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Supplementary information

Safety and tolerability of AAV8 delivery of a broadly neutralizing antibody in adults living with HIV: a phase 1, dose-escalation trial

In the format provided by the authors and unedited

Supplemental Tables and Figures.

Supplemental Table 1. Summary of reasons for ineligibility	
Cause	Number of Candidates Found Ineligible
Hypertension	11
AAV8 Ab positive*	10
Active drug use	9
Elevated creatinine	6
Weight > 115 kg	5
Inadequate venous access	4
Renal disease	3
Cardiovascular disease	3
VL > 50 copies/mL	3
Significant chronic pain	3
Chronic hepatitis	2
Unstable ARV regimen	2
Mental health concerns	2
Unable to provide ID	1
Previous receipt of mAb	1
CD4+ < 300	1
Stargdardt's disease	1
Epilepsy	1
Autoimmune condition	1
Neurosyphilis	1
Elevated ALT	1
Chronic respiratory condition	1

*Criteria for AAV8 seropositivity was modified by protocol amendment because of NHP data suggesting that low level pre-existing AAV8 seropositivity did not affect transduction. Based on these data the final exclusion criteria for pre-existing seropositivity was increased to a titer >1:90. Data presented here includes disqualification of volunteers for values of <1:90.

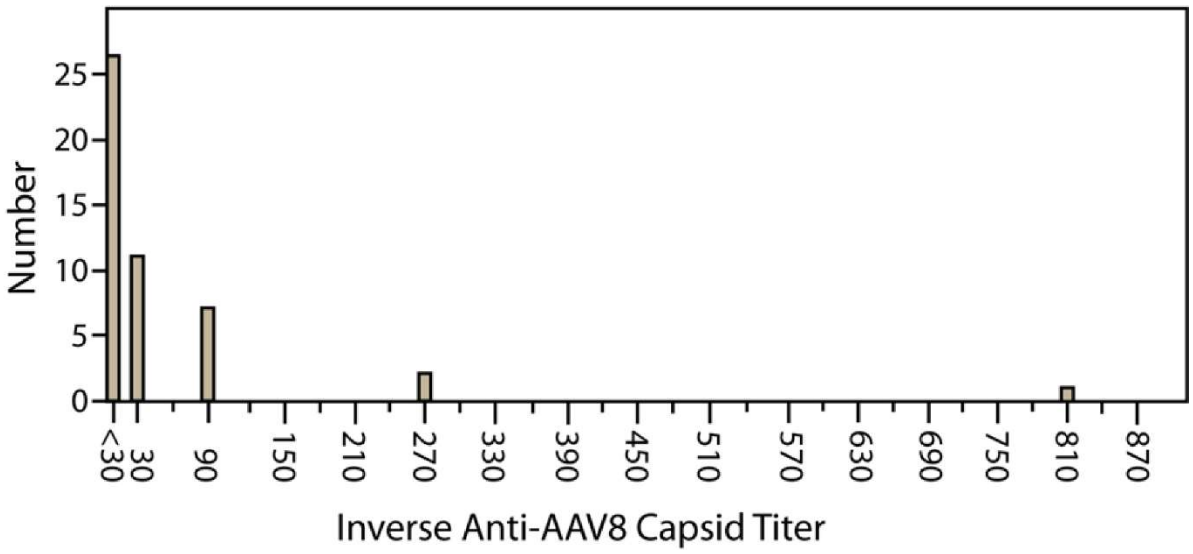
Supplemental Table 2. Demographic characteristics of study participants					
Category	Subcategory	Group 1 (N=3)	Group 2 (N=2)	Group 3 (N=3)	Overall (N=8)
		N (%)			
Gender	Male	3 (100)	2 (100)	1 (33)	6 (75)
	Female	0 (0)	0 (0)	2 (66)	2 (25)
Race	Asian	0 (0)	0 (0)	0 (0)	0 (0)
	Black/African	1 (33)	1 (50)	3 (100)	5 (62.5)
	White	2 (66.7)	1 (50)	0 (0)	3 (37.5)
Ethnicity	Non-Hispanic/Latino	3(100)	2(100)	3(100)	8(100)
	Hispanic/Latino	0(0)	0(0)	0(0)	0(0)
Median Age	Median Years (range)	56 (36-60)	41 (30-52)	52 (32-56)	52 (30-60)
Median Weight	Median Kg weight (range)	79 (66-82)	74 (73-75)	82 (71-91)	77 (66-91)

Supplemental Table 3. Clinical characteristic of participants at enrollment

Participant	CD4 (cells/µl)	VL (copies/ml)	Antiretroviral Therapy
Participant A	523	23	Tenofovir Alafenamide Emtricitabine Elvitegravir/Cobicistat
Participant B	446	<20	Abacavir Efavirenz Fosamprenavir/Ritonavir
Participant C	532	<20	Tenofovir Alafenamide Emtricitabine Rilpivirine
Participant D	950	<20	Abacavir Lamivudine Dolutegravir
Participant E	351	<20	Tenofovir Alafenamide Emtricitabine Darunavir/Ritonavir
Participant F	641	<20	Tenofovir Disoproxil Emtricitabine Rilpivirine
Participant G	407	<20	Tenofovir Alafenamide Emtricitabine Darunavir/Ritonavir
Participant H	797	<20	Abacavir Lamivudine Dolutegravir

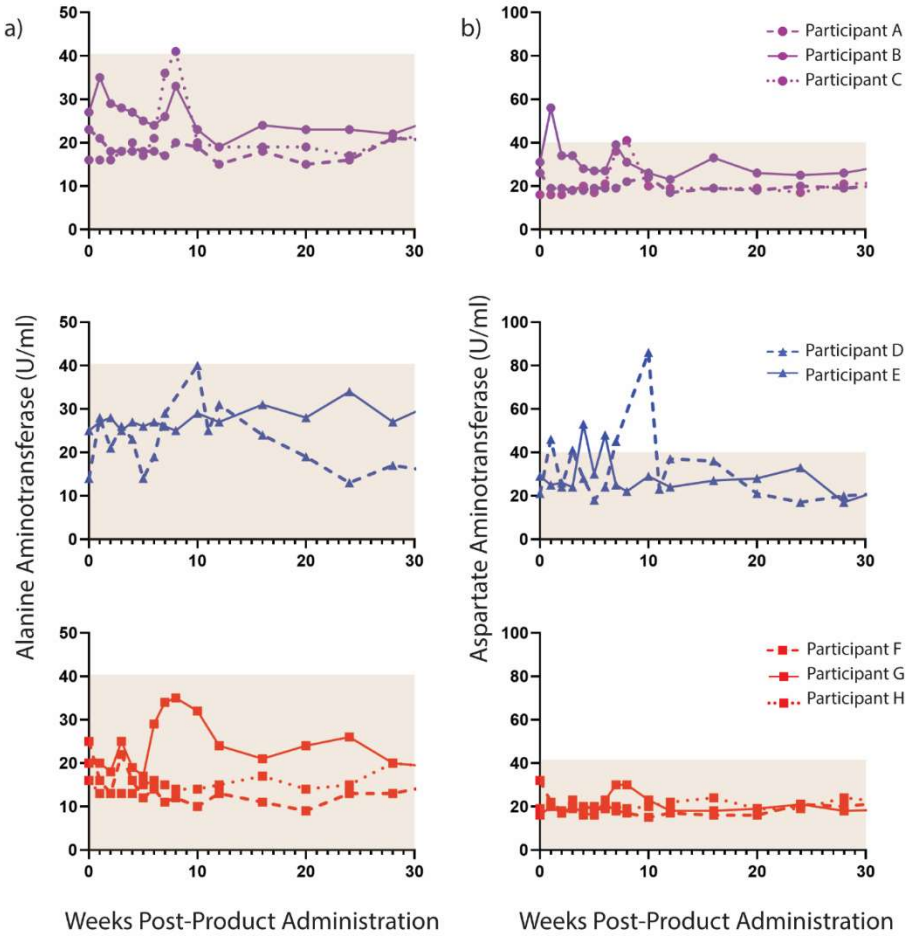
Supplemental Table 4. Participant IgG1 Allotype and Association with ADA			
Participant	GM1	GM3/17	ADA reponse
Participant A*	-/-	3+/3+	Tier 1/2+ Tier 3-
Participant B	-/-	3+/3+	Tier 1/2- Tier 3-
Participant C	+/+	17+/17+	Tier 1/2- Tier 3-
Participant D	-/-	3+/3+	Tier 1/2- Tier 3-
Participant E*	+/+	17+/17+	Tier 1/2+ Tier 3-
Participant F	+/+	17+/17+	Tier 1/2- Tier 3-
Participant G	+/+	17+/17+	Tier 1/2- Tier 3-
Participant H*	+/+	17+/17+	Tier 1/2+ Tier 3-
*Indicates Allotype from a participant with a positive VRC07 ADA response			

Supplemental Table 5. Geometric means ± 2 SD for *ex vivo* and *in vivo* produced VRC07			
Pseudovirus	Geometric mean	-2 SD	+ 2 SD
ID50s for *ex vivo* produce VRC07 (µg/ml)			
001428	0.0093	0.0032	0.0271
JRFLJB	0.0147	0.0038	0.0570
Q842	0.0304	0.0147	0.0632
T33-7	0.0146	0.0086	0.0247
TZBD	0.0355	0.0194	0.0647
ID50s from purified IgGs from all *in vivo* produced VRC07 (µg/ml)			
001428	0.0081	0.0018	0.0364
JRFLJB	0.0128	0.0026	0.0641
Q842	0.0193	0.0026	0.144
T33-7	0.0375	0.027	0.0521
TZBD	0.0357	0.0178	0.0711

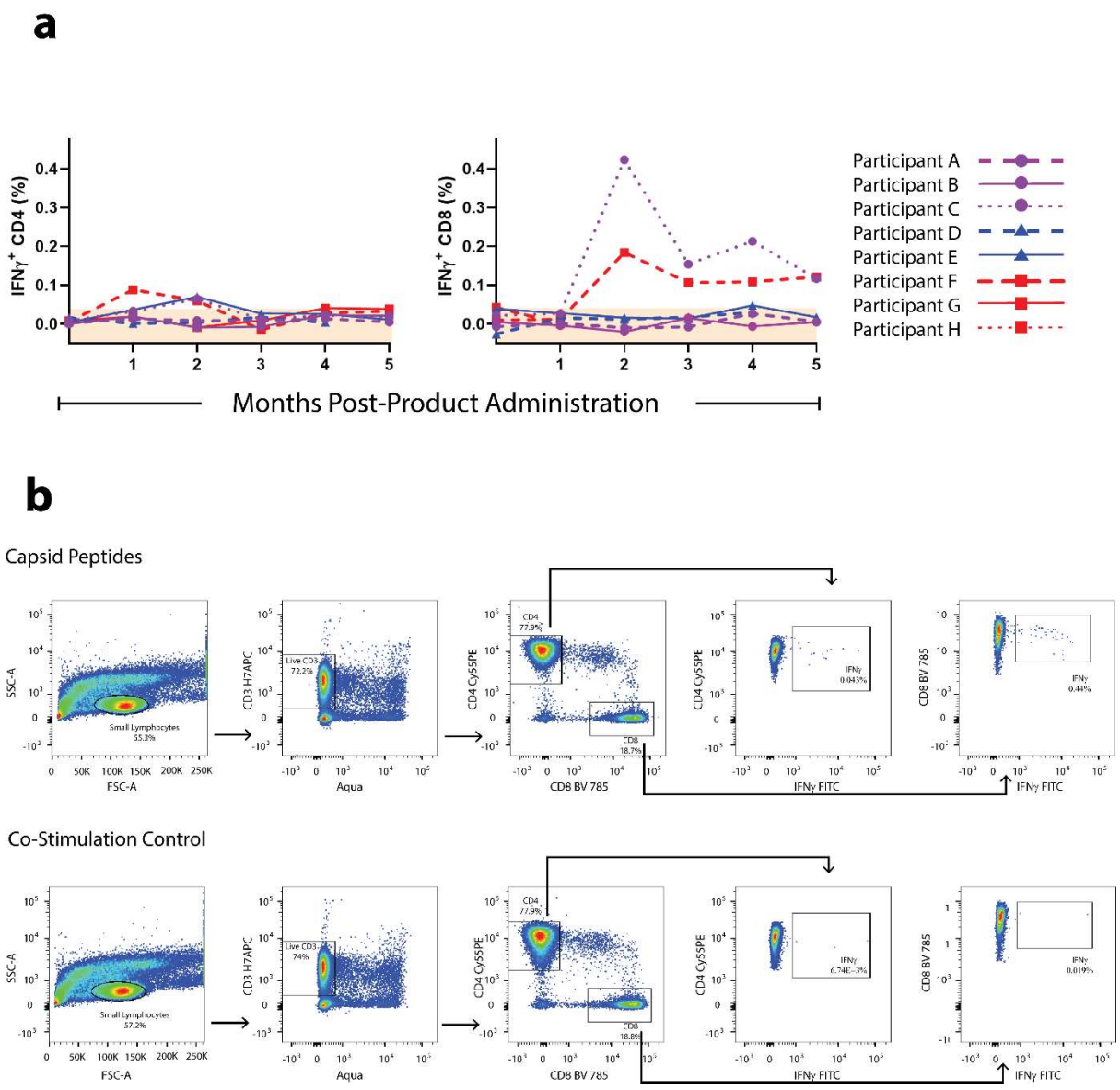


Supplemental Figure 1 AAV8 capsid serotiters for 48 pre-enrollment trial candidates. Titers are plotted as inverse serum dilution

Supplemental Fig. 2

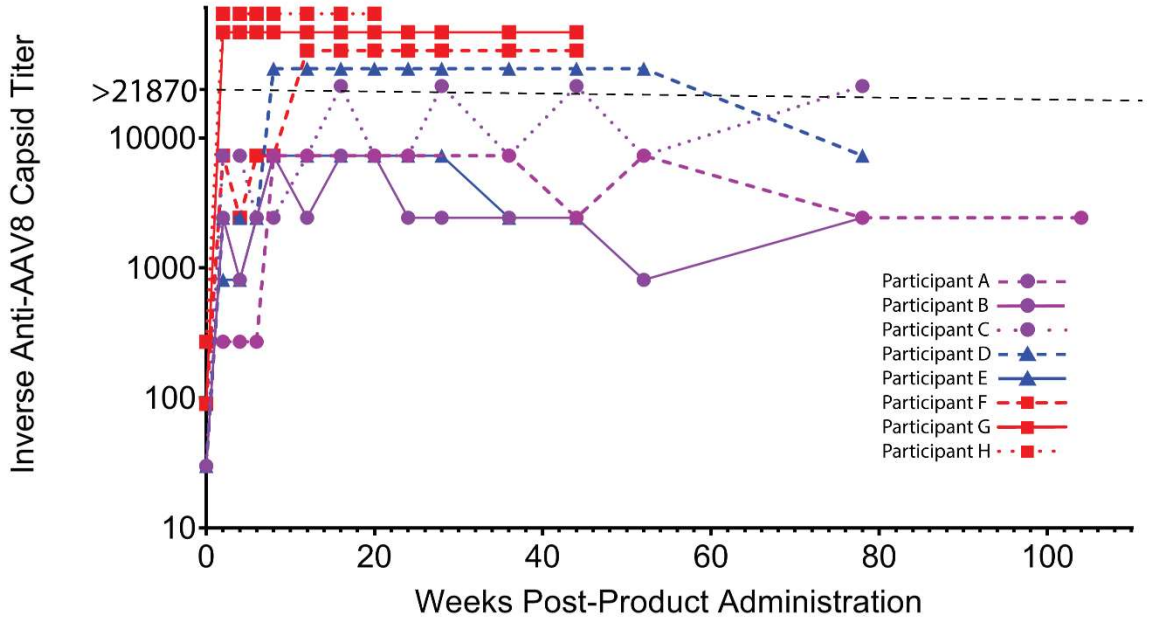


Supplemental Figure. 2 Longitudinal serum AST and ALT values after IM injection of 5 x 10^{10}, 5 x 10^{11} and 2.5 x 10^{12} AAV8-VRC07 vg/kg. Measured ALT is shown in column a), measured AST is shown in column b). Data from individual participants are as shown in the figure legend. Participants A-C received 5 x 10^{10}, Participants D and E received 5 x 10^{11}, and participants F-H received 2.5 x 10^{12} AAV8-VRC07 vg/kg. Limits of normal values for a) AST and b) ALT in males are shown in shading.

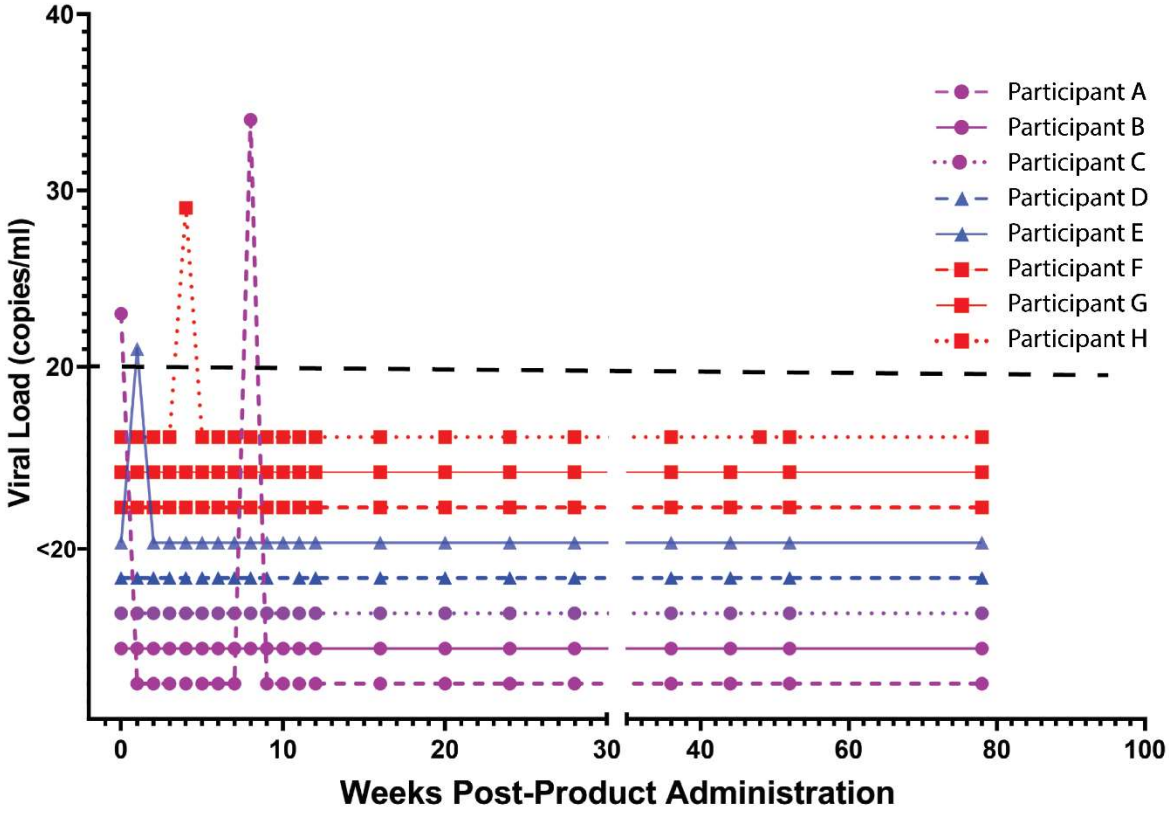


Supplemental Figure 3a Frequency (%) of CD4⁺ and CD8⁺ T cells producing IFNγ after stimulation with 15mer peptides overlapping by 11 amino acids covering capsid protein VP1, VP2 and VP3. Activity was measured using intracellular cytokine staining of cells incubated for 6h in the presence of monensin and brefeldin A. IFNγ responses for individual subjects are as indicated in the figure legend. Individual participants are as indicated in the figure legend. b) Gating strategy for the quantificaion of Intracellular IFNγ staining in CD4 and CD8 T cells in response to stimulation by an AAV8 peptide pool containing 15mer overlapping by 11 amino acids covering VP1, VP2, and VP3.

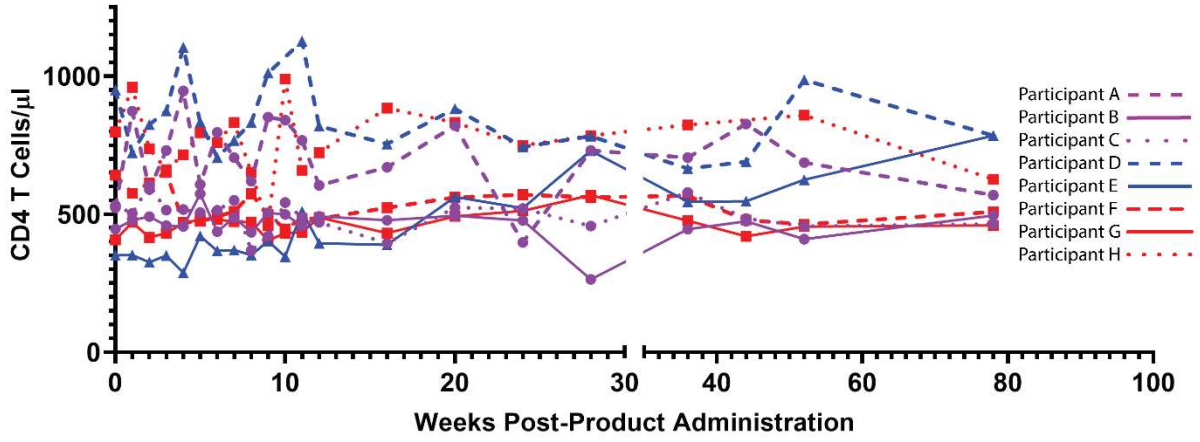
Supplemental Fig. 4



Supplemental Fig. 4 Longitudinal inverse anti-AAV8 capsid serotiters before and between 2 -104 weeks after IM injection of 5 x 10^{10}, 5 x 10^{11} and 2.5 x 10^{12} AAV8-VRC07 vg/kg. Individual participant values are as shown in the figure legend. Participants A-C received 5 x 10^{10}, participants D and E receive 5 x 10^{11} and participants F-H received 2.5 x 10^{12} vg AAV8-VRC07/kg.

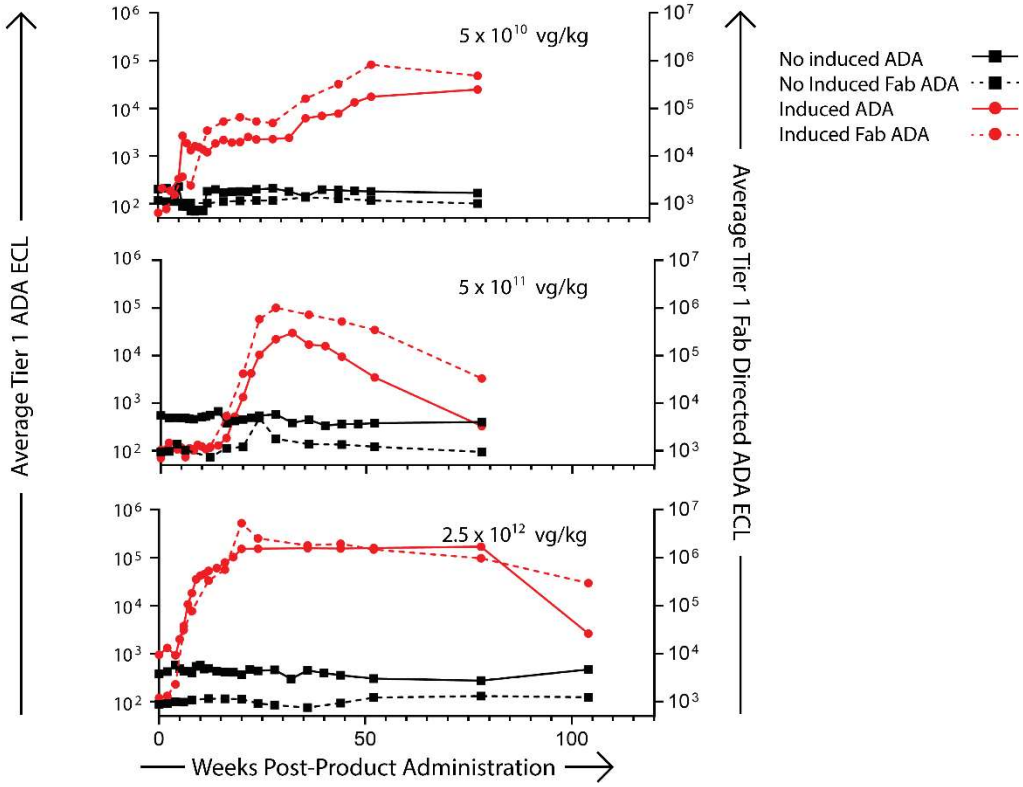


Supplemental Figure 5 Longitudinal HIV viral loads for participants in the 5×10^{10}, 5×10^{11} and 2.5×10^{12} AAV8-VRC07 vg/kg dose groups. Participants A-C received 5×10^{10}, participants D and E received 5×10^{11} and participant F-H received 2.5×10^{12} AAV8-VRC07 vg/kg. Data from individual participants are as indicated in the figure legend. Lower level of quantitation was 20 copies of HIV RNA/ml.



Supplemental Figure 6 Longitudinal CD4 T cell count for participants in 5 x 10^{10}, 5 x 10^{11} and 2.5 x 10^{12} AAV8-VRC07 vg /kg dose groups. Participants A-C received 5 x 10^{10}, participants D and E received 5 x 10^{11} and participants F-H received 2.5 x 10^{12} AAV8-VRC07 vg/kg. Individual participants are as shown in the figure legend.

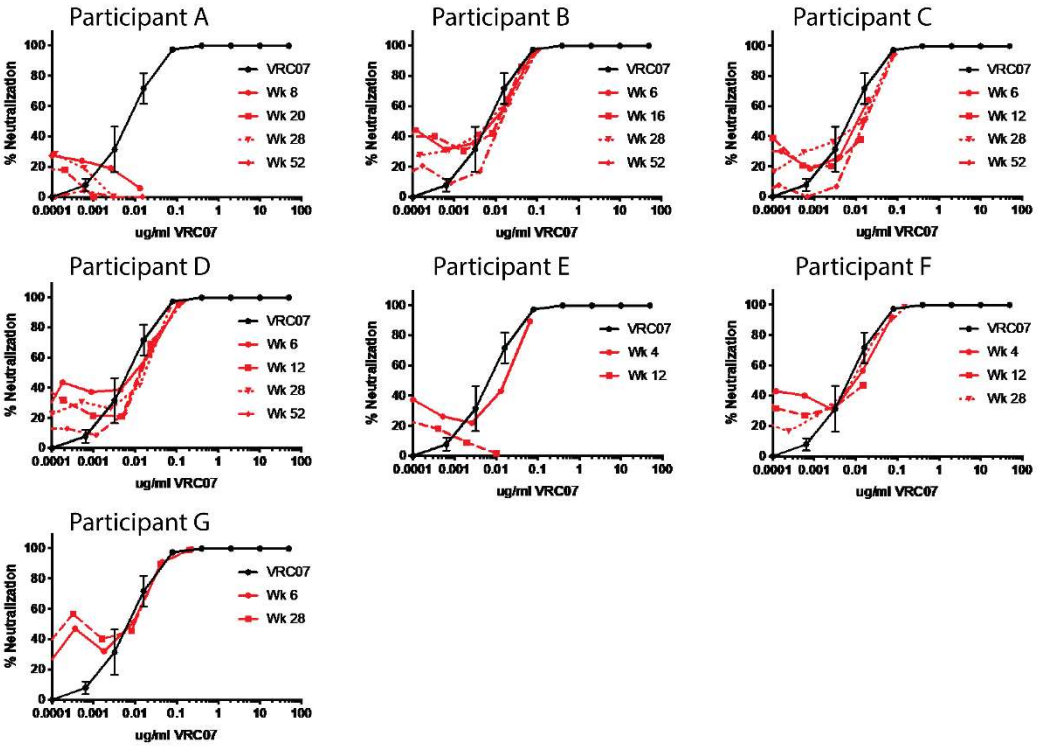
Supplemental Fig. 7



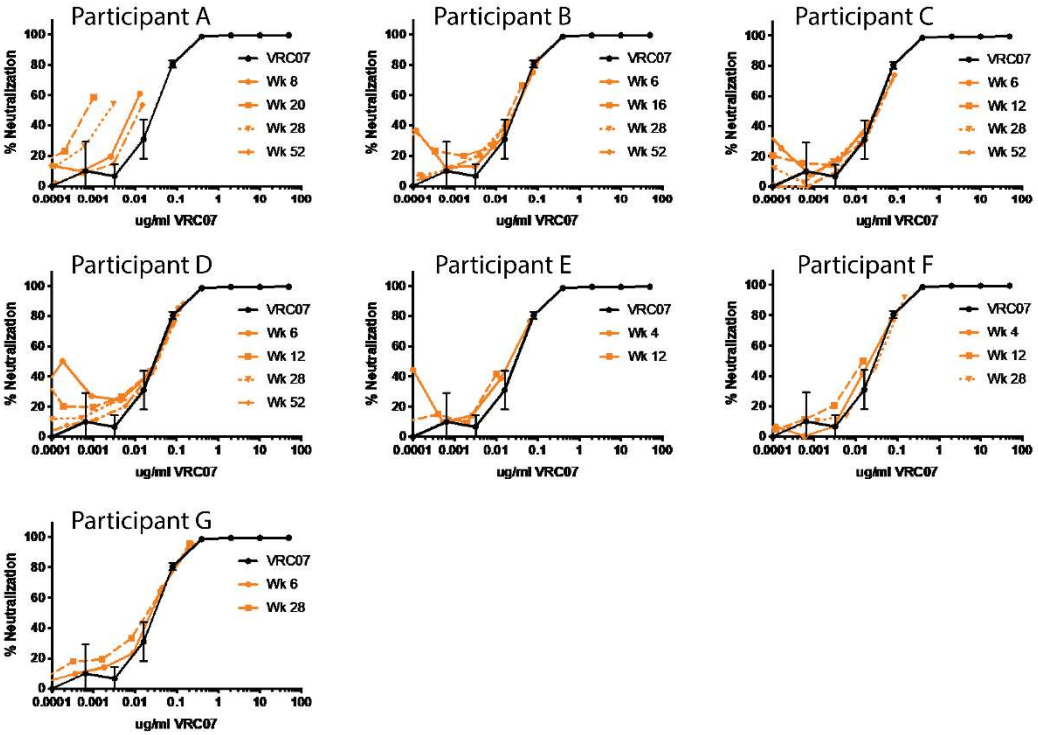
Supplemental Figure 7 Longitudinal tier 1 VRC07 ADA (left Y axis) and Fab directed ADA (right Y axis) activity. ADA activity is shown as measured electrochemiluminescence (ECL) in 1:30 dilutions of serum from participants in the 5 x 10^{10}, 5 x 10^{11} and 2.5 x 10^{12} AAV8-VRC07 vg/kg dose groups. Tier 1 ADA responses are shown on the left y axis, tier 1 ADA responses directed against the Fab fragment are shown on the right y axis.

Supplemental Figure 8

001428

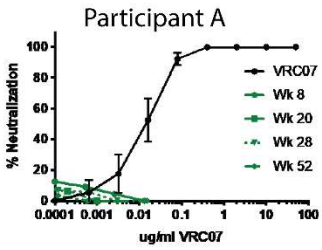


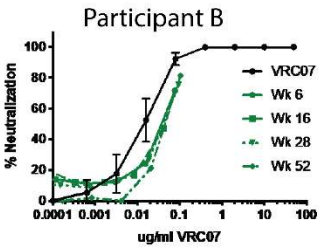
Q842

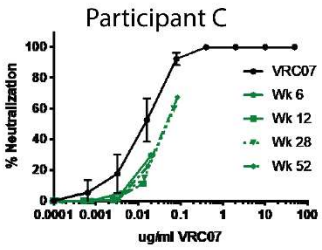


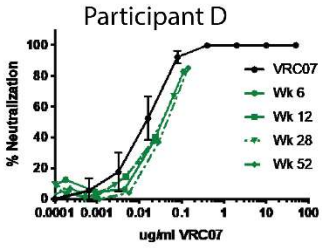
Supplemental Figure 8 (continued)

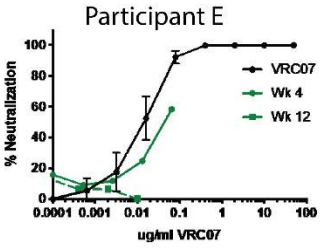
T33-7

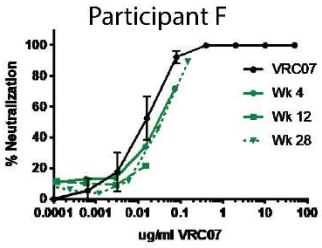


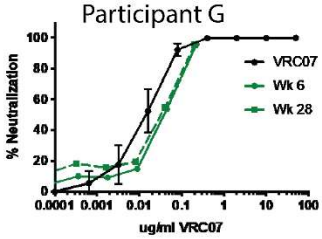




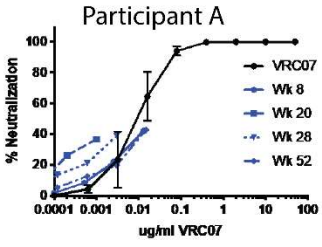


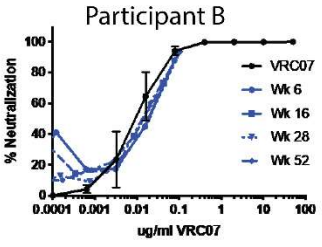


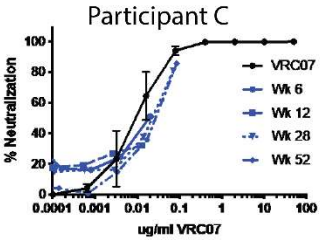


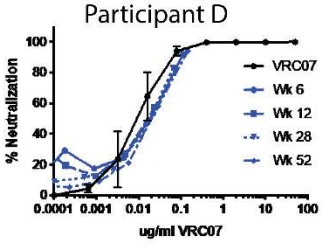


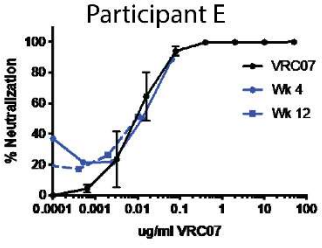
JRFL.JB

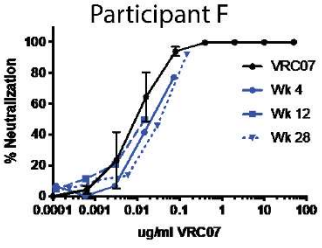


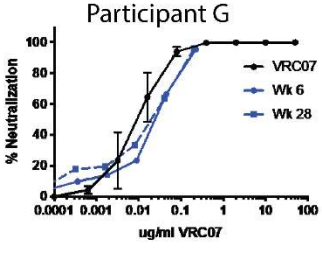






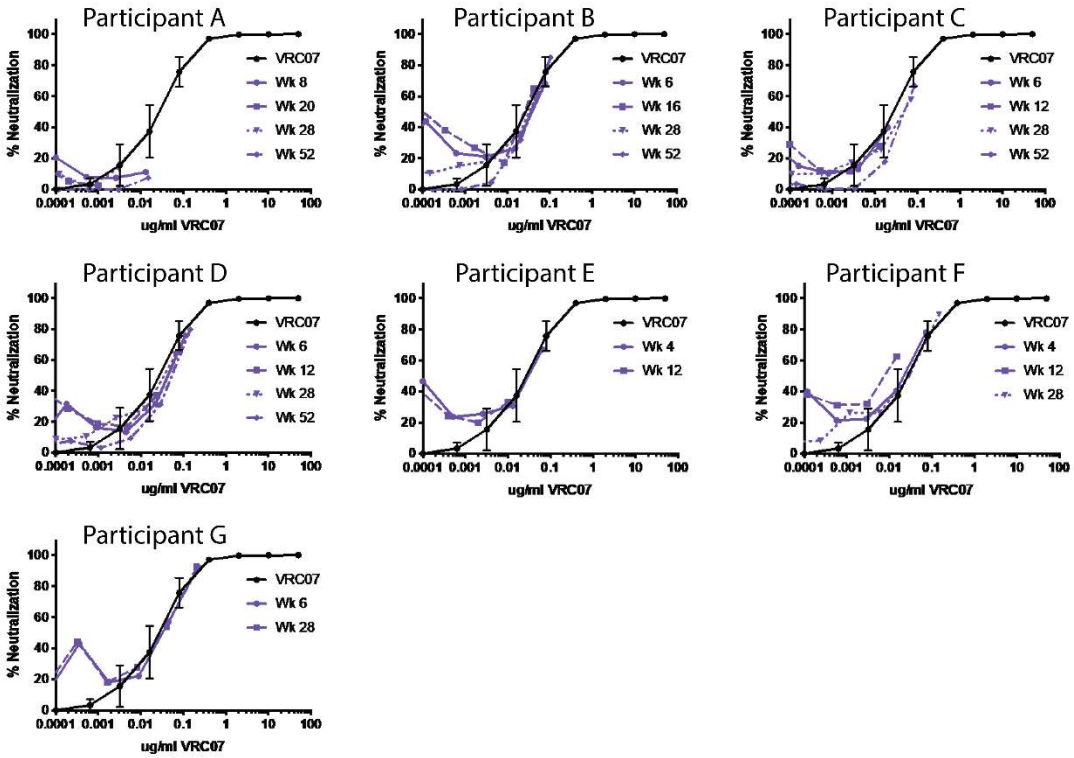




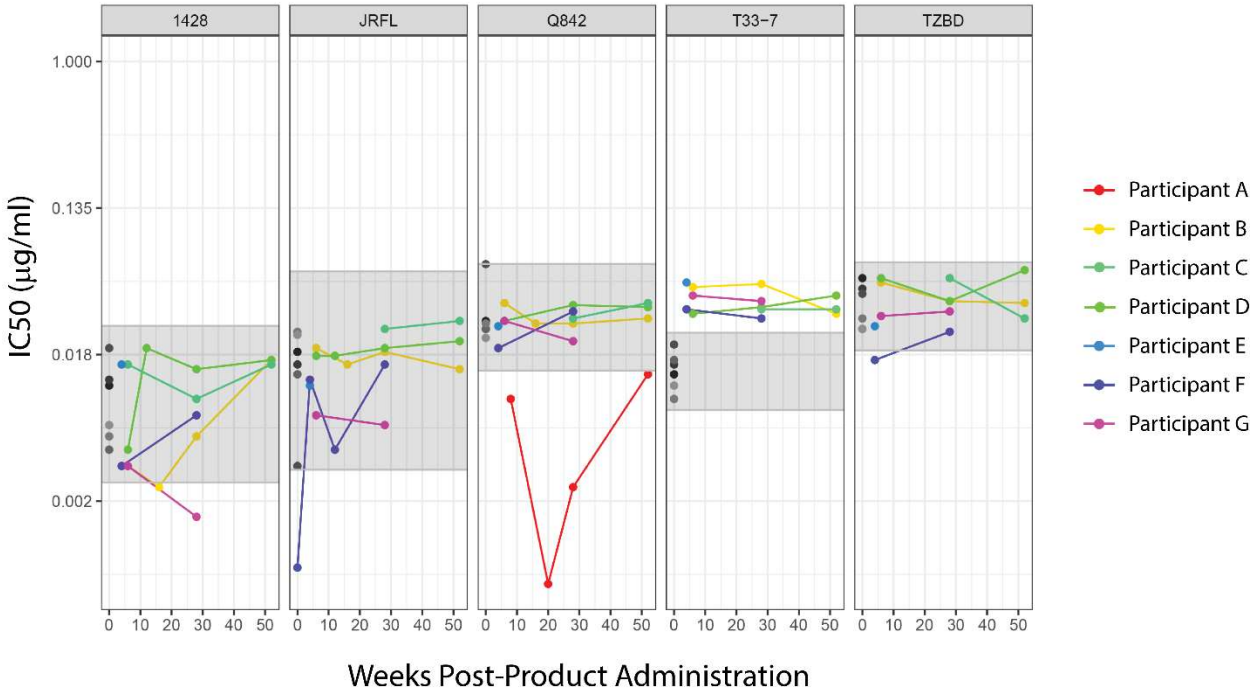


Supplemental Figure 8 (continued)

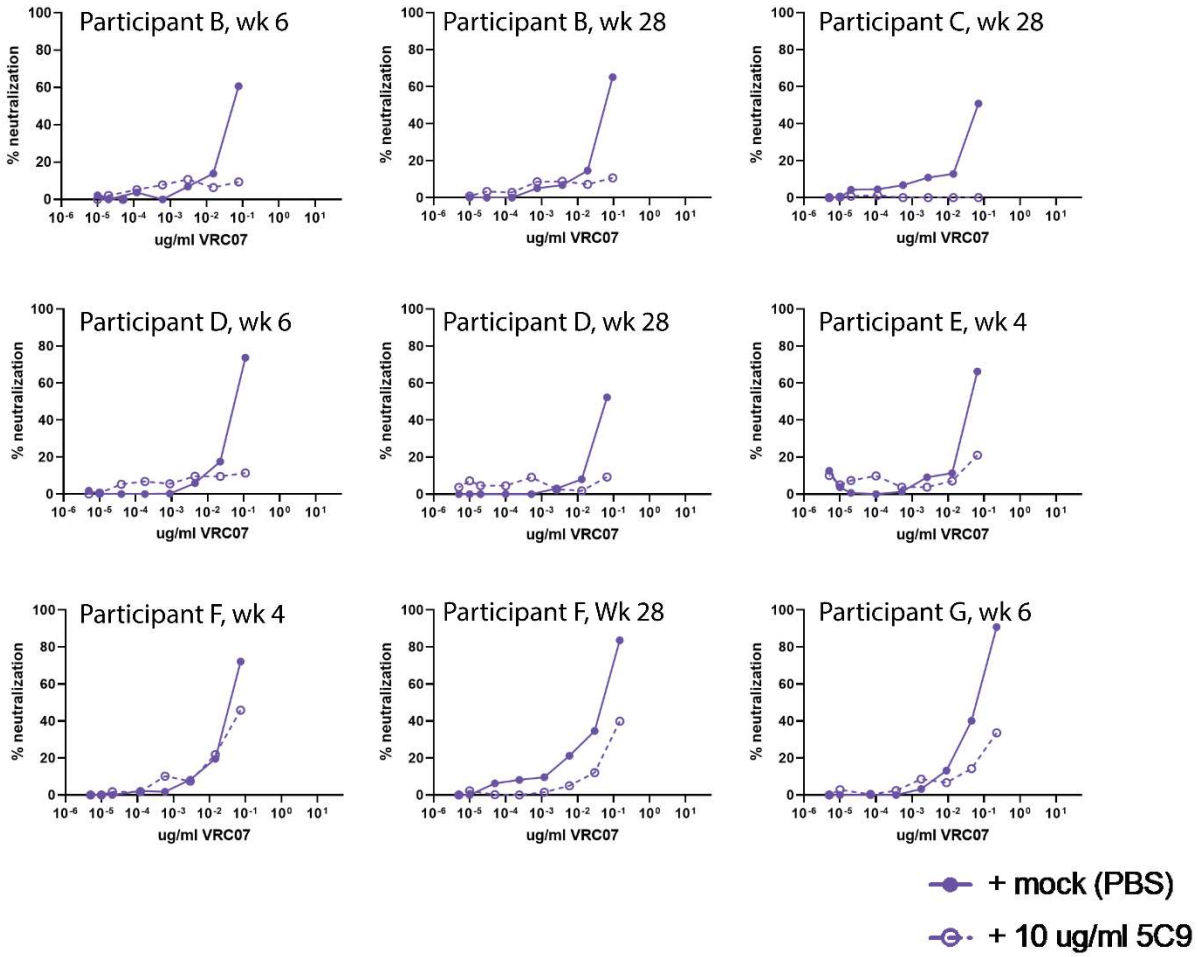
TZBD.02



Supplemental Figure 8 Longitudinal tier 2 pseudovirus neutralization by purified IgG containing VRC07. Percent neutralization of tier 2 pseudovirus in participant A-G, by purified IgG containing VRC07, is shown on the left axis. Measured VRC07 contained in each assay is shown on the x axis. Neutralization curves for each purified IgG sampled at the indicated timepoints are overlaid with the neutralization curve of *in vitro*-purified VRC07 (black lines). Pseudovirus neutralized is shown in the left margin. Data points for neutralization assays of ex vivo produced VRC07 represent the average of 6 replicate assays, error bars are ± SD. Data points for *in vivo* produced VRC07 represent a single assay.



Supplemental Fig. 9 Comparison of longitudinal pseudoviral IC50s for *in vivo* produced and *in vitro* produced and purified VRC07. Longitudinal IC50s determined for *in vivo* produced VRC07 from purified IgGs from seven different participants are as shown in the figure legend to the right of the graph as compared to IC50s determined for *ex vitro* produced VRC07 shown in black dots shown at the left of each segment. Shaded areas show ±2 SD around the geometric mean of the *ex vitro* samples. In total IgG was purified from 120 different plasma sample. From those samples 77 samples contained adequate VRC07 to determine the IC50s for pseudoviral neutralizations studies. Determinations of *ex vivo* produced and *in vitro* produced VRC07 IC50s were performed using the same pseudovirus lots. Each data point shown represents one assay.



Supplemental Figure 10 Longitudinal neutralization data and the effect of VRC07 paratope binding by 5C9 antibody on neutralization by purified IgG from study participants. Percent neutralization of TZBD.02 is shown on the y axis, measured concentration of VRC07 in purified IgG is shown on the x axis. Neutralization of TZBD.02 in the absence (-●-) and presence of 5C9 (- -○- -) are shown.