We appreciate the constructive comments provided by the Reviewers, and we have attempted to address them on a point-by-point basis below. The changes that we made were tracked in red in the main text.

**Reviewer #1**

1) The title of the paper suggests that it will focus on the use of whole-mount epifluorescence microscope which might be an area with more potential than dissection. Yet, this section was not very specific (a lot of it may vary).

**We described both embryonic heart dissection and whole-mount epifluorescence microscope to examine chamber specification during mouse embryogenesis using fluorescent reporter mice. Because successful dissection of embryonic hearts is a key to success of this protocol, we believe that it is important to describe the dissection method, in addition to epifluorescent microscope use.**

2) Figure 2 showed the ventricular specific expression of tdTomato. However, ventricles are easy to identify morphologically without using epifluorescence images. It is not clear how epifluorescence microscope images of ventricles are useful for analysis of embryonic cardiac formation.

**Although it is easy to identify ventricles in the post-natal heart, it is not straightforward to identify ventricles or future ventricles during early heart development. Our method will be helpful to study ventricular development or chamber specification during embryogenesis.**

3) The steps shown in Figure 1 are routine in laboratories handling mouse embryos. Therefore, general impact of this paper may not be high.

**We agree that the protocol described in this manuscript is not completely novel. However, it may fit into one of the aims in *JoVE*, “providing gold standard protocols that can serve trainees and other researchers trying to learn techniques”.**

4) Generation of the knock-in mouse model, such as vector construction, won't be a helpful content for a video. Perhaps those who want to perform electroporation, selection of ES cells, generation of mice from ES cells would be interested in seeing those procedures in a video.

**Generation of a knock-in reporter mouse line is not a topic that we focus on in this manuscript. However, we added an illustration of knock-in allele to describe genotyping primer designs (Figure 3A).**

5) All the important steps of the protocol were clearly stated and they lead to the outcomes presented in the paper.

**Thanks for your support.**

6) The main equipment and materials were listed with the company's name and the catalog number.  
**Thanks again for your support.**

7) The genotyping section was clearly organized and had all the details like the sequences of the primers. However, genotyping is already a well-established procedure, and it won't be a helpful content for a video.

**It may fit into one of the aims in *JoVE*, “providing gold standard protocols that can serve trainees and other researchers trying to learn techniques”.**

8) In the results, it was stated that tails were used for genotyping, but in the protocol it was stated that embryo's heads were used.  
**Thank you for pointing out the mistake. The embryo head was used for genotyping and the text has been changed accordingly.**

**Reviewer #2**

1) Epifluorescent imaging is a very useful tool, but it is limited to examine inside cardiac chambers. Since early mouse embryos are relatively transparent that allows confocal imaging without a clearing step, providing a wholemount cardiac chamber imaging protocol with a confocal microscope in addition to the current epifluorescent microscopy protocol would be additional benefits for this type of paper.  
**We agree that confocal imaging technique is a very useful tool to examine embryonic hearts. However, the main advantage of this protocol is to provide a simple tool for studying heart chamber specification in a regular lab setting without a need of expensive equipment.**

**Reviewer #3**

Although novelty is not required for publication, there are many such protocols available in the literature. The protocol is not a live imaging for embryonic hearts, nor is a three D imaging protocol.

**We agree that the protocol described in this manuscript is not completely novel. However, this article may fit into one of the aims in *JoVE*, “providing gold standard protocols that can serve trainees and other researchers trying to learn techniques”.**