**RESPONSE TO EDITORIAL COMMENTS**

Dear Prof. Lavie,  
  
Your manuscript JoVE54826R2 "Development and identification of a novel subpopulation of human neutrophil-derived giant phagocytes in-vitro" has been peer-reviewed and the following comments need to be addressed. Please keep our formatting requirements in mind while revising the manuscript to address peer review comments. Please maintain the overall manuscript changes, e.g., if formatting changes were made, commercial language was removed, etc. **DONE**  
Please track the changes to identify all of the manuscript edits. After revising the submission, please also upload a separate document that addresses each of the editorial and peer review comments individually with the revised manuscript. For each comment, please provide either (1) a description of how the comment was addressed within the manuscript or (2) a rebuttal describing why the comment was not addressed or out of the scope of this work for publication in JoVE. **DONE**  
  
Your revision is due by **Aug 18, 2016.**   
  
Please note that due to the high volume of JoVE submissions, failure to meet this deadline will result in publication delays. To submit a revision, go to the [JoVE Submission Site](http://www.editorialmanager.com/jove" \t "_blank) and log in as an author. You will find your submission under the heading "Submission Needing Revision".  
  
Sincerely,  
  
Mala Mani,  
Science Editor  
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**Editorial comments:**  
• Your manuscript has been modified by your editor, please maintain the current formatting throughout the manuscript. **Please use the updated manuscript located in your Editorial Manager account (under “File Inventory”) for all subsequent revisions**. **DONE**  
• Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammatical errors. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version. **DONE**  
• \*JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information. **DONE- except for refs 10, 17, 19 which do not have a doi number**  
  
• If you are re-using figures from a previous publication, please obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**DONE –** we used some pictures from our previous publication (references 14 (JLB) and 15(Hindawi Publishers). From both papers we have publishers permission to reuse data since both paper are free access and we attach the forms in supplemental files. We also cite the figures appropriately in the figure legend.

• Please remove Transwells (trademarked term) from Figure 8. **Removed**  
• 2.7: Please provide a reference for analyzing the slides by fluorescence microscopy. Mentioning the microscope details in the Table of Materials is not sufficient. **Corrected, we analyzed the data by software (ImageJ 1.49) which is specified in the table.**  
• Please update the access type to Open in the attached Author License Agreement (ALA). Scan the updated ALA and upload it with your manuscript files.   
•Formatting:  
-Unless some of the identification is to be shown in the video, please remove reference to it from the manuscript title. **N/A**

-Paraformaldehyde is toxic and requires a caution statement. **Corrected to** “…**with 100 µl 4% paraformaldehyde under a chemical hood…..”** Using such a low concentration and a small volume under hood is safe. All other instructions for handling paraformaldehyde are specified in manufacturer’s instructions.

•Grammar:  
-Line 66 – Do not begin sentences with conjunctives like “but” or “and”. **Corrected**-1.1 – “from informed consent young healthy adults”; “vain” **Corrected**-Line 185 – “allows to harvest at the same time mononuclear cells and neutrophils” – awkward phrasing - **Corrected**-1.2.15 – “IH-FCS” or “HI-FCS”? **It is correct as is “HI-FCS”**  
  
•Additional detail is required:  
-1.2.7 – About what volume of PMN layer is transferred? – The volume of the band containing the PMN varies between individuals and therefore we cannot add a specific value. As mentioned the bands described fig 2 are opaque and can be identified easily.

-1.2.10.1 – How does one check for contaminating RBC? If judging by the pellet, this step should appear after 1.2.11.

While the PMN are white blood cells and appear white, the red blood cells are RED. Therefore, are clearly noted in the pellet. We added to 1.2.10.1 “note: If contaminated by RBC, the PMN pellet appears reddish.”

-2.1 note – How would Gφ be detected in the removed medium? **Corrected in the note to 2.1**

-2.2 – How are washes performed? (ie volume, length of time, etc.). **Added a note.** The washing volume can vary depending on the perimeter of the cells that were cytospun on the slide and that depends on the specific centrifuge and rotor used. We used 100 µl, but in each lab this can vary according to the equipment used. The term “wash” it commonly used to be an immediate procedure of few seconds depending on the person capabilities for doing the washing. Again here we used 100 µl per wash but other labs can use more or less. The volume is not critical, only the perimeter of the cells is and that depends on the cytospin used.

-2.4, 2.5 – What are antibodies diluted in? **Added**

•Branding: 2.1, 2.1.1, 2.2, 3.9 plus 7 occurrences in figure legends – while cytospin is a method, it is also a trademarked term. We can allow the use in 2.1, but all other instances should use a generic term/phrase.

Unfortunately, I don’t know any other term to replace “cytospin”- unless I write a whole sentence to describe the procedure over and over, and that will be confusing. Although this term was originally used by “Shandon” centrifuges over 40 years ago, currently it is used as a generic term to describe centrifugation of cells onto slides for microscopy analysis and many companies hold centrifuge rotors for cytospin preparation. Definition of cytospin from the internet “The **cytospin process** is a simple **procedure**. Cells are washed in a serum and/or albumin based PBS or culture media solution. The cells are resuspended in up to 500 µl of this solution. A cytofunnel is attached to a glass slide and slide carrier.” Thus, currently it is a generic term and should remain as such in the paper.   
  
**Reviewers' comments:**  
**Reviewer #1:**  
*Manuscript Summary:*  
In their manuscript „ Development and identification of a novel subpopulation of human neutrophil-derived giant phagocytes in-vitro „ Lavie and colleagues describe preparation of a neutrophil-derived phagocytic cell population.  
  
*Major Concerns:*  
The MS is fine with me. I have no criticism (a seldom case).  
  
*Minor Concerns:*  
The MS is fine with me. I have no criticism (a seldom case).  
  
*Additional Comments to Authors:*  
N/A  
**Reviewer #2:**  
*Manuscript Summary:*  
This article describes an interesting new subpopulation of human neutrophil-derived giant phagocytes. The microscopy data is of an extremely high standard. The manuscript is enhanced by demonstrating this new cell population can undergo phagocytosis, autophagy and have an extended lifespan. The method is clear and well written.  
  
*Major Concerns:*  
No major concerns were raised in my review of this article.  
  
*Minor Concerns:*  
Please correct "vain" on in section 1.1. **Corrected**  
*Additional Comments to Authors:*  
N/A