Supporting Information

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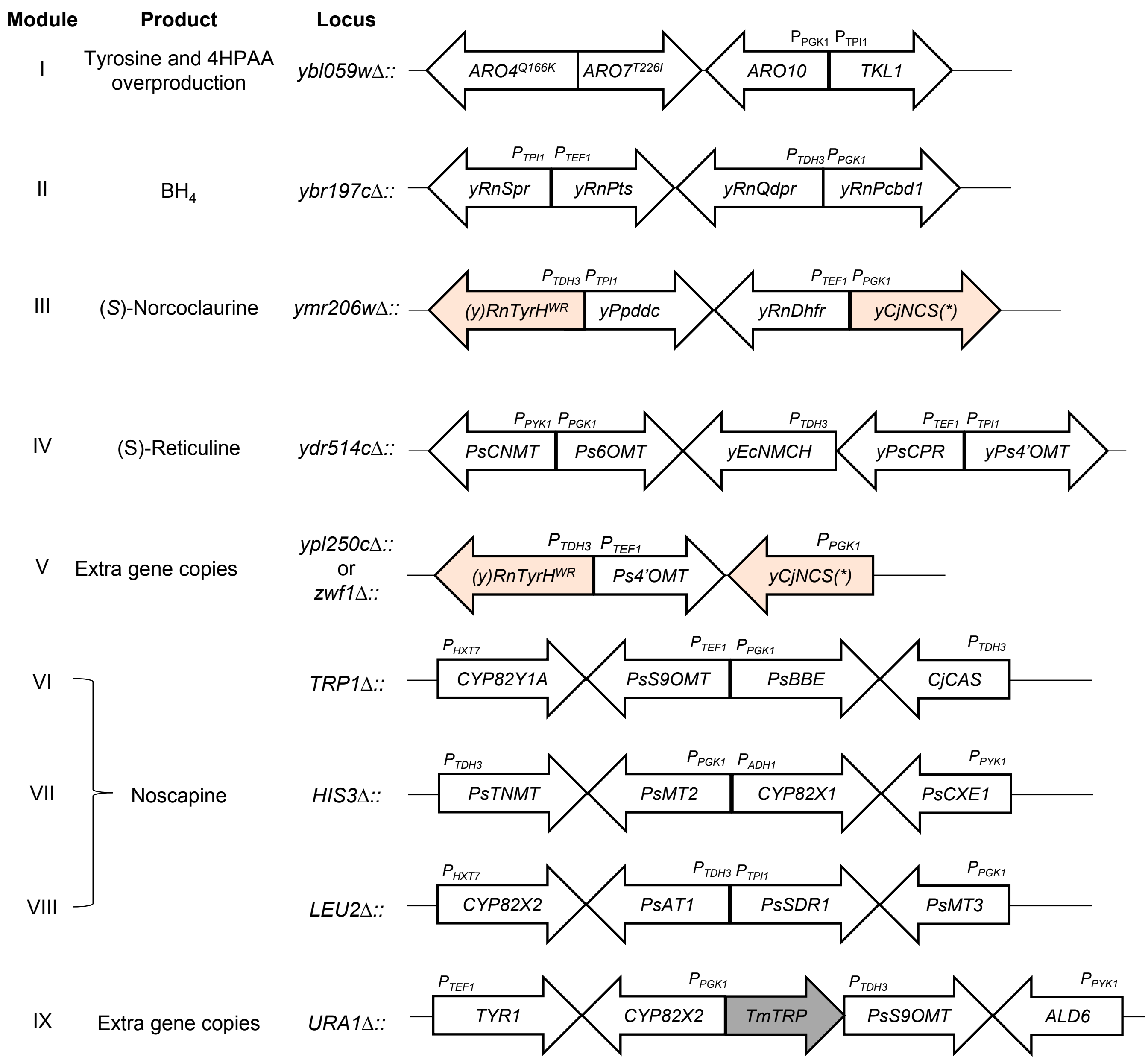


Fig. S1. Genetic design of pathway modules for yeast strain construction. Modules are designed to integrate into one of six chromosomal loci in the yeast genome. White block arrows indicate gene expression cassettes with a promoter, coding sequence, and terminator. Gray block arrows indicate loxP flanked selection markers. Orange block arrows indicate gene expression cassettes that encode for genes applied for gene modification during optimization process. For nonyeast enzymes, codon optimization is indicated by a leading "y," and the source genus and species are designated by the two letters immediately preceding the gene symbol. Asterisk indicates the N-terminal modification of CjNCS. See Tables S1 and S2 for expression cassette, strain number, genome assembly primers, and synthetic coding sequences.

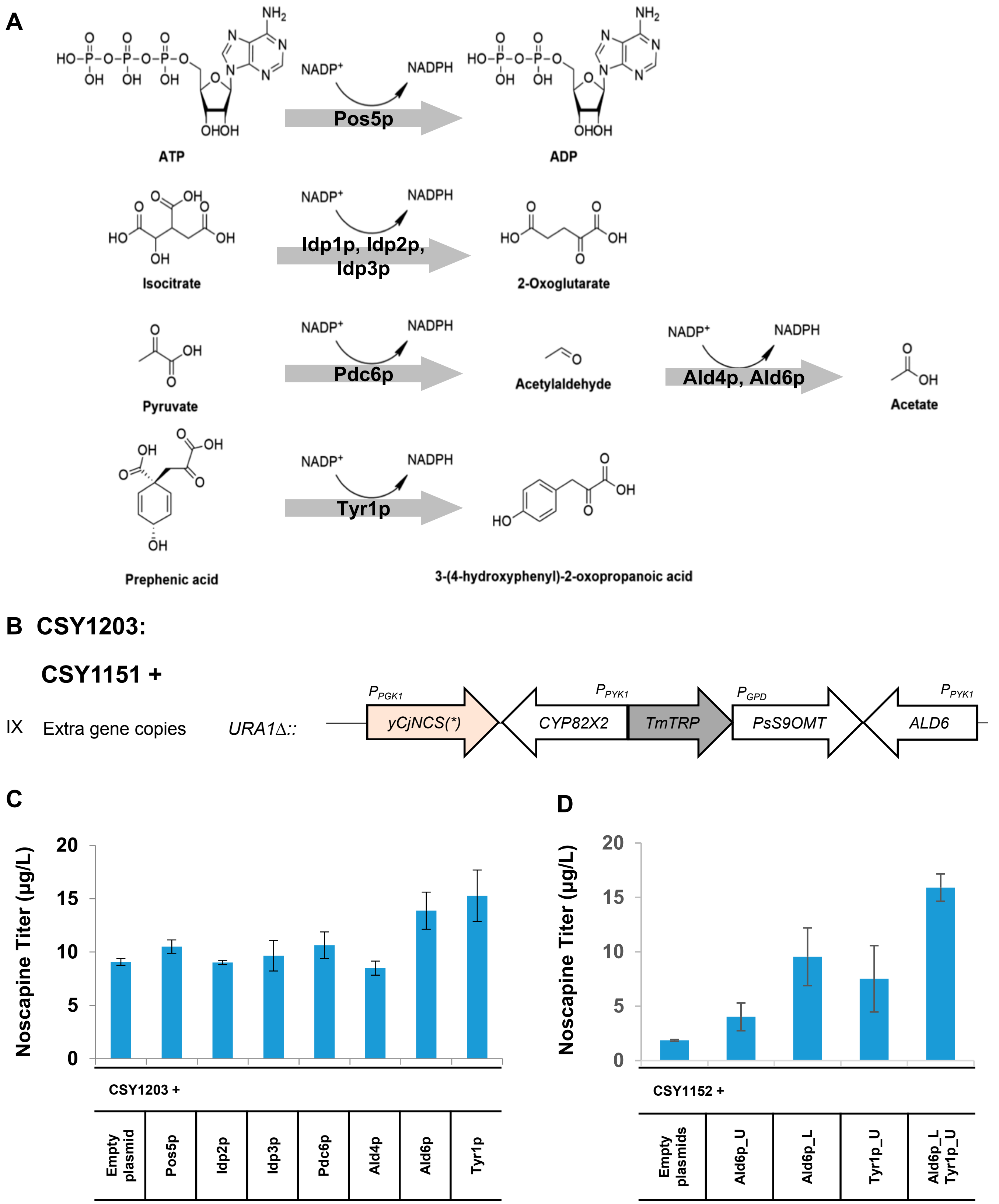


Fig. S2. Turning NADPH supply for higher noscapine titer. (*A*) Regeneration mechanism of selected *S. cerevisiae* endogenous enzymes: Pos5p, mitochondrial NADH kinase; Idp1p, Idp2p, and Idp3p, isocitrate dehydrogenase; Pdc6p, pyruvate decarboxylase; Ald4p and Ald6p, aldehyde dehydrogenase; Tyr1p, prephenate dehydrogenase. (*B*) Genotype of CSY1203. (*C*) Noscapine titer from CSY1203 expressing different NADPH regeneration enzymes. (*D*) Noscapine titer from CSY1152 expressing Ald6p, Tyr1p, or Ald6p and Tyr1p together. *S. cerevisiae* endogenous genes were expressed from a low-copy plasmid in CSY1203 and CSY1152. ALD6 was expressed from a low-copy plasmid harboring the selection marker LEU (Ald6p_L) or URA (Ald6p_U). TYR1 was expressed from a low-copy plasmid harboring the selection marker URA (Tyr1_U) (Table S1). All strains were cultured in SD with 2% dextrose, in the presence of 10 mM ascorbic acid at 25 °C for 72 h. Error bars represent SD of three biological replicates.

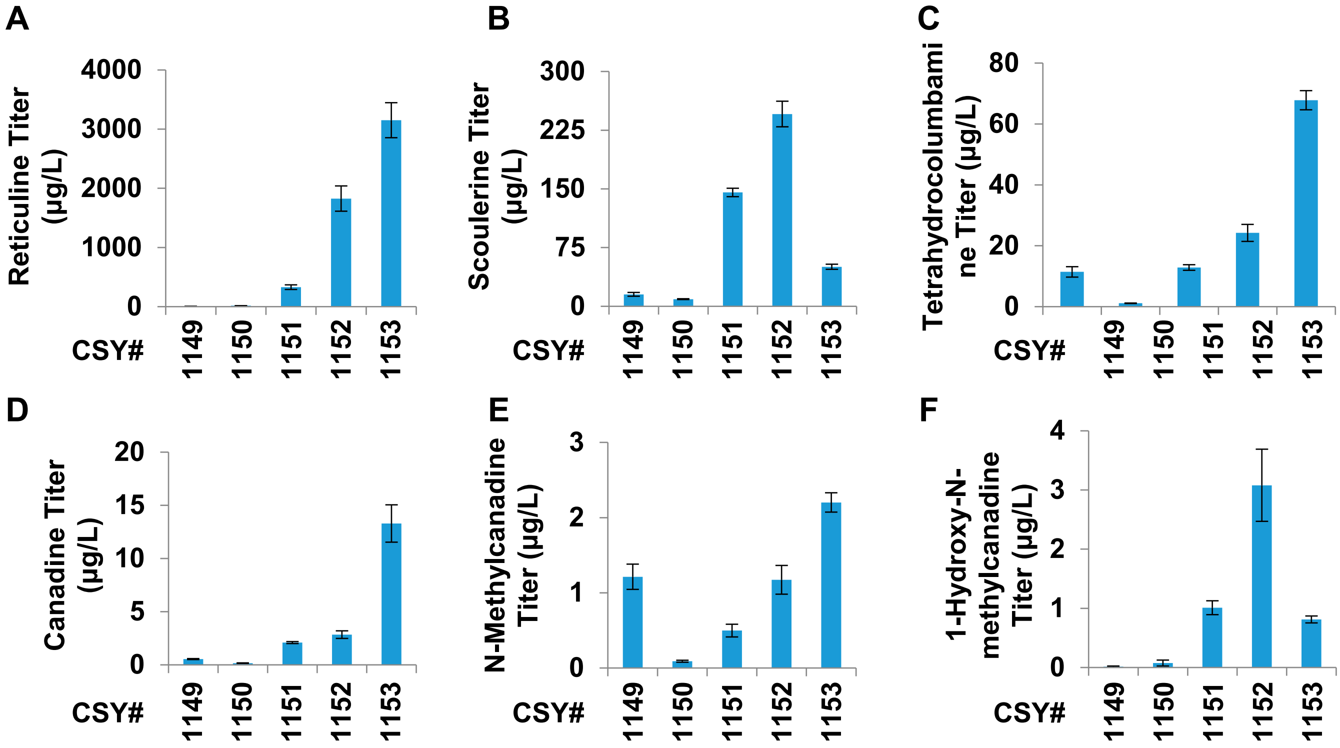


Fig. S3. Titer of (*A*) reticuline, (*B*) scoulerine, (*C*) tetrahydrocolumbamine, (*D*) canadine, (*E*) *N*-methylcanadine, and (*F*) 1-hydroxy-*N*-methylcanadine from engineered yeast strains. All strains were cultured in SD with 2% dextrose, in the presence of 10 mM ascorbic acid at 25 °C for 72 h. Error bars represent SD of three biological replicates.

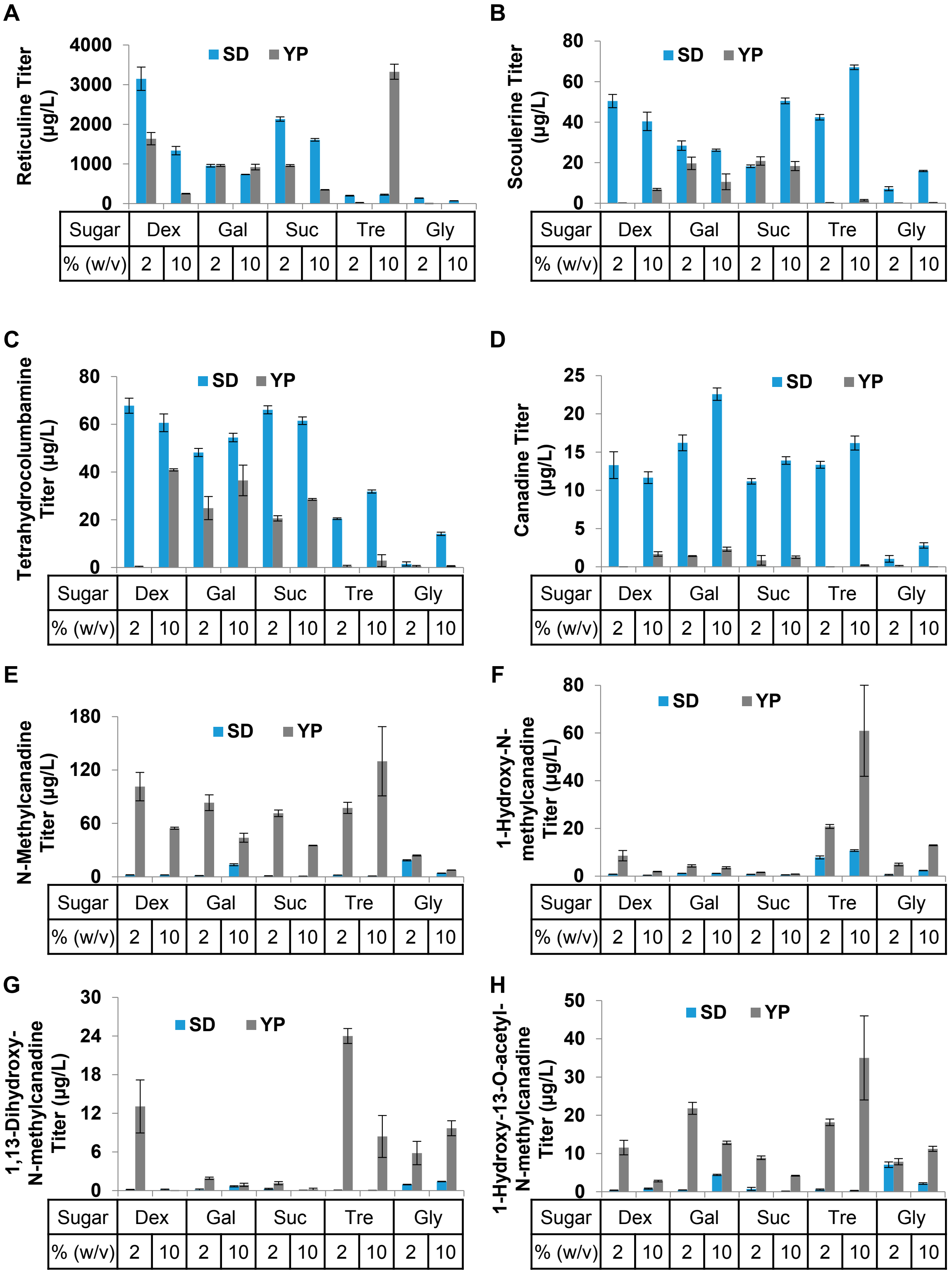


Fig. S4. Titer of (*A*) reticuline, (*B*) scoulerine, (*C*) tetrahydrocolumbamine, (*D*) canadine, (*E*) *N*-methylcanadine, (*F*) 1-hydroxy-*N*-methylcanadine, (*G*) 1,13-dihydroxy-*N*-methylcanadine, and (*H*) 1,hydroxyl-13-O-acetyl-*N*-methylcanadine from engineered yeast strains in different base medium and carbon sources. Base media are SD (synthetic defined) and YP (yeast extract and peptone). Carbon sources are 2% or 10% of dextrose, galactose, sucrose, trehalose, and glycerol. All noscapine titers were obtained from CSY1153 cultured at 25 °C for 72 h in the presence of 10 mM ascorbic acid. Error bars represent SD of at least three biological replicates.

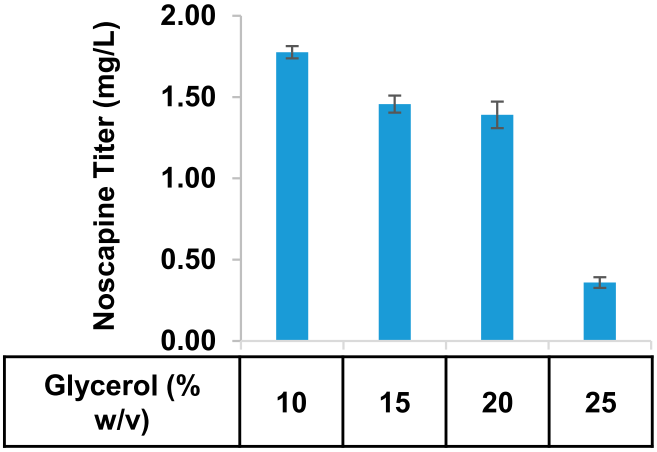


Fig. S5. Noscapine titers from CSY1153 under high glycerol concentrations. The 10%, 15%, 20%, or 25% glycerol were supplemented into YP with 2% dextrose. The strain was cultured at 25 °C for 72 h in the indicated media composition with 10 mM ascorbic acid. Error bars represent SD of three biological replicates.

Table S1. Engineered *S. cerevisiae* strains and yeast expression constructs used in this study

Strain	Plasmid no.	Fig.	*x*-axis label	Plasmid-based constructs	Genome modifications
CSY1060					ybr197cΔ::P_{TPI1}-yRnSpr-T_{STE2}, P_{TEF1}-yRnPts-T_{CYC1}, P_{GPD}-yRnQdpr-T_{ADH1}, P_{PGK1}-yRnPcbd1-T_{PHO5}; ydr514cΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{TDH3}- yEcNMCH-T_{ADH1}, P_{TEF1}-yPsCPR-T_{CYC1}, P_{TPI1}-yPs4′OMT-T_{STE2}; ymr206wΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TPI1}-yPpddc-T_{STE2}, P_{TEF1}-yRnDhfr-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}; ybl059wΔ::ARO4^{Q166K}, ARO7^{T226I}, P_{TEF1}-ARO10- T_{CYC1}, P_{TDH3}-TKL1-T_{ADH1}; ypl250cΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TEF1}-Ps4′OMT-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}
CSY1061					ybr197cΔ::P_{TPI1}-yRnSpr-T_{STE2}, P_{TEF1}-yRnPts-T_{CYC1}, P_{GPD}-yRnQdpr-T_{ADH1}, P_{PGK1}-yRnPcbd1-T_{PHO5}; ydr514cΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{TDH3}- yEcNMCH-T_{ADH1}, P_{TEF1}-yPsCPR-T_{CYC1}, P_{TPI1}-yPs4′OMT-T_{STE2}; ymr206wΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TPI1}-yPpddc-T_{STE2}, P_{TEF1}-yRnDhfr-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}; ybl059wΔ::ARO4^{Q166K}, ARO7^{T226I}, P_{TEF1}-ARO10- T_{CYC1}, P_{TDH3}-TKL1-T_{ADH1}; zwf1Δ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TEF1}-Ps4′OMT-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}
CSY1149		2*A* and S4			ybr197cΔ::P_{TPI1}-yRnSpr-T_{STE2}, P_{TEF1}-yRnPts-T_{CYC1}, P_{GPD}-yRnQdpr-T_{ADH1}, P_{PGK1}-yRnPcbd1-T_{PHO5}; ydr514cΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{TDH3}- yEcNMCH-T_{ADH1}, P_{TEF1}-yPsCPR-T_{CYC1}, P_{TPI1}-yPs4′OMT-T_{STE2}; ymr206wΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TPI1}-yPpddc-T_{STE2}, P_{TEF1}-yRnDhfr-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}; ybl059wΔ::ARO4^{Q166K}, ARO7^{T226I}, P_{TEF1}-ARO10- T_{CYC1}, P_{TDH3}-TKL1-T_{ADH1}; zwf1Δ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TEF1}-Ps4′OMT-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}; trp1Δ::P_{HXT7}-CYP82Y1A-T_{PGK1}, P_{TEF1}-PsS9OMT-T_{CYC1}, P_{PGK1}-PsBBE-T_{PHO5}, P_{GPD}-CjCAS-T_{ADH1}; HIS3Δ::P_{GPD}-PsTNMT-T_{CYC1}, P_{PGK1}-PsMT2-T_{PHO5}, P_{ADH1}-CYP82X1-T_{GAP1}, P_{PYK1}-PsCXE1-T_{MFA1}; leu2Δ::P_{HXT7}-CYP82X2-T_{CYC1}, P_{GPD}-PsAT1-T_{ADH1}, P_{TPI1}-PsSDR1-T_{STE2}, P_{PGK1}-PsMT3-T_{PHO5}
CSY1150		1 *B*, ii, and 2*A* and S4			ybr197cΔ::P_{TPI1}-yRnSpr-T_{STE2}, P_{TEF1}-yRnPts-T_{CYC1}, P_{GPD}-yRnQdpr-T_{ADH1}, P_{PGK1}-yRnPcbd1-T_{PHO5}; ydr514cΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{TDH3}- yEcNMCH-T_{ADH1}, P_{TEF1}-yPsCPR-T_{CYC1}, P_{TPI1}-yPs4′OMT-T_{STE2}; ymr206wΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TPI1}-yPpddc-T_{STE2}, P_{TEF1}-yRnDhfr-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}; ybl059wΔ::ARO4^{Q166K}, ARO7^{T226I}, P_{TEF1}-ARO10-T_{CYC1}, P_{TDH3}-TKL1-T_{ADH1}; ypl250cΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TEF1}-Ps4′OMT-T_{CYC1}, P_{PGK1}-yCjNCS-T_{PHO5}; trp1Δ::P_{HXT7}-CYP82Y1A-T_{PGK1}, P_{TEF1}-PsS9OMT-T_{CYC1}, P_{PGK1}-PsBBE-T_{PHO5}, P_{GPD}-CjCAS-T_{ADH1}; HIS3Δ::P_{GPD}-PsTNMT-T_{CYC1}, P_{PGK1}-PsMT2-T_{PHO5}, P_{ADH1}-CYP82X1-T_{GAP1}, P_{PYK1}-PsCXE1-T_{MFA1}; leu2Δ::P_{HXT7}-CYP82X2-T_{CYC1}, P_{GPD}-PsAT1-T_{ADH1}, P_{TPI1}-PsSDR1-T_{STE2}, P_{PGK1}-PsMT3-T_{PHO5}

Table S1. Cont.

Strain	Plasmid no.	Fig.	*x*-axis label	Plasmid-based constructs	Genome modifications
CSY1151		2*A* and S4			ybr197cΔ::P_{TPI1}-yRnSpr-T_{STE2}, P_{TEF1}-yRnPts-T_{CYC1}, P_{GPD}-yRnQdpr-T_{ADH1}, P_{PGK1}-yRnPcbd1-T_{PHO5}; ydr514cΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{TDH3}- yEcNMCH-T_{ADH1}, P_{TEF1}-yPsCPR-T_{CYC1}, P_{TPI1}-yPs4′OMT-T_{STE2}; ymr206wΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TPI1}-yPpddc-T_{STE2}, P_{TEF1}-yRnDhfr-T_{CYC1}, P_{PGK1}-truncated-yCjNCS-T_{PHO5}; ybl059wΔ::ARO4^{Q166K}, ARO7^{T226I}, P_{TEF1}-ARO10- T_{CYC1}, P_{TDH3}-TKL1-T_{ADH1}; ypl250cΔ::P_{GPD}-Rntyrh^{WR}-T_{ADH1}, P_{TEF1}-Ps4′OMT-T_{CYC1}, P_{PGK1}-truncated-yCjNCS-T_{PHO5}; trp1Δ::P_{HXT7}-CYP82Y1A-T_{PGK1}, P_{TEF1}-PsS9OMT-T_{CYC1}, P_{PGK1}-PsBBE-T_{PHO5}, P_{GPD}-CjCAS-T_{ADH1}; HIS3Δ::P_{GPD}-PsTNMT-T_{CYC1}, P_{PGK1}-PsMT2-T_{PHO5}, P_{ADH1}-CYP82X1-T_{GAP1}, P_{PYK1}-PsCXE1-T_{MFA1}; leu2Δ::P_{HXT7}-CYP82X2-T_{CYC1}, P_{GPD}-PsAT1-T_{ADH1}, P_{TPI1}-PsSDR1-T_{STE2}, P_{PGK1}-PsMT3-T_{PHO5}
CSY1152		2*A* and S4			ybr197cΔ::P_{TPI1}-yRnSpr-T_{STE2}, P_{TEF1}-yRnPts-T_{CYC1}, P_{GPD}-yRnQdpr-T_{ADH1}, P_{PGK1}-yRnPcbd1-T_{PHO5}; ydr514cΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{TDH3}- yEcNMCH-T_{ADH1}, P_{TEF1}-yPsCPR-T_{CYC1}, P_{TPI1}-yPs4′OMT-T_{STE2}; ymr206wΔ::P_{GPD}-yRntyrh^{WR}-T_{ADH1}, P_{TPI1}-yPpddc-T_{STE2}, P_{TEF1}-yRnDhfr-T_{CYC1}, P_{PGK1}-truncated-yCjNCS-T_{PHO5}; ybl059wΔ::ARO4^{Q166K}, ARO7^{T226I}, P_{TEF1}-ARO10- T_{CYC1}, P_{TDH3}-TKL1-T_{ADH1}; ypl250cΔ::P_{GPD}-yRntyrh^{WR}-T_{ADH1}, P_{TEF1}-Ps4′OMT-T_{CYC1}, P_{PGK1}-truncated-yCjNCS-T_{PHO5}; trp1Δ::P_{HXT7}-CYP82Y1A-T_{PGK1}, P_{TEF1}-PsS9OMT-T_{CYC1}, P_{PGK1}-PsBBE-T_{PHO5}, P_{GPD}-CjCAS-T_{ADH1}; HIS3Δ::P_{GPD}-PsTNMT-T_{CYC1}, P_{PGK1}-PsMT2-T_{PHO5}, P_{ADH1}-CYP82X1-T_{GAP1}, P_{PYK1}-PsCXE1-T_{MFA1}; leu2Δ::P_{HXT7}-CYP82X2-T_{CYC1}, P_{GPD}-PsAT1-T_{ADH1}, P_{TPI1}-PsSDR1-T_{STE2}, P_{PGK1}-PsMT3-T_{PHO5}
CSY1152	pAG416GPD-ccdB	2*B*	Control	P_{GPD}-ccdB-T_{CYC1}-URA1	
CSY1152	pCS3645	2*B*	POS5	P_{GPD}-POS5-T_{CYC1}-URA1	
CSY1152	pCS3649	2*B*	IDP1	P_{GPD}-IDP1-T_{CYC1}-URA1	
CSY1152	pCS3652	2*B*	IDP2	P_{GPD}-IDP2-T_{CYC1}-URA1	
CSY1152	pCS3646	2*B*	IDP3	P_{GPD}-IDP3-T_{CYC1}-URA1	
CSY1152	pCS3657	2*B*	PDC6	P_{GPD}-PDC6-T_{CYC1}-URA1	
CSY1152	pCS3647	2*B*	ALD4	P_{GPD}-ALD4-T_{CYC1}-URA1	
CSY1152	pCS3653	2*B*	ALD6	P_{GPD}-ALD6-T_{CYC1}-URA1	
CSY1152	pAG415GPD-ccdB, pAG416GPD-ccdB	S3C	Control	P_{GPD}-ccdB-T_{CYC1}-LEU2 P_{GPD}-ccdB-T_{CYC1}-URA1	
CSY1152	pAG415GPD-ccdB, pCS3653	S3C	ALD6_U	P_{GPD}-ccdB-T_{CYC1}-LEU2 P_{GPD}-ALD6-T_{CYC1}-URA1	
CSY1152	pCS4104, pAG416GPD-ccdB	S3C	ALD6_L	P_{GPD}-ALD6-T_{CYC1}-LEU2 P_{GPD}-ccdB-T_{CYC1}-URA1	
CSY1152	pAG415GPD-ccdB, pCS3655	S3C	TYR1_U	P_{GPD}-ccdB-T_{CYC1}-LEU2 P_{GPD}-TYR1-T_{CYC1}-URA1	
CSY1152	pCS4104, pCS3655	S3C	ALD6_L TYR1_U	P_{GPD}-ALD6-T_{CYC1}-LEU2 P_{GPD}-TYR1-T_{CYC1}-URA1	
CSY1152	pCS3655	2*B*	TYR1	P_{GPD}-TYR1-T_{CYC1}-URA1	

Table S1. Cont.

Strain	Plasmid no.	Fig.	*x*-axis label	Plasmid-based constructs	Genome modifications
CSY1153		2*A*, 3, 4*B*, 5, and 6 and S4 and S5			*ybr197cΔ::*P_{TPI1}*-yRnSpr-*T_{STE2}*,* P_{TEF1}*-yRnPts-*T_{CYC1}*,* P_{GPD}*-yRnQdpr-*T_{ADH1}*,* P_{PGK1}*-yRnPcbd1-*T_{PHO5}*; ydr514cΔ::*P_{PYK1}*-PsCNMT-*T_{MFa1}*,* P_{PGK1}*-Ps6OMT-*T_{PHO5}*,* P_{TDH3}*- yEcNMCH-*T_{ADH1}*,* P_{TEF1}*-yPsCPR-*T_{CYC1}*,* P_{TPI1}*-yPs4′OMT-*T_{STE2}*; ymr206wΔ::*P_{GPD}*-yRntyrh*^{WR}*-*T_{ADH1}*,* P_{TPI1}*-yPpddc-*T_{STE2}*,* P_{TEF1}*-yRnDhfr-*T_{CYC1}*,* P_{PGK1}*-truncated-yCjNCS-*T_{PHO5}*; ybl059wΔ::ARO4*^{Q166K}*, ARO7*^{T226I}*,* P_{TEF1}*-ARO10-* T_{CYC1}*,* P_{TDH3}*-TKL1-*T_{ADH1}*; ypl250cΔ::*P_{GPD}*-yRntyrh*^{WR}*-*T_{ADH1}*,* P_{TEF1}*-Ps4′OMT-*T_{CYC1}*,* P_{PGK1}*-truncated-yCjNCS-*T_{PHO5}*; trp1Δ::*P_{HXT7}*-CYP82Y1A-*T_{PGK1}*,* P_{TEF1}*-PsS9OMT-*T_{CYC1}*,* P_{PGK1}*-PsBBE-*T_{PHO5}*,* P_{GPD}*-CjCAS-*T_{ADH1}*; HIS3Δ::*P_{GPD}*-PsTNMT-*T_{CYC1}*,* P_{PGK1}*-PsMT2-*T_{PHO5}*,* P_{ADH1}*-CYP82X1-*T_{GAP1}*,* P_{PYK1}*-PsCXE1-*T_{MFA1}*; leu2Δ::*P_{HXT7}*-CYP82X2-*T_{CYC1}*,* P_{GPD}*-PsAT1-*T_{ADH1}*,* P_{TPI1}*-PsSDR1-*T_{STE2}*,* P_{PGK1}*-PsMT3-*T_{PHO5}*; ura1Δ::*P_{TEF1}*-TYR1-*T_{CYC1}*,* P_{PYK1}*-CYP82X2-*T_{PYK1}*, TmTRP,* P_{GPD}*-PsS9OMT-*T_{ADH1}*,* P_{PYK1}*-ALD6-*T_{MFA1}
CSY1203		S3*B*			*ybr197cΔ::*P_{TPI1}*-yRnSpr-*T_{STE2}*,* P_{TEF1}*-yRnPts-*T_{CYC1}*,* P_{GPD}*-yRnQdpr-*T_{ADH1}*,* P_{PGK1}*-yRnPcbd1-*T_{PHO5}*; ydr514cΔ::*P_{PYK1}*-PsCNMT-*T_{MFa1}*,* P_{PGK1}*-Ps6OMT-*T_{PHO5}*,* P_{TDH3}*- yEcNMCH-*T_{ADH1}*,* P_{TEF1}*-yPsCPR-*T_{CYC1}*,* P_{TPI1}*-yPs4′OMT-*T_{STE2}*; ymr206wΔ::*P_{GPD}*-Rntyrh*^{WR}*-*T_{ADH1}*,* P_{TPI1}*-yPpddc-*T_{STE2}*,* P_{TEF1}*-yRnDhfr-*T_{CYC1}*,* P_{PGK1}*-truncated-yCjNCS-*T_{PHO5}*; ybl059wΔ::ARO4*^{Q166K}*, ARO7*^{T226I}*,* P_{TEF1}*-ARO10-* T_{CYC1}*,* P_{TDH3}*-TKL1-*T_{ADH1}*; ypl250cΔ::*P_{GPD}*-Rntyrh*^{WR}*-*T_{ADH1}*,* P_{TEF1}*-Ps4′OMT-*T_{CYC1}*,* P_{PGK1}*-truncated-yCjNCS-*T_{PHO5}*; trp1Δ::*P_{HXT7}*-CYP82Y1A-*T_{PGK1}*,* P_{TEF1}*-PsS9OMT-*T_{CYC1}*,* P_{PGK1}*-PsBBE-*T_{PHO5}*,* P_{GPD}*-CjCAS-*T_{ADH1}*; HIS3Δ::*P_{GPD}*-PsTNMT-*T_{CYC1}*,* P_{PGK1}*-PsMT2-*T_{PHO5}*,* P_{ADH1}*-CYP82X1-*T_{GAP1}*,* P_{PYK1}*-PsCXE1-*T_{MFA1}*; leu2Δ::*P_{HXT7}*-CYP82X2-*T_{CYC1}*,* P_{GPD}*-PsAT1-*T_{ADH1}*,* P_{TPI1}*-PsSDR1-*T_{STE2}*,* P_{PGK1}*-PsMT3-*T_{PHO5}*; ura1Δ::* P_{PGK1}*-truncated-yCjNCS-*T_{PHO5}*,* P_{HXT7}*-CYP82X2-*T_{CYC1}*,* P_{GPD}*-PsS9OMT-*T_{ADH1}*,* P_{PYK1}*-Ald6-*T_{Mfa1}
CSY1203	pAG416GPD-ccdB	S3*B*	control	P_{GPD}*-ccdB-*T_{CYC1}*-URA1*	
CSY1203	pCS3645	S3*B*	POS5	P_{GPD}*-POS5-*T_{CYC1}*-URA1*	
CSY1203	pCS3649	S3*B*	IDP1	P_{GPD}*-IDP1-*T_{CYC1}*-URA1*	
CSY1203	pCS3652	S3*B*	IDP2	P_{GPD}*-IDP2-*T_{CYC1}*-URA1*	
CSY1203	pCS3646	S3*B*	IDP3	P_{GPD}*-IDP3-*T_{CYC1}*-URA1*	
CSY1203	pCS3657	S3*B*	PDC6	P_{GPD}*-PDC6-*T_{CYC1}*-URA1*	
CSY1203	pCS3647	S3*B*	ALD4	P_{GPD}*-ALD4-*T_{CYC1}*-URA1*	
CSY1203	pCS3653	S3*B*	ALD6	P_{GPD}*-ALD6-*T_{CYC1}*-URA1*	
CSY1203	pCS3655	S3*B*	TYR1	P_{GPD}*-TYR1-*T_{CYC1}*-URA1*	

Base strain is CEN.PK2-1D (*MATα; ura3-52*; *trp1-289*; *leu2-3,112*; *his3Δ1*; *MAL2-8*^{C}; *SUC2*), and constructs are expressed from low-copy plasmids. P, promoter; T, terminator.

Table S2. Plasmids used in this study

Plasmid no.	Genotype	Refs.
pAG416GPD-ccdB	*Centromeric URA3, attR1-PGPD-ccdB-attR2*	37
pCS3636	*attL1-POS5-attL1*	This work
pCS3642	*attL1-IDP1-attL1*	This work
pCS3643	*attL1-IDP2-attL1*	This work
pCS3637	*attL1-IDP3-attL1*	This work
pCS3663	*attL1-PDC6-attL1*	This work
pCS3640	*attL1-ALD4-attL1*	This work
pCS3639	*attL1-ALD6-attL1*	This work
pCS3644	*attL1-TYR1-attL1*	This work
pCS3125	*attL1-*P_{TEF1}*-CYP82Y1A-*T_{CYC1}*-attL2*	14
pCS3131	*attL1-*P_{PYK1}*-CYP82Y1A-*T_{MFA1}*-attL2*	14
pCS3146	P_{HXT7}*-CYP82Y1A-*T_{CYC1}*, leu2, template for integration cassette*	14
pCS3064	*attL1-*P_{TEF1}*-PsS9OMT-*T_{CYC1}*-attL2*	14
pCS3060	*attL1-*P_{PGK1}*-PsBBE-*T_{PHO5}*-attL2*	16
pCS3075	*attL1-*P_{GPD}*-CjCAS-*T_{ADH1}*-attL2*	16
pCS3145	P_{GPD}*-PsTNMT-*T_{CYC1}*, KanMX, template for integration cassette*	14
pCS3143	*attL1-*P_{PGK1}*-PsMT2-*T_{PHO5}*-attL2*	14
pCS2922	*hphNTI selection marker (HygR) flanked by loxP*	38
pCS3134	*attL1-*P_{ADH1}*-CYP82X1-*T_{GAP1}*-attL2*	14
pCS3141	*attL1-*P_{PYK1}*-PsCXE1-*T_{MFA1}*-attL2*	14
pCS3147	P_{HXT7}*-CYP82X2-*T_{CYC1}*, KanMX, template for integration cassette*	14
pCS3140	*attL1-* P_{GPD}*-PsAT1-*T_{ADH1}*-attL2*	14
pCS3142	*attL1-*P_{TPI1}*-PsSDR1-*T_{STE2}*-attL2*	14
pCS3244	*attL1-*P_{PGK1}*-PsMT3-*T_{PHO5}*-attL2*	14
pCS3659	*attL1-*P_{TEF1}*-TYR1-*T_{CYC1}*-attL2*	This work
pCS3136	*attL1-*P_{CYC1}*-CYP82X2-*T_{PYK1}*-attL2*	14
pCS2923	*TmtrpF selection marker (TYR) flanked by loxP*	38
pCS3661	*attL1-*P_{PYK1}*-ALD6-*T_{MFA1}*-attL2*	This work
pCS3638	*CRISPR Construct for removal of N terminus of CjNCS*	This work
pCS3658	*CRISPR Construct for replacement of codon-optimized Rntyrh*^{WR} *(yRntyrh*^{WR}*)*	This work

P, promoter; T, terminator.

Table S3. DNA sequences of genes used in this study

Gene	Codon-optimized or GenBank accession no.	DNA sequence
PsTNMT	DQ028579.1	
CYP82Y1A	Codon-optimized	ATGAGAACCGAATCCATCAAGACCAACAGACCAATGGATTTGTTGTTGCAATACTTACAACCTATCTCCGTTGCCTTGGTTGTTATTGCTTTGGTTTGGAATTACGGTAGAAGAAACCCAACTAAGAAATTGGCTCCTGAAGCTAGTGGTGCTTGGCCAGTTATTGGTCATTTGTTGTTGTTTATGAACGAAAACGACTTGAACCACGTTACTTTGGGTCACATGGCTGATAAGTATGGTCCAATTTTCTCATTGAGATTCGGTAGACATAGAACCTTGGTTGTTTCTTCATGGGAAATGGTCAAAGAATGTTTCACCGGTACAAACGACAAGTTGTTTTCTAACAGACCATCTTCCTTGGCCGTTAAGTTGATGTTTTACGACACTGAATCTTACGGTTTTGCCCCATACGGTAAATATTGGAGAGAATTGAGAAAGATCTCCACCCATAAGTTGTTGTCCAATCAACAATTGGAAAAGTTCAAGCACTTGAGAATCTCCGAAGTTGACAACTCCTTCAAAAAGTTGCATGAATTGTGCTCCAACAACAAGCAAGGTGGTGATACAACTTACGTTGCTTCTTTGGTTAGAATGGATGATTGGTTTGCCTACTTGACCTTCAACGTTATTGGTAGAATCGTCAGTGGTTTCCAATCTAATGCTGTTGCTGGTGCTACTAATTCCCAAGAAAAGTACAAGTTGGCCATCGATGAAGTCTCTAATTTGATGGCTACTTTCGCCGTTTCTGATGTTGTTCCAAGATTGGGTTGGATTGATAGATTGACTGGTTTGACCGGTAAGATGAAGAACTGTGGTAAAAAGTTGGATGCCGTTGTTGGTGATGCTGTTGAAGATCATAGACAAAAGAAATTGAAGATCTCTAGAAACAACACCGGTGCTTTGACTGAACATGAAGAAGAAGATTTCATCGACGTCTGCTTGTCCATTATGGAACAATCTCAAATTCCAGGTAACCACCCAGAAATCTCCGTTAAGTCTATTGCTTTGGATATGTTGTCTGGTGGTTCTGATACCACCAAGTTGATTATGACTTGGACCTTGTCTTTGTTATTGAACCACCCTGATATTTTGGACAAGGCCAAAGAAGAAGTTGATACCTACTTTGGTAAGAAAAAGATCTCTGATAACACCCCAGTTGTTGATGCTGCTGATGTTCCTAATTTGGTTTACATTCAAGCCATCATCAAAGAATCCATGAGATTATACCCAGCCTCTACCTTGATGGAAAGAATGACTTCTGATGATTGTGATGTCGGTGGTTTTCATGTTCCAGCTGGTACTAGATTGTGGGTTAATGTTTGGAAGATGCAAAGAGATCCAAGAGTTTGGAAAGACCCATTGGTTTTTTTGCCAGAAAGATTCTTGTCCAACGATAAGGGTATGGTTGATGTTAAGGGTCAAAACTACGAATTGATCCCATTTGGTACTGGTAGAAGAATTTGTCCAGGTGCTTCTTTTGCTTTAGAAGTCTTGCATTTGGTCTTGACCAGATTGATCTTGGAATTCGAAATGAAGGCTCCTGAAGGTAAGATTGATATGAGAGCTAGACCAGGTTTCTTCCACAACAAAGTTGTTCCATTGGATGTTCAATTGACCCCAAGAACTTTGGACTAA
PsS9OMT	Codon-optimized	ATGGCTACTAATGGTGAAATTTTCAACACCTACGGTCACAACCATCAATCTGCTACTGTTACTAAGATTACCGCCTCTAACGAATCTTCTAACGGTGTTTGTTACTTGTCTGAAACTGCCAACTTGGGTAAATTGATCTGTATTCCAATGGCTTTGAGAGCTGCTATGGAATTGAATGTTTTCCAATTGATCTCCAAGTTCGGTACTGATGCTAAAGTTTCCGCTTCTGAAATTGCTTCTAAGATGCCAAATGCTAAGAACAATCCTGAAGCTGCAATGTACTTGGACAGAATCTTGAGATTATTGGGTGCCTCCTCTATCTTGTCTGTTTCTACTACTAAGAAATCCATCAACAGAGGTGGTGATGATGTTGTTGTACACGAAAAGTTGTACGGTTTGACCAACTCTTCTTGTTGTTTGGTTCCAAGACAAGAAGATGGTGTTTCCTTGGTTGAAGAATTGTTGTTCACCTCCGATAAGGTTGTTGTTGACTCCTTCTTCAAATTGAAATGCGTCGTTGAAGAAAAGGACTCCGTTCCATTTGAAGTTGCTCATGGTGCTAAGATTTTTGAATACGCTGCTACTGAACCTAGAATGAATCAAGTTTTCAACGATGGTATGGCCGTTTTCTCTATCGTTGTTTTTGAAGCCGTTTTCAGAGTTTACGATGGTTTCTTGGACATGAAGGAATTATTGGATGTTGGTGGTGGTATCGGTACTTCCGTTTCTAAAATCGTTGCTAAGTACCCATTGATCAGAGGTGTTAATTTCGATTTGCCACACGTTATTTCTGTTGCTCCACAATATCCTGGTGTTGAACATGTTGCTGGTGATATGTTCGAAGAAGTTCCAAAAGGTCAAAACATGTTGTTGAAGTGGGTTTTACATGATTGGGGTGACGAAAGATGTGTCAAGTTGTTGAAAAACTGCTGGAACTCTTTGCCAGTTGGTGGTAAGGTTTTGATCATCGAATTTGTCTTGCCAAACGAATTGGGTAACAACGCTGAATCTTTCAACGCTTTGATTCCAGATTTGTTGTTAATGGCCTTGAACCCAGGTGGTAAAGAAAGAACTATTTCCGAATACGACGATTTGGGTAAAGCTGCTGGTTTCATTAAGACCATTCCAATCCCAATCTCCAACGGTTTACATGTTATCGAATTCCATAAGTAA
PsBBE	AF025430.1	

Table S3. Cont.

Gene	Codon-optimized or GenBank accession no.	DNA sequence
CjCAS	Codon-optimized	ATGGAAATGAACCCTTTGTTGGTTTGTGCTACTGTTGCTATCGTTTTCGCTACTACCACCATTATCAGAATCTTGTTCTCCTCTTCTTCCTTGCCACAAATGAAGTGGCCATCTGGTCCAAGAAAATTGCCAATTATTGGTAACTTGCACCAATTAGGTGATGATGTCTTGCATGTTGCTTTGGCTAAATTGGCAAAAGTTCACGGTTCTGTTATGACCATTTGGATTGGTTCTTGGAGACCAGTTATCGTTATCTCCGATATTGAAAAGGCTTGGGAAGTTTTGGTTAACAAGTCTGCTGATTATGGTGCTAGAGATATGCCAGAAATTACCAAGATTGCTTCTGCTTCTTGGCATACCATTTCTACATCTGATGCTGGTTCTTTCTGGCAAAATGTCAGAAAGGGTTTACAATCTGGTGCTATGGGTCCATTGAATGTTGCTGCTCAAAATCAATACCAAGAAAGAGACATGAAGAGATTGATCAAGGCCATGTCTGATGAAGCTGCTAACAACAATGGTATCGTTAAGCCATTGGATCACATCAAGAAGAACACCGTTAGATTATTGACCAGATTGATTTTCGGTCAAGCCTTCGATGATAACAAGTTCATCGAATCCATGCACTACGAAATCGAAGATATCATCAGAATTTCCGGTTACGCTAGATTGGCTGAAGCCTTTTATTACGCTAAATACTTGCCATCCCATAAGAAGGCTGAAAGAGAAGCCTTCTTGGTTAAGTGTAGAGTTGAAGAATTGGTCAGACCTTTGTTGTCATCTAAGCCACCAACTAACTCTTACTTGTACTTCTTGTTGTCCCAAAACTTTGAAGAAGAAGTTATCATCTTCTGCATCTTTGAATTATACTTGTTGGGTGTTGACTCCACTTCTTCTACTACTACATGGGCTTTGGCTTACTTGATTAGAGAACAAGGTGCTCAAGAAAAGTTGTACCAAGATATCAGAATGACCTTGGGTGATGTTGATTTGGTCAAGATCGAAGATGTCAACAAGTTGAAGTACTTGCAAGGTGTTGTCAAAGAAACCATGAGAATGAAGCCTATTGCTCCATTGGCTATTCCACATAAGACTGCTAAAGAAACTACCTTGATGGGTACTAAGGTTGCTAAGGGTACTAGAATCATGGTTAACTTGTACGCCTTGCACCACAATCAAAACATTTGGCCAGATCCATACAAATTCATGCCTGAAAGATTCTTGGAAGGTGAAACTGGTACTGCTTACAACAAAGCTATGGAACAATCTTTCTTGCCATTCTCTGCTGGTATGAGAATTTGTGCAGGTATGGATTTGGGTAAATTACAATTCGCTTTTGCCTTGGCCAACTTGGTTAATGCTTTTAAGTGGTCTTGTGTTGAAGAAGGTAAATTGCCAGATATGGGTGAAGAATTATCCTTCGTCTTGTTGATGAAGACTCCATTGGAAGCTAGAATTGCTGGTAGAAACGTTTAA
PsAT1	Codon-optimized	ATGGCTACCATGTCATCTGCTGCCGTTGAAGTTATTTCTAAAGAAACCATCAAGCCAAGAAACCCAACTCCATATCAATTGAGAAACTACAACATGTCCTTGTTGGACCAATACTCCTCTTTGGTTTACGTTCCAATCATCTTGTTTTACCCAGCTGCTTCTGATGCTAATTCCACTGGTTCTAAACATCATGATGACTTGCACTTGTTGAAGAGATCCTTGTCTGAAACTTTGGTTCACTTTTATCCAATGGCCGGTAGAATGAAGGATAACATGACTGTTGATTGCAACGATGAAGGTATCGACTTCTTTGAAGTTAGAATCAAGGGTAGAATGTGCGACTTCATGATGAAGAGTGATGCTCATTTGTCTTTGTTGTTGCCATCTGAAGTTGCTTCTACCAACTTTGTCAAAGAAGCCCAAGTTATCGTTCAAGTCAACATGTTTGATTGTGGTGGTACTGCTATTTGCTTCTGCATTTCTAACAAGATTGCTGATGCTTGCACCATGATTACCTTCATTAGATCTTTGGCTGGTACTACCAACATTGCTAGAAGAGGTTCTTCTATTGCTGCTCCAACTACCAATCAAAATTTGGTTCCTTCTTTCGACTCCACCTCTTTGTTTCCACCATCTGAACAATTGGCTTCCCAAGTTTCTTATCCAACTCAAGATTCTACCTCCGTTGACAAGTTGGTTTCTAAGAGATTTGTTTTCGATGCCGCCAAGATTACTTCTGCTAGAGAAAAATTGCAATCCTTGATGCACGATAAGTACAAGTGTCATAGACCAACCAGAGTTGAAGTTGTTTCTGCTTTGATTTGGAAGTCCGCTGTTAAGTCTGCTCCACCAGGTTCTATTTCTACTGTTACTCATGCTATGAACTTCAGAAAGAAGATGGATCCACCATTGCAAGATGCTTCATTTGGTAACTTGTGTGTTGTTGTTACTGCTGTTTTGCCAGCTACTACTGCTACTACAACTAATCCAGCTACTAAGAAGGTTTCCTCTACCTCTAATGAAGAACAAGTTGCCTTGGATGAATTGTCCGATTTTGTTGCTTTGTTGAGAAGAGAAATCGACAAGGTTAAGGGTGATAAGGGTTGCATGGAAAAGATCATCCAAAAGTTCATCTACGGTCATGATGCCTCTGTTGCTAAAGATTCTGATGTTGAAGATAAGGTTACCGCCTTGTTTATGACTTCTTGGTGTAAGTTTGGTTTCTACGAAGCTGATTTTGGTTGGGGTACTCCAGTTTGGGTTACTACTGTTCCATTGATTGAACCTAAGTACAAGAACATGGTTTTCATGAACGATATGAAGTGCGGTGAAGGTATTGAAGTTTGGGTTAACTTCTTGGAAGATGACATGACCAAGTTTGAACACCACTTGAGAGAAATCTTGCAATTATTCTAA

Table S3. Cont.

Gene	Codon-optimized or GenBank accession no.	DNA sequence
CYP82X1	Codon-optimized	ATGGAATTATTCATCAAGTTGCCATTCATCCAACCTATCCCATTCTCCATTATTTTGGTTACCACCGTTTCCATCGTCTTGTTGTACTCTGTTTTTTTCTGGGTTACCGACAAAAAGAAGAAGAGAAAAAAGGCTCCAAATGCTGCTGGTGCTTGGCCATTGATTGGTCATTTGAGATTATTGATGAACGACAAAGAACCATTATACAGAGCCTTGGGTTCTATGGCTGATAAGTATGGTCCAGCTTTCAACATCAGATTGGGTAATCAAGAAGTCTTGGTTGTCTCTAATTGGGAAATGGTTAAGCAATGCTTCGGTAATCAAAACGACAAGTTGTTCTCTAACAGACAAACTACTTTGGCTGCCAAGTACATGTTGAATCAAACTACCTCTTCTGGTTTTGCTCCATACGGTCCATATTGGAGAGAATTGAGAAAGATCATGGTTCAACAATTATTGTCCAAGCAATCCTTGGAATCTTGGAAGCACTTGAAGATCAAAGAAATGGATGCCTCCTTCTCCAAATTGAACGAATTGTGTAACAACAACGGTACTGGTACTGCTACCTTGATTAGAATGGACGAATGGTTTGCTGAATTGACCTTCAACGTTATTGCCAGAAATGTTTTCGGTTACCAATCTGGTGGTAGATCAACTGCTTTGACTAATGGTGATACTGAATCCAAGGGTGAAAGATACAAAAAGACTTTGGAAGAAGCCTTGCACTTGATGTCTATTTTCGCCGTTTCTGATATCTTCCCATCTTTGGAATGGGTTGATAGATTGAGAGGTTTGATCAGAAACATGAAGAGATTCGGTGACGAATTGAACTCTATTGCTGGTTGCTTGATCGAAGAACACAGACAAAAGAGATTACAATCCGTCAGTAAGTCCGATAAGGGTGTTGGTGATGAACAAGATTTCGTTGACGTTTTGTTGTCCGTTGCTGAAAAATCTCAATTGCCAGGTGATGATCCAGATTTGGTTATCAAGTCCATGATTTTGGAAATCGTCAGTGGTGGTTCTGAAACCACTTCTTCTACTTTGACTTGGGCTTTGTGTTTGTTATTGAACCATCCACACGTTTTGAAGAAGGCCAAAGAAGAATTGGATACTCACGTTGGTAAGGATAGACACGTTGAAGAATCTGATACTCCAAAGTTGGTTTACATCAACGCCATCATCAAAGAATCCATGAGATTATACCCAAACGGTGCCATGTTGGATAGATTGGCTTTAGAAGAATGTGAAGTTGGTGGTTTTCATGTTCCAGCCGGTGGTAGATTATTTGTTAACGTTTGGAAGATCCAAAGAGATCCATCTGTTTGGGAAAACCCATTGGAATTCAAACCAGAAAGATGGTTCTTGTCCAACGGTGAAAAAATGGATGTTGATTACAAGGGTCACAACCACGAATTCATTCCATTTGGTATTGGTAGAAGAATGTGCGCTGGTATGTTGTGGGCTTCTGAAGTTATTCATTTGGTCTTGCCAAGATTGATCCACGGTTTTGATATGAAGGCTGCTTCAGCTAATGGTAAGGTTGATATGGCTGAAATGGCTGGTATGGTTATCTGTTTCAAGAAAACCCCTTTGGAAGTTATGGTCAACCCAAGAGAATGA
PsCXE1	Codon-optimized	ATGGCCGATCCATACGAATTCTTGATGTGTATTCATAACCCAGAAGAAGATACCTTGACCAGAAATTTTCCAATTCCAGCTACTCCATTGGACCAAAACACTAAGGATATTTCTTTGAACCCAGACAGAAAGACCTCCTTGAGAATTTTTAGACCACCTACAAAAGAACCACCAGTCACTAAGAACAAGTTGTTGCCAATTATCATCTACTTCCATGGTGGTGGTTTCATTTTGTTCAACGCTGATTCTACCATGAACCACGATTTCTGTCAATCTATTGCTACCCATATTCCAGCCTTGGTTGTTTCTGTTGATTATAGATTGGCTCCAGAAAACAGATTGCCAGCTGCTTATGATGATGCTGTTGATGCTTTGAATTGGGTTAAGGATCAAGGTTTGGGTAAATTGAACAACTCCGAAGTCTGGTTGAAAGAATACGGTGATTTCTCCAAGTGCTTTATCATGGGTTGTTCTTCAGGTGCTAATGTTGCTTATCATGCTTCCTTGAGAGCCATCGAAATGGATTTGGAACCAGCTAAAATCAACGGTTTGATCTTGCATTGTCCATTCTTCGGTTCTTTGGAAAGAACTGAATCCGATTCCAAGGTCATCAACAATCAAGATTTGCCATTGGCCGTTAGAGATGTTATGTGGGAATTGGCTTTGCCATTAGGTTCTACTAGAGATCATGTTTACTGCAACCCAAACATCGATCATGATGGTTCTTCATCTGGTAACATGGTTGGTTTGATTGAAAGATGCTTCGTTGTTGGTTTTTACGGTGATCCATTGATCGACAGACAAATCCAATTGGTCAAGATGTTGGAAGAAAAGGGTGTTAAGGTTGAAACCTGGATTGAACAAGGTGGTTATCATGGTGTTTTGTGCTTCGATCCAATGATCAGAGAAACCTTCTTGGAAAAGTTGAAGCACTTCATCTTGAACGACGAATTCATCTACTAA

Table S3. Cont.

Gene	Codon-optimized or GenBank accession no.	DNA sequence
PsSDR1	Codon-optimized	ATGCACGGTCAAAAGAACATCTCCGAAAGATACCAAAAGTTCAAAGAAATGGAAGGTACTGGTAAGATCGTTTGTGTTACTGGTGGTGCTGGTTATTTGGCTTCTTGGTTGATTATGAGATTATTGGAAAGAGGTTACTCCGTTAGAACCACCGTTAGATCTGATCCAAAGTTCAGAGAAGATGTCTCTCATTTGAAAGCTTTGCCAGAAGCTACTGAAAAGTTGCAAATTTTTGAAGCCGACTTGGAAAACCCAGAATCTTTTGATGATGCTATCAACGGTTGTGTCGGTGTTTTTTTGGTTGCTCAAGGTATGAATTTCGCCGAAGAATACACTTTGGAAAAGATCATTAAGACCTGCGTCGAAGGTACTTTGAGAATCTTGCAATCTTGCTTGAAGTCCAAGACCGTTAAGAAGGTTGTTTACACTTCTTCTGCTGATGCTGCCATGATGATTTCTAATTTGAAGGCCGTAAAAGAAATCGACGAAACCATTTGGTCTGAAGTCGATAACTTCATCTCTAAGCCAGAACAAGTTATTCCAGGTTTGCCATCTTACGTTGTTTCTAAGGTTTTGACCGAAAGAGCCTGTTTGAAGTTCTCTGAAGAACATGGTTTGGATGTTGTTACCATTTTGCCACCATTGGTTGTTGGTCCTTTTATTACTCCACATCCACCACCATCTGTTTCCATTGCTTTGTCTATTATCTCCGGTGATGTCTCTATGATGTTGGGTGTTAGATTGGAAAACGCCGTTCATATTGATGATGTTGCTTTGGCCCATATCTTCGTTTTCGAATGTGAAAAAGCTAAGGGTAGACACATCTGCTCCTCTGTTGATTTTCCAATGCATGATTTGCCTAAGTTCATCTCTGAAAACTACCCAGAATTCAACGTTCCAACCGATTTGTTGAAGGACATCGAAGAACAAGAACCAGTTCATTTGTCCTCCGATAAGTTGTTGTCTATGGGTTTCCAATTCAAGTACGACTTCGCTGAAATTTTCGGTGATGCTATTAGATGCGCTAAAGAAAAGGGTTTCTTGTAA
PsMT2	Codon-optimized	ATGGAAATCCACTTGGAATCCCAAGAACAAGAAATGAAGTACCAATCCCAAATCTGGAATCAAATCTGCGGTACTGTTGATACCTCTGTTTTGAGATGTGCTATCCAATTGGGTATTTTCGATGCCATTCATAACTCTGGTAAGCCAATGATTACCTTGACCGAATTGTCATCCATAGTCTCTTCTCCATCCTCCTCTTCTATTGAACCATGTAACTTGTACAGATTGGTCAGATACTTGTCCCAAATGGACTTGATTTCTATCGGTGAATGTTTGAACGAAGCCACTGTTTCTTTGACTGGTACTTCTAAGTTGTTGTTGAGAAATCAAGAAAAGTCCTTGATCGATTGGGTTTTGGCTATTTCTTGCGAAATGATGGTTGTTGTCTGGCACGAATTATCCTCTTCAGTTTCTACTCCAGCTGATGAACCACCAATTTTCCAAAAAGTTCATGGTAAGAACGCTTTGGAATTGGCTGGTGAATTTCCAGAATGGAACGATTTGATTAACAACGCTATGACTTCTGACACCTCTGTTACAAAACCAGCTTTGATTCAAGGTTGCGGTAAGATTTTGAACGGTGTTACCTCTTTGATTGATGTTGGTGGTGGTCATGGTGCTACTATGGCTTATATCGTTGAAGCTTTCCCACATATTAAGGGTGCCGTTATTGATTTGCCACATGTTGTTGAAGCTGCTCCAGAAAGACCAGGTGTTGAATTCATTTCTGGTGACATCTTCAAGTCTATCTCTAACGCTGATGCTGTCTTGTTGAAATACGTCTTGCATAACTGGGAAGATACCGAATGTGTTAACTTGTTGAAGAGATGCAAAGAAGCTGTTCCAGCAGATAAGGGTAAGGTTATTATCATGGATTTGGTTATCGATGACGACGACAACTCTATTTTGACCCAAGCTAAATTGTCCTTGGATTTGACCGTTATGAATCACGGTGGTGGTAGAGAAAGAACAAAAGAAGATTGGAGAAACTTGATCGAAATGTCCGGTTTTTCCAGACACGAAATTATTCCAATTTCCGCCATGCCATCTATTATCGTTGCTTACCCATAA
PsMT3	Codon-optimized	ATGGAAGTTGTCTCCAAGATCGATCAAGAAAATCAAGCCAAGATCTGGAAGCAAATTTTCGGTTTTGCTGAATCCTTGGTTTTGAAGTGTGCTGTTCAATTGGAAATTGCCGAAACCTTGCATAACAACGTTAAGCCAATGTCCTTGTCTGAATTGGCTTCTAAATTGCCAGCTCAACCAGTTAACGAAGATAGATTATACAGAATCTTGCACTTCTTGGTCCACATGAAGTTGTTTAACAAGGATGCTACTACCCAAAAGTACTCTTTAGCTCCACCAGCTAAATACTTGTTGAAAGGTTGGGAAAAGTCCATGGTTCCATCCATTTTGTCTGTTACCGATAAGGATTTTACTGCTCCATGGAATCATTTGGGTGATGGTTTGACTGGTAACTGTAATGCTTTTGAAAAGGCTTTGGGTAAGGGTATCAGAGTCTACATGAGAGAAAATCCAGAAAAGGACCAATTATTCAACGAAGGTATGGCTTGCGATACCAGATTATTTGCTTCTGCTTTGGTTAACGAATGCAAGTCCATTTTCTCCGATGGTATTAACACTTTGGCTGGTGTTGGTAGAGGTACTGGTACTGCTGTTAAGGCTATTTCTAAAGCTTTCCCAGATATCAAGTGCACCATTCATGATTTGCCAGAAGTTACCTCTAAGAACTCCAAGATTCCAAGAGATGTTTTCAAGTCTGTTCCATCTGCTGATGCCATTTTCATGAAGTCTATATTGCACGAATGGAACGACGAAGAATGCATCCAAATTTTGAAGAGATGCAAAGAAGCCATTCCAAAGGGTGGTAAGGTTATTATTGCCGATGTTGTTATCGATATGGATTCCACTCATCCATACTCCAAATCAAGATTGGCTATGGATTTGGCCATGATGTTGCATACTGGTGGTAAAGAAAGAACCGAAGAAGATTGGAAGAAGTTGATTGACGCTGCTGGTTTTGCTTCTTGTAAGATTACAAAATTGTCCGCCTTGCAATCCGTTATTGAAGCTTATCCACATTAA

Table S3. Cont.

Gene	Codon-optimized or GenBank accession no.	DNA sequence
CYP82X2	Codon-optimized	ATGAAGTCGTTAATGATGAACAAGTTATTATTTCTCCAACGGATTACTGATTCTCCTTCGACCACCATTATCAGTACTTTTATTGTTACAATAATATCCATTGTTTTTCTCTACACTGTCTTGTTGATAAGGACGACTAAGAATAAGCAGAAGATAGCAGCACCAAAAGCATCGGGGGCGTGGCCGTTCATAGGTCATCTCAAACTATTCATGAAACAAGATACTCAGTTTTACAGAACTCTAGGAACCATGTCTGATAAATACGGGTCGGTGTTCACACTTCGATTAGGAAACCAAGCAATCCTAGTTGTGAGCAACTGGGAGATGGTAAAAGAATGTTTCACAACAAACGACAAGTCATTCTCGAATCGTCCAAGTACGTTAAGCACTAAATACATGCTGAATGACACTAATTCTGTCGTGTTTTCACCTTACGGAACGTATTGGAGAGAAATGCGGAAGATATTGGTGCAAAAACTACTGATCTCTAACCAAAGATCAGAGGCATTGAAAAATCTGAAAACGAAAGAAATCGACAACTCGTTTGTAAAGCTTAATGATTTATGCAACAACGATGTCAGTGGAGGAGGCACAAAAGTTAGGATGGACGAATGGTTGGCTGACATGATGTTCAACATTATTGCTAGGATTACATTTGGTTACCAAAGCGGAGGAGGCGATGCACCTGGCGCTTCTACAACATCCAAGAATGTCGAGAGATACAAGAAAACGTTGGACGAGATGTTTGTTGTTTTAGCGACGAGGTTTGCAGTTTCAGATATATTTCCATCTCTGGAGTTTATAGACCGATTGAGAGGTCTTGTAAAGGATATGAAAATCTTGGGAGACGAATTAAACTCCATTGCTGGATGTTTTATTGAAGAACATCGTCAAAAGAGACGAGAATCATTATCCTCATTGTTATCTTTGTCAAATGAATCCGTTGGTGATGAACAAGATTTCATTGATGTTCTCTTGTCAATAATGGATCAGTCACGGCTTCCCGGAGATGACCCAGATTTTATTATCAAAATTATGATCCTGGAAGCTTTTGCAGGTGGGACGGACAGTTTAAGTGCAACCTTAACTTGGGTCCTCTCTCTACTGCTGAACCACCCAAACGTGTTAAAGAGGGCAAGGGAGGAAATAGATAGGCATGTGGAAAACGGTAAGCAAGTGGAAGTGTCTGATATTCCGAAGCTCGGATACATTGATGCAATAATCAAAGAGACGATGAGATTGTATCCAGTCGGAGCATTAAGCGAACGATACACGACTGAAGAATGCGAGGTTGGTCGGTTTAACGTACCCGCTGGCACACGCTTACTGGTGAATATATGGAAGATCCACAGAGACCCAAGTGTGTGGGAGAATCCATCAGATTTTCAACCAGAGAGGTTTTTGTGCAGCGATAAGGTGGGTGTGGATTTATATGGCCAGAATTATGAGCTGATACCATTTGGGGCCGGTAGGAGGGTATGTCCGGCTATAGTTTCATCACTGCAGACGATGCATTATGCGTTGGCGCGTCTTATTCAAGGATATGAAATGAAATCAGCCAGCCTCGATGGGAAGGTGAATATGGAAGAAATGATAGCCATGTCGTGCCACAAGATGAGCCCTCTTGAAGTTATTATCAGTCCTCGGGAGCCGAGGCGGAGTTAA
N-terminal truncated CjNCS	Codon-optimized	ATGAACGGTAGACCTTTGTTGCACAGAGTCACCAAAGAAGAAACTGTTATGTTGTACCACGAATTGGAAGTTGCTGCTTCTGCTGATGAAGTTTGGTCTGTTGAAGGTTCTCCAGAATTGGGTTTACATTTGCCAGATTTGTTGCCAGCTGGTATTTTTGCCAAGTTCGAAATTACTGGTGATGGTGGTGAAGGTTCCATTTTGGATATGACTTTTCCACCAGGTCAATTCCCACATCATTACAGAGAAAAGTTCGTCTTTTTCGACCACAAGAACAGATACAAGTTGGTCGAACAAATCGATGGTGATTTCTTCGATTTGGGTGTTACTTACTACATGGATACCATTAGAGTTGTTGCTACTGGTCCAGATTCTTGCGTTATTAAGTCTACTACTGAATACCACGTCAAGCCAGAATTTGCTAAAATCGTTAAGCCATTGATCGATACCGTTCCATTGGCTATTATGTCTGAAGCTATTGCCAAGGTCGTCTTGGAAAACAAACATAAGTCATCTGAATGA

Table S3. Cont.

Gene	Codon-optimized or GenBank accession no.	DNA sequence
TyrH^{WR}	Codon-optimized	ATGCCAACTCCATCTGCTCCATCTCCACAACCAAAGGGTTTCAGAAGAGCTGTTTCTGAACAAGACGCTAAGCAAGCTGAAGCTGTTACTTCTCCAAGATTCATCGGTGAAGAACAATCTTTGATCGAAGACGCTAGAAAGGAAAGAGAAGCTGCTGCTGCTGCTGCTGCTGCTGTTGCTTCTTCTGAACCAGGTAACCCATTGGAAGCTGTTGTTTTCAGAAGAGAAGAAAGAGACGGTAACGCTGTTTTGAACTTGTTGTTCTCTTTGAGAGGTACTAAGCCATCTTCTTTGTCTAGAGCTGTTAAGGTTTTCGAAACTTTCGAAGCTAAGATCCACCACTTGGAAACTAGACCAGCTCAAAGACCATTGGCTGGTTCTCCACACTTGGAATACTTCGTTAGATTCGAAGTTCCATCTGGTGACTTGGCTGCTTTGTTGTCTTCTGTTAGAAGAGTTTCTGACGACGTTAGATCTGCTAGAGAAGACAAGGTTCCATACTTCCCAAGAAAGGTTTCTGAATTGGACAAGTGTCACCACTTGGTTACTAAGTTCGACCCAGACTTGGACTTGGACCACCCAGGTTTCTCTGACCAAGTTTACAGACAAAGAAGAAAGTTGATCGCTGAAATCGCTTTCCAATACAAGCACGGTGAACCAATCCCACACGTTGAATACACTGCTGAAGAAATCGCTACTTGGAAGGAAGTTTACGTTACTTTGAAGGGTTTGTACGCTACTCACGCTTGTAGAGAACACTTGGAAGGTTTCCAATTGTTGGAAAGATACTGTGGTTACAGAGAAGACTCTATCCCACAATTGGAAGACGTTTCTAGATTCTTGAAGGAAAGAACTGGTTTCCAATTGAGACCAGTTGCTGGTTTGTTGTCTGCTAGAGACTTCTTGGCTTCTTTGGCTTTCAGAGTTTTCCAATGTACTCAATACATCAGACACGCTTCTTCTCCAATGCACTCTCCAGAACCAGACTGTTGTCACGAATTGTTGGGTCACGTTCCAATGTTGGCTGACAGAACTTTCGCTCAATTCTCTCAAGACATCGGTTTGGCTTCTTTGGGTGCTTCTGACGAAGAAATCGAAAAGTTGTCTACTGTTTACTGGTTCACTGTTGAATTCGGTTTGTGTAAGCAAAACGGTGAATTGAAGGCTTACGGTGCTGGTTTGTTGTCTTCTTACGGTGAATTGTTGCACTCTTTGTCTGAAGAACCAGAAGTTAGAGCTTTCGACCCAGACACTGCTGCTGTTCAACCATACCAAGACCAAACTTACCAACCAGTTTACTTCGTTTCTGAATCTTTCAACGACGCTAAGGACAAGTTGAGAAACTACGCTTCTAGAATCCAAAGACCATTCTCTGTTAAGTTCGACCCATACACTTTGGCTATCGACGTTTTGGACTCTCCACACACTATCCAAAGATCTTTGGAAGGTGTTCAAGACGAATTGCACACTTTGGCTCACGCTTTGTCTGCTATCTCTTGA
ARH1	KZV12618.1	
IDP1	KZV12165.1	
IDP2	KZV09419.1	
IDP3	KZV08616.1	
PDC1	KZV09290.1	
PDC6	KZV11313.1	
ALD4	KZV08266.1	
ALD6	KZV07457.1	
TYR1	KZV13247.1	

Table S4. Tyrosine derivatives tested in this study in addition to 3-halo-tyrosines

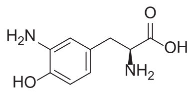
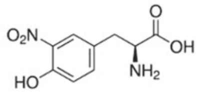
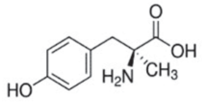
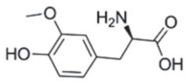
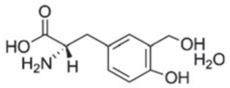
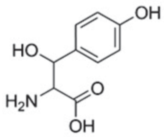
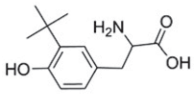
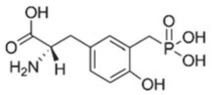
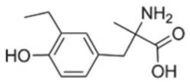
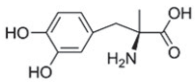
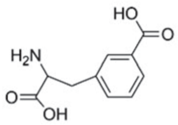
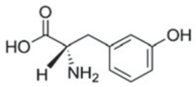
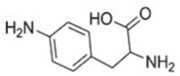
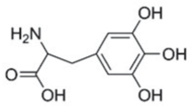
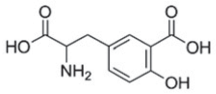
Name	PubChem CID	Structure
3-amino-L-tyrosine	160450	
3-nitro-L-tyrosine	65124	
α-methyl-L-tyrosine	441350	
3-O-methyl-DOPA	13206354	
3-(hydroxymethyl)- L-tyrosine	10081872	
2-amino-3-hydroxy-3-(4-hydroxyphenyl)propanoic acid	13309269	
2-amino-3-(3-tert-butyl-4-hydroxyphenyl)propanoic acid	18352476	
(2*S*)-2-amino-3-[4-hydroxy-3-(phosphonomethyl)phenyl]propanoic acid	15045708	
2-amino-3-(3-ethyl-4-hydroxyphenyl)-2-methylpropanoic acid	18544337	
D-α-methyl DOPA	721860	
3-(2-Amino-2-carboxyethyl)benzoic acid	265274	
L-m-tyrosine	6950578	
4-aminophenylalanine	95174	
2-amino-3-(3,4,5-trihydroxyphenyl)propanoic acid	22283619	
3-carboxytyrosine	583884	

Table S5. MRM transitions used to quantify alkaloids in LC–MS/MS analysis

	Quantifier MRM transition			Qualifier MRM transition		
Compound	Precursor → product ion	Fragmentor	Collision energy	Precursor → product ion	Fragmentor	Collision energy
Reticuline	330 → 192	120	19	330 → 137	120	31
Scoulerine	328 → 151	135	30	328 → 178	135	29
Tetrahydrocolumbamine	342 → 178	135	29	342 → 163	135	29
Noscapine	414 → 220	138	23	414 → 353	138	23
Canadine	340 → 176	135	29	340 → 149	135	25
N-methylcanadine	354 → 190	135	25	354 → 188	135	25
1-Hydroxy-*N*-methylcanadine	370 → 206	135	25	–*	–*	–*
1,13-Dihydroxy-*N*-methylcanadine	386 → 206	135	25	–*	–*	–*
1-Hydroxy-13-O-acetal-*N*-methylcanadine	428 → 206	135	25	428 → 368	135	25
4′-O-desmethyl-3-O-acetylpapaveroxine	444 → 206	138	23	444 → 398	138	23
Narcotolinehemiacetal	402 → 206	138	23	–*	–*	–*
Narcotoline	400 → 206	138	23	400 → 339	138	23

*The qualifier transitions of 1-hydroxy-*N*-methylcanadine, 1,13-dihydroxy-*N*-methylcanadine, and narcotolinehemiacetal are not determined; the identities of 1-hydroxy-*N*-methylcanadine, 1,13-dihydroxy-*N*-methylcanadine, and narcotolinehemiacetal are confirmed by the retention time.