

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Aequorin or luciferase 24 h rhythms data were collected automatically using the softwares associated to the photon counting cameras, IndiGO software (version 2.0.5.0, Berthold technologies) or Image32 software (version 5.33, Photek). Ct values for qPCR experiments were collected using Rotor-Gene Q series software (version 1.7, QIAGEN). Aequorin luminescence values for determination of Ca²⁺ concentration were obtained using FLUORstar Optima software (BMG Labtech, Germany).

Data analysis

Luciferase and aequorin image analyses were done using IndiGO software (version 2.0.5.0, Berthold technologies) or Image32 software (version 5.33, Photek). Leaf movement images were analyzed using MetaMorph (version 7.6.0.0). Circadian rhythms data from luciferase imaging, aequorin imaging and leaf movement were analysed using the BRASS (version 3.0) plug-in for MS excel (<http://www.amillar.org>) to carry out Fast Fourier Transform Non-Linear Least Squares (FFT-NLLS) analysis. Raw intensity data from microarray were normalized across chips using RMA (<http://www.bioconductor.org>). qPCR primers efficiencies were calculated using LinRegPCR (product version 1.0.0.0). Statistical analysis were performed in SigmaPlot (version 11.0 and 13.0).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that the data supporting the findings of this study are available within the paper (Figures 1 to 6) and its supplementary files (Supplementary Figure 1 to 5 and Supplementary Tables 2 and 3). Additionally, microarray data are available at NASC Arrays (<http://arabidopsis.info/affy>), experiment reference NASCARRAYS-529.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size was chosen based in previous experiments and experience, but it is not reported in the manuscript.
Data exclusions	For microarray, the array 1-22_Calcium_12h_Rep2_ATH1 was removed from the analysis after failing hybridization quality control, this criteria was pre-established. For the analysis of circadian period, only the plants/leaves considered rhythmic by BRASS were used to calculate period, this is a pre-established criteria. For Ct values, if one out of the three technical replicates had a difference greater than 0.5, the data was excluded. This is a pre-established criteria.
Replication	Microarray experiment was done once, but each data point/sample included two biological replicates (but the one mentioned in data exclusions section). To validate the microarray finding about CHE, we used a qPCR experiment. The qPCR experiments were done twice, including 3 biological replicates each and each one of these included three technical replicates; in both attempts the replication was successful. Leaves movements experiments for the screen showed in Table S2 were done once. Just those lines that were promising were analyzed further, including larger number of samples and then the experiments were repeated at least twice. Replication was successful. Flowering time experiments and camera experiments were done at least twice and replication was successful.
Randomization	Randomization of samples in camera, leaf movement and flowering time experiments is the normal way to do it and were assumed, so it is not specifically reported in the manuscript.
Blinding	Leaf movements experiments were performed and analyzed in blind without the experimenter knowledge of seeds lines. For these particular experiments this is the normal way to do it. This is to avoid bias during the data analysis, where rhythmic traces have to be selected by eye in an early step of the analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging