Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. Ecotype accession Columbia-0 (Col-0) was used for the wild-type in this study. *prr9 prr7 prr5* triple mutants, *PRR5pro:FLAG-PRR5-GFP/prr5*, *PRR7pro:FLAG-PRR7-GFP/prr7*, *PRR9pro:FLAG-PRR9-GFP/prr9*, and *PRR5-ox* was reported previously (1–3). To generate *35Spro:PRR5-FLAG* plants, the coding region of *PRR5* (*PSEUDO-RESPONSE REGULATOR 5*) was amplified using primers (5′-CACCATGACTAGTAGCGAGGAAG-3′ and 5′-TGGAGCTTGTGTGGATTGGAC-3′) with Prime Star DNA polymerase (Takara). Amplified DNA was cloned into pENTR/D-TOPO (Invitrogen) to construct an entry plasmid pENTR/D-PRR5. The plasmid was treated with Gateway LR clonase enzyme (Invitrogen) to generate pBS-PRR5-FLAG using pBS-FLAG GW (in which the *35S CaMV* promoter, Gateway reading frame cassette, *3-FLAG*, and *NOS* terminator were assembled 5′ to 3′ in pBlueScript) as a destination vector. The *PRR5-FLAG* region of pBS-PRR5-FLAG was then amplified using primers (5′-CACGGGGGACTCTAGAATGACTAGTAGCGAGGAAG-3′, and 5′-TTCGAGCTGCGGCCGCTTACTTGTCGTCATCGTCTTTG-3′), and cloned into the binary vector pSK1, in which a gene is driven under the control of *35SCaMV* promoter (4), between the XbaI and NotI sites using an In-Fusion HD kit (Takara), generating pSK1-PRR5-FLAG. To create *PRR5mtA* and *PRR5mtB*, a Quick Change Site-Directed Mutagenesis Kit (Agilent Technologies) was used with the appropriate primer sets and pENTR/D-PRR5 (for *PRR5mtA*; 5′-CGAGAGCCGGAAGAAATTAGTAGAGCAACGACCACGAATCAAAG-3′, and 5′-CTTTGATTCGTGGTCGTTGCTCTACTAATTTCTTCCGGCTCTCG-3′, for *PRR5mtB*; 5′- GAAATTAGCAGAGCAACGACCACACATCAAAGGCCAATTCGTTCG-3′, 5′-CGAACGAATTGGCCTTTGATGTGTGGTCGTTGCTCTGCTAATTTC-3′). Resultant pENTR/D-PRR5mtA or pENTR/D-PRR5mtB was then used to make pBS-PRR5mtA-FLAG, pSK1-PRR5mtA-FLAG, pBS-PRR5mtB-FLAG, and pSK1-PRR5mtB-FLAG. To generate the PRR5-VP fusion construct, *PRR5* coding sequence was integrated into the pBS-35S-VP vector (in which the *35S CaMV* promoter, Gateway reading frame cassette, two copies of *VP16*, and *NOS* terminator were assembled 5′ to 3′ in pBlueScript) using LR clonase. *PRR5-VP* region was then amplified using primers (5′-CACGGGGGACTCTAGAATGACTAGTAGCGAGGAAG-3′, and 5′-TTCGAGCTGCGGCCGCCTACCCACCGTACTC-3′), and cloned into pSK1 between the XbaI and NotI sites using an In-Fusion HD kit (pSK1-PRR5-VP). The binary vectors were used to transform Col-0 via an *Agrobacterium*-mediated method (5). Ten independent T1 transformants were selected, and Western blotting was performed at the T2 generation to verify that transgenic plants expressing exogenous protein. Two independent T2 plants were selected and used for further analyses. Plants were grown on MS (6) containing 2% (wt/vol) sucrose and 0.3% gellan gum with or without 20 $\mu g \cdot L^{-1}$ of Hygromycin B, under 12-h white light/12-h dark conditions (70 $\mu mol \cdot s^{-1} \cdot m^{-2}$).

Transient Transfection Assays and Detection of Luciferase Activity in *Arabidopsis* Seedlings. Transient assays and detection of luciferase activity were performed as described previously (2). To generate effector plasmids harboring truncated PRR5 (#1~#4), PRR9#3 and PRR7#3, the corresponding regions were cloned into pENTR/D-TOPO and integrated into pBS-FLAG GW using LR clonase. The truncated PRR5, PRR9, and PRR7 regions are: PRR5#1 (extending from Met of 1 to Phe of 180), PRR5#2 (Met of 1 to Gly of 501), PRR5#3 (Thr of 173 to Pro of 558), PRR5#4 (His of 328 to Pro of 558), PRR7#3 (Trp of 197 to Ser of 727), and PRR9#3 (Trp of 156 to Ser of 468). Note that a Met codon was added at the 5′ terminus of PRR5#3, PRR5#4, PRR7#3, and PRR9#3. The procedures used to generate pBS-PRR5mt-FLAG and pBS-PRR5-VP are described in *Plant Materials and Growth Conditions*, above.

ChIP-Seq Analysis. Eight grams of *PRR5pro:FLAG-PRR5-GFP/prr5* plants were harvested at Zeitgeber time (ZT) 12 and fixed in 300 mL of 1% (vol/vol) formaldehyde under vacuum for 40 min. The reaction was stopped by washing twice with ice-cold 0.3 M glycine. Plants were stored at −80 °C until use. Two grams of frozen plant material were then ground into powder in liquid nitrogen and lysed in 40 mL of nuclear isolation buffer [1 M hexylene glycol, 50 mM Tris·HCl pH 7.5, 10 mM $MgCl_2$, 0.5% Tritone X-100, 5 mM 2-mercaptoethanol, protease inhibitor mixture (P9599; Sigma-Aldrich), 50 μM 26S protease inhibitor MG132 (C2211; Sigma-Aldrich)]. Nuclei were separated by filtration through a 125-μm filter and Miracloth (Calbiochem). Isolated nuclei were suspended in 6 mL of lysis buffer (50 mM Tris·HCl pH 7.5, 100 mM NaCl, 0.1% Triton X-100, 50 μM MG132, and protease inhibitor mixture), and sonicated. Immunoprecipitation of FLAG-PRR5-GFP by anti-GFP antibody, and isolation of ChIP DNA were performed as described previously (2). ChIP DNA and input DNA were processed to generate a DNA library for deep sequencing as described (7). The resulting ChIP and input DNA libraries were sequenced with an Illumina Genome Analyzer (GA) II (Fig. S2).

ChIP-Seq Data Analysis. Basecalls of sequence reads were done by the Illumina GA II pipeline. To map sequence reads on the *Arabidopsis* genome, ChIP and input DNA sequence data in the FASTQ format were analyzed by CLC-bio Genomics Workbench (CLC bio) against the reference genome TAIR9. Peaks significantly appearing in ChIP DNA compared with the input [false-discovery rate (FDR) $q < 10^{-50}$] were annotated as binding loci of PRR5 (a total of 542 loci). Model-based Analysis of ChIP-Seq (MACS 2) was used for validating forward- and reverse-peak distribution (8). The data for MACS2 was drawn by R (www.r-project.org). Peaks from forward and reverse strands were within 200 bp, indicating that DNA fragment sizes in the ChIP library were acceptable, and that PRR5-GFP associates within a very close region of these peaks (Fig. S3). Five-hundred thirty-six genes have PRR5-bound loci within 5 kbp 5′ to corresponding putative transcription start site, 346 genes have bound loci within 5 kbp 3′ from the termination codon, and 170 genes have bound loci within the coding region (Fig. S3). Mapping reads (bam format from CLC-bio data analysis) were visualized with Integrative Genomics Viewer (www.broadinstitute.org/igv).

Microarray Analysis. Samples were harvested from wild-type and PRR5-VP–expressing plants grown under 12-h light/12-h dark conditions at ZT12, and total RNA was prepared from three biological replicates with an RNeasy Plant Mini Kit (Qiagen). The RNA samples were subjected to microarray analyses as described previously (9). Row CEL files were normalized by Robust Multi-array Average algorithm (10) with Bioconductor Simpleaffy package 2.10 in R (www.r-project.org) (11). Genes whose signal intensities were significantly changed (FDR $q < 0.01$) compared with wild-type were identified. Heat maps in Figs. 3 and 4 were generated by R (www.r-project.org), as described previously (12). The detection call for genes ("present", "marginal," and "absent") were used for validation for signal intensity of each gene. If a dataset is called "absent" for all samples, the gene was regarded as

invalidated in the dataset. Accessions numbers of microarray data are National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) GSE3416 for LDHH_ST (Fig. 3*A*) (13), NASCARRAYS-60 for LDHH_SM (Fig. 3*B*) (14), GSE8365 for LL12_LDHH (Fig. 3*C*) (15), NASCARRAYS-108 for LL23_LDHH (Fig. 3*D*) (16), NASCARRAYS-421 for *prr9 prr7 prr5* (Fig. 4*D*) (9), and NASCARRAYS-420 for *PRR5-ox* (Fig. S10). In Fig.3, signal intensities for *At1g02340*, *At1g09350* (*ATGOLS3*), *At3g19270* (*CYP707A4*), *At3g62690* (*ATL5*), *At4g25470* (*DREB1C/CBF2*), *At4g25490* (*DREB1B/CBF1*), *At4g36700*, *At5g15940*, and *At5g54470* (*BBX29*) were invalid in the LDHH_ST dataset (Fig. 4*A*). Signal intensities for *At1g18330*, *At1g69570* (*CDF5*), *At2g46790* (*PRR9*), *CYP707A4*, *DREB1B/CBF1*, *At4g36700*, and *At5g15940* were invalid in the LDHH_SM dataset (Fig. 4*B*). Signal intensities for *ATGOLS3*, *CDF5*, *CYP707A4*, *At3g55580* (*RCC1*), *DREB1B/CBF1*, *At5g15940*, and *BBX29* were invalid in the LL12_LDHH dataset (Fig. 4*C*). Signal intensities for *ATGOLS3*, *At1g18330*, *CDF5*, *PRR9*, *CYP707A4*, *RCC1*, *At3g62690* (*ATL5*), *DREB1C/CBF2*, *DREB1B/CBF1*, *At5g15490*, and *BBX29* were invalid in the LL23_LDHH dataset (Fig. 4*D*).

RT-Quantitative PCR. Seedlings were grown on MS plate containing 2% sucrose, under 12-h light and 12-h dark conditions for 18 d and released into constant light of 70 $\mu mol \cdot s^{-1} \cdot m^{-2}$. RNA was isolated with an RNeasy Plant Mini Kit. RT-quantitative PCR (RT-qPCR) was done as described previously (2) using Applied Biosystems StepOnePlus Real-Time PCR system (Life Technologies). Primer sets used in this study was shown in Dataset S5.

Flowering-Time Measurement. Flowering time was counted as described previously (17).

Elucidation of Enriched DNA Sequences in ChIP-DNA. DNA sequences of 500-bp length around peak reads near the PRR5 direct targets were sorted and identified with the MEME-ChIP open Web tool which finds common patterns in input DNA sequences (meme.sdsc.edu/meme/intro.html).

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16. Edwards KD, et al. (2006) FLOWERING LOCUS C mediates natural variation in the high-temperature response of the *Arabidopsis* circadian clock. *Plant Cell* 18:639–650.
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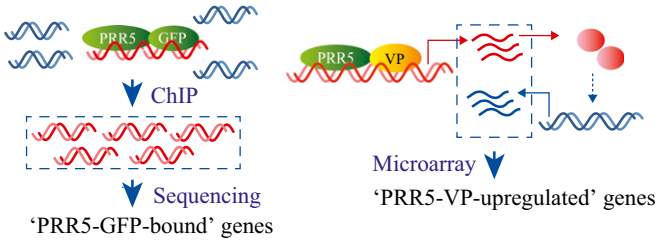


Fig. S1. Flowchart for screening the direct target genes of PRR5. ChIP-seq can identify genes bound by PRR5-GFP protein at the genomic scale (PRR5-bound genes) (*Left*), but the bound genes may not necessarily be the genes regulated by PRR5. Up-regulated genes in PRR5-VP–expressing plants compared with the wild-type can be referred to as "PRR5-VP up-regulated" genes (*Right*). The PRR5-VP up-regulated gene set not only include its direct target genes (wavy lines in red) but also contains genes up-regulated indirectly (wavy lines in blue). The comparison of PRR5-bound genes and PRR5-VP up-regulated genes defines the group of "PRR5 direct-target" genes.

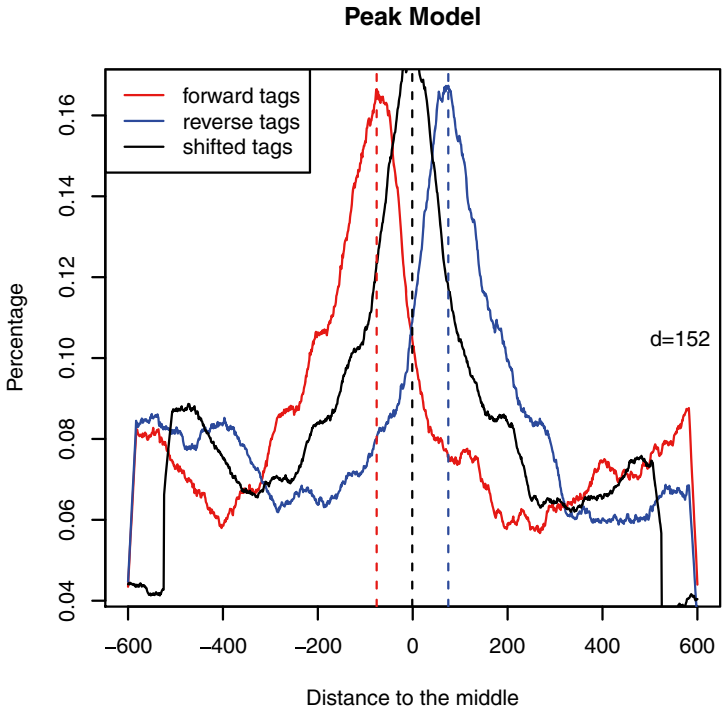
Sequence reads of ChIP DNA

	Count	Average length	Total bases
Reads	25,649,423	35.82	918,860,418
Matched	14,512,038	35.91	521,181,373
Not matched	11,137,385	35.71	397,679,045
References	7	17,095,392	119,667,750

Sequence reads of input DNA

	Count	Average length	Total bases
Reads	7,351,327	35.71	262,497,123
Matched	7,068,875	35.73	252,604,228
Not matched	282,452	35.03	9,892,895
References	7	17,095,392	119,667,750

Fig. S2. Sequence read numbers of ChIP and input DNA libraries. Sequence read numbers of DNA libraries indicate that about 97% of reads were successfully read to 36 bp (maximal length for the GAII platform). TAIR9 was used as the reference genome ("References," including five chromosomes, a mitochondrial genome, and a chloroplast genome). "Matched" and "Not Matched" indicate numbers of reads matched or not matched to the reference genome.



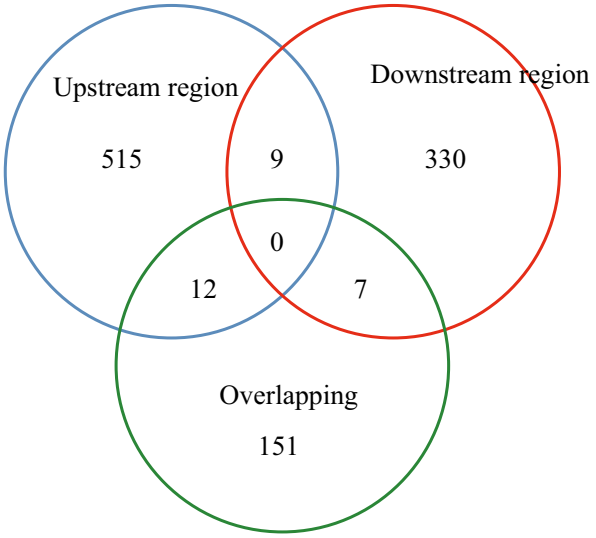


Fig. S3. Peak model for ChIP DNA library and location of the peak relative to the gene. The 5′ ends of sequence reads in all peaks were aligned by the center of forward and reverse strands (*Left*). "d" indicates the distance between the modes of the forward and reverse peaks. Venn diagram for peak location relative to the gene-model (TAIR9) (*Right*). Upstream, downstream, and overlapping indicates a gene having peaks for reads in ChIP within 5 kbp of 5′ side of relative to its TSS, a gene having peaks for reads in ChIP within 5 kbp of 3′ side of relative to its stop codon, and a gene having peaks within the coding sequence, respectively.

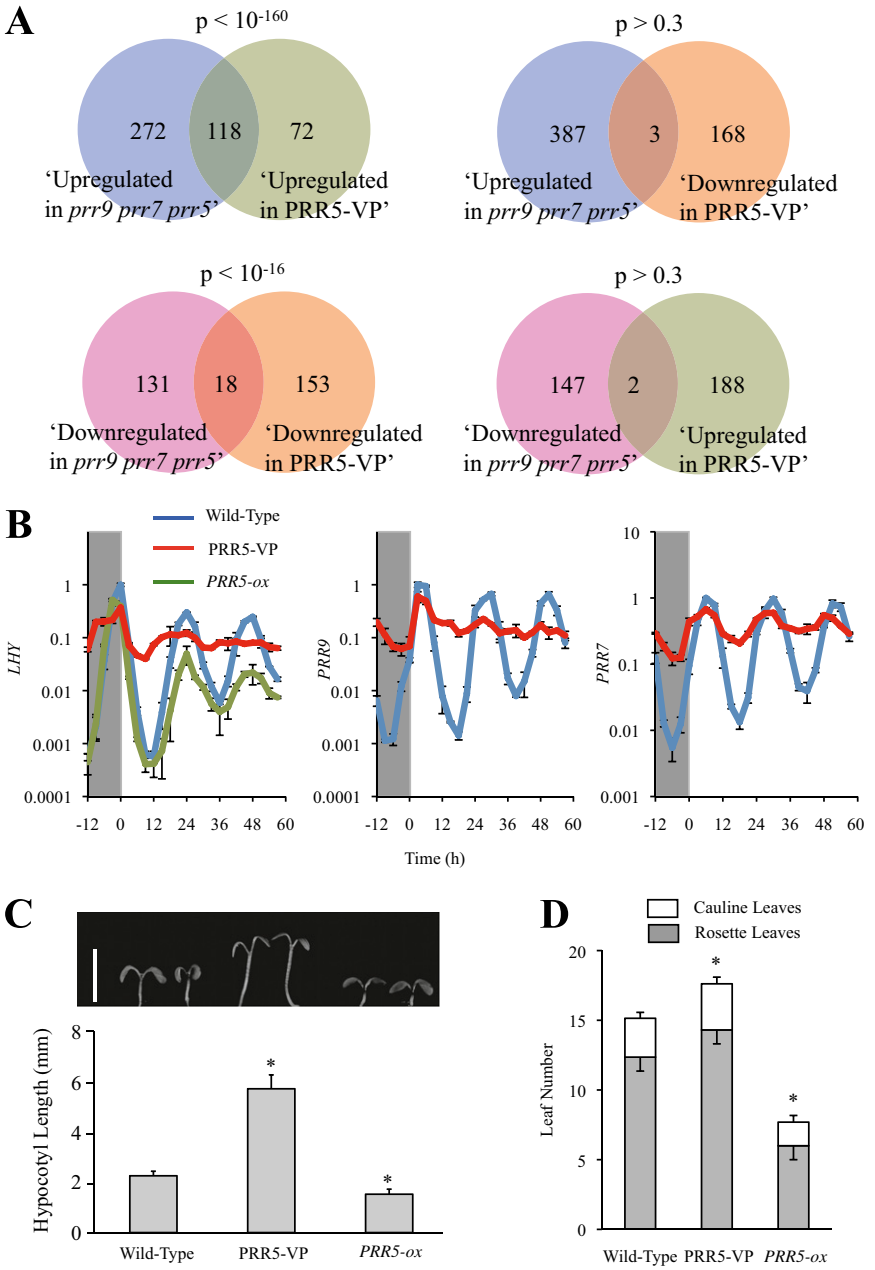


Fig. S4. Gene expression, hypocotyl length, and flowering time in PRR5-VP–expressing plants. (*A*) Comparisons of genome-wide gene expression in PRR5-VP–expressing plants and the *prr9 prr7 prr5* triple mutant. Fisher's exact test *P* values were obtained between two genes groups in which gene expression was significantly different in PRR5-VP–expressing plants or *prr9 prr7 prr5* compared with the wild-type (FDR q < 0.01). (*B*) mRNA expression of *LHY*, *PRR9*, and *PRR7* in PRR5-VP plants under 12-h light/12-h dark conditions and following constant-light conditions. Time indicates hours after the start of illumination. Gray areas are dark periods. Error bars indicate the SD of three biological replicates. (*C*) Hypocotyl length of PRR5-VP plants under 12-h light/12-h dark conditions. Typical seedlings are shown (*Upper*). (Scale bar, 5 mm.) Error bars indicate the SD of 20 biological replicates. (*D*) Flowering time of PRR5-VP plants under 16-h light/8-h dark conditions. Rosette and cauline leaves were counted when flower stems reached 1-cm high. Error bars indicate the SD of 12 plants. Asterisks in *C* and *D* indicate statistical differences compared with the wild-type (Student *t* test; *P* < 0.01). Note that similar results from *B*–*D* were obtained from an independent transgenic line of PRR5-VP–expressing plants.

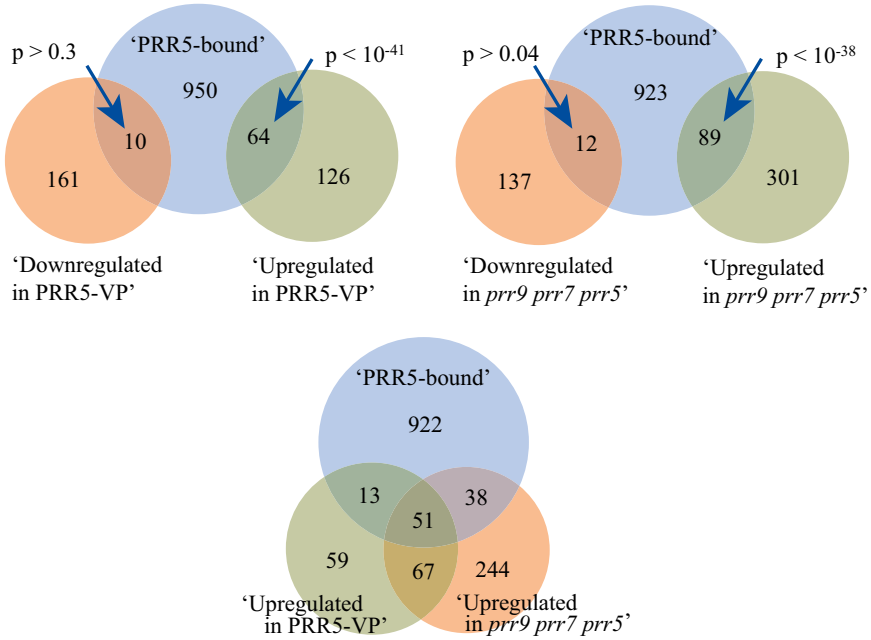


Fig. S5. Overlap between PRR5-bound genes and genes whose expression were significantly changed in PRR5-VP–expressing plants or *prr9 prr7 prr5*. Fisher's exact tests between PRR5-bound genes and genes whose expression were significantly increased or decreased in PRR5-VP–expressing plants or *prr9 prr7 prr5* triple mutants compared with wild-type (FDR $q < 0.01$) was conducted. The overlap between PRR5-bound genes and up-regulated genes was significant ($P < 10^{-41}$ or 10^{-38}), whereas that between PRR5-bound genes and down-regulated genes was not ($P > 0.01$).

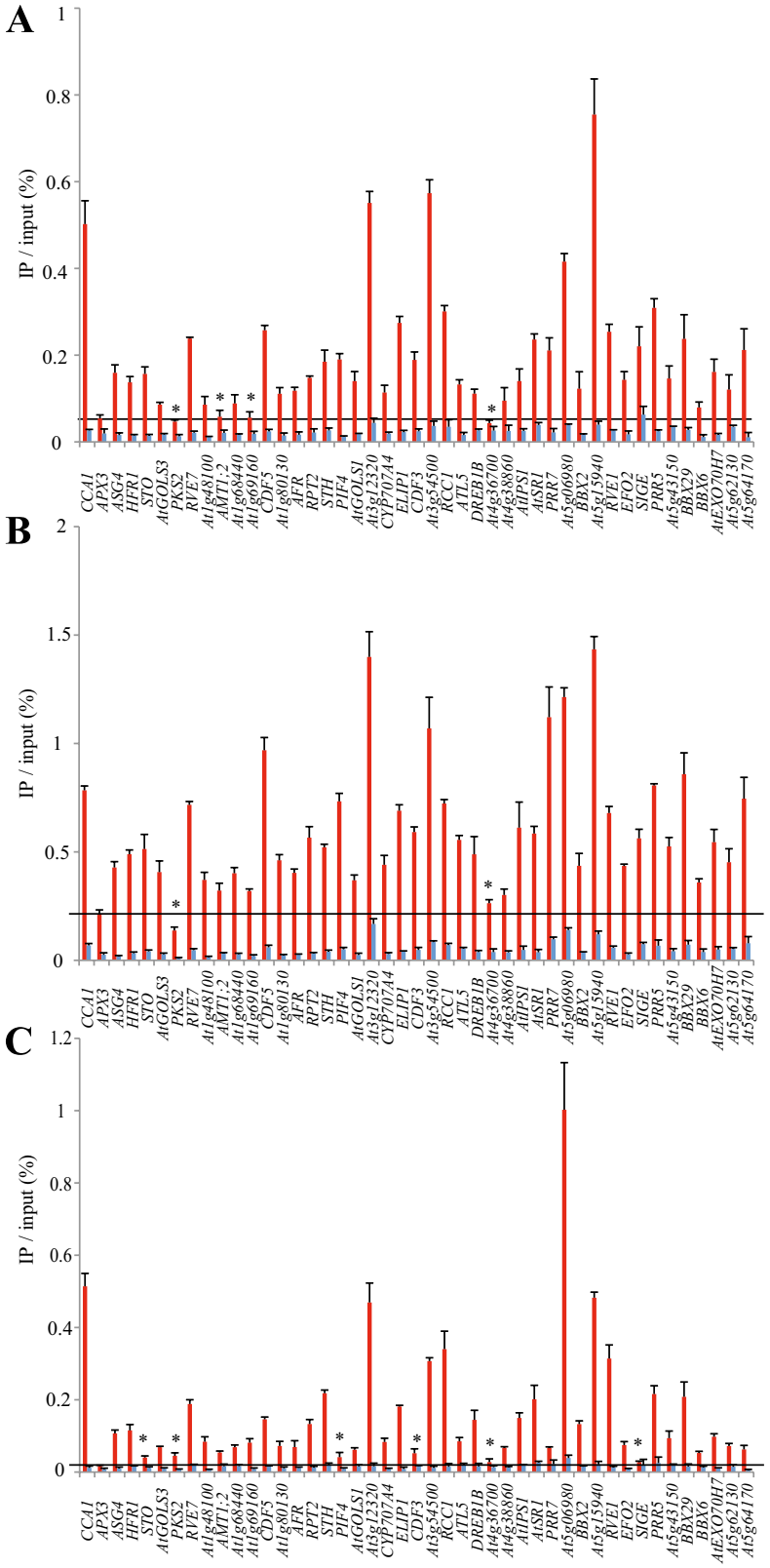


Fig. S6. ChIP-qPCR for target genes of PRR5. ChIP-qPCR for targets of PRR5 were performed for *PRR5pro:FLAG-PRR5-GFP/prr5* (*A*), *PRR7pro:FLAG-PRR7-GFP/prr7* (*B*), and *PRR9pro:FLAG-PRR9-GFP/prr9* (*C*). Percentages of amplicons coimmunoprecipitated with anti-GFP antibody (ChIP DNA) relative to input DNA are plotted. Error bars indicate the SD of three technical replicates. Red bars indicate ChIP enrichment when each PRR is expressed (ZT10 for *A* and *B*, and ZT4 for *C*). Blue bars indicate ChIP enrichment at ZT22, when three PRRs are not expressed. Asterisks indicate nonsignificant enrichment of the corresponding locus compared with the *ASCORBATE PEROXIDASE 3* (*APX3*) locus ($P > 0.01$). The horizontal lines indicate ChIP enrichment of *APX3* locus (negative control) when each PRR is expressed.

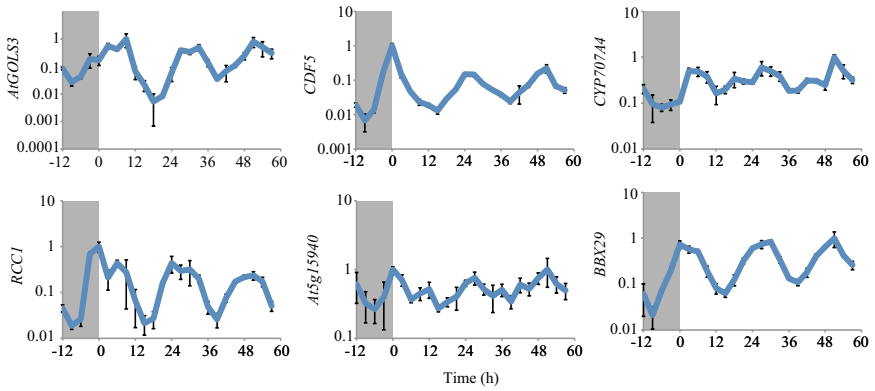


Fig. S7. Expression of "invalid genes in DIURNAL" by RT-qPCR. Plants were grown under 12-h light/12-h dark cycles for 18 d and transferred into constant light conditions. Gene expression was normalized with *APX3* mRNA. Error bars indicate the SD of three biological replicates. A 0 h indicates the time at the beginning of illumination. The gray area is the dark period. Signal intensities of *AtGOLS3*, *CDF5*, *RCC1*, and *BBX29* are invalid in constant-light conditions (LL12_LDHH and LL23_LDHH) in DIURNAL. Signal intensities of *CYP707A4* and *At5g15940* are invalid in constant light conditions and light and dark conditions (LDHH_ST and LDHH_SM). Expression of *DREB1B/CBF1* was also invalid in DIURNAL, but is already known to be under clock control (1).

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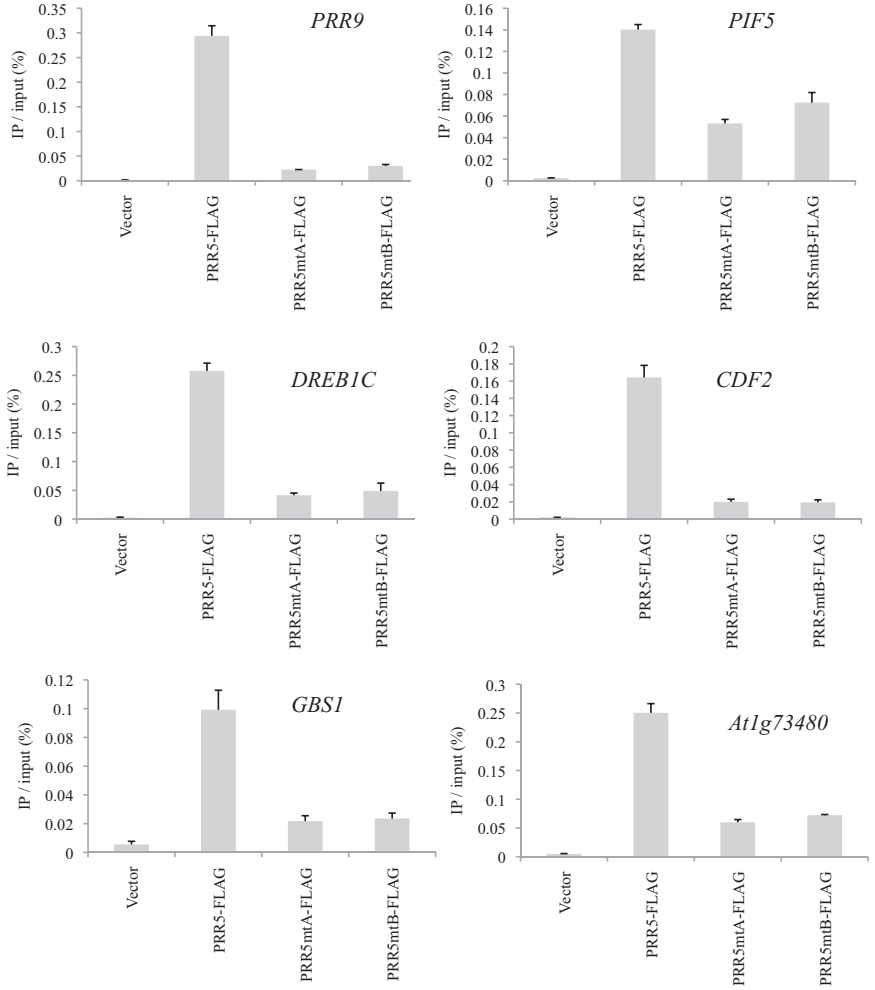

Fig. S8. PRR5 associates with direct target genes although it is CCT. ChIP-qPCR assays for representatives of the direct target genes in transgenic plants expressing PRR5-FLAG or PRR5mt-FLAG. Percentages of amplicons coimmunoprecipitated with anti-FLAG antibody (ChIP DNA) relative to input DNA are plotted. Error bars indicate the SD of three technical replicates.

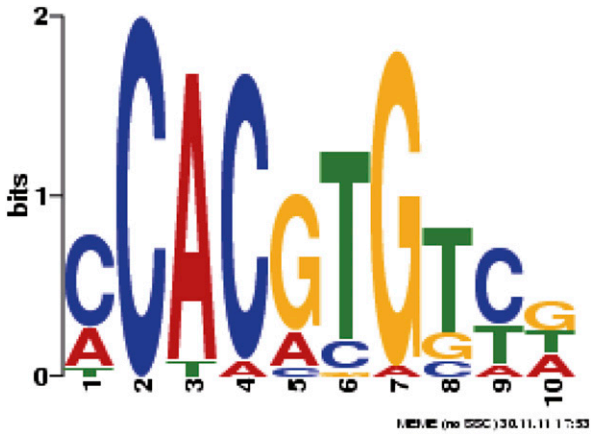

Fig. S9. Enriched sequences in ChIP-DNA. Enriched sequences in ChIP-DNA were searched using a motif-finding software MEME-ChIP open web tool. CACGTG elements were found in 37 sites ($P < 2.4e^{-18}$) among 64 genes.

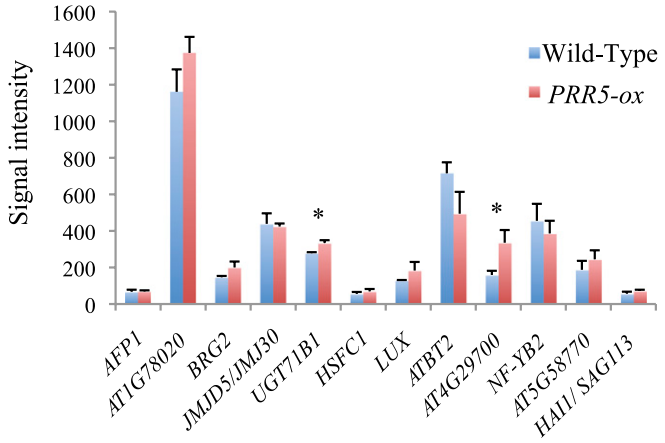

Fig. S10. Expression of 12 potential activated targets of PRR5. Gene expression data were derived from microarray dataset of NASCARRAYS-420. Error bars indicate the SD of three biological replicates. An asterisk indicates statistical up-regulation of the corresponding gene in *PRR5-ox* compared with the wild-type ($P < 0.01$).

Dataset S1. PRR5-bound genes

Dataset S1

The peaks of sequence reads statistically enriched in ChIP DNA compared with input DNA (FDR q < $10E^{-50}$) were annotated using reference genome *Arabidopsis* TAIR9.

Dataset S2. PRR5-VP up-regulated genes

Dataset S2

Up-regulated genes in PRR5-VP–expressing plants compared with the wild-type (FDR q < 0.01) were annotated using TAIR9. "fc" indicates fold-change of GeneChip signal intensity between PRR5-VP and wild-type.

Dataset S3. PRR5-direct-target genes

Dataset S3

The common genes between PRR5-bound genes and PRR5-VP up-regulated genes are listed.

Dataset S4. Shared targets of PRR5 and TOC1

Dataset S4

The common genes between PRR5-targets and TOC1-targets are listed.

Dataset S5. Primers used for ChIP-qPCR and RT-qPCR

Dataset S5

TSS indicates transcription start site according to TAIR9.