## nature research

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## **Reporting Summary**

Nature Research 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 Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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FOR	statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	onfirmed
$\boxtimes$	The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
$\boxtimes$	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. <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 P values as exact values whenever suitable.</i>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\times$	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 <u>statistics for biologists</u> contains articles on many of the points above.

## Software and code

Policy information about availability of computer code

Data collection No software was used for data collection, as publically available datasets were used.

Data analysis

Data was preprocessed using the Python software Scanpy (version 1.7.1), available at https://github.com/theislab/scanpy. Main analysis was done with our new Python software Squidpy (version 1.0.0), available at https://github.com/theislab/squidpy .

Additional software used was the following: anndata>=0.7.4  $\,$ 

dask-image>=0.5.0 dask[array]>=2021.02.0 docrep>=0.3.1 leidenalg>=0.8.2 omnipath>=1.0.5

pandas>=1.2.0 scanpy>=1.8.0 scikit-image>=0.17.1 statsmodels>=0.12.0 tqdm>=4.50.2 typing\_extensions xarray>=0.16.1

zarr>=2.6.1

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 Research guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

Human research participants

Dual use research of concern

Clinical data

All manuscripts must include a <u>data availability statement</u>. 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

Pre-processed datasets have been deposited at https://doi.org/10.6084/m9.figshare.c.5273297.v1 and they are all conveniently accessible in Python via the

squidpy.dataset mod	dule.					
Field-specific reporting						
Please select the o	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.					
\times Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences					
For a reference copy of	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	sclose on these points even when the disclosure is negative.					
Sample size	Sample size was determined by the previously published datasets that were used for this study. Datasets were chosen in order to describe the functionality of the software.					
Data exclusions	No data were excluded from analysis.					
Replication	All attempts at replication of data analysis were successful. No replication of experimental data was performed since we did not collect any experimental data.					
Randomization	Randomization of samples is not applicable to our study as we do not collect any experimental data.					
Blinding	Blinding was not relevant to our study as we report an analysis software as main finding					
Reportin	g for specific materials, systems and methods					
	ion from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ted 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 & ex	perimental systems Methods					
n/a Involved in th	ne study n/a Involved in the study					
Antibodies	ChIP-seq					
Eukaryotic						
	logy and archaeology MRI-based neuroimaging					
Animals ar	Animals and other organisms					