**Reply letter for editorial and production comments**

Changes to be made by the Author(s) regarding the written manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Reply: Revised.  
2. Please revise the title to be more concise.

Reply: The revised title: Using EGFP-expressing Escherichia coli to assess mouse peritoneal macrophage phagocytosis.  
3. Please add a Summary section before the Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

Reply: Revised.  
4. Please spell out each abbreviation the first time it is used.

Reply: Revised.  
5. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

Reply: Revised.  
6. Please include a space between all numerical values and their corresponding units: 15 mL, 37 °C, 60 s; etc.

Reply: Revised.  
7. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).

Reply: Revised.  
8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Alexa Fluor 633, PHrodo™, etc.

Reply: Revised.  
9. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Reply: Revised.  
10. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

Reply: Revised.  
11. 1.3, etc.: Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary.

Reply: Revised.  
12. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Some examples:  
Please ensure that conditions and primers are listed all PCR procedures.

Reply: Sure. The essential details were added into the video and the text. More details were given in the supplementary file.  
2.1: Please specify the age, gender and strain of mouse. Is the mouse anesthetized before injection?

Reply: Revised. The added text made it clearly now.  
2.3: How large is the dish?

Reply: 10 cm diameter. Added in the text.  
13. Please include single-line spaces between all paragraphs, headings, steps, etc.

Reply: Revised.  
14. Figure 2: Please define the error bars in the figure legend.

Reply: Revised.  
15. Please upload each Table individually to your Editorial Manager account as an .xls or .xlsx file.

Reply: Revised.  
16. Table 2: Please change uL to µL and include a space between the numerical value and the temperature unit (37 °C).

Reply: Revised.  
17. References: Please do not abbreviate journal titles.

Reply: Revised.  
18. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

Reply: Revised.  
  
Changes to be made by the Author(s) regarding the video:  
1. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

Reply: Sure. We have fully reviewed the text and video scripts.  
2. Please use the same subtitles in the video and in the written manuscript so that the protocol is easier to follow. Currently, step 1 in the video is to culture the EGFP-expressing E. coli while in the written protocol step 1 is to construct the pET-SUMO-EGFP plasmid and induce EGFP expression.

Reply: Thanks for advice. We've added some essential details to section 1.  
3. 01:50-02:13: This part in the video is hard to follow with the step 1.3 of the written protocol.

Reply: Revised.  
4. 03:20: Please change “50um” to “50 µm”.

Reply: Revised.  
5. The details in the video are not the same as the details in the written manuscript. Please cross-reference the video narration with the protocol text. Some examples:  
03:24-04:02: Such details in the video are not stated in the written manuscript.  
04:13: The video says 3 days while the written protocol states 3-5 days (step 2.1).  
05:01: The written protocol says “Perform a gentle massage on two sides of the mouse abdomen” while the video does not mention it.  
05:11: The video says 2-3 times while the written protocol states twice.  
05:33, 05:44: The video says “5×10”, while it should be “5 x 106” or “5 x 105” as stated in the written protocol (step 2.7).  
06:49: The voice over says 3-5 times while the written protocol and caption line say 3 times.  
07:07: The video says phallodin-iFluor 633 while the written protocol says phalloidin-Alexa Fluor 633.  
07:27: The video says with same volume of distilled water, while the written protocol says 200-500 μL (step 3.8).

Reply: Revised.  
6. 05:16: Please include a space between the values and their units (400 x g, 10 min, 4-8 °C).

Reply: Revised.  
7. 06:22: Please change uL to µL.

Reply: Revised.  
8. 06:37: Please make the number in CO2 a subscript. Please include a space between the numerical value and the temperature unit (37 °C).

Reply: Revised.  
9. 09:23, 09:52: Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).

Reply: Revised.  
10. 4:05: The anesthetized mouse clearly reacts to being rubbed here.

Reply: In fact, this clip wanted to tell the mouse was still alive. Because C57 mouse strain was susceptible to the sevoflurane, the mouse may pass out easily by overdosed sevoflurane. By showing the mouse reacts to being rubbed in the video, we wanted to tell the audience the mouse was still alive.

But the audience may raise other questions: 1. Did the mouse suffer the pain? 2. What's the purpose by doing that?

For the first question, the answer is no. According to the stages of anesthesia, we can tell the mouse was in anesthesia stage II, that is unconscious but may have body response to the rub. Actually, after the injection, the mouse stayed unconscious for 3-6 minutes until it was recovered from anesthesia. In the next 3 days after injection, the mouse general conditions were normal and the body weight did not changed markedly.

For the second question, what's the purpose of doing this. Peritoneal injection had a high risk of injuring the bowel, especially when the mouse struggling during injection. Therefore, by inducing anesthesia, the peritoneal injection can be easily performed and reduce the risk of injuries to the internal organs caused by injection.

We also explained briefly in the text.  
11. Please use the same figures for presenting results in the video and the written manuscript.

Reply: Figure 1 had Revised.   
12. 4:43, 4:57, 6:53, 8:08, 8:53, 9:04, 9:09, 9:11, 9:31, 9:41 - The edits here are jump cuts, which tend to have a jarring effect on the viewer. They should be smoothed out with crossfades instead.

Reply: Revised.  
13. Please upload a revised high-resolution video here:  
<http://www.jove.com/files_upload.php?src=17919103>  
Reply: The revised file named JOVE58751-HD-R2.mov has been uploaded.