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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	Libraries were sequenced using Illumina Next Seq platform and processed with Illumina commercial software. Confocal images were acquired with Leica Application Suite X (LAS X) v.3.5.5.19976 (Leica). Concentrations of RNA were determined by Nanodrop 1000 (Thermo Fisher Scientific). qPCR data was collected using QuantStudio™ Design & Analysis Software version 1.5.1 (Thermo Fisher Scientific). Western blotting was acquired with ChemiDoc™ Imaging System v. 2.3.0.07 (Bio-Rad). Steady-state metabolomics and metabolic flux analysis analysis was performed on an API-4000 triple quadrupole mass spectrometer (AB Sciex) coupled with a HPLC system (Agilent) and CTC PAL HTS autosampler (PAL System). The cell sorting was performed by FACSAria IIIu cell sorter (BD Biosciences, San Josè, CA). Cytofluorimetric analysis was performed using BD FACSCanto™ II Cell Analyzer (BD Biosciences). Fluorescent signals and absorbance in microplates were collected using i-control™ software version 2.0.10 (Tecan). Imaging on stereomicroscope was performed using Leica Application Suite V4.13 (Leica). AFM force indentation measurements were performed using a XE Bio - AFM (Park Systems, South Korea) equipped with an inverted microscope (Nikon Eclipse Ti).
Data analysis	FIJI version 2.1.0 (used for analyzing fluorescence intensity, physical parameters of colonies/tumorspheres, and counting invading cells) GraphPad Prism version 8.2.1 (used to generate graphs and perform statistical analysis) Living Image® Software (IVIS Imaging Systems) version 4.7.3 (used for the analysis of fluorescent signals in whole organs) Next Generation Diagnostic srl proprietary NEGEDIA Digital mRNA-seq pipeline version 2.0, ROSALIND® (https://rosalind.bio/), and DESeq2 version 1.30.1 (used for RNA-seq analysis) ShinyGO version 0.76.3 (http://bioinformatics.sdstate.edu/go/) (Used for Gene Ontology (GO) and KEGG pathway enrichment analysis) Image Lab Software version 6.0 (used for Western blot quantitative analysis) MultiQuant™ software version 3.0.2 (used for data analysis of metabolite and peak review of chromatograms). FACSDiva Software program version 9.0.1 (BD Biosciences) (used for cell sorting analysis and cytofluorimetric analysis)

Excel version 2311 (used for fold-change calculation and data normalization)
Images were treated (crop, overlay, colors) with FIJI version 2.1.0 or Adobe Photoshop CC2019 according to NPG's image integrity rules.

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

The raw and processed RNA sequencing data generated in this study have been deposited in the GEO database under accession code GSE229792 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE229792).
The RNA expression and associated clinicopathological data from the METABRIC cohort used in this study are available in cBioPortal under accession id brca_metabric [https://www.cbioportal.org/study/summary?id=brca_metabric].
Human GRCh38 and mouse GRCm39 reference genomes (primary genome assembly) used for the alignment of RNA sequencing data are available on GENCODE [https://www.gencodegenes.org/].
Publicly available RNA expression and associated clinicopathological data of 1904 patients from the METABRIC (Molecular Taxonomy of Breast Cancer Consortium) dataset 3 were retrieved from cBioPortal (http://www.cbioportal.org/).
All data are available in the manuscript, in the Supplementary Information or in the Source Data files. Source data are provided with this paper.

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	For in vitro and in vivo experiments sample size was chosen based on standards in the field and is indicated either in figure legends throughout the manuscript or directly shown as dots in the graphs.
Data exclusions	Data were not excluded from the analysis of in vitro and in vivo experiments for reasons other than technical mistakes.
Replication	All data are the result of independently-repeated experiments with independent biological samples. All experimental repetitions were carried out by thawing a new aliquot of cells deriving from the original stock. All qPCR experiments have been repeated at least three times independently, and each biological replicate was quantified by using at least 2 technical replicates (which were excluded from statistical analyses). All immunoblots and immunofluorescences have been repeated at least two times independently. Clear statements have been put into Methods section and Figure legends.
Randomization	The mice were randomly put into separate/groups cages for experiments.
Blinding	For mice, metabolite, and AFM studies, the experiments were performed in a blinded fashion when possible, which means that people performing the assays were not aware of the treatment groups until the data analyses were completed. Immunofluorescences and stainings were acquired by randomly selecting cells based on nuclear counterstain.

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

Western blot:
UBIAD1 (1:1000, Atlas Antibodies, Cat# HPA044862, RRID: AB_2679117),
UBIAD1/TERE1 (1:250, Santa Cruz Biotechnology, clone H8, Lot. #I2313, Cat.# sc-377013),
VINCULIN (1:5000, Sigma Cat.# V9131, RRID:AB_477629),
β-ACTIN (1:5000, Sigma Cat.# A5316, RRID:AB_476743),
p-FAK (Tyr397) (1:1000, GeneTex Cat.# GTX129840, RRID:AB_2886103),
FAK (1:200, Santa Cruz Cat.# sc-558, RRID:AB_2300502),
AKT(pan) (1:1000, Cell Signaling Cat.# 4691, RRID:AB_915783),
p-AKT1 (Ser473) (1:1000, Cell Signaling Cat.# 9018, RRID:AB_2629283),
p-AKT2 (Ser474) (1:1000, Cell Signaling Cat.# 8599, RRID:AB_2630347),
GSK-3β (1:1000, Cell Signaling Cat.# 9832, RRID:AB_10839406),
p-GSK-3β (Ser9) (1:1000, Cell Signaling Cat.# 9323, RRID:AB_2115201),
ERK1/2 (1:1000, Santa Cruz Cat.# sc-514302, RRID:AB_2571739),
p-ERK1/2 (Thr202/Tyr204) (1:1000, Cell Signaling Cat.#9101, RRID:AB_331646),
FSP1/AMID (1:1000, Santa Cruz Cat.# sc-377120),
GPX4 (1:1000, Abcam Cat.# 41787, RRID:AB_941790),
HMGCR (1:500, Santa Cruz Biotechnology Cat. #sc-271595, clone C-1, RRID: AB_10650274),
ERα (1:500, Santa Cruz Biotechnology Cat.# sc-542, clone MC-20, RRID:AB_631470),
HRP-conjugated anti-rabbit immunoglobulin G (IgG) (Sigma Cat. #A6154, RRID: AB_258284),
and HRP-conjugated anti-mouse IgG (Sigma Cat. #A4416, RRID: AB_258167).

Immunohistochemistry:
UBIAD1 (0.17µg/mL, ProteoGenix Home-made. Peptide sequence: cys-FRSQAFNKLPQRTAK),

Immunofluorescence:
CD31 (1:20, Dianova Cat.# DIA-310, RRID:AB_2631039),
Ki-67 (1:100, Cell Signaling Cat.# 9129-S, RRID:AB_2687446),
APC-Cy7 CD45 (1:100, BD Biosciences Cat.# 557659, RRID:AB_396774),
Paxillin (1:400, Transduction Laboratories Cat. #P13520),
FAK (1:50, Santa Cruz Cat.# sc-558, RRID:AB_2300502),
Phalloidin (1:300, Sigma-Aldrich Cat. #P1951, RRID:AB_2315148),
anti-mouse Alexa Fluor 488 (0.75µg/ml, Thermo Fisher Scientific, A-11001),
and anti-rat Alexa Fluor 488 (5µg/ml, Thermo Fisher Scientific, A-11006).

Validation

All primary antibodies are commercially available and validated by the manufacturer. Only anti-UBIAD1 from ProteoGenix was custom-made and not commercially available.

The following primary antibodies were used for Western blot experiments:

1) anti-UBIAD1 (Atlas Antibodies), validated with Western blot analysis in human fallopian tube tissue. Further validation via WB analysis in our hands using UBIAD1-overexpressing and UBIAD1-Knock out MDA-MB-231 cell lines. https://www.atlasantibodies.com/products/antibodies/primary-antibodies/triple-a-polyclonals/ubiad1-antibody-hpa044862/
2) anti-UBIAD1/TERE1 (Santa Cruz Biotechnology), validated with Western blot analysis in HeLa whole cell lysate. Further validation via WB analysis in our hands using UBIAD1-overexpressing and UBIAD1-Knock out MDA-MB-231 cell lines. https://www.scbt.com/p/tere1-antibody-h-8
3) anti-VINCULIN, validated with Western blot analysis in DLD-1 and HCT-116 mock or treated with miR-145; further validation in HeLa, COS7, NIH-3T3, RAT2, CHO, MDBK and MDCK. https://www.sigmaaldrich.com/US/en/product/sigma/v9131
4) anti-Beta-Actin, validated with Western blot analysis in a variety of tissues and species. https://www.sigmaaldrich.com/catalog/product/sigma/a5316?lang=it&region=IT
5) anti-p-FAK Tyr397, validated with Western blot analysis in HUVEC whole cell treated with Pervanadate; HeLa whole cell extracts transfected with PTK2 shRNA; HepG2 whole cell extracts treated with CIP or EGF. https://www.genetex.com/Product/Detail/FAK-phospho-Tyr397-antibody/GTX129840
6) anti-FAK, validated with Western blot analysis in HeLa whole cell lysate. https://www.scbt.com/p/fak-antibody-c-20
7) anti-AKT, validated with Western blot analysis in extracts from HeLa, NIH-3T3, C6 and COS. https://www.cellsignal.com/products/primary-antibodies/akt-pan-c67e7-rabbit-mab/4691
8) anti-p-AKT1 Ser473, validated with Western blot analysis in extracts from LNCaP cells, transfected with a construct expressing Akt1 shRNA or Akt2 shRNA, uninduced or doxycycline-induced; purified recombinant phospho-Akt1, phospho-Akt2 and phospho-Akt3 proteins; extracts from Akt1 (-/-) mouse embryonic fibroblast (MEF) or Akt2 (-/-) MEF, untreated or stimulated with hPDGF. https://

www.cellsignal.com/products/primary-antibodies/phospho-akt1-ser473-d7f10-xp-rabbit-mab-akt1-specific/9018
9) anti-p-AKT2 Ser474, validated with Western blot analysis in extracts from Akt1 (-/-) mouse embryonic fibroblasts (MEF) or Akt2 (-/-) MEF, untreated or treated with Human Platelet-Derived Growth Factor AA (hPDGF-AA); extracts from LNCaP cells, transfected with a construct expressing Akt1 shRNA or Akt2 shRNA, uninduced or doxycycline-induced; purified recombinant phospho-Akt1, phospho-Akt2 and phospho-Akt3 proteins. https://www.cellsignal.com/products/primary-antibodies/phospho-akt2-ser474-d3h2-rabbit-mab-akt2-specific/8599
10) anti-GSK-3β, validated with Western blot analysis in extracts from wild-type, GSK-3α (-/-), and GSK3β (-/-) mouse embryonic fibroblasts (MEFs) and HeLa cells. https://www.cellsignal.com/products/primary-antibodies/gsk-3b-3d10-mouse-mab/9832
11) anti-p-GSK-3β Ser9, validated with Western blot analysis in extracts from NIH/3T3 cells, λ-phosphatase- or PDGF-treated; extracts from wild type, GSK-3α (-/-) and GSK-3β (-/-) mouse embryonic fibroblast cells (MEF), untreated or PDGF treated. https://www.cellsignal.com/products/primary-antibodies/phospho-gsk-3b-ser9-5b3-rabbit-mab/9323
12) ERK1/2, validated with Western blot analysis in U-2 OS, ALL-SIL, HEL 92.1.7, M1, TK-1, RAW 264.7, HEK293, THP-1, DU 145, 3611-RF, K-562, MCF7 and Jurkat whole cell lysates; https://www.scbt.com/p/erk-1-2-antibody-c-9?gclid=Cj0KCQjw3a2iBhCFARIsAD4jQB0f9Imr0_aIH4ahu3ULWA3KA8iX5cvI9iXz2-66nG4XcS2e0jfhg7EaArQcEALw_wcB
13) p-ERK1/2 Thr202/Tyr204, validated with Western blot analysis of purified phosphorylated and nonphosphorylated p42 MAP kinase; whole-cell extracts from unstarved wild-type mouse embryonic fibroblasts (MEFs) treated with the indicated combinations of basic Fibroblast Growth Factor (bFGF #9952, 100 ng/ml for 30 minutes), Platelet-Derived Growth Factor (PDGF #9909, 100 ng/ml for 30 minutes), MEK1 Inhibitor (PD98059 #9900, 50 µM, 2 hour pre-treatment), and MEK1/2 Inhibitor (U0126 #9903, 10 µM, 2 hour pre-treatment). https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-antibody/9101
14) anti-FSP1/AMID, validated with Western blot analysis in whole cell lysates of Hep G2, NIH/3T3, HEK293 and A-673. https://www.scbt.com/p/amid-antibody-b-6
15) anti-GPX4, validated with Western blot analysis in extracts of HeLa Knockout or WT; different human tissues. https://www.abcam.com/products/primary-antibodies/glutathione-peroxidase-4-antibody-ab41787.html
16) anti-HMGCR, validated with Western blot analysis in J774.A1 whole cell lysate; in non-transfected (sc-117752) and mouse HMGCR transfected (sc-120842) 293T whole cell lysates. https://www.scbt.com/p/hmgcr-antibody-c-1
17) anti-ERα, validated with Western blot analysis of human recombinant ERα. https://www.scbt.com/p/eralpha-antibody-mc-20

The following primary antibodies were used for immunohistochemistry experiments:

1) anti-UBIAD1 (ProteoGenix), validated with immunohistochemistry analysis on tissue samples of human mammary gland and breast cancer.

The following primary antibodies were used for immunofluorescence experiments:

1) anti-CD31, tested by WB in J558L (CD31-) cells, murine lung and Lend cells (CD31+) and by IHC (murine lung, skeletal muscle, spinal cord, liver, and murine adenocarcinoma). https://www.dianova.com/downloads/dianova/en/dia310englv2.pdf
2) anti-Ki-67, validated with confocal immunofluorescent analysis of the ventricular zone in P21 mouse brain and HeLa cells; https://www.cellsignal.com/products/primary-antibodies/ki-67-d3b5-rabbit-mab/9129
3) anti-CD45 APC-Cy7, not validated by manufacturer; cited in 213 publications. https://www.citeab.com/antibodies/2408484-557659-bd-pharmingen-apc-cy-7-rat-anti-mouse-cd45
4) anti-Paxillin (1:400, Transduction Laboratories Cat. #P13520), validated in Western blot on a A431 lysate and in immunofluorescence in human fibroblastsr; cited in 5 publications. https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.610051.pdf
5) anti-FAK (1:50, Santa Cruz Cat.# sc-558, RRID:AB_2300502), validated in immunofluorescence in methanol-fixed HeLa cells; cited in 8 publications. https://datasheets.scbt.com/sc-558.pdf
6) Phalloidin (1:300, Sigma-Aldrich Cat. #P1951, RRID:AB_2315148), validated in human podocytes with disrupted actin filaments upon treatment with cytochalasin D; cited in 332 publications. https://www.sigmaaldrich.com/US/en/product/sigma/p1951?gclid=CjwKCAiApuCrBhAuEiwA8VJ6Jhd8cqd_oi3-tgrQ6GxHH4IdK4LZblxkFZNIlkyCLtbJUcBB6PVm0xoCOtwQAvD_BwE

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	MCF10A (ATCC Cat.# CRL-10317™), MCF7 (ATCC Cat.# HTB-22™), T-47D (ATCC Cat.# HTB-133™), BT474 (ATCC Cat.# HTB-20™), HCC202 (ATCC Cat.# CRL-2316™), HCC1143 (ATCC Cat.# CRL-2321™), MDA-MB-438 (ATCC Cat.# HTB-132™), MDA-MB-436 (ATCC Cat.# HTB-130™), MDA- MB-231 (ATCC Cat.# HTB-26™), Hs578T (ATCC Cat.# HTB-126™), and HEK293T (ATCC Cat.# CRL-11268) were obtained from ATCC. JIMT-1 were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Cat.# ACC-589).
Authentication	All cell lines were already authenticated by the vendors. MDA-MB-231 cell line was further verified by PCR-single-locus-technology analyzing independent PCR-systems (Eurofins Genomics).
Mycoplasma contamination	The cell lines were not contaminated by mycoplasma as determined by using the N-GARDE Mycoplasma PCR Reagent set PCR kit (Euroclone EMK090020)
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	The mice used in this study were female 6-40-week-old. Mice with C57BL/6J background were used for the genetic models of breast cancer, while NSG mice were used for xenografts. All mice were maintained on a 12hr light/dark cycle in a temperature-controlled environment (room temperature, 22°C) with 40-60% humidity and given free access to water and food. Immunodeficient mice (NSG) were kept under specific pathogen free (SPF) environment. The following mice lines were used: MMTV-PyMT (The Jackson Laboratory JAX #022974), MMTV-NeuT/Erbb2 (The Jackson Laboratory JAX #005038), Ubiad1+/- (here generated), Ubiad1f/f (Custom-made by Cyagen), K14-Cre; Brca1f/f; Tp53f/f (KBP; obtained from Prof. Bouwman at The Netherlands Cancer Institute), and NOD/SCID common γ chain knockout (NSG; Charles River). All mouse experiments were conducted in a certified animal facility under the control of the institutional organism for animal welfare and ethical approach to animals in experimental procedures (Unipd OBPA). Animal studies were conducted with the approval of Italian Ministry of Health and were performed in accordance with the Italian law (D.lgs. 26/2014), which enforces Dir. 2010/63/EU (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes) and EU 86/609 directive.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All experiments were performed in accordance with the European and Italian Legislations (Directive 2010/63/EU) and with permission for animal experimentation from the Ethics Committee of the University of Padua and the Italian Ministry of Health (Authorization number 336/2019-PR).

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	IEO breast cancer patients from years 2000-2012 randomized to metronomic CM (cyclophosphamide+methotrexate) maintenance chemotherapy or no chemotherapy derive from an internationally registered clinical trial of the International Breast Cancer Study Group: IBCSG 22-00 (published in Colleoni M. et al., JCO 2016).
Study protocol	IEO breast cancer patients from years 2000-2012 randomized to metronomic CM (cyclophosphamide+methotrexate) maintenance chemotherapy or no chemotherapy derive from an internationally registered clinical trial of the International Breast Cancer Study Group: IBCSG 22-00 (published in Colleoni M. et al., JCO 2016). - A cohort of IEO breast cancer patients from years 2000-2012 enrolled in an internationally registered randomized clinical trial of the International Breast Cancer Study Group (IBCSG 22-00, Colleoni M. et al., JCO 2016) was used for the evaluation of CDK12 as a predictive biomarker of response to methotrexate-based therapy (described in Fig. 8c and Supplementary Fig. 5b). Clinical endpoint: cumulative incidence of distant metastasis. - The cohort used for UBIAD1 immunohistochemistry and quantitative RT-PCR analysis is a retrospective consecutive collection of patients enrolled at IEO between years 1997-2000. Detailed description of the selection criteria and clinicopathological characteristics of this cohort have been previously described in Pece S. et al., EBioMedicine 2019. This cohort was used to assess the clinical value of UBIAD1 as a prognostic biomarker associated to different types of chemotherapy. Clinical endpoint: cumulative incidence of distant metastasis.
Data collection	For IBCSG 22-00 patients, clinical and follow-up data were collected according to prespecified criteria, as described in Colleoni M. et al., JCO 2016). For all the cohort studies, available clinical and pathological information included age, date at surgery, tumor characteristics (histological type, tumor size, nodal involvement, grade, perivascular infiltration, Ki67 proliferative index, estrogen-receptor and progesterone receptor status), and treatment modality (type of surgery, adjuvant radiotherapy, adjuvant endocrine therapy or chemotherapy, neoadjuvant chemotherapy). Patients were followed up with physical examination every 6 months, annual mammography and breast ultrasound, blood test every 6-12 months and further evaluation only in case of symptoms. When possible the status of women not presenting at the institute for scheduled follow-up visits for more that one year was obtained by telephone contact.
Outcomes	Cumulative incidence of Distant Metastasis was defined as the time from surgery to the appearance of distant metastasis or death from breast cancer as a first event.

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	At sacrifice, mouse blood was collected via cardiac puncture using a syringe with 25G needle containing 50µL of 500U/mL Heparin (Sigma). After centrifugation at 1,100g for 5min at RT, the upper layer of plasma was discarded and the underlying buffy coat layer containing WBC and Circulating Tumor Cells was collected. The buffy coat was then washed with Red Bood Cell Lysis Buffer (Sigma, Cat. #11814389001) according to manufacturer instructions to remove any remaining red blood cells. WBC and CTCs were resuspended in 3%BSA in PBS and sorted with FACSAria™ IIIu Cell Sorting to isolate only CTC-eGFP+ cells. After blood collection, organs and tumors were harvested, cut into 2mm pieces, resuspended in 1mg/mL Collagenase A (Sigma, Cat. #11088793001) in DMEM (Thermo Fisher), and dissociated at 37°C for 30min. After red blood cells were lysed, cells were resuspended in 3%BSA in PBS and sorted with FACSAria™ IIIu Cell Sorting to isolate only eGFP+ cancer cells. Gates were set based on dissociated organs from not-injected mice (negative control) and eGFP+ cells pre-injection. 5 x 105 cells/well were seeded in 6-well plates and allowed to adhere overnight. Cells were then treated as indicated. As positive control, cells were treated with 100 µM of cumene hydroperoxide (CH) for 30 min. Then, medium was removed, and cells were incubated for 15min with 3µM BODIPY® 576/589 C11 in PBS + 5%FBS. The solution was then removed, cells were washed with PBS, trypsinized, and collected in tubes. After 3 washes in PBS, cells were resuspended in FACS tubes in 300µl of PBS + 5%FBS. Flow cytometry analysis was performed using BD FACSCanto™ II Cell Analyzer (BD Biosciences). Non-treated cells without BODIPY C11 staining were used as blank. The ratio between oxidized (FITC-A) and reduced (PE-A) BODIPY C11 was measured.
Instrument	FACSAria IIIu cell sorter (BD Biosciences, San Josè, CA) BD FACSCanto™ II Cell Analyzer (BD Biosciences, San Josè, CA)
Software	FACSDiva Software program (BD Biosciences) v 9.0.1
Cell population abundance	Minimum 300,000 events were acquire per sample. All sorted populations were confirmed as highly pure by post-sorts (>95%). For the cytofluorimetric analysis, a minimum of 20,000 events were recorded per sample.
Gating strategy	For cell sorting: cells were separated from debris by plotting FSC-A vs. SSC-A; single cells were identified by plotting FSC-A vs. FSC-W and, subsequently, SSC-A vs. SSC-W. Gates for selecting eGFP positive cells were defined using negative and positive control samples. For cytofluorimetric analysis: single cells were identified by plotting FSC-A vs. FSC-H and cells were separated from debris and dead cells by plotting FSC-A vs. SSC-A. Gates for FITC-A and PE-A were defined based on the negative and positive control samples.

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