

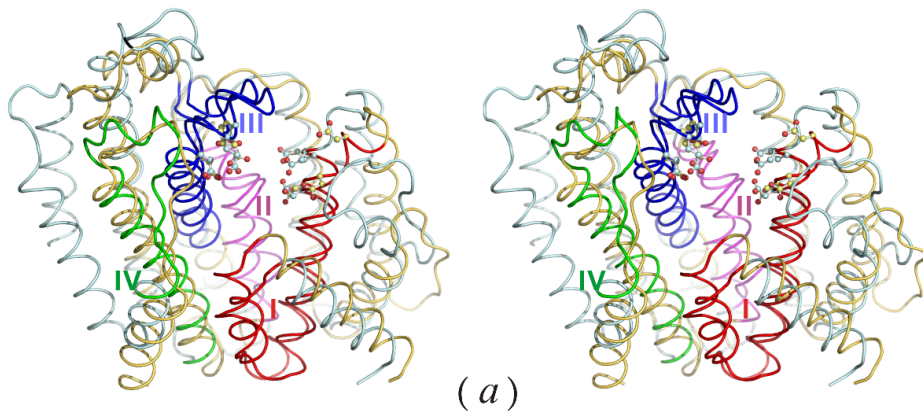
## Supplementary Material

**Supplementary Table S1.** Primers used to construct human SQS mutants in this study.

Mutant name	Primer sequence
<b>R52A</b>	5'-GTATCTCAATCAGACCAGTGCCAGTTTCGCAGCTGTTATCC-3'
<b>R52E</b>	5'-GTATCTCAATCAGACCAGTGAGAGTTTCGCAGCTGTTATCC-3'
<b>Y73A</b>	5'-CGCAACGCAGTGTGCATATTTGCTCTGGTTCTCCGAGCTCTGGA-3'
<b>R77A</b>	5'-ATATTTTATCTGGTTCTCGCGGCTCTGGACACACTGGA-3'
<b>K117A</b>	5'-GAGAGCAAGGAGGCAGATCGCCAGGTGCT-3'
<b>Q212L</b>	5'-TCTATGGGCCTGTTTCTGCTGAACACA AACATCATCCG-3'
<b>Q212N</b>	5'-TCTATGGGCCTGTTTCTGAATAAAACAAACATCATCCG-3'
<b>Q212E</b>	5'-TCTATGGGCCTGTTTCTGGAGAAAACAAACATCATCCG-3'
<b>R218A</b>	5'-CAGAAAACAAACATCATCGCGGACTATCTGGAAGACCA -3'
<b>R228A</b>	5'-GAAGACCAGCAAGGAGGAGCAGAGTTCTGGCCTCAAGA-3'
<b>F288A</b>	5'-GAAACCAGAGTGTGTTTAACGCCTGTGCTATTCCACAGGTG-3'
<b>F288L</b>	5'-GAAACCAGAGTGTGTTTAACCTCTGTGCTATTCCACAGGTGATG-3'
<b>K315E</b>	5'-GGTGTTCAAAGGGGCAGTGGAGATTGGAAAGGGCAAGC -3'
<b>R317E</b>	5'-CAAAGGGGCAGTGAAGATTGAGAAAGGGCAAGCAGTGACCC -3'
<b>K315E+R317E</b>	5'-GGTGTTCAAAGGGGCAGTGGAGATTGAGAAAGGGCAAGCAGTGACCC-3'
<b>K318E</b>	5'-GGGGCAGTGAAGATTCGGGAAGGGCAAGCAGTGACCCTG -3'
<b>K315E+K318E</b>	5'-GGTGTTCAAAGGGGCAGTGGAGATTGGGAAGGGCAAGCAGTGACCCTG -3'

**Supplementary Table S2.** Effects of mutation on retained activity of *hSQS* in this study.

Human SQS	%	
	First-step	Second-step
Wild type	100	100
R52A	59.7	37.8
R52E	91.7	9.1
Y73A	N/A	9.7
R77A	55.5	14.5
K117A	N/A	100
Q212L	N/A	4
Q212N	53.1	8.1
Q212E	N/A	85.4
R218A	93.6	22.4
R228A	85.3	70.2
F288L	N/A	53.2
F288A	N/A	22.7
K315E	89.6	40.5
R317E	N/A	82.6
K318E	93.6	86.9
K315E/R317E	N/A	9.2
K315E/K318E	94.1	7.4

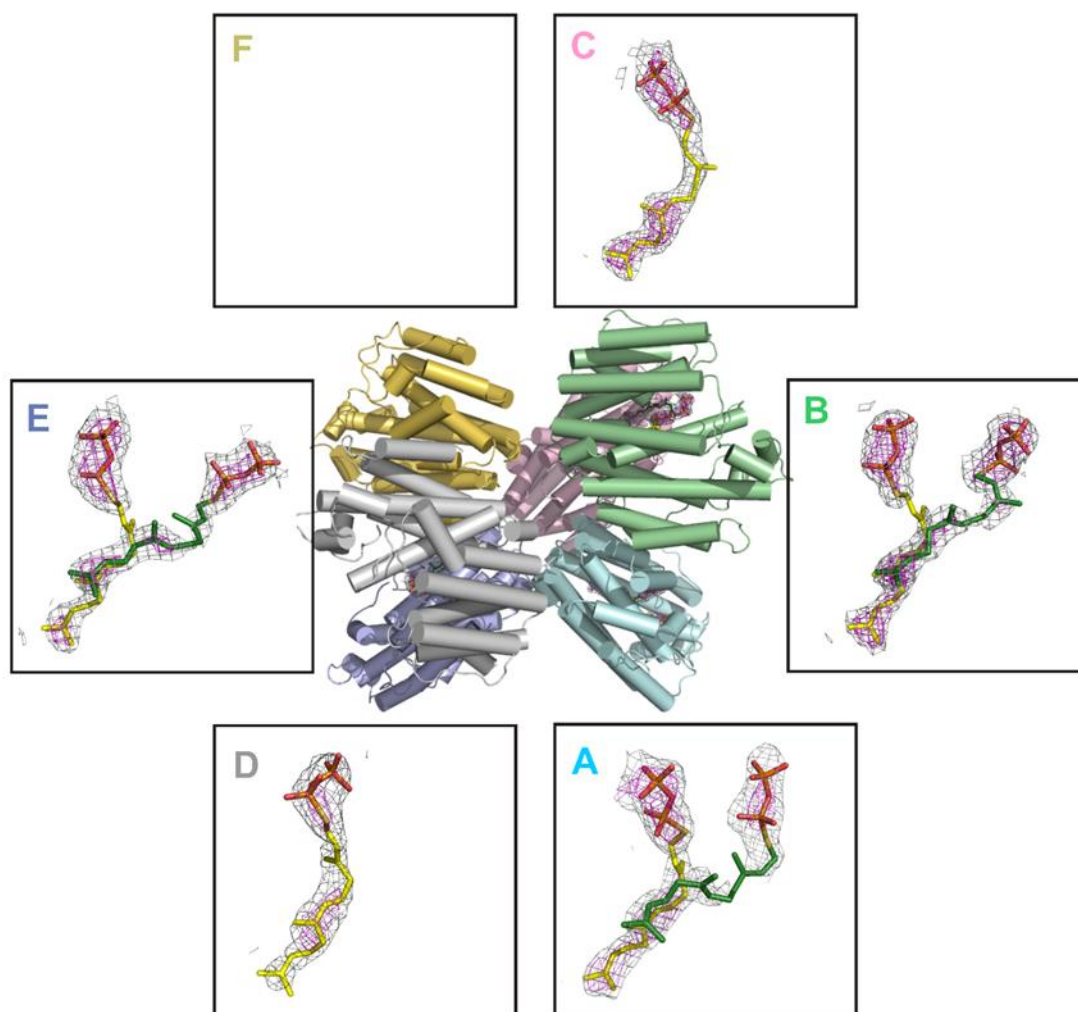


(a)

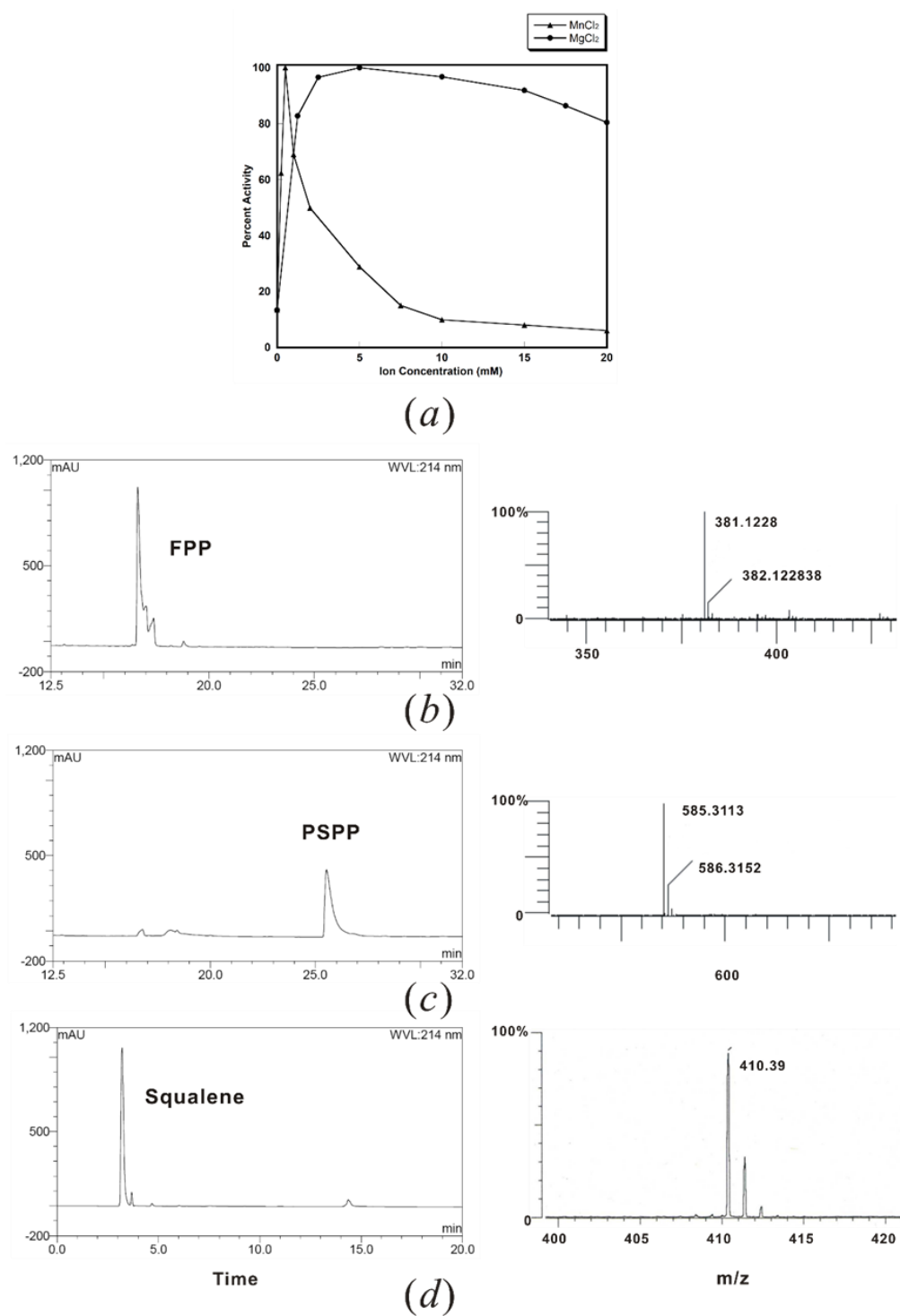
human	1	-MEFVKCLGHPEEFYNLVRFRIGGKRKVMKMDQDLSLSLKTCKYKLNQTSRSFAAVIQALDGEIRNAV	69
Rat	1	-MEFVKCLGHPEEFYNLVRFRMGGRNRFIPKMDRNSLSNSLKTCKYKLDQTSRSFAAVIQALDGDIRHAV	69
Yeast	1	MGKLLQLALHPVEMKAALKLFC--RTPLFSIYDQSTSPYLLHCFELLNLTSSRSFAAVIRELHPFLRNCV	68
Tc	1	-----MEYMEELVAMVRMKWRLRIEKGIAACND--EDLRFQVDILQAVSRSFAAVIMELDEEMDAV	60
Clustal Consensus		.....MEYMEELVAMVRMKWRLRIEKGIAACND--EDLRFQVDILQAVSRSFAAVIMELDEEMDAV	
human	70	CI FYLVLRALDTLEDDMTISVEKQVPLHNFHSFYQPDWRFMEE--SKEKDRQVLEDFPTISLEFRNLAE	137
Rat	70	CVFYLLLRALMDTVEDDMAISVEKKIPLLRNFHTFYEPDWRFMEE--SKEKDRVLEDFPTISLEFRNLAE	137
Yeast	69	TLFYLLLRALDTEDDMSIEHDLKIDLRHFHEKLLLTWSEEDGNAPVDKRAVLDFFESILLFEHKLKP	138
Tc	61	CI FYLVLRALDTEDDMTIPVDFKLRLELPKFEHLHDTTWCMMSG-VGVGRELELLERYTHVTRAYSRLGK	129
Clustal Consensus		CI FYLVLRALDTEDDMTIPVDFKLRLELPKFEHLHDTTWCMMSG-VGVGRELELLERYTHVTRAYSRLGK	
human	138	KYQTVADICRRMGIGMAEFLDKH-----VTSEQEWK YCHYVAGLVGIGLSR LFSASEFEDPLVGEDT	201
Rat	138	KYQTVADICRRMGCGMAEFLDKD-----VTSKQDWDK YCHYVAGLVGIGLSR LFSASEFEDPLVGEDT	201
Yeast	139	EYQEVVKEIKMGNMADYILDENYNLNGLOTVHDYD VYCHYVAGLVGDGLTRLLVIKAFANESLYSNE	208
Tc	130	AYQDVSIGGERMANGMCDFLTRK-----VETKADYDLYCHYVAGLVGHGLTLLYVSSGLDEVRLLADDL	193
Clustal Consensus		AYQDVSIGGERMANGMCDFLTRK-----VETKADYDLYCHYVAGLVGHGLTLLYVSSGLDEVRLLADDL	
human	202	ERANSMGLFLQKTNIRDYLEDQQGG--REFWPEVWVSRVYVKKLGDFAKPNIDLAVQCLNELITNALH	269
Rat	202	ECANSMGLFLQKTNIRDYLEDQQGG--RFQWPEVWVGRVYVKKLEDVFKPENVDVAVKCLNELITNALQ	269
Yeast	209	EYQEVVKEIKMGNMADYILDENYNLNGLOTVHDYD VYCHYVAGLVGDGLTRLLVIKAFANESLYSNE	276
Tc	194	TNANMGLFLQKTNIRDFYEDIREVPPRVFWPRITWEKYTDDLHAFKDELHEAKAVECLNAMVADALV	263
Clustal Consensus		TNANMGLFLQKTNIRDFYEDIREVPPRVFWPRITWEKYTDDLHAFKDELHEAKAVECLNAMVADALV	
human	270	IPDVIITYLRLRNSQSVNFCAIPQVMAIATLAACYNNQVFRKGVKIRKQAVTLMMDATNMPAVKAIY	339
Rat	270	IPDVIITYLRLRNSQSVNFCAIPQVMAIATLAACYNNHQVFRKGVKIRKQAVTLMMDATNMPAVKAIY	339
Yeast	277	VIDVLTYLALGIEHQSITQFCIPQVMAIATLALVFNRRNREVLHGNVVKIRKGTTCYLILKSRTRLQGCVEIFD	346
Tc	264	VPHVVEYVLA SLRDPVTFSAIPQVMAIATLSLVFNRRNREVLHGNVVKIRKGTTCYLILKSRTRLQGCVEIFD	333
Clustal Consensus		VPHVVEYVLA SLRDPVTFSAIPQVMAIATLSLVFNRRNREVLHGNVVKIRKGTTCYLILKSRTRLQGCVEIFD	
human	340	QYMEEYVYHRIPDSDP-----SSSKTRQIISTIRTONLPN-----CQLISRSHYSPIYLSFVML	392
Rat	340	QYIEEYVYHRVPNSDP-----SASKAKQLISNIRTQSLPN-----CQLISRSHYSPIYLSFVML	392
Yeast	347	YYLRDLKSKLAVQDPNFLKLNQISKIEQFMEEYQDKLPPNVKPNETPIFLKVKERSRMDDELVPVTOQE	416
Tc	334	YTLRLAARMNAQDA-----CYDRIEHLVNDAIRAMES-----HQPNGESVARSMMLR	382
Clustal Consensus		YTLRLAARMNAQDA-----CYDRIEHLVNDAIRAMES-----HQPNGESVARSMMLR	
human	393	LALSWQYLTTLTSLQVTEDEVVQTGEH---	417
Rat	393	LALSWQYLTTLTSLQVTEDEVVQREH---	416
Yeast	417	EYKFNMLVSLITLTVLLGFFYIYTLHRA	444
Tc	383	YALGGHLYTLVDNVVGYLGLK-----	404
Clustal Consensus		YALGGHLYTLVDNVVGYLGLK-----	

(b)

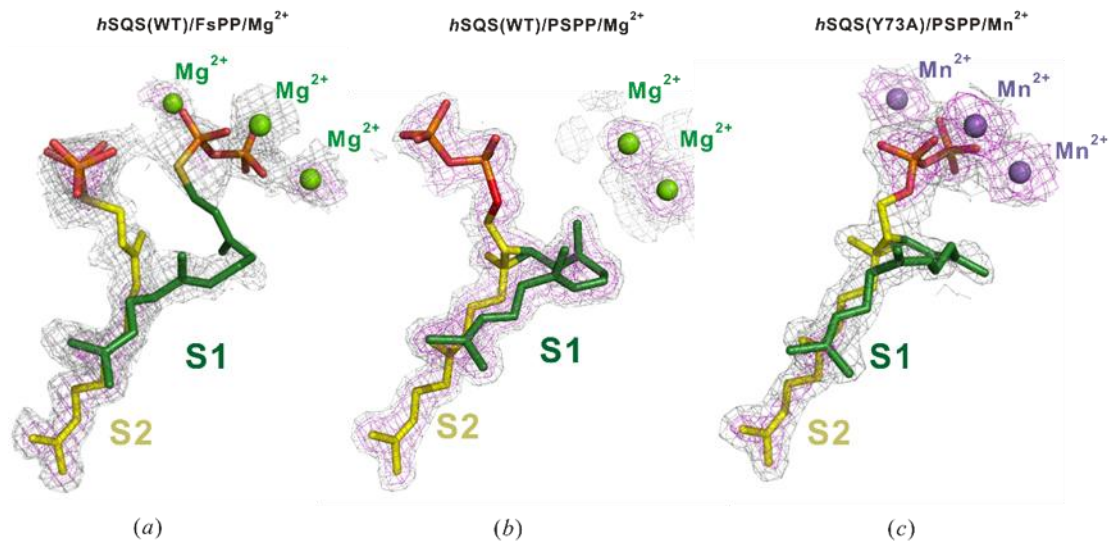
**Supplementary Fig. S1.** (a) Stereo view of the alignment of *hSQS* with *SaCrtM*, indicating the highly conserved regions are located at Regions I (red), II (magenta), and III (blue). Region IV (green), constituted NAD(P)H binding site, is an unique and conserved feature among SQSs and absent in *SaCrtM*. The negative residues on both asp-rich motifs are shown in ball and stick. *hSQS* and *SaCrtM* are presented in palecyan and yellow, respectively. (b) Alignment of SQS protein sequences. The protein sequences for human (AAP36671), rat (AAA42179), yeast (CAA42583), and *T. cruzi* (XP806809) were aligned using Clustal program with manual adjustment. Identities among the five species are marked by (\*) while homologous are indicated by (.) and conserved substitution by (:). Four conserved regions are labeled in boxes. Two DXXED motifs as the substrate binding sites are shown. The circles indicate the residues involved in NADPH recognition.



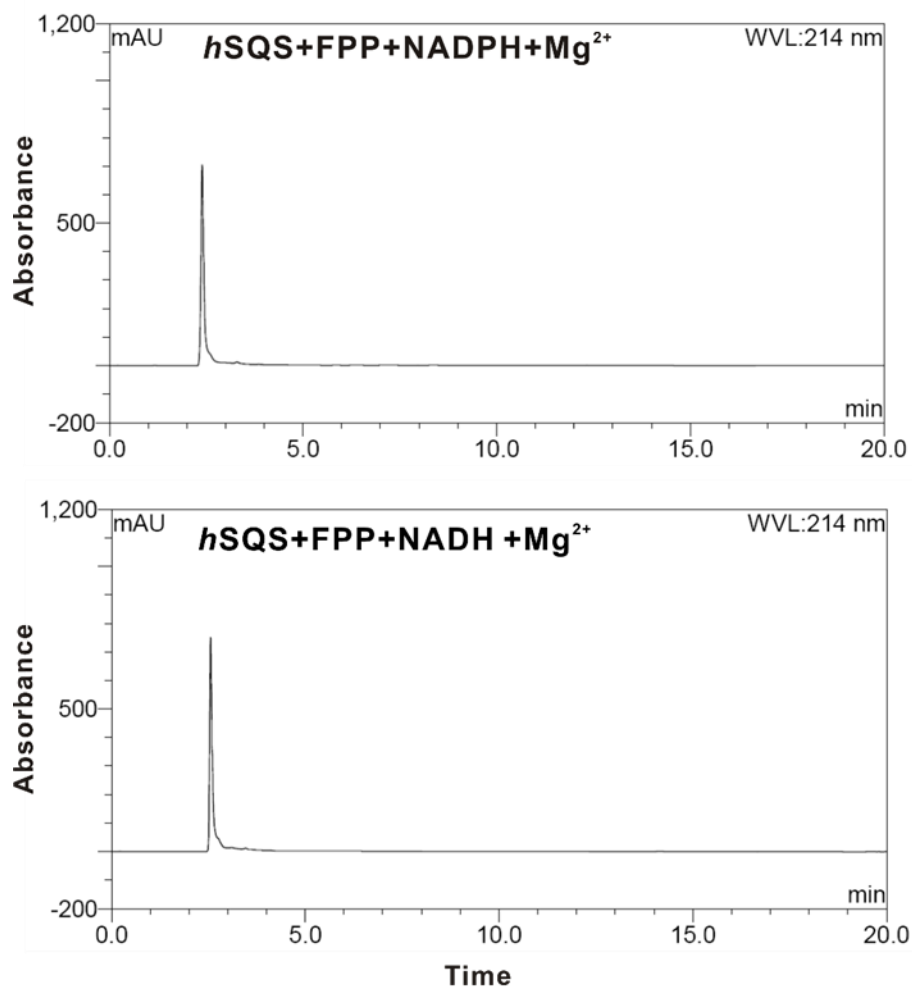
**Supplementary Fig. S2.** The final  $2Fo-Fc$  electron densities map (gray contoured at  $1.0\sigma$ , red at  $2.0\sigma$ ) for FsPP without  $Mg^{2+}$  in the active site. FsPP in S1 site is labeled in green, FsPP in S2 site is labeled in yellow. In their active sites, two  $C_{15}$ -FsPP molecules are in chains A, B and E; one molecule in chains C and D; and chain F is empty.



**Supplementary Fig. S3.** Activity Assays. (a) Both Mg<sup>2+</sup> and Mn<sup>2+</sup> stimulate enzyme activity. Reverse phase HPLC trace of FPP (b) and PSPP (c). The retention times of FPP and PSPP were 17.5 and 27.0 min. (d) Normal phase HPLC trace of squalene. The retention time of squalene was 3 min. Mass spectral analysis of FPP, PSPP and squalene are shown the molecular ions at  $m/z$  381, 585 and 410, respectively.

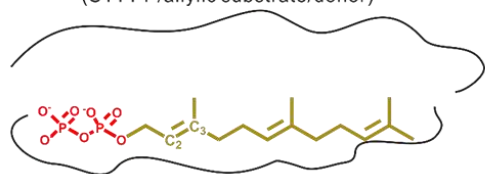


**Supplementary Fig. S4.** Substrate models in the active site. The  $2F_o - F_c$  electron density maps for all ligands are contoured  $1\sigma$  (in gray) and  $3\sigma$  (in red). (a) FsPP with  $Mg^{2+}$ . (b) PSPP with  $Mg^{2+}$ . (c) PSPP with  $Mn^{2+}$ . The catalytic  $Mg^{2+}$  and  $Mn^{2+}$  are shown as green and purple balls, respectively.

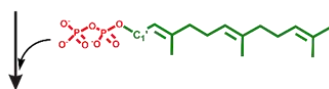


**Supplementary Fig. S5.** *hSQS* not only uses NADPH but NADH as reducing agents.

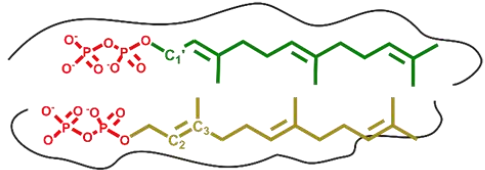
Activating binding site/two asp-rich motifs (ionization)  
(S1 FPP/allylic substrate/donor)



Nonactivating binding site  
(S2 FPP/alkene substrate/acceptor)



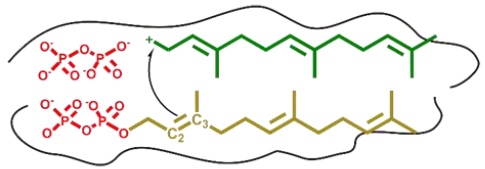
Activating binding site



Nonactivating binding site

↓ Mg<sup>2+</sup>-dependent ionization

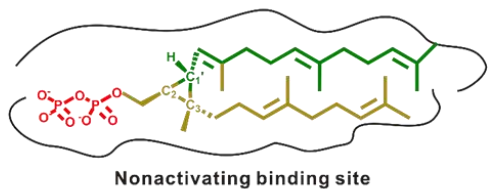
Activating binding site



Nonactivating binding site

↓ Mg<sup>2+</sup>-dependent condensation  
H<sup>+</sup>

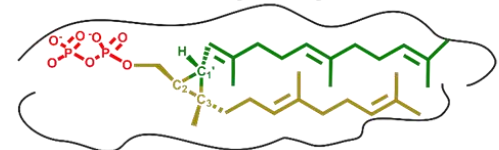
Activating binding site



Nonactivating binding site

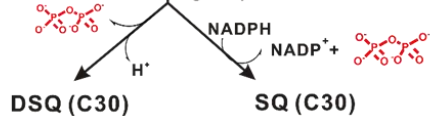
↓ Mg<sup>2+</sup>-dependent translocation

Activating binding site



Nonactivating binding site

↓ Mg<sup>2+</sup>-dependent ionization



DSQ (C30)      SQ (C30)

**Supplementary Fig. S6.** Proposed cartoon model of ordered incorporation of substrates in *hSQS* and *SaCrtM*.