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Dear Chair of the Thesis Committee,

This is to let you know of the supervisor's comments about the student Bc. Jan Kotal and about his thesis entitled as 'Production and functional characterization of tick salivary protease inhibitors'.

Bc. Jan Kotal performed his Master thesis research in the Laboratory of Genomics and Proteomics of Disease Vectors where I serve as the Principal Investigator. As his supervisor, at the beginning of his thesis I suggested his involvement in the artificial over-expression of different cysteine and serine protease inhibitors originating mainly from the salivary glands of the castor bean tick *Ixodes ricinus*. Bc. Jan Kotal immediately understood his role and involvement in the project (which receives funding support from GACR) and he did a tremendous body of work, thus managing to over-express in a prokaryotic system of gene over-expression (and in quantities exceeding a dozen of mgrs of pure protein) numerous tick protease inhibitors. This was possible mainly due to his passion for biochemical research and due to its devotion to the specific project; as a result he managed to optimize protein refolding in solution by using novel methodologies and kits.

Bc. Jan Kotal has proved to be very intelligent, resolute and highly motivated young scientist who can work completely individually and with his own initiative. During the work on his research project he has mastered a great number of important techniques which will definitely be of importance in his future research plans. Thanks to his high level of knowledge of scientific English he was able to travel abroad and familiarize himself with a repertoire of immunological methods at the cellular and organismal level, while visiting for two weeks the Technical University of Dresden and a laboratory in the front line of Biomedical research headed by Professor Triantafyllos Chavakis. Jan was able not only to plan his own experiments, but to successfully interpret the obtained results and to correlate them with already published data and the project progress. Due to its profound background of knowledge Bc. Jan Kotal fulfilled all the goals of his individual project and thereby he has prepared the fundamental basis for the continuation and successful completion of numerous other projects; one publication with his co-authorship is already under preparation, while numerous other publications will be prepared based on the biochemical and functional characterization of the proteins that he successfully over-expressed during his Master thesis. Accordingly, he is more than welcome to continue his PhD thesis research in our group, assuming that he wishes to do so and assuming that a successful outcome in his related exams will be achieved.

While spending the maximum possible time in the laboratory, Jan has been proven as a friendly and easy-going person, always ready to help and support the other members of the group. He responded exceptionally well in all my very demanding comments and requirements during the research experimentation and the writing of his Master thesis. In conclusion this is to recommend his thesis defence and I kindly suggest an excellent grade for it.

Yours sincerely,

Michail Kotsyfakis PhD