

***New Phytologist* Supporting Information Figs S1-S18, Methods S1 and Tables S1-S7**

Article title: The complete functional characterization of the terpene synthase family in tomato

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The following Supporting Information is available for this article:

Fig. S1 Annotated sequences of *TPS51* and *TPS52*.

Fig. S2 SDS-PAGE analysis of the purified recombinant His-tagged TPS proteins.

Fig. S3 Mass spectra of identified terpenes from Figs 3-5 and 10.

Fig. S4 GC-MS analysis of elemol and (+)-hedycaryol.

Fig. S5 GC-MS analysis of the products formed in planta by transiently co-expressing TPS with CPT genes in *Nicotiana benthamiana* leaves.

Fig. S6 Analysis of tomato terpene synthases with isoprene synthases from other plants.

Fig. S7 Sequence alignment of the proteins encoded by the functional tomato TPS-c genes and CPS genes from other plants.

Fig. S8 Predictions of subcellular localization for TPS and prenyltransferase proteins made by TargetP 1.1 (a) and ChloroP 1.1 (b).

Fig. S9 Phylogenetic analysis of tomato TPT homologs and TPTs identified in other plants.

Fig. S10 GC-MS analysis of the products formed in planta by transiently co-expressing TPS with TPT genes in *Nicotiana benthamiana* leaves.

Fig. S11 Sequence alignment of five Arabidopsis sesterterpene synthases and tomato TPSs from TPS-a, TPS-b and TPS-g clades.

Fig. S12 Sequence alignment of the proteins encoded by the functional tomato TPS-a genes.

Fig. S13 Sequence alignment of the proteins encoded by the functional tomato TPS-b and TPS-g genes.

Fig. S14 Sequence alignment of the proteins encoded by the functional tomato TPS-e/f

genes.

Fig. S15 Sequence analysis of TPT homologs from tomato and other plants.

Fig. S16 GC-MS analysis of terpenes extracted from tomato leaves.

Fig. S17 Correlation analysis of terpene biosynthesis genes and volatile terpenes from tomato.

Fig. S18 Terpene biosynthesis gene clusters identified from tomato genome by plantiSMASH.

Table S1 Primers used in this study.

Table S2 A list of terpene compounds identified from Figs 3-5 and 10.

Table S3 A list of tomato terpene synthase genes, their characteristics, and the enzymes they encode.

Table S4 A list of tomato terpene synthase pseudogenes.

Table S5 A list of the *trans*-prenyltransferase genes in tomato.

Table S6 A list of terpene compounds produced by all the functional tomato terpene synthases.

Table S7 A list of Arabidopsis terpene synthase genes, their characteristics, and the enzymes they encode.

Methods S1 Details on experimental procedures.

Fig. S1 Annotated sequences of *TPS51* and *TPS52*. Exons are shading in yellow, gaps in the reference genome (https://solgenomics.net/organism/Solanum_lycopersicum/genome; the 2017 release of version SL3.0) are underlined and filled by sequencing the cloned genomic DNA. Stop codons are shown in red. The obtained genomic sequences of *TPS51* and *TPS52* have been deposited to GenBank under accession numbers MN366354 and MN366355, respectively. The genomic sequences of other genes studied in this work can be found in the tomato genome database under loci listed in Tables S3-S5.

>TPS51 genomic sequence

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Fig. S2 SDS-PAGE analysis of the purified recombinant His-tagged TPS proteins. T, total protein after IPTG induction; S, soluble fraction; P, purified fraction with Ni-NTA agarose as described in Methods S1.

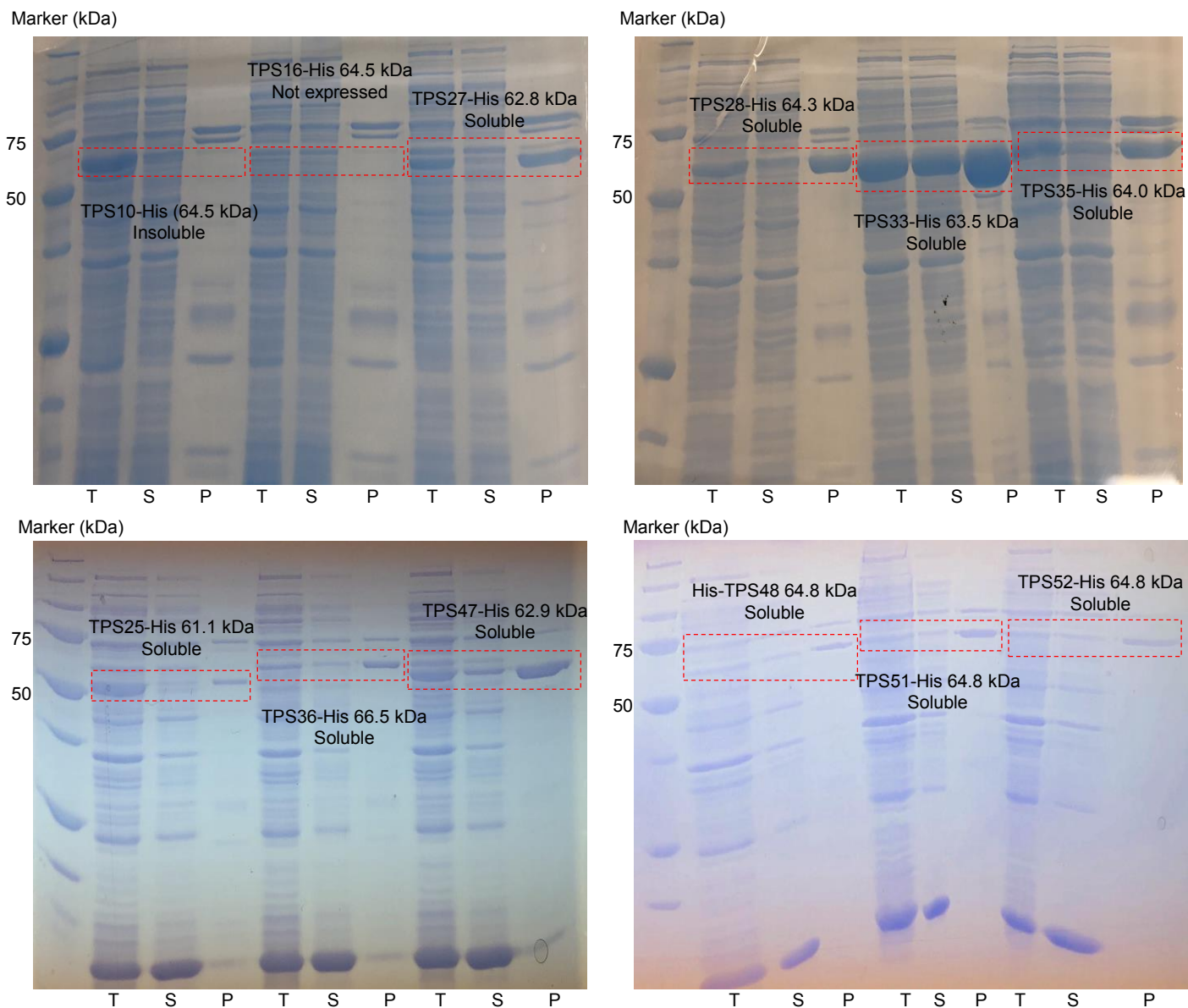
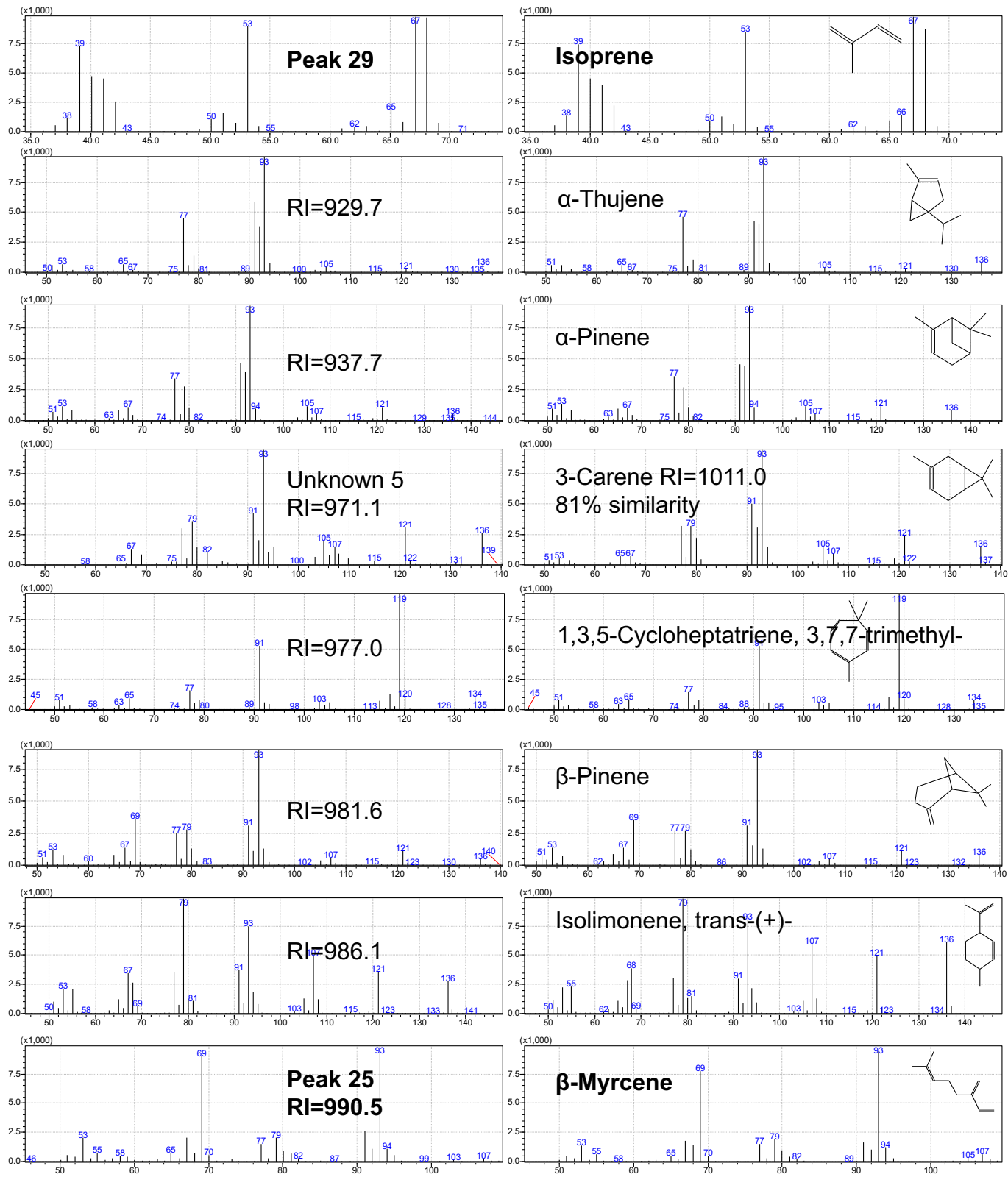
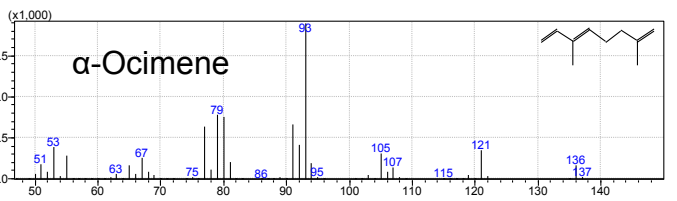
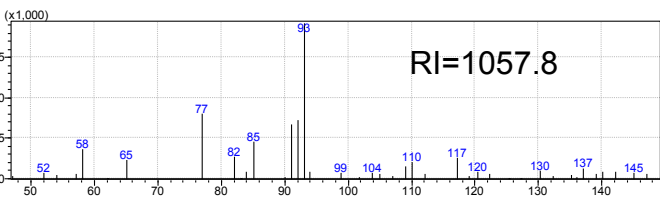
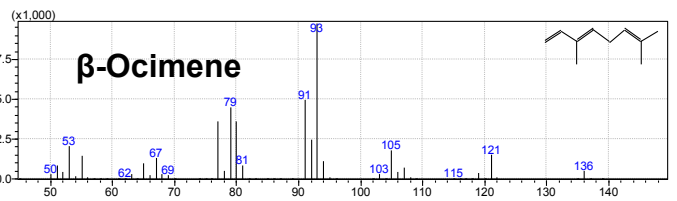
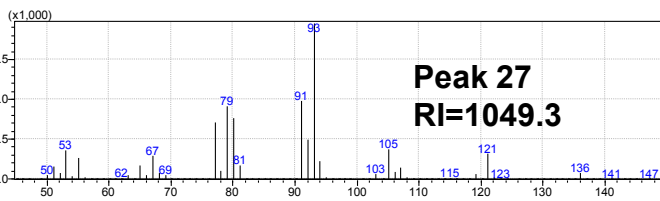
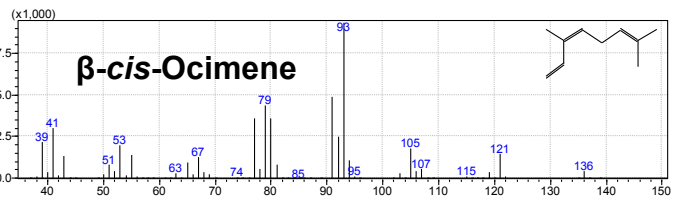
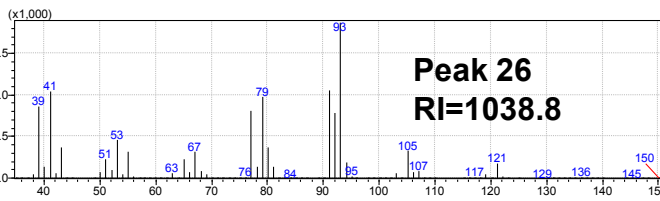
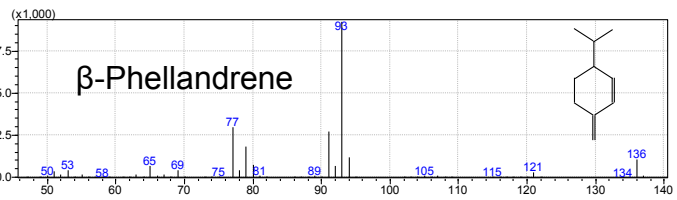
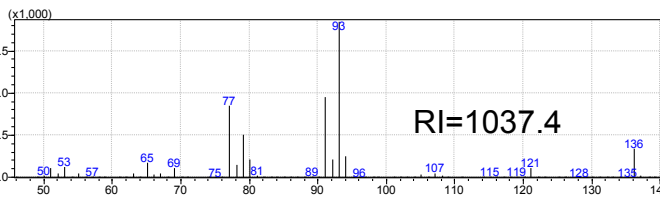
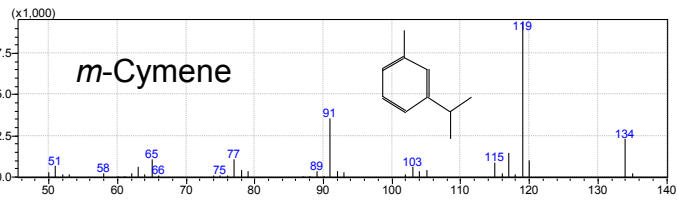
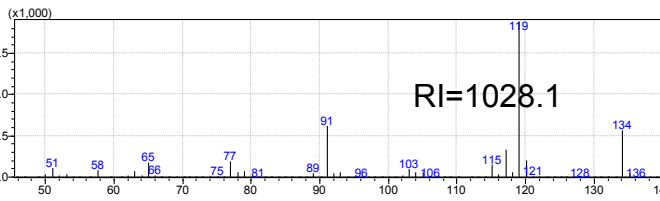
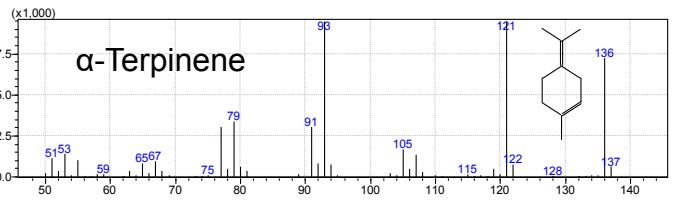
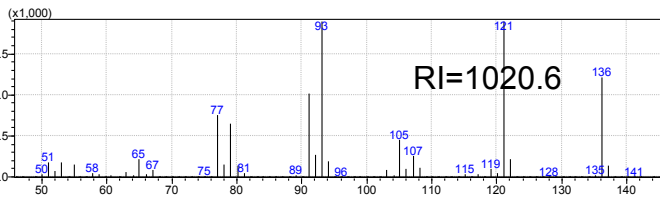
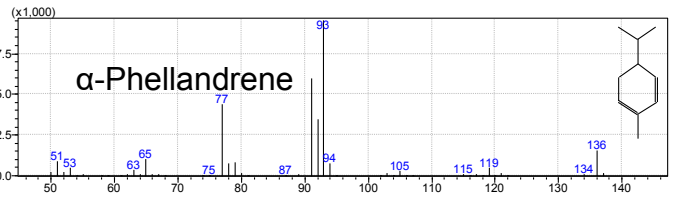
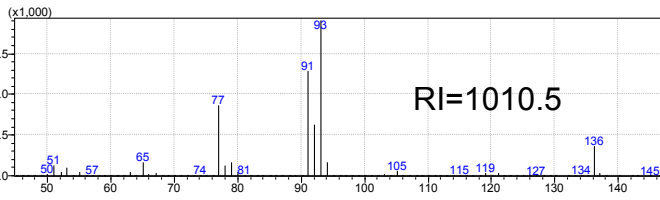
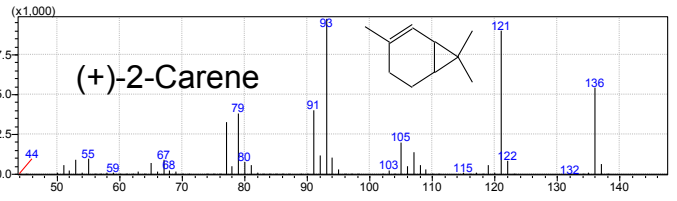
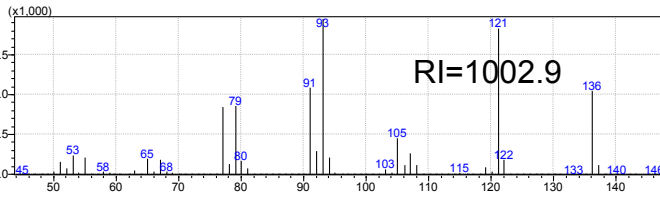
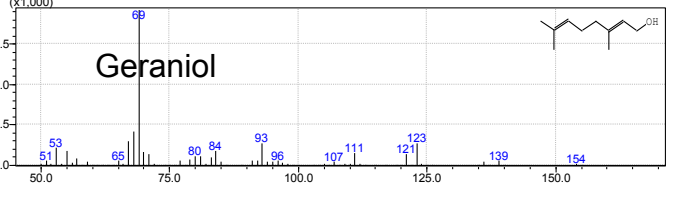
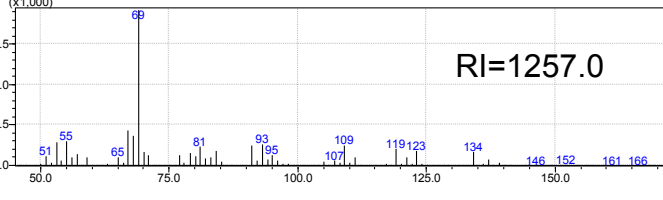
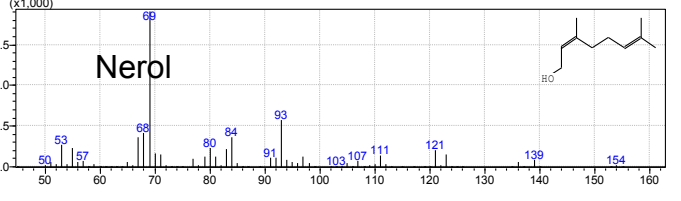
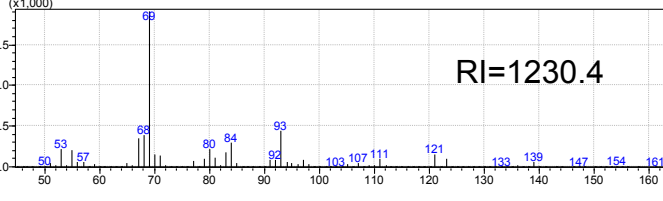
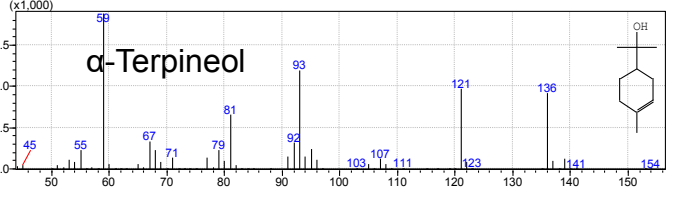
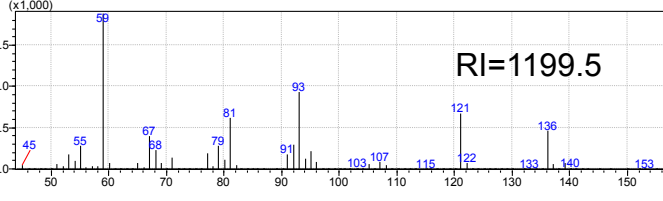
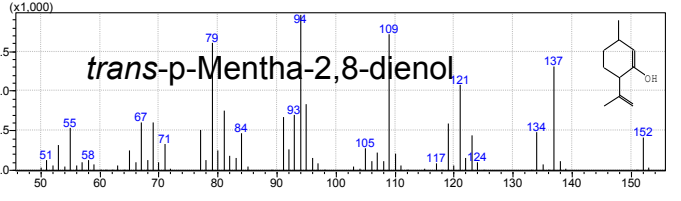
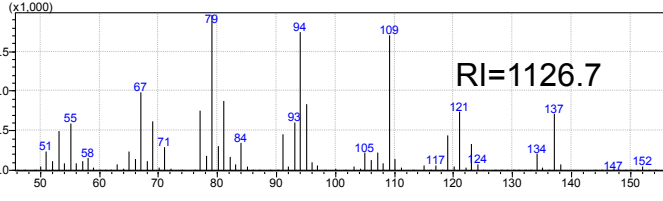
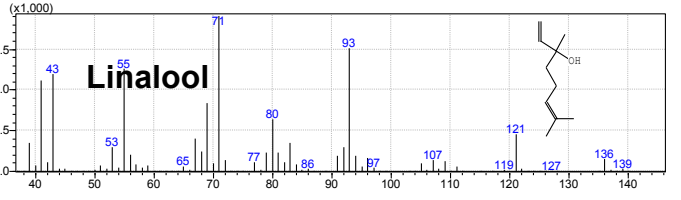
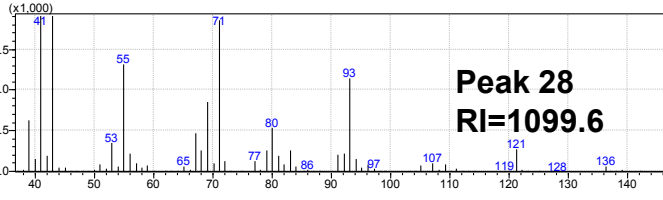
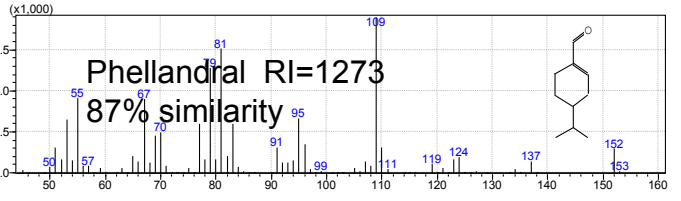
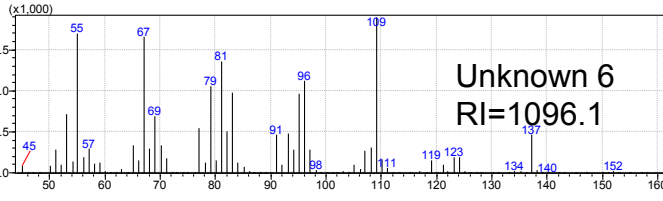
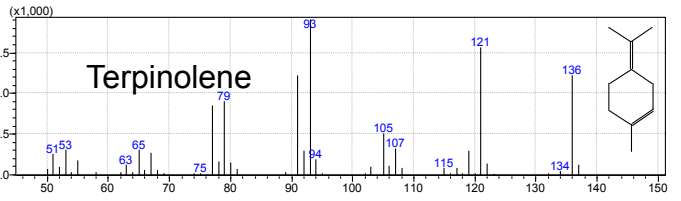
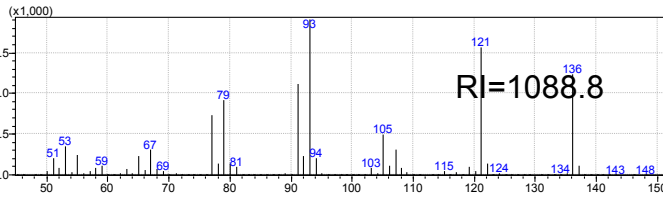
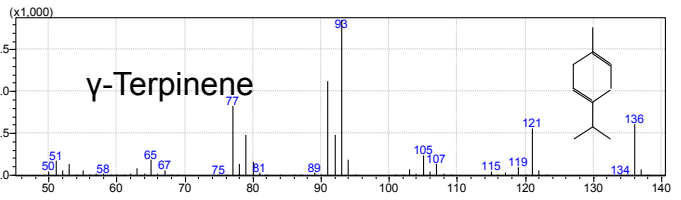
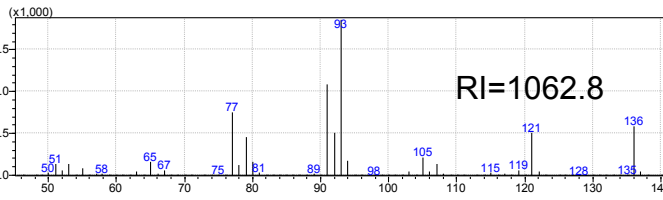
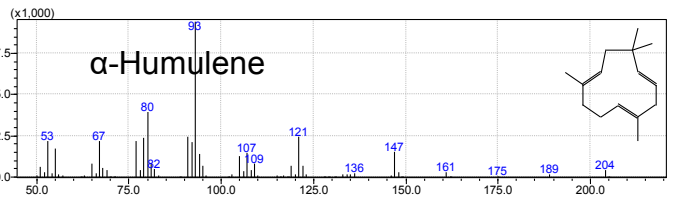
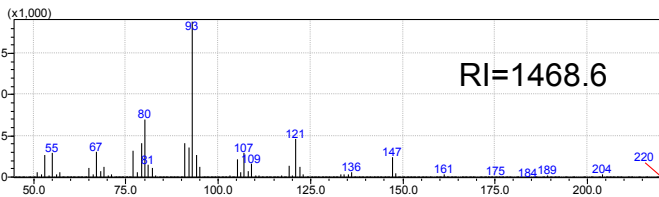
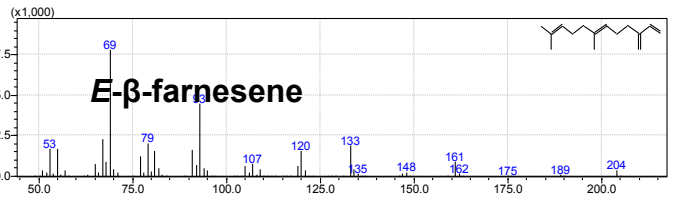
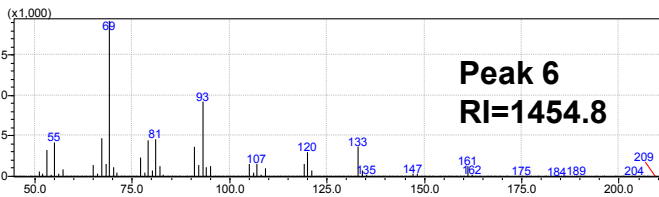
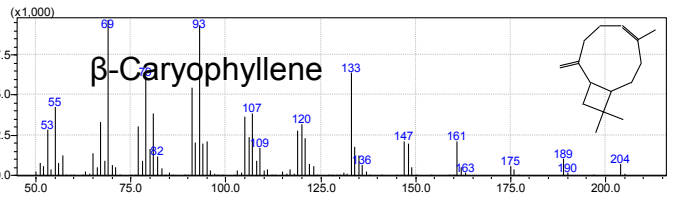
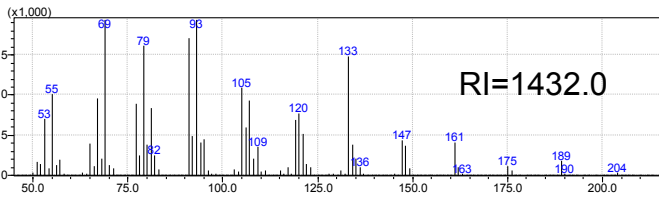
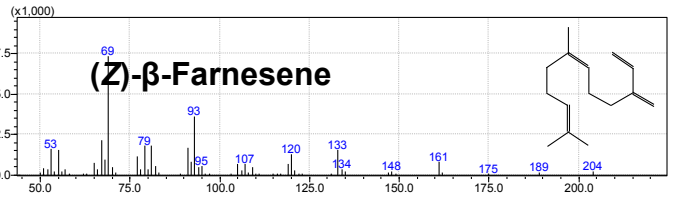
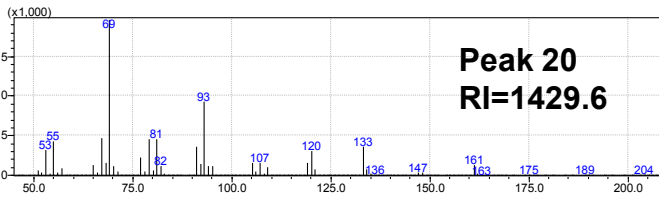
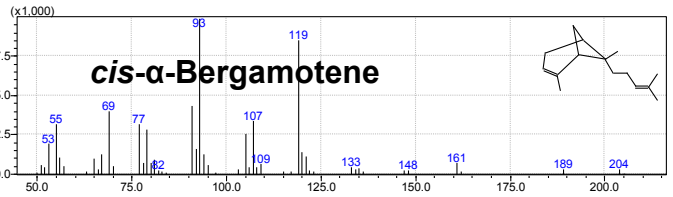
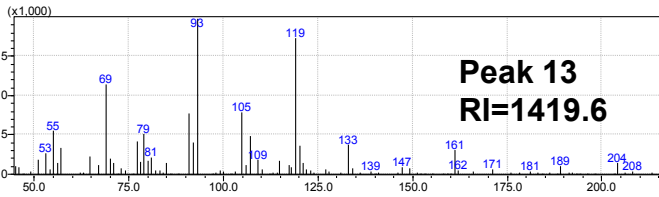
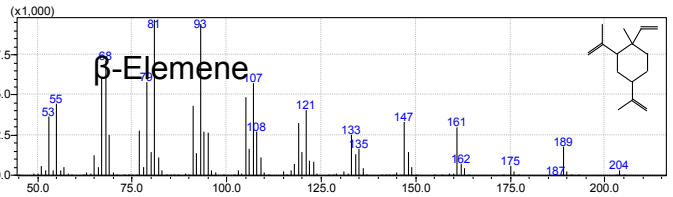
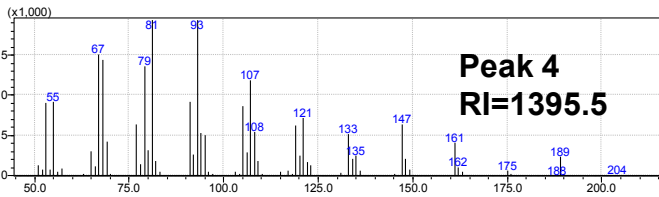
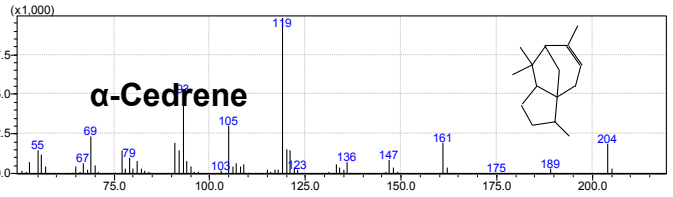
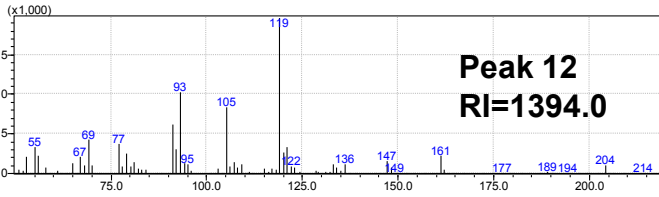
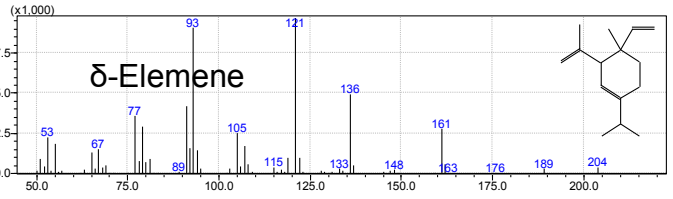
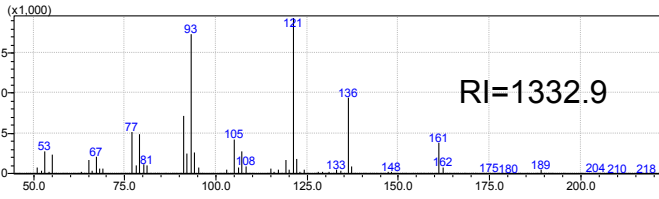


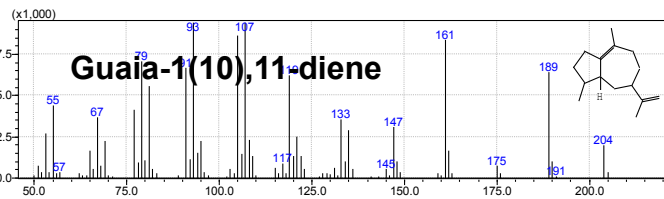
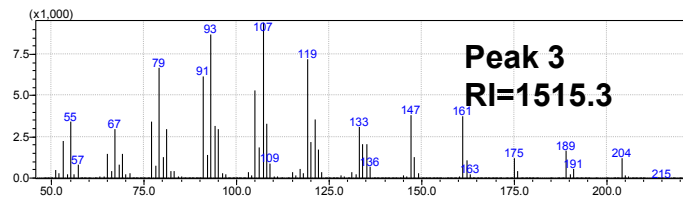
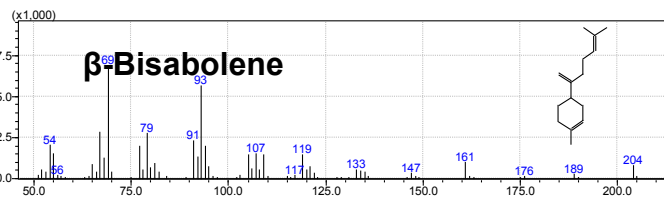
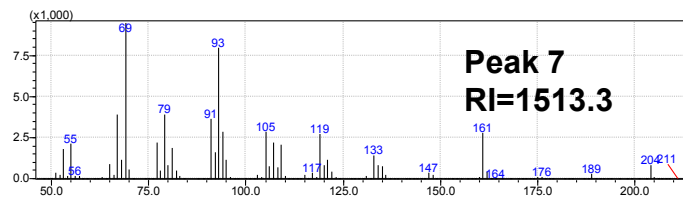
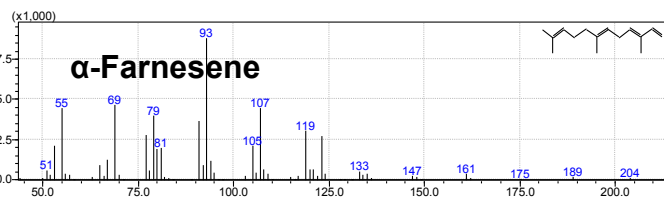
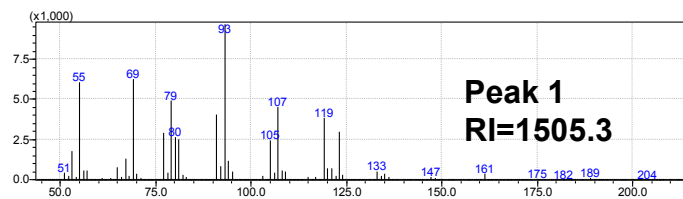
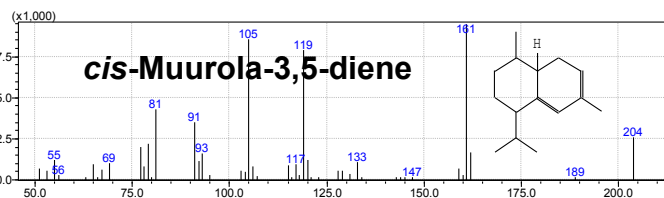
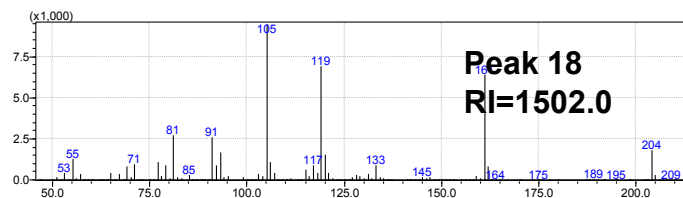
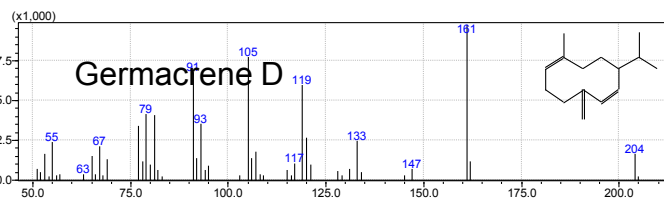
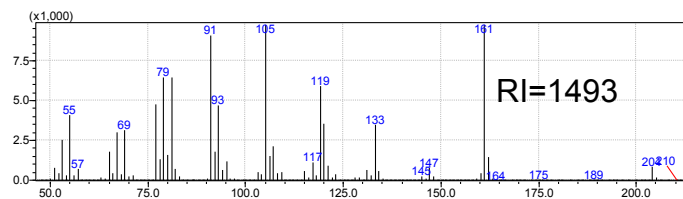
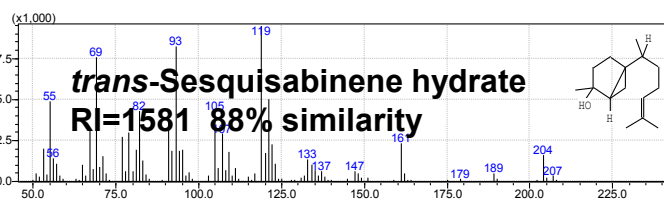
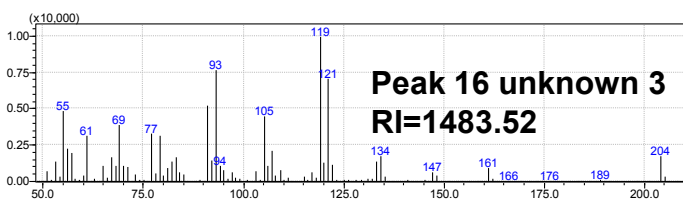
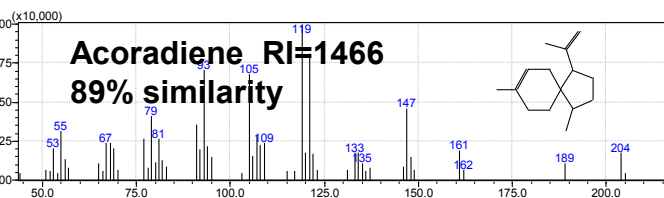
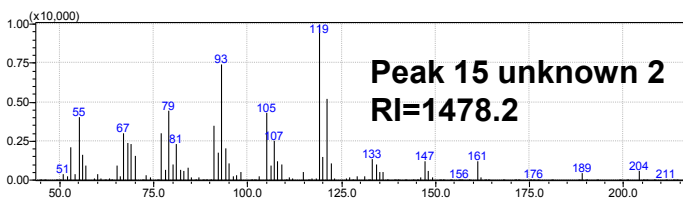
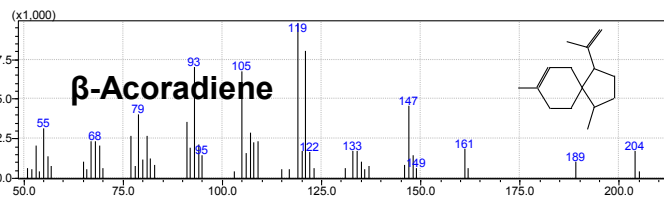
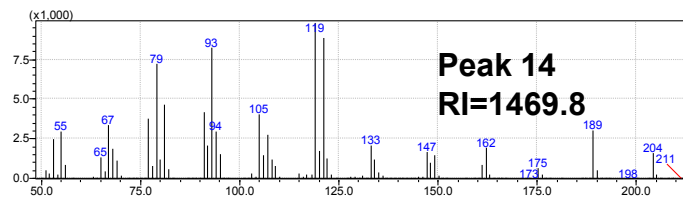
Fig. S3 Mass spectra of identified terpenes from Figs 3-5 and 10. The mass spectra of identified terpenes from NIST library are shown on the right. Compounds listed in order of elution from the Rxi-5Si1 MS column, the enzymatic products are shown in bold. Detailed information can be found in Table S2.

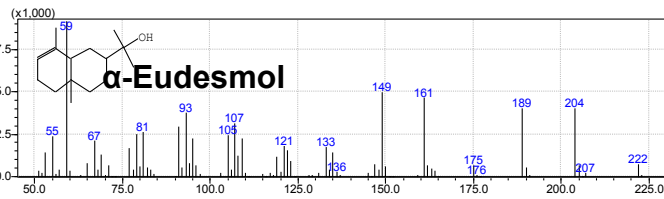
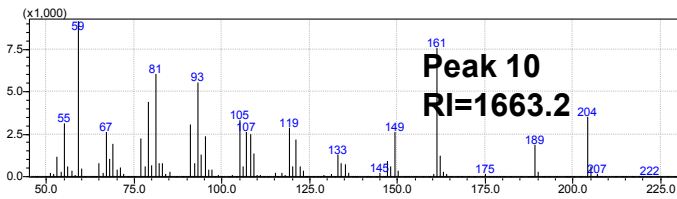
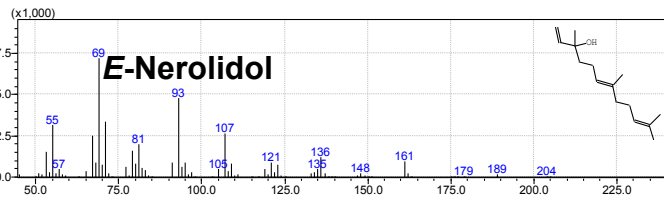
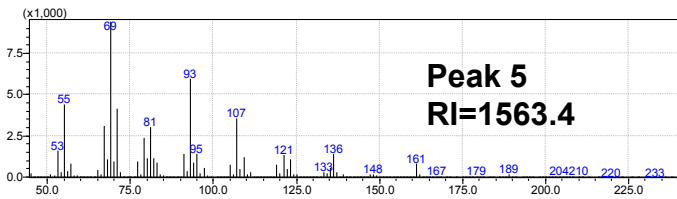
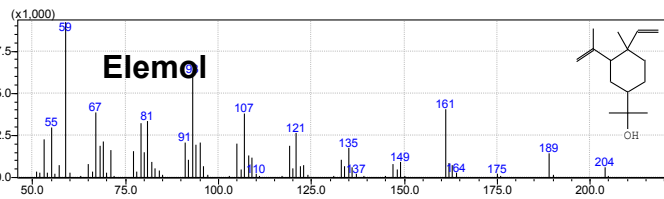
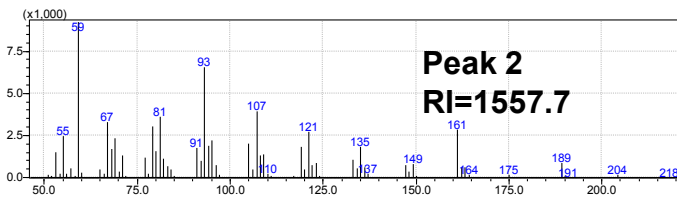
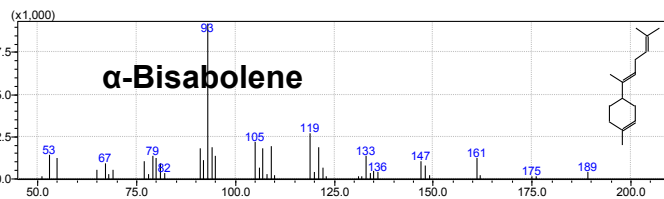
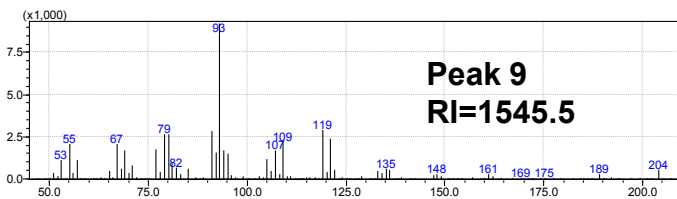
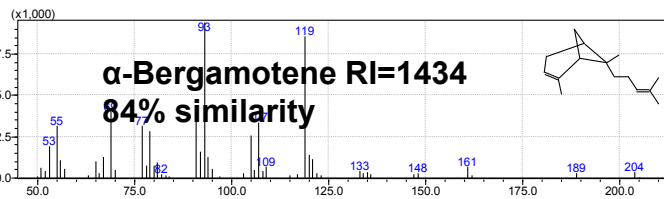
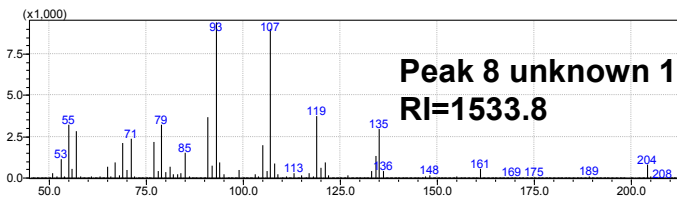
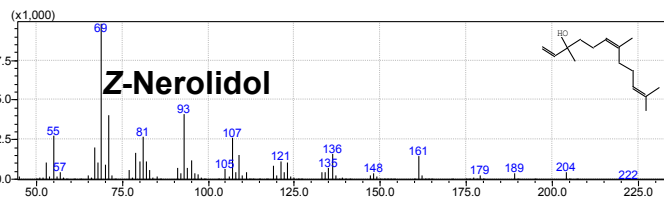
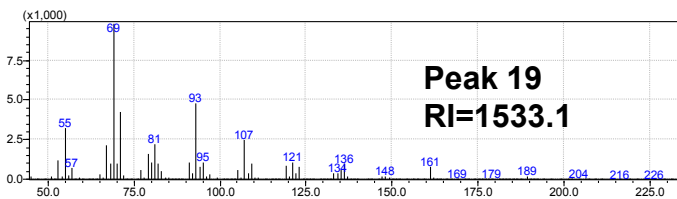
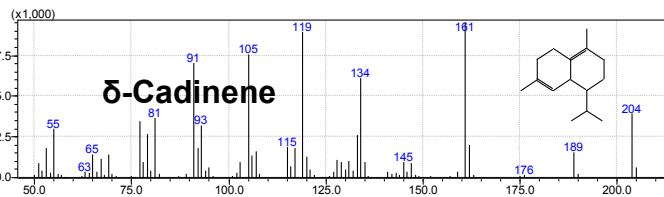
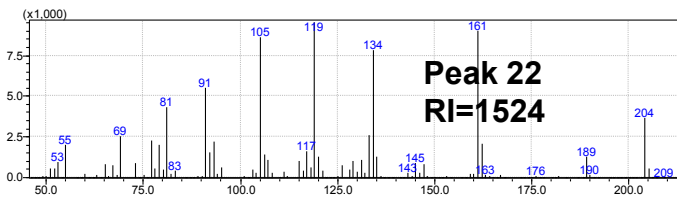
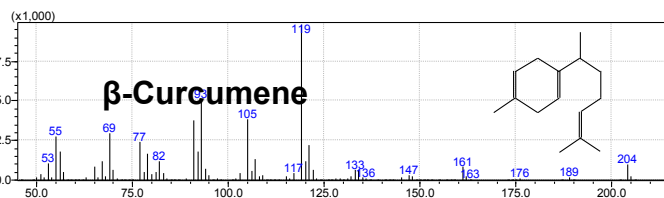
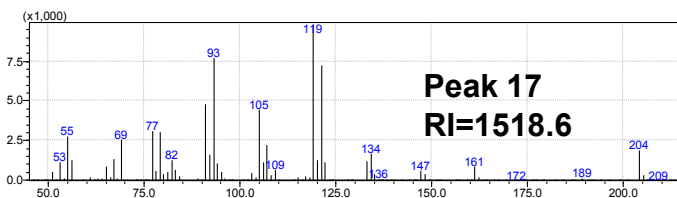












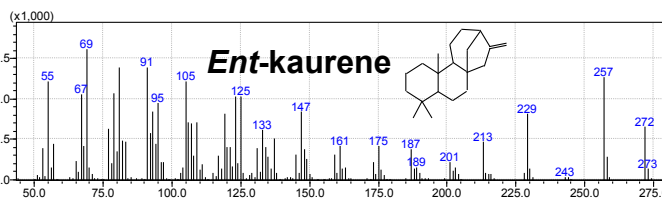
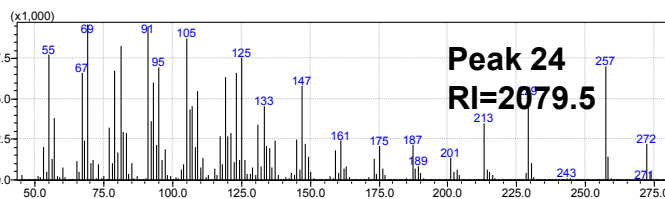
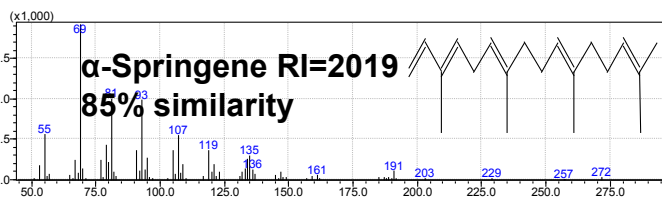
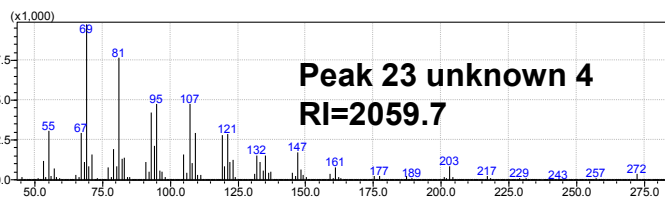
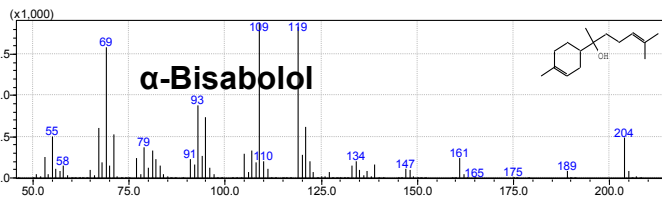
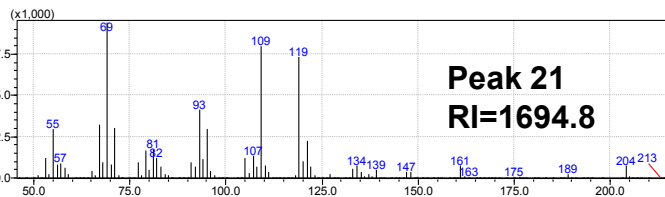
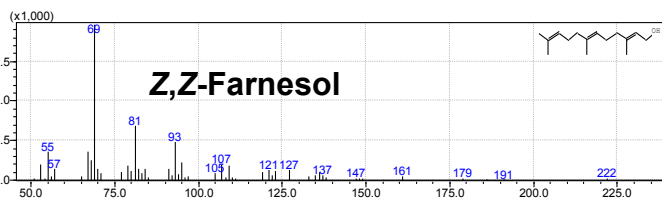
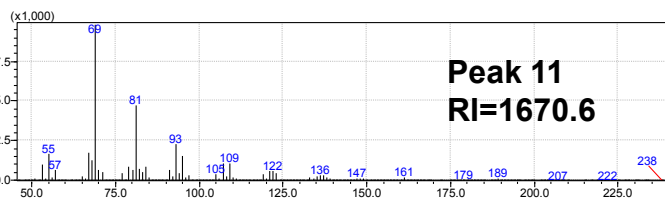


Fig. S4 GC-MS analysis of elemol and (+)-hedycaryol. Elemol is the thermal breakdown product of (+)-hedycaryol, which can be detected by lowering the injection port temperature.

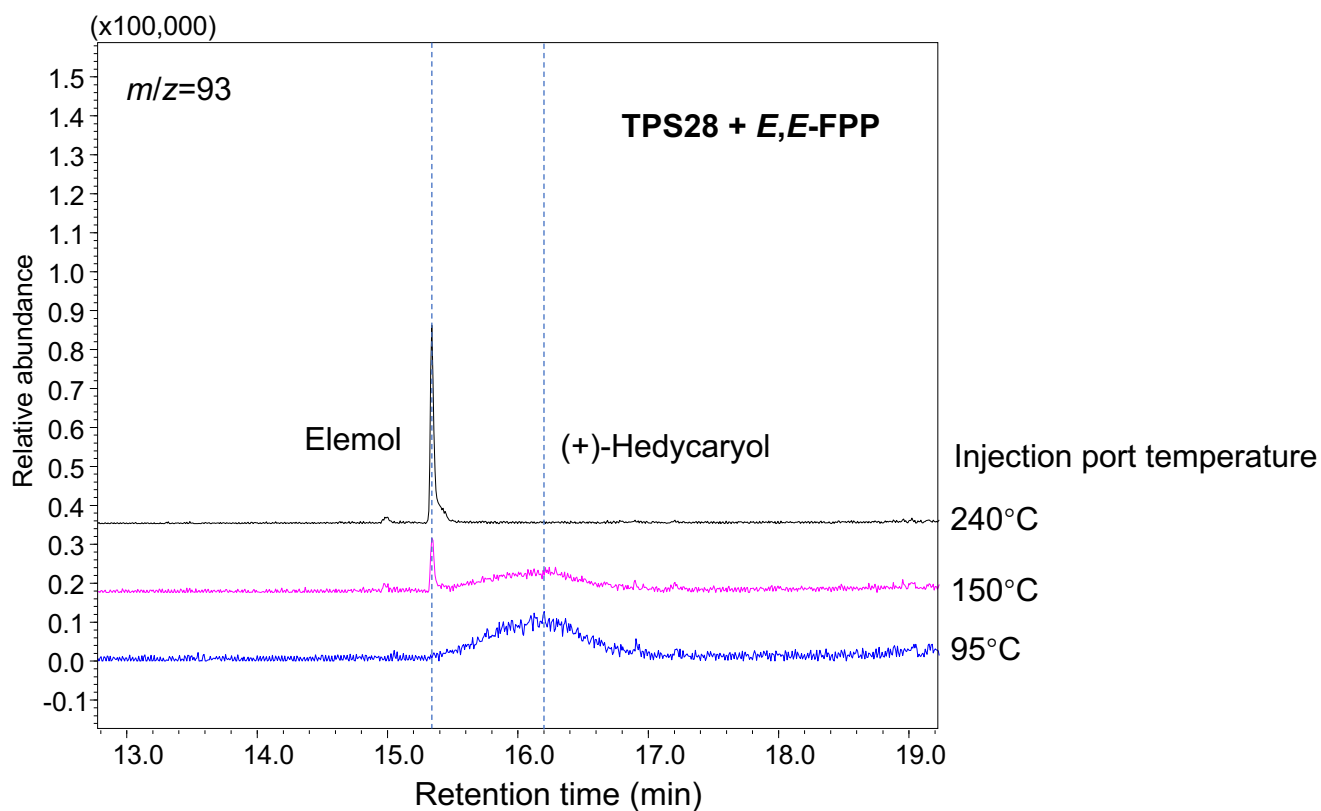


Fig. S5 GC-MS analysis of the products formed in planta by transiently co-expressing TPS with CPT genes in *Nicotiana benthamiana* leaves.

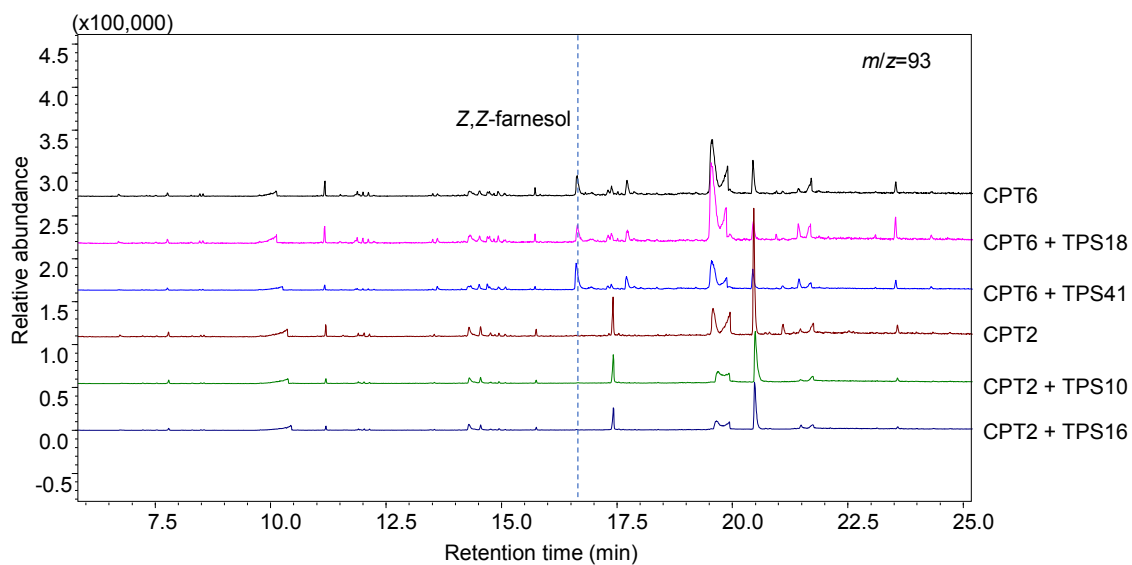
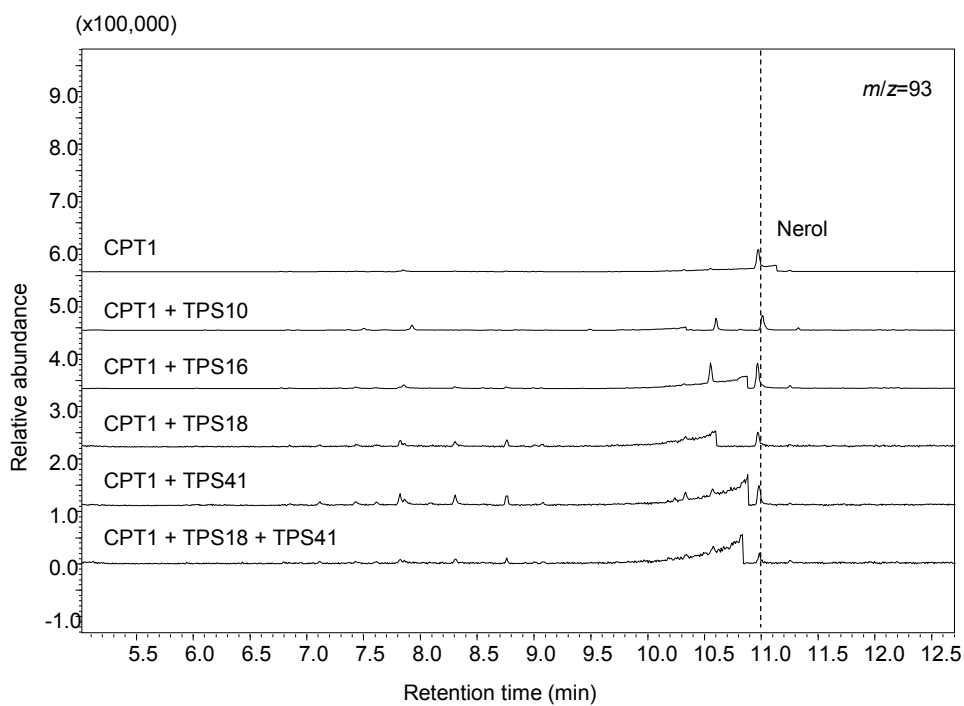


Fig. S6 Analysis of tomato terpene synthases with isoprene synthases from other plants. (a) Phylogenetic analysis of 34 tomato TPSs with three functionally characterized isoprene synthases. The three TPSs clustered with known isoprene synthase are shown in bold and shaded in green. (b) Multiple sequence alignment tomato TPSs from TPS-a, TPS-b and TPS-g clades with isoprene synthases. Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. Amino acids belonging to the ‘isoprene score’ amino acids are shown in red, the calculated “isoprene score” is shown next to each TPS. Among the calculated tomato TPSs, TPS47 has the highest isoprene score and is shown in bold. PalspS, isoprene synthase from *Populus alba* (AB198180); EgIspS, isoprene synthase from *Eucalyptus globulus* (BAF02831.1); PmIspS, isoprene synthase from *Pueraria montana* (AY316691.1).

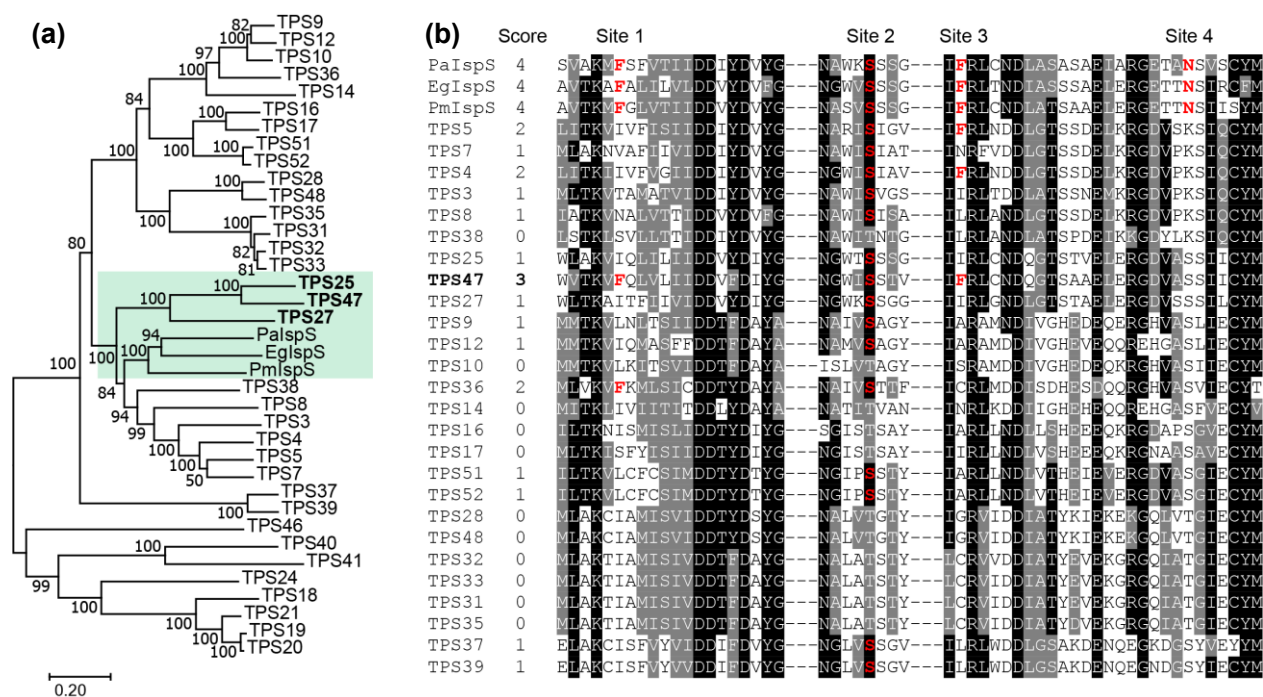
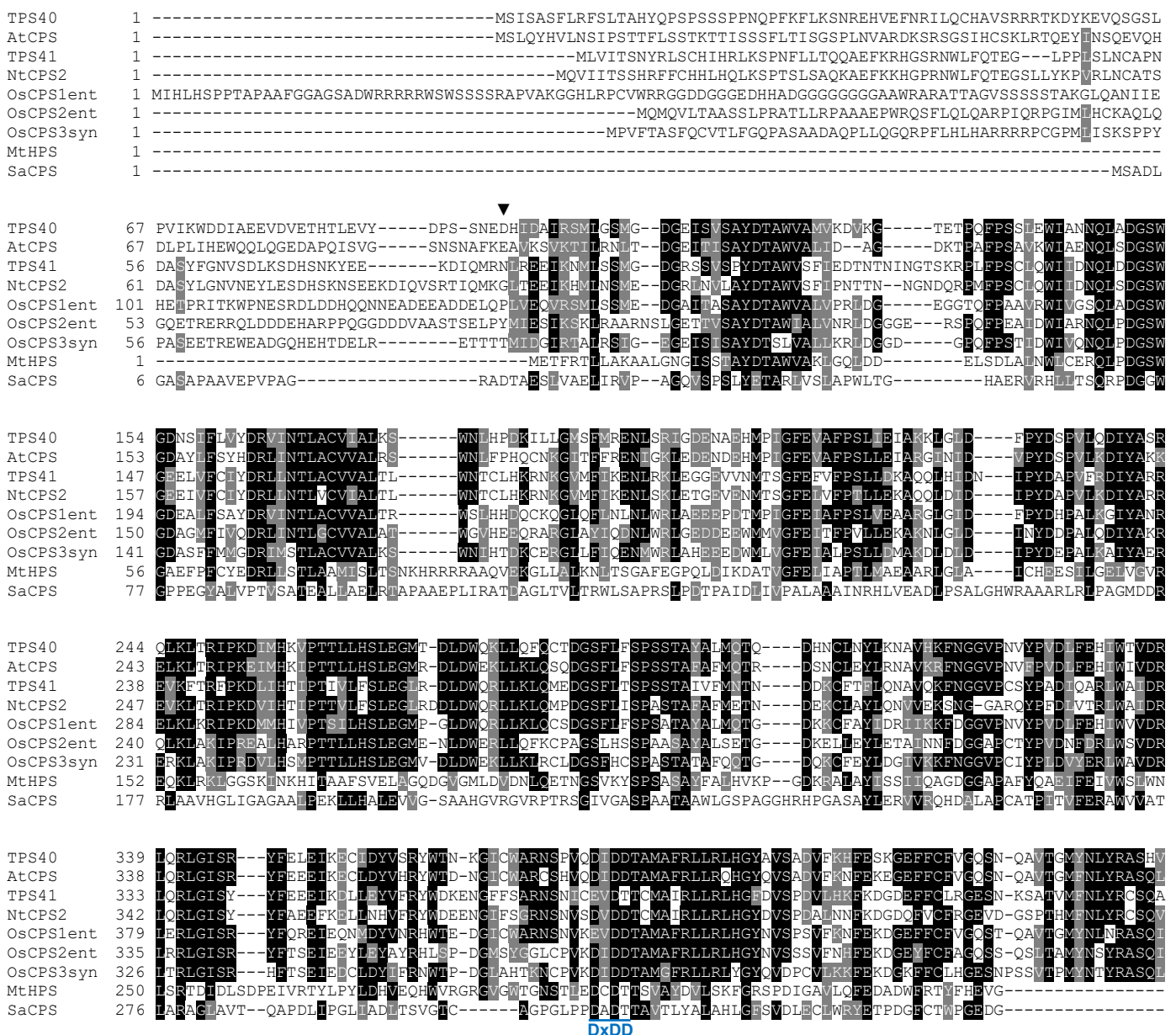


Fig. S7 Sequence alignment of the proteins encoded by the functional tomato TPS-c genes and CPS genes from other plants. Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. The conserved DxDD motif is underlined. The truncation sites for protein expression are indicated with triangle. AtCPS, copalyl diphosphate synthase from *Arabidopsis thaliana* (At4g02780); NtCPS2, 8-hydroxy-copalyl diphosphate from *Nicotiana tabacum* (HE588139.1); OsCPS1_{ent}, ent-copalyl diphosphate synthase 1 from *Oryza sativa* (Q6ET36.1); OsCPS2_{ent}, ent-copalyl diphosphate synthase 2 from *Oryza sativa* (Q6Z5I0.1); OsCPS3_{syn}, syn-copalyl diphosphate synthase 3 from *Oryza sativa* (Q0JF02.1); MtHPS, halimadienyl diphosphate synthase from *Mycobacterium tuberculosis* (NP_217894); SaCPS, copalyl diphosphate synthase from *Salinispora arenicola* (WP_012181499.1).



TPS40 434 MFSGEK-ILENAKIFISNYLREKRAQNOILLDKWITTKDLPGEVGYALDVPWYASLPRLEIRFFLEHYGGEEDVWIGKTLYRMPVNNNSIYLELAKSDYNN
 AtCPS 433 AFRFE-IKNAKEFSYNYLLEKREREREIDKWIIMKDLPEIGFALEIPWYASLPRVEIRFYLDQYGGENDVWIGKTLYRMPVNNNGYLELAKQDYNN
 TPS41 429 LFPGEI-ICEEAKNFTYNYFLHQYLANNOQSKDKWVIKADIPGEIRYALEFCWYASLPRVEIRLYLDQYGGADETWISKTLYRMPDVSNVYLELAKLDYNN
 NtCPS2 438 LFPGEK-ILEEAKNFTYNYFLQOCLANNRCLDKWVIKADIPGEIRYALEFCWYASLPRVEIRYLYLDQYGGADETWIGKTLYRMPDVNNNVYLELAKLDYNN
 OsCPS1ent 474 SFPGED-ILQARNFSEYFLREBAQGTIHDKWIISKDLPGEVGYLDFPWYASLPRVEIRTYLDQYGGNDVWIGKTLYRMPVNNNATYLELAKQDFNR
 OsCPS2ent 430 VFGDDGLEQIRACRAFLERERATGNIMDKWVIANGLESVEYALDFPWASLPRVEIRVYLEQYGADEDAWIKGLYRMTLVNNDYLELAKADFTN
 OsCPS3syn 422 KFPGDGVLGRAEVEFCRSFLQDRRCNSRMKDKWVIKADIPGEVGYAMDYPWASLPRLEIRLYLDQYGGSGVWIGKVLFRMTLFCNDYLELAKADESN
 MthPS 334 -----PSISTNVHVLGALKQAGYDR
 SaCPS 352 -----FSLSTNAHVLDVGLILTTDPGADRRH

TPS40 533 COALHQEFWRRIKRWIYECGLGEPG----LSEKRLLVYIYLCASIFEAQRSTERMAWVKTAALMDCVRSQFGSPQVSAAAFCEFAHYSSTALNSRYN
 AtCPS 532 COAQHQLEWDLIHKWWEENRLSEWG----VRRSELLECYLAAATIFESERSHERMVWAKSSVLVKIISSEFGESSDSRRSFSQDFHEYIANARSDHH
 TPS41 528 CQSQRHFEWLIHQEWEKGNFQKFG----ISKKEVLVSEFLAASSIFEVERSRQLAWAKSCHLCKMITSYINQEATVWN---SFLMEFKNYRDMSIKK
 NtCPS2 537 CQSQRHFEWLIHQEWEKCNFQOFG----ISKKYLLVSYFLAAASIFEVERSRERLAWAKSRIICCKMITSYINDEATVWTRNSLLMEFKVSHDPTRKN
 OsCPS1ent 573 COALHQHLELQGLQKWIENGLEAFG----MTPEDVLRAYFLAAACIFEPNRAERLAWARVSVLANTIIRHFYSMDSMKRMERFMWSSLYEENGNVLG
 OsCPS2ent 530 FORLSRLEWLSIKRWIIRNNLQAHG----VTEQSVLRAYFLAAANIPEPNRAAERLAWARTAILAEIASHLRQYSANGAADGMTERLISGLASHDWDW
 OsCPS3syn 522 FQKECRVLELNGIRRWILRSNLEKFGGT---DPQTTLMTSYFLASANIPEPNRAAERLAWARVALADAVSSHFRRIGGPKNSTNLEELISLVPFDAYS
 MthPS 354 CHPRVRKVLFTIRSSKEPGRFCWRDKW---HRSAYYTAHLICAAISNYDDALCSDAIGWILNTQRPDGSGWFFDQQAETAETAYCIQALAHWQRHSG---
 SaCPS 379 VTAARRLADALRCRQQADGSQDRWHASPHYATMCCALALAGFPGPGTAVTSLARAASWIVDTQRANCSWGRMKGTVEETAYAVQVLATVGRG-----

TPS40 628 TEDR-----LVGVLGTLNHLNLSALLTHG--RDVHHYLRHAWENWLLTVGEGEGEGEGG-----ABELIIRTLNLC
 AtCPS 627 FNDNRMLDRPGSVQASRLAGVIGTLNQMSFELFMSHG--RDVNNLLYLSGWDWKEKWKLYGDEGE-----LMVKMIILMKN
 TPS41 620 SNET-----KEIILNNNICQFLHQLIKETYQDLG--KDIHHQLHNVWEWLEENN---TTCQEA-----AVLLVQITNLS
 NtCPS2 632 GNET-----KEIILNKNRQFLRQLSEETFEDLG--KDIHHQLQNAWETWLVFLREKKNACQEE-----TELLVVRTINLS
 OsCPS1ent 668 LEGYAKDG-----ILARTICQLDLLSOETPPVREGQKCHHNLIRCAWIEWMMQINMKDGRYDKGRVMHPGSCVHNKETCLLIAQIVEICAGRI
 OsCPS2ent 625 RESK-----DSAARSLLYALDELIDLHAFGNASDSLREAWKQWLMSTWNE--SQGSTG-----DTALLMVRTIEICS
 OsCPS3syn 619 G-----SLREAWKQWLMMAWTAKESSQESIEG-----DTAILLMRAIEIFG
 MthPS 448 -----
 SaCPS 472 -----

TPS40 692 SVHWISEEILLSHPTQKLEITNRVSHRIR---LYKGHSEKQVGLMTFSEBIEGDMQQLAELVLSHSDASEIDANIKDFTLTVAKSFYYSAYCDDRTIN
 AtCPS 705 NDLTN---FFTHTHVRLAEIINRICLPRQ---YLKARRNDEK----EKTIKSMKEKMGKMWELALSESDFRVDVSIITFLDVAKAFYYPALCGD-HIQ
 TPS41 685 S-GHMTHEILS-----KYTNKVCHEMIN---EFCN---DQICNSKARDIELHMQALVKLVFSNTSSNNINQGIEDTFKVVKTFYYTAHVSEETIN
 NtCPS2 700 G-GYMTHEILFDADVENISNLTNKVCGRLN---ELQN---DKVTGGSKNTNIELDMQALVKLVFGNTSSN-INQDIKQTFFAVVKTFYYSAHVSEETIN
 OsCPS1ent 759 EEASMINNTEGSWFIQLASSICDSLHAKVYL---LSCDTKKNETTINQIDKIEELGMOELAQYELPRVDDRRINNKTKQTFLSLVKSCYAAANCSPHMLD
 OsCPS2ent 691 GRHGSAEQSLKNSADVARLEQIASSMCKSLATKILAQ--NGGSMNDVEGIDQEVVDEMKELIQRYVYSS-SNDVSSVTRQTFLDVVKSEFCYVAHCSPETID
 OsCPS3syn 659 GRHVLTGQRPD-LWEMSQLBQLTSSICCKLISRRVLACENGESTEKEVEEIQQVDIEMQELTRRVLQG--CSAINRLTRBTFLVVKSEFCYVAYCSPETID
 MthPS 448 -----TSLSAQISRAGGWLSQHCEPPYAPLWIAKTLVYCSATVVVKAAIL
 SaCPS 472 -----RPGADEAIRRGRAYLTEGTTAHDGPPPLWHDKDLVYRPMIVRAAVV

TPS40 789 FHIAKVLFEERVV--
 AtCPS 792 THISKVLFQKV---
 TPS41 770 NHISKVLFQKA---
 NtCPS2 792 FHISKVLFQQV---
 OsCPS1ent 856 QHISEVLEEQVI--
 OsCPS2ent 789 GHISKVLFEDVN--
 OsCPS3syn 756 SHIDKVLFEQDVI--
 MthPS 491 SARIRLVDESQ---
 SaCPS 518 AARHLAGAGPATA

Fig. S8 Predictions of subcellular localization for TPS and prenyltransferase proteins made by TargetP 1.1 (a) and ChloroP 1.1 (b).

(a) ### targetp v1.1 prediction results #####
 Number of query sequences: 42
 Cleavage site predictions not included.
 Using PLANT networks.

Name	Len	cTP	mTP	SP	other	Loc	RC
TPS3	607	0.781	0.218	0.044	0.036	C	3
TPS4	590	0.756	0.205	0.040	0.055	C	3
TPS5	609	0.563	0.315	0.087	0.018	C	4
TPS7	592	0.499	0.155	0.233	0.054	C	4
TPS8	597	0.375	0.099	0.136	0.546	-	5
TPS9	548	0.050	0.182	0.180	0.693	-	3
TPS10	556	0.166	0.244	0.083	0.313	-	5
TPS12	548	0.053	0.301	0.139	0.461	-	5
TPS14	568	0.113	0.093	0.089	0.861	-	2
TPS16	553	0.075	0.144	0.059	0.858	-	2
TPS17	554	0.091	0.149	0.063	0.774	-	2
TPS18	773	0.024	0.900	0.009	0.207	M	2
TPS19	778	0.612	0.323	0.014	0.133	C	4
TPS20	778	0.612	0.323	0.014	0.133	C	4
TPS21	784	0.661	0.215	0.022	0.122	C	3
TPS24	820	0.930	0.035	0.075	0.070	C	1
TPS25	547	0.272	0.086	0.089	0.713	-	3
TPS27	554	0.214	0.166	0.081	0.576	-	4
TPS28	554	0.169	0.129	0.125	0.725	-	3
TPS31	555	0.243	0.057	0.145	0.727	-	3
TPS32	548	0.113	0.199	0.078	0.627	-	3
TPS33	552	0.171	0.123	0.193	0.514	-	4
TPS35	556	0.211	0.073	0.142	0.692	-	3
TPS36	591	0.548	0.061	0.144	0.293	C	4
TPS37	560	0.547	0.206	0.055	0.092	C	4
TPS38	546	0.157	0.177	0.046	0.816	-	2
TPS39	563	0.055	0.151	0.252	0.584	-	4
TPS40	800	0.921	0.093	0.038	0.044	C	1
TPS41	780	0.077	0.730	0.022	0.145	M	3
TPS46	821	0.231	0.055	0.112	0.891	-	2
TPS47	562	0.096	0.239	0.083	0.649	-	3
TPS48	560	0.327	0.105	0.160	0.528	-	4
TPS51	550	0.054	0.154	0.074	0.876	-	2
TPS52	551	0.038	0.359	0.059	0.720	-	4
FPPS1	342	0.027	0.053	0.378	0.864	-	3
SSU1	315	0.019	0.278	0.057	0.600	-	4
SSU2	334	0.366	0.190	0.170	0.100	C	5
GGPPS3	360	0.505	0.132	0.010	0.423	C	5
TPT1	372	0.158	0.866	0.013	0.029	M	2
TPT2	373	0.178	0.785	0.009	0.053	M	2
GGPPS2	363	0.322	0.082	0.028	0.170	C	5
GGPPS1	365	0.823	0.085	0.074	0.119	C	2
cutoff		0.000	0.000	0.000	0.000		

(b) ### chlorop v1.1 prediction results #####
 Number of query sequences: 42

Name	Length	Score	cTP	CS-score	cTP-length
TPS3	607	0.564	Y	2.430	28
TPS4	590	0.550	Y	-0.472	38
TPS5	609	0.523	Y	-0.094	42
TPS7	592	0.538	Y	5.945	45
TPS8	597	0.443	-	-1.415	8
TPS9	548	0.437	-	1.897	2
TPS10	556	0.486	-	1.526	4
TPS12	548	0.446	-	3.181	38
TPS14	568	0.436	-	7.857	68
TPS16	553	0.428	-	7.547	9
TPS17	554	0.427	-	1.932	9
TPS18	773	0.454	-	0.863	28
TPS19	778	0.545	Y	8.925	36
TPS20	778	0.545	Y	8.925	36
TPS21	784	0.528	Y	5.963	36
TPS24	820	0.548	Y	4.184	53
TPS25	547	0.443	-	-0.394	8
TPS27	554	0.446	-	-0.085	33
TPS28	554	0.433	-	-2.312	16
TPS31	555	0.433	-	4.268	3
TPS32	548	0.438	-	-0.872	12
TPS33	552	0.442	-	6.280	3
TPS35	556	0.436	-	6.941	3
TPS36	591	0.485	-	3.693	41
TPS37	560	0.505	Y	8.499	30
TPS38	546	0.432	-	-2.033	13
TPS39	563	0.444	-	-0.156	86
TPS40	800	0.548	Y	2.548	46
TPS41	780	0.462	-	-0.630	26
TPS46	821	0.446	-	-1.743	13
TPS47	562	0.436	-	5.568	61
TPS48	560	0.469	-	0.624	42
TPS51	550	0.427	-	1.403	29
TPS52	551	0.428	-	2.759	34
FPPS1	342	0.432	-	1.534	12
SSU1	315	0.439	-	7.229	22
SSU2	334	0.512	Y	5.161	17
GGPPS3	360	0.567	Y	5.148	65
TPT1	372	0.506	Y	2.832	13
TPT2	373	0.507	Y	3.003	13
GGPPS2	363	0.528	Y	4.003	63
GGPPS1	365	0.553	Y	3.695	43

Fig. S9 Phylogenetic analysis of tomato TPT homologs and TPTs identified in other plants. The maximum likelihood phylogenetic tree was constructed using MEGA7 with default settings. The first two letters in each of the homologs are abbreviations for the species: Aa, *Artemisia annua*; Ag, *Abies grandis*; At, *Arabidopsis thaliana*; Am, *Antirrhinum majus*; Cb, *Clarkia breweri*; Cr, *Catharanthus roseus*; Hb, *Hevea brasiliensis*; Hl, *Humulus lupulus*; La, *Lupinus albus*; Mi, *Mangifera indica*; Mp, *Mentha piperita*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Pa, *Picea abies*; Sa, *Sinapis alba*; Vv, *Vitis vinifera*; Zm, *Zea mays*. The GenBank accession numbers of the proteins used in the phylogenetic analysis can be found in Methods S1. The tomato TPT homologs are shown in bold. TPT1, TPT2 and the four Arabidopsis GFPP synthases are shaded in red.

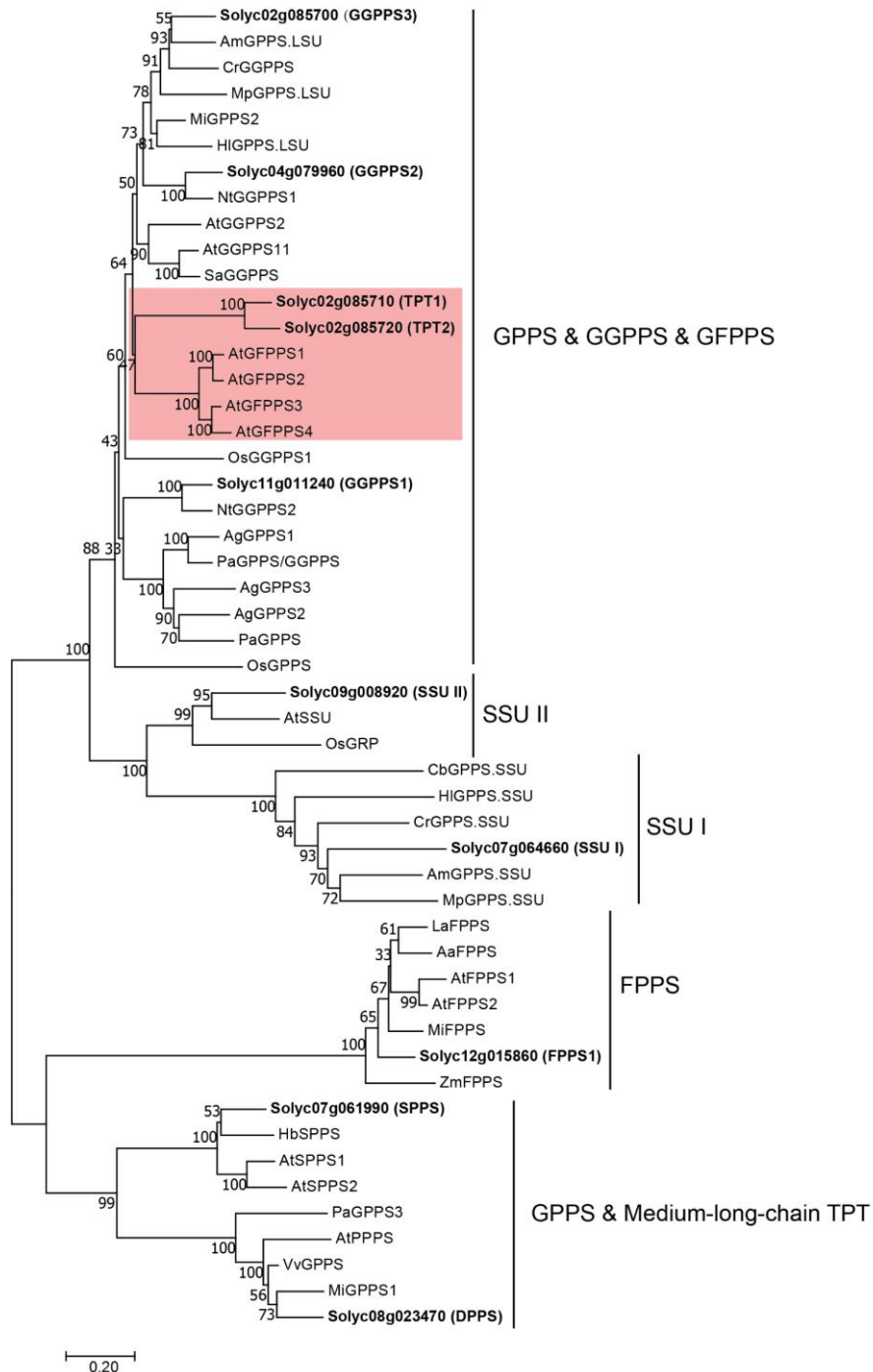


Fig. S10 GC-MS analysis of the products formed in planta by transiently co-expressing TPS with TPT genes in *Nicotiana benthamiana* leaves. Transit peptide truncated versions (“d”) of all tested genes were transiently expressed in tobacco cytosol, co-expression of *AtGFPPS2* with *AtTPS19* was used as a positive control to produce the sesterterpene (-)-retigeranin B (Shao *et al.*, 2017).

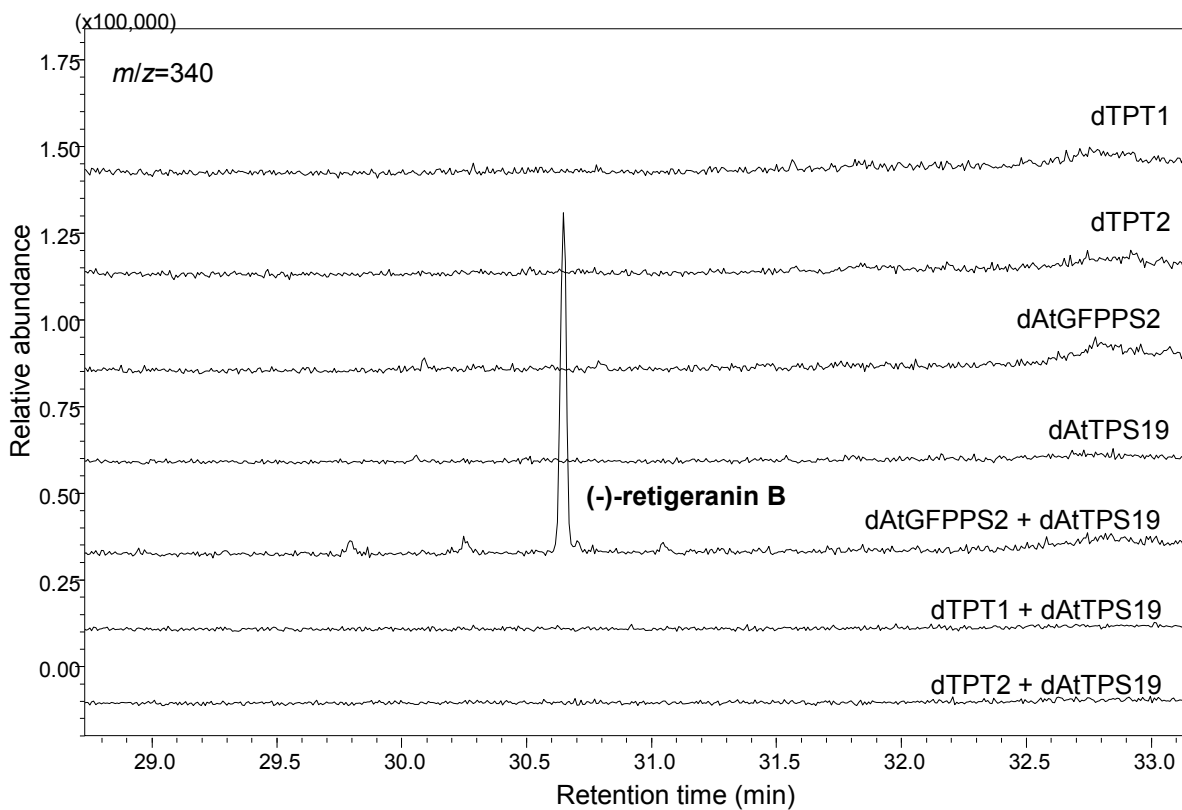


Fig. S11 Sequence alignment of five Arabidopsis sesterterpene synthases and tomato TPSs from TPS-a, TPS-b and TPS-g clades. (a) Key residues determining the substrate specificity of sesterterpene synthases are shown in red, the corresponding residues of tomato TPSs are shown in blue. Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. (b) Amino acids with large side chain (shaded in red) from tomato TPSs. (c) Amino acids with small side chain (shaded in yellow) from Arabidopsis sesterterpene synthases.

(a)	TPS5	323	F-SRDIIVENMF	FWAVGALFEPQHS
	TPS7	305	F-SRDRLVEDF	FWSVGLAFAEPQHS
	TPS4	303	F-SRNRILVENLF	FWAGTINFEPQHS
	TPS3	315	F-ARDRIVEAF	QWITGMIFESQEN
	TPS8	286	F-ARDRIVENF	FWITGVNIFLPOYG
	TPS38	259	F-VRDRIVEGEF	FWAVGFTPNPQNG
	TPS25	265	F-TRRIRIVESF	FWAGTASEGEHG
	TPS47	273	F-TRDRIVECF	YAGGIGSKPQOG
	TPS27	269	F-SRDRIVESF	LWSVGVAFAEPQHS
	AtTPS30	317	C-VRDRIVET	YFPTLGLYFEPRES
	AtTPS17	315	Y-IRDRIVEV	YFGALALYFEPQYS
	AtTPS25	316	Y-SIRERNVET	YLGGLGVLFEPQYS
	AtTPS18	317	Y-IRDRIVES	HLGGLGPFYFEPQYS
	AtTPS19	314	Y-IRDRSVES	HLGGLGPFYFEPQYS
	TPS9	261	Y-ARDRIVE	CYFWILGVYFEPQYS
	TPS12	260	Y-ARDRIVE	CYFWILGVYFEPQYS
	TPS10	266	Y-ARDRIVE	CYFWILGVYFEPQYS
	TPS36	301	Y-ARDKIVE	GYFWTLGVYFEPQYS
	TPS14	280	Y-ARDKIVE	CYFWATGVYFGPQYK
	TPS16	264	Y-VRDRIAE	VYFWSLGVYFEPQYS
	TPS17	267	Y-ARDRIVE	CYFWCLGPFYFEPQYS
	TPS51	262	F-ARDRVV	ELYFWSLGVYFEPQYS
	TPS52	263	F-ARDRVV	ELYFWSLGVYFEPQYS
	TPS28	266	Y-VRDRM	VCEFWTVGVYFEPQYS
	TPS48	272	Y-VRDRM	VCEFWAVGVYFEPQYS
	TPS32	262	Y-ARDRAV	ECYFWTMGVYAEQYS
	TPS33	266	Y-ARDRAV	ECYFWTMGVYAEQYS
	TPS31	269	Y-ARDRAV	ECYFWTMGVYAEQYS
	TPS35	270	Y-ARDRAV	ECYFWTMGVYAEQYS
	TPS37	279	L-IRDQPL	KWYFWPMALTDPRKMS
	TPS39	280	L-IRDQPL	KWYFWPMALTDPRKMS

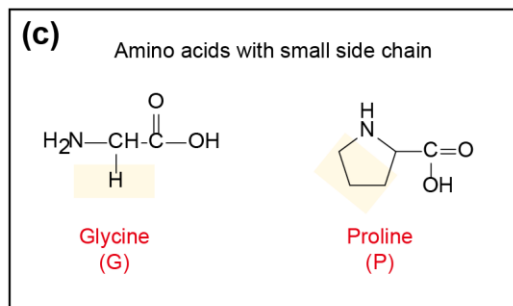
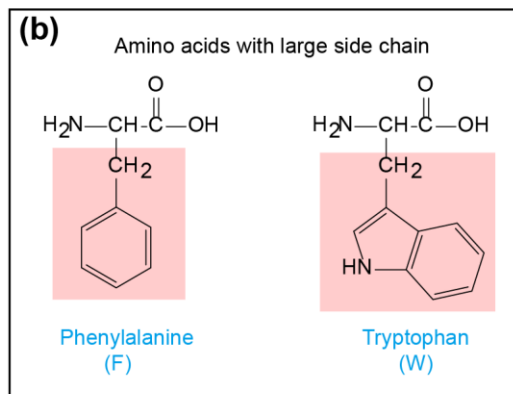
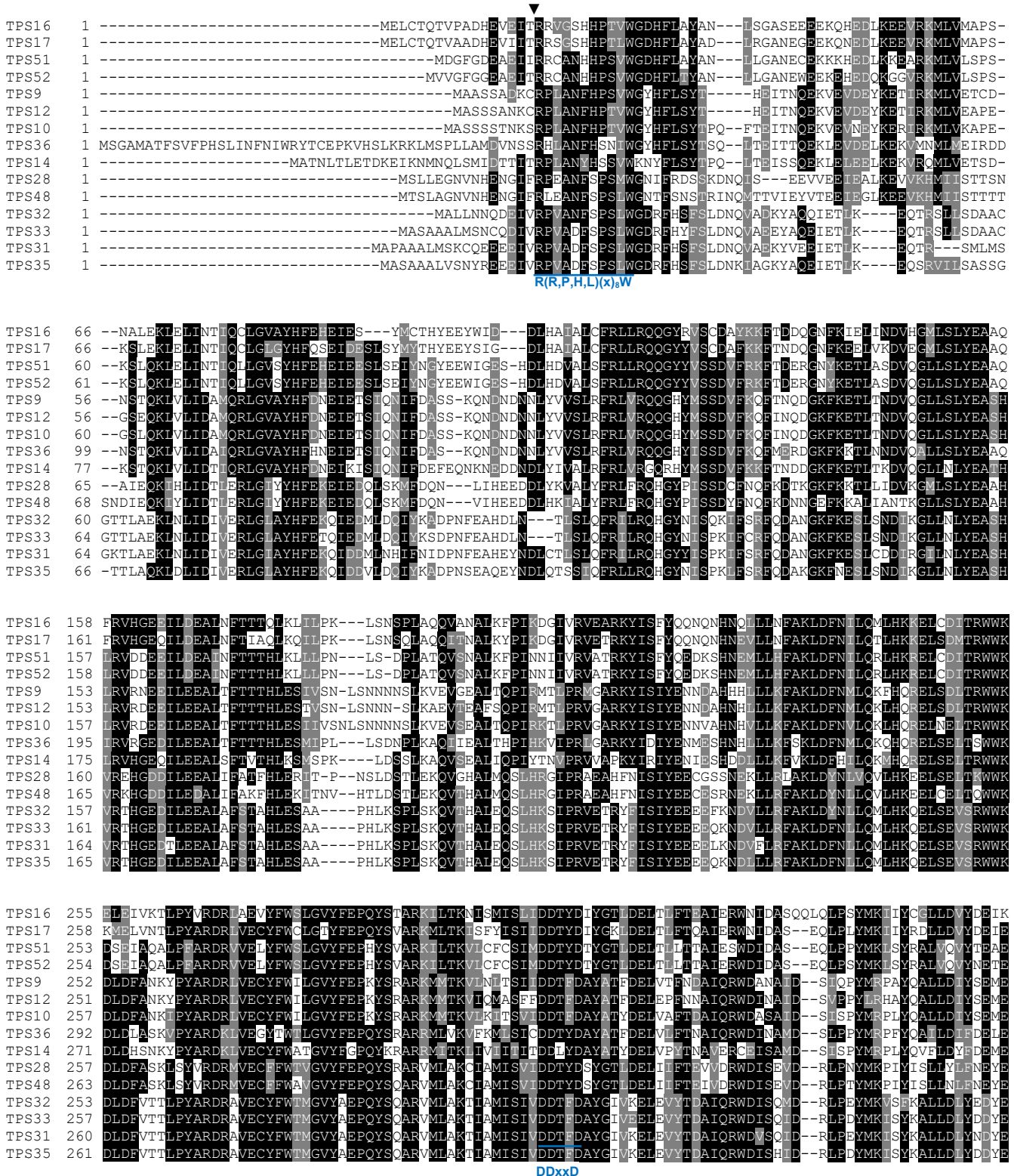


Fig. S12 Sequence alignment of the proteins encoded by the functional tomato TPS-a genes. Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. The conserved R(R,P,H,L)(x)₈W and DDxxD motifs are underlined. The truncation sites for protein expression in *E. coli* are indicated with triangle.



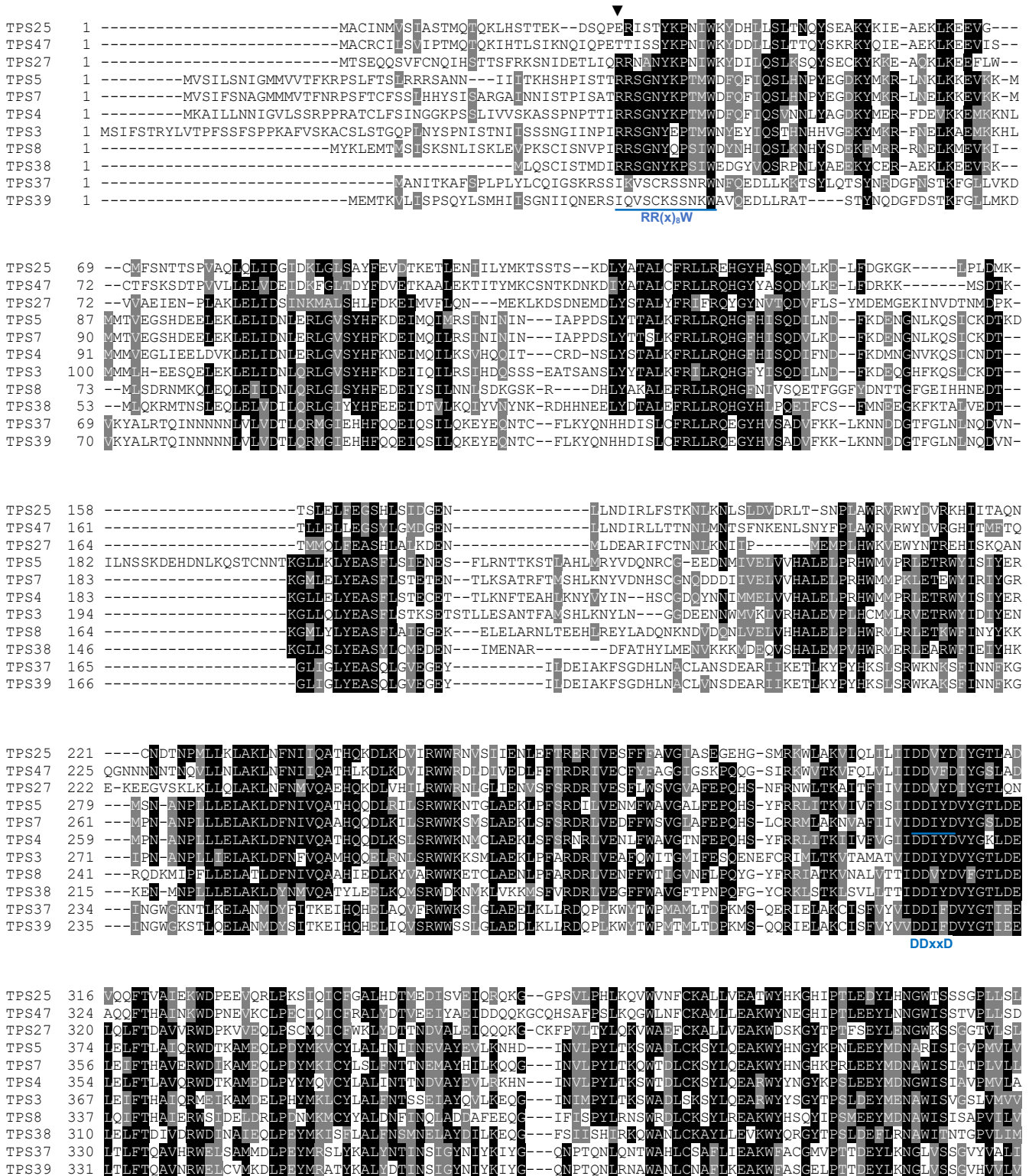
TPS16 355 KDLANE--NKSFLVNSINEMKKVMAYFOEAKWY-GKTI PKMEEYKSGISTSAVQVATTSWLGMGNVATKDSFDWVNEPFIIVASSI IARLINDL
TPS17 356 KELANE--NKSFLVNSINEMKKVVRGYFOEAKWY-GKKVPTMEQYKNGISTSAVILLTTTSLWLMGNVATKDAFDWVATEPPIIVASCYIIRLINDL
TPS51 351 RELENLGNKMTFRVKYSINEMKKLIRAYFOEAKWYH-GKDVPTMEQYKNGIPSSITYLLATTSWLGMGDVAIKDAFDWVSNPEFIIVASSI IARLINDL
TPS52 352 KELENLGNKMTYRVKYSINEMKKLIRAYFOEAKWYH-GKDVPTMEQYKNGIPSSITYLLATTSWLGMGDVAIKDAFDWVSNPEFIIVASSI IARLINDL
TPS9 350 QVLSKDG--KLDRVVYAKNEMKKLVRAYFKEAQWLNDCDHPKYEEHQENAVSAGYMMISTTCLVGEFEFISHETFEWLMNESVIWRASAI IARANDI
TPS12 349 QALAKEF--KSDRVVYAKNEMKKLVRAYFKEAQWLNDCDHPKYEEHQENAVSAGYMMGATTCLVGEFEFISKETFEWLINEPLIWRASSI IARAMDDI
TPS10 355 QVLSNEG--KLDRVVYAKNEMKKLVRAYFKEAQWLNDCDHPKYEEHQENAVSAGYMMGATTCLVGEFEFISKETFEWLMNESVIWRASAI IARANDI
TPS36 390 EELTKEG--KSDRVVYAKNEMKKLVRAYFKEAQWLNDCDHPKYEEHQENAVSAGYMMGATTCLVGEFEFISKETFEWLMNESVIWRASAI IARANDI
TPS14 369 EELTKDG--KAHYVYAKNEMKKLVRAYFKEAQWLNDCDHPKYEEHQENAVSAGYMMGATTCLVGEFEFISKETFEWLMNESVIWRASAI IARANDI
TPS28 355 REINE--QDRFNGVNVYKEMKELVRNYIEAEWFI-EGKIPSEFEYINNAVVTGTYLLAPASLLGMESTS-KRTFDWMMKPKIIVASAI IGRVIDDI
TPS48 361 IELTELE-QDRFNGVNVYKEMKELVRNYIEAEWFI-EGKIPSEFEYINNAVVTGTYLLAPASLLGMESTS-KRTFDWMMKPKIIVASAI IGRVIDDI
TPS32 351 KELSK--DGRSDVVQYAKERMKEIVRNYFVEAKWEI-EGYMPVSEYLSNALATSTYYLLTTTSLYLGKVSAT-KEDFEWLAKNPKILBANVTLCRVDDI
TPS33 355 TELS K--DGRSDVVQYAKERMKEIVRNYFVEAKWEI-EGYMPVSEYLSNALATSTYYLLTTTSLYLGKVSAT-KEDFEWLAKNPKILBANVTLCRVDDI
TPS31 358 TELS N--DGRSDVVQYAKERMKEIVRNYFVEAKWEI-EGYMPVSEYLSNALATSTYYLLTTTSLYLGKVSAT-KEDFEWLAKNPKILBANVTLCRVDDI
TPS35 359 TELS K--DGRSDVVQYAKERMKEIVRNYFVEAKWEI-EGYMPVSEYLSNALATSTYYLLTTTSLYLGKVSAN-KEDFEWLAKNPKILBANVTLCRVDDI

TPS16 452 LSHBEEQKRGDAPSGVECYMKEYGVTKEEAHKIRNTIENSWKDLYEEYFVNGTITPRVLLMCHINLARVIEFFIYK-DEDAYTFPKNNLKDVIYRILLD
TPS17 453 VSHBEEQKRGNAASAVECYMNEYSVTKEEAHKIRNTIENYWKDLYEEYFVNDMIITPRVLLMCHINLARVIEFFIYK-DEDAYTFSKNNLKDVISDILLVD
TPS51 450 VTHEIEVBERGDVASGIECYMNEYGATKEEAYMETRKTIEENWKLNRGCLKP--ITVPRVLLMPVNLNLRVAEFFYK-DEDAYTFSTNNLKDVISLVLVD
TPS52 451 VTHEIEVBERGDVASGIECYMNEYGATKEEAYMEIRKLIENWKLNRGCLKP--ITVPRVLLMPVNLNLRVAEFFYK-DEDAYTFSKNNLKDVISMLLVD
TPS9 448 VGHDEDEQRGHVASTIECYMKDYGASKQETYSKFLKEVINAWKDINKOFFRP--TEVPMFVLERVNLNLRVADTIYK-EKDTYNAKGLKNMINSILIE
TPS12 447 VGHVEQOREHGASIECYMKDYGVSKQEAAYVKFQKEVINWMDINREFFCPD-VEVPKFVLERVNLNLRVINTIYK-EKDEYTNKSGKFKNMIIISLLVE
TPS10 453 VGHDEDEQRGHVASTIECYMKEYGASKQEAAYAKFKKEVINWWDINKOFFRP--TEVPMFVLERALNLFARVITDIYQ-EVDGYTNSKGLLKDVLNSSLIE
TPS36 487 SDHSDQQRGHVASTIECYMKEYGASKQEAAYVKFRKEVKDAWKGNKALLRP--IEVPIFVLRILNLRVARTMDFHQQDEEDGYTNSNSKCKDITVTLILLVD
TPS14 466 IGHHEQOREHGASIECYMKEYGASKQEAAYVEARRQIANAWKDINTDYLHA--TOVPTFVLPALNLSRLVDIIEQE---DDETDSONFLKDTIKLIFVD
TPS28 451 ATYKTEKKGQVITGIECYMDENNLSVEKASQALSEIAEAWKDLNKECIKTTSNTIPNEILMRVNLRLRIDVYKNNODGYSNPKNNVKSVEIALLVN
TPS48 458 ATYKTEKKGQVITGIECYMDENNLSVEEASQALSEIAEAWKDLNKECIKSTDS-MPTEILMRVNLRLRIDVYKNNODGYSDPKNNVKAMIEALLVN
TPS32 447 ATYVEKGRGQIATGIECYMRDYGVSIOVAMDKFQEAETAWKDVNEGILR--PTPVSTELTRILNLRARIIDVYKNNODGYTHPEKVLKPHIALLVD
TPS33 451 ATYVEKGRGQIATGIECYMRDYGVSIOVAMDKFQEAETAWKDVNEGILR--PTPVSTELTRILNLRARIIDVYKNNODGYTHPEKVLKPHIALLVD
TPS31 454 ATYVEKGRGQIATGIECYMRDYGVSIOVAMDKFQEAETAWKDVNEGILR--PTPVSAKILTRILNLRARIIDVYKNNODGYTHPEKVLKPHIALLVD
TPS35 455 ATYVVEKGRGQIATGIECYMRDYGVSIEAEMKFEAEETAWKDVNEGILR--PTPVSTELTRILNLRARIIDVYKNNODGYTHPEKVLKPHIALLVD

TPS16 551 PII-----
TPS17 552 PII-----
TPS51 547 PITT----
TPS52 548 PIKA----
TPS9 545 SVKI----
TPS12 545 SVEI----
TPS10 550 SVKTSIS-
TPS36 585 SVTIGRS-
TPS14 561 SVNSTSCG
TPS28 551 PINM----
TPS48 557 SIKL----
TPS32 545 SIEI----
TPS33 549 SVEI----
TPS31 552 SIEI----
TPS35 553 SIEI----

Fig. S13 Sequence alignment of the proteins encoded by the functional tomato TPS-b and TPS-g genes.

Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. The conserved RR(x)₈W and DDxxD motifs are underlined. The truncation sites for protein expression in *E. coli* are indicated with triangle.



TPS25 414 HVILGLTN----ENLHLCK-NCOEIIYYTSLIIRLCNDQGTSAEELRGRDVASSIICYMH-QENVSEEDVAREHIESIILNSWEKTN-YHFNRLSTSHRK
TPS47 424 YVIWGLTNNKIITNESLDSSN-NCOEIIYHTSVIFRLCNDQGTSAEELRGRDVASSIICYMQ-QENVSEEDVAREHIESIILDSWKKIN-YHFNTLSMSHRE
TPS27 419 HVLLGLAODFS--QDYFLE-NERDLIYYSSLIIRLGNLDGTSIAELRGRDVSSSILCYMR-KENVKEDVARKHIEEMVLETWKKMN-RHCFENSS---P
TPS5 471 HSLFLVTNQITKEALDLSLTN--YPDIIIRWSAIIIRLNDLGTSSDELKRGDVSKSIQCYMN-ENGASEEEAEIHEIEFLIQETWEAMN-TAQSKNSPLSET
TPS7 453 HAFIFLTNPIITQALESLNN--YPDIIIRRCALINRFVDDLGTSSDELKRGDVPKSIQCYMN-DTGASEEEAREHINLLIREMWEVMNKDQISKQVLFSEE
TPS4 451 HAFIFLVDPIITKEALESLTN--YPDIIIRCSAIIIRLNDLGTSSDELKRGDVPKSIQCYMN-ENGASEEEAREHIFRLIKETWRKFMNTAHHKEKSLFCET
TPS3 464 NAFFLVTNPIITKEVLEYLFSNKYPDIIIRWPAIIIRLTDLLATSSNEMKRGDVPKSIQCYMK-ENGASEEEARKHINLMIKETWRKMINTAQHND-SLFCEK
TPS8 434 HAYFIVANFVNKEALHYLEN-NYHDIIRCSAIIIRLANDLGTSSDELKRGDVPKSIQCYMN-ETQASEEEARQYIRLLISQETWRKLNENAHWLAADPFPKI
TPS38 407 HAYFCITNPIITKEDELQRIN--HYPAITVSPSIIIRLANDLATSPEDETKKGDYLYKSIQCYMH-DSKSCSEENARNYIKKLIETWKKMNR-DILRDESLSKD
TPS37 427 HLFYILGLGVS---SMHLQD--ISLMSTSIKIIIRLWDDLGSARDENQEGKDGSYWEYMKENKSSMELAREHYIKLIEDEWRQLNKEHFCLMSQSTRS
TPS39 428 HMFYILGFGLNQNSIYLED--SSAMASSVAIIIRLWDDLGSARDENQEGNDGSYIECYMKGQKNASIELAREHYVVKLIEDEWRQLNKKHFNLNMGSLGS

TPS25 506 IMKHVINBARMAHVMYLSGDGFGVQDGETQDQVILINLVQSI I-----
TPS47 521 IAKHVINTARMGHVMYQFGDGFVQDGKTRDQILINLMEPI T-----
TPS27 511 LTKYIMNLARVTHFTYQNGDGFVQDRETRQQLSSLVQSIPI N-----
TPS5 567 FIEVAKNITKASHFMYLHSD-----VKSSIISKILFEPIIISNVAFALK-----
TPS7 550 FIKIVFNFSRTSHCVYQHGDGHGQNSHITNRIISKILFEPII T-----
TPS4 548 FVEIAKNITATTACHMYLKGDSHGQNTDVKNSISNLLFHPIT I-----
TPS3 562 FMGCANLARTGQTYQHGDGHGQNYKIQRNRIISKILFEPII T SMP-----
TPS8 532 FVTCAMNLARMAQCMYQHGDGHGQNSTTKNHIMALLFESVPIGHKHSAAEKEDHSMVNYREKFMI
TPS38 503 FRRITSMNLARIAQCMYQHGDGFGIIPDRETKDRILSLFFQPIPI T-----
TPS37 522 FSKASLNSARVSLMYSYDDKQSLPILQEYIKSMVDGNL-----
TPS39 526 YSKASLNLARMVPLMYNYDDKQSLHVLQEYINTVLYDV-----

Fig. S14 Sequence alignment of the proteins encoded by the functional tomato TPS-e/f genes. Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. The truncation sites for protein expression are indicated with triangle.

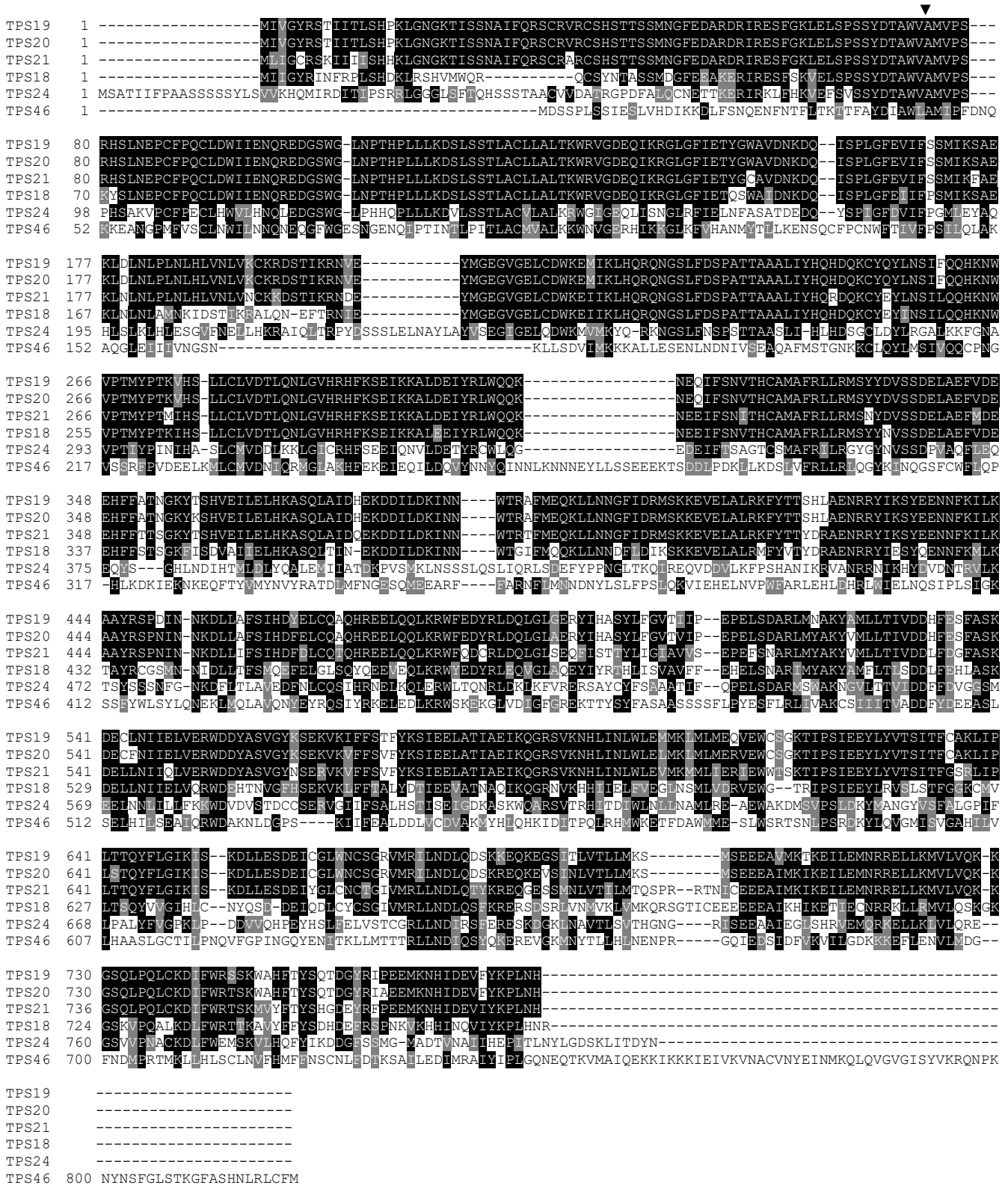


Fig. S15 Sequence analysis of TPT homologs from tomato and other plants. Black shading indicates identical amino acid residues and grey shading are the conserved or semi-conserved substitutions observed and dashes indicate gaps. The two aspartate-rich motifs (FARM, first aspartate-rich motif; SARM, second aspartate-rich motif) are underlined, the black arrow indicates the site of truncation of the N-termini for protein expression in this study. AtGGPPS11, geranylgeranyl diphosphate synthase 11 from *Arabidopsis thaliana* (At4g36810); AtGFPPS1, geranylgeranyl diphosphate synthase 1 (At1g49530); OsGGPPS1, geranylgeranyl diphosphate synthase 1 from *Oryza sativa* (Os07g39270); OsGPPS, geranyl diphosphate synthase (Os01g14630); AtSSU, small subunit of geranyl(geranyl) diphosphate synthase (At4g38460); OsGRP, GGPPS recruiting protein (Os02g44780); AmSSU, small subunit of geranyl diphosphate synthase from *Antirrhinum majus* (AAS82859).

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Solyc07g061990/SPPS 1 -----MMSVTCNLEIGRTPLESLACGCSFSKGV-VLRNVRSRFSGKLLSQRQEFGRISTKASLTGLAVLDLNKSEKPISLTNVFEVADD
Solyc08g023470/DPPS 1 -----MIFSKGLAQISRNRFSRCLWFLSLRIPQLHQSNHIDHPKVLGCRVHISWVSN--ALSGIGQOIHQQSTAVAEQVPPFLWADE
Solyc02g085700/GGPPS3 1 -----MSLSTITITWGYTHHPFSDVGNKGRSR---FRSPGFMPHLKMKFTTNPSSLVSVALTLTKQES---KSKKQAMFFKELYLE
AtGGPPS11 1 -----MASVTGLGSWVIVHHHHHPHSILTKSRSRSCPITLTKPIFRSKRTVSSSSSIVSSSVTKEDNLR---QSEPSSEFMVYIIT
AtGFPPS1 1 -----MATTVHLSSSLFQSRGRDRNSISSVKSRLKRTVLSLSSALTQDAGHIQPEGKS---NDNNSAFPDKLYLIRK
Solyc02g085710/TPT1 1 -----MSMLKRVINYLVKLSRGTTPNRSRSAAGTKLLLSSEETAEVIFRPKARAFCSNSTDLSKNESEVKNHEDILGEAGKTT-SVPLFKSYVLO
Solyc02g085720/TPT2 1 -----MSMRKGVIHNLARLSTRGTPKRFRSAGTKLLLSSEETAEVIFRPKARAFCSNSTDLSKNESEVKNHEDILGEAGKTT-SVPLFKSYVLO
Solyc04g079960/GGPPS2 1 -----MRSMNLDVSWGQACLVINQSLPYNSFNLGKINSKNRKILQQSLSYRTFSSVTVSAIATNEKVVMEKEEFNFKVVYAE
OsGGPPS1 1 -----MAAFPPLAASRVRIISPLIPAAAMAGTAGAAAASYAQRHRRRFAIVATAAASVPVAAAAAATGFNFVAYGE
Solyc11g011240/GGPPS1 1 -----MAFLATISGLDNLFNTNPNNFASRKLPPSQSYFLHKKIHASDVANSFQTFQVKERDVSKAKEFILLPEFQEVYVT
OsGPPS 1 -----MHVLAQSTAVAKVAASGLRRSPNPVSVTFQRSPSLLSFAACRRRCRRGCSVSVDVRCVSLGAMTEPLNGDVGVCVGGSGPFQRYVSA
Solyc09g008920/SSU II 1 -----MVFMSVMFSPSCLPRSRMVMQKAIQCSSSVSTASESVKFLKLYWTTL
AtSSU 1 -----MLFSGSAIFLSSFCSLPEKPHLTPMKISPAAIRSSSSSAPGSLNPLRLRYWTTL
OsGRP 1 -----MALSSFSMSLPPFAKLPSTKSTRFLPIRASSAAAAASPFLRLRYWTTL
Solyc07g064660/SSU I 1 -----MARILYFSSINGSSLSFYRPIEAKMGQNOEYWASI
AmSSU 1 -----MAHGLTHFNKSLGFLPSITKSKTRPSTRFVILAMTRTOYRATI
Solyc12g015860/FPFS1 1 -----MADLKKKFLDVYSVLKSDLLEDTAFFETDSDSRKWVDKMLDYNVPGKLNRLGSLVLSLGLKDG

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Solyc07g061990/SPPS 87 LLTINKNHNHNGAENF--VMSRAEQIFPG---GKVRPFAVFLVSRATHEMSG-----LKELTNHRRAEIEHMIHTASLHDDLDLSDSTR----
Solyc08g023470/DPPS 85 LSLITNIRSRMVAEVP--KASRAEYFFKIGVE---GKRFRPTVLLMATAINVQIPSPASQVDVDSFSGDLRTRQCCIAEITEMIHVASLHDDLDLSDADTR----
Solyc02g085700/GGPPS3 77 AVSNKRALDSAMSEKRP-VMHEISMRYSLLAG---KRIRPVLCAACELVGGVES-----TAMPAACAVEMIHHTMSLHDDLPCMDND----
AtGGPPS11 84 AEIVNKRALDSAMSEKRP-LKHEAMRYSLLAG---KRVRPVLCAACELVGGVES-----TAMPAACAVEMIHHTMSLHDDLPCMDND----
AtGFPPS1 74 AESVNRALDSAMSEKRP-LTQHEAMRYSLLAG---KRVRPVLCAACELVGGDEA-----TAMPAACAVEMIHHTMSLHDDLPCMDND----
Solyc02g085710/TPT1 89 IKSNNALDRAMPSEPK-IKFHEAMRYSLLEG---KRRCVPLCAACELVGGQES-----TAMPAACAVEMIHHTMSLHDDLPCMDND----
Solyc02g085720/TPT2 90 VKSVNALDRAMPSEPK-IKFHEAMRYSLLEG---KRRCVPLCAACELVGGQES-----TAMPAACAVEMIHHTMSLHDDLPCMDND----
Solyc04g079960/GGPPS2 80 AICVNRALDSAMSEKRP-PKHHEAMRYSLLAG---KRVRPVLCAACELVGGQNG---NAMPAAACAVEMIHHTMSLHDDLPCMDND----
OsGGPPS1 74 AAANVNRALDSAMSEKRP-PAAHEAMRYSLLAG---KRVRPVLCAACELVGGREA-----WAMPAAACAVEMIHHTMSLHDDLPCMDND----
AtGFPPS1 83 AIKVNKRALDSAMSEKRP-IKVHEAMRYSLLAG---KRVRPVLCAACELVGGDES-----LAPAAACAVEMIHHTMSLHDDLPCMDND----
OsGPPS 92 ADANVNRALDRAMPSEPK-ERKCEAMRYSLLAG---KRVRPVLCAACELVGGDAA-----AAPAAACAVEMIHHTMSLHDDLPCMDND----
Solyc09g008920/SSU II 51 ISDINOKLDRAMPSEPK-NQIYEAMRYSLAG---AKRSPVPLCAACELVGGNRL-----AAPAAACAVEMIHHTMSLHDDLPCMDND----
AtSSU 55 ITEINOKLDRAMPSEPK-AGIYEAMRYSLLAG---AKRSPVPLCAACELVGGDRL-----AAPAAACAVEMIHHTMSLHDDLPCMDND----
OsGRP 50 IADVEALDRAMPSEPK-ERHSAMRYSLLAG---GNEGTAAPVPLCAACELVGGPRE-----AAPAAACAVEMIHHTMSLHDDLPCMDND----
Solyc07g064660/SSU I 36 ESDIEAHLKKAISERAP-ESVFEPMHLLTFTTP---KSTAPVPLCAACELVGGDRD-----RAMPAASAHIMHVAVNYTHQHMTARTPGPVIE
AmSSU 46 ESDIEAHLKKAISERAP-ESVFEPMHLLTFTTP---KSTAPVPLCAACELVGGDRS-----DAMPAAAVHIMHVAVNYTHQHMTARTPGPVIE
Solyc12g015860/FPFS1 65 KELTADIEKASALGWC---EELWQAEVFLVDD---IMPGSHTRRGGQFVWYLNLEKVG-----MIAINDGILLRNIITRIKKYFRPESYVV----

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Solyc07g061990/SPPS 173 -----RGEIHLQVYCTRVAVLAGEFLFAQSSWYLANLEN-----LEWHLISQIIDFSSGEIKQSNPDCDVG-----LEY
Solyc08g023470/DPPS 184 -----RGTGLNFMGNKRLAVLAGDELRSKCVADASLKN-----TEVYCLEATVVEHIVGETQMOTSSDDEKCS-----EY
Solyc02g085700/GGPPS3 158 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VSSDRIVVITGELANCTGACGLVAGQVVDISEG-----ISDVTDKH
AtGGPPS11 165 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VVSFVVRVRAVGLANAGCTGGLVAGQVVDISEGGLDLDNVGLBHL
AtGFPPS1 155 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----LVAPKMIARAVGLARAGCTGGLVAGQVVDISEGGLDLDNVGLBHL
Solyc02g085710/TPT1 170 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VHFPTMVARAVGLARLGGPGAAAGQVVDLCCG--NSDITGLEEL
Solyc02g085720/TPT2 171 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VHFPTMVARAVGLARLGGPGAAAGQVVDLCCG--NSDITGLEEL
Solyc04g079960/GGPPS2 161 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VSPSRILVAVARLARSVGGGLVAGQVVDLCCG--KFNVTGLEML
OsGGPPS1 156 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VPPQRVVCAIGELSSAGSGLVAGQVVDLCCG--KQVSLTELE
Solyc11g011240/GGPPS1 164 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VPPQRVVCAIGELSSAGSGLVAGQVVDLCCG--KQVSLTELE
OsGPPS 173 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----VPPQRVVCAIGELSSAGSGLVAGQVVDLCCG--KQVSLTELE
Solyc09g008920/SSU II 133 -----TRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----SDIVPEDRVLRVITETARAVGSGAGAACQFIDIE-----GGPNAV
AtSSU 137 -----LRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----PDLVPRATLRLITEARAVGSGAGAACQVVDIE-----GGPFPL
OsGRP 137 -----TRRGKPNHKTIVGEDAVLAGDALSLAFEFHIAATKSG-----PDLVPRATLRLITEARAVGSGAGAACQFIDIE-----GGPFPL
Solyc07g064660/SSU I 122 HKFGPDGILVESQAKGLGPARNIRIDIEEPEKKGPNIEHLGDSHPPFELVVKAMSPAR-----KNSDKINSVTHETRAEVSSGQEGCYRELEWAQ--SGNDEETF
AmSSU 125 -----PMSISEIQKEDPNIEHLGDSHPPFELVVKAMSPAR-----NNPDRILHRAHIELETRAEVSSGQEGCYRELEWAQ--SGNDEETF
Solyc12g015860/FPFS1 145 -----DLDDLNFVGFQTAGSQMIDPDTTVGKDLRKYK-----LSLRRRIVQIKTAYSYFLVFCALVGVGEN-----LTKH

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FARM

Solyc07g061990/SPPS 243 LLKSYKTAASLAASTKGAIFSEVGSISEQMFQYGRNLGLSFOVDDILDITCSAAQLGKFAAGSDLAKGNLAFVVFALKEPNLRNIESEFHDAGSLEEAINLVKS
 Solyc08g023470/DPPS 254 MQKTYKTAASLANSCKAIALLAGHSARVSVLAFYGRNLGLAFQLDDLDITCSATLGRGSLSDIRHGIVTAFILYAMBEFPQLRTLVDGRGFDVNVVEIALDYLK
 Solyc02g085700/GGPPS3 239 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 AtGGPPS1 250 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 AtGFPPS1 239 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 Solyc02g085710/TPT1 251 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 Solyc02g085720/TPT2 252 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 Solyc04g079960/GGPPS2 242 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 OsGGPPS1 245 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 Solyc11g011240/GGPPS1 244 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 OsGPPS 255 EPIHSHKTAALLESVVIIGAILGGAPDEDVEKLRKFRARCGLLFQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 Solyc09g008920/SSU II 210 DFVQEKRFEMGFCSAVCGALLGGSDEEIQHMRYGRAGVGLYQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 AtSSU 214 SFVQEKRFEMGFCSAVCGALLGGSDEEIQHMRYGRAGVGLYQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 OsGRP 219 MKVLTKRFEMGFCSAVCGALLGGSDEEIQHMRYGRAGVGLYQVDDILDITKSSQQLGKTACKDILVADKVTYPKLIGLE-----
 Solyc07g064660/SSU I 225 EYVCKKRESETHACCAACGAILGGCDEDEKELRRFGLYGTQCGDING-----GRNYNK-----
 AmSSU 205 EYVCKKRESETHACCAACGAILGGCDEDEKELRRFGLYGTQCGDING-----GRNYNK-----
 Solyc12g015860/FPSP1 215 VDKKILDMGKLYFQVDDYLDLDFDPEVIEKIGTIDQFKCSHLYKALTELCNEEQKLLFENYKDNAAICAKIKALYND-----

SARM

Solyc07g061990/SPPS 353 CGGIQRAQDLAREKADLQMQLKCLPSSP-----FOALAEIVRYNLERIE-----
 Solyc08g023470/DPPS 364 SRGIQRTEDARKHASTLSAANDSLPESDDEEVQSRRAVEITHRWITRTK-----
 Solyc02g085700/GGPPS3 321 ----KSEFAEELNKBAQALVGFQEKI-----APLFAIANYLAYREN-----
 AtGGPPS1 332 ----KSEFAEELNKBAQALVGFQEKI-----APLFAIANYLAYREN-----
 AtGFPPS1 321 ----GSEVFAEKLRREBEQLLGFDPSEK-----APLFAIANYLAYREN-----
 Solyc02g085710/TPT1 353 ----KSEFAEQKLNKBAEQLVGFDPSEK-----APLFAIANYLAYREN-----
 Solyc02g085720/TPT2 334 ----KSEFAEQKLNKBAEQLVGFDPSEK-----APLFAIANYLAYREN-----
 Solyc04g079960/GGPPS2 324 ----KSEFAEELNGBAQQLAALFDSHK-----APLFAIANYLAYREN-----
 OsGGPPS1 327 ----KSEFAEELLSDAEQLSGFQETH-----APLFAIANYLAYREN-----
 Solyc11g011240/GGPPS1 326 ----KARQVAGELMARQMNESYFDYAK-----APLFAIANYLAYREN-----
 OsGPPS 337 ----KARQVAGELMARQMNESYFDYAK-----APLFAIANYLAYREN-----
 Solyc09g008920/SSU II 287 ----KAVVADDLRAQAKRELDELKYG----DKVMPYSFVLYAADRGFSDGQV---
 AtSSU 279 ----MNEVAPLKEKAKKELQVFNKYGG--DTLVMPYSFVLYAADRGFSLPLPL-
 OsGRP 291 ----EALGVBELKAQAKMEADRQCDKYE---ERVLPYSFVLYAADRGFELQDAATTP
 Solyc07g064660/SSU I 279 ----KMEKRVNDRILAMKELNTSFKGRK-----LKKQASLMEES-----
 AmSSU 257 ----GFEGRIKELKELAVKELESKGGKTI-----ELTRGVFEHSLAGV-----
 Solyc12g015860/FPSP1 297 ----IKLEEVFTEYEKTSYEKHTTSIAAHP--SKAVQVLLSFLGKLYKQK-----

Fig. S16 GC-MS analysis of terpenes extracted from tomato leaves. No significant differences between terpenes were observed from three extraction methods, data present in Fig. 10 was obtained by using MTBE dipping method as described in Methods S1. Terpene signals are identified by comparison of their mass spectra and retention indices with authentic standards and NIST libraries. Monoterpenes and sesquiterpenes are indicated with red and blue dotted lines, respectively. Non-terpene compounds are indicated with asterisks. Terpenes identified from left to right are as follows: α -thujene, α -pinene, unknown 5, 1,3,5-cycloheptatriene, 3,7,7-trimethyl-, β -pinene, isolimonene, β -myrcene, (+)-2-carene, α -phellandrene, α -terpinene, m-cymene, β -phellandrene, β -ocimene, α -ocimene, γ -terpinene, terpinolene, unknown 6, *trans*-p-mentha-2,8-dienol, α -terpineol, nerol, geraniol, δ -elemene (germacrene C), β -elemene (germacrene A), β -caryophyllene, α -humulene, germacrene D, cis-muurolo-3,5-diene, α -farnesene, E-nerolidol. Mass spectra and retention indices of the terpene compounds can be found in Fig. S3 and Table S2, respectively.

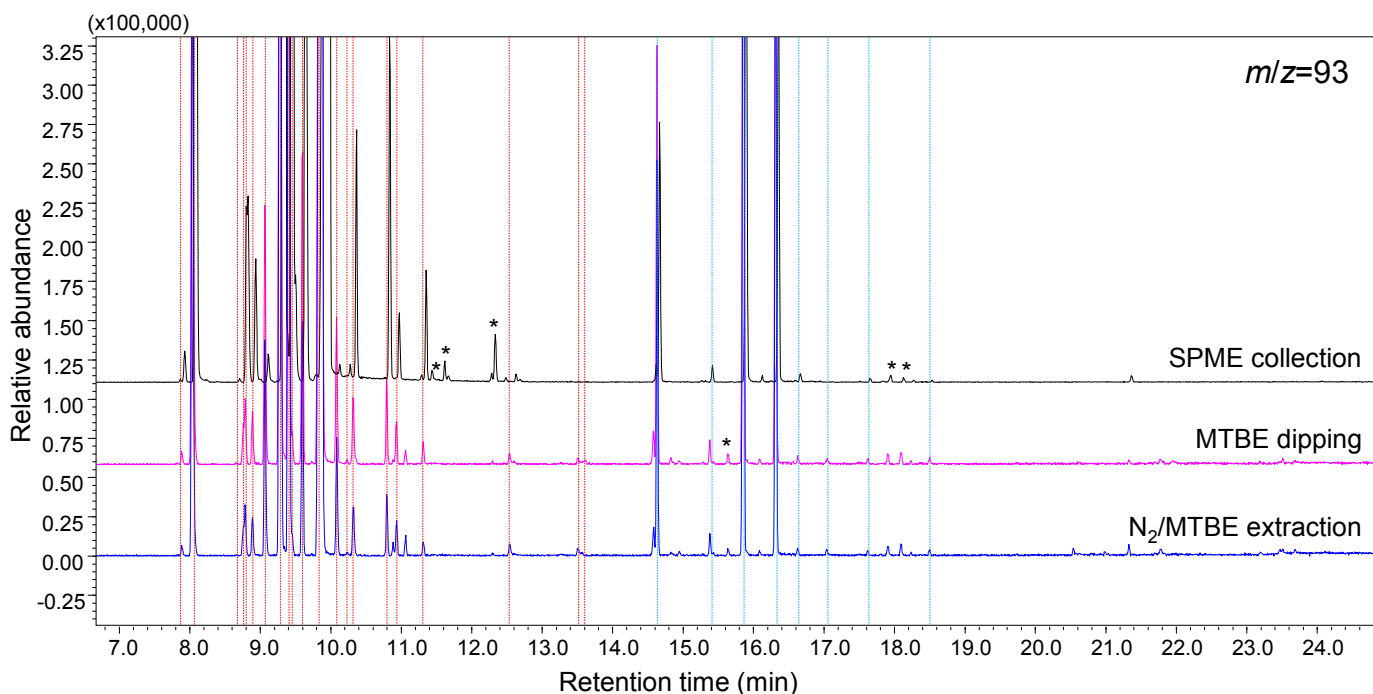


Fig. S17 Correlation analysis of terpene biosynthesis genes and volatile terpenes from tomato. The Spearman correlation coefficients were calculated using gene expression data and terpene profiling data as indicated in Fig. 9 and Fig. 10, respectively. Genes characterized in this work are shown in bold. The hierarchical clustering was performed using average linkage method by Cluster 3.0.

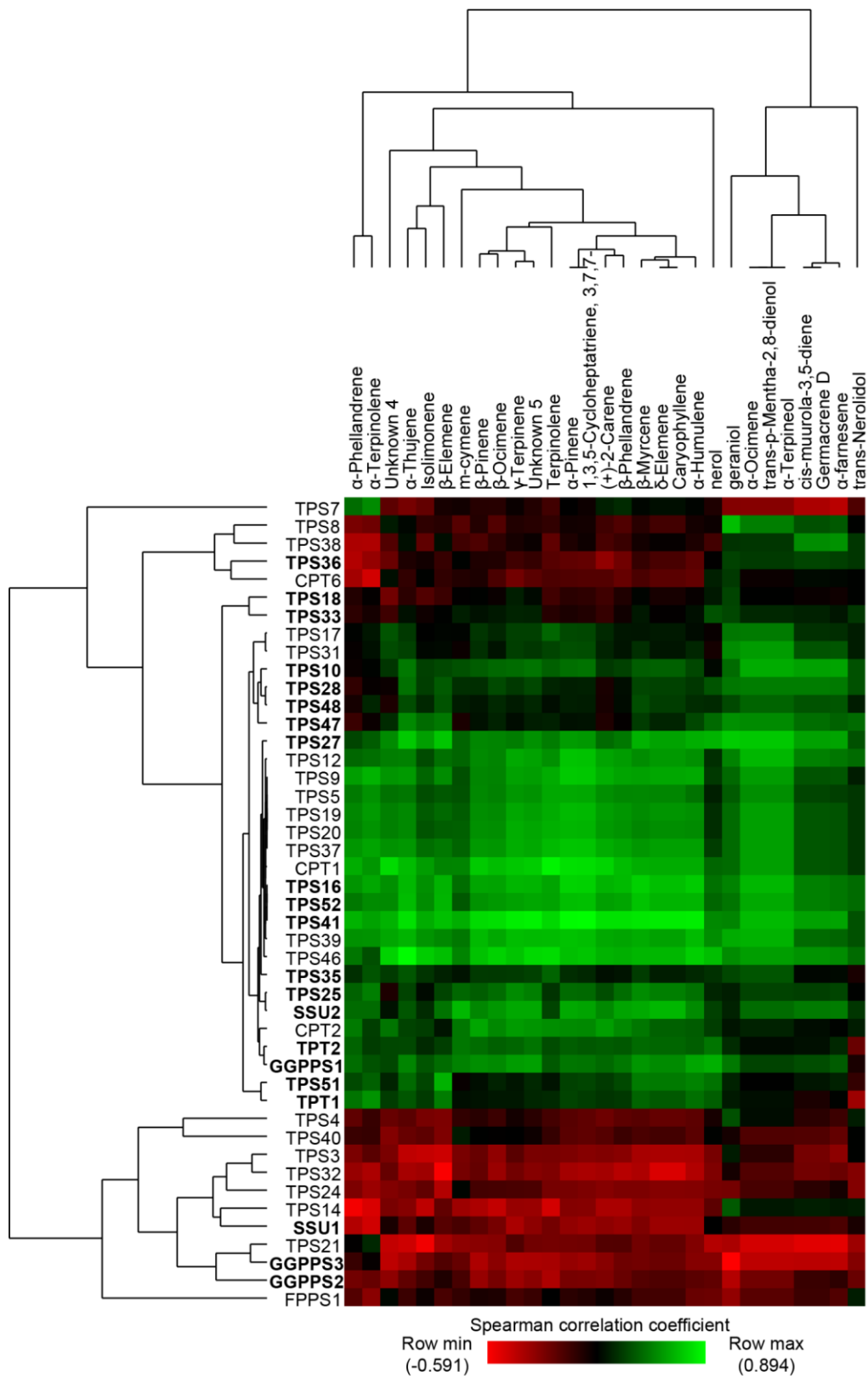


Fig. S18 Terpene biosynthesis gene clusters identified from tomato genome by plantiSMASH. The functional TPS genes are shown in bold. The eight clusters (7, 10, 17, 18, 26, 36, 42 and 43; numbered in the analysis of plant secondary metabolite clusters for *Solanum lycopersicum*) are obtained from plantiSMASH website (http://plantismash.secondarymetabolites.org/precalc/precalc/Solanum_lycopersicum/).

SL2.50ch01 - Gene Cluster 7. Type = terpene. Location: 93839435 - 93979803 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



SL2.50ch02 - Gene Cluster 36. Type = terpene. Location: 44246088 - 44308948 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



SL2.50ch06 - Gene Cluster 42. Type = terpene. Location: 37803946 - 38001175 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



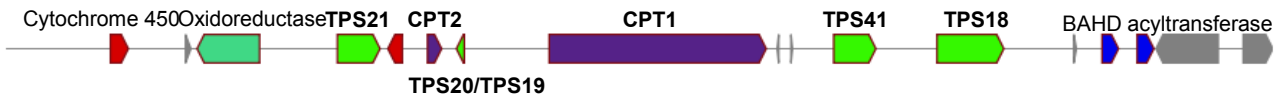
SL2.50ch06 - Gene Cluster 43. Type = terpene. Location: 38130615 - 38191506 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



SL2.50ch07 - Gene Cluster 17. Type = lignan-terpene. Location: 60595725 - 60709207 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



SL2.50ch08 - Gene Cluster 26. Type = terpene. Location: 464459 - 617526 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



SL2.50ch09 - Gene Cluster 10. Type = saccharide-terpene. Location: 71529135 - 71576652 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



SL2.50ch12 - Gene Cluster 18. Type = saccharide-terpene. Location: 836959 - 1084067 nt. [Click on genes for more information.](#)
[Show pHMM detection rules used](#)



Legend:

Cytochrome 450	Terpene synthase	Glycosyltransferase	BAHD acyltransferase	Epimerase
Dioxygenase	Amino oxidase	Aminotransferase	(Other) Biosynthetic Genes	Other Genes
biosynthetic genes	transport-related genes	regulatory genes	other genes	

Table S1 Primers used in this work.

Primer name ¹	Sequence (5'-3') ²
<i>For cloning of genomic DNA and sequencing</i>	
TPS47-F	CACACTTGATGTACCTTCCAATGTAC
TPS47-R	TTATGTAATAGGCTCCATCAAGTTGATTAGG
TPS48-F	GTATTATCACAATATAGCAACAATGAC
TPS48-R	TTACAGCTTGATGGAGTTGACGAGTAAAGC
TPS49-F	ATGGAATACTTAAACAAATATCC
TPS49-R	TAATTGAGACAATATGCATTGAG
TPS50-F	ATGGAGATTAACAATATGAATGTTC
TPS50-R	GTCACATTCATACATAAATACATAATG
TPS51-F	TGTTGGTTCCTTTCTTTCACC
TPS51-R	TTCATCATTGAGTAATAATTCTTTG
TPS52-F	ATGGTCGTTGGATTTGGTGGTGAAGC
TPS52-R	CAAGCTTTTAATCTTGACTATATTTTC
TPS53-F	CTACTTGGGTTCAATTCTAAGTAATTATTACAC
TPS53-R	CTCAAACCTTAATCATTACTCTACCTC
<i>For cloning in pET32b and creating His-tagged recombinant protein</i>	
TPS10-opti-NdeIF	GCTAATCCATATGCGATCCTCCAGTAGTACG
TPS10-opti-XhoIR-	CCGCTCGAGACTGATGGAAATTTTACGCTC
TPS16-opti-NdeIF	GCTAATCCATATGCGCCGCGTGGGGTCCCACCAC
TPS16-opti-XhoIR-	CCGCTCGAGGATAATTGGGTCGATAAGGATACG
TPS25-NdeIF	GCTAATCCATATGGAAAGGATTTCTACCTATAAACCAAAC
TPS25-NotIR-	TTCACGCGGCCGCTATAATAGACTGTACCAAGTTTATAAG
TPS27-NdeIF	GCTAATCCATATGCGACGAAATGCTAATTACAAGCC
TPS27-XhoIR-	CCGCTCGAGATTGAGAGGAAGTGATTGAACC
TPS47-opti-NdeIF	GCTAATCCATATGACTACGATTTTCATCCTATAAGCCC
TPS47-opti-XhoIR-	CCGCTCGAGAGTAATTGGCTCCATAAGATTAATTAATC
TPS51-opti-NdeIF	GCTAATCCATATGGACGGCTTCGGGGATGAGG
TPS51-opti-XhoIR-	CCGCTCGAGGGTTGTGATAGGATCGATTAAG
TPS52-opti-NdeIF	GCTAATCCATATGGTGGTAGGATTTGGCGGCG
TPS52-opti-XhoIR-	CCGCTCGAGTGCCTTGATCGGGTCGATAAGTACC
dGGPPS1-NdeIF	GCTAATCCATATGCCTGAGTTTGTGATTTCAAGAATAC
GGPPS1-XhoIR-	CCGCTCGAGATTCTGTGATTTGCAATATAAC
dGGPPS2-NdeIF	GCTAATCCATATGGAAAAAGAAGATTTAATTTTC
GGPPS2-XhoIR-	CCGCTCGAGATTTTGACGATTAGCAATGTAATC
dGGPPS3-NdeIF	GCTAATCCATATGGAGTTTAAAGAATACGTTCTTG
GGPPS3-XhoIR-	CCGCTCGAGATTCTCTCTGTAAGCAATATAATTTGC
dTPT1-XbaIF	GCTCTAGATGAGTGTCTTTGATTTCAAAGTTAC
TPT1-EcoRIR-	CGGAATTCATTTTTGCCGATGAAGAACG

dTPT2-XbaIF	GCTCTAGATGAGTGTCTTTGATTTGAAAAGTTAC
TPT2-EcoRIR-	CGGAATTCATTTTTGCCGATGAAGAAG
<i>To create non-tagged protein in pET32b and co-express with pET28a-SSU I/SSU II</i>	
GGPPS1-XhoIR+ (with dGGPPS1-NdeIF)	CCGCTCGAGTCAATTCTGTGCGATTTGCAATATAAC
GGPPS2-XhoIR+ (with dGGPPS2-NdeIF)	CCGCTCGAGTTAATTTTGACGATTAGCAATGTAATC
GGPPS3-XhoIR+ (with dGGPPS3-NdeIF)	CCGCTCGAGTTAATTCTCTCTGTAAGCAATATAAATTGC
<i>For cloning in pET28a and creating His-tagged recombinant protein</i>	
TPS28-NcoIF	CATGCCATGGTTCGCCAGAGGCTAATTTCTCTC
TPS28-XhoIR-	CCGCTCGAGCATATTGATAGGATTCACGAGTAAAG
TPS33-NcoIF	CATGCCATGGTTCGCCCTGTTGCTGATTTCTCTCC
TPS33-XhoIR-	CCGCTCGAGAATCTCAACAGAATCTACCAACAAGGC
TPS35-NcoIF	CATGCCATGGGCCCTGTTGCTGACTTCTCTCC
TPS35-XhoIR-	CCGCTCGAGAATTTCAATAGAGTCCACCAACAACGC
TPS36-NheIF	TTCACGCTAGCATGGATGTTAACTCTTACGTC
TPS36-NotIR-	TTCACGCGGCCGCTTATGATCGTCCTATAGTAAC
TPS48-NcoIF	CATGCCATGGACCGTCTAGAGGCTAATTTTCTCCTAG
TPS48-XhoIR-	CCGCTCGAGCAGCTTGATGGAGTTGACGAGTAAAG
dSSUI-NdeIF	GCTAATCCATATGGGCCAAAATCAATCTTATTG
SSUI-EcoRIR-	CGGAATTCAGACTCTTCAACAAGACTAGC
dSSUII-NdeIF	GCTAATCCATATGACAGCATCTGAGTCCGTCAAG
SSUII-EcoRIR-	CGGAATTCGACTTGCCATCAATGCTAAAAC
<i>For cloning in pEZS-NL and subcellular localization analysis</i>	
TPS3-XhoIF	CCGCTCGAGATGTCAATATTCTCCACAAGATATTTAG
TPS3-BamHIR-	CGGGATCCCGACGCCTAATAGGATTAATTATTCATTG
TPS4-EcoRIF	CGGAATTCATGAAGGCAATATTATTGAACAACATAGG
TPS4-BamHIR-	CGGGATCCCGCATAGTAGGTTTGTAATTTCTGAAACG
TPS5-XhoIF	CCGCTCGAGATGGTTTCAATATTGAGTAACATAGGG
TPS5-BamHIR-	CGGGATCCCGAGGCTTGTAATTCCTGAACGCCTTG
TPS7-EcoRIF	CGGAATTCATGGTTTCAATATTCAGTAACGCAGGG
TPS7-BamHIR-	CGGGATCCCGAGGCTTGTAATTCCTCGAACGCCTTG
TPS8-XhoIF	CCGCTCGAGATGTATAAACTGGAAATGACAATGTC
TPS8-BamHIR-	CGGGATCCCG TTTCTCGTCTGAATAATGATTCTTCAGTG
TPS9-XhoIF	CCGCTCGAGATGGCTGCTTCTTCTGCTGATAAGTGTC
TPS9-BamHIR-	CGGGATCCCGCAA AAA TGT TTT GAA TGG ATG TTT C
TPS10-EcoRIF	CGGAATTCATGGCTTCTTCTTCTTCTACTAATAAG
TPS10-BamHIR-	CGGGATCCCGCGCGTCGATCAACACAAGCTTTTG
TPS12-XhoIF	CCGCTCGAGATGGCTTCTTCTTCTGCTAATAAGTGTCG

TPS12-BamHIR-	CGGGATCCCGCAAAAATGTTTTGAATGGATGTTTC
TPS16-EcoRIF	CGGAATTCATGGAGCTGTGCACACAAACCGTTC
TPS16-BamHIR-	CGGGATCCCGGTAACCTCAATCTCATGTTCAAATG
TPS17-XhoIF	CCGCTCGAGATGGAGTTGTGCACACAAACCGTTGC
TPS17-BamHIR-	CGGGATCCCGGATTCATCAATCTCACTTTGAAAATG
TPS18-EcoRIF	CGGAATTCATGATAATTGGCTATAGAATCAATTTTC
TPS18-BamHIR-	CGGGATCCAAGGTAGGGTTTAGTCCCAAGATC
TPS19-XhoIF	CCGCTCGAGATGATAGTTGGCTATAGAAGCACAATC
TPS19-BamHIR-	CGGGATCCCGAGAAAGTGAGTCCTTTAGAAGCAATGG
TPS20-XhoIF	CCGCTCGAGATGATAGTTGGCTATAGAAGCACAATC
TPS20-BamHIR-	CGGGATCCCGAGAAAGTGAGTCCTTTAGAAGCAATGG
TPS21-XhoIF	CCGCTCGAGATGCTAATTGGCTGTAGAAGCAAAAATC
TPS21-BamHIR-	CGGGATCCCGAGAAAGTGAGTCCTTTAGAAGCAATGG
TPS24-XhoIF	CCGCTCGAGATGTCTGCTACCATTATTTTCTG
TPS24-BamHIR-	CGGGATCCCGATCTTCAAGTTGATTATGCAACACCC
TPS25-EcoRIF	CGGAATTCATGGCTTGCATAAACATGGTGTC
TPS25-BamHIR-	CGGGATCCCTTTGTATCAACCTCAAAGTAAGCAC
TPS27-EcoRIF	CGGAATTCATGACAAGTGAGCAGCAATCTG
TPS27-BamHIR-	CGGGATCCCGTAGAAACACCATGATTCCTTGTC
TPS28-EcoRIF	CGGAATTCATGAGTTTATTGGAAGGAAATGTAAACC
TPS28-BamHIR-	CGGGATCCCGCTTGCTTAGTTGATCTTCAATCTCC
TPS31-XhoIF	CCGCTCGAGATGGCCCCAGCTGCTGCATTGATGAG
TPS31-BamHIR-	CGGGATCCCGTCAACATGTGCTCTATTTGTTTTTC
TPS32-XhoIF	CCGCTCGAGATGGCCCTACTTAACAACCAAGATGAG
TPS32-BamHIR-	CGGGATCCCGCCAACATGTCCTCTATTTGTTTTTC
TPS33-EcoRIF	CGGAATTCATGGCCTCAGCTGCTGCATTGATG
TPS33-BamHIR-	CGGGATCCCGGTCTCTATTTGTGTCTCAAAATG
TPS35-EcoRIF	CGGAATTCATGGCCTCAGCTGCTGCATTGGTG
TPS35-BamHIR-	CGGGATCCCGTTTGTAATTTGATCCAACACATCG
TPS37-XhoIF	CCGCTCGAGATGGCAAATATTACTAAAGCTTTCTCCC
TPS37-BamHIR-	CGGGATCCCG GCGATTATAGCTCGTTTGAAGATAAGAGG
TPS38-XhoIF	CCGCTCGAGATGCTTCAAAGTTGCATATCAACTATG
TPS38-BamHIR-	CGGGATCCCGAATTCTAAAGCTGTATCATACAACCTC
TPS39-XhoIF	CCGCTCGAGATGGAAATGACTAAAGTCTTGATTCCC
TPS39-BamHIR-	CGGGATCCCGGTACGTGGAGTTGCTCTGAGAAGATC
TPS40-XhoIF	CCGCTCGAGATGTCGATCTCAGCTTCTTTTTTAAG
TPS40-BamHIR-	CGGGATCCCGCGATCGAATAGCATCGATATGGTC
TPS41-SalIF	ACGCGTTCGACATGCTGGTAATTACATCCAATTAC
TPS41-BamHIR-	CGGGATCCAAGTGAATATCCTTTTCTTCATACTTG
TPS46-XhoIF	CCGCTCGAGATGGATTCCTCACCATTGTCTTCTATTG

TPS46-BamHIR-	CGGGATCCCGTACCATGCATGCAAGAGTAATAGGGAG
TPS47-XhoIF	CCGCTCGAGATGGCTTGCAGATGCATATTATCC
TPS47-BamHIR-	CGGGATCCCGTGGAGTACTCTTTGAAAACGTG
TPS48-XhoIF	CCGCTCGAGATGACTTCACTGGCTGGAAATG
TPS48-BamHIR-	CGGGATCCCGCGTCATCTGATTAATCCGAGTAG
TPS51-XhoIF	CCGCTCGAGATGGATGGATTTGGTGATGAGGCAGAG
TPS51-BamHIR-	CGGGATCCCGTTATAGATTTCACTCAATGATTCCTC
TPS52-XhoIF	CCGCTCGAGATGGTCGTTGGATTTGGTGGTGAAGCGGAG
TPS52-BamHIR-	CGGGATCCCGATTATAGATTTCACTCAATGATTCCTC
FPPS1-XhoIF	CCGCTCGAGATGGCTGATCTGAAGAAGAAATTTTGG
FPPS1-BamHIR-	CGGGATCCCGCTTCTGCCTCTTATAAATCTTTCCCAAG
GGPPS1-EcoRIF	CGGAATTCATGGCATTTTTAGCTACCATTTCTGG
GGPPS1-BamHIR-	CGGGATCCCAATTCTGTCGATTTGCAATATAACTAG
GGPPS2-XhoIF	CCGCTCGAGATGAGATCTATGAACCTTGTTGATTC
GGPPS2-SalIR-	ACGCGTCGACTGATTTGACGATTAGCAATGTAATCTGC
GGPPS3-EcoRIF	CGGAATTCATGAGTCTTCAACAACAATTACAAC
GGPPS3-BamHIR-	CGGGATCCCGATTCTCTCTGTAAGCAATATAATTTGC
SSU I-EcoRIF	CGGAATTCATGGCAAGAATTTTATATTTTCTCTCC
SSU I-BamHIR-	CGGGATCCCGAGACTCTTCAACAAGACTAGCAATTTG
SSU II-EcoRIF	CGGAATTCATGGTTTTCTCCATGGTGATGAGC
SSU II-BamHIR-	CGGGATCCCGGACTTGGCCATCAATGCTAAAACC
TPT1-EcoRIF	CGGAATTCATGGCCATACTAAGTCAAATAGG
TPT1-BamHIR-	CGGGATCCCATTTTTGCCGATGAAGAACGAAATC
TPT2-EcoRIF	CGGAATTCATGTCTATGCGAAAAGGTGTAATC
TPT2-BamHIR-	CGGGATCCCATTTTTGCCGATGAAGAAGAAAATC
<i>For cloning in pEAQHT and tobacco transient expression</i>	
TPS10-NruIF	CTAGTCGCGAATGGCTTCTTCTTCTACTAATAAG
TPS10-XmaIR	TCCCCCGGGTATTGATATTTGACAGATTCAATCAG
TPS16-NruIF	CTAGTCGCGAATGGAGCTGTGCACACAAACCG
TPS16-XhoIR	CCGCTCGAGCTATATAATAGGATCAATAAGTATCC
TPS18-NruIF	CTAGTCGCGATGATAATTGGCTATAGAATCAATTC
dTPS18-NruIF	CTAGTCGCGATGGTCCCTTCAAATATTTCACTAAATG
TPS18-XmaIR	TCCCCCGGGTTCATCGATTATGGAGTGGTTTGTAATGAC
TPS41-NruIF	CTAGTCGCGAATGCTGGTAATTACATCCAATTAC
dTPS41-NruIF	CTAGTCGCGAATGAGGAATTTGAGAGAAGAGATA
TPS41-XmaIR	TCCCCCGGGGGCTTTCTGAAAAAGAACTTTGG
CPT1-NruIF	CTAGTCGCGAATGAGTTCTTTGGTTCTTCAATGTTG
CPT1-XmaIR	TCCCCCGGGATATGTGTGTCCACCAAACGTCTATG
CPT2-NruIF	CTAGTCGCGATGAACTCTTCAATAGTGTCTCAAC
dCPT2-NruIF	CTAGTCGCGATGCAAATCTAAAGCTGCCTCTAACAATGTC

CPT2-XmaIR	TCCCCCGGGTCAATATGTGTGTCCACCAAACGTC
CPT6-NruIF	CTAGTCGCGATGAACTCTTTGTTTGTAGGGAGGCC
CPT6-XmaIR	TCCCCCGGGTCAATATGTGTGTCCACCAAACGTCTATG
CPS-NruIF	CTAGTCGCGAATGTGCATCTCAGCTTCTTTTTTAAG
dCPS-NruIF	CTAGTCGCGATGGGTTCGTTGCCGGTTATCAAGTGG
CPS-XmaIR	TCCCCCGGGAACACTCTTTCAAATAGCACTTTGGC
KS-NruIF	CTAGTCGCGAATGTCTGCTACCATTATTTTTCTGCC
dKS-NruIF	CTAGTCGCGATGCGAGGACCGGATTTTGCTCTTCAATG
KS-XmaIR	TCCCCCGGGATTGTAATCAGTAATTAGTTTGAATCCC
GGPS1-NruIF	CTAGTCGCGAATGAGTCTTTCAACAACAATTACAAC
dGGPS1-NruIF	CTAGTCGCGATGGAGTTAAAGAATACGTTCTTGAAAAG
GGPS1-XmaIR	TCCCCCGGGATTCTCTCTGTAAGCAATATAATTTG
dTPT1-AgeIF	CCGACCGGTATGACAGTTTTTCCAAGAATGAAAG
TPT1-XmaIR	TCCCCCGGGTTTTTGCCGATGAAGAACGAAATC
dTPT2-AgeIF	CCGACCGGTATGACAGATCTTTCCAAGAATGAATC
TPT2-XmaIR	TCCCCCGGGTTTTTGCCGATGAAGAAGAAAATC
dAtTPS19-NruIF	CTAGTCGCGATGCGTCCCTTCGACGTACTTTTC
AtTPS19-XmaIR	TCCCCCGGGTGAAAGGGTATTGGGTGGACGTAC
dAtGFPS2-NruIF	CTAGTCGCGAATGATTCCACCAGAGGGAAAATGCAATG
AtGFPS2-XmaIR	TCCCCCGGGTGTGTCTGCAAGCGATGTAG
<i>For qRT-PCR</i>	
TPS3-QF	GTTCATGGGTTGTGCAGTAAAT
TPS3-QR	TAGGGCATGGAAATAGTGATGG
TPS4-QF	TGCTGTTCAAAGATGGGATACA
TPS4-QR	GTAGGGTAGGACGTTGATGTTG
TPS5-QF	GAGGTGACGTTTCCAAATCAATAC
TPS5-QR	GCTTCCCATGTCTCCTGTATC
TPS7-QF	AGCAGCACACCAACAAGA
TPS7-QR	GCTAATCCCACTGACCAGAAA
TPS8-QF	ACCTAGCAAGAATGGCACAA
TPS8-QR	CCTAGAGGAACGGATTCAAAGAG
TPS9-QF	ATCCAGAGATGGGATGCTAATG
TPS9-QR	CACGGTCCAGTTTACCTTCTT
TPS10-QF	GGTGGGAGCAAGGAAATACATATC
TPS10-QR	AAGTAACACTCAACCAGTCTGTCC
TPS12-QF	GAGACAGGTTGGTTGAGTGTTA
TPS12-QR	CATCAAAGAAGGAGGCCATTTG
TPS14-QF	GATTGTTGCGGGCGAATTTAT
TPS14-QR	GCTCTCTTTGTTGTTTCGTGTTT
TPS16-QF	CAACGACGTGCATGGAATGTTAAG

TPS16-QR	TTCCTTGCTTCTACCCTCACAAATAC
TPS17-QF	CCACCAATAGTTGTTGCTTCTTG
TPS17-QR	GCTTCTTCCTTTGTAACGCTATATTC
TPS18-QF	GTAATAGAAGAAAGTTGCTAAGGATGG
TPS18-QR	ATGACTTGATTGATATGATGCTTGAC
TPS19-QF	TCTTGAAGCTCCACAAAGCATCAC
TPS19-QR	CAAAGCAAGTTCCACCTCCTCT
TPS20-QF	TTCCCACTATGTATCCAACAAAGG
TPS20-QR	TGTTGCCATAGCCTGTATATTTTCATC
TPS21-QF	AACTCAAGAGGTGGTTTCAAGATTG
TPS21-QR	CTCAGGTCAGACACAACAGCTATG
TPS24-QF	AGTTTCACCCAGCATTCCAGTTC
TPS24-QR	CAACCCAGGCAGTATCGTATGAAG
TPS25-QF	ATGTTTCAGAGGATGTAGCTCG
TPS25-QR	GACCCCAAATCCATCTCCAG
TPS27-QF	CATGTTCTTCTTGGTTTGGCTCAAG
TPS27-QR	GATGAAGACACGTCACCTCTTTCC
TPS28-QF	CCATGAAGAAGATGATCTATAACAAGG
TPS28-QR	CTAACATGTGCTGCTTCATACAAAC
TPS31-QF	GAAGTATCAAGGTGGTGGAAAGA
TPS31-QR	GCCTGAGAGTATTGAGGTTTCAG
TPS32-QF	TTTGGACGATGGGAGTGTATG
TPS32-QR	CGGTGTAAACCTCCAGTTCTT
TPS33-QF	TAGGGATAGAGCAGTGGAGTGTTAC
TPS33-QR	ACCTCTGTATGGCATCGGTATAAAC
TPS35-QF	TCATGGAGAAGATATTTTGAAGAGG
TPS35-QR	TCGTAGATAGAGATGAAGTATCGTGTC
TPS36-QF	GGTAAATCAGACCGCGTCTAC
TPS36-QR	GTTGTTCCAAGCGCCATAAAGGTAG
TPS37-QF	GGGCTTGTTAGTTCTGGAGTTT
TPS37-QR	GCTATGGAAGTGGACATGAGTG
TPS38-QF	GCTTCTAGCTCTCTTCAACTC
TPS38-QR	GTTAGCCCACTGCTTCCCTTAT
TPS39-QF	CTGGAGTTCATGTGGTCTTAGTT
TPS39-QR	ATCATCCCAAAGGCGAAGG
TPS40-QF	GGTAGGAGAAGGAGAAGGAGAAGG
TPS40-QR	GGAGTCGGTGAGAACTCTATTGG
TPS41-QF	CACTTTGGATATGTGATTGTTGATTG
TPS41-QR	GCATGTGACAGACTTTATTGGTGTAC
TPS46-QF	AAGGTGGGATGCAAAGAATC

TPS46-QR	CATTTTGGCAACATCACCA
TPS47-QF	TTGGCCTCACTGATTACTTCGACG
TPS47-QR	CCATGCCCAAATATGACCCTTCC
TPS48-QF	ATGGAAGGATTTGAACAAAGAGTGC
TPS48-QR	AAAGCTTCGATTACAGCTTTCACG
TPS51-QF	ATGGATGGATTTGGTGTATGAGGCAGAG
TPS51-QR	GATTTGGAAGGAGAAAGCACTAGC
TPS52-QF	ATGGTCGTTGGATTTGGTGGTG
TPS52-QR	GATTTGGAAGGAGAAAGCACTAGC
FPPS1-QF	CGC CGG ATT GTC CAG TAT AAA AC
FPPS1-QR	GGT CAG CAA AGC AGT CCA GAT AAT C
GGPPS1-QF	CAGCGGGTAAGGACCTAATAAC
GGPPS1-QR	TGCCTTTGCATAGTCGAAGTAG
GGPPS2-QF	CGCTACTTGCTTTCGCATTC
GGPPS2-QR	CCAGCTACTAACCCTTCAGTTC
GGPPS3-QF	GGGATGCACTTCTTGCATTAG
GGPPS3-QR	CTACAACCTGACCAGCTACAA
SSU I-QF	GGACAGCTAGAAGGCCAATATC
SSU I-QR	GCTCCACATGCATGAATTTCC
SSU II-QF	TCGAGTCCTCCGGGTATTA
SSU II-QR	CACTCACCCATTTACCATATTTTC
TPT1-QF	GCGTCCGAGAAGGAGATTAATAG
TPT1-QR	GCTCAGAGGATTTAGTCACATCA
TPT2-QF	TTCGTGCTGTTGGAGAAGTAG
TPT2-QR	GCTCTTCCAATCCAGTATCACA
CPT1-QF	CAAAGGGCAACAAAGGACTTCACC
CPT1-QR	CAGGATTTGGACACTTGCTTTCAAG
CPT2-QF	GAACAAGAAGCTTGAAAGCAAGTGTC
CPT2-QR	CGTCTATGCCTTTGTTGAAAGTTAAG
CPT6-QF	TACCACCAAGTGTGCTAAATTCCC
CPT6-QR	ATGAGAGCTTCTTCTCCAAAGTCAG
Ubi-QF	CAGCTCGCTTTCTCAGTTCC
Ubi-QR	AGCTCGACAAACAGACTGGA

¹+, has a stop codon in the sequence; -, does not contain a stop codon in the sequence. d, for truncating the N-terminal transit peptide. opti, codon optimized version.

²Restriction sites are underlined.

Table S2 A list of terpene compounds identified from Figs 3-5 and 10.

Identified terpenes ¹	MS similarity to NIST library	Identification method	Retention indices ²	Reference retention indices ³	Source of reference
29. Isoprene	99%	b, c	n.c.	n.r.	Standard
α -Thujene	97%	a, b, c	929.7	929.7	Mandarin oil
α -Pinene	98%	a, b, c	937.7	937.7	Standard
Unknown 5	81% to 3-carene	-	971.1	3-carene RI=1011.0	Standard
1,3,5-Cycloheptatriene, 3,7,7-trimethyl	97%	a, b	977.0	972.6	NIST 2014
β -Pinene	97%	a, b, c	981.6	981.6	Standard
Isolimonene	95%	a, b, c	986.1	986.1	Standard
25. β-Myrcene	93%	a, b, c	990.5	990.5	Standard
(+)-2-Carene	96%	a, b, c	1002.9	1002.9	Standard
α -Phellandrene	97%	a, b, c	1010.5	1010.5	Standard
α -Terpinene	96%	a, b, c	1020.6	1020.6	Standard
<i>m</i> -cymene	97%	a, b, c	1028.1	1028.1	Standard
β -Phellandrene	95%	a, b, c	1037.4	1037.4	SIPHS1 (Schillmiller <i>et al.</i> , 2009)
26. β-cis-Ocimene	94%	a, b, c	1038.8	1038.8	Standard

27. β-trans-Ocimene	99%	a, b, c	1049.3	1049.3	Standard
α -Ocimene	90%	a, b	1057.8	1056	Jordán <i>et al.</i> (2013)
γ -Terpinene	98%	a, b, c	1062.8	1062.8	Standard
Terpinolene	95%	a, b, c	1088.8	1088.8	Standard
Unknown 6	87% to phellandral	-	1096.1	Phellandral RI=1273	Babushok <i>et al.</i> (2011)
28. Linalool	93%	a, b, c	1099.6	1099.6	Standard
<i>trans</i> -p-Mentha-2,8-dienol	95%	a, b	1126.7	1127.5	Babushok <i>et al.</i> (2011)
α -Terpineol	93%	a, b, c	1199.5	1199.5	Standard
nerol	95%	a, b, c	1230.4	1230.4	Standard
geraniol	90%	a, b, c	1257.0	1257.0	Standard
δ -Elemene	95%	a, b	1332.9	1333.8	Zizovic <i>et al.</i> (2007)
12. α-Cedrene	92%	a, b, c	1394.0	1394.0	Standard
4. β-Elemene	96%	a, b, c	1395.5	1395.5	Standard
13. <i>cis</i>-α-Bergamotene	90%	a, b	1419.6	1419.5	NIST 2014
20. (<i>Z</i>)-β-Farnesene	94%	a, b, c	1429.6	1429.6	Standard
β -Caryophyllene	95%	a, b, c	1432.0	1432.0	Standard
6. (<i>E</i>)-β-Farnesene	96%	a, b, c	1454.8	1454.8	Standard
α -Humulene	95%	a, b, c	1468.6	1468.6	Standard

14. β-Acoradiene	91%	a, b	1469.8	1466	Babushok <i>et al.</i> (2011)
15. Unknown 2	89% to β -acoradiene	-	1478.2	β -Acoradiene RI=1466	Babushok <i>et al.</i> (2011)
16. Unknown 3	88% to trans-Sesquisabinene hydrate	-	1483.5	trans-Sesquisabinene hydrate RI=1581	Babushok <i>et al.</i> (2011)
Germacrene D	90%	a, b, c	1493.0	1493.0	Ginger oil
18. <i>cis</i>-muurolo-3,5-diene	93% to <i>cis</i> -muurolo-3,5-diene 92% to α -cubebene	a, b	1494.0	n.r. α -cubebene RI=1351	Babushok <i>et al.</i> (2011)
1. (<i>E,E</i>)-α-Farnesene	97%	a, b, c	1505.3	1505.3	Standard
7. β-Bisabolene	94%	a, b, c	1513.3	1513.3	Ginger oil
3. Guaia-1(10),11-diene	94%	a, b	1515.3	1515	Novak <i>et al.</i> (2010)
17. β-Curcumene	93%	a, b	1518.6	1516	Kahriman <i>et al.</i> (2011)
22. δ-Cadinene	92%	a, b	1523.1	1524	Novak <i>et al.</i> (2010)
19. <i>Z</i>-Nerolidol	94%	a, b, c	1533.1	1533.1	Standard
8. Unknown 1	84% to α -Bergamotene	-	1533.8	α -Bergamotene RI=1434	Babushok <i>et al.</i> (2011)
9. α-Bisabolene	90%	a, b	1545.5	1541	Babushok <i>et al.</i> (2011)
2. Elemol	96%	a, b	1557.7	1557	Pinheiro <i>et al.</i> (2013)
5. <i>E</i>-Nerolidol	95%	a, b, c	1563.4	1563.4	Standard

10. α-Eudesmol	90%	a, b	1663.2	1664	Watanabe <i>et al.</i> (2005)
11. (Z,Z)-Farnesol	95%	a, b, c	1670.6	1670.6	SICPT6 (Akhtar <i>et al.</i> , 2013)
21. α-Bisabolol	93%	a, b, c	1694.8	1694.8	Standard
23. Unknown 4	85% to α -springene	-	2059.7	α -springene RI=2019	Setzer <i>et al.</i> (2006)
24. ent-Kaurene	95%	a, b, c	2079.5	2079.5	SITPS40 (Falara <i>et al.</i> , 2011)

¹Compounds listed in order of elution from the Rxi-5Sil MS column, the enzymatic terpene products are shown in bold. ²Retention indices relative to C₇-C₄₀ n-alkanes on the Rxi-5Sil MS column. ³Reference retention indices were obtained using the similar nonpolar column.

a, identification based on retention index; b, identification based on comparison with mass spectral data; c, identification based on comparison with authentic compounds; n.c. not calculated; n.r. no reference available from the similar column.

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Table S3 A list of tomato terpene synthase genes, their characteristics, and the enzymes they encode.

Chromosome	Locus	TPS	TPS-clade	No. of Exons	Full-length cDNA obtained	Protein size (aa)	Subcellular localization	Substrate	Main product
1	Solyc01g101170	31	a	6	Yes	555	Cytosol ^c	<i>E,E</i> -FPP	Viridiflorene ^a
	Solyc01g101180	32	a	6	Yes	548	Cytosol ^c	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	Viridiflorene ^b Unidentified ^b
	Solyc01g101190	33	a	6	Yes	552	Cytosol^c	<i>E,E</i>-FPP <i>Z,Z</i>-FPP	Guaia-1(10),11-diene^c β-Acoradiene^c
	Solyc01g101210	35	a	6	Yes	556	Cytosol^c	<i>E,E</i>-FPP <i>Z,Z</i>-FPP	Guaia-1(10),11-diene^c (<i>Z,Z</i>)-Farnesol^c
	Solyc01g105870	3	b	7	Yes	607	Plastid ^c	GPP	Camphene, tricyclene ^b
	Solyc01g105880	4 (MTS2)	b	7	Yes	590	Plastid ^c	GPP	β -Phellandrene ^d
	Solyc01g105890	5 (MTS1)	b	8	Yes	609	Plastid ^c	GPP	Linalool ^d
	Solyc01g105920	7	b	7	Yes	592	Plastid ^c	GPP	β -Myrcene, limonene ^b
	Solyc01g105960	8	b	7	Yes	597	Cytosol ^c	GPP NPP	1,8-Cineole ^b 1,8-Cineole ^b
2	Solyc02g079840	38	b	7	Yes	546	Cytosol ^c	<i>E,E</i> -FPP	α -Bergamotene ^b
	Solyc02g079890	25	b	7	Yes	547	Cytosol^c	GPP	β-Ocimene^c
	Solyc02g079910	27	b	7	Yes	554	Cytosol^c	<i>E,E</i>-FPP	α-Farnesene^c
	Solyc00g154480								
3	Solyc03g006550	46	e/f	11	Yes	821	Cytosol ^c	GGPP	Geranylinalool ^e
	Solyc03g007730	47	b	7	Yes	562	Cytosol^c	DMAPP	Isoprene^c
4	Solyc04g051620	48	a	8	Yes	560	Cytosol^c	<i>E,E</i>-FPP <i>Z,Z</i>-FPP	Hedycaryol (Elemol)^c Unidentified^c
	Solyc04g054380	28	a	7	Yes	554	Cytosol^c	<i>E,E</i>-FPP <i>Z,Z</i>-FPP	Hedycaryol (Elemol)^c Unidentified^c
6	Solyc06g059885	9 (Sst1)	a	7	Yes	548	Cytosol ^c	<i>E,E</i> -FPP	Germacrene ^f

	Solyc06g059910 Solyc06g059920	10	a	7	Yes	556	Cytosol^c	Z,Z-FPP	α-Bisabolol^c
	Solyc06g059930	12 (CAHS)	a	7	Yes	548	Cytosol ^c	<i>E,E</i> -FPP	β -Caryophyllene, α -humulene ^g
	Solyc06g060180	36	a	7	Yes	591	Mitochondria^b	Z,Z-FPP	<i>cis</i>-Muurola-3,5-diene^c
	Solyc06g084240	40 (CPS1)	c	15	Yes	800	Plastid ^c	GGPP	Copalyl diphosphate ^h
7	Solyc07g008680 Solyc07g008690	16	a	7	Yes	553	Cytosol^c	<i>E,E</i>-FPP	δ-Cadinene^c
	Solyc07g052120 Solyc07g052130	51	a	7	Yes	550	Cytosol^c	<i>E,E</i>-FPP Z,Z-FPP	<i>E</i>-Nerolidol^c α-Bisabolol^c
	Solyc07g052140 Solyc07g052150	52	a	7	Yes	551	Cytosol^c	<i>E,E</i>-FPP Z,Z-FPP	<i>E</i>-Nerolidol^c α-Bisabolol^c
	Solyc07g066670 Solyc07g066675	24 (KS)	e/f	15	Yes	820	Plastid ^c	CPP	<i>ent</i> -Kaurene ^b
8	Solyc08g005640	21	e/f	14	Yes	784	Plastid ^c	NNPP	Lycosantalene ⁱ
	Solyc08g005665	20 (PHS1)	e/f	14	Yes	778	Plastid ^c	NPP	β -Phellandrene ^j
	Solyc08g005670	19	e/f	14	Yes	778	Plastid ^c	NPP	β -Myrcene, β -ocimene ⁱ
	Solyc08g005710	41	c	15	Yes	780	Mitochondria^c	GGPP	Copalyl diphosphate^c
	Solyc08g005720	18	e/f	14	Yes	773	Mitochondria^c	NNPP	Unidentified^c
9	Solyc09g092470	14	a	7	Yes	568	Cytosol ^b	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	β -Bisabolene ^b α -Bisabolene ^b
10	Solyc10g005390	39	g	7	Yes	563	Cytosol ^c	GPP <i>E,E</i> -FPP	Linalool ^b <i>E</i> -Nerolidol ^b
	Solyc10g005410	37	g	7	Yes	560	Cytosol ^c	GPP <i>E,E</i> -FPP	Linalool ^b <i>E</i> -Nerolidol ^b
12	Solyc12g006570	17	a	7	Yes	554	Cytosol ^c	<i>E,E</i> -FPP	Valencene ^a

^aBleeker *et al.* (2011). ^bFalara *et al.* (2011). ^cThis work. ^dvan Schie *et al.* (2007). ^eFalare *et al.* (2014). ^fColby *et al.* (1998). ^gSchilmiller *et al.* (2010). ^hBensen and Zeevaart, (1990). ⁱMatsuba *et al.* (2013). ^jSchilmiller *et al.* (2009).

TPS gene numbers are adopted from Falara *et al.* (2011), terpene synthases characterized in this work are shown in bold.

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Table S4 A list of tomato terpene synthase pseudogenes.

Chromosome	Locus	TPS	No. of Exons	Type of mutation	Putative translated protein (aa)
1	Between Solyc01g101190 and Solyc01g101210	34	(-)3	Deletion of exons	No in frame start codon was found
	Solyc01g101220	30	(-)3(-)1	Deletion of exons	No in frame start codon was found
	Solyc01g105850 Solyc01g105860	1	7	Aberrant splicing, incorrect 3' splice sequence at the end of intron 4, cloned cDNAs skip exon 5 and part of exon 6	400(-)
	Solyc01g105900	6	7	Deletion of about 80nt in exon 3 to cause frame shift	165(-)
	Solyc01g105910	2	3(-)	Deletion of exons and stop codons in frame	131(-)
	Solyc01g105930 Solyc01g105940 Solyc01g105950	22	7	Large insertion in exon 2 and numerous stop codons in frame	52(-)
2	Solyc02g079900 Solyc02g079895	26	(-)4	Deletion of exons and mutation of 3' splice site	No in frame start codon was found
3	Solyc03g112175	49	(-)2	Only the last two exons were found.	(-)92
4	Between Solyc04g005100 and Solyc04g005120	50	2(-)2	Deletion of exons	51(-)
5	Solyc05g026590 Solyc05g026600	43	7	Insertion of an extra T in first exon to cause frame shift	30(-)
6	Between Solyc06g059885 and Solyc06g059880	23	(-)1	Deletion of exons	No in frame start codon was found
	Part of Solyc06g059930	11	2(-)2(-)	Deletion of exons	50(-)
	Solyc06g060010 Solyc06g060013	13	7	Insertion in exon 2 to cause frame shift	156(-)
7	Solyc07g051940	15	7	The end of the first exon has a deletion including	29(-).

				the splice site, so the intron is not spliced correctly, cloned cDNA has stop codon in frame	
9	Solyc09g065230	44	(-)3(-)	Deletion of exons	No in frame start codon was found
10	Solyc10g005420	42	(-)4(-)	Deletion of exons to cause frame shift	15(-)
	Solyc10g052745	53	(-)2	Deletion of exons	No in frame start codon was found
12	Solyc12g019240	29	(-)1(g)2(g)	Deletion of exons	No in frame start codon was found

TPS gene numbers and type of mutation are adopted from Falara *et al.* (2011), the new terpene synthase pseudogenes identified in this work are shown in bold.

Table S5 A list of the *trans*-prenyltransferase genes in tomato.

TPT	Locus	No. of exons	Predicted product ¹	Main Product	Subcellular Localization
FPPS1	Solyc12g015860	12	C15	FPP ^a	Cytosol ^b
GGPPS1	Solyc11g011240	1	C20	GGPP ^{b, c}	Plastid ^b
GGPPS2	Solyc04g079960	1	C20	GGPP ^{b, c}	Plastid ^b
SPPS	Solyc07g061990	6	C20	SPP ^d	Plastid ^d
DPPS	Solyc08g023470	12	≥C30	DPP ^d	Cytosol ^d
GGPPS3	Solyc02g085700	1	C20	GGPP ^b	Plastid ^b
TPT1	Solyc02g085710	1	C20	n.d. ^b	Mitochondria ^b
TPT2	Solyc02g085720	1	C20	n.d. ^b	Mitochondria ^b
SSU I	Solyc07g064660	1	C20	n.d. ^b	Plastid ^b
SSU II	Solyc09g008920	2	C20	n.d. ^b	Plastid ^b

¹Prediction of *trans*-prenyltransferase activity was made by the well-established “three floors” model of TPT product chain-length determination mechanism (Wang *et al.*, 2016).

^aGaffe *et al.* (2000). ^bThis work. ^cAment *et al.* (2006). ^dJones *et al.* (2013). n.d., not detected. Enzymes characterized in this work are shown in bold.

Ament K, Van Schie CC, Bouwmeester HJ, Haring MA, Schuurink RC. 2006. Induction of a leaf specific geranylgeranyl pyrophosphate synthase and emission of (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene in tomato are dependent on both jasmonic acid and salicylic acid signaling pathways. *Planta* **224**(5): 1197-1208.

Gaffe J, Bru JP, Causse M, Vidal A, Stamitti-Bert L, Carde JP, Gallusci P. 2000. *LeFPPS1*, a tomato farnesyl pyrophosphate gene highly expressed during early fruit development. *Plant Physiology* **123**(4): 1351-1362.

Jones MO, Perez-Fons L, Robertson FP, Bramley PM, Fraser PD. 2013. Functional characterization of long-chain prenyl diphosphate synthases from tomato. *Biochemical Journal* **449**(3): 729-740.

Wang CY, Chen QW, Fan DJ, Li JX, Wang GD, Zhang P. 2016. Structural analyses of short-chain prenyltransferases identify an evolutionarily conserved GFPPS clade in Brassicaceae plants. *Molecular Plant* **9**(2): 195-204.

Table S6 A list of terpene compounds produced by all the functional tomato terpene synthases.

TPS	Substrate	Products	Reference
3	GPP	Camphene , tricyclene, β -Myrcene, limonene, α -pinene, sabinene, β -pinene, unidentified	Falara <i>et al.</i> (2011)
4	GPP	β-Phellandrene , β -myrcene, sabinene	Van Schie <i>et al.</i> (2007)
5	GPP <i>E,E</i> -FPP	Linalool <i>E</i>-Nerolidol	Van Schie <i>et al.</i> (2007)
7	GPP	β-Myrcene , limonene, α -pinene, β -pinene, linalool, sabinene	Falara <i>et al.</i> (2011)
8	GPP NPP	1,8-Cineole , α -pinene 1,8-Cineole , α -pinene	Falara <i>et al.</i> (2011)
9	GPP <i>E,E</i> -FPP <i>Z,Z</i> -FPP	β-Myrcene , limonene, α -pinene, (+)-2-carene, α -terpinene, terpinolene Germacrene C , germacrene A, germacrene B, germacrene D Germacrene C , α -humulene, β -bisabolene, germacrene A, germacrene B	Bleeker <i>et al.</i> (2011) Colby <i>et al.</i> (1998) Bleeker <i>et al.</i> (2011)
10	<i>Z,Z</i> -FPP	α-Bisabolol	This work
12	GPP <i>E,E</i> -FPP <i>Z,Z</i> -FPP	β-Myrcene , limonene, terpinolene, <i>E</i> - β -ocimene β-Caryophyllene , α-humulene γ-Curcumene , β-bisabolene , <i>Z</i> - γ -bisabolene	Bleeker <i>et al.</i> (2011) Schilmiller <i>et al.</i> (2010)
14	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	β-Bisabolene , <i>E</i> -nerolidol, <i>E</i> - γ -bisabolene, <i>Z</i> - γ -bisabolene, <i>E</i> - α -bisabolene <i>E</i>-α-Bisabolene , β -bisabolene, <i>E</i> - γ -bisabolene, <i>Z</i> - γ -bisabolene,	Falara <i>et al.</i> (2011)
16	<i>E,E</i> -FPP	δ-Cadinene	This work
17	GPP <i>E,E</i> -FPP <i>Z,Z</i> -FPP	β-Myrcene , (+)-2-carene, limonene, terpinolene, <i>E</i> - β -ocimene, <i>Z</i> - β -ocimene, γ -terpinene, linalool Valencene , γ -gurjunene, <i>E</i> - β -farnesene, α -farnesene <i>Z</i>-γ-bisabolene , <i>E</i> - α -bergamotene, <i>Z</i> - α -bergamotene, <i>E</i> - γ -bisabolene, β -bisabolene	Bleeker <i>et al.</i> (2011)
18	NNPP	Unidentified	This work
19	NPP	β-Ocimene , β -myrcene	Matsuba <i>et al.</i> (2013)
20	NPP	β-Phellandrene , (+)-2-carene, limonene, α -phellandrene, α -terpinene	Schilmiller <i>et al.</i> (2009)
21	NNPP	Lycosantalene	Matsuba <i>et al.</i> (2013)
24	CPP	<i>Ent</i>-Kaurene	Falara <i>et al.</i> (2011)
25	GPP	<i>E</i>-β-ocimene , <i>Z</i> - β -ocimene, linalool, β -Myrcene	This work
27	<i>E,E</i> -FPP	α-Farnesene	This work
28	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	Elemol (hedycaryol) Unidentified , β -bisabolene, <i>E</i> - α -bisabolene, α -eudesmol, <i>Z,Z</i> -farnesol	This work
31	<i>E,E</i> -FPP	Viridiflorene	Bleeker <i>et al.</i> (2011)
32	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	Viridiflorene Unidentified	Falara <i>et al.</i> (2011)
33	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	Guaia-1(10),11-diene β-Acoradiene , β -curcumene, α -cedrene, <i>Z</i> - α -bergamotene, unidentified	This work
35	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	Guaia-1(10),11-diene , β -elemene (germacrene A) <i>Z,Z</i>-Farnesol , β -acoradiene	This work
36	<i>Z,Z</i> -FPP	<i>cis</i>-Muurolo-3,5-diene , β -acoradiene	This work

37	GPP <i>E,E</i> -FPP	Linalool <i>E</i> -Nerolidol	Falara <i>et al.</i> (2011)
38	<i>E,E</i> -FPP	<i>E</i> - α -Bergamotene	Falara <i>et al.</i> (2011)
39	GPP <i>E,E</i> -FPP	Linalool <i>E</i> -Nerolidol	Falara <i>et al.</i> (2011)
40	GGPP	Copalyl diphosphate	Bensen and Zeevaart, (1990)
41	GGPP	Copalyl diphosphate	This work
46	GGPP	Geranylinalool	Falara <i>et al.</i> (2014)
47	DMAPP	Isoprene	This work
48	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	Elemol (hedycaryol) Unidentified	This work
51	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	<i>E</i> -Nerolidol, <i>E</i> - β -farnesene α -Bisabolol, <i>Z</i> -nerolidol, <i>E</i> - α -bisabolene, β -bisabolene, <i>Z</i> - β -farnesene	This work
52	<i>E,E</i> -FPP <i>Z,Z</i> -FPP	<i>E</i> -Nerolidol, <i>E</i> - β -farnesene α -Bisabolol, <i>Z</i> -nerolidol, <i>E</i> - α -bisabolene, β -bisabolene, <i>Z</i> - β -farnesene	This work

The main product of each TPS enzyme using the corresponding substrate is shown in bold. The preferred substrates are shown in bold, considering their subcellular localizations. Structures of terpene compounds can be found in Fig. 11. TPS enzymes characterized in this work are shown in bold. Elemol is the thermal breakdown product of (+)-hedycaryol (Hattan *et al.*, 2016); β -elemene and δ -elemene are the thermal rearrangement of germacrene A and germacrene C, respectively (Colby *et al.*, 1998). References can be found in Table S3.

Table S7 A list of Arabidopsis terpene synthase genes, their characteristics, and the enzymes they encode.

Chromosome	AGI No.	TPS	TPS-clade	No. of Exons	Protein size (aa)	Subcellular localization	Substrate	Main product	Reference
1	At1g31950	29	a	7	607	M/P	Unknown	-	-
	At1g33750	22	a	6	603	M/P	<i>E,E</i> -FPP	(<i>E</i>)- β -Farnesene, α -farnesene	Wang <i>et al.</i> (2016)
	At1g48800	28	a	7	603	M/P	Unknown	-	-
	At1g61120	4	e/f	12	877	<u>C</u>	GGPP	Geranylinalool	Herde <i>et al.</i> (2008)
	At1g61680	14	g	7	569	<u>P</u>	GPP	Linalool	Chen <i>et al.</i> (2003)
	At1g66020	26	a	7	598	M/P	GGPP	Unknown diterpenes	Wang <i>et al.</i> (2016)
	At1g70080	6	a	7	611	M/P	GGPP	Dolabelladienol	Wang <i>et al.</i> (2016)
	At1g79460-KS1	32	e/f	14	785	<u>P</u>	<i>Ent</i> -CPP	<i>Ent</i> -kaurene	Yamaguchi <i>et al.</i> (1998); Helliwell <i>et al.</i> (2001)
2	At2g23230	5	a	7	606	M/P	Unknown	-	-
	At2g24210	10	b	7	591	<u>P</u>	GPP	(<i>E</i>)- β -ocimene, myrcene	Bohlmann <i>et al.</i> (2000)
3	At3g14490	17	a	7	601	P	GFPP	Sesquiterpene blend	Huang <i>et al.</i> (2017)
	At3g14520	18	a	7	605	<u>P</u>	GFPP	(+)-thalianatriene	Shao <i>et al.</i> (2017); Chen <i>et al.</i> (2019)
	At3g14540	19	a	7	602	<u>P</u>	GFPP	(-)-retigeranin B	Shao <i>et al.</i> (2017); Chen <i>et al.</i> (2019)
	At3g25810	24	b	7	598	P	GPP	Monoterpene blend	Chen <i>et al.</i> (2003)
	At3g25820	27	b	7	600	<u>P</u>	GPP	1,8-Cineole	Chen <i>et al.</i> (2004); Roos <i>et al.</i> (2015)
	At3g25830	23	b	7	600	<u>P</u>	GPP	1,8-Cineole	Chen <i>et al.</i> (2004)
	At3g29110	16	a	7	595	P	Unknown	-	-
	At3g29190	15	a	7	601	P	Unknown	-	-
	At3g29410	25	a	7	603	<u>P</u>	GFPP	(-)-ent-quiannulatene, (-)-variculatriene A	Huang <i>et al.</i> (2017); Chen <i>et al.</i> (2019)
	At3g32030	30	a	7	604	<u>P</u>	GFPP	(+)-Astallatene	Huang <i>et al.</i> (2017); Chen <i>et al.</i> (2019)
4	At4g02780-CPS	31	c	15	802	<u>P</u>	GGPP	<i>Ent</i> -CPP	Sun and Kamlya, 1997; Helliwell <i>et al.</i> (2001)

	At4g13280	12	a	8	524	C	<i>E,E</i> -FPP	(Z)- γ -bisabolene	Ro <i>et al.</i> (2006)
	At4g13300	13	a	7	554	C	<i>E,E</i> -FPP	(Z)- γ -bisabolene	Ro <i>et al.</i> (2006)
	At4g15870	1	a	7	598	P	Unknown	-	-
	At4g16730-Ws	2	b	7	589	<u>P</u>	GPP <i>E,E</i> -FPP	(E)- β -ocimene, myrcene (E,E)- α -farnesene	Huang <i>et al.</i> (2010)
	At4g16740	3	b	7	565	<u>P</u>	GPP <i>E,E</i> -FPP	(E)- β -ocimene, myrcene (E,E)- α -farnesene	Fäldt <i>et al.</i> (2003)
	At4g20210	8	a	7	600	<u>P</u>	GGPP	Rhizathalene A	Vaughan <i>et al.</i> (2013)
	At4g20230	9	a	7	607	M/P	GGPP	Unidentified diterpene	Wang <i>et al.</i> (2016)
	At4g20200	7	a	7	603	P	Unknown	-	-
5	At5g23960	21	a	7	547	C	<i>E,E</i> -FPP	(E)- β -caryophyllene	Chen <i>et al.</i> (2003)
	At5g44630	11	a	7	557	C	<i>E,E</i> -FPP	Several sesquiterpenes	Tholl <i>et al.</i> (2005)
	At5g48110-Cvi	20	a	8	592	<u>P</u>	GGPP	Dolathaliatriene, dolabelladienol	Wang <i>et al.</i> (2016)

TPS gene numbers are adopted from Aubourg *et al.* (2002). Underlined letters indicate the experimentally proved subcellular localizations and the other localizations were predicted by the TargetP and ChloroP programs. Ws, *Arabidopsis thaliana* Wassilewskija ecotype; Cvi, *A. thaliana* Cape Verde Islands ecotype. P, plastid; M, mitochondria; C, cytosol.

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1 **Methods S1 Details on experimental procedures.**

2 **Plant growth conditions**

3 Tomato seeds were germinated on moistened filter paper. After germination, the
4 seedlings were transplanted and grown in soil in a controlled growth room maintained
5 under 16 h of light ($300 \mu\text{E m}^{-2}\text{S}^{-1}$; Valoya LED grow lights, Helsinki, Finland) at 28°C
6 and 8 h of dark at 20°C . *Nicotiana benthamiana* and *Arabidopsis thaliana* (Col-0
7 ecotype) seeds were sown in potting soil, after germination the seedlings were transferred
8 and grown in soil in a growth chamber under controlled conditions of light intensity of
9 $200 \mu\text{E m}^{-2}\text{S}^{-1}$ (mixed cool-white and incandescent bulbs) for 16 h light period, and
10 day/night temperature of $25/20^\circ\text{C}$.

11

12 **RNA isolation, cDNA synthesis and quantitative real-time PCR**

13 Total RNA was isolated using the E.Z.N.A Plant RNA Kit (Omega Bio-tek, Norcross,
14 GA, USA) and incubated with DNase I (Promega, Madison, WI, USA) to avoid genomic
15 DNA contamination according to the manufacture's protocol. One microgram of total
16 RNA was used to synthesize cDNA by using the High Capacity cDNA Reverse
17 Transcription Kit (Thermo Fisher, Waltham, MA, USA). For quantitative real-time PCR
18 (RT-qPCR), $1 \mu\text{L}$ of the 10-fold diluted cDNA and gene-specific primers were applied to
19 $10 \mu\text{L}$ SYBR Green reactions (PowerUp SYBR Green Master Mix, Thermo Fisher),
20 which were run on a Real-Time PCR System (StepOnePlus, Thermo Fisher) with the
21 following cycles: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1
22 min, and a melting curve analysis. Gene expression values were calculated according to
23 the previously described comparative C_T method (Schmittgen & Livak, 2008). For
24 normalizing gene expressions, *Ubiquitin (Solyc11g071260)* was used as a control. Three
25 biological replicates were used for each point with three or more technical replicates.
26 Primers used are listed in Table S1.

27

28 **Protein expression in *Escherichia coli* and purification**

29 For enzymatic assays, TPS genes without their fragments corresponding to putative
30 transit peptides were cloned upstream and in-frame of the (His)₆-tag sequences in
31 pET32b to express TPS-His recombinant proteins. Restriction sites and primers used are
32 detailed in Table S1. The resulting recombinant plasmids were sequence verified and
33 transformed into *E. coli* Rosetta2(DE3)pLysS cells (Novagen, Burlington, MA, USA).
34 Single positive colonies were used to inoculate 4 mL LB medium containing 34 ng μL^{-1}
35 chloramphenicol and 100 ng μL^{-1} ampicillin. One mL of the overnight culture was
36 transferred to 100 mL of the same medium and continued to grow at 37°C to reach an
37 OD₆₀₀ of approximately 0.3. Protein expression was induced by adding isopropyl β -
38 thiogalactopyranoside (IPTG) to a final concentration of 1 mM. After 16 h incubation at
39 16°C, cells were harvested by centrifugation and stored at -80°C until use. The
40 recombinant His-tagged proteins were affinity purified using nickel-nitrilotriacetic acid
41 (Ni-NTA) agarose (Qiagen, Hilden, Germany) according to the manufacturer's manuals.
42 Protein concentration was determined using the Bradford method (Bradford, 1976) with
43 BSA as a standard.

44

45 **GC-MS analysis of terpenes**

46 The analysis of terpene products (C₁₀-C₂₅) was performed as described previously (Falara
47 *et al.*, 2011). One microliter of sample was auto-injected onto a Shimadzu (Kyoto, Japan)
48 QP-2010 SE GC-MS system equipped with the Rxi-5Sil column (30 m length, 0.25 mm
49 i.d., and 0.25 μm film thickness; Restek, Bellefonte, PA, USA). The injection port was
50 set to 240°C in splitless mode, and the flow rate of the He carrier gas was set at 1.4 mL
51 min⁻¹. The GC oven temperature was programmed as follows: 45°C for 2 min, 10°C min⁻¹
52 ¹ up to 250°C and hold for 3 min, 20°C min⁻¹ up to 320°C and hold for 5 min. The

53 analysis of prenyltransferase assay products was performed using an EC-WAX column
54 (30 m length, 0.32 mm i.d., and 0.25 μm film thickness; Grace, Columbia, MD, USA).
55 The injection port was set to 220°C in splitless mode, and the flow rate of the He carrier
56 gas was set at 1.5 mL min^{-1} . The GC oven temperature was programmed as follows: 45°C
57 for 2 min, 10°C min^{-1} up to 260°C and hold for 5 min. Interface temperature was 280°C
58 and ion source temperature was set to 260°C. The detector was activated after a 4.5-min
59 solvent delay. Mass spectra were obtained using the scan mode (total ion count, 45-450
60 mz^{-1}). The Kováts retention indices (Babushok *et al.*, 2011) of each terpene compounds
61 were determined based on a comparison with (C₇-C₄₀) n-alkanes. Products were
62 identified using the mass spectra database NIST11 and their mass spectra and retention
63 indices were compared with authentic compounds or the literature (Table S2).

64

65 Analysis of isoprene was performed by directly injecting the SPME fiber into the
66 Shimadzu QP-2010 SE GC-MS system equipped with the Rxi-5Sil column (30 m length,
67 0.25 mm i.d., and 0.25 μm film thickness; Restek). The injection port was set to 220°C in
68 splitless mode, and the flow rate of the He carrier gas was set at 1.0 mL min^{-1} . The GC
69 oven temperature was programmed as follows: 40°C for 3 min, 10°C min^{-1} up to 100°C
70 and hold for 3 min, 20°C min^{-1} up to 260°C and hold for 3 min. Isoprene was identified
71 by comparison with the authentic standard.

72

73 **Correlation analysis of tomato terpene synthase genes and terpene volatiles**

74 The Spearman correlation coefficients were calculated by GraphPad Prism 6 using gene
75 expression data and terpene profiling data as indicated in Fig. 9 and Fig. 10, respectively.
76 Based on the Spearman correlation coefficient, the hierarchical clustering was performed
77 using average linkage method by Cluster 3.0 and a heatmap was built using TreeView
78 3.0.

79

80 **Accession numbers**

81 The GenBank accession numbers of the proteins used in the phylogenetic analysis (Fig.
82 S9) are as follows: AgGPPS1, AF513111; AgGPPS2, AF513112; AgGPPS3, AF513113;
83 PaGPPS, EU432047; PaGPPS/GGPPS, GQ369788; PaGPPS3, EU432048; MiGPPS1,
84 JN035297; MiGPPS2, JN035298; MiFPPS, JN035296; AmGPPS.LSU, AAS82860;
85 AmGPPS.SSU, AAS82859; CbGPPS.SSU, AY534745; CrGGPPS, JX417183;
86 CrGPPS.SSU, JX417184; HIGPPS.LSU, FJ455407; HIGPPS.SSU, ACQ90681;
87 MpGPPS.LSU, AF182828; MpGPPS.SSU, AF182827; VvGPPS, AAR08151; LaFPPS,
88 U15777; AaFPPS, U36376; ZmFPPS, L39789; NtGGPPS1, GQ911583; NtGGPPS2,
89 GQ911584; SaGGPPS, CAA67330; HbSPPS, DQ437520; OsGGPPS1, Os07g39270;
90 OsGPPS, Os01g14630; OsGRP; Os02g44780. AGI numbers for *A. thaliana* TPTs are as
91 follows: AtPPPS, At2g434630; AtFPPS1, At5g47770; AtFPPS2, At4g17190; AtSPPS1,
92 At1g78510; AtSPPS2, At1g17050; AtGGPPS11, At4g36810; AtGGPPS2, At2g18620;
93 AtSSU, At4g38460. Tomato TPT homologs are listed in Table S5.

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