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Corresponding author(s): David A. Agard

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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 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	Cryo-EM data collection was done on a Titan Krios (Thermo Fischer Scientific) electron microscope (300kV) with a Gatan K2 direct electron detector (Gatan). Automatic data collection was done with SerialEM v.3.8-beta.
Data analysis	EM data processing was done with RELION v.3.0.8, UCSF MotionCor2 (MotionCor2_1.1.0-Cuda80), Gautomatch (Gautomatch-v0.53_sm_20_cu7.5_x86_64), CTFFIND v.4.1, PyMOL 1.8.4.2, UCSF Chimera v.1.14, and UCSF ChimeraX v.0.94. Model building was done using Rosetta v.3.11. Hsp70 binding site predictions were done using BiPPred server (https://www.bioinformatics.wzw.tum.de/bippred/submit/) and ChaperISM (https://github.com/BioinfLab/ChaperISM).

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 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

The cryo-EM maps generated in this study have been deposited in the Electron Microscopy Data Bank (EMDB) under the accession numbers EMD-23050 (GR:Hsp90:Hsp70:Hop; global map), EMD-23053 (Hsp90A-NTD-MD:Hsp70C-NBD; focused map), EMD-23054 (Hsp90B-NTD-MD: Hsp70S-NBD; focused map), EMD-23056 (Hsp90B-NTD-MD:Hsp70S-NBD:Hop-TPR2A-TPR2B-DP2; focused map), EMD-23055 (Hsp70S-NBD:Hop-TPR2A), and EMD-23051 (Hsp90AB-CTD:Hsp70S-SBD-β:Hop-DP2:GR-Helix 1). The atomic coordinates have been deposited in the PDB under the accession number 7KW7 (GR:Hsp90:Hsp70:Hop). Atomic models

used in model building are (also listed in Supplementary Table 2): 3T0H, 3Q6M, 5FWK for Hsp90; 3AY9, 4PO2, 4EZQ for Hsp70; 3UQ3 and 2LLW for Hop; 1M2Z for GR.

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 statistical method was used for sample size calculation. The amount of proteins used for the biochemical experiments was chosen based on previous experiences with this specific type of experiments and commonly used sample sizes in the field of research. For single particle cryoEM reconstructions, sample sizes were determined by available electron microscopy time and the number of particles on each micrograph obtained during the collection time.
Data exclusions	No data was excluded.
Replication	The number of the replications is stated in the Figure Legends.
Randomization	No randomization was performed, since this study did not allocate experimental groups.
Blinding	No blinding was performed, since it is not a common procedure for the methods used.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Anti-MBP (maltose-binding protein) (New England BioLabs; catalog: E8032S; dilution=1:10,000), anti-Hsp70 (Enzo Life Sciences; catalog: ADI-SPA-811-D; dilution=1:10,000), and anti-STIP1 (Proteintech/ThermoFisher; catalog: 15218-1-AP; dilution: 1:10,000) antibodies.
Validation	The Anti-MBP antibody from NEB was verified using both Western blotting and ELISA as described on the product's website (https://www.neb.com/products/e8032-anti-mbp-monoclonal-antibody#Product%20Information). The anti-Hsp70 antibody was validated using Western blot as described on product's website (https://www.enzolifesciences.com/ADI-SPA-811/hsp70-hsp72-polyclonal-antibody/). The anti-STIP1 anti-body was validated using Western blot as described on the product's website (https://www.thermofisher.com/antibody/product/STIP1-Antibody-Polyclonal/15218-1-AP).