Dear Professor Puta,

It is a pleasure to provide you with an overview and evaluation of the Master thesis of Jitka Simandlova who conducted her work in my laboratory at the Institute of Molecular Genetics AVCR v.i.i.

This Master thesis deals with the role of F-box helicase 1 (FBH1) in the regulation of homologous DNA recombination (HR) in human cells. HR provides an error-free mechanism for repair of DNA double-strands breaks that occur in S-phase of the cell cycle. However, HR has also the potential to generate chromosomal rearrangements if it occurs in an inappropriate way. A critical reaction during HR is the formation of RAD51 filament on single-stranded DNA (ssDNA). This nucleoprotein structure mediates homology search and strand exchange, allowing copying the damage DNA sequence from the donor DNA molecule. Several DNA helicases including FBH1 have been implicated in the regulation of RAD51 filament assembly. Previous studies in S. pombe have indicated that Fbh1 limits Rad51-dependent recombination at blocked replication forks. In human cells, FBH1 accumulates at sites of DNA damage and prevents loading of RAD51 on chromatin to suppress HR. In this Master thesis, Jitka Simandlova provides biochemical evidence suggesting that human FBH1 interacts physically with the RAD51 recombinase and possesses the ability to disrupt RAD51-ssDNA filaments in a manner dependent on its ssDNA-translocase activity and the presence of ssDNA-binding factor RPA. These are very important findings that further our
understanding of the molecular mechanisms underlying the regulation of HR in mammalian cells.

The thesis is generally well written. It begins with a comprehensive review of the current knowledge of the mechanisms of homologous recombination and its regulation, which provides the reader with a thorough overview of the concept addressed in the thesis. The experimental procedures and the results are clearly described, and the data presented are of a high quality. Discussion contains a critical evaluation of the results in the context of published work on regulation of homologous recombination. Moreover, based on the current data, the author proposes a model for FBH1 function in HR and outlines future directions to test its validity.

In summary, scientific content of the Master thesis of Jitka Simandlova is very high. The presented data form the basis for a future publication in a good scientific journal. I recommend that the Faculty of Science of the Charles University in Prague accept the Master thesis of Jitka Simandlova without reservation.

Yours sincerely,

Pavel Janscak