

Table S1. The *de novo* (+)-nootkatone biosynthesis genes used in this study.

Gene	Accession number	Description	Origin
<i>CnVS</i>	JX040471	valencene synthase	<i>Callitropsis nootkatensis</i>
<i>HPO</i>	EF569601.1	premnaspirodiene oxygenase	<i>Hyoscyamus muticus</i>
<i>mHPO</i>		<i>HPO</i> V482I, A484I	
<i>CnVO</i>	JX518290	valencene oxidase	<i>Callitropsis nootkatensis</i>
<i>AtCPR</i>	NM_118585.3	cytochrome P450 reductase	<i>Arabidopsis thaliana</i>
<i>ADH1</i>	NC_001147	alcohol dehydrogenase	CEN.PK2-1Ca
<i>ADH3</i>	NC_001145	alcohol dehydrogenase	CEN.PK2-1Ca
<i>tHMG1</i>	NM_001182434.1	3-hydroxy-3-methylglutaryl-coenzyme A reductase gene	CEN.PK2-1Ca
<i>ERG9</i>	NC_001140.6	squalene synthase	CEN.PK2-1Ca
<i>ROX1</i>	NC_001148.4	oxygen-responsive repressor	CEN.PK2-1Ca

Table S2. Plasmids used in this study

Plasmid	Characteristics	References
YEplac181	<i>S. cerevisiae-E. coli</i> shuttle vector, High-copy (2 μ), <i>LEU2</i> , <i>Amp</i> ^R	1
YEp352	<i>S. cerevisiae-E. coli</i> shuttle vector, High-copy (2 μ), <i>URA3</i> , <i>Amp</i> ^R	2
p181-HPO	YEplac181 derived, containing P _{GAL1} - <i>HPO</i> -T _{CYC1}	This study
p181-mHPO	YEplac181 derived, containing P _{GAL1} - <i>mHPO</i> -T _{CYC1}	This study
p181-CnVO	YEplac181 derived, containing P _{GAL1} - <i>CnVO</i> -T _{CYC1}	This study
p181-HC	YEplac181 derived, containing P _{GAL1} - <i>HPO</i> -T _{CYC1} and P _{GAL1} - <i>AtCPR</i> -T _{CYC1}	This study
p352-CPR	YEp352 derived, containing P _{GAL1} - <i>AtCPR</i> -T _{CYC1}	This study
p352-HC	YEp352 derived, containing P _{GAL1} - <i>HPO</i> -T _{CYC1} and P _{GAL1} - <i>AtCPR</i> -T _{CYC1}	This study
p352-HC2	YEp352 derived, containing P _{HXT7} - <i>HPO</i> -T _{TP1} and P _{HXT7} - <i>AtCPR</i> -T _{TP1}	This study
p352-HC3	YEp352 derived, containing P _{HXT7} - <i>HPO</i> -T _{TP1} and P _{CYC1} - <i>AtCPR</i> -T _{CYC1}	This study

p352-A1HC	YEplac352 derived, containing $P_{TDH3-ADH1-T_{ADH1}}$, $P_{GAL1-HPO-T_{CYC1}}$ and $P_{GAL1-AtCPR-T_{CYC1}}$	This study
p352-A1HC3	YEplac352 derived, containing $P_{TDH3-ADH1-T_{ADH1}}$, $P_{HXT7-HPO-T_{TP1}}$ and $P_{CYC1-AtCPR-T_{CYC1}}$	This study
p352-A3HC	YEplac352 derived, containing $P_{TDH3-ADH3-T_{ADH1}}$, $P_{GAL1-HPO-T_{CYC1}}$ and $P_{GAL1-AtCPR-T_{CYC1}}$	This study
p181-V1	YEplac181 derived, containing $P_{TDH3-VS-T_{ADH1}}$	This study
p181-V2	YEplac181 derived, containing $P_{TEF1-VS-T_{ADH1}}$	This study
p181-V1V2	YEplac181 derived, containing $P_{TDH3-VS-T_{ADH1}}$ and $P_{TEF1-VS-T_{ADH1}}$	This study
p181-V1H	YEplac181 derived, containing $P_{TDH3-VS-T_{ADH1}}$ and $P_{TDH3-tHMG1-T_{ADH1}}$	This study
p181-V2H	YEplac181 derived, containing $P_{TEF1-VS-T_{ADH1}}$ and $P_{TDH3-tHMG1-T_{ADH1}}$	This study
p181-V1HV2	YEplac181 derived, containing $P_{TEF1-VS-T_{ADH1}}$, $P_{TDH3-tHMG1-T_{ADH1}}$ and $P_{TEF1-VS-T_{ADH1}}$	This study

Table S3. Strains used in this study.

Strain	Parent strain	Plasmids/genotype	Sources
<i>E. coli</i>			
DH5 α		F ⁻ ϕ 80 <i>lacZ</i> Δ M15 Δ (<i>lacZYA-argF</i>) U169 <i>recA1 endA1 hsdR17</i> (r _K ⁻ , m _K ⁺) <i>phoA supE44 λ thi-1 gyrA96 relA1</i>	Invitrogen
<i>S. cerevisiae</i>			
CEN.PK2-1Ca		<i>MATa trp1-289 leu2-3 leu2-112 ura3-52 his3Δ1</i>	ATCC® MYA-1108™
BY4741		<i>MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Invitrogen
BJ5464		<i>MATα ura3-52 trp1 leu2Δ1 his3Δ200 pep4::<i>HIS3</i> prb1Δ1.6R</i> <i>can1 GAL</i>	ATCC® 208288™, ³
PK2-C	CEN.PK2-1Ca	YEplac181 and YEp352	This study
PK2-01	CEN.PK2-1Ca	p181-HPO and p352-CPR	This study

PK2-02	CEN.PK2-1Ca	p181-mHPO and p352-CPR	This study
PK2-03	CEN.PK2-1Ca	p181-CnVO and p352-CPR	This study
PK2-30	CEN.PK2-1Ca	p181-HC	This study
PK2-31	CEN.PK2-1Ca	p352-HC	This study
PK2-32	CEN.PK2-1Ca	p352-A1HC	This study
PK2-33	CEN.PK2-1Ca	p352-A3HC	This study
PK2-34	CEN.PK2-1Ca	p352-HC2	This study
PK2-35	CEN.PK2-1Ca	p352-HC3	This study
BY-32	BY4741	BY4741 with pYEp352-A1HC	This study
BJ-32	BJ5464	BJ5464 with pYEp352-A1HC	This study
PK2-11	CEN.PK2-1Ca	p181-V1	This study
PK2-12	CEN.PK2-1Ca	p181-V1, <i>erg9::Δ-220--176 rox1::mut</i>	This study

PK2-13	CEN.PK2-1Ca	p181-V1H, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-14	CEN.PK2-1Ca	p181-V2H, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-15	CEN.PK2-1Ca	p181-V1HV2, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-16	CEN.PK2-1Ca	p181-V2H, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-17	CEN.PK2-1Ca	p181-V1V2	This study
PK2-18	CEN.PK2-1Ca	p181-V1HV2, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-19	CEN.PK2-1Ca	p181-V1 and p352-A1HC	This study
PK2-20	CEN.PK2-1Ca	p181-V1 and p352-A1HC, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-21	CEN.PK2-1Ca	p181-V1H and p352-A1HC, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-22	CEN.PK2-1Ca	p181-V2 and p352-A1HC	This study
PK2-23	CEN.PK2-1Ca	p181-V2 and p352-A1HC, <i>erg9::Δ-220--176 rox1::mut</i>	This study
PK2-24	CEN.PK2-1Ca	p181-V2H and p352-A1HC, <i>erg9::Δ-220--176 rox1::mut</i>	This study

PK2-25

CEN.PK2-1Ca

p181-V2H and p352-A1HC3, *erg9::Δ-220--176 rox1::mut*

This study

Table S4. The primers used in this study*.

Primers	Sequence (5'-3')
Gal1-F1	GTTACCGAATTCTTTACCGTCGACAGTACGGATTAGAAGCCGCC
Gal1-R1	GTTACCCTGCAGTTTACCCTCGAGGCAAATTAAGCCTTCGAGCGT
VS-U	GTTACCGAGCTCGTTACCGGATCCATGGCTGAAATGTTCAACGGA
VS-D	GAGCGATCTAGAGAGCGACCCGGGTTAGGGAATAATCGGTTCGAC
CnVO-U	GTTACCGGATCCATGGACATGTCAACGATATGGT
CnVO-D	GAGCGACCCGGGTTATTCATATAAGTTGGGATATAACA
HPO-U	GTTACCGGATCCATGCAGTTCTTCTCACTGGTT
HPO-D	GAGCGACCCGGGTTATTCACGGGAGGGTTGATA
AtCPR-F	GTTACCGAGCTCGTTACCGGATCCATGACTTCTGCCTTGTATGCC
AtCPR-R	GAGCGATCTAGAGAGCGACCCGGGTCACCAGACATCTCTCAAGTA

TEF2-F	GAGCGAGTCGACATTACCCATAAGGTTGTTTGT
TEF2-R	GTTACCGAGCTCGTTTAGTTAATTATAGTTCGTTG
ADH1T-F	GAGCGATCTAGAAGCTTTGGACTTCTTCGCCA
ADH1T-R	TTTACACTCGAGCATAGGGTAGGGGAATTCGA
TDH3-F	GAGCGAGTCGACACAGTTTATTCCTGGCATCC
TDH3-R	GTTACCGAGCTCTTTGTTTGTATATGT
HPO-482UM	GAATTAGTTGGCATTACAATTGCCAGGAAATCCGA
HPO-482DM	TCGGATTCCTGGCAATTGTAATGCCAACTAATTC
ADH1-U	GTTACCGAGCTCGTTACCGGATCCATGTCTATCCCAGAACTCAA
ADH1-D	GAGCGATCTAGAGAGCGACCCGGGTTATTTAGAAGTGTCAACAACGTA
ADH3-U	GTTACCGAGCTCGTTACCGGATCCATGTTGAGAACGTCAACATTGT
ADH3-D	GAGCGATCTAGAGAGCGACCCGGGTTATTTACTAGTATCGACGACG

tHMG1-U	GTTACCGAGCTCGTTACCGGATCCATGGACCAATTGGTGAAAACCTGAA
tHMG1-D	GAGCGATCTAGAGAGCGACCCGGGTTAGGATTTAATGCAGGTGAC
ADH3-UM	TGACATGCGAGTTCTGTGAATCA
ADH3-DM	TGATTCACAGAACTCGCATGTCA
tHMG1-F	GCCTGCAGTTTACCCTCGGGACAGTTTATTCCTGG
tHMG1-R	CTCCAGCTTTTGTCGTCGACCATAGGGTAGGGGAA
TEF1-U	GTCGACGACAAAAGCTGGAGCTCATAGCTTCAAAATGTTTCT
Backbone-R	CCCGAGGGTAAACTGCAGGCATGCAAGCTTGGCGTAATC
TEF1-U	GTCGACGACAAAAGCTGGAGCTCATAGCTTCAAAATGTTTCT
2 μ -R	GTTCATAGGGTAGAATTAATTCTCATGTTTGACAGCTTATC
2 μ -F	TGATAAGCTGTCAAACATGAGAATTAATTCTACCCTATGA
TEF1-R	GAGCTCCAGCTTTTGTCGTCGACCCCGAGGGTAAACTGCAGGC

Tong-F	TAATAATGGTTTCTTAGTATGA
Tong-R	ACTAAGAAACCATTATTATCAT
ROX1-F	ACAGGATCTTAATAGACGAAGTTTTAGAGCTAGAAATA
ROX1-R	TTCGTCTATTAAGATCCTGTGATCATTTATCTTTCCTG
ERG9-F	TTTTCCACTGCACTTTGCATGTTTTAGAGCTAGAAATAGCA
ERG9-R	ATGCAAAGTGCAGTGGAAAAGATCATTTATCTTTCCTG
Tong2-F	GAACAAAAGCTGGAGCT
Vector2-R	AGCTCCAGCTTTTGTTCAAGACATAAAAAACAAAAAAG
Vector2-F	TTAACCTCACTAAAGGTCTTTGAAAAGATAATGTATGA
Tong2-R	CCTTTAGTGAGGGTTAA
ROX1-M-F	GCATTTATTCTGTTCCAGACAGCACTACCACAGGATCTTAATAGACGAATAACCGCTCA
	<u>A</u>

ROX1-M-R	AAATGTTTGAATTATGGGGTATTTCCACACCTTGAGCGGTTATTCGTCTATTAAGATCC
UAS-M-F	CTCTGACTCAGTACATTTTCATAGCCCATCTTCAACAACAATACCGACTTATCGGAAGG <u>C</u>
UAS-M-R	GCTCGTTTAGGCACTAAACCCAAAACCGATAACGCCTTCCGATAAGTCGGTATTGTT
CYC1p-F	GAGCGAGAATTCTTGGAAAACCAAGAAATG
CYC1p-R	TTTACCGAGCTCTATTAATTTAGTGTGTGTATTG
CYC1t-F	GAGCGACCCGGGGGGCCGCATCATGTAATTAG
CYC1t-R	GTTACCGGATCCGCAAATTAAGCCTTCGAG
HXT7p-F	GAGCGAGAATTCCTCGTAGGAAAAATTCGG
HXT7p-R	TTTACCGAGCTCTTTTTGATTAATAATTAATAAAAAACT
TPI1t-F	GAGCGACCCGGGGATTAATATAATTATATAAAAAATATTATCT
TPI1t-R	GTTACCGGATCCCTATATAACAGTTGAAAT

HPO-U1	TTTACAGAGCTCATGCAGTTCTTCTCAC
HPO-D1	TTTACACCCGGGTTATTCACGGGAGGGTT
AtCPR-F1	GAGCGATCTAGAATGACTTCTGCCT
AtCPR-R1	TTTACACTGCAGTCACCAGACATCTCTC
CAC-F	CAACTGTTATATAGGGATCCTTGGAAAACCAAGAAATGAATTA
CAC-R	CGACGGCCAGTGCCAAGCTTGCAAATTAAGCCTTCGAG
HA-F	TTCCCCTACCCTATGCTCGTAGGAAAAATTCGGGC
HA-R	CAGTTTACCCTCGAGGCAAATTAAGCCTTCGAGC

*The homologous arms are indicated in bold, and the complementary sequences are underlined.

References

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- 3 Jones, E. W., *Methods in enzymology*, 1991, **194**, 428-453.