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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.

No statistical methods were used to predetermine samples sizes.

2. Data exclusions

Describe any data exclusions.

None

3. Replication

Describe whether the experimental findings were reliably reproduced.

Experiments were repeated at least three times in the laboratory with the following exceptions, the RNAi screen and deep sequencing based competition assays (Fig. 1a-b, Extended Data Figure 1 c,d), immunohistochemical staining of NFS1 in human/mouse tissue (Fig 2b,c, Extended Data Fig. 4), and effects of cyst(e)inase or SSA/BSO on tumour growth (Fig. 4), which were performed once.

4. Randomization

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

To study the effects of cyst(e)inase or SSA/BSO on tumour growth, animals were randomly assigned into a treatment group with the constraint that the starting tumour burden in the treatment and control groups were similar.

5. Blinding

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

Studies were not conducted blind except analysis of the number of metastatic foci and immunohistochemical analysis of NFS1 levels in human tissue.

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.	Microsoft Excel, Prism, R, FlowJo

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.	No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibodies to NFS1 (sc-365308) from Santa Cruz, CD31 (77699), RPS6 (2217), TFRC (13208), FTH1 (3998), SOD1 (2770), and SOD2 (13141) from Cell Signaling Technologies, Ki67 (M3062) from Spring Biosciences; and RFP (600-401-379) from Rockland. Antibodies were validated by suppression of the intended target gene by RNAi and verification of the loss of a band of the predicted molecular weight by immunoblotting.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	MCF10DCIS.com cells from the Karmanos Cancer Center, Michigan; MDA-MB-231, SW900, NCI-H196, A549, NCI-H2170, NCI-H647, 786-O, A498 NCI-H838, NCI-H460 and SK-MES-1 from ATCC; NCI-H322 from Sigma
b. Describe the method of cell line authentication used.	STR based profiling
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were tested for mycoplasma contamination
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 used

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.	4-8 week old NOD/Scid female mice used in this study were purchased from Jackson Labs

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.	Not applicable

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Cells were plated in 6 well plates the day prior to incubation with indicated treatments. For all flow experiments involving DCIS cells, 150,000 cells/ well were plated. For 231 cells, 200,000 cells/well were plated for experiments involving BSO and 100,000 for all other experiments. With the exception of experiments involving tbHP, cells were incubated under indicated conditions, washed twice with 1X PBS and stained with 10 µM of CM-H2DCFDA or 10 µM BODIPY 581/591 C11 diluted in PBS for 20 minutes in an incubator at 37° and 21% O2. Following staining, cells were washed with PBS, trypsinized, and collected in 500 µL of PBS. For tbHP experiments, cells were washed twice, stained with the indicated probes, treated with tbHP in serum-containing media for 4 hrs, washed again, trypsinized, and collected. For experiments performed at 3% oxygen, cells were equilibrated for 1 week prior to assessing ROS level.
6. Identify the instrument used for data collection.	Life Technologies Attune NxT Acoustic Focusing Cytometer
7. Describe the software used to collect and analyze the flow cytometry data.	Data was collected using Attune NxT Software v2.5. Analysis of all data was performed on FlowJo V10 software.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Cells were not sorted.
9. Describe the gating strategy used.	Live cells were selected from the starting cell population on a FSC/SSC plot. Live cells were selected from the starting cell population on a FSC/SSC plot. Then, single cells were selected using a FSC-A/FSC-H plot from the population of live cells. Data displayed represents populations of live, singlet cells. To collect ROS measurement data for both CM-H2DCFDA and BODIPY 581/591 C11, an excitation wavelength of 488nm was used with an emission filter of 530/30nm. Percent of cells above the fluorescence of the maximum 1% of the control is indicated.

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