30.8.2021

RE: Evaluation report – Doctoral Thesis by Matej Fabišik

Dear Committee Members,

The presented thesis by Matej Fabišik deals with the important topic of signal transduction via adaptor proteins in the context of immune cells with the focus on finding the mechanistic role of newly described members of this protein family. The thesis has a standard format and it consist of a short introductory part, describing briefly cell-signaling mechanism and modes of signaling regulation, this is followed by also brief introduction of four adaptor proteins in the central focus of the thesis, re-prints of four publications authored by Matej Fabišik with their summary and finally the Conclusions part. The thesis is written in English with the minimum of spelling errors (with the exception of the List of abbreviations) and it is really easy to follow. However, as I personally do not consider myself "cell-signaling enthusiast" I feel that I would sometimes benefit from a more in-depth introduction or from scheme(s) summarizing the signaling cascade or for instance the domain structure of a particular adaptor protein.

Matej Fabišik is the co-author of 3 publications and the first author of the publication in *Frontiers in Immunology* journal. This is a recognizable achievement for the PhD student.

I would like to take this opportunity and ask several questions arising from reading the thesis. I will mainly restrict this to the publication in *Frontiers in Immunology* journal:

1) The expression level and the cellular source of *Lst1* expression is mainly determined by qPCR analysis. I am wondering if the recent advent of single cell genomics did not revealed *Lst1* expression in different subsets of hematopoietic cells?

2) Following the same line, you are mentioning that *Lst1* expression was reported to be increased in the intestinal biopsies of IBD patients. I am wondering if you checked how the expression level and cellular source of *Lst1* changes under the inflammatory conditions?

3) You are using the whole body knockout of Lst1 in your work and inspecting the impact only in the immune cells. Can you think about a more precise experimental setup on how to determine the impact

FACULTY OF SCIENCE
Jan Dobeš, PhD
Department of Cell Biology
address: Viničná 7, 128 00 Praha 2 **phone:** +420 221 951 624 **e-mail:** jan.dobes@natur.cuni.cz
VAT N. : CZ00216208 **web:** dobeslab.org

of Lst1 solely in the immune system, ruling out the effect of its potential presence in epithelial cells of the intestine in case of DSS induced colitis treatment?

4) You are observing a mild increase in Tnf-α and Il6 effector cytokines in the plasma of the Lst1 knockout mice after DSS treatment. Given the nature of these cytokines, I am wondering if you measured only the frequencies of T-cells in your cohort or also their activation or polarization status?

5) You are observing a striking decrease in NK and NKT cell frequencies in Lst1 deficient animals. Can you speculate about the mechanism, how this can be potentially achieved (and how would you test this)?

To summarize, the thesis itself clearly fulfilled all the requirements of our PhD program and I am highly recommending this thesis for the defense.

With best regards

Jan Dobeš

FACULTY OF SCIENCE
Jan Dobeš, PhD
Department of Cell Biology
address: Viničná 7, 128 00 Praha 2 **phone:** +420 221 951 624 **e-mail:** jan.dobes@natur.cuni.cz
VAT N. : CZ00216208 **web:** dobeslab.org