Institute of Pathological Physiology
First Faculty of Medicine
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Head: Prof. Martin Vokurka MD, PhD.

Examiner's report on PhD. thesis
Name of student: Dana Sovilj, MSc.
Title of thesis: Functional assessment of Bcl-2 family proteins in mitochondrial metabolism and beyond

The thesis of Dana Sovilj is well structured, written in English in a standard generally acceptable for the discipline. The theses focus mainly on the function of pro-apoptotic members of Bcl-2 family, Bax and Bak, and their role in the regulation of energy metabolism and behavior of cancer cells and function of the mitochondria.

The Introduction (p. 12-35) shows that the author collated extensive knowledge and became an expert in the studied field. Author clearly described the role of the Bcl2 protein family in the mitochondria biogenesis, bioenergetics, apoptosis, and cancerogenesis. The introduction is complemented with figures which help the reader to understand the background of the studied topic.

The hypothesis of the project is clearly formulated. To address the hypothesis author proposed 3 specific aims (p. 36-38).

The range of the most relevant methods for the thesis are briefly described in the Materials and Methods (p.38-43). For the complete list of methods and detailed description is reader referred to the authors publications listed in the results section. Used cell biology, molecular biology, and imaging methods are well selected to achieve relevant and original results.

Main results from four articles are concisely presented in the Result section (p. 44-52) and contains data that were already published. For detailed results the reader is referred to the published manuscripts in scientific journals with significant impact factor. The results are obtained from well designed and executed experiments using various tumor cell lines, CRISPR/Cas9-meditaed target gene editing, and evaluation of the *in vitro* and *in vivo* metabolic functions and growth of tumor cells with inactivated Bax and/or Bak. The result section of the thesis addresses the individual specific aims.

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In discussion (p. 52-61) Dana Sovilj shows that she is able critically evaluate obtained results, compare them to current knowledge, and formulate original conclusions. The references are well-chosen with balanced composition between original and recent articles.
Summary:

The presented work in its entirety is of considerable scientific and medical importance.

The thesis of Dana Sovilj fulfilled all the criteria for doctoral thesis. She exhibits expectation for independent scientific work, and after successfully defending her dissertation she deserves to be awarded the academic title PhD.

Questions:

1. Are there human inherited disorders/phenotypes associated with Bax or Bak mutations?
2. From the mid 90-ties several Bax and Bak deficient and transgenic mice models have been established. These models show several tissue specific non-apoptotic effects of Bax and Bak. Is there a knowledge about tissue specific binding partners outside of Bcl-2 family for Bax and Bak which can explain their tissue specific effects?

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