

Metabolic engineering of yeast for the production of carbohydrate-derived foods and chemicals from C₁₋₃ molecules

In the format provided by the authors and unedited

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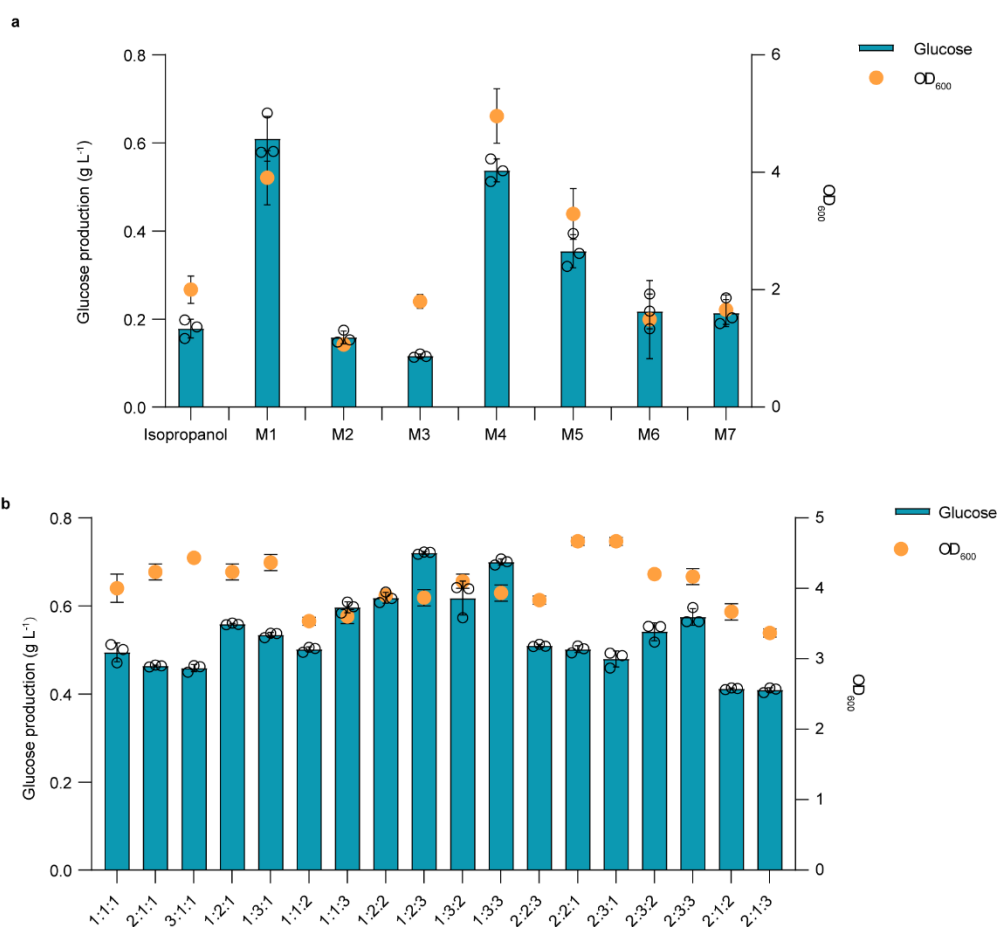
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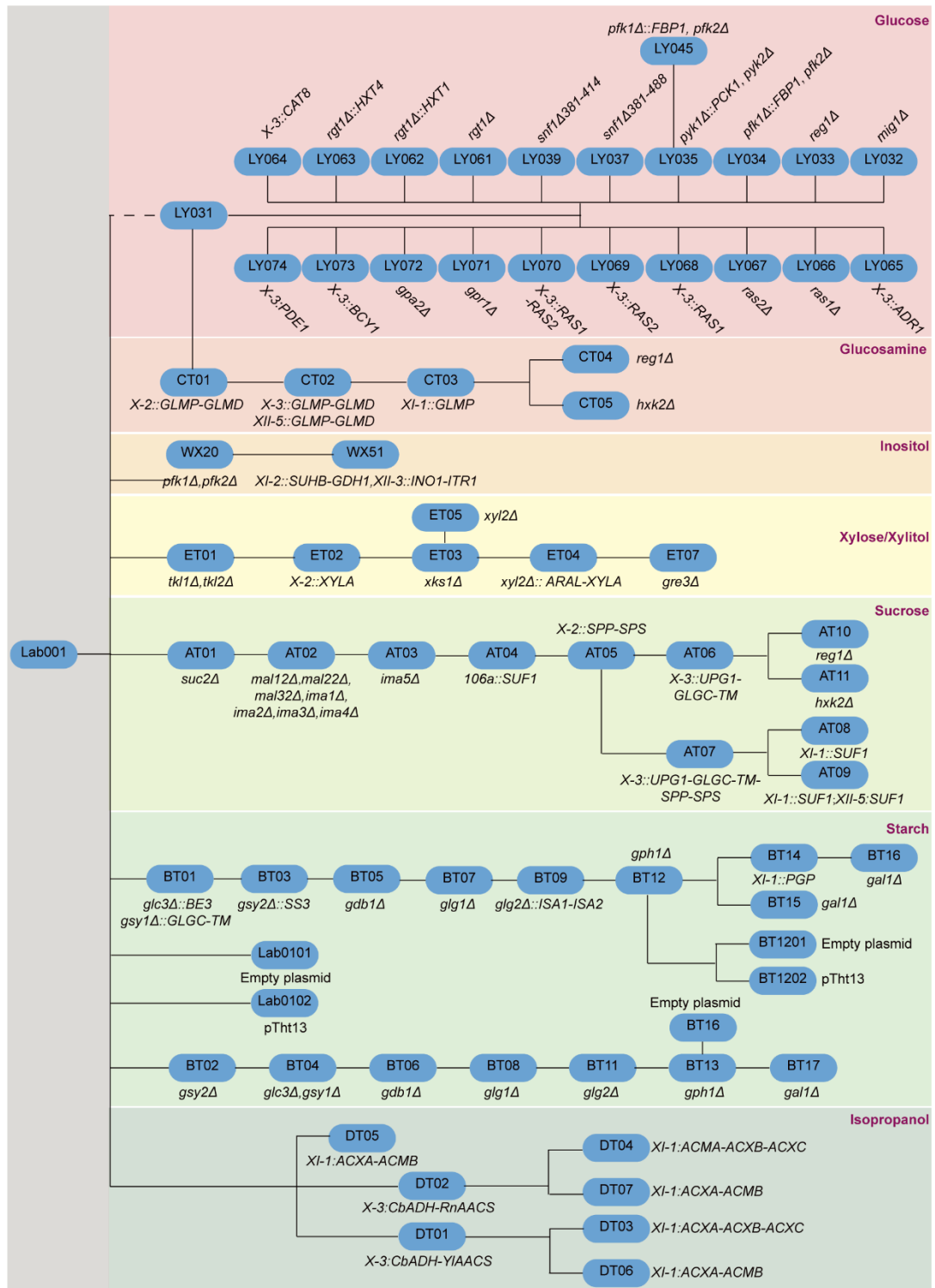
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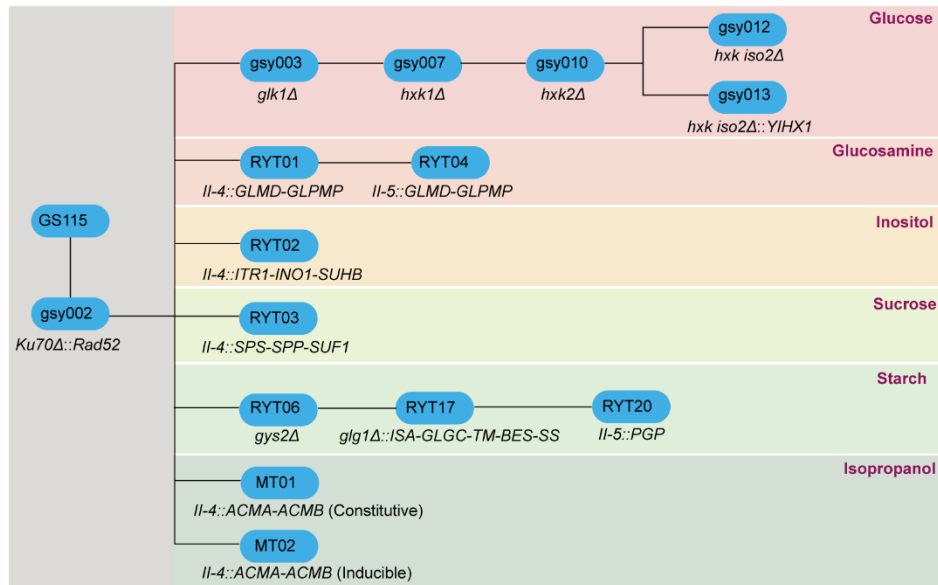
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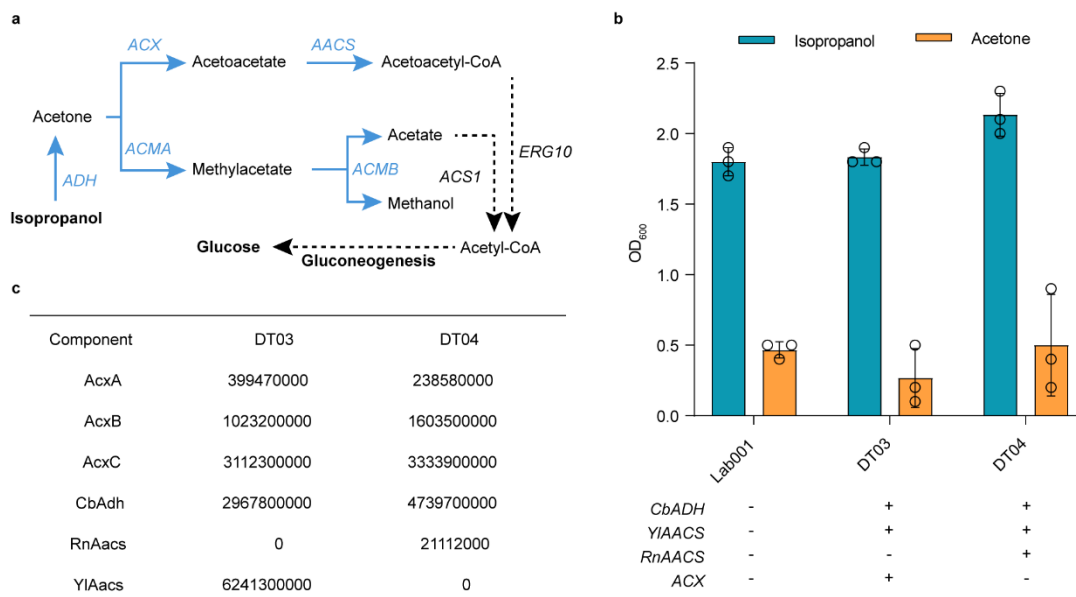
Supplementary Fig. 1 The effect of different substrate mixtures (a) and mix ratios (b) on cell growth and glucose production. *S. cerevisiae* strain LY031 was cultivated in minimal medium with 10 g L⁻¹ yeast extract, containing a total mixture of carbon sources at a concentration of 10 g L⁻¹ for 120 h. The data of cell growth and glucose production were subtracted from the background in absence of carbon source. M1, Isopropanol+Propionate; M2, Ethylene glycerol+Isopropanol; M3, Ethylene glycerol+Propionate; M4, Ethylene glycerol+Propionate+Isopropanol; M5, Ethylene glycerol+Propionate+Isopropanol+Oxalic acid; M6, Ethylene glycerol+Propionate+Isopropanol+ Oxalic acid+Methanol; M7, Ethylene glycerol+Propionate+Isopropanol+ Oxalic acid+Methanol+ Formate. All data are presented as mean \pm SD of biological triplicates (n=3).



Supplementary Fig. 2 Construction of recombinant strains by metabolic engineering of *S. cerevisiae* for production of carbohydrate derived products using different substrates.

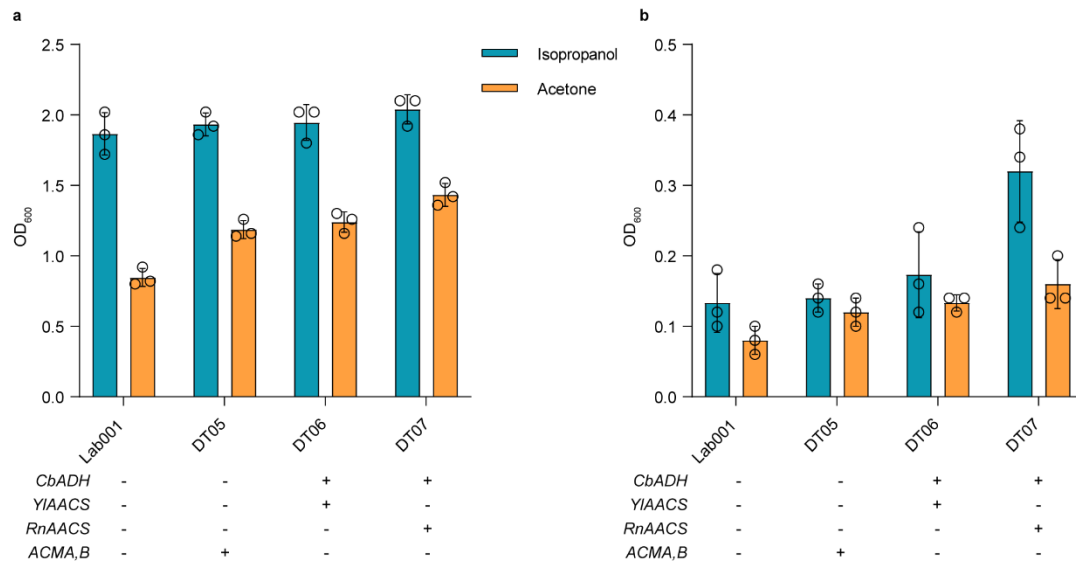


Supplementary Fig. 3 Construction of recombinant strains by metabolic engineering of *P. pastoris*.

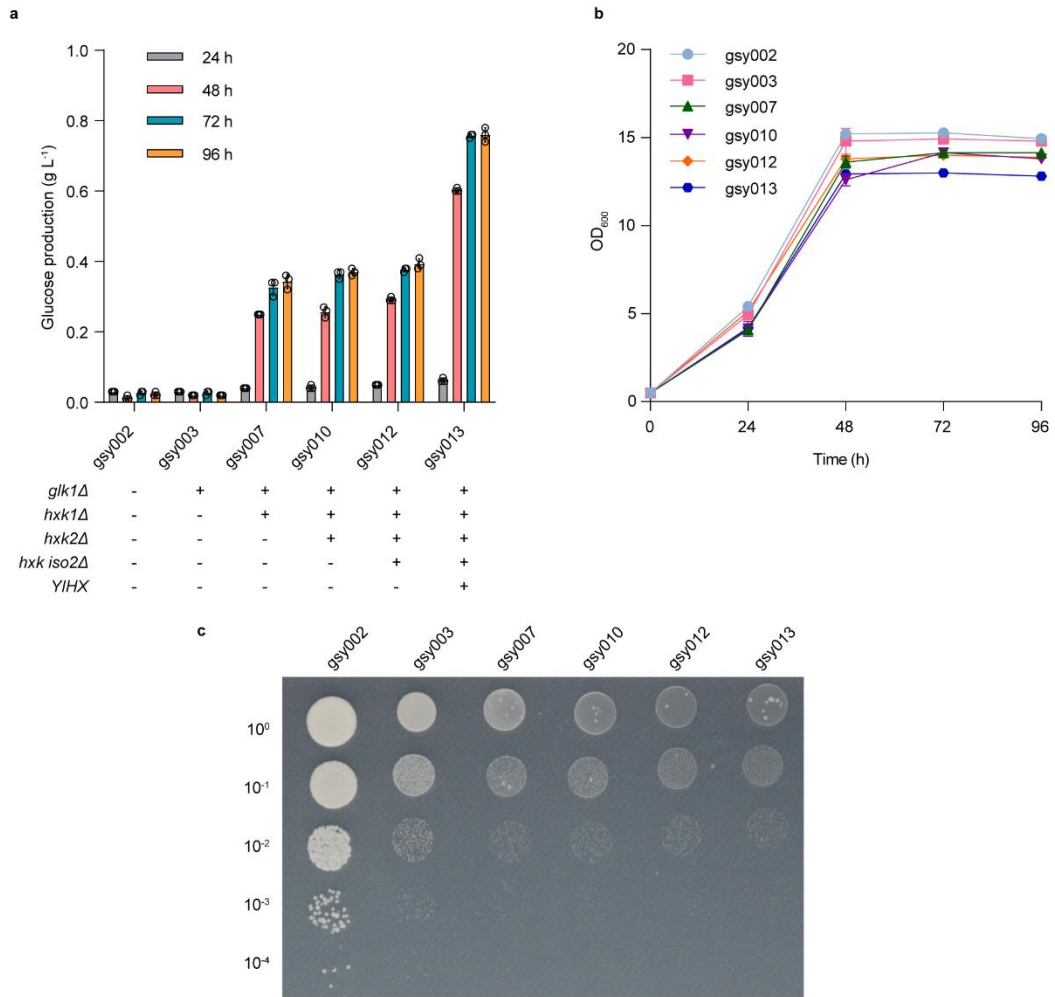


Supplementary Fig. 4 Isopropanol degradation in the engineered *S. cerevisiae* strains. a. Construction of synthetic pathways for isopropanol degradation. Blue arrows, overexpressed genes. Isopropanol is converted to acetyl-CoA by expressing alcohol dehydrogenase (Adh) from *Clostridium beijerinckii*, acetone carboxylase complex (Acx) from *Xanthobacter autotrophicus*, acetoacetyl-CoA synthetase (Aacs) from *Rattus norvegicus* (RnAacs) and *Yarrowia lipolytica* (YIAacs). AcmA is acetone monooxygenase from *Gordonia sp.* (strain TY-5), AcMB is methyl acetate hydrolase from *Gordonia sp.* (strain TY-5). **b.** Cell growth of the engineered strains using isopropanol or acetone as carbon source. The data of cell growth were subtracted from the background in absence of carbon source. **c.** The expression of heterologous enzyme components in recombinant strains were analyzed by proteomic analysis. All data are

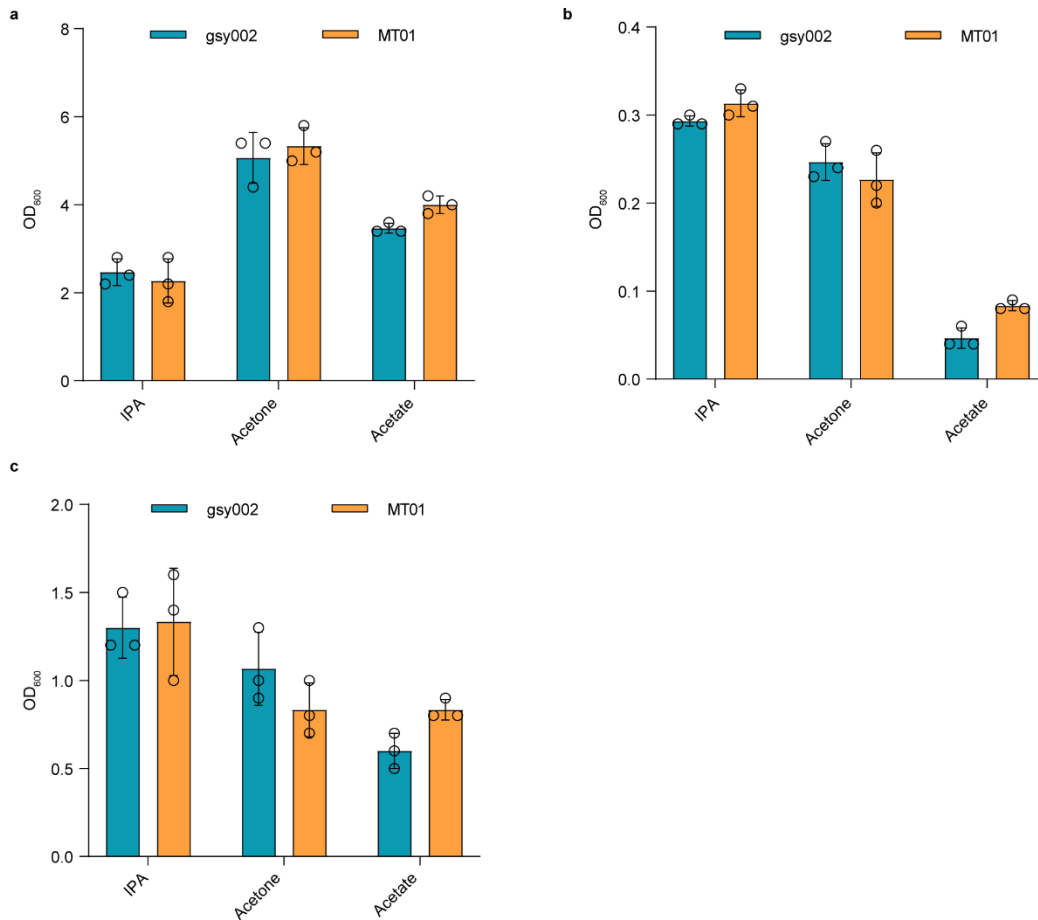
presented as mean \pm SD of biological triplicates (n=3).



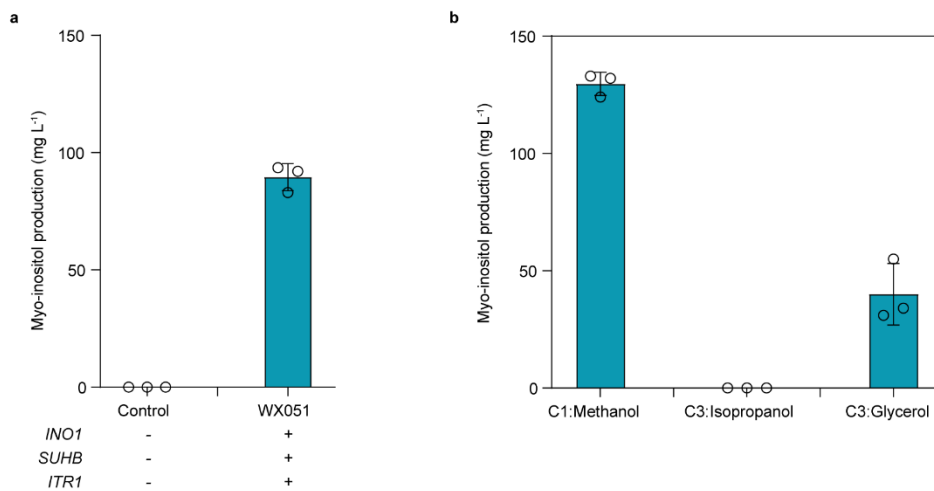
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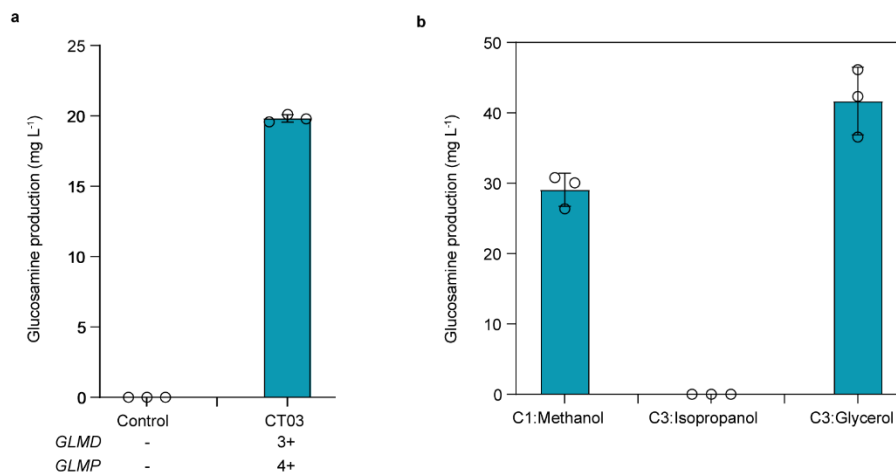
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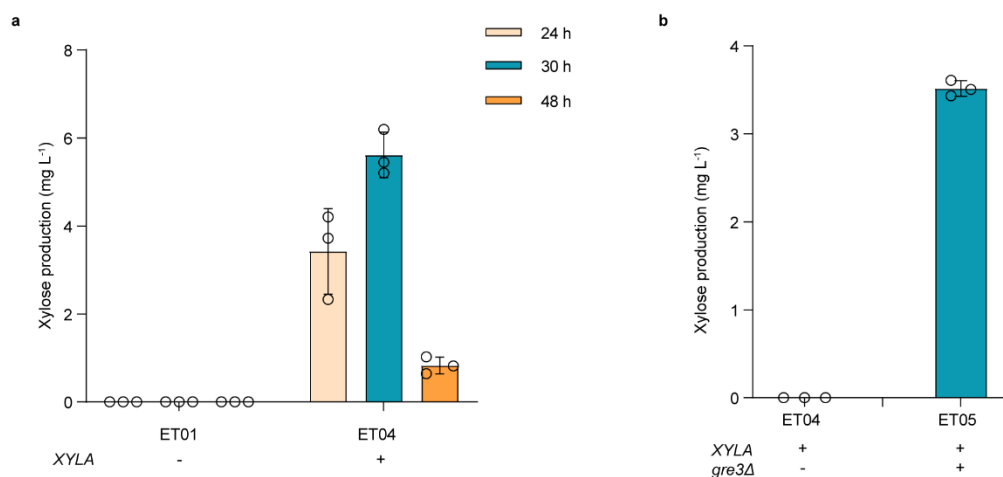
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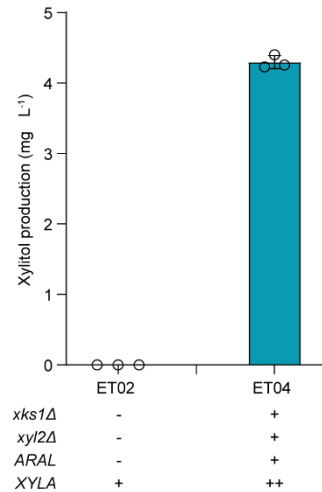
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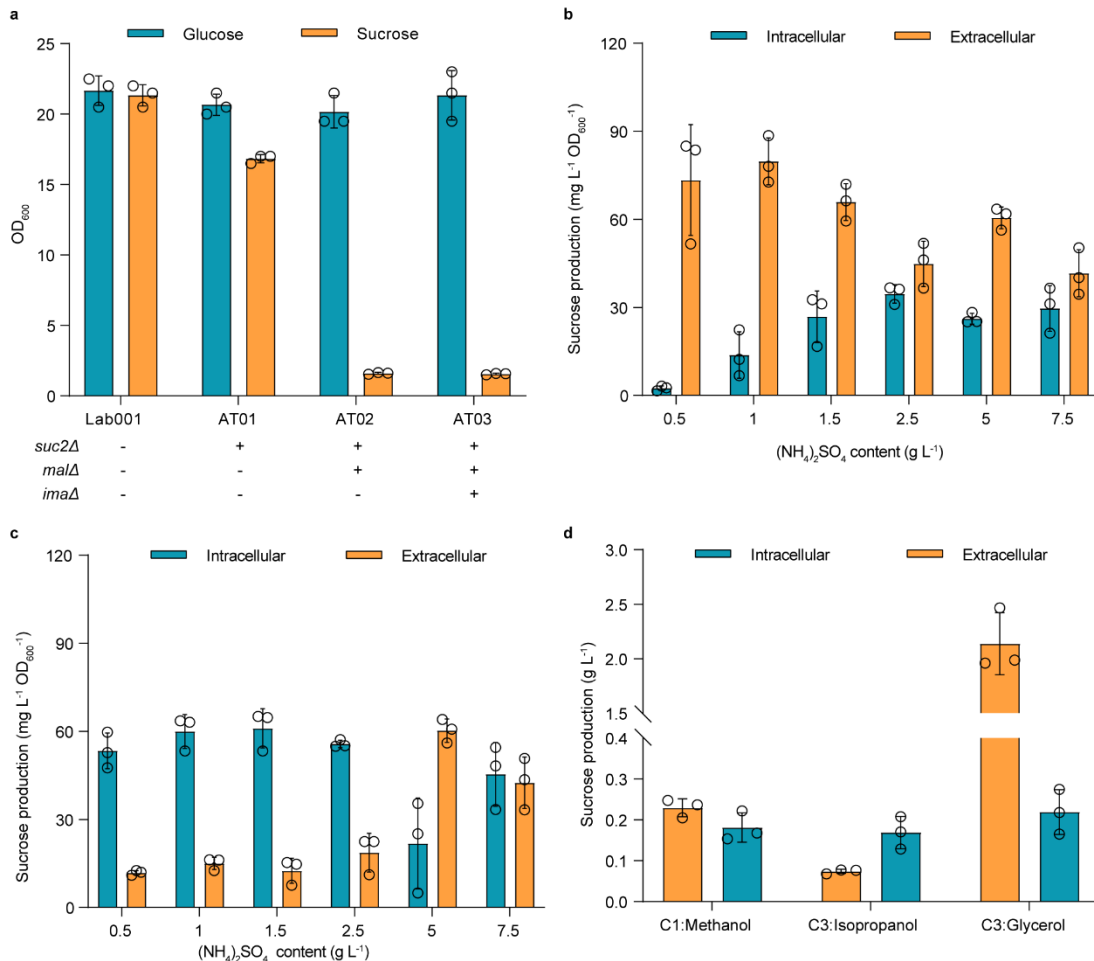
Supplementary Fig. 9 Production of glucosamine in yeast. **a.** Glucosamine production in minimal medium with 20 g L⁻¹ ethanol. **b.** Glucosamine production from different substrates. The initial OD₆₀₀ was 0.5 when 20 g L⁻¹ methanol or glycerol was used as carbon sources in YP medium. The initial OD₆₀₀ was 4 when 20 g L⁻¹ isopropanol was used as a carbon source in YP medium. All data are presented as mean ± SD of biological triplicates (n=3).



Supplementary Fig. 10 Production of xylose in *S. cerevisiae*. **a.** Xylose production at different fermentation time detected by D-xylose assay kit. **b.** Xylose production from *GRE3* deletion strain at 120 h. All data are presented as mean ± SD of biological triplicates (n=3).

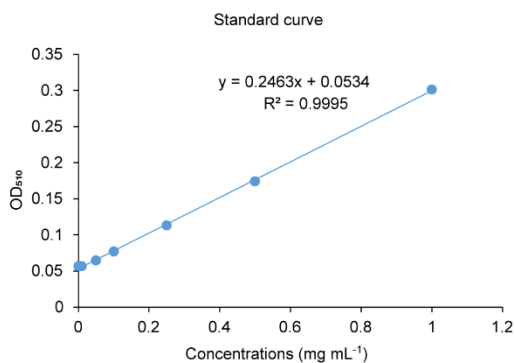


Supplementary Fig. 11 Production of xylitol in *S. cerevisiae*. All data are presented as mean \pm SD of biological triplicates (n=3).

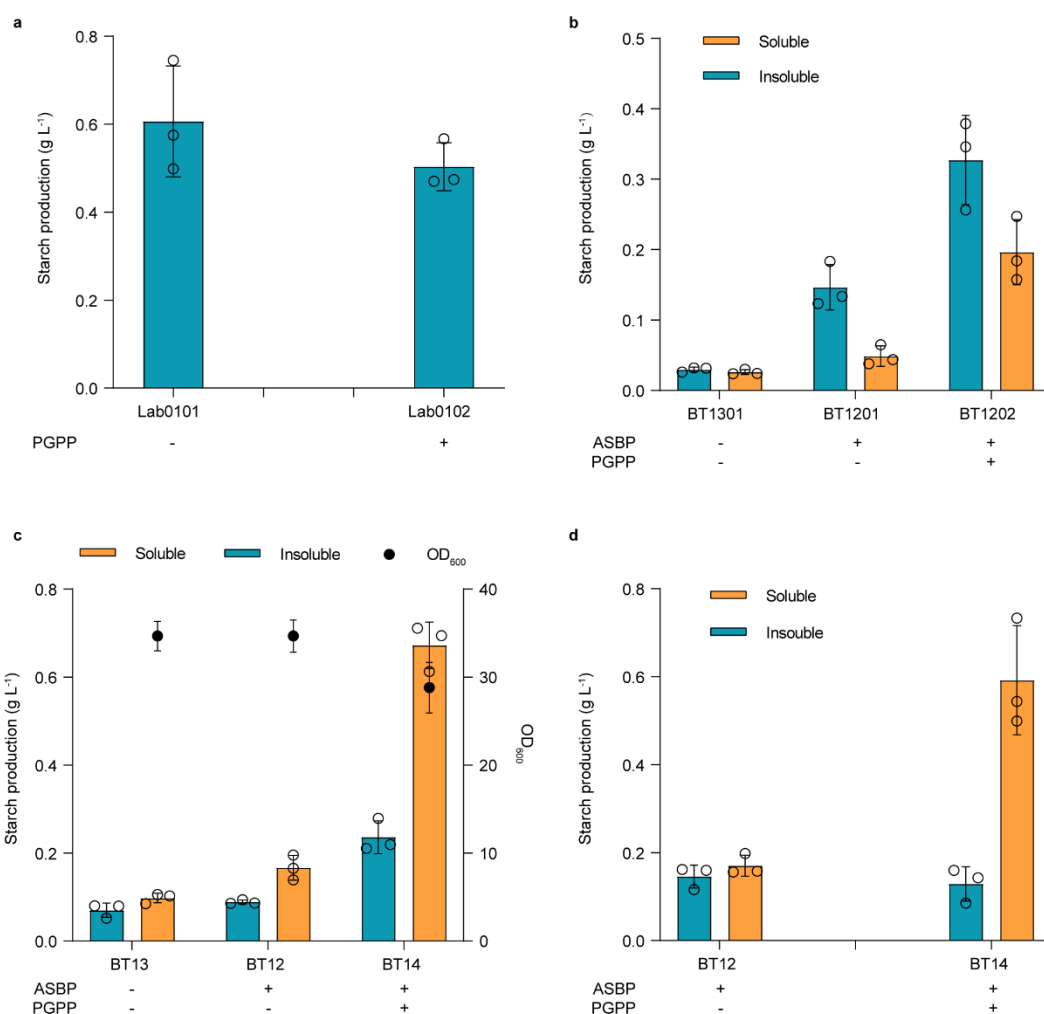


Supplementary Fig. 12 Production of sucrose in *S. cerevisiae*. **a.** Cell growth of the engineered strains with the disruption of sucrose degradation pathway. **b.** The effect of nitrogen supply on sucrose production at 72 h. **c.** The effect of nitrogen supply on sucrose production at 120 h. The effect of nitrogen supply was performed by using Delft medium with 30 g L⁻¹ ethanol

with different $(\text{NH}_4)_2\text{SO}_4$ content. **d.** Sucrose production using different substrates including methanol, isopropanol and glycerol. The initial OD_{600} was 0.5 when 20 g L^{-1} methanol or glycerol was used as carbon sources in YP medium. The initial OD_{600} was 4 when 2% isopropanol was used as a carbon source in YP medium. All data are presented as mean \pm SD of biological triplicates ($n=3$).

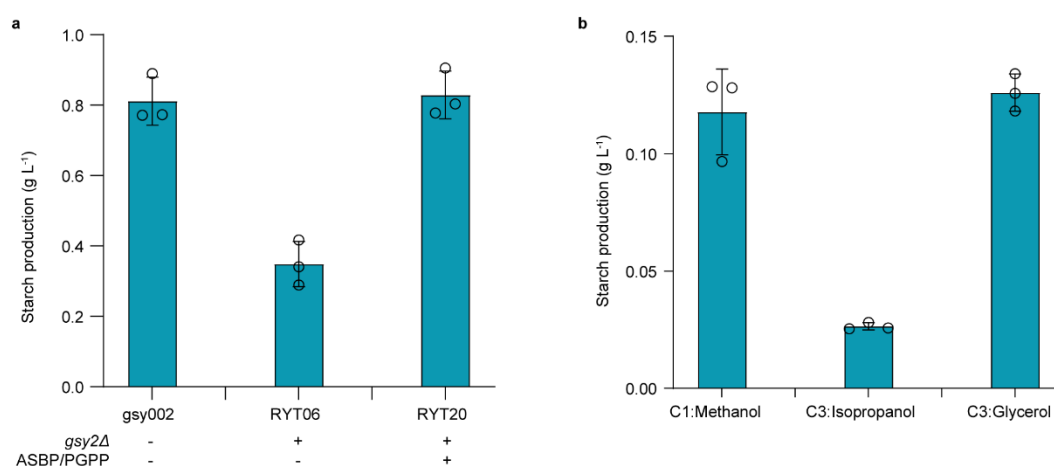


Supplementary Fig. 13 Standard curve of starch assays.

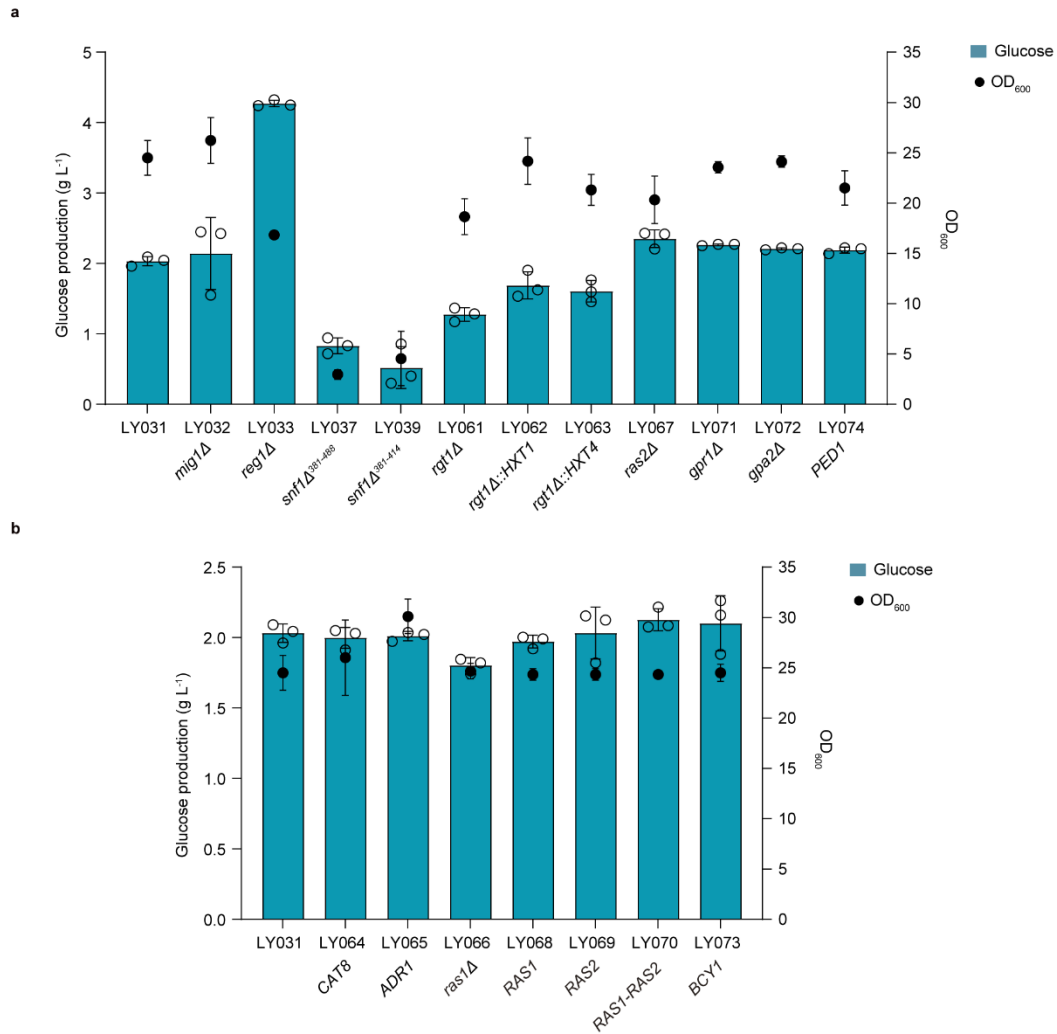


Supplementary Fig. 14 Starch production in *S. cerevisiae*. **a.** Starch production with

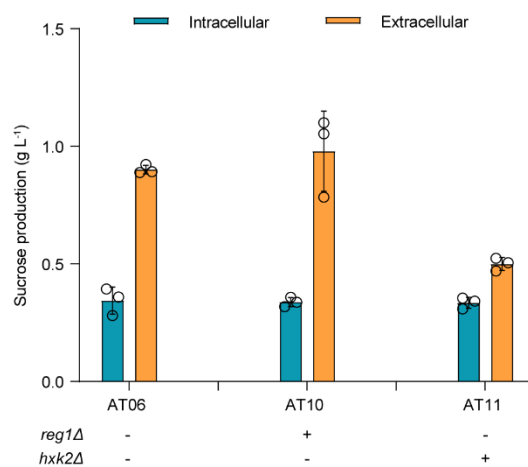
expression Pgp using high-copy plasmid. Empty plasmid was transformed into Lab01 to construct Lab0101 as the control. pTht13 with Pgp expression was transformed into Lab01 to construct Lab0102 for starch synthesis. **b.** Starch production in (glycogen)_n metabolism disruption strains with expression Pgp using high-copy plasmid. Empty plasmid was transformed into BT12 and BT13 to construct BT1201 and BT1301. pTht13 with Pgp expression was transformed into BT12 to construct BT1202. SC medium without uracil containing glucose was used for starch production. **c.** Starch production in (glycogen)_n metabolism disruption strains with integrating *PGP*. YPD medium was used for starch production. **d.** Starch production using ethanol as carbon source. All data are presented as mean \pm SD of biological triplicates (n=3).



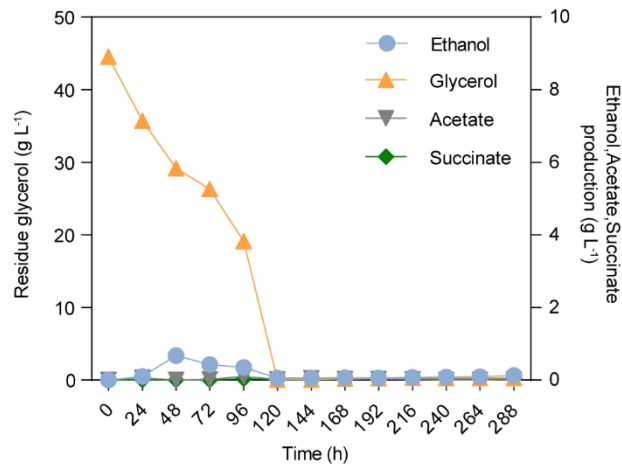
Supplementary Fig. 15 Starch production from different carbon sources, including methanol, isopropanol and glycerol. **a.** Starch production from *P. pastoris* strain RYT20 using glucose as the carbon source. The fermentation was conducted for 24 h in YP medium with an initial OD₆₀₀ of 0.2 and 20 g L⁻¹ glucose was used as the carbon source. **b.** Starch production using different substrates including methanol, isopropanol and glycerol. The fermentation was performed for 24 h in YP medium with an initial OD₆₀₀ of 0.5 and a carbon source composition of 20 g L⁻¹ methanol and 5 g L⁻¹ glucose. For 20 g L⁻¹ glycerol, the fermentation lasted for 120 h with an initial OD₆₀₀ of 0.5 in YP medium for 120 h fermentation. In the case of 20 g L⁻¹ isopropanol, the fermentation was carried out for 120 h with an initial OD₆₀₀ of 4 in YP medium. All data are presented as mean \pm SD of biological triplicates (n=3).



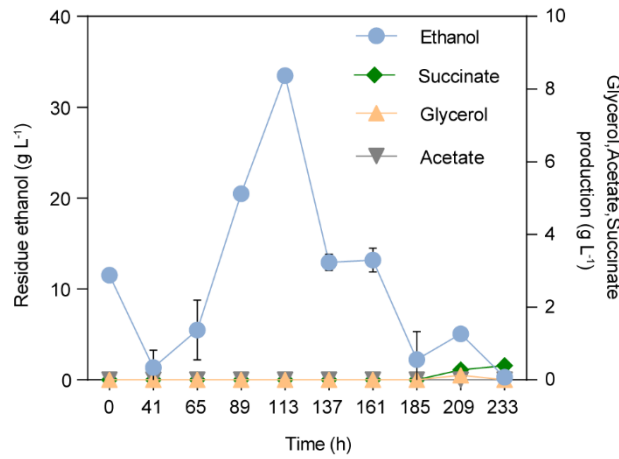
Supplementary Fig. 16 Manipulating regulatory genes in glucose repression pathway for glucose production (a and b). All data are presented as mean \pm SD of biological triplicates (n=3).



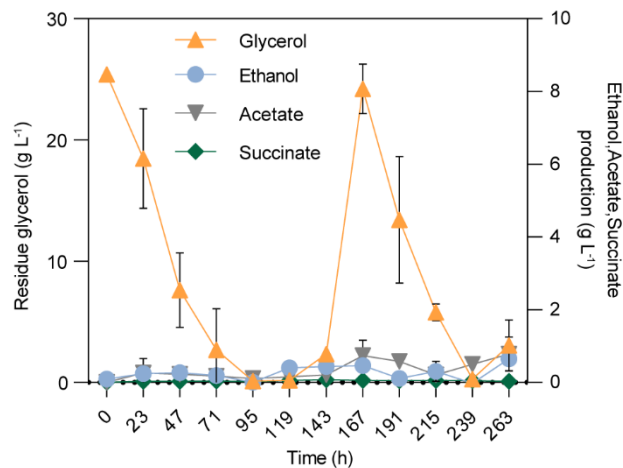
Supplementary Fig. 17 The effect of *reg1Δ* and *hsk2Δ* on sucrose production. All data are presented as mean \pm SD of biological triplicates (n=3).



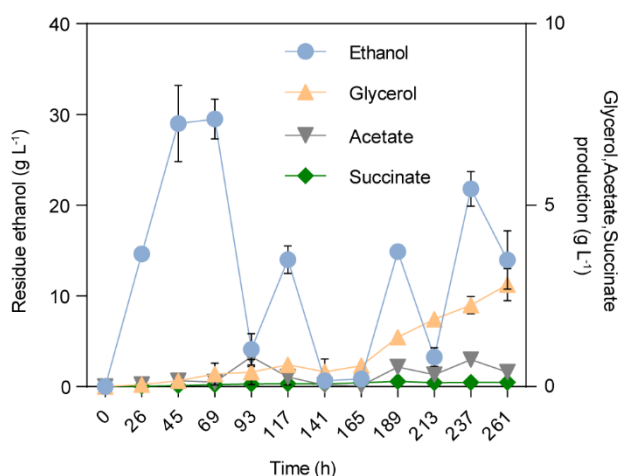
Supplementary Fig. 18 Analysis of metabolites of gsy013 for glucose production form C₁ substrate methanol during fed-batch fermentation. All data are presented as mean ± SD of biological triplicates (n=3).



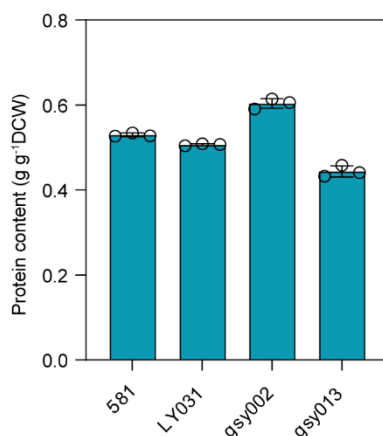
Supplementary Fig. 19 Analysis of metabolites of LY033 for glucose production form C₂ substrate ethanol during fed-batch fermentation. All data are presented as mean ± SD of biological duplicates (n=3).



Supplementary Fig. 20 Analysis of metabolites of gsy013 for glucose production form C₃ substrate glycerol during fed-batch fermentation. All data are presented as mean \pm SD of biological triplicates (n=3).



Supplementary Fig. 21 Analysis of metabolites of AT10 for sucrose production form ethanol during fed-batch fermentation. All data are presented as mean \pm SD of biological triplicates (n=3).



Supplementary Fig. 22 Protein content of the engineered strains. All data are presented as mean \pm SD of biological triplicates (n=3).

Supplementary tables

Supplementary Table 1 Plasmids used in this study.

Plasmid	Relevant characteristics	Reference
pJFE3	2 μ m, AmpR, URA3	1
pQC005	2 μ m, AmpR, URA3, gRNA-X-3	Lab collection
pQC029	2 μ m, AmpR, URA3, gRNA-X-2	Lab collection
pQC030	2 μ m, AmpR, URA3, gRNA-XI-1	Lab collection
pQC135	2 μ m, AmpR, URA3, gRNA-XI-2, gRNA-XII-3	Lab collection
pQC190	2 μ m, AmpR, URA3, gRNA-X-3, gRNA-XII-5	Lab collection
pQC035	2 μ m, AmpR, URA3, gRNA-XI-1, gRNA-XII-5	Lab collection

pTht001	2 μ m, AmpR, URA3, gRNA- <i>SUC2</i>	This study
pTht002	2 μ m, AmpR, URA3, gRNA- <i>MAL</i> , gRNA- <i>IMA</i>	This study
pTht003	2 μ m, AmpR, URA3, gRNA- <i>IMA5</i>	This study
pTht004	2 μ m, AmpR, URA3, gRNA- <i>GLC3</i> , gRNA- <i>GSY1</i>	This study
pTht005	2 μ m, AmpR, URA3, gRNA- <i>GSY2</i>	This study
pTht006	2 μ m, AmpR, URA3, gRNA- <i>GDB1</i>	This study
pTht007	2 μ m, AmpR, URA3, gRNA- <i>GLG1</i>	This study
pTht008	2 μ m, AmpR, URA3, gRNA- <i>GLG2</i>	This study
pTht009	2 μ m, AmpR, URA3, gRNA- <i>GPH1</i>	This study
pTht010	2 μ m, AmpR, URA3, gRNA- <i>GAL1</i>	This study
pTht011	2 μ m, AmpR, URA3, gRNA- <i>TKL1</i> , gRNA- <i>TKL2</i>	This study
pTht012	2 μ m, AmpR, URA3, gRNA- <i>XKS1</i>	This study
pTht013	2 μ m, AmpR, URA3, gRNA- <i>XKL2</i>	This study
pTht014	2 μ m, AmpR, URA3, gRNA- <i>GRE3</i>	This study
pTht015	2 μ m, AmpR, URA3, <i>GAL1p-PGP-FBA1t</i>	This study
pWX01	2 μ m, AmpR, URA3, gRNA- <i>PFK1</i> , gRNA- <i>PFK2</i>	This study
pLY006	2 μ m, ampR, URA3, gRNA1- <i>MIG1</i>	This study
pLY007	2 μ m, ampR, URA3, gRNA1- <i>REG1</i>	This study
pLY008	2 μ m, ampR, URA3, gRNA1- <i>RGT1</i>	This study
pLY009	2 μ m, ampR, URA3, gRNA1- <i>SNF1 (381-414)</i>	This study
pLY010	2 μ m, ampR, URA3, gRNA1- <i>SNF1 (381-488)</i>	This study
pLY011	2 μ m, ampR, URA3, gRNA1- <i>PYK1</i> , gRNA2- <i>PYK2</i>	This study
pLY012	2 μ m, ampR, URA3, gRNA1- <i>RAS1</i>	This study
pLY013	2 μ m, ampR, URA3, gRNA1- <i>RAS2</i>	This study
pLY014	2 μ m, ampR, URA3, gRNA1- <i>GPR1</i>	This study
pLY015	2 μ m, ampR, URA3, gRNA1- <i>GPA2</i>	This study
Based vector	2 μ m, hphMX, hphMX, Cas9	Prof. Gao
pGsy001	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>ku70</i>	This study
pGsy002	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>GLK1</i>	This study
pGsy003	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>HXX1</i>	This study
pGsy004	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>HXX2</i>	This study
pGsy005	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>HXX iso2</i>	This study
pGsy006	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>GSY</i>	This study
pGsy007	2 μ m, hphMX, hphMX, Cas9, gRNA- <i>GLG</i>	This study
pGsy008	2 μ m, hphMX, hphMX, Cas9, gRNA-II-4	This study
pGsy009	2 μ m, hphMX, hphMX, Cas9, gRNA-II-5	This study

Supplementary Table 2 Strains used in this study.

Name	Genotype	Base strain	Reference
Lab001	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3</i>	CEN.P K 113- 11C	Lab collection

Glucose in *S. cerevisiae*

LY031	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t)</i>	LY031	²
LY032	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) mig1Δ</i>	LY031	This study
LY033	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) reg1Δ</i>	LY031	This study
LY034	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) pfk1Δ::(TEF1p-FBP1-FBA1t) pfk2Δ</i>	LY031	This study
LY035	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) pyk1Δ::(TEF1p-PCK1-FBA1t) pyk2Δ</i>	LY031	This study
LY037	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) Snf1(381-488)Δ</i>	LY031	This study
LY039	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) Snf1(381-414)Δ</i>	LY031	This study
LY045	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) pyk1Δ::(TEF1p-PCK1-FBA1t) pyk2Δ pfk1Δ::(TEF1p-FBP1-FBA1t) pfk2Δ</i>	LY031	This study
LY061	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) rgt1Δ</i>	LY031	This study
LY062	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) rgt1Δ::(TEF1p-HXT1-FBA1t)</i>	LY031	This study
LY063	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) rgt1Δ::(TEF1p-HXT4-FBA1t)</i>	LY031	This study
LY064	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(CCW12p-CAT8-ADH1t)</i>	LY031	This study
LY065	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(CCW12p-ADR1-ADH1t)</i>	LY031	This study
LY066	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) ras1Δ</i>	LY031	This study
LY067	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ</i>	LY031	This study

	<i>hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) ras2Δ</i>		
LY068	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(ENO2p-RAS1-ADH1t)</i>	LY031	This study
LY069	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(TEF1p-RAS2-PYK1t)</i>	LY031	This study
LY070	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(PYK1t-RAS2-TEF1p-ENO2p-RAS1-ADH1t)</i>	LY031	This study
LY071	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) gpr1Δ</i>	LY031	This study
LY072	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) gpa2Δ</i>	LY031	This study
LY073	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(CCW12p-BCY1-ADH1t)</i>	LY031	This study
LY074	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 glk1Δ hxx1Δ hxx2Δ emi2Δ::(TEF1p-YHIX-DIT1t) YLR446WΔ::(CCW12p-AGPP-PYK1t) X3::(CCW12p-PDE1-ADH1t)</i>	LY031	This study

Glucose in *P. pastoris*

GS115	<i>his4</i>		Lab collection
gsy002	<i>his4 Δku70::RAD52</i>	GS115	This study
gsy003	<i>his4 Δku70::RAD52 Δglk1</i>	gsy002	This study
gsy007	<i>his4 Δku70::RAD52 Δglk1 Δhxx1</i>	gsy003	This study
gsy010	<i>his4 Δku70::RAD52 Δglk1 Δhxx1 Δhxx2</i>	gsy007	This study
gsy012	<i>his4 Δku70::RAD52 Δglk1 Δhxx1 Δhxx2 Δhxx iso2</i>	gsy010	This study
gsy013	<i>his4 Δku70::RAD52 Δglk1 Δhxx1 Δhxx2 Δhxx iso2::YHIX1</i>	gsy010	This study

Myo-inositol

WX020	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 Δpfk1 Δpfk2</i>	Lab01	This study
WX051	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 Δpfk1 Δpfk2 XI-2::(TDH3p-SUHB-CYC1t)+(TEF1Pp-GDH2-ADH2t) XII-3::(PGK1p-INO1-PYK1t)+(CCW12p-ITR1-FBA1t)</i>	WX02 0	This study
RYT02	<i>his4 Δku70::RAD52 II-4::(ADH2t-ITR1-GAP)+(DAS1t-INO1-DAS2p)+(AOX1p-SUHB-PMP20t)</i>	gsy002	This study

Glucosamine

CT01	LY031, X2::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-FBA1t)		
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CT02	LY031, <i>FBA1t</i>	<i>X2::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>X3::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>XII-5::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>FBA1t)</i>	CT01	This study
CT03	LY031, <i>FBA1t</i>	<i>X2::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>X3::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>XII-5::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>FBA1t) XI-1::(CCW12p-GLMP-FBA1t)</i>	CT02	This study
CT04	LY031, <i>FBA1t</i>	<i>X2::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>X3::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>XII-5::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>FBA1t) XI-1::(CCW12p-GLMP-FBA1t) <i>reg1Δ</i></i>	CT03	This study
CT05	LY031, <i>FBA1t</i>	<i>X2::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>X3::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>XII-5::(TDH3p-GLMD-ADH1t)+(CCW12p-GLMP-</i> <i>FBA1t) XI-1::(CCW12p-GLMP-FBA1t) <i>hvk2Δ</i></i>	CT04	This study
RYT01	<i>his4 Δku70::RAD52</i>	<i>II-4::(PMP20t-GLMD-TEF1)+(GAP1p-</i> <i>GLMP-ADH2t)</i>	gsy002	This study
RYT02	<i>his4 Δku70::RAD52</i>	<i>II-4::(PMP20t-GLMD-TEF1)+(GAP1p-</i> <i>GLMP-ADH2t) II-5::(PMP20t-GLMD-TEF1)+(GAP1p-</i> <i>GLMP-ADH2t)</i>	RYT01	
Xylose and xylitol				
ET01	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 tkl1Δ</i> <i>tkl2Δ</i>	Lab01	This study
ET02	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 tkl1Δ</i> <i>tkl2Δ X-2::(CCW12p-XYLA-FBA1t)</i>	ET01	This study
ET03	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 tkl1Δ</i> <i>tkl2Δ xks1Δ X-2::(CCW12p-XYLA-FBA1t)</i>	ET02	This study
ET04	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 tkl1Δ</i> <i>tkl2Δ xks1Δ xyl2Δ::(ADH1t-ARAL-TDH3p)+(CCW12p-XYLA-</i> <i>FBA1t)</i>	ET03	This study
ET05	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 tkl1Δ</i> <i>tkl2Δ xks1Δ xyl2Δ::(ADH1t-ARAL-TDH3p)+(CCW12p-XYLA-</i> <i>FBA1t) <i>gre3Δ</i></i>	ET04	This study
Sucrose				
AT01	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 suc2Δ</i>	Lab01	This study
AT02	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ</i>	AT01	This study
AT03	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i>	AT02	This study
AT04	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t)</i>	AT03	This study
AT05	<i>MATa ura3-52 can1Δ::cas9-natNT2</i>	<i>TRP1 LEU2 HIS3 suc2Δ</i>	AT04	This study

	<i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-ADH1t)+</i> <i>(CCW12p-SPP-FBA1t)</i>		
AT06	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) X-3::(TEF1p-GLGC-</i> <i>PYK1t)+(ENO2p-UGP1-UGP1t)</i>	AT05	This study
AT07	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) X-3::(TEF1p-GLGC-</i> <i>PYK1t)+(ENO2p-UGP1-UGP1t)+(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t)</i>	AT06	This study
AT08	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) X-3::(TEF1p-GLGC-</i> <i>PYK1t)+(ENO2p-UGP1-UGP1t)+(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) XI-1::(TEF1p-SUF1-PGK1t)</i>	AT07	This study
AT09	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) X-3::(TEF1p-GLGC-</i> <i>PYK1t)+(ENO2p-UGP1-UGP1t)+(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) XI-1::(TEF1p-SUF1-PGK1t)</i> <i>XII-5::(TEF1p-SUF1-PGK1t)</i>	AT08	This study
AT10	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) X-3::(TEF1p-GLGC-</i> <i>PYK1t)+(ENO2p-UGP1-UGP1t)+(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) XI-1::(TEF1p-SUF1-PGK1t)</i> <i>XII-5::(TEF1p-SUF1-PGK1t) Δreg1</i>	AT09	This study
AT11	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 suc2Δ</i> <i>mal12Δ mal22Δ mal32Δ ima1Δ ima2Δ ima3Δ ima4Δ ima5Δ</i> <i>106a::(TEF1p-SUF1-PGK1t) X-2::(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) X-3::(TEF1p-GLGC-</i> <i>PYK1t)+(ENO2p-UGP1-UGP1t)+(TDH3p-SPS-</i> <i>ADH1t)+(CCW12p-SPP-FBA1t) XI-1::(TEF1p-SUF1-PGK1t)</i> <i>XII-5::(TEF1p-SUF1-PGK1t) Δhck2</i>	AT10	This study
RYT03	<i>his4 Δku70::RAD52 II-4::(PGI1p-SUF1-DAS1t)+(ADH2t-SPS-</i> <i>GAP1p)+(TEF1p-SPP-PMP20t)</i>	gsy002	This study

Starch

BT01	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) GLC3::(TEF1p-BE3-PGK1t)</i>	Lab01	This study
BT02	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ</i>	Lab01	This study
BT03	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t)</i>	BT01	This study
BT04	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ</i>	BT02	This study
BT05	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ</i>	BT03	This study
BT06	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ gdb1Δ</i>	BT04	This study
BT07	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ</i>	BT05	This study
BT08	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ gdb1Δ glg1Δ</i>	BT06	This study
BT09	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t)</i>	BT07	This study
BT11	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ gdb1Δ glg1Δ Δglg2</i>	BT08	This study
BT12	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t) gph1Δ</i>	BT09	This study
BT13	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ gdb1Δ glg1Δ Δglg2 gph1Δ</i>	BT11	This study
BT14	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t) gph1Δ XI-1::(GAL1p-PGP-FBA1t)</i>	BT12	This study
BT15	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t) gph1Δ gal1Δ</i>	BT12	This study
BT16	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ:: (TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t) gph1Δ XI-1::(GAL1p-PGP-FBA1t) gal1Δ</i>	BT14	This study

BT17	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ gdb1Δ glg1Δ Δglg2 gph1Δ gal1Δ</i>	BT13	This study
Lab0101	<i>MATa ura3-52 can1::cas9-natNT2 TRP1 LEU2 HIS3</i> with pJFE3	Lab01	This study
Lab0102	<i>MATa ura3-52 can1::cas9-natNT2 TRP1 LEU2 HIS3</i> with pTht013	Lab01	This study
BT1201	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ::(TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t) gph1Δ</i> with pJFE3	BT12	This study
BT1202	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ::(TDH3p-GLGC-ADH1t) glc3Δ::(TEF1p-BE3-PGK1t) gsy1Δ::(TDH3p-SS3-PGK1t) gdb1Δ glg1Δ glg2Δ::(TDH3p-ISA2-ADH1t)+(CCW12-ISA1-FBA1t) gph1Δ</i> with pTht013	BT12	This study
BT1301	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 gsy2Δ glc3Δ gsy1Δ gdb1Δ glg1Δ Δglg2 gph1Δ</i> with pJFE3	BT13	This study
RYT06	<i>his4 Δku70::RAD52 Δgsy</i>	gsy002	This study
RYT17	<i>his4 Δku70::RAD52 Δgsy Δglg::(PMP20t-ISA1-PET9p)+(PGI1p-GLGC-DAS1t)+(GAP1t-BE3-TEF1p)+(GAP1p-SS3-ADH2t)</i>	RYT06	This study
RYT20	<i>his4 Δku70::RAD52 Δgsy Δglg::(PMP20t-ISA1-PET9p)+(PGI1p-GLGC-DAS1t)+(GAP1t-BE3-TEF1p)+(GAP1p-SS3-ADH2t) II-5::(GAP1p-PGP-PMP20t)</i>	RYT17	This study

Isopropanol

DT01	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TDH3p-YIAACS-TDH2t)+(TEF1p-CbADH-PGK1t)</i>	Lab01	This study
DT02	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TDH3p-RnAACS-TDH2t)+(TEF1p-CbADH-PGK1t)</i>	Lab01	This study
DT03	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TDH3p-YIAACS-TDH2t)+(TEF1p-CbADH-PGK1t) X-2::(TDH3p-ACXB-ADH1t)+(CCW12p-ACXA-FBA1t)+(TEF1p-ACXC-PYK1t)</i>	DT01	This study
DT04	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TDH3p-RnAACS-TDH2t)+(TEF1p-CbADH-PGK1t) X-2::(TDH3p-ACXB-ADH1t)+(CCW12p-ACXA-FBA1t)+(TEF1p-ACXC-PYK1t)</i>	DT02	This study
DT05	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XI-1::(TDH3p-ACMA-FBA1t)+(CCW12p-ACMB-DIT1t)</i>	Lab01	This study
DT06	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TDH3p-YIAACS-TDH2t)+(TEF1p-CbADH-PGK1t) XI-1::(TDH3p-ACMA-FBA1t)+(CCW12p-ACMB-DIT1t)</i>	DT01	This study
DT07	<i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TDH3p-RnAACS-TDH2t)+(TEF1p-CbADH-PGK1t) XI-1::(TDH3p-ACMA-FBA1t)+(CCW12p-ACMB-DIT1t)</i>	DT02	This study

MT01	<i>his4 ku70Δ::RAD52 XII-4::(PGI1p-CbADH-DAS1t)+(GAP1p-ACMA-ADH2t)+(TEF1p-ACMB-PMP20t)</i>	gsy002	This study
MT02	<i>his4 ku70Δ::RAD52 XII-4::(PGI1p-CbADH-DAS1t)+(AOX1p-ACMA-ADH2t)+(DAS2p-ACMB-PMP20t)</i>	gsy002	This study

Supplementary Table 3 Codon optimized genes in this study.

Gene	Sequence (5'-3')
<i>SUF1</i>	<p>ATGGATAATCCATCTACTAATGAATCTTCAAATATTTCTTCAATTCATCTAGAA TCAGCTAGTAATAGAAAACCAACTCCATTAATTAAGATGATTGCTGTTGCTTC TATTGCTGCTGGTATTCAATTTGGATGGGCTTTACAATTGTCATTATTAECTCC TTATATTCAATTATTAGGTGTTCCACATAAATGGGCTGCAAATATATGGTTGTG TGGTCCAATTTCTGGTATGATTATTCAACCAATAGTTGGTTACTATAGTGATCG TAATAGATCTAGGTTTGGTAGAAGGAGACCTTTTATTTTCTTTGGTGCTATAGC TGTTGCTGTTGCTGTATTTTGGATTGGTTTTGCTGCTGATATTGGACATTCTTTT GGTGACGATTTGAAGAAAAGACTAGACCTAAAGCTGTTGTAATTTTGTGTTTT TGGTTTTTGGATTTTAGATGTTGCTAATAACATGTTGCAAGGTCCATGTAGAGC TTTTATTGGTGACTTAGCTGCTGATGATCATAGAAGAATGAGAACAGGTAATG CTTTATTTTCATTTTTCATGACTGTTGGTAATGTTTTAGGTTACGCTGCTGGTAG TTATAGAAAATTGTTTATGATGTTACCATTCACTAAAACCTGAAGCTTGTAAATG AATTTTGTGCAAACCTTAAAAACATGTTTCTTTATTGCTATATTTTGTGATTTT GTTGTCTACTTTCGCTTTATTGTACGTTGAAGATATTCCATTACCATCTATTGA ATCTCAATCTCAAACCTCAAACACAAACACAATCTGAACCTGAACAACAAGTTT CTTGTTTCGGTGAAATTTTAGGTGCTTTTAATGGTTTACAAAACCTATGTGGA TGTTGATGTTAGTTACAGCTATTAATTGGATTGCTTGGTTTCCTTTCTTTTATT TGATACTGATTGGATGGGTCATGAAGTTTACGGTGGAATCCAGGAGATGATG CTTATAATAGAGGTGTTAGAGCTGGTGCAATGGGTTTAATGATAAACGCTGTA GTTTTAGCTTTAATGTCTTTAGCAGTTGAACCTTTAGGTAGATTTGTAGGCGGT GCTAAAAGATTGTGGGGTATTGTTAATATTATTTTAGCAGTTGGTTTAGCAAT GACAATTGTTATTACTAAAGCTGCTCAACATGAAAGACATGTATCTAATGGTA ATACTCCAAGTGCAGGTATTTCTGCTGCTAGTTTTGCATTTTTCGCTTTGTTAG GTATTCTTTAGCTATTAATTTTTCAGTTCCTTTTGCTTTGGCATCTATATATTC ATCTGCTAGTGGTGCAGGTCAAGGTTTATCCTTGGGTGTTTTGAATATTGCAAT TGTTGTTCCACAAATGATTGTATCTGCTTTATCAGGTCCATGGGATTCATTGTT TGGTGGTGGTAACTTGCCAGCTTTTGTGTTGGTATTGGTGCAGCTGTTATCTC TGGTGTTTTAGCAATTATTATTTTACCAACTCCAAAAGCTACTGATGTTGCTAA AGTTCCAATTGCTGGTGGTTTTTCATTA</p>
<i>SPP</i>	<p>ATGAGACAATTGTTGTTGATTCTGATTTGGATAAACACTTGGGTCGGCGATCA ACAAGCTTTGGAACATTTGCAAGAATACTTGGGTGACAGAAGAGGTAACCTTTT ACTTGGCTTACGCTACTGGTAGATCCTACCCTCTGCTAGAGAATTGCAAAAAG CAAGTTGGTTTGATGGAACCAGATTACTGGTTGACTGCTGTTGGTTCTGAAAT CTATCATCCAGAAGGTTTAGATCAACACTGGGCTGATTACTTGTCCGAACATT GGCAAAGAGATATTTTGAAGCTATTGCTGATGGTTTTTGAAGCCTTGAAGCCA CAATCTCCATTAGAACAAAACCCATGGAAAATTTCTTATCACTTAGATCCACA AGCTTGTCCAACCGTTATTGATCAATTGACCGAAATGTTGAAGGAAACTGGTA</p>

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SPS

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GLMP

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GLMD

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XYLA

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PGP

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ACXB

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GAAAATCCAACCTGGGATGCTATGTTACCTGATGCACAAATCAAAGAGATA
AACAAAGCTATTACTACTGAAGAAATGTTTTCTGATTATGATTTGTAAGTAAAT
ATATGAGAGGTGGACCTGGTTTTGGCGATCCATTGGATAGAGAACCTCAAGCT
GTTGCTGATGATATTAATGGTGGTTATGTTTTAGAAAGATTTGCTGGTGAAGTT
TACGGTGTAGTTGTTAGAAAAGGTGCTGATGGTCAATATGGTGTGATGAAGC
AGGTACTGCTGCTGCTAGAGCACAATTAAGAAAAGATAGATTAGCTAAGTCT
GTTCCAGTTTCTGAATGGATGAAAGGTGAAAGAGAAAAGATTTTAGCTAAAG
ATGCTGGTACACAAGTTAGACAAATGTTTGGTGCATCTTTTAAATTAGGTCT
AGATTTGAAAAGATTTTAGAACTTTTTGGTCATTGCCTGATTCTTGGACTTTA
CCAGAAGAAGAAATTTGGTGTCCAACCTACGGTTCTAGATATCAATGGATAT
TAGTGAATTGCCTGATGTTCACTGTTCAATTTGTTGAAGAATAA
ATGGTTAATGTTCCAGTAGGTCATTTGAGAAATGTTCAAGTTTTGGGTATTGAT
GCTGGTGGTACTATGACTGATACTTTCTTTGTTGATCAAGATGGTGACTTTGTT
GTTGGTAAAGCTCAATCTACTCCACAAAATGAAGCTTTAGGTTTGATTGCTTCT
AGTGAAGATGGTTTAGCTAATTGGGGAATGTCTTTGCATGAAGCTTTAGCTCA
ATTACAACTGGTGTACTCTGGTACTGCTATGTTGAATAGAGTTGTCCAAA
GAAAGGGTTTTAAAATGTGGTTTAATTGTTAATAGAGGTATGGAAGATTTTCAT
AGAATGGGTAGAGCTGTTCAATCTCACTTAGGTTATGCATATGAAGATAGAAT
TCACTTGAATACCCATAGATATGATCCTCCATTGGTACCTAGACATTTGACAA
GAGGTGTTGTTGAAAGAAGTATGATGATTGGTACACAAGTTATTCCATTAAGA
GAAGATACTGCTAGAGATGCAGCTAGAGATTTGATTGCTGCTGATGCTGAAGG
TATTGTTATTTCTTTATTGCATTCTTACAAGAATCCAGAAAATGAAAGAAGAG
TTAGAGATATAGTTTTAGAAGAAGTTGAAAAATCTGGAAAGAAAATTCCAGT
CTTTGCTAGTCTGATTATTATCCAGTTAGAAAGGAAACACATAGAACTAATA
CAACTATTTTAGAAGGTTACGCTGCTGAACCATCTAGGCAAACCTTATCAAAA
ATTTCTAATGCCTTTAAAGAAAGAGGAACTAAATTTGATTTTAGAGTCATGGC
CACCCATGGTGGTACTATATCATGGAAAGCTAAGGAATTAGCTAGAACAAATA
GTTTCTGGTCCAATAGGTGGAGTTATTGGTGCTAAATATCTAGGTGAAGTTTT
AGGTTATAAAAATATTGCTTGTTCAGATATTGGTGGTACTTCATTTGATGTAGC
TTTGATTACTCAAGGCGAAATGACTATTAAGAATGATCCTGATATGGCTAGAT
TAGTTTTGTCTTTACCATTAGTTGCTATGGACTCTGTAGGTGCAGGTAGAGGTA

ACXA

GTTTTATAAGGTTAGATCCATATACTAGAGCTATAAAAATTAGGTCCTGATTCT
GCTGGTTATAGAGTAGGTGTTTGTGGAAAGAATCAGGTATTGAAACTGTAAC
AATTCAGATTGTCATATGGTTTTAGGTTATCTAAATCCAGATAACTTTTTAGG
TGGTGCTGTAAATTGGATAGACAAAGATCTGTTGATGCTATAAAAGCTCAA
TAGCTGATCCATTAGGTTTATCAGTTGAAGATGCAGCTGCTGGTGTATAGAG
TTATTAGATTCTGATTTGAGAGATTATTTGAGAAGTATGATTTCTGGTAAAGGT
TATTCCCCAGCATCTTTTGTGGTTTTCTTATGGTGGTGCAGGTCCAGTTCAC
ACATATGGTTATACTGAAGGATTAGGTTTTGAAGATGTTATTGTTCCAGCATG
GGCTGCTGGTTTTTCAGCTTTTGGTTGTGCTGCAGCTGATTTTGAATATAGATA
TGATAAATCTTTAGATATTAATATGCCAACTGAAACCCAGATACTGATAAAG
AAAAAGCTGCTGCAACTTTACAAGCTGCATGGGAAGAATTAATAAAAATGT
ATTAGAAGAATTCAAATTGAATGGTTATTCTGCTGATCAAGTAACTTTACAAC
CTGGTTATAGAATGCAATATAGAGGTCAATTGAATGATCTAGAAATTGAATCT
CCTTTAGCACAAAGCACATACTGCTGCAGATTGGGATCAATTAAGTATGCTTT
TAATGCAACTTACGGTAGAGTTTATGCAGCTTCTGCTAGATCTCCAGAATTAG
GTTATAGTGTTACTGGTGCTATTATGAGAGGTATGGTTCCTATTCCAAAGCCA
AAAATCCAAAAGAACCAGAAGAAGGTGAAACACCACCTGAATCAGCTAAAA
TTGGAAGTAGGAAATTTTATAGAAAAAGAGATGGGTTGATGCTCAATTATAT
CATATGGAATCATTAAAGACCAGGTAATAGAGTTATGGGTCCAGCTGTTATTGA
ATCTGATGCAACAACCTTTTGTAGTTCCAGATGGTTTTGAAACTTGGTTAGATG
GTCATAGATTATTTCAATTTGAGAGAAGTTTAA

ACXC

ATGGCTTATACTAGATCTAAAATTGTTGATTTGGTTGATGGTAAAATTGATCC
AGATACTTTGCATCAAATGTTGTCTACTCCAAAAGATCCAGAAAGATTTGTTA
CTTATGTTGAAATTTTGAAGAAAGAATGCCATGGGATGATAAAATTATTTTG
CCTTTAGGTCCAAAATTATTCATTGTTCAACAAAAAGTTTCTAAAAAATGGAC
TGTTAGATGTGAATGTGGTCATGATTTTTGTGATTGGAAAGATAATTGGAAAT
TGTCTGCTAGAGTTCATGTTAGAGATACTCCACAAAAAATGGAAGAAATCTAT
CCAAGATTGATGGCTCCAACCTCATCTTGGCAAGTTATTAGAGAATATTTTTGT
CCAGAATGTGGTACTTTGCATGATGTTGAAGCTCCAACCTCATGGTATCCAGT
TATTCATGATTTTTCTCCAGATATTGAAGGTTTTTATCAAGAATGGTTGGGTTT
GCCAGTTCCAGAAAGAGCTGATGCTTAA

ACMA

ATGTCTACTACTTTGGATGCTGCTGTTATTGGTACAGGTGTCGCTGGTTTG
TATGAATTACATATGTTAAGAGAACAGGGTTTAGAAGTTAGAGCTTATGATAA
AGCTTCTGGTGTAGGAGGTACTTGGTATTGGAATAGATATCCAGGTGCTAGAT
TTGATAGTGAAGCATATATATATCAATATTTGTTTGTGATGAAGATTTGTATAAG
GGTTGGTCATGGTCTCAAAGATTTCCAGGTCAAGAAGAAATTGAAAGATGGTT
GAATTATGTTGCTGATTCAATTGGATTTGAGAAGAGATATTTCAATGGAACTG
AAATTACTTCAGCAGTTTTTGTGATGAAGATAGAAATAGATGGACTTTGACTACT
GCTGATGGCGATACTATTGATGCTCAATTTTTAATTACTTGTGTTGTTGTTG
TCAGCTCCAATGAAAGACTTATTTCTGGTCAATCTGATTTTGGTGGTCAATTA
GTTCATAACCGCTAGATGGCCAAAAGAAGGTATTGATTTTCGAGGTAAAAGAG
TCGGTGTATTGGTAATGGTGCTACTGGTATTCAAGTTATTCAATCAATTGCAG
CTGATGTTGATGAATTGAAGGTTTTTATAAGAACTCCACAATATGCATTACCT
ATGAAGAATCCTAGTTATGGTCCAGATGAAGTAGCTTGGTATAAATCTAGATT

ACTATTAGAAAGTCTTACCCACAACATACTATCATTACTGAAGAATCTGGTGA
ATTGGAAGGTACTGATCAAGATGTTCAATGGGTATTGATCCATTGGATGGTA
CTACTAACTTTATTAAGAGATTGCCACATTTCCGCCGTTTCTATTGCTGTTAGAA
TTAAGGGTAGAACCGAAGTCGCTGTTGTTTACGATCCTATGCGTAACGAATTG
TTCCTGCTACTAGAGGTCAAGGTGCTCAATTGAACGGTTATAGATTAAGAGG
TTCTACTGCTAGAGACTTAGATGGTACCATTTTAGCTACTGGTTTCCCTTTCAA
GGCTAAGCAATACGCTACTACTTACATCAACATTGTTGGTAAATTGTTCAACG
AATGTGCTGATTTTCAGACGCTACTGGTTCCGCTGCTTTGGATTTGGCTTACGTTG
CTGCTGGTAGAGTTGACGGTTTCTTTGAAATTGGTTTGAGACCATGGGATTTT
GCTGCTGGTGAATTGTTGGTTAGAGAAGCTGGTGGTATTGTTTCCGATTTTACT
GGTGGTCATAACTATATGTTGACCGGTAATATCGTTGCTGGTAATCCAAGAGT
TGTTAAGGCTATGTTGGCTAACATGAGAGATGAATTGTCTGATGCTTTGAAGA
GATAA

YIHX ATGTTGTACATTTTCGATTTGGGTAACGTTATTGTTGATATTGATTTCAACAGA
GTTTTGGGTGCTTGGTCTGATTTGACTAGAATTCCATTAGCTTCTTTGAAGAAG
TCTTTCCACATGGGTGAAGCTTTCCATCAACATGAAAGAGGTGAAATTTCTGA
TGAAGCTTTTCGCTGAAGCTTTGTGTCATGAAATGGCTTTGCCATTGTCTTACGA
ACAATTCTCTCATGGTTGGCAAGCTGTTTTCGTTGCTTTGAGACCTGAAGTTAT
TGCTATTATGCATAAGTTGAGAGAACAAGGTCATAGAGTTGTTGTTTTGTCTA
ACACTAATAGATTGCATACTACTTTCTGGCCAGAAGAATACCCAGAAATTAGA
GATGCTGCTGATCATATCTACTTGTCTCAAGATTTGGGTATGAGAAAGCCAGA
AGCTAGAATCTACCAACATGTTTTGCAAGCTGAAGGTTTCTCTCCATCTGATA
CTGTTTTCTTCGATGATAACGCTGATAACATTGAAGGTGCTAACCAATTGGGT
ATTACTTCTATTTTAGTTAAGGATAAGACTACTATTCCAGATTACTTCGCTAAG
GTTTTGTGTTAA

Supplementary Table 4 List of chemicals used in this study.

Chemical	Product code	Source
Glucosamine	346299	Merck Millipore
Xylose	A600998-0100	Sangon Biotech
Xylitol	A500997-0100	Sangon Biotech
Sucrose	V900116	Sigma-Aldrich
Glucose	V900392	Sigma-Aldrich
Myo-inositol	I0040	Sigma-Aldrich
Acetonitrile	100029	Merck Millipore
Methanol	106035	Merck Millipore
Ethanol	64-17-5	Thermo Fisher Scientific
Glycerol	A501745-0500	Sangon Biotech
Formate	S817616	Shanghai Macklin Biochemical
Ethylene glycol	E103322	Shanghai Aladdin Biochemical Technology
Oxalic acid	J33351	Shanghai Jinsui Bio-Technology
Isopropanol	67-63-0	Shanghai Lingfeng Chemical Reagent
Propionate	S12434	Shanghai Jinsui Bio-Technology
Yeast Extract Power	LP0021	Oxiod

Bacto™ Peptone	211677	Gibco
SD-Ura	PM2273	Coolaber Science & Technology
Bacto™ Agar	214010	Bacto
5-fluoroorotic acid	703-95-7	Shanghai Yuanye Bio-Technology

Supplementary Table 5 Primers used in this study.

Primers	Primers for Glucose (Sequences 5'-3')
mig1-up-F	GATTTTATGGAGTGTGATGAATG
mig1-up-R	GGCTATGGTAGTATGTCGTCTC
mig1-dn-F	TACGCTGACAAGTTTTTGGCG
mig1-dn-R	CATGAATGAGAGTTATTCTCTTGAC
mig1-VF	GTGCCTAATTCGTAATATCTCCAC
mig1-VR	CCTCAAAACTCTACTCCATGTTC
reg1-up-F	GTGGATATTGAAGGAAGGAATCAG
reg1-up-R	TTTTGGATTTTTCTTATCTCGTCTTCG
reg1-dn-F	AAGAAAGAATTTTGAAGTCAACATGAAG
reg1-dn-R	GGTGTTTCGCTTATTGACATTG
reg1-VF	GCCTCTTTTCTGACATAATATTGG
reg1-VR	CCCTCAGGAACAACAATTTTAGG
snf1-D-up-F	GCTTTTCAAGAATATCAGCAACG
snf1-D-up-R	CCTAATTTTCGTTGAATGCAGG
snf1-DI-dn-F	CCTGCATTCAACGAAATTAGGGCCAACTTTTCAACAACAAAGC
snf1-DA-dn-F	CCTGCATTCAACGAAATTAGGGCTGCCTCTAAAATATCTCCTC
snf1-D-dn-R	GGGTAGGCTGAAAAGTACTC
snf1-SF	GGCTGTTTCAATAATCATAGCG
snf1-SR	TCAAGATTGTTTACTTTATACAAAGGG
pfk1-up-F	CTCTTGATATGTGGGCTAAATC
pfk1-up-R(TEF1p)	GAGTAGAAACATTTTGAAGCTATCTTTGATATGATTTTGTTC GATTTTTTATA
TEF1p-F	ATAGCTTCAAATGTTTCTACTCC
TEF1p-R	TTTGTAATTAAACTTAGATTAGATTGC
fbp1-f(TEF1p)	CTAATCTAAGTTTTAATTACAAAATGCCAACTCTAGTAAATGG AC
fbp1-R(FBA1t)	CTATATCAATTAATTTGAATTAACCTACTGTGACTTGCCAATAT GG
FBA1t-F	GTTAATTCAAATTAATTGATATAGTTTTTTAATG
FBA1t-R	AGTAAGCTACTATGAAAGACTTTAC
pfk1-dn-F(FBA1t)	GTAAAGTCTTTCATAGTAGCTTACTATGATTGCAATGAAAAGTT TAAG
pfk1-dn-R	GTATTCGTAGACCGATGACAATAC
pfk1-VF	CTCTCTAATATCGAGCACACTG
pfk1-VR	CCGATTATCGGCCTAGTTTC
pfk2-up-F	GAATCTGACGGCACAAGAG
pfk2-up-R	GTGTAATAAAAGGTCATTTTCTTTGCGTATGGTTAGTTCTTGG

pfk2-dn-F	GCCAAGAACTAACCATACGCAAAGAAAATGACCTTTTATTACA CTTTC
pfk2-dn-R	GACGAAAAATGTAGACCTTGTATC
pfk2-VF	GTCATTATATACGATACCGTCCAG
pfk2-VR	CTTTGTTTCTCCCCTTGATAAAAC
pyk1-up-F	CTTAATCCAGAACTGGCACTT
pyk1-up-R(TDH3p)	GGCAGTATTGATAATGATAAACTCGATGTGATGATGTTTTATTT GTTTTG
tdh3p-F	TCGAGTTTATCATTATCAATACTGCC
tdh3p-R	TTTGTTTGTATGTGTGTTTATTCCG
pck1-up-F(tdh3p)	GAATAAACACACATAAAACAAAATGTCCCCTTCTAAAATG AATG
pck1-dn-R(eno2t)	CTAATAATTCTTAGTTAAAAGCACTTTACTCGAATTGAGGACC AGC
ENO2t-F	AGTGCTTTTAACTAAGAATTATTAGTC
ENO2t-R	AGGTATCATCTCCATCTCCCA
pyk1-dn-F(ENO2t)	CATATGGGAGATGGAGATGATACCTAAAAAGAATCATGATTGA ATGAAGATA
pyk1-dn-R	GATTAAACCACCAAACGAAGGC
pyk1-VF	GCTCTATTGTTTTCCATCTCTCG
pyk1-VR	GAAACGCGAATAAATTTAGGACAC
pyk2-up-F	CGAGGAGAAATCAAGAGAAATTG
pyk2-up-R	GTAATAAAAAATAAGGACTTTAATTTTTACGATAGTGCTTTTTGTT GTAATCTTAC
pyk2-dn-F	GATTACAACAAAAGCACTATCGTAAAAATTAAAGTCCTTATTT TTTTTAC
pyk2-dn-R	GAGAGAAGAGAGCCTACAGAAC
pyk2-VF	GATTTTCATTGCTGCCTACATCA
pyk2-VR	GGAAGAACAGTTTATGAATGAACTAC
rgt1-up-F	CATCGTTTATCGTTTTCTGTGAC
rgt1-up-R	GAAGGGAGCATAGTTACCTGAATTTGAAATATATTGGAGTTTG AGAG
rgt1-dn-F	CTCTCAAACCTCCAATATATTTCAAATTCAGGTAACCTATGCTCCC TTC
rgt1-dn-R	CAGGGAAAGGAAATGCAAAAAC
rgt1-VF	CACTTTATTCGGTGTGATTGC
rgt1-VR	CATGGTACCTGCTGGAAGAAC
Ku70_1_sgRNA_F	TGAAGACGCCATGGGGGTCCTGATGAGTCCGTGAGGACGAAA CGAGTAAGCTCGTCGACC
Ku70_2_sgRNA_F	AAACGAGTAAGCTCGTCGACCCCGGAGCTTCACGCGCGTTTTTA GAGCTAGAAATAGCAAG
GLK1_1_sgRNA_F	TGAAGACGCCATGAGTTCCTGATGAGTCCGTGAGGACGAAAC GAGTAAGCTCGTCGGA
GLK1_2_sgRNA_F	AAACGAGTAAGCTCGTCGGAACCTGGGTCACGTAGGCGTTTTTA

	GAGCTAGAAATAGCAAG
HXX iso2_1_sgRNA_F	TGAAGACGCCATGTATGAGCTGATGAGTCCGTGAGGACGAAAC GAGTAAGCTCGTCCTCA
HXX iso2_2_sgRNA_F	AAACGAGTAAGCTCGTCCTCATAAAGATTGCTCGTTAGTTTTA GAGCTAGAAATAGCAAG
HXX1_1_sgRNA_F	TGAAGACGCCATGCTTTCTCTGATGAGTCCGTGAGGACGAAAC GAGTAAGCTCGTCAGAA
HXX1_2_sgRNA_F	AAACGAGTAAGCTCGTCAGAAAGGTGTTGGCTAGTTAGTTTTA GAGCTAGAAATAGCAAG
HXX2_1_sgRNA_F	TGAAGACGCCATGGGATGTCTGATGAGTCCGTGAGGACGAAAC GAGTAAGCTCGTCACAT
HXX2_2_sgRNA_F	AAACGAGTAAGCTCGTCACATCCCAATGCTACCTACCGTTTTA GAGCTAGAAATAGCAAG
D_sgRNA_stru_Fw	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT TATCAACTTGAAAAAGT
A_sgRNA_stru_Rw	GGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAA CATGCTTCGGCATGGCG
B_sgRNA_stru_Rw	AGAAGACGCAAGCAGTCCAAAGCTGTCCCATTCGCCATGCCGA AGCATGTTGCCAGCCG
C_sgRNA_stru_Rw	AGGCTGGGACCATGCCGGCCAAAAGCACCGACTCGGTGCCACT TTTTCAAGTTGATAACG
Ku70 up-F	GCTGGAATTGAATTTAGGTTAGGAAC
Ku70 up-R(Rad52-1)	CTTTCTTTCTCTTTAGCCCCAACCTACTTGCAATGCTTTTTAT TATTCTCTGTGTTA
Ku70 dn-F(Rad52-2)	CAGAGCTATATGAATTTTTCCAGTCTATTTTCGTGTTCTTACTTT TTCCTCGCAACGTG
Ku 70 dn-R	AAATAAGAAGAGCAGCGTCTTGAATCAGGC
Ku70-F	ATGAGTGTTGTCAGCAAGCAATAC
Ku70-R	TCAGGCCTTAGTCTCTTTGAACTTC
Ku70 VF	GGTGCTAAAGGATTGTGTGTCGATG
Ku70 VR	GACGGTATTTGAAAATTAGCTATGCAC
Rad52-1-R	TAGGTTGGGGGCTAAAGAGGAAAGAAAGC
Rad52-1-F	TGTTTGTAGTGTAGGAGAGGACAACTTG
Rad52-2-F(1-p)	CAAGTTGTCCTCTCCTACACTACAAACAAGCCGTCTTTGGAGG GGAAATCGAAGGTTTTG
Rad52-2-R	ATAGACTGGAAAATTCATATAGCTCTGTAAATAG
HXX1 dn-F	TTTATGTTTAGAGCTAGAGAATAAGGG
HXX1 dn-R	ATGTTAAATGTTTTACTCTACTTCCTTTC
HXX1 up-R(HXX1 dn)	GAAAGGAAGTAGAGTAAAACATTTAACATTATGGTGTGATGAA GTAGCAGAAGCTCTAG
HXX1 up-F	TTTAGACCTAATCTTTTTCTCTCTGATCCTC
HXX1-F	CTAGGCTTTTTTGTAAGCTTCAGCCAATC
HXX1-R	ATGCCTATTGCTAAACCAGCTAACCAGGT
HXX1 VF	ACCTTTGATAATGGTTGCCCCAG

HXK1 VR	CGAAATCCAACGTAGGCCTTCC
GLK1 up-F	TTTGCTGGAGAGTCTCCCACTAATTCATG
GLK1 up-R	AATAATTGTAGATTTCCGGGAGAAGAGG
GLK1 dn-F(GLK1 up)	CCTCTTCTCCCCGAAATCTACAATTATTGTACCCTTACTATTAC GTAATATGTATG
GLK dn-R	TTCTTGCCAAATTCATTTGAAAGCTCTTC
GLK1-F	ATGTCTTTGCAAGAAGCTGTAAAGG
GLK1-R	TTAGATTGATACTGGCACAAAGAGC
GLK1 VF	GTCTCTTGCTAAGTATGAGCTTATTG
GLK1 VR	CAGAGAATCTTAATGATGCTGTAC
HXK2 up-F	CTGAATGACTTTGTCAACCAATGCAGCG
HXK2 up-R	TGGGATGTTAGAGCTGCTTGATTTCAAGCC
HXK2 dn-F(HXK2 up)	GAAATCAAGCAGCTCTAACATCCCATCCCAGATCTGCTTATGT AACGAAATATTTAC
HXK2 dn-R	ACATCACACATCTTTTCTCTATTCTTG
HXK2-F	ATGCTACCTACCTGGGTGATGGATTTC
HXK2-R	TTAGTTACTATCCTTTCCATCTTGAGGGTG
HXK2 VF	GGAGCTAAATCGGTTGCCCTTTCCAAGTG
HXK2 VR	CGAACTGTTTCGAAATCAGCTGTTC
HXK iso2 up-F	GGCAAACCTTGAATAATGTCGACAATATG
HXK iso2 up-R	GTTTCGTTCTAATCTTAAGTTCTTTACTTAAG
HXK iso2 dn-F(iso2 up)	GTAAAGAACTTAAGATTAGAACGAACTTAGATTAGTTAGTATG CATCGCGCGATCAG
HXK iso2 dn-R	GCTTAATAATATTATTCAATATAACATCTTGTATG
HXK iso2-F	ATGGTTCCTGTTTTGACCCCACAATTTTC
HXK iso2-R	TCATTCTGGTGTGAAGACTGAAACTGC
HXK iso2-dn-F-2(DAS1t)	CACTGATGATTACAATTTGGTTAGATTAGTTAGTATGCATCG
HXK iso2-dn-R-2	GCTTAATAATATTATTCAATATAACATCTTGTATGATTC
PpDAS1t-R	CCAAATTGTAATCATCAGTGATTATG
PpDAS1t-F	ACGGGAAGTCTTTACAGTTTTAGTTAG
Yihx-R(PpDAS1t)	TAACATAAACTGTAAAGACTTCCCGTTTAACACAAAACCTTAG CGAAGTAATCTGG
Yihx-F(PpPET9p)	CAACAACAAGTCTAACTTCTTCGTCGACTTCATGTTGTACATTT TCGATTTGGGTAAC
PpPET9p-R	GAAGTCGACGAAGAAGTTAGACTTGTTG
PpPET9p-F	AGTACGGGCCCTAGAAAATTCACCACTG
HXK iso2-VF	CGATTTACTCAATAAGGTGGCACC
HXK iso2-VR	GGTTATACATCTTGAGAATGACTCTG
gpr1-gRNA1	CGCAGTGAAAGATAAATGATCAAAAACAATTTTATACCAGTAGT TTTAGAGCTAGAAATAGCAAGT
gpa2-gRNA1	CGCAGTGAAAGATAAATGATCATAATATTGTCAACAGTAGAGT TTTAGAGCTAGAAATAGCAAGT
RAS1-gRNA1	CGCAGTGAAAGATAAATGATCATTCAATCATACTTTGGTT TTAGAGCTAGAAATAGCAAGT

RAS2-gRNA1	CCGCAGTGAAAGATAAATGATCCTGTATGAATCCTCAATTGTG TTTTAGAGCTAGAAATAGC
RAS2-gRNA2	CCGCAGTGAAAGATAAATGATCCACCAATATAGACAATTCCAG TTTTAGAGCTAGAAATAGC
RGT1-gRNA1	CGCAGTGAAAGATAAATGATCAATAAACATACTAATGATTTCGT TTTAGAGCTAGAAATAGCAAGT
RGT1-gRNA2	CGCAGTGAAAGATAAATGATCGTCTTGCAAAGCTTGAAATTGT TTTAGAGCTAGAAATAGCAAGT
Reg1-gRNA2	CGCAGTGAAAGATAAATGATCAAAAATCCTACTAATTTTACAGT TTTAGAGCTAGAAATAGCAAGT
gRNA1-SNF1 (381-414)	CGCAGTGAAAGATAAATGATCATCTTTGATCAAGGATATGAGT TTTAGAGCTAGAAATAGCAAGT
gRNA1-SNF1 (381-488)	CGCAGTGAAAGATAAATGATCATCTTTGATCAAGGATATGAGT TTTAGAGCTAGAAATAGCAAGT
MIG1-gRNA1	CGCAGTGAAAGATAAATGATCCGTATTTAAATCTGGTATACGT TTTAGAGCTAGAAATAGCAAGT
MIG1-gRNA2	CGCAGTGAAAGATAAATGATCAAAAATCAGAGTCAGATTTCGTGT TTTAGAGCTAGAAATAGCAAGT
PFK1-gRNA1	TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATA AATGATCGTGGCTGGAATCAAACACATGTTTTAGAGCTAGAAA TAGCAAGTTAAAATAAG
PFK2-gRNA1	TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATA AATGATCTTTTCGTTAACAGCAATCAAGTTTTAGAGCTAGAAA TAGCAAGTTAAAATAAG
PYK1-gRNA1	CGCAGTGAAAGATAAATGATCGTCTTGTTACTAATATGATAGT TTTAGAGCTAGAAATAGCAAGT
PYK2-gRNA1	CGCAGTGAAAGATAAATGATCTATCAACTTCGGTATTGAAAGT TTTAGAGCTAGAAATAGCAAGT
X-3 up fw	CGAGATCTTTGTGTTCCGGTTACC
enoX3-UP-R	GCAAGTTGGTGCACGTCGTTAGTGACATAACGCCGCGTGTCTC GTATGTCGGCTCTCGC
x3ENO2-F	GCGAGAGCCGACATACGAGACACGCGGCGTTATGTCACTAAC
ENO2p-R	TATTATGTATGTTATAGTATTAGTTG
RAS1-F	ACCAAGCAACTAATACTATAACATAACAATAAATGCAGGGAA ATAAATCAACTATAAG
RAS1-R	AAATCGCTCCCCATTTACCCAATTGTAGATATGCTCAACAAAT TATACAACAACCACC
ADH1t-F	GCATATCTACAATTGGGTGAAATG
ADH1-F	GCGAATTTCTTATGATTTATGATTT
adh1X3-down-F	TTATTTAATAATAAAAATCATAAATCATAAGAAATTCGCTGTG TCCGCGTTTCTAAGGC
X-3 down rv	GAGGTGGTTATTGATCACCGGA
pykX3-UP-R	GAACAACTTGGAAGGTTATACATGGGTACATAAATGCGTCTC GTATGTCGGCTCTCGC

PYK1t-F	GCATTTATGTACCCATGTATAACC
PYK1-R	AAAAAGAATCATGATTGAATGAAG
RAS2-F	TCAAAAAATAATATCTTCATTCAATCATGATTCTTTTTTTAAAC TTATAATACAACAGC
RAS2-R	AGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGCC TTTGAACAAGTCGAAC
TEF1p-F	TTTGTAATTA AAACTTAGATTAGATTG
TEF1p-R	ATAGCTTCAAAATGTTTCTACTC
tefX3-down-F	TGGAAGAGTAAAAAAGGAGTAGAAACATTTTGAAGCTATTGTG TCCGCGTTTCTAAGGC
tefENO2p-F	GAAGAGTAAAAAAGGAGTAGAAACATTTTGAAGCTATACGCG GCGTTATGTCACTAAC
ccwX3-UP-R	AACGTATTAAGTTTCTTTATTTTGCTTTGCCCTGGTTGTCTCGT ATGTCGGCTCTCGC
CCW12-F	AACCAGGGCAAAGCAAATAAAAAG
catCCW12-R	GTCGATCAGAATTATTATTTGCCATTATTGATATAGTGTTTAAG CGAATG
CAT8-F	ATGGCAAATAATAATTCTGATCGACAAGGTTTGGAAACCCA
CAT8-R	TTATTTGGCGTTTTGCCATTGGAATAAATCAGATACATTA
catADH-F	TATTCCAATGGCAAACGCCAAATAAGCGAATTTCTTATGATT TATGATT
CCW12-R	TATTGATATAGTGTTTAAGCGAATG
PDE1-F	CTGTCATTCGCTTAAACACTATATCAATAATGGTTGTATTCGAA ATAACTATACT
PDE1-R	TAAAAATCATAAATCATAAGAAATTCGCTTATAGAAACAAAGT GTGGCCTTCT
BCY1-F	CTGTCATTCGCTTAAACACTATATCAATAATGGTATCTTCTTTG CCCAAGGAA
BCY1-R	AAAATCATAAATCATAAGAAATTCGCTTAATGTCTTGTAGGAT CATTGAGCT
adrCCW12-R	GTTTGGTTTTTCTACGTTAGCCATTATTGATATAGTGTTTAAGC GAATGAC
ADR1-F	ATGGCTAACGTAGAAAAACCAAACGATTGTTTCAGGCTTTC
ADR1-R	TCAACTGTTTCCCTTTAGATGATTTTCCAAAGTGTTGAAAT
adrADH-F	GAAAATCATCTAAAGGAAACAGTTGAGCGAATTTCTTATGAT TTATGATTT
rgt1-up-F	CATCGTTTATCGTTTTTCTGTGAC
rgt1(TEF1p)-R	GGAGTAGAAACATTTTGAAGCTATAATTTGAAATATATTGGAG TTTGAGAG
TEF1p-F	ATAGCTTCAAAATGTTTCTACTCC
TEF1p-R	TTTGTAATTA AAACTTAGATTAGATTGC
hxt1(TEF1p)-F	GCAATCTAATCTAAGTTTTAATTACAAAATGAATTCAACTCCCG ATCTAATATC
hxt1(FBA1t)-R	CTATATCAATTAATTTGAATTA ACTTATTTTCTGCTAAACAAAC

	TCTTG
FBA1t-F	GTAAATCAAATTAATTGATATAGTTTTTTAATG
FBA1t-R	AGTAAGCTACTATGAAAGACTTTAC
rgt1(FBA1t)-F	GTAAAGTCTTTCATAGTAGCTTACTCAGGTAAGTATGCTCCCTT
	C
rgt1-dn-R	CAGGGAAAGGAAATGCAAAAAC
HXT4(TEF1p)-F	GCAATCTAATCTAAGTTTTAATTACAAAATGTCTGAAGAAGCT
	GCCTATC
HXT4(FBA1t)-R	CTATATCAATTAATTTGAATTAACCTACTTTTTTCCGAACATCT
	TCTTG
gpr1-up-F	CGAAAAGTAATCTGTATATATGAAGGTC
gpr1-up-R	CAAACATCGCGATACAAAACTTTTGAGTTGGAGAGTTTGCTT
	TATTC
gpr1-dn-F	GAATAAAGCAAACCTCTCCAACCTCAAAGTTTTTGTATCGCGAT
	GTTTG
gpr1-dn-R	GCGATTTTTTTGATTGAGTTCGC
gpr1-VF	CTAATTGAACCACGGTGGTAC
gpr1-VR	CTGGATCTAGTTGTCTATGTTTCTC
gpa2-up-F	CTGAATGAAAAAGTGAAAATGGAG
gpa2-up-R	GTTTTGTCTCTGTTTTAACTGTGCATGATATTTGCTTGAAAATA
	CGCG
gpa2-dn-F	CGCGTATTTTCAAGCAAATATCATGCACAGTTAAAACAGAGAC
	AAAAC
gpa2-dn-R	GATCTTCTCCGCATATTTCTTCTC
gpa2-VF	GATCGATAATACCACCTGTTTTATGC
gpa2-VR	CTCCTCCATATGAATCTTGGGTC
ras1-up-F	GCTAGGAATATGTATTACCCGCAC
ras1-up-R	CATATCAAGAGAGCAGGATCATTTGTCGTTCTAAAAAGGGAAA
	ATTTTG
ras1-dn-F	CAAAATTTTCCCTTTTTAGAACGACAAATGATCCTGCTCTCTTG
	ATATG
ras1-dn-R	GTATTATTAGGGGTGATCCAGTGTAC
RAS1-VF	GACCAAAAAATGGCAGGAATGAC
RAS1-VR	GATTTTCAAGAACTTTCAAGACTTC
ras2-up-F	GATGAAATGACTTGTACGCGC
ras2-up-R	GTTTCTACAACCTATTTCCCTTTTTATTTTTTTTCTGTATATCTCCTT
	TCAATTC
ras2-dn-F	GAAAGGAGATATACAGAAAAAAAATAAAAAGGAAATAGTTGT
	AGAAACGC
ras2-dn-R	CATGTGATACCAAGACCTTTTC
RAS2-VF	GATAAAATAATCAAATCGATGGTGC
RAS2-VR	CAGAAATTCAAAGGTGGAACGATAC

Primers

Primers for Myo-inositol (Sequences 5'-3')

PFK1-VER-F

TCCGATTTGAGATCGACTTG

PFK1-VER-R	TAGTTTCCATTTTTCCAGCG
PFK1-repair-F	ACTTTAGCACTATTTGGGAAAGCTTTTATATAAAAAATCTGAA ACAAAATCATATCAAAGATGATTGCAATGAAAAGTTTAAGTTA AGCAAAAGGAGGTAAAAATGGCATGCACTTTAAT
PFK1-repair-R	ATTAAAGTGCATGCCATTTTTACCTCCTTTTGCTTAACTTAAAC TTTTCATTTGCAATCATCTTTGATATGATTTTGTTTCAGATTTTTT ATATAAAAGCTTTCCCAAATAGTGCTAAAGT
PFK2-VER-F	TCGTTCCAAATGGCGTCCAC
PFK2-VER-R	TTCCTGAGAGTTATCAGACG
PFK2-repair-F	TGAACAATAGAAGTACTAGTTTAGAGACTAGTTTAGCATTGGCCA AGAACTAACCATACGCAAAGAAAATGACCTTTTATTACACTTT CTATTATTAATGTCAATTAATGTTAACCCATGTT
PFK2-repair-R	AACATGGGTTAACATTAATTGACATTAATAATAGAAAGTGTA TAAAAGGTCATTTTCTTTGCGTATGGTTAGTTCTTGCCAATGC TAAACTAGTCTCTAAATCTAGTTCTATTGTTCA
XI-2 VER-F	GTTTGTAGTTGGCGGTGGAG
XI-2 VER-R	GAGACAAGATGGGGCAAGAC
XI-2 UP-F	TAACCTTCGTATGAGGATTTTC
XI-2-up(cyc1)-R	TTAATTTGCGGCCGGTACCCTTCTATGGCACATTTTTCTGTTG
CYC1(XI-2up)-R	CAGAAAAATGTGCCATAGAAGGGTACCGGCCGCAAATTA
suhB(CYC1)-R	ATAACTAATTACATGACTCGAGGTGACGGTATCTTATCTCTTC AAAGCATCAGAC
CYC1-F	GATACCGTCGACCTCGAGTCAT
suhB(tdh3)-F	TTAGTTTCGAATAAACACACATAAACAAACAAAATGATGCACC CTATGCTTAACATAG
TDH3-R	CATTTTGTGTTGTTTATGTGTG
TEF1p-f(TDH3RC)	ATTGATAATGATAAACTCGAATAGCTTCAAAATGTTTCTAC
TDH3p(TEF1)-F	TAGAAACATTTTGAAGCTATTCGAGTTTATCATTATCAAT
GDH2-f	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGATG CTTTTTGATAACAAAAAT
TEF1-r	CATTTTGTAAATTAACCTTAG
ADH2(GDH2)-F	AAGCGGAGGCAAGTGCTTGAGCGAATTTCTTATGATTTATGAT TT
GDH2(ADH2)-R	ATAAATCATAAGAAATTCGCTCAAGCACTTGCCTCCGCTT
XI-2 dn-F	TTCACCCAATTGTAGATATGCCACAAGTAAAGCTCGTTGAC
ADH2(XI-2dn)-R	CAACGAGCTTTACTTGTGGGCATATCTACAATTGGGTGAA
XI-2-dn-R	ATGGTTGAAAAGGTTACAGAGG
XII-3 VER-R	TGGCCAATTGTTTCAAGTCAAG
XII-3 VER-F	TGGGCAGCCTTGAGTAAATC
XII-3-up-F	TGTGCCCTTAAAATTCATATAC
pyk1t-R	TAAGGGGTACCTGCTCATTCGCATTTATGTACCCATGTATAAC
XII-3-up-R	ATACATGGGTACATAAATGCGAATGAGCAGGTACCCCTTA
INO1(pyk1t)-R	AAATAATATCTTCATTCATCATGATTCTTTTTTTTACAACAATC TCTCTTCGAAT

PYK1t-F	AAAAAGAATCATGATTGAATGAAGATA
INO1(pgk1t)-F	GTAATTATCTACTTTTTACAACAAATATAACAAAATGACAGAA GATAATATTGCTCC
PGK1p-R	TTTGTTATATTTGTTGTAAAAAG
PGK1p-F	TATTTTGCTTTGCCCTGGTTACGCACAGATATTATAACATC
CCW12(pgk1)-f	ATGTTATAATATCTGTGCGTAACCAGGGCAAAGCAAATAAAAA G
ITR1(ccw12)-F	TAATCTTCTGTCAATTCGCTTAAACACTATATCAATAATGGGAAT ACACATACCATATC
CCW12-r	TATTGATATAGTGTTTAAGCGAATG
ITR1(fba1t)-R	TCATTAAAAACTATATCAATTAATTTGAATTAACCTATATATC CTCTATAATCTCTTG
FBA1t(xii3dn)-R	CAAACCTAATTAGCTCTATGCAGTAAGCTACTATGAAAGACT
FBA1t-f	GTAAATTCAAATTAATTGATATAGTTT
XII-3-dn-F	AGTCTTTCATAGTAGCTTACTGCATAGAGCTAATTAGGTTTG
XII-3-dn-R	GAACCTACAAGCTGATTTTGGT
II-4-gRNA-f1	AAACGAGTAAGCTCGTCCCTAAATACTACCTAACAGGTTTTTA GAGCTAGAAATAGCAAG
II-4-gRNA-f2	tgaagacgcatgTTTAGGCTGATGAGTCCGTGAGGACGAAACGAGT AAGCTCGTC
T1-R	GATTCTTCTCCAGATCTAGCGACTCTGGCAGATAAGATCGTGT GCGCGACAACACAGT
T1-F	TTGGGGTTGTTAGTACGAGAAGCT
T2-F	TGGACCATTACTGTGTTGTGCGCACACGATCTTATCTGCCAGA GTCGCTAG
T2-R	GCCGAATAGTTTGTATACGTCTTATGT
T3-F	ACTCATTACATAAGACGTATACAAACTATTCGGCTTATTGCGTT CGTTGTCTTTGT
T3-R	tATTTGTCCCTATTTCAATCAATTGAACAACCTATATGGATGAAG AATCAACAGTACC
T4-F	CAAGAACGGTACTGTTGATTCTTCATCCATATAGTTGTTCAATT GATTGAAATAGGG
T4-R	TCTTGACATAATCACTGATGATTACAATTTGGTTTTTTGTAGAAA TGTcttggtgcc
T5-F	acgaggacaccaagACATTTCTACAAAACCAAATTGTAATCATCAGT GATTATG
T5-R	ATGAGCTGAGATTTGAGGAGAGATTACAGTAAACGGGAAGTCT TTACAGTTTTAG
T6-F	TCCTAACTAAAACGTAAAGACTTCCCGTTTACTGTAATCTCTC CTCAAATC
T6-R	ctaaagttcactcttatacaactatcaaacatcaaaaATGACTATTCAATACACTCCTA
T7-F	ttttgatgttgatagttgataagagtgaac
T7-R	attactgtttgggcaatcctgttgataagac
T8-F	gtcttatcaacaggattgccccaaacagtaatgatctaacaatccaaagacgaaag

T8-R	cgtttcgaataaattagttgtttttg
T9-F	tgagaagatcaaaaaacaactaattattcgaacgATGCACCCTATGCTTAACATAG
T9-R	CATTAGTTTAGTCTTAAACTAAGCGAAACTACGTACTTATCTCT TCAAAGCATCAGAC
T10-F	GTACGTAGTTTCGCTTAGTTTAAGACTAAAC
T10-R	CTTGTCGTTAGTTCAATGACCTGGTGTC
T11-F	CCAGACACCAGGTCATTGAACTAACGACAAGGAATAGTAAGG ACCGGGTTCAGTTGACT
T11-R	GCTTGTCGTTGCGTTTTAGATGTC
JD-II-4-fw	CATGAGTCACGGCAGAGTT
JD-II-4-rv	CAGATAAAGGAGGCTCTCCAT

Primers	Primers for Glucosamine (Sequences 5'-3')
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X-2 up fw	CGTCTATGAGGAGACTGTTAGTTG
X-2 up rv	GACCACTTCGAGAGCAAGTTG
X-2 down fw	CCTGCATAATCGGCCTCAC
X-2 down rv	CTCGCCAAGGCATTACCATC
x2ADH1t-F	CAACTTGCTCTCGAAGTGGTCGCGAATTTCTTATGATTTATG
ADH1t-R	GCATATCTACAATTGGGTGAAATG
CCW12-F	TTGAAATGGCAGTATTGATAATGATAAACTCGAAACCAGGGCA AAGCAAAATAAAAAG
CCW12-R	TATTGATATAGTGTTTAAGCGAATGAC
FBA1t-F	GTAAATCAAATTAATTGATATAG
x2FBA1t-R	GTGAGGCCGATTATGCAGGAGTAAGCTACTATGAAAGACTTTA C
GlmD-F	CAAATCGCTCCCCATTTACCCAATTGTAGATATGCTTATGGTC TCAAAGAAGCAGC
GlmD-R	AACTTAGTTTCGAATAAACACACATAAACAAAACAAAATGAAG GTTATGGAATGTCAAAC
GlmP-F	AATCTTCTGTCAATTCGCTTAAACACTATATCAATAATGATTAAG GTTTTGTTGTTGG
GlmP-R	ACTCATTAAAAACTATATCAATTAATTTGAATTAACTTAAATA ACACCGAAATGCTTC
TDH3-F	TTTGTGTTGTTTATGTGTGTTTATTCG
TDH3-R	TCGAGTTTATCATTATCAATACTG
X-3 up fw	CGAGATCTTTGTGTTCCGGTTACC
adhX3-UP-R	TATTTAATAATAAAAATCATAAATCATAAGAAATTCGCGTCTC GTATGTCGGCTCTCGC
fba1X3-down-F	TCTTCGAGTTCTTTGTAAAGTCTTTCATAGTAGCTTACTTGTGTC CGCGTTTCTAAGGC
X-3 down rv	GAGGTGGTTATTGATCACCGGA
XII-5 up F	GTAGTGATCATTGGCTTAACG
adh1XII5-UP-R	ATTTAATAATAAAAATCATAAATCATAAGAAATTCGCGTGACA ATAAATTCAAACCGGT
fba1XII5-down-F	TCTTCGAGTTCTTTGTAAAGTCTTTCATAGTAGCTTACTCAACT

	CAGAAGTTTGACAGC
XII-5 down R	CTCTTTTGCCTTTCAAAAAAG
XI-1 up F	ATTTGTGTGAAGGAATAGTGACG
XI-1 up R	CAATGGGCTTGGTATTCCG
XI-1 down F	TTTCTTGGCATTGGCAAATC
XI-1 down R	AAGAGCCGAGTCCCCATCAG
XI1GlmP-F	CGGAATACCAAGCCCATTGAACCAGGGCAAAGCAAATAAAAA G
XI1GlmP-R	GATTTGCCAATGCCAAGAAAAGTAAGCTACTATGAAAGACTTT AC
II-5-gRNA-f1	AAACGAGTAAGCTCGTCAACTTTGAAACAAAAGAAGGGTTTTA GAGCTAGAAATAGCAAG
II-5-gRNA-f2	tgaagacgccatgAAAGTTCTGATGAGTCCGTGAGGACGAAACGAGT AAGCTCGTC
II5-V-F	TTGATGGTTTGTGATCAACTTGC
II5-V-R	TTGGAACGCATCAAATACCATCTG
T35-F	CCTGATTGGTCAAAGACAAGTG
T35-R	AAACAATCCAGACACCAGGTCATTGAACTAACGACAAGGCAT GGACCTGCTCTTATCTC
T36-F	AAAAGTTTTAAAGATGAGATAAGAGCAGGTCCATGCCTTGTCG TTAGTTCAATGACCTG
T67-R	GATTGATAGAGAAGCTGCTTCTTTGAGACCATAAGTACGTAGT TTCGCTTAGTTTAAAG
T68-F	ATTAGTTTAGTCTTAAACTAAGCGAACTACGTACTIONTATGGTCT CAAAGAAGCAGCTTC
T68-R	CTCACTACATACATTTTAGTTATTCGCCAACATGAAGGTTATGG AATGTCAAACCTTACG
T69-F	CTTCGTAAGTTTGACATTCCATAACCTTCATGTTGGCGAATAAC TAAAATGTATGTAG
T44-R	attggacgaggacaccaagACATTTCTACAAAAATAACTGTGCGCCTCTT TTATCTGC
T45-F	CAGTGCGGCAGATAAAAGAGGCGACAGTTATTTTTTTGTAGAAA TGTcttgggtgcctc
T70-R	CATCAACATCCAACAACAAAACCTTAATCATATAGTTGTTCAA TTGATTGAAATAGGG
T71-F	ttATTTGTCCCTATTTCAATCAATTGAACAACCTATATGATTAAGG TTTTGTTGTTGG
T71-R	CATTACATAAGACGTATACAACTATTCGGCTTAAATAACACC GAAATGCTTCAAAGC
T72-F	TACAAGGCTTTGAAGCATTTCGGTGTTATTTAAGCCGAATAGTT TGTATACGTCTTATG
T47-R	TCCCTTTTTATGATTAAATTAGATGACTACTTCTCTTATCTGCCA GAGTCGCTAGATC
T48-F	AGATCTAGCGACTCTGGCAGATAAGAGAAGTAGTCATCTAATT

X-2 down rv	CTCGCCAAGGCATTACCATC
XYL2-F	GTCATTGCTTAAACACTATATCAATAATGACTGCTAATCCATC TTTAG
XYL2-V-F	CCTACAATTCACGTCATCCTTG
XYL2-UP-F	CCACTATGGTGAACATTGGGT
xyl2ADH-R	TATTCATACTGCCTTGAGGATAGAGAACACCATTGAGCGCGAA TTTCTTATGATTTATG
adhXYL2-up-R	TTAATAATAAAAATCATAAATCATAAGAAATTCGCGCTCAATG GTGTTCTCTATCCTC
ARAL-R	CAAATCGCTCCCCATTTACCCAATTGTAGATATGCTCATATCA GAATCCCCTCCTCAG
aralADH-F	ACCAAATTGGCTGAGGAGGGGATTCTGATATGAGCATATCTAC AATTGGGTGAAATGG
aralTDH-R	CAGGCGTATCATGACTGGCCATAATACGCATTTTGTGTTGTTTAT GTGTGTTTATTCG
ARAL-F	AACTTAGTTTTCGAATAAACACACATAAAACAAACAAAATGCGTA TTATGGCCAGTCATG
tdhCCW12-F	TTGAAATGGCAGTATTGATAATGATAAACTCGAAACCAGGGCA AAGCAAAATAAAAAG
ccwTDH-F	AGTTTCTTTTATTTTGCTTTGCCCTGGTTTCGAGTTTATCATTAT CAATACTG
XylA-F	TCTGTCATTGCTTAAACACTATATCAATAATGGAATTCAATAT GCAAGCTTAC
CCW12-R	TATTGATATAGTGTTTAAGCGAATGAC
XylA-R	AACTATATCAATTAATTTGAATTAACTTATTTATCAAACAAATA ATGATTAACC
FBA1t-F	GTTAATTCAAATTAATTGATATAG
fbaxYL1-down-F	CGAGTTCTTTGTAAAGTCTTTCATAGTAGCTTACTAGGAAAGA AAATGTAACTTTTGGC
xylFBA1-R	CATCTTGATGCCAAAAGTTACATTTTCTTTCCTAGTAAGCTAC TATGAAAGACTTTAC
XYL2-DOWN-R	CGAACCTCATGAATGGCTTTG
XYL2-V-R	AGTAGCGGCAGACTGAATAG
XYL2-gRNA1	CCGCAGTGAAAGATAAATGATCTGTGCGCAGCAAATTTTAAATG TTTTAGAGCTAGAAATAGC
XYL2-gRNA2	CCGCAGTGAAAGATAAATGATCTTACGTGGTAGAATCACCAAG TTTTAGAGCTAGAAATAGC
GRE3-gRNA1	CCGCAGTGAAAGATAAATGATCGTAATTTACAAAACACTCAACTG TTTTAGAGCTAGAAATAGC
GRE3-gRNA2	CCGCAGTGAAAGATAAATGATCATTATGAAGCTATCAAATTG TTTTAGAGCTAGAAATAGC
GRE3-V-F	TTGTAACGTGAATGACCAGAG
GRE3-UP-F	GTAGACGCAGATACTGTAAATGC

GRE3-UP-R	CGTTGAGTATGGATTTTACTGGCTGGACTTTAAAAAATTTCCAA TTTTCTTTACG
GRE3-DOWN-F	CGTAAAGGAAAATTGGAAATTTTTTAAAGTCCAGCCAGTAAAA TCCATACTC
GRE3-DOWN-R	TGTGAGTCAATGTCGAGTTCAG
GRE3-V-R	TGGCTAGTGCTATCATTGCTG
Primers	Primers for Sucrose (Sequences 5'-3')
6006	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC
SUC2-gRNA1	CCGCAGTGAAAGATAAAATGATCGCATTACTAATGTTCAATATG TTTTAGAGCTAGAAATAGC
SUC2-gRNA2	CCGCAGTGAAAGATAAAATGATCTACATTAGCTATTCTCTTGAG TTTTAGAGCTAGAAATAGC
HoSUC2-up-F	GCCTAAGGGCTCTATAGTAAAC
HoSUC2-up-R	GACAATAAGTTTTATAACCTCATATACGTTAGTGAAAAGAAAA G
HoSUC2-down-F	CTTTTCTTTTCACTAACGTATATGAGGTTATAAACTTATTGTC
HoSUC2-down-R	GTAGTGTAAGGCAACTACATTAC
VSUC2-F	TCGAGGAATGCTTAAACGAC
MAL32-gRNA	CCGCAGTGAAAGATAAAATGATCTATAAGGAACTTATGATTTAG TTTTAGAGCTAGAAATAGC
IMA-gRNA	CCGCAGTGAAAGATAAAATGATCTTCTTCTGGAGACCTCCTAAG TTTTAGAGCTAGAAATAGC
HomoIMA-com-R	TTTTCGTAGTTGGCAATATCGTAACCCATATCATCTTGTGGCGA GTCG
HomoIMA-com-F	CAATGGTCTCGTGAGGAGCCAAATGCTGGTTTTTCTGGTCCTA
HomoIMA-F	CGACTCGCCACAAGATGATATGGGTTACGATATTGCCAACTAC GAAAAGGTCTGGCCAACATGCTAGAACACCTATG
HomoIMA-R	TAGGACCAGAAAAACCAGCATTGGCTCCTCACGAGACCATTG CATAGGTGTTCTAGCATGTTGGCCAGACC
IMA-verify-F	ATGACTATTTCTTCTGCACATCC
IMA-Verify-R	ATGGCTTCAATGTTCTGGAAG
HomoMAL-com-F	CCTCCCAAGAGAAGGTGCTTCTTTATCTTTTATTCTTGAAAA
HomoMAL-F	TTTTAAAGACTCCAATAACGATGGCTGGGGTGATTTAAAAGGT ATCACTTCCAAGTTGCAGCGAAGAAATTGAATTCAG
HomoMAL-R	TTTCCAAGAATAAAAAGATAAAGAAGCACCTTCTCTTGGGAGGC TGAATTCAATTTCTTCGCTGCAACTTGGAAGTG
HomoMAL-com-R	ATACCTTTTAAATCACCCAGCCATCGTTATTGGAGTCTTTAAA A
MAL-Verify-F	ATGACTATTTCTGATCATCCAG
MAL-Verify-R	ATGGTTTCAAACTCTGGAG
IMA5-gRNA1	CCGCAGTGAAAGATAAAATGATCATGAATGAATCATTTAGAGAG TTTTAGAGCTAGAAATAGC
IMA5-gRNA	CCGCAGTGAAAGATAAAATGATCTGGCCAACCTGATTTCAATTG TTTTAGAGCTAGAAATAGC

HomoIMA5-down-F	GTGCTCAATATTGAAAAGAAAAACCGAGCACATTTAACATAAA CG
HomoIMA5-down-R	GTTAGGGTGAGAATAGTCGAATG
HomoIMA5-up-F	AGTTGCGTGAAAGCGTAAATG
HomoIMA5-up-R	CGTTTATGTAAATGTGCTCGGTTTTTCTTTCAATATTGAGCA C
IMA5-Verify-F	GATGCTCTAGGCTGCTTTCTC
106a-up-F	GAGGGTCTGTCCAGCGAATAAG
tef106a-up-R	GGAGTAGAAACATTTTGAAGCTATCACAACCGACGATCCGGGG TC
pgk106a-down-f	GAAAATTCTGCGTTCGTTAGCTAATTTTTCCGGCAGAAAG
106a-down-R	GAAGGAAAGGAAATCACTTGG
106a-TEF-F	CCGGATCGTCGGTTGTGATAGCTTCAAATGTTTCTACTCC
sufTEF-R	GATTCATTAGTAGATGGATTATCCATTTTGTAAATTTAACTTAG ATTAG
sufPGK-F	CAATTGCTGGTGGTTTTTCATTAAATTGAATTGAATTGAAATCGA TAGATC
106a-PGK-R	CTTTCTGCCGAAAAATTAGCTAACGAACGCAGAATTTTC
SUF1-F	CTAATCTAAGTTTTAATTACAAAATGGATAATCCATCTACTAAT GAATC
SUF1-R	GATCTATCGATTTCAATTCAATTCAATTTAATGAAAACCACCAG CAATTG
106a-verify-F	CCACATATTTCCCTTTCTCTC
106a-verify-R	GGAAGACACTAAAGGTACCTAGC
X-2 up fw	CGTCTATGAGGAGACTGTTAGTTG
X-2 up rv	GACCACTTCGAGAGCAAGTTG
X-2 down fw	CCTGCATAATCGGCCTCAC
X-2 down rv	CTCGCCAAGGCATTACCATC
x2ADH1t-F	CAACTTGCTCTCGAAGTGGTCGCGAATTTCTTATGATTTATG
ADH1t-R	GCATATCTACAATTGGGTGAAATG
CCW12-F	TTGAAATGGCAGTATTGATAATGATAAACTCGAAACCAGGGCA AAGCAAATAAAAAG
CCW12-R	TATTGATATAGTGTTTAAGCGAATGAC
FBA1t-F	GTTAATTCAAATTAATTGATATAG
x2FBA1t-R	GTGAGGCCGATTATGCAGGAGTAAGCTACTATGAAAGACTTTA C
SPP-F	AATCTTCTGTCATTTCGCTTAAACACTATATCAATAATGAGACAA TTGTTGTGATTTTC
SPP-R	ATTAAAAACTATATCAATTAATTTGAATTAACTTAAGACAAG AAATCAAATGAGC
SPS-F	CGCTCCCCATTTACCCAATTGTAGATATGCTTAAACTGGGTCT AACAATTCG
SPS-R	CTTAGTTTCGAATAAACACACATAAACAAACAAAATGTCTTAC TCCTCTAAGTAC

TDH3-F	TTTGTTTGTATTATGTGTGTTTATTCG
TDH3-R	TCGAGTTTATCATTATCAATACTG
X-2 det fw	TGCGACAGAAGAAAGGGAAG
X-2 det rv	GAGAACGAGAGGACCCAACAT
X-3 up fw	CGAGATCTTTGTGTTCCGGTTACC
pykX3-UP-R	GAACCTAATTGGAAGGTTATACATGGGTACATAAATGCGTCTC GTATGTCGGCTCTCGC
X-3 down rv	GAGGTGGTTATTGATCACCGGA
ugpX3-down-F	GATCCTTTTCCTATTTTTCTCCTCTGTGTCCGCGTTTCTAAGGC
PYK1t-F	GCATTTATGTACCCATGTATAACC
PYK1-R	AAAAAGAATCATGATTGAATGAAG
glgCTM-F	CAAAAAATAATATCTTCATTCAATCATGATTCTTTTTTTATCG CTCCTGTTTATGCC
glgCTM-R	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGTT AGTTTAGAGAAGAACG
TEF1p-F	TTTGTAATTA AAACTTAGATTAGATTG
TEF1p-R	ATAGCTTCAAAATGTTTCTACTC
tefENO2p-F	GAAGAGTAAAAAAGGAGTAGAAACATTTTGAAGCTATACGCG GCGTTATGTCACTAAC
ENO2p-R	TATTATTGTATGTTATAGTATTAGTTG
UGP1-F	ATAACACCAAGCAACTAATACTATAACATAACAATAAATGTC CACTAAGAAGCACACC
UGP1-R	GCCTTAGAAACGCGGACACAGAGGAGAAAAATAGGAAAAGGA TC
adhUGP1-R	TTAATAATAAAAATCATAAATCATAAGAAATTCGCGAGGAGAA AAATAGGAAAAGGATC
ADH1-F	GCGAATTTCTTATGATTTATGATTT
FBA1t-R	AGTAAGCTACTATGAAAGACTTTAC
fbax3-down-F	TCTTCGAGTTCTTTGTAAAGTCTTTCATAGTAGCTTACTTGTGTC CGCGTTTCTAAGGC
X-3 Verify fw	TGACGAATCGTTAGGCACAG
X-3 Verify rv	CCGTGCAATACCAAAATCG
XI-1 up F	ATTTGTGTGAAGGAATAGTGACG
XI-1 up R	CAATGGGCTTGGTATTCCG
XI-1 down F	TTTCTTGGCATTGGCAAATC
XI-1 down R	AAGAGCCGAGTCCCCATCAG
XI-1-TEF-F	CGGAATACCAAGCCCATGATAGCTTCAAAATGTTTCTACTCC
XI-5-PGK-R	GATTTGCCAATGCCAAGAAATAACGAACGCAGAATTTTCG
XI-1 det fw	CTTAATGGGTAGTGCTTGACACG
XI-1 det rv	GAAGACCCATGGTTCCAAGGA
XII-5 up F	GTAGTGATCATTGGCTTAACG
XII-5 up R	GTGACAATAAATTCAAACCGGT
XII-5 down F	CAACTCAGAAGTTTGACAGC
XII-5 down R	CTCTTTTGCCTTTCAAAAAAG

XII-5-TEF-F	ACCGGTTTGAATTTATTGTACATAGCTTCAAAATGTTTCTACT CC
XII-5-PGK-R	GCTGTCAAACCTTCTGAGTTGTAACGAACGCAGAATTTTCG
XII-5 det fw	CCACCGAAGTTGATTTGCTT
XII-5 det rv	GTGGGAGTAAGGGATCCTGT
XII-5 det rv	GTGGGAGTAAGGGATCCTGT
pgiXII4-up-R	ATCACAGACATCCGACATCTAGTAACACAGGTGACCTATCGTG TGCGCGACAACACAGT
PGIp-F	TAGGTCACCTGTGTTACTAGATG
pgiSUF1-F	CCGGAATAATTCAACTCCAACCAATTGATAAAAATGGATAATCC ATCTACTAATGAATC
PGIp-R	TTTATCAATTGGTTGGAGTTG
SUF1-verify-F	GTATCTGCTTTATCAGGTCCATG
das1SUF1-R	TAAGGGCTCCTAACTAAAAGTGTAAAGACTTCCCGTTTAATGA AAACCACCAGCAATTG
DAS1t-F	ACGGGAAGTCTTTACAGTTTTAG
DAS1t-R	TTCTTCCAGATCTAGCGACTCTGGCAGATAAGACCAAATTGTA ATCATCAGTGATTATG
ADH2t-R	TCTTATCTGCCAGAGTCGCTAGAT
adhSPS-F	GAAACTCATTACATAAGACGTATACAACTATTCGGCTTAAAC TGGGTCTAACAATTCCG
ADH2t-f	GCCGAATAGTTTGTATACGTCTTATGT
gapSPS-R	aatttATTTGTCCTATTTCAATCAATTGAACAACCTATATGTCTTAC TCCTCTAAGTAC
GAP-R	ATAGTTGTTCAATTGATTGAAATAG
TEF1-F	gattggacgaggacaccaagACATTTCTACAAAAATAACTGTGCCTCT TTTATCTGC
GAP-F	TTTTTGTAGAAATGTcttgggtg
tefSPP-F	TTATTCTCACTACATACATTTTAGTTATTCGCCAACATGAGACA ATTGTTGTTGATTTT
TEF1p-r	GTTGGCGAATAACTAAAATGTATGT
pmp20SPP-R	ATTAGTTTAGTCTTAACTAAGCGAACTACGTACTTAAGACA AGAAATCAAAATGAGC
PMP20t-F	GTACGTAGTTTCGCTTAGTTTAAG
pmp20II4-dwon-F	AACAATCCAGACACCAGGTCATTGAACTAACGACAAGGAATA GTAAGGACCGGGTTCAG
PMP20t-r	CTTGTCGTTAGTTCAATGACCTGGTGTC
Primers	Primers for Starch (Sequences 5'-3')
GLC3-gRNA	CCGCAGTGAAAGATAAATGATCGATTCCCGTCTTTTAAATTAGT TTTAGAGCTAGAAATAGC
GSY1-gRNA	CCGCAGTGAAAGATAAATGATCCAATCTACAGTATTTTGATGG TTTTAGAGCTAGAAATAGC
GSY2-gRNA1	CCGCAGTGAAAGATAAATGATCAATTTGTAAAAAAGACAAGA GTTTTAGAGCTAGAAATAGC

GSY2-gRNA2	CCGCAGTGAAAGATAAATGATCACAAATAACTGCTTTTGAAGG TTTTAGAGCTAGAAATAGC
GLC3-down-F	CCAAGTATAAAGAACCGTCAAGACTCTATACTATATTGCTTAC
GLC3-down-R	TGTTGCAATCAATGAACAACC
GLC3-UP-F	GCACTCATCAACAATGTGATAG
GLC3-UP-R	GTAAGCAATATAGTATAGAGTCTTGACGGTTCTTTATACTTGG
GLC3-verify-F	CGGTGAATTCTGTAGTCTGTC
GLC3-verify-R	CCCAACAATTACATCAAAATCC
GSY1-down-F	GCCTGGAAACCTGTGAAGAAAAACCATCTTAACTTCTGCTAAC
GSY1-down-R	TGTGAGTTCTCTACGAGAATTG
GSY1-up-F	GACCATTTCCCTGCCACAGAAC
GSY1-UP-R	GTTAGCAGAAGTTAAGATGGTTTTTCTTCACAGGTTTCCAGGC
GSY1-verify-F	CACAATTTCCCGATCGGTAC
GSY1-verify-R	GTCGTCCATAGTAATTGACTCG
GSY2-down-F	GCATCACTGAGAAGTGGTAGCCATAAAGACATACGACATTTCCG
GSY2-down-R	AATGCCAGGTATGATGTAACG
GSY2-UP-F	GTTCTTGCAGCTTACCAATTC
GSY2-UP-R	CGAAATGTCGTATGTCTTTATGGCTACCACTTCTCAGTGATGC
GSY2-verify-F	CACGATGCTGACTTAGTACTAT
GSY2-verify-R	CGAAGTTGCTATTGTTGATGC
BE3-F	TCTAATCTAAGTTTTAATTACAAAATGGCTTCTCTGAGGAAGG ACTCTCG
BE3-R	CTATCGATTTCAATTCAATTCAATCTAAACATCTTCGGGTAACA GG
be3PGK-F	CCTGTTACCCGAAGATGTTTAGATTGAATTGAATTGAAATCGA TAG
be3TEF-R	CGAGAGTCCTTCCTCAGAGAAGCCATTTTGTAATTA AAACTTA GATTAGA
glc3PGK-R	GTAAGCAATATAGTATAGAGTTAACGAACGCAGAATTTTCG
glc3TEF-F	CCAAGTATAAAGAACCGTCAAGATAGCTTCAAAATGTTTCTAC TCC
tefGLC3-UP-R	GGAGTAGAAACATTTTGAAGCTATCTTGACGGTTCTTTATACTT GG
pgkGLC3-down-F	CGAAAATTCTGCGTTCGTTAACTCTATACTATATTGCTTAC
gsy1ADHt-R	GTTAGCAGAAGTTAAGATGGTTGCATATCTACAATTGGGTGAA ATG
gsy1TDH3-F	GCCTGGAAACCTGTGAAGAAATCGAGTTTATCATTATCAATAC TG
TM-F1	AAACACACATAAACAACAAAATGGTTAGTTTAGAGAAGAAC G
TM-F2	CTGCATCAACTCCGGGATCAAACGTATGGGCGTGATCACCCAG
TM-F3	CTCGATCTGGCCTCTGTGGTGGATAAACTGGATATGTACGATC GC
TM-F4	GACCCTTAACTCACTGGTTTTCCGATGGTTGTGTGATCTCCGGTT

	CG
TM-R1	CTGGGTGATCACGCCATACGTTTGATCCCGGAGTTGATGCAG
TM-R2	GCGATCGTACATATCCAGTTTATCCACCACAGAGGCCAGATCG
	AG
TM-R3	CGAACCGGAGATCACACAACCATCGGAAACCAGTGAGTTAAG
	GGTC
TM-R4	AAATCATAAATCATAAGAAATTCGCTTATCGCTCCTGTTTATGC
	CC
tmADH-F	GGGCATAAACAGGAGCGATAAGCGAATTTCTTATGATTTATGA
	T
tmTDH3-R	CGTTCTTCTCTAAACTAACCATTTTGTGTTTATGTGTGTTTAT
	TCG
tdhGSY1-UP-R	GCAGTATTGATAATGATAAACTCGATTTCTTACAGGTTTCCAG
	GC
adhGSY1-down-F	CATTTACCCAATTGTAGATATGCAACCATCTTAACTTCTGCTA
	AC
ss3PGK-F	CTATCACTCTGCACGCAAGTAAATTGAATTGAATTGAAATCGA
	TAG
gsy2PGK-R	CGAAATGTTCGTATGTCTTTATGGTAACGAACGCAGAATTTTCG
gsy2TDH3-F	GCATCACTGAGAAGTGGTAGTCGAGTTTATCATTATCAATACT
	GCC
SS3-F	CGAATAAACACACATAAAACAAAATGGGAAGTGCTCAGA
	AAAGAACTC
SS3-R	CTATCGATTTCAATTCAATTCAATTTACTTGCGTGCAGAGTGAT
	AG
ss3TDH3-R	GAGTTCTTTTCTGAGCACTTCCCATTTTGTGTTTATGTGTGTT
	TATTCG
tdhGSY2-UP-R	GGCAGTATTGATAATGATAAACTCGACTACCACTTCTCAGTGA
	TGC
pgkGSY2-down-F	CGAAAATTCTGCGTTCGTTACCATAAAGACATACGACATTTTCG
GDB1-gRNA1	CCGCAGTGAAAGATAAATGATCATAATTAAGGCGAAATAACTG
	TTTTAGAGCTAGAAATAGC
GDB1-gRNA2	CCGCAGTGAAAGATAAATGATCGTACATTACTTTTCATCGTAG
	TTTTAGAGCTAGAAATAGC
GDB1-UP-R	GCAAAAAGGGACCGTTCAATTAAAGCAGTTATATACTAAGGAC
	G
GDB1-down-F	CGTCCTTAGTATATAACTGCTTTAATTGAACGGTCCCTTTTTGC
GDB1-UP-F	TCAGGAACTTGGTTTTCAACC
GDB1-down-R	TTGATTGTAACACGCAAACGG
GDB1-verify-F	GTCTGTCCCTATTCTAATCCTC
GDB1-verify-R	CTCGCCTCTTTCTGTAAGTGC
GLG1-gRNA1	CCGCAGTGAAAGATAAATGATCAAGTCATTAGTAGAAACTG
	TTTTAGAGCTAGAAATAGC
GLG1-gRNA2	CCGCAGTGAAAGATAAATGATCTAAAGTGCCATTAATAGAGG

	TTTTAGAGCTAGAAATAGC
GLG1-UP-F	TTTTAGACAACCGGAACCTG
GLG1-down-F	GTGGAGACTTAGTAGGGATTCTACTTCCACGCACATATTAG
GLG1-UP-R	CTAATATGTGCGTGGAAGTAGAATCCCTACTAAGTCTCCAC
GLG1-down-R	TAGAAGGAGGCCGTTTAACG
GLG1-verify-F	CAGACTAATAGGTAAGTACG
GLG1-verify-R	TGGTTTGAATTTGGGATTCTTC
GLG2-gRNA1	CCGCAGTGAAAGATAAATGATCGTGTGAAGTAGTTACTTAAGG
	TTTTAGAGCTAGAAATAGC
GLG2-gRNA2	CCGCAGTGAAAGATAAATGATCCTGTACATTGCTGTATTACAG
	TTTTAGAGCTAGAAATAGC
GLG2-UP-R	CTAAGAGGTAGAGGGATGGCAAGCTCCGTAACAAACCTTAC
GLG2-down-F	GTAAGGTTTGTACGGAGCTTGCCATCCCTCTACCTCTTAG
GLG2-UP-F	CTTACGTACATGCAACAACACTAC
GLG2-down-R	GTCATAGTTGGTTTGAATTTGGG
GLG2-verify-F	CGTTTATTCAACGTTTCCCTATC
GLG2-verify-R	ACAAGTTTTCGTCCAATTCC
adhGLG2-up-R	CATAAATCATAAGAAATTCGCAGCTCCGTAACAAACCTTAC
fba1GLG2-down-F	GTAAAGTCTTTCATAGTAGCTTACTTGCCATCCCTCTACCTCTT
	AG
glg2ADHt-F	GTAAGGTTTGTACGGAGCTGCGAATTTCTTATGATTTATG
glg2FBAt-R	AAGAGGTAGAGGGATGGCAAGTAAGCTACTATGAAAGACTTT
	AC
ISA1-F	GTCATTCGCTTAAACACTATATCAATAATGGCAAAGGACAGAA
	GAAGCAACG
ISA1-R	CTATATCAATTAATTTGAATTAACCTCAGGGGTCTTTAATTGGTG
isa1CCW12-R	CGTTGCTTCTTCTGTCCCTTGCCATTATTGATATAGTGTTTAAGC
	GAATGAC
isa1FBA1t-F	CACCAATTAAGACCCCTGAGTTAATTCAAATTAATTGATATA
	G
ISA2-F	CGAATAAACACACATAAACAAACAAAATGGCAAGGCTTTTTTAC
	TGGTAGG
ISA2-R	CATTTACCCAATTGTAGATATGCCTAAGCGGTAGTATTGATG
	G
isa2ADHt-R	CCATCAATACTACCGCTTAGGCATATCTACAATTGGGTGAAAT
	G
isa2TDH3-F	CCTACCAGTAAAAAGCCTTGCCATTTTGTGTTTATGTGTGTT
	TATTCG
GPH1-gRNA1	CCGCAGTGAAAGATAAATGATCTCCACAATCAAAGGTCAGAA
	GTTTTAGAGCTAGAAATAGC
GPH1-gRNA2	CCGCAGTGAAAGATAAATGATCCTGTGTTGTATCCAAACGATG
	TTTTAGAGCTAGAAATAGC
GPH1-UP-R	GAGAAGGGTGCCAAAGAGATAAGTTGAAAGCTGCTTTACTG
GPH1-down-F	CAGTAAAGCAGCTTTCAACTTATCTCTTTGGCACCCCTTCTC

GPH1-UP-F	GATAAGGAAGGAACACCGAC
GPH1-down-R	AACGTCAGTACATCCTACCTG
GPH1-verify-R	CTCGCTCTTTTCAAACAATCC
GPH1-verify-F	CAGCACCTTCTATGTTTCAAGG
XI-1-PGP-F	CGGAATACCAAGCCCATTGTAAACCAATTTTATTTGAACTTGCC C
XI-1-PGP-R	GATTTGCCAATGCCAAGAAAAGTAAGCTACTATGAAAGACTTT AC
GAL1-g1	TGAAAGATAAATGATCAACCACTATAATTTAAGAGGTTTTAG AGCTAGAAATAGCAAG
GAL1-g2	TGAAAGATAAATGATCTTTTCTAAAGAACTTGCACGTTTTAG AGCTAGAAATAGCAAG
GAL1-LB-F	CGCTTAACTGCTCATTGCTATATTG
GAL1-LB-R	TTGTTCTGAACAAAGTAAAAAAGAAGTATACTATAGTTTTT TCTCCTTGACGTAA
GAL1-RB-F	GTATACTTCTTTTTTTTACTTTGTT
GAL1-RB-R	TTGTCTACTAAAATCTGAATTGTCC
GAL1-VF	AAAACCTTCTCTTTGGAACCTTCAG
GAL1-VR	GAATTTTTCTGTCATTTCTTTTCCT
II-5-gRNA-f1	AAACGAGTAAGCTCGTCAACTTTGAAACAAAAGAAGGGTTTTA GAGCTAGAAATAGCAAG
II-5-gRNA-f2	tgaagacgcatgAAAGTTCTGATGAGTCCGTGAGGACGAAACGAGT AAGCTCGTC
Gsy-sgRNA-f2	AAACGAGTAAGCTCGTCTCTTACTGACTGGCGTCGTAGTTTTAG AGCTAGAAATAGCAAG
Gsy-sgRNA-f1	tgaagacgcatgAAGAGACTGATGAGTCCGTGAGGACGAAACGAGT AAGCTCGTCTCTC
Glg-sgRNA-f2	AAACGAGTAAGCTCGTCTGCCTGAAAACATTAGCGCTGTTTTA GAGCTAGAAATAGCAAG
Glg-sgRNA-f1	tgaagacgcatgCAGGCACTGATGAGTCCGTGAGGACGAAACGAGT AAGCTCGTCTGCC
GSY-V-F	ATCAGAATGCTGGTGAGGACAG
GSY-UP-F	GCAACGAACTTGACGAATTTG
GSY-DOWN-F	CTCGTCTATCAAGGATAAAAATAATCATATCTAAAGCTGAATTTT GTAGTATCCATATTC
GSY-UP-R	GAATATGGATACTACAAAATTCAGCTTTAGATATGATTATTTTA TCCTTGATAGACGAG
GSY-DOWN-R	GGCATCTCTCTTTCTCCACTG
GSY-V-R	CATTGGGTTTTGTTGTGTTAGGAG
A92-F	GCAGCACGATCATAGAATAGAAC
B1-R	AACAATCCAGACACCAGGTCATTGAACTAACGACAAGCGCAAT TGAGGAGAGTGTGGAG
B2-F	CTACATAATGTTACTCCACACTCTCCTCAATTGCGCTTGTCTGTT

AGTTCAATGACCTG
 T51-R ATTCTCCTTCTTTACCAATTAAGACCCCTGAGTACGTAGTTT
 CGCTTAGTTTAAG
 T52-F ATTAGTTTAGTCTTAACTAAGCGAACTACGTACTCAGGGGT
 CTTTAATTGGTGAAAG
 T52-R GACAACAACAAGTCTAACTTCTTCGTCGACTTCATGGCAAAGG
 ACAGAAGAAGCAACG
 T53-F TTCAGCTTCGTTGCTTCTTCTGTCCTTTGCCATGAAGTCGACGA
 AGAAGTTAGACTTG
 T38-R CACAGACATCCGACATCTAGTAACACAGGTGACCTAAGTACGG
 GCCCTAGAAAATTCAC
 T39-F TTCCGACAGTGGTGAATTTTCTAGGGCCCGTACTTAGGTCACCT
 GTGTTACTAGATGTC
 T54-R AGTGATCGTTCTTCTCTAACTAACCATTTTATCAATTGGTTGG
 AGTTGAATTATTCCG
 T55-F TTCTTCCGGAATAATTCAACTCCAACCAATTGATAAAAATGGTTA
 GTTTAGAGAAGAACG
 T55-R AGGGCTCCTAACTAAAAGTAAAGACTTCCCCTTTATCGCTCC
 TGTTTATGCCCTAAC
 T56-F GCTACGGAAGTTAGGGCATAAACAGGAGCGATAAACGGGAAG
 TCTTTACAGTTTTAG
 T41-R GGCTTCACTCGTGGATCTATAATTGAACATGCCAAATTGTAATC
 ATCAGTGATTATGTC
 T42-F CTTGACATAATCACTGATGATTACAATTTGGCATGTTCAATTAT
 AGATCCACGAGTG
 T57-R TGTCCCATAGGCCTGTTACCCGAAGATGTTTAGATCGATTTGT
 ATGTGAAATAGCTG
 T58-F TTTTCGAATTTAGCTATTTACATACAAATCGATCTAAACATC
 TTCGGGTAACAGG
 T58-R TCTCACTACATACATTTTAGTTATTCGCCAACATGGCTTCTCTG
 AGGAAGGACTCTCG
 T59-F AAGAACGAGAGTCCTTCCTCAGAGAAGCCATGTTGGCGAATAA
 CTAATAATGTATGTAG
 T44-R attggacgaggaccaagACATTTCTACAAAAATAACTGTCGCCTCTT
 TTATCTGC
 T45-F CAGTGCGGCAGATAAAAAGAGGCGACAGTTATTTTTGTAGAAA
 TGTcttggtgcctc
 T60-R TTCTTCTTCTGAGTTCTTTTCTGAGCACTTCCATAGTTGTTCAAT
 TGATTGAAATAGGG
 T61-F ttATTTGTCCCTATTTCAATCAATTGAACAACATGGAAGTGCTC
 AGAAAAGAACTCAG
 T61-R AAACTCATTACATAAGACGTATACAAACTATTCGGCTTACTTG
 CGTGCAGAGTGATAG
 T62-F CTTGAGCTCTACTCTGCACGCAAGTAAGCCGAATAGTTTGT

	ATACGTCTTATG
T62-R	CCAGCTAAATACGAAAATGATGCTATCTTATACCTCTTATCTGC CAGAGTCGCTAGATC
T63-F	TCCAGATCTAGCGACTCTGGCAGATAAGAGGTATAAGATAGCA TCATTTTCGTATTTAG
A93-R	TGTGAACGTTATTCCTGTTATTGC
GLG-V-F	CAATAATAACCTTGATTTCGGAAAC
GLG-V-R	GGACTGAGAAACCTTGACATACA
T86-R	cctgattggacgaggacaccaagACATTTCTACAAAAGCATGGACCTGCT CTTATCTC
T87-F	TTTTTGTAGAAATGTcttgggtgcc
T87-R	ATAGTTGTTCAATTGATTGAAATAGGG
T88-F	taatttATTGTCCCTATTTCAATCAATTGAACAACCTATATGGCTAC CGCTAACGGTGC
T17-R	AACATTAGTTTAGTCTTAAACTAAGCGAAACTACGTACTTAAG CAATTTCAACGGCTTC
T89-F	CAATCCAGACACCAGGTCATTGAACTAACGACAAGGAAGTAGT CATCTAATTTAATCAT

Primers	Primers for IPA (Sequences 5'-3')
X-3 up fw	CGAGATCTTTGTGTTTCGGTTACC
X-3 down rv	GAGGTGGTTATTGATCACCGGA
TDH2t-F	ATTTAACTCCTTAAGTTACTTTAATG
TDH3-R	TTTGTTTGTTTATGTGTGTTTATTCG
X3-TDH3-F	GCGAGAGCCGACATACGAGACTCGAGTTTATCATTATCAATAC TGC
X3-TDHt-R	GCCTTAGAAACGCGGACACAGCGAAAAGCCAATTAGTGTG
YIAACS-F	CGAATAAACACACATAAACAAACAAAATGTTTAGTGTTAGAAA ACATATTAC
YIAACS-R	CATTAAAGTAACTTAAGGAGTTAAATTTACAAAACATCAATAG TTTTTAAG
RnAACS-F	CGAATAAACACACATAAACAAACAAAATGTCTAAATTAGCTAG ATTAGAAAAG
RnAACS-R	CTAAATCATTAAAGTAACTTAAGGAGTTAAATTTAAAAATCTT GTAATTCAGG
X3-PGK-F	GCGAGAGCCGACATACGAGACTAACGAACGCAGAATTTTCGA G
PGKt-R	ATTGAATTGAATTGAAATCGATAG
CbADH-F	TTGATCTATCGATTTC AATTCAATTTACAAAATAACAAC TGCTTTTATC
CbADH-R	CATAGCAATCTAATCTAAGTTTTAATTACAAAATGAAAGGTTTT GCTATGTTG
TEF1-F	TTTGTAATTTAAACTTAGATTAGA
tdh3TEF-R	TGAAATGGCAGTATTGATAATGATAAACTCGAATAGCTTCAA ATGTTTCTACTCC

TDH3-F	TCGAGTTTATCATTATCAATACTG
X-2 up fw	CGTCTATGAGGAGACTGTTAGTTG
adhX2-up-r	CATAAATCATAAGAAATTCGCGACCACTTCGAGAGCAAGTTG
x2ADH-F	CAACTTGCTCTCGAAGTGGTCGCGAATTTCTTATGATTTATG
acxbADH-R	CTGTTCAATTTGTTGAAGAATAAGCATATCTACAATTGGGTGA AATG
ACXB-R	AACTTAGTTTCGAATAAACACACATAAAACAAAACAAAATGAATG TTACAGTTGATCAATC
ACXB-F	CAAATCGCTCCCCATTTACCCAATTGTAGATATGCTTATTCTT CAACAAATTGAACAG
acxbTDH-F	GTAGATTGATCAACAGTAACATTCATTTTGTGTTTATGTGTG TTTATTCG
acxaCCW12-R	GACCTACTGGAACATTAACCATTATTGATATAGTGTTTAAGCG AATG
ACXA-F	CATTCGCTTAAACACTATATCAATAATGGTTAATGTTCCAGTAG GTC
ACXA-R	ACTATATCAATTAATTTGAATTAACCTTAACTTCTCTCAAATGA AATAATC
acxaFBA1t-f	GATTATTTCAATTTGAGAGAAGTTTAAGTTAATTCAAATTAATTG ATATAG
pyk1FBA1t-r	GGAAGGTTATACATGGGTACATAAATGCAGTAAGCTACTATGA AAGACTTTAC
fbapyk1t-f	GTAAAGTCTTTTCATAGTAGCTTACTGCATTTATGTACCCATGTA TAACC
acxcPYK1t-r	GTTCCAGAAAGAGCTGATGCTTAAAAAAGAATCATGATTGAA TGAAG
ACXC-F	CTTCATTCAATCATGATTCTTTTTTTAAGCATCAGCTCTTTCTGG AAC
ACXC-R	CAATCTAATCTAAGTTTTAATTACAAAATGGCTTATACTAGATC TAAAATTG
acxcTEF-F	CAATTTTAGATCTAGTATAAGCCATTTTGTAATTAAAACTTAGA TTAGATTG
x2TEF-R	GTGAGGCCGATTATGCAGGATAGCTTCAAATGTTTCTACTC
tefX2-down-f	GAGTAGAAACATTTTGAAGCTATCCTGCATAATCGGCCTCAC
X-2 down rv	CTCGCCAAGGCATTACCATC
XI-1 up F	ATTTGTGTGAAGGAATAGTGACG
XI-1 up R	CAATGGGCTTGGTATTCCG
x1-FBA1t-F	CGGAATACCAAGCCATTGAGTAAGCTACTATGAAAGACTTTA CAAAGAACTCGAAGAG
acmaFBA1t	TGCTGGTTATAAAGGTTTTGCATTATCTTAGTTAATTCAAATTA ATTGATATAGTTTTT
acmA-F	TAAGATAATGCAAACCTTTATAACCAGCA
acmA-R	TCGAATAAACACACATAAAACAAAACAAAATGATGTCTACTACTA CTTTGGATGCTGCTGT

TDH3-F	CATTTTGTGTTTATGTGTGTTTATTCTGA
ccwTDH3-R	AAGTTTCTTTTATTTTGCTTTGCCCTGGTTTCGAGTTTATCATTATCAATACTGCCATT
acmBCCW12-R	ACATCTAAAGAAGAAAAAGTAGAAGTCATTATTGATATAGTGT TTAAGCGAATGACAGA
CCW12-F	AACCAGGGCAAAGCAAATAAAAAGAACTT
acmB-F	ATGACTTCTACTTTTTCTTCTTTAGATGT
acmB-R	AAGGTAGACCAATGTAGCGCTCTTACTTTATTAAGATTTCAAA GCATCATAAAAAGCAG
DIT1t-F	TAAAGTAAGAGCGCTACATTGGTCTACCTT
x1DIT1t-R	GATTTGCCAATGCCAAGAAAGTTACTCCGCAACGCTTTTCTGA ACGCCCCGCTTCGCCT
XI-1 down F	TTTCTTGGCATTGGCAAATC
XI-1 down R	AAGAGCCGAGTCCCCATCAG
acmA-F	TTTATTTGTCCCTATTTCAATCAATTGAACAATATATGTCTAC TACTACTTTGGATGC
acmA-R	CTCATTACATAAGACGTATACAACTATTCGGCTAAGATAATG CAAAACCTTTATAACC
acmB-F	CTCACTACATACATTTTAGTTATTCGCCAACATGACTTCTACTT TTTCTTCTTTAGATG
acmB-R	AGTTTAGTCTTAACTAAGCGAACTACGTACTTAAGATTTCA AAGCATCATAAAAAGC
CbADH-F	TTCTTCCGGAATAATTCAACTCCAACCAATTGATAAAAATGAAA GGTTTTGCTATGTTGG
CbADH-R	AGGGCTCCTAACTAAAAGTAAAGACTTCCCGTTTACAAAAT AACAACTGCTTTTATC
ADH2t-f	GCCGAATAGTTTGTATACGTCTTATGT
ADH2t-R	TCTTATCTGCCAGAGTCGCTAGAT
DAS1t-F	ACGGGAAGTCTTTACAGTTTTAG
DAS1t-R	TTCTTCCAGATCTAGCGACTCTGGCAGATAAGACCAAATTGTA ATCATCAGTGATTATG
GAP-F	TTTTTGTAGAAATGTCTTGGTG
GAP-R	ATAGTTGTTCAATTGATTGAAATAG
PGIp-F	TAGGTCACCTGTGTTACTAGATG
PGIp-R	TTTATCAATTGGTTGGAGTTG
pgiXII4-up-R	ATCACAGACATCCGACATCTAGTAACACAGGTGACCTATCGTG TGCGCGACAACACAGT
pmp20II4-dwon-F	AACAATCCAGACACCAGGTCATTGAACTAACGACAAGGAATA GTAAGGACCGGGTTCAG
PMP20t-F	GTACGTAGTTTCGCTTAGTTTAAG
PMP20t-r	CTTGTCGTTAGTTCAATGACCTGGTGTC
TEF1-F	GATTGGACGAGGACACCAAGACATTTCTACAAAAATAACTGT CGCCTCTTTTATCTGC
TEF1p-r	GTTGGCGAATAACTAAAATGTATGT

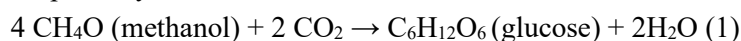
II4-up-f	TTGGGGTTGTTAGTACGAGAAGCT
II4-down-r	GCTTGTCGTTGCGTTTTAGATGTC
II4-verify-r	CAGATAAAGGAGGCTCTCCAT
II4-verify-F	CATGAGTCACGGCAGAGTT
aoxAcmA-F	ACTTGAGAAGATCAAAAAACAACATAATTATTCGAAAATGTCTA CTACTACTTTGGATGC
AOX1p-r	TTTCGAATAATTAGTTGTTTTTTGATCTTCTCAAGTTGTC
AOX1p-f	AGATCTAACATCCAAAGACGAAAGG
aoxDAS2p-f	TCATTCAACCTTTCGTCTTTGGATGTTAGATCTATTACTGTTTTG GGCAATCCTGTTG
DAS2p-r	TTTTGATGTTTGATAGTTTGATAAGAGTGAACCTTAG
dasAcmB-F	TTCACTCTTATCAAACATCAAACATCAAAAATGACTTCTACTT TTTCTTCTTTAGATG

Supplementary Note

Feasibility analysis

Take the conversion of methanol to glucose as an example, substrate costs and products prices as of 2019-2023:

Theoretically, combined methanol dehydrogenase dependent formaldehyde and CO₂ fixation pathway³:



Methanol cost is approximately \$0.35/Kg or 0.0112/mol (<https://www.procurementresource.com/resource-center/methanol-price-trends>). With the development of new energy technology in the future, methanol is expected to be produced at a lower cost and in a green /sustainable way⁴. The approximate price range for glucose is US\$ 1/Kg (0.18/mol) (<https://www.selinawamucii.com/insights/prices/united-kingdom/glucose/>). The cost of raw materials for fermentations can account for 70% of the total value of the product, particularly for bulk chemicals⁵. According to the price of methanol and glucose, the transformation has certain advantages and feasibility.

Other products:

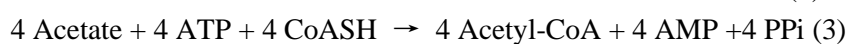
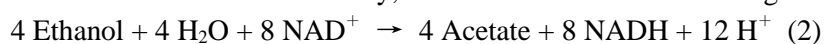
Glucosamine (\$11.53/Kg) is approximately 18.88-fold more expensive than glucose (www.chemanalyst.com/Pricing-data/glucosamine-1287).

Myo-inositol (\$10.00-\$15.00) is more expensive than glucose (www.alibaba.com/showroom/inositol-price.html).

Sucrose (\$2.80~\$3.80) is more expensive than glucose (www.alibaba.com/showroom/sucrose-price.html).

Maize starch (\$0.63) or potato starch (\$1.04) is also expensive than glucose (<https://www.selinawamucii.com/insights/prices/united-states-of-america/maize-starch/>; <https://www.selinawamucii.com/insights/prices/united-states-of-america/potato-starch/>).

Take the conversion of ethanol to glucose as an example for substrate costs and products prices as of 2019-2023. Theoretically, the conversion of ethanol into glucose as follows:



$4 \text{ Acetyl-CoA} + 4 \text{ H}_2\text{O} + 2 \text{ NAD}^+ \rightarrow 2 \text{ Succinate} + 4 \text{ CoASH} + 2\text{NADH} + 2 \text{ H}^+$
(Glyoxylate cycle) (4)

$2 \text{ Succinate} + 2 \text{ NAD}^+ + 2 \text{ FAD}^+ + 2 \text{ H}_2\text{O} \rightarrow 2 \text{ Oxaloacetate} + 2 \text{ NADH} + 2 \text{ FADH}_2 + 2 \text{ H}^+$ (TCA cycle) (5)

$2 \text{ Oxaloacetate} + 4 \text{ ATP} + 3 \text{ H}_2\text{O} + 2 \text{ NADH} + 2 \text{ H}^+ \rightarrow \text{G6P} + 4 \text{ ADP} + 2 \text{ CO}_2 + 2 \text{ NAD}^+ + 3 \text{ Pi}$ (Gluconeogenesis I) (6)

$\text{G6P} \rightarrow \text{G1P} + \text{H}_2\text{O} \rightarrow \text{Glu} + \text{Pi}/\text{G6P} + \text{H}_2\text{O} \rightarrow \text{Glu} + \text{Pi}$ (7)

Total:

$4 \text{ Ethanol} + 8 \text{ ATP} + 14 \text{ H}_2\text{O} + 2 \text{ FAD}^+ + 10 \text{ NAD}^+ \rightarrow \text{Glu} + 10 \text{ NADH} + 2 \text{ FADH}_2 + 2 \text{ CO}_2 + 4 \text{ AMP} + 4 \text{ PPI} + 4 \text{ ADP} + 4 \text{ Pi} + 14 \text{ H}^+$ (8)

1.25 was used as the P/O ratio for *S. cerevisiae* for the calculation^{6,7}.

In conclusion,

$4 \text{ Ethanol} + 14 \text{ H}_2\text{O} \rightarrow \text{Glu} + 2 \text{ CO}_2 + 14 \text{ H}^+ + 2 \text{ NADH} + 2 \text{ FADH}_2$ (9)

The theoretical yield of ethanol to glucose is 75%: the cell consumes 4 molecules of ethanol to form 1 glucose (see the calculation of theoretical yield). In 2023, the approximate price for ethanol is US\$ 1.75/Kg (0.081/mol) (<https://www.selinawamucii.com/insights/prices/united-states-of-america/ethyl-alcohol/>). The approximate price range for glucose is US\$ 1/Kg (0.18/mol) (<https://www.selinawamucii.com/insights/prices/united-kingdom/glucose/>). The cost of raw materials for fermentations can account for 70% of the total value of the product, particularly for bulk chemicals⁸. According to the price of ethanol and glucose, the catalytic process poses certain difficulties.

In conclusion, this technology can be applied to deep space exploration. In the future, we envision that this platform could be comparable to the photosynthesis in commercial sugar and its derivatives production and could mitigate CO₂-driven climate change.

Supplementary references

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