nature research

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Last updated by author(s):	Mar 16, 2021

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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
\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
\boxtimes	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
\boxtimes	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.
\boxtimes	A description of all covariates tested
\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
\boxtimes	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)
\boxtimes	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	\square Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Sof	ftware and code

Policy information about <u>availability of computer code</u>

Data analysis

RELION 3.1, MotionCor2 v. 1.1.0, Gctf v. 1.06, Phenix suite v 1.16, Coot (v. 0.8.9.1), MolProbity 4.5.1, Buccaneer (v. 1.6.10) CCP-EM (v. 1.4.1), UCSF Chimera (v. 1.14), GraphPad Prism (v. 8.1.2), PyMOL (v. 2.3.0), UCSF ChimeraX (v. 1.0), EMAN2 (v. 2.3.1)

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 <u>availability of data</u>

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 data supporting the findings of this manuscript are available from the corresponding authors upon reasonable request. A Reporting Summary for this Article is available as a Supplementary Information file. Source data are provided with this paper. Cryo-EM maps and models have been deposited in the Electron Microscopy Data Bank and the Protein Data Bank, respectively: WNVKUN (PDB ID: 7KVA, EMD-23044); bWNVKUN (PDB ID: 7KV9, EMD-23043); bMVEV (PDB ID: 7KVB, EMD-23045); and bDENV-2 (PDB ID: 7KV8, EMD-23042). Where published structures are mentioned in the text, the PDB code is provided.

Fig2 includes all four structures WNVKUN, bWNVKUN, bDENV2, bMVEV. Validation reports attached.

Fig3 included analysis of WNVKUN and bWNVKUN structures

Fig4 includes bMVEV, bWNVKUN and published JEV structure PDB:5WSN

gs includes bMVFV, bMVNKUN and published JEV structure PDB:5WSN get includes bMVFV, bMVNKUN and published MV structure PDB: 12TX g8 includes bDENV-2 and published DENV-2 structure: PDB: 403 groundes bDENV-2 and published DENV-2 structure: PDB: 403 groundes bDENV-2 and published DENV-2 structure: PDB: 403 groundes bDENV-2 and published DENV-2 structure: PDB: 401 groundes bDENV-2 structures PDB: 3127 and PDB: 4UTC ield-specific reporting gase 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 a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summaneflat.pdf ife sciences study design studies must disclose on these points even when the disclosure is negative. Sample size Cryo-EM sample size was not predetermined. Each cryo-EM dataset contained thousands of virus particles, from a single biological sample. The size of each dataset was based on instrument availability. Relevant statistics for image/particle numbers are provided under the cryo-EM section and in Fig. 7. Data exclusions Cryo-EM data collection was performed once for each sample. Fig 9c Individual data points represent mean value from two independent viral itrations. Replication Cryo-EM data collection was performed once for each sample. Fig 9c Individual data points represent mean value from two independent viral itrations. Bilinding Bilinding not relevant, analysis and collection of cryo-EM data did not require statistical interpretation and human bias is mitigated by established validation metrics. exporting for specific materials, experimental systems and methods used in many studies. Here, indicate whether each material, ten 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.
ield-specific reporting bease 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 a reference copy of the document with all sections, see nature com/documents/nr-reporting-summary-flat.pdf if esciences study design Istudies must disclose on these points even when the disclosure is negative. Cryo-EM sample size was not predetermined. Each cryo-EM dataset contained thousands of virus particles, from a single biological sample. The size of each dataset was based on instrument availability. Relevant statistics for image/particle numbers are provided under the cryo-EM section and in Fig. 7. Data exclusions Cryo-EM images of poor quality were removed based on visual inspection and assessment of the contrast transfer function. Replication Cryo-EM data collection was performed once for each sample. Fig 9c Individual data points represent mean value from two independent viral titrations. Randomization Cryo-EM data were split randomly into two half-datasets and independently refined. Fourier Shell Correlation between two half-datasets was used to provide a "gold-standard" estimate of the resolution of the cryo-EM maps. Blinding Blinding not relevant, analysis and collection of cryo-EM data did not require statistical interpretation and human bias is mitigated by established validation metrics. eporting for specific materials, systems and methods used in many studies. Here, indicate whether each material,
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stem 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 Methods
/a Involved in the study n/a Involved in the study
Antibodies ChIP-seq
Eukaryotic cell lines Flow cytometry
Palaeontology and archaeology MRI-based neuroimaging Animals and other organisms
Animals and other organisms Description Human research participants Human research partic
Clinical data
Dual use research of concern
<u> </u>
ntibodies

Antibodies used

4G4 (hybridoma culture supernatant and recombinant expiCHO); E16 (recombinant expiCHO); Goat anti-mouse Immunoglobulins antibody, HRP conjugate (Dako, Cat: P0447); IRDye 800CW Goat anti-human IgG secondary (LiCor, Cat: 926-32232); AlexaFluor 488conjugated goat anti-mouse IgG (H+L) secondary (Invitrogen; Cat: A-11001)

Validation

4G4 mab - Clark et al. J Gen Virol. 2007 Apr;88(Pt 4):1175-83.

Can be found here: https://eshop.uniquest.com.au/ns1-protein-monoclonal-antibody-mmabs-4g4/

E16 mAb - Nybakken et al. Nature. 2005. 437, 764-769, doi:10.1038/nature03956

Goat anti-mouse Immunoglobulins antibody, HRP conjugate - https://www.agilent.com/en/product/immunohistochemistry/ antibodies-controls/secondary-antibodies/goat-anti-mouse-immunoglobulins-hrp-(affinity-isolated)-153239

IRDye 800CW Goat anti-human - https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-human-igg-secondary-antibody AlexaFluo 488-conjugated goat anti-mouse - https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) C6/36 cells (Aedes albopictus) - sourced ATCC CRL1660

BSR cells (Mesocricetus auratus, baby hamster kidney) - sourced ATCC CCL-10

Vero cells (Cercopithecus aethiops, African green monkey kidney) - sourced ATCC CRL1586

Authentication Cell lines were not genetically confirmed, but their morphologies were visually confirmed.

Mycoplasma contamination Cell lines tested negative for mycoplasma.

Commonly misidentified lines (See <u>ICLAC</u> register)

No commonly misidentified cell lines were used.