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Dear PLoS Biology Editors,

Please accept this manuscript entitled “Automated identification and tracking of focal adhesions reveals phosphorylation of paxillin at serine 178 as a key regulator of adhesion dynamics” by Matthew E. Berginski, Eric A. Vitriol, Klaus M. Hahn and Shawn M. Gomez for publication in PLoS Biology.

Focal adhesions are dynamic cellular structures that are key players in cell signaling and motility, as well as processes such as cancer metastasis, embryonic development and wound healing. In this work we make two major contributions towards improving our understanding of these structures. First, we provide a comprehensive characterization of the spatiotemporal dynamics of focal adhesions within living cells (NIH 3T3 fibroblasts). This characterization is enabled through the development of a computational system that is capable of tracking through time all adhesions within an individual living cell. Unlike current approaches that have focused on characterizing behavior for 1-2 dozen adhesions in total, here we quantify properties for over 200,000 adhesions. While some recent automated approaches quantify adhesion properties as well, these analyses have been performed on fixed cells and thus cannot quantify many of the dynamics described here. Properties we quantify include where and when an adhesion is born and dies, the rates at which they assemble and disassemble, changes in area, etc. To our knowledge, this analysis provides the most comprehensive characterization of adhesion populations and their dynamics to date.

Second, we further demonstrate the power and sensitivity of this approach by quantifying differences in adhesion phenotypes between wild-type paxillin and a mutant, where serine 178 has been replaced by alanine. This site is phosphorylated by c-Jun N-terminal kinase and the inactivation of this site has been shown to disrupt cell motility. Here, we quantify an over 40% decrease in the rate of adhesion assembly arising from this mutation along with other changes that help to explain the potential mechanism behind the observed motility defects.

Together, we feel the results presented in this work constitute a significant advance in the methodologies used to quantify adhesion dynamics as well as improving our understanding of adhesion function under normal and perturbed conditions. As such, we feel this work would be of broad general interest to the biological community. Recent papers relevant to this work include:

- Donna J. Webb, Karen Donais, Leanna A. Whitmore, Sheila M. Thomas, Christopher E. Turner, J. Thomas Parsons & Alan F. Horwitz. (2004) FAK–Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. Nature Cell Biology 6, 154-161.

- Cai Huang, Zenon Rajfur, Christoph Borchers, Michael D. Schaller and Ken Jacobson. (2003) JNK phosphorylates paxillin and regulates cell migration. Nature 424, 219-223.

- Sabina E. Winograd-Katz, Shalev Itzkovitz, Zvi Kam, and Benjamin Geiger. (2009) Multiparametric analysis of focal adhesion formation by RNAi-mediated gene knockdown. The Journal of Cell Biology, Vol. 186, No. 3, 423-436.

As a final note, we do have material that may serve as a suitable striking cover image.

Thank you for your consideration.

Sincerely,

Shawn M. Gomez

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