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Corresponding author(s): Cyrus M. Ghajar

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Reporting Summary

Nature Research 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 Research 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 (*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 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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever 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 level 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 biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Acquired LC-MS metabolomic data was converted from raw to mzXML file format using Mass Matrix (Cleveland, OH). Metabolite assignments, isotopologue distributions, and correction for expected natural abundances of deuterium, 13C, and 15N isotopes were performed using MAVEN (Princeton, NJ).
Data analysis	Metaboanalyst5.0 (open source, metaboanalyst.ca) was used for Metabolite Set Enrichment Analysis. CellProfiler (open source, cellprofiler.org) was used for quantification of ROS intensity with an unbiased analysis pipeline. FIJI ImageJ (2.1.0, open source) was used for quantification of tumour area fraction and oxidation state. An Image J macro designed to measure Grx1-roGFP2 oxidation state by the laboratory of Tobias Dick was used, thereby eliminating user bias. Living Image Software (Perkin Elmer) was used to quantify bioluminescent tumour burden in mice. Statistical analyses were conducted using PrismGraphpad version 7 (GraphPad Software, CA). Morpheus (open source, https://software.broadinstitute.org/morpheus) was used to create heatmaps.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Raw values for the metabolomics in Figure 3 and Extended Data Figure 3 can be found in Supplementary Table 1. The full metabolomics dataset has been deposited

in Metabolomics Workbench (Study ID: ST002058) and can be directly accessed via its project DOI: http://dx.doi.org/10.21228/M89135.

Raw data for Figs. 1e, 1f, 1h; 2c, 2e-h; 3d-e, 3g-i; 4b-d, 4f-h, 4j; 5a-b, 5e, 5g, 5j; 6a-b, 6d, 6g, 6i, 6n, 7c-f, 7h, 7j-k; 8a, 8c, 8d, 8f, and 8g-h and Exnteded Data Fig. 2b, 2d, 3b-e, 4b-c, 4e, 5b, 5d-f, and 5h-j have been provided as individual Source Data files.

Additional details pertaining to the AluYb8 qPCR method and the studies described in Figure 1 and Extended Data Figure 2 are provided as Supplementary Table 2.

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	We conducted most culture experiments a minimum of three independent times, at minimum in triplicate. Most co-culture studies included at least 5 technical replicates. There is one exception where a co-culture experiment was not performed thrice: We performed our co-culture studies with C57BL/6-derived myocytes (WT and MCAT) in duplicate, with five wells per condition. Since we derived two unique MCAT myocyte lines from two different mice, we treated these samples as biological replicates. We felt that two independent co-culture studies that included two biological replicates (of 5 wells each) sufficiently tested our hypothesis - especially given the fact that in these studies, our measurements were made on a cell-by-cell basis, so we were able to record hundreds of unique data points from the conditions described above. All animals studies had a minimum of five mice per cohort. Sample size in mouse studies were based on pilot studies that measured the penetrance of tumor cells/tumor colonization in skeletal muscle (via tumor burden measurements). Sample size was calculated with the tumor burden measurements from the two cohorts (uninoculated and incoculated), with 80% power at least, and accepting P=0.05 as significant. Subsequent experimental studies used sufficient mice per cohort to reach statistical significance between the cohorts.
Data exclusions	No data were excluded from analysis or depiction within the manuscript.
Replication	Culture studies were replicated in triplicate (minimum). All attempts at replication were successful. Mouse studies contained sufficient mice to reach statistical significance with confidence. Immunofluorescent staining of culture wells and tissue sections were performed on at least three wells/slides per sample, and 5 cells of interest, at the very least, were sampled. Tumor cells in lung, brain and bone marrow were sampled with greater frequency due to their greater abundance in the tissue compared to skeletal muscle. All attempts at replication were successful.
Randomization	Randomization most directly applies to the animal studies in the manuscript. Experimental cohorts were assigned at random at the onset of the study.
Blinding	Blinding was not performed for cell culture experiments. These have automated inputs and outputs that remove bias. The individuals conducting animal experiments were blinded during data analysis. For mouse studies, investigators were not blinded to the allocation of differing cell lines used upon injection. Blinding was not necessary because bioluminescent imaging readouts were performed and are not subjective. Additionally, BLI analysis, metastatic foci counting, and DTC abundance analysis was performed blinded and region of interest sizes and image settings were kept consistent across groups.

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
☐	☒ Human research participants
☒	☐ 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
GFP : Chicken/IgY Polyclonal, Abcam ab13970, 10 mg/mL stock, used at 1:1000, Lot GR3190550-3, GR3190550-30, GR236651-3, amongst other lots
GFP : Chicken/IgY Polyclonal, Aves Labs Inc. GFP-1010, 10 mg/mL stock, used at 1:250, Lot GFP3717982, Used in whole mount
Ki67 : Mouse, Clone B56, BD BioSciences 561126, unknown stock conc., used at 1:200, Lots 5324782 and 9262114, Antibody conjugated to A647
Pan-laminin: Rabbit, Polyclonal, Sigma L9393, 0.5mg/mL stock, used at 1:500, Lot 099M-4886V
CD31: Armenian Hamster, Clone 2H8, Millipore MAB1398z, 0.5mg/mL stock, used at 1:300, Lot 3574365
Myosin: Mouse, Clone MY-32, Sigma M4276, unknown stock conc., used at 1:400, Lot 025M4773V
CD49f: Rat, Clone GoH3, BD BioSciences 555734 0.5mg/mL stock, used at 1:200, Lot 9206836
RFP: Rabbit, Polyclonal, Rockland 600-401-379, 1mg/mL stock, used at 1:200, Lot 42896
Laminin alpha-2: Rat, Clone 4H8-2, Sigma L0663, 2mg/mL stock, used at 1:300, Lot 2828586
Catalase: Rabbit, Polyclonal, Abcam ab16731, 1mg/mL stock, used at 1:2000 Lot GR3218544-1, Used for Western blot
Beta-actin: Mouse, Clone AC-15, Sigma A5441, unknown stock conc., used at 1:7500 Lot 127M4866V, Used for Western blot

Secondary Antibodies
Phalloidin (F-actin): Alexa Fluor 488 and Alexa Fluor 568, Fisher Scientific A12370, A12380, 1:500 dilution for IF
Goat anti-chicken IgY: Alexa Fluor 488 Abcam ab150169 2 mg/mL 1:500 dilution for IF, 1:400 for whole mount
Goat anti-rabbit IgG: Alexa Fluor 488 Invitrogen/Fisher Scientific A11008 2 mg/mL stock, 1:500 dilution for IF
Goat anti-rabbit IgG: Alexa Fluor 568 Invitrogen/Fisher Scientific A11011 2 mg/mL stock, 1:500 dilution for IF
Goat anti-rabbit IgG: Alexa Fluor 647 Invitrogen/Fisher Scientific A21244 2 mg/mL stock, 1:500 dilution for IF, 1:400 for whole mount
Goat anti-mouse IgG: Alexa Fluor 568 Invitrogen/Fisher Scientific A11031 2 mg/mL stock, 1:500 dilution for IF
Goat anti-mouse IgG: Alexa Fluor 647 Invitrogen/Fisher Scientific A21235 2 mg/mL stock, 1:500 dilution for IF
Goat anti-Armenian hamster IgG: Alexa Fluor 568 Invitrogen/Fisher Scientific AB175716 2 mg/mL stock, 1:500 dilution for IF
Goat anti-rat IgG: Alexa Fluor 647 Invitrogen/Fisher Scientific A21247 2 mg/mL stock, 1:500 dilution for IF

Validation

GFP and RFP antibodies were validated using isotype controls and also by staining mice that were not inoculated with GFP+ and RFP+ cells with the entire staining protocol (including GFP or RFP antibody) ensure that staining was null. All other staining antibodies used were chosen due to extensive use in prior publications, and validated independently for specificity by using isotype controls. Human cells in culture or mouse tissue sections with known expression of the protein of interest were stained to further validate the antibodies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Primary human skeletal muscle and lung fibroblast cells were obtained commercially from Lonza. Human breast cancer cell lines (MDA-MB-231, Hs578T, BT549, HCC70, MDA-MB-468), human 293T and Phoenix-AMPHO cells and mouse stromal cells (OP-9) were purchased commercially (ATCC). Mammary cancer cell line 4T1 was obtained from Karmanos Cancer Institute. EO771 were a gift from H.K. Lyerly (Duke University). Original commercial source for 4T1 and EO771 cells is ATCC.

Authentication

All cell lines were authenticated routinely by their characteristic 2D and 3D morphologies.

Mycoplasma contamination

Cells are mycoplasma tested upon arrival and every 6 months thereafter in the Ghajar Laboratory. No cell that we have maintained, including any of the cell lines or primary cells used in this study, have become contaminated with mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

We used mus musculus (mice) for our studies. NOD/SCID(NOD.CB17-Prkdcscid/J(NOD.SCID) (NOD-SCID, Strain 394) and BALB/c (Strain 028) female mice were purchased from Charles River and enrolled in study at six to eight weeks of age. C57BL/6NJ (005304) and B6.Cg-Tg(CAG-OTC/CAT)4033Prab/J ("mCAT", 016197) female mice were purchased from Jackson Laboratories and enrolled in study at six to ten weeks of age. Female NSG (NOD-SCID-Il2rg−/−) mice were obtained from the FHCRC CCEH core and enrolled in study at six weeks of age. Skeletal muscle specimens from female B6.Cg-Tg(CAG-OTC/CAT)4033Prab/J mice, aged 11 weeks, were also given as a gift from the Rabinovitch lab at the University of Washington.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All animal work was performed in accordance with institutional, IACUC (specifically, Fred Hutchinson Cancer Research Centre protocol 51075) and AAALAS guidelines and ethical regulations.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Skeletal muscle specimens (tibialis anterior, quadriceps, gastrocnemius) utilized in this study were collected from two female patients who had a diagnosis of metastatic breast cancer and provided informed consented to donate tissue post-mortem to the BROCADE rapid autopsy project. The two participants in our study were aged 54 and 61, with documented ER+ and PR+ disease at the time of diagnosis. The human specimens are de-identified and additional details have not been disclosed.
Recruitment	As enrolment in a rapid autopsy program is a sensitive topic, patients were recruited by their oncologist, based on their understanding of the patient's prognosis, state of mind & wishes to participate in research. These clinicians were investigators or collaborators of the Brocade program.
Ethics oversight	IRB approval was obtained for the use of human biospecimens (Peter MacCallum Cancer Centre IR # 10474) and all guidelines have been followed.

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