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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	LC-MS and LC-MS2 data were collected using XCalibur (Thermo Fisher Scientific); UV-Vis absorbance data for enzymatic assays were collected using Softmax Pro 6.22 (Molecular devices). Acquisition of ITC data was performed using the MicroCal Origin software (Malvern Panalytical).
Data analysis	LC-MS data of metabolomic samples were analyzed using XCalibur QuanBrowser 2.2 (Thermo Fisher Scientific). LC-MS2 data of proteomic samples were searched using Proteome Discoverer 2.1 (Thermo) and Mascot version 2.4.1 (Matrix Science) for sequence identification. X-ray diffraction data were indexed, integrated, and scaled using HKL2000 and molecular replacement performed using PHASER. The model was refined using PHENIX with manual model building in COOT. ITC traces were integrated and fitted to indicated binding models using MicroCal Origin data analysis module for VP-ITC (Malvern Panalytical). Anion-exchange chromatograms were integrated using Unicorn 6.4 (GE Healthcare). Regression and plotting were performed using PRISM (Graphpad). Structures were visualized using PyMOL 2.2.1 (Schordinger LLC). Figures were prepared using Adobe Illustrator.

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 upon request. 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

Structural data for the PurF-ppGpp complex used to generate Fig. 4d-f, 5a and Supplementary Fig. 4b-d have been deposited in RCSB PDB (https://www.rcsb.org/) with an identifier 6CZF. Raw proteomic LC-MS2 data as sources of Tables 1, Fig. 2c and the Supplementary Dataset have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD010402 and 10.6019/PXD010402. All other data generated or analyzed during this study are included in this published article (and its supplementary information files) or are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No sample size calculation is performed. We performed the capture-identification experiment in three biological replicates to assess the reproducibility of peptide identification. We did not make any statistical inference from these replicates. For biochemical or metabolomic experiment, a small sample size (2 or 3) was chosen due to the significant and consistent differences between groups.
Data exclusions	Prior to SILAC quantification, peptides with a MASCOT score less than 25 or an isolation interference greater than 30 were excluded due to the uncertainty of their sequences. The isolation interference-based criterion has been established by Sandberg et al. (J. Proteomics, 2014, 96, pp. 134-44). We further arbitrarily excluded proteins identified by only one unique peptide or by 2 or more unique peptides only once out of three biological replicates for the lack of reliability. In rare occasions, autosampler malfunction during LC-MS analysis of metabolomic samples may give rise to datasets with abnormally high signals for all internal standards. To avoid ion suppression caused by overload, such datasets are excluded and same samples are re-analyzed on the same instrument to provide substitutes.
Replication	As described above, we replicated all biochemical and metabolite-profiling experiments. All replicate experiments are successful.
Randomization	This study does not involve subjects that require randomization.
Blinding	This study does not involve procedures that require blinding.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Unique biological materials
☐	☒	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology
☒	☐	Animals and other organisms
☒	☐	Human research participants

Methods

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

Antibodies

Antibodies used	Immunoblotting for 6XHis-tagged proteins was performed using 6XHis-tag monoclonal antibody (ThermoFisher Scientific, MA1-21315)
Validation	Validation information of the 6XHis-tagged monoclonal antibody is available on the vendor's website at https://www.thermofisher.com/antibody/product/6x-His-Tag-Antibody-clone-HIS-H8-Monoclonal/MA1-21315