Supplementary Information

Metabolic Engineering and Genome-Wide Adaptive Evolution for Efficient Reduction of Glycerol in Industrial *Saccharomyces cerevisiae*

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Supplementary Figures

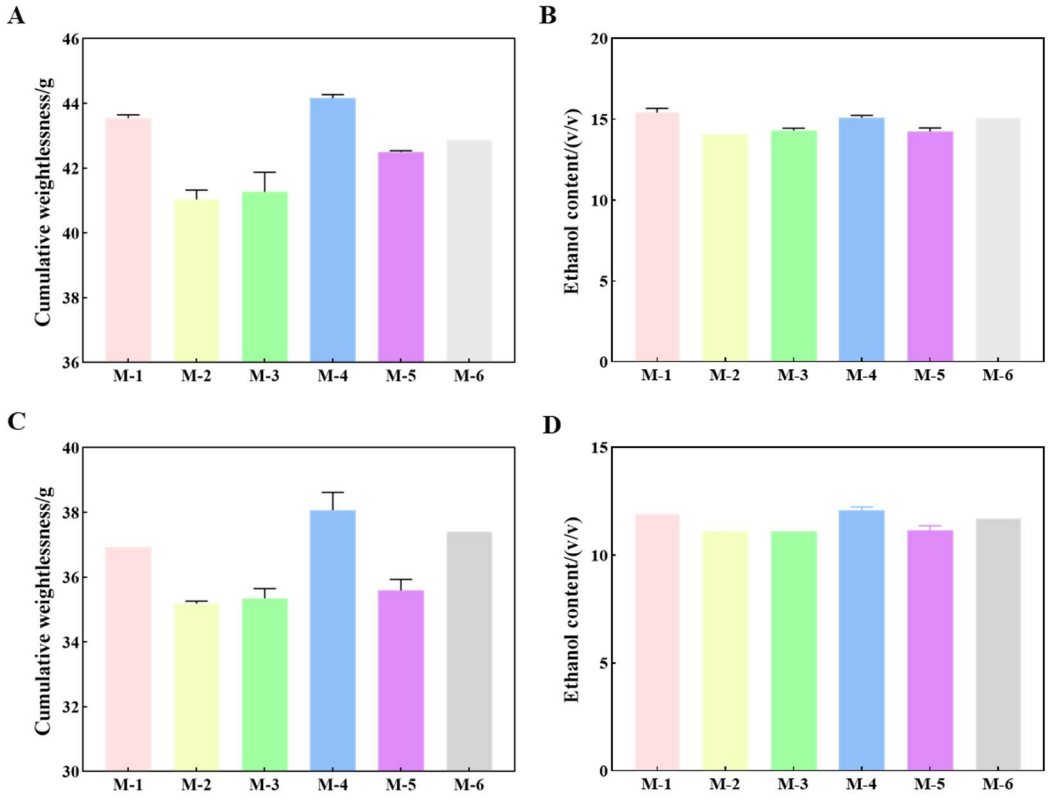

Figure S1. Cumulative weightlessness and ethanol yield in engineered strains. (A) Difference in cumulative weightlessness at 36°C. (B) Difference in ethanol yield at 36°C. (C) Difference in cumulative weightlessness at 39°C. (D) Difference in ethanol yield at 39°C.

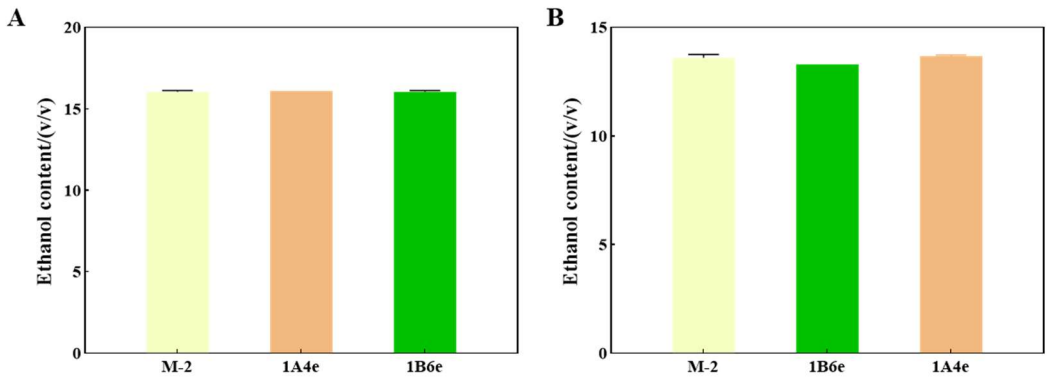

Figure S2. The ethanol yield of the evolved strains and the parental strain. (A) Ethanol yield of the evolved strains and the parental strain fermented at 35°C. (B) Ethanol yield of the evolved strains and the parental strain fermented at 38°C.

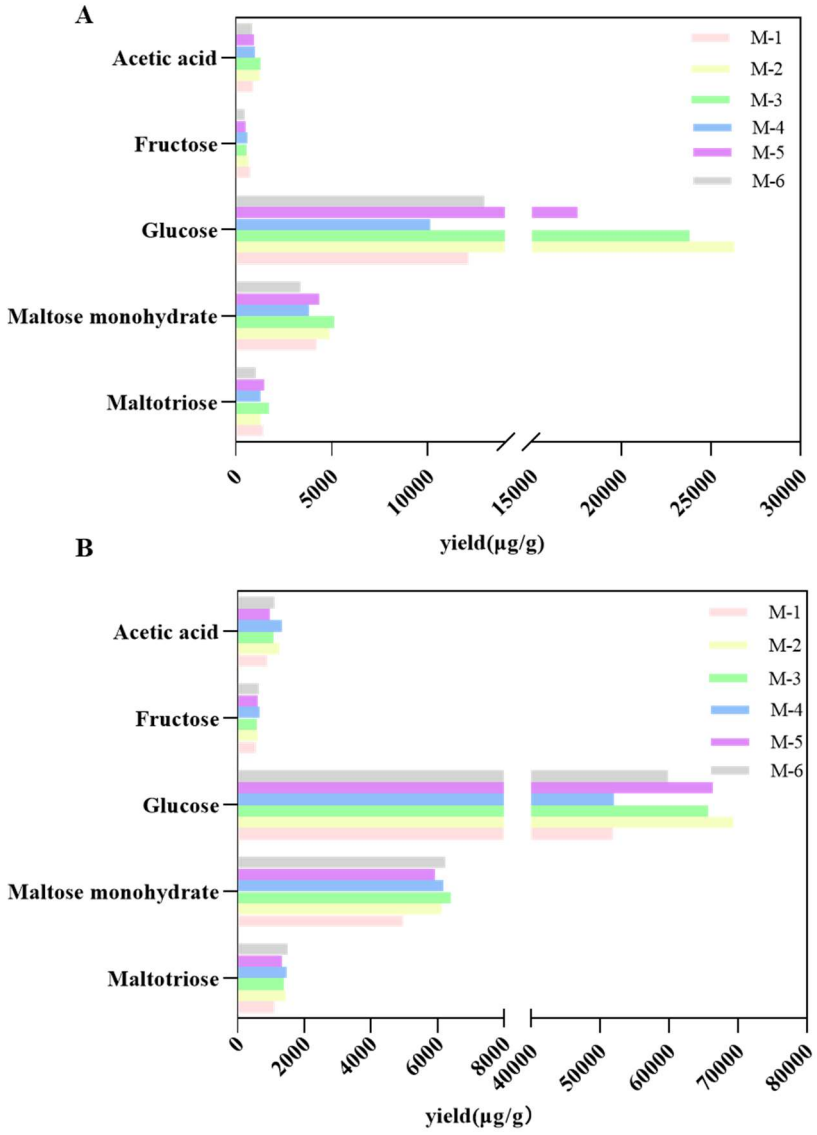

Figure S3. Residual sugar and acetic acid in fermentation of engineered strains. (A) Residual sugar and acetic acid in fermentation of engineered strains at 36°C. (B) Residual sugar and acetic acid in fermentation of engineered strains at 39°C.

Supplementary Tables

Supplementary Table S1. A list of plasmids constructed in this study.

Name	Description	Source
PSCM-H-gRNA-site5	*S. cerevisiae* M, HygB, *SNR52p*-site5-gRNA	
PSCM-H-gRNA-site10	*S. cerevisiae* M, HygB, *SNR52p*-site10-gRNA	
PSCM-G-gRNA- site19	*S. cerevisiae* M, G418, *SNR52p*-site19-gRNA[a]	
PSCM-G-gRNA- site21	*S. cerevisiae* M, G418, *SNR52p*-site21-gRNA	
PSCM-H-gRNA-*FPSp*	*S. cerevisiae* M, HygB, *SNR52p-FPSp*-gRNA	
PSCM-H-gRNA-*ΔADH2*	*S. cerevisiae* M, HygB, *SNR52p-ΔADH2*-gRNA	
PSCM-H-gRNA-*Δade2*	*S. cerevisiae* M, HygB, *SNR52p-Δade2*-gRNA	
pHCas9	2μ; *AmpR; TEF1p-Cas9-CYC1t*	This study
PUC- *TEF1p-Rim15-ADH1t*	2μ; *AmpR; TEF1p-Rim15-ADH1t*	This study
PUC-*PRK-Cbbm*	2μ; *AmpR; PGKp-PRK-ADH1t-CIT2p-Cbbm-CYC1t*	This study
PUC-*EroES-EroEL*	2μ; *AmpR; GAP1p-GroES-CYC1t-TEF1p-GroEL-ADH1t*	This study
p*Sp*dCas9-*polI5M*-gRNA-*mre11*	2μ; *KanR; TEFp-dCas9-polI5M*, tRNAphe-HDV-*SNR52p-mre11*-gRNA	This study
p*Sp*dCas9-*polI5M*-gRNA-*msh2*	2μ; *KanR; TEFp-dCas9-polI5M*, tRNAphe-HDV-*SNR52p-msh2*-gRNA	This study

Note: [a] HygB, Hygromycin B; G418, Geneticin; Amp, Ampicillin; Kan, Kanamycin.

Supplementary Table S2. A list of primers used in this study.

Oligo	Sequences (5'-3')
Site5-gRNA-F	gatcgctaataaagaggtaacggt
Site5-gRNA-R	aaacaccgttacctctttattagc
Site10-gRNA-F	gatccatgagcagccactgtatcg
Site10-gRNA-R	aaaccgatacagtggctgctcatg
Site19-gRNA-F	gatcgttgaaatataagtaaccct
Site19-gRNA-R	aaacagggttacttatatttcaac
Site21-gRNA-F	gatcacggactaaataacaccagg
Site21-gRNA-R	aaaccctggtgttatttagtccgt
Δ*ADH2*-gRNA-F	gatctggcataccgaccaaaacgg
Δ*ADH2*-gRNA-R	aaacccgttttggtcgctatgcca
FPSp-gRNA-F	gatccataaccgtaggaaggtacg
FPSp-gRNA-R	aaaccgtaccttcctacggttatg
Δ*ade2*-gRNA-F	gatcgtataatgtccagagttgtg
Δ*ade2*-gRNA-R	aaaccacaactctggacattatac
HR-UP-site5	ACATTAGATTGGAATTAGAGCTTAAGTGGTACAAACTAGGAtaagcgtccttctgtggtttaga
HR-DW-site5	TATTCCGCTTAAAAGTGCTTAACATCGGTGCCACACAATAGcaaattaaagccttcgagcgtcc
HR-UP-site10	CCTCTATGTGACGCTGTGTATTCTTTGTTGTAGTTATGCTgagcgacctcatgctatac
HR-DW-site10	TGTACGCTATACATTTACGTGCTGAGCTCCTAGGAAAGCTgcaaattaaagccttcgag
HR-UP-site19	TGAAAATTGTGCCGGATATTCAAGACTAAGAGATGTACAaccacacaccatagcttcaa
HR-DW-site19	AAATCGACATGTTAATGATCTTACGACAGAGTAGTTTATGgagcgacctcatgctatac
HR-UP-site21	ATAGCACGAGAAAAAACTCGTTCAACTAAGTCTAGACACAgagcgacctcatgctatac
HR-DW-site21	AGAGCTGTTTGAGAGCTCGTAGGCTTTGGTTGTTAAGAGAgcaaattaaagccttcgag

Supplementary Table S3. A list of heterologous genes coding sequences used in this study.

Gene	Coding sequences (5'-3')
PRK	ATGGCTGTTTGTACTGTTTATACTATTCCTACTACAACTCATTTGGGTTCTTCTTTCAATCAA AACAATAAGCAAGTTTTCTTCAATTATAAAAGATCTTCATCTTCTAATAACACATTGTTTAC AACTAGACCATCTTATGTTATTACTTGTTCTCAACAGCAAACTATTGTTATTGGTTTGGCTG CTGATTCTGGTTGTGGTAAATCTACTTTTATGAGAAGATTGACTTCTGTTTTTGGTGGTGCT GCTGAACCACCAAAAGGTGGTAATCCTGATTCTAATACTTTGATTTCTGATACAACAACTG TTATCTGTTTGGACGATTTTCATTCTTTGGACAGAAATGGTAGAAAAGTTGAAAAAGTTAC TGCTTTGGATCCAAAAGCTAATGATTTTGATTTGATGTATGAACAAGTTAAGGCTTTGAAA GAAGGTAAAGCTGTTGATAAACCAATTTATAATCATGTTTCTGGTTTGTTGGATCCACCTGA ATTGATTCAACCACCAAAAATTTTGGTTATTGAAGGTTTGCATCCAATGTACGATGCAAGA GTTAGAGAATTGTTGGACTTTTCAATCTACTTGGACATTTCTAATGAGGTCAAATTTGCTTG GAAAATTCAAAGAGATATGAAAGAAAGAGGTCACTCTTTGGAATCTATTAAGGCTTCTATT GAATCTAGAAAACCTGATTTTGATGCTTATATTGATCCACAAAAACAACATGCTGATGTTGT TATTGAAGTTTTGCCAACTGAATTGATTCCTGATGACGATGAAGGTAAAGTCTTAAGAGTC AGAATGATCCAAAAAGAAGGTGTCAAATTCTTCAATCCTGTTTATTTGTTTGATGAAGGTT CTACTATTTCTTGGATTCCATGTGGTAGAAAATTGACTTGTTCTTATCCTGGTATTAAATTTT CTTATGGTCCTGATACTTTTTATGGTAATGAAGTTACTGTTGTTGAAATGGATGGAATGTTT GATAGGTTGGATGAATTGATCTACGTTGAATCACATTTATCAAACTTGTCAACTAAATTTTA TGGTGAAGTCACACAACAAATGTTGAAGCATCAAAACTTTCCTGGTTCTAACAATGGTAC TGGTTTCTTTCAAACTATTATTGGTTTAAAGATCAGAGATTTGTTTGAACAATTGGTTGCTT CTAGATCTACTGCTACTGCTACTGCTGCTAAAGCTTAA
Cbbm	ATGGATCAATCTGCTAGATATGCTGATTTGTCTTTGAAAGAAGAAGATTTGATTAAAGGTG GTAGACATATTTTGGTTGCTTATAAAATGAAACCAAAATCTGGTTATGGTTATTTGGAAGCT GCAGCTCATTTTGCTGCTGAATCTTCTACTGGTACTAATGTTGAAGTTTCTACTACTGATGA TTTTACTAAAGGTGTTGATGCTTTGGTTTATTATATTGATGAGGCTTCTGAAGATATGAGAAT TGCTTATCCATTGGAATTGTTTGATAGAAATGTTACTGATGGTAGATTTATGTTGGTTTCTTT TTTGACTTTGGCTATTGGTAATAATCAAGGTATGGGTGATATTGAACATGCTAAAATGATTG ATTTTTATGTTCCTGAAAGATGTATTCAAATGTTTGATGGTCCTGCTACTGATATTTCTAATT TGTGGAGAATTTTGGGTAGACCTGTTGTTAATGGTGGTTATATTGCTGGTACTATTATTAAA CCAAAATTGGGTTTGAGACCTGAACCATTTGCTAAAGCTGCTTATCAATTTTGGTTGGGTG GTGATTTTATTAAAAATGATGAACCACAAGGTAATCAAGTTTTTTGTCCATTGAAAAAAGT TTTGCCATTGGTTTATGATGCTATGAAAAGAGCTCAAGATGATACTGGTCAAGCTAAATTGT TTTCTATGAATATTACTGCTGATGATCATTATGAAATGTGTGCTAGAGCTGATTATGCTTTGG AAGTTTTTGGTCCTGATGCTGATAAATTAGCTTTTTTGGTTGATGGTTATGTTGGTGGTCCT GGTATGGTTACTACTGCTAGAAGACAATATCCTGGTCAATATTTGCATTATCATAGAGCTGG TCATGGTGCTGTTACTTCTCCATCTGCTAAAAGAGGTTATACTGCTTTTGTTTTGGCTAAAA TGTCTAGATTGCAAGGTGCTTCTGGTATTCATGTTGGTACTATGGGTTATGGTAAAATGGAA GGTGAAGGTGATGATAAAATTATTGCTTATATGATTGAAAGAGATGAATGTCAAGGTCCTG TTTATTTTCAAAAATGGTATGGTATGAAACCAACTACTCCAATTATTTCTGGTGGTATGAAT GCTTTGAGATTGCCTGGTTTTTTTGAAAATTTGGGTCATGGTAATGTTATTAATACTGCTGG TGGAGGTTCTTATGGTCATATTGATTCTCCTGCTGCTGGTGCTATTTCTTTGAGACAATCTTA TGAATGTTGGAAACAAGGTGCTGATCCAATTGAATTTGCTAAAGAACATAAAGAATTTGCT AGAGCTTTTGAATCTTTTCCAAAAGACGCTGATAAGTTGTTTCCTGGTTGGAGAGAAAAAT TGGGTGTTCATTCTTAA
GroES	ATGAATATTAGACCATTGCATGATAGAGTTATTGTTAAAAGAAAAGAAGTTGAAACTAAAT CTGCTGGTGGTATTGTTTTGACTGGTTCTGCTGCAGCTAAATCTACTAGAGGTGAAGTTTT GGCTGTTGGTAATGGTAGAATTTTGGAAAATGGTGAAGTTAAACCATTGGATGTTAAAGTC GGTGATATTGTTATTTTCAATGATGGTTATGGTGTCAAATCTGAGAAAATTGATAATGAAGA AGTTTTGATCATGTCTGAATCTGATATTTTGGCTATTGTTGAAGCTTAA
GroEL	ATGGCTGCTAAAGATGTTAAATTTGGTAATGATGCTAGAGTTAAAATGTTGAGAGGTGTTA ATGTTTTGGCTGATGCTGTTAAAGTTACTTTGGGTCCAAAAGGTAGAAATGTTGTTTTGGA TAAATCTTTTGGTGCTCCAACTATTACTAAAGATGGTGTTTCTGTTGCTAGAGAAATTGAAT TGGAAGATAAATTTGAAAATATGGGTGCTCAAATGGTTAAAGAAGTTGCTTCTAAAGCTAA TGATGCTGCTGGTGATGGTACTACAACTGCTACTGTTTTGGCTCAAGCTATTATTACTGAAG GTTTGAAAGCTGTTGCTGCTGGTATGAATCCAATGGATTTGAAAAGAGGTATTGATAAAGC TGTTACTGCAGCTGTTGAAGAATTGAAGGCTTTGTCTGTTCCATGTTCTGATTCTAAAGCT ATTGCTCAAGTTGGTACTATTTCTGCTAATTCTGATGAAACTGTTGGTAAATTGATTGCTGA AGCTATGGATAAAGTTGGTAAAGAAGGTGTTATTACTGTTGAAGATGGTACTGGTTTGCAA GATGAATTGGATGTTGTTGAAGGTATGCAATTTGATAGAGGTTATTTGTCTCCATATTTTATT AATAAACCTGAAACTGGTGCTGTTGAGTTGGAATCTCCATTTATCTTGTTGGCTGATAAAA AAATCTCTAATATTAGAGAAATGTTGCCTGTTTTGGAAGCTGTTGCTAAAGCTGGTAAACC ATTGTTGATTATTGCTGAAGATGTTGAAGGTGAGGCTTTGGCTACTTTGGTTGTTAATACTA TGAGAGGTATTGTTAAAGTTGCTGCTGTTAAAGCTCCTGGTTTTGGTGATAGAAGAAAAGC TATGTTGCAAGATATTGCTACTTTGACTGGTGGTACTGTTATTTCTGAGGAGATTGGTATGG

	AATTGGAAAAAGCTACTTTAGAGGATTTGGGTCAAGCTAAAAGAGTTGTTATTAATAAAGA TACTACAACTATTATTGATGGTGTTGGTGAAGAAGCTGCTATTCAAGGTAGAGTTGCTCAA ATTAGACAACAAATTGAAGAAGCTACTTCTGATTATGATAGAGAAAAATTGCAAGAAAGA GTTGCTAAATTAGCTGGTGGAGTTGCTGTTATTAAAGTTGGTGCTGCTACTGAAGTTGAAA TGAAAGAAAAAAAAAGCTAGAGTTGAAGATGCTTTGCATGCTACTAGAGCTGCTGTTGAAG AGGGTGTTGTTGCTGGTGGTGGAGTTGCATTGATTAGAGTCGCATCTAAATTGGCTGACTT GAGAGGTCAAAATGAAGATCAAAATGTTGGTATTAAAGTTGCTTTGAGAGCTATGGAAGC TCCATTGAGACAAATTGTTTTGAATTGTGGTGAAGAACCATCTGTTGTTGCTAATACTGTTA AAGGTGGTGATGGTAATTATGGTTATAATGCAGCTACAGAAGAATATGGTAATATGATTGAT ATGGGTATTTTGGATCCAACTAAAGTTACTAGATCTGCTTTGCAATATGCTGCTTCTGTTGC TGGTTTGATGATTACTACTGAATGTATGGTTACTGATTTGCCAAAAAACGATGCTGCTGATT TGGGTGCTGCTGGTGGTATGGGTGGTATGGGAGGAATGGGAGGTATGATGTAA