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March 19th, 2019

Dear Dr. Upponi,

Please find enclosed our manuscript entitled, "Isolation of Macrophage Subsets and Stromal Cells from Human and Mouse Myocardial Specimens," which we are pleased to submit *JoVE*.

Immune cells are increasingly recognized to impart important effects on the heart under homeostasis and in the context of various cardiac diseases including myocardial infarction and heart failure. Among such immune populations, monocytes and macrophages have been implicated in coronary development, cardiac conduction, heart regeneration, and adverse left ventricular remodeling. Given these findings, there has been intense interest in this area and an expansion of laboratories studying cardiac immunology. At present, techniques to analyze and isolate cardiac immune and stromal cells vary considerably between laboratories.

In this manuscript, we present a universal protocol to isolate immune and stromal populations from the mouse and human myocardium. This technique is suitable for a variety of downstream applications including flow cytometry analysis and sorting, *ex vivo* activity assays, and transcriptomic profiling. We further discuss key protocol details and potential pitfalls. We believe that this manuscript will prove informative and useful to both established and new investigators interested in studying cardiac immune cell diversity and function.

This manuscript has not been submitted elsewhere, and all of the authors have seen and approved of this manuscript in its final form. We look forward to your comments on this manuscript.

Sincerely,

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