nature portfolio

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Last updated by author(s): Jan 13, 2022

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	Sequence information was obtained from TAIR (https://arabidopsis.org/) and Arabidopsis 1,001 Genome Browser (http://signal.salk.edu/atg1001/3.0/gebrowser.php). Sanger sequencing was performed by Eurofins Genomics. For gel documentation, the automated imaging system GEL iX IMager (Intas) was used. An epifluorescence microscope (BX-61, Olympus), equipped with UPlan(F), 100x/1.30 objective lens (Olympus) and a cooled black/white CCD camera (ORCA-R2, Hamamatsu) under the control of the microscope software CellSens Dimension (Olympus), was used to obtain microscopy pictures. The following fluorescence filters were used: FITC-2024B-000, Txred-4040C-000n and Sp. Aqua HC (Sembrock).
Data analysis	Sanger sequencing data was analyzed using ApE (v2.0.55). For evaluation of the TaqMan SNP genotyping assays, the LightCycler® 480 1.5 Software was used. Black and white microscopy pictures were pseudo-coloured using the Adobe Photoshop 2020 software. Graphs were made using R Studio (Version 1.1.423), Microsoft Excel 2016 and CorelDRAW 2020 (Version 22.1.1.523).

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

The authors declare that the data supporting the findings are available within the paper and Supplementary information or are available from the corresponding author upon reasonable request. Raw data for Figure 1d, Figure 2a and Supplementary Figure 4 are provided as Source Data file.

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	No sample-size calculation was performed. To determine plants harboring the inversion, rather large sample sizes were chosen due to the expected low inversion frequency (between 0.5 and 2%; see Schmidt, C., Pacher, M. & Puchta, H. Efficient induction of heritable inversions in plant genomes using the CRISPR/Cas system. Plant J 98, 577–589; 10.1111/tpj.14322 (2019)) and to randomize bias from integration of the Cas9 nuclease. We decided to screen 1600 plants to be able to detect inversions occuring with frequencies of about 0.1%.
Data exclusions	No data were excluded.
Replication	True biological replicates (i.e., independent plants) were used as replicates for statistical analyses. The number of replicates is stated in the Figure legends.
Randomization	All independent transgenic lines and all individual plants were randomly picked for analysis.
Blinding	Not required for most analyses, as samples were processed identically through standard procedures that should not bias the outcome. Phenotypical and fertility analyses were performed by randomly numbering the plant lines.

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
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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