Referee #1

Assembly and release of infectious hepatitis C virus involving an unusual organization of the secretory pathway (Triyatni M et al)

This manuscript builds on a previously published work by the authors (PLoS Path 2011) on a model to produce infectious HCV in an attempt to understand the mechanisms of the process. However, it is still important to be elaborative in the following sections:

1- The introduction does not offer enough background of the topic but rather lists the results of the previous manuscript on the new model for production of infectious HCV without the replication requirement

2- The introduction should be clearer about the new component of the model and what is it adding to the background paper (PLoS Path, 2011).

3- The methods require reshaping and major rewriting. For example:

a- The authors start by A and B under cell cultures as well as later in the methods when describing the Effect of RAB1: delete this formatting and start by introductory statements.

b- Add the country for the companies from where the reagents and products are purchased

c- Define the abbreviations before using acronyms (across the whole manuscript)

d- The authors are urged not to refer to Figures in the methods. The results are the platform for figures unless a mechanistic flow or chart is referred to (and this is not the case here).

e- The authors are urged to add introductory statement when describing the methods (this applies for all sections).

f- To add the company and country for the RT-TaqMan PCR

4- Results :

a- Figure 2 seems to the reviewer a slightly different version of Figure 2 of Reference 8. The description of the results on pages 13 and 14 confirms this whereby the authors refer to this part as if it is a duplication of the results. The reviewer suggests rewriting this part.

b- The results are not clear and require major revisions. The reviewer suggests explaining the purpose of each part of the results before launching into listing the former.

c- The authors do not refer to how representative are the experiments (How many times these were reproduced?) significance? Even though the figures are well done.

d- The authors suggest certain mechanisms without an in-depth analysis of the results

5- Discussion:

a- The reviewer was not able to discern any link between the results and the discussion. The reviewer suggests rewriting this part with a sequential order of a discussion and an interpretation of the results. It is also critical to link the findings of this paper to the current literature. The write-up as such does not clearly interpret the results.

b- Delete data not shown from the discussion. If data not shown, it is preferable not to interpret it in the discussion

c- Add a reference for the last paragraph on page 24. Why this paragraph was added when the authors did not even test the impact of cytokines. The authors could link this to recommendations/suggestion for the field.

d- What are the limitations of this study? And how do they stand in the field of HCV replication? How would this impact treatment (which is what the authors tried to suggest in the introduc tion and never discussed the latter again?

e- The reviewer suggests to refer to the new references in the field

We thank the referee for his/her comments. Our point-by-point reply is detailed below and/or directly in the manuscript:

Points 1 & 2: Sentences have been added to contextualize the introduction.
Points 3a-f: We modified the Materials and Methods and hope it clarifies the issues raised by the referee.
Point 4a: We modified the text describing this result to eliminate any confusion. Together with the IF result, Figure 2 shows that mitochondria are surrounding the assembly compartment but are not taking any direct part in it, which had not previously been documented. Obviously, the other components of the assembly compartment did not differ from what had already been reported.
Point 4b: We modified the manuscript to clarify as many issues as possible.
Point 4c: All experiments were repeated at least once, but the shown results are representative of three or more experiments. A dozen laser-scanning confocal microscopy cell stacks were analyzed for each IF condition.
Point 4d: We are not sure what mechanism(s) the referee is referring to (cf. our reply to point 4b).
Point 5a: The production of infectious HCV particles by Huh-7.5 cells is believed to involve a classical secretion pathway; it has also been reported to involve lipoproteins and exosomes. Secretion of HCV particles by BHK-WNV cells neither involved lipoproteins nor exosomes. The question, therefore, was: could it still follow a conventional pathway? Our results show that secretion of HCV particles went through a re-organized and re-wired pathway bypassing the conventional ERGIC and involving components of the inflammasome.
Such unusual secretion route of HCV could be coincidental in BHK-WNV cells. However, lipoprotein production (involving MAMs) and exosome secretion (implicated in intercellular transmission between hepatocytes) have also been linked to the cellular immuno-inflammatory response. In addition, with time HCV-infected Huh-7.5 cells tended to develop a cytoplasmic compartment resembling that observed in BHK-WNV cells. Therefore, the HCV production made possible by the prior replication of WNV in BHK cells could be somewhat related to that observed in hepatocytes.

It remains to be determined whether it reflects a more complex than anticipated organization of HCV production in hepatocytes or the existence of extra route(s) of HCV secretion.

Point 5b: Data previously stated as "not shown" are now available in Supplementary data.

Point 5c: We added a reference.

Point 5d: A paragraph has been added at the end of the discussion. In the introduction, we also raised questions regarding aspects other than treatment.

Point 5e: The median year of publication is 2009 with a first quartile within 2016-2014. We cited several original publications instead of more recent works/reviews deriving from them. Fifty years after the identification of non-A, non-B hepatitis, we are still in need of a vaccine to protect against HCV infections.

Referee #2

Comments to authors,

Scientific question proposed in the manuscript has been clearly presented in the introduction section, Methods and techniques have been clearly presented in the methods section and the manuscript provides adequate details of methods. The source of the presented data is reliable and the information in the results section indicates the academic significance of the main findings. Discussion section is satisfactory to answer the questions about whether the results response the proposed scientific question, achieved the aim of the study, or confirmed or rejected the hypothesis proposed in the manuscript. Conclusions of the manuscript have been clearly presented in the conclusion section. The manuscript cites all important, relevant and timely references. A good study to open up new possibilities for studying the assembly of native HCV virions.

We thank the referee for his/her nice comments.

Referee #3

Assembly and release of infectious hepatitis C virus involving an unusual organization of the secretory pathway. (Triyatni M et al)
ESPS Manuscript NO: 24677

In this manuscript, the authors aimed to examine whether the cellular factors including Calnexin, RAB1 and alpha-tubulin were involved in the production of HCV particles by BHK-WNV cells.
Moreover, it brings an interesting contribution to the current literature.
This manuscript is although very good but still require some minor changes, which are as follows.
Comments;
1. This study is designed as a continuation of the previous study (reference 8) of the authors. However, introduction of present manuscript is filled with the results of the previous study. The reviewer suggests minor rewriting for this part.
2. The explanation of abbreviations should be provided.
3. The country of the companies from where the reagents and products are purchased should be added.
4. It is not proper to interpret the data which are not shown ('not shown' repeated 6 times in the manuscript). If data was available, authors could add these data as supplementary material.
5. The authors should mention the limitations of their study.
6. The potential effects of the study results on knowledge of the pathogenesis of hepatitis C, current literature, and maybe current clinical applications or medication could be mentioned in discussion section.

We thank the referee for his/her nice comments:

Point 1: Sentences have been added to contextualize the introduction.
Points 2 & 3: We modified the text accordingly.
Point 4: Data stated as "not shown" are now available in Supplementary data.
Point 5: A paragraph has been added at the end of the discussion.
Point 6: Potential applications of structural and immunological studies could, of course, include the development of a vaccine; unfortunately, such possibility may still be remote.