

## SUPPLEMENTARY INFORMATION

### **Engineering yeast for production of plant terpenoids using synthetic biology approaches**

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## SUPPLEMENTARY TABLES

**Table S1: Reports on engineering approaches for production of plant terpenoids.**

<b>Production Platform</b>	<b>Biosynthetic pathway</b>	<b>Target Terpenoid</b>	<b>Titer</b>	<b>Ref.</b>
<i>Salvia miltiorrhiza</i>	Hairy roots cultures	tanshinones	2.51-2.89-fold increase of major tanshinones	<sup>1</sup>
<i>Centella asiatica</i>	Cell suspensions derived from callus cultures	centellosides, madecassoside, asiaticoside	900 µg/g DW	<sup>2</sup>
<i>Nicotiana benthamiana</i>	Heterologous expression of lipid transfer proteins	artemisinin	3 µg/g DW	<sup>3</sup>
<i>N. benthamiana</i>	Organelle compartmentalization	geraniol	129 µg/g FW	<sup>4</sup>
<i>N. benthamiana</i>	Metabolic pathway shunting	taxa-4(5),11(12)-diene	48 µg/g DW	<sup>5</sup>
<i>Physcomitrella patens</i>	Heterologous expression	taxa-4(5),11(12)-diene	0.05% fresh weight of tissue	<sup>6</sup>
<i>Chlamydomonas reinhardtii</i>	Redirecting the metabolic flux through the MEP pathway	sclareol	up to 656 mg L <sup>-1</sup> with a maximal productivity of 78 mg L <sup>-1</sup> day <sup>-1</sup>	<sup>7</sup>
<i>E. coli</i>	Multivariate-modular approach	taxa-4(5),11(12)-diene	1.02 g L <sup>-1</sup>	<sup>8</sup>
<i>E. coli</i>	CRISPR/Cas9 mediated genome integration	β-carotene	2.0 g L <sup>-1</sup>	<sup>9</sup>
<i>in vitro</i> platform	Synthetic crotonyl-coenzyme (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA cycle (CETCH)	limonene farnesene	0.22 mg L <sup>-1</sup> h <sup>-1</sup>	<sup>10</sup>
<i>in vitro</i> platform	cell-free system	cannabinoids cannabigerolic acid (CBGA)	~0.5 g L <sup>-1</sup>	<sup>11</sup>

**Table S2: Overview of heterologous biosynthetic pathway expression for sesquiterpenoid production in *S. cerevisiae*.** Examples of terpenoids produced by industrial fermentation are denoted with \*.

Product	Biosynthetic pathway	Approach	Titer	Ref.
nerolidol	Amorpha-4,11-diene synthase (ADS) from <i>Artemisia annua</i>	Compartmentalization in the mitochondria	427 mg L <sup>-1</sup>	<sup>12</sup>
nerolidol	Amorpha-4,11-diene synthase (ADS) and Cytochrome P450 monooxygenase (CYP71AV1) from <i>Artemisia annua</i>	Multiple metabolic engineering approaches	25 g L <sup>-1</sup>	<sup>13</sup>
$\alpha$ -santalene	Santalene synthase (SaSSy) from <i>Santalum album</i> and SanSyn from <i>Clausena lansium</i>	SanSyn (F441V), deletion of the yeast transcriptional repressor ROX1 and knockout of the genes encoding the enzymes involved in farnesyl diphosphate (FPP) consumption	842 mg L <sup>-1</sup>	<sup>14</sup>
santalol	Santalene synthase (SaSSy) from <i>Santalum album</i> and SanSyn from <i>Clausena lansium</i> , and CYP736A167 from <i>S. album</i>	SanSyn (F441V), deletion of the yeast transcriptional repressor ROX1 and knockout of the genes encoding the enzymes involved in farnesyl diphosphate (FPP) consumption	1.07 g L <sup>-1</sup>	<sup>14</sup>
<i>trans</i> -caryophyllene	<i>trans</i> -caryophyllene synthase from <i>S. fruticosa</i>	Tandem heterozygous deletions positive genetic interactors of HMG2	125 mg L <sup>-1</sup>	<sup>15</sup>
$\beta$ -farnesene*	$\beta$ -farnesene synthase (FS) from <i>Artemisia apiacea</i>	Genome scale modification to reprogram yeast central metabolism	130 g L <sup>-1</sup>	<sup>16</sup>
patchoulol	Patchoulol synthase (PTS) from <i>Pogostemon cablin</i>	Fusion of farnesyl diphosphate synthase (FPPS) and PTS in ERG9-repressed strain	23 mg L <sup>-1</sup>	<sup>17</sup>
kauniolide	Germacrene A (GAS), costunolide (COS), kauniolide (KAS) synthases, germacrene A oxidase (GAO)	Multigene Crispr/Cas9 mediated chromosomal integration	nd	<sup>18</sup>
<i>trans</i> -nerolidol	<i>trans</i> -nerolidol synthase (NES1) from <i>Actinidia chinensis</i>	Chromosomal gene amplification driven by gen haploinsufficiency	~2 g L <sup>-1</sup>	<sup>19</sup>
<i>trans</i> -nerolidol	<i>trans</i> -nerolidol synthase (NES1) from <i>Actinidia chinensis</i>	PEST sequence-dependent endoplasmic reticulum-associated protein degradation (ERAD) system established for Erg9p	~100 mg L <sup>-1</sup>	<sup>20</sup>

**Table S3: Overview of heterologous biosynthetic pathway expression for monoterpene production in yeast.** The majority of case studies listed below are engineered in *S. cerevisiae* terpene-producing platforms. Examples of terpenoids produced in *Y. lipolytica* are marked with \*, while in *R. toruloides* are shown with \*\*.

Product	Biosynthetic pathway	Approach	Titer	Ref.
limonene	Limonene synthase from <i>Citrus limon</i> (CILimS)	Orthogonal pathway using NPP as isomeric alternative substrate	166 mg L <sup>-1</sup> in semi-fed batch 917 mg L <sup>-1</sup> in fed batch	<sup>21</sup> <sup>22</sup>
limonene	Limonene synthase from <i>Citrus limon</i> (CILimS)	Compartmentalization in peroxisomes	2.6 g L <sup>-1</sup> in semi-fed batch	<sup>23</sup>
limonene*	Limonene synthase from <i>Citrus limon</i> (CILimS)	Overexpressing YALI0F19492p	5.11 mg L <sup>-1</sup>	<sup>24</sup>
limonene**	Limonene synthase from <i>Citrus limon</i> (CILimS)	Fusion of CILimS with <i>cis</i> prenyl transferase NPPS from <i>Solanum lycopersicum</i>	15.45 g L <sup>-1</sup>	<sup>25</sup>
sabinene	Sabinene synthase from <i>Salvia pomifera</i> (SpSabS)	Orthogonal pathway using NPP as isomeric alternative substrate	113 mg L <sup>-1</sup> in semi-fed batch	<sup>21</sup>
<i>trans</i> -sabinol	Sabinene synthase from <i>Salvia pomifera</i> (SpSabS), and Sabinene hydroxylase CYP750B1 from <i>Thuja plicata</i>	Orthogonal pathway using NPP as isomeric alternative substrate	nd	<sup>21</sup>
$\alpha$ -pinene	$\alpha$ -pinene synthase from <i>Pinus taeda</i> (PtPinS)	Orthogonal pathway using NPP as isomeric alternative substrate	nd	<sup>21</sup>
1,8-cineole	1,8-cineole synthase from <i>Salvia fruticosa</i>	Orthogonal pathway using NPP as isomeric alternative substrate	nd	<sup>21</sup>
geraniol	Geraniol synthase from <i>Ocimum basilicum</i>	Compartmentalization in peroxisomes	5.52 g L <sup>-1</sup> in semi-fed batch	<sup>23</sup>
linalool	Linalool synthase from diterpene <i>Mentha citrate</i> (McLIS)	Iterative design–build–test–learn engineering cycle	~0.3 mg L <sup>-1</sup>	<sup>26</sup>

**Table S4: Overview of heterologous expression of terpene synthase and decoration enzymes for diterpenoid production in *S. cerevisiae*.**

Product	Terpene synthase	Decoration enzyme	Approach	Titer	Ref.
miltiradiene	Labdadienyl/copalyl diphosphate synthase ( <i>SmCPS</i> ) and kaurene synthase-like ( <i>SmKSL</i> ) from <i>Salvia miltiorrhiza</i>		Fusion of <i>SmCPS</i> and <i>SmKSL</i> and fusion of <i>Bts1p</i> and <i>Erg20p</i>	365 mg L <sup>-1</sup>	<sup>27</sup>
sclareol	Class II diterpene synthase and sclareol synthase from <i>Salvia sclarea</i>		Global metabolic rewiring	11.4 g L <sup>-1</sup> in fed-batch fermentation	<sup>28</sup>
manool	Copalyl diphosphate synthase from <i>S. fruticosa</i> and sclareol synthase from <i>S. sclarea</i>		Combinatorial biosynthesis	96 mg L <sup>-1</sup>	<sup>29</sup>
manoyl oxide	8-hydroxy copalyl diphosphate synthase ( <i>CcCLS</i> ) from <i>Cistus creticus</i> and miltiradiene synthase from <i>S. pomifera</i> ( <i>SpMILS</i> )		Combinatorial biosynthesis	36 mg L <sup>-1</sup>	<sup>29</sup>
ferruginol	Copalyl diphosphate synthase from <i>S. fruticosa</i> and miltiradiene synthase from <i>S. pomifera</i> ( <i>SpMILS</i> )	CPR/CYP76AH24 from <i>Salvia pomifera</i>	Gene mining, pathway elucidation	21.2 mg L <sup>-1</sup>	<sup>27</sup>
carnosic acid	Copalyl diphosphate synthase from <i>S. fruticosa</i> and miltiradiene synthase from <i>S. pomifera</i> ( <i>SpMILS</i> )	CPR/CYP76AH24 and CYP76AK6 from <i>S. pomifera</i>	Gene mining, pathway elucidation	1 mg L <sup>-1</sup>	<sup>27</sup>
pisiferic acid	Copalyl diphosphate synthase from <i>S. fruticosa</i> and miltiradiene synthase from <i>S. pomifera</i> ( <i>SpMILS</i> )	Cytochrome P450s from <i>S. pomifera</i> CYP76AH24 (F112L) and CYP76AK6	Rational mutagenesis of CYP76AH24	2.65 mg L <sup>-1</sup>	<sup>30</sup>
salviol	Copalyl diphosphate synthase from <i>S. fruticosa</i> and miltiradiene synthase from <i>S. pomifera</i> ( <i>SpMILS</i> )	Cytochrome P450s from <i>S. pomifera</i> CYP76AH24 (F112L) and CYP71BE52	Rational mutagenesis of CYP76AH24	mg L <sup>-1</sup>	<sup>30</sup>
forskolin	CfTPS2 and CfTPS3 from <i>Coleus forskohlii</i>	NADPH-dependent cytochrome P450 oxidoreductase (CfPOR), CfCYP76AH15, CfCYP76AH11, CfCYP76AH16 and CfACT1-8	Pathway elucidation	40 mg L <sup>-1</sup>	<sup>31</sup>
casbene	Geranylgeranyl diphosphate synthase from <i>Phomopsis</i>		Ergosterol and glucose-	108.5 mg L <sup>-1</sup>	<sup>32</sup>

	<i>amygdali</i> (PaGGPPS) and casbene synthase from <i>Ricinus communis</i> (RcCBS)		sensitive promoter-controlled expression of ERG1, ERG9 and ERG20		
taxadiene	Taxadiene synthase (TASY) from <i>Taxus cuspidata</i>		Multi-chromosomal integrations and improved enzyme solubility	129 mg L <sup>-1</sup> in shake-flask fermentation	<sup>33</sup>
taxadien-5 $\alpha$ -ol	Taxadiene synthase (TASY) from <i>Taxus cuspidata</i>	Taxadiene-5 $\alpha$ -hydroxylase (CYP725A4) from <i>Taxus cuspidata</i>	Optimization of substrate channeling through heterologous pathway	10.6 mg L <sup>-1</sup>	<sup>34</sup> <sup>35</sup>
taxa-4(20),11(12)-dien-5 $\alpha$ -acetoxy-10 $\beta$ -ol (putative)	Taxadiene synthase from <i>T. brevifolia</i>	Taxadiene 5 $\alpha$ -hydroxylase, Taxadien-5 $\alpha$ -ol acetyltransferase (TAT) and taxane 10 $\beta$ -hydroxylase (10 $\beta$ CYP) from <i>Taxus cuspidata</i>	<i>E. coli</i> and <i>S. cerevisiae</i> co-culture system	1 mg L <sup>-1</sup>	<sup>36</sup>
triptonide	Miltiradiene biosynthetic pathway	CYP82D213, CYP82D274, CYP71BE86 and CYP71BE85 from <i>T. willfordii</i>	Pathway reconstructon	3.79 mg L <sup>-1</sup>	<sup>37</sup>

**Table S5: Overview of heterologous expression of terpene synthase and decoration enzymes for triterpenoid production in yeast.** Most case studies listed below are engineered in *S. cerevisiae* terpene-producing platforms. Examples of terpenoids produced in *Y. lipolytica* are marked with \*.

Product	Terpene synthase	Decoration enzyme	Approach	Titer	Ref.
$\beta$ -amyrin	$\beta$ -amyrin synthase (SAD1) from <i>Avena strigosa</i>		Site directed mutagenesis of SAD1	nd	<sup>38</sup>
friedelin	Oxidosqualene cyclase (OSC4) from <i>T. willfordii</i>		Deletion of ubiquitin conjugating enzyme UBC7	0.5 mg L <sup>-1</sup>	<sup>39</sup>
polpunic acid	Oxidosqualene cyclase (OSC4) from <i>T. willfordii</i>	CYP712K1 from <i>T. willfordii</i>	Deletion of ubiquitin conjugating enzyme UBC7	1.4 mg L <sup>-1</sup>	<sup>39</sup>
protopanaxadiol	Dammarenediol-II synthase (DDS) from <i>Panax ginseng</i>	Protopanaxadiol synthase (PPDS) from <i>P. ginseng</i>	Upstream pathway optimization	1189 mg L <sup>-1</sup> by two-phase fermentation	<sup>40</sup>
betulinic acid*	Lupeol synthase from <i>R. communis</i> (RcLus)	Lupeol C-28 oxidase <i>BPLO</i> (CYP716A180) from <i>Betula platyphylla</i>	Upstream pathway optimization and improved CYP/CPR interactions in <i>Y. lipolytica</i>	51.87 mg L <sup>-1</sup>	<sup>41</sup>
lupeol	Lupeol synthase from <i>Taraxacum koksaghyz</i>		Enhanced metabolic flux through MVA pathway	0.16 mg g <sup>-1</sup> CDW	<sup>42</sup>
ginsenosides Rg3	Dammarenediol-II synthase (DDS) from <i>P. ginseng</i>	Protopanaxadiol synthase (PPDS) and <i>PgUGT74AE2</i> and <i>PgUGT94Q2</i> from <i>P. ginseng</i>	Pathway discovery	1.3 mg L <sup>-1</sup>	<sup>43</sup>
ginsenosides Rd	Dammarenediol-II synthase (DDS) from <i>P. ginseng</i>	Protopanaxadiol synthase (PPDS) and <i>PgUGT71A27</i> , <i>PgUGT74AE2</i> and <i>PgUGT94Q2</i> from <i>P. ginseng</i>	Pathway discovery	nd	<sup>43</sup>
ginsenosides Rh1 and F1	Dammarenediol-II synthase (DDS) from <i>P. ginseng</i>	Protopanaxadiol synthase (PPDS) and <i>PgUGTPg100</i> from <i>P. ginseng</i>		92.8 mg L <sup>-1</sup> , 42.1 mg L <sup>-1</sup> , respectively	<sup>44</sup>
ginsenosides Rh2	Dammarenediol-II synthase (DDS) from <i>Panax ginseng</i>	Protopanaxadiol synthase (PPDS) from <i>P. ginseng</i> and <i>UGTPn50</i> from <i>P. notoginseng</i>	Directed evolution of <i>UGTPn50</i> from <i>P. notoginseng</i>	2.25 g L <sup>-1</sup> in fed-batch fermentation	<sup>45</sup>
ginsenosides	Dammarenediol-II	Protopanaxadiol	Pathway Optimization	1.3 g L <sup>-1</sup>	<sup>46</sup>

Rg2	synthase (DDS) from <i>Panax ginseng</i>	synthase (PPDS), CYP716A53v2 and <i>PgUGT71A54</i>			
ginsenosides Re	Dammarenediol-II synthase (DDS) from <i>Panax ginseng</i>	protopanaxadiol synthase (PPDS), CYP716A53v2 and <i>PgUGT71A53</i> , <i>PgUGT71A54</i>	Pathway Optimization	3.6 g L <sup>-1</sup>	<sup>46</sup>

**Table S6: Overview of heterologous expression of terpene synthase and decoration enzymes for carotenoid production in yeast.** Most case studies listed below are engineered in *S. cerevisiae* terpene-producing platforms. Examples of terpenoids produced in *Y. lipolytica* are marked with \*.

Product	Terpene synthase	Decoration enzyme	Approach	Titer	Ref.
lycopene	Phytoene synthase (CrtB)	Phytoene desaturase (CrtI)	Improve lipid oil-triacylglycerol (TAG) metabolism	2.37 g L <sup>-1</sup> in fed-batch fermentation	<sup>47</sup>
β-carotene	Phytoene synthase/ lycopene cyclase (CrtYB)	Phytoene desaturase (CrtI)	Enzyme fusion strategy creating tridomain CrtYBI enzyme	~3 mg L <sup>-1</sup> DCW	<sup>48</sup>
β-carotene*	Phytoene synthase/ lycopene cyclase (CrtYB)	Phytoene desaturase (CrtI), β-carotene ketolase (CrtW), β-carotene hydroxylase (CrtZ), β-carotene ketolase (OBKT)	Overexpressing of GGPP synthase, ERG13, and HMG	4.5 g L <sup>-1</sup> in fermentation	<sup>49</sup>
astaxanthin	Phytoene synthase/ lycopene cyclase (CrtYB)	Phytoene desaturase (CrtI), β-carotene ketolase (CrtW), β-carotene hydroxylase (CrtZ), β-carotene ketolase (OBKT)	Rational mutagenesis of β-carotene ketolase, overexpression of CrtE03M, tHMG1, CrtI and CrtYB	47.18 mg L <sup>-1</sup>	<sup>50</sup>
zeaxanthin	GGPP synthase (CrtE), phytoene synthase (CrtB) lycopene cyclase (CrtY)	Phytoene desaturase (CrtI)	Combined ligase cycling reaction with DNA assembler for larger plasmid assembly <i>in vivo</i>	2.4 mg L <sup>-1</sup>	<sup>51</sup>
canthaxanthin*	Phytoene synthase/ lycopene cyclase (carRP)	Phytoene dehydrogenase (carB) and β-carotene/zeaxanthin 4-ketolase (CrtW)	Chromosomal expression of β-carotene biosynthetic pathway	36.1 mg L <sup>-1</sup>	<sup>52</sup>



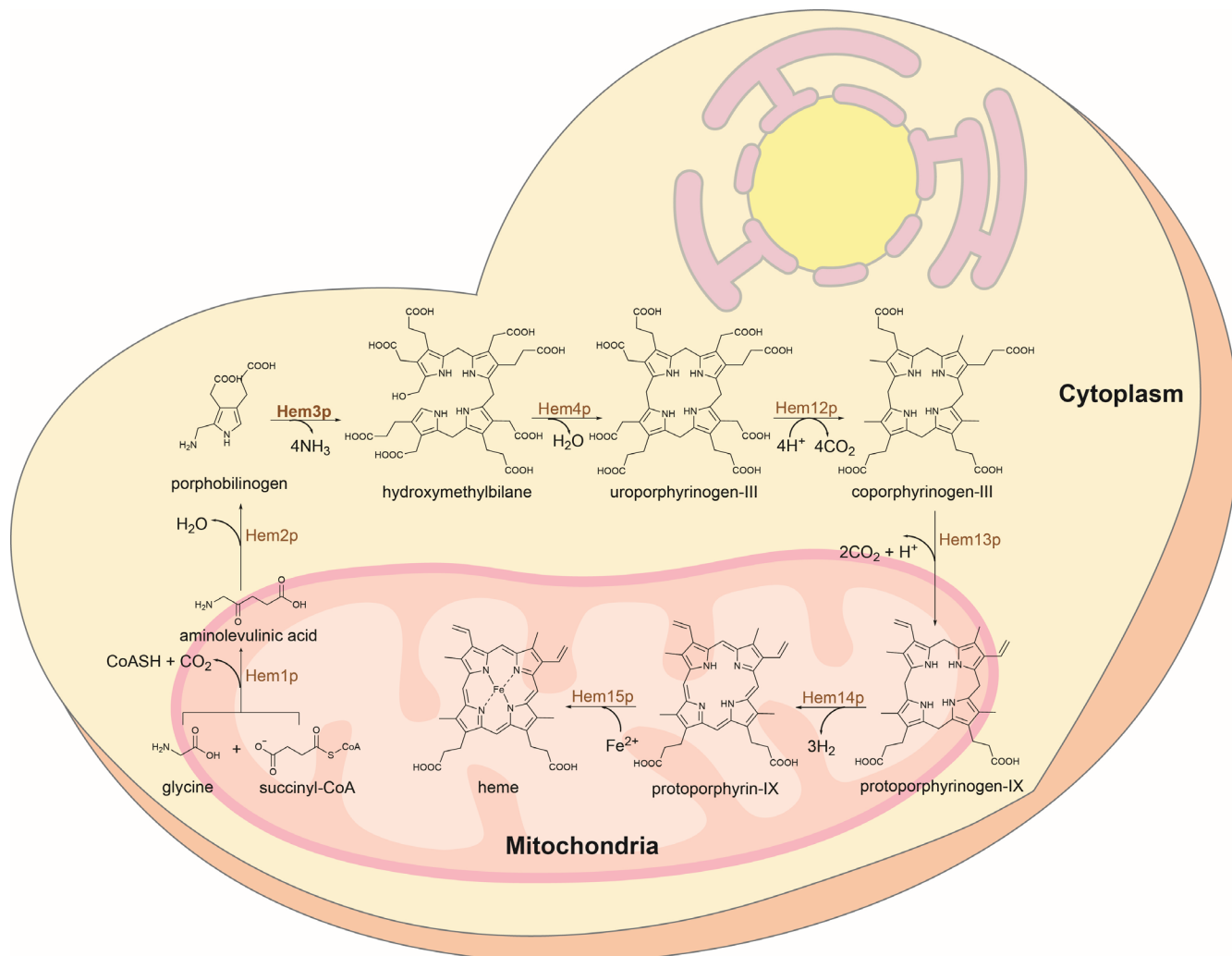
**Table S7: Overview of strategies for engineering biosynthetic enzymes involved in terpene upstream pathway.**

Wild-type enzyme	Protein engineering strategy	Engineered mutations	Engineered activity	Ref.
Farnesyl pyrophosphate synthetase (Erg20p)	Site-directed mutagenesis	F96W-N127W	Dominant negative GPP synthase 343-fold improvement in sabinene production	53
Farnesyl pyrophosphate synthetase (Erg20p)	Site-directed mutagenesis	F96C	GGPP synthase 70-fold improvement in sclareol production	54
Farnesyl pyrophosphate synthetase (Erg20p)	Degradation signal addition	N-terminal degron K3K15 addition	Decreased half-life 27-fold improvement in monoterpene production	55
<i>Citrus lemon</i> Limonene synthase (C <i>LimS</i> )	Site-directed mutagenesis	H570F or Y	NPP dedicated variant Shift substrate preference from GPP to NPP	21
<i>S. pomifera</i> Sabinene synthase (S <i>pSabS</i> )	Site-directed mutagenesis	H561F	NPP dedicated variant. 7-fold increase in sabinene titer	21
<i>Citrus lemon</i> Limonene synthase (C <i>LimS</i> )	Site-directed mutagenesis	H570V, L or I	Noncanonical C11 variant Accepts 2-methyl-GPP to produce 2-methyllimonene	56
<i>Salvia fruticosa</i> 1,8-cineole synthase (S <i>fCinS</i> )	Site-directed mutagenesis	F571Y	Noncanonical C11 variant Accepts 2-methyl-GPP to produce 2-methyl- $\alpha$ -terpineol	56
<i>S. fruticosa</i> 1,8-cineole synthase (S <i>fCinS</i> )	Site-directed mutagenesis	N388S-I451A	Noncanonical C11 variant Accepts 2-methyl-GPP to produce 2-methylmyrcene	56
<i>Pinus taeda</i> Pinene synthase (P <i>tPinS</i> )	Site-directed mutagenesis	F579V	Noncanonical C11 variant Accepts 2-methyl-GPP to produce 2-methylinalool	56
<i>Ocimum basilicum</i> Myrcene synthase (O <i>bMyrS</i> )	Site-directed mutagenesis	F607L	Noncanonical C11 variant Accepts 2-methyl-GPP to produce 2-methylinalool	56
<i>Avena strigosa</i> $\beta$ -amyrin synthase (SAD1)	Site-directed mutagenesis	S728F	Preferentially converts dioxidosqualene (over oxidosqualene) to dammarane	38
<i>Arabidopsis thaliana</i> Lupeol synthase (A <i>tLUP1</i> )	Site-directed mutagenesis	T729F	Preferentially converts dioxidosqualene to epoxydammaranes	38
<i>Clausena lansium</i> Santalene synthase	Site-directed mutagenesis	F441V	Changed product specificity; Sandalwood oil-like sesquiterpene profile	14
Isoprene synthase (ISPS)	Combinatorial mutagenesis	F338-V341-F485	4-fold improvement in isoprene yield	57

**Table S8: Reports of engineering cytochrome P459s involved in terpene biosynthesis.**

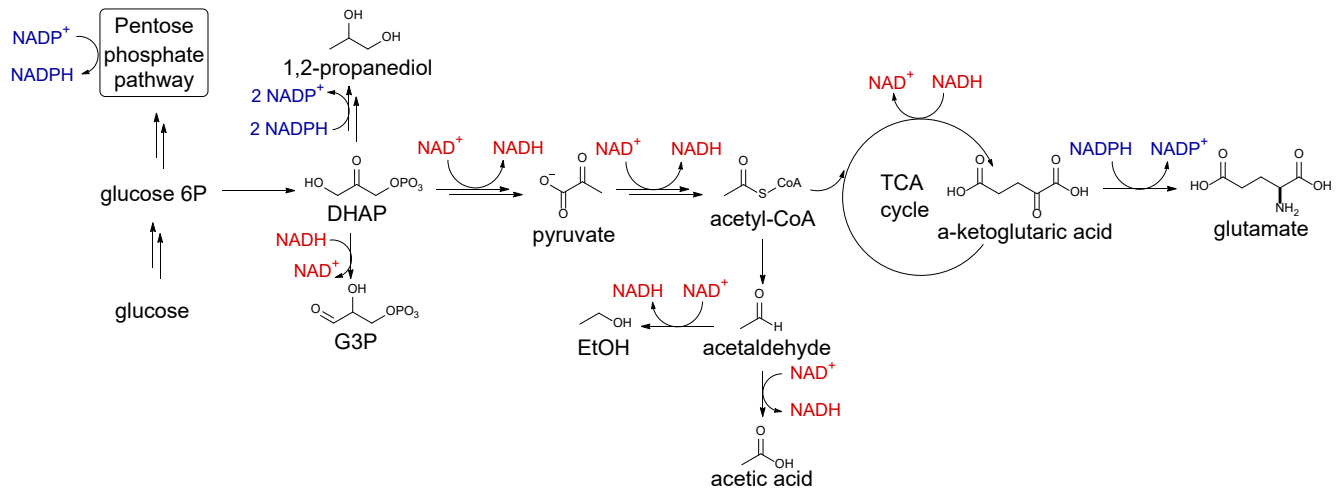
<b>Wild-type enzyme</b>	<b>Wild-type activity</b>	<b>Protein engineering strategy</b>	<b>Engineered mutations</b>	<b>Engineered activity</b>	<b>Ref.</b>
<i>Salvia pomifera</i> CYP76AH24	Miltiradiene/ Abietatriene oxidase	Site-directed mutagenesis	F112L	Improved product specificity for ferruginol; Increased production of downstream minor products by 14-24-fold	<sup>30</sup>
<i>Pinus taeda</i> CYP720B1	Abietadienol/ abietadienal oxidase	Rational mutagenesis	G359A	Improved product specificity for 18- hydroxy-miltiradiene; 2- fold higher efficiency than wild-type enzyme.	<sup>29</sup>
<i>Pinus taeda</i> CYP720B1	Abietadienol/ abietadienal oxidase	Rational mutagenesis	T295S	Relaxed product specificity	<sup>29</sup>
<i>Pinus taeda</i> CYP720B1	Abietadienol/ abietadienal oxidase	Rational mutagenesis	T295S-I223G	Improved product specificity for 19- hydroxy-miltiradiene	<sup>29</sup>
<i>Pinus taeda</i> CYP720B1	Abietadienol/ abietadienal oxidase	Rational mutagenesis	L123V-I223L	Improved product specificity for 3 $\beta$ - hydroxy-manool	<sup>29</sup>
<i>Pinus taeda</i> CYP720B1	Abietadienol/ abietadienal oxidase	Rational mutagenesis	I223G-L466M	Improved product specificity for 19- hydroxy-manool	<sup>29</sup>
<i>Coleus forskolii</i> CYP76AH15	13R-manoyl oxide oxidase	Site-directed mutagenesis	A99I S235G-Y236F L366F L366E	Improved catalytic activity; Enhanced production of 11-oxo-13R-manoyl oxide	<sup>58</sup>

## SUPPLEMENTARY FIGURES



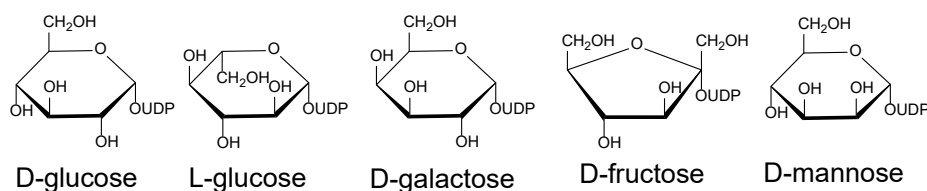
**Figure S1. Heme biosynthesis in yeast.** Heme biosynthesis starts with the decarboxylation of glycine and condensation reaction with succinyl-CoA and glycine to produce aminolaevulinic acid (ALA) in mitochondria. Subsequently, two ALA molecules are exported from the mitochondria to the cytosol to form porphobilinogen (PBG). Four units of PBG molecules are polymerized through deamination processes, and the chain is closed to form the characteristic coproporphyrinogen ring in heme structures. The coproporphyrinogen ring is transported to the mitochondria and goes through a series of modifications at its acetate and propionate side chains. Lastly, the insertion of a ferrous iron atom in the middle of the ring produces the final heme product. Rate limiting steps in heme biosynthesis are catalyzed by Hem3p followed by Hem2p and Hem12p<sup>59</sup>. Overexpression of HEM3 gene in yeast was found beneficial for terpene production<sup>27, 30, 60</sup>. Yeast enzymes are shown in brown, overexpressed enzymes are shown in bold.

## SUPPLEMENTARY SCHEMES

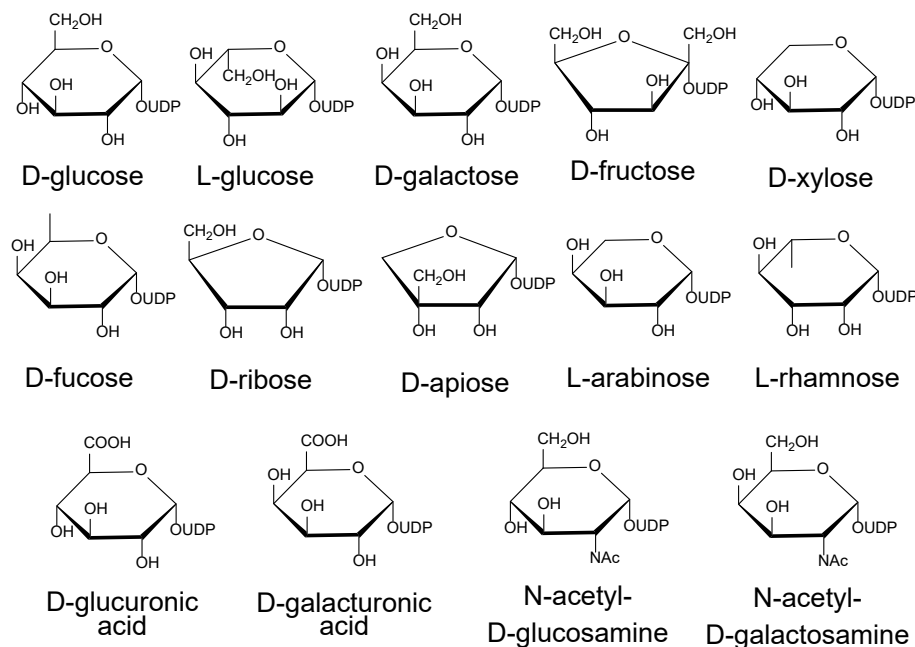


**Figure S2. Redox metabolism of NADH and NADPH in yeast.** NADH and NADPH cofactors donate electrons and hydrogens to reactions involved in yeast central metabolism. The ratio of NADH/NAD<sup>+</sup> (shown in red) and NADPH/NADP<sup>+</sup> (shown in blue) is crucial for redox reactions performed by different native enzymes. Any change to the intracellular redox balance could affect yeast metabolic network. Optimizing cofactor regeneration supports improved production of terpenoids.

### UDP-sugars synthesized in yeast



### UDP-sugars synthesized in plants



**Figure S3. UDP- sugars synthesized in yeast and plant.** Plant UDP-sugars, as essential components in the cell wall, have a highly diverse profile (bottom panel) in comparison with yeast counterparts (top panel). UDP-D-glucose is a common sugar highly conserved from bacteria to human and can be modified into different other UDP-sugars. With a rich cytosolic pool of UDP-D-glucose, yeast is a suitable platform for reconstruction of plant UDP-sugars that can further provide the fitted glycosylation residues for production of plant terpene glycosides or saponins.

## References:

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