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Supplementary Materials for

A specialized flavone biosynthetic pathway has evolved in the medicinal plant, *Scutellaria baicalensis*

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The PDF file includes:

Supplementary Information

fig. S1. Multiple alignment of CPY93B6, CPY93B24, and CPY93B25.

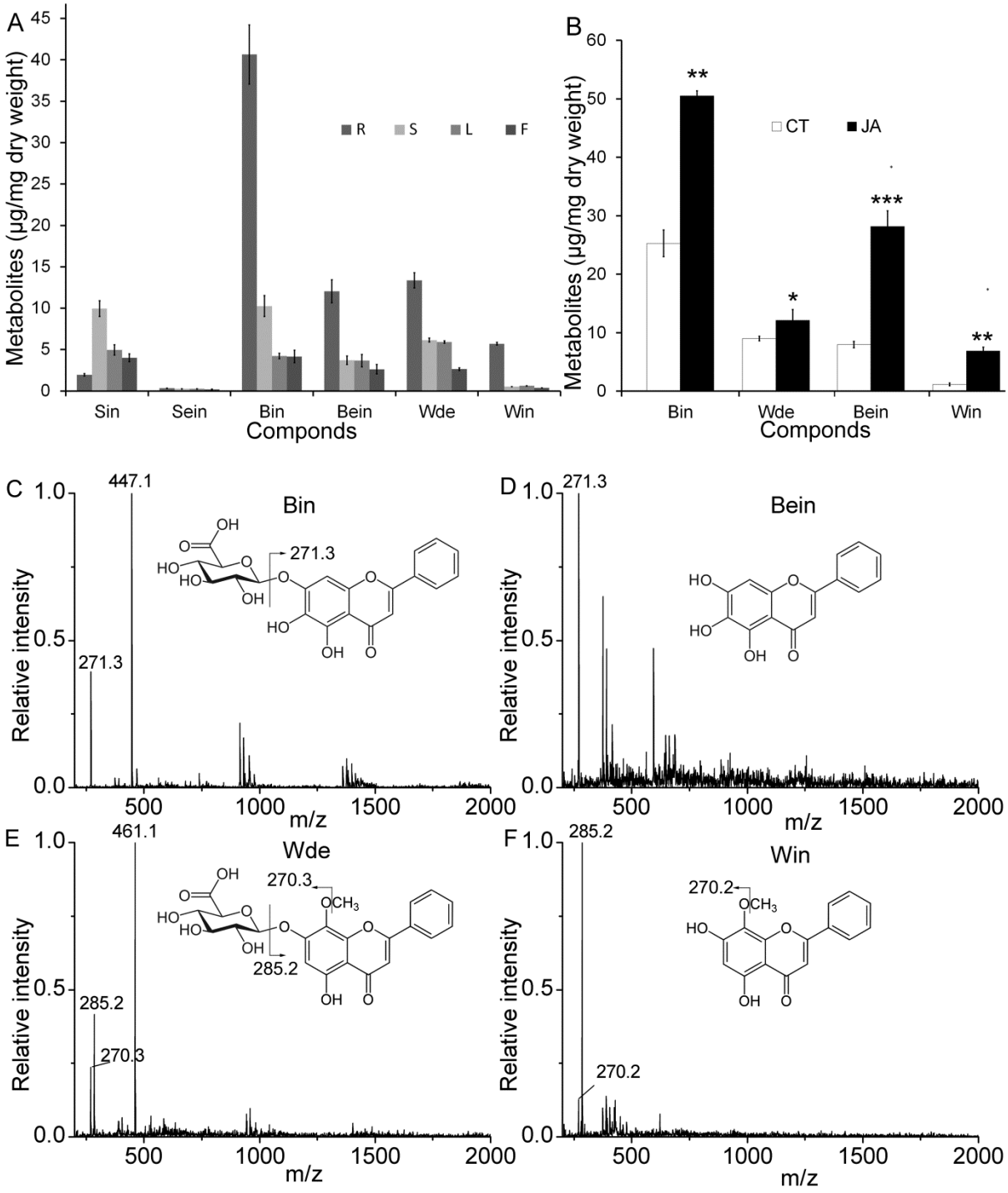


fig. S2. Flavone accumulation patterns in *S. baicalensis.* (**A**) Measurements of flavones from different organs of 6 month old *Scutellaria* plants. R, roots; S, stem; L, leaves; F, flowers. (**B**) Measurements of *Scutellaria* RSFs from hairy root lines subjected to MeJA treatment for 24h and MS profiles of baicalin (**C**), baicalein (**D**), wogonoside (**E**) and wogonin (**F**). Baicalin (bin), baicalein (bein), wogonoside (wde) and wogonin (win). SEs were calculated from 3 biological replicates. Differences were considered significant (Student's *t*-test) with *P<0.05, **P<0.01, ***P<0.001.

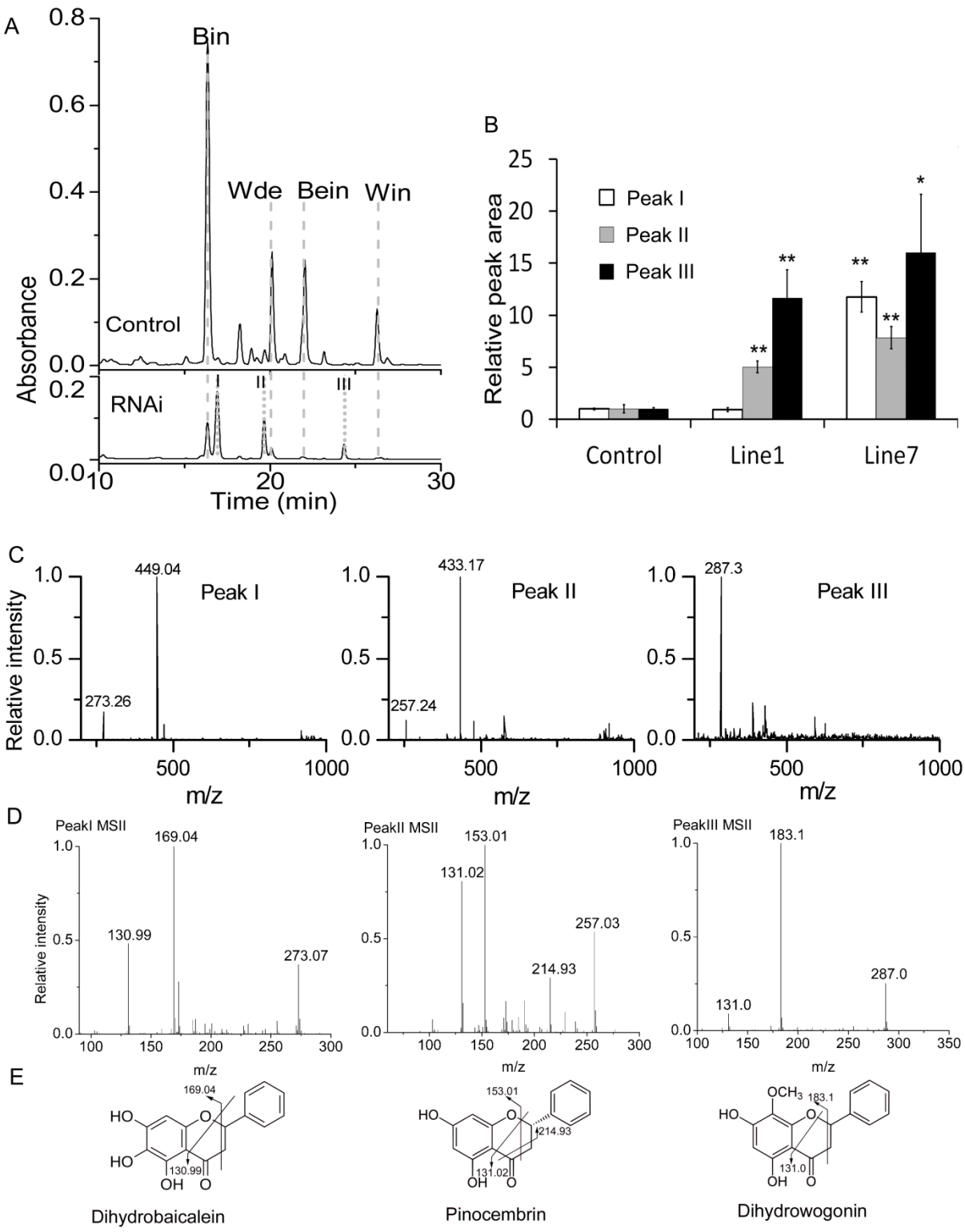


fig. S3. RNAi of *SbFNS II-2* in hairy root cultures of *S. baicalensis.* (**A**) HPLC profiles of flavone metabolites in SbFNSII-2-silenced hairy root lines compared with the profiles obtained from hairy roots transformed with an empty vector as control. Bin, baicalin; Wde, wogonoside; Bein, baicalein; Win, wogonin. (**B**) Relative area of the three new peaks in SbFNSII-2 RNAi lines. SEs were

calculated from 3 biological replicates. Significant differences were considered with *P<0.05, **P<0.01. (**C**) MS I of peaks I, II and III detected from FNSII-2 RNAi lines (**D**) MS/MS of m/z 273.26, 257.24 and 287.30 detected from MS I. (**E**) Structures and fragmentation patterns for MS/MS of m/z 273.26, 257.24 and 287.30 detected from MS I

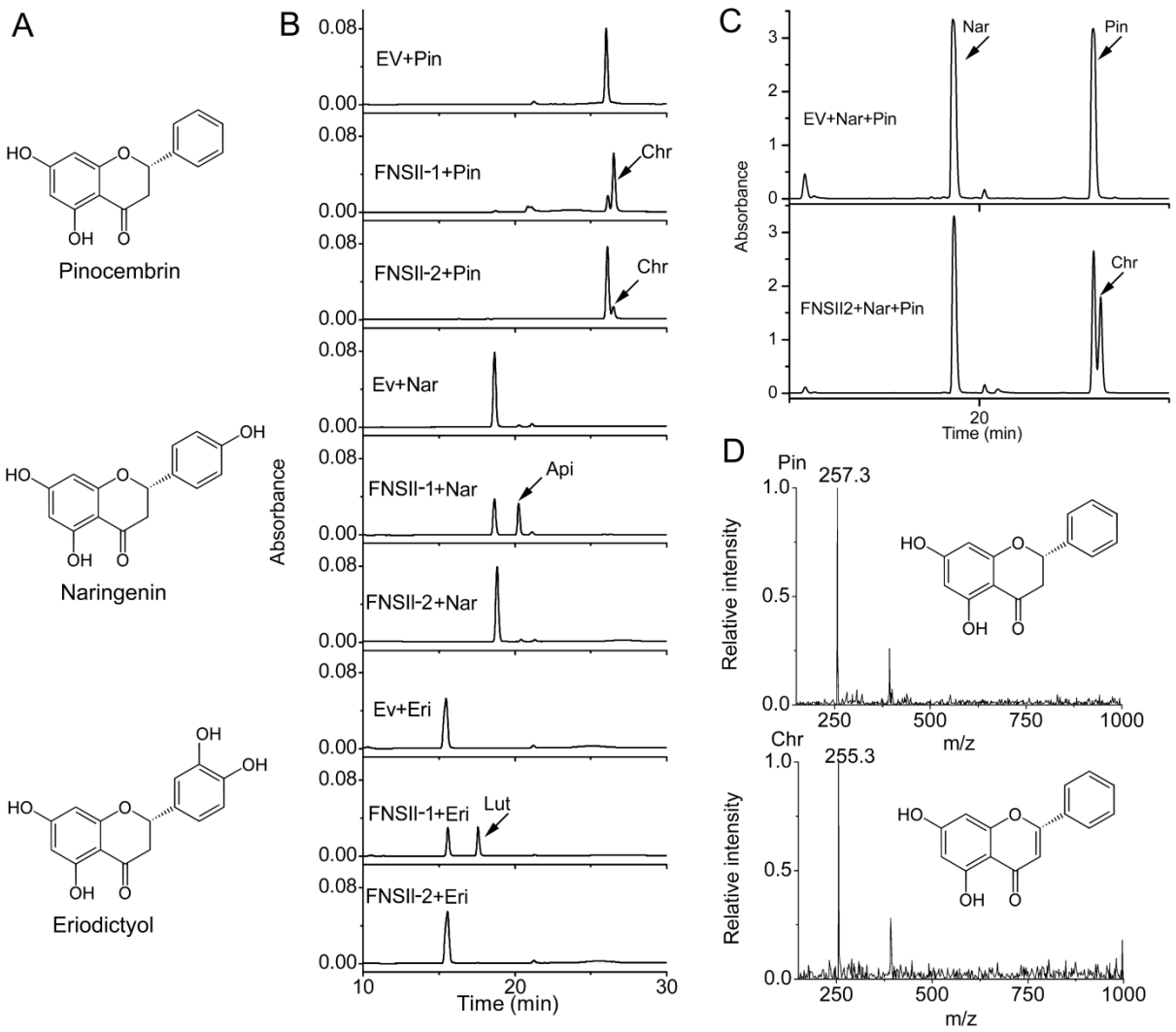


fig. S4. In vitro assay of SbFNSII-1 and SbFNSII-2 and in vivo assay of SbFNSII-2. (**A**) Structure of flavanones used for in vitro assay. (**B**) Metabolite profiles of assays of extracts of yeast carrying empty vector (EV), SbFNSII-1 or SbFNSII-2 assayed with pinocembrin, naringenin and eriodictyol as substrates, respectively. Pin, pinocembrin; Nar, naringenin; Eri, eriodictyol; Chr, chrysin; Api, apigenin; Lut, luteolin. (**C**) Yeast in vivo assay, both pinocembrin and naringenin were added to yeast media. (**D**) MS I of pinocembrin and chrysin.

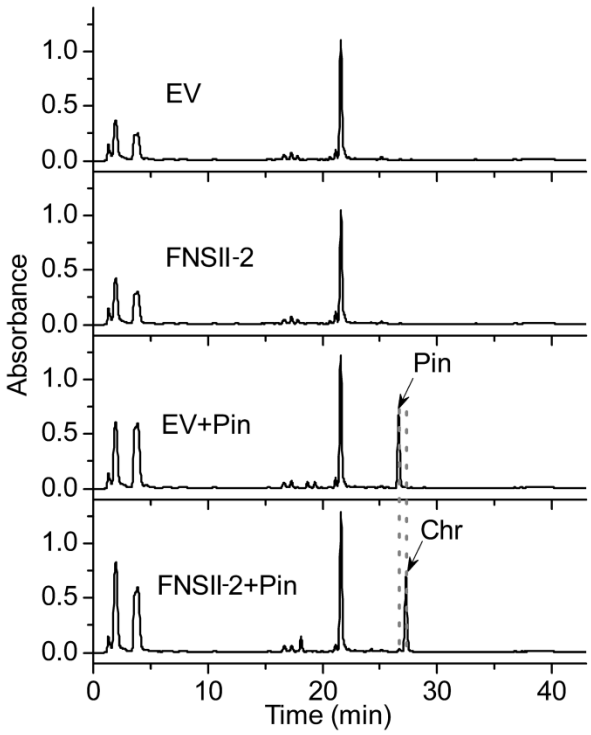
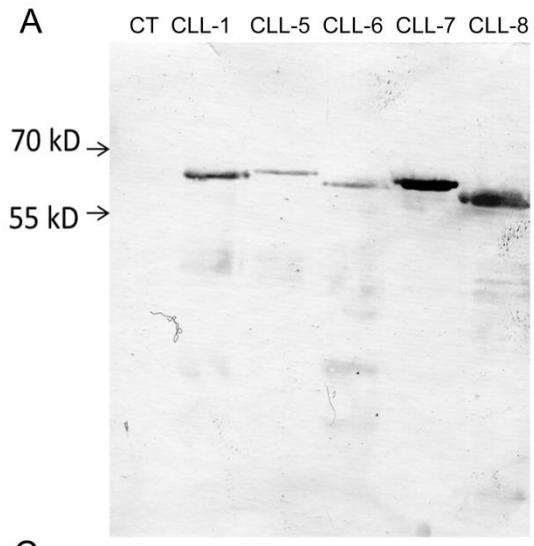


fig. S5. HPLC metabolite profiles of *Arabidopsis* plants carrying empty vector or a representative *SbFNSII-2* line, grown on MS with or without supplementation of pinocembrin.



B

	At4CL1	At4CL2	At4CL3	At4CLl7	SbCLL-7
At4CL1		92	70	41	42
At4CL2			70	40	43
At4CL3				40	41
At4CLl7					67
SbCLL-7					

C

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          Box1                                                    *
At4CL1   210SSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFHSDDVILCVLPMFHIYALNSIMLCG
At4CL2   203SSGTTGLPKGVMLTHKGLVTSVAQQVDGENPNLYFNRDDVILCVLPMFHIYALNSIMLCS
At4CL3   213SSGTTGLPKGVVLTHKSLITSVAQQVDGDNPNLYLKSNDVILCVLPLFHIYSLNSVLLNS
SbCLL-7  198SSGTTGMSKGVILTHRNFIASSLMITADQ--ESAGEMHNVFLCVLPMFHVFGLAVIMLSQ
At4CLl7  198SSGTTGTSKGVELTHGNFIAASLMVTMDQ--DLMGEYHGVFLCFLPMFHVFGLAVITYSQ

At4CL1   270LRVGAAILIMPKFEINLLLELIQRCKVTVAPMVPPIVLAIAKSSETEKYDLSSIRVVKSG
At4CL2   263LRVGATILIMPKFEITLLLEQIQRCKVTVAMVVPPIVLAIAKSPETEKYDLSSVRMVKSG
At4CL3   273LRSGATVLLMHKFEIGALLDLIQRHRVTIAALVPPLVIALAKNPTVNSYDLSSVRFVLSG
SbCLL-7  256LQRGNTIVSMSKFDFEMILKTVEKYGITHMWVVPPIILALAKNNVVKKYNLSSLQQIGSG
At4CLl7  256LQRGNALVSMARFELELVLKNIEKFRVTHLWVVPPVFLALSKQSIVKKFDLSSLKYIGSG

At4CL1   330AAPLGKELEDAVNAKFPNAKLGQGYGMTEAGPVLAMSLGFAKEPFPVKSGACGTVVRNAE
At4CL2   323AAPLGKELEDAISAKFPNAKLGQGYGMTEAGPVLAMSLGFAKEPFPVKSGACGTVVRNAE
At4CL3   333AAPLGKELQDSLRRRLPQAILGQGYGMTEAGPVLSMSLGFAKEPIPTKSGSCGTVVRNAE
SbCLL-7  316AAPLGRELMQECAKNFPQAVVVQGYGMTETCGIVSVENVYAGP---RHSGSAGTLVPGVE
At4CLl7  316AAPLGKDLMEECGRNIPNVLLMQGYGMTETCGIVSVEDPRLGK---RNSGSAGMLAPGVE

                  Box2
At4CL1   390MKIVDPDTGDSLSRNQPGEICIRG 413
At4CL2   383MKILDPDTGDSLPRNKPGEICIRG 406
At4CL3   393LKVVHLETRLSLGYNQPGEICIRG 416
SbCLL-7  373CQIVSVDKLKPLPPGQLGEICVRG 396
At4CLl7  373AQIVSVETGKSQPPNQQGEIWVRG 396
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fig. S6. Western blot analysis of the recombinant SbCLLs and SPB domain analysis of At4CLs and SbCLL-7. (**A**) Five SbCLLs were expressed as fusion proteins with an N-terminal His_6-tag in E. coli. The total proteins (10 µg each lane), were separated by SDS-PAGE, transferred to nitrocellulose membranes and probed with His-tag antibody. Protein extracts from bacteria containing the empty expression vector served as a control. Protein sizes are: SbCLL-1, 59.96 kD; SbCLL-5, 61.40 kD; SbCLL-6, 59.39 kD; SbCLL-7, 59.33 kD ; SbCLL-8, 56.12 kD. (**B**) The amino acid identity within the SPB domain of SbCLL-7 to 4CL-like proteins from *Arabidopsis thaliana*. (**C**) Multiple alignment of At4CL 1,2, 3, At4CLl7 and SbCLL-7. * Indicates Asn-256 of At4CL2 and Ala-249 of SbCLL-7 respectively.

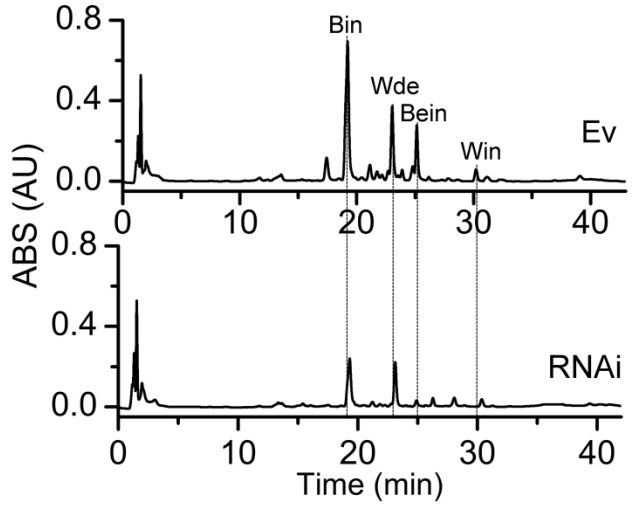

fig. S7. Metabolite profiles by HPLC from empty vector line and a representative *SbCLL-7* RNAi line.

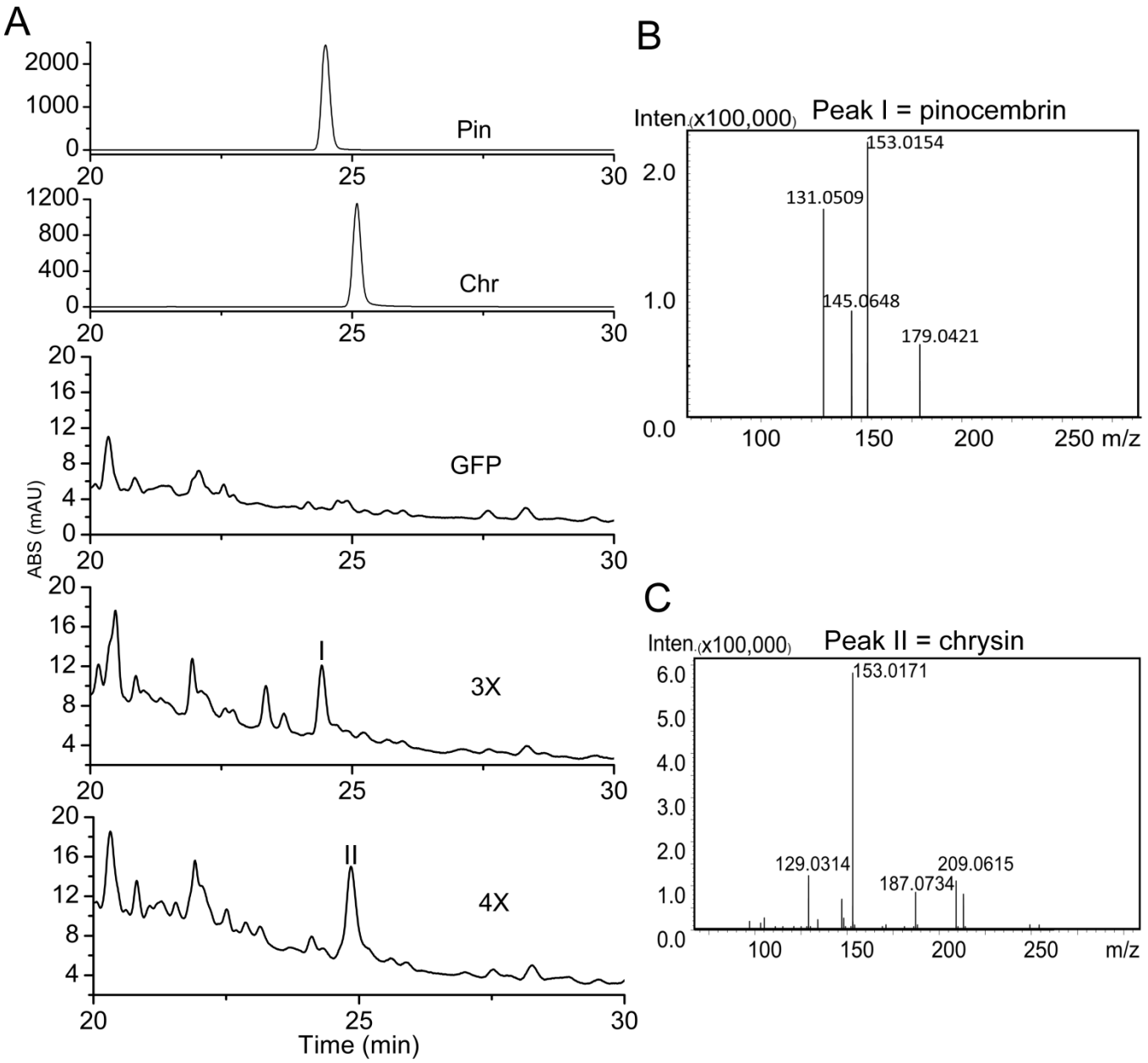

fig. S8. Metabolite profiles of HPLC analysis of infiltrated *N. benthamiana* leaves. (**A**) Metabolite profiles of infiltrated tobacco leaf without supplementation of cinnamate. Pin and Chr means pinocembrin and chrysin standards, respectively; GFP, *N. benthamiana* leaf infiltrated with GFP as a control; 3X, leaf infiltrated with *SbCLL-7*, *SbCHS-2* and *SbCHI*. 4X, leaf infiltrated with *SbCLL-7*, *SbCHS-2*, *SbCHI* and *SbFNSII-2*. (**B**) MS/MS of peak I, which was identical to the pinocembrin standard. (**C**) MS/MS of peak II, which was identical to the chrysin standard.

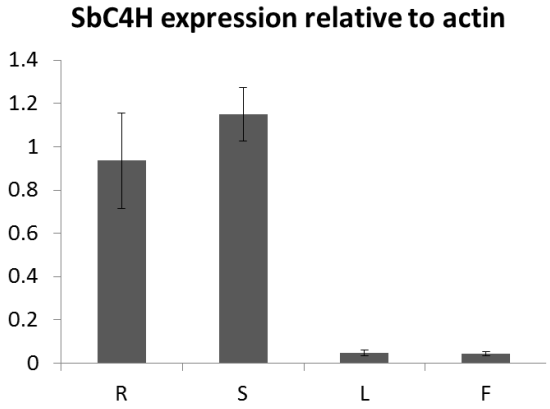

fig. S9. Transcript levels of *SbC4H* relative to actin. SEs were calculated from 3 independent biological replicates. R = roots, S = stems, L = leaves, F = flowers.

table S1. Primers used in this study. Underlined sequences mean recombination cites for Gateway cloning.

Names	Used for	Sequences
AP	3'RACE	GGCACGCGTCGACTAGTACT$[T]_{16}$
3'RACE AUAP	3'RACE	GGCCACGCGTCGACTAGTAC
SbFNSII1 GSP 1	3'RACE	GCTGGAAAAGATTATCACCGACAG
SbFNSII1 GSP 2	3'RACE	CCAGAAAGTGGTGGGATCTTGTAG
SbFNSII1 SP1	5'RACE	GCAAATCGAAGTTCTTACAGAACCAG
SbFNSII1 SP2	5'RACE	GCAAATCGAAGTTCTTACAGAACCAG
SbFNSII1 SP3	5'RACE	GATTCGCTCTTCTCCATGAGAAGC
FNSII1 CDS F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTC ATGGACTTAGTAGAAGTCACAC
FNSII1 CDS R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTAGAAAAC AATTTTCGGGTCAACC
FNSII1 QPCR F	QPCR	AAACCAACGAGCTGGTGTTC
FNSII1 QPCR	QPCR	CGGAGAAAAGTGTGGACCTC
SbFNSII1 RNAi F	RNAi	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACTTCTCAT GGAGAAGAGCGAATC
SbFNSII1 RNAi R	RNAi	GGGGACCACTTTGTACAAGAAAGCTGGGTAACAAGATCC CACCACTTTCTGG
SbFNSII 2 CDS F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGATGGA AGTCACACTGAATGTGG
SbFNSII 2 CDS R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTACTCTCCATA AAAGCAGCAGCAACAG
Sb FNSII2 RNAi F	RNAi	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGATGTGAT GGCTATGGGGAAGG
Sb FNSII2 RNAi R	RNAi	GGGGACCACTTTGTACAAGAAAGCTGGGTACTCTCTTCC AACCACTCTGTCC
SbFNSII 2 QPCR F	QPCR	TCACCTATGGCGTCTCCTTC
SbFNSII 2 QPCR R	QPCR	CAGCTCCTCAGTGACGTTGA
SbCLL1 F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAGA CTGTAGAAAACCACG
SbCLL1 R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAATTTGA

		AAGAGTAGCTGCTTTTG
SbCLL5 F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTTGTC GGTGGCCTCCGTG
SbCLL5 R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAGATGA GGATGGTGGAGTAGCAG
SbCLL6 F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGG AATTGATAGATCCACACAG
SbCLL6 R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTTCACATC TTGGAGAGAGCAAGC
SbCCL7 F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAGA AATCGGGCTATGG
SbCCL7 R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATAACTT CGATCGGACCTTTTC
SbCCL8 F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTAT TTCAACCCTAACCGGTTTG
SbCCL8 R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAGCTCC AAACTTAGGGACCTTAGC
SbCCL1 F	QPCR	ATAATCAAGTACAAAGGGTTCCA
SbCCL1 R	QPCR	ACCTGTTTGGATATAAATTGCTT
SbCCL5 F	QPCR	TGGAAACCCAGAATCCAGAG
SbCCL5 R	QPCR	GTTTCCGACAAAGAGGCAAG
SbCCL7 F	QPCR	ATTGAGGCCACCGTTGTATC
SbCCL7 R	QPCR	CGACTTTTGAAACCGTGGAT
SbCCL7 RNAi F	RNAi	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCATGGTT TACTTCAACTGG
SbCCL7 RNAi R	RNAi	GGGGACCACTTTGTACAAGAAAGCTGGGTTGCAGCTGTA TCATCTTGCTTG
SbCHS1 CDS F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTGAG CGTCGAAGAGTTC
SbCHS1 CDS R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGTTGAG AGGCACACTACGAAG
SbCHS1 QPCR F	QPCR	AACTGCCTCCACCAGTCCACC
SbCHS1 QPCR R	QPCR	GTCAACATCTCCTGCCTGACG
SbCHS2 CDS F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTGAC AGTTGAAGAATTCCAC

SbCHS2 CDS R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAATTGAG AGGCACACTATGC
SbCHS2 QPCR F	QPCR	GCAGTCCACTTATGCTGATTAC
SbCHS2 QPCR R	QPCR	GTGAAGTTGTCGTTCTCCTTC
SbCHI CDS F	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGC TTCGCCATCCGT
SbCHI CDS R	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAAACAA CTCCGATAGTCTTGC
SbCHI QPCR F	QPCR	AAGGCAGTAATAGAGAACAAACAG
SbCHI QPCR R	QPCR	TTAAAACAACTCCGATAGTCTTG
SbC4H F	QPCR	GCCGATTCTCTGTATCACTATC
SbC4H R	QPCR	ATGATTAAAATGATCTTGGCTTT