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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

Name of the Reviewer: Petr Sergiev

I confirm the absence of any conflict of interest	**Date: 07-25-2022**

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

With the development of next generation sequencing toolbox it became clear the pervasive transcription of the mammalian genome results in the synthesis of the multitude of long non-coding RNA. While a fraction of those appeared later to be peptide-encoding and many appeared as purely junk, still, thousands of lncRNAs are functional. The work presented by Evgeniia Shcherbinina goes about the study of one of such lncRNA, namely, LL35 on the model of live mice liver and hepatocytes cell culture.

In an academic literature review, Evgeniia summarized existing knowledge on the function of non-coding RNAs. The review is comprehensive and, I would say, encyclopedic. All role that were ascribed to lncRNAs in the scientific literature have been thoroughly reported. In addition, quite a complete review on lipid metabolism is also provided in the review. I would recommend to publish this work in a couple of separate review papers.

The amin study goes about the phenotype of cells and mice where the expression of LL35 have been lowered with the help of antisense oligonucleotides. This method was chosen due to inefficient RNA interference in the nucleus, where the major share of LL35 is localized. I would rather suggest to make a complete knockout using a pair of sgRNAs and Cas9, but this is just a matter of personal choice.

Following the confirmed LL35 downregulation, the cells and livers were subjected to a wide set of analysis, starting from systems biology-like techniques, such as transcriptome and metabolome. Several findings as well as hypotheses have been additionally checked by RT qPCR and western blotting. On top of that, a number of functional assays have been performed, such as respiration and glycolysis assay with the help of Seahorse device? Proliferation and mobility (wound healing) assay, cell cycle assay, histopathology analysis, mitochondrial potential staining, lipid peroxidation analysis etc.

To identify protein partners of LL35 author applied immunoprecipitation of candidate proteins, as well as RNA co-precipitation/fishing. As a result, a potential partner has been found, hnRNP A2B1.

I have to emphasize that a set of methods applied and efforts invested into the project seems enormous. It is a really huge work done with top-edge armory of techniques.

There are several questions, that might be raised while reading the thesis.

1. Why there are so small overlap between differentially expressed genes in vivo and in vitro? Might the smaller number of differentially expressed genes in the liver be explained by less efficient LL35 downregulation?
2. How one could explain opposite effects of LL35 downregulation on several genes in vivo and in vitro (e.g. Chek1, Cenpi, Ggt6, Gstm2 etc.)?
3. Metabolome analysis and changes in the glucose metabolism suggested to check differential expression for a number of genes. How well the results correlate with transcriptome analysis?
4. How proteins were selected for pull-down analysis of LL35?
5. Many RNP complexes are formed efficiently in the cell, rather than in the extracts. Would it be better to fish out naturally formed LL35 RNPs by e.g. complementary biotynt=ylated oligonucleotide?
6. Micro RNA sponge function was assumed to lower the concentration of miRNAs. Why is it so? Sponge should lower the concentration of *free* miRNA, but presumably not total.
7. Downregulation of LL35 expression was shown to decrease proliferation and migration of cells. How does it correspond with decrease in LL35 expression in cancer and upon liver regeneration?

8. Choice of E-cadherin as a marker for cell mobility is somewhat arbitrary. Might a set of genes related to the mobility be found as significantly enriched in differentially axpressed genes upon LL35 downregulation?

As a summary, I have to state that the work of Evgeniia Shcherbinina which was described in her thesis is a significant advancement of our understanding of lncRNA functioning in general and the function of LL35 in particular. The results were published in four papers in decent journals in the field. It is clear that Evgeniia Shcherbinina should defend the thesis by means of a formal thesis defense.

Provisional Recommendation

☒ *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*