

Supplemental Information

**AAV-Mediated Expression of Broadly Neutralizing
and Vaccine-like Antibodies Targeting
the HIV-1 Envelope V2 Region**

Fiona T. van den Berg, Nigel A. Makoah, Stuart A. Ali, Tristan A. Scott, Rutendo E. Mapengo, Lorraine Z. Mutsvunguma, Nonhlanhla N. Mkhize, Bronwen E. Lambson, Prudence D. Kgagudi, Carol Crowther, Salim S. Abdool Karim, Alejandro B. Balazs, Marc S. Weinberg, Abdullah Ely, Patrick B. Arbutnot, and Lynn Morris

SUPPLEMENTAL INFORMATION

Table S1. Expression levels of secreted antibodies in cell media.

Antibodies secreted in cell media following infection of HEK293T cells with AAV2- or AAV8-antibody vectors were detected using ELISA and quantified using a purified IgG antibody to generate a standard curve.

Antibody	Concentration (µg/ml)	
	AAV2	AAV8
VRC01	14.3	1.2
PGT121	0.6	0.3
CAP256.08	1.9	0.6
CAP256.09	7.6	0.6
CAP256.16	23.0	0.6
CAP256.21	12.6	1.3
CAP256.25	15.7	0.9
CAP228-16H	18.7	1.6
CAP228-19F	19.6	1.3

Table S2. Summary of correlation between antibody and anti-antibody expression.

Correlation between antibody expression (µg/ml) and anti-antibody expression (OD₄₅₀) was assessed using a Pearson correlation test (two-tailed p-value, $\alpha = 0.05$). p value: ns: not significant, $p > 0.05$; *: $0.01 > p \leq 0.05$; **: $0.001 > p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$

Summary						
Antibody group	Week	Number of Mice	Correlation coefficient r	r squared	p Value	Significance
All	2	53	-0.57	0.33	<0.0001	****
	24	53	-0.28	0.08	0.041	*
All CAP256	2	33	-0.44	0.19	0.0110	*
	24	33	-0.43	0.19	0.0123	*
All CAP228	2	15	-0.39	0.15	0.1557	ns
	24	15	-0.40	0.16	0.1449	ns
VRC01	2	5	-0.67	0.45	0.214	ns
	24	5	-0.72	0.52	0.168	ns
CAP256.08	2	9	-0.87	0.76	0.0021	**
	24	9	-0.49	0.24	0.185	ns
CAP256.09	2	6	-0.33	0.11	0.526	ns
	24	6	-0.67	0.45	0.143	ns
CAP256.16	2	5	-0.84	0.70	0.0765	ns
	24	5	-0.70	0.49	0.186	ns
CAP256.21	2	6	-0.90	0.82	0.0134	*
	24	6	-0.25	0.06	0.633	ns
CAP256.25	2	7	-0.56	0.32	0.188	ns
	24	7	-0.27	0.07	0.565	ns
CAP228.16H	2	7	-0.53	0.29	0.216	ns
	24	7	-0.37	0.13	0.418	ns
CAP228.19F	2	8	0.52	0.27	0.184	ns
	24	8	-0.29	0.09	0.481	ns

Table S3. Primer sequences used to amplify common elements of the antibody expression cassette.

Primer	Sequence (5' – 3')
SS F	GTCCTGGGTGACGAACAGGC
SS R	TGCCGAGCCCTCCTGTAACCA
HC G F	GTTACAGGAGGGCTCGGCACGAGACGGATATCCGTCTCAGCGAGACCAAGGGCCCATCGGTC
HC G R	GAGGGGGCAGCCTTGGGCTGACCCGAGACGTTTCGCGACGTCTCTTGCCGAGCCCTTTGGAGCC
LC F	GGTCAGCCCAAGGCTGCC
LC R	TGTAATCCAGAGGTTGATTAGGATCCTTTATGAACATTCTGTAGGGGCCAC

BsmBI recognition sites are underlined.

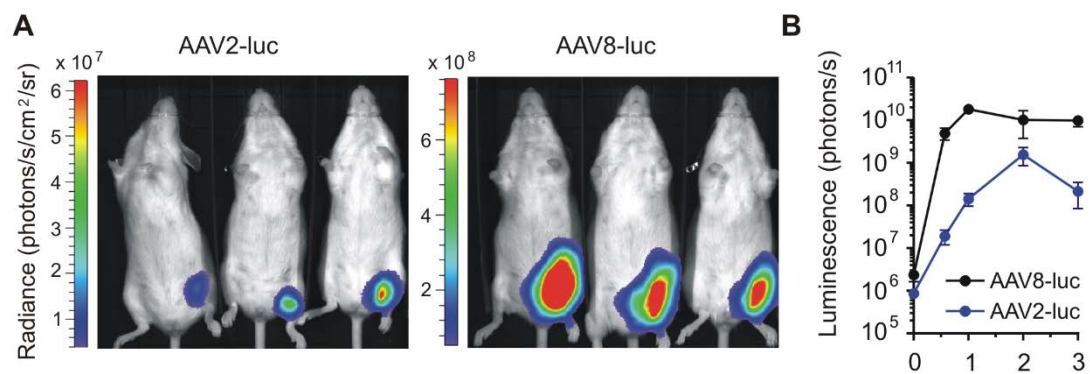
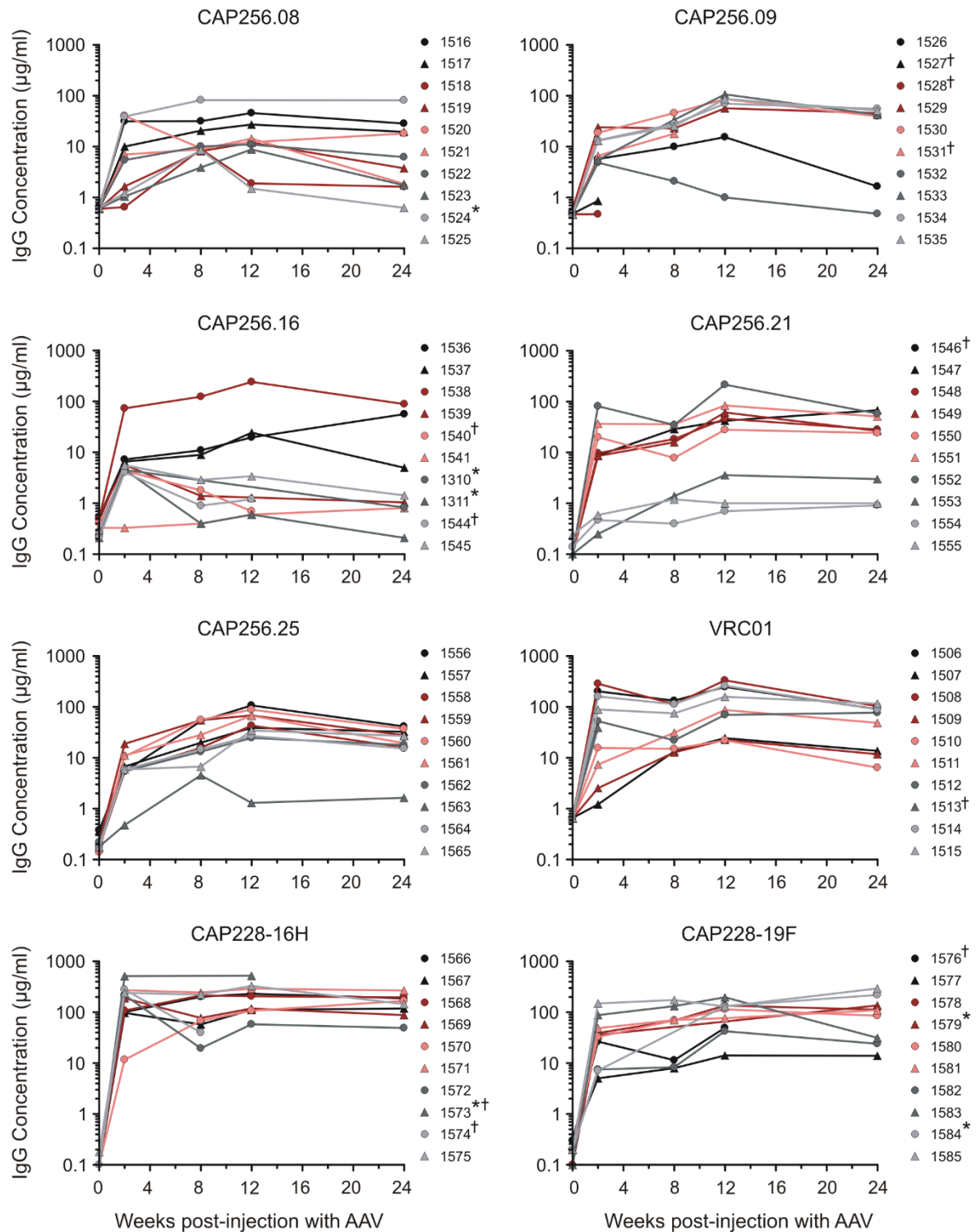


Figure S1. Bioluminescence of mice treated with AAV2-Fluc or AAV8-Fluc.

A. Representative bioluminescence images with 5 s exposure of mice at week 1 that received 1×10^{10} viral genome copies of the AAV2-Fluc or AAV8-Fluc. **B.** Mean luminescence for the AAV2-Fluc ($n = 6$) and AAV8-Fluc ($n = 6$) control groups. Luminescence was measured using unsaturated images acquired with a 5 s exposure and quantified as total flux (photons/s). Error bars: standard error of the mean.



† Mice died or were euthanized.
 * No sample available at one or more time points.

Figure S2. Human IgG antibody levels in individual mice over 24 weeks.

The antibody serum concentrations for NMRI mice were measured by sandwich ELISA over 24 weeks post-AAV8 infection. Results are shown for individual mice in each treatment group (n = 7 – 10). Ten mice were euthanized for several reasons or died with several causes identified by post-mortem: failure to recover from anaesthetic (3); enteritis (1); non-healing skin wound (1); serious skin reaction to metal ear tag (1); abdominal tumour (2); apparent hind limb paralysis (1) and gastrointestinal abnormalities (1). No serum sample was available at certain time points for some mice because a low volume of blood was collected. Alternatively, a mouse was omitted from a specific blood sample collection point because of concerns over health or weight.