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Author of the thesis: Kateřina Jeřábková, MSc.
Title of the thesis: The roles of Trim15 and UCHL3 in the ubiquitin-mediated cell cycle regulation.
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The presented PhD thesis by Kateřina Jeřábková is interested in the ubiquitin-mediated cell cycle regulation by studying the roles of an E3 ubiquitin ligase TRIM15 and a deubiquitinating hydrolase UCHL3. It is interesting and important topic because deregulation of ubiquitin-mediated signaling often occurs in cancer cells. The thesis is written in the full-length format.

Chapter *Introduction* provides a well-written overview of ubiquitin-mediated signaling in the context of cell cycle regulation. Also a well-focused and informative introduction to mitosis is provided. To this part, I have only one question:

Q1: On page 38, first paragraph author discusses the restriction point as a point of commitment to the next cell cycle transition. In the view of recent works (e.g. Cell. 2016 Jun 30;166(1):167-80.) I would like ask for discussion whether the restriction point is really the point of commitment (the point of no return) as presented in the text.

In the chapter *Projects* own research entitled *UCHL3 controls the chromosome segregation during mitosis* is presented. In this work author used high-content screen using siRNA library targeting about 500 genes for deubiquitinating enzymes and other ubiquitin factors. UCHL3 was identified as a top hit producing irregularly shaped nuclei as a consequence of defective mitosis. Further work validates this finding using two single siRNA and small molecule inhibitor TCID in HeLa cells. Next UCHL3 is described as an important factor for proper chromosome alignment in metaphase. The specify of mitotic phenotype after UCHL3 siRNA mediated downregulation is proved by the rescue experiment with catalytically active UCHL3. Additionally author shows that UCHL3 does not regulate the spindle assembly checkpoint (SAC), but it is involved in the chromosome alignment. The involvement of UCHL3 in chromosome alignment is further confirmed not only in HeLa cells but also in human primary lung fibroblasts cells using TCID inhibitor and in colorectal adenocarcinoma cells (Dld1) using shRNA and time-lapse microscopy. Finally, it is shown that UCHL3 stabilizes microtubule-kinetochore attachment by facilitating Astrin and CENP-E recruitment and UCHL3 interacts with Aurora B and deubiguitinates it. By the end of this part of PhD thesis discussion of obtained results is provided and possible model of UCHL3 function is proposed.

To this part of PhD thesis, I have the following questions:

Q2) On page 71 the high content siRNA screen is described and Table 9 on page 172 lists all tested candidates genes. Although it is mentioned that the resulting hit-list contains genes involved in chromosome segregation and cytokinesis, the basic result of this experiment (the hit-list) is not presented. I suggest author to include this hit list into the result section. It is important part of the work.

Q3) The whole genome siRNA screen on the regulator of mitosis was already published by the Mitocheck Project Consortium (Nature. 2010 Apr 1;464(7289):721-7.). The entire high-content data set from this work is freely available at https://www.mitocheck.org/. What was the rationale to run a new high content siRNA screen? How are results (mainly for UCHL3) from this screen in agreements with data from the Mitocheck project?

Q4) The percentage of cells with irregular nuclei is shown in Figure 14C. Which UCHL3 siRNA was used? It must be clearly stated in the figure legend or text.

Q5) In many experiments focusing on the chromosome alignment the synchronization protocol using Monastrol is used (Figure 16, 17, 19, 21 and 22). Did you try to observe the chromosome misalignment phenotype without synchronization on the continuously growing cells? Time-lapse imaging of HeLa Kyoto H2B-mCherry cells allows to do it relatively simply. Is it possible that synchronization has some effects on the observed phenotype?

Q6) Are differences on the Figures 22F and G statistically significant? It must be clearly stated in the figure, legend, or text.

Q7) How author can explain that although UCHL3 inhibition results in problems with chromosome alignments this problem is not detected by SAC and anaphase entry is not delayed (Figure 22 D)?

Q8) Figure 24 shows the effect of UCHL3 siRNA on the microtubules and their attachments to kinetochores. Are changes described in the text on page 105 significant? The proper quantification and statistic must be included.

Q9) Did you try to use the cold stable microtubule assay to better evaluate microtubule-kinetochore attachment? Which IF protocol was used for Figure 24A, B? How were you able to visualize stable microtubule-kinetochore attachments using standard IF protocol?

Q10) Are changes in Figure 25D significant? The proper statistic must be included.

Q11) In the chapter 2.3.10, page 113 the CRISPR/Cas9 generated UCHL3 knock-out HeLa cells are used. Do these cells produce the same or stronger phenotype in comparison to siRNA treated cells? What was the rationale to use CRISPR/Cas9 approach when all remaining data are based on siRNA?

Q12) On page 113 the Mass Spectrometry analysis of putative UCHL3 binding partner is described, and Aurora B is mentioned as one of many identified UCHL3 binding partners. Which other proteins were identified in this experiment? The complete list of identified proteins should be included.

Q13) In the figure legend for Figure 26 monastrol is mentioned for synchronization, but in the corresponding text on page 114, another Eg5 inhibitor STLC is mentioned. Which inhibitor was used?

Q14) On Figure 26A, western blot for Aurora B with 55 and 75 kDa molecular markers is shown. The second line shows the increase of probably ubiquitinated Aurora B (75 kDa) in the time 0 after the release. What is the situation in the time 45 when only small and cut western blot for Aurora B is shown? The description for Figure 26C is not clear. What is in individual lines?

Q15) Author has shown problem with chromosome alignment and chromosome segregation after acute downregulation of UCHL3 by siRNA. On the other hand it is mentioned in the text that mice deficient for UCHL3 are viable. How it possible to explain it? In the last part of the work UCHL3 knock-out HeLa cells generated by CRISPR/Cas9 technology are used. Is possible compensation in action in these permanent knock-out cells in comparison to acute siRNA knock-down cells?

In conclusion and from my perspective, the above mentioned part of the thesis focused on UCHL3 represents significant amount of work and data. However, before its final publishing, this work requires the major revision. This my requirement for revision is of course not relevant in the moment when UCHL3 work is published as primary research paper.

Chapter 3 is entitled: "*Trim15 implication in the cell cycle progression and migration*". It is mentioned in the thesis in the *Project Outlook* chapter (page 25) that this part of the work will not be published because other papers on the similar/same topic were already published by other groups.

The chapter *List of publications and communications* is on page 165. Following publications are listed:

1. Jerabkova K, Sumara I: Cullin 3, a cellular scripter of the non-proteolytic ubiquitin code. Semin Cell Dev Biol. 2018 Dec 28. pii: S1084-9521(18)30033-8.
DOI: 10.1016/j.semcdb.2018.12.007

2. Katerina Jerabkova, Yongrong Liao, Sadek Fournane, Charlotte Kleiss, Matej Durik, Laurent Brino, Radislav Sedlacek and Izabela Sumara: Deubiquitinating enzyme UCHL3 controls genome segregation in human cells. (Manuscript in preparation)

3. Marina Peralta, Katerina Jerabkova, Tommaso Lucchesi*, Laia Ortiz Lopez, Benjamin Vitre, Dong Han, Chaitanya Dingare, Izabela Sumara, Nadia Mercader, Virginie Lecaudey, Benedicte Delaval, Sigolène M. Meilhac and Julien Vermot: Intraflagellar transport complex B proteins regulate the Hippo effector Yap1 during cardiogenesis. (Manuscript is ready to resubmission after revision in Plos Biology)*

4. Jakub Ziak, Romana Weissova, Kateřina Jeřábková*, Martina Janikova, Roy Maimon, Tomas Petrasek, Barbora Pukajova, Mengzhe Wang, Monika S. Brill, Marie Kleisnerova, Petr Kasparek, Xunlei Zhou, Gonzalo Alvarez-Bolado, Radislav Sedlacek, Thomas Misgeld, Ales Stuchlik, Eran Perlson and Martin Balastik.*
(Manuscript in revision, EMBO Reports)

The first paper in the review article and it is already published. The second work that covers the main part of this thesis is stated as manuscript in preparation. The remaining third and fourth works are of completely different topics, and I do not understand how these works are related to the aims declared in the chapter *Aims of the study* (page 57). These works are not commented in the thesis.

The rules at Faculty of Science, Charles University requires that the PhD thesis is based on at least one first-author paper with original research (not review article). Unfortunately, the manuscript of this work is not included in the thesis, and only manuscript in preparation is mentioned. If this problem is not solved until the date of PhD defense it represents from my point of view the critical issue precluding successful defense of this PhD thesis. For this reason I am asking Board for Doctoral Study of Cell and Developmental Biology at Faculty of Science, Charles University (head Assoc. Prof. Dr. Petr Folk, Ph.D) to answer the question whether this PhD thesis fulfill the publication criteria needed for the PhD thesis defense.

In Liběchov, September 30, 2019.

Assoc. Prof. Dr. Petr Šolc, Ph.D.