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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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 was collected with SerialEM version 4.0.6.
ELISA data was collected by Tecan microplate reader (Tecan Life Sciences) with software SPARKCONTROL version 2.2.

Data analysis

CryoSPARC v4.2.1 and v4.5.3, and Relion 4.0 were used for cryo-EM single-particle analysis.
The SWISS-MODEL web server was used for protein model building.
MolView web server was used to build the heparin tri-saccharide ligand.
Phenix 1.21.1 and Coot 0.9.8 were used for model building, refinement, and validation.
GraphPad Prism 10 was used for statistical analysis of TEER and ELISA data.
UCSF Chimera 1.14 and ChimeraX 1.7.1 were used for visual presentation of cryo-EM maps and coordinates, as well as figure preparation.
AlphaFold 3 was used to generate models for DENV1 NS1, DENV3 NS1, and WNV NS1.
The APBS web server (server.poissonboltzmann.org), with APBS v3.4.1 and PDB2PQR v3.6.1, was used for electrostatic surface analysis.

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](#)

Cryo-EM density maps generated in this study have been deposited in the Electron Microscopy Data Bank (EMDB) under accession codes: EMD-65563 [<https://www.ebi.ac.uk/emdb/EMD-65563>] (dimer), EMD-65562 [<https://www.ebi.ac.uk/emdb/EMD-65562>] (loose tetramer 1), EMD-65558 [<https://www.ebi.ac.uk/emdb/EMD-65558>] (loose tetramer 2), EMD-65561 [<https://www.ebi.ac.uk/emdb/EMD-65561>] (stable tetramer 1), EMD-65560 [<https://www.ebi.ac.uk/emdb/EMD-65560>] (stable tetramer 2), EMD-65564 [<https://www.ebi.ac.uk/emdb/EMD-65564>] (stable tetramer:heparin complex), EMD-65565 [<https://www.ebi.ac.uk/emdb/EMD-65565>] (sNS1-mVenus), EMD-65566 [<https://www.ebi.ac.uk/emdb/EMD-65566>] (sNS1-mVenus Class 1), and EMD-65559 [<https://www.ebi.ac.uk/emdb/EMD-65559>] (sNS1-mVenus Class 2). Corresponding atomic coordinates have been deposited in the RCSB Protein Data Bank (PDB) under accession codes: 9W22 [<https://doi.org/10.2210/pdb9W22/pdb>] (dimer), 9W21 [<https://doi.org/10.2210/pdb9W21/pdb>] (loose tetramer 1), 9W1X [<https://doi.org/10.2210/pdb9W1X/pdb>] (loose tetramer 2), 9W20 [<https://doi.org/10.2210/pdb9W20/pdb>] (stable tetramer 1), 9W1Z [<https://doi.org/10.2210/pdb9W1Z/pdb>] (stable tetramer 2), 9W23 [<https://doi.org/10.2210/pdb9W23/pdb>] (stable tetramer:heparin complex), 9W24 [<https://doi.org/10.2210/pdb9W24/pdb>] (sNS1-mVenus), 9W25 [<https://doi.org/10.2210/pdb9W25/pdb>] (sNS1-mVenus Class 1), and 9W1Y [<https://doi.org/10.2210/pdb9W1Y/pdb>] (sNS1-mVenus Class 2).

The mass spectrometry proteomics data generated in this study have been deposited to the ProteomeXchange Consortium via the PRIDE45 partner repository with the dataset identifier PXD075984 [<https://doi.org/10.6019/PXD075984>]. The lipidomics mass spectrometry data have been deposited in the MassIVE repository with the dataset identifier MSV000101237 [<https://doi.org/10.25345/C5TH8C19H>].

Datasets generated and/or analyzed during this study are provided with the paper or included as Supplementary Information/Source Data file.
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

Reporting on sex and gender is not relevant as there are no research involving human participants, their data, or biological material in this study.

Reporting on race, ethnicity, or other socially relevant groupings

Reporting on race, ethnicity, or other socially relevant groupings is not relevant as there are no research involving human participants, their data, or biological material in this study.

Population characteristics

Population characteristics is not relevant as there are no research involving human participants, their data, or biological material in this study.

Recruitment

Recruitment is not relevant as there are no research involving human participants, their data, or biological material in this study.

Ethics oversight

Ethics oversight is not relevant as there are no research involving human participants, their data, or biological material in this study.

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](https://www.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

A total of 4,845, 10,236, 9,464, and 6,328 movies were collected for the sNS1 dimer fraction, the sNS1 tetramer-enriched fraction, the sNS1:Hp complex, and the sNS1-mVenus sample, respectively. From these, 18,923; 27,103; 25,540; 40,552; 55,807; 304,449; 61,580; 13,098; and 11,657 particles were selected to generate the final maps of the sNS1 dimer, loose tetramer 1, loose tetramer 2, stable tetramer 1, stable tetramer 2, sNS1-mVenus, sNS1-mVenus class 1, and sNS1-mVenus class 2, respectively. These particle numbers were sufficient to obtain high-quality cryo-EM reconstructions.

Sample sizes were chosen based on previous similar studies and standard practices in the field; no formal calculation was performed, and these sizes were sufficient to achieve reproducible results.

Data exclusions

Poor-quality micrographs and unsuitable particles were excluded during cryo-EM data processing prior to final map reconstruction.

Replication	All cryo-EM structures represent averages of more than ten thousand particles picked from thousands of micrographs. TEER and ELISA assays were performed using biologically independent protein preparations. ELISA assays were conducted three times independently. TEER assays comparing the HMWC and tetramer-enriched fractions were performed three times in HUVEC cells and three times in HDMEC cells. TEER assays testing the effect of heparin on NS1 binding were performed three times in HUVEC cells and twice in HDMEC cells. TEER assays comparing sNS1-mVenus and sNS1 were performed three times in both HUVEC and HDMEC cells. All experimental repeats were successful.
Randomization	Randomization was applied during Cryo-EM data processing in cryoSPARC, which randomly assigned particles into two independent half-sets for 3D reconstruction to assess resolution.
Blinding	Blinding is not relevant as there is no experiment subject to bias in this study.

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	Human antibody 5E3 (anti-DENV2 NS1 MAbs) was used as primary antibody for Western Blot in this study. This antibody was isolated and purified by Gavin Screation's lab. Goat anti-Human IgG (H+L) Secondary Antibody, HRP (Life Technologies; Catalog # H10307) was used as a secondary antibody in the Western blot. Antibodies were used at the following dilutions: primary antibody 1ug/mL, secondary antibody 1:2000.
Validation	These antibodies were validated and used routinely in the lab. 5E3 antibody has been published previously: Shu, B. et al. CryoEM structures of the multimeric secreted NS1, a major factor for dengue hemorrhagic fever. Nat Commun 13, 6756 (2022). https://doi.org/10.1038/s41467-022-34415-1 . The secondary antibody is a commercial antibody: https://www.thermofisher.com/antibody/product/Goat-anti-Human-IgG-H-L-Secondary-Antibody-Polyclonal/H10307 .

Eukaryotic cell lines

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

Cell line source(s)	All cell lines used were obtained from commercial sources. Expi293F cells (Thermo Fisher Scientific) Human umbilical vein endothelial cells (HUVECs) (Lonza Bioscience, catalogue no. C2517A) sex: Male Human dermal microvascular endothelial cells (HDMEC) (Lonza Bioscience, catalogue no. CC-2543) sex: Female
Authentication	Commercial cell lines were not authenticated.
Mycoplasma contamination	Cell line was not tested for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines was used in this study.

Plants

Seed stocks	Seed stocks are not relevant as no plant was used in this study.
Novel plant genotypes	Novel plant genotypes are not relevant as no plant was used in this study.
Authentication	Authentication of seed stocks and novel plant genotypes are not relevant as no plant was used in this study.