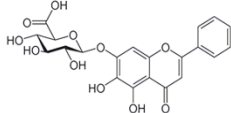
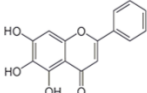
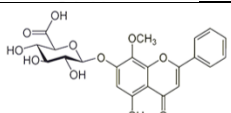
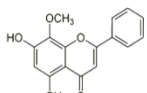
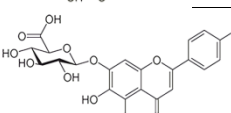
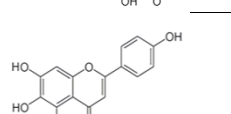
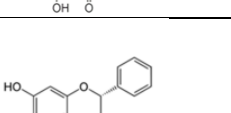
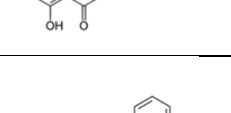
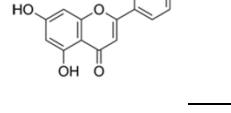
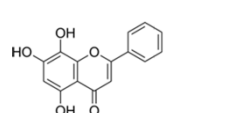
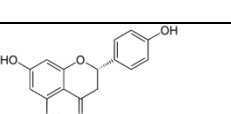


**Supplementary Table 1. Structures of Compounds related to this work**

Compounds related to this work	Structures
baicalin	
baicalein	
wogonoside	
wogonin	
scutellarin	
scutellarein	
pinocembrin	
chrysin	
norwogonin	
naringenin	
apigenin	

**Supplementary Table 2. FPKM of candidate flavone hydroxylase genes**

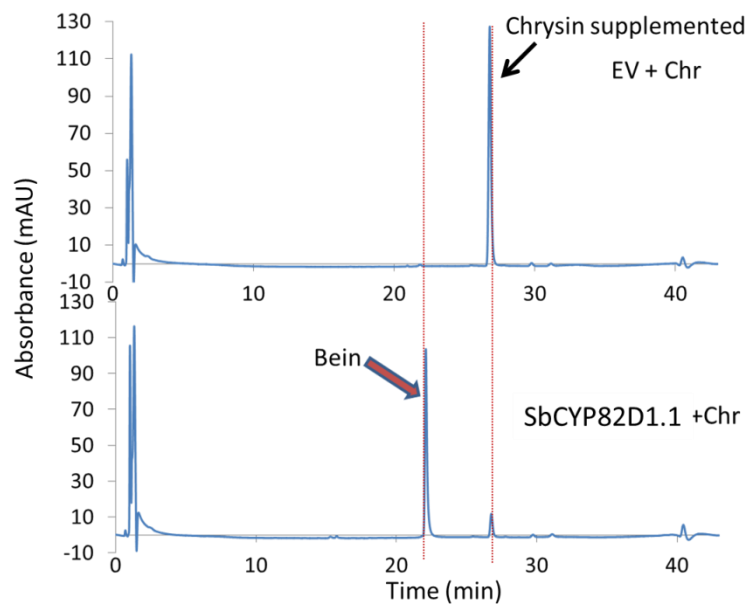
<b>Gene ID</b>	<b>Expression (FPKM) HairyRoots</b>	<b>Expression (FPKM) Flowers</b>
SbCYP82D1 CL5673.Contig4_All	59.7978	10.7494
SbCYP82D2 CL5510.Contig2_All	195.0871	3.3727
SbCYP71D1 CL3280.Contig1_All	3.464	1.9377
SbCYP71D2 Unigene16719_All	127.4283	8.5701

**Supplementary Table 3. Primers used in this study**

Names	Used for	Sequences
SbCYP82D1 CDS F	Cloning	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATG</u> GAGTTGAGCTCTGTCATCTATG
SbCYP82D1 CDS R	Cloning	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTTTAA</u> TATAGAGTAGGTGACAACCTTGG
SbCYP82D2 CDS F	Cloning	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATG</u> CTATATGACAACAGCCAATAC
SbCYP82D2 CDS R	Cloning	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTTCTAG</u> TAAAGAGTGGGAGACAAC
SbCYP71D1 CDS F	Cloning	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGGACATCCAATTTCCCTCACTC
SbCYP71D1 CDS R	Cloning	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTAT</u> CATGGCAAAGGTCTTGTAATAATAAGAG
SbCYP71D2 CDS F	Cloning	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGGGGTTTCCTCATTCTCCTCTC
SbCYP71D2 CDS R	Cloning	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTAC</u> TTCTAGTCCCTCTCTCTTTGCAAC
SbRTO CDS F	Cloning	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGGAAGCTCTCAAAGCTTCATC
SbRTO CDS R	Cloning	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTTT</u> CAACGGAAGGCATGGTCATAG
SbCYP82D1.1 QPCR F	qPCR	CTACGACGGAGCCTCGCTTG
SbCYP82D1.1 QPCR R	qPCR	TGGCTCGCTGCAGCTCGATC
SbCYP82D2 QPCR F	qPCR	ATTGGACGGAATTGTTGGA
SbCYP82D2 QPCR R	qPCR	TCACTTCCACCCACAATCAA
SbCYP71D1 QPCR F	qPCR	GTGAATTGCACGAAGAAGCA
SbCYP71D1 QPCR R	qPCR	GTCCACTGTTGTGGATGACG
SbCYP71D2 QPCR F	qPCR	CGACACCATTCTTGAGAGCA
SbCYP71D2 QPCR R	qPCR	CTCGGTTTCTTCACCAGCTC
SbRTO QPCR F	qPCR	CCAGAGAGTGTGAAGGCTGA
SbRTO QPCR R	qPCR	TTTGGTTGCACTTGAGGACG
SbActin QPCR F	qPCR	TCTTGATCTTGCTGGTTCGTG
SbActin QPCR R	qPCR	CACTGCAGAGCTGGTCTTTG
SbCYP82D1 RNAi F	RNAi	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ACATGAAGCAGTGGCTGGGGAAC
SbCYP82D1 RNAi R	RNAi	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTTT</u> GATGGTATCAGCATCGTAGTGGAG
SbCYP82D2 RNAi F	RNAi	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATCTCATGGCTGGTGGCACCAC
SbCYP82D2 RNAi R	RNAi	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTTC</u> ACACTGTCCGAACCCTCACTTC
SbRTO RNAi F	RNAi	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> ATGGAAGCTCTCAAAGCTTCATC
SbRTO RNAi R	RNAi	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTTC</u> ACCAACCATGGTACACACAC

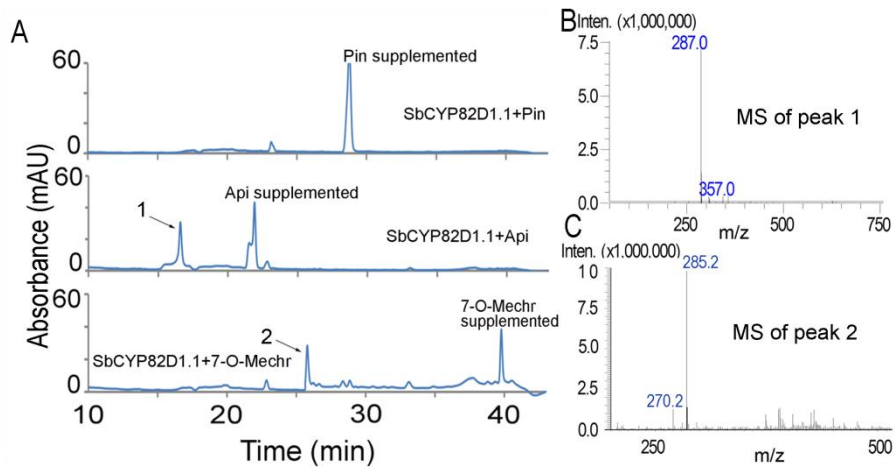
Underlined sequences represent Gateway® primers

**Supplementary Figure 1. HPLC profile of *in vitro* enzyme assay of CYP82D1.1 in yeast**



EV = Empty Vector; Chr = Chrysin; SbCYP82D1.1 = yeast expressing SbCYP82D1.1

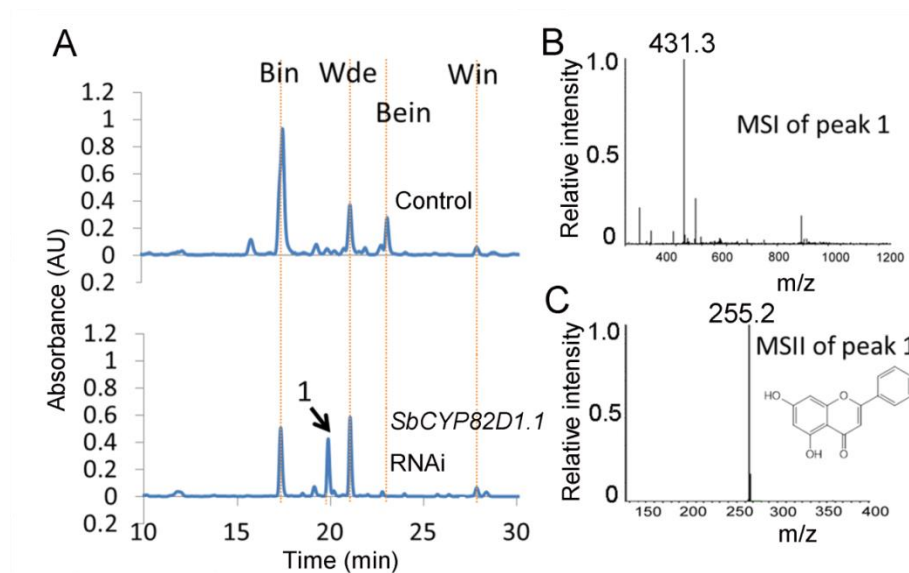
**Supplementary Figure 2. HPLC profiles of *in vitro* enzyme assays of CYP82D1.1 supplied with different substrates**



**Supplementary Figure 2. *In vitro* enzyme assays of CYP82D1.1 supplied with different substrates.**

(A) HPLC analysis of CYP82D1.1 with: Top; pinocembrin (Pin), Middle; apigenin (Api) and Bottom; 7-O-methylchrysin supplied as substrates. Peak 1 (scutellarein) and Peak 2 (negletein) were detected from reactions with apigenin and 7-O-methylchrysin, respectively. (B) MS of Peak 1 confirming this to be scutellarein and (C) Peak 2 confirming this to be negletein.

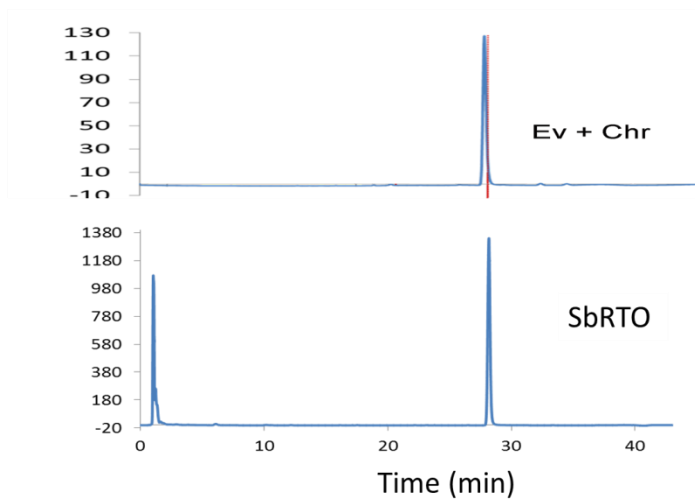
**Supplementary Figure 3. LC-MS analysis of flavonoids in *SbCYP82D1.1* RNAi hairy roots**



**Supplementary Figure 3. LC-MS analysis of flavonoids in *SbCYP82D1.1* RNAi hairy roots**

(A) HPLC analysis of empty vector control and *SbCYP82D1.1* RNAi hairy roots. Peak 1 was detected only in *SbCYP82D1.1* RNAi hairy roots. (B) MSI of Peak 1 and (C) MSII of Peak 1 showing this to be chrysin glucuronide. Bin = Baicalin; Bein = Baicalein; Win = Wogonin; Wde = Wogoniside.

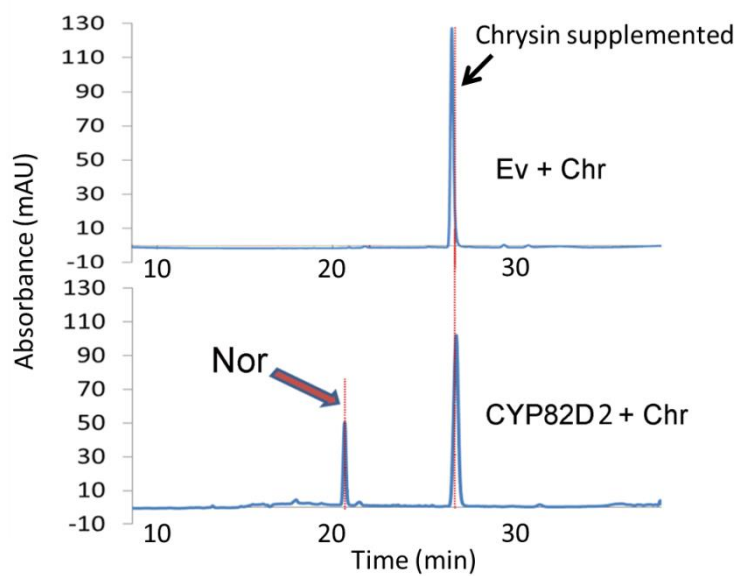
**Supplementary Figure 4. *In vivo* assay of SbRTO with chrysin in yeast**



**Supplementary Figure 4. *In vivo* assay of SbRTO with chrysin in yeast**

No new products were observed following feeding of yeast expressing SbRTO with chrysin, showing that SbRTO can not use chrysin as a substrate. Ev = Empty vector; Chr = chrysin

**Supplementary Figure 5. *In vitro* enzyme assay of CYP82D2 expressed in yeast**

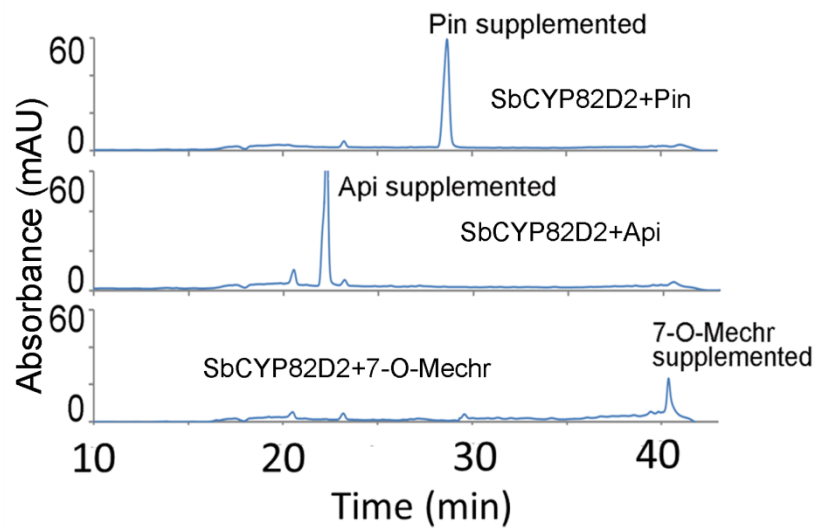


**Supplementary Figure 5. *In vitro* enzyme assay of CYP82D2 expressed in yeast**

Microsomes from yeast expressing SbCYP82D2 and supplied with chrysin (Chr) formed a new peak of norwogonin (Nor) compared to microsomes from empty vector control (Ev) yeast.



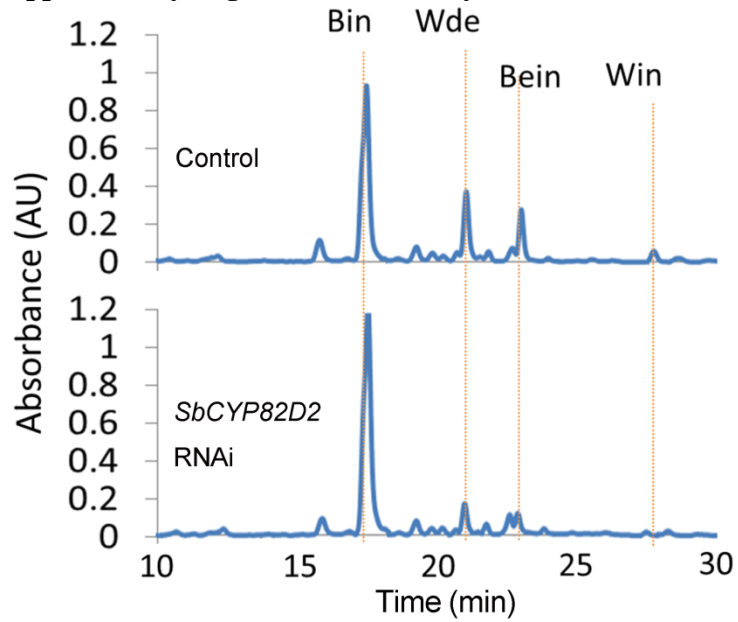
**Supplementary Figure 6. *In vitro* enzyme assays of CYP82D2 with different substrates**



**Supplementary Figure 6. *In vitro* enzyme assays of CYP82D2 with different substrates**

Microsomes prepared from yeast expressing SbCYP82D2 failed to make any new products when supplied with Pinocembrin (Pin), Apigenin (Api) or 7-Methylchrysin (7-O-MeChr).

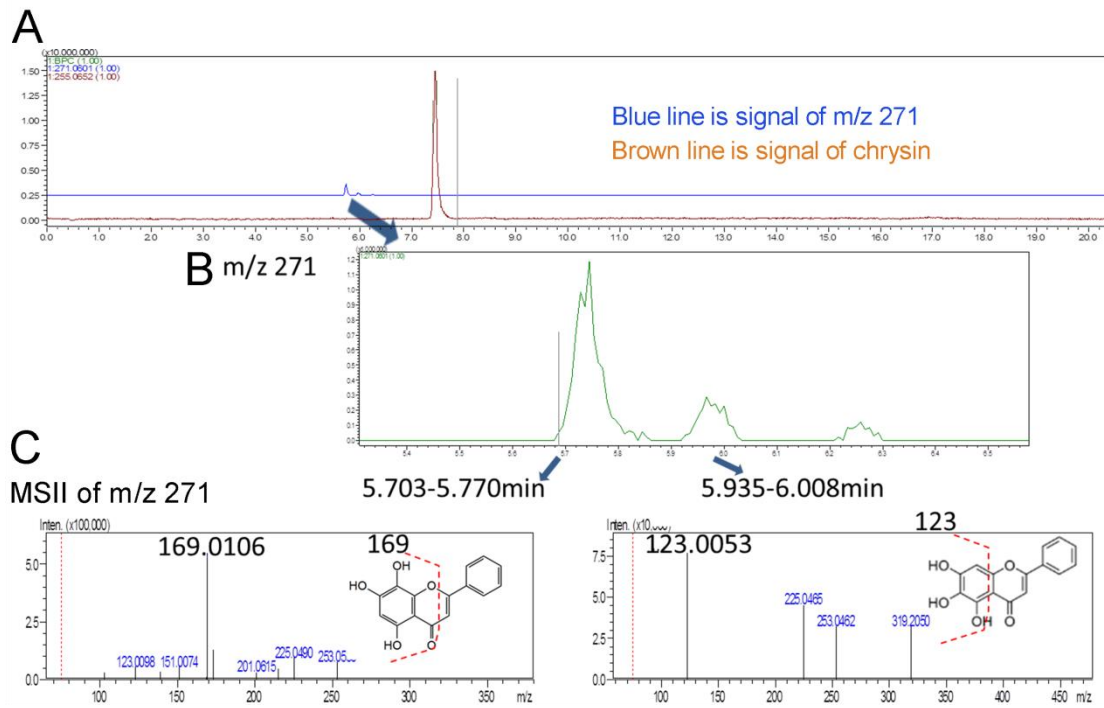
**Supplementary Figure 7. HPLC analysis of flavonoids in *SbCYP82D2* RNAi hairy roots**



**Supplementary Figure 7. HPLC analysis of flavonoids in *SbCYP82D2* RNAi hairy roots**

Levels of Baicalein (Bein), Baicalin (Bin), Wogonin (Win) and Wogoniside (Wde) in hairy roots silenced for *SbCYP82D2*, compared to an empty vector control.

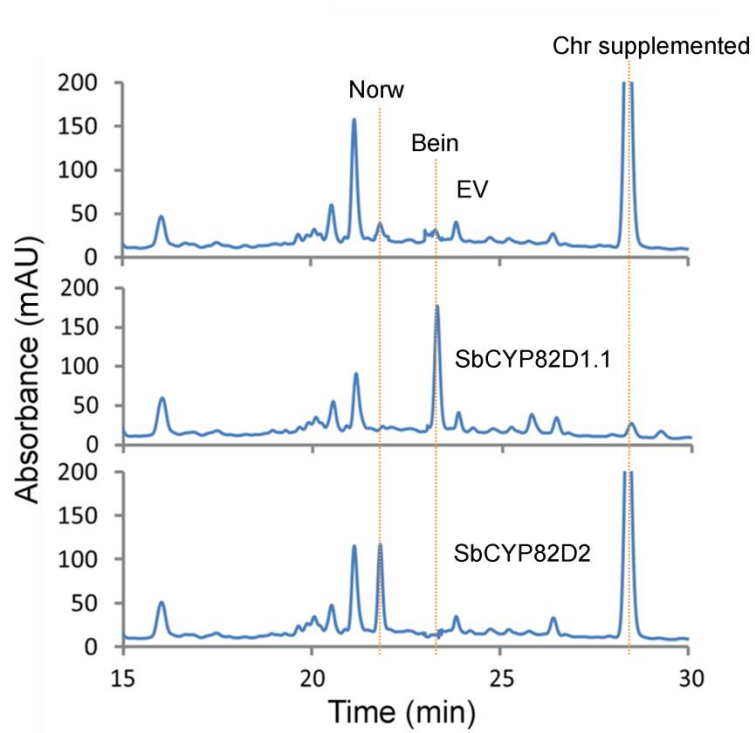
**Supplementary Figure 8. SbCYP82D2 can also produce baicalein in small amounts**



**Supplementary Figure 8. SbCYP82D2 can also produce baicalein in small amounts**

- (A) HPLC trace of flavonoids made following *in vivo* assay of CYP82D2 expressed in yeast and fed chrysin. Blue line shows new products compared to brown line showing chrysin peak
- (B) Higher resolution profile of new peaks of m/z I of 271
- (C) MSII of first peak with m/z 271 showing this to be norwogonin, and of second peak showing this to be baicalein

**Supplementary Figure 9. HPLC analysis of flavonoids in transgenic Arabidopsis expressing either SbCYP82D1.1 or SbCYP82D2 and fed chrysin**



**Supplementary Figure 9. HPLC analysis of flavonoids in transgenic Arabidopsis expressing either SbCYP82D1.1 or SbCYP82D2 and fed chrysin**

Norw = Norwogonin; Bein = Baicalein; EV = Empty Vector; Chr = Chrysin



CYP82G1 -----MTFLFSTLQLSFLSALVIFGYIFLKRQLSRCEVDSSTIP- EPLGALPLFGHLHLRKG- ---KLLCKLAAMSQKHGPIFSLKLGFYRLVWASDPKTVKDCFTNDLATATRNPIAFGRVYGVYVNASLTLAPYGDYWR  
CYP82E4v1 -----MVFPIEALVIG- ---LVTFTLFFLWTKKSKPKPLPKIPGGMPVIGHLFHFNDGG- ---DORPLARKLGDADKYGPFVTRGLGLPLVLVVSSYEAVKDCFTSDINDAFISRRPFLYGDYLVNMAHLFLAHYGYWRK  
SBCYP82D1 MLYDYSQYLAMEFSSAIVG- AIAFLFLYYCLLYTSSK- PHTFRAYKAPP EAGGARLFSGLHLRAG- ---GTJGELPHMLNLADKHGPFVTRGLGVRRLVWSSMEKELFTTCDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
SIN\_1025398\_Sind -----MEFSSALYA- AITFELLYYLVSRAK- LSTQKSSP EAGGARFGLHLRAG- ---SAGLSLPHMLNLADKYGPFVTRGLGVRRLVWSSMEKELFTTCDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
SM1\_00003468-RA\_Sal -----MEFSSALYA- AVALSLLFYVYLFFKSSNPKHAARAPPAQAGGARLLTGLHMLVWEEGSSKLPFHMLGDADKHGPFVTRGLGVRRLVWSSMEKELFTTCDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
CYP82D62 -----MDFNAVCA- ALAFISLISLTYLWLRASD- ---THQAPP EAGGAMPGLHLNIISSG- ---HTGLPHVSLNLADKHGPFVTRIGVHRAVWSSVEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
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CYP82D33 -----MEFISFVYV- LIAFSSLLFYVYLWESAKPKTT- THKAPP EAGGAMPGLHLNIISSG- ---PSAGLPHVSLNLADKHGPFVTRIGVHRAVWSSVEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
SBCYP82D2.2 -----MELSSVYV- AIALSLLFYVYLHFSPKPK- ---SSLNAPP EAGGAMPGLHLNIISSG- ---SASDKLPHVSLNLADKHGPFVTRIGVHRAVWSSMEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
CYP82A2 -----MEKLVNSTIGVGVVSLILLFLYLRGGSHK- ---SGEGLP TVAGAMPGLHLNIISSG- ---SKTPHKLGLADKHGPFVTRIGVHRAVWSSMEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
CYP82B1 -----MEKPIILLQLQAGLGLLALICFLYYVKSLSLSTRNQLVKHPQEAAGSNPVLGHLPLQVGS- ---GKPLFRVLDGMADKGFVTRIGVHRAVWSSMEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
CYP82Q1 -----MEFSLLLTVAITLVVTAFFLQISKRKVDTKNKL- ---PPKAGWAMPGLHLNIISSG- ---NRIAHVSLNLADKHGPFVTRIGVHRAVWSSMEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
CYP82C2 -----MDSLFS- LVPVILVVFVIALFKSK- ---KPKHVKAP APSGAMPGLHLNIISSG- ---EQLVYRVLGMDQYGPAMSLRGSSETFVSSFEVAKDCFTSDINDAFISRRPFLYGDYLVNMAHLFLAHYGYWRK  
CYP82H1 -----MITCEMGLYQMDIILFSLVFFSTLILWRIFSTVIR- ---KKTCSGPP EAGGARFGLHLRAG- ---SKLTHHLLGMDQYGPAMSLRGSSETFVSSFEVAKDCFTSDINDAFISRRPFLYGDYLVNMAHLFLAHYGYWRK  
CYP82N2v2 -----MDSLMLAYLFFPISVAS- IIAFVFLYMLFSSRLK- ---NKKIRTA MATGAMPGLHLNIISSG- ---GELPHMLNLADKHGPFVTRIGVHRAVWSSMEKELFTTNDVAVSSRRPMAAKLLGVDFAMFGAPYGYWRE  
CYP93B6 -----NALYAALLSAAVRSVLDKRRGR- ---PPY PGFPPLPIGHLHLGPR- ---LHQTHDLSQRYGLMQLRLLGSIRVIAASPELAKELCKLTVLSSRRKSHTAIDIVYDSS- FAFSPYGPYKWF

CYP82G1 LRK1VTVHLSNHSIEMGLHRSSEVNTIKHLYK- GNGTGS- ---IVKIDMLFEFLFNIIILRKMVGKRIEFG- ---VNSDEWR KEALKHCEYLAVIPMIDG- ---VIPMLGLD- FAKNSQ- MKRFLKELDSVN  
CYP82E4v1 NRK1LVIQVLSASRLKFKHVRFAIQAISKNLYTRIDGNS- ---TINLTDGDLLELNLVLMVWAGKRYIGP- ---GDEQVFR FKAFKDFMLSMFVLDMAFPDPLFKWVD- FQGHVKAMKRTFKDIDSDV  
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SIN\_1025398\_Sind LRK1LSVELLSRRELLESHVRYSETLQSVNELYKLEEKRDGSG- ---CHLVDMKRFQGLTINLVLSHWAGKRYCGV- ---ADAEETR CHQVLEFFFLYAGHVFVAD- ---AMPYGLMD- LGGHEKRRKTAEEHDIV  
SM1\_00003468-RA\_Sal IRK1LVTTEL SARLELQSRVVAETARSVRKLEHAEAGRDGSG- ---RVLVDMKRFQGLTINLVLSHWAGKRYCGV- ---ADAEETR CHQVLEFFFLYAGHVFVAD- ---AMPYGLMD- LGGHEKRRKTAEEHDIV  
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SBCYP82D2.2 IRK1LVTTEL SARLELQSRVVAETARSVRKLEHAEAGRDGSG- ---RVLVDMKRFQGLTINLVLSHWAGKRYCGV- ---ADAEETR CHQVLEFFFLYAGHVFVAD- ---AMPYGLMD- LGGHEKRRKTAEEHDIV  
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CYP82C2 ERWLNHR- ---RKRRESGDE- KADQFMDVMSLVQ- ---EDGSE YDVTI IKATCGTIIAGDGTSSVVAFTWALALLNLRHVRKKAQEEIDTKVGRDRWEESDIQMLYQAIIVKETHRYPYAPL TGREAREDCFVGGYR  
CYP82H1 GSCLKEHQ- ---QKRTNLSMHEEDDFIVMSLAMDGN- QFPGDITD- ---TATGCTLSLILGGYDTSATLWALSLLNLRHVRKKAQEEIDTKVGRDRWEESDIQMLYQAIIVKETHRYPYAPL TGREAREDCFVGGYR  
CYP82N2v2 ENWLEHR- ---KKNISVAE- ---SQDFMDVMSLVQAN- PTLQYDADITIKATCGTIIAGDGTSSVVAFTWALALLNLRHVRKKAQEEIDTKVGRDRWEESDIQMLYQAIIVKETHRYPYAPL TGREAREDCFVGGYR  
CYP93B6 KEIITDREK- ---QRTHGGGGGGAEDFLDMFLMESG- ---KAEV KFRHEH.KALLDFFTAGDTTVAICENATAEVIINPNVLEKKAQEEIANI VGFDRILQESDAPHLPYQLIKETFRHLPPMLAR- KS15SDVCDGVM

CYP82G1 VEKGTRLVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82E4v1 IPKGTRLVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
SBCYP82D1 VPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
SIN\_1025398\_Sind VPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
SM1\_00003468-RA\_Sal VPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82D62 IPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
SM1\_00005725-RA\_Sal IPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82D33 IPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
SBCYP82D2.2 IPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82A2 IQKGTFLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82B1 IKGTGTRLLVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82Q1 IPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82C2 VPRGTRMLVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82H1 VPRGTRMLVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP82N2v2 VPKGTWLIVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-  
CYP93B6 IPANTLLVNLNKHHRDPKINP- DPKTFKPERFMEDSQ- ---CEKSNFEYIPFGSGRRSCPGVNLGRVHVLARLLQGFELHKSDE- PLDMAEGP- GLALPKINPVEVVMVPRDLPKYLSLHPP-

## Multiple alignment of CYP82D proteins