

## Supporting Information

### **1, 10/1, 11 Cyclization catalyzed by diverged plant sesquiterpene synthases is dependent on a single residue**

Jin-Quan Huang<sup>‡a,b</sup>, Dong-Mei Li<sup>‡a,c</sup>, Jian-Xu Li<sup>b</sup>, Jia-Ling Lin<sup>b,d</sup>, Xiu Tian<sup>b</sup>, Ling-Jian Wang<sup>b</sup>,  
Xiao-Ya Chen<sup>b</sup>, and Xin Fang<sup>c\*</sup>

*<sup>a</sup> Yunnan University, Kunming, P.R. China*

*<sup>b</sup> National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology/CAS Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai 200032, PR China.*

*<sup>c</sup> State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences Kunming, Yunnan 650201, PR China*

*<sup>d</sup> School of Life Science and Technology, ShanghaiTech University, Shanghai 200031, China*

\* To whom correspondence should be addressed:

E-mail: [xinfang@mail.kib.ac.cn](mailto:xinfang@mail.kib.ac.cn)

<sup>‡</sup> These authors contributed equally to this work.

## Contents of Supporting Information

<b>Experimental section</b>	3
<b>Fig. S1</b> Alignment of 1,10-cyclases.	5
<b>Fig. S2</b> Alignment of 1,11-cyclases.	7
<b>Fig. S3</b> Alignment of 1,6-cyclases.	8
<b>Fig. S4</b> Mass spectra of products generated by enzymes used in the manuscript.	9
<b>Fig. S5</b> Effect of substitution of Cys at <sup>pre</sup> NSE/DTE position of SmSTPS3 with Ser.	14
<b>Fig. S6</b> Effect of substitution of Ser at <sup>pre</sup> NSE/DTE position of AaGAS with Cys.	14
<b>Fig. S7</b> Effect of substitution of Cys at <sup>pre</sup> NSE/DTE position of AaCES with Gly.	15
<b>Table S1</b> Plant sesquiterpene synthases used for the phylogenetic analysis.	16
<b>Table S2</b> Oligo-nucleotide primers used in this study.	19
<b>Table S3</b> Product profiles of SmSTPS3, AaGAS and their mutants (%).	20
<b>Table S4</b> Product profiles of AtTPS21 and its mutants (%).	20
<b>Table S5</b> Product profiles of PTSiso and its mutants (%).	20
<b>Table S6</b> Retention indices of new products generated by mutant enzymes.	21
<b>Table S7</b> Kinetic parameters of SmSTPS3, AaGAS, AtTPS21, PTSiso and their mutants with FDP.	21

## **Experimental section**

### **Phylogenetic analysis**

The protein sequences of 163 sesquiterpene synthases (Table S1) were obtained by searching the NCBI GenBank database. These sequences were aligned by the Clustal W program implemented in MEGA with default parameters. Sequences including the <sup>pre</sup>NSE/DTE residue and NSE/DTE motif were submitted to MEME web site (<http://meme-suite.org/>) to analyze amino acids frequency at the <sup>pre</sup>NSE/DTE site.

### **Plant materials**

*Salvia miltiorrhiza* (Lamiaceae) and *Artemisia annua* cv. Qiute (Asteraceae) plants were grown in a greenhouse at 25°C under a light intensity of 150  $\mu\text{mol}$  of photons  $\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and a 14-h light/10-h dark cycle. *Arabidopsis thaliana* (ecotype Columbia-0) (Brassicaceae) plants were grown at 22°C under a long-day photoperiod (16 h light/8 h dark) and *Pogostemon cablin* (Lamiaceae) plants were grown under similar conditions at 26°C. All plants were grown in a nursery at the Shanghai Institute of Plant Physiology and Ecology.

### **Gene isolation, expression, and sequence analysis**

Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA), and 1- $\mu\text{g}$  RNA was reverse transcribed into cDNA using an RNA PCR (AMV) kit (TaKaRa). The coding regions of *SmSTPS3*, *AaGAS*, *AtTPS21*, *PTSiso*, and *AaECS* were amplified from the cDNA library of each plant and cloned into the pET32a vector. *Escherichia. coli* BL21 (DE3) was used for protein expression.

### **Mutagenesis and protein preparation**

Site-directed mutagenesis of sesquiterpene synthases was performed by polymerase chain reaction (PCR) as previously reported <sup>[1]</sup> using an overlap extension strategy with the respective primers. The primers used for gene cloning and mutagenesis are listed in Table S2.

After adding 0.5 mM isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) to induce protein expression, *E. coli* BL21 (DE3) cells (50 mL) harboring the recombinant plasmids were incubated overnight at 20°C with shaking (200 rpm). The following day, the cells were collected, resuspended in 7 mL of cell lysis buffer (2.5 mM Tris-HCl, 30 mM NaCl, and 0.2 mM imidazole, pH 7.0), and disrupted using a constant cell disruption system at a pressure of 16.0 Kpsi. The supernatant was centrifuged,

passed through a Ni-NTA spin column (Qiagen) pre-equilibrated with lysis buffer, and washed with 10 mL wash buffer (2.5 mM Tris-HCl, 30 mM NaCl, and 0.4 mM imidazole, pH 7.0). Next, 2 mL of elution buffer (2.5 mM Tris-HCl, 30 mM NaCl, and 40 mM imidazole, pH 7.0) was added and the purified proteins were collected and determined for concentrations using the Bradford method with bovine serum albumin serving as a standard.

### **Enzyme assays**

TPS activities were assayed by adding 10 µg of the purified proteins to 450 µL of reaction buffer [25 mM HEPES (pH 7.0), 5 mM MgCl<sub>2</sub>, 5 mM DTT, and 40 µM FDP] in 2-mL screw-top glass vials (Agilent). The reaction mixture was overlaid with 500 µL of hexane and incubated at 37°C for 4 h. The organic phase was passed through a short column of Na<sub>2</sub>SO<sub>4</sub>, and then subjected to gas chromatography-mass spectrometry analysis (GC-MS: Agilent 6890 Series GC System coupled to an Agilent 5973 Network Mass Selective Detector) at an initial temperature of 60°C (5-min hold), which was subsequently increased to 240°C at 10°C/min. New products were identified by comparing their mass spectra (Fig. S4) and retention indices (Table S6) with those of reported known terpenes<sup>[2-9, 11, 12]</sup> and the National Institute of Standards and Technology (NIST) libraries.

### **Enzyme kinetics**

The kinetic parameters of SmSTPS3, AaGAS, AtTPS21, PTSiso, and the mutant enzymes were determined via a malachite green method by measuring the release of pyrophosphate<sup>[10]</sup>. Briefly, reactions were carried out in 50-µL reaction systems containing 25 mM MES, 25 mM CAPS, 50 mM Tris, 2.5 mU of the coupling enzyme inorganic pyrophosphatase (*Saccharomyces cerevisiae*, Sigma), and 5 mM MgCl<sub>2</sub> at pH 7.5. The reaction was initiated by adding a fixed concentration (10–15 nM) of the purified enzyme. Each assay contained different concentrations of FDP in the range from 1.0 to 100 µM. The reaction was allowed to proceed for 15 min at 30°C and then terminated by adding 12 µL of a malachite green development solution, followed by a further 15-min incubation. A<sub>623</sub> (UV absorbance at 623 nm) was measured using a NanoDrop 2000 microvolume spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 µmol of product per minute under the aforementioned conditions. Values were obtained using GraphPad Prism 5 based on the fitting to Michaelis–Menten curves.

**Fig. S1** Alignment of 1,10-cyclases. <sup>pre</sup>NSE/DTE residues are outlined in red.

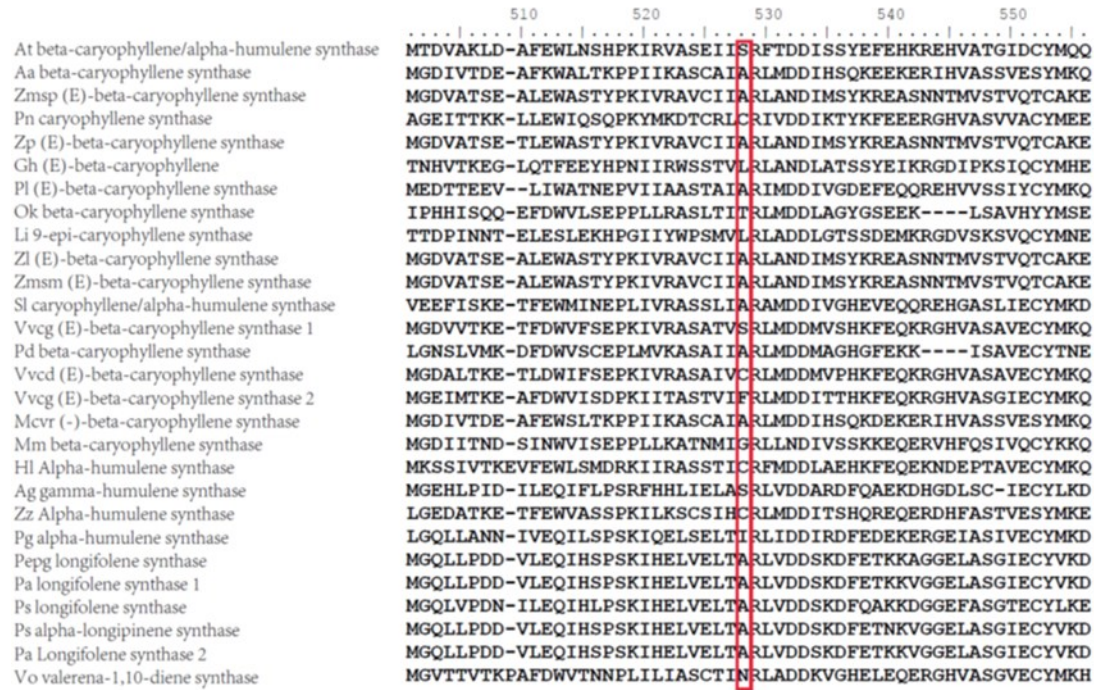




**Fig. S1** continued Alignment of 1,10-cyclases. <sup>pre</sup>NSE/DTE residues are outlined in red.

Aa (-)-germacrene D synthase	SEDSFKWALTNPLLIKAACA	CR	TMDDIVGHKEEKERGHVASFVCEYMKQHNVT
Aa germacrene D synthase 4	TEESFKWIDTFPPVVKASSAV	CR	IMDDIVGHKEEQQRGHVASSVESHMKQHDLT
Aa germacrene A synthase 1	SEDALAWYESHPKTLQASELI	SRL	QDDVMTYQFERERERQSATGVDAIKTYGVS
Aa germacrene A synthase 2	SEDALAWYESHPKTLQASELI	SRL	QDDVMTYQFERERERQSATGVDAIKTYGVS
Hi (-)-germacrene D synthase	TKETFWEVWSNEPLIVRASSVI	CR	LMDDMVGYGIESK----FTAVECYMNENDAL
Lv germacrene A synthase	VREDFEWLSKPKMLVAGLLI	CR	VIDD IATYEVEKDRGQIATGIESYMRDNGAT
Tp germacrene A synthase	SEDALAWYESHPKTLQASELI	SRL	QDDVMTYQFERERERQSATGVDAIKTYGVS
Cs germacrene A synthase	SEHALAWYESHPKTLQASELI	SRL	QDDVMTYQFERERERQSATGVDSYIKTYGVS
To germacrene A synthase	DEEALAWFETHPKILKASELI	SRL	QDDVMTYQFERERERQSATGVDAIKTYGVS
To germacrene A synthase	GEDALAWYESHPKTLQASELI	SRL	QDDVMTYQFERERERQSATGVDSYIKTYGVS
Do (-)-germacrene D synthase	KDEVFSWACSPFKIIEIAAIN	CR	LMDDIAGHENETLRSYTRRFEMSSTMVNL
Sa germacrene D-4-ol synthase	SKEAFDWLFSHPVIEASESV	CR	LMDDMRSHKFEQERGHVASGIECYMKQYGV
Cn germacrene A synthase	GEDALAWYESHPKTLQASELI	SRL	QDDVMTYQFERERERQSATGVDAIKTYGVS
Pc Germacrene A synthase	TKEDFEWFSKPKRIAVATQII	TR	VIDD IATYEVEKEKQSATGIDCYMKEHGVS
Hi Bicyclogermacrene synthase	TEEAFQWVLGSPDPVKAASV	CR	LQDDIVGYKFDKQRDHVSAIDCYMKDNINS
Ov Bicyclogermacrene synthase	SKADFDWIVNEPLIVRASCVI	CR	LMDDLVGDEYEEK----PSSVLCYMKQYVVS
Vv (+)-valencene synthase	TKEAFDWTSDPKIMSSSNFI	TR	LMDDIKSHKFEQKRGHVTSAVECYMKQYGV
Cs valencene synthase	TKEVFEWISNNPKVVKAAASVI	CR	LMDDMQGHEFEQKRGHVASAIECYTKQHGVS
Mn valencene synthase	SNDVFKWLFNDPKIVRAAALI	CR	LMDDIAGHKFEQERKRVASGVECYMEQYGV
Tu Aristolochene synthase	GYDVFNWAI SFPKI IENAAMV	SRL	MDDISGHECEKERSNVATAVDCYIKEHGVT
Nt 5-epi-aristolochene Synthase	TEQDFEWLSKNPKILEASVI	CR	VIDD TATYEVEKSRGQIATGIECCMRDYGIS
Na 5-epi-aristolochene synthase	AEQDFEWLSKNPKILEASVI	CR	VIDD TATYEVEKSRGQIATGIECCMRDYGVS
Cava 5-epi-aristolochene synthase	KEQQFEWLSKNPKILEGCVTI	CR	VIDD IATYEVEKNRGQLSTGIECYMRDYSVS
Ca 5-epi-aristolochene synthase	TEQDFEWLSKNPKILKANATI	CR	VIDD IATYEVEKSRGEIATGIECYRREYGV
Sm 5-epieremophilene synthase 1	VEEDFEWLANKPKMLVAGLLI	CR	VIDD IATYEVEKERGQSATGIESYMRDNNAT
Sm 5-epieremophilene synthase 2	VEEDFQWLAKKPKMLVAGLLI	CR	VIDD IATYEVEKERGQSATGIESYMRDNNAT
Sm 5-epieremophilene synthase 3	VEEDFQWLAKKPKMLVAGLLI	CR	VIDD IATYEVEKERGQSATGIESYMRDNNAT
Hm Vetispiradiene Synthase	TKEHFEWLATNPKILEANATI	CR	VDD IATYEVEKGRGQIATGIECYMRDYGVS
Am delta-guaiene synthase	LKETLDWASTIPEMVKASSLI	ARY	IDLQTYKAEERGETVSAVRCYMRREYGV
Ac Delta-guaiene synthase	LKETLDWASTIPEMVKASSLI	ARY	IDLQTYKAEERGETVSAVRCYMRREYDVS
Tvvl guaiene synthase	TVGTCKWATKKPKAVFAAERH	GRL	LINDIVGYEEHSHRPHVATSIDCYMKEYGVS
Cb hedycaryol synthase	TTEAFEWILTTPKPMVAASGAI	GRL	VDDVMSNDEEQERGHVATGLDCYMKQHGVS
Pc patchoulol synthase	TKEAFDWFVSRPFFIEATLI	AR	LVDITGHEFEKKREHVRTAVECYMEEHKVG
Pc patchoulol synthase isoform	TKEAFDWFVSRPFFIEATLI	AR	LVDITGCEFENKREHVRTAVECYMEEHKVG
Zmsm beta-selinene synthase	TREAFEWADSAAPMVLASGEV	GR	FLNDMASYKLGKKNKDVANAHECYMKEYGAT
Ob selinene synthase	TKKDFDWIRNRPLLQVAEVI	TR	MDDIAGHGTEKK----TTAVSCYMKEYECS
Ag Delta-selinene synthase	PDNILEQIHSPSKILDLELTI	GRI	ADDLKDFEDEKERGEMASSLQCYMKENPES
At delta-cadinene synthase	KLDAFEWLSSHPIRVASEII	SR	FDDISSYEFEHKREHVATGIDCYMQQFGVS
Vv gamma-cadinene synthase	TNEAFDWTSDPKIMSSSNFI	AR	LMDDIKSHKFEQKRGHVASAVECYMKQYGV
Pd alpha-copaene/delta-cadinene synthase	KREHFDWVLSDPDFVMASCI	CR	LADDIVGHEFEQTRDHI PSSVECYTQEHKTS
Rc (+)-delta-cadinene synthase	TKEAYEWASEDPKIRASSIV	CR	LMDDIVSHEFEQTRKHVASGVECYIKQYGAS
Ga (+)-delta-cadinene synthase	TPETFKWAASDPKIKASTII	CR	FMDIAEHKFNHRREDDCSAIECYMKQYGV
Gh (+)-delta-cadinene synthase	TPETFKWAANDPKIIQASTII	CR	FMDVTEHKFKHRREDDCSAIECYMEEYGV
Ob Gamma-cadinene synthase	TQEAIDWIKNEPNFAVKAGLI	GRY	WDDIGSHKRESKGGEMLTVMDCYMKQYSVS
Cm (+)-gamma-cadinene synthase	TNEVLQWLSNGPQIIKASTII	CR	LMDDIASHKFEQEREHVASAVECYMKQYDCS
Mg Beta-cubebene synthase	TKEVLEWAIHPTMLRITCSIV	AR	LMDDIPSNKLEQERKHVSSSVECYMKEHGTS
Pn copaene synthase	TKDVFEWAIKRPNIIVVAASMI	CR	NRDDIVGHKEEQERGDVPSGVECYTKDHGCT

**Fig. S2** Alignment of 1,11-cyclases. <sup>pre</sup>NSE/DTE residues are outlined in red.



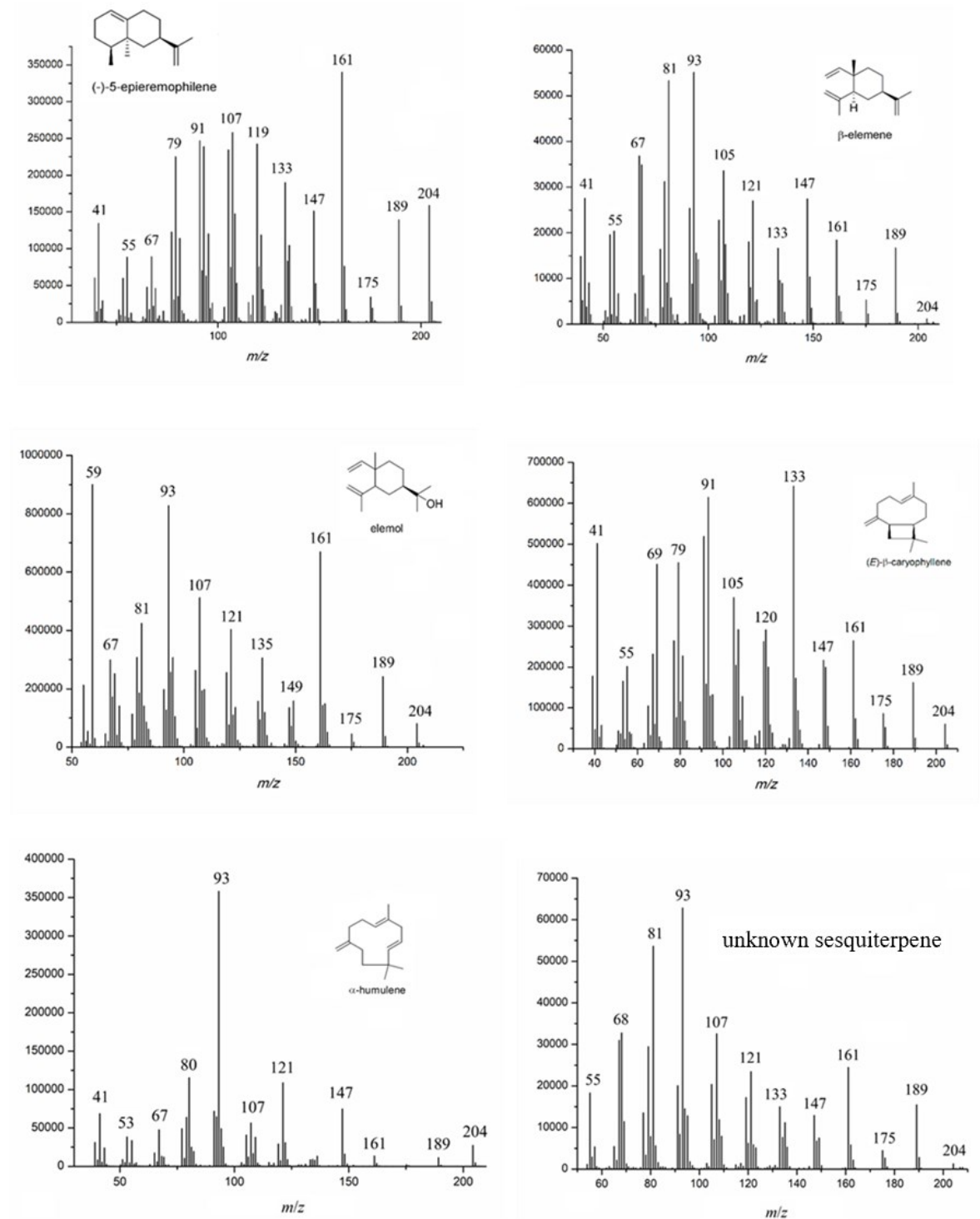


**Fig. S3** Alignment of 1,6 cyclases. <sup>pre</sup>NSE/DTE residues are outlined in red.

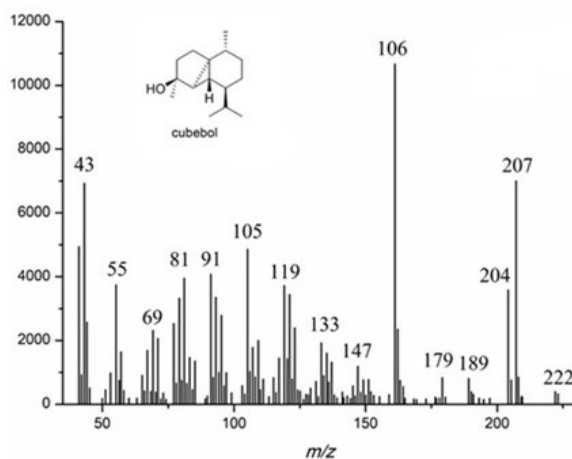
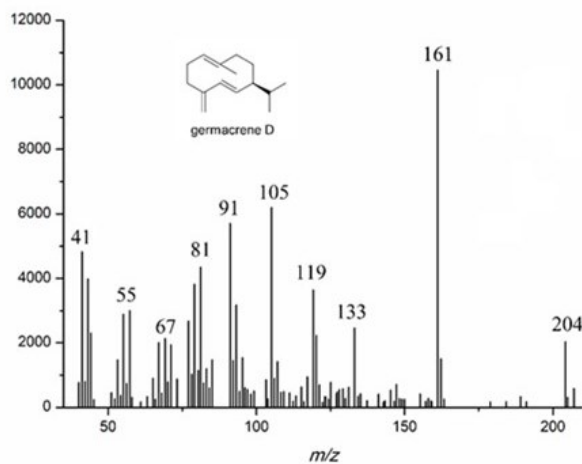
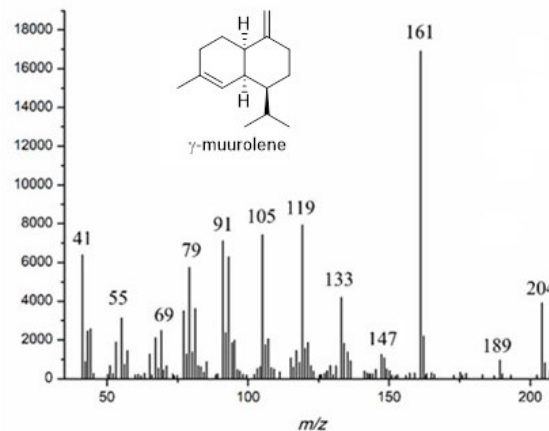
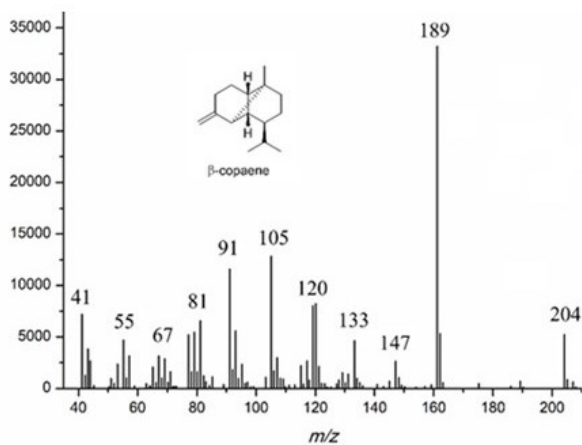
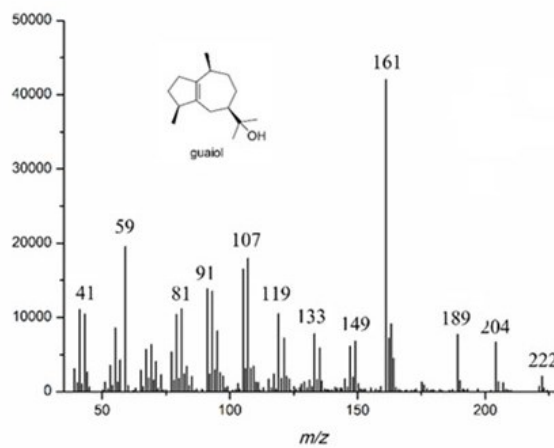
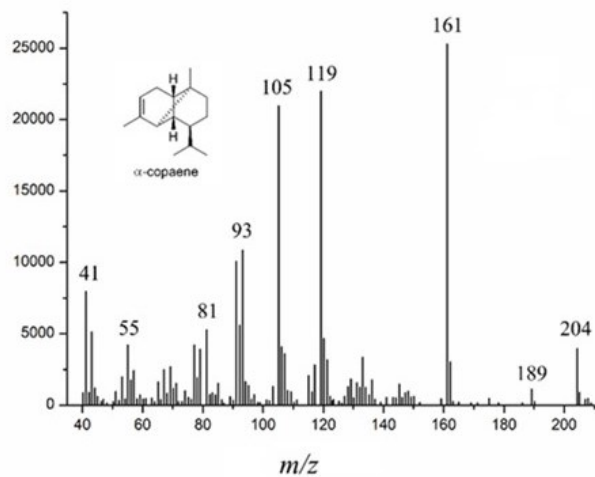
	710	720	730	740
Zm 7-epi-sesquithujene synthase	..... ..... ..... ..... ..... ..... .....	TFEWALSFPPQFIRTFGSEFVRLSNDVVSTKREQTKDHSPTVHCY		
Ag Alpha-bisabolene synthase		KVDYPGR-RVLTENLSLISRLADDTKTYKAEKARGELASSIECY		
Zm tps5-Del1		TFEWALSFPPQFIRTFGSEFVRLSNDVVSTKREQTKDHSPTVHCY		
Zo(S)-beta-bisabolene synthase		AFEWFASFPKIIEACATIIIRITNDITSMEREQKRAHVASTVDCY		
At (Z)-gamma-bisabolene synthase		AYEWRVSRPRLIKSLAAGRLMDDITDFDSDMSNGFAANAINYY		
Gb alpha bisabolene synthase		QVDLPGHDPKLIELNCIISRLSDDTKTFQAEKARGELASSIECY		
Pm(E)-gamma-bisabolene synthase		KIDYPERSRVLMEQICLISRLADDTQSYKAEKARGELASGIECY		
Sa Beta-bisabolene synthase		SAAYVNSVPPPLVRYSGLLNRLYNDLGTSSAEIARGDTLKS IQCY		
Ss Alpha-bisabolol synthase		SAAYVNSVPPPLVRYSGLLNRLYNDLGTSSAEIARGDTLKS IQCY		
Pa E-alpha-bisabolene synthase		KVDYPGR-RVLTENLSLISRLADDTKTYKAEKARGELASSIECY		
Zo (S)-beta-bisabolene synthase		AFEWFASFPKIIEACATIIIRITNDITSMEREQKRAHVASTVDCY		
Sb Beta-sesquiphellandrene synthase		ILDWLLSYPKLLKSMTTFFVRLSNDIASTKREQTGGHHAHVQCY		
Ss a-bisabolol synthase		SAAYVNSVPPPLVRYSGLLNRLYNDLGTSSAEIARGDTLKS IQCY		
Zo sesquiterpene synthase		AFEWFASFPKIVEACAIIVRITNDITSKEREQKREHVASTVDCY		
Ag E-alpha-bisabolene synthase		KVDYPGR-RVLTENLSLISRLADDTKTYKAEKARGELASSIECY		
Sa b-bisabolene synthase		SAAYVNSVPPPLVRYSGLLNRLYNDLGTSSAEIARGDTLKS IQCY		
Ha (Z)-gamma-bisabolene synthase K11		IFKWLSNYPPIVKASSLILRYMNDLSTRKDEQERNHVASSVKCY		
Ha (Z)-gamma-bisabolene synthase K7		IFKWLSNYPPIVKASSLILRYMNDLSTRKDEQERNHVASSVKCY		
Zm (S)-beta-macrocarpene synthase 1		IMKWVMSDAELVKSFGIFVRLSNDIVSTKREQREKHCVSTVQCY		
Zm(S)-beta-macrocarpene synthase 2		IMEWVMSDAELVKSFGIFVRLSNDIVSTKREQREKHCVSTVQCY		
Ee bisabolol synthase		TFKWVATHPPPLVKASCLILRLMDDIATHKEEQERGHVASSIECY		
Pd(+)-epi-alpha-bisabolol synthase		HILDRLTNPPVIKWVSIIVRLADDLGTSTDELKRGDNPKSIQCH		
Aa bisabolol synthase		AFEWAVSEPPPLRYKGIIGRRLNDLAGHKKEEQERKVVSSSVESY		
Am (+)-alpha-bisabolol synthase		AWEWAVSEPPPLRYKGIIGRRLNDLAGHKKEEQERKVVSSSVESY		
Ak (+)-alpha-bisabolol synthase		AWEWAVSEPPPLRYKGIIGRRLNDLAGHKKEEQERKVVSSSVESY		
Mcvr a-bisabolol synthase		AFKWVATHPPIVKAACKILRLMDDIATHKEEQERGHVASSIECY		
Aabs koidzumiol synthase		AWEWAVSEPPPLFRYSGILGRRLNDLVSHKDEQERKHISSSVESY		
Aabs koidzumiol synthase		AWEWAVSEPPPLFRYSGILGRRLNDLVSHKDEQERKHISSSVESY		
Aa Amorpha-4,11-diene synthase		SVEWAVSAPPLFRYSGILGRRLNDLMTHKAEQERKHSSSSLESY		
Aa epi-cedrol synthase		SFEWVFTNPPLVNACLLCRTMDDLGSKGGEQDRKQHVASTIECY		
Mcvr sesquiterpene synthase 1		AFKWVATHPPIVKAACKILRLMDDIATHKEEQERGHVASSIECY		
Mcvr sesquiterpene synthase 4		PFKWALANPPLVRATCAICRFMDDIVGHEDEQQRNHVSVVECY		
Vvcpn beta-curcumenene synthase		AFDWVLNGPKIVRACSTIIRLMDDMASHKFEQERGHVASSVECY		
Pc Gamma-curcumenene synthase		NIDWLLSQPRLASSTAIVMRCCNDLGSNQRESKGGVMTSLDCY		
Vvcg (E)-alpha-bergamotene synthase		VFDWLQONPKIVRASSKVIIRLMDDMATHKFEQERGHVASSIECY		
Pd Trans-alpha-bergamotene synthase		ELQYLEQYPGIIRWPSTVLRRLADDLGTASDEIKRGDVPKSIQCY		
Sa santalene bergamotene synthase 1		ALDKVHPLPDLHYSSLLSRLINDMGTSSDELERGDNLKSIQCY		
Sa santalene bergamotene synthase 2		ALDKVHPLPDLHYSSLLSRLINDIGTSPDEMARGDNLKSIHCY		
Ss santalene synthase		ALDKVHPLPDLHYSSLLSRLINDMGTSPDEMARGDNLKSIHCY		
Sau santalene synthase		ALDKVHPLPDLHYSSLLSRLINDIGTSPDEMARGDNLKSIHCY		
Sb Zingiberene synthase		VLDWLLTYPELLKCFITFFVRLSNDITSTKREQTGGHHAHVQCY		
Ob Alpha-zingiberene synthase		ELQSLERHAHSL---SMILRLADDLGTSSDEMKRGDVPKAIQCF		
At Alpha-barbatene synthase		AFEWLI SRPKLVRI LGAKTRLMDDIADFEEDMEKGYTANALNYY		



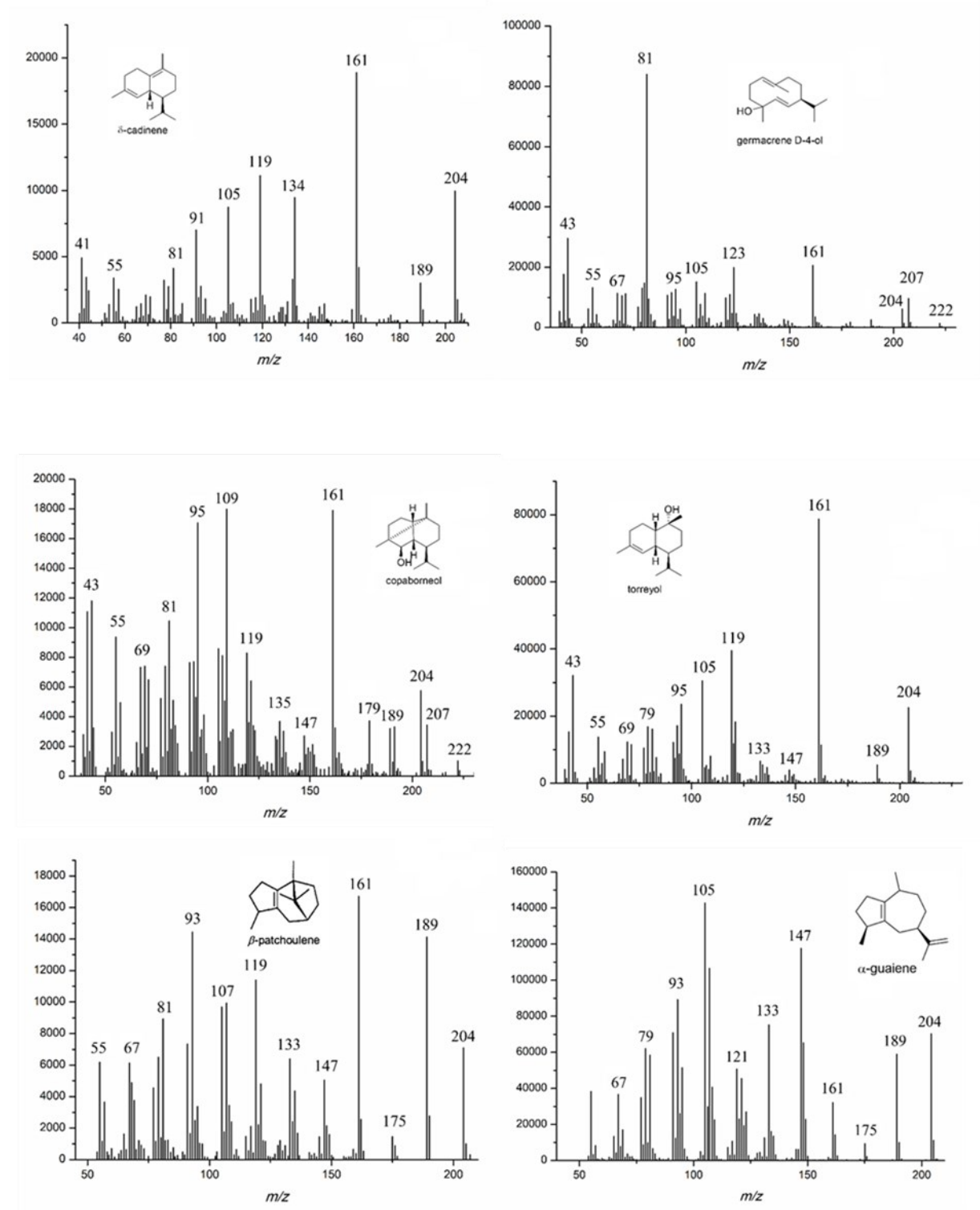
**Fig. S4** Mass spectra of products generated by enzymes used in the manuscript. The most of sesquiterpenes are yielded by Wt enzymes [2-7], and by compared with known terpenes in reported enzyme assay [8, 9].



**Fig. S4 continued** Mass spectra of products generated by enzymes used in the manuscript.

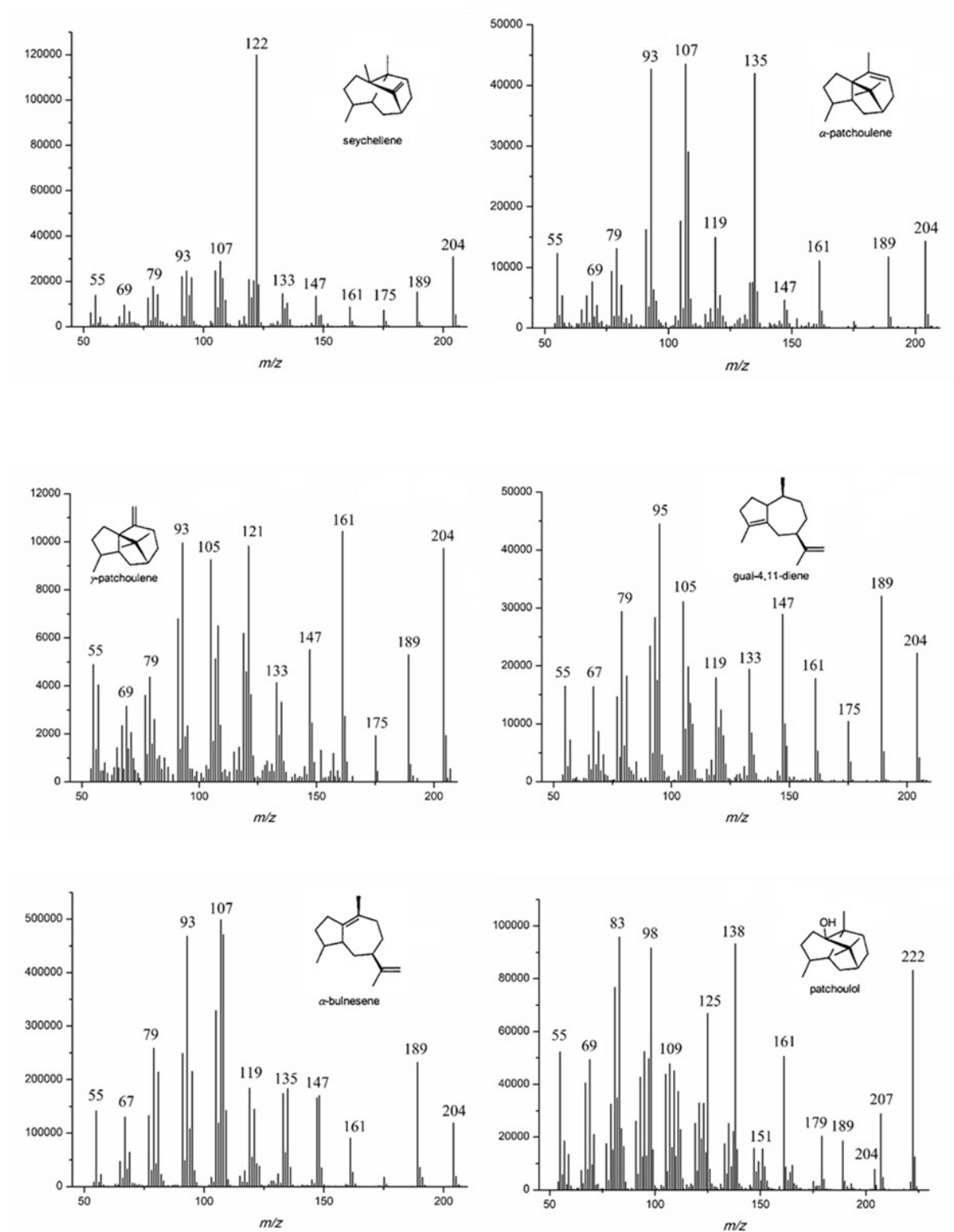


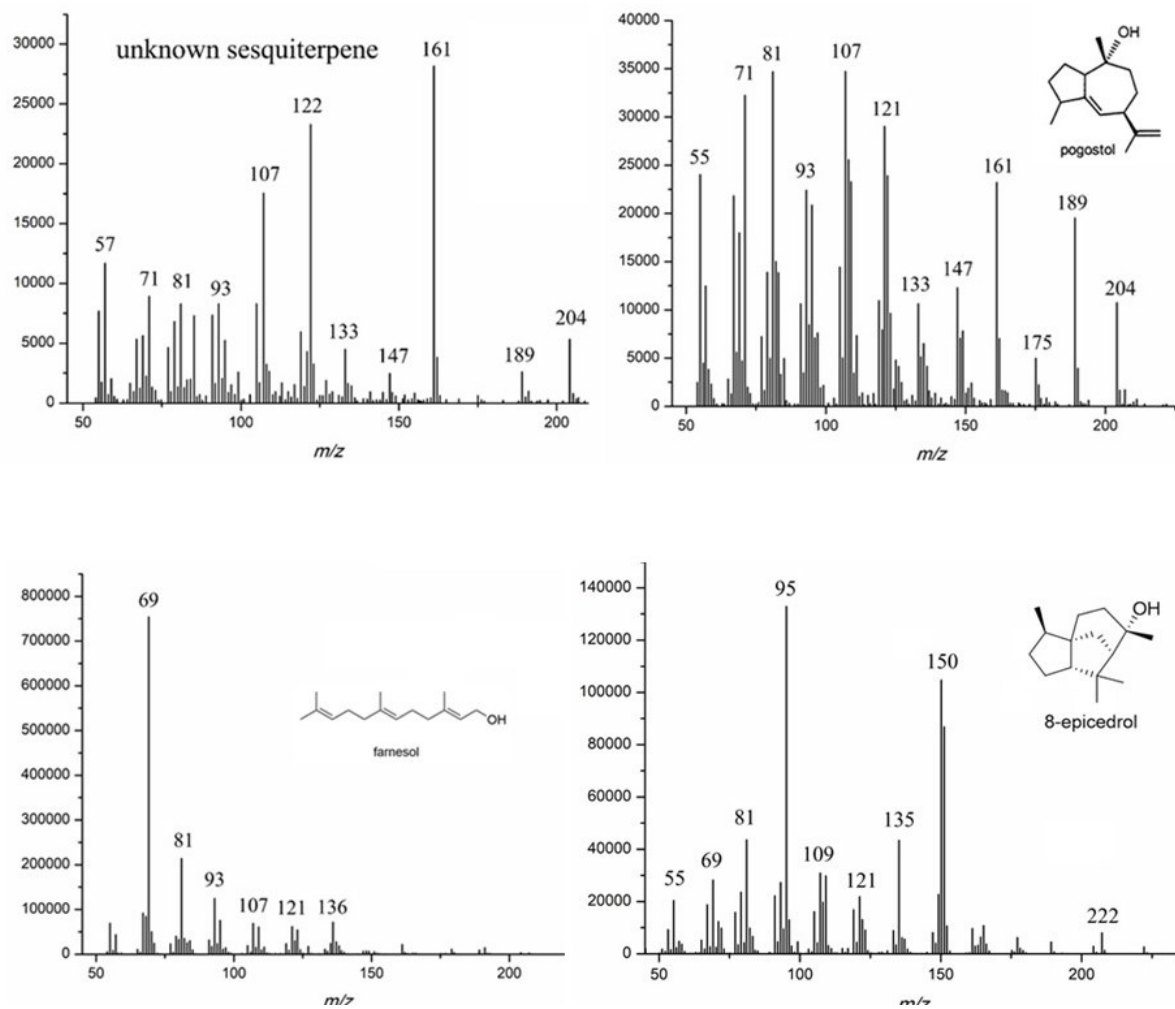
**Fig. S4 continued** Mass spectra of products generated by enzymes used in the manuscript.



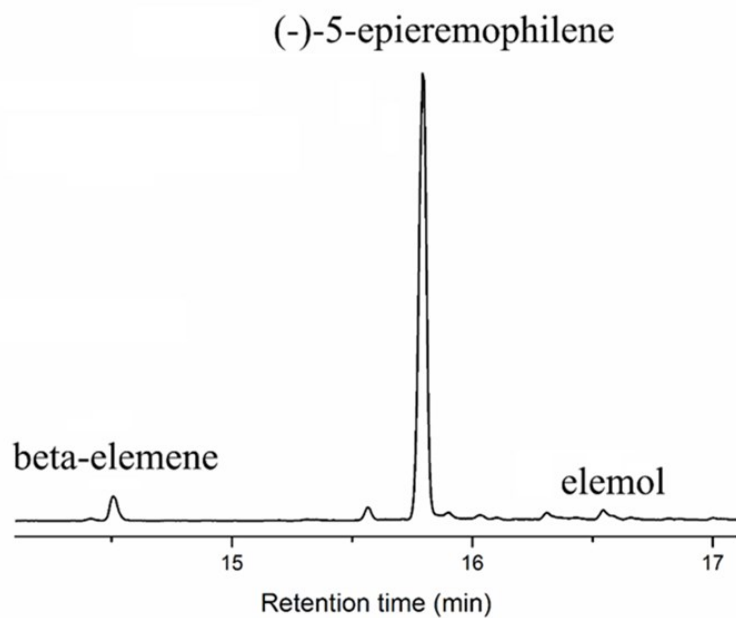


**Fig. S4 continued** Mass spectra of products generated by enzymes used in the manuscript.

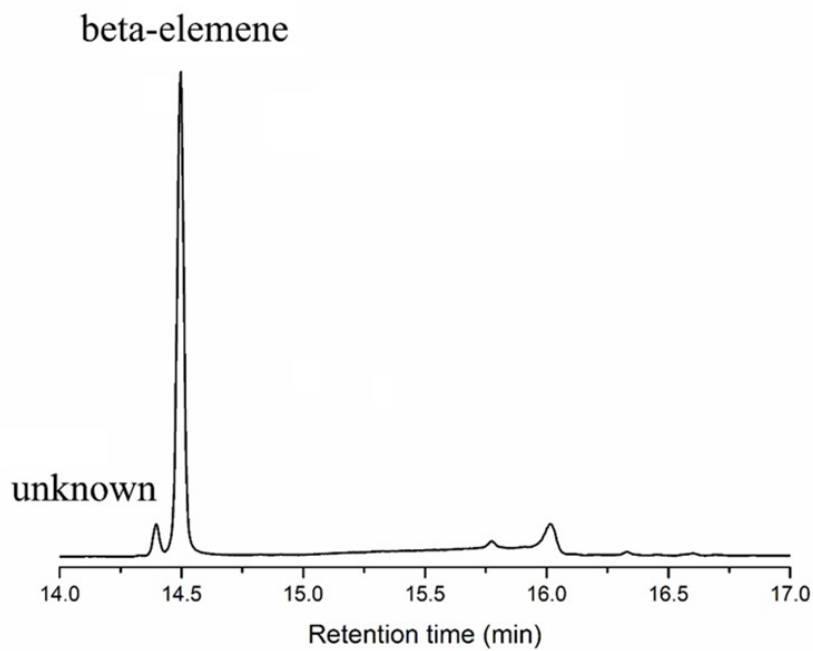




**Fig. S4 continued** Mass spectra of products generated by enzymes used in the manuscript.

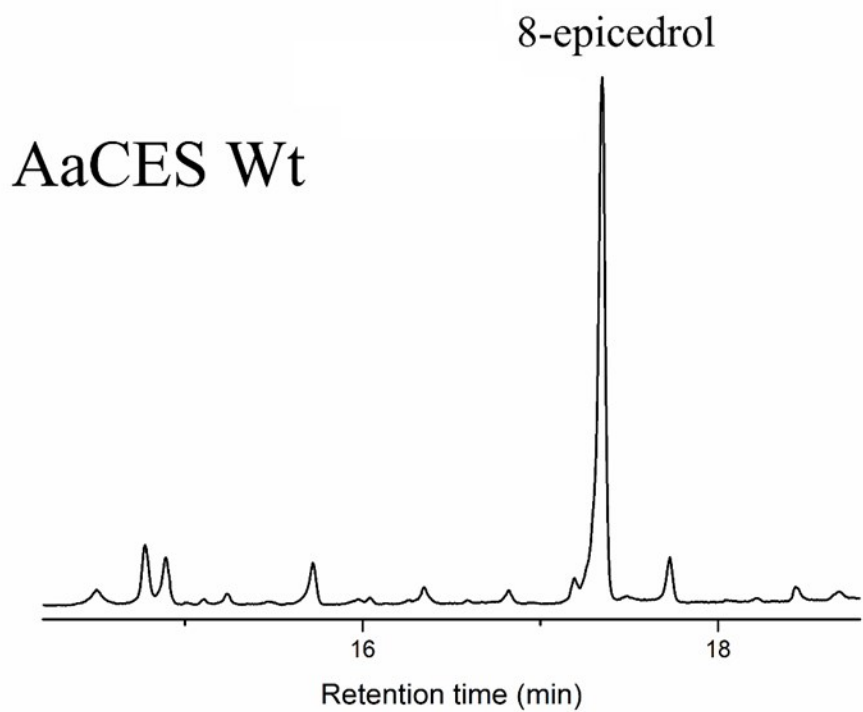
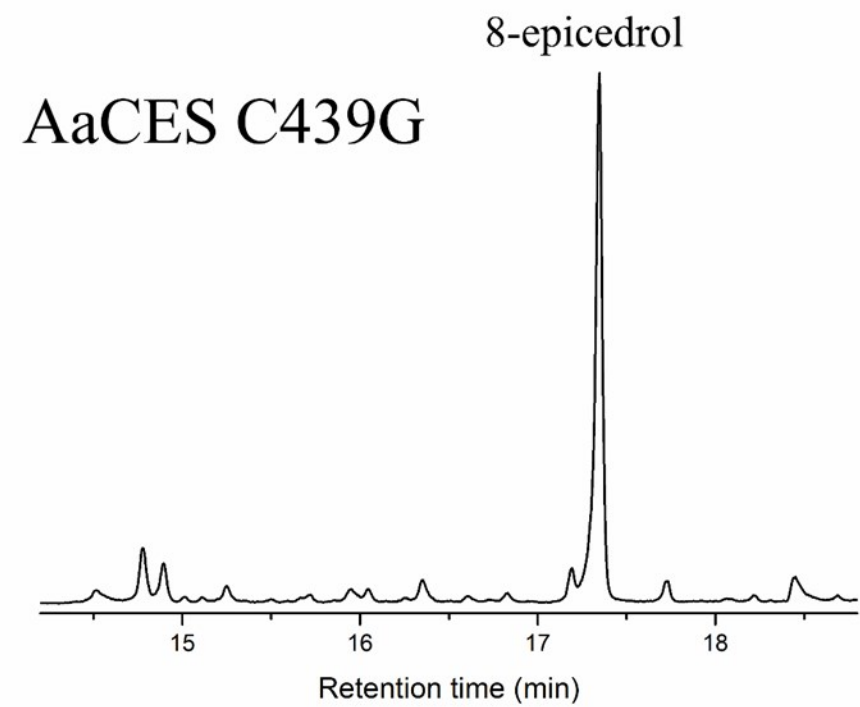


**Fig. S5** Effect of substitution of Cys at <sup>pre</sup>NSE/DTE position of SmSTPS3 with Ser.



**Fig. S6** Effect of substitution of Ser at <sup>pre</sup>NSE/DTE position of AaGAS with Cys.





**Fig. S7** Effect of substitution of Cys at <sup>pre</sup>NSE/DTE position of AaCES with Gly.

**Table S1** Plant sesquiterpene synthases used for the phylogenetic analysis, their corresponding protein ids, and organism names.

Sesquiterpene Synthases	Protein ID	Organism
Dc (-)-germacrene D synthase	PKU80372.1	<i>Dendrobium catenatum</i> (Orchidaceae)
Vv (-)-germacrene D synthase	AY561842.1	<i>Vitis vinifera</i> (Vitaceae)
Vv germacrene D synthase	HM807378.1	<i>Vitis vinifera</i> (Vitaceae)
Le germacrene C synthase	AF035631.1	<i>Lycopersicon esculentum</i> cv. VFNT (Solanaceae)
Ha germacrene A synthase 1	AAAY41421.2	<i>Helianthus annuus</i> (Asteraceae)
Ha (-)-germacrene D synthase	OTG28618.1	<i>Helianthus annuus</i> (Asteraceae)
Sc germacrene A synthase	CAC36896.1	<i>Solidago canadensis</i> (Asteraceae)
Ci germacrene A synthase	AAM21658.1	<i>Cichorium intybus</i> (Asteraceae)
Ls germacrene A synthase 1	AF489965.1	<i>Lactuca sativa</i> (Asteraceae)
Ls germacrene A synthase 2	AF489964.1	<i>Lactuca sativa</i> (Asteraceae)
Ls germacrene A synthase 3	AOT80657.1	<i>Lactuca sativa</i> (Asteraceae)
Bs germacrene A synthase 1	AIX97167.1	<i>Barnadesia spinosa</i> (Asteraceae)
Bs germacrene A synthase 2	AIX97168.1	<i>Barnadesia spinosa</i> (Asteraceae)
Am germacrene A synthase	AGD80135.1	<i>Achillea millefolium</i> (Asteraceae)
Ccvs germacrene A synthase	AET95645.1	<i>Cynara cardunculus</i> var. scolymus (Asteraceae)
Cz germacrene B synthase	BBE32340.1	<i>Curcuma zedoaria</i> (Zigiberaceae)
Cs germacrene D synthase	BBE32332.1	<i>Chengiopanax sciadophylloides</i> (Araliaceae)
Ac germacrene A synthase	BBE38101.1	<i>Aralia cordata</i> (Araliaceae)
Zp germacrene D synthase	BBD88589.1	<i>Zanthoxylum piperitum</i> (Rutaceae)
Tvvl germacrene D synthase	AMU19318.1	<i>Thapsia villosa</i> var. <i>laciniata</i> (Apiaceae)
Xs germacrene A synthase	AJT60315.1	<i>Xanthium strumarium</i> (Asteraceae)
Vo germacrene B/C/D synthase	AFR42417.1	<i>Valeriana officinalis</i> (Valerianaceae)
Vo germacrene C synthase	AGB05616.1	<i>Valeriana officinalis</i> (Valerianaceae)
Ga (-)-germacrene D synthase	KHG04103.1	<i>Gossypium arboreum</i> (Malvaceae)
Ha germacrene A synthase 2	EU327785.1	<i>Helianthus annuus</i> (Asteraceae)
Ha germacrene A synthase 1	DQ016667.2	<i>Helianthus annuus</i> (Asteraceae)
Ha germacrene A synthase 3	GU176380.1	<i>Helianthus annuus</i> cultivar HA300 (Asteraceae)
Zo germacrene D synthase	AAX40665.1	<i>Zingiber officinale</i> (Zigiberaceae)
Sl germacrene synthase	AEM05858.1	<i>Solanum lycopersicum</i> (Solanaceae)
Sl germacrene C synthase	AAC39432.1	<i>Solanum lycopersicum</i> (Solanaceae)
Sl epidermal germacrene C synthase	AAC39431.1	<i>Solanum lycopersicum</i> (Solanaceae)
Pc germacrene D synthase	AY508729.1	<i>Pogostemon cablin</i> (Lamiaceae)
Pc germacrene A synthase	AY508728.1	<i>Pogostemon cablin</i> (Lamiaceae)
Pc germacrene D synthase	AY508727.1	<i>Pogostemon cablin</i> (Lamiaceae)
Sc (-)-germacrene D synthase	AAR31145.1	<i>Solidago canadensis</i> (Asteraceae)
So (-)-germacrene D synthase	AQY54367.1	<i>Salvia officinalis</i> (Lamiaceae)
Sc (+)-germacrene D synthase	AAR31144.1	<i>Solidago canadensis</i> (Asteraceae)
Ob germacrene D synthase	AAV63786.1	<i>Ocimum basilicum</i> (Labiatae)
Gs (-)-germacrene D synthase	RZB75747.1	<i>Glycine soja</i> (Fabaceae)
Ls germacrene A synthase 2	AAM11627.1	<i>Lactuca sativa</i> (Asteraceae)
Ls germacrene A synthase 1	AAM11626.1	<i>Lactuca sativa</i> (Asteraceae)
Cmfk germacrene D synthase 1	RWR76602.1	<i>Cinnamomum micranthum</i> f. <i>kanehirae</i>
Aa germacrene D synthase 2	PWA81675.1	<i>Artemisia annua</i> (Asteraceae)
Aa germacrene D synthase 3	PWA51042.1	<i>Artemisia annua</i> (Asteraceae)

Aa (-)-germacrene D synthase	PWA68010.1	<i>Artemisia annua</i> (Asteraceae)
Aa germacrene D synthase 4	PWA58326.1	<i>Artemisia annua</i> (Asteraceae)
Aa germacrene A synthase 1	DQ447636.1	<i>Artemisia annua</i> (Asteraceae)
Aa germacrene A synthase 2	PWA48097.1	<i>Artemisia annua</i> (Asteraceae)
Hi (-)-germacrene D synthase	PIN14350.1	<i>Handroanthus impetiginosus</i> (Bignoniaceae)
Lv germacrene A synthase	AGN72806.1	<i>Lavandula viridis</i> (Lamiaceae)
Tp germacrene A synthase	AEH41844.1	<i>Tanacetum parthenium</i> (Asteraceae)
Cs germacrene A synthase	ABB00361.1	<i>Crepidiastrum sonchifolium</i> (Asteraceae)
To germacrene A synthase	ALY05869.1	<i>Taraxacum officinale</i> (Asteraceae)
To germacrene A synthase	ALY05868.1	<i>Taraxacum officinale</i> (Asteraceae)
Do (-)-germacrene D synthase	OEL12694.1	<i>Dichantherium oligosanthes</i> (Poaceae)
Sa germacrene D-4-ol synthase	B5A435.1	<i>Santalum album</i> (Santalaceae)
Cn germacrene A synthase	AMN10097.1	<i>Chamaemelum nobile</i> (Asteraceae)
Pc germacrene A synthase	Q49SP5.1	<i>Pogostemon cablin</i> (Lamiaceae)
Hi bicyclogermacrene synthase	PIN24805.1	<i>Handroanthus impetiginosus</i> (Bignoniaceae)
Ov bicyclogermacrene synthase	E2E2N7.1	<i>Origanum vulgare</i> (Lamiaceae)
Vv (+)-valencene synthase	AY561843.1	<i>Vitis vinifera</i> (Vitaceae)
Cs valencene synthase	AF441124.1	<i>Citrus sinensis</i> (Rutaceae)
Mn valencene synthase	XP_010092295.1	<i>Morus notabilis</i>
Tu aristolochene synthase	EMS62091.1	<i>Triticum Urartu</i> (Poaceae)
Nt 5-epi-aristolochene Synthase	5EAU_A	<i>Nicotiana tabacum</i> (Asteraceae)
Na 5-epi-aristolochene synthase	AAP05762.1	<i>Nicotiana attenuate</i> (Asteraceae)
Cava 5-epi-aristolochene synthase	CAA06614.1	<i>Capsicum annuum</i> var. <i>annuum</i> (Solanaceae)
Ca 5-epi-aristolochene synthase	NP_001311620.1	<i>Capsicum annuum</i> (Solanaceae)
Sm 5-epieremophilene synthase 1	KY432512.1	<i>Salvia miltiorrhiza</i> (Lamiaceae)
Sm 5-epieremophilene synthase 2	KY432513.1	<i>Salvia miltiorrhiza</i> (Lamiaceae)
Sm 5-epieremophilene synthase 3	KY432514.1	<i>Salvia miltiorrhiza</i> (Lamiaceae)
Hm vetispiradiene Synthase	5JO7_D	<i>Hyoscyamus muticus</i> (Solanaceae)
Am delta-guaiene synthase	ALR84836.1	<i>Aquilaria malaccensis</i> (Thymelaeaceae)
Ac delta-guaiene synthase	D0VMR8.1	<i>Aquilaria crassna</i> (Thymelaeaceae)
Tvvl guaiene synthase	AZL40028.1	<i>Thapsia villosa</i> var. <i>laciniata</i> (Apiaceae)
Cb hedyacryol synthase	BAU68096.1	<i>Camellia brevistyla</i>
Pc patchoulol synthase	AY508730.1	<i>Pogostemon cablin</i> (Lamiaceae)
Pc patchoulol synthase variant	AHL24448.1	<i>Pogostemon cablin</i> (Lamiaceae)
Zmsm beta selinene synthase	ATI25530.1	<i>Zea mays</i> subsp. <i>mays</i> (Poaceae)
Ob selinene synthase	AAV63785.1	<i>Ocimum basilicum</i> (Labiatae)
Ag delta-selinene synthase	O64404.1	<i>Abies grandis</i> (Pinaceae)
At delta-cadinene synthase	AJ544238.1	<i>Arabidopsis thaliana</i> (Brassicaceae)
Vv gamma-cadinene synthase	HM807380.1	<i>Vitis vinifera</i> (Vitaceae)
Pd alpha-copaene/delta-cadinene synthase	AFR23368.1	<i>Phyla dulcis</i> (Verbenaceae)
Rc (+)-delta-cadinene synthase	B9S9Z3.1	<i>Ricinus communis</i> (Euphorbiaceae)
Ga (+)-delta-cadinene synthase	Q43714.1	<i>Gossypium arboreum</i> (Malvaceae)
Gh (+)-delta-cadinene synthase	U88318.1	<i>Gossypium hirsutum</i> (Malvaceae)
Ob gamma-cadinene synthase	Q5SBP5.1	<i>Ocimum basilicum</i> (Labiatae)
Cm (+)-gamma-cadinene synthase	B2KSJ5.1	<i>Cucumis melo</i> (Cucurbitaceae)
Mg beta-cubebene synthase	B3TPQ6.1	<i>Magnolia grandiflora</i> (Magnoliaceae)
Pn copaene synthase	AVY53326.1	<i>Piper nigrum</i> (Piperaceae)
At beta-caryophyllene/alpha-humulene synthase	AF497491.1	<i>Arabidopsis thaliana</i> (Brassicaceae)
Aa beta-caryophyllene synthase	AF472361.1	<i>Artemisia annua</i> (Asteraceae)
Zmsp (E)-beta-caryophyllene synthase	EU259642.1	<i>Zea mays</i> subsp. <i>Parviglumis</i> (Poaceae)



Pn caryophyllene synthase	ARB08605.1	<i>Piper nigrum</i> (Piperaceae)
Zp (E)-beta-caryophyllene synthase	ABY79214.1	<i>Zea perennis</i> (Poaceae)
Gh (E)-beta-caryophyllene	XP_016727799.1	<i>Gossypium hirsutum</i> (Malvaceae)
Pl (E)-beta-caryophyllene synthase	ARQ83254.1	<i>Phaseolus lunatus</i> (Fabaceae)
Ok beta-caryophyllene synthase	AKA94109.1	<i>Ocimum kilimandscharicum</i> (Labiatae)
Li 9-epi-caryophyllene synthase	AGU13712.1	<i>Lavandula x intermedia</i> (Lamiaceae)
Zl (E)-beta-caryophyllene synthase	ABY79211.1	<i>Zea luxurians</i> (Poaceae)
Zmsm (E)-beta-caryophyllene synthase	EU259641.1	<i>Zea mays subsp. Mexicana</i> (Poaceae)
Sl caryophyllene/alpha-humulene synthase	GU647162.1	<i>Solanum lycopersicum</i> (Solanaceae)
Vvcg (E)-beta-caryophyllene synthase 1	HM807374.1	<i>Vitis vinifera</i> (Vitaceae)
Pd beta-caryophyllene synthase	AFR23370.1	<i>Phyla dulcis</i> (Verbenaceae)
Vvcd (E)-beta-caryophyllene synthase	JF808010.1	<i>Vitis vinifera</i> cultivar deying 84-1 (Vitaceae)
Vvcg (E)-beta-caryophyllene synthase 2	HM807373.1	<i>Vitis vinifera</i> isolate VvGwECar1 cultivar Gewurztraminer (Vitaceae)
Mcvr (-)-beta-caryophyllene synthase	I6RAQ6.1	<i>Matricaria chamomilla</i> var. recutita
Mm beta-caryophyllene synthase	ACN67535.1	<i>Mikania micrantha</i> (Asteraceae)
Hl alpha-humulene synthase	B6SCF5.1	<i>Humulus lupulus</i> (Cannabaceae)
Ag gamma-humulene synthase	AAC05728.1	<i>Abies grandis</i> (Pinaceae)
Zz alpha-humulene synthase	B1B1U3.1	<i>Zingiber zerumbet</i> (Zigiberaceae)
Pg alpha-humulene synthase	ADZ45513.1	<i>Picea glauca</i> (Pinaceae)
Pepg longifolene synthase	ADZ45515.1	<i>Picea engelmannii x Picea glauca</i> (Pinaceae)
Pa longifolene synthase 1	AAS47695.1	<i>Picea abies</i> (Pinaceae)
Ps longifolene synthase	ABV44454.1	<i>Pinus sylvestris</i> (Pinaceae)
Ps alpha-longipinene synthase	ADZ45516.1	<i>Picea sitchensis</i> (Pinaceae)
Pa longifolene synthase 2	Q675L0.1	<i>Picea abies</i> (Pinaceae)
Vo valerena-1,10-diene synthase	AGB05610.1	<i>Valeriana officinalis</i> (Valerianaceae)
Zm 7-epi-sesquithujene synthase	Q6JD73.1	<i>Zea mays</i> (Poaceae)
Ag alpha-bisabolene synthase	O81086	<i>Abies grandis</i> (Pinaceae)
Zm tps5-Dell	Q6JD70.1	<i>Zea mays</i> (Poaceae)
Zo(S)-beta-bisabolene synthase	D2YZP9	<i>Zingiber officinale</i> (Zigiberaceae)
At (Z)-gamma-bisabolene synthase	Q9T0J9.2	<i>Arabidopsis thaliana</i> (Brassicaceae)
Gb alpha bisabolene synthase	AIU94290.1	<i>Ginkgo biloba</i> (Ginkgoaceae)
Pm(E)-gamma-bisabolene synthase	Q4QSN4	<i>Pseudotsuga menziesii</i> (Pinaceae)
Sa beta-bisabolene synthase	E3W205	<i>Santalum austrocaledonicum</i> (Santalaceae)
Ss alpha-bisabolol synthase	E3W206	<i>Santalum spicatum</i> (Santalaceae)
Pa E-alpha-bisabolene synthase	AAS47689	<i>Picea abies</i> (Pinaceae)
Zo (S)-beta-bisabolene synthase	BAI67934.1	<i>Zingiber officinale</i> (Zigiberaceae)
Sb beta-sesquiphellandrene synthase	C5YHI2	<i>Sorghum bicolor</i> (Poaceae)
Ss a-bisabolol synthase	ADO87004.1	<i>Santalum spicatum</i> (Santalaceae)
Zo sesquiterpene synthase	BAI67935.1	<i>Zingiber officinale</i> (Zigiberaceae)
Ag E-alpha-bisabolene synthase	AAC24192.1	<i>Abies grandis</i> (Pinaceae)
Sa b-bisabolene synthase	ADO87003.1	<i>Santalum austrocaledonicum</i> (Santalaceae)
Ha (Z)-gamma-bisabolene synthase K11	AME16498.1	<i>Helianthus annuus</i> (Asteraceae)
Ha (Z)-gamma-bisabolene synthase K7	AME16497.1	<i>Helianthus annuus</i> (Asteraceae)
Zm (S)-beta-macrocarpene synthase 1	AAS88576.1	<i>Zea mays</i> (Poaceae)
Zm(S)-beta-macrocarpene synthase 2	Q1EG72.1	<i>Zea mays</i> (Poaceae)
Ee bisabolol synthase	AYJ71561.1	<i>Eremanthus erythropappus</i>
Pd(+)-epi-alpha-bisabolol synthase	J7LH11	<i>Phyla dulcis</i> (Verbenaceae)
Aa bisabolol synthase	AFV40969.1	<i>Artemisia annua</i> (Asteraceae)
Am (+)-alpha-bisabolol synthase	BAW34954.1	<i>Artemisia maritima</i> (Asteraceae)
Ak (+)-alpha-bisabolol synthase	BAW34955.1	<i>Artemisia kurramensis</i> (Asteraceae)

Mcvr a-bisabolol synthase	AIW00681.1	<i>Matricaria chamomilla</i> var. <i>recutita</i>
Aabs koidzumiol synthase	BAW34956.1	<i>Artemisia absinthium</i> (Asteraceae)
Aabs koidzumiol synthase	BAW34957.1	<i>Artemisia absinthium</i> (Asteraceae)
Aa amorpho-4,11-diene synthase	Q9AR04.2	<i>Artemisia annua</i> (Asteraceae)
Aa epi-cedrol synthase	CAC08805.1	<i>Artemisia annua</i> (Asteraceae)
Mcvr sesquiterpene synthase 1	AIG92846.1	<i>Matricaria chamomilla</i> var. <i>recutita</i> (Asteraceae)
Mcvr sesquiterpene synthase 4	AIG92848.1	<i>Matricaria chamomilla</i> var. <i>recutita</i> (Asteraceae)
Vvcpn beta-curcumene synthase	HM807381.1	<i>Vitis vinifera</i> isolate VvPNbCur cultivar Pinot Noir (Vitaceae)
Pc gamma-curcumene synthase	AY508726.1	<i>Pogostemon cablin</i> (Lamiaceae)
Vvcg (E)-alpha-bergamotene synthase	HM807376.2	<i>Vitis vinifera</i> isolate VvGwaBer cultivar Gewurztraminer (Vitaceae)
Pd trans-alpha-bergamotene synthase	J7LQ09.1	<i>Phyla dulcis</i> (Verbenaceae)
La exo-alpha-bergamotene synthase	Q2XSC4.1	<i>Lavandula angustifolia</i> (Lamiaceae)
Sa santalene bergamotene synthase 1	ADP30866.1	<i>Santalum album</i> (Santalaceae)
Sa santalene bergamotene synthase 2	ADP30867.1	<i>Santalum album</i> (Santalaceae)
Ss santalene synthase	ADO87002.1	<i>Santalum spicatum</i> (Santalaceae)
Sau santalene synthase	ADO87001.1	<i>Santalum austrocaledonicum</i> (Santalaceae)
Sb zingiberene synthase	C5YHH7	<i>Sorghum bicolor</i> (Poaceae)
Ob alpha-zingiberene synthase	Q5SBP4.1	<i>Ocimum basilicum</i> (Labiatae)
At alpha-barbatene synthase	Q4KSH9.2	<i>Arabidopsis thaliana</i> (Brassicaceae)

**Table S2** Oligo-nucleotide primers used in this study.

Name	Sequence (5'-3')	Purpose
pET SmSTPS3-F:	TTCCATGGCTATGGCTACAACAAGTAGAAAT	Protein expression
pET SmSTPS3-R:	ACGCGTCGACCTACATCTTGATAGGATCCACGAA C	Protein expression
pET AaGAS-F:	CGGGAGCTCATGGCAGCGGTTCAAGCTAATG	Protein expression
pET AaGAS-R:	CGGCTCGAGTTACACGGGGTAGAGAACCCAC	Protein expression
pET AtTPS21-F:	CGGGATCCATGGGGAGTGAAGTCAACCG	Protein expression
pET AtTPS21-R:	CGGAGCTCTCAAATGGGTATAGTTTCAATGAGCA	Protein expression
pET PTSiso-F:	TTTGGATCCATGGAGTTGTATGCCCAAAGTGTTG	Protein expression
pET PTSiso-R:	TTTGAGCTCATATGGAACAGGGTGAAGGTACAAC T	Protein expression
pET AaCES-F:	CGCGGATCCATGTCTCTTATAGTAGAGGATGTC	Protein expression
pET AaCES-R:	AAAGAGCTCTCATGTAATGATGGCATCAAC	Protein expression
SmSTPS3-C438G-F:	GGTCTCCTCATTGGCCGAGTCAT	mutagenesis
SmSTPS3-C438G-R:	ATGACTCGGCAATGAGGAGACC	mutagenesis
SmSTPS3-C438S-F:	GGTCTCCTCATTAGCCGAGTCAT	mutagenesis
SmSTPS3-C438S-R:	ATGACTCGGCTAATGAGGAGACC	mutagenesis
AaGAS-S454G-F:	CTGAGTTGATTGGAAGACTTCAAG	mutagenesis
AaGAS-S454G-R:	CTTGAAGTCTTCCAATCAACTCAG	mutagenesis
AaGAS-S454C-F:	CTGAGTTGATTGTAGACTTCAAG	mutagenesis
AaGAS-S454C-R:	CTTGAAGTCTACAAATCAACTCAG	mutagenesis
AtTPS21-S441G-F:	CTGAGATAATAGGTCGATTCACA	mutagenesis
AtTPS21- S441G-R:	TGTGAATCGACCTATTATCTCAG	mutagenesis
AtTPS21- S441C-F:	CTGAGATAATATGTCGATTCACA	mutagenesis
AtTPS21- S441C-R:	TGTGAATCGACATATTATCTCAG	mutagenesis
PTSiso-A445G-F:	CGTTAATCATTGGTAGGCTCATC	mutagenesis
PTSiso-A445G-R:	GATGAGCTACCAATGATTAACG	mutagenesis
PTSiso-A445C-F:	CGTTAATCATTGTAGGCTCATC	mutagenesis
PTSiso-A445C-R:	GATGAGCTACAAATGATTAACG	mutagenesis
PTSiso-A445S-F:	CGTTAATCATTAGTAGGCTCATC	mutagenesis
PTSiso-A445S-R:	GATGAGCTACTAATGATTAACG	mutagenesis

AaCES-C439G-F:  
AaCES-C439G-R:

GTTGTCTACTTGGTAGAACCAT  
ATGTTCTACCAAGTAGACAAC

mutagenesis  
mutagenesis

**Table S3** Product profiles of SmSTPS3, AaGAS and their mutants (%).

Compounds	SmSTPS3 Wt	SmSTPS:3C438G	SmSTPS:3C438S	AaGAS Wt	AaGAS:S454G	AaGAS:S454C
(-)-5-epieremophilene	100.0	39.4	92.2	—	—	—
$\beta$ -elemene	— <sup>a</sup>	5.9	5.3	93.5	19.5	93.8
elemol	—	54.7	2.5	—	74.4	—
unknown sesquiterpene	—	—	—	6.5	0.8	6.2
$\beta$ -caryophyllene	—	—	—	—	4.3	—
$\alpha$ -humulene	—	—	—	—	1.0	—

**Table S4** Product profiles of AtTPS21 and its mutants (%).

Compounds	AtTPS21 Wt	AtTPS21:S441G	AtTPS21:S441C
$\beta$ -caryophyllene	92.9	24.5	52.9
$\alpha$ -humulene	7.1	3.4	10.0
elemol	— <sup>a</sup>	69.2	—
guaiol	—	2.9	—
$\alpha$ -copaene	—	—	6.2
$\beta$ -copaene	—	—	3.3
$\gamma$ -muurolene	—	—	1.9
germacrene D	—	—	1.3
cubebol	—	—	2.4
$\delta$ -cadinene	—	—	1.6
germacrene D-4-ol	—	—	6.7
copaborneol	—	—	5.1
torreyol	—	—	8.6

**Table S5** Product profiles of PTSiso and its mutants (%).

Compounds	PTSiso Wt	PTSiso:A445S	PTSiso:A445G	PTSiso:A445C
$\beta$ -patchoulene	1.8	1.9	1.2	3.2
$\beta$ -elemene	6.4	11.3	6.4	37.5
$\beta$ -caryophellene	3.2	2.0	1.8	4.6
$\alpha$ -guaiene	14.6	7.0	9.6	7.2
seychellene	6.4	3.4	2.7	1.2
$\alpha$ -patchoulene	3.4	3.0	1.8	1.8
$\gamma$ -patchoulene	1.4	2.4	0.8	1.6
guai-4,11-diene	4.9	6.0	3.6	4.2
$\alpha$ -bulnesene	26.4	15.4	30.3	11.4
unknown sesquiterpene	1.6	1.7	1.0	5.6
elemol	— <sup>a</sup>	—	4.8	1.4
unknown sesquiterpene	—	—	—	5.3
alcohol				
pogostol	2.6	2.6	3.4	2.6
patchoulol	27.2	43.3	12.4	11.1

**Table S6** Retention indices of new products generated by mutant enzymes.

Compounds	RI recorded	RI of reference
elemol	1550	1550 <sup>[11]</sup>
guaiol	1597	1597 <sup>[12]</sup>
$\alpha$ -copaene	1377	1377 <sup>[9]</sup>
$\beta$ -copaene	1430	1430 <sup>[9]</sup>
$\gamma$ -muurolene	1478	1478 <sup>[9]</sup>
germacrene D	1481	1481 <sup>[9]</sup>
cubebol	1517	1517 <sup>[9]</sup>
$\delta$ -cadinene	1525	1525 <sup>[9]</sup>
germacrene D-4-ol	1577	1577 <sup>[9]</sup>
copaborneol	1608	1608 <sup>[9]</sup>
torreyol	1647	1647 <sup>[9]</sup>
farnesol	1700	1700 <sup>[11]</sup>

**Table S7** Kinetic parameters of SmSTPS3, AaGAS, AtTPS21, PTSiso and their mutants with FDP.

	<i>K<sub>m</sub></i> ( $\mu$ M)	<i>k<sub>cat</sub></i> ( $s^{-1}$ )	<i>k<sub>cat</sub>/K<sub>m</sub></i> ( $s^{-1} \text{ mM}^{-1}$ )
SmSTPS3 WT	6.56 $\pm$ 0.43	(0.44 $\pm$ 0.02) $\times 10^{-1}$	6.71 $\pm$ 0.45
C486G	5.87 $\pm$ 0.61	(0.34 $\pm$ 0.03) $\times 10^{-1}$	5.79 $\pm$ 0.56
AaGAS WT	5.39 $\pm$ 0.37	(0.56 $\pm$ 0.04) $\times 10^{-1}$	10.39 $\pm$ 0.72
S454G	5.57 $\pm$ 0.51	(0.48 $\pm$ 0.03) $\times 10^{-1}$	8.62 $\pm$ 0.49
AtTPS21 WT	3.43 $\pm$ 0.32	(0.17 $\pm$ 0.02) $\times 10^{-1}$	4.91 $\pm$ 0.41
S439G	3.26 $\pm$ 0.30	(0.12 $\pm$ 0.02) $\times 10^{-1}$	3.68 $\pm$ 0.37
S439C	5.13 $\pm$ 0.45	(0.06 $\pm$ 0.01) $\times 10^{-1}$	1.17 $\pm$ 0.09
PTSiso WT	6.83 $\pm$ 0.52	(0.32 $\pm$ 0.02) $\times 10^{-2}$	0.46 $\pm$ 0.03
A455G	6.46 $\pm$ 0.48	(0.43 $\pm$ 0.04) $\times 10^{-2}$	0.66 $\pm$ 0.05
A455S	4.14 $\pm$ 0.58	(0.22 $\pm$ 0.01) $\times 10^{-2}$	0.53 $\pm$ 0.04
A455C	9.85 $\pm$ 0.49	(0.34 $\pm$ 0.03) $\times 10^{-2}$	0.34 $\pm$ 0.03

## References

- [1] Fang X, Li JX, Huang JQ, et al., Systematic identification of functional residues of *Artemisia annua* amorpho-4,11-diene synthase, *Biochem J* 2017; 474: 2191–2202.
- [2] Fang X, Li CY, Yang Y, et al., Identification of a novel (-)-5-epieremophilene synthase from *Salvia miltiorrhiza* via transcriptome mining. *Front Plant Sci* 2017; 8: 627.

- [3] Berteau CM, Voster A, Verstappen FW, et al., Isoprenoid biosynthesis in *Artemisia annua*: cloning and heterologous expression of a germacrene A synthase from a glandular trichome cDNA library. Arch Biochem Biophys 2006; 448: 3–12.
- [4] Tholl D, Chen F, Petri J, et al., Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from *Arabidopsis* flowers. Plant J 2005; 42: 757–771.
- [5] Hartwig S, Frister T, Alemdar S, et al., Expression, purification and activity assay of a patchoulol synthase cDNA variant fused to thioredoxin in *Escherichia coli*. Protein Express Purif 2014; 97: 61–71.
- [6] Deguerry F, Pastore L, Wu S, et al., The diverse sesquiterpene profile of patchouli, *Pogostemon cablin*, is correlated with a limited number of sesquiterpene synthases. Arch Biochem Biophys 2006; 448: 123–136.
- [7] Hua L, Matsuda SPT, The molecular cloning of 8-epicedrol synthases from *Artemisia annua*. Arch Biochem Biophys 1999; 369: 208–212.
- [8] Liang J, Liu J, Brown R, et al., Direct production of dihydroxylated sesquiterpenoids by a maize terpene synthase. Plant J 2018; 94: 847–856.
- [9] Garms S, Köllner TG, Boland W, A multiproduct terpene synthase from *Medicago truncatula* generates cadalane sesquiterpenes via two different mechanisms. J Org Chem 2010; 75: 5590–5600.
- [10] Vardakou M, Salmon M, Faraldos JA, et al., Comparative analysis and validation of the malachite green assay for the high throughput biochemical characterization of terpene synthases, MethodsX 2014; 1: 187–196.
- [11] Xie Y, Wang J, Yang F, et al., Comparative analysis of essential oil components of two *Cryptomeria* species from China. Ind Crop Prod 2011; 34: 1226–1230.
- [12] Dural H, Bagci Y, Ertugrul K, et al., Essential oil composition of two endemic *Centaurea* species from Turkey, *Centaurea mucronifera* and *Centaurea chrysantha*, collected in the same habitat. Biochem Syst Ecol 2003; 31: 1417–1425.