High-throughput screening of an octanoic acid producer strain library enables detection of new targets for increasing titers in *Saccharomyces cerevisiae*

Leonie Baumann,[1] Stefan Bruder,[2] Johannes Kabisch,[2] Eckhard Boles[1] and Mislav Oreb*[1]

[1]Institute of Molecular Biosciences, Faculty of Biological Sciences, Goethe University Frankfurt, Max-von-Laue Straße 9, 60438 Frankfurt am Main, Germany

[2]Department of Biology, Computer-aided Synthetic Biology, Technical University Darmstadt, Schnittspahnstr. 10, 64287 Darmstadt, Germany

*Corresponding author: m.oreb@bio.uni-frankfurt.de

SUPPORTING INFORMATION

Table S1. Plasmids used in this study.

Plasmid	Characteristics	Reference
Plasmids from library		
pGP564 ("control")	2µ, *LEU2*, *KanR*	[2]
Library	pGP564, yeast genomic DNA fragments of ~ 10 kb size	[2]
Plate A10, well F1 ("*FAS1"*)	pGP564, [*YKL183C-A*]*, [*LOT5*], *FAS1*, [*PRS1*]*	[2]
Plate A16, well E2 ("*FAS2"*)	pGP564, [*SSO1*]&, *FAS2*, *YPL230W,* [*YPL229W*]*	[2]
Plate A1, well F6 ("F7-E2.8")	pGP564, [*YBL046W*]&, *COR1*, *YBL044W*, *ECM13*, *FUI1*, [*PRE7*]*	[2]
Plate A13, well A3 ("F7-E3.1")	pGP564, [*ESC1*]&, *ERG8*, *FMP42*, *FSH2*, *UBP8*, *MRE11*, *MRPL44*, *YMR226C*, *TAF7*, [*MTF1*]*	[2]
Plate A1, well C1 ("F8-E2.4")	pPGP564, *GDH3*, [*YAL061W*]*	[2]
Plate A3, well E7 ("F8-E2.7")	pPGP564, [*PSF1*], *RAD61*, *HED1*, *DAD1*, *KCS1*	[2]
Plate A12, well G7 ("F8-E2.10")	pPGP564, [*YML023C*]*, *APT1*, *UNG1*, *YML020W*, *OST6*, *YML018C*, [*PSP2*]*	[2]
Plate A13, well B3 ("*TAF7-MTF1*")	pPGP564, [*YMR226C*]*, *TAF7*, *MTF1*, *RRP5*, *RPS10B*, *YMR230W-A*, [*PEP5*]*	[2]
Plasmids for CRISPR		
pRCC-K	2µ, *kanMX*, *AmpR, pROX3-Cas9^{opt}-tCYC1*, *pSNR52-gRNA-tSUB4*	[3]
pRCC-N	2µ, *natNT2*, *AmpR*, *pROX3-Cas9^{opt}-tCYC1*, *pSNR52-gRNA-tSUB4*	[3]
pRCC-K-LBV32	pRCC-K with *gRNA* for *pWAR1* locus (TTGCTCCTACATTTATCGGA)	This study
pRCC-N-LBV47	pRCC-N with *gRNA* for *URA3* locus (AACGTTACAGAAAAGCAGGC)	This study
pRCC-K-LBV103	pRCC-K with *gRNA* for *PYK2* locus (ATGTCTTTGGCGGACAAGGG)	[1]
pRCC-N-SHV42	pRCC-N with *gRNA* for *FAA2* locus (GAAGATTTTGAAACCTTACG)	[4]
Other plasmids		
LBV14	2µ, *URA3*, *AmpR*, *pPDR12-EnvyGFP-tCYC1*	[1]
LBV91 ("*[PSF1]-RAD61*")	pGP564, [*PSF1*], *RAD61*	This study
LBV92 ("*HED1-DAD1-KCS1*")	pGP564, *HED1*, *DAD1*, *KCS1*	This study
LBV96 ("*[ESC1]&-ERG8*")	pGP564, [*ESC1*]&, *ERG8*	This study
LBV97 ("*FMP42-FSH2*")	pGP564, *FMP42*, *FSH2*	This study
LBV98 ("*UBP8-MRE11*")	pGP564, *UBP8*, *MRE11*	This study
LBV99 ("*MRPL44-YMR226C*")	pGP564, *MRPL44*, *YMR226C*	This study
LBV104 ("*HED1*")	pGP564, *HED1*	This study
LBV105 ("*DAD1*")	pGP564, *DAD1*	This study
LBV106 ("*KCS1*")	pGP564, *KCS1*	This study
LBV111 ("*FMP42*")	pGP564, *FMP42*	This study
LBV112 ("*FSH2*")	pGP564, *FSH2*	This study
LBV113 ("*KCS1-FSH2*")	pGP564, *KCS1*, *FSH2*	This study
LBGV023	ConLS'-gfp dropout-ConRE'-*LEU2*-CEN6-ARS4-*KanR*-ColE1	[5]
FWV26	pRS313, CEN6-ARS4, AmpR, *HIS3*	[6]
RPB34 ("*FAS1*")	pRS315, CEN6-ARS4, AmpR, *LEU2*, pFAS1-FAS1wt	[6]
RPB36 ("*FAS1^{RK}*")	pRS315, CEN6-ARS4, AmpR, *LEU2*, pFAS1-FAS1RK	[6]
RPB38 ("*FAS2*")	pRS313, CEN6-ARS4, AmpR, *HIS3*, pFAS2-FAS2wt	[6]

Table S2. Oligonucleotides used in this study.

Primer	Sequence 5'-3'	Application
Insertion of biosensor in *ura3* locus		
MRP130	CGCCTGCTTTTCTGTAACGTTGATCATTTATCTTTCACTGCGGAG	Amplification of pRCC-N with gRNA sequence for cutting in *ura3*
MRP131	AACGTTACAGAAAAGCAGGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	
WGP234	CTTGGTGGTGTTCGTCGTATCTCTTAATCATAGAAGCAGACAATGGAG	
WGP235	TGTTGTCTGACATTTTGAGAGTTAACACCGAAATTACCAAGGCTC	
LBP195	GTATACATGCATTTACTTATAATACAGTTTTGATATCTTTGTTTTGCATTTTACATTC	Amplification of biosensor (*pPDR12-EnvyGFP-tCYC1*) from LBY27 genomic DNA
LBP196	CTTTAATTTGCGGCCGGTACCCAATTCGCC	
MRP141	AACGCATGAAATCCTTCATTTG	Amplification for proof of successful integration
MRP142	CGAAGGAAGGAGCACAGAC	
Replacement of *pWAR1* by *pPDR12*		
WGP234	CTTGGTGGTGTTCGTCGTATCTCTTAATCATAGAAGCAGACAATGGAG	Amplification of pRCC-K with gRNA sequence for cutting in *pWAR1*
WGP235	TGTTGTCTGACATTTTGAGAGTTAACACCGAAATTACCAAGGCTC	
LBP199	TTGCTCCTACATTTATCGGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	
LBP200	TCCGATAAATGTAGGAGCAAGATCATTTATCTTTCACTGCGGAG	
LBP201	GAATTCTGTTGTTGTAATTGTCATAACTATTGAGCGATATCTTTGTTTTGCATTTTAC	Amplification of *pPDR12* with overhangs to *pWAR1* up- and downstream regions
LBP202	GACGGCAACGCCAGTTATTGCAATCTGCGTGTCCATTTTTTTATTAATAAGAACAATAAC	
LBP203	GACGCCACTGATATAAATCG	Amplification for proof of successful integration
LBP97	GCTTCATAATAGTTCCTCTGG	
Knockout of *FAA2*		
RPP266	GAAGTCCCGGTGTCCCTGACGTTATTGTAG	Amplification donor DNA for *faa2* knockout from SHY34 genomic DNA & proof of successful knockout
RPP267	GTGACCCATGTACTCCGCTAGATTGACCAG	
SHP80	TTAGCCGGTTACACCAAAGG	Amplification of an internal part of *FAA2*
LBP275	GCCACGAATTTGCAGTTC	
Cloning of LBV91-92, 96-99, 104-106, 111-113		
LBP375	GTGGCGGCCGCTCTAGAACTAGTGGATC	Amplification of fragment for LBV91 from F8-E2.7
LBP376	TATCGAATTCCTGCAGCCCGGGGGATCTCGTCTACCCGAAGTACTCTAGGCTTCCTATGC	
LBP377	CGGTGGCGGCCGCTCTAGAACTAGTGGATCTCCGCACCTTTTAAAAAAGGTTGAAAGGGC	Amplification of fragment for LBV92 from F8-E2.7
LBP393	CGGTATCGATAAGCTTGATATCGAATTCC	
LBP383	GCTTGATATCGAATTCCTGCAGCCCGGGGGATCGTGCACACTTTCAAGCTAACACGCAC	Amplification of fragment for LBV96 from F7-E3.1
LBP390	GGCGGCCGCTCTAGAACTAGTG	
LBP384	CACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCTTAAGGTAGATAATAGTGGTCCAT	Amplification of fragment for LBV97 from F7-E3.1
LBP385	GTGAGCGCGCGTAATACGACTCACTATAGGAATTATTAATAACAAATAAAAAAAGCAGGG	
LBP386	GCGGTGGCGGCCGCTCTAGAACTAGTGGATCGGTTACAGCTATTAAATCTTATAGTCTTG	Amplification of fragment for LBV98 from F7-E3.1
LBP387	GCTTGATATCGAATTCCTGCAGCCCGGGGGATCGAAAATAAAGGCATCTACAAATCTCAT	

LBP388	GCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCAGTCG AGTTTTATCGGATCTG	Amplification of fragment for LBV99 from F7-E3.1
LBP394	GTATCGATAAGCTTGATATCGAATTCCGTTCTCTTTATCATCTATA TATTACTCTTATAC	
LBP377	CGGTGGCGGCCGCTCTAGAACTAGTGGATCTCCGCACCTTTTAAA AAAGGTTGAAAGGGC	Amplification of fragment for LBV104 from F8-E2.7
LBP410	GTCGACGGTATCGATAAGCTTGATATCGAATTCAGTTGCGGTTCC CTCTGTCTCTC	
LBP411	CCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGTTCAAAGAGGA GAACGTTTG	Amplification of fragment for LBV105 from F8-E2.7
LBP412	GTATCGATAAGCTTGATATCGAATTCAAAGAAAGGATAGAACTA ATGAATATTCT	
LBP413	CCGCGGTGGCGGCCGCTCTAGAACTAGTGATGTACATATATCCTC ACACGTCCG	Amplification of fragment for LBV106 from F8-E2.7
LBP393	CGGTATCGATAAGCTTGATATCGAATTCC	
LBP384	CACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCTTAAGGTA GATAATAGTGGTCCAT	Amplification of fragments for LBV111 from F7-E3.1
LBP431	CTGACACAATGGACAATTAAATAAAATTAAGTAAAAAAAATAAG GACTTTAATTTTTAAACGGTGAGTAAGGAAAG	
LBP385	GTGAGCGCGCGTAATACGACTCACTATAGGAATTATTAATAACAA ATAAAAAAAGCAGGG	Amplification of fragment for LBV112 from F7-E3.1
LBP433	CACCGCGGTGGCGGCCGCTCTAGAACTAGTGTACAGTCTTTACTG CCCTAATA	
LBP413	CCGCGGTGGCGGCCGCTCTAGAACTAGTGATGTACATATATCCTC ACACGTCCG	Amplification of fragments for LBV113 from F8-E2.7 and F7-E3.1
LBP436	GGCCAGTATTAGGGCAGTAAAGACTGTACGAAAAACACTTTTCT GTTCTTGTTTGTC	
LBP385	GTGAGCGCGCGTAATACGACTCACTATAGGAATTATTAATAACAA ATAAAAAAAGCAGGG	
LBP437	GACAAACAAGAACAGAAAAGTGTTTTTCGTACAGTCTTTACTGCC CTAATA	

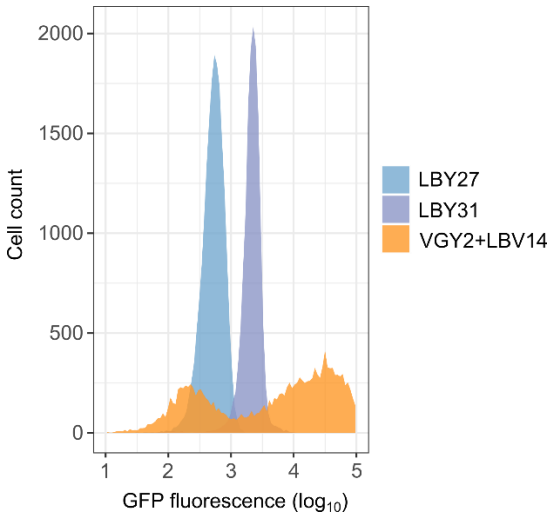

Figure S1. Fluorescence signal of genomically integrated vs. plasmid-based biosensor. The two strains LBY27 and LBY31 contain a stable genomic integration of the biosensor *pPDR12-GFP* whereas VGY2 was transformed with a multi-copy plasmid (LBV14) containing *pPDR12-GFP*. All strains were inoculated to an OD_{600} = 0.1 and grown in buffered (selective) SCD medium. Aliquots were taken after 4 h for GFP fluorescence measurement in a flow cytometer (common logarithmic transformation of fluorescence area plotted). LBY27 (non-producer). VGY2 (producer): *FAS1*^{RK}. LBY31 (producer): *FAS1*^{RK}, Δ*pWAR1*::*pPDR12*.

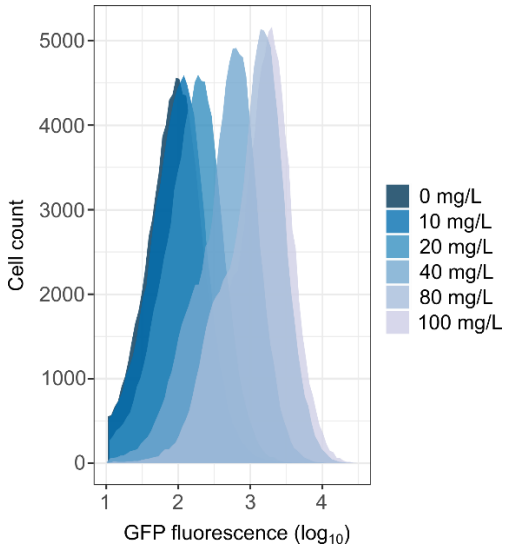

Figure S2. Evaluation of biosensor response to supplemented octanoic acid. Strain LBY27, which contains a stable integration of the biosensor *pPDR12-GFP*, was inoculated to an OD_{600} = 0.1 and cultivated until stationary phase. The indicated octanoic acid amounts were added, and the cultures were cultivated for another 3 h before measurement of GFP fluorescence in a flow cytometer (common logarithmic transformation of fluorescence area plotted).

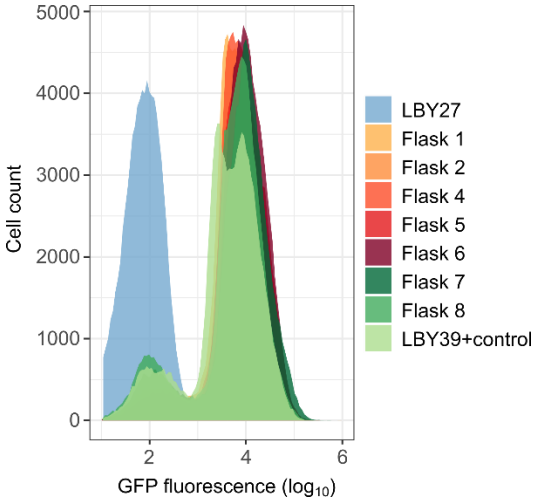

Figure S3. Fluorescence signals of library populations (E1 sorting). Strain LBY27 (non-producer) and LBY39+control (producer + empty vector) serve as controls. All contain a stable integration of the biosensor *pPDR12-GFP*. Populations of Flasks 1-8 (LBY39 with library) were grown for 48 h in buffered SCD^{-leu} medium before measurement in a flow cytometer (common logarithmic transformation of fluorescence area plotted). The top 10 % of fluorescent events of each of the Flasks 1-8 were sorted. LBY39: *FAS1*^{RK}, Δ*faa2*.

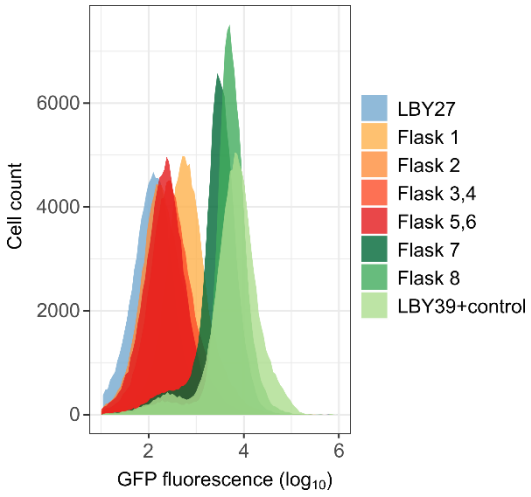

Figure S4. Fluorescence signals of library populations (E2 sorting). Strain LBY27 (non-producer) and LBY39+control (producer + empty vector) serve as controls. All contain a stable integration of the biosensor *pPDR12-GFP*. Populations of Flasks 1-8 (LBY39 with library) were grown for 66 h in buffered SCD^{-leu} medium before measurement of GFP fluorescence in a flow cytometer (common logarithmic transformation of fluorescence area plotted). The top 7.5 % of fluorescent events of Flask 7 and Flask 8, respectively, were sorted. LBY39: *FAS1*^{RK}, Δ*faa2*.

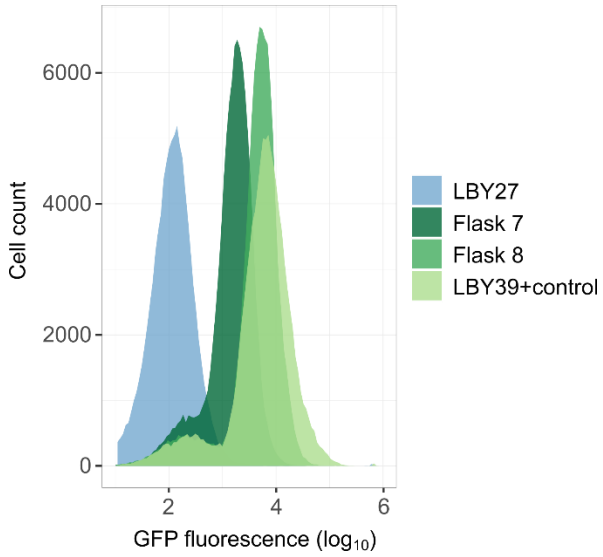

Figure S5. Fluorescence signals of library populations (E3 sorting). Strain LBY27 (non-producer) and LBY39+control (producer + empty vector) serve as controls. All contain a stable integration of the biosensor *pPDR12-GFP*. Populations of Flasks 7-8 (LBY39 with library) were grown for 66 h in buffered SCD^{-leu} medium before measurement of GFP fluorescence in a flow cytometer (common logarithmic transformation of fluorescence area plotted). The top 5 % and top 1% of fluorescent events of Flask 7 and Flask 8, respectively, were sorted. LBY39: $FAS1^{RK}$, Δ*faa2*.

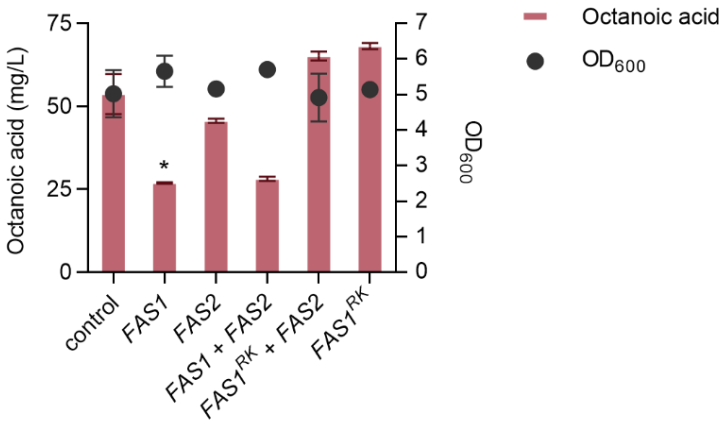

Figure S6. Effect of additional expression of *FAS* genes in LBY38 ($FAS1^{RK}$) background strain. OD_{600} (dots) and octanoic acid titers (bars) of octanoic acid producer strain LBY38 with plasmids containing indicated genes (+ empty plasmid LBGV023 or FWV26, for those with only one gene indicated). As control served LBY38 carrying empty vectors FWV26 and LBGV023. Fatty acids were extracted, methylated and quantified via GC 72 h after inoculation in buffered $SCD^{-leu\text{-}his}$ medium. LBY38: $FAS1^{RK}$, Δ*faa2*. *n* = 2, error bars = ± SD. Statistical analysis (indicated column vs. control) was performed using two-tailed unpaired *t* test (∗ $P < 0.05$).

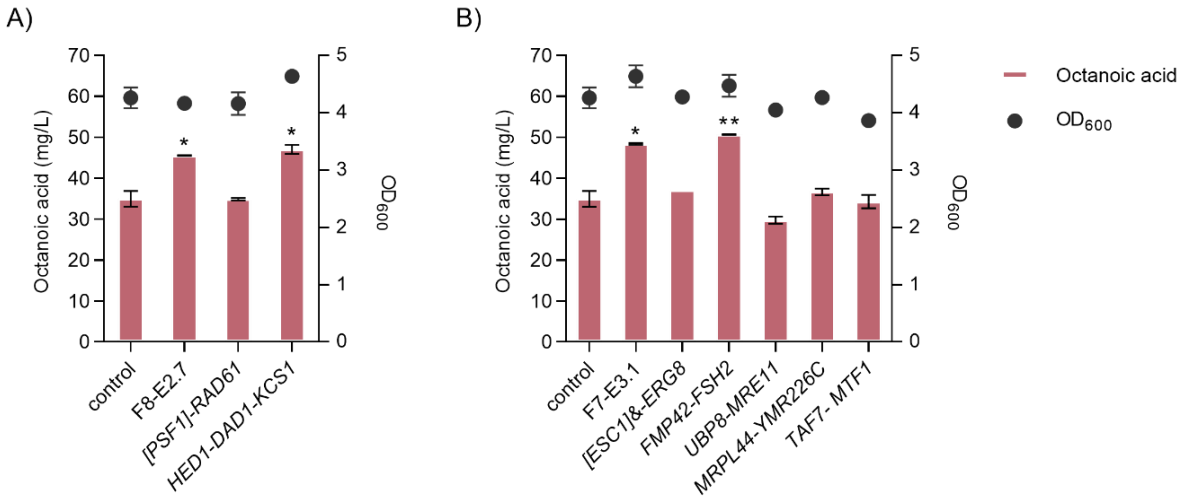

Figure S7. Identification of genes increasing octanoic acid titers. OD_{600} (dots) and octanoic acid titers (bars) of octanoic acid producer strain LBY38 containing either the vector control or plasmids with the indicated genes. (A) Plasmid F8-E2.7 contains all five genes indicated on the x-axis (B) Plasmid F7-E3.1 contains all ten genes indicated on the x-axis. Fatty acids were extracted, methylated and quantified via GC 48 h after inoculation in buffered SCD^{-leu} medium. LBY38 (producer): *FAS1*^{RK}, Δ*faa2*. *[]*: intact ORF, possibly missing up- or downstream regions. *[]&:* 5‘ end of gene is missing. *n* = 2, error bars = ± SD. Statistical analysis (indicated column vs. control) was performed using two-tailed unpaired *t* test (∗ $P < 0.05$, ∗∗ $P < 0.01$).

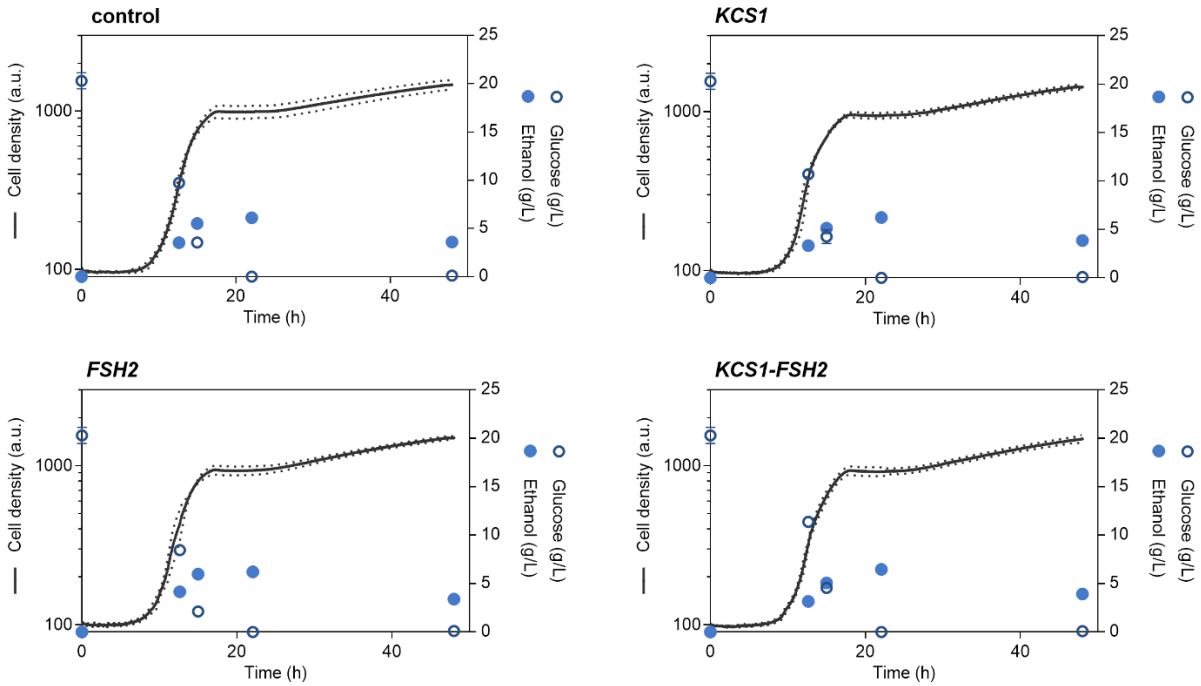

Figure S8. Growth and metabolic performance of selected strains. Cell densities and glucose and ethanol titers of strain CEN.PK2-1C containing either the vector control or plasmids with the indicated genes. Strains were grown in buffered SCD^{-leu} medium in shake flasks on a cell growth quantifier and samples were taken for HPLC measurement over the course of fermentation. *n* = 3, error bars = ± SD.

REFERENCES

(1) Baumann, L., Rajkumar, A. S., Morrissey, J. P., Boles, E., and Oreb, M. (2018) A yeast-based biosensor for screening of short- and medium-chain fatty acid production. *ACS Synth. Biol. 7*, 2640–2646.

(2) Jones, G. M., Stalker, J., Humphray, S., West, A., Cox, T., Rogers, J., Dunham, I., and Prelich, G. (2008) A systematic library for comprehensive overexpression screens in *Saccharomyces cerevisiae*. *Nat. Methods 5*, 239–241.

(3) Generoso, W. C., Gottardi, M., Oreb, M., and Boles, E. (2016) Simplified CRISPR-Cas genome editing for *Saccharomyces cerevisiae*. *J. Microbiol. Methods 127*, 203–205.

(4) Henritzi, S., Fischer, M., Grininger, M., Oreb, M., and Boles, E. (2018) An engineered fatty acid synthase combined with a carboxylic acid reductase enables de novo production of 1-octanol in *Saccharomyces cerevisiae*. *Biotechnol. Biofuels 11*.

(5) Lee, M. E., DeLoache, W. C., Cervantes, B., and Dueber, J. E. (2015) A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly. *ACS Synth. Biol. 4*, 975–986.

(6) Gajewski, J., Pavlovic, R., Fischer, M., Boles, E., and Grininger, M. (2017) Engineering fungal *de novo* fatty acid synthesis for short chain fatty acid production. *Nat. Commun. 8*, 14650.