Electronic Supplementary Material

Development of a dual temperature control system for isoprene biosynthesis in *Saccharomyces cerevisiae*

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Table S1 Yeast strains used or constructed in this study

Strain	Genotype/Description	Plasmid	Reference
BY4741	*MATα*, *his3Δ1*, *leu2Δ0* , *met15Δ0*, *ura3Δ0*	None	[1]
BY4742	*MATα*, *his3Δ1*, *leu2Δ0* , *lys2Δ0*, *ura3Δ0*	None	[1]
YXWP-113	BY4741, Δ*gal80::LEU2*	None	[2]
YXWP-111	YXWP-113, Δ*ho::*T_{TPS1}-*tHMG1*-P_{GAL7}-P_{GAL2}-*crtYB11M*-T_{PGK1}-T_{CYC1}-*crtI*-P_{GAL1}-P_{GAL10}-*crtE03M*-T_{ADH1}	None	[2]
YEGFP-TS0	YXWP-113, Δ*gal4::HIS3*	None	[3]
YCS113-URA	YEGFP-TS0, Δ*ho::*T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}-*URA3*-T_{CYC1}	None	This study
Y113EGFP-0	YXWP-113	pESC-URA-P_{ERG9}-EGFP	This study
Y113EGFP-1	YXWP-113	pESC-URA-1HSE-EGFP	This study
Y113EGFP-2	YXWP-113	pESC-URA-2HSE-EGFP	This study
Y113EGFP-S	YXWP-113	pESC-URA-SSA4-EGFP	This study
Yelacz	YEGFP-TS0, Δ*dpp1::*T_{ADH1}-*EGFP*-P_{GAL10}-P_{GAL1}-*LacZ*-T_{CYC1}	None	This study
Yelacz-ERG9-WT	Yelacz	pESC-URA-P_{ERG9}-*GAL4*	This study
Yelacz-SSA4-WT	Yelacz	pESC-URA-P_{SSA4}-*GAL4*	This study
BY4742-M-04-HIS	BY4742, *his3::HIS3*, Δ*lpp1::*T_{CYC1}-*ERG10-MLS*-P_{GAL1}-P_{GAL10}-*MLS-HMGS*-T_{ADH1}, Δ*ho::*T_{TPS1}-*tHMG1-MLS*-P_{GAL7}-P_{GAL2}-*MLS-ERG12*-T_{PGK1}, Δ*dpp1::*T_{CYC1}-*tHMG1-MLS*-P_{GAL1}-P_{GAL10}-*MLS-PMK*-T_{ADH1}, Δ*gal80::*T_{TPS1}-*MVD1-MLS*-P_{GAL7}-P_{GAL2}-*MLS-IDI1*-T_{PGK1},	None	[4]
BY4742-M-04-GAL4-HIS	BY4742-M-04-HIS, Δ*gal1-7::*P_{GAL4}-*GAL4*-T_{CYC1}	None	This study
BY4742-M-08-HIS3	BY4742-M-04-HIS3, Δ*ura3::*T_{ADH1}-*MVD1-MLS*-P_{GAL10}-P_{GAL1}-*MLS-IDI1*-T_{CYC1} Δ*lys2::*T_{ADH1}-*MCS*-P_{GAL10}-P_{GAL1}-*MLS-IDI1*-T_{CYC1}	None	[4]
M08H-CS	BY4742-M-08-HIS3, Δ*gal4::LEU2*	None	This study
M08H-CS-MLN-WT	M08H-CS	p416XWP01-*MLSLN-GAL4WT*	This study
M08H-CS-MLN-ep19	M08H-CS	p416XWP01-*MLSLN-GAL4ep19*	This study

M08H-CS-MLN-SSA4-WT	M08H-CS	p416XWP01-*MLSLN*-P_{SSA4}-*GAL4WT*	This study
M08H-CS-MLN-SSA4-ep19	M08H-CS	p416XWP01-*MLSLN*-P_{SSA4}-*GAL4ep19*	This study

Table S2 Primers used in this study

Primer name	Primer sequence	Description
URA3-LF	CGGGATCCATGTCGAAAGCTACATATAAGG	Amplify *URA3* and facilitate its insertion into pMRI-31
URA3-LR	GCGTCGACTTAGTTTTGCTGGCCGCATC	
ERG9-F2	GAAGAGCAGAAGCGGAAAAC	For Gal4p mutants library construction
GAL4-DR1	TTGTGAAAACTTGTAAGAGCC	
MET15nheI-F	CTAGCTAGCCAACAATTAAATCCAAGTTCACCTATTCTG	Amplify *MET15* and facilitate its insertion into p416XWP-P_{ERG9}-*GAL4*
MET15sacII-R	TCCCCGCGGGGATGACTAATTAAGTTAGTCAAGGCGCCA	
GAL4-LEU2XF1	CTATCCGTAATCATGGTCGGTTAAGCAAGGATTTTCTTAA	For construction of *GAL4* disruption cassette
GAL4-LEU2XR1	GCCTTTTTCTGTTTTATGAGCTAAACTGTGGGAATACTCAGGT	
LEU2XF2	ACCTGAGTATTCCCACAGTTTAGCTCATAAAACAGAAAAAGGC	
GAL4-XinR	AACCCAGAATCCCCTATACTAA	
GAL4-LEU2-R1	TTAAGAAAATCCTTGCTTAACCGACCATGATTACGGATAG	
GAL4-XinF1	AATTGGATCTCCCAAGAGTA	
Perg9416ecor-F1	GCCGCCCTTTAGTGAGGGTTGAATTCTGTGTGTGTGTGATATGTGACG	Amplify P_{ERG9}-P_{GAL1} bidirectional promotor
Perg9-gal1-R1	GCAGTGCGGCGCGAGGCACACGCTCTAACTCCGCAGGAAC	
Pgal1-ERG9-F1	GTTCCTGCGGAGTTAGAGCGTGTGCCTCGCGCCGCACTGC	
Pgal141601-R1	CCTATAGTGAGTCGTATTACGGATCCGGGGTTTTTTCTCCTTGACGTTAA	
MLS26sal1-F1	CTCACTATAGGGCCCGGGCGTCGACATGCTTTCACTACGTCAATCTATAAG	Amplify *MLS-ISPSLN* fragment
ISPhind3-R1	CTTAGCTAGCCGCGGTACCAAGCTTTTATCTTTCGAATG	
PQ-NotI-R	AGTCGCGGCCGCGATTATTGTTTTGTTTATTTTTTTTCTTGTTTTTGTTATTTAC	Amplify P_{SSA4}-P_{GAL1} bidirectional promoter

PQ-speI-Pgal1-F'	CAGTGCGGCGCGAGGCACAACTAGTAGATCTGCTAATATATTTTACGTAAGATTCGTTG	
PGAL1-speI-PQ-F	CTTACGTAAAATATATTAGCAGATCTACTAGTTGTGCCTCGCGCCGCACTGCTCC	
PGAL1-smaI-R	ACTGCCCGGGGTTTTTTCTCCTTGACGTTAAAGTATAGAGGTATATTAAC	
P1HSE-NOTI-R	GAAGACAGTAGCTTCATGCGGCCGCGATTATTGTTTTGTTTATTTTTTTTCTTG	
BAMH-1HSE-F	CCTATAGTGAGTCGTATTACGGATCCGAAGCTCCTTAGTTTGACGACAG	Amplify P_{SSA4-1}, P_{SSA4-2}, P_{SSA4} promotors to facilitate insersion
BAMH-2HSE-F	CCTATAGTGAGTCGTATTACGGATCCCGAGCAATGAAGTACATTCTAGAAGTTC	into pESC-URA
QUETA-BamH1-F	CTATAGTGAGTCGTATTACGGATCCAGATCTGCTAATATATTTTACGTAAGATTCGTTG	
lacZ-BamHI-F	CAAGGAGAAAAAACCCCGGATCCATGACCATGATTACGGATTCACTGG	Amplify *LacZ* to facilitate insersion into
lacZ-SacII-R	GAGCGGATCTTAGCTAGCCGCGGTTATTTTTGACACCAGACCAACTGGTAATG	pUMRI-11-EGFP-LacZ

29 **Table S3** Plasmids used or constructed in this study

Plasmids	Genotype/Description	Reference
pESC-LEU	*f1 ori*, *2μ ori*, *LEU2*, *Amp*, T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}-MCS2-T_{CYC1}	Agilent Technologies
pMRI-31	T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}-MCS2-T_{CYC1}, *HO* homologous arm	[5]
pMRI-31-URA3	T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}-*URA3*-T_{CYC1}, *HO* homologous arm	This study
p416XWP-P_{ERG9}-*GAL4*	*CEN/ARS*, *URA3*, P_{ERG9}-*GAL4*-T_{ADH1}	[3]
p416xwp-*MET*-P_{ERG9}-*GAL4*	*CEN/ARS*, *Met15*, P_{ERG9}-*GAL4*-T_{ADH1}	This study
p416XWP01	*CEN/ARS*, *URA3*, T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}-MCS2-T_{CYC1}	[6]
p416XWP01-*MISPSLN*	*CEN/ARS*, *URA3*, T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}- *MLS-ISPSLN* -T_{CYC1}	[4]
p416XWP01-P_{ERG9}	*CEN/ARS*, *URA3*, T_{ADH1}-MCS1-P_{ERG9}-P_{GAL1}-MCS2-T_{CYC1}	This study
p416XWP01-*MLSLN*-P_{ERG9}	*CEN/ARS*, *URA3*, T_{ADH1}-MCS1-P_{ERG9}-P_{GAL1}-*MLS-ISPSLN*-T_{CYC1}	This study
p416XWP01-*MLSLN-GAL4WT*	*CEN/ARS*, *URA3*, T_{ADH1}-*GAL4WT*-P_{ERG9}-P_{GAL1}-*MLS-ISPSLN*-T_{CYC1}	This study
p416XWP01-*MLSLN-GAL4ep19*	*CEN/ARS*, *URA3*, T_{ADH1}-*GAL4ep19*-P_{ERG9}-P_{GAL1}-*MLS-ISPSLN*-T_{CYC1}	This study
p416XWP01-*MLSLN*-P_{SSA4}-*GAL4ep19*	*CEN/ARS*, *URA3*, T_{ADH1}-*GAL4ep19*-P_{SSA4}-P_{GAL1}-*MLS-ISPSLN*-T_{CYC1}	This study
p416XWP01-*MLSLN*-P_{SSA4}-*GAL4WT*	*CEN/ARS*, *URA3*, T_{ADH1}-*GAL4WT*-P_{SSA4}-P_{GAL1}-*MLS-ISPSLN*-T_{CYC1}	This study
pESC-URA	*f1 ori*, *2μ ori*, *URA3*, *Amp*, T_{ADH1}-MCS1-P_{GAL10}-P_{GAL1}-MCS2-T_{CYC1}	GenBank: AF063585
pESC-URA-P_{ERG9}	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{ERG9}-MCS-T_{ADH1}	This study
pESC-URA-P_{ERG9}-EGFP	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{ERG9}-*EGFP*-T_{ADH1}	This study
pESC-URA-1HSE-EGFP	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{1HSE}-*EGFP*-T_{ADH1}	This study
pESC-URA-2HSE-EGFP	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{2HSE}-*EGFP*-T_{ADH1}	This study
pESC-URA-SSA4-EGFP	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{SSA4}-*EGFP*-T_{ADH1}	This study
pESC-URA-P_{ERG9}-*GAL4*	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{ERG9}-*GAL4*-T_{ADH1}	This study
pESC-URA-P_{SSA4}-*GAL4*	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{SSA4}-*GAL4*-T_{ADH1}	This study
pESC-URA-P_{SSA4}-*GAL4ep19*	*f1 ori*, *2μ ori*, *URA3*, *Amp*, P_{SSA4}-*GAL4ep19*-T_{ADH1}	This study
pUMRI-11-EGFP	T_{ADH1}-*EGFP*-P_{GAL10}-P_{GAL1}-*MCS2*-T_{CYC1}, *DPP1 homologous arm*	[3]
pUMRI-11-EGFP-LacZ	T_{ADH1}-*EGFP*-P_{GAL10}-P_{GAL1}-*LacZ*-T_{CYC1}, *DPP1 homologous arm*	This study

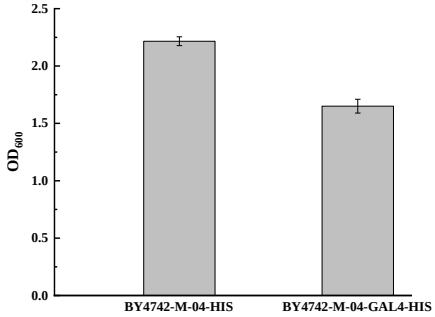

Fig. S1 Effect of *GAL4* overexpression on biomass. The effect of *GAL4* overexpression on biomass in strain BY4742-M-04-HIS [7] harboring a mitochondrial isoprene synthetic pathway. The data presented are the means of three biological replicates. Error bars represent the standard deviations.

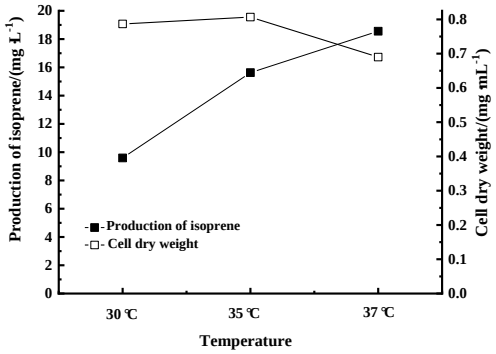

Fig. S2 Effect of temperature on isoprene production and cell growth. The effects of temperature (30 ℃, 35 ℃, 37 ℃) on isoprene production and cell growth. The data presented are the means of three biological replicates.

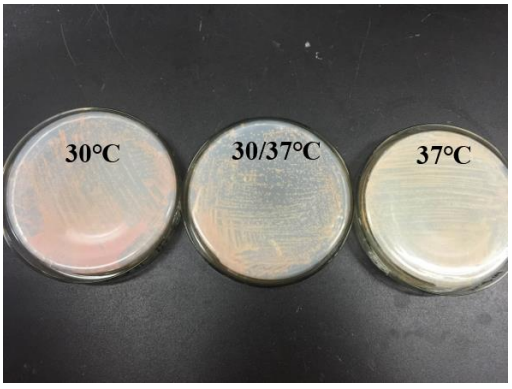

Fig. S3 Biosynthesis of lycopene in engineered yeast at different temperatures. Strain Yxwp111 expressing the lycopene pathway grown at 30 ℃, 30 ℃/37 ℃, 37 ℃ respectively.

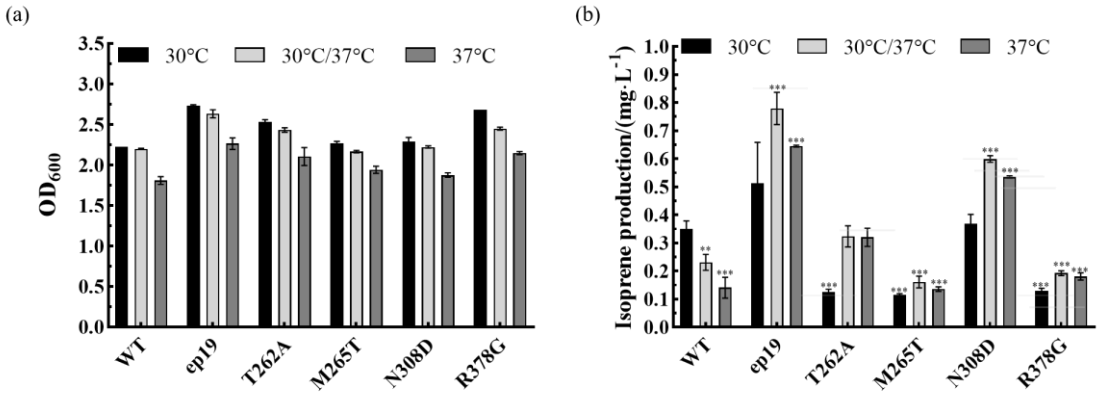

Fig. S4 Isoprene production and growth of strains expressing Gal4p variants with single-point mutations. (a) The growth of strains expressing Gal4p with single-point mutations. (b) The isoprene production of strains expressing Gal4p with single-point mutations. Wild-type Gal4p, Gal4ep19 and Gal4p variants carrying T262A, M265T, N308D, R378G single mutations were transformed respectively into M08H-CS. The isoprene production and biomass were compared after 48 h of shake-flask fermentation. Cells were cultured in sealed vials. The data presented are the means of three biological replicates. Error bars represent the standard deviations. Significant levels of t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

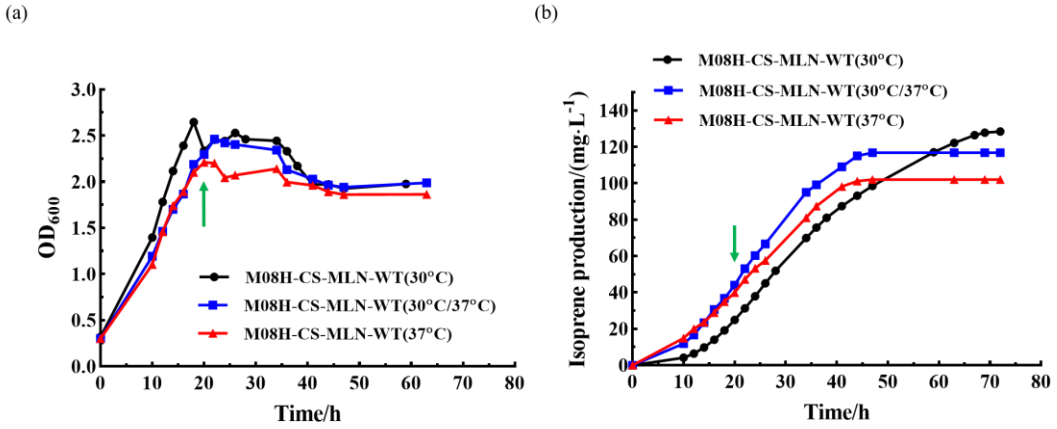

Fig. S5 Aerobic batch fermentation of strain M08H-CS-MLN-WT at different temperatures. The isoprene production and biomass were compared when cultured at 30 ℃, 30 ℃/37 ℃, 37 ℃ after 72 h of aerobic batch fermentation. Green arrow indicated the time point of temperature shift from 30 ℃ to 37 ℃. (a) Biomass of strain M08H-CS-MLN-WT at different temperatures. (b) The isoprene production of strain M08H-CS-MLN-WT at different temperatures.

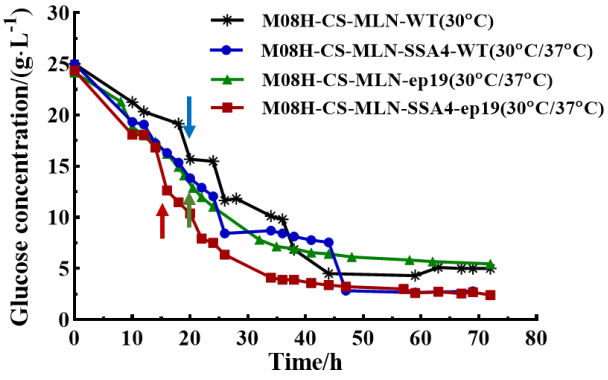

Fig. S6 Sugar consumption during aerobic batch fermentation of recombinant yeast strains. The arrows indicated the time points of temperature shift from 30 ℃ to 37 ℃.

References

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