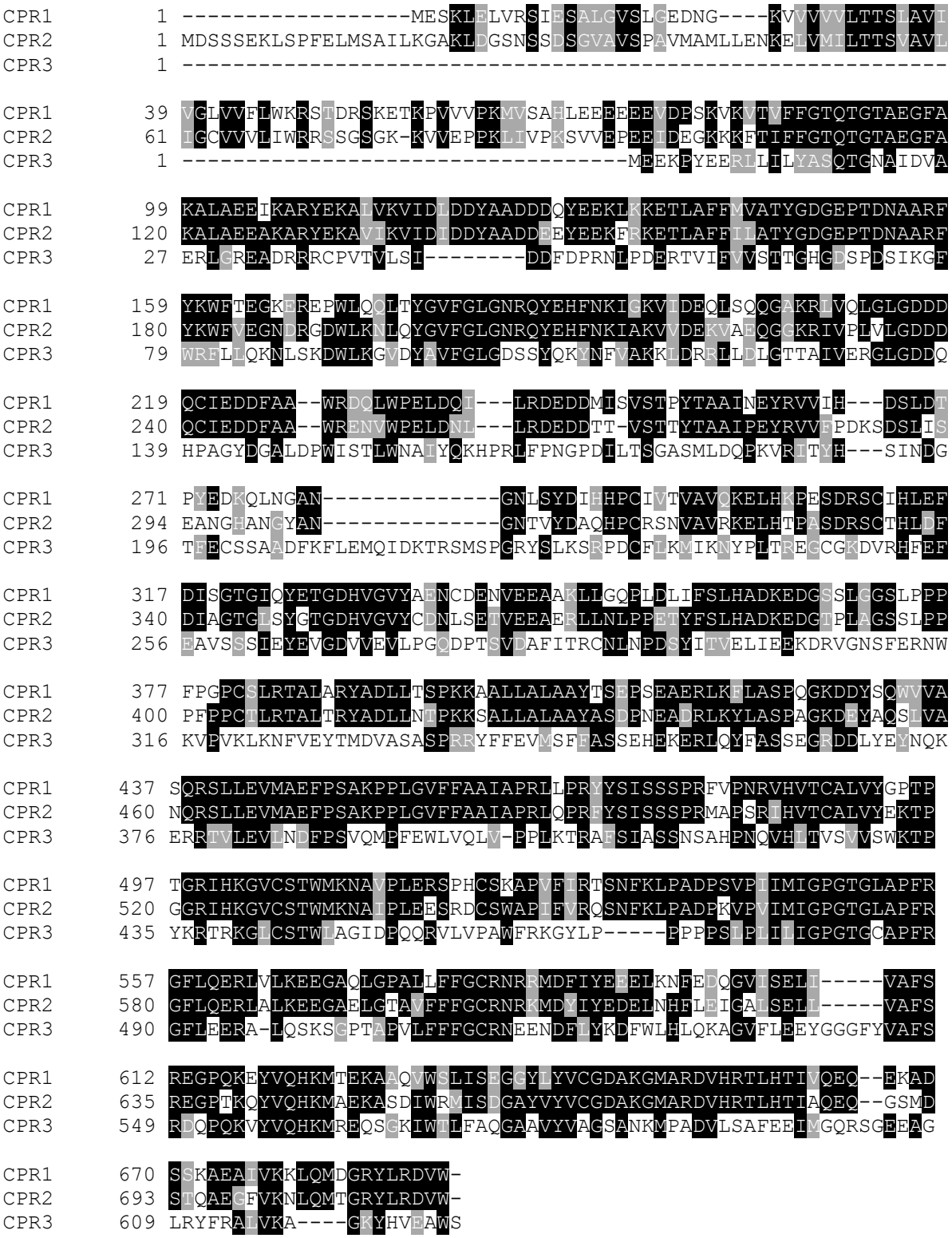
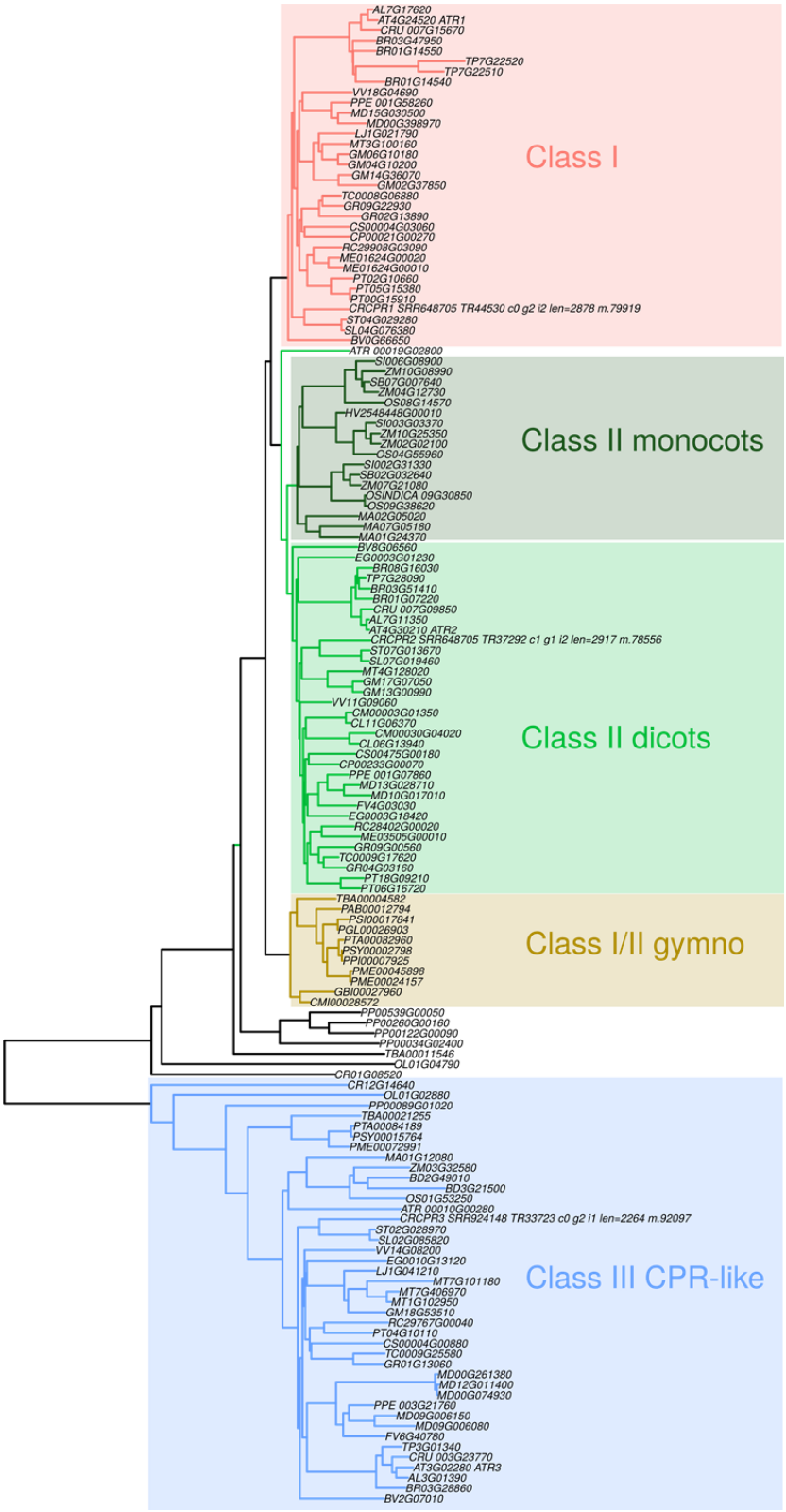
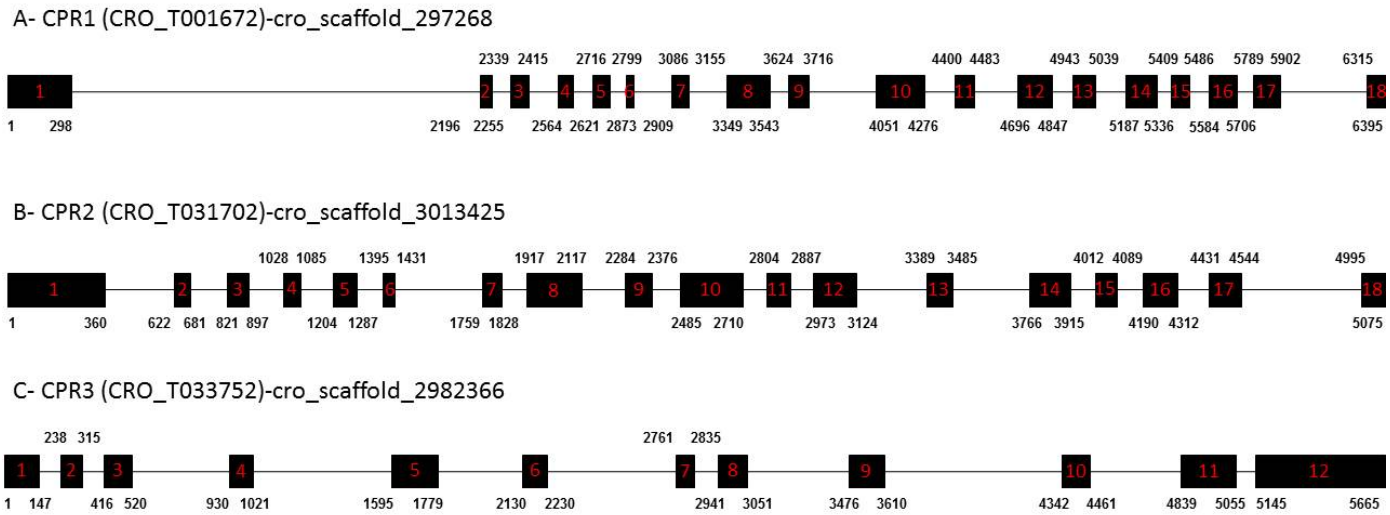


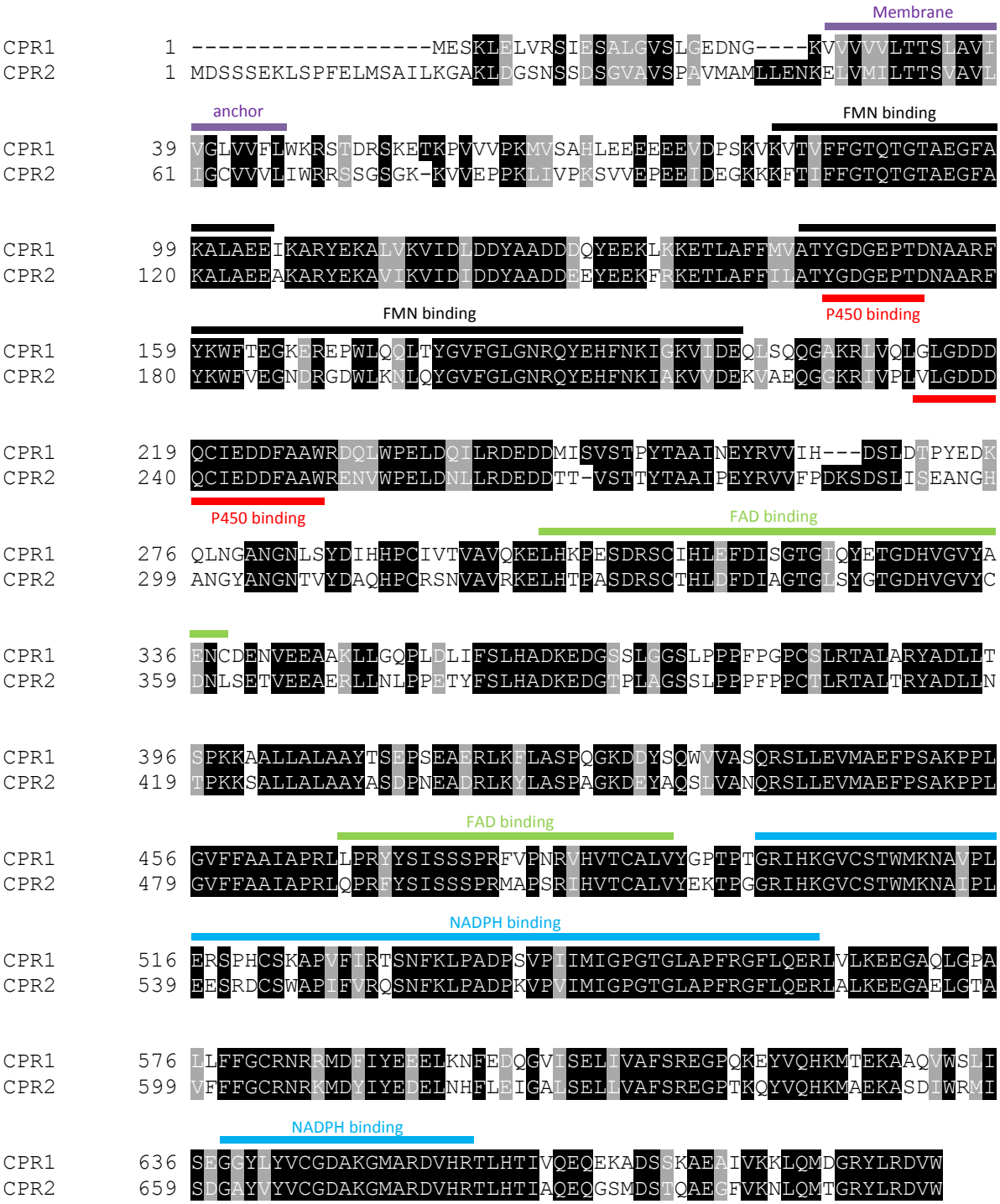
Supplemental Figure 1. Alignment of periwinkle CPR1, CPR2 and CPR3 deduced amino-acid sequences.
Identity and similarity are show with black and grey highlighting respectively.



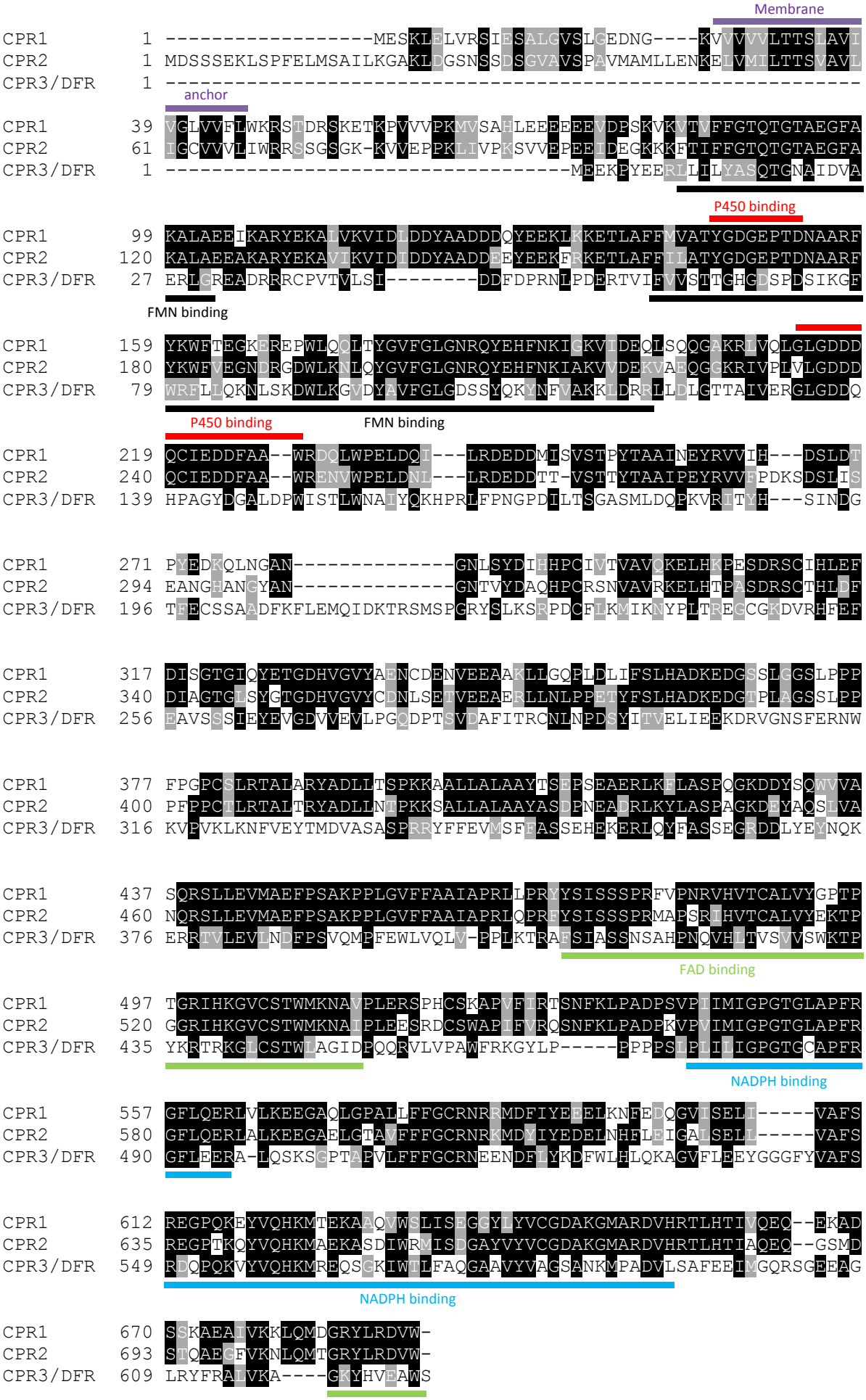
Supplemental Figure 2. CPR phylogenic tree. CPR sequences from Arabidopsis (ATR1 AT4G24520, ATR2 AT4G30210 and ATR3 AT3G02280) were used to find orthologs (blastx) in the PLAZA Dicotyledons v3.0, Monocotyledons v3.0 and Gymnosperm v1.0 databases (http://bioinformatics.psb.ugent.be/plaza/). Protein sequences of orthologs were obtained from pre-computed subfamilies ORTHO03D000287, ORTHO03D000526,ORTHO03D003816, ORTHO03D005981, ORTHO03M000764, ORTHO03M005434, ORTHO03M144957, ORTHO03M151063, ORTHO03M193943. Unique identifiers and sequences were prepared with the 'seqinr' R package. A phylogenetic tree was built with NCBI COBALT tool using a Fast Minimum Evolution tree (Desper R and Gascuel O, Mol Biol Evol 21:587-98, 2004) and default parameters. CRCPR=CPR sequences from Catharanthus roseus. All other identifiers are described on the web server of PLAZA.



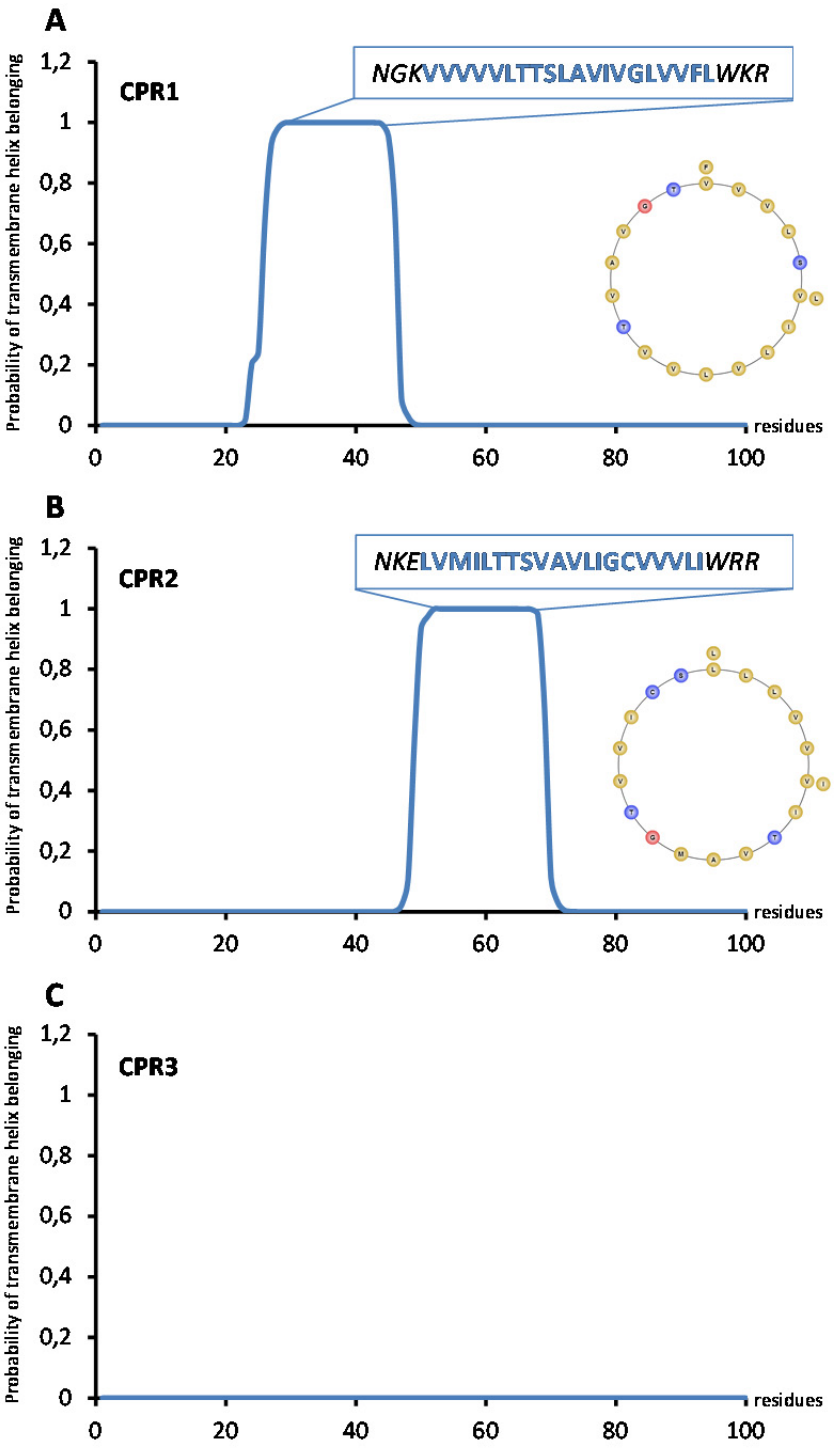
Supplemental Figure 3. Gene organizations of CPR1, CPR2 and CPR3/DFR. Exons and introns are indicated by boxes and solid lines, respectively.



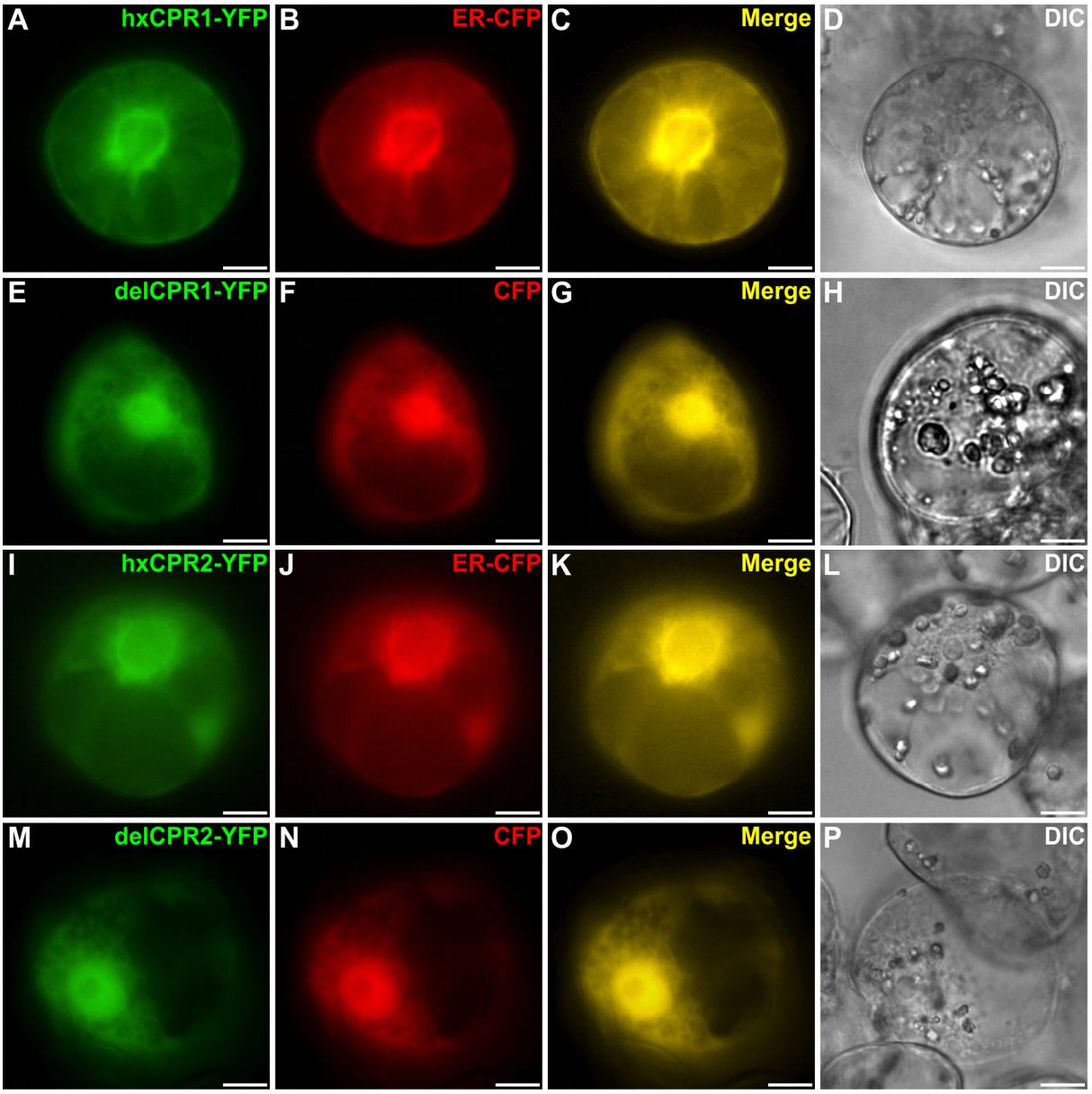
Supplemental Figure 4. Alignment of CPR1 and CPR2 amino-acid sequences highlighting characteristic membrane anchor, FMN, FAD, NADPH and P450-binding domains.



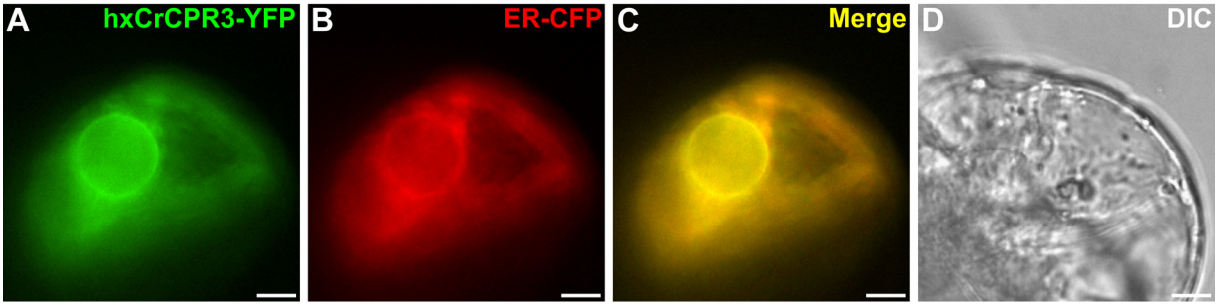
Supplemental Figure 5. Alignment of periwinkle CPR1, CPR2 and CPR3/DFR deduced amino-acid sequences and determination of the putative functional domains of CPR3 according to Varadarajan et al. (2010).



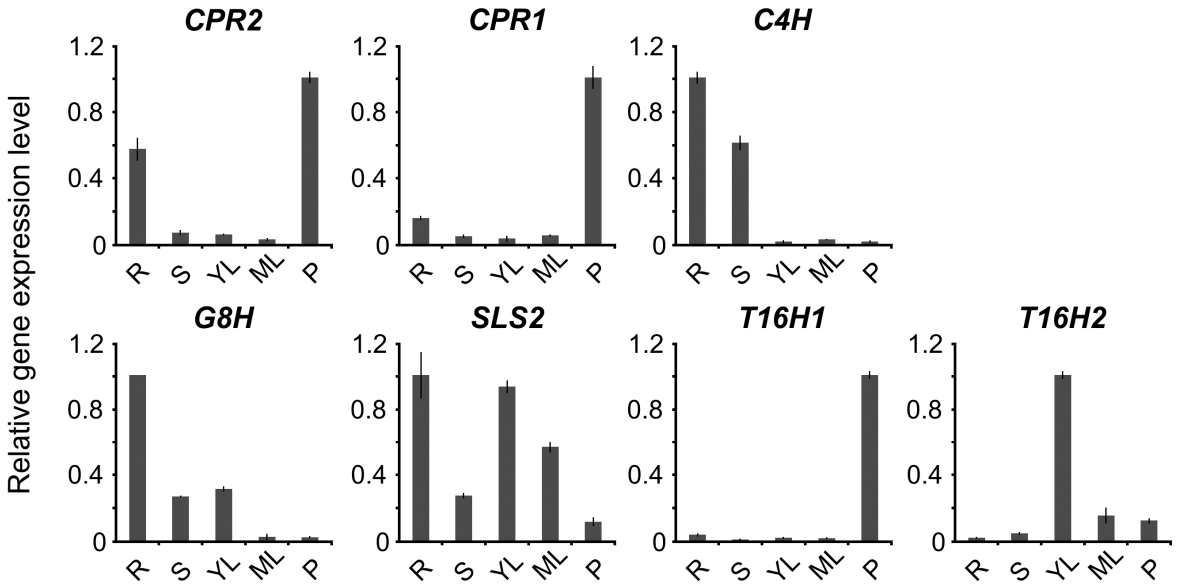
Supplemental Figure 6. Detection of a putative transmembrane helix at the N-terminal end of CPR1 (A), CPR2 (B) and CPR3/DFR (C). Probability of a residue to belong to a transmembrane helix as calculated for the 100-first amino acids of each CPR with a Markov model by the TMHMM server. Projection of the helical wheel has been done using http://www-nmr.cabm.rutgers.edu/bioinformatics/Proteomic_tools/Helical_wheel/.



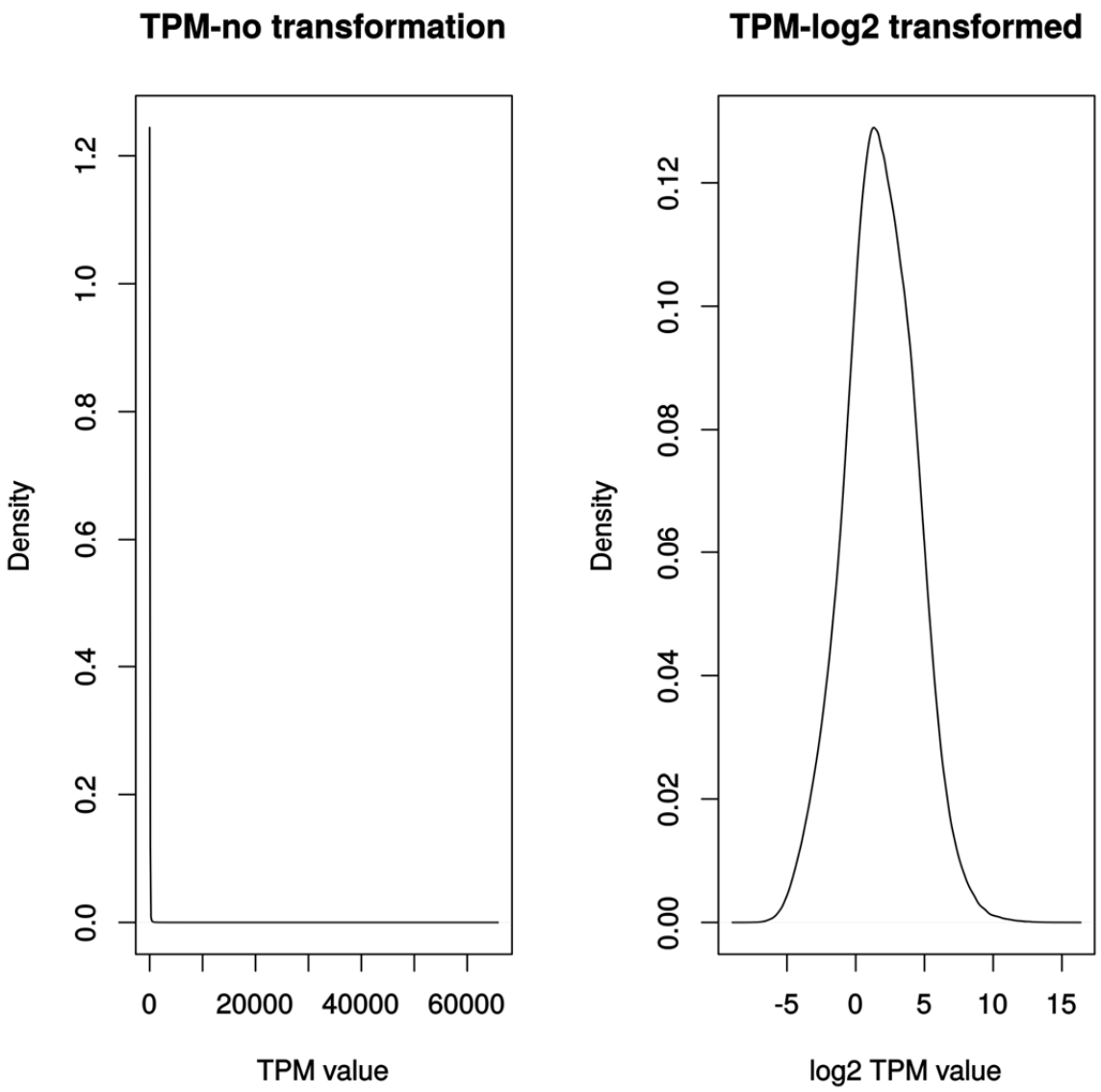
Supplemental Figure 7. Characterization of the membrane anchoring domain of CPR1 and CPR2. *C. roseus* cells were transiently transformed with plasmids expressing hxCPR1-YFP (A) corresponding to the first 53 residues of CPR1 fused to YFP, delCPR1-YFP corresponding to the remaining part of CPR1 (AA 54-691) fused to YFP (E), hxCPR2-YFP (I) corresponding to the first 74 residues of CPR2 fused to YFP or delCPR2-YFP corresponding to the remaining part of CPR2 (AA75-715) fused to YFP (M) in combination with plasmids expressing either an ER-CFP marker ("ER"-CFP; B, I) or a nucleocytosolic marker (CFP; F, N). Colocalization of the fluorescent signals appears on the merged images (C, G, K, O). Cell morphologies (D, H, L, P) were observed with differential interference contrast (DIC). Bars, 10 µm.



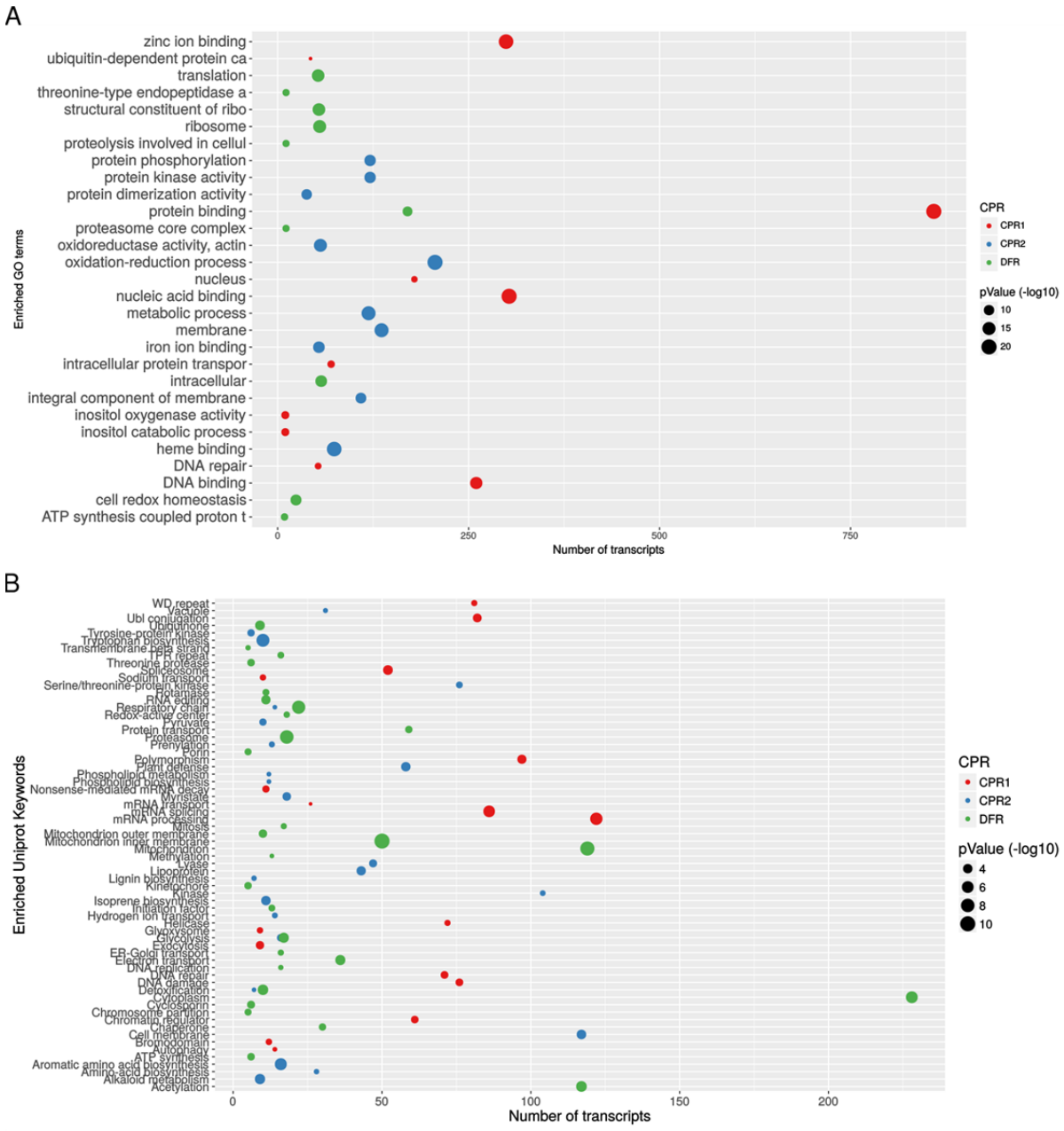
Supplemental Figure 8. Addition of the CPR2 membrane anchoring domain to CPR3 enables ER anchoring. *C. roseus* cells were transiently transformed with the plasmid pSCA-hxCPR3-YFP hxCPR1-YFP (A) in combination with the plasmid expressing the ER-CFP marker ("ER"-CFP; B). Colocalization of the fluorescent signals appears on the merged images (C). Cell morphology (D) was observed with differential interference contrast (DIC). Bars, 10 µm.



Supplemental Figure 9. Transcript distribution of CPR1, CPR2, C4H, G8H, SLS2, T16H1 and T16H2 in various *C. roseus* organs.
Relative expression of each gene was determined by real-time RTPCR analyses performed on total RNA extracted from various *C. roseus* organs including roots (R), stems (S), young leaves (YL), mature leaves (ML) and petals (P). RPS9 was used as a reference gene.



Supplemental Figure 10. Distribution of TPM. Normality was observed after transforming TPM with log2.



Supplemental Figure 11. Functional classification of nearest co-expressed genes with *C. roseus* CPR. (A). GO Molecular functions. GO terms were obtained after Pfam domain attribution with hmmerscan and with GO annotation in Uniprot. This graph presents for each CPR the 10 GO terms with more than 3 genes that were significantly enriched (hypergeometric distribution, FDR-adjusted p-value). (B). Keywords from Uniprot. Homologies to Uniprot entries were obtained by Blastx with the Uniprot database. Keywords from the resulting sequences were retrieved by mapping their names to the database (http://www.uniprot.org/uploadlists/).

contig	CPR type	consensus transcriptome (CDF97)	MPRG database	Phytometasyn database
contig CPR candidate 1	CPR1 (Class I)	SRR648705_TR44530_c0_g2_i2_len=2878	cra_locus_2963_iso_4_2763_ver_3 cra_locus_2963_iso_9_2760_ver_3 cra_locus_2963_iso_2_2285_ver_3 cra_locus_2963_iso_1_2148_ver_3 cra_locus_2963_iso_8_2587_ver_3	cro.CROLV1VD_velvet--Contig7081 cro.CROWL1VD_velvet--Contig5894 cro.CROAJ1VD_velvet--Contig11587 cro.CROWL1VD_velvet--Contig16079 cro.CRO1L1VD_velvet--Contig19083
Original CPR (Meijer et al., 1993)	CPR2 (Class II)	SRR648705_TR37292_c1_g1_i2_len=2917	cra_locus_1734_iso_3_2168_ver_3 cra_locus_1734_iso_1_1343_ver_3 cra_locus_1734_iso_6_1219_ver_3 cra_locus_1734_iso_5_988_ver_3 cra_locus_1734_iso_2_709_ver_3	cro.CROWL1VD_mira--CROWL1VD_rep_c2241 cro.CROLV1VD_velvet--Singlet39068 cro.CROLV1VD_velvet--Contig11364 cro.CROLV1VD_velvet--Contig11853 cro.CROLV1VD_velvet--Contig14514
contig CPR candidate 2	CPR3 (Class III)	SRR924148_TR33723_c0_g2_i1_len=2264	cra_locus_17065_iso_1_1838_ver_3 cra_locus_17065_iso_3_1961_ver_3 cra_locus_17065_iso_1_1975_ver_3	cro.CRO1L1VD_velvet--Singlet4709 cro.CRO1L1VD_velvet--Singlet66479 cro.CROWL1VD_velvet--Singlet981 cro.CROLV1VD_velvet--Singlet17019 cro.CRO1L1VD_velvet--Contig20495

Supplemental Table 1. Identification of contigs potentially encoding CPR candidates in the MPGR database (http://medicinalplantgenomics.msu.edu/index.shtml), phytometasyn database (http://www.phytometasyn.ca/) and in the *C.roseus* consensus transcriptome (Dugé de Bernonville et al., 2015a).

Yeast Strain	WATT 11	WT303	WT303	WT303	WT303	WT303
Expressed CPR	ATR	CPR1	CPR2	CPR3/DFR	hxCPR3	none*
pYeDP60	0	-	-	-	-	0
pYeDP60-T16H1	64.8	46	47.3	0	0	0
pYeDP60-T16H2	83.1	60.9	60.2	0	0	0
pYeDP60	0	-	-	-	-	0
pYeDP60-C4H	72.2	55.3 ± 0.8	50.3 ± 1.61	-	-	24.1
pYeDP60	0	-	-	-	-	0
pYeDP60-SLS2	57.5	52.6	66.1	24	29	27
pYeDP60	0	-	-	-	-	0
pYeDP60-G10H	95	77.2	82.3	-	-	12

- : not tested ; *basal CPR activity provided by the endogenous yeast CPR

Supplemental Table 2. Evaluation of the efficiency of P450 reduction by CPR1, CPR2, CPR3/DFR, hxCPR3/DFR, the yeast endogenous CPR (WT303 yeast strain) and the codon-optimized ATR1 of the WAT11 yeast strain. Substrate conversion rates (%) were determined using crude protein extract of WT303 yeast strain expressing each CPR/P450 pairs (C4H, SLS2, T16H1 and T16H2 assays) or microsomes (G8H assays) by addition of NADPH as electron donor. The hxCPR3 was created by fusion of the 74 first residues of CPR2 including the membrane spanning domain. Similar reactions were performed in the WAT11 yeast strain expressing the codon optimized ATR1 from Arabidopsis. Yeast endogenous CPR activity was also estimated by measuring P450 activity in similar conditions without expression of the periwinkle CPRs, and was subtracted from activities measured with CPR1 and CPR2. Control reactions aiming at evaluating the potential consumption of substrates by yeast endogenous enzymes were carried out using yeast strains transformed with the empty pYeDP60 vector. All assays were conducted independently 3 times with at least three technical replicates. nt, not tested.

Supplemental Table 5. Primers used for cDNA cloning.

Primers	Sequence	Applicatio	Restriction Site	Plasmid
CPRnewfor	CTGAGAACTAGTATGGAATCGAAGTTGGAGTTGGTGAG		*Spe*I	pSCA-YFP et SPYNE
CPRnewrev	CTGAGAACTAGTCCAGACGTCCCTGAGATATCGTC		*Spe*I	
CPRoldfor	CTGAGAGCTAGCATGGATTCTAGCTCGGAGAAGTTGTC		*Nhe*I	pSCA-YFP et SPYNE
CPRoldrev	CTGAGAGCTAGCCCAGACATCTCGGAGATACCTTCC		*Nhe*I	
CPR3pESC-For	CTGAGAACTAGTATGGAGGAGAAGCCGTACGAGGAGA		*Spe*I	pSCA-YFP et SPYNE
CPR3-YFP-Rev	CTGAGAACTAGTAGACCAGGCTTCCACATGATACTTACCG		*Spe*I	
G10Hfor	CTGAGAAGATCTATGGATTACCTTACCATAATATTAAC		*Bgl*II	SPYCE
G10Hnosp_Rev	CTGAGAACTAGTAAGGGTGCTTGGTACAGCA		*Spe*I	
SLS2for	CTGAGAACTAGTATGGAGATGGATATGGATATCATTAGAAAG		*Bgl*II	SPYCE
SLS2rev	CTGAGAACTAGTAAAATTCTGTCTCTCAAGCTTCTTGTAGATA		*Spe*I	
T16H1SpeI_FOR	CTGAGAACTAGTATGGAATTCTATTATTTTCTCTACTTGG		*Spe*I	SPYCE
T16H1nostREV	CTGAGAACTAGTAGCAGGAGAAGAGGAAGAAAAATTA	***Full length ORF cloning , YFP fusion construct- BiFC analysis***	*Spe*I	
T16H2-YFPfor	GCAGATCTATGGAGTTGTATTATTTTTCCACCTTTGC		*Bgl*II	SPYCE
T16H2nostREV	CTGAGAACTAGTATATTTACCTTTGAGAGAAGAAGCAG		*Spe*I	
C4Hfor	CTGAGAAGATCTATGGATCTTCTCCTCTTAGAGAAGA		*Bgl*II	SPYCE
C4Hnost_REV	CTGAGAAGATCTAAAAGTCCTAGGCTTGAGTACAATA		*Bgl*II	
helixCPR1-for	CTGAGAACTAGTATGGAATCGAAGTTGGAGTTGGTGAG		*Spe*I	pSCA-YFP
helixCPR1-rev	CTGAGAACTAGTGCTCCGATCAGTCGATCTCTTCCATA		*Spe*I	
delCPR1	CTGAGAACTAGTATGAAAGAAACGAAGCCCGTGGTTGT		*Spe*I	pSCA-YFP
helixCPR2-for	CTGAGATCTAGAATGGATTCTAGCTCGGAGAAGTTGTCG		*Xba*I	pSCA-YFP
helixCPR2-rev	CTGAGAACTAGTTCCGGAAGATCGCCGCCATATC		*Spe*I	
delCPR2	CTGAGAGCTAGCATGTCGGGTAAAAAAGTCGTGGAGCCT		*Nhe*I	pSCA-YFP
G10Hfor	CTGAGAAGATCTATGGATTACCTTACCATAATATTAAC		*Bgl*II	pYeDP60 -BamHI
G10Hrev	CTGAGAAGATCTTTAAAGGGTGCTTGGTACAG		*Bgl*II	
SLS2-pYEfor	CTGAGAAGATCTATGGAGATGGATATGGATATCATTAGAAAG		*Bgl*II	pYeDP60 – BamHI
SLS2-pYErev	CTGAGAAGATCTTTAAAAATTCTGTCTCTCAAGCTTCTTGTAGAT		*Bgl*II	
T16H1for	CTGAGAAGATCTATGGAGTTGTATTATTTTTCCACCTT		*Bgl*II	pYeDP60 – BamHI
T16H1rev	CTGAGAAGATCTCTAATATTTACCTTTGAGAGAAGAAG		*Bgl*II	
T16H2for	GCAGATCTGATGGAATTCTATTATTTTCTCTACTTGGCC		*Bgl*II	pYeDP60 – BamHI
T16H2rev	GCAGATCTCTAATATTTACCTTTGAGAGAAGAAGCAGAAT	***Yeast expression***	*Bgl*II	
C4Hfor	CTGAGAAGATCTATGGATCTTCTCCTCTTAGAGAAGA		*Bgl*II	pYeDP60 – BamHI
C4Hrev	CTGAGAAGATCTCTAAAAAGTCCTAGGCTTGAGTACA		*Bgl*II	
CPRnew-pESCfor	CTGAGAACTAGTATGGAATCGAAGTTGGAGTTGGTGAG		*Spe*I	pESC-Leu SpeI
CPRnew-pESCrev	CTGAGAACTAGTTCACCAGACGTCCCTGAGATATCGTC		*Spe*I	
CPRold-pESCfor	CTGAGAGCTAGCATGGATTCTAGCTCGGAGAAGTTGTC		*Nhe*I	pESC-Leu SpeI
CPRold-pESCrev	CTGAGAGCTAGCTCACCAGACATCTCGGAGATACCTTCC		*Nhe*I	
CPR3pESC-For	CTGAGAACTAGTATGGAGGAGAAGCCGTACGAGGAGA		*Spe*I	pESC-Leu SpeI
CPR3pESC-Rev	CTGAGAACTAGTTTAAGACCAGGCTTCCACATGATACTTACCG		*Spe*I	
helixCPR2-for	CTGAGATCTAGAATGGATTCTAGCTCGGAGAAGTTGTCG		*Xba*I	pESC-Leu SpeI
helixCPR2-rev	CTGAGAACTAGTTCCGGAAGATCGCCGCCATATC		*Spe*I	
CPR1fw	GGCGCGAUATCAACTGTGGCCTGAATTAG			pTRV2
CPR1rev	GGTTGCGAUTCTAAAGGTTGGCCTAATAATTTTGC	***VIGS***		
CPR2fw	GGCGCGAUCATCTTGGCCACGTATGGAG			pTRV2
CPR2rev	GGTTGCGAUTGCTTCTGAAATAAGTGAATCTGA			

Supplemental Table 5. Primers used for cDNA cloning.

Supplemental Table 6. Primers used for qPCR studies.

Primers	Sequence	Application
qCPR1for	ACTGAAAAGGCCGCCCAAGTA	
qCPR1rev	CAGCAAACAATTTCTAGAAAGGAACTGA	
qCPR2for	GCAGAAAAGGCTTCTGATATTTGGAGGA	
qCPR2rev	TTGGAACAGACGGAGGCAATACT	
qG10Hfor	CATTTATTAGGCGACCAACC	
qG10Hrev	GAACTTCTTTCGCCATTGTT	
qSLS2for	GGTCCATGTCAGTAAAACTCCAGTA	*Gene expression in periwinkle organs*
qSLS2rev	AACAAGGATCCCATGAAGTTGA	
qT16H1for	GCCCAAAACAGCCAATATTCAAACC	
qT16H1rev	ATGTGATGAGTATGGCCACCGC	
qT16H2for	GATCAACTCACAGTGGCAGTC	
qT16H2rev	GACTTGAGGACTTGTGATTGGC	
qC4Hfor	CTAAGATTGATACTAGTGAGAAAGGT	
qC4Hrev	ACTCAAATCTGCAGCGGAGATTCA	
qRPS9 for	TTACAAGTCCCTTCGGTGGT	
qRPSp rev	TGCTTATTCTTCATCCTCTTCATC	
qRTPCR-CPR1fw	CTCTTGTGAAAGTGATTGACTTG	
qRTPCR-CPR1rev	GAATGCTAAGGTCTCTTTCTTCAG	
qRTPCR-CPR2fw	GGGAGCTAAATTAGATGGGTCTAACTC	*CPR silencing characterization*
qRTPCR-CPR2rev	ACTGAAGTAGTCAAAATCATCACTAACT	
qRTPCR-Rbps9fw	TTGAGCCGTATCAGAAATGC	
qRTPCR-Rbps9rv	CCCTCATCAAGCAGACCATA	

Supplemental Table 6. Primers used for qPCR analyses.

Supplemental Material - VIGS

For silencing experiments the pTRV2u vector was transformed with a short section of the target gene (see below). For CPR2 VIGS constructs, a 400 bp section was chosen of the open reading frame, and for *CPR1* a 373 bp long section was chosen. The nucleotide sequence of these inserts is shown below with the primer regions highlighted in yellow.

>VIGS_CPR1 (construct a)

ATCAACTGTGGCCTGAATTAGATCAAATACTCAGAGATGAGGATGATATGATCTCTGTTTCCACCCCATATACAGCTGCAATCAA
TGAATACCGAGTAGTGATTCATGATTCTTTGGATACACCATACGAAGATAAGCAGTTAAACGGGGCTAATGGAAATCTTTCATA
TGATATTCATCATCCATGCATAGTTACTGTTGCGGTTCAAAAAGAGCTTCACAAACCTGAATCCGATCGTTCATGCATTCACCTG
GAATTTGATATTTCTGGAACAGGCATCCAATATGAAACTGGAGATCATGTGGGGGTATATGCTGAAAATTGTGATGAAAATGTT
GAAGAAGCAGCAAAATTATTAGGCCAACCTTTAGA

>VIGS_CPR2 (construct a)
CATCTTGGCCACGTATGGAGATGGTGAGCCAACCGACAATGCTGCAAGGTTCTACAAATGGTTTGTAGAGGGAAATGATAGAG
GGGACTGGCTAAAGAATCTGCAATATGGAGTTTTTGGCCTTGGTAACAGACAATATGAGCATTTCAACAAGATTGCTAAAGTGG
TGGATGAGAAAGTTGCTGAACAGGGTGGTAAGCGGATTGTTCCATTGGTTCTGGGAGACGATGACCAGTGCATTGAAGATGAC
TTTGCTGCATGGCGTGAGAATGTATGGCCTGAGTTGGATAACTTGCTCCGGGATGAGGATGATACAACTGTTTCTACAACCTAC
ACTGCTGCTATTCCAGAATATCGTGTTGTGTTCCCTGACAAATCAGATTCACTTATTTCAGAAGCA

The two *CPR* genes share 68.9% identity at thenucleotide level. Alignment of the sections chosen for constructs a show that some sequence identity could not be avoided due to the similarity of both genes. Sequence identity for the *CPR2* VIGS fragment to the corresponding region of the *CPR1* gene was 71.2%, and conversely, the identity of the *CPR1* VIGS fragment with the *CPR2* gene was 65.4%. The longest identical section of the *CPR2* VIGS section to the *CPR1* gene is a single stretch of 17 bp that should not cause cross-silencing.

```
>Nucleotide alignment 2 Alignment of 2 sequences: CPR1 (ORF), CPR2 (VIGS ORF)

Score = 931.0, Identities = 277/389 (71%),
Positives = 277/389 (71%), Gaps = 3/389 (0%)

CPR1 (ORF)        579 CATGGTGGCAACGTATGGAGATGGAGAGCCAACTGATAATGCTGCTAGGTTTTATAAATG 638
                      CAT  TGGC ACGTATGGAGATGG GAGCCAAC GA AATGCTGC AGGTT TA AAATG
CPR2 (VIGS ORF)     1 CATCTTGGCCACGTATGGAGATGGTGAGCCAACCGACAATGCTGCAAGGTTCTACAAATG  60

CPR1 (ORF)        639 GTTTACTGAGGGAAAAGAGAGGGAGCCATGGCTTCAGCAACTCACATATGGTGTATTTGG 698
                      GTTT   GAGGGAAA GA AG G G   TGGCT  AG A CT   ATATGG GT TTTGG
CPR2 (VIGS ORF)    61 GTTTGTAGAGGGAAATGATAGAGGGGACTGGCTAAAGAATCTGCAATATGGAGTTTTGG 120

CPR1 (ORF)        699 TTTGGGTAACCGTCAATATGAGCATTTCAATAAGATTGGGAAGGTAATCGATGAGCAACT 758
                        T GGTAAC G CAATATGAGCATTTCAA AAGATTG  AA GT  T GATGAG AA T
CPR2 (VIGS ORF)   121 CCTTGGTAACAGACAATATGAGCATTTCAACAAGATTGCTAAAGTGGTGGATGAGAAAGT 180

CPR1 (ORF)        759 CAGTCAACAAGGTGCAAAAAGATTGGTTCAGCTCGGTCTTGGGGATGATGATCAATGTAT 818
                        T AACA GGTG  AA  G  T GTTC   T G TCT GG GA GATGA CA TG AT
CPR2 (VIGS ORF)   181 TGCTGAACAGGGTGGTAAGCGGATTGTTCCATTGGTTCTGGGAGACGATGACCAGTGCAT 240

CPR1 (ORF)        819 CGAGGATGATTTTGCTGCTTGGCGGGATCAACTGTGGCCTGAATTAGATCAAATACTCAG 878
                      GA GATGA TTTGCTGC TGGCG GA  A  T TGGCCTGA TT GAT A  T CTC G
CPR2 (VIGS ORF)   241 TGAAGATGACTTTGCTGCATGGCGTGAGAATGTATGGCCTGAGTTGGATAACTTGCTCCG 300

CPR1 (ORF)        879 AGATGAGGATGATATGATCTCTGTTTCCACCCCATATACAGCTGCAATCAATGAATACCG 938
                      GATGAGGATGATA  A   CTGTTTC AC  C TA AC GCTGC AT    GAATA CG
CPR2 (VIGS ORF)   301 GGATGAGGATGATACAA---CTGTTTCTACAACCTACACTGCTGCTATTCCAGAATATCG 357

CPR1 (ORF)        939 AGTAGTGATTCATGATTCTTTGGATACAC 967
                      GT GTG T C TGA    T  GAT CAC
CPR2 (VIGS ORF)   358 TGTTGTGTTCCCTGACAAATCAGATTCAC 386


>Nucleotide alignment Alignment of 2 sequences: CPR2 (ORF), CPR1 (VIGS ORF)

Score = 665.0, Identities = 236/361 (65%),
Positives = 236/361 (65%), Gaps = 12/361 (3%)

CPR2 (ORF)         838 TATGGCCTGAGTTGGATAACTTGCTCCGGGATGAGGATGATACAA---CTGTTTCTACAA  894
                       T TGGCCTGA TT GAT A  T CTC G GATGAGGATGATA  A   CTGTTTC AC
CPR1 (VIGS ORF)      7 TGTGGCCTGAATTAGATCAAATACTCAGAGATGAGGATGATATGATCTCTGTTTCCACCC   66

CPR2 (ORF)         895 CCTACACTGCTGCTATTCCAGAATATCGTGTTGTGTTCCCTGACAAATCAGATTCACTTA  954
                       C TA AC GCTGC AT    GAATA CG GT GTG T C TGA    T  GAT CAC
CPR1 (VIGS ORF)     67 CATATACAGCTGCAATCAATGAATACCGAGTAGTGATTCATGATTCTTTGGATACAC---  123

CPR2 (ORF)         955 TTTCAGAAGCAAATGGCCATGCCAATGGTTATGCTAATGGCAACACCGTATATGATGCCC 1014
                         CA A G A AT   CA    AA GG    GCTAATGG AA      ATATGAT   C
CPR1 (VIGS ORF)    124 ---CATACGAAGATAAGCAGTTAAACGGG---GCTAATGGAAATCTTTCATATGATATTC  177

CPR2 (ORF)        1015 AGCATCCTTGCAGATCTAATGTTGCAGTGAGGAAGGAGCTTCATACTCCAGCATCTGATC 1074
                       A CATCC TGCA A  TA TGTTGC GT    AA GAGCTTCA A  CC G ATC GATC
CPR1 (VIGS ORF)    178 ATCATCCATGCATAGTTACTGTTGCGGTTCAAAAAGAGCTTCACAAACCTGAATCCGATC  237

CPR2 (ORF)        1075 GTTCTTGCACCCATTTGGATTTTGACATTGCTGGCACTGGCCTTTCATATGGAACTGGAG 1134
                       GTTC TGCA  CA  TGGA TTTGA ATT CTGG AC GGC T   ATATG AACTGGAG
CPR1 (VIGS ORF)    238 GTTCATGCATTCACCTGGAATTTGATATTTCTGGAACAGGCATCCAATATGAAACTGGAG  297

CPR2 (ORF)        1135 ATCATGTTGGAGTGTACTGTGATAATCTATCTGAAACCGTGGAGGAGGCTGAGAGATTAC 1194
                       ATCATGT GG GT TA   TGA AAT     TGAAA  GT GA GA GC G  A ATTA
CPR1 (VIGS ORF)    298 ATCATGTGGGGGTATATGCTGAAAATTGTGATGAAAATGTTGAAGAAGCAGCAAAATTAT  357

CPR2 (ORF)        1195 T 1195
                       T
CPR1 (VIGS ORF)    358 T  358
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Eight week old seedlings of *C. roseus* variety "SunStorm Apricot" (Syngenta) were used for all VIGS experiments. All plants were grown in a walk in growth chamber at 25^{o}C under a 12 h light/12 h dark regime using the John Innes compost mix No.2 (peat based). Stems of plants were pinched just under the last emerging fresh pair of leafs and tissue harvested 21 days later. The harvested material, approximately 50 mg of the latest emerging leaf pair, was frozen in liquid nitrogen, milled (RETSCH mill, Germany) and the sample divided into two fractions, one for RNA extraction and one for LCMS measurement.

The metabolite profile of silenced leaves was assessed by LCMS. Between 10-25 mg of ground tissue of each plant was weighed, collected into 200 µl methanol and incubated at 57 °C for 2 hr. After a 30 minute centrifugation step at 5,000 x *g* an aliquot of the supernatant, mixed with an equal volume of water, was analysed by LC-MS. A Shimadzu LCMS-IT-TOF Mass Spectrometer was used with a Phenomenex Kinetix 5µ C18 100A (100 × 2.10 mm, 5 µm) column using a binary solvent system consisting of acetonitrile (ACN) and 0.1% formic acid in water. The elution program was the following: a 5 min gradient from 10% up to 25% ACN, a 1 min gradient up to 100% ACN, 1.5 min isocratic at 100% ACN, 0.5 min down to at 10% ACN, 2min isocratic at 10% ACN. To assess changes to alkaloid production levels the four alkaloids vindoline, vindorosine, serpentine and catharanthine were chosen. The four compounds represent the end products of the individual alkaloid class branches in *C. roseus* leaf tissue and accumulate to relatively high levels in wild type leaf tissue.

Automated peak detection and extraction of 7 EV samples, as well as 8 samples for CPR1 and CPR2 was conducted using the profiling solutions software (Shimadzu) with standard extraction parameters.

To assess the level of silencing, material from six independently silenced plants was compared to six plants treated with the empty vector control plasmid. The q RT PCR reaction was performed in technical duplicate. **All q RT PCR primers were tested to ensured optimal efficiency. All melting curves generated in each experiment were analyzed to ensure that the primer pair is specific. RNA and water controls were included in the measurements in triplicate. The cDNA concentration was optimized according to the results of the primer testing to ensure that the Cq value for each gene analysed in the experiment would at between 20-30 cycles.** The relative quantification of gene expression was calculated using the expression values of a reference gene for normalisation of the data. Reference gene was in all cases the *40S Ribosomal protein 9* (*Rps9*). **All q RT PCR measurements were done using the** CFX96 touch Real-Time PCR system (BioRad) and the SYBR Green I dye. Each reaction was performed in a total volume of 25 µl consisting of a normalized concentration of cDNA, 0.2 mM of appropriate forward and reverse primer and the SsoAdvanced SYBR Green Supermix (BioRad, cat. No: **1725271) that contains dNTPs, Sso7d fusion polymerase, $MgCl_2$, SYBR® Green I and ROX normalization dyes. The qPCR reaction was initiated by a denaturation step at 95 ^{o}C for 10 min followed by 40 cycles at 95 ^{o}C for 15 s and 60 ^{o}C for 1 min. The resulting data was analyzed using the CFX software. The previously determined efficiency for the primer (100.8% for CPR 2 and 101.0% for CPR 1) was considered in this calculation.**

Three independent VIGS experiments were conducted and results were consistent among all experiments (albeit with varying statistical significance). Each silencing experiment consisted of plants silenced for CPR1 and CPR2 and included an equal amount of plants treated with an EV control. Between 7 and 8 plants were assessed for each silencing experiment.