

A public broadly neutralizing antibody class targets a membrane-proximal anchor epitope of influenza virus hemagglutinin

Jenna J. Guthmiller^{1,14*}, Julianna Han^{2,14}, Henry A. Utset¹, Lei Li¹, Linda Yu-Ling Lan³, Carole Henry^{1,4}, Christopher T. Stamper³, Olivia Stovicek¹, Lauren Gentles^{5,6}, Haley L. Dugan³, Nai-Ying Zheng¹, Sara T. Richey², Micah E. Tepora¹, Dalia J. Bitar¹, Siriruk Changrob¹, Shirin Strohmeier⁷, Min Huang¹, Adolfo García-Sastre^{7,8,9,10}, Raffael Nachbagauer^{4,7}, Peter Palese⁷, Jesse D. Bloom^{5,6,11,12}, Florian Krammer⁷, Lynda Coughlan¹³, Andrew B. Ward^{2*}, Patrick C. Wilson^{1,3,15*}

¹Department of Medicine, Section of Rheumatology, University of Chicago, Chicago, IL 60637, USA

²Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037, USA

³Committee on Immunology, University of Chicago, Chicago, IL 60637, USA

⁴Present address: Moderna Inc., Cambridge, MA 02139, USA

⁵Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

⁶Department of Microbiology, University of Washington, Seattle, WA 98195, USA

⁷Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

⁸Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

⁹Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

¹⁰The Tisch Cancer Center, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

¹¹Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA

¹²Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

¹³Department of Microbiology and Immunology and Center for Vaccine

University of Maryland School of Me

¹⁴These authors

¹⁵Lead Contact
*Correspondence: jguthmiller@uchicago.edu (J.J.G.); andrew@scripps.edu (A.B.W.);

31 **Summary**

32 Broadly neutralizing antibodies against influenza virus hemagglutinin (HA) have the potential to
33 provide universal protection against influenza virus infections. Here, we report a distinct class of
34 broadly neutralizing antibodies targeting an epitope toward the bottom of the HA stalk domain
35 where HA is “anchored” to the viral membrane. Antibodies targeting this membrane-proximal
36 anchor epitope utilized a highly restricted repertoire, which encode for two conserved motifs
37 responsible for HA binding. Anchor targeting B cells were common in the human memory B cell
38 repertoire across subjects, indicating pre-existing immunity against this epitope. Antibodies
39 against the anchor epitope at both the serological and monoclonal antibody levels were potently
40 induced in humans by a chimeric HA vaccine, a potential universal influenza virus vaccine.
41 Altogether, this study reveals an underappreciated class of broadly neutralizing antibodies against
42 H1-expressing viruses that can be robustly recalled by a candidate universal influenza virus
43 vaccine.

44

45 **Keywords:** broadly neutralizing antibodies, influenza, hemagglutinin stalk, universal vaccine,
46 public clones

47 **Introduction**

48 Influenza viruses remain a global health problem, with antigenically drifting seasonal viruses and the
49 constant risk of zoonotic influenza virus spillovers into humans. Antibodies against the major surface
50 glycoprotein hemagglutinin (HA) are critical for providing protection against influenza virus infection (Ng et
51 al., 2019). HA is divided into two domains: the globular head and the stalk. Most epitopes of the HA head
52 are highly variable and rapidly mutate to circumvent host humoral immunity (Henry et al., 2019; Kirkpatrick
53 et al., 2018). In contrast, the HA stalk is relatively conserved within and across influenza subtypes (Krystal
54 et al., 1982). Antibodies against the head and the stalk both independently correlate with protection against
55 influenza virus infection (Aydillo et al., 2020; Ng et al., 2019; Ohmit et al., 2011). Therefore, vaccine
56 formulations that preferentially induce antibodies against conserved epitopes of the HA head and stalk
57 domains could provide broad and potent protection against a wide array of influenza viruses.

58 Several broadly neutralizing epitopes have been identified on the HA of H1N1 viruses, including
59 the receptor-binding site (RBS) and lateral patch on the HA head (Ekert et al., 2012; Raymond et al., 2018;
60 Whittle et al., 2011) and the broadly neutralizing (BN) epitope on the HA stalk domain (Ekert et al., 2009;
61 Wrammert et al., 2011). Current seasonal influenza virus vaccines poorly induce antibodies against broadly
62 neutralizing epitopes of HA (Andrews et al., 2015; Corti et al., 2010). Therefore, new vaccine platforms
63 that preferentially drive antibodies against these conserved epitopes are desperately needed to increase
64 vaccine effectiveness against drifted strains and limit influenza morbidity and mortality. It is critically
65 important to drive the humoral immune response simultaneously against multiple conserved epitopes of
66 HA to avoid the generation of viral escape mutants. Notably, escape mutants near the lateral patch
67 (Linderman et al., 2014; Raymond et al., 2018) and the BN stalk epitope (Park et al., 2020) have been
68 shown to evade neutralizing antibodies at these epitopes. Hence, identification of additional broadly
69 neutralizing epitopes of HA that can be efficiently targeted remains an important pursuit to improve vaccine
70 effectiveness while avoiding escape mutants.

71 Humans are exposed to influenza viruses throughout their lifetime and reuse memory B cells
72 (MBCs) from prior exposures to provide defense against drifted and novel strains. Seasonal influenza virus
73 vaccines often recall MBCs targeting variable epitopes of the HA head rather than MBCs targeting
74 conserved epitopes of HA (Andrews et al., 2015; Dugan et al., 2020). In the absence of pre-existing
75 immunity against variable epitopes of the HA head, humans can recall MBCs targeting conserved epitopes
76 of the HA head and stalk domains (Andrews et al., 2015). Notably, first exposure to the 2009 pandemic
77 H1N1 virus (pH1N1) robustly recalled MBCs against conserved epitopes of the HA stalk domain (Andrews
78 et al., 2015; Wrammert et al., 2011). Additionally, exposure to influenza viruses of zoonotic origin can recall
79 MBCs targeting conserved epitopes of the HA stalk (Ellebedy et al., 2014; Henry Dunand et al., 2016;
80 Nachbagauer et al., 2014).

81 Several leading universal influenza virus candidates function to induce antibodies specifically
82 against the stalk domain. The chimeric HA (cHA) vaccine strategy utilizes the head domain from a zoonotic

83 influenza virus, for which humans have little pre-existing immunity, and the stalk domain from pH1N1
84 (Krammer et al., 2013; Pica and Palese, 2013). A phase I clinical trial has shown cHA vaccination robustly
85 drives protective antibodies against the stalk domain (Bernstein et al., 2020; Nachbagauer et al., 2020). In
86 addition to the cHA vaccine strategy, several groups have generated headless HA antigens that potently
87 induce B cells against the HA stalk in animal models while eliminating the potential of inducing B cells
88 against the HA head domain (Impagliazzo et al., 2015; Yassine et al., 2015). The full spectrum of distinct
89 epitopes on the HA stalk targeted by these vaccine antigens remains to be determined.

90 By analyzing the specificities of B cells targeting the H1 stalk through the generation of monoclonal
91 antibodies (mAbs), we identified a class of antibodies targeting an anchor epitope of HA near the viral
92 membrane. Antibodies targeting this epitope are broadly neutralizing across H1-expressing viruses and
93 potently protective *in vivo*. Additionally, we showed anchor epitope targeting antibodies were recalled in
94 humans via vaccination with both the 2009 monovalent influenza virus vaccine and by seasonal influenza
95 virus vaccination. Furthermore, we identified that the cHA vaccine platform robustly induced antibodies
96 against the anchor epitope. In contrast, membrane anchor targeting mAbs could not bind mini-HA, a
97 headless HA antigen, potentially due to trimer splaying of the rHA that used a GCN4 trimerization domain.
98 Anchor epitope targeting mAbs utilized a highly restricted repertoire and public clonotypes that encoded
99 for two conserved motifs in the kappa chain CDR3 (K-CDR3) and heavy chain CDR2 (H-CDR2). Lastly,
100 we identified that anchor targeting B cells are common within the human MBC pool. Together, our study
101 reveals a novel class of broadly neutralizing antibodies against the anchor epitope, a previously
102 unappreciated epitope. Our study additionally provides valuable insight into the binding and repertoire
103 features of anchor epitope targeting B cells and how cHA, a potential universal influenza virus vaccine,
104 potently induces antibodies against this epitope.

105 **Results**

106 **Identification of antibodies targeting the anchor epitope**

107 To dissect conserved HA stalk domain epitopes, we generated mAbs from acutely activated plasmablasts
108 isolated from subjects who received licensed or experimental influenza virus vaccines or were naturally
109 infected with pH1N1 during the 2009 pandemic (Table S1). Notably, plasmablasts found in the blood of
110 subjects after infection or vaccination derive from pre-existing MBCs (Andrews et al., 2015), and generation
111 of mAbs from plasmablasts allows for the dissection of how distinct influenza viruses recall pre-existing
112 immunity. We also generated mAbs from sorted HA⁺ B cells one month following vaccination with an
113 experimental cHA vaccine that utilized the head domain from an avian influenza virus and stalk domain
114 from pH1N1 (Bernstein et al., 2020). We specifically focused our studies on mAbs targeting the stalk
115 domain of H1-expressing viruses, as prior studies have shown first exposure to the 2009 pandemic H1N1
116 virus induce antibodies against the stalk domain (Li et al., 2012; Wrammert et al., 2011). To define
117 antibodies as targeting the H1 stalk, mAbs were tested for binding to cH5/1, which utilizes the head domain
118 from H5-expressing viruses and the stalk domain from the pH1N1 virus (Hai et al., 2012), and for

119 hemagglutination inhibition (HAI) activity against pH1N1 (A/California/7/2009), a feature of head binding
120 antibodies. MAbs that bound the cHA and that lacked HAI activity were classified as those binding the HA
121 stalk domain. Of all mAbs tested, nearly 49% targeted the HA stalk domain, whereas 40% targeted the HA
122 head domain (Figure S1A). To investigate what proportion were binding the BN stalk domain epitope, we
123 competed the stalk binding mAbs from our cohorts with CR9114, a well-defined antibody targeting the BN
124 stalk epitope (Dreyfus et al., 2012). We identified that only 21% of mAbs targeting the stalk domain had
125 greater than 80% competition with CR9114 (Figure S1B), indicating most H1 stalk domain targeting
126 antibodies were binding other epitopes of the HA stalk.

127 To investigate which epitopes the remaining 79% of mAbs were targeting on the stalk domain, we
128 performed negative stain electron microscopy with two stalk domain binding mAbs. Both mAbs bound an
129 epitope near the anchor of the HA stalk, towards the lower portion of the HA protomer (Figure 1A-B; Figure
130 S1C-D). Both mAbs were oriented at an upward angle towards the epitope (Figure 1A-B), suggesting this
131 epitope may be partially obstructed by the lipid membrane and may only be exposed for antibody binding
132 as the HA trimers flex on the viral membrane (Benton et al., 2018). FISW84, a recently identified anchor
133 binding mAb, (Benton et al., 2018), overlap with both 047-09 4F04 and 241 IgA 2F04 (Figure 1C),
134 suggesting this epitope is a common target of stalk binding antibodies. The footprint of several BN stalk
135 epitope binding mAbs (CR9114 and FI6v3) did not overlap with those of 047-09 4F04 and 241 IgA 2F04
136 (Figure 1D; Figure S1E), indicating the anchor epitope and the BN stalk epitope are distinct epitopes on
137 the HA stalk. To understand what proportion of stalk binding mAbs were binding to the anchor epitope, we
138 competed 047-09 4F04 mAb with the remaining stalk binding mAbs that did not compete with CR9114. In
139 total, we identified 50 distinct mAbs that competed for binding to the anchor epitope from a total of 21
140 subjects (Table S2) and accounted for 28% of all stalk mAbs identified (Figure 1E-F). Together, these data
141 indicate that the anchor epitope is a common target of antibodies binding the H1 stalk domain.

142 **Antibodies binding the anchor are broadly reactive amongst H1 viruses**

143 As the stalk domain is conserved amongst influenza viruses, we next determined the viral binding breadth
144 of antibodies targeting the anchor epitope. Anchor mAbs were broadly reactive amongst H1-expressing
145 viruses, including a swine origin H1N2 virus, but rarely cross-reacted with other influenza subtypes (Figure
146 2A; Figure S2A-B), as often occurs for antibodies targeting the BN stalk epitope (Figure 2A; Figure S2B).
147 While highly conserved amongst H1 viruses, the anchor epitope was poorly conserved across divergent
148 group 1 viral subtypes (Figure S2C). Anchor epitope targeting mAbs had nearly a 2-fold higher affinity for
149 pH1N1 virus than mAbs targeting the BN stalk epitope (Figure 2B). Because the anchor epitope is partially
150 obstructed by the lipid membrane, we next tested whether anchor binding mAbs had reduced affinity for
151 whole virus relative to recombinant HA (rHA). MAbs binding the anchor epitope and the BN stalk epitope
152 both exhibited reduced affinity for the whole virus (A/California/7/2009) relative to rHA from the same virus,
153 whereas mAbs targeting the HA head domain had similar affinity for whole virus and rHA (Figure S2D).

154 These data indicate that antibodies targeting the anchor epitope are broadly reactive amongst H1-
155 expressing viruses.

156 **Antibodies targeting the anchor epitope maintain binding to HA mutants in the stalk domain**

157 H1N1 viruses have acquired several mutations within the HA stalk domain, likely due to antibody
158 selective pressures or to increase stability (Cotter et al., 2014). To understand whether these mutations
159 have affected antibody binding to the anchor epitope, we screened mAbs against naturally occurring
160 mutants and experimentally identified viral escape mutants of BN stalk epitope binding mAbs (Figure 2C-
161 D; Figure S3A-B; Table S3). Anchor epitope binding mAbs were mostly unaffected by the mutants tested,
162 whereas most of the mAbs targeting the BN stalk epitope were affected by at least one mutant, notably
163 Q42E mutation in HA2 (Figure 2D). Regardless of mAb specificity, most antibodies had reduced binding
164 to A44V of HA2, which was recently shown to preferentially grow in the presence of mAbs against the BN
165 stalk epitope (Park et al., 2020). While A44 is distant from the anchor epitope, the A44V mutation was
166 shown to affect the conformation of the HA stalk (Park et al., 2020) and could explain the broad reduction
167 of HA binding by antibodies targeting either the anchor epitope or the BN stalk epitope. Furthermore,
168 A/Michigan/45/2015 acquired mutations at S124N and E172K of HA2 (Clark et al., 2017), which lay near
169 the binding footprint of 047-09 4F04 and 241 IgA 2F04 (Figure S3C). Despite this, mAbs targeting the
170 anchor epitope or BN stalk epitope bind A/California/7/2009 (S124, E172) with nearly identical affinity as
171 they do to A/Michigan/45/2015 (N124, K172; Figure S3D-E), indicating these mutations were likely not
172 driven by selective pressures of mAbs targeting these residues. Together, these data indicate that known
173 mutations within the HA stalk largely do not affect the binding of antibodies to the anchor epitope.

174 **Antibodies targeting the anchor epitope are broadly neutralizing against H1N1 viruses and are
175 potently protective *in vivo***

176 We next determined whether mAbs targeting the anchor epitope were neutralizing. All mAbs
177 targeting the anchor epitope and the BN stalk epitope were neutralizing against the pH1N1 virus
178 (A/California/7/2009) and had similar neutralizing potency relative to mAbs binding the BN stalk epitope
179 (Figure 3A). Furthermore, anchor targeting mAbs were broadly neutralizing against historical and recent
180 H1N1 viruses, as well as a swine H1N2 (A/swine/Mexico/AVX8/2011) virus (Figure 3B). Together, these
181 data indicate that antibodies against the anchor epitope and other broadly neutralizing epitopes could work
182 in tandem to be potently neutralizing against antigenically drifted and shifted H1-expressing viruses.

183 To test whether mAbs targeting the anchor epitope were protective *in vivo*, we prophylactically
184 administered a cocktail of 5 mAbs targeting the anchor epitope or the BN stalk epitope to mimic a polyclonal
185 response against these epitopes and infected mice with a lethal dose of a mouse-adapted pH1N1 virus
186 (A/Netherlands/602/2009; Figure 3C). Mice that received either cocktail lost a similar amount of weight and
187 experienced similar mortality (Figure 3D-E). Notably, mice were completely protected at 5 mg/kg of mAb
cocktail, whereas only 60-80% of animals survived at 1 mg/kg (Figure 3E). Together, these data reveal

189 mAbs targeting the anchor epitope are protective *in vivo* and could provide broad protection against H1-
190 expressing viruses.

191 **Anchor epitope targeting antibodies are induced by seasonal influenza virus vaccines**

192 Antibodies induced by influenza vaccination are biased toward variable epitopes of the HA head (Figure
193 4A). However, novel exposure to the 2009 pH1N1 virus robustly recalled MBCs targeting the conserved
194 epitopes of the HA head and stalk domains, likely because subjects had low pre-existing antibody titers
195 against the variable epitopes of the HA head (Andrews et al., 2015; Guthmiller et al., 2020; Li et al., 2012;
196 Wrammert et al., 2011). In contrast, subjects that have been repeatedly exposed to the pH1N1 virus tend
197 to recall MBCs targeting the variable epitopes of the HA head (Guthmiller et al., submitted for publication).
198 Consistent with this, subjects that received the 2009 monovalent influenza virus (MIV) vaccine robustly
199 induced a plasmablast response against the stalk domain (38%), as determined by generated mAbs,
200 whereas only 15% of mAbs isolated from subjects that received the seasonal vaccine 2014 quadrivalent
201 influenza virus vaccine (QIV) targeted the stalk domain (Figure 4A). Most subjects in each cohort had at
202 least one stalk domain-targeting mAb isolated, although the frequency of stalk domain-binding mAbs per
203 subject was higher in the subjects that received the 2009 MIV relative to subjects in the 2014 QIV cohort
204 (Figure S4A-B). When broken down by the specific stalk epitopes targeted, nearly 40% of stalk binding
205 mAbs isolated targeted the BN stalk epitope (Figure 4B). A larger proportion of mAbs (57%) targeting the
206 anchor epitope were isolated from subjects that received the 2014 QIV relative to those subjects that
207 received the 2009 MIV (16%; Figure 4B). Anchor epitope binding mAbs were detected in two out of six
208 subjects in the 2014 QIV cohort (33.3%) and four out of eleven subjects in the 2009 MIV cohort (36%;
209 Figure 4C), demonstrating that this epitope is commonly targeted after influenza virus vaccinations.

210 To confirm that anchor epitope targeting mAbs generated from plasmablasts were representative
211 of the serum antibody response, we performed electron microscopy polyclonal epitope mapping (EMPEM)
212 to dissect the targets of the polyclonal serum antibody response mounted by subjects 236 and 241 from
213 the 2014 QIV cohort. Both subjects had detectable antibodies targeting the anchor epitope at days 7 and
214 14 post vaccination, whereas only subject 241 had detectable antibodies against the BN stalk epitope at
215 day 14 (Figure 4D-E; Figure S4C-D). Notably, subject 241 had more complexes with antibodies targeting
216 the anchor than the BN stalk epitope (Figure S4D), suggesting this subject more readily mounted an
217 antibody response against the anchor epitope. Comparison of anchor epitope binding polyclonal antibodies
218 (pAbs) identified in subjects 236 and 241 revealed the 241 IgA 2F04 mAb strongly overlapped with the 241
219 pAb from the same donor (Figure 4E-F) while the 236 pAb sat slightly anterior to the HA trimer, similar to
220 047-09 4F04 (Figure 4G-H; Figure S4E). Together, these data indicate influenza virus vaccination can
221 recall MBCs targeting the anchor epitope.

222 **The CHA universal influenza virus vaccine candidate robustly induced antibodies against the**
223 **anchor epitope**

224 Several experimental universal influenza virus vaccine candidates currently being tested in clinical trials
225 are intended to induce antibodies against the stalk domain. Notably, a cHA vaccine platform was shown
226 to specifically induce antibodies against the stalk domain (Bernstein et al., 2020; Nachbagauer et al.,
227 2020). To investigate whether subjects who received the cHA vaccine mounted an antibody response
228 against the anchor epitope and the BN stalk epitope, we adapted the competition ELISA to detect serum
229 antibody responses that could compete for binding with 047-09 4F04 and CR9114, respectively. Subjects
230 enrolled in the cHA clinical trial received a prime-boost regimen of cHA, with the prime being a cH8/1
231 inactivated influenza virus vaccine with an adjuvant (IIV+AS03) or cH8/1 live-attenuated influenza virus
232 (LAIV) followed by a boost 3 months later with a cH5/1 IIV with or without adjuvant (Figure 5A). On the
233 prime vaccination, subjects that received the cH8/1 IIV+AS03 had a dramatic increase in serum antibody
234 responses against the anchor epitope and BN stalk epitope relative to the placebo group (Figure 5B) and
235 had a 3-fold increase in antibodies binding the anchor epitope over the day 0 time point (Figure S5A-B).
236 However, these titers drastically dropped after 3 months post-prime (Figure 5B). Subjects that received
237 the cH8/1 LAIV did not have an increase in serum antibody responses against either the anchor epitope
238 or the BN stalk epitope (Figure 5B; Figure S5A-B). After the cH5/1 boost, subjects in the LAIV/IIV+AS03
239 group dramatically increased antibody titers against both the anchor epitope and BN stalk epitope, whereas
240 subjects in the LAIV/IIV cohort with no adjuvant did not have a substantial increase in serum antibodies
241 compared to the placebo cohort (Figure 5B). Subjects that received the cH8/1 IIV+AS03 followed by the
242 cH5/1 IIV+AS03 also boosted antibody responses against both the anchor and BN stalk epitopes
243 compared to the placebo controls (Figure 5B), although the fold-change in titers was not statistically greater
244 than the placebo (Figure S5A-B). Furthermore, only subjects in the LAIV/IIV+AS03 cohort had a significant
245 fold-increase in antibodies targeting both the anchor and BN stalk epitopes relative to pre-boost titers
246 (Figure S5A-B). At a 1-year time point, subjects within the LAIV/IIV+AS03 and the IIV+AS03/IIV+AS03 had
247 a significant decrease in serum antibodies against the anchor epitope and subjects within the
248 LAIV/IIV+AS03 cohort had a significant decrease in serum antibodies against the BN stalk epitopes (Figure
249 S5C-D). Furthermore, we identified and generated anchor epitope targeting mAbs from acutely activated
250 plasmablasts isolated from one subject that received the cH8/1 IIV+AS03 prime and one subject that
251 received the cH5/1 IIV+AS03 boost (Table S1 and Table S2). Together, these data indicate that the cHA
252 vaccine strategy can robustly induce antibodies against the anchor epitope.

253 Headless HA antigens, or mini-HAs, are attractive universal influenza virus vaccine antigens, as
254 these antigens lack the immunodominant epitopes of the HA head (Impagliazzo et al., 2015; Yassine et
255 al., 2015). We next tested whether the anchor epitope was present on the recombinant mini-HA antigen
256 (Impagliazzo et al., 2015) by performing ELISAs with the anchor epitope targeting antibodies. Only 1 out
257 of 50 anchor antibodies bound the mini-HA antigen, whereas all anchor epitope binding mAbs bound cH6/1
258 (Figure 5C; Figure S5E). In contrast, all but one BN stalk epitope targeting mAbs were capable of binding
259 to both the mini-HA and the cH6/1 (Figure S5E), suggesting the anchor epitope is specifically disrupted on

260 this antigen. Compared to full-length HA, the membrane proximal region of the mini-HA splays by
261 approximately 14.5 Å (Impagliazzo et al., 2015), suggesting this splaying may disrupt the antigenicity of
262 the anchor epitope. Notably, the mini-HA antigen utilizes a GCN4 trimerization domain, whereas the cH6/1
263 utilizes a fibritin trimerization domain. Therefore, we next tested whether the loss of antigenicity of the
264 anchor epitope could be due to the utilization of the GCN4 trimerization domain. We identified that anchor
265 epitope targeting antibodies could bind A/California/7/2009 rHA with a fibritin trimerization domain, but not
266 A/California/7/2009 rHA with a GCN4 trimerization domain (Figure S5F), indicating a GCN4 trimerization
267 domain affected the antigenicity of this epitope. To understand whether anchor epitope targeting antibodies
268 could bind the mini-HA in a more native setting, we modified the mini-HA antigen to remove the
269 trimerization domain and include a transmembrane domain, which would lead to the HA being membrane-
270 bound. Transfected HEK293T cells were stained with mAbs targeting H1 head epitopes, the BN stalk
271 epitope, or the anchor epitope, and flow cytometry was performed. MAbs targeting the anchor and BN
272 stalk epitopes readily bound both the full-length membrane-bound A/California/7/2009 HA (Cal09) and the
273 membrane-bound mini-HA, whereas the H1 head-specific mAbs only bound the full-length Cal09 HA
274 (Figure 5D). These data indicate that the anchor epitope is antigenic when HA is trimerized more similarly
275 to membrane-bound HA. Additionally, these data indicate native-like HA antigens are likely to recall MBCs
276 targeting the anchor epitope, such as the cHA vaccine candidate.

Anchor epitope targeting mAbs utilize a restricted antibody repertoire

277 We next investigated the repertoire features of mAbs targeting the anchor epitope. All mAbs that targeted
278 the anchor epitope utilized one of four VH3 genes: VH3-23, VH3-30/VH3-30-3, and VH3-48, with over
279 three-quarters utilizing VH3-23 (Figure 6A-B). MAbs targeting the BN stalk epitope commonly used VH1
280 genes, of which the vast majority used VH1-69 (Figure 6A, C). Anchor epitope targeting mAbs used a
281 variety of DH genes (Table S2) and JH genes, although 70% of anchor targeting mAbs utilized JH4 (Figure
282 S6A). Amongst the anchor targeting mAb heavy chain sequences, 75% were non-clonal (Figure 6D),
283 indicating most anchor targeting utilize similar but distinct heavy chain VDJ recombinations. Similar to the
284 heavy chain, anchor epitope binding mAbs utilized a highly restricted light chain repertoire relative to the
285 BN stalk binding mAbs, with all mAbs utilizing a combination of VK3-11 or VK3-15 combined with JK4 or
286 JK5 (Figure 6E-G; Figure S6B). In contrast, mAbs targeting the BN stalk epitope used a wide array of
287 VK/VL genes (Figure 6E) and JK/JL genes (Figure S6B). Furthermore, all but one light chain of the anchor
288 targeting mAbs were clonal (Figure 6H), indicating the light chains were very similar across mAbs and
289 subjects. By determining paired heavy and light chain clones, we identified 4 distinct clonal expansions,
290 with one public clonal expansion found across multiple subjects (Figure 6I-J; Figure S6C). Anchor epitope
291 targeting mAbs were mutated to a similar extent as mAbs targeting the BN stalk epitope (Figure S6D). The
292 K-CDR3 length of anchor epitope binding mAbs was highly restricted, with all K-CDR3s being ten amino
293 acids in length (Figure S6E). Together, these data indicate that anchor epitope targeting mAbs utilize a
294 highly restricted repertoire, particularly for the light chain.

296 FISW84 (Benton et al., 2018) similarly uses VH3-23/VK3-15 and largely makes interactions with
297 the epitope via an NWP motif within the K-CDR3 loop and a tyrosine (Y) immediately following the H-CDR2
298 (Figure S6F). We identified that all anchor targeting mAbs possessed this NWP motif at the exact same
299 location within the K-CDR3, which was present in the germline sequence of the various VK/JK pairings
300 (Figure 6K). Moreover, all anchor binding mAbs utilized a germline encoded tyrosine at position 59 (Figure
301 6L), suggesting this residue could have led to the selection of B cells utilizing these particular VH3 genes.
302 Despite this, nearly 2/3 of VH3 genes utilize a tyrosine at this exact position (Figure S6G), suggesting other
303 features of the heavy chain may lead to the preferential selection of these particular VH3 genes into the B
304 cell repertoire against the anchor epitope. Together, these data reveal B cells targeting the anchor epitope
305 utilized a highly restricted V(D)J gene repertoire, and these specific features of the repertoire are likely
306 critical for binding the anchor epitope.

307 **Humans possess MBCs with features of anchor epitope targeting antibodies**

308 Due to the restricted repertoire features of anchor targeting mAbs, we next determined the relative
309 proportion of B cell subsets with these features by integrating single-cell RNA-sequencing and repertoire
310 sequencing of HA-specific B cells isolated from 22 subjects following cH5/1 vaccination (d112; Figure 5A).
311 Notably, most B cells isolated likely target the H1 stalk domain as we sequenced sorted cH5/1⁺ B cells and
312 humans have no measurable pre-existing immunity against the H5 head domain (Han et al., 2020).
313 However, subjects may have recruited naïve B cells against the H5 head component of the cH5/1 vaccine,
314 therefore the isolated B cell pool is likely a heterogenous population of mostly H1 stalk domain-reactive B
315 cells and some H5 head domain-reactive B cells. To investigate the proportion of B cells with repertoire
316 features of B cells targeting the anchor epitope, we selected B cells that used VH3-23/VH3-30/VH3-30-
317 3/VH3-48, VK3-11/VK3-15, JK4/JK5, a 10 amino acid length K-CDR3, and possessed an NWP motif within
318 the K-CDR3. For reference, we additionally segregated out B cells expressing VH1-69 and a kappa chain,
319 as these are the dominate repertoire features of B cells targeting the BN stalk epitope (Figure 6A, C, E).
320 We identified that B cells with features of antibodies binding the anchor epitope were abundant within the
321 human B cell repertoire, with 6% of all B cells identified fitting within this defined repertoire (Figure 7A). Of
322 subjects with ten or more VDJ⁺ B cells (n=20), we identified anchor targeting B cells in all but one subject
323 (Figure 7B). The anchor targeting B cell pool largely used VH3-23/VK3-15 pairing (Figure 7C). Additionally,
324 we generated 34 mAbs from the selected anchor targeting B cell list and 31 of these mAbs competed with
325 047-09 4F04 (Figure 7D). The anchor epitope B cells had a similar number of mutations as VH1-69/kappa
326 B cells (Figure 7E) and were largely class-switched to IgG1 and IgG3 (Figure 7F), indicative of prior class-
327 switch recombination and B cell selection within germinal centers. Together, these data indicate that the
328 anchor epitope is a common target of the human MBC repertoire against HA. Moreover, this study indicates
329 that most adults have pre-existing immunity against this epitope that can be harnessed by a potential
330 universal influenza virus vaccine candidate to provide broad protection against H1-expressing viruses.

332 **Discussion**

333 In this study, we identified a class of antibodies targeting a broadly neutralizing epitope of hemagglutinin
334 stalk domain near the viral membrane of H1-expressing influenza viruses. The stalk domain is conserved
335 within and often across influenza virus subtypes. Anchor epitope targeting antibodies showed broad
336 neutralizing activity against H1-expressing influenza viruses but rarely cross-reacted with other influenza
337 subtypes. The anchor epitope was poorly conserved across influenza virus subtypes, which could explain
338 the H1 subtype specificity of the anchor epitope targeting antibody class identified in this study. However,
339 the broadly neutralizing activity of anchor epitope targeting mAbs against pre- and post-pH1N1 viruses
340 and a swine-origin H1-expressing virus indicates the anchor epitope is an important target for pan-subtype
341 neutralizing antibodies. Anchor epitope targeting antibodies have the potential to provide protection against
342 antigenically drifted H1N1 viruses and zoonotic spillovers of H1-expressing viruses. Furthermore, stalk
343 binding antibodies are an independent correlate of protection against influenza virus infection and lower
344 respiratory symptoms (Aydillo et al., 2020; Ng et al., 2019). Whether antibodies against distinct stalk
345 domain epitopes are independent correlates of protection against influenza virus infection is yet to be
346 determined.

347 A striking feature of anchor epitope binding antibodies was the angle of approach, with the antibody
348 Fab tilting up towards the epitope and sterically clashing with the viral membrane. However, our data
349 indicate anchor epitope antibodies can bind HA in the context of the viral membrane, as these antibodies
350 could bind intact virus and were neutralizing *in vitro*. These data are consistent with a dynamic HA on the
351 membrane, tilting up to 52° along its three-fold axis (Benton et al., 2018), allowing for the exposure of
352 epitopes proximal to the membrane. As HA flexes on the viral membrane surface, the epitope may become
353 available, allowing for antibody binding at the observed angle. However, likely only 1-2 of the anchor
354 epitopes of the HA trimer is accessible as HA flexes, limiting the avidity of antibodies binding the anchor
355 epitope. Moreover, influenza viruses are densely decorated with the surface glycoproteins HA and NA
356 (Gallagher et al., 2018; Wasilewski et al., 2012), further limiting access to the anchor epitope. Further
357 research is needed to understand how the anchor epitope can be made more accessible for antibody
358 binding. Moreover, it is of interest to understand whether anchor epitope binding antibodies possess
359 membrane binding capabilities, similar to antibodies binding to membrane proximal external region
360 (MPER) of gp41 of HIV (Cardoso et al., 2005; Ofek et al., 2004).

361 The anchor targeting mAbs utilized a highly restricted repertoire that were public clonotypes across
362 subjects, with all antibodies possessing two conserved motifs near the H-CDR2 and a NWP motif within
363 the K-CDR3. In addition, VH3-23 and VH3-48 utilizing mAbs targeted the anchor epitope slightly differently,
364 with the VH3-48 mAb (047-09 4F04) sitting more anterior and superior on a single HA protomer relative to
365 the VH3-23 mAb (241 IgA 2F04). However, more antibodies need to be studied to further understand how
366 the slight differences in repertoire usage affect HA binding. Multiple classes of antibodies against the BN
367 stalk epitope of H1-expressing viruses have been identified (Joyce et al., 2016; Sui et al., 2009) and often

368 cross-react with other group 1 virus subtypes (i.e. H2, H5), and occasionally group 2 viruses (Henry
369 Dunand et al., 2015). Notably, each antibody class targets the BN stalk epitope at slightly different angles
370 and have slightly different binding footprints (Joyce et al., 2016; Wu and Wilson, 2020). As a result of this,
371 viral mutations that arise to circumvent one antibody class may have a minimal effect on viral binding
372 breadth and neutralization potential of other classes. Moreover, naturally occurring mutations within the
373 footprint of the anchor antibodies have not been observed, whereas mutations against the BN stalk epitope
374 have been observed (Wu et al., 2020).

375 Our study showed that humans have pre-existing immunity against the anchor epitope and
376 influenza virus vaccination can recall MBCs to secrete antibodies against this epitope. However, vaccine
377 HA antigens must have a native confirmation near the transmembrane domain, as our study showed that
378 trimer splaying potentially due to the GCN4 trimerization domain ablates antibody binding at the anchor
379 epitope. Split vaccines, HA-decorated nanoparticles, and mRNA vaccines that induce expression of
380 membrane-bound HA should possess a native anchor epitope that can be recognized by MBCs targeting
381 this epitope. Moreover, our study highlights that the cHA vaccine strategy was able to recall MBCs against
382 the anchor epitope and the BN stalk epitope, while avoiding the recruitment of MBCs targeting the variable
383 epitopes of the HA head (Nachbagauer et al., 2020). Similarly, the mini-HA/headless HA vaccine strategy
384 has the potential to also recall MBCs against multiple epitopes of the HA stalk domain, if folded natively
385 (van der Lubbe et al., 2018; Yassine et al., 2015). However, an optimal pan-H1 vaccine should strive to
386 induce antibodies against both conserved epitopes of the HA stalk domain and head domain, including the
387 RBS, lateral patch, and trimer interface in order to limit potential viral escape mutants. Vaccines that
388 strategically glycosylate variable epitopes have the potential to induce antibodies against conserved
389 epitopes of the HA head and stalk domains, while limiting B cell recruitment against variable epitopes (Bajic
390 et al., 2019; Boyoglu-Barnum et al., 2020; Eggink et al., 2014; Weidenbacher and Kim, 2019). Moreover,
391 mosaic antigens that replace the variable epitopes with those from avian influenza virus subtypes also
392 have the potential to induce broadly protective antibodies against HA (Broecker et al., 2019; Liu et al.,
393 2018; Sun et al., 2019). Together, our study indicates that novel influenza vaccination strategies have the
394 capability to robustly induce antibodies against the previously unappreciated anchor epitope that can
395 provide broad protection against H1-expressing viruses.

396 **Acknowledgments**

397 We are thankful to all subjects who participated in this study. We thank Sarah Andrews, Rafi Ahmed, Jens
398 Wrammert, and Karlynn Neu for initiating studies on the 2009 MIV, 2010 TIV, and 2014 QIV cohorts. We
399 thank Chiara Mariottini, Jodi Feser, Daniel Stadlbauer, and Anna-Karin Palm for their help on the cHA
400 vaccine trial. We thank Ian Wilson and Alec Freyn for fruitful discussion and feedback on experimental
401 design. We are thankful to the teams at PATH, GSK, Cincinnati Children's Hospital Medical Center, and
402 Duke University for their work on the chimeric HA vaccine trial (NCT03300050), which was funded in part
403 by the Bill and Melinda Gates Foundation (OPP1084518). The findings and conclusions contained within
404 are those of the authors and do not necessarily reflect positions or policies of the Gates Foundation. This
405 project was funded in part by the National Institute of Allergy and Infectious Diseases; National Institutes
406 of Health grant numbers U19AI082724 (P.C.W.), U19AI109946 (P.C.W.), U19AI057266 (P.C.W.), P01
407 AI097092 (P.P.), R01AI145870-01 (P.P.), R21AI146529 (L.C.), and T32AI007244-36 (J.H.), and the NIAID
408 Centers of Excellence for Influenza Research and Surveillance (CEIRS) grant number
409 HHSN272201400005C (P.C.W.), and HHSN272201400008C (L.C., F.K., A.G.-S., P.P.). This work was
410 also partially supported by the National Institute of Allergy and Infectious Disease (NIAID) Collaborative
411 Influenza Vaccine Innovation Centers (CIVIC; 75N93019C00051, F.K., A.G.-S., P.P., A.B.W., P.C.W.).

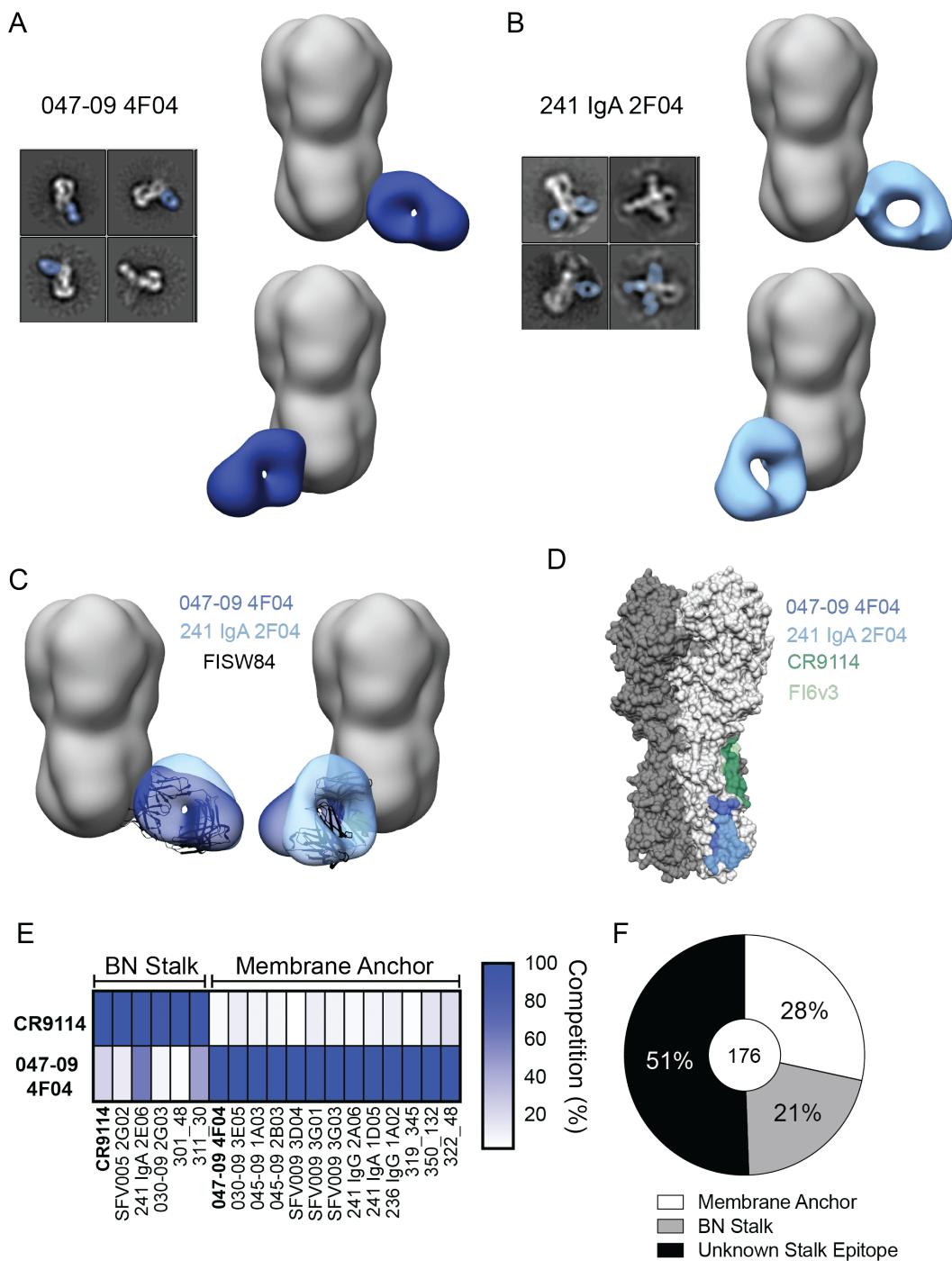
412 **Author contributions**

413 J.J.G. designed the study, characterized mAbs, analyzed the data, and wrote the manuscript. J.H.
414 generated structures, performed mapping experiments, analyzed data, and edited the manuscript. H.A.U.
415 performed characterization ELISAs. L.L. analyzed single-cell RNA-sequencing data. L.Y.L. sorted cH5/1⁺
416 B cells and generated RNA-sequencing data. C.H. and C.T.S. generated mAbs from cHA vaccine trial.
417 O.S., D.J.B., and S.C. performed virus-specific ELISAs. J.J.G., H.L.D., M.E.T., C.T.S., and H.A.U.
418 performed infection challenge studies. L.G. and J.D.B. generated HA mutant data on 045-09 2B06. N.-
419 Y.Z. grew and purified influenza viruses. S.T.R. helped perform EMPEM studies. M.H. performed mAb
420 cloning. S.S. and F.K. provided recombinant proteins. F.K., P.P., A.G.-S., and R.N. designed and
421 orchestrated cHA vaccination trial. L.C. provided recombinant protein and plasmids for membrane-bound
422 HA. A.B.W. supervised structural analyses and provided critical feedback on experimental design, P.C.W.
423 supervised the work and edited the manuscript. All authors provided feedback on the manuscript.

424 **Declaration of Interests**

425 The Icahn School of Medicine at Mount Sinai has submitted patent applications on universal influenza virus
426 vaccines naming R.N., A.G.-S. P.P. and F.K as inventors.

Figure 1

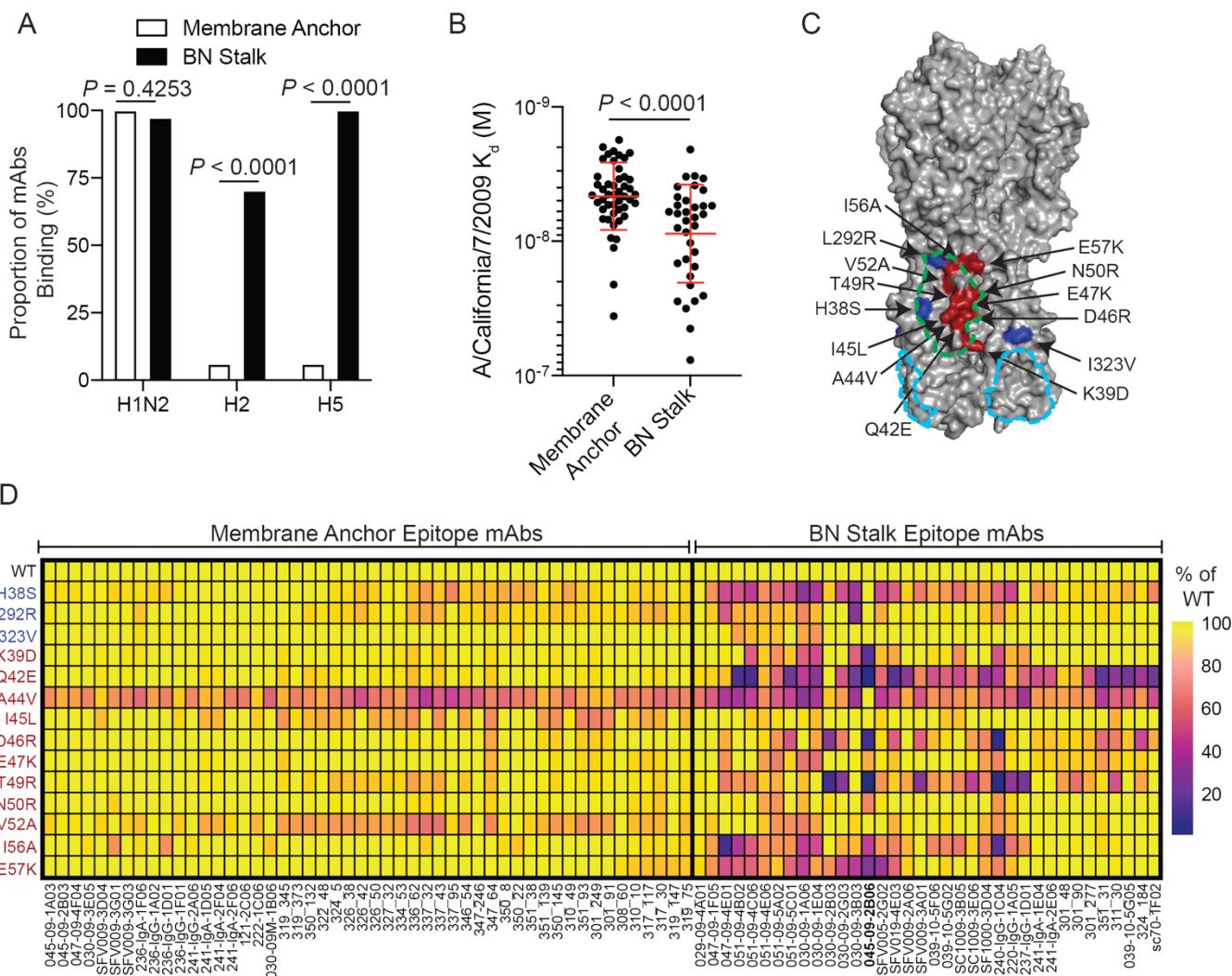


427

428 **Figure 1: The anchor epitope is a common target of HA stalk binding antibodies. (A-B)**, Negative
429 stain EM 2D class averages and 3D reconstructions of negative stain EM of 047-09 4F04 Fab **(A)** and 241
430 IgA 2F04 Fab **(B)** binding to A/California/4/2009 HA. **(C)** Overlay of 047-09 4F04, 241 IgA 2F04, and
431 FISW84 (PDB: 6HJQ) Fabs binding the anchor epitope of A/California/04/2009 HA. **(D)** Binding footprints
432 of 047-09 4F04, 241 IgA 2F04, CR9114, and FI6v3 on A/California/04/2009 HA. **(E)** Competition of stalk
433 binding mAbs with CR9114 or 047-09 4F04. **(F)** Proportion of mAbs binding to the anchor epitope, the BN

434 stalk epitope, or an unknown stalk epitope based on competition with 047-09 4F04 or CR9114. Number in
435 the center of the pie graph represents the number of mAbs tested. See also **Figure S1 and Table S2**.

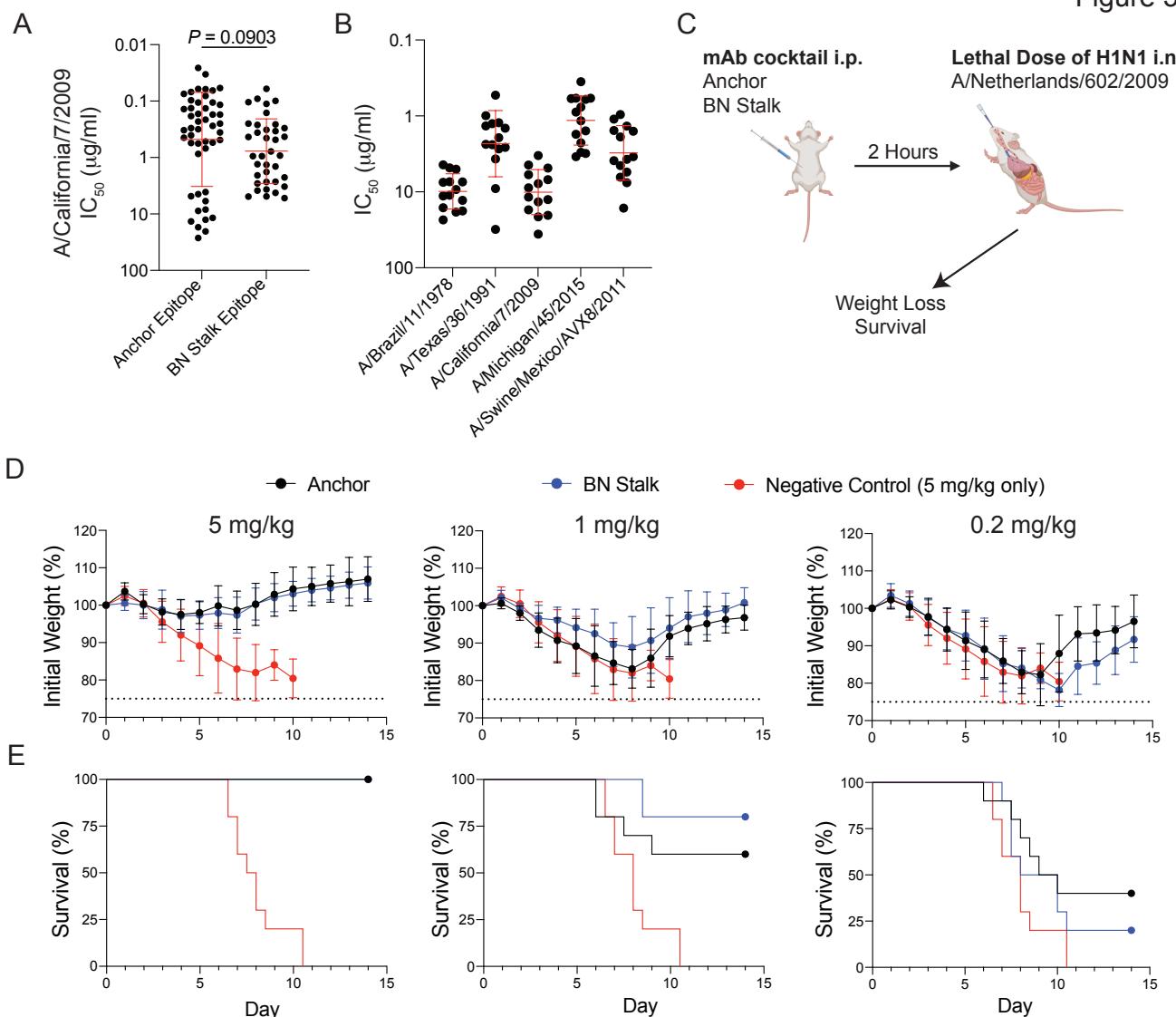
Figure 2



436
437 **Figure 2: Anchor epitope targeting antibodies are broadly reactive amongst H1 expressing viruses.**

438 (A) Proportion of anchor epitope and BN stalk epitope targeting mAbs binding to other group 1 subtypes.
439 (B) Apparent affinity of anchor and BN stalk binding mAbs to A/California/7/2009 virus. Data are
440 represented as mean \pm S.D. (C-D) Anchor and BN stalk binding mAbs were tested for binding to
441 A/California/7/2009 HA with naturally occurring mutations and experimentally determined mutations
442 induced by 045-09 2B06, a BN stalk epitope binding mAb. (C) Location of mutations modeled on
443 A/California/04/2009 HA (PDB: 4JTV). Residues in blue are located on HA1 and residues in red are located
444 on HA2. Outlines represent binding footprints of 047-09 4F04 (sky blue) and CR9114 (green). (D) Heatmap
445 of mAb binding to WT and mutant HAs shown as the proportion of signal relative to mAb binding to the WT
446 HA. Data in A were analyzed by Fisher's Exact tests and B were analyzed by unpaired non-parametric
447 Mann-Whitney test. See also **Figure S2, Figure S3, Table S3**.

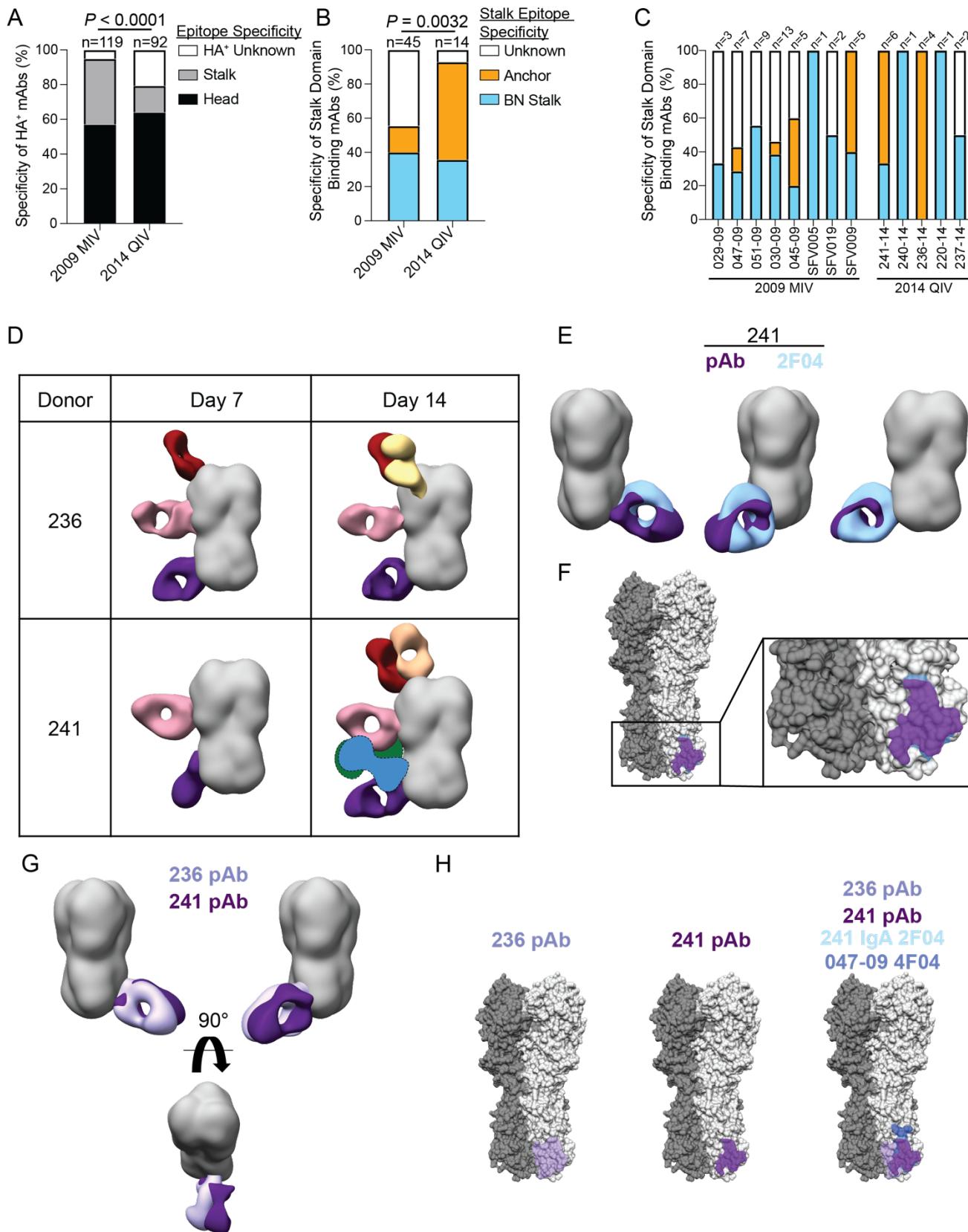
Figure 3



448

449 **Figure 3: Anchor epitope targeting mAbs are broadly neutralizing amongst H1 viruses and potentially**
 450 **protective *in vivo*.** (A) Neutralization potency of mAbs binding the anchor or BN stalk epitope against
 451 A/California/7/2009 H1N1. (B) Neutralization potency of anchor epitope binding mAbs against H1-
 452 expressing viruses. (C-E) Mice were prophylactically administered i.p. a cocktail of mAbs (n=5
 453 mAbs/cocktail) against the anchor epitope or BN stalk epitope, or an anthrax specific antibody. Mice were
 454 infected 2 hours later with 10 LD_{50} of A/Netherlands/602/2009 H1N1. (C) Experiment design. Weight loss
 455 (D) and survival (E) of mice in each treatment group. N=10 mice per treatment group and are pooled from
 456 two independent experiments. Data in A, B, and D are represented as mean \pm S.D. Data in A were
 457 analyzed by unpaired non-parametric Mann-Whitney test. See also Table S4.

Figure 4

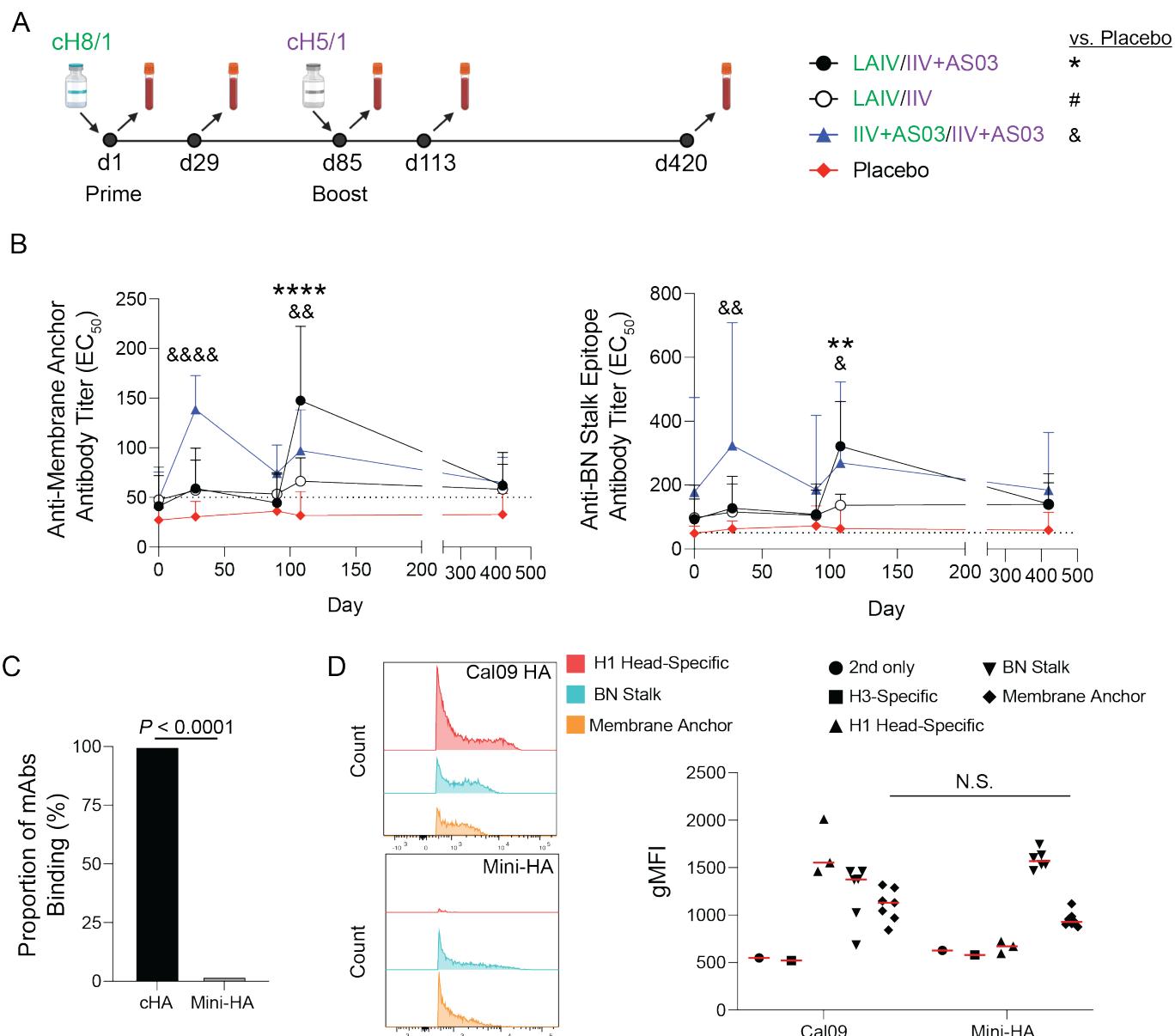


458

459 **Figure 4: Anchor epitope targeting B cells are induced by licensed influenza virus vaccines. (A-C)**
460 MAbs were generated from plasmablasts isolated 7 days after influenza virus vaccination with the 2009

461 MIV and the 2014 QIV. **(A)** Domain binding of HA⁺ mAbs. **(B-C)** Epitope specificity of stalk domain binding
462 mAbs by vaccine cohort **(B)** and by subjects **(C)**. **(D-F)** EMPEM of serum collected at day 7 and 14 following
463 2014 QIV in subjects 236 and 241 binding to A/Michigan/45/2015 HA. **(D)** Summary of pAbs at day 7 and
464 d14. **(E)** Overlap of 241 IgA 2F04 fab and pAb binding anchor epitope from subject 241. **(F)** Binding
465 footprint of 241 IgA 2F04 (sky blue) and pAb from subject 241 (purple). **(G)** Overlap of anchor epitope
466 binding pAbs from subjects 236 (lavender) and 241 (purple). **(H)** Binding footprint of pAbs from subjects
467 236 (lavender) and 241 (purple) relative to 241 IgA 2F04 and 047-09 4F04. Data in **A** and **B** were analyzed
468 using Chi-square tests. See also **Figure S4**.

Figure 5



469

470 **Figure 5: cHA vaccination in humans recalls MBCs targeting the anchor epitope. (A-B)** Subjects
471 enrolled in a phase 1 clinical trial received a prime-boost of cHA vaccine, where the prime used cH8/1 and
472 the boost used cH5/1. On the prime, subjects either received a LAIV or IIV with adjuvant (AS03). On the
473 boost, subjects received the IIV with or without adjuvant (AS03). Serum was collected before and after
474 vaccination and monitored for competing serum antibodies against the anchor epitope (047-09 4F04) and
475 BN stalk epitope (CR9114). LAIV/IIV+AS03 (n=10); LAIV/IIV (n=7); IIV+AS03/IIV+AS03 (n=7); Placebo
476 (n=6). **(A)** Trial design. **(B)** EC50s of serum antibodies competing for binding with 047-09 4F04 for binding
477 to the anchor epitope (left) and CR9114 for binding to the BN stalk epitope (right). Data are mean + S.D.
478 **(C)** Proportion of anchor epitope binding mAbs binding to cHA or mini-HA. **(D)** MAb binding to HEK293T
479 cells expressing full length A/California/7/2009 HA (Cal09) or mini-HA with a transmembrane domain.
480 Representative flow cytometry plots of mAbs binding to Cal09 HA and mini-HA (left) and geometric mean

481 fluorescence intensity (gMFI) of mAbs binding to Cal09 and mini-HA (right). Data represent the median
482 and each symbol represents a distinct mAb. Data in **B** were analyzed using a two-way ANOVA testing for
483 simple effects within rows, data in **C** were analyzed by Fisher's Exact test and data in **D** were analyzed by
484 unpaired non-parametric Mann-Whitney test. See also **Figure S5**.

Figure 6

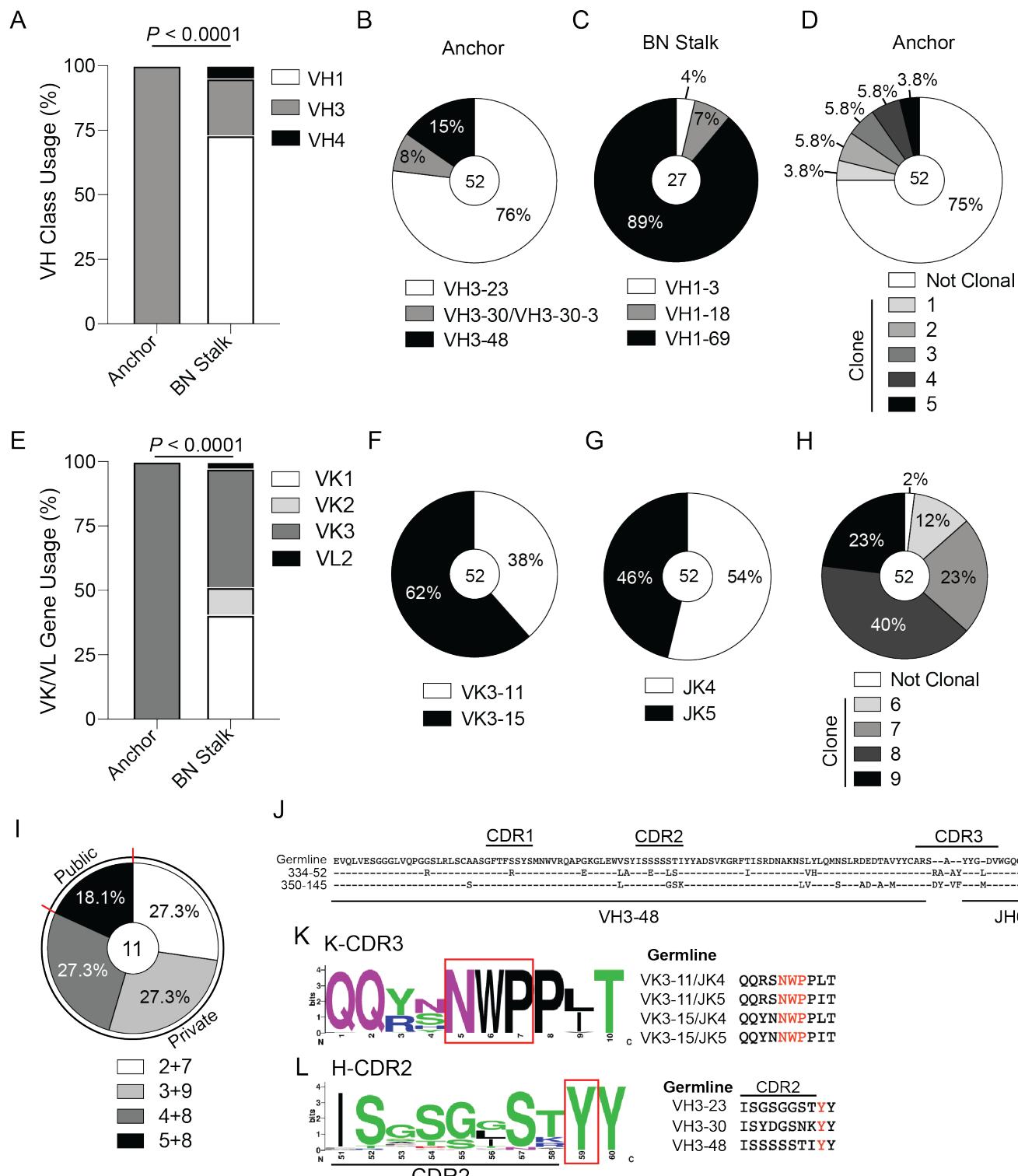


Figure 6: Anchor targeting mAbs use a highly restricted repertoire and possess a conserved binding motif within the K-CDR3. (A-C) Heavy chain VH classes (A) and gene usage of mAbs binding the anchor epitope (B) and the BN stalk epitope (C). Only VH1 gene usage of BN stalk epitope binding mAbs is shown in C. (D) Heavy chain clonality of mAbs binding the anchor epitope. (E) Light chain VK/VL

490 classes usage of mAbs binding the anchor epitope or BN stalk epitope. (**F-G**) VK (**F**) and JK (**G**) gene
491 usage of mAbs binding the anchor epitope. (**H**) Light chain clonality of mAbs binding the anchor epitope.
492 (**I**) Private and public clones that share heavy and light chains. (**J**) Alignment of VDJ of the VH3-48 public
493 clone. (**K**) Sequence logo of the K-CDR3 and the germline sequence of the K-CDR3 of VK3-11/VK3-15
494 combined with JK4/JK5. NWP motif is highlighted. (**L**) Sequence logo of the H-CDR2 with the tyrosines
495 directly following the H-CDR2 and the germline sequence of the H-CDR2 of VH3-23, VH3-30, and VH3-
496 48. Data in **A** and **E** were analyzed using Chi-square tests. See also **Figure S6**.

Figure 7

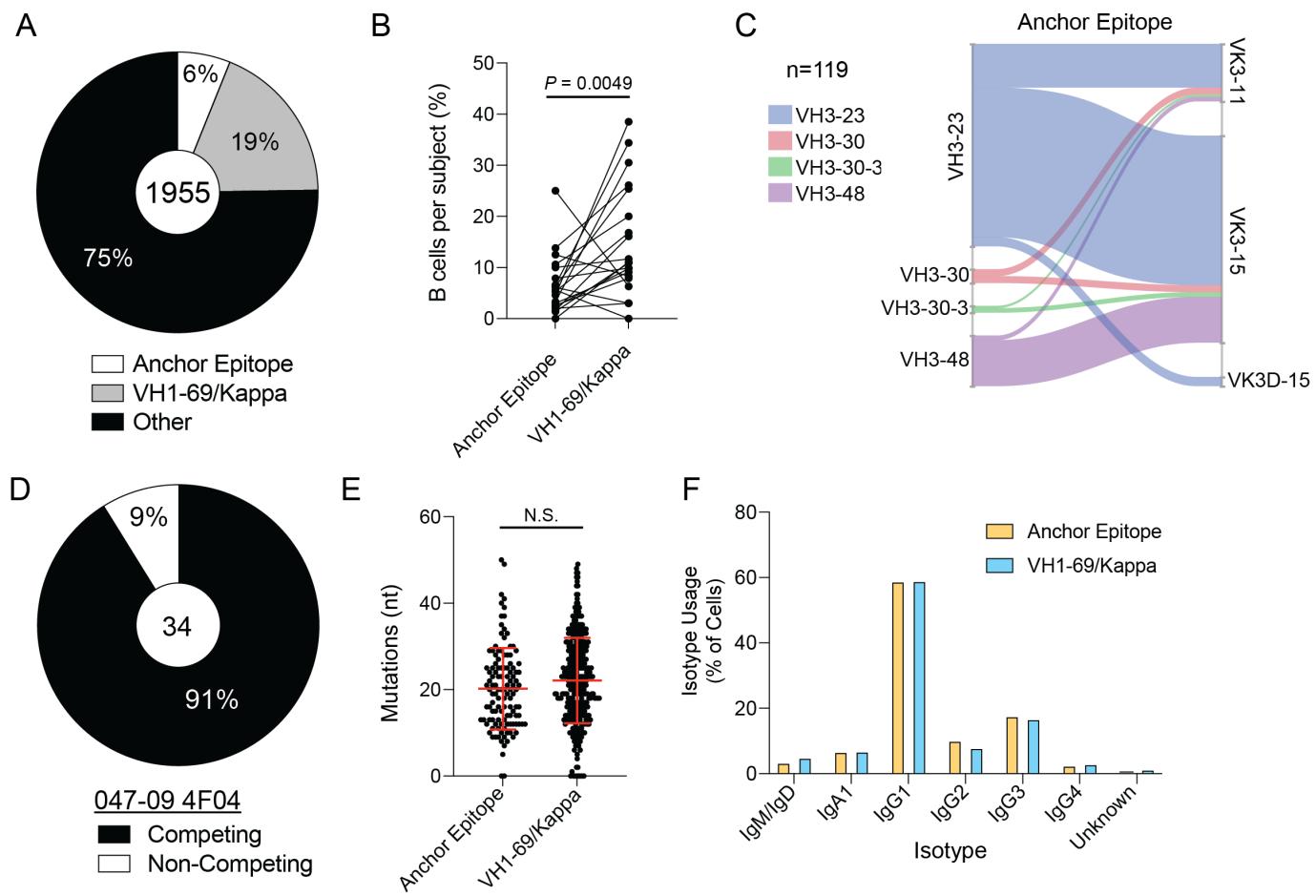


Figure 7: Humans possess MBCs targeting the anchor epitope. ch5/1⁺ B cells from PBMCs were sorted from subjects 28 days following a booster with the ch5/1 and were subjected to single-cell RNA-sequencing. **(A)** Proportion of all B cells with features of anchor antibodies, VH1-69/kappa (BN stalk epitope), or with other repertoire features. **(B)** Proportion of B cells with anchor binding antibody features or that use VH1-69/kappa chain by subject. Lines connect the same subject. **(C)** VH/VK pairing of B cells with features of anchor epitope binding antibodies. **(D)** 34 mAbs with anchor epitope binding mAb repertoire features and tested for competing for binding with 047-09 4F04. **(E-F)** number of heavy chain mutations **(E)** and isotype usage **(F)** of B cells with repertoire features of anchor binding antibodies or VH1-69/kappa. Data in **E** are represented as mean \pm S.D. Data in **D** were analyzed using a paired non-parametric Wilcoxon matched-pairs signed rank test and data in **E** were analyzed by unpaired non-parametric Mann-Whitney test.

509

STAR METHODS

510

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human CD19 PE-AF610 conjugate	Invitrogen/Thermo Scientific	Cat# MHCD1922 RRID: AB_10373379
Anti-human CD27 R-PE conjugate	Invitrogen/Thermo Scientific	Cat# MHC2704 RRID: AB_10392393
Anti-human CD38 APC-Cy5.5 conjugate	Invitrogen/Thermo Scientific	Cat# MHCD3819 RRID: AB_10371760
Anti-human CD3 FITC conjugate	Invitrogen/Thermo Scientific	Cat# MHCD0301 RRID: AB_10376003
Anti-human CD20 FITC conjugate	Invitrogen/Thermo Scientific	Cat# MHCD2001 RRID: AB_10373690
RosetteSep human B cell enrichment cocktail	StemCell Technologies	Cat#15064
HRP-conjugated goat anti-human IgG antibody	Jackson Immuno Research	Cat# 109-035-098 RRID:AB_2337586
Streptavidin-HRP	Southern Biotech	Cat#7100-05
Streptavidin-PE	Biolegend	Cat#405203
Anti-Influenza A Antibody, nucleoprotein, clone A3, biotin-conjugated	Sigma/Millipore	Cat#MAB8258B-5
Bacteria and Virus Strains		
NEB® 5-alpha Competent E. coli	NEB	Cat#C2988J
A/Solomon Islands/6/2006 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/California/7/2009 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/New Caledonia/20/1999 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/Brazil/11/1978 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/Chile/1/1983 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/Texas/36/1991 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/Michigan/45/2015 (H1N1)	Patrick Wilson's laboratory stock	N/A
A/swine/Mexico/AVX8/2011 (H1N2)	Patrick Wilson's laboratory stock	N/A
A/Wisconsin/57/2005 (H3N2)	Patrick Wilson's laboratory stock	N/A
A/Hong Kong/4801/2014 (H3N2)	Patrick Wilson's laboratory stock	N/A
B/Phuket/3073/2013 (B/Yamagata/16/1988-like lineage)	Patrick Wilson's laboratory stock	N/A
B/Brisbane/60/2008 (B/Victoria/2/1987-like lineage)	Patrick Wilson's laboratory stock	N/A
A/Netherlands/602/2009 (H1N1)	Patrick Wilson's laboratory stock	N/A

Biological Samples		
Human PBMC	This study	N/A
Chemicals, Peptides, and Recombinant Proteins		
A/California/7/2009 (H1N1) HA with fibritin trimerization domain	Florian Krammer's laboratory stock and Patrick Wilson's laboratory stock	N/A
A/California/7/2009 (H1N1) HA with GCN4 trimerization domain	Lynda Coughlan's laboratory stock and Andrew Ward's laboratory stock	N/A
A/Michigan/45/2015 HA	Patrick Wilson's laboratory stock	N/A
A/Ann Arbor/6/1960 (H2N2) HA with fibritin trimerization domain	Florian Krammer's laboratory stock	N/A
A/Indonesia/5/2005 (H5N1) HA with fibritin trimerization domain	Florian Krammer's laboratory stock	N/A
A/California/7/2009 (H1N1) HA with fibritin trimerization domain, single point mutations	Patrick Wilson's laboratory stock	N/A
Chimeric H6/1 HA (H6 head from A/mallard/Sweden/81/2002 combined with H1 stalk from A/California/04/2009)	Florian Krammer's laboratory stock	N/A
Chimeric H5/1 HA (H5 head from A/mallard/Sweden/24/2002 combined with H1 stalk from A/California/4/2009) with Y98F mutation	Florian Krammer's laboratory stock	N/A
Mini-HA (H1 stalk domain from A/Brisbane/59/2007)	Lynda Coughlan's laboratory stock	N/A
Mutant H1 proteins	Patrick Wilson's laboratory stock	N/A
PEI 25K, Transfection Grade	Polysciences	Cat# 23966-2
Super Aquablue ELISA substrate	ThermoFisher	Cat# 00-4203-58
EZ-link Sulfo-NHS-Biotin	ThermoFisher	Cat# 21217
Trypsin, TPCK treated	Sigma-Aldrich	Cat# T8802
Pierce™ Protein A agarose	ThermoFisher	Cat# 20334
Ni-NTA Agarose	Qiagen	Cat# 30210
Lymphocyte Separation Medium	Corning	Cat# 25-072-CV
Advanced DMEM	Invitrogen	Cat# 12491-023
DMEM	Invitrogen	Cat# 11965118
PFHM-II protein free hybridoma medium	Invitrogen	Cat# 12040-077
FBS	Invitrogen	Cat# 16000-044
Ultra Low FBS	Invitrogen	Cat# 16250078
Bovine Serum Albumin (Wilson)	Sigma-Aldrich	Cat# A9418
Bovine Serum Albumin (Bloom)	ThermoFisher	Cat# 15260-037
Opti-MEM	ThermoFisher	Cat# 31985-088
L-glutamine	Invitrogen	Cat# 25030-164
Penicillin-streptomycin	ThermoFisher	Cat# 15140163
Antibiotic/Antimycotic	ThermoFisher	Cat# 15240-112
Experimental Models: Cell Lines		
MDCK cells	ATCC	Cat# CCL-34
HEK293T Cell Line	ATCC	Cat# CRL-11268
MDCK-SIAT1	Jesse Bloom's laboratory stock	
Experimental Models: Organisms/Strains		
BALB/cJ	The Jackson Laboratory	RRID:IMSR_JAX:000651

Recombinant DNA		
IgG-AbVec	Patrick Wilson's laboratory stock	N/A
Igκ-AbVec	Patrick Wilson's laboratory stock	N/A
Igλ-AbVec	Patrick Wilson's laboratory stock	N/A
Deposited Data		
Negative stain reconstruction of 4F04 Fab bound to CA09 H1 HA	EMDataBank	D_100025433
Negative stain reconstruction of 2F04 Fab bound to CA09 H1 HA	EMDataBank	D_1000254374
Negative stain reconstruction of donor 236 day 7 polyclonal Fabs targeting the anchor and esterase epitopes of CA09 H1 HA	EMDataBank	D_1000254375
Negative stain reconstruction of donor 236 day 7 polyclonal Fabs targeting the RBS of CA09 H1 HA	EMDataBank	D_1000254376
Negative stain reconstruction of donor 236 day 14 polyclonal Fabs targeting the top of the head of CA09 H1 HA	EMDataBank	D_1000254377
Negative stain reconstruction of donor 236 day 14 polyclonal Fabs targeting the esterase epitope of CA09 H1 HA	EMDataBank	D_1000254378
Negative stain reconstruction of donor 236 day 14 polyclonal Fabs targeting the RBS of CA09 H1 HA	EMDataBank	D_1000254383
Negative stain reconstruction of donor 236 day 14 polyclonal Fabs targeting the anchor epitope of CA09 H1 HA	EMDataBank	D_1000254384
Negative stain reconstruction of donor 241 day 7 polyclonal Fabs targeting the esterase epitope of CA09 H1 HA	EMDataBank	D_1000254385
Negative stain reconstruction of donor 241 day 7 polyclonal Fabs targeting the anchor epitope of CA09 H1 HA	EMDataBank	D_1000254386
Negative stain reconstruction of donor 241 day 14 polyclonal Fabs targeting the anchor epitope of CA09 H1 HA	EMDataBank	D_1000254388
Negative stain reconstruction of donor 241 day 14 polyclonal Fabs targeting the esterase epitope of CA09 H1 HA	EMDataBank	D_1000254379
Negative stain reconstruction of donor 241 day 14 polyclonal Fabs targeting the top of the head of CA09 H1 HA	EMDataBank	D_1000254391
Negative stain reconstruction of donor 241 day 14 polyclonal Fabs targeting the RBS of CA09 H1 HA	EMDataBank	D_1000254382
Software and Algorithms		
GraphPad Prism (version 8.4.3)	GraphPad Software Inc	http://www.graphpad.com RRID: SCR_002798
IgBlast	NCBI	http://www.ncbi.nlm.nih.gov/igblast/ RRID: SCR_002873
JMP Pro 15.1.0	SAS Institute Inc.	https://www.jmp.com/en_us/home.html RRID: SCR_014242
Clustal Omega	EMBL-EBI	http://www.ebi.ac.uk/Tools/msa/clustalo/ RRID: SCR_001591

UCSF Chimera	Resource for Biocomputing Visualization and Informatics	https://www.cgl.ucsf.edu/chimera/ RRID: SCR_004097
Unicorn 7.0	GE Healthcare	https://www.gelifesciences.com/
Leginon	Suloway et al., 2005	N/A
Appion	Lander et al., 2009	N/A
DoG Picker	Voss et al., 2009	N/A
Relion	Scheres, 2012	N/A
PyMOL	Schrodinger	RRID: SCR_000305
FlowJo 10.7.1	Beckton, Dickson, & Company	RRID: SCR_008520
R	The R Foundation for Statistical Computing	http://www.R-project.org
RStudio		https://www.rstudio.com/ RRID: SCR_000432
Seurat 3.2		https://www.cell.com/cell/fulltext/S0092-8674(19)30559-8
ggplot2 3.3.2		https://ggplot2.tidyverse.org
cowplot 1.1.0		https://github.com/wilkelab/cowplot
CellRanger 3.0.2	10x Genomics	https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger
WebLogo	University of California, Berkeley	https://weblogo.berkeley.edu/logo.cgi

511

512 **Resource Availability**

513 **Lead Contact**

514 Further information and requests for resources and reagents should be directed to the Lead contact,
515 Patrick C. Wilson (wilsonp@uchicago.edu).

516 **Materials Availability**

517 There are restrictions to the availability of mAbs from this study due to the lack of an external centralized
518 repository for its distribution and our need to maintain the stock. We are glad to share mAbs with
519 reasonable compensation by requestor for its processing and shipping.

520 **Data and Code Availability**

521 Repertoire data generated from single cell RNA-sequencing data is deposited at NCBI GenBank under
522 accession numbers (in process of being deposited). Electron microscopy maps were deposited to the
523 Electron Microscopy DataBank under accession IDs: D_100025433, D_1000254374, D_1000254375,

524 D_1000254376, D_1000254377, D_1000254378, D_1000254383, D_1000254384, D_1000254385,
525 D_1000254386, D_1000254388, D_1000254379, D_1000254391, and D_1000254382. All next
526 generation sequencing data for 045-09 2B06 deep mutational scanning can be found on the Sequence
527 Read Archive under BioProject accession number PRJNA494885.

528 EXPERIMENTAL MODEL AND SUBJECT DETAILS

529 Human Materials

530 Human PBMCs were obtained from multiple subjects from multiple cohorts, which is outlined in Table S1.
531 All studies were performed with the approval of the University of Chicago Institutional Review Board (ID
532 #09-043-A). The chimeric HA vaccine study cohort is identified as clinical trial NCT03300050.

533 Cell Lines

534 Human Embryonic Kidney HEK293T (female, # CRL-11268) and Madin Darby canine kidney MDCK
535 (female, # CCL-34, NBL-2) cells were purchased and authenticated by the American Type Culture
536 Collection (ATCC). All cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C. HEK293T
537 cells were maintained in Advanced-DMEM supplemented with 2% ultra-low IgG fetal bovine serum (FBS)
538 (Invitrogen), 1% L-glutamine (Invitrogen) and 1% antibiotic-antimycotic (Invitrogen). MDCK cells were
539 maintained in DMEM supplemented with 10% FBS (Invitrogen), 1% L-glutamine (Invitrogen) and 1%
540 penicillin-streptomycin (Invitrogen).

541 METHOD DETAILS

542 Monoclonal antibody production

543 Monoclonal antibodies were generated as previously described (Guthmiller et al., 2019; Smith et al., 2009;
544 Wrammert et al., 2008). Peripheral blood was obtained from each subject approximately 7 days after
545 vaccination or infection or obtained 28+ days post-vaccination. Lymphocytes were isolated and enriched
546 for B cells using RosetteSep. Total PBs (CD3⁻CD19⁺CD27^{hi}CD38^{hi}; all cohorts except 2014 QIV), IgG⁺ PBs
547 (CD3⁻CD19⁺IgM⁻CD27^{hi}CD38^{hi}IgG⁺IgA⁻; 2014 QIV), IgA⁺ PBs (CD3⁻CD19⁺IgM⁻CD27^{hi}CD38^{hi}IgG⁻IgA⁺; 2014
548 QIV cohort), or HA⁺ bait-sorted MBCs (CD3⁻CD19⁺CD27⁺CD38^{lo/+}HA⁺, for 030-09M 1B06) were single-cell
549 sorted into 96-well plates. Immunoglobulin heavy and light chain genes were amplified by reverse
550 transcriptase polymerase chain reaction (RT-PCR), sequenced, cloned into human IgG1, human kappa
551 chain, or human lambda expression vectors, and co-transfected into HEK293T cells. Secreted mAbs were
552 purified from the supernatant using protein A agarose beads. For mAbs generated from the 2014 QIV
553 cohort, mAb names include the original isotype of the sorted PB, and all mAbs were expressed as human
554 IgG1. cH5/1-binding B cells (CD19⁺CD27⁺cH5/1⁺) were sorted from subjects 28 days after cH5/1
555 vaccination (NCT03300050). Cells were sorted with A/California/04/2009 HA (for 030-09M 1B06) or cH5/1
556 probe with a Y98F mutation to ablate non-specific binding to sialic acids on B cells. MAb heavy chain and
557 light chain sequences were synthesized from single-cell RNA-sequencing data of cH5/1-baited B cells
558 (IDT), and cloned into the human IgG1, human kappa chain, or human lambda expression vectors. B cell

559 clones were determined by aligning all the V(D)J sequences sharing identical progenitor sequences, as
560 predicted by IgBLAST using our in-house software, VGenes. Consensus sequence analysis was
561 performed using WebLogo (Crooks et al., 2004) and sequence alignments were determined using Clustal
562 Omega .

563 **Viruses and recombinant proteins**

564 Influenza viruses used in all assays were grown in-house in specific pathogen free (SPF) eggs, harvested,
565 purified, and titered. The A/swine/Mexico/AVX8/2011 H1N2 virus (Mena et al., 2016) was provided by
566 Ignacio Mena, Adolfo García-Sastre, and Sean Liu at Icahn School of Medicine at Mount Sinai.
567 Recombinant HA, cHA, and mini-HA were obtained from BEI Resources or kindly provided by the Krammer
568 laboratory at Icahn School of Medicine at Mount Sinai, the Coughlan laboratory at The University of
569 Maryland School of Medicine, or the Wilson laboratory at the University of Chicago. Recombinant HA
570 mutant proteins used in Figure 2 were generated with identified mutations from the deep mutational
571 scanning experiments (see below) or with known mutations that have arisen naturally or were identified in
572 other studies (Figure S3). All mutations were made on HA from A/California/7/2009. Mutant HAs were
573 expressed in HEK293T cells and purified using Ni-NTA agarose beads (Qiagen).

574 **Antigen Specific ELISA**

575 High protein-binding microtiter plates (Costar) were coated with 8 hemagglutination units (HAU) of virus in
576 carbonate buffer or with recombinant HA, including HA mutants described below, at 2 μ g/ml in phosphate-
577 buffered saline (PBS) overnight at 4°C. Plates were washed the next morning with PBS 0.05% Tween and
578 blocked with PBS containing 20% fetal bovine serum (FBS) for 1 hr at 37°C. Antibodies were then serially
579 diluted 1:3 starting at 10 μ g/ml and incubated for 1.5 hr at 37°C. Horseradish peroxidase (HRP)-conjugated
580 goat anti-human IgG antibody diluted 1:1000 (Jackson Immuno Research) was used to detect binding of
581 mAbs, and plates were subsequently developed with Super Aquablue ELISA substrate (eBiosciences).
582 Absorbance was measured at 405 nm on a microplate spectrophotometer (BioRad). To standardize the
583 assays, control antibodies with known binding characteristics were included on each plate, and the plates
584 were developed when the absorbance of the control reached 3.0 OD units. All ELISAs were performed in
585 duplicate twice. To determine mAb affinity, a non-linear regression was performed on background
586 subtracted ODs and K_d values were reported. To classify antigen-specificity, mAbs that did not definitively
587 bind the HA head or stalk are listed as binding unknown HA⁺ epitopes. Affinity measurements, as
588 represented as K_d at a molar concentration (M), were calculated using Prism 9 (Graphpad) by performing
589 a non-linear regression.

590 **Deep mutational scanning for stalk domain mutants**

591 The mutant libraires used herein were previously described (Doud and Bloom, 2016). The libraries consist
592 of all single amino-acid mutations to A/WSN/1933 (H1N1). The experiments were performed by using
593 biological triplicate libraries. The mutational antigenic profiling of the 045-09 2B06 was performed as
594 previously outlined (Doud et al., 2017). In brief, 10⁶ TCID₅₀ of two of the virus library biological replicates

595 was diluted in 1mL in IGM (Opti-MEM supplemented with 0.01% FBS, 0.3% BSA, and 100 μ g/ml calcium
596 chloride) and incubated with an equal volume of 045-09 2B06 antibody at a final concentration of 50 or 25
597 μ g/mL for 1.5 hours at 37°C. MDCK-SIAT1 cells were infected with the virus antibody mixtures. 2 hours
598 post-infection, the media was removed, the cells washed with 1 ml PBS, and 2 ml of fresh IGM was added.
599 15 hours post-infection, viral RNA was extracted, reverse-transcribed using primers WSNHA-For (5'-
600 AGCAAAAGCAGGGAAAATAAAACAC-3') and WSNHA-Rev (5'-
601 AGTAGAAACAAGGGTCTTCTTCTTCTG-3'), and PCR amplified according to the barcoded-
602 subamplicon library preparation as previously described (Doud and Bloom, 2016). The overall fraction of
603 virions that survive antibody neutralization was estimated using qRT-PCR targeting the viral nucleoprotein
604 (NP) and cellular GAPDH as previously described (Doud et al., 2017). Using 10-fold serial dilutions of the
605 virus libraries, we infected cells with no antibody selection to serve as a standard curve of infectivity. qPCR
606 Ct values from the standard curve samples compared to the virus-antibody mix samples are determined
607 for NP and GAPDH. We then generate a linear regression to fit the difference between the NP and GAPDH
608 Ct values for the standard curve samples, and then use this curve to interpolate the fraction surviving for
609 the antibody-virus selection samples. Across the three library replicates the fraction of virus surviving
610 antibody selection was 0.17, 0.1, and 0.14.

611 Illumina(R) deep sequencing data was analyzed using dms_tools2 version 2.4.12 software
612 package (Bloom, 2015) which can be found at https://github.com/jbloomlab/dms_tools2. All of the
613 computer code used is at https://github.com/jbloomlab/2B06_DMS, and the Jupyter notebook that
614 performs most of the analysis is at
615 https://github.com/jbloomlab/2B06_DMS/blob/master/analysis_notebook.ipynb. The sequencing counts
616 were processed to estimate the differential selection for each mutation, which is the log enrichment of that
617 mutation in the antibody-selected condition versus the control (Doud et al., 2017). The numerical
618 measurements of the differential selection that 2B06 imposes on each mutation can be found here:
619 https://github.com/jbloomlab/2B06_DMS/blob/master/results/diffsel/tidy_diffsel.csv.

620 Competition ELISAs

621 Plates were coated with 50 μ l of A/California/7/2009 HA at a concentration of 1 μ g/ml and incubated
622 overnight at 4°C. To biotinylate the antibodies with known epitope specificities, CR9114 and 047-09-4F04,
623 were incubated at 4°C with EZ-Link™ Sulfo-NHS-Biotin (Thermo Scientific) for 24h or 48h prior to use,
624 respectively. After blocking the plates with PBS 20% FBS for 1h at 37°C, serum samples were incubated
625 (starting dilution of 1:50 for human serum or 20 μ g/ml for mAbs) in the coated wells for 2h at room
626 temperature. Either biotinylated CR9114 or 047-09-4F04 was then added at a concentration equal to twice
627 its K_d and incubated in the wells with the serum or mAbs for 2h at room temperature. The biotinylated
628 antibodies were desalting before addition to remove free biotin using Zeba™ spin desalting columns, 7k
629 MWCO (Thermo Scientific). After washing the plates, wells were incubated with HRP-conjugated
630 streptavidin (Southern Biotech) at 37°C for 1h for detection of the biotinylated antibody. Super Aquablue

631 ELISA substrate (eBiosciences) was then added and absorbance was measured at 405nm on a microplate
632 spectrophotometer (Bio-Rad). To standardize the assays, biotinylated CR9114 or TS-09-4F04 was
633 incubated in designated wells on each plate without any competing serum or mAb, and data were recorded
634 when the absorbance of these wells reached an optical density (OD) of 1 to 1.5 units. After subtracting
635 background, percent competition by serum samples was then determined by dividing a sample's observed
636 OD by the OD reached by the positive control, subtracting this value from 1, and multiplying by 100. For
637 the serum data, ODs were log transformed and analyzed by non-linear regression to determine EC₅₀ values
638 using Prism software (Graphpad). For Figure 5 and Figure S4, only subjects with serum for all timepoints
639 were included.

640 **Microneutralization Assays**

641 Microneutralization assays for mAb characterization were carried out as previously described (Chen et al.,
642 2018; Henry Dunand et al., 2015). MDCK cells were maintained in DMEM supplemented with 10% FBS,
643 1% penicillin-streptomycin, and 1% L-glutamine at 37°C with 5% CO₂. The day before the experiment,
644 25,000 MDCK cells were added to each well of a 96-well plate. Serial two-fold dilutions of mAb were mixed
645 with an equal volume of 100 TCID₅₀ of virus for 1 hr and added to MDCK cells for 1 hr at 37°C. The mixture
646 was removed, and cells were cultured for 20 hrs at 37°C with 1X MEM supplemented with 1 µg/ml tosyl
647 phenylalanyl chloromethyl ketone (TPCK)-treated trypsin and appropriate mAb concentration. Cells were
648 washed twice with PBS, fixed with 80% ice cold acetone at 20°C for at least 1 hr, washed 3 times with
649 PBS, blocked for 30 min with 3% BSA, and then treated for 30 min with 2% H₂O₂. Cells were incubated
650 with a mouse anti-nucleoprotein antibody (1:1000; Millipore) in 3% BSA-PBS for 1 hr at room temperature
651 (RT), followed by goat anti-mouse IgG HRP (1:1000; Southern Biotech) in 3% BSA-PBS for 1 hr at RT.
652 The plates were developed with Super Aquablue ELISA substrate at 405 nm until virus only controls
653 reached an OD of 1. The signal from uninfected wells was averaged to represent 100% inhibition. The
654 signal from infected wells without mAb was averaged to represent 0% inhibition. Duplication wells were
655 used to calculate the mean and SD of neutralization, and inhibitory concentration 50 (IC₅₀) was determined
656 by a sigmoidal dose response curve. The inhibition ratio (%) was calculated as below: ((OD Pos. Control
657 – OD Sample) / (OD Pos. Control – OD Neg. Control)) * 100. The final IC₅₀ was determined using Prism
658 software (GraphPad).

659 ***In vivo* challenge infections**

660 MAbs cocktails (Table S4) were passively transferred into 6- to 8-week-old female BALB/c mice (Jackson
661 Laboratories) by intraperitoneal injection of 0.2, 1, and 5 mg/kg mAb cocktail, which are further detailed in
662 Figure S4. Negative control mice received 5 mg/kg of the anthrax-specific mAb 003-15D03 as an isotype
663 control. Two hours post-mAb injection, mice were anesthetized with isoflurane and intranasally challenged
664 with 10 LD₅₀ of mouse-adapted A/Netherlands/602/2009 H1N1 virus, with 10 µl of virus administered into
665 each nostril (20 µl total). As a read out, survival and weight loss were monitored 1-2 times daily for two
666 weeks. Mice were euthanized upon 25% weight loss or at the end of the experiment (14 days post

667 challenge). All experiments were done in accordance with the University of Chicago Institutional Animal
668 Care and Use Committee.

669 **HA footprint mapping**

670 The footprints of three mAbs (FISW84 (PDB: 6HJQ), CR9114 (PDB: 4FQI), and FI6v3 (PDB: 3ZTN)) were
671 mapped onto one HA protomer (A/California/4/2009, PDB: 4M4Y) using UCSF Chimera (Pettersen et al.,
672 2004) and Adobe Photoshop. EM maps of HA:fab complexes were aligned in UCSF Chimera and footprints
673 were mapped onto one HA protomer. Individual protomers of the HA trimer are indicated in different shades
674 of gray.

675 **Negative stain EM**

676 Immune complexes were prepared by incubating Fab with HA (A/California/04/2009 with E47K or E47G
677 stabilizing mutations) at greater than 3:1 molar ratio for 2 hours at room temperature (RT). Samples were
678 deposited at ~10 μ g/mL on glow-discharged, carbon-coated 400 mesh copper grids (Electron Microscopy
679 Sciences, EMS) and stained with 2% w/v uranyl formate. Samples were imaged at 52,000x magnification,
680 120kV, on a Tecnai Spirit T12 microscope equipped with an Eagle CCD 4k camera (FEI) or 62,000
681 magnification, 200kV, on a Tecnai T20 microscope equipped with a CMOS 4k camera (TVIPS).
682 Micrographs were collected with Leginon, single particles were processed with Appion and Relion,
683 footprints were mapped with UCSF Chimera, and figures were made with UCSF Chimera (Lander et al.,
684 2009; Pettersen et al., 2004; Scheres, 2012; Suloway et al., 2005).

685 **EMPEM**

686 Human serum samples were heat-inactivated at 55°C for 30min before incubating on Capture Select IgG-
687 Fc (ms) Affinity Matrix (Fisher) to bind IgG at 4°C for 72 hours on a rotator. Samples with IgG bound to
688 resin were centrifuged at 4,000 rpm and supernatant was collected. IgG samples were washed 3 times
689 with PBS followed by centrifugation to remove supernatant. Samples were buffer exchanged into buffer
690 containing 100mM Tris, 2mM EDTA, and 10mM L-cysteine through centrifugation with Amicon filters, then
691 incubated with papain for 4 hours at 37°C shaking at 80 rpm. The digestion reactions were quenched with
692 50mM iodoacetamide, buffer exchanged to TBS, and separated by size-exclusion chromatography (SEC)
693 with a Superdex 200 increase 10/300 column (GE Healthcare). Fab and undigested IgG were collected
694 and concentrated and 500 μ g Fab was complexed with 10 μ g HA for 18 hours at room temperature.
695 Reactions were purified by SEC and immune complexes were collected and concentrated. Negative stain
696 EM grids were prepared as described above.

697 **Membrane-bound HA and mAb staining**

698 HEK293T cells were plated into a 6-well plate and transfected overnight with 0.2 μ g of plasmid and 10
699 μ g/ml PEI. After 12-16 hours, media was replaced with PFHM-II and cells were rested for 3 days.
700 Transfected cells were trypsinized, washed, and aliquoted. Cells were stained with 10 μ g/ml of individual
701 mAbs for 30 minutes. Cells were washed and stained with anti-human IgG Fc-BV421 for 30 minutes. Cells
702 were washed 2 times and run on a BD LSRFortessa X-20. Data were analyzed using FlowJo v10.

703 **Single-cell RNA-seq and repertoire analysis**

704 cH5/1⁺ memory B cells (CD19⁺CD27⁺HA⁺) were bulk sorted and partitioned into nanoliter-scale Gel Bead-
705 In-Emulsions (GEMs) to achieve single cell resolution using the 10x Genomics Chromium Controller and
706 according to the manufacturer's instruction (10x Genomics). The sorted single cells were processed
707 according to 5' gene expression and B cell Immunoglobulin (Ig) enrichment instruction to prepare the
708 libraries for sequencing. Libraries were sequenced using an Illumina HiSeq 4000 at Northwestern
709 University or an Illumina NextSeq 500 at the University of Chicago. Cellranger Single-Cell Software Suite
710 (version 3.0) was used to perform sample de-multiplexing, barcode processing, and single-cell 5' and
711 V(D)J counting, and Cellranger mkfastq was used to de-multiplex raw base call (BCL) files into sample-
712 specific fastq files. Subsequently, reads were aligned to the GRCh38 human genome. Cellranger counts
713 and Cellranger vdj package were used to identify gene expression and assemble V(D)J pairs of antibodies.

714 Single cell datasets were analyzed using Seurat 3 toolkit. We performed conventional pre-process
715 steps for all 22 subjects including cell quality control (QC), normalization, identification of highly variable
716 features, data scaling, and linear dimensional reduction. More specifically, we only kept cells with more
717 than 200 and less than 2500 detected genes for QC step. We normalized the RNA data using conventional
718 log normalization. We identified 2000 highly variable genes for each dataset and performed principle
719 component analysis (PCA) in linear dimensional reduction step. We then integrated all 22 single cell
720 datasets from vaccinated subjects to remove batch effects. In this analysis, we filtered our dataset and
721 only kept cells with both transcriptome and full length and paired heavy and light chain V(D)J sequences
722 (n=1955). From these cells, we identified a group of "VH1-69/Kappa" B cells that used the VH1-69 gene
723 and kappa light chain. We also identified a group of "anchor epitope" B cells by the following rules: 1) VH
724 locus: VH3-23, VH3-30, VH3-30-3, or VH-3-48; 2) VK locus: VK3-11 or VK3-15; 3) JK locus: JK4 or JK5;
725 4) K-CDR3 length equal to 10; 5) a "NWP" pattern in K-CDR3 peptide.

726 **HA conservation modeling**

727 To generate the group 1 HA conservation model, we selected one representative sequence for each group
728 1 HA subtype from FluDB (<https://www.fludb.org/>; Table S5) according to a prior study (Burke and Smith,
729 2014). A multiple sequence alignment from these HA protein sequences was generated using MUSCLE
730 (Edgar, 2004) and the conservation of each residue was quantified using an entropy model (Crooks et al.,
731 2004). HA conservation was visualized on a H1 protein (PDB: 4JTV) using PyMOL (Schrodinger).

732 **Statistical analysis**

733 All statistical analyses were performed using Prism software (Graphpad Version 7.0) or R. Sample sizes
734 (n) for the number of mAbs tested are indicated in corresponding figures or in the center of pie graphs.
735 Number of biological repeats for experiments and specific tests for statistical significance used are
736 indicated in the corresponding figure legends. P values less than or equal to 0.05 were considered
737 significant. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P < 0.0001.

738

References

- 739 Andrews, S.F., Huang, Y., Kaur, K., Popova, L.I., Ho, I.Y., Pauli, N.T., Henry Dunand, C.J., Taylor, W.M., Lim,
740 S., Huang, M., *et al.* (2015). Immune history profoundly affects broadly protective B cell responses to
741 influenza. *Sci Transl Med* 7, 316ra192.
- 742 Aydillo, T., Escalera, A., Strohmeier, S., Aslam, S., Sanchez-Cespedes, J., Ayllon, J., Roca-Oporto, C., Perez-
743 Romero, P., Montejo, M., Gavalda, J., *et al.* (2020). Pre-existing Hemagglutinin Stalk Antibodies Correlate
744 with Protection of Lower Respiratory Symptoms in Flu-Infected Transplant Patients. *Cell Rep Med* 1,
745 100130.
- 746 Bajic, G., Maron, M.J., Adachi, Y., Onodera, T., McCarthy, K.R., McGee, C.E., Sempowski, G.D., Takahashi,
747 Y., Kelsoe, G., Kuraoka, M., *et al.* (2019). Influenza Antigen Engineering Focuses Immune Responses to a
748 Subdominant but Broadly Protective Viral Epitope. *Cell Host Microbe* 25, 827-835 e826.
- 749 Benton, D.J., Nans, A., Calder, L.J., Turner, J., Neu, U., Lin, Y.P., Ketelaars, E., Kallewaard, N.L., Corti, D.,
750 Lanzavecchia, A., *et al.* (2018). Influenza hemagglutinin membrane anchor. *Proc Natl Acad Sci U S A* 115,
751 10112-10117.
- 752 Bernstein, D.I., Guptill, J., Naficy, A., Nachbagauer, R., Berlanda-Scorza, F., Feser, J., Wilson, P.C.,
753 Solorzano, A., Van der Wielen, M., Walter, E.B., *et al.* (2020). Immunogenicity of chimeric
754 haemagglutinin-based, universal influenza virus vaccine candidates: interim results of a randomised,
755 placebo-controlled, phase 1 clinical trial. *Lancet Infect Dis* 20, 80-91.
- 756 Bloom, J.D. (2015). Software for the analysis and visualization of deep mutational scanning data. *BMC
757 Bioinformatics* 16, 168.
- 758 Boyoglu-Barnum, S., Hutchinson, G.B., Boyington, J.C., Moin, S.M., Gillespie, R.A., Tsybovsky, Y.,
759 Stephens, T., Vaile, J.R., Lederhofer, J., Corbett, K.S., *et al.* (2020). Glycan repositioning of influenza
760 hemagglutinin stem facilitates the elicitation of protective cross-group antibody responses. *Nat Commun*
761 11, 791.
- 762 Broecker, F., Liu, S.T.H., Suntruwong, N., Sun, W., Bailey, M.J., Nachbagauer, R., Krammer, F., and
763 Palese, P. (2019). A mosaic hemagglutinin-based influenza virus vaccine candidate protects mice from
764 challenge with divergent H3N2 strains. *NPJ Vaccines* 4, 31.
- 765 Burke, D.F., and Smith, D.J. (2014). A recommended numbering scheme for influenza A HA subtypes.
766 *PLoS One* 9, e112302.
- 767 Cardoso, R.M., Zwick, M.B., Stanfield, R.L., Kunert, R., Binley, J.M., Katinger, H., Burton, D.R., and Wilson,
768 I.A. (2005). Broadly neutralizing anti-HIV antibody 4E10 recognizes a helical conformation of a highly
769 conserved fusion-associated motif in gp41. *Immunity* 22, 163-173.
- 770 Chen, Y.Q., Wohlbold, T.J., Zheng, N.Y., Huang, M., Huang, Y., Neu, K.E., Lee, J., Wan, H., Rojas, K.T.,
771 Kirkpatrick, E., *et al.* (2018). Influenza Infection in Humans Induces Broadly Cross-Reactive and Protective
772 Neuraminidase-Reactive Antibodies. *Cell* 173, 417-429 e410.
- 773 Clark, A.M., DeDiego, M.L., Anderson, C.S., Wang, J., Yang, H., Nogales, A., Martinez-Sobrido, L., Zand,
774 M.S., Sangster, M.Y., and Topham, D.J. (2017). Antigenicity of the 2015-2016 seasonal H1N1 human
775 influenza virus HA and NA proteins. *PLoS One* 12, e0188267.
- 776 Corti, D., Sugitan, A.L., Jr., Pinna, D., Silacci, C., Fernandez-Rodriguez, B.M., Vanzetta, F., Santos, C.,
777 Luke, C.J., Torres-Velez, F.J., Temperton, N.J., *et al.* (2010). Heterosubtypic neutralizing antibodies are
778 produced by individuals immunized with a seasonal influenza vaccine. *J Clin Invest* 120, 1663-1673.
- 779 Cotter, C.R., Jin, H., and Chen, Z. (2014). A single amino acid in the stalk region of the H1N1pdm
780 influenza virus HA protein affects viral fusion, stability and infectivity. *PLoS Pathog* 10, e1003831.
- 781 Crooks, G.E., Hon, G., Chandonia, J.M., and Brenner, S.E. (2004). WebLogo: a sequence logo generator.
782 *Genome Res* 14, 1188-1190.

- 783 Doud, M.B., and Bloom, J.D. (2016). Accurate Measurement of the Effects of All Amino-Acid Mutations
784 on Influenza Hemagglutinin. *Viruses* **8**.
- 785 Doud, M.B., Hensley, S.E., and Bloom, J.D. (2017). Complete mapping of viral escape from neutralizing
786 antibodies. *PLoS Pathog* **13**, e1006271.
- 787 Dreyfus, C., Laursen, N.S., Kwaks, T., Zuidgeest, D., Khayat, R., Ekiert, D.C., Lee, J.H., Metlagel, Z., Bujny,
788 M.V., Jongeneelen, M., *et al.* (2012). Highly conserved protective epitopes on influenza B viruses.
789 *Science* **337**, 1343-1348.
- 790 Dugan, H.L., Guthmiller, J.J., Arevalo, P., Huang, M., Chen, Y.Q., Neu, K.E., Henry, C., Zheng, N.Y., Lan,
791 L.Y., Tepora, M.E., *et al.* (2020). Preexisting immunity shapes distinct antibody landscapes after influenza
792 virus infection and vaccination in humans. *Sci Transl Med* **12**.
- 793 Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput.
794 *Nucleic Acids Res* **32**, 1792-1797.
- 795 Eggink, D., Goff, P.H., and Palese, P. (2014). Guiding the immune response against influenza virus
796 hemagglutinin toward the conserved stalk domain by hyperglycosylation of the globular head domain. *J
797 Virol* **88**, 699-704.
- 798 Ekiert, D.C., Bhabha, G., Elsliger, M.A., Friesen, R.H., Jongeneelen, M., Throsby, M., Goudsmit, J., and
799 Wilson, I.A. (2009). Antibody recognition of a highly conserved influenza virus epitope. *Science* **324**, 246-
800 251.
- 801 Ekiert, D.C., Kashyap, A.K., Steel, J., Rubrum, A., Bhabha, G., Khayat, R., Lee, J.H., Dillon, M.A., O'Neil,
802 R.E., Faynboym, A.M., *et al.* (2012). Cross-neutralization of influenza A viruses mediated by a single
803 antibody loop. *Nature* **489**, 526-532.
- 804 Ellebedy, A.H., Krammer, F., Li, G.M., Miller, M.S., Chiu, C., Wrammert, J., Chang, C.Y., Davis, C.W.,
805 McCausland, M., Elbein, R., *et al.* (2014). Induction of broadly cross-reactive antibody responses to the
806 influenza HA stem region following H5N1 vaccination in humans. *Proc Natl Acad Sci U S A* **111**, 13133-
807 13138.
- 808 Gallagher, J.R., McCraw, D.M., Torian, U., Gulati, N.M., Myers, M.L., Conlon, M.T., and Harris, A.K.
809 (2018). Characterization of Hemagglutinin Antigens on Influenza Virus and within Vaccines Using
810 Electron Microscopy. *Vaccines* (Basel) **6**.
- 811 Guthmiller, J.J., Dugan, H.L., Neu, K.E., Lan, L.Y., and Wilson, P.C. (2019). An Efficient Method to
812 Generate Monoclonal Antibodies from Human B Cells. *Methods Mol Biol* **1904**, 109-145.
- 813 Guthmiller, J.J., Lan, L.Y., Fernandez-Quintero, M.L., Han, J., Utset, H.A., Bitar, D.J., Hamel, N.J., Stovicek,
814 O., Li, L., Tepora, M., *et al.* (2020). Polyreactive Broadly Neutralizing B cells Are Selected to Provide
815 Defense against Pandemic Threat Influenza Viruses. *Immunity*.
- 816 Hai, R., Krammer, F., Tan, G.S., Pica, N., Eggink, D., Maamary, J., Margine, I., Albrecht, R.A., and Palese, P.
817 (2012). Influenza viruses expressing chimeric hemagglutinins: globular head and stalk domains derived
818 from different subtypes. *J Virol* **86**, 5774-5781.
- 819 Han, J., Schmitz, A.J., Richey, S.T., Dai, Y.-N., Turner, H.L., Mohammed, B.M., Fremont, D.H., Ellebedy,
820 A.H., and Ward, A.B. (2020). Polyclonal epitope cartography reveals the temporal dynamics and diversity
821 of human antibody responses to H5N1 vaccination. *bioRxiv*, 2020.2006.2016.155754.
- 822 Henry, C., Zheng, N.Y., Huang, M., Cabanov, A., Rojas, K.T., Kaur, K., Andrews, S.F., Palm, A.E., Chen, Y.Q.,
823 Li, Y., *et al.* (2019). Influenza Virus Vaccination Elicits Poorly Adapted B Cell Responses in Elderly
824 Individuals. *Cell Host Microbe* **25**, 357-366 e356.
- 825 Henry Dunand, C.J., Leon, P.E., Huang, M., Choi, A., Chromikova, V., Ho, I.Y., Tan, G.S., Cruz, J., Hirsh, A.,
826 Zheng, N.Y., *et al.* (2016). Both Neutralizing and Non-Neutralizing Human H7N9 Influenza Vaccine-
827 Induced Monoclonal Antibodies Confer Protection. *Cell Host Microbe* **19**, 800-813.

- 828 Henry Dunand, C.J., Leon, P.E., Kaur, K., Tan, G.S., Zheng, N.Y., Andrews, S., Huang, M., Qu, X., Huang, Y.,
829 Salgado-Ferrer, M., *et al.* (2015). Preexisting human antibodies neutralize recently emerged H7N9
830 influenza strains. *J Clin Invest* 125, 1255-1268.
- 831 Impagliazzo, A., Milder, F., Kuipers, H., Wagner, M.V., Zhu, X., Hoffman, R.M., van Meersbergen, R.,
832 Huizingh, J., Wanningen, P., Verspuij, J., *et al.* (2015). A stable trimeric influenza hemagglutinin stem as a
833 broadly protective immunogen. *Science* 349, 1301-1306.
- 834 Joyce, M.G., Wheatley, A.K., Thomas, P.V., Chuang, G.Y., Soto, C., Bailer, R.T., Druz, A., Georgiev, I.S.,
835 Gillespie, R.A., Kanekiyo, M., *et al.* (2016). Vaccine-Induced Antibodies that Neutralize Group 1 and
836 Group 2 Influenza A Viruses. *Cell* 166, 609-623.
- 837 Kirkpatrick, E., Qiu, X., Wilson, P.C., Bahl, J., and Krammer, F. (2018). The influenza virus hemagglutinin
838 head evolves faster than the stalk domain. *Sci Rep* 8, 10432.
- 839 Krammer, F., Pica, N., Hai, R., Margine, I., and Palese, P. (2013). Chimeric hemagglutinin influenza virus
840 vaccine constructs elicit broadly protective stalk-specific antibodies. *J Virol* 87, 6542-6550.
- 841 Krystal, M., Elliott, R.M., Benz, E.W., Jr., Young, J.F., and Palese, P. (1982). Evolution of influenza A and B
842 viruses: conservation of structural features in the hemagglutinin genes. *Proc Natl Acad Sci U S A* 79,
843 4800-4804.
- 844 Lander, G.C., Stagg, S.M., Voss, N.R., Cheng, A., Fellmann, D., Pulokas, J., Yoshioka, C., Irving, C., Mulder,
845 A., Lau, P.W., *et al.* (2009). Appion: an integrated, database-driven pipeline to facilitate EM image
846 processing. *J Struct Biol* 166, 95-102.
- 847 Li, G.M., Chiu, C., Wrammert, J., McCausland, M., Andrews, S.F., Zheng, N.Y., Lee, J.H., Huang, M., Qu, X.,
848 Edupuganti, S., *et al.* (2012). Pandemic H1N1 influenza vaccine induces a recall response in humans that
849 favors broadly cross-reactive memory B cells. *Proc Natl Acad Sci U S A* 109, 9047-9052.
- 850 Linderman, S.L., Chambers, B.S., Zost, S.J., Parkhouse, K., Li, Y., Herrmann, C., Ellebedy, A.H., Carter,
851 D.M., Andrews, S.F., Zheng, N.Y., *et al.* (2014). Potential antigenic explanation for atypical H1N1
852 infections among middle-aged adults during the 2013-2014 influenza season. *Proc Natl Acad Sci U S A*
853 111, 15798-15803.
- 854 Liu, S.T.H., Behzadi, M.A., Sun, W., Freyn, A.W., Liu, W.C., Broecker, F., Albrecht, R.A., Bouvier, N.M.,
855 Simon, V., Nachbagauer, R., *et al.* (2018). Antigenic sites in influenza H1 hemagglutinin display species-
856 specific immunodominance. *J Clin Invest* 128, 4992-4996.
- 857 Mena, I., Nelson, M.I., Quezada-Monroy, F., Dutta, J., Cortes-Fernandez, R., Lara-Puente, J.H., Castro-
858 Peralta, F., Cunha, L.F., Trovao, N.S., Lozano-Dubernard, B., *et al.* (2016). Origins of the 2009 H1N1
859 influenza pandemic in swine in Mexico. *Elife* 5.
- 860 Nachbagauer, R., Feser, J., Naficy, A., Bernstein, D.I., Guptill, J., Walter, E.B., Berlanda-Scorza, F.,
861 Stadlbauer, D., Wilson, P.C., Aydillo, T., *et al.* (2020). A chimeric hemagglutinin-based universal influenza
862 virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled
863 phase I trial. *Nat Med*.
- 864 Nachbagauer, R., Wohlbold, T.J., Hirsh, A., Hai, R., Sjursen, H., Palese, P., Cox, R.J., and Krammer, F.
865 (2014). Induction of broadly reactive anti-hemagglutinin stalk antibodies by an H5N1 vaccine in humans.
866 *J Virol* 88, 13260-13268.
- 867 Ng, S., Nachbagauer, R., Balmaseda, A., Stadlbauer, D., Ojeda, S., Patel, M., Rajabhathor, A., Lopez, R.,
868 Guglia, A.F., Sanchez, N., *et al.* (2019). Novel correlates of protection against pandemic H1N1 influenza A
869 virus infection. *Nat Med* 25, 962-967.
- 870 Ofek, G., Tang, M., Sambor, A., Katinger, H., Mascola, J.R., Wyatt, R., and Kwong, P.D. (2004). Structure
871 and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with
872 its gp41 epitope. *J Virol* 78, 10724-10737.
- 873 Ohmit, S.E., Petrie, J.G., Cross, R.T., Johnson, E., and Monto, A.S. (2011). Influenza hemagglutination-
874 inhibition antibody titer as a correlate of vaccine-induced protection. *J Infect Dis* 204, 1879-1885.

- 875 Park, J.K., Xiao, Y., Ramuta, M.D., Rosas, L.A., Fong, S., Matthews, A.M., Freeman, A.D., Gouzoulis, M.A.,
876 Batchenkova, N.A., Yang, X., *et al.* (2020). Pre-existing immunity to influenza virus hemagglutinin stalk
877 might drive selection for antibody-escape mutant viruses in a human challenge model. *Nat Med* **26**,
878 1240-1246.
- 879 Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E.
880 (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**,
881 1605-1612.
- 882 Pica, N., and Palese, P. (2013). Toward a universal influenza virus vaccine: prospects and challenges.
883 *Annu Rev Med* **64**, 189-202.
- 884 Raymond, D.D., Bajic, G., Ferdman, J., Suphaphiphat, P., Settembre, E.C., Moody, M.A., Schmidt, A.G.,
885 and Harrison, S.C. (2018). Conserved epitope on influenza-virus hemagglutinin head defined by a
886 vaccine-induced antibody. *Proc Natl Acad Sci U S A* **115**, 168-173.
- 887 Scheres, S.H. (2012). RELION: implementation of a Bayesian approach to cryo-EM structure
888 determination. *J Struct Biol* **180**, 519-530.
- 889 Smith, K., Garman, L., Wrammert, J., Zheng, N.Y., Capra, J.D., Ahmed, R., and Wilson, P.C. (2009). Rapid
890 generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat Protoc* **4**, 372-
891 384.
- 892 Sui, J., Hwang, W.C., Perez, S., Wei, G., Aird, D., Chen, L.M., Santelli, E., Stec, B., Cadwell, G., Ali, M., *et al.*
893 (2009). Structural and functional bases for broad-spectrum neutralization of avian and human influenza
894 A viruses. *Nat Struct Mol Biol* **16**, 265-273.
- 895 Suloway, C., Pulokas, J., Fellmann, D., Cheng, A., Guerra, F., Quispe, J., Stagg, S., Potter, C.S., and
896 Carragher, B. (2005). Automated molecular microscopy: the new Leginon system. *J Struct Biol* **151**, 41-
897 60.
- 898 Sun, W., Kirkpatrick, E., Ermler, M., Nachbagauer, R., Broecker, F., Krammer, F., and Palese, P. (2019).
899 Development of Influenza B Universal Vaccine Candidates Using the "Mosaic" Hemagglutinin Approach. *J
900 Virol* **93**.
- 901 van der Lubbe, J.E.M., Huizingh, J., Verspuij, J.W.A., Tettero, L., Schmit-Tillemans, S.P.R., Mooij, P.,
902 Mortier, D., Koopman, G., Bogers, W., Dekking, L., *et al.* (2018). Mini-hemagglutinin vaccination induces
903 cross-reactive antibodies in pre-exposed NHP that protect mice against lethal influenza challenge. *NPJ
904 Vaccines* **3**, 25.
- 905 Wasilewski, S., Calder, L.J., Grant, T., and Rosenthal, P.B. (2012). Distribution of surface glycoproteins on
906 influenza A virus determined by electron cryotomography. *Vaccine* **30**, 7368-7373.
- 907 Weidenbacher, P.A., and Kim, P.S. (2019). Protect, modify, deprotect (PMD): A strategy for creating
908 vaccines to elicit antibodies targeting a specific epitope. *Proc Natl Acad Sci U S A* **116**, 9947-9952.
- 909 Whittle, J.R., Zhang, R., Khurana, S., King, L.R., Manischewitz, J., Golding, H., Dormitzer, P.R., Haynes,
910 B.F., Walter, E.B., Moody, M.A., *et al.* (2011). Broadly neutralizing human antibody that recognizes the
911 receptor-binding pocket of influenza virus hemagglutinin. *Proc Natl Acad Sci U S A* **108**, 14216-14221.
- 912 Wrammert, J., Koutsonanos, D., Li, G.M., Edupuganti, S., Sui, J., Morrissey, M., McCausland, M.,
913 Skountzou, I., Hornig, M., Lipkin, W.I., *et al.* (2011). Broadly cross-reactive antibodies dominate the
914 human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* **208**, 181-193.
- 915 Wrammert, J., Smith, K., Miller, J., Langley, W.A., Kokko, K., Larsen, C., Zheng, N.Y., Mays, I., Garman, L.,
916 Helms, C., *et al.* (2008). Rapid cloning of high-affinity human monoclonal antibodies against influenza
917 virus. *Nature* **453**, 667-671.
- 918 Wu, N.C., Thompson, A.J., Lee, J.M., Su, W., Arlian, B.M., Xie, J., Lerner, R.A., Yen, H.L., Bloom, J.D., and
919 Wilson, I.A. (2020). Different genetic barriers for resistance to HA stem antibodies in influenza H3 and H1
920 viruses. *Science* **368**, 1335-1340.

- 921 Wu, N.C., and Wilson, I.A. (2020). Influenza Hemagglutinin Structures and Antibody Recognition. *Cold*
922 *Spring Harb Perspect Med* **10**.
- 923 Yassine, H.M., Boyington, J.C., McTamney, P.M., Wei, C.J., Kanekiyo, M., Kong, W.P., Gallagher, J.R.,
924 Wang, L., Zhang, Y., Joyce, M.G., *et al.* (2015). Hemagglutinin-stem nanoparticles generate
925 heterosubtypic influenza protection. *Nat Med* **21**, 1065-1070.
- 926