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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 Authors & Referees 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	BD FACSDiva Software was used for flow cytometry
Data analysis	Microsoft Excel for Mac Version 16.43 GraphPad Prism 8.4.1 Fiji 1.52v MetaboAnalyst 4.0 ChimeraX 1.0 Morpheus FCS Express 6 Flow 6.05.0028 BD FACSDiva 8.0.1

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/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

Datasets generated during this current study are included in the published article as supplementary data or as publicly available datasets. Chemical screen data has been deposited in PubChem and proteomic data in PRIDE with accession PXD023882. Any additional data not included in this manuscript is available upon contact

with the corresponding author upon request.

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	A sample size of three replicates per condition was selected for initial experiments. Additional experiments were performed and biological replicates were often pooled as stated in text. For the majority of our techniques, biologically meaningful differences are detected with 3-8 replicates, indicating these sample sizes are sufficient.
Data exclusions	No data was excluded from analysis.
Replication	Experiments were replicated to confirm effect and effect sizes. Typically, experimental replicates were repeated weeks to months apart to ensure robustness of the data. All replicates were successful.
Randomization	Since no human or animal subjects were used in this study, randomization of samples or inclusion of co-variates were not performed.
Blinding	Blinding was not performed in this study mainly due to the lack of qualitative analyses. However, small-molecule screening by C.F.B. and metabolomics and lipidomics were run by collaborators in a blinded fashion.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	The following antibodies were used for western blot analysis: anti-SDHA (Abcam, ab14715, 1:3000), anti-ATP Synthase alpha (Life Technologies, 459240, 1:1000), anti-MTCO1 (Abcam, ab14705, 1:3000), anti-NDUFA9 (Abcam, ab14713, 1:1000), anti-NDUFS2 (Abcam, ab192022, 1:2000), anti-UQCRB (Proteintech, 10756-1-AP, 1:1000), anti-COX7A2L/SCAF1 (STJ110597, 1:1000), anti-FAR1 (ThermoFisher Scientific, PA553585, 1:1000), anti-AGPS (Santa Cruz Biotechnology, SC-374201, 1:1000), anti-HIGD2A (Santa Cruz Biotechnology, SC-390505, 1:1000), anti-DHODH (Cell Signaling, 166348, 1:1000), anti-CTPS1 (Cell Signaling, 98287S, 1:1000), anti-PEX14 (Proteintech, 10594-1-AP, 1:1000) and anti-β-Tubulin (Cell Signaling, 2146, 1:5000).
Validation	For antibodies not used routinely in the literature, CRISPR knockout cell line were generated for validation. Manufacturer information and publications were also used when KO cell lines were unavailable.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	293T and A375P cells were obtained from ATCC. U2OS/control cybrids were obtained from Rutger Vogel and Jan Smeitink Radbound University Medical Centre, Netherlands.
Authentication	Cell lines were not authenticated.

Mycoplasma contamination	Cell lines used in this study tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

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	Cells were trypsinized, diluted in DMEM, and resuspended in PBS before flow cytometry.
Instrument	BD FACSCanto II
Software	BD FACSDiva Software
Cell population abundance	The abundance of final analyzed cell population was ~90% of total starting population.
Gating strategy	Gating involved two steps. First, doublets and debris were removed from analysis using FSC (AxH) and SSC (AxH) plots and selecting the a rectangular gate around single cell events (identical gate for DMSO and drug treated samples). Second, to determine GFP+ cell population a marker gate was selected that differentiated GFP+ and GFP- cells (~99% purity). Since drug treatment increases autofluorescence in cells, a distinct marker gate was drawn for this population.

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