

Title	A yeast-based biosensor for screening of short- and medium-chain fatty acid production
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Publication date	2018-10-19
Original Citation	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 synthetic biology, 7(11), [7pp]. DOI:10.1021/acssynbio.8b00309
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://pubs.acs.org/doi/10.1021/acssynbio.8b00309 - 10.1021/acssynbio.8b00309
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Download date	2024-07-18 12:20:35
Item downloaded from	https://hdl.handle.net/10468/8434



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A Yeast-Based Biosensor for Screening of Short- and Medium-Chain Fatty Acid Production

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SUPPORTING INFORMATION

Table S1. Yeast strains and plasmids used in this study.

Strain	Characteristics	Reference
CEN.PK113-7D	<i>MATa</i> ; <i>MAL2-8c</i> ; <i>SUC2</i>	Euroscarf, Frankfurt am Main, Germany
CEN.PK113-11C	<i>MATa</i> ; <i>MAL2-8c</i> ; <i>SUC2</i> ; <i>ura3-52</i> ; <i>his3Δ1</i>	Euroscarf, Frankfurt am Main, Germany
LBV27	CEN.PK113-11C Δ <i>pyk2</i> :: <i>pPDR12</i> -GFP	This study
RPY21/FAS ^{R1834K}	<i>MATa</i> ; <i>ura3Δ0</i> ; <i>his3Δ0</i> ; <i>leu2Δ0</i> ; <i>TRP1</i> ; <i>lys2Δ0</i> ; <i>MET15</i> ; Δ <i>FAS1</i> :: <i>kanMX4</i> ; Δ <i>FAS2</i> :: <i>kanMX4</i> ; Δ <i>faa2</i> ; transformed with plasmids pRS315-FAS1 ^{R1834K} and pRS313-FAS2	¹
Plasmid	Characteristics	Reference
Plasmids used for fermentations		
p426pMET25-GFP	2 μ , <i>URA3</i> , <i>Amp^r</i> , <i>pMET25</i> -GFP- <i>tCYC1</i>	This study
p426pPDR12-GFP	2 μ , <i>URA3</i> , <i>Amp^r</i> , <i>pPDR12</i> -GFP- <i>tCYC1</i>	This study
pRS42H	2 μ , <i>hphNT1</i> , <i>Amp^r</i> , multiple cloning site including <i>EcoRV</i>	²
LBV17	pRS42H with <i>pPGK1-TPO1-tTPO1</i> integrated in <i>EcoRV</i> site	This study
LBV20	pRS42H with <i>pPYK1-ACC1^{S659AS1157A}-tACC1</i> integrated in <i>EcoRV</i> site	This study
pRS315-FAS1 ^{R1834K}	<i>CEN6/ARS4</i> , <i>LEU2</i> , <i>Amp^r</i> , <i>pADH2-FAS1^{R1834K}-tFAS1</i>	³
pRS313-FAS2	<i>CEN6/ARS4</i> , <i>HIS3</i> , <i>Amp^r</i> , <i>pADH2-FAS2-tFAS2</i>	³
Plasmids used for CRISPR/Cas9		
pRCC-K	2 μ , <i>kanMX</i> , <i>Amp^r</i> , <i>pROX3-Cas9^{opt}-tCYC1</i> , <i>pSNR52-gRNA</i>	⁴
pRCC-K-PYK2	pRCC-K with <i>gRNA</i> for <i>PYK2</i> locus	This study

Table S2. Oligonucleotides used in this study.

Primer	Sequence 5'-3'	Application
Plasmid construction or sequencing		
LBP63	CAATTAACCCTCACTAAAGGGGAACAAAAGCTGGAGCTGATATCTT TGTTTTGCATTTTAC	Amplification of <i>pPDR12</i> from CEN.PK113-11C with overhangs to the p426pMET25-GFP backbone
LBP64	CTACACCTGTAAACAATTCCTCGCCTTTAGACATTTTTTTATTAATA AGAACAATAAC	
LBP103	CTAATGTAGGCCATGGAAC	Sequencing of GFP
LBP76	GGTCGACGGTATCGATAAGCTTGATCCCGGGATAGTAGAAAAA AAGGGGATATCACTAC	Amplification of <i>TPO1-tTPO1</i> from CEN.PK113-11C with overhangs to pRS42H and <i>pPGK1</i> , respectively
LBP77	GTAATTATCTACTTTTTACAACAAATATAAAACAATGTCGGATCAT TCTCCATTCTAA	
LBP78	TTAGAAATGGGAGAATGATCCGACATTGTTTTATATTGTTGTA AAAGTAGATAATTAC	Amplification of <i>pPGK1</i> from CEN.PK113-11C with overhangs to <i>TPO1</i> and pRS42H, respectively
LBP79	GGTGGCGGCCGCTCTAGAACTAGTGGATCCCCGGGAATTACCG TCGCTCGTGATTG	
LBP80	GCTACTGCTGAGAACCTG	Sequencing of LBV17
VSP159	CGTGTGACAACAACAGCC	
LBP81	GACTCACTATAGGGCGAATTGGGTACCGGGCCCCGACAGATTGG GAGATTTTCATAGTAG	Amplification of <i>pPYK1</i> from CEN.PK113-11C with overhangs to pRS42H and <i>ACC1</i> , respectively
LBP82	GAAGACTCGAATAAGCTTTCTTCGCTCATTGTGATGATGTTTTATT TGTTTTGATTGGTG	
LBP83	CACCAATCAAAACAATAAAACATCATCAATGAGCGAAGAAA GCTTATTCGAGTCTTC	Amplification of <i>ACC1</i> with <i>S659A</i> and <i>S1157A</i> and <i>tACC1</i> with overhangs to <i>pPYK1</i> and pRS42H, respectively
RPP108	CTATGGCAATCAAAAGACCACCATCAGCTAGTTGAC	
RPP107	GATATCATACTGCGTCAACTAGCTGATGG	
RPP088	CATATGACAAATCTGAAACAGCAACAGCCCTGTTTCATAC	
RPP087	ATGGGTATGAACAGGGCTGTTGCTGTTTCAGATTTGTCATATGTT G	
LBP84	GTAATCTGAAGGATCTGTTTGAGCGCTTCCATCGGGCCCATCGAA TTCCTGCAGCCCGGG	
LBP98	CTTGTCCATCCAATCTGTTC	
LBP99	CCAAATAAGCACCGATACC	Sequencing of LBV20
LBP100	GCAACCATTCCTTAACAGG	
LBP101	GACATACAGAACTTCCAGG	
LBP102	GGAACATAGTTTGCAGTAGG	
RPP89	TTCGAAACCTTCTGTAGAAGCAACACAAAC	
RPP90	CGGTCAAGGAAAGAAGTGAACAAATTGAAC	
RPP109	TCCAACCTTGCCGTCATTGCT	
RPP056	CACACAGGAAACAGCTATGAC	Sequencing of p426pPDR12-GFP, LBV17 and LBV20
LBP85	CGTTACCCAACCTAATCGCC	
Insertion of <i>pPDR12-GFP</i> in <i>PYK2</i>		
LBP108	GTCCATTGTAAGATTACAACAAAAGCACTATCGGGCGAATTGGG TACCGG	Amplification of <i>pPDR12-GFP-tCYC1</i> from p426pPDR12-GFP with overhangs to <i>PYK2</i>
LBP109	ATTAAGTAAAAAATAAGGACTTTAATTTTTAGATATCTTTGTTT TGCATTTTACATTC	
LBP118	CAGAGCGGTGAAACGCAAC	Amplification and sequencing of Δ <i>pyk2::pPDR12-GFP-tCYC1</i>
LBP119	CGCAGTTTGCGAACATTACCTG	
Amplification of CRISPR/Cas9 plasmid pRCC-K		
WGP234	CTTGGTGGTGTTCGTCGTATCTCTTAATCATAGAAGCAGACAATG GAG	Amplification of pRCC-K with gRNA for <i>PYK2</i>
WGP235	TGTTGTCTGACATTTTGAGAGTTAACACCGAAATTACCAAGGCTC	
MGP193	TTGCAATTCGGAGTCCGCAAGTTTTAGAGCTAGAAATAGCAAGTT AAAATAAGG	

MGP194	CTTGCGGACTCCGAATTGCAAGATCATTTATCTTTCCTGCGGAG	
gRNA sequences used for deletion of <i>PYK2</i>		
<i>PYK2</i> gRNA	TTGCAATTCGGAGTCGCAA	

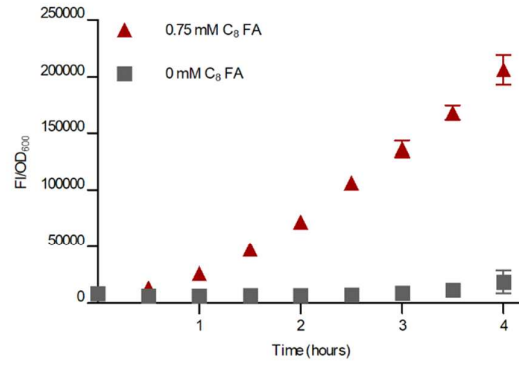


Figure S1. Time-dependent response of the biosensor in SCD medium with and without C₈ fatty acids. Relative fluorescence intensity (fluorescence intensity (FI) divided by OD₆₀₀) of the plasmid-based sensor in response to 0 (grey squares) and 0.75 mM (red triangles) C₈ fatty acids (FA), respectively. The background fluorescence of the biosensor strain not exposed to C₈ FA is very low over the entire time course. Error bars represent two technical replicates.

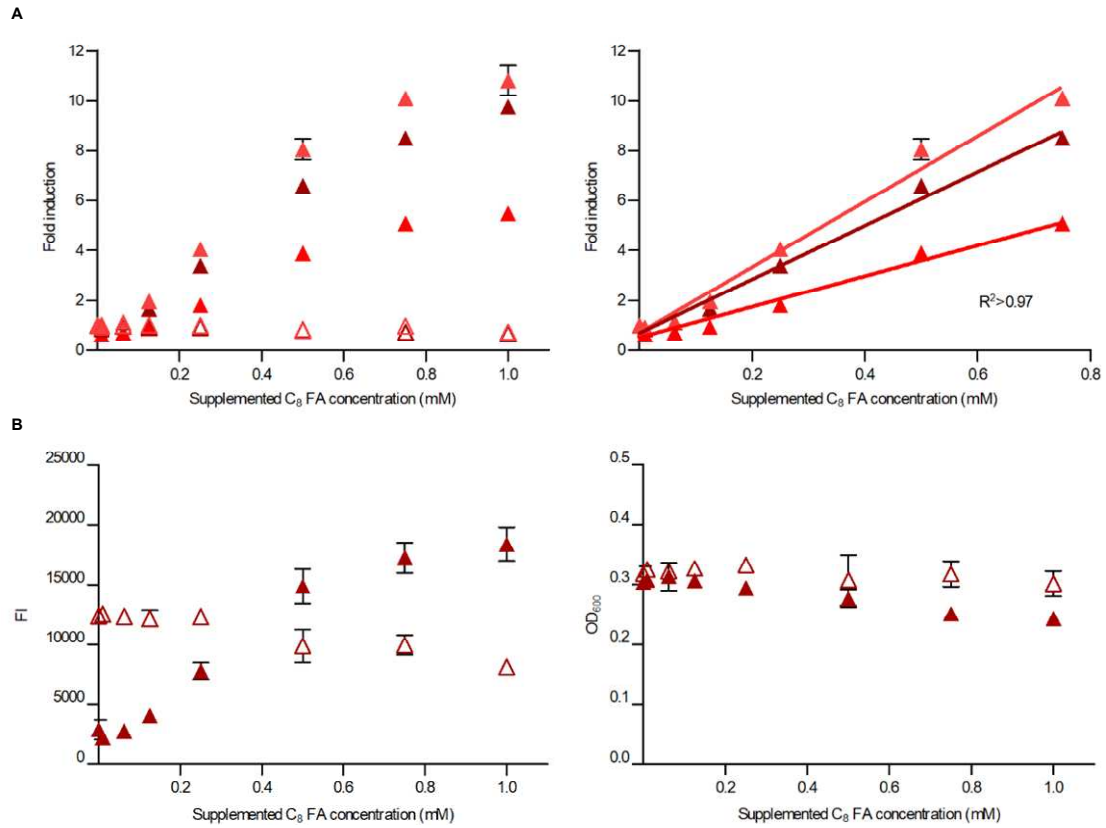


Figure S2. C₈ fatty acid-dependent response of the biosensor in SCD medium. (A) Response (left) and linear range (right) of the biosensor after 2 hours incubation with supplemented C₈ fatty acids (FA) in SCD medium. Shown are three biological replicates with two technical replicates each. For fold induction values, fluorescence intensities (FI) were divided by optical densities (OD₆₀₀) and normalized to FI/OD₆₀₀ values of samples without C₈ FA. (B) FI (left) and OD₆₀₀ (right) of all three biological replicates. Filled triangles: CEN.PK113-11C + p426pPDR12-GFP. Clear triangles: CEN.PK113-11C + p426pMET25-GFP.

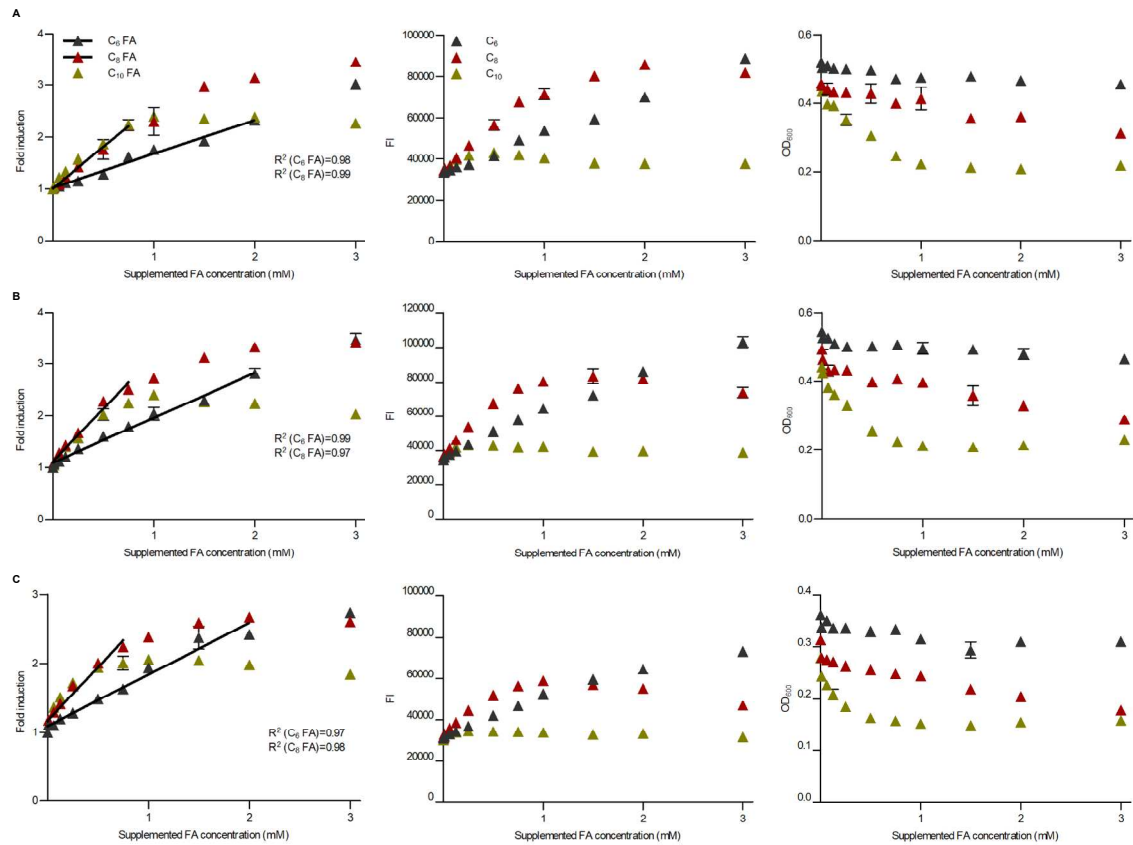


Figure S3. C_6 , C_8 and C_{10} fatty acid-dependent response of the biosensor in YPD medium of three biological replicates (A, B, C). Response and linear range (left), fluorescence intensities (FI; middle) and optical densities (OD_{600} ; right) after 4 hours incubation with supplemented C_6 , C_8 or C_{10} fatty acids (FA) of all three biological replicates. Linear ranges were only observed in response to C_6 and C_8 FAs (left). Error bars represent two technical replicates. For fold induction values, FIs were divided by OD_{600} values and normalized to FI/ OD_{600} values of samples without FA.

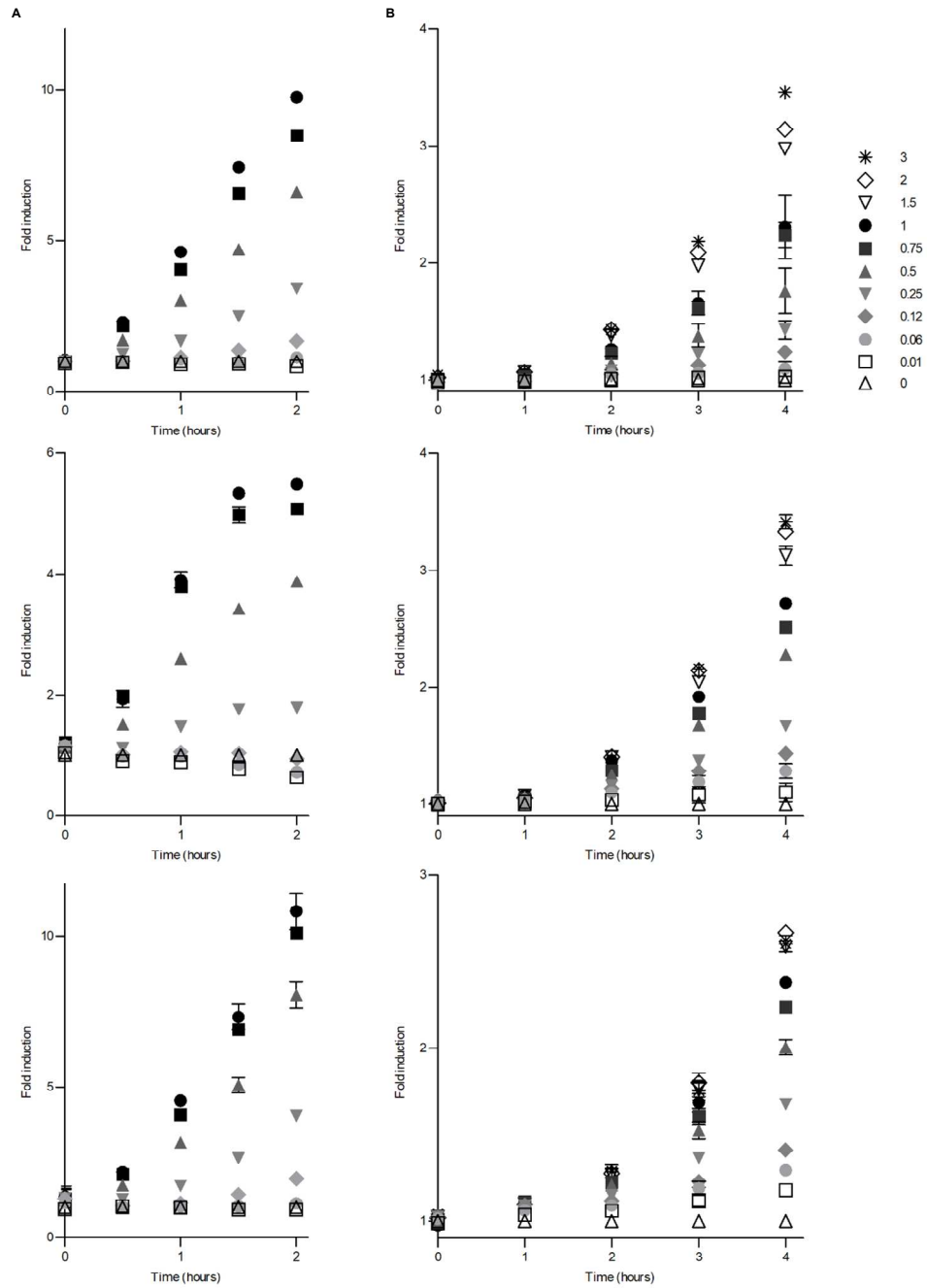


Figure S4. Time-dependent response of the biosensor to different C₈ fatty acid concentrations. Response over a 2 hour incubation period to supplemented 0-1 mM C₈ fatty acids (FA) in SCD (A) and over a 4 hour incubation period to supplemented 0-3 mM C₈ FA in YPD medium (B) of three biological replicates. Error bars represent two technical replicates. For fold induction values, fluorescence intensities (FI) were divided by optical densities (OD₆₀₀) and normalized to FI/OD₆₀₀ values of samples without C₈ FA (0 mM).

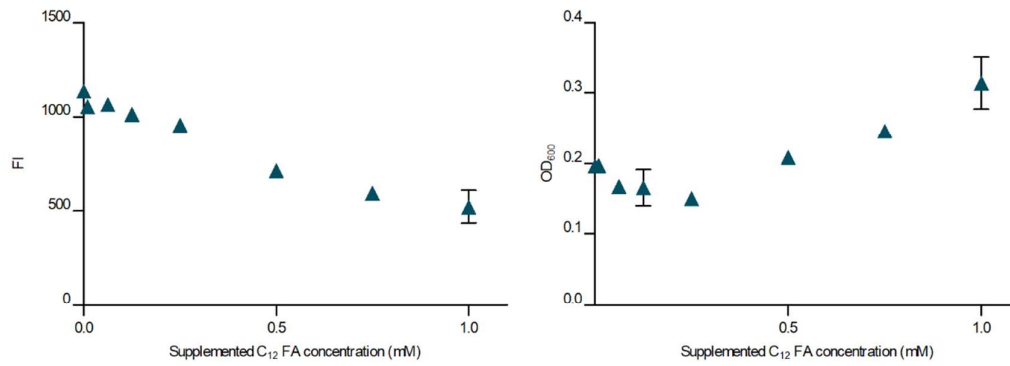


Figure S5. C₁₂ fatty acid-dependent growth and fluorescence of the biosensor. Fluorescence intensities (FI; left) and optical densities (OD₆₀₀; right) in response to supplementation with C₁₂ fatty acids (FA) after 4 hours incubation in YPD medium. Error bars represent two technical replicates.

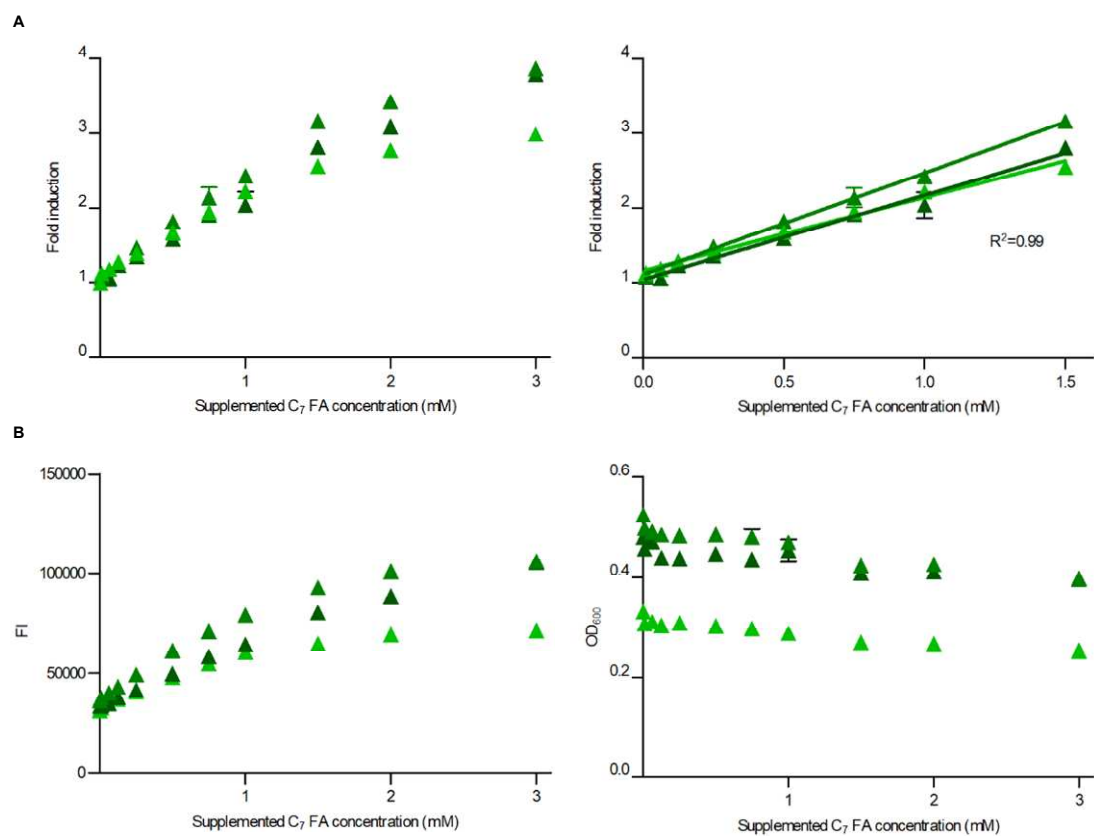


Figure S6. C₇ fatty acid-dependent response of the biosensor. (A) Response (left) and linear range (right) of the biosensor after 4 hours incubation with supplemented C₇ fatty acids (FA) in YPD medium. For fold induction values, fluorescence intensities (FI) were divided by optical densities (OD₆₀₀) and normalized to FI/OD₆₀₀ values of samples without C₇ FA. (B) FI (left) and OD₆₀₀ (right) values of all three biological replicates. Shown are three biological replicates with error bars representing two technical replicates.

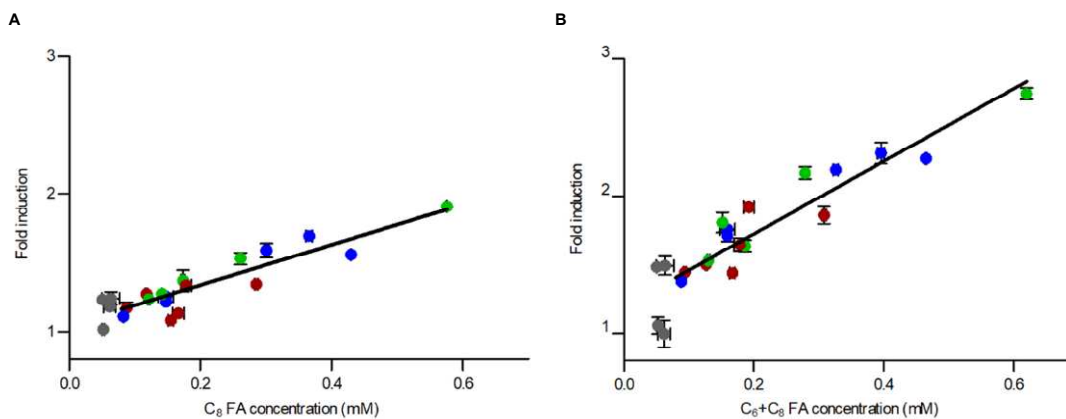


Figure S7. Biosensor response to fatty acids in *S. cerevisiae* culture supernatants and correlation to GC measurement. (A) Linear correlation of the fold induction of biosensor signal in 0.25 dilutions of culture supernatants with GC measurements of C₈ fatty acids (FA) of the same supernatants. (B) Linear correlation of the fold induction of biosensor signal in 0.5 dilutions of culture supernatants with GC measurements of C₆ and C₈ FA of the same supernatants. Strains: CEN.PK113-7D (grey), RPY21/FAS^{R1834K}/ pRS42H (red), RPY21/FAS^{R1834K}/ LBV17 (blue), RPY21/FAS^{R1834K}/ LBV20 (green).

REFERENCES

- (1) Henritzi, S., Fischer, M., Grninger, 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* 11:150.
- (2) Taxis, C., and Knop, M. (2006) System of centromeric, episomal, and integrative vectors based on drug resistance markers for *Saccharomyces cerevisiae*. *Biotechniques* 40, 73–78.
- (3) Gajewski, J., Pavlovic, R., Fischer, M., Boles, E., and Grninger, M. (2017) Engineering fungal *de novo* fatty acid synthesis for short chain fatty acid production. *Nat. Commun.* 8, 14650.
- (4) 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.