Supplemental information

B cell genomics behind cross-neutralization of SARS-CoV-2 variants and SARS-CoV

<table>
<thead>
<tr>
<th>Subject</th>
<th>Name used in Raw Sequence Files</th>
<th>Gender</th>
<th>Age</th>
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<th>Days Between Test and Blood Draw</th>
<th>Symptoms</th>
<th>Serum ID50</th>
<th>Serum ID80</th>
<th>RBD ELISA</th>
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<td>DON_1</td>
<td>M</td>
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<td>48</td>
<td>fever, malaise, chest tightness, loss of smell</td>
<td>655</td>
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<td>IgG+/IgM+</td>
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**Table S1.** Subject Characteristics, Related to Figure 1 and Figure S1. Shown are the subject IDs, gender, age at time of diagnosis (in years), result of clinical COVID-19 PCR test result, days between the COVID test and blood draw for this study, clinical symptoms at presentation, serum ID50 and ID80 values in a SARS-CoV-2 pseudovirus assay and results in serum ELISA against SARS-CoV-2 RBD. Subjects highlighted in red font were selected for mAb production; n/a = not available.
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<th>ISOTYPE</th>
<th>VH GENE</th>
<th>CDRH3 HEAVY CHAIN</th>
<th>LIGHT CHAIN</th>
<th>VK/VL GENE</th>
<th>CDRH3 LIGHT CHAIN</th>
<th>SORT BAIT Name</th>
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<th>PV IC50 (µg/ml)</th>
<th>AV IC50 (µg/ml)</th>
<th>AV IC50 (µg/ml)</th>
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<td>&gt;50</td>
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<td>&gt;50</td>
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<td>&gt;50</td>
<td>&gt;50</td>
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</table>

Table S2. Selected mAbs for functional evaluation, Related to Figure 2 and Figure S2. Shown are isotype, VH gene, CDRH3 amino acid sequence, light chain kappa or lambda light chain use, V kappa/V lambda gene, light chain CDR3 amino acid sequence, sorting bait used to identify the cell, mAb name, IC50 in µg/ml in SARS-CoV-2 pseudovirus neutralization assay (PV), IC80 in µg/ml in SARS-CoV-2 pseudovirus neutralization assay, IC50 in µg/ml in SARS-CoV-2 authentic virus neutralization assay (AV), IC80 in µg/ml in SARS-CoV-2 authentic virus neutralization assay for mAbs from subject BG1, BG4, BG7 and BG10 as indicated by the mAb names. All neutralization results in this table are from mAbs expressed as IgG1.
Table S3. mAb Binding to SARS-CoV-2 S trimer and RBD, related to Figures 2, 4 and Figure S2.

Heat map summarizing the area under the curve (AUC) values from ELISAs testing for binding to SARS-CoV-2, SARS-CoV and MERS-CoV RBD and S trimer respectively. Shown are also mAb polyreactivity as defined by binding to at least two of the four antigens ssDNA, dsDNA, LPS or insulin (STAR Methods), mAb reactivity in a baculovirus (BV) lysate assay (STAR Methods), SARS-CoV-2 neutralizer status, TC and isotype of the cell of origin and the number of clonal members for mAbs from BG1, BG4, BG7 and BG10. Red fields indicate high binding in ELISA (AUC > 1), orange fields intermediate binding (AUC 0.25-1) and white fields low or non-detectable binding (AUC < 0.25). (P) indicates polyreactive mAbs, (NP) indicates non-polyreactive mAbs, (X) indicates presence and (0) absence of neutralizing activity, (mA) indicates monomeric IgA, (dA) indicates dimeric IgA, (G) indicates non-polyreactive mAbs, (X) indicates presence and (0) absence of neutralizing activity in BV lysate assay or neutralizing activity in SARS-CoV-2 pseudovirus assay as indicated. When neutralizing activity was detected, the neutralizing form of the mAb (IgG, monomeric IgA or dimeric IgA (dA)) is indicated (Figure S2J, Table S2).

mGO53 = negative control mAb (Wardemann et al., 2003), 3B12 = positive control mAb for MERS-CoV S and MERS-CoV RBD (Tang et al., 2014), S227.14 = positive control mAb for SARS-CoV S and SARS-CoV RBD (Rockx et al., 2008), NIH45-46 (Scheid et al., 2011) and 45-46m2 (Diskin et al., 2013) are positive control mAbs and 3BC117 (Scheid et al., 2011) a negative control mAb for the baculovirus lysate assay (STAR Methods).

<table>
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<tr>
<th>NAME</th>
<th>CoV2 RBD</th>
<th>CoV2 S</th>
<th>CoV RBD</th>
<th>SARS-CoV</th>
<th>MERS S</th>
<th>Polyreactive</th>
<th>BV Lysate</th>
<th>Neutralizer</th>
<th>Cluster</th>
<th>Isotype</th>
<th># Mem.</th>
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<td>0.189</td>
<td>0.143</td>
<td>0.112</td>
<td>0.097</td>
<td>0.076</td>
<td>X (mA, dA)</td>
<td>0.209</td>
<td>0.162</td>
<td>NP</td>
<td>0</td>
<td>0</td>
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<tr>
<td>BG4</td>
<td>0.194</td>
<td>0.117</td>
<td>0.117</td>
<td>0.089</td>
<td>0.064</td>
<td>X (G)</td>
<td>0.177</td>
<td>0.134</td>
<td>NP</td>
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<td>BG4-1</td>
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<td>0.094</td>
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<td>X (mA, dA)</td>
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<td>BG4-4</td>
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<td>0.106</td>
<td>X (mGO53)</td>
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<td>0.162</td>
<td>NP</td>
<td>0</td>
<td>0</td>
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<tr>
<td>BG4-7</td>
<td>0.114</td>
<td>0.068</td>
<td>0.152</td>
<td>0.080</td>
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<td>X (mGO53)</td>
<td>0.120</td>
<td>0.091</td>
<td>NP</td>
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<td>0</td>
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<tr>
<td>BG4-14</td>
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<td>X (mGO53)</td>
<td>0.177</td>
<td>0.134</td>
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<td>0.053</td>
<td>X (mGO53)</td>
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<td>X (mGO53)</td>
<td>0.173</td>
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<td>X (mGO53)</td>
<td>0.177</td>
<td>0.134</td>
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3B12: mAb neutralization in BV lysate assay (AUC > 1), orange fields intermediate binding (AUC 0.25-1) and white fields low or non-detectable binding (AUC < 0.25). (P) indicates polyreactive mAbs, (NP) indicates non-polyreactive mAbs, (X) indicates presence and (0) absence of neutralizing activity in BV lysate assay or neutralizing activity in SARS-CoV-2 pseudovirus assay as indicated. When neutralizing activity was detected, the neutralizing form of the mAb (IgG, monomeric IgA or dimeric IgA (dA)) is indicated (Figure S2J, Table S2). mGO53 = negative control mAb (Wardemann et al., 2003), 3B12 = positive control mAb for MERS-CoV S and MERS-CoV RBD (Tang et al., 2014), S227.14 = positive control mAb for SARS-CoV S and SARS-CoV RBD (Rockx et al., 2008), NIH45-46 (Scheid et al., 2011) and 45-46m2 (Diskin et al., 2013) are positive control mAbs and 3BC117 (Scheid et al., 2011) a negative control mAb for the baculovirus lysate assay (STAR Methods).
For each of the 6 transcription factor clusters, the top 50 genes that are differentially upregulated are displayed. Genes are included only if their minimum log fold change is greater than 0.25 with a false discovery rate (FDR) < 0.05. Columns show the gene name, transcription factor cluster, average log fold change (avg_logFC) and BH adjusted P values (p_val_adj) respectively.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cluster</th>
<th>avg_logFC</th>
<th>p_val_adj</th>
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<td>EMP3</td>
<td>0</td>
<td>0.742674423</td>
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<td>CEB1</td>
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<td>0.704189386</td>
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<td>LAGS1</td>
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<td>0.648771538</td>
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Table S4. Transcription cluster marker genes, Related to Figure 4 and Figure S4. For each of the 6 transcription factor clusters the top 50 genes that are differentially upregulated are displayed. Genes are included only if their minimum log fold change is greater than 0.25 with a false discovery rate (FDR) < 0.05. Columns show the gene name, transcription factor cluster, average log fold change (avg_logFC) and BH adjusted P values (p_val_adj) respectively.
Table S5. Cryo-EM data collection and refinement statistics, Related to Figures 3, 5, and 6.
### Table S6. X-ray data collection and refinement statistics, Related to Figure 5.

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#### Data collection\(^a\)

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<tr>
<td>Redundancy</td>
<td>6.1 (4.2)</td>
</tr>
<tr>
<td>CC(_{1/2}) (%)</td>
<td>97.2 (25.9)</td>
</tr>
<tr>
<td>(&lt;I/\sigma I&gt;)</td>
<td>4.0 (0.7)</td>
</tr>
<tr>
<td>Mosaicity (°)</td>
<td>0.23</td>
</tr>
<tr>
<td>(R_{merge}) (%)</td>
<td>27.1 (135.8)</td>
</tr>
<tr>
<td>(R_{pim}) (%)</td>
<td>12.5 (88.6)</td>
</tr>
<tr>
<td>Wilson (B)-factor</td>
<td>60.9</td>
</tr>
</tbody>
</table>

#### Refinement and Validation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (Å)</td>
<td>38.8 - 3.1</td>
</tr>
<tr>
<td>Number of atoms</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>8,183</td>
</tr>
<tr>
<td>Ligand</td>
<td>60</td>
</tr>
<tr>
<td>Waters</td>
<td>0</td>
</tr>
<tr>
<td>(R_{work}/R_{free}) (%)</td>
<td>21.5/26.2</td>
</tr>
<tr>
<td>R.m.s. deviations</td>
<td></td>
</tr>
<tr>
<td>Bond lengths (Å)</td>
<td>0.01</td>
</tr>
<tr>
<td>Bond angles (°)</td>
<td>1.6</td>
</tr>
<tr>
<td>MolProbity score</td>
<td>1.89</td>
</tr>
<tr>
<td>Clashscore (all atom)</td>
<td>18.9</td>
</tr>
<tr>
<td>Poor rotamers (%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Ramachandran plot</td>
<td></td>
</tr>
<tr>
<td>Favored (%)</td>
<td>94.7</td>
</tr>
<tr>
<td>Allowed (%)</td>
<td>5.3</td>
</tr>
<tr>
<td>Disallowed (%)</td>
<td>0</td>
</tr>
<tr>
<td>Average (B)-factor (Å)</td>
<td>73.5</td>
</tr>
</tbody>
</table>

\(^a\)Numbers in parentheses correspond to the highest resolution shell
Data S1 (includes this page and the following 15 pages), Related to Figure 4 and Tables S2 and S3. UMAP plots displaying clonal members of all validated mAbs (Tables S2 and S3). Each plot represents where the tested mAb along with its clonally related cells are mapped on the transcriptome landscape.

Clones of the tested antibodies

Potently Neutralizing Antibodies

![UMAP plots](image-url)
Low Neutralizing Antibodies

Antibody BG1-6 of donor DON_1

Antibody BG1-12 of donor DON_1

Antibody BG1-15 of donor DON_1

Antibody BG1-17 of donor DON_1

Antibody BG1-26 of donor DON_1

Antibody BG1-27 of donor DON_1
Non-Neutralizing Binding Antibodies
Non-Neutralizing Low-Binding Antibodies
Data S1, Related to Figure 4 and Tables S2 and S3. UMAP plots displaying clonal members of all validated mAbs (Tables S2 and S3). Each plot represents where the tested mAb along with its clonally related cells are mapped on the transcriptome landscape.