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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

For disease-specific survival in MDA-MB-231 experiments, power analysis indicated that 10 mice per group will be sufficient to detect a difference at relative hazard ratios of <0.2 or >5 with 80% power and 95% confidence, given a median disease-specific survival of 3 months in the control group and a total follow up period of 250 days. For the 4T1 experiments, Power analysis indicates that 10 mice per group will be sufficient to detect a difference at relative hazard ratios of <0.25 or >4.0 with 80% power and 95% confidence, given a median survival of 58 days in the control group and a total follow up period of 180 days.

2. Data exclusions

Describe any data exclusions.

No data were excluded

3. Replication

Describe whether the experimental findings were reliably reproduced.

No attempts for replication failed. Immunofluorescence and immunoblot experiments were performed in three or more biological replicates (see figure legends). Key animal experiments were performed in up to 5 independent experiments

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No method of randomization was used

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded to group allocation.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Bulk RNA seq code is made publicly available (link in the methods section). Single cell RNAseq was analyzed using Python and already-published data and was appropriately referenced in the methods section. Wound healing assay (surface area) was analyzed using ImageJ. Single cell analysis was performed using Python 3.0. Cell migration using microfluidics devices was performed using MATLAB 2016a. RNAseq data was performed using R

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	There are no restrictions for distribution of research materials produced during this study.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Pre-validated antibodies were purchased from reputable sources (Cell signaling technology and abcam). Antibodies and their catalog numbers are listed in Supplementary information. Most antibodies were further validated when the target protein was depleted using shRNA.

Supplementary Table 1. Antibodies used in immunofluorescence.

Antibody against Company Catalog number
α-tubulin Sigma Aldrich T9026
β-actin Abcam ab8227
β-catenin Abcam ab16051
cGAS Sigma Aldrich HPA031700
Cox IV Abcam ab16056
dsDNA (Extended Fig. 8d) Abcam AB27156
dsDNA (Fig. 5f) Thermo Fisher Scientific MAB1293MI
Human centromere proteins Antibodies Incorporated 15-234-0001
IRF3 Abcam ab68481
p65 Abcam ab16502
Pericentrin Abcam ab4448
RelB Abcam ab180127
ssDNA Thermo Fisher Scientific MAB3299MI
Vimentin Abcam ab201637
STING Abcam ab181125

Supplementary Table 2. Antibodies used in immunoblots.

Antibody against Company Catalog number
β-actin Abcam ab8227
cGAS Sigma Aldrich HPA031700
GFP Life Technologies A11122
IRF3 Abcam ab68481
p100/p52 Cell Signaling Technology 4882
p65 Abcam ab16502
phospho-IRF3 Cell Signaling Technology 4947
phospho-p100 Abcam 194919
phospho-p65 Cell Signaling Technology 3033
phospho-TBK1 Cell Signaling Technology 5483
RelB Cell Signaling Technology 4922
STING Cell Signaling Technology 13647
TBK1 Cell Signaling Technology 3013
TRAF2 Cell Signaling Technology 4712
TRAF3 Cell Signaling Technology 4729
STAT1 Abcam ab47425

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

ATCC

b. Describe the method of cell line authentication used.

None as directly purchased from ATCC

c. Report whether the cell lines were tested for mycoplasma contamination.

Yes. Cells were tested each 4-6 months.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

None of the cell lines used are listed in the ICLAC database.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Animal experiments were performed in accordance with protocols approved by the Weill Cornell Medicine Institutional Animal Care and Use Committee. There was no need to randomize animals. Investigators were not blinded to group allocation. Intracardiac injection was performed as previously described31. Briefly, cells were trypsinized and washed with PBS and a 1x105 cells (in 100 µl of PBS) were injected into the left cardiac ventricle of female athymic 6-7 week old athymic nude (nu/nu) mice (Jackson Laboratory strain 002019). 2x105 cells were injected into the tail vein cohort of animals. Mice were then immediately injected with D-luciferin (150 mgkg-1) and subjected to bioluminescence imaging (BLI) using tan IVIS Spectrum Xenogen instrument (Caliper Life Sciences) to ensure systemic dissemination of tumor cells. Metastatic burden was measured 5-7 weeks after injection using BLI. BLI images were analyzed using Living Image Software v.2.50. Disease-specific survival endpoint was met when the mice died or met the criteria for euthanasia under the IACUC protocol and had radiographic evidence of metastatic disease. For Orthotopic tumor implantation, 2.5x105 cells in 50µl of PBS were mixed 1:1 with Matrigel (BD Biosciences) and injected into the fourth mammary fat pad. Only one tumor was implanted per animal. MDA-MB-231 primary tumors were surgically excited before they reached ~1.5 cm in the largest dimension (which was the maximum allowable under our IACUC protocol) and metastatic dissemination was assessed using BLI imaging at 1-3 week intervals for up to 30 weeks. Distant metastasis-free survival endpoint was met when BLI signal was seen outside of site of primary tumor transplantation. 4T1 tumors were excised 9 days after implantation. To derive short-term culture from primary tumors and metastases, anesthetized animals (isofluorane) were imaged then sacrificed. Ex-vivo BLI was subsequently performed on harvested organs to define the precise location of the metastatic lesion. Primary tumors and metastases were subsequently mechanically dissociated and cultured in DMEM with selection media (G418 or hygromycin) to select for tumor cells and exclude host cells. All subsequent assays (karyotyping, RNAseq, immunofluorescence, and subcellular fractionation) were performed after a single passage from the primary sample. To assess chromosome missegregation from primary tumor-derived and metastases-derived cells, we performed high-resolution immunofluorescence analysis on passage #1 cells, staining for DNA (DAPI) and centromeres (ACA). Cells with DNA or centromere staining in the middle of the anaphase plate was taken as evidence of chromosome missegregation.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

All human samples reported in the manuscript were from studies that were previously published and are adequately referenced in the manuscript. Details of patient characteristics - if available - is listed in the referenced manuscript.