Response Letter

Dear editor,

We would like to thank you and the reviewers for your efforts in evaluating our manuscript entitled “Establishment of DR3 Overexpression Cell Line to Assess the Apoptotic Response to Anti-mitotic Therapeutics” (JOVE-58705).

We were grateful for the constructive comments by all the reviewers. Below please see additional data and the point-by-point response to editorial and reviewers’ comments. We have also made changes in the manuscript accordingly and highlighted the major changes.

We hope that you will find the revised manuscript acceptable for publication in *JOVE*.

Sincerely yours,

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Below is the point-by-point response to editor’s and reviewers’ comments.

**Editorial Comments:**

We appreciate the editorial comments.

*Textual Overlap:* We re-wrote the indicated text in red.

*Abstracts:* The short abstract was added before the long abstract as following: Cell models for gene overexpression are important tools for studying gene function. Picking single clones after retroviral infection is a direct way to generate homogeneous and pure stable cell lines. In this article, HT29-DR3 cell lines generated in this way help reveal the mechanisms by which DR3 contributes to anti-mitotics-induced apoptosis.

*Protocol Language:* we checked the language and wrote the protocol section in imperative tense.

*Protocol Detail:* We added protocol details and split 3.4 to 4 sub steps to depict the animal experiments.

*Protocol Highlight:* We highlighted about 2 pages of text that should be visualized in yellow.

*Figures:* Figures have been renewed as following:

Figure 2



Figure 3



These data have not been published.

*Commercial Language:* All commercial products have been sufficiently referenced in the table of materials. We replaced the commercial sounding language with generic names.

**Responses to Reviewer #1:**

**Reviewer #1:**  
*Minor Concerns: Although this looks like a promising study, the authors have just briefly provided their results and they have mostly regarded their figures. I would recommend them to emphasize their important results in the text, besides figures and tables. They should compare the efficacy of their method with a conventional one.*

Thanks for the constructive suggestions, we elaborate the data in Results section. As Figure 1 shows, different clones express variant levels of DR3 after infection, picking single clones ensure gene stability and homogeneity. In addition, pool of the cells probably could not reflect gene function significantly. By comparison with a conventional approach, such as limited dilution, this approach is simple and efficient.

**Responses to Reviewer #2:**

**Reviewer #2:**  
Manuscript Summary:  
*The manuscript described a procedure on how to establish a stable cell line with DR3 overexpression. This cell line can then be used as a cell line model to study anti-mitotic drugs. Overall, the experimental procedures were clearly written and the reagents were also listed.*

We are thankful for the comments from the reviewer.  
 *Major Concerns:  
1) In the introduction, more references are required to better justify the importance of establishing DR3 overexpressed cell line.*

DR3 is a totally novel molecule that discovered to be involved in anti-mitotic drugs induced apoptosis. Gain-of function is classical and direct way to study gene function. Therefore, overexpression cell line is the essential model to identify the molecular mechanisms of DR3 mediated apoptosis after treatment with anti-mitotic agents. As requested, we added references (Reference 10-13) to justify the importance of establishing DR3 overexpressed cell line.

*2) Step 2.3, line 132, usually the semi dry transfer wouldn't need hours for a complete transfer. The authors claimed the transfer was performed at 400 mA for 2 hrs. The authors should double check the experimental condition to ensure the accuracy.*

Step 2.3, To ensure sufficient transfer of protein of various sizes, we usually transfer the protein onto PVDF membrane by Wet Transfer at constant current of 400 mA for 2 h.  *Minor Concerns:  
1) In the abstract, line 22, "picked up" should be replaced with "picked".  
2) In table 1, "invitrogene" should be changed to "Invitrogen".*

We replaced “picked up” with “picked” in line 22, and the "invitrogene" with "Invitrogen" in table 1.