nature portfolio

Corresponding author(s): Jitao Zhang, Giuliano Scarcelli

Last updated by author(s): Dec 3, 2022

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	Brillouin shift in samples was imaged using the line-scan Brillouin microscope (see methods) which was controlled by a custom built LabVIEW ver.2021 program.
Data analysis	Spectral data was analyzed using custom scripts written in Matlab R2021b or later version. Image analysis was performed using a combination of Fiji (version 2.1.0/1.53c) and Matlab, and statistical analysis was performed in GraphPad Prism 8 (see methods). AFM indentation data was processed using the JPK data processing software v6 provided by Brucker, the instrument manufacturer. Matlab code examples for spectrum analysis and image fusion are provided as Supplementary code.

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

The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary note files.

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	Sample sizes and experimental replication scheme were chosen based on our previous experience, and to be reasonably large to demonstrate the feasibility of the technique for biological application. These choices were based based on the current best practices in the field: see Mahajan, Vaibhav, et al. Cancers 13.21 (2021): 5549. or Nikolić, M., et al. Biophysical Journal 121.19 (2022): 3586-3599.
Data exclusions	No data were excluded from the analysis.
Replication	All replications were successful and gave similar results. All AFM and Brillouin microscopy experiments were repeated independently at least three times (from a fresh culture flask of cells, with fresh aliquots of ingredients). Cell viability and proliferation rate experiments were replicated in multiple spheroids in the same culture well, i.e. in one independent preparation of the cell culture well.
Randomization	All spheroids at certain culturing day, in a given experimental condition were selected at random, in random fields of view during data acquisition. Identical and independent culture wells were prepared for each experimental condition, therefore no special allocation procedure was necessary.
Blinding	Investigators were not blinded during data acquisition and analysis. Blinding was not necessary since data analysis was automated and standardized across all experimental conditions.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	MCF10A cell line was purchased from American Type Culture Collection. MCF10AT1k.cl2 cell line was obtained from Dr. Lisa Polin from Barbara Ann Karmanos Cancer Institute at Wayne State University (Detroid, MI, USA).
Authentication	Cells were not authenticated. Only cells with low passage number (<15) were used after obtaining them directly from the supplier.
Mycoplasma contamination	Prior to the experiments cell lines were tested for mycoplasma with the MycoFluor Mycoplasma Detection Kit (Thermofisher) and were confirmed to be negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	None.