nature portfolio

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Last updated by author(s): Jul 15, 2022

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
☐	☒ 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	For batch 1 of A375 siControl and siGCDH RNA-seq (Figure 2), libraries were prepared from total RNA using the QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina from Lexogen. Barcoded libraries were pooled and single end sequenced (1×75) on the Illumina NextSeq 500 using the High output V2.5 kit (Illumina Inc., San Diego CA). For second batch of A375, SK-Hep1, and SKBR3 (Figure S6), RNA-seq, polyA RNA was isolated using the NEBNext® Poly(A) mRNA Magnetic Isolation Module and barcoded libraries were made using the NEBNext® Ultra II™ Directional RNA Library Prep Kit for Illumina®(NEB, Ipswich MA). Libraries were pooled and single end sequenced (1X75) on the Illumina NextSeq 500 using the High output V2 kit (Illumina Inc., San Diego CA).
Data analysis	Data were analyzed using GGraphPad Prism version 8.0.0 (224) (graphing, statistical analysis), FlowJo 10.8.0 and BD FACSDiva 8.0.1(flow cytometry). Data were analyzed using FASTQC (v0.11.5), https://www.bioinformatics.babraham.ac.uk/projects/fastqc/; CUTADAPT (v2.3), https://github.com/marcelm/cutadapt; STAR (v2.7.0d_0221), https://github.com/alexdobin/STAR; RSEM (v1.3.1), https://github.com/deweylab/RSEM; DESeq2 (v1.22.2), https://bioconductor.org/packages/release/bioc/html/DESeq2.html; ggplot2 version 3.3.5; Ingenuity Pathway Analysis (IPA), http://www.ingenuity.com: Batch 1, A375 siGCDH vs. siControl, IPA version Q2 2019 (09-18-2019) and Batch 2, A375 +SKBR3+SK-Hep1 siGCDH vs. siControl, IPA version Q3 2021 (11-11-2021).

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

Databases/datasets used in the study for human genome and gene annotations:
Human genome hg38, http://ftp.ensembl.org/pub/release-84/fasta/homo_sapiens/dna/
Ensembl human gene annotations version 84, http://ftp.ensembl.org/pub/release-84/gtf/homo_sapiens/

RNA–seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE200424.

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 animal experiments, using 8 animals per each of our experimental groups, power analysis indicate the probability is 90 percent that each trial will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.389 times the standard deviation of the measured parameters for each trial. Unexpectedly dead animals were excluded. For in vitro experiments, a minimum of 3 samples were chosen as a sample size to ensure adequate power, unless stated otherwise. Sample sizes and statistical data are reported in figure legends.
Data exclusions	The data from unexpectedly dead animals or no tumor growth were excluded. No data were excluded from in vitro analysis.
Replication	Experiments were repeated at least 2 or 3 times independently (unless otherwise stated) and were reproducible. The number of repeats were indicated in figure legends.
Randomization	No randomization was performed during group allocation. Animals of same age and sex (littermates where possible) were used to control for covariates.
Blinding	The investigators were blinded to group allocation during experiments and outcome assessment.

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

Antibodies Purchased from Cell Signaling Technology (MA, USA):
NRF2 (D1Z9C), (dilution 1:1,000) (Cat#12721)
ATF4 (D4B8), (dilution 1:1,000) (Cat#11815)
DDIT3 (L63F7) (dilution 1:1,000) (Cat##2895)
p21 Waf1/Cip1 (12D1) (dilution 1:1,000) (Cat#2947)
HO1 (E9H3A) (dilution 1:1,000) (Cat #86806)
Cleaved Caspase-3 (Asp175) (5A1E) (dilution 1:1,000) (Cat #9664)
XBP-1s (D2C1F) (dilution 1:1,000) (Cat #12782)
ATF-3 (D2Y5W) (dilution 1:1,000) (Cat #33593)
PERK (D11A8), (dilution 1:1,000) (Cat #5683)
Phospho-PERK (Thr980) (16F8) (dilution 1:1,000) (Cat #3179)
IRE1α (14C10) (dilution 1:1,000) (Cat #3294)
eIF2α (D7D3)) (dilution 1:1,000) (Cat #5324)
Phospho-eIF2α (Ser51) (D9G8) (dilution 1:1,000) (Cat #3398)
ATF-6 (D4Z8V) (dilution 1:1,000) (Cat #65880)
HRP-conjugated anti-Mouse (dilution 1:10,000) (Cat #7076)
anti-Rabbit (dilution 1:10,000) (Cat #7074)

Antibodies Purchased from Santa Cruz Biotechnology, (TX, USA)
Bcl-2 (C-2) (dilution 1:1,000) (Cat #sc-7382)
Mcl-1 Antibody (22) (dilution 1: 1,000) (Cat #sc-12756)
HSP90 (F-8) (dilution 1: 4,000) (Cat #sc-13119)
Antibodies Purchased from Abcam
GCDH/GCD antibody (dilution 1:1,000) (Cat #ab232774)
DHTKD1 antibody (dilution 1:1,000) (Cat #ab230392)
AADAT antibody (dilution 1:1,000) (Cat #ab254661)

Antibodies Purchased from PTM Biolab LLC.
Pan Anti-glutaryllysine (dilution 1:1,000) (Cat #PTM-1151)

Antibodies Purchased from Proteintech
CHAC1 (dilution 1:1,000) (Cat #15207-1-AP)
ECHS1 antibody (dilution 1:1,000) (Cat #11305-1-AP)

Antibodies Purchased from Novus Biologicals, LLC
IRE1 alpha [p Ser724] (dilution 1:5,00) (Cat #NB100-2323)
Antibodies Purchased from Sigma-Aldrich
AASS (dilution 1:2,000) (Cat #HPA020728)
Anti-pan Phospho-Serine/Threonine (dilution 1:2,000) (Cat #SAB5700525)
HA-tag (clone HA-7) (dilution 1:2,000) (Cat #05-902R)

Validation

All antibodies were validated by the manufacturer (links below). We validated antibodies by detecting proteins at the expected sizes, which were consistent with the literature reports, confirming specific binding to the target proteins. Additionally, our use of RNAi and signaling inhibitors further validated some antibodies (as noted below)

Further own/manufacturer validation:
NRF2, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=12721&images=1

ATF4, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=11815&images=1

DDIT3, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=2895&images=1

p21 Waf1/Cip1 (12D1)
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=2947&images=1

HO1 (E9H3A)
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=86806&images=1

Cleaved Caspase-3 (Asp175) (5A1E), validated by caspase inhibitor in this study.
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=9664&images=1

XBP-1s (D2C1F)
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=12782&images=1

ATF-3 (D2Y5W), validated by knockdown in this study
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=33593&images=1

PERK (D11A8), validated by knockdown in this study
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=5683&images=1

Phospho-PERK (Thr980) Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=3179&images=1

IRE1α (14C10)
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=3294&images=1

eIF2α (D7D3) validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=5324&images=1

Phospho-eIF2α (Ser51) (D9G8)
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=3398&images=1

ATF-6 (D4Z8V)
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=65880&images=1

HRP-conjugated anti-Mouse
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=7076&images=1

HRP-conjugated anti-Rabbit
Validation Refs. from the manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=7074&images=1

Bcl-2 (C-2)
Validation Refs. from the manufacturer's datasheet: https://datasheets.scbt.com/sc-7382.pdf

Mcl-1 Antibody (22)
Validation Refs. from the manufacturer's datasheet: https://datasheets.scbt.com/sc-12756.pdf

HSP90 (F-8)
Validation Refs. from the manufacturer's datasheet: https://datasheets.scbt.com/sc-13119.pdf

GCDH/GCD antibody, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.abcam.com/gcdhgcd-antibody-ab232774.html

DHTKD1 antibody, validated by knockdown in this study
Validation Refs. from the manufacturer's datasheet: https://www.abcam.com/dhtkd1-antibody-ab230392.html

AADAT antibody, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.abcam.com/kat2aadat-antibody-ab254661.html

Pan Anti-glutaryllysine
Validation Refs. from the manufacturer's datasheet: https://www.ptmbiolabs.com/product/ptm-1151/

CHAC1, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.ptglab.com/products/pictures/pdf/15207-1-AP.pdf

ECHS1, validated by knockdown in this study.
Validation Refs. from the manufacturer's datasheet: https://www.ptglab.com/products/pictures/pdf/11305-1-AP.pdf

IRE1 alpha [p Ser724]
Validation Refs. from the manufacturer's datasheet: https://www.novusbio.com/products/ire1-alpha-antibody_nb100-2323#datasheet

AASS, validated by knockdown in this study
Validation Refs. from the manufacturer's datasheet: https://www.sigmaaldrich.com/US/en/product/sigma/hpa020728

Anti-pan Phospho-Serine/Threonine
Validation Refs. from the manufacturer's datasheet: https://www.sigmaaldrich.com/US/en/product/sigma/sab5700525

HA-tag (clone HA-7), validated by HA-NRF2 overexpression in this study
Validation Refs. from the manufacturer's datasheet: https://www.sigmaaldrich.com/US/en/product/mm/05902r

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cancer cell lines (breast: SKBR3 and MCF7; prostate: PC-3 and DU145; liver: SK-HEP1 and PLC and melanoma: A375 and 1205LU; human embryonic kidney : HEK293T) were obtained from ATCC. WM1346 and WM1366 and WM3629 melanoma

	cell lines were a gift from M. Herlyn (Wistar Institute) and human patient derived UACC-903 melanoma cell line was generated and obtained from the University of Arizona Cancer Center.
Authentication	Cell lines were obtained from verified sources and authenticated in our Genomic Shared Resources at SBP using short tandem repeat (STR) analysis. Allele profiles for each line were matched those maintained in the Expasy Cellosaurus STR database (https://web.expasy.org/cellosaurus/) using CLASTR v1.4.4
Mycoplasma contamination	All the cell lines tested negative for mycoplasma contamination (MycoAlert-Lonza).
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	All mice used were NOD/SCID (NOD.CB17-Prkdcscid/J) genetic background and 6–8-weeks-old male mice. Animals were housed in 4 mice per cage and maintained under controlled temperature (22.5°C) and illumination (12 h dark/light cycle) conditions.
Wild animals	No wild animals were used in this study.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All experimental animal procedures were approved by the Institutional Animal Care and Use Committee of Sanford Burnham Prebys Medical Discovery Institute (approval AUF 19-082).

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

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	SubG1 DNA content analysis was performed for determination of apoptotic population, analyzed by propidium iodide staining (Sigma Aldrich). Briefly, 1 × 106 cells were washed twice with cold PBS and fixed in 70% ethanol in PBS at 4°C overnight. Cells were washed, pelleted by centrifugation, and treated with RNase A (100 µg/mL) and propidium iodide (40 µg/mL) at room temperature for 30 min. Cell cycle distribution was assessed by flow cytometry (BD FACSCanto™, BD Biosciences), and data was analyzed using flowJo 10.8.0 and BD FACSDiva 8.0.1 software.
Instrument	All data were collected on an BD FACSCanto™ BD Biosciences.
Software	Data was collected and analyzed using BD FACSDiva 8.0.1 software.
Cell population abundance	Sorting was not performed
Gating strategy	Cell cycle analysis: PI

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