Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Control of 1,2-rearrangement process by oxidosqualene cyclase during triterpene biosynthesis

Shohei Takase, Yusuke Saga, Nozomi Kurihara, Shingo Naraki, Kenta Kuze, Genki Nakata, Takeshi Araki and Tetsuo Kushiro

School of Agriculture, Meiji University, 1-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan,

Table S1. Primers used for construction of PSX and CPQ mutants. S: sense primer, A: antisense primer

PeaPSX Y118L-A	5'- GAAACATGGGACCTCCAAGATCTCCTGGC -3'
PeaPSX I365L-A	5'- CTTATTTACAGGACCTAGGCAAATATAACG -3'
PeaPSX P480L-S	5'- CTTATTTACAGGACCTAGGCAAATATAACG -3'
PeaPSX T531S-S	5'- GGTGGCCTTGCATCATATGAGCTCACACG -3'
PeaPSX G617A-S	5'- GCTTTACATATGCTACTTGGTTTGGGATA -3'
CPQ L125Y-A	5'- CAAGGGCCCTCCATAATCCGAGGCCCAATT -3'
CPQ L373I-A	5'- GACTGGGCCAATACATATGTATCGACTATT -3'
CPQ L488P-S	5'- CGAGATCATGGATGGCCCATCTCCGACTG -3'
CPQ S539T-S	5'- GGTGGATTTGCAACATACGAGTTGACGAG -3'
CPQ A625G-S	5'- GTTTGTTTTACGTATGGGGGTTGGTTTGGC -3'

Table S2. N- and C-terminal primers for PSX and CPQ.

CPQ-Kpn-N	5'- AAATTAGGTACCATGTGGAGGCTGAAGGTGGGA -3'
CPQ-Xho-C	5'- AAGCAGCTCGAGTCATTCAGTAAGAACCCGATG -3'
PSX-Bam-N	5'- GTTTGGATCCAAAAATGTGGAAGCTCAAGGTTGCGG -3'
PSX-Xho-C	5'- AATAACTCGAGATTAGCAGGCCTGCAATACACGGCG -3'

Table S3. Primers used for constructing CPQ mutant with 13 mutations (CPQ 13-mut).

CPQ-1,2,3-mut-A	5'-CGAGACCCGGGATTAAAAACAAGGGCCCTCCATAATCCCCGGCCCAATT -3'
CPQ-4-mut-A	5'- CCAAGTACGGACAGCCACATTTTTCCCCAC -3'
CPQ-7,8-mut-S	5'-GTTAATGTTATCCTTTCTTTGCAAAATGATAATGGTGGATTTGCAACATACG -3'
CPQ-9,10,11-mut-S	5'- GCACCGCAGCAGCAATTCAAGCACTGACG -3'
CPQ-12,13-mut-S	5'- GGTACGGGTCTTGGGGGGTTTGTTTTACGTATGGGGGTTGG -3'

Fig. S1. The 1,2-rearrangement process during cyclization of oxidosqualene to produce various migrated oleanane type triterpenes.

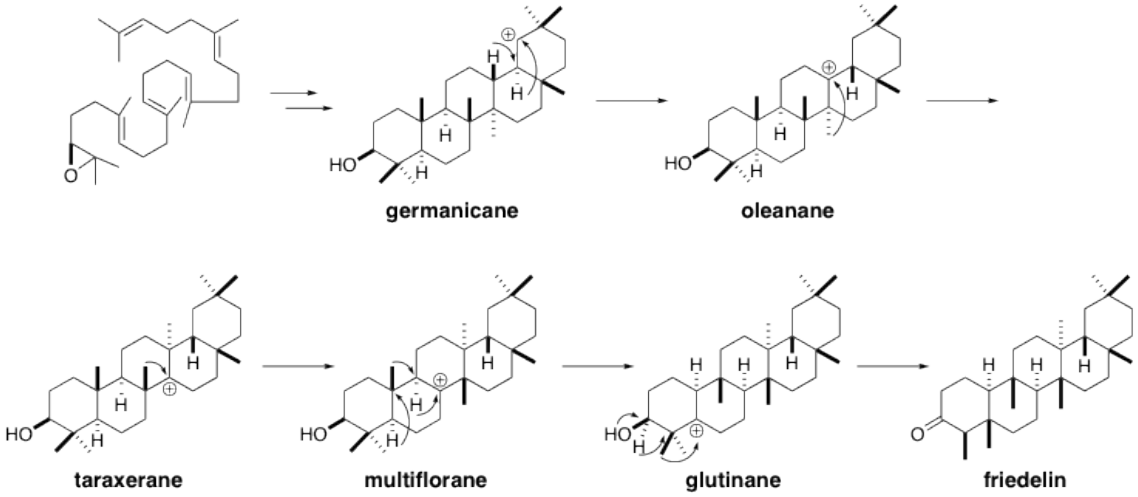


Fig. S2. Amino acid sequence alignments among cycloartenol synthases (CASs) and cucurbitadienol synthases (CBSs). PSX: *Pisum sativum* CAS, CPX: *Cucurbita pepo* CAS, CPQ: *Cucurbita pepo* CBS, McQ: *Momordica charantia* CBS. Five residues selected for mutation studies are highlighted in red boxes and each residue number for PSX is indicated.

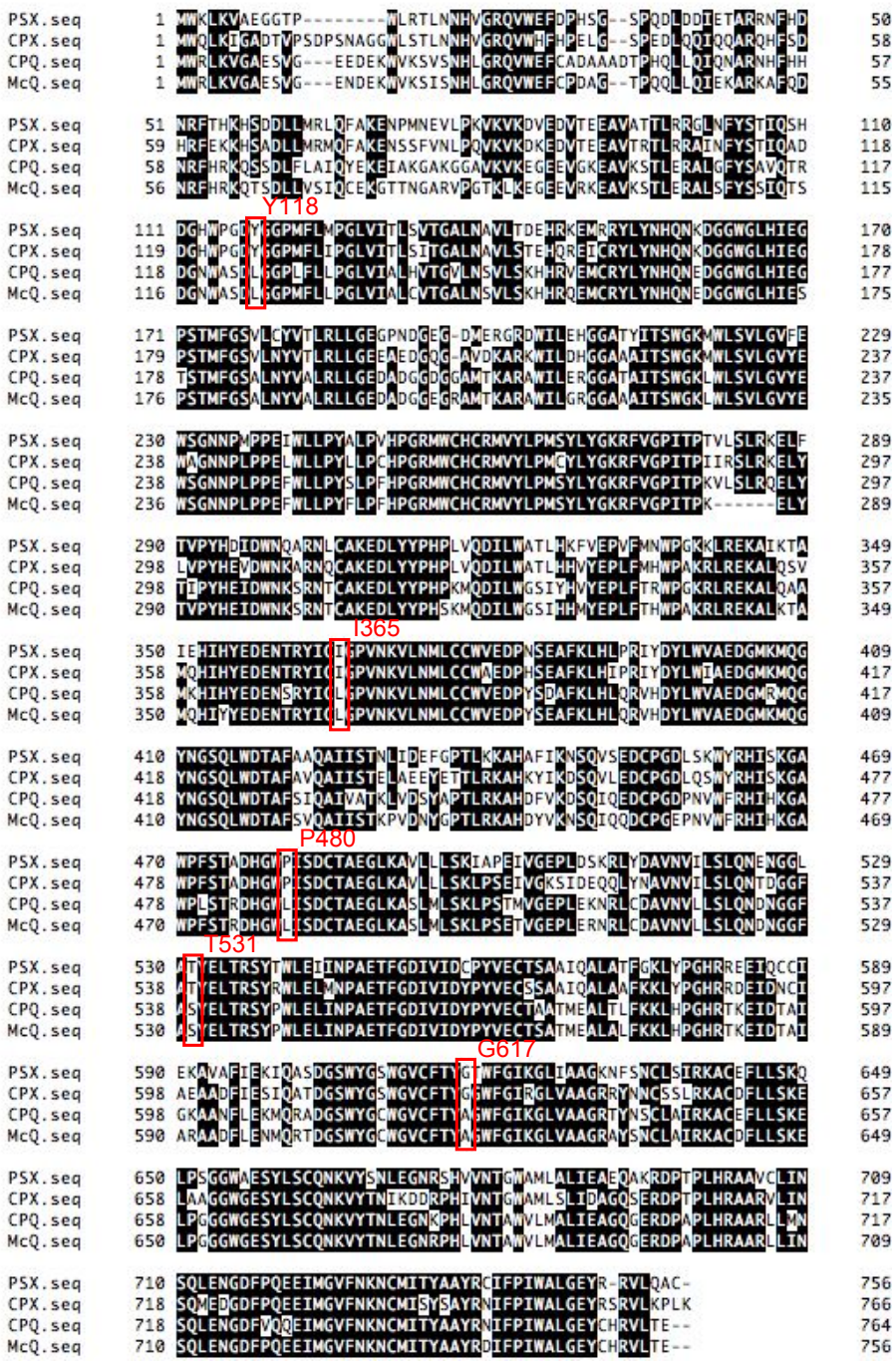


Fig. S3. The active site structure of human LAS in complex with the product lanosterol. Human LAS residues that corresponded to five residues selected for mutation studies in CAS and CBS are shown. hLAS numbering shown in white and PSX numbering shown in magenta in parentheses. Neighboring residues, Val453, Tyr503 and Tyr587 are also shown. The lanosterol molecule is shown in green.

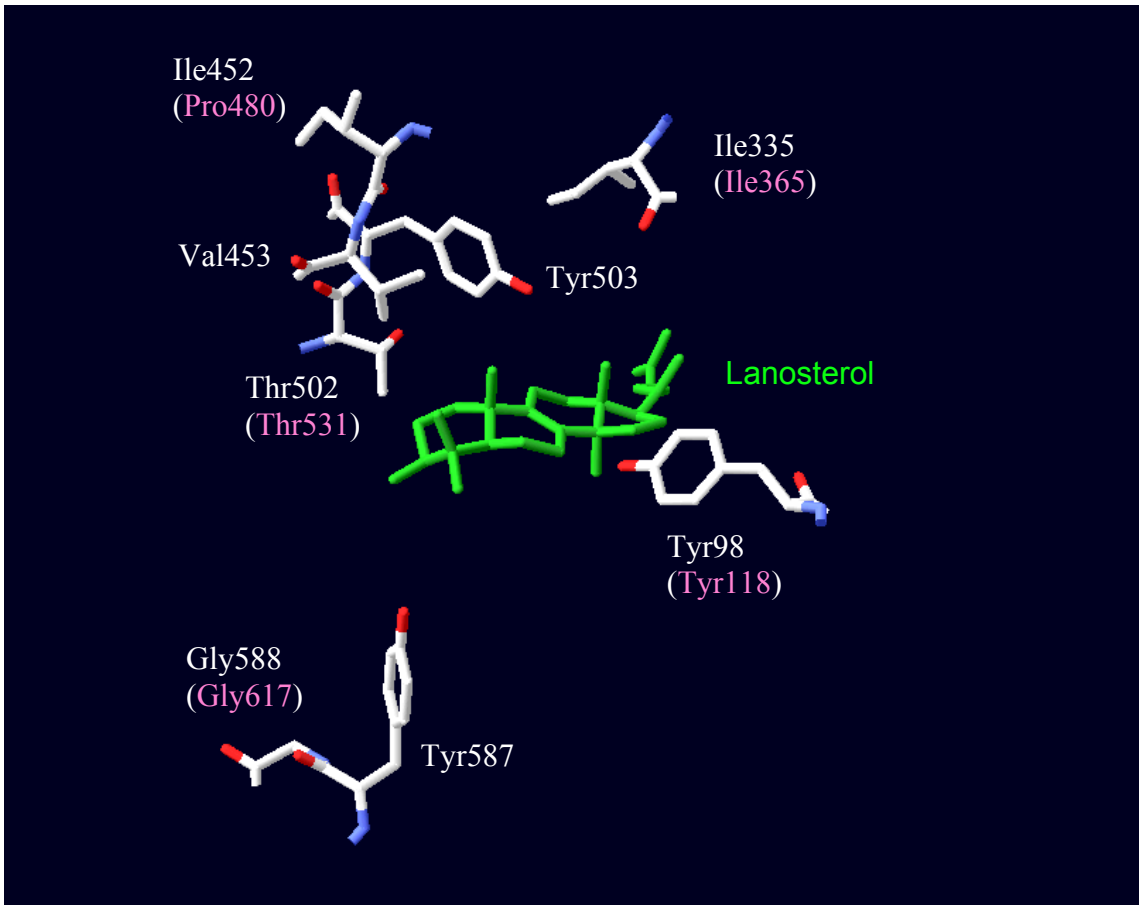


Fig. S4. TLC analysis of hexane extracts of the PSX Y118L mutant showing the production of cucurbitadienol. Lane 1: PSX wild type, Lane 2: PSX Y118L mutant, Lane 3: CPQ wild type.

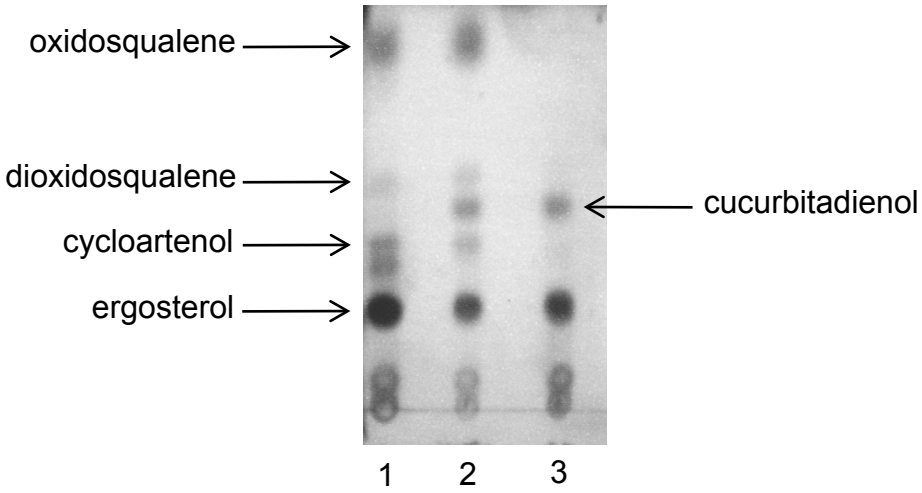


Fig. S5. TLC analysis of hexane extracts of other PSX mutants. Lane 1: Y118L mutant, Lane 2: I365L mutant, Lane 3: P480L mutant, Lane 4: T531S mutant, Lane 5: G617A mutant. Blue arrow corresponds to a position of cycloartenol while red arrow corresponds to a position of cucurbitadienol.

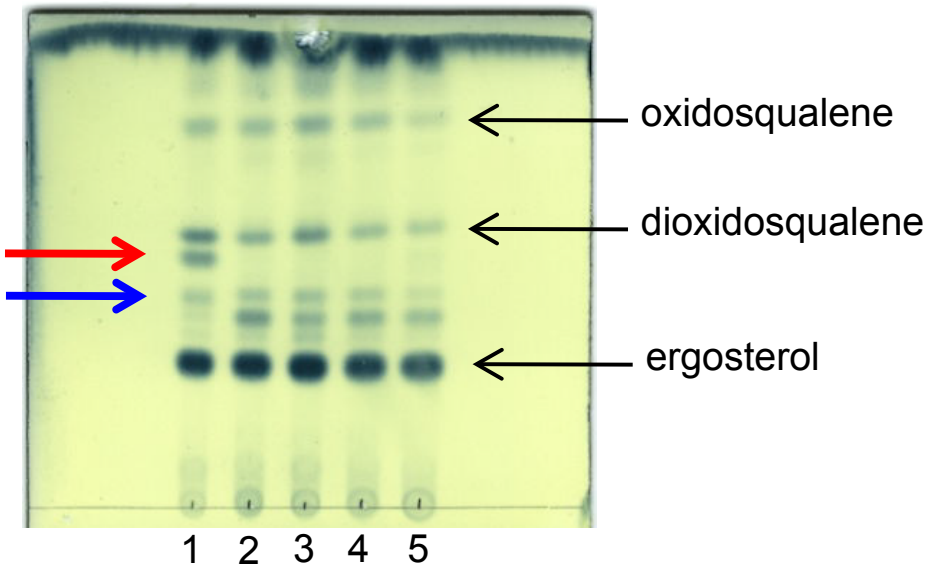


Fig. S6. A MS spectrum of cucurbitadienol produced by the PSX Y118L mutant.

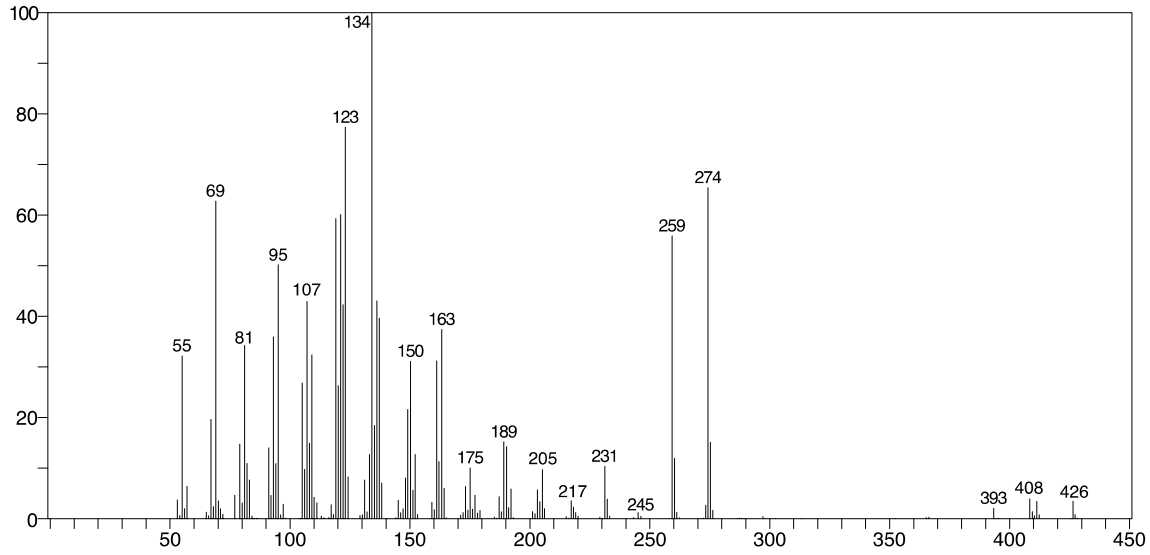


Fig. S7. ^{1}H-NMR spectrum (300 MHz, in $CDCl_3$) of cucurbitadienol extracted from the PSX Y118L mutant. A cross mark in red indicate impurities arising from a lipid (~ 1.2 ppm).

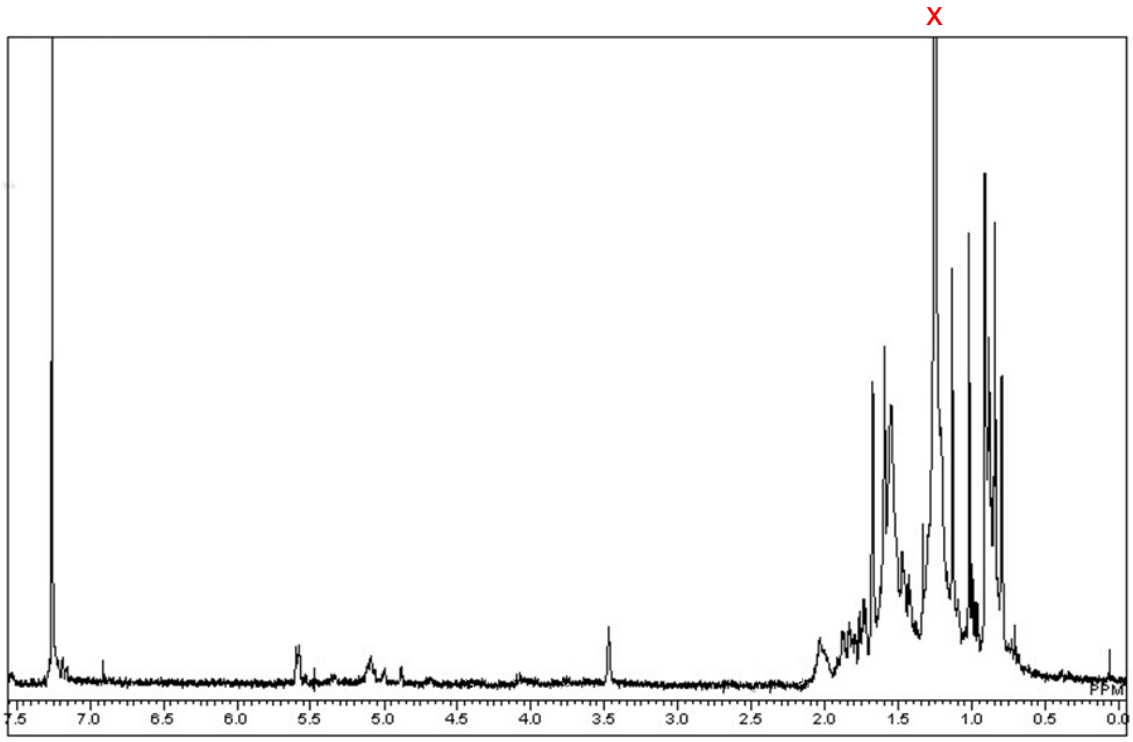
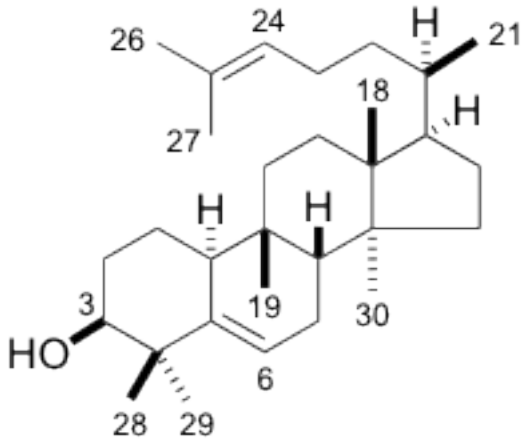


Fig. S8. Assignments of ^{1}H-NMR chemical shifts for cucurbitadienol.

	Product of Y118L	Cucurbitadienol*
position	^{1}H (ppm)	^{1}H (ppm)
3	3.473	3.472
6	5.587	5.589
18	0.844	0.848
19	0.914	0.916
21	0.901	0.900
24	5.091	5.092
26	1.677	1.679
27	1.595	1.596
28	1.134	1.134
29	1.021	1.022
30	0.799	0.804



cucurbitadienol

***** Literature values from: L. J. Goad and T. Akihisa, *Analysis of Sterols*, Chapman & Hall, London, 1997.

Fig. S9. TLC analysis of hexane extracts of CPQ mutants. Lane 1: L125Y mutant, Lane 2: L373I mutant, Lane 3: L488P mutant, Lane 4: S539T mutant, Lane 5: A625G mutant. Blue arrow corresponds to a position of cucurbitadienol while red arrow corresponds to a position of a typical triterpene monoalcohol such as cycloartenol.

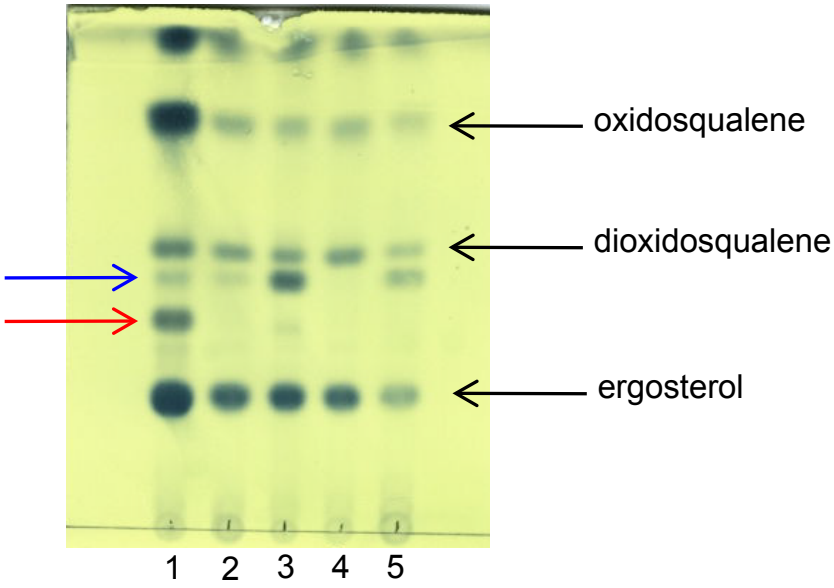


Fig. S10. A MS spectrum of parkeol produced by the CPQ L125Y mutant.

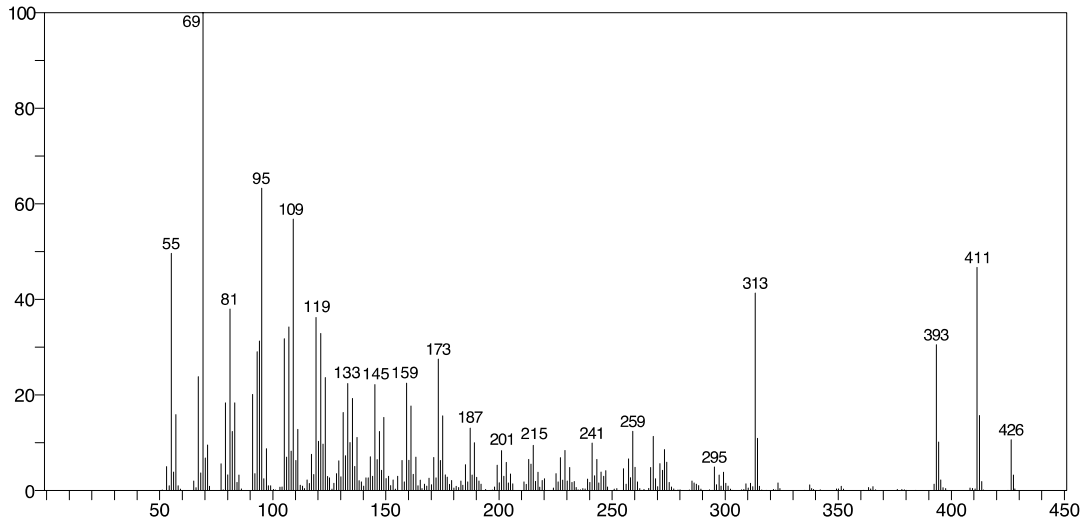
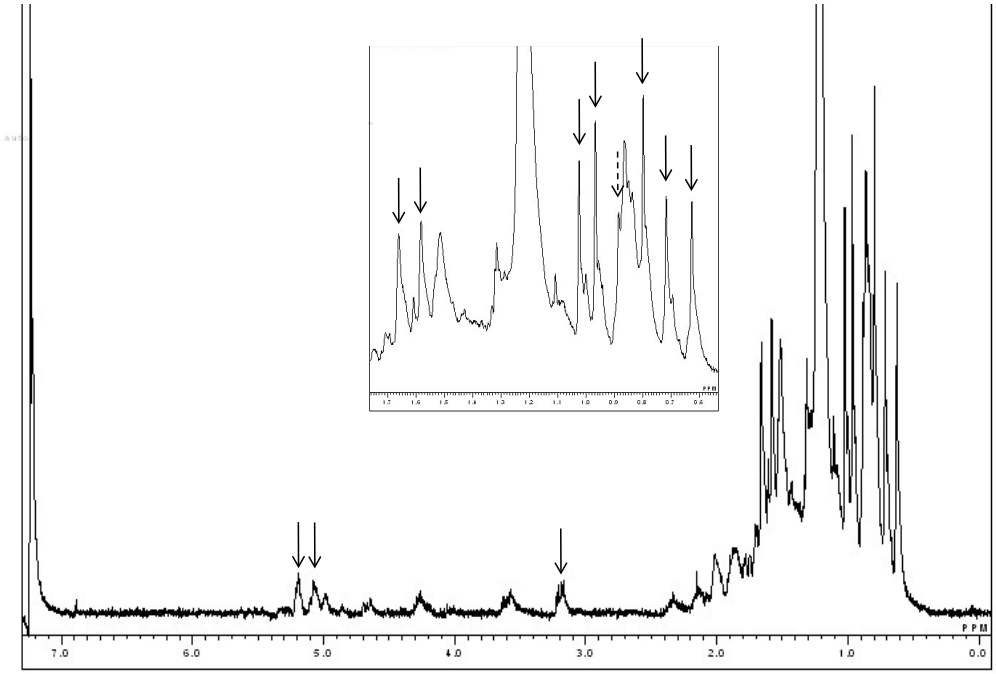


Fig. S11. ^{1}H-NMR spectrum (300 MHz, in $CDCl_3$) of parkeol produced by the CPQ L125Y mutant.



Product of L125Y	Parkeol[11]
^{1}H (ppm)	^{1}H (ppm)
0.646	0.65 (3H, s)
0.737	0.74 (3H, s)
0.818	0.82 (3H, s)
-	0.90 (3H, d)
0.987	0.99 (3H, s)
1.044	1.04 (3H, s)
1.603	1.60 (3H, s)
1.681	1.68 (3H, s)
3.227	3.22 (1H, dd)
5.086	5.09 (1H, t)
5.214	5.22 (1H, d)

Fig. S12. Position of residues mutated in CPQ for CPQ 13-mut. Each mutated position is marked by red dot under amino acid sequence alignment of CPQ, PSX and human LAS.

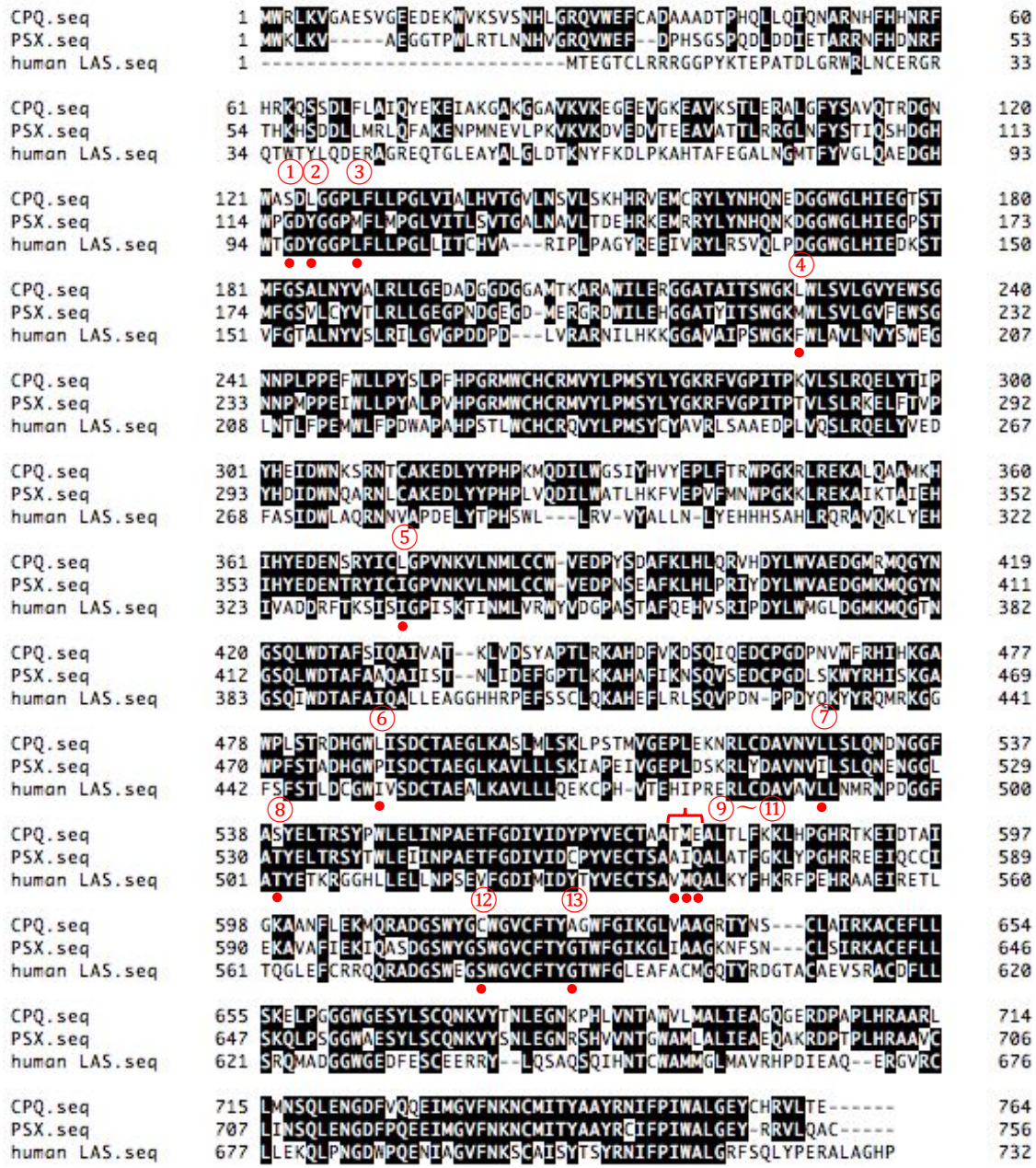


Fig. S13. Position of residues mutated in CPQ for CPQ 13-mut shown on the active site structure of human LAS complexed with the product lanosterol. For clarity, only the rear half of the active site is shown. Lanosterol is shown in green. Residues shown in cyan are those mutated in CPQ. Numbers corresponds to those indicated in Fig. S10. Only mutated residues No. 1~3 are not shown that are located in the fore front. Residues shown in red are those located within 6 Å away from the lanosterol molecule and these residues form the interior of the active site. Other residues shown are located within 12.5 Å away from the lanosterol molecule and are considered "second-sphere" residues. With all these mutations introduced in CPQ, all the residues shown in the figure becomes identical with CAS.

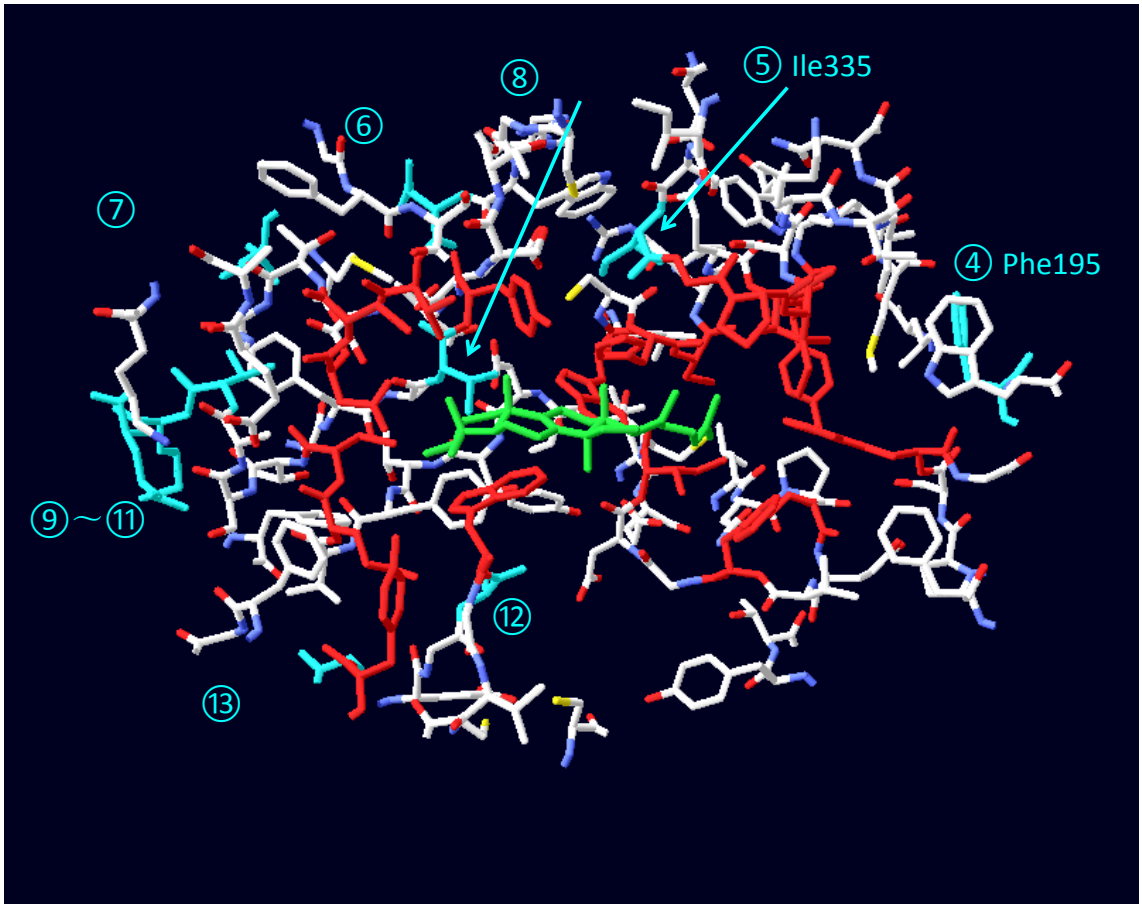


Fig. S14. TLC analyses of hexane extract of CPQ 13-mut and CPQ 3-mut. Lane 1: PSX wild type, Lane 2: CPQ L125Y mutant, Lane 3: CPQ 3-mut, Lane 4: CPQ 13-mut, Lane 5: CPQ wild type. Blue arrow indicates cucurbitadienol while red arrow indicates a typical triterpene monoalcohol position.

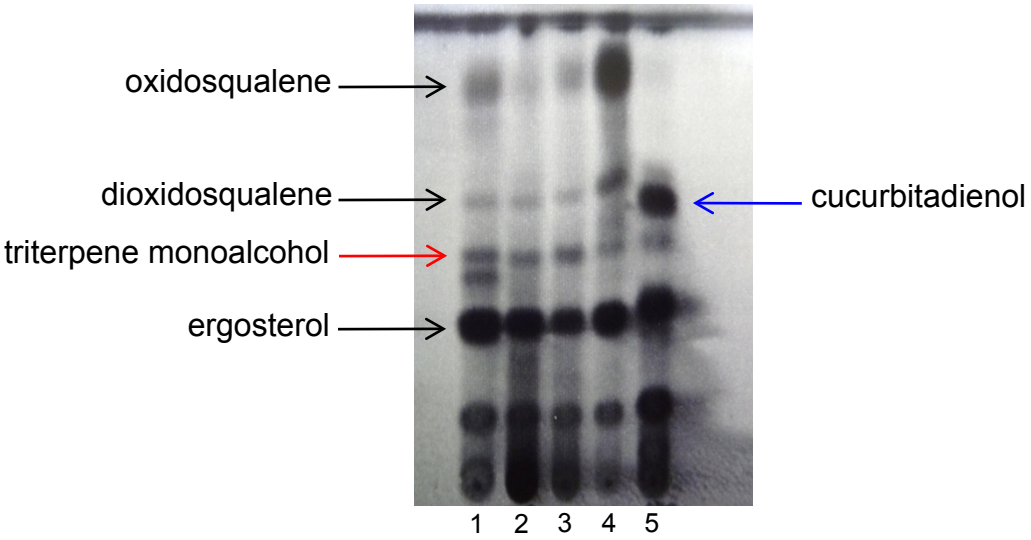


Fig. S15. GC-MS analyses of hexane extract of CPQ 13-mut and CPQ 3-mut. Dotted line indicates the position of parkeol. Peak for parkeol is not visible in CPQ 13-mut in this scale.