nature portfolio

Corresponding author(s): Sohail Tavazoie

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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.

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

- [x] The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- [x] A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [x] 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.
- [x] A description of all covariates tested
- [x] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [x] 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)
- [x] 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 whenever suitable.
- [x] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [x] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [x] 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	No custom or open-sourced software was used for data collection
Data analysis	Analysis of previously published whole-exome sequencing data (TCGA, METABRIC) was performed using RStudio v1.1.3, R v3.5, samtools/ bcftools 1.8, and the R packages 'TCGAbiolinks'/'survival'/'survminer as outlined in the methods section. Gene set enrichment analysis was performed using the R package clusterProfiler v3.12. Immunofluorescent and phase contrast images, and Western blot quantification were analyzed using Fiji/ ImageJ (v2.14.0). Optically cleared tumors were analyzed using Imaris. All other graphs and statistical analyses were generated using Graphpad Prism v8. Microsoft Excel v16 was used for data processing. Flow cytometry data was collected on the Attune software and analyzed using FlowJo (v10.10.0).

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

Raw sequencing data and count tables for transcriptional profiling of 4T1-derived spheroids have been deposited at the Gene Expression Omnibus under accession number GSE267958. Reads were mapped to the mouse genome assembly GRCm38. Data for the METABRIC study are publicly available under the EGA accession number EGAS00000000083; data from the TCGA study are publicly available from https://portal.gdc.cancer.gov.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	All human samples used in the study were from female breast cancer patients (>99% of all breast cancer cases).
Population characteristics	No data on population characteristics has been used in the manuscript.
Recruitment	Fresh and FFPE breast tumor samples were obtained from Cooperative Human Tumor Network (CHTN).
Ethics oversight	All research involving human samples at The Rockefeller University is supervised by a Institutional Review Board (IRB). All samples in this study were fully anonymized before being received at The Rockefeller University. The Rockefeller University IRB determined that our study was IRB-exempt.

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	No statistical methods were used to predetermine sample sizes. Sample sizes were arrived based on having at least three independent biological replicates.
Data exclusions	No data exclusions used.
Replication	Studies were repeated and successfully replicated at least twice (biological replicates).
Randomization	Allocation to different experiments was random. For in-vivo experiments, mice were age- and gender-matched; litter mates were used when possible and animals were randomized to each experimental cohort.
Blinding	Tumor measurements and metastasis quantification was performed in a blinded manner. Additionally, all MFI quantifications were performed in a blinded manner.

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

Primary antibodies used for Western blotting included mouse βIII-tubulin (1:1000; Biolegend, MMS-435P, Clone TUJ1), rabbit GAPDH (1:1000; Cell Signaling 2118, 14C10), mouse HSP-60 (1:1000; Santa Cruz Biotechnology, sc-13115), and rabbit TACR1 (1:1000; ThermoFisher, PA1-32229).

Primary antibodies used for immunofluorescence experiments include rabbit anti-CGRP (1:250, Cell Signaling, mAb#14959), mouse anti-βIII-tubulin (1:200, Biolegend, MMS-435P, Clone TUJ1), rabbit anti-Ki-67 (1:250, abcam, ab15580), rabbit anti-substance P (1:200, Sigma-Aldrich, AB1566), mouse anti-neurofilament-L (1:400, Cell Signaling, #2837), mouse anti-dsRNA (1:200, Axxora, JBS-RNT-SCI-10010200) and phalloidin (1:200, ThermoFisher Scientific, A12379). Nuclei were counterstained using Hoechst (1:500, ThermoFisher Scientific, H3579). Secondary antibodies include Donkey AlexaFluor IgG (H+L) conjugates (1:200, ThermoFisher Scientific, A21206, A21202, A31573, A31572, A31571, A31570, A21208).

Validation

All antibodies used have been validated by the manufacturers to be specific to our protein of interest for use against mouse (or human) tissue. Validation data is available at each manufacturer's website by searching under the provided catalog numbers. Manufacturer validation includes assessing cells known to express or not to express the target protein and cross-referencing the expression pattern with available literature or by orthogonal validation using an antibody independent strategy. All antibodies were also used successfully in previous peer-reviewed publications. For TACR1 and TLR7, the antibody was also validated in-house in knockdown cells.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

4T1, EO771, HEK293T, Py8119 and MDA-MB-231 cells were obtained from American Tissue Type collection (ATCC). 4T07 and 67NR cells were a generous gift from W. P. Schiemann (Case Comprehensive Cancer Center).

Authentication

No independent authentication was perfromed.

Mycoplasma contamination

All cell lines were tested for mycoplasma contamination every ~3 months. Cells used in this study were confirmed to be negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used in this study

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

Age and strain of all mice used in the study are described in the results and/ or methods section. All female mice were used in the study. Genetic strains used in the study include BALB/c, C57B6J, and NSG. For mammary fat pad injections and intraductal injections, 5-8 week old mice were used. 6-10 week old female mice were used for tail vein experiments. DRG neurons were isolated from 4-8 week old female mice.

Wild animals

Study did not involve wild animals

Reporting on sex

Only female mice were used in the study.

Field-collected samples

Study did not involve field-collected samples

Ethics oversight

All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the Rockefeller University.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NA
Study protocol	NA
Data collection	NA
Outcomes	NA

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	67NR cancer cells were washed with PBS several times and detached from culture plates using gentle pipetting (no trypsin was used). Cells were stained with TACR1 (extracellular) primary antibody and AF555 secondary antibody. Cells were then resuspended at a concentration of 2-5 x 10^6 / ml and run on the flow cytometer.
Instrument	Attune flow cytometer
Software	The Attune software was used for data collection and Flowjo software v10.10 was used for data analysis.
Cell population abundance	No cell sorting was performed.
Gating strategy	An initial gate based on basal scatter characteristics served to exclude debris followed by singlet gates based on FSC-A and FSC-H. Unstained cells were used to gate for TACR1 positivity. Gating strategy is provided in the Supplementary Information.

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