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	Data taken from clinical visits and laboratory data from the hospitals was collected on paper clinical report forms and entered using Excel spreadsheets (version 18025.20160). Experimental data was collected using ImageJ (version 1.54c), Imaris (version 10.0) and Thermo Xcalibur software (version 2.1).
Data analysis	GraphPad Prism (version 8.0.2); KneadData (version 0.5.1) FastQC (version 0.11.9) HUMAnN (version 3.6) ShortBred (version 0.9.5) MetaPhlAn4 (version 4.0.2) BlastP (version 2.10.1+) CD-hit (version 4.8.1) HMMER (version 3.1b2) Mafft (version 7.450) Fasttree (version 2.1.10) Fastp (version 0.20.0) QIIME 2 (versions 2017.9 and 2018.8) BBDuk (version 37.90) STAR (version 2.5.2b) Salmon (version 0.7.2) R (version 4.2.2)

Meta (version 7.0-0) R package
Survival (version 3.5-7) R package
Psych (version 4.2.3) R package
Mediation (version 4.5.0) R package
SSN analysis, https://efi.igb.illinois.edu/efi-est/
Clustal Omega, https://www.ebi.ac.uk/Tools/msa/clustalo/
Evolview, https://evolgenius.info//evolview-v2/
CIBERSORTx, https://cibersortx.stanford.edu/
Metascape, https://metascape.org/
All code is available in the lab's GitHub page: https://github.com/ZJJY-Bioinformatics/Cirrhosis-AADC.

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 metagenomic datasets for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB65440. The 16S rRNA sequences datasets for this study have been deposited in the ENA at EMBL-EBI under accession number PRJEB66346. All relevant sample-level patient data used in this study were provided Supplementary table 17.
The six previously published studies used are available under accession number: PRJEB6337; PRJEB14215 (Supplementary table 19), and GSE84044, GSE48452, GSE49541, GSE41919. Associated sample-level clinical metadata for external datasets were collected from their relevant publications. Additional public database used in this study include MetaCyc (https://biocyc.org/download.shtml); Uniref90 database (https://ftp.uniprot.org/pub/databases/uniprot/current_release/); GRCm38, M11(https://www.gencodegenes.org/mouse/release_M11.html); NCBI37 human genome (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.25/).

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	We have used the term sex in this manuscript to indicate a biological attribute as was suggested in the publishing guidelines provided by Nature Medicine. We considered sex in our study design and our findings apply to both male and female. In animal models, a mixture of male and female mice was employed in all experiments, and the exact distribution is stated in Supplementary table 18. Due to the small sample sizes in these experiments, no sex-based analysis was conducted. In human cohorts, both male and female participants were collected and were not significantly different between groups, and the exact distribution is stated in Supplementary table 14, 16, 17. Information about the sex of human participants was collected by the clinicians who directly interacted with these patients. This was carried out in accordance with the laws and practices in place at their relevant medical institutions. Sex was comparable between groups (Manuscript Fig.1b and e) or controlled for as a potentially confounding variable (Manuscript Fig.6e, i and j).
Reporting on race, ethnicity, or other socially relevant groupings	This study did not include, analyze, or report data based on these criteria.
Population characteristics	Data from 3 cohorts were used in this study. Cirrhosis cohort consists of cirrhotic patients (n = 31) and age- and sex-matched healthy subjects (n = 48). Patients diagnosed with liver cirrhosis based on clinical-pathologic features-including liver history, endoscopic, or radiologic findings (the presence of clinical cirrhosis was adjudicated in a blinded fashion by two separate hepatology experts; in order to be included both experts were required to agree on the presence of clinical cirrhosis. No clinically overt HE was reported in these patients, and their basic clinical data were provided in Supplementary table 17. HE cohort consists of cirrhotic patients with HE (n = 40), cirrhotic patients without HE (n = 41), and age and sex-matched healthy subjects (n = 22) cirrhotic patients. HE was carefully assessed by two independent hepatology experts and adjudicated to be present in the setting of a sudden change in mental state (from a state of previously normal consciousness) in the absence of another cause as described. Samples were collected before initiating HE treatment. TIPS cohort consists of cirrhotic patients that have clinically indicators for TIPS surgery. Clinically indicated for TIPS surgery to manage complications of portal hypertension, such as variceal bleeding or refractory ascites.
Recruitment	Cirrhosis cohort was recruited from the Department of Gastroenterology, West China Hospital. The inclusion criteria for cirrhosis were diagnosed with liver cirrhosis, have evidence of clinical liver disease for at least 6 months and were required to have an identifiable etiology of liver disease. The Exclusion criteria included the following: Individuals treated with antibiotics, probiotics or proton pump inhibitors within one month prior to sample collection; Any subject undergoing a gastrointestinal surgical procedure or colonoscopy within 6 months prior to sample collection; Any individual with uncontrolled infection; Individuals with uncontrolled systolic blood pressure. No clinically overt HE was reported in these patients, and their basic clinical data were provided in Supplementary table 17. HE cohort was recruited from the Department of Gastroenterology and Hepatology, Tianjin Third Central Hospital. The inclusion criteria for cirrhosis were the same as those for the cirrhosis cohort above. Clinical HE was carefully assessed by two

independent hepatology experts and adjudicated to be present in the setting of a sudden change in mental state. The inclusion criteria for healthy subjects were as follows: 1) Age and sex-matched with the cirrhotic patients from the "Health Checkup Center"; 2) Without clinically diagnosed chronic disease. Exclusion criteria were the same as those for the cirrhosis cohort described above. Samples were collected before initiating HE treatment.

TIPS cohort was recruited from the Department of Gastroenterology, West China Hospital. The inclusion criteria were: 1) diagnosed with liver cirrhosis based on clinical-pathologic features including liver history, endoscopic, or radiologic findings (the presence of clinical cirrhosis was adjudicated in a blinded fashion by two separate hepatology experts); 2) Clinically indicated for TIPS surgery to manage complications of portal hypertension; 3) Ability and willingness to provide informed consent for participation in the study. The Exclusion criteria were: 1) Severe cardiac, pulmonary, renal, or neurological diseases that could confound the study outcomes; 2) individuals treated with antibiotics, probiotics or proton pump inhibitors within one month prior to sample collection; 3) lost follow-up in the three-months follow-up period.

Ethics oversight

For the cirrhosis cohort, the study and all procedures were approved by the Medical Ethics Committee of West China Hospital and all participants gave written informed consent (ClinicalTrials.gov ID: NCT03990753).
For the HE cohort, the study and all procedures were approved by the Medical Ethics Committee of Tianjin Third Central Hospital and all participants gave written informed consent (ClinicalTrials.gov ID: NCT05140837).
For the TIPS cohort, the study and all procedures were approved by the West China Hospital and all participants gave written informed consent (ClinicalTrials.gov ID: NCT03177499).

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	In order to ensure the analysis power, the study includes as much as subjects as possible from the three cohorts. Thus no sample size calculation was performed. For each cohort we used samples that had both metagenomic and relevant data available: cirrhosis cohort: n = 79; HE cohort: n = 103; TIPS cohort: n = 60.
Data exclusions	In human cohorts, participants who did not provided both serum and fecal samples were excluded in some analysis. In animal experiments, no additional data were excluded from the analyses, but some figure panels include representative examples (e.g. immunofluorescence image).
Replication	In human cohorts, we used all available samples for analysis. For the experiments, replication was performed in all experiments and samples when possible. In vivo experiments were replicated with similar results, and the specific number of experiments performed during this study is provided in the Figure legends. For the in vitro screening of PDCI, each concentration of each inhibitor was performed three times. For the analysis of substrate specificity in AADCs, each experiment was performed at three times.
Randomization	In human cohorts, the sample collection, sequencing or LC-MS/MS analysis were performed in a random order. For screening of PDCI, or substrate specificity analysis in AADCs, the order in which samples were placed in 96-well plates or eppendorf tubes was randomized. The order in which samples were detected was also randomized. For animal experiments, mice were randomly assigned to groups according to their similar bodyweight and age prior to treatment. For behavioral experiment, mice were randomly selected depending on the group.
Blinding	For human samples, blinding was not applicable as it was an observational study. Detailed information on disease status, laboratory results and microbiota indices were required to conduct the analyses. For bacterial and animal experiments, investigators were not blinded to groups as this was necessary information to carry out studies. Data was analyzed in an objective fashion.

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

Antibodies

Antibodies used

Alexa Fluor™ 488-coated mouse anti-GFAP; Thermo Fisher Cat# 53-9892-82; RRID: AB_10598515; (1:400 for immunohistochemical staining)
Anti-rabbit IgG secondary antibody, Alexa Fluor™ 488; Invitrogen Cat# A-11008; RRID: AB_143165; (1:800 for immunohistochemical staining)
Anti-rabbit IgG secondary antibody, Alexa Fluor™ 633; Invitrogen Cat# A-21070; RRID: AB_2535731; (1:800 for immunohistochemical staining)
Rabbit anti-NeuN; Proteintech Cat# 26975-1-AP; RRID: AB_2880708; (1:400 for immunofluorescent staining)
Rabbit anti-MAP-2; Proteintech Cat# 17490-1-AP; RRID: AB_2137880; (1:500 for immunofluorescent staining)
Rabbit anti-Iba-1; Abcam Cat# ab178846; RRID: AB_2636859; (1:500 for immunofluorescent staining)
Mouse IgG Isotype control; Thermo Fisher Cat# 10400C; RRID: AB_2532980; (10 μg/g body weight for mice treatment)
Mouse Anti-Phenylethylamine monoclonal antibody, clone PEA (PEA-mab); Creative Diagnostics Cat# CABT-L2510; (10 μg/g body weight for mice treatment)

Validation

All antibodies are from commercial companies as following:
Alexa Fluor™ 488-coated mouse anti-GFAP: https://www.thermofisher.com/antibody/product/GFAP-Antibody-clone-GA5-Monoclonal/53-9892-82
Anti-rabbit IgG secondary antibody, Alexa Fluor™ 488: https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008
Anti-rabbit IgG secondary antibody, Alexa Fluor™ 633: https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21070
Rabbit anti-NeuN; Proteintech Cat# 26975-1-AP: https://www.ptgcn.com/products/NeuN-Antibody-26975-1-AP.html
Rabbit anti-MAP-2; Proteintech Cat# 17490-1-AP: https://www.ptgcn.com/products/MAP2B-Antibody-17490-1-AP.html
Rabbit anti-Iba-1; Abcam Cat# ab178846: https://www.abcam.com/products/primary-antibodies/iba1-antibody-epr16588-ab178846.html
Mouse IgG Isotype control; Thermo Fisher Cat# 10400C: https://www.thermofisher.com/antibody/product/Mouse-IgG-Isotype-Control/10400C
Mouse Anti-Phenylethylamine monoclonal antibody, clone PEA (PEA-mab); Creative Diagnostics Cat# CABT-L2510: https://www.creative-diagnostics.com/phenylethylamine-antibody-263061-144.html

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

Germ-free C57BL/6J mice (6-8 weeks old, maintained in sterilized vinyl isolators, kept at a constant temperature 20-26°C and humidity 40-60%).
Specific pathogen free C57BL/6J mice (6-8 weeks old, mice had free access to sterilized water and food ad libitum, under a strict 12-h light/dark cycle at a controlled temperature23°C ± 2°C, humidity 45 ± 5%).

Wild animals

No wild animals were used in this study.

Reporting on sex

The animal experiments were not specifically designed to address sex as a variable; however, equal numbers of males and females were included. The exact sex distribution is provided in Supplementary table 18. Due to the small sample sizes in these experiments, no sex-based analysis was conducted.

Field-collected samples

No samples collected from the field.

Ethics oversight

The ethics committee of Southern Medical University approved the animal protocols and animal care and experiments were done strictly adherence to the Southern Medical University animal care guidelines (ID: LAEC-2021-197).

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	For the cirrhosis cohort, ClinicalTrials.gov ID: NCT03990753. For the HE cohort, ClinicalTrials.gov ID: NCT05140837. For the TIPS cohort, ClinicalTrials.gov ID: NCT03177499.
Study protocol	https://classic.clinicaltrials.gov/
Data collection	Three cohorts were included in this study (Methods): The cirrhosis cohort, HE cohort and TIPS cohort. For the cirrhosis cohort, the clinical data and fecal samples were approved by the Medical Ethics Committee of West China Hospital, No clinically overt HE was reported in these patients, and their basic clinical data were provided in Supplementary table 17. For the HE cohort, the clinical data, fecal samples and serum samples were approved by the Medical Ethics Committee of Tianjin Third Central Hospital. Samples were collected before initiating HE treatment. For the TIPS cohort, the clinical data and serum samples were approved by the Medical Ethics Committee of West China Hospital. All participants gave written informed consent.
Outcomes	The main outcome is hepatic encephalopathy (HE) within three month after transjugular intrahepatic portosystemic shunts (TIPS). Clinical HE was carefully assessed by two independent hepatology experts and adjudicated to be present in the setting of a sudden change in mental state (from a state of previously normal consciousness) in the absence of another cause as described. Overall, 30% (18/60) developed overt HE within three month after TIPS. Serum samples were collected during TIPS surgery (Methods).

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA