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	*MATa*; *MAL2-8c*; *SUC2*	Euroscarf, Frankfurt am Main, Germany
CEN.PK113-11C	*MATa*; *MAL2-8c*; *SUC2*; *ura3-52*; *his3Δ1*	Euroscarf, Frankfurt am Main, Germany
LBY27	CEN.PK113-11C Δ*pyk2*::*pPDR12*-GFP	This study
RPY21/FAS^{R1834K}	*MATα; ura3Δ0*; *his3Δ0*; *leu2Δ0*; *TRP1*; *lys2Δ0*; *MET15*; Δ*FAS1*::*kanMX4*; Δ*FAS2*::*kanMX4; Δfaa2*; transformed with plasmids pRS315-FAS1^{R1834K} and pRS313-FAS2	[1]
Plasmid	**Characteristics**	**Reference**
Plasmids used for fermentations		
p426pMET25-GFP	2μ, *URA3*, *Amp^r*, *pMET25*-GFP-*tCYC1*	This study
p426pPDR12-GFP	2μ, *URA3*, *Amp^r*, *pPDR12*-GFP-*tCYC1*	This study
pRS42H	2μ, *hphNT1*, *Amp^r*, multiple cloning site including *Eco*RV	[2]
LBV17	pRS42H with *pPGK1-TPO1-tTPO1* integrated in *Eco*RV site	This study
LBV20	pRS42H with *pPYK1-ACC1^{S659AS1157A}-tACC1* integrated in *Eco*RV site	This study
pRS315-FAS1^{R1834K}	*CEN6/ARS4*, *LEU2*, *Amp^r*, *pADH2-FAS1^{R1834K}-tFAS1*	[3]
pRS313-FAS2	*CEN6/ARS4*, *HIS3*, *Amp^r*, *pADH2-FAS2-tFAS2*	[3]
Plasmids used for CRISPR/Cas9		
pRCC-K	2μ, *kanMX*, *Amp^r*, *pROX3-Cas9^{opt}-tCYC1*, *pSNR52-gRNA*	[4]
pRCC-K-PYK2	pRCC-K with *gRNA* for *PYK2* locus	This study

Table S2. Oligonucleotides used in this study.

Primer	Sequence 5'-3'	Application
Plasmid construction or sequencing		
LBP63	CAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTGATATCTTTGTTTTGCATTTTAC	Amplification of *pPDR12* from CEN.PK113-11C with overhangs to the p426pMET25-GFP backbone
LBP64	CTACACCTGTAAACAATTCCTCGCCTTTAGACATTTTTTTATTAATAAGAACAATAAC	
LBP103	CTAATGTAGGCCATGGAAC	Sequencing of GFP
LBP76	GGTCGACGGTATCGATAAGCTTGATCCCGGGATAGTAGAAAAAAAAGGGGATATCACTAC	Amplification of *TPO1-tTPO1* from CEN.PK113-11C with overhangs to pRS42H and *pPGK1*, respectively
LBP77	GTAATTATCTACTTTTTACAACAAATATAAAACAATGTCGGATCATTCTCCCATTTCTAA	
LBP78	TTAGAAATGGGAGAATGATCCGACATTGTTTTATATTTGTTGTAAAAAGTAGATAATTAC	Amplification of *pPGK1* from CEN.PK113-11C with overhangs to *TPO1* and pRS42H, respectively
LBP79	GGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGAATTACCGTCGCTCGTGATTTG	
LBP80	GCTACTGCTGAGAACCTG	Sequencing of LBV17
VSP159	CGTGTGACAACAACAGCC	
LBP81	GACTCACTATAGGGCGAATTGGGTACCGGGCCCCGACAGATTGGGAGATTTTCATAGTAG	Amplification of *pPYK1* from CEN.PK113-11C with overhangs to pRS42H and *ACC1*, respectively
LBP82	GAAGACTCGAATAAGCTTTCTTCGCTCATTGTGATGATGTTTTATTTGTTTTGATTGGTG	
LBP83	CACCAATCAAAACAAATAAAACATCATCACAATGAGCGAAGAAAGCTTATTCGAGTCTTC	Amplification of *ACC1* with *S659A* and *S1157A* and *tACC1* with overhangs to *pPYK1* and pRS42H, respectively
RPP108	CTATGGCAATCAAAAGACCACCATCAGCTAGTTGAC	
RPP107	GATATCATACTGCGTCAACTAGCTGATGG	
RPP088	CATATGACAAATCTGAAACAGCAACAGCCCTGTTCATAC	
RPP087	ATGGGTATGAACAGGGCTGTTGCTGTTTCAGATTTGTCATATGTTG	
LBP84	GTACTCTGAAGGATCTGTTTGAGCGCTTCCATCGGGCCCATCGAATTCCTGCAGCCCGGG	
LBP98	CTTGTCATCCAATCTGTTC	Sequencing of LBV20
LBP99	CCAAATAAGCACCGATACC	
LBP100	GCAACCATTCCTTAACAGG	
LBP101	GACATACAGAACTTCCAGG	
LBP102	GGAACATAGTTTGCAGTAGG	
RPP89	TTCGAAACCTTCTGTAGAAGCAACACAAAC	
RPP90	CGGTCAAGGAAAGAACTGAACAAATTGAAC	
RPP109	TCCAACTCTTGCCGTCATTTGC	
RPP056	CACACAGGAAACAGCTATGAC	Sequencing of p426pPDR12-GFP, LBV17 and LBV20
LBP85	CGTTACCCAACTTAATCGCC	
Insertion of *pPDR12*-GFP in *PYK2*		
LBP108	GTCCATTGTAAGATTACAACAAAAGCACTATCGGGCGAATTGGGTACCGG	Amplification of *pPDR12*-GFP-*tCYC1* from p426pPDR12-GFP with overhangs to *PYK2*
LBP109	ATTAAGTAAAAAAAATAAGGACTTTAATTTTTAGATATCTTTGTTTTGCATTTTACATTC	
LBP118	CAGAGCGGTGAAACGCAAC	Amplification and sequencing of Δ*pyk2*::*pPDR12*-GFP-*tCYC1*
LBP119	CGCAGTTTGCGAACATTACCTG	
Amplification of CRISPR/Cas9 plasmid pRCC-K		
WGP234	CTTGGTGGTGTTCGTCGTATCTCTTAATCATAGAAGCAGACAATGGAG	Amplification of pRCC-K with gRNA for *PYK2*
WGP235	TGTTGTCTGACATTTTGAGAGTTAACACCGAAATTACCAAGGCTC	
MGP193	TTGCAATTCGGAGTCCGCAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	

MGP194	CTTGCGGACTCCGAATTGCAAGATCATTTATCTTTCACTGCGGAG	
gRNA sequences used for deletion of *PYK2*		
PYK2 gRNA	TTGCAATTCGGAGTCCGCAA	

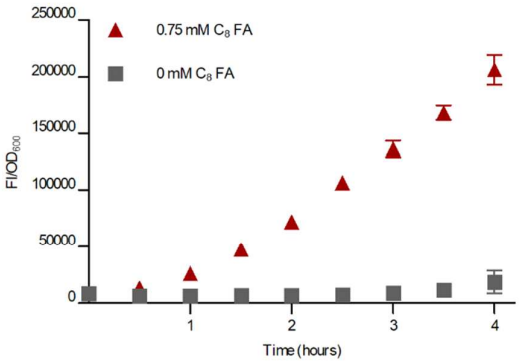

Figure S1. Time-dependent response of the biosensor in SCD medium with and without C_8 fatty acids. Relative fluorescence intensity (fluorescence intensity (FI) divided by OD_{600}) of the plasmid-based sensor in response to 0 (grey squares) and 0.75 mM (red triangles) C_8 fatty acids (FA), respectively. The background fluorescence of the biosensor strain not exposed to C_8 FA is very low over the entire time course. Error bars represent two technical replicates.

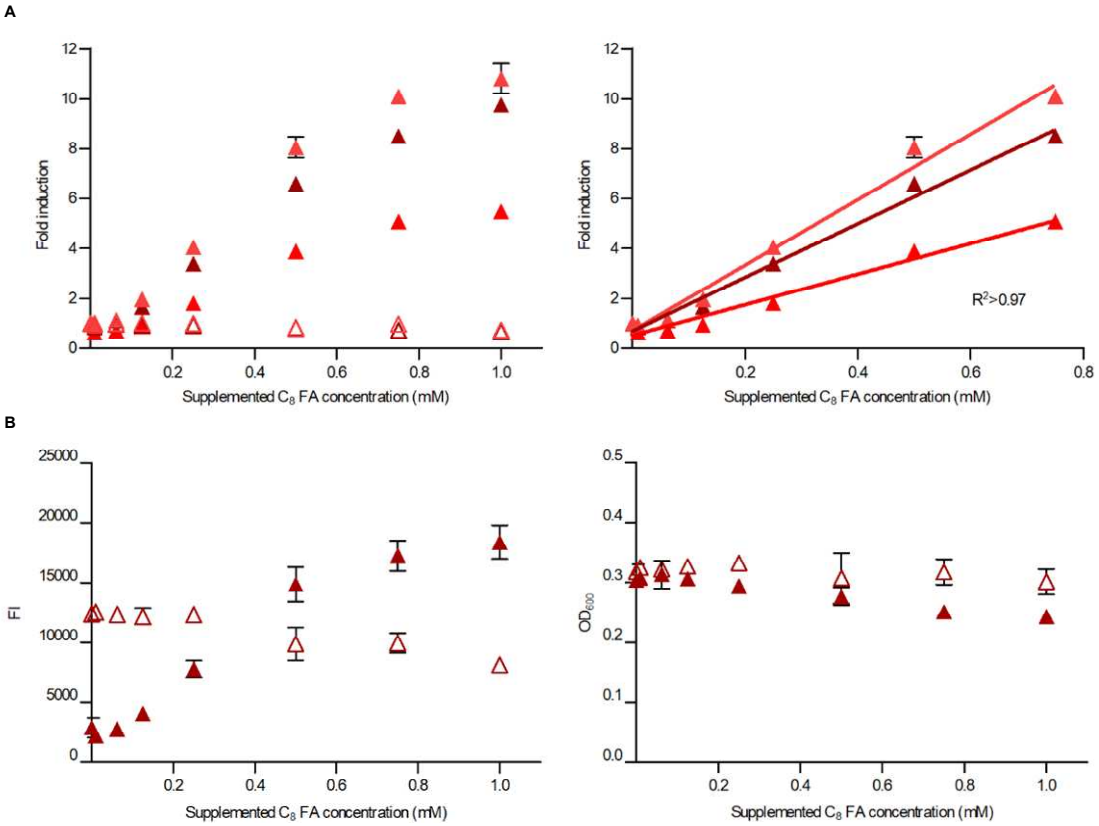

Figure S2. C_8 fatty acid-dependent response of the biosensor in SCD medium. (A) Response (left) and linear range (right) of the biosensor after 2 hours incubation with supplemented C_8 fatty acids (FA) in SCD medium. Shown are three biological replicates with two technical replicates each. For fold induction values, fluorescence intensities (FI) were divided by optical densities (OD_{600}) and normalized to FI/OD_{600} values of samples without C_8 FA. (B) FI (left) and OD_{600} (right) of all three biological replicates. Filled triangles: CEN.PK113-11C + p426pPDR12-GFP. Clear triangles: CEN.PK113-11C + p426pMET25-GFP.

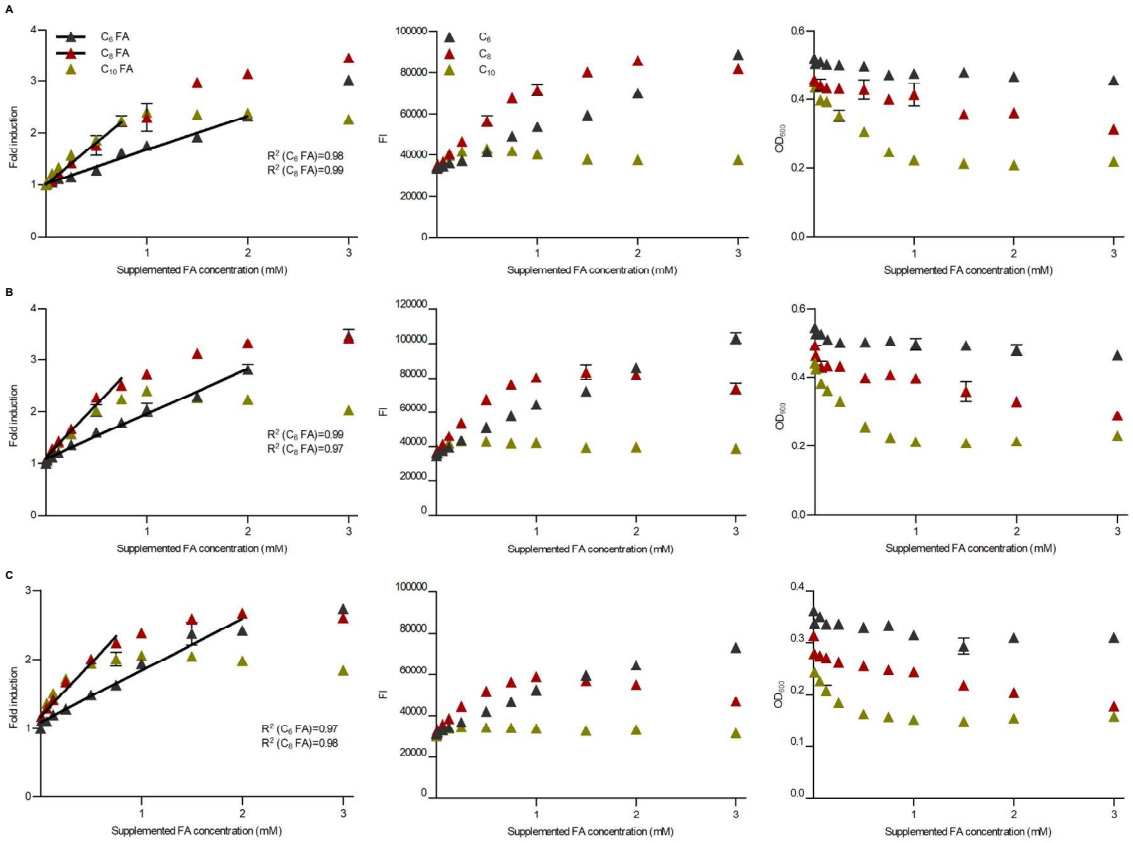

Figure S3. C_6, C_8 and C_{10} fatty acid-dependent response of the biosensor in YPD medium of three biological replicates (A, B, C). Response and linear range (left), fluorescence intensities (FI; middle) and optical densities (OD_{600}; right) after 4 hours incubation with supplemented C_6, C_8 or 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.

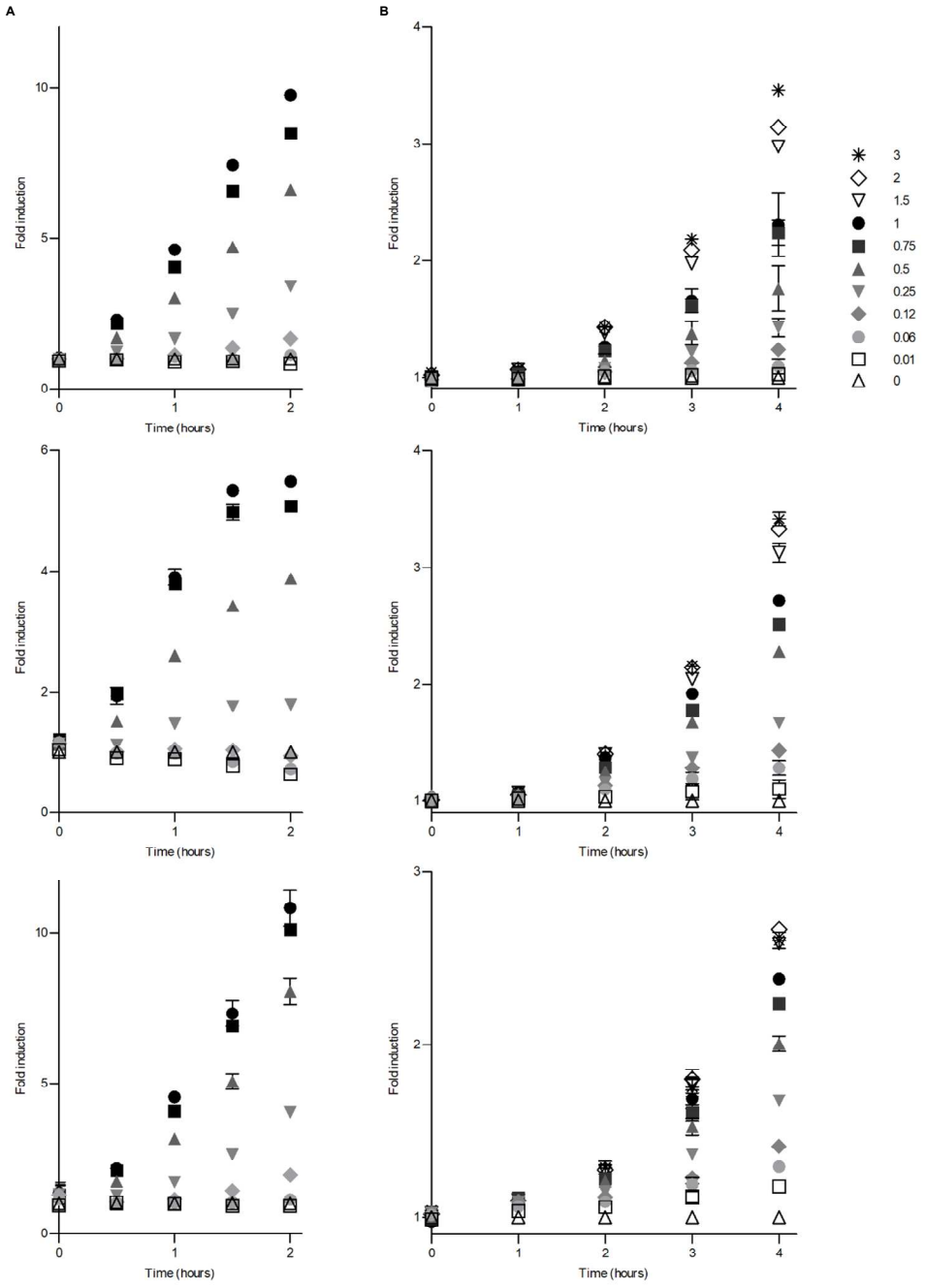

Figure S4. Time-dependent response of the biosensor to different C_8 fatty acid concentrations. Response over a 2 hour incubation period to supplemented 0-1 mM C_8 fatty acids (FA) in SCD (A) and over a 4 hour incubation period to supplemented 0-3 mM C_8 FA in YPD medium (B) of three biological replicates. Error bars represent two technical replicates. For fold induction values, fluorescence intensities (FI) were divided by optical densities (OD_{600}) and normalized to FI/OD_{600} values of samples without C_8 FA (0 mM).

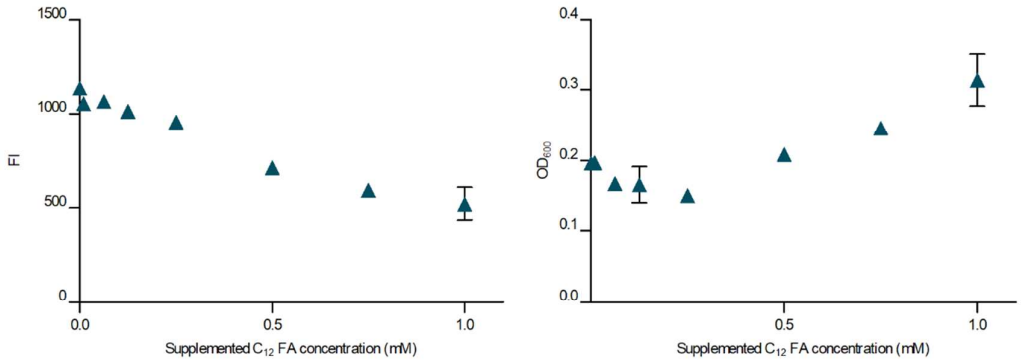

Figure S5. C_{12} fatty acid-dependent growth and fluorescence of the biosensor. Fluorescence intensities (FI; left) and optical densities (OD_{600}; right) in response to supplementation with C_{12} fatty acids (FA) after 4 hours incubation in YPD medium. Error bars represent two technical replicates.

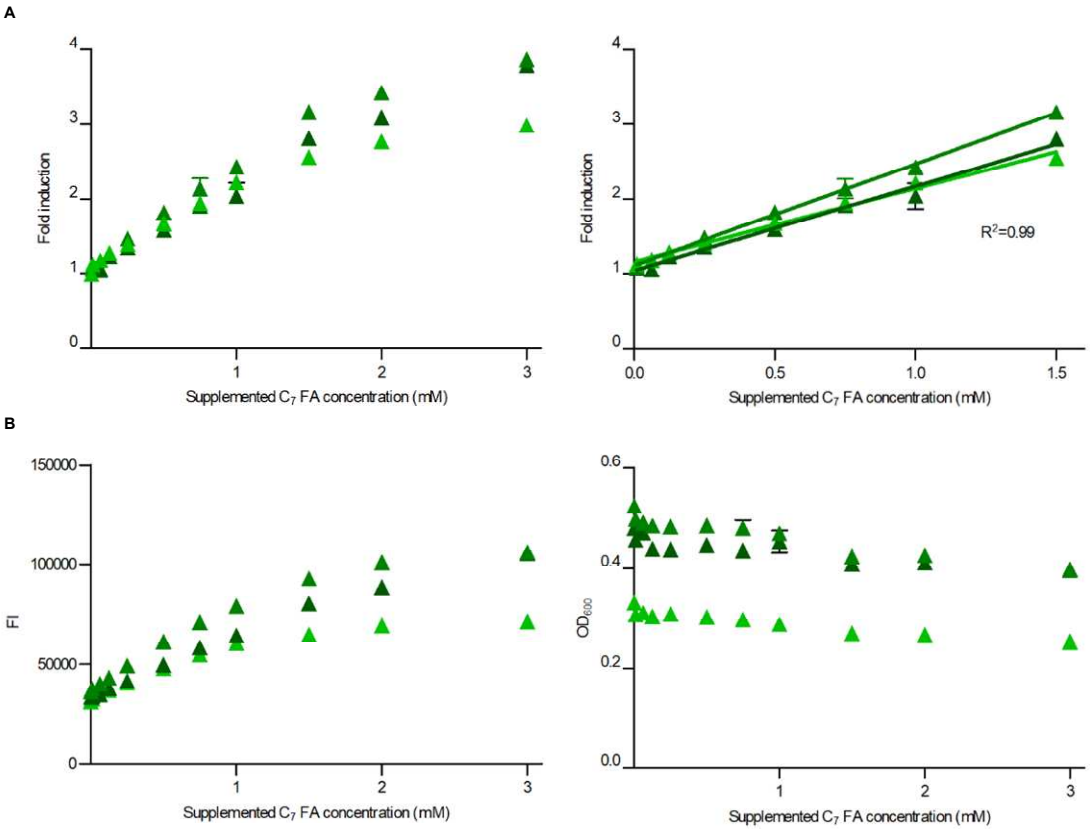

Figure S6. C_7 fatty acid-dependent response of the biosensor. (A) Response (left) and linear range (right) of the biosensor after 4 hours incubation with supplemented C_7 fatty acids (FA) in YPD medium. For fold induction values, fluorescence intensities (FI) were divided by optical densities (OD_{600}) and normalized to FI/OD_{600} values of samples without C_7 FA. (B) FI (left) and OD_{600} (right) values of all three biological replicates. Shown are three biological replicates with error bars representing two technical replicates.

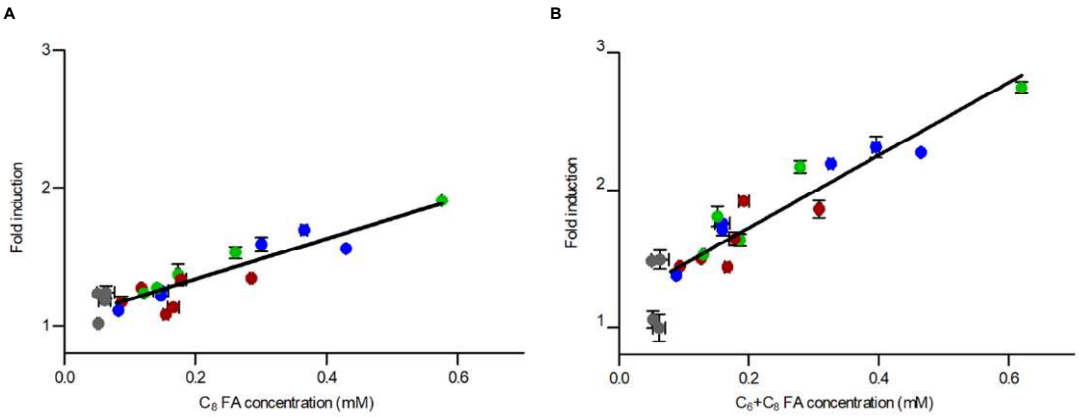

Figure S7. Biosensor response to fatty acids in *S. cerevisiae* culture supernatants and correlation to GC measurement. (A) Linear correlation of the fold induction of biosensor signal in 0.25 dilutions of culture supernatants with GC measurements of C_8 fatty acids (FA) of the same supernatants. (B) Linear correlation of the fold induction of biosensor signal in 0.5 dilutions of culture supernatants with GC measurements of C_6 and C_8 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).

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(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.

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(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.