

Overproduction of α -farnesene in *Saccharomyces cerevisiae*

by farnesene synthase screening and metabolic engineering

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

Strain	Genotype	Resource/Reference
YPH499	<i>MAT a, his3-Δ200, leu2-Δ1, trp1-Δ63, ura3-52, lys2-801, ade2-101</i>	ATCC
31906	<i>Saccharomyces cerevisiae</i> used for wine	CICC
WH4	YPH499 <i>gal80Δ::loxp, his3::HIS3, P_{GAL10}-HMG1, trp1::TRP1, P_{GALI}-HMG1, lys2::LYS2, P_{GAL10}-HMG1</i>	This study
WH4H	YPH499 <i>gal80Δ::loxp, his3::HIS3, trp1::TRP1, P_{GALI}-HMG1, lys2::LYS2, P_{GAL10}-HMG1</i>	This study
WH5	YPH499 <i>gal80Δ::loxp, his3::HIS3, P_{GAL10}-tHMG1, trp1::TRP1, P_{GALI}-tHMG1, lys2::LYS2, P_{GAL10}-tHMG1</i>	This study
WH6	YPH499 <i>gal80Δ::loxp, lys2::LYS2, P_{GAL10}-HMG1</i>	This study
WH7	YPH499 <i>gal80Δ::loxp, trp1::TRP1, P_{GALI}-HMG1, lys2::LYS2, P_{GAL10}-HMG1</i>	This study
WH8	YPH499 <i>gal80Δ::loxp, lys2::LYS2, P_{GAL10}-tHMG1</i>	This study
WH9	YPH499 <i>gal80Δ::loxp, trp1::TRP1, P_{GALI}-tHMG1, lys2::LYS2, P_{GAL10}-tHMG1</i>	This study
WHE	YPH499 <i>gal80Δ::loxp, his3::HIS3, P_{GAL10}-HMG1 P_{GALI}-ERG20, trp1::TRP1, P_{GAL10}-HMG1, P_{GALI}-IDII, lys2::LYS2, P_{GAL10}-HMG1, P_{GALI}-ERG10, ura3::URA3, P_{GAL10}-ERG8, P_{GALI}-ERG19, leu2::LEU2, P_{GAL10}-ERG12, P_{GALI}-ERG13</i>	This study
WHtE	YPH499 <i>gal80Δ::loxp, his3::HIS3, P_{GAL10}-tHMG1, P_{GALI}-ERG20, trp1::TRP1, P_{GAL10}-tHMG1, P_{GALI}-IDII, lys2::LYS2, P_{GAL10}-tHMG1, P_{GALI}-ERG10, ura3::URA3, P_{GAL10}-ERG8, P_{GALI}-ERG19, leu2::LEU2, P_{GAL10}-ERG12, P_{GALI}-ERG13</i>	This study
WHF3	YPH499 <i>gal80Δ::P_{TDH3}-Fsap-T_{TPH}, G418</i>	This study
WHF4	YPH499 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}, G418</i>	This study
WHF5	YPH499 <i>gal80Δ::P_{TDH3}-Fsaa-T_{TPH}, G418</i>	This study
WHF6	YPH499 <i>gal80Δ::P_{TDH3}-Fscj-T_{TPH}, G418</i>	This study
WHF7	WHE <i>gal80Δ::P_{TDH3}-Fsap-T_{TPH}, G418</i>	This study
WHF8	WHE <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}, G418</i>	This study
WHF9	WHE <i>gal80Δ::P_{TDH3}-Fsaa-T_{TPH}, G418</i>	This study
WHF10	WHE <i>gal80Δ::P_{TDH3}-Fscj-T_{TPH}, G418</i>	This study
WHF11	WHtE <i>gal80Δ::P_{TDH3}-Fsap-T_{TPH}, G418</i>	This study
WHF12	WHtE <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}, G418</i>	This study
WHF13	WHtE <i>gal80Δ::P_{TDH3}-Fsaa-T_{TPH}, G418</i>	This study
WHF14	WHtE <i>gal80Δ::P_{TDH3}-Fscj-T_{TPH}, G418</i>	This study
WHF17	WH4 <i>gal80Δ::P_{TDH3}-Fsap-T_{TPH}, G418</i>	This study
WHF18	WH4 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}, G418</i>	This study
WHF19	WH4 <i>gal80Δ::P_{TDH3}-Fsaa-T_{TPH}, G418</i>	This study
WHF20	WH4 <i>gal80Δ::P_{TDH3}-Fscj-T_{TPH}, G418</i>	This study
WHF21	WH5 <i>gal80Δ::P_{TDH3}-Fsap-T_{TPH}, G418</i>	This study
WHF22	WH5 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}, G418</i>	This study
WHF23	WH5 <i>gal80Δ::P_{TDH3}-Fsaa-T_{TPH}, G418</i>	This study

WHF24	WH5 <i>gal80Δ::P_{TDH3}-Fscj-T_{TPH}</i> , G418	This study
WH10	WH6 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}</i> , G418	This study
WH11	WH7 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}</i> , G418	This study
WH12	WH8 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}</i> , G418	This study
WH13	WH9 <i>gal80Δ::P_{TDH3}-Fssso-T_{TPH}</i> , G418	This study
WH18	WH6 <i>gal80Δ::P_{GALI}-Fssso-T_{TPH}</i> , G418	This study
WH19	WH7 <i>gal80Δ::P_{GALI}-Fssso-T_{TPH}</i> , G418	This study
WH31	YPH499 <i>gal80Δ::P_{GALI}-Fssso-T_{TPH}</i> , G418	This study
499L	YPH499 <i>lys2::LYS2</i>	This study
WH62	WH18 <i>dpp1Δ</i>	This study
WH66	WH19 <i>dpp1Δ</i>	This study
WH62S	WH62 <i>his3::HIS3, trp1::TRP1, ura3::URA3, leu2::LEU2</i>	This study

Table S2 Plasmids used in this study

Plasmid	Features	Resource/ Reference
Ts-80aa	Single integration vector targeted at <i>GAL80</i> locus, expression cassette P_{TDH3} - <i>Fsaa</i> - T_{TPH} , marker loxp- <i>KANMX</i> -loxp	This study
Ts-80ap	Single integration vector targeted at <i>GAL80</i> locus, expression cassette P_{TDH3} - <i>Fsap</i> - T_{TPH} , marker loxp- <i>KANMX</i> -loxp	This study
Ts-80cj	Single integration vector targeted at <i>GAL80</i> locus, expression cassette P_{TDH3} - <i>Fscj</i> - T_{TPH} , marker loxp- <i>KANMX</i> -loxp	This study
Ts-80so	Single integration vector targeted at <i>GAL80</i> locus, expression cassette P_{TDH3} - <i>Fsso</i> - T_{TPH} , marker loxp- <i>KANMX</i> -loxp	This study
Ts-HIS3	Single integration vector targeted at <i>HIS3</i> locus	This study
Ts-TRP1	Single integration vector targeted at <i>URA3</i> locus	This study
Ts-URA3	Single integration vector targeted at <i>URA3</i> locus	This study
Ts-LEU2	Single integration vector targeted at <i>LEU2</i> locus	This study
Ts-URA3-12P13	Single integration vector targeted at <i>URA3</i> locus, expression cassette <i>ERG12</i> - P_{GAL10} - P_{GALI} - <i>ERG13</i>	This study
Ts-LEU2-8P19	Single integration vector targeted at <i>LEU2</i> locus, expression cassette <i>ERG8</i> - P_{GAL10} - P_{GALI} - <i>MVD1</i>	This study
Ts-HIS3-tPE	Single integration vector targeted at <i>HIS3</i> locus, expression cassette <i>tHMG1</i> - P_{GAL10} - P_{GALI} - <i>ERG20</i>	This study
Ts-TRP1-tPI	Single integration vector targeted at <i>TRP1</i> locus, expression cassette <i>tHMG1</i> - P_{GAL10} - P_{GALI} - <i>IDII</i>	This study
Ts-LYS2-tP10	Single integration vector targeted at <i>LYS2</i> locus, expression cassette <i>tHMG1</i> - P_{GAL10} - P_{GALI} - <i>ERG10</i>	This study
Ts-HIS3-HPE	Single integration vector targeted at <i>HIS3</i> locus, expression cassette <i>HMG1</i> - P_{GAL10} - P_{GALI} - <i>ERG20</i>	This study
Ts-TRP1-HPI	Single integration vector targeted at <i>TRP1</i> locus, expression cassette <i>HMG1</i> - P_{GAL10} - P_{GALI} - <i>IDII</i>	This study
Ts-LYS2-HP10	Single integration vector targeted at <i>LYS2</i> locus, expression cassette <i>HMG1</i> - P_{GAL10} - P_{GALI} - <i>ERG10</i>	This study
Ts-HIS3-HP	Single integration vector targeted at <i>HIS3</i> locus, expression cassette <i>HMG1</i> - P_{GAL10} - P_{GALI}	This study
Ts-TRP1-HP	Single integration vector targeted at <i>TRP1</i> locus, expression cassette <i>HMG1</i> - P_{GAL10} - P_{GALI}	This study
Ts-LYS2-HP	Single integration vector targeted at <i>LYS2</i> locus, expression cassette <i>HMG1</i> - P_{GAL10} - P_{GALI}	This study
Ts-HIS3-tP	Single integration vector targeted at <i>HIS3</i> locus, expression cassette <i>tHMG1</i> - P_{GAL10} - P_{GALI}	This study
Ts-TRP1-tP	Single integration vector targeted at <i>TRP1</i> locus, expression cassette <i>tHMG1</i> - P_{GAL10} - P_{GALI}	This study
Ts-LYS2-tP	Single integration vector targeted at <i>LYS2</i> locus, expression cassette <i>tHMG1</i> - P_{GAL10} - P_{GALI}	This study
Ts-80101so	Single integration vector targeted at <i>GAL80</i> locus, expression	This study

	cassette P _{GALI} - <i>FssO</i> -T _{TPH} , marker loxp- <i>KANMX</i> -loxp	
pHCas9-Nours	<i>Cas9</i> expression	Yu Jiang
BTS-LPP1	Vector expressing gRNA used for <i>LPP1</i>	This study
BTS-DPP1	Vector expressing gRNA used for <i>DPP1</i>	This study

Table S3 Primers used in this study

Number	Primer name	Sequence (5' to 3')	Restriction site
F1	HIS3- <i>Sac</i> II-1	TCCCCGCGGGGAGGTGTCCATGTCGCCTAG	<i>Sac</i> II
F2	HIS3- <i>Sma</i> I-2	CTGTGTTATGACTTCCCTGACCCGGGCTAATGCCGT GTTCAAACGA	<i>Sma</i> I
F3	HIS3- <i>Sma</i> I-3	TCGTTTGAACACGGCATTAGCCCGGGTCAGGGAAGT CATAACACAG	<i>Sma</i> I
F4	HIS3- <i>Sac</i> II-4	TCCCCGCGGGGAGACAGCAGTTGGGTAGGC	<i>Sac</i> II
F5	HMG1- <i>Sma</i> I-1	TCCCCCGGGGGATAGTTATGACAATTACAACA	<i>Sma</i> I
F6	P10-P1-2(HIS3)	CTAATTTCCTTTTTCTGAAGCCATTATAGTTTTTCTCC TTGACGTTA	
F7	ERG20-1	TAACGTCAAGGAGAAAAAACTATAATGGCTTCAGA AAAAGAAATTAG	
F8	ERG20- <i>Sma</i> I-2	TCCCCCGGGGGACATGGTCCTTATCTAGTTTG	<i>Sma</i> I
F9	TRP1- <i>Sac</i> II-1	TCCCCGCGGGGAGATGACGAGTTGGTGGAGC	<i>Sac</i> II
F10	TRP1- <i>Bam</i> HI-2	CGGGATCCCGATATATGTGTACTTTGCAGT	<i>Bam</i> HI
F11	TRP1- <i>Afl</i> II-3	CGTGCTTAAGTGTGAGCTCTTTTAGATCGG	<i>Bsp</i> TI
F12	TRP1- <i>Sac</i> II-4	TCCCCGCGGGGAGAGGCTGATGGTGTATTATGC	<i>Sac</i> II
F13	HMG1- <i>Bam</i> HI-1	CGGGATCCCGTAGTTATGACAATTACAACA	<i>Bam</i> HI
F14	P10-P1-2(TRP1)	GCATACTATTGTTGTGCGGCAGTCATTATAGTTTTTTC TCCTTGACGTTA	
F15	IDI1-1	TAACGTCAAGGAGAAAAAACTATAATGACTGCCGA CAACAATAGTATGC	
F16	IDI1- <i>Afl</i> II-2	CGTGCTTAAGGCGTAAATAAAGAAAATAAAGTT	<i>Bsp</i> TI
F17	LYS2- <i>Sma</i> I-1	TCCCCCGGGGGATTGAAGGGTGGCTATGT	<i>Sma</i> I
F18	LYS2- <i>Sma</i> I-2	TCCCCCGGGGGATAAACCAGTGAACCTAACG	<i>Sma</i> I
F19	LYS2- <i>Bam</i> HI-3	CGGGATCCCGTTGTGCCTTTGTTACGTC	<i>Bam</i> HI
F20	LYS2- <i>Sal</i> I-4	ACGCGTCGACGTGACCATCAACCAGGAAGT	<i>Sal</i> I
F21	HMG1- <i>Sal</i> I-1	ACGCGTCGACTAGTTATGACAATTACAACA	<i>Sal</i> I
F22	P10-P1-2(LYS2)	ATACAATGTAAACGTTCTGAGACATTATAGTTTTTTC TCCTTGACGTTA	
F23	ERG10-1	TAACGTCAAGGAGAAAAAACTATAATGTCTCAGAA CGTTTACATTGTAT	
F24	ERG10- <i>Bam</i> HI-2	CGGGATCCCGAAAAGTAAGTCAAAAGGCAC	<i>Bam</i> HI
F25	tHMG1-2 (HTL)	AATTTTTGAAAATTCAATATAAATGGCTGCAGACCA ATTGGTGAAAAC	
F26	P10,1-1 (HTL)	GTTTTACCAATTGGTCTGCAGCCATTTATATTGAAT TTTCAAAAATT	
F27	HMG1- <i>Afl</i> II-1	CGTGCTTAAGTAGTTATGACAATTACAACA	<i>Afl</i> II
F28	P10,1- <i>Sal</i> I-2	ACGCGTCGACTATAGTTTTTCTCCTTGACGTTA	<i>Sal</i> I
F29	P10,1- <i>Bam</i> HI-2	CGGGATCCCGTATAGTTTTTCTCCTTGACGTTA	<i>Bam</i> HI
F30	HMG1-2	AATTTTTGAAAATTCAATATAAATGCCGCCGCTATT CAAGGGACTG	

F31	P10-P1-1	CAGTCCCTTGAATAGCGGCGGCATTTATATTGAATT TTCAAAAATT	
F32	URA3-SacII-1	TCCCCGCGGGGACATCAATCCGTGTAAGCAG	<i>SacII</i>
F33	URA3-SacII-2	TCCCCGCGGGGACTTGGTTCTGGCGAGGTA	<i>SacII</i>
F34	URA3-AflII-3	CGTGCTTAAGCAAACCGAAGTTATCTGATG	<i>BspTI</i>
F35	URA3-SalI-4	ACGCGTCGACCTTCGGTTTGTATCATCGTC	<i>SalI</i>
F36	ERG12-SalI-1	ACGCGTCGACATGGTCTGCTTAAATTTTCAT	<i>SalI</i>
F37	ERG12-2	AATTTTGA AAAATTCAATATAAATGTCATTACCGTTC TTAACTTCTGCA	
F38	P10-P1-1(URA3)	TGCAGAAGTTAAGAACGGTAATGACATTTATATTGA ATTTTCAAAAATT	
F39	P10-P1-2(URA3)	AACAAAGTTTAGTTGAGAGTTTCATTATAGTTTTTTC TCCTTGACGTTA	
F40	ERG13-1	TAACGTCAAGGAGAAAAAACTATAATGAAACTCTC AACTAAACT TTGTT	
F41	ERG13-AflII-2	CGTGCTTAAGTATATATATA TCATTGTTAT	<i>BspTI</i>
F42	LEU2-SacII-1	TCCCCGCGGGGATTTGGCCGAGCGGTCTAAG	<i>SacII</i>
F43	LEU2-SacII-2	TCCCCGCGGGGAAAGATAGTGGCGATAGGGT	<i>SacII</i>
F44	LEU2-SalI-3	ACGCGTCGACAGAGGTCGCCTGACGCATA	<i>SalI</i>
F45	LEU2-BamHI-4	CGGGATCCCGACCGCTCGGCCAAACAAC	<i>BamHI</i>
F46	ERG8-BamHI-1	CGGGATCCCGTAGCTTGTACCCATTAAAAGAATTTT ATCATGCCG	<i>BamHI</i>
F47	ERG8-2	AATTTTGA AAAATTCAATATAAATGTCAGAGTTGAG AGCCTTCAGTG	
F48	P10-P1-1(LEU2)	CACTGAAGGCTCTCAACTCTGACATTTATATTGAAT TTTCAAAAATT	
F49	P10-P1-2(LEU2)	GGTAACGGATGCTGTGTAAACGGTCATTATAGTTTT TTCTCCTTGACGTTA	
F50	ERG19-1	TAACGTCAAGGAGAAAAAACTATAATGACCGTTTAC ACAGCATCCGTTACC	
F51	ERG19-SalI-2	ACGCGTCGACTTTCTCATTCAAGTGGTAAC	<i>SalI</i>
F52	GAL80-SacII-1	TCCCCGCGGGGAAGGAGCAAGCAACTGACC	<i>SacII</i>
F53	GAL80-SacII-2	TCCCCGCGGGGAATTGGGCGTTCTATGAGG	<i>SacII</i>
F54	GAL80-XbaI-3	CATGTCTAGAAAGCATCTTGCCCTGTGCT	<i>XbaI</i>
F55	GAL80-XbaI-4	CATGTCTAGAGACGGGAGTGGAAGAAGACG	<i>XbaI</i>
F56	GAL80-AflII-3	CGTGCTTAAGAAAGCATCTTGCCCTGTGCT	<i>BspTI</i>
F57	GAL80-AflII-4	CGTGCTTAAGGACGGGAGTGGAAGAAGACG	<i>BspTI</i>
F58	XTP-AflII-5	CGTGCTTAAGTTCTATGTTCGGGTTTCAGC	<i>BspTI</i>
F59	XTP-AflII-6	CGTGCTTAAGATACTAGCGTTGAATGTTAGCGTCA	<i>BspTI</i>
F60	XTP-SalI-7	ACGCGTCGACTTTGTTTGTATGTGTGTTTATTC	<i>SalI</i>
F61	XTP-NheI-8	CTAGCTAGCTAGGATTAATATAATTATATAAAAAATA T	<i>NheI</i>
F62	XTP-BamHI-7	CGGGATCCCGTTTGTGTTTATGTGTGTTTATTC	<i>BamHI</i>
F63	XTP-SalI-8	ACGCGTCGACTAGGATTAATATAATTATATAAAAAAT	<i>SalI</i>

		AT	
F64	XXK- <i>Xba</i> I-1	GCTCTAGAGCTTCTATGTTTCGGGTTTCAGC	<i>Xba</i> I
F65	XXK-2	TATCCAAATTTCAACTGTTATATAGATGACCATGAT TACGAACTC	
F66	T _{TPH} -1	GAGTTCGTAATCATGGTCATCTATATAACAGTTGAA ATTTGGATA	
F67	T _{TPH} -2	GAATAAACACACATAAAACAAACAAAGATTAATATA ATTATATAAAAAATAT	
F68	P _{TDH3} -1	ATATTTTTATATAATTATATTAATCTTTGTTTGTTTAT GTGTGTTTATTC	
F69	P _{TDH3} - <i>Xba</i> I-2	GCTCTAGAGCATACTAGCGTTGAATGTTAGCGTCA	<i>Xba</i> I
F70	T _{TPH} -2	TAACGTCAAGGAGAAAAAACTATAGATTAATATAA TTATATAAAAAATAT	
F71	P101-1	ATATTTTTATATAATTATATTAATCTATAGTTTTTTCT CCTTGACGTTA	
F72	P101- <i>Xba</i> I-2	CATGTCTAGATTATATTGAATTTTCAAAAATT	<i>Xba</i> I
F73	XTP101- <i>Eco</i> RI-5	CGGAATTCCGTTTGTGTTTATGTGTGTTTATTC	<i>Eco</i> RI
F74	XTP101- <i>Bam</i> HI-6	CGGGATCCCGATTAATATAATTATATAAAAAATAT	<i>Bam</i> HI
F75	Fsso- <i>Eco</i> RI-1	CGGAATTCCGATGAATCACTCTTATGCTAACC	<i>Eco</i> RI
F76	Fsso- <i>Bam</i> HI-2	CGGGATCCCGTTATCTCAATGGCTCAACAACC	<i>Bam</i> HI
F77	471F	AAGGAGGGTATTCTGGGCCTCCATGTC	
F78	471R	TCTGCAGAATTCGTCGACGAGCTCGGTAC	
F79	DPP1-302R	CCCATTGATGATCCTCTCCGATCATTATCTTTTAC TGCGGAG	
F80	DPP1-149F	GGAAGAGGATCATCAATGGGGTTTTAGAGCTAGAA ATAGCAAG	
F81	ACT1-U	TTGGTAACGAAAGATTCAGAGCCC	
F82	ACT1-D	AAGATAGAACCACCAATCCAGACG	
F83	ERG10-U	TTACTGCCGCTAACGCTTCTC	
F84	ERG10-D	GCCTCACCCCAACCTTTGATAAT	
F85	ERG13-U	TTTTCTTACGGTTCCGGTTTAGC	
F86	ERG13-D	TCCTTTGGAGTTTCGGTGATTC	
F87	HMG1-U	GCTGGGTCTGTTGGTGGATT	
F88	HMG1-D	CGATGGATGGCATGGATACG	
F89	ERG12-U	ACCGATGACGAGGCTGTAGAAA	
F90	ERG12-D	CAGGATGAGAAACACCGATTGAG	
F91	ERG8-U	CAAAACAGGGCTGGGCTCCT	
F92	ERG8-D	CGCTACATCAAACCCGCTTCC	
F93	ERG19-U	CGTGGCAACCTCCGAATATT	
F94	ERG19-D	ACAGCATTTGGACCTGCATCA	
F95	ID11-U	CTACATCGTGCATTCTCCGTCTT	
F96	ID11-D	CCCCTTGTCTTAGTTTCATCTTCTG	
F97	ERG20-U	ACTATTCTTTCTACTTGCTGTCGC	
F98	ERG20-D	GTCTTCTTACCGTAATTTTCGTCT	

F99	ERG9-U	ATGGTTTGACCCGTTTGATTGT
F100	ERG9-D	AACCCCAGTTGTTCGTTTTCAG
F101	Fsso-U	AGAAGTTAAAGATTTGTCCCGTTGG
F102	Fsso-D	TGACTGCTTTATACTTTGGCTCGA

Table S4 Comparison of a previous study and our study

Name	Previous study	This study
Host	<i>S. cerevisiae</i> CEN.PK113-5D	<i>S. cerevisiae</i> YPH499
Expression manner	Episomal plasmid	Integrated into chromosome
Promoter	TEF1 promoter	TDH3 promoter
Fermentation time	48 h	120 h
Medium	Minimal medium (20 g/L glucose, 5 g/L (NH ₄) ₂ SO ₄ , 3 g/L KH ₂ PO ₄ , 0.5 g/L MgSO ₄ ·7H ₂ O, 1 ml/L trace element solution)	YPD (20 g/L glucose, 10 g/L yeast extract, 20 g/L peptone)
<i>Fsso</i>	/	69.66 mg/L α -farnesene
<i>Fsap</i>	4.0 mg/L α -farnesene	3.2 mg/L α -farnesene
<i>Fsaa</i>	3.0 mg/L β -farnesene	22.3 mg/L β -farnesene
<i>Fscj</i>	3.5 mg/L β -farnesene	17.5 mg/L β -farnesene

SI Materials and Methods

Construction of plasmids for overexpressing the MVA pathway

Integration plasmids used for the overexpression of the MVA pathway were constructed as follows. Fragments prepared by primers F1-2 and F3-4 with CICC31906 genomic DNA were fused and it was cloned into 19T simple vector (TaKaRa, Dalian, China), this plasmid was named Ts-HIS3. The genes and terminators of *tHMG1* and *ERG20* were amplified from YPH499 genomic DNA by primers F5, 25 and F7-8. P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F26, 6. *tHMG1*, *ERG20* and P_{GALI} - P_{GAL10} were fused and digested by *Sma*I, it was inserted into Ts-HIS3. The resulting plasmid was named Ts-HIS3-tPE. Fragment prepared by primers F9 and F12 with CICC31906 genomic DNA was cloned into 19T simple vector, this plasmid was named Ts-TRP1. The genes and terminators of *tHMG1* and *ID11* were amplified from YPH499 genomic DNA by primers F13, 25 and F15-16. P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F26 and F14. *tHMG1*, *ID11* and P_{GALI} - P_{GAL10} were fused and digested by *Bam*HI and *Bsp*TI, it was inserted into vector, which was prepared by primers F10-11 with Ts-TRP1. The resulting plasmid was named Ts-TRP1-tPI. Fragment prepared by primers F17-18 with CICC31906 genomic DNA was cloned into 19T simple vector, this plasmid was named Ts-LYS2. The genes and terminators of *tHMG1* and *ERG10* were amplified from YPH499 genomic DNA by primers F21, 25 and F23-24. P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F26 and F22. *tHMG1*, *ERG10* and P_{GALI} - P_{GAL10} were fused and

digested by *Bam*HI and *Sal*I, it was inserted into vector, which was prepared by primers F19-20 with Ts-LYS2. The resulting plasmid was named Ts-LYS2-tP10. *HMGI*, *ERG20* and P_{GALI} - P_{GALI0} were amplified from YPH499 genomic DNA by primers F5, 30 and F7-8 and F31, 6. *HMGI*, *ERG20* and P_{GALI} - P_{GALI0} were fused and digested by *Sma*I, it was inserted into Ts-HIS3. The resulting plasmid was named Ts-HIS3-HP20. *HMGI*, *IDII* and P_{GALI} - P_{GALI0} was amplified from YPH499 genomic DNA by primers F27, 30, F15-16 and F31, 14. *HMGI*, *IDII* and P_{GALI} - P_{GALI0} were fused and digested by *Bam*HI and *Bsp*TI, it was inserted into vector, which was prepared by primers F10-11 with Ts-TRP1. The resulting plasmid was named Ts-TRP1-HPI. *HMGI*, *ERG10* and P_{GALI} - P_{GALI0} was amplified from YPH499 genomic DNA by primers F21, 30, F23-24 and F31, 22. *HMGI*, *ERG10* and P_{GALI} - P_{GALI0} were fused and digested by *Bam*HI and *Sal*I, it was inserted into vector, which was prepared by primers F19-20 with Ts-LYS2. The resulting plasmid was named Ts-LYS2-HP10. Fragment prepared by primers F32-33 with CICC31906 genomic DNA was cloned into 19T simple vector, this plasmid was named Ts-URA3. The genes and terminators of *ERG12* and *ERG13* were amplified from YPH499 genomic DNA by primers F36-37 and F40-41. P_{GALI} - P_{GALI0} was amplified from YPH499 genomic DNA by primers F38-39. *ERG12*, *ERG13* and P_{GALI} - P_{GALI0} were fused and digested by *Bsp*TI and *Sal*I, it was inserted into vector, which was prepared by primers F34-35 with Ts-URA3. The resulting plasmid was named Ts-URA3-12P13. Fragment prepared by primers F42-43 with CICC31906 genomic DNA was cloned into 19T simple vector, this plasmid was named Ts-LEU2. The genes and terminators of *ERG8* and *MVDI* were amplified from YPH499 genomic DNA by

primers F460-47 and F50-51. P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F48-49. *ERG8*, *MVD1* and P_{GALI} - P_{GAL10} were fused and digested by *Bam*HI and *Sal*I, it was inserted into vector, which was prepared by primers F44-45 with Ts-LEU2. The resulting plasmid was named Ts-LEU2-8P19.

tHMG1 and P_{GALI} - P_{GAL10} were amplified from YPH499 genomic DNA by primers F5, 25 and F26, 28. *tHMG1* and P_{GALI} - P_{GAL10} were fused and digested by *Sma*I and *Sal*I, it was inserted into Ts-HIS3. The resulting plasmid was named Ts-HIS3-tP. *tHMG1* and P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F27, 25 and F26, 29. *tHMG1* and P_{GALI} - P_{GAL10} were fused and digested by *Bam*HI and *Bsp*TI, it was inserted into vector, which was prepared by primers F10-11 with Ts-TRP1. The resulting plasmid was named Ts-TRP1-tP. *tHMG1* and P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F21, 25 and F26, 29. *tHMG1* and P_{GALI} - P_{GAL10} were fused and digested by *Bam*HI and *Sal*I, it was inserted into vector, which was prepared by primers F19-20 with Ts-LYS2. The resulting plasmid was named Ts-LYS2-tP.

HMG1 and P_{GALI} - P_{GAL10} were amplified from YPH499 genomic DNA by primers F5, 30 and F31, 28. *HMG1* and P_{GALI} - P_{GAL10} were fused and digested by *Sma*I and *Sal*I, it was inserted into Ts-HIS3. The resulting plasmid was named Ts-HIS3-HP. *HMG1* and P_{GALI} - P_{GAL10} was amplified from YPH499 genomic DNA by primers F27, 30 and F31, 29. *HMG1* and P_{GALI} - P_{GAL10} were fused and digested by *Bam*HI and *Bsp*TI, it was inserted into vector, which was prepared by primers F10-11 with Ts-TRP1. The resulting plasmid was named Ts-TRP1-HP. *HMG1* and P_{GALI} - P_{GAL10} was amplified

from YPH499 genomic DNA by primers F21, 30 and F31, 29. *HMG1* and P_{GALI} - P_{GALI} were fused and digested by *Bam*HI and *Sal*I, it was inserted into vector, which was prepared by primers F19-20 with Ts-LYS2. The resulting plasmid was named Ts-LYS2-HP.

Construction of plasmids for producing farnesene

A part of YPH499 genomic DNA were amplified by PCR using primers F52-53, and then it was cloned into 19T simple vector, this plasmid was named Ts-80S. *Loxp-KANMX-loxp* was amplified from pMGKR by primers F58, 65, T_{TPII} and P_{TDH3} were amplified from YPH499 genomic DNA by primers F66-67 and F68, 59, *Loxp-KANMX-loxp*, T_{TPII} and P_{TDH3} were fused and then it was cloned into 19T simple vector, this plasmid was named Ts-XTP(A). After the digestion of pUC-Fsaa and Puc-Fscj by *Sal*I and *Nhe*I, they were inserted into vector, which was prepared by primers F60-61 with Ts-XTP(A), the resulting plasmids were named Ts-XTPaa and Ts-XTPcj. After the digestion of *Bsp*TI, they were inserted into vector, which was prepared by primers F56-57 with Ts-80S, the resulting plasmids were named Ts-80aa and Ts-80cj. *Loxp-KANMX-loxp* was amplified from pMGKR by primers F64-65, T_{TPII} and P_{TDH3} were amplified from YPH499 genomic DNA by primers F66-67 and F68-69, *Loxp-KANMX-loxp*, T_{TPII} and P_{TDH3} were fused and then it was cloned into 19T simple vector, this plasmid was named Ts-XTP(X). After the digestion of pUC-Fsap by *Sal*I and *Bam*HI, they were inserted into vector, which was prepared by primers F62-63 with Ts-XTP(X), the resulting plasmid was named Ts-XTPap. After the digestion of *Xba*I, they were inserted into vector, which was prepared by primers F54-55 with Ts-80S, the resulting

plasmid was named Ts-80ap. After the digestion of pUC-Fsso by *SalI* and *NheI*, it was inserted into vector, which was prepared by primers F62-63 with Ts-XTP(X), the resulting plasmid was named Ts-XTPso. After the digestion of *XbaI*, they were inserted into vector, which was prepared by primers F54-55 with Ts-80S, the resulting plasmid was named Ts-80so. *Loxp-KANMX-loxp* was amplified from pMGKR by primers F58 and F65, *T_{TPH}* and *P_{GALI}-P_{GALI0}* were amplified from YPH499 genomic DNA by primers F66, 70 and F71-72. *Loxp-KANMX-loxp*, *T_{TPH}* and *P_{GALI}-P_{GALI0}* were fused and then it was cloned into 19T simple vector, this plasmid was named Ts-XTP101. *Fsso* was amplified from plasmid pUC-Fsso by primers F75-76, after the digestion of *EcoRI* and *BamHI*, it was cloned into the vector, which was prepared by primers F73-74 with Ts-XTP101, the resulting plasmid was named Ts-XTP101so. After the digestion of *XbaI* and *AflII*, *loxp-KANMX-loxp-T_{TPH}-Fsso-P_{GALI}-P_{GALI0}* was cloned into vector, which was prepared by primers F54 and F57 with Ts-80S, the resulting plasmid was named Ts-80101so.

Construction of plasmids for deleting genes

Fragment DPP1-302 was amplified from plasmid 302 by primers F77 and F79, fragment DPP1-149 was amplified from plasmid 149 by primers F78 and F80. Fragment DPP1-302 and fragment DPP1-149 were fused and then cloned into plasmid BTS, both of which were digested by *BamHI* and *BcuI*, the resulting plasmid was named BTS-DPP1.

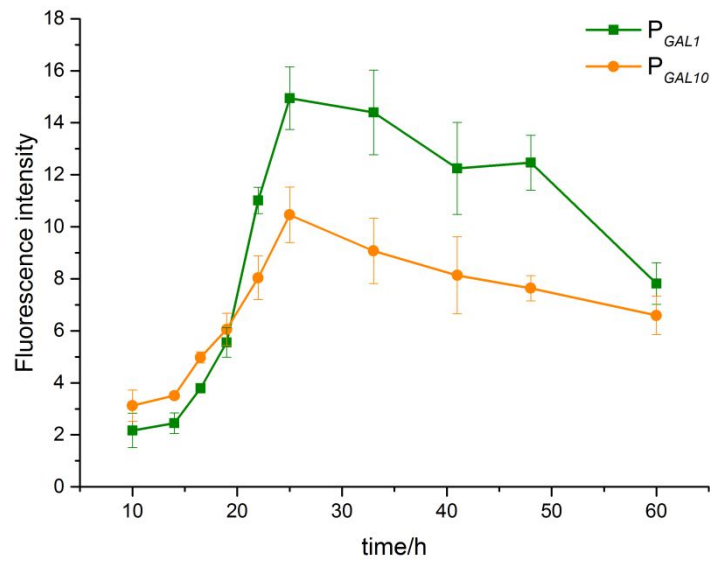


Figure S1. GFP fluorescence of GAL promoter-*EGFP-T_{TP11}* strains in YPD medium



Figure S2. GC-MS total ion chromatograms of synthesis product from engineered strain expressed *Fsso*

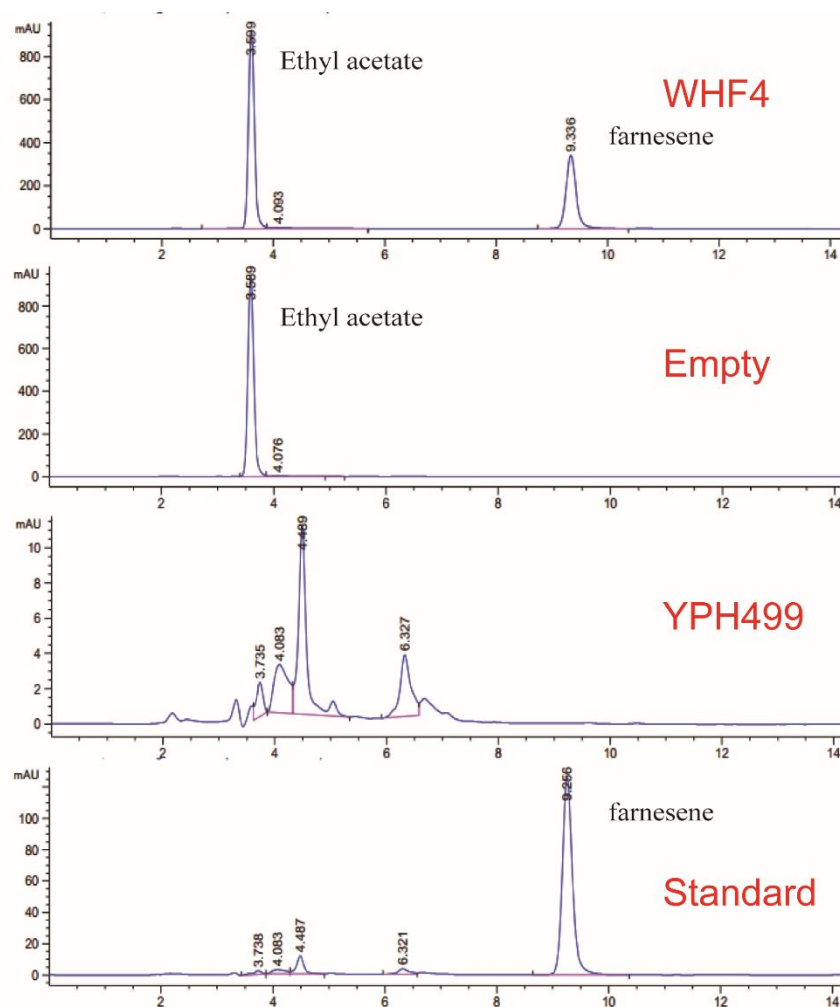


Figure S3. HPLC of synthesis product from engineered strain expressed *Fsso*

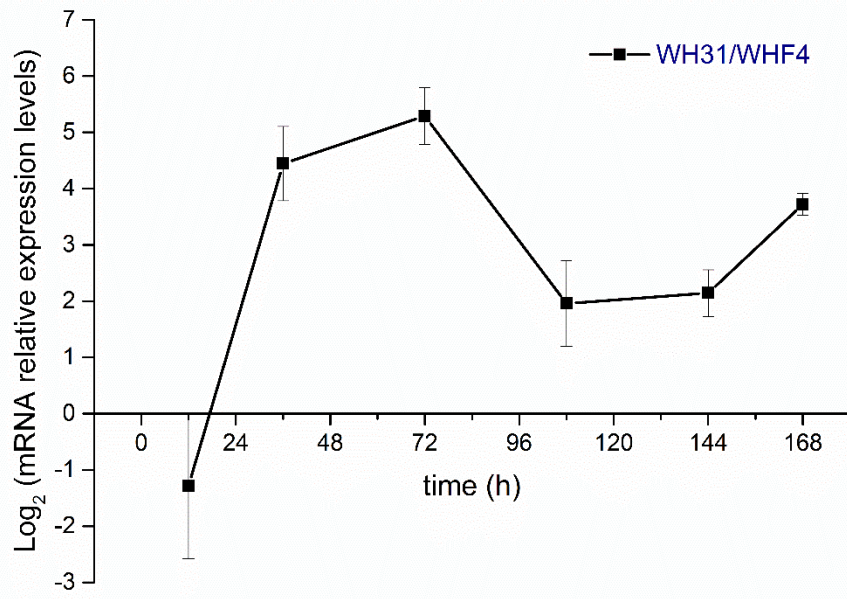


Figure S4. The influence of GAL and TDH3 promoter on transcriptional level of *Fss0* cultured in 250 mL shake flasks with 50 mL YPD medium.

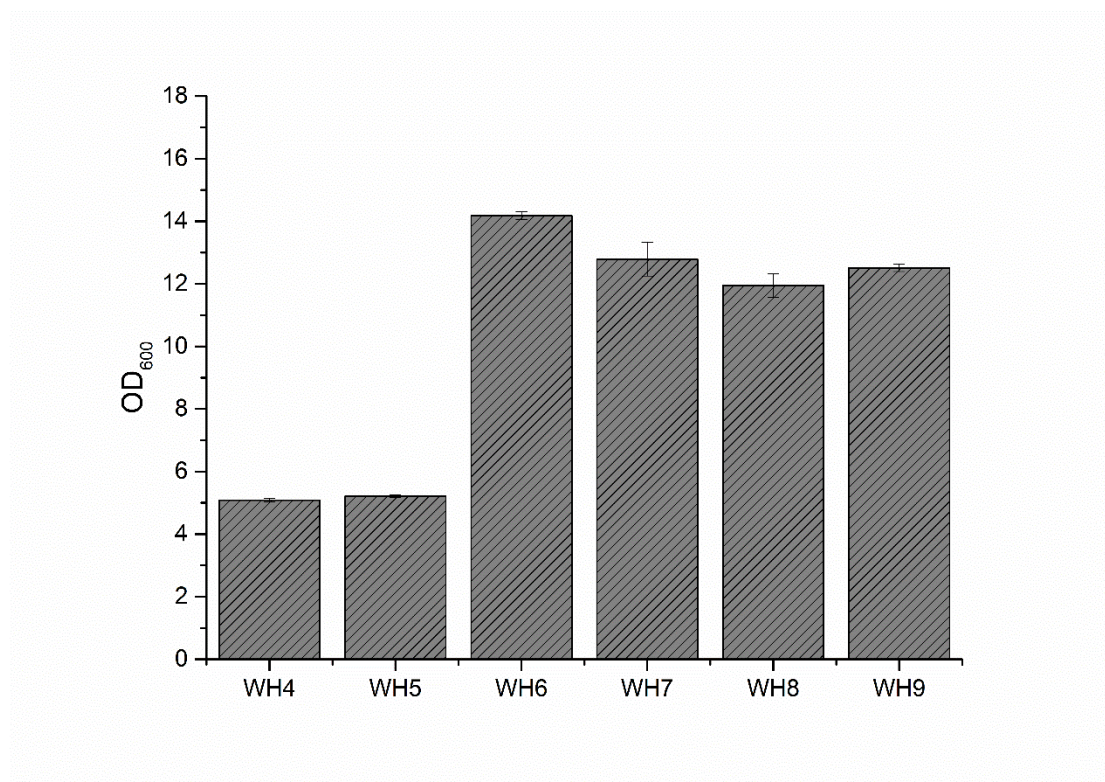


Figure S5. Cell growth of WH4, WH5, WH6, WH7, WH8 and WH9 culture in 50 mL YPD medium for 168 h.

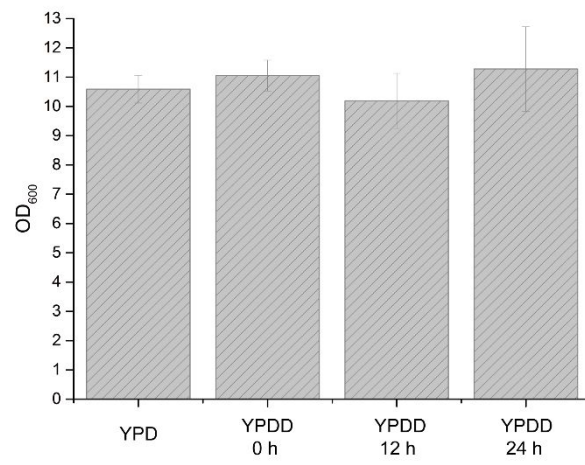


Figure S6. The effects of addition of dodecane at 0 h, 12 h and 24 h on cell growth of WH11 culture in 30 mL medium for 168 h.

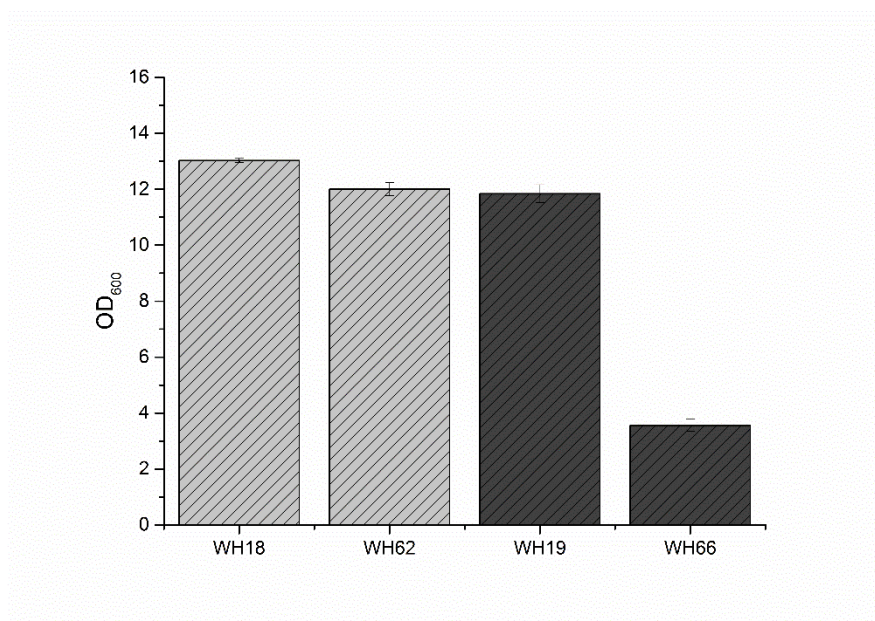


Figure S7. Cell growth of WH18, WH62, WH19 and WH66 culture in 30 mL YPDD medium for 168 h.

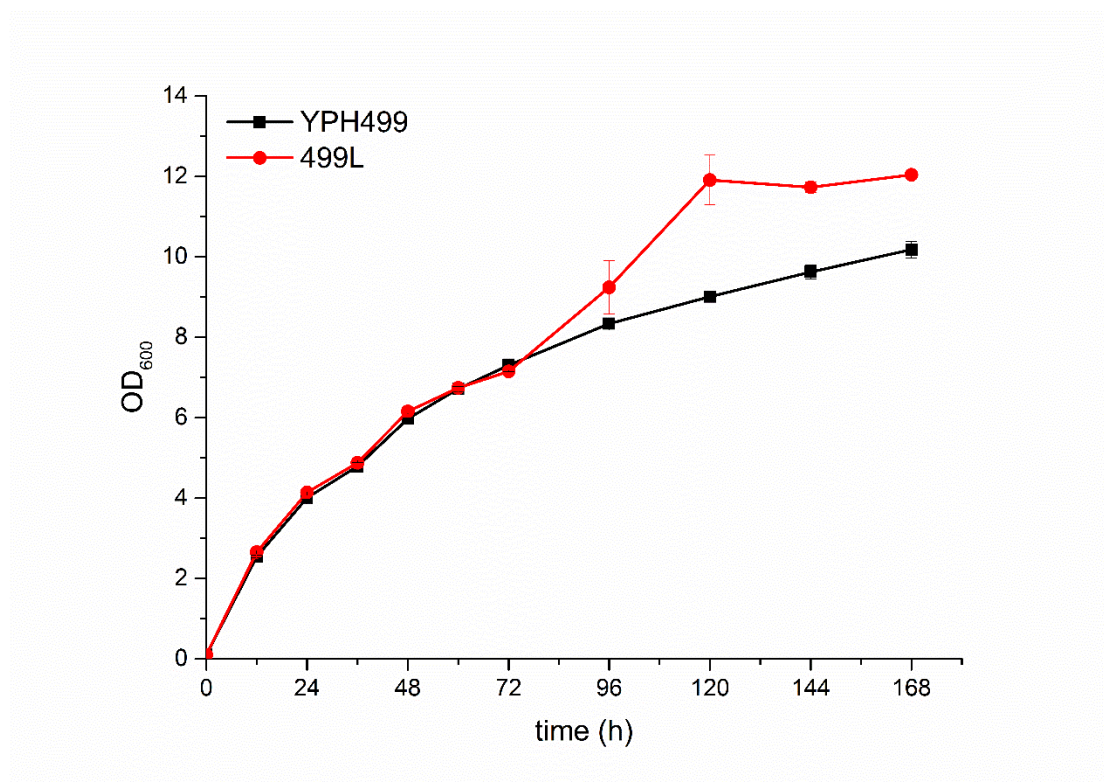


Figure S8. Cell growth of YPH499 and 499L culture in 50 mL YPD medium for 168 h.

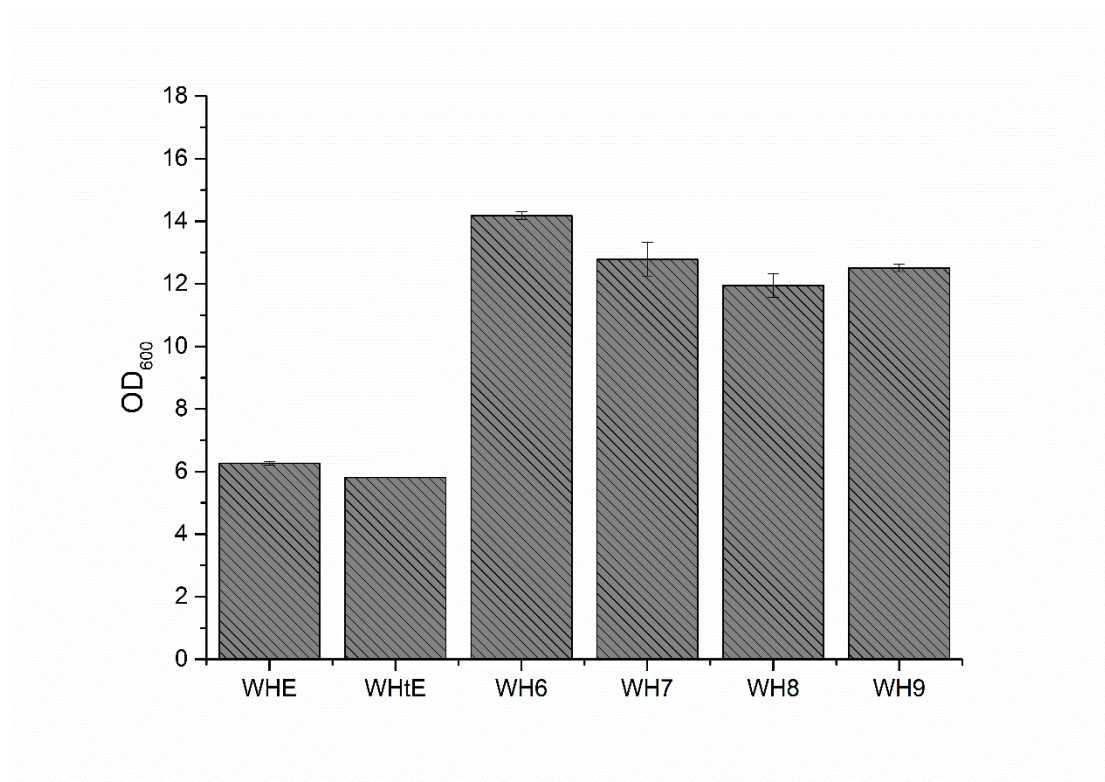


Figure S9. Cell growth of WHE, WHtE, WH6, WH7, WH8 and WH9 culture in 50 mL YPD medium for 168 h.