

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give <i>P</i> values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Sequencing data was collected using the operating system software for the Illumina HiSeq 1500, NextSeq 500 or Novaseq 6000; bcl2fastq. Confocal images were collected using a Nikon Ti-E inverted microscope with Nikon A1Si spectral detector confocal system or a Nikon A1RMP confocal microscope, controlled by the NIS-Elements software.
Data analysis	Data analysis was performed using the following softwares and packages: broom.mixed (v0.2.9.6) Cell Ranger (v7.1.0) dbscan (v1.1.11) DESeq2 (v1.16.0) DropletUtils (v1.20.0) Fiji (with the Grid/collection stitching, Cell counter, and MorphoLibJ plugins) (v2.16.0/1.54p) GenomicRanges (v1.52.0) ggplot2 (v3.5.1) hdWGCNA (v0.4.1) lme4 (v1.1.36) lmerTest (v3.2.1) macs3 (v3.0.4) mclust (v6.0.0) Monocle 3 (v1.3.7) pheatmap (v1.1.12) R (v4.3.1)

sccomp (v1.99.18)
 scDbfFinder (v1.14.0)
 SCP (v0.5.6)
 sctools (v0.8.0)
 Seurat (v5.0.0)
 SeuratWrappers (v0.4.0)
 ShinyGO (v0.82)
 Signac (v1.14.0)
 snapatac2 (v2.6.4)
 splines (v4.3.1)
 STAR (v2.5.3a)
 Trimmomatic (v1.10.0)
 velociraptor (v1.10.0)
 velocity (v0.17.17)
 All processing code is available at https://github.com/Listerlab/root_ERS_dichotomy.

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 raw FASTQ files have been deposited in the European Nucleotide Archive (ENA) under project accession PRJEB100815 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB100815>). Processed Seurat objects have been deposited on Zenodo under the DOIs 10.5281/zenodo.17411120 and 10.5281/zenodo.17411065. The previously published datasets re-analysed in this study are available from the Gene Expression Omnibus (GEO) under accession numbers GSE155304 (Farmer et al.; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155304>), GSE141730 (Wendrich et al.; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141730>) and GSE152766 (Shahan et al.; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE152766>). Source data are provided with this paper.

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

No statistical methods were used to predetermine sample size. Each single-cell RNA-seq replicate contains gene expression data from approximately 10,000 cells derived from hundreds of individual Arabidopsis seedlings. Hundreds of seedlings were pooled for each replicate to obtain sufficient cells for single-cell RNA-seq, and we aimed to recover approximately 10,000 cells per library, the maximum number of cells that can be processed per library using the Chromium Controller (10x Genomics), to maximise cell numbers and representation of different

cell populations. Multiple independent biological replicates were performed for each experimental condition to assess reproducibility across experiments.

Data exclusions No data were excluded from the analyses.

Replication We have replicate experiments for all our biological conditions/genotypes [Col-0 full MS (6), Col-0 half MS (3), Cvi (3), Ler (3), Ws-2 (3), C24 (3), aba2-3 (3), pyl11458 (3), scr-3 (3), Col-0 control drought (2), Col-0 drought 50% water (2), Col-0 drought 20% water (2)]. However, each experiment is composed of cells that are derived from hundreds of different Arabidopsis seedlings. Whole-mount HCR results were independently reproduced in at least two separate experiments.

Randomization N/A. Randomization was not applicable because individual seedlings were not assigned to experimental groups. For each single-cell RNA-seq experiment, hundreds of seedlings grown under the same genotype and experimental condition were pooled to generate a single-cell suspension and processed together as one biological replicate. Comparisons were subsequently performed between independently generated biological replicates from predefined genotypes or growth conditions.

Blinding N/A. Blinding was not relevant to our study because we didn't allocate samples into experimental groups.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

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

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
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No Yes

- | | | |
|-------------------------------------|--------------------------|---|
| ☒ | ☐ | Demonstrate how to render a vaccine ineffective |
| ☒ | ☐ | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| ☒ | ☐ | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| ☒ | ☐ | Increase transmissibility of a pathogen |
| ☒ | ☐ | Alter the host range of a pathogen |
| ☒ | ☐ | Enable evasion of diagnostic/detection modalities |
| ☒ | ☐ | Enable the weaponization of a biological agent or toxin |
| ☒ | ☐ | Any other potentially harmful combination of experiments and agents |

Plants

Seed stocks

aba2-3 (ABRC stock number CS3834), pyl11458 (ABRC stock number CS72390), scr-3 (NASC stock code N3997)

Novel plant genotypes

N/A

Authentication

All mutants obtained from seed stocks displayed their expected phenotypes.
The different Arabidopsis ecotypes maintained in our laboratory carried the expected SNPs (see Cell genotyping method).