

## HABILITATION THESIS REVIEWER'S REPORT

### Masaryk University

**Applicant**

Mgr. Lukáš Čajánek, PhD

**Habilitation thesis**

Molecular Control of Primary Cilium by Distal Appendages and Associated Proteins

**Reviewer**

Mgr. Jan Mašek, PhD

**Reviewer's home unit,  
institution**

Dpt. Of Cell Biology, Faculty of Sciences, Charles University, Prague

Dear members of the habilitation committee,

It is my genuine pleasure to provide this assessment in support of the habitation of Mgr. Lukáš Čajánek, PhD., applying for the title of Docent at Masaryk University.

Upon returning to Brno and establishing his group in 2017, Dr. Čajánek has demonstrated a clear and consistent record of research independence. As demonstrated by the presented habitation thesis and track record, his group has made meaningful contributions to the mechanistic understanding of ciliogenesis, with a particular focus on the kinase-driven events that control the initiation of primary cilia assembly. His work on TTBK2 — including characterization of novel substrates such as KIF2a, DVL3 and others, as well as detailed phosphosite mapping on CEP164 — has significantly advanced the field's understanding of how cilia formation is triggered and regulated at the distal appendage of the mother centriole.

More recently, his lab has opened an important new direction by demonstrating that the related kinase TTBK1 can regulate ciliogenesis in the context of human pluripotent stem cell-derived neural rosettes, placing his research squarely at the intersection of basic cilia cell biology and clinically relevant human cellular models. The group's methodological repertoire — combining proteomics, CRISPR gene editing, super-resolution microscopy, live-cell imaging, and stem cell differentiation assays — reflects both ambition and real technical capability. If there is a "missing piece of a puzzle" in the thesis as well as in the experimental repertoire of Dr. Čajánek is the *in vivo* aspect. Where, when and to what extent primary cilia, and related mechanisms matter in development and homeostasis, and to what extent can be the cell line-based findings extrapolated to those. While far from trivial, such interconnection is crucial for complete understanding of the topic.

Dr. Čajánek leads a group that includes multiple post-doctoral researchers and graduate students, and he does by all accounts take his mentoring task seriously, as I could repeatedly witness firsthand at several conferences, and from the successful PhD defences of his former students. Dr. Čajánek His ability to repeatedly secure independent international and national funding, confirms his independence as a group leader.

Beyond his own laboratory's output, Dr. Čajánek has invested genuine effort in building a broader research community. His active participation in and co-founding of CiliaNet CZ — a network connecting cilia and flagella researchers across Czech institutions — reflects an understanding that science advances through collaboration as much as through individual

laboratory output. This kind of community-building work is often undervalued in formal assessments, yet it is precisely the sort of contribution that strengthens a national research environment and creates lasting benefit for junior researchers and interdisciplinary projects alike. Dr. Čajánek has contributed to this network not as a peripheral participant but as one of its driving forces.

In summary, Mgr. Lukáš Čajánek, PhD. presents a strong case for habilitation. He has established an internationally recognised research group at Masaryk University, demonstrated sustained independence in research leadership and funding acquisition, contributed actively to the scientific community beyond his immediate group, and maintained a genuinely supportive environment for the next generation of researchers. From the materials I received, I cannot assess Dr. Čajánek's involvement in the teaching and thus leave this part of assessment on the committee.

**Reviewer's questions for the habilitation thesis defence** (number of questions up to the reviewer)

- 1) Would you say TTBK1 could be involved in priming the TTBK2-mediated phosphorylation or facilitate/enhance the TTBK2 binding to CEP164 by contributing to the otherwise TTBK2-mediated phosphorylation? Can CEP164 phosphorylation occur elsewhere then on the mother centriole?
- 2) How, in your view, match the recent reports of TTBK2-induced phase separation the previously established INPP5E-PIPK1 $\gamma$  mechanism of TTBK2-CEP164 binding regulation.
- 3) From wider perspective, how makes phase separation sense with environment surrounding the mother centriole and the basal body – as low cytoplasmic circulation and dense cytoskeleton were shown to prevent it. Could the changes during the basal body maturation indicate when the phase separation could play role and when not?
- 4) Considering the unknown role of CEP164–TTBK2 module in transitional zone gating, did the proximity biotinylation assays you performed provided some hints, and if not why?
- 5) Would you say that primary cilia play more prominent role in development when proliferation and cell movement are much more pronounced, compared with homeostasis, when terminally differentiated cells reside in solid organs?
- 6) Is, in terminally differentiated cell, the daughter centriole a rudiment, or is its complex structure justified functionally?
- 7) Curiosity question, related to the Wnt/Dvl connection with primary cilia, do primary cilia polarize in tissue along the Wnt/PCP- induced pattern, does the reported effects on Wnt activity and vice-versa change with serum starvation?

**Conclusion**

The habilitation thesis entitled "Molecular Control of Primary Cilium by Distal Appendages and Associated Proteins" by Mgr. Lukáš Čajánek, PhD **fulfils** requirements expected of a habilitation thesis in the field of Molecular Biology and Genetics.

Date: 7/5/2026

Signature:

