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.

☑️ 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 coefficients) 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 wherever 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 error terms 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 editors contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

CD values of CCK8 and luminescence intensities of CTG were read using a Multi-Mode Detection Platform (SpectraMax Paradigm; Molecular Devices) and analyzed using Microsoft Excel software. Transwell images were collected using fluorescence microscopy (Leica, DMi8) and analyzed using ImageJ software. Immunofluorescence images were obtained using a laser scanning confocal microscope (OLYMPUS FLUOVIEW 1000). Bioluminescence was observed on a Bruker in-Vivo Xtreme system. Biosensor interferometry was performed using ForteBio Octet RED (Sartorius, Germany).

Data analysis

All statistical analyses were performed using GraphPad Prism V8.0 for Windows (acquired from graphpad prism.cn). Immunofluorescence images were analyzed using ZEN and ImageJ software. Immunoblotting bands were analyzed using ImageJ software. Bioluminescence was analyzed using Bruker ML software. CD values of CCK8 and luminescence intensities of CTG were analyzed using Microsoft Excel software.

For manuscripts utilizing custom algorithms or software that are not widely available, describe and cite software available publicly or from a third party and give the data necessary to reproduce the results.

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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 provided in the Source Data file. The protein data of GIT2/β-Pix used in this study is available in PDB database under accession code 6JMT [http://doi.org/10.2210/pdb6JMT/pdb]. The GIT1 structure is available in Alpha Fold under accession code Q9Y2X7 [https://alphafold.com/entry/Q9Y2X7].

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.

[X] 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      | Sample sizes are determined according to previous experience and literatures (see related reference: Jie, M. et al. CircMRPS35 suppresses gastric cancer progression via recruiting KAT7 to govern histone modification. Mol. Cancer 2020, 19, 56). |
|------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions  | No data were excluded.                                                                                                                                                                      |
| Replication      | For in vitro experiments, the repeats were performed using cells (or cell lysates) from at least 3 wells, each well indicate one repeat. For in vivo experiments, the repeats were performed using 6 mice in each group, one mouse represents one repeat. All attempts at replication were successful. |
| Randomization    | Samples and animals were randomly allocated to groups for in vitro and in vivo experiments.                                                                                                       |
| Blinding         | The experiments were not performed in blind. Blinding is not feasible in the experiments for only animals were used in the treatments.                                                       |

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 |
|     | Paleontology 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                  |
|-----------------------------|
| GIT1, Rabbit polyclonal, GIT1 | Cell Signaling Technology (2919), 1:1000 dilution |
| Rac1/2/3, Rabbit polyclonal, Rac1/Thr38, Cell Signaling Technology (2465), 1:1000 dilution |
| Cdc42, Rabbit polyclonal, Cdc42, lys135, Cell Signaling Technology (2462), 1:1000 dilution |
| GAPDH, Rabbit monoclonal, GAPDH (4C10), Cell Signaling Technology (2118), 1:1000 dilution |
| Myc-Tag, Rabbit monoclonal, Myc (71D17), Cell Signaling Technology (2278), 1:1000 dilution |
| HA-Tag, Rabbit monoclonal, Y Cloud, MV, YHPD4QA (E2964A), Cell Signaling Technology (17324), 1:2000 dilution |
| Flag-Tag, Rabbit monoclonal, DYT3, DKKD (DKW38), Cell Signaling Technology (14793), 1:1000 dilution |
| Lys labeled, Goat Anti-Rabbit (GG-HP), Biosciences (170651), 1:800 dilution |
| Goat Anti-Rabbit (GG-HP), Biosciences (170651), 1:3000 dilution |
| Goat Anti-Mouse (GG-HP), Biosciences (170651), 1:3000 dilution |

Validation

All antibodies used were validated by the suppliers for their particular application, and also validated in the lab by staining the cells with or without the specific antigen expression. Validation statements are relevant references for the used antibodies can be found on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines and sex and gender in research

Cell line source(s)

Human gastric cancer cell lines MGC803, MKN45, SGC790, AGS, SGC7901, human gastric epithelial cell line FES-1, and human embryonic kidney cell line HEK 293T were provided by our collaborator, Department of Gastroenterology, Xinhua Hospital, Third Military Medical University, which were originally purchased from American Type Culture Collection (ATCC), National Collection of Authenticated Cell Cultures (Shanghai, China), or other commercial suppliers.

Authentication

All cell lines were routinely examined and confirmed by the morphology and growing behaviors under microscopy, which are consistent with the respective phenotypes showed by the suppliers. Authentication such as STR profiles of the cell lines could be obtained from the suppliers.

Mycoplasma contamination

All cell lines were tested negative for mycoplasma.

Commonly misidentified lines

(SGC7901) is a misidentified cell line registered in ICLAC. As SGC7901 was traditionally considered as a gastric cancer cell line and has been widely used in China. We performed the experiments using SGC7901 as one of the gastric cancer cell lines together with other authenticated lines. We believe that the results would not influence the conclusion of the present study. And we can delete the results involving SGC7901 if necessary.

Animals and other research organisms

Policy information about studies involving animals - ARRIVE guidelines recommended for reporting animal research, and Sex and gender in research

Laboratory animals

Four-week-old female nude mice were purchased from Vital River Company (Beijing, China) and housed in specific pathogen-free (SPF) animal facility (26-71.6°C temperature and 50%-60% humidity) and operated in the Center of Animal Experiments, Third Military Medical University, throughout the experiment.

Wild animals

This study did not involve wild animals.

Reporting on sex

Only female nude mice were used in the study.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

All animal experiments were approved by the Animal Care Committee of Third Military Medical University. All animal experiments were conducted in compliance with all relevant ethical regulations.

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