OREGON HEALTH & SCIENCE UNIVERSITY

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Dear Editor,

We write to submit our manuscript entitled 'Photoactivated localization microscopy with bimolecular fluorescence complementation (BiFC-PALM)' for consideration as a *video produced by JoVE*, thanks to a kind invitation by Associate Editor Joseph Petrick.

In this manuscript, we describe a new approach for imaging protein-protein interactions (PPIs) in a cell with nanometer spatial resolution, by combining BiFC with PALM. BiFC is commonly used to probe PPIs in cells for its high sensitivity, subcellular resolution, and compatibility with high-throughput assays, but its spatial resolution is limited. BiFC-PALM overcomes this limitation simply by using a photoactivatable fluorescent protein for the BiFC assay. By design, BiFC mediated by PPIs will reconstitute a complete photoactivatable fluorescent protein, which then enables PALM imaging of the PPI at the nanoscale. While conceptually simple and straightforward, BiFC-PALM or similar strategies have not been reported.

We demonstrated BiFC-PALM by splitting PAmCherry1, a photoactivatable mCherry widely used in PALM imaging experiments. To our excitement, PAmCherry1 BiFC showed superior performance over most existing BiFC systems, such as low background and efficient fluorophore reconstitution at 37 °C. These merits make PAmCherry1 BiFC well suited for studying PPIs with high specificity and in physiologically relevant settings. More generally, BiFC-PALM should work with many other photoactivatable fluorescent proteins that have been tested for PALM.

We believe that BiFC-PALM will be a powerful and widely adopted technique for many biological applications. In the manuscript we showed an example of using BiFC-PALM to study Ras-Raf interaction. Specifically, we were able to resolve the nanoscale clustering and diffusion of individual Ras-Raf complexes in an intact or living cell. This example was derived from an ongoing effort in our lab on using PALM and related techniques to uncover spatial mechanisms that regulate Ras oncogenic signaling. Additionally, we are already applying the technique to study Her2-Her3 heterodimerization and signaling in breast cancer, among others, where nanoscale membrane domains again play a critical role.

Author contributions are as follows. Conceived and designed the experiments: XN. Performed the experiments: AN TH LJL. Analyzed the data: XN AN TH. Wrote the paper: XN AN TH.

We tentatively suggest the following reviewers,

- Dr. Tom Kerppola, HHMI and University of Michigan
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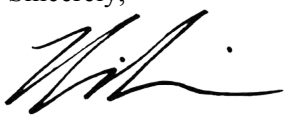
Thank you for your time and kind consideration.

Sincerely,



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