Comments for reviewers.

Thank you for the timely and thoughtful reviews. We have made text edits in the attached manuscript (with track changes and listed below) and have addressed your other comments below:

* We have titered virus without polybrene and have noticed it is not vital, but does increase titers slightly (<2-fold) to include polybrene. We have not tested neutralization assays in the absence of polybrene.
* We chose to transduce 293Ts with hACE2 via infection with VSV G-pseudotyped lentivirus packaging the hACE2 gene as this is generally a fairly stable way to express a gene of interest in target cells. This method should be more stable than propagating transfected cells and allowed us to create 293T-ACE2 cells quickly. We did not include a fluorescent selectable marker in our hACE2 plasmid to avoid potential conflicts with additional fluorophores in future experiments. Similarly, we did not use antibiotic selection \_\_\_\_\_. Importantly, Fig. 2A confirms that expression of hACE2 in these cells remains high after many passages (up to ~12 passages and ~6 weeks post-sort at the time of writing). As such, we do not expect loss of hACE2 to be a big problem for the typical use of these cells. Nonetheless, we recognize that expression could decrease overtime and recommend that labs using these cells freeze down stocks of these cells at a low passage number and, if passaging for a long time, periodically confirm high levels of expression via staining for hACE2. We have added text to suggest this.
* We are in the process of directly comparing IC50s from our assay to IC50s obtained by another lab using a BSL-3 neutralization assay with the SARS-CoV-2 virus. We do not have those results yet (and do not think it is necessary to delay publication in order to get them), but the IC50s we have gotten with our pseudotyped-lentivirus neutralization assay are within the range of IC50 values that have typically been reported for other pseudotyped neutralization assays.

1. Most recombinant lentivirus production systems contain envelope plasmid, packaging plasmid and transfer plasmid, as described in the article. However, only two types of plasmids, including envelope plasmid and backbone plasmid was used in packaging of SARS-CoV-2 pseudotype lentiviral particles in some other researches (Emerg Microbes Infect. 2020 Dec;9(1):680-686. Cell Res. 2020 Apr;30(4):343-355. ). What the advantages of the method in this research than previously reported?

The first paper referenced uses a VSV-pseudotyped system and the second paper uses a NL3.4 lentivirus system. All three systems (the two the reviewer mentions) and ours should all work well. In fact, it is likely that the VSV system may allow for higher titers of SARS-CoV-2-pseudotyped virus than our system. We do have some anecdotal evidence that the lentiviral pseudotyping system we discuss here may work better than some other commonly used lentiviral systems, but we have not thoroughly tested all lentiviral systems, and many should work. The main advantage of our system is that we have made the protocol and reagents widely available to facilitate other researchers running this assay. Previous papers (included the two mentioned) have fairly sparse methods sections that would make it difficult for other researchers to adapt and use their assays. The main advantage of our manuscript is the robust methods section and the public availability of our reagents.

2. 1 ug of lentiviral backbone plasmids, 0.22 ug of packaging plasmids, and 0.34 ug of envelope plasmids were used to transfect 293T cells and viruses were harvested in supernatant. Did you optimized the ratios of these plasmids to get higher titers of viruses?

We slightly optimized these ratios so that we were adding the same molar amount of Spike plasmid as we had been adding of other envelop plasmids for other lentiviral pseudotyping work going on in our lab. However, we did not test other ratios than these. These ratios work well and yield sufficient titers of lentivirus, but we acknowledge that further optimization of these ratios could potentially improve titers.

3. Generally, Huh-7 (human hepatoma cell) and Vero E6 (monkey kidney cell) cell lines were the natural target cells for SARS-CoV-2 and widely used in neutralization assay. If used the two cell lines as target cells, is it work for your measurement system?

In very preliminary tests, we tried infecting Vero E6 cells with somewhat equivocal results (but things were not fully optimized), but got much better infection with the 293T-ACE2 cells, so moved forward with those. We have not tried infecting Huh-7 cells with this system, but have heard anecdotally that they also do not work as well as the 293T-ACE2 cells. In theory, any cell line susceptible to SARS-CoV-2 infection **and** that can be readily transduced by HIV-based lentiviruses should work for this system, but the 293T-ACE2 cells seem to work best.

4. As mentioned in Material and methods, the titer of pesudovirus could be calculated by flow cytometry or luciferase. Fluorescent signal of ZsGreen is detected by flow cytometry and relative luciferase units is detected using luciferase. It is confusing that the higher titers of relative luciferase units are observed using the Luciferase-IRES-ZsGreen backbone but the fluorescent signal of ZsGreen is lower.

First, it is difficult to directly compare luciferase titers as measured in relative luciferase units per mL to ZsGreen titers as measured by transduction units per mL. One RLU per mL is not directly equivalent to one TU per mL. Indeed, a single cell transduced with the luciferase-containing backbone is likely contributing more than one RLU, whereas any cell transduced with a ZsGreen backbone can only be counted as one positive cell in the flow cytometry readout of ZsGreen. This contributes to the higher titers as measured by luciferase versus ZsGreen. Furthermore, the ZsGreen signal is lower from the Luciferase-IRES-ZsGreen backbone than the ZsGreen only backbone because expression is typically decreased following an IRES compared to expression from a single-gene construct (such as the ZsGreen only backbone). Thus, the differences in ZsGreen expression between the backbones are more an artifact of the differences in construct design than any biological differences in titer.

Comment 2. Competitive assays using ACE2 recombinant protein in the neutralization assay with the plasma of SARS-CoV-2 patient would have provided more insight on the specificity of the assay.

4. Specify the “human IgG” in line 416.

I asked Tyler and will ask Neil/David’s labs if Tyler doesn’t know.

5. Check reference 7, 38, 44

I’m not sure what the issue with these references is. I tested all the DOIs and the references looked fine to me by eye?

Below is a list of all changes made to the manuscript:

* Page 3, line 98: Changed “his” to “this.”
* Figure 3A: Noted that a representative example is shown.
* Page 6, line 183: Deleted "is"
* Page 6, line 187: Changed "neutralized" to "neutralize"
* 4.3.4:
  + Specified type of filter used
  + Made suggestion to freeze virus in aliquots to avoid freeze/thaw cycles.
* 4.4.6:
  + Please see above comment regarding testing polybrene.
* 4.5.8:
  + Added a line describing analysis in slightly more detail.
* Line 210:
  + Explicitly added comparison to neutralization titers with both other pseudotyped lentivirus systems and full SARS-CoV-2 virus.
* Line 157 & 159: Fixed figure number references to correctly refer to Figure 3.
* Line 140: Fixed figure number reference to correctly refer to Figure 3.
* Line 102: Corrected spelling of expression.
* Lines 207-225: Bolded references to Figure 4 to be consistent with other figure references.
* Throughout: Corrected uL/ug/um measurement notation to μL/μg/μm.