Review 2: "GRP78 binds SARS-CoV-2 Spike protein and ACE2 and GRP78 depleting antibody blocks viral entry and infection in vitro"

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Published on: Jun 17, 2021

DOI: 10.1162/2e3983f5.bc73c03f

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RR:C19 Evidence Scale rating by reviewer:

- **Strong.** The main study claims are very well-justified by the data and analytic methods used. There is little room for doubt that the study produced has very similar results and conclusions as compared with the hypothetical ideal study. The study's main claims should be considered conclusive and actionable without reservation.

Review:

The viral attachment to the cell surface receptor is a key factor for the progression of viral infection. The authors of the pre-print paper entitled "GRP78 binds *SARS-CoV-2* Spike protein and ACE2 and GRP78 depleting antibody blocks viral entry and infection in vitro", report results from in vitro models that demonstrate the role of cellular GRP78 as a co-factor in the interaction of *SARS-CoV-2* Spike protein and ACE2 on the cell surface. Using three dominant negative mutants of GRP78, the authors show the key role of the substrate-binding domain in the interaction with *SARS-CoV-2* Spike protein and, together with the ATP domain, for binding to ACE2 (GRP78-ACE2). In addition, the results from the immunofluorescence and Proximity Ligation Assay suggest a possible role of GRP78 as a scaffold for the interaction of the viral S protein and cell surface ACE2. The authors used a model including the human lung epithelial cell line H1299, *Vesicular stomatitis virus*, and the monoclonal antibody hMAb159, and obtained correlation between the decrease in the levels of cell surface (cs) GRP78 and those of csACE2, and associated GRP78 with the trafficking, localization, and stability of ACE2 on the cell surface.

The results pave the way for further studies on the role of GRP78 in the production of the viral S protein in the endoplasmic reticulum and the development of a therapy targeted at controlling the GRP78 levels.

The experimental approach is original, the results are presented concisely, clearly, and to the point. In its present form, the manuscript could be accepted and published as a short communication, after removing the subheadings of all the parts.

What could expand this preprint into a Full Research Article is in part already done by the authors: to separate the results from the discussion, to add to the figures, references, etc. ([https://www.jbc.org/article/S0021-9258\(21\)00552-4/fulltext](https://www.jbc.org/article/S0021-9258(21)00552-4/fulltext)). In addition, the manuscript would benefit from adding more specific information in the

Introduction and the Discussion, for example, in some of the following directions or others: the physiological function of GRP78 and ACE2 and potential side effects from the application of drugs for prophylaxis and/or treatment targeted at controlling the levels of csGRP78 and csACE2, respectively; about the expression and structure of GRP78 and ACE2 in other species and their interaction with SARS-CoV-2, in the context of cross-species transmission; or comparison of the interaction mechanism of other coronaviruses with the host cell ACE2 receptor.