**Editorial 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.  
2. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .svg, .eps, .psd, or .ai file.  
3. Figure 5: Please add a panel label to each graph so they can be better identified in the manuscript.  
4. Please remove the embedded table(s) from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.  
5. Please provide an email address for each author. Done  
6. Keywords: Please provide at least 6 keywords or phrases. Done  
7. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. Done  
8. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations. Done  
9. Please include single-line spaces between all paragraphs, headings, steps, etc. Done  
10. 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:  
Lines 68-69: Under what conditions are the cells propagated? Done  
Line 71: Please specify centrifugation parameters (force and time). Please specify throughout the protocol. Done  
Line 153: Please add more details here.  
Line 162: Please specify the age, gender and strain of mouse used. Done  
Line 166: Please specify the euthanasia method. Done  
Line 168: Please specify the size of the petri dish used. Done  
11. 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.  
12. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:  
a) Critical steps within the protocol  
b) Any modifications and troubleshooting of the technique  
c) Any limitations of the technique  
d) The significance with respect to existing methods  
e) Any future applications of the technique  
13. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Done  
14. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references. Done  
15. Table of Equipment and Materials: Please provide lot numbers and RRIDs of antibodies, if available. Done  
  
**Reviewers' comments:**  
  
Reviewer #1:  
  
This manuscript provides a protocol and representative data to evaluate how Listeria monocytogenes escapes from cells after replicating in the cytoplasm. This is an important step in Listeria pathogenesis and the authors provide a reasonable description of the protocol and provide data to support most of their conclusions. There are, however, a few major and multiple minor comments that should be addressed.  
  
Major Comments to the authors:  
1) In figure 2A, the authors state that there is no phalloidin signal in the uninfected samples, but clearly there is a fair amount of Actin-Rhodamine staining. Additionally, since the dot plot is showing S/N from uninfected samples, there should not be any events at all. Showing the FSC vs. SSC would be helpful, because in the uninfected S/N the events must be coming from debris, exosomes, air bubbles, etc. and not from Listeria. To strengthen these findings, microscopy should be shown.

We have added the FSC vs SSC plot in Fig. 2A

2) In this reviewer's opinion, figures 5 and 6 do not add very much value to the manuscript. However, if they are shown, a better description should be provided and the gating for figure 6 should be shown. Additionally, are other cells, including DC's macrophages, neutrophils, and hepatocytes infected with Listeria? Previous publications have implicated all of these cell types in either phagocytosing Listeria or becoming infected. Without comparisons to other cells, showing monocytes in isolation does not allow for a proper interpretation.

In lines 265-266 we have done a better job of explaining why the in vivo infections fit with the in vitro infection model. We only detect Listeria-derived GFP signal in the Ly6C monocytes.  
  
Minor Comments to the authors:  
1) List the other supplements in the DMEM media. There were no other supplements other than serum.  
2) When describing centrifugation steps, provide the RCF instead of stating "low-speed centrifugation". Done  
3) Clearly state when media contains antibiotic or not. Done  
4) On line 106, describe how to "harvest some wells". Does this mean S/N, cells or everything? Clarified  
5) On line 113, will cells be harvested?Clarified  
6) Define PFA. Done  
7) Describe and/or list the flow cytometer and analysis software used. Done  
8) List the vendors and clone names/numbers for all antibodies used. Done  
9) Line 245 should say "left panel". Done  
10) Describe how to isolate the peritoneal exudate macrophages. The appropriate literature is cited  
11) The protocol used for figure 4 should be described in the protocol section, not the figure legend. The experiment is somewhat complicated and, in our opinion, would not fit well in the protocol section.  
12) All of the strains of Listeria should be listed in a table with a description, antibiotic resistance and a reference to the original article. These strains are extensively described in the Results section and are cited appropriately.   
13) The original paper showing that specific depletion of neutrophils reduces host resistance to Listeria (EJI: 2011: vol 41, pp. 2666-2676) should be referenced on line 279. Done  
14) How reproducible are the data? How many times was each experiment repeated? Triplicate wells do not equal "n=3" and should not be used for statistical analysis purposes. This information is add to the figure legend.  
15) In the figure legends, the use of the term mouse macrophages is ambiguous. Are these primary cells or cell lines? The cell line is now identified (RAW 264.7).  
  
Reviewer #2:  
  
Manuscript Summary:  
The manuscript describes methods to monitor Lm emergence in in vitro and in vivo samples. The manuscript is well written and provides detailed procedures that can be easily followed by others and adapted to other intracellular, cytoplasmic pathogens.  
  
Major Concerns:  
The authors should consider adding some description of flow cytometry approaches to the introduction to compliment the introduction to CFU assays.  
  
Minor Concerns:  
1. Line 83: Add speed of shaker. Done  
2. Line 128: Clarify what you mean by step 1. Done  
3. Line 142: Clarify what you mean by described above (list step numbers). Done  
4. Line 164: Add an "and" between "plates" and "incubate". Done  
5. Line 230: It would be helpful for the reader to include a description of prf\* strain here (e.g., that this is a hypervirulent strain). This will help understand the results without reading the discussion. Done  
6. Figure 4: The legend describes this a competitive index that would result in a ratio of WT to mutant strains. However the data in the graph is represented as the total number of bacteria (CFU) per 10^6 cells (not sure which bacteria of the mixed population are shown). Please reconcile. Recommend showing as a competitive index, as this would be the most appropriate format to display the data.. Mistake on our part, has been corrected.  
  
Several places in the results, Ref. numbers need to be processed through your ref. management software. As these are different format from rest of the manuscript, I am not sure they refer to the correct reference as listed in Bibliography. Plese verify.