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1 *Research Article*

2 **TITLE**

3 Novel monoclonal antibodies showing broad neutralizing activity for SARS-CoV-2 4 variants including Omicrons BA.5 and BA.2.75

5

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36 **Summary**

37 We identified novel neutralizing monoclonal antibodies against SARS-CoV-2 variants
38 (including Omicron) from individuals received two doses of mRNA vaccination after they
39 had been infected with wildtype. We named them MO1, MO2 and MO3. MO1 shows high
40 neutralizing activity against authentic variants: D614G, Delta, BA.1, BA.1.1, BA.2, and
41 BA.2.75 and BA.5. Our findings confirm that the wildtype-derived vaccination can induce
42 neutralizing antibodies that recognize the epitopes conserved among the SARS-CoV-2
43 variants (including BA.5 and BA.2.75). The monoclonal antibodies obtained herein could
44 serve as novel prophylaxis and therapeutics against not only current SARS-CoV-2 viruses but
45 also future variants that may arise.

46

47 **Keywords**

48 Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), Omicron variants, human
49 monoclonal antibody, broadly neutralizing activity, spike, vaccine

50

51 **Introduction**

52 Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) caused the explosive
53 Coronavirus disease 2019 (COVID-19) pandemic that began in 2019; SARS-CoV-2 has

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54 infected over 577 million people worldwide and is responsible for more than 6.4 million
55 deaths as of August 4, 2022 (https://covid19.who.int/). The Omicron variant (BA.1;
56 B.1.1.529) of SARS-CoV-2 was first reported in South Africa in November 2021 and had
57 spread worldwide
58 (https://www.cdc.gov/coronavirus/2019-ncov/variants/Omicron-variant.html) (Dejnirattisai et
59 al., 2022). The spread of the Omicron variant has caused an increase of COVID-19 infections,
60 and the Omicron BA.5 variant has spread worldwide (Tegally et al., 2022). We and other
61 research groups have shown that three doses of the mRNA vaccine can protect individuals
62 against the BA.1 and BA.2 variants of SARS-CoV-2 (Muik et al., 2022) (Furukawa et al.,
63 2022) (Tjan et al., 2022). We have also observed that neutralizing antibodies against Omicron
64 variants are increased in the sera of individuals received a two-dose mRNA vaccine after they
65 had been infected with wildtype SARS-CoV-2 (D614G) (Kurahashi et al., 2022a). We
66 therefore screened and searched those individuals' B cells, as we suspected that it is possible
67 that these B cells could produce antibodies that have broad neutralizing activity against
68 SARS-CoV-2 variants including Omicron BA.5. In the present study, we identified three
69 novel monoclonal antibodies (mAbs) that are broadly effective against SARS-CoV-2 variants
70 including Omicron BA.5.
71

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72 **RESULTS**

73 **B cells that produce antibodies with broad neutralizing activity against SARS-CoV-2**
74 **were obtained from the PBMCs of SARS-CoV-2-infected, and subsequently**
75 **two-dose-vaccinated patients**

76 To isolate mAbs targeting common epitopes of SARS-CoV-2 variants, we searched for
77 antibody genes from PBMCs of infected and subsequently vaccinated patients. We selected
78 three PBMCs from the patients who showed high neutralizing activity against all of the
79 D614G, Delta, and Omicron BA.1 variants previously we reported (Kurahashi et al., 2022a).
80 In the single B cells isolated from the PBMCs, ten antibody genes were further selected based
81 on the ELISA findings of Fabs produced by the Ecobody technology (Ojima-Kato et al.,
82 2017), and we tested the purified recombinant IgG antibodies with the V genes. Ten mAbs
83 were examined for neutralizing activity, and we used the three (which we named MO1, MO2,
84 and MO3) that showed neutralizing activity in the present study.
85

86 **Broadly neutralizing mAbs against SARS-CoV-2 variants**

87 As depicted in Figure 1, we observed that the three neutralizing mAbs for D614G, i.e., MO1,
88 MO2, and MO3, they recognize the SARS-CoV-2 spike protein of D614G. MO1 recognized
89 the spike protein of five variants: D614G, Delta, BA.1, BA.2 and BA.5. MO2 recognized the

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90 spike protein of four of these variants (not BA.5). MO3 recognized three of the variants (not
91 Delta or BA.5). We next assessed whether these three mAbs had neutralizing activity against
92 SARS-CoV-2 variants. As shown in Figures 1B, C and D, the mAb MO1 inhibited all five
93 variants and BA.1.1 variant with the following IC_{50} values: D614G (23.62 ng/mL), Delta
94 (15.84 ng/mL), BA.1 (4.0 ng/mL), BA.1.1 (10.64 ng/mL), BA.2 (20.31 ng/mL), and BA.5
95 (15.67 ng/mL). MO2 inhibited four variants (BA.5 was the exception), with the following
96 IC_{50} values: D614G (65.81 ng/mL), Delta (88.24 ng/mL), BA.1 (17.71 ng/mL), BA.1.1
97 (36.05 ng/mL) and BA.2 (151.2 ng/mL). MO3 suppressed three variants (Delta and BA.5
98 were the exceptions), with the following IC_{50} values: D614G (231.57 ng/mL), BA.1 (594.63
99 ng/mL), and BA.2 (701.95 ng/mL). Furthermore, all of three mAbs could neutralize BA.2.75
100 as well (data not shown).
101

102 **The novel neutralizing mAb MO1 has high affinity against the SARS-CoV-2 BA.5 spike**
103 **protein**

104 The affinity between the SARS-CoV-2 BA.2 spike protein and the MO1 and MO2 mAbs was
105 next evaluated by the Bio-layer interferometry (BLI) method. The prefusion-stabilized BA.2
106 spike ectodomain trimer hardly dissociated from MO1 or MO2, indicating high avidity due to
107 multivalent binding sites on the spike trimer (data not shown). Both MO1 and MO2 showed

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108 high affinity with the BA.2 spike RBD, with the dissociation constants (K_D) of 3.3 nM and
109 2.0 nM, respectively (Figures 2A, C). MO1 also has high affinity with the BA.5 spike RBD
110 with the K_D of 11 nM (Figures 2B, C), whereas MO2 showed no binding to the BA.5 spike
111 RBD, which is consistent with its loss of the neutralizing activity for BA.5.
112

113 **DISCUSSION**

114 The emergence of SARS-CoV-2 variants has prolonged the COVID-19 pandemic. The
115 novel neutralizing mAb that we identified in the present study and named MO1 has strong
116 neutralizing potency against all of the major SARS-CoV-2 variants described to date,
117 including the Omicron BA.5 variant that is raging worldwide. The Omicron variants BA.1
118 and BA.2 are able to escape from the majority of the known neutralizing antibodies (Ai et al.,
119 2022; Yamasoba et al., 2022a), and the escape ability of BA.5 is strengthened due to
120 additional mutations such as F486V around the binding site of class 1 antibodies and the
121 mutation site L452R around the binding site of class 3 antibodies as reported (Cao et al.,
122 2022). Indeed, the neutralizing antibody MO2 identified herein, which can strongly neutralize
123 both BA.1 and BA.2, lost neutralizing activity for BA.5. In contrast, MO1 has maintained
124 neutralizing activity against BA.5. A conserved epitope avoiding the position of the L452R or
125 F486V mutation sites is expected for MO1, considering the broad neutralizing activity of

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126 MO1 (Figure 1). Structural analysis by cryoelectron microscopy (cryo-EM) is under progress
127 and the details of the MO1 epitope are under investigation.

128 It has been reported that the antibodies bebtelovimab (LY-CoV1404 (Westendorf et al.,
129 2022)) and cilgavimab (AZD1061 (Dong et al., 2021)) have neutralizing activity against
130 Omicron BA.1, BA.2, and BA.5, and that cilgavimab showed reduced activity against BA.5
131 (Yamasoba et al., 2022b). The binding affinity and neutralizing function of MO1 to BA.5 are
132 high (Figures 1, 2). Our preliminary cryo-EM analysis indicated that MO1 accesses to the
133 RBD from the similar side as the bebtelovimab and cilgavimab, while the binding site is
134 substantially different from them.

135 MO1 is derived from PBMCs of individuals who were infected with a SARS-CoV-2
136 variant — presumably the wildtype with only the D614G mutation in the spike — and
137 then received two doses of an mRNA vaccine encoding the spike gene of the ancestral
138 SARS-CoV-2 (Kurahashi et al., 2022a). Our finding that MO1 can broadly neutralize the
139 early SARS-CoV-2 variants and the Omicron BA.1, BA.1.1, BA.2 and BA.5 variants
140 demonstrates that immunity against the spike with the ancestral SARS-CoV-2 RBD sequence
141 can protect humans by inducing neutralizing antibodies, like MO1, that recognize conserved
142 epitopes. Indeed, our serological study regarding the sustainability of the neutralizing
143 antibodies after early SARS-CoV-2 (D614G) infection indicated that cross-reactive

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144 neutralizing antibodies are sustained for >6 months, although neutralizing antibodies specific
145 for SARS-CoV-2 (D614G) decline (Kurahashi et al., 2022a; Kurahashi et al., 2022b). It is
146 also noteworthy that the booster dose (three-dose) vaccination using the mRNA vaccine
147 based on wildtype SARS-CoV-2 can induce neutralizing antibodies against Omicron BA.1
148 and BA.2 (Furukawa et al., 2022; Tjan et al., 2022).

149 It is speculated that MO1 recognizes a conserved epitope shared among SARS-CoV-2
150 variants considering its broad neutralizing activity. Isolation of MO1 from human memory
151 B-cells may demonstrate that target sites remain available for neutralizing antibodies even in
152 the spike of Omicron variants. Thus, the key task to develop effective immunity against a
153 broad range of SARS-CoV-2 variants is thus to determine how to effectively induce
154 antibodies against the conserved epitope. One of our previous studies revealed that patients
155 who were infected with wildtype SARS-CoV-2 and then received a two-dose mRNA
156 vaccination acquired high neutralizing antibody titers against Omicron BA.1 (Kurahashi et al.,
157 2022a), and the neutralizing antibodies that were isolated in the present study were derived
158 from donors with this background. Repeated exposure to the SARS-CoV-2 spike protein,
159 irrespective of the variation in the sequence, may induce broadly neutralizing antibodies.

160 Cao et al. reported that BA.1-derived vaccine boosters may not achieve
161 broad-spectrum protection against new Omicron variants because Omicron may evolve

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162 mutations to evade the humoral immunity elicited by BA.1 (Cao et al., 2022). In addition, all
163 antibodies were found to be able to neutralize BA.2.75 variant newly appeared (Tan et al.,
164 2022). The MO1 binding site observed by preliminary cryoelectron micrography is distant
165 from mutation sites of the BA.2.75 variants (data not shown).

166 Our present findings demonstrate that the booster vaccination for the wildtype can
167 induce neutralizing antibodies that may recognize common epitopes that are conserved
168 among SARS-CoV-2 variants. It can also be speculated that a three- or four-dose vaccination
169 based on the wildtype may stimulate memory B cells that can produce antibodies that have
170 common epitopes conserved, showing broad neutralizing activity, and that booster
171 vaccinations should thus be required even for previously infected individuals. The three new
172 monoclonal antibodies identified in the present study could serve as novel therapeutics
173 against not only the current SARS-CoV-2 variants but also new variants that arise in the
174 future.

175

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182 Research Alliance Laboratories for providing the SARS-CoV-2 B2 strain used as the D614G
183 variant herein. We also thank the National Institute of Infectious Disease Japan for providing
184 the SARS-CoV-2 of Pango lineages AY.122, BA.1.18, BA.1.1, BA.2, BA.2.75 and BA.5 used
185 here as the Delta, Omicron (B.1.159) BA.1, BA.1.1, BA.2, BA2.75 and BA.5 variants,
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197 **Author contributions:**

198 Conceptualization, HI, MN, LHT and YM.; Methodology, HI, MN, HS, KK and YM.;

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199 Sample collection: TN, SN, SS and SI.; Formal analysis, HI, MN, LHT, SS, MIM, GBE, HS,
200 KK, NH, KA, YK, KF, MS TN, JA and YM.; Funding acquisition: YM.; Project
201 administration, HI, MN, YM.; Supervision, YM.; Writing – original draft.; HI, MN and YM.;
202 Writing – review & editing, HI, MN and YM.
203
204 **Declaration of interests**

205 The authors declare no conflicts of interest in this research.
206
207 **Figure legends**

208 **Figure 1.** Identification of broadly neutralizing mAbs against SARS-CoV-2 variants. **(A)** The
209 binding of three mAbs to the SARS-CoV-2 spike ectodomains of the D614G, Delta, BA.1,
210 BA.2, and BA.5 variants as revealed by ELISAs. **(B)** The neutralizing activity of the three
211 mAbs, MO1, MO2 and MO3 against D614G, Delta, BA.1, BA.1.1, BA.2 or BA.5 as
212 evaluated by the plaque reduction neutralization test (PRNT). **(C)** The 50% inhibitory
213 concentrations (IC50) of the mAbs MO1, MO2 and MO3 against the SARS-CoV-2 variants
214 calculated from the above neutralization data (B) are shown. **(D)** A presentation of plaque
215 reduction in MO1's PRNT test against BA.5.
216

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217 **Figure 2.** Analysis of the affinity between the three novel mAbs and spike antigens by
218 biolayer interferometry (BLI). **(A)** The sensorgram for the BA.2 spike RBD's binding to the
219 mAb MO1 or MO2. *Dashed lines:* the fitting curves. **(B)** The same BLI analysis as in (A)
220 between the BA.5 spike RBD and MO1. **(C)** Summary of the BLI kinetics evaluated from the
221 curve fitting.

222

223 **Materials and Methods**

224 **Collection of human samples**

225 Blood samples were collected at Hyogo Prefectural Kakogawa Medical Center (Kakogawa,
226 Japan) from patients who were infected with COVID-19 during the 5-month period of
227 July–November 2020 and then received a two-dose messenger (m)RNA vaccine. In our
228 earlier investigation (Kurahashi et al., 2022a), we identified the sera that had high
229 neutralizing activity against SARS-CoV-2, and in the present study we used those patients'
230 (donors') sera and data.

231

232 **Isolation of human antibodies' genes from the donors' PBMCs**

233 In order to isolate human antibody genes from the above-described donor PBMCs, the sera's
234 memory B cells that expressed anti-SARS-CoV-2 spike Abs were screened and then sorted as

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235 single cells. Next, the anti-spike reactivity of the antibody Fab domain expressed from each V
236 gene as shown by Ecobody technology (Ojima-Kato et al., 2017) was evaluated by an ELISA
237 (enzyme-linked immunosorbent assay) (iBody, Nagoya, Japan).

238 The sequences of the selected V genes were determined and then subcloned into
239 human immunoglobulin (IgG) mAb-expressing vectors. We used a capillary electrophoresis
240 (CE) sequencer (model DS3000, Hitachi High-Tech, Tokyo) to confirm the sequences.

241

242 **Antibody expression and purification**

243 With polyethyleneimine and the two plasmids containing the heavy-chain and light-chain
244 sequences of the antibodies, recombinant mAbs were expressed in HEK293T (human kidney)
245 cells by transfection.

246 For the purification of the antibodies, we added rProtein A Sepharose® (Cytiva,
247 Marlborough, MA) to the culture supernatant. The trapped mAbs was eluted with sodium
248 citrate buffer (i.e., 40 mM trisodium citrate, pH 3.4). The solution was then immediately
249 neutralized to ~pH 7.0 by the addition of 1 M Tris-HCl buffer.

250

251 **Enzyme-linked immunosorbent assay (ELISA)**

252 Our earlier study (Ren et al., 2022) also describes the ELISA performed. The optical density

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253 was measured at wavelength 405 nm (OD_{405}) by a microplate photometer (Multiskan™ FC,
254 Thermo Fisher Scientific).
255
256 **Viruses**
257 BIKEN Innovative Vaccine Research Alliance Laboratories (Osaka, Japan) provided the
258 SARS-CoV-2 strain that contains the spike D614G mutation (DNA Data Bank of Japan
259 [DDBJ]: accession no. LC644163), and in the present study we refer to this strain as strain
260 D614G. Japan's National Institute of Infectious Disease (Tokyo) provided the SARS-CoV-2
261 strains of the Pango lineage AY.122 (EPI_ISL_2158617) which we used as the Delta variant,
262 BA.1.18 (EPI_ISL_7418017) which we used as the Omicron BA.1 variant, BA1.1
263 (EPI_ISL_7571618) which we used as the BA1.1 variant, BA.2 (EPI_ISL_9595859) which
264 we used as the BA.2 variant, BA.2.75 (EPI_ISL_13969765), which we used as the BA.2.75
265 variant, and BA.5 (EPI_13241867), which we used as the BA.5 variant. We propagated the
266 viruses by the infection of Vero E6 (TMPRSS2) cells in 2% FBS containing DMEM
267 (Matsuyama et al., 2020) in order to create a stock of each virus (Furukawa et al., 2021) .
268
269 **Plaque reduction neutralization test (PRNT)**
270 To conduct a plaque reduction neutralization test (PRNT) and determine the neutralization

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271 percentage for each virus strain, we seeded Vero E6/TMPRSS2 cells (2 × 10^5 cells/well) on
272 12-well plates (Corning) and cultivated them for 24 hr with 5% CO_2 at 37°C. The cell
273 monolayers were then washed one time with DMEM (without FBS). Each antibody diluted in
274 DMEM (without FBS) was mixed with 100 plaque forming units (PFU) of SARS-CoV-2 and
275 incubated for 1 hr at 37°C. We then added the virus–antibody mixture to the Vero
276 E6/TMPRSS2 cells and cultured the cells for 1 hr with 5% CO_2 at 37°C. After the removal of
277 the inoculum, the infected cells were washed twice with PBS and incubated for 3–6 days at
278 37°C with 5% CO_2 together with DMEM containing 2% FBS and 1.6% methylcellulose.
279 After the removal of the culture medium, PBS was used to wash the cells two times,
280 and the cells were fixed with 80% methanol for 1 hr at room temperature. The remaining cells
281 were stained with 1% crystal violet in 50% methanol for the visualization of plaques, which
282 were counted manually. The ratio of neutralization was obtained by dividing the number of
283 plaques obtained without antibody by the number of plaques obtained with antibody.
284

285 **Bio-layer interferometry**

286 The affinity between each antibody and spike protein was measured by bio-layer
287 interferometry (BLI) with a BLItz Biolayer Interferometer System (Sartorius, Goettingen,
288 Germany).

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289

290 **Ethics statement**

291 The Kobe University Graduate School of Medicine's Ethics Committee approved the
292 collection and use of the COVID-19 patients' blood samples (approval code: B200200). The
293 patients' written informed consent for this use was obtained.

294

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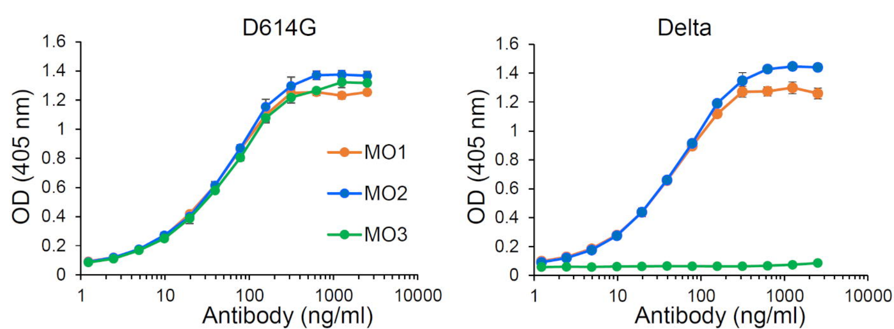
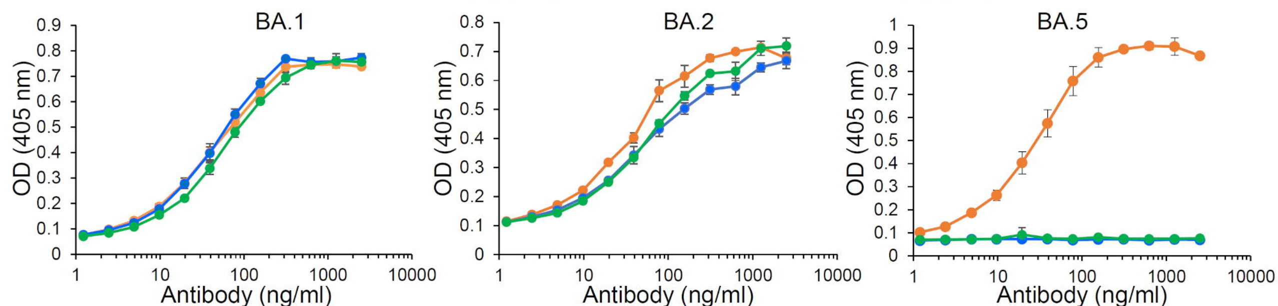
359
360
361

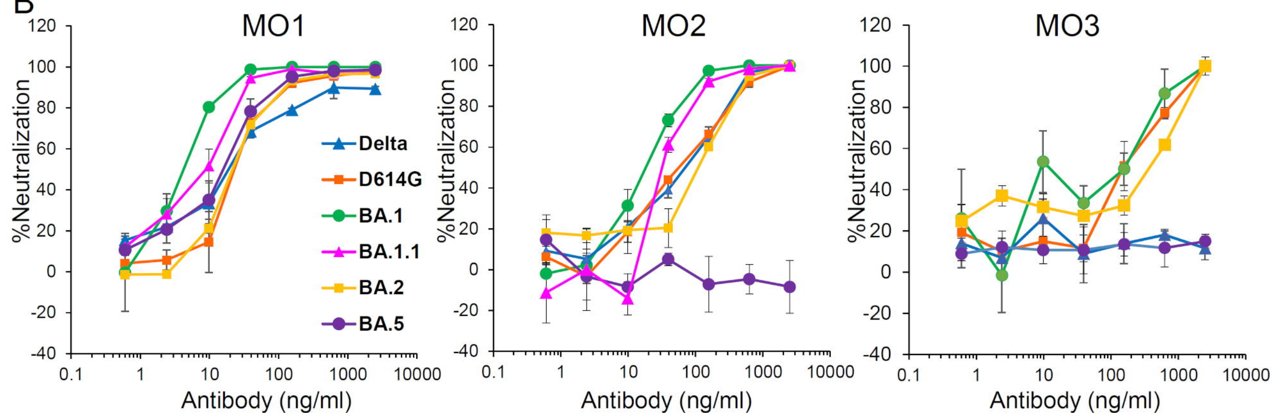
Fig. 1

A





B



C

IC50 (ng ml-1)	D614G	Delta	BA.1	BA.1.1	BA.2	BA.5
MO1	23.62	15.84	4.00	10.64	20.31	15.67
MO2	65.81	88.24	17.71	36.05	151.2	-
MO3	231.57	-	594.63	ND*	701.95	-

* ND : Not determined

D

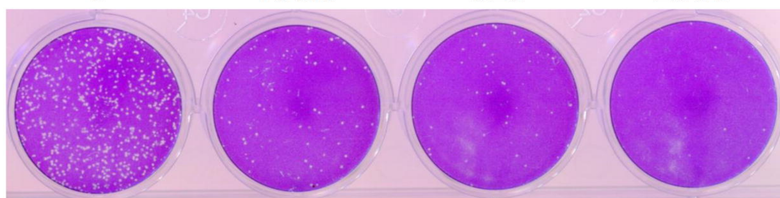
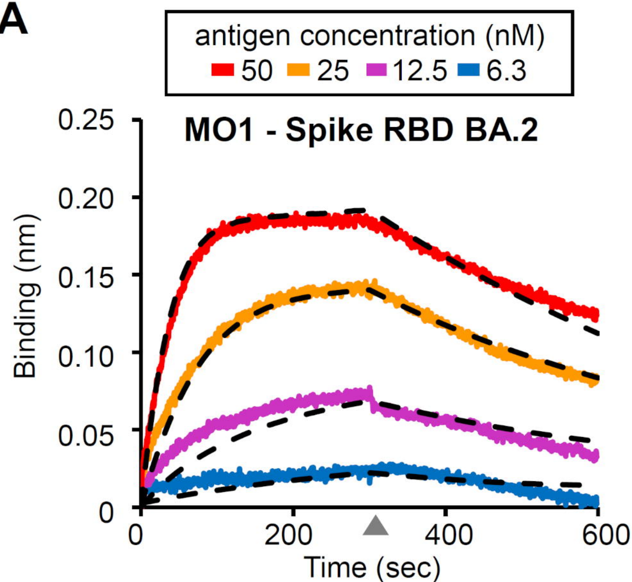
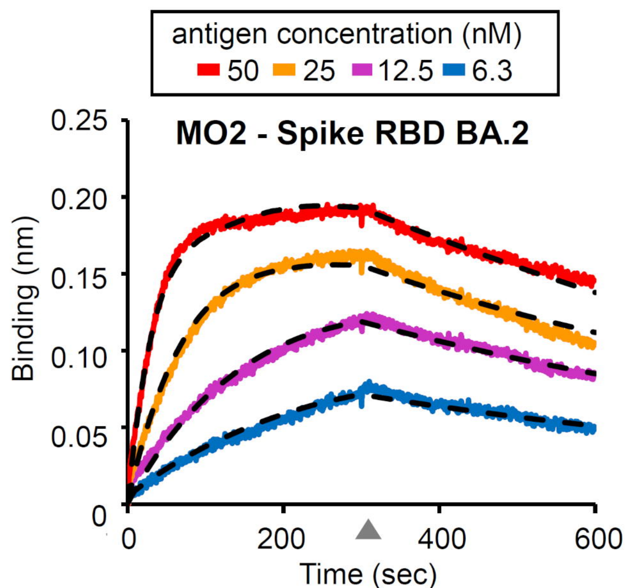


Fig. 2

A





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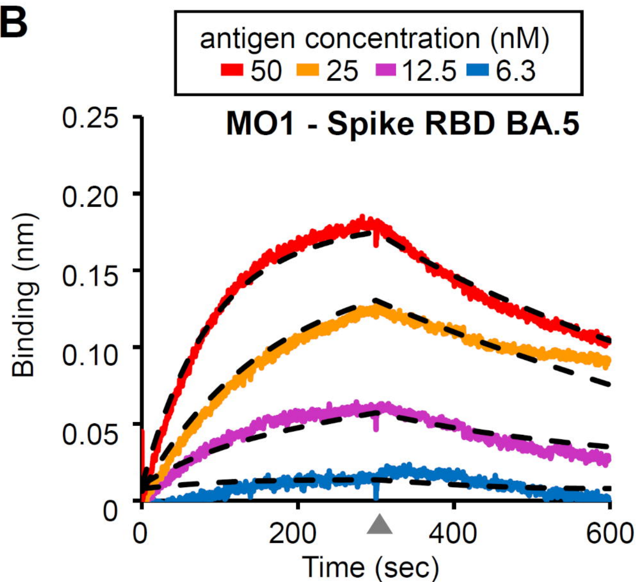

C

Ligand (antibody)	Analyte (antigen)	KD (M)	Ka(1/Ms)	kd(1/s)	R^2
MO1	Spike RBD BA.2	3.3×10^{-9}	5.3×10^{5}	1.9×10^{-3}	0.999
MO1	Spike RBD BA.5	1.1×10^{-8}	1.6×10^{5}	1.8×10^{-3}	0.987
MO2	Spike RBD BA.2	2.0×10^{-9}	5.5×10^{5}	1.1×10^{-3}	0.999
MO2	Spike RBD BA.5	No binding			