DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY

UNIVERSITY of ROCHESTER MEDICAL CENTER

MEDICINE of THE HIGHEST ORDER

February 20th, 2019

Dear Editor(s),

We have submitted our manuscript **"A luciferase-fluorescent reporter influenza virus for live imaging and quantification of viral infection"** by Chiem et al., which we would like to be considered for publication at Journal of Visualized Experiments (JoVE).

Influenza cause yearly epidemics that severely burdens the global healthcare system, leading to over 500,000 deaths worldwide. Therefore, it is crucial to understand the mechanism of influenza viral infection and hastily determine the pathogenicity and transmissibility of new viral isolates. In the last few decades, researchers have been using replication-competent reporter-expressing influenza viruses to track the presence of the virus in infected cells and/or animal models of infection, which have expanded the knowledge in the influenza field. However, these viruses have traditionally expressed a single reporter gene, either a fluorescent or a bioluminescent protein. Fluorescent proteins are better suited to observe viral dynamics and localization at the cellular level, while luciferase proteins are more robust than fluorescent proteins for *in vivo* studies such as live imaging in animals. As a consequence, recombinant influenza viruses expressing only a single reporter gene are limited in their practicality and convenience depending on the chosen experimental approach.

We nullified this limitation through the generation of a novel recombinant replication-competent bi-reporter influenza virus (BIRFLU). BIRFLU was constructed by introducing two reporter genes, NanoLuc luciferase (NLuc) and the fluorescent Venus, into the hemagglutinin (HA) and non-structural (NS) viral segments, respectively, of A/Puerto Rico/08/1934 H1N1 (PR8). This novel recombinant virus is able to exploit the benefits of both fluorescence and bioluminescence signals and is applicable to a wider range of experimental approaches. *In vitro*, we found BIRFLU to be stable and with comparable fitness to the wild-type PR8 virus. *In vivo,* BIRFLU dissemination could be directly visualized and quantified in same live animals through time, or *ex vivo* in lungs from infected animals, using an *in vivo* imaging system (IVIS). Importantly, BIRFLU was also stable *in vivo* and fluorescent and bioluminescent reporter signals were comparable and correlated with viral titers in the lungs of infected animals. Ultimately, the dual reporter approach will allow researchers to use BIRFLU to study influenza and to facilitate the development of novel therapeutic approaches.

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Because of their expertise and knowledge on influenza virus biology, we would like to suggest the following reviewers to evaluate our manuscript:

- Dr. Munir Iqbal, The Pirbright Institute: munir.iqbal@pirbright.ac.uk;
- Dr. Anice Lowen, Emory University School of Medicine: anice.lowen@emory.edu; and,
- Dr. Richard Plemper, Georgia State University: rplemper@gsu.edu.

Thank you for your time and consideration.

I look forward to hearing from you.

Sincerely,

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