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December 2, 2010

PLoS Biology
Public Library of Science
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Dear PLoS Biology editors:

Please consider this revised manuscript titled "High-Resolution Quantification of Focal Adhesion Spatiotemporal Dynamics in Living Cells" by Matthew E. Berginski, Eric A. Vitriol, Klaus M. Hahn and Shawn M. Gomez for publication in *PLoS Biology.* We note that this is a previous interaction (10-PLBI-RA-6319). We greatly appreciate the reviewer's thoughtful comments and have tried to thoroughly address their questions and concerns.

Highlights of some of our changes relative to specific reviewer comments follow below. A full, point-by-point response is included after this summary section:

- Reviewer 1 had a major concern regarding the availability of the software, stating that "*Taken together, I would recommend acceptance of the paper, under the condition that the software is made available to the scientific community (ideally – downloadable from the Journal's website).*"
    - We were not clear enough in our original submission and have clarified it in this revision – the software is freely available under an open-source license and can be downloaded from our lab website (http://gomezlab.bme.unc.edu/tools) and includes documentation and sample data on which the software can be run. In addition, we would be happy to have the software available on the PLoS' website. Continued updates to the software will be made available on the lab website.
    - A number of other concerns were also addressed.
- Reviewer 2 was perhaps most interested in us elucidating the mechanisms and role of the phosphorylation event on Paxillin that we describe. While we do agree that this is a very interesting and important area worth pursuing, it was not the main focus of this manuscript. We have addressed all other Reviewer concerns as well as possible and have further altered the title to deemphasize the "key regulator" aspect of the S178 Paxillin mutant used in this work.
- Reviewer 3 was very favorable towards the work, with the primary critique being to provide supporting measurements using another adhesion marker. We used FAK as an alternative marker of adhesions, replicating relevant aspects of the statics and dynamics analysis using this marker. We found identical rates of assembly and disassembly for adhesion regardless of whether Paxillin or FAK was used as the adhesion marker.

Again, we greatly appreciate the reviewers' comments and questions and feel that they have greatly improved the quality of the manuscript. We would like to use the same list of potential reviewers identified in our prior interaction (10-PLBI-RA-6319). We believe this work provides a novel picture of adhesion dynamics in living cells as well as a tool for use by the broader adhesion research community. We look forward to moving ahead towards publication of this manuscript.

Sincerely,

Shawn M. Gomez & Klaus M. Hahn
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