

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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	EPU (FEI/ThermoFisher) was used for automated collection of cryo-EM data
Data analysis	Cryo-EM data was analyzed through the cisTEM 1.0.0-beta package (including Unblur and CTFFIND 4.1 procedures), Relion 2.1 and 3.0 and cryoSPARC v1. Modelling was performed using COOT 0.8.9.1, secondary structure prediction from RaptorX and Namdinator. Phenix 1.14-3260 was used for refinement, Molprobidity, EMRinger and Mtriage were used for validation. LocScale was used for scaling of maps for visualization. All of the software is published and referenced in the supplementary. All but cryoSPARC are open source. No custom algorithms were used.

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

PDB entry codes (6ROH, 6ROI, and 6ROH) and EMBD entry codes (EMD-4972, EMD-4973, and EMD-4974) have been included

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	For Drs2 mutants (Figure 3), activity was measured from 3 different samples (1 trace out of the 3 measurements is displayed for each species in extended data Figure 4). We consider this sample size is sufficient because: 1/ activity measurement is based on continuous monitoring of NADH oxidation, resulting in accurate measurement of the rate of ATP hydrolysis, as compared with end-point measurements and 2/ the 3 values obtained were very similar to each other
Data exclusions	No data were excluded to plot results displayed in any tables or Figures
Replication	All attempts were included as replicates to plot data displayed in Figure 3 and the reproducibility between the various measurements is illustrated by error bars (standard deviation) and scatter plots. All experiments were reproducible.
Randomization	No group allocation was performed in this study
Blinding	Investigators were not blinded to group allocation as the present study is not a clinical research trial

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

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Saccharomyces cerevisiae expression strain W303.1b/GAL4 (a, leu2-3, his3-11, trp1-1::TRP1-GAL10-GAL4, ura3-1, ade2-1, canr, cir+)
Authentication	n.a.
Mycoplasma contamination	n.a.
Commonly misidentified lines (See ICLAC register)	n.a.