

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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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

No software was used.

Data analysis

Mass spectrometry data were analyzed with Proteome Discoverer (v2.4.1.15 SP1), Sequest HT, Percolator (v3.04), DEP, proDA, imp4p, pRoloc, CORREP, ClusterProfiler (v3.0.4), and REVIGO. ATAC-seq data were processed with TrimGalore (v0.6.10), Bowtie2 (v2.5.1), Samtools (v1.16), Picard (v2.23.8), deepTools (v3.5.1), MACS2 (v2.2.8), GenomicRanges (v4.4), csaw, edgeR, ClusterProfiler (v3.0.4), AnnotationDbi, ChIPSeeker, DESeq2, Circlize, and ComplexHeatmap. Metabolomics data were analyzed using MassHunter, DEP, and decoupleR (run_mean).

All code to produce the figures relevant to proteomics analysis in this paper can be found at https://github.com/Skourtis/Rito_Fabio.

Images were analyzed with custom-written Fiji (v1.54f) or MatLab (vR2018b) scripts. All other code used in this manuscript can be found at <https://github.com/SdelciLab/CINAPS>.

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](#)

DATA AVAILABILITY

All data relevant for the conclusions of this work have been made available through the main text or the supplementary information. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE100 partner repository with the dataset identifier PXD043524. The ATAC-Seq data have been deposited to the GEO Repository (GSE248846 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE248846>]). Additional source data for plots presented in this work are available as Source Data 1 through 7. Due to size limitations Larger Source Data L1 and L2 can be downloaded from Zenodo (<https://zenodo.org/records/15641870>). Please refer to the description of source data files and our code availability statement for further information.

CODE AVAILABILITY

All code to produce the figures relevant to proteomics analysis in this paper can be found at https://github.com/Skoutis/Rito_Fabio. All other code to produce figures from Source Data used in this manuscript can be found at <https://github.com/SdelciLab/CINAPS>.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Breast cancer tissue microarrays (Tissue Array, BR725) were analysed for samples from 17 individuals all of whom were females (sex). HeLa (female), U2OS (female), MIA PaCa-2 (male), and MDA-MB-231 (female) cell lines were used in this study. Sex (female/male) is based on donor origin as reported by ATCC; gender is not applicable to cell lines. Sex and gender related information is not applicable for this study.

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

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

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](https://www.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

The sample size for each experiment is specified in the respective figure legend. No statistical method was used to predetermine sample size. The study focuses on acute confinement (<30 min of confinement on average). Sample size was determined by the maximum samples imageable within this time frame per replicate, for at least 3 independent biological replicates.

Data exclusions

No data were excluded.

Replication

All experiments were performed with at least 3 biological replicates in independent experiments. All experimental findings were reliably reproducible.

Randomization

Randomization into groups was not applicable as defined treatments (e.g., Latrunculin, Oligomycin, BAM15, SMIFH2) were compared to controls. Where applicable, high-throughput assays (e.g., cell cycle, DNA damage) were performed in randomized wells to minimize plate effects. Where possible, data acquisition was performed by multiple investigators and imaged in different orders for treatment groups.

Blinding

Investigators were not blinded during data acquisition, but data analysis was largely automated using scripts, macros, or high-throughput software to minimize bias.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	For Western blot analysis (1:1000) - β -actin (A1978, Sigma-Aldrich), Vinculin (Santa Cruz Biotechnology sc-25336 or Cell Signalling Technology 13901S), Tubulin (Sigma-Aldrich T9026), H3 (Cell Signalling Technology 14269), H3K27me3 (Diagenode C15410195), H3K9me3 (Diagenode C15410193), γ H2AX (Sigma Aldrich 05-636), Fis1 (GTX111010 GeneTex), Drp1 (8570 D6C7, Cell Signalling), and Mfn1 (14739 D6E2S, Cell Signalling). For immunofluorescence - α -Tubulin primary antibody used at 1:1000 (Sigma Aldrich T6199), Phalloidin for actin staining at 1:40 (Thermo Fisher Scientific A12379), Alexa Fluor 633 secondary antibody at 1:250 (Thermo Fisher Scientific A-21050), γ H2AX at 1:500 (Sigma Aldrich 05-636), SHMT2 at 1:600 (Proteintech 11099-1-AP), and MTHFD2 at 1:500 (Abcam ab151447). For tissue microarray analysis - SDHB mouse antibody at 1:25 (Abcam ab14714) and Alexa Fluor 555-conjugated anti-mouse secondary antibody at 1:200. Additionally, DAPI was used at 1:1000 for nuclear staining (Sigma-Aldrich MBD0015).
Validation	Antibodies were validated using manufacturer-provided validation data, prior literature, and expected specificity based on known molecular weight and subcellular localization. Where applicable, signal specificity was confirmed by knock-outs, drug treatments, or absence of signal in negative controls. Consistency across replicates and alignment with published results further supported antibody reliability.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HeLa (CCL-2, ATCC), U2OS (HTB-96, ATCC), MIA PaCa-2 (CRL-1420, ATCC) and MDA-MB-231 (HTB-26, ATCC) cells were used in this study.
Authentication	Cell lines were not authenticated in house, but were obtained commercially from ATCC.
Mycoplasma contamination	All cell lines were negative for routine mycoplasma testing.
Commonly misidentified lines (See ICLAC register)	N/A

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	N/A
Study protocol	N/A
Data collection	No clinical trial was conducted as part of this study, but commercially available tissue microarray for breast carcinoma (BR725) was used.
Outcomes	N/A

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A