

Comparative Analysis of Deep Mutational Scanning Datasets in Enteroviruses A and B Identifies Functional Divergence and Therapeutic Targets

Corresponding Author: Dr Patrick Dolan

Version 0:

Decision Letter:

14th October 2025

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Dr Dolan,

Your manuscript entitled "Comparative Analysis of Deep Mutational Scanning Datasets in Enteroviruses A and B Identifies Functional Divergence and Therapeutic Targets" has now been seen by two reviewers, whose comments are attached. The reviewers have raised a number of concerns which will need to be addressed before we can offer publication in Nature Ecology & Evolution. We will therefore need to see your responses to the criticisms raised, along with a revised manuscript, before we can reach a final decision regarding publication.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

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* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at <http://www.nature.com/natecolevol/info/final-submission>. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

* Extended Data Figures - please ensure that any supplementary figures and tables that are crucial to the manuscript's conclusions are converted into Extended Data figures and tables to increase visibility of these data. Extended Data figures and tables are online-only (present in the online PDF and full-text HTML versions of the paper), peer-reviewed display items that provide essential background to the article but are not included in the main article due to space constraints. A maximum of ten Extended Data display items (figures and tables) is permitted.

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We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us

know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Ecology & Evolution or published elsewhere.

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Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

[redacted]

Reviewer expertise:

Reviewer #1: evolution of viral pathogens, theory and computational modelling, fitness constraints

Reviewer #2: viral genetic analysis, comparative genomics, host-pathogen interactions

Reviewer comments:

Reviewer #1 (Remarks to the Author):

In this work, Alvarez-Rodriguez, Bakhache, and collaborators reanalyze deep mutational scanning (DMS) data sets for enterovirus A and B viruses to highlight similarities and differences in viral fitness constraints, at the level of both virus species and types. Broadly, they observed similar fitness constraints across these two data sets despite significant sequence divergence, but fitness effects differed in regions subject to different functional constraints (e.g., binding to different host proteins) and in regions involved in immune escape or antagonism. They also compared mutational tolerance in DMS experiments with natural sequence variation, finding broad agreement between the two, but again with some focused differences that may be explained by host-pathogen interactions that were not present in the experimental conditions. The authors also combined their data with an algorithm for identifying potential drug targets to identify possible binding pockets under exceptional mutational constraints.

Overall, the authors have made clever use of these data sets (and auxiliary analyses) to gain a better understanding of mutational constraint and divergence in EV-A and EV-B. The whole of their analyses is more than the sum of their parts, as differences in fitness effects between the two data sets can distinguish between functional constraints that are specific to one virus versus ones that apply more broadly to enteroviruses in general. In this thorough study, the authors use a variety of biological features – including physicochemical properties of amino acids, protein secondary structure, and location relative to important host protein binding sites – to explain the experimental results and place them in context. This is excellent work.

Main comments

1. Several parts of the analysis rely on sequence alignments. The Methods describe how the alignments were produced, but they don't include statistics like the number of sequences in each alignment or subalignment. This is especially important for features like entropy, which can be sensitive to sampling depth. Similarly, the sequences used for analysis should also be checked for other possible pathologies (e.g., bias in the distribution due to a large number of samples from a single time/location). If I understand correctly, the step in the natural diversity analysis pipeline that applies cd-hit should cluster highly similar sequences together. The authors should verify that similar numbers of "redundant" sequences are removed for the different species/type alignments, or if these are very different, they should explain why this clustering gives a good representation of natural sequence diversity (or, alternately, why their results do not depend strongly on different clustering conditions).

2. While readers can refer back to prior work, given the centrality of the DMS experiments used in this paper, it would be helpful to expand their description a bit in the main text and to be a bit more precise in the Methods. Based on the description in the Methods, is it correct that the mutational fitness effects (MFEs) defined here are enrichment ratios? Preferences, a normalized version of enrichment ratios, are also commonly computed in analyses using dms_tools. When statistically comparing differences in MFEs between data sets, the authors should also account for experimental uncertainty in the MFEs, which could be quantified through differences in estimates between experimental replicates.

3. In lines 212-216, the authors suggest that targeting of surface-exposed loops on the viral capsid by the adaptive immune system could be one reason why divergent mutational tolerances are observed in these regions. This needs more explanation. If I understand correctly, antibodies are not included in the DMS experiments, and thus selection for immune escape should not be directly reflected in the DMS data. In principle, one might expect that the exposed parts of the capsid

could face similar pressures to accommodate escape mutations. Are there known differences in antigenic evolution or variability between A and B enteroviruses?

Minor comments

1. For plots like Fig. 2C (also Fig. 3A, Fig. 5D), it would be helpful to show at least in supplementary a histogram or other more detailed way of visualizing the distribution. Some differences in distribution appear subtle for the box and whisker plots as currently shown.
2. Line 440: should this read "lower variability" instead of "lower conservation"? This would be consistent with the potential binding pocket being conserved.

Reviewer #2 (Remarks to the Author):

In this work, the authors build on their previous DMS analyses to compare the MFEs of two enteroviruses. Most analyses are appropriate (although some relevant details are missing) and the manuscript is well written. This said, I think that the presented results represent an incremental follow-up of their previous works rather than really novel insights.

Major concerns

HEV frequently recombine. Therefore, the meaning of the phylogenetic analyses is limited. The authors may want to consider using identity matrices instead

I don't think it is particularly unexpected that there is a correlation in MFE between the two viruses. This result was somehow expected given their previous works and in general it reflects the idea that distinct proteins are differentially tolerant to change. After all, these proteins perform the same function and I guess they share very similar structures in the two viruses. Maybe this section can be shortened.

Why was site MFE difference not available for some sites (marked as NA in figure 2)?

Concerning the analysis of the receptor binding sites, the authors imply that CAR is more expressed in HeLa than DAF. Where was this information obtained from? Based on the human protein atlas data it seems to be the opposite. This is relevant for their interpretation of the results.

Concerning the AlphaFold3 models of GBF1 with the 3A dimers there is no indication of the reliability of the prediction (e.g., pTM and ipTM scores). Without this information it is impossible to assess the results of their analyses and whether the conclusions are supported

Concerning the druggable pocket in protein 2C, they mention one in their previous Plos Bio paper. Is it the same pocket? From that previous manuscript, it seems that it had 16% of neutral sites. Is this compatible with it being a promising target?

The discussion reiterates parts of the results and can be shortened.

For the natural variation analyses, how many virus sequences were analyzed?

*****END*****

Version 1:

Decision Letter:

17th November 2025

Dear Dr. Dolan,

Thank you for submitting your revised manuscript "Comparative Analysis of Deep Mutational Scanning Datasets in Enteroviruses A and B Identifies Functional Divergence and Therapeutic Targets" (NATECOLEVOL-25082982A). It has now been seen again by the original reviewers and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Ecology & Evolution, pending minor revisions to satisfy the reviewers' final requests and to comply with our editorial and formatting guidelines.

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Thank you again for your interest in Nature Ecology & Evolution. Please do not hesitate to contact me if you have any questions.

[redacted]

Reviewer #1 (Remarks to the Author):

I thank the authors for their revisions, which have addressed my previous questions. I have no further comments to add.

Reviewer #2 (Remarks to the Author):

Thank you for addressing my comments. I think that, for the sake of transparency, the authors should include a sentence disclosing that low ipTM scores are associated with poorly supported models. In this case, other data support their conclusions, but when no additional information is available ipTM scores of 0.2 do not allow much inference.

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Reviewer comments:

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In this work, Alvarez-Rodriguez, Bakhache, and collaborators reanalyze deep mutational scanning (DMS) data sets for enterovirus A and B viruses to highlight similarities and differences in viral fitness constraints, at the level of both virus species and types. Broadly, they observed similar fitness constraints across these two data sets despite significant sequence divergence, but fitness effects differed in regions subject to different functional constraints (e.g., binding to different host proteins) and in regions involved in immune escape or antagonism. They also compared mutational tolerance in DMS experiments with natural sequence variation, finding broad agreement between the two, but again with some focused differences that may be explained by host-pathogen interactions that were not present in the experimental conditions. The authors also combined their data with an algorithm for identifying potential drug targets to identify possible binding pockets under exceptional mutational constraints.

Overall, the authors have made clever use of these data sets (and auxiliary analyses) to gain a better understanding of mutational constraint and divergence in EV-A and EV-B. The whole of their analyses is more than the sum of their parts, as differences in fitness effects between the two data sets can distinguish between functional constraints that are specific to one virus versus ones that apply more broadly to enteroviruses in general. In this thorough study, the authors use a variety of biological features – including physicochemical properties of amino acids, protein secondary structure, and location relative to important host protein binding sites – to explain the experimental results and place them in context. This is excellent work.

Main comments

1. Several parts of the analysis rely on sequence alignments. The Methods describe how the alignments were produced, but they don't include statistics like the number of sequences in each alignment or subalignment. This is especially important for features like entropy, which can be sensitive to sampling depth. Similarly, the sequences used for analysis should also be checked for other possible pathologies (e.g., bias in the distribution due to a large number of samples from a single time/location). If I understand correctly, the step in the natural diversity analysis pipeline that applies cd-hit should cluster highly similar sequences together. The authors should verify that similar numbers of "redundant" sequences are removed for the different species/type alignments, or if these are very different, they should explain why this clustering gives a good representation of natural sequence diversity (or, alternately, why their results do not depend strongly on different clustering conditions).

We thank the reviewer for bringing up this point and apologize for not including more detail in the original manuscript. We have added the number of full-length sequences downloaded and the number of sequences analyzed after clustering to the Methods (lines 839-859). The reviewer is correct in noting that cd-hit clustering was used to mitigate redundancy in our dataset. Entropy calculations were performed on sequences clustered at 98% nucleotide identity, which resulted in a 73% reduction in EVA sequences (4034 to 1081) and a 54% reduction in EVB sequences (1644 to 762). This difference is due to significant oversampling of EV-A71 and CV-A6 relative to other EVA and EVB types. By clustering, we are able to reduce the impact of this oversampling while also retaining the overall genetic diversity of our dataset. We also checked our data for overrepresentation of samples from specific countries and years, which revealed a relatively large number of sequences from China, particularly in the EVA dataset. However, the vast majority of these sequences are EV-A71 and CV-A6, so this overrepresentation was also addressed via clustering. We found no major biases in sample dates.

To address the concern that entropy calculations may be sensitive to sampling depth, we performed entropy calculations on sequences clustered at a range of nucleotide identities (98%, 95%, 90%, 85%, and 80%). Comparing the results of these calculations reveals highly similar entropy patterns under all clustering conditions indicating that our results are not dependent on sampling strategy. We have added the results of this analysis as Supplementary Figure 5C, D.

2. While readers can refer back to prior work, given the centrality of the DMS experiments used in this paper, it would be helpful to expand their description a bit in the main text and to be a bit more precise in the Methods. Based on the description in the Methods, is it correct that the mutational fitness effects (MFEs) defined here are

enrichment ratios? Preferences, a normalized version of enrichment ratios, are also commonly computed in analyses using dms_tools. When statistically comparing differences in MFEs between data sets, the authors should also account for experimental uncertainty in the MFEs, which could be quantified through differences in estimates between experimental replicates.

We thank the reviewer for this helpful comment. We have expanded the description of the DMS experiments in both the main text and the Methods (lines 156-175 and 744-758). The reviewer is correct that the mutational fitness effects (MFEs) in this study are defined as enrichment ratios between mutant and wild-type counts across conditions. We have clarified this point in the main text (lines 163-175).

As mentioned by the reviewer, preferences represent a standardized measure of MFE that scales the effect of each mutation at each site to a value between 0 and 1. We have utilized this metric for the phyDMS analysis, as required by the program. However, by standardizing all MFEs to range from 0-1, the ability to identify strong differences between sites is lost. We therefore utilize enrichment ratios for the rest of the work.

Regarding experimental uncertainty, we note that the MFEs reported in this study represent the average across experimental replicates from each dataset. We chose this metric for this comparative study as differences in experimental methodologies used in each dataset (including both the mutagenesis and sequencing methods) as well as the different number of experimental replicates can introduce additional sources of error into the analysis. That said, experimental variability and associated errors were carefully addressed in the original publications from which each dataset was obtained and showed high reproducibility between experimental replicates. In this study, we now report the correlation of mutation-level MFE values using the same analysis pipeline for both viruses (Pearson's $R = 0.7-0.95$ for CVB3 and $R = 0.89-0.96$ for EVA71) in the Methods (Lines 788-790) to help clarify the experimental error associated with the data.

3. In lines 212-216, the authors suggest that targeting of surface-exposed loops on the viral capsid by the adaptive immune system could be one reason why divergent mutational tolerances are observed in these regions. This needs more explanation. If I understand correctly, antibodies are not included in the DMS experiments, and thus selection for immune escape should not be directly reflected in the DMS data. In principle, one might expect that the exposed parts of the capsid could face similar pressures to accommodate escape mutations. Are there known differences in antigenic evolution or variability between A and B enteroviruses?

Thank you for pointing out this ambiguity. Indeed, the current DMS experiments do not include antibody selection, and therefore immune escape pressures are not directly captured in our data. We apologize for the confusion. We have now clarified that immune pressure in nature could be responsible but requires further experimental validation in EVA71 (Lines 255-257: "Moreover, the mutational flexibility of surface loops may also be shaped by type-specific host immune pressures, as previously described for CVB3 , although this possibility remains to be validated in EVA71.").

Minor comments

1. For plots like Fig. 2C (also Fig. 3A, Fig. 5D), it would be helpful to show at least in supplementary a histogram or other more detailed way of visualizing the distribution. Some differences in distribution appear subtle for the box and whisker plots as currently shown.

Thank you for this comment. We agree that showing the data distributions more explicitly would be helpful. We included density plots in the supplementary figures (Supplementary Figures 3A-C and 5H) to better visualize the underlying distributions and highlight subtle differences between datasets.

2. Line 440: should this read "lower variability" instead of "lower conservation"? This would be consistent with the potential binding pocket being conserved.

Thank you for catching this. You are correct, the intended meaning is "lower variability" (or lower entropy), which is consistent with the potential binding pocket being conserved. We will revise this wording in the main text accordingly.

Reviewer #2 (Remarks to the Author):

In this work, the authors build on their previous DMS analyses to compare the MFEs of two enteroviruses. Most analyses are appropriate (although some relevant details are missing) and the manuscript is well written. This said, I think that the presented results represent an incremental follow-up of their previous works rather than really novel insights.

We thank the reviewer for this feedback. However, we believe that by providing the first full proteome comparative analysis of deep mutational scanning (DMS) datasets for two related viruses, spanning a diverse array of structurally and functionally diverse proteins, our analysis is the first to establish comparative approaches for DMS datasets from distinct viruses, addressing the key question of how well such studies extrapolate between related species and is thus of broad utility to the scientific community. Specifically, our results and the analysis framework reveal that such comparison can uncover conserved and divergent constraints on protein function that cannot otherwise be obtained by examining single DMS datasets. Moreover, it highlights the potential of this approach to uncover hotspots for virus-host interactions which differ between related species. Finally, our comparison of both DMS datasets with evolution in nature helps place the results of DMS studies in the broader evolutionary context, which has not been extensively done in the past.

Major concerns

HEV frequently recombine. Therefore, the meaning of the phylogenetic analyses is limited. The authors may want to consider using identity matrices instead

Thank you for your comment. It is certainly true that human enteroviruses recombine frequently and that this recombination confounds many phylogenetic analyses. It is important to note, however, that recombination in enteroviruses is confined to intraspecies exchanges in all regions except the 5'-UTR. Thus, recent recombination should not be an issue for the phylogenetic trees in Figure 1 as they were 1) constructed using capsid and 3Dpol sequences, and 2) depict between-species, rather than within-species, evolutionary relationships. We believe that these trees are valuable additions to the manuscript as they show the evolutionary relationship between EVA and EVB, which is important context in a comparative analysis like ours. We include both a capsid and a 3Dpol tree to account for recombination in the deeper evolutionary history of enteroviruses (pre-speciation) which resulted in these regions of the genome having different evolutionary relationships. A sentence addressing this has been added to the revised manuscript (lines 142-145). Similar phylogenetic trees showing the evolutionary relationships between all *Picornaviridae* species can be found on the International Committee for the Taxonomy of Virus (ICTV) website.

Ultimately, we include phylogenetic trees to provide context on the evolutionary relationship between EVA and EVB, but our results are not dependent on these trees.

I don't think it is particularly unexpected that there is a correlation in MFE between the two viruses. This result was somehow expected given their previous works and in general it reflects the idea that distinct proteins are differentially tolerant to change. After all, these proteins perform the same function and I guess they share very similar structures in the two viruses. Maybe this section can be shortened.

Thank you for your comment. We agree that a correlation in MFE between the two viruses is not unexpected, as their proteins share similar functions and overall structures. However, this analysis serves as an important quality control step to verify that our experimental and computational methods produce consistent and comparable MFE measurements across both Enterovirus genomes. This is particularly relevant because the mutational scans were performed independently in different laboratories, using different experimental approaches, and in different cell lines. We have clarified this in the main text (lines 243-245).

Establishing this correlation gives us confidence that the observed differences in MFE reflect true biological variation rather than technical artifacts. Moreover, the capsids of these viruses are known to use distinct receptors and exhibit very different binding modes, which is consistent with the differences

in MFEs we observe. Similarly, our results suggest that replication proteins, although performing comparable functions, may achieve these functions through different binding mechanisms.

Why was site MFE difference not available for some sites (marked as NA in figure 2)?

The site MFE difference was not available for some sites (marked as NA in Figure 2) because those regions could not be structurally aligned between the two Enteroviruses. Insertions and deletions (InDels) caused gaps in the alignment, so we could not compare the MFE at those sites. Therefore, these regions were marked as NA. This is indicated in lines 307-310 and 827-829 .

Concerning the analysis of the receptor binding sites, the authors imply that CAR is more expressed in HeLa than DAF. Where was this information obtained from? Based on the human protein atlas data it seems to be the opposite. This is relevant for their interpretation of the results.

We apologize for the confusion. We have revised the text to clarify that our interpretation refers to receptor usage mechanisms rather than expression levels. In particular, CVB3 is known to efficiently enter HeLa-H1 cells via CAR alone (Chung et al. 2005). This may reduce dependence on DAF and alleviate structural constraints associated with co-receptor usage, potentially explaining the mutational flexibility observed in this region. This has now been clarified in lines 324-328.

Concerning the AlphaFold3 models of GBF1 with the 3A dimers there is no indication of the reliability of the prediction (e.g., pTM and iptM scores). Without this information it is impossible to assess the results of their analyses and whether the conclusions are supported

Thank you for your feedback regarding the AlphaFold3 models. We have now included the pTM, iptM, and ranking scores for the GBF1–3A dimer models in the revised manuscript (Line 436-437). The iptM scores are relatively low, which likely reflects the limited structural information available for this type of membrane–host interaction and the inherent complexity of modeling GBF1 (a very large and flexible protein) together with the membrane-associated 3A protein.

Despite these limitations, several independent lines of evidence support the distinct binding modes suggested by our AlphaFold3 predictions. GBF1 is a well-established host factor for enteroviruses (Belov et al. 2008). As mentioned in the text, the $\alpha 2$ helix of CVB3 3A has been shown not to be required for GBF1 interaction (Wessels et al. 2007), whereas our differential MFE tolerance indicates that this region plays a role in EVA71 3A–GBF1 interaction. Consistently, in a parallel preprint from our group, deep mutational scanning of EVA71 3A under GBF1 inhibition revealed an enrichment of mutations within the $\alpha 2$ helix, supporting its involvement in this interaction and reinforcing the idea of a different binding mode (Dolan, Bakhache, and Symonds-Orr 2025) . Together, these modeling, experimental, and previously published data support the conclusion that EVA71 and CVB3 3A proteins engage GBF1 through distinct mechanisms. We would like to note that we had already included the following statement in the manuscript to acknowledge these limitations and to emphasize the need for future structural validation:

“While our predictions explain differences in MFEs between EVA71 and CVB3 3A, more structural studies are needed to map the distinct interaction surfaces between enterovirus 3A proteins and GBF1.”

We included this statement to make it clear that, although our predictions are consistent with multiple independent lines of evidence, additional experimental structural work will be required to confirm and refine these proposed interaction differences.

Concerning the druggable pocket in protein 2C, they mention one in their previous Plos Bio paper. Is it the same pocket? From that previous manuscript, it seems that it had 16% of neutral sites. Is this compatible with it being a promising target?

We thank the reviewer for this question. The pocket described in our previous *PLOS Biology* paper for CVB3 is distinct from the one analyzed in the current manuscript. The earlier pocket, identified using an older version of Sitemap and AlphaFold2-predicted structures, was larger (25 residues, 130 Å²). In the present study, we used the updated Sitemap version and AlphaFold3-predicted structures, which

identified a smaller pocket in CVB3 2C (20 residues, 77 Å²) that shares 13 residues with the previously defined pocket.

In the *PLOS Biology* paper, we used a very stringent definition of mutational tolerance, classifying each site based on the single most tolerated mutation (i.e., the mutation with the highest MFE at that position). Using this criterion, 16% of the residues in the pocket (4 out of 25) were classified as neutral. Of these four sites, three are not part of the newly identified pocket and only one overlaps. In contrast, in the current study we assess the average site MFE, which provides a more representative measure of the overall mutational effects at each site. Using this more integrative metric, all 20 residues in the newly defined pocket exhibit average deleterious effects.

Together with the entropy analysis presented in the manuscript, these results indicate that residues in the 2C pocket are highly mutationally constrained, supporting that this pocket represents a promising therapeutic target.

The discussion reiterates parts of the results and can be shortened.

We thank the reviewer for this suggestion. We have revised the Discussion to reduce redundancy with the Results section and focused on interpretation and broader implications.

For the natural variation analyses, how many virus sequences were analyzed?

We thank the reviewer for pointing out this oversight in our Methods section. After clustering at 98% sequence similarity to mitigate oversampling of some types, we were left with 1081 EVA sequences, 762 EVB sequences, 292 EVC sequences, and 71 EVD sequences. We have added these numbers to the Methods (line 839-859).

Belov, George A., Qian Feng, Krisztina Nikovics, Catherine L. Jackson, and Ellie Ehrenfeld. 2008. "A Critical Role of a Cellular Membrane Traffic Protein in Poliovirus RNA Replication." *PLoS Pathogens* 4 (11): e1000216.

Chung, Sun-Ku, Joo-Young Kim, In-Beom Kim, Sang-Ick Park, Kyung-Hee Paek, and Jae-Hwan Nam. 2005. "Internalization and Trafficking Mechanisms of Coxsackievirus B3 in HeLa Cells." *Virology* 333 (1): 31–40.

Dolan, Patrick, William Bakhache, and Walker Symonds-Orr. 2025. "Genotype-by-Inhibitor Interactions to Dissect Enterovirus Replication." *Research Square*. <https://doi.org/10.21203/rs.3.rs-7660613/v1>.

Wessels, Els, Daniël Duijsings, Kjerstin H. W. Lanke, Willem J. G. Melchers, Catherine L. Jackson, and Frank J. M. van Kuppeveld. 2007. "Molecular Determinants of the Interaction between Coxsackievirus Protein 3A and Guanine Nucleotide Exchange Factor GBF1." *Journal of Virology* 81 (10): 5238–45.

NATECOLEVOL-25082982B

Reviewer #1:

Remarks to the Author:

I thank the authors for their revisions, which have addressed my previous questions. I have no further comments to add.

Thank you for your constructive comments and suggestions.

Reviewer #2:

Remarks to the Author:

Thank you for addressing my comments. I think that, for the sake of transparency, the authors should include a sentence disclosing that low ipTM scores are associated with poorly supported models. In this case, other data support their conclusions, but when no additional information is available ipTM scores of 0.2 do not allow much inference.

Thank you for this suggestion, we agree. We have included a statement in lines 265-8.

“Although these predictions exhibit low ipTM scores (~ 0.2), the predicted interfaces correspond to regions of differential selection in EVA71 and CVB3. Notably, recent work has shown that virus-host protein interactions are often associated with similarly low ipTM scores (Baptista et al. 2025).”

Reference:

Baptista, Delora, Lidia Gomez-Lucas, Jürgen Jänes, Nevan J. Krogan, Maria João Amorim, Ylva Ivarsson, and Pedro Beltrao. 2025. “AlphaFold Models of Host-Pathogen Interactions Elucidate the Prevalence and Structural Modes of Molecular Mimicry.” *BioRxiv.org*. <https://doi.org/10.1101/2025.06.04.657796>.