nature portfolio

Peer Review File

Human Telomerase Reverse Transcriptase Supports Respiratory Syncytial Virus Replication

Corresponding Author: Dr Hoang Huynh

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This manuscript from Dr. Kahn's group, which dissects RSV replication mechanisms, offers several important advances. The data are rigorous, the statistical analyses are sound, and the combined use of gene knockouts with specific inhibitors provides a thorough examination of each host factor. Including a positive-strand RNA virus as a control is also commendable. The following suggestions should strengthen the manuscript:

Update RSV mortality data. The current background cites a 2005 study conducted before vaccines and monoclonal-antibody prophylaxis became widespread. Please replace it with more recent global and U.S. estimates.

Clarify the role of A549 cells (page 6). A549 cells support RSV replication but are not typically used for high-titer stock preparation, which is usually done in HEp-2 or Vero cells. Consider describing A549 cells as "permissive for viral replication" rather than "used to propagate virus."

Validate TERT findings in primary cells. Because A549 is a cancer line, TERT-related effects could be cell-line-specific. Replicating key experiments in primary human airway epithelial cells would confirm the relevance of TERT in a physiologic context.

Discuss (or plan) in vivo studies. Animal experiments are not strictly necessary for this manuscript, but outlining planned in vivo validation (e.g., in a small-animal RSV model) would underscore the translational potential of the findings.

Expand on nuclear import versus inclusion bodies. The enrichment of viral RNAs in nuclear fractions suggests that nuclear import may play a larger role in RSV biology than cytoplasmic inclusion bodies, which are comparatively sparse for RSV/hMPV. A brief comparison with other negative-strand RNA viruses and discussion of possible functional implications would be valuable.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
The manuscript by Huynh et al. presents intriguing findings regarding the potential role of human TERT in RSV RNA synthesis and protein expression. The authors demonstrate that at higher concentrations of ALS-8112 (an RSV L polymerase inhibitor), viral mRNA levels significantly increase at 48 hours compared to 24 hours, despite complete suppression of viral protein expression. Through TERT knockdown and pharmacological inhibition experiments, the study provides compelling evidence for TERT's involvement in RSV replication. While the experimental work is substantial, the central claim that TERT functions as an RSV RdRp requires additional supporting evidence.

Major Concerns:

1.Mechanistic Evidence for TERT-RNA Interaction:
As TERT is predominantly nuclear, the manuscript lacks critical data demonstrating how viral RNA (cytoplasmic) interacts with TERT.

2. While negative-sense RNA viruses typically form RNP complexes with N, P, and M2-1 proteins, the immunostaining shows minimal RSV N/P protein signals in the nuclear. Some Strong evidence to show TERT function as RdRp is needed.

Suggested experiment: RNA FISH to validate TERT co-localization with viral RNA.

Minor Concerns:

1. The observation that Episilvestrol inhibits influenza NP/NA protein expression without reducing viral RNA levels (MOI=0.3) requires clarification, as viral propagation would be expected in vehicle controls at low MOI infection.

2. The half-life of ALS-8112 in culture medium should be provided to properly interpret the 48-hour timepoint results.

3. Figures 6F and 6G show conflicting nuclear localization patterns for RSV N/P proteins.

Recommendation: Use a cytoplasmic-specific marker (rather than β-actin) for proper compartmentalization analysis.

4. The nuclear/cytoplasmic fractionation assay using RPL19 RNA as reference may not accurately reflect viral RNA distribution.

Alternative approach: RNA FISH would provide more reliable spatial resolution of viral RNA localization.

Reviewer #4

(Remarks to the Author)
This manuscript proposes that the human telomerase reverse transcriptase catalytic subunit (hTERT) acts as an auxiliary RNA dependent RNA polymerase (RdRp) for respiratory syncytial virus (RSV) when the viral L polymerase is inhibited. The idea that a canonical host reverse transcriptase can be co opted by a negative strand RNA virus for genome replication is novel and, if fully validated, would open an unexpected therapeutic avenue. The authors present extensive cell based genetic and pharmacological experiments to support their model, but the evidence remains largely indirect. Below I detail the major concerns, and minor comments.

Major Concern
1， Lack of direct biochemical evidence for hTERT RdRp activity on RSV RNA. All claims rest on RNA and protein readouts in cells. Purify recombinant hTERT (± hTERC) and demonstrate de novo synthesis or primer dependent extension on an RSV derived RNA template in vitro; include catalytically inactive hTERT mutants as negative controls.
2， Specificity of pharmacological tools. BIBR 1532 can affect telomere independent hTERT functions and potentially other polymerases at the doses used. Provide side by side toxicity curves, show that BIBR 1532 does not inhibit a panel of other viral polymerases, and rescue BIBR 1532 inhibition with ectopic, inhibitor resistant hTERT mutants.
3， mRNA–protein discordance(?). Viral mRNAs accumulate while viral proteins drop under hTERT inhibition—opposite to the expectation for a polymerase defect. Measure nascent transcription, mRNA stability, and translation efficiency (polysome profiling) to clarify where the block occurs.

Minor Comments
1， Statistics and Replicates – Clearly state the number of biological repeats and the statistical test used beneath each figure panel.
2， Nomenclature – Use “hTERT” consistently (currently alternates with “TERT”).
3， Discussion – Compare results to reports of TERT RdRp activity in the RMRP pathway and in yeast telomerase complexes; clarify whether those precedents were template dependent or de novo.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have diligently addressed all the comments from me. Thanks!

Reviewer #3

(Remarks to the Author)
The reviewer highly appreciates the authors' efforts to improve the quality of the manuscript. The revised version has addressed most of the concerns raised previously, yet several critical issues remain open to discussion.
The authors claim that human telomerase reverse transcriptase (hTERT) can function as an RNA-dependent RNA polymerase (RdRp) to support respiratory syncytial virus (RSV) replication. They have verified this hypothesis via hTERT knockdown and the application of hTERT inhibitors, but these experiments only serve as supportive evidence. For a protein to act as an RdRp, its localization to the viral replication complex and physical interaction with viral RNA are indispensable prerequisites. The authors stated that no satisfactory hTERT antibody was available to perform the aforementioned experiments. To resolve this limitation, the reviewer recommends two alternative strategies:
(1) Introducing an epitope tag to the endogenous hTERT protein via the gene-editing knock-in technology;
(2) Overexpressing epitope-tagged recombinant hTERT protein in target cells.
In addition, in Figure 8A, the authors reported the nuclear enrichment of RSV RNA using nuclear-cytoplasmic fractionation coupled with quantitative PCR (qPCR). Given the inherent limitations of this approach, the reviewer suggests validating the subcellular distribution of RSV RNA by RNA fluorescence in situ hybridization (RNA FISH). Notably, RNA exhibits uneven distribution between the nucleus and cytoplasm. Even though RPL19 is a ribosomal RNA, it cannot be used as a reference for uniform nuclear-cytoplasmic distribution. In contrast, RNA FISH enables direct visualization of the subcellular localization of RSV RNA in situ.
Minor issues requiring correction are listed below:
(1) Page 7: The phrase "These observations suggest RSV viral replication" should be revised to "viral protein synthesis".
(2) Pages 12 and 13: The authors stated "These results suggested that viral proteins N and P could redundantly interfere the regulations of eIF2α by eIF2α kinases during RSV infections through physical interactions." However, the detected protein-protein interactions alone are insufficient to demonstrate that the N and P proteins block both the activation of eIF2α kinases and the subsequent phosphorylation of eIF2α.

Reviewer #4

(Remarks to the Author)
This revised submission has successfully addressed the concerns raised in the previous review.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This manuscript from Dr. Kahn's group, which dissects RSV replication mechanisms, offers several important advances. The data are rigorous, the statistical analyses are sound, and the combined use of gene knockouts with specific inhibitors provides a thorough examination of each host factor. Including a positive-strand RNA virus as a control is also commendable. The following suggestions should strengthen the manuscript:

Update RSV mortality data. The current background cites a 2005 study conducted before vaccines and monoclonal-antibody prophylaxis became widespread. Please replace it with more recent global and U.S. estimates.

Thank you for your comment. We have updated the global and U.S. estimates on page 3.

Clarify the role of A549 cells (page 6). A549 cells support RSV replication but are not typically used for high-titer stock preparation, which is usually done in HEp-2 or Vero cells. Consider describing A549 cells as "permissive for viral replication" rather than "used to propagate virus."

Thank you for your suggestion. We have modified the wording on page 6.

Validate TERT findings in primary cells. Because A549 is a cancer line, TERT-related effects could be cell-line-specific. Replicating key experiments in primary human airway epithelial cells would confirm the relevance of TERT in a physiologic context.

Thank you for your suggestion. We have added new data **Figure 6** for primary human small airway epithelial cells (HSAEC), and revision on page 11-12.

Discuss (or plan) in vivo studies. Animal experiments are not strictly necessary for this manuscript, but outlining planned in vivo validation (e.g., in a small-animal RSV model) would underscore the translational potential of the findings.

Thank you for your comment. We have updated the discussion on page 25-26.

Expand on nuclear import versus inclusion bodies. The enrichment of viral RNAs in nuclear fractions suggests that nuclear import may play a larger role in RSV biology than cytoplasmic inclusion bodies, which are comparatively sparse for RSV/hMPV. A brief

comparison with other negative-strand RNA viruses and discussion of possible functional implications would be valuable.

Thank you for your comment. We have updated the discussion on page 24.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (Remarks to the Author):

The manuscript by Huynh et al. presents intriguing findings regarding the potential role of human TERT in RSV RNA synthesis and protein expression. The authors demonstrate that at higher concentrations of ALS-8112 (an RSV L polymerase inhibitor), viral mRNA levels significantly increase at 48 hours compared to 24 hours, despite complete suppression of viral protein expression. Through TERT knockdown and pharmacological inhibition experiments, the study provides compelling evidence for TERT's involvement in RSV replication. While the experimental work is substantial, the central claim that TERT functions as an RSV RdRp requires additional supporting evidence.

Major Concerns:

1.Mechanistic Evidence for TERT-RNA Interaction:
As TERT is predominantly nuclear, the manuscript lacks critical data demonstrating how viral RNA (cytoplasmic) interacts with TERT.

Thank you for your comment. We agreed that the interaction between TERT and viral RNA is important to define. RNA FISH and/or immunofluorescence microscopy is probably best means to obtain the data. However, there is no validated/reliable anti-hTERT antibody for immunofluorescence or Western Blot analyses commercially available. We obtained 3 different commercially available antibodies and, in our hands, none were satisfactory. We would be happy to provide the reviewer with the data (though we do not believe that it would be appropriate to place such data in the supplemental figures). Nonetheless, we show that RSV proteins do enter the nucleus (see Figure 7).

2. While negative-sense RNA viruses typically form RNP complexes with N, P, and M2-1 proteins, the immunostaining shows minimal RSV N/P protein signals in the nuclear. Some Strong evidence to show TERT function as RdRp is needed.

Independent studies have shown that hTERT can function as RdRp (Maida and Masutomi, 2011; Maida et al., 2009), as we have cited in the manuscript (see page 9, 26).

Suggested experiment: RNA FISH to validate TERT co-localization with viral RNA.

Thank you for your comment as we addressed in comment #1.

Minor Concerns:

1. The observation that Episilvestrol inhibits influenza NP/NA protein expression without reducing viral RNA levels (MOI=0.3) requires clarification, as viral propagation would be expected in vehicle controls at low MOI infection.

Thank you for your comment. We hypothesize that Episilvestrol, a RNA helicase inhibitor, should not have a profound impact on viral mRNA synthesis early in infection (in this case 24 hpi). Rather, pharmacological inhibition of the separation of ds RNA would impact ribosome access and hence reduce protein expression, which was observed in our data. We have addressed this point in the manuscript, page 20.

2. The half-life of ALS-8112 in culture medium should be provided to properly interpret the 48-hour timepoint results.

Thank you for your comment. The half-life of ALS-8112 in culture medium is 29 hours (DeVincenzo et al., 2015), and revision on page 9.

3. Figures 6F and 6G show conflicting nuclear localization patterns for RSV N/P proteins.

Thank you for your comment. We noted approximately 5% of RSV N and P proteins could also be detected in the nucleus of infected cells. Low level of nuclear localization was observed possibly due to various factors, including sensitivity of assay, m.o.i, and time. As we have shown in Figure 6A (now Figure 7A), we could detect the interaction of viral proteins with host kinase PKR at 48, but not at 24 hpi (m.o.i = 0.2). This is probably due to scant amounts of viral proteins at 24 hpi, and the sensitivity of Western Blot. In general, we have observed viral protein expression at 48 hours is approximately 4-10 folds higher than 24 hpi, and correlated with ~10 folds higher of RNA expression.

Therefore, we have decided to use 48 hpi of m.o.i = 0.2 for Western Blot on Figure 6F (now Figure 7F). On the other hand, high expression of viral proteins also increased background for immunofluorescence; thus, 24 hpi of m.o.i = 0.1 was performed on Figure 6G (now Figure 7G) for better visualization. These observations supporting the notion that these viral proteins could redundantly interfere the eIF2α kinases in the cytoplasm and nucleus. We have revised on page 23.

Recommendation: Use a cytoplasmic-specific marker (rather than β-actin) for proper compartmentalization analysis.

Thank you for your comment. eIF2α is known as cytoplasmic protein marker (Wek, 2018), and Lamin B1 is nuclear protein marker (Sobo et al., 2024). β-actin is known to present in both cytoplasmic and nuclear (Szabo et al., 2025). We have included all 3 markers on Figure 6F (now Figure 7F), see top paragraph on page 13.

4. The nuclear/cytoplasmic fractionation assay using RPL19 RNA as reference may not accurately reflect viral RNA distribution.

Thank you for your comment. We have tried GAPDH, β-actin, and RPL19 as house-keeping genes (Fiddler and Clarke, 2021). RPL19, a ribosomal protein subunit of 60S, gave the best distribution.

Alternative approach: RNA FISH would provide more reliable spatial resolution of viral RNA localization.

Thank you for your comment. We agreed that the interaction between TERT and viral RNA is important. RNA FISH and/or immunofluorescence microscopy is probably best to obtain the data. However, there is no validated/reliable anti-hTERT antibody for immunofluorescence or Western Blot commercially available as we have tried 3 different antibodies (see our response to the comments of reviewer #1.).

Reviewer #4 (Remarks to the Author):

This manuscript proposes that the human telomerase reverse transcriptase catalytic subunit (hTERT) acts as an auxiliary RNA dependent RNA polymerase (RdRp) for respiratory syncytial virus (RSV) when the viral L polymerase is inhibited. The idea that a canonical host reverse

transcriptase can be co opted by a negative strand RNA virus for genome replication is novel and, if fully validated, would open an unexpected therapeutic avenue. The authors present extensive cell based genetic and pharmacological experiments to support their model, but the evidence remains largely indirect. Below I detail the major concerns, and minor comments.

Major Concern

1, Lack of direct biochemical evidence for hTERT RdRp activity on RSV RNA. All claims rest on RNA and protein readouts in cells. Purify recombinant hTERT (± hTERC) and demonstrate de novo synthesis or primer dependent extension on an RSV derived RNA template in vitro; include catalytically inactive hTERT mutants as negative controls.

Thank you for your comment. Previous studies have shown hTERT could function as RdRp as we have cited in the manuscript (Maida et al., 2009). Currently there is no validated/reliable anti-hTERT antibody commercially available as we have tried 3 different antibodies (as mentioned above, we would be happy to provide the data). While it is true that our data is dependent on RNA and protein readouts, we have used a variety of approaches to test the hypothesis that hTERT is involved in RSV replication. The experiments proposed by the reviewer are valid though beyond the scope of this study.

2, Specificity of pharmacological tools. BIBR 1532 can affect telomere independent hTERT functions and potentially other polymerases at the doses used. Provide side by side toxicity curves, show that BIBR 1532 does not inhibit a panel of other viral polymerases, and rescue BIBR 1532 inhibition with ectopic, inhibitor resistant hTERT mutants.

Thank you for your comment. BIBR-1532 is known as selective hTERT inhibitor (Pascolo et al., 2002). Treatment with BIBR-1532 at 40 μM for 48 hrs did not have significant toxicity on A549 cells, and no toxicity at 20 μM for 6 days (Ding et al., 2019). This is consistent with our observations for 10 and 30 μM treatment. The selectivity of BIBR-1532 was also assessed with various DNA and RNA polymerases, including HIV reverse transcriptase (Damm et al., 2001). Consistent with these observations, BIBR-1532 did not exerted negative effects on influenza RNA expression (Supplementary Figure 4). We have revised on page 21.

3, mRNA–protein discordance(?). Viral mRNAs accumulate while viral proteins drop under hTERT inhibition—opposite to the expectation for a polymerase defect. Measure nascent transcription, mRNA stability, and translation efficiency (polysome profiling) to clarify where the block occurs.

Thank you for your comments. We think these suggestions are interesting; however, we think it is suitable for future works.

Minor Comments

1, Statistics and Replicates – Clearly state the number of biological repeats and the statistical test used beneath each figure panel.

Thank you for your comments. The biological repeats are stated in each Figure Legend located at the end of the manuscript. The statistical test was described throughout the manuscript and described in the experimental procedures section.

2, Nomenclature – Use "hTERT" consistently (currently alternates with "TERT").

Thank you for your suggestions. We have modified to hTERT throughout the manuscript.

3, Discussion – Compare results to reports of TERT RdRp activity in the RMRP pathway and in yeast telomerase complexes; clarify whether those precedents were template dependent or de novo.

Thank you for your comment. Although the present data in our manuscript cannot directly compare to reports of hTERT RdRp activity in the RMRP and in yeast, we have added discussion and speculated a potential mechanism on page 26-27.

Damm, K., Hemmann, U., Garin-Chesa, P., Hauel, N., Kauffmann, I., Priepke, H., Niestroj, C., Daiber, C., Enenkel, B., Guilliard, B., *et al.* (2001). A highly selective telomerase inhibitor limiting human cancer cell proliferation. Embo J *20*, 6958-6968.

DeVincenzo, J.P., McClure, M.W., Symons, J.A., Fathi, H., Westland, C., Chanda, S., Lambkin-Williams, R., Smith, P., Zhang, Q., Beigelman, L., *et al.* (2015). Activity of Oral ALS-008176 in a Respiratory Syncytial Virus Challenge Study. N Engl J Med *373*, 2048-2058.

Ding, X., Cheng, J., Pang, Q., Wei, X., Zhang, X., Wang, P., Yuan, Z., and Qian, D. (2019). BIBR1532, a Selective Telomerase Inhibitor, Enhances Radiosensitivity of Non-Small Cell Lung Cancer Through Increasing Telomere Dysfunction and ATM/CHK1 Inhibition. Int J Radiat Oncol Biol Phys *105*, 861-874.

Fiddler, J.L., and Clarke, S.L. (2021). Evaluation of candidate reference genes for quantitative real-time PCR analysis in a male rat model of dietary iron deficiency. Genes Nutr *16*, 17.

Maida, Y., and Masutomi, K. (2011). RNA-dependent RNA polymerases in RNA silencing. Biol Chem *392*, 299-304.

Maida, Y., Yasukawa, M., Furuuchi, M., Lassmann, T., Possemato, R., Okamoto, N., Kasim, V., Hayashizaki, Y., Hahn, W.C., and Masutomi, K. (2009). An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. Nature *461*, 230-235.

Pascolo, E., Wenz, C., Lingner, J., Hauel, N., Priepke, H., Kauffmann, I., Garin-Chesa, P., Rettig, W.J., Damm, K., and Schnapp, A. (2002). Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. J Biol Chem *277*, 15566-15572.
Sobo, J.M., Alagna, N.S., Sun, S.X., Wilson, K.L., and Reddy, K.L. (2024). Lamins: The backbone of the nucleocytoskeleton interface. Curr Opin Cell Biol *86*, 102313.
Szabo, A., Borkuti, P., Kovacs, Z., Kristo, I., and Vilmos, P. (2025). Recent advances in nuclear actin research. Nucleus *16*, 2498643.
Wek, R.C. (2018). Role of eIF2alpha Kinases in Translational Control and Adaptation to Cellular Stress. Cold Spring Harb Perspect Biol *10*.

Dear Dr. Georgieva,

We are grateful for the reviews and for the consideration of our work for publication in your journal. Below please find a point-by-point response to the critiques of each of the reviewers (our responses below are in *blue italics*).

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors have diligently addressed all the comments from me. Thanks!

- *We appreciate the reviewer's comments.*

Reviewer #3 (Remarks to the Author):

The reviewer highly appreciates the authors' efforts to improve the quality of the manuscript. The revised version has addressed most of the concerns raised previously, yet several critical issues remain open to discussion.
The authors claim that human telomerase reverse transcriptase (hTERT) can function as an RNA-dependent RNA polymerase (RdRp) to support respiratory syncytial virus (RSV) replication. They have verified this hypothesis via hTERT knockdown and the application of hTERT inhibitors, but these experiments only serve as supportive evidence. For a protein to act as an RdRp, its localization to the viral replication complex and physical interaction with viral RNA are indispensable prerequisites. The authors stated that no satisfactory hTERT antibody was available to perform the aforementioned experiments. To resolve this limitation, the reviewer recommends two alternative strategies:
(1) Introducing an epitope tag to the endogenous hTERT protein via the gene-editing knock-in technology;

- *We agreed that it would be ideal to have evidence of hTERT directly interacting with RSV RNA though, as noted in our initial response acknowledged by the reviewer, a satisfactory hTERT antibody is not available, as far as we know. We appreciate the review's suggestion. However, introducing an epitope tag to the endogenous hTERT protein as a potential alternative approach to overcome the limitation of availability of commercial hTERT antibody may modify the native structure or function of endogenous hTERT. This approach would necessitate, among other requirements, that the tagged endogenous hTERT is fully functional and since the potential interactions between hTERT and viral RNA are not yet know, it would be difficult to determine whether a tag is interfering with that activity. We believe that this would be beyond the scope of the current study though would be suitable for future work. Nonetheless, we agreed that our current data serve as supportive evidence. Therefore, we have revised the manuscript title to "Human Telomerase Reverse Transcriptase Supports Respiratory Syncytial Virus Replication", and revised the wording on page 3 (top bullet point) and page 9 (last paragraph header).*

(2) Overexpressing epitope-tagged recombinant hTERT protein in target cells.

- *Please see our rebuttal to the previous point.*

In addition, in Figure 8A, the authors reported the nuclear enrichment of RSV RNA using nuclear-cytoplasmic fractionation coupled with quantitative PCR (qPCR). Given the inherent limitations of this approach, the reviewer suggests validating the subcellular distribution of RSV RNA by RNA fluorescence in situ hybridization (RNA FISH). Notably, RNA exhibits uneven distribution between the nucleus and cytoplasm. Even though RPL19 is a ribosomal RNA, it cannot be used as a reference for uniform nuclear-cytoplasmic distribution. In contrast, RNA FISH enables direct visualization of the subcellular localization of RSV RNA in situ.

- *We agreed that our approach has inherit limitations though we attempted to address these limitations by repeating the experiments at a different time points. The level of RPL19 RNA within each compartment was comparable between 24 hpi (Figure 8A) and 4 hpi (Figure 8B): Ct value of 17.2 (24hpi) vs 17.6 (4hpi) for total lysate, Ct value of 16.4 (24hpi) vs 17 (4hpi) for cytosolic fraction, and Ct value of 19 (24hpi) vs 20 (4hpi) for nuclei fraction. Relative to 24hpi, viral mRNAs were enriched in the nuclei at 4hpi (Figure 8B). We believe that these data support our findings of the distribution of viral RNA during infection.*

Minor issues requiring correction are listed below:
(1) Page 7: The phrase "These observations suggest RSV viral replication" should be revised to "viral protein synthesis".

- *We have modified the wording as suggested (see page 7, top).*

(2) Pages 12 and 13: The authors stated "These results suggested that viral proteins N and P could redundantly interfere the regulations of eIF2α by eIF2α kinases during RSV infections through physical interactions." However, the detected protein-protein interactions alone are insufficient to demonstrate that the N and P proteins block both the activation of eIF2α kinases and the subsequent phosphorylation of eIF2α.

- *We agreed that protein-protein interactions alone are not sufficient to demonstrate that the N and P proteins block both the activation of eIF2α kinases and the subsequent phosphorylation of eIF2α. The regulation of eIF2α was also suggested by contribution of phosphatase PP2A (see Groskreutz et al., 2010 cited and below). We have revised the manuscript to highlight this point. (page 13, top).*
- *Groskreutz, D.J., Babor, E.C., Monick, M.M., Varga, S.M., and Hunninghake, G.W. (2010). Respiratory syncytial virus limits alpha subunit of eukaryotic translation initiation factor 2 (eIF2alpha) phosphorylation to maintain translation and viral replication. J Biol Chem 285, 24023-24031.*

Reviewer #4 (Remarks to the Author):

This revised submission has successfully addressed the concerns raised in the previous review.

- *We appreciate the reviewer's comments.*

In summary, we believe that we have adequately addressed the critiques of the reviewer and appreciate your consideration of our manuscript.

Hoang Huynh