nature portfolio

Corresponding author(s): Sarah Taylor

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

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		
	\bigvee 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 Only common tests should be described solely by name; describe more complex techniques in the Methods section.		
\checkmark	A description of all covariates tested		
\checkmark	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons		
\checkmark	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)		
\checkmark	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>		
\checkmark	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings		
\checkmark	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes		
	\checkmark 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 | Pipeline versions of CellRanger, SpaceRanger, and Xenium are provided in the manuscript

Data analysis Methods provide links to 10x analysis guides for step-by-step instructions

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 data is publicly available in GEO (GSE243280) and here: https://www.10xgenomics.com/products/xenium-in-situ/preview-datasethuman-breast

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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.

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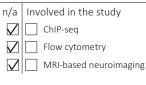
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Materials & experimental systems



Methods



Antibodies

Antibodies used	CD20 (AB219329; 1:200), HER2 (AB134182; 1:1500), Ms IgG (A-11029; 1:500), Rb IgG (AB150088; 1:500)
Validation	Spatial concordance with in situ RNA data