Supplemental Materials

The blue light-dependent phosphorylation of the CCE domain determines the photosensitivity of Arabidopsis CRY2

Qin Wang[1,2,6,7], William D. Barshop[3,7], Mingdi Bian[4,7], Ajay A. Vashisht[3], Reqing He[2], Xuhong Yu[6], Bin Liu[5], Paula Nguyen[6], Xuanming Liu[2], Xiaoying Zhao[2]*, James A. Wohlschlegel[3], Chentao Lin[6]*

1 Basic Forestry and Proteomics Research Center, Fujian Agriculture and Forestry University, Fuzhou, China; 2 Hunan Province Key Laboratory of Plant Functional Genomics and Developmental Regulation, College of Biology, Hunan University, Changsha 410082, China; 3 Department of Biological Chemistry, University of California, Los Angeles, CA 90095, USA; 4 Laboratory of Soil and Plant Molecular Genetics, College of Plant Science, Jilin University, Changchun 130062, China; 5 Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China, 6 Department of Molecular, Cell & Developmental Biology, University of California, Los Angeles, CA90095, USA.

7. Those authors contributed equally

*: Corresponding authors

Supplemental Table 1

Modified Residue(s)	Phosphopeptide Localization	XCorr	ΔCn	Δppm	phosphoRS Probability	Normalized Ratio
pS(598)	NLEGIQD**S**SDQITTSLGK	4.233	0.585	2.2	99.7433	2.2606
pS(599)	NLEGIQDS**S**DQITTSLGK	4.683	0.527	1.0	99.8314	3.3265
pS(605)	NLEGIQDSSDQITT**S**LGK	4.418	0.586	2.0	99.9999	3.3448
pS(598)/pS(605)	NLEGIQD**S**SDQITT**S**LGK	2.894	0.539	2.7	99.5195	17.0327

Table S1. Blue light-induced accumulation of the phosphopeptides of CRY2.

Sites of phsosphorylation are indicated by amino-acid residue position(s). All included peptides have an estimated spectrum-level false positive rate of less than 5% and a phosphosite localization probability of greater than 99% by the phosphoRS algorithm. Metrics of identification quality, XCorr, ΔCn and Δppm were taken from the ProLuCID search outputs. Normalized peak ratios were calculated between blue light and dark conditions (see Methods).

Supplemental Table 2

	WT	*cry1cry2*	CRY2	*4sA*	*6sA*	*8sA*	*10sA*	*13sA*
Hypocotyl length (mm)	2.25±0.4	8.78±0.7	1.43±0.4	2.0±0.1**	3.3±0.5***	4.1±0.2***	6.18±0.5***	6.25±0.5***
Days to flower (d)	33.0±1.9	62.3±4.9	32.93±1.8	32.9±1.6	33.0±1.9	33.5±1.5	37.0±3.3**	37.1±3.2**
Leaf number	13.7±0.8	38.0±2	14±0.6	13.8±1.2	14±1.3	14.2±1.1	17.4±1.9***	16.5±1.6***
				4sD	***6sD***	***8sD***	***10sD***	***13sD***
Hypocotyl length (mm)	– –	– –	– –	1.96±0.1**	1.93±0.4*	4.1±0.6***	5.43±0.5 ***	5.4±0.4***
Days to flower (d)	– –	– –	– –	32.9±2.3	32.9±1.8	33.6±1.6	35.0±2.1*	35.2±2.3*
Leaf number	– –	– –	– –	14.2±0.6	13.5±1.1	14.2±0.8	14.4±1.2	14.1±1.7

Table S2. A statistical analysis of the effects of serine substitution mutations in CRY2 on hypocotyl elongation in *Arabidopsis*

Results represent the mean ± SD (n= 15 to 20), the p values of Turkey's LSD Test for the comparison between CRY2 and serine substitution mutations are shown.

Supplemental Figure 1

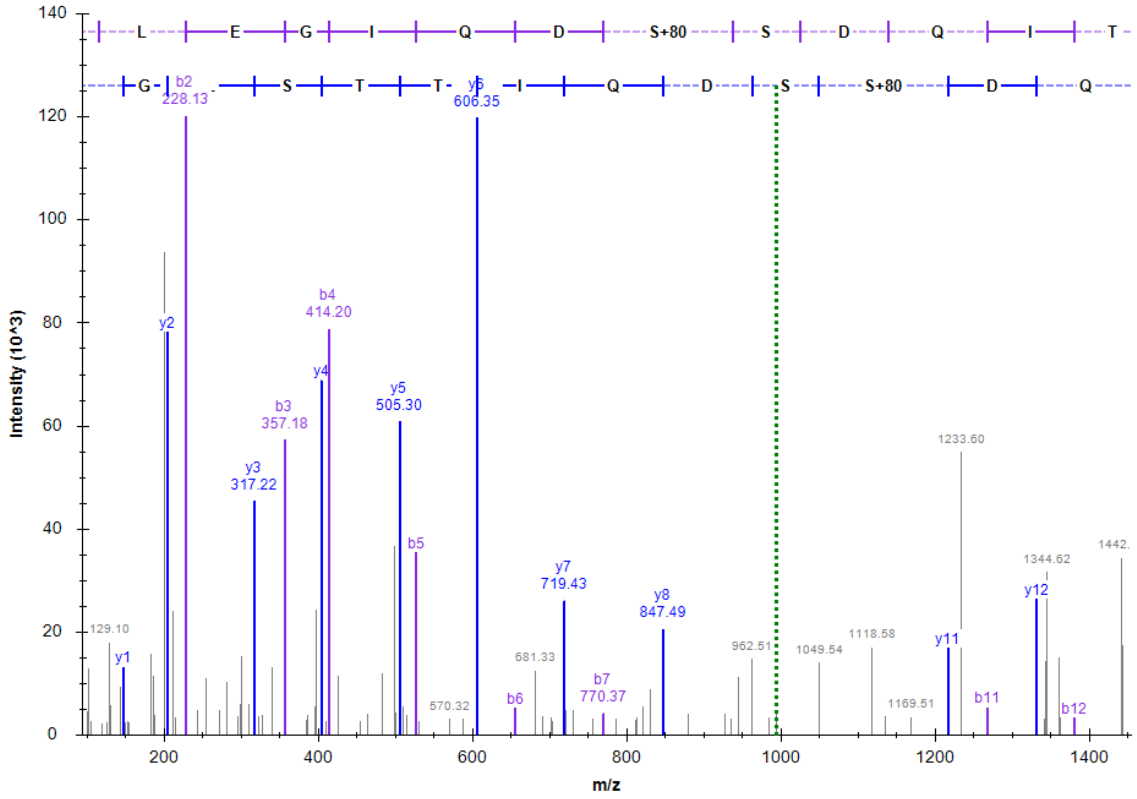

Fig. S1. A representative tandem mass spectrum of the NLEGIQD**p**SSDQITTSLGK peptide (pS598)

Fragments matched within 10ppm are annotated as either the B-ions (purple) or Y-ions (blue) series. The green dashed line indicates precursor m/z.

Supplemental Figure 2

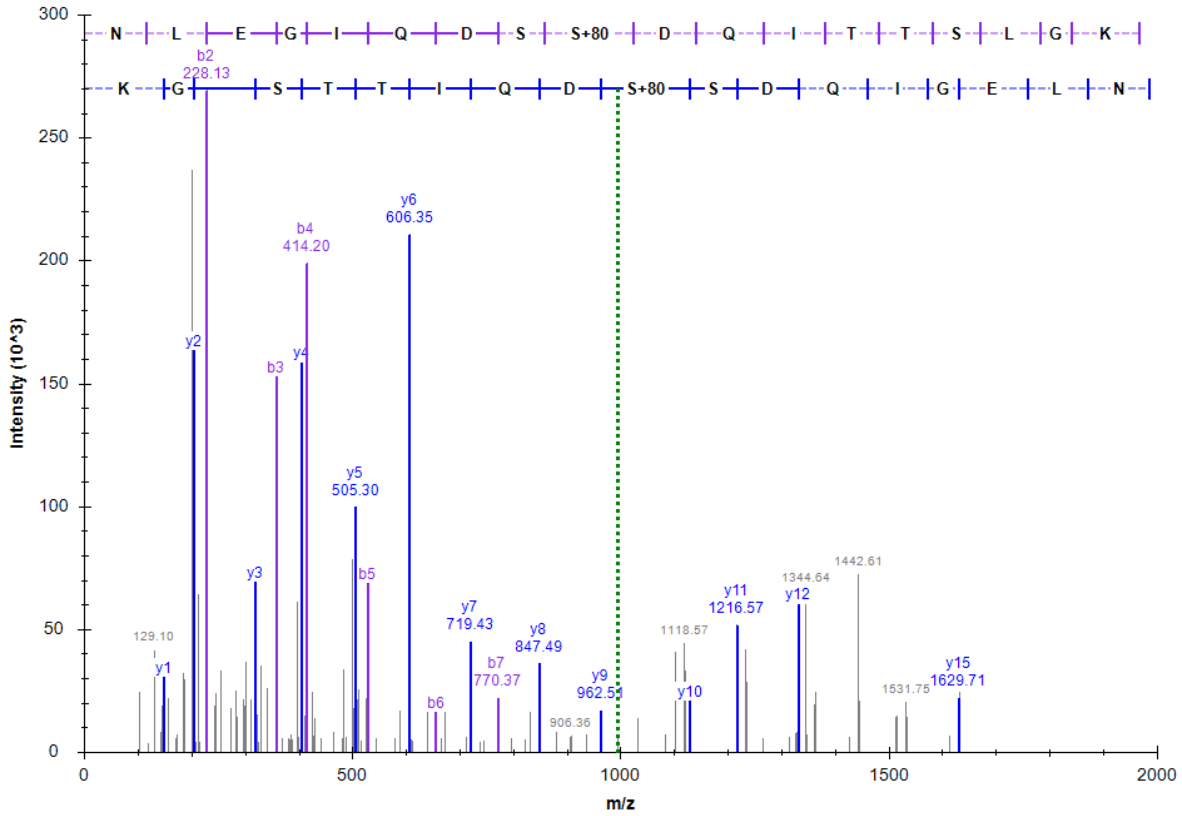

Fig. S2. A representative tandem mass spectrum of the NLEGIQDS**p**SDQITTSLGK peptide (pS599)

Fragments matched within 10ppm are annotated as either the B-ions (purple) or Y-ions (blue) series.

Supplemental Figure 3

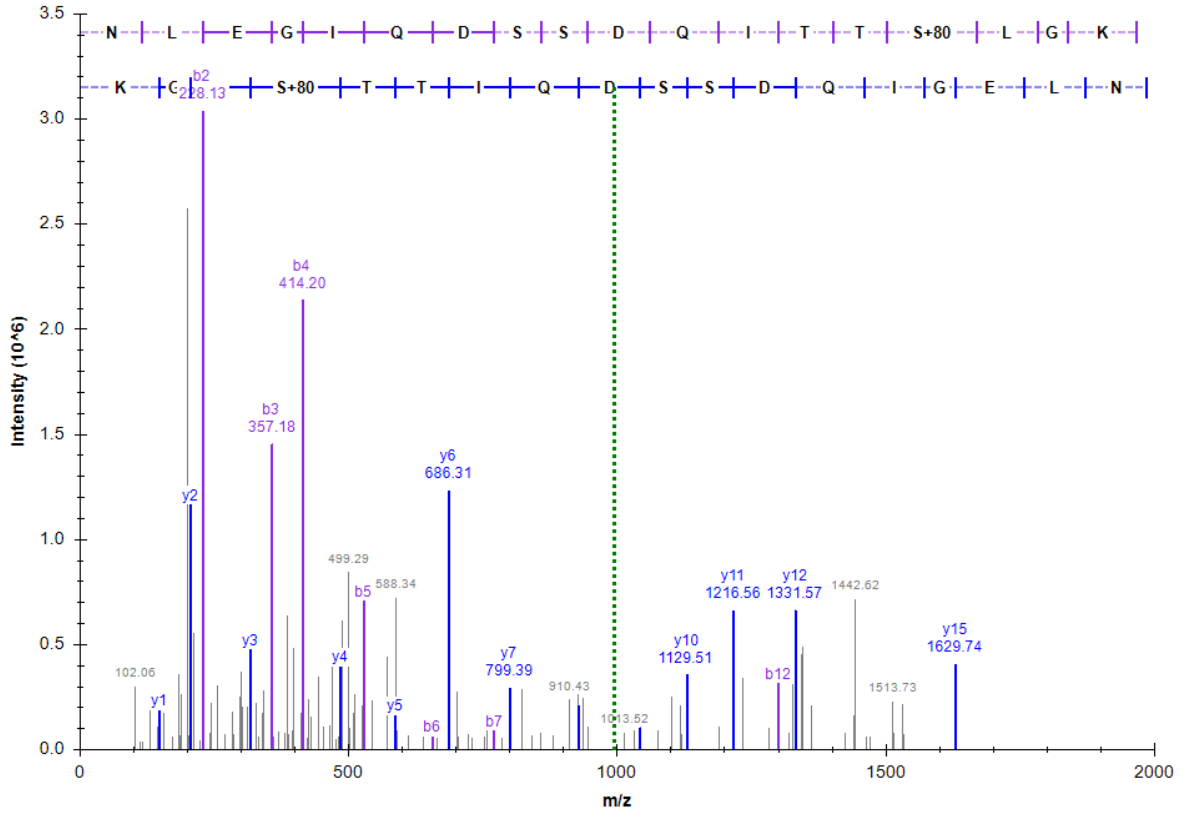

Fig. S3. A representative tandem mass spectrum of the NLEGIQDSSDQITT**p**SLGK peptide (pS605)

Fragments matched within 10ppm are annotated as either the B-ions (purple) or Y-ions (blue) series..

Supplemental Figure 4

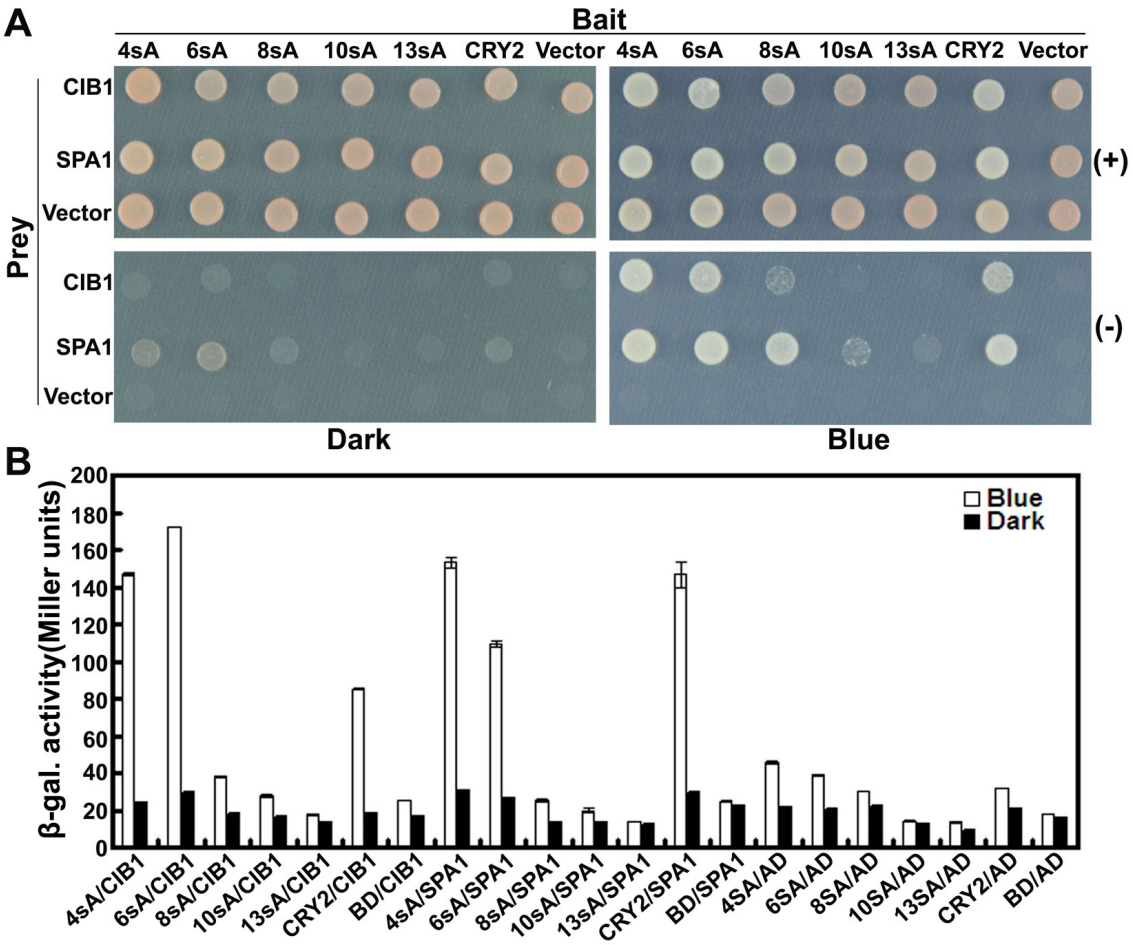

Fig. S4. Yeast two-hybrid assays showing that the serine-substitution mutations of CRY2 impaired its activity to interact with SPA1 and CIB1 in yeast cells.
(A) Histidine auxotrophy assay showing interaction between serine-substitution mutations of CRY2 and SPA1 or CIB1 in blue light (Blue, 25 μmol m^{-2} s^{-1}) or in the dark (Dark). The positive controls of the CRY2-SPA1 pair and the CRY2-CIB1 pair, and the negative control of pGADT7 (prey vector)-pBridge (bait vector) pair are included.
(B) β-galactosidase activity liquid assay showing interaction between serine-substitution mutations of CRY2 and SPA1 and CIB1 in blue light (Blue, 75 μmole m-2 s-1) or in the dark (Dark). Miller units were calculated to present the activity of β-galactosidase.