**REBUTTAL**

**Reviewer #1:**

*Minor Concerns:*  
*1. The order of procedures in the protocol should follow the order discussed in the text and on the video*.

All portions of the text have been re-ordered to match the order in the video. In particular, the introduction has been revised so that the order is the same as in the video and throughout the manuscript.

*2. The authors may want to suggest filtering of cells through 40-70 µm mesh prior to injecting into the facial vein to make sure particulates are removed. Injecting 108 splenocytes in a 50µl volume seems like quite a high concentration and may lead to clumping which the authors indicate results in blockage of blood vessels and death. Have you actually injected this many cells in the past?*

We agree that injecting 108 splenocytes in 50µl would be quite high (probably unacceptably high). As a general rule, we do not exceed a concentration of 2 x 108 cells/ml for injection into neonatal mice. Therefore, we do not routinely filter the cells because this results in non-specific losses of unknown numbers of cells. The text in the discussion has been corrected to indicate this important point.   
  
*3. In section 2.3 on systemic immunization it reads as if only 25µl can be injected in the peritoneum of 7 day old mice. This seems rather small even for a 7 day old mouse.*

Volumes larger than 25µl can be injected and, indeed, there are reports in the literature of i.p. injection of 50µl, even into 1 day old pups. However, there is little “open space” in the peritoneal cavities of murine pups. We have found that injection volumes exceeding 25µl result in leakages of unknown amounts at unacceptably high frequencies (≥20% of injections). Therefore, to ensure 100% delivery, we use the conservative volume of 25µl. This has been explained in the discussion in the revised manuscript.  
  
*4. In section 2.1 it indicates that the lack of leakage is an indicator of a successful subcutaneous injection. Is there a trick to preventing leaking?*

The only “tricks” are stated in the written procedure – i.e., that the injection and needle removal should be performed slowly.

*5. In section 3.5 it is stated that no needle is attached when bacteria are drawn into a 1 ml syringe prior to injecting through tubing. Since this could result in bacteria on the hub and exposed outside the syringe, it might be better to use a large gauge needle to draw up the bacteria and then switch to the needle with the attached tubing. This will reduce the likelihood of contacting the bacteria and potential exposure of personnel. This is only a suggestion for working with infectious agents.*

This is a good suggestion but adding it into the protocol at this point would require us to re-film the video footage. Although our current procedure hasn’t caused any obvious problems, we will consider incorporating this practice into our protocols in the future.