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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

For Cryo-EM data collection:
EPU v1.5.0.32 software (FEI) and SCIPION v3.0 wrapper was used for data collection

Data analysis

Luminescence was read on a Tristar2 LB 942 (Berthold technologies)
Graph Prism 9.5.1 was used to perform data analysis
Confocal microscopy data were processed and analyzed with Image J 2.9.0/1.53t

For Cryo-EM data analysis:
MotionCor2 1.4.0 and Gctf 1.06 were used for motion and CTF correction, respectively.
Helixplorer-1 software (<https://rico.ibs.fr/helixplorer/>) was used for determination of the helical parameters
cryoSPARC v4.1.1 was used for data processing for the generation of electron density maps.
Coot 0.9.6 was used for model hand editing
Phenix v1.20.1-4487 was used for structure coordinates refinement

For structure prediction and analysis:
ChimeraX version 1.4.dev202204260114 was used for map and model visualization and generation of figures

AlphaFold2 was used for CHIKV model coordinates prediction through ColabFold.
 PISA server (<https://www.ebi.ac.uk/pdbe/pisa/>) was used for structure analysis
 Toffee (<https://tcoffee.crg.eu/apps/tcoffee/>) was used for amino acid sequence alignments.

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](#)

The data that support this study are available from the corresponding authors upon request.

The electron density maps and models generated in this study have been deposited in the Electron Microscopy and Protein Data Banks, respectively, as follows: The helical reconstruction map at 2.53 Å and model coordinates (EMD: 17729 [<https://www.ebi.ac.uk/emdb/EMD-17729>], PDB: 8PK7 [<https://www.rcsb.org/structure/8PK7>]). The focus refinement map at 2.35 Å resolution and the refined model coordinates: (EMD: 17678 [<https://www.ebi.ac.uk/emdb/EMD-17678>], PDB: 8PHZ [<https://www.rcsb.org/structure/8PHZ>]). For the map at showing the densities corresponding to the macrodomain with a resolution of 2.98 Å and the 3D focused classification map at 6.84 Å (EMD: 17730 [<https://www.ebi.ac.uk/emdb/EMD-17730>]). All maps and models will be publicly available from 14 Aug 2024.

The source data generated in this study for western blot, viral titrations, microscopy statistical analysis and luminescence read outs are provided in the Source Data file and Supplementary information. The nsP3 protein sequences used in this study are available in the Uniprot database under accession codes Q8JUX6 [<https://www.uniprot.org/uniprotkb/Q8JUX6/>] for CHIK, P13886 [<https://www.uniprot.org/uniprotkb/P13886/>] for O'nyong-nyong virus (ONNV), P13887 [<https://www.uniprot.org/uniprotkb/P13887/>] for Ross river virus (RRV), Q8QZ73 [<https://www.uniprot.org/uniprotkb/Q8QZ73/>] for Mayaro virus (MAYV), Q4QXJ8 [<https://www.uniprot.org/uniprotkb/Q4QXJ8/>] for Eastern equine encephalitis virus (EEEV), P36327 [<https://www.uniprot.org/uniprotkb/P36327/>] for Venezuelan equine encephalitis virus (VEEV), P87515 [<https://www.uniprot.org/uniprotkb/P87515/>] for Barmah forest virus (BFV), P03317 [<https://www.uniprot.org/uniprotkb/P03317/>] for Sindbis virus (SINDO), Q86924 [<https://www.uniprot.org/uniprotkb/Q86924/>] for Aura virus (AURAV) and Q8JX1 [<https://www.uniprot.org/uniprotkb/Q8JX1/>] for Sleeping disease virus (SDV).

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

No experiments presented in this study required sample size to be determined. All the experiments with statistical analysis were repeated at least two independent times with at least two technical replicates.

Data exclusions

No data were excluded

Replication

All cell culture experiments were repeated at least two independent times with multiple technical replicates as mentioned in the figure legends. For these experiments cells were inoculated with different multiplicity of infection (MOI) and data presented in figures showed results for one representative MOI in the linear range of infection.

Randomization	No experiments presented in this study required randomization
Blinding	No experiments presented in this study required blinding

Behavioural & social sciences study design

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

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing	N/A
Data exclusions	N/A
Non-participation	N/A
Randomization	N/A

Ecological, evolutionary & environmental sciences study design

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

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	N/A
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☒ No

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

Antibodies

Antibodies used	anti-Flag M2 mouse monoclonal antibody (Fs1804, Sigma), anti-dsRNA J2 mouse monoclonal antibody (Scicons), anti-nsP1, anti-nsP2, anti-nsP3 and anti-Capsid rabbit polyclonal antibodies were provided by Andres Merit (University of Tartu, Estonia), anti-CHIKV E2 mouse monoclonal antibody 3E4 was provided by V. Choumet (Pasteur Institute, France), anti-GAPDH mouse monoclonal antibody (SC-47724, Santa Cruz Biotechnology), anti-FHL1 mouse monoclonal antibody (MAB5938, R&D Systems) and anti-G3BP1 mouse monoclonal antibody (H-10 sc-365338, Santa Cruz Biotechnology). Cells infected with CHIKV were stained with either mAb anti E2 (1/700), mAb anti-Capsid (1/100), mAb anti G3BP1 (1/200), mAb anti-FHL1 (1/200) or mAb anti-dsRNA (1/500) followed by staining with Alexa Fluor AF488-conjugated Goat anti-mouse IgG (ThermoFisher, 1/1,000). Cells were also stained with rabbit anti nsP1 or nsP2 (1/1,000) followed by staining with Alexa Fluor AF488-donkey anti-rabbit IgG (ThermoFisher, 1:1,000). For immunoblot analyses, membrane were immunostained with rabbit anti-nsP1, nsP2, nsP3 or nsP4 (1/10,000) followed by immunostaining with HRP-conjugated goat anti-rabbit IgG (1/10,000). Cells transfected with FLAG-tagged constructs were stained with anti-FLAG M2 (1/) followed by staining with Alexa Fluor AF488- or Alexa Fluor 647-conjugated Goat anti-mouse IgG (ThermoFisher, 1/1,000) for immunofluorescence or with anti-FLAG M2 (1/5,000) followed by HRP-conjugated rabbit anti-mouse IgG (Dako Cytomotion, P0260, 1/5,000).
Validation	All the antibodies have been used in previous studies.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK-293T (CRL-3216) and Fibroblast BJ-5ta cells (CRL-4001) were purchased from ATCC, Vero E6 were provided by Marc Lecuit (Pasteur Institute Paris, France), BHK-21 were provided by Beate M. Kümmerer (University of Bonn and German Centre for Infection Research, Germany) and U2OS cells were provided by Andres Merits (University of Tartu, Estonia)
Authentication	none
Mycoplasma contamination	All cell lines were tested every two weeks for mycoplasma contamination using the MycoAlert Myoplasma detection kit from Lonza Primary cell lines were not tested
Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used in this study