

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the 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
- A statement on 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 statistical parameters 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

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

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

Data collection No software was used

Data analysis

Data was aligned using Hisat2 v 2.2.1

Data was analyzed using HOMER v.4.11 software (<http://homer.ucsd.edu/homer/>) and all commands including parameters used are listed within the methods section. Some data was analyzed using HOMER2 software which is "in principle offered to be published" at nature (2022-03-04864B). What was analyzed utilizing this software was noted within the methods section.

METASCAPE (<https://metascape.org/gp/index.html>) software was utilized for gene ontology analysis.
MEIRLOP (<https://github.com/npdeloss/meirlop>) software was utilized for covariant centric motif analysis.

The following python libraries were also used to run the code:

matplotlib (<https://matplotlib.org/>) v 3.9
pandas (<https://pandas.pydata.org/>) v 2.2.2
numpy v. 1.25
scipy v 1.7.3
statsmodel v 0.13.2
sys v 3.7.12
seaborn v 0.12.2

Any custom code used for analysis was listed within the methods section with a description of how it was done. This code requires Python 3 or higher to run.

All custom code and commands from HOMER software are described in detail for each of the steps it was involved within. These sections are: csRNA-seq Data Analysis, 5'GRO-seq and GRO-seq analysis, RNA-seq analysis, ChIP-seq analysis, ATAC-seq analysis, Motif correlation of stable and unstable TSRs, Transcript stability switch analysis, Mapping stats calculation, Histograms and annotation of TSS to captured reads, Tag distribution histograms, Hexamer Analysis, RNA processing-related motif finding, Pausing index, Gene Ontology Analysis, and STARR-seq analysis.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All raw and processed data generated for this study can be accessed at NCBI Gene Expression

Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/eo/>) accession number GSE233927.

All data can be browsed at: https://labs.wsu.edu/duttke/mcdonaldbr_ernaplants_2024/

All data generated and analyzed are summarized in Supplementary Table 3.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size

Data exclusions

Replication

Randomization

Blinding

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Plants |

Methods

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> ChIP-seq |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

- | | |
|--|--|
| Cell line source(s) | <input type="text" value="Drosophila S2 - male sex, purchased from ATCC"/> |
| Authentication | <input type="text" value="Morphology and replication rate was compared to parameters provided by ATCC"/> |
| Mycoplasma contamination | <input type="text" value="Cell lines were not tested for mycoplasma"/> |
| Commonly misidentified lines
(See ICLAC register) | <input type="text" value="No commonly misidentified lines were used."/> |

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

- | | |
|-------------------------|----------------------------------|
| Laboratory animals | <input type="text" value="N/A"/> |
| Wild animals | <input type="text" value="N/A"/> |
| Reporting on sex | <input type="text" value="N/A"/> |
| Field-collected samples | <input type="text" value="N/A"/> |
| Ethics oversight | <input type="text" value="N/A"/> |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | | |
|-------------------------------------|---|
| No | Yes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Public health |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> National security |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Crops and/or livestock |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Ecosystems |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Any other significant area |

Experiments of concern

Does the work involve any of these experiments of concern:

- | No | Yes | |
|-------------------------------------|--------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Demonstrate how to render a vaccine ineffective |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Increase transmissibility of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Alter the host range of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enable evasion of diagnostic/detection modalities |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Enable the weaponization of a biological agent or toxin |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Any other potentially harmful combination of experiments and agents |

Plants

Seed stocks

A.thaliana - Col-0
Physcomitrium (Physcomitrella) patens - Gransden
Selaginella moellendorffii was purchased from Plant Delights Nursery

Novel plant genotypes

Not applicable

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

ChIP-seq

Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

N/A

Files in database submission

N/A

Genome browser session

(e.g. [UCSC](#))

N/A

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.