# nature portfolio

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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 Editorial Policies and the Editorial Policy Checklist.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

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n/a	Confirmed
	$oxed{x}$ 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. <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>
x	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	$oxed{x}$ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	$\blacksquare$ 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

Perkin Elmer Lambda 35 spectrophotometer, FluoroMax-4(Horiba), Bruker Avance III 400 M NMR spectrometer, Agilent 6540 Q-TOF mass spectrometer, JEOL JEM-1011 transmission electron microscope (Japan), ZEISS Laser Scanning Microscope (LSM 710), BD LSRFortessa Cell Analyzer, PerkinElmer IVIS Lumina K Series III in vivo imaging system.

Data analysis

Mean fluorescence intensity (MFI) were obtained by Zen software on confocal microscope Zeiss LSM710. NMR spectra were analyzed using MestReNovel Lite software (Mestre Research S.L). Average area intensity of mice were recored by the Living Image software on PerkinElmer IVIS Lumina K Series III in vivo imaging system. Image J (Java  $1.8.0_{-}172$ ) was used to analysis the western blot data. FlowJo\_v10.6.2 was used to analyses the Flow Cytometry data. All data were analyzed and statistically calculated using Microsoft Excel 2016 software (Microsoft, Redmond, WA). All statistical data were presented as mean  $\pm$  SD. The DFT calculations were performed with the Gaussian 09 W program package.

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 <u>data availability statement</u>. 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 the source data underlying Figures 2a-f, 4c-e, 5b-c, 7b, 7d, 10c-e and Supplementary Figures 18a-c, 19a-f, 20a-d, 21e-h, 23a-f, 24a-j, 29a-i, 31a-b, 33, 34, 36b, 36d, 38a-b are provided as a Source Data file. All other data supporting the findings of this study are available ether in the article and/or its Supplementary Information files.

ield-spe	ecific reporting	
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<b>x</b> Life sciences	ces Behavioural & social sciences Ecological, evolutionary & environmental sciences	
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_ife scie	nces study design	
All studies must d	isclose on these points even when the disclosure is negative.	
Sample size	Simple sizes of 2-5 biologically independent samples or animals per group were used for in vitro and in vitro studies, respectively, as indicated for specific experiments in Figure captions. We adhered to sample size requirements necessary for determining statistical significance with reference to the numbers used in recent relevant publications (Huang, Y., Guan, Z., Dai, X. et al. Nat. Commun 2021, 12, 2390; Zhang, W., Hu, X., Shen, Q. et al. Nat. Commun. 2019. 10, 1704)	
Data exclusions	No data were excluded from the analyses.	
Replication	All data presented were repeated for at least twice times with similar results as indicated in figure legends.	
Randomization	The samples and animals used in this paper were randomly distributed into several groups for the further experiments.	
Blinding	The experiment data were performed automatically with machine setups independently to the observer. Image acquisition and data analysis were automated and not subject to human bias. For quantitative analysis results (mean fluorescence intensity), we asked other researchers who were either involved or not involved in the study to examine blinded samples for biological effects. Occasionally blinding was deployed,	

## 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.

as in tumor size measurement, with the measuring person having no knowledge on the identity of the mice being measured.

Materials & experimental systems	Methods
n/a Involved in the study	n/a   Involved in the study
Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
Animals and other organisms	·
Human research participants	
Clinical data	
Dual use research of concern	

#### **Antibodies**

Antibodies used

Anti-p115-RhoGEF (Abcam, Catalog no: ab223759, 1:100 dilution,), Anti-GM130 (Abcam, Catalog no: ab52649, 1:1500 dilution), Anti-p53 (Abcam, Catalog no:ab1431, 1:1000 dilution), Anti-p53 (Abcam, Catalog no:ab1431, 1:1000 dilution), Anti-PUMA (Abcam, Catalog no:ab33906, 1:2000 dilution), Bcl-2 (Abcam, Catalog no:ab182858, 1:200 dilution), Bax (Abcam, Catalog no:ab32503, 1:5000 dilution), caspase-3 (Abcam, Catalog no:ab184787, 1:200 dilution) and Anti-GAPDH (Abcam, Catalog no:ab8245, 1:10000 dilution) were purchased from Abcam. Anti-rabbit HRP secondary antibody (Cell Signaling Technology, Catalog no:70745, 1:2000

All antibody were verified by the supplier and each lot has been quality tested. Validation details of the antibodies are available on the manufacture's websites:

https://www.abcam.cn/puma-antibody-ep512y-ab33906.html

https://www.abcam.cn/p15-rhogef-antibody-ab223759.html.

https://www.abcam.cn/p53-phospho-s15-antibody-ab1431.html

https://www.cellsignal.cn/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074?

\_=1642406733512&Ntt=7074&tahead=true

https://www.abcam.cn/gapdh-antibody-6c5-loading-control-ab8245.html

https://www.abcam.cn/caspase-3-antibody-epr18297-ab184787.html

https://www.abcam.cn/bax-antibody-e63-ab32503.html

https://www.abcam.cn/bcl-2-antibody-epr17509-ab182858.html

https://www.abcam.cn/p53-antibody-pab-240-ab26.html

https://www.abcam.cn/p53-antibody-ep892y-cis-golgi-marker-ab52649.html.

#### Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) The human cervical cancer cell line HeLa were purchased from Stem Cell Bank, Chinese Academy of Science (Shanghai, China)

Authentication Cell lines were authenticated by short tandem repeat (STR) testing.

Mycoplasma contamination Cells lines were tested negative for mycoplasma contamination.

Commonly misidentified lines (See <u>ICLAC</u> register)

No commonly misidentified lines were used.

#### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

BALB/C-nu female male mice aged 6–8 weeks of specific pathogen free (SPF) grade were purchased from GemPharmatech Co., Ltd.,

license No.: SCXK (Jiangsu) 2018-0008. The mice were housed in animal-holding units in a pathogen-free environment with

temperature at 22  $\pm$  2 °C and 55  $\pm$  5% humidity, under the 12 h/12 h dark/light cycle.

Wild animals No wild animals were used in this work.

Field-collected samples No field-collected samples were used in this work.

Ethics oversight All the animal experiments in this study were approved by the Institutional Animal Care and Use Committees of GemPharmatech,

approval No.: SYXK (Jiangsu) 2018-0027.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

#### Flow Cytometry

## Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

🗶 A numerical value for number of cells or percentage (with statistics) is provided.

#### Methodology

Sample preparation HeLa cells were seeded in a 6 cm plate (Corning) at a density of 2x105 cells mL-1 and incubated overnight to 60–70%

confluence. Then the culture medium was replaced with medium containing TPE-PyT-CPS (0.2  $\mu$ M). Photoirradiation was imposed with a 532 nm laser (65 mW cm-2, 2 min) after 6 h of incubation. Apoptosis assay was performed 24 h after irradiation. Cells were detached by trypsin and washed with PBS. The cell precipitation was suspended into the 1× binding buffer (100  $\mu$ L) containing 5  $\mu$ L Annexin V-FITC (BD Biosciences) and incubated at room temperature for 1 h in the dark. The

analysis was performed by BD FACSCalibur flow cytometer within 1 h after adding 400  $\mu$ L 1  $\times$  binding buffer.

Instrument BD LSRFortessa Cell Analyzer

Software FlowJo\_V10

Cell population abundance

The flow cytometry was performed on each sample to a total cell number for at least 100000 events

Gating strategy

Briefly, the single cells were selected by FSC and SSC plot. The The fluorescence signal of annexin V was used as an index to detect apoptosis HeLa cells.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.