Dear Editors,

Attached you will find our manuscript “Human gut microbiota are associated with HIV-reactive immunoglobulin at baseline and following HIV vaccination”. This research article examines and compares microbiome and immunogenicity data from a cohort of HIV uninfected participants from a clinical study of an experimental HIV vaccine regimen.

Previous analysis of this clinical cohort (Pantaleo et al. *In prep.*) showed that most participants, even at baseline, before administration of vaccines, had antibodies that bound to the HIV gp41 envelope protein. The concentration of these antibodies appeared to vary between participants. Vaccination lead to an increase in gp41 binding antibodies as well the appearance IgG antibodies to gp120 envelope proteins and gp70.v1-v2, a hypervariable envelope protein analogue. The magnitude of these vaccine responses were variable. One possible mechanism driving this variability relates to the microbiome: Previous analyses by Williams et al. (2015, 2018) have suggested that gp41 and gp120 HIV envelope proteins are cross reactive with microbiome peptides, suggesting that antibodies to these two proteins could also be reaching with the microbiome. Towards this end, we investigated whether participants microbiomes were statistically associated with baseline antibody concentrations to gp41 as well as the magnitude of gp41, gp120 and v1v2 HIV envelope proteins.

We found that both gp41 binding IgG antibodies at baseline and gp41, gp120,and gp70 v1-v2 binding IgG antibodies all appeared to associate with the microbiome, either 6 or 12 months post immunization. Specifically, participants with similar microbiomes also had similar baseline and vaccine related immunogenicity. Interestingly, microbiomes that associated with low gp41 concentrations at baseline also associated with high gp41, gp120 and v1v2 concentrations 6 and 12 months after immunization.

We coarsely characterized which elements of the microbiome – within our cohort—appear to associate with immune response to vaccines. Specifically, we observed that family level groups fall into two co-occurring modules, one of which associates with high baseline gp41 antibodies and low vaccine response, while the other module associates with low gp41 antibodies at baseline and high vaccine response.

Our study provides promising indication that the microbiome may affect response to these HIV vaccines. Our cohort contains only 21 participants and so statistical power is low. Nevertheless, we see a strong signal and have generated hypotheses that will be verifiable with future clinical studies. We expect this study to be of particular interest to those interested in understanding interactions between the human microbiome and the immune system as well as those interested in understanding variability in vaccine response.

Any editor with experience with either vaccines immunogenicity or the human microbiome would be in a good position to coordinate the review of this manuscript. We suggest the following members of your editorial board: **Erwin G Zoetendal , Yiping Han, Ryan M. Thomas, Holger Till.**

We thank you for your consideration.

Sincerely,

Jacob Cram et al.