nature portfolio

Corresponding author(s): Peng Gong

Last updated by author(s): Aug 6, 2026

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	Serial-EM software (4.1) was used for cryo-EM data collection.
Data analysis	cryoSPARC (v4.2.1); UCSF Chimera (1.15); UCSF ChimeraX (1.8); Coot (0.8.9.2); Phenix (1.19); PyMol (3.0.4); AlphaFold 3; THESEUS (3.3.0); APBS (1.5); SrufRace (5.0); WebLogo (2.8.2); GraphPad Prism (8.3.0).

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 cryo-EM density maps and atomic coordinates have been deposited to the Electron Microscopy Data Bank (EMDB) and the Protein Data Bank (PDB), respectively. The accession numbers are listed as follows: apo structure (25XL, EMD-80447); cRNA-bound structure (25XM, EMD-80448); EC14 structure (25XN, EMD-80449); EC14b structure (25XO, EMD-80450). Source data are provided with this paper.

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	27,140 micrographs were used for determining CCHFV L apo and 5′-cRNA bound. The structure of CCHFV RdRP elongation complex (EC14 and EC14b) was solved using 34,552 micrographs. No statistical methods were used to predetermine sample size. Sample size for the cryo-EM study was determined by the requirement to ensure high-resolution reconstruction. For minigenome assay, sample size are reported in the figure legends. Generally, experiments were done individually for three biological replicates. For biochemical experiments, sufficient sample sizes were used to ensure experimental reproducibility. Exact sample sizes are indicated in the methods and figure legends.
Data exclusions	Cryo-EM particles that are of low-resolution or are not having the shape or size of CCHFV L were excluded during 2D and 3D classification.
Replication	The data shown in micrographs in Figs. 1a, 3b, 4b-c and Extended Data Fig. 8c were repeated at least three times independently with similar results. The data shown in micrographs in Fig. 1c and Extended Data Figs. 3e and 9d-f were repeated twice independently with similar results. Note that all enzymology experiments were reproducible across different protein preparations.
Randomization	Randomization was not performed. This study involved biochemical assays, minigenome assay, and structural determination. In each case, samples were processed in parallel under identical conditions without allocation to distinct experimental groups. For comparisons involving mutant constructs, at least three independent replicates were performed.
Blinding	Investigators were not blinded to group allocation during data collection or analysis. The primary readouts are objective, including instrument-based measurements for biochemical, minigenome assays and structural analyses.

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: The mouse anti-DDDDK monoclonal antibody (ABclonal, AE005); the mouse anti-β-actin monoclonal antibody (ABclonal, AC004); HRP-conjugated goat anti-mouse antibody (Invitrogen, G-21040) .

Validation: The mouse anti-DDDDK monoclonal antibody (ABclonal, AE005) was used at a 1:3000 dilution to determine the expression levels of the flag-tagged CCHFV L protein and its mutants via western blot analysis. And the mouse anti-β-actin monoclonal antibody (ABclonal, AC004) was used at a 1:3000 dilution as the internal control. Immunoreactive bands were detected using HRP-conjugated goat anti-mouse antibody (Invitrogen, G-21040) at a 1:5000 dilution. The results showed that these antibodies allow detection of the specific corresponding protein with expected size.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): Sf9 cells for baculorvirus expression system (CCTCC, GDC0008). BSR-T7/5 cells for CCHFV-minigenome assay (Cellverse Co., Ltd, iCell-c054).

Authentication: Cells are routinely used in our labs and were not specifically validated for these studies outside of the manufacturer's specifications. Sf9 cells were authenticated via polymerase chain reaction (PCR) assay and isoenzyme analysis. Isoenzyme analysis was performed to determine glucose-6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LD). BSR-T7/5 cells were authenticated via short tandem repeat (STR) profiling.

Mycoplasma contamination: Cell lines tested negative for mycoplasma, bacterial and fungal.

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell lines were used in this study.

Plants

Seed stocks: No seed stocks were used in this study.

Novel plant genotypes: No novel plant genotypes were generated in this study.

Authentication: This is not applicable to this study.