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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

RNA quality and concentration was determined with the Fragment Analyzer Standard Sense RNA kit. RNA-seq libraries were prepared using the NEBNextUltra 34 II Directional RNA Library Prep Kit for Illumina with 1000 ng of input material. Samples were pooled for sequencing on the NovaSeq instrument.

Multiplex transcript expression levels were measured by nCounter (Nanostring Technologies, Seattle, WA) with a custom panel containing the 95 ISR CLIC genes.

Image acquisition of lipid droplets, stress granules, CHOP immunofluorescence, and OP-Puromycin incorporation was performed on an Opera Phenix High Content Screening system (Perkin Elmer).

Cell confluence images were acquired on an Incucyte SX-5 Live-Cell Analysis (Sartorius)

Metabolomics samples were analyzed in both positive and negative ESI-LC-MS methods on Vanquish UPLCs coupled to Q-Exactive Plus mass spectrometers.Metabolites were separated using a SeQuant® ZIC-pHILIC column (5 µm, 200 Å, 150 × 2.1 mm) with the Mobile phase A was 20 mM ammonium carbonate in water (pH 9.2) and mobile phase B was 95:5 (v/v) acetonitrile/mobile A. Lipidomics samples were analyzed in both positive and negative ion modes using the same LC-MS method consisting of a Vanquish UPLC coupled to a Q-Exactive Plus mass spectrometer. Lipids were separated using a Thermo Scientific Accucore C30 column (2.6 µm, 150 Å, 2.1 × 250 mm) at a flow rate of 200 µL/min.

Bioanalyzer experiments were run on a Seahorse Bioanalyzer.

Data analysis

RNA-seq analysis was executed using an in-house, web-based platform, consisting of the following steps. Sequencing quality control was performed using FastQC (v0.11.5). Transcript expression was then quantified using Salmon (v0.9.1) in pseudo-alignment mode, without adapter trimming, producing transcript-per-million (TPM) estimates, using the Ensembl (GRCh38) transcriptome. Time course analysis was performed using built-in functions in R. Hierarchical clustering was performed and heatmaps were generated using the heatmap.2 package in R. Overrepresentation analysis of Gene Ontology-Biological Process terms was performed using the R function enrichGO from the ClusterProfiler library. Differential expression analysis was performed using the DESeq2 package in R producing q values and effect sizes for the comparison between basal states of WT and ATF4KO Dmr-PERK cells.

Multiplex transcript expression levels were measured by nCounter (Nanostring Technologies, Seattle, WA) with a custom panel containing the 95 ISR CLIC genes. Data analysis was performed on GraphPad Prism and plotted with the gplots package in R.

Image analysis, including nucleus, cell, and spot segmentation, was performed using Harmony software (Perkin Elmer) for lipid droplet, stress granule, CHOP immunofluorescence, and OP-Puromycin measurements. For high-content imaging experiments, Nuclei, cytoplasm and spot segmentation were performed in the Harmony software on maximum intensity projections of 3 slices for
each field while data plotting and statistical analysis were performed in Python. LD radius quantification was performed in Python 3.10.

Cell confluence was quantified with Incucyte (Sartorius) software using label-free segmentation methods for viability measurements. Data visualization and statistics were performed in Graphpad Prism software.

Metabolomics and Lipidomics datasets were processed with the software package MAVEN2 (https://github.com/eugenemel/maven_core). Natural 13C isotope abundance was corrected using the IsoCorrectoR R package. For lipidomics analysis, raw files were converted to mzML files using msconvert from ProteoWizard, using vendor centroiding on all scans. Analysis of the lipidomics and metabolomics data was performed using Calico Lipidomics and Metabolomics Analysis (ClamR) package (https://github.com/calico/claman) in R. Heatmaps were generated using the pheatmap package in R. A linear model was fit to the data and a pairwise comparison between treatments at individual time points was performed. The p value was calculated using a two-tailed t-test; the q value was calculated based on multiple hypothesis testing using the J.D. Storey qvalue package in R.

Bioenergetics experiments were analyzed with Wave software (Agilent) and data visualization and statistics were performed in Graphpad Prism software.

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

The data generated in the current study are available in the supplementary information or in the accompanying Source Data file. Raw RNA-seq data and annotated gene counts are available in Gene expression omnibus under accession codes GSE273599, GSE273600, and GSE273601. Raw untargeted LC-MS metabolomics and lipidomics data are available in Metabolomics Workbench under study ID ST003405 and ST003398, respectively.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*

Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For all independent experiments, a minimum of two replicates were used per condition.
Data exclusions	No data was excluded.
Replication	To ensure replication, all RNA-seq, metabolomics, lipidomics, bioenergetics, and alphalisa experiments were performed with three technical replicates. Quantification of lipid droplet content and growth curves assays were performed with at least two technical replicates. Lipid droplet quantification, growth curve assays, protein content quantification by western blot or alphalisa, and bioenergetics were repeated on 2-3 independent occasions. Replication was successful for all data presented.
Randomization	Experimental group randomization was not relevant to this study as all experiments were performed using immortalized cell lines. Randomization was applied to high-content imaging and metabolomics LC-MS runs using a custom script so as not to bias between groups should there be changes to sample plate position (imaging) or instrumentation signal (LC-MS) over the course of the analysis.
Blinding	Investigators were not blinded to group allocations. Sample processing, acquisition, and analysis were automated to prevent bias between 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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	anti-G3BP1, BD cat# 611126 anti-CHOP, Cell Signaling cat# L63F7 anti-p70S6K, Cell Signaling cat# 9202 anti-phospho-p70S6K (T389), Cell Signaling cat# 9205 anti-ATF4, Cell Signaling cat# 11815 anti-GADD34, Proteintech cat# 104491-1-AP anti-phospho-eIF2a (S51), Cell Signaling cat# 3398 anti-eIF2a Cell Signaling cat# 5324 anti-cleaved caspase-3, Cell Signaling cat# 9662s anti-cleaved caspase-8, Cell signaling cat# 9746t anti-actin, Cell signaling cat# 3700
Validation	anti-G3BP1 was validated for IF by evaluating signal co-localization with a U2OS Dmr-PERK cell lines stably expressing G3BP1-GFP

Validation	anti-CHOP was validated for IF by evaluating lack of staining in cells treated with siRNA against CHOP anti-p70S6K and anti-phospho-p70S6K were validated for western blot using lysates from U2OS cells treated with the mTORC inhibitor Torin anti-ATF4 was validated for western blot using lysates from ATF4 KO U2OS cells anti-GADD34 was validated for western blot using lysates from GADD34 KO U2OS cells anti-eIF2a and anti-phospho-eIF2s were validated for western blot using lysates from U2OS cells treated with ISR activators thapsigargin, tunicamycin and oligomycin anti-cleaved caspase-3 and -8 were validated using lysates from U2OS that underwent prolonged treatment with ISR activators.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	U2OS and HCT116 cells were obtained from ATCC
Authentication	Authentication was provided by ATCC
Mycoplasma contamination	All cells tested negative for mycoplasma during routine monthly testing
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*