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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was chosen based on the standards commonly accepted in the field. Relevant to the sample size consideration in this study was the flowering time analysis based on the leaf number counting. We analyzed the number of leaves at the flowering from more than 23 plants for each line. Six independent T2 lines for each transgene were used. Statistical methods were not used to pre-determine the sample size.

2. Data exclusions

Describe any data exclusions.

No data were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

For mass spectrometry, in vitro pull down, ITC, and Split-LUC assays, two independent replicates were performed with reproducible results.

ChIP-seq experiments using pooled plants were performed two independent replicates for EBSΔC FLAG ChIP-seq and H3K4me3 ChIP-seq for EBS and EBSΔC. The two biological replicates were highly reproducible. For the EBS and CLF FLAG ChIP-seq, we performed the replicates but failed due to either a technical issue or unknown reasons. Thus, we do not have replicates for them, however, EBS, CLF, EBSΔC have very similar binding patterns, which confirmed each other.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For flowering time assay, we randomly arranged the plants with different genotypes in the growth chamber to minimize the potential position effects of light and humidity from the growth chamber. Randomization was not required for other types of experiments conducted in this study.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were not blinded to group allocation during data collections and analysis.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Bowtie (v2.1.0); SICER (V1.1); BEDTools (2.17.0); R (3.2.3); IGV genome browser (v2.3); HKL2000/3000, diffraction data processing; Phenix, structure determination and refinement; Coot, model building; Procheck, structure geometry analysis; Pymol, structure figures; Origin 7.0; ITC data fitting. SEQUEST (1.3.0.339); GenePix Pro 6.1; MACS (1.4.2); Image studio (LI-COR); Student's t test was conducted using the Excel.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The genetic materials generated in this study are available from the authors upon request without restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Anti-H3 antibody (Abcam, ab1791), anti-H3K4me3 antibody (Millipore, 04-745), anti-H3K27me3 antibody (Millipore, 07-449), anti-FLAG antibody (Sigma, A8592), anti-GFP antibody (Roche, 11814460001), and anti-GST antibody (Thermo Fisher, CAB4169) were commercially available and were validated for use by corresponding companies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used.
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used.
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No eukaryotic cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	No human participants were used in this study.

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ChIP-seq Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data deposition

1. For all ChIP-seq data:

☒ a. Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ b. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

2. Provide all necessary reviewer access links. *The entry may remain private before publication.*	GEO access code of GSE101428
3. Provide a list of all files available in the database submission.	EBS-FLAG EBSΔC-FLAG-Rep1 EBSΔC-FLAG-Rep2 CLF-FLAG H3 Col-H3K4me3 EBS-H3K4me3-Rep1 EBS-H3K4me3-Rep2 EBSΔC-H3K4me3-Rep1 EBSΔC-H3K4me3-Rep2
4. If available, provide a link to an anonymized genome browser session (e.g. UCSC).	N/A

Methodological details

5. Describe the experimental replicates.	Two independent replicates for EBSΔC FLAG ChIP-seq and H3K4me3 ChIP-seq for EBS and EBSΔC were performed. For the EBS and CLF FLAG ChIP-seq, we do not have replicate.
6. Describe the sequencing depth for each experiment.	All ChIP-seq experiments were done using 1x50bp length and single-end sequencing method with total reads covering minimum of 20x coverage of the Arabidopsis genome.
7. Describe the antibodies used for the ChIP-seq experiments.	Anti-FLAG antibody (Sigma, A8592), anti-H3 antibody (Abcam, ab1791), and anti-H3K4me3 antibody (Millipore, 04-745) were used for all ChIP-seq experiments.
8. Describe the peak calling parameters.	P value less than 0.001 is used to call the significant peaks.
9. Describe the methods used to ensure data quality.	Student's t-test and the hypergeometric test were used for significance.
10. Describe the software used to collect and analyze the ChIP-seq data.	Bowtie2, SICER, BEDTools, and R