October 6th, 2018

Dear Editor,

Please, find enclosed our revised manuscript, entitled "**In vitro differentiation model of human normal memory B cells to long-lived plasma cells**" to be considered for publication in Jove.

We thank the reviewers for their comments that help us to improve our manuscript. We have answered all comments and we hope that our manuscript could be published in Jove.

The manuscript has been seen and approved by all listed authors.

The authors have no conflict of interest to declare.

With my best regards,

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***Editorial Comments:***

*• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.*

*•* ***Protocol Language:*** *Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.*

We checked the manuscript.

*•* ***Protocol Detail:*** *Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video.* ***Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. All of your steps lack explicit details and are too general to film or resproduce.*** *There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:*

*1) Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.*

An ethics statement was included in the manuscript.

*2) Line 87: Unclear how peripheral blood cells are obtained. Please describe the stepd from the time of blood draw to when the PBC are obtained (e.g. centrifugation etc). Mention all centrifugation speeds in g and report durations and temperatures.*

The manuscript was modified with more details included (p 3, lines 89-97)

*3) Line 88: Unclear exactly is done. What is the bead concentration? How is the purification done? Describe the gating strategy.*

We described these points with more details.

*4) Lines 91-99: Mention culture duration and environmental conditions.*

We modified the manuscript including these informations.

*5) Line 103: Unclear how these steps are performed as most details are absent.*

We included more details within the manuscript.

*•* ***Protocol Numbering:*** *Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.*

The numbering was modified.

*•* ***Protocol Highlight:*** *Please highlight ~1-2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.*

*1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.  
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.*

*3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.  
4) Notes cannot be filmed and should be excluded from highlighting.  
5) Please bear in mind that software steps without a graphical user interface/calculations/ command line scripting (such as the entire section II of the protocol) cannot be filmed.*

We highlighted parts of the manuscript accordingly.

*•* ***Discussion:*** *JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol*.

The discussion was extended.

*•* ***Figures:****1) Please increase the font size on axis ticks in fig 2.*

We modified the figure.

*2) Please remove the text “Figure #..” from the figure files.*

This was removed.

*•* ***Commercial Language:****JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Invitrogen, Sigma-Aldrich, R&D Systems, Peprotech, AbCys SA, FACSAria sorter, Becton Dickinson, IntronA, etc.*

*1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.*

We modified the manuscript.

*2) While this primarily applies to commercially available products, we ask our authors to sparingly use any specific product names in order to reduce the appearance of any bias. To this end, we ask that you please reduce the number of instances of "\_\_\_\_ GenomicScape \_\_\_" within your text.*

GenomicScape is a freely available webtool developed by our group. This resource is available for all the scientific community.

• Please define all abbreviations at first use.

• Please use standard abbreviations and symbols for SI Units such as μL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must 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]."

**Comments from Peer-Reviewers:**

**Reviewer #1:**

*In this manuscript by Jourdan et al. authors propose a strategy that enables in vitro MBC to plasma cell differentiation in humans. This offers a convenient alternative to obtaining differentiated cells from subjects which are both rare and remotely located. While the idea behind the manuscript is useful, article falls short in multiple aspects.*

*Major comments:  
1-In vitro Plasma cell generation has already been very well documented (Le Gallou et al. Journal of Immunology 2012; Cocco et al. Journal of Immunology 2012) Unfortunately authors, despite using a similar procedure to those described in these articles, do not refer to these publications and discuss why we need another method on top of theirs.*

As indicated in the manuscript, our protocol was defined in 2009 (Jourdan et al. Blood 2009) and improved in 2011 and 2014 (Jourdan et al. Journal of Immunology 2011 and Jourdan et al. Leukemia 2014). We discussed the plasma cell generation studies published in 2012 and the different methodologies p 6 to 7, lines 245 to 266.

*2-The differences in the protocol described in this manuscript compared to the previously published protocols is the use of IL15. The authors should justify the need to use these cytokines while others can generate PCs without them. Also authors should discuss the requirement for each reagent with relevant citations so that readers can have a better understanding of the protocol.*

We modified the manuscript p 6 to 7, lines 257 to 260.

*3-The functionality of the plasma cells generated through this strategy has not been shown. Is there a life expectancy limit for these cells? Also, how are their antibody production rates compare with equal amounts of in vivo generated plasma cells? There is no single demonstration of antibody production which should be included with representative ELISAs for each stage of differentiation.*

The goal of the manuscript is to present an illustrate the in vitro model of B to PC differentiation and GEP analyses using tools developed by the group. Antibody production rates were previously fully described by the team for the different differentiation steps (Jourdan et al, Blood, 2009; Jourdan et al. JI, 2011 and Jourdan et al. Leukemia, 2014). We modified the manuscript p 4, lines 144 to 147 and p 7 lines 273 to 277.

Other comments:  
*1-The term 'Epigenetic genes' should be replaced. This is not an existing term and authors should avoid generating new terminology.*

We modified the manuscript.

*2-Why are only certain genes compared but not the entire transcriptome?*

Entire transcriptional analyses were already done and published. Here, we focused on

*3-The methodological details of PCA analysis are irrelevant and can be excluded. This is a method that describes plasma cell generation.*

The method reported also a molecular atlas of B to PC differentiation using publicly available tools developed by our group. This molecular atlas includes publicly available GEP data related to B to PC differentiation (murine and human GEP data) (kassamabara et al. Plos Comp Biol. 2015).

*4-Authors should check the manuscript for grammatical errors and typos.*

The manuscript was reviewed.

**Reviewer #2:**

Manuscript Summary:

The authors described the cell culture method that would recapture in vivo differentiation process from human memory B cells to plasma cells. They also represent the bioinformatics tool for analyzing the gene expression data during memory B cell to plasma cell differentiation.

Major Concerns: None

Minor Concerns:  
The description of 'I - In vitro normal plasma cell differentiation model' would better be more precise. For example, the plating cell number was not described for the culture stage 2, 3 and 4. Also the explanation on 'Resto6 stromal cell' would be necessary. If the culture efficiency depends on the donor conditions etc, it should also be noted. This referee is interested whether the system is applicable to single cell culture.

We defined the part 1 of the protocol more precisely (p 3, lines 89-116). We also extended the description of Resto 6 stromal cells p 7, lines 266 to 270. We also add more details within the discussion p 7, lines 271-277.

We never tried to transpose the system to single cell culture. This is an interesting question that we will investigate in the future.