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1 **Boosting with ALVAC-HIV and AIDSVAX B/E enhances Env constant region 1 and 2**
2 **antibody-dependent cellular cytotoxicity**

3 By

4 David Easterhoff^{1*}, Justin Pollara¹, Kan Luo¹, William D. Tolbert¹², Brianna Young¹¹, Dieter
5 Mielke¹, Shalini Jha¹, Robert J. O’Connell³, Sandhya Vasan^{2,3,10}, Jerome Kim^{2**}, Nelson L.
6 Michael^{2,10}, Jean-Louis Excler^{2,10**}, Merlin L. Robb^{2,10}, Supachai Rerks-Ngarm⁵, Jaranit
7 Kaewkungwal⁶, Punnee Pitisuttithum⁶, Sorachai Nitayaphan⁷, Faruk Sinangil⁸, James Tartaglia⁹,
8 Sanjay Phogat^{9#}, Thomas B. Kepler⁴, S. Munir Alam¹, Kevin Wiehe¹, Kevin O. Saunders¹, David
9 C. Montefiori¹, Georgia D. Tomaras¹, M. Anthony Moody¹, Marzena Pazgier¹², Barton F.
10 Haynes^{1*} and Guido Ferrari^{1*}

11
12 ¹Duke University, Durham, NC; ²US Military HIV Research Program, Walter Reed Army
13 Institute of Research, Silver Spring, MD ; ³U.S. Army Medical Directorate, AFRIMS,
14 Bangkok, Thailand; ⁴Boston University, Boston, MA; ⁵Thai Ministry of Public Health,
15 Nonthaburi, Thailand; ⁶Mahidol University, Bangkok, Thailand; ⁷Royal Thai Army
16 Component, AFRIMS, Bangkok, Thailand; ⁸Global Solutions of Infectious Diseases,
17 South San Francisco, CA; ⁹Sanofi Pasteur, Swiftwater, PA; NH; ¹⁰The Henry M.
18 Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD;
19 ¹¹Department of Biochemistry and Molecular Biology of University of Maryland,
20 Baltimore, MD USA . ¹²Infectious Diseases Division, Uniformed Services University of
21 the Health Sciences, Bethesda, MD, USA,

22 ** Current affiliation: International Vaccine Institute, Seoul, Republic of Korea

23 # Current affiliation: Glaxosmithkline, USA

24 *Correspondence: david.easterhoff@duke.edu (D.E.), barton.haynes@duke.edu (B.F.H.) and
25 guido.ferrari@duke.edu and (G.F.)

26 **Significance**

27 Over one million people become infected with HIV-1 each year making the development
28 of an efficacious HIV-1 vaccine an important unmet medical need. The RV144 human
29 HIV-1 vaccine-regimen is the only HIV-1 clinical trial to date to demonstrate vaccine-
30 efficacy. An area of focus has been on identifying ways by which to improve upon
31 RV144 vaccine-efficacy. The RV305 HIV-1 vaccine-regimen was a follow-up boost of
32 RV144 vaccine-recipients that occurred 6-8 years after the conclusion of RV144. Our

33 studies focused on the effect of delayed boosting in humans on the vaccine-induced
34 antibody repertoire. It was found that boosting with a HIV-1 Env vaccine increased
35 antibody-mediated effector function potency and breadth.

36 **Abstract**

37 Induction of protective antibodies is a critical goal of HIV-1 vaccine development. One
38 strategy is to induce non-neutralizing antibodies that kill virus-infected cells as these
39 antibody specificities have been implicated in slowing HIV-1 disease progression and in
40 protection. HIV-1 Env constant region 1 and 2 (C1C2) antibodies frequently contain
41 potent antibody dependent cellular cytotoxicity (ADCC) making them a vaccine target.
42 Here we explore the effect of delayed and repetitive boosting of RV144 vaccinee
43 recipients with ALVAC/AIDSVAX B/E on the C1C2-specific antibody repertoire. It was
44 found that boosting increased clonal lineage specific ADCC breadth and potency. A
45 ligand crystal structure of a vaccine-induced broad and potent ADCC-mediating C1C2-
46 specific antibody showed that it bound a highly conserved Env gp120 epitope. Thus,
47 rationally designed boosting strategies to affinity mature these type of IgG C1C2-
48 specific antibody responses may be one method by which to make an improved HIV
49 vaccine with higher efficacy than seen in the RV144 trial.

50 **INTRODUCTION**

51 CD4-inducible (CD4i) epitopes within HIV-1 envelope (Env) constant regions 1 and
52 2 (C1C2) are targets for antibodies that mediate antibody dependent cellular cytotoxicity
53 (ADCC) [1]. C1C2-specific antibody epitopes have been termed Cluster A [1] and defined
54 by two Env-targeted monoclonal antibodies (mAbs), A32 [2] and C11 [1]. Structural
55 analyses of antigen complexes formed by A32, A32-like [3-5] and C11-like antibodies [6]

56 indicate that these antibodies bind distinct Env epitopes. The A32 epitope involves a
57 discontinuous sequence within layers 1 and 2 of the inner domain [4, 5] while the C11
58 epitope maps to the inner domain eight-stranded β sandwich [6]. Importantly, both
59 antibodies are non-neutralizing for tier 2 HIV strains, but are capable of broad and potent
60 ADCC [1, 2].

61 The secondary analysis of HIV-1 infection risk in RV114 (NCT00223080)
62 indicated that ADCC in the presence of low anti-Env IgA responses correlated with
63 decreased HIV-1 acquisition [7]. While antibodies representative of the Env variable
64 region 2 (V2) response inversely correlated with HIV-1 acquisition [7], we previously
65 demonstrated that synergy between A32-blockable C1C2-specific antibodies and V2-
66 specific antibodies increased the potency of V2 antibodies induced in the RV144 trial
67 [8].

68 Here we have studied the effects of late boosting of RV144 vaccinees on affinity
69 maturation and potency of C1C2-specific ADCC antibodies in the RV305 HIV-1 vaccine
70 trial (NCT01435135). We found that ALVAC/AIDS VAX B/E immunizations induced
71 C1C2-specific antibodies and that late booster immunizations increased C1C2-specific
72 antibody variable heavy and variable light ($V_H + V_L$) chain gene mutation frequencies
73 and increased their ADCC breadth and potency.

74 **RESULTS**

75 **AIDS VAX B/E N-terminal deletion alters C1C2-specific antibody responses.**

76 AIDS VAX B/E protein used in the RV144 and RV305 HIV-1 vaccine trial had an eleven
77 amino acid N-terminal deletion [9] that removed a majority of the C11-like antibody
78 epitope [6], whereas CRF_01_AE_gp140_Env_92TH023 in ALVAC (vCP1521) did have

79 the gp120 N-terminal 11 amino acids [10]. To determine if C11 could bind to gp120
80 proteins with an 11 amino acid N-terminal deletion, we assayed A32 and C11 antibodies
81 for binding to full length AE.A244gp120 or to AE.A244gp120 Δ 11 (N-terminal 11 aa
82 deleted). Antibody A32 bound to full length AE.A244gp120 and A32 binding was
83 enhanced on AE.A244gp120 Δ 11 (**Fig. 1A**) [9]. In contrast, antibody C11 only bound to
84 the full length AE.A244gp120 (**Fig 1A**). From these data we concluded that C11-like
85 antibody responses were unlikely to be boosted by AIDSVAX B/E.

86 A total of 19 RV305-derived NNABs isolated from four individuals (**Table S1 &**
87 **S2**) were identified that blocked the C1C2 mAb A32 binding to AE.A244gp120 Δ 11 (**Fig**
88 **1B**). Compared to previously published RV144 C1C2-specific antibodies [11] the RV305
89 C1C2-specific antibodies had significantly more V_H and V_L chain gene mutations
90 (Wilcoxon rank sum test $P < 0.0001$) (**Fig 1C**) suggesting that RV305 boosting induced
91 additional somatic mutations in C1C2-specific antibodies.

92 To determine if RV305 boosted A32 blockable antibodies contained a binding
93 epitope similar to A32, we used the A32 ligand crystal structure [5] to identify critical
94 A32 antibody contact residues, and then designed an AE.A244gp120 Δ 11 mutant
95 protein (AE.A244gp120 Δ 11 F35S, H72L, V75A, E106K, D107H, S110A, Q114L) to
96 eliminate A32-like antibody binding (**Fig 1A**). In ELISA, the RV305 antibody, DH838,
97 was the only antibody with binding eliminated by mutating the A32 epitope (**Fig 1D**).
98 Likewise, DH838 was the only antibody that used a VH3 family gene while all other
99 ALVAC/AIDSVAX B/E – induced C1C2-specific antibodies used VH1 genes (**Table S1**).
100 Thus, as in RV144, ALVAC/AIDSVAX B/E boosting preferentially expanded VH1 gene

101 C1C2-specific antibodies [11] and these antibodies bound epitopes distinct from A32
102 but in close enough proximity to be sterically cross-blocked by A32 (**Fig 1B**).

103 **Boosting increased C1C2-specific ADCC breadth and potency.** RV305

104 C1C2-specific antibodies and a subset of RV144 C1C2-specific antibodies were next
105 assessed for ADCC against a cross-clade panel of HIV-1 infectious molecular clone
106 (IMC) infected CD4+ T cells (HIV-1 AE.CM235, B.WITO, C.TV-1, C.MW965, C.1086C,
107 C.DU151 and C.DU422). Antibodies were ranked using an ADCC score (See methods)
108 that accounted for ADCC breadth and potency. Apart from the RV144-derived A32
109 blockable antibody CH38, which was naturally an IgA antibody but tested here as a
110 recombinant IgG1 antibody, 16/19 RV305 antibodies ranked higher than the RV144
111 antibodies (**Table 1**). Next RV305 derived C1C2-specific antibody heavy chain gene
112 mutation frequency was used as a proxy for responsiveness to boosting and compared
113 to the ADCC score. The V_H mutation frequency (% nucleotide) inversely correlated with
114 the ADCC score (Spearman Correlation -0.5599; p value = 0.0127) (**Fig S1**). However
115 the V_H mutation frequency of those antibodies with the highest ADCC scores were
116 above the average heavy chain gene mutation frequency for RV144 (**Fig S1 Fig 1C**).
117 Thus, while a RV144 boosting regimen was necessary to increase C1C2-specific ADCC
118 breadth and potency, additional boosting with same immunogens may not be beneficial.

119 **Boosting of RV144 vaccinees in the RV305 trial increased ADCC breadth and**
120 **potency of the RV144 derived C1C2-specific, DH677 clonal lineage.** Next the C1C2-

121 specific DH677 memory B cell clonal lineage was used to study affinity maturation and
122 ontogeny of ALVAC/AIDS VAX B/E-induced ADCC responses. B cell clonal lineage
123 member DH677.1 arose after the original RV144 trial (ALVAC + AIDS VAX B/E) and the

124 DH677.2, DH677.3 and DH677.4 clonal lineage members were isolated after delayed
125 and repetitive boosting with AIDSVAX B/E alone (RV305 Group II). Thus, this B cell
126 clonal lineage belongs to a long-lived memory B cell pool started by the RV144 vaccine-
127 regimen and boosted many years later with the RV305 vaccine-regimen (**Fig 2**). The
128 DH677 clonal lineage was assayed by surface plasmon resonance for binding to the
129 AIDSVAX B/E proteins - AE.A244gp120 Δ 11 and B.MNgp120 Δ 11 – as well as full length
130 AE.A244gp120. DH677 unmutated common ancestor (UCA) did not bind to
131 B.MNgp120 Δ 11, had minimal binding to the full length AE.A244gp120 and this binding
132 was enhanced with AE.A244gp120 Δ 11 (**Fig 2 and Fig S2**). The RV305 boosts more
133 than doubled the V_H chain gene mutation frequency from 1.04% (DH677.1; RV144) up
134 to 4.51% (DH677.4; RV305) which resulted in 100-fold increase in apparent affinity for
135 the AIDSVAX B/E proteins (DH677.1 AE.A244gp120 Δ 11 K_D= 45.2 & B.MNgp120 Δ 11
136 K_D =219 to DH677.4 AE.A244gp120 Δ 11 K_D= 0.49 & B.MNgp120 Δ 11 K_D =2.86) and also
137 improved binding to full length AE.A244gp120 (**Fig 2 and Fig S2**).

138 The ontogeny of vaccine-induced ADCC was studied by assaying the DH677
139 clonal lineage against a cross-clade panel of IMC infected cells (AE.CM235, B.WITO,
140 C.TV-1, C.MW965, C.1086C, C.DU151 and C.DU422). The RV144 prime-boost
141 immunization regimen minimally increased ADCC breadth and potency (DH677 UCA
142 ADCC Score = -2.32; DH677.1 ADCC Score = -2.20 (see methods)). Conversely,
143 RV305 boosting substantially increased ADCC breadth and potency (DH677.3 ADCC
144 Score = 4.56) (**Fig 2 and Fig S3**). These data indicated that the RV144 prime-boost
145 regimen was insufficient to fully affinity mature this C1C2-specific B cell clonal lineage.

146 Rather RV305 trial boosting of this particular RV144 vaccinee profoundly enhanced
147 DH677 lineage ADCC breadth and potency.

148 **Crystal structure of the potent ADCC-mediating antibody DH677.3.** We next
149 determined the crystal structures of the antigen binding fragment (Fab) of the highest
150 ranking RV305 ADCC antibody DH677.3 (**Table 1**) - alone and in complex with clade AE
151 gp120_{93TH057} core_e plus the CD4-mimetic M48-U1 (**Table S3**). DH677.3 Fab-gp120_{93TH057}
152 core_e-M48U1 complex (**Fig 3**) showed that, similar to other Cluster A antibodies, DH677.3
153 approaches gp120 at the face that is buried in the native Env trimer [3-5] and binds the
154 C1C2 region exclusively within the gp120 inner domain. gp120 residues involved in
155 DH677.3 binding map to the base of the 7-stranded β -sandwich (residues 82, 84, 86-87,
156 222-224, 244-246, and 491-492) and its extensions into the mobile layers 1 (residues 53,
157 60, 70-80) and 2 (residues 218-221). By docking at the layer 1/2/ β -sandwich junction
158 the antibody buried surface area (BSA) utilizes 248 \AA^2 of the β -sandwich, 542 \AA^2 of layer
159 1 and 135 \AA^2 of layer 2 (**Table S4**). The majority of contacts providing specificity involve
160 a network of hydrogen bonds and a salt bridge (**Fig 3A, inset**) contributed by the antibody
161 heavy chain and gp120 side chain atoms of layer 1 (α turn connecting the $\beta 7$ -- $\beta 0$ strands,
162 D⁷⁸ and N⁸⁰) and the 7-stranded- β -sandwich (strand $\beta 7$, Q²⁴⁶). The contacts provided by
163 the light chain are less specific and consist of hydrogen bonds to the gp120 main chain
164 atoms and hydrophobic contacts within a hydrophobic cleft formed at the layer 1/2/ β -
165 sandwich junction (**Fig 3B and C**). Overall DH677.3 utilizes all six of its complementary
166 determining regions (CDRs), and relies approximately equally on both heavy chain and
167 light chain with a total buried surface area (BSA) of 973 \AA^2 : 498 \AA^2 for the light chain and
168 475 \AA^2 for the heavy chain (**Table S4 and Fig 3C and S5**). Interestingly, 25 of 29 gp120

169 contact residues are conserved in >80% of sequences in the HIV Sequence Database
170 Compendium
171 (<https://www.hiv.lanl.gov/content/sequence/HIV/COMPENDIUM/compendium.html>) with
172 15 of 29 being effectively invariant (>99% conserved) (**Fig 3B**).

173 **Comparison of the DH677.3 mode of binding and epitope footprint to Cluster A**
174 **prototype antibodies.** Antigen complex structures of mAb A32 and N12-i3 (C11-like) [3,
175 6], antibodies isolated from HIV-1-infected individuals, confirm that DH677.3 recognized
176 a unique epitope between the A32 and C11 antibody-binding sites involving Env epitope
177 elements of both (**Fig 4**). While the A32 antibody epitope consists exclusively of gp120
178 mobile layers 1 and 2 (76% and 24% of gp120 BSA, respectively; **Table S4, Fig 4 B and**
179 **C**), DH677.3 relies less on layers 1 and 2 (53% and 14% of gp120 BSA, respectively) and
180 effectively utilizes the gp120 7-stranded β -sandwich (24% of gp120 BSA) (**Table S4, Fig**
181 **4 B and C**). The ability to recognize the 7-stranded β -sandwich renders DH677.3 similar
182 to the C11-like antibody N12-i3, which almost exclusively depends on the β -sandwich for
183 binding (94% of its total gp120 BSA; **Table S4, Fig 4 B and C**). Interestingly, N12-i3 and
184 other C11-like antibodies require the N-terminus of gp120 for binding and recognize a
185 unique gp120 conformation formed by docking of the gp120 N-terminus as an 8th strand
186 to the β -sandwich to form an 8-stranded- β -sandwich structure [6]. The DH677.3 complex
187 crystals were obtained with gp120_{93TH057} core_e which lacks the N-terminus (Δ 11 aa
188 deletion) and therefore the direct judgment, based on structure, whether or not the 8th
189 strand is involved in binding was not possible (**Fig 3**). However, we were able to model
190 the N/C-termini-gp120_{93TH057} core_e from the N12-i3 Fab complex structure (PDB code:
191 5W4L) to the DH677.3 Fab-gp120_{93TH057} core_e-M48U1 complex without any steric

192 clashes (**Fig 4A, inset**). Both the conformation and orientation of CDR H1 and 2 of
193 DH677.3 allowed easy access to the 8-stranded- β -sandwich structure and enabled
194 contacts to the 8th strand. These data indicated that DH677.3 is capable of
195 accommodating both the 7 and 8-stranded- β -sandwich conformations of gp120 with
196 effective contacts to the 8th strand. Thus, the DH677.3 C1C2 antibody has a unique
197 binding angle to the C1C2 region compared to C1C2 antibodies C11 and A32.

198 **DH677 lineage antibodies mediate ADCC against CD4 downmodulated HIV-1**

199 **infected cells.** During natural infection the HIV-1 accessory protein Nef downregulates
200 CD4 expression on the surface of virus infected cells [12, 13]. Cell surface expressed
201 CD4 facilitates the exposure of CD4i Env epitopes – like C1C2 - by binding to co-
202 expressed cell surface Env [14]. The analyses of ADCC breadth was performed using
203 target cells infected with IMCs containing the *Renilla* luciferase (LucR) reporter gene,
204 which restricts Nef expression leading to incomplete CD4 downregulation [15].
205 Nevertheless, Vpu expression can compensate for Nef function and induce CD4
206 downregulation during the 72 hour incubation of the target cells before assays were
207 performed. To exclude any possible impact of this technical aspect of IMCs with LucR
208 on our ADCC results, full length IMCs (n=7) that do not contain a report gene were used
209 to evaluate ADCC of the affinity matured RV305 C1C2-specific antibodies DH677.3 and
210 DH677.4 and A32 [2], against target cells positive for intracellular p24 (p24+) and with
211 downregulated CD4 (CD4-). As clade CRF01_AE possess a histidine at Env HXB2
212 position 375 that influences sensitivity to CD4i antibody binding and ADCC [16, 17] only
213 clade B and clade C isolates were used.

214 When evaluating elimination of total p24+ cells no significant difference
215 (Wilcoxon rank sum test; $p > 0.05$) in specific killing was noted among the three different
216 antibodies (**Fig 5A**). However, when infected cells were separated into p24+CD4+ (**Fig**
217 **5B**) and p24+CD4- (**Fig 5C**) it was found that the RV305-boosted DH677.3 antibody
218 was significantly better (Wilcoxon rank sum test $p = 0.03$) at mediating ADCC against
219 p24+ CD4- infected cells (**Fig 5C**) when compared to A32. These data indicate that the
220 DH677 clonal lineage epitope was more frequently exposed on Env conformers on the
221 surface of IMC infected cells even in the context of CD4 downmodulation making this
222 epitope a highly desirable NNAb vaccine target and important consideration in the
223 setting of cure AIDS initiatives.

224 **DISCUSSION**

225 In this study it was found that late boosting of RV144 vaccinees increased C1C2-
226 specific antibody $V_H + V_L$ chain gene mutation frequency and increased clonal lineage
227 specific ADCC breadth and potency (**Table 1, Fig 2**). Most RV305 derived antibodies
228 had broader and more potent ADCC activity than the RV144 derived antibodies (**Table**
229 **1**) but V_H chain gene mutation frequency and the ADCC score did not directly correlate
230 (**Fig S1**). While RV305 was necessary to mature C1C2-specific antibody responses,
231 additional boosting with ALVAC/AIDSVAX B/E would not increase ADCC breadth and
232 potency. Likely rationally designed sequential [18] boosting immunogens to select for
233 critical mutations [19] that directionally affinity mature highly functional
234 ALVAC/AIDSVAX B/E-induced C1C2-specific antibodies are needed.

235 Improving vaccine-induced NNAb effector function will also require more detailed
236 immunological studies on the timing and frequency of boosting. In VAX003

237 (NCT00002441) and VAX004 (NCT00002441) trials frequent protein immunizations
238 skewed Env-specific antibody subclass usage from the highly functional IgG3 to IgG4
239 [20-22]. The RV305 boosts that were studied here occurred several years (6-8yrs) later,
240 unlike previous HIV-1 vaccine trials. Whether the boosting interval can be shortened
241 without skewing antibody subclass usage is not known, but it is possible that boosting
242 with long rest intervals ($\geq 1-2$ years) will be necessary.

243 The AIDSVAX B/E protein used for boosting in the RV144 and RV305 HIV-1
244 vaccine trial contained an N-terminal 11 amino acid deletion with important implications
245 for NNAb induction. Previously it was shown that this modification enhanced exposure
246 of the C1C2 region and V2 loop [9]. Here we show that this modification disrupts C11-
247 like antibody binding (**Fig 1**) but does creates a germline-targeting immunogen for
248 DH677-like B cell lineages (**Fig S2**). Ligand crystal structure analysis found that
249 DH677.3 recognized a unique C1C2 epitope that involves parts of epitope footprints of
250 C1C2 Cluster A antibodies A32 and N12-i3 (C11-like) as well as new elements of the
251 inner domain Layer 1 and the 7-stranded- β -sandwich (**Fig 3 and 4**). The DH677.3
252 epitope is positioned midway between the A32 and N12-i3 binding sites with most
253 residues being highly conserved. Interestingly, DH677.3 binds at the edge of the gp120
254 inner domain 7-stranded β -sandwich and with layers 1 and 2 with a binding mode that
255 allows it to accommodate the addition of the N-terminus as the 8th strand to the 7-
256 stranded- β -sandwich, a gp120 conformation emblematic of the late stages of HIV entry
257 and recognized by C11 and C11-like antibodies [6]. Most likely this feature allows
258 DH677.3 to recognize a broader range of Env targets, emerging in both the early (when
259 A32 epitope becomes available) and late stage (when C11 epitope becomes available)

260 of the viral entry process. Identification of a stage 2A of the HIV-1 Env expressed on
261 the surface of infected cells in presence of the CD4 molecule or CD4 mimetics reiterate
262 the importance of targeting these epitopes by vaccine induced responses as detected in
263 our assays [23]. In addition, a model of DH677.3 in complex with gp120 antigen bound
264 to a CD4 of a target/infected cell confirms that the recognition site and angle of
265 approach position the DH677.3 IgG for easy access for effector cell recognition and Fc-
266 effector complex formation (**Fig 4A**).

267 ADCC-mediating antibodies have been shown to reduce mother-to-child HIV-1
268 transmission [24-26], slow virus disease progression [26-28] and in RV144 correlated
269 with reduced risk of infection in vaccine recipients with lower anti-Env plasma IgA
270 responses [7]. Synergy between the RV144 C1C2 and V1V2 mAbs suggest a role for
271 the C1C2 plasma responses that could not be directly identify by the correlates of
272 protection study. Based on the data reported in this study and by Zoubchenok et al [16]
273 , it is clear that the magnitude of Env susceptibility to ADCC by the C1C2-specific Ab
274 responses is not consistent as suggested by the conserved sequence of this region and
275 varies according to the conformational stage of the HIV-1 envelope. That DH677.3 was
276 better than A32 at mediating ADCC against HIV-1 clade B and C CD4 down-modulated
277 cells (**Fig 5**) make this antibody an attractive candidate for targeting HIV-1 infected cells
278 *in vivo* in the setting of HIV-1 infection. We have previously shown that the C1C2
279 antibody A32 when formulated as a bi-specific antibody can potently opsonize and kill
280 HIV-1 infected CD4+ T cells [29]. Whether DH677.3-type of antibodies are superior to
281 A32 for targeting virus-infected cells remains to be determined.

282 In summary, our data demonstrate that if the RV144 vaccine trial had been
283 boosted, ADCC-mediating antibodies would have undergone affinity maturation for both
284 ADCC potency and breadth of recognition of HIV-1-infected CD4+ T cells. Rationally
285 designed subsequent boosting strategies to immunofocus IgG C1C2-specific response
286 towards DH677-like antibody specificities may be one method by which to provide
287 greater protection than observed in the RV144 HIV-1 vaccine trial.

288 **METHODS**

289 ***Ethics Statement.*** The RV305 clinical trial (NCT01435135) was a boost given to
290 162 RV144 clinical trial participants (NCT00223080) six-eight years after the conclusion
291 of RV144 [30]. Donors used in this study were from groups boosted either with
292 AIDSVAX B/E + ALVAC-HIV (vCP1521) (Group I) or AIDSVAX B/E alone (Group II).
293 The RV305 clinical trial (NCT01435135) received approvals from Walter Reed Army
294 Institute of Research, Thai Ministry of Public Health, Royal Thai Army Medical
295 Department, Faculty of Tropical Medicine, Mahidol University, Chulalongkorn University
296 Faculty of Medicine, and Siriraj Hospital. Written informed consent was obtained from all
297 clinical trial participants. The Duke University Health System Institutional Review Board
298 approved all human specimen handling.

299 ***Antigen-specific single-cell sorting.*** 1×10^7 peripheral blood mononuclear
300 cells (PBMCs) per vaccine-recipient were stained with AE.A244gp120 Δ 11 fluorescently
301 labelled proteins and a human B cell flow cytometry panel. Viable antigen-specific B
302 cells (AqVd- CD14- CD16- CD3- CD19+ IgD-) were single-cell sorted with a BD
303 FACS Aria II- SORP (BD Biosciences, Mountain View, CA) into 96 well PCR plates and
304 stored at -80°C for RT-PCR.

305 **Single-cell reverse transcriptase PCR.** Single B cell cDNA was generated with
306 random hexamers using SSIII. The antibody variable heavy and light chain variable
307 regions were PCR amplified using AmpliTaq360 Master Mix (Applied Biosystems). PCR
308 products were purified (Qiagen, Valencia, CA) and sequenced by Genewiz. Gene
309 rearrangements, clonal relatedness, unmutated common ancestors and intermediate
310 ancestor inferences were made using Cloanlyst [31]. DH677 clonal lineage tree was
311 generated using FigTree.

312 **Monoclonal antibody production.** PCR-amplified heavy and light chain gene
313 sequences were transiently expressed as previously described [32]. Ig containing cell
314 culture supernatants were used for ELISA binding assays. For large scale expression,
315 V_H and V_L chain genes were synthesized (V_H chain in the IgG1 4A backbone) and
316 transformed into DH5α cells (GeneScript, Piscataway, NJ). Plasmids were expressed in
317 Luria Broth, purified (Qiagen, Valencia, CA) and Expi293 cells were transfected using
318 ExpiFectamine™ (Life Technologies, Carlsbad, CA) following the manufacturers
319 protocol. After five days of incubation at 37°C 5% CO₂ the Ig containing media was
320 concentrated, purified with Protein A beads and the antibody buffer exchanged into
321 PBS.

322 **Antibody binding and blocking assays.** Direct ELISAs were performed as
323 previously described [32]. Briefly, 384-well microplates were coated overnight with
324 30ng/well of protein. Antibodies were diluted and add for one hour. Binding was
325 detected with an anti-IgG-HRP (Rockland) and developed with SureBlue Reserve TMB
326 One Component (KPL). Plates were read on a plate reader (Molecular Devices) at

327 450nm. A32-blocking assays were performed by adding the RV305 antibodies followed
328 by biotinylated A32 and detecting with streptavidin HRP.

329 **Neutralization assays.** TZM-bl neutralization assays were performed in the
330 Montefiori lab as previously described [33]. Data are reported as IC₅₀ titers for
331 antibodies.

332 **Infectious molecular clones (IMC).** The HIV-1 reporter virus used were
333 replication-competent IMC designed to encode the *env* genes of CM235 (subtype A/E;
334 GenBank No. AF259954.1), WITO (subtype B; GeneBank No. JN944948), 1086.c
335 (subtype C; GeneBank No. FJ444395), TV-1 (subtype C; GeneBank No. HM215437),
336 MW96.5 (subtype C; GeneBank No.), DU151 (subtype C; GeneBank No. DQ411851),
337 DU422 (subtype C; GeneBank No. DQ411854) in *cis* within an Nef deficient isogenic
338 backbone that expresses the *Renilla* luciferase reporter gene [34]. The subtype AE
339 Env-IMC-LucR viruses used were the NL-LucR.T2A-AE.CM235-ecto (IMC_{CM235})
340 (plasmid provided by Dr. Jerome Kim, US Military HIV Research Program), and clinical
341 *env* isolates from the RV144 trial that were built on the 40061-LucR virus backbone. All
342 the other IMCs were built using the original NL-LucR.T2A-ENV.ecto backbone as
343 originally described by [35] Reporter virus stocks were generated by transfection of
344 293T cells with proviral IMC plasmid DNA, and virus titer was determined on TZM-bl
345 cells for quality control [35]

346 **Infection of CEM.NKR_{CCR5} cell line with HIV-1 IMCs.** CEM.NKR_{CCR5} cells were
347 infected with HIV-1 IMCs as previously described [36]. Briefly, IMCs were titrated in
348 order to achieve maximum expression within 48-72 hours post-infection as determined
349 by detection of Luciferase activity and intra-cellular p24 expression. IMC infections

350 were performed by incubation of the optimal dilution of virus with CEM.NKR_{CCR5} cells for
351 0.5 hour at 37°C and 5% CO₂ in presence of DEAE-Dextran (7.5 µg/ml). The cells were
352 subsequently resuspended at 0.5x10⁶/ml and cultured for 48-72 hours in complete
353 medium containing 7.5µg/ml DEAE-Dextran. For each ADCC assay, we monitored the
354 frequency infected target cells by intracellular p24 staining. Assays performed using
355 infected target cells were considered reliable if cell viability was ≥60% and the
356 percentage of viable p24⁺ target cells on assay day was ≥20%.

357 ***Luciferase ADCC Assay.*** ADCC activity was determined by a luciferase (Luc)-
358 based assay as previously described [8, 37] Briefly, CEM.NKR_{CCR5} cells (NIH AIDS
359 Reagent Program, Division of AIDS, NIAID, NIH from Dr. Alexandra Trkola) [38] were
360 used as targets after infection with the HIV-1 IMCs. PBMC obtained from a HIV-
361 seronegative donor with the heterozygous 158F/V and 131H/R genotypes for Fc_γR3A
362 and Fc_γR2A [39, 40], respectively, were used as a source of effector cells, and were
363 used at an effector to target ratio of 30:1. Recombinant mAbs were tested across a
364 range of concentrations using 5-fold serial dilutions starting at 50 µg/mL. The effector
365 cells, target cells, and Ab dilutions were plated in opaque 96-well half area plates and
366 were incubated for 6 hours at 37°C in 5% CO₂. The final read-out was the luminescence
367 intensity (relative light units, RLU) generated by the presence of residual intact target
368 cells that have not been lysed by the effector population in the presence of ADCC-
369 mediating mAb (ViviRen substrate, Promega, Madison, WI). The % of specific killing
370 was calculated using the formula: percent specific killing = [(number of RLU of target
371 and effector well – number of RLU of test well)/number of RLU of target and effector
372 well] ×100. In this analysis, the RLU of the target plus effector wells represents

373 spontaneous lysis in absence of any source of Ab. The ADCC endpoint concentration
374 (EC), defined as the lowest concentration of mAb capable of mediating ADCC in our in
375 vitro assay, was calculated by interpolation of the mAb concentration that intersected
376 the positive cutoff of 15% specific killing. The RSV-specific mAb Palivizumab was used
377 as a negative control.

378 **ADCC Score.** Antibodies were tested across a range of concentrations using 5-fold serial
379 dilutions starting at 50 µg/mL. Since the dilution curves are not monotonic due to pro-
380 zone effect of mAbs, non-parametric area under the curve (AUC) was calculated using
381 trapezoidal rule with activity less than 15% set to 0 %. For calculating a weighted average
382 to obtain a score for ADCC activity explaining both potency and breadth of the mAbs, in
383 this study we have used Principal Component Analysis (PCA) to compute an ADCC
384 score. PCA is the most commonly used method to reduce the dimensionality of the data
385 set [41]. It uses Eigen vector decomposition of the correlation matrix of the variables,
386 where each variable is represented by a viral isolate. Most of the shared variance of the
387 correlations of ADCC AUC is explained by first principal component (PC1) [42]. Ideally,
388 one would want to explain 70% of the variance but should not be at the expense of adding
389 principal components with an Eigenvalue less than 1 [43].

390 In this study we a panel of 7 HIV isolates was tested which implies that our data set has
391 seven dimensions. ADCC activity was measured as AUC . In our analysis PC1 and PC2
392 have Eigen values above 1 and together account for 80.57% variance (**Table S5**). Scores
393 obtained from the first Principal Component can be interpreted as weighted average of
394 the 7 isolates that would account for both potency as well as breadth of the mAbs [43].
395 Higher PC1 score would mean that mAb has a higher breadth as well as potency for

396 ADCC activity. To calculate the ADCC score, the standardized AUC value for each
397 monoclonal antibody is calculated for each viral isolate, multiplied by factor loading of the
398 given viral isolate and then these products are added together. Standardized AUC values
399 imply zero mean and unit standard deviation. The AUC values below the value of mean
400 AUC will result in negative PC1 scores.

401 ***Infection of primary cells with HIV-1 IMCs.*** Infectious molecular clones
402 encoding the full-length transmitted/founder sequence of seven individuals infected with
403 either subtype B or C viruses from the CHAVI acute infection cohort (CH77, CH264,
404 CH0470, CH042, CH185, CH162 and CH236) were constructed as previously described
405 [44, 45] and used to infect primary CD4+ cells. To infect cells, cryopreserved peripheral
406 blood mononuclear cells (PBMCs) were thawed and stimulated in R20 media (RPMI
407 media (Invitrogen) with 20% Fetal Bovine Serum (Gemini Bioproducts), 2mM L-
408 glutamine (Invitrogen), 50 U /mL penicillin (Invitrogen), and 50 µg/mL Gentamicin
409 (Invitrogen)) supplemented with IL-2 (30U/mL, Proleukin), anti-CD3 (25ng/mL clone
410 OKT-3, Invitrogen) and anti-CD28 (25ng/mL, BD Biosciences) antibodies for 72 hours at
411 37°C in 5% CO₂. CD8 cells were depleted from the PBMCs using CD8 microbeads
412 (Miltenyi Biotec, Germany) according to the Manufacturer's instructions and 1.5 x 10⁶
413 cells were infected using 1 mL virus supernatant by spinoculation (1125 x g) for 2 hours
414 at 20 °C. After spinoculation, 2 mL of R20 supplemented with IL-2 was added to each
415 infection and infections were left for 72 hours. Infected cells were used if viability was
416 >70% and more than 5% of cells were p24+.

417 **Infected Cell Elimination Assay.** HIV-1-infected or mock-infected CD8-depleted

418 PBMCs cells were used as targets and autologous cryo-preserved PBMCs rested
419 overnight in R10 supplemented with 10ng/ml of IL-15 (Miltenyi Biotec) were used as a
420 source of effector cells. Infected and uninfected target cells were labelled with a
421 fluorescent target-cell marker (TFL4; OncoImmunin) and a viability marker (NFL1;
422 OncoImmunin) for 15 min at 37 °C, as specified by manufacturer. The labeling of the
423 target cells with these two markers allowed to clearly identify only the live viable cells in
424 our gating strategy and exclude artifacts related to the presence of dead cells staining.
425 Cells were washed in R10 and adjusted to a concentration of 0.2×10^6 cells/mL. PBMCs
426 were then added to target cells at an effector/target ratio of 30:1 (6×10^6 cells/mL). The
427 target/effector cell suspension was plated in V-bottom 96-well plates and co-cultured
428 with 10 µg/mL of each mAb. Co-cultures were incubated for 6 h at 37 °C in 5% CO₂.
429 After the incubation period, cells were washed and stained with anti-CD4-PerCP-Cy5.5
430 (eBioscience, clone OKT4) at a final dilution of 1:40 in the dark for 20 min at room
431 temperature (RT). Cells were then washed, resuspended in 100 µL/well
432 Cytofix/Cytoperm (BD Biosciences), incubated in the dark for 20 min at 4 °C, washed in
433 1x Cytoperm wash solution (BD Biosciences) and co-incubated with anti-p24 antibody
434 (clone KC57-RD1; Beckman Coulter) to a final dilution of 1:100, and incubated in the
435 dark for 25 min at 4 °C. Cells were washed three times with Cytoperm wash solution
436 and resuspended in 125 µL PBS-1% paraformaldehyde. The samples were acquired
437 within 24 h using a BD Fortessa cytometer. The appropriate compensation beads were
438 used to compensate the spill over signal for the four fluorophores. Data analysis was
439 performed using FlowJo 9.6.6 software (TreeStar). Mock-infected cells were used to
440 appropriately position live cell p24^{+/-} and CD4^{+/-} gates.

441 Specific killing was determined by the reduction in % of viable p24+ cells in the
442 presence of mAbs after taking into consideration non-specific killing, and was calculated
443 as:

$$444 \frac{\text{p24\% (target + effector cells)} - \text{p24\% (targets + effectors + mAb/plasma)}}{\text{p24\% (target + effector cells)}}$$

445 CH65 (an anti-influenza monoclonal antibody, kindly provided by Dr. Moody) was used
446 as negative control. To remove background signal, the highest value of percent specific
447 killing induced by CH65 was subtracted from the calculated reduction in % of p24+ cells
448 and then negative values were rounded to 0%.

449 **Surface plasmon resonance** – The binding and kinetic rates measurement of gp120
450 proteins against RV305 mAbs were obtained by surface plasmon resonance (SPR)
451 using the Biacore 3000 instrument (GE Healthcare). SPR measurements were
452 performed using a CM5 sensor chip with anti-human IgG Fc antibody directly
453 immobilized to a level of 9000-11000RU (response unit). Antibodies were then
454 captured at 5ul/min for 60s to a level of 100-300RU. For binding analyses, the gp120
455 proteins were diluted to approximately 1000nM in PBS and injected over the captured
456 antibodies for 3 minutes at 30ul/min. For kinetics measurements, the gp120 proteins
457 were diluted from 5-750nM and injected using a high performance kinetics injection for 5
458 minutes at 50uL/min. This was followed by a dissociation period of 600s and surface
459 regeneration with Glycine pH2.0 for 20s. Results were analyzed using the Biacore
460 BiaEvaluation Software (GE Healthcare). Negative control antibody (Ab82) and blank
461 buffer binding were used for double reference subtraction to account for non-specific
462 protein binding and signal drift. Subsequent curve fitting analysis was performed using a

463 1:1 Langmuir model with a local Rmax and the reported rate constants are
464 representative of two measurements.

465 ***Protein preparation and complex crystallization*** DH677.3 Fab alone was grown and
466 crystallized at concentration ~10 mg/ml. The structure was solved by molecular
467 replacement with PDB ID 3QEG in space group P2₁ to a resolution of 2.6 Å. Clade A/E
468 93TH057 gp120 core_e, (gp120_{93TH057} core_e, residues 42-492 (Hxbc2 numbering)), lacking
469 the V1, V2 and V3 variable loops and containing a H375S mutation to allow binding of
470 the CD4 mimetic M48U1 [46] was used to obtain crystals of DH677.3 Fab-antigen
471 complex. gp120_{93TH057} core_e was prepared and purified as described in [3].
472 Deglycosylated gp120_{93TH057} core_e was first mixed with CD4 mimetic peptide M48U1 at a
473 molar ratio of 1:1.5 and purified through gel filtration chromatography using a Superdex
474 200 16/60 column (GE Healthcare, Piscataway, NJ). After concentration, the gp120_{93TH057}
475 core_e-M48U1 complex was mixed with a 20% molar excess of DH677.3 Fab and passed
476 again through the gel filtration column equilibrated with 5 mM Tris-HCl buffer pH 7.2 and
477 100 mM ammonium acetate. The purified complex was concentrated to ~10 mg/ml for
478 crystallization experiments. The structure was solved by molecular replacement using
479 the DH677.3 Fab and PDB ID 3TGT as searching models in space group P1 to a
480 resolution 3.0 Å. The final R_{factor}/R_{free} (%) for the Fab structure is 19.9/26.1 and the final
481 R_{factor}/R_{free} for the complex is 21.4/27.4 (**Table S3**). The PDB IDs for the deposited
482 structures are 6MFJ and 6MFP respectively. In each case the asymmetric unit of the
483 crystal contained two almost identical copies of Fab or the Fab- gp120_{93TH057} core_e
484 complex (**Fig S2**)

485 **Crystallization and Data collection** Initial crystal screens were done in vapor-diffusion
486 hanging drop trials using commercially available sparse matrix crystallization screens
487 from Hampton Research (Index), Emerald BioSystems (Precipitant Wizard Screen) and
488 Molecular Dimensions (Proplex and Macrosol Screens). The screens were monitored
489 periodically for protein crystals. Conditions that produced crystals were then further
490 optimized to produce crystals suitable for data collection. DH677.3 Fab crystals were
491 grown from 20% PEG 3000, 100 mM HEPES pH 7.5, and 200 mM sodium chloride.
492 DH677.3 complex crystals were grown from 25% PEG 4000 and 100 mM MES pH 5.5.
493 Crystals were briefly soaked in crystallization solution plus 20% MPD before being flash
494 frozen in liquid nitrogen prior to data collection.

495 **Data collection and structure solution** Diffraction data were collected at the Stanford
496 Synchrotron Radiation Light Source (SSRL) at beam line BL12-2 equipped with a Dectris
497 Pilatus area detector. All data were processed and reduced with HKL2000 [47].
498 Structures were solved by molecular replacement with Phaser [48] from the CCP4 suite
499 [49]. The DH677.3 Fab structure was solved based on the coordinates of the N12-i2 Fab
500 (PDB: 3QEG), and the DH677.3 complex was then solved with coordinates from the
501 DH677.3 Fab model, gp120 (PDB: 3TGT), and M48U1 (PDB: 4JZW). Refinement was
502 carried out with Refmac [50] and/or Phenix [51]. Refinement was coupled with manual
503 refitting and rebuilding with COOT [52]. Data collection and refinement statistics are
504 shown in **Table 1**.

505 **Structure validation and analysis** The quality of the final refined models was
506 monitored using the program MolProbity [53]. Structural alignments were performed
507 using the program lsqkab from the CCP4 suite [49]. The PISA [54] webserver was used

508 to determine contact surfaces and residues. All illustrations were prepared with the
509 PyMol Molecular Graphic suite (<http://pymol.org>) (DeLano Scientific, San Carlos, CA,
510 USA). Conservation of the DH677.3 epitope was calculated using the HIV Sequence
511 Database Compendium
512 (<https://www.hiv.lanl.gov/content/sequence/HIV/COMPENDIUM/compendium.html>)
513 comparing gp120 residues relative to Clade B Hxhc2. Only unique sequences in the
514 database having an equivalent residue at each position were included in the calculated
515 percentage representing approximately 32,000 sequences on average.

516 **Statistical Methods** For luciferase based ADCC assay background correction was
517 performed by subtracting the highest value of percent specific killing induced by CH65
518 and then rounding off the negative values to zero. In order to assess if two groups have
519 different response pairwise comparisons between groups was conducted using
520 Wilcoxon rank sum test. Statistical analysis was performed using SAS software (SAS
521 Institute Inc., Cary, N.C.).

522

523 **FIGURE LEGENDS**

524 **Figure 1. Identification of RV305 C1C2-specific antibodies.** (A) The C1C2-specific
525 antibodies A32 or C11 were assayed by direct binding ELISA for reactivity with full
526 length AE.A244gp120 or AE.A244g120 Δ 11. An A32-specific mutant protein was
527 designed (AE.A244g120 Δ 11 F35S H72L V75A E106K D107H S110A Q114L) to identify
528 A32-like antibody responses. 19B antibody was used as a positive control and CH65 as
529 a negative control. (B) RV305 non-neutralizing antibodies were assayed for A32-
530 blocking by ELISA. (C) RV305 non-neutralizing A32-blockable antibody heavy and light

531 chain gene sequence mutation frequencies were analyzed by Cloanalyst (Kepler et al.,
532 2014) and compared to previously published RV144 heavy and light chain gene
533 sequence mutation frequencies (% nucleotide) (Bonsignori et al., 2012). Statistical
534 significance was determined using a Wilcoxon rank sum test. Red bar represents that
535 mean (D) RV305 non-neutralizing A32-blockable antibody were assayed by direct
536 binding ELISA to AE.A244g120Δ11 and AE.A244g120Δ11 F35S H72L V75A E106K
537 D107H S110A Q114L. Data are expressed as % binding the mutant protein relative to
538 WT. Shown are the mean with standard deviation of two independent experiments.

539 **Figure 2. RV305 boosting increased the apparent affinity and antibody dependent**
540 **cellular cytotoxicity breadth and potency of the C1C2-specific RV144 derived**
541 **DH677 memory B cell clonal lineage.** DH677.1 antibody was isolated by antigen-
542 specific single-cell sorting PBMC from the RV144 vaccine trial. DH677.2, DH677.3 and
543 DH677.4 were isolated by antigen-specific single-cell sorting PBMC collected after the
544 second boost AIDSVAX B/E (RV305 Group II) given in RV305 (~7yrs later). The
545 intermediate ancestors and unmutated common ancestor was inferred using Clonalyst
546 [31]. Recombinantly expressed antibodies were assayed by biolayer interferometry for
547 binding to the AIDSVAX B/E proteins - AE.A244g120Δ11 + B.MNg120Δ11- and for
548 antibody dependent cellular cytotoxicity (ADCC) against AE.C235, B.WITO, C.TV-1,
549 C.MW965, C.1086C, C.DU151 and C.DU422 Renilla luciferase reporter gene infectious
550 molecular clone infected cells. An ADCC score (see methods) was used to account for
551 breadth and potency.

552 **Figure 3. Crystal structure of the DH677.3 Fab-gp120_{93TH057} core_e-M48U1 complex.**
553 (A) The overall structure of the complex is shown as a ribbon diagram (left) and with the

554 molecular surface displayed over the Fab molecule (middle), colored based on
555 electrostatic charge, red negative and blue positive. The gp120 outer domain is gray
556 and inner domain colored to indicate inner domain mobile layer 1 (yellow), 2 (cyan), 3
557 (light orange) and the 7-stranded b-sandwich (magenta). Complementary determining
558 regions (CDRs) are colored: CDR H1 (light blue), CDR H2 (dark green), CDR H3
559 (black), CRL1 (light green), CDR L2 (blue) and CDRL3 (brown). A blow-up view shows
560 the network of hydrogen (H) bonds formed at the Fab-gp120 interface. H-bonds
561 contributed by side chain and main chain atoms of gp120 residues are colored in
562 magenta and blue, respectively. **(B)** Antibody buried surface area (BSA) and gp120
563 residues forming DH677.3 epitope are shaded in blue according to BSA (antibody) and
564 percent conservation of gp120 residues (Env). gp120 main chain (blue) and side chain
565 (red) hydrogen bonds (H) and salt bridges (S) are shown above the residue. **(C)** The
566 DH677.3 Fab-gp120_{93TH057} core_e interface. CDRs are shown as ribbons (left) and balls-
567 and-sticks of residues contributing the binding (right) over the gp120 core. The
568 molecular surface of gp120 is colored as in **(A)** (left) and by electrostatic potential
569 (right).

570 **Figure 4. Recognition of HIV-1 Env by DH677.3 and other Cluster A antibodies. (A)**

571 The overlay of DH677.3 and Cluster A antibodies A32 and N12-i3 (C11-like) bound to
572 the gp120 core. Crystals structures of the gp120 antigen in complex with the Fab of
573 DH677.3, A32 (PDB code 4YC2) and N12-i3 (PDB code 5W4L) were superimposed
574 based on gp120. The d1 and d2 domains of the target cell receptor CD4 was added to
575 replace peptide mimetic M48U1 of the DH677.3 Fab-gp120_{93TH057} core_e-M48U1
576 complex. Molecular surfaces are displayed over Fab molecules and colored in lighter

577 and darker shades of brown, blue and green for the heavy and light chains of DH677.3,
578 A32 and N12-i3, respectively. A blow up view shows details of the DH677.3 interaction
579 with the 8-stranded β -sandwich of the gp120 inner domain. The 8th strand (colored in
580 blue) formed by the 11 N-terminal residues of gp120 in the N12-i3 bound conformation
581 (PDB: 5W4L) was modeled into the DH677.3 Fab-gp120_{93TH057} core_e-M48U1 complex.
582 CHR H1 and 2 of DH677.3 are colored light blue and dark green, respectively. **(B)** and
583 **(C)** Comparison of DH677.3, A32 and N12-i3 epitope footprints. In **(B)** the DH677.3
584 epitope footprint (shown in red) is plotted on the gp120 surface with layers colored as in
585 Figure 1 with the A32 and N12-i2 epitope footprints shown in black. In **(C)** the DH677.3,
586 A32 and N12-i3 gp120 contact residues are mapped onto the gp120 sequence. Side
587 chain (+) and main chain (-) contact residues are colored green for hydrophobic, blue for
588 hydrophilic and black for both as determined by a 5 Å cut off value over the
589 corresponding sequence. Buried surface residues as determined by PISA are shaded.
590 The DH677.3 epitope footprint overlays with the epitopes of both A32 and N12-i3.

591 **Figure 5. RV305 derived C1C2-specific antibody DH677.3 is significantly better**
592 **than A32 at mediating antibody-dependent cellular cytotoxicity against CD4 down**
593 **modulated infectious molecular clone infected cells.** Cells were infected with clade
594 B and clade C full length infectious molecular clones (IMC) that do not contain a
595 reporter gene. Surface CD4 expression was analyzed by flow cytometry and p24
596 expression was measured in live/viable (A) all p24+ (B) p24+ CD4+ and (C) p24+ CD4-
597 IMC infected cell populations. Data are shown with the mean and standard deviation.

598 **Table1: Ranking C1C2-specific antibodies by ADCC breadth and potency.** RV305
599 and RV144 C1C2-specific antibodies were assayed for antibody-dependent cellular

600 cytotoxicity against AE.CM235, B.WITO, C.TV-1, C.MW965, C.1086C, C.DU151 and
601 C.DU422 infectious molecular clone infected cells. Antibodies were ranked using an
602 ADCC Score that accounts for breadth and potency (see methods). Number of strains
603 recognized was determined by ADCC endpoint concentration > 40µg/mL.

604 **Figure S1. RV305 C1C2-specific antibody ADCC score inversely correlate with**
605 **antibody mutation frequency.** Correlation between RV305 antibody heavy chain
606 gene mutation frequency (% nucleotide; Cloanlyst) [31] and ADCC score (see
607 methods) was calculated with SAS (Spearman Correlation = -0.5599; p value = 0.0127).

608 **Figure S2. RV305 boosting increased affinity of the C1C2-specific RV144 derived**
609 **DH677 memory B cell clonal lineage to the AIDSVAX B/E proteins.** DH677.1
610 antibody was isolated by antigen-specific single-cell sorting PBMC from the RV144
611 vaccine trial. DH677.2, DH677.3 and DH677.4 were isolated by antigen-specific single-
612 cell sorting PBMC collected after the second boost AIDSVAX B/E (RV305 Group II)
613 given in RV305 (~7yrs later). The intermediate ancestors and unmutated common
614 ancestor was inferred using Clonalayst [31]. Recombinantly expressed antibodies were
615 assayed by surface plasmon resonance for binding to the AIDSVAX B/E proteins -
616 AE.A244g120Δ11 + B.MNg120Δ11- and to full length AE.A244gp120.

617 **Figure S3. Boosting in RV305 increased DH677 B cell clonal lineage cross-clade**
618 **antibody-dependent cellular cytotoxicity breadth and potency.** Recombinantly
619 expressed DH677 clonal lineage members were assayed for antibody-dependent
620 cellular cytotoxicity (ADCC) against AE.CM235, B.WITO, C.TV-1, C.MW965, C.1086C,
621 C.DU151 and C.DU422 Renilla luciferase reporter gene infectious molecular clone

622 infected cells. Data are shown as radar plots with an ADCC score (see methods) that
623 accounts for ADCC breadth and potency.

624 **Figure S4. Comparison of the two copies of the DH677.3 Fab-gp120_{93TH057} core-**
625 **M48U1 complex and the two Fab copies in the apo Fab structure from the**
626 **asymmetric unit of crystals. (A)** The root mean square deviation (RMSD) between
627 complex copies is 0.946 Å for main chain residues. **(B)** The RMSD between the Fab
628 copies in the apo Fab structure is 0.540 Å for main chain residues. **(C)** Comparison of
629 the free and bound DH677.3 Fab. The α -carbon backbone diagram of superposition of
630 the structures of DH677.3 Fab alone (dark cyan-heavy chain and light cyan-light chain)
631 and N5-i5 Fab bound to CD4-triggered gp120 (dark brown-heavy chain and light brown-
632 light chain). The average RMSD between free and bound Fabs is 0.818 Å for main
633 chain residues.

634 **Figure S5. Antibody contact residues.** mAb side chain (+) and main chain (-) contact
635 residues colored green for hydrophobic, blue for hydrophilic and black for both as
636 determined by a 5 Å cut off value over the corresponding sequence. CDRs are colored
637 as in Figure 1 and buried surface residues as determined by PISA are shaded.

638 **Table S1. Immunogenetics of non-neutralizing A32-blocking RV305 C1C2-specific**
639 **antibodies.** RT-PCR amplified variable heavy and variable light chain genes were
640 Sanger sequenced (Genewiz) and analyzed with Cloanalyst [31].

641 **Table S2. A32-blocking antibodies do not neutralize HIV-1.** (A) Recombinantly
642 expressed antibodies were assayed in the TZM-bl neutralization assay against

643 autologous and heterologous Tier 1 and Tier 2 isolates. No neutralization was detected.
644 Data are shown as EC50 ($\mu\text{g}/\text{mL}$)

645 **Table S3. DH677.3 structural data collection and refinement statistics**

646 **Table S4. Details of the DH677.3, A32, and N12-i3 interfaces based on the**
647 **DH677.3-gp120_{93TH057}core_e-M48U1, A32 Fab-ID2_{93TH057}, and N12-i3 Fab-**
648 **gp120_{93TH057}core_e+N/C-M48U1 structures** as calculated by the EBI PISA server
649 (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver). The two copies in the
650 asymmetric unit of the DH677.3, A32, and N12-i3 complexes are averaged in the table.

651

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678

679 **DECLARATION OF INTEREST**

680

681 B.F.H., G.F. and D.E. have patents submitted on antibodies listed in this paper.

682

683 **DISCLAIMER**

684

685 The views expressed are those of the authors and should not be construed to represent
686 the positions of the Uniformed Services University, U.S. Army, Department of Defense
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689

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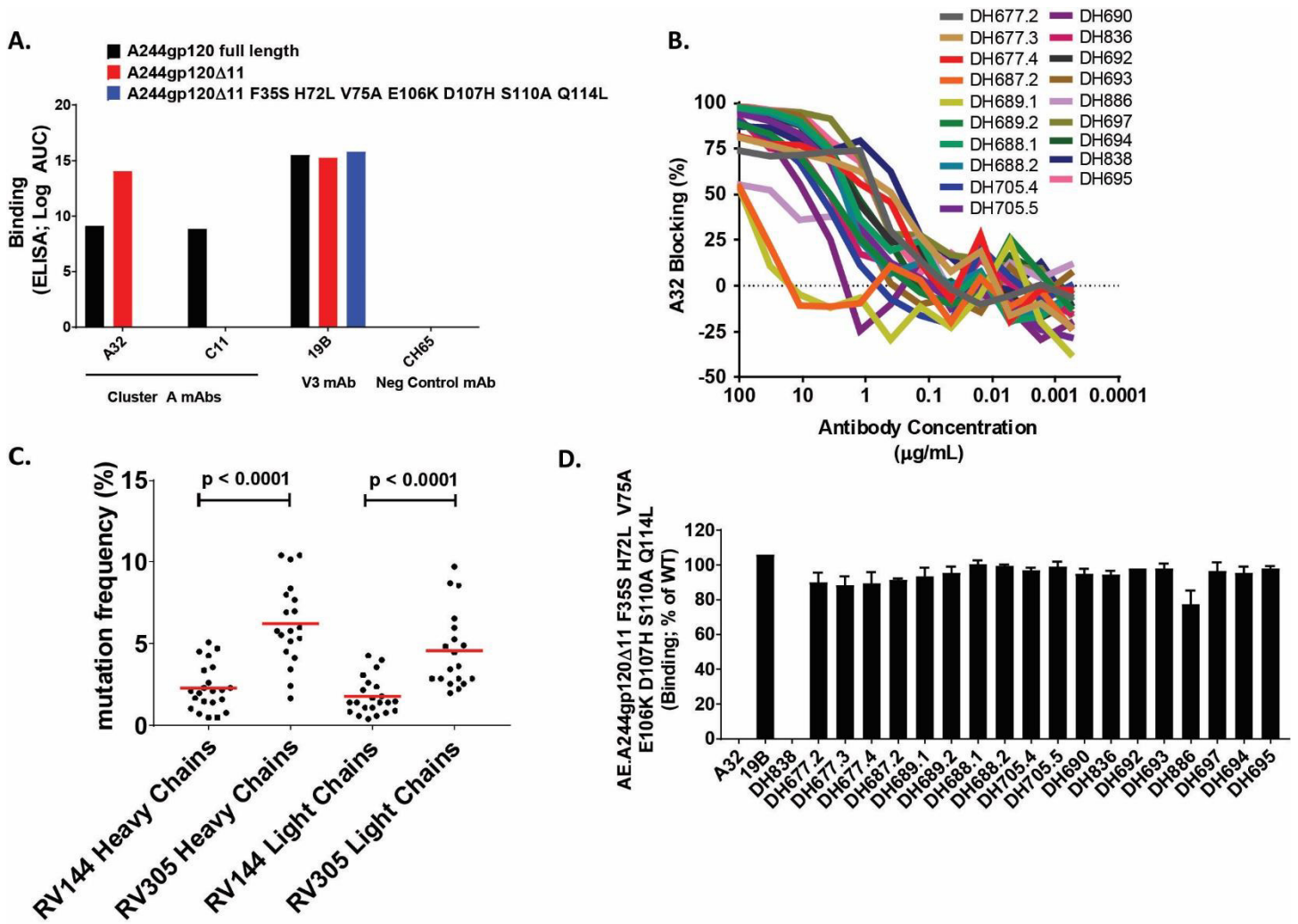


Figure 1.

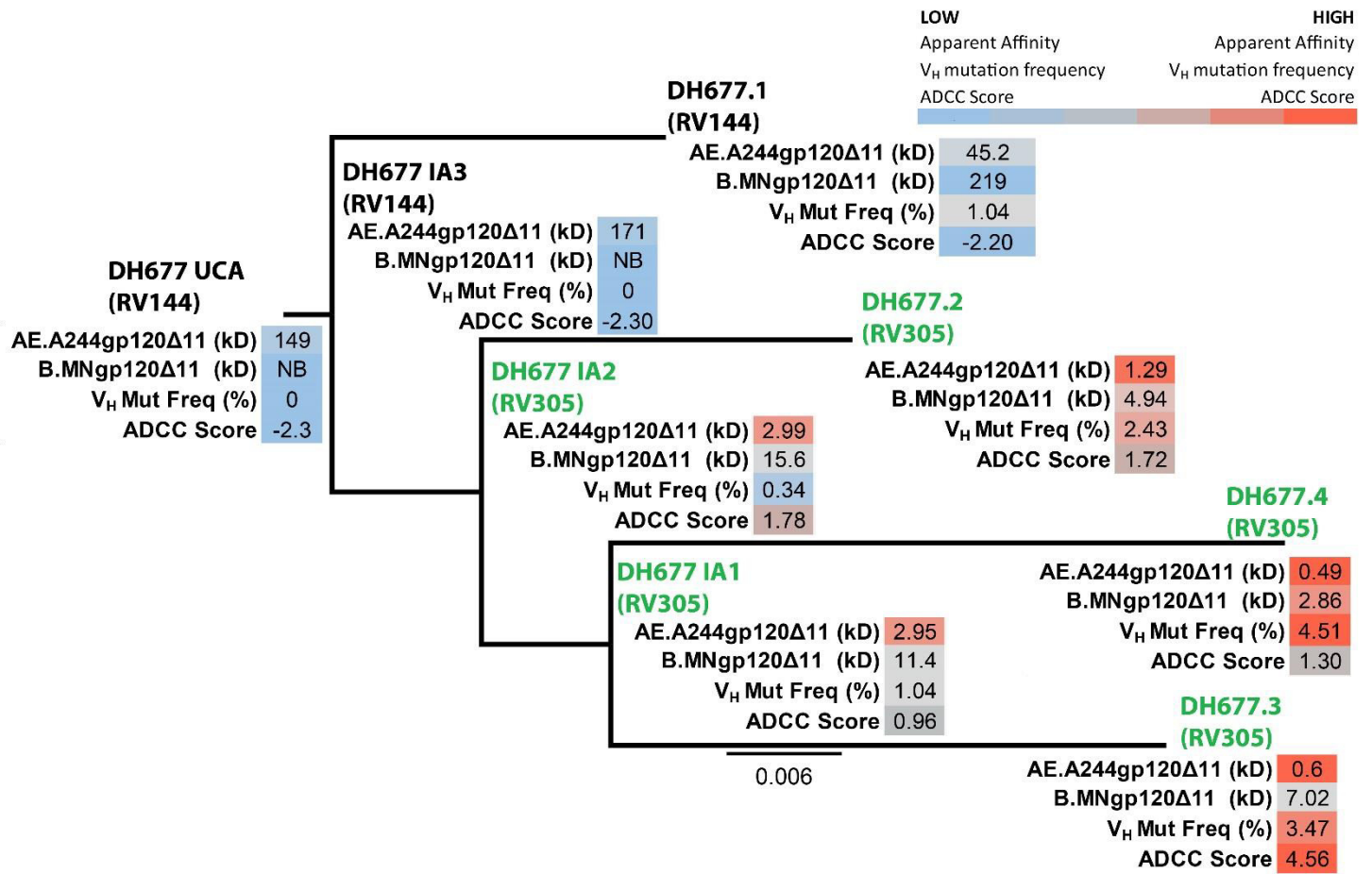


Figure 2.

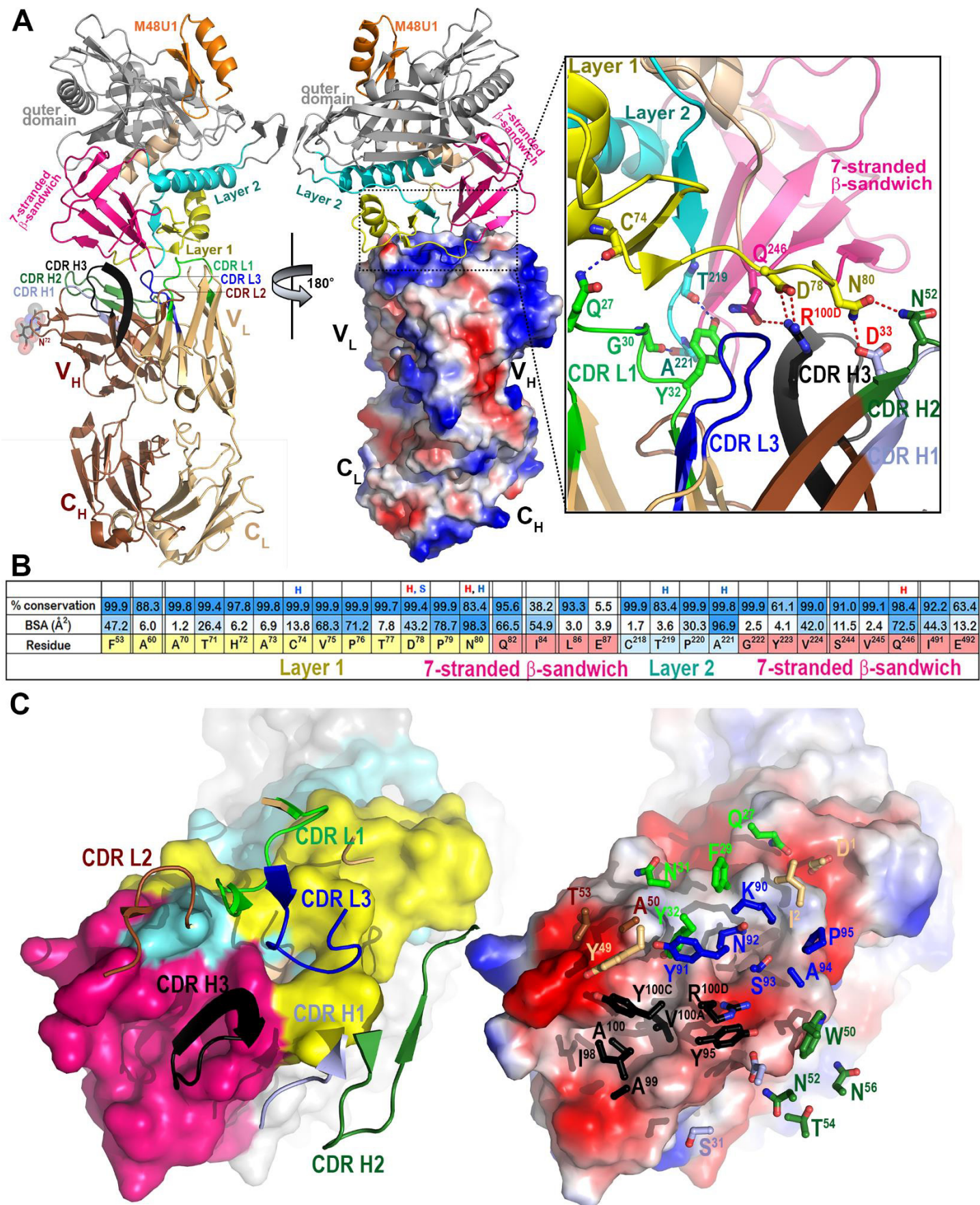


Figure 3.

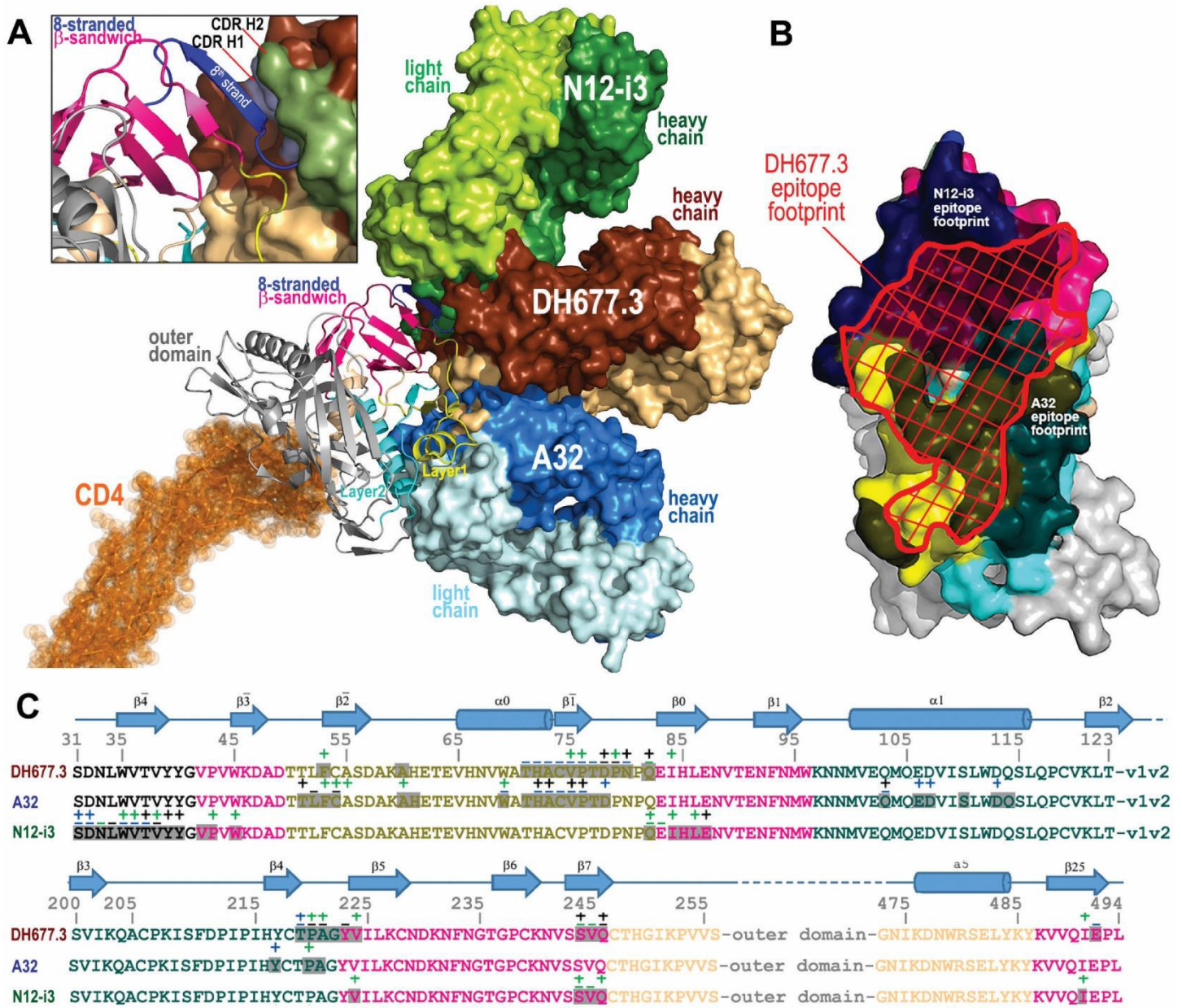


Figure 4.

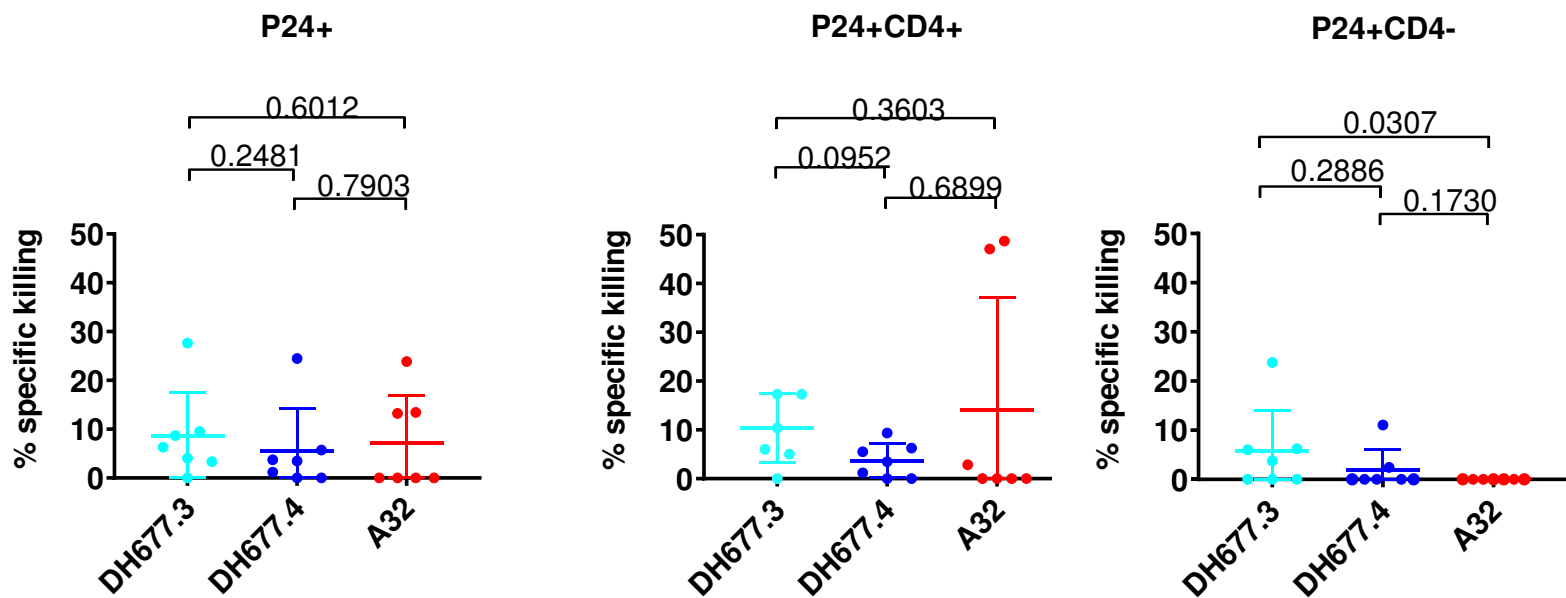


Figure 5.

Table1: Ranking C1C2-specific antibodies by ADCC breadth and potency

Rank	Antibody	Study	Score	Average AUC	Number of strains recognized
1	A32		6.62	198.70	7
2	CH38	RV144	6.28	186.56	6
3	DH677.3	RV305	4.56	146.90	7
4	DH697	RV305	2.70	111.98	7
5	DH677.2	RV305	1.72	111.76	5
6	DH838	RV305	1.62	107.24	4
7	DH677.4	RV305	1.30	94.00	6
8	DH695	RV305	0.86	82.94	7
9	DH688.1	RV305	0.66	77.94	6
10	DH694	RV305	0.58	80.18	5
11	DH705.5	RV305	0.52	75.84	5
12	DH705.4	RV305	0.46	75.46	6
13	DH690	RV305	0.36	67.44	7
14	DH692	RV305	0.08	65.14	6
15	DH693	RV305	0.06	64.54	7
16	DH886	RV305	-0.08	67.96	5
17	DH688.2	RV305	-0.30	60.42	5
18	DH836	RV305	-0.32	62.52	5
19	CH57	RV144	-0.48	53.22	5
20	CH90	RV144	-1.06	38.98	5
21	CH54	RV144	-1.20	34.58	5
22	DH689.2	RV305	-1.42	24.54	6
23	DH689.1	RV305	-1.68	26.48	6
24	DH677.1	RV144	-2.20	16.38	2
25	DH687.2	RV305	-2.70	0.16	2