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Assessment of Bachelor thesis of Tamara Gajovská "Effects of mTOR and AMPK pathways on specification of inner cell mass (ICM) cell lineages during mouse preimplantation development"

Formal aspects. The thesis is written in English in a classical format combining the following sections: introduction, project aims, material and methods, results, discussion, conclusion, references, and appendix. In total it covers impressive 79 pages, however, the author decided to use a rather unusually narrow format with lines reaching up to three-quarters of the typical length. Not only do not I understand why it is presented in this format, but it is especially inconvenient for reading. It doesn't look good either.

The good news is that if it were properly formatted, it would be still reasonably long and sufficient (so I really wonder why....). The introduction is detailed, written in really complex English, albeit sometimes quite technical and very dense. The introductory text is supported with several figures, all of them properly cited. The material and method section clearly describes how experiments were performed.

Results are sometimes a little bit difficult to digest. There is a lot of abbreviations, which is probably necessary, but sometimes it is a bit thought to read through. Don't take me wrong; as a non-native speaker, I do not even dare to criticize the grammar, which seems to be perfectly fine. It is rather the readability of the text that bothers me, further exacerbated by relatively complicated, perhaps unavoidable, terminology of the field.

The first page of results is written almost as it is a part of material and methods. On the other hand, the purpose of a bachelor thesis is to get a bit familiar with writing and I think Tamara Gajovská had a really difficult and complex topic to deal with, and yet she managed to so more than sufficiently.

The second positive fact is that Tamara Gajovská performed a remarkable amount of work using difficult techniques. Immunohistochemistry experiments are demanding, and experiments were performed in a reasonable number of replicates. That is, for a bachelor student, well above what is required. Good for her.

Questions:

- 1. When I look at Fig. 8 (or 6) legend, I read: Immuno-fluorescent staining of DMSO control (upper n=18) and GSK621 (n=19). Can you please explain the "n" in the figure legend?
- 2. Could you please describe Torin1?
- 3. I was wondering, if your data might have any potential wider impaction(s) regarding the culture of human embryos, such as those resulting from *in vitro* fertilization, prior to transfer back to the mother's uterus?

4. The third aim was "To compare the effects of AMPK activation and mTOR inhibition and assess whether AMPK activation is a phenocopy mTOR inhibition (as hypothesized)." Could you please comment on the fact that you actually observed a phenotype related to reduced PrE formation (with little effect on the EPI)? Can you explain why you think the effect of AMPK activation was only restricted to PrE in the wider context of ICM cell differentiation?

Taken together, I am convinced that the presented work more than fulfills the criteria for a bachelor thesis at our or any other faculty, and the mark should be somewhere in the range of excellent or very good. The actual grade should better be defined after the defense.

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