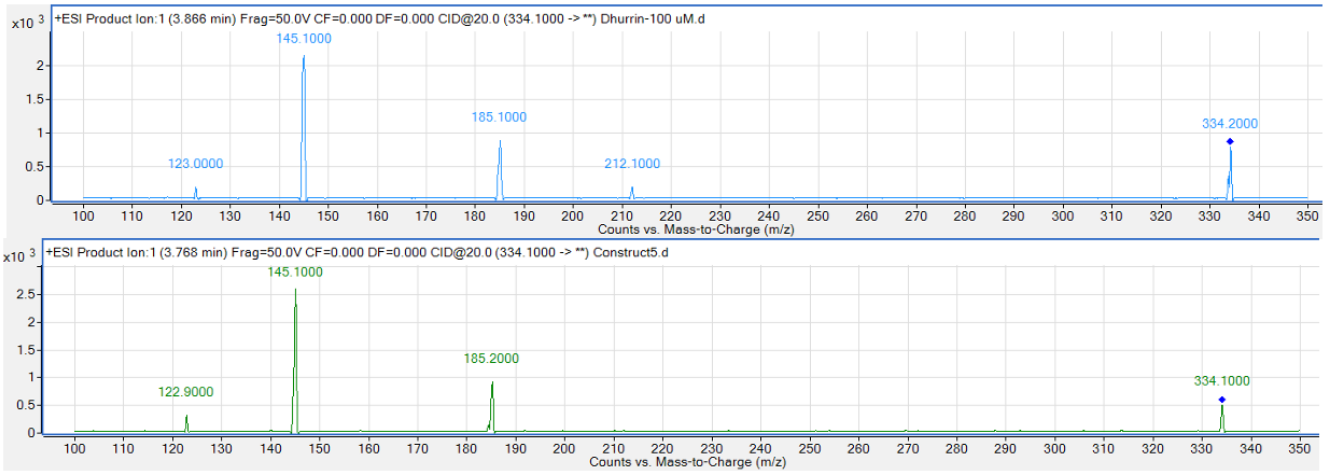

Figure S1: Mass spectrum of yeast-derived dhurrin and authentic standard. Above: Product ion mass spectrum of 100 µM dhurrin standard (Sigma). Below: Product ion mass spectrum of CSY1214 culture medium after 48 hours growth.

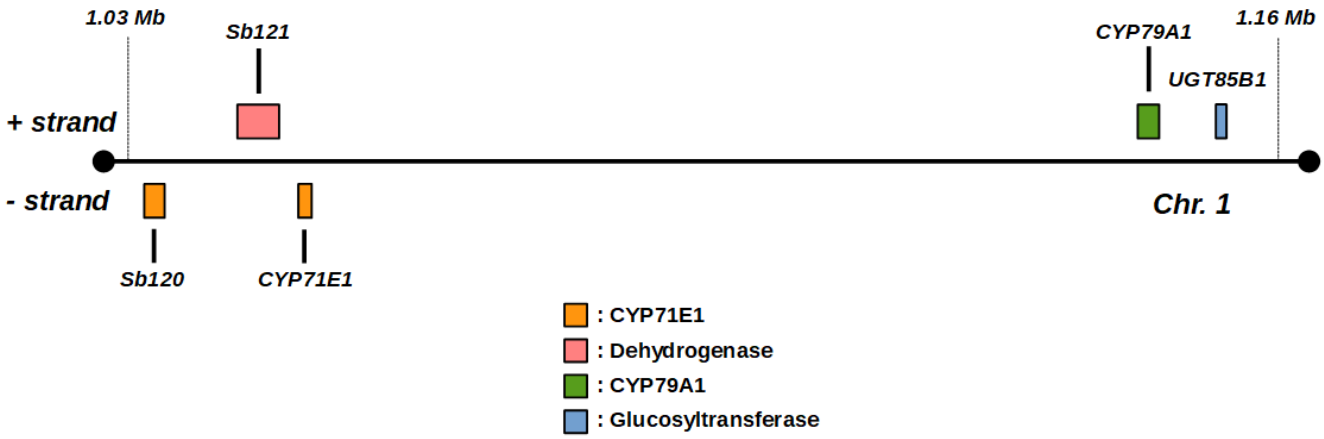

Figure S2: Illustration of the genomic context of the dhurrin pathway in *S. bicolor*. Enzymes in the dhurrin pathway and others characterized in this work are shown as they appear in the *S. bicolor* genome; color-coding indicates enzymes' predicted classification.

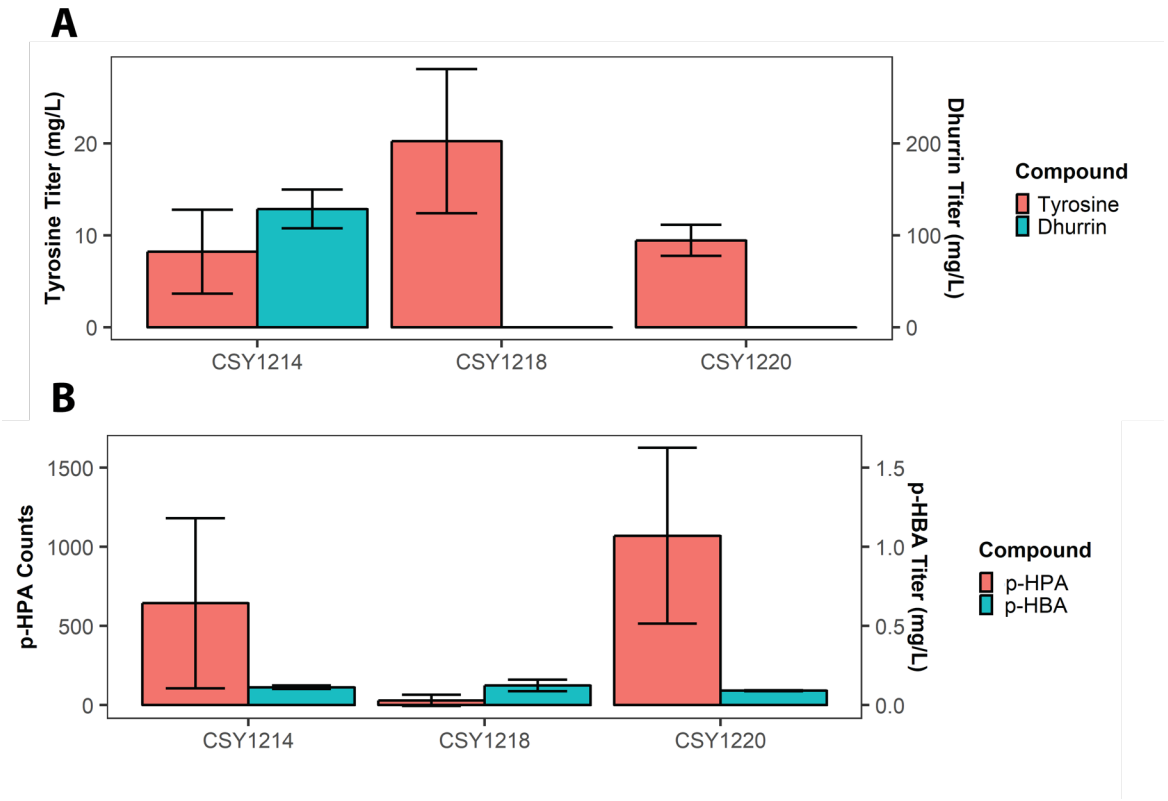

Figure S3: Effects of replacing CYP71E1 with Sb120 in a dhurrin pathway module. A: Tyrosine and dhurrin titers after 48 hours growth in plate culture. **B:** *p*-hydroxyphenylacetaldoxime ("p-HPA") ion counts and p-hydroxybenzylaldehyde ("p-HBA") titers after 48 hours growth in plate culture. CSY1214 is the complete dhurrin pathway expression strain, CSY1218 is the selection marker-matched control strain, and CSY1220 is identical to CSY1214, except that it expresses Sb120 in place of CYP71E1.

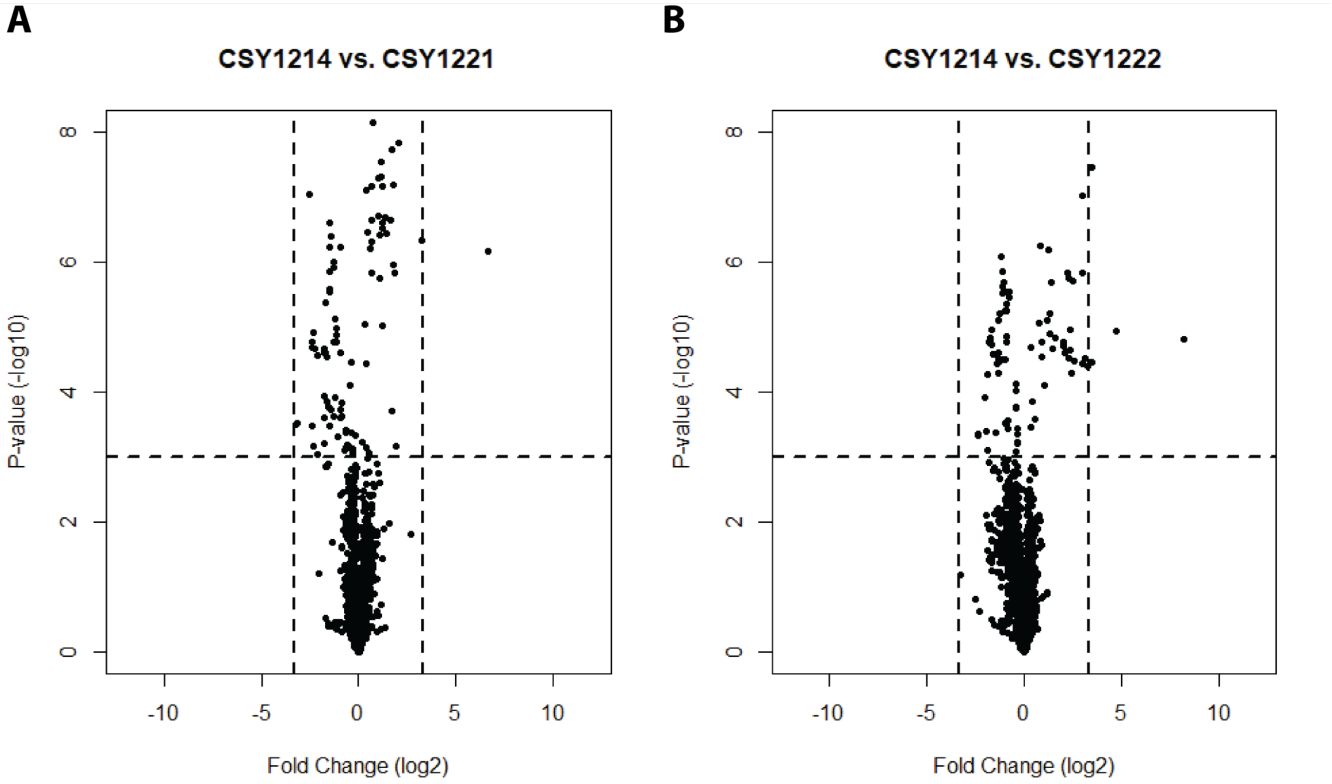

Figure S4: Untargeted metabolomic analysis of Sb120, Sb121 expression in dhurrin-producing strains. A: Volcano plot of fold changes and *t*-test significance values for detected ion abundances in qTOF analysis of CSY1221 (CSY1214 + Sb120 expression strain) culture supernatant, using CSY1214 (complete dhurrin pathway expression strain) as the reference for comparison. **B:** as in A, but analyzing CSY1222 (CSY1214 + Sb121 expression strain) with CSY1214 as the reference. Vertical lines indicate a tenfold change in mean ion abundance; horizontal lines mark the threshold of a Bonferonni-corrected significance test (original p-value threshold 0.05).

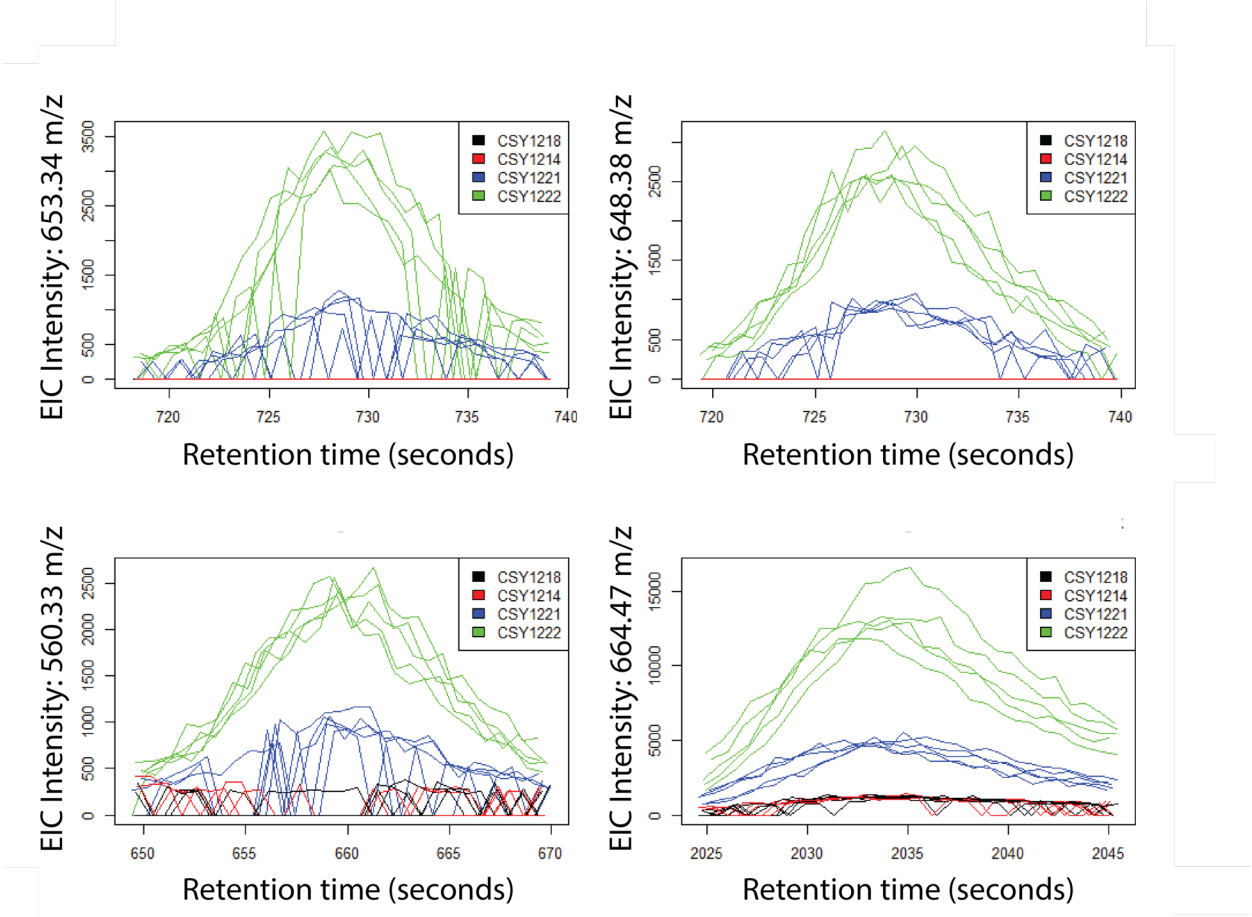

Figure S5: Chromatograms of candidate differentially produced ions in Sb120, Sb121 expression in dhurrin-producing strains. Extracted ion chromatograms are displayed for the four ions identified as potentially differentially expressed by untargeted metabolomics; strains tested are CSY1214 (dhurrin pathway expression strain), CSY1218 (selection marker-matched control strain with no *Sorghum* enzymes expressed), CSY1221 (CSY1214 + Sb120 expression strain), and CSY1222 (CSY1214 + Sb121 expression strain).

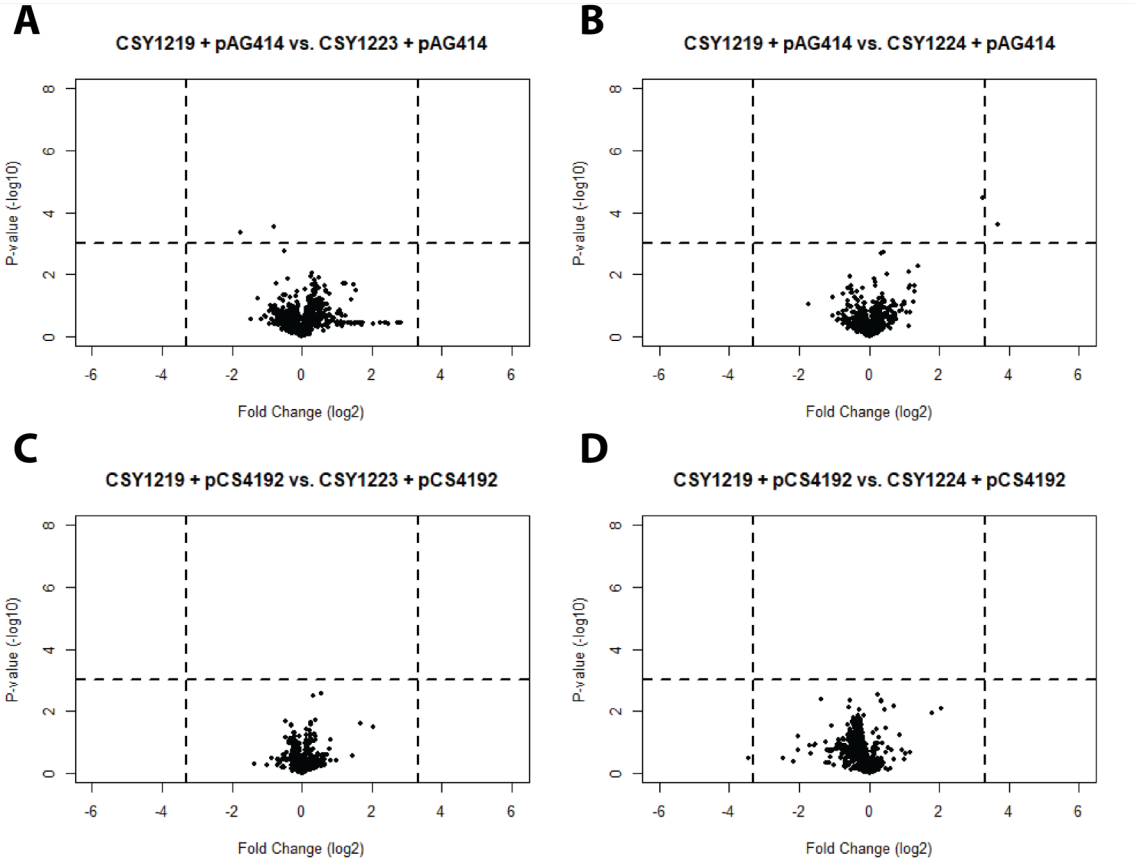

Figure S6: Untargeted metabolomic analysis of Sb120, Sb121 expression in partial dhurrin pathways. A: Volcano plot of fold changes and *t*-test significance values for detected ion abundances in qTOF analysis of CSY1223 (partial dhurrin pathway strain expressing SbCYP79A1 and Sb120) + pAG414 (selection marker control plasmid) culture supernatant, using CSY1219 (partial dhurrin pathway strain expressing SbCYP79A1) + pAG414 as the reference for comparison. **B:** Volcano plot of fold changes and *t*-test significance values for detected ion abundances in qTOF analysis of CSY1224 (partial dhurrin pathway strain expressing SbCYP79A1 and Sb121) + pAG414 culture supernatant, using CSY1219 + pAG414 as the reference for comparison. **C:** Volcano plot of fold changes and *t*-test significance values for detected ion abundances in qTOF analysis of CSY1223 + pCS4192 (plasmid expressing CYP71E1) culture supernatant, using CSY1219 + pCS4192 as the reference for comparison. D**:** Volcano plot of fold changes and *t*-test significance values for detected ion abundances in qTOF analysis of CSY1224 + pCS4192 culture supernatant, using CSY1219 + pCS4192 as the reference for comparison. Vertical lines indicate a tenfold change in mean ion abundance; horizontal lines mark the threshold of a Bonferonni-corrected significance test (original p-value threshold 0.05).

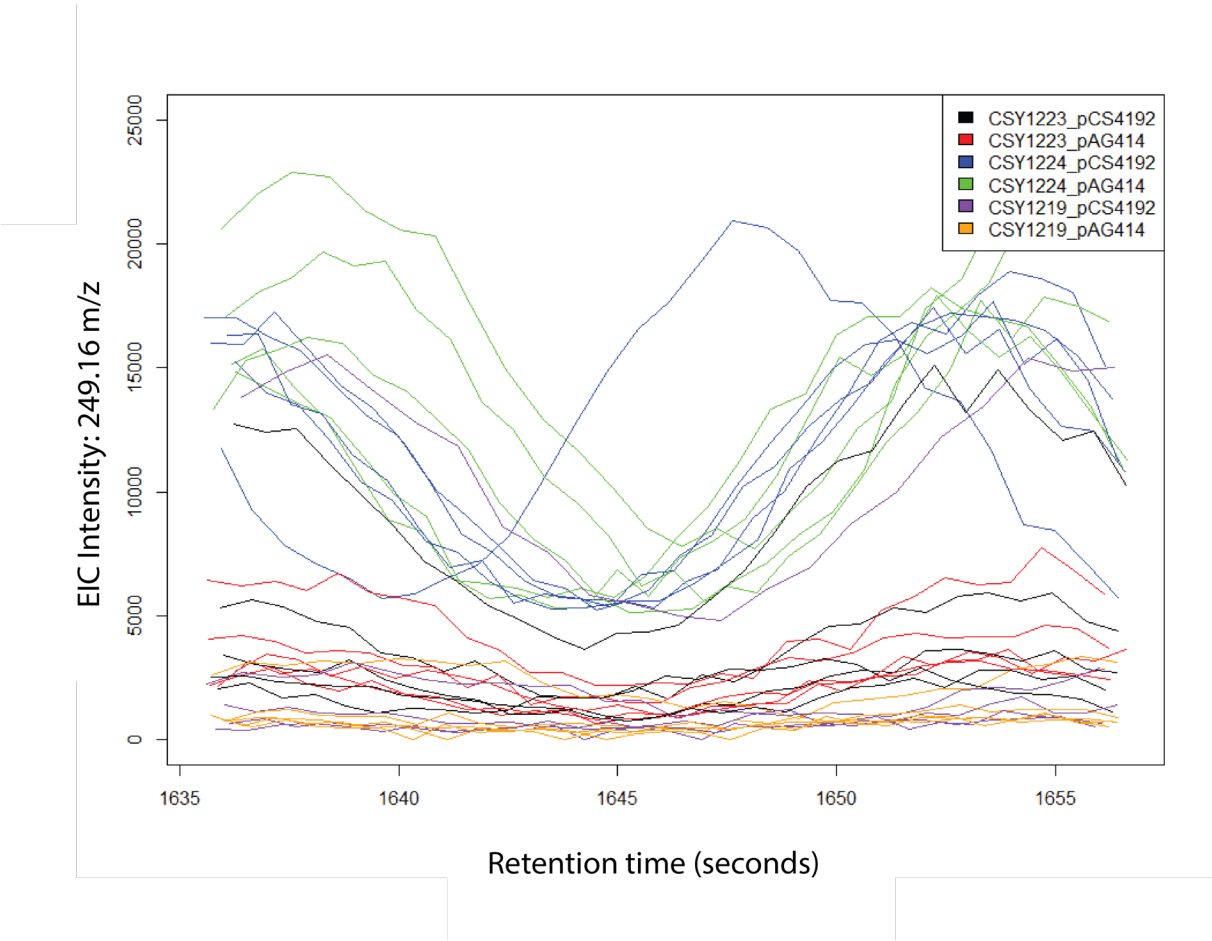

Figure S7: Chromatograms of the candidate differentially produced ion in Sb121 expression in partial dhurrin pathway strains. An extracted ion chromatogram is displayed for the ion identified as potentially differentially expressed by untargeted metabolomics. Strains tested are CSY1223 (partial dhurrin pathway strain expressing SbCYP79A1 and Sb120) + pCS4192 (plasmid expressing CYP71E1), CSY1223 + pAG414 (selection marker control plasmid), CSY1224 (partial dhurrin pathway strain expressing SbCYP79A1 and Sb121) + pCS4192, CSY1224 + pAG414, CSY1219 (partial dhurrin pathway strain expressing SbCYP79A1) + pCS4192, and CSY1219 + pAG414.

Table S1: *S. bicolor* gene sequences, codon-optimized for yeast expression.

Gene	Codon-Optimized Sequence
CYP71E1	ATGGACTCTATCGGTAAATGCATGGATCCACAATTGCCATTGGAAATTTC TGTTCCAGGTGTTCCAAGAGACAGAAGAAGAACTAACACTAATACCAA CACTAACCCAAGAGGTAGAGAACCAGCTTCTTCTTACATTATGCCAACT AACCATACCCAACAATCCGAAGCTAAACAAGCTGAATTGGAAGCTGCTC ATACTCCACATTCTTCTAGACCAGCTATTCCACAAGGTAAACAAGGTCAT GGTCAATTGACTATGGCTACTACTGCTACTCCACAATTATTGGGTGGTTC AGTTCCACAACAATGGCAAACTTGTTTGTTGGTTTTGTTGCCAGTTTTGT TGGTCAGTTACTACTTGTTGACCTCCAGATCTAGAAACAGATCAAGATCT GGTAAATTAGGTGGTGCTCCAAGATTGCCACCAGGTCCAGCTCAATTGC CAATTTTGGGTAACTTGCATTTGTTGGGTCCATTGCCACATAAGAACTTG AGAGAATTGGCTAGAAGATACGGTCCAGTTATGCAATTGAGATTGGGTA CTGTTCCAACCGTTGTTGTTTCTTCTGCTGAAGCTGCTAGAGAAGTTTTG AAGGTTCATGATGTTGATTGCTGTTCTAGACCTGCTTCTCCAGGTCCAAA AAGATTGTCTTACGATTTGAAGAACGTTGGTTTCGCTCCATATGGTGAAT ATTGGAGAGAAATGAGAAAGTTGTTCGCCTTGGAATTATTGTCCATGAG AAGAGTTAAGGCTGCTTGTTACGCTAGAGAACAAGAAATGGATAGATTG GTTGCCGATTTGGATAGAGCTGCTGCTTCTAAAGCTTCTATCGTTTTGAA CGATCATGTTTTCGCTTTGACCGATGGTATTATTGGTACTGTTGCTTTCGG TAACATCTACGCTTCTAAACAATTCGCCCACAAAGAAAGATTCCAACAC GTTTTGGATGACGCTATGGATATGATGGCTTCTTTTAGTGCTGAAGATTTC TTTCCAAATGCTGCTGGTAGATTGGCTGATAGATTGTCTGGTTTTTTGGC CAGAAGAGAAAGAATTTTCAACGAATTGGACGTTTTCTTCGAAAAGGTT ATCGATCAACATATGGATCCAGCTAGACCAGTTCCAGATAATGGTGGTGA TTTGGTTGATGTCTTGATCAACTTGTGCAAAGAACACGATGGTACTTTG AGATTCACTAGAGATCATGTTAAGGCCATTGTCTTGGATACTTTCATTGG TGCTATTGACACCTCCTCTGTTACTATTTTGTGGGCTATGTCTGAATTGAT GAGAAAGCCACAAGTTTTGAGAAAGGCTCAAGCTGAAGTTAGAGCTGC AGTTGGTGATGATAAGCCAAGAGTTAATTCTGAAGATGCTGCCAAGATT CCATACTTGAAGATGGTTGTCAAAGAAACCTTGAGATTGCATCCACCAG CTACTTTGTTAGTTCCTAGAGAAACTATGAGAGATACCACCATTTGTGGT TATGATGTTCCAGCTAACACCAGAGTTTTTGTTAATGCTTGGGCTATTGG TAGAGATCCTGCTTCATGGCCAGCTCCAGATGAATTCAATCCAGATAGAT TCGTTGGTTCCGATGTTGACTATTACGGTTCCCATTTTGAATTGATTCCAT TTGGTGCCGGTAGAAGAATTTGTCCAGGTTTAACTATGGGTGAAACTAA CGTTACTTTTACCTTGGCCAACTTGTTGTACTGTTATGATTGGGCTTTGCC AGGTGCTATGAAGCCTGAAGATGTTTCTATGGAAGAAACTGGTGCTTTG ACTTTCCACAGAAAAACTCCATTGGTTGTTGTCCCAACTAAGTACAAGA ATAGAAGAGCTGCTTAA
CYP79A1	ATGGCTACCATGGAAGTTGAAGCTGCTGCTGCTACTGTTTTGGCTGCTC CTTTGTTATCTTCTTCAGCCATTTTGAAGTTGTTGTTGTTCGTTGTCACCT TGTCTTATTTGGCTAGAGCTTTGAGAAGACCAAGAAAGTCTACTACCAA GTGTTCTTCTACTACTTGTGCTTCACCTCCAGCTGGTGTTGGTAATCCAC CATTGCCACCAGGTCCAGTTCCATGGCCAGTTGTTGGTAACTTGCCAGA AATGTTGTTGAACAAGCCAGCTTTCAGATGGATTCACCAAATGATGAGA GAAATGGGTACTGATATTGCCTGTGTTAAGTTGGGTGGTGTTCATGTTGT TTCTATTACCTGTCCAGAAATCGCCAGAGAAGTTTTGAGAAAACAAGAT

	GCCAACTTCATCTCTAGACCATTGACTTTTGCCTCTGAAACTTTTTCTGG TGGTTACAGAAATGCCGTTTTGTCTCCATATGGTGATCAATGGAAAAAG ATGAGAAGAGTCTTGACCTCCGAAATTATCTGTCCATCTAGACATGCTTG GTTGCATGATAAGAGAACTGATGAAGCTGATAACTTGACCAGATACGTT TACAACTTGGCTACAAAAGCTGCTACAGGTGATGTTGCTGTTGATGTTA GACATGTTGCTAGACATTACTGCGGTAACGTCATTAGAAGATTGATGTTC AATAGAAGATACTTCGGTGAACCACAAGCTGATGGTGGTCCAGGTCCAA TGGAAGTTTTACACATGGATGCTGTTTTCACCTCTTTGGGTTTGTTGTAC GCTTTCTGTGTTTCTGATTATTTGCCATGGTTGAGAGGTTTGGATTTGGAT GGTCACGAAAAGATTGTCAAAGAAGCTAACGTTGCCGTTAACAGATTAC ACGATACCGTTATTGATGATAGATGGAGACAATGGAAGAGTGGTGAAAG ACAAGAAATGGAAGATTTCTTGGATGTCTTGATCACCTTGAAAGATGCT CAAGGTAATCCTTTGTTGACCATCGAAGAAGTTAAGGCCCAATCTCAAG ATATTACTTTTGCTGCCGTTGACAATCCATCTAATGCTGTTGAATGGGCTT TGGCTGAAATGGTTAACAATCCAGAAGTTATGGCTAAGGCTATGGAAGA ATTGGATAGAGTTGTAGGTAGAGAAAGATTGGTCCAAGAATCCGATATT CCAAAGTTGAACTACGTTAAGGCCTGCATTAGAGAAGCCTTTAGATTGC ATCCAGTTGCTCCATTCAATGTTCCACATGTTGCATTGGCTGATACAACT ATTGCTGGTTATAGAGTTCCAAAGGGTTCCCATGTTATTTTGTCTAGAAC TGGTTTGGGTAGAAACCCAAGAGTTTGGGATGAACCATTGAGATTTTAC CCAGATAGACATTTGGCTACTGCTGCTTCAGATGTTGCTTTAACTGAAAA CGACTTGAGATTCATCTCATTCTCCACTGGTAGAAGAGGTTGTATTGCTG CTTCTTTGGGTACTGCTATGTCTGTTATGTTGTTCGGTAGATTATTGCAAG GTTTCACTTGGTCTAAACCAGCCGGTGTTGAAGCAGTTGATTTGTCTGA ATCTAAGTCCGATACTTTCATGGCTACCCCATTGGTTTTACATGCTGAAC CTAGATTGCCAGCTCACTTGTACCCATCTATTTCTATCTGA
UGT85B1	ATGGGTTCTAATGCTCCACCACCACCAACTCCACATGTTGTTTTGGTTCC ATTTCCAGGTCAAGGTCATGTTGCTCCATTGATGCAATTGGCTAGATTAT TGCATGCTAGAGGTGCTAGAGTTACTTTCGTTTACACTCAATACAACTAC AGAAGATTATTGAGAGCTAAGGGTGAAGCTGCTGTTAGACCACCAGCTA CTTCATCTGCTAGATTCAGAATTGAAGTTATCGACGACGGTTTGTCTTTG TCTGTTCCACAAAATGATGTTGGTGGTTTGGTTGACTCTTTGAGAAAGA ACTGTTTACATCCATTCAGAGCCTTGTTGAGAAGATTGGGTCAAGAAGT TGAAGGTCAAGACGCTCCACCAGTTACTTGTGTTGTTGGTGATGTTGTT ATGACTTTTGCTGCTGCTGCAGCTAGAGAAGCTGGTATTCCAGAAGTTC AATTTTTCACTGCTTCTGCTTGTGGTTTGTTGGGTTACTTGCATTATGGTG AATTGGTCGAAAGAGGTTTGGTCCCTTTTAGAGATGCTTCTTTGTTGGCT GATGATGATTACTTGGATACTCCATTGGAATGGGTTCCAGGTATGTCACA TATGAGATTGAGAGATATGCCAACCTTCTGTAGAACTACTGATCCAGATG ATGTTATGGTTTCTGCTACCTTGCAACAAATGGAATCTGCTGCTGGTTCT AAGGCTTTGATTTTGAATACCTTGTACGAATTGGAAAAGGATGTTGTTG ATGCCTTGGCTGCTTTTTTTCCACCAATCTATACTGTTGGTCCATTGGCTG AAGTTATTGCCTCTTCTGATTCTGCTTCAGCTGGTTTGGCTGCTATGGATA TTTCTATTTGGCAAGAAGATACCAGATGCTTGTCTTGGTTGGATGGTAAA CCAGCAGGTTCTGTTGTTTACGTTAATTTCGGTTCTATGGCTGTTATGAC AGCTGCTCAAGCAAGAGAATTTGCTTTGGGTTTGGCTTCTTGTGGTTCT CCATTTTTGTGGGTAAAAAGACCAGATGTCGTAGAAGGTGAAGAAGTTT TGTTGCCTGAAGCTTTGTTGGATGAAGTTGCAAGAGGTAGAGGTTTAGT TGTTCCATGGTGTCCACAAGCTGCAGTTTTGAAACATGCTGCTGTTGGT

	TTGTTCGTTTCTCATTGTGGTTGGAACTCATTATTGGAAGCTACAGCTGC TGGTCAACCAGTTTTGGCTTGGCCATGTCATGGTGAACAAACTACAAAC TGTAGACAATTGTGTGAAGTTTGGGGTAATGGTGCTCAATTGCCAAGAG AAGTAGAATCTGGTGCTGTTGCTAGATTGGTTAGAGAAATGATGGTCGG TGATTTGGGTAAAGAAAAGAGAGCTAAAGCTGCAGAATGGAAAGCTGC TGCCGAAGCTGCCGCTAGAAAAGGTGGTGCTTCTTGGAGAAATGTTGA AAGAGTTGTTAACGACTTGTTATTGGTCGGTGGTAAGCAATGA
Sobic.001G012000	ATGGCTGCTGTTCATGTTATAGTTGAAGGTGGTTCTTCTTCCTCTCCACA ACATCAACACCAACAATGGAGATTGACTTTGGTTTTGGCTGTTGTTGTTC CATTGGTTTTCTTGATTTTGTTGGCTAGAACTAGAACCAGAAGAAGAGC TAAATCTGGTTCCTCTACTGAAAAGGGTAGAAGATTATTGCATTTGCCAC CAGGTCCACCAACTTTGCCAATTTTGGGTAACTTGCATCAATTGGGTGCT TTGCCACATCAATCTTTGAGAGAATTAGCTAGAAGACACGGTCCAGTTA TGTTGTTGAGATTGGGTTCTGTTCCAACCTTGGTTGTTTCTTCTGCTGAA GCTGCTAGAGAAGTTATGAAAACTAGAGATGCTGATTGCTGCTCTAGAC CAGATACTCCAGGTGCTAGAAGATTGTCTTATGGTCATAAGGATGTTGCC TTTTCTCCATACGGTGATTATTGGAGAGATATGAGAAAGTTGTTCGTCGT CGAATTCTTGTCTGCAAGAAGAGTTAGAGCTGCAGATTATGCAAGAGAA GCTGAAGTTGATAAGTTGATCGGTAGATTGTCCTTGTCATCTTCAGCTGG TGGTAGACCAGTTAGATTGGAAGATCATATCTTCAGATTGATGGATGGTG TTATTGGTACTGTTGCTTTCGGTAATATCTACGGTACTGAACAATTCGCCC ATAAGAAACATTTCCACGATGTTTTGGATGAAGCTATGTCTGCTAAAGCT GGTTTTTCCGCTGAAGATTACTATCCAAATGCTGCCGGTAGATTATTAGAT AGATTGACTGGTGCTGCTGCCAGAAGAGAAAGAGTTTTTAGAGATTTGG ATGCCTTCTTCGATACCATCATCGATCAACATTTGGTTAATCCACCACCAT CTAGAGCTACTACACCAGGTGGTGCTGGTCATGGTCCAGATTTGATTGAT GTTTTTGTCGATTTGATGGAAATGGAAGAAAGACAAGTTGACGGTTCTT TCAGATTCACTAGAGATCACATTAAGGGTTTGTTGTCCAACGTTTTCACT GCTTCTGTTGATACTTCTTCCGTTACTATGGTTTGGGCTATGGCTGAATTG ATGAGAAGACCAGCTATGTTGAGAAAGGCTCAAGAAGAAGTAAGATCT GTTGTAGGTGGTGGTGGTAGAGAAACTGAAAGAGTTCATCCAGACGAC GTTGCTAAATTGAGATACTTGAAAGCCGTTGTCAAAGAAACCTTGAGAT TGCATCCAGCTGCACCTTTGTTGTTGCCAAGAGAAACTTTAAGACAAGT CTCCATTTGCGGTTATGATGTTCCAGCTAAAACCAGAGTTTTGGTTAACG CTTGGGCTATTGGTAGAGATCCAAGATCATGGGGTGATAGACCTGAAGA ATTTGATCCAGATAGATTCAATGACGGTGGTGGTGTTGGTTTTAATGGTA CTCATTTTGAATTGGTTCCATTCGGTGCTGGTAGAAGAATGTGTCCAGGT ATGGGTATGGGTGTTGCTACTGTTGAATTCACTTTGGCTAACTTGTTGTA CTGCTTCGATTGGGAATTGCCAGATGGTGTAGGTGTTGACGATGTTTCTA TGCAAGAAGCCGGTGGTTTGTCTGTTCATAAGAAAACCCCTTTGTTATTA GTCCCAACCAGATACAAGTGCAGATCAAGAGATCAATCCTTGGTTTCTA CCCAATGA
Sobic.001G012101	ATGGGTGATGCTTACGCTAAGAGAGTTTTGTTGACTGCTGCTGGTGATG CTGTTTCTAGAGGTATTGCTTCTACTTTGGCTAAACATGGTTGCAGATTG GTTTTGTTGGGTGATGAAGGTGCTTTGGCTGCTACTGCTGAAGAAGCTA GAAGATGTGGTGACGGCGGTGGTGGTGTTGAATTGGTTGGTTTGGATTT GGAAGCTTGTGGTGAAGCTGCTGCAGATGCTGCTGTTGATAGAGCTTGG AGATGTTTTGCTGGTTTAGATGCTTTCGTTAACTGCTACTCTTACGAAGG TGAAGTTCAAGATTGCTTGTCCATCTCTGAAGATGAATACAGAAAGACC

	ATCAAGGTTAACGTTGTTACTCCATGGTTTTTGATGAAGGCTATTGCCAA GAGATTCCAAGATACAAAATCTGGTGGTTCCATCGTTTTCTTGACCCAA ATTATTGGTGCTGAAAGAGGTTTGTATCCAGGTGCTGCTGCTTATGGTAC TTCTTTGGGTGCTATTCATCAATTGGTTAGATTGTCCGCTATGGAATTGGG TAAGCACAAGATTAGAGTTAACGCTGCTTGTAGAGGTTTACACTTGCAA GATAAGTTCCCAGTTTCCGTCGGTAAAGAAAAAGCTGAAAAAGCAACT GCTGTTGTTATGCCATTGAGAAGATGGTTGGATCCAGAAAAAGATTTGG CTTCTATGGTCTTGTACTTGGTTGGTGACGAATCTAGATATATGACCGGTA CTACCATTTTCGTTGATGGTGCTCAATCTATCGTTAGACCAAGAATGAGA TCCTTCATGTAA

Table S2: Plasmids used in this work.

Plasmid	Description	Ref.
pAG414GAL-ccdB	Centromeric TRP1, attR1-pGAL-ccdB-attR2	24
pAG414GPD-ccdB	Centromeric TRP1, attR1-pGPD-ccdB-attR2	24
pCS1056	Centromeric KanR, attR1-pGPD-ccdB-attR2	30
pCS1748	Centromeric URA3, pTEF1-GFP-tADH1; pTEF1-mCherry-tCYC1	25
pCS4182	pGPD-SbCYP71E1-tADH1	This work
pCS4183	pTEF1-SbCYP79A1-tCYC1	This work
pCS4184	pTPI1-SbUGT85B1-tSTE2	This work
pCS4185	pPGK1-Sobic.001G012000-tPHO5	This work
pCS4186	pPYK1-Sobic.001G012101-tMFA1	This work
pCS4187	Centromeric KanR, CRISPR construct for LEU2 locus insertion	This work
pCS4188	Centromeric KanR, CRISPR construct for LYP1 locus insertion	This work
pCS4189	Centromeric KanR, CRISPR construct for YBR197C locus insertion	This work
pCS4190	attL1-SbCYP71E1-tADH1-attL2	This work
pCS4191	Centromeric TRP1, pGAL1-attB1-SbCYP71E1-tADH1-attB2	This work
pCS4192	Centromeric TRP1, pGPD-attB1-SbCYP71E1-tADH1-attB2	This work

Table S3: Primers used to assemble *S. bicolor* promoter-gene-terminator cassette plasmids.

Final Plasmid	Backbone	Gene	Primer Binding Site	Primer Sequence
pCS4182	pCS2656	SbCYP71E1	Backbone Forward Primer	agaatagaagagctgcttaacgcgccacttctaaataagcgaatttct
pCS4182	pCS2656	SbCYP71E1	Backbone Reverse Primer	catttaccgatagagtccattgtataggatccactagttctagaatccgtcg
pCS4182	pCS2656	SbCYP71E1	Gene Forward Primer	gaactagtggatcctatacaatggactctatcggtaaatgcatggatcc
pCS4182	pCS2656	SbCYP71E1	Gene Reverse Primer	gcttatttagaagtggcgcgttaagcagctcttctattcttgtacttagttgggac
pCS4182	pCS2656	SbCYP71E1	Promoter Sequencing Primer	ccttctattaccttctgctctctctgatttgg
pCS4182	pCS2656	SbCYP71E1	Terminator Sequencing Primer	cctacaggaaagagttactcaagaataagaatttttcgt
pCS4182	pCS2656	SbCYP71E1	Gene Midpoint Sequencing Primer	gagagaattggctagaagatacggtccag
pCS4183	pCS2657	SbCYP79A1	Backbone Forward Primer	acccatctatttctatctgactcgagtcatgtattagttatgtcacgcttacat
pCS4183	pCS2657	SbCYP79A1	Backbone Reverse Primer	tcaacttccatggtagccattgtatacctaggaaaacttagattagattgctatgctttct
pCS4183	pCS2657	SbCYP79A1	Gene Forward Primer	Taagttttcctaggtatacaatggctaccatggaagttgaagctg
pCS4183	pCS2657	SbCYP79A1	Gene Reverse Primer	taactaatacatgactcgagtcagatagaaatagatgggtacaagtgagctgg
pCS4183	pCS2657	SbCYP79A1	Promoter Sequencing Primer	cgatgacctcccattgatatttaagttaataaacgg
pCS4183	pCS2657	SbCYP79A1	Terminator Sequencing Primer	cctagacttcaggttgtctaactccttcc
pCS4183	pCS2657	SbCYP79A1	Gene Midpoint Sequencing Primer	cagaaatgccgttttgtctccatatggtg
pCS4184	pCS2661	SbUGT85B1	Backbone Forward Primer	tggtcggtggtaagcaatgatcaaaatttacggctttgaaaaagtaatttc
pCS4184	pCS2661	SbUGT85B1	Backbone Reverse Primer	ggtggagcattagaacccattgtatattttagtttatgtatgtgtttttttgtagttatagat
pCS4184	pCS2661	SbUGT85B1	Gene Forward Primer	tacataaactaaaatatacaatgggttctaatgctccaccacca
pCS4184	pCS2661	SbUGT85B1	Gene Reverse Primer	ttcaaagccgtaaattttgatcattgcttaccaccgaccaataacaag

pCS4184	pCS2661	SbUGT85B1	Promoter Sequencing Primer	cagggaatataaagggcagcataatttaggagt
pCS4184	pCS2661	SbUGT85B1	Terminator Sequencing Primer	gaatgtggtgcatctgatgagcac
pCS4184	pCS2661	SbUGT85B1	Gene Midpoint Sequencing Primer	cttttgctgctgctgcagctagaga
pCS4185	pCS2663	Sobic.001G012000	Backbone Forward Primer	ccttggtttctacccaatgattttgtataactaaataatattggaaactaaatacgaatacc
pCS4185	pCS2663	Sobic.001G012000	Backbone Reverse Primer	ataacatgaacagcagccattgtatatatttgttgtaaaaagtagataattacttccttg
pCS4185	pCS2663	Sobic.001G012000	Gene Forward Primer	ttttacaacaaatatatacaatggctgctgttcatgttatagttgaagg
pCS4185	pCS2663	Sobic.001G012000	Gene Reverse Primer	tattatttagttatacaaaatcattgggtagaaaccaaggattgatctcttg
pCS4185	pCS2663	Sobic.001G012000	Promoter Sequencing Primer	ctaaccaaggggtggtttagtttagtagaac
pCS4185	pCS2663	Sobic.001G012000	Terminator Sequencing Primer	catccatttacctgtttcggatatctctgc
pCS4185	pCS2663	Sobic.001G012000	Gene Midpoint Sequencing Primer	gttgccttttctccatacggtgattattgg
pCS4186	pCS2664	Sobic.001G012101	Backbone Forward Primer	gaatgagatccttcatgtaagcccgactgataacaacagtgtagatgta
pCS4186	pCS2664	Sobic.001G012101	Backbone Reverse Primer	ttagcgtaagcatcacccattgtataatgttttatttgttttgattggtgtcttgtaaatagaaacaa
pCS4186	pCS2664	Sobic.001G012101	Gene Forward Primer	aacaaataaaacattatacaatgggtgatgcttacgctaagagagtttt
pCS4186	pCS2664	Sobic.001G012101	Gene Reverse Primer	actgttgttatcagtcgggcttacatgaaggatctcattcttggtctaacgatagattg
pCS4186	pCS2664	Sobic.001G012101	Promoter Sequencing Primer	gatttcctttccttcccatatgatgctaggt
pCS4186	pCS2664	Sobic.001G012101	Terminator Sequencing Primer	gggaaaacatgttgtttacggagaaatgaaaag
pCS4186	pCS2664	Sobic.001G012101	Gene Midpoint Sequencing Primer	tggatttggaagcttgtggtgaagc

Table S4: Other PCR primers used in this work.

Oligo Name	Sequence	Use
M13F	gtaaaacgacggccagtcttaagc	Sequence-verify pCS4182-8 and pCS4192
M13R-extended-seq-R	agagctgccaggaaacagctatgac	Sequence-verify pCS4182-8 and pCS4192
attB-Sb122gt-F	ggggacaagtttgtacaaaaaagcaggctcctatacaatggactctatcggtaaatgcatggatcc	Generate pCS4192
attB-Sb122gt-R	ggggaccactttgtacaagaaagctgggtcgatctgccggtagaggtgtggtc	Generate pCS4192
pCS1056-LYP1-F	gcttgaaccatttttttatgccaagcccttaaacgccgcgaatatgcatagcttcaaaatgtttctactccttttttactct	Integrate AtATR1 cassette from pCS1056 into LYP1 locus
pCS1056-LYP1-R	gccaaaaactgccgtgcggcacagtatgtatgtttttgagcaaattaaagccttcgagcgtcccaa	Integrate AtATR1 cassette from pCS1056 into LYP1 locus
LYP1-cPCR-F	gctcctattgccattggagaaaaccc	Colony PCR test of AtATR1-LYP1 integration
LYP1-cPCR-R	gcatgggtaatttgttgttgatgtgcg	Colony PCR test of AtATR1-LYP1 integration
pCS1056-cPCR-N_terminus	acttgagctgcttaaacaaatcggaagc	Colony PCR test of AtATR1-LYP1 integration
pCS1056-cPCR-C_terminus	gcagaggctatagttaagaaacttcaaaccg	Colony PCR test of AtATR1-LYP1 integration
pCS4183-LEU2-F	aggtatttactttggtaagagaaaggaagacaaattaaagccttcgagcgtccc	Integrate SbCYP79A1 cassette from pCS4183 into LEU2 locus
pCS4184-LEU2-F	aggtatttactttggtaagagaaaggaagacggaccttaatacattcagacact	Integrate SbUGT85B1 cassette from pCS4184 into LEU2 locus
pCS4184-pCS4183-R	gaaggctttaatttgtgaactcgtaaaagcaaaggtggt	Amplify SbUGT85B1 cassette from pCS4184 with homology to pCS4183 for DNA assembler
pCS4183-pCS4184-F	gcttttacgagttcacaaattaaagccttcgagcgtccc	Amplify SBCYP79A1 cassette from pCS4183 with homology to pCS4184 for DNA assembler
pCS4183-pCS1748-R	aataatggtttcttaatatgcatagcttcaaaatgtttc	Amplify SBCYP79A1 cassette from pCS4183 with

		homology to pCS1748 for DNA assembler
pCS1748-pCS4183-F	tgaagctatgcatattaagaaaccattattatcatgaca	Amplify URA3 cassette from pCS1748 with homology to pCS4183 for DNA assembler
pCS1748-LEU2-R	ttcactatcccaagcgacaccatcaccatccactcaaccctatctcggtctatt	Integrate URA3 cassette from pCS1748 into LEU2 locus
pCS1748-pCS4182-R	ctctaccggcagatccactcaaccctatctcggtctatt	Amplify URA3 cassette from pCS1748 with homology to pCS4182 for DNA assembler
pCS4182-pCS1748-F	agatagggttgagtggatctgccggtagaggtgtggtca	Amplify SbCYP71E1 cassette from pCS4182 with homology to pCS1748 for DNA assembler
pCS1748-pCS4185-R	aaaaggaagagtgaacactcaaccctatctcggtctatt	Amplify URA3 cassette from pCS1748 with homology to pCS4185 for DNA assembler
pCS4185-pCS1748-F	agatagggttgagtgttcactcttccttttttcatttt	Amplify Sobic.001G012000 cassette from pCS4185 with homology to pCS1748 for DNA assembler
pCS4182-LEU2-R	ttcactatcccaagcgacaccatcaccatcgagctcttcgagtttatcattatc	Integrate SbCYP71E1 cassette from pCS4182 into LEU2 locus
pCS4185-LEU2-R	ttcactatcccaagcgacaccatcaccatcaggcatttgcaagaattactcgtg	Integrate Sobic.001G012000 cassette from pCS4185 into LEU2 locus
LEU2-cPCR-F	caagccacaatttgctaaaggtactgacttc	Colony PCR test of dhurrin cluster-LEU2 integration
LEU2-cPCR-R	ggccaaaacattagctttatccaaggacc	Colony PCR test of dhurrin cluster-LEU2 integration
pCS1748-cPCR-C	tcgcgttaaatttttgttaaatcagctcatttttaacc	Colony PCR test of dhurrin cluster-LEU2 integration
pCS1748-cPCR-N	gaattacaatcaataccgacgaaagggcc	Colony PCR test of dhurrin cluster-LEU2 integration
pCS4182-cPCR-N (pGPD)	gtgtcatcatttactccaggcaggttg	Colony PCR test of dhurrin cluster-LEU2 integration
pCS4182-cPCR-C	agcctgaagatgtttctatggaagaaactgg	Colony PCR test of dhurrin cluster-LEU2 integration
pCS4183-cPCR-N	gtagtagaagaacacttggtagtagactttcttgg	Colony PCR test of dhurrin cluster-LEU2 integration

pCS4183-cPCR-C	gaagcagttgatttgtctgaatctaagtccg	Colony PCR test of dhurrin cluster-LEU2 integration
pCS4184-cPCR-N	tggaaccaaaacaacatgtggagttgg	Colony PCR test of dhurrin cluster-LEU2 integration
pCS4184-cPCR-C	gaaaagagagctaaagctgcagaatggaaag	Colony PCR test of dhurrin cluster-LEU2 integration
pCS4185-cPCR-N	ccaaagtcaatctccattgttggtgttgatg	Colony PCR test of dhurrin cluster-LEU2 integration, pCS4185-YBR197C integration
pCS4185-cPCR-C	gaattgccagatggtgtaggtgttgac	Colony PCR test of dhurrin cluster-LEU2 integration, pCS4185-YBR197C integration
pCS4186-cPCR-N (pPYK1)	ccatttagcttgccggaaaaactttcgg	Colony PCR test of pCS4186-YBR197C integration
pCS4186-cPCR-C	ggtgctcaatctatcgttagaccaagaatgag	Colony PCR test of pCS4186-YBR197C integration
pCS4185-YBR197C-F	ccacgcctccagcactaaacccccttaacgaccagcattagaggcatttgcaagaattactcgtgagtaagg	Integrate Sobic.001G012000 cassette from pCS4185 into YBR197C locus
pCS4185-YBR197C-R	gctaccatttacgcttctttcgtatgcagtattacccctattcactcttccttttttcatttttgcgatgcc	Integrate Sobic.001G012000 cassette from pCS4185 into YBR197C locus
pCS4186-YBR197C-F	ccacgcctccagcactaaacccccttaacgaccagcattagaaaatagccgccatgaccccgg	Integrate Sobic.001G012101 cassette from pCS4186 into YBR197C locus
pCS4186-YBR197C-R	gctaccatttacgcttctttcgtatgcagtattacccctagaattctcttaggattcgattcacattcatctttttttagc	Integrate Sobic.001G012101 cassette from pCS4186 into YBR197C locus
YBR197C-cPCR-F	tggttcaattaaaggcaaagagcgacaaag	Colony PCR test of integrations into YBR197C
YBR197C-cPCR-R	gtatcgatttcagaccatgatacgtcttgc	Colony PCR test of integrations into YBR197C

Table S5: Yeast strains used in this work.

Strain	Genotype
CEN.PK2	MATα URA3-52; TRP1-289; LEU2-3/112; HIS3Δ1; MAL2-8C; SUC2
CSY1212	CEN.PK2 LEU2Δ::pTPI1-SbUGT85B1-tSTE2, pTEF1-CYP79A1-tCYC1, URA3, pGPD-CYP71E1-tADH1
CSY1213	CSY1212 LYP1Δ::pTEF1-AtATR1-tCYC1
CSY1214	CSY1213 YBL059WΔ::ARO4 Q166K, ARO7 T226I, hphNTI, pGPD-TKL1-tADH1
CSY1215	CEN.PK2 YBL059WΔ::ARO4 Q166K, ARO7 T226I, hphNTI, pGPD-TKL1-tADH1
CSY1216	CSY1215 LEU2Δ::pTPI1-SbUGT85B1-tSTE2, pTEF1-CYP79A1-tCYC1, URA3, pGPD-CYP71E1-tADH1
CSY1217	CSY1215 LYP1Δ::pTEF1-AtATR1-tCYC1
CSY1218	CSY1217 LEU2Δ::URA3
CSY1219	CSY1217 LEU2Δ::pTEF1-CYP79A1-tCYC1
CSY1220	CSY1217 LEU2Δ::pTPI1-SbUGT85B1-tSTE2, pTEF1-CYP79A1-tCYC1, URA3, pPGK1-Sobic.001G012000-tPHO5
CSY1221	CSY1214 YBR197CΔ::pPGK1-Sobic.001G012000-tPHO5
CSY1222	CSY1214 YBR197CΔ::pPYK1-Sobic.001G012101-tMFA1
CSY1223	CSY1219 YBR197CΔ::pPGK1-Sobic.001G012000-tPHO5
CSY1224	CSY1219 YBR197CΔ::pPYK1-Sobic.001G012101-tMFA1