

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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

Analysis of mRNA expression levels of NSUN6-targeted genes in patients was conducted using the TCGA online portal (<https://www.cbioportal.org/>).
Kaplan-Meier survival analysis was collected using <http://gepia2.cancer-pku.cn/>

Data analysis

All statistical analysis were performed with GraphPad Prism v10.2.0 (also mentioned in the manuscript within the methods section)
SHAPE data was analyzed using Qushape (<https://weekslab.org/software/qushape>)

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](#)

All data generated in this study are available in the manuscript/Supplementary Information/Source Data file. Raw and processed data of RNA-seq and the UBS-sequencing data which were deposited at NCBI GEO under accession: GSE279973 and GSE280051. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Sex and gender were not focus of our analysis. However, our study samples were breast cancer cell lines whose primary cell sources were all female.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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	N/A
Data exclusions	Data were not excluded from analysis.
Replication	All the experimental data in this study were replicated at least three times, and the attempts of replication were successful and gave similar results.
Randomization	N/A
Blinding	N/A

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	NSUN6 Rabbit pAb (ABclonal, Cat#A13454), used at 1:1500 β-Actin Mouse mAb (ABclonal, Cat#AC004), used at 1:5000 DYKDDDDK Tag (D6W5B) Rabbit mAb (Cell Signaling, Cat#14793), used at 1:5000 Anti-Digoxigenin-AP, Fab fragments from sheep (Roche, Cat#11093274910), used at 1:10000
Validation	NSUN6 Rabbit pAb (ABclonal, Cat#A13454): The manuscript provides validation of the antibody for Western Blot (WB) in NSUN6 knockout MDA-MB-231 cells in Extended Data Fig. 4c. β-Actin Mouse mAb (ABclonal, Cat#AC004): The manufacturer's website indicates validation for use in human cell lines for WB. Validation results are also provided in Extended Data Fig. 4c, h of the manuscript. DYKDDDDK Tag (D6W5B) Rabbit mAb (Cell Signaling, Cat#14793): The manufacturer's website indicates WB validation for mock transfected or transfected with DYKDDDK-GFP in 293T cells. Anti-Digoxigenin-AP, Fab fragments from sheep (Roche, Cat#11093274910): For the detection of digoxigenin-labeled compounds, the validation results are provided in Fig. 4c, d.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	MDA-MB-231: the sex of primary cell line is female. HEK293T: the sex of primary cell line is female. BT-549 cell line: the sex of primary cell line is female. MDA-MB-468 cell line: the sex of primary cell line is female. All the cell lines were obtained from the Cell Resource Center of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.
Authentication	All cell lines have a correct STR report from the Cell Resource Center of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.
Mycoplasma contamination	No mycoplasma contamination for all the cell lines used in this study.
Commonly misidentified lines (See ICLAC register)	This study did not contain any of the commonly misidentified cell lines.

Plants

Seed stocks	None
Novel plant genotypes	None
Authentication	None