

Structural basis for protocadherin-1 recognition by New World hantaviruses

Corresponding Author: Dr Pablo Guardado-Calvo

Any redactions in this file are there to maintain patient confidentiality, the confidentiality of unpublished data, or to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)

Battini et al. report the structural basis for recognition of the cadherin superfamily protein, protocadherin-1 (PCDH1), by the New World hantavirus Andes virus. The authors use cryo-electron microscopy to determine the structure of the Andes virus glycoprotein complex bound to PCDH1, determining a high-resolution structure of a complex that includes soluble tetramerized Gn/Gc, as well as a lower-resolution structure of VLPs bound to the receptor.

They show that receptor binding is asymmetric, with one copy of receptor binding the tetrameric viral glycoprotein. The binding site is a deep central pocket that is formed by Gn tetramers. The authors use mutagenesis to identify three Gn loops as determinants for receptor binding. They also identify mechanisms of antibody neutralization, including direct occlusion of the receptor-binding site on Gn and steric occlusion. Overall, the structural work is exciting as it represents a major advance in our understanding of how hantaviruses bind to cellular receptors. The work is also timely given recent concerns for Andes virus human-to-human spread during the small outbreak earlier this year. There are, however, some important issues as noted below that should be addressed before publication.

MAJOR

1. The authors should do an extensive comparison of the binding mode shown for structures obtained with VLP and their tetramerized construct. With a stated resolution of 3.9 for the VLP bound complex, the authors should be able to dock PCDH1 EC1 and Gn/Gc into density, generate a model, and provide comparisons of receptor bound to their Gn/Gc tetramer as compared to the receptor bound to VLPs. Is it possible that the receptor-binding mode on the actual VLP is more flexible than in their tetramerized construct?

2. "We observed specific binding (Figure 1D), but with a low affinity inconsistent with reported values for the viral particles binding to PCDH1, suggesting that only a small proportion of soluble spikes can bind to the receptor."

More information should be provided here, including the prior reported affinity and the associated reference. Were the prior assays done with VLPs? The rationale for the findings "suggesting that only a small proportion of soluble spikes can bind to the receptor" is also unclear. Having a small proportion of soluble spikes binding to the receptor on the sensor tip surface would likely result in a low response signal but would be less likely to impact the association and dissociation of molecules and estimates of on and off rates. Could the authors clarify the basis for their interpretation here? Performing BLI assays with immobilized VLPs would allow for direct comparison of values.

3. Some of the neutralization phenotypes are weak in Figure 3C. A negative control protein should have been included in assays to confirm that the inhibition is specific. Could the authors also specify what statistical test was used?

Minor

1. “we designed a soluble version of the ANDV spike tetramer by adding a hetero-octamerization domain at the C-terminal end of the Gn and Gc ectodomains and cloned both genes into a bicistronic plasmid”

More information should be provided here on the strategy used to promote oligomerization.

2. The Figure S1 legend should describe what is shown in (A) and (B).

3. “Previous studies have identified PCDH1 mutations that inhibit virus binding¹⁷, and all are in the BCEC1 loop or nearby regions, likely altering the conformation of the loop. In line with the structural data, mutations in the tip of the FG loop have little (D142A, D142R) or no effect (L143A, V144A, Q145A) on virus binding¹⁷, because none of them introduce steric restrictions.”

This text is difficult to follow without a figure showing these features. In Figure 1G, I would recommend adding annotation of the PCDH1 loops in a figure. I would also recommend adding clarification of which loops are labeled on top of the panels showing zoom-in views in Figure 1G (loops DE, CD, and AB). It was difficult to ascertain that these loops were on Gn.

4. “We fused EC1 to the N-terminus of the M segment via a flexible linker and transfected FreeStyle293 cells to produce VLPs.”

Can the authors provide a diagram of the protein construct? It would also be helpful to clarify the rationale for using this approach, particularly given that the binding mode is not one receptor to one Gn/Gc protomer. Had the authors tried adding soluble PCDH1 EC1 to the VLP preparation?

5. “We identified two amino acid substitutions (L36F, Q38E) in the GnH AB loop of SEOV, DOBV, and HTNV and one in the GnH DE loop of HTNV (G418L) that are predicted to cause steric clashes with PCDH1.”

From the presentation provided in Figure 2A, it is unclear how the Q38E substitution in HTNV would cause steric clashes.

6. “In contrast, both the sequence and structure of the GnH CD loop vary substantially across hantaviruses—it is an extended and flexible loop in NWHs but a compact and ordered helix in OWHs (Figure 3A and Extended Data Figure S4)”

Can the authors clarify how they know the loop is “flexible” in New World hantaviruses?

7. “The sensitivities to PCDH1 inhibition of the ANDV-SNV chimeras (SNV $\approx$ ANDVSNV-All < ANDVSNV-CD < ANDVSNV-CD) and the SNV-ANDV chimeras...”

It seems like the ANDV SNV-CD is listed twice in a row here.

8. “Although this binding mode is unique, the use of a pocket accessible only in the complete spike has been reported in the encephalitis alphavirus Western equine encephalitis virus (WEEV)²³, which engages two receptors, the very low-density lipoprotein receptor (VLDLR) and protocadherin-10 (PCDH10), through the same surface.”

The concept of a receptor engaging a surface that is only formed on assembled virus spike glycoproteins is one that was also established from work demonstrating how other alphaviruses bind their receptors, including chikungunya virus' binding mode with MXRA8, and the VEEV binding mode with LDLRAD3. Note also that WEEV binds avian MXRA8 through the same cleft, so a third receptor can be engaged through the same cleft.

The authors only cite Raju et al. 2025, but multiple papers should be cited here in terms of WEEV recognition of alternate receptors (40402741, 37804831, 40187345, and 40628733).

It would also be interesting to compare the binding loops/surfaces on PCDH10 that bind WEEV E2–E1 with the binding loops/surfaces on PCDH1 that bind Gn/Gc. Do PCDH1 and PCDH10 use similar surfaces/loops to bind viral glycoproteins?

9. Is anything known about the natural oligomeric state of PCDH1? Does it form homophilic dimers in trans, and if so, would dimerization interfere with glycoprotein binding?

10. “Sequence analysis of the recent ANDV outbreak strain (LS-CH2016/2016-12-23/Switzerland) revealed only a few amino acid substitutions in the Gn ectodomain, none of which mapped to the PCDH1-binding pocket or altered receptor interaction in the DMS experiments (Extended Data Figures S4–S5)”. A figure showing the position of the substitutions on the receptor-bound glycoprotein structure would be useful for the reader here, as it is difficult to decipher those positions just by looking at the sequence alignment. Additionally, are any substitutions in the footprints of the human antibodies the authors analyzed in their manuscript?

11. The authors claim that there is only a slight 3° rotation of the GnH domain relative to GnB between the soluble and VLP-derived spike models. I cannot find these data in Extended Data Figure S2, which only shows map-model correlations between the soluble spike map and the model built in this study. Please provide additional figures that align the two models and highlight any observable conformational changes.

12. Figure 2C, the right panel seems to have electrostatic coloring on the receptor surface? This should be described in the figure legend.

13. PDB validation reports should be provided for the cryo-EM structure(s).

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #3

(Remarks to the Author)

The manuscript by Battini and colleagues describes the structural basis for binding of Andes virus, which is a New World hantavirus, to its cellular receptor protocadherin-1 (PCDH1). This study reveals an interesting strategy for the virus to engage the receptor in a deep central pocket that is formed by four Gn molecules. Interestingly, PCDH1 binds asymmetrically to three Gn loops that form the receptor binding site. This major observation explains the need for intact particles or soluble tetramers to bind PCDH1. Mutational analyses of these loops demonstrated the specificity of binding between Old World and New World hantaviruses. The authors go on to examine the binding of four neutralizing antibodies and show they function in different ways to block spike access or occlude the receptor binding site. The authors speculate that this is an example of the canyon hypothesis proposed in the 1980s to explain exclusion of antibody access to a cellular receptor binding site but permitting the receptor to bind to a virus. This study provides interesting insight into virus-receptor interactions, mechanisms of antibody neutralization and hints at therapeutic interventions. The conclusions are appropriate and they do justice to previous work in their citations. This is a well performed study that will be of significant general interest.

Specific comments:

Figure 1: It would be useful to show the Gn/Gc tetrameric spike in the absence of bound PCDH1. For Panel E it might be useful to state the view orientation. Also, at the end of the Figure legend (also in the text) it says the EC1 domain was fused to the N terminus of the M segment but Panel A doesn't show the M segment as it shows Gn and Gc proteins separately. This should be clarified.

Antibody characterization – in the section that describes “Mechanistic insights into antibody-mediated neutralization...” the authors describe different assays for the four antibodies. This is somewhat confusing – why didn't they use a standard set of assays for all four neutralizing antibodies?

Figure 4, Panel A – I think it would be more informative if the authors could show enlarged views of the clashes between the Fab and PCDH1.

I was surprised that the authors didn't discuss the asymmetric binding site for PCDH1 in more detail. It is interesting that receptor engagement requires a quaternary interaction that 4 Gn molecules create.

Referee #4

(Remarks to the Author)

This manuscript describes, using cryoEM, how New World hantaviruses, exemplified by Andes virus (ANDV), structurally engages the cellular receptor protocadherin 1 (PCDH1) and provides information on how neutralizing antibodies may disrupt this interaction. The authors solve a near atomic resolution structure of the ANDV Gn/Gc spike tetramer bound to the PCDH1 ectodomain, focusing on the EC1 domain previously implicated in receptor usage. They show that PCDH1 asymmetrically inserts into a deep central pocket formed by four Gn protomers within the spike, a configuration that explains why only intact viral particles or engineered soluble tetrameric spikes, but not monomeric Gn/Gc heterodimers, can bind PCDH1 detectably. The paper is a highly valuable addition to our molecular understanding of the biology of ANDV.

My main concern with the paper is the interpretation of the neutralizing antibody (nAb) data. The authors give great prominence to the “canyon hypothesis”:

“Together, these findings define the structural determinants of hantavirus-receptor recognition, provide a blueprint for therapeutic design, and offer a new and especially dramatic example of the “canyon hypothesis,” in which a virus evades antibody surveillance by burying its receptor-binding site in a surface depression that the receptor, but not an antibody, can readily access.”

Rossmann's canyon hypothesis was formulated from the 3D structure of rhinovirus HRV14, which showed a deep depression (“canyon”) around each fivefold axis of the capsid. He proposed that the cell receptor binds in this canyon, whose floor residues are conserved but sterically hidden from the bulky antigen-binding site of antibodies, allowing the virus to conserve receptor-contact residues while letting exposed surfaces accumulate escape mutations. This idea generalized to the notion that many viruses might hide critical receptor-binding sites in recessed or shielded regions (e.g., depressions on influenza HA and NA spikes), thereby reducing immune pressure on those functional sites.

However, Smith and colleagues solved structures of HRV14 with neutralizing Fabs and demonstrated that at least some antibodies can penetrate the receptor-binding canyon, directly contacting conserved residues implicated in ICAM-1 binding. Their Nature paper “Neutralizing antibody to human rhinovirus 14 penetrates the receptor-binding canyon” explicitly contradicted the strict steric-exclusion aspect of the original hypothesis and showed that antibody footprint and receptor-binding surface can substantially overlap. Indeed, many studies since, notably in HIV recognition by nAbs, show that antibodies can recognize recessed regions, indeed almost any surface on a protein. I accept that this may reduce the frequency of nAb precursor B cells making induction of such nAbs more difficult but the nuances here need to be discussed

in detail and the paper not used to resurrect the simplistic canyon hypothesis, especially not in the abstract.

A second concern I have with the paper is in relation to the discussion of nAb mechanism that I find ignores a fair body of literature that indicates the complexity of Ab neutralization of viruses. Blocking of receptor binding is just one of many mechanisms and examples of neutralization by simply binding to a virus surface protein distant from a receptor site abound. I refer the authors to a review by Parren and Burton from as far back as 2001 (doi: 10.1016/s0065-2776(01)77018-6) or more recently from Burton (doi: 10.1038/s41577-023-00858-w.). The authors should note the studies of Desrosiers, Sodroski and Burton all that showed one could place a tag almost anywhere on the HIV surface glycoprotein and an anti-tag antibody would neutralize the virus. The authors are welcome to disagree with the papers quoted but a more sophisticated discussion is warranted in a paper of this potential prominence.

Finally, I emphasize that this is a fine paper in my opinion revealing important molecular details of how a New World hantavirus recognizes its receptor target and will be widely appreciated and quoted by those working in this field.

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)

The authors have addressed all of our comments. We only have a minor suggestion for Figure 3C. We recommend adding labeling to the top and bottom panels with the molecules used in assays (e.g., WT vs. mutant soluble EC1) to make it easier for the reader to grasp what is shown from looking at the figure, rather than having this information only in the figure legend.

Referee #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #3

(Remarks to the Author)

This is a revised manuscript that examines the structure of the cellular receptor, protocadherin-1, for Andes virus. The response to reviewer queries was thorough and has significantly improved this manuscript. From my current review of the response, and the revised manuscript, I suggest the manuscript makes a significant addition to our knowledge of hantaviruses and their use of cellular receptor proteins. With the revised figures, the manuscript is accessible to a general audience and is well written.

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

Referee #1 (Remarks to the Author):

Battini et al. report the structural basis for recognition of the cadherin superfamily protein, protocadherin-1 (PCDH1), by the New World hantavirus Andes virus. The authors use cryo-electron microscopy to determine the structure of the Andes virus glycoprotein complex bound to PCDH1, determining a high-resolution structure of a complex that includes soluble tetramerized Gn/Gc, as well as a lower-resolution structure of VLPs bound to the receptor.

They show that receptor binding is asymmetric, with one copy of receptor binding the tetrameric viral glycoprotein. The binding site is a deep central pocket that is formed by Gn tetramers. The authors use mutagenesis to identify three Gn loops as determinants for receptor binding. They also identify mechanisms of antibody neutralization, including direct occlusion of the receptor-binding site on Gn and steric occlusion. Overall, the structural work is exciting as it represents a major advance in our understanding of how hantaviruses bind to cellular receptors. The work is also timely given recent concerns for Andes virus human-to-human spread during the small outbreak earlier this year. There are, however, some important issues as noted below that should be addressed before publication.

We sincerely thank the reviewer for their thoughtful and constructive comments. All the suggestions have been very helpful in clarifying key aspects of our study and have significantly improved the quality and clarity of the revised manuscript.

MAJOR

1. The authors should do an extensive comparison of the binding mode shown for structures obtained with VLP and their tetramerized construct. With a stated resolution of 3.9 for the VLP bound complex, the authors should be able to dock PCDH1 EC1 and Gn/Gc into density, generate a model, and provide comparisons of receptor bound to their Gn/Gc tetramer as compared to the receptor bound to VLPs. Is it possible that the receptor-binding mode on the actual VLP is more flexible than in their tetramerized construct?

We thank the reviewer for this comment and for encouraging a deeper comparison between the receptor-bound structures obtained with eVLPs and our soluble tetrameric spike.

Although the cryo-EM map of the VLP-bound complex has an overall resolution of 3.9 Å, the local resolution in the region corresponding to PCDH1 EC1 is substantially lower. The map is sufficient to show that the model obtained from the soluble spike complex fits well into the eVLP density, but the local density around EC1 is not of sufficient quality to support atomic modeling. To clarify this point, we have added a new panel (Figure S5C) comparing the cryo-EM densities of EC1 in the soluble spike complex and in the eVLP spike. As shown there, the EC1 polypeptide chain can be traced unambiguously in the soluble spike map, whereas the corresponding density in the eVLP map is too weak for de

novo model building, although it is fully compatible with the model derived from the soluble complex.

In contrast, the density corresponding to the viral spike itself in the eVLP maps is of sufficient quality to allow modeling. This allowed us to perform direct comparisons between the spike in eVLPs without receptor (PDB 9P3I), bound to PCDH1, and the soluble spike bound to PCDH1. These comparisons show that receptor binding induces only a small rotation of the Gn head domains (Figure S3D and S5D). Further, in the map of the soluble spikes, we also detected asymmetries induced by PCDH1 binding among the four Gn subunits, which we now show in the Figures S3B and S3C. All these structural details and comparisons are now discussed in the revised manuscript.

lines 169-175 : *"The map revealed clear densities for Gn and Gc and an additional density at the center of the spike (Figure 1H). This central density was compatible in size and position with the PCDH1-EC1 domain observed in the soluble spike complex. However, the quality of this density did not allow unambiguous building of the EC1 domain, preventing a detailed comparison of the VLP:receptor interface with that obtained using the soluble spike (Extended Data Figure S5C). This work nevertheless confirmed that PCDH1-EC1 occupies the central region of the Gn/Gc tetramer in viral particles."*

Lines 151-156: *"Notably, the densities corresponding to the DE and CD loops are weaker in the protomers that do not engage EC1 than in the receptor-contacting protomers (Extended Data Figure S3B). This suggests that these loops are flexible and that PCDH1 binding stabilizes specific conformations of these regions. Indeed, comparison of the four protomers reveals that EC1 binding induces a conformational change in the CD and DE loops of one protomer, creating asymmetry within the pocket (Extended Data Figure S3C)."*

2. "We observed specific binding (Figure 1D), but with a low affinity inconsistent with reported values for the viral particles binding to PCDH1, suggesting that only a small proportion of soluble spikes can bind to the receptor."

More information should be provided here, including the prior reported affinity and the associated reference. Were the prior assays done with VLPs? The rationale for the findings "suggesting that only a small proportion of soluble spikes can bind to the receptor" is also unclear. Having a small proportion of soluble spikes binding to the receptor on the sensor tip surface would likely result in a low response signal but would be less likely to impact the association and dissociation of molecules and estimates of on and off rates. Could the authors clarify the basis for their interpretation here? Performing BLI assays with immobilized VLPs would allow for direct comparison of values.

We sincerely thank the reviewer for these helpful comments and the opportunity to clarify these points. Our bio-layer interferometry (BLI) experiments were performed to confirm the presence of specific binding between the soluble ANDV spike and PCDH1 fragments, with the main objective of validating the interaction for subsequent structural analyses rather than deriving biologically meaningful affinity constants.

Previous studies reported higher apparent affinities used VLPs of Maporal virus, a different New-World hantavirus. However, it is difficult to compare those measurements directly with ours, as they involved different hantaviruses and VLP-based assays involved many spikes presented on the particle surface, making it challenging to disentangle the contribution of avidity from the apparent affinity. Although we agree with the reviewer that BLI assays using immobilized ANDV VLPs would facilitate a more direct comparison, our ANDV VLP preparations are too heterogeneous and are not good enough for reliable measurements.

One of the points we initially tried to make with the BLI data was that our soluble spike preparation is conformationally dynamic, in line with the cryo-EM data. However, after carefully considering the reviewer's comment, we agree that such conformational heterogeneity would be expected to affect the response amplitude rather than the kinetic rate constants, and therefore this conclusion cannot be drawn from the BLI data.

For these reasons, we have re-written the sentence to remove the reference to the VLP-based affinities to avoid potentially misleading comparisons.

Lines 117-121: "Further, we used bio-layer interferometry (BLI) to demonstrate specific spike-EC1 binding. Specifically, sEC1-4WT or sEC1-4MUT were immobilized on sensors, which were then dipped into solutions containing increasing concentrations of soluble spike (Figure 1D). BLI assays performed in the opposite orientation corroborated these findings (Extended Data Figure S1B)."

3. Some of the neutralization phenotypes are weak in Figure 3C. A negative control protein should have been included in assays to confirm that the inhibition is specific. Could the authors also specify what statistical test was used?

We thank the reviewer for the opportunity to strengthen this observation. The revised manuscript now includes neutralization curves with a control soluble PCDH1 EC1-2 protein containing mutations that abrogate its interaction with the ANDV spike. These data, now included in Figure 3C, clearly demonstrate the inhibition observed with WT PCDH1 EC1-2 is specific. We have also added descriptions of the statistical analyses used for both panels to the figure legend.

Minor

1. "we designed a soluble version of the ANDV spike tetramer by adding a hetero-octamerization domain at the C-terminal end of the Gn and Gc ectodomains and cloned both genes into a bicistronic plasmid"

More information should be provided here on the strategy used to promote oligomerization.

We thank the reviewer for this helpful suggestion. To clarify the oligomerization strategy, we have added a more detailed description to the results section and a new panel A in Figure S1. In short, the hetero-octamerization domain was generated by splitting HALC4_136, a de novo-designed tetramerization scaffold with the symmetry and spacing required to approximate the native hantavirus spike. The first two helices of each HALC4_136 protomer were fused to the C terminus of Gn, whereas the third helix was fused to the C terminus of Gc. We also engineered a disulfide bond between the Gn- and Gc-linked helices to stabilize the hetero-assembly. Co-expression of the two ectodomains therefore reconstituted a hetero-oligomeric (Gn/Gc)₄ complex.

lines 107-111: *"The octamerization sequence was derived from HALC4_136, a de novo tetramerization domain comprising three antiparallel helices per protomer¹⁹. We split this scaffold by fusing the first two helices to Gn and the third helix to Gc, so that co-expression of Gn and Gc would reconstitute a hetero-oligomeric assembly with the stoichiometry of the native spike (Extended Data Figure S1A)."*

2.The Figure S1 legend should describe what is shown in (A) and (B).

We thank the reviewer for pointing this out. In the revised version, we have expanded the legend of the Figure S2 (previous Figure S1) to provide a more detailed description of all panels.

3. "Previous studies have identified PCDH1 mutations that inhibit virus binding¹⁷, and all are in the BCEC1 loop or nearby regions, likely altering the conformation of the loop. In line with the structural data, mutations in the tip of the FG loop have little (D142A, D142R) or no effect (L143A, V144A, Q145A) on virus binding¹⁷, because none of them introduce steric restrictions."

This text is difficult to follow without a figure showing these features. In Figure 1G, I would recommend adding annotation of the PCDH1 loops in a figure. I would also recommend adding clarification of which loops are labeled on top of the panels showing zoom-in views in Figure 1G (loops DE, CD, and AB). It was difficult to ascertain that these loops were on Gn.

We thank the reviewer for this suggestion. In the revised version, we have updated the Figure 1G to clearly label the relevant PCDH1 loops and residues, and to identify the DE, CD, and AB loops in the zoom-in panels using a consistent color code. We think that these changes make the structural features discussed in the text easier to visualize.

4. "We fused EC1 to the N-terminus of the M segment via a flexible linker and transfected FreeStyle293 cells to produce VLPs."

Can the authors provide a diagram of the protein construct? It would also be helpful to clarify to the rationale for using this approach, particularly given that the binding mode is not one receptor to one Gn/Gc protomer. Had the authors tried adding soluble PCDH1 EC1 to the VLP preparation?

We thank the reviewer for this helpful observation. In the revised version, we have clarified the rationale for fusing PCDH1 EC1 to the N-terminus of the M segment and have added a schematic representation of this construct in a new panel in Figure 1A.

As described in the new version of the manuscript, we did not attempt to decorate pre-formed VLPs with soluble receptor because flow cytometry experiments showed only weak binding of PCDH1 EC1 to cell-surface-displayed ANDV glycoproteins. We reasoned that increasing the local receptor concentration near assembling spikes would favor productive complex formation. For this purpose, we fused PCDH1 EC1 to the N-terminus of the M segment through using a flexible (GGGS)₅ linker.

lines 161-166 : *"To validate the model with viral particles, we studied the interaction between PCDH1 and virus-like particles (VLPs). We did not attempt to decorate preformed VLPs with soluble receptor because flow cytometry revealed only weak binding of EC1 to ANDV glycoproteins presented on the cell surface. Instead, reasoning that increasing the local concentration of receptor near the spike could enhance binding, we fused EC1 to the N terminus of the M segment via a flexible linker (Figure 1A)."*

5. "We identified two amino acid substitutions (L36F, Q38E) in the GnH AB loop of SEOV, DOBV, and HTNV and one in the GnH DE loop of HTNV (G418L) that are predicted to cause steric clashes with PCDH1."

From the presentation provided in Figure 2A, it is unclear how the Q38E substitution in HTNV would cause steric clashes.

We thank the reviewer for noting this point. The reviewer is correct, the Q38E substitution in HTNV does not introduce any steric clash with PCDH1. We appreciate the opportunity to correct this error and have removed all references to this residue both from the main text and from the corresponding figure.

6. "In contrast, both the sequence and structure of the GnH CD loop vary substantially across hantaviruses—it is an extended and flexible loop in NWHs but a compact and ordered helix in OWHs (Figure 3A and Extended Data Figure S4)"

Can the authors clarify how they know the loop is "flexible" in New World hantaviruses?

We thank the reviewer for this comment. Our original statement that the CD loop is "flexible" in New World hantaviruses was based on observations from our cryo-EM maps, which show that the CD loop is poorly resolved in the absence of PCDH1 (Figure S3B), as well as on the elevated B-factors for this region in previously determined ANDV GnH

structures. In contrast, the corresponding loop in the HTNV GnH structure displays B-factors similar to the protein average, consistent with a more ordered conformation. However, we agree that this information cannot be generalized to all New- and Old-World hantaviruses. Accordingly, we have removed this broad statement from the text and now specify that the CD loop appears flexible in ANDV in the absence of receptor binding.

Lines 151-156: *"Notably, the densities corresponding to the DE and CD loops are weaker in the protomers that do not engage EC1 than in the receptor-contacting protomers (Extended Data Figure S3B). This suggests that these loops are flexible and that PCDH1 binding stabilizes specific conformations of these regions. Indeed, comparison of the four protomers reveals that EC1 binding induces a conformational change in the CD and DE loops of one protomer, creating asymmetry within the pocket (Extended Data Figure S3C)."*

7. "The sensitivities to PCDH1 inhibition of the ANDV-SNV chimeras (SNV $\approx$ ANDVSNV-All < ANDVSNV-CD < ANDVSNV-CD) and the SNV-ANDV chimeras..."
It seems like the ANDV SNV-CD is listed twice in a row here.

We thank the reviewer for catching this mistake, which has been corrected in the revised version of the manuscript.

Lines 238-242: *"The sensitivities to PCDH1 inhibition of the ANDV-SNV chimeras (SNV $\approx$ ANDVSNV-All < ANDVSNV-CD < ANDV), the SNV-ANDV chimeras (SNV < SNVANDV-CD < SNVANDV-All < ANDV), and the HTNV-ANDV chimeras (HTNV $\approx$ HTNVANDV-CD < HTNVANDV-All < ANDV) were also concordant with our binding results."*

8. "Although this binding mode is unique, the use of a pocket accessible only in the complete spike has been reported in the encephalitis alphavirus Western equine encephalitis virus (WEEV)²³, which engages two receptors, the very low-density lipoprotein receptor (VLDLR) and protocadherin-10 (PCDH10), through the same surface." The concept of a receptor engaging a surface that is only formed on assembled virus spike glycoproteins is one that was also established from work demonstrating how other alphaviruses bind their receptors, including chikungunya virus' binding mode with MXRA8, and the VEEV binding mode with LDLRAD3. Note also that WEEV binds avian MXRA8 through the same cleft, so a third receptor can be engaged through the same cleft.

The authors only cite Raju et al. 2025, but multiple papers should be cited here in terms of WEEV recognition of alternate receptors (40402741, 37804831, 40187345, and 40628733).

It would also be interesting to compare the binding loops/surfaces on PCDH10 that bind WEEV E2-E1 with the binding loops/surfaces on PCDH1 that bind Gn/Gc. Do PCDH1 and PCDH10 use similar surfaces/loops to bind viral glycoproteins?

We thank the reviewer for these excellent observations and for pointing out the additional relevant literature. We have revised the discussion to include citations to the recent studies showing that alphaviruses engage different receptors (PCDH10, VLDLR, and MXRA8)

through a common quaternary surface. We also expanded the comparative analysis between PCDH1 and PCDH10, clarifying that although both receptors belong to the protocadherin family and interact with their respective viral glycoproteins via the membrane-distal EC1 domain, they use distinct binding modes and surfaces: PCDH10- EC1 binds laterally to the alphavirus spike, whereas PCDH1 EC1 inserts axially into the central cavity of the hantavirus spike.

We have now included a new paragraph in the Discussion section:

Lines 299-310: "The concept that receptors can recognize quaternary surfaces formed only in assembled viral glycoprotein complexes is not unique to hantaviruses, as the receptor-binding pocket for multiple alphaviruses is accessible only in the complete spike. For example, Western equine encephalitis virus (WEEV) can accommodate multiple receptors, including protocadherin-10 (PCDH10)²⁶⁻²⁹, the very low-density lipoprotein receptor (VLDLR)^{28,29} and MXRA8^{26,30}, using the same pocket formed by adjacent E2/E1 heterodimers. It is interesting to compare the interactions of ANDV with PCDH1 with those of WEEV with PCDH10 because both receptors are non-clustered δ -cadherins³¹ and use their membrane-distal EC1 domain for viral engagement. However, the two viruses use distinct binding modes and protocadherin-binding surfaces. PCDH10 EC1 engages the alphavirus spike laterally through its A, G, F, and C β -strands, whereas PCDH1 EC1 inserts axially into the central cavity of the hantavirus spike using its BC and FG loops."

9. Is anything known about the natural oligomeric state of PCDH1? Does it form homophilic dimers in trans, and if so, would dimerization interfere with glycoprotein binding?

We thank the reviewer for this question, which gave us the opportunity to include this important information. Biochemical and structural studies indicate that the soluble ectodomain of PCDH1 exists in a monomer–dimer equilibrium in solution, but the oligomeric state of the full-length protein at the plasma membrane remains unknown. Crystallographic data showed that PCDH1 can form antiparallel homodimers through an EC1–EC4 interface. We have performed a structural alignment and observed that this dimeric configuration is incompatible with the receptor-binding mode described here, as the EC4 domain would not fit into the pocket formed by the viral spike. Consistent with this, previous mutagenesis studies found that monomeric PCDH1 retained its ability to interact with rVSV-ANDV, whereas mutations that stabilized the dimer significantly reduced binding. These points and their implications have been incorporated into the revised discussion section:

Lines 287-296: "PCDH1 is a calcium-dependent cell-cell adhesion protein. Biochemical studies with soluble ectodomain fragments showed that PCDH1 can exist in a monomer–dimer equilibrium in solution^{17,25}; however, the oligomeric distribution of full-length PCDH1 at the plasma membrane is not known. Structural studies showed that PCDH1 can form antiparallel homodimers through an EC1-EC4 interface²⁵, a configuration that is incompatible with the receptor-binding mode described herein, because EC4 would not

fit into the pocket. Previous studies with recombinant PCDH1 mutants engineered to remain monomeric did not reduce interaction with rVSV-ANDV, whereas stabilization of the dimer decreased binding¹⁷. Overall, these data suggest that the virus engages monomeric PCDH1 at the cell surface, although dynamic PCDH1 dimers may also contribute if they transiently expose the EC1 domain. ”

10. ”Sequence analysis of the recent ANDV outbreak strain (LS-CH2016/2016-12-23/Switzerland) revealed only a few amino acid substitutions in the Gn ectodomain, none of which mapped to the PCDH1-binding pocket or altered receptor interaction in the DMS experiments (Extended Data Figures S4–S5)”. A figure showing the position of the substitutions on the receptor-bound glycoprotein structure would be useful for the reader here, as it is difficult to decipher those positions just by looking at the sequence alignment. Additionally, are any substitutions in the footprints of the human antibodies the authors analyzed in their manuscript?

We thank the reviewer for this helpful suggestion. In the revised version, we have added a new panels in the Figures S4 and S7 showing the location of the amino acid substitutions identified in the recent Hondius outbreak strain mapped onto the spike structure to show that none of these substitutions lie within the PCDH1-binding pocket (Figure S4D) or within the epitopes of the four human antibodies analyzed in this study (Figure S7B). We also added a corresponding sentence in the discussion:

Lines 198-201: ”Interestingly, none of the Gn ectodomain substitutions observed in the recent Hondius outbreak isolate of ANDV (LS-CH2016/2016-12-23/Switzerland) mapped to the PCDH1-binding pocket or were selected in the DMS experiments, indicating that this virus likely resembles previous isolates of the virus in its capacity to recognize and use PCDH1 (Extended Data Figures S4, S6 and S7).”

11. The authors claim that there is only a slight 3° rotation of the GnH domain relative to GnB between the soluble and VLP-derived spike models. I cannot find these data in Extended Data Figure S2, which only shows map-model correlations between the soluble spike map and the model built in this study. Please provide additional figures that align the two models and highlight any observable conformational changes.

We thank the reviewer for this observation. In response, we have added a new panel in Figure S3D comparing the spike structures in the absence and presence of PCDH1.

12. Figure 2C, the right panel seems to have electrostatic coloring on the receptor surface? This should be described in the figure legend.

We thank the reviewer for pointing out this issue. We have rewritten the legend to specify that the receptor surface in the right panel is colored according to electrostatic potential.

13. PDB validation reports should be provided for the cryo-EM structure(s).

We have now included the final validation reports for both cryo-EM structures.

Referee #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

We thank the reviewer for taking the time to evaluate our manuscript and for contributing to the review process.

Referee #3 (Remarks to the Author):

The manuscript by Battini and colleagues describes the structural basis for binding of Andes virus, which is a New World hantavirus, to its cellular receptor protocadherin-1 (PCDH1). This study reveals an interesting strategy for the virus to engage the receptor in a deep central pocket that is formed by four Gn molecules. Interestingly, PCDH1 binds asymmetrically to three Gn loops that form the receptor binding site. This major observation explains the need for intact particles or soluble tetramers to bind PCDH1. Mutational analyses of these loops demonstrated the specificity of binding between Old World and New World hantaviruses. The authors go on to examine the binding of four neutralizing antibodies and show they function in different ways to block spike access or occlude the receptor binding site. The authors speculate that this is an example of the canyon hypothesis proposed in the 1980s to explain exclusion of antibody access to a cellular receptor binding site but permitting the receptor to bind to a virus. This study provides interesting insight into virus-receptor interactions, mechanisms of antibody neutralization and hints at therapeutic interventions. The conclusions are appropriate and they do justice to previous work in their citations. This is a well performed study that will be of significant general interest.

We sincerely thank the reviewer for the encouraging comments and we appreciate the positive assessment of our structural analyses. We think that all the comments helped us to further improve the clarity and presentation of the manuscript.

Specific comments:

Figure 1: It would be useful to show the Gn/Gc tetrameric spike in the absence of bound PCDH1.

We fully agree with the reviewer that comparing the spike in the presence and absence of PCDH1 is important, and we appreciate this suggestion. In the revised manuscript, we have added new panels in Extended Data Figures S3D and S5D comparing the spike with and without receptor. At the level of the overall spike, receptor binding induces a slight rotation of the Gn head domains. At a more local scale, analysis of the cryo-EM map of the soluble spike also revealed conformational changes associated with receptor binding, which are now highlighted in Extended Data Figures S3B and S3C. We have also added a panel in

Figure S1 showing the cryoEM density obtained from the soluble spikes in absence of PCDH1.

Lines 151-156: *"Notably, the densities corresponding to the DE and CD loops are weaker in the protomers that do not engage EC1 than in the receptor-contacting protomers (Extended Data Figure S3B). This suggests that these loops are flexible and that PCDH1 binding stabilizes specific conformations of these regions. Indeed, comparison of the four protomers reveals that EC1 binding induces a conformational change in the CD and DE loops of one protomer, creating asymmetry within the pocket (Extended Data Figure S3C)."*

For Panel E it might be useful to state the view orientation. Also, at the end of the Figure legend (also in the text) it says the EC1 domain was fused to the N terminus of the M segment but Panel A doesn't show the M segment as it shows Gn and Gc proteins separately. This should be clarified.

We have also revised the legend of Figure 1 to explain the viewing orientations of the two views in panel E and to clarify the constructs used to generate the PCDH1/eVLP complex.

Figure 1. Structure of the ANDV spike in complex with PCDH1. (A) The upper panels show a schematic of the soluble spike construct. Gn and Gc ectodomains were fused to a C-terminal tetramerization domain (TD). Cysteines in the Gn and Gc TD domains were engineered to form a disulfide bond. N-glycosylation sites are shown in green, and the locations of the signal peptides (SP) and fusion loop (FL) are indicated. The lower panel shows a schematic of the construct used to produce the eVLPs fused to the PCDH1 EC1 domain. (B) ELISA binding of soluble spikes to immobilized WT or double-mutant PCDH1 EC1-4 (DM: Y81A/F83A). Data are mean $\pm$ s.d. of triplicate wells from three independent experiments (n = 9) (C) Competition ELISA. The soluble spike tetramer was preincubated with serial dilutions of soluble WT or DM sEC1 and then added to plates coated with WT PCDH1 EC1-4. Data are mean $\pm$ s.d. of triplicate wells from two independent experiments (n = 6) (D) BLI analysis of soluble spikes tetramer binding to His-tagged WT or DM sEC1-4 immobilized on NTA biosensors (E) Cryo-EM maps obtained after co-expression of the soluble spike tetramers with PCDH1 EC1-4. The left panel is a top-view of the map along the fourfold axis. The right panel is the same map rotated 45° around the y-axis, as indicated by the central arrow. Gn is shown in red, Gc in yellow, and PCDH1 EC1 in blue. (F) Atomic model of ANDV spike bound to PCDH1 EC1, colored as in panels E (G) Contacts of Gn and PCDH1 EC1. Gn is shown in pink and PCDH1 EC1 in light blue, with contacting residues (buried surface area > 5 Å²) highlighted in darker colors. (H) Cryo-EM map obtained by fusing the PCDH1 EC1 domain to the N-terminus of the M segment, colored as in panel E and as indicated in the lower schematic of panel A.

Antibody characterization – in the section that describes "Mechanistic insights into antibody-mediated neutralization..." the authors describe different assays for the four antibodies. This is somewhat confusing – why didn't they use a standard set of assays for all four neutralizing antibodies?

We thank the reviewer again for this helpful comment and apologize for not making this point clearer in the original text. We analyzed four human neutralizing antibodies (ANDV5, ANDV34, MIB22, and ADI-65534), which are amongst the most potent anti-ANDV antibodies described to date. These antibodies were developed by different groups and had previously been characterized using different experimental assays to assess receptor blocking. ANDV5 and MIB22 were shown to inhibit the binding of soluble PCDH1 to spike-expressing cells in a FACS-based assay, whereas ANDV34, tested in the same assay, did not block receptor interaction. ADI-65534, in contrast, was shown to inhibit PCDH1 binding in an ELISA-based format.

In our study, to enable direct comparison, we have standardized the analyses by using a single ELISA-based assay for all four antibodies under identical conditions (Figure 4B). In the current version, we have also characterized all four antibodies in a standardized rVSV-ANDV neutralization assay to allow a side-by-side comparison of their inhibitory activities (Figure 4C).

We have revised the text to make it clearer.

Lines 249-253: "To explore their mechanisms of action, we analyzed four well-studied human nAbs: ANDV-522 and MIB2222,23, which have been reported to interfere with the binding of soluble PCDH1 to spike-expressing cells; ANDV-3422, which does not block this interaction; and ADI-655348, which was reported to block the binding of rVSVs to PCDH1 EC1-2 immobilized on ELISA plates.."

Lines 261-266: "In parallel, we produced the four nAbs as both full-length antibodies (IgGs) and single-chain variable fragments (scFvs) lacking the antibody constant regions and compared their capacity to block rVSV-ANDV:PCDH1 binding by ELISA (Figure 4B). We also compared the neutralizing activity of the four full-length antibodies against rVSV-ANDV in HUVECs, which express higher levels of PCDH1 than many immortalized cell lines of non-endothelial origin¹⁶ (Figure 4C)."

Figure 4, Panel A – I think it would be more informative if the authors could show enlarged views of the clashes between the Fab and PCDH1.

We thank the reviewer for this useful suggestion. In the new version, we have added a new column of panels in Figure 4A showing zoomed views of the antibody binding sites to show the clashes between the antibody fragments and PCDH1.

I was surprised that the authors didn't discuss the asymmetric binding site for PCDH1 in more detail. It is interesting that receptor engagement requires a quaternary interaction that 4 Gn molecules create.

We thank the reviewer for this comment. As noted in our response to the first reviewer, we have added a few lines and panels in the Figure S3 describing the asymmetry induced by PCDH1 binding. We have also expanded the discussion to address the possible

biological implications of this quaternary receptor-binding site. We suggest that such organization may confer some flexibility to bind different receptors through a limited number of aminoacid changes. We also draw parallels with alphaviruses, where receptor binding can influence the pH threshold for fusion, and speculate a possible link between receptor binding and fusion activation in hantaviruses.

Lines 310-326: *"By analogy with WEEV, we propose that the PCDH1-binding pocket of ANDV and other NWHs can accommodate distinct receptors for OWH and other non-PCDH1-using hantaviruses. Consistent with this idea, we found that variations in the receptor-binding loops of HTNV Gn, which normally cannot recognize PCDH1, enable a rVSV displaying the HTNV spike to bind PCDH1. Structural, sequence, and mutagenesis studies further showed that the GnH CD loop is flexible, variable, and tolerant to multiple mutations, suggesting it could adapt to recognize alternative receptors. Deep-mutational scanning data indicated that substitutions in the GnH CD and AB loops can alter affinity for PCDH1. Further, the fact that the pocket is formed by four subunits may also facilitate viral adaptation to different receptors through a limited number of amino acid substitutions.*

Another interesting parallel with alphaviruses concerns fusion activation. In alphaviruses, the quaternary interactions that form the receptor-binding site must be disrupted to allow membrane fusion, and MXRA8 binding has been proposed to modulate the pH threshold for fusion activation³². In contrast, the PCDH1-binding site does not involve the hantavirus fusion protein Gc, suggesting that receptor binding and membrane fusion by hantaviruses might be independent events. However, it has been reported that Gn adopts a different tetrameric arrangement at acidic pH5, raising the possibility that PCDH1 engagement may also influence fusion activation."

Referee #4 (Remarks to the Author):

This manuscript describes, using cryoEM, how New World hantaviruses, exemplified by Andes virus (ANDV), structurally engages the cellular receptor protocadherin 1 (PCDH1) and provides information on how neutralizing antibodies may disrupt this interaction. The authors solve a near atomic resolution structure of the ANDV Gn/Gc spike tetramer bound to the PCDH1 ectodomain, focusing on the EC1 domain previously implicated in receptor usage. They show that PCDH1 asymmetrically inserts into a deep central pocket formed by four Gn protomers within the spike, a configuration that explains why only intact viral particles or engineered soluble tetrameric spikes, but not monomeric Gn/Gc heterodimers, can bind PCDH1 detectably. The paper is a highly valuable addition to our molecular understanding of the biology of ANDV.

We sincerely thank the reviewer for their positive assessment and constructive comments, which helped us to strengthen and clarify the discussion, especially on the neutralization mechanisms and the immune evasion.

My main concern with the paper is the interpretation of the neutralizing antibody (nAb) data. The authors give great prominence to the "canyon hypothesis":

"Together, these findings define the structural determinants of hantavirus-receptor recognition, provide a blueprint for therapeutic design, and offer a new and especially dramatic example of the "canyon hypothesis," in which a virus evades antibody surveillance by burying its receptor-binding site in a surface depression that the receptor, but not an antibody, can readily access."

Rossmann's canyon hypothesis was formulated from the 3D structure of rhinovirus HRV14, which showed a deep depression ("canyon") around each fivefold axis of the capsid. He proposed that the cell receptor binds in this canyon, whose floor residues are conserved but sterically hidden from the bulky antigen-binding site of antibodies, allowing the virus to conserve receptor-contact residues while letting exposed surfaces accumulate escape mutations. This idea generalized to the notion that many viruses might hide critical receptor-binding sites in recessed or shielded regions (e.g., depressions on influenza HA and NA spikes), thereby reducing immune pressure on those functional sites.

However, Smith and colleagues solved structures of HRV14 with neutralizing Fabs and demonstrated that at least some antibodies can penetrate the receptor-binding canyon, directly contacting conserved residues implicated in ICAM-1 binding. Their Nature paper "Neutralizing antibody to human rhinovirus 14 penetrates the receptor-binding canyon" explicitly contradicted the strict steric-exclusion aspect of the original hypothesis and showed that antibody footprint and receptor-binding surface can substantially overlap. Indeed, many studies since, notably in HIV recognition by nAbs, show that antibodies can recognize recessed regions, indeed almost any surface on a protein. I accept that this may reduce the frequency of nAb precursor B cells making induction of such nAbs more difficult but the nuances here need to be discussed in detail and the paper not used to resurrect the simplistic canyon hypothesis, especially not in the abstract.

We thank the reviewer for this important comment. We fully agree that, in the original version of the manuscript, our discussion of the canyon hypothesis was too simplistic and did not reflect the important debates that have followed the original proposal.

In the revised manuscript, we have removed the reference to the canyon hypothesis from the abstract and substantially expanded the discussion regarding this point. We now describe the hantavirus receptor-binding cavity as reminiscent of canyon-like clefts observed in picornaviruses, while explicitly noting that recessed receptor-binding sites do not preclude antibody recognition, as shown by the work of Smith and colleagues. We also discuss the possibility that such recessed pockets may provide a different selective advantage by allowing escape through substitutions in more exposed, peripheral antibody-contact residues while preserving the more buried receptor-binding interface. Indeed, MIB22 provides a good example of this. Finally, we mention that identifying antibodies capable of reaching the base of the cavity, which may exist even if they are likely to be rare, could be of considerable therapeutic interest.

Lines 348-365: *"This recessed receptor-binding site is reminiscent of the canyon-like clefts described in picornaviruses, which inspired Rossmann's "canyon hypothesis." In its original*

form, this hypothesis proposed that receptor-binding residues located deep within a narrow cleft could remain accessible to the receptor but not to conventional antibodies, thereby reducing the immune pressure on the most conserved residues^{41,42}. However, the subsequent description by Smith and co-workers⁴² of HRV14-specific neutralizing antibodies whose Fabs can penetrate the receptor-binding cleft showed that virus-host arms races could indeed produce antiviral solutions to this strategy. The preceding observations with HRV14 raise an interesting alternative possibility also noted by these authors⁴²: that the recessed receptor-binding site affords the virus a second line of defense by allowing it to escape neutralization through amino acid substitutions that disrupt a more extensive and surface-exposed virus-antibody interface while sparing a smaller and more buried virus-receptor interface. Indeed, ANDV escape mutations to MIB-22 are at residues distinct from, and more peripheral to, those critical for ANDV-PCDH1 recognition. Our work suggests that access to conserved sequences at the base of the receptor-binding cavity in the hantavirus spike is likely to require antibodies with extended CDR loops, single-domain antibodies, or biologics designed to fit into the cavity. The identification of such molecules could yield exceptionally potent and broad antiviral inhibitors."

A second concern I have with the paper is in relation to the discussion of nAb mechanism that I find ignores a fair body of literature that indicates the complexity of Ab neutralization of viruses. Blocking of receptor binding is just one of many mechanisms and examples of neutralization by simply binding to a virus surface protein distant from a receptor site abound. I refer the authors to a review by Parren and Burton from as far back as 2001 (doi: 10.1016/s0065-2776(01)77018-6) or more recently from Burton (doi: 10.1038/s41577-023-00858-w.). The authors should note the studies of Desrosiers, Sodroski and Burton all that showed one could place a tag almost anywhere on the HIV surface glycoprotein and an anti-tag antibody would neutralize the virus. The authors are welcome to disagree with the papers quoted but a more sophisticated discussion is warranted in a paper of this potential prominence.

We thank the reviewer again for this important comment and for drawing our attention to the relevant literature. We agree that our original discussion of antibody-mediated neutralization was again overly simplistic. In the revised manuscript, we have expanded the discussion to emphasize that blocking receptor attachment is only one of several non-mutually exclusive mechanisms of neutralization. We now also cite studies showing that antibodies binding exposed, non-receptor-binding regions of viral glycoproteins can still neutralize infectivity through steric interference. In this context, we present our findings on ADI-65534 and ANDV-34 as an example of this broader principle as they do not directly occlude the PCDH1-binding pocket, but by binding to accessible regions of the spike can prevent receptor engagement through steric hindrance. Finally, we note that this mechanism suggests potential therapeutic strategies aimed at increasing antibody occupancy on accessible spike surfaces.

Lines 48-51 in the abstract: *"Structural and functional analyses of four neutralizing antibodies revealed that they block receptor binding through two distinct mechanisms—*

occluding access to the PCDH1-binding site or by sterically preventing receptor engagement.”

Lines 327-346: *“The neutralizing antibody response is essential for controlling hantavirus infections, as neutralizing titers correlate with HCPS severity³³ and administration of human ANDV immune plasma reduced case fatality rate in a small trial³⁴. Antibodies may neutralize enveloped viruses by several non-mutually exclusive mechanisms, including by blocking viral attachment, membrane fusion or egress, inducing virion aggregation and, in vivo, by recruiting Fc-dependent effector functions^{35,36}. Several neutralizing monoclonal antibodies targeting the ANDV spike have been described^{22,23,37}. Some block viral membrane fusion by preventing dissociation of the prefusion heterodimer or the formation of the postfusion trimer. Others interfere with PCDH1 engagement, but the lack of structural information has prevented a mechanistic understanding of how they do so. The structural data presented here show that the PCDH1-binding pocket is too narrow to readily accommodate a conventional antibody. We therefore analyzed a panel of four human neutralizing antibodies to understand how they block receptor binding. We observed that ADI-65534 and ANDV-34 do not directly compete with PCDH1 but instead inhibit receptor binding through a steric interference mechanism mediated by the antibody constant region. This model of neutralization, in which antibody binding to accessible spike surfaces can prevent virus attachment without directly targeting the receptor-binding sites, has been reported for other viruses^{36,38-40}, and suggests that strategies to increase antibody occupancy at the virion surface, such as combining non-competing antibodies targeting accessible epitopes, or focusing the response on exposed loops in the most apical regions of the tetramer, may enhance neutralizing activity against hantaviruses. ”*

Finally, I emphasize that this is a fine paper in my opinion revealing important molecular details of how a New World hantavirus recognizes its receptor target and will be widely appreciated and quoted by those working in this field.

We sincerely thank the reviewer for this very positive assessment of our work. We are grateful for the very thoughtful and constructive comments, which have helped us substantially improve the manuscript.

Referees' comments:

We thank all reviewers for their constructive comments, which really helped us to improve our manuscript.

Referee #1 (Remarks to the Author):

The authors have addressed all of our comments. We only have a minor suggestion for Figure 3C. We recommend adding labeling to the top and bottom panels with the molecules used in assays (e.g., WT vs. mutant soluble EC1) to make it easier for the reader to grasp what is shown from looking at the figure, rather than having this information only in the figure legend.

We thank the reviewer for this final comment. We have included labels in panel 3C to make the figure easier to read.

Referee #3 (Remarks to the Author):

This is a revised manuscript that examines the structure of the cellular receptor, protocadherin-1, for Andes virus. The response to reviewer queries was thorough and has significantly improved this manuscript. From my current review of the response, and the revised manuscript, I suggest the manuscript makes a significant addition to our knowledge of hantaviruses and their use of cellular receptor proteins. With the revised figures, the manuscript is accessible to a general audience and is well written.

We thank the reviewer for the positive and constructive comments throughout the review process.

Editorial comments

1. Please provide the manuscript in .docx format. Currently it is in PDF format. [Done](#)
2. Please add references to the abstract. [Done](#)
3. Please create a separate reference list for any methods references, making sure that the numbering continues from the main text references. [Done](#)
4. Please note that the legends for the main figures should not exceed 300 words. If it is not possible to reduce the length accordingly, please ensure that the final legends are as close as possible to 300 words. [Done](#)
5. Please reduce subheadings to 40 characters (with spaces) or less. [Done](#)
6. You have more than one account on EJP. Please contact Nature Manuscripts (naturemanuscripts@nature.com) to confirm all accounts which belong to you, and your primary email address. [Done](#)
7. Please provide a supplementary information guide as a separate word document. [Done](#)

8. Please reduce the total number of Extended Data items to 10. These ED can be multi-panelled but all panels should fit on a single page. If the existing ED cannot be consolidated into 10 items then please move less important panels to SI. [Done](#)
9. There are potential third party rights issues in the figures. Please check the sources of all illustrations and clarify whether permissions are needed to adapt or reproduce them. Please make sure to include the relevant details in third party rights table when you resubmit (more information below). If Biorender or a similar software has been used, please also ensure to provide relevant licenses. In particular please check figure/s: [There is no third party rights in the figures](#)
10. Please make sure to provide a third party rights table (more information below) when you resubmit. If Biorender or similar software has been used, please also ensure to provide relevant licenses. [Done](#)
11. Flagging that there are display items in the SI (Supplementary table)- please check if any of these materials should be extended data and notify us if they require further reproducibility checks. [They should be keep it as SI](#)
12. Please re-supply the Extended data figures individually in EPS, JPEG or TIF format. [Done](#)
13. Please ensure that the text size in all figures is at least 5 pt Arial. [Done](#)
14. The funding information has been merged with the acknowledgment section; please provide it as a separate section. [Done](#)
15. Flagging that Extended data Figures are labelled as "Figure S1, Figure S2.." Please make them Extended Data figures. [Done](#)
16. The data deposited to the PDB and EMDB with the dataset identifiers PDB-30XO, PDB-30XT and EMD-58134, EMD-77314 are currently not available. [All are now available or will be released in the next round of structure releases.](#)