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	Tth MAG lipase reductive methylation: EMBL Proteomics Core Facility, Heidelberg, Germany X-ray data collection, structure solution, and validation: synchrotron radiation beamlines BM14, ESRF, Grenoble and BW7A, DORIS III, EMBL/DESY, Hamburg Analysis of crystal content: Chiralix, Nijmegen, The Netherlands
Data analysis	Tth MAG lipase reductive methylation: MaxEnt1/MassLynx 4.1. (Waters) X-ray data collection, structure solution, and validation: HKL suite v 1.97.9; SHELXD E 2008/9; Phenix 1.9_1692; Coot 0.8.9; ARP-wARP v7; PDBeFOLD v2.58; ColabFold v1.5.2-patch (AlphaFold2, MMseqs2); PYMOL v2.5 Monoacylglycerol (MAG) ester assay: GraphPad Prism 10.0.1 Mono p-nitrophenyl acyl ester (pNP) assay: GraphPad Prism 9.1.2 Cellular Tth MAG lipase activity by cell extract analysis: Chemidoc Imaging and Image Lab 6.1. (Biorad) GraphPad Prism 5.04 Nano differential scanning fluorimetry: PR.ThermControl (Nanotemper); MoltenProt (EMBL Hamburg Sample Preparation and Characterization Facility) Circular dichroism: CONTIN-CD (s-provencher.com)

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 coordinates of the X-ray structures determined in this contribution have been deposited in the Protein Data Bank under accession codes 7Q4J [https://doi.org/10.2210/pdb7Q4J/pdb], 7Q4H [https://doi.org/10.2210/pdb7Q4H/pdb], 8B9S [https://doi.org/10.2210/pdb8B9S/pdb]. All other data generated in this study are provided in the Supplementary Information and a Source Data file.
The coordinates of the following X-ray structures used for analyzing the results in this work are also available from the Protein Data Bank with accession codes: 3S2Z [https://doi.org/10.2210/pdb3S2Z/pdb], 2WTM [https://doi.org/10.2210/pdb2WTM/pdb], 7D79 [https://doi.org/10.2210/pdb7D79/pdb], 3HJU [https://doi.org/10.2210/pdb3HJU/pdb], 3LLC [https://doi.org/10.2210/pdb3LLC/pdb], 4KE8 [https://doi.org/10.2210/pdb4KE8/pdb], 4KE9 [https://doi.org/10.2210/pdb4KE9/pdb], 3PFB [https://doi.org/10.2210/pdb3PFB/pdb], 3PFC [https://doi.org/10.2210/pdb3PFC/pdb], 5CML [https://doi.org/10.2210/pdb5CML/pdb], and 6QE2 [https://doi.org/10.2210/pdb6QE2/pdb])

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample was determined by its unique sequence (UNIPROT code: I9KU82). For statistically significant functional characterization, seven different variants of the enzyme were created and analyzed (E43A, E43K, E72R, S113A, Y154A, Y154R,Lid). Structural characterization was carried out using the wild-type enzyme and using a version where methionine residues were substituted by seleno-methionine, to allow experimental phasing. The latter one was structurally analyzed as active and PMS-inhibited versions. MAGs of different lengths (C2, C4, C6, C8, C10, C12, C14, C16, C18), C8 DAG and C8 TAG, and ferulate were used as substrates for statistically significant functional characterization.
Data exclusions	No data were excluded.
Replication	Monoacylglycerol (MAG) ester assay: N = 3 or 4 Mono p-nitrophenyl acyl ester (pNP) assay: N = 3 or 4 Cellular Tth MAG lipase activity by cell extract analysis: N = 3, 4, 6, 7 Nano differential scanning fluorimetry: N = 3 Circular dichroism: N = 3
Randomization	No randomization was applied as the sample was unique.
Blinding	Blinding was not relevant as only one unique sample was used.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging