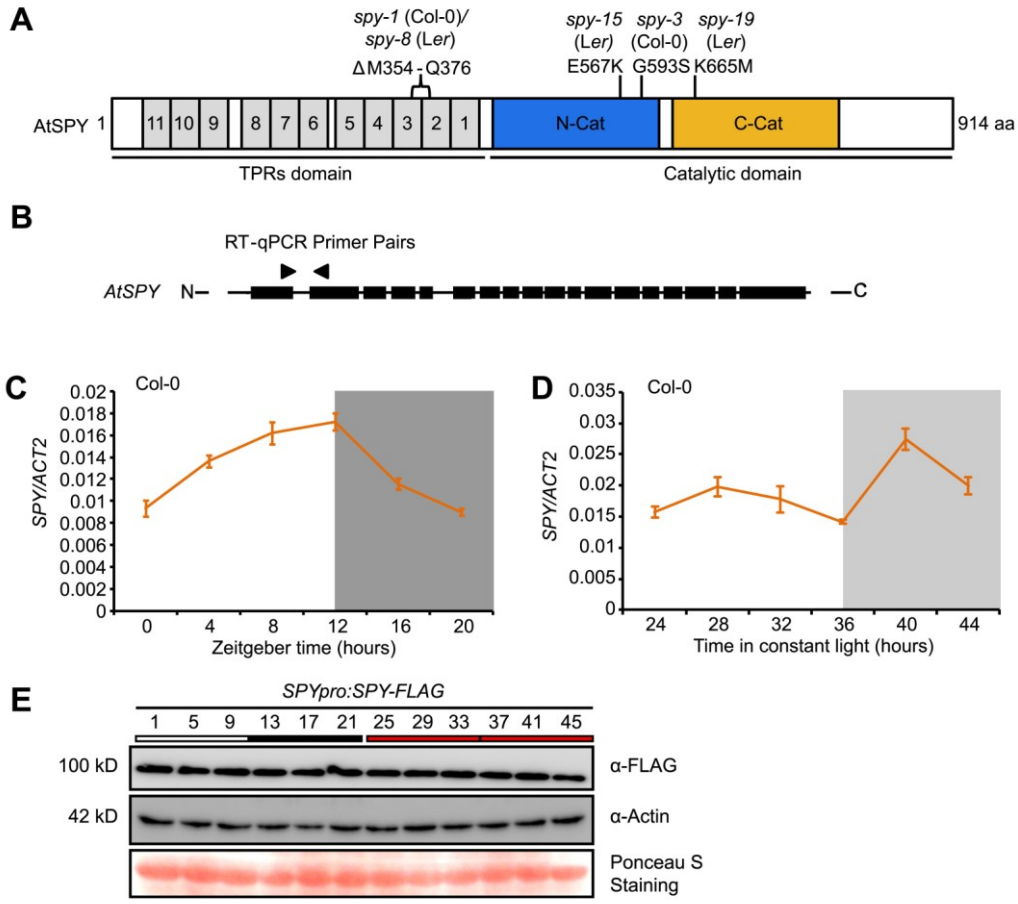


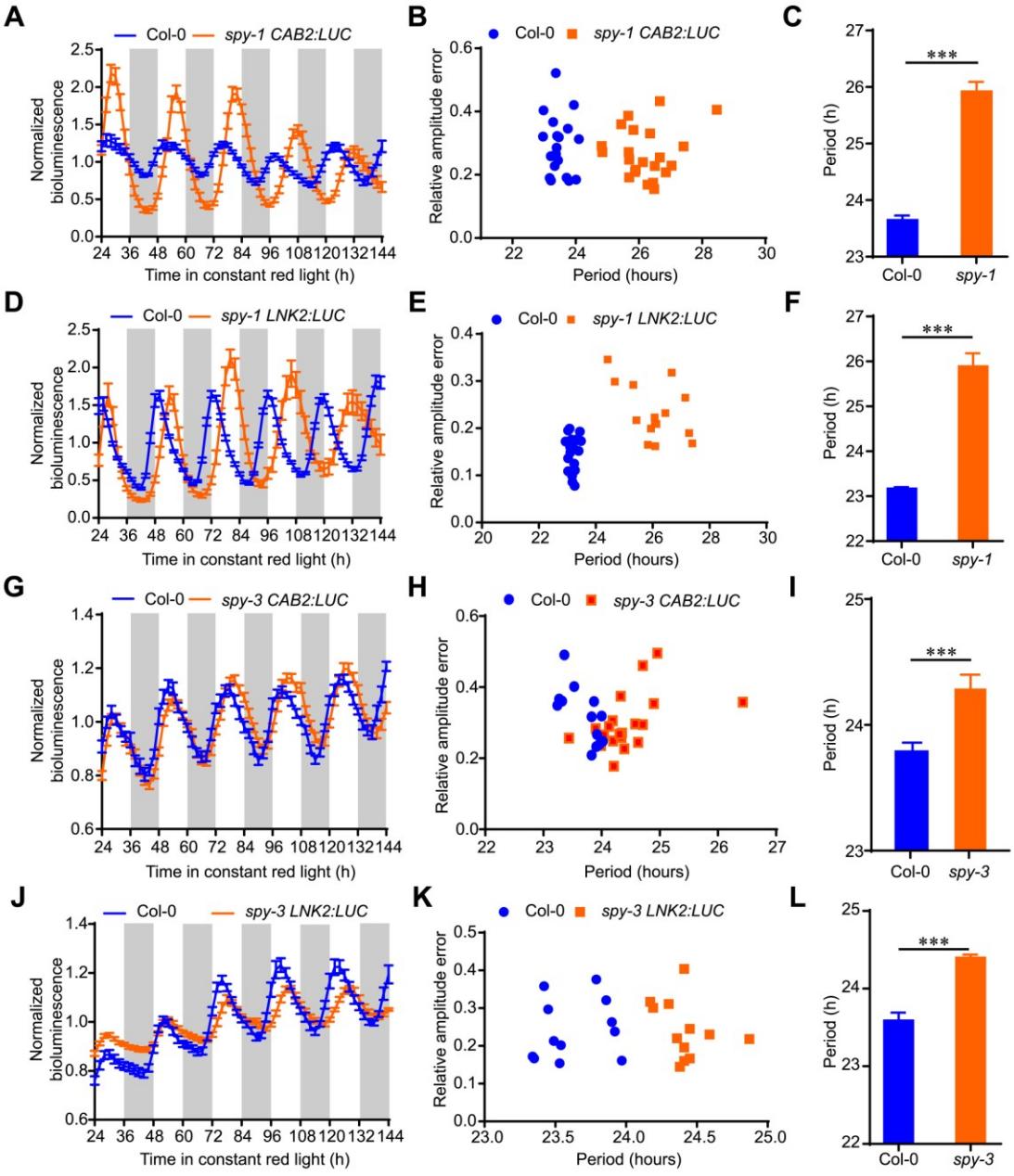
Supplemental Figure 1. *SPY* is not feedback regulated by circadian clock.

(**A**) Schematic diagrams of domain organization of SPY protein and its respective mutations. The amino acid deletion or change and the mutant background are indicated in each mutant allele.

(**B**) Diagram indicating the position of primers used for RT-qPCR to determine *SPY* mRNA levels.

(**C and D**) The temporal expression pattern of *SPY* in LD (**D**) and LL (**E**) conditions. Data represent mean ± s.d. (n = 3).

(**E**) Western blot analysis showing protein abundance of SPY in a time course experiment.



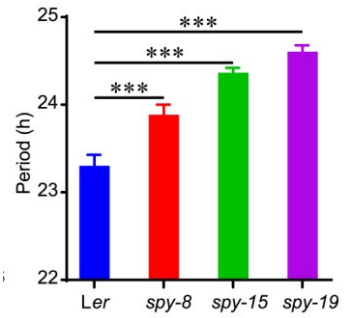
Supplemental Figure 2. Circadian phenotypes of *spy* mutants with additional reporters.

(**A, B and C**) Circadian phenotype of *spy-1* mutant determined with *CAB2pro:LUC* as a reporter in constant red light. Data represent the mean ± s.e. (n = 20 seedlings).

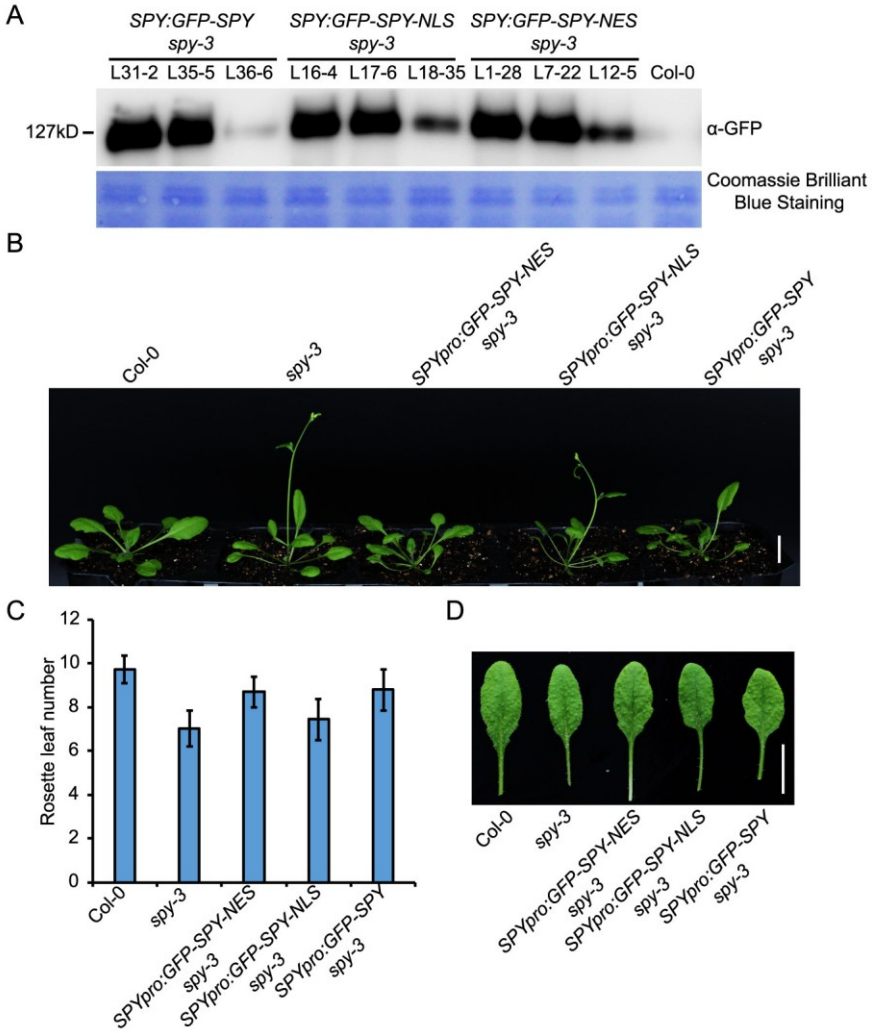
(**D, E and F**) Circadian phenotype of *spy-1* mutant determined with *LNK2pro:LUC* as a reporter in constant red light. Data represent the mean ± s.e. (n = 20 seedlings).

(**G, H and I**). Circadian phenotype of *spy-3* mutant determined with *CAB2pro:LUC* as a reporter in constant red light. Data represent the mean ± s.e. (n = 20 seedlings).

(**J, K and L**) Circadian phenotype of *spy-3* mutant determined with *LNK2pro:LUC* as a reporter in constant red light. Data represent the mean ± s.e. (n = 20 seedlings). *t*-test, ***$p < 0.001$.



Supplemental Figure 3. The *spy* mutants displays the lengthened circadian period.
The estimated circadian period of A. Data represent mean ± s.e. (n=18). *** $p < 0.001$ (Student *t*-test).



Supplemental Figure 4. Identification of respective SPY transgenic lines and characterization of their physiological phenotypes.

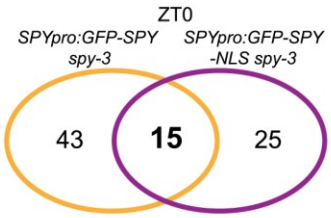
(A) Western blot determining SPY protein level in selective T3 progeny of homozygous transgenic lines.

(**B**) Representative transgenic lines showing *SPYpro:GFP-SPY-NES* can rescue the flowering time phenotype of *spy-3* mutant, but not *SPYpro:GFP-SPY-NLS*. Scale bars=1 cm.

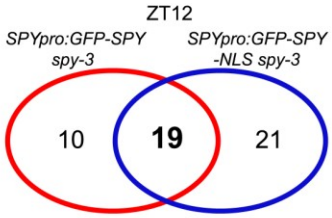
(**C**) Statistical analysis of the flowering time phenotype shown in (B). Data represent mean ± s.e. (n=12).

(**D**) Representative transgenic lines showing *SPYpro:GFP-SPY-NES* can rescue the leaf serration phenotype of *spy-3* mutant, but not *SPYpro:GFP-SPY-NLS*. Scale bars=1 cm.

A



C



B

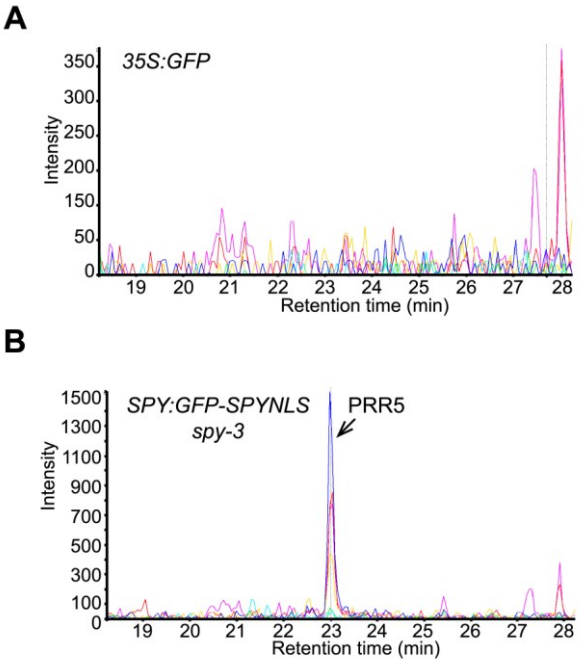
NO.	Peak name	Gene ID	Gene Name
1	**W8QNP6**	***At3g11540***	**Glycosyltransferase**
2	A0A178WNI0	*At1g80670*	RAE1
3	A0A178W2Q0	*At1g10470*	ARR4
4	Q9LU63/no	*At5g51110*	MWD22_5
5	A8MR56	*At3g53930*	Autophagy-related protein 1b
6	A0A178W507	*At1g70770*	SmD3
7	F4KGV5	*At5g10450*	14-3-3-like protein GF14 lambda
8	A0A178V3F1	*At4g03780*	Serine/threonine-protein phosphatase
9	A0A178UK47	*At5g22330*	RuvB-like helicase
10	A0A178W3T8	*At1g13360*	ERF1-2
11	A0A178V716	*At3g47620*	**TCP14**
12	A0A178V1U6	*At4g38280*	HDA14
13	A0A178UYV3	*At4g38680*	GRP2
14	A0A178VTU6	*At2g44170*	Uncharacterized protein
15	F4JPL2	*At4g36080*	TRA1B Phosphotransferases/inositol or phosphatidylinositol kinases

D

NO.	Peak name	Gene ID	Protein Name
1	**W8QNP6**	***At3g11540***	**Glycosyltransferase**
2	A0A178W2Q0	*At1g10470*	Uncharacterized protein
3	Q6LA42	*At5g24470*	APRR5
4	A0A178V2E7	*At4g25120*	SHI4
5	Q9FZJ2	*At1g27430*	F17L21.22
6	A0A178VW16	*At2g36720*	VTC1
7	A0A178WL38	*At1g10240*	NFXL1
8	A0A178UMG2	*At5g63860*	EXL2
9	A0A178UF05	*At5g62690*	Uncharacterized protein
10	A0A178V7W0	*At3g53110*	Uncharacterized protein
11	Q9SAF2	*At1g13190*	F3F19.21
12	A0A178UVL7	*At4g02890*	Endoglucanase
13	O22791	*At2g33410*	Putative RNA-binding protein
14	O64866	*At2g44310*	Calcium-binding EF -hand-containing protein
15	A0A178W6P4	*At1g53090*	RH22
16	A0A178V5T0	*At3g55510*	Uncharacterized protein
17	Q9ZUX4	*At2g27730*	Uncharacterized protein
18	Q9LHL2	*At3g13224*	Uncharacterized protein
19	C0Z3A1	*At2g30620*	Uncharacterized protein

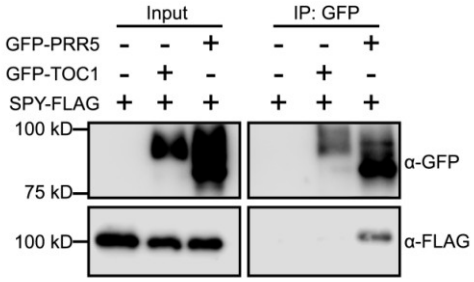
Supplemental Figure 5. Overlapped proteins identified by AP-MS between transgenic line of *SPYpro:GFP-SPY* and *SPYpro:GFP-SPY-NLS*.

(**A**) and (**C**) Venn diagram showing the number of overlapped proteins between *SPYpro:GFP-SPY* and *SPYpro:GFP-SPY-NLS*, with sampling at ZT0 (**A**) and ZT12 (**C**) respectively. (**B**) and (**D**) The list of overlapped proteins shown in A and C.



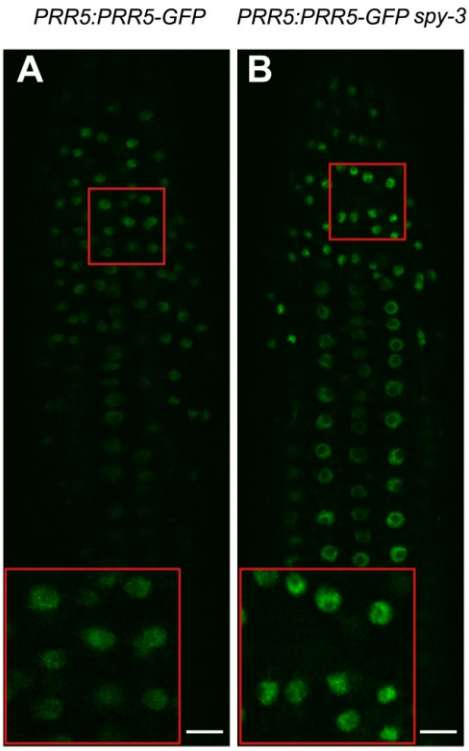
Supplemental Figure 6. Peptides of PRR5 protein were identified by affinity purification followed by mass spectrometry.

Seedlings of *SPY:GFP-SPY-NLS spy-3* and *35S:GFP* plants were used for affinity purification. The extract ion chromatography of peptide from PRR5 protein was found in the immunoprecipitates of *SPY:GFP-SPY-NLS spy-3* (**B**) but not that of *35S:GFP* (**A**).



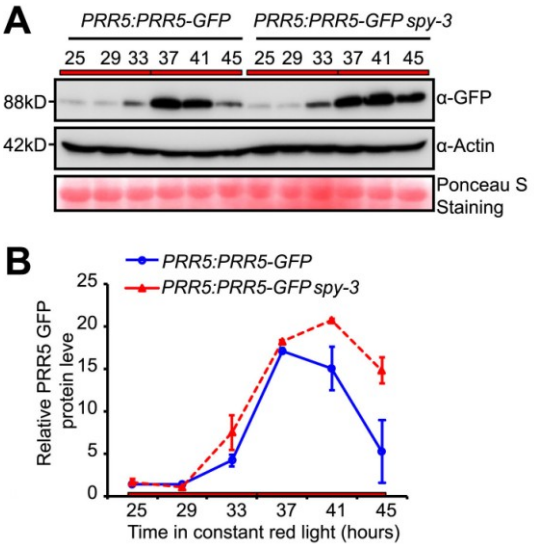
Supplemental Figure 7. TOC1 did not interact with SPY *in planta*.

Co-immunoprecipitation analysis of SPY-FLAG with GFP tagged TOC1 and PRR5 was performed with GFP-Trap beads to precipitate protein complexes extracted from co-infiltrated leaves of *N. benthamiana*.

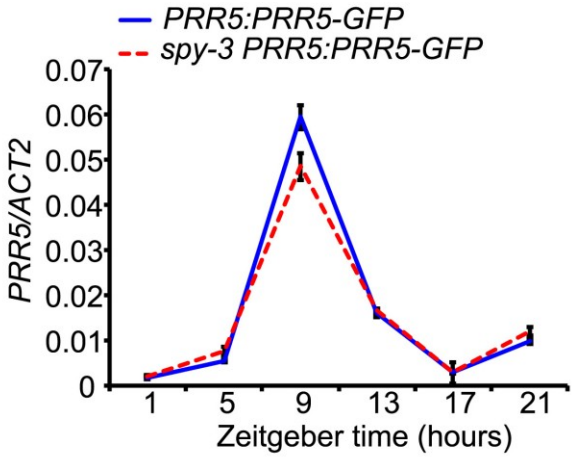


Supplemental Figure 8. PRR5 abundance, but not subcellular localization pattern, was altered in *spy-3* mutant.

Subcellular localization pattern of PRR5-GFP fusion protein in *PRR5:PRR5-GFP* and *PRR5:PRR5-GFP spy-3* was observed by confocal fluorescence microscopy. Scale bars = 20 μm. Close-up views are shown in the lower red boxes.

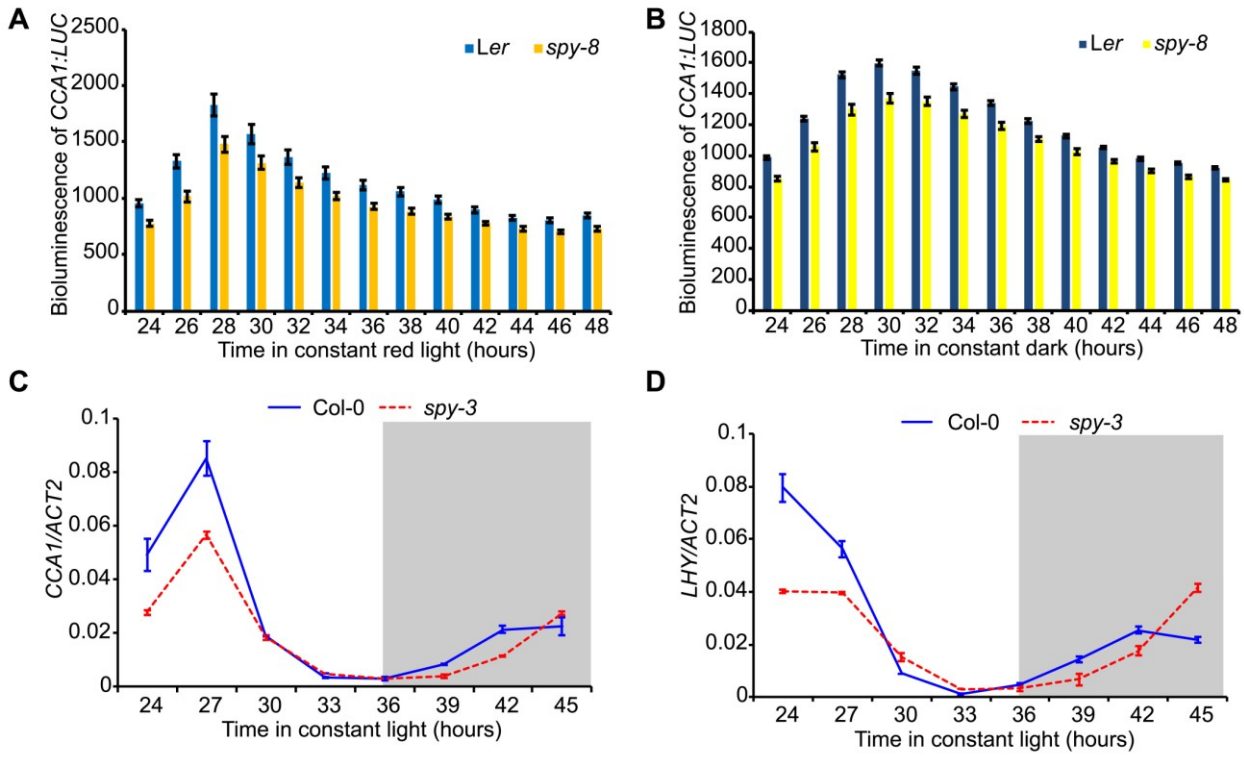


Supplemental Figure 9. Immunoblot analysis showing higher PRR5 protein accumulation in *spy-3* mutant in constant red light. Seedlings of *PRR5:PRR5-GFP* and *PRR5:PRR5-GFP spy-3* were grown in 12 h L/12 h D photocycles, then transferred to constant light condition. Samples were collected at indicated time points. PRR5-GFP protein abundance was detected with GFP antibody. Actin antibody and ponceau staining were used as loading controls. Error bar represents s.d. from three biological replicates.



Supplemental Figure 10. Transcript levels of PRR5 were not significantly changed in *spy-3* mutant.

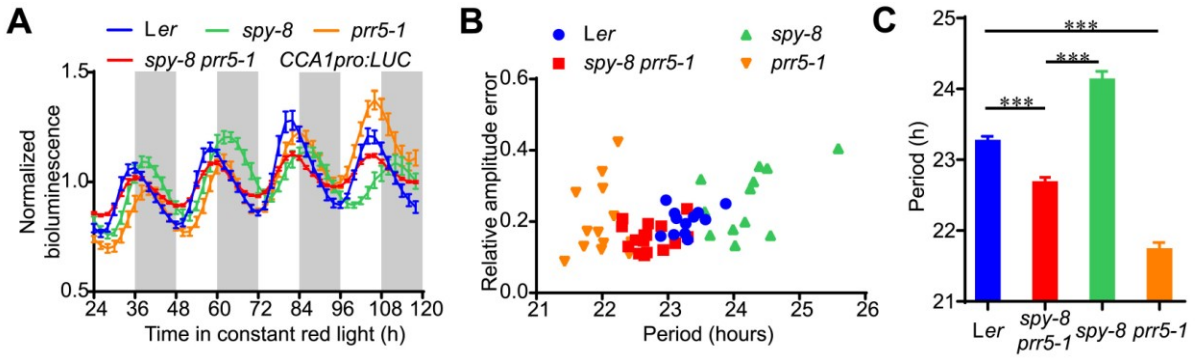
The same tissues in A were used for determine the transcript level of *PRR5* by RT-qPCR. The transcript levels of PRR5 were normalized by *ACT2* expression. Data represent mean ± s.e. (n = 3, biological replicates).



Supplemental Figure 11. Transcript levels of *CCA1* and *LHY* are decreased in *spy-3*.

(**A** and **B**) Bioluminescence of *CCA1:LUC* showing the lower transcription activity of *CCA1* promoter in *spy-8* from LD transferred into continuous red light (**A**) or darkness (**B**). Data represent mean ± s.e. (n = 15).

(**C** and **D**) Transcript levels of *CCA1* (**C**) and *LHY* (**D**) in *spy-3* mutant under LL condition. Data represent mean ± s.e. (n=3, biological replicates). The gene expression level was normalized by *ACT2* expression.

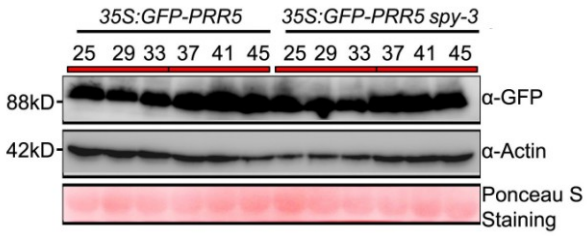


Supplemental Figure 12. The lengthened circadian period phenotype in *spy-8* could be reverted by null mutation of PRR5.

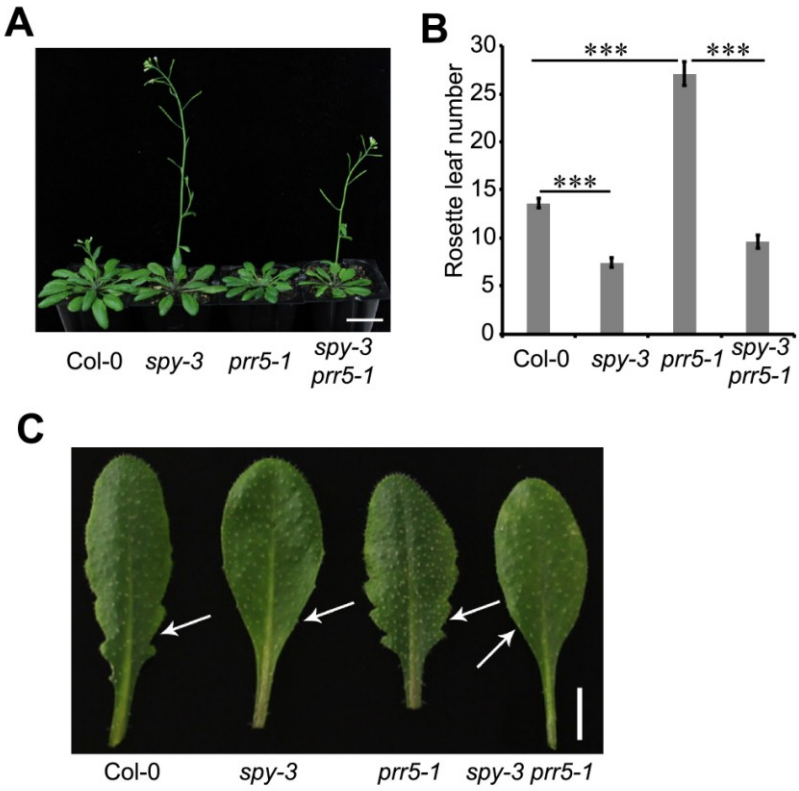
(**A**) Normalized bioluminescence activity of *CCA1:LUC* in *spy-8*, *prr5-1*, *spy-8 prr5-1* and L*er* plants in continuous monochromatic red light (20 µmol m^{-2} s^{-1}).

(**B**) Scatter plot showing that the lengthened circadian period phenotype of *spy-8* could be partially rescued by null mutation of PRR5, *prr5-1*.

(**C**) The estimated circadian period of *spy-8*, *prr5-1*, *spy-8 prr5-1* and L*er* plants. Data represent the mean ± s.e. (n = 15 seedlings). ***$p < 0.001$ (Student *t*-test).



Supplemental Figure 13. Immunoblot analysis showing PRR5 protein abundance in *35S:PRR5-GFP* and *35S:PRR5-GFP spy-3* in constant red light. Seedlings were grown in 12 h L/12 h D, then transferred to constant red light condition. Samples were collected at indicated time points. PRR5-GFP protein abundance was detected with GFP antibody, while actin antibody and ponceau staining were used as loading controls.



Supplemental Figure 14. The regulation on flowering time and leaf serration by SPY is independent of PRR5.

(**A**) The flowering time of *spy-3*, *prr5-1*, *spy-3 prr5-1* and Col-0 plants. Rosette leaf number were counted after bolting around 3 cm.

(**B**) Quantification the number of rosette leaves at bolting of *spy-3*, *prr5-1*, *spy-3 prr5-1* and Col-0 plants. Data represent the mean ± s.d. (n = 12). ***$p<0.001$ (Student *t*-test).

(**C**) Leaf serration phenotypes of *spy-3*, *prr5-1*, *spy-3 prr5-1* and Col-0 plants, showing the PRR5 mutation cannot genetically rescue the lack of leaf serration phenotype of *spy-3*. The fifth leaves of indicated plants were taken for photography. Scale bars = 1 cm.

Table S1. A list of oligonucleotides used in this study

Primer name	Sequence 5' to 3'	Purpose
qPCR Primers		
qCCA1-F	CCTTTTACAAACACCGGCTCTT	qPCR
qCCA1-R	AATCGGGAGGCCAAAATGA	
qLHY-F	AAATCAAGATGAGAATTGCTCGGG	
qLHY-R	ACTTGTTTCAATGTCGCCACTTACTTTC	
qPRR5-F	ATTCCGAATGAAGCGAAAGGA	
qPRR5-R	TCGTAACGAACCTTTTTCTCAT AACAT	
qSPY-F	TGCTGAGTCCTACCAGAAAGC	
qSPY-R	TCGGTCAAAACAATGGCTAA	
qACT2-F	GCTGAGAGATTCAGATGCCCA	
qACT2-R	GTGGATTCCAGCAGCTTCCAT	
Genotyping Primers		
LBa1	TTTTCGCCCTTTGACGTTGGAG	*prr5-1*
prr5-1-F	CGGCTTTCTGCTGTCCAACAC	*prr5-1*
prr5-1-R	TCGCGCTTAGAGTTTTGCTCG	
sec-5-F	TCATGAATCAATCCTTGAGCC	*sec-5*
sec-5-R	TTTCGATGTCCCTTCTTTGTG	
BP	ATTTTGCCGATTTCGGAAC	*sec-5*
spy-1-F	CTTGGGGTGGCTTATGGAGA	*spy-1* mutant for
spy-1-R	CAGCATTGCGAGAATCTGGA	sequencing
spy-3-F	GCAGATGCAAAAACATACAGG	*spy-3mutant for*
spy-3-R	GGGAACTGCACACAGTATCCTAGCC	*sequencing*
Cloning		
SPY-F	ATGGTGGGACTGGAAGATG	
SPY-R	CTAGCTAGTGGAGTCCATTC	35Spro:GFP-SPY
SPY-NLS-R	CTAAACCTTTCTCTTCTTCTTAGGGCTAGT GGAGTCCATTCTC	35Spro:GFP-SPY-NLS
SPY-NES-R	CTAAATATCAAGTCCAGCCAACTTAAGA GCAAGGCTAGTGGAGTCCATTCTC	35Spro: GFP-SPY-NES
GFP-SPY-F	CTCGACTCTAGAGGATCCCC	
GFP-SPY-R	CTAGCTAGTGGAGTCCATTC	SPYpro: GFP-SPY
GFP-SPY-NLS-R	CTAAACCTTTCTCTTCTTCTTAGG	
GFP-SPY-NES-R	CTAAATATCAAGTCCAGCCAACTTA	SPYpro: GFP-SPY-NES
PRR5 CDS-F	ATGACTAGTAGCGAGGAAGTAG	
PRR5 CDS-R	TGGAGCTTGTGTGGATTGGA	
SPY CDS-F	CGGGGTACCATGGTGGGACTGGAAGATG	
SPY CDS-R	CCATGGACCTTATCGTCATCGTCCTTGTAA	
SPY-FLAG-F	ATGGTGGGACTGGAAGATG	SPYpro:SPY-FLAG
SPY-FLAG-R	CTTATCGTCATCGTCCTTGTAA	

Primer name	Sequence 5’ to 3’	Purpose
GI CDS-F	ATGGCTAGTTCATCTTCATC	
GI CDS-R	TTGGGACAAGGATATAGTAC	
PRR5C-CDS1673-R	CTATGGAGCTTGTGTGGATTGG	
PRR5C-CDS514-F	ATGCAAACTTCACTTGCTCCTG	
PRR5M-CDS1524-R	CTATTGAAGAGATTGCTGAATTT	
PRR5M-CDS514-F	ATGCAAACTTCACTTGCTCCTG	
PRR5N-CDS1-F	ATGACTAGTAGCGAGGAAG	
PRR5N-CDS513-R	CTATCTTCTCCAGACATGCTG	
PRR7 CDS-F	ATGAATGCTAATGAGGAGGG	
PRR7 CDS-R	GCTATCCTCAATGTTTTTTA	
PRR9 CDS-F	ATGGGGGAGA TTGTGGTTTT	
PRR9 CDS-R	TGATTTTGTAGACGCGTCTG	
SPY^{CT} CDS1294-F	ATGAATGCTGGCCAGAACCGAT	
SPY^{CT} CDS-R	GCTAGTGGAGTCCATTCTCTTTG	
SPY^{NT} CDS1-F	ATGGTGGGACTGGAAGATGAT	
SPY^{NT} CDS-R	GCGAGAATCTGGATCTATCTTAAG	
TOC1 CDS-F	ATGGATTTGAACGGTGAGTGT	
TOC1 CDS-R	AGTTCCCAAAGCATCATCCT	