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Dear Phillip Steindel,

We are pleased to submit a revised version of the manuscript JoVE59139, entitled "Quantitative analysis of cellular composition in advanced atherosclerotic lesions of smooth muscle cell lineage tracing mice" for consideration for publication in JoVE.

We thank the Editor and the four Reviewers for their constructive comments. We have carefully edited the manuscript to address the majority of the Reviewer's concerns and comments. We have made significant modifications of the figures to increase the quality of the pictures.

We opted to develop a new protocol to perform single-cell counting using ImageJ, free software developed by the NIH and accessible to everybody. As such, our protocol can be performed by all investigator independently of the brand of confocal microscope used.

We illustrated single cell counting with ImageJ in Figure 5 by taking screen captures. Then, we gave two examples of single cell counting analyses with matching immunofluorescent staining: SMC and macrophage quantification in the fibrous cap area at two locations of the brachiocephalic artery in Figure 6 and the quantification of RUNX2+ chondrogenic cells in Figure 7.

We feel these modifications have increased the overall quality of the manuscript.

We are looking forward to hearing from the JoVE editorial team.

Sincerely,

Delphine Gomez