Opponent's report of Ondrej Ballek diploma thesis
RACK1 AS A CANDIDATE PROTEIN INVOLVED IN THE REGULATION OF TRANSLOCATION OF LCK TO LIPID RAFTS

In the presented diploma thesis Ondřej Ballek focused on the mechanism of translocation of non-receptor tyrosine protein kinase Lck to the lipid rafts during the activation of naïve T-cells. Specifically, he identified scaffold/adaptor protein RACK1 as Lck binding partner and showed that SH2, SH3 and C-terminal domains of Lck are necessary for efficient association of Lck with RACK1. He also demonstrated that RACK1 associates with Lck in T-cells and that this association is rapidly abolished when T-cells are activated. Furthermore, it was demonstrated that RACK1 and Lck co-redistribute to specific cellular structures. In the last part author focused on the dynamic of Lck redistribution to the lipid rafts and showed that both Lck and its partner RACK1 are present in high molecular weight complex and that after T-cell activation Lck translocates to lipid rafts.

Presented diploma thesis is very well written and I really enjoyed reading it. The introductory part has a logical structure which goes from description of the process of T-cells activation and formation of immunological synapse to description of main signaling events during this process. Author then highlights the role of protein kinase Lck and Lck translocation to lipid rafts during T-cells activation, and suggests that adaptor/scaffold protein RACK1 may regulate this process. In the result section, author tests this hypothesis. He adopted many methodological approaches including biochemical, biological and imaging techniques to analyze the role of Lck and RACK1 during T-cell activation. The results are then discussed point-by-point and the conclusions are largely justified.

Overall, this diploma thesis is very good quality and in my opinion represents solid foundation for the paper with high potential. I did not find any really weak points and there is no major concern about the data quality and results interpretation, although one part needs clarification.

My major question concerns the experiment concerning the structure-function analysis of Lck-RACK1 interaction. Although author states that RACK1 associates only with activated Lck but not with wild-type Lck the experiment shown in fig. 4.3 clearly shows association of wt-Lck with RACK1. Also the results shown in Fig. 4.5. and 4.6. show that T-cell activation results in Lck activation (as judged by tyrosine phosphorylation) and disruption of RACK1 – Lck complex suggesting that RACK1 may also associate with inactive Lck. Is it possible that RACK1 preferentially associates with "primed conformation" of Lck that is still inactive but susceptible for activation or can author come with alternative explanation?

I have also very general question. For me it is surprising that author did not use interfering RNA for knocking down RACK1 protein and estimating its role in T-cell

activation and Lck translocation to lipid rafts. Is there any technical limitation why this kind of experiment could not be done?

Minor points:
Figs.4.3. & 4.6., panels B: It is not described how quantification of western blots was done. Although these data are only supportive to western blots it should be done at least from duplicates to show the reproducibility of the results.

Figs. 4.8. & 4.9. The immunofluorescence figures are mediocre quality as printed and too small to allow careful inspection of the data. It would be better to print it on glossy paper and to show enlarged areas of interests in separate panels.

Editing and typos:
There are also some editing and typographical errors typical for this kind of work. These are mentioned for author's information and it is not necessary to answer them:

Pg. 5. "Divý typ" should be "Divoký typ"
Pg. 7. Lipofectamin should be Lipofectamine (also pg. 37 in the chapter heading)
Pg. 11. Phrase that RACK1 is "important cytoskeletal element" is over-statement.
Pg. 24. It is not stated what GM1 and GM3 mean (however, it is explained later on in the text)
Pg.29. Typo: 36k Da should be 36 kDa
Pg. 30 "RACK1 is express…" should be "RACK1 is expressed…"
Pg.34. I think that by "inactivated fetal calf serum" author meant "heat inactivated fetal calf serum"
Pg. 35 & 36. Incorrect manufacturers name (Milteney Biotech instead of Miltenyi Biotec, Gemomed instead of Genomed)
Pg. 38. Chapter 3.6 refers to itself.
Pg. 38 "were bounded" is grammatically incorrect
Pg. 39 "…buffer for (WB)" – parentheses do not make sense
Pg. 40. Typo: orthovanadatenatrium
Pg. 41. "then places" should be "then placed"
Pg.41. I think that instead of "procedure was implied…" author meant "procedure was applied". The same at page 51.
Pg. 43. List of primers include primers for MORG1 and EED which are not described anywhere in the text.
Pg.44. In the text the section 2.8.1.is referred. But this section does not exist in this thesis.
Pg.47. Figure is labeled as fig. 3 but it should be Fig. 4.3.
Pg. 51. Instead of "RACK1 is depend on" should be either "RACK1 is dependent on" or "RACK1 depends on"
Pg.60. Typo: Y50F-Lck
Pg. 64. Typo: moesin)

Conclusion:
From the results presented it is clear that the goal of this study has been largely achieved. I would like to stress out one more time that that the presented data are novel and

although "the story" has not been finished yet I believe that it will be finished soon considering the fact that Ondřej Ballek will continue his work in Dr. Filipp lab as PhD student. I also appreciate the fact that the thesis is written in English that further strengthens the potential of Ondřej Ballek as a future PhD candidate. Based on the quality of Ondřej Ballek diploma thesis I recommend this thesis to be fully accepted as the fulfillment of the requirements for the degree of Master of Science.

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