Supplemental Table 1. Sequences of PCR primers used to assemble expression and VIGS constructs, confirm TRV infection, and perform real-time quantitative (RT-q)PCR analysis.

***Pichia pastoris* expression constructs**

Primers used for sequence amplification and ligation into pPINK

Sequence name	Forward primer 5'-3' (*Stu*1)	Reverse primer 5'-3' (*Kpn*1)	Insert size (bp)
pPINK-FADOX1	GCGCAGGCCTATCGATACTGGCAAATTTC	GCGCGGTACCTCAAAATAATCCATCACTT	1509
pPINK-FADOX3	GCGCAGGCCTTTTGGAACTTTATCAACAA	GCGCGGTACCAGTGCATGCTTCAATAAGT	1551
pPINK-FADOX5	GCGCAGGCCTGCTAATAATTCACTTAATG	GCGCGGTACCCTACTGCTTGCCCCAAGTA	1536
pPINK-FADOX7	GCGCAGGCCTTCAAGTGAATATGAAGATGATTATAACTTT	GCGCGGTACCTTAATATGAAACCGCAGGAATACTCT	1518
pPINK-FADOX8	GCAGGCCTATCTTCTCGAATGTGTTGGCAACAA	GCGGTACCTTAAATCTTTAATACATAGGCAATACTTGGTAT	1539
pPINK-FADOX5-HIS	ATAGGCCTGCTAATAATTCACTTAATGGAGATTTTCTC	ATGGTACCCTAATGATGATGATGATGATGCTGCTTGCCCC AAGTACTAACA	1554
pPINK-BBE1-HIS	ATAGGCCTGGTGATGTTAATGATAATCTCCTCTC	ATGGTACCCTAATGATGATGATGATGATGCAATTCCTTCAA CATGTAAATTTCCTCAAAT	1626
pPINK-BBE2-HIS	ATAGGCCT GGTGATGTTAATGATAATCTCCTCTC	ATGGTACCCTAATGATGATGATGATGATGCAGTTCCTTCAA CATGTAATTTTCCTC	1626

VIGS constructs

Primers used for sequence amplification and ligation into pTRV2

Sequence name	Forward primer 5'-3' (*Bam*HI)	Reverse primer 5'-3' (*Xho*1)	Insert size (bp)
pTRV2-FADOX1	GGATCCCCATACGTTTCGAAGAAC	CTCGAGGACGGAGCACTGACTGGGTAA	341
pTRV2-FADOX3	GGATCCCCATTTGTTTCAAAATGT	CTCGAGACTGTAGAAAAATGTCAGA	392
pTRV2-BBE	GGATCCGGTGTCCGACTGTTGGTAG	CTCGAGTGGTATCTAATCCTGATAAGAAAGC	528
pTRV2-DBOX	GGATCCCGTTATGTTTCCAAGAATC	CTCGAGGAAAGACATAAACTCTTTC	410
pTRV2-FADOX8	GGATCCCCCTGTTTCTTGGGGATGCTAA	CTCGAGGTTTTTCGTTCCACGAAGATACATATAAAA	392

Confirmation of TRV infection

Primers used for the detection of TRV in *A. tumefaciens*-infiltrated plants

Sequence name	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
TRV-coat protein	ATGGGAGATATGTACGATG	TAGGGATTAGGACGTATC	613

RT-qPCR analysis of VIGS plants
Primers used to quantify gene expression

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Product Size (bp)
FADOX1	CATTCCAAGTGATGGATTATTTTGAA	GACGGAGCACTGACTGGGTAA	72
FADOX3	CATGCACTAGAGATGCTAATGTATCG	CATTGAGTTTTGACAGATTTCACAGTAG	73
BBE	CGGAAACAGCTTGGGTTGAA	CTGCGCAATCGCATAGTAGAGT	62
DBOX	GGTTTGGTGCCTTGGTACGA	GTGACAATGCGAGCATCAATG	67
FADOX8	CAATGCAGAGAGGAATGAGACTAGAA	CATCAGTTCATGACGGAATCAACT	78
Ubiquitin	CCATTTGGTGCTTCGTCTAC	CAAGCCATAGCTGAAACACC	276

RT-qPCR analysis of plant organs
Primers used to quantify gene expression

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Product Size (bp)
BBE	CGGAAACAGCTTGGGTTGA	CGGACACCAACCAGCAGTAA	101
DBOX	GTACTTGGGGCAAGCAGTAGAGAT	GCAGCTTATATGGACTGCACTGTATTA	132
Ubiquitin	CCATTTGGTGCTTCGTCTAC	CAAGCCATAGCTGAAACACC	276

***Saccharomyces cereviciae* expression constructs**
Primers used for sequence amplification and ligation into pYES2

Primer Name	Sequence (5'-3')
pYES2 HA Tag F	TACCCATACGATGTTCCAGATTACGCTTAAATCATGTAATTAGTTATGTCACGCTTAC
pYES2 HA Tag R	CTCCTTGACGTTAAAGTATAGAGG
DBOX F	ATATACCTCTATACTTTAACGTCAAGGAGAAAACAATGGCTAATAATTCACTTAATGGAG
DBOX HA Tag R	TTAAGCGTAATCTGGAACATCGTATGGGTACTGCTTGCCCCAAGTACTAAC
pYES F	CCTGCATTAATGAATCGGC
pYES R	ACTAGTGGATCATCCCCAC
6OMT F	GAAGATAAGAAAGATTTAATTATCAAAAAAACAATGGAAACAGTATCAAAGATCGAC
6OMT R	GAATGTAAGCGTGACATAACTAATTACATGATTAATATGGATAGGCTTCGATCACG
4OMT F	AGTTTTAAAACACCAAGAACTTAGTTTCGAAAAACAATGGGTTCCTTGGATGCG
4OMT R	GACCAAACCTCTGGCGAAGAAGTCCATTATGGAAAAGCTTCTATAACAGATTGTATTG
PMA1 F	GAGACTGCAGCATTACTTTGAGAAGACAGGCATTGCTGGGATCAC
PMA1 R	AGTAGATACACACTGACTCTGGACTTTGATAATTAAATCTTTCTTATCTTCTTATTCTTTTC
TDH3 F	GAGACTGCAGCATTACTTTGAGAAGTCGAGTTTATCATTATCAATAC
TDH3 R	AGTAGATACACACTGACTCTGGACTCGAAACTAAGTTCTTGGTG
CYC1 F	TCACTTACACGAGGAGATGCATTGTCATGTAATTAGTTATGTCACGCTTACATTC
CYC1 R	ACAACTCATGGTGATGTGATTGCCGCAAATTAAAGCCTTCGAGCGTC

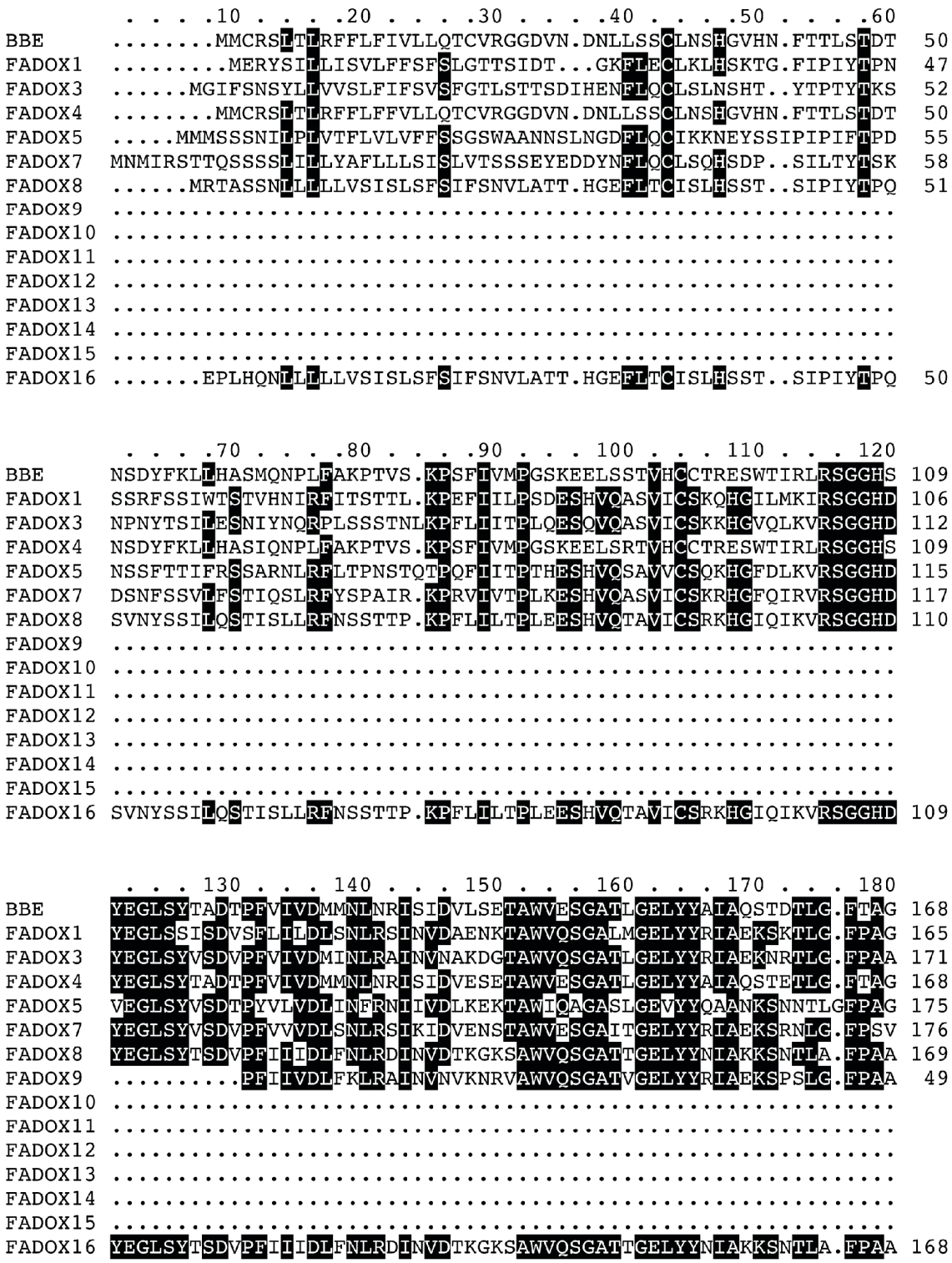
ADH1 F	TCACTTACACGAGGAGATGCATTGTGGACTTCTTCGCCAGAGGTTTG
ADH1 R	ACAACTCATGGTGATGTGATTGCCGCATGCCGGTAGAGGTGTGG
pYES:C1 F	CCGCCGCGCTTAATGGGGCGCTACAGGGCGCGTGGGGATGATCCACTAGTGAGACTGCAGCATTACTTTGAGAAG
PMA1 R2	TTGATACTGTTTCCATTGTTTTTTTGATAATTAAATCTTTCTTATCTTCTTATTCTTTTC
CYC1 F2	CGTGATCGAAGCCTATCCATATTAATCATGTAATTAGTTATGTCACGCTTACATTC
C6:H1 R	CAATCTCGCCCACAGCCCCTTTCTTTAATCATTCCGACCCCCGCCATGAGACAACTCATGGTGATGTGATTGCC
C1:H1 F	CTCATGGCGGGGGTCGGAATGATTAAAGAAAGGGGCTGTGGGCGAGATTGGAGACTGCAGCATTACTTTGAGAAG
TDH3 R2	GGCTTCGCATCCAAGGAACCCATTGTTTTTCGAAACTAAGTTCTTGGTGTTTTAAAACT
ADH1 F2	GAGCAATACAATCTGTTATAGAAGCTTTTCCATAATGGACTTCTTCGCCAGAGGTTTG
pYES:C6 R	CAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGGACAACTCATGGTGATGTGATTGCC

Supplemental Table 2. Compound list and CID spectra used for the identification and quantification of benzylisoquinoline alkaloids by LC-MS/MS in plants subjected to virus-induced gene silencing.

Compound	$[M+H]^+$	Retention time (min)	CE (eV)	ESI^+-CID, m/z (Relative abundance)	Reference
N-Methylcoclaurine	300	6.27	30	300 (18), 269 (26), 237 (16), 219 (3), 209 (15), 192 (10), 175 (13), 161 (8), 145 (14), 143 (31), 121 (40), 107 (100)	Schmidt et al. 2007[a]
Thebaine	312	6.49	30	312 (2), 281 (2), 266(4), 255 (1), 251 (11), 249 (2), 237 (1), 234 (2), 223 (2), 221 (7), 218 (4), 195 (2), 177 (1), 58 (100)	Authentic standard
Coptisine	320	12.63	30	320 (100), 318 (7), 292 (30), 290 (6), 277 (6), 262 (4), 249 (2), 234 (2)	Wang et al., 2004[b]
Stylopine	324	11.54	30	324 (24), 265 (1), 176 (100), 174 (3), 149 (26), 119 (2), 91 (2)	Authentic standard
Dehydroscoulerine	326	10.13	35	326 (14), 311 (59), 310 (100), 296 (8), 295 (7), 294 (17), 282 (52), 268 (14), 267 (20)	Inferred
Scoulerine	328	7.22	30	328 (25), 178 (100), 151 (5)	Authentic standard
Reticuline	330	5.25	30	330 (1), 299 (1), 298 (36), 267 (3), 239 (3), 227 (1), 207 (2), 192 (100), 175 (21), 151 (4), 145 (1), 143 (21), 137 (33)	Authentic standard
Sanguinarine	332	7.71	35	332 (76), 330 (24), 317 (72), 304 (58), 302 (26), 289 (13), 276 (16), 275 (17), 274 (100), 261 (7), 248 (12), 246 (49), 244 (21), 218 (25), 216 (15), 189 (4)	Authentic standard
Dihydrosanguinarine	334	12.81	35	334 (7), 319 (88), 318 (100), 276 (11)	Authentic standard
Berberine	336	6.40	35	336 (67), 321 (77), 320 (100), 306 (21), 304 (17), 292 (82), 278 (4), 275 (5)	Authentic Standard
Columbamine	338	9.53	35	338 (90), 323 (100), 322 (8), 308 (3), 294 (6), 279 (1)	Zhang et al., 2006[c]; Liscombe et al., 2009[d]
Papaverine	340	8.12	35	340 (89), 324 (65), 296 (10), 202 (100), 171 (13)	Authentic standard
Canadine	340	10.89	25	340 (13), 176 (100), 165 (3), 149 (9)	Authentic standard

Tetrahydrocolumbamine	342	8.50	20	342 (36), 192 (5), 178 (100), 165 (4), 151 (13)	Authentic standard
Tetrahydropapaverine	344	7.83	20	344 (2), 327 (11), 312 (2), 296 (6), 281 (5), 192 (100), 189 (36), 174 (6), 165 (3), 158 (2), 151 (27), 136 (5)	Authentic standard
Chelerythrine	348	7.82	25	348 (54), 333 (44), 332 (100), 318 (30), 316 (9), 315 (9), 304 (56), 303 (3), 290(12), 287 (5)	Authentic standard
Dihydrochelerythrine	350	12.72	25	350 (58), 335 (25), 334 (100), 320 (38), 306 (53), 292 (8)	Authentic standard
Palmatine	352	10.74	25	352 (50), 337 (53), 336 (100), 322 (28), 320 (17), 308 (69), 294 (7)	Wang et al., 2004[b]; Zhang et al., 2006[c]
Protopine	354	6.71	30	354 (100), 336 (8), 275 (19), 247 (9), 206 (22), 189 (70), 188 (65), 165 (11), 149 (31)	Authentic standard
Tetrahydropalmatine	356	9.98	20	356 (48), 192 (100), 165 (27), 150 (3)	Authentic standard
Noscapine	414	10.84	25	414 (4), 365 (5), 353 (21), 323 (5), 220 (100), 206 (4), 179 (8)	Authentic standard

[a] Schmidt, J., Boettcher, C., Kuhnt, C., Kutchan, T.M., and Zenk, M.H. (2007) *Phytochemistry* **68**, 189-202
[b] Wang, D., Liu, Z., Guo, M., and Liu S. (2004) *J. Mass Spec.* **39**, 1356-1365
[c] Zhang, Y., Shi, Q., Shi, P., Zhang, W., and Cheng, Y. (2006) *Rap. Comm. Mass Spectrom.* **20**, 2328-2342
[d] Liscombe DK, Ziegler J, Schmidt J, Ammer C, and Facchini PJ (2009) *Plant J.* **60**, 729-743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FADOX1 .....MERYSILLISVLFFSFSLGTTSIDT...GKFLECLKLHSKTG.FIPIYTPN 47
FADOX3 .....MGIFSNSYLLVVSLFIFSVSFGTLSTTSDIHENFLQCLSLNSHT..YTPTYTKS 52
FADOX4 .....MMCRSLTLRFFLFFVLLQTCVRGGDVN.DNLLSSCLNSHGVHN.FTTLSTDT 50
FADOX5 .....MMMSSSNILPLVTFLVLVFFSSGSWAANNSLNGDFLQCIKKNEYSSIPIPIFTPD 55
FADOX7 MNMIRSTTQSSSSLILLYAFLLLSISLVTSSSEYEDDYNFLQCLSQHSDP..SILTYTSK 58
FADOX8 .....MRTASSNLLLLLVSISLSFSIFSNVLATT.HGEFLTCISLHSST..SIPIYTPQ 51
FADOX9 .....
FADOX10 .....
FADOX11 .....
FADOX12 .....
FADOX13 .....
FADOX14 .....
FADOX15 .....
FADOX16 .....EPLHQNLLLLLVSISLSFSIFSNVLATT.HGEFLTCISLHSST..SIPIYTPQ 50
. . . .70 . . . .80 . . . .90 . . . 100 . . . 110 . . . 120
BBE NSDYFKLLHASMQNPLFAKPTVS.KPSFIVMPGSKEELSSTVHCCTRESWTIRLRSGGHS 109
FADOX1 SSRFSSIWTSTVHNIRFITSTTL.KPEFIILPSDESHVQASVICSKQHGILMKIRSGGHD 106
FADOX3 NPNYTSILESNIYNQRPLSSSTNLKPFLIITPLQESQVQASVICSKKHGVQLKVRSGGHD 112
FADOX4 NSDYFKLLHASIQNPLFAKPTVS.KPSFIVMPGSKEELSRTVHCCTRESWTIRLRSGGHS 109
FADOX5 NSSFTTIFRSSARNLRFLTPNSTQTPQFIITPTHESHVQSAVVCSQKHGFDLKVRSGGHD 115
FADOX7 DSNFSSVLFSTIQSLRFYSPAIR.KPRVIVTPLKESHVQASVICSKRHGFQIRVRSGGHD 117
FADOX8 SVNYSSILQSTISLLRFNSSTTP.KPFLILTPLEESHVQTAVICSRKHGIQIKVRSGGHD 110
FADOX9 .....
FADOX10 .....
FADOX11 .....
FADOX12 .....
FADOX13 .....
FADOX14 .....
FADOX15 .....
FADOX16 SVNYSSILQSTISLLRFNSSTTP.KPFLILTPLEESHVQTAVICSRKHGIQIKVRSGGHD 109
. . . 130 . . . 140 . . . 150 . . . 160 . . . 170 . . . 180
BBE YEGLSYTADTPFVIVDMMNLNRISIDVLSETAWVESGATLGELYYAIAQSTDTLG.FTAG 168
FADOX1 YEGLSSISDVSFLILDLSNLRSINVDAENKTAWVQSGALMGELYYRIAEKSKTLG.FPAG 165
FADOX3 YEGLSYVSDVPFVIVDMINLRAINVNAKDGTAWVQSGATLGELYYRIAEKNRTLG.FPAA 171
FADOX4 YEGLSYTADTPFVIVDMMNLNRISIDVESETAWVESGATLGELYYAIAQSTETLG.FTAG 168
FADOX5 VEGLSYVSDTPYVLVDLINFRNIIVDLKEKTAWIQAGASLGEVYYQAANKSNNTLGFPAG 175
FADOX7 YEGLSYVSDVPFVVVDLSNLRSIKIDVENSTAWVESGAITGELYYRIAEKSRNLG.FPSV 176
FADOX8 YEGLSYTSDVPFIIIDLFNLRDINVDTKGKSAWVQSGATTGELYYNIAKKSNTLA.FPAA 169
FADOX9 ..........PFIIVDLFKLRAINVNVKNRVAWVQSGATVGELYYRIAEKSPSLG.FPAA 49
FADOX10 .....
FADOX11 .....
FADOX12 .....
FADOX13 .....
FADOX14 .....
FADOX15 .....
FADOX16 YEGLSYTSDVPFIIIDLFNLRDINVDTKGKSAWVQSGATTGELYYNIAKKSNTLA.FPAA 168

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FADOX1    VCPTVGVGGLFSGGGYGTLLRKYGLAADNVIDAQIVDVNGKILDRESMGEDLFWAIRGGA 225
FADOX3    VCTTVGVGGQFSGGGYGSLLRKYGVAGDNVIDVRIVDAHGQILNKDTMGEDLFWAIRGGG 231
FADOX4    WCPTVGSGGHISGGGFGMMSRKYGLAADNVVDAILIDSTGSILDREKMGEDVFWAIRGGG 228
FADOX5    FCPTVGVAGHISGGGFGALVRKYGLASDQVIDARIVTVDGKIYTKETMGKDLYWAIRGGG 235
FADOX7    FCPTVGVGGSFQGGGYGNMLRKYGLAADNVIDARIVDAQGRVLDKESMGEDLFWAIRGGG 236
FADOX8    ICTTVGIGGHISGGGYGSMLRKYGTAGDNVIDARIVDVHGRILNRKSMGEGLFWAIRGGG 229
FADOX9    FCATVGVGGFLSGGGYGSMFRKYGLAADNVINARIVDVHGKILDKRSMGKDLFWAIRGGG 109
FADOX10   ..............................KITQIHICNQNNLLQIKSSICNTRNTV  27
FADOX11   ..............................................................
FADOX12   ..............................................................
FADOX13   ..............................................................
FADOX14   ..............................................................
FADOX15   .........QFSGGGYGSMLRKYGLADDNVIDIRVVDARGKILNKETMGKDLFWAIRGGG  51
FADOX16   ICTTVGIGGHISGGGYGSDVKEIWN.............................. 193

          . . . 250 . . . 260 . . . 270 . . . 280 . . . 290 . . . 300
BBE       GGVWGAIYAWKIKLLPVPEKLTVFRVTKNVGIED.ASSLLHKWQYVADELDEDFTVSVLG 287
FADOX1    PGSFGVILSWKIRLVYVPPTVTVFRIEKLLRDDADLTSLVYRWQEVAHKLPRELFIRAGI 285
FADOX3    GGSFGVVLSWKIKLVPVPPTVTMFTITKTLEE..GATGLVHKWQEVAPKFPNELFMRVIL 289
FADOX4    GGVWGAVYAWKIKLLPVPEKLTVFRVTKNVGIEG.ASSLLHKWQYVADELDEDFTVSVLG 287
FADOX5    ANNFGVLLSWKVKLVPVTPVVTVATISRTLEQG..ATDLVHKWQFVADRLHEDVYIGLTF 293
FADOX7    .MSFGIVVSWKIRLVYVPTNVTVFTINKNLDQ..GATKLVHRWQEVASELPHELFVRVSI 293
FADOX8    GGSFGIVLSWKLRLVSVPPTVTVFSVGKTLAEGATSLVHKWQDIAHKLPQELLIFLTLRV 289
FADOX9    GGSFGIVLSWKIRLVDVPPTVALCSVKKSQED..GATKIVHKWQEVAHKLPQEVFLDVDL 167
FADOX10   DVAKEIVLSWKIRLVSVPPTVTVFSVGKTLAER..ATSLVRKWQKVAHKLPHDLFIMLGV  85
FADOX11   .......SFMEDQIVTVPPVVTVFSVGKTLAEG..ATSLVHKWQQVAHKLPHDLFLQLGL  51
FADOX12   ..............................................................
FADOX13   ..............................................................
FADOX14   ...................VTVFSPGRVLEQD..ATKLVMKWQQVADKLPQDLFIRL..  36
FADOX15   GGSFGVVLSWKIKLVPVPPTVTIFTVTKTLEE..GATGLVHKWQEVAPKFPNELFMR... 106
FADOX16   ..............................................................

          . . . 310 . . . 320 . . . 330 . . . 340 . . . 350 . . . 360
BBE       GVNG.......NDAWLMFLGLHLGRKDAAKTIIDEKFPELGLVDKEFQEMSWGESMAFLS 340
FADOX1    APVG...TNERRTIKASFISLSLGDTKTLVPVMKEGFPELGLESKDCIEMSWIRSVLWYA 342
FADOX3    TLGTSTTQKGKKTIQASFNSMYLGTTTKLLLVMKERFPELGLTSKDCTETSWVRSTLYFG 349
FADOX4    GVNG.......NDAWLMFLGLYLGREDAAKTIIDEKFPELGSVEEEFQEMSWVASTAFLS 340
FADOX5    SVANS.SRAGGKTVSVQFAFLFLGGSDRLLELMEESFPELGLKRNETTEMKWVESHVYFY 352
FADOX7    SVVNSTTKEGQKTILASFPSLYLGNTENLLAVMKERFPELGLETKDCAEMTWIQSQLYIN 353
FADOX8    VNAT...TKDEKTVRATFRSLFLGDAKKLKIVMKQTFPELGLESKDSIEMSWIQSVLFLD 346
FADOX9    TAVN..ATGNNKTFLASFNSVYLGGVEKFQIVMKERFPELGLETKDCIEMSWIQSVLFSS 225
FADOX10   GVVDS.TPTGNKTILTTFTSLFLGDSKKLQTVMKERFPELGLEAKDCIEMSWLRSVLFLD 144
FADOX11   SVVDA.TPKEEKTILATFDSMYLGDAKKLQTIMQDKFPELGLESKDYIEMSWIQSVLLFD 110
FADOX12   ..............................................................
FADOX13   ..............................................................
FADOX14   VVQLG.TWNGKKTVQVVFNSLYLGGAQDLLKIMQQSFPELGLTTNDCKEMSWIDSVVNIG  95
FADOX15   ..............................................................
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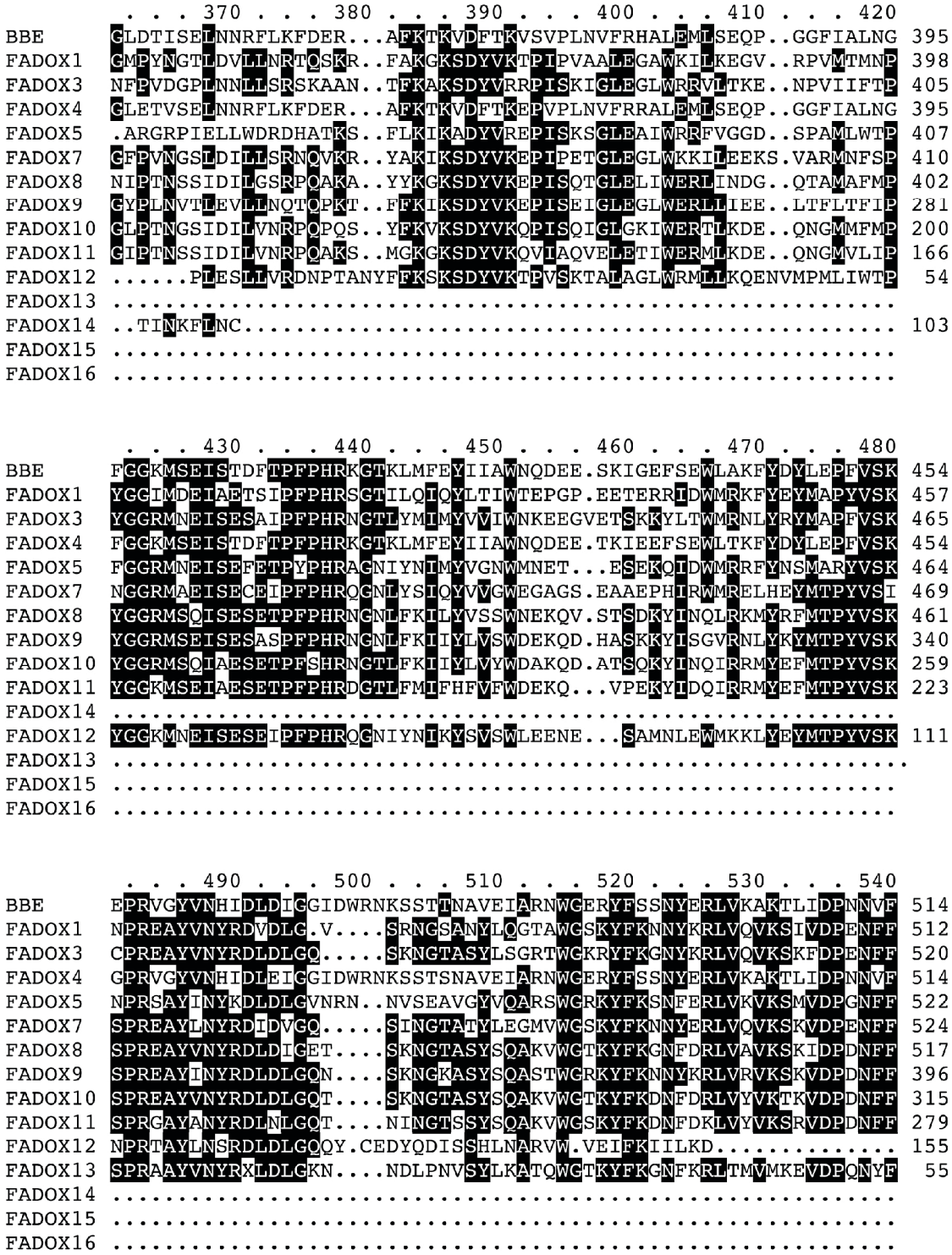
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FADOX1 GMPYNGTLDVLLNRTQSKR..FAKGKSDYVKTPIPVAALEGAWKILKEGV..RPVMTMNP 398
FADOX3 NFPVDGPLNNLLSRSKAAN..TFKAKSDYVRRPISKIGLEGLWRRVLTKE..NPVIIFTP 405
FADOX4 GLETVSELNNRFLKFDER...AFKTKVDFTKEPVPLNVFRRALEMLSEQP..GGFIALNG 395
FADOX5 .ARGRPIELLWDRDHATKS..FLKIKADYVREPISKSGLEAIWRRFVGGD..SPAMLWTP 407
FADOX7 GFPVNGSLDILLSRNQVKR..YAKIKSDYVKEPIPETGLEGLWKKILEEKS.VARMNFSP 410
FADOX8 NIPTNSSIDILGSRPQAKA..YYKGKSDYVKEPISQTGLELIWERLINDG..QTAMAFMP 402
FADOX9 GYPLNVTLEVLLNQTQPKT..FFKIKSDYVKEPISEIGLEGLWERLLIEE..LTFLTFIP 281
FADOX10 GLPTNGSIDILVNRPQPQS..YFKVKSDYVKQPISQIGLGKIWERTLKDE..QNGMMFMP 200
FADOX11 GIPTNSSIDILVNRPQAKS..MGKGKSDYVKQVIAQVELETIWERMLKDE..QNGMVLIP 166
FADOX12 ......PLESLLVRDNPTANYFFKSKSDYVKTPVSKTALAGLWRMLLKQENVMPMLIWTP 54
FADOX13 .....
FADOX14 ..TINKFLNC..... 103
FADOX15 .....
FADOX16 .....
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FADOX1 YGGIMDEIAETSIPFPHRSGTILQIQYLTIWTEPGP.EETERRIDWMRKFYEYMAPYVSK 457
FADOX3 YGGRMNEISESAIPFPHRNGTLYMIMYVVIWNKEEGVETSKKYLTWMRNLYRYMAPFVSK 465
FADOX4 FGGKMSEISTDFTPFPHRKGTKLMFEYIIAWNQDEE.TKIEEFSEWLTKFYDYLEPFVSK 454
FADOX5 FGGRMNEISEFETPYPHRAGNIYNIMYVGNWMNET...ESEKQIDWMRRFYNSMARYVSK 464
FADOX7 NGGRMAEISECEIPFPHRQGNLYSIQYVVGWEGAGS.EAAEPHIRWMRELHEYMTPYVSI 469
FADOX8 YGGRMSQISESETPFPHRNGNLFKILYVSSWNEKQV.STSDKYINQLRKMYRFMTPYVSK 461
FADOX9 YGGRMSEISESASPFPHRNGNLFKIIYLVSWDEKQD.HASKKYISGVRNLYKYMTPYVSK 340
FADOX10 YGGRMSQIAESETPFSHRNGTLFKIIYLVYWDAKQD.ATSQKYINQIRRMYEFMTPYVSK 259
FADOX11 YGGKMSEIAESETPFPHRDGTLFMIFHFVFWDEKQ...VPEKYIDQIRRMYEFMTPYVSK 223
FADOX14 .....
FADOX12 YGGKMNEISESEIPFPHRQGNIYNIKYSVSWLEENE...SAMNLEWMKKLYEYMTPYVSK 111
FADOX13 .....
FADOX15 .....
FADOX16 .....
. . . 490 . . . 500 . . . 510 . . . 520 . . . 530 . . . 540
BBE EPRVGYVNHIDLDIGGIDWRNKSSTTNAVEIARNWGERYFSSNYERLVKAKTLIDPNNVF 514
FADOX1 NPREAYVNYRDVDLG.V....SRNGSANYLQGTAWGSKYFKNNYKRLVQVKSIVDPENFF 512
FADOX3 CPREAYVNYRDLDLGQ.....SKNGTASYLSGRTWGKRYFKGNYKRLVQVKSKFDPENFF 520
FADOX4 GPRVGYVNHIDLEIGGIDWRNKSSTSNAVEIARNWGERYFSSNYERLVKAKTLIDPNNVF 514
FADOX5 NPRSAYINYKDLDLGVNRN..NVSEAVGYVQARSWGRKYFKSNFERLVKVKSMVDPGNFF 522
FADOX7 SPREAYLNYRDIDVGQ.....SINGTATYLEGMVWGSKYFKNNYERLVQVKSKVDPENFF 524
FADOX8 SPREAYVNYRDLDIGET....SKNGTASYSQAKVWGTKYFKGNFDRLVAVKSKIDPDNFF 517
FADOX9 SPREAYINYRDLDLGQN....SKNGKASYSQASTWGRKYFKNNYKRLVRVKSKVDPDNFF 396
FADOX10 SPREAYVNYRDLDLGQT....SKNGTASYSQAKVWGTKYFKDNFDRLVYVKTKVDPDNFF 315
FADOX11 SPRGAYANYRDLNLGQT....NINGTSSYSQAKVWGSKYFKDNFDKLVYVKSRVDPDNFF 279
FADOX12 NPRTAYLNSRDLDLGQQY.CEDYQDISSHLNARVW.VEIFKIILKD..... 155
FADOX13 SPRAAYVNYRXLDLGKN....NDLPNVSYLKATQWGTKYFKGNFKRLTMVMKEVDPQNYF 55
FADOX14 .....
FADOX15 .....
FADOX16 .....

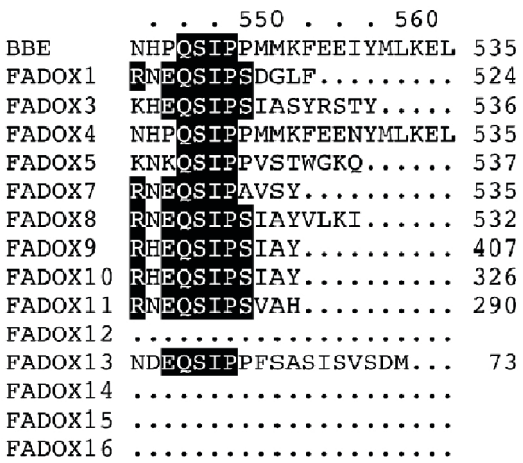

FIGURE S1. **Alignment of deduced amino acid sequences of opium poppy FAD-dependent oxidases (FADOXs) with opium poppy berberine bridge enzyme (BBE1).** Sequences were aligned using ClustalX. Shaded boxes indicate residues that are identical in at least 40% of the aligned proteins. Dots represent gaps introduced into sequences to maximize the alignment.

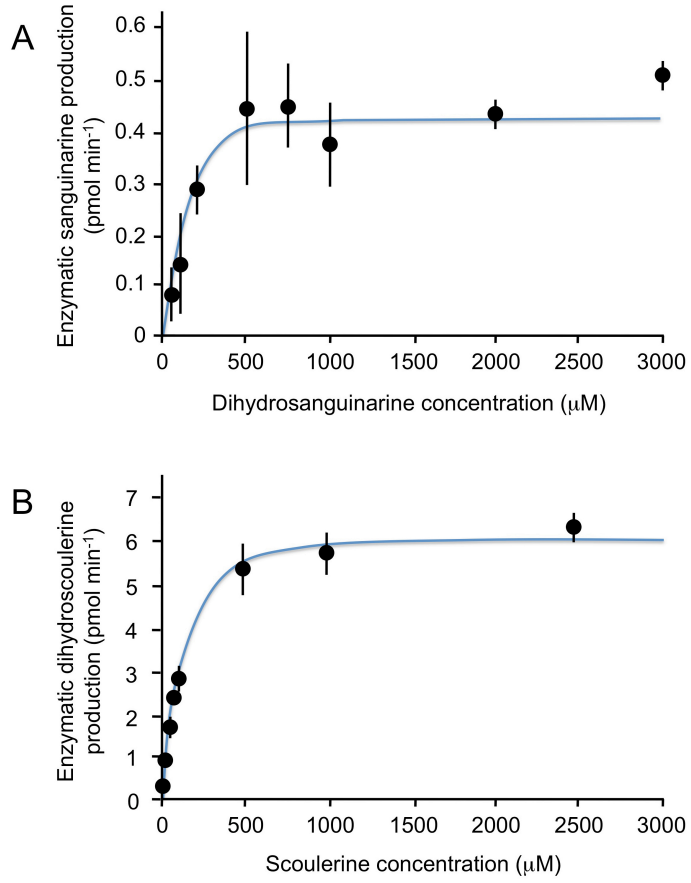

FIGURE S2. **Steady-state enzyme kinetics of recombinant DBOX with varying concentrations of (*S*)-scoulerine (*A*) and dihydrosanguinarine (*B*) substrates.** Enzyme assays were conducted as described in Experimental Procedures. Incubation time and protein quantity were adjusted prior to final analyses to ensure linear-range conditions. Points represent mean ± standard deviation of three (for scoulerine) and five (for dihydrosanguinarine) independent experiments. Curve-fitting and K_m determinations were performed using GraphPad Prism 5.

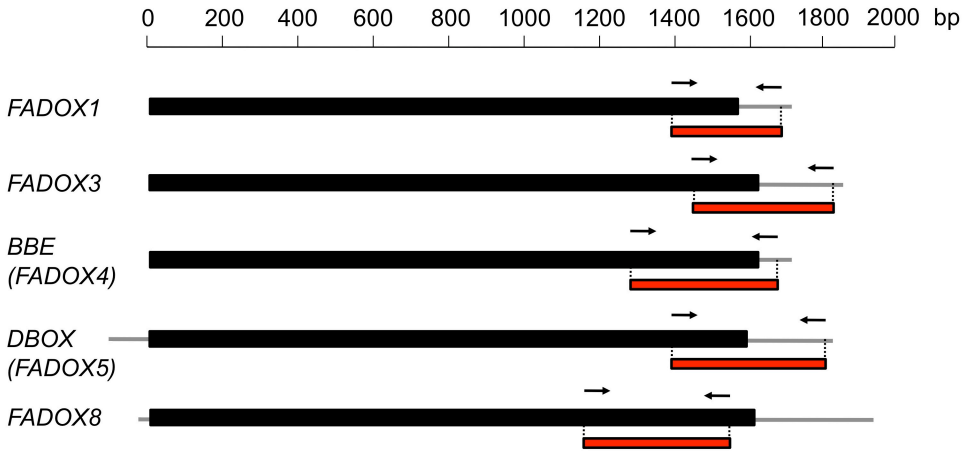

FIGURE S3. **Regions of various FADOX cDNAs used to construct virus-induced gene silencing (VIGS) vectors in pTRV2.** Coding regions of each cDNA are shown as thick black line elements, whereas non-coding 5'- and 3'-untranslated regions (UTRs) are shown as thin lines. Red segments represent unique regions in each cDNA used to construct gene-specific VIGS vectors. Sequence lengths are shown in base pairs (bp) with respect to the start codon in each cDNA. For pTRV2-BBE, a nearly identical region in *BBE1* and *BBE2* was selected.

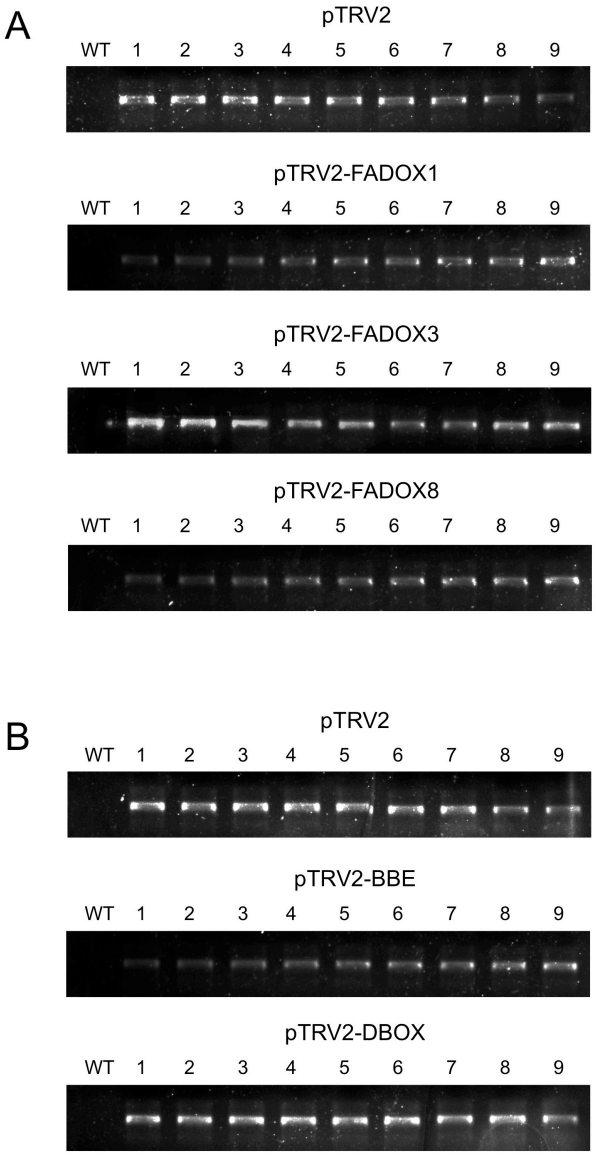

FIGURE S4. **Detection of tobacco rattle virus in individual opium poppy plants infiltrated with various pTRV2 vectors, and used for gene expression and alkaloid profile analyses.** RT-PCR was performed using primers listed in supplemental Table S1. The 613-base pair amplicon corresponding to tobacco rattle virus-coat protein transcripts was detected on agarose gels stained with ethidium bromide.

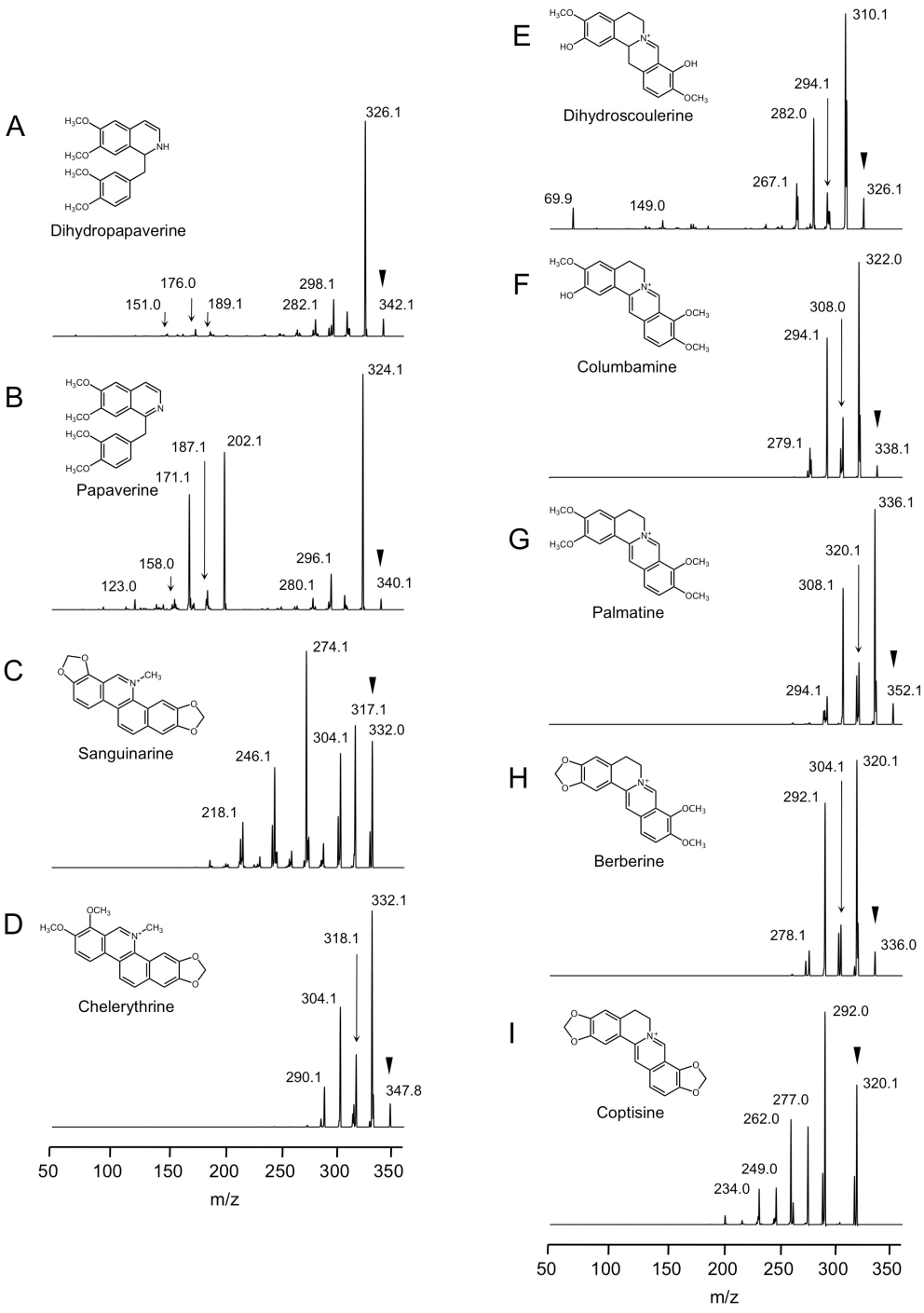

FIGURE S5. **Collision-induced dissociation mass spectra for reaction products of DBOX (FADOX5) enzyme assays.** Following liquid chromatography, molecular parent ions (arrowheads) were generated and focused using electrospray ionization (ESI) and subjected to mass spectrometry. To identify or characterize compounds, daughter ions were generated using argon gas collision at either -30 eV (benzophenanthridines) or -35 eV (1-benzylisoquinolines and protoberberines). Parent ion structures are shown. In panel A, the structure of only one isoform (1,2-dihydropapaverine) is shown, although CID analysis does not preclude the occurrence of 3,4-dihydropapaverine.

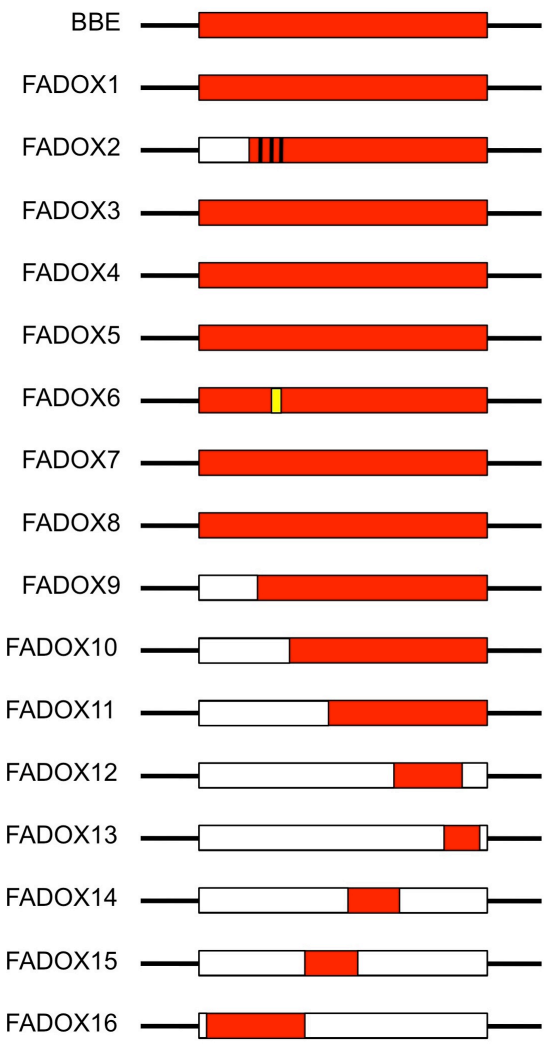

FIGURE S6. **Schematic representation of sequence coverage for sixteen independently assembled contigs representing opium poppy FAD-dependent oxidases (FADOXs) with homology to berberine bridge enzyme (BBE).** Red designates open reading frame (ORF) regions for which DNA sequence was available, whereas the remaining open-box regions indicate putatively missing ORF sequence. Black lines flanking each ORF represent untranslated regions (UTRs). FADOX2 contained three stop codons (black stripes) and FADOX6 showed a sequence gap (yellow stripe) compared with BBE.

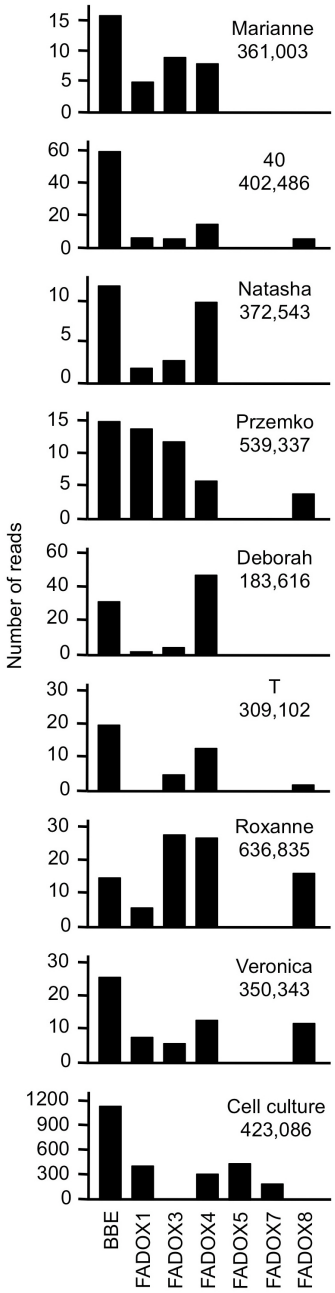

FIGURE S7. **Expression levels of opium poppy berberine bridge enzyme (BBE1) and FAD-dependent oxidoreductases (FADOXs) as indicated by the number of reads in nine different Roche 454-based sequence libraries.** BBE1 and FADOX read abundances are shown for stem databases representing eight different opium poppy chemotypes and a single sanguinarine-accumulating opium poppy cell culture database. The total read count for each library is indicated under the library name in each panel.

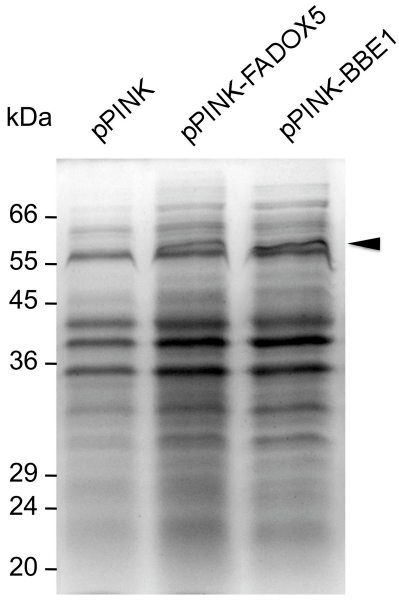

FIGURE S8. **SDS-PAGE of recombinant FADOX5 and BBE proteins produced in *Pichia pastoris*.** Each lane represents 4% (v/v) of total protein obtained from 100-mL of *P. pastoris* culture medium following 100 h of induction. Yeast harboring empty pPINKα-HC vector were included as a negative control. Coomassie Brilliant Blue staining was used to visualize proteins.

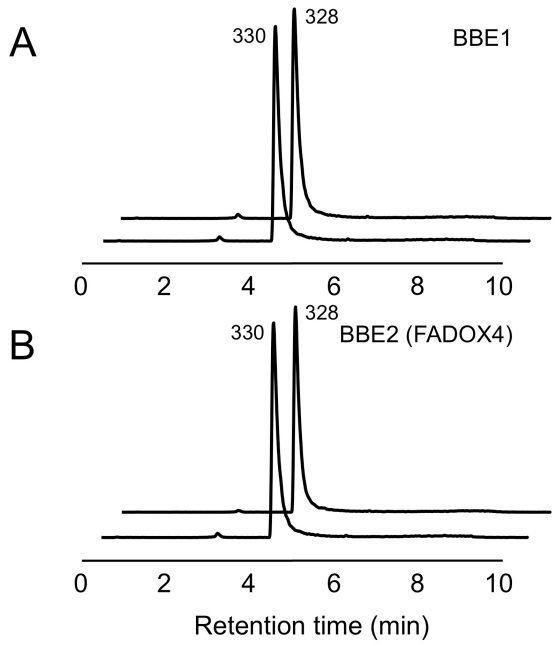

FIGURE S9. **Extracted ion chromatograms (EICs) showing the conversion of (*S*)-reticuline to (*S*)-scoulerine by BBE1 and BBE2 (FADOX4).** In each panel, the lower EIC corresponds to an assay conducted with the empty pPINKα-HC vector control, and the upper EIC shows an assay performed with recombinant enzyme. Parent ion mass-to-charge (*m/z*) values are indicated next to substrate and product peaks. The latter were subjected to collision-induced dissociation analysis for identification.

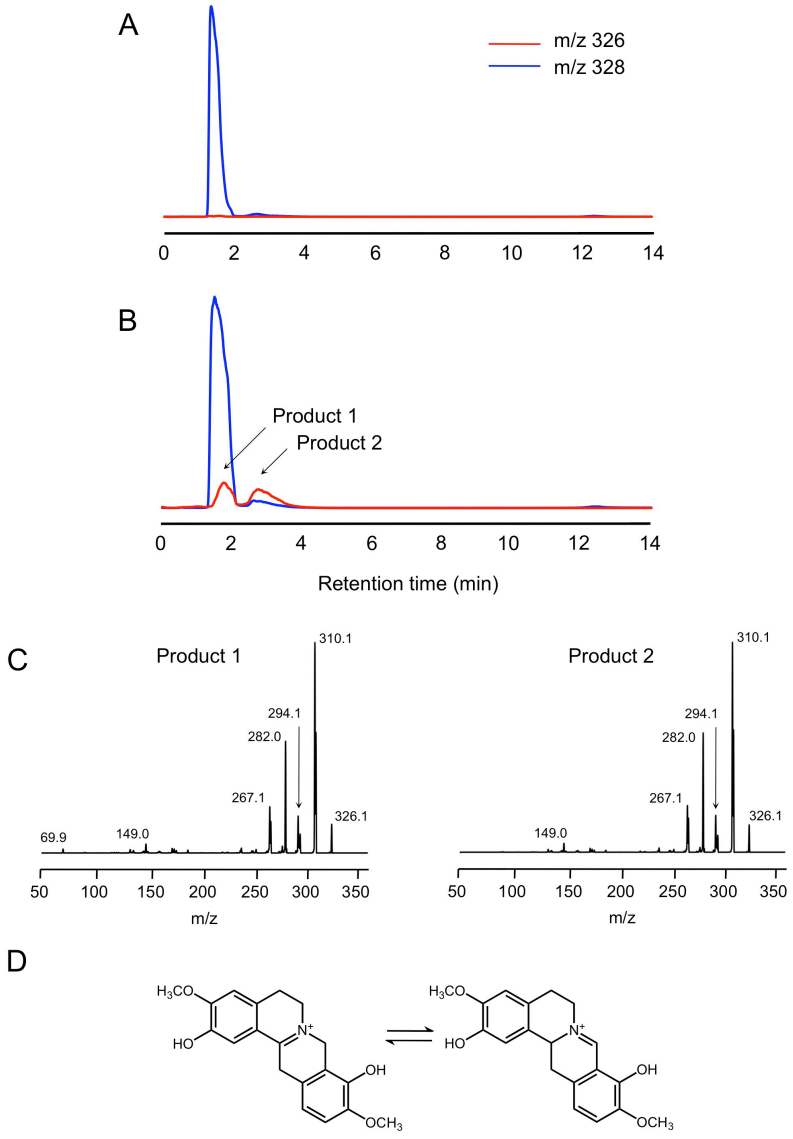

FIGURE S10. **DBOX catalyzes the formation of two reaction products with the same molecular ion mass from (*S*)-scoulerine substrate.** Extracted ion chromatograms corresponding to the (*S*)-scoulerine substrate (blue, *m/z* 328) and product (red, *m/z* 326) in standard enzyme assays containing culture medium proteins from *Pichia pastoris* (*A*) harboring empty pPinkα-HC vector and (*B*) producing opium poppy DBOX. Despite identical *m/z* values, DBOX reaction products eluted separately after liquid chromatography at 1.8 min (Product 1) and 2.8 min (Product 2), respectively, with Product 1 co-eluting with (*S*)-scoulerine. *C*, Collision-induced dissociation spectra obtained for each product using collision energy of -35 eV. *D*, Proposed isomers of dihydroscoulerine corresponding to Product 1 and Product 2.

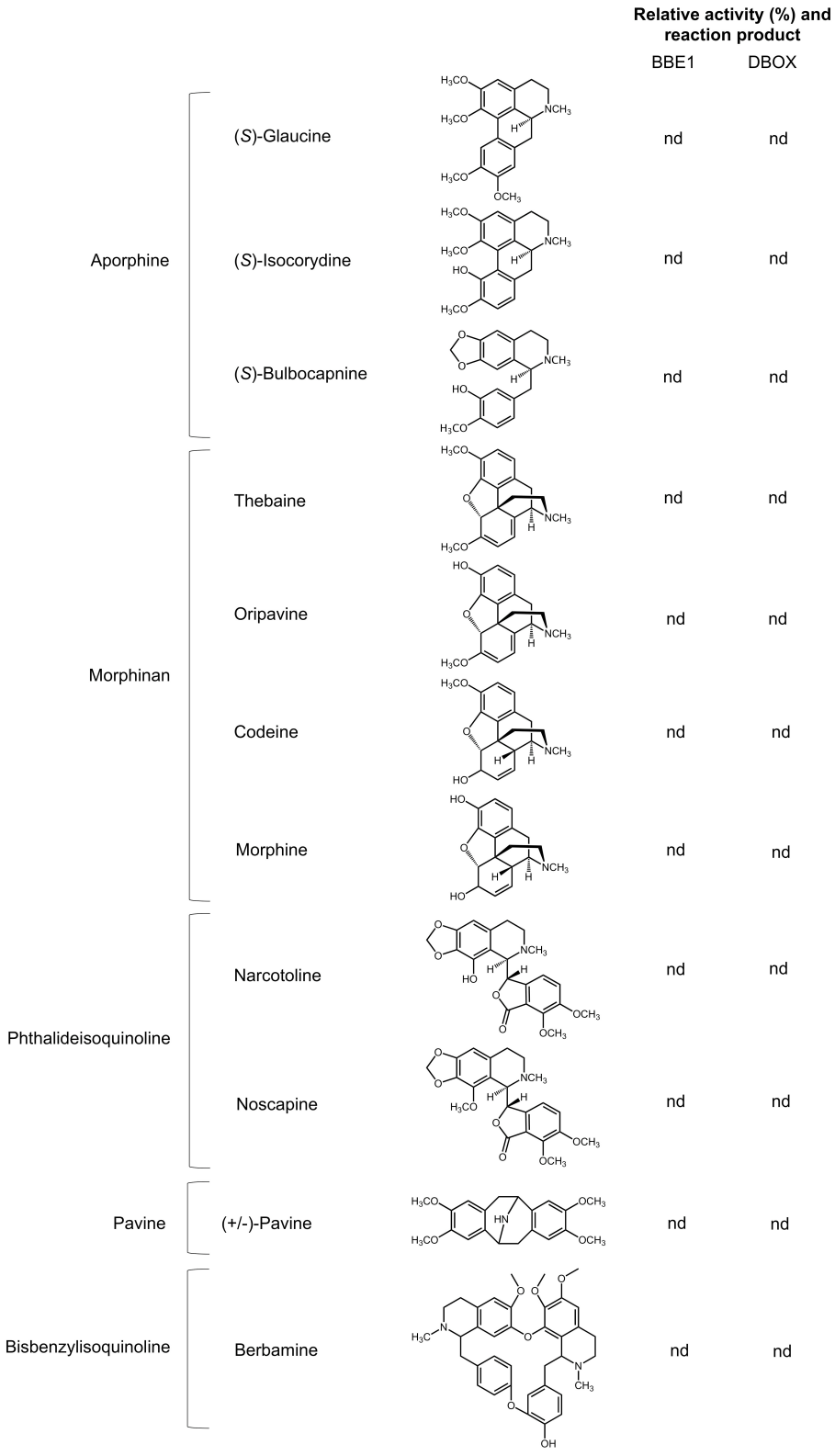

FIGURE S11. **Additional benzylisoquinoline alkaloids tested as substrates for BBE1 and DBOX (FADOX5).** Abbreviation: nd, not detected.

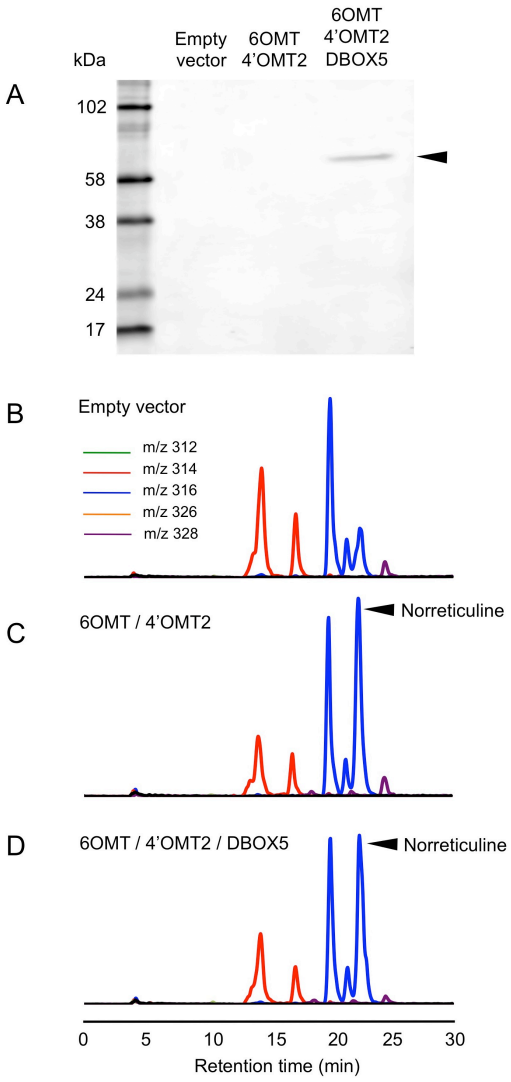

FIGURE S12. **Strains of *Saccharomyces cerevisiae* co-expressing *6OMT* and *4'OMT2*, or *6OMT*, *4'OMT2,* and *DBOX*, and batch-fed (*R,S*)-norlaudanosoline accumulate endogenous (*S*)-norreticuline.** The yeast strain expressing *DBOX* does not turnover norreticuline (*m/z* 316) to expected oxidation products (*m/z* 314, 312) or reaction byproducts (*m/z* 328, 326). *A,* Immunoblot analysis for HA-tagged, recombinant DBOX produced in *S. cerevisiae*. Yeast cell pellets were re-suspended in 200 µL of CelLytic Y (Sigma) containing the mini-complete protease inhibitor cocktail (Roche) and 10 mM dithiothreitol. Glass beads (~50 µL) were added and cells were incubated with shaking for 1 h at room temperature, followed by vortexing. Protein extracts (50 µg) were separated by SDS-PAGE and subsequently transferred to a nitrocellulose membrane for HA epitope detection. *B-D,* Extracted ion chromatograms (EICs) of culture medium corresponding to endogenous norreticuline (*m/z* 316), potential oxidation products (*m/z* 314, 312) and byproducts (*m/z* 328, 326) observed in enzyme assays using *Pichia pastoris* culture medium proteins. Culture medium was diluted five-fold in 50% (v/v)

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methanol containing 0.2% formic acid. Metabolites were separated on an Agilent Eclipse C18 (2.1 mm × 150 mm, 3.5 µm) column using a gradient of 0.2% (v/v) formic acid (solvent A) and acetonitrile containing 0.2% formic acid (solvent B). The solvent gradient was 0-3 min at 5% B, 3-55 min 5–45% B, 55–60 min 40–90% B, 60-62 min 90-5% B, followed by a 10 min equilibration at 5% B. Metabolites were detected using a Finnigan LTQ ion trap mass spectrometer (Thermo Scientific). Collision-induced dissociation analysis was used to identify norreticuline eluting at 22.5 minutes. Similar results were obtained using cell lysates.

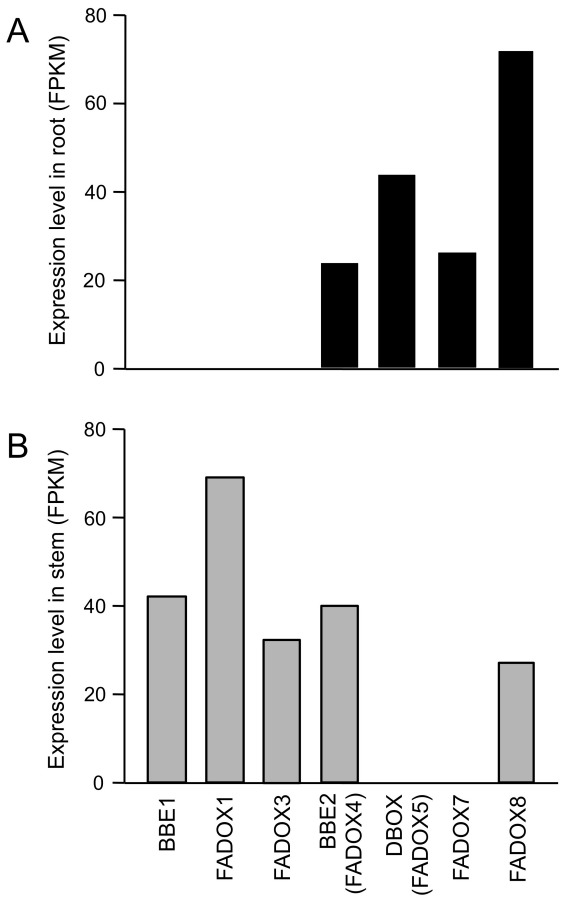

FIGURE S13. **BBE and DBOX (FADOX5) gene expression levels in stem (upper panel) and root (lower panel) of opium poppy determined by RNA-seq analysis.** The opium poppy chemotype used was Bea's Choice. FPKM denotes fragments per kilobase of exon model per million mapped reads.

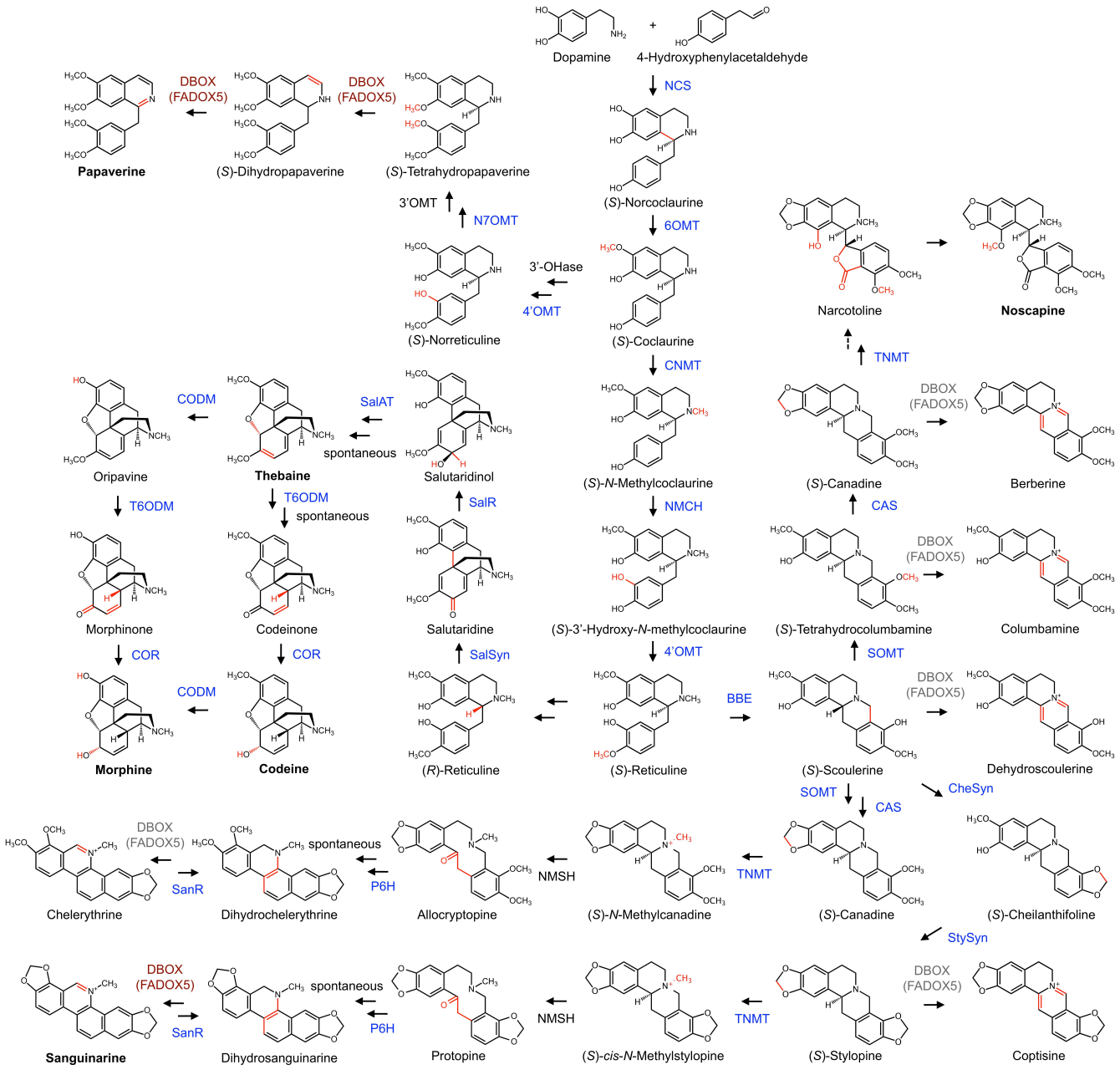

FIGURE S14. **Expanded benzylisoquinoline alkaloid metabolic map illustrating reactions catalyzed by DBOX (FADOX5).** Enzymes for which corresponding cDNAs have been isolated are shown in blue. DBOX-catalyzed reactions confirmed in opium poppy plants are shown in dark red. Compounds in bold are major alkaloids in opium poppy. Alterations within alkaloid structure resulting from each enzyme are highlighted (bright red). Two arrows denote two conversions whereas dashed arrows denote multiple

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steps. Abbreviations: 4'OMT, 3'-hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase; 6OMT, norcoclaurine 6-*O*-methyltransferase; BBE, berberine bridge enzyme; CAS, canadine synthase; CheSyn, cheilanthifoline synthase; CNMT, coclaurine *N*-methyltransferase; CODM, codeine *O*-demethylaseCOR, codeinone reductase; DBOX, dihydrobenzophenanthridine oxidase; NCS, norcoclaurine synthase; NMCH, *N*-methylcoclaurine 3'-hydroxylase; NMSH, *N*-methylstylopine 14-hydroxylase; P6H, protopine 6-hydroxylase; SalAT, salutaridinol 7-*O*-acetyltransferase; SalR, salutaridine:NADPH 7-oxidoreductase; SalSyn, salutaridine synthase; SOMT, scoulerine 9-*O* methyltransferase; STOX, (*S*)-tetrahydroxyprotoberberine oxidase; StySyn, stylopine synthase; T6ODM, thebaine 6-*O*-demethylase; TNMT, tetrahydroprotoberberine *cis*-*N*-methyltransferase; TPOX, tetrahydropapaverine oxidase.

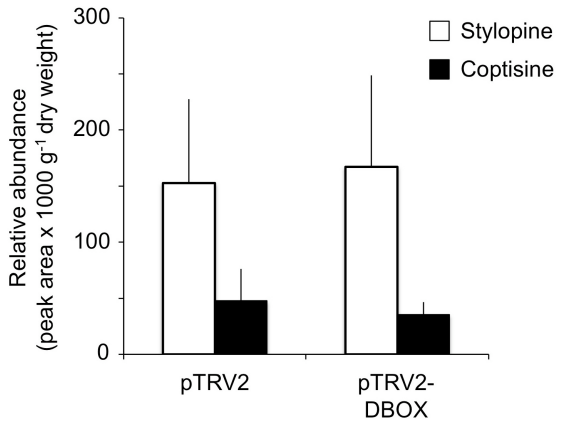

FIGURE S15. **Effect of virus-induced gene silencing (VIGS) on stylopine and coptisine levels in opium poppy roots.** Multiple reaction monitoring (MRM) was used at *m/z* 176, 149 (stylopine) and *m/z* 292, 318 (coptisine) whereby the first ion represents the quantifier and the second ion the qualifier, respectively. Paired t-test analysis indicated no significant difference in alkaloid levels between empty pTRV2 vector control and *DBOX*-silenced (pTRV2-DBOX) plants at $P < 0.05$.

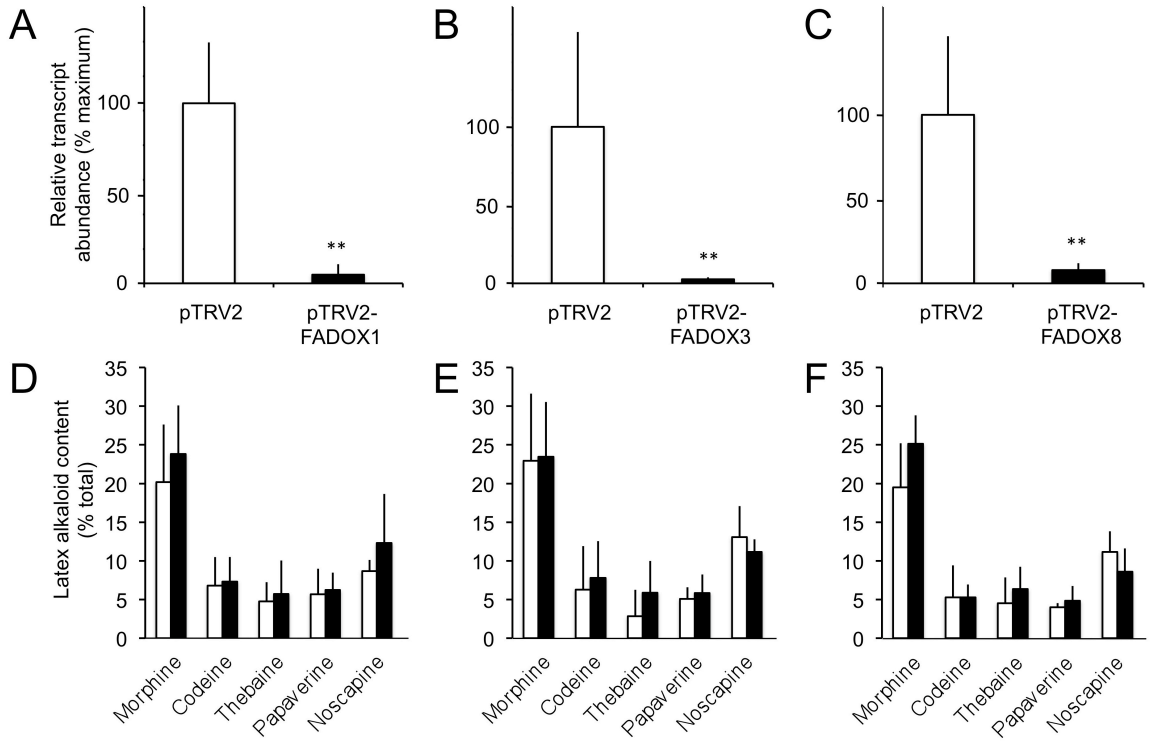

FIGURE S16. **Effect of suppressing FADOX1, FADOX3, and FADOX8 transcript levels by virus-induced gene silencing on latex alkaloid profiles.** *A-C,* Relative *FADOX* transcript abundance in stems of opium poppy chemotype Bea's Choice infiltrated with *Agrobacterium tumefaciens* harboring various pTRV2 constructs or the empty pTRV2 vector. *D-F,* Relative alkaloid levels in *FADOX*-silenced plants. Mean ± standard deviation was calculated for nine plants per construct. Paired t-test analysis indicated no significant differences in the major alkaloid content of empty vector controls (pTRV2) and *FADOX*-silenced plants at $P < 0.05$.