Supporting Information

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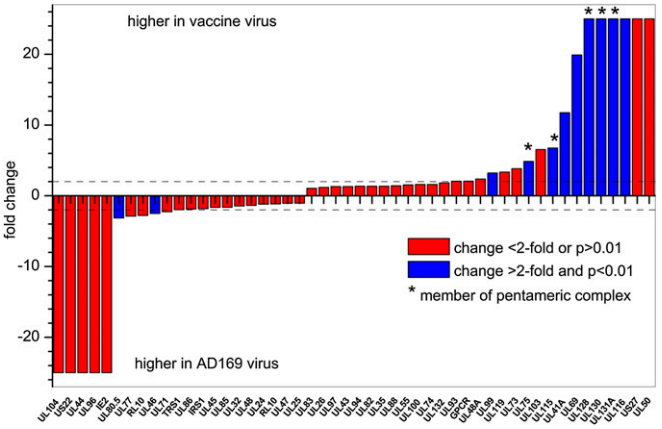
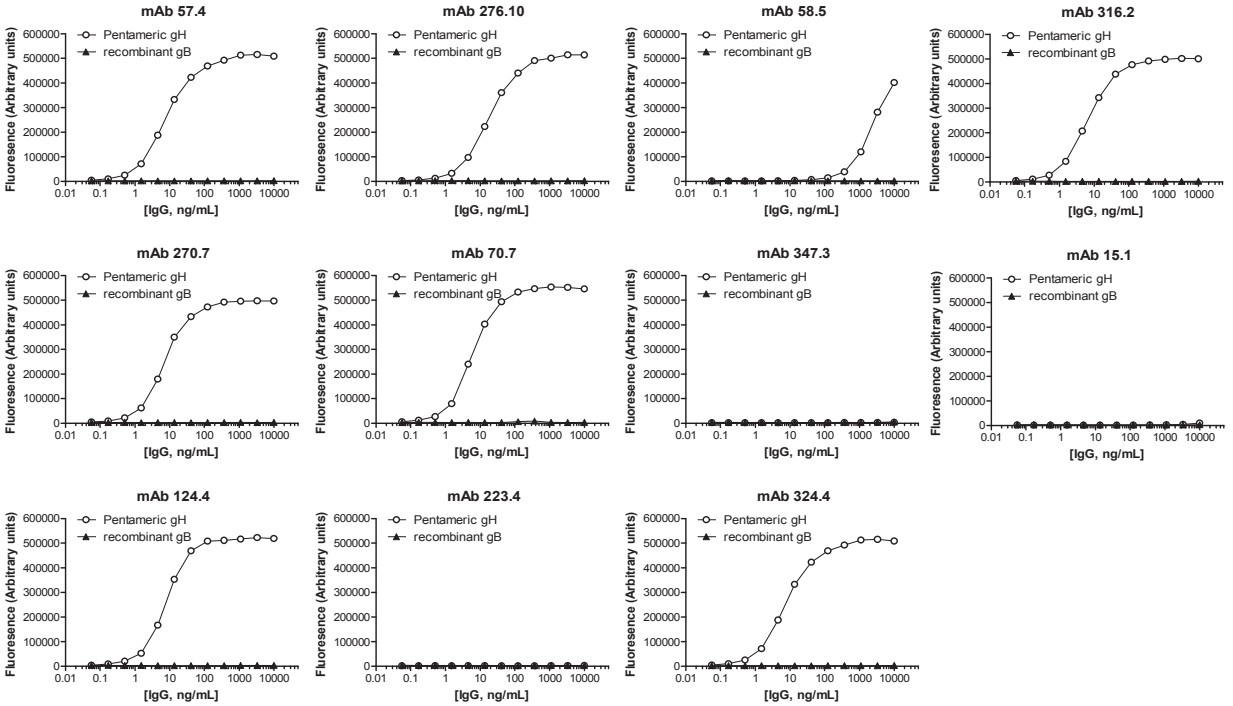


Fig. S1. Comparison of proteomic compositions of AD169 virus versus the vaccine virus. The protein composition of the parental strain AD169 and the experimental vaccine were determined by semiquantitative, label-free shotgun proteomics. Tryptic digests of each strain were analyzed in triplicate by nano-liquid chromatography-MS/MS. The analysis identified 50 viral proteins at a false-discovery rate of less than 0.5%. Label-free quantification was performed based on the peak height of identified MS signals and fold change values were calculated to differentiate AD169 and the experimental vaccine. An analysis of variance (ANOVA) was performed to identify statistically significant changes (P value < 0.01). Fold changes were considered to be significant if they were higher than twofold or if the P value of the ANOVA analysis was below 0.01. Fold changes shown in red bars are not significant. Fold changes shown in blue bars are significant. Proteins that are exclusively identified in either one of the samples were artificially set to a fold change of ± 25 to visualize them. Members of the pentameric glycoprotein H (gH) complex are indicated with an asterisk. The guiding dashed lines indicate twofold change limits.

A. Elite neutralizing antibodies



B. Elite binding antibodies

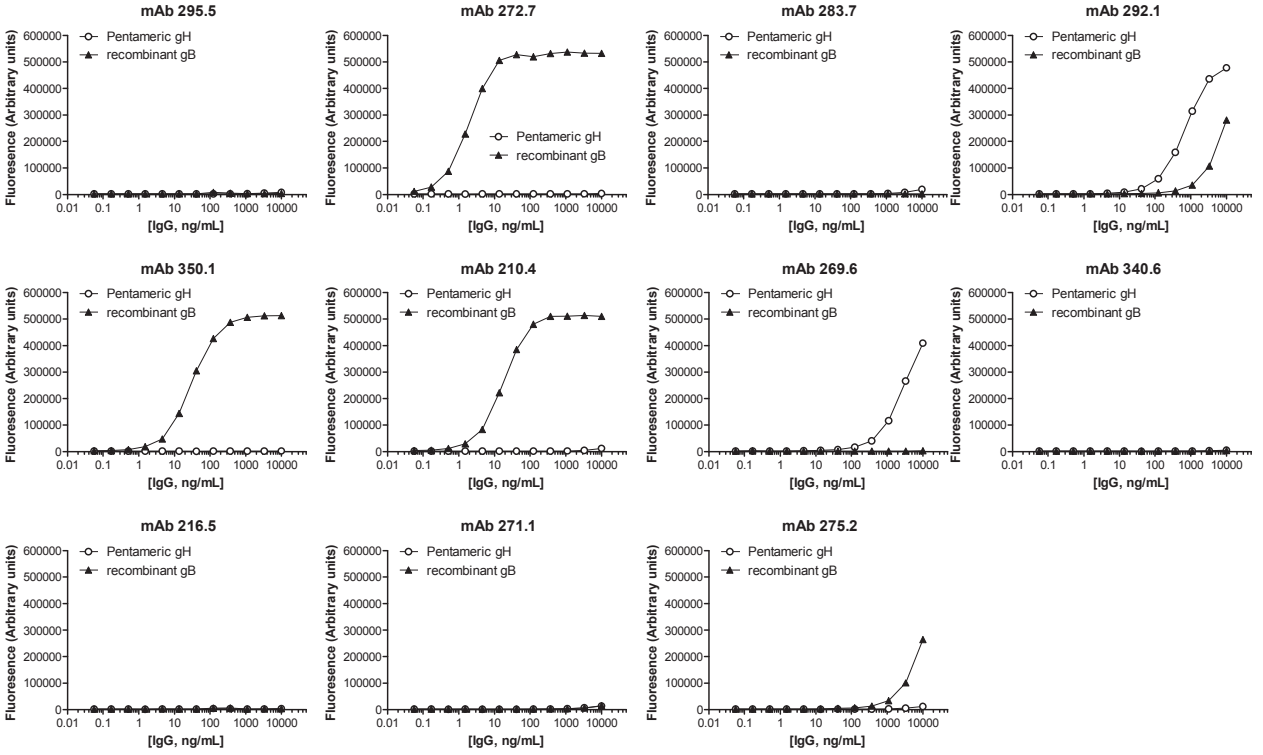
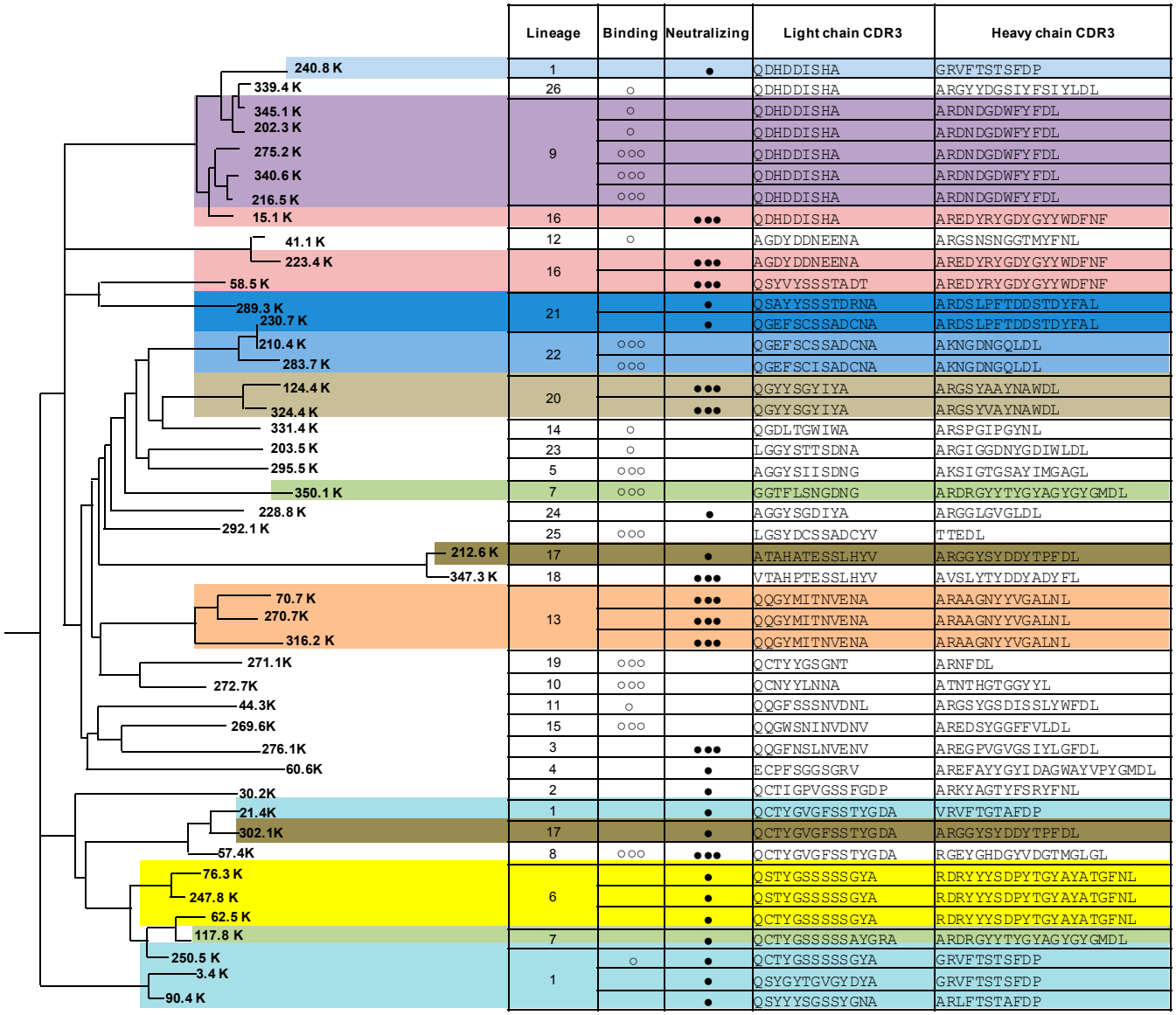


Fig. S2. Reactivity to recombinant glycoprotein B (gB) or recombinant pentameric gH complex by elite neutralizing and elite binding mAbs. Elite neutralizing antibodies (*A*) and elite binding antibodies (*B*) were tested for reactivity to recombinant gB or recombinant pentameric gH complex immobilized on plate (*Materials and Methods*).



Lineage	Binding	Neutralizing	Light chain CDR3	Heavy chain CDR3
1		●	QDHDDISHA	GRVFTSTSFDP
26	○		QDHDDISHA	ARGYYDGSIYFSIYLDL
9	○		QDHDDISHA	ARDNDGDWFYFDL
	○		QDHDDISHA	ARDNDGDWFYFDL
	○○○		QDHDDISHA	ARDNDGDWFYFDL
	○○○		QDHDDISHA	ARDNDGDWFYFDL
	○○○		QDHDDISHA	ARDNDGDWFYFDL
16		●●●	QDHDDISHA	AREDYRYGDYGYYWDFNF
12	○		AGDYDDNEENA	ARGSNSNGGTMYFNL
16		●●●	AGDYDDNEENA	AREDYRYGDYGYYWDFNF
		●●●	QSYVYSSSTADT	AREDYRYGDYGYYWDFNF
21		●	QSAYYSSSTDRNA	ARDSLPFTDDSTDYFAL
		●	QGEFSCSSADCNA	ARDSLPFTDDSTDYFAL
22	○○○		QGEFSCSSADCNA	AKNGDNGQLDL
	○○○		QGEFSCISADCNA	AKNGDNGQLDL
20		●●●	QGYYSGYIYA	ARGSYAAYNAWDL
		●●●	QGYYSGYIYA	ARGSYVAYNAWDL
14	○		QGDLTGWIWA	ARSPGIPGYNL
23	○		LGGYSTTSDNA	ARGIGGDNYGDIWLDL
5	○○○		AGGYSIISDNG	AKSIGTGSAYIMGAGL
7	○○○		GGTFLSNGDNG	ARDRGYYTYGYAGYGYGMDL
24		●	AGGYSGDIYA	ARGGLGVGLDL
25	○○○		LGSYDCSSADCYV	TTEDL
17		●	ATAHATESSLHYV	ARGGYSYDDYTPFDL
18		●●●	VTAHPTESSLHYV	AVSLYTYDDYADYFL
13		●●●	QQGYMITNVENA	ARAAGNYYVGALNL
		●●●	QQGYMITNVENA	ARAAGNYYVGALNL
		●●●	QQGYMITNVENA	ARAAGNYYVGALNL
19	○○○		QCTYYGSGNT	ARNFDL
10	○○○		QCNYYLNNA	ATNTHGTGGYYL
11	○		QQGFSSSNVDNL	ARGSYGSDISSLYWFDL
15	○○○		QQGWSNINVDNV	AREDSYGGFFVLDL
3		●●●	QQGFNSLNVENV	AREGPVGVGSIYLGFDL
4		●	ECPFSGGSGRV	AREFAYYGYIDAGWAYVPYGMDL
2		●	QCTIGPVGSSFGDP	ARKYAGTYFSRYFNL
1		●	QCTYGVGFSSTYGDA	VRVFTGTAFDP
17		●	QCTYGVGFSSTYGDA	ARGGYSYDDYTPFDL
8	○○○	●●●	QCTYGVGFSSTYGDA	RGEYGHDGYVDGTMGLGL
6		●	QSTYGSSSSSGYA	RDRYYYSDPYTGYAYATGFNL
		●	QSTYGSSSSSGYA	RDRYYYSDPYTGYAYATGFNL
		●	QCTYGSSSSSGYA	RDRYYYSDPYTGYAYATGFNL
7		●	QCTYGSSSSSAYGRA	ARDRGYYTYGYAGYGYGMDL
1	○	●	QCTYGSSSSSGYA	GRVFTSTSFDP
		●	QSYGYTGVGYDYA	GRVFTSTSFDP
		●	QSYYYSGSSYGNA	ARLFTSTAFDP

Fig. S3. Phylogenetic analysis of 45 rabbit mAbs and their lineages in correlation to their binding and neutralizing properties. Phylogenetic tree was constructed based on the entire light-chain variable-region amino acid sequence, and lineage groups were classified based on similarities in the heavy-chain complementarity-determining region 3 (CDR3) (Fig. 6). Lineage groups containing two or more mAbs are highlighted with different background colors. The solid dots identify neutralizing mAbs, with those of three dots indicating the elite neutralizing mAbs, whereas the open circles identify nonneutralizing mAbs, with those of three circles indicating the elite binding mAbs.

Table S1. Binding and neutralizing properties for all 45 rabbit mAbs

Rabbit mAb clone ID	Binding to virus (EC_{50} binding), μg/mL	Neutralization in ARPE-19 cells (EC_{50} neutralizing), μg/mL	Neutralization in MRC-5 cells (EC_{50} neutralizing), μg/mL
3.4	2.13	0.25	0.19
15.1	1.07	0.03	100.00
21.4	2.58	0.48	0.35
30.2	0.90	0.45	0.20
41.1	0.18	100.00	100.00
44.3	8.79	3.13	100.00
57.4/57.4	0.09	0.01	100.00
58.5	0.59	0.02	100.00
60.6	0.55	0.13	0.08
62.5	0.84	0.20	100.00
70.7	1.46	0.03	0.09
76.3	0.75	0.23	0.47
90.4	2.81	0.47	0.23
117.8	1.66	0.27	1.47
124.4	1.72	0.05	0.08
202.3	0.10	10.00	100.00
203.5	4.03	17.03	100.00
210.4	0.03	100.00	100.00
212.6	52.91	3.71	100.00
216.5	0.06	100.00	100.00
223.4	1.54	0.06	100.00
228.8	3.34	0.28	0.25
230.7	0.27	0.34	0.34
240.8	2.60	0.79	100.00
247.8	0.76	0.16	0.28
250.5	5.27	2.31	1.26
269.6	0.03	100.00	100.00
270.7	0.68	0.02	0.06
271.1	0.07	39.66	100.00
272.7	0.01	100.00	100.00
275.2	0.10	100.00	100.00
276.10	0.25	0.01	100.00
283.7	0.01	100.00	100.00
289.3	4.83	0.76	0.59
292.1	0.02	68.67	100.00
295.5	0.00	100.00	100.00
302.1	4.74	0.75	100.00
316.2	1.06	0.02	0.08
324.4	4.09	0.10	0.21
331.4	100.00	100.00	100.00
339.4	2.36	100.00	100.00
340.6	0.05	100.00	100.00
345.1	0.11	100.00	100.00
347.3	0.34	0.03	100.00
350.1	0.02	100.00	100.00

All antibodies are sorted based on their clone ID, numerically. Binding and neutralizing properties for each antibody are expressed as the IgG concentration to achieve 50% of the desired functional measure. All EC_{50} values were obtained by four-parameter curve fitting, with $R^2 \geq 0.90$. An arbitrary value of 100 μg/mL was assigned if the fitting was unconverged as described in *Materials and Methods*. Elite neutralizing and elite binding antibodies are marked in red and blue, respectively, and the designation is based on their ≥10-fold higher neutralizing or binding activity than those of CytoGam (Fig. 2 *A* and *B*). mAb 57.4 is an elite neutralizing and binding antibody.

Table S2. Somatic mutational rates for all 45 mAbs

	mAb ID	V_H, aa	No. changes, aa	% mutation		V_L, aa	No. changes, aa	% mutation	
Neutralizing mAb (listed in ascending order based on their EC_{50} neutralizing)	57.4.	106	33	0.31		113	21	0.19	
	276.1.	106	13	0.12		109	21	0.19	
	58.5.	106	10	0.09		111	17	0.15	
	316.2.	106	16	0.15		113	21	0.19	
	270.7.	106	16	0.15		113	16	0.14	
	70.7.	106	15	0.14		113	17	0.15	
	347.3.	105	10	0.10		115	8	0.07	
	15.1.	106	9	0.08		113	21	0.19	
	124.4.	106	14	0.13		112	11	0.10	
	223.4.	106	10	0.09		111	20	0.18	
	324.4.	106	11	0.10		112	8	0.07	
	60.6.	106	11	0.10		114	26	0.23	
	247.8.	103	30	0.29		114	17	0.15	
	62.5.	103	28	0.27		114	16	0.14	
	76.3.	103	28	0.27		114	18	0.16	
	3.4.	104	23	0.22		111	25	0.23	
	117.8.	106	5	0.05		114	15	0.13	
	228.8.	106	12	0.11		112	19	0.17	
	230.7.	106	10	0.09		115	11	0.10	
	30.2.	106	26	0.25		113	24	0.21	
	90.4.	104	18	0.17		112	15	0.13	
	21.4.	104	21	0.20		113	20	0.18	
	302.1.	106	5	0.05		113	22	0.19	
	289.3.	106	11	0.10		111	18	0.16	
	240.8.	104	24	0.23		112	24	0.21	
			Average =	0.16	(CI: 0.122, 0.187)		Average =	0.16	(CI: 0.142, 0.179)
Nonneutralizing mAb (listed in ascending order based on their EC_{50} binding)	295.5.	105	16	0.15		111	16	0.14	
	272.7.	104	21	0.20		110	16	0.15	
	283.7.	105	6	0.06		115	12	0.10	
	292.1.	105	14	0.13		115	14	0.12	
	350.1.	106	5	0.05		111	20	0.18	
	210.4.	105	6	0.06		115	11	0.10	
	269.6.	106	16	0.15		113	24	0.21	
	340.6.	106	22	0.21		113	23	0.20	
	216.5.	106	21	0.20		113	23	0.20	
	271.1.	106	12	0.11		111	18	0.16	
	275.2.	106	18	0.17		113	22	0.19	
	202.3.	106	20	0.19		113	23	0.20	
	345.1.	106	17	0.16		113	21	0.19	
	41.1.	106	14	0.13		111	19	0.17	
	339.4.	106	7	0.07		113	22	0.19	
	203.5.	105	11	0.10		111	16	0.14	
	250.5.	104	24	0.23		114	14	0.12	
	44.3.	106	17	0.16		114	23	0.20	
	212.6.	106	3	0.03		115	4	0.03	
	331.4.	106	11	0.10		111	14	0.13	
			Average =	0.13	(CI: 0.106, 0.161)		Average =	0.16	(CI: 0.134, 0.178)

Neutralizing mAbs are sorted based on their neutralizing capacity (EC_{50} neutralizing), whereas nonneutralizing mAbs are sorted based on their binding affinity (EC_{50} binding). Number of changes in V_H or V_L regions are determined based on IMGT database. CI, 95% confidence interval.