

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	All softwares used for data collection are publicly or commercially available and are described in details in the methods section. Briefly: for mass spectrometry data collection we used Thermo XCalibur 4.0; Moxi V Cell Counter (cell counting); Bio Rad iMark plate reader and Bio Rad MPM 6 software (v3.1.9) were used for the collection of cell proliferation data; Sequencing data were collected using an Illumina HiSeq2500 platform; Leica SP8 LIGHTNING confocal microscope and LasX software for confocal image acquisition; Applied Biosystems™ StepOne™ Real-Time PCR and software was used for quantitative PCR data collection; Synthetic lethality screen data was mined from the publicly available database The Cell Map and the online tool thecellmap.org; SLC25A38 structure was modeled using AlphaFold; Oxygen consumption rates (OCR) data was collected using a Seahorse XFe96 Analyzer (Agilent Technologies);
Data analysis	All softwares used for data analysis are publicly or commercially available and are described in details in the methods section. Briefly, for the CRISPRi pooled screening processing pipeline, we used python scripts (Python (v3.7.0)) from the Weissman lab (https://github.com/mhorlbeck/ScreenProcessing). All statistical analysis were performed on GraphPad Prism 9 and Microsoft Excel (v16.68). Fiji with ImageJ (v2.3.0/1.35q), Cell Profiler 4.2.5 and scripts in R (v4.1.2) were used for confocal image analysis and quantification; Thermo XCalibur 4.0 QualBrowser and QuanBrowser were used for mass spectrometry (LC-MS) peak detection and quantification; For the proteomics study, mass spectrometry raw files were searched against a Uniprot human database (6,563,750 entries) using MaxQuant version (2.03.01) and the Andromeda search engine. The label-free quantitation (LFQ) intensities were log2 transformed in Perseus version 2.0.7.0 and used in subsequent analysis. R scripts using the packages pOmics2 and Tidyverse70 to perform data cleaning, missing data imputation and normalization.

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 in this study are available within the manuscript, Figures, Supplementary Figures, Supplementary Data and Source Data files. The full tables reporting our genetic screen (CRISPRi) results as well as primers used for the targeted library construction are shown in Supplementary Data; Source data are provided with this paper. Mass spectrometry proteomics raw data have been deposited to the ProteomeXchange Consortium via the PRIDE71 partner repository with the dataset identifier PXD058312. The following plasmids generated in this study will be available via Addgene: pMXs_FLAG-SLC25A38 (#218412), pMXs_FLAG-SLC25A39 (#218413), pMXs-FLAG-SLC25A38-Arg134Cys (#218416), pMXs-FLAG-SLC25A38-Arg134His (#218417), pMXs-FLAG-SLC25A38-Asp188His (#218418), pMXs-FLAG-SLC25A38-Arg187Gln (#218419), pMXs-FLAG-SLC25A38-Arg282Ala (#218420), pMXs-FLAG-SLC25A38-Arg278Ala (#218421), pMXs-3xMyc-hem25 (#218414) and pMXs-bleoR-MitoTag (#218415).

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	N/A
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	Based on previous experience and standards in the field, we used a minimal sample size of n=3 for cell and in vitro assays to allow for higher reliability and sufficient size for statistical analysis. Each replicate was defined as an independent culture of cells.
Data exclusions	For the CRISPRi screen, we followed established protocols in the field and in which guides were excluded from the gene score if there were fewer than 50 reads initially ("day 0" timepoint). For the seahorse study shown on Fig 5L a few data points were not available due to failed oligomycin injection and/or premature injection of rotenone/antimycin. No data were excluded from cell/in vitro experiments presented here.
Replication	We have reproduced every cell/in vitro experiment shown here at least twice independently. We have not encountered problems in reproducibility of these results.
Randomization	When possible, samples were randomized both at collection time and while running LC-MS sequences to avoid instrument bias on data collection.
Blinding	Sample blinding was not performed for most experiments as they did not involve formal qualitative scoring or animal/human subjects.

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

Antibodies that recognize SLC25A38 (ab133614), total OXPHOS Rodent WB Antibody Cocktail (ab110413) and anti-ATP5a (Mouse, ab14748) from Abcam, SLC25A39 (14963-1-AP), PDXK (15309-1-AP) and PROSC (25154-1-AP) from Proteintech, SHMT2 (HPA020549), SFXN1 (HPA019543) from Atlas Antibodies, GAPDH (2118), VDAC (4661), SHMT1 (12612), Citrate Synthase (CS) (14309), Calreticulin (CalR) (12238), Catalase (12980), Cathepsin B (31718), GOLGIN-97 (13132), myc (2278), DYKDDDDK Tag (14793) and hemagglutinin (HA) epitopes (3724) from Cell Signaling Technology, Tom20 (HPA011562), FLAG (F1804) from MilliporeSigma and anti-HA raised in rat (11867423001) from Roche.

Validation

All antibodies used here are commercially available and have been validated by the manufacturer or previously validated by other groups (cited in the text). We have validated the following antibodies using Western Blots with our knockout lines (Extended Data Fig. 4): SLC25A39 (14963-1-AP), PDXK (15309-1-AP) and PROSC (25154-1-AP) from Proteintech and SLC25A38 (ab133614) from Abcam, SHMT2 (HPA020549), SFXN1 (HPA019543) from Atlas Antibodies and SHMT1 (12612) from Cell Signaling Technologies.

Eukaryotic cell lines

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

Cell line source(s)

K562 and HEK293 were purchased from ATCC; K562s expressing nuclease-dead Cas9 (dCas9-KRAB) to programmably interfere with gene expression when co-expressing sgRNAs (CRISPRi) (gift from Jonathan Weismann's lab) are fully characterized in Cell 159, 647–661 (2014) and Elife 5, (2016). Jurkat cells knockout for SLC25A38 and SFXN1 were a gift from Nora Kory's lab and are fully described in Science 362, (2018).

Authentication

None

Mycoplasma contamination

Cell lines were tested negative for Mycoplasma by PCR analysis.

Commonly misidentified lines
(See [ICLAC](#) register)

N/A

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A