



Basel, 15 September 2018

To the Editors of JoVE

Dear Editors,

Here we submit our article "**Analysis of endocytic uptake and retrograde transport to the trans-Golgi network using functionalized nanobodies in cultured cells**" for your consideration for *JoVE*.

Nanobodies are an emerging class of protein binders with many advantages over conventional antibodies (small size, non-crosslinking, no disulfide bonds and thus allowing bacterial expression). They have recently been implemented as intracellular or cell surface traps, and for high-resolution protein localization. In our previous study (Buser et al., 2018, PMID: 29915061), we developed bacterially expressed and derivatized anti-GFP nanobodies as tools to follow GFP-tagged cell surface proteins on their retrograde route to endosomes and the Golgi. They allow the quantitative analysis of protein uptake and transport to intracellular compartments either biochemically, by fixed- and live-cell imaging, or by electron microscopy. Our tool thus allows to dissect the retrograde fate and identify the molecular players involved in retrograde transport of any GFP fusion of interest. Our here submitted protocol allows a detailed methodological description of bacterial nanobody expression and their use to study endocytic uptake and retrograde transport to the Golgi in mammalian cells.

Since our study is of exceptional importance in the investigation of endocytic and retrograde traffic, and moreover is intelligible to a broad scientific audience, we consider submission of our protocol to *JoVE* as suited.

We hope that you will also consider our article to be of interest and are looking forward to your response.

Yours sincerely,

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