<u>Supporting Information for</u>:

Catalytic bases and stereo-control in Lamiaceae class II diterpene cyclases

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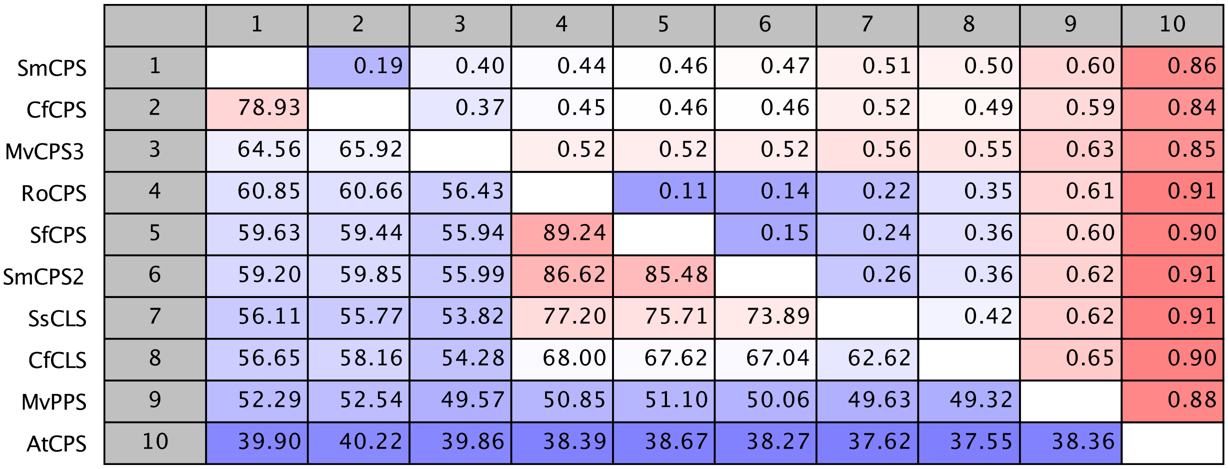
Figure S1. Identity (lower) and distance (upper) matrix for DTC alignment shown in Figure 1.



		1	2	3	4	5	6	7	8	9	10
SmCPS	1		0.19	0.40	0.44	0.46	0.47	0.51	0.50	0.60	0.86
CfCPS	2	78.93		0.37	0.45	0.46	0.46	0.52	0.49	0.59	0.84
MvCPS3	3	64.56	65.92		0.52	0.52	0.52	0.56	0.55	0.63	0.85
RoCPS	4	60.85	60.66	56.43		0.11	0.14	0.22	0.35	0.61	0.91
SfCPS	5	59.63	59.44	55.94	89.24		0.15	0.24	0.36	0.60	0.90
SmCPS2	6	59.20	59.85	55.99	86.62	85.48		0.26	0.36	0.62	0.91
SsCLS	7	56.11	55.77	53.82	77.20	75.71	73.89		0.42	0.62	0.91
CfCLS	8	56.65	58.16	54.28	68.00	67.62	67.04	62.62		0.65	0.90
MvPPS	9	52.29	52.54	49.57	50.85	51.10	50.06	49.63	49.32		0.88
AtCPS	10	39.90	40.22	39.86	38.39	38.67	38.27	37.62	37.55	38.36	

Figure S2. Mass spectra for enzymatic products and authentic standards (as indicated).

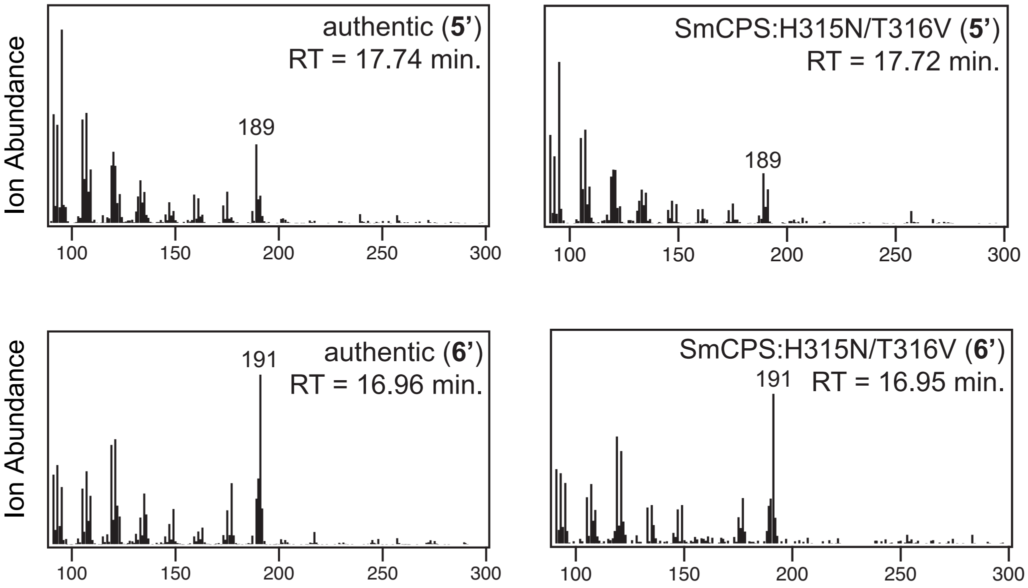


Figure S3. GC-MS chromatograms for indicated AtCPS mutants (**1’**, geranylgeraniol; **2’**, *ent*-copalol; **2***, acetylated *ent*-copalol; **3’**, *ent*-8α-hydroxy-copalol; **7’**, *ent*-kolavenol; +, contaminants).

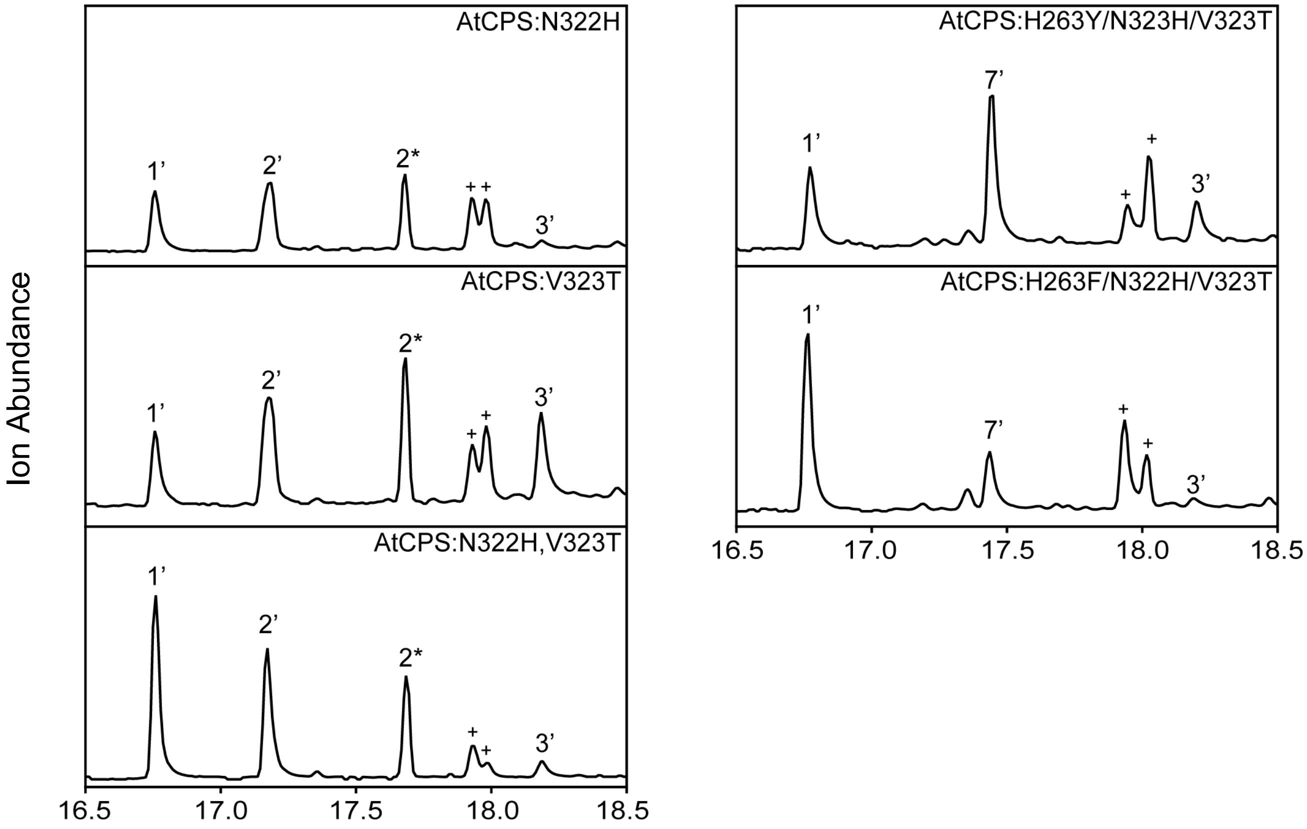
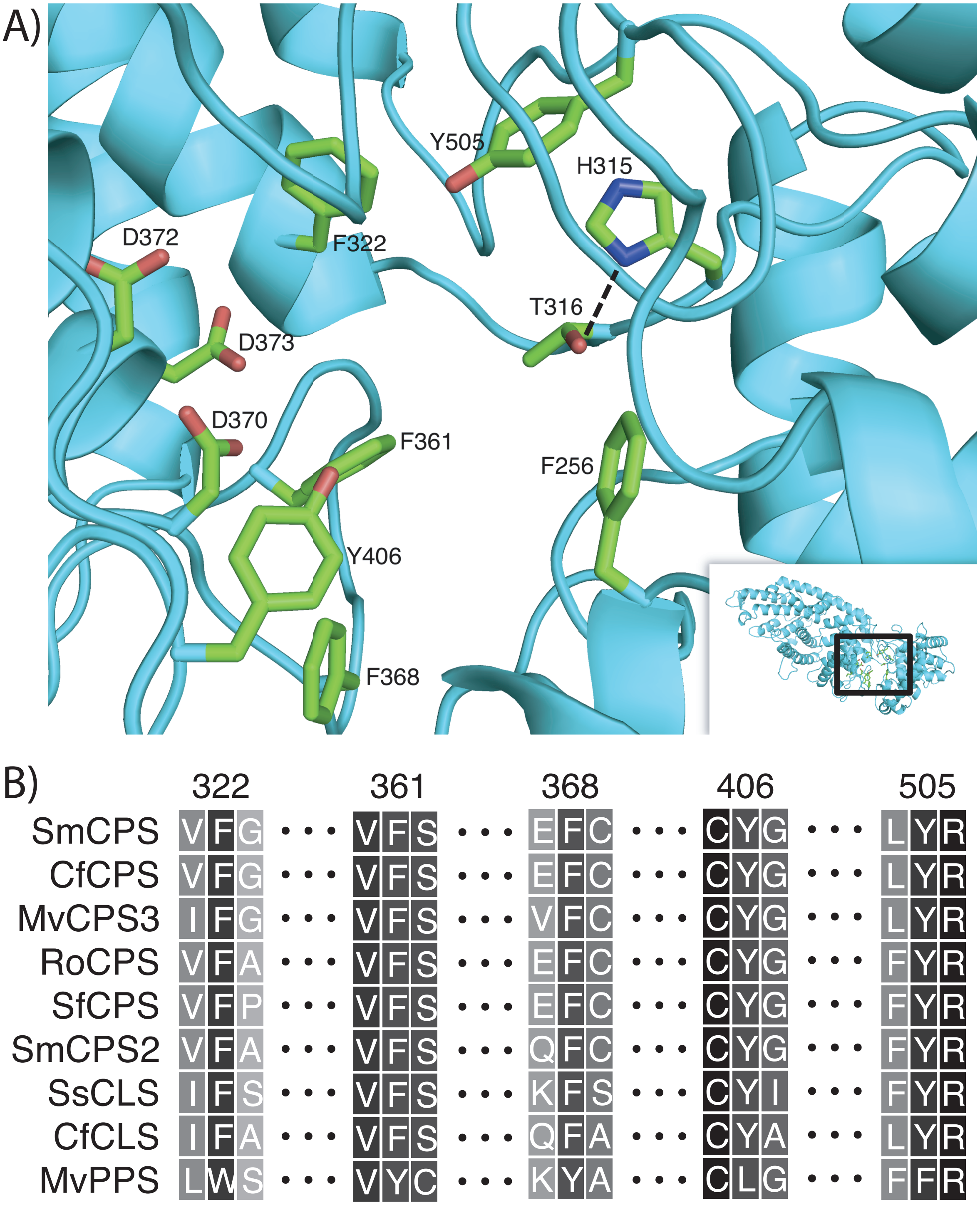
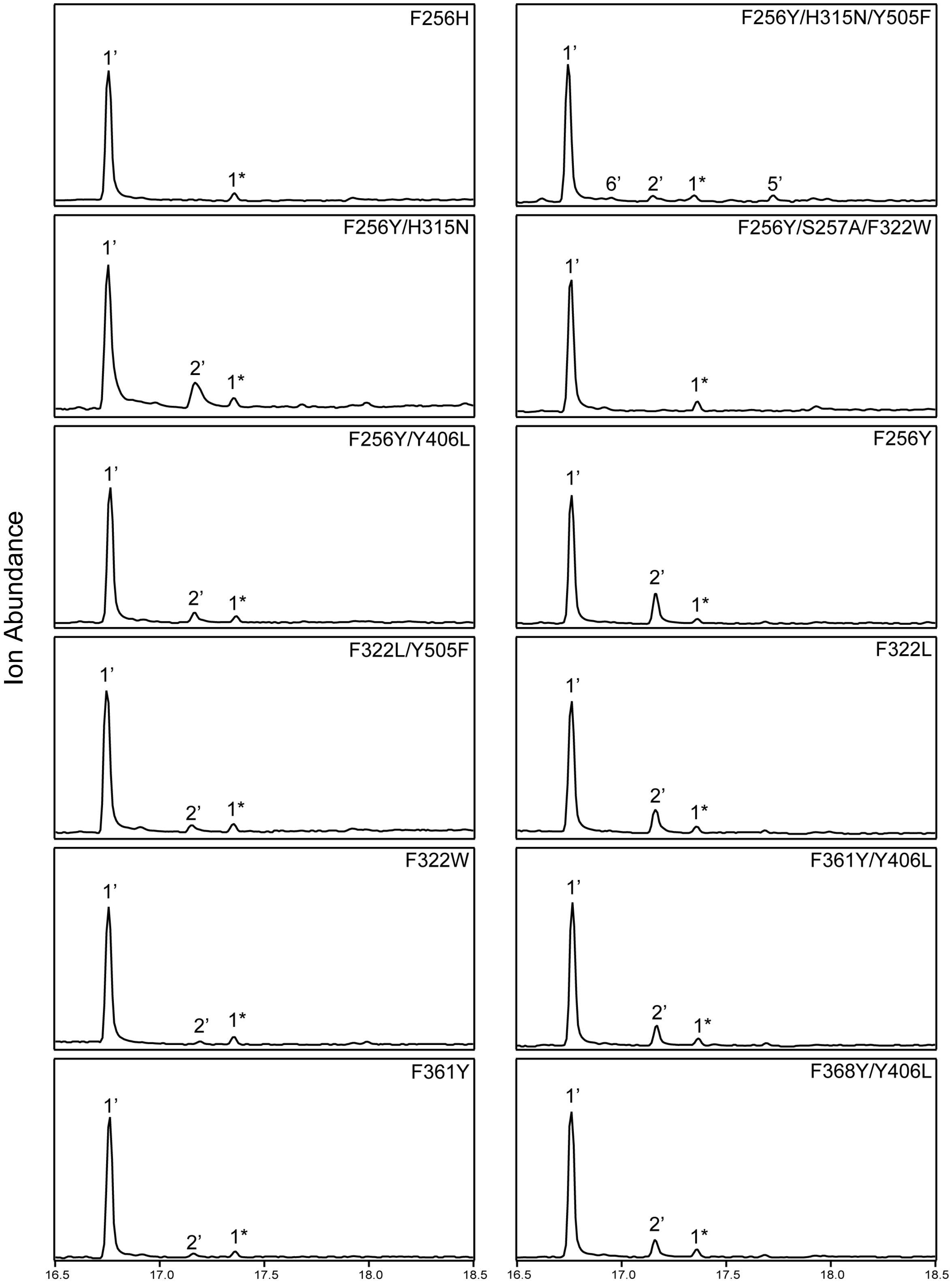


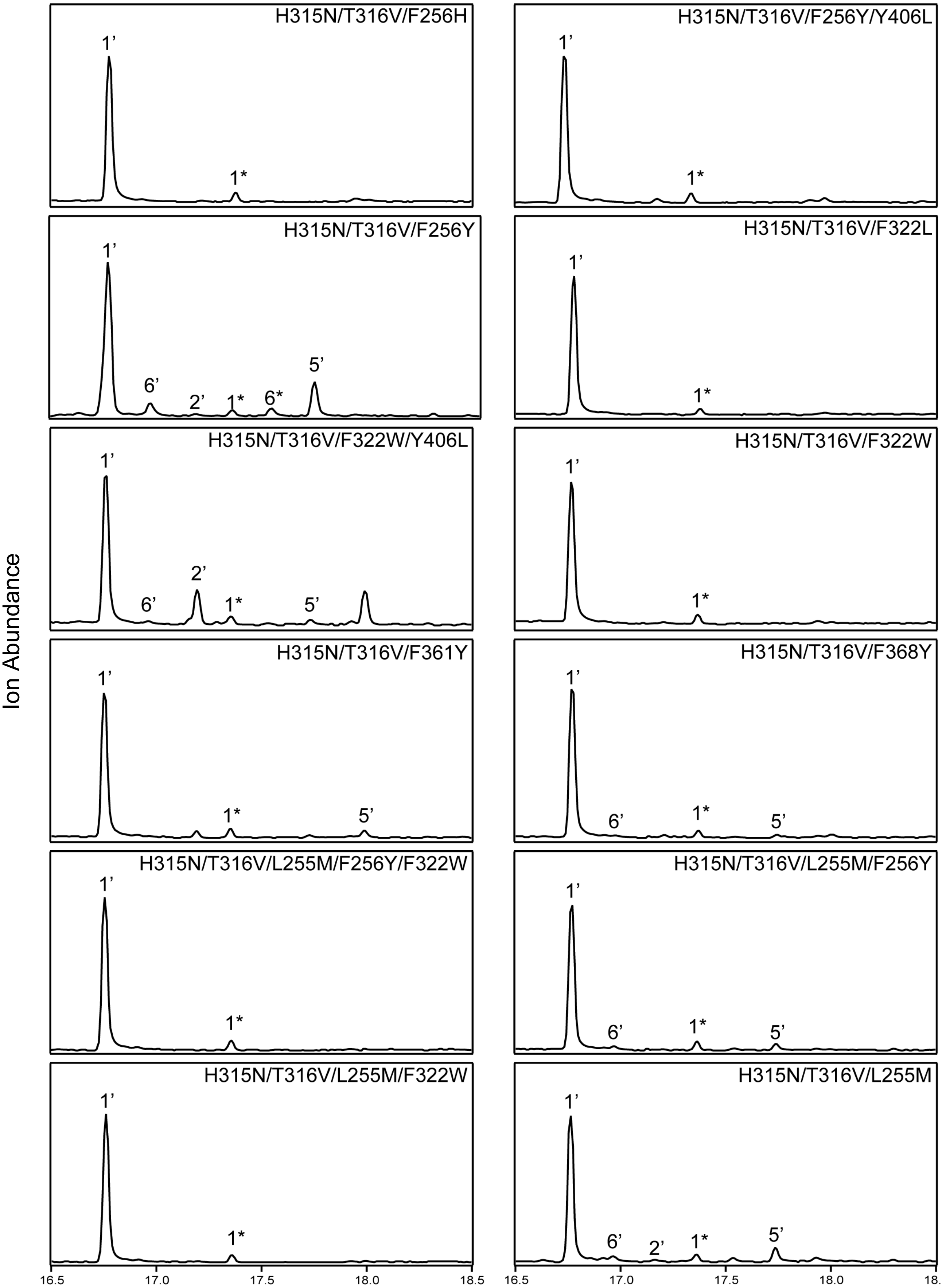
Figure S4. SmCPS active site. A) Modeled structure, with the side chains of the full set of residues targeted here, along with the aspartates from the DxDD motif that define the active site, shown and labeled. B) Conservation of the active site aromatic amino acids in the clade of Lamiaceae DTCs from more specialized metabolism.



	322		361		368		406		505
SmCPS	VFG	• • •	VFS	• • •	EFC	• • •	CYG	• • •	LYR
CfCPS	VFG	• • •	VFS	• • •	EFC	• • •	CYG	• • •	LYR
MvCPS3	IFG	• • •	VFS	• • •	VFC	• • •	CYG	• • •	LYR
RoCPS	VFA	• • •	VFS	• • •	EFC	• • •	CYG	• • •	FYR
SfCPS	VFP	• • •	VFS	• • •	EFC	• • •	CYG	• • •	FYR
SmCPS2	VFA	• • •	VFS	• • •	QFC	• • •	CYG	• • •	FYR
SsCLS	IFS	• • •	VFS	• • •	KFS	• • •	CYI	• • •	FYR
CfCLS	IFA	• • •	VFS	• • •	QFA	• • •	CYA	• • •	LYR
MvPPS	LWS	• • •	VYC	• • •	KYA	• • •	CLG	• • •	FFR

Figure S5. GC-MS chromatograms for indicated SmCPS mutants (**1’**, geranylgeraniol; **1***, acetylated geranylgeraniol; **2’**, copalol; **3’**, 8α-hydroxy-copalol; **5’**, terpentedienol; **6’**, *syn*-halima-5,13*E*-dienol; **6***, acetylated *syn*-halima-5,13*E*-dienol; **8’**, *endo*-copalol).




Ion Abundance
H315N/T316V/F256H
1’
1*
H315N/T316V/F256Y/Y406L
1’
1*
H315N/T316V/F256Y
1’
6’
2’
1*
6*
5’
H315N/T316V/F322L
1’
1*
H315N/T316V/F322W/Y406L
1’
6’
2’
1*
5’
H315N/T316V/F322W
1’
1*
H315N/T316V/F361Y
1’
1*
5’
H315N/T316V/F368Y
1’
6’
1*
5’
H315N/T316V/L255M/F256Y/F322W
1’
1*
H315N/T316V/L255M/F256Y
1’
6’
1*
5’
H315N/T316V/L255M/F322W
1’
1*
H315N/T316V/L255M
1’
6’
2’
1*
5’
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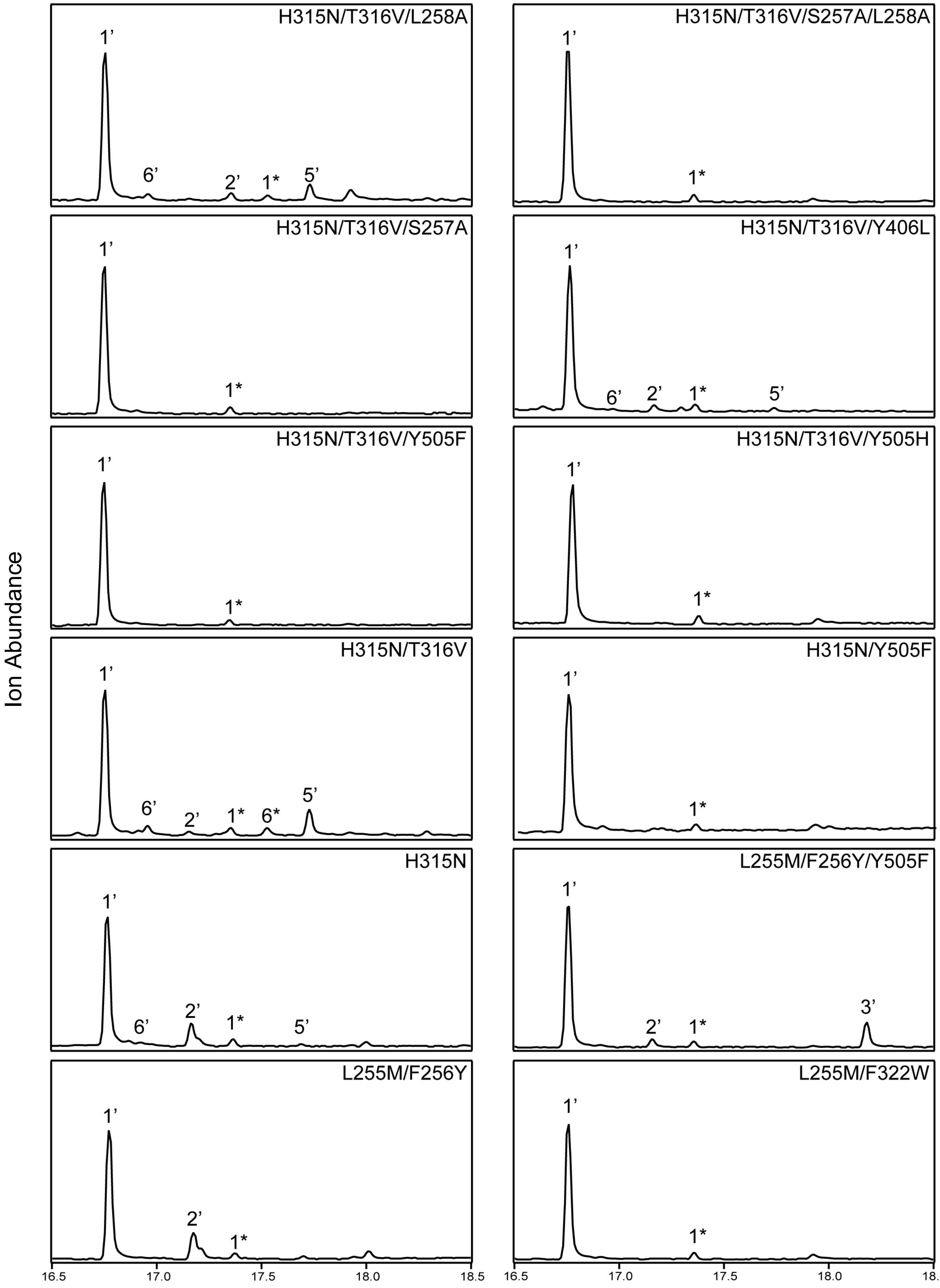
17.0
17.5
18.0
18.5
16.5
17.0
17.5
18.0
18.5


Ion Abundance
H315N/T316V/L258A
1’
6’
2’
1*
5’
H315N/T316V/S257A/L258A
1’
1*
H315N/T316V/S257A
1’
1*
H315N/T316V/Y406L
1’
6’
2’
1*
5’
H315N/T316V/Y505F
1’
1*
H315N/T316V/Y505H
1’
1*
H315N/T316V
1’
6’
2’
1*
6*
5’
H315N/Y505F
1’
1*
H315N
1’
6’
2’
1*
5’
L255M/F256Y/Y505F
1’
2’
1*
3’
L255M/F256Y
1’
2’
1*
L255M/F322W
1’
1*
16.5
17.0
17.5
18.0
18.5
16.5
17.0
17.5
18.0
18.5

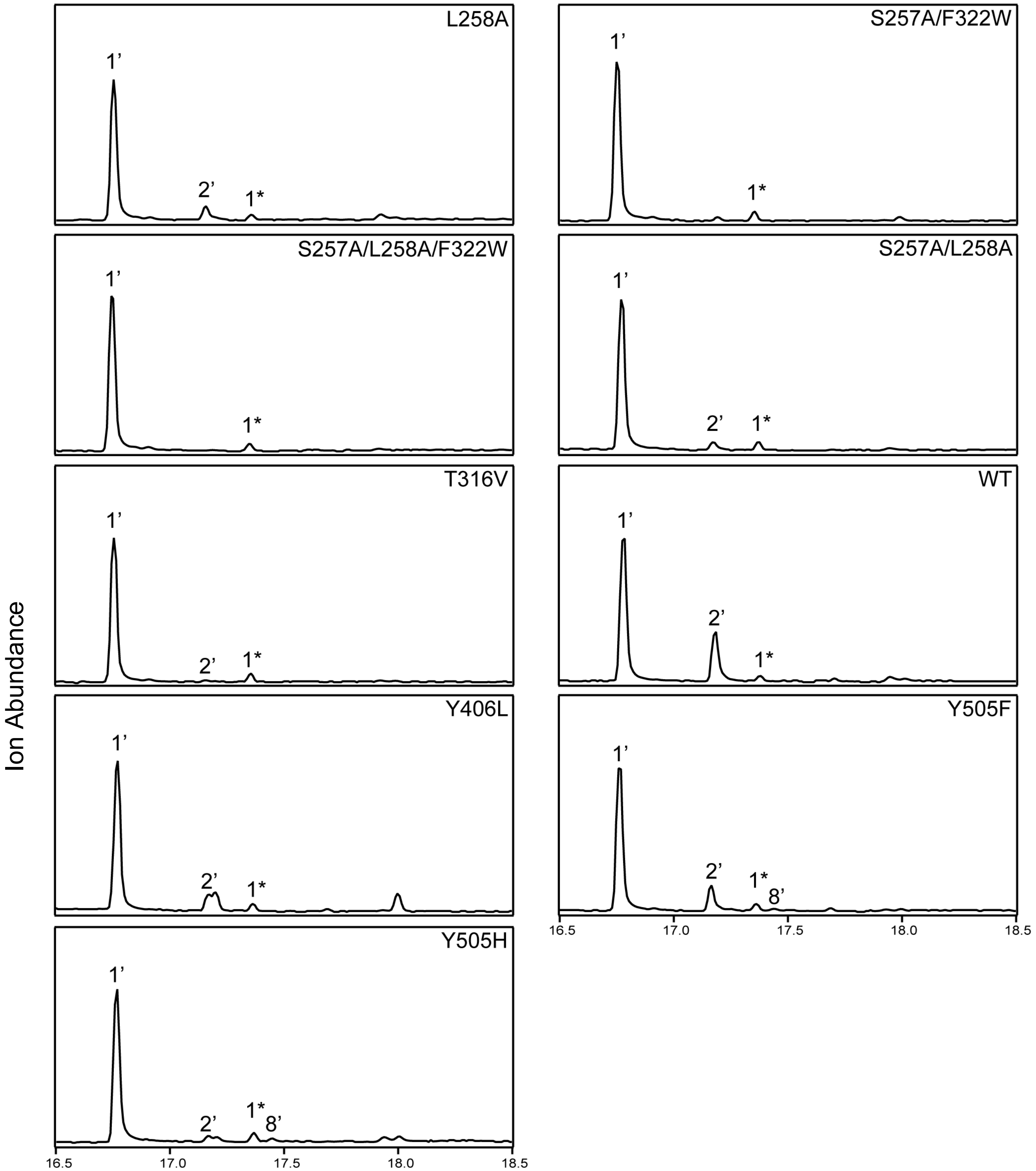

Ion Abundance
L258A
1’
2’
1*
S257A/F322W
1’
1*
S257A/L258A/F322W
1’
1*
S257A/L258A
1’
2’
1*
T316V
1’
2’
1*
WT
1’
2’
1*
Y406L
1’
2’
1*
Y505F
1’
2’
1*
8’
Y505H
1’
2’
1*
8’
16.5
17.0
17.5
18.0
18.5
16.5
17.0
17.5
18.0
18.5

Table S1. Primers used here (bold/underlined highlights introduced changes).

Gene	Mutant/use	Primer
SmCPS	sub-cloning F	**CACC**ATGCATCAAGGCCATGATGCG
	sub-cloning R	TCACGCGACTGGCTCGAAAAG
	L255M F	CCGACATCATTA**ATG**TTTAGTTTGGAAGGGCTCG
	L255M R	CGAGCCCTTCCAAACTAAA**CAT**TAATGATGTCGG
	F256H F	CCGACATCATTATTA**CA**TAGTTTGGAAGGGCTC
	F256H R	GAGCCCTTCCAAACTA**TG**TAATAATGATGTCGG
	F256Y F	CCGACATCATTATTAT**A**TAGTTTGGAAGGGCTCG
	F256Y R	CGAGCCCTTCCAAACTA**T**ATAATAATGATGTCGG
	S257A F	CCGACATCATTATTATTT**GCG**TTGGAAGGGCTCG
	S257A R	CGAGCCCTTCCAA**CGC**AAATAATAATGATGTCGG
	S257G F	CCGACATCATTATTATTT**GGC**TTGGAAGGGCTCG
	S257G R	CGAGCCCTTCCAA**GCC**AAATAATAATGATGTCGG
	S257N F	CCGACATCATTATTATTTA**AC**TTGGAAGGGCTCG
	S257N R	CGAGCCCTTCCAA**GT**TAAATAATAATGATGTCGG
	L258A F	CGACATCATTATTATTTAGT**GC**GGAAGGGCTCG
	L258A R	CGAGCCCTTCC**GC**ACTAAATAATAATGATGTCG
	H315N F	GAGGAGCGCCA**A**ACACTTATCCCGTC
	H315N R	GACGGGATAAGTGT**T**TGGCGCTCCTC
	T316V F	GAGCGCCACAC**GTG**TATCCCGTCGAC
	T316V R	GTCGACGGGATA**CAC**GTGTGGCGCTC
	F322L F	CGTCGACGTG**CTG**GGAAGGCTCTGG
	F322L R	CCAGAGCCTTCC**CAG**CACGTCGACG
	F322W F	CGTCGACGTGT**GG**GGAAGGCTCTGG
	F322W R	CCAGAGCCTTCC**CC**ACACGTCGACG
	F361Y F	CGGATAAGGGAGTTT**AT**AGTGGGAGAGAATCG
	F361Y R	CGATTCTCTCCCACT**AT**AAACTCCCTTATCCG
	F368Y F	GGAGAGAATCGGAGT**A**TTGCGACATTGACG
	F368Y R	CGTCAATGTCGCAA**T**ACTCCGATTCTCTCC
	Y406L F	GTAAATTCTCTTGC**CTG**GGCGGGCAGATGATC
	Y406L R	GATCATCTGCCCGCC**CAG**GCAAGAGAATTTAC
	Y505F F	GGAAAGACGCTGT**TT**AGGATGCCGGAG
	Y505F R	CTCCGGCATCCT**AA**ACAGCGTCTTTCC
	Y505H F	CGGAAAGACGCTG**CAT**AGGATGCCGGAG
	Y505H R	CTCCGGCATCCT**ATG**CAGCGTCTTTCCG
	L255M/F256Y F	CAAAATACCGACATCATTA**ATGTA**TAGTTTGGAAGGGCTC
	L255M/F256Y R	GAGCCCTTCCAAACTA**TACAT**TAATGATGTCGGTATTTTG
	L255M**+S257A** F	CGACATCATTAATGTTT**GCG**TTGGAAGGGCTCG
	L255M**+S257A** R	CGAGCCCTTCCAA**CGC**AAACATTAATGATGTCG
	L255M**+L258A** F	CGACATCATTAATGTTTAGT**GC**GGAAGGGCTCG
	L255M**+L258A** R	CGAGCCCTTCC**GC**ACTAAACATTAATGATGTCG
	F256H/S257N F	CAAAATACCGACATCATTATTA**CATAAC**TTGGAAGGGCTC
	F256H/S257N R	GAGCCCTTCCAA**GTTATG**TAATAATGATGTCGGTATTTTG
	F256Y/S257A F	GACATCATTATTAT**ATGCG**TTGGAAGGGCTCGAAAATTTG
	F256Y/S257A R	CAAATTTTCGAGCCCTTCCAA**CGCAT**ATAATAATGATGTC
	F256Y/S257G F	GACATCATTATTAT**ATGGC**TTGGAAGGGCTCGAAAATTTG
	F256Y/S257G R	CAAATTTTCGAGCCCTTCCAA**GCCAT**ATAATAATGATGTC
	F256Y**+L258A** F	CGACATCATTATTATATAGT**GC**GGAAGGGCTCG
	F256Y**+L258A** R	CGAGCCCTTCC**GC**ACTATATAATAATGATGTCG
	S257A/L258A F	GACATCATTATTATTT**GCGGC**GGAAGGGCTCGAAAATTTG
	S257A/L258A R	CAAATTTTCGAGCCCTTCC**GCCGC**AAATAATAATGATGTC
	H315N/T316V F	GAGGAGCGCCA**A**AC**GTG**TATCCCGTCG
	H315N/T316V R	CGACGGGATA**CAC**GT**T**TGGCGCTCCTC
	L255M/F256Y**+S257A/L258A** F	GACATCATTAATGTAT**GCGGC**GGAAGGGCTCGAAAATTTG
	L255M/F256Y**+S257A/L258A** R	CAAATTTTCGAGCCCTTCC**GCCGC**ATACATTAATGATGTC
AtCPS	H263Y F	CCGACATCATTA**ATG**TTTAGTTTGGAAGGGCTCG
	H263Y R	CGAGCCCTTCCAAACTAAA**CAT**TAATGATGTCGG
	H263F F	CCGACATCATTATTA**CA**TAGTTTGGAAGGGCTC
	H263F R	GAGCCCTTCCAAACTA**TG**TAATAATGATGTCGG
	N322H F	CCGACATCATTATTAT**A**TAGTTTGGAAGGGCTCG
	N322H R	CGAGCCCTTCCAAACTA**T**ATAATAATGATGTCGG
	V323T F	CCGACATCATTATTATTT**GCG**TTGGAAGGGCTCG
	V323T R	CGAGCCCTTCCAA**CGC**AAATAATAATGATGTCGG
	N322H/V323T F	CCGACATCATTATTATTT**GGC**TTGGAAGGGCTCG
	N322H/V323T R	CGAGCCCTTCCAA**GCC**AAATAATAATGATGTCGG