

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 (<i>n</i>) 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. <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>
☒	☐ 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 <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	ITC: VP-ITC microcalorimeter instrument (MicroCal) Fluorescent imaging: Nikon W1 spinning disk confocal microscopy, NIS-Elements z-stack images: ZEISS laser scanning confocal microscopy 710, ZEN 3.2 RNA Blot: Bio-rad gel doc, Bio-rad chemidoc, Li-cor Odyssey imaging system Cell attachment assay: ZEISS Observer 7, ZEN 3.1 pro
Data analysis	ITC: Origin Fluorescent imaging and quantification: Nikon NIS-Elements Viewer, ZEISS ZEN 3.6 (blue edition), ImageJ (FIJI, 2.9.0/1.53t [64-bit]) Colocalization assay: ImageJ (FIJI, 2.9.0/1.53t [64-bit]) with plugins (coloc 2 and JACoP) RNA Blot: Bio-rad image lab Cell attachment assay: ImageJ (FIJI) All the statistics calculation and graph making were performed with OriginPro 2021b or GraphPad Prism 8 Molecular dynamics simulation: oxDNA, oxView, tacoxDNA, oxDNA analysis tools, RNAfold, RNAComposer Scheme drawing: Biorender.

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 data were deposited in the public repository, Figshare, which can be accessed via: https://figshare.com/projects/Spatial_Imaging_of_GlycoRNA_in_single_Cells_with_ARPLA/164113

Spatial_Imaging_of_GlycoRNA_in_single_Cells_with_ARPLA/164113

The data generated and/or analyzed during the current study are also available from the corresponding authors on reasonable request.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

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](https://www.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 statistical methods were used to predetermine the sample sizes. All experiments were performed with at least 3 biological replicates, for each individual biological replicates, 3 technical repeats were performed (cell imaging, RNA gel blot), 6 technical repeats were performed (cell attachment assay).

Data exclusions

No data were excluded from the analysis.

Replication

All experiments (Gel-based assay, ITC, cell imaging and attachment assay) were performed at least 3 times independently. Different batches of materials and buffers were prepared separately. The sensors for cell imaging were prepared separately. And the biological repeats were performed in different days. All experimental data was reliable and reproducible from independent replicates.

Randomization

The primary focus of the in-test-tube experiments was to investigate the biochemical and biophysical interactions of the molecules under controlled conditions. These experiments were performed in a controlled environment, using purified reagents and precisely defined experimental conditions. Given the high level of control over experimental variables in these in vitro assays, randomization is not applicable. Furthermore, the experiments are often designed to measure specific molecular interactions or reactions, where randomization would not be beneficial.

For cell images collection, the samples were allocated into experimental groups based on the specific treatment or cell type under investigation. The allocation was not random, but each experimental group was subjected to identical experimental conditions, including cell culture conditions, incubation times, and reagent concentrations. For the images taking, the ROIs for capturing were chosen randomly and more than 10 frame views were imaged in each well to ensure a fair representation of the sample. To quantify the intracellular fluorescent signals, the ROIs of cells were picked randomly to avoid any bias in the selection process.

Blinding

During the experiments, the cell staining with ARPLA and confocal imaging were performed by two separate researchers, Y.M. and W.G. This process was carried out in a sequential manner, where Y.M. was responsible for the cell staining with ARPLA, and W.G. conducted the confocal imaging. In subsequent experiments, we switched the roles of the researchers, with W.G. performing the staining and Y.M. conducting the imaging, to further ensure unbiased experimental outcomes. For other experiments, blinding was not possible due to objective factors inherent to the nature of the tests. We are confident that this limitation does not compromise the conclusions of our study. The results presented in our manuscript are based on rigorous data analysis and a comprehensive interpretation of the findings.

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

Methods

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

Antibodies

Antibodies used

TSNARE1 Polyclonal Antibody (Invitrogen, # PA5-113146, RRID: AB_2867880), VTI1B Polyclonal Antibody (Invitrogen, # PA5-116120, RRID: AB_2900754), and Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody (Invitrogen, # A32794, RRID: AB_2762834)

Validation

TSNARE1 Polyclonal Antibody: <https://www.thermofisher.com/antibody/product/TSNARE1-Antibody-Polyclonal/PA5-113146>; https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FSLG%2FCertificate%2FCertificates-of-Analysis%2FPA5113146_WG3342203.PDF
 VTI1B Polyclonal Antibody: <https://www.thermofisher.com/antibody/product/VTI1B-Antibody-Polyclonal/PA5-116120>; https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FSLG%2FCertificate%2FCertificates-of-Analysis%2FPA5116120_XH3659143.PDF
 Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody: <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32794>

Eukaryotic cell lines

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

Cell line source(s)

Most of the cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) or Cancer Center at University of Illinois at Urbana-Champaign. The detailed sources information of each cell line is: HeLa CCL2 (ATCC), HEK293T (ATCC), PANC-1 (ATCC), sh-SY5Y (Gift from Dr. Liviu Mirica, UIUC), MCF-10A (Cancer Center, UIUC), MCF-7 (Cancer Center, UIUC), MDA-MB-231 (Gift from Dr. Aaron Baker at UT Austin, the original source was Cell Biolabs Inc.), HL-60 (Cancer Center, UIUC), THP-1 (Gift from Dr. Jefferson Chan at UIUC, the original source was Cancer Center, UIUC), HUVEC (PromoCell)

Authentication

The cells lines were authenticated by ATCC (HeLa, HEK, and PANC-1) or Cancer Center at UIUC (other cell lines) using their standard procedures, which include morphology and STR profiling. In our lab, we routinely check the cell morphology using microscope to ensure their authenticity. Primary cell line (HUVEC) authentication information can be requested from the vendor (PromoCell Inc.).

Mycoplasma contamination

All the cell lines have been tested routinely with mycoplasma PCR detection kit (VWR, Cat 76452-688). No contaminated cells were used in this study.

Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified cell lines were used.