

Supplementary information for:

Yarrowia lipolytica as a platform for punicic acid production

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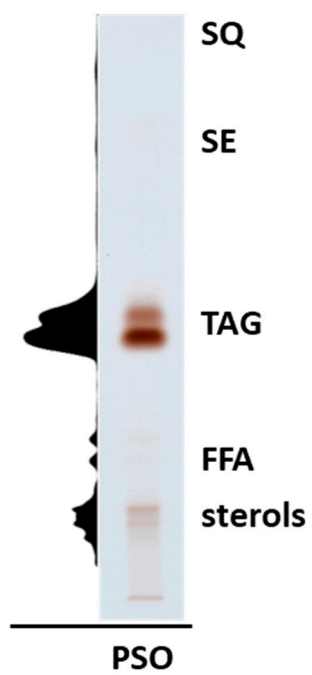
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Authors have jointly supervised the work

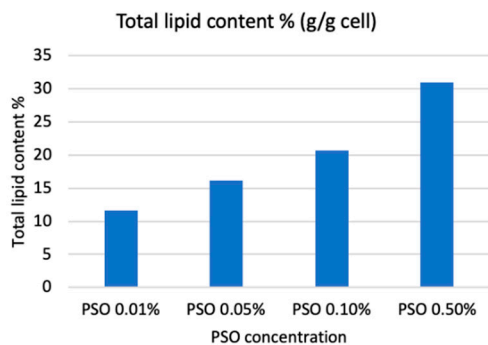
† These authors contributed equally to this work.

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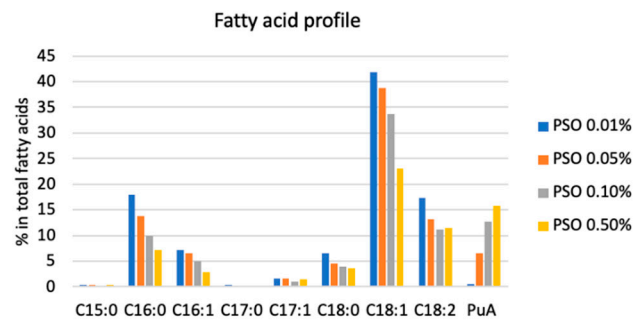


Supplementary Figure S1. Neutral lipid profile of PSO (densitogram (276 nm) and visualization). Abbreviation: SQ (squalene), SE (steryl ester), TAG (triacylglycerol), FFA (free fatty acid).

(a)

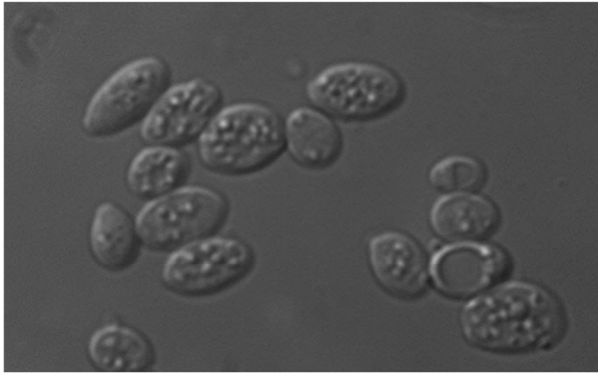


(b)

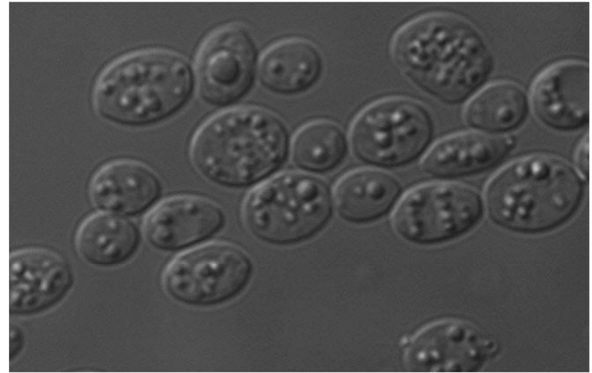


Supplementary Figure S2. Lipid content and fatty acid profiles of *Y. lipolytica* JMY3820 obese strain upon pomegranate seed oil (PSO) feeding. Yeast cells were grown for 72 hours in YPD (2% glucose) media supplemented with four different PSO concentrations (0.01%, 0.05%, 0.1% and 0.5%). (a) lipid content as gWW/ gDCW, (b) lipid profiles as a percentage of total lipid.

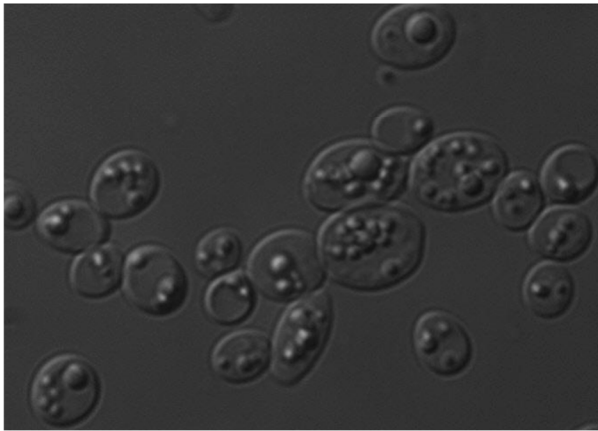
(a) 0.01% PSO



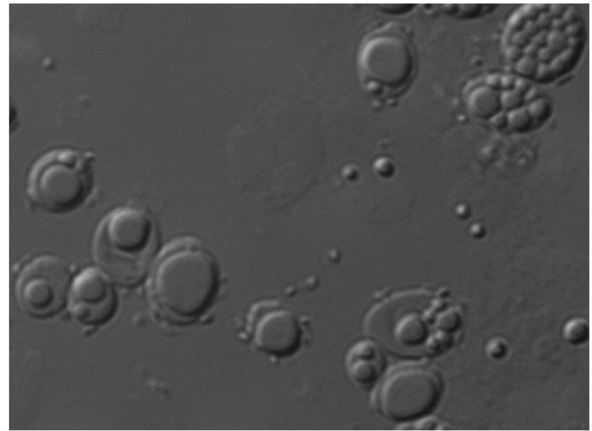
(b) 0.05% PSO



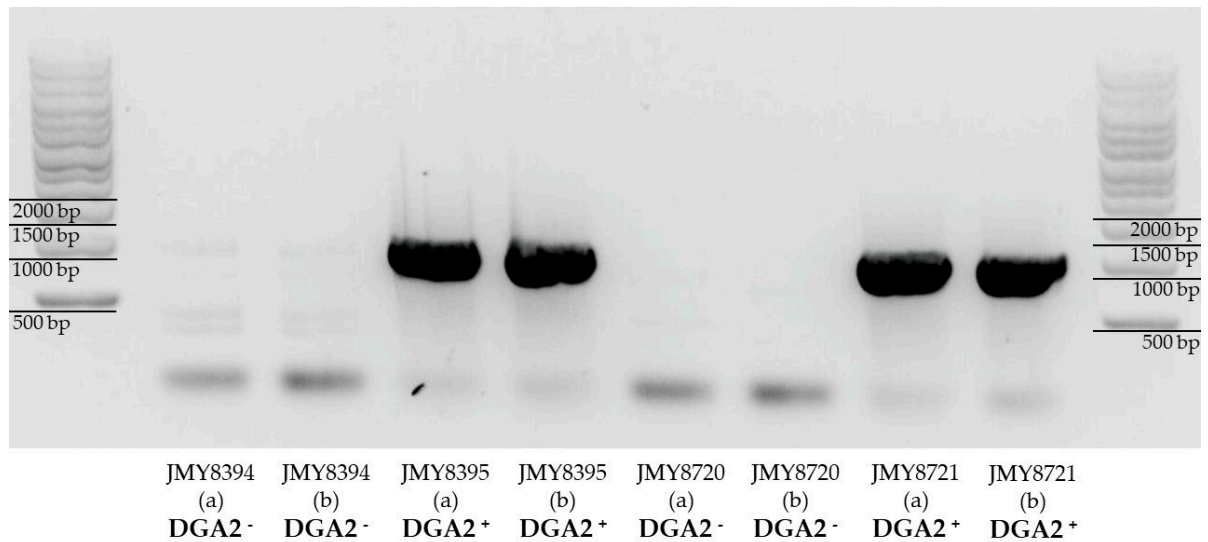
(c) 0.1% PSO



(d) 0.5% PSO



Supplementary Figure S3. Microscopic imaging of *Y. lipolytica* JMY3820 obese strain upon pomegranate seed oil (PSO) feeding. Yeast cells were grown for 72 hours in YPD (2% glucose) media supplemented with four different PSO concentrations: (a) 0.01%, (b) 0.05%, (c) 0.1%, (d) 0.5%. Images were obtained using a Zeiss Axio Imager M2 microscope (Zeiss, Le Pecq, France) with a 100× objective lens and Zeiss filter sets 45 and 46 for fluorescence microscopy. Axiovision 4.8 software (Zeiss, Le Pecq, France) was used for image acquisition.



Supplementary Figure S4. Verification of the presence of *pTEF-DGA2* cassette in the genome of JMY8394, JMY8395, JMY8720 and JMY8721 strains. The presence of *pTEF-DGA2* was confirmed by PCR with the pTEF-internal-Fw2 (5'-CCAATTGACCCCAAATTGACC-3') and DGA2-Rev (5'-ATGAGGCGAGTTTGAGAGACAG-3') primers and subsequent electrophoresis. The expected length of the fragment was 992 base pairs (bp). Two parallel experiments were performed for each strain.

Supplementary Table S1. Relative level of PuA in *Y. lipolytica* cells grown in media supplemented with PSO.

PSO concentration	PuA in lipids (%)	PuA in FFA (%)
0.5%	14.3 ± 1.7	42.8 ± 15.2
1.0%	21.9 ± 6.3	47.9 ± 11.1

results from three independent experiments

Supplementary Table S2. Cultivation of all constructed *PgFADX*-expressing *Y. lipolytica* strains for 72 hours on YPD medium containing 6% (w/v) glucose for JMY8381 (control strain), JMY8393 (*URA3ex-pTEF-PgFADX*), JMY8394 (*URA3ex-pTEF-PgFADX*), JMY8395 (*URA3ex-pTEF-PgFADX*), and on YPDE medium containing 6% (w/v) glucose and 2% (w/v) erythritol for JMY8710 (Δ *eyk1* control strain), JMY8714 (Δ *eyk1* + *URA3ex-pEYK1 4AB-PgFADX*), JMY8715 (Δ *eyk1* + *URA3ex-pEYK1 4AB-PgFADX*), JMY8716 (Δ *eyk1* + *URA3ex-pEYK1 4AB-PgFADX*), JMY8717 (Δ *eyk1* + *URA3ex-pEYD1-PgFADX*), JMY8718 (Δ *eyk1* + *URA3ex-pEYD1-PgFADX*), JMY8719 (Δ *eyk1* + *URA3ex-pEYD1-PgFADX*), JMY8720 (Δ *eyk1* + *URA3ex-pEYK1 4AB-coreTEF-PgFADX*), JMY8721 (Δ *eyk1* + *URA3ex-pEYK1 4AB-coreTEF-PgFADX*) and JMY8722 (Δ *eyk1* + *URA3ex-pEYK1 4AB-coreTEF-PgFADX*).

	<i>PgFADX</i> promoter	DCW (g/L)	TFA/DCW (%)	PuA (% of esterified FA)	PuA (μ g/mg DCW)
JMY8381 obese	-	19.1 \pm 0.1	34.2 \pm 1.9	-	-
JMY8393 obese ^x + <i>PgFADX</i>	<i>pTEF</i>	15.4 \pm 0.8	10.5 \pm 0.8	1.6 \pm 0.3	1.8 \pm 0.4
JMY8394 obese ^x + <i>PgFADX</i>	<i>pTEF</i>	13.5 \pm 0.3	9.2 \pm 0.2	1.6 \pm 0.0	1.5 \pm 0.0
JMY8395 obese + <i>PgFADX</i>	<i>pTEF</i>	18.6 \pm 0.6	33.5 \pm 1.4	0.4 \pm 0.2	0.9 \pm 0.0
JMY8710 obese + Δ <i>eyk1</i>	-	14.4 \pm 0.3	25.5 \pm 1.4		-
JMY8714 obese + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYK1 4AB</i>	14.7 \pm 0.2	27.9 \pm 4	0.3 \pm 0.0	0.9 \pm 0.4
JMY8715 obese + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYK1 4AB</i>	17.5 \pm 0.9	33.7 \pm 0.1	0.3 \pm 0.0	1.0 \pm 0.1
JMY8716 obese + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYK1 4AB</i>	18.3 \pm 0.3	30.9 \pm 3.6	0.3 \pm 0.0	1.0 \pm 0.1
JMY8717 obese + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYD1</i>	17.2 \pm 0.6	26.9 \pm 1.5	0.02 \pm 0.0	0.1 \pm 0.0
JMY8718 obese ^x + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYD1</i>	12.4 \pm 1.0	6.9 \pm 0.6	0.4 \pm 0.2	0.3 \pm 0.2
JMY8719 obese + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYD1</i>	19.2 \pm 0.6	32.1 \pm 1.4	0.1 \pm 0.0	0.2 \pm 0.1
JMY8720 obese ^x + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYK1 4AB-coreTEF</i>	13.7 \pm 0.1	8.8 \pm 0.7	3.1 \pm 0.2	2.8 \pm 0.3
JMY8721 obese + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYK1 4AB-coreTEF</i>	19.9 \pm 0.3	31.6 \pm 0.4	0.5 \pm 0.1	1.8 \pm 0.1
JMY8722 obese ^x + Δ <i>eyk1</i> + <i>PgFADX</i>	<i>pEYK1 4AB-coreTEF</i>	13.5 \pm 0.6	8.0 \pm 0.4	2.8 \pm 1.0	2.3 \pm 0.9

For each data point, we used three biological replicates and calculated average and standard deviation values. The “x” designation next to the word obese means that the strain has lost the *pTEF-DGA2* overexpression cassette.

Supplementary Table S3. Sequences of *FADX*-expressing cassettes used in this study: the *NotI* fragments used to transform *Y. lipolytica* comprising the **PgFADX** and **TkFADX** expression cassettes under the control of different promoters (*pTEF*, *pEYK1-4AB*, *pEYD1AB* or *pEYK1-4AB-coreTEF*) and the *LIP2* terminator, along with an expression cassette for the *URA3* auxotrophic selection marker (*URA3ex*), flanked by two Zeta sequences (*ZetaUp* and *ZetaDown*) that allow random integrations into *Y. lipolytica* genome.

[illegible]

FAD _X gene	<i>TrFAD_X</i>
-----------------------	--------------------------

[illegible]

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<i>FAD</i> X gene	<i>PgFAD</i> X
Promoter	<i>pEYD1AB</i>
<i>E. coli</i> strain	JME5335
<i>Y. lipolytica</i> strain	JMY8717
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FADX gene	<i>PgFADX</i>
Promoter	<i>pEYK1-4AB-coreTEF</i>
<i>E. coli</i> strain	JME5336
<i>Y. lipolytica</i> strain	JMY8721

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CCGCATTAAGCTCCACAGCCTTGCATAATCCGATGACCTGACTAGTGCAGTTGGGACTTTAGCCAAGGGTATAAAA
GACCACCGTCCCCGAATTACCTTTCCTCTCTTTTCTCTCTCTCTGTCAACTCACACCCGAAGGATCCACAAATG
GGCGCAGACGGTACTATGAGCCCCGTGCTTACGAAGCGACGACCTGATCAGGAAATTAATAAGCTCGATATTAAC
CCAACCACGAGGTCGATATTGCACGACGAGCACCCCACTCTAAGCCCCCTTTACTCTCAGCGATCTTCGTTCTGCT
ATTCTCCCCACTGTTTCCACCGTTCCTCTTATGTCTCCAGTACCTTATCCGGGATTTTGCTCTTGCAATTCCTTT
CTACCATTCGCTGTGACCTATATCCCCCTCCTTCTAAACCTCTCGCCTGTATGGCTTGGCCCGTGTACTGGTTTCTC
CAGGGATCTAATATGCTCGGAATTTGGGTATCGCCACGAATGCGGACATCAGGCCTTTAGCAATTACGGTTGGGT
CAACGACGCAGTCGGTTTCTTTCTGCATACCAGCCTCCTTGTGCCTTATTTCCCTTTCAAATACTCGCACAGACGTCA
CCACTCCAACACTAATAGCGTTGAGCAGCATGAGGTTTTTGTTCCTAGACACAAGGACGGCGTCCAATGGTATTATC
GGTCTTCAACAATACGCCTGGCCGTGCTCTCACACTGACTCTTACACTCCTGGTTGGATGGCCTTCTACCTTGCTTT
TAACGCTTCCGGTAGACCTTATGACGGTTTCGCTCGCATTACAATCCTAATGCCCAAATTTTAACCTCCGTGAGCG
TTTCTGGGTGCACGTGTCGAACATCGGCATTCTTGCTATCTACTATATCCTGTACCGACTCGCCACTACGAAGGGCT
TCCTTGGCTTCTTTCCATCTACGGCGTGCCCGTGCTCATCCTCAACGCTTTCGTTGTTCTCATCACATTCCTCCAGCAC
TCGCATCCTGCACTCCCCCACTACAATTCGACGAGTGGGATTGGCTTCGGGGTGCCCTTGAACAGTCGACCGAGA
TTATGGATTTCTGAACGAGGTTTTTCACGATATCACGGATACCCACGTTATTCATCACCTTTTCCCCACCATGCCCA
TTACAACGCTAAGGAGGCTACTGTGTCGATCCGGCCCATCCTGAAAGATTACTATAAGTTTGATCGTACACCCATT
GGAGAGCTCTCTGGCGGGAGGCCAAAGAATGTCTTTACGTGCAAGCCGATGGTACCGGCTCGAAGGGAGTCCTCTG
GTTTAAATCTAAATTTTAACTAGTGTCTGTGGTATCTAAGCTATTTATCACTCTTTACAACCTTCTACCTCAACTATCT
ACTTTAATAAATGAATATCGTTTATCTCTATGATTACTGTATATGCGTTCCTCTAAGACAGAGTCATGTGTAACACT
CGCTCTGGAGAGTTAGTCATCCGACAGGGTAACCTCTAATCTCCCAACACCTTATTAACCTCTGCGTAACCTGTAACCTT
CTTGCCACGTCGATCTTACTCAATTTTCTGCTCATCATCTGCTGGATTGTTGTCTATCGTCTGGCTCTAATACATTTAT
TGTTTATTGCCAAACAACCTTTCATTGCACGTAAGTGAATTGTTTTATAACAGCGTTCGCCAATTGCTGCGCCATCGT
CGTCCGGCTGTCTACCGTTAGGGGTAGTGTGTCTCACACTACCGAGGTTACTAGAGTTGGGAAAGCGATACTGCCT
CGGACACACCACCTGGGTCTTACGACTGCAGAGAGAATCGGCGTTACCTCTCTCACAAGCCCTTCAGTGGGGCCG
C

Supplementary Table S4. Primers used in the study.

Name	Sequence (5' – 3')
pTEF-internal-Fw	TCTGGAATCTACGCTTG TTCAG
pEYK-internal-Fw	GTACGTTTCAATCTGGGGAAGCGG
pEYD-internal-Fw	CCCATCGATGGAAACCTTAATAGGAGACTACTTCC
PgFADX-Rev	CCAGAGGACTCCCTTCGAGCC
TkFADX-Rev	CGCCTTTGCTGGAGCTCGATGC
pTEF-internal-Fw2	CCAATTGACCCCAAATTGACC
DGA2-Rev	ATGAGGCGAGTTTGAGAGACAG