

Figure S1. Elimination of IctAG synthesis and expression of *EaDAcT* does not affect growth on YPD. Cell density (C.D.; solid lines) and extracellular glucose concentrations (Glc; dashed lines) from indicated strains cultured aerobically in bioreactors containing YPD media. Values represent the mean \pm S.D. and are representative of three biological replicates.

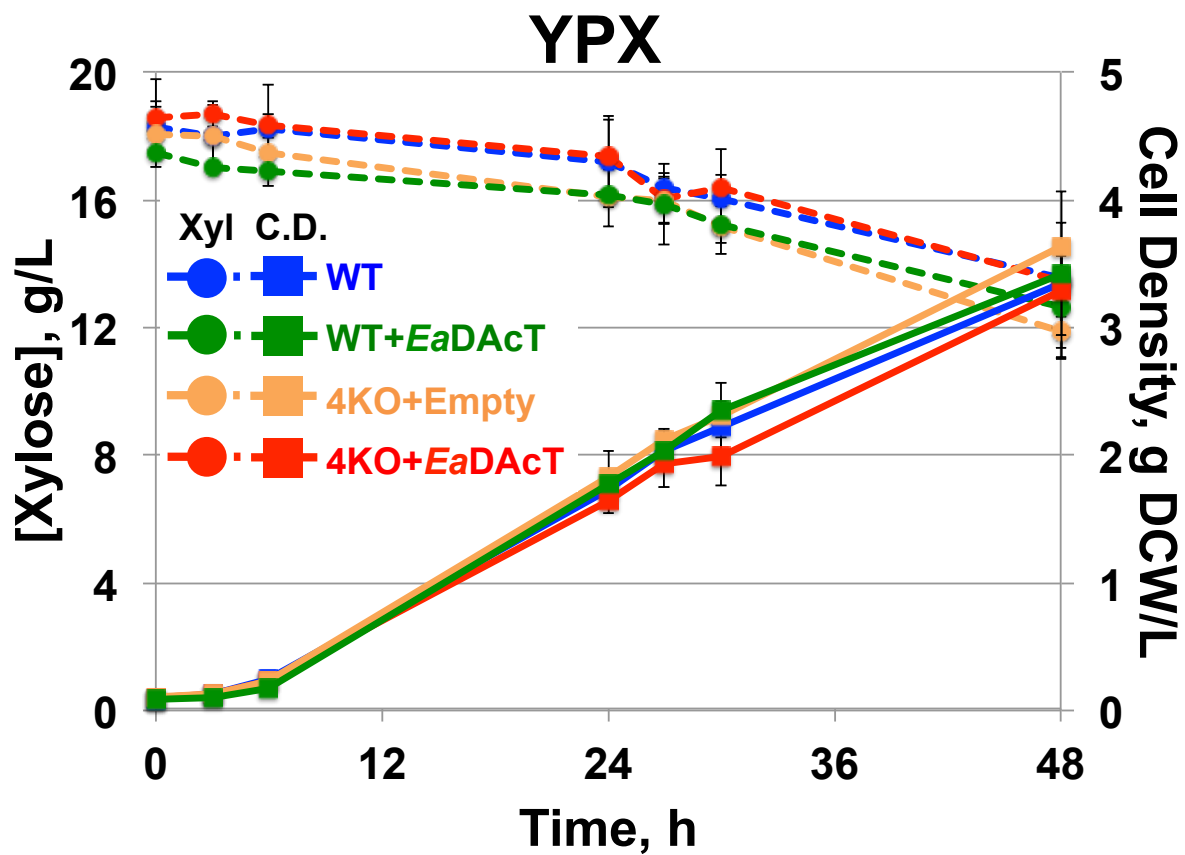


Figure S2. Acetyl-TAG producing yeast strains can consume and grow on xylose. Cell density (C.D.; solid lines) and extracellular xylose concentrations (Xyl, dashed lines) from indicated strains cultured aerobically in bioreactors containing YPX media. Values represent the mean \pm S.D. and are representative of three biological replicates.

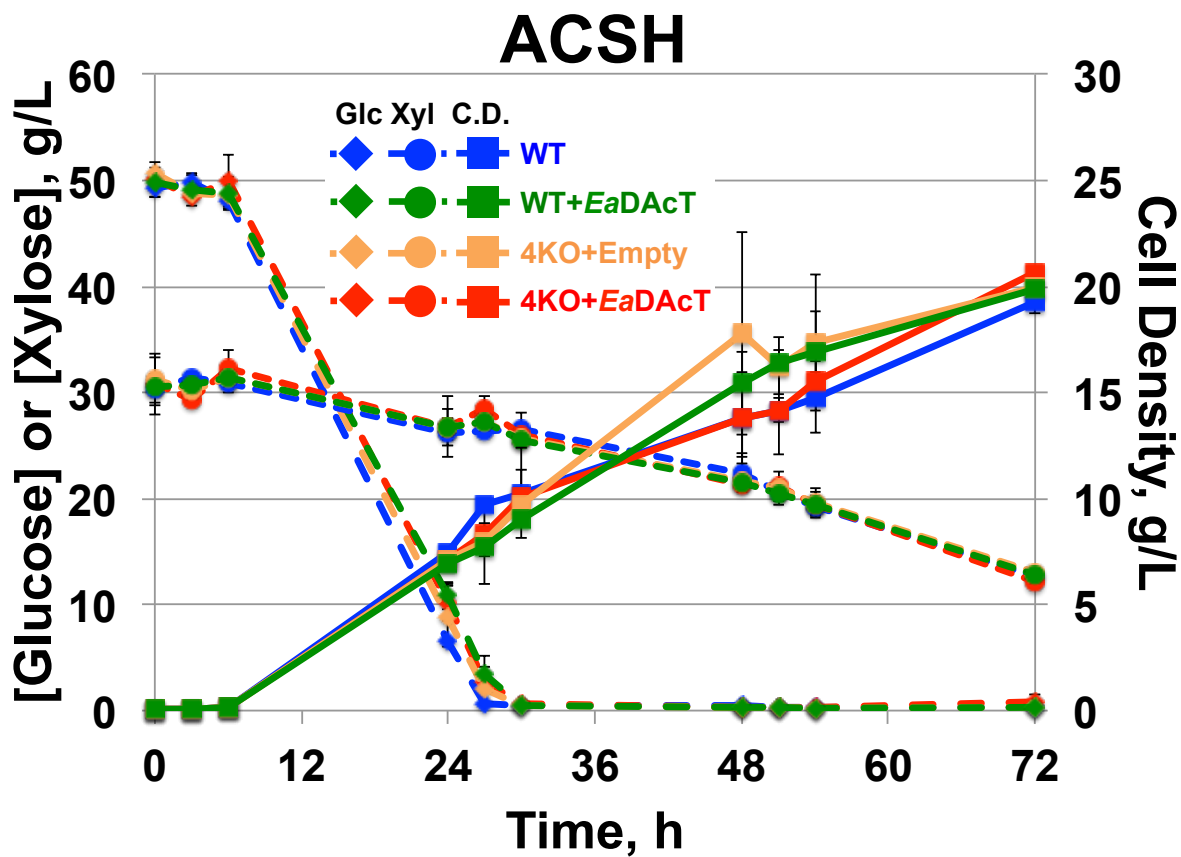


Figure S3. Acetyl-TAG producing yeast strains consume sugars in ACSH similarly to wild-type cells. Cell density (C.D.; solid lines) and concentrations of extracellular glucose (Glc; dashed lines) and xylose (Xyl; dotted lines) from indicated strains cultured aerobically in bioreactors containing ACSH. Values represent the mean \pm S.D. and are representative of three biological replicates.

Table S1. *S. cerevisiae* strains used in this study.

| Strain name | Genotype | Reference |
|-----------------------|---|------------------|
| SCY62 | MATa ADE2 | [21] |
| H1246 | MATa <i>are1Δ::HIS3 are2Δ::LEU2 dga1Δ::KanMX4</i> <i>lro1Δ::TRP1 ADE2</i> | [21] |
| GLBRCY2A | NRRL YB-210; MATα/MATa <i>HO/HOΔ::PGK1p-XYL1-TDH3t-TDH3p-XYL2-TEF2t-TEF2p-XYL3-CYC1t-LoxP-KanMX-LoxP</i> | [26] |
| GLBRCY27D | Haploid derivative of GLBRCY2A; MATα <i>HOΔ::PGK1p-XYL1-TDH3t-TDH3p-XYL2-TEF2t-TEF2p-XYL3-CYC1t-LoxP-KanMX-LoxP</i> | This study |
| WT, GLBRCY40 | GLBRCY27D with <i>loxP-KanMX-loxP</i> marker excised by Cre | This study |
| 4KO, GLBRCY375 | GLBRCY40 <i>are1Δ::LoxP are2Δ::LoxP dga1Δ::LoxP lro1Δ::LoxP</i> | This study |
| WT+EaDAcT, GLBRCY123 | GLBRCY40 <i>HOΔ::PGK1p-XYL1-TDH3t-TDH3p-XYL2-TEF2t-TEF2p-XYL3-CYC1t -ACT1p-TEF1t-TEF1p-EaDAcT-TUB1t-LoxP-HphMX4-LoxP</i> | This study |
| 4KO+Empty, GLBRCY122 | GLBRCY375 <i>HOΔ::PGK1p-XYL1-TDH3t-TDH3p-XYL2-TEF2t-TEF2p-XYL3-CYC1t -ACT1p-TEF1t-TEF1p-TUB1t-LoxP-HphMX4-LoxP</i> | This study |
| 4KO+EaDAcT, GLBRCY121 | GLBRCY375 <i>HOΔ::PGK1p-XYL1-TDH3t-TDH3p-XYL2-TEF2t-TEF2p-XYL3-CYC1t -ACT1p-TEF1t-TEF1p-EaDAcT-TUB1t-LoxP-HphMX4-LoxP</i> | This study |

In the genotype description, “p” denotes promoter sequence and “t” denotes terminator sequence.

Table S2. Primers used for genetic engineering.

| Primer name | Purpose | Sequence (5' to 3') |
|--------------------|---|---|
| ybDGA1LoxPFOR | Forward primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>DGA1</i> ORF | ATACATAAGGAAACGCAGAGG CATACAGTTTGAACAGTCA CATAA agctgaagcttcgtacgctgc |
| ybDGA1LoxPREV | Reverse primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>DGA1</i> ORF | CCTTATTTATTCTAACATATTTTGT GTTTTCCGATGAATTCATTA gcataggccactagtggatctg |
| ybLRO1LoxPFOR | Forward primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>LRO1</i> ORF | ACAAAAGGTTCTCTACCAACGA ATTTCGGCGACAATCGAGT AAAAA agctgaagcttcgtacgctgc |
| ybLRO1LoxPREV | Reverse primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>LRO1</i> ORF | TCTTTGAAATAATACACGGAT GGATAGTGAGTCAATGTCTG GTCAT gcataggccactagtggatctg |
| ybARE1LoxPFOR | Forward primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>ARE1</i> ORF | TGTTTCAGCACGGCTTGCAGC AAGAGCGCCAAAACAGATT GCAAGA agctgaagcttcgtacgctgc |
| ybARE1LoxPREV | Reverse primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>ARE1</i> ORF | TATCTATCAAGGGCTTGCAGG GGACACACGTGGTACGGTG GCAGT gcataggccactagtggatctg |
| ybARE2LoxPFOR | Forward primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>ARE2</i> ORF | CAAGGACACATTACGTTAGC AAAAGCAACAATAACAAACA CAACC agctgaagcttcgtacgctgc |
| ybARE1LoxPREV | Reverse primer to amplify LoxP-KanMX4-LoxP cassette for deletion of <i>ARE2</i> ORF | CTCCACAGAACAGTTGCAGG ATGCCTTAGAATGTCAAGTAC AACG gcataggccactagtggatctg |
| ScARE1-507FOR | Forward primer to verify deletion of <i>ARE1</i> , anneals 507 bp upstream of <i>ARE1</i> ORF | gctccaacgccctcagcgtc |
| ScARE1+998REV | Reverse primer to verify deletion of <i>ARE1</i> , anneals 998 bp downstream of <i>ARE1</i> ORF | agagcctttgtgctgggtcg |
| ScARE2-515FOR | Forward primer to verify deletion of <i>ARE2</i> , anneals 515 bp upstream of <i>ARE2</i> ORF | gcgccgctgggaaaacgcac |
| ScARE2+986REV | Reverse primer to verify deletion of <i>ARE2</i> , anneals 986 bp downstream | cttttggcgcgttttcgcc |

of *ARE2* ORF

| | | |
|---------------|---|-----------------------|
| ScDGA1-541FOR | Forward primer to verify deletion of <i>DGA1</i> , anneals 541 bp upstream of <i>DGA1</i> ORF | caatgtcgacgaagcgcatcg |
| ScDGA1+503REV | Reverse primer to verify deletion of <i>DGA1</i> , anneals 503 bp downstream of <i>DGA1</i> ORF | cctatagagcctattggtcgg |
| ScLRO1-534FOR | Forward primer to verify deletion of <i>LRO1</i> , anneals 534 bp upstream of <i>LRO1</i> ORF | cgacacattgctcggcacg |
| ScLRO1+520REV | Reverse primer to verify deletion of <i>LRO1</i> , anneals 520 bp downstream of <i>LRO</i> ORF | gctcctcattaggggaacagg |

Primer sequences in upper case denote sequences used for homologous recombination; lower case denotes annealing sequences.