## Additional File 1

# Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid short- and branched-chain alkyl esters biodiesel

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**Table S1:** List of primers used in this study.

| DNA Primer | Sequence (5' to 3') |
|---|---|
| Maqu-f-EcoR1 | AAGAGGAATTCATGACTCCATTGAACCCAACC |
| Maqu-r-Not1 | CTCTTGCGGCCGCTCAGTGATGG |
| ws-f-EcoR1 | AAGAGGAATTCATGAAGAGATTGGGTACTTTGG |
| ws-r-Not1 | CTCTTGCGGCCGCTTAGTGATGG |
| OPI1 KO-f | TACAGTGCTGATTAAAGCGTGTGTATCAGGACAGTGTTTTTAACGAAGATACTAGTCATTG ATGTCTGAAAATCAACGTTTAGGATTATCAGAGGAAGAGGCAGCTGAAGCTTCGTACGC |
| OPI1 KO-r | TATTATTCCGTATAATATTATTACTGGTGGTAATGCATGAAAGACCTCAATCTGTCTCGGTT AGTCCTTGCTATCCACGTTGTCCTGAGAGGGCTTTAGCATAGGCCACTAGTGGATCTG |
| RPD3 KO-f | ATGGTATATGAAGCAACACCTTTTGATCCGATCACGGTCACAGCTGAAGCTTCGTACGC |
| RPD3 KO-r | TCAATAGAATTCATTGTCATGCTCAACATGTAGGTCCCGCATAGGCCACTAGTGGATCTG |
| Kan-r | GCGTTTCCCTGCTCGCAGGT |
| OPI1 up-f | GCCAAGAAAGCATATCAGGCCAGAA |
| OPI1 mid-r | GAACTCATCGTCGTTGTAGTCGTAATCATC |
| RPD3 up-f | CGAAAGGGAAAAACAGAAAAGATACTAGTAGTTG |
| RPD3 mid-r | CATCAGTATGGAACTGACACATTTCTTGTTTCG |

**Table S2:** Codon optimized sequence of wax ester synthase genes used in this study.

| Gene | Sequence (5' to 3') |
|---|---|
| ***ws2*** | ATGAAGAGATTGGGTACTTTGGATGCTTCTTGGTTGGCTGTTGAATCTGAAGATACTCCAAT<br>GCATGTTGGTACTTTACAAATCTTCTCATTGCCAGAAGGTGCTCCAGAAACTTTTTTGAGAG<br>ATATGGTTACCAGAATGAAGGAAGCTGGTGATGTTGCTCCACCTTGGGGTTACAAATTGGC<br>TTGGTCTGGTTTTTTGGGTAGAGTTATTGCTCCAGCATGGAAGGTTGATAAGGATATTGATT<br>TGGACTACCACGTTAGACATTCTGCTTTGCCAAGACCAGGTGGTGAAAGAGAATTAGGTAT<br>TTTGGTTTCCAGATTGCACTCCAACCCATTGGATTTTTCAAGACCATTGTGGGAATGCCATG<br>TTATCGAAGGTTTGGAAAACAATAGATTCGCCTTGTACACCAAGATGCATCACTCTATGATT<br>GATGGTATCTCCGGTGTTAGATTGATGCAAAGAGTTTTGACTACCGATCCAGAAAGATGTAA<br>TATGCCACCACCTTGGACTGTTAGACCACATCAAAGAAGAGGTGCTAAGACTGACAAAGAA<br>GCCTCTGTTCCAGCTGCTGTTTCTCAAGCTATGGATGCTTTGAAATTGCAAGCTGATATGGC<br>TCCAAGATTGTGGCAAGCTGGTAATAGATTGGTTCATTCTGTTAGACATCCAGAAGATGGTT<br>TGACTGCTCCTTTTACTGGTCCAGTTTCTGTTTTGAACCATAGAGTTACAGCCCAAAGAAGA<br>TTCGCTACTCAACACTACCAATTAGACAGATTGAAGAACTTGGCTCATGCTTCTGGTGGTTC<br>TTTGAATGATATTGTCTTGTACTTGTGCGGTACTGCCTTAAGAAGATTTTTGGCTGAACAAAA<br>CAACTTGCCAGATACACCATTGACTGCTGGTATTCCAGTTAACATTAGACCAGCTGATGATG<br>AAGGTACTGGTACTCAAATCTCCTTTATGATTGCTTCTTTGGCTACCGATGAAGCTGATCCA<br>TTGAATAGATTGCAACAAATCAAGACCTCTACCAGAAGAGCTAAAGAACACTTGCAAAAGTT<br>GCCAAAGTCTGCTTTGACTCAATACACCATGTTGTTGATGTCCCCATATATCTTACAATTAAT<br>GTCCGGTTTGGGTGGTAGAATGAGACCAGTTTTTAACGTTACCATCTCTAATGTTCCAGGTC<br>CTGAAGGTACATTGTATTATGAAGGTGCTAGATTGGAAGCCATGTACCCAGTTTCTTTGATT<br>GCTCATGGTGGTGCCTTGAACATTACTTGTTTGTCTTATGCTGGTTCCTTGAACTTTGGTTT<br>CACTGGTTGTAGAGATACCTTGCCATCTATGCAAAAATTGGCTGTTTACACTGGTGAAGCCT<br>TGGATGAATTGGAATCTTTGATTTTGCCACCTAAGAAGAGAGCCAGAACTAGAAAACATCAT<br>CATCACCATCACTAA |
| ***Maqu_0168*** | *A*TGACTCCATTGAACCCAACCGACCAATTATTCTTGTGGTTGGAAAAAAGACAACAACCTAT<br>GCACGTTGGTGGTTTACAATTATTCAGTTTTCCTGAAGGTGCCCCAGATGATTATGTTGCTC<br>AATTAGCTGACCAATTAAGACAAAAGACTGAAGTTACCGCCCCATTCAATCAAAGATTGTCT<br>TATAGATTGGGTCAACCAGTTTGGGTTGAAGATGAACATTTGGATTTGGAACACCACTTTAG<br>ATTTGAAGCCTTGCCAACTCCAGGTAGAATCAGAGAATTATTGTCTTTCGTTTCCGCCGAAC<br>ATTCCCATTTGATGGATAGAGAAAGACCAATGTGGGAAGTTCATTTGATCGAAGGTTTGAAG<br>GATAGACAATTTGCCTTGTACACCAAGGTTCACCATTCTTTGGTTGATGGTGTTTCTGCTAT<br>GAGAATGGCTACTAGAATGTTGTCTGAAAACCCTGATGAACATGGTATGCCACCAATTTGG<br>GATTTGCCATGTTTGTCAAGAGATAGAGGTGAATCTGATGGTCATTCTTTGTGGAGATCAGT<br>TACTCATTTGTTGGGTTTGTCCGGTAGACAATTAGGTACTATTCCAACCGTTGCCAAAGAAT<br>TATTGAAAACCATCAATCAAGCCAGAAAGGACCCAGCTTACGATTCTATTTTTCATGCTCCA<br>AGATGCATGTTGAATCAAAAGATTACCGGTTCCAGAAGATTCGCTGCTCAATCTTGGTGTTT<br>GAAGAGAATTAGAGCTGTTTGTGAAGCCTACGGTACTACTGTTAATGATGTTGTTACAGCTA<br>TGTGTGCTGCTGCTTTGAGAACTTACTTGATGAATCAAGATGCCTTGCCAGAAAAACCATTG<br>GTTGCTTTTGTTCCAGTCTCCTTGAGAAGAGATGATTCTTCTGGTGGTAATCAAGTCGGTGT<br>TATTTTGGCTTCATTGCACACTGATGTTCAAGAAGCTGGTGAAAGATTATTGAAGATTCACC<br>ACGGTATGGAAGAAGCCAAACAAAGATATAGACACATGTCCCCAGAAGAAATCGTTAACTAT<br>ACTGCTTTGACTTTGGCTCCAGCTGCTTTCCATTTGTTGACTGGTTTAGCTCCAAAGTGGCA<br>AACTTTCAACGTTGTTATTTCTAACGTTCCAGGTCCATCCAGACCATTATATTGGAATGGTG<br>CTAAATTGGAAGGTATGTACCCAGTTTCCATCGATATGGATAGATTGGCTTTGAACATGACC<br>TTGACCTCTTACAACGATCAAGTCGAATTTGGTTTGATCGGTTGTAGAAGAACTTTGCCATC<br>ATTGCAAAGAATGTTGGACTACTTGGAACAAGGTTTGGCTGAATTGGAATTGAACGCTGGTT<br>TACATCATCATCACCATCACTGA |

**Table S3:** Growth parameters of engineered yeast strains.

| Strain | $\mu_{max}(h^{-1})$ | $Y_{sx}$ (g/g) |
|---|---|---|
| ws2 BY4742 | 0.337 ± 0.029 | 0.137 ± 0.002 |
| ws2 BY4742 *opi1*Δ | 0.380 ± 0.006 | 0.129 ± 0.002 |
| ws2 BY4742 *rpd3*Δ | 0.317 ± 0.023 | 0.115 ± 0.002 |
| Maqu_0168 BY4742 | 0.321 ± 0.029 | 0.128 ± 0.002 |
| Maqu_0168 BY4742 *opi1*Δ | 0.333 ± 0.016 | 0.116 ± 0.002 |
| Maqu_0168 BY4742 *rpd3*Δ | 0.274 ± 0.011 | 0.112 ± 0.003 |
| pESC BY4742 | 0.417 ± 0.017 | 0.129 ± 0.001 |
| ws2 BY4742-IB | 0.513 ± 0.012 | 0.091 ± 0.001 |
| ws2 BY4742-IB *opi1*Δ | 0.396 ± 0.055 | 0.101 ± 0.003 |
| Maqu_0168 BY4742-IB | 0.431 ± 0.006 | 0.056 ± 0.001 |
| Maqu_0168 BY4742-IB *opi1*Δ | 0.404 ± 0.049 | 0.069 ± 0.002 |
| pESC BY4742-IB | 0.424 ± 0.038 | 0.131 ± 0.002 |

$Y_{sx}$: yield of biomass on substrate

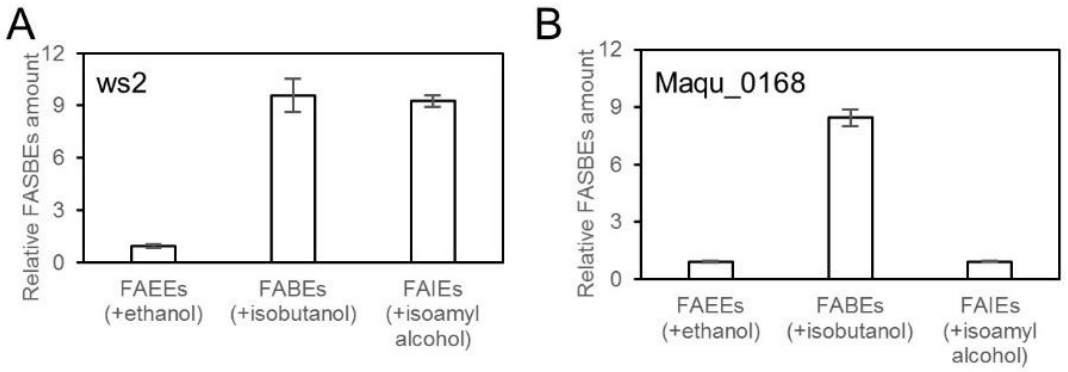


**Figure S1:** Relative FAEEs, FABEs or FAIEs production with exogenous alcohol feeding. Yeast cells expressing ws2 (A) and Maqu_0168 (B) with exogenous feeding of 0.1% ethanol, isobutanol or isoamyl alcohol were compared with no alcohol feeding cells. Values are the mean of biological duplicates ± SD after 48 h.

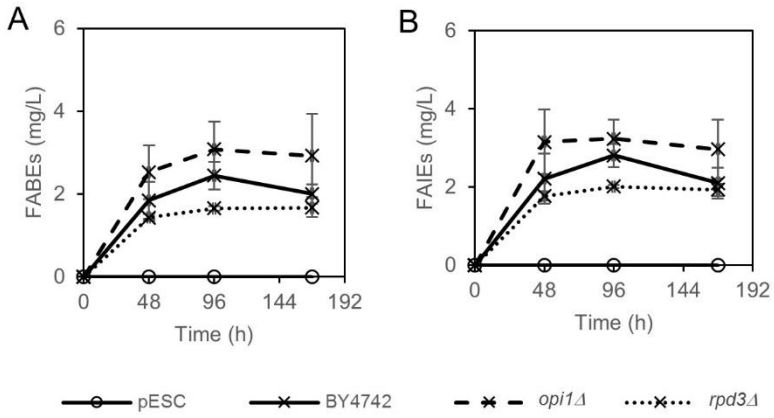


**Figure S2:** Production of fatty acid esters in engineered yeast expressing ws2. (A) FABEs (B) Total FAIEs. Figures are the same as Figure 3B and C, with adjusted y-axis scales.

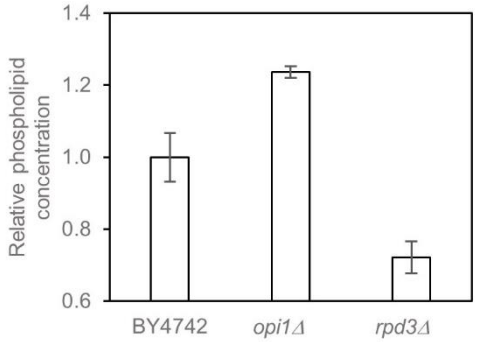


**Figure S3**: Relative phospholipid concentration of engineered yeast strains. Strains were transformed with empty pESC-URA plasmid and cultured in minimal medium lacking uracil with 0.2% glucose and 1.8% galactose. Absolute phospholipid concentration for BY4742 was 16.2 μM. Values are the mean of biological triplicates ± SD after 48 h.

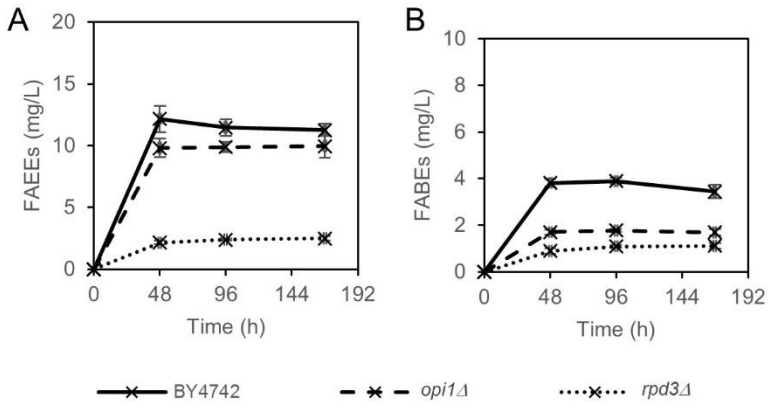


**Figure S4:** Production of fatty acid esters in engineered yeast expressing Maqu_0168. (A) FAEEs (B) FABEs. Figures are the same as Figure 4A and B, with adjusted y-axis scales.

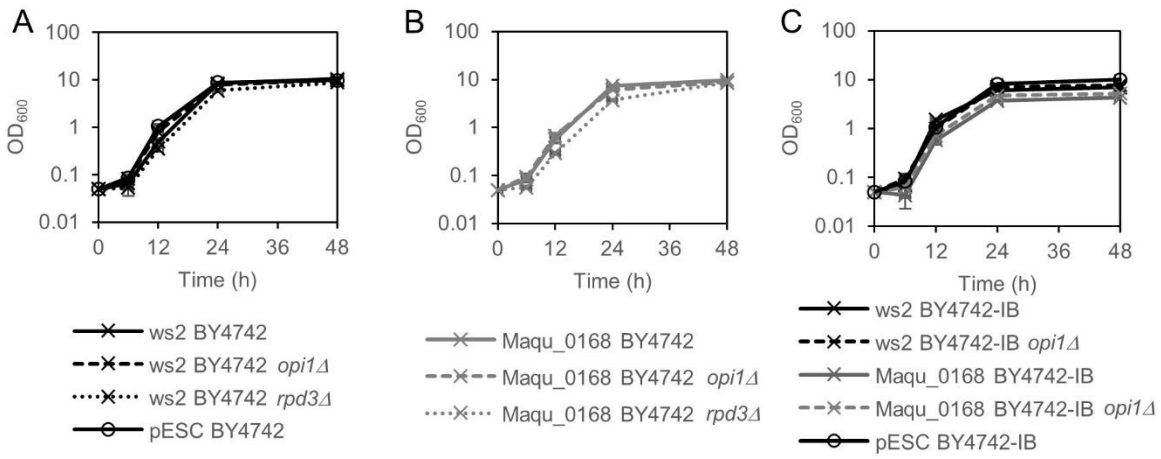


**Figure S5**: Growth curves for engineered cells. (A) BY4742 and mutants expressing ws2 (B) BY4742 and mutants expressing Maqu_0168 (C) BY4742-IB and OPI1 mutant expressing ws2 or Maqu_0168. Values are the mean of biological triplicates ± SD at 0,6,12,24 and 48 h.

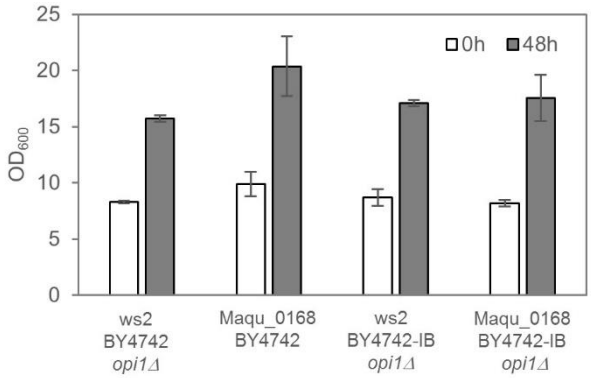


**Figure S6**: $OD_{600}$ for high cell density fermentation as shown in Figure 6. Values are the mean of biological triplicates ± SD at 48 h.

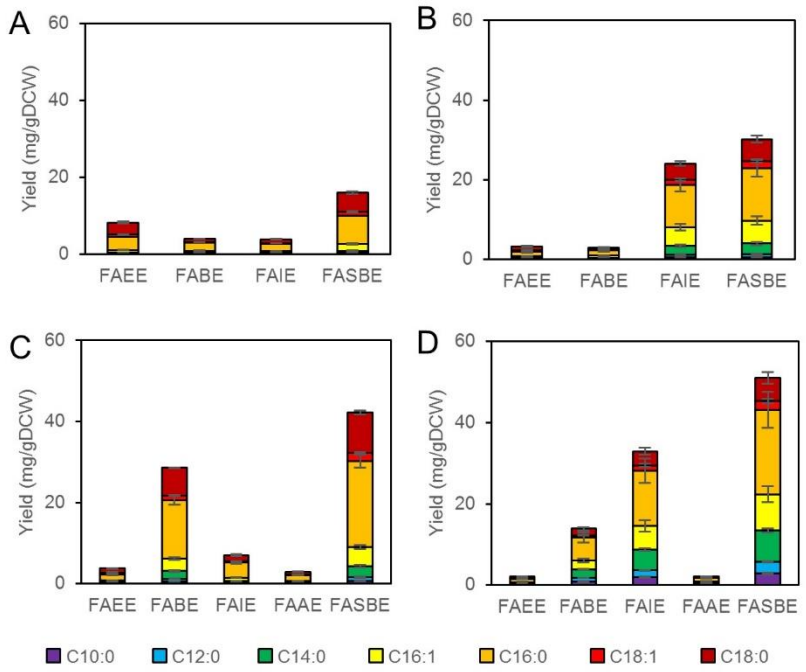


**Figure S7:** FASBEs production yield corresponding to Figure 6. (A) BY4741 *opi1Δ* expressing *ws2*, (B) BY4742 expressing *Maqu_0168*, (C) BY4742-IB *opi1Δ* expressing *ws2* and (D) BY4742-IB *opi1Δ* expressing *Maqu_0168*.