Skoltech
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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Dmitrii Smirnov

PhD Program: Life Sciences

Title of Thesis: Investigation of the role of SIRT6 in molecular mechanisms of the gene expression regulation, metabolism and aging

Supervisors:

Assistant Professor Ekaterina Khrameeva, Skoltech

Associate Professor Deborah Toiber, Ben-Gurion University

Name of the Reviewer: Sergey Dmitriev, Moscow State University

I confirm the absence of any conflict of interest	**Date: 03-11-2023**

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

Proteins of the sirtuin family, including SIRT6, are known to control genome integrity and chromatin structure through multiple mechanisms. Their role in these processes is of particular interest in the case of brain, especially in the context of cancer, as well as physiological and pathological aging. In his study, Dmitrii Smirnov studied transcriptional and metabolic dysregulation in the brain of mice bearing a knockout of the *Sirt6* gene, followed by the analysis of human datasets obtained from brain cancer tissues, as well as a cross-talk between SIRT6 and the YY1 transcription factor that occur on a specific promoter of the glioblastoma marker *TP73-AS1*, which is highly expressed in the aging brain. The study is preceded by the development of a bioinformatics pipeline for the analysis of mass spectrometry data.

Although a little bit diverse, three topics together represent solid basis for the PhD thesis and fit well with the declared topic of the thesis. Using both standard and self-developed bioinformatics pipelines, Dmitrii showed that brain-specific *Sirt6* knockout results in global transcriptional and metabolic dysregulation in the brain and these changes are primarily associated with mitochondrial dysfunction: decreased expression of mitochondria-related genes and abundance key metabolites, as well as reduction of mitochondrial content (a decreased mitochondrial biogenesis or increased degradation) and impaired oxidative phosphorylation. This contributed to our understanding of how SIRT6 protects brain health. Next, based on ChIP-Seq data analysis, he predicted that YY1 directly activates the *TP73-AS1* promoter to induce its expression, the statement which was then proven by gene reporter analysis. This finding is important to understand the mechanisms of brain cancer.

The results obtained by the author correspond well to the international level and current state of research in this area. Their impact to the field is significant. The conclusions are convincing and supported by the results. The study also links SIRT6 functions to a wide range of age-related brain diseases, including neurodegenerative diseases and brain tumors, making it attractive for therapeutic applications.

The author used standard bioinformatics approaches relevant to the study. For LC-MS data analysis, Dmitrii developed and thoroughly described a detailed workflow designed for untargeted lipidomics (and also applicable to the analysis of metabolomics data), i.e. step-by-step practical guidelines, which are undoubtedly useful for colleagues working in this field.

Thesis structure is adequate; the main content is separated to the three interrelated sections according to the three above-mentioned topics.

The papers published by the author (in: *Metabolites*, IF2022=4.1, 1st author, 2021; *Aging*, IF2022=5.2, 2nd author, 2021; *Cell Death Dis*, IF2022=9.0, 1st author, 2023) are cited a total of 17 times since 2021 according to Google Scholar. The paper published in *Cell Death Dis* (the main paper in the whole story), is of special importance, to my opinion: this study, in addition to bioinformatics approaches, required a large amount of "wet" laboratory work – but nevertheless, Dmitrii (who clearly performed only the bioinformatic part), is the first author in this paper. This reflects the crucial role of the analyses he performed for the conclusions made in this study. Besides, 4 conference reports, including one oral presentation, are declared (although two of them seem to be unrelated to the thesis's topic).

However, I would declare some issues to be addressed before/during the thesis defense:

1. It would be helpful to describe the mouse model in a little more fully: how the specificity of *Sirt6* knockout in the brain was achieved ("brain" is actually a quite complex organ in terms of histology, so a minimal information about a promoter used for *Cre* (?) expression, for example, would be useful);

2. p.3: "We showed that SIRT6 may interact with YY1 and… SIRT3 and SIRT4… to facilitate the

transcription of the mitochondria-related genes." – I found no direct evidence for the (direct) interaction of YY1 and sirtuins in this study, except binding to the same promoter regions (which may be quite long) – so it would be good to explain or state this more cautiously.

3. The novel pipeline for untargeted lipidomics data analysis: What is a difference compared to the "old" pipelines? – It should be clarified.

4. At least in humans, YY1 is one of the major transcription factors regulating the LINE-1 retrotransposon expression. It is also known from the studies by the Gorbunova lab that SIRT6 is critical for LINE-1 suppression. Did you try to analyze the effects of SIRT6 on LINE-1 transcript abundance in your study? It would be also interesting to look at YY1 and SIRT6 binding to LINE-1 promoters in ChIP-Seq data.

5. p.69: "Together, this data suggests that YY1 is a major TP73-AS1 regulator." – Please explain: I found no solid evidence in this study supporting the statement that YY1 is indeed the "major" regulator.

6. I would be glad to see the "Conclusions" section in the form of clear theses (points), rather than lengthy text, which would be more appropriate to the "Discussion" section.

Minor points:

7. "*M. musculus*" and gene names should be italized; nomenclature names of mouse genes should be given as follows: *Sirt6*.

8. Abbreviations should be spelled out on initial appearance in text (e.g. YY1 (TF Yin Yang 1) is spelled out only in the beginning of the 3rd part of the thesis).

9. p68: What is "the 824-promoter activity"?

10. References should start with the last names of the authors: this will be much more useful to the readers.

Despite the issues raised above, the quality of the thesis is clearly good enough that it could be defended in its current form, once all my points have been addressed during the formal 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*