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# Jury Member Report – Doctor of Philosophy thesis.

**Name of Candidate:** Evgeniia Shcherbinina

**PhD Program**: Life Sciences

**Title of Thesis:** Role of lncRNA LL35 in hepatocytes function

**Supervisor:** Dr. Timofei Zatsepin, Velocity Global Rus

**Name of the Reviewer:**

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|---|---|
| I confirm the absence of any conflict of interest | **Date:** 11-08-2022 |

*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 submit signed copy of the report 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 relevance of the topic of dissertation work to its actual content
- The relevance 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

Long non-coding RNAs (lncRNAs) are key regulators of gene expression for all main cellular pathways. The present work by Evgeniia Shcherbinina is focused on LL35 murine lncRNA. LL35 is a functional analogue of human DEANR1, which is known to be involved in upregulation of glycolysis and, thus, cell proliferation in pancreatic cancer cells. Thus, investigation of LL35 in mice models is important not only for basic science, but also for better understanding of functions of the human health-relevant DEANR1.

To study LL35 functioning, the author developed model systems based on antisense nucleotide-based depletion of LL35 in hepatocytes in vitro and in mice in vivo. LL35 knockdown was found to result in physiological changes of hepatocytes in vitro such as decreased cells proliferation and cell cycle arrest in S phase as well as decreased cell migration ability. Author analyzed changes at proteome and transcriptome levels. Moreover, these studies were complemented with lipidome and metabolome analyses. Combination of these approaches demonstrated that LL35 regulates lipid and glucose metabolism. In addition, Evgeniia showed that LL35 participates in the regulation of main cellular signaling cascades such as Notch and NF-κB pathways. Author concluded that LL35 expresses under normal conditions and supports survival and migration of hepatocytes.

The thesis is well prepared. In particular, it contains a deep literature review on mechanisms of action of lncRNA (about 70 pages, 24 illustrations, ~200 references). The only minor disadvantage is the all Figures are adapted from the published papers (are permissions required?), with no original drawing by the author. Methods and Results are clearly written and illustrated.

Evgeniia is a coauthor of four papers. The main part of the work has just been published in the Biomedicines journal (IF 4.8) with the first authorship of Evgeniia Shcherbinina. In addition, Evgeniia participated in publications in the International Journal of Molecular Sciences (IF 6.2), Journal of Cell Biology (IF 10.5), and Biochimie (IF 4.1). Thus, the requirements of Skoltech in publications for PhD defense are fully satisfied.

Minor points:

1. Page 95: “Libraries were sequenced by HiSeq4000 (Illumina, San Diego, USA) instrument in 5 nt single-read mode”. What does "5 nt" mean? Were only 5 nucleotides sequenced?
2. Materials and Methods: Company name, city, state and country is mentioned every time. This clutters up the text; for example see page 101, where “Avanti Lipids, Birmingham, AL, USA” repeats 10 times in one sentence. It is generally accepted to note city, state and country of a company for the first time only.
3. Page 113: “Center for Molecular and Cell Biology”. It should be Center for Molecular and Cellular Biology. “Institute of Biomedica Chemistry” -> Institute of Biomedical Chemistry.
4. In Fig. 29B,C, Fig. 31 and Suppl. Fig. S3 bars have different shades of gray. Is it meaningful?
5. Page 131: “mean fluorescent intensity for LL35 ASO = 104.9 ±26.1 pixels, mean for Luc ASO = 76.0 ±20.1 pixels”. Usually, fluorescence intensity in images are not measured in pixels.
6. Formatting of Supplementary Figures and their legends could be better if the legends are placed on the same page below the image.

| **Provisional Recommendation** |
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| ☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense* |
| ☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense 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 defense* |