

1 **Fcγ receptor-dependent antibody effector functions are required for vaccine**
2 **protection against infection by antigenic variants of SARS-CoV-2**

3

4 Samantha R. Mackin^{1,2}, Pritesh Desai¹, Bradley M. Whitener¹, Courtney E. Karl^{1,3}, Meizi Liu¹,
5 Ralph S. Baric⁴, Darin K. Edwards⁵, Taras M. Chiczc⁶, Ryan P. McNamara⁶, Galit Alter^{5,6,7}, and
6 Michael S. Diamond^{1,2,3,7,8¶}.

7

8 ¹Department of Medicine, Washington University School of Medicine, St. Louis, MO

9 ²Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO

10 ³Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO

11 ⁴Department of Epidemiology, University of North Carolina, Chapel Hill, NC,

12 ⁵Moderna, Inc., Cambridge MA

13 ⁶Ragon Institute of MGH, MIT and Harvard, Cambridge, MA.

14 ⁷Andrew M. and Jane M. Bursky the Center for Human Immunology and Immunotherapy Programs,
15 Washington University School of Medicine, St. Louis, MO

16 ⁸Center for Vaccines and Immunity to Microbial Pathogens, Washington University School of Medicine,
17 St. Louis, MO

18 ¶ **Corresponding author:** Michael S. Diamond, M.D., Ph.D., mdiamond@wustl.edu

19

20 **ABSTRACT**

21 Emerging SARS-CoV-2 variants with antigenic changes in the spike protein are
22 neutralized less efficiently by serum antibodies elicited by legacy vaccines against the ancestral
23 Wuhan-1 virus. Nonetheless, these vaccines, including mRNA-1273 and BNT162b2, retained
24 their ability to protect against severe disease and death, suggesting that other aspects of
25 immunity control infection in the lung. Although vaccine-elicited antibodies can bind Fc gamma
26 receptors (Fc γ R) and mediate effector functions against SARS-CoV-2 variants, and this
27 property correlates with improved clinical COVID-19 outcome, a causal relationship between Fc
28 effector functions and vaccine-mediated protection against infection has not been established.
29 Here, using passive and active immunization approaches in wild-type and Fc-gamma receptor
30 (Fc γ R) KO mice, we determined the requirement for Fc effector functions to protect against
31 SARS-CoV-2 infection. The antiviral activity of passively transferred immune serum was lost
32 against multiple SARS-CoV-2 strains in mice lacking expression of activating Fc γ Rs, especially
33 murine Fc γ R III (CD16), or depleted of alveolar macrophages. After immunization with the
34 preclinical mRNA-1273 vaccine, protection against Omicron BA.5 infection in the respiratory
35 tract also was lost in mice lacking Fc γ R III. Our passive and active immunization studies in mice
36 suggest that Fc-Fc γ R engagement and alveolar macrophages are required for vaccine-induced
37 antibody-mediated protection against infection by antigenically changed SARS-CoV-2 variants,
38 including Omicron strains.

39 INTRODUCTION

40 Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-
41 2) in late 2019, 638 million infections and 6.6 million deaths have been reported
42 (<https://covid19.who.int/>). As part of the global response to COVID-19, vaccines using multiple
43 different platforms (mRNA, adenoviral-vectored, subunit-based, and inactivated virion) were
44 generated and deployed resulting in reductions in symptomatic infections, hospitalizations, and
45 deaths. These SARS-CoV-2 vaccines all targeted the viral spike protein derived from strains
46 that circulated in early 2020. However, the continuing evolution of SARS-CoV-2, with increasing
47 numbers of amino acid changes in the spike protein amidst successive waves of infection, has
48 jeopardized the immunity generated by these vaccines and the control of virus infection and
49 transmission ¹.

50 SARS-CoV-2 vaccination can induce neutralizing antibodies that inhibit infection ^{2,3}.
51 Correlates of vaccine protection initially focused on the neutralizing activity of elicited anti-spike
52 antibodies ^{4,5}. Emerging variants of concern, which have amino acid substitutions in regions
53 targeted by neutralizing antibodies including the receptor binding domain (RBD) and N terminal
54 domain (NTD) ^{6,7}, have jeopardized vaccine-mediated protection against infection and prompted
55 the development of bivalent vaccine boosters ⁸. Indeed, a substantial decrease in the
56 neutralizing activity of serum antibodies elicited by vaccines against the ancestral Wuhan-1
57 virus has been observed against emerging variants, which has correlated with symptomatic
58 breakthrough infections, especially with Omicron lineage viruses ⁹⁻¹¹. The large number (>30) of
59 substitutions in the spike protein in Omicron lineage strains, which abrogates or reduces binding
60 of the majority of highly neutralizing vaccine-derived and therapeutic antibodies, has been
61 termed an antigenic shift ¹²⁻¹⁴. Despite the loss in serum neutralizing activity against variants
62 such as those in Omicron lineage, most individuals remain protected against severe disease
63 and death. The basis for this protection has not been fully determined but could be due to

64 beneficial effects of non-neutralizing antibodies, cross-reactive T cell responses, or anamnestic
65 memory B cell responses ^{6,15-18}.

66 Fc effector function activity of non-neutralizing, cross-reactive, anti-spike antibodies is
67 one hypothesized mechanism for protection against antigenically-shifted SARS-CoV-2 variants
68 ⁷. In patients with moderate to severe COVID-19, the ability of antibodies to bind Fc-gamma
69 receptors (Fc γ R) and mediate effector functions correlated with increased survival ¹⁹.
70 Interactions of the conserved Fc region of IgG antibodies with Fc γ R or complement can promote
71 clearance of virally-infected cells through antibody-dependent cellular cytotoxicity (ADCC),
72 antibody-dependent cellular phagocytosis (ADCP), or complement-dependent deposition and
73 phagocytosis or lysis. Indeed, monoclonal antibodies (mAbs) that lose their ability to neutralize
74 SARS-CoV-2 variants yet still bind the spike protein avidly enough to trigger Fc effector
75 functions retain protective activity ^{15,20}. Analogously, non-neutralizing antibodies induced by
76 SARS-CoV-2 vaccines have been linked to protection against variant Omicron strains by virtue
77 of their ability to engage Fc γ Rs and promote clearance ²¹⁻²³. Furthermore, the depletion of RBD-
78 specific antibodies from serum of mRNA-1273 or BNT162b2 vaccinated individuals did not
79 appreciably impact Fc-mediated effector function activity in cell culture ⁶, suggesting that
80 antibodies recognizing conserved, non-neutralizing epitopes may contribute to protection
81 against variant strains.

82 Although serum-derived anti-SARS-CoV-2 antibody-mediated Fc effector functions can
83 activate complement deposition, immune cell phagocytosis, and target cell killing *in vitro*, their
84 contribution to protection *in vivo* remains uncertain. Existing data on the role of Fc-Fc γ R
85 interactions in the context of vaccine-mediated protection against SARS-CoV-2 is largely
86 correlative. To address this gap, we evaluated the impact of Fc effector functions in the context
87 of passive transfer of vaccine-elicited antibodies or active immunization with mRNA-1273
88 vaccine using wild-type, C1q KO, and Fc γ R KO C57BL/6 mice and challenge with SARS-CoV-2

89 viruses. In passive serum transfer experiments, we found that activating FcγRs but not C1q
90 were required to control SARS-CoV-2 infection in the lower respiratory tract, and protection was
91 lost in mice depleted of alveolar macrophages but not neutrophils and monocytes. Experiments
92 with mice lacking individual FcγRs showed the protective effect of passively transferred serum
93 antibody on viral load reduction required expression of FcγR III. To determine the impact of
94 FcγRs in the context of active immunization, wild-type, FcγR I KO, FcγR III KO, and FcγR I/III/IV
95 KO mice were administered a two-dose primary vaccination series with mRNA-1273, evaluated
96 for immunogenicity, and then challenged with the antigenically shifted Omicron BA.5 strain.
97 Although the levels of anti-RBD antibody, neutralizing antibody, and spike-specific T cells were
98 similar in all tested strains of mice, protection against infection in the nasal turbinates and lungs
99 was lost in FcγR III KO and FcγR I/III/IV KO mice. Overall, our results in mice suggest that Fc-
100 FcγR interactions contribute to antiviral protection *in vivo* in the context of both passive and
101 active immunization with legacy vaccines, particularly when neutralizing antibody levels are low
102 against antigenically distant SARS-CoV-2 variant strains.

103 RESULTS

104 Immunoglobulin subclass and Fc γ R binding of vaccine-induced immune serum.

105 To begin to evaluate the contribution of Fc effector functions to antibody protection against
106 SARS-CoV-2 infection, we profiled vaccine-induced antibodies from sera pooled from
107 immunized C57BL/6 mice using a systems serology assay ²⁴. We measured the binding of
108 polyclonal antibodies to several spike proteins (Wuhan-1, B.1.617.2, BA.1, and BA.4/5) and
109 determined their IgG subclass specificity (IgG1, IgG2a, or IgG2c) and ability to interact with
110 specific Fc γ Rs (Fc γ R IIb, Fc γ R III, or Fc γ R IV). We used naïve sera and binding to influenza
111 hemagglutinin (HA) protein as negative controls (**Fig 1a-f**). Immune sera contained higher levels
112 of IgG1, IgG2b, and IgG2c antibodies against Wuhan-1, B.1.617.2, BA.1, and BA.4/5 but not
113 HA compared to naïve sera (**Fig 1a-c**). Anti-spike antibody binding to inhibitory (Fc γ R IIb) and
114 activating (Fc γ R III and Fc γ R IV) was higher in immune sera compared to naïve sera for all
115 SARS-CoV-2 spike variants (**Fig 1d-f**). We also assessed the effector function activity of
116 immune serum using assays that measure spike-specific antibody-dependent cellular
117 phagocytosis in murine monocytes (ADCP) and neutrophils (ADNP) (**Fig 1g-h and Extended**
118 **Data Fig 1**). Compared to naïve sera, vaccine-elicited immune sera promoted greater ADCP
119 and ADNP activity against the Wuhan-1 and BA.4/5 spike proteins. In comparison, immune
120 sera did not enhance antibody-dependent natural killer cell activation (CD107a expression, **Fig**
121 **1i**). Immune sera also promoted antibody-dependent complement deposition (ADCD) on beads
122 coated with spike proteins compared to HA protein (**Fig 1j**). Overall, these studies indicated that
123 our pooled vaccine sera have a diversity of antibodies against spike proteins that enables
124 binding to Fc γ Rs, and most Fc-mediated effector functions in cell culture.

125 Protection in the lungs conferred by passive sera transfer requires Fc-Fc γ R

126 **engagement.** To assess the impact of Fc effector functions *in vivo* in the context of polyclonal
127 immune anti-SARS-CoV-2 antibodies, pooled naïve or vaccine-elicited immune sera was

128 transferred passively to 12-week-old male wild-type, Fc γ R I/III/IV KO (common γ chain KO), or
129 C1q KO C57BL/6 mice; Fc γ R I/III/IV KO mice lack the common γ chain present in all activating
130 murine Fc γ Rs, whereas C1q KO mice lack C1q, a protein required for initiation of the antibody-
131 dependent complement activation pathway. One day after transfer, mice were inoculated with
132 SARS-CoV-2 MA-10²⁵, and 4 days post-infection (dpi), nasal wash, nasal turbinates, and lungs
133 were harvested (**Fig 2a**). We used the mouse-adapted MA-10 strain for these initial studies
134 because it spreads to the lungs of conventional C57BL/6 mice without a need for ectopic human
135 ACE2 expression. Pooled vaccine-induced immune sera neutralized MA-10 at a 1/3,300 dilution
136 titer, and one day after transfer, serum from recipient mice neutralized MA-10 with a titer of 1/36
137 (**Fig 2b**). In the nasal washes and nasal turbinates of the upper airway of wild-type, Fc γ R I/III/IV
138 KO, and C1q KO mice, we observed no significant differences in levels of viral RNA or
139 infectious virus among the three groups receiving naive or immune serum. Although there was a
140 trend towards less viral infection in the nasal turbinates of animals receiving immune compared
141 to naïve sera, the comparisons did not reach statistical significance (**Fig 2c-e**); these results are
142 consistent with the lower accumulation of IgG in the upper respiratory tract after passive transfer
143 by a systemic route²⁶. Results from lung tissues, however, showed a different pattern, with a
144 loss of protection against infection by immune sera (viral RNA levels and infectious virus) in
145 Fc γ R I/III/IV KO but not in C1q KO mice (**Fig 2f-g**). Thus, Fc γ R expression in the lower
146 respiratory tract appears important for control of infection after passive antibody transfer.

147 **Antibody protection in the lungs requires Fc γ R III engagement.** Because MA-10 is
148 not matched to the vaccine antigen, in the context of passive transfer, we might underestimate
149 the protection against infection afforded by serum neutralizing antibody. To address this issue
150 and also identify which Fc γ R contributed to the protective phenotype, we passively transferred
151 naïve or immune sera to 12-week-old male wild-type, Fc γ R I KO, Fc γ R II KO, Fc γ R III KO, and
152 Fc γ R I/III/IV KO congenic C57BL/6 male mice before inoculation with SARS-CoV-2 WA1/2020

153 N501Y/D614G (**Fig 3a**), a more closely matched virus; because this suite of Fc γ R-deficient
154 C57BL/6 mice lacks human ACE2 expression, we used a virus with a mouse-adapting N501Y
155 mutation^{27,28}. Pooled vaccine-elicited immune sera neutralized WA1/2020 N501Y/D614G more
156 efficiently than MA-10 at a 1/16,750 serum dilution, and one day after transfer, serum from
157 recipient mice neutralized WA1/2020 N501Y/D614G with a titer of 1/750 (**Fig 3b**), which
158 exceeds a ~1/50 presumptive correlate of protection in humans⁴. As seen with MA-10 infection,
159 in the nasal washes and nasal turbinates of the upper respiratory tract, we did not observe
160 serum antibody protection in wild-type C57BL/6 mice (**Fig 3c-e**); thus, we focused analysis on
161 the lung. Indeed, passive transfer of immune sera protected against SARS-CoV-2 infection in
162 the lungs of wild-type C57BL/6 mice as measured by viral RNA and infectious virus levels (**Fig**
163 **3f-g**). Similar levels of protection were observed in Fc γ R I KO and Fc γ R II KO. However,
164 protection against SARS-CoV-2 lung infection was diminished or lost in Fc γ R III and Fc γ R I/III/IV
165 KO mice. These data suggest that even in the context of passive transfer of immune sera with
166 neutralizing activity, protection against lower respiratory tract infection by SARS-CoV-2 is
167 mediated at least in part by Fc interactions with activating Fc γ Rs, particularly Fc γ R III.

168 **Vaccine-elicited immunity requires Fc-Fc γ R engagement to confer protection**
169 **against SARS-CoV-2 infection.** We next evaluated the dependence on Fc-Fc γ R engagement
170 on protection against SARS-CoV-2 infection in the context of vaccine-elicited immunity, which
171 induces both cellular and humoral responses. We immunized groups of nine-week-old male
172 wild-type, Fc γ R I KO, Fc γ R III KO, and Fc γ R I/III/IV KO C57BL/6 mice twice over four weeks with
173 0.25 μ g of control or preclinical mRNA-1273 vaccine (**Fig 4a**); we did not immunize Fc γ R II KO
174 mice, since the virological phenotypes in the context of passive antibody transfer were present
175 in mice lacking activating Fc γ Rs but not Fc γ R II (**Fig 2 and 3**). The 0.25 μ g dose of mRNA
176 vaccine was used because the B and T cell responses generated in C57BL/6 mice with this
177 dose approximate those observed in humans receiving 100 μ g doses^{29,30}. One potential

178 limitation of this experiment is that a loss of activating FcγRs could affect vaccine-induced
179 immune responses, which might confound interpretation of challenge studies. To evaluate this
180 first, twenty-four days following boosting, serum was obtained to measure binding and
181 neutralizing antibody against WA1/2020 N501Y/D614G and BA.5. As expected, higher levels of
182 serum IgG were detected against Wuhan-1 than BA.5 receptor binding domain (RBD) protein
183 (**Fig 4b-c**), consistent with the antigenic shift of Omicron lineage strains^{12,14}. However, no
184 statistical differences in binding titers were observed between the groups of vaccinated wild-
185 type and FcγR KO mice (**Fig 4b-c**). Similarly, lower neutralization titers were detected against
186 BA.5 than WA1/2020 N501Y/D614G, with no substantive differences observed between groups
187 of vaccinated wild-type and FcγR KO (**Fig 4d-e**). Thus, mRNA-1273 vaccination induced
188 relatively similar humoral immune responses in mice that were sufficient or deficient in FcγR
189 expression. We also measured spike-specific CD4⁺ and CD8⁺ T cell responses in vaccinated
190 mice using previously defined immunodominant peptides^{31,32}. As expected, antigen-specific T
191 cell responses were greater in animals given the mRNA-1273 vaccine compared to the control
192 mRNA vaccine. However, IFN-γ and TNF-α responses in CD4⁺ and CD8⁺ T cells after peptide
193 restimulation were equivalent in FcγR KO and wild-type mice after mRNA-1273 vaccination
194 (**Extended Data Fig 2**). These experiments establish that FcγR KO and wild-type mice have
195 similar serum antibody and T cell responses after mRNA vaccination.

196 To assess whether mice expressing FcγRs were differentially protected against SARS-
197 CoV-2 infection by vaccine-induced immunity, animals were challenged by intranasal route with
198 10³ FFU of BA.5, and infectious virus in the nasal turbinates and lungs was measured at 3 dpi
199 (**Fig 4a**). For these experiments, we used BA.5 as the challenge virus because: (i) it encodes a
200 mouse-adapting mutation (N501Y) that facilitates replication in mice lacking human ACE2
201 expression³³; (ii) it allowed us to assess protection against infection under conditions when high
202 levels of neutralizing antibody are absent (**Fig 4e**); and (iii) BA.5, and other Omicron variants

203 are circulating, so use of this strain could provide insight as to how legacy vaccines directed
204 against ancestral spikes protect against severe BA.5 disease in humans. Notably, we observed
205 protection against BA.5 infection in the upper and lower respiratory tract of wild-type and Fc γ R I
206 KO mice but not in Fc γ R III KO or Fc γ R I/III/IV KO mice (**Fig 4f-g**). Thus, protection elicited by
207 mRNA-1273 vaccine-induced immunity against the antigenically shifted BA.5 SARS-CoV-2
208 requires Fc-Fc γ R engagement, and Fc γ R III interactions in particular contribute to this
209 phenotype in mice.

210 **Alveolar macrophages are required for antibody protection against BA.5 infection**
211 **after passive immunization.** We next addressed which Fc γ R III-expressing immune cells in the
212 lung were important for mediating antibody protection in the context of passive transfer of
213 immune sera and BA.5 challenge (**Fig 5**). Mice that received vaccine-elicited immune sera had
214 detectable amounts of anti-BA.5 spike IgG but low levels of neutralizing activity (~1/10 titer), as
215 expected (**Extended Data Fig 3**). Flow cytometric analysis of CD45⁺ immune cells in the lungs
216 of wild-type C57BL/6 mice showed that murine monocytes, neutrophils, interstitial
217 macrophages, and alveolar macrophages express Fc γ R III (**Extended Data Fig 4 and**
218 **Supplementary Table 1**). We first assessed the role of neutrophils and monocytes by depleting
219 these cells in wild-type mice with an anti-Ly6C/Ly6G (Gr-1) antibody (**Fig 5a**). Depletion of these
220 cells in circulation (**Fig 5b-d, Extended Data Fig 5a**), which corresponds to depletion in the
221 lung³⁴, did not impact BA.5 infection in the nasal turbinates or lungs; decreased levels of
222 infectious BA.5 virus in the lungs were seen after immune sera transfer regardless of whether
223 neutrophils and monocytes were present (**Fig 5e-f**). We next depleted alveolar macrophages
224 (**Fig 5g-i, Extended Data Fig 5b**) in the lung using a previously described protocol of intranasal
225 administration of clodronate liposomes³⁵. Treatment with clodronate, but not control liposomes,
226 which depleted alveolar macrophages but not other Fc γ R-expressing immune cells in the lung,
227 was associated with a loss of protection against BA.5 infection after passive transfer of immune

228 but not non-immune (naïve) sera (**Fig 3j-k**). Together, these experiments establish an important
229 role for FcγR III-expressing alveolar macrophages in antibody-mediated control of BA.5 infection
230 in mice.

231 **DISCUSSION**

232 Despite the diminished neutralizing ability of vaccine-elicited antibodies against SARS-
233 CoV-2 variants with amino acid substitutions in the RBD and NTD ^{12,21}, protection against
234 severe disease is maintained in the majority of vaccine recipients ^{36,37}. Although neutralizing
235 activity of antibodies is a correlate of vaccine-mediated protection ⁴, the ability of monovalent
236 COVID-19 vaccines to protect against Omicron disease in the setting of waning serum antibody
237 neutralization suggests additional protective immune mechanisms. These include anamnestic B
238 cell responses that rapidly generate cross-reactive neutralizing antibodies, cross-reactive T cells
239 responses, and/or non-neutralizing, cross-reactive antibodies that promote Fc mediated effector
240 activities ^{6,16,18,38,39}. In our experiments, we focused on evaluating Fc mediated effector functions
241 as a possible mechanism of vaccine-mediated protection against antigenic variants. *In vitro*
242 studies with human convalescent sera have demonstrated that Fc effector functions are
243 retained against antigenically variant strains, and that sera of COVID-19 patients with more
244 severe disease have compromised FcγR binding abilities and effector functions ^{6,15,19,40}. Studies
245 in mice with passively transferred mAbs show that Fc effector functions contribute to protection
246 ^{41,42}, and this activity is maintained against antigenically distant strains even when neutralizing
247 capacity is compromised ²⁰. Here, our experiments in mice show that Fc-FcγR interactions
248 contribute to control of SARS-CoV-2 infection *in vivo* in the context of active or passive
249 immunization, and that alveolar macrophages are a key cell type required for this activity.

250 Pooled immune serum from vaccinated mice was profiled for anti-spike antibodies against
251 the ancestral SARS-CoV-2 strain and several variants of concern. The increased binding of
252 immune antibodies to spike proteins was associated with several Fc effector functions including
253 ADCP, ADNP, and ADCD. When immune serum was passively transferred to mice, protection
254 against infection by SARS-CoV-2 strains MA-10 or WA1/2020 N501Y/D614G in the lungs
255 required FcγR expression, particularly FcγR III, even though serum antibody neutralizing activity

256 was present after transfer. These data showing a requirement for Fc effector functions for
257 optimal antibody-mediated control of virus infection in the lung are consistent with studies in
258 mice and hamsters with neutralizing mAbs that bind epitopes in the RBD ^{20,41}. Moreover, Fc-
259 engineered anti-SARS-CoV-2 non-neutralizing and neutralizing mAbs binding the NTD and RBD
260 regions confer greater protection in mice and hamsters ^{43,44}. In the context of passive antibody
261 transfer, we observed less impact in the upper respiratory tract tissues, which could reflect the
262 diminished ability of IgG antibodies in sera to accumulate in airway spaces ²⁶. Nonetheless, we
263 observed Fc γ R-dependent reductions in viral load in the nasal turbinates after active mRNA
264 vaccination, which could be due to higher levels of systemic anti-spike IgG/IgA or production of
265 antibody by tissue-resident B cells. Persistent SARS-CoV-2 IgG antibodies in oral mucosal fluid
266 and upper respiratory tract specimens have been reported following mRNA vaccination ⁴⁵.

267 In wild-type and Fc γ R KO mice immunized with mRNA-1273, we observed similar levels of
268 neutralizing and RBD or spike-specific antibodies, and vaccine-induced CD4⁺ and CD8⁺ T cell
269 responses. Although these results contrast with the idea that Fc γ Rs have key roles in regulating
270 adaptive immunity ^{46,47}, they are consistent with studies showing a lack of impairment of
271 adaptive immune responses in Fc γ R KO mice to bacterial infection or IgG complexes ⁴⁸.
272 Indeed, in control mRNA vaccinated animals, SARS-CoV-2 viral loads were similar in wild-type
273 and Fc γ R KO mice. Thus, we attribute the diminished control of infection of the antigenically-
274 shifted BA.5 strain in the turbinates and lungs of mRNA-1273 vaccinated Fc γ R KO mice to the
275 loss of Fc-Fc γ R interactions that mediate antibody effector functions. Notwithstanding these
276 results, antigen-matched bivalent mRNA vaccines targeting BA.1 and BA.5 spike proteins can
277 induce higher levels of neutralizing antibodies against Omicron strains ^{8,33,49}, which might result
278 in less reliance on Fc effector functions for protection against infection. We also performed
279 cellular depletions to investigate the cell type responsible for the protection conferred by
280 passively transferred antibody. Although several myeloid cells (monocytes, neutrophils,

281 interstitial macrophages, and alveolar macrophages) in the lung express multiple Fc γ R
282 including Fc γ R III, depletion of alveolar macrophages compromised protection against infection.
283 This effect was antibody-dependent since in the absence of immune sera, clodronate-
284 dependent depletion of alveolar macrophages did not affect the viral burden in the lungs. These
285 results are consistent with studies of influenza virus in mice, which showed that protective
286 immunity conferred by non-neutralizing antibodies required alveolar macrophages and other
287 lung phagocytes³⁵.

288 **Limitations of study.** We note several limitations in our study. (i) The conclusions showing
289 an antiviral role for Fc effector functions of antibodies and Fc γ R III are based on experiments in
290 mice. We used mice because of the availability of animals deficient in specific Fc γ Rs, the
291 reagents to achieve immune cell depletions, and the ability to perform both passive and active
292 immunization, and BA.5 challenge. However, our results may not directly correlate with results
293 in humans because of species-dependent differences in Fc γ R subtypes, functions, and
294 expression on specific immune cells in the lung (**Supplementary Table 1**)^{50,51}. Future studies
295 with human Fc γ R transgenic mice⁵² lacking individual human Fc γ Rs may help to bridge this
296 gap. (ii) While we evaluated anti-spike antibody function in Fc γ R I KO, Fc γ R II KO, Fc γ R III KO,
297 and Fc γ R I/III/IV KO mice, we did not directly assess a role for Fc γ R IV, as we did not have
298 these KO mice. Passive and active immunization studies in Fc γ R IV KO mice⁵³ are warranted.
299 (iii) Although our studies in mice indicate an importance of alveolar macrophages and Fc γ R III
300 interactions with antibody, we did not identify a specific cellular mechanism of action. While our
301 serum profiling analysis is consistent with an antibody- and Fc-dependent phagocytic
302 mechanism, it remains unclear if this occurs with opsonized virus or infected cells. (iv) We
303 challenged mice with an antigenically-shifted BA.5 isolate. Future infection experiments using
304 BQ.1.1, BF.7, or other emerging strains may be informative for determining the breadth of this
305 mechanism of protection. (v) Although challenge of wild-type and Fc γ R KO C57BL/6 mice with

306 BA.5 allowed us to evaluate the effects on viral burden in the setting of low levels of transferred
307 or induced serum antibody neutralization, clinical disease and pathology do not develop
308 because Omicron strains are less virulent in C57BL/6 and other strains of mice ⁵⁴. Testing of
309 vaccinated wild-type and Fc γ R KO mice with antigenically-shifted yet more pathogenic SARS-
310 CoV-2 strains [for mice] might enable assessment of the contribution of polyclonal antibodies
311 and Fc effector functions to protection against lung inflammation. It will be important to
312 determine whether vaccine-elicited antibodies engage Fc γ Rs (e.g., Fc γ R I and Fc γ R III) on
313 specific myeloid cells and promote inflammation, as infection-induced antibodies from patients
314 enhanced SARS-CoV-2 uptake by monocytes and macrophages, and triggered inflammasome
315 activation, pyroptotic cell death, and COVID-19 pathogenesis ^{55,56}. However, these studies also
316 showed that immune plasma from mRNA vaccine recipients did not promote antibody-
317 dependent monocyte infection and inflammation.

318 In summary, our experiments in mice provide insight and help to explain human studies
319 that correlate Fc-Fc γ R interactions with clinical outcome against SARS-CoV-2 and emerging
320 variants of concern. We demonstrate the importance of particular murine Fc γ Rs in mediating
321 antibody protection against SARS-CoV-2 infection and identify alveolar macrophages as a key
322 contributing cell type in the context of passive immunization. Our results also provide an
323 explanation as to how Fc-Fc γ R interactions might contribute to monovalent vaccine-mediated
324 protection against severe infection by SARS-CoV-2 variants even in the setting when serum
325 neutralizing antibody activity is lost ⁷. They also suggest that targeting Fc effector functions in
326 the context of vaccine design could be a strategy to generate more broadly protective immune
327 responses ²³. Future studies are warranted to define the epitopes targeted by antibodies with
328 strong Fc effector functions and develop improved *in vitro* Fc effector function assays that
329 correlate better with protection *in vivo* against infection by SARS-CoV-2 and variants ⁵⁷.

330 **Acknowledgements.** This study was supported by the NIH (R01 AI157155, NIAID
331 Centers of Excellence for Influenza Research and Response (CEIRR) contracts
332 75N93021C00014 and 75N93019C00051, to M.S.D.; and R01 AI110700 to R.S.B.). We thank
333 Mehul Suthar for the BA.5 isolate used in this study and Taia Wang for critical comments on the
334 manuscript.

335 **Author contributions.** S.R.M. performed binding and neutralization assays,
336 immunization, passive transfer, depletion studies, challenge experiments, and flow cytometry.
337 P.D. performed and analyzed T cell responses. B.W. performed mouse experiments. C.E.K.
338 performed some of the flow cytometry experiments. M.L. performed immune cell processing and
339 staining. R.S.B. provided the mouse-adapted MA-10 strain. R.P.M., T.M.C., and G.A. designed,
340 performed, and analyzed the serological profiling experiments. D.K.E. provided mRNA vaccines
341 and helped to design vaccination experiments. S.R.M. and M.S.D. designed studies and wrote
342 the initial draft, with the other authors providing editorial comments.

343 **Competing interests.** M.S.D. is a consultant for Inbios, Vir Biotechnology, Senda
344 Biosciences, Moderna, and Immunome. The Diamond laboratory has received unrelated
345 funding support in sponsored research agreements from Vir Biotechnology, Emergent
346 BioSolutions, and Moderna. R.S.B is a member of the Scientific Advisory Board of VaxArt and
347 Adagio, has consulted for Takeda, and received unrelated funding from J&J and Pfizer. G.A. is
348 a founder/equity holder in Seroymx Systems and Leyden Labs and has served as a scientific
349 advisor for Sanofi Vaccines. G.A. has collaborative agreements with GlaxoSmithKline, Merck,
350 Abbvie, Sanofi, Medicago, BioNtech, Moderna, BMS, Novavax, SK Biosciences, Gilead, and
351 Sanaria. D.K.E. and G.A. are employees and shareholder in Moderna, Inc. All other authors
352 declare no conflicts of interest.

353 **FIGURE LEGENDS**

354 **Figure 1. Systems serology analysis of vaccine-induced immune sera. (a-c)** Levels
355 of IgG1 (a), IgG2b (b), IgG2c (c) that bind to SARS-CoV-2 spike [Wuhan-1, B.1.617.2, BA.1,
356 and BA.4/5], or influenza hemagglutinin (HA) in naïve and vaccine-induced immune sera. (d-f)
357 Levels of spike- or HA-binding IgG antibodies that engage FcγR IIb (d), FcγR III (e), or FcγR IV
358 (f) in naïve and vaccine-induced immune sera. (g-j) Antibody effector functions. Antibody-
359 mediated cellular phagocytosis with monocytes (ADCP, g) or neutrophils (ADNP, h) activity
360 using vaccine-induced immune (red) or naïve (white) sera and beads coated with SARS-CoV-2
361 Wuhan-1 and BA.4/5 spike proteins and murine monocytes (bars indicate median values; one-
362 way ANOVA with Tukey's post-test; ns, not significant; **P* < 0.05, ***P* < 0.01). (i) CD107a
363 surface expression on natural killer cells (ADNKA) after incubation with beads encoded with
364 Wuhan-1 or BA4/5 spike proteins and immune sera (bars indicate median values; one-way
365 ANOVA with Tukey's post-test; ns, not significant). (j) Deposition of complement (ADCD) on
366 beads coated with indicated SARS-CoV-2 spike or influenza HA proteins after treatment with
367 naïve or immune sera.

368 **Figure 2. Vaccine-derived immune sera protection against SARS-CoV-2 MA-10**
369 **infection in wild-type C1q KO, and FcγR I/III/IV KO mice. (a)** Scheme of passive transfer,
370 virus challenge and tissue harvest. (b) Neutralizing antibody responses against SARS-CoV-2
371 MA-10 using sera from naïve (circles) or Wuhan-1 spike protein vaccinated mice (pooled from
372 animals immunized and boosted with mRNA-1273 or ChAd-SARS-CoV-2-S) (squares). Also
373 shown is serum neutralizing antibody activity from recipient wild-type (black squares) and FcγR
374 I/III/IV KO (green squares) mice one day after transfer of immune sera. (c-g) Twelve-week-old
375 male wild-type, C1q KO, and FcγR I/III/IV KO C57BL/6 mice were passively transferred by
376 intraperitoneal injection 60 μL of naïve or vaccine-induced immune sera one day before
377 intranasal challenge with 10³ FFU of SARS-CoV-2 MA-10. At 4 dpi, viral RNA in the nasal wash

378 (c), nasal turbinates (d), and lungs (f) were quantified by qRT-PCR, and infectious virus in the
379 nasal turbinates (e) and lungs (g) was determined by plaque assay (bars indicate median
380 values; n = 6-7 mice per group, two experiments, dotted lines show limit of detection [LOD]).
381 One-way ANOVA with Tukey's post-test; ns, not significant; * $P < 0.05$, **** $P < 0.0001$).

382 **Figure 3. Vaccine-elicited immune sera protection against SARS-CoV-2 WA1/2020**
383 **N501/D614G infection in wild-type, FcγR I KO, FcγR II KO, FcγR III KO, and FcγR I/III/IV KO**
384 **mice.** (a) Scheme of passive transfer, virus challenge, and tissue harvest. (b) Neutralizing
385 antibody response against SARS-CoV-2 WA1/2020 N501Y/D614G using sera from naïve
386 (circles) or Wuhan-1 spike protein vaccinated (squares) mice. Also shown is serum neutralizing
387 antibody activity from recipient wild-type (black squares) and FcγR I/III/IV KO (green squares)
388 mice one day after transfer of immune sera. (c-g) Twelve-week-old male wild-type, FcγR I KO,
389 FcγR II KO, FcγR III KO, and FcγR I/III/IV KO mice were passively transferred by intraperitoneal
390 injection 60 μL of naïve or vaccine-immune sera one day before intranasal challenge with 10⁴
391 FFU of WA1/2020 N501Y/D614G. At 4 dpi, viral RNA and infectious virus was measured in the
392 upper respiratory tract (nasal wash) (c), nasal turbinates (d-e) or lungs (f-g) and quantified by
393 qRT-PCR and plaque assay. Panels c-e: wild-type mice only; panels f-g: wild-type, FcγR I KO,
394 FcγR II KO, FcγR III KO, and FcγR I/III/IV KO mice (bars indicate median; n = 9-18 mice per
395 group, three experiments, dotted lines show LOD). One-way ANOVA with Tukey's post-test (ns,
396 not significant; *** $P < 0.01$, ** $P < 0.001$, **** $P < 0.0001$).

397 **Figure 4. Protection against SARS-CoV-2 BA.5 infection after mRNA-1273**
398 **vaccination of wild-type and FcγR-deficient C57BL/6 mice.** (a) Scheme of immunization,
399 serum sampling, virus challenge, and tissue harvest. (b-c) Anti-Wuhan-1 (b) and BA.4/5 (c)
400 RBD IgG responses from serum of mice immunized with control or mRNA-1273 vaccines (n =
401 10-22, boxes illustrate geometric mean titers [GMT], dotted lines show LOD). (d-e) Neutralizing
402 antibody responses against WA1/2020 N501Y/D614G (d) and BA.5 (e) from serum collected

403 from wild-type, Fc γ R I KO, Fc γ R III KO, and Fc γ R I/III/IV KO mice 25 days after completion of a
404 two-dose primary vaccination series with control (closed circles) or mRNA-1273 (open circles).
405 **(f-g)** Nine-week-old male wild-type, Fc γ R I KO, Fc γ R III KO, and Fc γ R I/III/IV KO mice were
406 immunized twice at four-week intervals with control or mRNA-1273 vaccine via intramuscular
407 route. Four weeks after the primary vaccination series, mice were challenged via intranasal
408 route with 10⁴ FFU of BA.5. At 3 dpi, infectious virus in the nasal turbinates **(f)** and lungs **(g)** was
409 determined by plaque assay (bars indicate median values; n = 8-10 mice per group, two
410 experiments, dotted lines show LOD, one-way ANOVA with Dunnett's test (ns, not significant,
411 ***P* < 0.01, ****P* < 0.0001, *****P* < 0.0001).

412 **Figure 5. Depletion of alveolar macrophages impairs the protective activity of**
413 **passively-transferred immune sera against BA.5 infection.** **(a)** Scheme for depletion of
414 neutrophils and monocytes, passive transfer of immune sera, and BA.5 challenge. Wild-type
415 C57BL/6 twelve-week-old male mice were administered 500 μ g of anti-Gr-1 (Ly6C/Ly6G) or
416 isotype control antibody at Day -3 and -1 by intraperitoneal injection. On Day -1, mice were also
417 given 60 μ L of naïve or immune sera by intraperitoneal injection. On Day 0, mice were
418 inoculated with 10⁴ FFU of BA.5, and tissues were harvested for virological analysis on Day +3.
419 **(b-d)** Analysis of depletion of immune cell subsets in blood of mice receiving anti-Gr-1
420 (Ly6C/Ly6G) or isotype antibody at 3 dpi. Summary of different cell types **(b)** based on the
421 gating strategy (see **Extended Data Fig 5a**). Results are from two experiments (n = 6-10 mice
422 per group; Mann-Whitney test with Bonferroni post-test correction, ns, not significant; ***P* <
423 0.01, ****P* < 0.001, *****P* < 0.0001). Representative flow cytometry dot plots showing depletion of
424 neutrophils **(c)** and monocytes **(d)** with numbers indicating the cell population as a percentage
425 of CD45⁺ cells. **(e-f)** Analysis of infectious viral burden by plaque assay at 3 dpi in nasal
426 turbinates **(e)** and lungs **(f)** after BA.5 challenge (two experiments, n = 6-10 mice per group;
427 Mann-Whitney test, ns, not significant). **(g)** Scheme for depletion of alveolar macrophages,

428 passive transfer of immune sera, and BA.5 challenge. Wild-type C57BL/6 twelve-week-old male
429 mice were administered control or clodronate liposomes at Day -3 by an intranasal route. On
430 Day -1, mice were given 60 μ L of naïve or immune sera by intraperitoneal injection. On Day 0,
431 mice were inoculated with 10^4 FFU of BA.5, and tissues were harvested on Day +3. **(h)** Analysis
432 of depletion of immune cell subsets in lungs of mice receiving liposomes at 3 dpi. Summary of
433 different cell types **(h)** based on gating strategy (see **Extended Data Fig 5b**). Results are from
434 two experiments ($n = 7-12$ mice per group; Mann-Whitney test with Bonferroni post-test
435 correction, ns, not significant; $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$). **(i)** Representative flow
436 cytometry dot plots showing depletion of alveolar macrophages after liposome administration
437 with numbers indicating the cell population as a percentage of CD45⁺ cells. **(j-k)** Analysis of
438 infectious viral burden by plaque assay at 3 dpi in nasal turbinates **(j)** and lungs **(k)** after BA.5
439 challenge (two experiments, $n = 7-12$ mice per group; Mann-Whitney test, ns, not significant;
440 $****P < 0.0001$).

441

442 **EXTENDED DATA FIGURES**

443 **Extended Data Figure 1. Gating strategy for Luminex-based and Fc effector**
444 **function assays.** **(a)** Gating for Luminex-bead based antibody binding to spike-coated beads.
445 **(b)** Gating for ADNP assay showing CD66⁺ neutrophils with opsinophagocytosed beads. **(c)**
446 Gating for ADCP assay showing THP-1 monocytes and opsinophagocytosed beads. **(d)** Gating
447 for ADCD assay showing complement deposition on spike and antibody coated beads. **(e)**
448 Gating for NK cell activation assay showing CD107a expression.

449 **Extended Data Figure 2. T cell responses in mRNA-1273 vaccinated wild-type and**
450 **Fc γ R KO mice.** **(a)** Representative flow cytometry plots show gating scheme for quantification
451 of spike-specific CD4⁺ and CD8⁺ T cell responses in the spleen of wild-type, Fc γ R III KO, and
452 Fc γ R I/III/IV KO mice at day 10 after boosting with control or mRNA-1273 vaccines. **(b-c)** At day

453 10 after boosting, the spleen of wild-type and Fc γ R KO mice were harvested, and T cell
454 responses were measured *ex vivo* after spike peptide re-stimulation. Splenocytes were
455 incubated overnight with class I MHC (**b**) or class II MHC (**c**) immunodominant spike peptides,
456 and the percentages and numbers of IFN γ and TNF α positive CD8⁺ (**b**) or CD4⁺ (**c**) T cells were
457 quantified by intracellular staining and flow cytometry. Data are pooled from two experiments (n
458 = 9-10 per group). Comparisons were made between groups that received the mRNA 1273
459 vaccine (one-way ANOVA with Tukey's post-test; all comparisons were not significant; column
460 height indicates mean values).

461 **Extended Data Figure 3. Levels of anti-BA.5 antibody in mice passively transferred**
462 **vaccine-elicited serum antibody.** (a) Levels of anti BA.5 spike IgG in serum of mice that were
463 passively transferred naïve or immune sera. Amounts are compared to those in vaccine-elicited
464 immune serum before transfer (n = 5 mice per group, columns indicate mean values). (**b**)
465 Neutralizing antibody response against SARS-CoV-2 BA.5 using sera from naïve (circles) or
466 spike protein vaccinated (grey squares) mice. Also shown is serum neutralizing antibody activity
467 from recipient wild-type mice one day after transfer of immune sera (black squares). The data
468 are representative of results with n = 5 mice per group.

469 **Extended Data Figure 4. Fc γ R expression on myeloid cells in the lung.** Lung cells
470 from wild-type (black), Fc γ R I KO (purple), Fc γ R III KO (blue), and Fc γ R I/III/IV KO (green) mice
471 were strained with antibodies for Fc γ R I, Fc γ R III, or Fc γ R IV. After gating on live cells, alveolar
472 macrophages, neutrophils, and monocytes were defined (see **Extended Data Fig 5**). The data
473 are representative of results with n = 3 mice per group, and histograms are shown.

474 **Extended Data Figure 5. Gating scheme for analysis of cell populations in the**
475 **blood and lung.** (a) Immune cell populations in the blood of C57BL/6 mice were analyzed using
476 the indicated gating scheme and conjugated antibodies. After gating on live single cells,
477 monocytes (P5) were defined as CD45⁺ CD11b^{hi} Ly6C^{hi}; neutrophils (P6) were defined as CD45⁺

478 CD11b^{hi} Ly6G^{hi}; natural killer (NK) cells (P7) were defined as CD45⁺ NK1.1⁺; and B cells (P8)
479 were defined as CD45⁺ B220⁺ cells. (b) Immune cell populations in the lungs of C57BL/6 mice
480 were analyzed using the indicated gating scheme and conjugated antibodies. After gating on
481 live single cells, alveolar macrophages (P1) were defined as CD45⁺ CD11c⁺ Siglec-F⁺; interstitial
482 macrophages (P2) were defined as CD45⁺ CD64⁺, eosinophils (P3) were defined as CD45⁺
483 CD11b⁺ Siglec-F⁺; CD11b dendritic cells (P4) were defined CD45⁺ CD11b⁺ CD11c⁺, Siglec-F⁻,
484 MHC II⁺; monocytes (P5) were defined as CD45⁺ Ly6C^{hi}; neutrophils (P6) were identified
485 as CD45⁺ Ly6G⁺; natural killer (NK) cells (P7) were defined as CD45⁺ NK1.1⁺; and B cells (P8)
486 were defined as CD45⁺ B220⁺.

487 **Supplementary Table 1. Murine and human FcγR expression in immune cells of the lung**

Lung cell subset	Human FcγRs					
	FcγR I (CD64) activating	FcγR IIa (CD32a) activating	FcγR IIb (CD32b) inhibitory	FcγR IIc (CD32c) activating	FcγR IIIa (CD16a) activating	FcγR IIIb (CD16b) (GPI-anchored)
Alveolar macrophages	+	+	+	-	+	-
Interstitial macrophages	+	+	+	-	+	-
Ly6C ⁺ monocytes	+	+	+	-	-	-
Neutrophils	Inducible	+	+	-	-	+
Eosinophils	Inducible	+	+	-	-	Inducible
CD11b ⁺ Dendritic cells	+	+	+	-	Inducible	-
NK cells	-	-	-	+	+	-
B cells	-	-	+	-	-	-

488

Lung cell subset	Mouse FcγRs			
	FcγR I (CD64) activating	FcγR IIb (CD32b) inhibitory	FcγR III (CD16) activating	FcγR IV (CD16-2) activating
Alveolar macrophages	+	+	+	+
Interstitial macrophages	+	+	+	+
Ly6C ⁺ monocytes		+	+	+
Neutrophils	-	+	+	+
Eosinophils	-	+	Inducible	-
CD11b ⁺ Dendritic cells	+	+	+	-
NK cells	-	-	+	-
B cells	-	+	-	-

489

490 Table was compiled based on published results ^{51,58-60}.

491

492 **METHODS**

493 **Cells.** Vero-TMPRSS2⁶¹ and Vero-hACE2-TMPRSS2⁶² cells were cultured at 37°C in
494 Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS),
495 10 μM HEPES pH 7.3, 1 mM sodium pyruvate, 1× non-essential amino acids, and 100 U/mL
496 of penicillin–streptomycin. Vero-TMPRSS2 and Vero-hACE2-TMPRSS2 cells were
497 supplemented with 5 μg/mL of blasticidin and 10 μg/mL of puromycin, respectively. All cells
498 routinely tested negative for mycoplasma using a PCR-based assay.

499 **Viruses.** All SARS-CoV-2 strains used (WA1/2020 N501Y/D614G, mouse-adapted MA-
500 10, B.1.351, and BA.5) have been previously described^{25,33,62,63}. All viruses were subjected to
501 next generation deep sequencing to confirm presence and stability of substitutions. All
502 experiments with virus were performed in approved biosafety level 3 (BSL-3) facilities with
503 appropriate positive-pressure respirators, personal protective gear, and containment.

504 **Mice.** Animal studies were carried out in accordance with the recommendations in the
505 Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The
506 protocols were approved by the Institutional Animal Care and Use Committee at the Washington
507 University School of Medicine (assurance number A3381–01). Virus inoculations were
508 performed under anesthesia that was induced and maintained with ketamine hydrochloride and
509 xylazine, and all efforts were made to minimize animal suffering. Experiments were neither
510 randomized nor blinded. C57BL/6J mice (Cat # 000664) were obtained from The Jackson
511 Laboratory. FcγR I KO⁶⁴, FcγR II KO (Taconic Biosciences; Cat # 580), FcγR III KO (Jackson
512 Laboratory; Cat # 009637), FcγR I/III/IV (common γ-chain) KO (Taconic Biosciences; Cat # 583),
513 and C1q KO⁶⁵ were obtained commercially or collaborators and then backcrossed onto a
514 C57BL/6J background (>99%) using Speed Congenics (Charles River Laboratories) and single
515 nucleotide polymorphism analysis. Animals were housed in groups and fed standard chow diets.

516 **Preclinical mRNA and ChAd-SARS-CoV-2 vaccines.** A sequence-optimized mRNA
517 encoding prefusion-stabilized Wuhan-Hu-1 (mRNA-1273) was designed and synthesized *in*
518 *vitro* using an optimized T7 RNA polymerase-mediated transcription reaction with complete
519 replacement of uridine by N1m-pseudouridine⁶⁶. A non-translating control mRNA was
520 synthesized and formulated into lipid nanoparticles as previously described⁶⁷. The reaction
521 included a DNA template containing the immunogen open-reading frame flanked by 5'
522 untranslated region (UTR) and 3' UTR sequences and was terminated by an encoded polyA tail.
523 After RNA transcription, the cap-1 structure was added using the vaccinia virus capping enzyme
524 and 2'-O-methyltransferase (New England Biolabs). The mRNA was purified by oligo-dT
525 affinity purification, buffer exchanged by tangential flow filtration into sodium acetate, pH 5.0,
526 sterile filtered, and kept frozen at -20°C until further use. The mRNA was encapsulated in a lipid
527 nanoparticle through a modified ethanol-drop nanoprecipitation process described previously⁶⁸.
528 Ionizable, structural, helper, and polyethylene glycol lipids were briefly mixed with mRNA in an
529 acetate buffer, pH 5.0, at a ratio of 2.5:1 (lipid:mRNA). The mixture was neutralized with Tris-
530 HCl, pH 7.5, sucrose was added as a cryoprotectant, and the final solution was sterile-filtered.
531 Vials were filled with formulated lipid nanoparticle and stored frozen at -20°C until further use.

532 The ChAd-SARS-CoV-2 and ChAd-Control vaccine vectors were derived from simian
533 Ad36 backbones⁶⁹, and the constructing and validation has been described in detail previously
534⁷⁰. The rescued replication-incompetent ChAd-SARS-CoV-2-S were scaled up in HEK293 cells
535 and purified by CsCl density-gradient ultracentrifugation. For passive transfer studies, a large
536 batch (8 mL) of immune sera (obtained \geq 30 days post immunization) was pooled from C57BL/6
537 mice vaccinated with ChAd-SARS-CoV-2 or mRNA-1273.

538 **Viral antigens.** Recombinant RBD proteins from Wuhan-1 and BA.5 SARS-CoV-2
539 strains were expressed as described^{71,72}. Recombinant proteins were produced in Expi293F
540 cells (ThermoFisher) by transfection of DNA using the ExpiFectamine 293 Transfection Kit
541 (ThermoFisher). Supernatants were harvested 3 days post-transfection, and recombinant

542 proteins were purified using Ni-NTA agarose (ThermoFisher), then buffer exchanged into PBS
543 and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore).

544 **ELISA.** Recombinant Wuhan-1, BA.4/5 receptor binding domain (RBD), or BA.4/5 spike
545 protein (4 µg/mL) was immobilized on 96-well Maxisorp ELISA plates (Thermo Fisher) overnight
546 at 4°C in coating buffer (1X PBS supplemented with 0.05% Tween-20, 2% BSA, and 0.02%
547 NaN₃). Plates were washed with PBS and blocked with 4% BSA for one hour at 25°C. Serum
548 was serially diluted in 2% BSA and incubated on plates for 1 h at 25°C. After washing with PBS,
549 RBD or spike-bound serum antibodies were detected with horseradish peroxidase conjugated
550 goat anti-mouse IgG (1:500 dilution, Milipore Sigma) incubating for 2 h at 25°C. Plates were
551 washed and developed with 3,3'-5,5' tetramethylbenzidine substrate (Thermo Fisher), halted
552 with 2 N H₂SO₄ and read at 450 nm using a microplate reader (BioTek). Mean serum endpoint
553 titers were calculated with curve fit analysis of optical density (OD) values set as the reciprocal
554 value of the serum dilution equal to the mean plus six times the standard deviation of
555 background signal.

556 **Focus reduction neutralization test.** Serial dilutions of sera were incubated with 10²
557 focus-forming units (FFU) of WA1/2020 N501Y/D614G or BA.5 for 1 h at 37°C. Antibody-virus
558 complexes were added to Vero-TMPRSS2 cell monolayers in 96-well plates and incubated at
559 37°C for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM. Plates
560 were harvested 30 h (WA1/2020 N501Y/D614G and MA-10) or 72 h (BA.5) later by removing
561 overlays and fixed with 4% PFA in PBS for 20 min at room temperature. Plates were washed
562 and sequentially incubated with an oligoclonal pool (SARS2-02, -08, -09, -10, -11, -13, -14, -17,
563 -20, -26, -27, -28, -31, -38, -41, -42, -44, -49, , -57, -62, -64, -65, -67, and -71⁷³ of anti-S murine
564 antibodies (including cross-reactive mAbs to SARS-CoV) and HRP-conjugated goat anti-mouse
565 IgG (Sigma Cat # A8924, RRID: AB_258426) in PBS supplemented with 0.1% saponin and
566 0.1% bovine serum albumin. SARS-CoV-2-infected cell foci were visualized using TrueBlue

567 peroxidase substrate (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular
568 Technologies).

569 **Viral plaque assay.** Titration of infectious SARS-CoV-2 was performed as previously
570 described ⁷⁴. Briefly, lung and nasal turbinate homogenates were serially diluted and added to
571 Vero-TMPRSS2-hACE2 cell monolayers in 24-well tissue culture plates for 1 h at 37°C. Cells
572 were then overlaid with 1% (w/v) methylcellulose in MEM and incubated for 72 h (MA-10 and
573 WA1/2020 N501Y/D614G) or 96 h (BA.5). Subsequently, cells were fixed with 4%
574 paraformaldehyde in PBS for 20 min at room temperature before staining with 0.05% (w/v)
575 crystal violet in 20% methanol. Viral plaques were counted manually.

576 **Mouse experiments.** (a) Passive transfer studies. Twelve-week-old male C57BL/6,
577 FcγR I KO, FcγR II KO, FcγR III KO, FcγR I/III/IV KO, and C1q KO mice were administered 60 ul
578 of sera (naïve or pooled from mice immunized and boosted with mRNA-1273 or ChAd-SARS-
579 CoV-2-S) one day prior to challenge with 50 μl of 10³ FFU of MA-10 or 10⁴ FFU of WA1/2020
580 N501Y/D614G by intranasal administration. Lungs, nasal turbinates, and nasal washes
581 (collected in 500 μl of .5% bovine serum albumin in phosphate buffered saline) were harvested
582 four days after inoculation for virological analysis. (b) Immunization studies. Nine-week-old male
583 C57BL/6, FcγR I KO, FcγR III KO, and FcγR I/III/IV KO mice were immunized and boosted with
584 0.25 μg of control or mRNA-1273 vaccine by intramuscular injection four weeks apart. Animals
585 were bled twenty-four days after boosting for immunogenicity analysis of sera and then
586 challenged four days later with 50 μl of 10⁴ FFU of BA.5 by intranasal administration. Lungs,
587 nasal turbinates, and nasal washes (collected in 500 μl of .5% bovine serum albumin in
588 phosphate buffered saline) were harvested three days after inoculation for virological analysis.
589 In vivo studies were not blinded with mice randomly assigned to treatment groups.

590 **Immune cell depletions.** For monocyte and neutrophil depletions, anti-Ly6G/Ly6C
591 (BioXCell; clone RB8-8C5; 500 μg) or an IgG2b isotype control (BioXCell; clone LTF-2; 500 μg)

592 were administered to mice by intraperitoneal injection at days -3 and -1 relative to SARS-CoV-2
593 inoculation. For alveolar macrophage depletion, clodronate liposomes (Liposoma; 250 µg) or
594 control liposomes (Liposoma; 250 µg) were administered via intranasal route at day -3 relative
595 to SARS-CoV-2 inoculation.

596 For analysis of neutrophil and monocyte depletion, peripheral blood was collected on the
597 day of harvest. Erythrocytes were lysed with ACK lysis buffer (GIBCO) at room temperature for
598 3 min and resuspended in RPMI 1640. Single-cell suspensions were preincubated with Fc block
599 antibody (1:100; clone S17011E; Biolegend) in PBS + 2% heat-inactivated FBS + 1 mM EDTA
600 for 20 min at 4°C, stained with antibodies against CD45 (AF488; clone 30-F11; Biolegend),
601 CD11b (APC/Fire 810; clone M1/70; Biolegend), Ly6G (Spark Blue 550; clone 1A8; Biolegend),
602 Ly6C (APC-Fire 750; clone HK1.4; Biolegend), NK1.1 (BV570; clone PK136; Biolegend), B220
603 (Pacific Blue; clone RA3-6B2; Biolegend), fixable viability dye (eFluor 506; BD Biosciences),
604 True-Stain Monocyte Blocker (Biolegend; 5 µl/sample), and Brilliant Stain Buffer Plus (BD
605 Biosciences; 10 µl/sample), and then fixed with 4% paraformaldehyde in PBS for 20 min at
606 room temperature. All antibodies were used at a dilution of 1:200. The viability dye was used at
607 1:100. Absolute cell counts were determined using Precision Count Beads (Biolegend). Flow
608 cytometry data were acquired on a 3 laser Cytex Aurora cytometer (Cytexbio) and analyzed
609 using FlowJo software v10.8 (Treestar).

610 For analysis of lung tissues, mice were euthanized by ketamine overdose, perfused with
611 sterile PBS, and the right inferior lung lobes were digested in 50 µL of 5 mg/mL of Liberase Tm
612 (Roche) and 12.5 µL of 10 mg/mL of DNase I (Sigma-Aldrich) in 5 mL of HBSS at 37°C for 35
613 min. Single cell suspensions of lung digest were preincubated with Fc block antibody
614 (Biolegend) in PBS + 2% heat-inactivated FBS + 1 mM EDTA for 20 min at 4°C. Cells were then
615 stained with antibodies against CD45 (AF488), CD11b (APC/Fire 810), MHC II (BV711; clone
616 M5/114.15.2; Biolegend), CD11c (PE-Cy7; clone N418; Biolegend), CD64 (BV421; clone X54-

617 5/7.1; Biolegend), CD88 (Viogreen; clone REA1206; Miltenyi Biotec), Siglec-F (PE Dazzle 594;
618 clone S17007L; Biolegend), Ly6G (Spark Blue 550), Ly6C (APC-Fire 750), NK1.1 (BV570), CD3
619 (BV650; clone 145-2C11; BD Biosciences), B220 (Pacific Blue), CD16.2 (APC), CD16 (PE),
620 fixable viability dye (eFluor 506), True-Stain Monocyte Blocker (5 μ l/sample), and Brilliant Stain
621 Buffer Plus (10 μ l/sample) and then fixed with 4% paraformaldehyde in PBS for 20 min at room
622 temperature. All antibodies were used at a dilution of 1:200 with the exceptions of MHC II
623 BV711, which was used at 1:300 and the viability dye, used at 1:100. In experiments staining for
624 mouse Fc γ R III, Fc block was not used. Absolute cell counts were determined using Precision
625 Count Beads (Biolegend). Flow cytometry data were acquired on a 3 laser Cytex Aurora
626 cytometer (Cytexbio) and analyzed using FlowJo software (version 10.4.2).

627 **Measurement of viral burden.** Tissues were weighed and homogenized with zirconia
628 beads in a MagNA Lyser instrument (Roche Life Science) in 1 ml of DMEM medium
629 supplemented with 2% heat-inactivated FBS. Tissue homogenates were clarified by
630 centrifugation at 10,000 rpm for 5 min and stored at -80°C . RNA was extracted using the
631 MagMax mirVana Total RNA isolation kit (Thermo Fisher Scientific) on the Kingfisher Flex
632 extraction robot (Thermo Fisher Scientific). RNA was reverse transcribed and amplified using
633 the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher Scientific). Reverse transcription was carried
634 out at 48°C for 15 min followed by 2 min at 95°C . Amplification was accomplished over 50
635 cycles as follows: 95°C for 15 s and 60°C for 1 min. Copies of SARS-CoV-2 *N* gene RNA in
636 samples were determined using a published assay ⁷⁴.

637 **Antibody isotype and Fc-receptor binding profiling.** Serum samples were analyzed
638 by a customized Luminex assay to quantify the levels of antigen-specific antibody subclasses
639 and Fc γ R binding profiles, as previously described ^{75,76}. Briefly, antigens were coupled to
640 magnetic Luminex beads (Luminex Corp) by carbodiimide-NHS ester-coupling (Thermo Fisher).
641 The antigen-coupled microspheres were washed and incubated with heat-inactivated serum
642 samples at an appropriate sample dilution (1:100-1:400 for antibody isotyping and 1:1,000 for all

643 low-affinity FcγRs) overnight in 384-well plates with continuous shaking (Greiner Bio-One).
644 Unbound antibodies were washed away using the magnetic 384-well HydroSpeed Plate Washer
645 (Tecan) using 1X Luminex assay buffer (1X PBS, 0.1% BSA, 0.05% Tween-20). Secondary
646 antibodies (PE-coupled IgG1, IgG2b, IgG2c, IgG3) were added and incubated for 1 h at room
647 temperature with continuous shaking. Unbound complexes were washed away using the
648 magnetic 384-well Hydrospeed Plate Washer. Beads were resuspended in 40 μL of QSOL
649 buffer (Sartorius) and then run on the iQue Screener PLUS (Intellicyt) using a customized gating
650 strategy for each bead region (**Extended Data Fig 1**). Median fluorescence intensity was
651 calculated for all samples, which were run in technical replicates.

652 For FcγR binding, sera were incubated with antigen-coated beads and washed as
653 described above. Custom synthesized FcγR (FcγR2b, FcγR3, FcγR4; Duke Protein Production
654 facility) were biotinylated and then bound to PE-Streptavidin. The labeled FcγRs were then
655 incubated with the sera for 1 h at room temperature with continuous shaking. Unbound
656 complexes were washed as indicated above. Beads were resuspended in 40 μL of QSOL buffer
657 and then run on the iQue Screen PLUS (Intellicyt). All flow cytometry files were analyzed using
658 Intellicyt ForeCyt (v8.1).

659 All antigens and FcγRs were equilibrated in 1X PBS using Zeba-Spin desalting and size
660 exclusion chromatography columns (ThermoFisher) prior to bead coupling. Dilution curves for
661 each antibody isotype and subclass and FcγRs were performed for each antigen to ensure
662 reported values were within the linear range of detection. Binding for antigens was calculated as
663 the fold increase relative to naïve levels, which arbitrarily were set to 1.

664 **Evaluation of antibody-mediated effector functions.** ADCP and ADNP were
665 evaluated using a flow-cytometry-based phagocytic assay using fluorescently labeled
666 microspheres. In brief, fluorescent neutravidin microspheres were coupled with biotinylated
667 antigens and then incubated with the diluted serum to form immune complexes. Bead-bound
668 immune complexes were incubated with monocytes (THP-1 cells) or neutrophils overnight at 37

669 °C in 96 well, round-bottom plates (Corning). After overnight incubation, plates were centrifuged
670 and non-bound beads/immune complexes were removed. Cells were then fixed in 4%
671 paraformaldehyde and stained for indicated markers. Microsphere-positive cells were quantified
672 through flow cytometry and phagocytic scores were calculated as (the percentage of
673 microsphere positive cells * MFI of positive cells) /100,000.

674 ADCC was quantified through the coupling of biotinylated antigens to neutravidin
675 microspheres and then incubated with guinea pig complement at 37 °C for 50 min. Reactions
676 were quenched with 15 mM EDTA. Fluorescein-conjugated anti-C3b was added to the mixture
677 for 1 h with constant shaking. Plates were washed with 1X PBS and complexes were fixed with
678 4% paraformaldehyde and washed again with 1X PBS. Beads were resuspended in 35 µL of 1X
679 PBS and then analyzed by flow cytometry. Complement deposition was calculated as the fold
680 increase relative to naïve levels, which arbitrarily were set to 1.

681 ADNKA was quantified through the surface expression of CD107a (as a marker for
682 degranulation). In brief, buffy coats were obtained from healthy donors at Massachusetts
683 General Hospital and enriched using the RosetteSep NK enrichment kit (Stemcell) in the
684 presence of IL-15. Antigen-coated, 96-well ELISA plates were then incubated with serum and
685 the NK cell preparation. Plates were placed in a 37°C incubator for 2 h. Reactions were stopped
686 and cells were fixed and stained extracellularly with anti-CD107a-PeCy5, anti-CD3-PB, anti-
687 CD56-PE-Cy7, and anti-CD16-APC-Cy7. Cells were washed three times with 1X PBS and
688 resuspended in 30 µL of 1X PBS and analyzed by flow cytometry.

689 **T cell analysis.** Spleens were harvested from control or mRNA-1273 vaccinated wild
690 type or FcγR KO mice at day 10 after boosting, and single cell suspensions were generated
691 after tissue disruption and passage through a 70-µm cell strainer. Splenocytes were pelleted by
692 centrifugation, and erythrocytes were lysed using ACK lysis buffer (Thermo Fisher). Cells then
693 were re-suspended in RPMI 1640 media supplemented with 10% FBS, 1% HEPES, 1% L-

694 glutamine and 0.1% β -mercaptoethanol. For peptide stimulation, splenocytes were incubated
695 separately with class I MHC (VL8, peptide sequence S539-546: VNFNFGGL³²) or class II MHC
696 ((#62, peptide sequence S62-76: VTWFHAIHVSGTNGT³¹) immunodominant spike peptides (1
697 μ g/mL) overnight at 37°C in the presence of Brefeldin A (1:500, Invitrogen). The following day,
698 cells were washed and stained with Fc block (Clone 93; Cat: 101320; BioLegend), CD45
699 (BUV395; Clone 30-F11; Cat: 564279; BD Biosciences), CD8 β (PerCP/Cy5.5; Clone
700 YTS156.7.7; Cat: 126610; BioLegend), CD4 (FITC; Clone GK1.5; Cat: 100406; BioLegend),
701 CD44 (APC/Cy7; Clone IM7; Cat: 103028; BioLegend) for 30 min at 4°C in FACS buffer (1x
702 PBS with 2% FCS and 2 mM EDTA). Dead cells were excluded using Live/Dead (Thermo
703 Fisher) that was added concurrently with staining. Following this, cells were washed, fixed with
704 and stained for intracellular IFN- γ APC; Clone XMG1.2; Cat: 505810; BioLegend) and TNF- α
705 (PE/Cy7; Clone MP6-XT22; Cat: 25-7321-82; Invitrogen) using BD fixation/permeabilization kit
706 (BD Biosciences) according to the manufacturer's instructions. Cells were processed by flow
707 cytometry on a Cytex Aurora and analyzed using FlowJo software version 10.4.2).

708 **Statistical analysis.** Statistical significance was assigned when *P* values were < 0.05
709 using GraphPad Prism version 9.3. Tests, number of animals, median values, and statistical
710 comparison groups are indicated in the Figure legends. Changes in infectious virus titer, viral
711 RNA levels, or serum antibody responses were analyzed by one-way ANOVA with a post-test
712 correction when comparing three or more groups. When comparing two groups, a Mann-
713 Whitney test was performed and a Bonferroni correction was used to account multiple
714 independent comparisons. Best-fit lines were calculated using non-linear regression analyses.

715 **Materials availability.** All requests for resources and reagents should be directed to the
716 Lead Contact author. This includes viruses, proteins, vaccines, and primer-probe sets. All
717 reagents will be made available on request after completion of a Materials Transfer Agreement

718 (MTA). The preclinical mRNA vaccines (control and mRNA-1273) can be obtained under an
719 MTA with Moderna (contact: Darin Edwards, Darin.Edwards@modernatx.com).

720 **Data availability.** All data supporting the findings of this study are available within the
721 paper, its Extended Data, or Source Data files. Any additional information related to the study
722 also is available from the corresponding author upon request.

723 **Code availability.** No code was used in the course of the data acquisition or analysis.

724

725 REFERENCES

- 726
- 727 1. Krause, P.R., *et al.* SARS-CoV-2 Variants and Vaccines. *N Engl J Med* **385**, 179-186
728 (2021).
- 729 2. Amanat, F. & Krammer, F. SARS-CoV-2 Vaccines: Status Report. *Immunity* **52**, 583-589
730 (2020).
- 731 3. Bates, T.A., *et al.* Neutralization of SARS-CoV-2 variants by convalescent and
732 BNT162b2 vaccinated serum. *Nat Commun* **12**, 5135 (2021).
- 733 4. Khoury, D.S., *et al.* Neutralizing antibody levels are highly predictive of immune
734 protection from symptomatic SARS-CoV-2 infection. *Nat Med* **27**, 1205-1211 (2021).
- 735 5. Cromer, D., *et al.* Relating In Vitro Neutralization Level and Protection in the CVnCoV
736 (CUREVAC) Trial. *Clin Infect Dis* **75**, e878-e879 (2022).
- 737 6. Kaplonek, P., *et al.* mRNA-1273 vaccine-induced antibodies maintain Fc effector
738 functions across SARS-CoV-2 variants of concern. *Immunity* **55**, 355-365.e354 (2022).
- 739 7. Hederman, A.P., *et al.* SARS-CoV-2 mRNA vaccination elicits broad and potent Fc
740 effector functions to VOCs in vulnerable populations. *medRxiv : the preprint server for*
741 *health sciences* (2022).
- 742 8. Chalkias, S., *et al.* A Bivalent Omicron-Containing Booster Vaccine against Covid-19. *N*
743 *Engl J Med* **387**, 1279-1291 (2022).
- 744 9. Cele, S., *et al.* Omicron extensively but incompletely escapes Pfizer BNT162b2
745 neutralization. *Nature* **602**, 654-656 (2022).
- 746 10. Andrews, N., *et al.* Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529)
747 Variant. *N Engl J Med* (2022).
- 748 11. Elliott, P., *et al.* Rapid increase in Omicron infections in England during December 2021:
749 REACT-1 study. *Science* **375**, 1406-1411 (2022).
- 750 12. Wang, Q., *et al.* Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4
751 and BA.5. *Nature* **608**, 603-608 (2022).
- 752 13. Cao, Y., *et al.* Omicron escapes the majority of existing SARS-CoV-2 neutralizing
753 antibodies. *Nature* (2021).
- 754 14. Cameroni, E., *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron
755 antigenic shift. *Nature* **602**, 664-667 (2022).
- 756 15. Grunst, M.W. & Uchil, P.D. Fc effector cross-reactivity: A hidden arsenal against SARS-
757 CoV-2's evasive maneuvering. *Cell reports. Medicine* **3**, 100540 (2022).
- 758 16. Zhu, D.Y., *et al.* Defining the determinants of protection against SARS-CoV-2 infection
759 and viral control in a dose-down Ad26.CoV2.S vaccine study in nonhuman primates.
760 *PLoS Biol* **20**, e3001609 (2022).
- 761 17. Tarke, A., *et al.* SARS-CoV-2 vaccination induces immunological T cell memory able to
762 cross-recognize variants from Alpha to Omicron. *Cell* **185**, 847-859.e811 (2022).
- 763 18. Brasu, N., *et al.* Memory CD8(+) T cell diversity and B cell responses correlate with
764 protection against SARS-CoV-2 following mRNA vaccination. *Nat Immunol* **23**, 1445-
765 1456 (2022).
- 766 19. Zohar, T., *et al.* Compromised Humoral Functional Evolution Tracks with SARS-CoV-2
767 Mortality. *Cell* **183**, 1508-1519.e1512 (2020).
- 768 20. Case, J.B., *et al.* Resilience of S309 and AZD7442 monoclonal antibody treatments
769 against infection by SARS-CoV-2 Omicron lineage strains. *Nat Commun* **13**, 3824
770 (2022).
- 771 21. Bates, T.A., *et al.* BNT162b2-induced neutralizing and non-neutralizing antibody
772 functions against SARS-CoV-2 diminish with age. *Cell Rep* **41**, 111544 (2022).
- 773 22. Richardson, S.I., *et al.* SARS-CoV-2 Omicron triggers cross-reactive neutralization and
774 Fc effector functions in previously vaccinated, but not unvaccinated, individuals. *Cell*
775 *Host Microbe* **30**, 880-886.e884 (2022).

- 776 23. Richardson, S.I. & Moore, P.L. Targeting Fc effector function in vaccine design. *Expert*
777 *Opin Ther Targets* **25**, 467-477 (2021).
- 778 24. Ackerman, M.E., Barouch, D.H. & Alter, G. Systems serology for evaluation of HIV
779 vaccine trials. *Immunol Rev* **275**, 262-270 (2017).
- 780 25. Dinnon, K.H., 3rd, *et al.* A mouse-adapted model of SARS-CoV-2 to test COVID-19
781 countermeasures. *Nature* **586**, 560-566 (2020).
- 782 26. Cobb, R.R., *et al.* A combination of two human neutralizing antibodies prevents SARS-
783 CoV-2 infection in cynomolgus macaques. *Med (New York, N.Y.)* **3**, 188-203.e184
784 (2022).
- 785 27. Winkler, E.S., *et al.* SARS-CoV-2 causes lung infection without severe disease in human
786 ACE2 knock-in mice. *J Virol*, Jvi0151121 (2021).
- 787 28. Gu, H., *et al.* Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy.
788 *Science* **369**, 1603-1607 (2020).
- 789 29. Ying, B., *et al.* Boosting with variant-matched or historical mRNA vaccines protects
790 against Omicron infection in mice. *Cell* **185**, 1572-1587.e1511 (2022).
- 791 30. Ying, B., *et al.* Protective activity of mRNA vaccines against ancestral and variant SARS-
792 CoV-2 strains. *Sci Transl Med*, eabm3302 (2021).
- 793 31. Zhuang, Z., *et al.* Mapping and role of T cell response in SARS-CoV-2-infected mice. *J*
794 *Exp Med* **218**(2021).
- 795 32. Li, C., *et al.* Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech
796 BNT162b2 vaccine. *Nat Immunol* **23**, 543-555 (2022).
- 797 33. Scheaffer, S.M., *et al.* Bivalent SARS-CoV-2 mRNA vaccines increase breadth of
798 neutralization and protect against the BA.5 Omicron variant in mice. *Nat Med* (2022).
- 799 34. Chong, Z., *et al.* Nasally delivered interferon- λ protects mice against infection by SARS-
800 CoV-2 variants including Omicron. *Cell Rep* **39**, 110799 (2022).
- 801 35. Laidlaw, B.J., *et al.* Cooperativity between CD8+ T cells, non-neutralizing antibodies,
802 and alveolar macrophages is important for heterosubtypic influenza virus immunity.
803 *PLoS Pathog* **9**, e1003207 (2013).
- 804 36. Accorsi, E.K., *et al.* Association Between 3 Doses of mRNA COVID-19 Vaccine and
805 Symptomatic Infection Caused by the SARS-CoV-2 Omicron and Delta Variants. *Jama*
806 **327**, 639-651 (2022).
- 807 37. Chemaitelly, H., *et al.* mRNA-1273 COVID-19 vaccine effectiveness against the B.1.1.7
808 and B.1.351 variants and severe COVID-19 disease in Qatar. *Nat Med* **27**, 1614-1621
809 (2021).
- 810 38. Wang, Z., *et al.* Memory B cell responses to Omicron subvariants after SARS-CoV-2
811 mRNA breakthrough infection in humans. *J Exp Med* **219**(2022).
- 812 39. Kaplonek, P., *et al.* mRNA-1273 and BNT162b2 COVID-19 vaccines elicit antibodies
813 with differences in Fc-mediated effector functions. *Sci Transl Med* **14**, eabm2311 (2022).
- 814 40. Bowman, K.A., *et al.* Hybrid Immunity Shifts the Fc-Effector Quality of SARS-CoV-2
815 mRNA Vaccine-Induced Immunity. *mBio* **13**, e0164722 (2022).
- 816 41. Winkler, E.S., *et al.* Human neutralizing antibodies against SARS-CoV-2 require intact
817 Fc effector functions for optimal therapeutic protection. *Cell* **184**, 1804-1820.e1816
818 (2021).
- 819 42. Schäfer, A., *et al.* Antibody potency, effector function, and combinations in protection
820 and therapy for SARS-CoV-2 infection in vivo. *J Exp Med* **218**(2021).
- 821 43. Beaudoin-Bussi eres, G., *et al.* A Fc-enhanced NTD-binding non-neutralizing antibody
822 delays virus spread and synergizes with a nAb to protect mice from lethal SARS-CoV-2
823 infection. *Cell Rep* **38**, 110368 (2022).
- 824 44. Yamin, R., *et al.* Fc-engineered antibody therapeutics with improved anti-SARS-CoV-2
825 efficacy. *Nature* **599**, 465-470 (2021).

- 826 45. Mades, A., *et al.* Detection of persistent SARS-CoV-2 IgG antibodies in oral mucosal
827 fluid and upper respiratory tract specimens following COVID-19 mRNA vaccination. *Sci*
828 *Rep* **11**, 24448 (2021).
- 829 46. Heyman, B. Antibodies as natural adjuvants. *Curr Top Microbiol Immunol* **382**, 201-219
830 (2014).
- 831 47. Hamano, Y., Arase, H., Saisho, H. & Saito, T. Immune complex and Fc receptor-
832 mediated augmentation of antigen presentation for in vivo Th cell responses. *J Immunol*
833 **164**, 6113-6119 (2000).
- 834 48. Fransen, M.F., *et al.* A Restricted Role for FcγR in the Regulation of Adaptive Immunity.
835 *J Immunol* **200**, 2615-2626 (2018).
- 836 49. Davis-Gardner, M.E., *et al.* mRNA bivalent booster enhances neutralization against
837 BA.2.75.2 and BQ.1.1. *bioRxiv* (2022).
- 838 50. Pincetic, A., *et al.* Type I and type II Fc receptors regulate innate and adaptive immunity.
839 *Nat Immunol* **15**, 707-716 (2014).
- 840 51. Ravetch, J.V. & Bolland, S. IgG Fc receptors. *Annu Rev Immunol* **19**, 275-290 (2001).
- 841 52. Bournazos, S., DiLillo, D.J. & Ravetch, J.V. humanized mice to study FcγR function.
842 *Curr Top Microbiol Immunol* **382**, 237-248 (2014).
- 843 53. Nimmerjahn, F., *et al.* FcγRIV deletion reveals its central role for IgG2a and IgG2b
844 activity in vivo. *Proc Natl Acad Sci U S A* **107**, 19396-19401 (2010).
- 845 54. Halfmann, P.J., *et al.* SARS-CoV-2 Omicron virus causes attenuated disease in mice
846 and hamsters. *Nature* (2022).
- 847 55. Junqueira, C., *et al.* FcγR-mediated SARS-CoV-2 infection of monocytes activates
848 inflammation. *Nature* **606**, 576-584 (2022).
- 849 56. Sefik, E., *et al.* Inflammasome activation in infected macrophages drives COVID-19
850 pathology. *Nature* **606**, 585-593 (2022).
- 851 57. Goldblatt, D., Alter, G., Crotty, S. & Plotkin, S.A. Correlates of protection against SARS-
852 CoV-2 infection and COVID-19 disease. *Immunol Rev* **310**, 6-26 (2022).
- 853 58. Bournazos, S., Gupta, A. & Ravetch, J.V. The role of IgG Fc receptors in antibody-
854 dependent enhancement. *Nat Rev Immunol* **20**, 633-643 (2020).
- 855 59. Misharin, A.V., Morales-Nebreda, L., Mutlu, G.M., Budinger, G.R. & Perlman, H. Flow
856 cytometric analysis of macrophages and dendritic cell subsets in the mouse lung.
857 *American journal of respiratory cell and molecular biology* **49**, 503-510 (2013).
- 858 60. Schyns, J., Bureau, F. & Marichal, T. Lung Interstitial Macrophages: Past, Present, and
859 Future. *J Immunol Res* **2018**, 5160794 (2018).
- 860 61. Zang, R., *et al.* TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human
861 small intestinal enterocytes. *Sci Immunol* **5**(2020).
- 862 62. Chen, R.E., *et al.* Resistance of SARS-CoV-2 variants to neutralization by monoclonal
863 and serum-derived polyclonal antibodies. *Nat Med* (2021).
- 864 63. Chen, R.E., *et al.* In vivo monoclonal antibody efficacy against SARS-CoV-2 variant
865 strains. *Nature* (2021).
- 866 64. Ioan-Facsinay, A., *et al.* FcγRI (CD64) contributes substantially to severity of
867 arthritis, hypersensitivity responses, and protection from bacterial infection. *Immunity* **16**,
868 391-402 (2002).
- 869 65. Botto, M., *et al.* Homozygous C1q deficiency causes glomerulonephritis associated with
870 multiple apoptotic bodies. *Nat Genet* **19**, 56-59 (1998).
- 871 66. Nelson, J., *et al.* Impact of mRNA chemistry and manufacturing process on innate
872 immune activation. *Science advances* **6**, eaaz6893 (2020).
- 873 67. Corbett, K.S., *et al.* SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen
874 preparedness. *Nature* **586**, 567-571 (2020).
- 875 68. Hassett, K.J., *et al.* Optimization of Lipid Nanoparticles for Intramuscular Administration
876 of mRNA Vaccines. *Molecular therapy. Nucleic acids* **15**, 1-11 (2019).

- 877 69. Roy, S., *et al.* Creation of a panel of vectors based on ape adenovirus isolates. *J Gene*
878 *Med* **13**, 17-25 (2011).
- 879 70. Hassan, A.O., *et al.* A single intranasal dose of chimpanzee adenovirus-vectored
880 vaccine protects against SARS-CoV-2 infection in rhesus macaques. *Cell Reports*
881 *Medicine* (2021).
- 882 71. Amanat, F., *et al.* SARS-CoV-2 mRNA vaccination induces functionally diverse
883 antibodies to NTD, RBD, and S2. *Cell* **184**, 3936-3948.e3910 (2021).
- 884 72. Stadlbauer, D., *et al.* SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a
885 Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol* **57**, e100
886 (2020).
- 887 73. VanBlargan, L.A., *et al.* A potently neutralizing SARS-CoV-2 antibody inhibits variants of
888 concern by utilizing unique binding residues in a highly conserved epitope. *Immunity*
889 (2021).
- 890 74. Case, J.B., Bailey, A.L., Kim, A.S., Chen, R.E. & Diamond, M.S. Growth, detection,
891 quantification, and inactivation of SARS-CoV-2. *Virology* **548**, 39-48 (2020).
- 892 75. Brown, E.P., *et al.* Multiplexed Fc array for evaluation of antigen-specific antibody
893 effector profiles. *J Immunol Methods* **443**, 33-44 (2017).
- 894 76. Brown, E.P., *et al.* High-throughput, multiplexed IgG subclassing of antigen-specific
895 antibodies from clinical samples. *J Immunol Methods* **386**, 117-123 (2012).

896

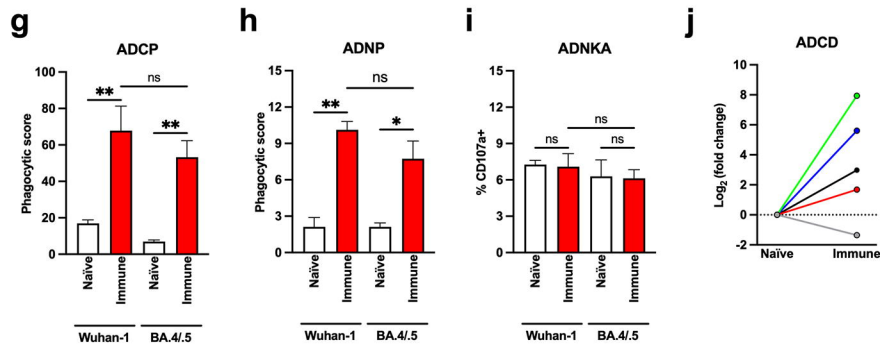
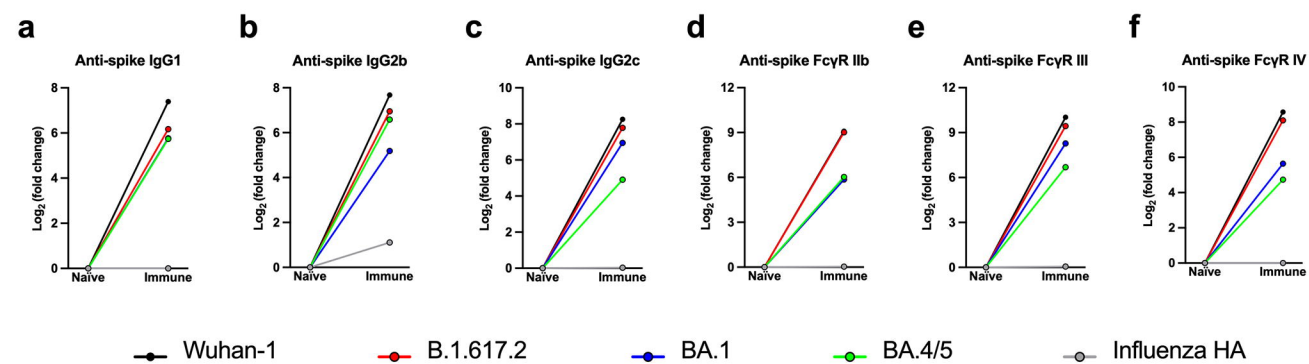


Figure 1

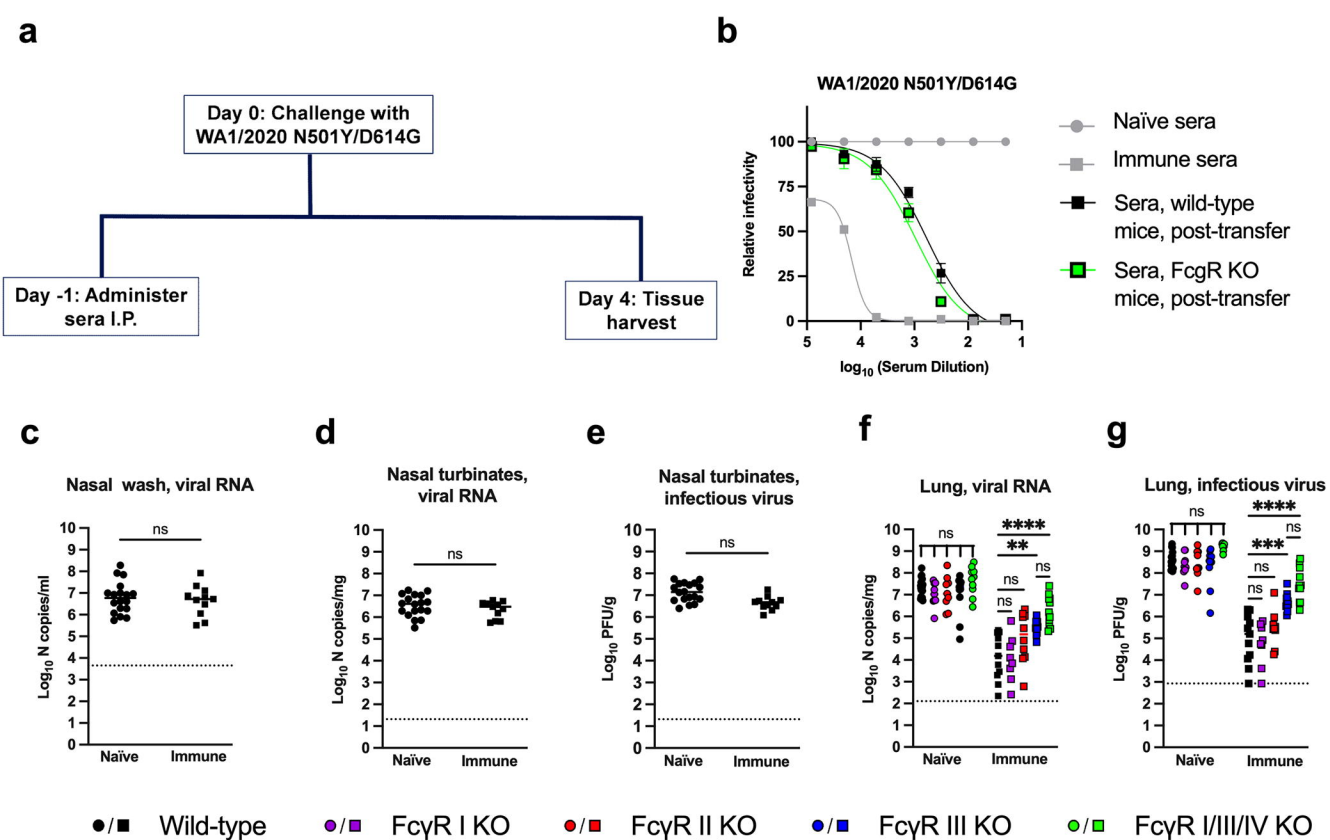
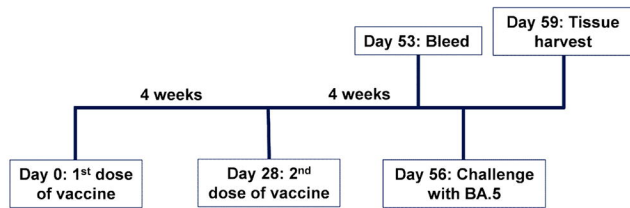
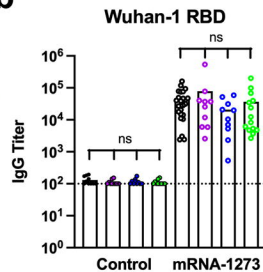
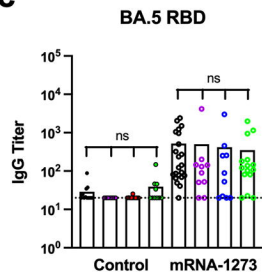
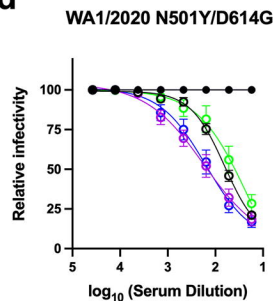
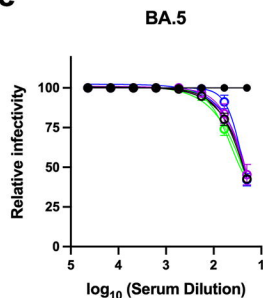
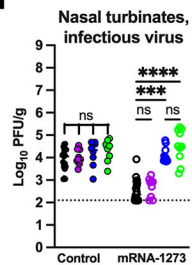
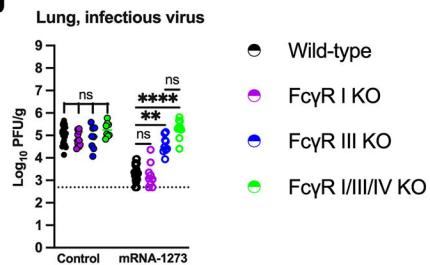


Figure 3

a**b****c****d****e****f****g**

- Wild-type
- FcγR I KO
- FcγR III KO
- FcγR I/III/IV KO

Figure 4

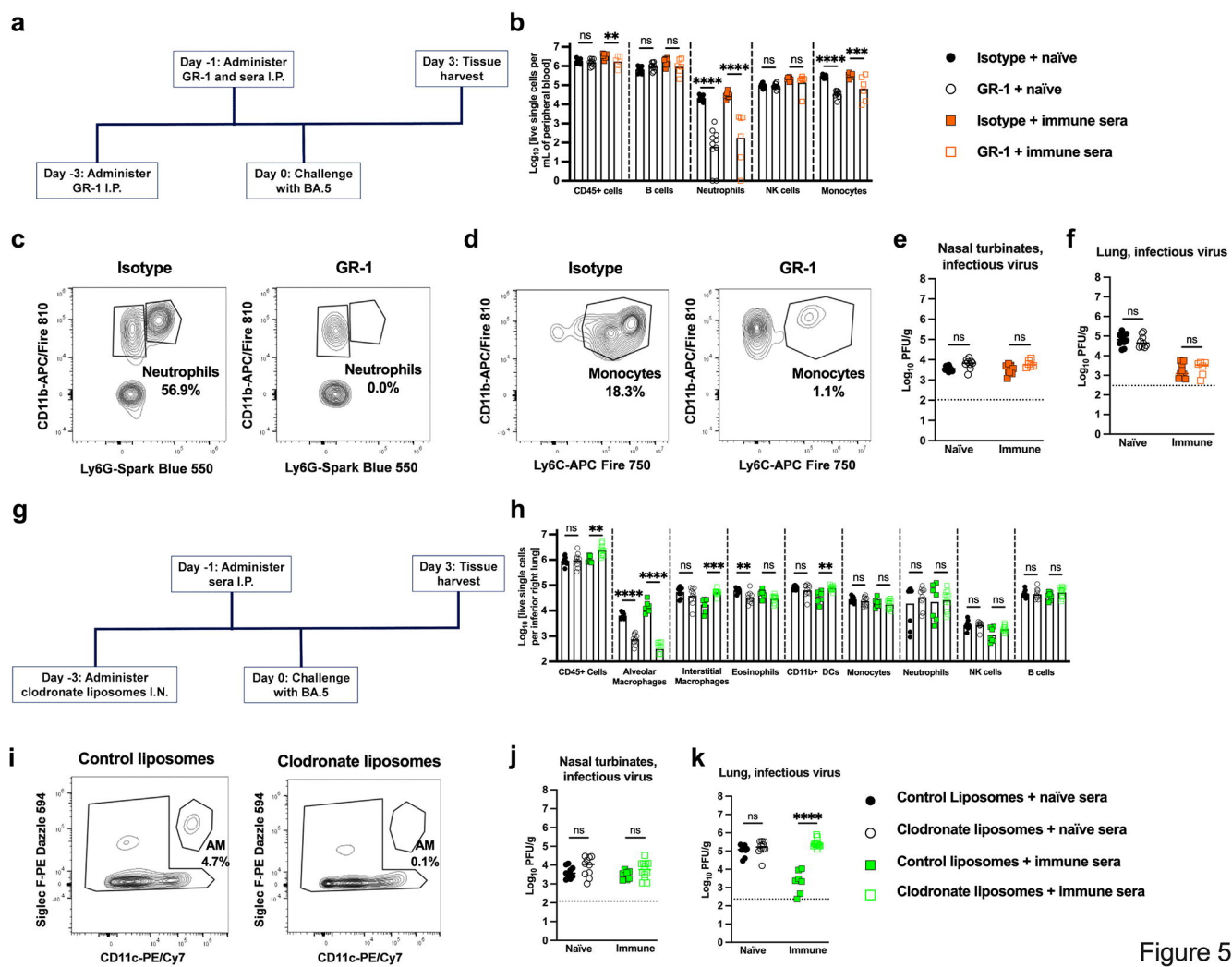


Figure 5