Skolkovo Institute of Science and Technology

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: Yuri Kotelevtsev

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**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 dissertation is well written in canonical way. It contains 202 pages, 39 Figures of which 13 expanded figures present major results of the study and 5 Supplementary figures. The literature review is well written and sets the state of the art of the functional role of lncRNA LL35, and of its human functional homolog DEANR1. The review logically leads to the aims and goals of the experimental study. The Materials and Methods section is written clearly and allows thorough reproduction of the results. Results are well presented and appropriately discussed. Conclusions are supported by the results

- The relevance of the topic of dissertation work to its actual content

  The content of the dissertation covers the investigation of functional role of murine long non coding RNA lncRNA LL35 which was the stated topic of the research.

- The relevance of the methods used in the dissertation

  Author applied a plethora of modern methods of cell biology including in vivo knock down using siRNA and evaluation of the results by the tour de force of transcriptomics, lipidomics and metabolomics approaches which were available through well developed facilities in Skoltech. The candidate demonstrates good understanding of all these methods, ability to present results in professional way and makes adequate conclusions.
- The scientific significance of the results obtained and their compliance with the international level and current state of the art

  The author obtained several principle results describing the functional role of LL35:

Expression of LL35 is downregulated in the in vivo model of liver fibrosis, in liver proliferation after partial hepatectomy, in hepatocellular carcinoma attributing cytostatic or possibly tumor suppressor role of LL35 similar to stated for DEANR1.

Gene expression profiles comparing wild type and LL35 knockdown AML12 cells *in vitro* and in liver *in vivo* established nearly 800 genes changing expression were detected (523 upregulated, 273 downregulated).

Proteomic analysis revealed regulation of proteins involved in cell-cycle. This was further investigated showing that LL35 hampered cell migration and proliferation leading to the cell-cycle arrest in S-phase.

Important and unexpected results were related to metabolic function of LL35. LL35 knockdown causes upregulation of G6PC and PEPCK, which potentially may increase insulin sensitivity. Although, the changes were small and insulin clump down experiments have not supported the role of LL35 in regulation of insulin sensitivity

- The relevance of the obtained results to applications (if applicable)

  The relevance of the results obtained on functional properties of LL35 for validation of DEANR1 as a potential pharmacological target requires further justification. This is a general issue arising from species specificity and sequence dissimilarity between mouse and human of majority lncRNA. I believe that the candidate will address this issue during oral defense.
- The quality of publications

Publications are of highest quality, with the main publication describing the results of the thesis in Biomedicines journal (IF 6.1). Co-author of three papers, published in Journal of Molecular Sciences (IF 5.9), Journal of Cell Biology (IF 10.5) and Biochimie (IF 4.1). All Skoltech requirements for PhD defense are satisfied.

The summary of issues to be addressed before/during the thesis

The relevance of the results obtained on functional properties of LL35 for validation of DEANR1 as a potential pharmacological target to be addressed during oral defense.

Provisional Recommendation

X☐ *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*