

## 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 MXCube (version 2), EPU (version 3.15.0.11609)

Data analysis XDS (version January 10, 2022), CCP4 (version 9), PHENIX (version 1.19.2-4158), PYMOL (version 3.0.3), Illustrators (v28.5), Prism (v11), ChimeraX (v1.6.1), BWioTek Gens Software (version 3.2), iBright Analysis Software (version 5.6.0), coot (v0.9.8.92), Burrows-Wheeler Alignment Tool (BWA, version 0.7.17), seaborn (v0.13.2), and a custom Python code used to process the raw FASTQ files to variant counts per position, which is available on GitHub ([https://github.com/Jwang30/DMS\\_Calling](https://github.com/Jwang30/DMS_Calling)).

Atomic coordinates of the reported structures have been deposited in the Protein Data Bank under accession codes 30XO and 30XT, and in the EMDB under accession code EMD-58134 and EMD-77314. The atomic coordinates of PCDH1, ANDV-34, ADI-65534, ANDV-5, the spike protein in eVLPs, the Gn head domain of PUUV, and HALC4136 used in this study are published in the PDB under accession codes 6BX7, 8DBZ, 6P3Y, 9P3I, 5FX4, 8D09. Raw Illumina sequencing data (FASTQ files) have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1501900. Processed variant-level and site-level score matrices are provided as an Excel workbook (PCDH1-binding DMS Supplementary Table). A companion supplementary document (PCDH1-binding DMS Supplementary analysis) describes the workbook contents, data fields and associated analyses.

## 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](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     | No statistical methods were used to predetermine sample size. Sample sizes were chosen based on previous experience with these assays. Experiments were repeated two to five times independently, as indicated in the figure legends.                                                                                                                                                                                                                                                                |
| Data exclusions | No independent experiments were excluded                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Replication     | All experiments were performed and verified in multiple replicates as indicated in figure legends                                                                                                                                                                                                                                                                                                                                                                                                    |
| Randomization   | Randomization was not relevant to this study because no animals, human participants or heterogeneous biological samples were studied. The study consisted of controlled in vitro experiments in which experimental conditions were predefined.                                                                                                                                                                                                                                                       |
| Blinding        | Data collection and analysis was not performed blind because it was not needed. The study is performed under controlled condition, using purified molecules, and cell lines. The experimental conditions were standardized and reproducible, ensuring that the outcomes were determined by the experimental variables rather than observer input. The data analysis methods, were predefined and computationally driven, eliminating the potential for investigator bias during data interpretation. |

## 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    |                                                           | Methods                             |                                                 |
|-------------------------------------|-----------------------------------------------------------|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                                     | n/a                                 | Involved in the study                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies            | <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines | <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    | <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                           |                                     |                                                 |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | Antibodies MIB22, ADI-65534, ANDV-34 and ANDV-5 were produced from synthetic plasmids (GenScript) as described in methods, cloning the corresponding variable sequence in an IgG1 backbone. Hantavirus Gc-specific antibodies include: rabbit polyclonal Ab anti-Gc "16281" from Pacific Immunology Corp. (custom antibody, internal identifier 16281; no clone or catalog number) and mouse monoclonal Ab anti-Gc (clone 2H4/F6) obtained from Nicole D. Tischler (Laboratorio de Virología Molecular, Centro Ciencia & Vida, Fundación Ciencia & Vida, Santiago, Chile, PMID: 24335294). Anti-Strep-tag monoclonal antibody (clone C23.21, Thermo Scientific, Cat. No. 910STRL). Anti-mouse HRP-conjugated antibody (Jackson ImmunoResearch, Cat. No. 115-035-146). High Sensitivity Streptavidin-HRP Pierce™, ThermoFisher, catalog No. 21130. Anti-rabbit-IgG-Alexa Fluor™ 680, ThermoFisher (RRID: AB_2535736, Cat. number A32734, ), anti-mouse-IgG-Alexa Fluor™ 680, ThermoFisher (RRID: AB_2535723), and anti-mouse-IgG-IRDye-800CW, LICOR (RRID: AB_621842), cat. number: 926-32210. goat anti-Mouse IgG, Alexa Fluor™ 680 (ThermoFisher, cat # A-21057) and anti-DDK(FLAG)-DyLight™-594 mAb (ThermoFisher, cat # 50-167-1194) |
| Validation      | All primary antibodies were validated as follows. The mouse monoclonal anti-Gc antibody 2H4/F6 has been validated for detection of hantavirus Gc by immunoblotting and immunostaining (PMID: 24335294). The recombinant human monoclonal antibodies ANDV-5, ANDV-34 have been previously validated for specific binding to ANDV Gn/Gc and for ANDV neutralization (PMID: 36971354). MIB22 was shown to bind ANDV glycoproteins (PMID: 30463919), and ADI-65534 was validated for binding to ANDV Gn/Gc by BLI, ELISA and for neutralization of ANDV (PMID: 38830098). The mouse monoclonal anti-Strep-tag antibody C23.21 (Thermo Fisher Scientific/PROGEN, Cat. No. 910STRL) was validated for detection of Strep-tagged proteins by immunoblotting by ThermoFisher.                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

## Eukaryotic cell lines

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

|                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                               | Drosophila melanogaster S2 cells (ThermoFisher Cat# R69007), Human Expi293F cells (ThermoFisher Cat# A14527), Human umbilical vein endothelial cells (HUVEC, ThermoFisher), African green monkey kidney Vero cells (ATCC), Human hepatocarcinoma Huh.7.5.1 cells (Jan Carette, Stanford University, CA), Human Freestyle™-293-F suspension cells (Thermo Fisher) and 293T cells (ATCC).          |
| Authentication                                                    | HUVECs were authenticated by Invitrogen by cell morphology and cell type-specific markers. 293-F suspension cells and Huh.7.5.1 cells were not authenticated. 293T cells were authenticated by ATCC by STR profiling. Vero cells were characterized by ATCC by karyotyping. Drosophila melanogaster S2 cells were authenticated by ThermoFisher Scientific by karyotyping and isozyme profiling. |
| Mycoplasma contamination                                          | All cell lines were subjected to routine mycoplasma testing and were found to be negative.                                                                                                                                                                                                                                                                                                       |
| Commonly misidentified lines (See <a href="#">ICLAC</a> register) | No misidentified cell lines were used in this study                                                                                                                                                                                                                                                                                                                                              |

## Plants

|                       |     |
|-----------------------|-----|
| Seed stocks           | N/A |
| Novel plant genotypes | N/A |
| Authentication        | N/A |