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Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Alexander Tyshkovskiy

PhD Program: Life Sciences

Title of Thesis: Molecular Signatures and Mechanisms behind Lifespan Extensions

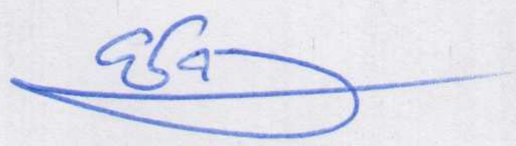
Supervisor: Prof. Philipp Khaitovich

Co-Supervisor: Prof. Vadim Gladyshev

Chair of PhD defense Jury: Prof. Mikhail Gelfand ***Email**: m.gelfand@skoltech.ru*

Date of Thesis Defense: 23 October 2018

Name of the Reviewer:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** **Georgii A. Bazykin**  **Date: 09-30-2018**

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

- **Brief evaluation of the thesis quality and overall structure of the dissertation.**

The thesis addresses the molecular mechanisms underlying senescence and lifespan and the levels of RNA expression and methylation. It consists of three main parts. In the first part, changes in expression in response to irradiation are compared between mouse and the naked mole rat – a model species which is known to demonstrate exceptionally longevity. The author shows massive changes in expression of multiple genes belonging to multiple pathways in response to irradiation, and interprets this as evidence for cellular senescence in both species. Using the "guilt by association" approach, he also shows that the same processes affected in mouse in response to irradiation are also affected in NMR. In the second part, the author studies changes in DNA methylation in the course of aging, demonstrating, among other things, the "smoothing" of the pattern of methylation with age (so that hypomethylated sites become more methylated, while hypermethylated sites become less methylated),

and the acceleration of this trend with age. Finally, in the third part, he obtains massive data on expression changes due to a variety of lifespan-extending interventions, and infers genes the expression changes in which can serve as "markers" of longevity change.

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

The title nicely sums up the diversity of subprojects covered in the thesis.

- **The relevance of the methods used in the dissertation**

Up to date methods are used, both bioinformatics and wet lab.

- **The scientific significance of the results obtained and their compliance with the international level and current state of the art**

The author has put together an exceptional dataset which may help address the nature of senescence - a pressing question indeed. Although some details of the interpretation can be controversial (see below), the value of the obtained data for further research is big.

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

The obtained results may inform therapy/drug design.

- **The quality of publications**

High: one co-first-authored paper in PNAS, and one paper in Aging Cell (one more paper submitted).

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

While the overall quality of the work is undoubtedly high, some issues need to be raised.

Some statements in the literature review are vague, poorly formulated, or lack logic. For example, the last para in p. 20 deals with gene expression associations with maximum lifespan, but the positive selection discussed here has affected gene sequence, not expression. The last phrase in p. 21 states that a "2-fold decrease in mutation rate may result in 100-fold increase in cell mass free from cancer"; this is formally correct (yes, it MAY result in such an increase), but it is not explained how these figures were obtained, and no reference is provided. The first phrase of Section 1.3 (and the section title) address the within-species variation, but most of this paragraph deals with between-species variation.

While the overall validity of the results is convincing, in some instances, their interpretation seems ambiguous, and the results seem to be somewhat overinterpreted (or the interpretation depends on other considerations not stated explicitly). It is unclear why the authors interpret a wider range of pathways perturbed in response to irradiation in NMR compared to mouse as evidence that the response in NMR is "more systematic and nonstochastic" (p. 48) and "more organized" (p. 50), and implies "higher robustness and control of their gene expression profile" (p. 53). I don't see how a diversity of response is an evidence of robustness and control. Conversely, one could argue that the diversity of processes involved in a response suggests deficit of organization rather than its excess. I also don't see why the deficit of the signal of apoptosis in NMR implies higher senescence in this species (p. 51, top). To me, this just indicates a lack of apoptosis. This conclusion also seems to be contradicted by the results of the experiment showing that senescence is attenuated in NMR compared to mouse (p. 54). If this interpretation depends on some additional results, this is totally fine but it should be stated so.

My other concern is associated with the effect of caloric restriction (CR) of the methylation change (p.

90). The author shows that under CR, the methylation changes in the course of aging (the "long-term cumulative changes", in their terminology) in the direction opposite to that of the the initial change in methylation due to CR (the "initial shift"; p.84). But is this long-tem change sufficient to compensate for the "deleterious" initial shift? In Fig. 14A and 14C, CR actually makes the methylome more "aged", not less "aged", at the endpoint. The author seems to suggest that CR helps fight methylation changes due to aging (p. 92), but this will only work if the net change is typically in the direction of the "young" state. Is this the case?

These criticisms in no way undermine the fact that the author has put a substantial amount of work into the projects, and has produced a solid thesis.

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*