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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: Pavel Ivanov, Ph.D.

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date:** **10-08-2022**

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

A variety of evidences has suggested that lncRNAs function as key regulators in crucial cellular functions, including proliferation, differentiation, apoptosis, migration, and invasion, by regulating the expression level of target genes via epigenomic, transcriptional, or post-transcriptional approaches. The dissertation by Evgeniia Shcherbinina is dedicated to study a specific lncRNA, murine lncRNA LL35 (LL35).

Murine lncRNA LL35/Falcor shares similar genomic locus as human DEANR1, lncRNA that has been shown to regulate cell proliferation, apoptosis, glucose metabolism and tumorigenesis. DEANR1 is also proposed to act as tumor suppressor in some cancer subtypes. Although lncRNAs are less conserved between species than protein-coding genes, based initially on the genome localization, this Ph.D. thesis work hypothesized that murine LL35 is a putative functional analog of DEANR1 in human cells. This thesis was aiming to probe this hypothesis using in vitro and in vivo settings. Significantly, if proven, murine LL35 offers significant advantages to study mechanisms of lncRNA-mediated regulation of its targets and to test potential therapies with implication to use findings in the context of human disease.

This work is timely and well justified. The thesis itself is well written and well structured, although some minor changes are requested by me below. It is clear that PhD candidate had opportunity to learn various techniques, which is a big plus. The thesis is technically sound, and there are no ethical points to consider. Conclusions are mostly supported by the data. The methods used are relevant and adequate for the actual goals of the dissertation.

Introduction is comprehensive (~80 pages), and contains references to the primary literature. I especially appreciate the parts of the introduction describing lipid and glucose metabolism in its relation to the published lncRNA literature.

Materials and Methods section (~25 pages) is adequate and contains detailed information.

Results section is mostly well written. No major concerns.

Specific questions about the work:

1) The RACE analysis identified 1193nt transcript that is different to the annotated sequences. What could be the reasons for it?
2) Loss-of-function approach has been chosen. Would overexpression studies complement these data, what are the limitations of these approaches?
3) How would you control unspecific side effects of ASO-mediated depletion studies? Why exactly 5 ASOs were chosen for these LOF studies?
4) A cut-off for differentially expressed genes (|log2foldchange|>0.8,adjustedp-value<0.1) has been chosen. How would justify this choice? Why only 5 differentially expressed genes were common between in vitro and invivo sets?
5) Putative hits from RNA-seq data were validated by qRT-PCR. Why western blotting was not used to validate hits on the protein level?
6) There is a good correlation between pathways found in «–omics» data. How many/what the fraction of individual genes/ proteins overlap between RNA-seq and proteomics analyses?
7) Sponging of microRNAs: how simalar human miR-222-3p and murine miR-22-3p and 23a-3p?

8) It is not clear whether total lysate or nuclear extracts were used for RIP and/or biotinylated LL35 pull down analyses.

Additional points and discussion:

I feel that Discussion section should be revised. The following points should be discussed: 1) What are the limitations of the study?; 2) Why specific cell line was chosen for in vitro studies?; 3) What are possible cytoplasmic functions of LL35, and whether cytoplasmic pool of LL35 is affected during LOF studies?; 4) Why RIP approach was chosen? Are any of putative binding proteins (GC1α ,STAT3, PKM1, CTNNB1, SIRT1, IGF2BP2) are known RNA-binding proteins?; and 5) Future directions

Minor points:

* NMD is a “nonsense-mediated mRNA decay” (and not ‘nonsense-mediated decay’).

- Figure 26. It would be useful to give more information in the figure legend (e.g., what are green boxes). Also exon numbering should be added.
- Fig 35C and its quantification: how many repeats were done for this analysis?

Conclusions are concise and supported by results.

The Ph.D. candidate has four publications, one of which she is a first author (Biomedicines 2022). I estimate them as good level, well suitable for PhD defense. No objections here.

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*