Zhao Chen, Ph.D.
Editor, JoVE

March 26, 2011

Dear Dr. Zhao Chen:

Enclosed please find our manuscript entitled “Cell memebrane and subcellular localization of Smoothened in Hedgehog signaling” for submission to *Journal of Visualized Experiments*.

As you are aware of, the seven-transmembrane protein Smoothened (Smo) is a critical component in the receptor complex of Hedgehog (Hh) signal transduction. Recently, it drew much attention from the research field as abnormal activation of Smo has been observed in several types of human cancers. However, Smo signaling mechanisms remain a long standing puzzle. Although it has been shown that Smo activation requires phosphorylation and cell-surface accumulation, it is unclear how Hh regulates the subcellular trafficking of Smo. Thus, it is very important to develop assays with which to monitor the localization of Smo that is regulated by upstream signal.

In this study, we provide detailed methods to monitor Smo cell surface accumulation and to track the intracellular movement of Smo. With these assays, we demonstrate that Smo is presumably localized in the early and late endosomes in the absence of Hh and is removed from the early endosomes and accumulated on the cell surface upon Hh stimulation. I believe our study not only sheds important light into the Hh signal transduction mechanism but also has more general implication on the control of signal transductions.

I would like to suggest the following referees as they are experts in the field: Xiaodong Cheng (xcheng@utmb.edu) and Jing Yang (Jing.Yang@nationwidechildrens.org). Please exclude Matthew Scott, Alan Zhu, Dan Kalderon, and Pascal Therond as their work overlap ours.

I look forward to your favorable decision.

Sincerely and best regards,

Jianhang Jia, Ph.D.