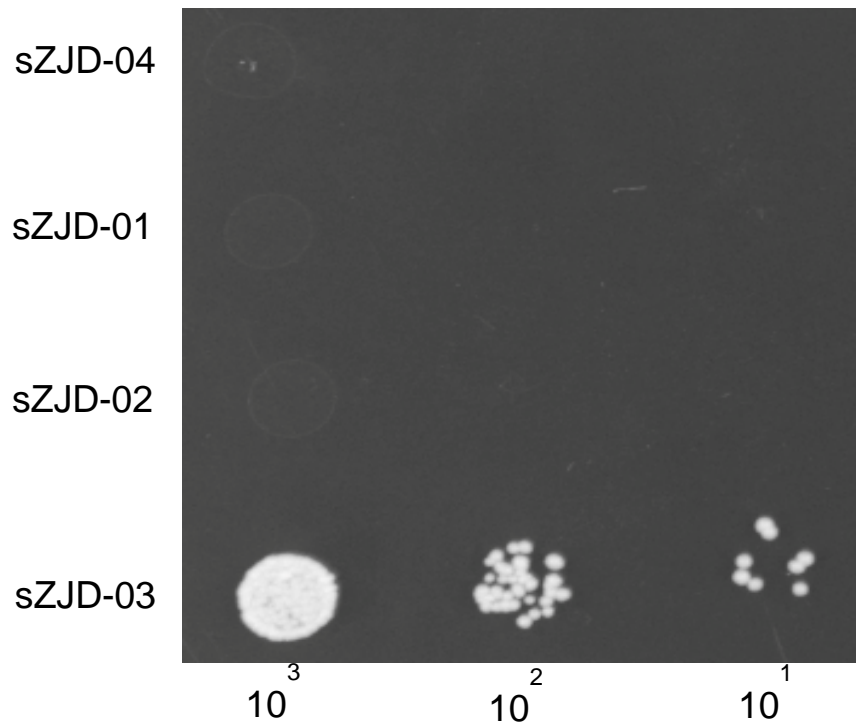
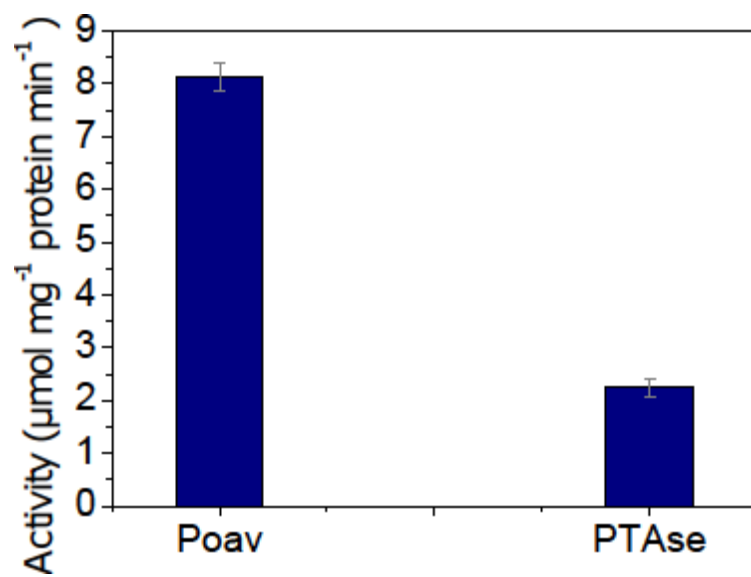


Global rewiring of cellular metabolism renders *Saccharomyces cerevisiae*  
Crabtree-negative

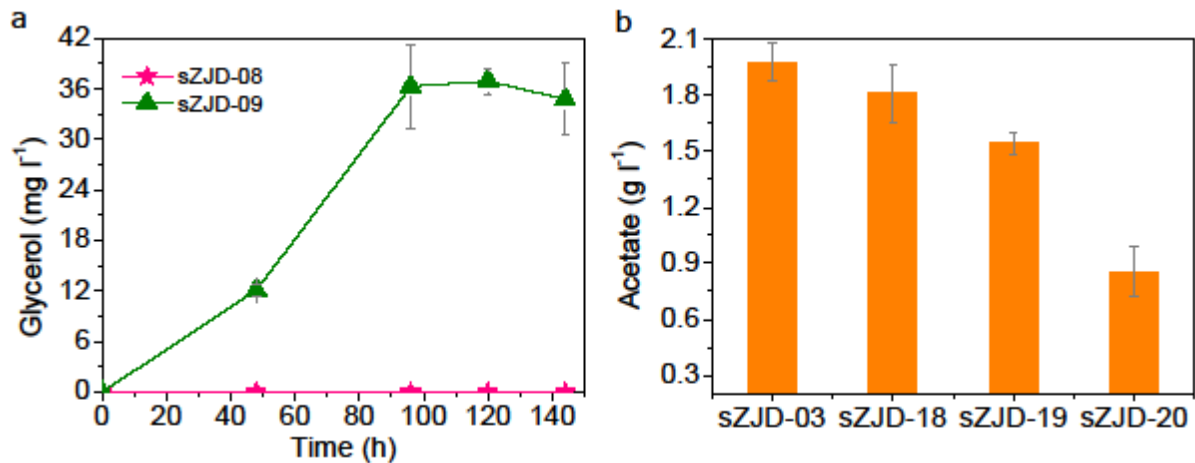
Dai *et al.*



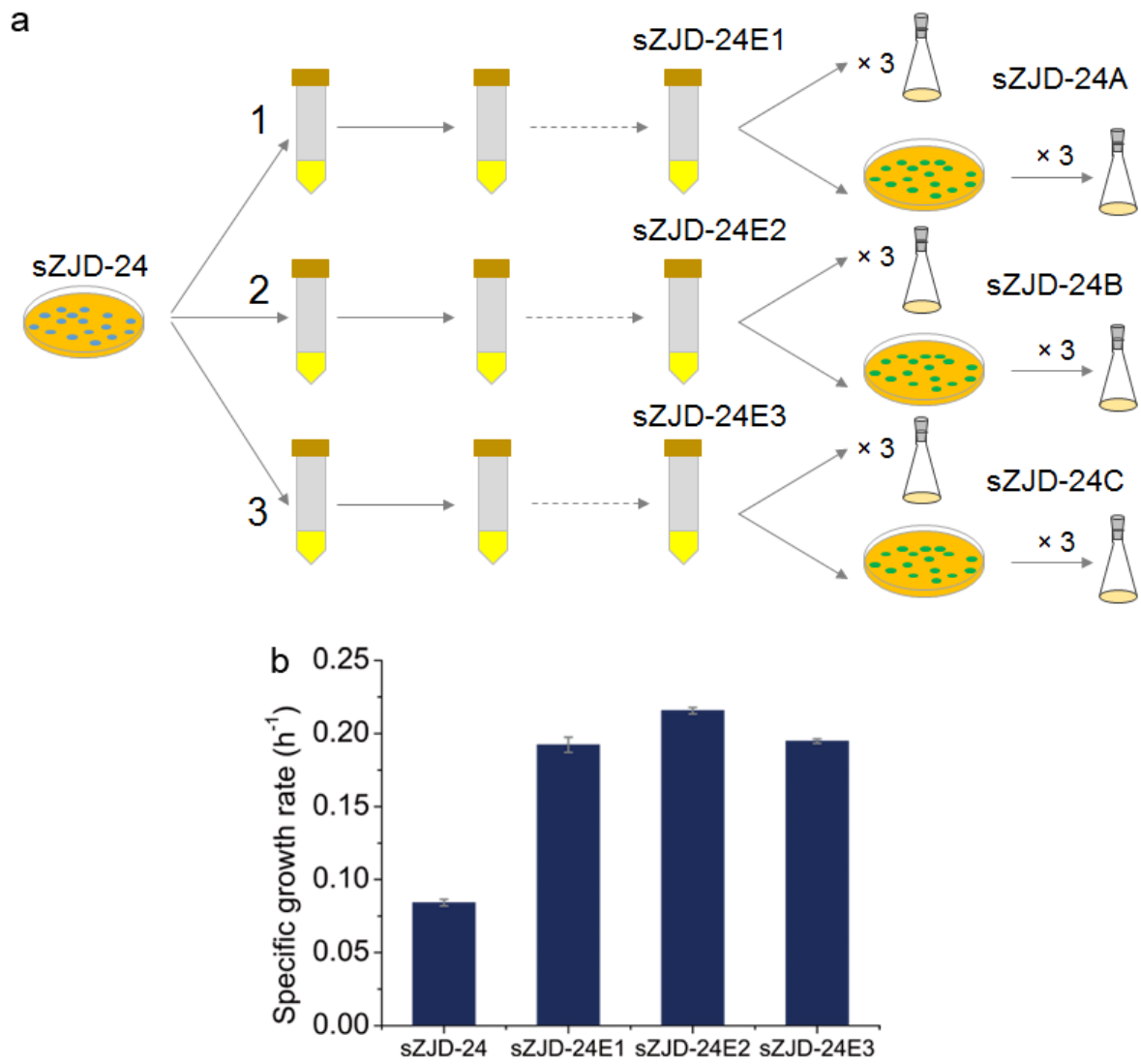
**Supplementary Figure 1.** Serial dilution of the different strains were grown on synthetic medium agar plates with 20 g l<sup>-1</sup> glucose. sZJD-01: strain expressing PO gene from *S. pneumoniae* and PTA gene from *S. enterica*. sZJD-02: strain expressing PO gene from *L. plantarum* and PTA gene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



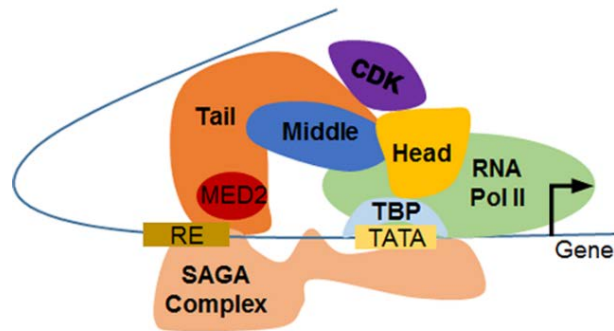
**Supplementary Figure 2.** Enzyme activity of pyruvate oxidase and phosphotransacetylase in strain sZJD-03 cell-free extract. The data represent the mean  $\pm$  s.d. of biological duplicates.



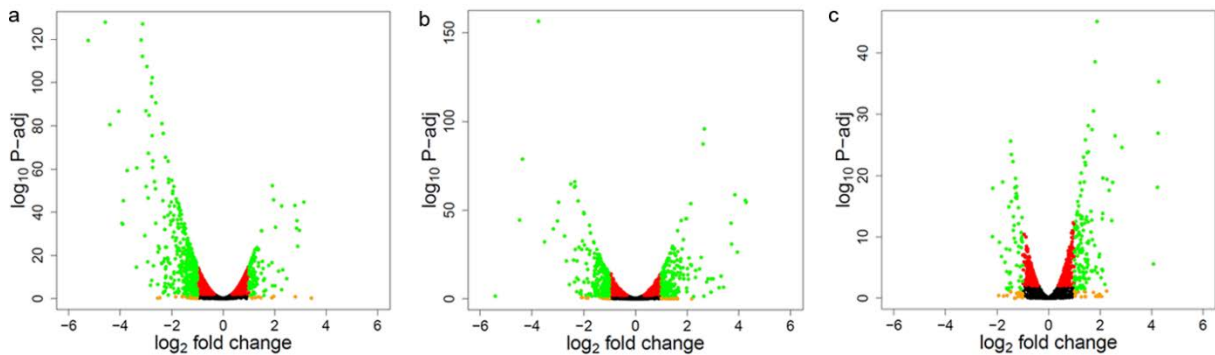
**Supplementary Figure 3.** Identification of promiscuous enzyme(s) for acetate accumulation. **(a)** Glycerol concentration in the medium of strain IMI076 harbouring the control plasmid (sZJD-08) and the POavPTase containing plasmid (sZJD-09), respectively. To identify the promiscuous enzyme(s) in Pdc<sup>-</sup> *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  $\pm$  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 3-phosphate to glycerol. The structure of acetate-phosphate is similar to that of glycerol 3-phosphate, and we therefore suspected that GPP might be promiscuous involved in converting acetyl-phosphate to acetate. **(b)** Extracellular acetate concentration of Pdc<sup>-</sup> strains with the POav/PTase pathway carrying deleting in promiscuous phosphatase genes *GPP1* and *GPP2* in a synthetic medium containing 20 g l<sup>-1</sup> glucose. sZJD-03: control, sZJD-18: *GPP1* single deletion, sZJD-19: *GPP2* single deletion, sZJD-20: *GPP1* and *GPP2* double deletion.



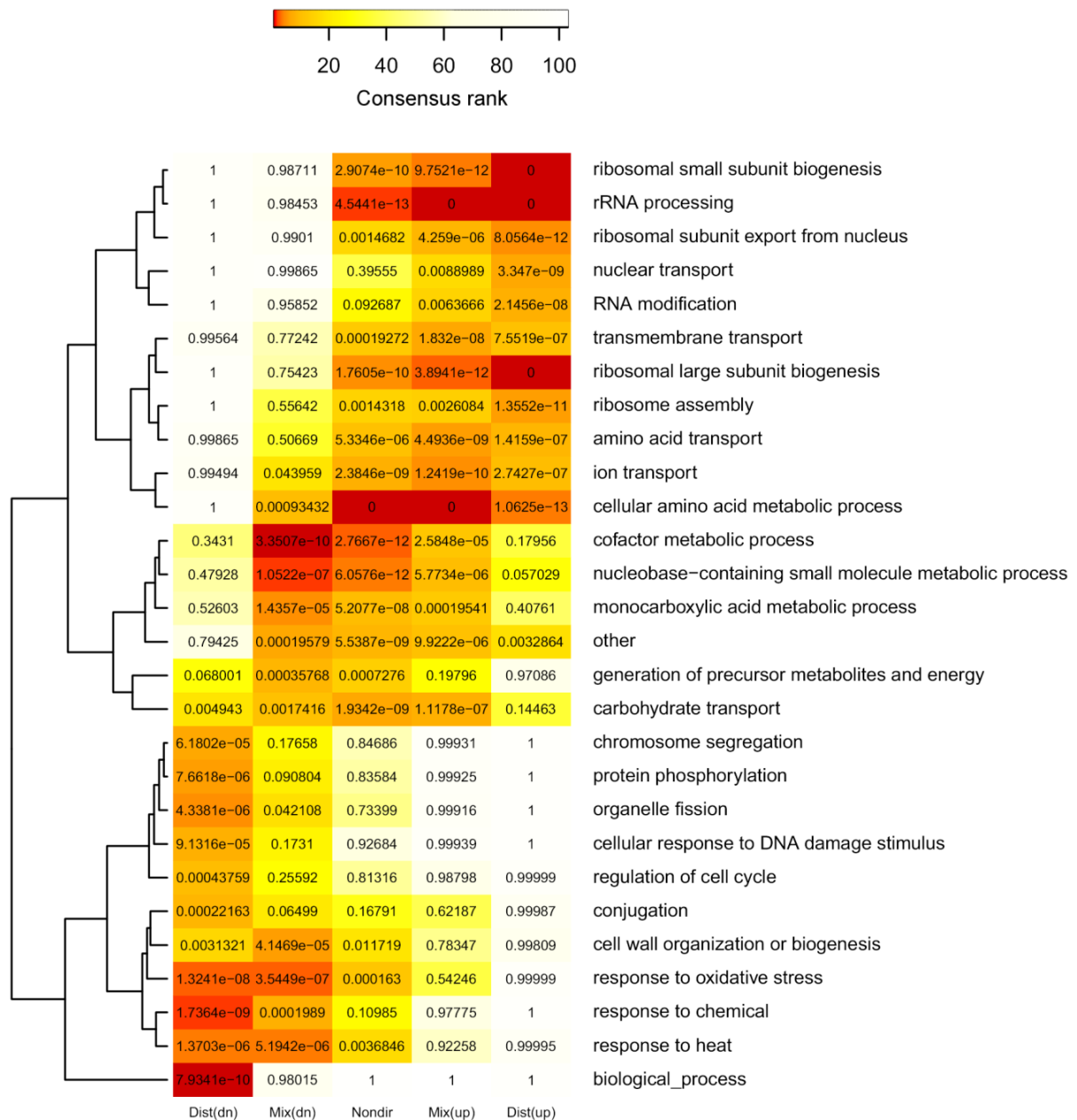
**Supplementary Figure 4.** ALE experimental setup and determination of specific growth rate. **(a)** Experimental procedure for ALE which generated three evolved populations (sZJD-24E1, sZJD-24E2 and sZJD-24E3).  $\times 3$  means 3 replicates and 3 clones were used for determine the specific growth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



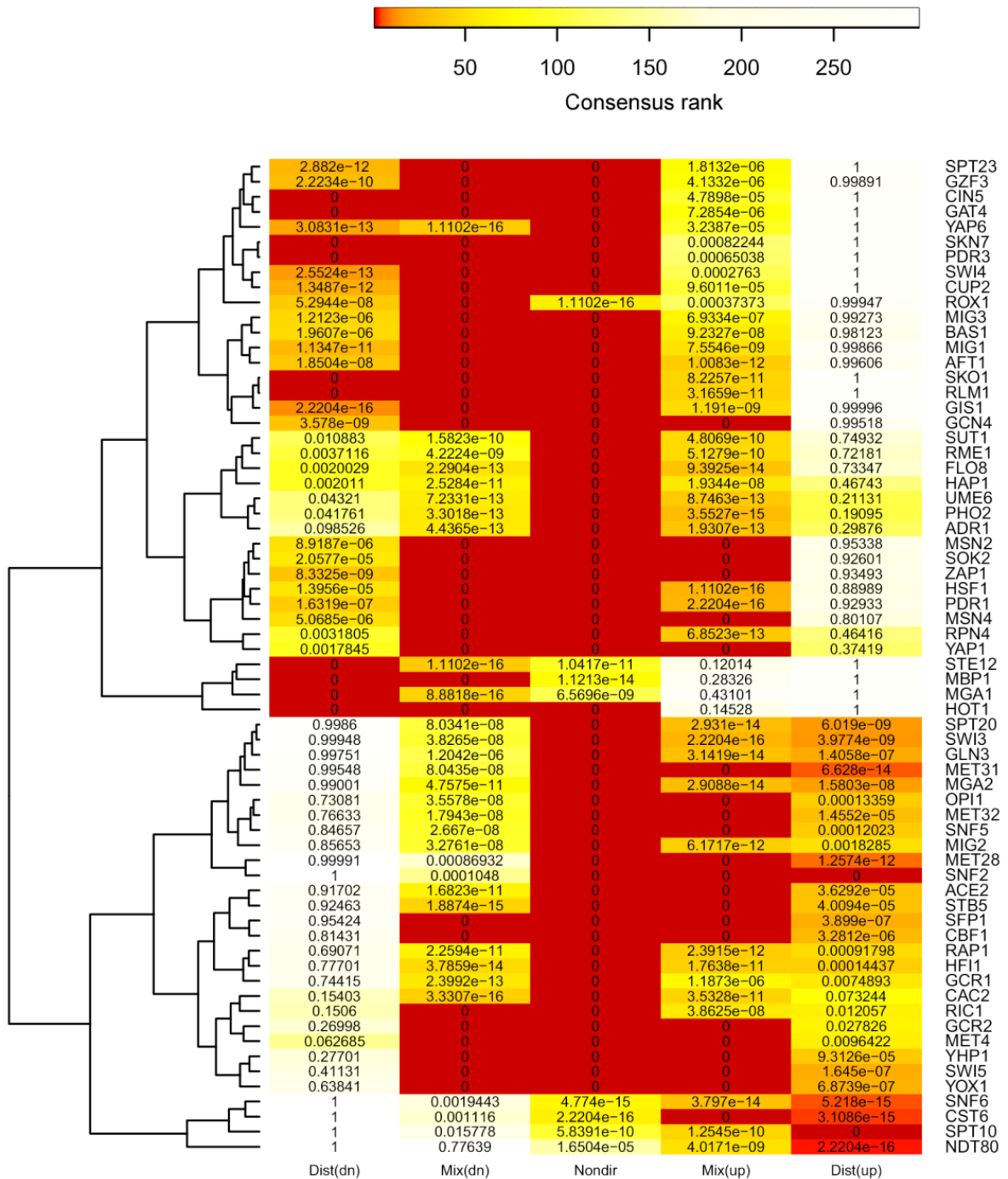
**Supplementary Figure 5.** Transcriptional activation mode of TATA-containing, SAGA-dependent genes, which requires tail module, SAGA complex, activators. Blue line indicates DNA.



**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:  $\text{padj} < 0.01$  &  $\text{abs}(\log_2 \text{FC}) > 1$ , red dots:  $\text{padj} < 0.01$  &  $\text{abs}(\log_2 \text{FC}) \leq 1$ , yellow dots:  $\text{padj} \geq 0.01$  &  $\text{abs}(\log_2 \text{FC}) > 1$ , black dots:  $\text{padj} \geq 0.01$  &  $\text{abs}(\log_2 \text{FC}) \leq 1$ .

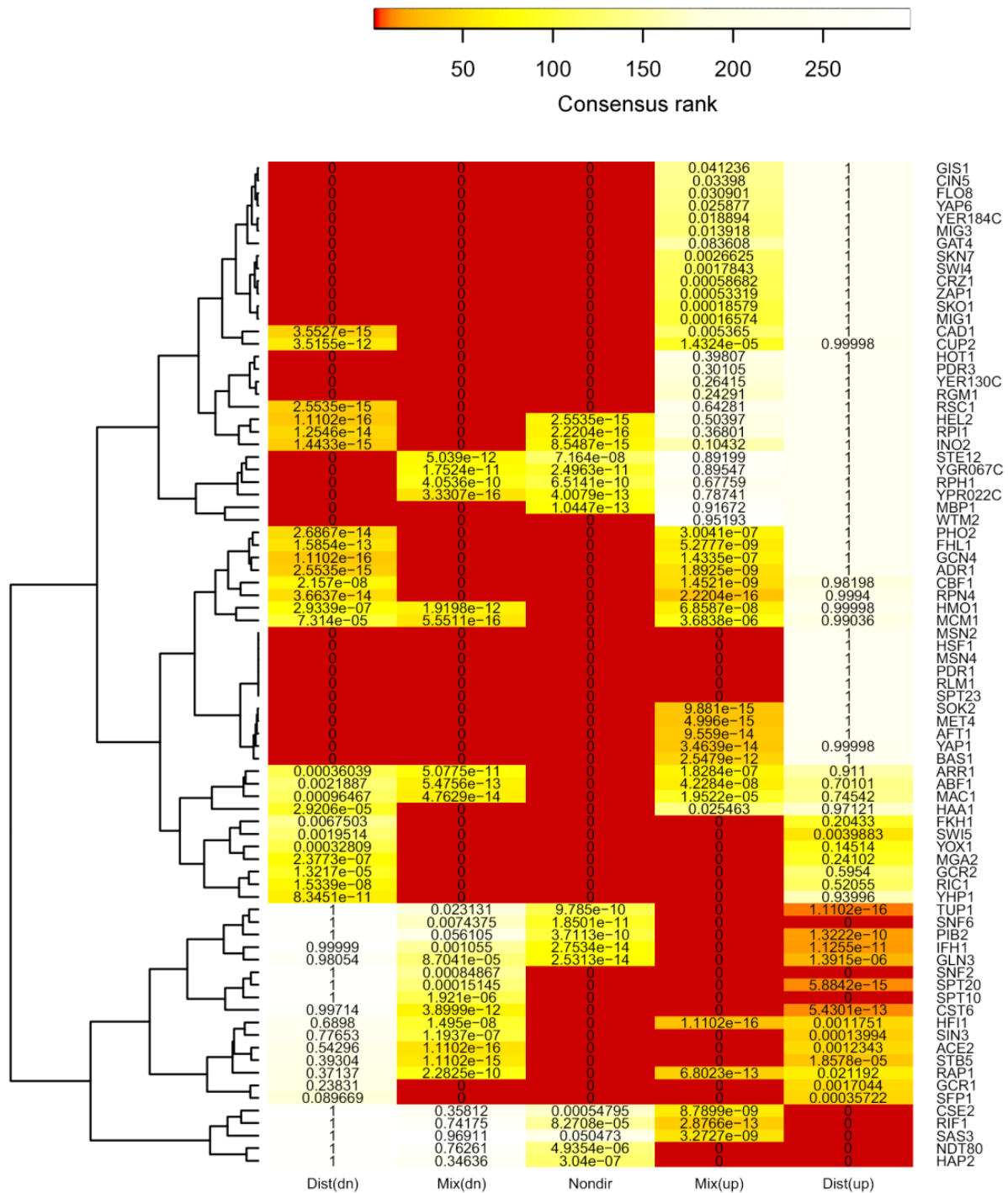


**Supplementary Figure 7.** Reporter GO term analysis of the transcription profiles of strain sZJD-27. The color key shows the rank of GO terms and the significance (p-value) of the GO term is included in each cell of the heatmap. GO terms that have a consensus rank  $\leq 10$  in any of the groups (distinct-directional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

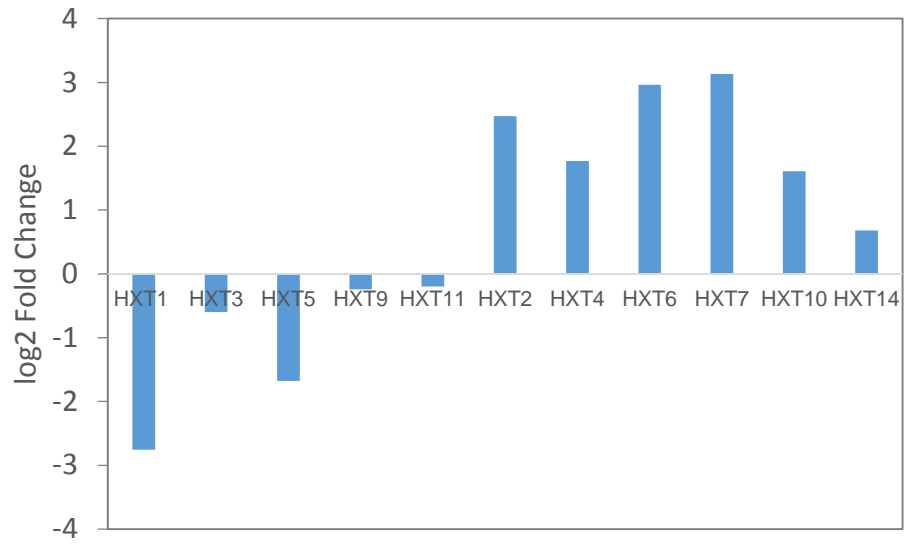


**Supplementary Figure 8.** Reporter transcription factors (TFs) analysis of strain sZJD-27 compared with control strain. The color key shows the rank of TFs and the significance (p-value) of the TF is included in each cell of the heatmap. TFs that have a consensus rank  $\leq 5$  in any of the groups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

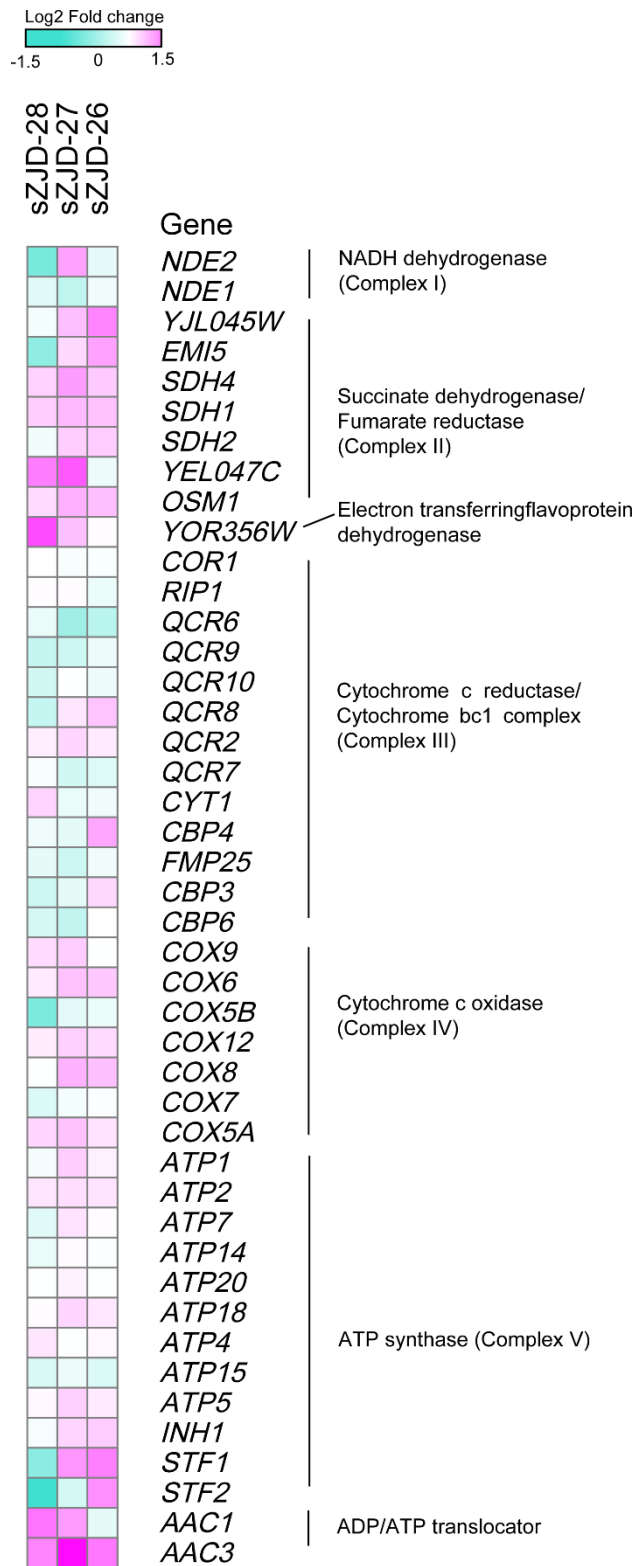




**Supplementary Figure 9.** Reporter transcription factors (TFs) analysis of strain sZJD-28 compared with control strain. The color key shows the rank of TFs and the significance (p-value) of the TF is included in each cell of the heatmap. TFs that have a consensus rank  $\leq 5$  in any of the groups (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.



**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 <i>ura3-52 his3-Δ1 pdc1Δ pdc5Δ pdc6Δ</i>	<sup>1</sup>
IMI076	MATa <i>pdc1Δ(-6,-2)::loxP pdc5Δ(-6,-2)::loxP pdc6Δ(-6,-2)::loxP ura3-52 MTH1-ΔT</i>	<sup>2</sup>
CEN.PK 113-11C	MATa <i>MAL2-8c SUC2 his3Δ1 ura3-52</i>	Kötter, University of Frankfurt, Germany
POspPTAse sZJD-01	CEN.PK YMZ-E1 with pZJD-01	This study
POlpPTAse sZJD-02	CEN.PK YMZ-E1 with pZJD-02	This study
POavPTAse sZJD-03	CEN.PK YMZ-E1 with pZJD-03	This study
Control sZJD-04	CEN.PK YMZ-E1 with pSP-GM1	This study
sZJD-08	IMI076 with pSP-GM1	This study
sZJD-09	IMI076 with pZJD-03	This study
sZJD-11	CEN.PK YMZ-E1( <i>acs2Δ::TEF1p-POav-ADH1t acs1Δ::TEF1p-PTAse-CYC1t</i> )	This study
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)	<sup>3</sup>
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>gpp1Δ</i> )	This study
sZJD-22	sZJD-11( <i>gpp2Δ</i> )	This study
sZJD-23	sZJD-11( <i>gpp1Δ gpp2Δ</i> )	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( <i>X-2::KanMX-TEF1p-Cas9-CYC1t</i> )	This study
sZJD-26	sZJD-25( <i>GPD1<sup>W71*</sup></i> )	This study
sZJD-27	sZJD-25( <i>MED2<sup>*432Y</sup></i> )	This study
sZJD-28	sZJD-25( <i>MED1<sup>*432Y</sup>GPD1<sup>W71*</sup></i> )	This study

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

Species	Yield: EtOH/Glu (g g <sup>-1</sup> )	Growth rate (h <sup>-1</sup> )
<i>S. cerevisiae</i> *	0.00	0.109
<i>Kluyveromyces nonfermentans</i> **	0.00	0.101
<i>Eremothecium sinicaudum</i> A2**	0.00	0.117
<i>E. sinicaudum</i> A1**	0.00	0.122
<i>K. Lactis</i> A2**	0.00	0.255
<i>K. Marxianus</i> B**	0.00	0.269
<i>K. Marxianus</i> A1**	0.00	0.314
<i>K. Wickerhamii</i> **	0.00	0.321
<i>Debaromyces vanriijiae</i> A1**	0.00	0.347
<i>K. aestuarii</i> **	0.00	0.429
<i>S. cerevisiae</i> A2**	0.38	0.289

yeast

\* Data from this study, \*\*Data from previous work<sup>4</sup>.**Supplementary Table 3.** Mutations in evolved *S. cerevisiae* strains isolated from three populations

Strain	Mutations						
sZJD-24A1	<i>MED2</i>	<i>HXK2</i>	<i>EBP2</i>	<i>BEM3</i>	<i>ALG14</i>	<i>FAB1</i>	<i>MGA1</i>
	*432Y	Q195*	T415S	N673H	R20S	A620S	M274R
	TAG-TAT	CAA-TAA	ACC-AGC	AAT-CAT	AGG-AGT	GCT-TCT	ATG-AGG
sZJD-24A2	<i>MED2</i>	<i>HXK2</i>	<i>MCH2</i>	<i>NUD1</i>	<i>DIT2</i>	<i>GTO1</i>	
	*432Y	Q195*	A63T	G195G	V24F	F280Y	
	TAG-TAT	CAA-TAA	GCT-ACT	GGC-GGT	GTC-TTC	TTT-TAT	
sZJD-24B1	<i>MED3</i>	<i>SIW14</i>	<i>MHO1</i>	<i>TPS2</i>			
	L156*	W234*	T160K	Δ3bp			
	TTG-TAG	TGG-TGA	ACG-AAG	2344-2346			
sZJD-24B2	<i>MED3</i>	<i>SIW14</i>	<i>MHO1</i>	<i>AGP2</i>			
	L156*	W234*	T160K	C204Y			
	TTG-TAG	TGG-TGA	ACG-AAG	TGT-TAT			
sZJD-24C1	<i>MED2</i>	<i>GPD1</i>	<i>DUN1</i>				
	*432Y	W71*	T57A				
	TAG-TAT	TGG-TAG	ACA-GCA				
sZJD-24C2	<i>MED2</i>	<i>GPD1</i>					
	*432Y	W71*					
	TAG-TAT	TGG-TAG					
sZJD-24C3	<i>MED2</i>	<i>GPD1</i>					
	*432Y	W71*					
	TAG-TAT	TGG-TAG					

**Supplementary Table 4.** Plasmids used in this study

Plasmids	Genotype or characteristic	Source
pSP-GM1	2 $\mu$ m, <i>AmpR</i> , <i>URA3</i> , TEF1p,ADH1t, PGK1p, CYC1t	5
pSP-GM2	2 $\mu$ m, <i>AmpR</i> , <i>URA3</i> , 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, <i>AmpR</i> , ori	7
pCfB2312	CEN/ARS, KanMX, <i>AmpR</i> , TEF1p, Cas9, CYC1t	8

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