



LUND UNIVERSITY
Faculty of Medicine

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Ronald Myers, PhD.
Science Editor JoVE

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Dear Dr. Ronald Myers,

I am pleased to resubmit the original research article entitled "Direct Lineage Reprogramming of Adult Mouse Fibroblast to Erythroid Progenitors" by Melissa Ilsley and colleagues for further consideration for publication in the *Journal of Visualized Experiments*. We thank the reviewers for their comments on our submitted manuscript. We have endeavored to address all concerns brought forward by the reviewers and yourself. In response to these comments, we have made a number of additions to the manuscript. One example is addition of microarray data comparing gene expression of successfully reprogrammed hemoglobinized cells with non-hemoglobinized cells, which suggest the non-hemoglobinized cells are macrophages.

Regarding major concerns Reviewer #1 and #2 express concern our method can not generate enough erythrocytes for transfusion and that we need to demonstrate how efficiency and yield from the reprogramming. We first would like to clarify that we propose the method described in this paper is first and foremost useful for studying aspects of erythroid development, which certainly could lead to discoveries enabling industrial production of erythrocytes. Direct reprogramming *per se* is likely not a scalable method for generating erythrocytes for transfusion and we therefore removed a sentence suggesting this from the discussion. We also agree with reviewers that information about robustness and yield is valuable to the manuscript and has attempted to address both of these concerns by providing details reprogramming ability of cells from set numbers of fibroblasts and included some further discussion about this concern.

Two other major concerns from reviewers are that reprogrammed cells proliferate poorly and have low enucleation efficiency. We now explain this is because cells are reprogrammed to primitive erythroid progenitors which are known to hardly proliferate *in vitro* and do not enucleate. We have corrected the grammatical issues pointed out in the manuscript and accompanying figures.

Finally, to simplify the process, I have attached a second document outlining our responses to all other comments.

Thank you for your consideration,

Sincerely,

Johan Flygare