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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 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	Gels were imaged with LI-COR Odyessey CLx using Image Studio v5.2 software. QuantStudio Real-Time PCR v1.2 was used to collect protein thermal stability (DSF) data. ATPase assay data were collected with Gen5 v3.02.2 (Biotek). Oxygen consumption rates were determined using the Seahorse XF96 software. HAP1 cell growth was collected using the Incucyte Base Analysis Software. Crystallography data sets for refinement were collected using JBluIce-EPICS V2019-3 beamline control software. Thermo Scientific Xcalibur was used for LC-MS data acquisition.
Data analysis	Tm values were determined using Protein Thermal Shift v1.3. Mass spectrometry results were analyzed using TraceFinder 5.1 (Thermo) and MaxQuant software package ver. 1.5.1.2. HAP1 cell growth was analyzed using the Incucyte Base Analysis Software. Crystallography data were reduced using autoPROC V1.0.5(20190923) and XDS V20210322. The structures were solved using molecular replacement in Phaser V2.8.3. Geometric restraints for CA157 were prepared with phenix.elbow. Structures were iteratively improved using model building in Coot V0.9, refinement in Phenix V1.20.1-4487, and sterochemical validation on MOLPROBITY V4.02-528. Final models were prepared for deposition using PDB_EXTRACT V3.27. SBGrid provided curated crystallographic software. Interaction diagrams were generated in LigPlot+ V2.2.5. General statistics were performed using GraphPad Prism Version 8.4.3.

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 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

LC-MS lipid measurement data that support the findings of this study were deposited into the MassIVE data repository under accession #MSV000090082. Structural data generated in this study have been deposited in the PDB with accession codes 7UDP and 7UDQ. Structural data that were used to support the findings in this study are available in the PDB with accession codes 5I35 and 4PED.

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	Sample sizes are noted in figure legends and methods. No statistical means were used to predetermine experimental sample size. Triplicate is a generally accepted standard for the minimal number of replicates needed to obtain conclusive evidence for these types of experiments.
Data exclusions	Two data points were excluded from the DSF analysis in Fig. 1a due to insufficient melt curves upon manual inspection.
Replication	All attempts at experimental replication were successful and all experiments contained at least three independent samples
Randomization	Randomization was not relevant to our study as our experiments were done quantitatively and data acquisition was performed by machines, mitigating investigator bias.
Blinding	As above, blinding was not relevant to our study as all measurements were derived from automated measurements or computational analyses.

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HAP1 WT, COQ8A KO, COQ8B KO, and COQ8A/B DKO cells were obtained from Horizon Discovery. HEK-293 cells were obtained from ATCC, CAT# CRL-1573. SUM159PT cells were a collaborative gift from Charles M. Perou (UNC).
Authentication	We did not perform cell line authentication.
Mycoplasma contamination	HAP1 cells were negative for mycoplasma contamination as tested by the commercial source. HEK-293 cells were tested

Mycoplasma contamination	mycoplasma negative using MycoAlert™ Mycoplasma Detection Kit (Lonza). SUM159PT cells are routinely checked for mycoplasma.
Commonly misidentified lines (See ICLAC register)	HEK-293 cells are listed as commonly misidentified. They were used as they are the standard for NanoBRET experiments per manufacturer instruction.