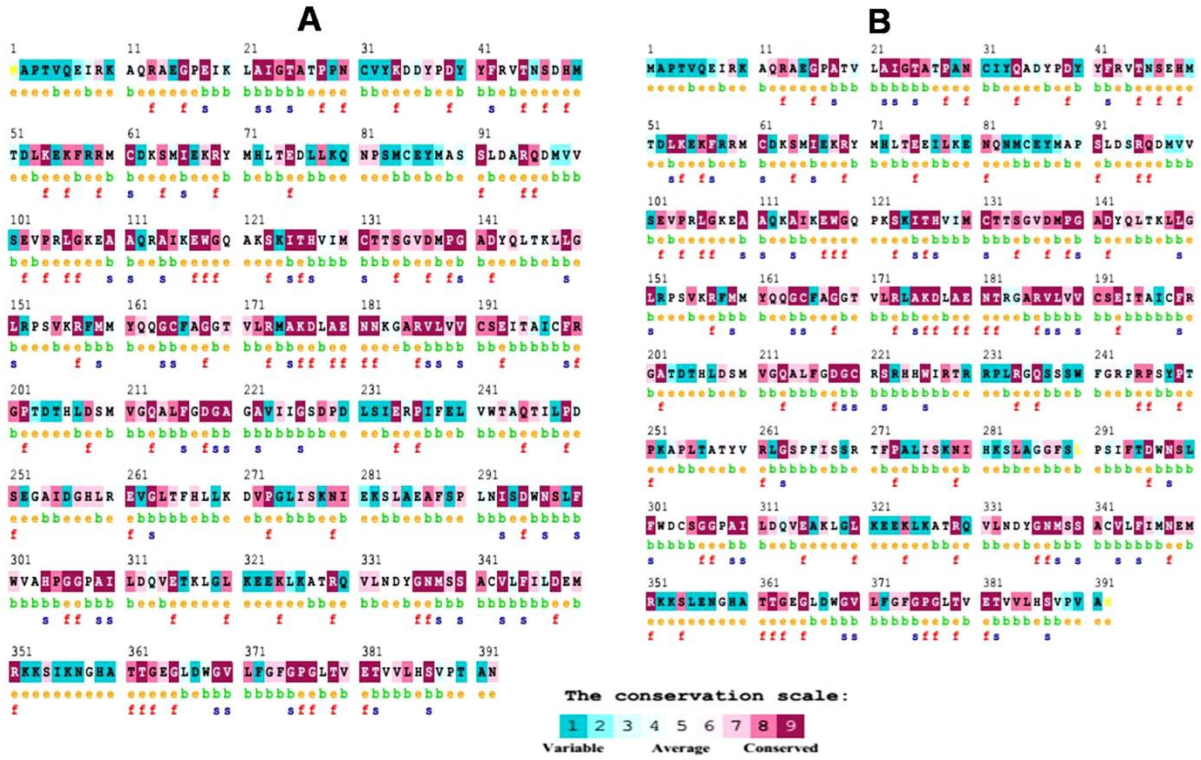
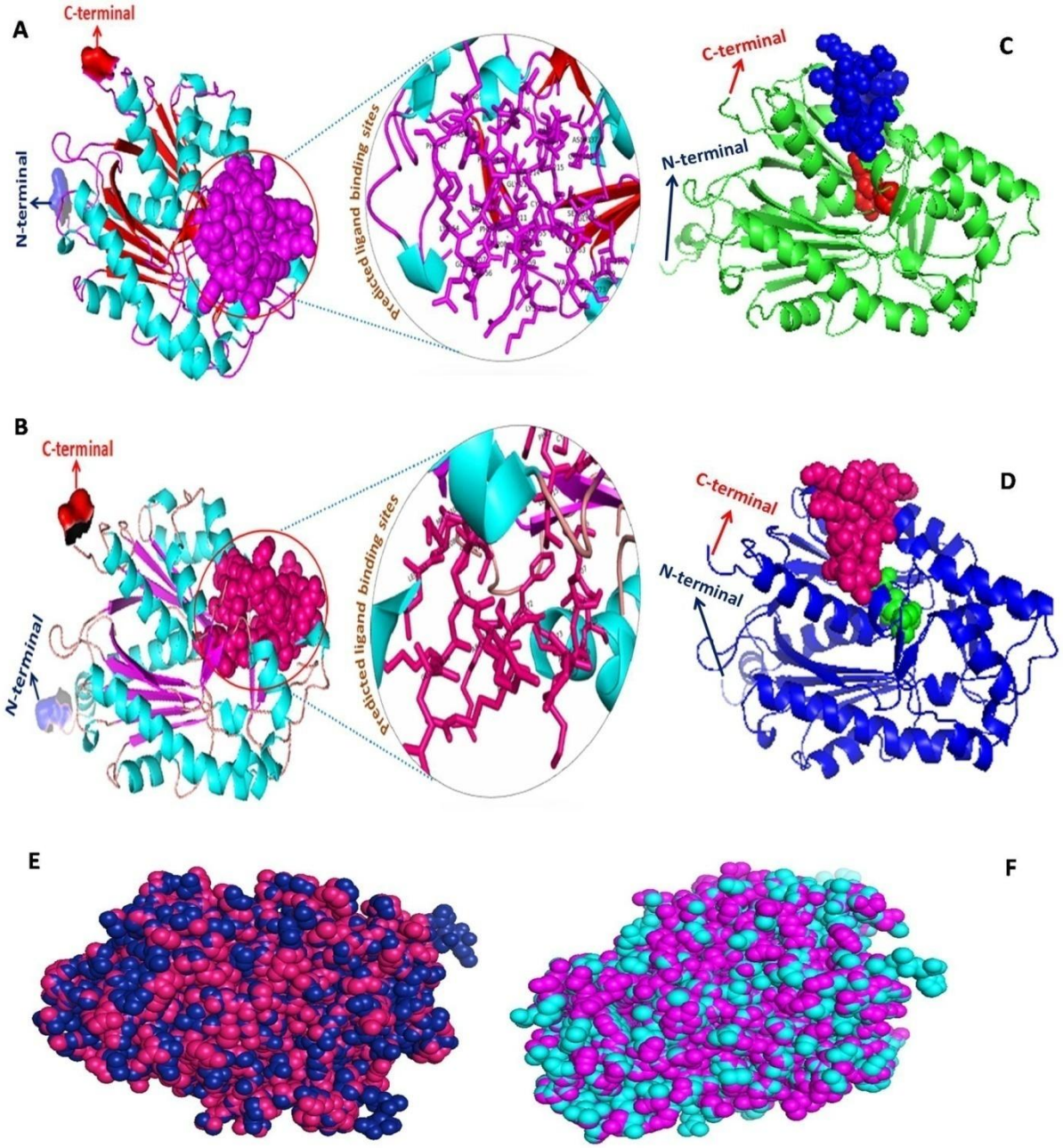
Supplementary material:

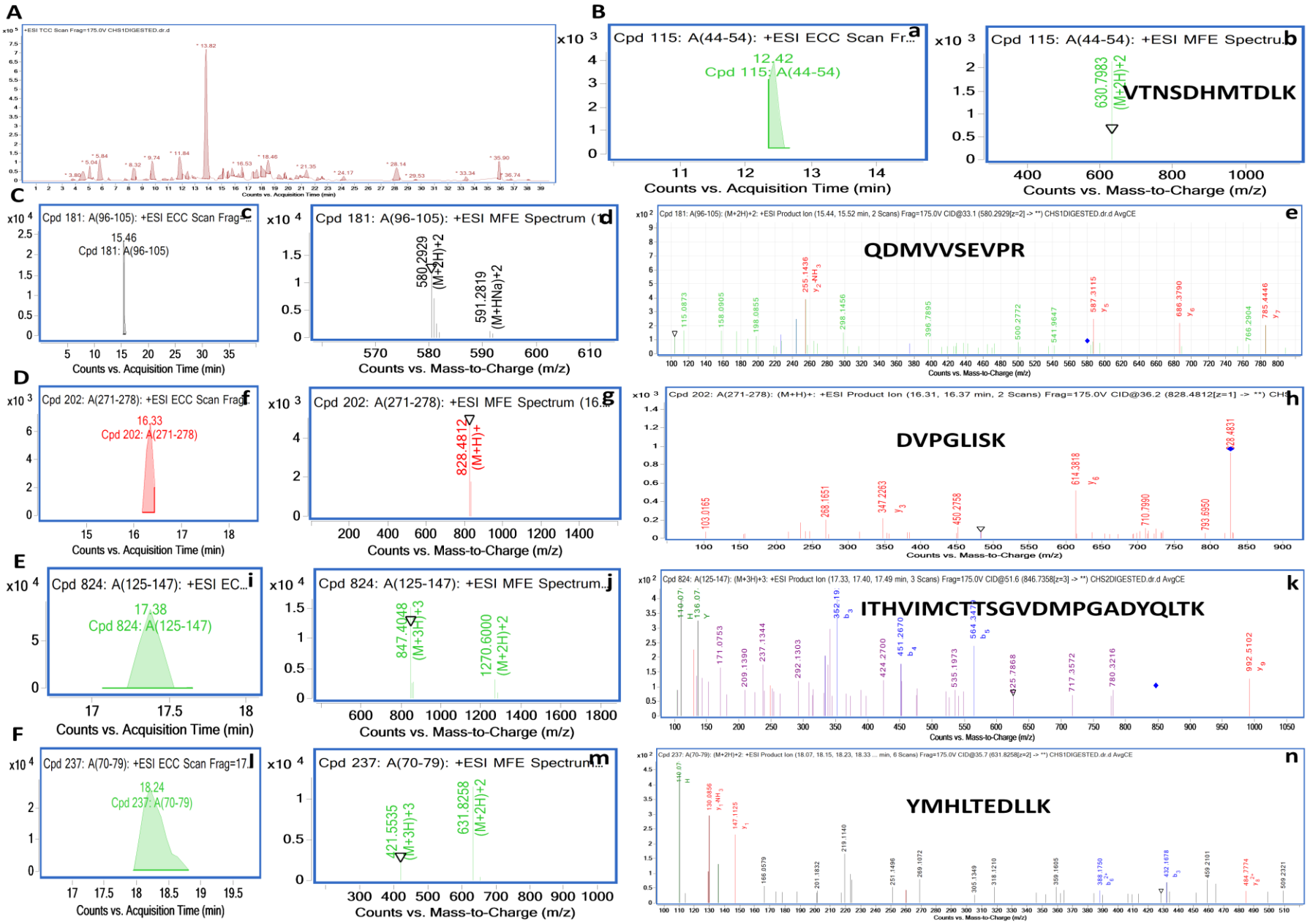


Supplemental Figure S1: Conserved residue prediction for *Re*CHSs: Conserved residue analysis of *Re*CHS1 (A) and *Re*CHS2 (B) were performed using ConSurf and ConSeq web servers. Residue conservation from variable to conserved is shown in blue (1) to purple (9). Abbreviations: e= exposed residue according to the neural-network algorithm; b= buried residue according to the neural-network algorithm; f= predicted functional residue (highly conserved and exposed); s= predicted structural residue (highly conserved and buried); and X= insufficient data, the calculation for this site was performed on less than 10% of the sequences.

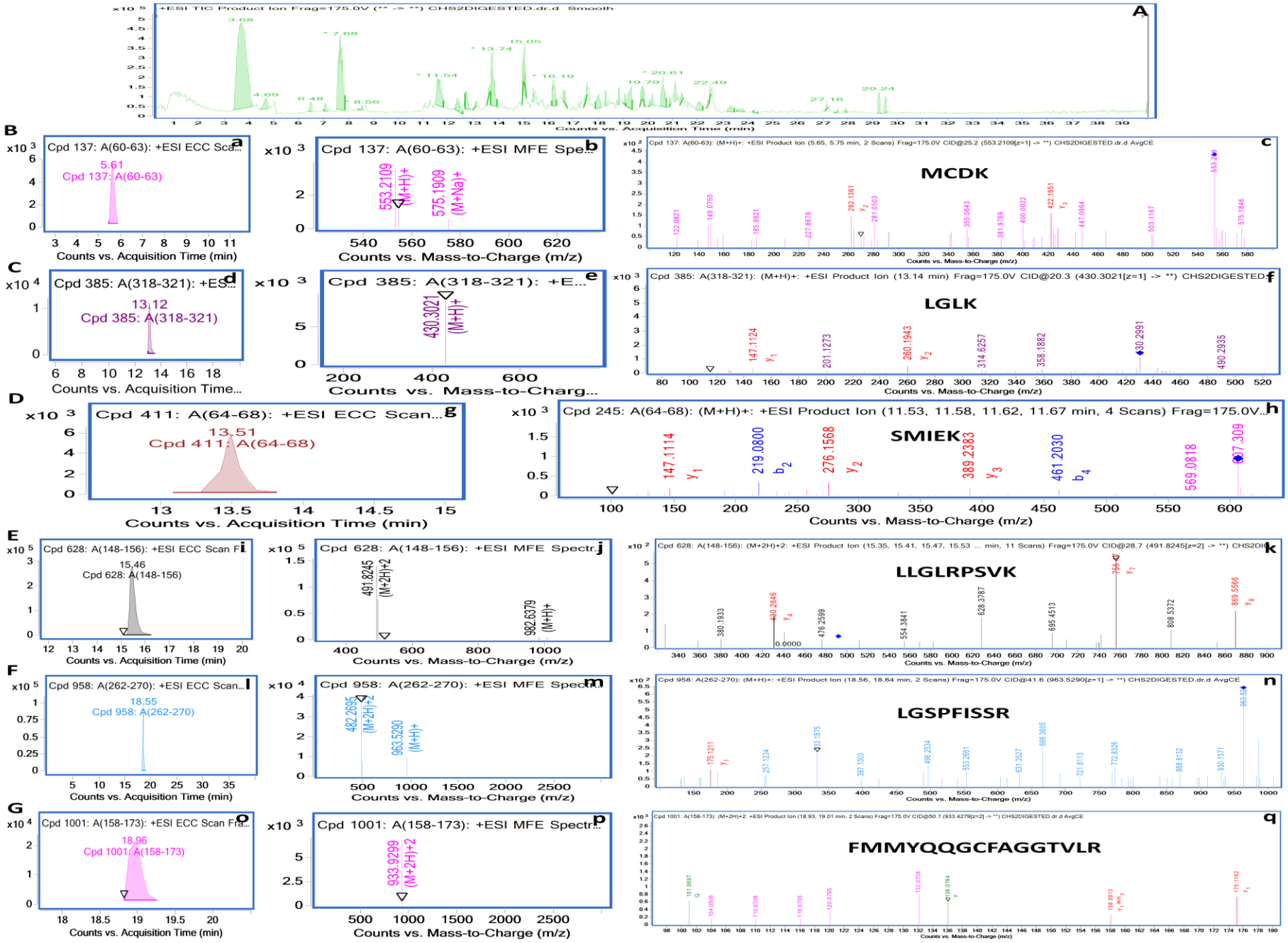


Supplemental Figure S2: Predicted three-dimensional models and ligand-binding sites of *Re*CHSs: (A-B); Ribbon model display of the three-dimensional structures of *Re*CHS1 (A) and *Re*CHS2 (C) as predicted by Phyre[2] web server, using the crystal structure of *Medicago sativa* CHS (PDB code c1cmla) as template. N- and C-terminal domains are shown as blue and red caps, respectively. The ligand binding sites as predicted by 3DLigandSite web server are depicted in the ribbon model (*Re*CHS1, magenta; *Re*CHS2, pink) and also highlighted as an inset. The predicted ligand binding sites for *Re*CHS1 were Lys^{34}, Asp^{35}, Asp^{36}, Tyr^{37}, Pro^{38}, Asp^{39}, Tyr^{41}, Phe^{42}, Lys^{54}, Glu^{55}, Lys^{56}, Phe^{57}, Arg^{58}, Arg^{59}, Met^{60}, Cys^{61}, Asp^{62}, Lys^{63}, Ser^{64}, Cys^{165}, Leu^{207}, Asp^{208}, Ser^{209}, Met^{210}, Val^{211}, Gly^{212}, Gln^{213}, Ala^{214}, Leu^{215}, Phe^{216}, Ile^{255}, Leu^{268}, Lys^{270}, Val^{272}, Pro^{273}, Gly^{306}, Gly^{307}, Ala^{309}, Ile^{310}, Asn^{337} and for *Re*CHS2 the residues include Glu^{55}, Lys^{56}, Phe^{57}, Arg^{58}, Arg^{59}, Met^{60}, Asp^{62}, Lys^{63}, Ser^{64}, Cys^{165}, Leu^{207},

Asp[208], Met[210], Val[211], Gly[212], Leu[215], Phe[216], Leu[255], Ser[268], Arg[270], Phe[272], Pro[273], Gly[306], Gly[307], Ala[309], Ile[310], Asn[337]. **(C-D)**; Ribbon display of 3D structures of *Re*CHS1 (C) and *Re*CHS2 (D) as predicted by I-TASSER server, using the crystal structure of *Medicago sativa* CHS (PDB code c1cmla) as template. The highly conserved catalytic triad (Cys-His-Asn) is shown in the central core of the structures (*Re*CHS1, pink; *Re*CHS2, violet). The malonyl CoA binding motifs are also depicted (*Re*CHS1, blue; *Re*CHS2, pink) **(E-F)**; Superimposition of 3D ribbon models of *Re*CHS1 (E, pink) and *Re*CHS2 (F, cyan) with Alfalfa (blue for *Re*CHS1 and violet for *Re*CHS2) using Pairwise Alignment Tool of FATCAT web server.


A
B
a
Cpd 115: A(44-54): +ESI ECC Scan Fr...
12.42
Cpd 115: A(44-54)
Counts vs. Acquisition Time (min)
b
Cpd 115: A(44-54): +ESI MFE Spectru...
630.7983
(M+2H)+2
VTNSDHMTDLK
Counts vs. Mass-to-Charge (m/z)
C
c
Cpd 181: A(96-105): +ESI ECC Scan Frag=...
15.46
Cpd 181: A(96-105)
Counts vs. Acquisition Time (min)
d
Cpd 181: A(96-105): +ESI MFE Spectrum (1...
580.2929
(M+2H)+2
591.2819
(M+HNa)+2
Counts vs. Mass-to-Charge (m/z)
e
Cpd 181: A(96-105): (M+2H)+2: +ESI Product Ion (15.44, 15.52 min, 2 Scans) Frag=175.0V CID@33.1 (580.2929[z=2] -> **) CHS1DIGESTED.dr.d AvgCE
QDMVVSEVPR
Counts vs. Mass-to-Charge (m/z)
D
f
Cpd 202: A(271-278): +ESI ECC Scan Frag=...
16.33
Cpd 202: A(271-278)
Counts vs. Acquisition Time (min)
g
Cpd 202: A(271-278): +ESI MFE Spectrum (16...
828.4812
(M+H)+
Counts vs. Mass-to-Charge (m/z)
h
Cpd 202: A(271-278): (M+H)+: +ESI Product Ion (16.31, 16.37 min, 2 Scans) Frag=175.0V CID@36.2 (828.4812[z=1] -> **) CHS...
DVPGLISK
Counts vs. Mass-to-Charge (m/z)
E
i
Cpd 824: A(125-147): +ESI EC...
17.38
Cpd 824: A(125-147)
Counts vs. Acquisition Time (min)
j
Cpd 824: A(125-147): +ESI MFE Spectrum...
847.4048
(M+3H)+3
1270.6000
(M+2H)+2
Counts vs. Mass-to-Charge (m/z)
k
Cpd 824: A(125-147): (M+3H)+3: +ESI Product Ion (17.33, 17.40, 17.49 min, 3 Scans) Frag=175.0V CID@51.6 (846.7358[z=3] -> **) CHS2DIGESTED.dr.d AvgCE
ITHVIMCTTSGVDMPGADYQLTK
Counts vs. Mass-to-Charge (m/z)
F
l
Cpd 237: A(70-79): +ESI ECC Scan Frag=17...
18.24
Cpd 237: A(70-79)
Counts vs. Acquisition Time (min)
m
Cpd 237: A(70-79): +ESI MFE Spectrum...
421.5535
(M+3H)+3
631.8258
(M+2H)+2
Counts vs. Mass-to-Charge (m/z)
n
Cpd 237: A(70-79): (M+2H)+2: +ESI Product Ion (18.07, 18.15, 18.23, 18.33 ... min, 6 Scans) Frag=175.0V CID@35.7 (631.8258[z=2] -> **) CHS1DIGESTED.dr.d AvgCE
YMHLTEDLLK
Counts vs. Mass-to-Charge (m/z)

Supplemental Figure S3: LC-MS/MS of tryptic digest of *Re*CHS1: (A) Positive ESI TIC spectrum of tryptic digest; **(B)** The extracted EIC chromatogram of +2 charged 630.7983 ion (a) and its spectrum with molecular ion peak eluting at 12.42 min (b); **(C)** extracted EIC chromatogram of +2 charged 580.2929 ion (c), its spectrum with molecular ion peak eluting at 15.46 min (d), MS/MS spectrum of 580.2929 $[M+2H]^{+2}$ (e); **(D)** extracted EIC chromatogram of +1 charged 828.4812 ion (f), its spectrum with molecular ion peak eluting at 16.33 min (g), MS/MS spectrum of 828.4812 $[M+H]^{+1}$ (h); **(E)** extracted EIC chromatogram of +3 charged 847.4048 ion (i), its spectrum with molecular ion peak eluting at 17.38 min (j), MS/MS spectrum of 847.4048 $[M+2H]^{+3}$ (k); **(F)** extracted EIC chromatogram of +3 charged 421.5535 ion (l), its spectrum with molecular ion peak eluting at 18.24 min (m), MS/MS spectrum of 421.5535 $[M+2H]^{+3}$ (n). The chromatograms were acquired in the positive ionisation mode and the identified peptides are depicted on the respective tandem mass spectra of precursor ions. The QTOF-MS spectra gave less than 1 ppm error from the theoretical accurate mass (*m/z*) for all the identified peptides (Table I). Fragmentation during MS analysis yielded numerous b- and y- ions, which permitted sequencing of the peptide. The screenshot views of matched b- and y- fragment ions were also generated using the Agilent Mass Hunter BioConfirm version B.06.00 software (not shown).


A
+ESI TIC Product Ion Frag=175.0V (** -> **) CHS2DIGESTED.dr.d Smooth
Counts vs. Acquisition Time (min)
B
a
Cpd 137: A(60-63): +ESI ECC Sca...
5.61
Cpd 137: A(60-63)
Counts vs. Acquisition Time (min)
b
Cpd 137: A(60-63): +ESI MFE Spe...
553.2109 (M+H)+
575.1909 (M+Na)+
Counts vs. Mass-to-Charge (m/z)
c
Cpd 137: A(60-63): (M+H)+: +ESI Product Ion (5.65, 5.75 min, 2 Scans) Frag=175.0V CID@25.2 (553.2109[z=1] -> **) CHS2DIGESTED.dr.d AvgCE
MCDK
Counts vs. Mass-to-Charge (m/z)
C
d
Cpd 385: A(318-321): +ES...
13.12
Cpd 385: A(318-321)
Counts vs. Acquisition Time...
e
Cpd 385: A(318-321): +E...
430.3021 (M+H)+
Counts vs. Mass-to-Charg...
f
Cpd 385: A(318-321): (M+H)+: +ESI Product Ion (13.14 min) Frag=175.0V CID@20.3 (430.3021[z=1] -> **) CHS2DIGESTED
LGLK
Counts vs. Mass-to-Charge (m/z)
D
g
Cpd 411: A(64-68): +ESI ECC Scan...
13.51
Cpd 411: A(64-68)
Counts vs. Acquisition Time (min)
h
Cpd 245: A(64-68): (M+H)+: +ESI Product Ion (11.53, 11.58, 11.62, 11.67 min, 4 Scans) Frag=175.0V...
SMIEK
Counts vs. Mass-to-Charge (m/z)
E
i
Cpd 628: A(148-156): +ESI ECC Scan Fi...
15.46
Cpd 628: A(148-156)
Counts vs. Acquisition Time (min)
j
Cpd 628: A(148-156): +ESI MFE Spectr...
491.8245 (M+2H)+2
982.6379 (M+H)+
Counts vs. Mass-to-Charge (m/z)
k
Cpd 628: A(148-156): (M+2H)+2: +ESI Product Ion (15.35, 15.41, 15.47, 15.53 ... min, 11 Scans) Frag=175.0V CID@28.7 (491.8245[z=2] -> **) CHS2DIG
LLGLRPSVK
Counts vs. Mass-to-Charge (m/z)
F
l
Cpd 958: A(262-270): +ESI ECC Scan...
18.55
Cpd 958: A(262-270)
Counts vs. Acquisition Time (min)
m
Cpd 958: A(262-270): +ESI MFE Spectr...
482.2695 (M+2H)+2
963.5290 (M+H)+
Counts vs. Mass-to-Charge (m/z)
n
Cpd 958: A(262-270): (M+H)+: +ESI Product Ion (18.56, 18.64 min, 2 Scans) Frag=175.0V CID@41.6 (963.5290[z=1] -> **) CHS2DIGESTED.dr.d AvgCE
LGSPFISSR
Counts vs. Mass-to-Charge (m/z)
G
o
Cpd 1001: A(158-173): +ESI ECC Scan Fra...
18.96
Cpd 1001: A(158-173)
Counts vs. Acquisition Time (min)
p
Cpd 1001: A(158-173): +ESI MFE Spectr...
933.9299 (M+2H)+2
Counts vs. Mass-to-Charge (m/z)
q
Cpd 1001: A(158-173): (M+2H)+2: +ESI Product Ion (18.93, 19.01 min, 2 Scans) Frag=175.0V CID@50.7 (933.4279[z=2] -> **) CHS2DIGESTED.dr.d AvgCE
FMMYQQGCFAGGTVLR
Counts vs. Mass-to-Charge (m/z)

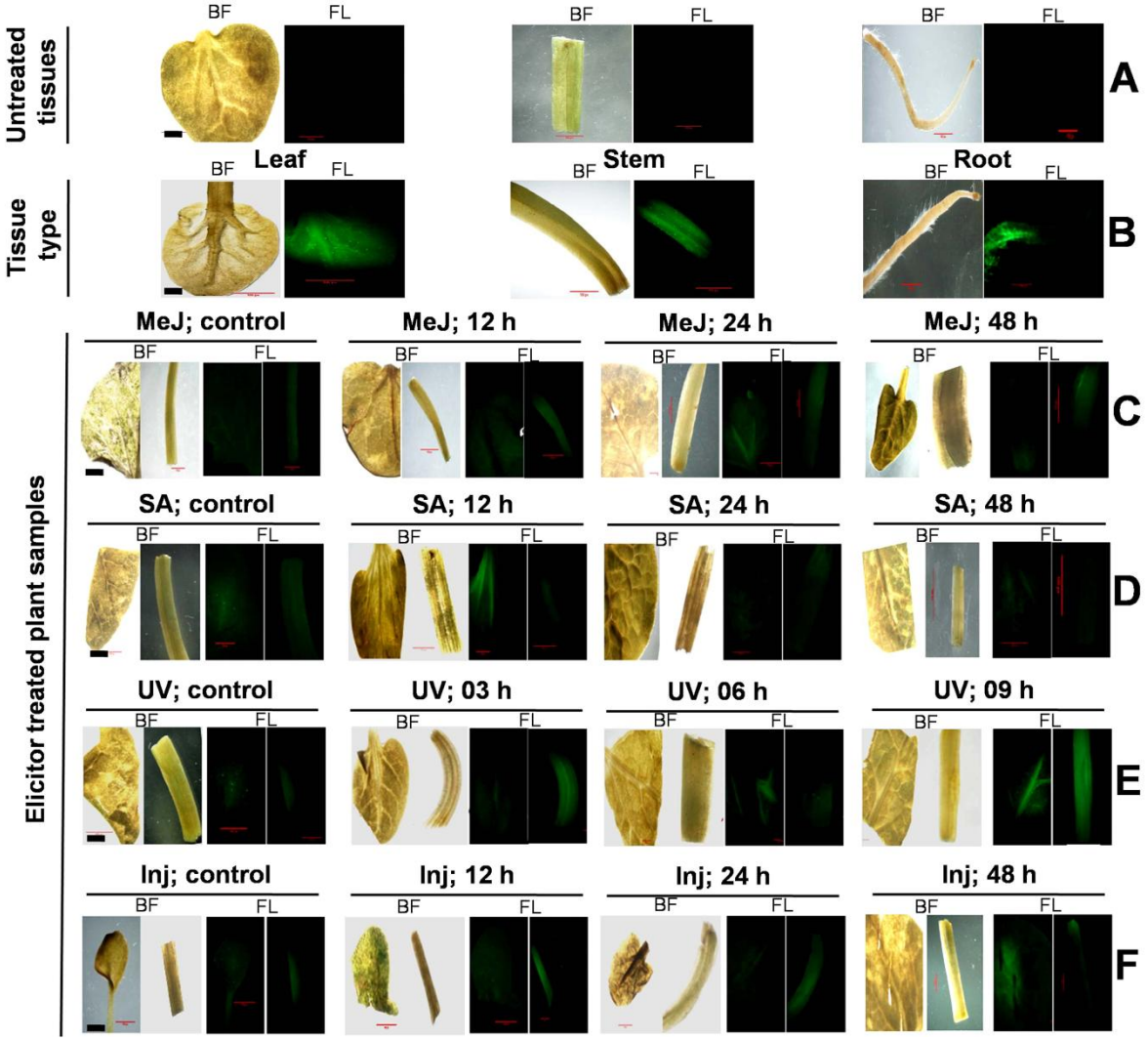
Supplemental Figure S4: LC-MS/MS of tryptic digest of *Re*CHS2: (A) Positive ESI TIC spectrum of tryptic digest; **(B)** The extracted EIC chromatogram of +1 charged 553.2109 ion (a), its spectrum with molecular ion peak eluting at 5.61 min (b), MS/MS spectrum of 553.2109 $[M+H]^{+1}$ (c); **(C)** extracted EIC chromatogram of +1 charged 430.3021 ion (d), its spectrum with molecular ion peak eluting at 13.12 min (e), MS/MS spectrum of 430.3021 $[M+H]^{+1}$ (f); **(D)** extracted EIC chromatogram of +1 charged 607.309 ion (g), MS/MS spectrum of 607.309 $[M+H]^{+1}$ (h); **(E)** extracted EIC chromatogram of +2 charged 491.8245 ion (i), its spectrum with molecular ion peak eluting at 15.46 min (j), MS/MS spectrum of 491.8245 $[M+2H]^{+2}$ (k); **(F)** extracted EIC chromatogram of +1 charged 963.5290 ion (l), its spectrum with molecular ion peak eluting at 18.55 min (m), MS/MS spectrum of 963.5290 $[M+H]^{+1}$ (n). **(G)** extracted EIC chromatogram of +2 charged 933.9299 ion (o), its spectrum with molecular ion peak eluting at 18.96 min (p), MS/MS spectrum of 933.9299 $[M+2H]^{+2}$ (q). The chromatograms were acquired in the positive ionisation mode and the identified peptides are depicted on the respective tandem mass spectra of precursor ions. The QTOF-MS spectra gave less than 1 ppm error from the theoretical accurate mass (*m/z*) for all the identified peptides except 963.5290 $[M+H]^{+1}$ ion where ppm error was 5.13 (Table I). Fragmentation during MS analysis yielded numerous b- and y- ions, which permitted sequencing of the peptide. The screenshot views of matched b- and y- fragment ions were also generated using the Agilent Mass Hunter BioConfirm version B.06.00 software (not shown).


+ CTATAGGGCA CGCGTGGTCG ACGGCCTGTA ATACGACTCA CTATGGGGCA CGCGTGGTCG ACGGCCTGTA
- GATATCCCGT GCGCACCAGC TGCCGGACAT TATGCTGAGT GATACCCCGT GCGCACCAGC TGCCGGACAT
+ ATACGACTTA CTATAGGGCA CGCGTGGTCG ACGGCCCGGG CTGGTCCTTG ACGGCTTTGA TGTGATTTAT
- TATGCTGAAT GATATCCCGT GCGCACCAGC TGCCGGGCCC GACCAGGAAC TGCCGAAACT ACACTAAATA
CGTCA-motif
TGACG-motif
+ TGTTGTGTTG AACTGTTTAG TTAGCACTTT TATGACGTGC TTAAGTTTAC CAACATTTGT CATGTTTCCT
- ACAACACAAC TTGACAAATC AATCGTGAAA ATACTGCACG AATTCAAATG GTTGTAAACA GTACAAAGGA
G-box
+ ATAAGATTCA TTTCGTTGGA ATCTTCTGTG GGATTGATTG CTGTAAAGAC CCGGTCTTTC AGAATCATCT
- TATTCTAAGT AAAGCAACCT TAGAAGACAC CCTAACTAAC GACATTTCTG GGCCAGAAAG TCTTAGTAGA
W-box
+ CTTGATCATG CCTTTTCATT ATTCCAGTAA AAGTATGAAG GTTGACTAGC GTACTCGGTT ATTACTCGAG
- GAACTAGTAC GGAAAAGTAA TAAGGTCATT TTCATACTTC CAACTGATCG CATGAGCCAA TAATGAGCTC
TIS
TATA box
+ GGACGATAAG TCTAACGTAA TACAACTACT TCGAACCATC TGCTTTGTGT CCTTGCATGG TCTATTCATT
- CCTGCTATTC AGATTGGATT ATGTTGATGA AGCTTGGTAG ACGAAACACA GGAACGTACC AGATAAGTAA
5 UTR
+ AGACTAACCA ATCGCATTTG TTATCTGCAT TGTTGGCTGG TGTCATCCGT TCGATT
- TCTGATTGGT TAGCGTAAAC AATAGACGTA ACAACCGACC ACAGTAGGCA AGCTAA
5 UTR

Fig S5 (A)

Fig S5 (B)

Supplemental Figure S5. Nucleotide sequences of *Re*CHS1 (A) and *Re*CHS2 (B) gene promoters. The putative core promoter consensus sequences and the motifs with significant similarity to the previously identified *cis*-acting elements are highlighted and the names are given. TIS represent the transcription initiation site. 5' untranslated regions (UTRs) are depicted with an arrow.



Supplemental Figure S6: Flavonoid accumulation in different tissues of *R. emodi* as determined by diphenylboric acid-2-aminoethyl ester (DPBA) staining: (A) Tissue samples without DPBA staining; **(B)** Relative flavonoid detection in leaf, stem and root tissues of the plant; **(C-F)** Time course effect of abiotic elicitor treatments on flavonoid accumulation in response to 0.1 mM MeJ (C), 0.1 mM SA (D), UV-B radiation (E) and wounding/injury (F). The elicited tissues were harvested at different time intervals ranging from 12 to 48 h as depicted (except for UV for which incubation periods were 3, 6 and 9 h). Fluorescence of flavonoids is enhanced by diphenylboric acid-2-aminoethyl ester (DPBA). Fluorescence was visualized after DPBA staining with blue light under a stereo microscope (Nikon SMZ1000) equipped with a green fluorescent protein (GFP) filter unit (excitation: 480/40 nm, barrier filter: 535/50 nm). For elicitor treated samples, leaf and intermodal segments (2-3 cm above basal portion) were used for imaging and the pictures were taken both in bright- (BF) and fluorescence- (FL) fields. The leaves were irradiated from the abaxial side and bright field images (BF) were taken for leaf morphology interpretation. All the images were obtained under same conditions and assembled using Adobe Photoshop 6 software (Adobe Systems Inc., USA) software without altering the colour parameters. Bar = 100 µm.

Supplemental Table S1: Synonymous and non-synonymous mutations of *Re*CHSs

Sequence name	S	N	ds	dn	ds/dn
*Re*CHS1 versus *Re*CHS2	267.83	911.17	0.889 ± 0.10	0.195 ± .05	4.558

*S mean number of synonymous sites, N mean number of non-synonymous sites, ds mean synonymous substitutions per synonymous site, dn mean non-synonymous substitutions per non-synonymous site

Supplemental Table S2: Concentrations of major flavonoid and anthraquinone constituents from different tissues, plant samples from four different geographical locations of North Western Himalayas and elicitor treated tissues of *Rheum emodi*.

Sample	Flavonoid content (% DWB)				Anthraquinone content (% DWB)	
	Kaempferol	Naringenin	Quercetin	Rutin	Emodin	Chrysophanol
Leaf	0.0236±0.004	3.2633±0.53	0.216±0.022	12.896±0.989	1.873±0.221	3.707±0.188
Stem	0.0198±0.004	3.152±0.46	0.0501±0.004	5.8372±0.511	0.659±0.158	3.338±0.135
Root	0.0126±0.003	4.671±0.439	0.099±0.003	5.5802±0.546	0.5646±0.12	3.0198±0.137
Bonera	0.0145±0.003	12.834±0.853	1.058±0.047	14.305±1.056	***	***
Yarikhah	0.0703±0.004	7.431±0.443	0.734±0.058	11.603±1.413	***	***
Pen se La	0.156±0.005	13.844±0.903	1.431±0.414	15.139±1.034	***	***
Nyoma	0.251±0.01	15.266±1.043	3.915±0.879	17.887±1.114	***	***
Control UV-B	0.0068±0.001	2.806±0.607	0.104±0.006	8.4012±0.49	0.094±0.007	0.278±0.007
UV-3h	0.063±0.005	3.9862±0.437	0.1956±0.005	15.254±0.664	0.812±0.041	2.7118±0.136
UV-6h	0.066±0.005	4.564±0.391	0.1668±0.011	16.705±0.732	0.652±0.034	1.228±0.03
UV-9h	0.0141±0.004	5.0182±0.66	0.044±0.006	12.715±0.52	0.436±0.007	1.719±0.135
Control Wounding	0.0068±0.001	2.806±0.607	0.104±0.006	7.401±1.443	0.090±0.012	0.282±0.021
W-12h	0.0831±0.008	3.007±0.722	0.093±0.007	10.305±1.295	0.578±0.403	0.991±0.064
W-24h	0.056±0.005	3.515±0.547	0.078±0.006	14.794±1.206	0.488±0.034	1.057±0.056
W-48h	0.0214±0.003	5.3313±0.883	0.0309±0.004	17.569±1.619	0.627±0.0372	1.075±0.067
Control SA	0.011±0.003	1.4723±0.024	0.0916±0.003	11.198±0.803	0.2534±0.009	0.292±0.011
SA-12h	0.1637±0.022	3.304±0.254	0.3193±0.031	13.376±0.703	0.505±0.022	1.526±0.046
SA-24h	0.038±0.002	3.6474±0.451	0.185±0.016	16.402±0.817	1.0565±2.761	2.762±0.107
SA-48h	0.0075±0.001	1.425±0.313	0.1335±0.024	15.2103±0.406	0.2091±0.0104	0.273±0.068
Control MeJ	0.011±0.003	1.4723±0.024	0.0916±0.003	11.197±0.803	0.247±0.010	0.298±0.021
MeJ-12h	0.035±0.003	5.722±0.402	0.186±0.01	15.348±0.642	0.439±0.374	1.821±0.127

MeJ-24h	0.075±0.004	8.507±0.637	0.622±0.033	17.086±0.321	0.291±0.028	0.588±0.026
MeJ-48h	0.0124±0.002	2.545±0.212	0.131±0.019	13.3102±0.556	0.581±0.021	2.165±0.216

***Refer to the our earlier report (http://dx.doi.org/10.1016/j.sajb.2014.07.012)

Supplemental Table S3: Primers used in this study.

Name	Sequence	Application
Degenerate primers		
DegCHS1_F	5'-TGACCACATGACCGACTTGA	Core amplification
DegCHS1_R	5'-ACCATGGAGTCCAGGTGAGT	Core amplification
DegCHS2_F	5'-TTGAAGGCCATCGAAGAGTG	Core amplification
DegCHS2_R	5'-GTGAGCAATCCAGAAGAGTGA	Core amplification
5' and 3' RACE primers		
GeneRacer RNA Oligo	5′-CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA	RACE programme
GeneRacer Oligo dT	5′-GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{24}$	RACE programme
5' RACE_OUT*	5′-CGACTGGAGCACGAGGACACTGA	RACE programme
5' RACE_INN*	5′-GGACACTGACATGGACTGAAGGAGTA	RACE programme
3' RACE_OUT*	5′-GCTGTCAACGATACGCTACGTAACG	RACE programme
3' RACE_INN*	5′-CGCTACGTAACGGCATGACAGTG	RACE programme
5' CHS1_OUT	5′-TCTCAATCATCGACTTATCACACATGCG	RACE programme
5' CHS1_INN	5′-TCCTTCAAGTCGGTCATGTGGTCA	RACE programme
3' CHS1_OUT	5′-CCGACACTCACCTGGACTCCATGGTA	RACE programme
3' CHS1_INN	5′-GAGCTCGAATTCACTGGCCGTCGTT	RACE programme
5' CHS2_OUT	5′-ATGAAGCGCTTGACGGAAGGGCGCA	RACE programme
5' CHS2_INN	5′-TCGACGCCCGAGGTGGTGCACATGAT	RACE programme
3' CHS2_OUT	5′-CTCCCCTCAATATTTACTGATTGGAAC	RACE programme
3' CHS2_INN	5′-CTAAGAACATCCACAAGAGTCTTGCGG	RACE programme
Full-length primers		
FulCHS1_F	5'-*ATG*GCGCCAACCGTGCAGGAGAT	Full length cloning
FulCHS1_R	5'-*TCA*ATTGGCGGTGGGAACACTGTGGA	Full length cloning
FulCHS2_F	5'-*ATG*GCACCGACGGTCCAGGAGAT	Full length cloning

FulCHS2_R	5'-*CTA*GTGAGCAACTGGTACACTGTGT	Full length cloning
Expression primers		
ExpCHS1_F	5'-GGATCC*ATG*GCGCCAACCGTGCAGGAGAT	Expression analysis
ExpCHS1_R	5'-GAATTCC*TCA*ATTGGCGGTGGGAACACTGTGGA	Expression analysis
ExpCHS2_F	5'-GGATCC*ATG*GCACCGACGGTCCAGGAGAT	Expression analysis
ExpCHS2_R	5'-GAATTCC*CTA*GTGAGCAACTGGTACACTGTGT	Expression analysis
Real-Time primers		
Actin_F	5'-GAGAGTTTTGATGTCCCTGCCATG	Real-Time analysis
Actin_R	5'-CAACGTCGCATTTCATGATGGAGT	Real-Time analysis
RtCHS1_F	5'-GACCACATGACCGACTTGAA	Real-Time analysis
RtCHS1_R	5'-TCAGAAGATCCTCCGTCAGAT	Real-Time analysis
RtCHS2_F	5'-GGCCCTCATCTCTAAGAACATC	Real-Time analysis
RtCHS2_R	5'-CCTGAGCAATCCCAGAAGAG	Real-Time analysis
Promoter primers		
Walker-AP1*	5'-GTAATACGACTCACTATAGGGC	Promoter analysis
Walker-AP2*	5'-ACTATAGGGCACGCGTGGT	Promoter analysis
PmCHS1_OUT	5'-TGTTGGTGACCCTGAAGTAGTAGTC	Promoter analysis
PmCHS1_INN	5'- CCGTGCCAATGGCCAGTTTTATTT	Promoter analysis
PmCHS2_OUT	5'- GGTAGTCCGCTTGGTAGATGCAGTTG	Promoter analysis
PmCHS2_INN	5'- TCCGCCCTCTGCGCCTTCCTGATCT	Promoter analysis

*Primers provided with the kit # Start/stop codon's are italicized and enzyme sites are underlined

Supplemental Table S4: Correlation of mRNA levels with flavonoid and anthraquinone content in different tissues of *Rheum emodi*. Pearson correlation for the comparison of transcript levels and metabolite (total flavonoid and total anthraquinone) content was performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) to test the statistical significance of the relationship. * indicates $p < 0.1$; ** indicates $p < 0.05$; and *** indicates $p < 0.01$.

	Variables	***Re*CHS1**	***Re*CHS2**	**Total flavonoids**	**Total anthraquinones**
Leaf tissue	*Re*CHS1	-	-0.64*	0.39**	-0.53*
	*Re*CHS2	-0.64*	-	-0.53*	0.04
	Total flavonoids	0.39**	-0.53*	-	-0.34
	Total anthraquinones	-0.53*	0.04	-0.34	-
Stem tissue	*Re*CHS1	-	0.09	0.41	-0.31*
	*Re*CHS2	0.09	-	-0.55*	0.93**
	Total flavonoids	0.41	-0.55*	-	0.81*
	Total anthraquinones	-0.31*	0.93**	0.81*	-
Root tissue	*Re*CHS1	-	0.99***	0.60*	0.36
	*Re*CHS2	0.99***	-	-0.60*	0.34
	Total flavonoids	0.60*	-0.60*	-	0.51*
	Total anthraquinones	0.36	0.34	0.51*	-

Supplemental Table S5: Correlation of mRNA levels with flavonoid and anthraquinone content in plant samples of *Rheum emodi* from different geographic locations (1600-4500 m asl). Pearson correlation for the comparison of transcript levels and metabolite (total flavonoid and total anthraquinone) content was performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) to test the statistical significance of the relationship. * indicates $p < 0.1$; ** indicates $p < 0.05$; and *** indicates $p < 0.01$.

	Variables	***Re*CHS1**	***Re*CHS2**	**Total flavonoids**	**Total anthraquinones**
Bonera, Pulwama	*Re*CHS1	-	0.95**	0.58*	0.24
	*Re*CHS2	0.95**	-	-0.66**	0.40*
	Total flavonoids	0.58*	-0.66**	-	0.32**
	Total anthraquinones	0.24	0.40*	0.32**	-
Yarikhah, Srinagar	*Re*CHS1	-	-0.59***	0.71**	0.39
	*Re*CHS2	-0.59***	-	-0.33	0.66**
	Total flavonoids	0.71**	-0.33	-	-0.89*
	Total anthraquinones	0.39	0.66**	-0.89*	-
Pense La, Ladakh	*Re*CHS1	-	-0.26	0.47*	0.21
	*Re*CHS2	-0.26	-	-0.09	0.49*
	Total flavonoids	0.47*	-0.09	-	-0.68**
	Total anthraquinones	0.21	0.49*	-0.68**	-
Nyoma, Ladakh	*Re*CHS1	-	0.89***	0.64**	-0.36
	*Re*CHS2	0.89***	-	-0.68**	0.30*
	Total flavonoids	0.64**	-0.68**	-	-0.73***
	Total anthraquinones	-0.36	0.30*	-0.73***	-

Supplemental Table S6: Correlation of mRNA levels with flavonoid and anthraquinone content in MeJ treated plants of *Rheum emodi*. Pearson correlation for the comparison of transcript levels and metabolite (total flavonoid and total anthraquinone) content was performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) to test the statistical significance of the relationship. * indicates $p < 0.1$; ** indicates $p < 0.05$; and *** indicates $p < 0.01$.

	Variables	***Re*CHS1**	***Re*CHS2**	**Total flavonoids**	**Total anthraquinones**
MeJ control	*Re*CHS1	-	-0.94**	0.25	-0.34
	*Re*CHS2	-0.94**	-	0.57*	0.44*
	Total flavonoids	0.25	0.57*	-	0.30
	Total anthraquinones	-0.34	0.44*	0.30	-
MeJ 12 h	*Re*CHS1	-	-0.03	0.48***	-0.59*
	*Re*CHS2	-0.03	-	-0.89*	0.21**
	Total flavonoids	0.48***	-0.89*	-	0.44*
	Total anthraquinones	-0.59*	0.21**	0.44*	-
MeJ 24 h	*Re*CHS1	-	-0.83**	0.12	-0.47*
	*Re*CHS2	-0.83**	-	0.45*	0.38
	Total flavonoids	0.12	0.45*	-	-0.46*
	Total anthraquinones	-0.47*	0.38	-0.46*	-
MeJ 48 h	*Re*CHS1	-	0.59*	0.36**	-0.38
	*Re*CHS2	0.59*	-	0.52*	0.29
	Total flavonoids	0.36**	0.52*	-	-0.80***
	Total anthraquinones	-0.38	0.29	-0.80***	-

Supplemental Table S7: Correlation of mRNA levels with flavonoid and anthraquinone content in SA treated plants of *Rheum emodi*. Pearson correlation for the comparison of transcript levels and metabolite (total flavonoid and total anthraquinone) content was performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) to test the statistical significance of the relationship. * indicates $p < 0.1$; ** indicates $p < 0.05$; and *** indicates $p < 0.01$.

	Variables	***Re*CHS1**	***Re*CHS2**	**Total flavonoids**	**Total anthraquinones**
SA control	*Re*CHS1	-	0.94**	0.50*	-0.41
	*Re*CHS2	0.94**	-	0.55	0.34*
	Total flavonoids	0.50*	0.55	-	0.33
	Total anthraquinones	-0.41	0.34*	0.33	-
SA 12 h	*Re*CHS1	-	0.95***	0.21	-0.39
	*Re*CHS2	0.95***	-	0.23	0.39*
	Total flavonoids	0.21	0.24	-	0.91*
	Total anthraquinones	-0.39	0.39*	0.91*	-
SA 24 h	*Re*CHS1	-	-0.94**	0.73*	0.63
	*Re*CHS2	-0.94**	-	0.30*	0.62**
	Total flavonoids	0.73*	0.30*	-	0.79*
	Total anthraquinones	0.63	0.62**	0.79*	-
SA 48 h	*Re*CHS1	-	-0.86*	0.61**	0.45*
	*Re*CHS2	-0.86*	-	-0.46***	0.39
	Total flavonoids	0.61**	-0.46***	-	-0.92**
	Total anthraquinones	0.45*	0.39	-0.92**	-

Supplemental Table S8: Correlation of mRNA levels with flavonoid and anthraquinone content in UV treated plants of *Rheum emodi*. Pearson correlation for the comparison of transcript levels and metabolite (total flavonoid and total anthraquinone) content was performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) to test the statistical significance of the relationship. * indicates $p < 0.1$; ** indicates $p < 0.05$; and *** indicates $p < 0.01$.

	Variables	***Re*CHS1**	***Re*CHS2**	**Total flavonoids**	**Total anthraquinones**
UV control	*Re*CHS1	-	0.97**	0.65**	0.05
	*Re*CHS2	0.97**	-	0.05	0.90*
	Total flavonoids	0.65**	0.05	-	0.15
	Total anthraquinones	0.05	0.90*	0.15	-
UV 3 h	*Re*CHS1	-	0.75*	-0.30	0.40
	*Re*CHS2	0.75*	-	0.40	0.74***
	Total flavonoids	-0.30	0.24	-	-0.66**
	Total anthraquinones	0.40	0.74***	-0.66**	-
UV 6 h	*Re*CHS1	-	0.59***	0.43**	0.56*
	*Re*CHS2	0.59***	-	0.56*	0.56**
	Total flavonoids	0.43**	-0.47*	-	-0.09
	Total anthraquinones	0.56*	0.56**	-0.09	-
UV 9 h	*Re*CHS1	-	-0.95**	0.76***	0.34
	*Re*CHS2	-0.95**	-	0.53*	-0.12
	Total flavonoids	0.76***	0.53*	-	-0.45*
	Total anthraquinones	0.34	-0.12	-0.45*	-

Supplemental Table S9: Correlation of mRNA levels with flavonoid and anthraquinone content in wounded plants of *Rheum emodi*. Pearson correlation for the comparison of transcript levels and metabolite (total flavonoid and total anthraquinone) content was performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) to test the statistical significance of the relationship. * indicates $p < 0.1$; ** indicates $p < 0.05$; and *** indicates $p < 0.01$.

	Variables	***Re*CHS1**	***Re*CHS2**	**Total flavonoids**	**Total anthraquinones**
Wound control	*Re*CHS1	-	0.55	0.07	-0.33
	*Re*CHS2	0.55	-	0.45*	0.68**
	Total flavonoids	0.07	0.45*	-	0.94**
	Total anthraquinones	-0.3	0.68**	0.94**	-
Wound 12 h	*Re*CHS1	-	0.99***	0.41*	-0.39**
	*Re*CHS2	0.99***	-	0.37	0.36*
	Total flavonoids	0.41*	0.37	-	0.89*
	Total anthraquinones	-0.39**	0.36*	0.89*	-
Wound 24 h	*Re*CHS1	-	0.99***	0.57*	0.39
	*Re*CHS2	0.99***	-	-0.34**	0.43***
	Total flavonoids	0.57*	-0.34**	-	0.09
	Total anthraquinones	0.39	0.43***	0.09	-
Wound 48 h	*Re*CHS1	-	-0.69***	0.41	0.22
	*Re*CHS2	-0.69***	-	-0.46*	0.16
	Total flavonoids	0.41	-0.46*	-	0.79***
	Total anthraquinones	0.22	0.16	0.79***	-