

SUPPORTING INFORMATION

De Novo* Biosynthesis of Caffeic Acid from Glucose by Engineered *Saccharomyces cerevisiae

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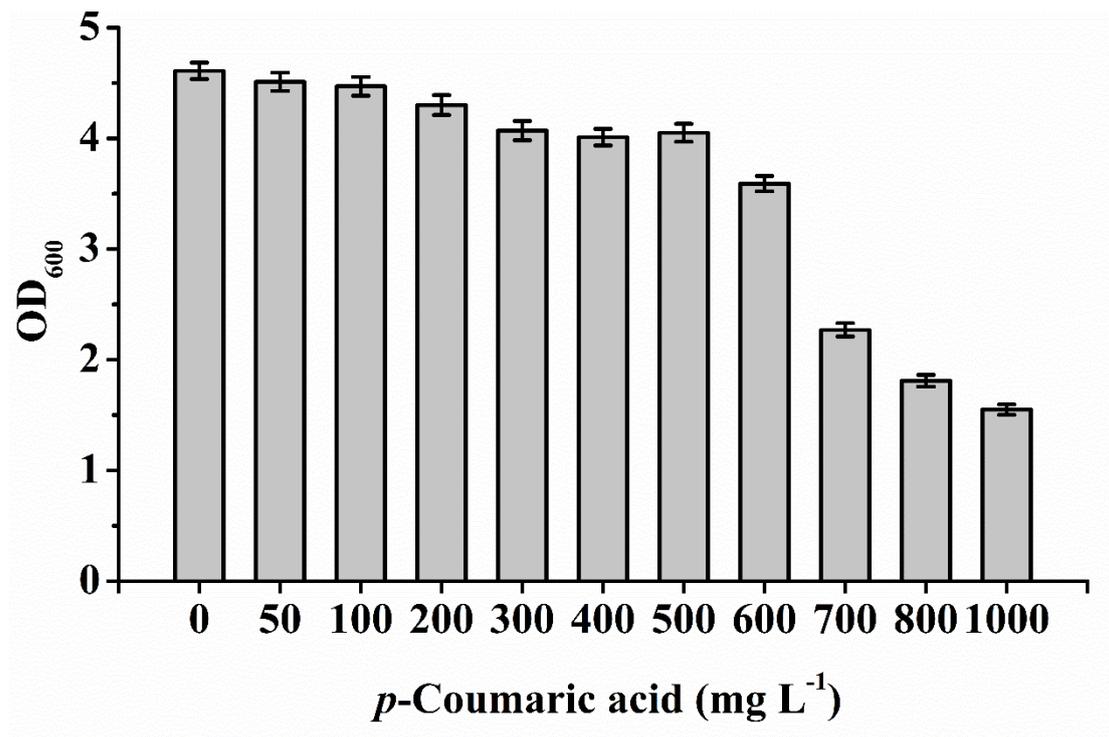


Figure S1. Effects of *p*-coumaric acid supplement concentrations on cells growth.

Average \pm standard deviations were calculated from three biological replicates.

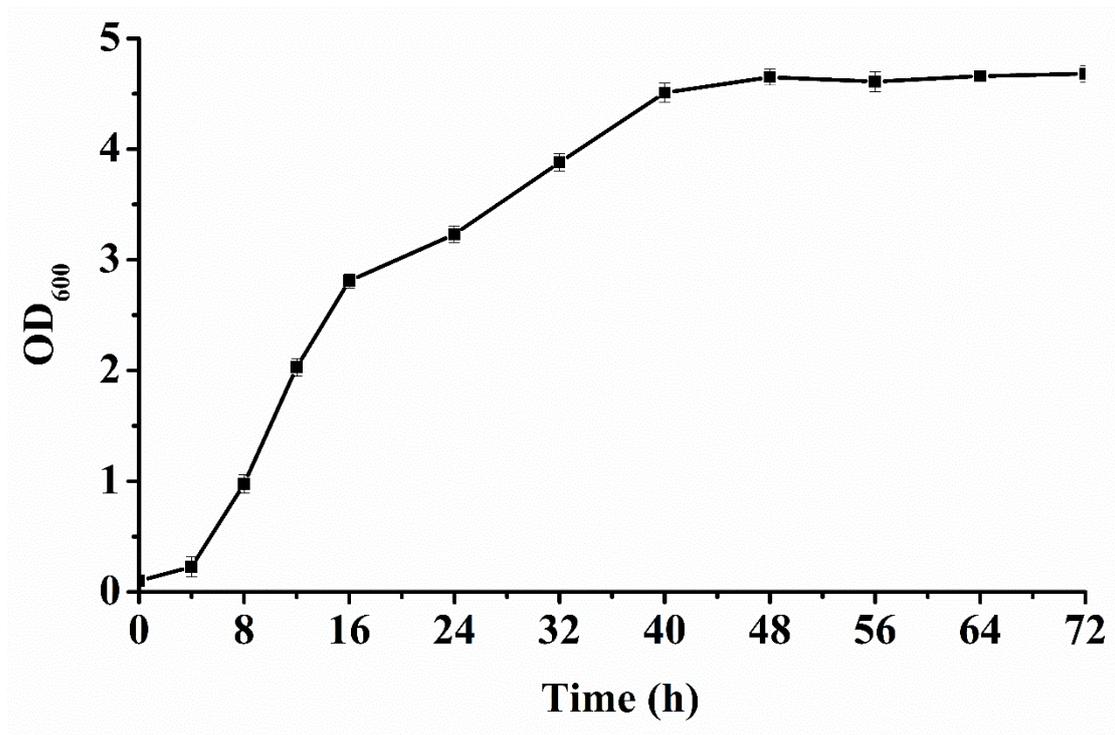


Figure S2. Growth curve of NKC2 strain in SC-His medium. Average \pm standard deviations were calculated from three biological replicates.

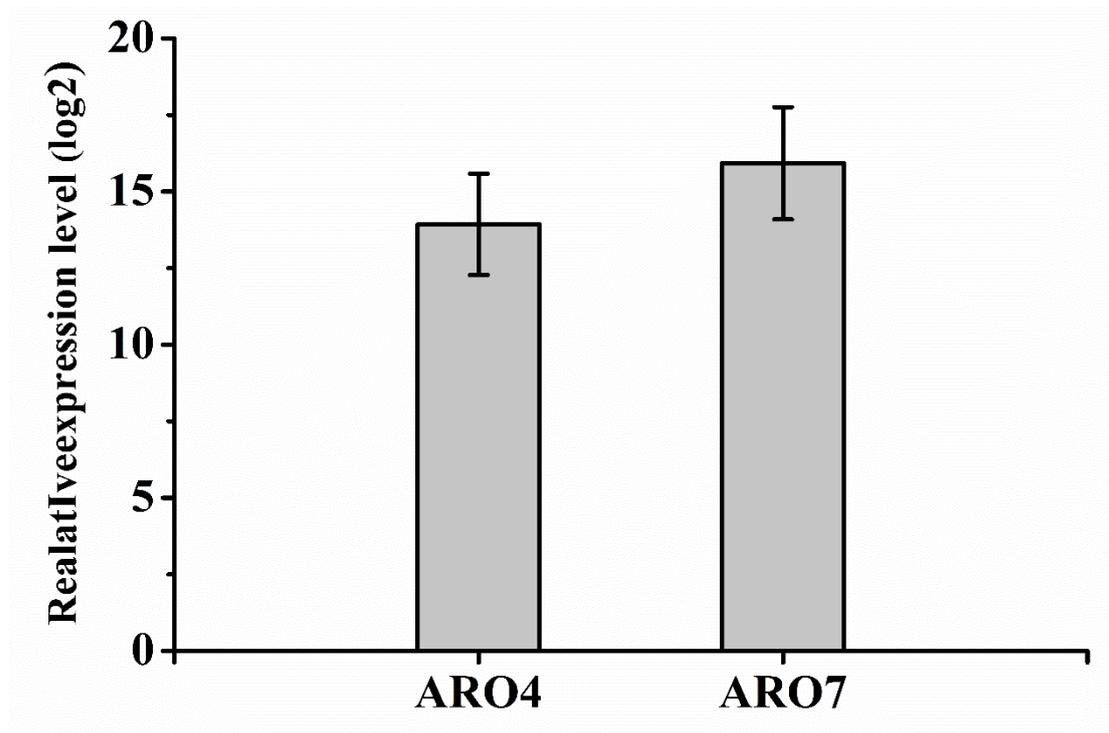


Figure S3. RT-qPCR analysis of gene expression levels of the engineered strain NKC6 compared with BY4742 strain. Average \pm standard deviations were calculated from three biological replicates.

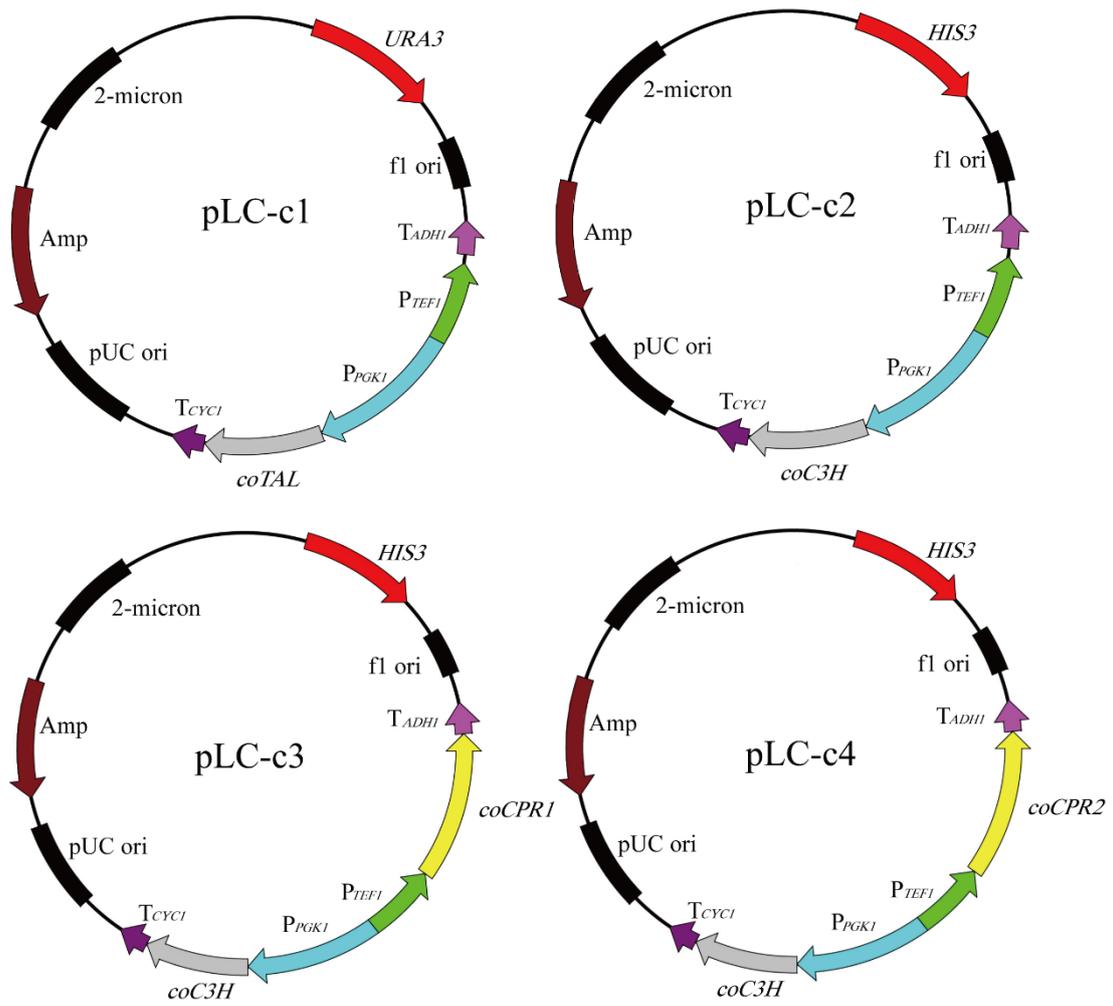


Figure S4. The structure charts of plasmids pLC-c1, pLC-c2, pLC-c3 and pLC-c4.

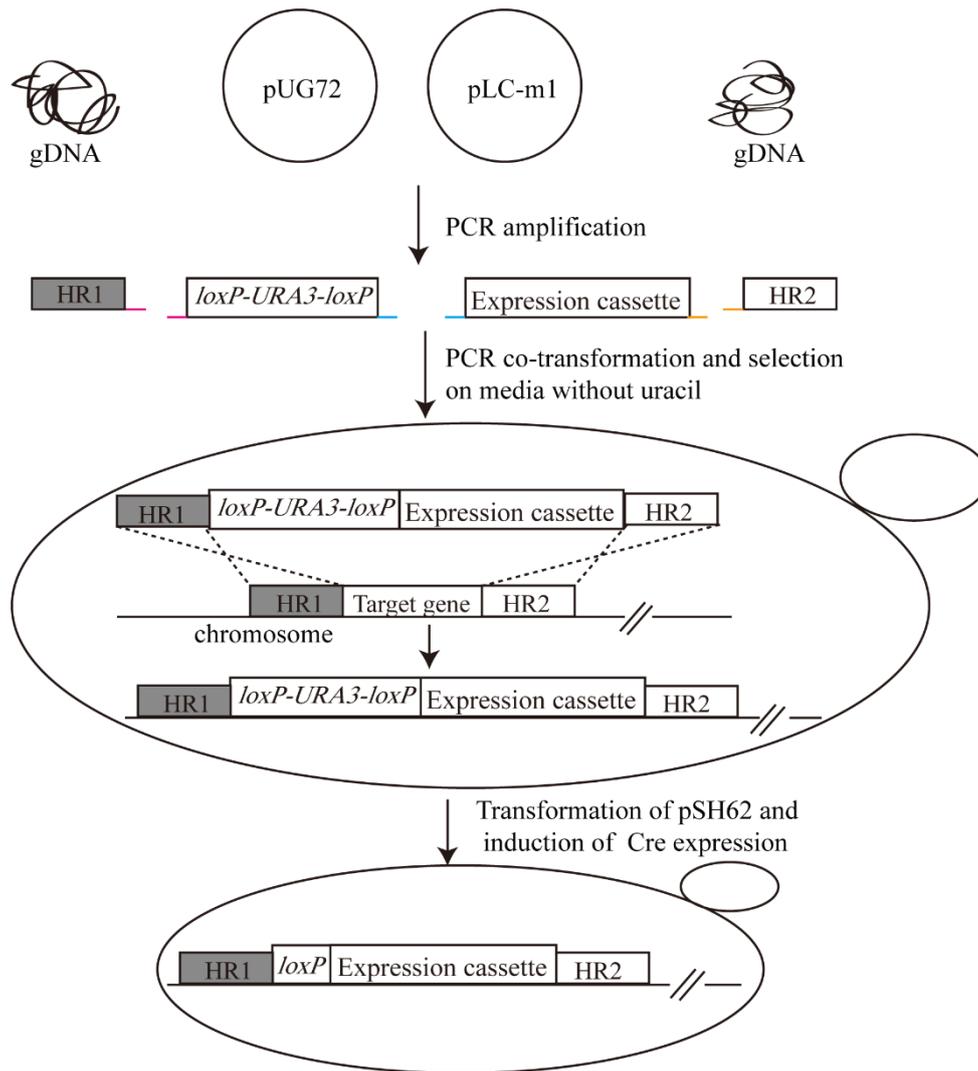


Figure S5. Schematic representation of the replacement of a gene that needed to be knocked out with the overexpression cassette in *S. cerevisiae*. HR1 and HR2 represent upstream homologous region and downstream homologous region of the target gene that would be knocked out, respectively. The overexpression cassette was P_{TEF1} - $ARO4^{br}$ - T_{ADH1} or P_{PGK1} - $ARO7^{br}$ - T_{CYC1} . First, HR1, *URA3* cassette, overexpression cassette and HR2 were individually amplified via PCR and co-transformed into yeast cell to form the functional cassette via chromosomal integration; second, Cre recombinase was induced with galactose to remove *URA3* selection marker; finally, the target gene was replaced by overexpression cassette.

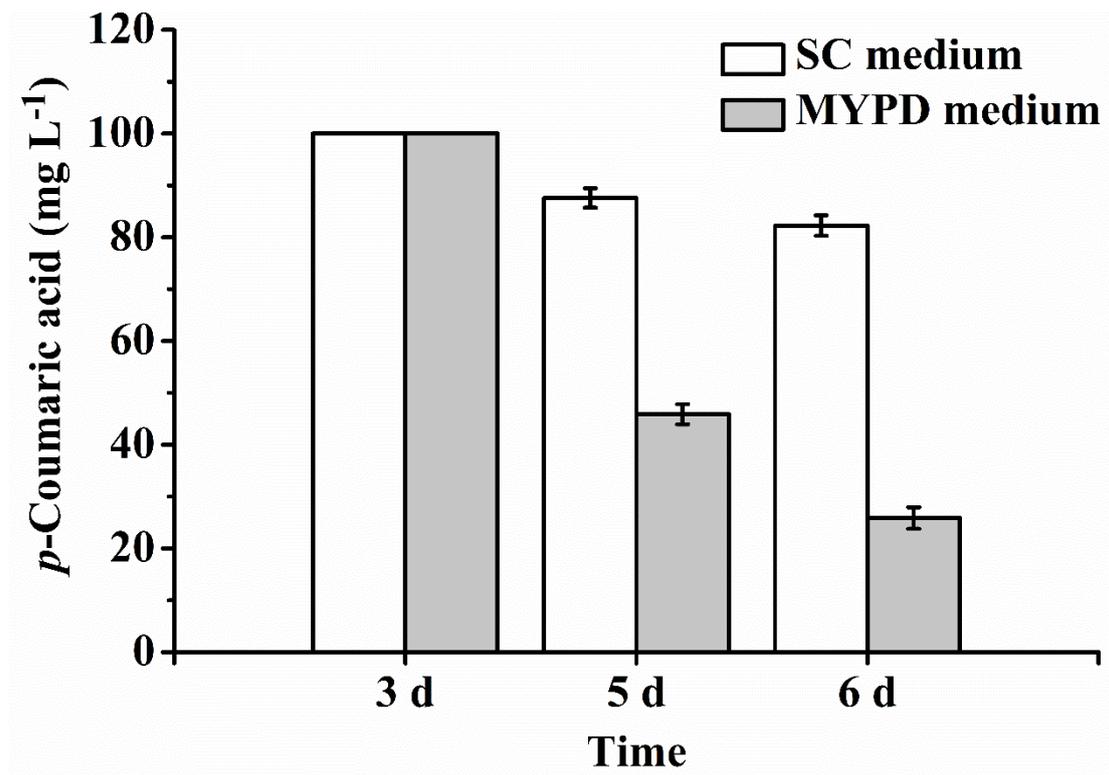


Figure S6. *p*-Coumaric acid consumptions in SC medium and MYPD medium. BY4742 strain was cultured in SC medium and MYPD medium for 6 d, respectively, and 100 mg L⁻¹ *p*-coumaric acid was added at 3 d. Average \pm standard deviations were calculated from three biological replicates.

Table S1. Gene Sequences of TAL, C3H, CPR1 and CPR2 with Codon**Optimization**

gene	sequence
<i>coTAL</i>	ATGACTTTGCAATCACAGACTGCAAAGGATTGCTTGGCTTTGGACGGAGC ATTGACTTTGGTCCAATGCGAAGCTATAGCTACTCATAGATCTAGAATTTCT TGTCCTCCAGCTTTGAGAGAGAGATGTGCTAGGGCACATGCTAGGTTGG AGCACGCTATAGCAGAACAGAGACACATATACGGAATTACTACTGGTTTC GGTCCTTTGGCAAACAGATTGATTGGTGCTGACCAGGGTGTGAATTGCAA CAAAATTAATATAACCACTTAGCTACAGGTGTCGGTCCTAAGTTGTCTTGG GCAGAAGCTAGGGCTTTGATGTTAGCTAGATTGAACTCTATTTTGCAAGGT GCTTCTGGTGCTTCTCCAGAGACAATAGACAGAATTGTTGCTGTTTTGAAC GCTGGTTTTCGCACAGAGGTCCAGCTCAAGGTAAGTGTGCTGCTTCTGGT GACTTGACACCATTGGCTCATATGGTTTTGGCATTGCAAGGAAGAGGTAG AATGATTGATCCTTCTGGTAGAGTCCAAGAGGCTGGTGCTGTTATGGACAG ATTATGCGGTGGTCCATTGACATTGGCTGCTAGGGACGGTTTTGGCTTTGGT TAACGGAACCTTCTGCTATGACTGCTATTGCTGCTTTGACAGGTGTTGAGGC AGCTAGGGCTATTGACGCTGCATTGAGACACTCTGCTGTCTTGATGGAAGT CTTATCTGGTCATGCTGAGGCTTGGCACCCAGCATTTCAGAAATTGAGGCC TCATCCAGGTCAGTTGAGAGCTACTGAGAGGTTAGCTCAGGCTTTGGATGG TGCAGGTAGAGTCTGCAGAACTTTGACTGCTGCTAGGAGGTTAACTGCTGC AGACTTGAGACCAGAGGATCATCCAGCTCAAGACGCTTACTCTTTAAGGG TCGTCCACAGTTGGTTGGTGCAGTTTGGGACACATTGGACTGGCATGACA GAGTCGTTACTTGTGAATTAATTCTGTTACTGATAATCCAATTTTCCCAG AGGGTTGCGCTGTTCCCTGCTTTGCATGGTGGTAACTTTATGGGTGTCCACG TCGCATTGGCTTCTGACGCTTTGAACGCAGCTTTGGTCACTTTGGCTGGTTT GGTCGAAAGGCAGATTGCAAGATTGACAGATGAGAAGTTGAACAAAGGTT TGCCAGCTTTCTTGACGGTGGTCAGGCTGGTTTGCAATCTGGTTTCATGG GTGCACAGGTCACAGCTACTGCATTGTTGGCTGAGATGAGGGCTAACGCA ACTCCTGTCTCTGTTTCAGTCTTTGTCTACAAATGGTGCAAACCAGGACGTC GTCTCTATGGGTACAATTGCTGCAAGGAGGGCAAGAGCACAGTTGTTGCC ATTGTCACAAATTCAAGCAATTTAGCTTTGGCTTTGGCACAGGCTATGGA CTTGTTAGACGATCCAGAGGGTCAAGCAGGTTGGTCTTTGACTGCTAGGGA CTTGAGGGACAGAATAAGGGCAGTCTCTCCAGGATTGAGAGCTGACAGAC CTTTGGCTGGTCACATTGAGGCTGTCGCACAAGGTTTGAGACATCCATCTG CTGCAGCTGATCCACCAGCTTAA

coC3H ATGTCTTGGTTTTTAATTGCAGTTGCAACTATAGCTGCAGTTGTTTCTTATA
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CAATTGTTGGTAATTTATACGATATTAACCAGTCAGATTTAGATGTTACT
ATGAATGGGCACAATCTTATGGTCCTATTATTTTCAGTTTGGATTGGTCTAT
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GCACGACCAAAAGTTGGCTGACAGGCACAGGAACAGATCTACTGAAGCTT
TTTCTAGAAATGGTCAAGATTTGATATGGGCTGACTACGGTCCACATTACG
TTAAAGTTAGAAAAGTTTGTACATTGGAATTATTACACCAAAGAGATTAG
AATCTTTGAGACCAATTAGAGAGGACGAGGTCACTGCTATGGTTGAGTCT
GTCTTCAGAGATTGTAATTTGCCAGAAAATAGAGCTAAAGGATTACAATT
GAGAAAGTATTTAGGTGCTGTTGCATTCAATAATTACTIONAGATTGGCTTT
CGGTAAGAGGTTTCATGAATGCTGAGGGTGTGTCGACGAACAGGGTTTGG
AATTCAAGGCTATTGTTTCTAATGGTTTAAAATTAGGTGCTTCTTTGTCTAT
TGCTGAGCATATTCCATGGTTGAGGTGGATGTTCCCAGCTGATGAGAAGGC
ATTTCGAGAACACGGTGTAGGAGAGACAGATTGACAAGAGCAATTATGG
AGGAACATACTTTGGCTAGACAAAATCATCTGGTGCAAAACAACATTTCT
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CACGACTTCAGATTGTTGCCTTTTCGGTGCAGGTAGAAGGGTTTGGCCAGGT
GCACAATTGGGTATTAACCTGGTTACTTCTATGATGTCACACTTATTGCATC
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CTGAAAATCCTGGTTTGGTCACATATATGAGAACACCAGTTCAGGCTGTTG
CTACTCCAAGATTGCCATCTGATTTATATAAAAAGGGTCCATATGATATGT
AA

coCPR1

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AACTGATAACGCTGCAAGATTCTATAAGTGGTTTACAGAAGAGAATGAAA
GAGATATTAAGTTACAACAATTAGCTTACGGTGTTTTTGCATTGGGTAATA
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CTTCTTACCAAGATTGGCTCCTTCTAGGGTCCATGTTACTTCTGCTTTGGT
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coCPR2

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GGTAA

Table S2. Primers Used for the Construction of Plasmids and Strains

primers	sequences (5'-3')	applications
Construction of plasmids		
1	CAAGGAAGTAATTATCTACTTTT TACAACAAATATAAAACAAGGA TCCGCCACCATGACTTTGCAATC ACAGACTG	Amplify <i>coTAL</i> cassette for pLC-c1 construction from <i>coTAL</i> fragment synthesized by Gene Art, forward primer
2	GTTCCATGTCGACGCCCGGGCCC TATAGTGAGTCGTATTACGGATC CTTAAGCTGGTGGATCAGCTG	Amplify <i>coTAL</i> cassette for pLC-c1 construction from <i>coTAL</i> fragment synthesized by Gene Art, reverse primer
3	CAAGGAAGTAATTATCTACTTTT TACAACAAATATAAAACAAGGA TCCATGTCTTGGTTTTTAATTGCA GTTG	Amplify <i>coC3H</i> cassette for pLC-c2 construction from <i>coC3H</i> fragment synthesized by Gene Art, forward primer
4	GTTCCATGTCGACGCCCGGGCCC TATAGTGAGTCGTATTACGGATC CTTACATATCATATGGAACCC	Amplify <i>coC3H</i> cassette for pLC-c2 construction from <i>coC3H</i> fragment synthesized by Gene Art, reverse primer
5	GCAATCTAATCTAAGTTTTAATT ACAAGCGGCCGCATGACTTCAGC TTTGTACGCATC	Amplify <i>coCPR1</i> cassette for pLC-c3 construction from <i>coCPR1</i> fragment synthesized by Gene Art, forward primer
6	CGTCATCCTTGTAATCCATCGAT ACTAGTGCGGCCGCTTACCAAAC ATCTCTCAAGTATC	Amplify <i>coCPR1</i> cassette for pLC-c3 construction from <i>coCPR1</i> fragment synthesized by Gene Art, reverse primer
7	GCAATCTAATCTAAGTTTTAATT ACAAGCGGCCGCATGTCTTCTTC ATCTTCTTCATC	Amplify <i>coCPR2</i> cassette for pLC-c4 construction from <i>coCPR2</i> fragment synthesized by Gene Art, forward primer
8	CGTCATCCTTGTAATCCATCGAT ACTAGTGCGGCCGCTTACCAAAC GTCTCTTAAATATCTAC	Amplify <i>coCPR2</i> cassette for pLC-c4 construction from <i>coCPR2</i> fragment synthesized by Gene Art, reverse primer
Replacing <i>ARO10</i> with <i>P_{PGKI}-ARO7^{br}-T_{CYCI}</i> cassette		
9	CCTCTTCTTCTTGTGTGTTTAAGC	Amplify <i>ARO10</i> upstream homologous region for <i>ARO7^{br}</i> overexpression and <i>ARO10</i> deletion from <i>S. cerevisiae</i> genome, forward primer.
10	CGACCTGCAGCGTACGAAGCTTC AGCTGGCTTAAGGGAGTTTCTTT GTTATCTTG	Amplify <i>ARO10</i> upstream homologous region for <i>ARO7^{br}</i> overexpression and <i>ARO10</i> deletion from <i>S. cerevisiae</i> genome, reverse primer.
11	CAAGATAACAAAGAACTCCCTT AAGCCAGCTGAAGCTTCGTACGC TGCAGGTCG	Amplify <i>URA3</i> selection marker for <i>ARO7^{br}</i> overexpression and <i>ARO10</i> deletion from pUG72, forward primer.

12	GATAAGACCCCATTTCTTTGAAGG TACTTCCCGGCCGCATAGGCCAC TAGTGGATCTG	Amplify <i>URA3</i> selection marker for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from pUG72, reverse primer.
13	CAGATCCACTAGTGGCCTATGCG GCCGGGAAGTACCTTCAAAGAAT GGGGTCTTATC	Amplify P _{PGK1} - <i>ARO7^{fb}</i> -T _{CYC1} cassette for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from pLC-m1, forward primer.
14	CACACGATAGGAATGACAGAAA AAAGCTTCGAGCGTCCCAAACC TTCTCAAGC	Amplify P _{PGK1} - <i>ARO7^{fb}</i> -T _{CYC1} cassette for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from pLC-m1, reverse primer.
15	GCTTGAGAAGGTTTTGGGACGCT CGAAGCTTTTTTCTGTTCATTCCTA TCGTGTG	Amplify <i>ARO10</i> downstream homologous region for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from <i>S. cerevisiae</i> genome, forward primer.
16	CGAAGTATTTCCGGACTCTTTCTTC	Amplify <i>ARO10</i> downstream homologous region for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from <i>S. cerevisiae</i> genome, reverse primer.

Replacing *PDC5* with P_{TEF1}-*ARO4^{fb}*-T_{ADH1} cassette

17	GTTGAAAATGACGACGAGCCTG	Amplify <i>PDC5</i> upstream homologous region for <i>ARO4^{fb}</i> overexpression and <i>ARO10</i> deletion from <i>S. cerevisiae</i> genome, forward primer.
18	CGACCTGCAGCGTACGAAGCTTC AGCTGGTGTAAATAAGAAAGAGA GGAAAGGAC	Amplify <i>PDC5</i> upstream homologous region for <i>ARO4^{fb}</i> overexpression and <i>ARO10</i> deletion from <i>S. cerevisiae</i> genome, reverse primer.
19	GTCCTTTCCTCTCTTTCTTATTAC ACCAGCTGAAGCTTCGTACGCTG CAGGTCG	Amplify <i>URA3</i> selection marker for <i>ARO4^{fb}</i> overexpression and <i>PDC5</i> deletion from pUG72, forward primer.
20	GCTTTCTCAGGTATAGCATGAGG TCGCTCCGGCCGCATAGGCCACT AGTGGATCTG	Amplify <i>URA3</i> selection marker for <i>ARO4^{fb}</i> overexpression and <i>PDC5</i> deletion from pUG72, reverse primer.
21	CAGATCCACTAGTGGCCTATGCG GCCGGAGCGACCTCATGCTATAC CTGAGAAAGC	Amplify P _{TEF1} - <i>ARO4^{fb}</i> -T _{ADH1} cassette for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from pLC-m1, forward primer.
22	CCTAAACATCTATAACCTTCAAA AGGCACACACCATAGCTTCAAAA TGTTTCTAC	Amplify P _{TEF1} - <i>ARO4^{fb}</i> -T _{ADH1} cassette for <i>ARO7^{fb}</i> overexpression and <i>ARO10</i> deletion from pLC-m1, reverse primer.
23	GTAGAAACATTTTGAAGCTATGG TGTGTGCCTTTTGAAGGTTATAG ATGTTTAGG	Amplify <i>PDC5</i> downstream homologous region for <i>ARO4^{fb}</i> overexpression and <i>PDC5</i> deletion from <i>S. cerevisiae</i> genome, forward primer.

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CTCTTGCTTGTTCTGGAGTTC

Amplify *PDC5* downstream homologous region for *ARO4^{br}* overexpression and *PDC5* deletion from *S. cerevisiae* genome, reverse primer.

Table S3. Primers Used for Real-time Quantitative PCR

primers	sequences (5'-3')	applications
1	GTGAACAATTACACAGCTCCTAT AG	Amplify partial cDNA of <i>S. cerevisiae</i> <i>ALG9</i> gene for real-time PCR, forward primer.
2	CCTATGATTATCTGGCAGCAGGA AAG	Amplify partial cDNA of <i>S. cerevisiae</i> <i>ALG9</i> gene for real-time PCR, reverse primer.
3	CGCTAACGGTGAAAACGCCATTA CC	Amplify partial cDNA of <i>S. cerevisiae</i> <i>ARO4</i> gene for real-time PCR, forward primer.
4	CGTCTTCAGTAGTTTCCCAACCT ATAC	Amplify partial cDNA of <i>S. cerevisiae</i> <i>ARO4</i> gene for real-time PCR, reverse primer.
5	GAGAGGTCGCATTTGCCACATG	Amplify partial cDNA of <i>S. cerevisiae</i> <i>ARO7</i> gene for real-time PCR, forward primer.
6	CAGGTGATTCGAATCTTCTGATG CG	Amplify partial cDNA of <i>S. cerevisiae</i> <i>ARO7</i> gene for real-time PCR, reverse primer.