nature research

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Last updated by author(s): Aug 12, 2019

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Generic Data Acquisition (GDA) Software v8.52
Data analysis	XIA2 v0.4.0.342-ge8fbd7c-dials-1.2 -3d pipeline, PHASER v2.6.1, COOT v0.8.2, REFMAC v5.8.0151, CCP4i v7.0.014, PYMOL v1.2r1, MolProbity v4.3, GraphPad Prism 8.2.0, Pymol 2.3.2, Chemdraw 18.2, Jalview 2.10, DALI server, MolProbity ver. 4.3, Modeller 9.18, Chimera 1.12, Amber 16, Desmond 5.1

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Atomic coordinates and structure factors for recombinant, DON-modified human ASNS have been deposited in the Protein Data Bank with accession number 6GQ3. Coordinates for the computational models of the 1a/MgPPi/ASNS, 1b/MgPPi/ASNS and β–aspartyl-AMP/MgPPi/ASNS complexes, MD simulation trajectories and I/O files for the free energy calculations, and raw data for protein purification and kinetic assays are available from Professor Nigel Richards (RichardsN14@cardiff.ac.uk) on request. Requests for plasmids and other reagents needed to obtain the ASNS variants used in this study should be sent to Professor Yuichiro Takagi (ytakagi@iu.edu). Raw data for the chemoproteomic profiling experiments can be obtained by contacting Dr. Tyzoon Nomanbhoy (tyzoonn@ACTIVX.com).

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 ex-vivo experiments were preformed. This study uses purely in-vitro data.
Data exclusions	No data was excluded
Replication	Crystal growth was not replicated as sufficient data with high redundancy could be collected from one crystal. All kinetics measurements were performed in triplicates.
Randomization	Not applicable.
Blinding	Not applicable. We were not doing blinded trials.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Sf9 cells (Spodoptera frugiperda) were acquired from Expression Systems, LLC. HCT-116 cells were acquired from ATCC, CCL-247™
Authentication	None of the cell lines were authenticated
Mycoplasma contamination	Cells were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used