RESPONSES TO REVIEWERS

I thank all four reviewers for their favorable comments and constructive suggestions towards my manuscript. Below are the point-by-point responses to issues raised by the reviewers.

Reviewer #1 (Reviewer's code: 02446947)

Major corrections:

1) ICP0 has been detected in the tegument of virions, and may be recruited (along with ICP4) initially to intranuclear capsids (via the E3 ubiquitin ligase domain) as well as in the cytoplasm to capsids during HSV-1 assembly, and is therefore part of incoming viral proteins delivered during entry (studies by the labs of Lippe and Nicola). This should be mentioned along with the possible role (eg targeting capsids to the nucleus during entry, Delboy and Nicloa 2011 J Virol) of incoming ICP0 prior to de novo synthesis.

A paragraph has been added in *Section 2* of "ICP0 protein: Domains and functions", highlighted in yellow. In this paragraph, the incorporation of ICP0 is discussed as a part of potential functions of the nuclear-to-cytoplasmic translocation of ICP0.

2) A comment on ICP0 homologues found in other Herpesviridae members would be helpful

Although ICP0 orthologues from other *Herpesviridae* members play somewhat similar roles in counteractions against host defenses, their biochemical properties are not necessary the same. For example, the RING domain of VZV ORF61 does not work as an E3 and does not degrade PML, but the ORF61 protein can partially rescue ICP0 activity. In CMV, the function of ND10 dispersal is fulfilled by two proteins, IE1 and pp71. Therefore, in order to focus on the in-depth discussion of ICP0 domains and functions, the ICP0 orthologues are not included to avoid distraction and confusion.

3) Need to incorporate recent publications on ICP0 listed below into the review Pozhidaeva AK, Mohni KN, Dhe-Paganon S, Arrowsmith CH, Weller SK, Korzhnev DM, Bezsonova I. Structural Characterization of Interaction between Human Ubiquitin Specific Protease 7 and Immediate Early Protein ICP0 of Herpes Simplex Virus-1. J Biol Chem. 2015 Jul 29. pii: jbc.M115.664805. [Epub ahead of print] Smith S, Weller SK. HSV-I and the cellular DNA damage response. Future Virol. 2015 Apr;10(4):383-397. Sloan E, Tatham MH, Groslambert M, Glass M, Orr A, Hay RT, Everett RD. Analysis of the SUMO2 Proteome during HSV-1 Infection. PLoS Pathog. 2015 Jul 22;11(7):e1005059. Taylor KE, Mossman KL. Cellular Protein WDR11 Interacts with Specific Herpes Simplex Virus Proteins at the trans-Golgi Network To Promote Virus Replication. J Virol. 2015 Oct 1;89(19):9841-52.

The paper of Pozhidaeva et al. has been added on Page 12, highlighted in yellow.

The paper of Smith et al. is a review focusing on HSV-1 & DNA damage responses. Although some of the original studies have been discussed in the present review and the relevant original

papers are included in the references, the review paper of Smith et al is not included in the references.

The paper of Sloan et al. focuses on identifying cellular proteins modified by SUMO-2. Although this is an important paper helping to put the cellular/viral protein-interaction network together, the SUMO-2 proteome has not provided direct relevance to ICP0 functions. Therefore it is not included as a reference of the present review.

The paper of Taylor et al. has identified a new interaction to ICP0. It is now discussed in *Section 6.4*, highlighted in yellow.

4) For the ICP0 interactions listed were they confirmed by more than one assay and if so this should be highlighted in the text.

Most of the ICP0 interactions were identified by one method and they are likely transient and weak interactions. The only confirmed strong binding of ICP0 is to the USP7 protein, which has been discussed in extensive details in *Section 5.3*.

5) In figure 1 need to illustrate that there are actually 2 copies of the ICP0 gene in the HSV genome

Corrected. See the new Figure 1.

6) It would be very desirable to have an additional figure summarizing the interaction partners of ICP0, where they bind ICPO, and the function of the interaction in the context of viral replication.

Table 1 is inserted.

Minor corrections:

(relevant section names in italics): Done

1) Abstract "gene product" to "gene products" "the HSV-1 pathogenicity" to "HSV-1 pathogenicity"

Corrected.

2) Introduction "opportunistic pathogen" to "opportunistic pathogens" clarify the term "unusual shift of ICP0" "a yeast-2-hybrid screenings" to "yeast 2-hybrid screenings" "to coordination" to "coordination"

Corrected.

3) ICP0 gene structure when referring to “ICP0 gene” in this section change to “the ICPO gene” “to latency-associated” to “the latency-associated”

Corrected.

4) 1. RING finger domain and E3 ubiquitin ligase activity Clarify the statement: “The structure of ICP0 RING finger has been demonstrated by nuclear magnetic resonance (NMR) (34), but the crystal structure has not yet been solved.” Is there an issue with the NMR structure that there is an absolute need for a crystal structure? “for at least 1000 folds” to “at least 1000 fold” “over viral outcome” to “overall viral outcome”

In the sentence “The structure of ICP0 RING finger has been demonstrated by nuclear magnetic resonance (NMR) (34), but the crystal structure has not yet been solved” the second half is now taken out.
Typos are corrected.

5) 2. Nuclear localization domain and ICP0 nuclear/cytoplasmic translocation “presence of NLS. Once inside nucleus” to “presence of the NLS. Once inside the nucleus” “see section for” to “see section on” Clarify if there is an NES motif in ICP0?

Corrected.
Added: “So far, a functional NES has not been identified.”

6) 3. Proline-rich region and ND10-fusion “proline-rich region” to “proline-rich regions” “ND10-fucion” to “ND10-fusion” “with SH3” to “with an SH3” “indicate the importance of ND10-fusion” to “indicates the importance of the ND10-fusion”

Corrected.

7) 4. SUMO interaction motif and ICP0 substrate recognition “SUMO moiety” to “The SUMO moiety” “SUMO-interaction” to “the SUMO-interaction” “contain SIM” to “contain a SIM” “scattering throughout ICPO” to “scattered throughout the ICP0” “ICP0 ability” to “the ability of ICP0” “PML (108) suggesting” to “PML (108). This suggests”

Corrected.

8) 5. ICP0 C-terminus and a diverse array of functions “The C-terminus of ICP0, br

Corrected.

Reviewer #2 (Reviewer’s code: 00681914)

The reviewer would recommend to add a Table listing the important facts in a simple overview (Domain Localization (aa.) Activity Function)

Table 1 is inserted.

the specific features of latency established in semipermissive neurons are not mentioned. The HSV DNA in them may go dormant from the very beginning in the absence of any virus replication. What is the precise role of ICP0, in contrast to ICP4, in neuronal latency? According to reviewer`s experienece, ICP4 mRNA but not ICP0 mRNA could be found in non-cultured ganglia, i.e. prior to reactivation. ICP0 gene transcription is an indicator of the onset of reactivation (see Rezuchová I et al., in Intervirology 2003, 46: 25-34).

The requirement of ICP0 in latency reactivation has been discovered for 25 years. However the mechanism of how ICP0 functions to reactivate the latently infected HSV is not yet clear. In my opinion, a careful dissection of ICP0 functional domains and the delineation of domain coordination is essential for understanding the role of ICP0 in latent infection. I have pointed out the importance of ICP0 in latency reaction on Pages 5 and 16, highlighted in yellow, but detailed functions of ICP0 domains in latency reactivation are currently unavailable.

Reviewer #3 (Reviewer's code: 02773782)

I think the author did collect and read a huge number of recent references and also try to present a significant review in this filed. However, it is better to avoid a literature review to be an annotated bibliography in which you summarize briefly each article, and the readers could not find the key point and focus of the article. Since ICP0 is a multifunctional protein, it is hard to include all aspects in one manuscript. I would suggest the author could consider reorganizing the manuscript, narrowing the scope and selecting one or two aspects as a focus to expound. Meanwhile, ICP0 has an important role in latent infection establishment and recurrent infection. But in this review, the author only described the role of ICP0 in the acute phase of infection, and did not mention the function in the latency.

The key point of the present review is to come up with a detailed outline of the known ICP0 properties. Through dissecting the important ICP0 functional domains, I hope to draw a roadmap toward deciphering the complex functionality of this protein. To select one or two aspects as suggested by Reviewer #3 will defeat the purpose of the present review.

As mentioned above in responses to Reviewer #2, the importance of ICP0 in latency reactivation has been pointed out on Pages 5 and 16, highlighted in yellow, but detailed functions of ICP0 domain in latency reactivation are currently unavailable.

In addition, charts and diagrams are recommended to use to help readers understand more clearly.

Table 1 is inserted.

Reviewer #4 (Reviewer's code: 00504063)

Information on the transcriptional, post-transcriptional regulation of ICP0 could be added to the manuscript to make a more complete delineation.

This review focuses on the functional domains of ICP0. Although a potential alternative splicing and the microRNA regulation are briefly mentioned on Pages 6 and 17, they are discussed in the context of ICP0 protein functions. The transcriptional regulation of ICP0 is regulated by multiple viral proteins such as VP16 and ICP4, and itself can become an entire topic for an independent review. Therefore, that is beyond the scope of the present review and has been left out to avoid distraction.