Supplementary Material of Combinatorial Metabolic Engineering in *Saccharomyces cerevisiae* for the Enhanced Production of the FPP-Derived Sesquiterpene Germacrene

a)

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LPP1_WT           ATGATCTCTGTCATGGCGGATGAGAAACATAAGGAGTATTTTAAGCTATACTACTTTCAG
LPP1_delta        ATGATCTCTGTCATGGCGGATGAGAAACATAAGGAGTATTTTAAGCTATACTACTTTCAG
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LPP1_WT           TACATGATAATTGGTCTATGTACGATATTATTCCTCTATTCGGAGATATCCCTGGTACCT
LPP1_delta        TACATGATAATTGGTCTATGTACGATATTATTCCTCTATTCGGAGATATCCCTGGTACCT
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LPP1_WT           AGGGGCCAAAACATCGAATTTAGTCTTGATGACCCCAGTATATCAAAACGTTATGTACCT
LPP1_delta        AGGGGCCAAAACATCGAATTTAGTCTTGATGACCCCAGTATATCAAAACGTTATGTACCT
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LPP1_WT           AACGAACTCGTGGGCCCACTAGAATGTTTGATTTTGAGTGTTGGACTGAGTAACATGGTC
LPP1_delta        AACGAACTCGTGGGCCCACTAGAATGTTTGATTTTGAGTGTTGGACTGAGTAACATGGTC
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LPP1_WT           GTCTTCTGGACCTGCATGTTTGACAAGGACTTACTGAAGAAGAATAGAGTA-AAGAGACT
LPP1_delta        GTCTTCTGGACCTGCATGTTTGACAAGGACTTACTGAAGAAGAATAGAGTATAAGAGACT
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LPP1_WT           AAGAGAGAGGCCGGACGGAATCTCGAACGATTTTCACTTCATGCATACTAGCATTCTATG
LPP1_delta        AAGAGAGAGGCCCGACGGAATCTCGAACGATTTTCACTTCATGCATACTAGCATTCTATG
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LPP1_WT           TCTGATGCTGATTATAAGCATAAATGCTGCCCTAACAGGCGCCTTAAAGTTGATTATAGG
LPP1_delta        TCTGATGCTGATTATAAGCATAAATGCTGCCCTAACAGGCGCCTTAAAGTTGATTATAGG
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LPP1_WT           AAACTTGAGGCCTGACTTTGTTGATAGATGTATACCTGACCTCCAAAAGATGAGTGATTC
LPP1_delta        AAACTTGAGGCCTGACTTTGTTGATAGATGTATACCTGACCTCCAAAAGATGAGTGATTC
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LPP1_WT           AGATTCTTTGGTTTTTGGCTTGGACATTTGCAAGCAGACTAACAAATGGATTCTATACGA
LPP1_delta        AGATTCTTTGGTTTTTGGCTTGGACATTTGCAAGCAGACTAACAAATGGATTCTATACGA
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LPP1_WT         AGGCTTAAAAAGCACTCCAAGCGGACATTCAAGTTTCATAGTCAGTACCATGGGCTTTAC
LPP1_delta      AGGCTTAAAAAGCACTCCAAGCGGACATTCAAGTTTCATAGTCAGTACCATGGGCTTTAC
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LPP1_WT         ATATCTTTGGCAAAGGGTTTTCACCACACGCAATACAAGAAGTTGCATTTGGTGCCCTTT
LPP1_delta      ATATCTTTGGCAAAGGGTTTTCACCACACGCAATACAAGAAGTTGCATTTGGTGCCCTTT
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LPP1_WT         ATTAGCTCTAGTAGTAATGGTTTCAAGGGTTATCGATCACAGACATCATTGGTACGATGT
LPP1_delta      ATTAGCTCTAGTAGTAATGGTTTCAAGGGTTATCGATCACAGACATCATTGGTACGATGT
                ************************************************************

LPP1_WT         TGTCTCTGGAGCTGTTCTAGCATTTTTAGTCATTTATTGTTGCTGGAAATGGACATTTAC
LPP1_delta      TGTCTCTGGAGCTGTTCTAGCATTTTTAGTCATTTATTGTTGCTGGAAATGGACATTTAC
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LPP1_WT         AAACTTGGCGAAAAGAGACATACTTCCTTCACCGGTTAGTGTTTAG
LPP1_delta      AAACTTGGCGAAAAGAGACATACTTCCTTCACCGGTTAGTGTTTAG
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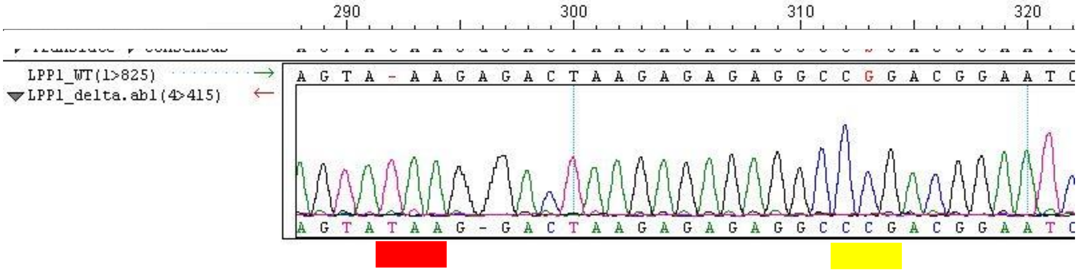

b)

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DPP1_WT         ATGAACAGAGTTTCGTTTATTAAAACGCCTTTCAACATAGGGGCGAAATGGAGATTAGAA

DPP_delta       ATGAACAGAGTTTCGTTTATTAAAACGCCTTTCAACATAGGGGCGAAATGGAGATTAGAA

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DPP1_WT         GATGTCTTTTTGCTCATTATCATGATACTTCTTAACTACCCAGTGTATTACCAACAACCG

DPP_delta       GATGTCTTTTTGCTCATTATCATGATACTTCTTAACTACCCAGTGTATTACCAACAACCG

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DPP1_WT         TTCGAACGTCAGTTTTACATTAACGATCTCACTATATCGCATCCTTATGCGACAACTGAA

DPP_delta       TTCGAACGTCAGTTTTACATTAACGATCTCACTATATCGCATCCTTATGCGACAACTGAA

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DPP1_WT         CGTGTAAATAACAACATGTTGTTTGTTTATAGTTTTGTCGTGCCATCTTTAACCATATTG

DPP_delta       CGTGTAAATAACAACATGTTGTTTGTTTATAGTTTTGTCGTGCCATCTTTAACCATATTG

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DPP1_WT         ATAATTGGTTCCATTTTGGCCGATAGAAGACATTTGATT---TTTATTTTGTACACATCT

DPP_delta       ATAATTGGTTCCATTTTGGCCGATAGAAGACATTTGATTTAATTTATTTTGTACACATCT

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DPP1_WT         CTCCTTGGTTTATCACTCGCTTGGTTCAGTACGAGTTTCTTTACAAACTTCATCAAGAAT

DPP_delta       CTCCTTCGTTTATCACTCGCTTGGTTCAGTACGAGTTTCTTTACAAACTTCATCAAGAAT

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DPP1_WT         TGGATTGGAAGACTAAGACCAGATTTTCTAGATCGTTGCCAACCTGTTGAAGGCTTGCCA

DPP_delta       TGGATTGGAAGACTAAGACCAGATTTTCTAGATCGTTGCCAACCTGTTGAAGGCTTGCCA

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DPP1_WT         TTGGACACTTTATTTACTGCAAAAGATGTGTGTACGACTAAGAATCACGAACGTCTGTTG

DPP_delta       TTGGACACTTTATTTACTGCAAAAGATGTGTGTACGACTAAGAATCACGAACGTCTGTTG

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DPP1_WT         GATGGGTTTAGGACAACTCCGTCAGGTCATTCAAGTGAAAGCTTTGCAGGACTGGGTTAT

DPP_delta       GATGGGTTTAGGACAACTCCGTCAGGTCATTCAAGTGAAAGCTTTGCAGGACTGGGTTAT

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DPP1_WT         TTGTACTTCTGGCTATGTGGGCAACTTTTGACTGAATCACCGTTGATGCCTTTATGGAGA

DPP_delta       TTGTACTTCTGGCTATGTGGGCAACTTTTGACTGAATCACCGTTGATGCCTTTATGGAGA

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DPP1_WT         AAAATGGTGGCCTTTCTACCACTGTTAGGAGCTGCACTAATTGCTCTATCCAGAACTCAA

DPP_delta       AAAATGGTGGCCTTTCTACCACTGTTAGGAGCTGCACTAATTGCTCTATCCAGAACTCAA

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DPP1_WT         GATTACAGACATCATTTCGTCGATGTAATTTTAGGGTCTATGTTGGGTTATATAATGGCA

DPP_delta       GATTACAGACATCATTTCGTCGATGTAATTTTAGGGTCTATGTTGGGTTATATAATGGCA

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DPP1_WT         CACTTTTTCTACAGAAGAATCTTCCCACCCATTGATGATCCTCTTCCGTTCAAACCATTG

DPP_delta       CACTTTTTCTACAGAAGAATCTTCCCACCCATTGATGATCCTCTTCCGTTCAAACCATTG

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DPP1_WT         ATGGACGATTCAGATGTCACCCTGGAGGAAGCAGTCACCCATCAGAGGATCCCGGATGAG

DPP_delta       ATGGACGATTCAGATGTCACCCTGGAGGAAGCAGTCACCCATCAGAGGATCCCGGATGAG

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DPP1_WT         GAATTACATCCTTTGTCCGATGAAGGTATGTAA

DPP_delta       GAATTACATCCTTTGTCCGATGAAGGTATGTAA

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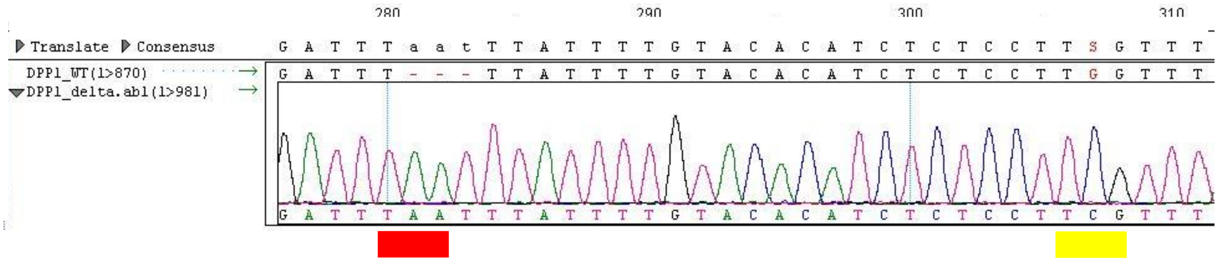

Figure S1. Sequence alignments and sequencing data corresponding to the *LPP1*- and *DPP1*-knockout strains. **a**) Sequence alignment and corresponding sequence data of the *S. cerevisiae LPP1* wildtype-allele (LPP1_WT) and *LPP1* knockout-allele (LPP1_delta) resulting from the integration of a premature stop codon. **b**) Sequence alignment and corresponding sequence data of the *S. cerevisiae DPP1* wildtype-allele (DPP1_WT) and *DPP1* knockout-allele (DPP1_delta) resulting from the integration of a premature stop codon. Red boxes indicate the integrated stop codon; yellow boxes indicate the modified PAM; underlined sequences correspond to the presented sequencing results; alignments of sequences were generated using the Clustal MUSCLE algorithm (http://www.ebi.ac.uk/Tools/msa/muscle/); Sequencing data were analysed using Lasergene SeqMan Pro (DNASTAR).

Table S1. Oligonucleotides used to generate the guide RNA expression cassette in pML104 plasmids or for stop codon integration. Italic letters indicate 20mer guide sequences; bold letters indicate the integrated stop codon; italic and bold letters indicate the modified protospacer-adjacent motif (PAM) to prevent further Cas9 activity.

Oligonucleotide Name	Sequence (5´to 3´)	Used for
***ROX1*-Guide RNA**	GAT C*TG AGG GGT CGA GTT AGC CCT* GTT TTA GAG CTA G	ligation in pML104
P_{erg9}-Guide RNA	GAT C*CG TGT TCT CGC TGC TCG TTT* GTT TTA GAG CTA G	ligation in pML104
***LPP1*-Guide RNA**	GAT C*AG ACT AAG AGA GAG GCC GGA* GTT TTA GAG CTA G	ligation in pML104
***DPP1*-Guide RNA**	GAT C*AT TTT GTA CAC ATC TCT CCT* GTT TTA GAG CTA G	ligation in pML104
***LPP1*-Stop**	TTT GAC AAG GAC TTA CTG AAG AAG AAT AGA GTA **TAA** GAG ACT AAG AGA GAG GC***C CG***A CGG AAT CTC GAA CGA TTT T	integration of stop codon
***DPP1*-Stop**	ATT TTG GCC GAT AGA AGA CAT TTG ATT **TAA** TTT ATT TTG TAC ACA TCT CTC CT***T CG***T TTA TCA CTC GCT TGG TTC AG	integration of stop codon

Table S2. Yeast strains used to enhance the metabolic flux towards sesquiterpenoid FPP and the corresponding sterol- and farnesol/germacrene-A measurements. Values are represented in g/g CDW.

Yeast Strain	**Short Name Yeast Strain**	**Initial pH**	**CuSO$_4$ (µM)**	**Squalene**	**2,3-Oxidosqualene**	**lanosterol**	**Ergosterol**	***Trans-Trans-*Farnesol**	**Germacrene-A**
WT		6.5	0	0.0032 (±0.000877)	0.000015 (±0.000003)	0.00424 (±0.000691)	0.018667 (±0.001036)	-	-
WT ***P_{GAL1}-TkGAS2***	ST0	6.5	0	0.004219 (±0.00099)	0.000011 (±0.000002)	0.007663 (±0.001554)	0.020636 (±0.002723)	0.000716 (±0.000023)	0.000004 (±0.000006)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13	ST1	6.5	0	0.150902 (±0.044394)	0,000096 (±0.000031)	0.127558 (±0.043748)	0.082939 (±0.02365)	-	-
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 P_{GAL1}-TkGAS2	ST3	6.5	0	0.077391 (±0.011625)	0.000063 (±0.000008)	0.029367 (±0.004729)	0.016594 (±0.002407)	0.003338 (±0.000284)	0.000393 (±0.000038)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}***	ST2	6.5	0	0.100485 (±0.011249)	0.000109 (±0.00003)	0.028349 (±0.003271)	0.038247 (±0.004749)	-	-
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}***	ST2	6.5	150	0.001521 (±0.000204)	0.000782 (±0.000056)	0.003958 (±0.000602)	0.024172 (±0.002494)	-	-
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}***	ST2	6.5	375	0.001337 (±0.00023)	0.001044 (±0.000239)	0.005181 (±0.001151)	0.022969 (±0.002797)	-	-
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}*** ***P_{GAL1}-TkGAS2***	ST4	6.5	150	0.001268 (±0.000067)	0.000387 (±0.000029)	0.004861 (±0.0001324)	0.011888 (±0.001612)	0.122461 (±0.003535)	0.001097 (±0.000044)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}*** ***lpp1Δ*** ***P_{GAL1}-TkGAS2***	ST5	4.5	15	0.002755 (±0.000917)	0.003491 (±0.000512)	0.007104 (±0.001601)	0.03149 (±0.005712)	0.11631 (±0.003323)	0.007454 (±0.000317)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}*** ***lpp1Δ*** ***P_{GAL1}-TkGAS2***	ST5	6.5	150	0.001315 (±0.000284)	0.001117 (±0.000549)	0.003273 (±0.000744)	0.013671 (±0.001356)	0.111314 (±0.003375)	0.00414 (±0.00015)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}*** ***lpp1Δ*** ***P_{GAL1}-TkGAS2***	ST5	8.5	150	0.041856 (±0.005932)	0.000837 (±0.000153)	0.02078 (±0.003467)	0.038452 (±0.004871)	0.032799 (±0.009024)	0.003457 (±0.001213)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$P_{erg9\Delta}$::P_{CTR3}*** ***dpp1Δ*** ***P_{GAL1}-TkGAS2***	ST6	6.5	150	0.00102 (±0.000091)	0.000732 (±0.000117)	0.003713 (±0.002224)	0.009354 (±0.003776)	0.091141 (±0.002983)	0.000922 (±0.000069)
rox1::P_{GAL1}-tHMGR P_{GAL10}-ERG13 ***$Pe_{rg9\Delta}$::P_{CTR3}*** ***lpp1Δ dpp1Δ*** ***P_{GAL1}-TkGAS2***	ST7	6.5	150	0.000961 (±0.00032)	0.000545 (±0.003278)	0.002599 (±0.001472)	0.013053 (±0.003278)	0.048922 (±0.005419)	0.000743 (±0.000307)

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