# Reply to Rubinstein: Is Lgr4 essential for VSV– and VSV-G–pseudotyped lentiviral vector entry to cells?

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*This is a response to a letter by Rubinstein (1).*

In response to the concerns delineated in a letter by Menachem Rubinstein (1), we found that protein truncation without an endogenous signal peptide is commonly performed in co-immunoprecipitation (2). Furthermore, there was no aggregation or precipitation of misfolded protein in our co-immunoprecipitation assay (Fig. 5*B* of Ref. 3), and the truncated LGR4-ECD (25–528 amino acids) has well-demonstrated bioactivity, as shown in our previous paper (4). Moreover, the data from our pull-down (Fig. 5*C*) and Biacore assays (Fig. 5, *D* and *E*) confirmed that a strong interaction exists between Lgr4 and intact VSV particles (3).

On the question of binding affinity, according to the affinity model, fast estimation of $K_D$ is reasonable only when the binding is saturated. In our case, it was hard to reach saturation for every concentration curve. Thus, as in other studies (see, for example, Ref. 5), the kinetics model was the only choice. Upon kinetic regression, the results showed that $K_{on} = 15{,}450\ \text{M}^{-1}\ \text{s}^{-1}$, $K_{off} = 0.001067\ \text{s}^{-1}$, and $K_D = K_d/K_a = 69.05$ nM.

Finally, the $IC_{50}$ for LDLR-ECD cited by Dr. Rubinstein refers to sLDLR-mediated inhibition of vesicular stomatitis virus (VSV)–induced cytopathic effects in human WISH cells (Fig. 1*A* of Ref. 6). In contrast, we detected inhibition of VSV replication by LGR4-ECD in mouse MEF cells by quantitative PCR (Fig. 6*A* of Ref. 3). It is highly problematic to compare $IC_{50}$ values determined using different assays, different cells, different expression systems, and different proteins.

Taken together, we have shown a series of findings that support Lgr4 as a key factor involved in facilitating VSV infection. We, therefore, stand by our conclusion that Lgr4 is essential for VSV infection.

The authors declare that they have no conflicts of interest with the contents of this article.

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