CHARLES UNIVERSITY
Faculty of science

In Prague 12.1.2021

**Review of the diploma thesis**

The diploma thesis of Bc. Miriama Pekľanská is focused on the CRISPR Cas9 approach concerning the fluorescently tagged knock-in of Wwc2, Mapk13, Mapk14 genes and tracking their expression during early embryonic development of mouse embryos. The Wwc2 gene is employed in the first cell fate decision (ICM/TE) via Hippo signaling pathway and Mapk13 and Mapk14 are key for the differentiation of primitive endoderm (PrE) thus separation of hypoblast from the remaining ICM cell mass.

Diploma thesis is written on 82 pages and divided into standard parts in accordance with general rules. The Abstract is clear and consistent. On the other hand I would appreciate the specification of studied genes here and also short description of achieved results, since abstract should represent compressed version of diploma thesis containing all important parts (what is known in the studied field, why I performed my research, by which methods and what are my outcomes). The Literary research is well structured, clear and contains all important information for the reader to grasp the scientific and experimental background. All figures are in a good resolution and cited in the text. I must appreciate the care with which they were prepared. Also figure legends are well arranged and self-explanatory. Project aims are clear. The chapter Material and methods is described in detail and offer the reader all necessary information to repeat experiments. I have only one critic comment. I would appreciate the figure containing gene sequences with depicted sgRNA target and PAMs and the 5´ and 3´ arm borders. Also, I miss the information if Cas9 target the last exon or intron sequence. The second approach would prevent the problems with potential indel frame shits caused by Cas9 mediated integration of target sequence. In the chapter Results author describes the construction of gene target vectors, i.e. cloning of 5´arm of Wwc2 and 5´a and 3´arms of Mapk13 and Mapk14 genes. Then the preparation of

**FACULTY OF SCIENCE**
Assoc.prof. Vladimir Krylov, PhD.

**address:** Vinicna 7, 128 43 Prague 2 **phone:** 221 951773 **e-mail:** vkrylov@natur.cuni.cz

biotinylated amplificates, the construction of sgRNA vectors and production of sgRNAs and streptavidin conjugated Cas9 RNA for the subsequent microinjection into one blastomere of two-cell mouse embryos. On page 53 you wrote: "However, after this second round of BIO-PCR using gel extracted template, the expected product was not obtained, possibly due to unfavorable ionic conditions in the template preparation after gel extraction ". Could you please explain what do you exactly mean by this statement? Even construction of all three target gene constructs and production of sgRNAs and Cas9 RNA were successful the second aim of the diploma thesis was not accomplished, and only weak fluorescence signal was observed in one embryo with targeted Mapk13 gene. This negative result is of course not a problem and one must admire the enormous work the author did. What I must criticize is the following discussion which is written on 3.5 pages with only one cited paper (Gu et al., 2018). This chapter should not be a summary of the author's views without any reference to the available literature. I miss the discussion of other CRISPR Cas9 knock-in approaches (for example intron targeting, if relevant). Have you tested the ability of Cas9 to cleave the target sequence? And what about the sgRNA design? Are you sure that sgRNAs were well designed and function? Have you tried to inject more than one sgRNA targeting the different sequence in the studied gene? These above-mentioned aspects should be well discussed in the diploma thesis using the relevant literature.

In general, the diploma thesis is well written and arranged. As I wrote above, I must admire the enormous experimental work the author performed and how carefully prepared and check the text and figures. The weak point is chapter Discussion which should be the most important part of diploma thesis showing how the student is able to put her results into broader context, grasp the negative outcomes and offer their feasible solution.

Despite the critical remarks, I fully recommend the diploma thesis for a successful defense and subsequent award of the title of Mgr.

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Questions:

1) How the anterior- posterior polarity of EPI cell lineage is established in the mouse embryo?
2) How the E and P-cadherin expression is regulated in the early mouse embryo resulting in the separation TE and ICM cell lineages?

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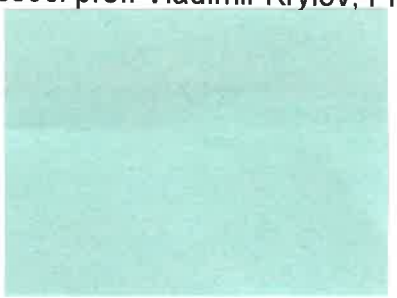

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