nature portfolio

Corresponding author(s): Duyoung Min

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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	LabVIEW (version 19.0f2) for the magnetic tweezer data; Tecan i-control (version 2.0.10.0) for the absorbance and fluorescence data; Xcalibur (version 4.3) for the LC-MS/MS data; GROMACS MD software package (version 2021.4) for the MD trajectory data; Voronoia (version 1.0) for protein cavity data; the GAMESS program package for the DFT calculation data; Win-EPR for the EPR data;
Data analysis	MSFragger (version 3.8), FragPipe (version 20.0), Sequest Sorcerer platform (Sagen-N Research) and MODplus (modification search tool; version 1.02) used for the LC-MS/MS data; Visual Molecular Dynamics (version 1.9.3) used for visualization of MD simulation trajectory files; Python 3.9.12 (https://www.python.org), NumPy 1.22.4 (https://numpy.org), Pandas 1.4.2 (https://pandas.pydata.org), biopandas 0.4.1 (https://biopandas.github.io/biopandas/), matplotlib 3.5.1 (https://matplotlib.org), seaborn 0.11.2 (https://seaborn.pydata.org), and MDAnalysis 2.6.1 (https://www.mdanalysis.org) used for MD simulations and MC analysis; PANTHER classification system (version 18.0) used for protein classification; OriginPro 2020 (ver.9.7.0.185) for other data. Analysis codes are available in Code Ocean (https://doi.org/10.24433/CO.6042424.v4).

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 data that support the findings of this study are available in the main text, Supplementary Information, and Source Data, as well as corresponding author(s) upon request. The Source Data are provided with this paper. The mass spectrometry data for single proteins have been deposited to the ProteomeXchange consortium via the jPOST repository under the accession codes PXD056895 for ProteomeXchange [https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD056895] and JPST003425 for jPOST [https://repository.jpostdb.org/entry/JPST003425]. The mass spectrometry data for whole-cell proteomics were deposited to the ProteomeXchange consortium via the PRIDE partner repository under the accession code PXD038746 [https://dx.doi.org/10.6019/PXD038746]. Other raw data such as the MD simulation trajectory files and bioinformatic/structural analyses for cellular proteomes have been deposited to figshare, which are available in [https://doi.org/10.6084/m9.figshare.24751755].

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	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

Life sciences study design

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

Sample size	The sample sizes of single-molecule tweezer experiments were chosen based on previous studies (Naqvi, M. M. et al. Sci Adv 8, eabl6293 (2022); Min, D. et al. Nat chem biol 14, 489-496 (2018); Kim, S. et al. Elife 12 (2023)). For other experiments such as mass spectrometry and spectrophotometric assays, the experiments were performed in triplicate, which is the typical sample size in biological sciences. The specific numbers for sample sizes are described in the corresponding figure captions.
Data exclusions	No data were excluded.
Replication	All replication attempts were successful, with no null measurements observed. The single-molecule tweezer experiments were performed five to twelve times for each condition. The ROS measurements, the LC-MS/MS, and the EPR measurements were performed in triplicate for each condition.
Randomization	Randomization is not applicable to our study because all experiments involve specific sample conditions that cannot be randomized.
Blinding	Blinding is not applicable to our study because group allocation is not relevant to our experiments.

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HeLa (ATCC, CCL-2)
Authentication	The cell line was authenticated by the commercial vendor ATCC. Example morphology, STP profiling, Karyotype, or other information about the cell line are provided in a website (https://www.atcc.org/products/ccl-2).
Mycoplasma contamination	The cell lines were tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	None

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a