

Vaccine induction of CD4-mimicking broadly neutralizing antibody precursors in macaques

3
4
5 Kevin O. Saunders^{1,2,3,4,14}, James Counts^{1,5}, Victoria Stalls^{1,5}, Robert Edwards^{1,5}, Kartik
6 Manne^{1,5}, Xiaozhi Lu^{1,5}, Bhishem Thakur^{1,5}, Katayoun Mansouri^{1,5}, Yue Chen^{1,5}, Rob Parks^{1,5},
7 Maggie Barr^{1,5}, Laura Sutherland^{1,5}, Joena Bal^{1,5}, Nicholas Havill^{1,6}, Haiyan Chen^{1,5}, Emily
8 Machiele^{1,5}, Nolan Jamieson^{1,5}, Bhavna Hora^{1,5}, Megan Kopp^{1,5}, Katarzyna Janowska^{1,5}, Kara
9 Anasti^{1,5}, Chuancang Jiang^{1,5}, Sravani Venkatayogi^{1,5}, Amanda Eaton^{1,5}, Rory Henderson^{1,5},
10 Christopher Barbosa⁷, S. Munir Alam^{1,5}, Sampa Santra⁸, Drew Weissman^{9,10}, M. Anthony
11 Moody^{1,11}, Derek W. Cain^{1,5}, Ying Tam⁷, Mark Lewis¹², Wilton B. Williams^{1,2,3}, Kevin Wiehe^{1,5},
12 David Montefiori^{1,2}, Priyamvada Acharya^{1,2,13}, Barton F. Haynes^{1,3,4,5},

13
14 ¹Duke Human Vaccine Institute, Duke University School of Medicine, Durham, NC 27710,
15 USA

16 ²Department of Surgery, Duke University School of Medicine, Durham, NC 27710, USA.

17 ³Department of Immunology, Duke University School of Medicine, Durham, NC 27710, USA.

18 ⁴Department of Molecular Genetics and Microbiology, Duke University School of Medicine,

¹⁹ ⁵Department of Medicine, Duke University School of Medicine, Durham, NC 27710, USA.

20 ⁶Department of Biology, Davidson College, Davidson, NC 28035, USA

21 7Acuitas LLC, Vancouver, BC

²² ⁸Beth Israel Deaconess Medical Center, Boston, MA 02215, USA.

23 ⁹Department of Microbiology

24 ¹⁰Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

25 ¹¹Department of Pediatrics, Duke University School of Medicine, Durham, NC 27710, USA.

26 ¹²BIOQUAL, Rockville, MD 20850, USA.

27 ¹³Department of Biochemistry, Duke University School of Medicine, Durham, NC 27710, USA.

28 ¹⁴Lead contact

29

30 [#]Correspondence: kevin.saunders@duke.edu (K.O.S.); Priyamvada.acharya@duke.edu (P.A.);

31 barton.haynes@duke.edu (B.F.H.)

32

33 **Key words: HIV vaccine, broadly neutralizing antibodies, rhesus macaques, CD4 binding**

34 **site**

35 **SUMMARY**

36 The CD4 binding site (CD4bs) is a conserved epitope on HIV-1 envelope (Env) that can be
37 targeted by protective broadly neutralizing antibodies (bnAbs). HIV-1 vaccines have not elicited
38 CD4bs bnAbs for many reasons, including the CD4bs is occluded by glycans, immunogen
39 expansion of appropriate naïve B cells, and selection of functional antibody mutations. Here, we
40 demonstrate immunization of macaques with a CD4bs-targeting immunogen elicits neutralizing
41 bnAb precursors with structural and genetic features of CD4-mimicking bnAbs. Structures of the
42 CD4bs nAbs bound to HIV-1 Env demonstrated binding angles similar to human bnAbs and
43 heavy chain second complementarity determining region-dependent binding characteristic of all
44 known human CD4-mimicking bnAbs. Macaque nAbs were derived from variable and joining
45 gene segments orthologous to the genes of human V_H1-46-class bnAbs. This vaccine study
46 initiated the B cells from which derive CD4bs bnAbs in primates, accomplishing the key first
47 step in development of an effective HIV-1 vaccine.

48 **INTRODUCTION**

49 Broadly neutralizing antibodies (bnAbs) can protect against sensitive viruses in humans
50 and animal models of HIV-1 infection ¹⁻⁴, and are a primary goal of HIV-1 vaccine
51 development⁵. BnAbs target one of seven conserved epitopes on HIV-1 Env⁶. Among these Env
52 conserved sites is the binding site for CD4 (CD4bs)⁶. Monoclonal antibody isolation from people
53 living with HIV-1 has identified two classes of bnAbs that mimic CD4 in the manner in which
54 they bind Env⁶⁻¹⁰. The first class of bnAbs are derived from the VH1-2*02 germline gene
55 segment and includes VRC01, CH31, and 3BNC117 ^{10,11}. This type of CD4 mimicking antibody
56 uses beta strands in its heavy chain second complementarity determining region (HCDR2) to
57 recapitulate the beta strands of CD4 allowing both proteins to fit similarly into the CD4bs^{7,10}.

58 Extensive studies have been conducted to characterize the frequency of this type of CD4
59 mimicking antibody in the human repertoire, and found the precursors to be relatively rare¹². The
60 second class of CD4 mimicking bnAbs are derived from the VH1-46 gene segment and include
61 bnAbs such as CH235.12, 8ANC131, and 1-18^{6,11,13,14}. Like VH1-2*02-derived bnAbs, these
62 antibodies mimic CD4 using beta strands in their HCDR2^{13,15}. Vaccine design efforts have been
63 focused on the VRC01 class antibodies¹⁶⁻²⁰, and relatively less investigation has aimed to elicit
64 VH1-46 class bnAbs^{15,21,22}. However, the potent and broad neutralization of VH1-46-derived
65 bnAbs, the lack of insertions and deletions in their genes, heterogenous light chain gene usage,
66 and normal LCDR3 lengths make this type of bnAb a desirable vaccine design target.

67 It has been proposed that the first step in eliciting bnAbs with vaccination is for Env
68 immunogens to bind the bnAb precursor—termed the unmutated common ancestor (UCA)
69 antibody of the bnAb B cell clone²³⁻²⁵. Therefore, one approach to eliciting VH1-46-class CD4bs
70 bnAbs is to engineer high affinity Env immunogens that bind to the VH1-46-derived, unmutated
71 B cell receptor of naïve B cells that target the CD4bs^{5,23-26}. To enable design of such
72 immunogens for VH1-46-class CD4bs bnAbs, we previously isolated the CH235 bnAb lineage
73 and contemporaneous HIV-1 Env sequences from the same individual, named CH505^{13,27,28}. We
74 engineered an Env that bound the UCA antibody of the CH235 lineage (CH235 UCA) by
75 introducing N279K and G458Y substitutions into the HIV-1 Env inferred to have initiated the
76 infection in the CH505 individual^{15,21}. This engineered Env, called CH505 M5.G458Y, induced
77 serum autologous neutralizing CD4bs antibodies and selected for functional somatic mutations
78 needed for neutralization breadth in CH235 UCA knock-in mice²¹. Similarly, immunization of
79 rhesus macaques with M5.G458Y Env trimer conjugated to ferritin nanoparticles generated
80 CD4bs serum neutralizing antibodies²¹. These macaque serum CD4bs antibodies showed

81 hallmarks of the CH235 lineage in that the neutralizing antibodies were dependent upon the
82 N279K and/or G458Y substitutions engineered into the Env to promote CH235 UCA binding²¹.
83 However, it was unknown whether these serum neutralizing CD4bs antibodies were IGHV1-
84 derived, CD4 mimicking neutralizing antibodies.

85 Here, we vaccinated rhesus macaques with M5.G458Y Env trimers and a novel lipid
86 nanoparticle adjuvant and elicited serum CD4bs autologous tier 2 virus neutralizing antibodies.
87 The serum antibodies bound to HIV-1Env with orientations comparable to CH235. Isolated
88 monoclonal neutralizing CD4bs antibodies from multiple macaques utilized rhesus VH gene
89 segments orthologous to human VH1-46 and had angles of approach to Env similar to the
90 CH235 bnAb. A high-resolution structure of one of these vaccine-induced antibodies showed it
91 mimicked CD4 utilizing structural features similar to VH1-46 bnAbs. The hallmark amino acids
92 identified to mediate binding of human CD4bs bnAbs were also functionally required in rhesus
93 macaque CD4bs nAbs. Lastly, the inferred precursor of this vaccine-induced CD4bs nAb bound
94 to the vaccine immunogen consistent with the vaccine goal of targeting specific VH1-46-like
95 germline antibodies. Thus, this study demonstrates induction of CD4 mimicking, CD4bs nAbs in
96 rhesus macaques with paratope structures, immunogenetics, and neutralization signatures
97 recapitulating human VH1-46-type bnAb precursors.

98

99 **RESULTS**

100 **Vaccine induction of serum CD4bs nAbs**

101 To elicit VH1-46 bnAb-like antibody responses, three rhesus macaques were vaccinated
102 six times with the CH505 M5.G458Y HIV-1 Env engineered to bind to the CH235 UCA^{15,21}
103 (**Figure 1A**). M5.G458Y Env was enriched for Man₅GlcNAc₂ glycans and adjuvanted with a

104 lipid nanoparticle that has been shown to boost antibody production for both mRNA and protein
105 immunogens²⁹. Serum IgG responses to the autologous or vaccine-matched Env arose after a
106 single immunization and peaked after two immunizations, with Man₅GlcNAc₂-enrichment on
107 Env improving serum IgG binding (**Figure 1B**). We performed competition assays with serum
108 and CH235.12 to determine whether the Env-specific serum antibody response was targeted to
109 the CD4bs. Serum antibody throughout the first five immunizations showed increasing ability to
110 block the binding of CD4bs bnAb CH235.12, but exhibited low blocking of N332-glycan bnAb
111 2G12 (**Figure 1C**). Thus, immunization elicited a substantial CD4bs binding antibody response
112 compared to N332 glycan-directed antibodies.

113 The neutralizing antibody ID50 titer against the vaccine-matched virus was high, peaking
114 at a group geometric mean of 1:339,853 serum dilution after the fifth immunization (**Figure 1D**).
115 Heterologous neutralization was modest against CH235-sensitive viruses (**Figure S1A,B**).
116 CH235 lineage antibodies at the beginning of affinity maturation exhibit a neutralization
117 signature where they potently neutralize viruses that have both N279K (also called M5) and
118 G458Y substitutions, moderately neutralize viruses with either N279K or G458Y, and weakly
119 neutralize viruses lacking both substitutions¹⁵. After six immunizations serum neutralizing
120 antibodies were most potent when virion-associated Env included both N279K and G458Y.
121 Neutralization was weaker if only one of these substitutions was present, and weakest if neither
122 substitution was present (**Figure 1E**). Thus, the serum neutralization signature matched that of
123 the CH235 lineage^{15,21}. The serum neutralization of M5.G458Y virus was also sensitive to a
124 CD4bs amino acid change from Asn to Asp at position 280 (N280D), which is known to
125 knockout CH235 lineage antibody neutralization early in development²¹. As the CH235 lineage
126 evolves it becomes less affected by N280D substitution (**Figure S1C**). Serum neutralization

127 showed the same pattern, since after three immunizations the N280D substitution reduced
128 neutralization titer 78-fold, but after six immunizations only reduced neutralization titer 7-fold
129 (**Figure 1F and G**).

130 Glycans proximal to the CD4bs can hinder neutralization by CD4bs antibodies^{30,31}. The
131 CH235 lineage antibodies bind in the vicinity of the N197 glycan. While the N197 glycan is
132 disordered in the cryo-EM structure of the CH235 UCA complex (PDB ID 6UDA)¹⁵, cryo-EM
133 structures of CH235.12 bound to CH505.N279K (or M5) SOSIP Env trimers, showed well-
134 defined density for glycan N197 making close contact with a region of the antibody heavy chain
135 near K19T, E81D and T70Y substitutions (**Figure S2 and Table S1**), suggesting
136 accommodation of glycan N197 by the maturing CH235 lineage through the acquisition of
137 somatic mutations. A K19T substitution in CH235 UCA improved its binding to CH505 Envs,
138 and removal of the N197 glycosylation site improved CH235 UCA antibody neutralization
139 (**Figure S2 and S3**)¹⁵. Consistent with elicitation of CH235-like antibodies, vaccinated macaque
140 serum neutralization potency also increased upon removal of the N197 glycosylation site (**Figure**
141 **1H**).

142 We next visualized Env epitopes targeted by the elicited antibodies using antigen binding
143 fragments (fabs) prepared from total IgG purified from the serum of each vaccinated macaque,
144 and performed negative stain electron microscopic polyclonal epitope mapping (EMPEM) of the
145 fabs bound to the M5.G458Y Env trimer. Three dimensional (3D) classifications from all three
146 animals showed robust polyclonal Fab binding to the CD4bs, as well as binding to the trimer
147 base, the N611 glycan epitope, and the V1/V3 epitope (**Figure S4**). Masked 3D classifications
148 focused on the CD4bs demonstrated all NHPs developed antibodies that bind to the CD4bs with
149 a horizontal approach such that the flat plane of the Fab is approximately parallel to the plane of

150 the page when seen in top view (**Figure 1I**). All NHPs also developed antibodies that bound the
151 CD4bs with an angle of approach more similar to canonical VH1-46-type of CD4bs bnAbs
152 (**Figure 1J**). Rigid-body fitting of the Env-CH235 UCA complex into the EMPEM maps shows
153 that this latter subset of serum CD4bs antibodies have binding modes similar to CH235 UCA at
154 this resolution (**Figure 1K**). Hence, structural studies indicated vaccine elicitation of CD4bs
155 antibody responses targeting the CH235 epitope.

156 **Induction of lymph node TFH responses**

157 In addition to the serum antibody response, we analyzed vaccine-induced TFH cell
158 responses in lymph nodes harvested prior to vaccination and one week after the 3rd and 4th
159 immunizations (weeks 9 and 13 after immunization). We quantified Env-specific TFH cells
160 using an Activation-Induced Marker Assay^{32,33}, using an overlapping peptide pool spanning
161 CH505TF gp140 to stimulate lymph node cells ex vivo (**Supplementary Figure 5A**). Env-
162 specific TFH cells were not detected in lymph node biopsies from any monkey at pre-
163 immunization time points but were readily detected in all four NHP after the 3rd and 4th
164 immunizations (**Figure S5B**), indicative of vaccine-induced Env-specific CD4 T cell help for
165 humoral responses. Similarly, Env-specific B cells were undetectable in lymph nodes prior to
166 vaccination, but steadily increased in frequency after the 3rd and 4th immunizations (**Figure**
167 **S5C and S5D**). Vaccination with CH505 M5 G458Y GnTI- SOSIP nanoparticles/LNP elicited
168 robust humoral responses that were supported by cognate CD4 T cell help.

169 **Distinct phenotypic classes of recombinant CD4bs neutralizing antibodies**

170 To compare vaccine-induced antibodies more specifically to VH1-46 bnAbs, we isolated
171 antibody sequences from blood memory B cells for phenotypic and genotypic characterization as
172 recombinant IgG antibodies (Abs) (**Figure 2A and S6A**). M5.G458Y Env trimer-specific single

173 B cells were sorted from two rhesus macaques (RM7193 and RM7196), and their corresponding
174 B cell receptors were sequenced. Approximately 30% of the immunoglobulin heavy chain
175 variable regions were derived from IGHV1 in each macaque (**Figure S6B**). Using the KIMDB
176 database of *Macaca mulatta* sequences we assigned IGHV gene segments to immunoglobulin
177 sequences. Three clonal lineages were derived from rhesus IGHV1-105, which is orthologous to
178 human IGHV1-46 (**Figure 2B**). The most abundant macaque IGHV used by the antigen-specific
179 B cells was IGHV1-84, which was only 78% identical to both rhesus IGHV1-105 and human
180 IGHV1-46 (**Figure 2B and S6C**). The three VH1-105 utilizing antibodies displayed common
181 distributions of mutation percentages and CDR3 lengths (**Figure S6D and S6E**).

182 Fifty-three recombinant antibodies were selected for further study based on derivation
183 from IGHV1 gene segments and/or initial Env binding screens. Antibodies were assessed to
184 determine whether Env reactivity was dependent on Man₅GlcNAc₂ glycosylation or amino acid
185 substitutions at N280, N279K, or G458Y. Of the fifty-three antibodies, thirty-one (58%)
186 neutralized the autologous, vaccine-matched virus, Man₅GlcNAc₂-enriched M5.G458Y (**Figure**
187 **2C and S7**). Overall, Man₅GlcNAc₂ enrichment enhanced antibody neutralization, but had a
188 minor effect on binding to soluble Env trimers (**Figure 2C and 2D**). Twenty-one of thirty-one
189 (68%) neutralizing antibodies exhibited at least a five-fold reduction in neutralization in the
190 presence of a N280D substitution (**Figure 2C**). The same percentage of antibodies were
191 dependent on N279K or G458Y for neutralization of the vaccine-matched virus. Generally, both
192 of these phenotypes were concordant with observed loss of binding to N280D versions of
193 M5.G458Y soluble Env or to Env gp120 without N279K or G458Y (**Figure 2D**). Twenty-four
194 antibodies showed a 50% or greater reduction in Env binding magnitude in the presence of
195 CH235.12 (**Figure 2D**). Altogether, the binding phenotype coupled with the competition with

196 CH235.12 for binding to Env indicated the majority (68%) of nAbs exhibited a CD4bs
197 specificity similar to CH235 lineage antibodies (**Figure 2C-2E**).

198 Seven of the fifty-three antibodies (DH1285 and DH1389-DH1394) were capable of
199 neutralizing all of the CH505 viruses tested regardless of the presence of N279K or
200 G458Y(**Figure 2C and Figure S7A**). For these seven nAbs, Man₅GlcNAc₂ enrichment
201 improved M5.G458Y recombinant Env binding as well as pseudovirus neutralization (**Figure 2C**
202 **and 2D**). Additionally, these seven recombinant antibodies bound to both M5.G458Y and
203 CH505 TF soluble Env trimers (**Figure 2E**). However, only 1 of the 7 nAbs, DH1285, competed
204 with CH235.12 for binding to Env and bound to Env gp120 monomers with or without N279K
205 and G458Y substitutions (**Figure 2D**). These results suggested DH1285 was a CD4bs antibodies
206 that no longer required N279K or G458Y, whereas the other 6 Man₅GlcNAc₂-enriched CH505
207 TF nAbs targeted a different epitope (**Figure 2 and S7**).

208 **IGHV1-105-derived macaque nAbs exhibit a CH235-like Env binding mode**

209 From the 53 monoclonal antibodies, we found four nAbs (DH1285, DH1357, DH1358,
210 and DH1359) that competed with CH235.12 for binding to Env and were derived from the
211 macaque gene ortholog of human VH1-46, IGHV1-105. DH1285 was isolated from NHP7193
212 and DH1357, DH1358, and DH1359 were isolated from NHP7196 (**Figure 2A and S6A**).
213 DH1357 and DH1358 were clonally related. DH1358 and DH1359 had the same IGHV
214 sequence, but different light chains (**Figure 3A and S8**). DH1285 had a 15 amino acid HCDR3
215 like CH235.12, whereas the other 3 VH-105 rhesus antibodies had 19 amino acid HCDR3s
216 (**Figure 3A**).

217 We performed negative stain electron microscopy (NSEM) to determine whether these
218 antibodies exhibited Env binding modes similar to CH235.12 (**Figure 3B**). The vaccine-induced

219 macaque Fabs DH1357, DH1358 and DH1359 bound to closed trimers and exhibited angles of
220 approach that were highly similar to each other and to the human bnAb CH235.12 (**Figure 3B**).
221 The structure of DH1285 was solved in complex with four different stabilized versions of CH505
222 TF or M5.G458Y gp140 Env. Three of the structures showed DH1285 bound to partially open
223 Env conformations and one structure with M5.G458Y showed it bound to a closed Env (**Figure**
224 **3B and S9**). Although the individual variable loops cannot be resolved by NSEM, the overall
225 density and shape of the partially open Env protomers in the DH1285-bound structure were
226 consistent with an open-occluded Env trimer in which the protomers rotate away from one
227 another to open the trimer, but the V1V2 loops remain in their closed position occluding the V3
228 co-receptor binding site³⁴ (**Figure 3B and S9A-C**). Altogether, the collection of structures
229 showed DH1285 bound to both open and closed Env conformations, demonstrating that DH1285
230 can bind multiple Env conformations and that a partially open conformation was not required for
231 binding (**Figure S9**). A high-resolution model of the CH235.12 complex fit into the NSEM
232 density maps for DH1357, DH1358 and DH1359 with only small deviations in the constant
233 regions of the Fabs, thus confirming their similarity to CH235.12 (**Figure 3C**). In contrast, when
234 the CH235.12 complex is modeled into the DH1285 structure it can be seen that DH1285 targets
235 the same epitope as CH235.12, but with a different angle of approach that is most apparent when
236 viewed from the top looking down the trimer axis (**Figure 3C**).

237 Next, we compared the binding affinity of the vaccine-induced antibodies to that of
238 CH235.12 lineage members. DH1285 showed the highest affinity (0.049 nM) for Man₅GlcNAc₂-
239 enriched M5.G458Y among all macaque and human antibodies tested (**Figure 3D**). Both
240 DH1358 and CH235.12 bound to Man₅GlcNAc₂-enriched M5.G458Y with approximately 0.2
241 nM affinity (**Figure 3D**). DH1357 and DH1359 Fab affinity for Man₅GlcNAc₂-enriched

242 M5.G458Y were most similar to the later intermediates (CH235 I39 and I35) in the CH235.12
243 lineage (**Figure 3D**). For the CH505 TF Env trimer, CH235 I39, CH235 I35, and CH235.12 had
244 detectable affinity, while DH1285 was the only macaque antibody that bound the CH505 TF Env
245 (**Figure 3D**). The DH1285 affinity (2,070 nM) was 100-fold weaker than CH235 I39 or CH235
246 I35 for CH505 TF Env (**Figure 3D**). Overall, vaccine-induced rhesus antibodies had extremely
247 high affinities for the vaccine immunogen but were most similar to early intermediate antibodies
248 in the CH235 lineage when binding to a wildtype Env.

249 We next determined antibody neutralization of a panel of CH505 TF viruses that included
250 combinations of Env modifications that enabled CH235 precursor binding to Env. Of the
251 vaccine-induced antibodies, DH1285 showed the broadest neutralization of the variant CH505
252 viruses. The neutralization potency of DH1285 closely resembled the potency of CH235.12.
253 DH1358 exhibited the second broadest neutralization, showing a preference for either
254 Man₅GlcNAc₂-enrichment or G458Y to be present in the virus. The neutralization profile of
255 rhesus CD4bs Ab DH1359 was most similar to CH235 UCA in that it was still highly dependent
256 on N279K and G458Y. Four of the five viruses DH1359 neutralized had both N279K and
257 G458Y. DH1357 and DH1359 showed similar neutralization patterns, although neutralization
258 was more potent for DH1357. DH1285 and DH1357 were the highest affinity antibodies and
259 were the least dependent on Env modifications that enabled CH235 precursor binding such as
260 N197 glycan removal, N279K, G458Y, and Man₅GlcNAc₂ enrichment. Thus, of the four
261 CH235.12-blocking antibodies, the binding phenotypes of DH1285 and DH1357 have
262 progressed the furthest from the precursor stage.

263

264 **DH1285 exhibits the canonical CD4 mimicking structure of VH1-46 and VRC01 bnAb**
265 **classes**

266 To obtain higher resolution definition of DH1285 binding to HIV-1 Env, we determined
267 cryo-EM structures of the DH1285 Fab bound to a stabilized CH505 TF SOSIP Env (**Figure 4A**,
268 **S10, S11, and Table S2**). The particles in the cryo-EM dataset, picked and sorted using
269 reference-free 2D classification, revealed DH1285 Fabs bound to the Env trimer in the 2D class
270 averages. A heterogeneous mix of particles were observed, from which *ab initio* models were
271 generated and particles were sorted via multiple rounds of heterogeneous refinements, yielding
272 distinct classes showing compositional heterogeneity arising from varied occupancy of the
273 DH1285 Fabs per Env trimer, as well as conformational heterogeneity arising from different
274 relative orientations of the Env protomer. Cryo-EM reconstructions of DH1285 Fab bound to the
275 CH505 Env trimer were resolved to a global resolution ranging from 5-6 Å (**Figure 4A, S10**,
276 **S11 and Table S2**). Local refinement of the DH1285 Fab interface with the Env yielded a 4.5 Å
277 resolution reconstruction, enabling unambiguous placement of the Fab and resolution of
278 interfacial loops and secondary structures, including the CD4 binding loop, and Loops D and V5
279 of Env gp120 and the antibody CDR loops (**Figure 4C**).

280 Human CD4bs bnAbs that mimic CD4 bind with stereotypical paratope structures that
281 resemble portions of CD4^{9,13,15}. Specifically, each human bnAb has a HCDR2 in a beta strand
282 conformation that contacts the CD4 binding loop, and an arginine at position 71 in the V_H that
283 forms a salt bridge with aspartic acid at position 368 in Env (**Figure 4D**). DH1285 antibody
284 interacts with gp120 using both heavy and light chain complementary determining regions
285 (CDRs). The DH1285 HCDR2 interacts with the CD4 binding loop of gp120 mimicking the
286 gp120-CD4 receptor interaction in this region, with residue Asp168 of the CD4 binding loop

287 positioned to make a salt bridge with Arg 71 in the antibody framework region 3. Therefore,
288 DH1285 exhibits the conserved structural and interactive signatures of the VH1-2 and VH1-46
289 CD4-mimetic antibodies.

290 The DH1285 HCDR3 and LCDR3 regions contacted both gp120 Loop D and Loop V5,
291 respectively (**Figure 4C and 4E**), with the DH1285 HCDR3 interaction with Loop D most
292 closely resembling that of CH235.12. The HCDR3 also contacted structural elements in the
293 gp120 inner domain with the gp120 Trp96 side chain stacking against the HCDR3 tip. Loop D
294 also contacted the light chain LCDR1 and LCDR3 regions as well as Trp 50 of the HCDR2,
295 making Loop D a key interactive region that contacted spatially separated regions of the
296 antibody. While LCDR2 showed no direct contact with the epitope, it contacted the LCDR1 and
297 LCDR3 loops and may play a role in stabilizing the conformations of these paratope loops and
298 influencing their presentation. Comparison of gp120-CD4 (PDB: 1GC1), gp120-VRC01(PDB:
299 3NGB), gp120-8ANC131(PDB: 4RWY), gp120-CH235(PDB: 5F9W) on gp120-DH1285
300 complex reveals their relative orientations and highlights the similarity of their interactions
301 centered on HCDR2-CD4 binding loop (**Figure 4E**). Taken together, the structural data confirm
302 that antibody DH1285 binding to HIV-1 Env recapitulates the key structural signatures that are
303 hallmarks of VH1-2 and VH1-46 germline derived CD4-mimetic HIV-1 bnAbs.

304

305 **DH1285 and CD4-mimicking human bnAbs share molecular immunology features for Env**
306 **binding**

307 The combination of high-resolution structures of VRC01 and CH235 precursors have
308 identified W50, R71, and N58 as germline V_H sequence-encoded amino acids that mediate
309 contact within the CD4bs on Env^{9,15,35-37}. These amino acids are postulated to be the genetic

310 basis for why specific IGHV gene segments are used by CD4 mimicking bnAbs. Also, V_H amino
311 acids at position 54 substantially affect binding affinity of CD4 mimicking antibodies by
312 inserting their side chains into the cavity on Env usually occupied by F43 in CD4, termed the
313 Phe43 cavity³⁸. We compared the V_H sequence and structure of DH1285 to known VRC01 class
314 and VH1-46 class bnAbs, identified that the same amino acids at positions 50, 54, and 71 as
315 VH1-46 or VRC01 class antibodies. At position 58, DH1285 had a K58H substitution that
316 differed from the human bnAbs examined (**Figure 5A and B**). In addition to the R71 described
317 above, the DH1285 HCDR2 was positioned similarly to human CD4bs bnAbs such that the
318 DH1285 VH W50 and Y54 contacted the CD4bs in a manner akin to VRC01, NIH45-46, CH235
319 UCA, 1-18, and 3BNC117 (**Figure 5A and B**). Alanine substitution of W50, Y54, and R71
320 showed that not only were they shared amino acids, but they also were required for optimal
321 binding to CH505 TF Env trimer and $\text{Man}_5\text{GlcNAc}_2$ -enriched CH505 TF neutralization (**Figure**
322 **5A and C**). Alanine scanning mutagenesis of the entire HCDR2 further showed that N52, P52a,
323 and N56 were required for optimal Env binding and neutralization of the same protein and virus,
324 respectively (**Figure 5C**). Given the proximity of R73 to R71, we also substituted alanine at this
325 position, although it should be noted that R73 is a result of somatic mutation. R73A substitution
326 moderately reduced CH505 TF neutralization (**Figure 5C**). Binding and neutralization of
327 $\text{Man}_5\text{GlcNAc}_2$ -enriched M5.G458Y was only affected by R71 substitution, suggesting the Env
328 engineering to improve affinity for CH235-like antibodies compensated for most single amino
329 acid substitutions (**Figure S12A and S12B**).

330 DH1285 also shared identity with nine amino acids that resulted from somatic mutation
331 in VH1-46 class bnAbs (**Figure 5D and S12C**). While some of these amino acids may be shared
332 due to hotspots for somatic mutations, we hypothesized that a subset of the nine mutations were

333 shared because they improved Env binding for this class of antibody. Examination of the CH235
334 UCA and DH1285 Fabs in complex in Env supported this hypothesis since four of the amino
335 acids were located at the antigen-antibody interface (**Figure 5E**). We changed the shared amino
336 acids to the germline VH1-46 amino acids to determine whether the shared amino acid
337 contributed to antibody binding or neutralization (**Figure 5F, S12D, and S12E**). Six single
338 amino acid changes had no effect on binding or neutralization (**Figure 5F, S12D, and S12E**).
339 R73T and E23K showed a 4-fold and 23-fold decrease in Man₅GlcNAc₂-enriched CH505 TF
340 neutralization potency respectively, but both changes caused minor reductions in recombinant
341 Env binding (**Figure 5F, S12D, and S12E**). In contrast, D31S substitution completely abrogated
342 CH505 TF gp140 trimer binding and Man₅GlcNAc₂-enriched CH505 TF neutralization. The
343 structural basis for the dependence on D31 was that D31 interacted with K474 in the CD4bs of
344 HIV Env (**Figure 5G**). It also coordinated the overall HCDR1 conformation through interactions
345 with Y27, T28, and T30 in the first framework of DH1285 (**Figure 5G**). DH1285 E23 did not
346 mediate direct contact with Env but coordinated the beta sheet conformation that ultimately
347 positions R71 to interact with the CD4bs loop (**Figure 5H**). Altogether, DH1285 has functional
348 canonical germline-encoded and somatic mutation-encoded amino acids found in CD4
349 mimicking human bnAbs.

350

351 **Env engineering for CH235 UCA binding enables binding by the DH1285 UCA**

352 The M5.G458Y immunogen was designed to bind to the precursor of CH235 lineage.
353 However, it is unknown how well it can bind to orthologous precursors in nonhuman primates
354 that are not exactly the same sequence as the CH235 UCA. To infer the UCA antibody that gave
355 rise to the DH1285 lineage, we performed two independent MiSeq next-generation sequencing

356 (NGS) runs of macaque VH1 and VK1 regions of peripheral blood B cells. We used Cloanalyst
357 and Partis to assign clonality to the recovered heavy chain and light chain sequences and inferred
358 a DH1285 UCA. We ran IgDiscover using our MiSeq sequences and found no evidence for a
359 new IGHV1-105 allele being present in macaque 7193³⁹. Thus, the UCA is composed of
360 previously known V gene segments. We examined the binding affinity of the UCA for the
361 immunogen designed to target CH235-like unmutated antibodies. The apparent affinity of the
362 DH1285 IgG was 36.8 nM, which was 8-fold weaker than the CH235 UCA apparent affinity of
363 4.5 nM (**Figure 6A, S13A, and S13B**). A fast on-rate between the antigen and B cell receptor is
364 associated with the ability of the B cell receptor to signal⁴⁰. We found the on-rate between the
365 CH235 and DH1285 UCA were similar, differing by only 5-fold (6.86E4 and 1.35E4
366 respectively) (**Figure 6A and S13B**). Neither UCA bound to the CH505 TF (**Figure 6A**). Thus,
367 antibody precursors with 8-fold weaker apparent binding affinity and 5-fold slower on-rates than
368 the CH235 precursor can be engaged and expanded by the engineered Env M5.G458Y.

369 To understand how the heavy chain evolved from the UCA, we inferred heavy chains
370 within the DH1285 lineage using NGS sequences that were observed in two independent
371 sequencing runs (**Figure 6B and S13A**). The NGS-derived DH1285 lineage V_H sequences were
372 paired with the DH1285 light chain and produced as recombinant IgGs for phenotypic
373 characterization. The DH1285 UCA bound to Man₅GlcNAc₂-enriched M5.G458Y Env trimer by
374 ELISA. The binding magnitude for Man₅GlcNAc₂-enriched M5.G458Y Env trimer increased by
375 less than 2-fold in logAUC as the DH1285 clone continued to evolve, with the major increase in
376 binding magnitude occurring at the first intermediate antibody (I3; **Figure 6B and S13C**).
377 Overall, binding magnitude to Man₅GlcNAc₂-enriched M5.G458Y Env trimer increased as the
378 V_H region acquired more amino acid changes (**Figure 6B**). Showing the importance of the Env

379 modifications that promote CH235 precursor binding, the DH1285 UCA showed negligible
380 binding to CH505 TF Env trimer lacking these modifications (**Figure 6B**). Thus, the
381 modification of Env to enable CH235 precursor binding also enabled vaccine-induced rhesus
382 CD4bs DH1285 UCA binding. Within the DH1285 antibody clone, CH505 TF Env reactivity
383 was only detected by DH1285, DH1285 I1 and DH1285.13044, which were all from the same
384 clade of the phylogeny (**Figure 6B**). In contrast to M5.G458Y binding, CH505 TF binding did
385 not strongly correlate with number of antibody amino acid changes suggesting the requirement
386 for specific critical amino acid substitutions rather than a particular number of amino acid
387 changes (**Figure S13C, and S13D**). Comparison of the VH sequences of the CH505 TF-reactive
388 antibodies to the other clonally related V_H sequences that lacked CH505 TF binding showed
389 amino acid changes N54Y, T68A, and Y102S (**Figure 6D and S13E**). Alanine substitution of
390 Y54 demonstrated that this tyrosine was required for CH505 TF Env binding (**Figure 5C**).
391 Hence, DH1285 UCA BCR precursor engaged the engineered immunogen in vivo after macaque
392 immunization and affinity matured to include known key somatic mutations such as N54Y, that
393 enabled interaction with wildtype envelope.

394

395 **DISCUSSION**

396 Here we demonstrate here that an HIV-1 immunogen designed to engage the VH1-46 class
397 CH235 bnAb lineage selects for similar BCR in outbred nonhuman primates defined by
398 structural mode of Env binding, amino acid mutations acquired, and usage of the rhesus macaque
399 ortholog of human VH1-46 gene . Thus, BCRs that can give rise to a VH1-46 bnAb as antibodies
400 derived for an orthologous immunoglobulin heavy chain variable gene segment, contains the
401 critical R71_{VH}, CD4bs-dependent binding, and HCDR2-mediated binding to the HIV-1 Env CD4

402 binding loop-- criteria selected based on immunogenetic and structural studies of the VH1-46-
403 derived CD4bs bnAbs^{6,11,13,14}. These characteristics are also hallmarks of most if not all CD4-
404 mimicking bnAbs including VRC01 and 3BNC117. Remarkably, our structural and
405 immunogenetic studies demonstrate that DH1285 possessed all four of these criteria for
406 classifying it as a VH1-46-like bnAb precursor. In addition to R71_{VH}, the VH1-105-derived
407 antibodies also possessed W50_{VH} in HCDR2, which is another shared trait of CD4-mimicking
408 CD4bs antibodies such as VRC01 and 3BNC117³⁵⁻³⁷. Interestingly, CH235 is thought to
409 somatically mutate to encode W50, although the germline gene of the HIV-infected individual
410 was not sequenced to confirm W50 was not present in its allele of IGHV1-46¹³. The shared
411 amino acids encoded by human IGHV1-46 and macaque IGHV1-105 highlight the genetic
412 similarities between humans and macaques. It has been posited that macaques lack the necessary
413 germline encoded gene segments to express CD4-mimicking neutralizing antibodies. To the
414 contrary, we find here that indeed macaques can make CD4bs antibodies with sufficient germline
415 amino acids that form the initial, critical contacts made with Env. Although we only
416 administered an Env designed to engage and expand germline antibodies similar to the VH1-46
417 bnAbs from humans, we found that solated BCRs that were expressed as recombinant antibodies
418 had begun the process of affinity maturation to include residues similar to the VH1-46 bnAbs.
419 Thus, the engineered HIV-1 Env immunogen was successful in eliciting the desired target CD4bs
420 antibodies and began to select for an affinity maturation pathway similar to human VH1-46 class
421 bnAbs. It should also be noted that the neutralizing CD4bs antibodies elicited in the macaques
422 could be detected in the serum. Therefore, a substantial portion of the serum neutralizing
423 antibody response was directed to the CD4bs.

424 Three vaccine-induced IGHV1-105-derived antibodies showed highly similar angles of
425 approach to CH235. A fourth IGHV1-105-derived antibody showed a distinct angle of approach
426 but same contact site on Env. This result is significant since structural studies of the CH235
427 lineage has demonstrated that the CH235 angle of approach is determined at the UCA stage of
428 the lineage and is unchanged during affinity maturation¹³. This observation suggests that human
429 VH1-46 bnAb precursors will also need to adopt the correct binding angle and orientation at
430 their precursor stage. For macaque or human antibodies whose angles of approach differ from
431 CH235.12, it is uncertain whether they can evolve to bind to Env in a manner similar to CH235.
432 The observation that the macaque antibodies described here have the appropriate initial binding
433 mode is encouraging for trying to select for higher affinity-matured bnAb intermediates with
434 greater neutralization breadth.

435 The germline targeting approach where immunogens are designed to interact with high
436 affinity to one or a few known bnAb antibody precursors is a major strategy for HIV-1 vaccine
437 development^{5,23-25}. The G001 trial has provided proof-of-concept for targeting putative VRC01-
438 class CD4bs antibodies in humans although structural confirmation of HCDR2 binding of
439 precursors was not reported⁴¹. VRC01 affinity maturation includes selection of rare deletions in
440 the light chain that may prove difficult for vaccine elicitation⁴². Thus, vaccine designs targeting
441 additional CD4bs bnAb classes are warranted. The CH235 lineage is an advantageous vaccine
442 target since it lacks rare nucleotide deletions or an unusually short light chain third
443 complementarity determining region observed in the VRC01 lineage^{10,13,28}. We show here that
444 such CD4bs bnAb precursor B cells can be expanded with the Man₅GlcNAc₂-enriched
445 M5.G458Y immunogen. Although the immunogen was designed based on the human CH235
446 precursor antibody, it was able to bind with nanomolar apparent affinity to a rhesus macaque

447 inferred germline CD4bs IgG antibody. This cross-species germline targeting of CD4bs bnAbs
448 shows the immunogen is quite adept at interacting with these precursors, as well as shows the
449 validity of using rhesus macaques as a model for development of vaccine immunogens that can
450 target VH1-46 CD4bs bnAb lineages. This precise targeting of CD4bs antibodies may explain
451 why we observe extremely high titers of CD4bs-dependent neutralizing antibodies exhibiting the
452 CH235 neutralization signature in the serum. Overall, this study supports the notion that
453 immunogens designed based on a known antibody lineage can elicit similar antibody lineages in
454 unrelated individuals or even different primate species. The HVTN309 Phase I trial will test this
455 hypothesis in humans by administering M5.G458Y envelope nanoparticles with ionizable LNP
456 as an adjuvant to elicit VH1-46 class bnAb precursors.

457 Due to the protracted affinity maturation process for bnAb development, a single Env is
458 not expected to select for affinity maturation from germline antibody to bnAb^{23,43}. However, the
459 antibodies isolated after the priming immunization can inform the next steps in sequential
460 vaccine design. We found here that the processed glycan at position 197 on CH505 TF Env
461 impeded DH1285 neutralization of natively glycosylated CH505 TF pseudovirus. Vaccine
462 immunogens that select for affinity matured DH1285 that can accommodate processed glycan at
463 position 197 would be a logical next step in sequential vaccine design. There are clues from the
464 VH1-46 bnAb class as to how affinity maturation can accommodate this glycan. The CH235
465 lineage makes improbable K19T, E81D and T70Y amino acid substitution in its heavy chain
466 precisely where the glycan juxtaposes the antibody framework region. Vaccine immunogens that
467 select for such BCR mutations in DH1285 antibodies may enable it to neutralize natively
468 glycosylated wildtype viruses. In the initial antibody-virus coevolution study of the individual
469 who generated the CH235 lineage, Envs with elongated and glycosylated fifth variable regions

470 arose over time²⁷. Selecting for antibodies that can accommodate changes in the fifth variable
471 region of the CD4bs is hypothesized to be one pathway towards developing neutralization
472 breadth.

473 Adjuvants play a critical role in the vaccine-induced immune response. In a previous
474 study in mice, we showed that LNP were potent adjuvants when mixed with protein immunogens
475 and induced robust Tfh responses²⁹. Here, LNP were potent inducers of serum antibody
476 responses with as little as two immunizations and induced robust Tfh and GC B cell responses
477 after three immunizations. While Env trimers have been viewed as less immunogenic than
478 nanoparticle vaccines⁴⁴⁻⁴⁶, lipid nanoparticles mixed with HIV-1 Env trimers were able to elicit
479 potent neutralizing antibodies.

480 We acknowledge the study limitations are that it is difficult to determine whether a
481 particular antibody will develop into a bnAb based on its early stages of development. For
482 previous germline-targeting HIV vaccines, the bnAb precursors are usually non-neutralizing⁴¹,
483 however the bnAb precursors elicited here demonstrate nanogram per milliliter IC80
484 neutralization titers. Thus, the neutralization potency combined with the favorable genetic and
485 structural traits make development into a CD4bs bnAb a plausible outcome. We also
486 acknowledge one limitation is that we immunized six times which is not conducive to
487 heterologous prime boost sequential vaccines. Lastly, we tested only one dose of LNP as an
488 adjuvant. The adjuvanting effect of other doses of LNP still needs to be investigated in future
489 studies.

490 In summary, proof of concept for VH1-46-type, CD4 mimicking, CD4bs bnAb
491 precursors has been achieved in outbred non-human primates, providing evidence for the
492 possibility that fully affinity-matured bnAbs will be able to be induced in both rhesus macaques

493 and in healthy humans by judicious choice of a series of boosting immunogens that can
494 sequentially select for functional improbable mutations necessary for full bnAb potency and
495 breadth.

496

497 **MATERIALS AND METHODS**

498 **Animals and Immunization.** Indian-origin rhesus macaques were housed in AAALAC-
499 accredited facilities and all veterinary and study procedures performed in accordance with Duke
500 University IACUC-approved protocols and Bioqual (Rockville, MD) standard operating
501 procedures. All macaques were 2 years and 4 months old at the start of the study, and weighed
502 between 5.2 and 6.8 kg at the end of the study. The study began with 4 macaques, but one female
503 macaque, NHP 7194 was removed from the study for animal medicine reasons, leaving 3 (2
504 female and 1 male) macaques that completed the study. The first 5 immunizations were spaced
505 every four saweeks with a final immunization 14 weeks after the 5th immunization. Vaccine was
506 administered intramuscularly by injecting 750 µL of protein plus adjuvant mixture in both the
507 left and right quadriceps (total 1.5 mL injection per animal per immunization). Each
508 immunization consisted of 100 µg CH505.M5.G458Y Man₅GlcNAc₂-enriched Env trimer
509 formulated with nucleoside-modified mRNA encoding luciferase encapsulated in lipid
510 nanoparticles from Acuitas. Throughout the duration of the study, whole blood and serum were
511 drawn on the day of vaccination and one and/or two weeks post-vaccination.

512

513 **HIV Env Protein Production.** Previously described chimeric CH505 TF and
514 CH505.M5.G458Y SOSIP gp140 Env proteins^{15,21} were stabilized with E64K and A316W

515 stabilizing mutations⁴⁷. The codon-optimized genes expressing the trimers were encoded by the
516 VRC8400 vector.

517 For expression, Freestyle 293F (ThermoFisher) or 293S GnT1⁻ cells were diluted at the
518 time of transfection to 1.25×10^6 cells/mL with fresh Freestyle293 (ThermoFisher) media in 500
519 mL batches. Co-Transfection was performed with plasmid DNA (650 µg SOSIP trimer plasmid
520 and 150 µg furin expressing plasmid per 1L of culture volume) complexed with 293fectin in
521 OPTI-MEM. After 6 days, cell cultures were harvested by centrifugation of the cells for 60
522 minutes at 4000 rpm on a Sorvall table top centrifuge. Supernatant was filtered through 0.8 µm
523 filter and concentrated to approximately 100 mL with Vivaflow 200 cassettes (Sartorius) with a
524 30 kDa MWCO. Concentrates were again filtered to 0.8 µm, then purified with positive selection
525 on a 10 mL affinity column containing PGT145 conjugated to CnBr-activated Sepharose
526 (Cytvia), buffered with PBS. Following loading and washing, Env trimers were eluted using 3M
527 MgCl₂, and immediately equilibrated with 5 volumes of 10 mM tris, pH8. The eluate was filtered
528 to 0.2 µm and concentrated to 2 mL with a centricon-70 10 kDa MCWO. If biotinylated (for
529 immunochemistry), Avi-tagged Env proteins at 25 µM were dialyzed into Tris pH8 and
530 incubated for 5 hours with mild agitation at 30 °C using the BirA biotin-protein ligase reaction
531 kit (Avidity LLC), then reconcentrated prior to size-exclusion chromatography. Two milliliters
532 of concentrated protein was purified on a Superose6 16/600 column (Cytvia) in 500 mM NaCl
533 buffer with 10 mM tris, pH 8 to isolate trimeric protein. All chromatography steps were
534 conducted on a an AKTA Pure (Cytvia). The trimeric pool of protein fractions was pooled,
535 filtered to 0.2 µm, and snap frozen for long-term storage at -80 °C.

536 Recombinant gp120s were produced via transient transfection using Freestyle 293F
537 (ThermoFisher) or 293S GnT1⁻ cells and 293Fectin using the same conditions as SOSIP gp140s.

538 Following five days of culture, cells were harvested via centrifugation and filtered to 0.8 μ m.
539 Cell-free supernatant was concentrated using a 10 kDa MWCO Vivaflow 50 (Sartorius). The
540 concentrate was mixed with *Galanthus nivalis* lectin agarose resin (Vistar Labs) incubated
541 overnight at 4 °C. The resin beads were repetitively pelleted via centrifugation and washed twice
542 by resuspension with MES buffer, before protein was eluted with methyl- α -pyranoside.
543 Monomeric protein was purified using Superdex200 (GE Healthcare) size-exclusion
544 chromatography column on an AKTA Pure (Cytvia).

545

546 **Site-Directed Mutagenesis.** Antibody heavy chain plasmids were mutated using the
547 QuikChange Lightning (Agilent) kit, following manufacturer recommended reaction conditions.
548 Oligos introducing site-specific mutations were designed with the QuikChange Primer Design
549 Tool (Agilent), synthesized and purified via standard desalting by Integrated DNA Technologies.
550 Cloning was performed in XL-10 gold cells and sequence integrity was determined with Sanger
551 sequencing (Azenta) and subsequent DNA alignment using Geneious (BioMatters).

552

553 **Recombinant Antibody Production.** Monoclonal antibodies and Fabs were encoded in heavy
554 and light chain plasmids and co-transfected into Expi293F (293i) cells using expifectamine.
555 Briefly, 293i cells were diluted to 2.5×10^6 cells/mL with fresh Expi293 media in 100 mL
556 batches and incubated for 4 hours prior to transfection. Fifty micrograms of each plasmid was
557 mixed with expifectamine in OPTI-MEM I, then added to the transfection culture. Expifectamine
558 kit enhancers were added approximately 16-18 hours post-transfection and cultures were
559 incubated for 5 days before harvest. The cultures were centrifuged at 4000 rpm in a Sorval table
560 top centrifuge and supernatant was filtered through 0.8 μ m filter. The cell-free supernatant was

561 incubated overnight with either protein A resin (ThermoFisher) for IgG1 or LambdaFabSelect or
562 KappSelect resin (Cytvia) for lambda or kappa containing Fabs, respectively at 4°C. The bead
563 slurry was centrifuged at 1200 rpm for 10 minutes, then aspirated before washing via gravity
564 filtration with 20 mM tris (pH 7) and 350 mM NaCl buffer and subsequently eluting with 2.5%
565 acetic acid. The eluate was neutralized with Trizma (pH 8), then buffer exchanged through
566 repetitive centrifugation in a Vivaspin Turbo-15 concentrator with 25 mM citrate and 125 mM
567 NaCl buffer (pH 6) before final storage at -80 °C.

568 **AIM assay measuring TFH responses.** Env-specific TFH cells were quantified using an
569 Activation-Induced Marker Assay^{32,33}, using an overlapping peptide pool spanning CH505 TF
570 gp140 to stimulate lymph node cells *ex vivo*. TFH cells were identified by flow as viable
571 lymphocytes that were CD4⁺ CD8⁻ CXCR5^{hi} PD1^{hi}. Env specificity was measured as the
572 frequency of TFH cells co-expressing OX40 and CD25 after *ex vivo* stimulation with an Env
573 peptide pool, after background subtraction of frequency in unstimulated conditions.

574

575 **Enzymatically-Digested Antibody Fabs.** Antibody Fabs were generated by papain digestion
576 using the PierceTM Fab Preparation Kit (ThermoFisher Catalog No: 44985). The manufacturer's
577 protocol was followed except IgG antibodies were dialyzed into PBS for 2h prior to digestion
578 and IgG was digested for 16-18 h. SDS-PAGE and Coomassie staining was used to examine the
579 digestion of IgG into Fabs. Fabs were run through a Superdex200 10/300 column in 25mM Citric
580 Acid with 125mM NaCl (Cytiva) to remove any aggregates. Fabs were stored frozen at -80 °C in
581 25mM Citric Acid 125mM NaCl pH 6 Buffer.

582 **HIV-1 neutralization assays.** Neutralizing antibody assays were performed with HIV-1 Env-
583 pseudotyped viruses and TZM-bl cells (NIH AIDS Research and Reference Reagent Program

584 contributed by John Kappes and Xiaoyun Wu) as described previously^{48,49}. Neutralization titers
585 are the reciprocal sample dilution (for serum) or antibody concentration in µg/mL (for mAbs) at
586 which relative luminescence units (RLU) were reduced by 80% or 50% (ID80/IC80 and
587 ID50/IC50 respectively) compared to RLU in virus control wells after subtraction of background
588 RLU in cell only control wells. Serum samples were heat-inactivated at 56 °C for 30 minutes
589 prior to assay.

590

591 **Fab-Env complex formation, negative staining, and data analysis.**

592 For polyclonal serum Fabs, ~1 mg of polyclonal Fabs at ~10 mg/ml were mixed with 20 µg of
593 M5.G458Y Env trimer and incubated overnight at 4 °C. To remove excess unbound Fab, the
594 mixture was separated by size exclusion chromatography on a Superose 6 Increase 10/300
595 column and fractions eluting at the expected volume for the complex were combined and
596 concentrated with a 100-kDa molecular weight cutoff spin concentrator to a nominal trimer
597 concentration of ~1 mg/ml. Concentrated sample was then diluted to 0.4 mg/ml with HEPES-
598 buffered saline (HBS), containing 150 mM NaCl, 20 mM HEPES, pH 7.4, augmented with 8
599 mM glutaraldehyde for crosslinking and incubated for 5 minutes at room temperature.
600 Unreacted glutaraldehyde was quenched by addition of 1 M Tris, pH 7.4 to a final Tris
601 concentration of 80 mM. As needed, quenched samples were diluted to 0.1-0.2 mg/ml with HBS
602 augmented with 5% glycerol or applied without dilution to glow-discharged carbon films on 300
603 mesh copper EM grids for negative staining. After blotting excess sample, samples were stained
604 for 1 minute with 2% uranyl formate, blotted and allowed to air dry.

605

606 For monoclonal FabS, 36 μ g of Fab was mixed with 10 μ g M5.G458Y Env trimer in a total
607 volume of 100 μ l of HBS and incubated overnight at 4 °C. Fab-trimer complexes were then
608 diluted with 400 μ l of HBS augmented with 10 mM glutaraldehyde, incubated for 5 minutes at
609 room temperature, and quenched by addition of 1 M Tris to 80 mM final concentration.
610 Quenched samples were then concentrated with a 100-kDa molecular weight cutoff spin
611 concentrator, which allows excess unbound Fabs to pass the filter and retains the Fab-trimer
612 complex. Concentrated sample was then diluted and negatively stained as described above.

613
614 Negatively stained grids were examined on a Philips EM420 electron microscope operating at
615 120 kV, 49,000x nominal magnification, and ~0.5 μ m defocus. Images were acquired on a 76-
616 megapixel CCD camera, corresponding to a nominal calibration of 2.4 $\text{\AA}/\text{pixel}$. Datasets were
617 typically ~100 or ~500 images for monoclonal or polyclonal samples, respectively. Image
618 analysis was performed with standard protocols in Relion 3.0⁵⁰, beginning with automated
619 particle picking, followed with two rounds of 2D classification/selection, and then by 1-2 rounds
620 of 3D classification/selection to discard junk particles and select Fab-bound trimer particles. For
621 monoclonal samples, particle stacks from well-resolved 3D classes were chosen and final 3D
622 refinements with post-processing performed. For polyclonal samples, the initial 3D
623 classifications were used to estimate the epitope occupancy for each polyclonal sample (Figure
624 S4), and the particles from all Fab-bound classes were combined and further analyzed as
625 described below.

626
627 Subsequent analysis of polyclonal samples follows that of Antanasijevic et al.⁵¹ with slight
628 modifications. Briefly, the entire particle stack was refined to a single structure with C3

629 symmetry imposed and then symmetry expanded. The symmetry expanded particle stack was
630 subjected to focused 3D classification without alignment using a 100-Å diameter spherical mask
631 centered on the Fabs bound to the CD4bs of one protomer. Classes showing a Fab-like density
632 were selected and their particles combined and subjected to an unmasked C1 refinement with
633 local angular searches only. The location of the 100-Å mask was adjusted as needed to contain
634 all Fabs observed, and a second round of masked 3D classification without alignment performed.
635 Fab-containing classes were selected and their combined particles were then subjected to a C1
636 refinement with a shaped Fab-trimer mask and local angular searches only, followed by a third
637 round of 3D classification without alignment using a shaped Fab-trimer mask. 3D classes that
638 displayed distinct orientations of the Fab relative to the Env protomer were individually selected,
639 and/or classes deemed sufficiently similar were combined, and their particles subjected to
640 individual refinements with local angular searches only and post-processing.

641
642 **Cryo-EM.** Purified HIV-1 Env stabilized CH505 TF chimeric SOSIP Env trimer (CH505 TF
643 chTrimer) preparations were diluted to a final concentration of about 1 mg/mL in 2 mM Tris pH
644 8.0, 200 mM NaCl and 0.02% sodium azide, were mixed with 6-fold molar excess of DH1285
645 Fab and incubated for 2 hours at room temperature. 2.5 µL of protein was deposited on a
646 Quantifoil 1.2/1.3 holey carbon grid that had been glow discharged for 30 seconds in a PELCO
647 easiGlow Glow Discharge Cleaning System. After a 30 second incubation in > 95% humidity,
648 excess protein was blotted away for 2.5 seconds before the grid was plunge frozen into liquid
649 ethane using a Leica EM GP2 plunge freezer (Leica Microsystems). Cryo-EM data were
650 collected on a FEI Titan Krios microscope (Thermo Fisher Scientific) operated at 300 kV. Data
651 were acquired with a Gatan K3 detector operated in counting mode. Data processing was

652 performed within cryoSPARC⁵² including particle picking, multiple rounds of 2D classification,
653 *ab initio* reconstruction, heterogeneous and homogeneous map refinements, local refinement,
654 and non-uniform map refinements. ChimeraX⁵³, Coot⁵⁴, Isolde⁵⁵ and Phenix⁵⁶ were used for
655 model-building and refinement.

656

657 **Epitope-Specific Single B Cell Sorting.** Peripheral blood B cell sorting was performed as
658 described previously^{57,58}. Briefly, cryopreserved PBMC were stained with viability dye, CD14,
659 CD16, CD20, CD3, CD27, IgD, fluorophore-labeled Man₅GlcNAc₂-enriched
660 CH505.M5.G458Y, and fluorophore-labeled Man₅GlcNAc₂-enriched CH505.M5.G458Y.N280D
661 Env Trimer. Env trimers with C-terminal avi-tags (Avidity) were biotinylated and conjugated to
662 streptavidin labeled with different fluorochromes. Live, IgD-negative single B cells that bound to
663 CH505.M5.G458Y but not CH505.M5.G458Y.N280D were sorted into cell lysis buffer and 5X
664 first-strand synthesis buffer in individual wells of a 96-well PCR plate. Plates were frozen on dry
665 ice and ethanol immediately and stored at -80 °C until reverse transcription of RNA.

666

667 **Rhesus Immunoglobulin RT-PCR.** Immunoglobulin genes were amplified as previously
668 described^{57,58}. Immunoglobulin genes from a single B cell were reverse transcribed with
669 Superscript III (ThermoFisher) and constant region-specific reverse primers. Five microliters of
670 complementary DNA were used for two rounds of nested PCR. PCR amplicons were purified
671 and sequenced with 4 µM of forward and reverse primers. Contigs of the forward and reverse
672 antibody sequences were made by the Duke automated sequence analysis pipeline (Duke ASAP).
673 Immunogenetics of rhesus macaque immunoglobulin genes were determined with the macaque
674 heavy and light chain reference library in Cloanalyst, where IGHV1-h is the closest v-gene

675 segment based on sequence identity to human IGHV1-46. Rhesus IGHV gene segments were
676 subsequently annotated using the macaque sequence database in KIMDB where genes denoted
677 IGHV1-h by Cloanalyst were called as IGHV1-105*01^{39,59}. Recombination summaries of the
678 immunogenetics of identified antibodies were partitioned into clones and an unmutated common
679 ancestor (UCA) was inferred from select clones with the infer UCA function in Cloanalyst. A
680 phylogenetic tree for the DH1285 lineage was generated using the heavy chain sequences since
681 light chain gene clonality has a certain degree of uncertainty due to the single V and J junction
682 used to determine clonal relatedness. Each antibody with interpretable sequencing was expressed
683 via a linear DNA cassette in Expi293F cells (Thermo Fisher Scientific, Cat No. A14527). Cell
684 culture media was tested for binding to HIV-1 envelope. Antibodies with binding or
685 immunogenetics of interest were synthesized and cloned into gamma, kappa, or lambda
686 expression vectors (GenScript). Plasmids were prepared for transient transfection of Expi293F
687 cells using the MidiPrep plasmid plus kit (Qiagen). Antibodies were produced in Expi293F cells
688 (Thermo Fisher Scientific, Cat No. A14527).

689
690 **Next generation sequencing of antibody genes.** Illumina MiSeq sequencing of antibody heavy
691 chain VDJ and VK sequences was performed on peripheral B cells from week 32 as previously
692 described⁴⁹. Total RNA was isolated from PBMCs and two independent cDNA samples were
693 generated for library construction. We expect sequences error due to NGS sample preparation to
694 be less than four base pairs (<1%) based on previous experiments. Thus, inference of the
695 unmutated common ancestor and intermediate antibodies was performed with sequences
696 observed in sequencing runs of both cDNA samples. V, D, and J gene segment inference, clonal

697 relatedness testing and reconstruction of clonal lineage trees were performed using the
698 Cloanalyst software package⁵⁹.

699

700 **Biolayer interferometry (BLI).** Biolayer interferometry was performed as previously
701 described⁶⁰. BLI ligand titration and binding kinetics assays were performed on an Octet Red96e
702 system (Sartorius) at 30°C with an orbital shake speed of 1000 rpm. Assays were performed in
703 flat bottom 96-well plates with assays (Greiner) using 0.22μm-filtered Phosphate buffered saline
704 supplemented with 0.05% Tween 20 and 0.1% bovine serum albumin (PBS-T-BSA). For the
705 ligand titration experiment, two-fold serial dilutions of the biotinylated avi-tagged CH505 TF
706 chSOSIPv4.1 and Man₅GlcNAc₂-enriched CH505 M5.G458Y chSOSIPv4.1 Env trimers were
707 immobilized on hydrated Streptavidin (SA) Tips (Octet[®] Sartorius) for 120 seconds. Env
708 immobilization concentration started at 10 μg/mL. After a 60 second wash and 180 second
709 Baseline step in PBS-T-BSA, the tips were incubated with 1000nM or 500 nM of IgG or Fab for
710 300 seconds. The optimal Env concentrations for affinity studies were analyzed from the
711 sensorgram traces and binding responses using Data Analysis HT 12.0 software (Forte Bio).
712 Ligand loading conditions where ligand loading was linear and Fab association reached
713 approximately 0.4 nm were selected. Binding kinetics experiment were performed with optimal
714 Env concentrations. Env was immobilized on hydrated SA biosensor tips and incubated with
715 two-fold serial dilutions of antibody IgG or Fabs for 300 seconds followed by a 600-second-long
716 dissociation step in PBS-T-BSA. The binding response sensorgram curves were globally fitted
717 using a 1:1 Binding Model and the rate constants k_a, k_d and K_d were calculated from 4 or more
718 curves using Data Analysis HT 12.0 software (Forte Bio).

719

720 **Indirect ELISA Using Env Trimers.** Corning 384-well plates were coated overnight at 4 °C
721 with 15 μ L of either streptavidin (for biotinylated proteins) or the base-binding RM19R,
722 expressed with a human IgG constant region diluted to 2 μ g/mL in 0.1M NaCO₃. Plates were
723 washed with PBS-T (PBS + 0.05% Tween-20), then all sample wells were blocked for 1 hour at
724 room temperature (all subsequent steps) with 40 μ L blocking buffer (PBS + 15% v/v goat serum
725 + 4% w/w whey protein + 0.05% v/v Tween-20). Plates were again washed, then incubated with
726 15 μ L/well Env diluted to 2 μ g/mL in blocking buffer for 1 hour. Plates were washed again, then
727 incubated with 10 μ L of a serial dilution of rhesus antibodies or serum beginning at a
728 concentration of 100 μ g/mL or 1:30, respectively in blocking buffer for 1.5 hours. Samples were
729 again washed, then incubated with anti-rhesus IgG conjugated to HRP (Southern Biotech Cat.
730 No.: 4700-05) diluted in blocking buffer for 1 hour. Plates were again washed, then developed
731 with 20 μ L/well of a tetramethylbenzidine peroxidase substrate (SeraCare) for 15 minutes, then
732 quenched with equal volume 1% HCl. Absorbance was measured at 450 nm on a SpectraMax
733 340 PC.

734

735 **Indirect ELISA Using Env gp120.** Corning 384-well plates were coated overnight at 4 °C with
736 15 μ L of gp120 diluted to 2 μ g/mL in 0.1M NaCO₃. Plates were washed with PBS-T, then all
737 sample wells were blocked for 1 hour at room temperature (all subsequent steps) with 40 μ L
738 blocking buffer. Plates were washed again, then incubated with 10 μ L of a serial dilution of
739 rhesus antibodies or serum beginning at a concentration of 100 μ g/mL or 1:30, respectively in
740 blocking buffer for 1.5 hours. Samples were again washed, then incubated with anti-rhesus IgG
741 conjugated to HRP (Southern Biotech Cat. No.: 4700-05) diluted in blocking buffer for 1 hour.
742 Plates were again washed, then developed with 20 μ L/well of a tetramethylbenzidine peroxidase

743 substrate (SeraCare) for 15 minutes, then quenched with equal volume 1% HCl. Absorbance was
744 measured at 450 nm on a SpectraMax 340 PC.

745
746 **Competition ELISAs.** Corning 384-well plates were coated overnight at 4 °C with 15 µL
747 streptavidin diluted to 2 µg/mL in 0.1M NaCO₃. Plates were washed with PBS-T, then all sample
748 wells were blocked for 1 hour at room temperature (all subsequent steps) with 40 µL blocking
749 buffer (PBS + 15% v/v goat serum + 4% w/w whey protein + 0.05% v/v Tween-20). Plates were
750 again washed, then incubated with 15 µL/well Env diluted to 2 µg/mL in blocking buffer for 1
751 hour. Plates were washed again, then incubated with 10 µL/well 2G12 or CH235.12 (human
752 IgG) diluted to 50 µg/mL in blocking buffer. Plates were washed again, then incubated with 10
753 µL of a serial dilution of rhesus antibodies or serum beginning at a concentration of 100 µg/mL
754 or 1:30, respectively in blocking buffer for 1.5 hours. Samples were again washed, then
755 incubated with anti-rhesus IgG conjugated to HRP (Southern Biotech Cat. No.: 4700-05) diluted
756 in blocking buffer for 1 hour. Plates were again washed, then developed with 20 µL/well of a
757 tetramethylbenzidine peroxidase substrate (SeraCare) for 15 minutes, then quenched with equal
758 volume 1% HCl. Absorbance was measured at 450 nm on a SpectraMax 340 PC.

759

760 **List of Supplementary Materials**

761 Figure S1 to S11

762 Table S1

763 **References**

- 764 1. Corey, L., Gilbert, P.B., Juraska, M., Montefiori, D.C., Morris, L., Karuna, S.T., Edupuganti, S., Mgodi,
765 N.M., deCamp, A.C., Rudnicki, E., et al. (2021). Two Randomized Trials of Neutralizing Antibodies to
766 Prevent HIV-1 Acquisition. *N Engl J Med* 384, 1003-1014. 10.1056/NEJMoa2031738.
- 767 2. Moldt, B., Rakasz, E.G., Schultz, N., Chan-Hui, P.Y., Swiderek, K., Weisgrau, K.L., Piaskowski, S.M.,
768 Bergman, Z., Watkins, D.I., Poignard, P., and Burton, D.R. (2012). Highly potent HIV-specific antibody

769 neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc
770 Natl Acad Sci U S A 109, 18921-18925. 10.1073/pnas.1214785109.

771 3. Mascola, J.R., Stiegler, G., VanCott, T.C., Katinger, H., Carpenter, C.B., Hanson, C.E., Beary, H., Hayes,
772 D., Frankel, S.S., Birx, D.L., and Lewis, M.G. (2000). Protection of macaques against vaginal transmission
773 of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 6, 207-
774 210. 10.1038/72318.

775 4. Ko, S.Y., Pegu, A., Rudicell, R.S., Yang, Z.Y., Joyce, M.G., Chen, X., Wang, K., Bao, S., Kraemer, T.D.,
776 Rath, T., et al. (2014). Enhanced neonatal Fc receptor function improves protection against primate SHIV
777 infection. Nature 514, 642-645. 10.1038/nature13612.

778 5. Haynes, B.F., Wiehe, K., Borrrow, P., Saunders, K.O., Korber, B., Wagh, K., McMichael, A.J., Kelsoe, G.,
779 Hahn, B.H., Alt, F., and Shaw, G.M. (2022). Strategies for HIV-1 vaccines that induce broadly neutralizing
780 antibodies. Nat Rev Immunol, 1-17. 10.1038/s41577-022-00753-w.

781 6. Kwong, P.D., and Mascola, J.R. (2018). HIV-1 Vaccines Based on Antibody Identification, B Cell
782 Ontogeny, and Epitope Structure. Immunity 48, 855-871. 10.1016/j.immuni.2018.04.029.

783 7. Zhou, T., Georgiev, I., Wu, X., Yang, Z.Y., Dai, K., Finzi, A., Kwon, Y.D., Scheid, J.F., Shi, W., Xu, L., et
784 al. (2010). Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 329,
785 811-817. 10.1126/science.1192819.

786 8. Wu, X., Zhou, T., Zhu, J., Zhang, B., Georgiev, I., Wang, C., Chen, X., Longo, N.S., Louder, M., McKee,
787 K., et al. (2011). Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep
788 sequencing. Science 333, 1593-1602. 10.1126/science.1207532.

789 9. Zhou, T., Lynch, R.M., Chen, L., Acharya, P., Wu, X., Doria-Rose, N.A., Joyce, M.G., Lingwood, D.,
790 Soto, C., Bailer, R.T., et al. (2015). Structural Repertoire of HIV-1-Neutralizing Antibodies Targeting the
791 CD4 Supersite in 14 Donors. Cell 161, 1280-1292. 10.1016/j.cell.2015.05.007.

792 10. Zhou, T., Zhu, J., Wu, X., Moquin, S., Zhang, B., Acharya, P., Georgiev, I.S., Alatae-Tran, H.R., Chuang,
793 G.Y., Joyce, M.G., et al. (2013). Multidonor analysis reveals structural elements, genetic determinants, and
794 maturation pathway for HIV-1 neutralization by VRC01-class antibodies. Immunity 39, 245-258.
795 10.1016/j.immuni.2013.04.012.

796 11. Scheid, J.F., Mouquet, H., Ueberheide, B., Diskin, R., Klein, F., Oliveira, T.Y., Pietzsch, J., Fenyo, D.,
797 Abadir, A., Velinzon, K., et al. (2011). Sequence and structural convergence of broad and potent HIV
798 antibodies that mimic CD4 binding. Science 333, 1633-1637. 10.1126/science.1207227.

799 12. Jardine, J.G., Kulp, D.W., Havenar-Daughton, C., Sarkar, A., Briney, B., Sok, D., Sesterhenn, F., Ereno-
800 Orbea, J., Kalyuzhniy, O., Deresa, I., et al. (2016). HIV-1 broadly neutralizing antibody precursor B cells
801 revealed by germline-targeting immunogen. Science 351, 1458-1463. 10.1126/science.aad9195.

802 13. Bonsignori, M., Zhou, T., Sheng, Z., Chen, L., Gao, F., Joyce, M.G., Ozorowski, G., Chuang, G.Y.,
803 Schramm, C.A., Wiehe, K., et al. (2016). Maturation Pathway from Germline to Broad HIV-1 Neutralizer
804 of a CD4-Mimic Antibody. Cell 165, 449-463. 10.1016/j.cell.2016.02.022.

805 14. Schommers, P., Gruell, H., Abernathy, M.E., Tran, M.K., Dingens, A.S., Gristick, H.B., Barnes, C.O.,
806 Schoofs, T., Schlotz, M., Vanshylla, K., et al. (2020). Restriction of HIV-1 Escape by a Highly Broad and
807 Potent Neutralizing Antibody. Cell 180, 471-489 e422. 10.1016/j.cell.2020.01.010.

808 15. LaBranche, C.C., Henderson, R., Hsu, A., Behrens, S., Chen, X., Zhou, T., Wiehe, K., Saunders, K.O.,
809 Alam, S.M., Bonsignori, M., et al. (2019). Neutralization-guided design of HIV-1 envelope trimers with
810 high affinity for the unmutated common ancestor of CH235 lineage CD4bs broadly neutralizing antibodies.
811 PLoS Pathog 15, e1008026. 10.1371/journal.ppat.1008026.

812 16. Jardine, J., Julien, J.P., Menis, S., Ota, T., Kalyuzhniy, O., McGuire, A., Sok, D., Huang, P.S.,
813 MacPherson, S., Jones, M., et al. (2013). Rational HIV immunogen design to target specific germline B cell
814 receptors. Science 340, 711-716. 10.1126/science.1234150.

815 17. Tian, M., Cheng, C., Chen, X., Duan, H., Cheng, H.L., Dao, M., Sheng, Z., Kimble, M., Wang, L., Lin, S.,
816 et al. (2016). Induction of HIV Neutralizing Antibody Lineages in Mice with Diverse Precursor
817 Repertoires. Cell 166, 1471-1484 e1418. 10.1016/j.cell.2016.07.029.

818 18. Sok, D., Briney, B., Jardine, J.G., Kulp, D.W., Menis, S., Pauthner, M., Wood, A., Lee, E.C., Le, K.M.,
819 Jones, M., et al. (2016). Priming HIV-1 broadly neutralizing antibody precursors in human Ig loci
820 transgenic mice. Science 353, 1557-1560. 10.1126/science.aah3945.

821 19. Dosenovic, P., von Boehmer, L., Escalano, A., Jardine, J., Freund, N.T., Gitlin, A.D., McGuire, A.T., Kulp,
822 D.W., Oliveira, T., Scharf, L., et al. (2015). Immunization for HIV-1 Broadly Neutralizing Antibodies in
823 Human Ig Knockin Mice. Cell 161, 1505-1515. 10.1016/j.cell.2015.06.003.

824 20. Medina-Ramirez, M., Garces, F., Escolano, A., Skog, P., de Taeye, S.W., Del Moral-Sanchez, I., McGuire, A.T., Yasmeen, A., Behrens, A.J., Ozorowski, G., et al. (2017). Design and crystal structure of a native-like
825 HIV-1 envelope trimer that engages multiple broadly neutralizing antibody precursors in vivo. *J Exp Med*
826 214, 2573-2590. 10.1084/jem.20161160.

827 21. Saunders, K.O., Wiehe, K., Tian, M., Acharya, P., Bradley, T., Alam, S.M., Go, E.P., Scearce, R.,
828 Sutherland, L., Henderson, R., et al. (2019). Targeted selection of HIV-specific antibody mutations by
829 engineering B cell maturation. *Science* 366. 10.1126/science.aay7199.

830 22. Gristick, H.B., Hartweger, H., Loewe, M., van Schooten, J., Ramos, V., Oliveira, T.Y., Nishimura, Y.,
831 Koranda, N.S., Wall, A., Yao, K.H., et al. (2023). CD4 binding site immunogens elicit heterologous anti-
832 HIV-1 neutralizing antibodies in transgenic and wild-type animals. *Sci Immunol* 8, eade6364.
833 10.1126/sciimmunol.ade6364.

834 23. Haynes, B.F., Kelsoe, G., Harrison, S.C., and Kepler, T.B. (2012). B-cell-lineage immunogen design in
835 vaccine development with HIV-1 as a case study. *Nat Biotechnol* 30, 423-433. 10.1038/nbt.2197.

836 24. Klein, F., Mouquet, H., Dosenovic, P., Scheid, J.F., Scharf, L., and Nussenzweig, M.C. (2013). Antibodies
837 in HIV-1 vaccine development and therapy. *Science* 341, 1199-1204. 10.1126/science.1241144.

838 25. Stamatatos, L., Pancera, M., and McGuire, A.T. (2017). Germline-targeting immunogens. *Immunol Rev*
839 275, 203-216. 10.1111/imr.12483.

840 26. Hoot, S., McGuire, A.T., Cohen, K.W., Strong, R.K., Hangartner, L., Klein, F., Diskin, R., Scheid, J.F.,
841 Sather, D.N., Burton, D.R., and Stamatatos, L. (2013). Recombinant HIV envelope proteins fail to engage
842 germline versions of anti-CD4bs bNAbs. *PLoS Pathog* 9, e1003106. 10.1371/journal.ppat.1003106.

843 27. Liao, H.X., Lynch, R., Zhou, T., Gao, F., Alam, S.M., Boyd, S.D., Fire, A.Z., Roskin, K.M., Schramm,
844 C.A., Zhang, Z., et al. (2013). Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus.
845 *Nature* 496, 469-476. 10.1038/nature12053.

846 28. Gao, F., Bonsignori, M., Liao, H.X., Kumar, A., Xia, S.M., Lu, X., Cai, F., Hwang, K.K., Song, H., Zhou,
847 T., et al. (2014). Cooperation of B cell lineages in induction of HIV-1-broadly neutralizing antibodies. *Cell*
848 158, 481-491. 10.1016/j.cell.2014.06.022.

849 29. Alameh, M.G., Tombacz, I., Bettini, E., Lederer, K., Sittplangkoon, C., Wilmore, J.R., Gaudette, B.T.,
850 Soliman, O.Y., Pine, M., Hicks, P., et al. (2022). Lipid nanoparticles enhance the efficacy of mRNA and
851 protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. *Immunity* 55,
852 1136-1138. 10.1016/j.jimmuni.2022.05.007.

853 30. Zhou, T., Doria-Rose, N.A., Cheng, C., Stewart-Jones, G.B.E., Chuang, G.Y., Chambers, M., Druz, A.,
854 Geng, H., McKee, K., Kwon, Y.D., et al. (2017). Quantification of the Impact of the HIV-1-Glycan Shield
855 on Antibody Elicitation. *Cell Rep* 19, 719-732. 10.1016/j.celrep.2017.04.013.

856 31. McGuire, A.T., Hoot, S., Dreyer, A.M., Lippy, A., Stuart, A., Cohen, K.W., Jardine, J., Menis, S., Scheid,
857 J.F., West, A.P., et al. (2013). Engineering HIV envelope protein to activate germline B cell receptors of
858 broadly neutralizing anti-CD4 binding site antibodies. *J Exp Med* 210, 655-663. 10.1084/jem.20122824.

859 32. Havenar-Daughton, C., Reiss, S.M., Carnathan, D.G., Wu, J.E., Kendric, K., Torrents de la Pena, A.,
860 Kasturi, S.P., Dan, J.M., Bothwell, M., Sanders, R.W., et al. (2016). Cytokine-Independent Detection of
861 Antigen-Specific Germinal Center T Follicular Helper Cells in Immunized Nonhuman Primates Using a
862 Live Cell Activation-Induced Marker Technique. *J Immunol* 197, 994-1002. 10.4049/jimmunol.1600320.

863 33. Reiss, S., Baxter, A.E., Cirelli, K.M., Dan, J.M., Morou, A., Daigneault, A., Brassard, N., Silvestri, G.,
864 Routy, J.P., Havenar-Daughton, C., et al. (2017). Comparative analysis of activation induced marker (AIM)
865 assays for sensitive identification of antigen-specific CD4 T cells. *PLoS One* 12, e0186998.
866 10.1371/journal.pone.0186998.

867 34. Yang, Z., Dam, K.A., Bridges, M.D., Hoffmann, M.A.G., DeLaitisch, A.T., Gristick, H.B., Escolano, A.,
868 Gautam, R., Martin, M.A., Nussenzweig, M.C., et al. (2022). Neutralizing antibodies induced in
869 immunized macaques recognize the CD4-binding site on an occluded-open HIV-1 envelope trimer. *Nat
870 Commun* 13, 732. 10.1038/s41467-022-28424-3.

871 35. West, A.P., Jr., Diskin, R., Nussenzweig, M.C., and Bjorkman, P.J. (2012). Structural basis for germ-line
872 gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120. *Proc Natl Acad
873 Sci U S A* 109, E2083-2090. 10.1073/pnas.1208984109.

874 36. Scharf, L., West, A.P., Jr., Gao, H., Lee, T., Scheid, J.F., Nussenzweig, M.C., Bjorkman, P.J., and Diskin,
875 R. (2013). Structural basis for HIV-1 gp120 recognition by a germ-line version of a broadly neutralizing
876 antibody. *Proc Natl Acad Sci U S A* 110, 6049-6054. 10.1073/pnas.1303682110.

877

878 37. Scharf, L., West, A.P., Sievers, S.A., Chen, C., Jiang, S., Gao, H., Gray, M.D., McGuire, A.T., Scheid, J.F.,
879 Nussenzweig, M.C., et al. (2016). Structural basis for germline antibody recognition of HIV-1
880 immunogens. *Elife* 5. 10.7554/eLife.13783.

881 38. Diskin, R., Scheid, J.F., Marcovecchio, P.M., West, A.P., Jr., Klein, F., Gao, H., Gnanapragasam, P.N.,
882 Abadir, A., Seaman, M.S., Nussenzweig, M.C., and Bjorkman, P.J. (2011). Increasing the potency and
883 breadth of an HIV antibody by using structure-based rational design. *Science* 334, 1289-1293.
884 10.1126/science.1213782.

885 39. Vazquez Bernat, N., Corcoran, M., Nowak, I., Kaduk, M., Castro Dopico, X., Narang, S., Maisonasse, P.,
886 Dereuddre-Bosquet, N., Murrell, B., and Karlsson Hedestam, G.B. (2021). Rhesus and cynomolgus
887 macaque immunoglobulin heavy-chain genotyping yields comprehensive databases of germline VDJ
888 alleles. *Immunity* 54, 355-366 e354. 10.1016/j.immuni.2020.12.018.

889 40. Hossain, M.A., Anasti, K., Watts, B., Cronin, K., Derking, R., Groschel, B., Kane, A.P., Edwards, R.J.,
890 Easterhoff, D., Zhang, J., et al. (2022). B cells expressing IgM B cell receptors of HIV-1 neutralizing
891 antibodies discriminate antigen affinities by sensing binding association rates. *Cell Rep* 39, 111021.
892 10.1016/j.celrep.2022.111021.

893 41. Leggat, D.J., Cohen, K.W., Willis, J.R., Fulp, W.J., deCamp, A.C., Kalyuzhniy, O., Cottrell, C.A., Menis,
894 S., Finak, G., Ballweber-Fleming, L., et al. (2022). Vaccination induces HIV broadly neutralizing antibody
895 precursors in humans. *Science* 378, eadd6502. 10.1126/science.add6502.

896 42. Bonsignori, M., Scott, E., Wiehe, K., Easterhoff, D., Alam, S.M., Hwang, K.K., Cooper, M., Xia, S.M.,
897 Zhang, R., Montefiori, D.C., et al. (2018). Inference of the HIV-1 VRC01 Antibody Lineage Unmutated
898 Common Ancestor Reveals Alternative Pathways to Overcome a Key Glycan Barrier. *Immunity* 49, 1162-
899 1174 e1168. 10.1016/j.immuni.2018.10.015.

900 43. Haynes, B.F., Burton, D.R., and Mascola, J.R. (2019). Multiple roles for HIV broadly neutralizing
901 antibodies. *Sci Transl Med* 11. 10.1126/scitranslmed.aaz2686.

902 44. Sliepen, K., Ozorowski, G., Burger, J.A., van Montfort, T., Stunnenberg, M., LaBranche, C., Montefiori,
903 D.C., Moore, J.P., Ward, A.B., and Sanders, R.W. (2015). Presenting native-like HIV-1 envelope trimers
904 on ferritin nanoparticles improves their immunogenicity. *Retrovirology* 12, 82. 10.1186/s12977-015-0210-
905 4.

906 45. Tokatlian, T., Read, B.J., Jones, C.A., Kulp, D.W., Menis, S., Chang, J.Y.H., Steichen, J.M., Kumari, S.,
907 Allen, J.D., Dane, E.L., et al. (2019). Innate immune recognition of glycans targets HIV nanoparticle
908 immunogens to germinal centers. *Science* 363, 649-654. 10.1126/science.aat9120.

909 46. He, L., Kumar, S., Allen, J.D., Huang, D., Lin, X., Mann, C.J., Saye-Francisco, K.L., Copps, J., Sarkar, A.,
910 Blizzard, G.S., et al. (2018). HIV-1 vaccine design through minimizing envelope metastability. *Sci Adv* 4,
911 eaau6769. 10.1126/sciadv.aau6769.

912 47. de Taeye, S.W., Ozorowski, G., Torrents de la Pena, A., Guttman, M., Julien, J.P., van den Kerkhof, T.L.,
913 Burger, J.A., Pritchard, L.K., Pugach, P., Yasmeen, A., et al. (2015). Immunogenicity of Stabilized HIV-1
914 Envelope Trimers with Reduced Exposure of Non-neutralizing Epitopes. *Cell* 163, 1702-1715.
915 10.1016/j.cell.2015.11.056.

916 48. Li, M., Gao, F., Mascola, J.R., Stamatatos, L., Polonis, V.R., Koutsoukos, M., Voss, G., Goepfert, P.,
917 Gilbert, P., Greene, K.M., et al. (2005). Human immunodeficiency virus type 1 env clones from acute and
918 early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol*
919 79, 10108-10125. 10.1128/JVI.79.16.10108-10125.2005.

920 49. Saunders, K.O., Nicely, N.I., Wiehe, K., Bonsignori, M., Meyerhoff, R.R., Parks, R., Walkowicz, W.E.,
921 Aussedat, B., Wu, N.R., Cai, F., et al. (2017). Vaccine Elicitation of High Mannose-Dependent
922 Neutralizing Antibodies against the V3-Glycan Broadly Neutralizing Epitope in Nonhuman Primates. *Cell*
923 Rep 18, 2175-2188. 10.1016/j.celrep.2017.02.003.

924 50. Zivanov, J., Nakane, T., Forsberg, B.O., Kimanis, D., Hagen, W.J., Lindahl, E., and Scheres, S.H. (2018).
925 New tools for automated high-resolution cryo-EM structure determination in RELION-3. *Elife* 7.
926 10.7554/eLife.42166.

927 51. Antanasijevic, A., Sewall, L.M., Cottrell, C.A., Carnathan, D.G., Jimenez, L.E., Ngo, J.T., Silverman, J.B.,
928 Groschel, B., Georges, E., Bhiman, J., et al. (2021). Polyclonal antibody responses to HIV Env
929 immunogens resolved using cryoEM. *Nat Commun* 12, 4817. 10.1038/s41467-021-25087-4.

930 52. Punjani, A., Rubinstein, J.L., Fleet, D.J., and Brubaker, M.A. (2017). cryoSPARC: algorithms for rapid
931 unsupervised cryo-EM structure determination. *Nat Methods* 14, 290-296. 10.1038/nmeth.4169.

932 53. Goddard, T.D., Huang, C.C., Meng, E.C., Pettersen, E.F., Couch, G.S., Morris, J.H., and Ferrin, T.E.
933 (2018). UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Sci* 27, 14-25.
934 10.1002/pro.3235.

935 54. Emsley, P., Lohkamp, B., Scott, W.G., and Cowtan, K. (2010). Features and development of Coot. *Acta
936 Crystallogr D Biol Crystallogr* 66, 486-501. 10.1107/S0907444910007493.

937 55. Croll, T.I. (2018). ISOLDE: a physically realistic environment for model building into low-resolution
938 electron-density maps. *Acta Crystallogr D Struct Biol* 74, 519-530. 10.1107/S2059798318002425.

939 56. Afonine, P.V., Poon, B.K., Read, R.J., Sobolev, O.V., Terwilliger, T.C., Urzhumtsev, A., and Adams, P.D.
940 (2018). Real-space refinement in PHENIX for cryo-EM and crystallography. *Acta Crystallogr D Struct
941 Biol* 74, 531-544. 10.1107/S2059798318006551.

942 57. Saunders, K.O., Edwards, R.J., Tilahun, K., Manne, K., Lu, X., Cain, D.W., Wiehe, K., Williams, W.B.,
943 Mansouri, K., Hernandez, G.E., et al. (2022). Stabilized HIV-1 envelope immunization induces neutralizing
944 antibodies to the CD4bs and protects macaques against mucosal infection. *Sci Transl Med* 14, eab05598.
945 10.1126/scitranslmed.abo5598.

946 58. Wiehe, K., Easterhoff, D., Luo, K., Nicely, N.I., Bradley, T., Jaeger, F.H., Dennison, S.M., Zhang, R.,
947 Lloyd, K.E., Stolarchuk, C., et al. (2014). Antibody light-chain-restricted recognition of the site of immune
948 pressure in the RV144 HIV-1 vaccine trial is phylogenetically conserved. *Immunity* 41, 909-918.
949 10.1016/j.jimmuni.2014.11.014.

950 59. Kepler, T.B. (2013). Reconstructing a B-cell clonal lineage. I. Statistical inference of unobserved ancestors.
951 F1000Res 2, 103. 10.12688/f1000research.2-103.v1.

952 60. Alam, S.M., Aussedat, B., Vohra, Y., Ryan Meyerhoff, R., Cale, E.M., Walkowicz, W.E., Radakovich,
953 N.A., Anasti, K., Armand, L., Parks, R., et al. (2017). Mimicry of an HIV broadly neutralizing antibody
954 epitope with a synthetic glycopeptide. *Sci Transl Med* 9. 10.1126/scitranslmed.aai7521.

955

956

957 **Acknowledgments:** We thank Victoria Gee-Lai, Margaret Deyton, Advaiti Khanore, Giovanna
958 Hernandez, and Aja Sanzone for technical assistance. We thank Elizabeth Donahue for
959 program management and assistance with manuscript preparation. Illumina NGS was
960 performed by the DHVI Viral Genetics Analysis Core Facility. Flow cytometry was
961 performed by the DHVI Flow Cytometry Core Facility.

962

963 **Funding:** This study was funded by the National Institutes of Health, National Institute of
964 Allergy and Infectious Diseases, Division of AIDS grant UM1AI144371 for the Duke
965 Consortia for HIV/AIDS Vaccine Development (BFH), U54 AI170752 (PA), and R01
966 AI145687 (PA).

967

968 **Author contributions:**

969 KOS, DW and BFH conceived the macaque immunogenicity study. ML, SS, and LS
970 administered the immunogenicity study and processed samples from the macaque study. JC, RP,
971 MB and NH characterized antibody binding and analyzed results. XL isolated and sequenced the
972 antibodies. JC, NJ, MK, RH, and JB performed protein production for antibodies and envelopes.
973 RE and KatM performed negative stain electron microscopy experiments. VS, KJ, KarM, BT,
974 and PA determined cryo-electron microscopy structures. YC, BH, WBW performed next-
975 generation sequencing of antibody genes. KW and SV analyzed antibody sequences and
976 performed bioinformatic inferences of clonality. KA and SMA performed binding kinetics
977 assays with surface plasmon resonance. JB and KOS performed binding kinetic assays with
978 biolayer interferometry. YT and CB provided lipid nanoparticle adjuvant. CJ, AE, and DCM
979 generated and analyzed serum and monoclonal antibody neutralization results. MAM and DWC
980 performed fluorescence-activated cell sorting and flow cytometry phenotyping experiments.
981 BFH, KOS, PA, BT, RE, and JC wrote the first draft of the manuscript, which was edited by all
982 coauthors. KOS, JC, and BFH reviewed all study data. BFH provided funding for the study.
983

984 **Competing interests:** YT and CB are employees of Acuitas Therapeutics. Acuitas Therapeutics
985 had no role in the execution of the study, data collection, or data interpretation. KOS,
986 DCM, RH, PA, and BFH have patents concerning the envelope immunogens used in this
987 study. All remaining authors declare no competing interests.

988 **Data and materials availability:** All data are available in the main text or the supplementary
989 materials. Cryo-EM data sets have been deposited in the Protein database and electron
990 microscopy database under accessioning numbers EMD-27621 and EMD-27622. All
991 unique reagents generated in this study are available from the lead contact with a
992 completed materials transfer agreement between the donor and recipient institutions.

993 **Lead contact:** Further information and requests for resources and reagents should be directed to
994 and will be fulfilled by the lead contact, Kevin Saunders (kevin.saunders@duke.edu).

995

996 **FIGURE LEGENDS**

997 **Figure 1. CH505.M5.G458Y stabilized gp140 Trimers Induce Serum CD4 Binding Site-
998 Directed Antibodies In Rhesus Macaques.** **(A)** Vaccination of rhesus macaques with
999 Man₅GlcNAc₂-enriched CH505.M5.G458Y Env trimers formulated with ionizable lipid
1000 nanoparticles. **(B)** Serum IgG binding magnitude to Man₅GlcNAc₂-enriched (blue) and
1001 heterogeneously glycosylated (red) vaccine-matched Env. Values are reported as the group mean
1002 and standard error for the area under the log₁₀ transformed concentration curve (logAUC) over
1003 time. Immunization time points indicated by arrows. **(C)** Serum blocking of CD4bs bnAb
1004 CH235.12 (blue) and N332 glycan bnAb 2G12 (gray) binding to CH505.M5.G458Y Env. **(D)**
1005 Serum neutralization ID50 titer against vaccine-matched tier 2 pseudotyped virus increases over
1006 the course of vaccination. Trend line shows group geometric mean (n=3 macaques). **(E)** Week 32
1007 (post-6 immunizations) neutralization activity depends upon germline-targeting mutations
1008 N279K (M5) and G458Y, as well as Man₅GlcNAc₂ enrichment. In **E-H**, different glycoforms of
1009 pseudovirus are color-coded as in **B**. Bars represent group geometric mean titers. Values for
1010 individual macaques are shown as symbols. **(F,G)** Weeks 10 (F, post 3 immunizations) and 32
1011 (G) serum neutralization of CH505.M5.G458Y pseudovirus is greatly diminished in the presence
1012 of CD4bs KO mutation N280D. Values reported above the bars in F and G are fold change in the
1013 group geometric mean titer. **(H)** Removal of the N-linked glycosylation site at Env position 197,
1014 which shields the CD4bs, improves serum neutralization of heterogeneously glycosylated
1015 CH505.M5.G458Y pseudovirus by both the CH235 UCA and vaccinated macaque serum. **(I-K)**

1016 3D reconstruction of negative stain electron microscopy images of serum-derived Fabs bound to
1017 CH505.M5.G458Y Env trimers. **(I)** Top and side view of serum CD4bs antibodies with binding
1018 orientations such that both Fab chains are visible when looking down from the trimer. **(J)** Top
1019 and side view of serum CD4bs antibodies with binding orientations similar to CH235. The Fab
1020 orientation is rotated 90 degrees compared to the Fabs in **I**. **(K)** Superposition of the CH235-
1021 bound Env structure (spheres representation) into the observed density for serum Fabs bound to
1022 Env (surface representation).

1023

1024 **Figure 2. CH505.M5.G458Y-Specific Monoclonal Antibodies from Vaccinated Macaques**
1025 **Demonstrate CD4bs-Directed Binding and Neutralization.** **(A)** Fluorescence-activated sorting
1026 of Env reactive single B cells that lack binding in the presence of CD4 KO substitution N280D.
1027 **(B)** IGHV gene segments used by unique B cell clones isolated from NHP7193 and 7196.
1028 IGHV1-105 is highlighted yellow since it is orthologous to the human IGHV1-46 gene segment
1029 used by CD4bs bnAbs. **(C)** Pseudotyped virus neutralization IC50 titer against autologous virus
1030 with and without $\text{Man}_5\text{GlcNAc}_2$ enrichment. Neutralization was mapped to the CD4bs by
1031 removal of CD4bs bnAb targeting substitutions (N279K or G458Y) or CD4bs knockout
1032 substitution (N280D). **(D)** Percent loss of macaque antibody binding due to competition with
1033 CH235.12 binding to Env (blue), the presence of CD4bs knockout substitution N280D (green),
1034 or heterogenous Env glycosylation (pink) as determined by ELISA. Blocking magnitude or
1035 decrease in binding is determined relative to binding to CH505.M5.G458Y/GnT1⁻. Error bars
1036 represent the standard deviation of three replicates. **(E)** Binding reactivity, reported as logAUC,
1037 for SOSIP trimers (orange) (N=3 independent experiments) or gp120 versions of HIV-1 Env by
1038 ELISA (N=2 independent experiments). Neutralizing antibodies that are derived from IGHV1-

1039 105 and compete with CH235.12 for binding to envelope are marked with stars beside their
1040 names.

1041

1042

1043 **Figure 3. Characterization of Four CH235-like Precursors Isolated from Two Vaccinated**
1044 **Macaques. (A)** Immunogenetics of putative macaque antibody precursors (left) and HCDR3
1045 alignment compared to human CD4bs bnAbs (right). **(B)** Negative stain electron microscopy
1046 shows approach angles for different Fabs binding to the CD4bs of CH505.M5.G458Y. Human
1047 CD4-mimicking bnAb CH235.12 is show for comparison. The gp120 axis is indicated by a black
1048 arrow. Note the gp120 is rotated in the DH1285 bound Env. **(C)** Superposition of CH235.12 and
1049 rhesus Fab in complex with Env. CH235.12 bound to Env is the structure shown in spheres
1050 representation. **(D)** Comparison of Fab binding affinity for M5.G458Y (top) or TF (bottom)
1051 stabilized gp140 Env trimers. Dashed lines indicate limit of detection. **(E)** Macaque nAb IC50
1052 neutralization titers against CH505 pseudovirus variants with different CH235 enabling
1053 substitutions. Macaque nAb titers are compared to the CH235 lineage bnAb putative precursor
1054 (CH235 UCA) or bnAb CH235.12.

1055

1056 **Figure 4. Cryo-EM Structure of DH1285 Bound to CH505 TF Env Demonstrates Antibody**
1057 **Mimicry of CD4 like Human BnAbs. (A)** Cryo-EM reconstruction of DH1285 Fab (heavy
1058 chain in dark blue light chain in light blue) in complex with CH505 TF Env SOSIP (gp120 in
1059 light gray, gp41 in black). **(B)** Cryo-EM reconstruction (shown as a blue mesh) from local
1060 refinement of the gp120/Fab interface, with underlying fitted model shown in cartoon
1061 representation. **(C)** Three views of DH1285 bound to CH505 TF gp120; gp120 colored gray with
1062 the CD4 binding loop colored red, Loop D colored cyan, and Loop V5 colored orange. DH1285
1063 heavy chain colored dark blue with HCDR1, HCDR2 and HCDR3 colored magenta, green and
1064 brown, respectively. DH1285 heavy chain colored dark blue with HCDR1, HCDR2 and HCDR3

1065 colored magenta, green and brown, respectively. DH1285 light chain colored light blue with
1066 LCDR1, LCDR2 and LCDR3 colored pink, light green, and light brown, respectively. **(D)**
1067 Interactions of the CD4 binding loop (red) shown with, (from left to right) CD4, DH1285, ,
1068 CH235, 8ANC131, and VRC01. Residue Asp 368 in gp120, Arg 71 in the antibody heavy
1069 chains and Arg 59 in CD4 are shown in stick representation. The salt bridge between Asp 368
1070 and Arg 71 in the antibodies or between Asp 368 and Arg 59 in CD4 are shown as dashed lines.
1071 **(E)** Surface representation showing the interactions of gp120 Loop D (cyan) with the bound
1072 antibody. Antibody heavy chain is shown in dark blue and light chain in light blue; HCDR1,
1073 HCDR2 and HCDR3 colored magenta, green and brown, respectively

1074 **Figure 5. Molecular Features Are Conserved Among DH1285 And Human CD4-Mimicking**

1075 **BnAbs. (A,B)** DH1285 has the key amino acids for interaction with the CD4bs (antibody amino
1076 acids 50, 54, 58, and 71) that were previously identified in human CD4-mimicking bnAbs. **(A)**
1077 Antibody amino acid interactions by human bnAbs and DH1285 with Env are superimposed
1078 (PDBs:6UDA, 5V8M, 6UDJ, 5WDU, 6V8X). Of note are the interactions between the D loop
1079 and W50, the Phe43 cavity and Y54, the CD4bs loop and R71, and the V5 loop and H58. **(B)**
1080 Comparison of residue identity at key sites listed in **(A)** and their conservation among V_H gene
1081 segment-restricted CD4bs bnAbs. Relevant human and rhesus germlines are shown in the first
1082 three rows. Shared amino acids with DH1285 are shown in bold and highlighted yellow. **(C)**
1083 Binding and neutralization activity of the DH1285 HCDR2, R71, and R73 alanine mutants.
1084 CH505.TF Env trimer binding mean log AUC of three independent experiments and standard
1085 error of mean are shown. $\text{Man}_5\text{GlcNAc}_2$ -enriched CH505 TF pseudovirus neutralization IC_{80}
1086 titers for the DH1285 alanine mutant antibodies are shown under the binding magnitude graph.
1087 **(D)** V_H amino acids encoded for by somatic mutations that are conserved between DH1285 and
1088 VH1-46 bnAbs are shown in colored boxes. Boxes with periods indicate amino acids that are
1089 identical to the CH235 UCA. **(E)** Structural location of conserved DH1285 and VH1-46 bnAb
1090 mutations relative to the HIV-1 gp120 interface. The conserved amino acids are shown as
1091 spheres within the DH1285 variable region and are color-coded as in **(D)**. **(F)** CH505 TF Env
1092 trimer binding by antibodies with conserved amino acids reverted to the VH1-46 germline amino
1093 acid. Binding magnitude is shown as the mean logAUC from triplicate experiments with
1094 standard error of the mean. The heatmap under the bar graph shows the neutralization IC_{80} titer
1095 for the same mutant antibodies against $\text{Man}_5\text{GlcNAc}_2$ -enriched CH505 TF pseudovirus.
1096 Neutralization titer is color-coded as in **C**. **(G)** DH1285 cryo-EM structure showing $\text{D31}_{\text{DH1285}}$

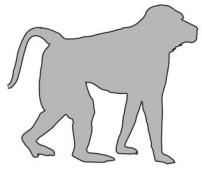
1097 contacts with K474_{Env}. (H) DH1285 cryo-EM structure showing positioning of E23_{DH1285} relative
1098 to other framework region residues with potential structural coordination of the positioning of
1099 the R71 for contact with the CD4bs loop.
1100

1101

1102 **Figure 6. DH1285 Ontogeny Shows the Putative DH1285 Precursor Antibody Binds the**
1103 **Vaccine Immunogen and the Emergence of CH505 TF Env Recognition. (A)** (Top) Apparent
1104 binding affinity and (bottom) association-rate of DH1285 UCA and CH235 UCA IgG binding to
1105 Man₅GlcNAc₂-enriched CH505.M5.G458Y or TF Env trimer. **(B)** Phylogenetic tree of DH1285
1106 clonally-related VH sequences. Terminal nodes show observed sequences with internal nodes
1107 indicating inferred intermediates. Binding magnitude for Man₅GlcNAc₂-enriched
1108 CH505.M5.G458Y and CH505.TF gp140 was determined for each DH1285 clonal member
1109 when paired with the DH1285 light chain. Antibodies that react with CH505 TF Env trimer are
1110 colored blue. Values are the mean of 2 independent experiments. **(C)** Spearman's correlation of
1111 M5.G458Y (blue) Env trimer log₁₀ EC₅₀ binding titer and the number of DH1285 VH amino acid
1112 substitutions. **(D)** Amino acid residues at three sites that mutate when CH505 TF Env trimer
1113 binding is first observed in the DH1285 lineage.

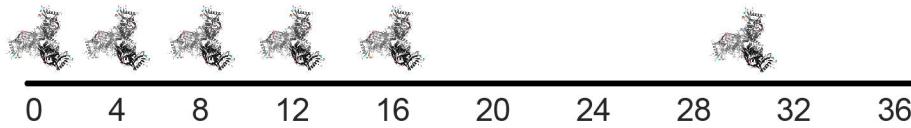
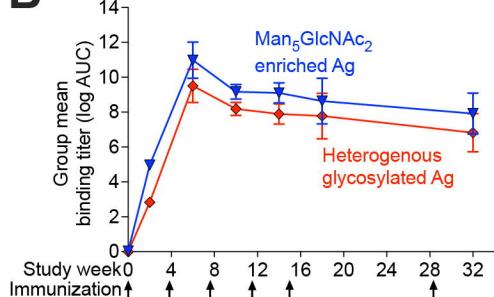
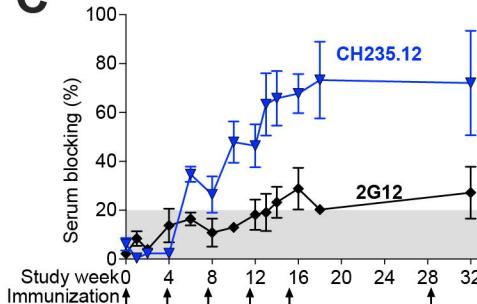
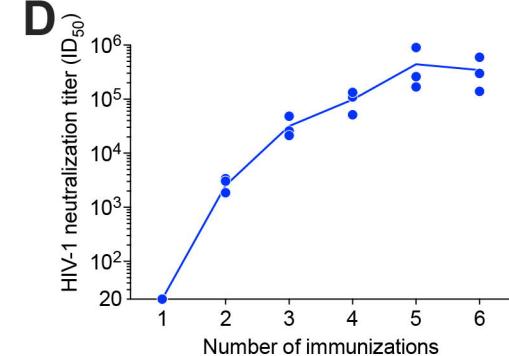
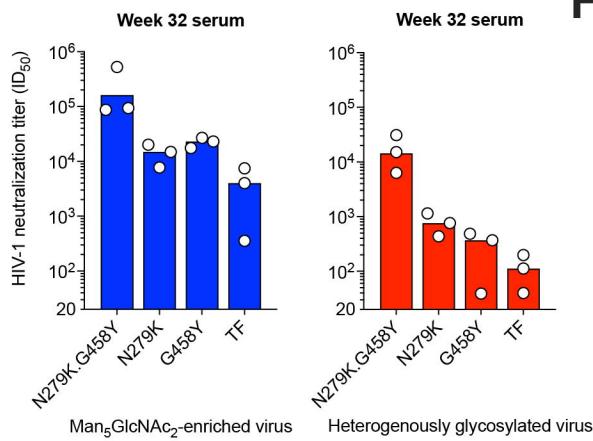
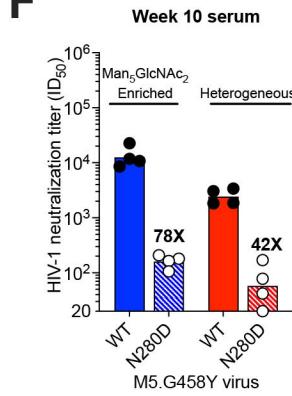
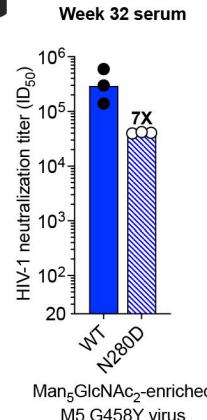
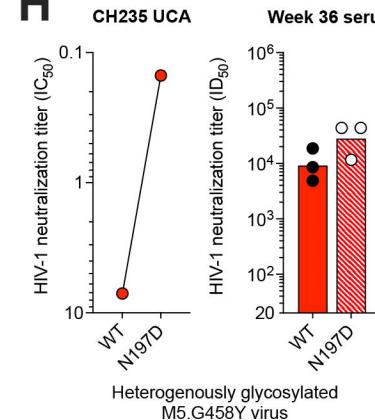
1114

Figure 1

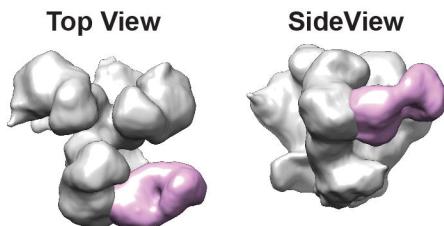
A

Rhesus macaque immunogenicity (n=4)

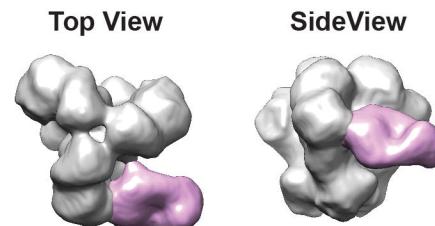
Env + LNP
Immunization
Study week

**B****C****D****E****F****G****H****I**

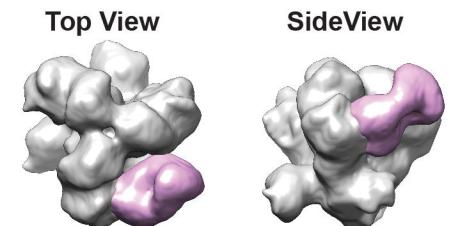
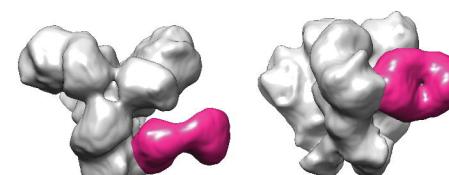
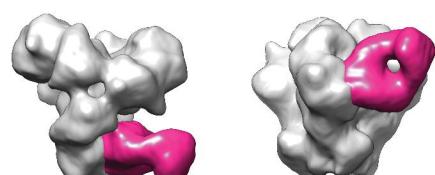
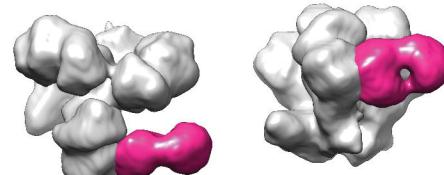
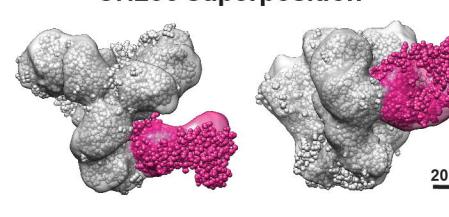
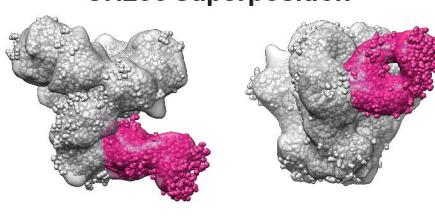
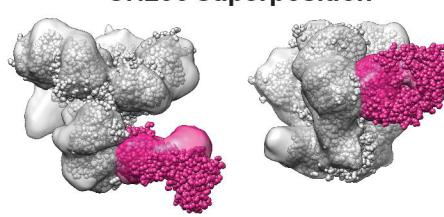
NHP 7193



NHP 7196



NHP 7183

**J****K**

20 Å

Figure 2

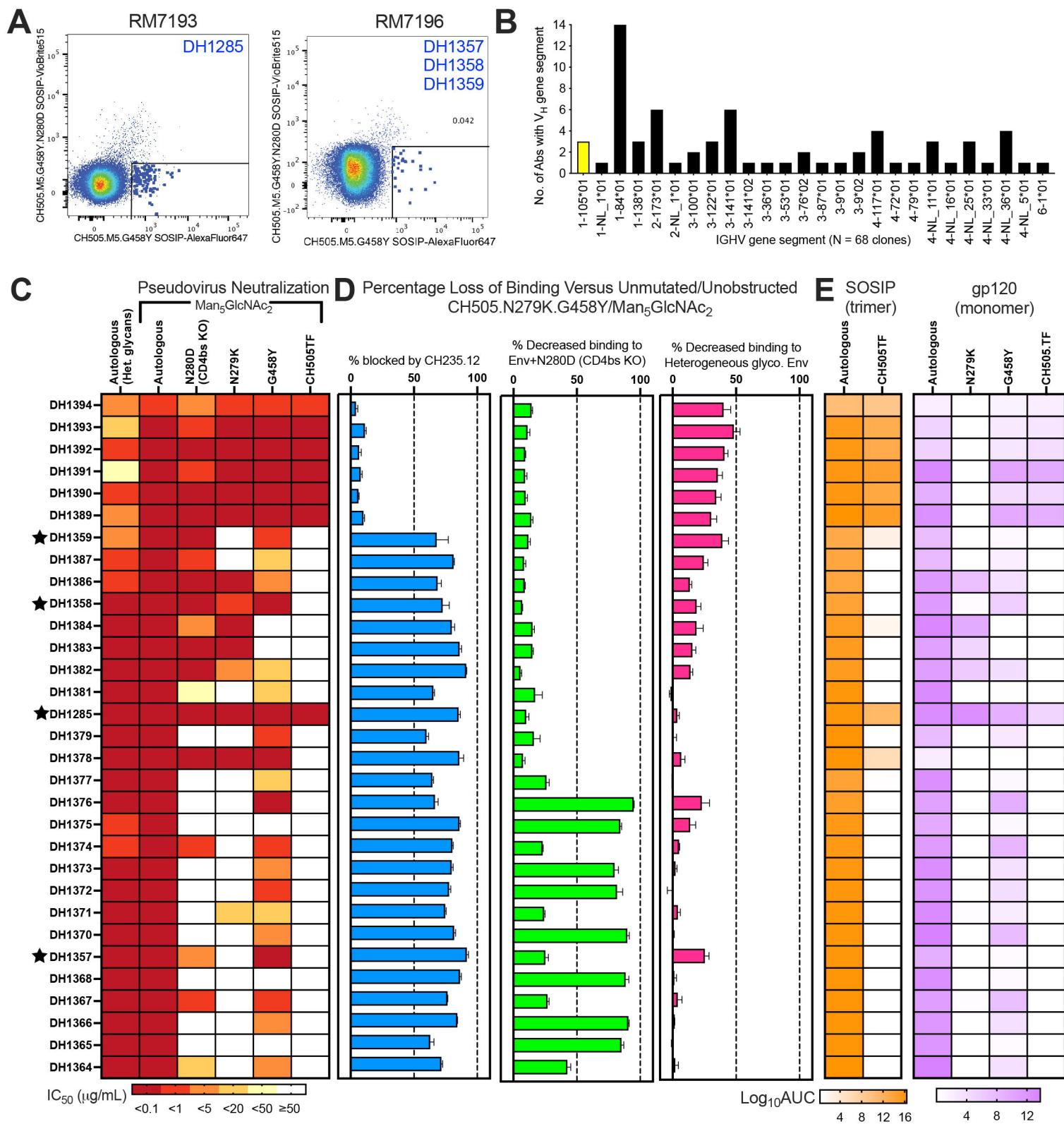


Figure 3

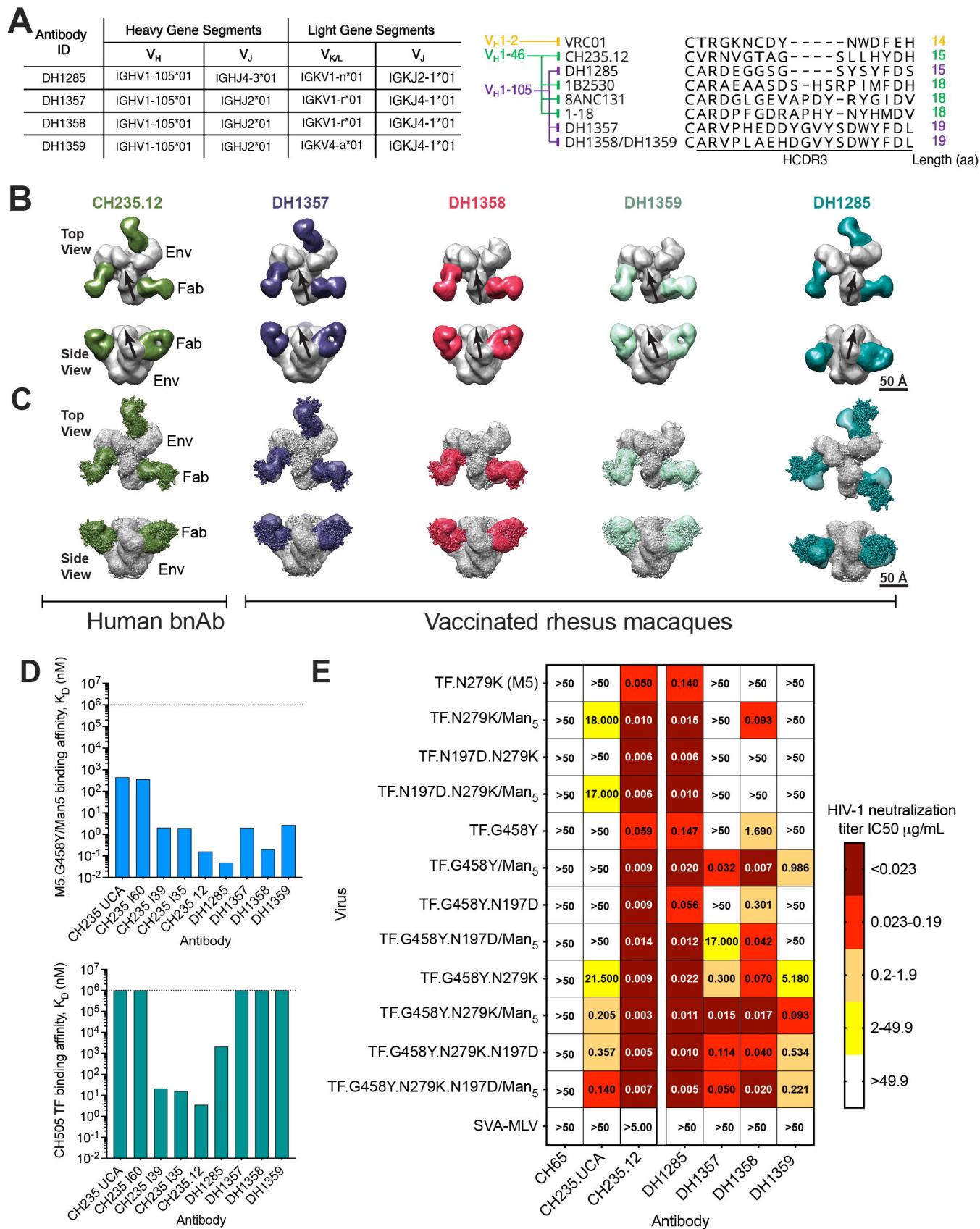


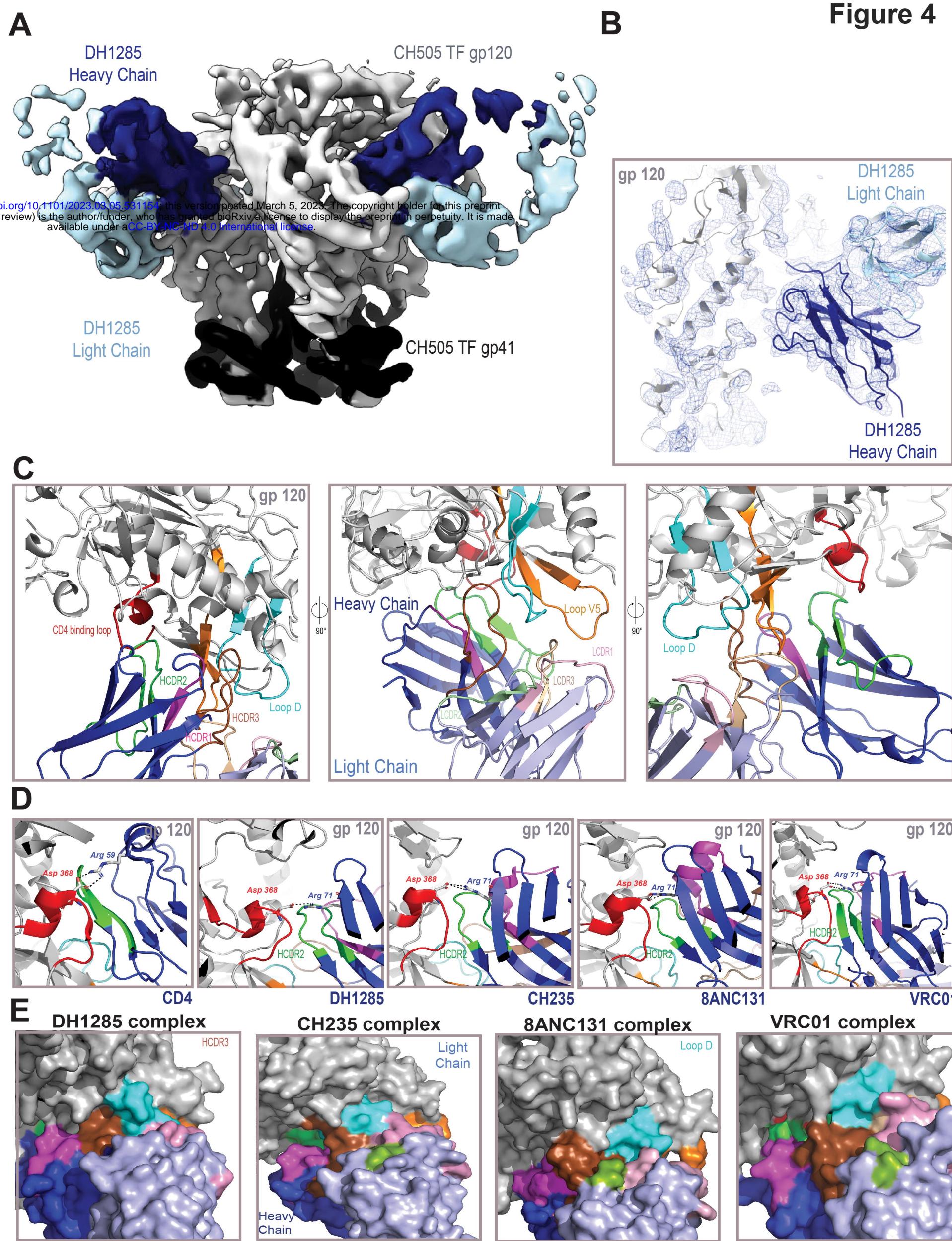
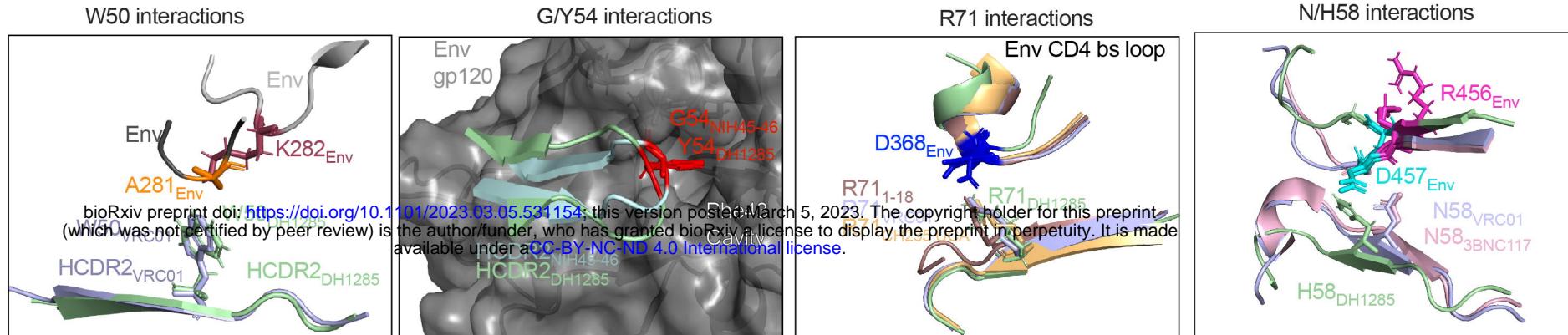
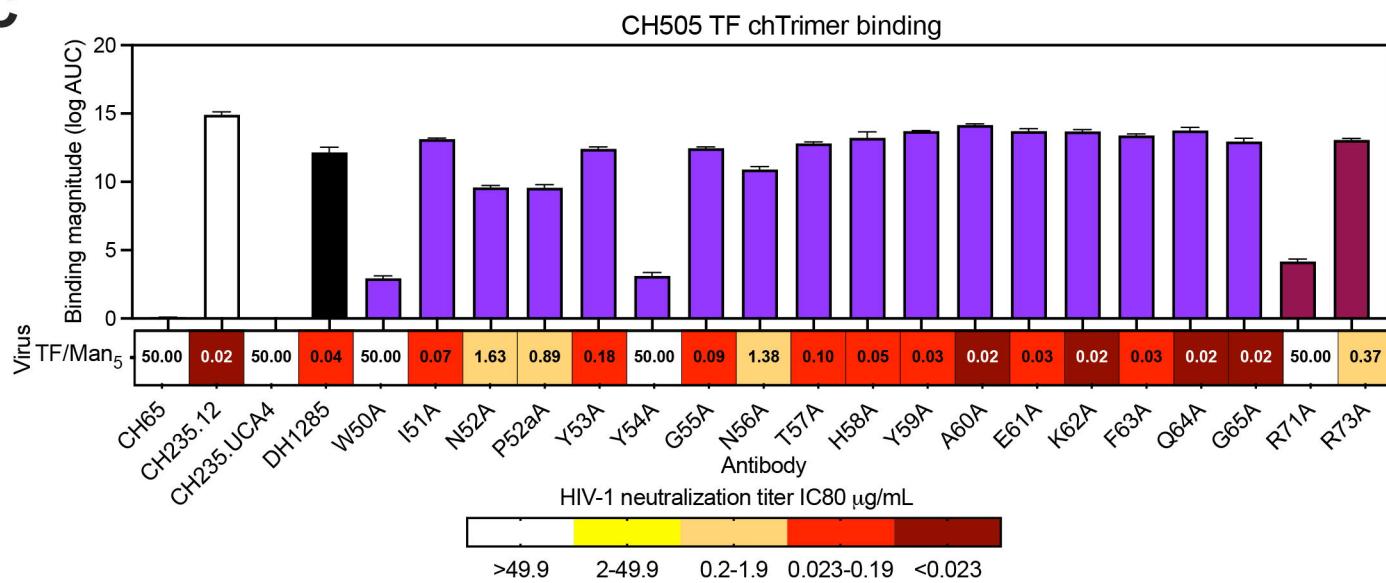
Figure 4

Figure 5

A

B

IGHV Segment	AA position			
	50	54	58	71
IGHV1-2*02	W	S	N	R
IGHV1-46*01	I	G	S	R
RM IGHV1-105*01	W	N	K	R
VRC01	W	G	N	R
NIH45-46	W	G	N	R
12A12	W	Y	N	R
3BNC117	W	T	N	R
IOmA	W	R	K	R
CH235	W	W	N	R
CH235 UCA	I	G	S	R
CH235.12	Y	N	D	R
561-1-18	V	F	I	R
1B2530	M	R	W	R
DH1285	W	Y	H	R

C

D

Antibody	V _H amino acid position								
	19	20	23	31	34	74	76	81	86
CH235 UCA	K	V	K	S	M	T	S	E	R
DH1285	R	L	E	D	I	R	T	D	T
CH235.12	T	L	V	D	I	K	E	D	.
8ANC131	T	I	L	E	I	R	G	.	.
1NC9	T	.	E	N	I	I	E	H	.
8ANC134	T	I	L	E	I	R	G	.	.
1B2530	T	L	Q	K	I	.	E	T	K
561-9-17	.	I	V	K	L	I	T	.	T
561-1-23	.	I	E	K	L	I	T	.	T
561-1-18	R	I	R	K	T	S	T	.	Q

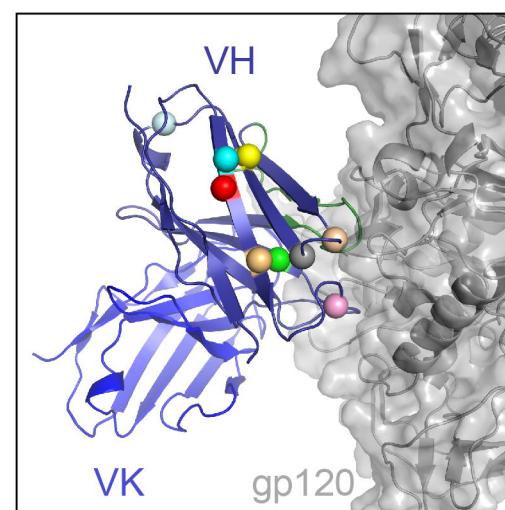
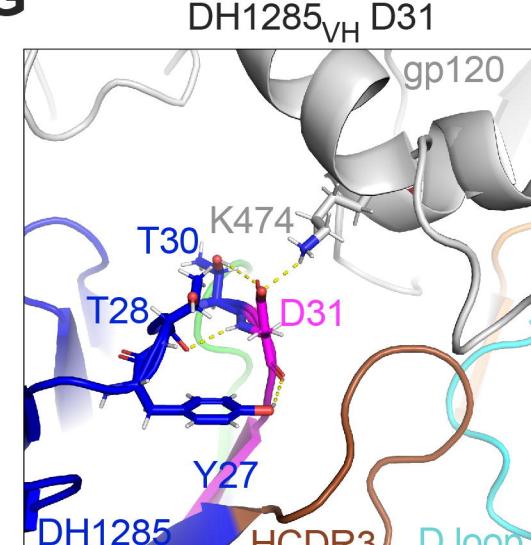
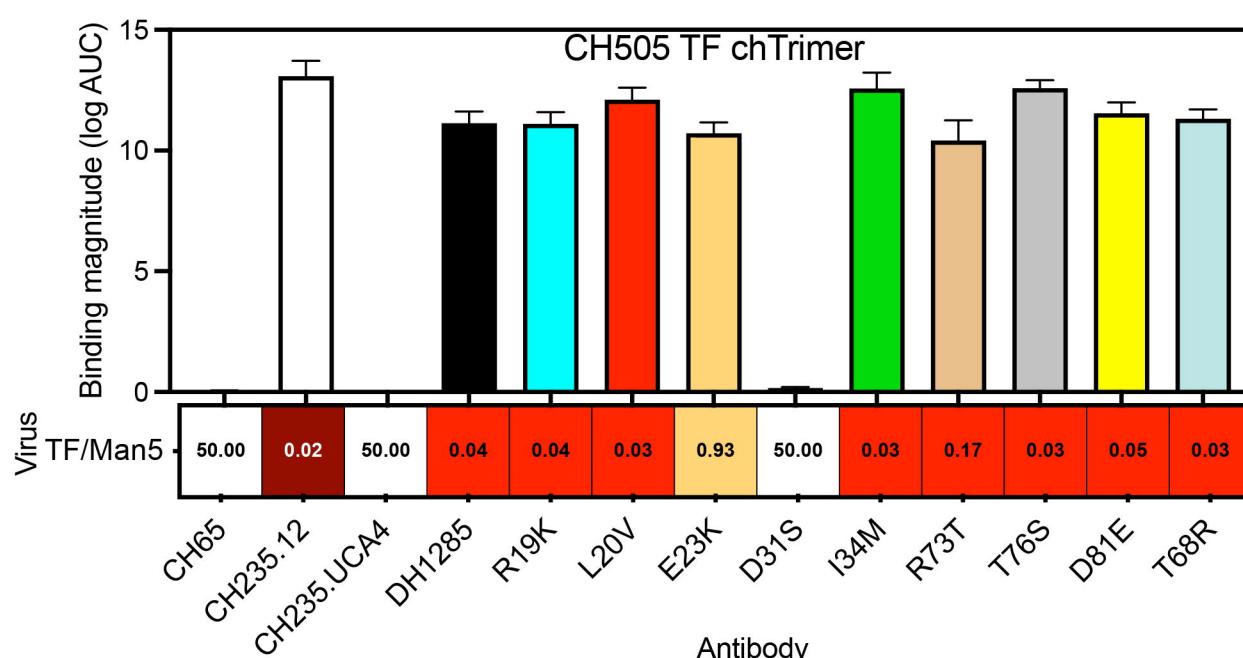
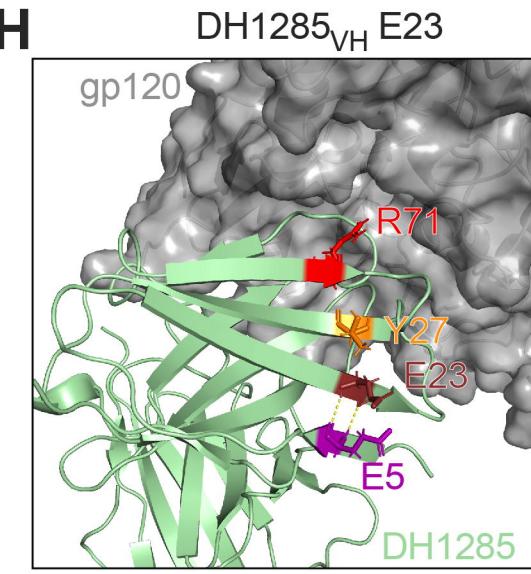
E

G

F

H


Figure 6

