nature portfolio

Corresponding author(s): Eliezer Van Allen

Last updated by author(s): 2025/09/09

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	Single nuclei RNA-Seq and ATAC-Seq reads were mapped to the GRCh38 human reference genome and processed into gene count marries and unique ATAC seq fragment captured by the assay with CellRanger (6.0.1) and CellRanger Arc(2.0.0).
Data analysis	Data analysis Data analysis was performed using Python (3.8.12) and R (4.1) with the following packages: Cellbender (0.3.0) eliminating technical artifacts from snRNA-seq data. Scrublet (0.2.3) remove doublets from snRNA-seq data scanpy (1.9.1) for quality control, clustering, and annotation scPipe (https://github.com/jingxinfu/scPipe/blob/hr16466_stable) the pipeline for single cell/nuclei RNA-seq and ATAC-seq quality control, clustering, and annotation, which include following packages: scenicplus (1.0.1.dev6+ge5ba6fc) for gene regulatory networks (GRNs) construction VISION (3.0.1) for gene signature calculation CytoSig (0.0.3) for cytokine activity inference scSHC (0.1.0) produce clusters corresponding to distinct cell populations multinichenetr (2.0.0) for cell-cell comunication analysis MACS2 (2.2.6) for peaks calling in sn-ATAC-seq data pycisTopic(1.0.3.dev21+ge9b0e1a) for quality control and cistopic modeling in sn-ATAC-seq data nimfa (1.4.0) for NMF-based recurrent program detection. GSEApy (1.1.3) for Gene set enrichment analysis. Packages used for visualization include: seaborn (0.13.2), PyComplexHeatmap (1.6.6), matplotlib (3.7.5) Description of the data analysis can be found at https://github.com/jingxinfu/hr_brca_single_cell

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

Raw sequencing reads of all single-cell experiments (snRNA-seq and multiomic GEX and ATAC profiling) of this study have been deposited under restricted access in the database of Genotypes and Phenotypes (dbGaP) with data accession number phs002419.v2. To access the raw sequencing from dbGaP, researchers must be approved by a specific Data Access Committee (DAC). The Source data, including processed datasets, Source data are available at this link: https://zenodo.org/records/15799566. The publicly available TCGA bulk RNA-Seq data and corresponding clinical information used in this study are downloaded from (https://gdc.cancer.gov/about-data/publications/pancanatlas). Bulk RNA-Seq data and clinical annotations from metastatic HR+ breast cancer are retrieved from the Source Data of the referenced study19. The remaining data are available within the Article, Supplementary Information or Source Data file. Source data for confidential variables are excluded. Additional data or material requests will be reviewed by the senior authors for intellectual property or confidentiality considerations.

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	As breast cancer is a leading cause of cancer death among women, only female patients were enrolled.
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	All patients involved in this study were females with early stage hormone positive breast cancer. Detailed clinical information can be found in Supplementary Data 1.
Recruitment	Patients who fit the clinical criteria and consented to the study were selected for inclusion for single nuclei RNA-seq and multiomics analysis.
Ethics oversight	Early-stage HR+/HER2- breast cancers (Supplementary Table 1) were collected with written consent from all patients. The study (NCT02999477) was approved by the institutional review board of Dana-Farber/Harvard Cancer Center, and was conducted in accordance with the principles of the Declaration of Helsinki.

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	Given the exploratory nature of our study, no statistical methods were applied to determine sample size. The number of tumor tissues processed in this study reflect the availability and accessibility of the patients cohort in the clinical trial.
Data exclusions	We removed non-cell barcodes determined by CellBender package. In addition, we excluded low quality cells with gene and unique molecular identifier (UMI) counts less than 250 and 500, respectively, and a mitochondrial percentage larger than 5%. We also removed doublets determined by the scrublet package.
Replication	The single nuclei RNA-seq and ATAC-seq experiments described in this study consisted of independent single replicate per patient tumor. This was due to the limited tissue samples collected from clinical specimens and funding limitations.
Randomization	Participants were randomized 1:1 to receive a two-week window of either nabˇpaclitaxel or pembrolizumab, with pre- and post-window breast biopsies. Subsequently, all participants received a combination of neoadjuvant nabˇpaclitaxel and pembrolizumab, followed by assessment of response at surgery.
Blinding	Investigators were not blinded to the clinical information, as this was required to guide and design the analysis.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov identifier: NCT02999477
Study protocol	This research study is exploring chemotherapy in combination with immunotherapy (a Form: nr-reporting-summary 4 therapy that uses the body's own immune system to control cancer) as a possible treatment for hormone receptor-positive breast cancer.
Data collection	Participants underwent tumor biopsies at baseline, Week 3 Day 1 (post-monotherapy), Week 7 Day 1 (post-combination therapy), before any off-trial neoadjuvant therapy (pre-AC), and at surgery.
Outcomes	The primary objective of the protocol was to characterize changes in PD-L1 expression after treatment with either nab-paclitaxel(Arm A) or pembrolizumab(Arm B) monotherapy, i.e., to evaluate changes in PD-L1 levels between breast biopsy tissue at baseline and at week 3 day 1.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*