Supplementary materials for *de novo* biosynthesis of sex pheromone components of *Helicoverpa armigera* through an artificial pathway in yeast

This profile includes:

Method details

Table S1-4

Figure S1-5

1.1 Optimization of fermentation conditions

For optimization of fermentation conditions, a $L^{16}(4^3)$ orthogonal table was designed to obtain best combination of these conditions. To be specific, pH of medium varied from 5 to 6.5 in step of 0.5 adjusted by 1M KOH, concentration of $(NH_4)_2\ SO_4$ varied from 2 g/L to 10 g/L in step of 3.7 g/L and concentration of KH_2PO_4 varied from 2 g/L to 15 g/L in step of 4.3 g/L. Specific combinations were shown below (Table S2 & S3). Through extremum difference analysis (Fig. 4D), best theoretical combination is pH=6, 7.4 g/L $(NH_4)_2\ SO_4$, 6.3g/L KH_2PO_4.

1.2 Extraction of metabolites

The detailed methods of free fatty acid, fatty aldehydes and alcohols from fermentation broth were shown below:

For free fatty acids, simultaneous extraction and methylation was conducted as described previously[1]. Briefly, cell cultures from flask fermentation were diluted twofold and those from batch were diluted 4-fold in total 0.4 mL in 4-mL glass vials. Then, 20 μL of 40% $(C_4H_9)_4N^+OH^-$ was added into vials followed with 400 μL pre-mixed CH_2Cl_2 solution containing 0.2M CH_3I and 50 mg/L pentadecanoic acid as an internal standard. The mixtures were shaken for 60 min at 1500 rpm on a mixer. After centrifugation, organic phases were transferred into 1.5 mL centrifugal tubes and evaporated to dryness in Concentrator Plus (Eppendorf, Germany). In the end, 50 μL hexane was added to suspend methyl esters and transferred into glass inserts for gas chromatography (GC) or gas chromatography-mass spectrum (GC-MS) analysis.

For fatty aldehydes, cell pellets were collected by centrifuging 10 mL cell culture from flask fermentation or 2 mL from batch fermentation. After washed by 20 mL of 50 mM Tris-HCl (pH 7.5), cell pellets were suspended in 0.5 mL of 6.7% Na_2SO_4 and transferred into 4-mL glass vials. Then, 2 mL mixture of isopropanol and hexane in ratio of 2:3 (v/v) was added into vials, and the mixture was shaken for 120 min at 1500 rpm on a mixer. After centrifugation, organic phases were transferred into new 4-mL vials and evaporated to dryness. Finally, 50 μL hexane was added to suspend extracts and transferred into glass inserts for GC or GC-MS analysis. As for fermentation with dodecane cover, after centrifugation to promote phase separation, upper dodecane layer was directly transferred into glass inserts for analysis. Rest of cell culture were dealt with same methods mentioned above if needed.

For raw purification of fermentation extracts, cell culturte from fed-batch fermentation was directed extracted by 1L hexane:isopropanol (3:2, v/v) overnight. After phase separation in funnel, near 640 mL organic phase was concentrated 40-fold for thin layer chromatography (TLC). Seventy microliter of concentrated sample was loaded onto a TLC plate (Silica gel 60, Merck, Germany) and separated with a mobile phase of hexane/diethyl ether/HAc (85:15:1, v/v/v)[2]. The bands were visualized by spraying 20% H_2SO_4-EtOH solution, followed by heating. Target gel areas were collected and suspended with 4 mL hexane. The extracts were centrifuged, and supernatant was transferred into a new vial for evaporation to dryness. One hundred microliter of hexane was added to dissolve the compounds, followed by analysis by GC-FID to quantify final concentration of (Z)-11-hexadecenal.

1.3 Dimethyldisulphide (DMDS) derivatization of samples

To verify the position of double bond in products, DMDS derivatization was conducted as described previously[3]. Briefly, near 1μg aldehydes in *n*-hexane (20-50 μL) was derivatized by addition of 50 μL of DMDS and 5 μL iodine solution (60 mg in 1 mL of ether). The reaction was conducted in 1 mL vials and kept at 40℃ overnight. Then samples were diluted by 400 μL of *n*-hexane, followed with another 100 μL of $Na_2S_2O_3$ solution (5% in ddH_2O) to remove the iodine. The organic phase was removed and concentrated into 50 μL. A 2 μL aliquot was analyzed by GC-MS.

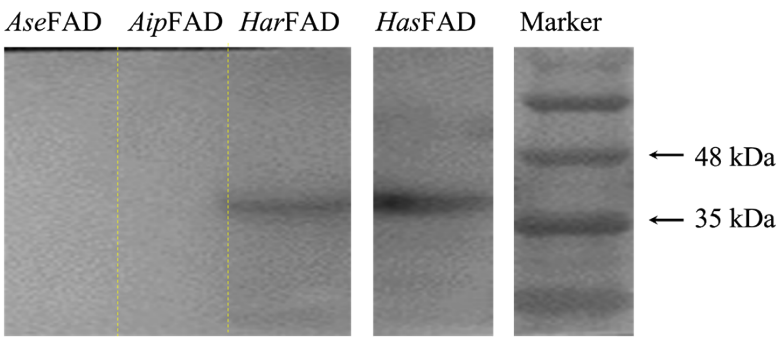

Figure S1: Western-blot analysis of FADs in strain FA3

After Integration with C-terminal 6 × His-tags, molecular weight of *Ase*FAD, *Aip*FAD, *Har*FAD and *Has*FAD is 39.69, 39.82, 38.85 and 39.57 kDa, respectively. Expression of *Ase*FAD and *Aip*FAD is below the detection limit of western-blot analysis. Under the same total protein load, the expression of *Har*FAD is slightly lower than *Has*FAD.

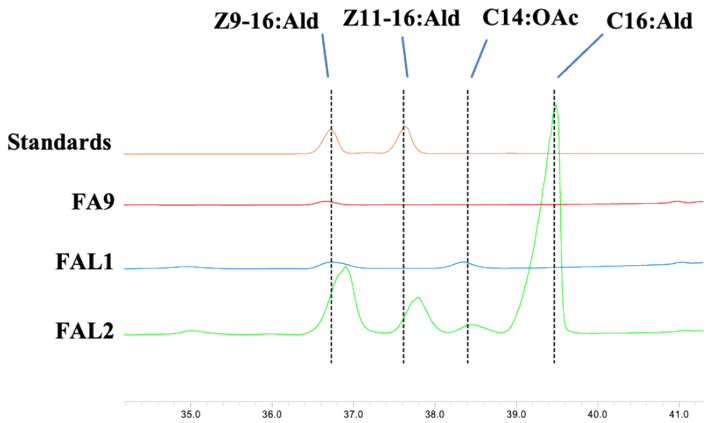

Figure S2: Gas-chromatography analysis of target products in strain FA9, FAL1,FAL2

Gas-chromatography analysis of interested compounds in raw extracts of strain FA9, FAL1,FAL2 after 4-day fermentation. Target products and other peaks around were illustrated. As shown in Fig. S2, after *HFD1* deletion, strain FAL2 could produce Z11-16:Ald.

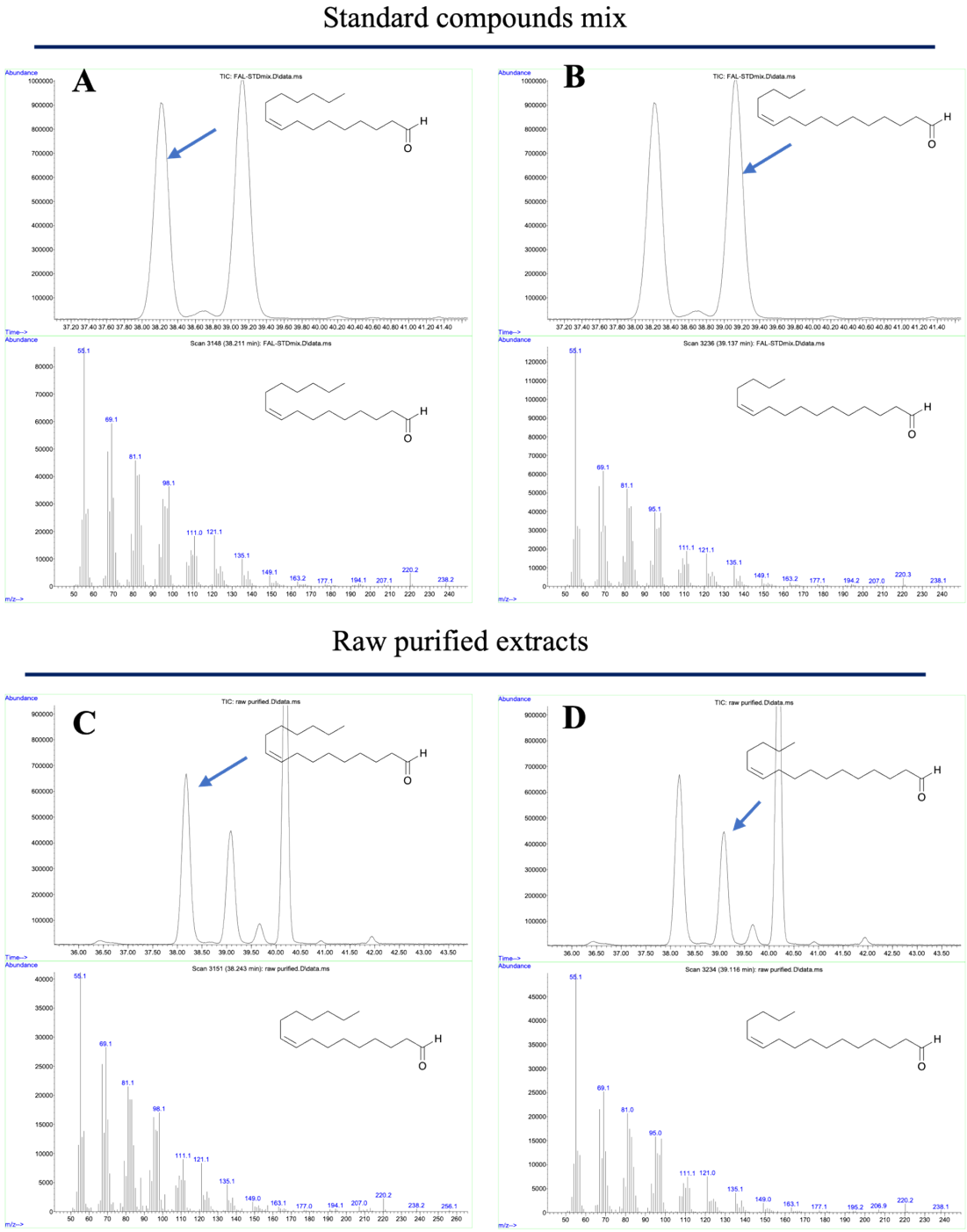

Figure S3: GC-MS spectrum of aldehyde standard mixture and raw extracts of fermentation

As shown above, retention time of candidate peaks in raw extracts of fermentation (Fig. S3C-D) corresponded to that of commercial standards in mixture (Fig. S3A-B). The fragments distribution is also similar to the corresponding, respectively.

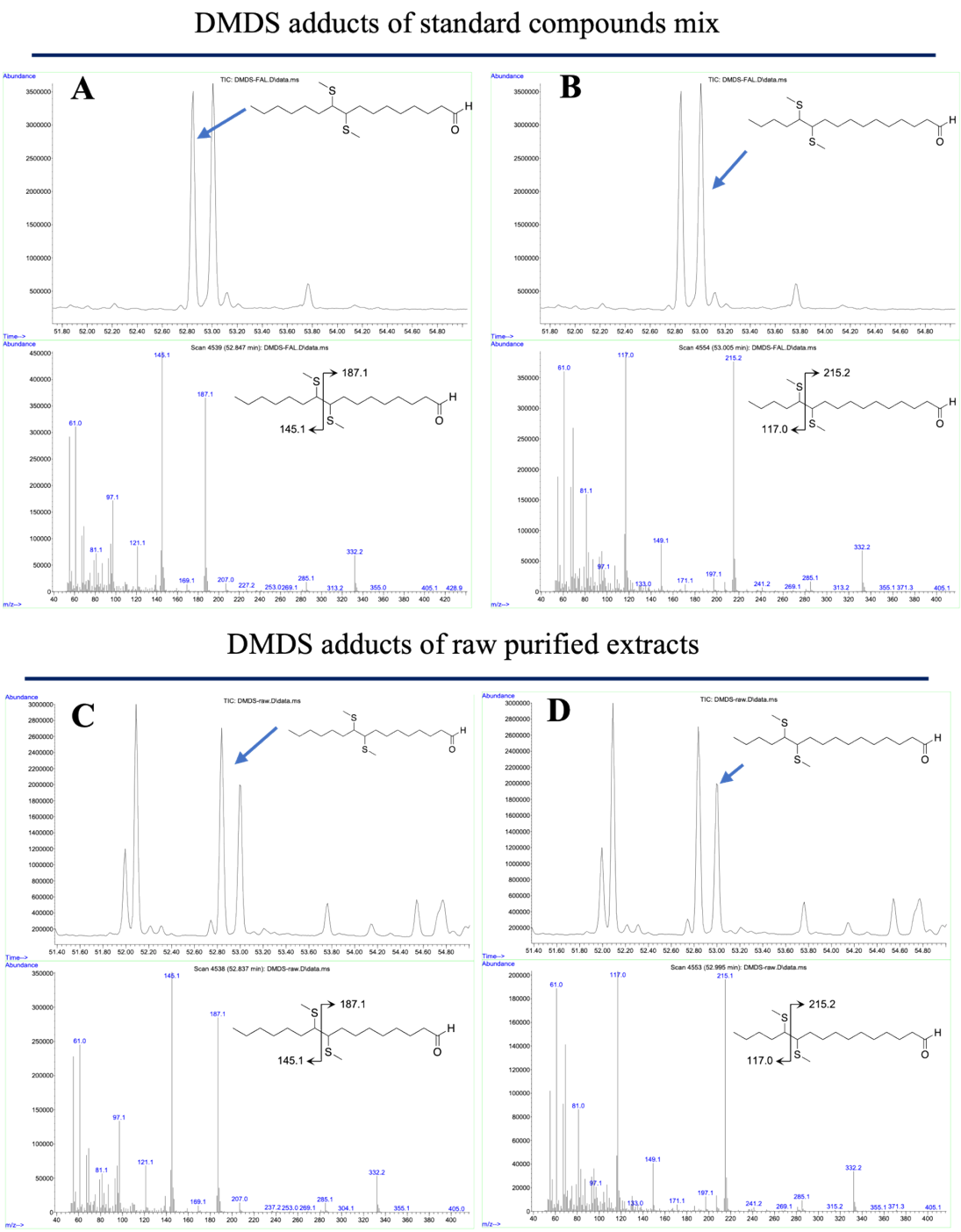

Figure S4: GC-MS spectrum of dimethyldisulphide (DMDS) adducts of aldehyde standard mixture and raw purified extracts of fermentation

After derivatization of standard mixture, a molecular ion of DMDS-adducts Z9-16:Ald and its Z11 isomer appeared at *m/z* 332. Taking *m/z* 145/187 as a pair of diagnostic ions for DMDS-adducts Z9-16:Ald and *m/z* 117/215 for DMDS-adducts Z11-16:Ald, production of Z9-16:Ald and Z11-16:Ald by fermentation of strain FAL2 was verified.

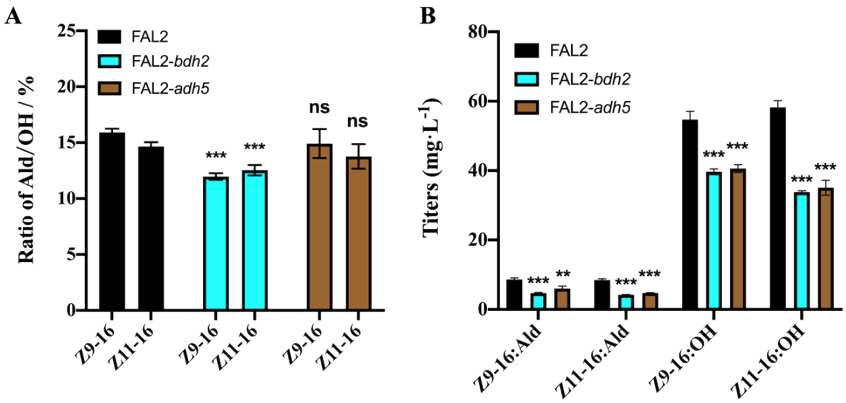

Figure S5: Ratio of aldehydes to alcohols and titers of aldehydes and alcohols in strain FAL2-*adh5* and -*bdh2*

(A) Ratio of aldehydes to alcohols in strain FAL2, FAL2-*bdh2* and FAL2-*adh5*. The ratio was calculated by dividing titers in mmol/L of aldehydes into titers in mmol/L of corresponding alcohols. (B) Titers of Z9-16:Ald, Z11-16:Ald, Z9-16:OH and Z11-16:OH in strain FAL2, FAL2-*bdh2* and FAL2-*adh5*. The standard deviation was calculated from three biological replicates. Differences in titer and ratio between mutants and strain FAL2 are indicated: ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$.

Table S1: Primers for strain construction

Primer name	Primer sequence, 5'->3'
POX1 target RNA-fw	GCTATTTCTAGCTCTAAAACATGAAATTACAAAGGAATCAGATCATTTATCTTTCACTG
POX1 target RNA-rv	CAGTGAAAGATAAATGATCTGATTCCTTTGTAATTTCATGTTTTAGAGCTAGAAATAGC
POX1 repair oligo fw	AAAGAAAATATAATAAATTAGTATTGCGATGTAGAGGTTTCCTGTTTTCCTTCGAACCC
POX1 repair oligo rv	GGGTTCGAAGGAAAACAGGAAACCTCTACATCGCAATACTAATTTATTATATTTTCTTT
POX1 dg fw	GGAATTCGGTCATTAGCGGC
POX1 dg rv	ATCGTTGTGGCCGATGAAAC
FAA1 target RNA-fw	TCCTCATTCTTATAATATTCGTTTTAGAGCTAGAAATAGCAAG
FAA1 target RNA-rv	GAATATTATAAGAATGAGGAGATCATTTATCTTTCACTGCGG
FAA1 repair oligo fw	AGACAAAAAAAGACAACAATTGGATCAACATTTCCATGATAGG
FAA1 repair oligo rv	ATCATGGAAATGTTGATCCAATTGTTGTCTTTTTTTGTCTTTTGTG
FAA1 dg fw	CTTAGAATATGGATGATGCAGCCC
FAA1 dg rv	GGTTGCTATGGTTTGTCTTCC

FAA4 target RNA-fw	AATAAACCTTAAGTTTCCACGTTTTAGAGCTAGAAATAGCAAG
FAA4 target RNA-rv	GTGGAAACTTAAGGTTTATTGATCATTTATCTTTCACTGCGG
FAA4 repair oligo fw	ACTAAGAAGTACGCATCAAAAGGAAGACATAGTTTTTTACTTTCC
FAA4 repair oligo rv	GTAAAAAACTATGTCTTCCTTTTGATGCGTACTTCTTAGTTTTTC
FAA4 dg fw	CGGCTTTTTGGCTGCGCGTC
FAA4 dg rv	CAAACTTGGTGTACTATAGTGC
GAL80 target RNA-fw	GCAGTGAAAGATAAATGATCGTTTATAAAAGTAACATGATGTTTTAGAGCTAGAAATAG
GAL80 targetRNA-rv	GCTATTTCTAGCTCTAAAACATCATGTTACTTTTATAAACGATCATTTATCTTTCACTG
GAL80 repair oligo fw	GGTTTCCCGTTCTTTCCACTCCCGTCAAGCATCTTGCCCTGTGCTTGGCCC
GAL80 repair oligo rv	GGGGCCAAGCACAGGGCAAGATGCTTGACGGGAGTGGAAAGAACGGGAAACC
GAL80 dg fw	CCTGCTGGTCTTCTGGTCTG
GAL80 dg rv	TATGATGGAAGGATGCCCGC
HFD1 targetRNA fw	GCAGTGAAAGATAAATGATCAGGGTAAAATCATTCCAATAGTTTTAGAGCTAGAAATAG
HFD1 targetRNA rv	CTATTTCTAGCTCTAAAACTATTGGAATGATTTTACCCTGATCATTTATCTTTCACTGC
HFD1 repair oligo fw	ATAGCCATAGTAATTTATCACCAACACGTAATGTTTAAGGTTAATTAATTATTTGATG
HFD1 repair oligo rv	AATTAATTAACCTTAAACATTACGTGTTGGTGATAAATTACTATGGCTATG
HFD1 dg fw	TTCCAGTACAGGCACACCAG
HFD1 dg rv	GGGTGATGGTTTGGCGAATG
ADH5 targetRNA fw	CAATTGAAATTTCCATTAATGTTTTAGAGCTAGAAATAGCAAG
ADH5 targetRNA rv	ATTAATGGAAATTTCAATTGGATCATTTATCTTTCACTGCGG
ADH5 repair oligo fw	ATCTACAAAATCAAAGCATCTCTTTTGTAACGAATTTGATGAATATA
ADH5 repair oligo rv	ATCAAATTCGTTACAAAAGAGATGCTTTGATTTTGTAGATATGTAG
ADH5 dg fw	AGTTCTCCTTTCGCGGAAGG
ADH5 dg rv	TGGCTAGCTTTGGGACGATC
BDH2 targetRNA fw	AGTGTGAAAAACTTGAAAGTGTTTTAGAGCTAGAAATAGCAAG
BDH2 targetRNA rv	ACTTTCAAGTTTTTCACACTGATCATTTATCTTTCACTGCGG
BDH2 repair oligo fw	TTCATTGAACATATTTCAGATTGTGATTGAGTACTCACGTTC
BDH2 repair oligo rv	ACGTGAGTACTCAATCACAATCTGAAATATGTTCAATGAATTTATTGTTA
BDH2 dg fw	GATATGATAAGGAGAACTAAGCA
BDH2 dg rv	GTCACACGAAGTATCTGCC
XI-3 target RNA-fw	GCAGTGAAAGATAAATGATCATATGTCTCTAATTTTGGAAGTTTTAGAGCTAGAAATAG
XI-3 target RNA-rv	CTATTTCTAGCTCTAAAACTTCCAAAATTAGAGACATATGATCATTTATCTTTCACTGC
XI-3 up-repair fw	AGTTACTTGCTCTATGCGTTTGC

XI-3 up-repair rv	CCGCTCGGCGGCTTCTAATCCGTACTAATCAGACGCACGCTTGGC
XI-3 dn-repair fw	GAGAAGGTTTTGGGACGCTCGAAGTTACGTGGATTGAGCCAGCA
XI-3 dn-repair rv	TGAGAATCCGGACCAGCAGAT
XI-3-up-Gal1p fw	ATTGACGCCAAGCGTGCGTCTGATTAGTACGGATTAGAAGCCGCC
GAL1p rv	GGGTTTTTTCTCCTTGACG
CYC1t fw	ATCCGCTCTAACCGAAAAGG
XI-3-dn-CYC1t rv	ATCTGTATTGCTGGCTCAATCCACGTAACTTCGAGCGTCCCAAAACCT
Delta target RNA-fw	TGATCTGAGGATTCCTATATCCTCGGTTTTAGAGCTAGAAATAGCAAG
Delta target RNA-rv	TCTAAAACCGAGGATATAGGAATCCTCAGATCATTTATCTTTCACTGCGGAGAAG
Delta up-repair fw	TGTTGGAATAGAAATCAACTAT
Delta up-repair rv	GGATATAGGAATCCTCAAAATG
G418-Delta dn-repair fw	TGTCAAGGAGGGTATTCTGGGCCTCCATGTCGCTGTCGAGGAGAACTTCTAGTATATTC
Delta dn-repair rv	ATGAAGCAGGTGTTGTTGTCTGTTGAG
G418p-fw	CAGCGACATGGAGGCCCAGAAT
G418t-rv	TCGACACTGGATGGCGGCGTTA
Delta-GAL1p-fw	GATTCCATTTTGAGGATTCCTATATCCAGTACGGATTAGAAGCCGCCGAGC
G418t-CYC1t-rv	CTAACGCCGCCATCCAGTGTCGACTTCGAGCGTCCCAAAACCTTCTCAAG
AipFAD-fw	ATGGCTCAAGGCGTCCAAACG
AipFAD-rv	TTAATTGTCTTTCCAGATTTTCAAAAT
GAL1p-AipFAD fw	CTCTATACTTTAACGTCAAGGAGAAAAAACCCATGGCTCAAGGCGTCCAAACGACTACG
CYC1t-AipFAD rv	TGTCTAACTCCTTCCTTTTCGGTTAGAGCGGATTTAATTGTCTTTCCAGATTTTCAA
CYC1t-AipFAD-His rv	GTCTAACTCCTTCCTTTTCGGTTAGAGCGGATTTAGTGATGGTGATGATGGTGATTGTC
HasFAD-fw	ATGGCCCAAAGCTATCAATCAAC
HasFAD-rv	TTAACTTAATTTATCGTTTACACCCGATCC
GAL1p-HasFAD fw	TATACTTTAACGTCAAGGAGAAAAAACCCATGGCCCAAAGCTATCAATCAACTACGG
CYC1t-HasFAD rv	TGTCTAACTCCTTCCTTTTCGGTTAGAGCGGATTTAACTTAATTTATCGTTTACACC
CYC1t-HasFAD-His rv	AACTCCTTCCTTTTCGGTTAGAGCGGATTTAGTGATGGTGATGATGGTGACTTAATTTA
HarFAD-fw	ATGGCCCAAAGCTATCAATC
HarFAD-rv	TTAACTTGATTTATCTTTTACATCCC
GAL1p-HarFAD fw	ACCTCTATACTTTAACGTCAAGGAGAAAAAACCCATGGCCCAAAGCTATCAATCAA
CYC1t-HarFAD- rv	TTGTCTAACTCCTTCCTTTTCGGTTAGAGCGGATTTAACTTGATTTATCTTTTACATC
CYC1t-HarFAD-His- rv	TCCTTTTCGGTTAGAGCGGATTTAGTGATGGTGATGATGGTGACTTGATTTATCTTTTA
AseFAD-fw	ATGGCTCAAGGTGTTCAAACTAC

AseFAD-rv	TTAATTATCTTTCCAGATCT
GAL1p-AseFAD fw	TATACTTTAACGTCAAGGAGAAAAAACCCATGGCTCAAGGTGTTCAAACTACAACTAT
CYC1t-AseFAD rv	GTCTAACTCCTTCCTTTTCGGTTAGAGCGGATTTAATTATCTTTCCAGATCTTCAA
CYC1t-AseFAD-His rv	TAACTCCTTCCTTTTCGGTTAGAGCGGATTTAGTGATGGTGATGATGGTGATTATCTTT
OLE1p target RNA-fw	TAAATGATCATAGCCGTAACAATAGGGATGTTTTAGAGCTAGAAATAGCAAGTTAAAAT
OLE1p target RNA-rv	AGCTCTAAAACATCCCTATTGTTACGGCTATGATCATTTATCTTTCACTGCGGAGAAGT
OLE1p-up fw	GTTCTGAGGTATTCGTATCGC
OLE1p-up rv	TCTATGGTAACCGGCAGTAAT
CTR3p-fw	GTATTCCAATGAGAATCGCTAG
CTR3p-rv	CTTTGTATAGCCCTTAAATGTG
CTR3p-OLE1pOUT-UP-rv	CATTTCTAGCGATTCTCATTGGAATACATAGTCACGGAGAATCTTGACG
CTR3p-OLE1-fw	GAAAACACATTTAAGGGCTATACAAAGATGCCAACTTCTGGAACTACTA
MET3p-fw	TGGTATAAGGTGAGGGGGTCCACAG
MET3p-rv	GAATACCACCGTGAGGAGCAGGCATG
MET3p-OLE1pOUT-UP-rv	CTGTGGACCCCCTCACCTTATACCAATAGTCACGGAGAATCTTGACGT
MET3p-OLE1-fw	CATGCCTGCTCCTCACGGTGGTATTCATGCCAACTTCTGGAACTACTA
CYC1p-fw	TCATTGGCGAGCGTTGGTT
CYC1p-rv	ATTTAGTGTGTGTATTTGTGTTTG
CYC1p-OLE1pOUT-up-rv	ACCAACCAACGCTCGCCAAATGAATAGTCACGGAGAATCTTGACGT
CYC1p-OLE1-fw	ACACAAACACAAATACACACACTAAATATGCCAACTTCTGGAACTACT
PDA1p-fw	GAAATTCAAAACTCTCCAGACAA
PDA1p-rv	ATTGGCACAAATGTGGTTTCCT
PDA1p-OLE1pOUT-UP-rv	CTTTGTCTGGAGAGTTTTGAATTTCATAGTCACGGAGAATCTTGACGT
PDA1p-OLE1-fw	GAAAGGAAACCACATTTGTGCCAATATGCCAACTTCTGGAACTACTA
YMRW15 target RNA-fw	GATGGCATAATATTGAGAAAGTTTTAGAGCTAGAAATAGCAAG
YMRW15 target RNA-rv	TTTCTCAATATTATGCCATCGATCATTTATCTTTCACTGCGGAGAAG
YMRW15-up-fw	TCAATCAAAGCAACCCACAA
YMRW15-up-rv	AGTAAAAAAGGAGTAGAAACATTTTGAAGCTATGGCGCGTGCGGTGTAAGAAAATGACA
TCDp-fw	CTGTTTCTCAAACTTTATGTCATTTTCTTACACCGCACGCGCCATAGCTTCAAAATGTT
TCDp-rv	TTCTTTCCAATCTTTCTTCTCTGGTAATTGGGGACATTTTGTTTGTTTATGTGTGTTTA
MmaCAR-fw	GAACTTAGTTTCGAATAAACACACATAAACAAACAAAATGTCCCCAATTACCAGAGAAG
MmaCAR-rv	TACTTGACCTGAACTTAACGATTTGCTCTCAATCCGCTTACAACAAGCCCAACAGCCTC
RBLt-fw	GTACGTTTCTGATTTGAGGCTGTTGGGCTTGTTGTAAGCGGATTGAGAGCAAATCGTTA

RBLt-rv	GCGTATTTTAAGTTTAATAACTCGAAAATTCTGCGTTAGCCGAAAATCTTTCAAGCACG
PGK1t-fw	AATGAATCTTTCTGTCGTGCTTGAAAGATTTTCGGCTAACGCAGAATTTTCGAGTTATT
PGK1t-rv	ACCATGTGCTACTGGTGTATGTAACTGCTTGTCTTGAGATCTCCCATGTCTCTACTGGT
npgA-fw	CAAAGAAGCACCACCACCAGTAGAGACATGGGAGATCTCAAGACAAGCAGTTACATACA
npgA-rv	GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGTTCAAGATACCTCTTCTG
TEF1p-fw	TTGGAGAGGTAGAAGCAGAAGAGGTATCTTGAACCATTTTGTAATTAAAACTTAGATTA
TEF1p-rv	GAAGATTTATAATGGTTTATCGGTTGCATTTTCCATGAGTGATCCCCCACACACCATAG
YMRW15-dn-fw	GTAGAAACATTTTGAAGCTATGGTGTGTGGGGGATCACTCATGGAAAATGCAACCGATA
YMRW15-dn-rv	GCCGTCCTCATGATGTGTTA

Table S2: Levels set in pH and concentration of phosphate and nitrogen source

Levels	pH	$(NH_4)_2$ SO_4 g/L	KH_2PO_4 g/L
1	5	2	2
2	5.5	4.7	6.3
3	6	7.4	10.6
4	6.5	10	15

Table S3: Combinations in optimization of fermentation conditions

Number	Combinations	pH	$(NH_4)_2$ SO_4 g/L	KH_2PO_4 g/L
1	111	5	2	2
2	122	5	4.7	6.3
3	133	5	7.4	10.6
4	144	5	10	15
5	212	5.5	2	6.3
6	223	5.5	4.7	10.6
7	234	5.5	7.4	15
8	241	5.5	10	2
9	313	6	2	10.6
10	324	6	4.7	15
11	331	6	7.4	2
12	342	6	10	6.3

13	414	6.5	2	15
14	421	6.5	4.7	2
15	432	6.5	7.4	6.3
16	443	6.5	10	10.6

Table S4: Concentration of Z9-16: Ald and Z11-16: Ald in tested samples in dual-choice assay

Samples	Z9-16: Ald (mg/L)	Z11-16: Ald (mg/L)
Standard blend	1.5	48.5
Raw extracts of FAL2	91.9	45.3
Raw extracts of WT	0	0
Raw purified of FAL2	75.2	47.5

References

1 Y. J. Zhou, N. A. Buijs, Z. Zhu, J. Qin, V. Siewers and J. Nielsen, *Nat. Commun.*, 2016, **7**, 11709.

2 B. J. Ding, P. Hofvander, H. L. Wang, T. P. Durrett, S. Stymne and C. Löfstedt, *Nat. Commun.*, 2014, **5**, 1–7.

3 H. R. Buser, H. Arn, P. Guerin and S. Rauscher, *Anal. Chem.*, 1983, **55**, 818–822.