

Aschoff's Rules on Circadian Rhythms Orchestrated by Blue Light Sensor

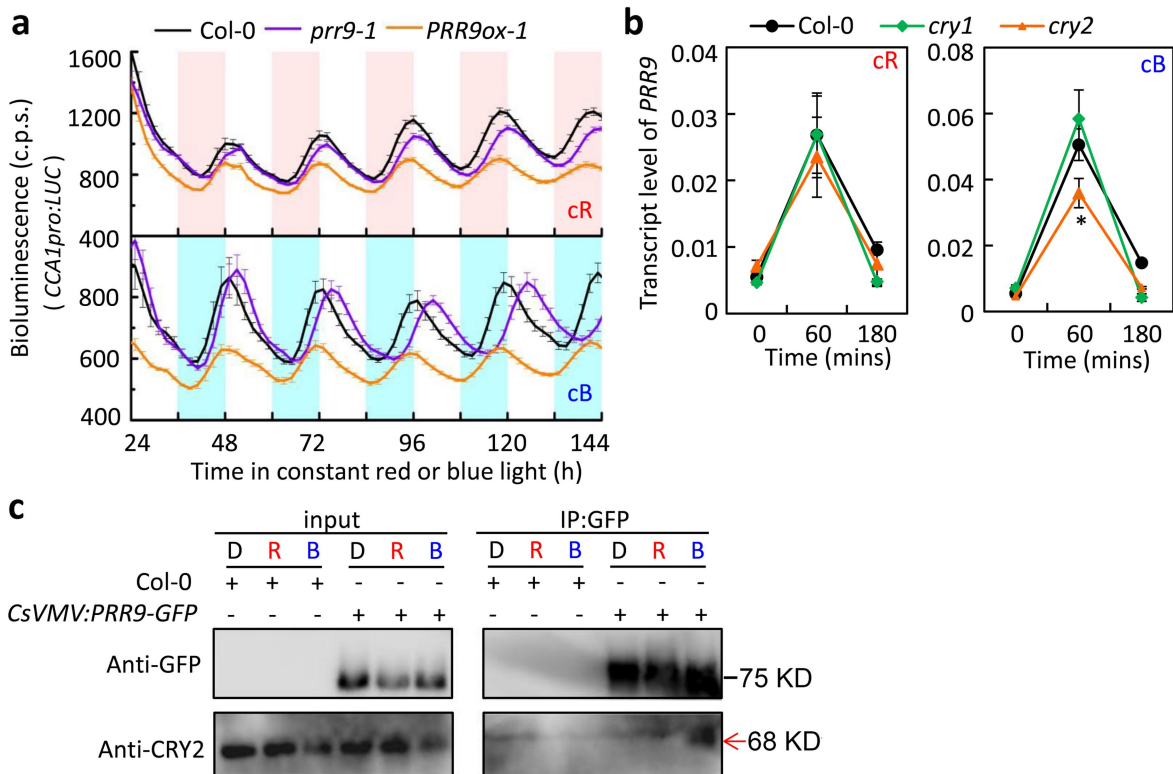
CRY2 and Clock Component PRR9

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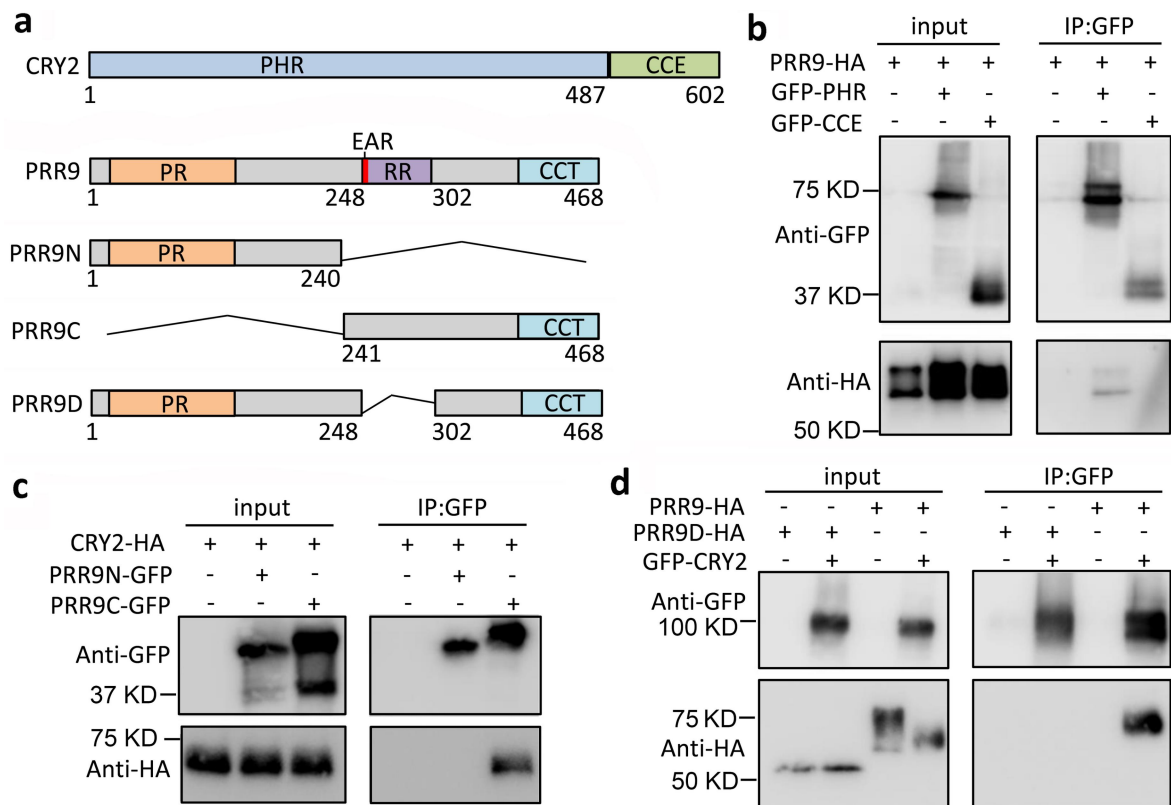


Supplementary Fig. 1: PRR9 is involved into blue light input.

a Bioluminescence trace of *CCA1pro:LUC* in Col-0, *prr9-1* and *PRR9ox-1* plants in constant 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ red light (cR) (n=20, 20, 19 respectively) or blue light (cB) (n=20, 20, 20 respectively) conditions. Representative data from three biological repeats. Data represent mean $\pm$ s.e.m. **b** Expression profile of *PRR9* in Col-0, *cry1* or *cry2* mutant determined by RT-qPCR. Seedlings are grown in LD condition for 10 days, then transferred to red or blue light (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for indicated durations. Data are mean $\pm$ s.d., n=3, technical repeats.

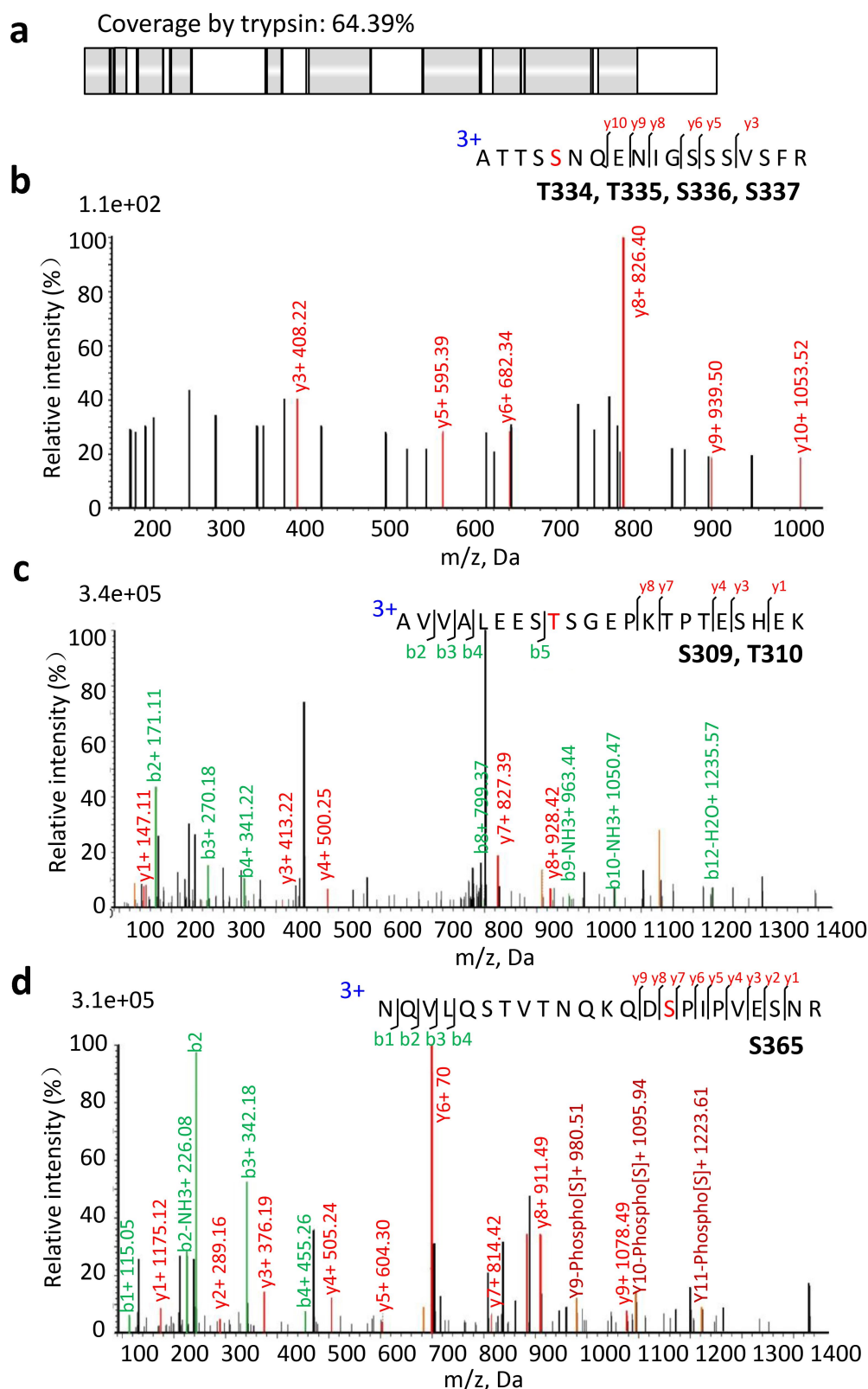
17 (* $p < 0.05$, Student's t -test) **c** Co-IP assay showing CRY2 interacted with PRR9 under blue
18 light in *Arabidopsis*. 7-day-old Col-0 and *CsVMV:PRR9-GFP* seedlings were exposed to
19 dark, red (R) and blue light (B) ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) respectively for 10 mins. Red arrow indicates
20 endogenous CRY2 recognized by CRY2 antibody. Representative data from three
21 independent biological repeats.

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Supplementary Fig. 2: PHR domain of CRY2 is required for interacting with C-terminal of PRR9.

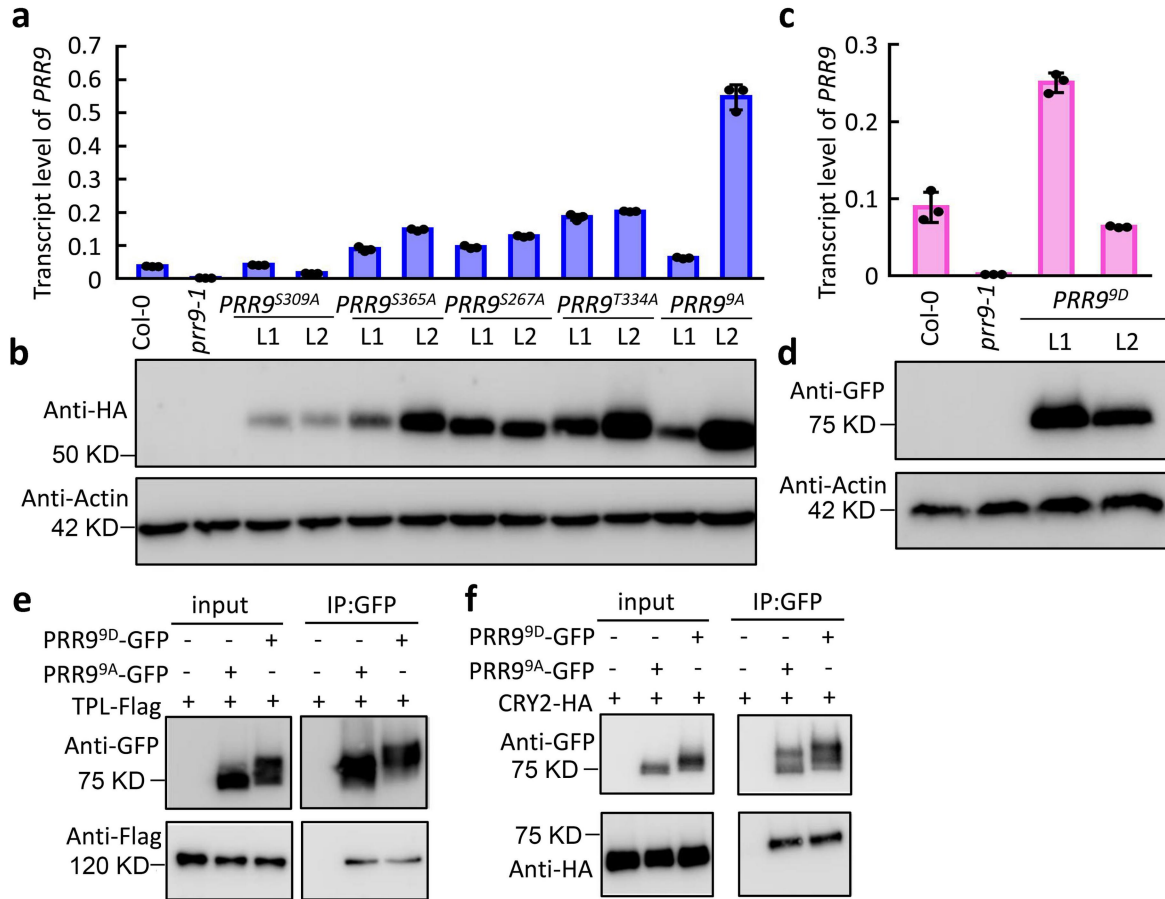
a Schematic diagram showing domain structure of CRY2, PRR9 and their truncated proteins used for CoIP assays. Numbers indicated the amino acid position. **b** PHR domain of CRY2 interacted with PRR9 in *N. benthamiana* leaves by Co-IP assay. The IPed (PHR or CCE) and co-IPed signals (PRR9) were detected in immunoblots probed with GFP and HA antibodies, respectively. **c** PRR9C-GFP interacted with CRY2 in *N. benthamiana* leaves by Co-IP assay. The IP (PRR9N or PRR9C) and co-IP signals (CRY2) were detected in immunoblots with GFP and HA antibodies, respectively. **d** Co-IP assay showing PRR9D can not interact with CRY2 in *N. benthamiana* leaves. The IP (CRY2) and co-IP signals (PRR9 or PRR9D) were detected in immunoblots with GFP and HA antibodies, respectively. For figure (b-d), similar results were observed from three independent biological repeats.



Supplementary Fig. 3: PRR9 peptides coverage and higher-energy collisional dissociation spectra of the phosphopeptides identified by mass spectrometry.

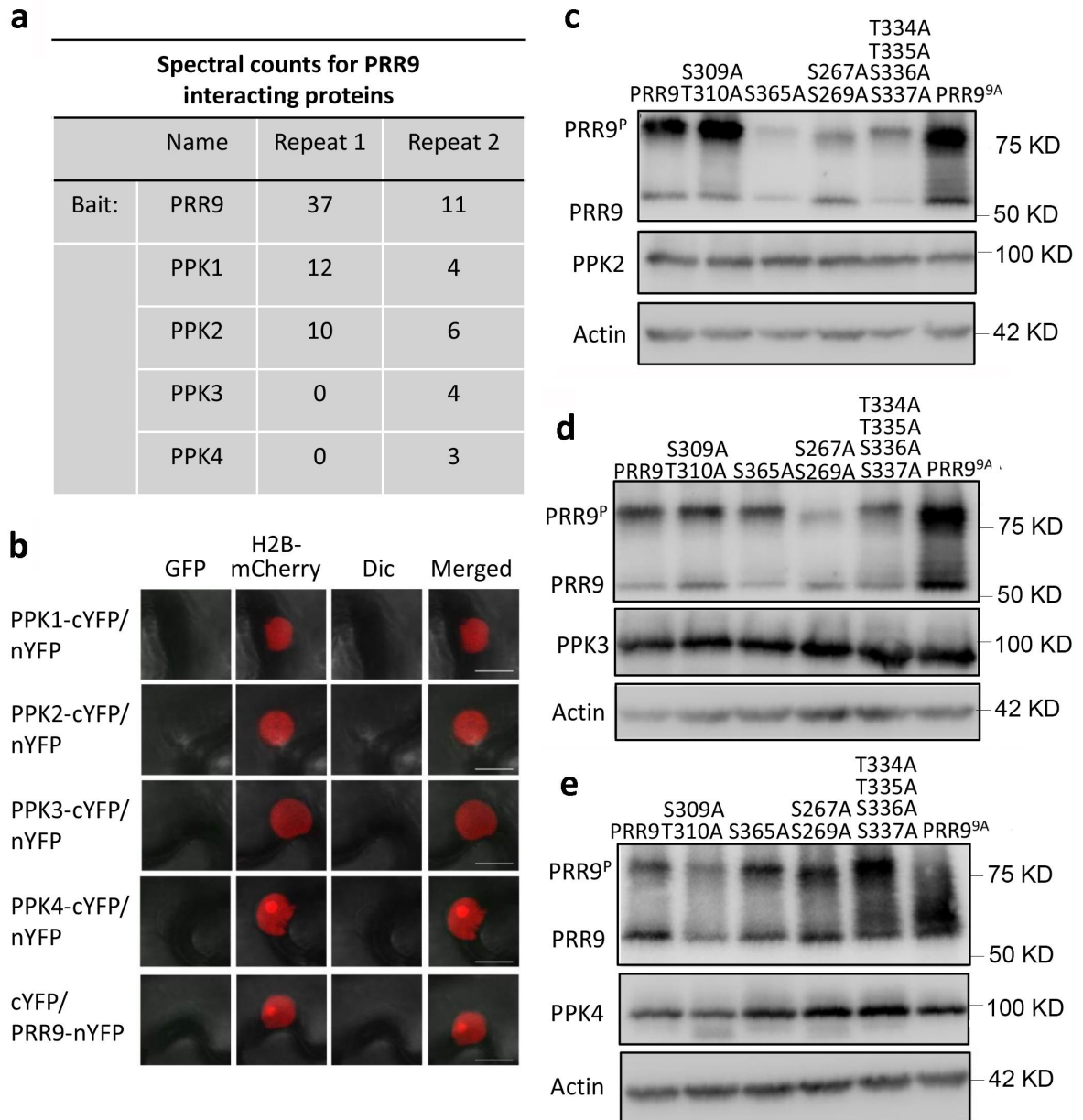
a Mass spectrometry coverage of PRR9 digested by trypsin from three biological replicates.

41 Regions in gray were identified by mass spectrometry while blank regions were not detected.
42 **b-d** Mass spectrogram of three representative phosphopeptides ATTSSNQENIGSSSVSFR
43 (b), AVVALEESTSGEPKTPTESHEK (c) and NQVLQSTVTNQKQDSPIPVESNR (d). Black
44 bold characters are putative phosphosites on the phosphopeptide.



Supplementary Fig. 4: Characterization of PRR9 phospho-mutants.

a, b *PRR9* transcript levels at ZT1 (a) and protein levels at ZT5 (b) from Col-0, *prrr9-1* and respective two independent lines of *PRR9* phosphorylation-point mutation variants driven by native promoter tagged with HA in *prrr9-1* background were detected. Data in (a) are mean $\pm$ s.d., n=3, technical repeats. Actin was used as a protein loading control in (b). **c, d** *PRR9* transcript levels at ZT1 (c) and protein levels at ZT5 (d) from Col-0, *prrr9-1* and *PRR9:PRR9^{9D}-GFP* in *prrr9-1* background (*PRR9^{9D}*). Data in (c) are mean $\pm$ s.d., n=3, technical repeats. Actin was used as a loading control in (d). **e, f** Co-IP assay showing phosphorylation of *PRR9* has no effect on its interaction with TPL (e) and CRY2 (h) in planta from three biological repeats.



Supplementary Fig. 5: PPKs interact with PRR9 *in vitro* and *in vivo*.

a PRR9-interacting proteins identified by IP-MS analysis. Spectral counts were from two biological replicates. **b** Negative controls for BiFC assay. PPKs-cYFP and cYFP were individually co-expressed with nYFP or PRR9-nYFP as indicated in *N. benthamiana* leaves. H2B-mCherry as a nucleus marker. Bars, 20 μ m. (n=12, 12, 12, 12 cells/2 leaves) **c-e** Immunoblot showing GFP tagged-PPK2 (c), PPK3 (d) and PPK4 (e) catalyzed phosphorylation of PRR9 and series of PRR9 mutants fused with HA tag. Total protein lysates were analyzed by immunoblot with GFP and HA antibodies, respectively. Actin was

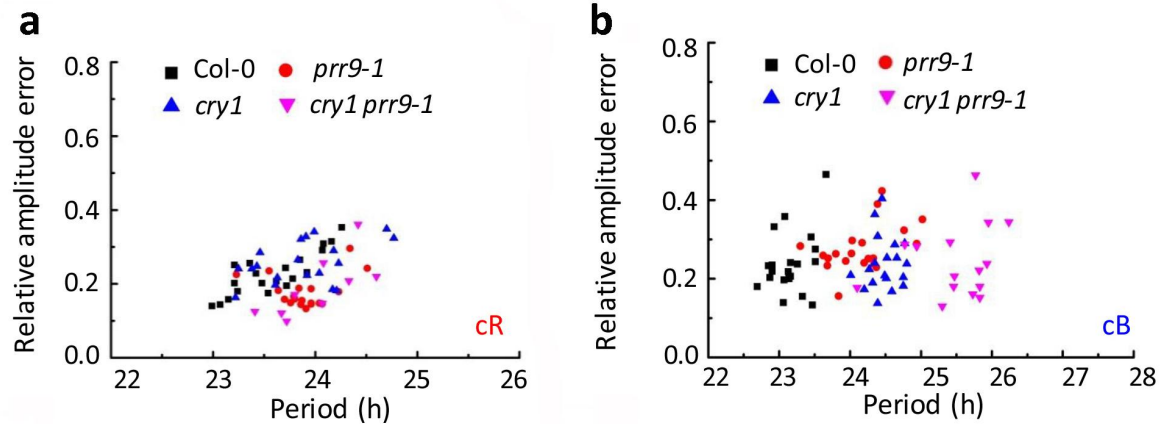
65 used as a loading control. Similar results were observed from three independent biological
66 repeats.

Supplementary Table 1: Circadian period length of Col-0, *prp9-1*, *cry2* and *cry2 prp9-1* under constant blue light with different light quantity.

	Period (h) $\pm$ S.E.M			
cB ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Col-0	<i>prp9-1</i>	<i>cry2</i>	<i>cry2 prp9-1</i>
2.5	24.33 $\pm$ 0.11	26.55 $\pm$ 0.13	25.77 $\pm$ 0.24	26.22 $\pm$ 0.18
10	24.41 $\pm$ 0.07	26.86 $\pm$ 0.07	26.02 $\pm$ 0.14	26.83 $\pm$ 0.11
20	23.62 $\pm$ 0.07	25.65 $\pm$ 0.14	24.45 $\pm$ 0.14	25.39 $\pm$ 0.12

Supplementary Table 2: Circadian period length of Col-0, *cry2*, *PRR9ox-1* and *cry2 PRR9ox-1* under constant blue light with different light quantity.

	Period (h) $\pm$ S.E.M			
cB ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Col-0	<i>cry2</i>	<i>PRR9ox-1</i>	<i>cry2 PRR9ox-1</i>
2.5	24.54 $\pm$ 0.11	25.24 $\pm$ 0.24	24.30 $\pm$ 0.21	23.80 $\pm$ 0.12
10	24.43 $\pm$ 0.10	24.97 $\pm$ 0.19	23.96 $\pm$ 0.08	23.81 $\pm$ 0.09
20	23.90 $\pm$ 0.05	24.42 $\pm$ 0.08	23.68 $\pm$ 0.08	23.57 $\pm$ 0.13



Supplementary Fig. 6: CRY1 and PRR9 act additively in regulating circadian period in constant blue light.

a, b Scatter plot of the estimated circadian period and relative amplitude error of Col-0, *prr9-1*, *cry1* and *cry1 prr9-1* plants in $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous red ($n=20, 19, 19, 18$ respectively) (a) or blue light ($n=20, 19, 20, 18$ respectively) (b). The analysis was carried out three biological repeats with similar results.

81 **Supplementary Table 3: Primers used in this assay.**

	Primer Sequence (5'-3')
BiFC assay	
35S:PRR9-nYFP-F	ATTACGAACGATAGTTAATTAAATGGGGGAGATTGTGGTTTT
35S:PRR9-nYFP-R	ACTGCCACCTCCTCC ACTAGT TGATTTTGTAGACGCGTCTG
35S:CRY1-cYFP-F	ATTACGAACGATAG TTAATTAA ATGTCTGGTTCTGTATCTGG
35S:CRY1-cYFP-R	ACTGCCACCTCCTCC ACTAGT CCCGGTTTGTGAAAGCC
35S:CRY2-cYFP-F	ATTACGAACGATAG TTAATTAAATGAAGATGG AAAAAAGAC
35S:CRY2-cYFP-R	ACTGCCACCTCCTCC ACTAGT TTTGCAACCATTTTTTC
35S:PPK1-cYFP-F	ATTACGAACGATAGTTAATTAAATGCCGGAGCTTCGCCGTGG
35S:PPK1-cYFP-R	ACTGCCACCTCCTCCACTAGTAGTAGATACAGTTCCGCCATAG
35S:PPK2-cYFP-F	ATTACGAACGATAG TTAATTAAATGCCAGAGT TAAGAAGTGG
35S:PPK2-cYFP-R	ACTGCCACCTCCTCCACTAGTAGTGCAAAGTGTCTCCCAAAC
35S:PPK3-cYFP-F	ATTACGAACGATAG TTAATTAAATGCCAGAGT TAAGAAGTGG
35S:PPK3-cYFP-R	ACTGCCACCTCCTCCACTAGTAGTGCAAAGTGTCCGACCATAC
35S:PPK4-cYFP-F	ATTACGAACGATAG TTAATTAAATGCCTGAGC TGCCTAGCAA
35S:PPK4-cYFP-R	ACTGCCACCTCCTCC ACTAGTAGTTGACACAGTTCCGACCATA
Split Nano LUC assay	
CsVMV:PRR9-NlucN-R	TAACCTGCCATGGATCCCGGGGTTTCATAGGATCTCCTCGAAGAG TCGATAGCCGGTGACTGATTTTGTAGACGCGTCTG
CsVMV:CRY1-NlucC-R	GAGGGTGAAGACCATCCCGGGCCCGGTTTGTGAAAGCC
CsVMV:CRY2-NlucC-R	GAGGGTGAAGACCATCCCGGGTTCGCAACCATTTTTTC
Co-IP assay	
CsVMV:PRR9-HA-F	AATTCTGCAGTCGACGGTACC ATGGGGGAGA TTGTGGTTTT
CsVMV:PRR9-HA-R	TAACCTGCCATGGATCCCGGGGTTGATTTTGTAGACGCGTCTG
CsVMV:PRR9-GFP-F	AATTCTGCAGTCGACGGTACC ATGGGGGAGA TTGTGGTTTT
CsVMV:PRR9-GFP-R	TACTCATCCATGGAT CCCGGGC TGATTTTGTAGACGCGTCTG
CsVMV:CRY1-GFP-F	AATTCTGCAGTCGACGGTACC ATGTCTGGTTCTGTATCTGG
CsVMV:CRY1-GFP-R	TACTCATCCATGGAT CCCGGGC CCCGGTTTGTGAAAGCC
CsVMV:CRY2-GFP-F	AATTCTGCAGTCGACGGTACCATGAAGATGGACAAAAAGAC
CsVMV:CRY2-GFP-R	TACTCATCCATGGAT CCCGGGCTTTGCAACCATTTTTTC
CsVMV:CRY2-HA-F	AATTCTGCAGTCGACGGTACCATGAAGATGGACAAAAAGAC
CsVMV:CRY2-HA-R	TAACCTGCCATGGATCCCGGGGTTTTCGCAACCATTTTTTC
pENTR2B:CRY2-F	GCTGGGTCTAGATATCTCGAGTCACCCGGTTTGTGAAAGCC
pENTR2B:CRY2-R	GCTGGGTCTAGATATCTCGAGTCATTTGCAACCATTTTTTC
pENTR2B:PHR-R	GCTGGGTCTAGATATCTCGAGTCATCTTGAAATAGCTTTAGC
pENTR2:CCE-F	CAGTCGACTGGATCCGGTACCATGACCCGTGAAGCACAGATA
CsVMV:PRR9N-GFP-R	TACTCATCCATGGAT CCCGGGC ACGCTTGCAATTCACCAA
CsVMV:PRR9C-GFP-F	AATTCTGCAGTCGACGGTACCATGCCTGATAGTATTTATAAAGA
CsVMV:PRR9D-HA-F	CCTGATAGTA TTTATAAAGACAAGGCCGTCGTTG CTTTAGAGGA
CsVMV:PRR9D-HA-R	TCCTCTAAAGCAACGACGGCCTTGCTTTATAAATACTATCAGG
CsVMV:PPK1-GFP-F	AATTCTGCAGTCGACGGTACCATGCCGGAGC TTCGCCGTGG

CsVMV:PPK1-GFP-R	TACTCATCCATGGAT CCCGGGCAGATACAGTTCGGCCATAG
CsVMV:PPK2-GFP-F	AATTCTGCAGTCGACGGTACCATGCCAGAGT TAAGAAAGTGG
CsVMV:PPK2-GFP-R	TACTCATCCATGGAT CCCGGGC GCAAACGTCTCTCCCAAAGC
CsVMV:PPK3-GFP-F	AATTCTGCAGTCGACGGTACCATGCCAGAGTTAAGAAAGTGG
CsVMV:PPK3-GFP-R	TACTCATCCATGGAT CCCGGGCGCAAACGTCCGACCATAGC
CsVMV:PPK4-GFP-F	AATTCTGCAGTCGACGGTACCATGCCTGAGCTGCGTAGCAA
CsVMV:PPK4-GFP-R	TACTCATCCATGGATCCCGGGCTGACACAGTTCGACCATA
35S:PPK1-Flag-F	CGAGCTCATGCCGGAGCTTCGCCGTGG
35S:PPK1-Flag-R	CGCGGATCCGTGGTGATGGTGATGATGAGATACAGTTCGGCCAT AGC
Transient expression assay	
Green-0800-CCA1pro-LUC-F	GTCGACGGTATCGATAAGCTTTCATGCATGGTTAGCTTAGC
Green-0800-CCA1pro-LUC-R	CGCTCTAGAAGTAGTGATCCTCTCCATCACTAAGCTCCTCTAC
UC18-PRR9-3HA-F	ACAGTCGACCAATTGGGTACCATGGGGGAGA TTGTGGTTTT
UC18-PRR9-3HA-R	GTACATAGAGCGGCCCTCGAGTTTTGTAGACGCGTCTG
UC18-CRY2-3HA-F	ACAGTCGACCAATTGGGTACCATGAAGATGGACAAAAAGAC
UC18-CRY2-3HA-R	GTACATAGAGCGGCCCTCGAGTTTGCAACCATTTTTTC
Site-directed mutation in the primer	
PRR9 ^{S267AS269A} _F	TGAAAAGATCTTGCGCGGTAGCGTTTGAGAA
PRR9 ^{S267AS269A} _R	TTCTCAAACGCTACCGCGCAAGATCTTTTCA
PRR9 ^{S309AT310A} _F	TAGAGGA GAGTGCTGCA GGTGAGCCAA AGA
PRR9 ^{S309AT310A} _R	TCTTTGGCTCACCTGCAGCACTCTCCTCTA
PRR9 ^{T334AT335AS336AS337A} _F	GAAGCGCCGCAGCGGCCGCCAACCAGGAG
PRR9 ^{T334AT335AS336AS337A} _R	CTCCTGGTTGGCGGCCGCTGCGGCGCTTC
PRR9 ^{S365A} _F	CAGAAGCAAG ATGCACCCAT ACCGGTAGAA
PRR9 ^{S365A} _R	TTCTACCGGTATGGGTGCATCTTGCTTCTG
PPK1 ^{K175A} _F	TTCTCCTTTACTCATCCATGGAGATACAGTTCGGCCATAG
PPK1 ^{K175A} _R	TTCTCCTTTACTCATCCATGGGCAAACGTCTCTCCCAAAGC
qPCR primers	
qCCA1-F	CCTTTTACAAACACCGGCTCTT
qCCA1-R	AATCGGGAGGCCAAAATGA
qLHY-F	AAATCAAGATGAGAATTGCTCGGG
qLHY-R	ACTTGTTTCAATGTCGCCACTTACTTTC
qPRR9-F	GTTGAAGAGGAAAGATCGATGCTT
qPRR9-R	CTGCTCTGGTACCGAACCTTTT
qACT2-F	GCTGAGAGATTCAGATGCCCA
qACT2-R	GTGGATTCCAGCAGCTTCCAT
ChIP-qPCR primers	
CCA1-A1-F	CAGGTAGTCCCGGAACTCGTGG
CCA1-A1-R	CGGAAATGGAGAAATCTCAGCC
CCA1-A2-F	CTCCATTTCCGTAGCTTCTGG
CCA1-A2-R	CAAACAATAAGAAAGACCATGAC

LHY-A3-F	CACAGCTGGCACCGTACCCAC
LHY-A3-R	CTCAAGTTGCTTCTCTACGAGC
LHY-A4-F	GTTAGAGAGGATTTGAAGCAG
LHY-A4-R	CATCGCTCTCGCTGCTTTG
ChIP-ACT2-F	CGTTTCGCTTTCCCTTAGTGTTAGCT
ChIP-ACT2-R	AGCGAACGGATCTAGAGACTCACCTTG
Pull down assay	
pMAL2C:PRR9-MBP-F	AGGATTTTCAGAATTCGGATCCATGGGGGAGA TTGTGGTTTT
pMAL2C:PRR9-MBP-R	TGCCTGCAGGTCGACTCTAGACTATGATTTTGTAGACGCGTCG
Primers for PCR scoring	
ppk2-1-F	CCATGGCAAGGATATCAGGTCTGTTTTT
ppk2-1-R	AATAAAAAATGGCATTAAACCGGGGTGAG
ppk3-2-F	ACATCAAAGGTTTGTGCATGGAGAT
ppk3-2-R	TATTCGTAAGTGCCTCGAGGAACGCTT
prp9-1-F	GCTGTTTCTGATGGTTTAGC
prp9-1-R	AAACTCGTCTCTTTCATCCA
cry1-LP	AAATCCGCATCAAGAAGTGTG
cry1-RF	TCCACTGGTGCTTCTCAGATC
cry2-1-F	ATGAAGATGGACAAAAAGAC
cry2-1-R	ATACTCTCTTAAACCGATTCCCCTA
LB21	TGGTTCACGTAGTGGGCCATCG