## nature portfolio

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## **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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For all	statistical ar	halyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a C	onfirmed					
$\boxtimes \Box$	The exact	sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement				
	A stateme	ent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
	The statis Only comm	tical test(s) used AND whether they are one- or two-sided non tests should be described solely by name; describe more complex techniques in the Methods section.				
$\boxtimes   \Box$	A descript	tion of all covariates tested				
	A descript	tion 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 h	ypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted see as exact values whenever suitable.				
$\boxtimes \Box$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings					
$\boxtimes \Box$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
$\square$ Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated						
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.						
Soft	ware an	d code				
Policy	information	about <u>availability of computer code</u>				
Data	collection	CellRanger v3.1.0				
Data	analysis	R v3.6 Seurat v3.2.3 Trimmomatic v0.36 STAR v2.7.0b DESeq2 v1.24.0 featureCounts v1.6.4				

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

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- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The snRNA-seq and bulk RNA-seq data have been deposited in the GEO database under accession number GSE174599 and GSE174656, respectively.

Field-spe	ecific reporting
	ne 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 t	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>
Life scier	nces study design
All studies must dis	close on these points even when the disclosure is negative.
Sample size	For bulk RNA-seq, we used 3 biological replicates as typical in the field
Data exclusions	no data was excluded
Replication	Main statment of the snRNA-seq analysis was validated with microscopy analysis of specific reporter genes
Randomization	The samples were collected randomly
Blinding	N/a
We require informatis system or method list  Materials & ex  n/a Involved in the Antibodies Eukaryotic Palaeontol  Animals an Human res  Clinical dat	cell lines  cell lines  MRI-based neuroimaging  d other organisms  earch participants
Flow Cytome	etry
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	s state the marker and fluorochrome used (e.g. CD4-FITC).
	s are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
<del></del>	ontour plots with outliers or pseudocolor plots.
	alue for number of cells or percentage (with statistics) is provided.

## Methodology

Sample preparation

Inflorescences were gently crushed to pieces in liquid nitrogen using a mortar and a pestle and then transferred to a gentleMACS M tube. After liquid nitrogen evaporated totally, 5 ml of Honda buffer (2.5% Ficoll 400, 5% Dextran T40, 0.4 M sucrose, 10 mM MgCl2, 1  $\mu$ M DTT, 0.5% Triton X-100, 1 tablet/50 ml cOmplete Protease Inhibitor Cocktail, 0.4 U/ $\mu$ l RiboLock, 25 mM Tris-HCl, pH 7.4) was added to the tube. Nuclei were released at 4 °C by homogenizing the tissue on a gentleMACS Dissociator with a running program as described previously (Sunaga-Franze et al. 2021). The resulting homogenate was filtered through a 70  $\mu$ m strainer, and another 5ml Honda buffer was applied onto the filter to collect the remaining nuclei. Nuclei were then pelleted by centrifugation at 1000 g for 6 min at 4 °C and then resuspended gently in 500  $\mu$ l Honda buffer. The nuclei suspension was filtered again through a 30  $\mu$ m strainer, diluted by adding 500  $\mu$ l PBS buffer, and stained with 2  $\mu$ M DAPI. Ambion RNase Inhibitor and SUPERaseIn RNase Inhibitor were added to a final concentration of 0.4 U/ $\mu$ l and 0.2 U/ $\mu$ l, respectively. 200,000 events of single nuclei were selected on DAPI signals by a BD FACS Aria Fusion into a 1.5-ml tube with landing buffer (15  $\mu$ l 4% BSA in PBS with 80 U Ambion RNase Inhibitor and 80 U SUPERaseIn RNase Inhibitor).

Instrument BD FACSAria Fusion

Software FACSDiVa v8.0.2

Cell population abundance
The nuclei population is around 15% of total events for the 4 Day inflorescences and 50% for the 3 Day inflorescences. The purity of nuclei is determined by the highest intensity and sharp peaks of DAPI signal. Sample without DAPI was used as a control to set the DAPI gate to exclude auto-florescent components.

Muclei population cannot be distinguished by the preliminary SSC and FSC gate only, so outliers from the main population with extreme values (supposedly small debris and larger aggregates) are excluded. Nuclei doublets off the diagonal of the FSC-H vs FSC-A and SSC-H vs SSC-A plots are excluded. Nuclei with the highest DAPI intensity (two peaks representing 2c or 4 c of DNA amount ) in the DAPI-H vs DAPI-A plot are selected, while damaged nuclei containing only DNA/RNA residues with low DAPI signal are excluded.

| Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.