Review of the Bachelor thesis “The energy metabolism of dyskinetoplastic Trypanosoma parasites” by Julie Ebenstreitová

The Bachelor thesis of Julie Ebenstreitová, entitled “The energy metabolism of dyskinetoplastic Trypanosoma parasites”, is based on the knowledge we have from *Trypanosoma brucei brucei* and explores mitochondrial metabolism of related, dyskinetoplastic strains of trypanosomes. First of all, I would like to stress out that dyskinetoplastic trypanosomes are not as well established model as *T. b. brucei*, having several implications. Firstly, there is much less literature available about them, and knowledge is mostly transferred from the bloodstream form of *T. b. brucei.* Secondly, these strains are more difficult to cultivate and subsequently work with. Lastly, every finding about dyskinetoplastic trypanosomes is novel and interesting, which is definitely true also for results of this work.

Overall, the thesis is of sufficient extent, well structured, and contains all required information in appropriate chapters. I would like to acknowledge all the effort Julie went through and provided very thorough Introduction. She started quite broadly and described mitochondrial metabolism in kinetoplastic trypanosomes as we understand it currently, leading toward the assumptions we have about the dyskinetoplastic parasites. She built up the background very nicely in order to explain the rationale behind her work. Although there are some minor errors and sometimes the language could be more precise, I appreciate that she was brave and wrote such a comprehensive introductory chapter. The Methods part is very detailed, sometimes even too much. However, my major criticism here is about references, which are completely missing from this part. Especially when describing the strains of cells, plasmids and similar, they have to be referenced. The Results are more on the scarce side, but that can be explained by the technical difficulty of working with the dyskinetoplatic strains. There could have been growth curves of WT *T. evansi* included. The results are described in detail and properly explained, although some speculations would be more suitable for the Discussion. Lastly, there are some references missing from the Introduction and Discussion and completely missing from the Methods section. In addition, some reagents and protocols are included in the References in unsuitable formatting.

Minor comments

- p. 1. “Notably, of all the Trypanosomatids, only *T. b. brucei* is an extracellular parasite.” – not correct
- p. 2 “www.gla.ac.uk” is not a good reference, you could have used a review paper by Giordani *et al*., Parasitology, 2016.
- I believe Figures 2, 3, and 4 were adapted from your supervisor, so it should be noted.
- It would have been better to keep the Results section in the same order as the Aims, and start Results with validation of dyskinetoplastic cell lines.

Questions

1. Please, quantify the proteins SCoAS and ASCT detected on the western blot in Figure 6. It will strengthen the results and allow you to speak about fold changes in a precise manner.

2. Please, show a map of the generated plasmid pT7-v5-ASCT. Is the v5 tag at the N-, or C-terminus? Why is it important?

3. Regarding the phenotype observed in Figure 14 B in induced cells, you speculate that the cells “found a way to escape the ASCT expression”. How would you test that?

4. Can ASCT produce ATP on its own? Please, include a more detailed scheme of the reaction. What does your data imply about the regulation of the pathway?

Overall, I am satisfied with the thesis and I believe it exceeds expectations for Bachelor students. Based on the written thesis, I suggest the mark 1/excellent, however, the presentation makes up a substantial part of the final mark.

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