

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                                                                                                                                                                                                                                                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | Rosetta software suite (2019.01) ( <a href="https://www.rosettacommons.org/software">https://www.rosettacommons.org/software</a> )<br>PyRosetta-4 (release 2019.22) ( <a href="https://www.pyrosetta.org/downloads">https://www.pyrosetta.org/downloads</a> )<br>Source code and design scripts are available at: <a href="https://github.com/hanlunj/buttressed_loops.git">https://github.com/hanlunj/buttressed_loops.git</a> and <a href="https://doi.org/10.5281/zenodo.10999147">https://doi.org/10.5281/zenodo.10999147</a> |
| Data analysis   | autoPROC (1.0.5); Phaser (2.8.3); Coot (0.9.5 and 0.9.8.6); XDS (Feb 5, 2021); BUSTER (2.10.4); Phenix (1.19.1_4122 and 1.20.1_4487); Phenix AutoBuild (1.18.2_3874 and 1.19.2_4158); TLSMD (13 June 2012); Molprobit (implemented in Phenix 1.19.1_4122 and 1.20.1_4487); Python (3.7); seaborn (0.11.2); matplotlib (3.1.3); pandas (0.24.2); PyMOL (2.4.1)                                                                                                                                                                     |

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 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 the design models, protein sequences and DNA sequences are available at: [https://files.ipd.uw.edu/pub/2023\\_buttressed\\_loops/data.tar.gz](https://files.ipd.uw.edu/pub/2023_buttressed_loops/data.tar.gz) and Zenodo (<https://doi.org/10.5281/zenodo.10999147>). Crystal structures and reflection data have been deposited in the RCSB Protein Data Bank with accession IDs 8FRE (RBL4) and 8FRF (RBL7\_C2\_3). X-ray diffraction images have been deposited in the SBGrid Data Bank (8FRE and 8FRF).

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

|                             |                                  |
|-----------------------------|----------------------------------|
| Reporting on sex and gender | <input type="text" value="N/A"/> |
| Population characteristics  | <input type="text" value="N/A"/> |
| Recruitment                 | <input type="text" value="N/A"/> |
| Ethics oversight            | <input type="text" value="N/A"/> |

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](https://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     | <input type="text" value="We characterized in total 102 designed RBLs and 34 peptide-binding RBLs that passed our in silico filters which were used to predict the protein folding and protein-peptide interactions. These designs were of significant structural and sequence diversity."/> |
| Data exclusions | <input type="text" value="No data were excluded from analysis."/>                                                                                                                                                                                                                            |
| Replication     | <input type="text" value="Designed proteins were expressed and purified in multiple batches. The samples used for CD, SAXS and X-ray crystallography were independently prepared."/>                                                                                                         |
| Randomization   | <input type="text" value="We did not employ randomization, as all the samples were characterized by the same procedure and there was no treatment group in our study."/>                                                                                                                     |
| Blinding        | <input type="text" value="Blinding was not relevant to our study, as there was no treatment group in our study."/>                                                                                                                                                                           |

## 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                                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |

Methods

|                                     |                                                 |
|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |