nature research

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Reporting Summary

Nature Research 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 Research 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	HORIBA software package (LS6-NavSharp module and ViewSharp module) Renishaw software package (WiRE 5.0)
Data analysis	HORIBA software package (LS6-NavSharp module and ViewSharp module) Renishaw software package (WiRE 5.0) OriginPro 9

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The detailed raw data for Fig. 6 and its interpretation for the low-copy-number protein detection in single living cells by scPISA are available in the original publication (Liu, J., Yin, D. Y., Wang, S. S., Chen, H. Y., Liu, Z. Probing low-copy-number proteins in a single living cell. Wiley, DOI: 10.1002/anie.201608237, 2016.). In addition, the primary data for Figs. 5e and 5f are provided as source data files with this protocol.

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	No sample-size calculation was performed. All of individual cells were randomly selected in a culture dish.
Data exclusions	No data were excluded from the analyses.
Replication	All of measures were repeated at least three parallel tests to verify the reproducibility of the experimental findings.
Randomization	All of samples were randomly allocated into experimental groups.
Blinding	Because all of samples were single living cells, blinding was not relevant to this study.

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

Antibodies

Antibodies used	Polyclonal anti-survivin antibody (Abcam, cat. no. ab24479, RRID: AB_778189). Monoclonal anti-survivin antibody (Abcam, cat. no. ab134170, RRID: AB_1580809).
Validation	Polyclonal and monoclonal anti-survivin antibody have been validated by western blot, immunocytochemistry/immunofluorescence and immunohistochemistry in Abcam before sale (see https://www.abcam.cn/survivin-antibody-ab24479.html and https://www.abcam.cn/survivin-antibody-epr2675-ab134170.html).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HepG-2 cell line was obtained by a 15 years old adolescent male and purchased from KeyGEN BioTECH. MCF-7 cell line was obtained by a 69 years old adult female and purchased from KeyGEN BioTECH. HeLa cell line was obtained by a 31 years old adult female and purchased from KeyGEN BioTECH. L-02 cell line was obtained by a 40 years old adult female and purchased from KeyGEN BioTECH.
Authentication	All cell lines have been authenticated by KeyGEN BioTECH.
Mycoplasma contamination	All cell lines have been tested and confirmed as negative for mycoplasma contamination before use.
Commonly misidentified lines (See ICLAC register)	There are no commonly misidentified cell lines.