Reviewer's Report on the Ph.D. thesis of Anastasiya Klebanovych, M.Sc. entitled „REGULATORY MECHANISMS OF CENTROSOMAL MICROTUBULE NUCLEATION ".

The dissertation thesis of Anastasiya Klebanovych, M.Sc. was carried out under the guidance of doc. RNDr. Pavel Dráber, DSc. at the Laboratory of Biology of Cytoskeleton at the Institute of Molecular Genetics of the Czech Academy of Sciences in Prague. The candidate is the first author of two manuscripts published in *Cells* and *Life Science Alliance,* the first author of one manuscript deposited in bioRxiv, and co-author on three additional manuscripts. The Ph.D. thesis is presented as a short version with a general introduction and each manuscript discussed in a separate section. In the thesis, the author clearly defines the extent of her contribution to individual publications.

Anastasiya Klebanovych focused on the characterization of novel components and properties of centrosomal microtubule nucleation machinery. Although the basic mechanisms of the centrosomal microtubule nucleation are well understood, the actual regulatory mechanisms that control and fine-tune microtubule nucleation are still quite mysterious. A recurring theme in this work is the identification of novel components associating with centrosome, and by using established as well as newly developed assays determine their role in MT nucleation. In this matter, the thesis is not conceptually monothematic but rather presents several pieces of the MT nucleation puzzle.

The work presented in this thesis brings novel findings that substantially and non-trivially broaden our knowledge about microtubule nucleation in a different biological context. The attempt to characterize several factors involved in the microtubule nucleation – from protein phosphorylation through the protein modification to the protein association with centrosome - is impressive and represents the strength of presented thesis. From my point of view, the key and a very interesting part is the crosstalk of signaling pathways (SHP-1, ULF1 pathway, PAK1) with the centrosome and with the process of microtubule nucleation. In particular, the identification of ULF1 and C53 as negative regulators of microtubule nucleation involved in endoplasmic reticulum stress response is potentially a far-reaching observation that points to a new role of ufmylation in modulating microtubule organization.

The presented Ph.D. thesis in terms of content is very well written although I find it quite succinct. The condensed form of the text then often leads to generalized descriptions, and the text sometimes reads as a list of facts. As far as I can judge, the presented thesis contains a minimum number of grammatical errors, typos and stylistic clumsiness that are otherwise typical for this kind of work. Yet rarely, the work contains inaccurate statements, e.g. "*microtubules target small molecules called Tubulin-Binding Agents (TBAs), affecting microtubule dynamics"* (pg. 8), or that U2OS cells have epithelial morphology (pg. 23). In my opinion, the presentation of at least some manuscripts should be supported by a model

summarizing the findings. However, these minor shortcomings do not lessen the high formal level of work.

Conclusion:

Despite the abovementioned comments, the methodological and professional level of the work is very good. From the results presented it is clear that the goals of this study have been achieved. The multiple experimental approaches applied many procedures and techniques, as well as the analysis of obtained results, show the applicant's competence in conducting research. Taken together, it indicates that the author is fully prepared for the scientific carrier if she has chosen so. Based on the quality of Anastasiya Klebanovych's Ph.D. thesis I recommend it to be fully accepted as the fulfillment of the requirements for the degree of *philosophiæ doctor*.

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TOMÁŠ VOMASTEK, PhD
Institute of Microbiology ASCR
Vídeňská 1083
142 20 Prague-4
E-mail: vomy@biomed.cas.cz
tel: (+420) 241 062 166

I feel that the results are very strong in providing a fresh insight into the complex functions and the regulations of γ–tubulin and centrosomal tubulin polymerization. Considering the fact that the results are presented in the form of already reviewed and published manuscripts, my questions are more or less general:

1. The first question concerns the centrosomal and the non-centrosomal microtubule nucleation. As indicated in the text, centrosomal nucleation is typical for migrating cells. Indeed, the position of MTOC and radial microtubule arrays pointing to the leading edge are considered hallmarks of polarized mesenchymal motile cells such as fibroblasts. On the other hand, e.g. differentiating epithelial cells display typically a non-centrosomal mode of microtubule nucleation. I would like to ask whether it is known what happens during the epithelial-mesenchymal transition, the trans-differentiation process during which epithelial cells lose the epithelial characteristics and gain mesenchymal single-cell migratory phenotype? Is it known if there is a switch from non-centrosomal to centrosomal microtubule polymerization and if yes, how is such switch regulated?
2. The second question concerns the localization of SHP-1. On page 31 the author states that "*we could not detect SHP-1 at the centrosome using a limited set of commercial antibodies*". Moreover, in the bioRxiv manuscript, SHP-1 tagged with RFP was used as the MTOC-localization negative

control in U2OS cells. Has the author examined the localization RFP/GFP-tagged version of SHP-1 (or catalytically dead SHP-1) expressed in KO bone marrow-derived mast cells (BMMCs) activated by antigen or pervanadate?

In addition, it is shown that in SHP-1 KO BMMC cells there is increased tyrosine phosphorylation and delayed dephosphorylation of cellular proteins, as well as of GIT1 and Syk. Is the tyrosine phosphorylation of γ–Tubulin also affected?

Related question: Phospho-tyrosine antibodies are usually very good for immunofluorescence localization studies. Has the author examined phospho–Tyr signal in centrosome in SHP-1 KO cells or cells activated by antigen or pervanadate?

3. In the text, the author states "*Although the ufmylation process is analogous to ubiquitination, it differs functionally and serves as a non-proteolytic signal*" (page 18). On page 162 and related figure 4B, it is evident that C53 protein level is decreased in the cells deficient in UFM1-protein ligase 1 (UFL1). Does it mean that ULF1 stabilizes C53 protein by attachment of UFM1? Attachment of UFM1 should change the mobility of C53; has it been observed?

   Following this argument, ULF1 is in complex with γTuRC. Is any protein of γTuRC complex modified by UFM1?