Supplementary Table S1. Mutagenesis studies of OSCs.

Enzyme	Mutation site	Function Changed
AsAS	S728F [1]	Convertes AsAS into an enzyme that makes tetracyclic (dammarane) instead of pentacyclic products.
AtCAS1	Y410C, H477Y, A469V, I481T, Y532H [2]	Alters product specificity: Y410C and H477Y mutants produce lanosterol as the dominant product, whereas the A469V, I481T and Y532H mutants produce a mixture of lanosterol and achilleol A.
AtCYC	Y410T [3]	Converts cycloartenol synthase to an oxidosqualene cyclase that forms lanosterol as its major product
AtCYC	Y118L [4]	Produces of cucurbitadienol as a major product
AtCYC	Y410T, H477N, I481V [5]	alters the position or electronic properties of the tyrosine by interaction with the phenolic hydroxyl group
AtCBS	L125Y [4]	Produces of parkeol
AtCPI	W112L [6]	Extends substrate specificity
AtLSSl	N477H [7]	Promotes the biosynthesis of lanosterol (88%) and parkeol (12%) by relocating the polar His257 and Tyr410 residues close to C-9 and C-ll.
AtLSSl	V481I [7]	Facilitates lanosterol production (25% lanosterol, 21% parkeol, and 54% cycloartenol) by enlarging the active site and reducing steric control.
AtLUP1	T729F [1]	Similarly resulted with AsAS.
CcLSS	H234E, H234Y, H234F, D456E, D456N, D456H [8]	Inactive
EtAS	V483, W534, M729 [9]	The Gly and Ala variants with a smaller bulk size at position 483 predominantly afford monocyclic camelliol C. The Trp534 residue, with the largest steric bulk among all natural amino acids, is essential for high enzymatic activity. Various variants of Trp534 exhibit significantly decreased enzymatic activities and provide no aberrantly cyclized products. Altering the steric bulk at the Met729 position affords the pentacyclic skeletons.
EtAS	F474 [10]	The Gly and Ala mutants produce significantly larger amounts of the bicyclic products and a decreased amount of beta-amyrin, indicating that the F474 residue was located near the B-ring formation site.
EtAS	D485N, C564A [11]	No activity of the D485N variant and significantly decreased activity of the C564A variant were found.
EtAS	W612, L734, Y736 [12]	The aliphatic variants (Ala, Val, Met) of Trp612 show almost no activity, but the aromatic variants exhibit high activities. The L734G and L734A variants exhibit significantly decreased activities but yielded taraxerol in a high production ratio. The Val, Ile, and Met variants show markedly high activities (56-78% of wild-type activity). The aliphatic variants of Tyr736 show markedly decreased activities, but the Phe mutant exhibit high activity (67%), which indicates that the electrons are critical for catalysis.
OeLS	L256W [13]	Produces exclusively beta-amyrin with only minor amount of lupeol.
PgAS	W259L [13]	Produces lupeol as a major product together with beta-amyrin in 2:1 ratio.
PgAS	Y261H [13]	Produces dammara-18,21-dien-3beta-ol (as a 3:5 mixture of E/Z isomer) together with minor amount of dammara-18(28),21-dien-3beta-ol.
ScLSS	T384Y, V454I [14]	Alters the product structure
ScLSS	H234X [15]	Changes in product specificity from lanosterol formation to either protosta-12,24-dien-3beta-ol or parkeol production
ScLSS	F699T [16]	Produces novel protosta-13(17),24-dien-3beta-ol as the sole truncated rearrangement product

ScLSS	T384Y, Q450H, V454I [17]	Produces parkeol but not lanosterol as the sole end product
ScLSS	I705X [18]	Affects lanosterol's C/D ring stabilization including 6-6-5 tricyclic and protosteryl C-17 cations and 17α/β-exocyclic side chain stereochemistry
ScLSS	Y99X [19]	Two truncated intermediates were isolated from the Y99X mutants.
ScLSS	Y510W/K [20]	Fails to complement cyclase-deficiency
ScLSS	C703I/H [21]	Generates an iridal-type triterpenoid
ScLSS	C457G, T509G [22]	Generates achilleol A as the major product

Note: Abbreviation of enzyme: AsAs, beta-amyrin synthase of *Avena strigosa*; AtCYC, Cycloartenol synthase of *Arabidopsis thaliana*; AtCBS, Cucurbitadienol synthase of *Arabidopsis thaliana*; AtCPI, Cyclopropylsterol-cycloisomerase of *Arabidopsis thaliana*; AtLSS1, Lanosterol synthase of *Arabidopsis thaliana*; AtLUP1, Lupeol synthase 1 of *Arabidopsis thaliana*; CcLSS, Lanosterol cyclase of *Cephalosporium caerulens*; EtAS, beta-amyrin synthase of *Euphorbia tirucalli*; OeLS, Lupeol synthase of *Olea europaea*; PgAS, beta-Amyrin Synthase of *Panax ginseng*; ScLSS, Lanosterol cyclase of *Saccharomyces cerevisiae*.

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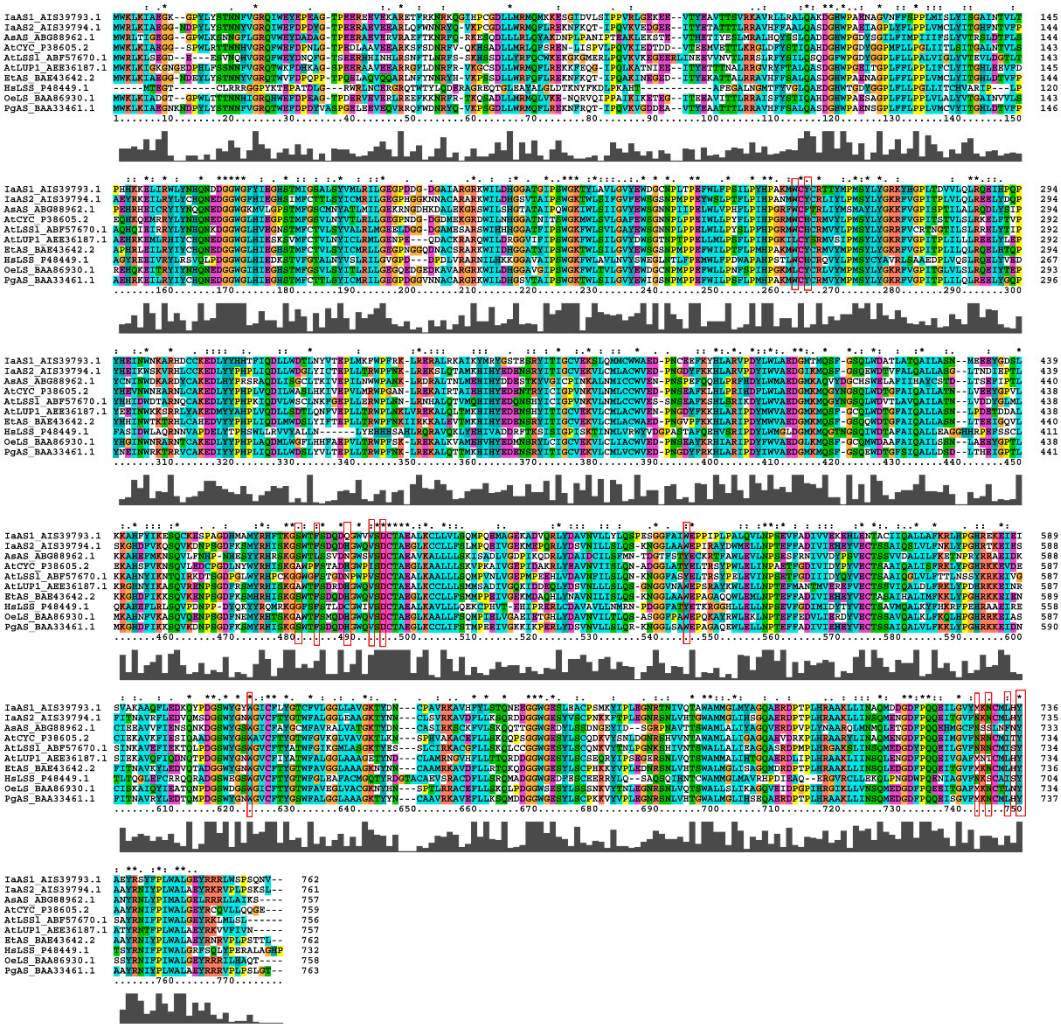

Supplementary Figure S1. Multi-sequence alignment of IaAS1, IaAS2 and other eight OSCs.

AsAS(ABG88962.1): beta-amyrin synthase of *Avena strigosa*, AtCYC(P38605.2): Cycloartenol synthase of *Arabidopsis thaliana*, AtLSSl(ABF57670.1): Lanosterol synthase of *Arabidopsis thaliana*, AtLUP1(AEE36187.1): Lupeol synthase 1 of *Arabidopsis thaliana*, EtAS(BAE43642.2): beta-amyrin synthase of *Euphorbia tirucalli*, HsLSS(P48449.1): Human Lanosterol synthase, OeLS(BAA86930.1): Lupeol synthase of *Olea europaea*, PgAS(BAA33461.1): beta-Amyrin Synthase of *Panax ginseng*. The reported site-directed mutations are marked in red boxes.