Title: HPV E6 and E7 oncoproteins cooperatively alter the expression of Disc Large 1 polarity protein in epithelial cells.

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“HPV E6 and E7 oncoproteins cooperatively alter the expression of Disc Large 1 polarity protein in epithelial cells”, by María Paula Dizanzo; Federico Marziali; Clarisse Brunet Avalos; Marina Bugnon Valdano Santiago Leiva; Ana Laura Cavatorta; Daniela Gardiol.

Dr. Erica Golemis
BMC Cancer Editorial Office

Dear Dr. Erica Golemis:

We are submitting the revised version of our manuscript entitled “HPV E6 and E7 oncoproteins cooperatively alter the expression of Disc Large 1 polarity protein in epithelial cells” article by Dizanzo, Marziali et al.

We have addressed the specific comments of the Editor and Reviewer 1, indicating the changes that have been made in this response letter and the manuscript section where the changes can be
viewed. The respective lane numbers specified below correspond to the revised Word version uploaded online.

Please note that this new submission also includes new supplementary figures.

Please also note that the Reference checking list included two not validated and one not checked references. We have revised this and the References were amended in the new manuscript version.

We are grateful to the reviewers for their helpful comments, which we feel have helped us to improve the quality of the manuscript, and we hope that it is now suitable for publication.

Editor Comments

1 –The manuscript was uploaded in the Cell and molecular biology section.

2--Please find attach the original uncropped blots regarding the different figures uploaded as Supplementary figures, as required. The relevant bands shown in the figures are indicated by a dashed line rectangle, representing the cropped areas. They were labeled as in the main text. In the figure legends is now stated that “full-long blots are presented in the Supplementary figure X”, as corresponds. Please note that only in figure 1 the results were captured using Rx films, meanwhile in the other Figures the Chemiluminescent reaction was acquired with a GE Amersham Imager 600 equipment. This is now clarified in Methods Section (lanes 178-9).

3- The cell catalogue numbers and the mycoplasma contamination test are indicated in the manuscript (Methods, lanes 143-147).

HEK293 cells are from the laboratory stock and A549 cells are a gift from the cell bank of the Instituto Nacional de Enfermedades Virales Humanas- Dr. Julio Maiztegui (Pergamino, Argentina, Cell culture section).

4- None of the cell lines used need ethics approval. This was included in Ethics approval and consent to participate section in the Declarations

5- Funding section. It was clarified that the grant was only for reagent purchasing

6- I have the permission of the acknowledged people: Dolores Campos and Rodrigo Vena.

Reviewer 1 comments
1- Figure 1. The results shown in Fig 1 A for the E6:DLG1 5:1 transfection ratio is representative of several independent experiments. As suggested by the reviewer we included the densitometry analysis for this condition as well. It can be observed the statically significant decrease of DLG1 abundance in the presence of high E6 levels (E6:DLG1 5:1). This analysis is described in the Legend to Figure 1, Page 20, Lanes 630-633.

2- Figure 2.

As suggested by the Reviewer it was included the label for the PCC chart’s x axis.

Figure 2 C represent results using the same transfection conditions. The quantification implies the analysis of a total of 90 micrographs corresponding to four independent experiments. This is described now in the legend to figure 2 C (Page 31, Ln 654-6).

3- As suggested, the right table describing the sequence of E6.18 mutant was omitted in the new version and this is now mentioned in the text (Methods Page 7, lane 150 and Results page 13, lanes 275).

4- As suggested, the PCC quantification results are shown for most of the immunofluorescence experiments to clarify the protein co-localization degree (Fig. 3 and Fig. 4A; Legend to Figure 3, page 32, lanes 670-1 and Legend to figure 4 page 31 lanes 683-5).

5- As suggested by the reviewer we included lung epithelial A549 cells as a new cell system beside the HEK293 cell line for the assays shown in Fig. 1. This new result is shown in Fig. S1, and described in the text (Pg. 12, Ln 247-249). This finding indicates that the effects of the E6 protein over DLG1 expression levels are independent of the epithelial cell line used in the experiment. These data strongly support the previously reported E618-mediated DLG1 degradation, and further demonstrate that this effect largely relies on high levels of E618 (Fig.S1). Altogether this new cell model demonstrates the consistency of our results.

Regarding the results shown in figure 5, and as previously described, the achievement of triple transfection in cell lines other than HEK293 is very difficult. However, we could show in figure 4B the effects of E6 and E7 on DLG1 expression by immunofluorescence methodology, since it is possible to identify the few triple transfected A549 cells with this technique. Nevertheless, with the experimental approach used for the experiments described in Figure 5, where whole cell lysates are used in the WB experiments, is very difficult to demonstrate the changes in DLG1 levels due to the few triple transfected cells.

Reviewer 2

No suggestions were received as this reviewer accepted the manuscript.
We declare that the manuscript is not under consideration for publication elsewhere and that its publication in the present form has been approved by all authors.

We look forward to hearing from you. Thank you very much in advanced.

Best regards,

Dr. Daniela Gardiol

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