nature portfolio

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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	Data were collected on a Talos Arctica electron microscope (Thermo Fisher Scientific) using the EPU Automated Data Acquisition Software for Single Particle Analysis (Thermo Fisher Scientific)
Data analysis	All image-processing steps were performed within the Scipion software framework. Movies were motion-corrected and dose-weighted with Motioncor. Aligned, non-dose-weighted micrographs were used to estimate the contrast transfer function (CTF) with Ctffind4 50. All subsequent image processing steps were performed using Relion 2.1. Particles were picked up with Xmipp. 2D and 3D classification was performed with Relion. Local resolution was estimated using MonoRes. The localized reconstruction method was done with the LocalRec plugin available in Scipion. Aomic models were calculated with Coot and refined in Phenix; quality of the obtained models was assessed with Molprobity and with the Worldwide PDB (wwPDB) OneDep System. Graphics were produced using UCSF Chimera. The electrostatic potential fwas calculated using the Coulombic surface coloring tool available within UCSF Chimera. RNA-capsid interactions were analyzed with the Find clashes/contacts tool within UCSF Chimera. Secondary structure prediction for the ssRNA genome of RV-B14 was done with the server RNAfold.

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 atomic coordinates and cryo-EM density maps were deposited in the Protein Data Bank and EM Data Bank with codes 8PNF and EMD-17781 for the full RV-B14 virion, and 8PNB and EMD-17780 for the empty RV-B14 capsid.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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	Sample size, 473,724 vitrified particle images, was approriate to calculate a 3D map of full virions with 313,305 images at 2.89 Å resolution and a 3D map of empty virions with 8,381 images at 3.77 Å resolution
Data exclusions	We did not exclude any data from consideration. All virus images were used in the initial 2D classification, and a and refinementsll selected images were used in the subsequent 3D classification and refinement.
Replication	3D maps were calculated with different software packages such as Relion and CryoSPARC, with different parameters, and the resulting maps were similar.
Randomization	Bias to calculate the 3D maps is minimized based on the gold-standard (FSC = 0.143) criterion by comparison of two entirely independent maps (i.e., the dataset is randomly split into two independent halves that are separately refined and reconstructed)
Blinding	N/A

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

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)	H1 HeLa cells (American Type Culture Collection [ATCC CRL-1958])
Authentication	ATCC
Mycoplasma contamination	Cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	N/A