

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 (<i>n</i>) 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. <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> |
| ☐ | ☒ 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 <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	For scRNAseq, base call files from Illumina were demultiplexed and converted to FASTQ files using bcl2fastq (v2.20.0.422) (Illumina). The Cell Ranger (10X Genomics v3.1.0) pipeline was used to align the sequence reads against the mm10 reference genome, deduplicate reads, call cells and generate cell x gene counts matrices for each library. Paired-end reads were processed and mapped to the mm10 mouse genome using Cell Ranger pipeline v4.0.0. Cell doublets were estimated using Scrublet v0.2.1 and DoubletDecon v1.1. For scATACseq, paired-end reads were processed and mapped to the mm10 mouse genome using the Cell Ranger ATAC pipeline v1.2.0. Doublets were removed using AMULET v1.0. Simple repeats, segmental duplications, repeat masker and blacklisted regions obtained from the UCSC Genome Browser and ENCODE project were filtered. For spatial transcriptomics, Illumina base call files for all libraries were converted to FASTQs using bcl2fastq v2.20.0.422 (Illumina). For each tissue section and corresponding library, the whole slide brightfield image and CytAssist image were aligned manually using the Loupe Browser (v6.4.1) via landmark registration. Each whole slide image was uploaded to a local OMERO server where a rectangular region of interest (ROI) containing just the tissue was drawn via OMERO.web and OMETIFF images of each ROI were generated using the OMERO Python API. FASTQ files, the image registration JSON file, and associated OMETIFF corresponding to high resolution bright field image were used for further processing, including alignment to the GRChm38 mm10-specific filtered probe set (10x Genomics Mouse Probeset v1.0.0) using the v2.1.0 Space Ranger count pipeline (10x Genomics).
Data analysis	For single-cell RNA-seq, filtered data matrices were then analyzed using Seurat v4. Harmony v0.1 was used for batch correction. DESeq2 v1.34.0 and Seurat v4 were used for DEG analysis. Cinar v0.2.4, Enrihr (web interface), and phyper were used for enrichment analysis. CellphoneDB was used to calculate ligand receptor interactions between different cell types in young and old mice.

For single nuclei ATAC-seq, paired-end reads were processed and mapped to the mm10 mouse genome using the Cell Ranger ATAC pipeline v1.2.0. Doublets were removed using AMULET. Filtered reads were analyzed using Signac v1.1. MACS2 was used for peak calling for each sample per cell type using the BAMPE option. We used the Diffbind197 package for generating the consensus sequences per cell type, which are then used for differential analyses between young and old using DESeq2 with absolute log2FC ≥ 1 within cinaR R package. The differential peaks were annotated with gene identities using ChIPSeeker198. We added motif information to the snATAC-seq object using the AddMotifs function in signac for the mm10 genome using the JASPAR2020 database. We then calculated a per cell motif activity score using chromVAR. We used these motif activity scores to conduct differential analysis using the FindMarkers function in Signac between old and young mice using the Wilcoxon Rank Sum test with no cutoffs being used for fold change or minimum percentage of cells expressing the motif.

For spatial transcriptomics, sequencing reads from Visium Spatial Gene Expression Slide were pre-processed with Scanpy v1.9.3. Raw read counts in each sample were normalized using the Pearson residual method. We used BBKNNv1.5.1 to remove batch effects.

Code is available on: https://github.com/UcarLab/Mammary_gland_aging

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](#)

Data has been deposited to GEO: GSE216542. We used the publicly available mm10 reference genome, TCGA data available through TCGA Biolinks, and human aging data from Sayaman, R. W. et al. Luminal epithelial cells integrate variable responses to aging into stereotypical changes that underlie breast cancer susceptibility. bioRxiv, doi:10.1101/2022.09.22.509091 (2022).

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

Life sciences study design

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

Sample size

Data exclusions

Replication

Randomization

Blinding

No blinding was performed as the mice were treated equally and downstream clustering methods were unsupervised.

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

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Virgin female young (3-4 months old) and older (17-18 months old) C57BL/6J mice (JAX #000664). All mice were bred and maintained in house under regular conditions, with 12hr/12hr light/dark cycle, and food (LabDiet 5K0G) and water ad libitum. Young and older animals were housed together for at least a month prior to tissue collection to eliminate environmental differences and to synchronize their estrous cycles.
Wild animals	Study did not involve wild animals
Reporting on sex	Mammary glands were collected only from female mice
Field-collected samples	Study did not involve field-collected samples
Ethics oversight	All animal work was performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at The Jackson Laboratory (IACUC #06005).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Study did not use plants
Novel plant genotypes	Study did not use plants
Authentication	Study did not use plants