Supporting Information

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SI Materials and Methods

Promoter Analysis. Natural variation [i.e., single nucleotide polymorphisms (SNPs)] around the HSE repeat was analyzed using the sequencing data of ∼500 *Arabidopsis* accessions at 1001genomes.org. Promoter sequences from other species were downloaded from GBrowse at Phytozome (www.phytozome.net). The following *PRR7* loci (with database IDs) were identified using BLASTP: *Arabidopsis lyrata* (939656), *Capsella rubella* (Carubv10000314m.g), *Thellungiella salsuginea* (*T. halophila*; Thhalv10012789m.g), and *Brassica rapa* (Bra009565). The sequences were processed and aligned in Geneious R6 (Biomatters; www.geneious.com).

Luciferase Imaging. Bioluminescence of single seedlings was monitored as described previously (1) or with a PIXIS 2048B back-illuminated digital CCD system (Princeton Instruments) with a Distagon T 28-mm large-format lens F/2.0 25-mm F/1.4 1" (Megapixel), at 15- to 25-min acquisitions every 1–2.5 h. For compensation assays, seedlings were entrained under 12L:12D at 22 °C and transferred to LL and 27 °C at lights on (ZT0). For salt stress, the 1× MS + 3% sucrose plates were supplemented with 100 mM NaCl. Circadian period estimates were based on the data recorded after the first 24–36 h under constant conditions by FFT-NLLS analysis in Biological Rhythms Analysis Software System (BRASS 3.0) (2). In gating assays, seedlings on 1× MS + 3% sucrose plates were sprayed with 5 M NaCl at the indicated times on the first day under LL, and 15-min acquisitions were performed every 1 h. Imaging experiments were performed two or three times, yielding similar results.

Gene Expression Analysis. Seedlings were entrained in a growth chamber (Percival) under a 12L:12D cycle at 22 °C and collected at the 1-wk-old stage. Whole seedlings were harvested, and total RNA extraction, cDNA synthesis, and qPCR were performed as described previously (1). For qPCR analysis, primer efficiencies (40–100%) were taken into account using the features of Bio-Rad CFX Manager 3.1 software. The qPCR data represent values from two or three biological replicates, each with three technical replicates. The graphs illustrate the expression from one biological replicate, with error bars denoting the SD or SEM of the biological replicates.

1. Helfer A, et al. (2011) *LUX ARRHYTHMO* encodes a nighttime repressor of circadian gene expression in the *Arabidopsis* core clock. *Curr Biol* 21(2):126–133.

2. Plautz JD, et al. (1997) Quantitative analysis of *Drosophila* period gene transcription in living animals. *J Biol Rhythms* 12(3):204–217.

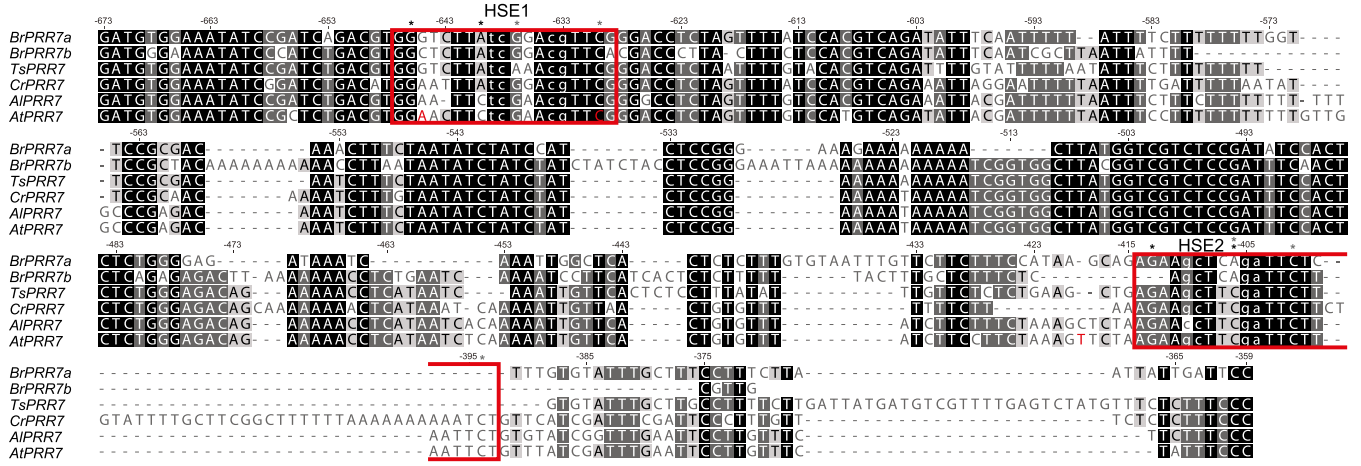

Fig. S1. The inverted HSE repeats in the *PRR7* promoter (HSE1 and HSE2; boxes) are conserved among relatives of *Arabidopsis*. The region shown is −673 bp to −328 bp (relative to translation ATG). Al, *Arabidopsis lyrata*; At, *A. thaliana*; Br, *Brassica rapa*; Cr, *Capsella rubella*; Ts, *Thellungiella salsuginea*. The mutation constructs (HSEmutA and HSEmutB) were mutated as indicated (*; black symbols for mutA, gray for mutB), with G or C replaced with T. The SNPs at positions −645, −630, and −420 bp (C to G, A to T/G, and T to C, respectively) are marked in red. Shading represents degree of similarity (≥70%).

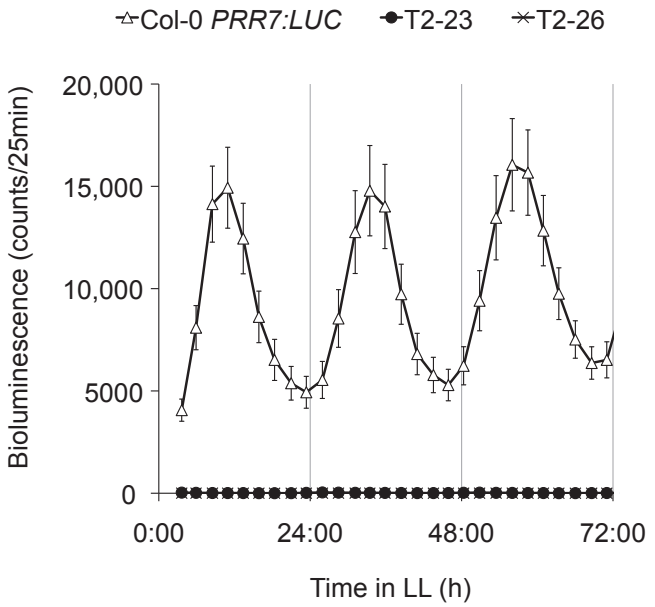

Fig. S2. Bioluminescence of WT *PRR7:LUC* compared with late-flowering *HsfB2b-ox* lines. Seedlings were entrained under a 12L:12D cycle for 1 wk before being transferred to constant light (LL). Two independent *HsfB2b-ox* T2 lines (#23 and #26) are shown. Error bars represent the SEM of technical replicates; $n = 10–15$.

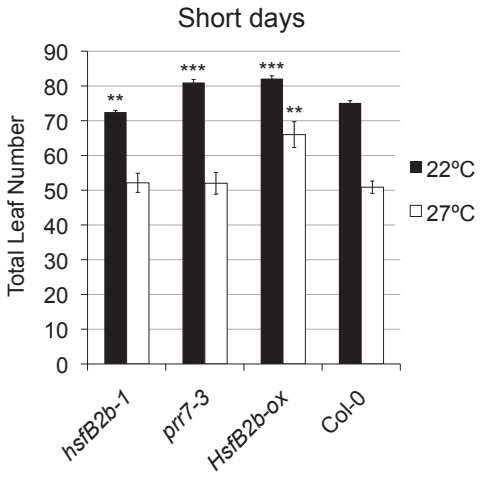

Fig. S3. Late flowering of *HsfB2b-ox* under short days. Plants were planted in soil and grown at 22 °C under 8L:16D short days for 2 wk before being transferred to 27 °C. The total leaf number was determined at the time of bolting. The experiment was repeated twice, yielding similar results ($n > 8$). Error bars represent SEM.

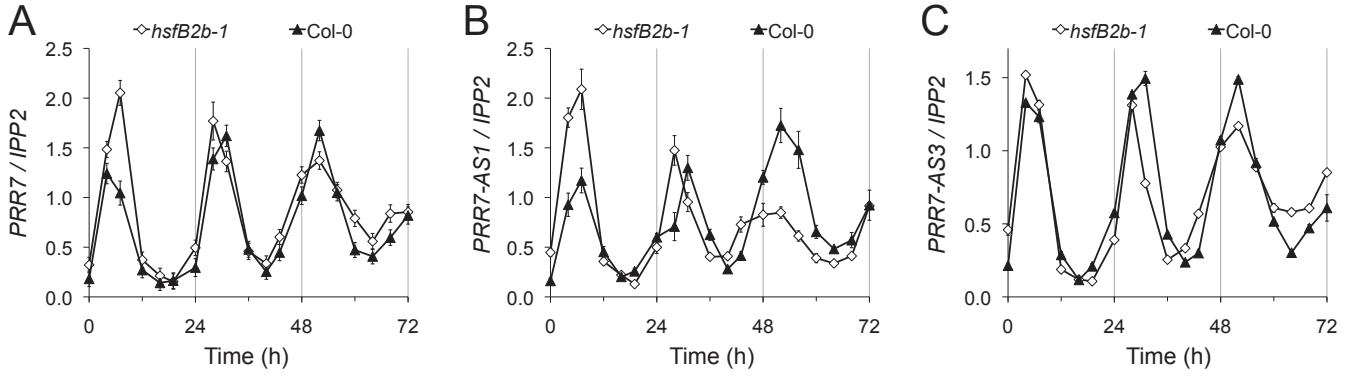

Fig. S4. *PRR7* transcript levels in the *hsfB2b-1* mutant background. Samples of whole seedlings were obtained under LL every 4 h after entrainment in a 12L:12D cycle for 1 wk. Three transcripts were measured: *PRR7* (*A*), *PRR7-AS1* (*B*), and *PRR7-AS3* (*C*). Error bars represent SEM of biological replicates.

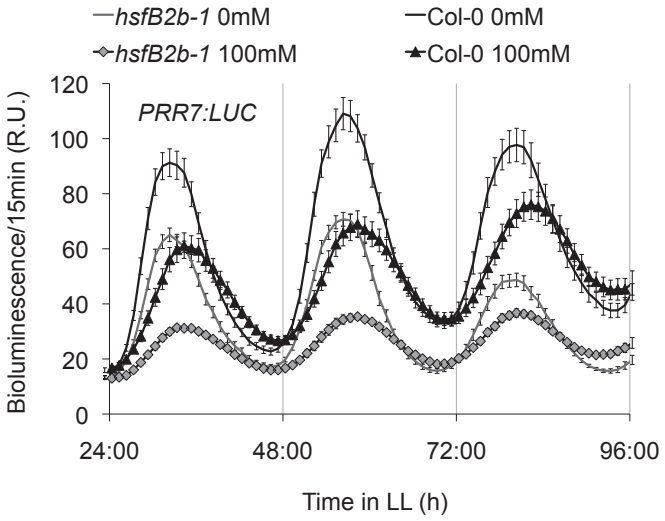

Fig. S5. Luminescence profiles of *PRR7:LUC* in *hsfB2b-1* and Col-0 on 100mM NaCl at 22 °C. Seedlings were entrained in 12L:12D for 1 wk before being transferred to LL and NaCl-supplemented medium at ZT0. Data are the same as in Fig. 4.

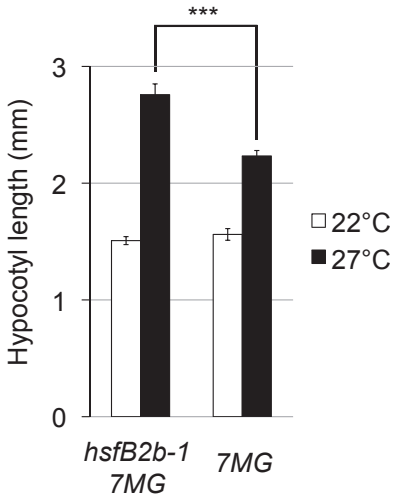

Fig. S6. Hypocotyl length of 6-d-old *hsfB2b-1 7MG* and *7MG* seedlings. The seedlings were grown under short-day photoperiods at room temperature (22 °C), or under warm short days at 27 °C. For the latter, seedlings were grown at 22 °C for the first 3 d and then transferred to 27 °C. Error bars represent SEM of technical replicates; $n > 18$.

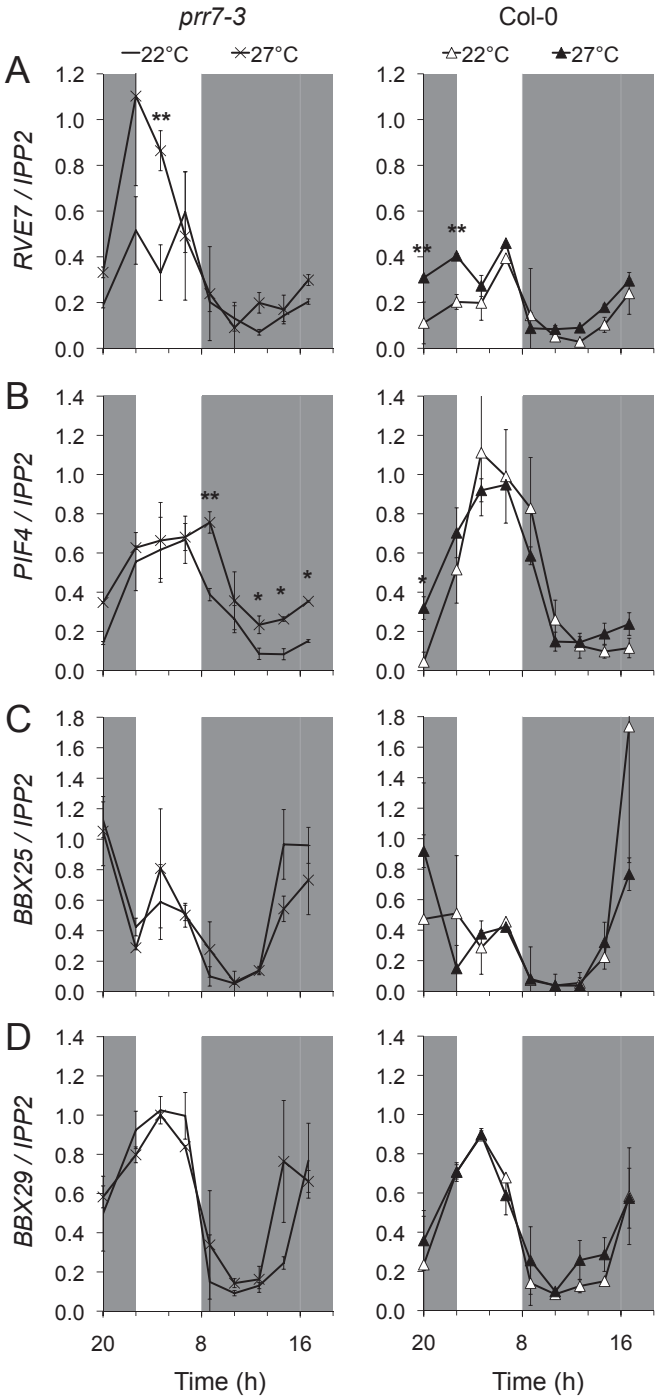

Fig. S7. Transcript levels, measured by qPCR, for *prr7-3* and Col-0, under 22 °C and 27 °C short days. (*A*) *RVE7*. (*B*) *PIF4*. (*C*) *BBX25*. (*D*) *BBX29*. Samples were prepared as in Fig. 5.

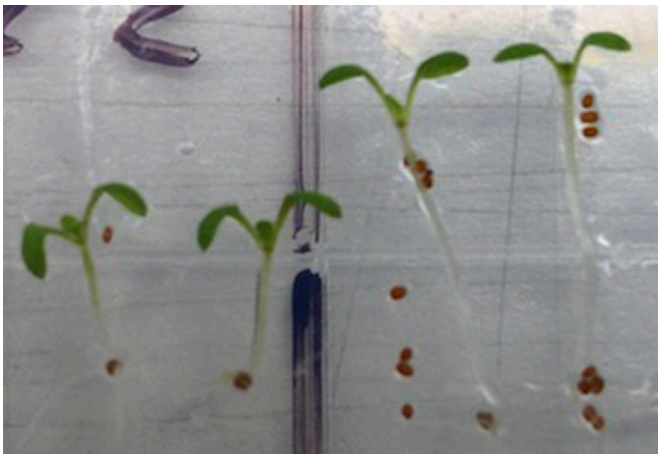

Fig. S8. Long hypocotyls of *35S::BBX29-ox*. The seedlings were grown under 22 °C short days for 1 wk. The control is shown on the left.

Table S1. *PRR7* target genes that have advanced phase in short days compared with long days (DIURNAL) (1)

Shift, h	Gene	AGI
1	*AHA1*	At2g18960
1	*bZIP59*	At2g31370
1	*STH*	At2g31380
1	*NF-YA4*	At2g34720
1	*CCA1*	At2g46830
1	*AFP4*	At3g02140
1	*AtDi19-4*	At3g06760
1	*SPD1*	At3g10420
1	*HYH*	At3g17609
1	*AtGH3.10*	At4g03400
1	*FER1*	At5g01600
1	*CIPK14*	At5g01820
1	*PRR7*	At5g02810
2	*PPR-like*	At1g18900
2	*JAZ6*	At1g72450
2	*MPK15*	At1g73670
2	*RBR1*	At3g12280
2	—	At3g12300
2	—	At3g12320
2	*LNK3*	At3g54500
2	*uORF*	At5g15948
2	*RVE2*	At5g37260
2	*LHCB3*	At5g54270
2	*ABA1*	At5g67030
3	*STO*	At1g06040
3	*RVE7*	At1g18330
3	*PIF4*	At2g43010
3	*PSI-P*	At2g46820
3	—	At3g46630
3	*BT2*	At3g48360
3	—	At3g56360
3	*PIF5*	At3g59060
3	*ATL5*	At3g62690
3	*LHCB5*	At4g10340
3	*BBX29*	At5g54470
3	*EXO70H7*	At5g59730
4	—	At1g06050
4	*GLX1*	At1g11840
4	—	At3g12290
5	*PIFI*	At3g15840
5	*LUX*	At3g46640
5	*FER3*	At3g56090
5	—	At5g09620
5	*OCP3*	At5g11270
6	*SCPL49*	At3g10410
7	*SIG4*	At5g13730
9	*HY5*	At5g11260

1. Michael TP, et al. (2008) Network discovery pipeline elucidates conserved time-of-day–specific *cis*-regulatory modules. *PLoS Genet* 4(2):e14.

Table S2. Primers used in this study

Gene, application(s)	Forward (5′-3′)	Reverse (5′-3′)
HsfA1b, CDS, TOPO	cacc ATG GAA TCG GTT CCC GAA T	TTA TTT CCT CTG TGC TTC TGA GG
HsfA3, CDS, TOPO	cacc ATG AGC CCA AAA AAA GAT GC	CTA AGG ATC ATT CAT TGG CG
HsfA6a, CDS, TOPO	cacc ATG GAT TAT AAC CTT CCA	TAT AAA ATG TTC CAC TAA ATC AC
hsfB2b-1, genotyping	CTG AAT CGG CAA GTG TTT TTC	AAG TAG TGG ATG TGG TGC TGG
HsfB2b 3′ UTR, qPCR	TCC AGA TCG TTG GGA GTT TT	GCC ATA GCA GGC TGA GAG AT
HsfB2b, CDS, TOPO	cacc ATG CCG GGG GAA CAA ACC	TTT TCC GAG TTC AAG CCA CG
ProHsfB2b, TOPO	cacc TTT TAG AGA AAT ATA AGA TAA CCA CCA	GAG TTA TAG ATC AAA AAT CTA AAC TTT CC
GFP, qPCR	CTG CTG CCC GAC AAC CA	TGT GAT CGC GCT TCT CGT T
IPP2, qPCR	GTA TGA GTT GCT TCT CCA GCA AAG	GAG GAT GGC TGC AAC AAG TGT
HSE1A, mutagenesis	ATC CGC TCT GAC GTG TAA CTT TTC GAA CGT TCG GGA C	GTC CCG AAC GTT CGA AAA GTT ACA CGT CAG AGC GGA T
HSE1B, mutagenesis	CTG ACG TGG AAC TTC TCT AAC GTT TGG GAC CTC TAG TTT TGT	ACA AAA CTA GAG GTC CCA AAC GTT AGA GAA GTT CCA CGT CAG
HSE2A, mutagenesis	CTG TGT TTA TCT TCC TTC TAA AGT TCT AAT AAG CTT TGA TTC TTA ATT CTG TTT ATC GAT TTG AAT	ATT CAA ATC GAT AAA CAG AAT TAA GAA TCA AAG CTT ATT AGA ACT TTA GAA GGA AGA TAA ACA CAG
HSE2B, mutagenesis	GTG TTT ATC TTC CTT CTA AAG TTC TAA GAA GCT TTG ATT TTT AAT TTT GTT TAT CGA TTT GAA TTC CTT GTT TCT	AGA AAC AAG GAA TTC AAA TCG ATA AAC AAA ATT AAA AAT CAA AGC TTC TTA GAA CTT TAG AAG GAA GAT AAA CAC
HSE-SNP-645, mutagenesis	TCC GCT CTG ACG TGG TAC TTC TCG AAC GTT C	GAA CGT TCG AGA AGT ACC ACG TCA GAG CGG A
HSE-SNP-420, mutagenesis	CTG TGT TTA TCT TCC TTC TAA AGC TCT AAG AAG CTT CGA TTC TTA AT	ATT AAG AAT CGA AGC TTC TTA GAG CTT TAG AAG GAA GAT AAA CAC AG
PRR7, qPCR	TTG GAG AAG ATG CCA AAG TTC T	GTT CCG CTC TCA CTT CCA CTA C
PRR7-AS1, qPCR	CGG TCT CTT GTG CAA GAT TT	AGA TGA TGA CAG GGA TGT TCC
PRR7-AS3, qPCR	TCC CTG TCA TCA GTG AGT TCT T	GCA TCT ACT TTC CAA CAT ACG C
proPRR7, 2kb, TOPO	cacc CTT TCT CTG CTG CAA TGA CAT TGA GAC TTG	CAC ACC AAC TCT GCT TCG CTG AAT TC
proPRR7, -673/-328bp, TOPO	cacc GAT GTG GAA ATA TCC GCT CTG ACG TGG AAC	GAA AAA TGG ATC TAG CAC AAT CAG CTA ACG
proUBQ10, Gateway B4/B1R	ggg gac aac ttt gta tag aaa agt tgg a AAG CTT CGA CGA GTC AGT AAT AA	ggg gac tgc ttt ttt gta caa act tgt ACT AGT CTG TTA ATC AGA AAA ACT CAG A
BBX25, qPCR	CTG CGA CAT CTG CCT TGA GAA G	AGA GCG AGT ATT TGG CGC ATG G
BBX28, qPCR	CAG ATC TTC TCT GCT CCG ATG ATG	GCG CCG TTC ATT CTG ACG ATA C
BBX29, qPCR	AAC CTC TCC GAT GAG GAG AAC C	ATC TTG ACA AAC CTT CGT CTC TCG
HY5, qPCR	CAC CAT GCA GGA ACA AGC GAC TAG CT	TCA AAG GCT TGC ATC AGC ATT AG
HYH, qPCR	TCA ACG GGA TAT AGT GGA AAA GA	TGG TTG GCT TAT AAA AGA ACA CG
PIF4, qPCR	TCT CCG ACC GGT TTG CTA GA	CGC GGC CTG CAT GTG T
RVE7, qPCR	CAG AAA TCT TCG GTG GAC GGA	TGA AAC GCT TTC TTG CTG CA

Overhangs for cloning are in lower-case type.