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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Aleksandra Strotskaya

PhD Program: Life Sciences

Title of Thesis: Effects of Targeting by the *Esherichia coli* I-E CRISPR-Cas System on Infection by Various Phages.

Supervisor: Professor Konstantin Severinov

Chair of PhD defense Jury: Professor Yuri Kotelevtsev ***Email:*** ***y.kotelevtsev@skoltech.ru***

Date of Thesis Defense: October 24, 2017

Name of Reviewer: Asst. Prof. Dmitriy Papatsenko, CDIBB

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** Papatsenko **Date: DD-MM-YYYY**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to forward a completed copy of this report to the Chair of the Jury at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevancy of the topic of dissertation work to its actual content
- The relevancy of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications
- The summary of issues to be addressed before/during the thesis defense

Thesis work by Strotskaya A. is devoted to investigation of the effects of type I-E CRISPR-Cas system of model organism Escherichia coli on the infection by diverse bacteriophages - M13, λ, T5, T7, T4 and R1-37. So far, there has been no systematic study of how a bacterium with CRISPR-Cas system can prevent infection by phages with different attack strategies.

Currently, CRISPR-Cas systems are in the focus of intensive investigation, great hopes are associated with CRISPR-Cas genome targeting and genome editing tools. Many research techniques aimed at genome screening or lineage tracing may also utilize CRISPR-Cas systems. Since there are many such systems are already present in bacteria, it is essential to explore this great natural diversity of molecular mechanisms and their responses to different phages. Clearly, some of the identified mechanisms can provide new insights on how the bacteria can fight the phage infections and help to improve the existing or introduce new CRISPR-Cas based tools and applications.

For the described reasons, the proposed direction of this thesis work and the major goals appear to be on the bleeding edge of current research and technology in biological sciences.

Major findings of this thesis work include discovery of distinct consequences of infection in the presence of active CRISPR-Cas system, observed for cells infected with different phages. Based on the data, it has been proposed that type I-E CRISPR-Cas systems acts not as a true immune systems, but rather cause altruistic death of cells infected with lytic viruses. In many cases, however, such cells release little or no phage progeny, so the infected cultures continue to grow and the infection process dies off unless escaper phage mutants emerge. So it appears that in many of the cases analyzed in the dissertation CRISPR immunity functions similarly to abortive infection mechanisms, not curing individual infected cells but preventing the spread of the virus through the population. These are very interesting findings, which may open new directions in the investigation of alternative strategies of immunity responses in general and diversity of CRISPR-Cas systems in particular.

The dissertation manuscript is very well written and contains substantial backgrounds and clear well-though diagrams and images illustrating major scientific findings. Very deep analysis of the existing literature has been performed and relevant conclusions have been derived from the results. The amount of the work, quality of the preformed research and quality of publications, along with significance of the selected research direction fully justify this work as a completed PhD thesis project.

Few minor wishes (which may be addressed at the time of defense) from the side of the reviewer: (a) There is a section entitled "novelty and practical application", while the novelty is described there, there is nothing written about technology etc. The author would probably need to think about possible practical applications, which may emerge from this study and discuss them at the time of dissertation defense. (b) The conclusions are clear, but it may be better to reduce this section a bit, since in its current format the conclusions are too long and resemble second discussion. Simply it is hard to "fish out" there the major, most essential findings. I assume that a corresponding slide may be prepared at the time of defense.

Provisional Recommendation

☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defence*

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defence only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defence*