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Review of the Doctoral Dissertation of Raghunandan Mahadeva

The doctoral dissertation "Iron Deficiency Uniquely Reprograms the Amino Acid and Lipid Metabolism of Red Pulp Macrophages to Support Enhanced Iron Recycling Functions" by Raghunandan Mahadeva was prepared under the supervision of Dr hab. Wojciech Pokrzywa (main supervisor) and Dr Katarzyna Mleczko-Sanecka (auxiliary supervisor). Raghunandan Mahadeva was enrolled in the Warsaw PhD School in Natural and BioMedical Sciences and the dissertation was submitted to the Doctoral Committee at the International Institute of Molecular and Cell Biology in Warsaw.

The main goal of the work described in the dissertation was to characterize the metabolic response of red pulp macrophages to systemic iron deficiency. The research direction and the results are novel and are part of the research program of Dr Katarzyna Mleczko-Sanecka. The title of the dissertation fits its topic which is relevant to development of the discipline of biological sciences, hematology and specifically erythrocyte biology and iron homeostasis.

The dissertation is in the form of two articles (Manuscript 1 prepublished in *bioRxiv* (2026). DOI: <https://doi.org/10.1101/2025.06.13.659526>, and Manuscript 2 published *EMBO Rep.* 2026 Feb;27(3):598-628. doi: 10.1038/s44319-025-00673-5. Epub 2026 Jan 6.). Additionally, the submitted thesis analyzes and discusses in detail material from Manuscript 1 and devotes pages 30-31 to a brief discussion of Manuscript 2. Outside of the two articles, there is an abstract in English and Polish, an extensive introduction (pages 11-21), briefly stated research objectives (page 21), review of the results (pages 22-29 for Manuscript 1 and pages 30-31 for Manuscript 2), discussion pertaining to Manuscript 1 (pages 32-36), future directions (pages 37-39) and a bibliography (pages 40-48). The mandatory declarations of the Candidate's contributions to the articles are included after each Manuscript. The dissertation is written in English, and is remarkably clear and well-organized. Overall, the writing is logical and methodical, and the reference list is appropriate.

The *Introduction* is well written, starting with a review of the role of iron in mammalian biology, then an incisive analysis of cellular and systemic iron homeostasis, followed by an introduction of the special features and functions of red pulp macrophages as specialized iron-recycling cells. Figure 1 summarizes these functions and related mechanisms. The following sections discuss the metabolic features of efferocytic macrophages, focusing on amino acids and lipids, and proposing red pulp macrophages as a special case of efferocytosis. It would have been helpful to have a few more explanatory figures.

The *Results* section describes the contents of the Manuscripts 1 with only a brief section on Manuscript 2. Both of these manuscripts have many authors but the candidate clearly describes

his substantial role in the research and analysis pertaining to each manuscript. The *Results* focus on aspects of research in which the candidate had a major role. Here too it would have been helpful to use a few more explanatory figures, especially one focused on the proposed schema by which iron deficiency intensifies erythrophagocytosis and its metabolic underpinnings.

As a whole, the commentary (pages 11-48) added to the two manuscripts demonstrates an excellent theoretical and experimental background of the Candidate in the biological sciences, in hematology, iron biology and metabolism, a broader range of subjects than is usually covered in PhD theses.

Both manuscripts are multiauthored but the specific contribution of the candidate is clearly documented and includes a substantial proportion of Manuscript 1 but only a smaller part in Manuscript 2. Both works have extensive documentation and represent a large number of experiments so the volume of work presented by the applicant is adequate for a PhD thesis. The quality of the experimental evidence is high, and the interpretation is insightful and appropriately restrained.

In "*Future Directions*" the candidate raises several reasonable options for extending the work further. I especially endorse the big physiologic question, the fate of the iron that is generated by intensified erythrophagocytosis during iron deficiency. It is possible that the crucial function of the recovered iron is to prevent severe iron deficiency from impacting vital nonerythroid functions, and that the supply of iron to erythropoiesis is secondary. To ask this question specifically, it would be necessary to test the iron distribution under conditions of increasingly severe iron deficiency, and to use isotopic iron to monitor how iron is allocated.

I also endorse the second big question, the specificity of the red pulp macrophage response for erythrophagocytosis, as compared to phagocytosis of other distinct cargoes. This question should be asked in the setting of iron deficiency to ascertain whether there is a second signal provided by erythrophagocytosis, and if so what the nature of that signal is.

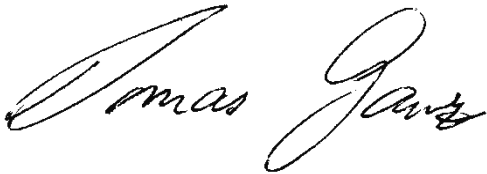
Finally, I think that the current version of Manuscript 1 has an undiscussed gap that needs to be addressed in the future. Although the roles of ferroportin and Syk kinase in the control of macrophage erythrophagocytosis and metabolic adaptations are well supported by experiments, it is not made clear how the two are connected. Syk is activated by receptor associated kinases but these are not known for ferroportin. This is another area that could be explored in much more detail.

My main criticism is not directed at the candidate but at the (international) system which values large manuscripts with broad claims, scope and impact. Such manuscripts make it difficult to make and document individual contributions. The candidate and his supervisor tried to do this well but ultimately there is no substitute for manuscripts which define smaller areas of research and involve fewer authors. Such smaller publications also provide more motivation and greater personal satisfaction for graduate students and postdocs.

In summary, this is an excellent PhD dissertation, containing impactful research which is well documented and presented. The subject matter, experimental evidence, discussion and presentation are at the top world class level in the area of erythrocyte biology and iron metabolism.

I, the undersigned, hereby state that the doctoral dissertation of Raghunandan Mahadeva meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Raghunandan Mahadeva to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

Sincerely,

A handwritten signature in black ink, reading "Tomas Ganz". The signature is fluid and cursive, with the first name "Tomas" and the last name "Ganz" clearly distinguishable.

Tomas Ganz, Ph.D., M.D.
Distinguished Professor of Medicine and Pathology