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Supplementary Materials for

Diversion of HIV-1 vaccine–induced immunity by gp41-microbiota cross-reactive antibodies

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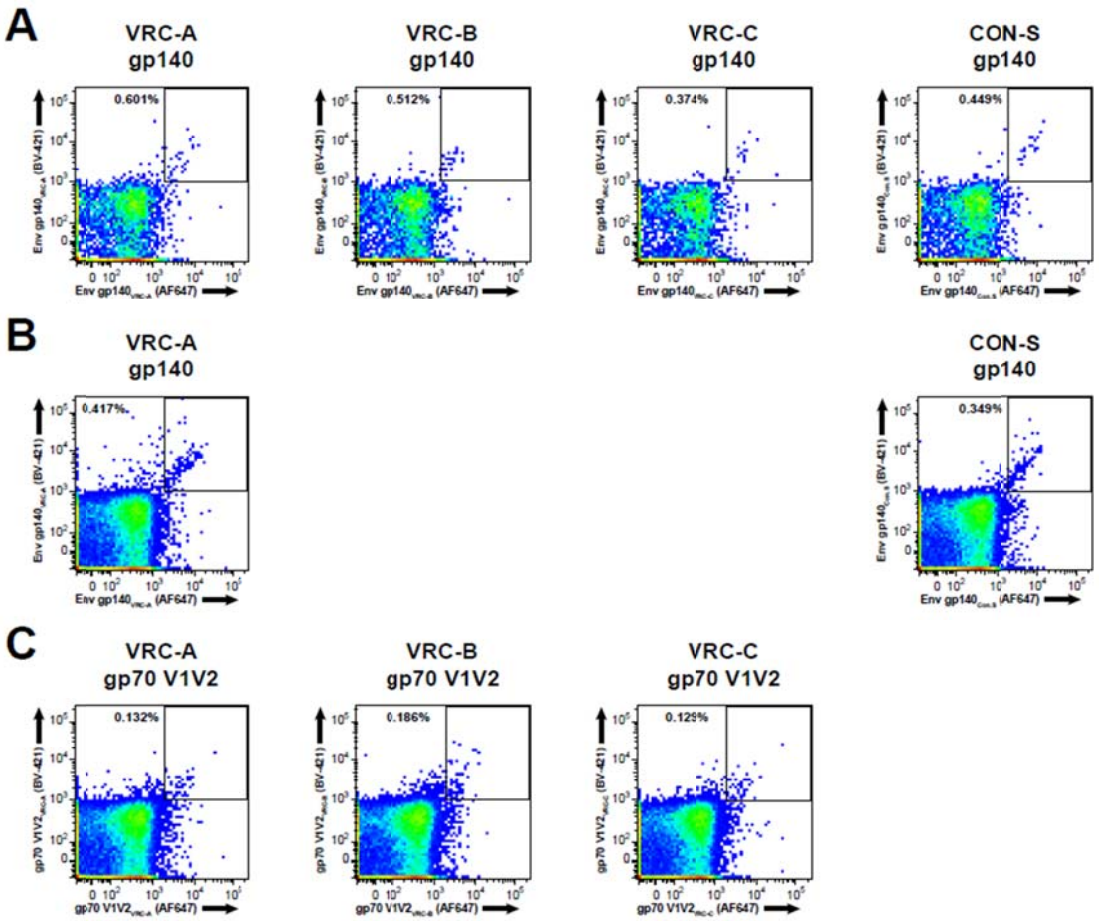
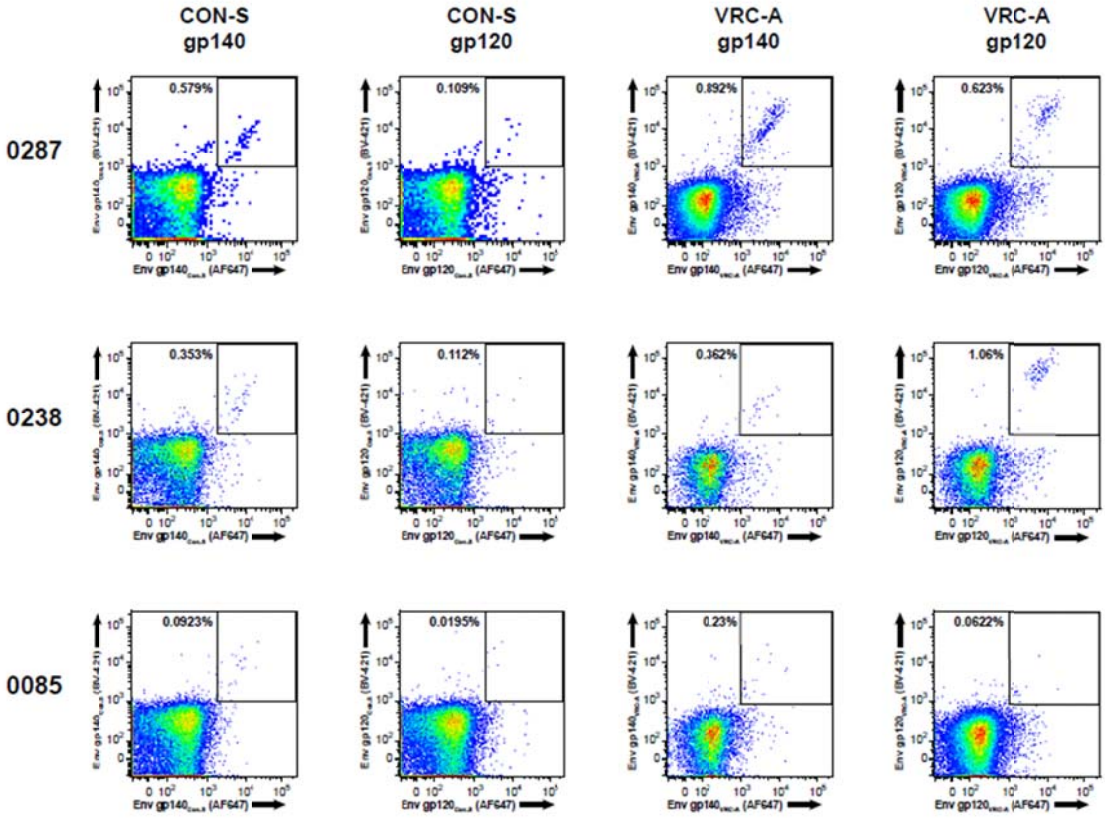


Figure S1. Profile of Env-reactive memory B cells detected by vaccine-strain and group M consensus Envs. (**A** and **B**) Representative sort plots of CON-S and vaccine-strain gp140 Envs were used to isolate Env-reactive memory B cells (square-shaped gates) from HVTN 204 trial participants, 204-013 (A) and 204-096 (B), using peripheral blood mononuclear cells (PBMCs) isolated at 4 weeks post rAd5 boost. (**C**) Representative sort plots of vaccine-strain gp70 V1V2 proteins used to isolate Env-reactive memory B cells (square-shaped gates) from HVTN 204 trial participant, 204-096, using PBMCs isolated at 4 weeks post rAd5 boost. Env-reactive memory B cells bound both AF647 (x-axis) and BV421 (y-axis) labeled Envs, and shown as double positive (DP) reactive cells in the square-shaped gates.



PTID	CON-S gp140		CON-S gp120		VRC-A gp140		VRC-A gp120	
	Mem B Cells	Env-DP Cells	Mem B Cells	Env-DP Cells	Mem B Cells	Env-DP Cells	Mem B Cells	Env-DP Cells
204-449	33806	175	39766	27	39414	309	45816	226
204-242	15803	48	12468	9	6938	23	10622	104
204-466	40604	31	25665	2	21449	44	37963	16
Total	90213	254	77899	38	67801	376	94401	346
Percent Env-DP cells	0.28		0.05		0.55		0.37	
Env-DP cells: gp140 vs. gp120	*P*-value <0.0001				*P*-value <0.0001			

Figure S2

Figure S2. Frequency of Env-reactive memory B cells detected by gp140 and gp120 proteins. (**Top**) Representative sort plots of CON-S and VRC-A gp140 and gp120 staining of Env-reactive memory B cells in 3 HVTN 204 trial participants (204-449, 204-242, 204-466). Env-reactive memory B cells bound both AF647 (x-axis) and BV421 (y-axis) labeled Envs and shown as double positive (DP) reactive cells in the square-shaped gates. (**Bottom**) The table shows the frequencies of Env-reactive memory B cells isolated from 3 vaccinees using CON-S and VRC-A gp140 and gp120 hooks. The number of total memory B cells and Env-reactive memory B cells were obtained from FACS analysis. Abbreviations: mem, memory; DP, double positive. Statistics – Cochran-Mantel-Haenszel Test, SAS. CON-S and VRC-A gp140 and gp120 hooks were validated for isolating gp120-reactive memory B cells from 3 RV305 vaccinees who received a gp120 immunogen (data not shown).

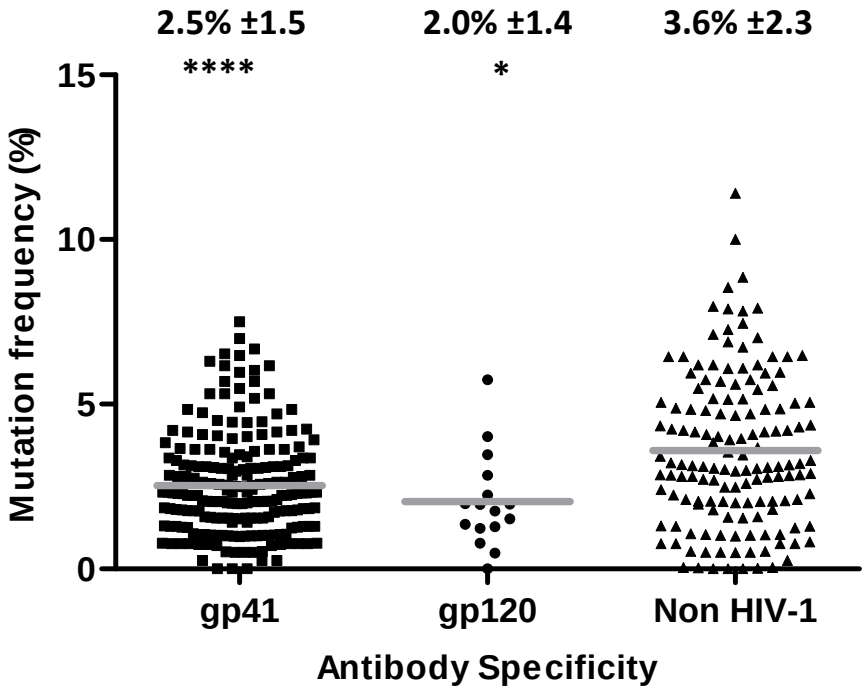
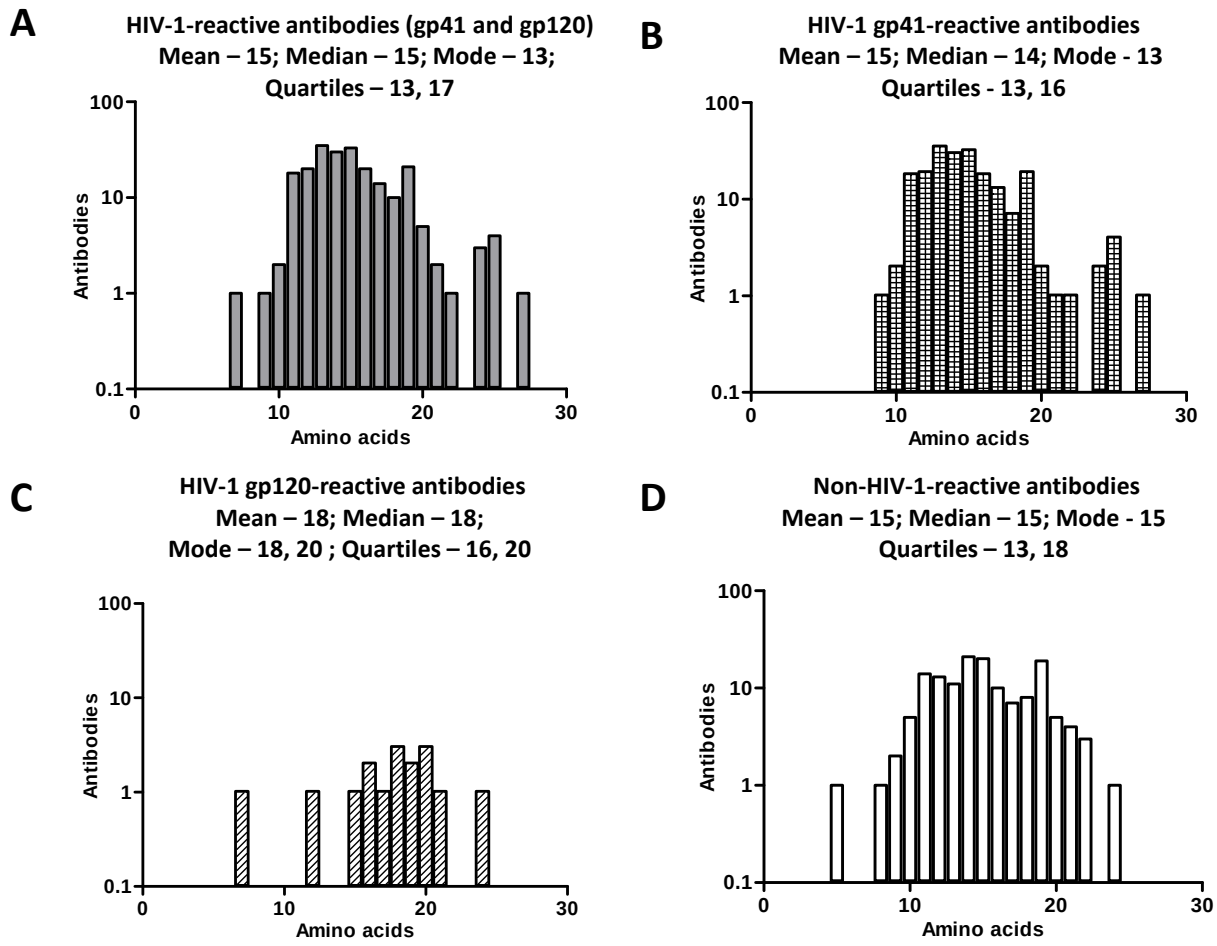


Figure S3. The mutation frequencies of IGHV chain sequences of antibodies (Abs) obtained from 8 HVTN 204 and 082 trial participants, as inferred by SODA (*69*)**.** IGHV sequences with inferred mutation frequencies: 205 gp41-reactive, 16 gp120-reactive, and 145 non HIV-1-reactive Abs. Statistics: ****$P <$ 0.0002 (gp41 vs. Non HIV-1), *P = 0.008 (gp120 vs. Non HIV-1), P = 0.33 (gp41 vs. gp120) (Differences of least square means, SAS). The mean and standard deviation were reported for each set of Abs; mean (gray line on graph).



E. Immunogenetics – Antibodies with long HCDR3 (≥22)

HVTN Trial	Vaccinee ID	Antibody binding	IGHV ID	V_H	D_H	J_H	H Mutation Frequency (%)	HCDR3 length	IGKV/ IGLV ID	IGKV/ IGLV	Ig Isotype and class
204	204-013	HIV-1 gp120	H6467	3-7*01	2-2*02	6*02	0.48	24	K4788	4-1*01	G1
204	204-096		H6566	1-69*04,09	2-2*01,02,03	6*03	2.42	22	L2486	1-5*03	G1
204	204-096		H6609	1-69*04,09	2-2*01,02,03	4*02	5.97	24	L2513	9-49*01	G1
204	204-096		H6608	3-33*05	3-10*01	6*02	2.39	24	K4916	2-28*01	G1
082	082-081	HIV-1	H6757	3-11*01	3-3*01	6*02	0.71	25	K5036	2-28*01	G1
082	082-081	gp41	H6758	3-11*01	3-3*01	6*02	1.42	25	K5037	2-28*01	G3
082	082-081		H6763	3-11*01	3-3*01	6*02	1.42	25	K5040	2-28*01	G3
082	082-081		H6824	3-11*01	3-3*01	6*02	0.71	25	K5086	2-28*01	M
082	082-081		H6717	1-2*02	3-3*01	6*02	6.31	27	K5005	1-39*01	G1
204	204-121		H6083	3-49*03	3-22*01	3*02	2.86	22	K4530	3-15*01	A1
204	204-121	Non	H6084	3-7*01	5-5*01	6*02	3.15	22	K4531	1-16*01	G1
204	204-165	HIV-1	H5819	4-31*03	3-10*01	3*02	0.00	22	K4371	1-5*03	D
204	204-096		H6609	1-69*04,09	2-2*01,02,03	4*02	5.97	24	K4917	2D-29*02	G1

Figure S4

Figure S4. The CDR3 length of IGHV chain sequences for antibodies (Abs) that were isolated from 8 HVTN 204 and 082 trial participants, as inferred by SODA (*69*). Ab IGHV sequences with inferred mutation frequencies: 221 HIV-1 (**A**), 205 gp41 (**B**), 16 gp120 (**C**), and 145 Non HIV-1 (**D**) Abs. The mean, median and quartiles (25th and 75th percentiles) were reported (GraphPad Prism). Statistics: Kolmogorov-Smirnov Test for differences in HCDR3 length distribution for Abs, SAS – gp120-reactive Abs vs. gp41-reactive Abs, P =0.002; gp120-reactive Abs vs. non HIV-1-reactive Abs, P =0.013; gp41-reactive Abs vs. non HIV-1-reactive, P =0.422; HIV-1-reactive Abs vs. non HIV-1-reactive Abs, P =0.875. (**E**) Metadata for Abs with long HCDR3 length ≥22.

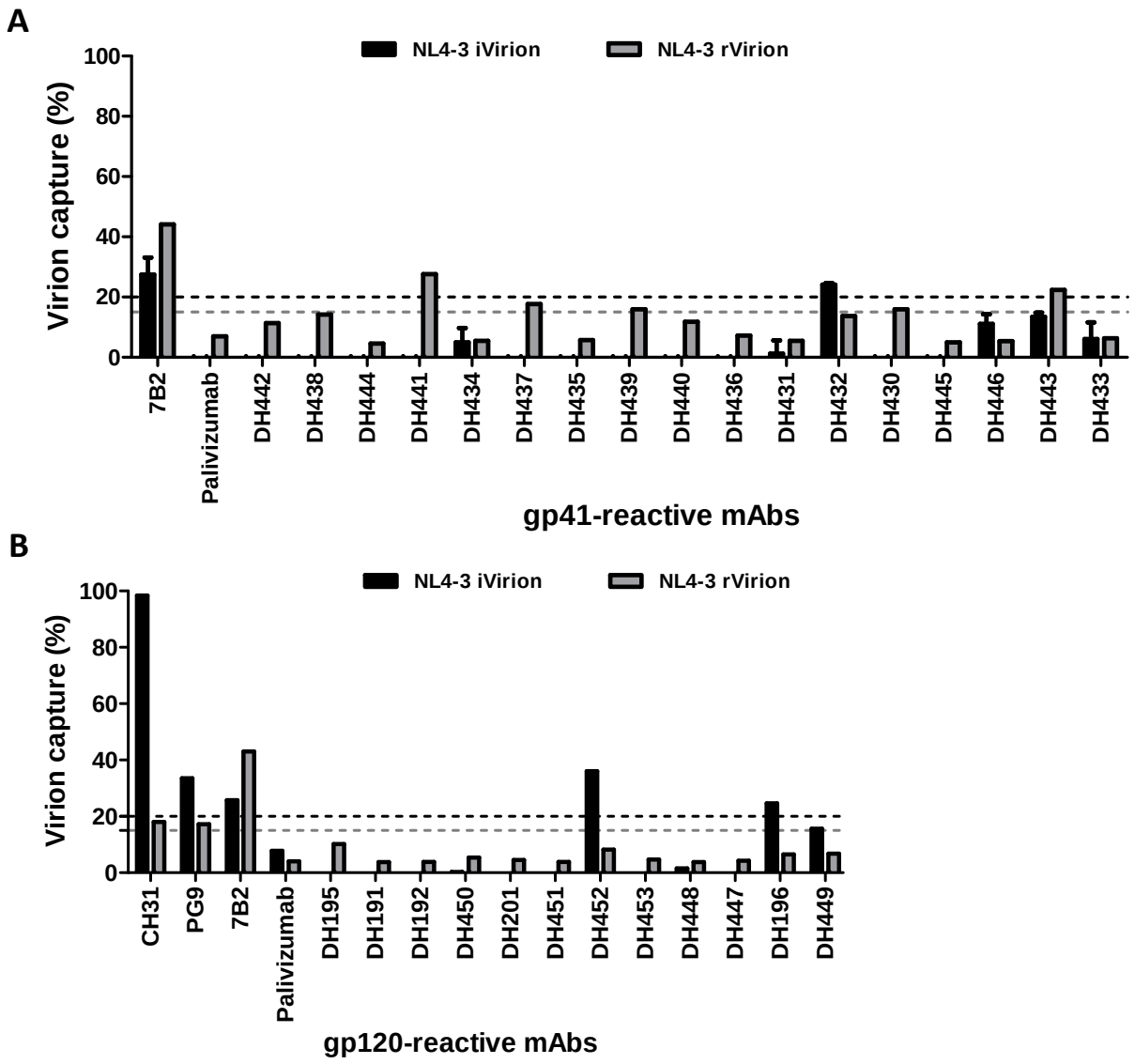


Figure S5. Virion capture profile of vaccine-induced monoclonal antibodies (mAbs). (**A**) Gp41-reactive and (**B**) g120-reactive mAbs capture of infectious-virion (black bars) and viral-particles (gray bars) from a representative assay. Controls: 7B2 (non-neutralizing gp41 Ab), palivizumab (anti-RSV Ab), CH31 and 2G12 (gp120 mAbs). Dash lines set positivity cutoffs – 20% (iVirion capture), 15% (rVirion capture). Error bars, SEM of triplicate virus capture technical replicates. Data shown are representative of two experiments.

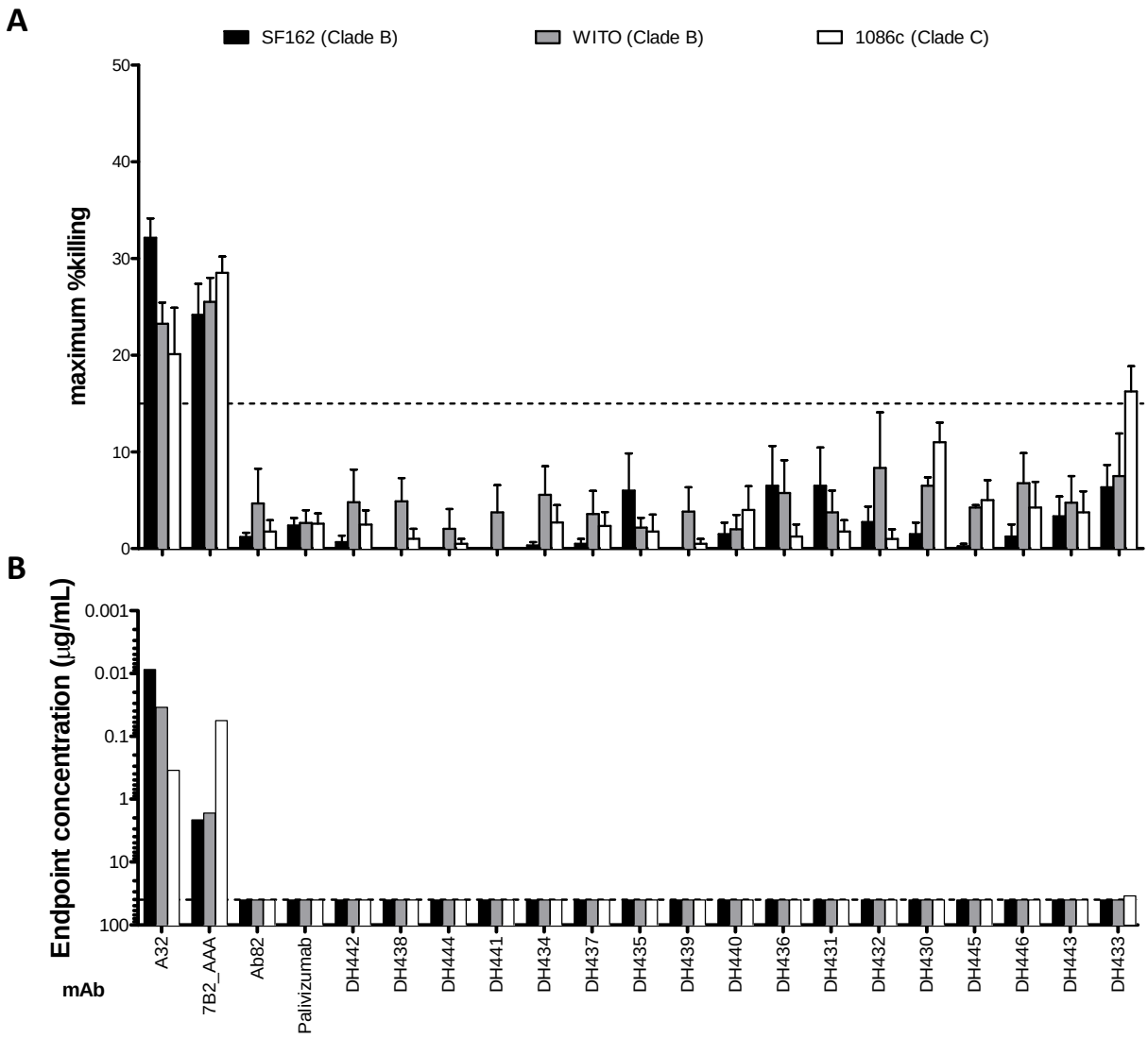


Figure S6. Antibody dependent cellular cytotoxicity (ADCC) profile of vaccine-induced gp41-reactive monoclonal antibodies (mAbs). MAbs were screened for ADCC activity against multiclade HIV-1 IMC-infected NKR targets in at least two experiments; (**A**) maximum Ab-mediated percent killing, and (**B**) Ab endpoint concentration required for ADCC activity. Data shown are averaged from replicate assays, with standard deviation represented by error bars.

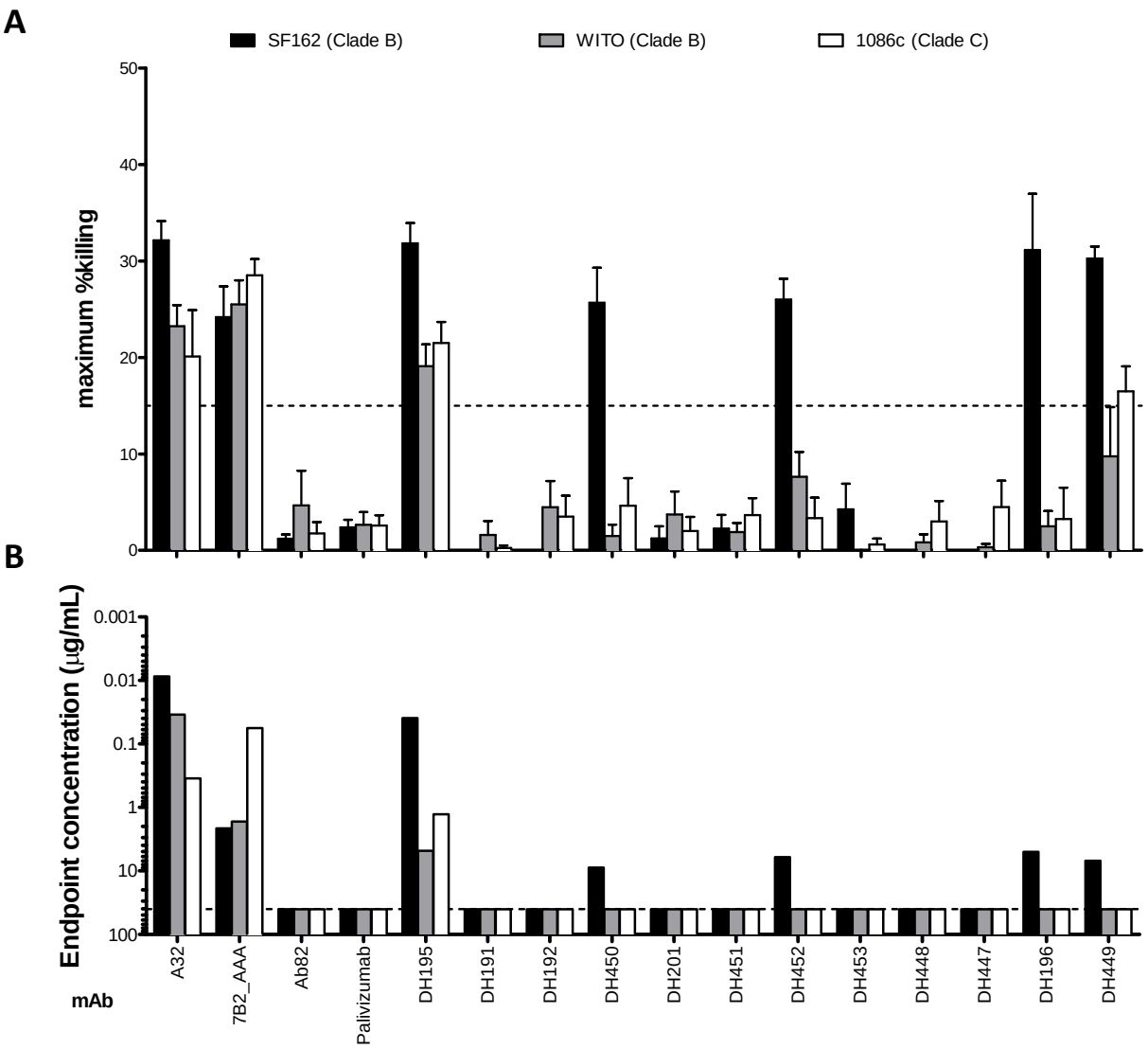


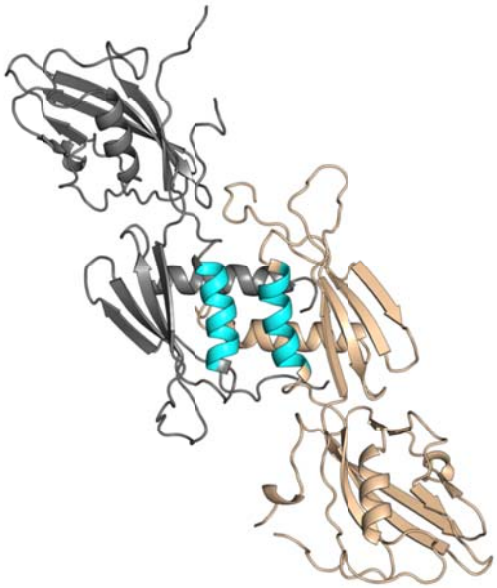
Figure S7. Antibody dependent cellular cytotoxicity (ADCC) profile of vaccine-induced gp120-reactive monoclonal antibodies (mAbs). MAbs were screened for ADCC activity against multiclade HIV-1 IMC-infected NKR targets in at least two experiments; (**A**) maximum Ab-mediated percent killing, and (**B**) Ab endpoint concentration required for ADCC activity. Data shown are averaged from replicate assays, with standard deviation represented by error bars.

A

Protein	Sequence
Gp41 (aa 554-560)	NLLRAIE
Bacterial RNA polymerase (aa 41-47)	NALRAIL

Gp41: VRC-A, B.MN;
Bacterial (*E. coli*) RNA polymerase, alpha-subunit homodimer

B ***Bacterial RNA polymerase homodimer***



C. ***Structural alignment of gp41 and RNA polymerase alpha subunit***

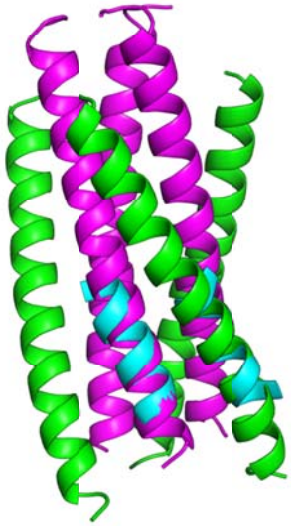
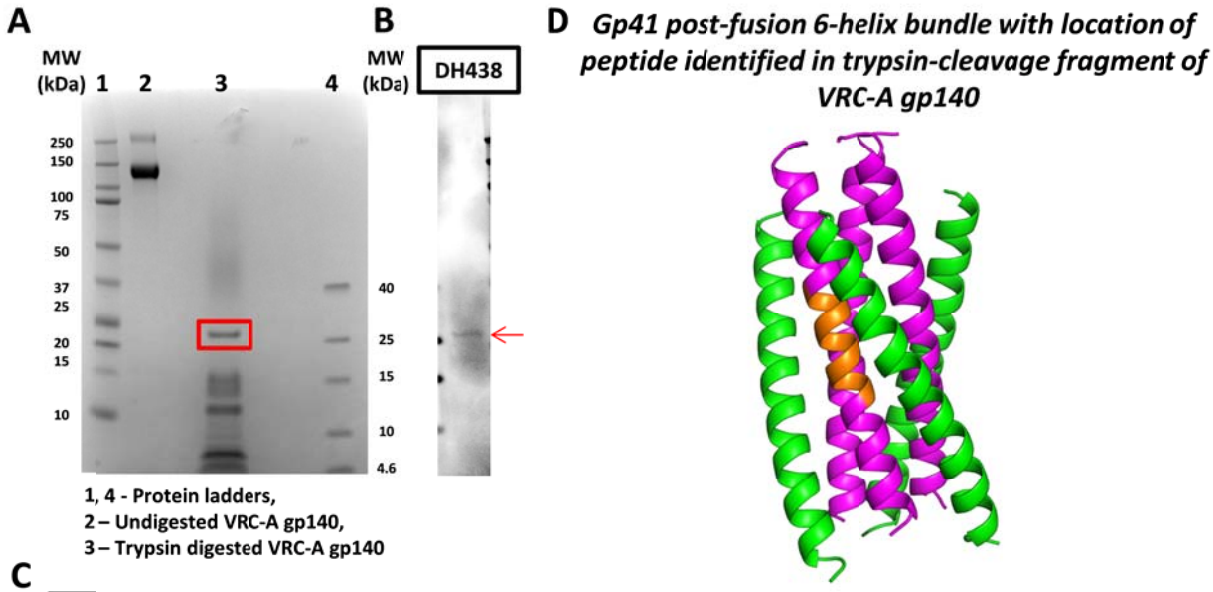


Figure S8

Figure S8. Sequence and structural similarities of HIV-1 Env gp41 and bacterial proteins. (**A**) Sequence similarity (underlined) was observed between a short segment of the HR1 region of gp41 [MN, VRC-A] and the H1 helix of the alpha subunit of bacterial (*E. coli*) RNA polymerase (cyan). (**B**) The orientation of the H1 helices (cyan) at the homodimer interface of *E. coli* RNA Polymerase (PDB: 1BDF). The orientation of the two H1 helices at the homodimer interface in bacterial RNA polymerase is similar to helical regions at the HR-1 (magenta) and HR-2 (green) interface in the gp41 six-helix bundle (PDB: 1AIK). (**C**) Structural alignment by superposition of the RNA polymerase H1 helices onto the gp41 six-helix bundle, which revealed good structural similarity (1.30Å backbone atom RMSD). Accession numbers for protein sequences: Gp41 - VRC-A [AY669706], MN (commercially obtained from ImmunoDX); bacterial RNA polymerase alpha-subunit homodimer [WP_001162094].



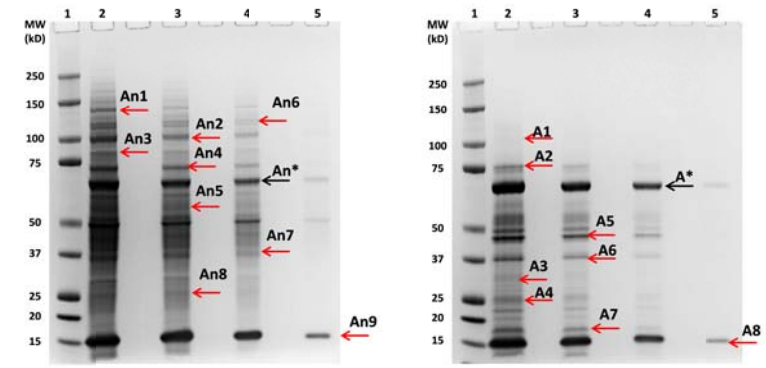
C

Protein	Overlapping sequence	Protein domain
Bacterial RNA polymerase (aa 41-47)	NALRAIL	α-subunit (helix)
Peptide from trypsinized gp140 (aa 554-567)	NLLRAIEAQQHLLQ	HR-1 (helix)

Bacterial (*E. coli*) RNA polymerase, alpha (α)-subunit;
Peptide (orange) from trypsinized VRC-A gp140

Figure S9. Proteomic analysis of VRC-A gp140 to identify gp41-reactive antibody (Ab) binding site. (**A**) Coomassie-stained SDS-PAGE gels showing trypsin-digested and undigested products of VRC-A gp140. An IM cross-reactive gp41-reactive Ab DH438 demonstrated binding to protein bands in western blot analysis that corresponded to a ~25 kDa protein band as indicated by the red arrow (**B**). The gp140 fragment band was cut from the SDS-PAGE gel (red boxed area) and further subjected to liquid chromatography-mass spectrometry (LC-MS) for analysis of sequences. (**C** and **D**) LC-MS sequence results of a peptide found in the 25 kDa protein band on the SDS-PAGE gel. Peptide sequence aa 558-567 AIEAQQHLLQ (shown in orange) identified by LC-MS was in the Env gp41 heptad repeat 1 (HR-1) region with the LRAI gp41 sequence of the HR-1 that is the putative cross-reactive region with bacterial RNA polymerase. Similar residues in the protein sequences in this region are underlined. Accession numbers for proteins: AY669706 (VRC-A), WP_001162094 (bacterial RNA polymerase).

A



(1) Protein ladder ; Anaerobe (An) intestinal microbiota, µg; (2) 100, (3) 50, (4) 25, (5) 5

(1) Protein ladder ; Aerobe (A) intestinal microbiota, µg; (2) 100, (3) 50, (4) 25, (5) 5

B

Candidate intestinal microbiota (IM) cross-reactive proteins	Protein Size (kDa)	Band; SDS-PAGE
Molecular chaperone DnaK - Heat shock protein 70	69.1	A2
Chain A, D-Ribose protein (E. coli)	28	A3
A-type flagellin (P. aeriginosa)	40	A3
Lysine, arginine, ornithine-binding periplasmic protein (E. coli)	28	A4
Cysteine transporter subunit (E. coli)	29	A4
Porin (P. aeruginosa PA01)	48	A5
Phosphate ABC transporter peripiasmic substrate-binding protein PstS (E. coli)	37	A6
Outer membrane protein A (E. coli)	37	A6
DNA starvation/stationary phase protection protein Dps (E. coli)	19	A7
30S ribosomal protein S7 (Shigella flexneri)	40	A7
Chain A, outer membrane protein Ompyx (E. coli)	16	A7
Pyruvate-flavodoxin oxidoreductase (M. eisdenii)	129	AN1
Cell surface protein (B. vulgatus)	100	AN2
Pyruvate formate lyase (E. coli)	80	AN3
Polyribonecleotide nucleotidyltransferase (P. asaccharolytica)	80	AN3
Dipeptidase PepV (C. perfringens)	55	AN5
D-lactate /malate dehydrogenase	37	AN7
Chaperonin GroEL (E. coli) (synagis reactive)	70	*A
Pyruvate carboxylase (P. asacchaorylytica) (synagis reactive)	70	*AN

C

Protein	Overlapping sequence	Protein domain
MN or VRC-A gp41 (aa 554-560)	NLLRAIE	HR-1 (helix)
Pyruvate-flavodoxin oxidoreductase (aa 499-505)	DLLRGIK	*ns*

Gp41: VRC-A, B.MN;
Pyruvate-flavodoxin oxidoreductase (M.eisdenii), *ns* – no structure available

Figure S10. Candidate intestinal microbiota (IM) proteins that cross-react with gp41-reactive Abs. (**A**) Coomassie-stained SDS-PAGE gel analysis of bacterial proteins in whole cell lysates (WCLs) of anaerobe and aerobe IM. Red arrows indicate protein bands which in western blot analysis (not shown) were blotted by at least one of 3 representative gp41-reactive mAbs tested; these bands were cut from gels for liquid chromatography-mass spectrometry (LC-MS) analysis. Black arrows indicate the protein band cross-reactive with negative control RSV-Ab, palivizumab. (**B**) Candidate IM proteins identified by tryptic fragments in LC-MS had >95% probability based on peptide-fragment LC-MS analysis. The protein size and marked band numbering on SDS-PAGE analysis of Anaerobe (An) or Aerobe (A) bacterial lysates are listed. (**C**) Sequence similarity of HIV-1 MN or VRC-A gp41 and pyruvate-flavodoxin oxidoreductase. The LLR sequence was found in the HR-1 domain of gp41 structure (*26*). Pyruvate-flavodoxin oxidoreductase does not have a published crystal structure for this type of analysis. Accession numbers for proteins: AY669706 (VRC-A), WP_022497386 (pyruvate-flavodoxin oxidoreductase).

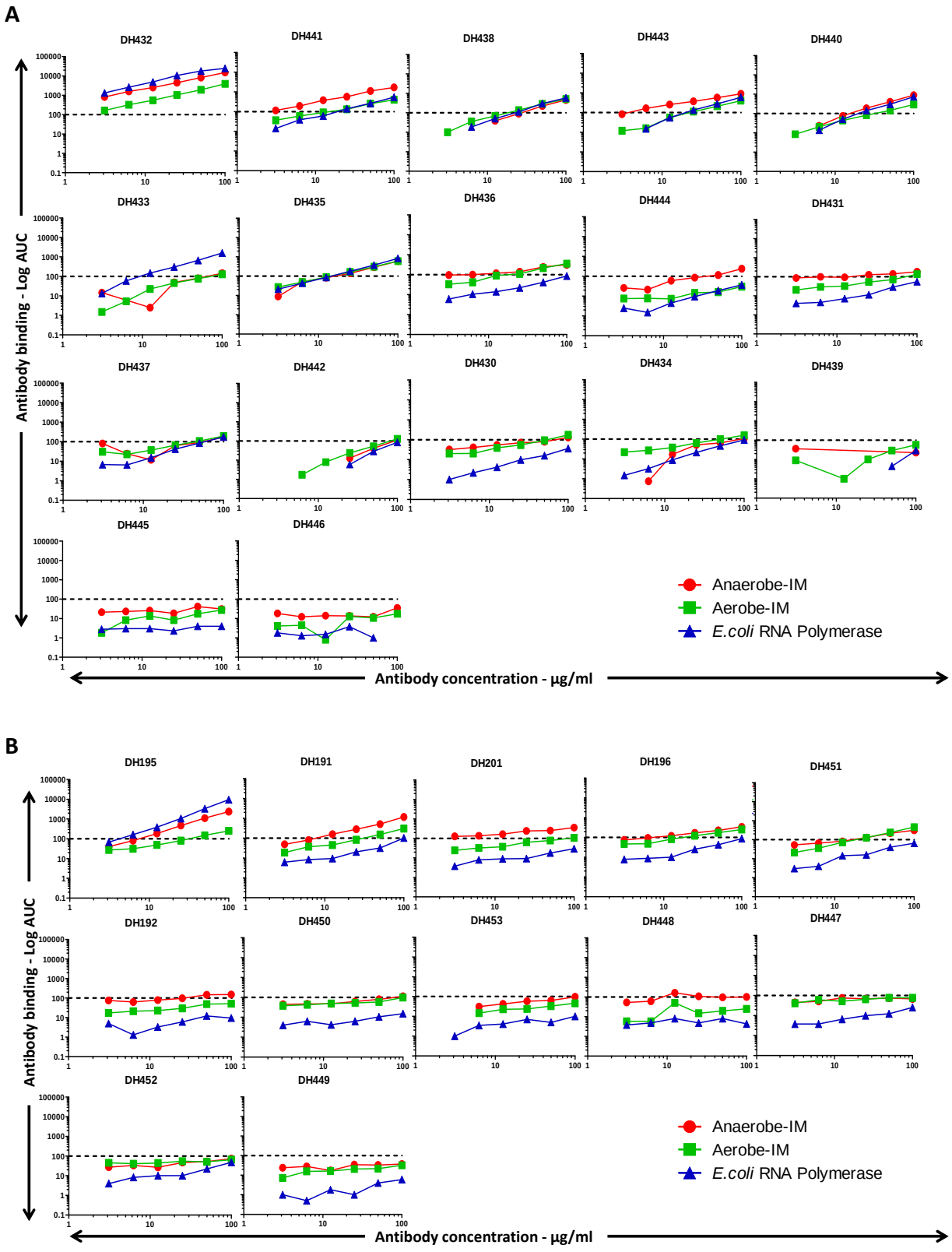

A
DH432
DH441
DH438
DH443
DH440
DH433
DH435
DH436
DH444
DH431
DH437
DH442
DH430
DH434
DH439
DH445
DH446
Antibody binding - Log AUC
Anaerobe-IM
Aerobe-IM
E.coli RNA Polymerase
Antibody concentration - µg/ml
B
DH195
DH191
DH201
DH196
DH451
DH192
DH450
DH453
DH448
DH447
DH452
DH449
Antibody binding - Log AUC
Anaerobe-IM
Aerobe-IM
E.coli RNA Polymerase
Antibody concentration - µg/ml

Figure S11

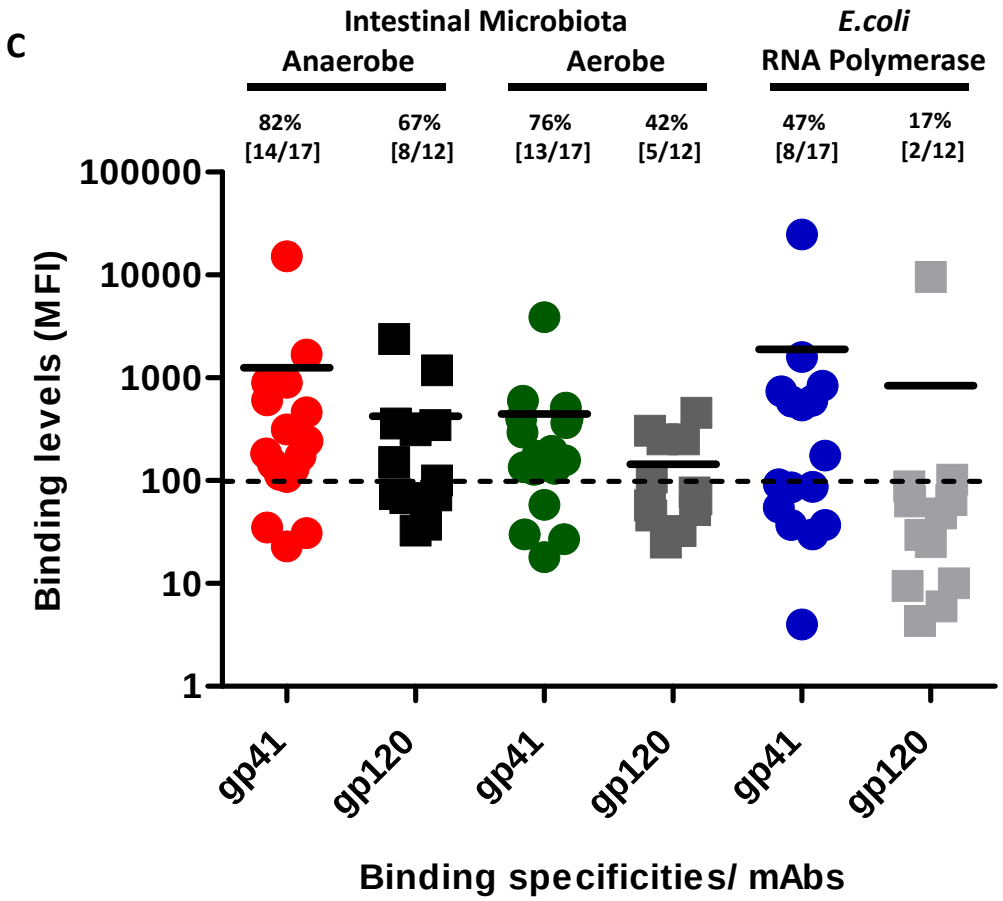


Figure S11. Polyreactivity of vaccine-induced monoclonal antibodies (mAbs). (**A**) 17 gp41-reactive mAbs representative of 17 different clonal lineages of 45 total Abs, and (**B**) 12 gp120-reactive mAbs representative of different epitopes targeted by HIV-1 were tested for binding anaerobe and aerobe whole cell lysates of intestinal microbiota (IM) (red and green symbols) and *E. coli* RNA polymerase (blue symbol). MAbs were screened at 2-fold dilutions from 6.25-100 µg/ml. (**C**) Binding is reported as mean fluorescence intensity (less background) for Abs screened at 100µg/ml from a single BAMA experiment. Ab-IM reactivity in BAMA was also observed in western blot and/or SPR (not shown). The positivity cutoff was set at 100MFI (black dotted line) (*2*).

```
DH477 CAGGTGCAGC TGGTGCAGTC TGGGGCTGAG GTGAAGAAGC CTGGGTCCTC GGTGAAGGTC TCCTGCAAGG CTTCT
DH476 ---------- ---------- ---------- ---------- ---------- ---------- ---------- -C--- 75

DH477 GGAGG CACCTTCAGC AGCTATGCTA TCAGCTGGGT GCGACAGGCC CCTGGACAAG GGCTTGAGTG GATGGGAGGG
DH476 ----- ----A----- ------A--T ---A------ ---------- ---------- ---------- -------A-- 150

DH477 ATCATCCCTA TCTTTGGTAC AGCAAACTAC GCACAGAAGT TCCAGGGCAG AGTCACGATT ACCGCGGACA AATCC
DH476 ---------- -TC--C---T ----GG---- ---------- ---------- -------G-- ---------- ----- 225

DH477 ACGAG CACAGCCTAC ATGGAGCTGA GCAGCCTGAG ATCTGAGGAC ACGGCCGTGT ATTACTGTGC GAGAGCGGAC
DH476 ----A --------T- ---------- C--A------ ------A--- ---------- -C-------- --C--G---T 300

DH477 GATATTTGGC CTCTTGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCAG
DH476 ---T-G---- ---------- ---------- ---------- ---T------ ----- 355
```

Figure S12. V_HDJ_H nucleotide sequence alignment of clonally-related antibodies DH477 and DH476. Antibody IDs - DH477 (pre-vaccine), DH476 (post-vaccine). Dash lines indicates site of shared nucleotides. Nucleotide sequences were numbered according to the sequence alignment publishing tool (HIV Los Alamos Database).

Table S1. Demographics of HVTN 204 and 082 trial participants. Patients were enrolled in the phase II HVTN 204 and phase Ib HVTN 082 HIV-1 vaccine trials, which studied the VRC DNA prime, rAd5 boost vaccine regimen.

Vaccinee ID	HVTN Trial	Gender	Age	Ad5 Sero-positivity	Location
204-096	204	Female	20	Negative**	USA
204-013	204	Female	22	Negative**	USA
204-121	204	Female	27	Negative**	USA
204-165	204	Female	24	Positive**	USA
082-006	082	Female	37	Negative*	USA
082-081	082	Female	37	Negative*	USA
082-001	082	Female	39	Negative*	USA
082-003	082	Female	18	Negative*	USA

Ad5 sero-positivity was measured at baseline *(prevaccine) or **(month 6).

Table S2. Binding specificities of HIV-1-reactive antibodies isolated from HVTN 204 and 082 trial participants. HIV-1-reactive antibodies (Abs) were isolated from single sorted memory B cells using fluorophore-labeled vaccine-matched and group M consensus HIV-1 Env proteins. Abs were screened against a panel of multiclade gp120 and clade B MN gp41 recombinant proteins; 16/16 (100%) gp120-reactive Abs and 195/205 (95%) gp41-reactive Abs were reactive with recombinant vaccine-strain Env proteins.

Vaccinee ID	HVTN trial	HIV-1-reactive antibodies	Gp120-reactive antibodies	Gp41-reactive antibodies
204-096	204	44	3	41
204-013	204	26	3	23
204-121	204	5	1	4
204-165	204	5	1	4
082-006	082	56	5	51
082-081	082	39	0	39
082-001	082	28	2	26
082-003	082	18	1	17
Number of Abs		221	16 (7%)	205 (93%)

Table S3. Unique HIV-1-reactive antibodies isolated from HVTN 204 and 082 trial participants. All HIV-1-reactive antibodies (Abs) isolated. Clones that contain ≥2 Abs with the same inferred V_H, J_H, and HCDR3 length, or a single Ab with no additional clone member were counted as one unique Ab type.

Vaccinee ID	HVTN Trial	Total HIV-1-reactive antibodies	Unique antibodies		
			HIV-1-reactive antibodies	Gp120-reactive antibodies	Gp41-reactive antibodies
204-096	204	44	21	3	18
204-013	204	26	13	3	10
204-121	204	5	3	1	2
204-165	204	5	5	1	4
082-006	082	56	27	5	22
082-081	082	39	23	0	23
082-001	082	28	21	2	19
082-003	082	18	18	1	17
Number of antibodies		221	131 (59%)*	16 (12%)†	115 (88%)‡

*Of the total Abs isolated, 59% were unique. Of the unique HIV-1 Abs in the fourth column, 12% were gp120-reactive† and 88% were gp41-reactive‡.

Table S4. Number of clones of HIV-1-reactive antibodies isolated from HVTN 204 and 082 trial participants. Total antibodies are all HIV-1-reactive antibodies (Abs) isolated. Unique Abs classified as clones, where each clone of ≥2 Abs, or a single Ab with no clonal lineage member, is counted as one Ab clone. Abs that used similar heavy chain gene, but separate kappa and lambda chains were counted as separate clones.

HIV-1-reactive antibodies	Total antibodies	Unique antibodies	Clones; ≥2 antibodies	Clones; single antibody
gp41	205/221 (93%)	115/131 (88%)	34	81
gp120	16/221 (7%)	16/131 (12%)	0	16
Number of Abs	221	131	34/131 (26%)	97/131 (74%)

Table S5. Clones of HIV-1 gp41-reactive antibodies from HVTN 204 and 082 trial participants.
Antibodies (Abs) were classified as being clonally related if they were inferred to use the same V_H and J_H gene segments, and have the same HCDR3 length. Heavy chain genes were statistically inferred for 205 gp41 Abs listed in table S5.

Vaccinee ID	Clone ID	Clone members	V_H	J_H	HCDR3 Length	Ig Isotype and class
082-081	TrH006717	1	1-2*02	6*02	27	G1
082-081	TrH006761	1	1-2*02	4*02	18	G1
082-003	TrH006968	1	1-2*02	4*02	9	G1
082-003	TrH006973	1	1-3*01	4*02	15	G1
204-096	TrH006532	2	1-3*01	5*02	12	G1
082-081	TrH006821	1	1-46*01	5*02	11	G1
082-003	TrH007152	1	1-46*01	5*02	11	G1
082-006	TrH007526	1	1-46*02	6*02	17	G1
082-081	TrH006827	1	1-69*01^F	4*02	18	M
082-001	TrH006882	1	1-69*01^F	4*02	16	G1
082-003	TrH007147	1	1-69*01^F	5*01	11	G3
204-013	TrH006483	1	1-69*01^F	4*02	15	G1
204-165	TrH005804	1	1-69*01^F	4*02	14	G1
082-081	TrH006838	1	1-69*01,06^F	6*03	17	G1
204-121	TrH005653	3	1-69*02^L	5*02	11	G1
082-081	TrH006766	4	1-69*02^L	6*02	12	G1
082-081	TrH006767	2	1-69*02^L	3*02	12	G1
082-081	TrH006772	1	1-69*02^L	6*02	13	G1
082-081	TrH006759	2	1-69*02^L	5*02	15	G1
082-081	TrH006831	1	1-69*02^L	6*02	17	G1
082-081	TrH006819	1	1-69*02^L	4*02	13	G3
082-001	TrH006881	1	1-69*02^L	6*03	14	G1
082-003	TrH006970	1	1-69*02^L	2*01	13	G1
082-003	TrH007149	1	1-69*02^L	4*02	13	G1
082-003	TrH006974	1	1-69*02^L	3*02	18	G1
082-003	TrH006975	1	1-69*02^L	4*02	11	G1
204-013	TrH006470	4	1-69*02^L	3*02	15	G3
204-013	TrH006464	5	1-69*02^L	6*02	19	G3
204-013	TrH006480	1	1-69*02^L	6*02	14	G1
204-013	TrH006466	1	1-69*02^L	3*01	15	G3
082-081	TrH006773	1	1-69*04,09^L	3*02	12	G1
082-001	TrH006876	2	1-69*04,09^L	5*02	14	G1
082-001	TrH006956	2	1-69*04,09^L	4*02	13	G1
082-001	TrH006960	2	1-69*04,09^L	6*03	11	G1
082-001	TrH006957	1	1-69*04,09^L	5*02	19	G1
082-001	TrH006880	1	1-69*04,09^L	5*01	19	G1
082-001	TrH006883	1	1-69*04,09^L	6*03	12	G3
204-096	TrH006521	4	1-69*04,09^L	4*02	16	G1
204-096	TrH006517	8	1-69*04,09^L	6*03	15	G3
204-096	TrH006520	7	1-69*04,09^L	6*03	12	G1
204-096	TrH006531†	3	1-69*04,09^L	4*02	15	G1
204-096	TrH006628†	2	1-69*04,09^L	4*02	15	G1

Table S5 contd.

204-096	TrH006609	1	1-69*04,09^{L}	4*02	24	G1
204-096	TrH006566	1	1-69*04,09^{L}	6*03	22	G1
204-096	TrH006540	1	1-69*04,09^{L}	4*02	17	G1
082-006	TrH007024†	7	1-69*04,09^{L}	3*01,02	13	G1
082-006	TrH007027†	1	1-69*04,09^{L}	3*01,02	13	G1
082-006	TrH007090†	1	1-69*04,09^{L}	4*02	13	G1
082-006	TrH007102†	1	1-69*04,09^{L}	4*02	13	G1
082-006	TrH007030	6	1-69*04,09^{L}	3*02	13	G1
082-006	TrH007026	9	1-69*04,09^{L}	6*02	14	G3
082-006	TrH007029	3	1-69*04,09^{L}	4*02	14	G1
082-006	TrH007025	1	1-69*04,09^{L}	4*02	12	G1
082-006	TrH007140	1	1-69*04,09^{L}	4*02	15	G1
082-081	TrH006762	7	1-69*04,09,10^{L}	6*02	16	G3
204-096	TrH006587	1	1-69*04,09,10^{L}	3*02	15	G1
082-006	TrH007032	4	1-69*04,09,10^{L}	3*02	13	G1
082-001	TrH006891	1	1-69*06^{F}	6*03	16	G3
082-001	TrH006886	1	1-69*04,09,10^{L}	6*03	14	G1
082-001	TrH006896	1	1-69*06^{F}	3*02	18	G1
082-003	TrH007148	1	1-69*06^{L}	4*02	11	G1
204-013	TrH006477	1	1-69*06^{F}	4*02	19	G1
082-081	TrH006829	1	1-69*08^{L}	6*02	13	G1
082-081	TrH006833	1	1-69*08^{L}	6*02	19	G1
204-013	TrH006469	3	1-69*08^{L}	6*02	13	G1
082-081	TrH006716	1	1-8*01	4*01,02,03	11	A1
082-001	TrH006885	1	1-8*01	4*02	14	G1
082-081	TrH006715	1	2-5*01	5*02	14	M
082-081	TrH006757	4	3-11*01	6*02	25	G1
082-001	TrH006889	3	3-23*01	4*02	17	G1
082-003	TrH006966	1	3-23*01	3*02	18	G1
082-003	TrH006967	1	3-23*01	4*02	17	G1
204-096	TrH006530	1	3-23*01	4*02	16	G1
204-096	TrH006522	1	3-23*01	6*03	21	G1
082-006	TrH007096	1	3-23*01	4*02	17	G1
082-006	TrH007132	1	3-23*01	5*02	18	G1
082-006	TrH007139	1	3-23*01	4*02	14	G1
204-096	TrH006563	2	3-23*03	4*02,03	13	G3
082-006	TrH007035	1	3-30*04	6*02	20	G1
082-001	TrH006895	1	3-30*18	6*03	10	G1
082-003	TrH006965	1	3-30*18	4*02	15	G3
082-003	TrH007151	1	3-30*18	6*02	14	G1
204-013	TrH006476	2	3-30*18	6*02	17	G1
082-081	TrH006826	2	3-30-3*01	6*03	15	A2
204-013	TrH006468	1	3-30-3*01	5*02	11	G1
204-165	TrH005939	1	3-30-3*01	4*02	14	G1
204-096	TrH006519	2	3-33*05	4*02	14	G1
204-096	TrH006608	1	3-33*05	6*02	24	G1
204-096	TrH006600	1	3-48*02	4*02	14	G1

Table S5 contd.

082-001	TrH006878	1	3-48*03	3*02	17	G1
204-096	TrH006633	1	3-48*03	6*03	13	G1
082-001	TrH006892	1	3-49*03	6*03	17	M
082-006	TrH007036	5	3-64*01	6*02	19	G1
082-001	TrH006887	3	3-74*01,02	5*02	19	G1
082-001	TrH006893	1	3-9*01	3*02	15	G1
082-001	TrH007285	1	3-9*01	3*01	15	G1
082-003	TrH007146	1	3-9*01	4*02	15	G1
082-006	TrH007089	1	3-9*01	1*01	16	G1
082-003	TrH006971	1	4-34*01,02	4*02	12	G1
082-006	TrH007092	2	4-34*01,02	3*02	11	G1
204-121	TrH006090	1	4-4*02	5*02	19	A1
204-013	TrH006465	4	4-4*07	3*02	14	G3
204-096	TrH006569	2	4-59*01	5*02	11	G3
082-006	TrH007023	1	4-59*01	6*02	18	G1
082-006	TrH007137	1	4-59*01	5*02	11	G1
082-081	TrH006754†	2	4-61*01,08	4*02	16	G1
082-081	TrH006756†	1	4-61*01,08	4*02	16	G1
082-081	TrH006818	1	4-61*02	5*02	13	G1
082-003	TrH007145	1	4-61*02	5*02	20	G1
082-081	TrH006768	1	5-51*01	4*02	13	G1
204-165	TrH005899	1	5-51*01	2*01	19	G1
082-006	TrH007099	1	5-51*01	6*02	15	G1
082-006	TrH007315	1	6-1*01,02	3*01	10	G1
082-003	TrH006969	1	7-4-1*02	6*02	15	G1
204-165	TrH005898	1	7-4-1*02	5*02	11	G1

†Abs with similar heavy chain genes, but used kappa and lambda chains were counted as separate clones. $^{F, L}$IGHV1-69-using Abs that encoded phenylalanine (F) or leucine (L) residues at position 54 in the HCDR2 region.

Table S6. Clones of HIV-1 gp120-reactive antibodies Antibodies (Abs) were defined as being clonally related if they were inferred to use the same V_H and J_H gene segments, and have the same HCDR3 length. Heavy chain genes were statistically inferred for 16 gp120-reactive Abs listed in table S6.

Vaccinee ID	Clone ID	Clone members	V_H	J_H	HCDR3 Length	Ig Isotype and class
204-121	TrH005656	1	3-30*18	4*02	18	A1
082-001	TrH006964	1	3-48*03	4*02	7	G1
082-001	TrH006894	1	5-51*01	5*02	20	G1
082-003	TrH006976	1	3-66*02	3*01	16	G1
204-096	TrH006614	1	3-33*05	1*01	12	G3
204-096	TrH006625	1	4-31*03	3*02	20	G1
204-096	TrH006631	1	3-53*01	3*02	21	G1
204-013	TrH006484	1	3-53*01	3*01	18	G1
204-013	TrH006481	1	3-53*01,02	3*02	20	G1
204-013	TrH006467	1	3-7*01	6*02	24	G1
204-165	TrH005803	1	1-3*01	4*02	17	G3
082-006	TrH007037	1	3-30*04	3*02	19	G1
082-006	TrH007039	1	3-66*01	3*02	18	G1
082-006	TrH007309	1	3-23*01	4*02	16	G1
082-006	TrH007308	1	5-51*01	2*01	19	G1
082-006	TrH007034	1	4-31*03	3*02	15	G1

Table S7. IGHV, IGKV, and IGLV gene segments for antibodies isolated from HVTN 204 and 082 trial participants. Heavy and light chain variable gene segments used by antibodies (Abs) that were isolated from 8 HVTN 204 and 082 trial participants. Heavy and light chain genes were statistically inferred for 205 gp41-reactive, 16 gp120-reactive, and 145 non HIV-1-reactive Abs.

IGHV gene segment	Abs			IGKV gene segment	Abs			IGLV gene segment	Abs		
	gp41-reactive	gp120-reactive	Non HIV-1-reactive		gp41-reactive	gp120-reactive	Non HIV-1-reactive		gp41-reactive	gp120-reactive	Non HIV-1-reactive
1-2	3 (1.5%)	0 (0%)	8 (5.5%)	1-5	17 (13%)	0 (0%)	11 (12%)	1-5	1 (1%)	0 (0%)	0 (0%)
1-3	3 (1.5%)	1 (6%)	2 (1%)	1-6	1 (1%)	0 (0%)	2 (2%)	1-40	4 (5%)	0 (0%)	0 (0%)
1-24	0 (0%)	0 (0%)	2 (1%)	1-9	1 (1%)	0 (0%)	2 (2%)	1-44	6 (8%)	0 (0%)	6 (11%)
1-46	3 (1.5%)	0 (0%)	3 (2%)	1-12	0 (0%)	0 (0%)	2 (2%)	1-47	5 (7%)	1 (14%)	2 (4%)
1-69	125 (61%)	0 (0%)	18 (12%)	1-16	0 (0%)	0 (0%)	1 (1%)	1-51	8 (10%)	0 (0%)	5 (9%)
1-8	2 (1%)	0 (0%)	4 (3%)	1-17	0 (0%)	0 (0%)	2 (2%)	10-54	0 (0%)	0 (0%)	2 (4%)
2-5	1 (0.5%)	0 (0%)	2 (1%)	1-27	1 (1%)	0 (0%)	1 (1%)	2-8	0 (0%)	1 (14%)	0 (0%)
2-70	0 (0%)	0 (0%)	4 (3%)	1-33	0 (0%)	0 (0%)	1 (1%)	2-11	6 (8%)	0 (0%)	3 (5.5%)
3-7	0 (0%)	1 (6%)	4 (3%)	1-39	17 (12%)	5 (56%)	9 (10%)	2-14	21 (29%)	0 (0%)	4 (7%)
3-9	4 (2%)	0 (0%)	7 (5%)	1-40	0 (0%)	0 (0%)	0 (0%)	2-18	0 (0%)	0 (0%)	1 (2%)
3-11	4 (2%)	0 (0%)	2 (1%)	1-44	0 (0%)	0 (0%)	0 (0%)	2-23	2 (3%)	0 (0%)	2 (4%)
3-15	0 (0%)	0 (0%)	6 (4%)	1-47	2 (1.5%)	0 (0%)	0 (0%)	3-1	5 (7%)	1 (14%)	3 (5.5%)
3-23	12 (6%)	1 (6%)	12 (8%)	1-D	0 (0%)	0 (0%)	2 (2%)	3-9	0 (0%)	0 (0%)	1 (2%)
3-30	10 (5%)	2 (12.5%)	17 (12%)	2-11	0 (0%)	0 (0%)	0 (0%)	3-10	2 (3%)	2 (29%)	1 (2%)
3-33	3 (1.5%)	1 (6%)	3 (2%)	2-14	0 (0%)	0 (0%)	0 (0%)	3-19	2 (3%)	0 (0%)	2 (4%)
3-48	3 (1.5%)	1 (6%)	1 (1%)	2-24	0 (0%)	0 (0%)	4 (4%)	3-21	0 (0%)	0 (0%)	10 (18%)
3-49	1 (0.5%)	0 (0%)	3 (2%)	2-28	9 (7%)	0 (0%)	4 (4%)	3-25	0 (0%)	1 (14%)	3 (5.5%)
3-53	0 (0%)	3 (19%)	0 (0%)	2-29	0 (0%)	0 (0%)	1 (1%)	4-1	0 (0%)	0 (0%)	1 (2%)
3-64	5 (2%)	0 (0%)	3 (2%)	2-30	0 (0%)	0 (0%)	2 (2%)	4-69	0 (0%)	0 (0%)	1 (2%)
3-66	0 (0%)	2 (12.5%)	2 (1%)	2-40	0 (0%)	0 (0%)	1 (1%)	5-37	3 (4%)	0 (0%)	0 (0%)
3-72	0 (0%)	0 (0%)	1 (1%)	2-D	0 (0%)	1 (11%)	1 (1%)	6-57	0 (0%)	1 (14%)	2 (4%)
3-73	0 (0%)	0 (0%)	2 (1%)	3-9	0 (0%)	0 (0%)	0 (0%)	7-46	0 (0%)	0 (0%)	3 (5.5%)
3-74	3 (1.5%)	0 (0%)	5 (3%)	3-11	7 (5%)	0 (0%)	7 (8%)	8-61	1 (1%)	0 (0%)	1 (2%)
4-4	5 (2%)	0 (0%)	9 (6%)	3-15	9 (6%)	0 (0%)	4 (4%)	9-49	7 (10%)	0 (0%)	2 (4%)
4-31	0 (0%)	2 (12.5%)	8 (5.5%)	3-20	67 (51%)	2 (22%)	21 (23%)				
4-34	3 (1.5%)	0 (0%)	2 (1%)	3-D	0 (0%)	0 (0%)	1 (1%)				
4-39	0 (0%)	0 (0%)	3 (2%)	4-1	0 (0%)	1 (11%)	9 (10%)				
4-59	4 (2%)	0 (0%)	2 (1%)	5-2	0 (0%)	0 (0%)	1 (0.5%)				
4-61	5 (2%)	0 (0%)	3 (2%)	5-45	0 (0%)	0 (0%)	0 (0%)				
5-51	3 (1.5%)	2 (12.5%)	4 (3%)	6-21	1 (1%)	0 (0%)	1 (1%)				
5-a	0 (0%)	0 (0%)	1 (1%)								
6-1	1 (0.5%)	0 (0%)	0 (0%)								
7-4-1	2 (1%)	0 (0%)	2 (1%)								
Total	205	16	145	Total	132	9	90	Total	73	7	55

Table S8. Light-chain variable gene segments for IGHV1-69-using antibodies isolated from HVTN 204 and 082 trial participants. Light-chain variable gene segments for IGHV1-69-using antibodies (Abs) that were isolated from 8 HVTN 204 and 082 trial participants. Light chain genes were statistically inferred for 125 gp41-reactive and 18 non HIV-1-reactive Abs that used IGHV1-69. IGHV1-69-using gp120-reactive Abs were not found.

IGVK gene segment	Antibodies		IGLV gene segment	Antibodies	
	gp41-reactive	Non HIV-1-reactive		gp41-reactive	Non HIV-1-reactive
1-5	12 (13%)	2 (14%)	1-5	1 (3%)	0 (0%)
1-27	1 (1%)	0 (0%)	1-40	1 (3%)	0 (0%)
1-33	0 (0%)	1 (7%)	1-44	0 (0%)	0 (0%)
1-39	14 (15%)	0 (0%)	1-47	1 (3%)	0 (0%)
2-28	2 (2%)	1 (7%)	1-51	1 (3%)	1 (25%)
2-29	0 (0%)	1 (7%)	2-11	1 (3%)	1 (25%)
2D	0 (0%)	1 (7%)	2-14	9 (29%)	0 (0%)
3-11	3 (3%)	0 (0%)	3-1	5 (16%)	0 (0%)
3-15	2 (2%)	0 (0%)	3-19	1 (3%)	1 (25%)
3-20	60 (64%)	7 (50%)	3-21	0 (0%)	1 (25%)
3D	0 (0%)	1 (7%)	5-37	3 (10%)	0 (0%)
			8-61	1 (3%)	0 (0%)
			9-49	7 (23%)	0 (0%)
Total	94	14	Total	31	4

Table S9. IGHV gene families for antibodies isolated from HVTN 204 and 082 trial participants. Heavy-chain variable gene families used by antibodies (Abs) that were isolated from 8 HVTN 204 and 082 trial participants. Heavy chain genes were statistically inferred for 205 gp41-reactive, 16 gp120-reactive, and 145 non HIV-1-reactive Abs.

IGHV gene family	gp41-reactive antibodies	gp120-reactive antibodies	Non HIV-1-reactive antibodies
IGHV1	136 (66%)	1 (6%)	37 (25.5%)
IGHV2	1 (0.5%)	0 (0%)	6 (4%)
IGHV3	45 (22%)	11 (69%)	68 (47%)
IGHV4	17 (8%)	2 (12.5%)	27 (19%)
IGHV5	3 (1.5%)	2 (12.5%)	5 (3%)
IGHV6	1 (0.5%)	0 (0%)	0 (0%)
IGHV7	2 (1.0%)	0 (0%)	2 (1%)
Total	205	16	145

Table S10. IGKV gene families for antibodies isolated from HVTN 204 and 082 trial participants. Kappa-chain variable gene families used by antibodies (Abs) that were isolated from 8 HVTN 204 and 082 trial participants. Kappa light-chain genes were statistically inferred for 132 gp41-reactive, 9 gp120-reactive, and 90 non HIV-1-reactive Abs.

IGKV genes	gp41-reactive antibodies	gp120-reactive antibodies	Non HIV-1-reactive antibodies
IGKV1	39 (29.5%)	5 (55.5%)	33 (36.7%)
IGKV2	9 (6.8%)	1 (11.1%)	13 (14.4%)
IGKV3	83 (62.9%)	2 (22.2%)	33 (36.7%)
IGKV4	0 (0%)	1 (11.1%)	9 (10%)
IGKV5	0 (0%)	0 (0%)	1 (1.1%)
IGKV6	1 (0.8%)	0 (0%)	1 (1.1%)
IGKV7	0 (0%)	0 (0%)	0 (0%)
Total	132	9	90

Table S11. IGLV gene families for antibodies isolated from HVTN 204 and 082 trial participants. Lambda-chain variable gene families used by antibodies (Abs) that were isolated from 8 HVTN 204 and 082 trial participants. Lambda light-chain genes were statistically inferred for 73 gp41-reactive, 7 gp120-reactive, and 55 non HIV-1-reactive Abs.

IGLV gene families	gp41-reactive antibodies	gp120-reactive antibodies	Non HIV-1-reactive antibodies
IGLV1	24 (32.9%)	1 (14.3%)	13 (23.6%)
IGLV2	29 (39.7%)	1 (14.3%)	10 (18.2%)
IGLV3	9 (12.3%)	4 (57.1%)	20 (36.4%)
IGLV4	0 (0%)	0 (0%)	2 (3.6%)
IGLV5	3 (4.1%)	0 (0%)	0 (0%)
IGLV6	0 (0%)	1 (14.3%)	2 (3.6%)
IGLV7	0 (0%)	0 (0%)	3 (5.5%)
IGLV8	1 (1.4%)	0 (0%)	1 (1.8%)
IGLV9	7 (9.6%)	0 (0%)	2 (3.6%)
IGLV10	0 (0%)	0 (0%)	2 (3.6%)
Total	73	7	55

Table S12. Repertoire of IGHV1-69–using heavy chain genes in 8 HVTN 204 and 082 trial participants. IGHV1-69, percentage of heavy chain genes that used IGHV1-69 gene segment. 54F-HCDR2, 54L-HCDR2, and non-54F/L-HCDR2 are percentages. Total seqs., Total number of heavy chain sequences (seqs.) that used IGHV1-69 gene segment. Only the sequences from the overlap duplicate sequences generated via NGS of prevaccination RNA transcripts from peripheral blood of 8 HVTN 204 and 082 vaccinees (Table S24) were used to determine the number and frequency of IGHV1-69-using heavy chain genes of IGM, IgG, and IgA isotypes.

Vaccinee ID	IgM					IgG					IgA				
	IGHV 1-69	54F-HCDR2	54L-HCDR2	Non-54F/L-HCDR2	Total seqs.	IGHV 1-69	54F-HCDR2	54L-HCDR2	Non-54F/L-HCDR2	Total seqs.	IGHV 1-69	54F-HCDR2	54L-HCDR2	Non-54F/L-HCDR2	Total seqs.
204-121	10.4	70.2	29.3	0.5	9506	5.0	36.0	13.5	50.5	4579	1.8	40.9	35.8	23.3	1851
204-013	5.5	79.6	19.5	0.8	2395	6.0	51.7	24.7	23.6	3316	4.4	61.3	7.5	31.2	3404
204-096	5.2	77.9	21.4	0.7	2601	2.6	60.3	26.9	12.8	2240	1.6	48.1	32.3	19.6	1359
204-165	10.2	99.1	0.4	0.5	4307	4.3	72.1	15.6	12.3	857	2.8	79.4	7.5	13.1	1277
082-081	5.8	73.7	26.1	0.2	4012	3.2	63.3	16.3	20.4	1994	1.3	35.0	34.4	30.6	180
082-001	9.2	77.4	21.3	1.3	3665	2.7	31.0	47.0	22.0	1336	2.9	44.2	23.5	32.4	1130
082-003	7.7	88.0	11.8	0.2	6250	8.2	39.3	57.8	2.9	5604	1.9	41.3	48.6	10.1	664
082-006	4.1	0.3	98.8	0.9	3024	0.7	7.7	72.7	19.6	311	0.3	6.3	75.2	18.5	222

Table S13. CLL archetypes in antibodies from different subjects. HIV-1 gp41-reactive Abs (HVTN204 and 082 trial participants). Healthy controls (*16*). CLL, chronic leukemia lymphocyte; IMGT database (www.imgt.org) as described (*23*). CLL archetypes (*25*). Statistics were performed using Fisher's exact test. No statistical difference in frequency of CLL archetypes observed between cohorts 1 and 2.

Cohorts	Number of VH1-69 Abs	Frequency of CLL archetypes (%) and *P* value
HIV-1 Vaccinees (gp41-reactive Abs)	125	2 (1.6%); *P = 0.002 (vs. CLL Abs)*
Healthy controls	960	22 (2.3%); *P = 1.7x10-5 (vs. CLL Abs)*
CLL patients	142	15 (10.6%)

Table S14. HIV-1 gp41-reactive antibodies from representative clones that were generated as purified monoclonal antibodies. Seventeen gp41-reactive monoclonal antibodies (mAbs) representing 17 clones and 45 total Abs were generated via large scale transfection.

Vaccinee ID	Clone	Number of antibodies	Monoclonal antibody
204-165	TrH005804	1	DH445
204-165	TrH005899	1	DH446
204-096	TrH006519	2	DH431
204-096	TrH006563	2	DH433
204-013	TrH006476	2	DH443
204-013	TrH006477	1	DH441
204-121	TrH005654	3	DH430
204-096	TrH006531	3	DH438
204-096	TrH006628	2	DH437
204-096	TrH006587	1	DH435
204-096	TrH006521	4	DH436
204-013	TrH006469	3	DH444
204-013	TrH006465	4	DH442
204-013	TrH006466	1	DH439
204-013	TrH006464	5	DH440
204-096	TrH006520	2	DH434
204-096	TrH006517	8	DH432

Table S15. HIV-1 gp120-reactive antibodies from representative clones that were generated as purified monoclonal antibodies. Twelve gp120-reactive monoclonal antibodies (mAbs) representing 12 clones were generated via large scale transfection. Epitope mapping was evaluated via binding to multiclade linear overlapping peptides in triplicate arrays. MAbs bound similar HIV-1 Env regions using peptides via ELISA (not shown). Non-linear epitope; no binding to linear peptides by mAbs. The peptide (HIV-1 isolate) was a 15-oligomer peptide with overlap by 11 amino acid peptides that were bound by the gp120 mAbs. NB, no binding.

Clone	Monoclonal antibody ID	Epitope	Peptide (HIV-1 isolate)
TrH006964	DH201	C1	AKAYDTEVHNVWATH (B.CON), DTEVHNVWATHACVP (B.CON)
TrH007308	DH191	V2	KDKKKNVYALFYKLD (ZM651), RDKKKQVYALFYKLD (D.CON)
TrH007309	DH192	V2	TELRDKKQKVHALFY (AE.92TH023, A244), TELKDKKHKVHALFY (1086C),
TrH005803	DH449	V3	TRPNNNTRKSIHIGP (B.CON), NNTRKSIHIGPGRAF (B.CON)
TrH006894	DH450	V3	NNTRKSIRIGPGQTF (1086C), RKSIRIGPGQTFYAT (1086C)
TrH006467	DH196	V3	RKSVRIGPGQAFYAT (TV1), NNTRKSIRIGPGQTF (1086C)
TrH007037	DH452	V3	RKRIHIGPGRAFYTT (MN), RKSIHIGPGRAFYAT (B.CON)
TrH006976	DH451	Non-linear	NB
TrH006631	DH448	Non-linear	NB
TrH006625	DH447	Non-linear	NB
TrH007039	DH453	Non-linear	NB
TrH007034	DH195	Non-linear	NB

Table S16. Binding specificities of representative vaccine-induced gp41-reactive monoclonal antibodies 6-helix bundle recombinant MN gp41, post-fusion protein (5-helix) (*21*)**, and recombinant vaccine-strain gp140 proteins.** Seventeen representative HIV-1 gp41-reactive monoclonal antibodies (mAbs) were screened for binding . MAb binding was measured via ELISA (EC_{50} (µg/ml) and SPR (RU, response unit). For SPR analysis, Env gp140 and MN gp41 proteins were injected at 200 and 40 µg/ml, respectively, for 5 minutes and binding responses were measured after 10s post-injection over a 10s window. NB - no binding. Data shown from ELISA were representative of two experiments, while SPR assay was performed once.

Monoclonal antibody ID	ELISA (EC_{50}, µg/ml)		SPR (RU)			
	MN gp41	Post-fusion gp41	MN gp41	VRC-A gp140	VRC-B gp140	VRC-C gp140
DH439	0.01	0.01	1160	887	635	942
DH432	0.02	0.01	326	631	428	851
DH433	0.01	0.01	958	449	201	337
DH430	0.01	0.005	391	654	485	842
DH434	0.03	0.01	722	758	620	740
DH440	0.04	0.01	1160	830	695	887
DH441	0.07	5.1	1160	920	2	934
DH436	0.05	0.01	1580	655	513	749
DH438	0.10	12.8	439	7	2	5
DH431	0.13	0.01	695	168	152	437
DH437	0.84	0.5	1090	707	473	843
DH443	0.36	0.03	623	1	54	29
DH445	3.39	0.01	17	549	193	146
DH442	0.45	0.01	458	763	484	840
DH435	1.34	0.62	162	184	170	308
DH444	0.04	0.004	127	371	291	360
DH446	29.1	NB	687	565	4	155

Table S17. Apparent binding affinities of vaccine-induced gp41-reactive monoclonal antibodies. Five representative gp41-binding monoclonal antibodies (**Table S14**) were measured by SPR analysis in a single experiment for rate constants (k_a, k_d) and K_d values for binding to recombinant MN gp41, and vaccine-strain (VRC-A) and group M consensus (M.CON-S) gp140 proteins. Env gp140 proteins were injected at concentrations ranging from 0.5-10 µg/ml over mAbs captured on an anti-IgFc immobilized surface as described previously (*58*). Double referencing with control palivizumab mAb and PBS buffer was used to subtract non-specific responses.

Monoclonal antibody ID	HIV-1 binding specificity	MN gp41			VRC-A gp140			M.CON-S gp140		
		k_a (Ms^{-1})	k_d (s^{-1})	K_d (nM)	k_a (Ms^{-1})	k_d (s^{-1})	K_d (nM)	k_a (Ms^{-1})	k_d (s^{-1})	K_d (nM)
DH439	gp41	$5.3x10^{2}$	$3.76x10^{-5}$	71	$2.32x10^{5}$	$1.9x10^{-4}$	0.82	$2.87x10^{5}$	$5.83x10^{-4}$	2.03
DH432	gp41	$1.67x10^{4}$	$1.43x10^{-5}$	0.86	$3.46x10^{5}$	$3.28x10^{-4}$	0.09	$2.69x10^{5}$	$3.38x10^{-4}$	1.26
DH434	gp41	$1.15x10^{3}$	$3.99x10^{-5}$	34.7	$5.23x10^{5}$	$2.87x10^{-4}$	0.55	$2.75x10^{5}$	$7.32x10^{-4}$	2.66
DH440	gp41	$1.14x10^{4}$	$1.09x10^{-5}$	0.95	$3.53x10^{5}$	$1.98x10^{-4}$	0.56	$8.69x10^{5}$	$6.26x10^{-4}$	0.72
DH441	gp41	$1.29x10^{4}$	$2.38x10^{-5}$	1.84	$3.16x10^{5}$	$2.57x10^{-4}$	0.81	$7.03x10^{4}$	$163x10^{-3}$	23.2

Table S18. Neutralization profile of HIV-1 gp41-reactive monoclonal antibodies. Gp41-reactive monoclonal antibodies (mAbs) were screened for HIV-1 neutralization via a TZM-bl assay. Positive control mAb - 4E10. Non HIV-1 control virus (SVA) - murine leukemia virus (MuLV). Neutralization color-key: IC50 <1 (red), 1-10 (orange); no color for negative neutralization.

Monoclonal	HIV-1 isolates (IC_{50}, µg/ml)					
antibody ID	A.92RW020	B.HXB2	B.BaL	B.MN	C.97ZA012	SVA
DH439	>50	>50	>50	>50	>50	>50
DH433	>50	>50	>50	>50	>50	>50
DH432	>50	>50	>50	>50	>50	>50
DH430	>50	>50	>50	>50	>50	>50
DH434	>50	>50	>50	>50	>50	>50
DH440	>50	>50	>50	>50	>50	>50
DH441	>50	>50	>50	>50	>50	>50
DH436	>50	>50	>50	>50	>50	>50
DH438	>50	>50	>50	>50	>50	>50
DH431	>50	>50	>50	>50	>50	>50
DH437	>50	>50	>50	>50	>50	>50
DH443	>50	>50	>50	>50	>50	>50
DH445	>50	>50	>50	>50	>50	>50
DH442	>50	>50	>50	>50	>50	>50
DH435	>50	>50	>50	>50	>50	>50
DH444	>50	>50	>50	>50	>50	>50
DH446	>50	>50	>50	>50	>50	>50
4E10	2.9	<0.02	2.4	<0.02	3.2	>50

Table S19. Neutralization profile of HIV-1 gp120-reactive monoclonal antibodies. Gp120-reactive monoclonal antibodies (mAbs) were screened for HIV-1 neutralization via a TZM-bl assay. Positive control mAb - CH01-31. Non HIV-1 control virus (SVA) - murine leukemia virus (MuLV). Neutralization color-key: IC50 <1 (red), 1-10 (orange); no color for negative neutralization.

Monoclonal antibody ID	HIV-1 isolates (IC_{50}, µg/ml)					
	A.92RW020	B.HXB2	B.BaL	B.MN	C.97ZA012	SVA
DH201	>50	>50	>50	>50	>50	>50
DH191	>50	>50	>50	>50	>50	>50
DH192	>50	>50	>50	>50	>50	>50
DH449	>50	>50	>50	<0.023	>50	>50
DH450	>50	>50	6.8	1.7	>50	>50
DH196	>50	>50	1.2	0.11	>50	>50
DH452	>50	>50	0.94	<0.023	>50	>50
DH451	>50	>50	>50	>50	>50	>50
DH448	>50	>50	>50	>50	>50	>50
DH447	>50	>50	>50	>50	>50	>50
DH453	>50	>50	>50	>50	>50	>50
DH195	>50	>50	>50	>50	>50	>50
CH01-31	0.05	0.16	0.14	0.51	0.16	>25

Table S20. Antibody reactivity with linear peptides containing LLRAIE HR-1 sequence. Gp41-reactive monoclonal antibodies (mAbs) DH440, DH432 and DH438 were tested for binding linear peptides containing the LLRAIE HR-1 sequence via peptide array (*57*). Data expressed as relative light fluorescence units reflecting degree of Ab reactivity; For DH440, DH432, and DH438, background subtraction was 1000. Data shown are representative of triplicate arrays.

Gp41 peptide sequence	DH440, 10 µg/ml	DH432, 10 µg/ml	DH438, 20 µg/ml
ARQLLSGIVQQQSNL	1	1	1
ARQLLSGIVQQQNNL	1	1	1
LLSGIVQQQSNLLRA	573	1	457
LLSGIVQQQNNLLRA	1	1	1
GIVQQQSNLLRAIEA	1	1	223
GIVQQQNNLLRAIEA	1	1	391
QQQSNLLRAIEAQQH	5742	113	20823
QQQNNLLRAIEAQQH	4626	1	14627
SNLLRAIEAQQHLLQ	12387	1	7436
SNLLRAIEAQQHLLK	9218	1	5372
NNLLRAIEAQQHLLQ	13071	1	9581
SNLLRAIEAQQHMLQ	12969	1246	20945
LRAIEAQQHLLQLTV	1670	1	1
LRAIEAQQHLLKLTV	1	1	1258
LRAIEAQQHMLQLTV	1232	251	4295
IEAQQHLLQLTVWGI	1	1	1119
IEAQQHLLKLTVWGI	1665	1	2360
IEAQQHMLQLTVWGI	1	1	585
QQHLLQLTVWGIKQL	1	1	1
QQHLLKLTVWGIKQL	1	1	1
QQHMLQLTVWGIKQL	1	1	1

Table S21. Poly-reactivity of vaccine-induced gp41-reactive and gp120-reactive monoclonal antibodies. Column 2,monoclonal antibodies (mAbs) were screened (@50µg/ml, except DH446 @48µg/ml) for binding auto-antigens by AtheNA (ANA-II Plus test system) (*29*). Positivity cutoff for binding in auto-reactivity assay was ≥121 (relative units); *11/17 (65%) gp41 and *4/12 (33%) gp120 Abs tested positive for auto-reactivity. Third column, mAbs were tested (@100µg/ml) for binding cardiolipin by ELISA (QUANTA Lite ACA IgG III). Positivity cutoff for cardiolipin binding was ≥0.18 (OD_{450nm}); 4/17 (23.5%) gp41 and 1/11 (9%) gp120 mAbs tested positive for binding cardiolipin. Control mAbs: 4E10 (anti-HIV), palivizumab (anti-RSV). Values of positive binding generated using commercially available kits were highlighted in orange.

Monoclonal antibody ID	HIV-1 reactivity	SSA	SSB	Sm	RNP	Scl70	Jo1	dsDNA	Cent B	Histone	CL
*DH438	gp41	424	320	418	245	260	341	11	187	338	0.42
*DH443	gp41	371	138	101	41	100	209	225	128	299	0.32
*DH440	gp41	467	197	46	139	32	233	52	141	231	0.23
*DH444	gp41	425	247	231	242	182	182	3	230	334	0.05
*DH441	gp41	418	247	23	247	13	213	62	165	211	0.21
*DH432	gp41	165	154	48	21	39	161	52	67	68	0.05
*DH430	gp41	148	217	169	84	66	173	0	82	104	0.07
*DH433	gp41	172	9	23	7	16	14	328	26	219	0.16
*DH435	gp41	11	8	19	8	11	17	400	208	52	0.17
*DH437	gp41	417	13	13	20	9	13	82	40	27	0.14
*DH434	gp41	4	4	19	9	18	16	175	54	65	0.10
DH436	gp41	15	20	24	8	32	110	105	82	107	0.14
DH431	gp41	58	5	15	12	5	14	77	96	66	0.10
DH442	gp41	76	4	13	107	11	24	15	74	57	0.05
DH439	gp41	5	2	11	8	11	11	54	24	15	0.06
DH445	gp41	13	5	6	2	2	3	6	4	5	0.10
DH446	gp41	2	6	3	3	3	2	1	5	3	0.08
*DH195	gp120	199	260	98	40	85	195	267	130	173	0.35
*DH201	gp120	403	24	23	7	9	5	426	262	291	0.08
*DH449	gp120	103	135	94	63	69	86	226	228	238	0.05
*DH196	gp120	12	6	13	175	4	3	25	20	24	0.04
DH448	gp120	10	4	4	3	2	3	3	4	4	0.05
DH447	gp120	11	5	8	8	3	94	16	10	13	0.05
DH191	gp120	15	11	15	8	16	6	21	7	18	0.13
DH192	gp120	11	4	10	4	3	4	12	2	9	0.04
DH453	gp120	14	5	11	3	3	5	18	8	10	0.05
DH451	gp120	11	4	19	17	10	4	19	14	15	0.15
DH450	gp120	5	3	8	11	6	3	19	5	8	0.06
DH452	gp120	18	4	9	4	4	3	78	35	44	0.07
*4E10	gp41	442	198	25	10.5	4.5	280	14	20	65	1.92
Palivizumab	RSV	5	2	2.5	1.5	2	3	1	1	2.5	0.04

Table S22. HEp-2 cell reactivity of vaccine-induced gp41-reactive and gp120-reactive monoclonal antibodies. mAbs were screened (@50 μg/ml) in HEp-2 cell immunofluorescence staining. Reactivity patterns are defined by Zeus Scientific (http://zeussscientific.com). Reference mAbs: 2F5, 17b.

Monoclonal antibody ID	HIV-1 reactivity	Hep-2 cell reactivity
DH438*	gp41	Granular
DH443*	gp41	Cytoplasmic
DH440*	gp41	Cytoplasmic
DH444*	gp41	cytoplasmic
DH441	gp41	Negative
DH432*	gp41	cytoskeletal
DH430	gp41	negative
DH433	gp41	negative
DH435	gp41	negative
DH437	gp41	negative
DH434	gp41	negative
DH436	gp41	negative
DH431	gp41	negative
DH442	gp41	negative
DH439	gp41	negative
DH445	gp41	negative
DH446	gp41	negative
DH195	gp120	negative
DH201*	gp120	cytoplasmic
DH449	gp120	negative
DH196	gp120	negative
DH448	gp120	negative
DH447	gp120	negative
DH191	gp120	negative
DH192	gp120	negative
DH453	gp120	negative
DH451	gp120	negative
DH450	gp120	negative
DH452	gp120	negative
2F5	gp41	cytoplasmic and nuclear
17b	gp41	negative

*MAbs with HEp-2 cell reactivity confirmed in duplicate assays: 5/17 (29%) gp41 and 1/12 (8%) gp120 mAbs demonstrated HEp-2 cell reactivity.

Table S23. Primers for cDNA amplification of heavy chain genes via illumina next generation sequencing. Primers used in first round cDNA amplification are listed here in table S23. The barcode-tagged primers used in the second round of PCR amplification are commercially available (Illumina Miseq). Reverse Ig isotype primers (IgA1, IgA2, IgG, IgD, IgM) were used for cDNA production as previously described (*13*). Nucleotides highlighted in primers; (pink) overlap with illumina-barcoded primers, (blue) Ig-specific sequences, and (black) the illumina sequencing primers.

Primer Name	Sequences (5-3)	Nucleotides	Tm
IGHV1-Ext__P5_1ST	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATGGACTGGACCTGGAGG	20	56.3
IGHV2-Ext__P5_1ST	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGGACATACTTTGTTCCA	19	41.9
IGHV3-Ext__P5_1ST	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATGGAGTTTGGGCTGAGC	20	56.8
IGHV4-Ext__P5_1ST	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGAAACACCTGTGGTTCTT	20	45.7
IGHV5-Ext__P5_1ST	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGGGGTCAACCGCCATCCT	20	59.8
IGHV6-Ext__P5_1ST	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGTCTGTCTCCTTCCTCAT	20	44.5
HUIGA_Deep_R__P7_1ST	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTCAGCGGGAAGACCTTG	19	54.7
HUIGG_Deep_R__P7_1ST	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGATGGGCCCTTGGTGGA	18	58.7
HUIGM_Deep_R__P7_1ST	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAGACGAGGGGGAAAAGG	19	54.8

Table S24. Pre-vaccine IGHV repertoire interrogated via illumina next generation sequencing. B cell values are the estimated total number of B-cells in each blood mononuclear cell sample analyzed by illumina next generation sequencing (NGS) from each vaccinee. With one unique IGHV sequence per B cell, then the estimated number of B cells would also represent the estimated number of unique IGHV sequences expected. The use of vaccine-induced IGHV sequence as a probe to search the IGHV repertoire for a clonally-related member contributed to the power of our analysis. In order to detect the frequency of IGHV1-69-using antibodies (Abs) and their allelic variants (**Table S12**), we used the number of overlap sequences from each duplicate Illumina MiSeq run. For detection of a pre-vaccine sequence reflective of a post-vaccine Ab, we sampled both duplicate runs for pre-vaccination Abs with the same V_HDJ_H recombination and HCDR3 length for clonal relatedness.

HVTN Trial	Vaccinee ID	Dataset	Sequences				Cells	
			IgA	IgG	IgM	Total	PBMC	B cells[1]
204	204-121	Run 1	1,110,364	1,089,016	2,059,638	4,259,018	15M	1.5M (10%)
		Run 2	866,608	621,247	903,521	2,391,376		
		Overlap	101,004	90,801	91,428	283,233		
204	204-013	Run 1	384,435	614,221	1,297,748	2,296,404	15M	600K (4%)
		Run 2	471,869	621,217	1,445,102	2,538,188		
		Overlap	77,625	54,819	43,371	175,815		
204	204-096	Run 1	877,214	1,034,254	711,775	2,623,243	15M	1.3M (8.9%)
		Run 2	884,236	886,671	1,041,686	2,812,593		
		Overlap	84,368	87,673	49,796	221,837		
204	204-165	Run 1	496,624	172,137	349,429	1,018,190	7.6M	755K (10%)
		Run 2	455,762	414,124	475,097	1,344,983		
		Overlap	44,883	20,126	42,375	107,384		
082	082-081	Run 1	316,869	664,925	782,420	1,764,214	7.9M	806K (10.2%)
		Run 2	83,509	585,688	558,573	1,227,770		
		Overlap	14,317	63,265	69,652	147,234		
082	082-001	Run 1	354,431	370,114	468,033	1,192,578	7.7M	765K (8.03%)
		Run 2	407,045	378,933	530,185	1,316,163		
		Overlap	38,320	50,067	39,972	128,359		
082	082-003	Run 1	548,746	699,065	954,528	2,202,339	7.8M	636K (8.2%)
		Run 2	233,845	467,916	724,364	1,426,125		
		Overlap	34,642	68,187	81,071	183,900		
082	082-006	Run 1	546,812	437,340	793,136	1,777,288	7.8M	837K (10.8%)
		Run 2	514,466	250,583	614,055	1,379,104		
		Overlap	65,755	46,077	72,981	184,813		

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Table S25. Pre and post-vaccine clonally related antibodies tested for cardiolipin reactivity.
Monoclonal antibodies (DH477, DH476) were screened for reactivity with cardiolipin, using an anti-cardiolipin ELISA (QUANTA Lite ACA IgG III). Positivity cutoff was optical density (OD) of 0.18.

Antibody ID	Vaccination schedule	Binding, OD (μg/ml)			
		100	33	11	3.7
DH477	PRE	1.04	0.44	0.18	0.16
DH476	POST	0.33	0.12	0.08	0.09

Table S26. Pre and post-vaccine clonally-related antibodies tested for binding auto-antigens (AtheNA).
Monoclonal antibodies (DH477, DH476) were screened for reactivity with host antigens via AtheNA (AtheNA Multi-Lyte System) (*29*). Positivity cutoff was 121.

Antibody ID	Vaccination schedule	SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
DH477	PRE	15	21	5	13	7	32	0	199	70
DH476	POST	75	25	2	3	2	156	38	30	18