SUPPLEMENTAL INFORMATION

DEFINING THE POTASSIUM BINDING REGION IN AN APPLE TERPENE SYNTHASE

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SUPPLEMENTARY FIGURES

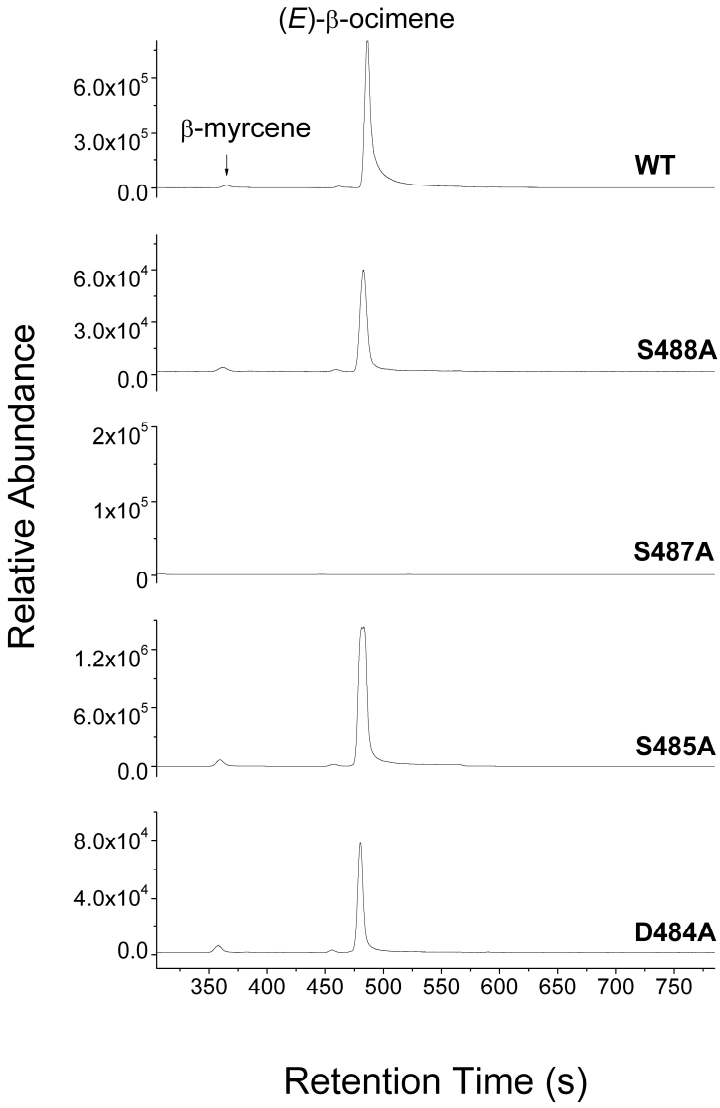

Fig. S1. Monoterpene volatile analysis of H-α1 loop mutants. GCMS analysis showing selected ion (*m/z* 93) traces for headspace volatiles produced from MdAFS1 (WT) and H-α1 loop mutant recombinant enzymes incubated with GDP. Equal amounts of protein (50 μg) were used, and incubation was for 30 min at 30°C prior to extraction of headspace volatiles using solid phase microextraction (SPME).

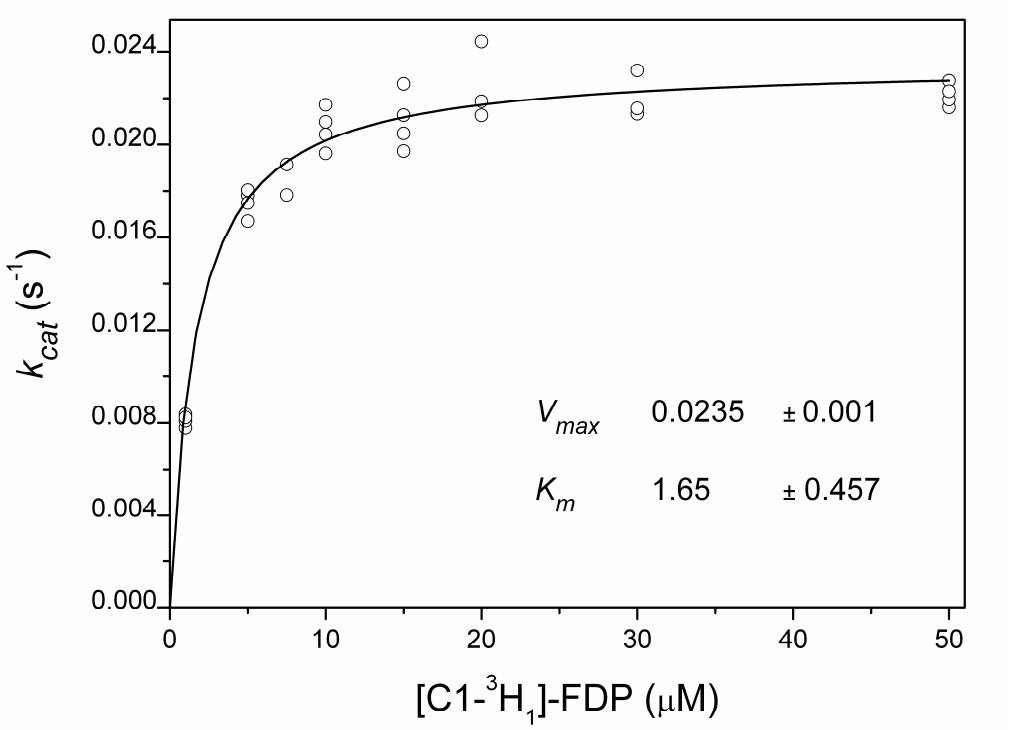

Fig. S2. MdAFS1-S487K mutant FDP kinetics. Showing the response curve for the S487K in the presence of varying [C1-$^3H_1$]-FDP concentrations The Michaelis constant (K_m) and maximal velocity (V_{max}) data (inset) were calculated from scintillation (dpm) data by non-linear regression of the Michaelis–Menten equation using the Origin 7.5 graphics package (Microcal Software Inc, USA). Data points for replicated assays at each substrate concentration are indicated by circles. Assays were set up according to the methods of Green et al 2006 (21) and experiments were carried out at least twice.

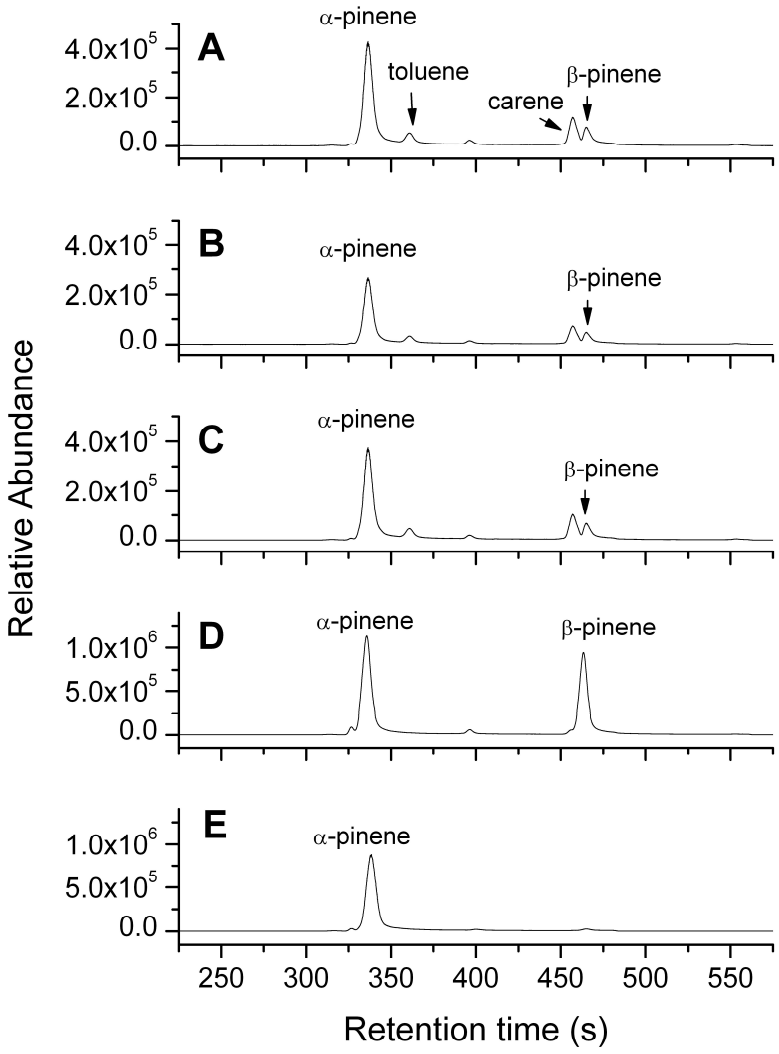

Fig. S3. PsTPS2 volatile analysis. GCMS analysis showing selected ion (*m/z* 93) traces for monoterpenes derived from enzymes following GDP feeding**.** Retention times for α- and β-pinenes produced from (A) PsTPS2, (B) PsTPS2-S538K and (C) PsTPS2-S538A were determined by comparison to authentic α- and β-pinene pinene standards (D) and (E). Additional peaks identified by MS include the monoterpene carene and a toluene contaminant.

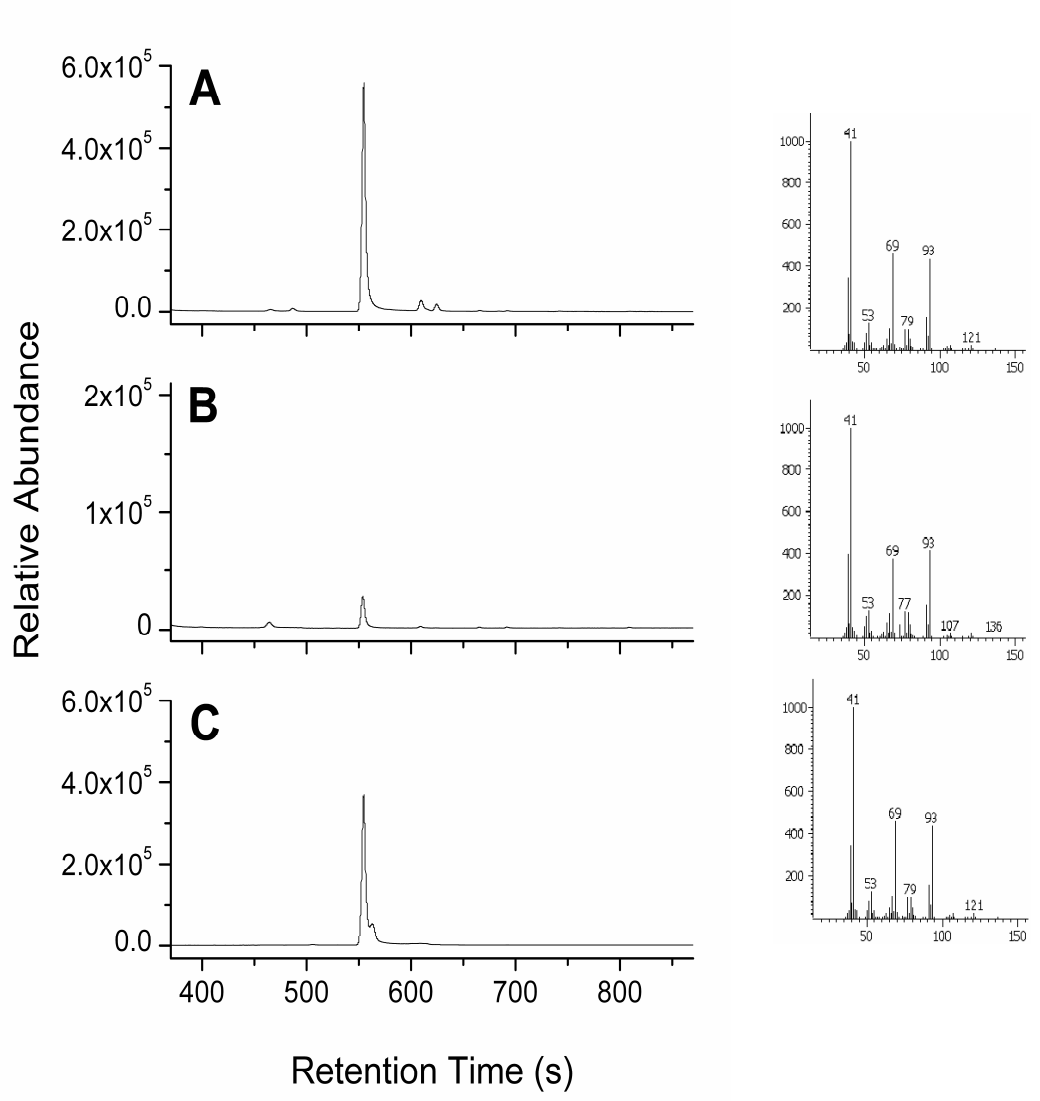

Fig. S4. AcTPS2 volatile analysis**.** GCMS analysis showing selected ion (*m/z* 93) traces for AcTPS2, and AcTPS2K-514A mono-TPS activity. Retention times for the predominant mono-TPS product (β-myrcene) were compared to an authentic standard (lower panel). Mass spectra for corresponding enzymatically produced β-myrcene and the authentic reference compound are also shown.

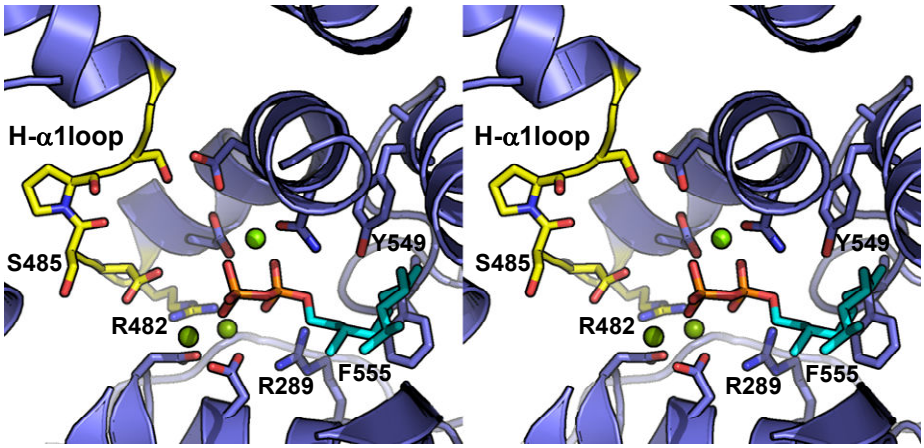

Fig. S5. MdAFS1 substrate binding pocket. Stereoview plot of residues speculated to play a role in the increased FDP and GDP catalysis observed in the S485A mutant. The S485 mutation could result in a minor rearrangement of the H-α1 loop region (yellow) allowing GDP substrate to interact more favourably with Y549 and F555 (carbocation stabilisation) or the R289 potentially positioned for substrate PPi interaction. Mg^{2+} (green spheres), and FDP precursor positions are equivalent to those presented in the paper (Fig. 2B).

SUPPLEMENTARY TABLES

Table S1. Terpene synthase mutagenic primers.

Target	Primer	Sequence (5′ to 3′)
MdAFS1-D484A	Forward	CAAGAGAGAGGGGCTTCTCCTTCATCAATC
MdAFS1-D484A	Reverse	GATTGATGAAGGAGAAGCCCCTCTCTCTTG
MdAFS1-S485A	Forward	CAAGAGAGAGGGGATGCTCCTTCATCAATC
MdAFS1-S485A	Reverse	GATTGATGAAGGAGCATCCCCTCTCTCTTG
MdAFS1-S487A	Forward	GAGGGGATTCTCCTGCATCAATCGTATGTTAC
MdAFS1-S487A	Reverse	GTAACATACGATTGATGCAGGAGAATCCCCTC
MdAFS1-S487K	Forward	GAGGGGATTCTCCTAAGTCAATCGTATGTTAC
MdAFS1-S487K	Reverse	GTAACATACGATTGACTTAGGAGAATCCCCTC
MdAFS1-S488A	Forward	GGGGATTCTCCTTCAGCAATCGTATGTTACATG
MdAFS1-S488A	Reverse	CATGTAACATACGATTGCTGAAGGAGAATCCCC
AcTPS2-K514A	Forward	GTGGCGATATTCCGGCAAGCATCCAGTGC
AcTPS2-K514A	Reverse	GCACTGGATGCTTGCCGGAATATCGCCAC
AcTPS2-K514S	Forward	GTGGCGATATTCCGAGTAGCATCCAGTGC
AcTPS2-K514S	Reverse	GCACTGGATGCTACTCGGAATATCGCCAC
PsTPS2-S538A	Forward	CCGTGGAGAAGAAGCTGCTAGTATATCTTG
PsTPS2-S538A	Reverse	CAAGATATACTAGCAGCTTCTTCTCCACGG
PsTPS2-S538K	Forward	CCGTGGAGAAGAAGCTAAGAGTATATCTTG
PsTPS2- S538K	Reverse	CAAGATATACTCTTAGCTTCTTCTCCACGG

MdAFS1, *Malus domestica* α-farnesene synthase; AcTPS2, *Actinidia chinensis* myrcene synthase; PsTPS2, *Picea sitchensis* pinene synthase.

Table S2. Summary of kinetic parameters for WT and S487K mutated MdAFS1 recombinant enzymes.

Enzyme	K_m (μM)	k_{cat} (s^{-1})	k_{cat} / K_m ($mM^{-1}.s^{-1}$)
WT	3.03 ± 0.05	0.053 ± 0.0011	17.5
S487K	1.65 ± 0.02	0.022 ± 0.0010	13.3

Analysis was carried out according to the methods of Green *et al* 2006 (21). K_m, the Michaelis constant; k_{cat}, turnover number of enzyme. Data for K_m and k_{cat} are presented as mean ± SEM, N=4.