Cross-reaction of current available SARS-CoV-2 MAbs against the pangolin-origin coronavirus GX/P2V/2017

Highlights
- Cross-reaction of 50 human SARS-CoV-2 MAbs against GX/P2V/2017 is assessed
- GX/P2V/2017 displays substantial immune difference from the SARS-CoV-2 infection
- The complex structures of the GX/P2V/2017 RBD with two MAbs are resolved

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In brief
Pangolin-origin CoVs pose a potential threat to humans. Jia et al. test the cross-reaction of current available SARS-CoV-2 MAbs against the pangolin-origin coronavirus GX/P2V/2017. Structural analysis of two MAbs provides further insight into the immune difference of the pangolin-origin CoV.
**Article**

**Cross-reaction of current available SARS-CoV-2 MAbs against the pangolin-origin coronavirus GX/P2V/2017**

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**SUMMARY**

Since the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, multiple SARS-CoV-2-related viruses have been characterized, including pangolin-origin GD/1/2019 and GX/P2V/2017. Our previous study indicated that both viruses have the potential to infect humans. Here, we find that CB6 (commercial name etesevimab), a COVID-19 therapeutic monoclonal antibody (MAb) developed by our group, efficiently inhibits GD/1/2019 but not GX/P2V/2017. A total of 50 SARS-CoV-2 MAbs divided into seven groups based on their receptor-binding domain (RBD) epitopes, together with the COVID-19 convalescent sera, are systematically screened for their cross-binding and cross-neutralizing properties against GX/P2V/2017. We find that GX/P2V/2017 displays substantial immune difference from SARS-CoV-2. Furthermore, we solve two complex structures of the GX/P2V/2017 RBD with MAbs belonging to RBD-1 and RBD-5, providing a structural basis for their different antigenicity. These results highlight the necessity for broad anti-coronavirus countermeasures and shed light on potential therapeutic targets.

**INTRODUCTION**

Emerging and re-emerging viruses pose significant threats to global public health and economic development.1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), keeps evolving into novel variants due to sustained global transmission,2–4 resulting in approximately 628.7 million laboratory-confirmed cases and over 6.6 million deaths worldwide as of November 4, 2022 (https://covid19.who.int/).

Although global efforts have been made, and animal origin is suspected, the natural reservoir and intermediate host remain unknown for SARS-CoV-2.5 Multiple SARS-CoV-2-related bat coronaviruses (CoVs) have been reported worldwide, including those identified in southern China (RaTG13, RaTG15, RmYN02, and RmYN04);6–10 Cambodia (RshSTT82 and RshST T200);11 Japan (Rc-ö139);12 Thailand (RacCS203);13 Bulgaria (BM48-31);14 Kenya (BkY72);15 and Laos (BANAL viruses).16 Notably, the majority of these related viruses were discovered in bats of the *Rhinolophus* genus,17 making *Rhinolophus* bat a potential reservoir host of SARS-CoV-2. A World Health Organization (WHO) report concluded that the zoonotic introduction of SARS-CoV-2 was possible to likely and considered that an intermediate host was very likely.17 Pangolins are one of the intermediate suspected hosts as SARS-CoV-2-related viruses have been detected in pangolins smuggled from unknown regions, albeit arguments against this are also held.18,19}

The gain of ability of a virus to bind to its receptor in other species is a prerequisite for interspecies transmission.20 The potential of SARS-CoV-2-related CoVs to cause infection in humans and other host species has been studied by us and other groups.7,10,11,21 For instance, the potential infectivity of bat CoVs (RaTG13 and BANAL viruses) in humans and other animals has been proposed.7,22 In addition, our previous study showed that two pangolin CoVs (GD/1/2019 and GX/P2V/2017) bind human angiotensin-converting enzyme 2 (ACE2) as efficiently as
SARS-CoV-2 and present a potentially broader host range. These findings indicate an urgent need to find therapeutic candidates against these SARS-CoV-2-related viruses.

SARS-CoV-2 uses the receptor-binding domain (RBD) of the spike (S) protein to recognize the host receptor ACE2. The epitopes of the most potent therapeutic monoclonal antibodies (MAbs) overlap with the ACE2-interacting surface of the RBD and block the interaction with ACE2.23,24 Recently, a global consortium study defined seven SARS-CoV-2 RBD-directed antibody communities with distinct epitopes and competition profiles.25 These communities contain MAbs that bind to the receptor-binding motif (RBM) (RBD-1, RBD-2, and RBD-3), the outer face of the RBD (RBD-4 and RBD-5), or the inner face of the RBD (RBD-6 and RBD-7), providing a basis for the study of the immunogenicity differences between RBDs of SARS-CoV-2 and related viruses.

In this study, we aimed to perform a prospective study in preparation for the potential infections caused by two pangolin-origin SARS-CoV-2-related CoVs (GD/1/2019 and GX/P2V/2017). First, the ability of the therapeutic MAb CB6 (commercial name etesevimab), previously developed by our group for emergency use against COVID-19, to cross-react with the two pangolin-origin CoVs was evaluated. Binding and neutralizing activity of CB6 to GD/1/2019 was observed but not that of GX/P2V/2017. We then screened the cross-reactivity of the seven classes of RBD-directed MAbs to GX/P2V/2017 and identified decreased neutralizing activities against GX/P2V/2017 in the majority of them. We further investigated the cross-reactivity mechanisms by solving the complex structures of the GX/P2V/2017 RBD with two MAbs that engage to different epitopes. Altogether, these results suggest that pangolin-origin CoV GX/P2V/2017 has the immune potential to escape the vaccine and MAbs against COVID-19, highlighting the great necessity for broad anti-CoV countermeasures and shedding light on the potential targets.

RESULTS

The cross-reactivity of CB6 to the pangolin-origin CoVs

Three RBDs—GX/P2V/2017, GD/1/2019, and SARS-CoV-2 RBDs—were prepared to assess the ability of SARS-CoV-2 RBD MAbs to cross-react with the pangolin-origin CoV RBDs (Figures S1A–S1D). First, the binding affinity of CB6 to the GX/P2V/2017 and GD/1/2019 RBDs was evaluated by surface plasmon resonance (SPR). As shown in Figure 1A, the binding affinity of CB6 to GD/1/2019 was observed but not that of GX/P2V/2017. Then, we screened the cross-reactivity of CB6 to SARS-CoV-2, GX/P2V/2017, and GD/1/2019 (Figures S1A–S1D). First, the binding affinity of CB6 to the RBDs was determined with an equilibrium binding assay. As shown in Figure 1B, the binding affinity of CB6 to the RBDs was determined with an equilibrium binding assay.
dissociation constant \((K_D)\) of 24.94 nM for SARS-CoV-2 RBD and 40.57 nM for GD/1/2019 RBD, while no binding to the GX/P2V/2017 RBD was detected. Interestingly, the neutralization activity of CB6 against pseudotyped GD/1/2019 was similar to that against pseudotyped SARS-CoV-2, with half-maximal inhibitory concentrations \((IC_{50})\) of 0.013 and 0.020 \(\mu\)g/mL, respectively. Consistently, CB6 showed no detectable effect in preventing the entry of GX/P2V/2017 pseudovirus even at 400 \(\mu\)g/mL (Figure 1B). Sequence alignments of RBDs of SARS-CoV-2, GD/1/2019, and GX/P2V/2017 were performed, and the CB6 binding site for SARS-CoV-2 RBD was labeled. In the CB6 binding surface, there are only one substitution \((K417R)\) for the GD/1/2019 RBD but eight substitutions for the GX/P2V/2017 RBD (Figure 1C).

**Binding of GX/P2V/2017 RBD/S protein to the seven communities of SARS-CoV-2 RBD-directed MAbs**

Because the CB6 MAb could not recognize the GX/P2V/2017 RBD, we evaluated the cross-reactivity of other SARS-CoV-2 MAbs. A total of 50 SARS-CoV-2 MAbs divided into seven communities based on their RBD epitope were chosen to test their binding affinities to GX/P2V/2017 RBD, and SARS-CoV-2 RBD was included as a control (Figure 2; Tables S5 and S6).

Overall, among the 50 MAbs tested, all showed decreased or lost binding to the GX/P2V/2017 RBD, compared with those to the SARS-CoV-2 RBD, with the exception of 47D11 (RBD-5) and CR3022 (RBD-7), which presented no more than 4-fold higher binding affinities to the GX/P2V/2017 RBD than to the SARS-CoV-2 RBD. Specifically, in the RBD-1 group, only 4 MAbs (BD-604, P2C-1F11, BD-629, and CC12.1) out of 16 showed detectable binding to the GX/P2V/2017 RBD, and the binding affinities decreased by ~310- to 2,600-fold compared with their corresponding binding strength for the SARS-CoV-2 RBD. Among the 11 MAbs in the RBD-2 group, only S2E12 could bind to the GX/P2V/2017 RBD, with ~24-fold lower affinity than that for the SARS-CoV-2 RBD. Similarly, in the RBD-3 group, ADI-56046 could bind to the GX/P2V/2017 RBD with ~40,000-fold lower affinity than that of the SARS-CoV-2 RBD. None of the seven RBD-4 MAbs bound to the GX/P2V/2017 RBD. Two RBD-5 MAbs interacted with the GX/P2V/2017 RBD with slightly higher (47D111) or lower (S309) affinities. In contrast to the above communities, all RBD-6 and RBD-7 MAbs could bind to both SARS-CoV-2 and GX/P2V/2017 RBDs, with binding affinities to GX/P2V/2017 RBDs ~2- to ~100-fold weaker except for the CR3022 MAbs.

Considering that GX/P2V/2017 has variations in key residues implicated in the gating mechanism of the RBD up/down and the interactions of S MAbs, we conducted an SPR binding assay between the trimeric GX/P2V/2017 S and fragment antigen-binding regions (Fab) of the MAbs. The data indicated that 11 GX/P2V/2017 RBD-targeted MAbs \((K_D < 1 \mu\)M) bound to the GX/P2V/2017 trimeric S protein with similar binding affinity to the RBD (Figures S3 and S4).

**Neutralization of MAbs against the pseudotyped GX/P2V/2017**

Next, we investigated the cross-neutralizing activities of 12 MAbs that maintained relatively high binding strength to GX/P2V/2017 RBD \((K_D < 1 \mu\)M) against the GX/P2V/2017 pseudoviruses (Figures 3A–3C). In line with their binding features, 47D11 and CR3022 had slightly stronger neutralizing activities against GX/P2V/2017 than against SARS-CoV-2. COVA1-16 also displayed slightly increased neutralizing activity with an \(IC_{50}\) enhancement of ~1.7-fold. Among the MAbs tested, only S2E12 (RBD-2), 47D11 (RBD-5), S309 (RBD-5), and COVA1-16 (RBD-6) maintained neutralizing activities with \(IC_{50} < 1 \mu\)g/mL, being ~0.06, ~0.38, ~0.07, and ~0.67 \(\mu\)g/mL, respectively.

Although the binding between RBD-7 MAbs and the GX/P2V/2017 RBD were at tens of nanomolar level, they showed low potencies against the GX/P2V/2017 pseudovirus, similar to the effect against SARS-CoV-2.

**Complex structures of GX/P2V/2017 RBD with the Fabs of P2C-1F11 and S309 MAbs**

To further elucidate the molecular mechanism underlying the immune escape of GX/P2V/2017 from SARS-CoV-2 MAb recognition, we solved the RBD-Fab complex structure of two MAbs engaging distinct epitopes (P2C-1F11 and S309), namely GX/P2V/2017 RBD-P2C-1F11 and GX/P2V/2017 RBD-S309 at a resolution of 2.8 and 3.3 Å, respectively (Tables S1–S4). Superimposition of the structure of GX/P2V/2017 RBD-P2C-1F11 onto that of the SARS-CoV-2 RBD bound to P2C-1F11 yielded a root-mean-square deviation (RMSD) of 0.822 Å (for 537 Ca atoms) (Figure 4A). The complex structure of GX/P2V/2017 RBD-S309 Fab was also similar to the SARS-CoV-2 RBD-S309 structure, with an RMSD of 0.999 Å (for 505 Ca atoms) (Figure 4D), indicating that the two phylogenetically related CoV RBDs have highly conserved structures and similar modes of interaction with the two neutralizing MAbs. P2C-1F11 shares similar GX/P2V/2017 RBD-human ACE2 (hACE2)-binding mimicry and spatial clashing with hACE2 (Figures 5A–5E).

In contrast, S309, which belongs to RBD-5, targeted the outer face of the GX/P2V/2017 RBD and bound away from the RRM (Figures 5A–5E).

Key residues contributing to the hydrogen bond and van der Waals (vdw) interaction between the GX/P2V/2017 RBD and the two neutralizing MAbs were also identified and labeled (Tables S1 and S2; Figures 4A–4F). Overall, 22 residues in the P2C-1F11 paratope interacted with the GX/P2V/2017 RBD, 18 of which were derived from the heavy chain (1 residue from HFR1, 7 residues from HCDR1, 4 residues from HCDR2, 1 residue from HCDR3, and 5 residues from HCDR3) and only 5 from the light chain (LCDR1) (Figure 4C). Furthermore, the S309 paratope was composed of five CDR loops and two FR loops, where HCDR3 and LCDR2 sandwiched the glycan of the GX/P2V/2017 RBD at position N343 (Figure 4D). Of the 24 paratope residues of S309 that interacted with the GX/P2V/2017 RBD, 17 were from the heavy chain (4 from HCDR1, 1 from HCDR2, and 12 from HCDR3) and 7 from the light chain (3 from LCDR1, 1 from LFR2, 1 from LFR2, and 2 from LFR3) (Figure 4F). The total interactions of the GX/P2V/2017 RBD with the Fabs of P2C-1F11 and S309 were 129 and 289, including 14 and 10 H-bonds, respectively (Tables S1 and S2).
| Antibodies | Affinity (nM) | RBDs | SARS-CoV-2 | GX/P2V/2017 |
|------------|--------------|------|------------|-------------|
| RBD-1      |              |      |            |             |
| RBD-2      |              |      |            |             |
| RBD-3      |              |      |            |             |
| RBD-4      |              |      |            |             |
| RBD-5      |              |      |            |             |
| RBD-6      |              |      |            |             |
| RBD-7      |              |      |            |             |
| BD-604     | <0.1         |      | 259.8±0.88 |             |
| CB6        | 24.94±1.15   |      |             |             |
| P2C-1F11   | 5.5±0.07     |      | 1795.7±169.06 |         |
| B38        | 167.8±6.9    |      |             |             |
| BD-236     | 4.92±0.04    |      |             |             |
| BD-629     | 0.22±0.03    |      | 171.7±7.45 |             |
| C102       | 33.2±0.33    |      |             |             |
| C105       | 12.8±0.33    |      |             |             |
| C1A-B3     | 30.8±0.52    |      |             |             |
| C1A-C2     | 7.7±0.38     |      |             |             |
| C1A-F10    | 9.2±0.38     |      |             |             |
| CC12.1     | 19.0±0.27    |      | 5887.0±125.45 |         |
| CC12.3     | 8.5±0.12     |      |             |             |
| COVA2-04   | 30.3±0.96    |      |             |             |
| CV30       | 5.8±0.07     |      |             |             |
| S2H14      | 110.2±5.3    |      |             |             |
| LY-COV555  | 2.2±0.3      |      |             |             |
| P2C-1A3    | 13.2±0.8     |      |             |             |
| REGN10933  | 1.2±0.0      |      |             |             |
| Ab23       | 1392.7±87.1  |      |             |             |
| COVA2-39   | 14.2±0.25    |      |             |             |
| C121       | 5.1±0.04     |      |             |             |
| C144       | 42.1±0.85    |      |             |             |
| H4         | 275.2±22.5   |      |             |             |
| S2E12      | 2.7±0.13     |      | 52.9±0.23 |             |
| S2M11      | 7±0.7        |      |             |             |
| 2-4        | 34.8±1.59    |      |             |             |
| ADI-56046  | 0.2±0.04     |      | 9130.9±936.77 |         |
| BD-368-2   | 9.0±0.13     |      |             |             |
| C002       | 57.1±0.96    |      |             |             |
| C104       | 60.0±3.04    |      |             |             |
| CV07-270   | 6.5±0.05     |      |             |             |
| P17        | 2.5±0.19     |      |             |             |
| P2B-2F6    | 122.3±2.5    |      |             |             |
| S2H13      | 461.4±30.16  |      |             |             |
| REGN10987  | 16.6±0.14    |      |             |             |
| S309       | <0.1         |      | 0.39±0.13 |             |
| C110       | 1.0±0.21     |      |             |             |
| C119       | 12.1±0.23    |      |             |             |
| C135       | 4.6±0.08     |      |             |             |
| 2H04       | 401.9±74.1   |      |             |             |
| 47D11      | 16.1±4.69    |      | 6.59±0.62 |             |
| S309       | <0.1         |      |             |             |
| C012       | 1.4±0.05     |      | 2.8±0.09 |             |
| 2-36       | 12.0±6.1     |      | 81.9±1.99 |             |
| CR3022     | 24.9±0.81    |      | 18.1±0.07 |             |
| EY6A       | 6.4±0.31     |      | 27.1±0.08 |             |
| H014       | 0.3±0.06     |      | 4.7±0.3  |             |
| S2A4       | 9.4±0.46     |      | 1051.6±17.28 |         |
| S304       | 3.9±0.05     |      | 24.4±0.1  |             |
GX/P2V/2017, respectively (Figure 4; Tables S1 and S2). Compared with the SARS-CoV-2 RBD, the GX/P2V/2017 RBD possessed four substitutions on the P2C-1F11 binding interface, namely R403K, K417V, N460K, and Q493E. Three substitutions (R403K, K417V, and Q493E) significantly decreased the H-bonds and vdW contacts, and another substitution (F486L) lost the interaction of L486 with P2C-1F11, which synchronically contributed to the weakening of the binding affinity (Figure 4A). Furthermore, six substitutions of the SARS-CoV-2 RBD and the GX/P2V/2017 RBD participated in the binding interface of S309, namely T345S, R346K, N440K, L441Q, K444L, and V445T (Figure 4D). The six substitutions increased multiple vdW and H-bond interactions with residues L110, I111, S30, S31, T32, S53, and S68 in the S309 paratope, resulting in a still-potent binding ability of the GX/P2V/2017 RBD to S309 (Figures 4E and 4F; Table S2).

**Cross-reactive immune response of SARS-CoV-2 to GX/P2V/2017**

The majority of MAbs that efficiently prevent SARS-CoV-2 infections analyzed in this study lost or greatly decreased their efficiencies against GX/P2V/2017. Therefore, we evaluated whether SARS-CoV-2 infection (convalescent serum) or COVID-19 vaccination (ZF2001 vaccinees) would stimulate cross-reactive immune responses against this pangolin-origin CoV. ZF2001, a recombinant tandem-repeat dimeric SARS-CoV-2 RBD-based protein subunit vaccine, has been widely used in China and several other countries. An enzyme-linked immunosorbent assay (ELISA) was developed to test the binding of the GX/P2V/2017 RBD to serum samples from COVID-19 convalescent donors and ZF2001 vaccinees. The SARS-CoV-2 antibodies in the sera of all convalescent donors and vaccinees could cross-react with the GX/P2V/2017 RBD with a high endpoint titer of 10^4–10^5, in which the GX/P2V/2017 RBD-specific antibody level in the convalescent serum was slightly higher than that of the SARS-CoV-2 RBD (Figure 6A). Furthermore, the cross-neutralizing activity of the serum samples from convalescent donors and vaccinees against SARS-CoV-2 and GX/P2V/2017 pseudoviruses was evaluated. The convalescent and vaccinee sera were significantly more effective in neutralizing the SARS-CoV-2 RBD virus than the GX/P2V/2017 RBD, indicating a lower cross-neutralizing antibody response to GX/P2V/2017 stimulated by SARS-CoV-2 infection or ZF2001 vaccination (Figures 6B and 6C). Notably, six of the 10 COVID-19 convalescent sera and two of the 12 vaccine recipient sera were below the neutralization titer detection line (Figures 6B–6D). In addition, we observed a similar 50% pseudovirus neutralization titer (pVNT50) change in sera from convalescent and vaccinees against the GX/P2V/2017 pseudovirus, with a 3- to 170-fold decrease, relative to SARS-CoV-2 pseudovirus (Figures 6B and 6C). Interestingly, although similar titers of cross-binding antibodies were observed in serum samples between convalescent patients and ZF2001 vaccinees, the cross-neutralizing activities against GX/P2V/2017 were slightly lower in samples of convalescent patients than those in vaccinees (Figure 6D).

**DISCUSSION**

Since the identification of SARS-CoV-2, the COVID-19 pandemic has been ongoing for more than 2 years, leading to serious global crises in public health, the economy, and the society. Various CoVs from bats and pangolins with high sequence homology to the SARS-CoV-2 have been identified, and some could use the same receptor as SARS-CoV-2 and SARS-CoV to enter human cells, thereby posing a great potential threat for public health and causing another epidemic or even pandemic. Thus, prospective studies and preparation of countermeasures against these CoVs are needed.

The two pangolin-origin CoVs, GD/1/2019 and GX/P2V/2017, represent potential threats. Our previous studies have revealed that the RBDs of these two pangolin-origin CoVs present a similar mode of interaction with the hACE2 receptor to SARS-CoV-2, which explains the similar binding affinities among the three CoV RBDs with the hACE2. Based on the phylogenetic similarities with the SARS-CoV-2, we asked whether the countermeasures developed against COVID-19 could be used to prevent and control a possible disease caused by the two pangolin-origin CoVs. Recent studies have reported that the SARS-CoV-2 infection induces strong cross-reactive antibodies to RaTG13, and a SARS-CoV-2 MAb, CB6, possessed a potent cross-reactive neutralizing activity against the RaTG13 pseudovirus. Here, we found that CB6 also binds to the GD/1/2019 RBD and efficiently prevents the transduction of pseudotyped GD/1/2019 into hACE2-expressing cells. However, CB6 had very limited effect, if any, to prevent GX/P2V/2017 infection. The reasons for the difference could be obvious, as the GD/1/2019 epitope has only one residue difference from SARS-CoV-2 RBD, whereas GX/P2V/2017 RBD has eight residue changes in the epitope for CB6 (Figure 1C).

A global consortium study defined and structurally illustrated seven SARS-CoV-2 RBD-directed antibody communities (RBD-1 to RBD-7) with distinct footprints and competition profiles. Accordingly, this study incorporated 50 MAbs divided into the seven antibody communities and found that all SARS-CoV-2 RBD-1, RBD-2, RBD-3, RBD-4, and RBD-5 MAbs lost or substantially decreased their binding strength to the GX/P2V/2017 RBD, with the exception of 47D11 in RBD-5, indicating a distinct antigenicity between RBDs from SARS-CoV-2 and GX/P2V/2017 at the epitopes covered by RBD-1 to RBD-5 MAbs. In contrast, most of the MAbs in RBD-6 and RBD-7 could bind to the GX/P2V/2017 RBD with similar or slightly decreased affinities compared with the SARS-CoV-2 RBD, which is due to the...
GX/P2V/2017 RBD containing only one substitution in the binding site of RBD-6 and RBD-7 MAbs but multiple substitutions in the binding sites of RBD-1 to RBD-5 (Figure S2).

Concerning the neutralizing activities, although RBD-6 and RBD-7 MAbs maintained strong interactions with the GX/P2V/2017 RBD, their potency to inhibit the entry of GX/P2V/2017 was low, similarly to the effects observed to prevent the infection of SARS-CoV-2. The most potent MAbs tested against GX/P2V/2017 were S2E12 (RBD-2) and S309 (RBD-5). At the polyclonal level, both ZF2001 vaccination and SARS-CoV-2 infection induced cross-binding of antibodies to the GX/P2V/2017 RBD with a titer similar to that of the SARS-CoV-2 RBD, while their neutralizing activities decreased by 1–2 logs, especially for the convalescent sera. Among the 10 donors included in this...
Figure 4. Complex structures of P2C-1F11 and S309 Fabs bound to the SARS-CoV-2 or GX/P2V/2017 RBD

(A) Overall structures of the SARS-CoV-2-RBD/P2C-1F11 and GX/P2V/2017 RBD/P2C-1F11 Fab complexes. The SARS-CoV-2 RBD is in gray and the GX/P2V/2017 RBD in orange. The heavy and light chains of P2C-1F11 Fab are indicated in cyan and magenta, respectively.

(B) Detailed interactions of the SARS-CoV-2 RBD with P2C-1F11 Fab.

(C) Detailed interactions of the GX/P2V/2017 RBD with P2C-1F11 Fab. Residues involved in the interaction are labeled, and H-bonds are shown as yellow dotted lines with a cutoff of 3.3 Å.

(D) Overall structure of SARS-CoV-2 RBD/S309 and GX/P2V/2017 RBD/S309 Fab complexes. The heavy and light chains of S309 Fab are indicated in hafnium or deep salmon, respectively.

(E) Detailed interactions of the SARS-CoV-2 RBD with S309 Fab.

(F) Detailed interactions of the GX/P2V/2017 RBD with S309 Fab. Residues involved in the interaction are labeled, and H-bonds are shown as yellow dotted lines with a cutoff of 3.5 Å.
Figure 5. Comparison of the antibody-binding sites of GX/P2V/2017 and SARS-CoV-2 RBDs
(A and B) Gray surface depiction of the RBD of the complex structures of RBDs bound to the Fabs. The Fabs of S309, P2C-1F11, and hACE2 are shown superimposed. S309 Fab is depicted as a cartoon with the heavy chain in hafnium and the light chain in deep salmon, P2C-1F11 Fab with the heavy chain in cyan and light chain in magenta, and hACE2 in green.
(C) Footprints of S309 Fab and hACE2 on the SARS-CoV-2 or GX/P2V/2017 RBD.
(D) Footprints of P2C-1F11 Fab and hACE2 on the SARS-CoV-2 or GX/P2V/2017 RBD.
(E) Sequence alignment of the RBD sequences from the SARS-CoV-2 and GX/P2V/2017. The blue and brown short lines indicate the binding sites of the SARS-CoV-2 and GX/P2V/2017 RBDs to human ACE2, respectively. Magenta triangles and ellipses indicate the binding sites of the SARS-CoV-2 and GX/P2V/2017 RBDs to MAb P2C-1F11, respectively. The orange triangles and ellipses indicate the binding sites of the SARS-CoV-2 and GX/P2V/2017 RBDs to MAb S309, respectively.
study, 6 showed undetectable inhibitory effects. Together with the discovery of the binding and neutralizing features of the seven RBD MAb communities on GX/P2V/2017, it is suggested that SARS-CoV-2 infection possibly induces more MAbs with lower neutralizing activities belonging to RBD-6 and RBD-7 than those of the other five communities with higher potencies. ZF2001, a protein subunit vaccine that uses the tandem-repeat SARS-CoV-2 RBD dimer as the antigen, blocked the epitopes of all MAbs tested from the RBD-7 group and part of the MAbs of the RBD-6 community. These results suggest that ZF2001 induces fewer MAbs targeting RBD-6 and RBD-7, which confer relatively less potency, but concentrates on the epitopes covering from RBD-1 to RBD-5, which contains the MAbs with higher potencies. Further studies are needed to explore this possibility, which would aid in the optimization of the immunogens.

Limitations of the study
Due to the limited availability of RBD-3 and RBD-6 when we set up the experiments, this study only contained one and three mAbs from either class, respectively. More MAb members in the two classes should be evaluated in further study for more accurate characterization of the immune difference of GX/P2V/2017. Furthermore, the pseudovirus system only represents the function of S protein, and other proteins of SARS-CoV-2/GX/P2V/2017 could also influence the infectivity and pathogenicity. The neutralizing assay against the authentic GX/P2V/2017 virus needs to be further studied.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:

Figure 6. Binding and neutralization activities of sera from ZF2001 vaccinees and COVID-19 convalescent donors against SARS-CoV-2 and GX/P2V/2017
(A) ELISA showing the immunoglobulin G (IgG) titers of sera from convalescent, ZF2001 vaccinees, and unvaccinated individuals against the SARS-CoV-2 or GX/P2V/2017 RBD. Data are presented as the mean ± SEM.

(B) Neutralization assay depicting the 50% pseudovirus neutralization titer (pVNT50) of twelve serum samples from ZF2001 vaccinees against SARS-CoV-2 or GX/P2V/2017 pseudovirus.

(C) Neutralization assay depicting the pVNT50 of ten serum samples from COVID-19 convalescent donors against SARS-CoV-2 or GX/P2V/2017 pseudovirus.

(D) Neutralization activities of serum samples from ZF2001 vaccinees and COVID-19 convalescent donors against GX/P2V/2017 pseudovirus. Results were obtained from at least two independent experiments (n = 2). Data are presented as the mean ± SEM. Statistical significance was calculated using two-tailed, unpaired Student’s t tests. Asterisks indicate p values: *p < 0.05, **p < 0.01, and ***p < 0.001.
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**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Anti-VSV-G antibody | I1- Hybridoma ATCC® | Cat#CRL2700 |
| goat anti-human IgG-HRP | OriGene | ZB-2304 |
| **Bacterial and virus strains** | | |
| Escherichia coli (E. coli) strain DH5a | TIANGEN | Cat# CB101-02 |
| **Biological samples** | | |
| ZF2001® vaccinees blood samples | N/A | N/A |
| SARS-CoV-2 prior infection donor blood samples | N/A | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| PEI | Alfa | A04043896-1g |
| SARS-CoV-2 RBD with his-tag, spike residues 319-541 | This paper | N/A |
| GX/P2V/2017 RBD with his-tag, spike residues 319-541 | This paper | N/A |
| GX/P2V/2017 spike ECD trimer-6P with his-tag, spike residues 1-1207 | This paper | N/A |
| GD/1/2019 RBD with his-tag, spike residues 319-541 | This paper | N/A |
| **Critical commercial assays** | | |
| HisTrap HP 5 mL column | GE Healthcare | Cat# 17524802 |
| HiLoad 16/600 Superdex 200 pg | GE Healthcare | Cat# 28989335 |
| Protein An HP 5 mL column | GE Healthcare | Cat#17040303 |
| Series S Sensor Chip Protein A | GE Healthcare | Cat#29127556 |
| Membrane concentrator | Millipore | UFC901096 |
| **Deposited data** | | |
| GX/P2V/2017 RBD complexed with the Fab fragment of P2C-1F11 | This paper | Protein DataBank: 7XNF |
| GX/P2V/2017 RBD complexed with the Fab fragment of S309 | This paper | Protein DataBank: 7XSW |
| **Experimental models: Cell lines** | | |
| HEK 293F cells | Gibco | Cat# 11625-019 |
| HEK 293T cells | ATCC | CRL-3216 |
| Vero cells | ATCC | CCL81 |
| **Recombinant DNA** | | |
| pCAGGS-MAbs | Huang et al.²⁹ | N/A |
| pCAGGS-VSV-ΔG-GFP | Li et al.³⁰ | N/A |
| pCAGGS-SARS-CoV-2 S | Niu et al.²¹ | N/A |
| pCAGGS-GX/P2V/2017 S | Niu et al.²¹ | N/A |
| pCAGGS-GX/P2V/2017 S ECD-6P | This paper | N/A |
| pCAGGS-GD/1/2019 S | Niu et al.²¹ | N/A |
| **Software and algorithms** | | |
| PyMOL software | Molecular Graphics System, Version 1.8 Schrödinger | https://pymol.org/2/ |
| BLAcore® 8K Evaluation software | GE Healthcare | N/A |
| HKL2000 software | Otwinowski and Minor³¹ | N/A |

(Continued on next page)
RESOURCE AVAILABILITY

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, George Fu Gao (gaof@im.ac.cn).

**Materials availability**
All unique/stable reagents generated in this study are available from the lead contact with a completed Materials Transfer Agreement.

**Data and code availability**
- The accession numbers for the atomic coordinates and diffraction data reported in this study are PDB: 7XNF (structure of the GX/P2V/2017 spike receptor-binding domain complexed with the Fab fragment of P2C-1F11) and 7XSW (structure of the GX/P2V/2017 spike receptor-binding domain complexed with the Fab fragment of S309).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

**Cells**
Human embryonic kidney (HEK) Expi293F cells (Gibco) were cultured at 37°C in SMM 293-TII expression medium with 5% CO2 in a shaking incubator (140 rpm). HEK 293T (ATCC CRL-3216) and Vero cells (ATCC CCL81) were cultured at 37°C in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 5% CO2.

**Human samples**
Serum samples of convalescents were provided by Ditan Hospital, Beijing, China. Serum samples of vaccinees were from volunteers who received the third immunization with the SARS-CoV-2 protein subunit vaccines, ZF2001. Studies were approved by the Ethics Committee of the Institute of Microbiology, Chinese Academy of Sciences (Project Number: SQIMCAS2021149). All candidates signed the written informed consent, and the information of the candidates is included in the Table S7.

METHOD DETAILS

**Gene cloning, protein expression, and purification**
The coding sequence of SARS-CoV-2 RBD (residues R319-F541, GISAID: EPI_ISL_402119), GX/P2V/2017 RBD (residues R319-F541, GISAID: EPI_ISL_410542), and GD/1/2019 RBD (residues R319-F541, GISAID: EPI_ISL_410721) was cloned into the pCAGGS vector with a C-terminal six-histidine tag using the EcoRI and BglII restriction sites. The variable regions of the 50 MAbs fused with the constant region of IgG1 were cloned into the pCAGGS vector. Gene sequence encoding GX/P2V/2017 spike ECD trimer (residues 1-1207) were synthesized with optimized codons and were cloned into pCAGGS vectors with a Strep-II tag and a His-tag. The "6P"-mutations (F814P, A889P, A896P, A939P, K983P, and V984P) were introduced to stabilize the profusion state. The RBDs of SARS-CoV-2, GX/P2V/2017, GD/1/2019 and GX/P2V/2017 spike ECD cloned in pCAGGS were expressed in Expi293F cells after plasmid transfection using the Sinofection Transfection Reagent (Sino Biological). Five days post-transfection, the culture supernatants were collected and filtered using a 0.22 μm filter. Soluble RBD proteins were purified with a 5 mL His-Trap HP column (GE Healthcare), and further purified by gel filtration using a HiLoad 16/600 Superdex 200 pg (GE Healthcare) with a buffer containing 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl. Soluble GX/P2V/2017 S trimer protein was purified by gel filtration using a Superose 6 Increase 10/300 GL column (GE Healthcare) with a same protein buffer as other protein.

The MAbs were expressed and purified from the culture supernatants of Expi293F cells using a Protein A affinity column (GE Healthcare), and further purified using HiLoad 16/600 Superdex 200 pg (GE Healthcare). Purified proteins were stored in a buffer containing 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl. The proteins used for ELISA and neutralization assays were transferred to...
phosphate-buffered saline (PBS). Proteins used for the SPR assay were transferred into a PBST solution containing 1.8 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$ (pH 7.4), 137 mM NaCl, 2.7 mM KCl, and 0.005% (v/v) Tween 20. Fabs were generated by papain digestion and further purified using a Protein A column and gel filtration using a Superdex$^\text{TM}$ 200 10/300 GL column.

**SPR analysis**

SPR-based measurements were performed by BIAcore8000 system (GE Healthcare) with protein A chip (GE Healthcare) at 25°C in single-cycle mode. All proteins used for the kinetic analyses were exchanged to PBST. The concentrated supernatant containing MAbs was captured on the protein A chip at more than 500 response units. Gradient concentrations of the RBD proteins were run across flow cell 2 of the chip, with flow cell 1 set as the control. After each cycle, the sensor chip was regenerated using glycine (pH 1.5). For spike trimmer-Fabs binding assay, GX/P2V/2017 S trimmer protein was biotinylated and immobilized on an SA chip to approximately 2,000 response units. Gradient concentrations of 11 Fab proteins were run across flow cell 2 of the chip, with flow cell 1 set as the control. After each cycle, the sensor chip was regenerated using glycine (pH 2.5). Binding kinetics were analyzed with the Biacore$^\text{TM}$ Insight software (GE Healthcare) using a 1:1 Langmuir binding model. These results were visualized using Origin 2021.

**ELISA**

ELISA plates were coated overnight at 4°C with 200 ng per well of SARS-CoV-2 RBD or GX/P2V/2017 RBD protein in 0.05 M carbonate-bicarbonate buffer (pH 9.6) and blocked with 5% skimmed milk in PBS. The serum was serially diluted and added to each well. After incubation at 37°C for 1 h, the plates were washed three times with 0.05% Tween 20 in PBS and incubated with goat anti-human IgG-HRP antibody (Thermo Fisher Scientific) for 1 h. After intensive washing, 3,3',5,5'-tetramethylebenzidine (TMB) substrate was added to each well. The reaction was stopped with 2 M hydrochloric acid and the absorbance was measured at 450 nm using a microplate reader (PerkinElmer, USA). The endpoint titer was defined as the highest reciprocal dilution of serum to give an absorbance greater than 2.1-fold of the background values. The antibody titers below the limit of detection were determined as half of the limit of detection.

**Production of pseudoviruses**

SARS-CoV-2, GX/P2V/2017 and GD/1/2019 pseudoviruses were constructed with a GFP-encoding replication-deficient vesicular stomatitis virus (VSV) vector backbone (VSV-ΔG-GFP) and the coding sequence of the corresponding spike proteins, as previously described. Briefly, HEK-293T cells were transfected with 30 μg of spike protein expression plasmids and the VSV-ΔG-GFP pseudovirus was added 24 h later. The inoculum was removed after incubating for 1 h at 37°C. After washing the cells with PBS, the culture medium was changed into DMEM supplemented with 10% FBS and 10 μg/mL of anti-VSV-G antibody (I1Hybridoma ATCC® CRL2700™). The pseudoviruses were harvested 20 h post inoculation, passed through a 0.45 μm filter (Millipore, Cat#SLHP033RB), aliquoted and stored at −80°C.

**Neutralization assay**

For the neutralization assay, 1 × 10$^4$ of Vero cells were plated in each well of a 96-well plate 24 h before infection. Four-fold serial dilutions of the MAbs or blood supernatants were incubated with an equal volume of supernatant containing 1,000 fluorescence focus units (FFU) of pseudovirus for 1 h at 37°C. The mixture was then added to the Vero cells in triplicate. Fifteen hours later, the number of infected cells was measured using a CQ1 Confocal Quantitative Image Cytometer (Yokogawa). IC$_{50}$ values were calculated using GraphPad Prism 7.0.

**Crystal screening and structure determination**

The complexes of GX/P2V/2017 RBD with either the P2C-1F11 Fab or the S309 Fab were crystallized using the vapor-diffusion sitting-drop method with 0.8 μL of protein mixing with 0.8 μL of reservoir solution at 18°C. A high-resolution crystal of the complex of GX/P2V/2017 RBD with P2C-1F11 Fab was obtained in a solution containing 0.1 M MES monohydrate (pH 6.0) and 20% w/v polyethylene glycol monomethyl ether 2,000, with a protein concentration of 10 mg/mL. The crystal of the complex of GX/P2V/2017 RBD with S309 Fab was obtained in a solution containing 0.2 M magnesium chloride hexahydrate, 0.1 M MES (pH 6.0), and 20% w/v PEG 6000, with a protein concentration of 10 mg/mL. Crystals were frozen with crystallization solution containing 20% glycerol. Diffraction data were collected at Shanghai Synchrotron Radiation Facility (SSRF) BL19U. Data were processed with the HKL2000 software. The complex structures of GX/P2V/2017 RBD with the Fabs were determined by the molecular replacement method using Phaser, using previously reported SARS-CoV-2 RBD/Fab complex structures (PDB: 7CD1 and 7R6X). The atomic models were built using Coot and refined using Phenix, and the stereochemical qualities of the final models were assessed using MolProbity. Data collection, processing, and refinement statistics are summarized in Tables S3 and S4. All structural figures were generated using Pymol software (https://pymol.org/2/).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Binding affinity analysis**

$K_a$, $K_d$ and $K_D$ values of SPR experiments were obtained with BlAcore 8K Evaluation Software (GE Healthcare), using a 1:1 binding model. The values indicate the mean ± SD of three replicated experiments.
Pseudovirus neutralization assays
Each group contained three replicates. And data are presented as the mean ± SD of three independent assays. Statistical significance was calculated using two-tailed, unpaired Student’s t-tests. Asterisks indicate p values: * (p < 0.05), ** (p < 0.01), and *** (p < 0.001). Analyses were performed with GraphPad Prism 9 software.
Supplemental information

Cross-reaction of current available SARS-CoV-2 MAbs against the pangolin-origin coronavirus GX/P2V/2017

Yunfei Jia, Sheng Niu, Yu Hu, Yan Chai, Anqi Zheng, Chao Su, Lili Wu, Pengcheng Han, Pu Han, Dan Lu, Zhimin Liu, Xinxin Yan, Di Tian, Zhihai Chen, Jianxun Qi, Wen-xia Tian, Qihui Wang, and George Fu Gao
Supplemental information

Figure S1
Figure S2

|     | Outer face | Inner face | RDMS |
|-----|------------|------------|------|
| RBD | 330 340 345 360 370 375 380 385 400 417 445 448 453 462 490 497 500 503 506 509 512 515 530 535 540 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 | 330 340 345 360 370 375 380 385 400 417 445 448 453 462 490 497 500 503 506 509 512 515 530 535 540 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 | 330 340 345 360 370 375 380 385 400 417 445 448 453 462 490 497 500 503 506 509 512 515 530 535 540 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 | 330 340 345 360 370 375 380 385 400 417 445 448 453 462 490 497 500 503 506 509 512 515 530 535 540 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 |
| Prototype | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY |
| GD2/12012 | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY |
| Beta      | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY |
| Omennna   | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY |
| Dalia     | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY | GTRNNLSKNYSASSSTDRKTVGLNSTVEFFQSGONGY |
| SA.1      | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH |
| SA.2      | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH |
| SA.3      | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH |
| SA.xBA.5  | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH | DTRRNKLSPNYLAFPTDNRKTVGLNKVAPFRSRYGGH |
| GA/F201195 | GSSALGSSSSTDSAVSNLSKONLY | GSSALGSSSSTDSAVSNLSKONLY | GSSALGSSSSTDSAVSNLSKONLY |
Figure S3

A

Raw curve for the indicated GX/P2V/2017 Spike to Fabs
Fitted curve for the indicated GX/P2V/2017 Spike to Fabs

B

| Affinity (nM) | GX/P2V/2017-ABD | GX/P2V/2017-5A |
|--------------|-----------------|-----------------|
| RBC-1        | 233.8100.86     | 591.11137.39   |
| RBC-2        | 179.76166.09    | 1070.46542.42  |
| RBC-3        | 171.735.45      | 416.265.53     |
| RBC-4        | 52.040.23       | 247.735.48     |
| RBD-1        | 9130.040.067.7  | 4911.040.123.45 |
| RBD-2        | 0.0920.13       | 0.8340.43      |
| RBD-3        | 6.900.52        | 4.024.29       |
| RBD-4        | 61.156.31       | 4814.04       |
| RBD-5        | 2.510.97        | 2.721.01       |
| RBD-6        | 81.984.39       | 143.764.24     |
| RBD-7        | 26.330.07       | 20.772.52      |
| EYWA         | 27.140.00       | 15.971.07      |
| G2498        | 24.470.01       | 19.291.16      |

Affinity not determined >1000 10-1000 1-10 <1
**Figure S1.** Protein expression and purification of RBDs of SARS-CoV-2, GX/P2V/2017 and GD/1/2019, Related to Figure 1.

(A-C) Absorbance curves at 280 nm of the gel filtration profiles of the SARS-CoV-2 RBD protein (A), GX/P2V/2017 RBD protein (B) and GD/1/2019 RBD protein (C) with HiLoad 16/600 Superdex 200 pg. (D) SDS-PAGE migration profiles of RBD-monomer proteins under non-reducing conditions.

**Figure S2.** Amino acid mutation mapping of RBDs from SARS-CoV-2 prototype, the variants and two pangolin-origin CoVs, Related to Figure 2.

Three major epitopes on SARS-CoV-2 RBD targeted by seven classes of MAbs (RBD-1–RBD-7), and residue mutation mapping of RBDs from different CoVs.

**Figure S3.** SPR characterization of the binding affinity of GX/P2V/2017 trimer spike protein to the Fabs of 11 MAbs, Related to Figure 2.

(A) The raw and fitted curves are shown as dotted and solid lines, respectively. (B) The binding affinity between GX/P2V/2017 trimer spike protein and the Fabs of 11 MAbs is shown. Mean ± SD represents the mean and standard deviation of three independent experiments.

**Figure S4.** Binding curves of 50 mAbs to RBDs from SARS-CoV-2 prototype and GX/P2V/2017, and the binding curves of 11 Fabs to GX/P2V/2017 trimer spike protein, Related to Figure 2.

The raw and fitted curves are shown as dotted and solid lines, respectively. One representative data are shown of three independent experiments.
Table S1. Comparison of P2C-1F11 Fab binding to SARS-CoV-2 RBD and GX/P2V/2017 RBD, Related to Figure 4.

| Chain | P2C-1F11 | GX/P2V/2017 RBD | SARS-CoV-2 RBD |
|-------|----------|----------------|----------------|
| Heavy |          |                |                |
| V2    | N487 (1) |                |                |
| G26   | G476 (2), S477 (2) | N487 (2, 1) |                |
| I27   | A475 (3), G476 (1), N487 (2) | A475 (1), N487 (3) |                |
| T28   | A475 (6), G476 (3) | A475 (4), G476 (1), S477 (1) |                |
| S30   | K458 (3, 1) |                |                |
| S31   | K458 (3, 1), Y473 (6, 1), Q474 (1) | K458 (1), Y473 (6, 1), Q474 (1) |                |
| N32   | A475 (6, 1) | A475 (5, 1) |                |
| Y33   | Y421 (2), L455 (5, 1) | Y421 (1), L455 (6, 1), F456 (2) |                |
| Y52   | G416 (2), V417 (4), D420 (1), Y421 (3) | G416 (1), K417 (7, 1), D420 (2), Y421 (2) |                |
|       | Y421 (4), R457 (3, 1), K458 (5, 1), Y473 (1) | Y421 (4), R457 (3, 1), K458 (6, 1), Y473 (2) |                |
|       | G54      | Y421 (2, 1), K460 (5) | Y421 (2, 1), K458 (2), S459 (1), N460 (5) |
|       | S56      | T415 (2), D420 (4, 1) | T415 (2), D420 (4, 1) |
|       | Y58      | T415 (3, 1), G416 (2) | T415 (5, 1), G416 (2) |
|       | R97      | N487 (2, 1), Y489 (4, 1) | A475 (1), F486 (3), N487 (4, 1), Y489 (2, 1) |
|       | L99      | Y489 (4) | F486 (1), Y489 (4) |
|       | V100     | L455 (3) |                |
|       | V101     | Y453 (1) | L455 (1) |
|       | Y102     | E493 (7, 1) | Q493 (4, 1) |
|       | D105     | F486 (2) |                |
| Light |          |                |                |
| I2    | Y505 (1) |                |                |
| S28   | Y505 (5, 1) |                | G502 (2), Y505 (2) |
| V29   | Y505 (8) |                | Y505 (1) |
| S30   | Y505 (4) |                | Y505 (4) |
| Y33   | K403 (1), Y453 (1) |                | R403 (3, 1), Y453 (1, 1) |
| Q91   | Y505 (1) |                |                |
| Y92   | Y505 (1) |                |                |
| Total | 129, 14  | 123, 15        |                |

The numbers without underlining in parentheses of GX/P2V/2017 RBD and SARS-CoV-2 RBD residues represent the number of vdw contacts between the indicated RBD residues and P2C-1F11 Fab. Underlined numbers indicate the number of potential H-bonds between pairs of residues. The vdw contacts were analyzed at a cutoff of 4.0 Å and H-bonds at a cutoff of 3.3 Å.


Table S2. Comparison of S309 Fab binding to SARS-CoV-2 RBD and GX/P2V/2017 RBD, Related to Figure 4.

| Chain | S309 | GX/P2V/2017 RBD | SARS-CoV-2 RBD |
|-------|------|----------------|---------------|
| Heavy |      |                |               |
| G26   | N331 (2) |                |               |
| Y27   | N331 (3) |                |               |
| P28   | L335 (4) | I332 (6), L335 (3) |               |
| T30   | N334 (4) | T333 (1), N334 (1) |               |
| S31   | L335 (5) | L335 (6, D), E340 (1) |               |
| R32   | FUC801 (4) |                |               |
| Y54   | N334 (4) |                |               |
| R98   | FUC801 (1) |                |               |
| Y100  | G339 (3), N343 (10, D), NAG601 (7), FUC801 (15) | G339 (6), E340 (1), N343 (8, D), NAG601 (12) |
| G103  | E340 (6) | E340 (1) |               |
| A104  | E340 (6) | E340 (10) |               |
| W105  | N334 (5), L335 (3), P337 (20), E340 (13, D), S359 (2), N360 (3), C361 (3) | L335 (3), P337 (22), E340 (15, D), S359 (2), C361 (3) |               |
| F106  | P337 (6), E340 (12), V341 (1), K356 (17), R357 (2), I358 (4) | P337 (4), E340 (11), V341 (1), K356 (17), R357 (2), I358 (1) |               |
| G107  | E340 (1) | E340 (1) |               |
| E108  | A344 (1), K356 (5, D) | N354(1), K356 (6, D) |               |
| S109  | A344 (3), S345 (13, D) | N343 (1), A344 (3), T345 (17, D) |               |
| L110  | E340 (7), N343 (5), A344 (4), S345 (15) | E340 (4), N343 (6), A344 (3), T345 (9) |               |
| I111  | N343 (8), A344 (4), S345 (7), Q441 (2), R509 (1) | N343 (8), A344 (4), T345 (9), L441 (1), R509 (1) |               |
| D115  | FUC801 (1) |                |               |
| S30   | K346 (6, D) |                |               |
| S31   | K440 (2, D), L444 (1) |                |               |
| T32   | S345 (9), Q441 (4) | T345 (5), L441 (4) |               |
| Light |      |                |               |
| Y50   | NAG601 (5), NAG701 (1), FUC801 (10, 1) | NAG601 (4) |               |
| S53   | K440 (4) |                |               |
| T57   | NAG701 (2), FUC801 (5, 1) |                |               |
| S68   | T445 (3, 1) |                |               |
| D93   | R346 (1) |                |               |
| Total | 289, 10 | 240, 7 |               |

The numbers without underlining in parentheses of GX/P2V/2017 RBD and SARS-CoV-2 RBD residues represent the number of vdw contacts between the indicated RBD residues and S309 Fab. Underlined numbers indicate the number of potential H-bonds.
between pairs of residues. The vdw contacts were analyzed at a cutoff of 4.5 Å and H-bonds at a cutoff of 3.5 Å.
Table S3. Crystallographic data collection and refinement statistics of the complex

GX/P2V/2017 RBD-P2C-1F11 Fab, Related to Figure 4.

| Data collection | GX/P2V/2017 RBD-P2C-1F11 Fab |
|-----------------|-------------------------------|
| **Space group** | P2₁2₁2₁                       |
| **Cell dimensions** |                               |
| a, b, c (Å)   | 55.722, 85.440, 183.739       |
| α, β, γ (°)   | 90.000, 90.000, 90.000        |
| **Wavelength (Å)** | 0.97930                     |
| **Resolution (Å)** | 50.00–2.80 (2.90–2.80)       |
| **Unique reflections** | 22549 (2186)                |
| Rmerge        | 0.153 (0.925)                |
| I/σI          | 11.905 (1.692)               |
| Completeness (%) | 99.8 (99.8)                 |
| Redundancy    | 6.6 (6.3)                    |
| **Refinement** |                               |
| Resolution (Å) | 41.61–2.79                   |
| No. of reflections | 21432                      |
| Rwork/Rfree   | 0.3209/0.3839                |
| **Number of Atoms** |                               |
| Protein       | 4446                         |
| Ligand/ion    | 0                            |
| Water         | 19                           |
| **B-factors** |                               |
| Protein       | 43.34                        |
| Ligand/ion    | 23.74                        |
| Water         |                               |
| **RMSDs**     |                               |
| Bond length (Å) | 0.002                       |
| Bond angles (°) | 0.501                       |
| **Ramachandran Statistics (%)** |                 |
| Most favored (%) | 96.80                       |
| Allowed (%)    | 3.20                         |
| Disallowed (%) | 0                            |
# Table S4. Crystallographic data collection and refinement statistics of the complex GX/P2V/2017 RBD-S309 Fab, Related to Figure 4.

| Data collection | GX/P2V/2017 RBD-S309 Fab |
|-----------------|--------------------------|
| Space group     | P₁                       |
| Cell dimensions |                          |
| a, b, c (Å)     | 54.542, 73.597, 105.359  |
| α, β, γ (°)     | 110.618, 92.175, 97.198  |
| Wavelength (Å)  | 0.978530                 |
| Resolution (Å)  | 50.00–3.30 (3.50–3.30)   |
| Unique reflections | 40975 (6611)               |
| Rmeans          | 3.421–3.303               |
| I/σI            | 1.9 (0.7)                 |
| Completeness (%)| 89.0 (89.2)               |
| Redundancy      | 4.94                     |
| Refinement      |                          |
| Resolution (Å)  | 48.54–3.30               |
| No. of reflections | 21703                   |
| Rwork/Rfree     | 0.2766/0.3216             |
| Number of Atoms |                          |
| Protein         | 9593                     |
| Ligand/ion      | 0                        |
| Water           | 0                        |
| B-factors       |                          |
| Protein         | 75                       |
| Ligand/ion      | 0                        |
| Water           | 0                        |
| RMSDs           |                          |
| Bond length (Å) | 0.002                    |
| Bond angles (°) | 0.543                    |
| Ramachandran Statistics (%) |          |
| Most favored (%)| 96.22                    |
| Allowed (%)     | 3.78                     |
| Disallowed (%)  | 0                        |
Table S7. The information of donors, Related to Figure 6.

| Donors | Gender | Age | Development stage  |
|--------|--------|-----|--------------------|
| 1      | Male   | 43  | Healthy            |
| 2      | Female | 40  | Healthy            |
| 3      | Male   | 34  | Healthy            |
| 4      | Female | 23  | Convalescent       |
| 5      | Male   | 42  | Convalescent       |
| 6      | Male   | 36  | Convalescent       |
| 7      | Male   | 45  | Convalescent       |
| 8      | Male   | 39  | Convalescent       |
| 9      | Female | 47  | Convalescent       |
| 10     | Female | 61  | Convalescent       |
| 11     | Female | 59  | Convalescent       |
| 12     | Female | 50  | Convalescent       |
| 13     | Male   | 49  | Convalescent       |
| 14     | Female | 29  | ZF2001 vaccinees   |
| 15     | Female | 28  | ZF2001 vaccinees   |
| 16     | Male   | 27  | ZF2001 vaccinees   |
| 17     | Male   | 30  | ZF2001 vaccinees   |
| 18     | Female | 30  | ZF2001 vaccinees   |
| 19     | Female | 38  | ZF2001 vaccinees   |
| 20     | Male   | 23  | ZF2001 vaccinees   |
| 21     | Female | 31  | ZF2001 vaccinees   |
| 22     | Male   | 27  | ZF2001 vaccinees   |
| 23     | Female | 26  | ZF2001 vaccinees   |
| 24     | Male   | 41  | ZF2001 vaccinees   |
| 25     | Female | 52  | ZF2001 vaccinees   |