

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
<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	Sequences were collected from the Imine Reductase Engineering Database version 3 [https://ired.biocatnet.de/].
Data analysis	The following softwares were employed: MAFFT v7 [https://mafft.cbrc.jp/alignment/server/], BOOSTER [https://booster.pasteur.fr/], Graphical Representation of Ancestral Sequence Predictions [http://grasp.scmdb.uq.edu.au/], PhyML3.0 [http://www.atgc-montpellier.fr/phyml/], PyMol2.5, Phenix1.20.1, WinCoot, AutoDock vina v1.1.1, AlphaFold2, Jalview v2.11.2.6, iTol v6 [https://itol.embl.de/], MAGELLAN [https://bioinfo.mnhn.fr/abi/public/magellan/Magellan.help.html]. 'epistasis' Python package, Adobe Illustrator 2020, PROMALS3D (http://prodata.swmed.edu/promals3d/promals3d.php), IQtree (version 2.3.6 for Linux x86 64-bit), Rstudio v4.4.1.

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 ancestral imine reductase sequences (N1-N5, N557-N560, AltN1-AltN5, and AltN557-AltN560) generated in this study is provided in the Supplementary

Information. Additionally, these ancestral sequences generated in this study have also been deposited in the Genbank database under accession codes: PP329371 (N1), PP329372 (N2), PP329373 (N3), PP329374 (N4), PP329375 (N5), PP329376 (N557), PP329377 (N558), PP329378 (N559), PP329379 (N560), PP329380 (AltN1), PP329381 (AltN2), PP329382 (AltN3), PP329383 (AltN4), PP329384 (AltN5), PP329385 (AltN557), PP329386 (AltN558), PP329387 (AltN559), and PP329388 (AltN560). The experimental data generated in this study are provided in the Supplementary Information and Source Data files. The protein structures generated in this study have been deposited in the Protein Data Bank (PDB) under accession codes: 8JKU https://www.rcsb.org/structure/8JKU(N559), 8HWYhttps://www.rcsb.org/structure/8HWY(N559-M6). The eight extant sequences in the study are provided in the Source Data file. The two β -hydroxyacid dehydrogenase sequences are available under 4GBJ https://www.rcsb.org/structure/4GBJ and 3PEF https://www.rcsb.org/structure/3PEF. All raw data generated in this study are provided in the Supplementary Data and Source Data files.

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	For ee and conversion measurements typically n=3 independent experiments.
Data exclusions	A sequence with X character was excluded.
Replication	The experiments were independently replicated and consistently yielded similar results. The number of replicates is specified in the figure legends.
Randomization	Randomization is not typically applied in enantiomeric excess (ee) of enzyme/biocatalysis assays.
Blinding	Blinding is not typically applied in enantiomeric excess (ee) of enzyme/biocatalysis assays.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A