

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                                                                                                                                                                                                                                                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                         |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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 | No software is used in data collecting.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Data analysis   | We used the newly Python package NODE for data analysis. NODE is described in Methods and Supplementary information and deposited at <a href="https://github.com/wzdrgr/NODE">github[https://github.com/wzdrgr/NODE]</a> (Python package 0.1.5). In addition, we also used the following software for comparative analysis. SpaTalk[ <a href="https://github.com/ZJUFanLab/SpaTalk">https://github.com/ZJUFanLab/SpaTalk</a> ](R package, version 1.0):A cell-cell communication inference approach for spatially resolved transcriptomic data and deconvolution. RCTD[ <a href="https://github.com/dmccable/spacexr">https://github.com/dmccable/spacexr</a> ](R package, version 2.2.0):A statistical method for robust decomposition of cell type mixtures in spatial transcriptomics data based on Poisson factor analysis model. Seurat[ <a href="https://github.com/satijalab/seurat">https://github.com/satijalab/seurat</a> ](R package, version 4.3.0):Seurat is an R package designed for QC, analysis, and exploration of single-cell RNA-seq data. deconvSeq[ <a href="https://github.com/rosedu1/deconvSeq">https://github.com/rosedu1/deconvSeq</a> ](R package, version 0.3.0):R package for performing cell type deconvolution of bulk RNA sequencing, single cell RNA sequencing, and bisulfite sequencing data. SPOTlight[ <a href="https://github.com/MarcElosua/SPOTlight">https://github.com/MarcElosua/SPOTlight</a> ](R package, version 0.1.7):A statistical method for decomposition of cell type mixtures in spatial transcriptomics data based on topic profile signatures and NMF model. CARD[ <a href="https://github.com/YMa-lab/CARD?tab=readme-ov-file">https://github.com/YMa-lab/CARD?tab=readme-ov-file</a> ](R package, version 1.0): R package for performing cell type deconvolution of spatial transcriptomics. GraphST[ <a href="https://github.com/JinmiaoChenLab/GraphST">https://github.com/JinmiaoChenLab/GraphST</a> ](Python package 1.1.1): Python package for clustering, integration, and deconvolution of spatial transcriptomics. SpaDecon[ <a href="https://github.com/kpcoleman/SpaDecon">https://github.com/kpcoleman/SpaDecon</a> ](Python package 1.1.2): Python package for performing cell type deconvolution of spatial transcriptomics. SpatialDWLS[ <a href="https://github.com/RubD/Giotto">https://github.com/RubD/Giotto</a> and <a href="https://github.com/rdong08/spatialDWLS_dataset">https://github.com/rdong08/spatialDWLS_dataset</a> ](R package, version 4.0):R tool for |

performing accurate deconvolution of spatial transcriptomic data.  
 SpatialPrompt[<https://github.com/swainasish/SpatialPrompt>](Python package 0.0.6): Python package for spot deconvolution and clustering in spatial transcriptomics.  
 SPADE[<https://github.com/anlingUA/SPADE?tab=readme-ov-file>](R package, version 0.0.0.9000): R package for performing cell type deconvolution of spatial transcriptomics.  
 In addition, we also used the following packages for data analysis: scipy[<https://github.com/scipy/scipy>](Python package, version 1.10.0)

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 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](#)

This study made use of publicly available datasets. These include the mouse olfactory bulb dataset (<https://www.spatialresearch.org>) human pancreatic ductal adenocarcinoma (PDAC) dataset (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111672>), human skin squamous cell carcinoma (SCC) dataset (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE144240>), developing human heart dataset (<https://www.spatialresearch.org/>). Those dataset and following scRNA-seq references used are also publicly available with details provided in supplementary tables 2.

Simulation scenarios 1-4 (two replicates) scRNA-seq reference dataset: Count matrices were download from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE144236>.

Mouse olfactory bulb scRNA-seq reference dataset: Count matrices were download from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121891>.

Human pancreatic ductal adenocarcinoma scRNA-seq reference dataset: Count matrices were download from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111672>.

Human skin squamous cell carcinoma scRNA-seq reference dataset: Count matrices were download from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE144236>.

Developing human heart scRNA-seq reference dataset: Count matrices were download from <https://data.mendeley.com/datasets/mbvhhf8m62/2>

## 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](https://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

To evaluate the deconvolution accuracy of NODE and other deconvolution methods, we performed simulations and each simulation was replicated for 2 times to check variability as well as stability of each methods we compared. All attempts at replication were successful.

Randomization

Randomization is not relevant to this study because each sample or slide was analyzed separately.

Blinding

Blinding is not relevant to our study because we don't compare any case/control 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                                 | Involvement in the study                               |
|-------------------------------------|--------------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input 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 checked="" type="checkbox"/> | <input type="checkbox"/> Plants                        |

### Methods

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

## Plants

Seed stocks

Not relevant

Novel plant genotypes

Not relevant

Authentication

Not relevant