# New Phytologist Supporting Information

Figs S1–S7 and Tables S1–S3

Article title: PHYTOCHROME INTERACTING FACTORS (PIFs) mediate metabolic control of the circadian system in Arabidopsis

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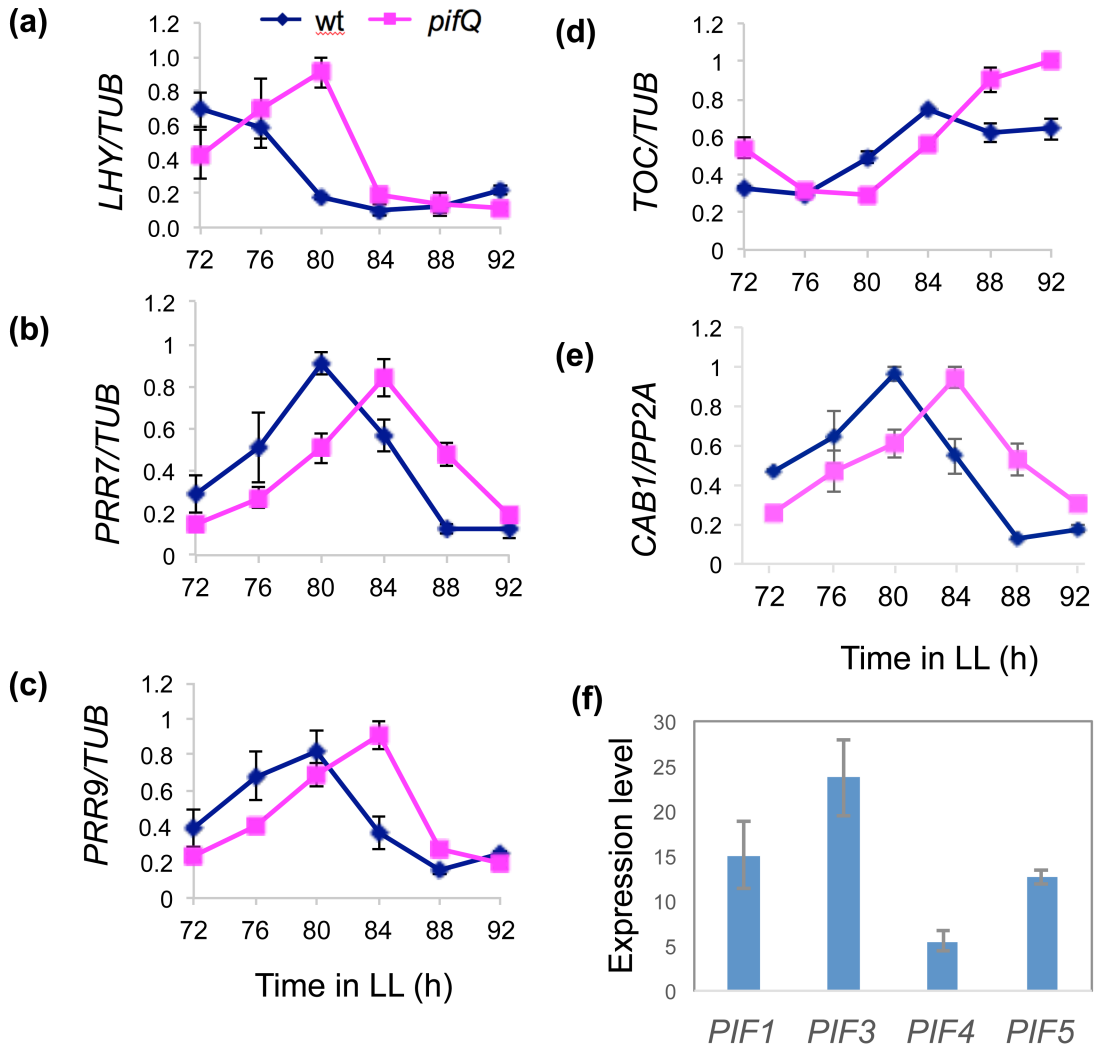
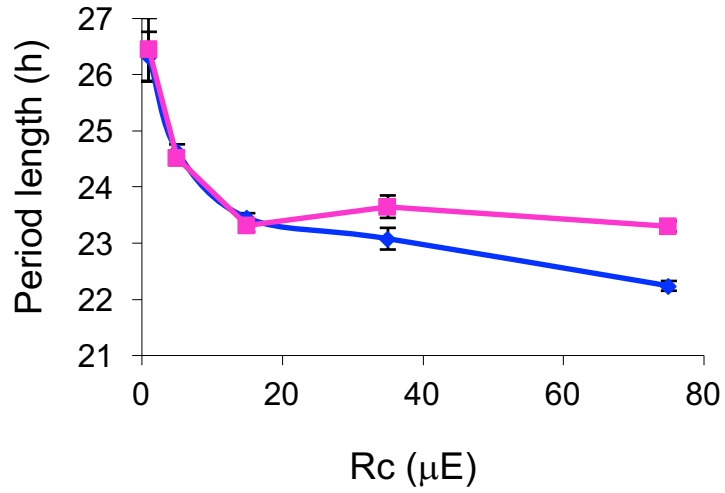



**Fig. S1** Circadian oscillator and output gene expression in *pifQ* and wt plants. *pifQ* and wt plants were grown on medium supplemented with 2% sucrose in 14 L:10 D 100μmolm$^{-2}$s$^{-2}$ at 23$^{o}$C for two weeks before being transferred to LL at the same intensity. Plants were harvested at indicated times for qRT-PCR assays. The average of 2-3 independent experiments is shown with the +/- SE. Gene expression of *LHY (a)*, *PRR7 (b)*, *PRR9 (c), TOC1 (d)* and *CAB1 (e)*. Expression level for a-d was normalized to *TUB* and *CAB2* was normalized to *PP2A*. The *pifQ* mutation effects expression of all the genes ($p<0.001$, two-way ANOVA time/genotype). f) Expression of four PIFs in the *PIF* overexpression lines used for phenotypic analysis. Seedlings were grown 7-days under white light and *PIF* gene expressions were quantified by qPCR analysis using *PP2A* as a control. Primers are listed in Table S1.

**(a)**

| | | Period length (h) | | SEM | |
|---|---|---|---|---|---|
| | Rc (µE) | wt | *pifQ* | wt | *pifQ* |
| 3% sucrose | 1 | 25.41 | 26.50 | 0.10 | 0.11 |
| | 5 | 24.05 | 24.78 | 0.05 | 0.08 |
| | 15 | 23.85 | 24.54 | 0.05 | 0.12 |
| | 35 | 23.36 | 24.37 | 0.04 | 0.04 |
| | 75 | 22.78 | 24.39 | 0.10 | 0.07 |
| 0% sucrose | 1 | 26.32 | 26.45 | 0.44 | 0.56 |
| | 5 | 24.64 | 24.52 | 0.12 | 0.13 |
| | 15 | 23.45 | 23.31 | 0.08 | 0.11 |
| | 35 | 23.08 | 23.65 | 0.19 | 0.20 |
| | 75 | 22.24 | 23.30 | 0.09 | 0.09 |

**(b)**




**(c)**

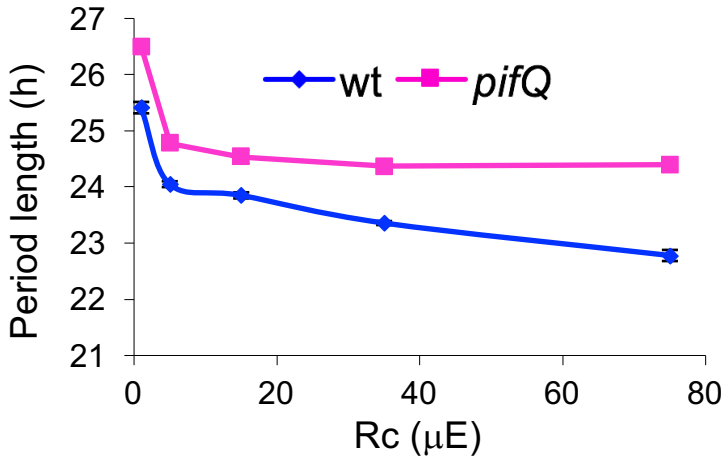


**Fig. S2** PIFs are involved in directly regulating signals from sucrose to the oscillator. (a) Table showing period lengths and SEM data for Fig. 2(a) (0% sucrose) and Fig. 3(a) (3% sucrose). (b and c) Data from Fig. 2(a) and Fig. 3(b) respectively replotted on a linear scale. Error bars indicate +/-SE.

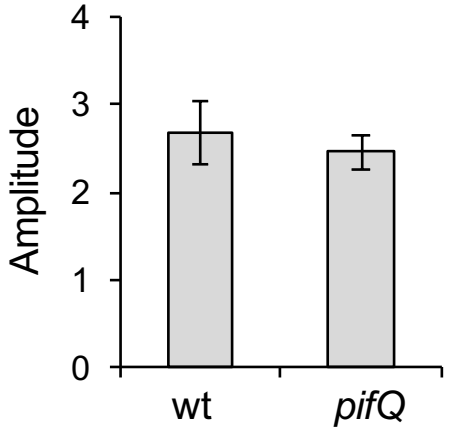



**Fig. S3** The amplitude of *CCA1*:*LUC* oscillations in wt and *pifQ* plants is similar. As described in Fig. 2 (c and d) *pifQ CCA1*:*LUC* and wt *CCA1*:*LUC* lines were entrained on medium without sucrose before being transferred to 50 μmol $m^{-2}s^{-1}$ continuous red light (Rc). The average of 2 independent experiments (n ≥40). Amplitude was calculated using BRASS software. Error bars indicate +/-SE.

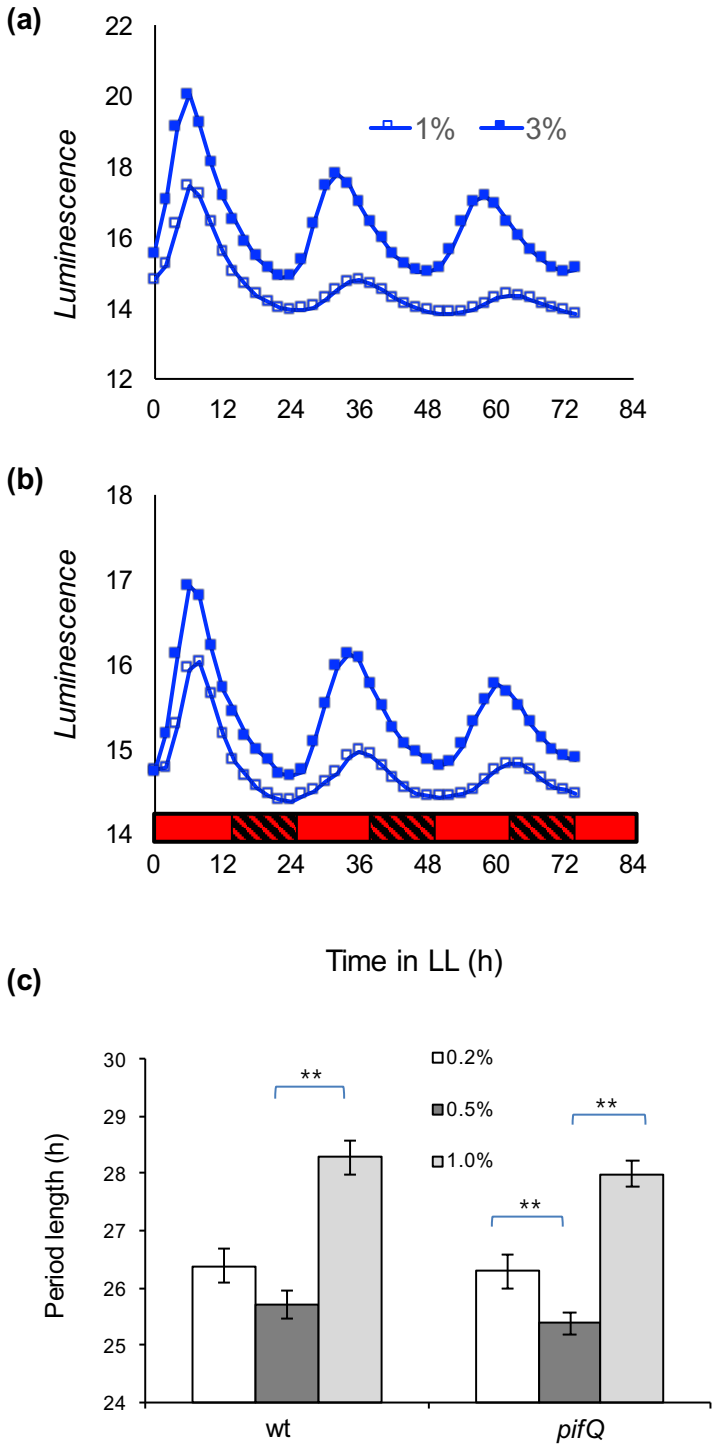



**Fig. S4** Sucrose affects rhythms in wt and *pifQ* plants. (a and b) Representative luciferase traces for (a) wt and (b) *pifQ* from Fig. 3(b). (c) Data from 0.2%, 0.5% and 1.0% sucrose in Fig. 3(b) replotted, (Student *t*-test ** p <0.01). Error bars indicate +/-SE.

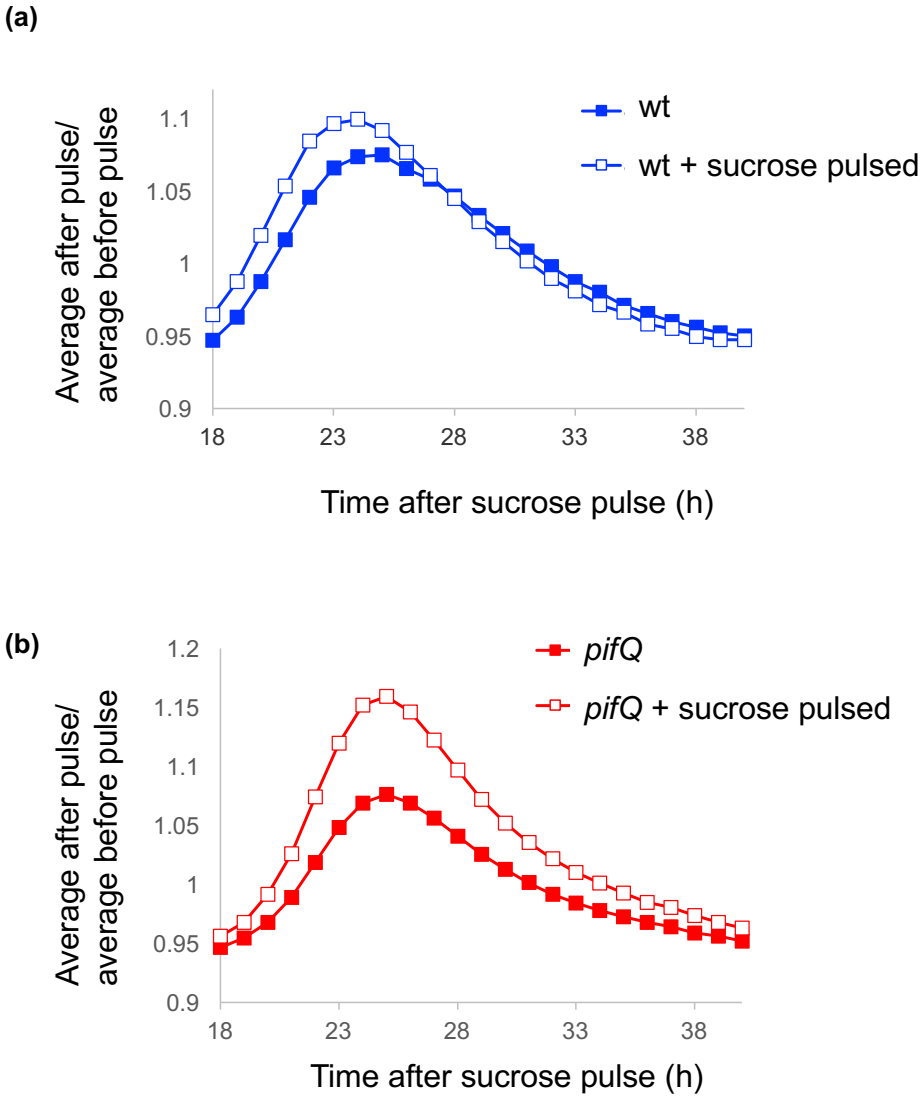



**Fig. S5** Sucrose pulses change the phase of wt but not *pifQ* plants. Phase changed in wt (a) in response to the sucrose pulse whereas in *pifQ* (b) phase remain the same. Sucrose pulse experiments were performed as described in Materials and Methods. The amplitudes of *CCA1*:*LUC* activity for 20 plants were calculated at each hourly time-point after the sucrose pulse and the results divided by the average amplitude 17 hours before the pulse were plotted.

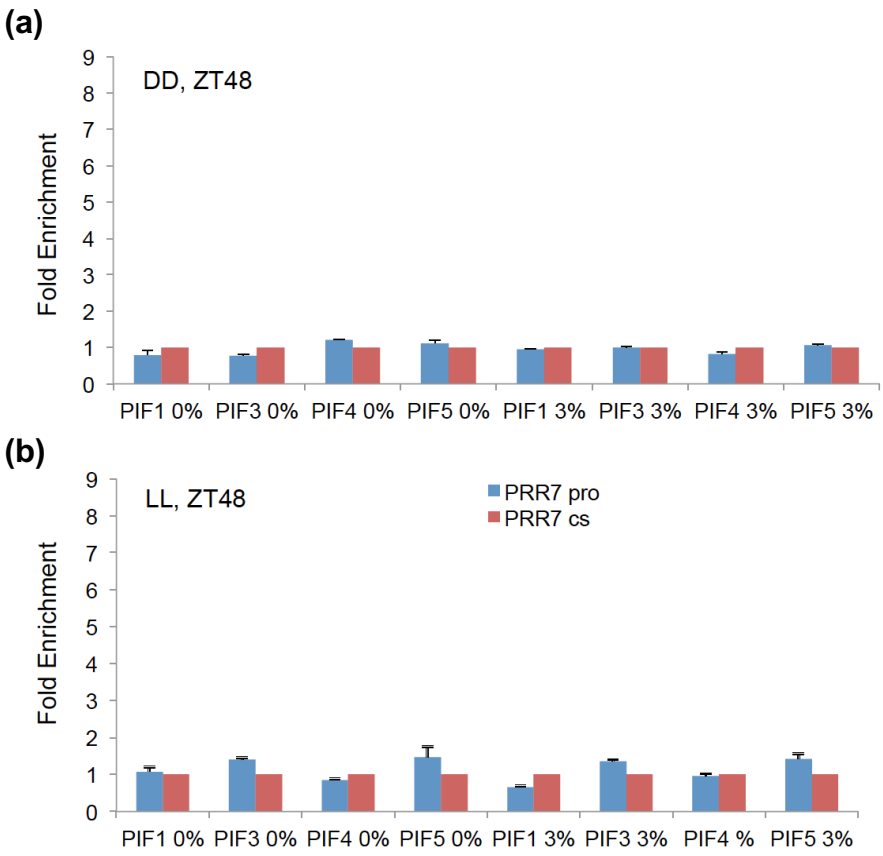
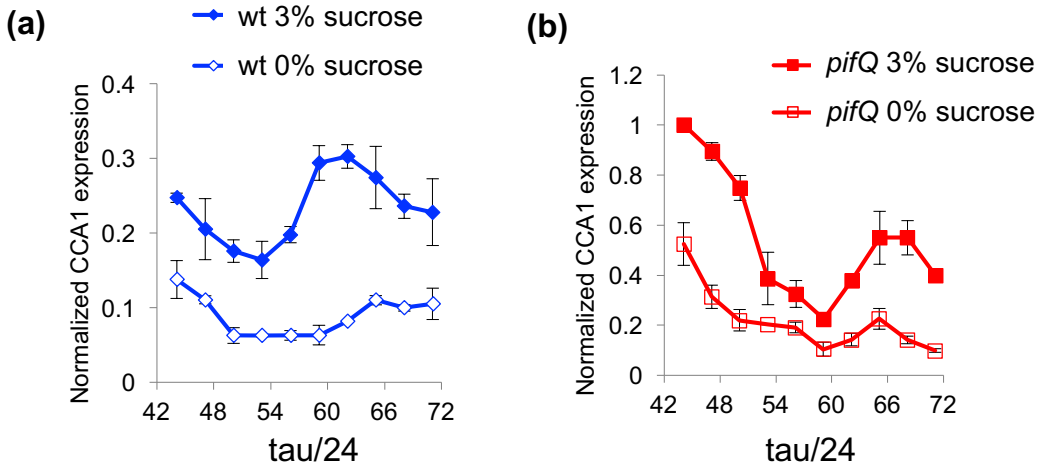



**Fig. S6.** PIFs do not directly regulate *PRR7* expression. ChIP assays on *PRR7* gene using four PIFs in the presence or absence of sucrose. Seedlings were grown with (3%) and without sucrose in 16 L:8 D for seven days and then transferred to (a) DD and (b, 75 μmol $m^{-2}s^{-1}$) LL. Samples were collected after 48 hours in DD or LL for the ChIP assays. A coding region sequence was used as control for normalization. cs, coding sequence; pro, promoter region containing G-box. Bar graph shows an average of at least 3 independent biological replicates with +/- SE.




(c)

| | tau/24 |
|---|---|
| wt 3% sucrose | 1.08 |
| wt 0% sucrose | 1.08 |
| pifQ 3% sucrose | 1.14 |
| pifQ 0% sucrose | 1.10 |

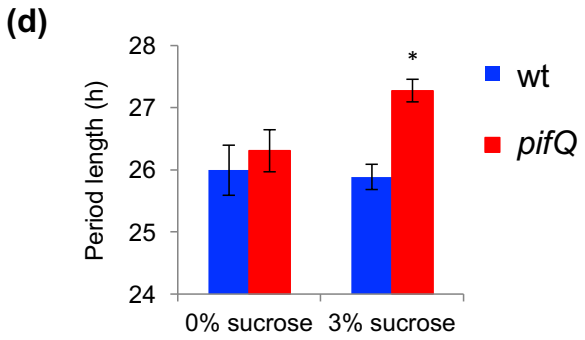



**Fig. S7.** Sucrose affects the timing of *CCA1* and *LHY* expression peaks in DD in wt plants. *pifQ* and wt plants were entrained for 10 days in 14 L:10 D 100 μmol $m^{-2}s^{-1}$ before being transferred to DD. Plants were harvested at indicated times for RT-qPCR assays for (a) *CCA1* and (b) *LHY*. Expression levels of *CCA1* and *LHY* was normalized with *PP2A* expression and then to the maximum for all the samples in the experiment and plotted against tau/24. (c) and (d) *pifQ CCA1*:*LUC* and wt *CCA1*:*LUC* lines were entrained on medium with or without 3% sucrose

before being transferred to DD and luciferase activity measured. (e) period length in DD (tau)/24 for each condition/genotype. (d) The period length average of 4 independent experiments (n ≥120). Student *t*-test * $p < 0.05$. Error bars indicate +/-SE.

**Supplementary Table S1**

Primer sequences used

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| **Cloning primers** | | |
| *pCCA1::Luc* cloning | cagaattcgttaacgagacgcg | aagagctctcctgctgagcctcg |
| **qRT-PCR primers** | | |
| *CCA1* | gcaacaaaaactgctgtcca | acttcccgtctttcgaggat |
| *LHY* | ttcagatcagaagtcatgcaca | gtccaaagcttggcaaacag |
| *TOC1* | gattccacgagtttgggaga | tcgatatcaggtccctctgc |
| *CAB1* | gcctcaacaatggctctctc | gcttggcaacagtcttcctc |
| *PIF1* | gccaccactactgatgaaactg | atgaacttcagcagcacgag |
| *PIF3* | gacggcgtgataggatcaac | catcgaagctttgtccacct |
| *PIF4* | aagtcgaaccaacgatcagg | ttgcaaagccttcattctctc |
| *PIF5* | cagaccagaagatgaattagt | acggttctctacgagcttgg |
| *PP2A* | aaacttgcgtgagggagaaa | ggaaaatcccacatgctgat |
| *TUB* | actcactacccccagctttg | gaccagggaacctcagacag |
| **ChIP qPCR Primers** | | |
| *CCA1 control* | cctcgtcagacacagacttcca | ccgcagtagaatcagctccaata |
| *CCA1 pro* | tgtagtgaaccgcacgagaa | tgtctgatacactagaaacatcagtgg |
| *LHY pro* | ttctggctcgtagagaagcaa | ctggaacagcaccaagggta |
| *LHY control* | ctcgaaagcctgggaacaac | tccaagaacgcctgattcaa |
| *PRR7 pro* | ccgccaaaatctattcaacggtccag | atggtatatcaaaaacagtcgttc |
| *PRR7 control* | ttggacgaaaaaagctgtggatg | caaatttatcatcatgttcttgag |

**Supplementary Table S2**

p values for pif mutants, compared with wt by ANOVA single factor analysis and by Student's t-test.

| | *pifQ* | *pif345* | *pif145* | *pif134* | *pif13* | *pif45* | *pif34* |
|---|---|---|---|---|---|---|---|
| ANOVA | 1.1981E-12 | 9.3421E-06 | 8.78531E-05 | 0.0004 | 0.2258 | 0.1384 | 0.0010 |
| t-test | 5.9613E-12 | 8.7008E-05 | 0.0002 | 0.0002 | 0.2836 | 0.1802 | 0.0025 |

**Supplementary Table S3**

Average period for *pif* mutant leaf movements calculated with and without outliers.

| With outliers | *pif34* | *pif45* | *pif13* | *pif134* | *pif145* | *pif345* | *pifQ* | wt |
|---|---|---|---|---|---|---|---|---|
| Average | 24.78 | 24.32 | 24.26 | 24.65 | 24.89 | 25.18 | 25.75 | 23.92 |
| SEM | 0.23 | 0.25 | 0.27 | 0.13 | 0.20 | 0.25 | 0.17 | 0.14 |
| Without outliers | | | | | | | | |
| Average | 24.66 | 24.37 | 24.26 | 24.65 | 24.73 | 25.02 | 25.71 | 24.00 |
| SEM | 0.17 | 0.16 | 0.27 | 0.13 | 0.12 | 0.20 | 0.12 | 0.11 |

| With outliers | wt | *PIF1-ox* | *PIF3-ox* | *PIF4-ox* | *PIF5-ox* |
|---|---|---|---|---|---|
| Average | 23.80 | 21.32 | 22.81 | 23.51 | 22.67 |
| SEM | 0.23 | 0.19 | 0.18 | 0.30 | 0.18 |
| Without outliers | | | | | |
| Average | 23.80 | 21.32 | 22.61 | 23.66 | 22.76 |
| SEM | 0.23 | 0.19 | 0.12 | 0.27 | 0.17 |