MS: MBE-19-0355

Are universally conserved residues universally required for stable protein expression or functions of cryptochromes?

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Supplementary Materials

Fig. S1

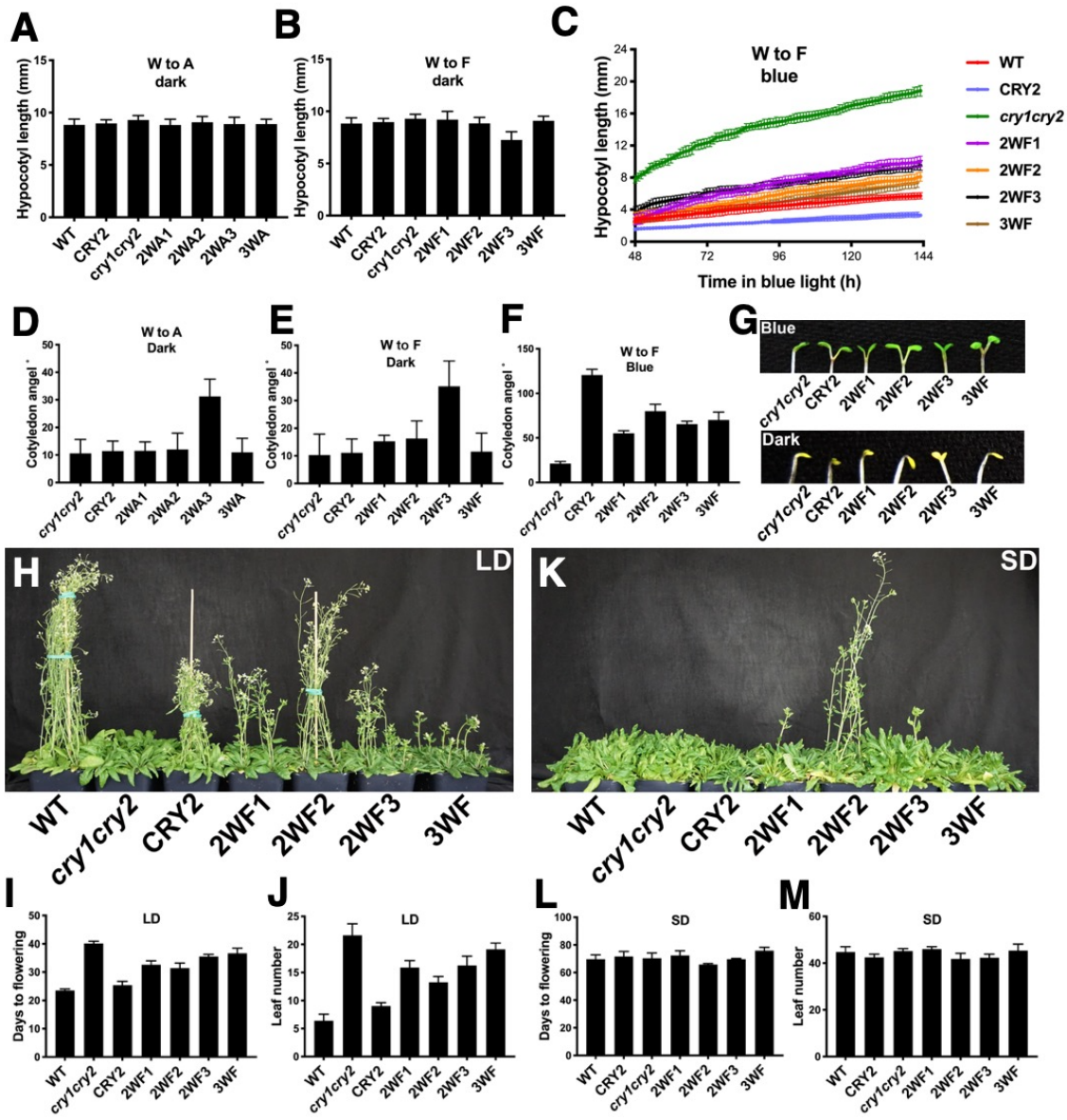

Fig. S1. The double and triple mutants of the universally conserved Trp-triad residues of CRY2 remained photobiologically active *in vivo*.

(**A-B**) Hypocotyl length of six-day-old seedlings expressing the respective wild-type (GFP-CRY2) or double and triple mutants of the Trp-triad residues of CRY2, and *cry1cry2* mutant or wild-type (WT) seedlings grown in continuous blue light (20 μmol m^{-2} s^{-1}). The residue replacements involved in each mutant were listed in table S1. Bar indicates SD of the mean (n≥20).

(**C**) The assay was carried out as described in Fig. 1B.

(**D-E**) Cotyledon unfolding angels of six-day-old seedlings of the indicated genotypes grown in dark were manually measured using Fiji (NIH). Bar indicates SD of the mean (n≥20).

(**F**) Angles between the two cotyledons, indicating the cotyledon unfolding phenotype, were measured from the images taken for Fig. S1C at 114 hours after germination. Bars indicates SD of the mean (n=3).

(**G**) The cotyledon unfolding phenotype. Representative images of 6-day-old seedlings grown in blue light (20 $\mu mol \bullet m^{-2} \bullet s^{-1}$) (upper) or darkness (lower) are shown.
(H-M) Images of 40 (H) or 60 (K) -day-old plants grown in in long-day (16 h day/ 8 h night) or short-day photoperiod (8 h day/ 16 h night). Days to flowering (I, L) and rosette leaf number (J, M) at flowering of the respective genotypes are shown. Bars indicates SD of the mean ($n \geq 8$). The wild-type (WT) and transgenic plants constitutively expressing the "wild-type" GFP-CRY2 (CRY2) fusion protein or three different double (2WF1, 2WF2, 2WF3) or triple (3WF) mutants of the Trp-triad residues of CRY2 as the GFP-CRY2 fusion proteins in the *cry1cry2* mutant background are indicated. See Table S1 for more detailed information.

Fig. S2

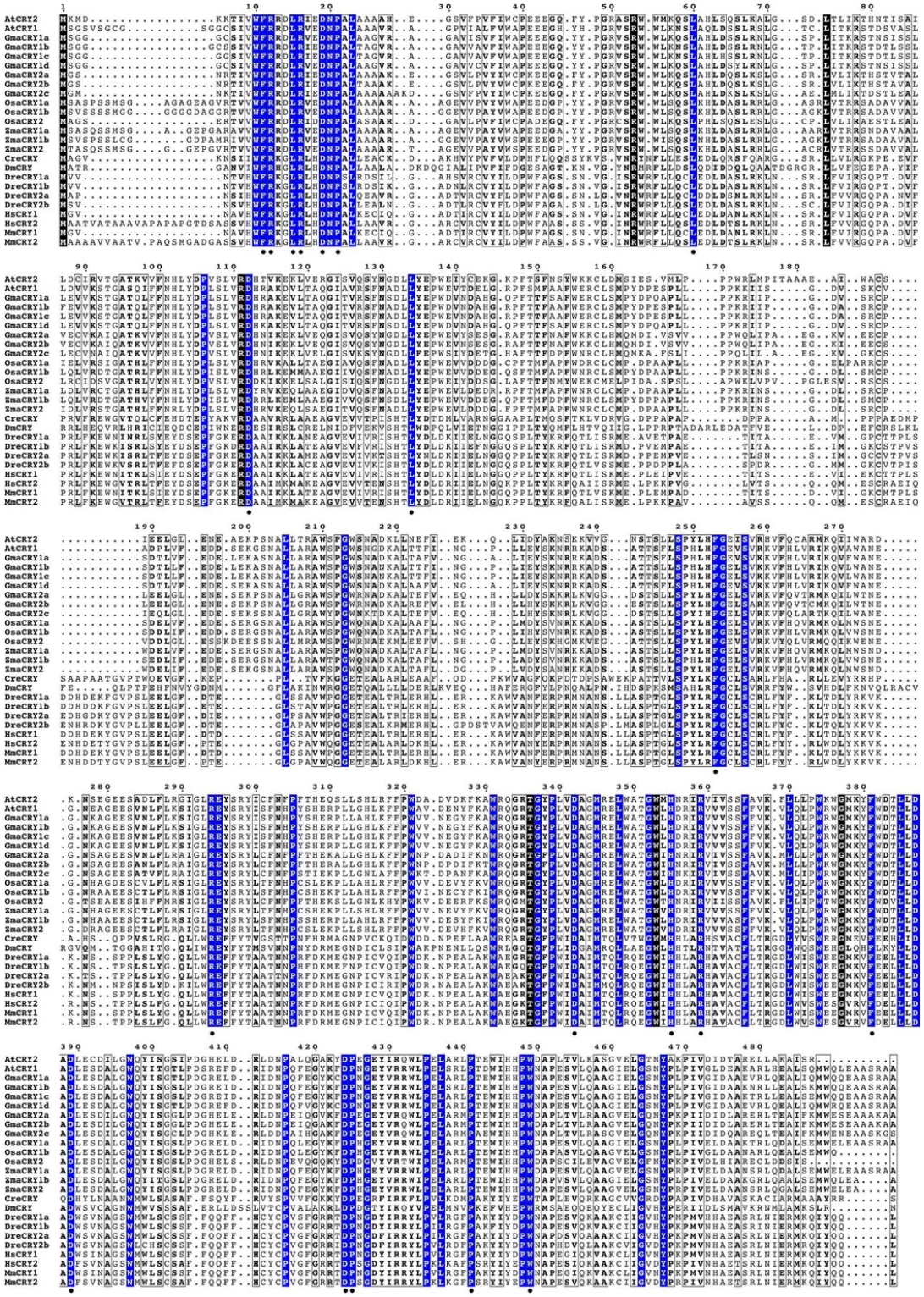

Fig. S2. Amino acid multiple sequence alignment of representative Eukaryotic Cryptochromes. The 57 universally conserved residues are labelled with blue (analyzed in the current study) or black background (not analyzed in the current study, excluding the start codon); the residues whose the alanine replacement were classified as “lack of protein” (Fig. 3 and table S4) are labelled with round dots. NCBI accession number and species of proteins used in the alignment are listed in table S2**.**

Fig. S3

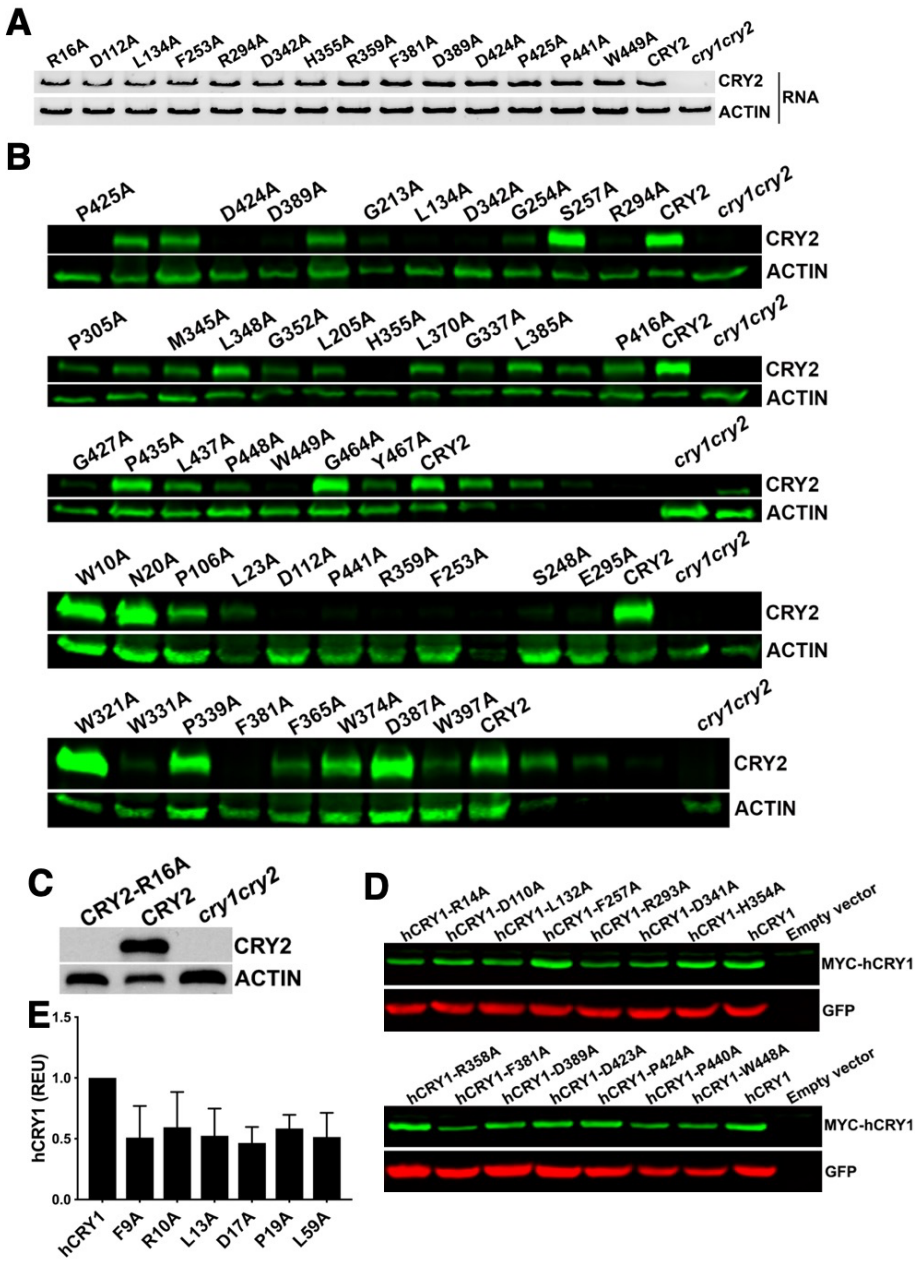

Fig. S3. RNA and protein expression of mutants of Arabidopsis CRY2 and hCRY1.

(**A**) RT-PCR analyses of mRNA expression of wild-type or mutant of *GFP-CRY2* (upper) and *ACTIN* (lower) genes of seven-day-old seedlings grown in the long-day (16 h day/ 8 h night) period in the genotypes indicated.

(**B**) Immunoblots showing wild-type or UCR mutant of GFP-CRY2 fusion proteins expressed in transgenic plants. Samples were extracted from seven-day-old seedlings grown in the long-day (16 h day/8 h night) period, fractionated in SDS/PAGE (10%), blotted, probed with anti-CRY2 antibody (CRY2) and anti-ACTIN antibody (ACTIN, control), then probed with fluorophore-conjugated secondary antibodies, and detected by the Odyssey CLx System (LI-COR).

(**C**) Immunoblots showing wild-type or the CRY2^{R16A} mutant of GFP-CRY2 fusion proteins expressed in transgenic plants. Samples were prepared, blotted, and probed primary antibodies as described in B,

then probed with HRP (horseradish peroxidase)-conjugated secondary antibodies and detected by the ECL (Enhance Chemiluminescence) method.
(**D**) Immunoblot showing stable protein expression of hCRY1 UCR mutant proteins, each being altered at the residue equivalent to the corresponding Arabidopsis CRY2 UCR "lack of protein) mutants. Samples were prepared from whole cell lysates of HEK293T cells co-transfected by two plasmids: the sample plasmid encoding the indicated MYC-hCRY1 protein mutated at the indicated UCR and the control plasmid encoding GFP as the transfection and immunoblot controls.
(**E**) Quantification of protein expression of hCRY1 in HEK293T cells from Fig. 2C. GFP-hCRY1 Signals were acquired from fluorescent immunoblot (LI-COR) and quantified by an internal method of Image Studio Lite software (LI-COR), and normalized to GFP signals, and presented as hCRY1 relative expression unit (REU). Bars indicates SD of the mean (n=3).

Fig. S4

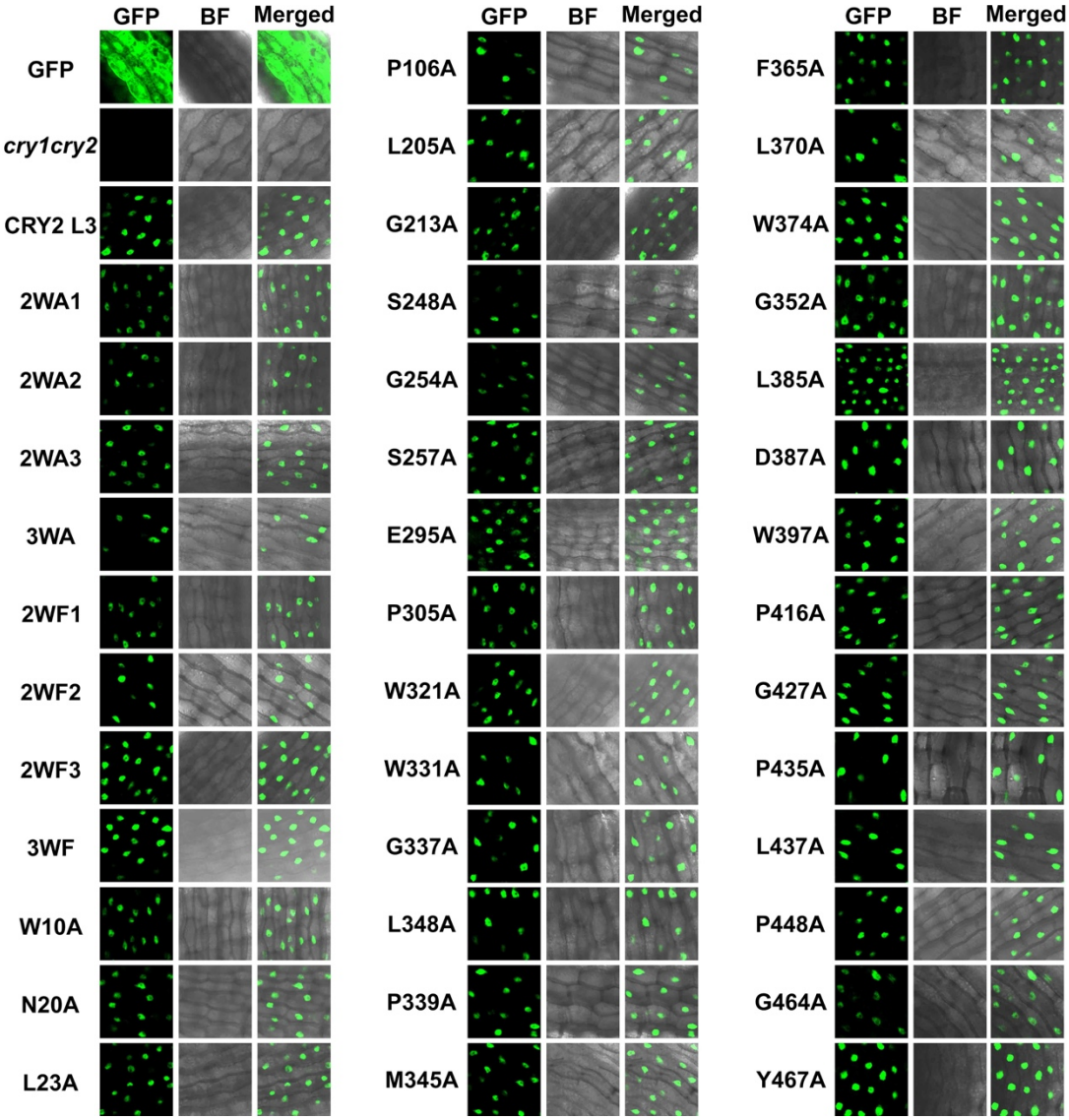

Fig. S4. Subcellular localization of the GFP-CRY2 mutants.
Seedlings were grown on MS medium in the long-day (16 h day/ 8 h night) period of white light. Roots of two-day-old seedlings were directed analyzed by a Zeiss LSM700 confocal fluorescence microscope, and processed by the Fiji (NIH) software.

Fig. S5

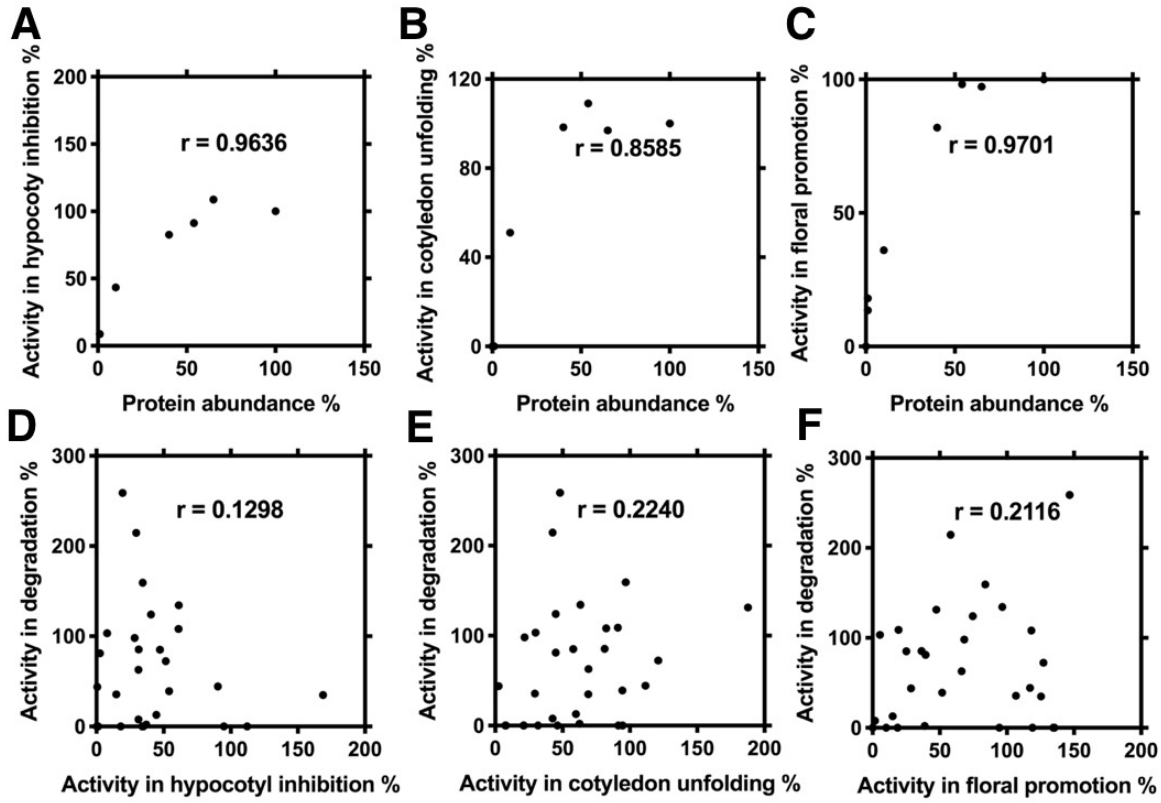

Fig. S5. Spearman's correlations.

(**A-C**) Correlations between protein abundance and the relative specific-activities of the blue-light dependent inhibition of hypocotyl elongation (*A*), cotyledon unfolding (*B*) and floral promotion (*C*) for wild-type GFP-CRY2 fusion proteins.

(**D-F**) Correlations between the blue-light dependent proteolysis activities (calculation detailed in SI Appendix, Materials and Methods) and the relative specific-activities of the blue-light dependent inhibition of hypocotyl elongation (*D*), cotyledon unfolding (*E*) and floral promotion (*F*) for transgenic plants of mutants of universally conserved GFP-CRY2 fusion proteins.

Fig. S6

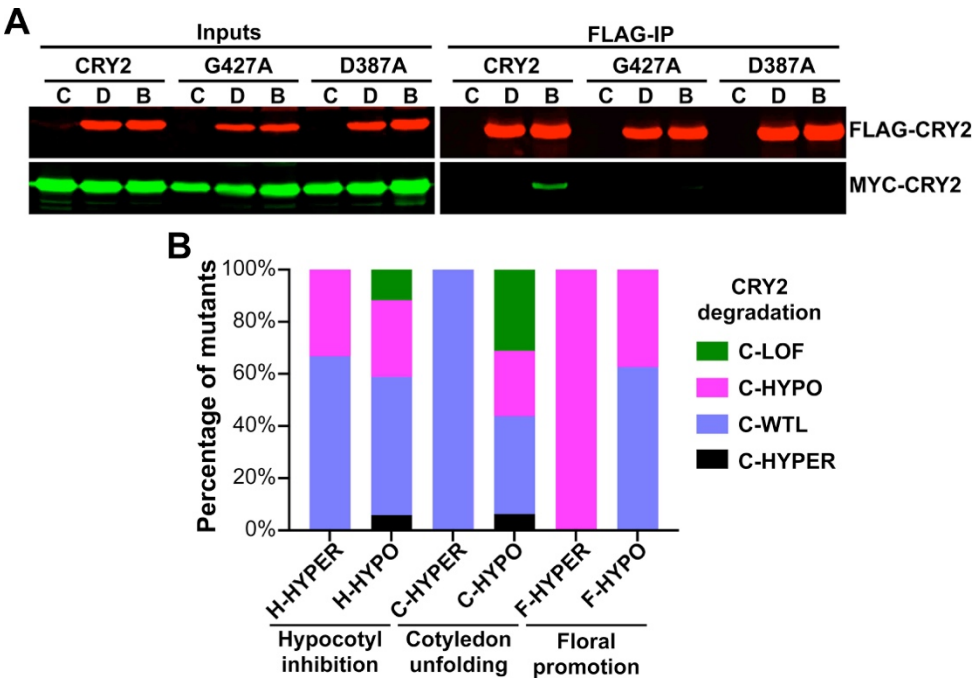

Fig. S6. Blue light-dependent CRY2 dimerization of the CRY2^{G427A} proteins and correlation between physiological and biochemical activities.

(A) HEK293T cells were cotransfected to express the indicated proteins, exposed to blue light (100 µmol m^{-1} s^{-1}) for 120 minutes, and immunoprecipitated by antibody to FLAG (FLAG-IP). The IP signal (FLAG-CRY2) or the co-IP signals (MYC-CRY2) were detected by immunoblots probed with antibodies to FLAG (upper panel) or to MYC (lower panel).

(B) Classification in blue light-induced proteolysis of the CRY2 mutants classified as hypermorphic or hypomorphic for hypocotyl inhibition (H-HYPER, H-HYPO), cotyledon unfolding (C-HYPER, C-HYPO) and floral promotion (F-HYPER, F-HYPO).

Fig. S7

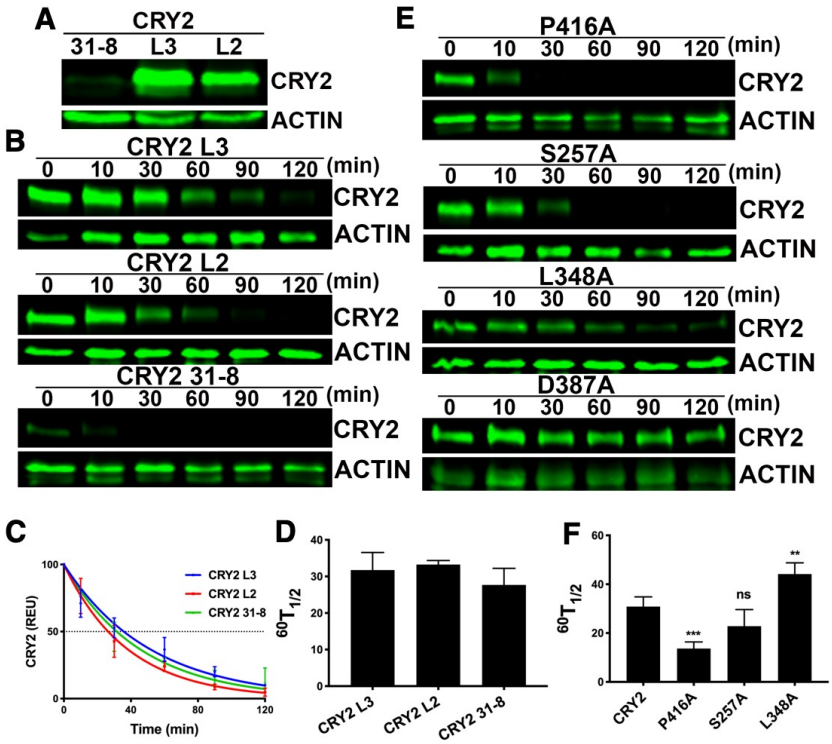

Fig. S7. Blue-light dependent proteolysis of CRY2.

(**A**) Immunoblots showing wild-type GFP-CRY2 fusion proteins expressed in transgenic plants. Samples were extracted from seven-day-old seedlings grown in the long-day (16 h day/8 h night) period, fractionated in SDS/PAGE (10%), blotted, probed with anti-CRY2 antibody (CRY2) and anti-ACTIN antibody (ACTIN, control), then probed with fluorophore-conjugated secondary antibodies, and detected by the Odyssey CLx System (LI-COR)

(**B**) Representative immunoblots of samples prepared from six-day-old etiolated seedlings exposed to blue light (60 µmol m^{-2} s^{-1}) for the indicated time in the indicated genotypes. Samples were probed with antibodies to CRY2 and ACTIN (control).

(**C**) Degradation curves of wild-type GFP-CRY2 fusion proteins. Assays were conducted as described in Fig. 3H. Bars indicates SD of the mean (n=2).

(**D**) Half-life ($^{60}T_{1/2}$) of proteolysis was calculated from degradation curves of C as described in Fig. 3J. Bars indicates SD of the mean (n=2).

(**E**) Representative immunoblots of samples prepared from six-day-old etiolated seedlings exposed to blue light (60 µmol m^{-2} s^{-1}) for the indicated time in the representative mutants of GFP-CRY2 classified as HYPER (P416A), WTL (S257A), HYPO (L348A) and LOF (D387A). Assays were conducted as described in *B*.

(**F**) Half-life ($^{60}T_{1/2}$) of representative mutants of GFP-CRY2 classified as HYPER (P416A), WTL (S257A), HYPO (L348A) was calculated from degradation curve in Fig. 2H as described in Fig. 3J. Three asterisks indicate P=0.0003, "ns" indicates P>0.05, double asterisks indicate P=0.0027, compared with half-life of wild-type GFP-CRY2 fusion proteins.

Fig. S8

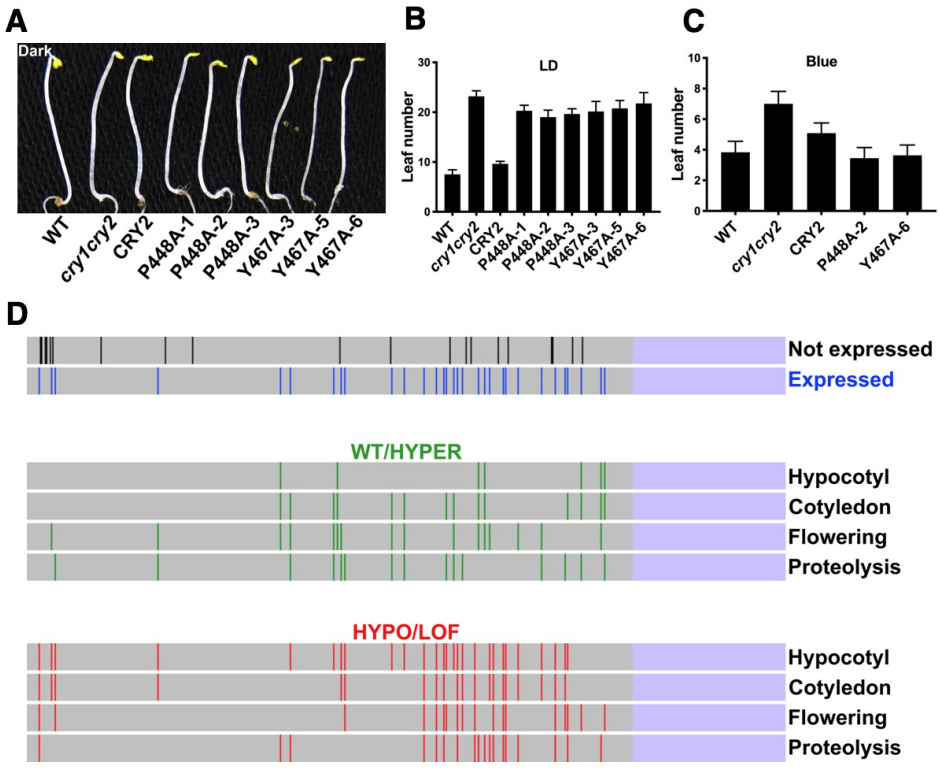

Fig. S8. Functional analyses of neighboring UCRs of CRY2.

(**A**) Six day-old seedlings expressing wild-type GFP-CRY2 or GFP- CRY2^{P448A} (P448A), CRY2^{Y467A} (Y467A) mutants, and *cry1cry2* mutant or wild-type seedlings grown in dark.

(**B-C**) Number of rosette leaves at the time of flowering of independent lines of CRY2^{P448A} and CRY2^{Y467A} mutants in the long-day (16 h day/ 8 h night) period (B), or in the continuous blue light (70-80 µmol•m^{-2}•s^{-1}) (C). Bars indicates SD of the mean (n≥8).

(**D**) Linear representation of mutants of CRY2. Grey and light blue color represents PHR and CCE domain, respectively. Black, blue, green, red, purple and pink lines indicate activities as labeled at the top of each panel.

Table S1

Residues	Mutant	Protein Abundance	Hypocotyl inhibition		Cotyledon unfolding		Floral initiation	
			Activity	Category	Activity	Category	Activity	Category
W321A + W374A	2WA1	117.43%	30.03%	HYPO	55.67%	HYPO	38.36%	HYPO
W321A + W397A	2WA2	344.10%	30.97%	HYPO	88.43%	HYPO	77.45%	HYPO
W374A + W397A	2WA3	46.09%	39.38%	HYPO	39.37%	HYPO	57.91%	HYPO
W321A + W374A + W397A	3WA	77.53%	51.94%	HYPO	74.10%	HYPO	87.19%	WTL
W321F + W374F	2WF1	26.98%	17.65%	HYPO	34.15%	HYPO	45.10%	HYPO
W321F + W397F	2WF2	106.56%	30.03%	HYPO	59.06%	HYPO	52.62%	HYPO
W374F + W397F	2WF3	38.20%	28.98%	HYPO	44.46%	HYPO	27.84%	HYPO
W321F + W374F + W397F	3WF	17.95%	29.83%	HYPO	49.15%	WTL	21.05%	HYPO

Table S1. Summary of the relative protein abundance and relative specific activities of CRY2 trp-triad mutants.

The protein abundance and physiological activities of double and triple mutants of Trp-triad expressed in the *cry1cry2* parental lines were shown. "HYPER", "WTL", "HYPO" and "LOF" represent hypermorph (increased activity), "wild-type"-like, hypomorph (reduced activity) and loss-of-function, respectively. Protein abundance and activities were normalized to L5 of wild-type GFP-CRY2 line (Fig. 3, A-C and table S3). Activities were categorized using the standard curves shown in Fig. 3 (A-C).

Table S2

Name	Group	Family	Species	NCBI	aa
AtCRY2	Plants-Dicots	Brassicaceae	*Arabidopsis thaliana*	NP_171935.1	1-487
AtCRY1	Plants-Dicots	Brassicaceae	*Arabidopsis thaliana*	NP_567341.1	1-501
GmaCRY1a	Plants-Dicots	Fabaceae	*Glycine max*	XP_025980601.1	1-494
GmaCRY1b	Plants-Dicots	Fabaceae	*Glycine max*	NP_001241002.1	1-495
GmaCRY1c	Plants-Dicots	Fabaceae	*Glycine max*	NP_001235205.1	1-494
GmaCRY1d	Plants-Dicots	Fabaceae	*Glycine max*	NP_001240855.1	1-494
GmaCRY2a	Plants-Dicots	Fabaceae	*Glycine max*	KRG92422.1	1-494
GmaCRY2b	Plants-Dicots	Fabaceae	*Glycine max*	XP_006588363.1	1-494
GmaCRY2c	Plants-Dicots	Fabaceae	*Glycine max*	NP_001241551.1	1-495
OsaCRY1a	Plants-Monocots	Poaceae	*Oryza sativa*	BAB70686.1	1-510
OsaCRY1b	Plants-Monocots	Poaceae	*Oryza sativa*	BAB70688.2	1-501
OsaCRY2	Plants-Monocots	Poaceae	*Oryza sativa*	BAC56984.1	1-486
ZmaCRY1a	Plants-Monocots	Poaceae	*Zea mays*	XP_008644161.1	1-506
ZmaCRY1b	Plants-Monocots	Poaceae	*Zea mays*	PWZ40305.1	1-502
ZmaCRY2	Plants-Monocots	Poaceae	*Zea mays*	XP_008677763.1	1-502
CreCRY	Plants-Green algae	Chlamydomonadaceae	*Chlamydomonas reinhardtii*	XP_001698054.1	1-493
DmCRY	Animals-Invertebrate	Drosophilidae	*Drosophila melanogaster*	NP_732407.1	1-514
DreCRY1a	Animals-Invertebrate	Cyprinidae	*Danio rerio*	NP_001070765.1	1-491
DreCRY1b	Animals-Invertebrate	Cyprinidae	*Danio rerio*	NP_571865.4	1-491
DreCRY2a	Animals-Invertebrate	Cyprinidae	*Danio rerio*	CAQ13306.1	1-491
DreCRY2b	Animals-Invertebrate	Cyprinidae	*Danio rerio*	NP_571867.2	1-494
HsCRY1	Animals-Vertebrate	Hominidae	*Homo sapiens*	NP_004066.1	1-491
HsCRY2	Animals-Vertebrate	Hominidae	*Homo sapiens*	NP_066940.3	1-510
MmCRY1	Animals-Vertebrate	Muridae	*Mus musculus*	NP_031797.1	1-491
MmCRY2	Animals-Vertebrate	Muridae	*Mus musculus*	NP_034093.1	1-509

Table S2. Eukaryote proteins used for multiple sequence alignment shown in fig. S2

Fifty-seven residues of CRY2 are universally conserved among the cryptochromes family of the eukaryotes. NCBI accession numbers, and residues forming the PHR domain of each protein used for alignment were shown in “NCBI” and “aa”, respectively.

6 Table S3

CRY2 residues	Mutant	Protein Abundance	Hypocotyl inhibition		Cotyledon unfolding		Floral initiation		Protein degradation		hCRY1 residues	Previously published mutants			
			Activity	Category	Activity	Category	Activity	Category	$^{60}T_{1/2}$	Category		Species	Protein	Mutant	References
Endogenous CRY2		4.159%	45.5%	N/A	99.88%	N/A	100%	N/A	N/A	N/A	N/A			N/A	
GFP-CRY2 L1		10.41%	43.5%	N/A	51.00%	N/A	36%	N/A	N/A	N/A	N/A			N/A	
GFP-CRY2 L2		40.07%	82.6%	N/A	98.31%	N/A	82%	N/A	33	N/A	N/A			N/A	
GFP-CRY2 L3		53.72%	91.3%	N/A	109.1%	N/A	98%	N/A	32	N/A	N/A			N/A	
GFP-CRY2 L4		64.67%	109%	N/A	96.97%	N/A	97%	N/A	N/A	N/A	N/A			N/A	
GFP-CRY2 L5		100.0%	100%	N/A	100.0%	N/A	100%	N/A	N/A	N/A	N/A			N/A	
W10	W10A	64.60%	1.17%	LOF	21.64%	HYPO	10%	LOF	>120	LOF	W8			N/A	
N20	N20A	72.71%	14.8%	HYPO	30.55%	HYPO	105%	WTL	59	HYPO	N18			N/A	
L23	L23A	24.72%	1.83%	LOF	35.70%	HYPO	28%	HYPO	36	WTL	L21			N/A	
P106	P106A	31.77%	48.8%	HYPO	54.51%	HYPO	75%	WTL	25	WTL	P104			N/A	
L205	L205A	10.67%	44.2%	WTL	49.35%	WTL	60%	WTL	119	HYPO	L205			N/A	
G213	G213A	8.971%	13.0%	HYPO	33.78%	WTL	26%	WTL	43	WTL	G213	*Mus musculus*	mCRY2	G230R	(McCarthy et al. 2009)
S248	S248A	6.436%	11.4%	HYPO	38.14%	WTL	26%	WTL	22	WTL	S252	*Mus musculus*	mCRY1	S252A/D	(Ode et al. 2017)
G254	G254A	13.22%	15.1%	HYPO	13.17%	HYPO	35%	WTL	31	WTL	G258	*Pisum sativum*	PsCRY1	G250E	(Platten et al. 2005)
												Arabidopsis thaliana	CRY2	G254R	(Guo et al. 1998)
S257	S257A	115.0%	43.6%	HYPO	49.58%	HYPO	79%	HYPO	26	WTL	S261	*Mus musculus*	mCRY1	S261A/D	(Lamia et al. 2009; Ode et al. 2017)
E295	E295A	5.963%	6.1%	LOF	17.96%	WTL	43%	WTL	14	HYPER	E294	*Mus musculus*	mCRY2	E312K	(McCarthy et al. 2009)
P305	P305A	20.71%	40.8%	HYPO	61.12%	WTL	76%	WTL	29	WTL	P304			N/A	
W321	W321A	414.1%	44.2%	HYPO	74.89%	HYPO	46%	HYPO	113	HYPO	W320	Previously published Trp-triad residues were not listed.			
W331	W331A	26.91%	5.84%	LOF	17.04%	HYPO	0.00%	LOF	>120	LOF	W330	*Arabidopsis thaliana*	CRY1	W334A/F	(Gao et al. 2015)
												Arabidopsis thaliana	CRY1	G340E	(Ahmad and Cashmore 1993; Ruckle et al. 2007)
G337	G337A	28.06%	33.9%	HYPO	49.61%	HYPO	11%	LOF	88	HYPO	G336	*Mus musculus*	mCRY1	G336D	(Czarna et al. 2013)
												Mus musculus	mCRY2	G354D	(McCarthy et al. 2009)
P339	P339A	85.98%	32.4%	HYPO	86.56%	WTL	37%	HYPO	35	WTL	P338	*Mus musculus*	mCRY2	P356L	(McCarthy et al. 2009)

M345	M345A	22.67%	43.4%	HYPO	93.04%	WTL	86%	WTL	39	WTL	M344			N/A	
L348	L348A	48.37%	0.460%	LOF	2.248%	LOF	25%	HYPO	53	HYPO	K347	*Arabidopsis thaliana*	CRY2	L348F	(Taslimi et al. 2016)
G352	G352A	23.09%	5.51%	LOF	23.16%	HYPO	3.6%	LOF	30	WTL	G351			N/A	
F365	F365A	38.09%	76.6%	WTL	101.3%	WTL	97%	WTL	53	HYPO	F364			N/A	
L370	L370A	30.35%	132%	HYPER	70.24%	WTL	95%	HYPER	60	HYPO	L370			N/A	
W374	W374A	69.86%	58.9%	HYPO	47.76%	HYPO	92%	WTL	>120	LOF	W374	Previously published Trp-triad residues were not listed.			
L385	L385A	26.59%	23.3%	HYPO	34.61%	HYPO	1.2%	LOF	98	HYPO	L385			N/A	
D387	D387A	99.56%	0.910%	LOF	8.592%	LOF	19%	LOF	>120	LOF	D387	*Drosophila melanogaster*	dCRY	D410N	(Stanewsky et al. 1998)
												Arabidopsis thaliana	CRY2	D387A	(Liu et al. 2008)
												Mus musculus	mCRY1	D387N	(Hitomi et al. 2009)
W397	W397A	16.94%	21.2%	HYPO	21.59%	HYPO	78%	WTL	>120	LOF	W397	Previously published Trp-triad residues were not listed.			
P416	P416A	11.88%	14.8%	HYPO	24.39%	HYPO	28%	WTL	17	HYPER	P415			N/A	
G427	G427A	6.763%	6.17%	LOF	3.049%	LOF	0.00%	LOF	>120	LOF	G426			N/A	
P435	P435A	26.13%	34.9%	HYPO	46.81%	HYPO	18%	HYPO	35	WTL	P434			N/A	
L437	L437A	13.33%	28.8%	HYPO	57.44%	WTL	26%	HYPO	57	HYPO	L436			N/A	
P448	P448A	19.19%	130%	HYPER	65.58%	WTL	12%	LOF	29	WTL	P447			N/A	
G464	G464A	85.24%	89.5%	WTL	81.70%	HYPO	92%	WTL	>120	LOF	G463			N/A	
Y467	Y467A	10.13%	145%	HYPER	98.32%	HYPER	20%	HYPO	25	WTL	Y466			N/A	

Table S3. Summary of the relative protein abundance and relative specific activities of UCR mutants of CRY2 with stable protein expression

The protein abundance and activities of wild-type and mutant GFP-CRY2 expressed in the *cry1cry2* parental lines were shown. "HYPER", "WTL", "HYPO" and "LOF" represent hypermorph (increased activity), "wild-type"-like, hypomorph (reduced activity) and loss of function, respectively. Protein abundance and activities were normalized to L5 of wild-type GFP-CRY2 line. Activities were categorized using the standard curves shown in Fig. 3 (A-C). N/A, not applied.

Table S4

CRY2 residues	Mutant	hCRY1 residues	Previously published mutants			
			Species	Protein	Mutant	References
F11	F11A	F9			N/A	
R12	R12A	R10			N/A	
L15	L15A	L13			N/A	
R16	R16A	R14			N/A	
D19	D19A	D17	Arabidopsis	CRY1	D21N	(Ruckle et al. 2007)
P21	P21A	P19			N/A	
L60	L60A	L59			N/A	
D112	D112A	D110			N/A	
L134	L134A	L132			N/A	
F253	F253A	F257	mouse	mCRY1	F257A	(Rosensweig et al. 2018)
R294	R294A	R293			N/A	
D342	D342A	D341			N/A	
H355	H355A	H354			N/A	
R359	R359A	R358	mouse	mCRY1	R358K	(Hitomi et al. 2009)
F381	F381A	F381			N/A	
D389	D389A	D389			N/A	
D424	D424A	D423			N/A	
P425	P425A	P424			N/A	
P441	P441A	P440			N/A	
W449	W449A	W448			N/A	

Table S4. Summary of UCR mutants of CRY2 without stable expression from the "lack of protein" group.

N/A, not applied.

Table S5

Arabidopsis CRY2 residue	Species	**Previously reported cryptochrome mutants** Protein	Mutation	Category	Phenotype	Reference
T7	Mm	mCRY2	S23L	Non-UCR	circadian	(McCarthy et al. 2009)
D19	At	CRY1	D21N (cry1-401)	UCR	hypocotyl	(Ruckle et al. 2007)
C39	Mm	mCRY1	D38A	Non-UCR	circadian	(Rosensweig et al. 2018)
P40	Mm	mCRY1	P39G	Non-UCR	circadian	(Rosensweig et al. 2018)
E42	Mm	mCRY1	F41S	Non-UCR	circadian	(Rosensweig et al. 2018)
R53	Mm	mCRY1	R51A	UCR	circadian	(Rosensweig et al. 2018)
S59	At	CRY1	S66N (cry1-388)	Non-UCR	hypocotyl	(Shalitin et al. 2003)
S72	Mm	mCRY1	S71A/D	Non-UCR	interaction	(Lamia et al. 2009)
S72	Mm	mCRY1	S71A/D	Non-UCR	circadian	(Ode et al. 2017)
Y104	Mm	mCRY1	S102A/D	Non-UCR	circadian	(Ode et al. 2017)
D105	Mm	mCRY1	E103K	Non-UCR	circadian	(Rosensweig et al. 2018)
D105	Mm	mCRY2	E121K	Non-UCR	circadian	(McCarthy et al. 2009)
V107	Mm	mCRY1	F105A	Non-UCR	circadian	(Rosensweig et al. 2018)
S108	Mm	mCRY1	G106R	Non-UCR	circadian	(McCarthy et al. 2009)
S108	Mm	mCRY1	G106R/W	Non-UCR	circadian	(Rosensweig et al. 2018)
R111	Mm	mCRY1	R109Q	UCR	circadian	(McCarthy et al. 2009)
L133	Mm	mCRY1	T131A/D	Non-UCR	circadian	(Ode et al. 2017)
K156	Mm	mCRY1	T155A/D	Non-UCR	circadian	(Ode et al. 2017)
L159	Mm	mCRY1	S158A/D	Non-UCR	circadian	(Ode et al. 2017)
G213	Mm	mCRY2	G230R	UCR	circadian	(McCarthy et al. 2009)
W214	Mm	mCRY1	E214K	Non-UCR	circadian	(McCarthy et al. 2009)
N216	Mm	mCRY1	E216	Non-UCR	circadian	(McCarthy et al. 2009)
A217	At	CRY1	G220D (hy4-6)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
A217	At	CRY1	G220D (hy4-6)	Non-UCR	flower	(Ahmad et al. 1998)
A217	Mm	mCRY1	A217V	Non-UCR	circadian	(McCarthy et al. 2009)

D218	Mm	mCRY1	L218F	Non-UCR	circadian	(McCarthy et al. 2009)
F224	Mm	mCRY1	H224E	Non-UCR	interaction	(Czarna et al. 2013)
Y232	Mm	mCRY1	A232T	Non-UCR	circadian	(McCarthy et al. 2009)
G241	Mm	mCRY1	S243A/D	Non-UCR	circadian	(Ode et al. 2017)
S243	Mm	mCRY1	S247A/D	Non-UCR	circadian	(Ode et al. 2017)
S243	Mm	mCRY1	S247D	Non-UCR	interaction	(Czarna et al. 2013)
S245	Mm	mCRY1	T249A/D	Non-UCR	circadian	(Ode et al. 2017)
S248	Mm	mCRY1	S252D	UCR	circadian	(Ode et al. 2017)
Y250	Mm	mCRY1	Y254A/D	Non-UCR	circadian	(Ode et al. 2017)
F253	Mm	mCRY1	F257A	UCR	circadian	(Rosensweig et al. 2018)
G254	At	CRY2	G254R	UCR	flower	(Guo et al. 1998)
E255	Mm	mCRY1	C259Y	Non-UCR	circadian	(McCarthy et al. 2009)
S257	Mm	mCRY1	S261A/D	UCR	circadian	(Ode et al. 2017)
K268	Mm	mCRY1	T270A/D	Non-UCR	circadian	(Ode et al. 2017)
S278	Mm	mCRY1	S280A/D	Non-UCR	interaction	(Lamia et al. 2009)
S278	Mm	mCRY1	S280A/D	Non-UCR	circadian	(Ode et al. 2017)
G280	At	CRY1	G283E (hy4-5)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
S283	At	CRY1	S286N (cry1-402)	Non-UCR	hypocotyl	(Ruckle et al. 2007)
D285	Mm	mCRY1	S285A/D	Non-UCR	circadian	(Ode et al. 2017)
R289	Mm	mCRY1	G288R	Non-UCR	circadian	(McCarthy et al. 2009)
E295	Mm	mCRY2	E312K	UCR	circadian	(McCarthy et al. 2009)
Q310	Mm	mCRY1	M309I	Non-UCR	circadian	(McCarthy et al. 2009)
G334	At	CRY1	G337D (hy4-4)	UCR	hypocotyl	(Ahmad and Cashmore 1993)
G334	Mm	mCRY2	G351D	UCR	circadian	(McCarthy et al. 2009)
G337	At	CRY1	G340E (cry1-404)	UCR	hypocotyl	(Ruckle et al. 2007)
G337	At	CRY1	G340E (hy4-1)	UCR	hypocotyl	(Ahmad and Cashmore 1993)
G337	Mm	mCRY1	G336D	UCR	circadian	(Czarna et al. 2013)

G337	Mm	mCRY2	G354D	UCR	circadian	(McCarthy et al. 2009)
P339	Mm	mCRY2	P356L	UCR	circadian	(McCarthy et al. 2009)
G344	At	CRY1	G347E (hy4-16)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
G344	At	CRY1	G347R (hy4-15)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
G344	At	CRY1	G347R (cry1-375)	Non-UCR	hypocotyl	(Shalitin et al. 2003)
L348	At	CRY2	L348F	UCR	interaction	(Taslimi et al. 2016)
W349	At	CRY2	W349R	Non-UCR	interaction	(Taslimi et al. 2016)
T351	Mm	mCRY2	E368K	Non-UCR	circadian	(McCarthy et al. 2009)
N356	Mm	mCRY1	H355E	Non-UCR	interaction	(Czarna et al. 2013)
V367	At	CRY2	V367M	Non-UCR	cotyledon	(Botto et al. 2003)
V367	At	CRY2	V367M	Non-UCR	flower	(El-Din El-Assal et al. 2001)
G377	At	CRY1	G380R (cry1-344)	UCR	hypocotyl	(Shalitin et al. 2003)
W382	Mm	mCRY1	E382A	Non-UCR	circadian	(Rosensweig et al. 2018)
D393	Mm	mCRY1	N393A/C	Non-UCR	circadian	(Ode et al. 2017)
Y399	At	CRY2	Y399A/F	Non-UCR	hypocotyl/degradation	(Eckel et al. 2018)
I404	At	CRY1	L407F	Non-UCR	flower/germination	(Exner et al. 2010)
I404	Mm	mCRY1	S404A/D	Non-UCR	circadian	(Ode et al. 2017)
P405	Mm	mCRY1	F405A	Non-UCR	interaction	(Czarna et al. 2013)
G420	Mm	mCRY2	G437D (cry1-305)	Non-UCR	circadian	(McCarthy et al. 2009)
W433	Mm	mCRY1	Y432A/D	Non-UCR	circadian	(Ode et al. 2017)
S459	At	CRY1	A462V (cry1-305)	Non-UCR	hypocotyl	(Shalitin et al. 2003)
S486	Mm	mCRY1	K485D/E	Non-UCR	interaction	(Czarna et al. 2013)
E490	At	CRY2	E490G	Non-UCR	interaction	(Taslimi et al. 2014)
N/A	At	CRY1	E508K (cry1-349)	Non-UCR	hypocotyl	(Shalitin et al. 2003)
N/A	At	CRY1	E515K (hy4-19)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
N/A	At	CRY1	E531K (hy4-20)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
N/A	At	CRY1	R536K (cry1-321)	Non-UCR	hypocotyl	(Shalitin et al. 2003)

N/A	At	CRY1	P549L (hy4-9)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
N/A	At	CRY1	G380R (cry1-344)	Non-UCR	hypocotyl	(Shalitin et al. 2003)
N/A	At	CRY2	K541R	Non-UCR	hypocotyl/degradation	(Zuo et al. 2012)
N/A	At	CRY1	E559L (hy4-22)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
N/A	At	CRY1	R581K (hy4-23)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
N/A	At	CRY1	611K (hy4-24)	Non-UCR	hypocotyl	(Ahmad et al. 1995)
N/A	At	CRY1	E623K (cry1-403)	Non-UCR	hypocotyl	(Ruckle et al. 2007)
N/A	Mm	mCRY1	S492A/D	Non-UCR	circadian	(Ode et al. 2017)
N/A	Mm	mCRY1	S588A/D	Non-UCR	circadian	(Ode et al. 2017)

Table S5. List of previously reported mutants of cryptochromes included in Fig. 2D.

Only residues not selected based on level of sequence conservation were included here. Different mutations in the same residue were considered as 2 hits (1) if the mutations were separately examined in two independent studies (e. g. residue S72 of Arabidopsis CRY2), or (2) if a residue was mutated into two different other residues in the same phenotype-based genetic screen in a study (e. g. mutant hy4-15 and hy4-16). In contrast, different mutations of the same residue were considered as 1 hit if the residue was rationally selected to be changed into different residues in the same study (e. g. Y104 of Arabidopsis CRY2). N/A, not applied because corresponding residues are not available. At, *Arabidopsis thaliana*. Mm, *Mus Musculus*.

Table S6

pFGFP-C-CRY2F	tccagctccaggatccATGAAGATGGACAAAAAGAC
pFGFP-C-CRY2R	GAGAAAGCTTGGATCC TCATTTGCAACCATTTTTTCCC
CRY2-W10A-36R	TCTAAACGCAACTATAGTCT
CRY2-W10A-22F	ATAGTTGCGTTTAGAAGAGA
CRY2-F11A-39R	TCTTCTAGCCCAAACTATAG
CRY2-F11A-25F	GTTTGGGCTAGAAGAGACCT
CRY2-R12A-42R	GTCTCTTGCAAACCAAACTA
CRY2-R12A-28F	TGGTTTGCAAGAGACCTAAGGA
CRY2-L15A-51R	AATCCTTGCGTCTCTTCTAA
CRY2-L15A-37F	AGAGACGCAAGGATTGAGGA
CRY2-R16A-54R	CTCAATTGCTAGGTCTCTTC
CRY2-R16A-40F	GACCTAGCAATTGAGGATAA
CRY2-D19A-63R	AGGATTAGCCTCAATCCTTA
CRY2-D19A-49F	ATTGAGGCTAATCCTGCATT
CRY2-N20A-66R	TGCAGGAGCATCCTCAATCC
CRY2-N20A-52F	GAGGATGCTCCTGCATTAGC
CRY2-P21A-69R	TAATGCAGCATTATCCTCAA
CRY2-P21A-55F	GATAATGCTGCATTAGCAGC
CRY2-L23A-75R	TGCTGCTGCTGCAGGATTAT
CRY2-L23A-61F	CCTGCAGCAGCAGCAGCTGC
CRY2-L60A-186R	GTGAGCAGCTGATTGTTTCA
CRY2-L60A-172F	CAATCAGCTGCTCACTTATC
CRY2-P106A-324R	CGAAACAGCATCATAGAGGT
CRY2-P106A-310F	TATGATGCTGTTTCGTTAGT
CRY2-D112A-342R	GGTATGGGCCCGAACTAACG
CRY2-D112A-328F	GTTCGGGCCCATACCGTAAA
CRY2-L134A-408R	TTCATACGCTAGATCTCCAT
CRY2-L134A-394F	GATCTAGCGTATGAACCGTG
CRY2-L205A-621R	AGTTAACGCCGCATTGCTCG
CRY2-L205A-607F	AATGCGGCGTTAACTAGAGC
CRY2-G213A-645R	GCTCCATGCTGGAGACCAAG
CRY2-G213A-613F	TCTCCAGCATGGAGCAATGC
CRY2-S248A-750R	ATACGGAGCAAGTAGTGAAG
CRY2-S248A-736F	CTACTTGCTCCGTATCTCCA
CRY2-F253-765R	TTCCCCGGCATGGAGATACG
CRY2-F253A-751F	CTCCATGCCGGGGAAATAAG
CRY2-G254-768R	TATTTCCGCGAAATGGAGAT
CRY2-G254A-754F	CATTTCGCGGAAATAAGCGT
CRY2-S257A-777R	TCTGACGGCTATTTCCCCGA
CRY2-S257A-763F	GAAATAGCCGTCAGACACGT
CRY2-R294A-888R	ATACTCTGCTAAACCGATTC

CRY2-R294A-874F	GGTTTAGCAGAGTATTCTCGGT
CRY2-E295A-891R	AGAATACGCTCTTAAACCGA
CRY2-E295A-877F	TTAAGAGCGTATTCTCGGTA
CRY2-P305A-921R	AGTAAACGCGAAGTTGAAAC
CRY2-P305A-907F	AACTTCGCGTTTACTCACGA
CRY2-W321A	
CRY2-W331A-999R	TTGTCTCGCGGCCTTGAACT
CRY2-W331A-1004F	AAGGCCGCGAGACAAGGCAG
CRY2-G337A-1017R	CGGATAAGCGGTCCTGCCTT
CRY2-G337A-1003F	AGGACCGCTTATCCGTTGGT
CRY2-P339A-1023R	CACCAACGCATAACCGGTCC
CRY2-P339A-1009F	GGTTATGCGTTGGTGGATGC
CRY2-D342A-1032R	TCCGCCAGCCACCAACGGAT
CRY2-D342A-1018F	TTGGTGGCTGCCGGAATGAGA
CRY2-M345A-1041R	CTCTCTCGCTCCGGCATCCA
CRY2-M345A-1027F	GCCGGAGCGAGAGAGCTTTG
CRY2-L348A-1050R	AGCCCAAGCCTCTCTCATTC
CRY2-L348A-1036F	AGAGAGGCTTGGGCTACCGG
CRY2-G352A-1062R	CATCCATGCGGTAGCCCAAA
CRY2-G352A-1048F	GCTACCGCATGGATGCATAA
CRY2-H355A-1071R	TCTGTTAGCCATCCATCCGGT
CRY2-H355A-1057F	TGGATGGCTAACAGAATAAGA
CRY2-R359A-1083R	AATCACTGCTATTCTGTTAT
CRY2-R359A-1069F	AGAATACGAGTGATTGTTTC
CRY2-F365A-1101R	CACAGCAGCGCTTGAAACAA
CRY2-F365A-1087F	TCAAGCGCTGCTGTGAAGTT
CRY2-L370A-1116R	AAGGAGAGCAAACTTCACAG
CRY2-L370A-1102F	AAGTTTGCTCTCCTTCCATG
CRY2-W374A-1128R	CCATTTGGCTGGAAGGAGAA
CRY2-W374A-1114F	CTTCCAGCCAAATGGGGAAT
CRY2-F381A-1149R	ATCCCAGGCATACTTCATTC
CRY2-F381A-1135F	AAGTATGCCTGGGATACACT
CRY2-L385A-1161R	ATCCAAAGCTGTATCCCAGA
CRY2-L385A-1147F	GATACAGCTTTGGATGCTGA
CRY2-D387A-1167R	ATCAGCAGCCAAAAGTGTAT
CRY2-D387A-1153F	CTTTTGGCTGCTGATTTGGA
CRY2-D389A-1173R	TTCCAAAGCAGCATCCAAAA
CRY2-D389A-1159F	GATGCTGCTTTGGAATGTGA
CRY2-W397A-1197R	ATACTGGGCGCCAAGGATGTC
CRY2-W397A-1183F	CTTGGCGCCCAGTATATCTC
CRY2-P416A-1254R	TAACGCGGCATTGTCCAAGC
CRY2-P416A-1259F	GACAATGCCGCGTTACAAGG

CRY2-D424A-1278R	TTCTGGGGCATATTTGGCGC
CRY2-D424A-1264F	AAATATGCCCCAGAAGGTGA
CRY2-P425A-1281R	ACCTTCTGCGTCATATTTGG
CRY2-P425A-1267F	TATGACGCAGAAGGTGAGTA
CRY2-G427A-1287R	GTACTCAGCTTCTGGGTCAT
CRY2-G427A-1273F	CCAGAAGCTGAGTACATAAG
CRY2-P435A-1311R	AAGCTCGGCAAGCCATTGCC
CRY2-P435A-1297F	TGGCTTGCCGAGCTTGCGAG
CRY2-L437A-1317R	TCTCGCAGCCTCGGGAAGCC
CRY2-L437A-1303F	CCCGAGGCTGCGAGATTGCC
CRY2-P441A-1315R	TTCAGTTGCCAATCTCGCAA
CRY2-P441A-1315F	AGATTGGCAACTGAATGGAT
CRY2-P448A-1350R	GTCCCATGCATGATGGATCC
CRY2-P448A-1336F	CATCATGCATGGGACGCTCC
CRY2-W449A-1353R	AGCGTCCGCTGGATGATGGA
CRY2-W449A-1339F	CATCCAGCGGACGCTCCTTT
CRY2-G464A-1398R	GTTTGTTGCGAGTTCCACAC
CRY2-G464A-1384F	GAACTCGCAACAAACTATGC
CRY2-Y467A-1407R	TTTCGCAGCGTTTGTTCCGA
CRY2-Y467A-1393F	ACAAACGCTGCGAAACCCAT

Table S6. Primers used for site-directed mutagenesis