Dear JoVE reviewers,

Thank you for the opportunity to revise our manuscript " Measuring Proliferation of Vascular Smooth Muscle Cells Using Click Chemistry". We appreciate the suggestions of the reviewers and believe their feedback has significantly improved the quality of the manuscript. Please find attached a point-by-point response to reviewer's concerns. Our responses to the reviewers' comments are in italics.
We hope that you find our responses satisfactory and that the manuscript is now acceptable for publication.

Sincerely,

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Reviewer 1 Major Concerns:

1. The first two paragraphs have no citations. Please include citations for historic/data-evident statements.
References have been added to the first 2 paragraphs.

2. Where were the vascular smooth muscle cells derived from (what species)? Include source (vendor, cat#), also state if they were a cell line or generated from other technology.
We use human vascular smooth muscle cells (VSMC) isolated from iliac arteries using an explant method. This information has been added to the protocol as a note under item 2.1, "Label vascular smooth muscle cells with EdU".

3. Were the VSMCs differentiated and characterized? What kind of VMSCs were used here? Was there any form of injury or intoxication besides PDGF? if yes, the responses could be altered.
The smooth muscle cells are propagated in media using serum and are therefore by definition proliferative or synthetic rather than differentiated or contractile. No other treatment was added prior to PDGF. The VSMC are characterized by the smooth muscle cell markers smooth muscle cell alpha actin and smoothelin. This information has been added to the text.

4. Have you performed experiments with shear stress in the 2 D/cell culture model?

We have not performed these experiments. In addition, because we are using VSMC as a means to demonstrate the use of click chemistry to measure proliferation in adherent cells we are focusing on this assay.

5.What particular disease indication are you working on? Details of that are also missing. Don't need elaborate details but it will justify use of this methodology for categorized disease functional areas.
Proliferation of VSMC contributes to intimal hyperplasia and atherosclerosis. This indication has been added to the introduction.

6. Section 2.2 Cite literature.
I am assuming that the reviewer wants the resazurin to be cited. A reference was added to the note below section 2.1 and to the results section where resazurin is mentioned.

7. Section 3-provide excitation emission wavelengths for fluorescence read outs.
Cy3 excitation and emission wavelengths have been added to item 4.1 number 2 in the protocol.

Minor Concerns:
8. Include photographs of fluorescence imaging. It is good to see quantified data but pictures of the staining also have value. Can be an added as a separate figure or together with quantified data.
An image of EdU positive cells has been added (new Figure 3).

9. Since this is a methods journal, it is extremely helpful if a flow-chart or pictorial guide is provided for the steps. Do this for all the proposed protocols.
A pictorial guide has been added (new Figure 1).

10. A little more detail on click chemistry can be provided.
More detail on click chemistry has been added to the introduction.

Reviewer #2:
Manuscript Summary:
The topic is very interesting and novel. The paper is well organized in general.

Minor Concerns:
As the author mentioned: "...we touch on the ways that this basic protocol can be modified for measuring other cell metabolites.", we hope to see more details in this aspect, especially the application in other types of cells.
This information has been added to the beginning of the discussion.