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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Smirnov Dmitrii

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: Dmitri Pervouchine

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 04-11-2023**

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

The dissertation "Investigation of the role of SIRT6 in the molecular mechanisms of the gene expression regulation, metabolism and aging" by Dmitrii Smirnov is devoted to studying the role of SIRT6 gene in orchestrating gene expression in the mouse brain using SIRT6 knockout models. The study provides important implications for understanding the processes of aging and neurodegeneration associated with SIRT6 dysfunction. The dissertation contains all necessary sections including Abstract, Introduction, Literature review, Results, which are presented as three different sections, Conclusions, and Bibliography.

The Literature review starts with mitochondrial dysfunction as a hallmark of aging, and the role played by SIRT6 in brain aging and neurodegeneration. The author describes the relationship between sirtuins

and mitochondrial activity, lipid metabolism, the cooperation between SIRT6 and another important factor YY1, and lastly associations between glioblastoma and brain aging, which however seems quite disconnected with the rest of Literature review. The results section is organized in three blocks, each having a mini-introduction, methods, specific results, and discussion. The first block, a novel pipeline for untargeted lipidomics data analysis, describes a methodology for measurements of lipidome vomposition LC-MS experimental workflow. Furthermore, the author discusses visualization of LC-MS data and applications of untargeted lipidomics. Overall, this section presents a novel method, which is a technical pre-requisite for the successive sections. The second block, the effect of SIRT6 deficiency on transcriptome and metabolome levels during normal and pathological aging, describes how the lack of SIRT6 alters gene expression levels in the mouse brain. The author demonstrates that SIRT6 regulates mitochondrial metabolism, and its deficiency leads to impaired oxidative phosphorylation. It is concluded that the lack of SIRT6 results in a reduction of mtDNA gene expression and mitochondrial content, and that the cooperation between sirtuins and YY1 axes promote oxidative phosphorylation in the brain. Lastly, the author speculates about the neuropathological role of SIRT6 considering its role in mitochondrial deregulation. The results of this block are novel and contribute greatly to the understanding of brain aging and neurodegeneration. The last, third block, the role of SIRT6' co-partner YY1 in aging and brain tumors, describes the response of 824 promoters in a luciferase activity assay upon overexpression of YY1, a cooperating partner of SIRT6. It is demonstrated that the expression of TP73-AS1, a TP73 antisense RNA transcript, is highly elevated upon SIRT6 overexpression.

In reading this manuscript, I have got the following comments and suggestions (mostly about the way of presentation).

1. It seems that the purpose of Abstract and Introduction have been misinterpreted by the defendant: Abstract must be a short summary of the work, while Introduction must be a pedestrian intro into the context of the problem. Here, the purposes of these two sections appear to be switched. The Abstract describes the background, while the Introduction merely consists of enumeration of thesis chapters. Instead, the Introduction should give the reader a broad understanding where this thesis stands scientifically rather than be just a manual on how to use this thesis.

2. On p.48 brain-specific SIRT6-knockout is mentioned, but the author never mentioned how this knockout was obtained. Is this a cell line or an animal model? Did the author contribute to the generation of this knockout? It would be great to have at least a paragraph on that page that discusses this issue (maybe it exists somewhere else, but I may overlooked it).

3. Some sections seem to be not very well connected with the others, e.g., switching to diseased old brains in Chapter 5 needs more motivation.

4. On p. 65 it is announced that TP73-AS1 is highly expressed in the aging brain. A curious reader wants to ask a question of what is the function of this antisense transcript, and how is it related to the function of the sense gene. I am sure that there is plenty of literature on this topic. Generally, I have an impression that the thesis here and later (especially in Conclusions) becomes a bit cryptic.

Nevertheless, the above comments are mostly cosmetic and do not detract from the scientific value of the thesis. The dissertation of Dmitrii Smirnov is an important and insightful journey into the functional characterization of SIRT6, it's role in aging and disease.

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*

Sincerely,

Dmitri D. Pervouchine