

July 6, 2024

Dear Editor and Reviewers,

We would like to submit the revised version of the manuscript entitled “**Immune Responses Induced by a Recombinant *Lactiplantibacillus plantarum* Surface-Displaying the gD Protein of Pseudorabies Virus**” (viruses-3035916) for your consideration of publication in *Viruses*.

The authors would like to thank all the reviewers and editors at *viruses* for carefully evaluating our manuscript and for all the helpful comments and suggestions for improvement. We have carefully addressed these concerns and properly revised the manuscript. We hope these changes and our response to the reviewers’ comments are satisfactory. For clarity, all the revisions are highlighted in blue in the revised manuscript. The point-by-point responses to the reviewers and a marked-up version of the manuscript have been provided as separate files for your reference.

We are looking forward to hearing from you soon.

Sincerely,

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Responses to Reviewer #2:

Introduction

1. “US6 antigen of PRV encoding gD protein” US6 is the name of gene. The description is unclear.

Answer: *US6* is the gene encoding the gD protein. The statement has been updated in Line 103 in the revised manuscript.

“This study is aimed at developing a recombinant NC8 (rNC8) for surface-expression of the gD antigen of PRV virus encoded by the *US6* gene”.

2. A dendritic cell-targeting peptide (DCpep) were introduced into the pMG36e vector for the I.V. delivery system. The description is appropriate.

Answer: The statement has been updated according to the suggestion in Lines 105-106 as “To enhance the bioavailability of the targeted immunogen, LPxTG surface-anchoring protein along with dendritic cell-targeting peptide (DCpep) (AYYKTASLAPAE) were cloned into the pMG36e vector to target the dendritic cells (DCs)”.

Materials and Methods

1. Freshly extracted T cells from mice spleens is repeated description in the 2.9.

Answer: We have deleted the repeated description in section 2.1 and modified in section 2.9 as “the freshly extracted T cells from mice spleens were harvested from immunized mice groups”.

2. days post-immunization (dpi) is repeated description.

Answer: The repeated statement has been deleted (Lines 213-216).

3. Detailed information of DCs should be supplied in the Materials and methods.

Answer: The mice DCs used for this study has been updated in the Materials and Methods Section 2.1 in Lines 116-118 as “The JAWS II cells (ATCC CRL-33612) are passaged cell line of mice DCs, it was cultured in MEM α (catalog no. 12571063;

ThermoFisher Scientific, China) with 25% FBS and 5 ng/mL GM-CSF at 37°C”.

4. The primers for EGFP fused with LPxTG and pMG36e-EGFP may be different. The primers for pMG36e-EGFP are not shown in the Table 1.

Answer: The primers for pMG36e-LPxTG-EGFP and pMG36e-EGFP have been updated in Table 1.

5. “The PRV antigen fragment was ligated by digesting the pMG36e-LP3065 vector with *Xho*I and *Bam*HI restriction sites at 37°C with homologous recombination enzyme.” PRV antigen fragment is not a gene. It cannot be ligated with a vector. Moreover, the vector is digested by homologous recombination enzyme?

Answer: The statement has been updated as “The pMG36e vector was digested at *Xho*I and *Bam*HI restriction sites, and US6 gene fragment was ligated with digested vector using homologous recombination enzyme” in (Lines146-148).

6. 2.4 is not a method.

Answer: We moved Section 2.4 into the figure legends of Figure 1b and added the flow cytometry protocols.

7. “Washed the pallet twice with phosphate-buffered saline (PBS) and 4% paraformaldehyde as tissue fixative was added and put at rotatory stirrer for 30 min”
The description is ambiguous.

Answer: The statement has been rephrased in Lines 166-167 as “The pallet was washed twice with phosphate-buffered saline (PBS) and fixed using 4% paraformaldehyde for 30 min at room temperature”.

8. Move primer sequences for qPCR into Table 1.

Answer: The primer sequences for qPCR have been listed in Table 1.

Results

1. Figure 1 c “Wester blotting” word error.

Answer: Corrected.

2. The experimental goals and conclusions are not consistent.

Answer: The conclusion section has been improved as per suggestions.

3. Figure.3A is not described in the Result.

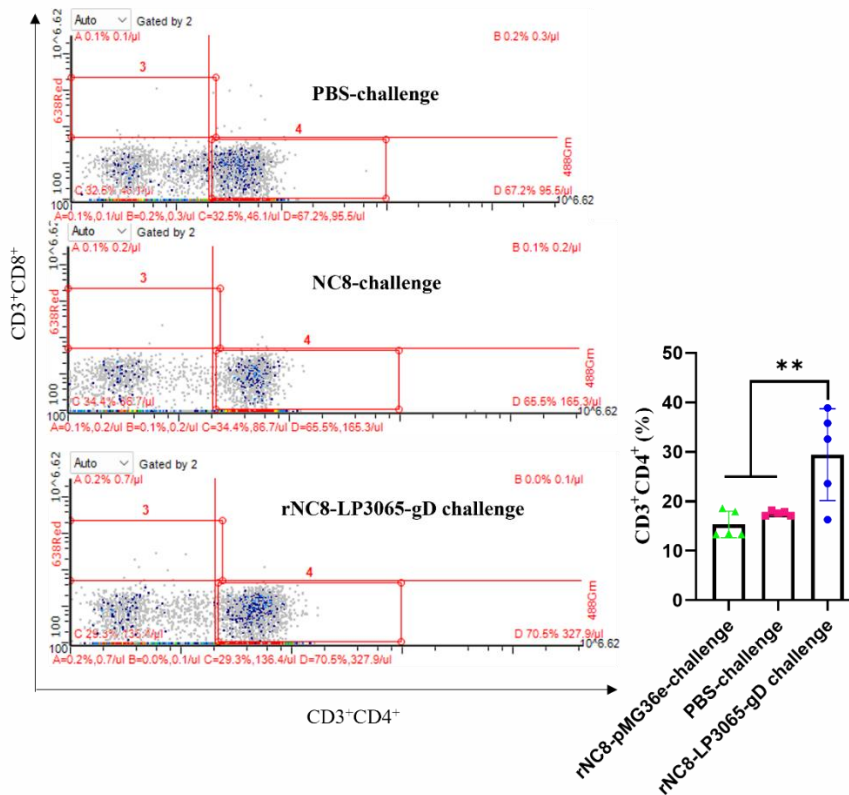
Answer: Figure 3a has been highlighted (Line 343).

4. The evaluation of immune effect of commercialized PRV vaccine by intravenous injection should be supplied.

Answer: In the previous study, the same experiments of administration of inactivated PRV vaccine with a probiotic strain intravenously have been conducted. Our results are consistent with the previous findings by protecting the mice from PRV lethal challenge and increasing the survival rate up to 80%. The related study reference has been included in the Discussion section in (Lines 536-537).

5. Figure 4B is not clear.

Answer: The figure has been updated and resized according to suggestions.



6. The time of figure 5B and 5C mismatch Figure 5A.

Answer: [Timeline for Figures 5b and c have been adjusted according to suggestions.](#)

7. Recommend moving Figure 5A to position Figure 4A.

Answer: [The figure has been relocated to 4A.](#)

Discussion

The discussion section only discussed the results of this article, without comparing or discussing it with other studies.

Answer: [In the Discussion, the results has been modified and added in Lines 518-522, 527-531, and 536-537.](#)