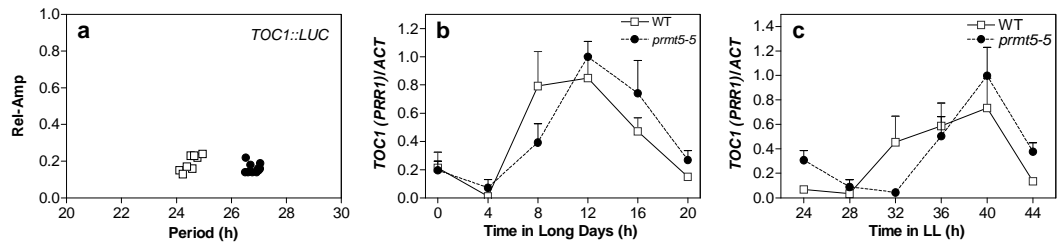
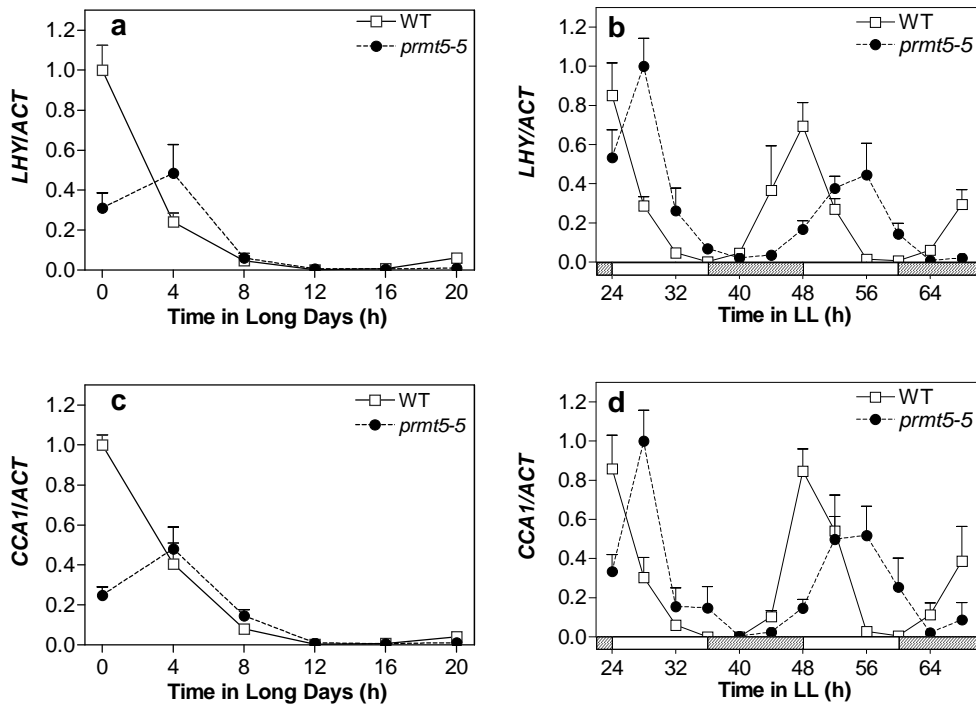


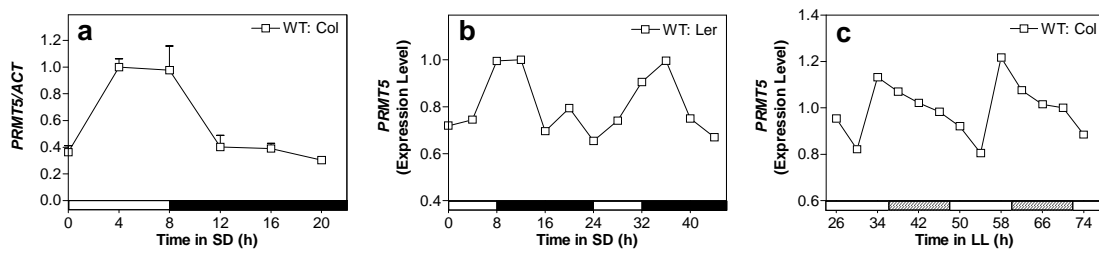
Supplementary Fig. 1. Structure of PRMT5 and physiological characterization of *prmt5* mutants. (a) Structure of the *PRMT5* gene and a scheme of the PRMT5 protein. Introns are represented by lines and exons by boxes (gray boxes indicates coding regions and black boxes, UTR's). T-DNA insertions in *prmt5-1* and *prmt5-2* mutants and nonsense mutation in *prmt5-5* are indicated. The premature stop codon generated in *prmt5-5* mutant spans exon 20's last base, in red, and the two bases at the beginning of exon 21, in black (Upper Panel). Diagram of the PRMT5 protein. Functional and conserved PRMT5 domain is in blue. The fragment absent in the protein from *prmt5-5* mutant is represented by crossed lines (Lower Panel). (b) Hypocotyl length of wild-type and *prmt5* seedlings grown under short days (SD, 8h light: 16h dark) relative to hypocotyl length of the same genotypes under constant dark conditions (n=6 replicates of 10 seedlings each). (c) Circadian rhythm of leaf movement under constant light (LL; h, hours) (n=6). □, wild-type (Columbia ecotype); ◆, *prmt5-1* mutant and ▼, *prmt5-2* mutant. (d) Period length of the circadian rhythm of leaf movement estimated for the indicated genotypes by using FFT-NLLS (h, hours; n=6). Error bars indicate +SEM (** P<0.01, *** P<0.001).



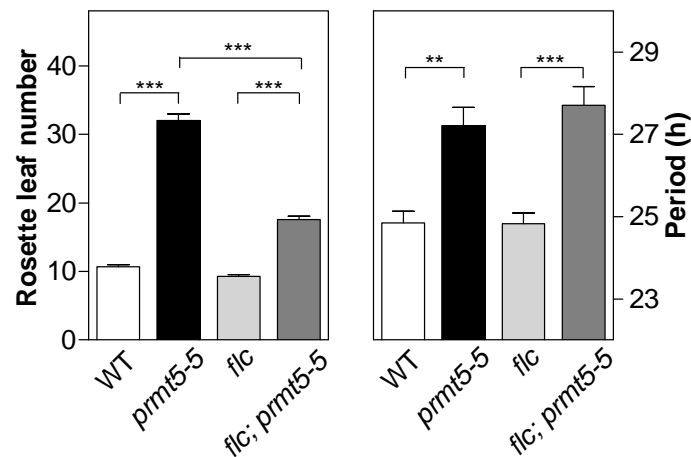
Supplementary Fig. 2. Circadian oscillations in *TOC1* expression in *prmt5* mutants. **a**, Period length and relative amplitude error estimated for *TOC1::LUC* bioluminescence rhythms by using FFT-NLLS analysis. Seedlings were entrained under 12h light: 12h dark cycles for 7 days before being released into constant light conditions for 5 days. *TOC1* expression measured by qPCR in seedlings kept under long day (16h light:8h darkness) conditions, $n=5$ (**b**) or under LL after entrainment under 12h light: 12h darkness (**c**); values are expressed relative to *ACTIN8* and normalized to the maximum value of each gene. Data represent average +SEM. □, wild-type (Columbia ecotype) and ●, *prmt5-5* mutant.



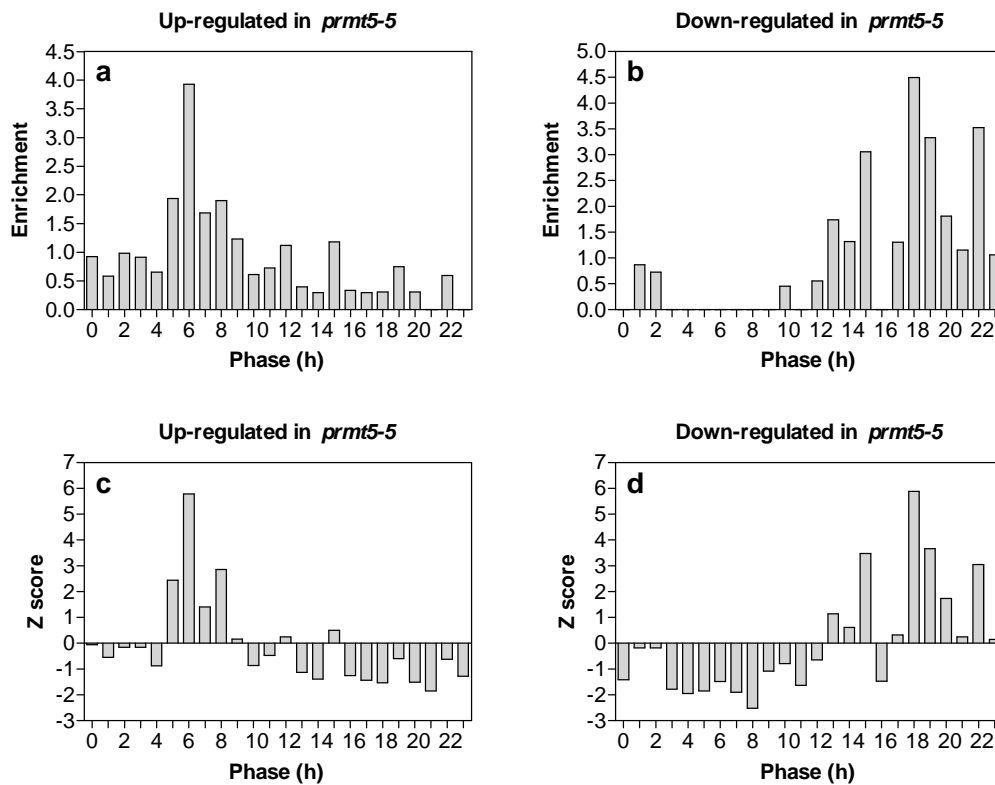
Supplementary Fig. 3. PRMT5 affects daily and circadian oscillations of *CCA* and *LHY* expression. **a, b**, *LHY* and **c, d**, *CCA1* expression measured by qPCR in seedlings kept under long day (16h light:8h darkness) conditions, $n=5$ (**a, c**) or under LL after entrainment under 12h light: 12h darkness, $n=4$ (**b, d**); values are expressed relative to *ACTIN8* and normalized to the maximum value of each gene. Data represent average +SEM. □, wild-type (Columbia ecotype) and ●, *prmt5-5* mutant.



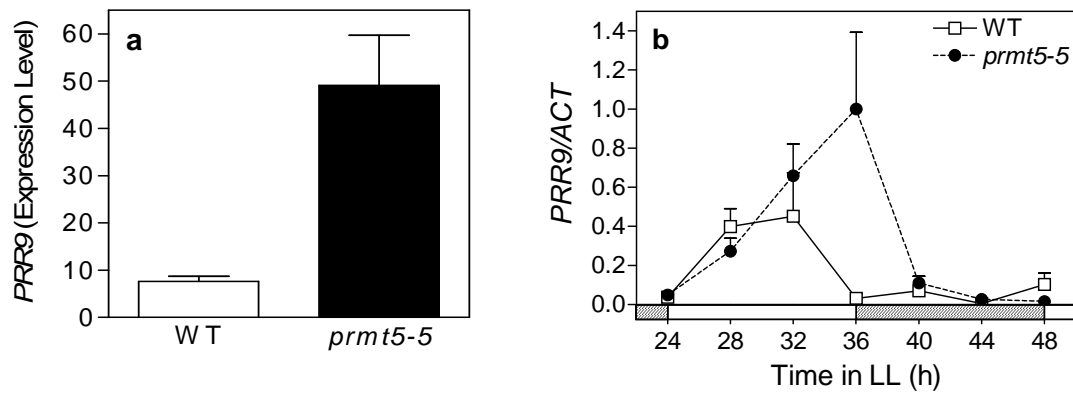
Supplementary Fig. 4. Daily and circadian regulation of *PRMT5* expression. (a) *PRMT5* expression measured by qPCR under short days (SD; 8h light: 16h darkness). Values (n=4) are relative to *ACTIN8* and represent average +SEM. □, wild-type (Columbia ecotype). (b) *PRMT5* expression levels determined by microarray analysis in plants grown under SD during two consecutive days. Data were normalized to the maximum value and were obtained from Michael, T.P. *et al. PLoS Genet.* **4**, e14 (2008). □, wild-type (Landsberg *erecta* ecotype). (c) Normalized *PRMT5* expression levels determined by microarray analysis in plants grown under LL after entrainment under 12h light: 12h darkness. Data were obtained from Edwards, K.D. *et al. Plant Cell* **18**, 639-650 (2006). □, wild-type. Open and closed boxes indicate light and dark period respectively. Lined boxes indicate subjective night.



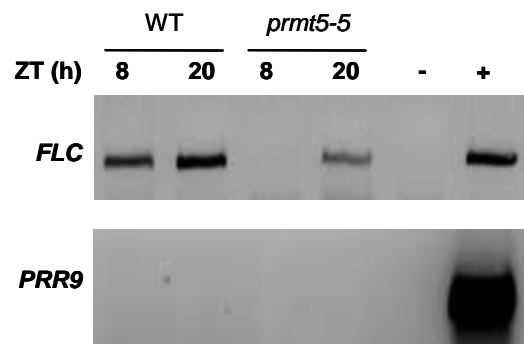
Supplementary Fig. 5. *FLC* is not responsible for the longer circadian period of the *prmt5-5* mutant. Flowering time measured as the number of rosette leaves at time of 1 cm high flower bolt, $n=22-24$ (left panel). ANOVA followed by a Tukey's Multiple Comparison Test was used to check for statistical significance in comparisons among genotypes. Free-running period of leaf movement under LL, $n=6$, h, hours. (right panel). *** $P<0.001$, ** $P<0.01$. Data represent average +SEM. Period estimates were calculated with Brass 3.0 software (Biological Rhythms Analysis Software System); available from (<http://www.amillar.org>) and analyzed with the FFT-NLLS suite of programs, as described previously (see Methods). ANOVA test followed by Tukey's Multiple Comparison Test were applied for comparisons.



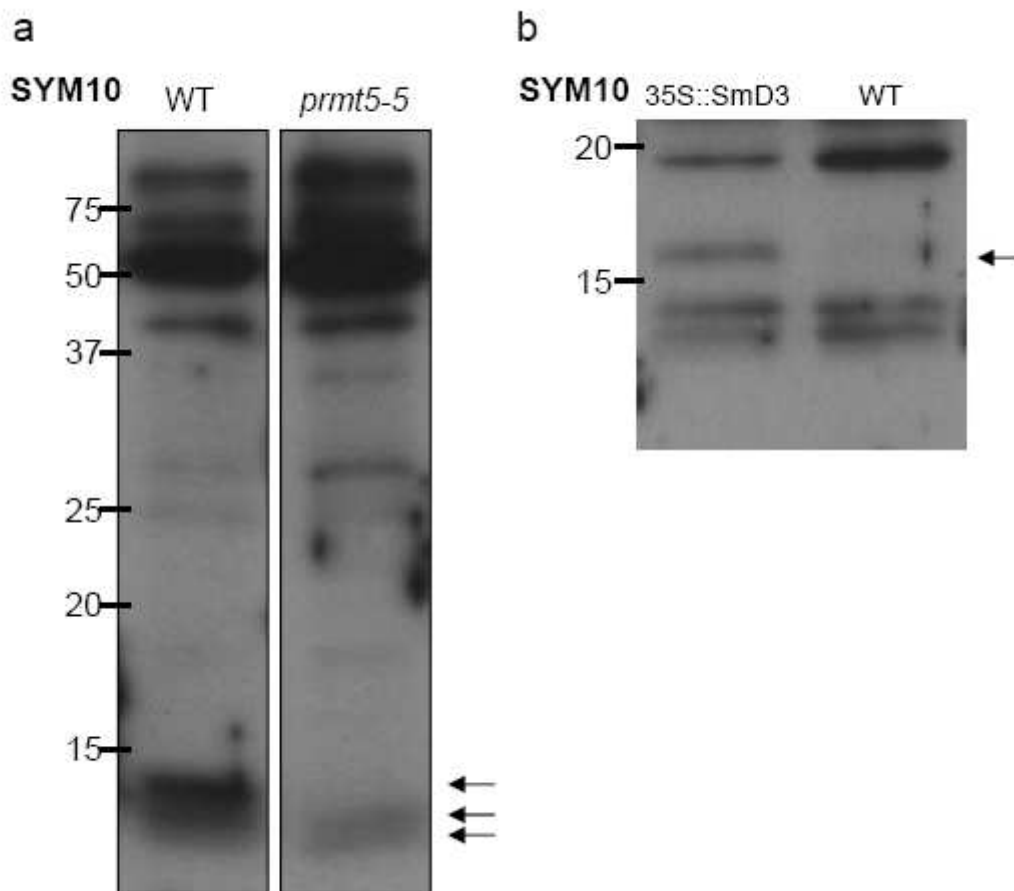
Supplementary Fig. 6. Phase distribution of clock-regulated genes whose expression was affected in *prmt5-5* mutant plants. Phase enrichment of circadian-regulated genes whose expression was up (a) or down-regulated (b) in *prmt5-5* mutants. Phase enrichment Z-score of genes up (c) or down-regulated (d) in *prmt5-5* mutants (h, hours). The analysis was performed with the genes identified as up or down regulated at least 1,5 fold in *prmt5* mutant plants (S. table I), which were also identified as clock regulated by Edwards *et al.* *Plant Cell*, **18**, 639 (2006). The phase over-representation analysis was conducted with Phaser (<http://phaser.cgrb.oregonstate.edu>), and was based on phases of expression estimated using the LL23 (i.e. 2nd and 3rd days under free-running conditions) dataset (Michael *et al.* *Plos Biology*, **6**, e225 (2008)).



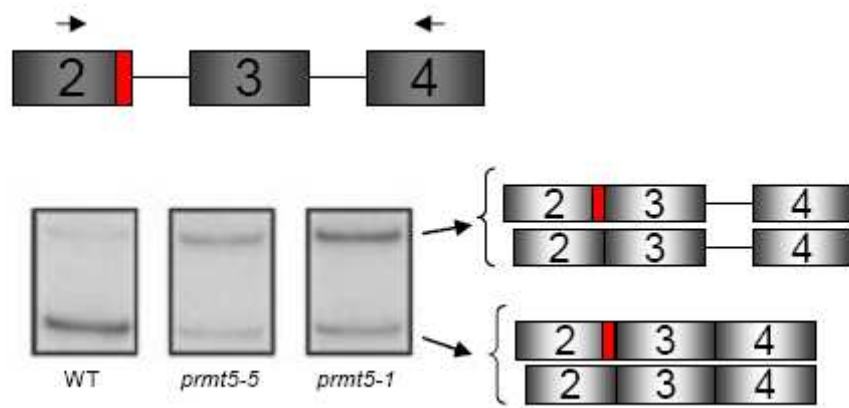
Supplementary Fig. 7. PRMT5 affects expression of the clock gene *PRR9*. **a**, *PRR9* expression measured by Affymetrix microarrays in plants grown always under LL (n=3). Data represents average +SEM. White bar, wild-type; black bar, *prmt5-5* mutant. **b**, *PRR9* expression measured by qPCR, in seedlings transferred to LL after entrainment under light:dark cycles (n=4). Data represents average +SEM. □, wild-type; ●, *prmt5-5* mutant. Open and lined boxes indicate subjective day or night period respectively.



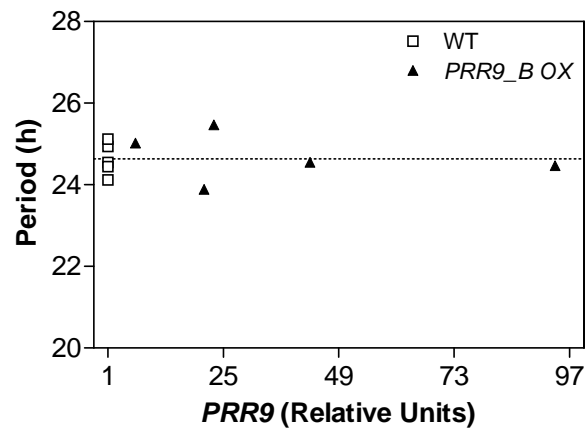
Supplementary Fig. 8. Symmetric dimethylation of histone 4 by PRMT5. Symmetric dimethylation of histone 4 arginines in the regulatory regions of *FLC* and *PRR9* was evaluated by Re-ChIP assays. Chromatin was immunoprecipitated using anti histone H4 followed by immunoprecipitation with anti-symmetric dimethyl-arginine antibodies. Representative PCR bands from Re-ChIP assays in seedlings entrained under 12h light:12h darkness are shown at two different times of the light/dark cycle. (-) without DNA control, (+) input DNA control.



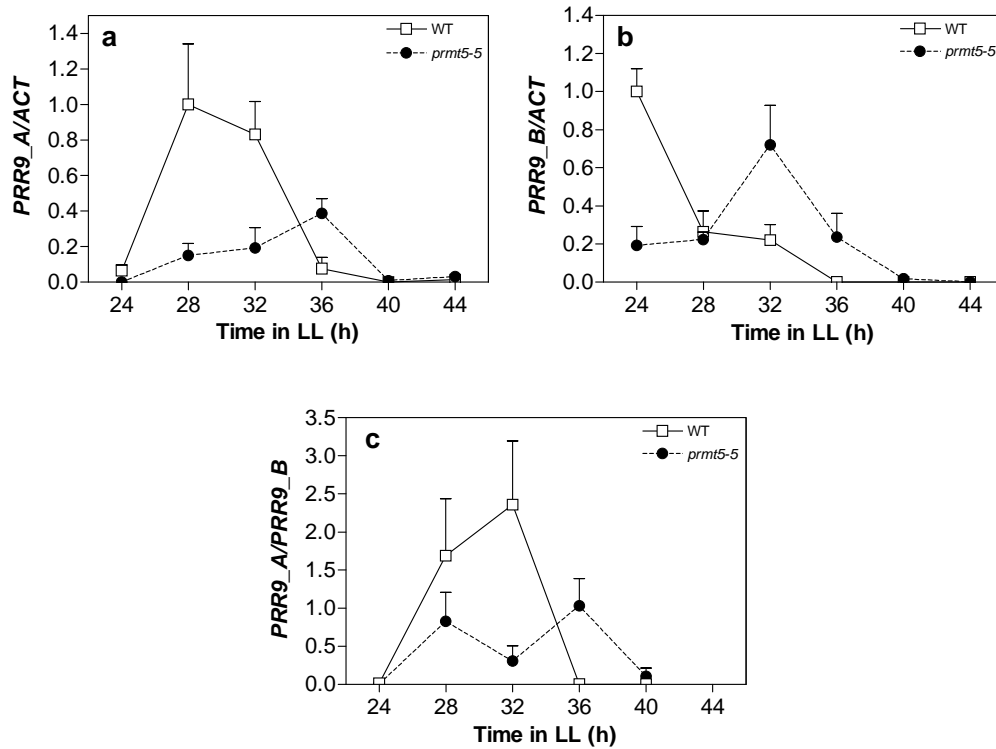
Supplementary Fig. 9. PRMT5 is required for symmetric arginine dimethylation of Sm proteins in Arabidopsis. **a**, Western blot analysis of lysates from WT and *prmt5-5* mutant seedlings grown under light/dark cycles, probed with the symmetric dimethyl arginine-specific antibody SYM10. This antibody detects symmetrically dimethylated arginines and recognized three small proteins in samples from WT but not, or to a lesser extent, in samples from *prmt5* mutant plants. Arrows show bands nearly absent, or significantly reduced, in the *prmt5-5* mutant. Based on similar findings with SYM10 in flies, these small proteins are likely Sm and/or LSm proteins. **b**, Comparison of SYM10 reactivity in samples from WT and *SmD3* over-expressing lines (35S::SmD3). The arrow indicates the location of SmD3. The higher reactivity obtained with SYM10 antibodies in the *SmD3* over-expressing lines indicates that SmD3 is indeed a target of PRMT5 activity in Arabidopsis. SmD3 levels in the WT plants used for these experiments were close to the limit of detection of the SYM10 antibody. Left numbers represent molecular weights (kDa).



Supplementary Fig. 10. Alternative splicing of the clock gene *PRR9* is affected in different *prmt5* mutant alleles. Alternative *PRR9* mRNA variants with intron 3 retained or spliced were measured by radioactive PCR in wild-type, *prmt5-5* and *prmt5-1* mutant plants grown under LL without any prior temperature or light entrainment. A representative slice of gel is shown.

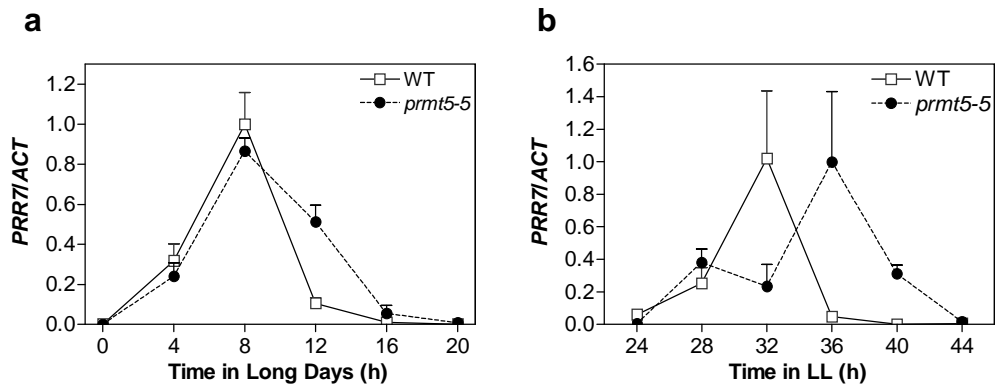


Supplementary Fig. 11. Overexpression of *PRR9_B* does not affect circadian rhythms in leaf movement in *Arabidopsis* plants. Period of circadian rhythm of leaf movement under constant light and temperature conditions in wild-type (□) or *PRR9_B* OX (▲) seedlings. *PRR9_B* over-expressing seedlings are independent T1 lines of transgenic WT plants carrying a construct that overexpress the truncated isoform of PRR9 originated by the premature stop codon present in both *PRR9_B* and *PRR9_D* isoforms (h, hours; n=5). Expression level of PRR9 was measured by qPCR and represents both, endogenous and exogenous PRR9 isoforms. Values are expressed relative to *PP2A*. Data is representative of three independent experiments. Dotted line indicates mean period value of WT plants.

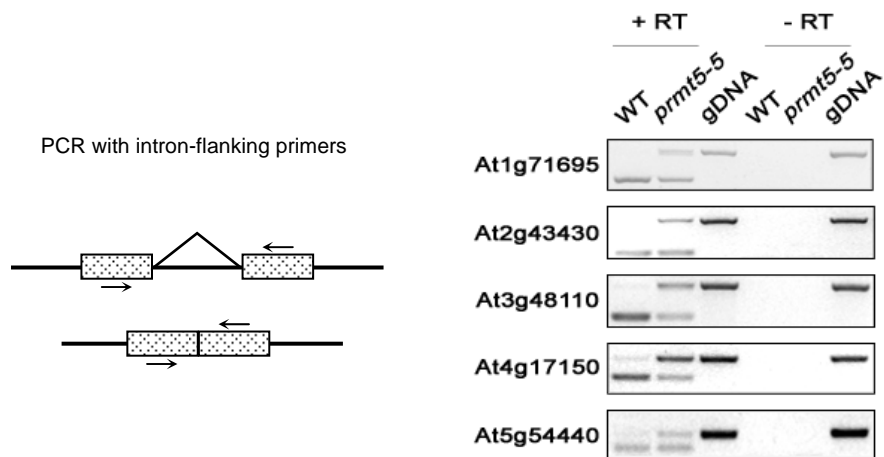


Supplementary Fig. 12. PRMT5 affects the alternative splicing of the clock gene *PRR9*.

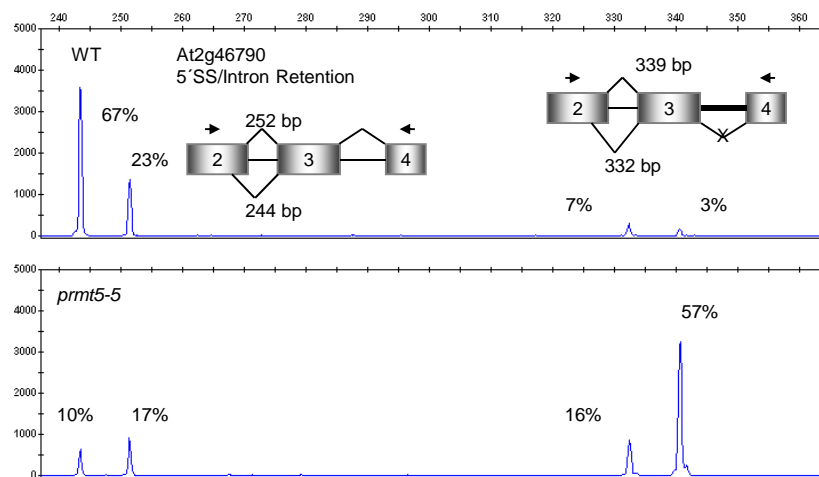
Expression of *PRR9_A* (a) and *PRR9_B* (b) by qPCR under LL (n=4). Values are relative to *ACTIN8*. c, A/B isoforms ratio as a measure of *PRR9*'s alternative splicing pattern. Data represents average +SEM. □, wild-type; ●, *prmt5* mutant.



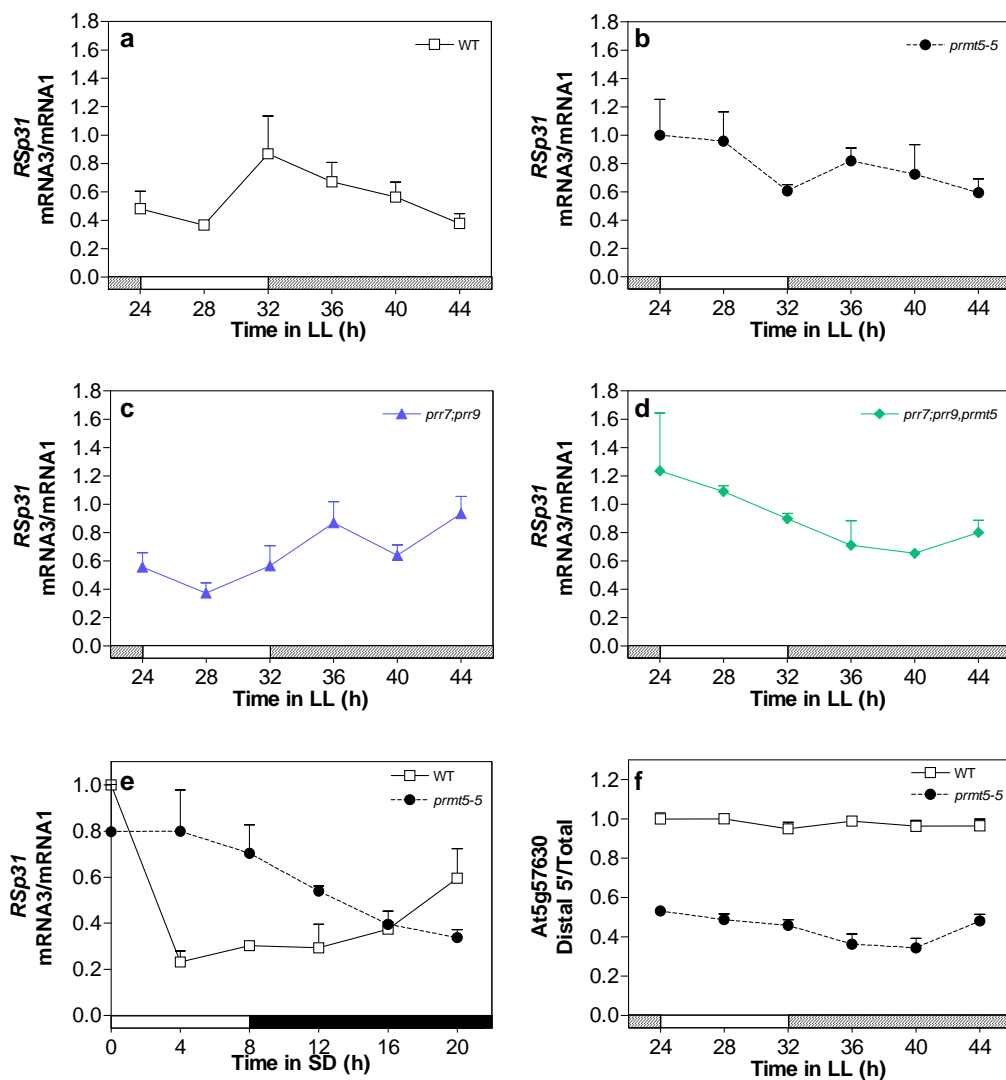
Supplementary Fig. 13. PRMT5 affects daily and circadian oscillations of *PRR7* expression. qPCR measurements of *PRR7* expression in seedlings kept under long day (16h light:8 h darkness) conditions, $n=5$ (**a**) or under LL after entrainment under 12h light: 12h darkness, $n=4$ (**b**); values are expressed relative to *ACTIN8* and normalized to the maximum value of each gene. Data represent average \pm SEM. \square , wild-type (Columbia ecotype) and \bullet , *prmt5-5* mutant.



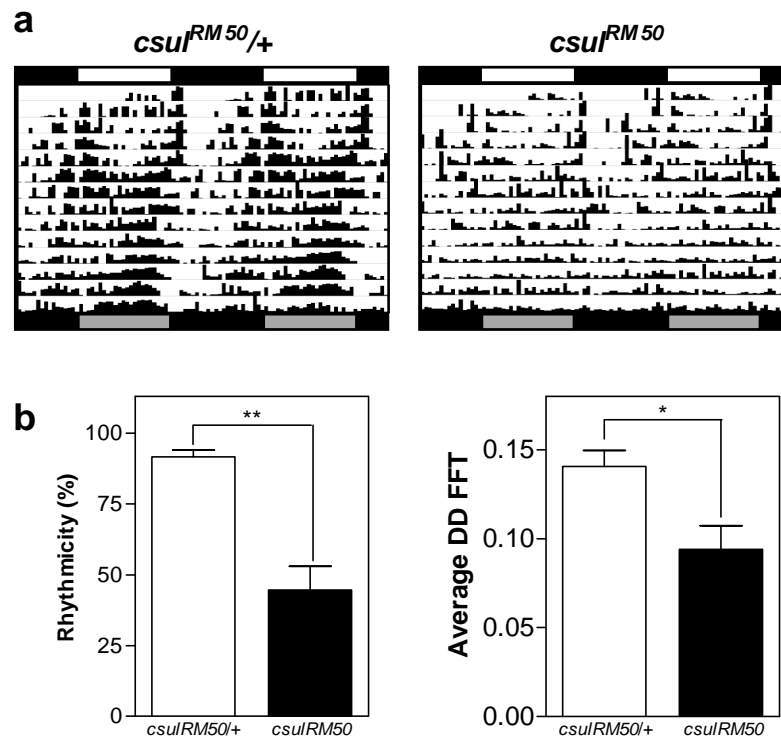
Supplementary Fig. 14. Validation by conventional RT-PCR of intron retention events identified using tiling arrays in *Arabidopsis*. gDNA, genomic DNA (control); “+RT” indicates reactions with reverse transcriptase (RT). As a control, reactions without RT (-RT) were performed. The upper band corresponds to the unspliced isoform (as in gDNA) and the lower band to the spliced one. Data is representative of three independent experiments.



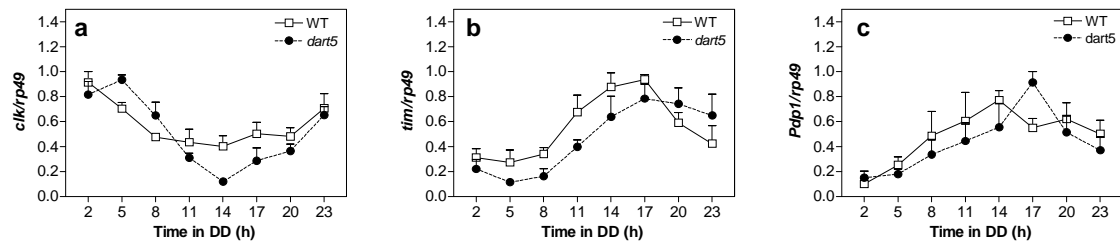
Supplementary Fig. 15. Electropherogram characterizing splicing pattern changes of *PRR9* (At2g46790) in wild-type and *prmt5-5* mutant plants. Schematic gene structures showing the expected alternative spliced isoforms and their corresponding sizes. Percentage of each isoform relative to the total is shown.



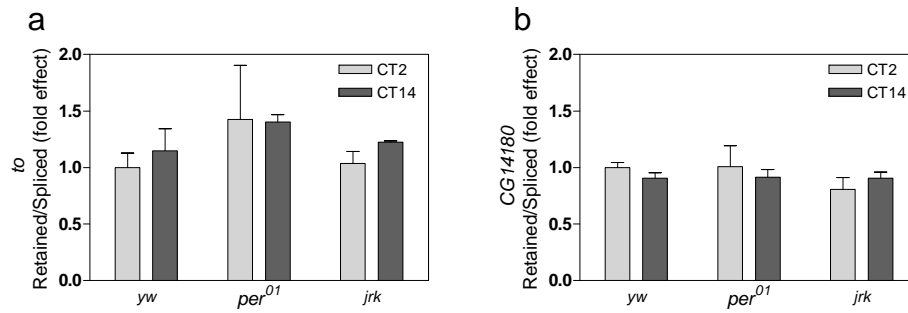
Supplementary Fig. 16. Alternative splicing of *RSp31* and *At5g57630* is affected in *prmt5* mutants. a-e, *RSp31* splice variants measured by radioactive RT-PCR in seedlings transferred to LL after entrainment under light:dark cycles (a-d) or in plants grown under SD (8h light: 16h darkness) (e). Different isoforms of this gene have been previously reported (Kalyna, M. *et al. Nucl. Acids Res.* **34, 4395-4405 (2006)). *At5g57630* splice variants measured by radioactive RT-PCR in seedlings transferred to LL after entrainment under light:dark cycles (f). Values are average +SEM (n=3). □, wild-type; ●, *prmt5-5*. ▲, *prr7;prr9*; ◆, *prr7;prr9;prmt5*. Open and closed boxes indicate light and dark period respectively. Lined boxes indicate subjective night.**



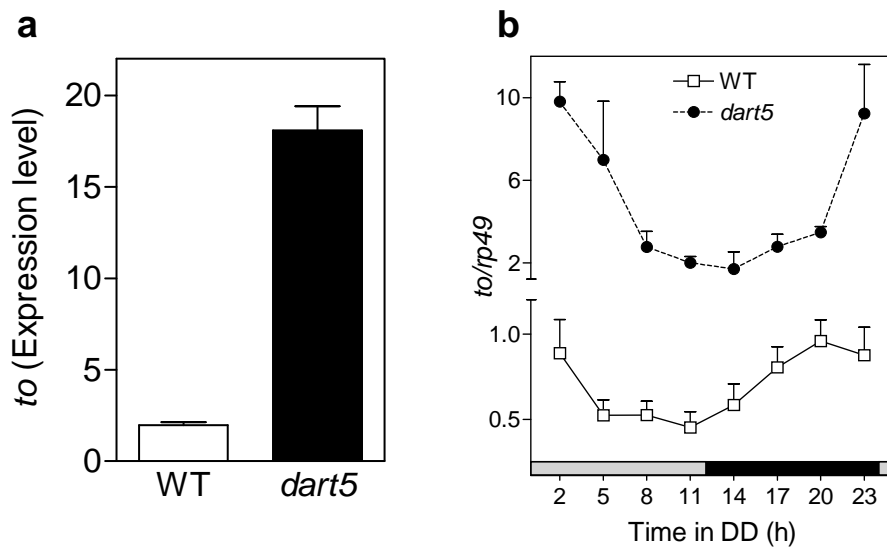
Supplementary Fig. 17. PRMT5 regulates circadian rhythms in locomotor activity in *Drosophila*. (a) Representative double-plotted actograms for the indicated genotypes. Transfer to constant conditions (DD) was performed after four days. The black and white boxes at the top of the actograms indicate night and day phases, respectively. The black and gray bars at the bottom of the actograms indicate subjective night and day respectively. (b) Percentage of rhythmic flies (left) and power FFT (right), a measure of the strength of the rhythm, in the different genotypes. Error bars indicate SEM. Error bars indicate +SEM (* $P < 0.05$, ** $P < 0.01$; $n = 53-73$).



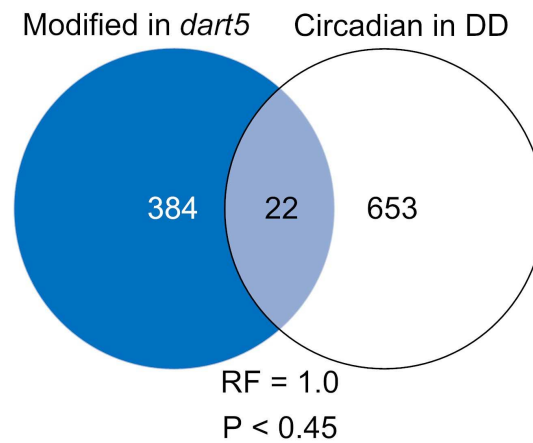
Supplementary Fig. 18. Circadian regulation of core-clock gene expression in *dart5* mutant flies. *clk* (a), *tim* (b) and *Pdp1* (c) expression in fly-heads measured by qPCR. Flies were entrained under 12h light:12h darkness cycles and then released into DD (h, hours). Values are expressed relative to *rp49* and normalized to the maximum of each time-course and each genotype. Data are average +SEM of 4 replicates. □, wild-type and ●, *dart5* mutant.



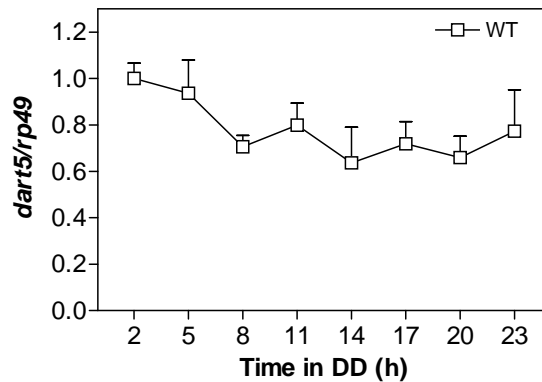
Supplementary Fig. 19. PRMT5-regulated alternative splicing events are not affected in arrhythmic clock mutants. *to* (a) and *CG14180* (b) splice variants in fly-heads measured by radioactive RT-PCR in DD; data are average +SEM, n=2 (representative of two independent experiments). *CG7199*, a gene whose alternative splicing was also affected in *dart5* (Fig. 4f) was also analyzed in arrhythmic clock mutants but was excluded from this analysis due to its low expression in the *yw* background.



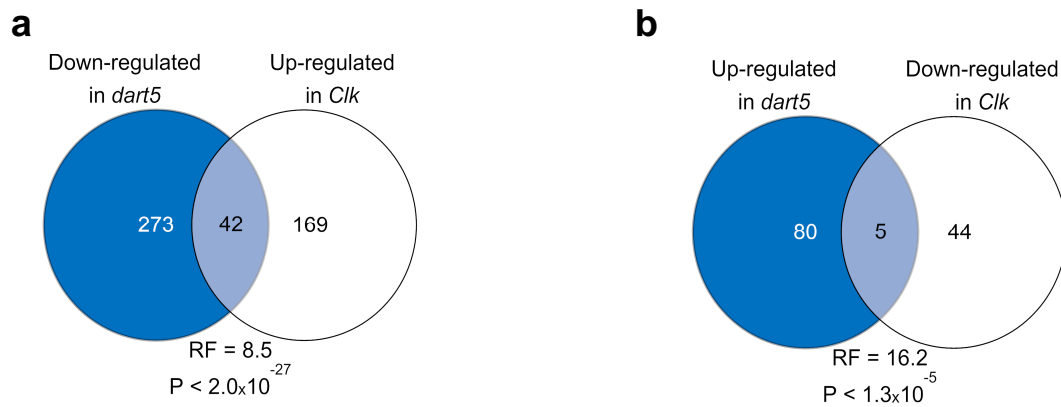
Supplementary Fig. 20. Expression of *takeout*, a clock-output gene, is altered in *dart5* mutant flies. *takeout* (*to*) expression measured using affymetrix arrays, $n=3$ (a) or by qPCR in DD, $n=4$ (b). Values are expressed relative to *rp49*. The black and gray bars at the bottom indicate subjective night and day respectively. □, wild-type (w^{1118}); ●, *dart5*. Data are average +SEM.



Supplementary Fig. 21. Overlap between genes under PRMT5 and circadian clock control in DD. Number of genes whose expression was altered in *dart5* and are under circadian clock control in constant darkness (Data were obtained from Keegan, K.P. *et al. PLoS Comput Biol* **3**, e208 (2007)). The overlap between these groups is shown. The representation factor (RF) is the observed/expected overlap, and p was calculated using the hypergeometric test.



Supplementary Fig. 22. *dart5* expression is not regulated by the circadian clock under constant darkness. *dart5* expression measured by qPCR in cDNA samples obtained from fly-heads. Flies were entrained in 12h light:12h darkness cycles and then released to DD (h, hours). Values are expressed relative to *rp49* and normalized to the maximum value. Data are average +SEM of 4 replicates. □, wild-type (w^{1118}).



Supplementary Fig. 23. Overlap between PRMT5 and CLOCK regulated genes. Number of genes controlled by *dart5* and *Clk* (*jrkl*), and the overlap between those up-regulated in *dart5* and down-regulated in *Clk* (**a**) and the other way around (**b**).