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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	1. The absorbance values for the determining enzyme activity, total phenolic content, free amino groups and thiol groups were obtained using an microplate reader AMR-100 (Allsheng, Hangzhou, China) or SuPerMax 3000FA (Shanpu, Shanghai, China). 2. HPLC was performed using the Agilent 1200 LC system equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector (RID, Agilent 1260). 3. GC-MS data were measured by GC-MS T8040NX (Shimadzu, Japan) equipped with a DB-5MS column (Agilent, J&W Scientific, 30 m × 0.25 mm × 0.25 μm). 4. LC-MS/MS data were acquired using an Ultimate 3000 system (Thermo Fisher, San Jose) coupled to a Thermo Scientific Q Exactive™ HF-X mass spectrometer, provided by Spectrum Biotech Co. (Suzhou, China). 5. CD data were collected on a Chirascan CD spectrometer (Applied Photophysics, Leatherhead, United Kingdom).
Data analysis	1. Statistical analysis was performed by GraphPad Prism (version 8). 2. The relative quantity of SDS-PAGE bands was analyzed by the Quantity One Software (version 4.6.2; BioRad, Shanghai, China). 3. For CaBglA identification, the MS spectra results were analyzed by the Mascot Software (Version 2.3.01, Matrix Science). 4. For PC-CaBglA crosslinking identification, software Byonic (Version 2.13, Protein Metrics, USA) was used for data analysis.

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

All data are contained in the paper and its Supplementary Information files. All data underlying this study are available from the corresponding author upon request. The protein mass spectrometry data generated in this study have been deposited in the ProteomeXchange Consortium database (https://proteomecentral.proteomexchange.org) via the iProX partner repository (https://www.iprox.cn/page/project.html) under accession code PXD073102. Source data are provided with this paper.

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	This is not relevant for this study
Reporting on race, ethnicity, or other socially relevant groupings	This is not relevant for this study
Population characteristics	This is not relevant for this study
Recruitment	This is not relevant for this study
Ethics oversight	This is not relevant for this study

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	No statistical methods were used to predetermine the sample size. As most experiments were designed to compare differences between two or more treatment groups, we typically employed n=3 biological replicates per group, with selected experiments using n=4. These sample sizes were determined based on common practice of the described experiments and the need to have sufficient statistical power.
Data exclusions	No data were excluded from any of the experiments.
Replication	All key experiments including enzyme incubation assays, pre- and post-incubation activity measurements, and quantification of various substrate components, were performed at least three biological replicates. All SDS-PAGE analyses were biologically replicated at least twice. LC-MS/MS analyses were carried out without replications, as a single experiment provided high peptide sequence coverage together with stringent false discovery rate (FDR) thresholds, provided sufficiently reliable data for the objectives of this study.
Randomization	Randomization was not relevant to this study. The allocation of samples into experimental groups was based directly on the treatment conditions. To ensure comparability across groups, all biological replicates within an experiment originated from the same source material, were processed in parallel, and were subjected to identical conditions (such as buffer, reaction time, and temperature), except for the variable under investigation.
Blinding	No blinding experiments are included in this study. Data was produced using objective quantitative methods to remove the influence of subjective bias.

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

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	This study did not involve laboratory animals. Two non-pathogenic bacterial species were utilized: Escherichia coli for molecular cloning and recombinant protein expression, and the thermophilic, anaerobic, cellulolytic bacterium Clostridium thermocellum for whole-cell saccharification of lignocellulose, and no other organisms were employed in this study.
Wild animals	No wild animals were used in this study.
Reporting on sex	Not applicable; sex is not a biological variable for the bacterial strain used.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	No ethical approval or guidance was required for this study, as it did not involve animals or human subjects, and the bacteria used are not infectious or pathogenic.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*