Reviewer 2:

As compared to hemagglutinin, neuraminidase and antibodies recognizing neuraminidase (NA) have received decidedly less attention. Yet, it is increasingly clear that a better understanding of NA-based immunity has important implications for the development of novel therapeutics. Innovative tools and techniques to study anti-NA antibodies are needed to help fill our gaps in knowledge. In the study described here, Gentles and colleagues describe a modified influenza A virus that is dependent on NA for entry. The experiments that follow are straightforward. Using a single-cycle infection assays, the authors demonstrate that a panel of anti-NA antibodies can neutralize this HA-binding deficient virus. The authors then go on to demonstrate that this modified IAV can be used to select antibody escape mutant for NA. The latter use offers perhaps the greatest utility going forward, given that enzyme-linked lectin assays (ELLA) can be used to identify antibodies that inhibit NA enzymatic activity. Minor revisions as suggested below are necessary before this article be considered for publication

The authors provide no explanation as to why the HAΔbind generated is an H3 HA(HK68) while the two other viruses used (NAbind/HAwt, NAwt/HAwt) are H1 HAs (Cal09). Similar mutations have been described in Cal09 (Y108F, Reference 1) and moreover the Y98F mutation is well described in other Group 1 HAs (References 2, 3). The rationale for the generating and H3N1 NAbind/HAΔbind should be addressed explicitly or better yet a modified H1N1 viruses should be generated and assessed.

The H3 HAΔbind has been rigorously tested in our lab showing that this combination of mutations completely prevents HA binding while still allowing for efficient viral growth in the presence of a receptor-binding NA. To our knowledge, this is the only HA that has been characterized in the literature as completely binding deficient. Although Y98F and other single mutations to HA greatly reduce receptor affinity, they retain enough binding activity to enable the virus to still grow in cell culture (although not mice); see for instance https://jvi.asm.org/content/82/10/5079. In our original work generating the H3 HAΔbind, we found it was necessary to introduce a large number of mutations (three receptor-binding point mutations, deletion of the 220 loop, and extra glycosylation) to make HA so completely binding deficient that it could not support any viral growth in the absence of a binding NA. We agree that it should be possible to generate a similar H1 HA, but since it requires engineering the HA so extensively we chose to just use the H3 HA. In particular, since all antibodies tested here are directed to the NA rather than HA removing any dependency on HA antigenicity, we felt it did not matter for the purpose of these experiments if the HA was H1 or H3.

We have added text in the Results explaining these facts.

Along similar lines, to demonstrate that HA sialic acid binding is indeed ablated in the NAbind/HAΔbind, a simple HAI should be performed.

The ablation of HA binding in the NAbind/HAΔbind virus identical to the one used here, and the lack of receptor-binding of this HA has been rigorously demonstrated by Hooper et. al 2015. That point is now cited clearly.

It would be helpful to include a summary table that describes the epitopes for the monoclonal antibodies utilized, as well as their relative NA inhibition in ELLA and single-cycle infection assays (i.e. + weakly neutralizing, +++ potent neutralization)

Added suggested table and refer to table in text.

Line 51 typo–missing words (s) “N1 NA to bind receptor”

Fixed wording for readability