# 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.

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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.
$\times$	A description of all covariates tested
$\times$	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>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\times$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\times$	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 Flow-cytomery data were collected with CytExpert (ver. 2.3.0.84). Confocal images were collected with FluoView31S (ver.2.3.1.163).

Data analysis

Flow-cytomery data were analyzed with CytExpert (ver. 2.3.0.84). 2D/3D autofluorescence imaging of NADH/FAD were analyzed with Imaris x64 (ver. 9.0.1); Statistical analysis was performed with Origin Pro (ver. 8.00.000) and GraphPad Prism (ver. 7.00); ImageJ (ver. 1.4.3.67) and Matlab R2017a (ver. 1.0.0.1) were used to analyze fluorescent grayscale. Alpha EaseFC 4.0 was used to analyze the WB.

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 <u>availability of data</u>

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 <u>policy</u>

The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information or from the authors upon request. The raw data generated in this study are provided in the Supplementary Information/Source Data file. A reporting summary for this article is available as a Supplementary Information file.

Field-spe	cific reporting				
Please select the or \( \sum \) Life sciences	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.  Behavioural & social sciences				
For a reference copy of t	he document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Life scier	ices study design				
All studies must dis	close on these points even when the disclosure is negative.				
Sample size	o ensure the accuracy of the experiments, at least three replicates were performed. In the characterization experiments, 3-4 samples were sed to analyze particle size and zeta potentials. In the cell experiments, 3-6 samples were used to analyze biocompatibility, toxicity, and herapeutic effect of probes. In antitumor experiments, 5 mice each group were used to analyze tumor volume, survival rate, body weight in hice. For other experiments, the sample size for each group was 3-5.				
Data exclusions	No data were excluded from the analyses.				
Replication	The experiments were reproduced successfully, the number of experiments was listed in corresponding figure legends.				
Randomization	The samples were allocated into experimental groups randomly.				
Blinding	The investigators were blinded to group allocation during data collection and analysis.				
Reportin	g for specific materials, systems and methods				
	on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ed 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.				
	perimental systems  Methods				
n/a Involved in th					
Antibodies	ChIP-seq				
Eukaryotic	cell lines				
Palaeontology and archaeology MRI-based neuroimaging					
Animals and other organisms					
Human research participants					
Clinical data					
Dual use research of concern					
Antibodies					
Antibodies used	Anti-HSP90 Rabbit pAb, P07900, P82995, P07901, diluted to 1:1000; Anti-HSP60 Rabbit pAb, P10809, P63039, P63038, diluted to 1:500; Anti-Integrin α4 Rabbit pAb, p13612, diluted to 1:1000; Anti-Integrin β1 Rabbit pAb, P09055, P49134, diluted to 1:1000; Cy3-labeled goat anti-rabbit IgG, FITC-labeled goat anti-rabbit IgG, diluted to 1:200; All the above were obtained from Wuhan service Biotechnology Co., Ltd.				
Validation	All information about antibodies can be found on the respective antibody website:  Anti-HSP90 Rabbit pAb, P07900, P82995, P07901, diluted to 1:1000, https://www.servicebio.cn/goodsdetail?id=1506  Anti-HSP60 Rabbit pAb, P10809, P63039, P63038, diluted to 1:500, https://www.servicebio.cn/goodsdetail?id=5537				

## Eukaryotic cell lines

Policy information about <u>cell lines</u>

Cell line source(s)

4T1 and C26 cell line was obtained from China Center for Type Culture Collection (Wuhan, China), Luc-4T1 cell line was

 $Anti-Integrin\ \beta 1\ Rabbit\ pAb,\ P09055,\ P49134,\ diluted\ to\ 1:1000,\ https://www.servicebio.cn/goodsdetail?id=2618$ 

Cy3-labeled goat anti-rabbit IgG, FITC-labeled goat anti-rabbit IgG, diluted to 1:200,https://www.servicebio.cn/goodsdetail?id=253;https://www.servicebio.cn/goodsdetail?id=259

obtained from Wuhan service Biotechnology Co., Ltd., RAW 264.7 cell line was was kindly provided by Dr. Degiang Deng Cell line source(s) (Britton Chance Center for Biomedical Photonics at Wuhan National Laboratory for Optoelectronics).

Authentication Each cell line was morphologically confirmed according to the information provided by the cell-source center, and the main 4T1 and RAW 264.7 cell line were authenticated with short tandem repeat (STR) analysis.

All cell lines were confirmed to be mycoplasma-negative by DAPI staining. Mycoplasma contamination

Commonly misidentified lines 4T1 and RAW 264.7 cell lines were of stable origin and there was little possibility of misidentification. (See ICLAC register)

## Animals and other organisms

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

BALB/c female nude mice (purchased as 4-week-old, used as 5-week-old, ~18 g), BALB/c female mice (purchased as 4-week-old, used Laboratory animals as 5-week-old, ~18 g), KM male mice (purchased as 5-week-old, used as 6-week-old, ~32 g) were purchased from Beijing Weitong

Lihua Biotechnology Co., Ltd. All mice were housed in an animal facility under constant environmental conditions (room temperature, 22±1°C, relative humidity, 40-70 % and a 12 h light-dark cycle). All mice had access to food and water ad libitum.

Wild animals The study did not involve wild animals.

The study did not involve samples collected from the field. Field-collected samples

All animal experiments were approved by the animal experiment ethics committee of Huazhong University of Science and Ethics oversight

Technology (IACUC Number: S904).

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

4T1 breast cancer cells were derived from mice, and the cells were cultured in a 5% CO2 incubator at 37 °C for 12 h before Sample preparation used

Instrument CytoFLEX flow cytometer

Software CytExpert (ver. 2.3.0.84) was used to collect and analyze the flow cytometry data.

After the cells were centrifuged, the pelleted cells were taken for flow cytometric analysis. With adjusting the gain and Cell population abundance

compensation, 10,000 cells were taken from each group for analysis.

The untreated cell group was taken, and the voltage was adjusted to make the intensity of FTIC and PI channels around 1000; Gating strategy the single-stained cell group was taken for compensation adjustment and deducting the mutual influence, finally

experimental groups were carry out. If the intensity value was greater than 1000, positive signal was considered to exist.

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