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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	NanoTemper analysis software; QuantStudio 6 Flex Real-time qPCR software
Data analysis	In addition to abovementioned tools also used for analysis, the following software was used: SigmaPlot (v.11.0).; GraphPad Prism (v.8.0); PKSolver PlugIn for MicrosoftExcel (v.2.0); Warp (v.1.0.7.); cryoSPARC (v.2.14.2); Relion (v.3.0.7); Coot (v.0.9.); Phenix (v.1.18); FreeStyle (v.1.6); MaxQuant (v.1.6.10.43); R-Studio (v.1.1.383.); TraceFinder (v.4.1); Progenesis QI Software (v.2.3); TargetLynx Software (v.4.1 SCN 950)

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

The central data generated and analysed during this study are included in this article. Source data used to generate graphs are provided with this manuscript. Uncropped gels can be found in Supplemental Figure 1. Publicly available datasets used in this study are: human proteome database (UP000005640, Uniprot), POLRMT structures (PBD IDs: 5OLA, 4BOC, Protein Data Bank). Further information and requests for unique reagents should be directed to the corresponding authors. Proteomics data and R scripts for analysis were deposited into PRIDE with the dataset identifier PXD018426. The electron microscopy maps were deposited with the Electron Microscopy Data Bank (accession code EMD-11679) and the structural model of POLRMT with IMT1B was deposited with the Protein Data Bank (accession code 7A8P).

nature research | reporting summary

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	In vitro, cell culture and metabolomics experiments were performed using sample sizes based on standard protocols in the field. For quantitative proteomics, we chose a sample size which we determined previously to be a highly reproducible value. For tumour xenograft studies, sample sizes were pre-determined by statistical analysis. For toxicity and pharmacokinetic panels, no sample size determination was performed, cohort sizes were chosen based on previous experience to balance an exploratory, observational study with needless use of excess animals.
Data exclusions	For qPCR tissue analysis, samples were excluded if there was no sufficient amount of starting material (RNA, DNA) to perform the standardized experiment. In mouse tumour volume analysis, no samples were excluded.
Replication	All experimental data was reliably reproduced as indicated in the method section and figure legends. For mouse xenograft studies, one independent DLD1 xenograft study and three independent A2780 xenograft studies were conducted, two of which are shown here. All of the tumor xenograft studies showed similar results. For toxicity panels and pharmacokinetics, one independent study was performed.
Randomization	Tumour-bearing mice were randomly allocated to treatment groups. For toxicity studies, mice were weighed and groups assigned according to a randomized block design such that mean starting weight was similar in all groups.
Blinding	For mouse studies, investigators were not blinded to group allocation or experimental outcome, as the compound was not provided blinded. Investigators were also not blinded during sample analysis.

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	Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam); anti-actin (ab3280, Abcam); anti-MTCO1 (ab14705, Abcam); anti-phospho-AMPK (T172) (#2535, Cell Signalling Technology); anti-AMPK (#2532, Cell Signalling Technology); anti-phospho-S6 ribosomal protein (#4856, Cell Signalling Technology); anti-S6 ribosomal protein (# 2217, Cell Signalling Technology); anti-LRPPRC (AgriSera Cat# LRPPRC_antibody, RRID:AB_2716302); anti-tubulin (#2125, Cell Signalling Technology); anti-tubulin (T9026, Sigma); anti-Vinculin (ab129002, Abcam); anti-HSP60 (#4870; Cell Signalling Technology); anti-hPOLRMT (sc-67350, Santa Cruz, kind gift from D. Temiakov); anti-cleaved PARP (used as part of the Cell Cycle and Apoptosis WB Cocktail, ab139417, Abcam), anti-PCNA (ab29, abcam); anti-mouse IgG (NXA931V, GE Healthcare); anti-rabbit IgG (NA9340V, GE Healthcare)
Validation	Primary antibodies for western blotting: - anti-LRPPRC: The self-made antibody against LRPPRC (AgriSera Cat# LRPPRC_antibody, RRID:AB_2716302) has been published previously and was shown to specifically detect LRPPRC by western blotting comparing protein levels in mitochondrial extracts of wild-type and LRPPRC knock-out samples. https://scicrunch.org/resolver/AB_2716302 - Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam): The Abpromise covers the use of the antibody for WB application. The antibody has been referenced in 592 publications. https://www.abcam.com/total-oxphos-rodent-wb-antibody-

cocktail-ab110413.html

- anti-actin (ab3280, Abcam): The Abpromise covers the use of the antibody for WB application. The antibody had been referenced in 399 publications, however, it has been discontinued.

- anti-MTCO1 (ab14705, Abcam): The Abpromise covers the use of the antibody for WB application. The antibody had been referenced in 310 publications. https://www.abcam.com/mtco1-antibody-1d6e1a8-ab14705.html

- anti-phospho-AMPK (T172) (#2535, Cell Signalling Technology): The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 1597 publications. https://www.cellsignal.com/products/primary-antibodies/phospho-ampka-thr172-40h9-rabbit-mab/2535?Ntk=Products&Ntt=2535

- anti-AMPK (#2532, Cell Signalling Technology): The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 1163 publications. https://www.cellsignal.com/products/primary-antibodies/ampka-antibody/2532?Ntk=Products&Ntt=2532

- anti-phospho-S6 ribosomal protein (#4856, Cell Signalling Technology): The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 184 publications. https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser235-236-2f9-rabbit-mab/4856?Ntk=Products&Ntt=4856

- anti-S6 ribosomal protein (# 2217, Cell Signalling Technology): The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 1087 publications. https://www.cellsignal.com/products/primary-antibodies/s6-ribosomal-protein-5g10-rabbit-mab/2217?Ntk=Products&Ntt=2217

- anti-tubulin (#2125, Cell Signalling Technology): The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 365 publications. https://www.cellsignal.com/products/primary-antibodies/a-tubulin-11h10-rabbit-mab/2125?Ntk=Products&Ntt=2125

- anti-tubulin (T9026, Sigma): The antibody was subjected to enhanced antibody validation and referenced in 3026 publications. https://www.sigmaaldrich.com/catalog/product/sigma/t9026?lang=de&region=DE

- anti-Vinculin (ab129002, Abcam): The Abpromise covers the use of the antibody for WB application. The antibody had been referenced in 105 publications. https://www.abcam.com/vinculin-antibody-epr8185-ab129002.html

- anti-HSP60 (#4870; Cell Signalling Technology): The antibody guarantee covers the use of the antibody for WB applications. The antibody has been referenced in 60 publications. https://www.cellsignal.de/products/primary-antibodies/hsp60-d307-antibody/4870?Ntk=Products&Ntt=4870

- anti-hPOLRMT (sc-67350, Santa Cruz): The antibody has been verified using siRNA knockdown of POLRMT in HeLa cells (Kühl, 2014 and this study), recombinant hPOLRMT was loaded as a control (see Supplementary Figure 1). The product seems to have been discontinued.

- anti-cleaved PARP (used as part of the Cell Cycle and Apoptosis WB Cocktail, ab139417, Abcam): The Abpromise covers the use of the antibody for WB application. https://www.abcam.com/cell-cycle-and-apoptosis-wb-cocktail-pcdkphh3actinparp-ab139417.html

- anti-PCNA (ab29, abcam): The Abpromise covers the use of the antibody for WB application. The antibody has been referenced in 368 publications. https://www.abcam.com/pcna-antibody-pc10-ab29.html

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Cell lines were derived from American Type Culture Collection (ATCC), European Collection of Authenticated Cell Cultures (ECACC), Cell Line Service GmbH (CLS), National Institutes of Cancer (NCI-DTP) and DSMZ-German Collection of Microorganisms and Cell Cultures GmbH.

HeLa (ATCC CCL-2), A2780 (ECACC 93112519), DLD-1 (ECACC 90102540), hPBMC (DRK West Blutspendedienst Hagen, Germany), primary human hepatocytes (X008052-P, BioreclamationIVT)

For cell panel: 5637 (ATCC HTB-9), CLS-439 (CLS; 300150), J82 (ATCC HTB-1), T24 (ATCC HTB-4), UMUC3 (ATCC CRL-1749), MG63 (ATCC CRL-1427), MHH-ES1 (CLS; 300136), RD-ES (ATCC HTB-166), SAOS2 (ATCC HTB-85), U2OS (ATCC HTB-96), SF-295 (NCI-DTP, SF-295), SK-N-AS (ATCC CRL-2137), SK-N-SH (ATCC HTB-11), SNB75 (NCI-DTP, SN-75), U87MG (ATCC HTB-14), BT-20 (ATCC HTB-19), Hs578T (ATCC HTB-126), JIMT1 (DSMZ, ACC 589), MCF7 (ATCC HTB-22), MDA-MB-231 (ATCC HTB-26), MDA-MB-436 (ATCC HTB-130), MDA-MB-468 (ATCC HTB-132), SKBR3 (ATCC HTB-30), C33A ATCC HTB-31), Ca Ski (ATCC CRM-CRL-1550), HeLa (ATCC CCL-2), Caco-2 (ATCC HTB-37), COLO 205 (ATCC CCL-222), COLO-678 (DSMZ, ACC 194), DLD-1 (ECACC,

90102540), HCT-116 (ATCC CCL-247), HCT-15 (ATCC CCL-225), HT-29 (ATCC HTB-38), LoVo (ATCC CCL-229), SW620 (ATCC CCL-227), HT-1080 (ATCC CCL-121), GRANTA-519 (DSMZ, ACC 342), HL-60 (DSMZ, ACC 3), K-562 (DSMZ ACC 10), Kasumi-1 (DSMZ ACC 220), L-363 (DSMZ, ACC 49), Mino (DSMZ, ACC 687), MV-4-11 (DSMZ, ACC 102), PBMC (donor specific), Ramos (DSMZ, ACC 602), SU-DHL-10 (DSMZ, ACC 576), SU-DHL-6 (DSMZ, ACC 572), THP-1 (DSMZ, ACC 16), WSU-NHL (DSMZ, ACC 58), 786-O (ATCC CRL-1932), ACHN (ATCC CRL-1611), Caki-1 (ATCC HTB-46), Hek293 (ATCC CRL-1573), UO-31 (NCI-DTP, UO-31), HepG2 (ATCC HB-8065), PLC/PRF/5 (ATCC CRL-8024), SK-HEP-1 (ATCC HTB-52), A549 (ATCC CCL-185), Calu-6 (ATCC HTB-56), IMR-90 (ATCC CCL-186), NCI-H292 (ATCC CRL-1848), NCI-H358M (NCI-DTP, NCI-H358M), NCI-H460 (ATCC HTB-177), NCI-H82 (ATCC HTB-175), A-204 (ATCC HTB-82), A-673 (ATCC CRL-1598), Hs729 (ATCC HTB-153), RD (ATCC CCL-136), A2780 (ECACC 93112519), EFO-21 (DSMZ, ACC 235), IGROV1 (NCI-DTP, IGR-OV1), OVCAR3 (ATCC HTB-161), OVCAR4 (NCI-DTP, OVCAR-4), SK-OV-3 (ATCC HTB-77), AsPC-1 (ATCC CRL-1682), BxPC-3 (ATCC CRL-1687), MIA PaCa-2 (ATCC CRM-CRL-1420), PANC-1 (ATCC CRL-1469), Panc 10.05 (ATCC CRL-2547), JAR (ATCC HTB-144), JEG-3 (ATCC HTB-36), 22Rv1 (ATCC CRL-2505), DU 145 (ATCC HTB-81), PC-3 (ATCC CRL-1435), A-375 (ATCC CRL-1619), A-431 (ATCC CRL-1555), SK-MEL-28 (ATCC HTB-72), SK-MEL-5 (ATCC HTB-70), SK-LMS-1 (ATCC HTB-88)

Authentication	All cell lines used here are commercially available and have been verified by the manufacturers by STR analysis of human cell lines. https://www.lgcstandards-atcc.org/Products/Cells_and_Microorganisms/Cell_Lines/Misidentified_Cell_Lines.aspx https://www.phe-culturecollections.org.uk/technical/cell-lines-faqs.aspx https://clsgmbh.de/faq.php#2 https://dtp.cancer.gov/organization/btb/docs/DCTDTumorRepositoryCatalog.pdf https://www.dsmz.de/collection/catalogue/human-and-animal-cell-lines/identity-control/authentication-of-cell-lines
Mycoplasma contamination	Cell lines were routinely examined for mycoplasma contamination (negative).
Commonly misidentified lines (See ICLAC register)	none

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Mus musculus: female NMRI:nu/nu, 7-9 weeks; female Balb/c nude (7-9 weeks); male CD-1 (7-9 weeks), male C57Bl6N (10-14 weeks)
Wild animals	No wild animals were used.
Field-collected samples	The study does not contain field-collected samples.
Ethics oversight	Local Animal Welfare authorities: Regional Office for Health and Social Affairs, Berlin; Tübingen Regional Council

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