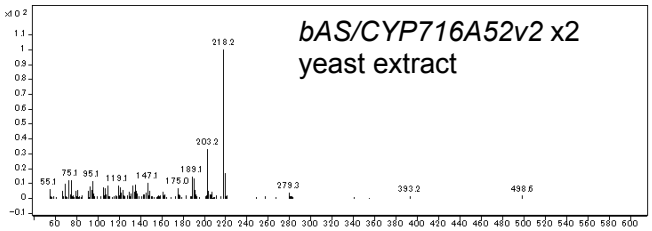
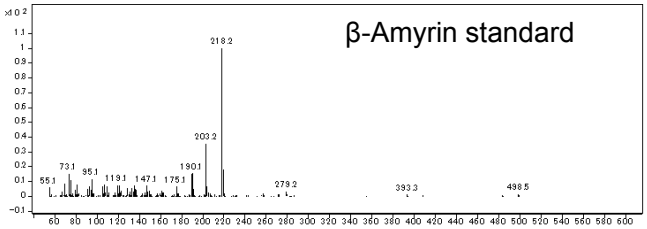
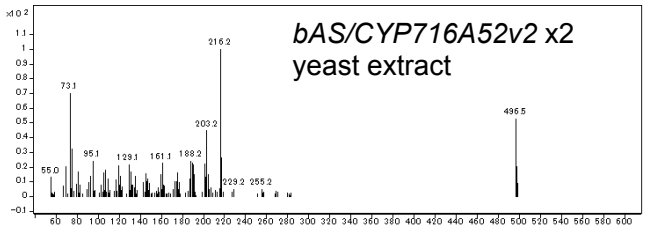
A

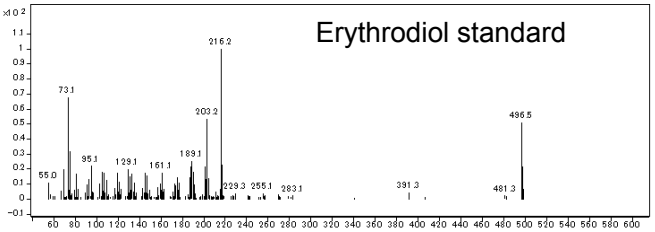
Peak 1 (Rt=29.04 min)



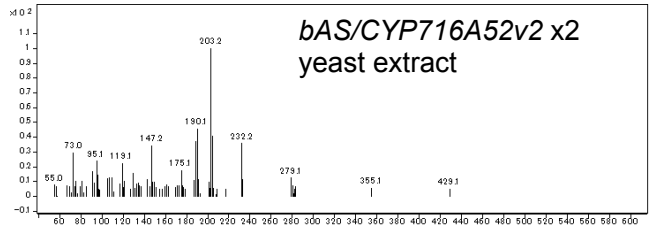


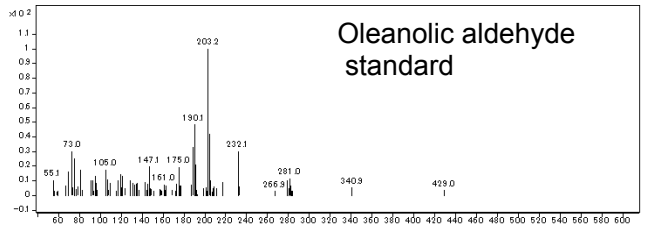
Peak 2 (Rt=34.44 min)



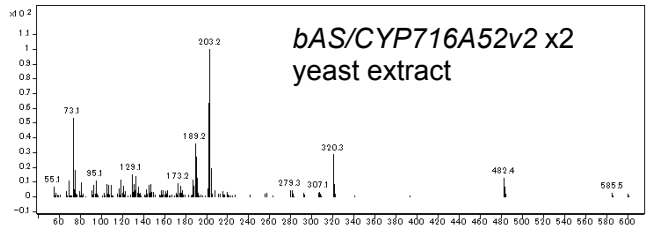


Peak 3 (Rt=35.79 min)





Peak 4 (Rt=36.57 min)



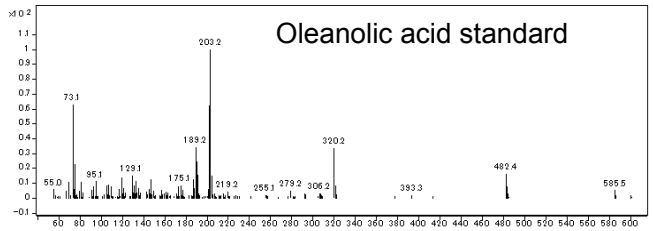
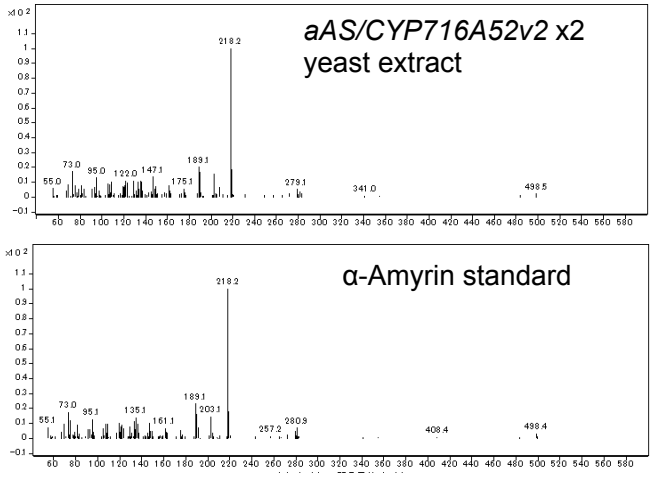


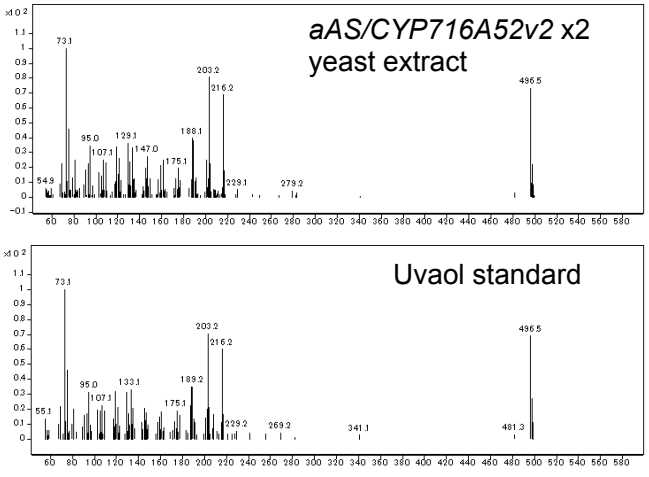
Figure S1. Mass spectra of target compounds and authentic standards shown in Figure 2. **A)** Mass spectra of β-amyrin derivatives. **B)** Mass spectra of α-amyrin derivatives. **C)** Mass spectra of lupeol derivatives.

B

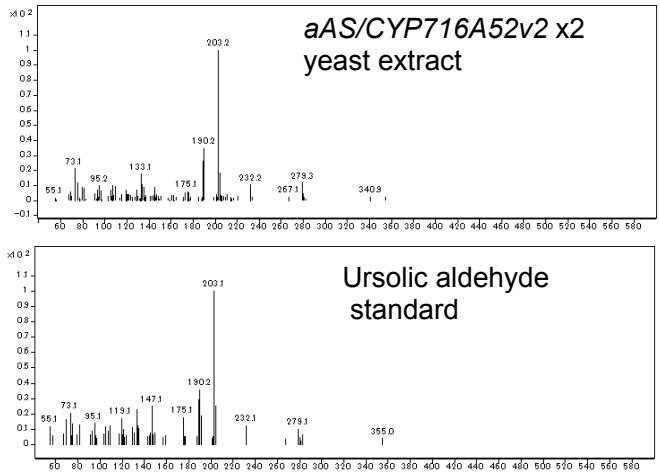
Peak 5 (Rt=29.93 min)



Peak 6 (Rt=35.63min)



Peak 7 (Rt=37.32 min)



Peak 8 (Rt=38.40 min)

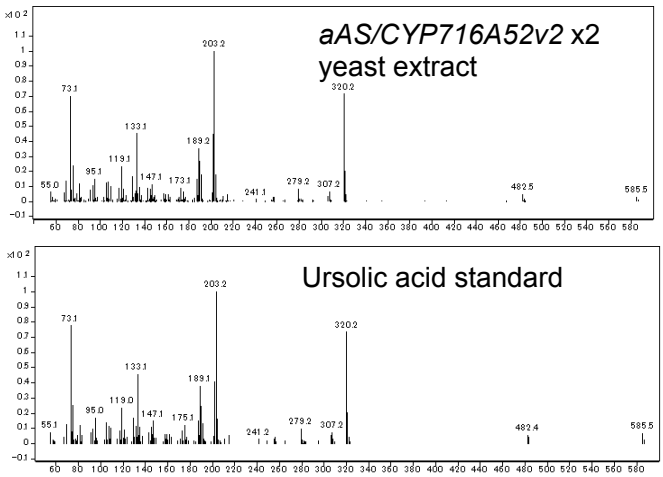
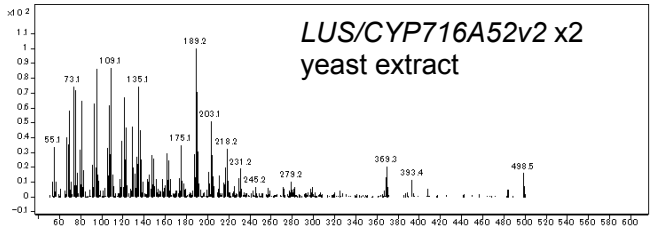
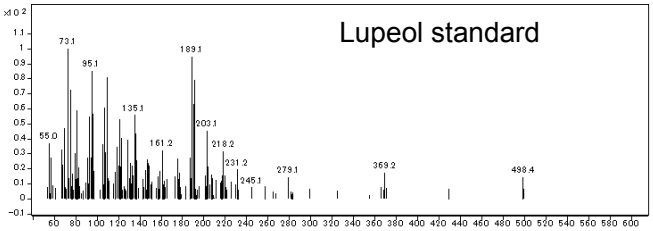


Figure S1. Cont.

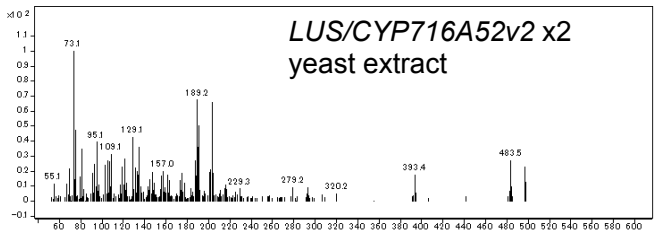
C

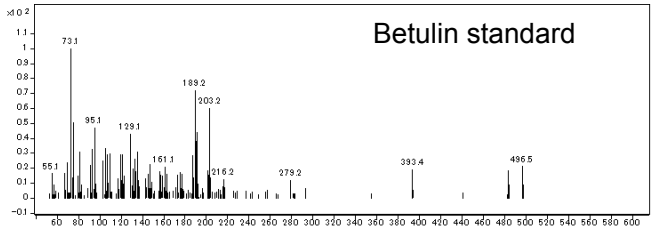
Peak 9 (Rt=30.21 min)



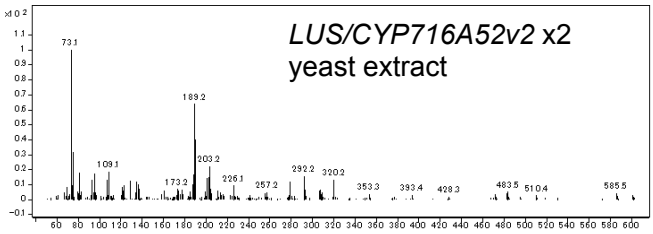


Peak 10 (Rt=36.33 min)





Peak 12 (Rt=37.29 min)



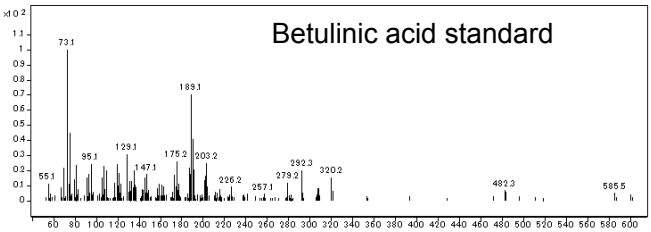
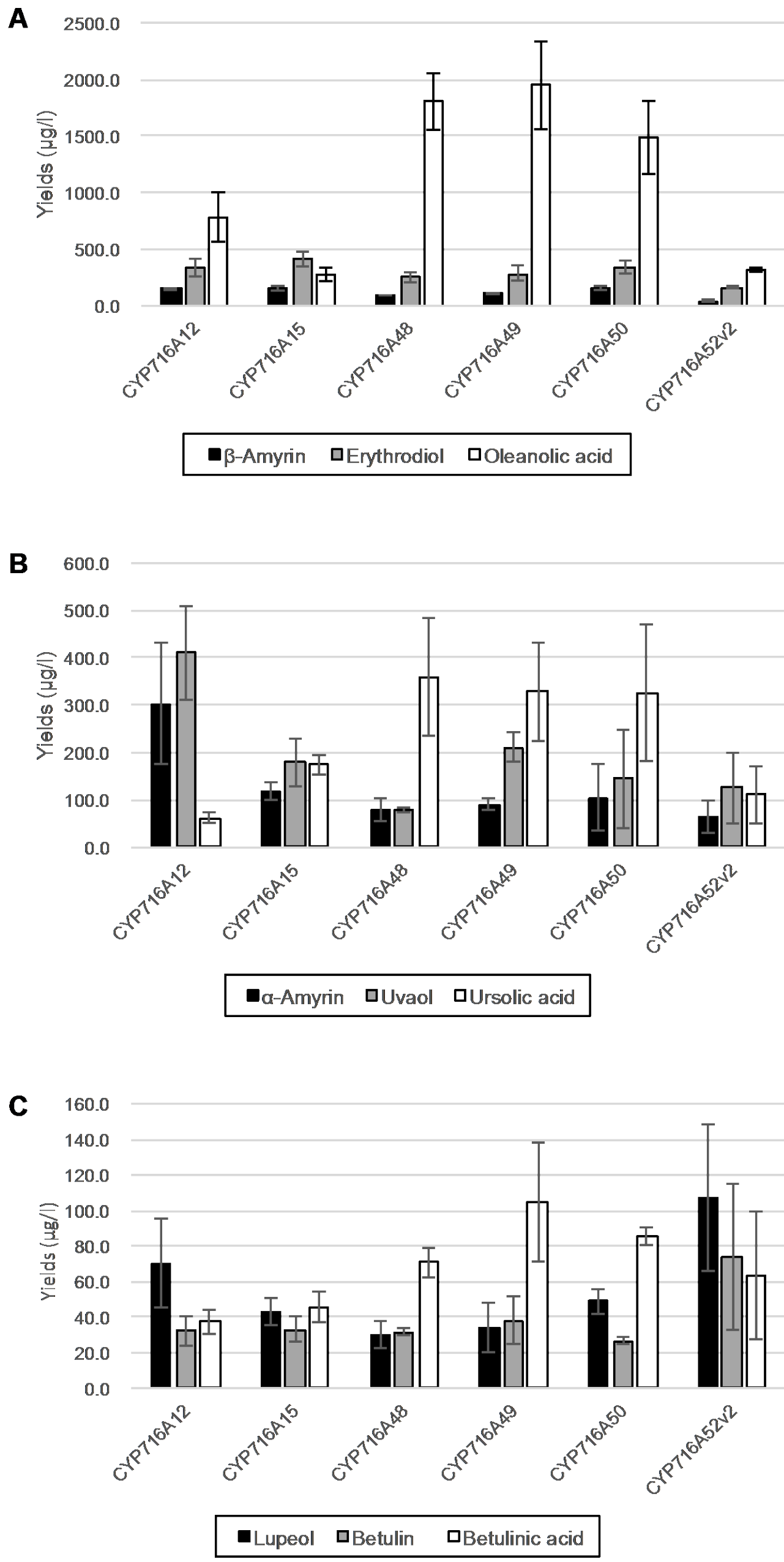
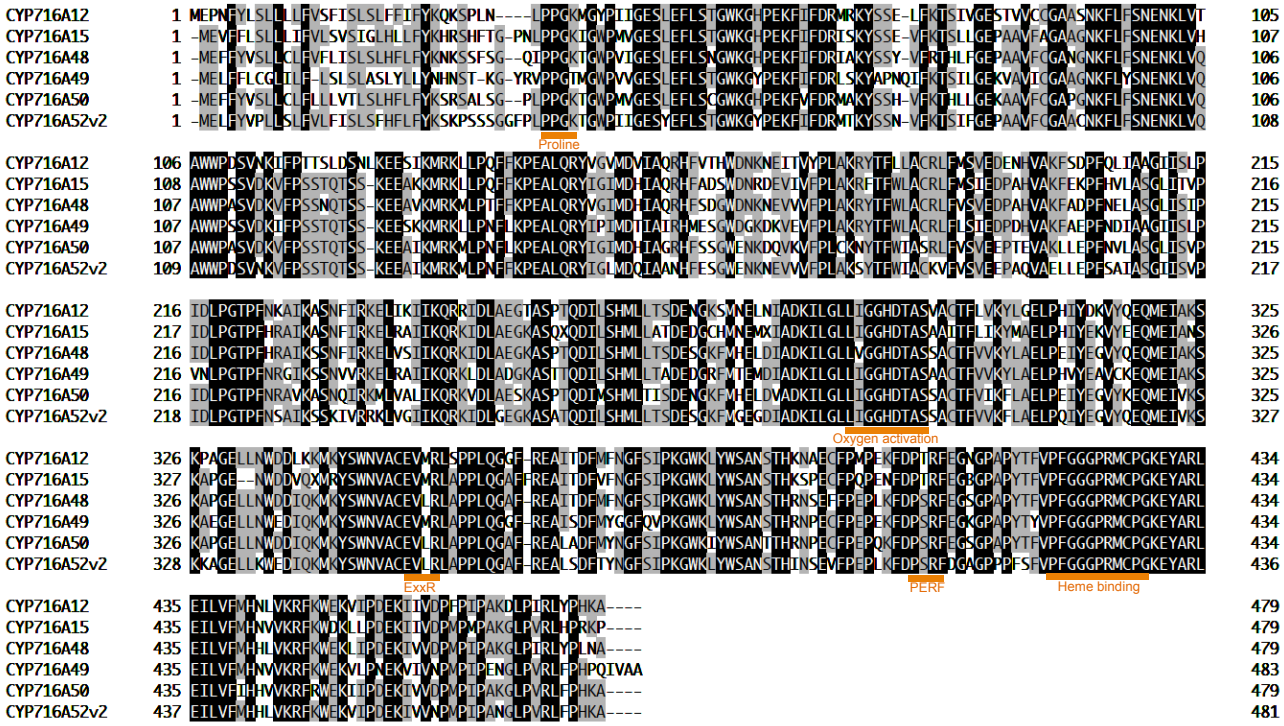


Figure S1. Cont.



Supplemental Figure S2. Absolute amounts of detected triterpenoids.
The amounts of (A) β-amyrin derivatives in *bAS/CPR/*CYP716A-expressing yeast extracts, (B) α-amyrin derivatives in *aAS/CPR/CYP716A*-expressing yeast extracts, and (C) lupeol derivatives in *LUS/CPR/CYP716A*-expressing yeast extracts are given. Quantitation and error bars correspond to the mean and standard deviation, respectively (n = 3).



Supplemental Figure S3. Protein alignment of CYP716A enzymes used in this study. Full primary sequences of CYP716s were aligned using GENETYX Mac (ver. 12.0). Common features found in plant P450s are highlighted.

Supplemental Table 1. List of primers used in this study

No.	Target seq	Sequence (5' to 3')
1	CYP716A48 F	CACCATGGAGTTCTTCTATGTCTCTCTTC
2	CYP716A48 R	TTAAGCATTAAGGGGATAAAGAC
3	CYP716A49 F	CACCATGGAGCTCTTCTTCCTTT
4	CYP716A49 R	TTAAGCAGCAACAATTTGAGG
5	CYP716A50 F1	CGCTCACAAACAATCTGGAA
6	CYP716A50 F2	CACCATGGAGTTTTTCTATGTCTCTTTG
7	CYP716A50 R	TTAGGCCTTGTGTGGAAAAA
8	CYP716A52v2 F1	CACCATGGAACTCTTCTATGTCCCTCT
9	CYP716A52v2 R	TTAGGCTTTGTGTGGAAATAGGC

The underlined sequences were added to facilitate the unidirectional cloning of the product into pENTR/D-TOPO (Invitrogen)

pplemental Table 2. Amino acid identities of the enzymes analyzed in this stu

	CYP716A12	CYP716A15	CYP716A48	CYP716A49	CYP716A50	CYP716A52v2
CYP716A12						
CYP716A15	74					
CYP716A48	77	79				
CYP716A49	71	74	74			
CYP716A50	71	75	83	73		
CYP716A52v2	73	70	81	73	79	