Supporting Information

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SI Materials and Methods

Hypocotyl Assay. Seeds were cold-treated at 4 °C for 3 d, sown on 1/2 strength Murashige and Skoog medium minus sucrose plates, and then were exposed to continuous white light for 12 h to induce uniform germination. The plates were transferred to continuous blue (470 nm) or red (670 nm) light at indicated fluence rates in a Percival E30 LED color light chamber (Percival Scientific) at 22 °C for 5 d.

β-Glucuronidase Staining. Histochemical β-glucuronidase (GUS) assays in stably transformed lines of *Arabidopsis* were performed as described (1) with minor alterations. Seedlings were immersed in GUS staining solution: 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 0.5% Triton X-100, 0.1% Tween 20, 10 mM EDTA (pH 8.0), and 2 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (Chemica Alta Ltd). Incubation was carried out at 37 °C until blue coloration appeared (usually between 16 and 24 h) before being cleared of chlorophyll by a series of 50–70% (vol/vol) ethanol solutions. The GUS-stained seedlings were photographed using a stereo microscope (Nikon SMZ1500).

Subcellular Localization of Protein arginine methyltransferase-GFP. Full-length Protein arginine methyltransferase 5 (*PRMT5*) cDNA was generated by PCR amplification (primers are given in Table S3) and fused to GFP coding sequences controlled by the cassava vein mosaic virus promoter (2). *Arabidopsis* mesophyll protoplasts were isolated from mature leaves of the wild-type plants and transfected with the PRMT5-GFP construct as described (3). Protoplasts were incubated further overnight at 22 °C under dim light and examined with an Axioplan2 fluorescence microscope (Carl Zeiss).

1. Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 6:3901–3907.
2. Verdaguer B, de Kochko A, Beachy RN, Fauquet C (1996) Isolation and expression in transgenic tobacco and rice plants, of the cassava vein mosaic virus (CVMV) promoter. *Plant Mol Biol* 31:1129–1139.
3. Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc Natl Acad Sci USA* 97:2940–2945.

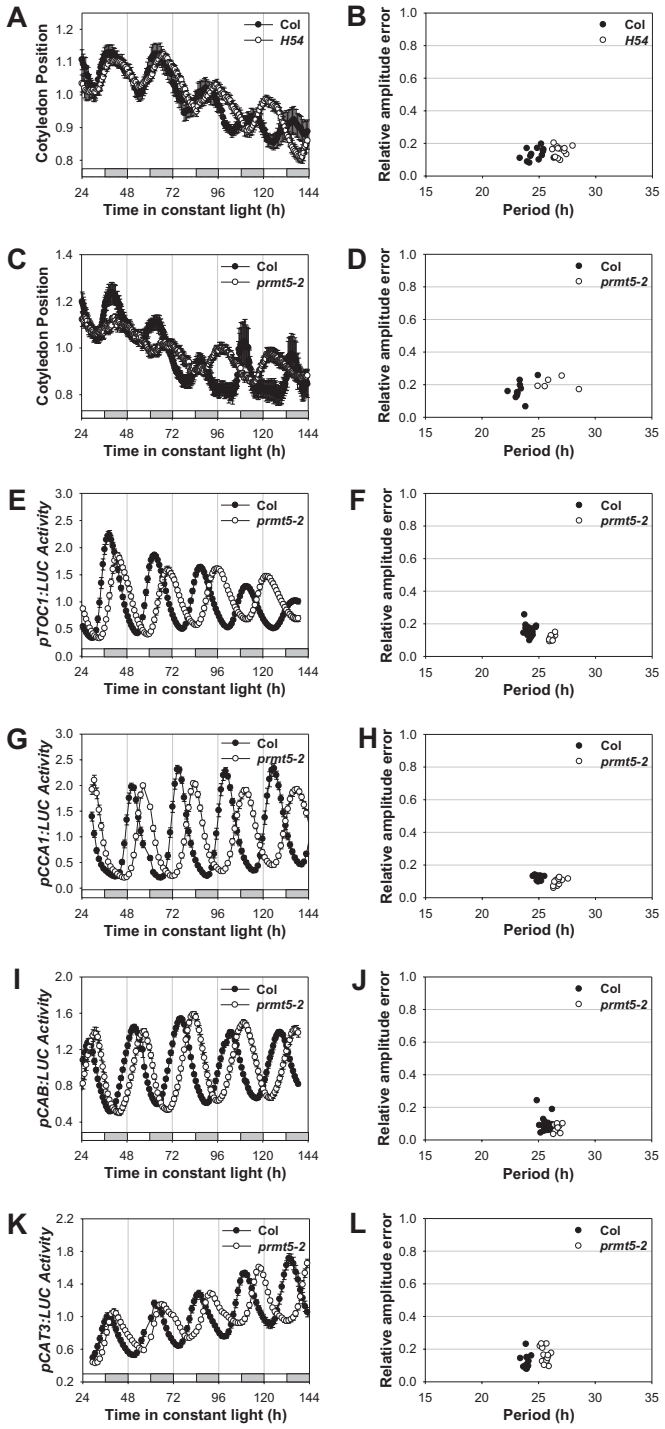

Fig. S1. *prmt5* mutations lengthen the period of multiple rhythms. Seedlings of the indicated genotypes were entrained to photocycles [light-dark (LD): 12/12 h) for 6 (*A*–*D*) or 7 (*E*–*L*) d before release into continuous light (LL). Average traces (mean ± SEM; n = 5–13) of cotyledon movement of wild type (Columbia, Col) versus the *prmt5-54* mutation (*H54*; *A* and *B*) or *prmt5-2* (*C* and *D*) or of LUC activity (mean ± SEM; n = 10–24) of transcriptional fusions of firefly Luciferase (LUC) to the Timing of CAB expression 1 promoter (*pTOC1:LUC*) (*E*), to the Circadian clock-associated 1 promoter (*pCCA1:LUC*) (*G*), to the Chlorophyll a/b Binding protein 2 promoter (*pCAB:LUC*) (*I*), or to the Catalase 3 promoter (*pCAT3:LUC*) (*K*) expression in *prmt5-2* and period versus relative amplitude error plots (*B*, *D*, *F*, *H*, *J*, and *L*) show the long period of *prmt5-54* (*H54*; *A* and *B*) and of *prmt5-2* (*C*–*L*). The white and gray bars in *A*, *C*, *E*, *G*, *I*, and *K* indicate subjective day and night, respectively.

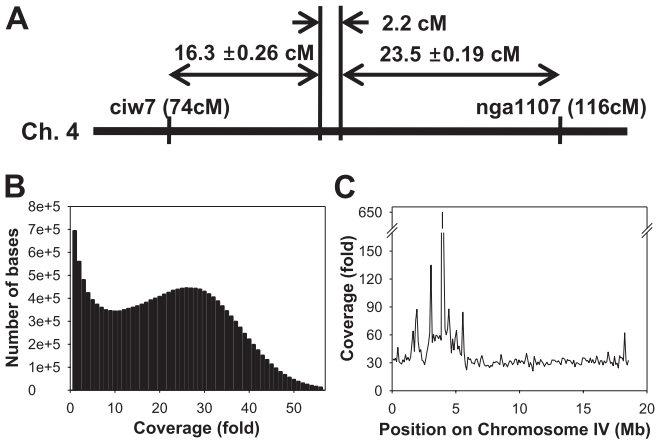

Fig. S2. Low-resolution mapping and resequencing of the *H54* mutation indicate it is a lesion in *PRMT5*. (*A*) Bulked segregant analysis maps *H54* to between 74 and 116 cM on chromosome 4. (*B*) Short reads were mapped to the *Arabidopsis* Columbia 0 (Col-0) reference genome allowing both unique and random (up to 10 hits in the genome) matches for an average 30-fold coverage of the genome. (*C*) Sequence coverage in sliding 500-bp windows across chromosome 4 shows that coverage is even except at highly repetitive regions such as the centromere.

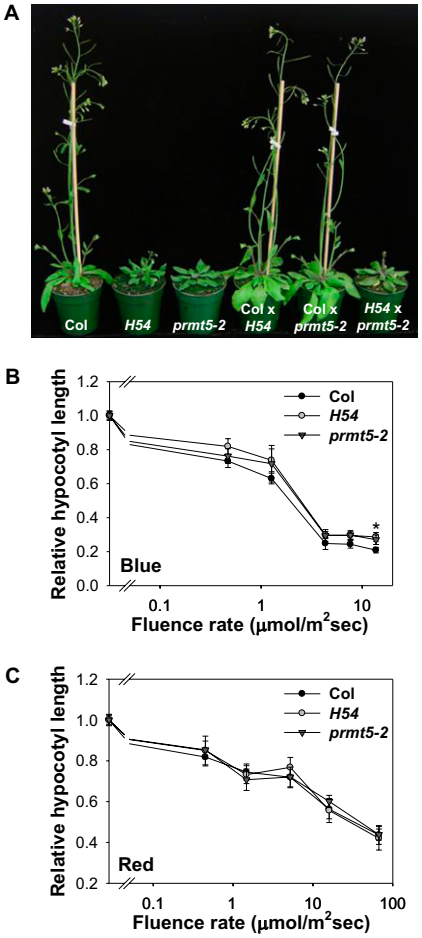

Fig. S3. *prmt5* mutation delays flowering time and reduces inhibition of hypocotyl elongation in blue light. (*A*) Flowering time is delayed in *H54* and *prmt5-2*. Both *H54* and *prmt5-2* are recessive, and the late-flowering phenotype seen on long (LD: 16/8 h) days is fully rescued in the F1 progeny of a back-cross to Col. *H54* and *prmt5-2* are allelic, because the F1 progeny of a cross between them is not complemented and shows the late-flowering phenotype. Images were taken 32 d after planting. (*B* and *C*) Fluence–response curves for inhibition of hypocotyl elongation in blue (*B*) or red (*C*) light following 5 d growth. Hypocotyl lengths (mean ± SD; $n = 12$) are presented relative to the length in the dark. Asterisks indicate lengths significantly longer than wild type (Col) in high-fluence blue light ($P < 0.0001$ as determined by Student's two-tailed t test).

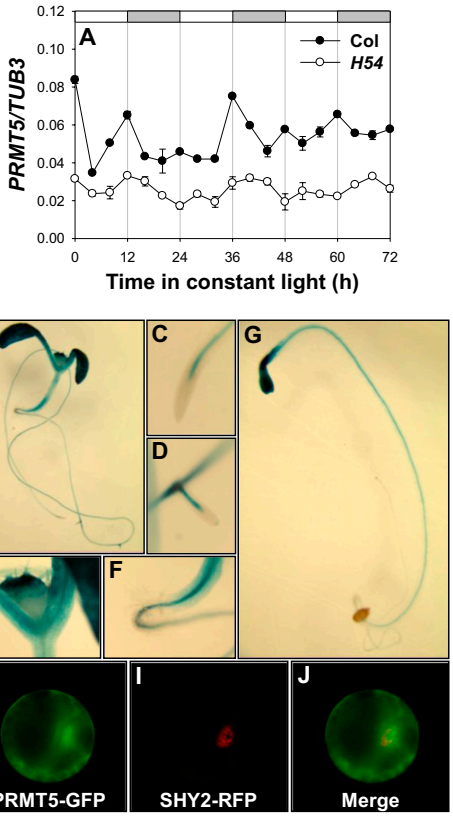

Fig. S4. *PRMT5* shows clock-regulated mRNA accumulation. Col and *H54* seedlings were entrained to photocycles (LD: 12/12 h) for 10 d before release into LL. Transcript levels (mean ± SEM from two independent experiments) of *PRMT5* were estimated by quantitative PCR and normalized to tubulin (*TUB3*) expression. White and gray bars indicate subjective day and night, respectively. (*B–G*) *PRMT5* promoter activity was examined by histochemical staining for GUS activity in *pPRMT5:GUS* transgenic seedlings. (*B*) A 7-d-old light-grown seedling: (*C*) root tip. (*D*) lateral root. (*E*) developing leaves. (*F*) hypocotyl–root junction. (*G*) A 7-d-old dark-grown seedling. Six independent lines were used in the staining analysis. (*H–J*) Subcellular localization of PRMT5-GFP (*H*), Short Hypocotyl 2 fused to RFP (SHY2-RFP) (*I*), a control nuclear marker protein (1), and merged image (*J*) in *Arabidopsis* protoplasts.

1. Kim HJ, et al. (2008) Control of plant germline proliferation by SCF(FBL17) degradation of cell cycle inhibitors. *Nature* 455:1134–1137.

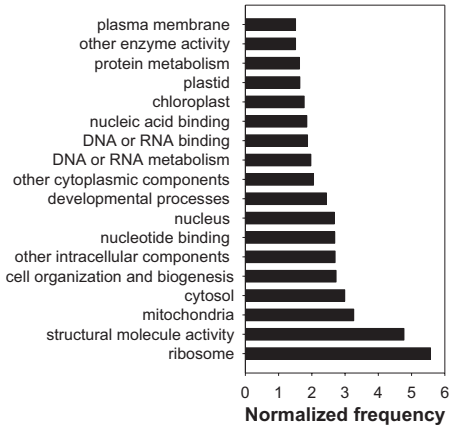

Fig. S5. Gene Ontology (GO) overrepresentation among the 1,253 circadian and diel *PRMT5*-coexpressed genes. The 1,253 circadian and diel *PRMT5*-coexpressed genes were searched for GO categories using the Classification Superviewer at the Bio-Array Resource for Plant Functional Genomics (http://bar.utoronto.ca/). The frequency is normalized against all *Arabidopsis* genes and is calculated as follows: $(N\_in\_Class_{input\_set}/N\_Classified_{input\_set})/(N\_in\_Class_{reference\_set}/N\_Classified_{reference\_set})$.

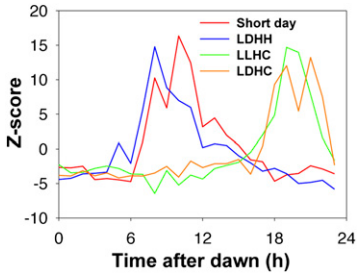

Fig. S6. The top 500 *PRMT5*-coexpressed genes are selectively phased by environmental conditions. The top 500 genes from the gene set of 1,253 circadian and diel *PRMT5*-coexpressed were used in PHASER (http://phaser.cgrb.oregonstate.edu/) to identify the time of day that these genes are overrepresented. The phase of peak expression of genes from plants grown in photocycles (LD: 12/12 h) at constant temperature (LDHH) or in short days (LD: 8/16 h) at constant temperature is overrepresented at 8–12 h after dawn. In contrast, the phase of peak expression of genes from plants grown under conditions with superimposed thermocycles, either constant light and hot/cold cycles (LLHC) or light/dark cycles and hot/cold cycles (LDHC) is overrepresented between 18 and 22 h after dawn. The Z-score is the significance of the overrepresentation at any given time of day.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)