Global rewiring of cellular metabolism renders Saccharomyces cerevisiae Crabtree-negative

Dai *et al.*



Supplementary Figure 1. Serial dilution of the different strains were grown on synthetic medium agar plates with 20 g l⁻¹ glucose. sZJD-01: strain expressing PO gene from *S. pneumoniae* and PTA gene from *S. enterica*. sZJD-02: strain expressing PO gene from *L. plantarum* and PTA gene from *S. enterica*. sZJD-03: strain expressing PO gene from *A. viridans* and PTA gene from *S. enterica*. sZJD-04: control strain.



Supplementary Figure 2. Enzyme activity of pyruvate oxidase and phosphotransacetylase in strain sZJD-03 cell-free extract. The data represent the mean \pm s.d. of biological duplicates.



Supplementary Figure 3. Identification of promiscuous enzyme(s) for acetate accumulation. (a) Glycerol concentration in the medium of strain IMI076 harbouring the control plasmid (sZJD-08) and the POavPTAse containing plasmid (sZJD-09), respectively. To identify the promiscuous enzyme(s) in Pdc minus background yeast, Pdc⁻ *S. cerevisiae* IMI076 was used, because it could grow in excess glucose media without introducing exogenous acetyl-CoA producing pathway and be a comparable control strain. The data represent the mean ± s.d. of biological triplicates. Glycerol was produced by strain IMI076 containing the POavPTAse pathway in contrast to IMI076 with an empty plasmid. Glycerol 3-phosphate phosphatase (GPP) is responsible for catalyzing the conversion of glycerol 3phosphate to glycerol. The structure of acetate-phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate-phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate phosphate is similar to that of glycerol 3phosphate to glycerol. The structure of acetate concentration of Pdc⁻ strains with the POav/PTAse pathway carrying deleting in promiscuous phosphatase genes *GPP1* and *GPP2* in a synthetic medium containing 20 g l⁻¹ glucose. sZJD-03: control, sZJD-18: *GPP1* single deletion, sZJD-19: *GPP2* single deletion, sZJD-20: *GPP1* and *GPP2* double deletion.



Supplementary Figure 4. ALE experimental setup and determination of specific growth rate. (a)
Experimental procedure for ALE which generated three evolved populations (sZJD-24E1, sZJD-24E2 and sZJD-24E3). ×3 means 3 replicates and 3 clones were used for determine the specific growth rate.
(b) The specific growth rate of three evolved populations and starting strain sZJD-24.



Supplementary Figure 5. Transcriptional activation mode of TATA-containing, SAGA-dependent genes, which requires tail module, SAGA complex, activators. Blue line indicates DNA.



Supplementary Figure 6. Volcano plot of transcriptional changes of strains sZJD-28 (a), sZJD-27(b) and sZJD-26 (c) compared with strain sZJD-25. Green dots: padj< 0.01 & abs (log2 FC) > 1, red dots: padj< 0.01 & abs (log2 FC) \leq 1, yellow dots: padj \geq 0.01 & abs (log2 FC) > 1, black dots: padj \geq 0.01 & abs (log2 FC) \leq 1.



			Г	1	0.98711	2.9074e-10	9.7521e-12	0	ribosomal small subunit biogenesis
			Ъ	1	0.98453	4.5441e-13	0	0	rRNA processing
			ļL	1	0.9901	0.0014682	4.259e-06	8.0564e-12	ribosomal subunit export from nucleus
				1	0.99865	0.39555	0.0088989	3.347e-09	nuclear transport
			L	1	0.95852	0.092687	0.0063666	2.1456e-08	RNA modification
	Г	_	Г	0.99564	0.77242	0.00019272	1.832e-08	7.5519e-07	transmembrane transport
			ſL	1	0.75423	1.7605e-10	3.8941e-12	0	ribosomal large subunit biogenesis
			Г	1	0.55642	0.0014318	0.0026084	1.3552e-11	ribosome assembly
		Ц	l	0.99865	0.50669	5.3346e-06	4.4936e-09	1.4159e-07	amino acid transport
			Г	0.99494	0.043959	2.3846e-09	1.2419e-10	2.7427e-07	ion transport
		-	L	1	0.00093432		0	1.0625e-13	cellular amino acid metabolic process
			Г	0.3431	3.3507e-10	2.7667e-12	2.5848e-05	0.17956	cofactor metabolic process
			Ļ	0.47928	1.0522e-07	6.0576e-12	5.7734e-06	0.057029	nucleobase-containing small molecule metabolic process
			ΗL	0.52603	1.4357e-05	5.2077e-08	0.00019541	0.40761	monocarboxylic acid metabolic process
	L		L	0.79425	0.00019579	5.5387e-09	9.9222e-06	0.0032864	other
			_	0.068001	0.00035768	0.0007276	0.19796	0.97086	generation of precursor metabolites and energy
				0.004943	0.0017416	1.9342e-09	1.1178e-07	0.14463	carbohydrate transport
			1	6.1802e-05	0.17658	0.84686	0.99931	1	chromosome segregation
			ſ	7.6618e-06	0.090804	0.83584	0.99925	1	protein phosphorylation
			٦L	4.3381e-06	0.042108	0.73399	0.99916	1	organelle fission
			⊣└	9.1316e-05	0.1731	0.92684	0.99939	1	cellular response to DNA damage stimulus
			L	0.00043759	0.25592	0.81316	0.98798	0.99999	regulation of cell cycle
	-	_	Г	0.00022163	0.06499	0.16791	0.62187	0.99987	conjugation
			Ļ	0.0031321	4.1469e-05	0.011719	0.78347	0.99809	cell wall organization or biogenesis
				1.3241e-08	3.5449e-07	0.000163	0.54246	0.99999	response to oxidative stress
_				1.7364e-09	0.0001989	0.10985	0.97775	1	response to chemical
			٦	1.3703e-06	5.1942e-06	0.0036846	0.92258	0.99995	response to heat
	L		—	7.9341e-10	0.98015	1	1	1	biological_process
				Dist(dn)	Mix(dn)	Nondir	Mix(up)	Dist(up)	

Supplementary Figure 7. Reporter GO term analysis of the transcription profiles of strain sZJD-27. The color key shows the rank of GO terms and the significance (p-value) of the GO term is included in each cell of the heatmap. GO terms that have a consensus rank \leq 10 in any of the groups (distinct-directional down, mixed-directional down, non-directional change, mix-directional up and distinct-directional up) are shown in the heatmap.



Supplementary Figure 8. Reporter transcription factors (TFs) analysis of strain sZJD-27 compared with control strain. The color key shows the rank of TFs and the significance (p-value) of the TF is included in each cell of the heatmap. TFs that have a consensus rank ≤5 in any of the groups (distinct-directional down, mixed-directional down, non-directional change, mix-directional up and distinct-directional up) are shown in the heatmap.



Supplementary Figure 9. Reporter transcription factors (TFs) analysis of strain sZJD-28 compared with control strain. The color key shows the rank of TFs and the significance (p-value) of the TF is included in each cell of the heatmap. TFs that have a consensus rank \leq 5 in any of the groups (distinct-directional down, mixed-directional down, non-directional change, mix-directional up and distinct-directional up) are shown in the heatmap.



Supplementary Figure 10. Fold changes profiles of strain sZJD-28 compared with control strain sZJD-25.

Log2 Fold cl	hange	
-1.5 0	1.5	
sZJD-28 sZJD-27 sZJD-26	0	
	Gene NDE2 NDE1 YJL045W	NADH dehydrogenase (Complex I)
	EMI5 SDH4 SDH1 SDH2 YEL047C	Succinate dehydrogenase/ Fumarate reductase (Complex II)
	OSM1 YOR356W1	Electron transferringflavoprotein
	COR1 RIP1 QCR6 QCR9 QCR10 QCR8 QCR2 QCR7 CYT1 CBP4 FMP25 CBP3	Cytochrome c reductase/ Cytochrome bc1 complex (Complex III)
	CBP6 COX9 COX6 COX5B COX12 COX8 COX7 COX5A ATP1 ATP2	Cytochrome c oxidase (Complex IV)
	ATP1 ATP14 ATP20 ATP18 ATP4 ATP15 ATP5 INH1 STF1 STF2	ATP synthase (Complex V)
	AAC1 AAC3	ADP/ATP translocator

Supplementary Figure 11. Transcriptional levels of genes related to oxidative phosphorylation in sZJD-28, sZJD-27 and sZJD-26 compared with sZJD-25.

Supplementary Table 1. Strains used in this study

Strains	Genotype or characteristic	Source
CEN.PK YMZ-E1	MATa ura3-52 his3-Δ1 pdc1Δ pdc5Δ pdc6Δ	1
IMI076	MATa pdc1Δ(-6,-2)::loxP pdc5Δ(-6,-2)::loxP	2
	pdc6Δ(-6,-2)::loxP ura3-52 MTH1-ΔT	
CEN.PK 113-11C	MATa MAL2-8c SUC2 his3∆1 ura3-52	Kötter, University of
		Frankfurt, Germany
POspPTAse	CEN.PK YMZ-E1 with pZJD-01	This study
sZJD-01		
POIpPTAse	CEN.PK YMZ-E1 with pZJD-02	This study
sZJD-02		
POavPTAse	CEN.PK YMZ-E1 with pZJD-03	This study
sZJD-03		
Control	CEN.PK YMZ-E1 with pSP-GM1	This study
sZJD-04		
sZJD-08	IMI076 with pSP-GM1	This study
sZJD-09	IMI076 with pZJD-03	This study
sZJD-11	CEN.PK YMZ-E1(acs2Δ::TEF1p-POav-ADH1t	This study
	acs1∆::TEF1p-PTAse-CYC1t)	
sZJD-12	sZJD-11(pIST05)	This study
sZJD-13	sZJD-11(pYC1)	This study
sZJD-14	CEN.PK 113-11C(pIST05)	This study
HPY01	CEN.PK 113-11C(pYC1)	3
sZJD-15	CEN.PK YMZ-E1(<i>gpp1Δ</i>)	This study
sZJD-16	CEN.PK YMZ-E1(<i>gpp2Δ</i>)	This study
sZJD-17	CEN.PK YMZ-E1(<i>gpp1Δ gpp2Δ</i>)	This study
sZJD-18	sZJD-15(pZJD-03)	This study
sZJD-19	sZJD-16(pZJD-03)	This study
sZJD-20	sZJD-17(pZJD-03)	This study
sZJD-21	sZJD-11(<i>gpp1∆</i>)	This study
sZJD-22	sZJD-11(<i>gpp2∆</i>)	This study
sZJD-23	sZJD-11(gpp1∆ gpp2∆)	This study
sZJD-24	sZJD-23(<i>XI-5::URA3 X-2::HIS3</i>)	This study
sZJD-24E1	Evolved sZJD-24	This study
sZJD-24E2	Evolved sZJD-24	This study
sZJD-24E3	Evolved sZJD-24	This study
sZJD-24A1	Clone from evolved sZJD-24E1	This study
sZJD-24A2	Clone from evolved sZJD-24E1	This study
sZJD-24A3	Clone from evolved sZJD-24E1	This study
sZJD-24B1	Clone from evolved sZJD-24E2	This study
sZJD-24B2	Clone from evolved sZJD-24E2	This study
sZJD-24B3	Clone from evolved sZJD-24E2	This study
sZJD-24C1	Clone from evolved sZJD-24E3	This study
sZJD-24C2	Clone from evolved sZJD-24E3	This study
sZJD-24C3	Clone from evolved sZJD-24E3	This study
sZJD-25	sZJD-23(X-2::KanMX-TEF1p-Cas9-CYC1t)	This study
sZJD-26	sZJD-25(<i>GPD1</i> ^{W71*})	This study
sZJD-27	sZJD-25(<i>MED2</i> ^{*432Y})	This study
sZJD-28	sZJD-25(<i>MED1</i> ^{*432Y} GPD1 ^{W71*})	This study

Species	Yield: EtOH/Glu (g g⁻¹)	Growth rate (h^{-1})
S. cerevisiae*	0.00	0.109
Kluyveromyces nonfermentans **	0.00	0.101
Eremothecium sinecaudum A2**	0.00	0.117
E. sinecaudum A1**	0.00	0.122
K. Lactis A2**	0.00	0.255
K. Marxianus B**	0.00	0.269
K. Marxianus A1**	0.00	0.314
K. Wickerhamii**	0.00	0.321
Debaromyces vanrijiae A1**	0.00	0.347
K. aestuarii**	0.00	0.429
S. cerevisiae A2**	0.38	0.289

Supplementary Table 2. Growth rate and ethanol yield on glucose of Crabtree negative and positive

yeast

 $\ensuremath{^*}$ Data from this study, $\ensuremath{^*}\xspace$ Data from previous work $\ensuremath{^4}\xspace$.

Supplementary Table 3. Mutations in evolved S. cerevisiae strains isolated from three populations

Strain	Mutations						
sZJD-24A1	MED2	HXK2	EBP2	BEM3	ALG14	FAB1	MGA1
	432Y	Q195	T415S	N673H	R20S	A620S	M274R
	TA <mark>G</mark> -TA <mark>T</mark>	CAA-TAA	A <mark>C</mark> C-A <mark>G</mark> C	AAT-CAT	AG <mark>G</mark> -AG <mark>T</mark>	GCT-TCT	ATG-A <mark>G</mark> G
sZJD-24A2	MED2	HXK2	MCH2	NUD1	DIT2	GTO1	
	432Y	Q195	A63T	G195G	V24F	F280Y	
	TA <mark>G</mark> -TA <mark>T</mark>	CAA-TAA	GCT-ACT	GG <mark>C</mark> -GGT	GTC-TTC	TTT-TAT	
sZJD-24B1	MED3	SIW14	MHO1	TPS2			
	L156*	W234*	T160K	∆3bp			
	TTG-T <mark>A</mark> G	TG <mark>G</mark> -TG <mark>A</mark>	A <mark>C</mark> G-A <mark>A</mark> G	2344-2346			
sZJD-24B2	MED3	SIW14	MHO1	AGP2			
	L156*	W234*	T160K	C204Y			
	TTG-T <mark>A</mark> G	TG <mark>G</mark> -TG <mark>A</mark>	A <mark>C</mark> G-A <mark>A</mark> G	TGT-TAT			
sZJD-24C1	MED2	GPD1	DUN1				
	432Y	W71	T57A				
	TA <mark>G</mark> -TA <mark>T</mark>	T <mark>G</mark> G-T <mark>A</mark> G	ACA-GCA				
sZJD-24C2	MED2	GPD1					
	432Y	W71					
	TA <mark>G</mark> -TA <mark>T</mark>	T <mark>G</mark> G-T <mark>A</mark> G					
sZJD-24C3	MED2	GPD1					
	432Y	W71					
	TA <mark>G</mark> -TA <mark>T</mark>	T <mark>G</mark> G-T <mark>A</mark> G					

Supplementary Table 4. Plasmids used in this study

Plasmids	Genotype or characteristic	Source
pSP-GM1	2 μm, AmpR, URA3, TEF1p,ADH1t, PGK1p, CYC1t	5
pSP-GM2	2 μm, AmpR, URA3, TEF1p, CYC1t, PGK1p, ADH1t	5
pZJD-01	pSP-GM1-(TEF1p-POsp-ADH1t, PGK1p-PTAse-CYC1t)	This study
pZJD-02	pSP-GM1-(TEF1p-POlp-ADH1t, PGK1p-PTAse-CYC1t)	This study
pZJD-03	pSP-GM1-(TEF1p-POav-ADH1t, PGK1p-PTAse-CYC1t)	This study
pIST05	pSP-GM1-(TEF1p-FarnSYNcj-ADH1t, PGK1p-tHMG1-CYC1t)	6
pYC1	pSP-GM2-(TEF1p-MCRca-CYC1t)	3
pUG6	loxP-pAgTEF1-kanMX-tAgTEF1-loxP, AmpR, ori	7
pCfB2312	CEN/ARS, KanMX, AmpR, TEF1p, Cas9, CYC1t	8

Supplementary References

- 1 Zhang, Y. *et al.* Adaptive mutations in sugar metabolism restore growth on glucose in a pyruvate decarboxylase negative yeast strain. *Microb. Cell Fact.* **14**, 116, doi:10.1186/s12934-015-0305-6 (2015).
- 2 Oud, B. *et al.* An internal deletion in *MTH1* enables growth on glucose of pyruvatedecarboxylase negative, non-fermentative *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **11**, 131, doi:10.1186/1475-2859-11-131 (2012).
- Chen, Y., Bao, J. C., Kim, I. K., Siewers, V. & Nielsen, J. Coupled incremental precursor and cofactor supply improves 3-hydroxypropionic acid production in *Saccharomyces cerevisiae*. *Metab. Eng.* 22, 104-109, doi:10.1016/j.ymben.2014.01.005 (2014).
- Hagman, A., Sall, T., Compagno, C. & Piskur, J. Yeast "Make-Accumulate-Consume" Life
 Strategy Evolved as a Multi-Step Process That Predates the Whole Genome Duplication. *Plos One* 8, e68734, doi:ARTN e68734 10.1371/journal.pone.0068734 (2013).
- 5 Chen, Y., Partow, S., Scalcinati, G., Siewers, V. & Nielsen, J. Enhancing the copy number of episomal plasmids in *Saccharomyces cerevisiae* for improved protein production. *FEMS Yeast Res.* **12**, 598-607, doi:10.1111/j.1567-1364.2012.00809.x (2012).
- 6 Tippmann, S., Scalcinati, G., Siewers, V. & Nielsen, J. Production of farnesene and santalene by *Saccharomyces cerevisiae* using fed-batch cultivations with RQ-controlled feed. *Biotechnol. Bioeng.* **113**, 72-81, doi:10.1002/bit.25683 (2016).
- 7 Guldener, U., Heck, S., Fiedler, T., Beinhauer, J. & Hegemann, J. H. A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res.* **24**, 2519-2524, doi:DOI 10.1093/nar/24.13.2519 (1996).
- 8 Stovicek, V., Borodina, I. & Forster, J. CRISPR–Cas system enables fast and simple genome editing of industrial *Saccharomyces cerevisiae* strains. *Metab. Eng. Commun.* **2**, 13-22 (2015).