nature portfolio

Corresponding author(s): **Yonghui Dong; Yue Pang**

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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	Cor	nfirmed
	x	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	X	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	X	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.
X		A description of all covariates tested
	X	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	X	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)
X		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.
X		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

 Policy information about availability of computer code

 Data collection

 Plots were generated using R package ggplot2 (v) under R (v4.2.0) environment and Inkscape (v1.1) software

 Data analysis

 Statistical analyses were performed under R (v4.2.0) environment. The lampreydb web database was organized

 Using MySQL (v.8.0) and Django (v3.0.6).

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 sequences files used in this study are available in the National Center for Biotechnology Information (accession number: ON814546, ON814547, ON814548, and ON814549). The metabolomics data have been archived in MetaboLights with MTBLS5857.

Human research participants

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

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
	N/A
Ethics oversight	

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.

x Lif	e sciences		Behavioural & social sciences		Ecological, evolutionary & environmental sciences
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For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

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

Sample size	For both targeted and untargted metabolomics studies, n=3 was chosen as the minimal replicate number
Data exclusions	Data were not excluded for any statistical analysis here
Replication	Five QC samples were included to check the data quality. A full QC report has been included as supplementary information along with this manuscript.
Randomization	The sample injection orders for both targeted and untargeted metabolomics studies have been randomized to minimize any possible within and between batch effects.
Blinding	Blinding was not possible during sample collection because sample tissues were clearly different from one another.

Behavioural & social sciences study design

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

Study description	N/A
Research sample	N/A
Sampling strategy	N/A

Data collection	N/A
Timing	N/A
Data exclusions	Ν/Α
Non-participation	N/A
Randomization	

Ecological, evolutionary & environmental sciences study design

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

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	N/A
Randomization	N/A
Blinding	N/A
Did the study involve field	d work? Yes X No

Field work, collection and transport

Field conditions	N/A
Location	N/A
Access & import/export	N/A
Disturbance	N/A

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

Methods	5
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n/a	Involved in the study	n/a	Involved in the study
X	Antibodies	X	ChIP-seq
X	Eukaryotic cell lines	X	Flow cytometry
X	Palaeontology and archaeology	X	MRI-based neuroimaging
	x Animals and other organisms		
X	Clinical data		
X	Dual use research of concern		

Antibodies

Antibodies used	N/A
Validation	N/A

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research	olicy information about	cell lines	and Sex and	Gender in	Research
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Cell line source(s)	N/A
Authentication	N/A
Mycoplasma contamination	N/A
Commonly misidentified lines (See <u>ICLAC</u> register)	N/A

Palaeontology and Archaeology

Specimen provenance	N/A			
Specimen deposition	N/A			
Dating methods	N/A			
Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.				
Ethics oversight	N/A			

Ethics oversight

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

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	The study did not involve laboratory animals
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Wild animals	The study did not involve laboratory animals		
Reporting on sex	The adult Japanese lampreys (L. japonica) at spawning migration stage were obtained in December 2020 in Songhua River in Heilongjiang province of China. The northeast lampreys (Lampetra morii) were obtained in December, 2020 in Yalu River in Liaoning province of China		
Field-collected samples	Lampreys were kept in fresh water at 10 ± 2 °C in dim light without feeding for 72 h. Then fourteen different lamprey tissues were carefully dissected, and subjected to spatial metabolomics analysis.		
Ethics oversight	The handling of lampreys was approved by the Animal Welfare and Research Ethics Committee of the Institute of Dalian Medical University (Permit number: AEE17013).		

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	N/A
Study protocol	N/A
Data collection	N/A
Outcomes	N/A

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:



- X Public health
- **x** National security
- X Crops and/or livestock
- **X** Ecosystems
 - Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No Yes

X

- Demonstrate how to render a vaccine ineffective
 Confer resistance to therapeutically useful antibiotics or antiviral agents
 Enhance the virulence of a pathogen or render a nonpathogen virulent
 Increase transmissibility of a pathogen
 Alter the host range of a pathogen
 Enable evasion of diagnostic/detection modalities
- **x** Enable the weaponization of a biological agent or toxin
- **X** Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

MA Confirm that both raw and final processed data have been deposited in a public database such as GEO.

MA Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	N/A
Files in database submission	N/A
Genome browser session (e.g. <u>UCSC</u>)	N/A

Methodology

Replicates	N/A
Sequencing depth	N/A
Antibodies	N/A
Peak calling parameters	N/A
Data quality	N/A
Software	N/A
Data quality	N/A

Flow Cytometry

Plots

Confirm that:

MA The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

MA The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

MA All plots are contour plots with outliers or pseudocolor plots.

N/A A numerical value for number of cells or percentage (with statistics) is provided.

N/A

Methodology

Sample preparation	(N/A
Instrument	N/A
Software	N/A
Cell population abundance	N/A
Gating strategy	N/A

MA Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

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N/A		
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X Not used		
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Preprocessing

Normalization

Acquisition

Imaging type(s)

Field strength

Area of acquisition Diffusion MRI

Preprocessing software

Normalization template

Noise and artifact removal

Volume censoring

Design specifications

Behavioral performance measures

Sequence & imaging parameters

Used

N/A

N/A

N/A

N/A

N/A

N/A

N/A

Statistical	modeling	&	inference

.		
Model type and settings	N/A	
Effect(s) tested	N/A	
Specify type of analysis: 🗌 Whole brain 📄 ROI-based 📄 Both		
Statistic type for inference (See <u>Eklund et al. 2016</u>)	N/A	
Correction	N/A	

Models & analysis

n/a Involved in the study		
X Functional and/or effective connectivity		
x Graph analysis		
X Multivariate modeling or predictive analysis		
Functional and/or effective connectivity	N/A	
Graph analysis	N/A	
Multivariate modeling and predictive analysis	N/A	