nature portfolio

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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	Zeiss Zen Blue 2.1
Data analysis	GraphPad Prism 9.2.0, FIJI 2.0.0, CellProfiler 3.1.9, MATLAB R2020a, MassLynx 4.1, R 3.4.4, XCMS 3.2.0, MSE Data Viewer 1.3

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 mass spectrometry raw data files are deposited to the Columbia Academic Commons and can be accessed at https://doi.org/10.7916/rphv-v394. Metlin can be accessed at https://massconsortium.com. LIPID MAPS can be accessed at https://www.lipidmaps.org. HMDB can be accessed at https://hmdb.ca. All other data is included as Source Data and can be accessed at https://doi.org/10.7916/hggm-7r90.

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	No sample size calculation was performed. For all viability experiments, a minimum sample size of 3 independent biological samples was used as is convention within the field and sufficient for calculating statistical significance. For imaging experiments, enough images were taken for sufficient cells to effectively evaluate statistical significance or lack thereof, resulting in at least 14 cells per condition for per cell experiments, or at least two images for Manders coefficient overlap. These sample sizes resulted in statistical significance with p values of <0.0001 indicating that sample size is likely sufficient. For all other studies, sample size of at least 3 was used, again as is convention in the field and resulting in statistically significant results.
Data exclusions	No data was excluded.
Replication	All attempts at replication reproduced the results. For all experiments a minimum of two independent replications was performed.
Randomization	Randomization was used in lipidomics for the order of running samples. For all other experiments, randomization was not required as there is no preferential allocation of certain cells in vitro, and thus the cells are inherently randomized.
Blinding	No in vivo studies were performed. Blinding does not typically occur in this type of in vitro experimentation, and many of the experiments were performed and analyzed by a single person making blinding not possible. Additionally, the quantitative output of the experiments is not affected by blinding as an observational study or survey would be.

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	Mouse monoclonal anti-ACSL4 (F-4) from Santa Cruz Biotechnology Cat# sc-365230. Rabbit monoclonal anti-Pan-Actin (D18C11) from Cell Signaling Technology, Cat# 8456. Mouse monoclonal anti-β-Actin (8H10D10) (Cell Signaling Technology), Cat #3700. Rabbit monoclonal anti-Cytochrome c (D18C7) (Cell Signaling Technology), Cat #11940. Rabbit Monoclonal anit-PDI (C81H6) (Cell Signaling Technology) #3501, IRDye Goat Anti-Mouse 680, IRDye Goat Anti-Rabbit 800 antibodies (LICOR), mouse anti-ACSL4 antibody (Invitrogen PA5-27137), rabbit anti-Calnexin antibody (Invitrogen MA3-027), Alexafluor 594-conjugated anti-mouse (Invitrogen A-11032) and Alexafluor 488-conjugated anti-rabbit antibody (Invitrogen A11006).
Validation	All commercial antibodies were rigorously validated by their distributors for functionality, sensitivity, and specificity. They are all demonstrated to be effective across multiple cell lines and cited in numerous publications. For Cell Signaling Technology, western blotting was validated by testing in multiple cell lines, using growth factors/chemical activators/inhibitors to modulate expression and confirm specificity, using siRNA or KO cell lines to verify specificity, side-by-side comparison for lot-lot consistency, and optimal dilution predetermination. Santa Cruz antibodies are validated by testing in multiple cell lines, including over-expression cell lines showing an increase in specific signal. LICOR antibodies are isolated by affinity chromatography and then confirmed for specifity by ELISE and flow cytometry, as well as tested for cross-reactivity by dot blot and/or solid phase adsorption, then specifically tested and qualified in Western blotting. Invitrogen antibodies are tested for specificity using at minimum one method including knockout, knockdown, cell treatment, relative expression, neutralization, peptide array, SNAP-ChIP, or Immunoprecipitation Mass Spectrometry, and then confirmed for effectiveness for their application of interest (in this case immunofluorescence imaging).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	All cell lines were obtained from ATCC. These cell lines include HT1080, PANC-1, HEK293T, N27, and HT22.
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	All cells are negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.