

Reporting Summary

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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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P 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 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

The simulated data was generated with the dyno R package, version 0.1.0 and the dynverse package, version 0.1. The mouse bone marrow case study dataset was downloaded from http://trapnell-lab.gs.washington.edu/public_share/valid_subset_GSE72857_cds2.RDS. The data is now available in our tradeSeqPaper GitHub repository, at <https://github.com/statOmics/tradeSeqPaper>. The olfactory epithelium dataset was downloaded from GEO with accession number GSE95601.

Data analysis

Data has been analyzed using R packages tradeSeq v0.9.0, edgeR v3.22.5, Monocle 3 v2.99.3, zinbwave v 1.2.0, Monocle 2 v2.9.0, slingshot v1.1.3, mgcv v1.8-23, stageR v1.5.1, clusterExperiment v2.2.0, ggplot2 v3.1.0, tidyverse v1.2.1, fgsea 1.6.0 and Python v3.6.7 module GPfates v1.0.0.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

The code to generate all simulated datasets is included in the GitHub repository of the paper at <https://github.com/statOmics/tradeSeqPaper>. The data for the bulk RNA-seq time course is available at https://github.com/376-daniel-spies/rna-seq_tcComp. The data for the mouse bone marrow case study were downloaded from http://trapnell-lab.gs.washington.edu/public_share/valid_subset_GSE72857_cds2.RDS and is now available in our GitHub repository at <https://github.com/statOmics/tradeSeqPaper>. The raw data for the olfactory epithelium case study are available on GEO with accession number GSE95601. The data for the 10X adipocyte case study are available on GEO with accession number GSE128889.

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](https://www.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	This study has re-used public data, and no sample size calculations were performed.
Data exclusions	In the mouse bone marrow case study, the dendritic and eosinophyl cell types were removed prior to the analysis, since these did not seem to contribute to the trajectory. In the mouse olfactory epithelium case study, only genes with at least five counts per million in at least 15 cells were retained for analysis. In the adipocyte differentiation case study, we only retain cells relevant to the trajectory using marker genes defined in the original manuscript, we only retain genes with a count of at least 2 in at least 400 cells.
Replication	Our study replicates findings of previous studies on public datasets.
Randomization	Not applicable for this study, since only public datasets were used.
Blinding	Not applicable for this study, since only public datasets were used.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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