Reviewer #1

The paper by Tedjakusuma et al conveys novel information on mucosal Ad5 based vaccines with SARS-COV2 spike. The study is well designed and the immunological responses and viral load responses are robust. There are some areas where the paper could be improved.

1. Did the authors asses nasal CD8+ TRM generation? As the Kohlmeier lab has recently shown in Nature that these responses can limit viral load as well as transmission in a Sendai virus model.

We thank the reviewer for their comments and for bringing this study to our attention. To measure the CD8+ TRM we would have needed to collect samples with nasal brushes. This is something we will consider implementing in future studies. While we did use nasal synthetic absorptive matrix (SAM) devices to collect mucosal secretions, these unfortunately do not work well for collecting cells. Therefore, investigating nasal CD8+ TRM generation following immunization is beyond the scope the current study.

2. The authors need to discuss this in the context of the recent Barouch NHP paper in the same December issue of Nature.

We thank the reviewer for this suggestion. The following sentence has been added to the discussion (lines 482-485):

“A recent study investigating intranasal or intratracheal boost vaccination with rAd26 found antibody and T cell responses generated in the lung following were the strongest immunologic correlate for protection against SARS-CoV-2 challenge in rhesus macaques (McMahan, Wegmann et al. 2023).

We also included another Barouch lab study in the discussion (lines 539 – 549)

“Other studies have investigated if a variant matched vaccine is critical for SARS-CoV-2 protective immunity. Boost intramuscular immunization with rAd26 containing either Wuhan Spike or Omicron BA.1 Spike were compared in NHPs previously immunized with rAd26 containing Wuhan Spike. While both regimens induce strong antibody responses, the Omicron BA.1 booster offered only a moderate advantage in Omicron-specific immunity. Furthermore, while neutralizing antibodies were shown to be critical for protection, cross reactive T cell responses also played a significant role in protection against Omicron challenge. This study suggests that while matched vaccines offer slightly better protection against homologous SARS-CoV-2 variants, Wuhan-based vaccines continue to provide effective protection against variant infections (Solforosi, Costes et al. 2023).”

3. The authors need to discuss limitations of Ad5 in populations that have pre-existing immunity

We thank the reviewer for raising a good point. Research suggests that oral administration of rAd5 is not adversely affected by pre-existing immunity in the way that intramuscular delivery is. A sentence has been added (lines 57 -60) to further review the effect of pre-existing immunity to rAd5 on immunogenicity.

“Orally administered rAd5 is not hindered by pre-existing adenoviral antibodies in humans (Liebowitz, Lindbloom et al. 2015), whereas intramuscular delivery can be significantly hindered by pre-existing immunity (Scallan, Tingley et al. 2013).”