

Electronic Supplementary Information

Biosynthesis of α -bisabolene from low-cost renewable feedstocks by peroxisome engineering and systems metabolic engineering of the yeast *Yarrowia lipolytica*

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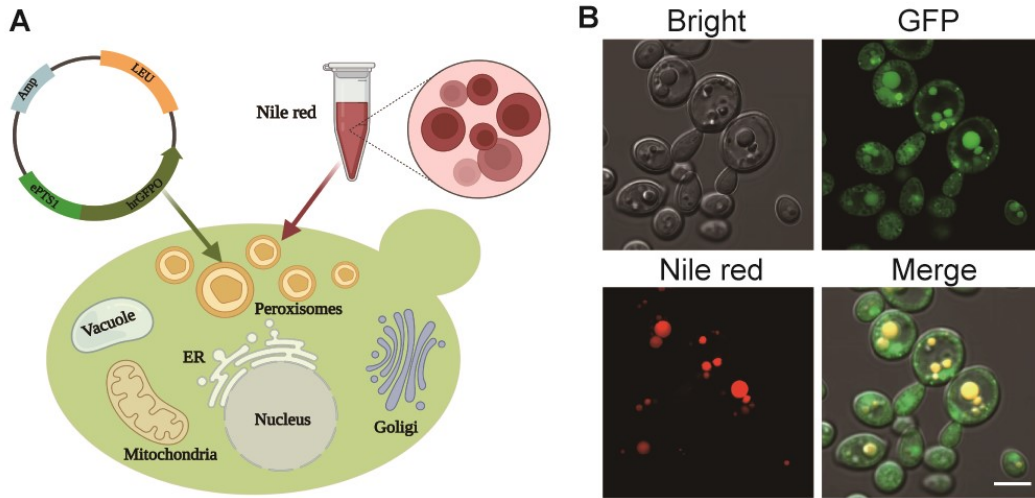


Fig. S1 Visualizing the location of peroxisomes.

(A) GFP-ePTS1 is used to indicate the location of peroxisomes in *Y. lipolytica*. Nile red is used to stain intracellular lipids, including those in peroxisomes.

(B) LSCM visualization of peroxisomes using Nile red and GFP-ePTS1. A single focal plane is shown for all micrographs. The scale bar is 5 μ m.

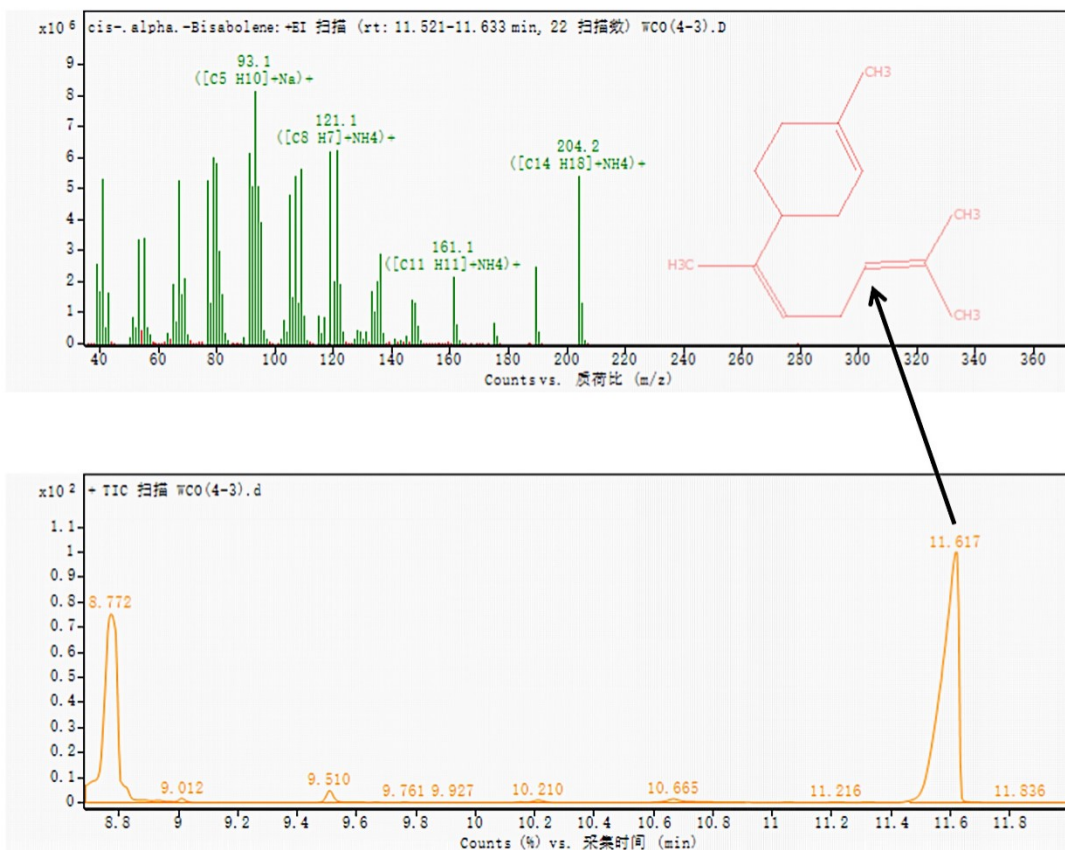


Fig. S2 A representative GC-MS profile of solvent overlay-extracted α -bisabolene from a culture of engineered *Y. lipolytica* strains.

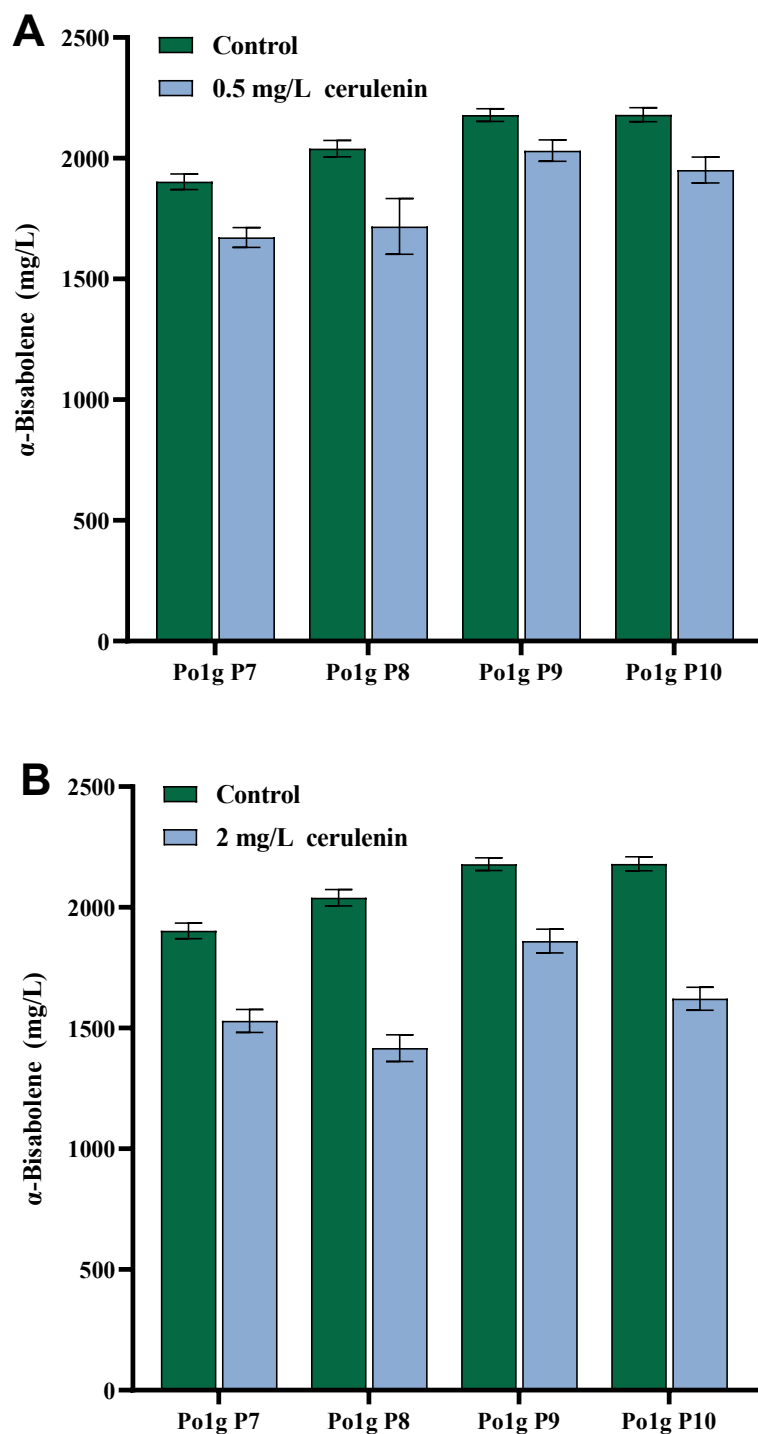


Fig. S3 Effects of addition of cerulenin on titers of α -bisabolene.

(A) Effect of 0.5 mg/L cerulenin on α -bisabolene production carried out for Po1g P7, Po1g P8, Po1g P9 and Po1g P10 strains compared to the same strains without supplementation of cerulenin (control).

(B) Effect of 2 mg/L cerulenin on α -bisabolene production carried out for Po1g P7, Po1g P8, Po1g P9 and Po1g P10 strains compared to the same strains without supplementation of cerulenin (control).

YIPex23p	MSD.....K EKKKSSATHAAFPPSTASQPOS.....MSP	31
Ylr324p	MSG.NTTNVHETRAKFAETLQPRI GGNTTKVI RAALEKNEAESGVSEDNDNGSLEKVNVA	64
Ygr004p	MSEI.NNENLEPTSSSTVAESTE.....SKNKHI R S ALRKRKGLS AOTYEE.....DQEAI	56
Ybr168pMDTNSKTKVQTENKKI KAKFI HN.....HGQKPS	31
ConsensusI	
YIPex23p	SSTPTPTVTKALAOAYFYI LASDKVLGLLTVTEDDQWOSFLLVAVYVTVVMYYEYLV	96
Ylr324p	TSPTPTI SKALVKLYFYLI LI DEF LNVTWTGKNI MSSVLMCLFI TTVEYFETLVKYF	129
Ygr004p	TSPTPTKTVSRSLVRLYFYLI VVDNFI S I TWSNDNVSANLLGI FLFTVCVLYFGFI	121
Ybr168p	QIPTPTM SSTIFHAYPLLLI FDNALANI MWSDDKCLTFI YLTSI WLTISFFI PV	96
Consensust p l y p d l w h	
YIPex23p	G.....FI WAFVYI RQSVERROTSEP SLDALVHTLTNVTTKANLLLLPI TSLSL	145
Ylr324p	A.....ILWGYSLLDNYI EGTLS SPTLEDI ALLMNRVSLKSDI LLSPMVNLG	177
Ygr004p	G.....I I WVYLLI DKHVQETNASCP SLDI I HVMDRVSMKSSAVLSPI TI LS	169
Ybr168p	TKI LRLWLG I S GSAFLF L S F M Y I V S L I A S L R D T E P P T L D E I V V L L E S V L D K L E V L R N E L N V W K K	161
Consensusp l i v k	
YIPex23p	TPRDVT.....RLAFTTLFLSPLLYMFGAYFFLGRKFLTTGVFFITVYHMAARVTRAVI	201
Ylr324p	.TODIQ.....RLLYTTVI LSP I YVM T W L L L P P R S L M L M V G M F L L T Y H S P M S K V A R R L L W	232
Ygr004p	.AQDVR.....RLLFIT I AFLSPVYI FLT V F V L S P N Y L M L I G G L Y V I T Y H S K L I R R M R R Y L W	224
Ybr168p	LKLSFDGVNKECSGKRLFCRLFLFGTI F Q I V I M R Y I S P G T Y T R F F I I T G L I Y N T S S F Q A T L R L L W	226
Consensusr l p l i y w	
YIPex23p	KSKAIRLVTFYLTGLDFSNTRNLGAF GFTOSPLSVQS.....KDGKPVRF TYVLYENORR	257
Ylr324p	KFKI RLLLVFYVTCLDLGGI NKDOGI F ATVOKQVKKLASTENS NGVLSDSKPI RF TYVLYENORR	297
Ygr004p	KFRV RLLLVFYI TGLDLGGP DNNRRL FASVNKKI RSFVWNEVGN. TSNTKKT VLFKVALFENORR	288
Ybr168p	RFTAVR...NFYYLGI E S F K I S S F L P K H L K M E Q I I P L S Q . G R A I T V P L V E V L P K L R D K K G D D H I H	288
Consensusr f g	
YIPex23p	WGI GWTANLLAYERTP WDEF LNEVTPPSEFKLPDTEGTGMKWVWDP TWRLDCTN. DCAI VI I	321
Ylr324p	WGI GWKPSMLS YERTP WDEF LNEAPSPENFHLPE. ETNTMVMRWVDKT WR L D M N . D C A I Q V P	360
Ygr004p	WGI GWTSTMLS YERAS WDEF LNTSPSEVFTLPE. EOSGM AEW H D K D W L D L T N . D C I I Q L P	351
Ybr168p	I L Q L L N E Q K D N F G N E D L K I L E I E V Y E N Q R R W Y Q N K . N W S T K L P Y E R Q N Y C I E I K N T D C T L T M R	352
ConsensusI n d g	
YIPex23p	GNKALSTP. DPSPSEGWY YDNTWKRPTADDSFSKYTRRRRWRTAELI TVTKPTDVVVTVEED.	384
Ylr324p	NSKARTSA. DPSPDEGFI YDNTWKKP SKEDSFSKYTRRRRWRTAELVKTSDFDDES VI NSNRNS	424
Ygr004p	ASAAKTKV. KP GADEGFI YDNTWNNP SATDTYKKYTRRRRW RTATVTTTYDDEPTVEKATPN.	414
Ybr168p	SCLPPDGLGEEELPNNWHWI NDNWDGTDWI YSDSAWKEI GOYSSLESFTRSRKWKRL FHL....	413
Consensusw	
YIPex23pGVTDAAG...DVEI I TTETEEK	403
Ylr324p	AI EOKVEENS TNGL TAEQELGS NKOEKDNAKVGEP TTEETKEF AEASNI NEGEFERI SSTDEEV	489
Ygr004p	SHALKSEEN.....NRVRK...RKV.....SFSTAN.....EVHI I PSSDSSK	449
Ybr168p	413
Consensus	
YIPex23p	VRRRKGI RFEEDS.....	416
Ylr324p	LKSRARDRLAKVLDDTEEKEQSNPTI GRDSKKA	522
Ygr004p	LI QI SDVSMSPSL.....	462
Ybr168p	413
Consensus	

Fig. S4 Sequence alignment of *Y. lipolytica* YIPex23p with the proteins Ylr324p, Ygr004p, and Ybr168p encoded by the *S. cerevisiae* genome. Amino acid sequences were aligned with the use of the DNAMAN program. Identical residues in four proteins are shaded black, whereas identical residues in at least three of the proteins are shaded pink, whereas identical residues in two proteins are shaded blue. Similarity rules: G = A = S; A = V; V = I = L = M; I = L = M = F = Y W; K = R = H; D = E = Q = N; and S = T = Q = N.

Table S1 Strains used in this study.

Strains	Genotype	Reference
Po1g <i>KU70</i> Δ	<i>MATA, xpr2-332, leu2-270, ku70⁻, ura3-302::URA3, Axp-2</i>	30
Po1g-GFP-ePTS1	Po1g <i>KU70</i> Δ, <i>GFP-ePTS1</i>	This study
Po1g KaBS	Po1g <i>KU70</i> Δ, <i>α-Bis</i>	4
Po1g P1	Po1g <i>KU70</i> Δ, <i>Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1</i>	This study
Po1g P2	Po1g P1, <i>ABC-G1</i>	This study
Po1g P3	Po1g P1, <i>ABC-G1-ePTS1</i>	This study
Po1g P4	Po1g P1, <i>ABC-G1, HMGR-ePTS1, α-Bis-ePTS1</i>	This study
Po1g P5	Po1g P1, <i>ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1</i>	This study
Po1g P6	<i>MATA, xpr2-332, leu2-270, ku70⁻, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, POX2</i>	This study
Po1g P7	<i>MATA, xpr2-332, leu2-270, ku70⁻, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, MFE1</i>	This study
Po1g P8	<i>MATA, xpr2-332, leu2-270, ku70⁻, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, POT1</i>	This study
Po1g P9	<i>MATA, xpr2-332, leu2-270, ku70⁻, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, MFE1, POT1</i>	This study

Po1g P10	<i>MATA, xpr2-332, leu2-270, ku70, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, MFE1, POT1, POX2</i>	This study
Po1g P11	<i>MATA, xpr2-332, leu2-270, ku70, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, MFE1, POT1, ANT1</i>	This study
Po1g P12	<i>MATA, xpr2-332, leu2-270, ku70, ura3-302::URA3, Axp-2, Erg10-ePTS1, Erg13-ePTS1, HMGR-ePTS1, Erg12-ePTS1, IDI-ePTS1, Erg8-ePTS1, Erg19-ePTS1, Erg20-ePTS1, α-Bis-ePTS1, ABC-G1-ePTS1, HMGR-ePTS1, α-Bis-ePTS1, MFE1, POT1, ANT1, ΔYIPex23p, GFP-ePTS1</i>	This study

Table S2 Plasmids used in this study.

Plasmids	Features	Reference
pYLEX1(leu2)	<i>Y. lipolytica-integrative plasmid, P_{hp4d}-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-hyg	<i>pYLEX1 variant with the hyg marker</i>	This study
pYLEX1-nat	<i>pYLEX1 variant with the nat marker</i>	This study
pYLEX1-GFP-ePTS1	<i>P_{hp4d}-GFP-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1- α -Bis	<i>P_{hp4d}-α-Bis-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1- α -Bis- ePTS1	<i>P_{hp4d}-α-Bis-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg10-ePTS1	<i>P_{hp4d}-Erg10-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg13-ePTS1	<i>P_{hp4d}-Erg13-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-HMGR-ePTS1	<i>P_{hp4d}-HMGR-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg12-ePTS1	<i>P_{hp4d}-Erg12-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg8-ePTS1	<i>P_{hp4d}-Erg8-ePTS1-T_{XPR2}, leu2, leu2, Ap^r</i>	This study
pYLEX1-Erg19-ePTS1	<i>P_{hp4d}-Erg19-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-IDI-ePTS1	<i>P_{hp4d}-IDI-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg20-ePTS1	<i>P_{hp4d}-Erg20-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-POX2	<i>P_{hp4d}-POX2-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-MFE1	<i>P_{hp4d}-MFE1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-POT1	<i>P_{hp4d}-POT1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-ANT1	<i>P_{hp4d}-ANT1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg10-Erg13- ePTS1	<i>P_{hp4d}-Erg10-ePTS1-T_{XPR2}, P_{hp4d}-Erg13-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg10-Erg13- HMGR-ePTS1	<i>P_{hp4d}-Erg10-ePTS1-T_{XPR2}, P_{hp4d}-Erg13-ePTS1-T_{XPR2}, P_{hp4d}-HMGR-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg10-Erg13- HMGR-Erg12-ePTS1	<i>P_{hp4d}-Erg10-ePTS1-T_{XPR2}, P_{hp4d}-Erg13-ePTS1-T_{XPR2}, P_{hp4d}-HMGR-ePTS1-T_{XPR2}, P_{hp4d}-Erg12-ePTS1-T_{XPR2}, leu2, Ap^r</i>	This study
pYLEX1-Erg10-Erg13-	<i>P_{hp4d}-Erg10-ePTS1-T_{XPR2}, P_{hp4d}-Erg13-ePTS1-T_{XPR2}, P_{hp4d}-HMGR-ePTS1-T_{XPR2}, P_{hp4d}-Erg12-ePTS1-T_{XPR2},</i>	This study

HMGR-Erg12-Erg8-ePTS1	$P_{hp4d}\text{-Erg8-ePTS1-}T_{XPR2}, leu2, Ap^r$	
pYLEX1-Erg10-Erg13- HMGR-Erg12-Erg8-Erg19- α -Bis-ePTS1	$P_{hp4d}\text{-Erg10-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg13-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-HMGR-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg12-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-Erg8-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg19-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-}\alpha\text{-Bis-ePTS1-}T_{XPR2}, leu2, Ap^r$	This study
pYLEX1-hyg-IDI-ePTS1	$P_{hp4d}\text{-IDI-ePTS1-}T_{XPR2}, hyg, Ap^r$	This study
pYLEX1-hyg-IDI-Erg20- ePTS1	$P_{hp4d}\text{-IDI-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg20-ePTS1-}T_{XPR2}, hyg,$ Ap^r	This study
pYLEX1-hyg-IDI-Erg20- ePTS1-ABC-G1	$P_{hp4d}\text{-IDI-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg20-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-ABC-G1-TXPR2}, hyg, Ap^r$	This study
pYLEX1-hyg-IDI-Erg20- ePTS1-ABC-G1-ePTS1	$P_{hp4d}\text{-IDI-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg20-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-ABC-G1-ePTS1-}T_{XPR2}, hyg, Ap^r$	This study
pYLEX1-hyg-IDI-Erg20- HMGR- α -Bis-ePTS1-ABC- G1	$P_{hp4d}\text{-IDI-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg20-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-HMGR-ePTS1-}T_{XPR2}, P_{hp4d}\text{-}\alpha\text{-Bis-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-ABC-G1-TXPR2}, hyg, Ap^r$	This study
pYLEX1-hyg-IDI-Erg20- HMGR- α -Bis-ABC-G1- ePTS1	$P_{hp4d}\text{-IDI-ePTS1-}T_{XPR2}, P_{hp4d}\text{-Erg20-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-HMGR-ePTS1-}T_{XPR2}, P_{hp4d}\text{-}\alpha\text{-Bis-ePTS1-}T_{XPR2},$ $P_{hp4d}\text{-ABC-G1-ePTS1-}T_{XPR2}, hyg, Ap^r$	This study
pYLEX1-nat-POX2	$P_{hp4d}\text{-POX2-}T_{XPR2}, nat, Ap^r$	This study
pYLEX1-nat-MFE1	$P_{hp4d}\text{-MFE1-}T_{XPR2}, nat, Ap^r$	This study
pYLEX1-nat-POT1	$P_{hp4d}\text{-POT1-}T_{XPR2}, nat, Ap^r$	This study
pYLEX1-nat-MFE1-POT1	$P_{hp4d}\text{-MFE1-}T_{XPR2}, P_{hp4d}\text{-POT1-}T_{XPR2}, nat, Ap^r$	This study
pYLEX1-nat-MFE1-POT1- POX2	$P_{hp4d}\text{-MFE1-}T_{XPR2}, P_{hp4d}\text{-POT1-}T_{XPR2}, P_{hp4d}\text{-POX2-}$ T_{XPR2}, nat, Ap^r	This study
pYLEX1-nat-MFE1-POT1- ANT1	$P_{hp4d}\text{-MFE1-}T_{XPR2}, P_{hp4d}\text{-POT1-}T_{XPR2}, P_{hp4d}\text{-ANT1-}$ T_{XPR2}, nat, Ap^r	This study

Table S3 Primers used in PCR.

Applications	Primer	Sequence (5'-3')
Amplify <i>GFP-ePTS1</i> gene from plasmid pYLEX1- <i>GFP-ePTS1</i> , forward primer	<i>Amplify GFP-ePTS1 gene from plasmid pYLEX1-GFP-ePTS1, F</i>	CCACACACATCCACaGGTACCaATGCTTTCACTAC GTCAATCTATAAGATTTTTCAAGCCAGCCACAAG AACTTTGTGTAGCTCTAGATATCTGCTTCAGggtacc atggtgtctaagcag
Amplify <i>GFP-ePTS1</i> gene from plasmid pYLEX1- <i>GFP-ePTS1</i> , reverse primer	<i>Amplify GFP-ePTS1 gene from plasmid pYLEX1-GFP-ePTS1, R</i>	ttagtttcgggtcccacgtgTTAGTGGTGGTGGTGGTGGTG
Amplify <i>Erg10</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>Erg10-ePTS1-F</i>	actttggtctactcgggtaccAATGGAGCCCGTCTACATTGTT
Amplify <i>Erg10</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>Erg10-ePTS1-R</i>	GGGACAGGCCATGGAGctacagcttgatcgctcctcgccca gacacttctcaacaatgatag
Amplify <i>HMGR</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>HMGR-ePTS1-F</i>	acaaccacacacatccacAATGCTACAAGCAGCTATTGGAA A
Amplify <i>HMGR</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>HMGR-ePTS1-R</i>	TTAGTTTCGGGTTCCCACctacagcttgatcgctcctcgccc agtgaccgtatgcaaatatcgc
Amplify <i>Erg13</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>Erg13-ePTS1-F</i>	acaaccacacacatccacgtgAATGTCGCAACCCGAGAACG
Amplify <i>Erg13</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>Erg13-ePTS1-R</i>	TTAGTTTCGGGTTCCgtgctacagcttgatcgctcctcgccca gctgcttgatctcgtactttc
Amplify <i>Erg12</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>Erg12-ePTS1-F</i>	ggaaccgaaactaaggatcAATGGACTACATCATTTTCGGC G
Amplify <i>Erg12</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>Erg12-ePTS1-R</i>	ACAAGTTCCGTAGTTGctacagcttgatcgctcctcgcccag atgggtccagggaccgat
Amplify <i>IDI</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>IDI-ePTS1-F</i>	actttggtctactccgAATGCTACTTGATCCACCGCC
Amplify <i>IDI</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>IDI-ePTS1-R</i>	GGGACAGGCCATGGAGGTACCTAcagcttgatcgctcctc tcggcccagatgacgacgtcttacgc
Amplify <i>α-Bis</i> gene from plasmid	<i>α-bisabolene-F</i>	cgcatagttaagccagtaGGGAGCTGCATGTGTTCAGAGG

pYLEX1- α -Bis, forward primer		
Amplify α -Bis gene from plasmid pYLEX1- α -Bis, reverse primer	<i>α-bisabolene-R</i>	tagcgatagcggagtgaCGCGCCGCATAGGCCAG
Amplify α -Bis gene from plasmid pYLEX1- α -Bis, forward primer	<i>α-bisabolene-ePTS1-F</i>	acaaccacacacatccacgtgAATGGCCGGTGTCTCTGCC
Amplify α -Bis gene from plasmid pYLEX1- α -Bis, reverse primer	<i>α-bisabolene-ePTS1-R</i>	TTAGTTTCGGGTTCCCACTTAcagcttgatcgtcgtcctcgcc ccagGTGGTGATGGTGGTGGTGGAG
Amplify <i>Erg19</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>Erg19-ePTS1-F</i>	acaaccacacacatccacgtgAATGATCCACCAGGCCTCCA
Amplify <i>Erg19</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>Erg19-ePTS1-R</i>	TTAGTTTCGGGTTCCgtgctacagcttgatcgtcgtcctcgccca gcttgcgttcttcagagaac
Amplify <i>Erg20</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>Erg20-ePTS1-F</i>	acaaccacacacatccacgtgAATGTCCAAGGCGAAATTCG
Amplify <i>Erg20</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>Erg20-ePTS1-R</i>	TTAGTTTCGGGTTCCgtgctacagcttgatcgtcgtcctcgccca gcttctgcgttgaatct
Amplify <i>Erg8</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>Erg8-ePTS1-F</i>	acaaccacacacatccacgtgAATGCTACTTGAACCCCTTCT CG
Amplify <i>Erg8</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>Erg8-ePTS1-R</i>	TTAGTTTCGGGTTCCgtgCTAcagcttgatcgtcgtcctcgccca agatgaccacctattcgctcc
Amplify <i>POX2</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>POX2-F</i>	acaaccacacacatccacAATGAACCCCAACAACACTGGC
Amplify <i>POX2</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>POX2-R</i>	ttagtttcgggtcccacgtgCTATTCCTCATCAAGCTCGCAA
Amplify <i>MFE1</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>MFE1-F</i>	actttggtactccggtacAATGTCTGGAGA ACTAAGATAC GACGG
Amplify <i>MFE1</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>MFE1-R</i>	gggacaggccatggaggtaccTTAGAGCTTAGCATCCTTGGG G
Amplify <i>POT1</i> gene form <i>Y. lipolytica</i> genomic DNA, forward primer	<i>POT1-F</i>	actttggtactccggtacATGGAGCCCGTCTACATTGTTT
Amplify <i>POT1</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>POT1-R</i>	gggacaggccatggaggtaccCTAACACTTCTCAACAATGAT AGAGGAA
Amplify <i>ANTI</i> gene form <i>Y. lipolytica</i>	<i>ANTI-F</i>	actttggtactccgAATGGCAGCTATTTCCAAAGACTAT

genomic DNA, forward primer		G
Amplify <i>ANT1</i> gene form <i>Y. lipolytica</i> genomic DNA, reverse primer	<i>ANT1-R</i>	gggacaggccatggaggtacTTATCCCTTGATCAAGGTGGG G
Amplify the gene expression cassette, forward primer	<i>PshAI-F</i>	agtcataagtgcggcgacgaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>PshA I-R</i>	gtgggcgcggggcatgactaGATAAGCTGTCAAACATGAGA ATTTCG
Amplify the gene expression cassette, forward primer	<i>FspA I-F</i>	gacaggagcacgatcatcgAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>FspA I-R</i>	gggtcctggccacgggtcgcATAAGCTGTCAAACATGAGAA TTCG
Amplify the gene expression cassette, forward primer	<i>BsaWI-F</i>	atcctgcgatgcagatccggaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>BsaWI-R</i>	cctgcaccattatgttccggGATAAGCTGTCAAACATGAGAA TTCG
Amplify the gene expression cassette, forward primer	<i>Nru I-F</i>	ccatccagcctcgcgtcgcgaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Nru I-R</i>	acgtcttgctggcgttcgGATAAGCTGTCAAACATGAGAAT TCG
Amplify the gene expression cassette, forward primer	<i>Sal I-F</i>	ctctcaaggcctcggtcgacGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Sal I-R</i>	cgcataagggagagcgtcgaGATAAGCTGTCAAACATGAGA ATTTCG
Amplify the gene expression cassette, forward primer	<i>EcoNI-F</i>	ctcccttatgcgactcctgcaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>EcoNI-R</i>	actactgggctgcttctaaGATAAGCTGTCAAACATGAGAA TTCG
Amplify the gene expression cassette, forward primer	<i>BstZ17 I-F</i>	cgcatagtaagccagtaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>BstZ17 I-R</i>	tagcgatagcggagtgaGATAAGCTGTCAAACATGAGAAT TCG

Amplify the gene expression cassette, forward primer	<i>Spe I-F</i>	cgaggcagcagatccactagGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Spe I-R</i>	gggccgcataggccaGATAAGCTGTCAAACATGAGAAT TCG
Amplify the gene expression cassette, forward primer	<i>Not I-F</i>	cgcatagttaagccagtaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Not I-F</i>	tagcgatagcggagtgtgGATAAGCTGTCAAACATGAGAAT TCG
Amplify the gene expression cassette, forward primer	<i>Aan I-F</i>	taatgaaatgatgatttaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Aan I-R</i>	gggacaggccatggaggtacTTATCCCTTGATCAAGGTGGG G
Amplify the gene expression cassette, forward primer	<i>FspA I-IDI-F</i>	gacaggagcacgatcatcgAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>FspA I-IDI-R</i>	gggtcctggccacgggtgcgATAAGCTGTCAAACATGAGAA TTCG
Amplify the gene expression cassette, forward primer	<i>Nru I-Erg20-F</i>	ccatccagcctcgcgtcggaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Nru I-Erg20-R</i>	acgtcttgctggcgttcgGATAAGCTGTCAAACATGAGAAT TCG
Amplify the gene expression cassette, forward primer	<i>EcoN I-ABC-G1- ePTS1-F</i>	ctcccttatgcgactcctgcaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>EcoN I-ABC-G1- ePTS1-R</i>	actactgggctgcttctaaGATAAGCTGTCAAACATGAGAA TTCG
Amplify the gene expression cassette, forward primer	<i>BstZ17 I-α- bisabolene-ePTS1- F</i>	cgcatagttaagccagtaGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>BstZ17 I-α- bisabolene-ePTS1- R</i>	tagcgatagcggagtgtgGATAAGCTGTCAAACATGAGAAT TCG
Amplify the gene expression cassette, forward primer	<i>FspA I-MFE1-F</i>	gacaggagcacgatcatcgAGGCCGTTGAGCACCGC

Amplify the gene expression cassette, reverse primer	<i>FspA I-MFE1-R</i>	gggtcctggccacgggtcgcATAAGCTGTCAAACATGAGAA TTCG
Amplify the gene expression cassette, forward primer	<i>Sal I-POT1-F</i>	ctctcaagggcatcggGAGGCCGTTGAGCACCGC
Amplify the gene expression cassette, reverse primer	<i>Sal I-POT1-R</i>	cgcataagggagagcgtcgaGATAAGCTGTCAAACATGAGA ATTCG
