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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   The only statistical comparison between states is a A vs. B comparison (Figure 6).

2. Data exclusions

   Describe any data exclusions.

   For the A vs. B comparison of figure 6, we ran six technical replicates and used only five (as described in Methods:Breast tumor xenograft sample). The first injection from each tumor was used to passivate the new LC column, and these files were not included in later analyses. This decision was made after looking at the data concordance with other replicates (it is common that these first LC runs are very distinct and discarded).

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   The only replicates were technical replicates, not experimental. Here we chose the number of technical replicates based on our previous experience with quantitative proteomics data. The final number of five was more than the expected number of replicates required. The data exclusion of the first run on these is described above.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   For the Breast tumor xenograft comparison, all samples were block-order randomized. Each sample was then analyzed by LC-MS.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Data analysis for the breast tumor xenograft comparison was not blinded. Our analysis goal was to find differential proteoforms between the two conditions, so we needed to group the samples in order to run the analysis.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	This is primarily a software paper. The methods section goes into great detail about the software, and it is open source on GitHub with appropriate tutorials and documentation.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The only materials used were tumor samples that are not available for wider distribution. There is insufficient material remaining to distribute. However, these samples were only used as a demonstration for the algorithm.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	no antibodies were used

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	no cell lines were used
b. Describe the method of cell line authentication used.	no cell lines were used
c. Report whether the cell lines were tested for mycoplasma contamination.	no cell lines were used
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	no cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	the human in mouse xenograft samples have been previously published and we cite that manuscript.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	The human ovarian tumor sample as described in the methods section is a pool of five female patients.