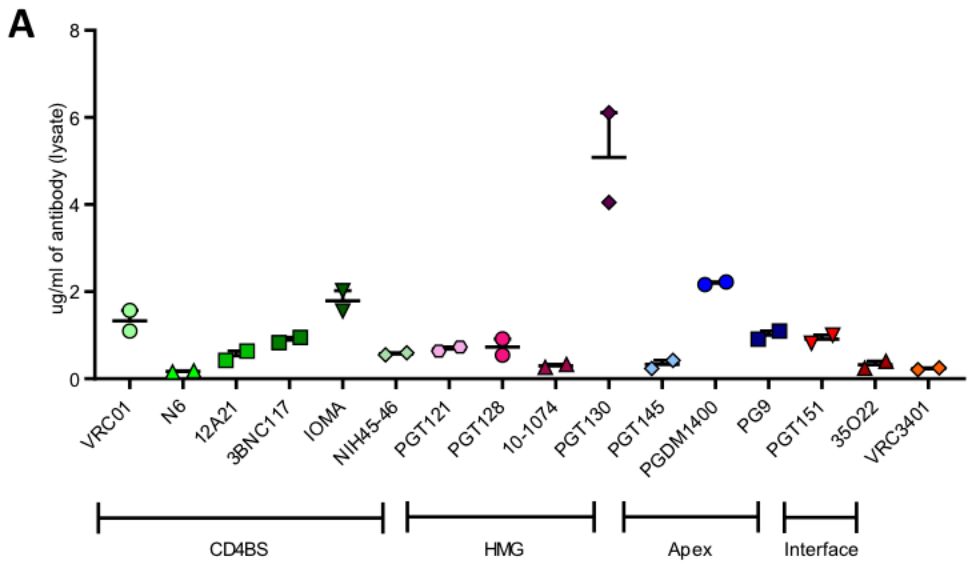
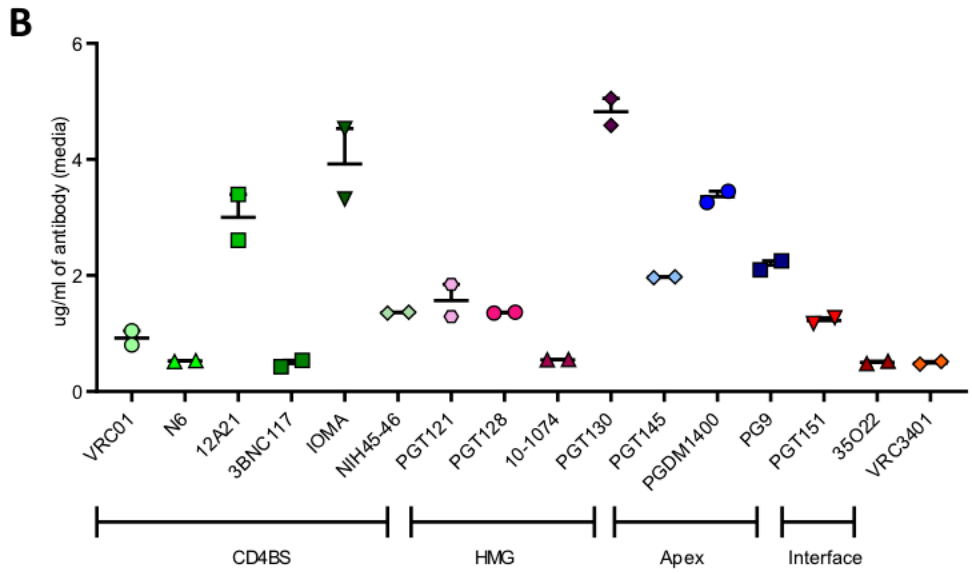
Supplemental Figures and Table





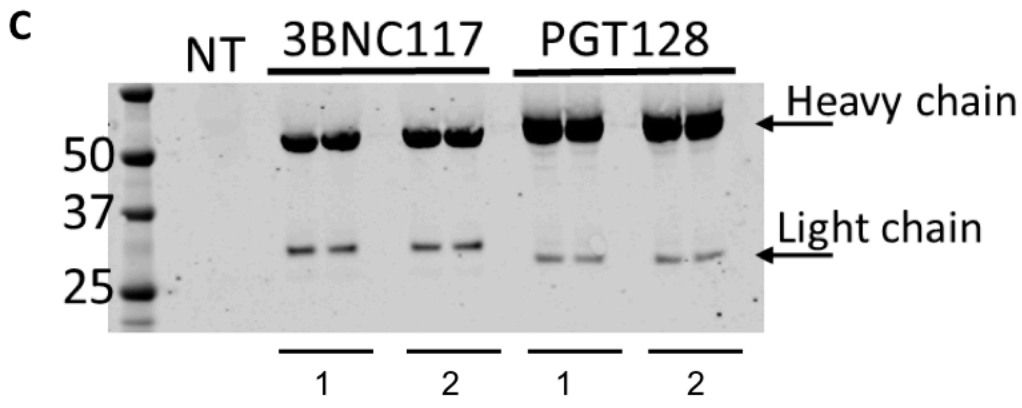


Figure S1: *In vitro expression of various HIV-1 specific dMAbs.* HEK293T cells were transiently transfected with plasmid dMAb constructs expressing 16 HIV-1 antibodies. Quantification of human IgG expression in cell lysate (**A**) and supernatant (media) (**B**) using two technical replicates but representative of two experimental replicates. (**C**) Example Western blot of two dMAbs, 3BNC117 and PGT128, in the media of transfected cells demonstrating expression of both the heavy and light chain. Lanes 1 and 2 for each dMAb were biological replicates.

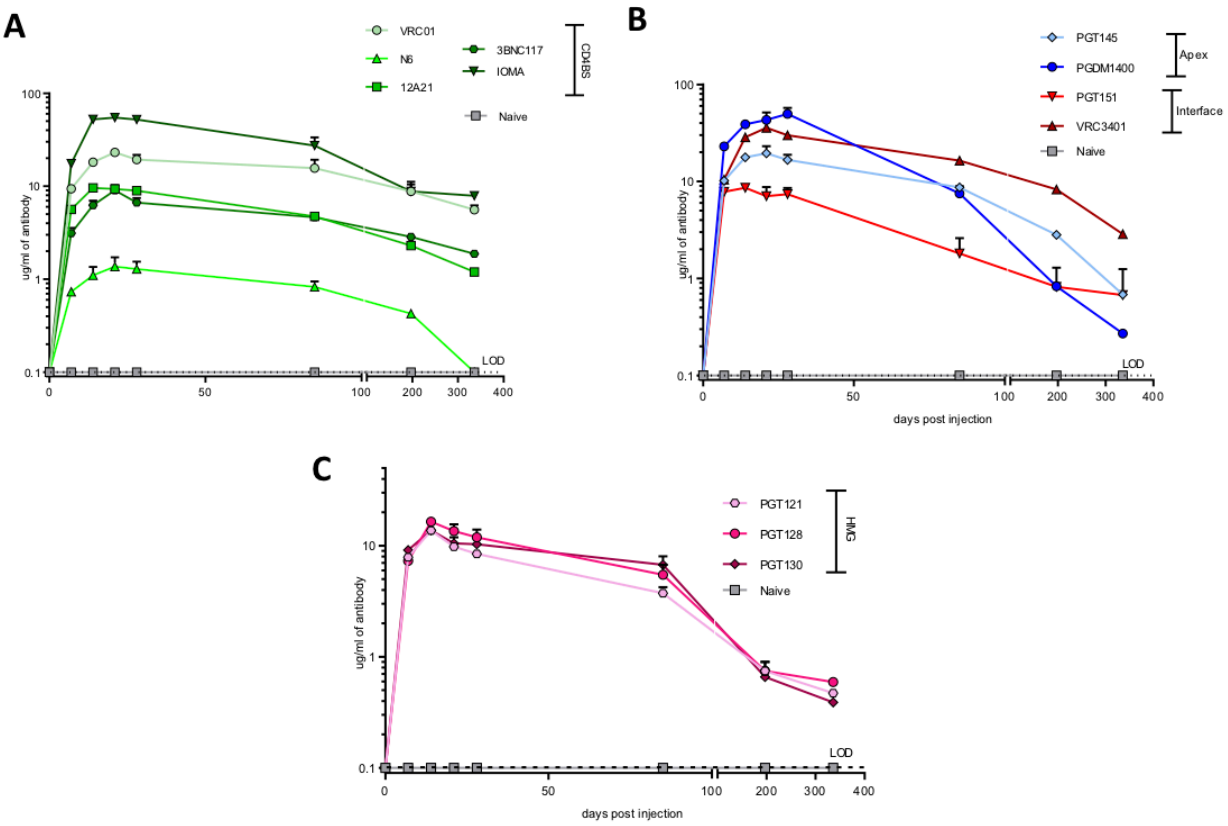


Figure S2: Time course expression of the CD4bs, Apex, HMG and Interface dMAbs in mice. Groups of mice (n=5) were transiently immunodepleted and delivered various dMAbs. Expression of dMAbs in the serum was followed over time for the CD4bs (A), apex and interface (B) and high mannose glycan (C) dMAbs as well as for naïve mice. Dots represent mean expression with bars displaying the standard error of the mean. Representative of two experimental replicates.

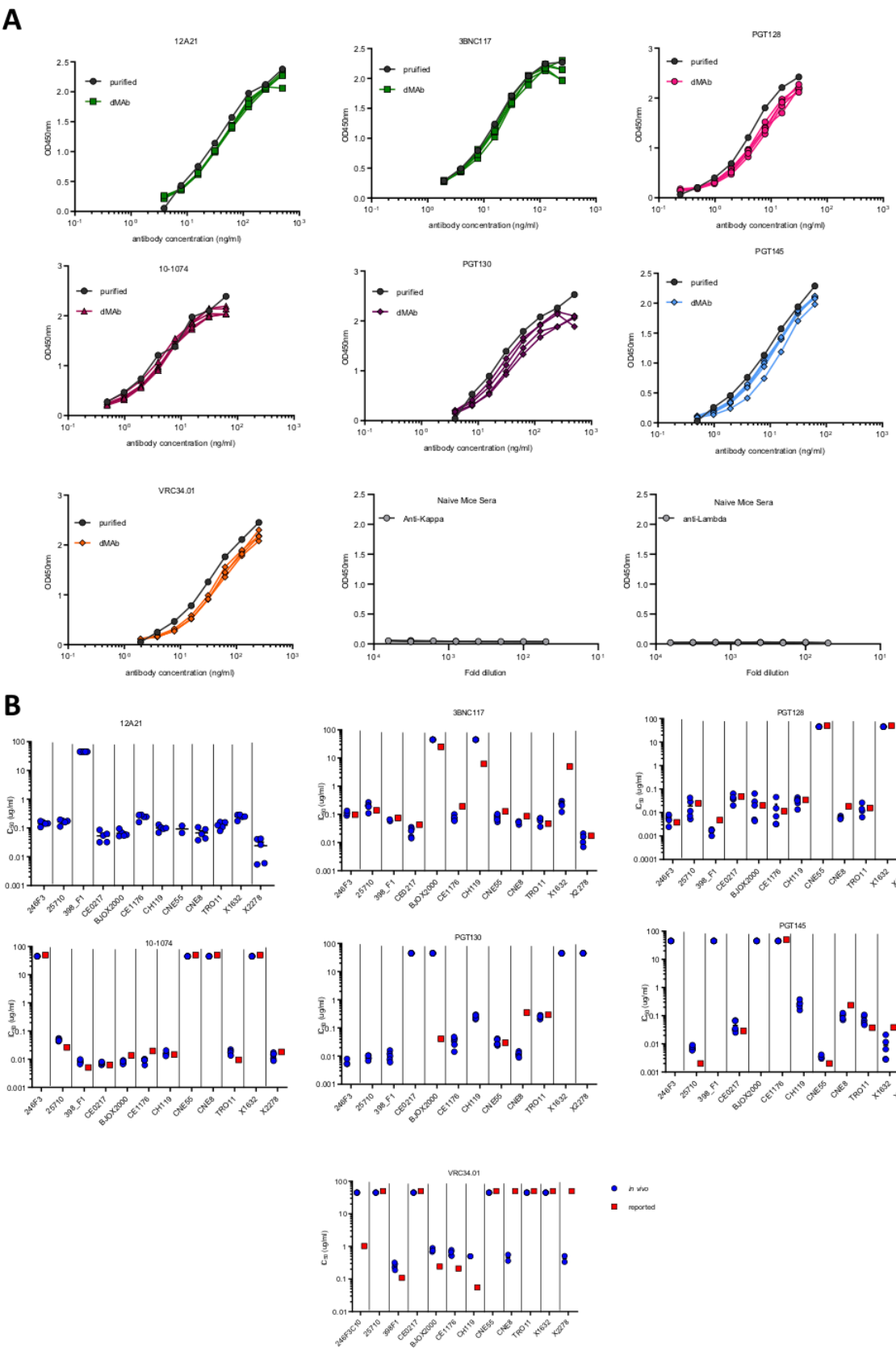

A
12A21
3BNC117
PGT128
10-1074
PGT130
PGT145
VRC34.01
Naive Mice Sera
purified
dMAb
Anti-Kappa
anti-Lambda
OD450nm
antibody concentration (ng/ml)
Fold dilution
B
12A21
3BNC117
PGT128
10-1074
PGT130
PGT145
VRC34.01
246F3
25710
398_F1
CE0217
BJOX2000
CE1176
CH119
CNE55
CNE8
TRO11
X1632
X2278
in vivo
reported

Figure S3: *Functionality of HIV-1 bNabs dMAbs produced in vivo*. (**A**) Binding curves of recombinant (black) and in vivo produced dMAbs (mice, Day 14, colors, n= 5) against HIV-1 Env trimer (BG505_MD39). No trimer binding was detected using naïve mouse serum using the two secondary antibodies. (**B**) Individual mouse IC_{50} across the 12-virus global panel (blue circles) vs values reported in the literature (red squares). Literature values gathered from Los Alamos CatNaber.

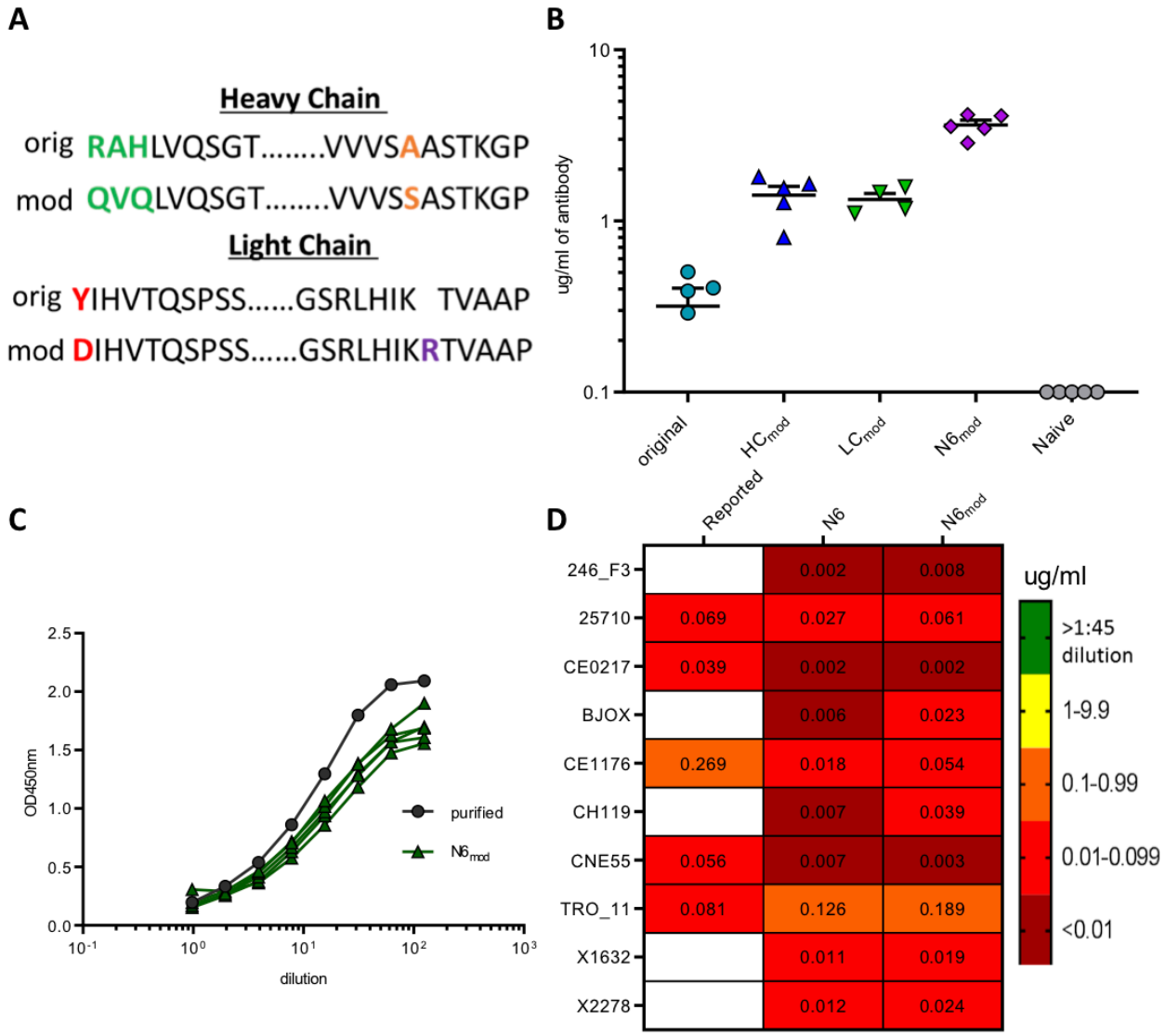
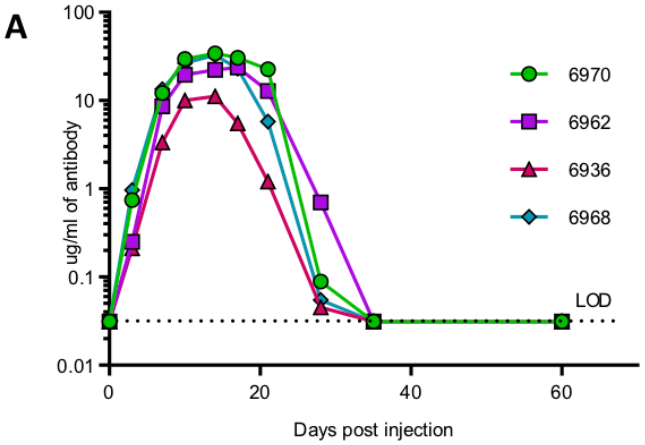
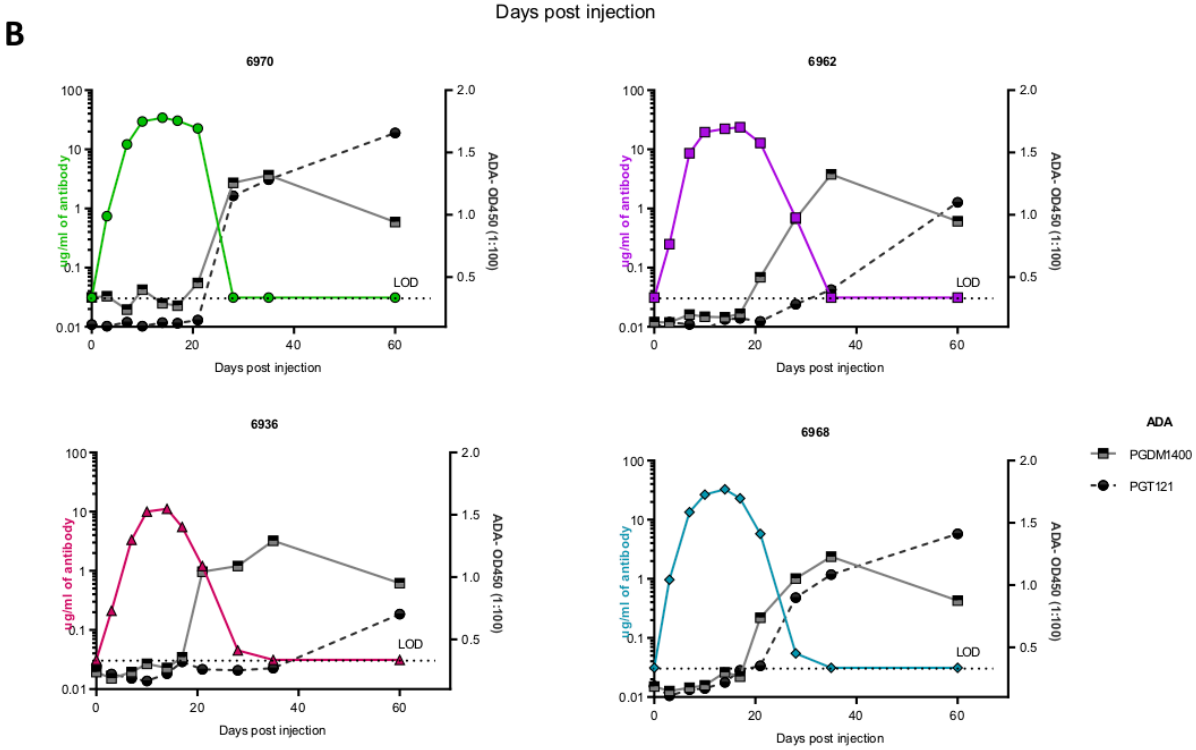


Figure S4: *Amino acid sequence modifications to HIV-1 broadly neutralizing antibody N6 improve dMAb expression in vivo without compromising N6 binding or function.* (**A**) Modifications to the beginning and end of the heavy and light chain amino acid sequence of human IgG1 monoclonal antibody N6 were produced. These modifications were selected to make the antibody sequence more similar to the human germline. (**B**) Groups of mice (n=5) were transiently immunodepleted and injected with plasmid DNA expressing original N6, heavy chain (HC) modified + light chain (LC) original (HC_{mod}), HC original + LC modified (LC_{mod}) or both HC and LC modified ($N6_{mod}$). Expression levels of serum dMAb was determined

on Day 14. (**C**) Serum binding of $N6_{mod}$ (green, triangle) to HIV-1 envelope trimer BG505_MD39 were compared to binding of purified monoclonal N6 original (black, circle). (**D**) Neutralization IC_{50} (μg/mL) of recombinant N6 original vs $N6_{mod}$ against HIV-1 envelope pseudotype viruses representing 10 of the global panel in a TZM-bl assay. Reported values gather from Los Alamos CatNaber.


A
100
10
1
0.1
0.01
ug/ml of antibody
0
20
40
60
Days post injection
6970
6962
6936
6968
LOD


B
6970
6962
6936
6968
ug/ml of antibody
ADA- OD450 (1:100)
Days post injection
LOD
ADA
PGDM1400
PGT121

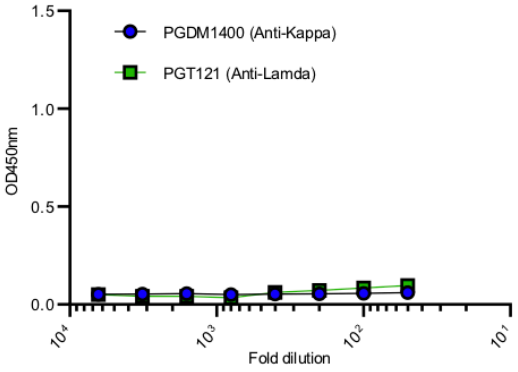

C
PGDM1400 (Anti-Kappa)
PGT121 (Anti-Lamda)
OD450nm
1.5
1.0
0.5
0.0
Fold dilution

Figure S5: *Individual expression of human IgG1 and ADA development in group 1 dMAb administered NHPs.* NHPs were injected on D0 with dMAb expressing PGDM1400 only. (**A**) Expression kinetics of human IgG1 in NHP serum for each of the four NHPs. (**B**) Expression of human IgG (left y-axis) vs ADA (right y-axis) against PGDM1400 (which was administered as dMAb) and PGT121 (which was not administered to group 1) over time. Expression and ADA levels representative of two replications. (**C**) Serum binding curves against HIV-1 Env trimer, BG505_MD39, using different secondary antibodies to establish the binding of PGDM1400 (human IgG1 kappa light chain, blue), and PGT121 (human IgG1 lambda light chain, green) for Day 0 pre-bleed serum.

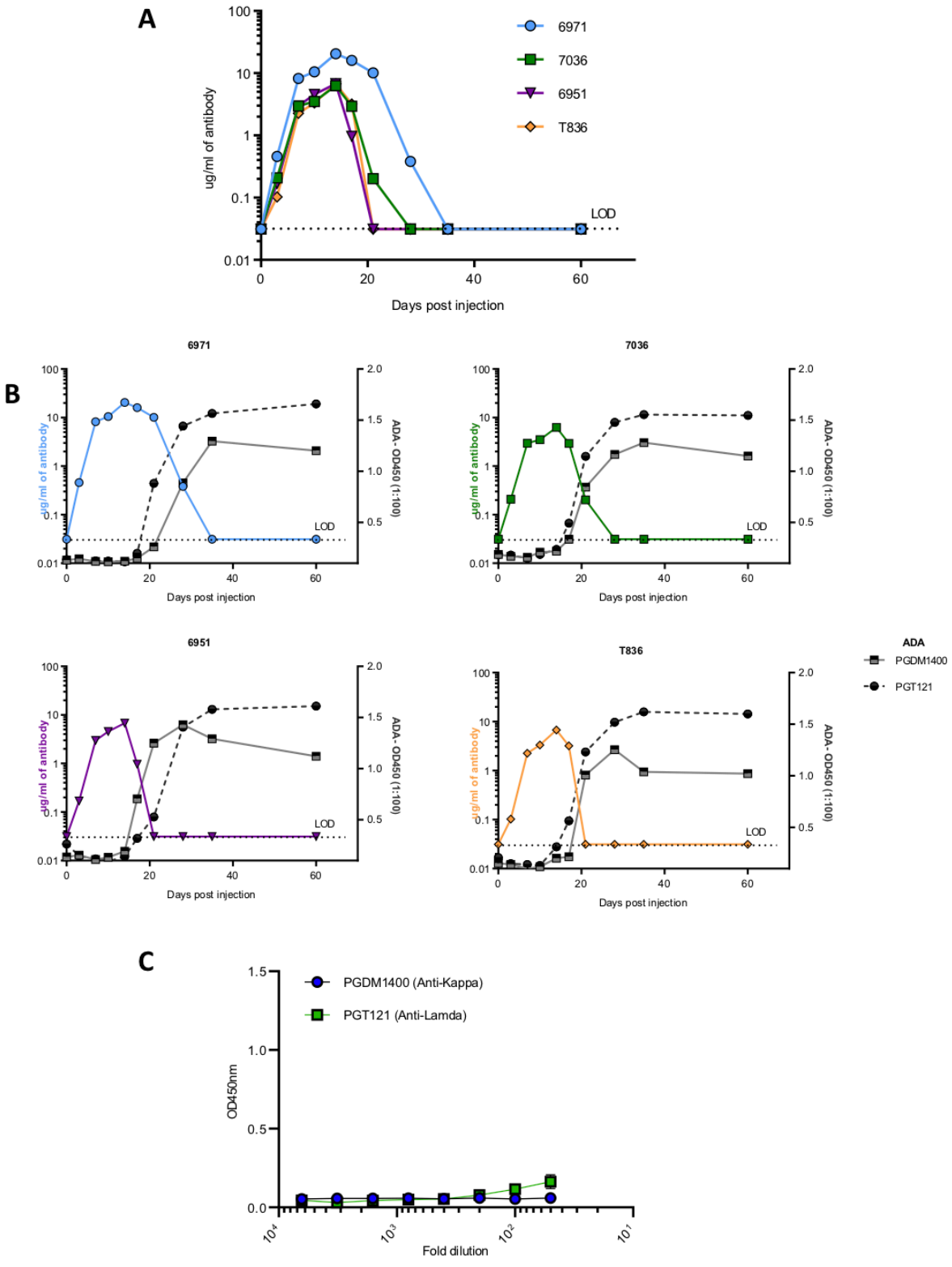


Figure S6: *Individual expression of human IgG1 and ADA development in group 2 dMAb administered NHPs.* NHPs were injected on D0 with dMAbs expressing PGDM1400 and PGT121. (**A**) Expression kinetics

of human IgG1 in NHP serum for each of the four NHPs. (**B**) Expression of human IgG (left y-axis) vs ADA (right y-axis) against PGDM1400 (dMAb administered) and PGT121 (dMAb administered) over time. Expression and ADA levels representative of two replications. (**C**) Serum binding curves against HIV-1 Env trimer, BG505_MD39, using different secondary antibodies to establish the binding of PGDM1400 (human IgG1 kappa light chain, blue), and PGT121 (human IgG1 lambda light chain, green) for Day 0 pre-bleed serum.

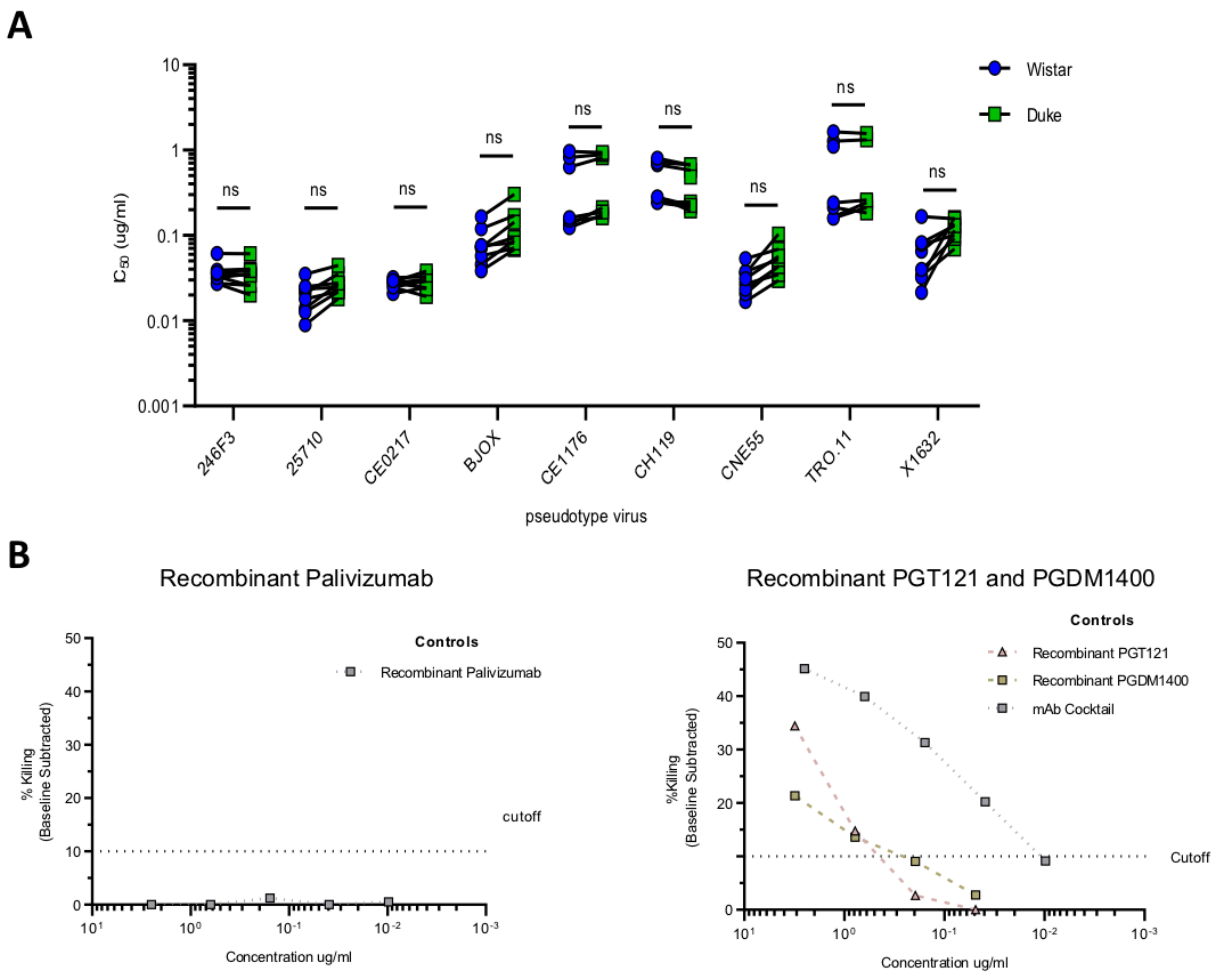
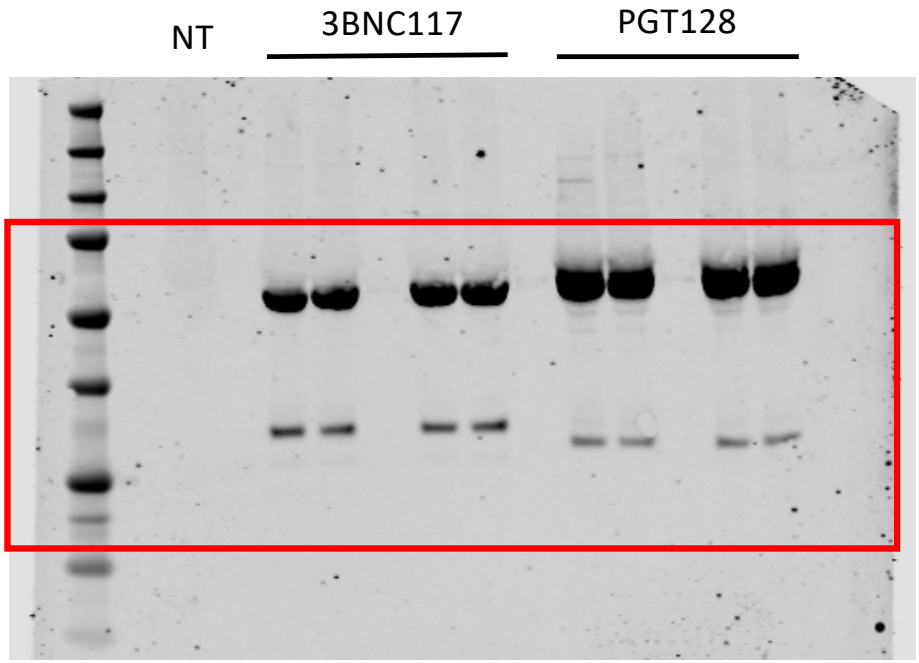


Figure S7: *Similar neutralization titers (IC_{50}) for groups 1 and 2 of NHP dMAb study between in-house and collaborator assays and ADCC controls.* **(A)** To validate neutralization titers observed at Wistar, serum collected from NHPs at Days 0 and 14 were sent to Dr. Montefiori's group at Duke to cross-validate data collected at Wistar. There was no significant difference between the IC_{50} titers determine between the two groups for all nine viruses selected from the global panel. A modified ANOVA with post-hoc Tukey test was performed to determine significant difference in titers between the two labs. $P < 0.05$ was considered significant. **(B)** The RSV-specific monoclonal antibody, Palivizumab, was used as a negative control and a mixture of A32, CH44, 2G12, and 7B2 monoclonal antibodies were used as positive controls for the ADCC assay.

	Heavy Chain				Light Chain			
	GeneBank	Family	CDR3 length	% AA somatic mutations	GeneBank	Family	Kappa or Lambda	Epitope
VRC01	GU980702	IGHV1-2	14	42	GU980703	IGLV1-33	Kappa	
N6	KX595108	IGHV1-2	15	33	KX595112	IGLV1-33	Kappa	
12A21	HE584541	IGHV1-2	15	31	HE58451	IGLV1-39	Kappa	
3BNC117	HE584537	IGHV1-2	12	35	HE584538	IGLV1-33	Kappa	
IOMA	KX610770	IGHV1-2	19	18	KX610771	IGLV2-23	Lambda	
NIH45-46	HE584543	IGHV1-2	18	41	HE584544	IGLV3-11	Kappa	CD4BS
PGT121	JN201894	IGHV4-59	26	24	JN201911	IGLV3-21	Lambda	
PGT128	JN201900	IGHV4-39	21	30	JN201917	IGLV2-8	Lambda	
10-1074	4FQ2_H	IGHV4-59	26	21	4FQ2_L	IGLV3-9	Lambda	
PGT130	JN201901	IGHV4-38	21	22	JN201918	IGLV2-8	Kappa	HMG
PGT145	JN201910	IGHV1-8	33	28	JN201927	IGLV2-28	Kappa	
PGDM 1400	KP006370	IGHV1-8	35	28	KP006383	IGLV2-28	Kappa	
PG9	GU272045	IGHV3-33	30	19	GU272046	IGLV2-14	Lambda	Apex
PGT151	KJ700282	IGHV3-30	28	28	KJ700290	IGLV2-29	Kappa	
35O22	KM001872	IGHV1-18	24	34	KM001880	IGLV2-14	Lambda	Interface
VRC3401	KU711816	IGHV1-2	15	20	KU711823	IGLV1-9	Kappa	Fusion

Table S1: *GenBank accession numbers used for the basis of HIV-1 dMAbs.*



Full unedited gel for Supplemental Figure 1C: Detection antibody used – IRDye-680 anti-human secondary antibody (LI-COR Bioscience)
Red box indicates the area used for the figure.