nature COMMUNICATIONS

ARTICLE

Received 11 Jan 2016 | Accepted 21 Apr 2016 | Published 25 May 2016

DOI: 10.1038/ncomms11709 OPEN

Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories

Yongjin J. Zhou[1,2], Nicolaas A. Buijs[1,†], Zhiwei Zhu[1,2], Jiufu Qin[1], Verena Siewers[1,2] & Jens Nielsen[1,2,3,4]

Sustainable production of oleochemicals requires establishment of cell factory platform strains. The yeast *Saccharomyces cerevisiae* is an attractive cell factory as new strains can be rapidly implemented into existing infrastructures such as bioethanol production plants. Here we show high-level production of free fatty acids (FFAs) in a yeast cell factory, and the production of alkanes and fatty alcohols from its descendants. The engineered strain produces up to 10.4 g l^{-1} of FFAs, which is the highest reported titre to date. Furthermore, through screening of specific pathway enzymes, endogenous alcohol dehydrogenases and aldehyde reductases, we reconstruct efficient pathways for conversion of fatty acids to alkanes (0.8 mg l^{-1}) and fatty alcohols (1.5 g l^{-1}), to our knowledge the highest titres reported in *S. cerevisiae*. This should facilitate the construction of yeast cell factories for production of fatty acids derived products and even aldehyde-derived chemicals of high value.

[1] Department of Biology and Biological Engineering, Chalmers University of Technology, Kemivägen 10, Gothenburg SE-41296, Sweden. [2] Novo Nordisk Foundation Center for Biosustainability, Chalmers University of Technology, Gothenburg SE41296, Sweden. [3] Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm DK2970, Denmark. [4] Science for Life Laboratory, Royal Institute of Technology, Stockholm SE-17121, Sweden. † Present address: Evolva Biotech, Lersø Parkallé 40-42, Copenhagen DK-2100, Denmark. Correspondence and requests for materials should be addressed to J.N. (email: nielsenj@chalmers.se).

Sustainable and cost-effective routes for renewable production of chemicals and fuels are needed to support the growing population and economy with a reduced carbon footprint[1,2]. Oleochemicals are substitutes of petrochemicals and are usually derived from plant oils and animal fats, which have limited availability[3]. Microbial fatty acid biosynthesis has captured much attention as it offers a way for renewable oleochemicals production[4]. There have been several reports on engineering the bacterium *Escherichia coli* for the production of various oleochemicals[5–11], including alkanes that can be used directly as biofuels[6]. On the other hand, for industrial scale production the yeast *Saccharomyces cerevisiae* is more suitable due to its robustness and tolerance towards harsh fermentation conditions, as well as its widespread use for bioethanol production[12,13]. This will allow transforming existing bioethanol production plants for production of these chemicals. The productivity and yield of oleochemicals produced by the well characterized model yeast *S. cerevisiae* is still relatively low[14–16]. Moreover, most biosynthetic pathways are designed to utilize the tightly regulated lipid biosynthesis intermediate fatty acyl-CoA[14] or fatty acyl carrier protein (ACP)[17], which limits the metabolic flux. Free fatty acids (FFAs) on the contrary can be accumulated to much higher levels (>200-fold higher than fatty acyl-CoA)[18] and used for the biosynthesis of alkanes and fatty alcohols through formation of a fatty aldehyde intermediate[7]. We thus explored the establishment of FFA-derived pathways for the production of alkanes and fatty alcohols, two classes of valued oleochemicals (Fig. 1).

We first constructed a plasmid-free yeast strain by blocking fatty acid activation and degradation, introducing an optimized acetyl-CoA pathway, expressing a more efficient fatty acid synthase (FAS) and overexpressing the endogenous acetyl-CoA carboxylase. The engineered strain produced up to $10.4\,g\,l^{-1}$ of FFAs in fed-batch fermentation. We then constructed biosynthetic pathways for production of alkanes and fatty alcohols by screening endogenous alcohol dehydrogenases/aldehyde reductases (ADH/ALRs) and pathway balancing, which resulted the highest titres of alkanes ($0.8\,mg\,l^{-1}$) and fatty alcohols ($1.5\,g\,l^{-1}$) in *S. cerevisiae*.

Results

Systematic engineering for free fatty acids production. We first started by establishing a platform strain that overproduces FFAs. In *S. cerevisiae*, fatty acids are mainly synthesized *de novo* by a cytosolic type I FAS[19] as activated fatty acids (fatty acyl-CoAs) by condensing acetyl-CoA and malonyl-CoA. FFAs are rapidly re-activated by fatty acyl-CoA synthetases to fatty acyl-CoAs, whose accumulation feedback inhibits fatty acid biosynthesis[20]. A wild-type strain therefore only produced $3\,mg\,l^{-1}$ FFAs (Fig. 2a). To circumvent this, we interrupted the reactivation process by deleting two of the main fatty acyl-CoA synthetase encoding genes *FAA1* and *FAA4*. To prevent fatty acid degradation through β-oxidation we also deleted *POX1* encoding the fatty acyl-CoA oxidase, which catalyses the first step of this pathway. The resulting strain YJZ06 produced $0.56\,g\,l^{-1}$ FFAs (Fig. 2a). This is consistent with earlier studies, which have shown that interruption of FFA activation is essential for FFA accumulation and secretion[21,22]. Our previous[15] and current studies (*vide infra*) showed that deletion of the aldehyde dehydrogenase-encoding gene *HFD1* is essential for the production of fatty aldehyde-derived alkanes and fatty alcohols. Thus, we used the *HFD1* knockout strain YJZ08 for further engineering. To further increase FFA production we expressed a truncated *E. coli* thioesterase encoding gene *'tesA* (refs 5,14) to increase FFA release from the FAS complex, which resulted in a titre of $0.67\,g\,l^{-1}$ (strain YJZ13).

Next we aimed on increasing the supply of the precursor cytosolic acetyl-CoA by introducing a synthetic chimeric citrate lyase pathway (Fig. 1), which has been proposed to play an important role in lipid accumulation in oleaginous yeasts[23]. In addition to expressing an ATP:citrate lyase (ACL) as described before[24], we here constructed and optimized the citrate lyase cycle (Figs 1 and 3a) by systematically comparing different heterologous ACLs and malic enzymes (MEs), two significant components of this pathway, and overexpressing the endogenous mitochondrial citrate transporter Ctp1 and malate dehydrogenase 'Mdh3. Introduction of the chimeric acetyl-CoA pathway, consisting of ACL and ME from *Rhodospuridium toruloides* combined with overexpression of Ctp1 and 'Mdh3, improved the growth of a pyruvate decarboxylase negative strain IMI076 with an internal deletion in MTH1 (Pdc− *MTH1-ΔT*)[25] (Fig. 3c). We further show that ACL from *Mus musculus* (MmACL) was better than the ones from *R. toruloides* (RtACL) and *Homo sapiens* (HsACL) in improving IMI076 growth (Fig. 3c) and ME from *R. toruloides* (RtME) was important for cell growth in addiction to ACL in IMI076 (Fig. 3d). Furthermore, the ACL-based acetyl-CoA pathway rescued the growth of pyruvate decarboxylase negative strain RWB837 (Fig. 3b), which is growth-deficient[25]. Consistently, plasmid-overexpression of these genes improved FFA production (Fig. 3e) and *MmACL* was better for FFA production compared with RtACL and HsACL (Fig. 3f). Since

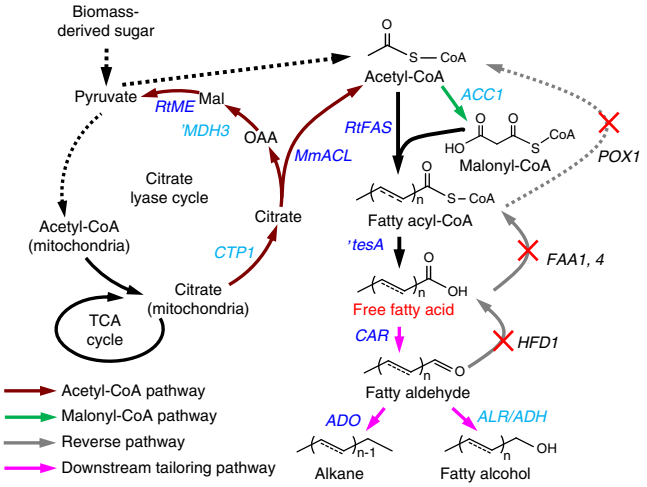


Figure 1 | Establishing a yeast fatty acid platform for production of oleochemicals and biofuels. The dotted lines indicate multiple steps and solid lines a single step. Overexpressed genes are shown in light blue (endogenous) or navy blue (heterologous). Reverse pathways were eliminated by deleting the corresponding genes (marked with X). For FFA production, fatty acyl-CoA synthetase encoding genes *FAA1* and *FAA4*, and fatty acyl-CoA oxidase encoding gene *POX1*, were disrupted. Furthermore, the truncated *E. coli* thioesterase 'TesA was overexpressed. For enhancing acetyl-CoA supply, a chimeric acetyl-CoA pathway, consisting of ACL (MmACL) from *Mus musculus*, ME (RtME) from *Rhodosporidium toruloides*, endogenous malate dehydrogenase with removed peroxisomal signal ('Mdh3) and citrate transporter Ctp1, was constructed and genome-integrated. For increased FFA biosynthesis, *R. toruloides* FAS encoding genes (*RtFAS1* and *RtFAS2*) were expressed through genome-integration and acetyl-CoA carboxylase encoding gene *ACC1* was overexpressed by promoter replacement. OAA, oxaloacetate; Mal, malate. For alkane/fatty alcohol production, a heterologous CAR from *Mycobacterium marinum* was introduced for reducing FFAs to fatty aldehydes, which were then transformed to alkanes by an ADO, or fatty alcohols by an ADH or an ALR.

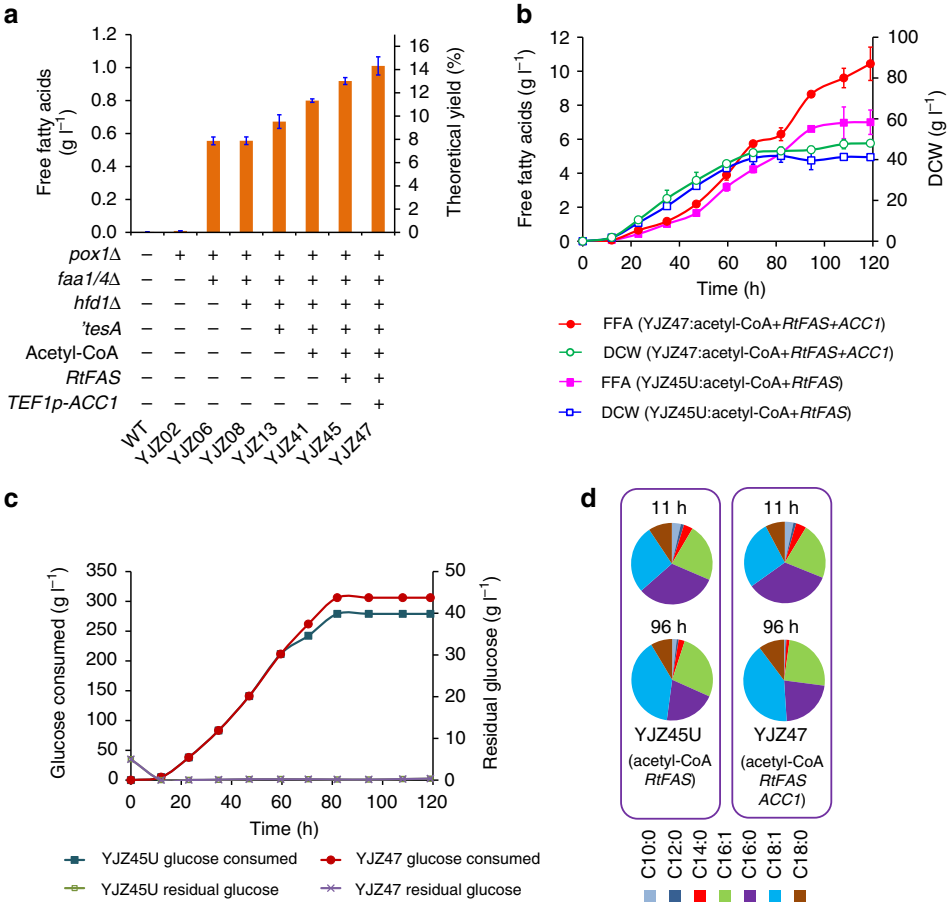


Figure 2 | FFA production by engineered *S. cerevisiae* strains. (**a**) FFA titres obtained with engineered strains in shake flasks after 72 h cultivation at 200 r.p.m., 30 °C. All data represent the mean ± s.d. of biological triplicates. (**b**) Fed-batch fermentation of strains YJZ45U and YJZ47. YJZ45U is a prototrophic strain with complementation of the *URA3* marker in YJZ45. Time courses of FFA titres (filled symbols) and cell mass (open symbols) are shown. (**c**) Glucose consumption profile and time courses of residual glucose during fed-batch fermentation. The data represent the mean ± s.d. of biological duplicates. (**d**) FFA profiles of the strain YJZ45 and YJZ47 at 11 h and 96 h.

plasmid-expression retarded the cell growth probably due to the metabolic burden (Supplementary Fig. 1), we thus genomic-integrated the optimized actyl-CoA pathway consisting of *MmACL*, *RtME*, *CTP1* and *'MDH3*, which improved FFA production to 0.80 g l^{-1} (strain YJZ41, Fig. 2a).

Then we enhanced fatty acid synthesis by expressing a *R. toruloides* FAS (RtFAS). This FAS has two ACP domains, which may improve fatty acid biosynthesis efficiency by increasing the intermediate concentration in its reaction chamber[23,26]. *RtFAS* was functionally expressed and increased the total lipid and FFA content (Supplementary Fig. 2). Genomic integration of both *RtFAS* and the acetyl-CoA pathway (YJZ45) increased the FFA titre to 0.92 g l^{-1} in shake flasks and the corresponding prototrophic strain YJZ45U reached 7.0 g l^{-1} in fed-batch cultivation. After ensuring sufficient acetyl-CoA supply and fatty acid synthesis, we wanted to evaluate whether increased supply of malonyl-CoA, another tightly regulated precursor, could increase FFA production. We first evaluated an acetyl-CoA carboxylase mutant (Acc1^{S1157A,S659A}, Acc1**)[27] in which regulation by phosphorylation is abolished. However, its expression resulted in a lower FFA titre with lower biomass yield in fed-batch cultivation and promoted longer-chain fatty acid biosynthesis (Supplementary Fig. 3). The latter is consistent with a previous study reporting a shift towards C18 fatty acids at a higher malonyl-CoA/acetyl-CoA ratio by an *in vitro* reconstituted FAS from *S. cerevisiae*[28]. Alternatively, we moderately enhanced the expression of the wild-type *ACC1* by replacing its native promoter with the *TEF1* promoter (strain YJZ47), which enabled an increase of FFA production to 1.0 g l^{-1} (333-fold higher than wild-type strain, 14.3% of theoretical yield) in shake flask cultivation. It should be emphasized that the heavily engineered strain YJZ47 had a similar biomass yield compared with wild-type strain (Supplementary Fig. 4). This robustness is very important for implementation in industrial processes. Glucose limited fed-batch cultivation of this strain resulted in a titre of 10.4 g l^{-1} FFAs (Fig. 2b,c), which was 49% higher than strain YJZ45U and also 20% higher than an engineered *E. coli* (8.6 g l^{-1}) in fed-batch culture[29] (Table 1). Interestingly, an increased percentage of oleic (C18:1) and stearic acid (C18:0) was observed in both strains during the fermentation (Fig. 2d and Supplementary Fig. 3c), which may be attributed to the upregulation of the fatty acid elongation system[30], since the yeast FAS has much higher level production of C16 fatty acids than C18 fatty acids *in vitro*[31].

Engineering a fatty acid pathway for alkane production. Subsequently, we wanted to exploit the FFAs for the production of alkanes, ideal drop-in biofuels[6]. We previously introduced a cyanobacterial fatty acyl-CoA-derived pathway, consisting of a *Synechococcus elongatus* fatty acyl-ACP/CoA reductase (AAR)

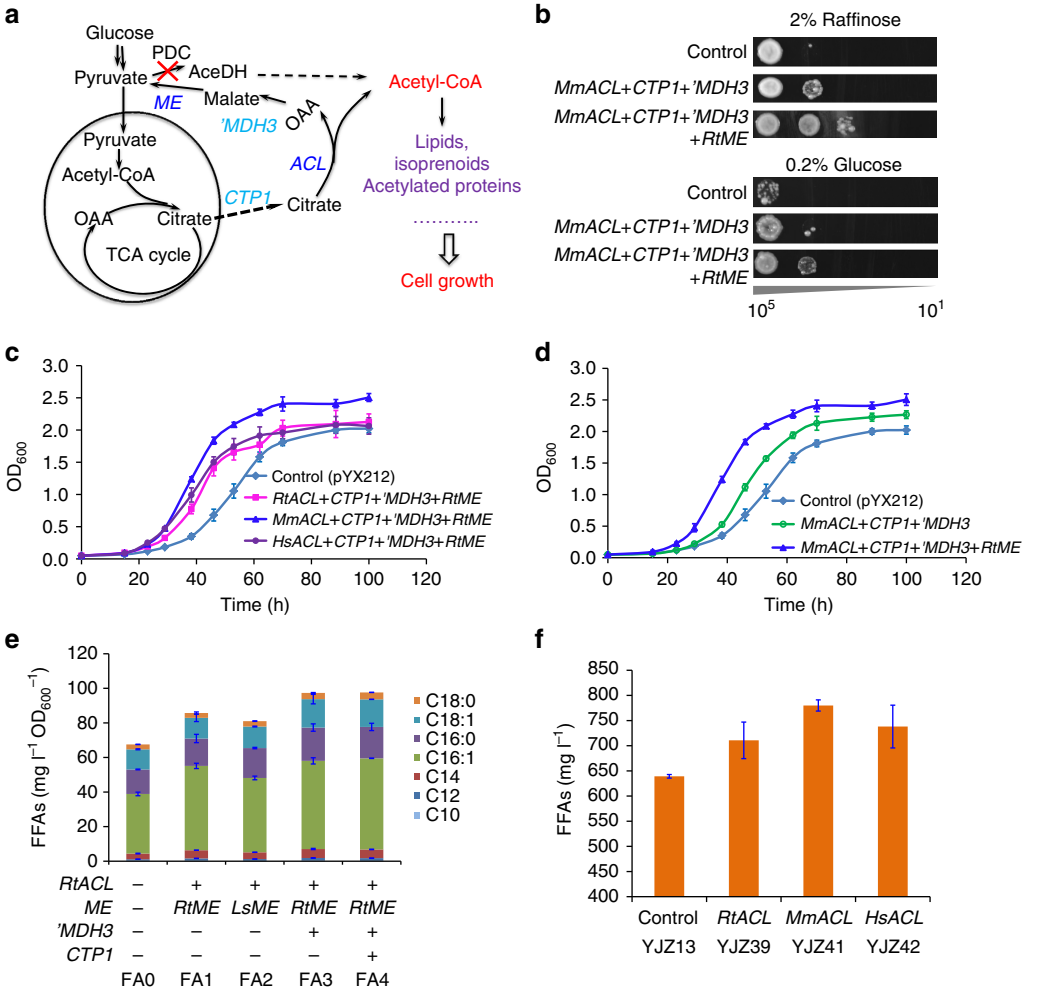


Figure 3 | Optimization and characterization of ACL-based acetyl-CoA pathways in *S. cerevisiae*. (**a**) Schematic illustration of the chimeric citrate lyase pathway for improved supply of acetyl-CoA, a key precursor for synthesis of cell building block. (**b**) The ACL-based acetyl-CoA pathway rescued the growth of PDC-negative strain RWB837, which is growth-deficient. (**c**) Introduction of the ACL-based acetyl-CoA pathway improved the growth of PDC-negative mutant strain *S. cerevisiae* IMI076. Cells were cultured with an initial OD_{600} of 0.05 in SC-URA at 30 °C, 200 r.p.m. (**d**) ME is beneficial for cell growth in addiction to ACL in the PDC-negative mutant strain *S. cerevisiae* IMI076. (**e**) Introduction of the heterologous citrate lyase by-pass pathway improved FFA production. The engineered strains were constructed by transforming YJZ08 with the corresponding plasmids as shown in Supplementary Table 3. (**f**) Effect of different ACLs on production of FFAs. *RtACL*, *MmACL* and *HsACL* represent the optimized ACL genes from *R. toruloides*, *M. musculus* and *H. sapiens*, respectively. The engineered strains were cultivated in shake flasks containing 15 ml minimal media for 72 h at 200 r.p.m., 30 °C. All data represent the mean ± s.d. of biological triplicates.

Table 1 | Comparison of cell factories for production of free fatty acids.

Microorganism	Media	Cultivation mode	Titre (g l^{-1})	Yield (% theoretical yield)	Reference
E. coli	MM	Shake flask	1.1	14	5
E. coli	SMM	Fed-batch	8.6	N.C.*	29
E. coli	SMM	Fed-batch	3.9	N.C.*	54
Y. lipolytica	MM	Shake flask	0.5	7	43
S. cerevisiae	MM	Shake flask	0.1-0.5	2-7	13,39,40
S. cerevisiae	YPD	Shake flask	2.2	N.C.*	21
S. cerevisiae	MM	Shake flask	1.0	14	This study
S. cerevisiae	MM	Fed-batch	10.4	9	This study

MM, minimal media; SMM, semi-minimal media containing complex media component such as yeast extract; YPD, complex media containing 20 g l^{-1} peptone, 10 g l^{-1} yeast extract and 20 g l^{-1} glucose.
*N.C.: not calculated due to containing complex media component such as yeast extract.

and fatty aldehyde deformylating oxygenase (*Se*ADO), in yeast and thereby demonstrated for the first-time production of alkanes in this organism[15]. The study, however, suggested that the AAR was inefficient in yeast. We therefore explored an alternative pathway by expressing a *Mycobacterium marinum* carboxylic acid reductase (MmCAR)[7] (Fig. 4a). For activation of MmCAR[7], we

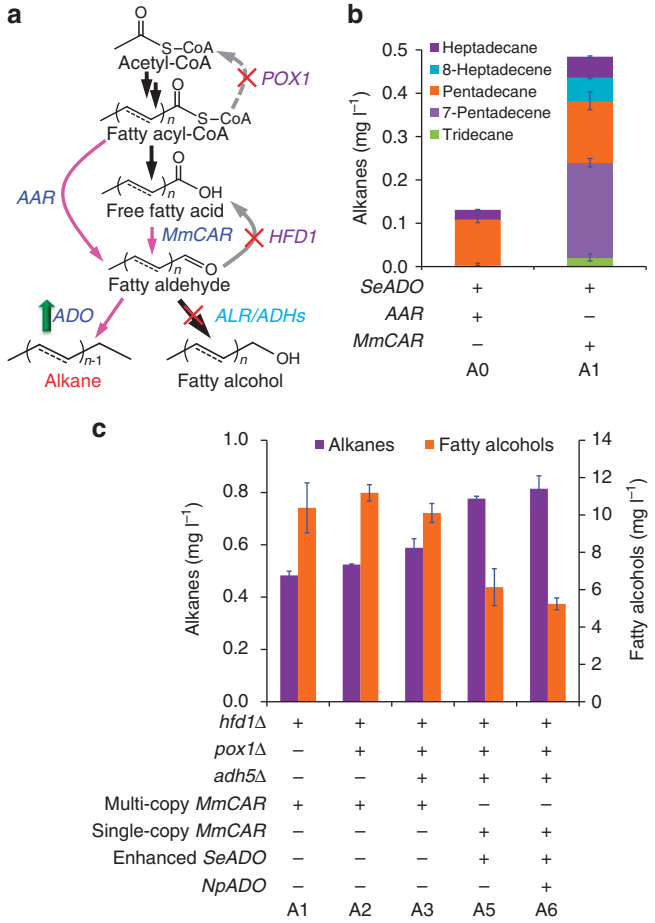


Figure 4 | Alkane production from FFAs. (**a**) The rewired metabolic pathways for enhancing alkane biosynthesis and decreasing accumulation of the by-product fatty alcohols. The alkane pathways are shown with pink arrows. (**b**) Alkane production by the FFA-derived (CAR and ADO) or the fatty acyl-CoA-derived (AAR and ADO) pathway. (**c**) Stepwise increasing alkane titres by eliminating competing pathways of aldehyde reduction and enhancing ADO expression, and corresponding fatty alcohol accumulation is also showed. The strains were cultivated in shake flasks for 72 h at 200 r.p.m., 30 °C. All data represent the mean ± s.d. of biological triplicates.

expressed 4′-phosphopantetheinyl transferase NpgA from *Aspergillus nidulans*. This FFA-based pathway enabled a 2.7-fold higher alkane production (0.48 mg l^{-1}) than the fatty acyl-CoA-based pathway in an *hfd1*Δ background (Fig. 4b). Deletion of *POX1* slightly increased alkane production to 0.52 mg l^{-1} (Fig. 4c). Further increasing the fatty acid supply did not increase the titre, but instead increased fatty alcohol production (Supplementary Fig. 6). Fatty alcohol accumulation might be caused by endogenous promiscuous aldehyde reductases (ALRs) and/or alcohol dehydrogenases (ADHs) that compete for the fatty aldehyde intermediates[32]. To solve this, we tried to identify the main competing enzymes by single deletion of 17 (putative) ALR/ADH-encoding genes (Supplementary Table 1). Of these, *ADH5* deletion led to an increased alkane production and decreased fatty alcohol accumulation (Fig. 4c and Supplementary Fig. 7). To further increase flux towards alkanes we increased the expression of the ADO by expressing *SeADO* under control of strong promoter UAS-TDH3p (ref. 33) and modulated *MmCAR* expression by single-copy genomic integration. The resulting strain A5 produced 50% more alkanes corresponding to 0.78 mg l^{-1} and had a 40% reduction in fatty alcohol accumulation, compared with the control strain A2 (Fig. 4c). Finally we evaluated additional expression of *Nostoc punctiforme NpADO* and this increased alkane production to 0.82 mg l^{-1} with a further reduction in fatty alcohol accumulation (Fig. 4c). Although the titre is still cannot be comparable to *E. coli*, it represent more the eightfold higher titre than our previous work[15].

Tailoring fatty acid for production of fatty alcohols. The accumulation of fatty alcohols in our alkane producing strains (Fig. 3) gave us confidence to further explore the production of fatty alcohols from FFAs (Fig. 5a). Fatty alcohols are widely used as detergents, cosmetic ingredients and for the formulation of pharmaceuticals. Current fatty alcohol production strongly relies on plant oils, and microbial production could ensure a stable supply, without competition with food oil production, and enables tailored production of specific fatty alcohols. As observed for alkane production (Fig. 3b), the CAR was more efficient for fatty alcohol production than *Acinetobacter baylyi* fatty acyl-CoA/ACP reductase (ACR) or AAR (Supplementary Fig. 8). Since deletion of *ADH5* decreased fatty alcohol production in our ALR/ADH screening (Supplementary Fig. 7b,c), we overexpressed *ADH5* to increase fatty alcohol production. Indeed, *Adh5* was more efficient for fatty alcohol synthesis than several other ADH/ALRs, that is, endogenous Sfa1, Adh6, Adh7 or heterologous YjgB from *E. coli* (Supplementary Fig. 7d). When increasing the FFA supply (strain FOH6), the fatty alcohol production reached a titre of 23.2 mg l^{-1} (Fig. 5b and Supplementary Fig. 9b). Allowing substrate channelling of the fatty aldehyde intermediates, by fusing MmCAR and Adh5, increased the fatty alcohol titre further by 26% (strain FOH21). However, enzyme fusion had a negative effect in the *HFD1* deletion strain FOH23 (Supplementary Fig. 9d), which may be attributed to the low activity of MmCAR in the fusion enzyme. Combining deletion of *HFD1* and blocking fatty acid degradation (strain FOH8) further increased fatty alcohol production to 61.2 mg l^{-1} (Fig. 5b). However, there was still an accumulation of intracellular C18 fatty aldehydes (Supplementary Fig. 10b), indicating that C18 aldehyde reduction was a limiting step. Since a previous study showed that the bi-functional fatty acyl-CoA reductase *FaCoAR* from *Marinobacter aquaeolei* VT8 (ref. 34) has high activity towards long-chain fatty-aldehydes, we expressed *FaCoAR* instead of *ADH5* together with *MmCAR* in FOH28 and this resulted in 77.1 mg l^{-1} fatty alcohols. Co-expression of *ADH5* and *FaCoAR* (strain FOH29) further improved fatty alcohol production to 81.8 mg l^{-1} (Fig. 5b). Expression of *FaCoAR* and *ADH5* resulted in ~80% reduction of the C18 fatty aldehyde (octadecanal and 9-octadecenal) content compared with *ADH5* overexpression (Supplementary Fig. 10b). We also evaluated fusion of *MmCAR* and *FacoAR*, but this decreased fatty alcohol production (Supplementary Fig. 10c). Our ADH/ALR knockout screening showed that *ADH6* deletion increases fatty alcohol production by 50% (Supplementary Fig. 7b). We therefore deleted *ADH6* (strain FOH31) resulting in increased fatty alcohol production to 89.5 mg l^{-1} (Fig. 4b).

We found that there was still a high accumulation of FFAs (>2-fold higher than fatty alcohols, Supplementary Fig. 11) in strain FOH31, which indicated that fatty acid biosynthesis was overflown and the downstream reduction needed to be enhanced. We thus genome-integrated an additional copy of *MmCAR* under control of a *GAL7* promoter (together with *GAL80* deletion to enable gene expression without galactose addition). The resulting strain FOH33 produced 28% more fatty alcohols (115 mg l^{-1}) with a 65% reduction in FFA accumulation (Fig. 5b and Supplementary Fig. 11). Glucose limited fed-batch cultivation

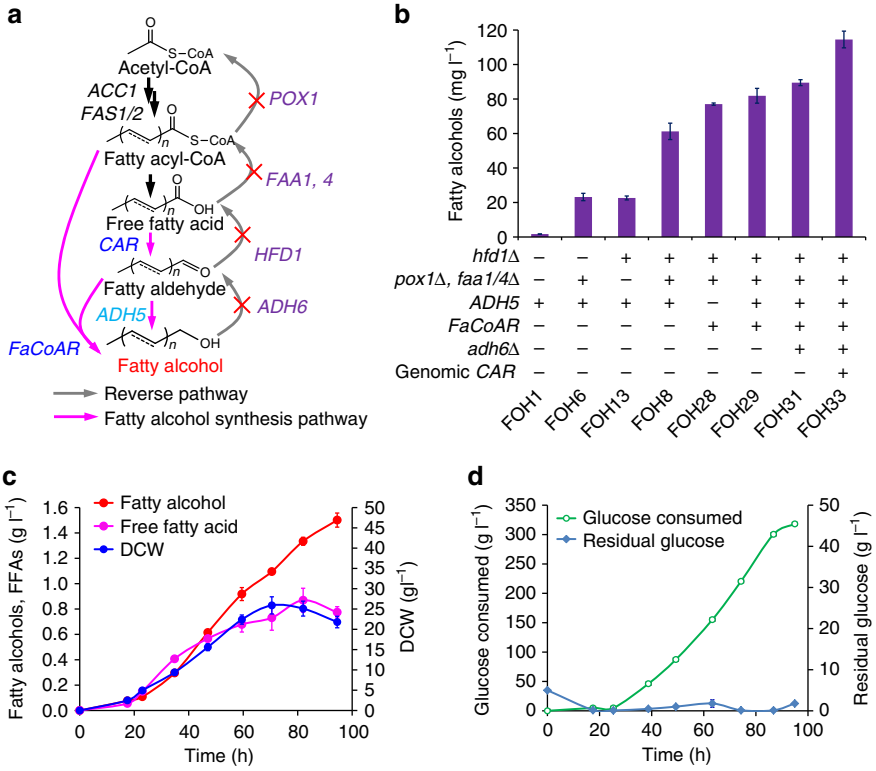


Figure 5 | Engineering fatty alcohol production from FFAs. (**a**) The rewired metabolic pathways for fatty alcohol production. Genes responsible for reverse reactions were deleted (marked with X), and genes related to fatty alcohol synthesis pathways were (over)-expressed. (**b**) Production of fatty alcohols in engineered strains in shake flasks, after 72 h cultivation at 200 r.p.m., 30 °C. All data represent the mean ± s.d. of biological triplicates. (**c**) Fed-batch fermentation of the best fatty alcohol producing strain FOH33 in 1 l bioreactor. (**d**) Glucose consumption profile and time courses of residual glucose during fed-batch fermentation. The data represent the mean ± s.d. of duplicates.

Table 2 | Comparison of cell factories for production of fatty alcohols.

Microorganism	Media	Cultivation mode	Titre (g l^{-1})	Yield (% theoretical Yield)	Reference
E. coli	MM	Fed-batch	0.75	6	17
E. coli	MM	Fed-batch	1.75	8	55
E. coli	MM	Fed-batch	1.65	35	46
S. cerevisiae	MM	Shake flask	0.10	1.4	14,45
S. cerevisiae	MM	Concentrated resting cells. Fed-batch	1.11	N.C.*	35
S. cerevisiae	MM	Shake flask	0.12	1.7	This study
S. cerevisiae	MM	Fed-batch	1.51	1.4	This study

MM, minimal media.
*N.C.: not calculated due to the concentration of the cells with unknown initial fatty alcohols.

(Fig. 5d) of FOH33 had a more significant improvement (onefold, Supplementary Fig. 11c) in production of fatty alcohols (1.5 g l^{-1}, Fig. 5c), which is the highest reported titre of fatty alcohols produced by *S. cerevisiae* to date[14,35]. The titre is also comparable to *E. coli* cells though the yield is still much lower (Table 2).

Discussion

The budding yeast *S. cerevisiae* is an attractive host for biosynthesis of specific products because of its robustness in industrial harsh conditions and easily transfer to existing bioethanol production plants. In this study, we undertook a major metabolic engineering effort to engineer *S. cerevisiae* for high-level production of FFAs and then their further transformation into alkanes and fatty alcohols. We demonstrated for the first time the significant conversion of FFAs to alkanes and fatty alcohols in yeast, and we also showed that this FFA dependent pathway is far more efficient than the earlier reported route from fatty acyl-CoA (Fig. 3b and Supplementary Fig. 8). The production of alkanes and fatty alcohols benefited from our effort to streamline the fatty acid overproduction by taking the advantage of high cellular FFA levels (>200-fold higher than fatty acyl-CoA).

Oleaginous yeasts have been engineered for high-level production of neutral lipids such as triacylglycerol[36,37], an ideal feedstock for biodiesel production through transesterification. However, the intracellular accumulation requires very high cell density fermentation and also makes it challenging to recover the products[38]. FFAs are another ideal feedstocks for deoxygenated production of renewable hydrocarbon-based biofuels that are entirely fungible with fossil fuels[39]. More importantly, FFAs can be secreted (Supplementary Fig. 4c), which is beneficial for high-

level production by decoupling it from the cell growth (Fig. 2b). Aiming to overproduce FFAs, several researchers disrupted FFA activation and enhanced FFA biosynthesis, for example, through expression of different thioesterases, which enabled FFA production at 0.1–0.5 g l^{-1} in minimal media in shake flask cultures (Table 1)[14,40,41]. More recently, disruption of FFA activation and neutral lipid recycle enabled production of 2.2 g l^{-1} in complex (YPD) medium[21]. However, due to its high costs, complex makeup and variable composition, YPD medium would not be suitable for industrial production. Furthermore, the final engineered strain had a 20% lower biomass level in YPD medium, which indicated that the combination of disrupting FFA activation and neutral lipid recycle was harmful to the cell, and might retard growth further in minimal media with lower and less diverse nutrient availability. In this study, we systematically optimized the primary metabolism by disrupting FFA activation, constructing a more efficient fatty acid synthesis system and a chimeric citrate lyase cycle for enhanced precursor supply. More importantly, we are the first to construct a plasmid-free FFA overproducing strain by integration of all pathway components into the genome, which is important for application in industrial processes. These strategies enabled high-level FFA production in yeast under shake flask with minimal media (Fig. 2a) without a decrease in the biomass yield (Supplementary Fig. 4a). Fed-batch cultivation not only led to accumulation of a high FFA titre (10.4 g l^{-1}), but also a high biomass titre of 48 g l^{-1}, which is at the same level as a wild-type CEN.PK strain in fed-batch cultivation[42]. Before our study, the highest FFA titre (8.6 g l^{-1}) was reached by an engineered *E. coli* in fed-batch culture[29]. This is the first time that *S. cerevisiae* surpassed *E. coli* in regards to oleochemical production. It is worthy to mention that the FFA titre is also higher than oleaginous yeast *Yarrowia lipolytica* of 0.5 g l^{-1} (Table 1)[43], which shows the potential of *S. cerevisiae* for FFA production.

Though lower in titre, the alkane production was much higher by using the FFA-based pathway compared with the fatty acyl-CoA-based pathway (Fig. 3b). By-product accumulation can hamper metabolic engineering endeavours. Because of the low ADO activity[44], the alkane titre remained low and fatty alcohols were being produced as major by-products (Fig. 3c). To overcome this problem, we first identified Adh5 as a key enzyme for conversion of fatty aldehydes to fatty alcohols by screening a series of ALR/ADH deletion strains. By deleting Adh5, we could significantly improve alkane production. However, their indispensable role in the biosynthesis of essential metabolites makes it impossible to delete all these enzymes. Increased expression of enzymes involved in conversion of fatty aldehydes to alkanes further increased alkane production, pointing to this step as having major flux control.

In contrast to alkane production, fatty alcohol biosynthesis relies on efficient reduction of fatty aldehyde (Fig. 5a). We therefore took advantage of our screening of different ALR/ADH deletion strains and found that overexpression of *ADH5* and deletion of *ADH6* could significantly improve fatty alcohol production (Supplementary Fig. 7). Combined with enhanced precursor supply, our final strain produced 1.5 g l^{-1} fatty alcohols in fed-batch culture, which to our knowledge is the highest reported titre by *S. cerevisiae*. Current heterologous fatty alcohol biosynthesis pathways in yeast are designed to utilize fatty acyl-CoA as precursor, which enabled producing ~90 mg l^{-1} fatty alcohols in shake flasks[14,45]. Recently, increasing acetyl-CoA supply and relieving the inhibition on fatty acyl-CoA biosynthesis, resulted in production of fatty alcohols at 330 mg l^{-1} in shake flask and 1.1 g l^{-1} in fed-batch cultivation with high concentrated cells[35]. In that study, concentrated cells were used in fed-batch cultivation, which might result in an overestimated titre since concentrated cells should carry high-level initial cellular fatty alcohols. Moreover, the higher titre compared with our study for shake flask cultures might be attributed to the use of a dodecane overlay, which has been shown to be beneficial for fatty alcohol production[46]. However, a dodecane overlay will result in higher costs for product recovery due the similar boiling points of fatty alcohols and dodecane. Here, our strain produced much more fatty alcohols in fed-batch culture without a dodecane overlay. In the future, identification of fatty alcohol transporters might realize *in situ* product separation and recovery.

In conclusion, we have developed yeast cell factories for the production of FFAs and fatty alcohols, as well as demonstrated the significant production of alkanes in yeast. These strains represent a starting point for establishing yeast-based commercial bioprocesses for the production of oleochemicals and advanced biofuels from renewable resources. Our metabolic engineering strategies of pathway balancing at the fatty aldehyde node not only facilitated the production of fatty aldehyde-derived products but also provide valuable insights for construction of yeast cell factories for production of other valuable aldehyde chemicals, for example, vanillin[47], because of the similarity of the competition from ALR/ADHs.

Methods

Strains and reagents. Plasmids and *S. cerevisiae* strains used are listed in Supplementary Tables 2 and 3. PrimeStar DNA polymerase was purchased from TaKaRa Bio. Taq DNA polymerase, restriction enzymes, DNA gel purification and plasmid extraction kits were purchased from Thermo Scientific. Yeast plasmid Miniprep I kits were purchased from Zymo Research. All oligonucleotides (Supplementary Table 4) were synthesized at Sigma-Aldrich. All chemicals including analytical standards were purchased from Sigma-Aldrich unless stated otherwise. All codon optimized heterologous genes were synthesized (Genscript) and listed in Supplementary Table 5.

Strain cultivation. Yeast strains for preparation of competent cells were cultivated in YPD consisting of 10 g l^{-1} yeast extract (Merck Millipore), 20 g l^{-1} peptone (Difco) and 20 g l^{-1} glucose (Merck Millipore). Strains containing *URA3*-based plasmids or cassettes were selected on synthetic complete media without uracil (SC-URA), which consisted of 6.7 g l^{-1} yeast nitrogen base (YNB) without amino acids (Formedium), 0.77 g l^{-1} complete supplement mixture without uracil (CSM-URA, Formedium), 20 g l^{-1} glucose (Merck Millipore) and 18 g l^{-1} agar (Merck Millipore). The *URA3* maker was removed and selected against on SC + FOA plates, which contained 6.7 g l^{-1} YNB, 0.77 g l^{-1} complete supplement mixture and 0.8 g l^{-1} 5-fluoroorotic acid. Strains containing the *kanMX* cassettes were selected on YPD plates containing 200 mg l^{-1} G418 (Formedium).

Shake flask batch fermentations for production of alkanes and fatty alcohols were carried out in minimal medium containing 5 g l^{-1} $(NH_4)_2SO_4$, 3 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$, 30 g l^{-1} glucose, trace metal and vitamin solutions[48] supplemented with 40 mg l^{-1} histidine and/or 60 mg l^{-1} uracil if needed. While for production of FFAs, the minimal media was modified by using lower glucose (20 g l^{-1}) and higher KH_2PO_4 (14.4 g l^{-1}), which was beneficial for FFA accumulation (Supplementary Fig. 5). Cultures were inoculated, from 24 h precultures, at an initial OD_{600} of 0.1 in 15 ml minimal medium and cultivated at 200 r.p.m., 30 °C for 72 h.

The batch and fed-batch fermentations for fatty acid and fatty alcohol production were performed in 1.0 l bioreactors, with an (initial) working volume of 0.4 l, in a DasGip Parallel Bioreactors System (DasGip). The initial batch fermentation was carried out in minimal medium containing 5 g l^{-1} $(NH_4)_2SO_4$, 3 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$, 10 g l^{-1} glucose, trace metal and vitamin solutions. The temperature, agitation, aeration and pH were monitored and controlled using a DasGip Control 4.0 System. The temperature was kept at 30 °C, initial agitation set to 600 r.p.m. and increased to maximally 1,200 r.p.m. depending on the dissolved oxygen level, aeration was provided at 30 sl h^{-1} and the dissolved oxygen level was maintained above 40%, the pH was kept at 5.6 by automatic addition of 4 M KOH and 2 M HCl. The aeration was controlled and provided by a DasGip MX4/4 module. The composition of the off-gas was monitored using a Dasgip Offgas Analyzer GA4. Addition of the acid, base, and glucose feed was carried out with Dasgip MP8 multi-pump modules (pump head tubing: 0.5 mm ID, 1.0 mm wall thickness). The pumps, pH and DO probes were calibrated before the experiment. During the fed-batch cultivation, the cells were fed with an 800 g l^{-1} glucose solution with a feed rate that was exponentially increasing ($\mu = 0.03$ h^{-1}) to maintain a constant biomass-specific glucose consumption rate. The initial feed rate was calculated using the biomass yield and concentration that were obtained

during prior duplicate batch cultivations with these strains. The feeding was started once the CO_2 levels dropped after the glucose was consumed.

Dry cell weight measurements were performed by filtrating 1 ml of broth through a weighed 0.45 μm filter membrane (Sartorius Biolab) and measuring the weight increase after drying for 48 h in a 65 °C oven. The filter was washed once before and three times after filtrating the broth with 5 ml deionized water.

Genetic manipulation. Seamless gene deletion was performed (Supplementary Fig. 12a) by using *Kluyveromyces lactis URA3* (*KlURA3*) as a selection marker, which was looped out by homologous recombination of the direct repeats, and selection on SC + FOA plates[49]. The deletion cassettes were constructed by fusing 200–600 nucleotide homologous arms with the *KIURA3*. For single gene deletion in identification of the ALRs and alcohol dehydrogenases, *kanMX* cassettes containing about 70 nucleotide homologous arms at both ends were used to transform strain YJZ03. *amdSYM* cassette[50] was used as a selection marker for genome-integration of FAS genes from *S. cerevisiae* (ScFAS) or *R. toruloides* (RtFAS). The pathways for alkane and alcohol production were assembled on a yeast chromosome or plasmid backbone pYX212 by using a modular pathway engineering strategy[51]. The gene expressing modules, consisting of a promoter, a structural gene, a terminator and the promoter of the next module for homologous recombination, were constructed by fusion PCR. Then the modules were gel purified and transformed to the *S. cerevisiae* with linearized plasmid pYX212.Genome-integration was performed by using a modular pathway integration strategy (Supplementary Fig. 12b). Taking the example of targeted integration of (*TPIp-MmCAR-FBA1t*) + (*PGK1p-EcFNR-CYC1t*) + (*TEF1p-EcFD-TDH2t*) + (*tHXT7p-npgA*) at the *ADH5* locus in YJZ03, the whole pathway was divided into three modules of AK1, 2 and 3. In detail, the upstream homologous arm *ADH5*-up (from position − 382 to + 3) was amplified from CEN.PK113-11C genomic DNA with primer pair p59/p60. The AK1 module of *ADH5*-up + (*TPIp-CAR-FBA1t*) + *CYC1t* was assembled by fusing the parts of ADH5-up, TPIp-CAR-FBA1t + CYC1t. The part *TPIp-CAR-FBA1t* + *CYC1t* was amplified from the pAlkane16 by using primer pair p19/p31. The AK2 module of (*CYC1t-EcFNR-PGK1p*) + (*TEF1p-EcFd-TDH2t*) was amplified pAlkane16 by using the primer pair p32/p34. The AK3 module of *TDH2t* + (*tHXT7*p-*npgA*) + *URA3* + *ADH5-3*' was assembled by fusing the DNA parts of *TDH2t, tHXT7p-npgA, KIURA3* and *ADH5*-3'. The *TDH2t* was amplified from yeast genome DNA by using primer pair p15/p63. The *tHXT7p-npgA* was amplified from pAlkane16 with primer pair p27/p64. Amplification of *KIURA3* was performed by using primer p65/p66 and pWJ1042 as a template. And downstream homologous arms *ADH5*-3' (from position + 579 to + 945) was amplified from CEN.PK113-11C genomic DNA by using primer pair p61/p62. Then the three modules (AK1, 2 and 3) were transformed into YJZ03 and transformants were selected on SC-URA plates (6.7 g l^{-1} YNB without amino acids, 0.77 g l^{-1} complete supplement mixture without uracil and 20 g l^{-1} glucose and 15 g l^{-1} agar). Clones were verified by colony PCR. Subsequently, 2–3 clones with correct module integration were cultivated overnight in YPD liquid medium and then plated on SC + FOA plates after wash for looping out of *URA3* and also the 3' end of the *ADH5* (from + 579 to + 1,056 that was left in place after the first round integration). All other pathways were integrated as above and the genetic arrangement is shown in Supplementary Fig. 12c

Metabolite extraction and analysis. FFAs were simultaneously extracted and methylated by dichloromethane containing methyl iodide as methyl donor[52]. Since the FFAs were secreted and cell culture formed an emulsion (Supplementary Fig. 4c), the cell culture should be mixed well before sample taking. Cell cultures from shake flask were diluted twofold with water and those from bioreactor were diluted 10-fold. Briefly, 200 μl aliquots of cell culture dilutions were taken into glass vials from 72 h incubated cultures, then 10 μl 40% tetrabutylammonium hydroxide (base catalyst) was added immediately followed by addition of 200 μl dichloromethane containing 200 mM methyl iodide as methyl donor and 100 mg l^{-1} pentadecanoic acid as an internal standard. The mixtures were shaken for 30 min at 1,400 r.p.m. by using a vortex mixer, and then centrifuged at 5,000*g* to promote phase separation. A 160 μl dichloromethane layer was transferred into a GC vial with glass insert, and evaporated 4 h to dryness. The extracted methyl esters were resuspended in 160 μl hexane and then analysed by gas chromatography (Focus GC, ThermoFisher Scientific) equipped with a Zebron ZB-5MS GUARDIAN capillary column (30 m × 0.25 mm × 0.25 μm, Phenomenex) and a DSQII mass spectrometer (ThermoFisher Scientific). The GC program was as follows: initial temperature of 40 °C, hold for 2 min; ramp to 130 °C at a rate of 30 °C per minute, then raised to 280 °C at a rate of 10 °C per min and hold for 3 min. The temperature of inlet, mass transfer line and ion source were kept at 280, 300 and 230 °C, respectively. The injection volume was 1 μl. The flow rate of the carrier gas (helium) was set to 1.0 ml min^{-1}, and data were acquired at full-scan mode (50–650 *m/z*). Final quantification was performed using the Xcalibur software.

For alkane and fatty alcohol quantification, cell pellets were collected from 5 ml (fatty alcohol) or 10 ml (alkane) cell culture and then freeze dried for 48 h. Metabolites were extracted by 2:1 chloroform:methanol solution[53], which contained hexadecane (alkanes) and pentadecanol (fatty alcohols) as internal standards. The extracted fraction was dried by rotary evaporation and dissolved in hexane (alkanes) or ethyl acetate (fatty alcohols). Quantification of fatty alcohols and alkanes was performed on the same GC–MS system as used for fatty acid analysis. The GC program for alkane analysis was as follows: initial temperature of 50 °C, hold for 5 min; then ramp to 140 °C at a rate of 10 °C per min and hold for 10 min; ramp to 310 °C at a rate of 15 °C per min and hold for 7 min. The GC program for fatty alcohol quantification was as follow: initial temperature of 45 °C hold for 2.5 min; then ramp to 220 °C at a rate of 20 °C per min and hold for 2 min; ramp to 300 °C at a rate of 20 °C per min and hold for 5 min. The temperature of inlet, mass transfer line and ion source were kept at 250, 300 and 230 °C, respectively. The flow rate of the carrier gas (helium) was set at 1.0 ml min^{-1}, and data were acquired at full-scan mode (50–650 *m/z*). Final quantification was performed with Xcalibur software.

The extracellular glucose, ethanol and organic acid concentrations were determined by high-performance liquid chromatography analysis. To that end, a 1 ml broth sample was filtered through a 0.2 μm syringe filter and analysed on an Aminex HPX-87G column (Bio-Rad) on an Ultimate 3000 HPLC (Dionex Softron GmbH). The column was eluted with 5 mM H_2SO_4 at a flow rate of 0.6 ml min^{-1} at 45 °C for 26 min.

Data availability. The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information file or available from the corresponding author upon reasonable request.

References

1. Peralta-Yahya, P. P., Zhang, F., del Cardayre, S. B. & Keasling, J. D. Microbial engineering for the production of advanced biofuels. *Nature* **488,** 320–328 (2012).
2. Ragauskas, A. J. *et al.* The path forward for biofuels and biomaterials. *Science* **311,** 484–489 (2006).
3. Biermann, U., Bornscheuer, U., Meier, M. A. R., Metzger, J. O. & Schafer, H. J. Oils and fats as renewable raw materials in chemistry. *Angew Chem. Int. Ed.* **50,** 3854–3871 (2011).
4. Lennen, R. M. & Pfleger, B. F. Microbial production of fatty acid-derived fuels and chemicals. *Curr. Opin. Biotechnol.* **24,** 1044–1053 (2013).
5. Steen, E. J. *et al.* Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. *Nature* **463,** 559–562 (2010).
6. Schirmer, A., Rude, M. A., Li, X., Popova, E. & del Cardayre, S. B. Microbial biosynthesis of alkanes. *Science* **329,** 559–562 (2010).
7. Akhtar, M. K., Turner, N. J. & Jones, P. R. Carboxylic acid reductase is a versatile enzyme for the conversion of fatty acids into fuels and chemical commodities. *Proc. Natl Acad. Sci. USA* **110,** 87–92 (2013).
8. Choi, Y. J. & Lee, S. Y. Microbial production of short-chain alkanes. *Nature* **502,** 571–574 (2013).
9. Kallio, P., Pasztor, A., Thiel, K., Akhtar, M. K. & Jones, P. R. An engineered pathway for the biosynthesis of renewable propane. *Nat. Commun.* **5,** 4731 (2014).
10. Dellomonaco, C., Clomburg, J. M., Miller, E. N. & Gonzalez, R. Engineered reversal of the beta-oxidation cycle for the synthesis of fuels and chemicals. *Nature* **476,** 355–359 (2011).
11. Liu, T., Vora, H. & Khosla, C. Quantitative analysis and engineering of fatty acid biosynthesis in *E. coli*. *Metab. Eng.* **12,** 378–386 (2010).
12. Mattanovich, D., Sauer, M. & Gasser, B. Yeast biotechnology: teaching the old dog new tricks. *Microb. Cell Fact.* **13,** 34 (2014).
13. Caspeta, L. *et al.* Altered sterol composition renders yeast thermotolerant. *Science* **346,** 75–78 (2014).
14. Runguphan, W. & Keasling, J. D. Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid-derived biofuels and chemicals. *Metab. Eng.* **21,** 103–113 (2014).
15. Buijs, N. A., Zhou, Y. J., Siewers, V. & Nielsen, J. Long-chain alkane production by the yeast *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **112,** 1275–1279 (2015).
16. Zhou, Y. J., Buijs, N. A., Siewers, V. & Nielsen, J. Fatty acid-derived biofuels and chemicals production in *Saccharomyces cerevisiae*. *Front. Bioeng. Biotechnol.* **2,** 32 (2014).
17. Liu, R. *et al.* Metabolic engineering of fatty acyl-ACP reductase-dependent pathway to improve fatty alcohol production in *Escherichia coli*. *Metab. Eng.* **22,** 10–21 (2014).
18. Schjerling, C. K. *et al.* Disruption of the gene encoding the acyl-CoA-binding protein (ACB1) perturbs acyl-CoA metabolism in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **271,** 22514–22521 (1996).
19. Leibundgut, M., Jenni, S., Frick, C. & Ban, N. Structural basis for substrate delivery by acyl carrier protein in the yeast fatty acid synthase. *Science* **316,** 288–290 (2007).
20. Faergeman, N. J. & Knudsen, J. Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. *Biochem. J.* **323,** 1–12 (1997).
21. Leber, C., Poison, B., Fernandez-Moya, R. & Da Silva, N. A. Overproduction and secretion of free fatty acids through disrupted neutral lipid recycle in *Saccharomyces cerevisiae*. *Metab. Eng.* **28,** 54–62 (2015).
22. Scharnewski, M., Pongdontri, P., Mora, G., Hoppert, M. & Fulda, M. Mutants of *Saccharomyces cerevisiae* deficient in acyl-CoA synthetases secrete fatty acids due to interrupted fatty acid recycling. *FEBS J.* **275,** 2765–2778 (2008).

23. Zhu, Z. *et al.* A multi-omic map of the lipid-producing yeast *Rhodosporidium toruloides*. *Nat. Commun.* **3,** 1112 (2012).
24. Tang, X., Feng, H. & Chen, W. N. Metabolic engineering for enhanced fatty acids synthesis in *Saccharomyces cerevisiae*. *Metab. Eng.* **16,** 95–102 (2013).
25. Oud, B. *et al.* An internal deletion in MTH1 enables growth on glucose of pyruvate-decarboxylase negative, non-fermentative *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **11,** 131 (2012).
26. Zhu, Z., Zhang, S., Lin, X., Liu, W. & Zhao, Z. K. Expression, purification and characterization of a novel fatty acid synthase from *Rhodosporidium toruloides*. *Sheng Wu Gong Cheng Xue Bao* **30,** 1414–1423 (2014).
27. Shi, S., Chen, Y., Siewers, V. & Nielsen, J. Improving production of malonyl coenzyme a-derived metabolites by abolishing Snf1-dependent regulation of Acc1. *mBio* **5,** e01130–14 (2014).
28. Sumper, M., Oesterhelt, D., Ripertinger, C. & Lynen, F. Die synthese verschiedener carbonsäuren durch den multienzymkomplex der fettsäuresynthese aus hefe und die erklärung ihrer bildung. *Eur. J. Biochem.* **10,** 377–387 (1969).
29. Xu, P. *et al.* Modular optimization of multi-gene pathways for fatty acids production in *E. coli*. *Nat. Commun.* **4,** 1409 (2013).
30. Tehlivets, O., Scheuringer, K. & Kohlwein, S. D. Fatty acid synthesis and elongation in yeast. *Biochim. Biophys. Acta* **1771,** 255–270 (2007).
31. Aprahamian, S. A., Arslanian, M. J. & Wakil, S. J. Comparative studies on the kinetic parameters and product analyses of chicken and rat liver and yeast fatty acid synthetase. *Comp. Biochem. Physiol. B* **71,** 577–582 (1982).
32. Rodriguez, G. M. & Atsumi, S. Toward aldehyde and alkane production by removing aldehyde reductase activity in *Escherichia coli*. *Metab. Eng.* **25,** 227–237 (2014).
33. Blazeck, J., Garg, R., Reed, B. & Alper, H. S. Controlling promoter strength and regulation in *Saccharomyces cerevisiae* using synthetic hybrid promoters. *Biotechnol. Bioeng.* **109,** 2884–2895 (2012).
34. Willis, R. M., Wahlen, B. D., Seefeldt, L. C. & Barney, B. M. Characterization of a fatty acyl-CoA reductase from *Marinobacter aquaeolei* VT8: a bacterial enzyme catalyzing the reduction of fatty acyl-CoA to fatty alcohol. *Biochemistry* **50,** 10550–10558 (2011).
35. Feng, X., Lian, J. & Zhao, H. Metabolic engineering of *Saccharomyces cerevisiae* to improve 1-hexadecanol production. *Metab. Eng.* **27,** 10–19 (2015).
36. Qiao, K. *et al.* Engineering lipid overproduction in the oleaginous yeast *Yarrowia lipolytica*. *Metab. Eng.* **29,** 56–65 (2015).
37. Zhang, S. *et al.* Engineering *Rhodosporidium toruloides* for increased lipid production. *Biotechnol. Bioeng.* **113,** 1056–1066 (2016).
38. Jin, G., Yang, F., Hu, C., Shen, H. & Zhao, Z. K. Enzyme-assisted extraction of lipids directly from the culture of the oleaginous yeast *Rhodosporidium toruloides*. *Bioresour. Technol.* **111,** 378–382 (2012).
39. Santillan-Jimenez, E. & Crocker, M. Catalytic deoxygenation of fatty acids and their derivatives to hydrocarbon fuels via decarboxylation/decarbonylation. *J. Chem. Technol. Biotechnol.* **87,** 1041–1050 (2012).
40. Li, X. *et al.* Overproduction of fatty acids in engineered *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **111,** 1841–1852 (2014).
41. Chen, L., Zhang, J., Lee, J. & Chen, W. N. Enhancement of free fatty acid production in *Saccharomyces cerevisiae* by control of fatty acyl-CoA metabolism. *Appl. Microbiol. Biotechnol.* **98,** 6739–6750 (2014).
42. Mazzoleni, S. *et al.* A novel process-based model of microbial growth: self-inhibition in *Saccharomyces cerevisiae* aerobic fed-batch cultures. *Microb. Cell. Fact.* **14,** 109 (2015).
43. Rutter, C. D., Zhang, S. & Rao, C. V. Engineering *Yarrowia lipolytica* for production of medium-chain fatty acids. *Appl. Microbiol. Biotechnol.* **99,** 7359–7368 (2015).
44. Andre, C., Kim, S. W., Yu, X. H. & Shanklin, J. Fusing catalase to an alkane-producing enzyme maintains enzymatic activity by converting the inhibitory byproduct H_2O_2 to the cosubstrate O_2. *Proc. Natl Acad. Sci. USA* **110,** 3191–3196 (2013).
45. Tang, X. & Chen, W. N. Enhanced production of fatty alcohols by engineering the TAGs synthesis pathway in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **112,** 386–392 (2015).
46. Youngquist, J. T. *et al.* Production of medium chain length fatty alcohols from glucose in *Escherichia coli*. *Metab. Eng.* **20,** 177–186 (2013).
47. Hansen, E. H. *et al.* *De novo* biosynthesis of vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*). *Appl. Environ. Microb.* **75,** 2765–2774 (2009).
48. Verduyn, C., Postma, E., Scheffers, W. A. & Van Dijken, J. P. Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcoholic fermentation. *Yeast* **8,** 501–517 (1992).
49. Akada, R. *et al.* PCR-mediated seamless gene deletion and marker recycling in *Saccharomyces cerevisiae*. *Yeast* **23,** 399–405 (2006).
50. Solis-Escalante, D. *et al.* amdSYM, a new dominant recyclable marker cassette for *Saccharomyces cerevisiae*. *FEMS Yeast Res.* **13,** 126–139 (2013).
51. Zhou, Y. J. *et al.* Modular pathway engineering of diterpenoid synthases and the mevalonic acid pathway for miltiradiene production. *J. Am. Chem. Soc.* **134,** 3234–3241 (2012).
52. Haushalter, R. W. *et al.* Production of anteiso-branched fatty acids in *Escherichia coli*; next generation biofuels with improved cold-flow properties. *Metab. Eng.* **26,** 111–118 (2014).
53. Khoomrung, S. *et al.* Rapid quantification of yeast lipid using microwave-assisted total lipid extraction and HPLC-CAD. *Anal. Chem.* **85,** 4912–4919 (2013).
54. Xu, P., Li, L., Zhang, F., Stephanopoulos, G. & Koffas, M. Improving fatty acids production by engineering dynamic pathway regulation and metabolic control. *Proc. Natl Acad. Sci. USA* **111,** 11299–11304 (2014).
55. Liu, A., Tan, X., Yao, L. & Lu, X. Fatty alcohol production in engineered *E. coli* expressing *Marinobacter* fatty acyl-CoA reductases. *Appl. Microbiol. Biotechnol.* **97,** 7061–7071 (2013).

Acknowledgements

This work was funded by Knut and Alice Wallenberg Foundation, Novo Nordisk Foundation, Vetenskapsrådet and FORMAS. This work is part of a collaborative project between Chalmers and Total. We thank Prof. Zongbao K. Zhao (Dalian institute of Chemical Physics, CAS, China) for kindly sharing the RtFAS encoding cDNA (*RtFAS1* and *RtFAS2*), Michael Gossing for help with constructing the *ACC1*** expression cassette, and Sakda Khoomrung and Julia Karlsson for their help with GC analysis. We also appreciate the helpful discussion with Mingtao Huang, Boyang Ji, Yun Chen, Paulo Teixeira, Paul Hudson (KTH, Stockholm, Sweden), Rahul Kumar and Guodong Liu.

Author contributions

Y.J.Z. and J.N. conceived the study; Y.J.Z. designed and performed all the experiments and analysed the data; N.A.B. assisted with experimental design, data analysis and bioreactor studies; J.Q. assisted with bioreactor studies; Z.Z. assisted with constructing the single gene deletion strains of ADHs/ALRs and verifying the RtFAS function; Y.J.Z., N.A.B., Z.Z., V.S. and J.N. wrote the manuscript.

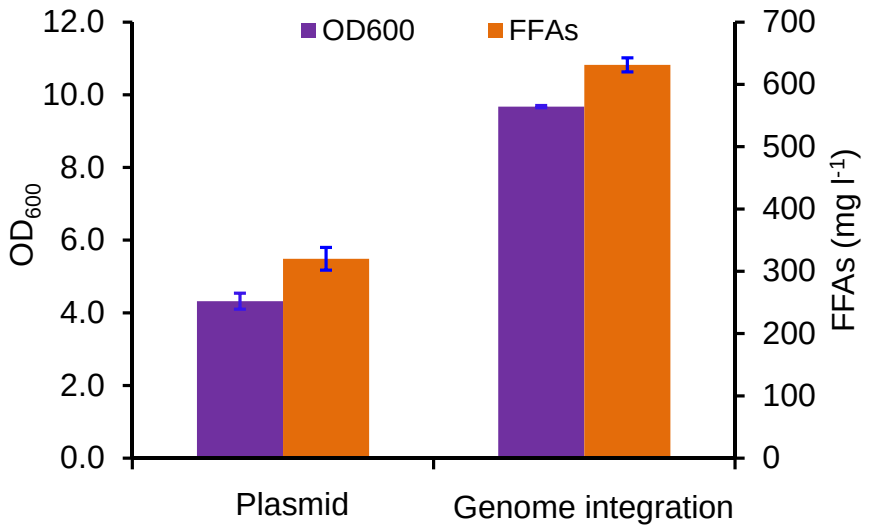
Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

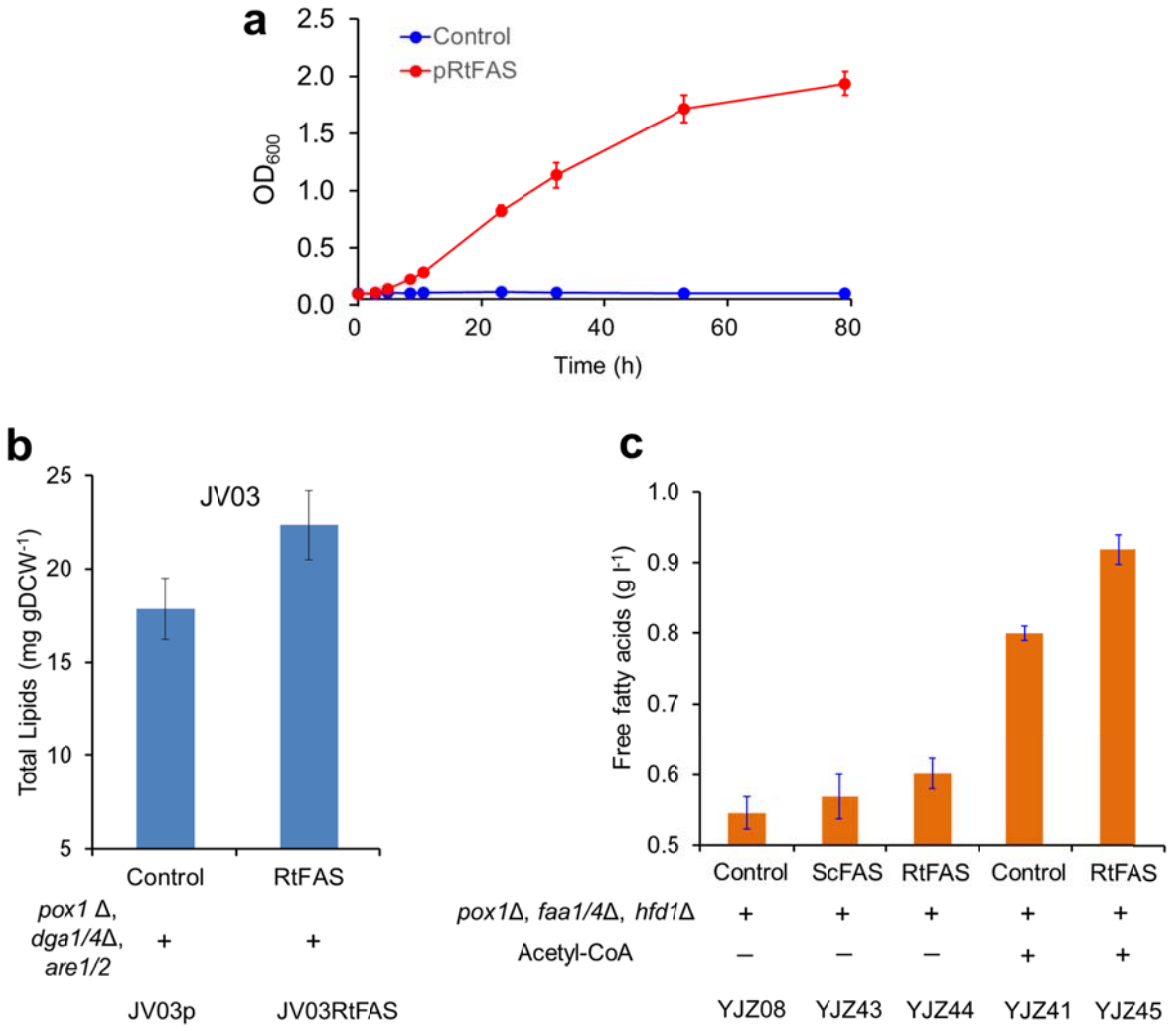
Competing financial interests: Y.J.Z., N.A.B., V.S. and J.N. have filed a patent (Engineering of hydrocarbon metabolism in yeast, No. WO2015057155 A1) for protection of part of the work described herein. All other authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

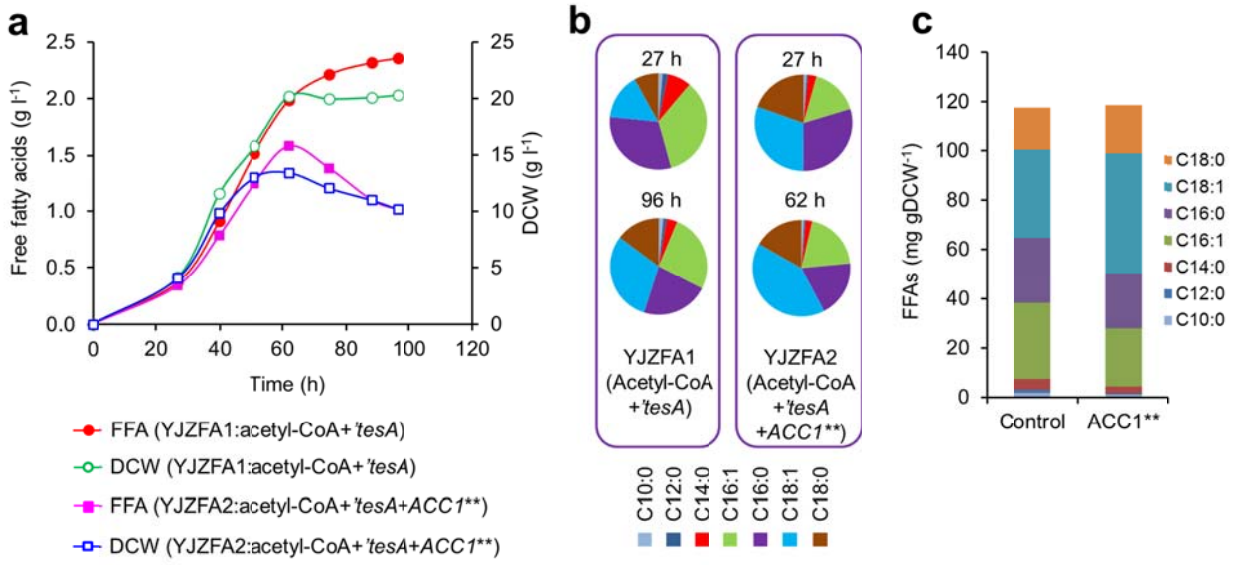
How to cite this article: Zhou, Y. J. *et al.* Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories. *Nat. Commun.* 7:11709 doi: 10.1038/ncomms11709 (2016).



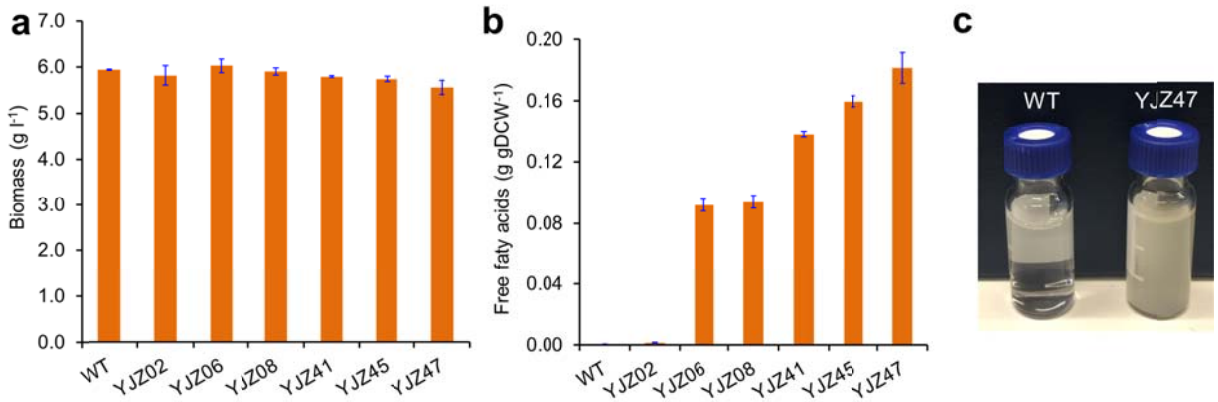
Supplementary Fig. 1. Genome integrated acetyl-CoA pathway had higher biomass and FFA titer compare to plasmid pathway. The plasmid pCoA4 transformed to YJZ08 and the same pathway was integrated to the YJZ08 genome. All data represent the mean ± s.d. of biological triplicates.



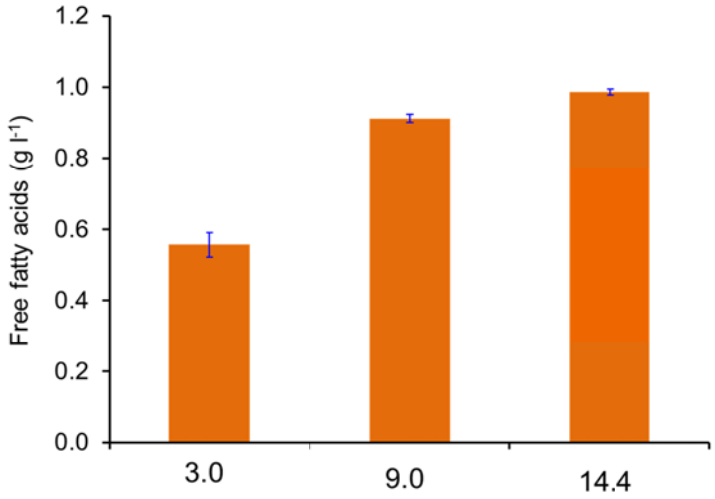
Supplementary Fig. 2. Expression of *R. toruloides* fatty acid synthase (RtFAS) increased the total lipid and FFA production. (A) RtFAS complemented the endogenous *FAS* deletion in *S. cerevisiae* PWY12 (*Δfas1::LEU2*, *Δfas2::HIS3*). (B) Expression of RtFAS increased the total lipid content in a JV03 background. (C) Expression of RtFAS was more efficient than overexpression of endogenous ScFAS in terms of increasing FFA production. All data represent the mean±s.d. of biological triplicates.



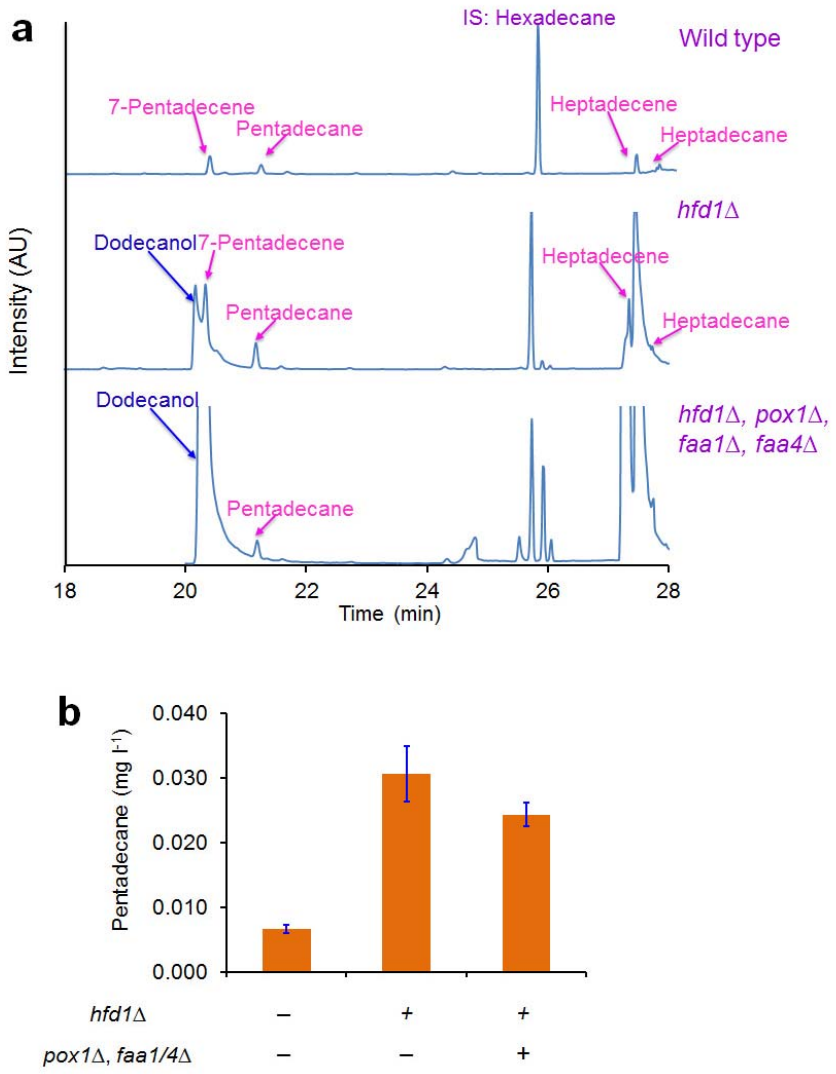
Supplementary Fig. 3. Effect of acetyl-CoA carboxylase mutant (Acc1^{S1157A,S659A}, Acc1**) on FFA production. (**a**) Fed batch fermentation of strains YJZFA1 and YJZFA2. Time courses of FFA titers (filled symbols) and cell mass (open symbols) are shown. YJZFA1 is a prototrophic strain harboring the ACL based pathway in plasmid pFab1 and YJZFA2 additionally expressesed *ACC1***. Here, 300 g l^{-1} glucose was fed and 2 M KOH was used for pH control other than described in Methods. (**b**) FFA profiles of the strain YJZFA1 and YJZFA2 at the time point where feeding was initiated and at the highest titer. (C) The maximal biomass specific free fatty acid titer.



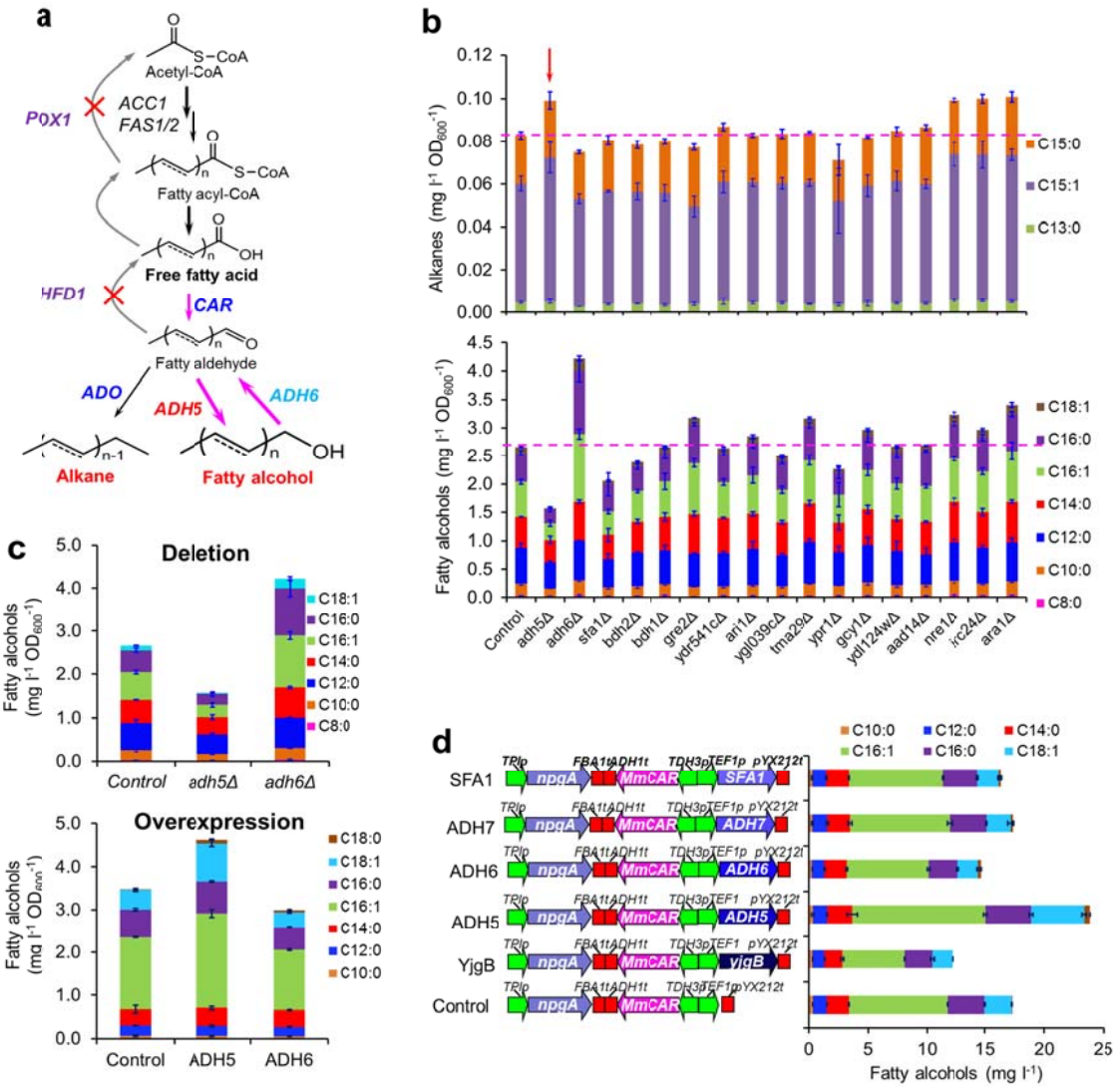
Supplementary Fig. 4 Comparison the biomass titers and specific FFA titers among recombinant *S. cerevisiae* variants. (**a**) The final biomass titers. (**b**) The specific FFA titers. (**c**) Comparison of cell culture between wild-type strain and final strain YJZ47. The cell cultures were centrifuged and the cell pellets were removed. The engineered strains were cultivated in shake flasks containing 15 mL optimized minimal media for 72 h at 200 rpm, 30 °C. All data represent the mean ± s.d. of biological triplicates.



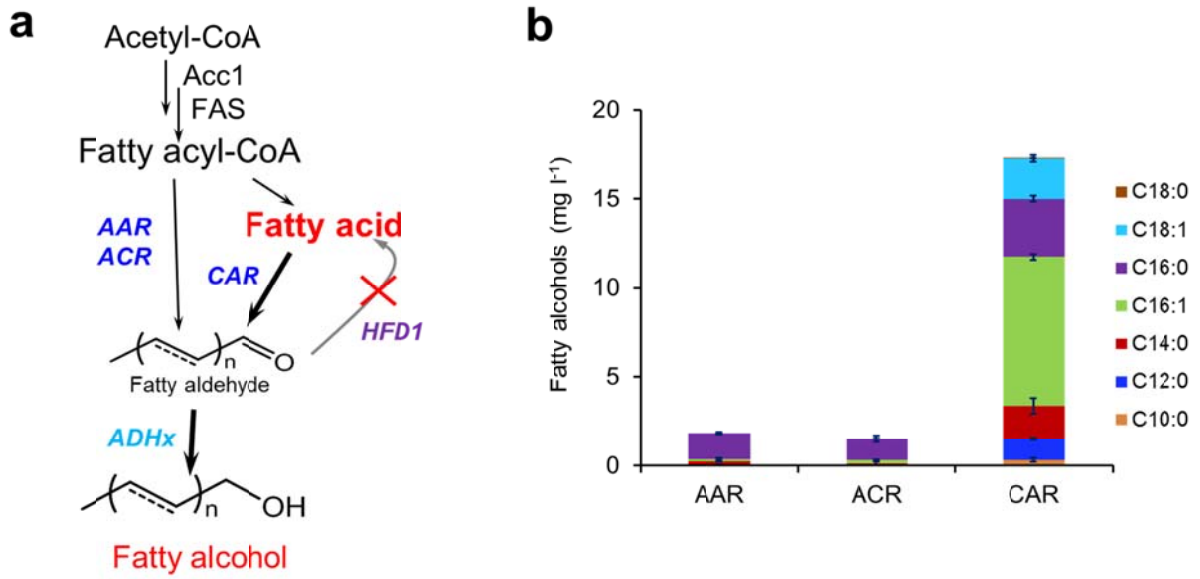
Supplementary Fig. 5 the effect of KH_2PO_4 level on FFA production. The other media components were 5 g l^{-1} $(NH_4)_2SO_4$, 0.5 g l^{-1} $MgSO_4 \bullet 7H_2O$, 30 g l^{-1} glucose, trace metal and vitamin solutions[1] supplemented with 40 mg l^{-1} histidine and/or 60 mg l^{-1} uracil. The engineered strain YJZ47were cultivated in shake flasks containing 15 mL media for 72 h at 200 rpm, 30 °C. All data represent the mean±s.d. of biological triplicates.



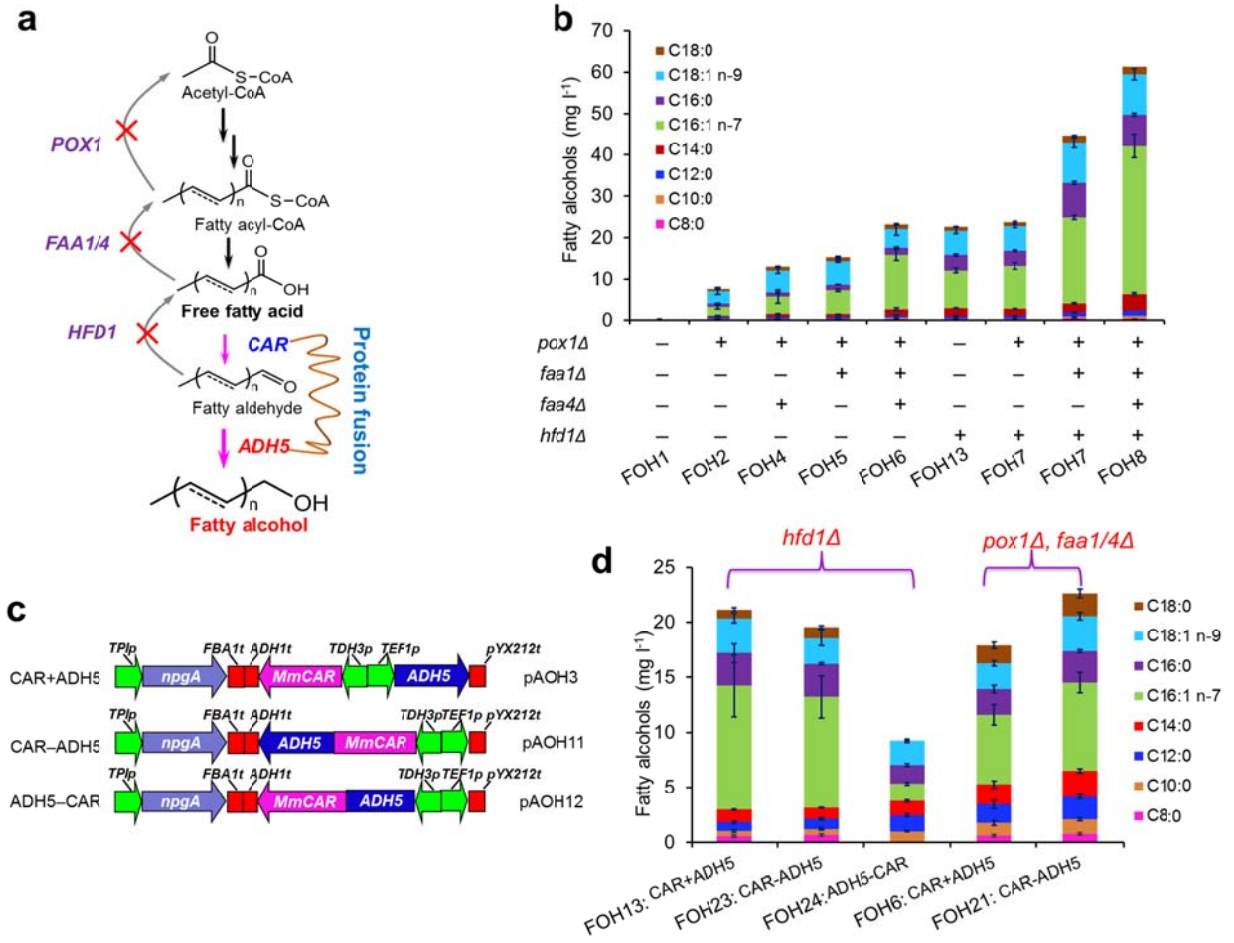
Supplementary Fig. 6. The ADO catalyzed step is rate limiting for alkane production, precursors are in sufficient supply. (a) GC chromatograms of extracted metabolites from different background strains harboring pAlkane16. *HFD1* deletion (middle chromatogram) increased the production of alkanes and fatty alcohols compared with the wild-type background (top chromatogram), which might be attributed to the blockage of fatty aldehyde dehydrogenation to fatty acids. Further increasing fatty acid supply (*hfd1*Δ *pox1*Δ *faa1*Δ *faa4*Δ) increased the amount of fatty alcohols, whose peaks covered the alkane peaks (bottom chromatogram). (b) Pentadecane production in the corresponding strains. The data represent the mean±s.d. of biological triplicates. As the peaks of 7-pentadecene and 8-heptadecene were covered by the fatty alcohols in the fatty acid overproducing strain, it became impossible to reliably quantify the alkanes. We thus compared the pentadecane titers, which indicated that a higher fatty acid supply did not increase the alkane but fatty alcohol production instead. These results indicated that the ADO is a limiting step and fatty acids might inhibit ADO activity, as the ADO has been shown to have fatty acid binding activity[2].



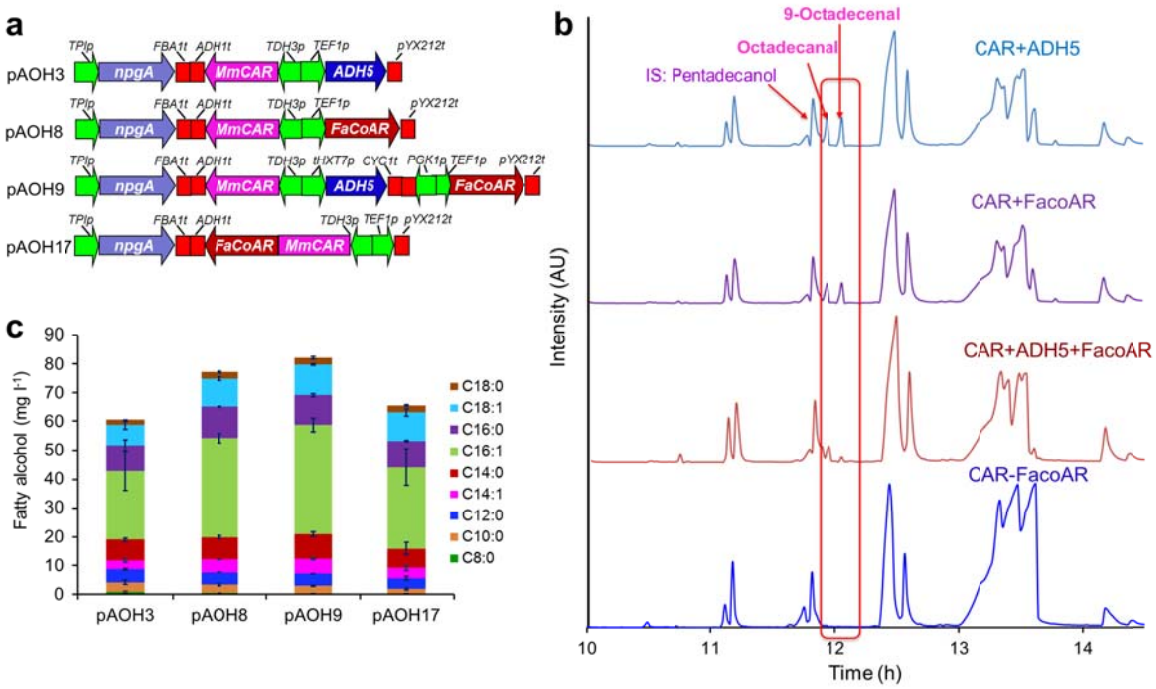
Supplementary Fig. 7 Engineering expression of alcohol dehydrogenase/aldehyde reductase genes for production of alkanes or fatty alcohols, (**a**) Schematic illustration of the engineered metabolic pathways of fatty alcohol biosynthesis. (**b**) The effect of single gene deletions on production of alkanes (top) and fatty alcohols (bottom). The targeted genes, encoding an alcohol dehydrogenase or aldehyde reductase, were selected based on the catalytic efficiency toward aldehyde reduction (Supplementary Table 1). And they were deleted in the YJZ03 background, and plasmid pAlkane16 was introduced for alkane production. Here, *ADH5* deletion showed increased alkane production and decreased fatty alcohol accumulation, and was considered as the first target for improving alkane production. (**c**) Opposite roles of alcohol dehydrogenases Adh5 and Adh6 in fatty alcohol biosynthesis. The effect of *ADH5* or *ADH6* deletion on fatty alcohol accumulation in strain YJZ03 (*hfd1*Δ and *pox1*Δ) harboring the plasmid pAlkane16 (top panel). The effect of *ADH5* or *ADH6* overexpression on fatty alcohol production in strain YJZ01 (*hfd1*Δ) harboring PAOH0 (bottom panel). (**d**) The amount of fatty alcohol produced with overexpression of genes encoding different alcohol dehydrogenase or aldehyde reductase in YJZ01. The data represent the mean ± s.d. of three independent clones.



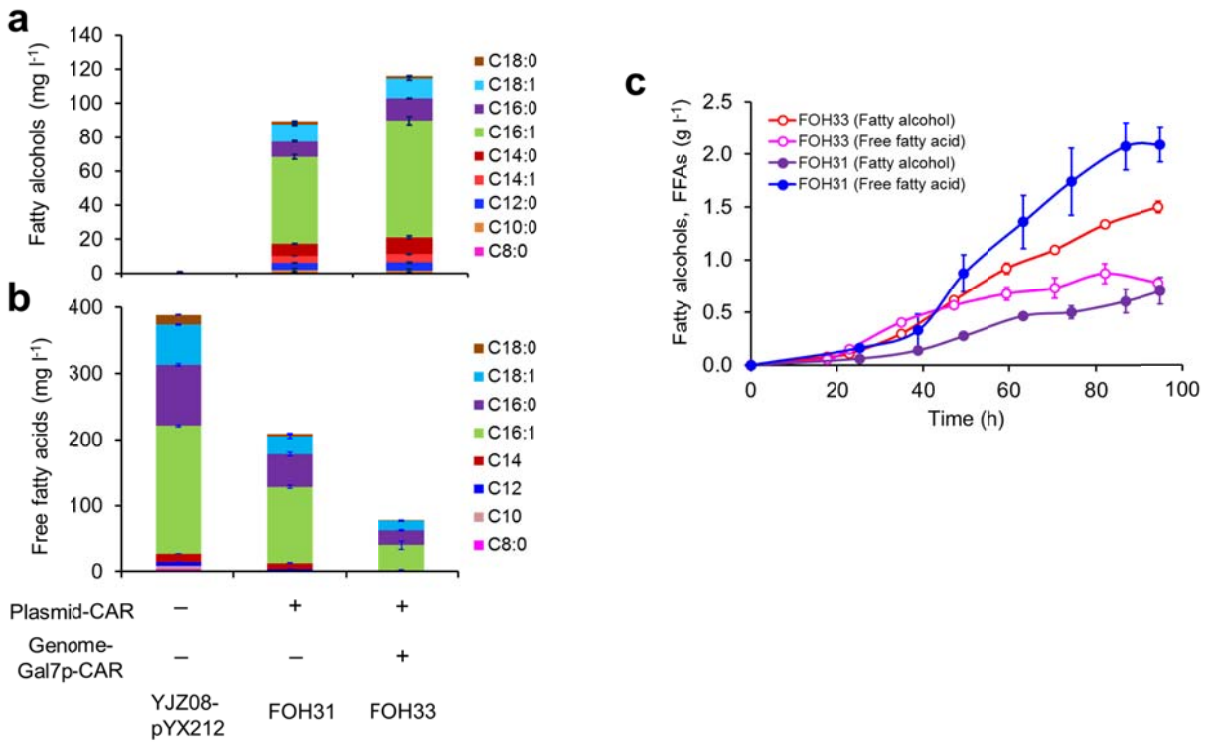
Supplementary Fig. 8 Comparison of fatty alcohol production with the expression of fatty acyl-CoA reductase ACR from *Acinetobacter baylyi*, fatty acyl-ACP/CoA reductase AAR from *Synechococcus elongatus* or carboxylic acid reductase CAR from *Mycobacterium marinum* (with its cofactor encoding gene *npgA* from *Aspergillus nidulans*). (**a**) Schematic illustration of the engineered metabolic pathways for fatty alcohol production. (**b**) CAR overexpression resulted in a much higher fatty alcohol level compared with overexpression of AAR and ACR. All data are presented as the mean±s.d. of biological triplicates.



Supplementary Fig. 9 Engineered fatty alcohol production by blocking the reverse reactions and enzyme fusion. (**a**) Schematic representation of the engineered fatty alcohol biosynthetic pathways. (**b**) Fatty alcohol production from the engineered strains with deletion of *POX1*, *FAA1*, *FAA4* and *HFD1*. (**c**) Schematic representation of gene fusion constructs for fatty alcohol biosynthesis, CAR+ADH5 represents non-fusion expression of *CAR* and *ADH5* (plasmid pAOH3); CAR-ADH5 represents the fusion expression of *CAR-ADH5* with the CAR at N-terminus (plasmid pAOH11), and ADH5-CAR represents the fusion expression of *ADH5-CAR* with the Adh5 at the N-terminus (plasmid pAOH12). The fusion enzymes encoding genes were constructed by inserting a widely used GGGS linker encoding sequence "GGT GGT GGT TCT" between the two corresponding genes. (**d**) The amount of fatty alcohol produced by the fatty acid overproducing strain (YJZ06, *Δpox1; Δfaa1; Δfaa4*) and *HFD1* deletion strain (YJZ01) harboring different plasmids represented in c. The strain variants were cultivated in shake flasks for 72 h, at 30°C, 200 rpm. The data represent the mean ± s. d. of three independent clones.

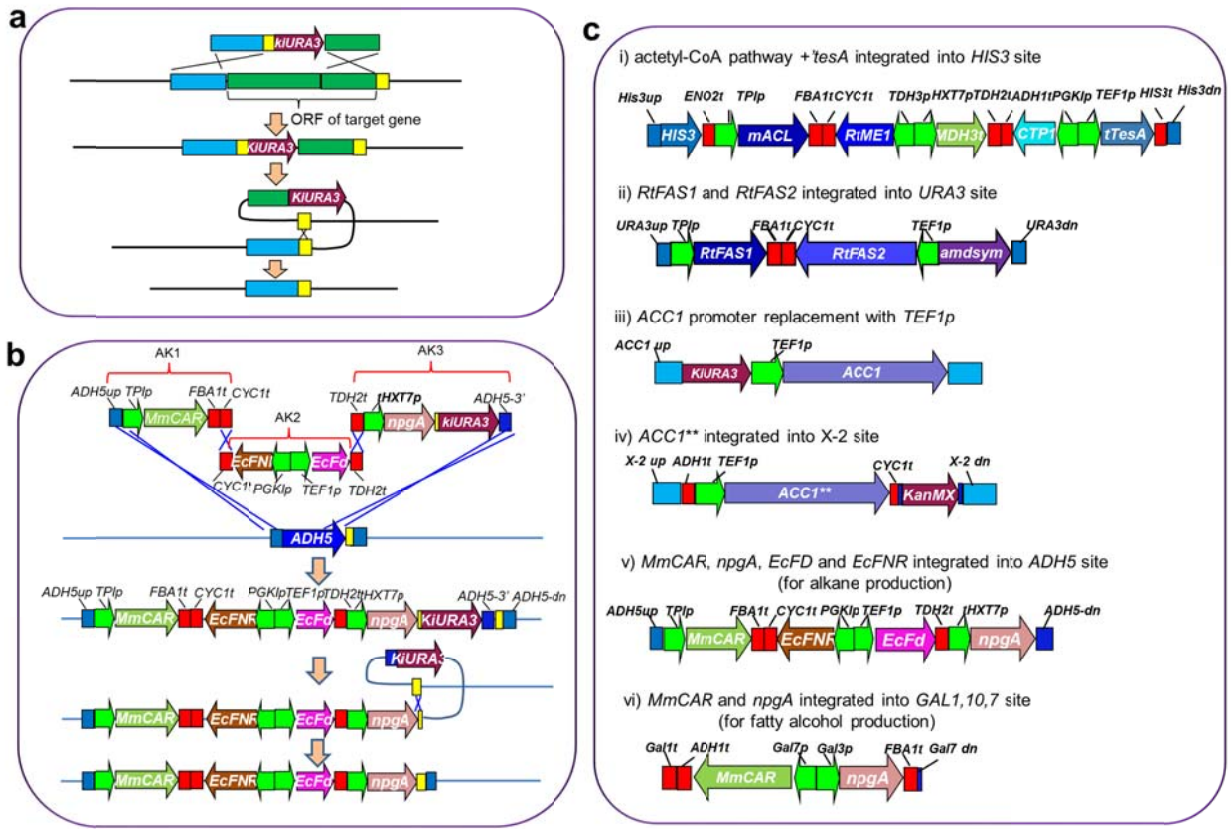


Supplementary Fig. 10 Overexpression of *FaCoAR* and *ADH5* increased long chain fatty alcohol production and decreased C18 fatty aldehyde accumulation. (**a**) Schematic representation of gene arrangement of the metabolic pathway for fatty alcohol biosynthesis. All these pathways were assembled on a pYX212 vector and then transformed into YJZ08 (*hfd1Δ pox1Δ faa1Δ faa4Δ*). (**b**) GC chromatograms of the extracts from the strains containing the corresponding pathways as shown in a. *FacoAR* expression decreased the accumulation of C18 fatty aldehyde octadecanal and 9-octadecenal. The fusion of CAR and FaCoAR avoided accumulation of octadecanal and 9-octadecenal completely, though the total fatty alcohol titer decreased by 15.2% as shown in c. (**c**) Titer of fatty alcohols from the strains harboring the corresponding plasmids. The data represent the mean ± s.d. of three independent clones.



111

112 Supplementary Fig. 11. Enhancing fatty acid reduction for fatty alcohol production. (**a**) Fatty alcohol titer
113 in shake flasks. (**b**) FFA titer from the strains harboring the corresponding pathways in shake flasks. The
114 strain variants were cultivated in minimal media for 72 h, at 30°C, 200 rpm. The data represent the mean
115 ± s.d. of three independent clones. (**c**) The accumulation of fatty alcohols and FFAs from FOH11 and
116 FOH33 in fed-batch fermentation.



Supplementary Fig. 12 Schematic illustration of genome engineering strategy. (**a**) The seamless gene deletion strategy. (**b**) Modular pathway integration strategy. (**c**) The genetic arrangement of genome-integrated pathways.

122 **Supplementary Table 1**. *S. cerevisiae* (putative) alcohol dehydrogenases and aldehyde reductases[a]

Enzyme	Descriptions	localization
Medium to long-chain alcohol dehydrogenase (alcohol formation)		
ADH3 (YMR083W)	Involved in shuttling of mitochondrial NADH to the cytosol under anaerobic conditions and ethanol production[4].	Mitochondria
ADH4 (YGL256W)	Adh4 is seemingly not expressed in laboratory *S. cerevisiae* strains.	Cytoplasm
ADH5 (YBR145W)	Overexpression of *ADH5* increased isobutanol production in *S. cerevisiae*[5].	Cytoplasm
ADH6 (YMR318C)	NADPH-dependent aldehyde reductase. k_{cat}/K_m is 1-3 × 10^5 mM^{-1} min^{-1} (Ref. 6); *ADH6* deletion decreased the reduction of vanillin to vanillyl alcohol[7].	Cytoplasm
ADH7 (YCR105W)	Involved in fusel alcohol synthesis or in aldehyde tolerance[8]. Absent in CEN.PK strains.	Cytoplasm
SFA1 (YDL168W)	Reduction of long chain and complex aldehydes to corresponding alcohols[9].	Cytoplasm/ Mitochondria
XYL2 (YLR070C)	Xylitol dehydrogenase that converts xylitol to D-xylulose[10].	Unknown
BDH1 (YAL061w)	NAD-dependent butanediol dehydrogenase, catalyzes reduction of acetoin to 2,3-butanediol[11,12].	Cytoplasm
BDH2 (Yal060w)	Homolog of *BDH1*	Cytoplasm
GRE2 (YOL151W)	Reduction activity toward complex aldehydes and ketones[13].	Cytoplasm
ARI1 (YGL157w)	Reduction activity toward furan aldehydes[14] and high activity (k_{cat}/K_m=550 min^{-1} mM^{-1}) toward phenylacetaldehyde[15].	Cytoplasm/ Nucleus
YGL039w	Reduction activity toward phenylacetaldehyde[15], short chain fatty aldehydes with a NADH preference[16].	Cytoplasm
YDR541c	Reduction activity toward fatty aldehydes (up to C8) and benzyl aldehydes with a NADPH preference[16].	Unknown
YBR159w	3-ketoreductase of the microsomal fatty acid elongase[17].	Endoplasmic reticulum
TMA29 (YMR226c)	Reduction activity toward aromatic α-ketoesters and aliphatic ketones[18].	Cytoplasm/ Nucleus
NRE1 (YIR035c)	Short chain alcohol dehydrogenase[19].	Cytosol
IRC24 (YIR036c)	Reduction activity toward benzyl (k_{cat}/K_m=44 min^{-1} mM^{-1}) and 1-phenyl-1,2-	Cytoplasm

	propanedione (k_{cat}/K_m=3 000 min^{-1} mM^{-1}) [20].	
AYR1 (YIL124w)	1-Acyldihydroxyacetone-phosphate reductase[21]	Lipid particles
YKL107w	Putative short-chain dehydrogenase/reductase, proposed to be a palmitoylated membrane protein	Unknown
AAD14 (YNL331c)	Aryl alcohol dehydrogenase[22], combination of Aad3, 4, 10, 14-16 may be involved in biosynthesis of long-chain and complex alcohols[9]	Unknown
AAD3 (YCR107w)	Homolog of *AAD14*	Unknown
AAD4 (YDL243c)	Homolog of *AAD14*	Unknown
AAD10 (YJR155w)	Homolog of *AAD14*	Unknown
AAD16 (YFL057c)	Homolog of *AAD14*	Unknown
AAD15 (YOL165c)	Homolog of *AAD14*	Unknown
Aldose reductase family		
YPR1 (YDR368w)	Reduction activity toward diacetyl and ethyl acetoacetate[19]	Cytoplasm/ Nucleus
GCY1 (YOR120w)	High activity toward dl-glyceraldehyde (k_{cat}/K_m =556 min^{-1} mM^{-1}) and nitrobenzaldehyde (k_{cat}/K_m =546 min^{-1} mM^{-1}) [23,24]	Cytoplasm/ Nucleus
YDL124w	Reduction activity toward dl-Glyceraldehyde (k_{cat}/K_m =17 min^{-1} mM^{-1}) and nitrobenzaldehyde k_{cat}/K_m =110 min^{-1} mM^{-1}) but lower than Gcy1[23]	Cytoplasm/ Nucleus
YJR096w	Much lower reduction activity than Gcy1 and YDL124Wp[23]	Cytoplasm/ Nucleus
ARA1 (YBR149w)	Showed reduction activity toward diacetyl[25] and acetoin[12]	Cytoplasm
GRE3 (YHR104w)	Involved in reduction of aldoses such as d-xylose[10,26].	Cytoplasm/ Nucleus
D-Hydroxyacid dehydrogenase family		
GOR1 (YNL274c)	Glyoxylate reductase, showed activity toward glyoxylate and hydroxypyruvate[27]	Cytoplasm/ Mitochondria
YPL113c	Glyoxylate reductase, showed activity toward glyoxylate and hydroxypyruvate, but much lower compared with Gcor1[27]	Unknown
YGL185c	Glyoxylate reductase, showed activity toward glyoxylate and hydroxypyruvate, but much lower compared with Gcor1[27]	Cytoplasm

FDH1 (YOR388c)	NAD^+-dependent formate dehydrogenase[28]	Cytoplasm
FDH2 (YPL275w)	NAD^+-dependent formate dehydrogenase[28]	Cytoplasm

123

124 a, Genes selected for deletion are indicated in bold

125 **Supplementary Table 2**. Plasmids used in this study[a]

Plasmids	Genotype or characteristic	Resource
pYX212	2 μm, AmpR, *URA3*, TPIp, pYX212t	R&D systems
pCoA1	pYX212-(*TPIp*-***RtACL***-*FBA1t*)+(*TDH3p*-***RtME***-*CYC1t*)	This study
pCoA2	pYX212-(*TPIp*-***RtACL***-*FBA1t*)+(*TDH3p*-***LsME***-*CYC1t*)	This study
pCoA3	pYX212-(*TPIp*-***RtACL***-*FBA1t*)+(*TDH3p*-***RtME***-*CYC1t*)+(tHXT7p-***'MDH3***-*pYX212t*)	This study
pCoA4	pYX212-(*TPIp*-***RtACL***-*FBA1t*)+(*TDH3p*-***RtME***-*CYC1t*)+(tHXT7p-***'MDH3***-*TDH2t)*+(*PGK1p*-***CTP1***-*ADH1t*)	This study
pFab1	pYX212-(*TPIp*-***RtACL***-*FBA1t*)+(*TDH3p*-***RtME***-*CYC1t*)+(tHXT7p-***'MDH3***-*TDH2t)*+(*PGK1p*-***CTP1***-*ADH1t*)+(*TEF1p*-***'tesA***-*pYX212t*)	This study
pFab3	pYX212-(*TPIp*-***RtACL***-*FBA1t*)+(*TDH3p*-***RtME***-*CYC1t*)+(tHXT7p-***'MDH3***-*TDH2t)*+(*PGK1p*-***CTP1***-*ADH1t*)+(*TEF1p*-***fadM***-*pYX212t*)	This study
pRtFAS	pYX212-(*TPIp*-***RtFAS1***-*FBA1t*)+ (*TEF1p*-***RtFAS2***-*CYC1t*)	This study
pAOH0	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)	This study
pAOH1	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*TEF1p*-***yjgB***-*pYX212t*)	This study
pAOH3	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*TEF1p*-***ADH5***-*pYX212t*)	This study
pAOH4	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*TEF1p*-***ADH6***-*pYX212t*)	This study
pAOH5	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*TEF1p*-***ADH7***-*pYX212t*)	This study
pAOH6	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*TEF1p*-***SFA1***-*pYX212t*)	This study
pAOH8	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*TEF1p*-***FacoAR***-*pYX212t*)	This study
pAOH9	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR***-*ADH1t*)+(*tHXT7p*-***ADH5***-*CYC1t*)+(*TEF1p*-***FacoAR***-*pYX212t*)	This study
pAOH11	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR-ADH5***-*ADH1t*)	This study
pAOH12	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***ADH5-MmCAR***-*ADH1t*)	This study
pAOH17	pYX212-(*TPIp*-***npgA***-*FBA1t*)+(*TDH3p*-***MmCAR-FacoAR***-*ADH1t*)	This study

pAlkane7	pYX212-(*TPIp*-***SeAAR***-*FBA1t*)+(PGK1p-***EcFNR***-*CYC1t*)+(*TEF1p*-***EcFD***-*TDH2t*)+(*TDH3p*-***SeADO***-*ADH1t*)	29
pAlkane16	pYX212-(*TPIp*-***MmCAR***-*FBA1t*)+(PGK1p-***EcFNR***-*CYC1t*)+(*TEF1p*-***EcFD***-*TDH2t*)+(*TDH3p*-***SeADO***-*ADH1t*)+(tHXT7p-***npgA***-*pYX212t*)	This study
pAlkane65	pYX212-(*UAS*-*TDH3p*-***SeADO***-*pYX212t*)	This study
pAlkane67	pYX212-(*UAS*-*TDH3p*-***SeADO***-*pYX212t)*	This study
pAlkane68	pYX212-(*GAL7p*-***NpADO***-*CYC1t*)+(*UAS*-*TDH3p*-**SeADO**-*pYX212t*)	This study

a, Expressed genes are indicated in bold

b, per1 means the peroxisome targeting peptide 1 encoding sequence: GGTGGTGGTTCTTCTAAACTA and per2 means peroxisome targeting peptide 1 encoding sequence: GGTGGTGGTTCTGCCGCTGTAAAACTATCGCAGGCAAAATCTAAACTA

Supplementary Table 3 *S. cerevisiae* strains used in this study.

Strain	Genotype or characteristic	Resource
Background strains/fatty acid producing strains		
CEN.PK 113-11C	*MATa MAL2-8c SUC2 his3Δ1 ura3-52*	Kötter, University of Frankfurt, Germany
EY1673	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 PEX3-mRFP+kanMX6*	[3]
YJZ01	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ*	[29]
YJZ02	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ*	This study
YJZ03	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ*	This study
YJZ04	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa4Δ*	This study
YJZ05	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa1Δ*	This study
YJZ06	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa1Δ faa4Δ*	This study
YJZ07	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ*	This study
YJZ08	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ*	This study
JV03	*MATa MAL2-8c SUC2 ura3-52 HIS3 are1Δ dga1Δ are2Δ lro1Δ pox1Δ*	[30]
JV03RtFAS	*MATaMAL2-8c SUC2 ura3-52 HIS3 are1Δ dga1Δ are2Δ lro1Δ pox1Δ* pRtFAS	This study
RWB837	*MATa pdc1Δ(-6,-2)::loxP pdc5Δ(-6,-2)::loxP pdc6Δ(-6,-2)::loxP ura3-52*	[31]
IMI076	*MATa pdc1Δ(-6,-2)::loxP pdc5Δ(-6,-2)::loxP pdc6Δ(-6,-2)::loxP ura3-52 MTH1-ΔT*	[31]
FA0	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ pYX212*	This study
FA1	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ pCoA1*	This study
FA2	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ pCoA2*	This study
FA3	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ pCoA3*	This study
FA4	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ pCoA4*	This study
YJZFA1	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ* pFab1, p413::HIS3	This study
YJZFA2	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ X2::*(*TEF1p-**ACC1****-CYC1t*) pFab1 p413::HIS3	This study
YJZ13	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ his3Δ::HIS3*+(*TEF1p-**'tesA**-HIS3t*)	This study
YJZ39	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ his3Δ::HIS3*+(*TPIp-**RtACL**-FBA1t*)+(*TDH3p-**RtME**-CYC1t*)+(tHXT7p-***'MDH3**-TDH2t)*+(*PGK1p-**CTP1**-ADH1t*)+(*TEF1p-**'tesA**-HIS3t*)	This study

YJZ41	*MATa; MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faaΔ1 faa4Δ his3Δ::HIS3*+(*TPIp-**MmACL**-FBA1t*)+(*TDH3p-**RtME**-CYC1t*)+(tHXT7p-***'MDH3**-TDH2t)*+(*PGK1p-**CTP1**-ADH1t*)+(*TEF1p-**'tesA**-HIS3t*)	This study
YJZ42	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ his3Δ::HIS3*+(*TPIp-**HsACL**-FBA1t*)+(*TDH3p-**RtME**-CYC1t*)+(tHXT7p-***'MDH3**-TDH2t)*+(*PGK1p-**CTP1**-ADH1t*)+(*TEF1p-**'tesA**-HIS3t*)	This study
YJZ43	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ ura3Δ::*(*TPIp-**ScFAS1**-FBA1t*)+ (*TEF1p-**ScFAS2**-CYC1t*)*+amdSym*	This study
YJZ44	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ ura3Δ::*(*TPIp-**RtFAS1**-FBA1t*)+ (*TEF1p-**RtFAS2**-CYC1t*)*+amdSym*	This study
YJZ45	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ his3Δ::HIS3*+(*TPIp-**MmACL**-FBA1t*)+(*TDH3p-**RtME**-CYC1t*)+(tHXT7p-***'MDH3**-TDH2t)*+(*PGK1p-**CTP1**-ADH1t*)+(*TEF1p-**'tesA**-HIS3t*) *ura3Δ::*(*TPIp-**RtFAS1**-FBA1t*)+ (*TEF1p-**RtFAS2**-CYC1t*)*+amdSym*	This study
YJZ45U	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ his3Δ::HIS3*+(*TPIp-**MmACL**-FBA1t*)+(*TDH3p-**RtME**-CYC1t*)+(tHXT7p-***'MDH3**-TDH2t)*+(*PGK1p-**CTP1**-ADH1t*)+(*TEF1p-**'tesA**-HIS3t*) *ura3Δ::*(*TPIp-**RtFAS1**-FBA1t*)+ (*TEF1p-**RtFAS2**-CYC1t*)*+amdSym p416::URA3*	This study
YJZ47	*MATa MAL2-8c SUC2 hfd1Δ pox1Δ faa1Δ faa4Δ his3Δ::HIS3*+(*TPIp-**MmACL**-FBA1t*)+(*TDH3p-**RtME**-CYC1t*)+(tHXT7p-***'MDH3**-TDH2t)*+(*PGK1p-**CTP1**-ADH1t*)+(*TEF1p-**'tesA**-HIS3t*) *ura3Δ::*(*TPIp-**RtFAS1**-FBA1t*)+ (*TEF1p-**RtFAS2**-CYC1t*)*+amdSym acc1::KlURA3+TEF1p+**ACC1***	This study
Fatty alcohol producing strains		
FOH1	CEN.PK 113-11C, pAOH3	This study
FOH2	*MATa MAL2-8cSUC2 his3Δ1 ura3-52 pox1Δ* pAOH3	This study
FOH3	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ* pAOH3	This study
FOH4	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa4Δ* pAOH3	This study
FOH5	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa1Δ* pAOH3	This study
FOH6	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa1Δ faa4Δ* pAOH3	This study
FOH7	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ* pAOH3	This study
FOH8	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ* pAOH3	This study
FOH10	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH0	This study
FOH11	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH1	This study
FOH13	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH3	This study

FOH14	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH4	This study
FOH15	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH5	This study
FOH16	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH6	This study
FOH21	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 pox1Δ faa1Δ faa4Δ* pAOH11	This study
FOH23	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH11	This study
FOH24	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAOH12	This study
FOH28	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ* pAOH8	This study
FOH29	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ* pAOH9	This study
FOH30	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ* pAOH17	This study
FOH31	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ adh6Δ::kanMX*, pAOH9	This study
FOH33	*MATa MAL2-8c SUC2 ura3-52 hfd1Δ pox1Δ faa1Δ faa4Δ*, *adh6Δ::kanMX*, *gal80Δ*, *gal1/10/7Δ::*(*GAL7p-**MmCAR**-ADH1t*)+(GAL3p-***npgA***-*FBA1t*) pAOH9	This study
Alkane producing strains		
ZW31	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ adh5Δ*	This study
YJZ60	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ Gal80Δ:: SeFNR+SeFd adh5Δ::* (*TPIp-**MmCAR**-FBA1t*)+(PGK1p-***EcFNR***-*CYC1t*)+(*TEF1p-**EcFD**-TDH2t*)+(tHXT7p-***npgA***-*ADH5t*)	This study
A0	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAlkane7 (previously named as KB19)	[29]
A1	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ* pAlkane06	This study
A2	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ* pAlkane06	This study
A3	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ adh5Δ* pAlkane06	This study
A5	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ Gal80Δ:: SeFNR+SeFd adh5Δ::* (*TPIp-**MmCAR**-FBA1t*)+(PGK1p-***EcFNR***-*CYC1t*)+(*TEF1p-**EcFD**-TDH2t*)+(tHXT7p-***npgA***-*ADH5t*) pAlkane67	This study
A6	*MATa MAL2-8c SUC2 his3Δ1 ura3-52 hfd1Δ pox1Δ Gal80Δ:: SeFNR+SeFd adh5Δ::* (*TPIp-**MmCAR**-FBA1t*)+(PGK1p-***EcFNR***-*CYC1t*)+(*TEF1p-**EcFD**-TDH2t*)+(tHXT7p-***npgA***-*ADH5t*) pAlkane68	This study

133 **Supplementary Table 4**. Primers used in this study

Primer No.	Name	Sequence (5'-3')
Primers for seamless gene deletion of *POX1*, *FAA1* and *FAA4*		
p1	POX1(up)-F	GATTCCTTCAGTTCCACTTTTTGC
p2	POX1(up)-R	GAATTGAAACAAAAGTCGCAAAACAGAGGGTTCGAAGGAAAACAGGAAACCTCTACTC ACATATCGCAATACTAATTTATTAT
p3	KIURA3-F1	CTTCGAACCCTCTGTTTTGCGACTTTTGTTTCAATTCAACTAGTGTCGCCAAGTTTTAACG TGATTCTGGGTAGAAGATCG
p4	KIURA3-R2	GAGCCAATAGTTGTGGCTGCACAACTTTAGAGATCCATCGATAAGCTTGATATCG
p5	POX1(dw)-F	GATCTCTAAAGTTGTGCAGCCAC
p6	POX1(dw)-R	CGCATTAGCTGCACCACCTAAC
p7	FAA1(up)-F	CACCCACCCATCGCATATCAGG
p8	FAA1(up)-R	CTGAAAAAGTGCTTTAGTATGATGAGGCTTTCCTATCATGGAAATGTTGATCCATTACA TATTGTTGTCTTTTTTTGTC
p9	KIURA3-F2	GATAGGAAAGCCTCATCATACTAAAGCACTTTTTCAGTTTTTTGCTTTAGAACTGCTACC GTGATTCTGGGTAGAAGATCG
p10	KIURA3-R2	CAACATATTCGTTAGATCTGTAAACGGACTCTAATTTCCATCGATAAGCTTGATATCG
p11	FAA1(dw)-F	GAAATTAGAGTCCGTTTACAGATC
p12	FAA1(dw)-R	GTCAAAGAACACTATGCCTGCTAG
p13	FAA4(up)-F	GTCCCCATCAATTAAGAACCCTC
p14	FAA4(up)-R	GAAAATGAAACGTAGTGTTTATGAAGGGCAGGGGGGAAAGTAAAAAACTATGTCTTCC TTTACATTTTGATGCGTACTTCTTAG
p15	KIURA3-F3	CTTTCCCCCCTGCCCTTCATAAACACTACGTTTCATTTTCTAAGAGCATCAATTTGCGTGA TTCTGGGTAGAAGATCG
p16	KIURA3-R4	GATATCACCGGTACGGAACCAGCCATCATCGGTAAAGGCATCGATAAGCTTGATATCG
p17	FAA4(dw)-F	CCTTTACCGATGATGGCTGGTTC
p18	FAA4(dw)-R	GATGTAACAAGACCGTTTTCTGGAG
Primers for episomal plasmid construction for fatty acid production		
p19	TPIp-F	GTTTAAAGATTACGGATATTTAACTTACTTAGAATAATG
p20	TPIp-R	CATTTTTAGTTTATGTATGTGTTTTTTGTAG
p21	PGK1p-F	CGCACAGATATTATAACATCTGCACAATAGG
p22	PGK1p-R	CATTTTGTTATATTTGTTGTAAAAAGTAGATAATTAC
p23	TEF1p-F	ATAGCTTCAAAATGTTTCTACTCCTTTTTTACTC
p24	TEF1p-R	CATTTTGTAATTAAAACTTAGATTAGATTGCTATGC
p25	TDH3p-F	CTCGAGTTTATCATTATCAATACTGCCATTTC
p26	TDH3p-R	GTTTGTTTATGTGTGTTTATTCGAAACTAAGTTCTTGGTG
p27	tHXT7p-F	GTATTCTTTGAAATGGCAGTATTGATAATGATAAACTCGAGCTCGTAGGAACAATTTCG
p28	tHXT7p-R	CATTTTTTGATTAAAATTAAAAAAACTTTTTGTTTTTGTG
p29	FBA1t-F	GTTAATTCAAATTAATTGATATAGTTTTTTAATGAG
p30	FBA1t-R	AGTAAGCTACTATGAAAGACTTTACAAAGAAC
p31	CYC1t-F	GATACCGTCGACCTCGAGTCATGTAATTAGTTATGTC

p32	CYC1t-R	GGGTACCGGCCGCAAATTAAAGCCTTCGAGCGTCC
p33	TDH2t-F	ATTTAACTCCTTAAGTTACTTTAATGATTTAGTTTTTA
p34	TDH2t-R	GCGAAAAGCCAATTAGTGTGATAC
p35	ADH1t-F	GCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAG
p36	ADH1t-R	GCATATCTACAATTGGGTGAAATGGGGAGCGATTTG
p37	pYX212t-F	TAGGGCCCACAAGCTTACGCGTCGACCCGGGTATCC
p38	pYX212t-R	GCCGTAAACCACTAAATCGGAACCCTAAAGG
p39	RtACL-F1	CTATAACTACAAAAAACACATACATAAACTAAAAATGTCCGCAAAGCCTATCAGAG
p40	RtACL-R1	CTCATTAAAAAACTATATCAATTAATTTGAATTAACTTATTGTCTTTGTTGGACTAAAATTC
p41	RtME1-F1	CAAGAACTTAGTTTCGAATAAACACACATAAACAAACAAAATGCCTGCTCATTTTGCCC
p42	RtME1-R1	GACATAACTAATTACATGACTCGAGGTCGACGGTATCTCATACTTTTCTCAATGGTC
p43	LsME1-F1	CAAGAACTTAGTTTCGAATAAACACACATAAACAAACAAAATGGCCCCTAAATCCTCCA
p44	LSME1-R1	GTGACATAACTAATTACATGACTCGAGGTCGACGGTATCTCATACTTTTCTCAATGGTC
p45	MDH3t-F1	GTTTTTTTAATTTTAATCAAAAAATGGTCAAAGTCGCAATTCTTG
p46	MDH3t-R1	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATCAAGAGTCTAGGATGAAACTC
p47	MDH3t-R2	TAAAAACTAAATCATTAAAGTAACTTAAGGAGTTAAATTCAAGAGTCTAGGATGAAAC
p48	CTP1-R	CTTATTTAATAATAAAAATCATAAATCATAAGAAATTCGCTCAGGCTAGCATAACTAAG
P49	CTP1-F	GAAGTAATTATCTACTTTTTACAACAAATATAACAAAATGTCCAGTAAAGCTACCAAAAG
P50	tTesA-F:	GCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGCCGATACTTTGTTAATTTTG
P51	tTesA-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATCAAGAATCGTGATTGACTAATG
P52	PGK1p-R2	CTTATTTAATAATAAAAATCATAAATCATAAGAAATTCGCTTTGTTATATTTGTTGTAAAAAG
P53	fadM-F	GCATAGCAATCTAATCTAAGTTTTAATTACAAAATGCAAACCCAAATTAAGGTTAG
P54	fadM-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTATTTTACCATTTGTTCTAAC
Primers for constructing pathways for alkane production		
p55	CAR-F2	CTATAACTACAAAAAACACATACATAAACTAAAAATGTCACCTATCACCAGAGAAG
p56	CAR-R2	CTCATTAAAAAACTATATCAATTAATTTGAATTAACTCACAACAAACCCAACAATCTC
p57	npgA-F4	CACAAAAACAAAAAGTTTTTTTAATTTTAATCAAAAAATGGTGCAAGACACATCAAG
p58	npgA-R4	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTAGGATAGGCAATTACACACC
p59	ADH5up-F	GAAAAATGACTGATGTCTACAGGAC
p60	ADH5up-R	CATTATTCTAAGTAAGTTAAATATCCGTAATCTTTAAACCATGATGCTTTGATTTTGTAGATATG
p61	ADH5dn-F	CGCCCTTGCTATGGGTTACAG
p62	ADH5dn-R	ACCTCTGGCGAAGAAATCTAAAGC
p63	TDH2t(tHXt7p)-R	GAAGAACACGCAGGGGCCCGAAATTGTTCCTACGAGCGAAAAGCCAATTAGTGTGATAC
p64	npgA-R5	GCTTATATAAAAAGTAAAAATATATTCATCAAATTCGTTACAAAAGATTAGGATAGGCAATTACACACC
p65	KIURA3-F6	CGAATTTGATGAATATATTTTTACTTTTTATATAAGCTATTTTGTAGATATTGACGTGATTCTGGGTAGAAGATCG

p66	KIURA3-R6	CGATACCAATGACCCTGTAACCCATAGCAAGGGCGCATCGATAAGCTTGATATCG
Primers for constructing pathways for fatty alcohol production		
p67	CAR-F1	CAAGAACTTAGTTTCGAATAAACACACATAAACAAACAAAATGTCACCTATCACCAGAGAAG
p68	CAR-R1	CTTATTTAATAATAAAAATCATAAATCATAAGAAATTCGCTTACAACAAACCCAACAATCTC
p69	npgA-F3	GCTTAAATCTATAACTACAAAAAACACATACATAAACTAAAAATGGTGCAAGACACATCAAG
p70	npgA-R3	CTCATTAAAAAACTATATCAATTAATTTGAATTAACTTAGGATAGGCAATTACACAC
p71	YjgB-F	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGTCAATGATAAAAAGTTAC
p72	YjgB-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTAGTGATGGTGATGGTGATGGTAATC
p73	ADH5-F	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGCCTTCGCAAGTCATTCCTGAAAAAC
p74	ADH5-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATCATTTAGAAGTCTCAACAACATATC
p75	ADH6-F	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGTCTTATCCTGAGAAATTTGAAG
p76	ADH6-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTAGTCTGAAAATTCTTTGTCGTAGC
p77	ADH7-F	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGCTTTACCCAGAAAAATTTCAGG
p78	ADH7-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTATTTATGGAATTTCTTATCATAATC
p79	SFA1-F	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGTCCGCCGCTACTGTTGGTAAAC
p80	SFA1-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTACTATTTTATTTCATCAGACTTCAAGACG
p81	CAR-R3:	CCACCCAACAAACCCAACAATCTCAAATC
p82	ADH5-F2	GTATCAGATTTGAGATTGTTGGGTTTGTTGGGTGGTGGTTCTGGTGGTGGTTCTATGCCTTCGCAAGTCATTCCTG
p83	ADH5-R2	CTTATTTAATAATAAAAATCATAAATCATAAGAAATTCGCTTATTTAGAAGTCTCAACAACATATC
p84	ADH5-F3	CAAGAACTTAGTTTCGAATAAACACACATAAACAAACAAAATGCCTTCGCAAGTCATTCCTG
p85	ADH5-R3	CTAATCTTTCTTCTCTGGTGATAGGTGACATAGAACCACCACCAGAACCACCACCTTTAGAAGTCTCAACAACATATC
p86	CAR-F3	GTGGTTCTATGTCACCTATCACCAGAGAAG
p87	ADH5-F4	CACAAAAACAAAAAGTTTTTTTAATTTTAATCAAAAAATGCCTTCGCAAGTCATTCCTGAAAAAC
p88	ADH5-R4	GACATAACTAATTACATGACTCGAGGTCGACGGTATCTCATTTAGAAGTCTCAACAACATATC
p89	FaCoAR-F1	GCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAATTATTTCTTGACAGGTG
p90	FaCoAR-R1	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTACCAATAGATACCTCTCA
p91	FaCoAR -F2	GTATCAGATTTGAGATTGTTGGGTTTGTTGGGTGGTGGTTCTGGTGGTGGTTCTATGAATTATTTCTTGACAGG
p92	FaCoAR -R2	CTTATTTAATAATAAAAATCATAAATCATAAGAAATTCGCTTACCAATAGATACCTCTCATAATG

P93	Gal10t-F2	CTGGGCTGCAGGAATTCGATATCAAGCTTATCGATGGGAGACACTATTGAGGGTACGGAG
P94	Gal10t-R	GTTTCACCGTTTTTCAAGGTTACAC
P95	MmCAR-F6	CATGATAAAAAAAAACAGTTGAATATTCCCTCAAAAATGTCACCTATCACCAGAGAAGAAAG
P96	npgA-F6	GAGAAAATAAAAGTAAAAAGGTAGGGCAACACATAGTATGGTGCAAGACACATCAAG
P97	Gal7p-F	TTTGCCAGCTTACTATCCTTCTTG
P98	Gal7p-R	CATTTTTGAGGGAATATTCAACTG
P99	Gal3p-F	GTGCATATTTTCAAGAAGGATAGTAAGCTGGCAAATTGCTAGCCTTTTCTCGGTCTTGC
P100	Gal3p-R	ACTATGTGTTGCCCTACCTTTTTAC
P101	FBA1t-URA3-R1	CATTCATATCATATTTTTTCTATTAACTGCCTGGTTTCTTTTAAATTTTTTATTGGTTGTCGCATCGATAAGCTTGATATCG
P102	URA3(Gal7)-F	CGAGGTCCTCCTTCACCATTTGGTTAAATTGGCTGTGATTCTGGGTAGAAGATCG
P103	Gal7(dn)-F	AGCCAATTTAACCAAATGGTGAAG
P104	Gal7(dn)-R	CAGTCTTTGTAGATAATGAATCTG
Primers for genomic integration for free fatty acid production		
P105	His3(up)-F	CTCTTGGCCTCCTCTAGTACACTC
P106	His-R3	GCAGAAAAGACTAATAATTCTTAGTTAAAAGCACTCTACATAAGAACACCTTTGGTGG
P107	ENO2t-F	AGTGCTTTTAACTAAGAATTATTAGTC
P108	ENO2t-R	AGGTATCATCTCCATCTCCCATATGCATATCA
P109	ENO2t-TPIp-F	CCACAGTGATATGCATATGGGAGATGGAGATGATACCTGATCTACGTATGGTCATTCTTC
P110	’TesA-R2	CGTATGCTGCAGCTTTAAATAATCGGTGTCATCAAGAATCGTGATTGACTAATG
P111	His3t-F	GACACCGATTATTTAAAGCTGCAG
P112	His3t-R	CTGTTATTTCTGGCACTTCTTGG
P114	MmACL-F	CTATAACTACAAAAAACACATACATAAACTAAAAATGTCCGCTAAAGCTATTTCC
P115	MmACL-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTACATACTCATGTGTTCAGG
P116	HsACL-F	CTATAACTACAAAAAACACATACATAAACTAAAAATGTCCGCAAAAGCCATTTCC
P117	HsACL-R	GGATACCCGGGTCGACGCGTAAGCTTGTGGGCCCTATTACATACTCATGTGTTCAGG
P118	URA3(up)-F	AAACGACGTTGAAATTGAGGCTACTGCG
P119	URA3(up)-R	GAAGAAGAATGACCATACGTAGATCCCCAATTCGGACTAGGATGAGTAGCAGCACGTTCC
P120	RtFAS1-F	CTATAACTACAAAAAACACATACATAAACTAAAAATGAACGGCCGAGCGACGCGGAG
P121	RtFAS1-R	CTCATTAAAAAACTATATCAATTAATTTGAATTAACTCAGAGCCCGCCGAAGACGTCGAG
P122	RtFAS2-F	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGTCGCGGCGCAGGACTTGC
P123	RtFAS2-R	GACATAACTAATTACATGACTCGAGGTCGACGGTATCCTACTTCTGGGCGATGACGACG
P124	TEF1p(URA3)-F:	GTTTTGCTGGCCGCATCTTCTCAAATATGCTTCCCAGCCATAGCTTCAAAATGTTTCTACTCC
P125	Amdsym-F	GAGTAAAAAAGGAGTAGAAACATTTTGAAGCTATAAGCTTCGTACGCTGCAGGTCG
P126	Amdsym-R	CTGGCCGCATCTTCTCAAATATGCTTCCCCGACTCACTATAGGGAGACCG
P127	URA3(dn)-F	GGGAAGCATATTTGAGAAGATGCGGC

P128	URA3(dn)-R	GGAAACGCTGCCCTACACGTTCGC
P129	ACC1(up)-F	CGTTACGCCCTCCAGAGTCACC
P130	ACC1(up)-R	CTGGGCTGCAGGAATTCGATATCAAGCTTATCGATGCTAGGCTATACTGTGCCAGAATACG
P131	TEF1p(ACC1)-F	CATCCAATGCAGACCGATCTTCTACCCAGAATCACATAGCTTCAAAATGTTTCTACTCC
P132	TEF1p(ACC1)-R	CTGTGGAGAAGACTCGAATAAGCTTTCTTCGCTCATTTTGTAATTAAAACTTAGATTAG
P133	ACC1-F	ATGAGCGAAGAAAGCTTATTCG
P134	ACC1(In)-R	GTACCACCTGGCACTTCAATG

134

135

Supplementary Table 5. Codon optimized genes used in this study.

Synthesized genes	Sequence (5'-3')
RtACL	ATGTCCGCAAAGCCTATCAGAGAATACGACGCCAAATTGTTGTTAGCCTATCACTTAGCAAG AGCCCCTACCGCAGGTTCCAAAGCAGTTGCAAGAGATGGTTTTCAATCTCCAGAAGTAAAA GTTGCCCAAGTCTCATGGGACCCTGAAACCAATCAAGTAACTCCAGATGCTGCATTGCCTCA TTGGGTTTTCACTGAAAAATTGGTTGTCAAGCCAGATCAATTGATTAAAAGACGTGGTAAAG CAGGTTTGTTAGCCTTAAACAAAACTTGGGCTGAAGGTAAACAATGGATAGCCGAAAGAGC TGGTAAACAAGTCCAAGTAGAAAAGACTACAGGTACATTGAACAACTTCATCGTTGAACCAT TCTGTCCACATCCTTCCGATGCTGAATACTACATTTGCATCAACAGTGTCAGAGAAGGTGAC GTAATTTTGTTTACTCACGAAGGTGGTGTTGATGTCGGTGACGTTGACGCCAAAGCATTGAC TTTGTTAGTACCAGTTGGTGGTGAATTGCCTTCCAGAGATGAAATCAGAAGTCAATTGTTGA AGCATGTTACAGGTGCAGAAAGACAAGAAGCCTTAATAGACTACATCATCAGATTGTACTCC GTCTACGTAGATTTGCACTTTGCTTACTTAGAAATCAATCCATTGGTTGCAGTCGAAAACCCT TCTACTGGTAAAACAGATATTTTCTATTTGGATATGGCCGCTAAGTTGGACCAAACTGCTGA ATACGTAGTTGGTCCAAAATGGGCAATAGCCAGAGATCCTTCAATCATTAATCCAGCAGCCG CTCCTATGTCTAACGGTAAAATCTCAGCTGATAAGGGTCCACCTATGTTTTGGCCACCTCCAT TCGGTAGAGACTTAACTAAGGAAGAAGCATATATTGCCAAGTTGGATGGTTCTACAGGTGC CTCATTGAAATTGACCGTATTAAATGCTGAAGGTAGAATATGGACAATGGTTGCTGGTGGT GGTGCATCCGTCGTATATAGTGATGCTATCGCAGCTCATGGTTTTGCCCACGAATTGGCTAA TTATGGTGAATACTCTGGTGCACCAACTCAAACACAAACCTATGAATACGCCAAAACTATTTT GGATTTGATGACCAGAGGTACTCCAAACCCTCAGGGTAAATTGTTGTTTATTGGTGGTGGTA TTGCAAATTTTACTAACGTTGCTGCAACATTCAAAGGTATCATCACAGCTTTGAAGGAATACC AACATAGATTGCAAGAACACAAAGTTAGAATCTTCGTCAGAAGAGGTGGTCCAAATTACCA AGAAGGTTTAAAGGCTATGAGATTGTTAGGTGAAACTTTGGGTGTAGAAATCCAAGTTTTC GGTCCAGAAACACATATTACCTCTATAGTTCCTTTGGGTTTAGGTTTGATTAAATCAGTTGAT GACGCCTTAAAGGTCCCAGGTGCTAGAGCTGCTGCTGATGCAACTGGTACATTAACCCCAGT TCCTGGTTCCCCAAAAAGTAGAGCCGCTCAATTGCCTACAGGTGCATCTACCCCATCAAGAC AACAACCTCAAGATAACATAGTATCCTTTAGTGATAAAGTTCATGCTCCAGACTCTGGTAGA CCTTGGTATAGACCTTTCGATGAAACCACTAGAAGTATAGTTTACGGTTTACAACCTAGAGC TATCCAAGGCATGTTGGATTTTGACTTCGCATGTGGTAGAGAAACACCATCTGTCGCAGCTA TGGTTTATCCTTTTGGTGGTCATCACGTTCAAAAATTCTACTGGGGTACTAAGGAAACATTGT TGCCAGTTTTTACTTCAATGAAGGAAGCTGTCGCAAAGTGCCCTGATGCCGACGTTGTCGTA AACTTCGCTTCTTCAAGATCAGTTTACCAATCTACTTTGGAAGCATTGGAATTTCCACAAATC AAAGCCATTGCTTTGATAGCTGAAGGTGTTCCTGAAAGACATGCAAGAGAAATTTTACACTT GGCCAAAAAGAAAGAAGTAATTATCATCGGTCCAGCTACTGTTGGTGGTATTAAACCAGGT TGTTTCAGAATCGGTAACACAGGTGGTATGAACGAAAACATCTTGTCCAGTAAATTGTATAG AGCTGGTTCTGTAGGTTACGTTTCTAAGTCAGGTGGCATGTCTAACGAATTAAACAACATAT TATCATTGACAACCGACGGTGCTTATGAAGGTATCGCAATTGGTGGTGACAGATACCCAGG TACTACTTTTATTGACCATTTGTTGAGATACGAAGCAGATCCTAACTGTAAGATGTTGGTTTT GTTGGGTGAAGTCGGTGGTGTAGAAGAATACAGAGTTATTGAAGCTGTCAAATCTGGTCAA ATTAAGAAACCAATCGTCGCATGGGCCATAGGTACTTGCGCAAAGATGTTTGCCACAGACG TACAATTCGGTCACGCTGGTTCTATGGCTAATTCTGATTTGGAAACAGCTGAAGCTAAAAAT AACGCAATGAGAGCTGCTGGTTTTATTGTTCCTCCAACCTTCGAAGAATTGCCACAAGTTTTG GCTGAAACATACCAAAAATTGGTCGGTGACGGTACTATTCAACCAAAGCCTGAAGTTCCTCC ACCTCAAATACCAATGGATTACAATTGGGCACAAACATTGGGTATGGTCAGAAAACCTGCC GCTTTTATCTCCACCATTAGTGACGAAAGAGGTCAAGAATTGTTATATGCTGGTATGCCAAT TTCTAAGGTTTTCGAAGAAGATATAGGTATCGGTGGTGTTGTCTCATTGTTGTGGTTCAAGA

	GAAGATTACCAGCCTACGCTACTAAGTTCTTGGAAATGGTTTTGATGTTGACTGCTGATCAT GGTCCTGCCGTCTCTGGTGCTATGACCACTGTAATTACAACCAGAGCCGGTAAAGATTTGGT ATCTTCATTAGTTGCTGGTTTGTTAACTATAGGTGACAGATTTGGTGGTGCTTTAGACGGTG CAGCCCAAGAGTTTACTAGAGCTTTCGAAGCAGGTTTGACTCCAAGAGAATTTGTTGATTCT ATGAGAAAGGCAAATAAGTTAATACCAGGTATCGGTCATAAGGTCAAATCAAAGGCTAATC CTGATAAAAGAGTTGAATTGGTCAAAAACTACGTTTTTAAACACTTCCCATCCGCAAAGTTG TTAGAATACGCATTAGCCGTAGAAGATGTTACCAGTGCTAAGAAAGACACTTTGATCTTGAA CGTTGATGGTGCTATTGCAGTCTCCTTTGTAGATTTGTTGAAAAATTCTGGTGCCTTCACCGC TGAAGAAGCTGCTGAATACATGAAGATCGGTACTTTGAACGGTTTGTTCGTTTTGGGTAGAT CAATCGGTTTCATAGCACATCACTTGGATCAAAAGAGATTGAAGCAACCATTATACAGACAT CCTGCTGACGACATTTTCATCCAACCATTCAACACTGACAGAATTTTAGTCCAACAAAGACAA TAA
MmACL	ATGTCCGCTAAAGCTATTTCCGAACAAACTGGTAAAGAATTATTATACAAGTACATTTGCAC CACCTCAGCCATACAAAACAGATTCAAGTATGCAAGAGTTACACCAGATACCGACTGGGCCC ATTTGTTACAAGATCACCCTTGGTTGTTATCTCAATCATTGGTTGTCAAACCTGACCAATTGA TTAAAAGACGTGGTAAATTGGGTTTAGTCGGTGTAAACTTGAGTTTAGATGGTGTTAAGTCT TGGTTGAAGCCAAGATTAGGTCATGAAGCTACAGTTGGTAAAGCAAAGGGTTTCTTGAAAA ATTTCTTGATCGAACCATTCGTACCTCACTCACAAGCTGAAGAATTTTACGTTTGTATCTATG CAACTAGAGAAGGTGACTATGTCTTGTTTCATCACGAAGGTGGTGTTGACGTCGGTGACGTT GACGCCAAAGCTCAAAAGTTGTTAGTAGGTGTTGATGAAAAGTTAAACACAGAAGACATCA AGAGACATTTGTTGGTACACGCCCCAGAAGATAAAAAGGAAGTTTTGGCTTCCTTTATAAGT GGTTTGTTTAATTTCTACGAAGATTTGTACTTCACCTACTTGGAAATTAACCCTTTAGTAGTTA CTAAGGATGGTGTCTATATATTGGACTTAGCTGCAAAAGTAGATGCAACTGCCGACTACATC TGTAAGGTTAAGTGGGGTGACATTGAATTTCCACCTCCATTCGGTAGAGAAGCATATCCAGA AGAAGCCTACATTGCTGATTTGGACGCAAAATCTGGTGCCTCATTGAAGTTAACATTGTTGA ACCCTAAGGGTAGAATATGGACTATGGTTGCTGGTGGTGGTGCAAGTGTCGTATATTCTGA TACAATCTGCGACTTGGGTGGTGTTAACGAATTAGCTAACTACGGTGAATACTCAGGTGCAC CATCCGAACAACAAACTTATGATTACGCTAAGACCATCTTGAGTTTAATGACTAGAGAAAAG CATCCTGAAGGTAAAATTTTGATCATCGGTGGTTCTATAGCAAACTTCACTAACGTTGCCGCT ACATTCAAGGGTATAGTCAGAGCTATCAGAGATTATCAAGGTCCATTGAAGGAACACGAAG TTACAATATTCGTCAGAAGAGGTGGTCCTAACTACCAAGAAGGTTTAAGAGTAATGGGTGA AGTTGGTAAAACTACAGGTATCCCAATTCATGTATTTGGTACTGAAACACACATGACTGCCA TCGTTGGTATGGCTTTAGGTCATAGACCAATTCCTAATCAACCTCCAACAGCAGCCCACACC GCCAATTTCTTGTTAAACGCTTCCGGTAGTACCTCTACTCCAGCACCATCAAGAACTGCCTCA TTCTCCGAAAGTAGAGCTGATGAAGTTGCTCCAGCTAAGAAAGCAAAACCAGCCATGCCTC AAGACTCCGTTCCAAGTCCTAGATCATTGCAAGGTAAATCAGCAACATTATTTTCCAGACAT ACCAAAGCCATTGTATGGGGTATGCAAACAAGAGCTGTTCAAGGCATGTTGGATTTCGACT ATGTTTGTAGTAGAGATGAACCATCTGTCGCTGCAATGGTATATCCTTTTACCGGTGACCAT AAACAAAAGTTCTACTGGGGTCACAAGGAAATATTAATCCCAGTTTTTAAAAACATGGCCGA TGCTATGAAAAAGCATCCTGAAGTTGATGTATTGATTAACTTCGCTTCATTAAGATCCGCTTA TGATTCTACTATGGAAACAATGAACTACGCACAAATTAGAACCATAGCTATCATTGCAGAAG GTATACCAGAAGCATTGACTAGAAAGTTAATCAAAAAGGCCGATCAAAAAGGTGTCACTAT AATCGGTCCAGCTACAGTAGGTGGTATAAAACCTGGTTGTTTTAAGATCGGTAATACTGGTG GCATGTTGGATAACATATTGGCATCAAAATTGTATAGACCAGGTTCCGTAGCTTACGTTTCA AGAAGCGGTGGTATGAGTAACGAATTGAACAACATAATTTCAAGAACCACTGATGGTGTTT ATGAAGGTGTCGCTATTGGTGGTGACAGATACCCAGGTTCTACTTTTATGGATCATGTTTTG AGATATCAAGACACACCTGGTGTCAAAATGATCGTTGTCTTAGGTGAAATAGGTGGTACTG AAGAATACAAAATTTGCAGAGGTATAAAGGAAGGTAGATTGACAAAACCAGTAGTTTGTTG

	GTGCATTGGTACTTGTGCAACTATGTTTTCTTCAGAAGTTCAATTCGGTCATGCAGGTGCCTG CGCTAATCAAGCATCTGAAACAGCAGTTGCCAAAAACCAAGCCTTAAAGGAAGCTGGTGTT TTTGTCCCTAGATCATTCGATGAATTGGGTGAAATCATTCAATCCGTATATGAAGACTTAGTT GCCAAGGGTGCTATTGTCCCAGCTCAAGAAGTACCTCCACCTACTGTTCCTATGGATTACTCA TGGGCAAGAGAATTGGGTTTGATCAGAAAGCCAGCTAGTTTTATGACCTCTATCTGTGATGA AAGAGGTCAAGAATTGATCTATGCTGGTATGCCTATCACTGAAGTCTTCAAGGAAGAAATG GGTATCGGTGGTGTATTGGGTTTGTTGTGGTTCCAAAGAAGATTACCAAAGTACTCATGTCA ATTCATAGAAATGTGCTTAATGGTTACAGCTGATCATGGTCCAGCTGTTTCTGGTGCCCACA ACACCATAATCTGCGCTAGAGCAGGTAAAGATTTGGTTTCTTCTTTGACCTCTGGTTTGTTAA CTATTGGTGACAGATTTGGTGGTGCATTGGACGCCGCTGCAAAAATGTTTTCAAAGGCTTTC GATTCCGGTATAATCCCAATGGAATTTGTTAATAAGATGAAAAAGGAGGGTAAATTAATCAT GGGTATCGGTCATCGTGTTAAGTCAATTAATAACCCTGATATGAGAGTCCAAATATTGAAGG ACTTCGTAAAGCAACACTTCCCAGCAACACCTTTGTTAGATTACGCCTTAGAAGTTGAAAAG ATTACAACCTCTAAAAAGCCAAATTTGATCTTGAACGTTGATGGTTTTATAGGTGTCGCTTTC GTAGACATGTTAAGAAACTGTGGTTCTTTTACTAGAGAAGAAGCCGATGAATATGTTGACAT TGGTGCTTTGAATGGTATATTTGTCTTAGGTAGATCAATGGGTTTTATTGGTCATTACTTGGA TCAAAAGAGATTAAAGCAAGGTTTGTATAGACACCCTTGGGACGATATTTCCTACGTTTTGC CTGAACACATGAGTATGTAA
HsACL	ATGTCCGCAAAAGCCATTTCCGAACAAACTGGTAAAGAATTATTATACAAGTTCATCTGCAC AACCTCAGCCATACAAAACAGATTCAAGTATGCAAGAGTTACTCCAGATACAGACTGGGCC AGATTGTTACAAGATCATCCTTGGTTGTTATCACAAAACTTGGTTGTCAAGCCTGACCAATTG ATTAAAAGACGTGGTAAATTGGGTTTAGTAGGTGTTAATTTGACATTAGATGGTGTTAAGTC CTGGTTGAAGCCAAGATTAGGTCAAGAAGCAACCGTCGGTAAAGCCACTGGTTTCTTGAAA AATTTCTTGATCGAACCATTCGTACCTCATTCTCAAGCTGAAGAATTTTACGTTTGTATATAC GCAACTAGAGAAGGTGACTATGTCTTGTTTCATCACGAAGGTGGTGTTGATGTAGGTGACG TAGACGCCAAAGCTCAAAAGTTGTTAGTTGGTGTCGATGAAAAATTGAACCCAGAAGACAT TAAAAAGCATTTGTTGGTTCACGCCCCTGAAGATAAAAAGGAAATATTGGCTTCTTTTATCTC AGGTTTGTTTAATTTCTACGAAGATTTGTACTTCACTTACTTGGAAATTAACCCATTGGTAGT TACAAAGGATGGTGTATACGTTTTGGACTTAGCTGCAAAGGTCGATGCAACAGCCGACTAC ATTTGTAAAGTAAAGTGGGGTGACATAGAATTTCCACCTCCATTCGGTAGAGAAGCATATCC AGAAGAAGCCTACATTGCTGATTTGGACGCCAAATCCGGTGCTAGTTTGAAGTTAACCTTGT TGAACCCTAAAGGTAGAATCTGGACTATGGTTGCAGGTGGTGGTGCCTCAGTCGTATATTCC GATACTATTTGCGACTTGGGTGGTGTTAACGAATTAGCTAACTACGGTGAATACAGTGGTGC ACCATCTGAACAACAAACCTATGATTACGCTAAGACTATCTTGAGTTTAATGACAAGAGAAA AGCATCCTGATGGTAAAATTTTGATCATCGGTGGTTCTATCGCTAACTTCACAAACGTCGCC GCTACCTTCAAAGGTATAGTAAGAGCAATCAGAGATTACCAAGGTCCATTGAAGGAACACG AAGTAACCATTTTTGTTAGAAGAGGTGGTCCTAACTACCAAGAAGGTTTAAGAGTCATGGG TGAAGTAGGTAAAACTACAGGTATCCCAATTCATGTATTTGGTACTGAAACTCACATGACTG CTATTGTTGGTATGGCATTAGGTCATAGACCAATACCTAATCAACCTCCAACTGCTGCTCACA CAGCTAATTTCTTGTTAAACGCATCTGGTTCAACATCCACCCCAGCCCCATCAAGAACAGCTA GTTTCTCTGAATCAAGAGCTGATGAAGTTGCTCCAGCTAAGAAAGCAAAACCAGCCATGCCT CAAGACTCTGTTCCATCACCTAGATCCTTGCAAGGTAAAAGTACCACTTTGTTTTCAAGACAT ACAAAGGCAATTGTTTGGGGTATGCAAACCAGAGCCGTCCAAGGCATGTTGGATTTCGACT ATGTTTGTTCAAGAGATGAACCATCCGTTGCTGCAATGGTCTATCCTTTTACTGGTGACCATA AACAAAAGTTCTACTGGGGTCACAAGGAAATATTAATCCCAGTTTTTAAAAACATGGCCGAT GCTATGAGAAAGCATCCTGAAGTTGATGTATTGATTAACTTCGCAAGTTTAAGATCAGCCTA TGATTCAACTATGGAAACTATGAACTACGCTCAAATCAGAACTATTGCTATCATTGCAGAAG GTATCCCAGAAGCATTGACAAGAAAATTAATTAAAAAGGCAGATCAAAAGGGTGTAACCAT AATCGGTCCAGCAACTGTTGGTGGTATCAAACCTGGTTGTTTTAAGATTGGTAATACAGGTG

	GCATGTTGGATAACATATTGGCTTCAAAATTGTATAGACCAGGTTCCGTCGCATACGTATCC AGAAGTGGTGGTATGAGTAACGAATTAAACAACATAATTTCAAGAACAACCGATGGTGTAT ATGAAGGTGTTGCTATTGGTGGTGACAGATACCCAGGTTCTACTTTTATGGATCATGTTTTG AGATATCAAGACACCCCTGGTGTCAAAATGATCGTTGTCTTAGGTGAAATAGGTGGTACAG AAGAATACAAAATTTGTAGAGGTATTAAGGAAGGTAGATTGACCAAACCAATTGTTTGTTG GTGCATAGGTACATGTGCTACCATGTTTTCTTCAGAAGTTCAATTCGGTCACGCAGGTGCCT GCGCTAATCAAGCATCTGAAACAGCAGTTGCCAAAAACCAAGCATTGAAGGAAGCAGGTGT TTTTGTCCCTAGATCATTCGATGAATTGGGTGAAATCATTCAATCCGTCTATGAAGACTTAGT AGCCAATGGTGTAATTGTTCCAGCTCAAGAAGTTCCTCCACCTACTGTCCCTATGGATTACTC TTGGGCTAGAGAATTGGGTTTAATCAGAAAACCAGCTTCTTTTATGACTTCCATTTGTGATGA AAGAGGTCAAGAATTGATCTATGCTGGTATGCCTATCACAGAAGTTTTCAAGGAAGAAATG GGTATAGGTGGTGTCTTGGGTTTGTTGTGGTTCCAAAAGAGATTGCCAAAGTACTCATGTCA ATTCATTGAAATGTGCTTAATGGTCACCGCTGATCATGGTCCTGCCGTATCCGGTGCTCACA ACACTATAATCTGCGCTAGAGCAGGTAAAGATTTGGTTTCTTCTTTGACTTCAGGTTTGTTAA CAATTGGTGACAGATTTGGTGGTGCTTTGGACGCCGCTGCAAAGATGTTTAGTAAGGCATTC GATTCTGGTATAATCCCAATGGAATTTGTTAATAAGATGAAAAAGGAGGGTAAATTAATCAT GGGTATCGGTCATCGTGTTAAGTCTATAAATAACCCTGATATGAGAGTACAAATCTTGAAGG ACTATGTTAGACAACACTTTCCAGCAACACCTTTGTTAGATTACGCCTTAGAAGTTGAAAAG ATTACTACATCTAAGAAACCAAATTTGATCTTGAACGTTGATGGTTTGATCGGTGTTGCTTTT GTTGATATGTTAAGAAACTGTGGTAGTTTCACTAGAGAAGAAGCCGATGAATATATTGACAT CGGTGCTTTGAACGGTATCTTCGTTTTGGGTAGATCAATGGGTTTTATTGGTCATTACTTGGA TCAAAAGAGATTAAAGCAAGGTTTGTATAGACACCCTTGGGATGATATTTCCTACGTTTTGC CTGAACACATGAGTATGTAA
RtME	ATGCCTGCTCATTTTGCCCCTTCACAACCATTACAAGGTGGTCCATCCCCTTCACAATTGGGT CCTAAAGAATTATTGATAGAAAGAGCATTGACAAGATTGAGATCAATCCCAAACGATTTGG AAAAATATACCTTTTTGGCCGGTTTAAGAGGTAGAAATCCTGATGTCTTCTACGGTTTAGTA GGTGGTAACATGAAGGAATGTTGCCCAATTATCTATACTCCTGTTATAGGTTTAGCTTGTCA AAATTGGTCCTTGATCCATCCACCTCCACCTGAAAGTGATCCAACAATTGACGCATTGTATTT GTCTTACTCAGATTTGCCAAACTTACCTCAATTGATCGGTGGTTTGAAGACTAGATTGCCTCA CGATCAAATGCAAATCTCCGTTGTCACAGACGGTAGTAGAGTATTGGGTTTGGGTGACTTG GGTGTTGGTGGTATGGGTATATCTCAGGGTAAATTGTCATTATACGTTGCTGCTGGTGGTGT CAATCCAAAGGCCACTTTACCTATCGCTATTGATTTTGGTACTGACAACGAAACTTTGTTAGC TGATCCATTGTACGTTGGTCAAAGAATTAGAAGATTATCTCAAGAAAAGTGTTTGGAGTTTA TGGAAGTTTTCATGAGATGCATGCATGAAACCTTCCCAAATATGGTTATTCAACACGAAGAC TGGCAAACTCCATTGGCTTTCCCTTTGTTGCATAAGAACAGAGATTTGTACCCTTGTTTCAAC GATGACATTCAAGGTACTGGTGCAGTAGTTTTAGCAGGTGCCATAAGAGCTTTTCACTTAAA CGGTGTTGCATTGAAGGATCAAAAGATTTTGTTTTTCGGTGCCGGTTCTTCAGGTGTTGGTG TCGCTGAAACAATATGCAAGTACTTCGAATTGCAAGGCATGTCTGAAGACGAAGCCAAATC AAAGTTCTGGTTGGTAGATTCAAAGGGTTTGGTTGCTCATAATAGAGGTGACACATTACCAT CTCACAAAAAGTATTTGGCAAGATCAGAACCAGATGCCCCTAAATTGAGAACCTTGAAGGA AGTCGTAGAACATGTTCAACCAACTGCTTTGTTAGGTTTATCTACAGTCGGTGGTACTTTTAC AAAGGAAATCTTGGAAGCTATGGCAACTTACAATAAGAGACCAATTGTCTTTGCTTTATCAA ACCCTGTAGCCCAAGCTGAATGTACCTTCGAAGAAGCTGTTGAAGGTACTGACGGTAGAGT CTTGTACGCATCCGGTAGTCCATTCGATCCTGTTGAATACAAGGGTAAAAGATACGAACCAG GTCAAGGTAATAACATGTATATCTTCCCTGGTTTAGGTATTGGTGCTATATTGGCAAGAGTC TCCAAAATTCCAGAAGAATTAGTACATGCATCCGCCCAAGGTTTAGCAGACAGTTTGACACC AGAAGAAACCGCCAGACACTTGTTGTACCCTGATATCGAAAGAATTAGAGAAGTTTCTATAA AAATCGCTGTAACAGTTATACAAGCCGCTCAAAAGTTAGGTGTTGATAGAAACGAAGAATT GCGTGGTAAATCCAGTGCAGAAATTGAAGCCTATGTCAGAAAAGGTATGTATCACCCATTAT

	TAGAAGCAGAACAACAAGCACAATGA
LsME	ATGGCCCCTAAATCCTCCACCAGAGTTCCATTATCCGTCAAAGGTCCAATAGACTGC CCTTATGAAGGTAAAGAAATGTTAAACTTACCTCAATTCAATAGAGGTACAGCCTTC ACCGCTGAAGAAAGAGATTTGTTTAATTTGGTCGGTAATTTGCCAGCTGCATTACAA ACTTTGCAAAATCAAGTAGACAGAGCCTATGATCAATACTCTTCAATTTCTACAGCTT TGGGTAAAAACACCTTTTTAATGTCATTGAAGGTCCAAAACGAAGTATTGTACTTCA AATTGTTACAAGATCATTTGAAGGAAATGTTCTCAATCATCTATACTCCAACAGAATC TGAAGCTATCGAACATTATTCAAGATTGTTTAGAAGACCAGAAGGTTGTTTCTTGAA CATCAACCACCCAGAATACATCGAAAGATCCTTAGCCGCTTGGGGTACAGAAGAAG ATATTGACTACATCATTGTTAGTGACGGTGAAGAAATTTTGGGTATAGGTGACCAA GGTGTCGGTGCTATAGGTATCTCCAGTGCAAAAGCCGTATTAATGACCTTGTGTGCA GGTGTTCATCCATCAAGATGCATTCCAGTTGCCTTAGACGTCGGTACTGATAACGAA CAATTGTTAGAAGATGAATTATATTTGGGTAACAGACACAACAGAGTTAGAGGTGG TAGATACGATAAATTTGTCGATGACTTCGTTCAATGTGTCAAAAAGTTATATCCAAG AGCCGTTTTGCATTTTGAAGACTTCGGTTTACCTAACGCAAGAAGATTGTTAGATAC TTACAGACCAAGATTGGCTTGCTTTAATGATGACGTCCAAGGTACTGGTGCAGTAAC TTTAGCAGCCTTGTCTTCAGCTGTCAGAGTAGCAGGTATCGATTTTAGAGACTTGAG AACAGTTATTTTCGGTGCAGGTACTGCCGGTACAGGTATAGCTGACCAATTAAGAG ATTTCTTGAATACCCAAGGTATCTCTAAACAACAAGTTATCGACCATATTTGGTTGGT CGATAAGCCTGGTTTGTTATTGAAATCCATGCACGATAAGTTGACTAGTGCACAAAG ACCATACGCTGCATCTGATGACAGATGGAAGGAAATAGATACAAAGTCCTTAAGTG AAATCGTTAAGAAAGTTAAGCCTCACGTTTTGATTGGTTGTTCTACTAAACCAAAGG CCTTCAACGAAGCTGTTTTAAGAGAAATGGCTAAGCATGTAGAAAGACCAATCGTTT TCCCTTTGTCAAACCCAACTAGATTGCACGAAGCTACACCAGCTGAAATTTTTAAATA CACCGATGGTAAAGCATTGGTAGCTACTGGTTCCCCATTCGATCCTGTTGACGGTAA AGAAATCGCTGAAAACAACAACTGCTTCGTTTACCCAGGTATCGGTATGGGTTCTAT TTTGTCAAGAGCAGATAGAGTTACCGAAACTATGATAGCCGCTGTTGTCAAAGAATT AGCATCCTTGGCCCCTAGTGAAAAAGATCCAACAGGTGCATTATTGCCTGATGTTGC CGACATAAGAGATATCTCTGCTAAAATTGCTACAGCAGTAGTTTTGCAAGCATTGGA AGAAGGTACTGCAAGAGTCGAAGAAATAGAAGGTATTAAAGTTCCAAGAGATAGA GACCATTGTTTGGAATGGGTAAAAGAACAAATGTGGCAACCTGAATACAGACCATT GAGAAAAGTATGA
'tesA	ATGGCCGATACTTTGTTAATTTTGGGTGACTCTTTATCAGCCGGTTATAGAATGTCC GCTAGTGCTGCATGGCCAGCATTGTTAAACGATAAATGGCAATCTAAGACTTCAGTT GTCAATGCATCTATATCAGGTGACACATCACAACAAGGTTTGGCCAGATTACCAGCT TTGTTAAAACAACATCAACCTAGATGGGTCTTGGTAGAATTAGGTGGTAACGATGG TTTGAGAGGTTTTCAACCTCAACAAACCGAACAAACTTTGAGACAAATCTTACAAGA TGTTAAGGCCGCTAATGCAGAACCATTGTTAATGCAAATTAGATTACCTGCCAACTA TGGTAGAAGATACAATGAAGCATTTTCTGCAATCTATCCAAAATTGGCAAAGGAATT TGATGTACCATTGTTGCCATTTTTCATGGAAGAAGTTTACTTAAAACCTCAATGGAT GCAAGATGACGGTATTCATCCAAACAGAGATGCTCAACCTTTTATAGCAGACTGGAT GGCCAAACAATTGCAACCATTAGTCAATCACGATTCTTGA
AAR	ATGTTCGGTTTAATAGGTCACTTAACAAGTTTAGAACAAGCCAGAGATGTCAGTAG AAGAATGGGTTACGATGAATACGCAGACCAAGGTTTAGAATTTTGGTCTTCAGCCC CACCTCAAATCGTAGATGAAATTACAGTTACCTCTGCTACTGGTAAAGTCATTCATG

	GTAGATACATCGAATCATGTTTCTTGCCAGAAATGTTGGCTGCAAGAAGATTCAAAA CTGCAACAAGAAAGGTTTTGAATGCAATGTCCCATGCCCAAAAGCACGGTATCGAT ATTTCCGCATTGGGTGGTTTTACAAGTATAATCTTCGAAAACTTCGATTTGGCTAGTT TGAGACAAGTTAGAGACACTACATTGGAATTCGAAAGATTCACCACTGGTAACACC CACACTGCTTACGTCATTTGTAGACAAGTAGAAGCCGCTGCAAAAACCTTGGGTATA GATATCACACAAGCCACCGTTGCTGTTGTCGGTGCTACTGGTGACATCGGTTCCGCA GTATGCAGATGGTTGGATTTGAAATTGGGTGTTGGTGACTTAATCTTGACAGCTAG AAACCAAGAAAGATTGGATAACTTGCAAGCAGAATTAGGTAGAGGTAAAATCTTGC CATTGGAAGCCGCTTTGCCTGAAGCCGATTTTATCGTTTGGGTCGCTTCTATGCCAC AAGGTGTAGTTATTGATCCAGCTACCTTAAAACAACCTTGCGTTTTGATAGACGGTG GTTATCCTAAAAATTTGGGTTCTAAGGTTCAAGGTGAAGGTATCTATGTCTTGAACG GTGGTGTCGTAGAACATTGTTTCGATATAGACTGGCAAATCATGTCAGCAGCCGAA ATGGCAAGACCTGAAAGACAAATGTTTGCCTGCTTCGCTGAAGCAATGTTGTTAGA ATTTGAAGGTTGGCACACTAATTTCTCTTGGGGTAGAAACCAAATTACAATAGAAAA GATGGAAGCCATCGGTGAAGCCTCTGTTAGACACGGTTTCCAACCTTTAGCCTTAGC AATCTGA
ACR1	ATGAATAAGAAGTTAGAAGCATTGTTTAGAGAAAATGTCAAGGGTAAAGTCGCTTT AATCACTGGTGCCTCCTCAGGTATCGGTTTAACTATCGCAAAAAGAATTGCTGCAGC CGGTGCCCATGTTTTGTTAGTCGCTAGAACTCAAGAAACATTGGAAGAAGTTAAGG CTGCAATCGAACAACAAGGTGGTCAAGCATCTATATTCCCATGTGATTTGACAGACA TGAATGCAATAGATCAATTATCCCAACAAATCATGGCCAGTGTAGATCATGTTGACT TTTTGATTAATAACGCAGGTAGATCTATAAGAAGAGCCGTTCATGAATCATTTGATA GATTCCACGACTTCGAAAGAACAATGCAATTAAACTACTTCGGTGCTGTCAGATTGG TATTGAACTTGTTGCCTCACATGATCAAGAGAAAGAATGGTCAAATTATAAACATCT CTTCAATCGGTGTATTGGCCAACGCTACCAGATTCTCTGCTTATGTTGCATCAAAAG CCGCTTTAGATGCTTTTTCCAGATGCTTGAGTGCAGAAGTTTTGAAGCATAAGATCT CTATAACTTCAATCTATATGCCATTGGTCAGAACACCAATGATCGCACCTACCAAAAT CTATAAGTACGTTCCAACATTGTCTCCTGAAGAAGCAGCCGATTTGATAGTTTATGC TATCGTCAAGAGACCTACCAGAATTGCCACTCACTTGGGTAGATTAGCTTCCATTAC CTACGCAATAGCCCCAGACATAAACAACATCTTGATGTCTATTGGTTTTAATTTGTTT CCTTCCAGTACTGCTGCATTAGGTGAACAAGAAAAATTGAACTTATTACAAAGAGCC TACGCAAGATTATTCCCTGGTGAACATTGGTGA
CAR	ATGTCACCTATCACCAGAGAAGAAAGATTAGAAAGAAGAATACAAGACTTATACGC CAACGATCCTCAATTCGCCGCTGCCAAGCCAGCAACAGCCATCACCGCTGCAATTGA AAGACCAGGTTTGCCATTGCCTCAAATCATCGAAACTGTTATGACAGGTTATGCTGA TAGACCTGCTTTGGCACAAAGATCAGTAGAATTTGTTACAGATGCAGGTACTGGTC ATACTACATTGAGATTGTTACCACACTTCGAAACTATCTCTTACGGTGAATTATGGG ACAGAATTTCTGCCTTGGCTGATGTTTTATCAACCGAACAAACTGTTAAACCTGGTG ACAGAGTCTGTTTGTTGGGTTTTAATTCTGTTGACTACGCAACTATAGATATGACATT GGCCAGATTAGGTGCAGTAGCCGTTCCATTGCAAACCTCTGCCGCTATTACTCAATT ACAACCAATAGTCGCTGAAACACAACCTACCATGATAGCAGCCTCTGTAGATGCTTT GGCAGACGCCACTGAATTGGCTTTATCAGGTCAAACTGCAACAAGAGTCTTAGTATT CGACCATCACAGACAAGTTGATGCCCATAGAGCTGCTGTTGAATCCGCTAGAGAAA GATTGGCAGGTAGTGCCGTTGTCGAAACTTTAGCTGAAGCAATAGCTAGAGGTGAC GTTCCAAGAGGTGCTTCTGCTGGTTCTGCTCCTGGTACAGACGTCTCCGATGACAGT

	TTGGCATTGTTAATCTATACCTCTGGTTCAACTGGTGCCCCAAAAGGTGCTATGTAC CCTAGAAGAAATGTTGCTACATTTTGGAGAAAGAGAACCTGGTTCGAAGGTGGTTA CGAACCATCTATCACTTTGAACTTCATGCCTATGTCACATGTTATGGGTAGACAAATC TTGTATGGTACTTTATGCAACGGTGGTACAGCATACTTTGTTGCCAAGTCTGACTTG TCAACATTATTCGAAGATTTGGCTTTAGTCAGACCAACTGAATTAACATTCGTCCCTA GAGTATGGGATATGGTTTTTGACGAATTTCAATCAGAAGTCGATAGAAGATTGGTA GATGGTGCTGACAGAGTAGCTTTAGAAGCACAAGTTAAGGCAGAAATAAGAAACG ATGTTTTGGGTGGTAGATATACATCTGCCTTAACCGGTTCTGCTCCAATATCAGACG AAATGAAGGCTTGGGTAGAAGAATTGTTAGATATGCATTTGGTTGAAGGTTACGGT TCAACTGAAGCTGGTATGATATTAATCGACGGTGCAATTAGAAGACCAGCCGTTTT GGATTATAAATTGGTTGATGTCCCTGACTTGGGTTACTTTTTAACTGATAGACCACA CCCTAGAGGTGAATTGTTGGTTAAGACAGATTCTTTGTTCCCAGGTTATTACCAAAG AGCTGAAGTTACAGCAGATGTCTTTGATGCTGACGGTTTCTATAGAACCGGTGACAT TATGGCAGAAGTCGGTCCTGAACAATTCGTATACTTAGATAGAAGAAACAACGTTTT GAAATTGTCTCAGGGTGAATTTGTAACTGTTTCAAAGTTGGAAGCTGTATTCGGTGA CTCTCCATTAGTTAGACAAATATATATATACGGTAATTCAGCCAGAGCTTATTTGTTA GCAGTCATAGTACCAACACAAGAAGCCTTGGATGCTGTTCCTGTCGAAGAATTGAA AGCCAGATTGGGTGACTCCTTGCAAGAAGTTGCAAAGGCCGCTGGTTTGCAAAGTT ACGAAATCCCAAGAGATTTCATCATCGAAACCACTCCTTGGACCTTAGAAAACGGTT TGTTAACTGGTATCAGAAAATTGGCTAGACCACAATTGAAAAAGCATTACGGTGAA TTGTTAGAACAAATATATACTGACTTGGCCCACGGTCAAGCTGATGAATTGAGATCC TTAAGACAAAGTGGTGCAGATGCCCCAGTATTAGTTACAGTCTGTAGAGCAGCCGC TGCATTGTTAGGTGGTTCCGCTAGTGATGTTCAACCTGACGCACATTTTACCGATTT GGGTGGTGACTCTTTGTCAGCTTTATCTTTTACAAATTTGTTGCACGAAATCTTCGAT ATAGAAGTACCAGTTGGTGTCATTGTATCACCTGCTAACGATTTGCAAGCATTGGCA GATTATGTTGAAGCCGCTAGAAAACCAGGTTCTTCAAGACCTACTTTTGCTTCTGTTC ATGGTGCATCAAATGGTCAAGTTACAGAAGTCCACGCTGGTGACTTGTCTTTGGATA AGTTCATTGATGCAGCCACTTTGGCCGAAGCTCCAAGATTACCTGCTGCAAACACTC AAGTAAGAACAGTTTTGTTAACCGGTGCTACTGGTTTCTTGGGTAGATATTTGGCAT TAGAATGGTTAGAAAGAATGGATTTGGTTGACGGTAAATTGATTTGCTTAGTCAGA GCAAAGTCCGACACTGAAGCAAGAGCCAGATTGGATAAAACATTCGATAGTGGTG ACCCAGAATTGTTAGCACATTACAGAGCTTTAGCAGGTGACCACTTGGAAGTTTTAG CCGGTGACAAGGGTGAAGCTGACTTGGGTTTAGATAGACAAACATGGCAAAGATT GGCTGATACCGTAGACTTAATCGTTGATCCAGCCGCTTTAGTCAACCATGTATTGCC ATACTCCCAATTGTTCGGTCCTAACGCATTGGGTACTGCTGAATTGTTGAGATTGGC TTTGACTTCTAAAATTAAGCCTTACTCCTACACCAGTACTATCGGTGTTGCAGATCAA ATTCCACCTTCAGCCTTCACTGAAGATGCTGACATAAGAGTCATCTCCGCAACAAGA GCCGTAGATGACAGTTATGCTAATGGTTACTCCAACAGTAAATGGGCAGGTGAAGT TTTGTTAAGAGAAGCCCATGATTTGTGTGGTTTACCAGTTGCTGTCTTTAGATGCGA CATGATTTTGGCAGATACAACCTGGGCCGGTCAATTGAACGTTCCAGATATGTTCAC AAGAATGATCTTGTCCTTAGCAGCCACCGGTATAGCTCCTGGTAGTTTCTATGAATT GGCTGCTGATGGTGCTAGACAAAGAGCACATTACGATGGTTTGCCAGTTGAGTTTA TTGCCGAAGCTATCTCCACCTTAGGTGCTCAAAGTCAAGATGGTTTCCATACTTATCA CGTAATGAATCCATACGATGACGGTATTGGTTTGGACGAATTTGTTGATTGGTTAAA CGAATCTGGTTGTCCTATTCAAAGAATAGCTGATTATGGTGACTGGTTACAAAGATT

	CGAAACTGCTTTGAGAGCATTACCAGATAGACAAAGACATTCCAGTTTGTTACCTTT GTTACACAATTACAGACAACCAGAAAGACCTGTCAGAGGTTCTATTGCTCCTACAGA TAGATTCAGAGCCGCTGTACAAGAAGCAAAAATAGGTCCAGATAAGGACATCCCTC ATGTTGGTGCTCCTATTATCGTAAAGTATGTATCAGATTTGAGATTGTTGGGTTTGTT GTAA
npgA	ATGGTGCAAGACACATCAAGCGCAAGCACTTCGCCAATTTTAACAAGATGGTACAT CGACACCCGCCCTCTAACCGCCTCAACAGCAGCCCTTCCTCTCCTTGAAACCCTCCAG CCCGCTGATCAAATCTCCGTCCAAAAATACTACCATCTGAAGGATAAACACATGTCT CTCGCCTCTAATCTGCTCAAATACCTCTTCGTCCACCGAAACTGTCGCATCCCCTGGT CTTCAATCGTGATCTCTCGAACCCCAGATCCGCACAGACGACCATGCTATATTCCACC CTCAGGCTCACAGGAAGACAGCTTCAAAGACGGATATACCGGCATCAACGTTGAGT TCAACGTCAGCCACCAAGCCTCAATGGTCGCGATCGCGGGAACAGCTTTTACTCCCA ATAGTGGTGGGGACAGCAAACTCAAACCCGAAGTCGGAATTGATATTACGTGCGTA AACGAGCGGCAGGGACGGAACGGGGAAGAGCGGAGCCTGGAATCGCTACGTCAA TATATTGATATATTCTCGGAAGTGTTTTCCACTGCAGAGATGGCCAATATAAGGAGG TTAGATGGAGTCTCATCATCCTCACTGTCTGCTGATCGTCTTGTGGACTACGGGTAC AGACTCTTCTACACTTACTGGGCGCTCAAAGAGGCGTATATAAAAATGACTGGGGA GGCCCTCTTAGCACCGTGGTTACGGGAACTGGAATTCAGTAATGTCGTCGCCCCGG CCGCTGTTGCGGAGAGTGGGGATTCGGCTGGGGATTTCGGGGAGCCGTATACGGG TGTCAGGACGACTTTATATAAAAATCTCGTTGAGGATGTGAGGATTGAAGTTGCTG CTCTGGGCGGTGATTACCTATTTGCAACGGCTGCGAGGGGTGGTGGGATTGGAGCT AGTTCTAGACCAGGAGGTGGTCCAGACGGAAGTGGCATCCGAAGCCAGGATCCCT GGAGGCCTTTCAAGAAGTTAGATATAGAGCGAGATATCCAGCCCTGTGCGACTGGG GTGTGTAATTGCCTATCCTAA
SeADO	ATGCCACAATTAGAAGCCTCCTTAGAATTAGACTTTCAATCAGAATCATATAAAGAT GCTTACAGTAGAATCAACGCAATCGTCATTGAAGGTGAACAAGAAGCATTTGATAA CTACAACAGATTGGCAGAAATGTTACCAGATCAAAGAGACGAATTGCATAAATTGG CCAAGATGGAACAAAGACACATGAAAGGTTTCATGGCTTGTGGTAAAAATTTGTCC GTTACTCCTGATATGGGTTTCGCACAAAAGTTTTTCGAAAGATTGCATGAAAACTTC AAAGCTGCAGCCGCTGAGGGTAAAGTTGTCACATGTTTGTTGATCCAATCTTTGATA ATCGAATGCTTTGCTATCGCAGCCTATAATATCTACATTCCAGTCGCTGATGCATTCG CCAGAAAGATTACCGAAGGTGTAGTTAGAGACGAATATTTGCACAGAAACTTCGGT GAAGAATGGTTGAAGGCAAACTTCGATGCTTCTAAGGCAGAATTGGAAGAAGCTA ATAGACAAAACTTGCCTTTAGTCTGGTTGATGTTAAATGAAGTAGCCGATGACGCTA GAGAATTGGGTATGGAAAGAGAATCATTAGTTGAAGACTTCATGATCGCATACGGT GAAGCCTTAGAAAACATCGGTTTTACTACCAGAGAAATAATGAGAATGTCCGCATA CGGTTTGGCAGCAGTCTAA
NpADO	ATGCAACAATTAACAGACCAATCAAAGGAATTAGACTTCAAATCAGAAACTTACAAA GATGCCTACTCCAGAATCAACGCAATCGTCATTGAAGGTGAACAAGAAGCACATGA AAACTACATCACCTTGGCCCAATTATTACCAGAATCCCATGATGAATTGATCAGATT GTCTAAGATGGAATCAAGACACAAAAAGGGTTTTGAAGCCTGTGGTAGAAATTTGG CTGTTACTCCTGACTTACAATTTGCCAAAGAATTTTTCTCTGGTTTGCACCAAAACTT CCAAACTGCTGCAGCCGAGGGTAAAGTTGTCACATGTTTGTTGATCCAATCATTAAT AATCGAATGCTTTGCTATCGCTGCATATAATATCTACATTCCAGTTGCCGATGACTTC GCTAGAAAAATTACAGAAGGTGTAGTTAAGGAAGAATATTCCCATTTGAACTTTGG

	TGAAGTCTGGTTAAAAGAACACTTCGCAGAGAGTAAGGCCGAATTGGAATTAGCAA ATAGACAAAACTTGCCTATCGTCTGGAAAATGTTAAATCAAGTAGAAGGTGACGCT CATACCATGGCAATGGAAAAGGATGCTTTGGTTGAAGACTTCATGATTCAATACGG TGAAGCATTATCAAACATAGGTTTTTCTACCAGAGACATTATGAGATTGAGTGCTTA CGGTTTGATAGGTGCTTGA

References

1 Verduyn, C., Postma, E., Scheffers, W. A. & Van Dijken, J. P. Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcoholic fermentation. *Yeast* **8**, 501-517 (1992).

2 Khara, B. *et al.* Production of propane and other short-chain alkanes by structure-based engineering of ligand specificity in aldehyde-deformylating oxygenase. *Chembiochem : a European journal of chemical biology* **14**, 1204-1208 (2013).

3 Huh, W. K. *et al.* Global analysis of protein localization in budding yeast. *Nature* **425**, 686-691 (2003).

4 Bakker, B. M. *et al.* The mitochondrial alcohol dehydrogenase Adh3p is involved in a redox shuttle in *Saccharomyces cerevisiae*. *J. Bacteriol.* **182**, 4730-4737 (2000).

5 Kondo, T. *et al.* Genetic engineering to enhance the Ehrlich pathway and alter carbon flux for increased isobutanol production from glucose by *Saccharomyces cerevisiae*. *J. Biotechnol.* **159**, 32-37 (2012).

6 Larroy, C., Fernandez, M. R., Gonzalez, E., Pares, X. & Biosca, J. A. Characterization of the *Saccharomyces cerevisiae* YMR318C (ADH6) gene product as a broad specificity NADPH-dependent alcohol dehydrogenase: relevance in aldehyde reduction. *Biochem J.* **361**, 163-172 (2002).

7 Hansen, E. H. *et al.* De novo biosynthesis of vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*). *Appl Environ Microb* **75**, 2765-2774 (2009).

8 Larroy, C., Pares, X. & Biosca, J. A. Characterization of a *Saccharomyces cerevisiae* NADP(H)-dependent alcohol dehydrogenase (ADHVII), a member of the cinnamyl alcohol dehydrogenase family. *Eur. J. Biochem.* **269**, 5738-5745 (2002).

9 Dickinson, J. R., Salgado, L. E. & Hewlins, M. J. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *The Journal of biological chemistry* **278**, 8028-8034 (2003).

10 Toivari, M. H., Salusjarvi, L., Ruohonen, L. & Penttila, M. Endogenous xylose pathway in *Saccharomyces cerevisiae*. *Appl Environ Microb* **70**, 3681-3686 (2004).

11 Gonzalez, E. *et al.* Characterization of a (2R,3R)-2,3-butanediol dehydrogenase as the *Saccharomyces cerevisiae* YAL060W gene product - Disruption and induction of the gene. *The Journal of biological chemistry* **275**, 35876-35885 (2000).

12 Gonzalez, E. *et al.* Role of *Saccharomyces cerevisiae* oxidoreductases Bdh1p and Ara1p in the metabolism of acetoin and 2,3-butanediol. *Appl Environ Microb* **76**, 670-679 (2010).

13 Muller, M., Katzberg, M., Bertau, M. & Hummel, W. Highly efficient and stereoselective biosynthesis of (2S,5S)-hexanediol with a dehydrogenase from *Saccharomyces cerevisiae*. *Org. Biomol. Chem.* **8**, 1540-1550 (2010).

14 Jordan, D. B. *et al.* Kinetic mechanism of an aldehyde reductase of *Saccharomyces cerevisiae* that relieves toxicity of furfural and 5-hydroxymethylfurfural. *BBA-Proteins Proteom.* **1814**, 1686-1694 (2011).

15 Hwang, J. Y. *et al.* Simultaneous synthesis of 2-phenylethanol and L-homophenylalanine using aromatic transaminase with yeast ehrlich pathway. *Biotechnol. Bioeng.* **102**, 1323-1329 (2009).
16 Moon, J. & Liu, Z. L. Direct enzyme assay evidence confirms aldehyde reductase function of Ydr541cp and Ygl039wp from *Saccharomyces cerevisiae*. *Yeast* **32**, 399-407 (2015).
17 Han, G. *et al.* The *Saccharomyces cerevisiae* YBR159w gene encodes the 3-ketoreductase of the microsomal fatty acid elongase. *The Journal of biological chemistry* **277**, 35440-35449 (2002).
18 Yang, Y., Zhu, D., Piegat, T. J. & Hua, L. Enzymatic ketone reduction: mapping the substrate profile of a short-chain alcohol dehydrogenase (YMR226c) from *Saccharomyces cerevisiae*. *Tetrahedron-Asymmetr.* **18**, 1799-1803 (2007).
19 Katz, M., Hahn-Hagerdal, B. & Gorwa-Grauslund, M. F. Screening of two complementary collections of *Saccharomyces cerevisiae* to identify enzymes involved in stereo-selective reductions of specific carbonyl compounds: an alternative to protein purification. *Enzyme Microb Tech* **33**, 163-172 (2003).
20 Maruyama, R., Nishizawa, M., Itoi, Y., Ito, S. & Inoue, M. The enzymes with benzil reductase activity conserved from bacteria to mammals. *J. Biotechnol.* **94**, 157-169 (2002).
21 Athenstaedt, K. & Daum, G. 1-Acyldihydroxyacetone-phosphate reductase (Ayr1p) of the yeast *Saccharomyces cerevisiae* encoded by the open reading frame YIL124w is a major component of lipid particles. *The Journal of biological chemistry* **275**, 235-240 (2000).
22 Delneri, D., Gardner, D. C., Bruschi, C. V. & Oliver, S. G. Disruption of seven hypothetical aryl alcohol dehydrogenase genes from *Saccharomyces cerevisiae* and construction of a multiple knock-out strain. *Yeast* **15**, 1681-1689 (1999).
23 Chang, Q., Griest, T. A., Harter, T. M. & Petrash, J. M. Functional studies of aldo-keto reductases in *Saccharomyces cerevisiae*. *BBA-Mol. Cell Res.* **1773**, 321-329 (2007).
24 Petrash, J. M. *et al.* Functional genomic studies of aldo-keto reductases. *Chem.-Biol. Interact.* **130**, 673-683 (2001).
25 van Bergen, B., Strasser, R., Cyr, N., Sheppard, J. D. & Jardim, A. alpha,beta-dicarbonyl reduction by *Saccharomyces* D-arabinose dehydrogenase. *BBA-Gen. Subjects* **1760**, 1636-1645 (2006).
26 Traff, K. L., Otero Cordero, R. R., van Zyl, W. H. & Hahn-Hagerdal, B. Deletion of the GRE3 aldose reductase gene and its influence on xylose metabolism in recombinant strains of *Saccharomyces cerevisiae* expressing the xylA and XKS1 genes. *Appl Environ Microb* **67**, 5668-5674 (2001).
27 Rintala, E., Pitkanen, J. P., Vehkomaki, M. L., Penttila, M. & Ruohonen, L. The ORF YNL274c (GOR1) codes for glyoxylate reductase in *Saccharomyces cerevisiae*. *Yeast* **24**, 129-136 (2007).
28 Overkamp, K. M. *et al.* Functional analysis of structural genes for NAD(+)-dependent formate dehydrogenase in *Saccharomyces cerevisiae*. *Yeast* **19**, 509-520 (2002).
29 Buijs, N. A., Zhou, Y. J., Siewers, V. & Nielsen, J. Long-chain alkane production by the yeast *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **112**, 1275-1279 (2015).
30 Valle-Rodriguez, J. O., Shi, S. B., Siewers, V. & Nielsen, J. Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid ethyl esters, an advanced biofuel, by eliminating non-essential fatty acid utilization pathways. *Appl Energ* **115**, 226-232 (2014).
31 Oud, B. *et al.* An internal deletion in MTH1 enables growth on glucose of pyruvate-decarboxylase negative, non-fermentative *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **11**, 131 (2012).