**Manuscript:**

**Responses to JOVE editorial comments are given in red:**

1. Please adjust the numbering of your protocol section to follow JoVE instructions for authors, 1. should be followed by 1.1) and then 1.1.1) if necessary and all steps should be lined up at the left margin with no indentations.

The numbering has been adjusted in the protocol section on pages 5-8 as instructed and all the steps are lined up at the LH margin with no indentation.  
  
2. Please add a one line space between each step and sub-steps of your protocol section.

A one line space has been added between steps and sub-steps on pages 5-8 of the protocol.  
  
3. Please specify the cell line used in the protocol where applicable.

We have specified which cell lines were used in step 1.1 on page 5 and step 2.1 on page 6. We have also emphasised that the protocol can be used for any adherent cells which produce sufficient ECM for analysis in step 1.1 on page 5 and with the statement “cells of interest” in steps 3.1 and 4.1 on page 7.  
  
4. In step 2 how are the cells washed? Please provide details.

The details of the washing step have been added in step 1.2 on page 5.  
  
5. Step 3 is a run-on sentence. Please split the sentence and provide details as to how the solution is prepared, how the PBS is removed, etc.

The sentence has been split in step 1.3 on page 5. Details have been provided as to how the solutions are prepared in the extractor hood and removed with a plastic transfer pipette.  
  
6. In step 4-5 what is the hydroxide-solubilized material is the author referring to?

An explanation of the hydroxide-solubilized material has been included in step 1.4 on page 5.

7. Please define all the abbreviations upon its first occurrence. PBS, EMC, etc.

All abbreviations are now defined upon first usage.  
  
8. In step 6 please provide a caution statement for paraformaldehyde.

A caution statement for paraformaldehyde is now included in step 1.6.  
  
9. In step 7 how are the washing carried out?

A description of the washing step is provided in step 1.2 on page 5. The reader is directed to this step from step 1.7.  
  
10. Is step 8 a NOTE?

Step 8 on page 5 has been changed to a note as it is not an essential part of the protocol but is referred to in the results section.  
  
11. Please re-write steps of your protocol section in imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.). Please try to avoid usage of phrases such as “should be”, “could be”, “would be” and write in the active/imperative style.

The tense has been changed where appropriate to the imperative.  
  
12. For instance see step 1 in section 2.

Please see response to point number 11.  
  
13. Prior to step 1, at what point are the cells counted and how?

Steps 2.1 and 2.2 on page 6 have been re-written to provide an explanation of the method for cell counting.   
  
14. Phenol red-free media is not mentioned previously in section 1 and seems discontinuous. Please provide the details.

A description of phenol red and the effect it has on imaging has been provided in step 2.2 on page 6.  
  
15. In step 4 is the temperature stabilized to 37 degree C? If so, please specify.

In step 2.3 on page 6 it is now specified that the temperature is stabilized to 37 degree C in the chamber.   
  
16. In step 5 how many cells?

A minimum number of cells is now specified in step 2.5 on page 6.  
  
17. In step 6 please specify the wavelengths used?

The wavelengths for maximum excitation and emission of RFP are now included in step 2.7 on page 6.  
  
18. If z-stack imaging step is to be filmed then please provide details as to how this is carried out. Optionally, please provide a reference.

More detail has been provided for the z-stack imaging process in step 2.6 on page 6.  
  
19. In step 8, how is this carried out?

More detail is provided on how the time-lapse microscopy images are captured In step 2.7 on page 6.  
  
20. In section 3, step 1, please specify the cell used.

We now note in step 3.1 on page 7 that any adherent cell types can be used and that the two cell populations can be different cells.  
  
21. In step 2 how are these prepared? Any precautions to be used?

More detail is provided in step 3.2 on page 7 on the constitution of the PBS. The reader is now referred to step 1.3 on page 5 for more information on preparations and precautions.  
  
22. In step 3 how are the cells washed?

In step 3.3 on page 7 the reader is now referred to step 1.2 on page 5 for more details on the washing step.  
  
23. In step 4 how is the PBS removed?

Details are now provided in step 3.4 on page 7 on how to remove the PBS.  
  
24. In step 5 please replace copious with the actual amount.

In step 3.5 on page 7 copious has been explained by “at least 20 ml per 100 mm dish”.  
  
25. In step 6, please specify the step number.

In step 3.6 on page 7, the step number is referred to is step 3.5.  
  
26. In step 7, what is the concentration of trypsin – EDTA used? how much is used? How long is it incubated in? Please provide details of the harvesting step.

The concentration of the trypsin-EDTA is now included as % weight by volume and details of the amount, timing of incubation and the harvesting step are included in step 3.7 on page 7.  
  
27. What happens post-centrifugation between step 7-8?

In step 3.8 on page 7 details of the resuspension and counting of cells post-centrifugation is now included.  
  
28. In step 8 how are cells counted?

The use of a hemocytometer to count the cells is stated in step 3.8 on page 7.  
  
29. Please provide a reference for step 9 and provide the different wavelengths used.

A reference is now provided for step 3.9 on page 7 along with the emission and excitation maxima for FITC and DAPI.  
  
30. Again in section 4, specify the cells.

In step 4.1 on page 7 it is stated that any adherent cells of interest may be used.  
  
31. In step 4 how is this heated?

In step 4.4 on page 8 it is specified that the SDS-PAGE sample buffer is heated to 95 °C using a heat-block.  
  
32. Please provide a citation for step 8, 9 10,11, 12.

Citations are now provided for steps 4.8, 4.9, 4.10, 4.11, and 4.12 on page 8.  
  
33. In step 12 please provide the antibody details, and dilutions. Please do not use brand names or commercial names.

Antibody details and dilutions are now included in step 4.12 on page 8.  
  
34. Please make a note that cited step should not be in the highlighting for visualization.

No highlighting has been included for any of the cited steps.  
  
35. After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10 page limit for the protocol text, but there is a 2.75 pages limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages (or less) of text to identify which portions of the protocol are most important to include in the video; i.e. which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVEs instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

Regarding the protocol steps important to be included in the video, we have highlighted the steps we consider would be crucial to show. These cover about 1.5 pages of text. There are additional steps that could be included, but we would need input from JoVE on how accessible these are for videoing. These steps include section 3.2-3.6, which are carried out in a laminar flow hood, also 2.5 and 2.8-2.9, to demonstrate how the ECM is identified on the same square of a gridded dish before and after cell imaging and removal of cells. We have not highlighted these sections, as we would need more information on how the video will be designed.

36. If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

The appropriate phrases have been included in the legends for figure 1 on page 13 and figure 4C on page 14 as they have both been published previously by our laboratory. Both Journals allow authors to reproduce figures from their papers without specific reprint permission.   
  
37. In the figures, please specify a scale bar and define the scale units in the figure legends.

Scale bars and units are specified in figures 1, 2 and 3.  
  
38. Please define all error bars (SD, SEM) in the legends of their respective figures.

There are no error bars in any of the figures.  
  
39. Please make sure that the “Discussion” is written under the following sections.  
a. Critical steps within the protocol.  
b. Modifications and troubleshooting.  
c. Limitations of the technique.  
d. Significance of the technique with respect to existing/alternative methods.  
e. Future applications or directions after mastering this technique.

The discussion on page 11-12 has been rearranged to reflect the sections listed above and the headings have been included.  
  
40. IMP: Please copyedit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

The entire manuscript has been copyedited by native English speakers and the text adjusted to American-English.  
  
41. NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

Please see all line-by-line responses above.