

Supporting Information

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

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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'- CGAGACCCGGGATTA AAAACAAGGGCCCTCCATAATCCCCGGCCCAA TT -3'
CPQ-4-mut-A	5'- CCAAGTACGGACAGCCACATTTTTCCCCAC -3'
CPQ-7,8-mut-S	5'- GTTAATGTTATCCTTTCTTTGCAAAATGATAATGGTGGATTGCAACA TACG -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.

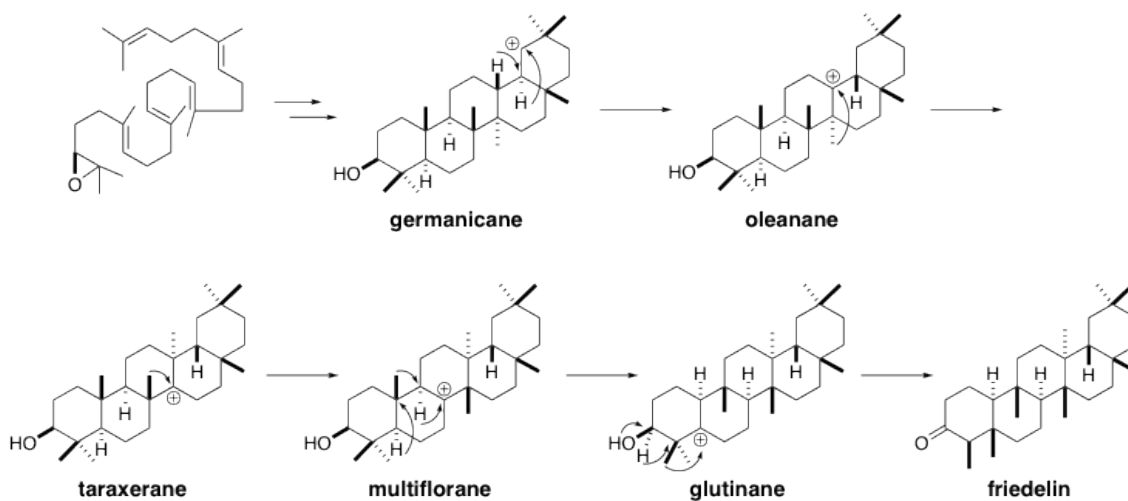


Fig. S2. Amino acid sequence alignments among cycloartenol synthases (CASs) and cucurbitadienol synthases (CBSs). PSX: *Pisum sativum* CAS, CPX: *Cucurbita pepo* CAS, CPQ: *Cucurbita pepo* CBS, McQ: *Momordica charantia* CBS. Five residues selected for mutation studies are highlighted in red boxes and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

PSX.seq	1	MNKLKVAEGGTP-----MLRTLNNHVGQVWEFDPHSG--SPQDLDDIETARRNFHD	50
CPX.seq	1	MNQLKAGADTPSDPSNAGGLSTLNNHVGQVWFHPELGG--SPEDLQIQQARQHFD	58
CPQ.seq	1	MNRLKVGAEVSG---EEDKMKVKSVSNHVGQVWEFCADAAADTPHQLLQIQARNHFHH	57
McQ.seq	1	MNRLKVGAEVSG---ENDEKMKVKSISNHVGQVWEFCADAG--TPQQLQIEKARKAFQD	55
PSX.seq	51	NRFTHKHSDDLRLQFAKENPMNEVLKVKVKDVEDVTEAVATTLRRLQNFYSTIQSH	110
CPX.seq	59	HRFEKHKHADLLMRMFAKENSFVNLQVKVKDKEDVTEAVTRTLRRADNFYSTIQAD	118
CPQ.seq	58	NRFHRRKQSDLEFLAIQYEKETAKGAKGGAVKVKEGEEVGGKAVKSTLERALGFYSAVQTR	117
McQ.seq	56	NRFHRRKQSDLLVSIQCEKGTNNGARVPGTKLKEGEEVRRKEAVKSTLERALSFYSIQTS	115
PSX.seq	111	DGHMPGTYGGPMFLPGLVITLSTVTGALNAVLTDEHRKEMRRYLYNHQNKGGWGLHIEG	170
CPX.seq	119	DGHMPGTYGGPMFLPGLVITLSTVTGALNAVLTSTEHRETCRYLYNHQNKGGWGLHIEG	178
CPQ.seq	118	DGNWASTLGGPFLPGLVIALVTVGLNSVLSKHHRVEMCRYLYNHQNKGGWGLHIEG	177
McQ.seq	116	DGNWASTLGGPMFLPGLVIALVTVGALNSVLSKHHRVEMCRYLYNHQNKGGWGLHIES	175
PSX.seq	171	PSTMFGSVLCYVTLRLLGEGPNDGEG-DMERGRDITLEHGATYITSWGKMLSVLGVVE	229
CPX.seq	179	PSTMFGSVLYVYTLRLLGEEAEDGGG-AVDKARKWILDHGGAATITSWGKMLSVLGVVE	237
CPQ.seq	178	TSTMFGSALNYVALRLLGEDADGGGGAMTKARAWILERGGATAITSWGKLWSVLGVVE	237
McQ.seq	176	PSTMFGSALNYVALRLLGEDADGGGGRAMTKARAWILGRGGAATITSWGKLWSVLGVVE	235
PSX.seq	230	WSGNNPYPPELWLLPYALPVHPGRMWCHCRMVYLPMSYLYGKRFVGPITPTVLSLRKELF	289
CPX.seq	238	WAGNNPLPPELWLLPYLPCHPGRMWCHCRMVYLPMSYLYGKRFVGPITPIIRSLRKELY	297
CPQ.seq	238	WSGNNPYPPELWLLPYSLPHHPGRMWCHCRMVYLPMSYLYGKRFVGPITPKVLSLRQELY	297
McQ.seq	236	WSGNNPYPPELWLLPYFLPHHPGRMWCHCRMVYLPMSYLYGKRFVGPITPK-----ELY	289
PSX.seq	290	TVPYHIDWNQARNLCAKEDLYPHPLVQDILWATLHKFVEPVMNWPQKRLREKALKTA	349
CPX.seq	298	LVPYHEDWNKARNQCAKEDLYPHPLVQDILWATLHHVYEPLFMHWPQKRLREKALQSV	357
CPQ.seq	298	TVPYHIDWNKSRNTCAKEDLYPHPKMDDILWGSYHMYEPLFTRWPQKRLREKALQAA	357
McQ.seq	290	TVPYHIDWNKSRNTCAKEDLYPHSKMDDILWGSYHMYEPLFTHWPQKRLREKALKTA	349
PSX.seq	350	IEHHIHYEDENTRYICLGPVNKVLNMLCCWVEDPNSFAFKLHLPRIDYDLWVAEDGMKMQG	409
CPX.seq	358	QHHIHYEDENTRYICLGPVNKVLNMLCCWVEDPNSFAFKLHLPRIDYDLWVAEDGMKMQG	417
CPQ.seq	358	MHHIHYEDENSRYLCLGPVNKVLNMLCCWVEDPYSDFAKLHLQRVHVDLWVAEDGMKMQG	417
McQ.seq	350	QHHIHYEDENTRYICLGPVNKVLNMLCCWVEDPYSFAFKLHLQRVHVDLWVAEDGMKMQG	409
PSX.seq	410	YNGSQLWDTAFAAQAIISTNLIDFQPTLKKAHAFIKNSQVSEDCPGDLSKNYRHISKGA	469
CPX.seq	418	YNGSQLWDTAFVQAIISTELAEETTLRKAHKYIKDSQVLEDCPGDLQSNYRHISKGA	477
CPQ.seq	418	YNGSQLWDTAFSIQAIIVATKLVDSYAPTLLRKAHDFVKDSIQIEDCPGDPNVWFRHISKGA	477
McQ.seq	410	YNGSQLWDTAFVQAIISTKPVQNYGPTLKAHDYVKNSSIQEQDCPGEPNVWFRHISKGA	469
PSX.seq	470	WPFSTADHGVPLSDCTAEGLKAVLLLSKIAPETVGEPLDSKRLYDAVNVILSLQNDGGF	529
CPX.seq	478	WPFSTADHGVPLSDCTAEGLKAVLLLSKLPSETVGSIDEQQLYNVAVNVILSLQNTDGGF	537
CPQ.seq	478	WPFSTADHGVPLSDCTAEGLKASLLLSKLPSTVGEPLKKNRLDAVNVILSLQNDGGF	537
McQ.seq	470	WPFSTADHGVPLSDCTAEGLKASLLLSKLPSETVGEPLERNRLDAVNVILSLQNDGGF	529
PSX.seq	530	ATYELTRSYRWLELNPAAETFGDIVIDCPYVECTSAAIQALATFQKLYPGHRRDEIQCCI	589
CPX.seq	538	ATYELTRSYRWLELNPAAETFGDIVIDYPYVECTSAAIQALAAFKLYPGHRRDEIDNCI	597
CPQ.seq	538	ATYELTRSYRWLELNPAAETFGDIVIDYPYVECTAATMEALTLFKKLYPGHRTKEIDTAI	597
McQ.seq	530	ATYELTRSYRWLELNPAAETFGDIVIDYPYVECTAATMEALTLFKKLYPGHRTKEIDTAI	589
PSX.seq	590	EKAARFIEKIQASDGSWYGSNGVCFYTGWFGIKGLVAAGKFNCSLIRKACDFLLSKE	649
CPX.seq	598	AEAADFIEESIQATDGSWYGSNGVCFYTGWFGIRGLVAAGRNYNCSLIRKACDFLLSKE	657
CPQ.seq	598	GKAARFLEKMQRADGSWYGSNGVCFYTGWFGIKGLVAAGRNYNSCLIRKACDFLLSKE	657
McQ.seq	590	ARAADFLENMQRADGSWYGSNGVCFYTGWFGIKGLVAAGRNYNSCLIRKACDFLLSKE	649
PSX.seq	650	LPSGGWAGESYLSQNKVYTNLEGNRSYVNTQWMLALTEAGQERDPTPLHRAARVLIN	709
CPX.seq	658	LAAAGWAGESYLSQNKVYTNLEKDDRPHIVNTQWMLSLIDAGSERDPTPLHRAARVLIN	717
CPQ.seq	658	LPGGWAGESYLSQNKVYTNLEGNRPHLVNTQWMLMALTEAGQERDPTPLHRAARVLIN	717
McQ.seq	650	LPGGWAGESYLSQNKVYTNLEGNRPHLVNTQWMLMALTEAGQERDPTPLHRAARVLIN	709
PSX.seq	710	SQLENGDFPQEEIMGVFNKNCMIYAAAYRDIFFIWAALGEYR-RVLQAC-	756
CPX.seq	718	SQLENGDFPQEEIMGVFNKNCMIYAAAYRNIFFIWAALGEYRSRVLKPLK	766
CPQ.seq	718	SQLENGDFPQEEIMGVFNKNCMIYAAAYRNIFFIWAALGEYCHRVLITE--	764
McQ.seq	710	SQLENGDFPQEEIMGVFNKNCMIYAAAYRDIFFIWAALGEYCHRVLITE--	756

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.

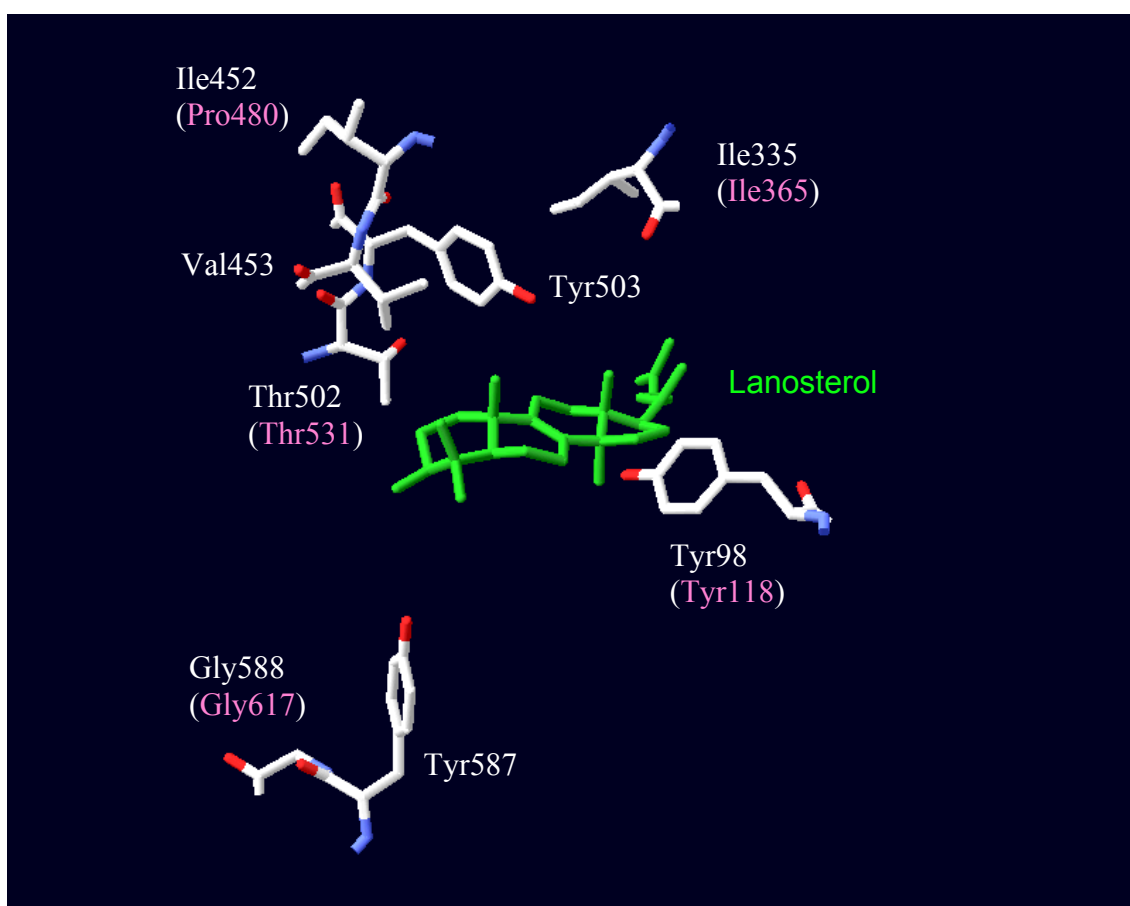


Fig. S4. TLC analysis of hexane extracts of the PSX Y118L mutant showing the production of cucurbitadienol. Lane 1: PSX wild type, Lane 2: PSX Y118L mutant, Lane 3: CPQ wild type.

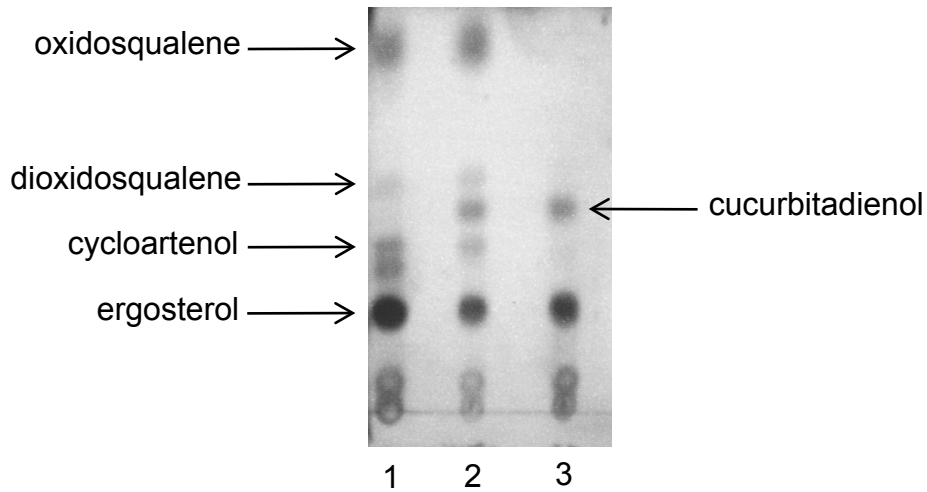


Fig. S5. TLC analysis of hexane extracts of other PSX mutants. Lane 1: Y118L mutant, Lane 2: I365L mutant, Lane 3: P480L mutant, Lane 4: T531S mutant, Lane 5: G617A mutant. Blue arrow corresponds to a position of cycloartenol while red arrow corresponds to a position of cucurbitadienol.

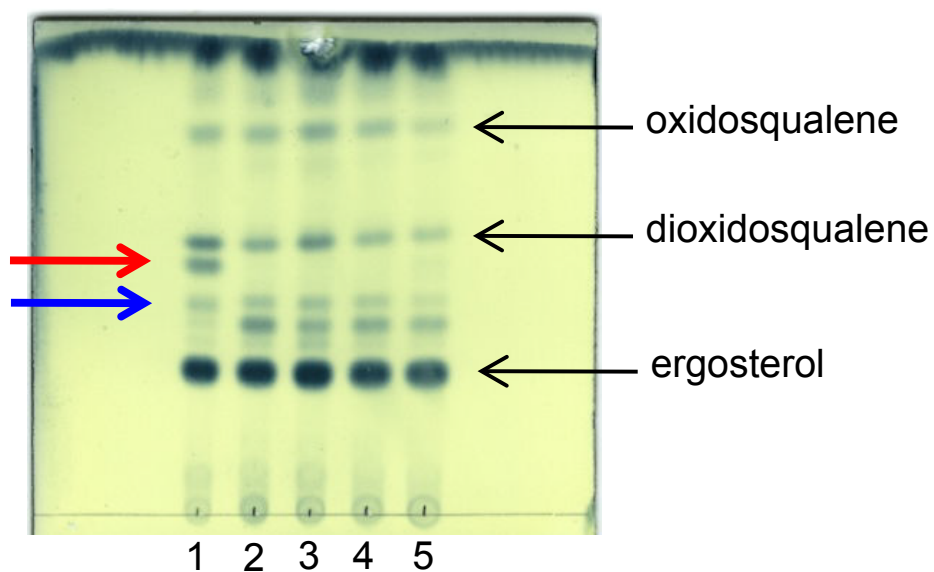


Fig. S6. A MS spectrum of cucurbitadienol produced by the PSX Y118L mutant.

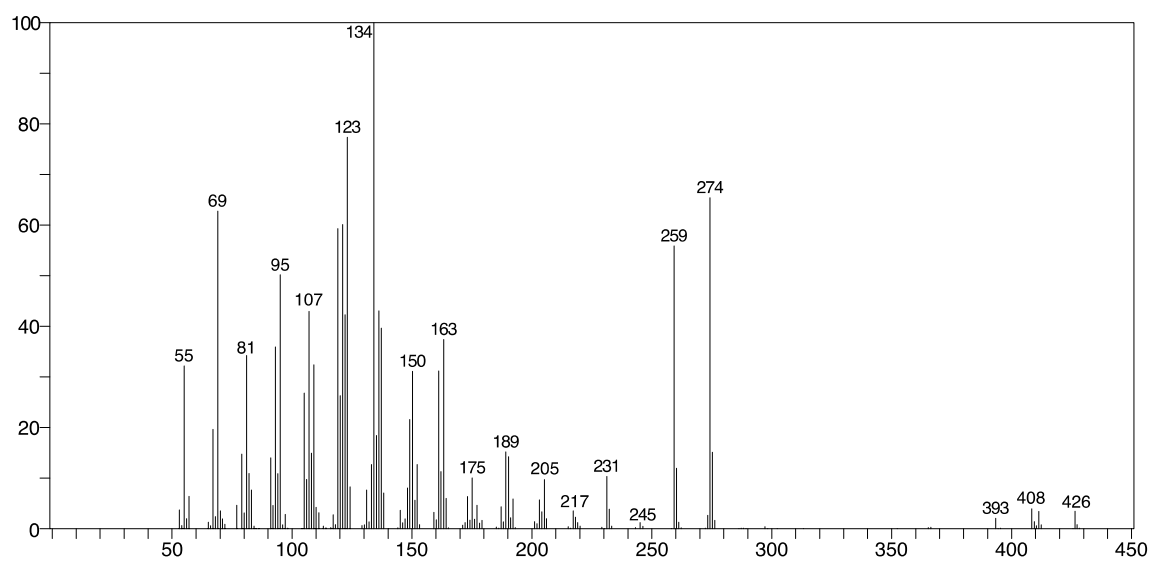


Fig. S7. ^1H -NMR spectrum (300 MHz, in CDCl_3) of cucurbitadienol extracted from the PSX Y118L mutant. A cross mark in red indicate impurities arising from a lipid (~ 1.2 ppm).

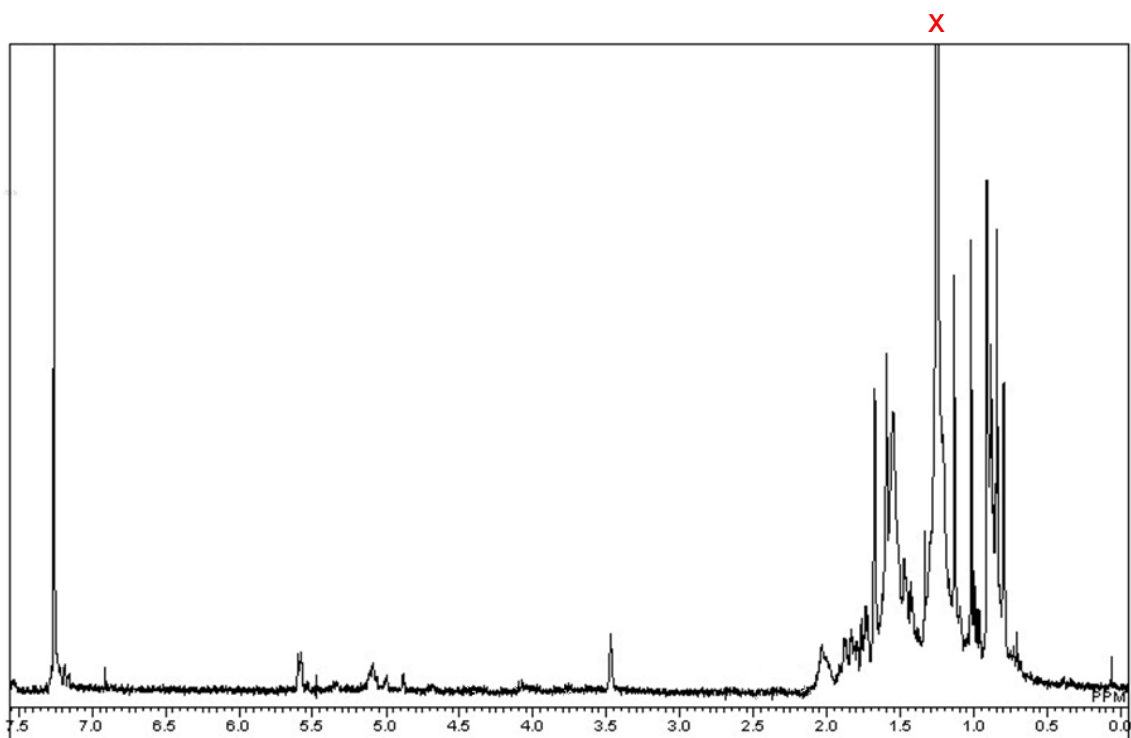
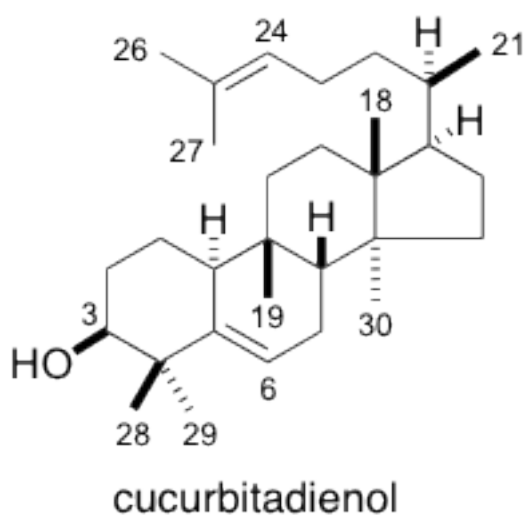


Fig. S8. Assignments of ^1H -NMR chemical shifts for cucurbitadienol.

	Product of Y118L	Cucurbitadienol*
position	^1H (ppm)	^1H (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



* 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.

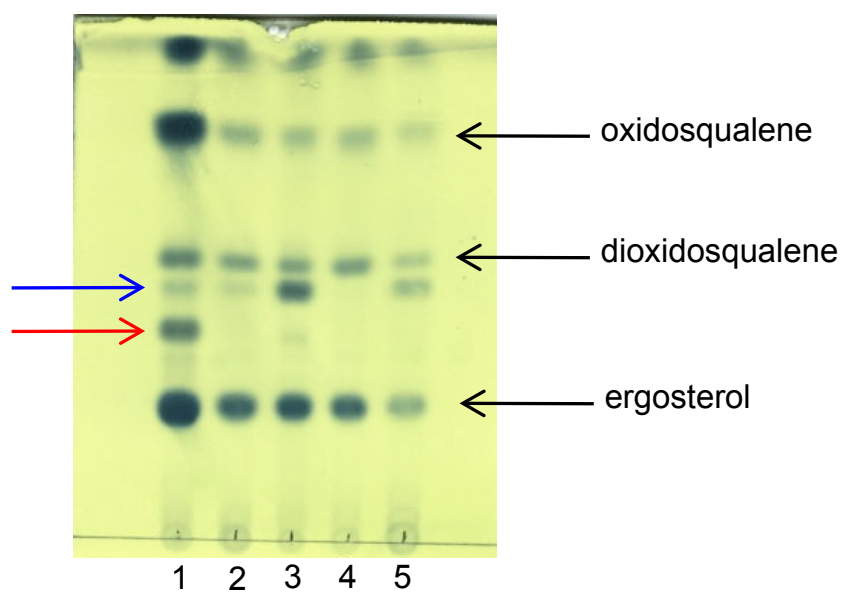


Fig. S10. A MS spectrum of parkeol produced by the CPQ L125Y mutant.

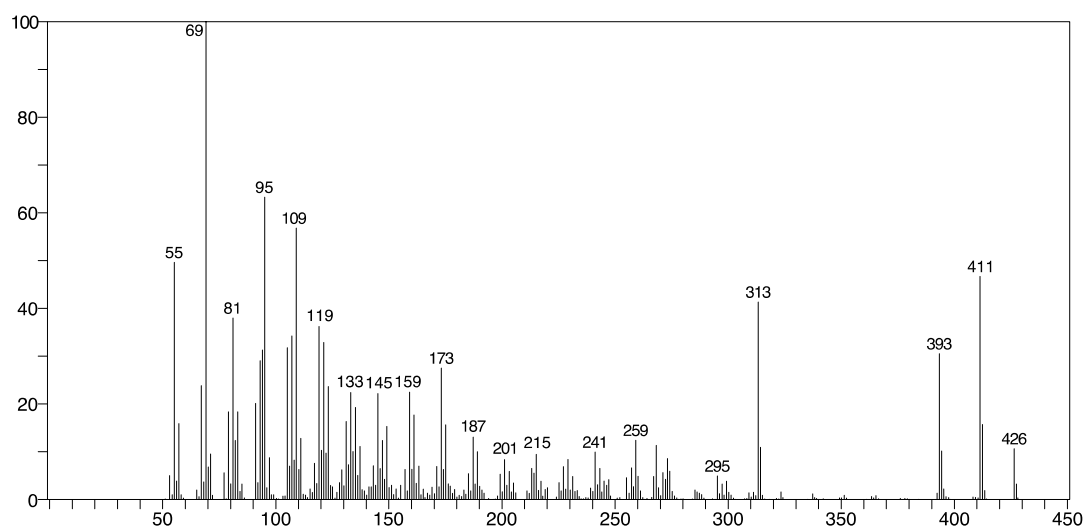
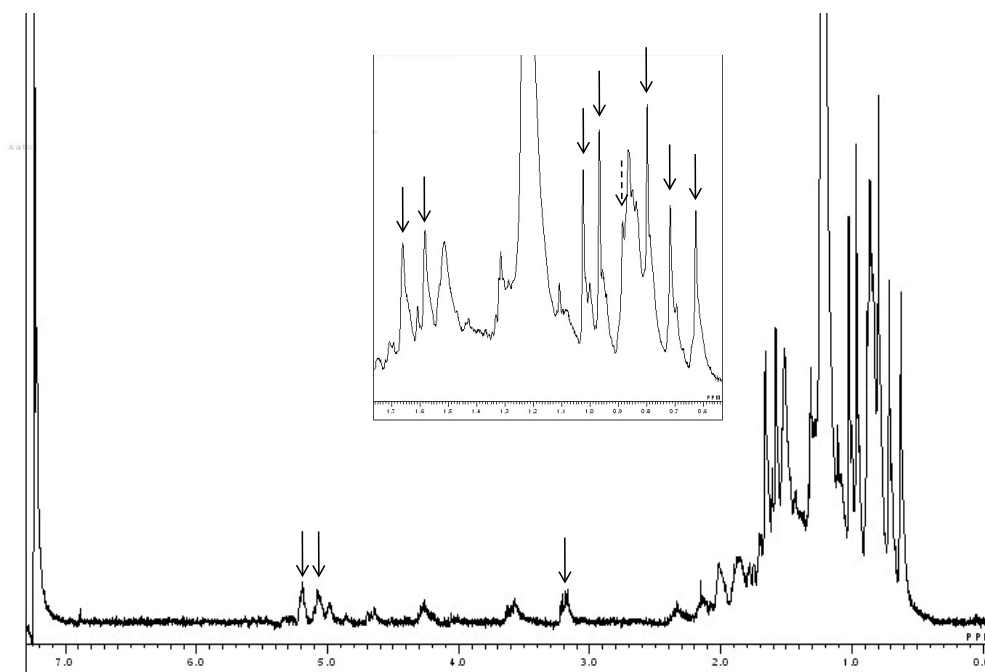


Fig. S11. ^1H -NMR spectrum (300 MHz, in CDCl_3) of parkeol produced by the CPQ L125Y mutant.



Product of L125Y	Parkeol ¹¹
^1H (ppm)	^1H (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.

CPQ.seq	1	MRRLKVGAEISVGEDEKNNVKSVS ¹ NHLGRQVWEFCADAAADTPHQLLQIQNARNHFHNR	60
PSX.seq	1	MRRLKV-----AEGGTPMLRRLNNHVGROVWEF--DPHSGSPQDLDDIETARRNFHNR	53
human LAS.seq	1	-----MTEGTCLRRRGGPYKTEPATDLGRWRLNCERGR	33
CPQ.seq	61	HRKQSSDLFLAIDQYEKEIAKGA ² GGAVKVKEGEEV ³ GVKEAVKSTLERALGFYSAVQTRDGN	120
PSX.seq	54	THKHSDDLMLRLQFAKENPMNEVLPKVKVKDVEDVTEAVATTLRRGLNFYSTIQSHDGH	113
human LAS.seq	34	QTWTYLQDERAGREQTGLEAYALGLD ⁴ TKNYFKDLPKAHTAFEGALN ⁵ GMTFYVGLQ ⁶ AEDGH	93
CPQ.seq	121	MASDLGGPLFLLPGLVIALHVTGV ⁷ LNSVLSKHHRVEMCR ⁸ RYLYNHQNE ⁹ DGGWGLHIEGTST	180
PSX.seq	114	MPGDYGGPFLPGLVITL ¹⁰ SVTGALNAVLTDEHRKEMR ¹¹ RYLYNHQNE ¹² DGGWGLHIEG ¹³ ST	173
human LAS.seq	94	WTGDYGGPLFLLPGLLIT ¹⁴ CHVA---RIPLPAGYREI ¹⁵ VRYLRSVQL ¹⁶ PDGGWGLHIE ¹⁷ DKST	150
CPQ.seq	181	MFGSALNYVALRLLGEDADGGGGAMTKARAWILERGGATAIT ¹⁸ SWGK ¹⁹ LWLSVLGVYEW ²⁰ SG	240
PSX.seq	174	MFGSVLQYVTLRLLGEGPN ²¹ DGEGD-MERGRDWILEHGGATYIT ²² SWGK ²³ WLSVLGVFEW ²⁴ SG	232
human LAS.seq	151	VFGTALNYVSLRLLGVGDD ²⁵ PD---LV ²⁶ RARNILHKKGGAVAT ²⁷ PSWGK ²⁸ FWLWLV ²⁹ LVY ³⁰ SWEG	207
CPQ.seq	241	NNPLPPEFNL ³¹ LPYSLP ³² HPGRMWCHCRMVYLPMSYLYGKRFVGPIT ³³ PKVLSLRQEL ³⁴ Y ³⁵ TIP	300
PSX.seq	233	NNPMPPEINL ³⁶ LPYALP ³⁷ HPGRMWCHCRMVYLPMSYLYGKRFVGPIT ³⁸ PKVLSLRKEL ³⁹ FT ⁴⁰ V ⁴¹ P	292
human LAS.seq	208	LNTLFPENWLPD ⁴² WAP ⁴³ HPSTLWCHCR ⁴⁴ YYL ⁴⁵ PMSYCYAV ⁴⁶ RLSAAED ⁴⁷ PLV ⁴⁸ SLRQEL ⁴⁹ Y ⁵⁰ VED	267
CPQ.seq	301	YHEIDWNKSRNTCAKEDLYPHPK ⁵¹ QDILWGSIT ⁵² YHVEPL ⁵³ FTRWPKR ⁵⁴ LREKALQAAMK ⁵⁵ H	360
PSX.seq	293	YHIDWNQARNLCAKEDLYPHPL ⁵⁶ VQDILWATLHKFV ⁵⁷ EPV ⁵⁸ FMN ⁵⁹ WPK ⁶⁰ LREKAIKTAIEH	352
human LAS.seq	268	FASIDWLAQRN ⁶¹ VAPDEL ⁶² YTPHSWL---LRV-VY ⁶³ ALLN-LYEH ⁶⁴ HS ⁶⁵ SAHL ⁶⁶ RQ ⁶⁷ RAV ⁶⁸ Q ⁶⁹ KLYEH	322
CPQ.seq	361	IHYEDENSR ⁷⁰ YICL ⁷¹ GPVNKVLNMLCCW- ⁷² VEDPYS ⁷³ DAFKLHL ⁷⁴ QRV ⁷⁵ HDYL ⁷⁶ VWAEDGMR ⁷⁷ MQGYN	419
PSX.seq	353	IHYEDENTRYICIG ⁷⁸ PVNKVLNMLCCW- ⁷⁹ VEDPNS ⁸⁰ EAFKLHL ⁸¹ PRTY ⁸² DYL ⁸³ VWAEDGMM ⁸⁴ QGYN	411
human LAS.seq	323	IVADDRFTK ⁸⁵ SI ⁸⁶ GPISK ⁸⁷ TINMLVR ⁸⁸ NYV ⁸⁹ DGPASTAF ⁹⁰ QEH ⁹¹ VSRI ⁹² PDYLW ⁹³ MGL ⁹⁴ DGMM ⁹⁵ QGTN	382
CPQ.seq	420	GSQLWD ⁹⁶ TAF ⁹⁷ SI ⁹⁸ QAI ⁹⁹ VAT--KL ¹⁰⁰ VD ¹⁰¹ SY ¹⁰² AP ¹⁰³ TL ¹⁰⁴ RKA ¹⁰⁵ DF ¹⁰⁶ V ¹⁰⁷ KD ¹⁰⁸ SQIQ ¹⁰⁹ EDCP ¹¹⁰ GD ¹¹¹ PN ¹¹² W ¹¹³ FR ¹¹⁴ HI ¹¹⁵ HKGA	477
PSX.seq	412	GSQLWD ¹¹⁶ TAF ¹¹⁷ AQAI ¹¹⁸ IST--NL ¹¹⁹ ID ¹²⁰ E ¹²¹ FG ¹²² PTL ¹²³ KKA ¹²⁴ H ¹²⁵ FI ¹²⁶ K ¹²⁷ NSQ ¹²⁸ VSE ¹²⁹ DCP ¹³⁰ GD ¹³¹ L ¹³² SK ¹³³ WY ¹³⁴ RH ¹³⁵ ISKGA	469
human LAS.seq	383	GSQIWD ¹³⁶ TAF ¹³⁷ AQAL ¹³⁸ LEAG ¹³⁹ GH ¹⁴⁰ RR ¹⁴¹ PE ¹⁴² FSSCL ¹⁴³ QKA ¹⁴⁴ H ¹⁴⁵ E ¹⁴⁶ FL ¹⁴⁷ RL ¹⁴⁸ SQ ¹⁴⁹ V ¹⁵⁰ PDN- ¹⁵¹ PPDY ¹⁵² Q ¹⁵³ K ¹⁵⁴ Y ¹⁵⁵ RR ¹⁵⁶ MR ¹⁵⁷ KG	441
CPQ.seq	478	WPLSTR ¹⁵⁸ DHG ¹⁵⁹ WLT ¹⁶⁰ SDCTA ¹⁶¹ EGLKASL ¹⁶² MLSK ¹⁶³ LPST ¹⁶⁴ MVGE ¹⁶⁵ PLEKN ¹⁶⁶ RLCD ¹⁶⁷ AVN ¹⁶⁸ VLL ¹⁶⁹ SL ¹⁷⁰ QND ¹⁷¹ GGF	537
PSX.seq	470	WPFSTADHG ¹⁷² WPI ¹⁷³ SDCTA ¹⁷⁴ EGLKAVLL ¹⁷⁵ LSKIA ¹⁷⁶ PEI ¹⁷⁷ VGE ¹⁷⁸ PLDSK ¹⁷⁹ RLY ¹⁸⁰ DAVN ¹⁸¹ VLL ¹⁸² SL ¹⁸³ QND ¹⁸⁴ GGF	529
human LAS.seq	442	FSFSTLD ¹⁸⁵ CG ¹⁸⁶ WIV ¹⁸⁷ SDCTA ¹⁸⁸ EAL ¹⁸⁹ KAVLL ¹⁹⁰ QEK ¹⁹¹ CPH-V ¹⁹² TEH ¹⁹³ IP ¹⁹⁴ RE ¹⁹⁵ RLC ¹⁹⁶ DAV ¹⁹⁷ WLL ¹⁹⁸ NMR ¹⁹⁹ NP ²⁰⁰ DGGF	500
CPQ.seq	538	AS ²⁰¹ YEL ²⁰² TR ²⁰³ SY ²⁰⁴ NLE ²⁰⁵ LIN ²⁰⁶ PAET ²⁰⁷ FGD ²⁰⁸ IVID ²⁰⁹ YP ²¹⁰ VECT ²¹¹ AA ²¹² T ²¹³ EA ²¹⁴ L ²¹⁵ T ²¹⁶ L ²¹⁷ F ²¹⁸ K ²¹⁹ KL ²²⁰ HP ²²¹ GH ²²² RT ²²³ KE ²²⁴ ID ²²⁵ TAI	597
PSX.seq	530	ATYEL ²²⁶ TR ²²⁷ SY ²²⁸ NLE ²²⁹ LIN ²³⁰ PAET ²³¹ FGD ²³² IVID ²³³ CP ²³⁴ VECT ²³⁵ SA ²³⁶ A ²³⁷ QAL ²³⁸ AT ²³⁹ FG ²⁴⁰ KLY ²⁴¹ PG ²⁴² H ²⁴³ RR ²⁴⁴ E ²⁴⁵ I ²⁴⁶ QCCI	589
human LAS.seq	501	ATYET ²⁴⁷ K ²⁴⁸ R ²⁴⁹ GGH ²⁵⁰ L ²⁵¹ EL ²⁵² LN ²⁵³ PE ²⁵⁴ VF ²⁵⁵ GDI ²⁵⁶ VID ²⁵⁷ TY ²⁵⁸ VECT ²⁵⁹ SA ²⁶⁰ V ²⁶¹ QAL ²⁶² KY ²⁶³ F ²⁶⁴ H ²⁶⁵ K ²⁶⁶ R ²⁶⁷ PE ²⁶⁸ HRA ²⁶⁹ E ²⁷⁰ IRE ²⁷¹ TL	560
CPQ.seq	598	GKAA ²⁷² NFL ²⁷³ E ²⁷⁴ K ²⁷⁵ Q ²⁷⁶ RAD ²⁷⁷ GS ²⁷⁸ WY ²⁷⁹ CG ²⁸⁰ W ²⁸¹ GV ²⁸² CFTY ²⁸³ AG ²⁸⁴ W ²⁸⁵ FG ²⁸⁶ IK ²⁸⁷ GL ²⁸⁸ V ²⁸⁹ AAG ²⁹⁰ RTY ²⁹¹ NS---CL ²⁹² A ²⁹³ IRK ²⁹⁴ ACE ²⁹⁵ FLL	654
PSX.seq	590	EKAV ²⁹⁶ A ²⁹⁷ F ²⁹⁸ E ²⁹⁹ K ³⁰⁰ IAS ³⁰¹ DGS ³⁰² WY ³⁰³ GS ³⁰⁴ W ³⁰⁵ GV ³⁰⁶ CFTY ³⁰⁷ GT ³⁰⁸ W ³⁰⁹ FG ³¹⁰ IK ³¹¹ GL ³¹² V ³¹³ AAG ³¹⁴ KN ³¹⁵ FSN---CL ³¹⁶ S ³¹⁷ IRK ³¹⁸ ACE ³¹⁹ FLL	646
human LAS.seq	561	TQGL ³²⁰ E ³²¹ FC ³²² RR ³²³ Q ³²⁴ Q ³²⁵ RAD ³²⁶ GS ³²⁷ W ³²⁸ EG ³²⁹ SW ³³⁰ GV ³³¹ CFTY ³³² GT ³³³ W ³³⁴ FG ³³⁵ LEAF ³³⁶ AC ³³⁷ M ³³⁸ GT ³³⁹ Y ³⁴⁰ RD ³⁴¹ GT ³⁴² CA ³⁴³ EV ³⁴⁴ SR ³⁴⁵ AC ³⁴⁶ DFLL	620
CPQ.seq	655	SK ³⁴⁷ EL ³⁴⁸ PG ³⁴⁹ GG ³⁵⁰ W ³⁵¹ GESY ³⁵² LSC ³⁵³ QNK ³⁵⁴ VY ³⁵⁵ TN ³⁵⁶ LEG ³⁵⁷ NK ³⁵⁸ PH ³⁵⁹ LV ³⁶⁰ NT ³⁶¹ M ³⁶² V ³⁶³ LM ³⁶⁴ AL ³⁶⁵ IEA ³⁶⁶ G ³⁶⁷ GER ³⁶⁸ DP ³⁶⁹ N ³⁷⁰ PL ³⁷¹ HRA ³⁷² ARL	714
PSX.seq	647	SKQL ³⁷³ PS ³⁷⁴ GG ³⁷⁵ W ³⁷⁶ ESY ³⁷⁷ LSC ³⁷⁸ QNK ³⁷⁹ VY ³⁸⁰ SN ³⁸¹ LEG ³⁸² NR ³⁸³ SH ³⁸⁴ V ³⁸⁵ NT ³⁸⁶ GW ³⁸⁷ AM ³⁸⁸ LM ³⁸⁹ AL ³⁹⁰ IEA ³⁹¹ EQ ³⁹² AK ³⁹³ RD ³⁹⁴ P ³⁹⁵ PL ³⁹⁶ HRA ³⁹⁷ AV ³⁹⁸ C	706
human LAS.seq	621	SR ³⁹⁹ Q ⁴⁰⁰ AD ⁴⁰¹ GG ⁴⁰² W ⁴⁰³ GE ⁴⁰⁴ DF ⁴⁰⁵ ES ⁴⁰⁶ CE ⁴⁰⁷ ERRY---L ⁴⁰⁸ Q ⁴⁰⁹ SA ⁴¹⁰ Q ⁴¹¹ SI ⁴¹² HN ⁴¹³ TC ⁴¹⁴ WAM ⁴¹⁵ ML ⁴¹⁶ MA ⁴¹⁷ VR ⁴¹⁸ HP ⁴¹⁹ DI ⁴²⁰ EA ⁴²¹ Q---E ⁴²² RG ⁴²³ VR ⁴²⁴ C	676
CPQ.seq	715	L ⁴²⁵ NSQL ⁴²⁶ ENG ⁴²⁷ DF ⁴²⁸ VQ ⁴²⁹ Q ⁴³⁰ EIM ⁴³¹ GV ⁴³² FN ⁴³³ KNC ⁴³⁴ MITY ⁴³⁵ AA ⁴³⁶ YRN ⁴³⁷ IF ⁴³⁸ P ⁴³⁹ I ⁴⁴⁰ WAL ⁴⁴¹ GEY ⁴⁴² CH ⁴⁴³ RVL ⁴⁴⁴ TE-----	764
PSX.seq	707	L ⁴⁴⁵ NSQL ⁴⁴⁶ ENG ⁴⁴⁷ DF ⁴⁴⁸ PQ ⁴⁴⁹ EEIM ⁴⁵⁰ GV ⁴⁵¹ FN ⁴⁵² KNC ⁴⁵³ MITY ⁴⁵⁴ AA ⁴⁵⁵ YR ⁴⁵⁶ IF ⁴⁵⁷ P ⁴⁵⁸ I ⁴⁵⁹ WAL ⁴⁶⁰ GEY ⁴⁶¹ RR ⁴⁶² V ⁴⁶³ L ⁴⁶⁴ QAC-----	756
human LAS.seq	677	L ⁴⁶⁵ LEK ⁴⁶⁶ QL ⁴⁶⁷ PN ⁴⁶⁸ GD ⁴⁶⁹ W ⁴⁷⁰ PQ ⁴⁷¹ EN ⁴⁷² IA ⁴⁷³ GV ⁴⁷⁴ FN ⁴⁷⁵ KSC ⁴⁷⁶ AIS ⁴⁷⁷ YS ⁴⁷⁸ YRN ⁴⁷⁹ IF ⁴⁸⁰ P ⁴⁸¹ I ⁴⁸² WAL ⁴⁸³ GR ⁴⁸⁴ FS ⁴⁸⁵ QL ⁴⁸⁶ Y ⁴⁸⁷ PERAL ⁴⁸⁸ AG ⁴⁸⁹ HP	732

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.

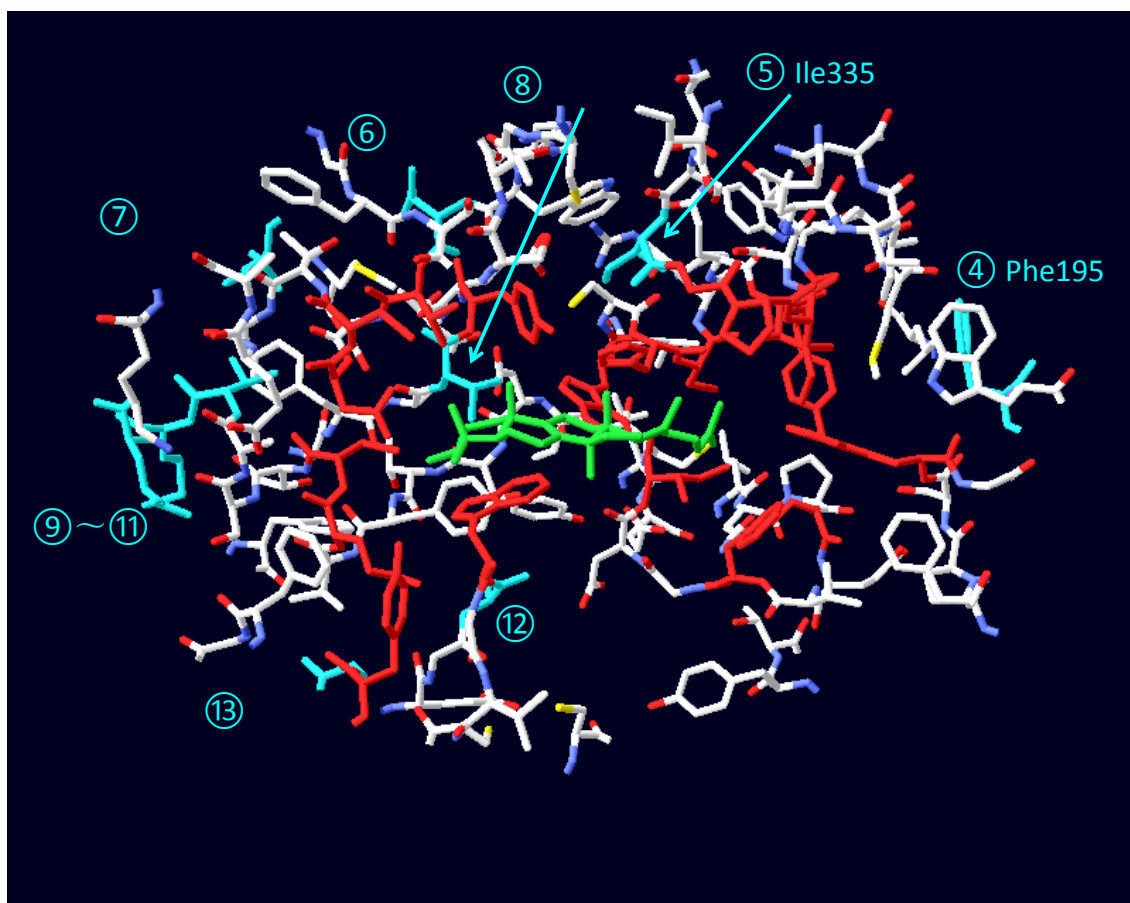


Fig. S14. TLC analyses of hexane extract of CPQ 13-mut and CPQ 3-mut. Lane 1: PSX wild type, Lane 2: CPQ L125Y mutant, Lane 3: CPQ 3-mut, Lane 4: CPQ 13-mut, Lane 5: CPQ wild type. Blue arrow indicates cucurbitadienol while red arrow indicates a typical triterpene monoalcohol position.

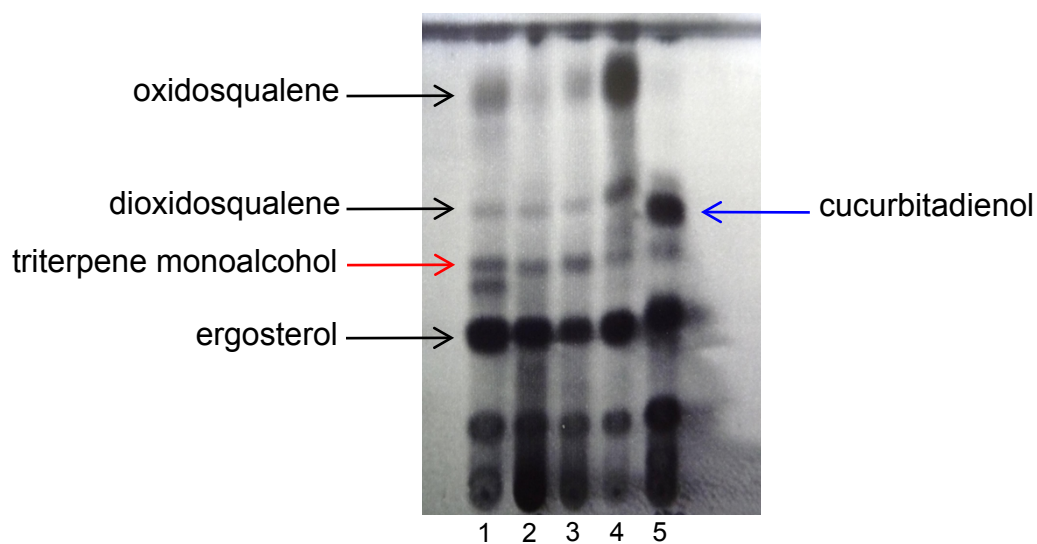


Fig. S15. GC-MS analyses of hexane extract of CPQ 13-mut and CPQ 3-mut. Dotted line indicates the position of parkeol. Peak for parkeol is not visible in CPQ 13-mut in this scale.

