

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 [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 (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	SAMtools (1.7), BWA (0.7.17), pysam (0.14.1), OPENANNO (1.0 http://health.tsinghua.edu.cn/openannotate/ or http://bioinfo.au.tsinghua.edu.cn/openness/anno/)
Data analysis	The RA3 R package is available at: https://github.com/cuhklinlab/RA3 (DOI: https://doi.org/10.5281/zenodo.4581063) The source code for reproduction is available at: https://github.com/cuhklinlab/RA3_source (DOI: https://doi.org/10.5281/zenodo.4581077) R packages: Rtsne (0.15), umap (0.2.5.0), ggplot2 (3.3.1) Python packages: scanpy (1.4.6), scikit-learn (0.22.2.post1), SciPy (1.4.1), NumPy (1.18.2), pandas (1.0.3) For the method comparison: scABC(0.99.0), Cusanovich2018 (http://atlas.gs.washington.edu/fly-atac/docs/), Scasat (https://github.com/ManchesterBioinference/Scasat), cisTopic (0.3.0), SCALE (1.0.3), SnapATAC(1.0.0) For GREAT analysis, trajectory inference and motif analysis: GREAT (4.0.4), slingshot (1.7.0), chromVAR (1.4.1)

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](#)

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 human bone marrow dataset and the corresponding reference ATAC-seq data were retrieved from NCBI Gene Expression Omnibus (GEO) with the accession

number GSE96772 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE96772]. Single-cell data of the GM/HEK and GM/HL datasets are available at GEO GSE68103 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68103]. The InSilico mixture dataset was collected from GEO with the accession number GSE65360 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65360]. BAM files of the reference data for GM/HEK, GM/HL and InSilico mixture datasets were obtained from ENCODE with the following accession number: ENCFF774HUB [https://www.encodeproject.org/files/ENCFF774HUB/], ENCFF775ZJX [https://www.encodeproject.org/files/ENCFF775ZJX/], and ENCFF783ZLL [https://www.encodeproject.org/files/ENCFF783ZLL/] for the GM/HEK dataset; ENCFF783ZLL [https://www.encodeproject.org/files/ENCFF783ZLL/], ENCFF775ZJX [https://www.encodeproject.org/files/ENCFF775ZJX/], ENCFF746RUG [https://www.encodeproject.org/files/ENCFF746RUG/], and ENCFF328JJT [https://www.encodeproject.org/files/ENCFF328JJT/] for the GM/HL dataset; and ENCFF328JJT [https://www.encodeproject.org/files/ENCFF328JJT/], ENCFF826DJP [https://www.encodeproject.org/files/ENCFF826DJP/], ENCFF923SKV [https://www.encodeproject.org/files/ENCFF923SKV/], ENCFF775ZJX [https://www.encodeproject.org/files/ENCFF775ZJX/], and ENCFF949CIK [https://www.encodeproject.org/files/ENCFF949CIK/] for the InSilico mixture dataset. The mouse forebrain dataset can be accessed in GEO with the accession number GSE100033 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE100033]. The MCA mouse brain dataset is available at <http://atlas.gs.washington.edu/mouse-atac>. The 10X PBMC dataset is available at <https://support.10xgenomics.com/single-cell-atac/datasets>. The reference data of fresh PBMCs was downloaded from GEO with the accession number GSE129785 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE129785].

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	No experiments in study.
Data exclusions	No experiments in study.
Replication	No experiments in study.
Randomization	No experiments in study.
Blinding	No experiments in study.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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