCTAAAGCTGCTGAACATGGTGCT GATATTGTTACTATTTTGGCTGCAGCTGAGGACGTTTCTATTAAAGGTGCTGTTGAGGA AGCTAAAAAATTGGGTAAAAAAATTTTGGTTGATATGATTGCTGTTAAAAATTTGGAA GAAAGAGCAAAGCAAGTTGACGAAATGGGTGTTGATTATATTTGTGTTCATGCTGGTT ATGATTTACAAGCTGTTGGTAAGAATCCATTGGATGATTTGAAAAGAATTAAAGCTGT TGTTAAAAATGCTAAAACTGCTATTGCTGGTGGTATTAAATTGGAAACTTTGCCTGAAG TTATTAAAGCTGAACCTGATTTGGTTATTGTTGGTGGAGGTATTGCTAATCAAACTGAT AAAAAAGCTGCAGCTGAAAAAATTAATAAATTGGTTAAACAAGGTTTGTAAACTTTAC GCCGAGAGGCTTTATAGGAAGGACGGTACCATCCCAGTCGACGAGGAAAATAGAATT AGAATCGATGACTGGGAACTTGAGGAGGACGTCCAGAAAGCCGTTTCTGCCTTGATGG AGAAGGTCACCGGTGAAAATGCCGAGAGTTTGACCGACTTGGCCGGTTACAGACACG ACTTCTTGGCCAGTAACGGTTTTGACGTTGAGGGAATCAACTACGAGGCCGAAGTTGA GAGGTTCGACAGAATCTAA
BmPHI	ATGTTGACTACTGAATTTTTGGCTGAAATTGTTAAAGAATTGAATTCTTCTGTTAATCA AATTGCTGATGAAGAAGCTGAAGCTTTGGTTAATGGTATTTTGCAATCTAAAAAAGTTT TTGTTGCTGGTGCTGGTAGATCTGGTTTTATGGCTAAATCTTTTGCTATGAGAATGATG CATATGGGTATTGATGCTTATGTTGTTGGTGAAACTGTTACTCCAAATTATGAAAAAGA AGATATTTTGATTATTGGTTCTGGTTCTGGTGAAACTAAATCTTTGGTTTCTATGGCTCA AAAAGCTAAATCTATTGGTGGTACTATTGCTGCTGTTACTATTAATCCTGAATCTACTA TTGGTCAATTGGCTGATATTGTTATTAAAATGCCTGGTTCTCCAAAAGATAAATCTGAA GCTAGAGAAACTATTCAACCAATGGGATCTTTGTTTGAACAGACATTGTTATTGTTCTA TGATGCTGTTATCTTGAGATTTATGGAAAAAAAAGGTTTGGATACTAAAACTATGTAT GGTAGACATGCTAATTTGGAATAA
Bmglpx	ATGAGAGAATTGAAGTCAGAAAAAAGAGTTCAATCTTTGGCTATGGAATTTTTGTCTG TTGCTCAACAAGCTGCTTTGGCTTCTTATCCATGGATTGGTAAAGGTAATAAAAATGAA GTTGATAGAGCTGGTACTGAAGCTATGAGAAATAGATTGAATTTGATTGATATGTCTG GTTTGATTGTTATTGGTGAAGGTGAAATGGATGAAGCTCCAATGTTGTATATTGGTGAA GAATTGGGTACTGGTAAAGGTCCACAATTGGATATTGCTGTTGATCCTGTTGATGGTAC TGGTTTGATGGCTAAAGGTATGGATAATTCTATTGCTGTTATTGCTGCTTCTACTAGAG GTTCTTTGTTGCATGCTCCTGATATGTATATGGAAAAAATTGCTGTTGGTCCAAAAGCT AAAGGTTGTGTTAATTTGGATGCTTCTTTGACTGAAAATATGAAATCTGTTGCTAAAGC TTTGGGTAAAGATTTGAGAGAATTAACTGTTATGATTCAAGATAGACCAAGACATGAT CATTTGATTCAACAAGTTAGAGATGTTGGTGCTAGATTGAAATTGTTTTCTGATGGTGA TGTTACTAGAGCTATTGGTACTGCTTTGGAAGAAGTTGATGTTGATATTTTGGTTGGTA CTGGTGGTGCTCCTGAAGGTGTTATCGCTGCTACTGCTTTGAAATGTTTGGGTGGTGAT TTTCAAGGTAGATTGGCTCCTCAAAATGAAGAGGAATTTGACAGATGTATCACTATGG GTATTACTGACCCTAGAAAAATCTTTACTATTGATGAAATTGTTAAATCAGATGATTGT TTTTTTGTTGCTACTGGTATTACAGATGGTTTGTTGATTAATGGTATTAGAAAAAAAGA AGACGGTTTGATGCAAACTCATTC
PpAOX	ATGGCTATCCCCGAAGAGTTTGATATCCTAGTTCTAGGTGGTGGATCCAGTGGATCCTG TATTGCCGGAAGATTGGCAAACTTGGACCACTCCTTGAAAGTTGGTCTTATCGAAGCA GGTGAGAACAACCTCAACAACCCATGGGTCTACCTTCCAGGTATTTACCCAAGAAACA TGAAGTTGGACTCCAAGACTGCTTCCTTCTACACTTCTAACCCATCTCCTCACTTGAAT GGTAGAAGAGCCATTGTTCCATGTGCTAACGTCTTGGGTGGTGGTTCTTCTATCAACTT CATGATGTACACCAGAGGTTCTGCTTCTGATTACGATGACTTCCAAGCCGAGGGCTGG AAAACCAAGGACTTGCTTCCATTGATGAAAAAGACTGAGACCTACCAAAGAGCTTGCA ACAACCCTGACATTCACGGTTTCGAAGGTCCAATCAAGGTTTCTTTCGGTAACTACACC TACCCAGTTTGCCAGGACTTCTTGAGGGCTTCTGAGTCCCAAGGTATTCCATACGTTGA CGACTTGGAAGACTTGGTTACTGCTCACGGTGCTGAACACTGGTTGAAGTGGATCAAC AGAGACACTGGTCGTCGTTCCGACTCTGCTCATGCATTTGTCCACTCTACTATGAGAAA CCACGACAACTTGTACTTGATCTGTAACACGAAGGTCGACAAAATTATTGTCGAAGAC GGAAGAGCTGCTGCTGTTAGAACCGTTCCAAGCAAGCCTTTGAACCCAAAGAAGCCAA GTCACAAGATCTACCGTGCTAGAAAGCAAATCGTTTTGTCTTGTGGTACCATCTCCTCT CCATTGGTTTTGCAAAGATCCGGTTTTGGTGACCCAATCAAGTTGAGAGCCGCTGGTGT TAAGCCTTTGGTCAACTTGCCAGGTGTCGGAAGAAACTTCCAAGACCACTACTGTTTCT TCAGTCCTTACAGAATCAAGCCTCAGTACGAGTCTTTCGATGACTTCGTCCGTGGTGAT GCTGAGATTCAAAAGAGAGTCTTTGACCAATGGTACGCCAATGGTACTGGTCCTCTTG CCACTAACGGTATCGAAGCTGGTGTCAAGATCAGACCAACACCAGAAGAACTCTCTCA AATGGACGAATCCTTCCAGGAGGGTTACAGAGAATACTTCGAAGACAAGCCAGACAA

	GCCAGTTATGCACTACTCCATCATTGCTGGTTTCTTCGGTGACCACACCAAGATTCCTC CTGGAAAGTACATGACTATGTTCCACTTCTTGGAATACCCATTCTCCAGAGGTTCCATT CACATTACCTCCCCAGACCCATACGCAGCTCCAGACTTCGACCCAGGTTTCATGAACG ATGAAAGAGACATGGCTCCTATGGTTTGGGCTTACAAGAAGTCTAGAGAAACCGCTAG AAGAATGGACCACTTTGCCGGTGAGGTCACTTCTCACCACCCTCTGTTCCCATACTCAT CCGAGGCCAGAGCCTTGGAAATGGATTTGGAGACCTCTAATGCCTACGGTGGACCTTT GAACTTGTCTGCTGGTCTTGCTCACGGTTCTTGGACTCAACCTTTGAAGAAGCCAACTG CAAAGAACGAAGGCCACGTTACTTCGAACCAGGTCGAGCTTCATCCAGACATCGAGTA CGATGAGGAGGATGACAAGGCCATTGAGAACTACATTCGTGAGCACACTGAGACCAC ATGGCACTGTCTGGGAACCTGTTCCATCGGTCCAAGAGAAGGTTCCAAGATCGTCAAA TGGGGTGGTGTTTTGGACCACAGATCCAACGTTTACGGAGTCAAGGGCTTGAAGGTTG GTGACTTGTCCGTGTGCCCAGACAATGTTGGTTGTAACACCTACACCACCGCTCTTTTG ATCGGTGAAAAGACTGCCACTTTGGTTGGAGAAGATTTAGGATACTCTGGTGAGGCCT TAGACATGACTGTTCCTCAGTTCAAGTTGGGCACTTACGAGAAGACCGGTCTTGCTAG ATTCTAA
PpCTA	ATGTCTCAACCACCTAAATcGGACAACATCAAATGGTGCTCCTGTCTCCGATGTATTTG CTACAGAGAGAGCCACCTTCGACAACGCTAATCACGCTAACAATGCCCCCAAAGTTGG GCCTCTGCTGCTACAAGACTTTCAATTGATTGATTCTCTTGCCCATTTCGACAGAGAAC GTATTCCAGAGAGAGTTGTGCATGCCAAAGGTGCGGGAGCTTTCGGTGAGTTTGAGGT GACTGACGACATATCTGACGTTTGTGCTGCCAAATTCTTGGATACTATTGGGAAGAAA ACCCGTATCTTCACTCGATTCTCCACCGTTGGAGGTGAGAAAGGTTCTGCCGACAGTGC CCGTGACCCAAGAGGGTTTTCCACCAAGTTTTACACAGAGGAAGGTAACTTGGACCTT GTGTACAACAACACTCCCATCTTCTTTATCAGAGATCCATCCAAGTTCCCACATTTCAT CCACACCCAAAAGAGGAATCCTGCTACTAACCTGAAGGACGCCAATATGTTCTGGGAT TACCTTGTTAACAACCAAGAGTCCATTCATCAGGTGATGTACTTGTTCTCAGATCGTGG AACTCCTGCTTCTCTTAGAAAAATGAACGGGTACTCTGGTCACACTTACAAATGGTAC AACAAGAAAGGTGAGTGGGTTTACGTACAAGTTCACTTCAAGTCGGACCTGGGTGTCG TAAACTTCAACAATGAAGAGGCTGGAAAGCTTGCTGGCGAGGATCCAGACTACCACAC CGGTGATCTTTTCAACGCCATTGAGAGAGGCGAGTATCCTTCATGGACTTGTTACATTC AGACCATGACTCAAGAACAAGCTGCCAAGCAACCATTTTCCGTGTTTGACTTGACCAA GGTTTGGCCACACAAAGACTTCCCATTGAGGAGATTTGGTAAATTCACTTTGAACGAA AACCCAAAGAACTACTTTGCCGAAGTTGAGCAAGCCGCCTTCTCTCCATCTCATACTAT CCCTAGTATGCAGCCATCTGCTGATCCAGTCCTTCAATCTCGACTGTTCTCCTACCCAG ATACTCACCGTCATAGATTGGGTGTTAACTATCAGCAGATTCCTGTCAATTGTCCAGTG GCTCCTGTTTTTACCCCTCAGATGCGTGATGGTTCCATGACTGTCAATGGCAACCTTGG GTCTACTCCCAACTACAAGTCTTCTTTCTGTCCATTCTCTACAGAGGCACAGATCCAGA CCAATTCACACACACCAGAAGAAGTTTTGGCTGCTCACACTGAGAAATTCCACTGGGG AGGTATCCTTGACAGTAAGAGCTACGATTTTGAACAGCCAAGAGCGCTTTGGAAAGTA TTCGGCAAAACTCCTGGTCAGCAACGTAACTTCTGCCACAACGTTGCCGTTCACGTTGC GGCTGCCAACCATGAAATTCAAGACAGGGTTTTTGAATACTTCTCCAAGGTTTACCCTG AAATTGGTGACCAGATTCGTAAAGAAGTATTGCAGCTATCTCCAAGAGGTGACTCTGC AGCAAGATTGTAG
PpDAK	ATGTCTAGTAAACATTGGGATTACAAGAAAGATCTGGTACTCAGCCATCTGGCTGGAT TATGTCAGTCCAACCCCCATGTAAGGCTAATTGAGTCCGAAAGAGTCGTCATCTCCGC CGAGAACCAGGAGGACAAGATTACCTTGATCTCTGGTGGAGGTTCAGGACACGAGCCT CTACATGCTGGCTTTGTCACCAAGGACGGTCTTTTGGATGCCGCTGTGGCCGGGTTCAT CTTTGCCTCGCCATCTACTAAACAGATATTTAGCGCAATCAAAGCCAAACCTTCGAAG AAGGGAACTTTGATCATCGTCAAGAACTATACAGGAGACATTCTTCACTTCGGTCTTGC CGCCGAAAAGGCCAAGGCCGAAGGTCTCAACGCAGAACTGCTGATTGTTCAGGACGA TGTCTCTGTTGGTAAAGCCAAGAATGGCCTGGTAGGGCGTAGAGGTTTGGCTGGAACT TCTTTAGTGCACAAGATTTTGGGTGCCAAAGCTTATTTACAAAAGGACAACCTGGAAT TGCATCAGCTGGTCACTTTCGGTGAGAAGGTCGTTGCCAACCTGGTTACAATTGGTGCT TCCTTGGACCATGTCACCATTCCAGCCAGAGCAAACAAACAGGAGGAAGATGATTCAG ATGACGAGCACGGCTACGAAGTGCTAAAGCACGATGAATTTGAAATCGGAATGGGTA TTCACAACGAACCAGGTATCAAGAAGTCGTCGCCAATTCCAACAGTAGACGAATTGGT GGCTGAGCTACTGGAGTACCTTCTTTCGACTACCGATAAGGACCGAAATTACGTCCAA TTTGACAAGAATGATGAAGTTGTGCTACTGATCAATAACTTGGGAGGAACCTCAGTGT TGGAATTGTACGCTATTCAGAACATTGTTGTCGACCAACTGGCCTCCAAATATTCCATT AAACCAGTCCGCATTTTCACTGGCACTTTCACCACATCACTGGATGGGCCCGGTTTTTC TATCACTTTATTGAATGCTACCAAGACAGGAGATAAAGATATCCTAAAGTTTTTAGAC CACAAGACTAGTGCCCCTGGCTGGAACTCCAACATATCAGACTGGTCTGGCAGGGTTG ACAATTTTATTGTGGCTGCCCCAGAAATAGACGAAGGAGACAGTTCATCTAAGGTATC TGTTGATGCTAAGCTGTATGCTGACCTTCTGGAGTCTGGTGTGAAGAAGGTCATTTCAA AAGAGCCAAAGATCACATTGTATGACACTGTTGCAGGAGATGGTGATTGTGGTGAAAC GTTGGCGAATGGTTCCAATGCCATTTTAAAGGCTCTGGCTGAAGGAAAGCTTGATCTC AAAGACGGTGTTAAATCACTGGTGCAAATTACAGACATCGTGGAAACTGCCATGGGTG GAACATCTGGAGGTCTGTACTCCATTTTCATCAGCGCTTTAGCAAAGTCTTTGAAAGAG

	AAGGAACTTTCCGAGGGTGCCTACACACTGACCCTTGAAACGATCTCTGGATCCTTAC AGGCAGCACTTCAGTCTCTATTCAAATACACAAGAGCACGTACTGGAGACAGAACTCT TATTGACGCTTTGGAGCCATTTGTAAAGGAATTCGCCAAGTCTAAGGATCTGAAACTT GCAAACAAAGCTGCCCATGATGGTGCTGAAGCCACAAGAAAGCTCGAAGCCAAGTTT GGAAGAGCATCTTATGTTGCCGAAGAAGAGTTCAAACAATTCGAAAGTGAAGGTGGA CTACCTGATCCTGGTGCTATTGGTCTTGCCGCTTTGATTTCAGGAATTACTGATGCATA CTTCAAATCTGAAACCAAGTTGTAG
PpDAS2	ATGGTTGACAACACTATCTATGCTATTGTTGGTGATGCTTGTTTGCAAGAGGGACCTGC TTTGGAATCGATTTCCTTAGCTGGTCACTTGGCCTTGGACAACCTTATTGTGATCTACG ACAACAACCAGGTTTGTTGTGATGGTTCCGTCGATGTTAACAACACCGAAGACATTTCT GCTAAGTTTAGAGCTCAGAACTGGAATGTCATTGAAGTCGAGAATGGTTCTAGAGATG TTGCTACCCTTGTCAAGGCCATCGAATGGGCCAAGGCTGAGAATGAGAGACCAACTCT GATCAACGTTAGAACTGAAATTGGACAGGATTCTGCTTTCGGTAACCACCACGCTGCT CACGGTTCTGCTCTTGGTGAGGAAGGTATCCGGGAGTTGAAGGCCAAGTACGGTTTCG ATGTCGCTAGAAAGTTCTGGTTCCCACAGGAGGTCTATGATTTCTTTGCTGAAAAACCA GCCGAGGGTGATCAACTAGTTGCTAACTGGAAGAAACTTTTGGATGAGTACGTTAAGA ACTATCCTCAAGAAGGTGAGGAATTAAAGGCCCGTATTAGAGGTGAACTTCCAAAGAA CTGGAAGAGTTTCATTCCACAGGACAAACCAACCGAGCCAACTGCTACCAGAACCTCT GCTAGAGAAATTGTTAGATCTCTGGGACAAAACCTTCCTCAGGTTATTGCTGGTTCTGG TGACTTGTCCGTGTCCATTCTTTTGAACTGGGGAGGAGTTAAGTACTTCTTCAACCCTA AGTTACAAACTTTCTGTGGATTGGGTGGTGACTACTCTGGTAGATATATTGAGTTTGGT ATCAGAGAACACTCTATGTGTGCTATTGCCAATGGTTTGGCTGCATACAACAAGGGTA CTTTCTTGCCTATTACCTCAACTTTCTACATGTTCTACCTGTATGCAGCACCTGCCTTGC GTATGGCTGCACTTCAAGAGTTGAAAGCAATTCACATTGCTACACACGACTCCATCGG AGCTGGTGAAGATGGTCCAACGCACCAGCCTATTGCTTTGTCTTCATTATTCAGAGCTA TGCCCAACTTCTACTACATTAGACCAGCCGATGCTACCGAGGTTGCAGCTCTGTTTGAA GTAGCTGTTGAGCTCGAGCACTCCACCTTGTTCTCTCTGTCCAGACACGAGGTTGAGCA ATACCCAGGTAAGACTTCGGCTGAGGGAGCCAAAAGAGGTGGTTACGTCGTTGAAGA CTGTGAGGGTAAGCCAGACGTCCAATTAATTGGTGCTGGTTCCGAATTGGAGTTTGCC GTCAAAACTGCTCGTTTGCTAAGACAACAGAAGGGATGGAAGGTCAGAGTTCTGTCAT TCCCATGTCAGAGACTGTTTGACCAACAATCCCTGGCATACAGACGTTCTGTCCTTAGA AGAGGAGAGGTTCCAACTGTCGTTGTTGAGGCCTATGTCGCATACGGATGGGAGAGAT ACGCCACTGCTGGTTACACCATGAACACCTTTGGTAAGTCTCTTCCTGTTGAGGATGTC TACAAATACTTCGGATACACTCCTGAGAAGATTGGTGAGAAGGTTGCTGCATACGTCA ACTCTATTAAGGCTAGTCCTCAAATCCTTTACGAATTCACCGATTTGAAGGGAAAACC AAAGCACGACAAACTATAA
FLS	ATGGCTATGATTACCGGTGGTGAATTGGTTGTTAGAACTTTGATTAAGGCTGGTGTTGA ACATTTGTTTGGTTTGCACGGTATCCACATTGATACCATTTTCCAAGCTTGTTTAGATCA CGATGTTCCAATTATTGATACTAGACACGAAGCCGCTGCTGGTCACGCCGCTGAAGGT TACGCTAGAGCTGGTGCTAAGTTAGGTGTTGCATTAGTCACTGCTGGTGGTGGTTTCAC TAACGCTGTTACTCCAATTGCTAATGCTCGTACTGATAGAACTCCAGTTTTGTTCTTGA CAGGTTCTGGTGCTTTAAGAGATGATGAAACTAACACTTTACAAGCTGGTATTGATCA AGTTGCTATGGCTGCTCCAATTACTAAGTGGGCCCATAGAGTTATGGCAACTGAACAT ATTCCAAGATTGGTCATGCAAGCTATTAGAGCTGCCTTGTCTGCTCCAAGGGGTCCAGT TTTGTTGGATTTGCCATGGGATATCTTGATGAACCAGATTGATGAAGATTCTGTTATCA TCCCTGATTTGGTTTTGTCCGCCCATGGTGCTCATCCAGACCCAGCTGATTTGGATCAA GCCTTGGCACTATTAAGAAAGGCTGAAAGACCAGTTATCGTTTTAGGTAGTGAAGCTT CAAGAACTGCTAGAAAGACCGCTTTGTCAGCTTTCGTTGCTGCCACTGGTGTCCCTGTT TTCGCTGACTATGAAGGTTTATCTATGTTATCTGGTTTGCCTGATGCTATGAGAGGTGG TTTAGTTCAAAACCTTTACTCTTTCGCTAAGGCCGATGCCGCTCCAGATCTAGTTTTGA TGTTGGGTGCTAGATTCGGTTTGAACACCGGTCATGGTTCTGGTCAATTAATTCCACAT TCTGCTCAAGTTATTCAAGTTGATCCAGACGCTTGTGAATTGGGTAGATTGCAAGGCAT TGCTTTAGGTATCGTTGCTGATGTTGGTGGTACCATAGAAGCTTTAGCTCAAGCAACCG CTCAAGACGCTGCTTGGCCAGATAGAGGTGACTGGTGTGCTAAAGTTACTGACTTAGC TCAAGAAAGATACGCTTCTATTGCTGCTAAGTCATCTTCTGAACATGCTTTGCATCCTT TCCACGCTTCCCAAGTTATTGCTAAACACGTTGATGCTGGTGTTACTGTTGTTGCCGAT GGTGGTTTGACTTACTTGTGGTTGTCAGAAGTTATGTCTCGTGTTAAGCCTGGTGGTTT CTTGTGTCATGGTTACTTGAACTTGATGGGTGTTGGTTTCGGTACAGCTTTAGGTGCTC AAGTCGCCGACTTAGAAGCCGGTCGTCGTACTATTTTGGTCACTGGTGACGCCTCTGTT GTTTACTCTATTGGTGAATTCGATACCTTGGTTAGAAAGCAATTACCATTGATTGTTAT TATCATGAACAATCAATCTTGGGGTTGGACTTTACATTTCCAACAATTAGCTGTCGGTC CAAATAGAGTTACTGGTACTAGATTAGAAAACGGTTCCTATCATGGTGTCGCTGCTGC CTTCGGTGCTGATGGTTACCATGTTGATTCTGTTGAATCCTTTTCAGCTGCTTTAGCTCA AGCTTTGGCTCATAACAGACCTGCTTGTATTAATGTTGCTGTCGCTTTGGACCCAATTC CACCAGAAGAATTGATTTTGATTGGTATGGACCCATTTGCTGGTTCTACTGAAAACTTG TACTTCCAATCCGGTGCTTAA

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