SUPPLEMENTARY INFORMATION

Supplementary Table 1: Strains, genes and plasmids used in this study.

Strain or plasmid	Description	Source/Accession #
Strains		
E. coli JM109	*recA1 supE44 endA1 hsdR17 ($r_k^-m_k^+$) gyrA96 relA1 thi*Δ(*lac-proAB [F'traD36 proAB$^+$ lacI^q lacZΔM15]*	New England Biolabs
E. coli BL21 (DE3)	*F$^-$ ompT hsdS$_B$($r_B^-m_B$)gal dxm* (DE3)	New England Biolabs
Genes		
COP3	Sesquiterpene cyclase from *C. cinereus*	XP_001832925
COP4	Sesquiterpene cyclase from *C. cinereus*	XP_001836356
COP6	Sesquiterpene cyclase from *C. cinereus*	XP_001832548
Plasmids		
pUCmodRBS	constitutive *lac* promoter, optimal RBS, Amp^r	(1)
pET-21	T7 promoter, for C-terminal fusion of His-tag, Amp^r	Novagen
pHIS8	Modified pET vector for N-terminal fusion of 8xHis-tag, Kam^r	(3)
pUCmod-Cop3	*COP3* cloned into BglII and NotI sites of pUCmodRBS	(1)
pHIS8-Cop6	*COP6* cloned into BamH1 and NcoI sites of pHIS8.	(4)
pET21-Cop4	*COP4* cloned into NdeI and XhoI sites of pET-21	(4)

Supplementary Table 2. Active site volumes of Cop sesquiterpene synthase homology model structures in the closed and open conformation. Active site volumes were calculated with CASTp (2) using the CASTpyMol version 2.0 as described in Methods.

	Radius (Å)	Closed (Å^3)	Open(Å^3)
Cop3	2.5	400	3353
Cop4	2.5	412	3376
Cop6	2.5	503	1695

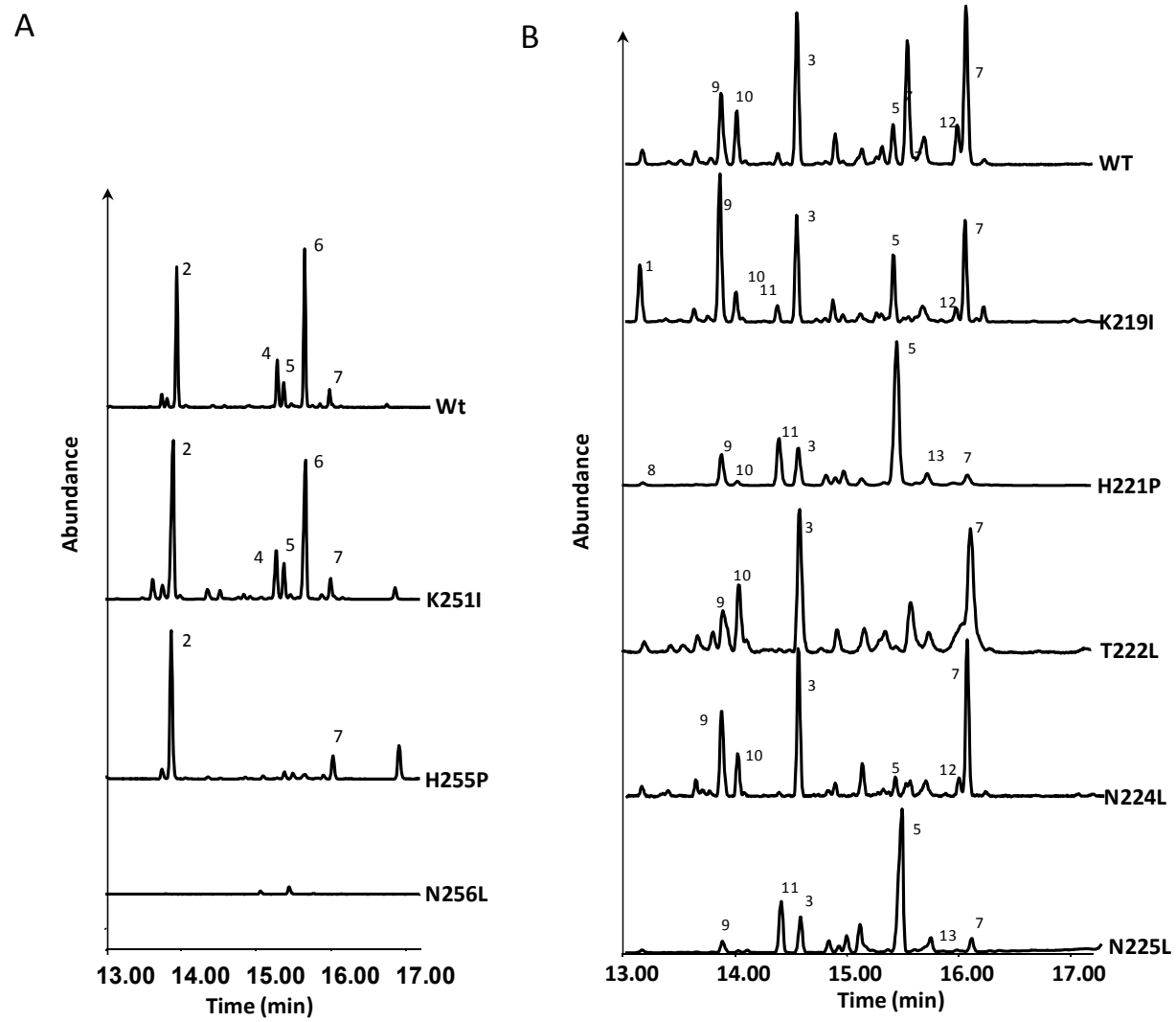
Supplementary Table 3. Sesquiterpenes produced by Cop6, Cop4 and Cop3 and their mutants under *in vitro* reaction conditions with FPP as substrate. Sesquiterpene product numbers correspond to the compound numbers shown in Figure 2 and Scheme 1. Compound 16 (γ-cadinene) is not produced *in vivo* and therefore not shown in Figure 2 and Scheme 1. The structures and mass spectra of compound 16 along with all other detected compounds are shown in Supplementary Figure 2. Relative amounts (%) of sesquiterpene products are shown. Percentages are averages calculated from the product profiles of three independent *in vitro* reactions. The error was less than 5% in all cases. Major sesquiterpene products of wild-type and mutant enzymes are highlighted in bold. U: sum of unidentified sesquiterpene compounds.

		Cop6		
Sesquiterpene[1]	**Wt**	**C236A**	**E237L**	**N240L**
1	**88.9**	**88.9**	**90.9**	**94.8**
2	3.9	4.6	3.8	2.9
U[2]	7.2	6.5	5.3	2.4

		Cop4				
Sesquiterpene	**Wt**	**K233I**	**H235P**	**T236L**	**N238L**	**N239L**
3	-	6.4	-	6.6	3.6	-
4	5.4	14.1	6.0	12.4	11.9	-
5	2.8	-	-	6.5	3.6	-
6	7.4	4.8	11.0	10.6	14.2	9
7	**29.3**	13.1	**57.0**	-	23	**60**
8	10.4	8	-	18.9	15.1	-
9	**28.2**	**43.3**	-	**44.9**	**22.7**	-
10	-	-	-	-	-	-
11	-	3.3	17.0	-	6	17
12	-	3.7	-	-	3.2	-
U	16.6	6.9	5.0	-	-	11

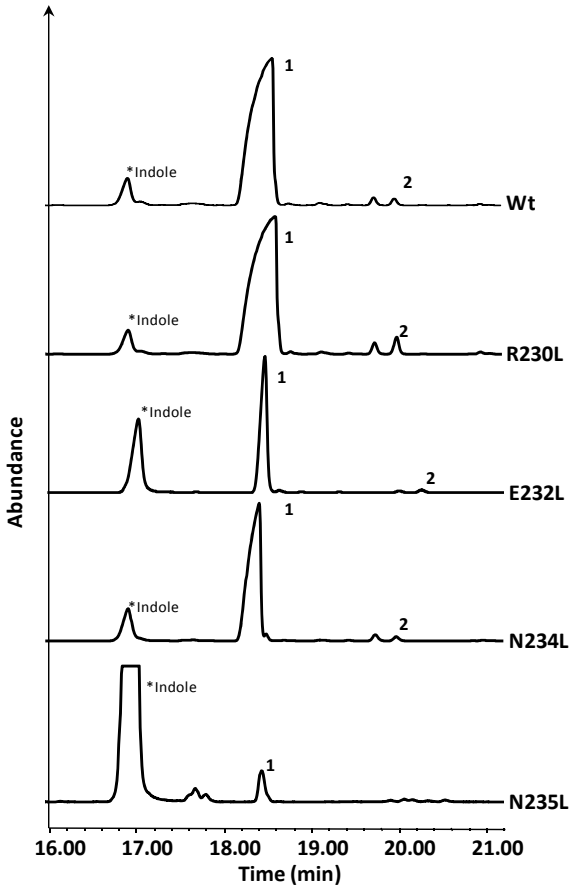
		Cop3		
Sesquiterpene	**Wt**	**K251I**	**H255P**	**N256L**
8	-	-	23	-
13	42	43	**50**	-
14	14	15	-	-
15	**43**	**43**	-	-
16	-	-	27	-

Supplementary Figure 1. GC/MS analysis of reaction products of *in vivo* assays of different H-α1 loop mutants for several Sesquiterpenes synthases. (A) Cop3. (B) Cop4 and (C) Cop6. Peaks are labeled with numbers that correspond to their identified structures shown in Figure 2 and Scheme 1. Compounds were identified by comparison of their mass spectra and RI values with data from published reference (MassFinder3, terpene library and authentic standards). Mass spectra of identified compounds are shown in Supplementary Figure 2.

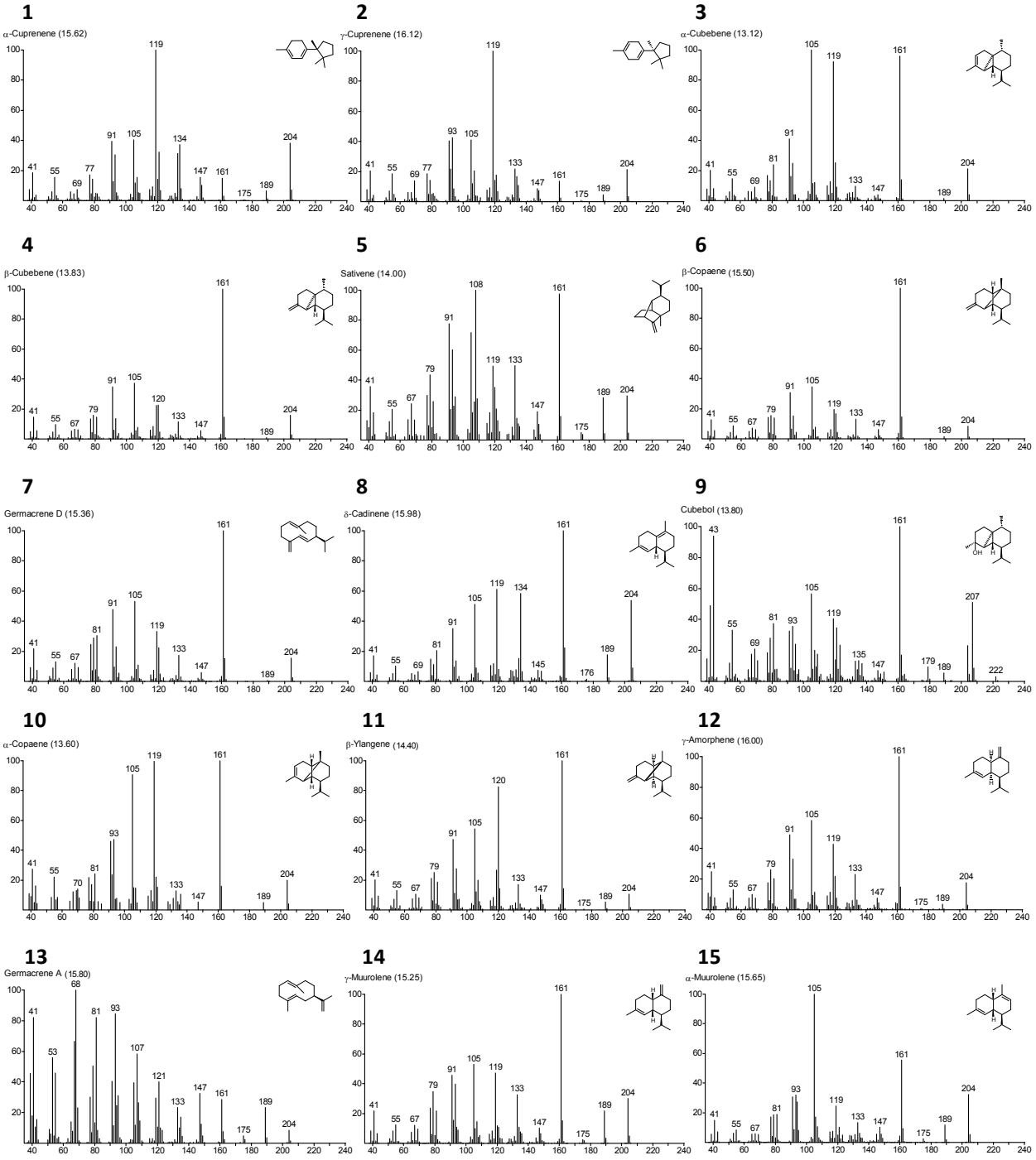


Supplementary Figure 1 continued

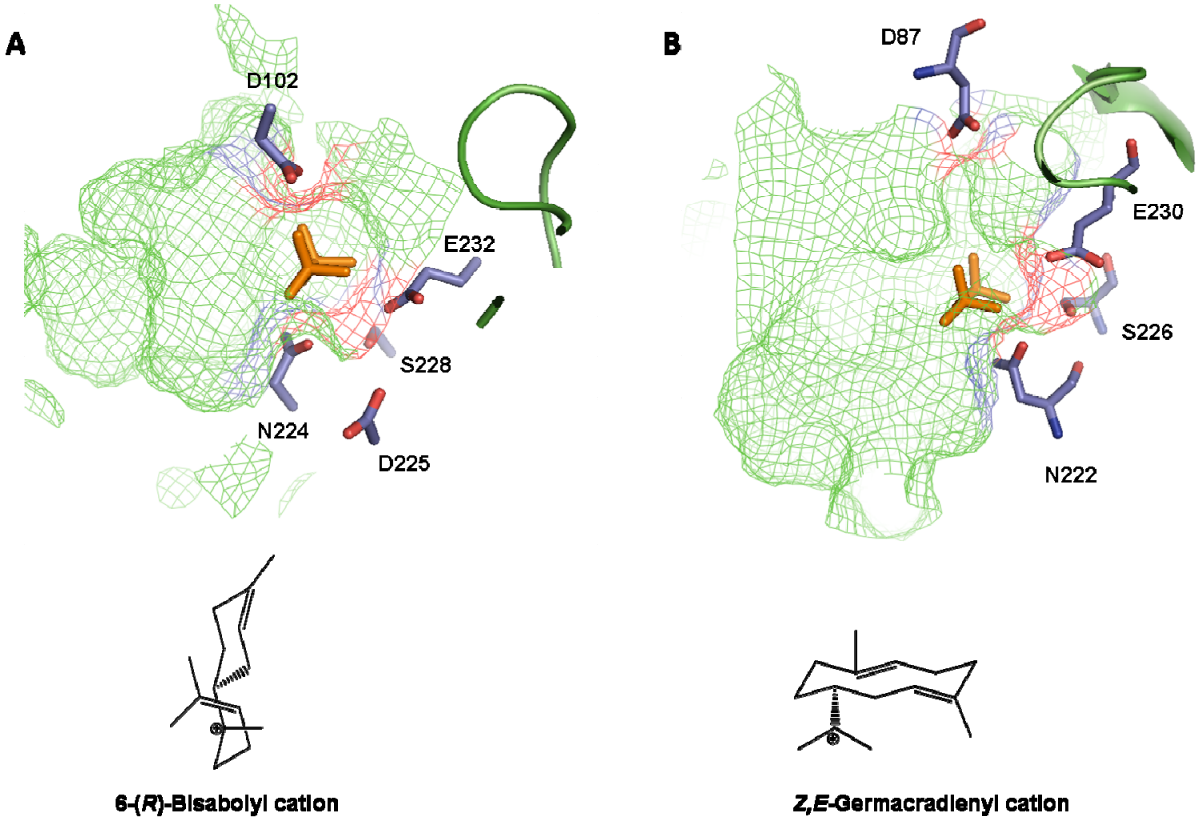
C



Supplementary Figure 2: Mass spectra of identified sesquiterpenoids. Numbers correspond to compound names shown in Figures 2 & 3 and Scheme 1. Numbers given in parentheses represent retention times of compounds.



Supplementary Figure 3: Active site contours of Cop6 (A) and Cop4 (B) homology models in the closed conformation. Active site contours are depicted by a mesh representation. Residues of the conserved metal-binding DDXXD and NSE/DTE motifs located at the active site entrance are shown in purple, while orange sticks indicate the bound pyrophosphate ligand. The H-α1 loop for each enzyme is depicted in green. The initial cyclic carbocation products of Cop6 (6-(*R*)-bisabolyl cation) and Cop4 (*Z,E*-germacradienyl cation) are also shown. The binding pocket of Cop6 is narrower, enforcing rearrangement of its initial 6-(*R*)-bisabolyl carbocation along one pathway to (-)-α-cuprenene **1**. In contrast, the wider binding pocket of Cop4 provides much less conformational restraint, allowing the formation of multiple cyclization products from its initial cyclic *Z,E*-germacradienyl carbocation.



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