nature portfolio

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Last updated by author(s): Nov 3, 2026

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	
☐	☒	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 *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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	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	No software was used

Data analysis	The custom code for data preprocessing, analysis, and visualization is publicly available via Github at https://github.com/shaochwu/atera_project. The package versions used for data analysis are listed in a table below: Package / tool Version Used for R 4.2 Preprocessing and cell-type annotation Seurat 4 Preprocessing, normalization, clustering, annotation Seurat 5.1.0 Myeloid separability / downsampling analysis Seurat 5.5.0 Spatial niche assignment (BuildAssayNiche) SpatialQM (R package; Python code adapted from it) 0.1.0.9000 Quality-control metrics inferCNVpy 0.6.1 Single-cell copy-number inference (Atera) BWA-MEM (bwa) 0.7.19 WGS read alignment SAMtools 1.23.1 Sorting and indexing alignments Snakemake 9.23.1 WGS workflow orchestration CNVkit 0.9.13 WGS copy-number profiling NumPy 2.4.4 Maximum-parsimony phylogeny (inferCNV clones) NumPy 2.4.2 Neighbor-joining phylogeny (WGS regions) igraph 2.3.2 Duct segmentation (connected components) RANN 2.6.2 Radius neighbor search (200 µm microenvironments) ASHLAR 1.19.0 Cross-round image registration (Orion mIF)

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

All raw and processed WGS data for the LCM regions have been deposited to SRA under the BioProject ID PRJNA1509685. The multiplex IF data have been deposited using TissueViewer29 under this link: https://tissueviewer.com/link/m3zkg5.

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	This study used female breast cancer samples.
Reporting on race, ethnicity, or other socially relevant groupings	All datasets used in this study are publicly available on 10x Genomics website and the race, ethnicity or other socially relevant groupings are not available. Xenium 280 data was obtained from the S1-Bottom section in https://www.10xgenomics.com/datasets/xenium-ffpe-human-breast-biomarkers. Pre-production Atera Whole Transcriptome Assay data was obtained from https://www.10xgenomics.com/datasets/atera-wta-ffpe-human-breast-cancer. Xenium 5K data was obtained from https://www.10xgenomics.com/datasets/xenium-prime-ffpe-human-breast-cancer
Population characteristics	See above.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This study used only one sample per comparison group because only one Atera Breast cancer sample was available at the time of the preparation of this study that aimed to provide a first independent benchmarking of this new technology.

Data exclusions	No data were excluded.
Replication	Not applicable.
Randomization	Not applicable because of the data availability.
Blinding	Not applicable.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

DC-LAMP EPR24265-8 62-1109-501
GZMB D6E9W 62-1220-501
CD8 AMC908 62-1366-501
CD20 L26 62-1004-601
CCR7 EPR23192-57 AB272938, Abcam
TCF1/7 C63D9 85942SF, Cell Signaling Technology
CD163 EPR14643 62-1441-601
FOLR2 OTI4G6 62-1025-601
CD3e D7A6E 62-1119-601
CD4 EPR6855 62-1098-701
FoxP3 236A/E7 62-1012-701
CD138 EPR6454 52-1059-701
LYVE1 EPR21857 62-1337-701
SMA 1A4 62-1046-801
PanCK C11/AE1/AE3 62-1015-801
HLA-DR EPR3692 62-1092-801
SPP1 Polyclonal 62-1022-501
CD68 D4B9C 15981-1-AP, Proteintech
IBA1 EPR16589 62-1304-601
KRT17 EPR1624Y Ab238953, Abcam
ISG15 Polyclonal 15981-1-AP, Proteintech
KRT19 4 E8 NBP22116, Novus Biologicals
CD56 MRQ-42 62-1060-601
LAMC2 E3 SC28330, Santa Cruz
CLEC9A EPR22324 AB223188, Abcam
CD141 E7Y9P 62-1065-701
KRT5 SP27 AB236216, Abcam
HER2 D8F12 62-1087-701
CD206 E2L9N 91992S, Cell Signaling Technology
Ki-67 D3B5 62-1123-801
IL4I1 EPR22070 62-1023-801

Validation

All antibodies have been first tested using immunohistochemistry on human tonsil sections and the staining pattern was interpreted by a pathologist. After that every antibody conjugated to a fluorophore was assessed as a single stain to verify that the staining pattern observed by IHC is mentained.

Plants

Seed stocks	Not applicable.
Novel plant genotypes	Not applicable.
Authentication	Not applicable.