Supplementary Materials for

Seasonal coronavirus-specific B-cells with limited SARS-CoV-2 cross-reactivity dominate the IgG response in severe COVID-19 patients

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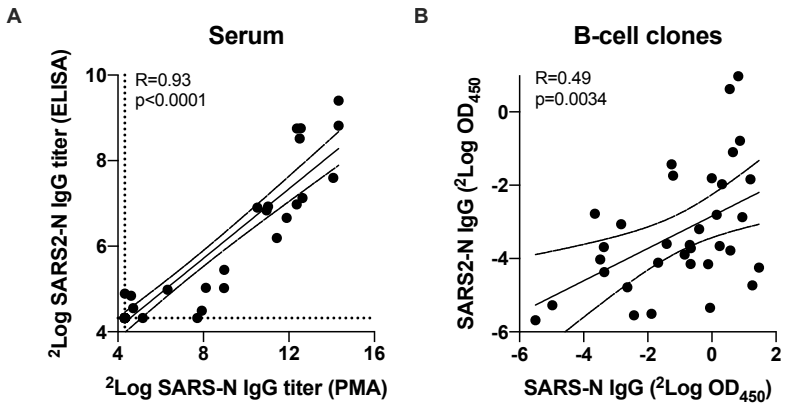
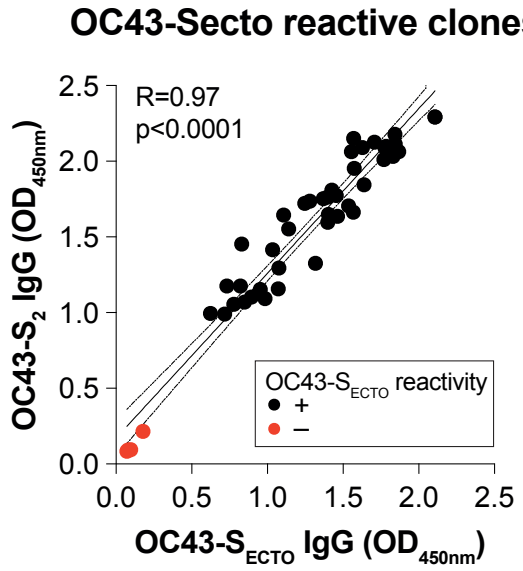
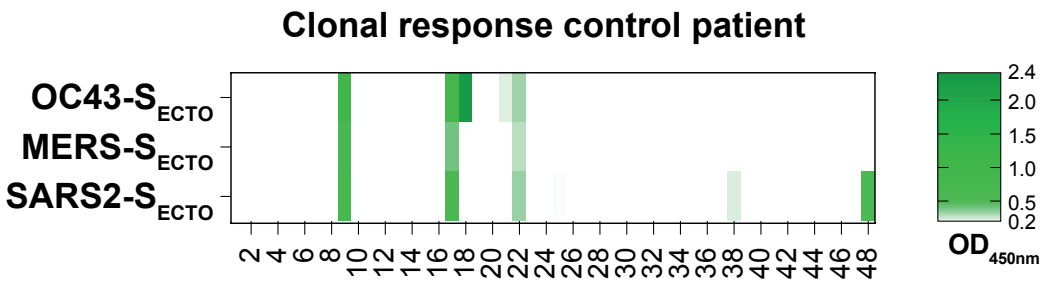


Supplementary figure 1. Serum IgG titers and IgG B-cell clone reactivity towards N of SARS-CoV and SARS-CoV-2 are strongly correlated. A) Serum IgG titers against nucleocapsid (N) of SARS-CoV (SARS-N) and SARS-CoV-2 (SARS2-N) were determined using protein microarray (PMA) and ELISA, respectively. Horizontal and vertical dashed lines indicate assay background of PMA and ELISA, respectively. **B**) Culture supernatants of selected SARS-N-reactive IgG B-cell clones were tested for SARS-N and SARS2-N using ELISA. Linear regression shows that both serum IgG titers and B-cell clones significantly correlated. Solid line depicts the best-fit regression coefficient. Dashed line shows the 95% confidence of the best-fit line.



Supplementary figure 2. Boosted OC43-S_{ECTO} clones recognize epitopes in OC43-S_2. A random selection of boosted OC43-S_{ECTO} reactive clones that did not show cross-reactivity towards OC43-S1 (black dots, n=43) were screened using OC43-S and OC43-S_2 on ELISA. Non-reactive clones were included as a negative control (red dots, n=3). The significant correlation suggests all selected boosted OC43-S_{ECTO} targeted an epitope within S_2 (linear regression analysis).



Supplementary figure 3. Clonal IgG response of a disease control patient towards OC43-, MERS- and SARS2-S_{ECTO}. B-cells were isolated from PBMC of a disease control patient and stimulated in vitro at limiting dilution to ensure clonal reactivity. Culture supernatants were screened for IgG reactivity towards the ectodomain of spike from HCoV-OC43, MERS-CoV and SARS-CoV-2 to supplement MERS-S_{ECTO} protein microarray results. Three clones that cross-react with MERS-S_{ECTO} were identified.

Supplementary Table 1. Antigens used in this study

Antigen	[a]Use	Source or reference	[b]Production system	Amino acid residues and ref sequence	[c]Tag
229E-N	P	Medix Biochemica (Cat.No: 610091)	E. coli		N-term His-tag
NL63-N	P	Medix Biochemica (Cat.No: 610041)	E. coli		N-term His-tag
HKU1-N	P	Medix Biochemica (Cat.No: 610042)	E. coli		N-term His-tag
OC43-N	P	Medix Biochemica (Cat.No: 610040)	E. coli		N-term His-tag
MERS-N	P	Sino biological (Cat.No: 40068-V08B)	BIC		C-term His-tag
SARS-N	E, P	Sino biological (Cat. No: 40143-V08B)	BIC		C-term His-tag
SARS2-N	E	Sino biological (Cat. No: 40158-V08B)	BIC		C-term His-tag
NL63-S_{ECTO}	P	Kindly provided by Dr. Berend Jan Bosch (*1*)	DS2	16-1291, UniProt Q6Q1S2	C-term GCN4, Strep-tag
HKU1-S_{ECTO}	P	Kindly provided by Dr. Berend Jan Bosch (*2*)	HEK293T	14-1266, GenBank HM034837	C-term T4T, mFCS, Strep-tag
OC43-S_{ECTO}	E, P	Kindly provided by Dr. Berend Jan Bosch (*3*)	HEK293F	15-1263, UniProt Q696P8	C-term GCN4, mFCS, Strep-tag
MERS-S_{ECTO}	P	Kindly provided by Dr. Berend Jan Bosch (*2*)	BIC	19-1262, GenBank YP_009047204.1	C-term T4T, mFCS, Strep-tag
MERS-S_{ECTO}	E	Sino biological (Cat.No: 40069-V08B)	BIC		C-term His-tag
SARS-S_{ECTO}	P	Kindly provided by Dr. Berend Jan Bosch (*4*)	HEK293F	1-1160, GenBank AAP13567.1	C-term T4T, mFCS, Strep-tag
SARS2-S_{ECTO}	P	Kindly provided by Dr. Berend Jan Bosch (*5*)	HEK293T	1-1200, GenBank QHD43416.1	C-term T4T, mFCS, Strep-tag
229E-S_1	P	Generated in-house (*6*)	HEK293T	1-537	Mouse Fc-tag
NL63-S_1	P	Generated in-house (*6*)	HEK293T	1-717	Mouse Fc-tag
HKU1-S_1	P	Generated in-house (*6*)	HEK293T	1-750	Mouse Fc-tag
OC43-S_1	P	Generated in-house (*6*)	HEK293T	1-760	Mouse Fc-tag
MERS-S_1	P	Generated in-house (*6*)	HEK293T	1-751	Mouse Fc-tag
SARS-S_1	P	Generated in-house (*6*)	HEK293T	1-676	Mouse Fc-tag
SARS2-S_1	P	Generated in-house (*6*)	HEK293T	1-674	Mouse Fc-tag
SARS2-S_{RBD}	P	Generated in-house (*6*)	HEK293T	329-538	Mouse Fc-tag
OC43-S_2	E	Sino biological (40607-V08B1)	BIC		His-tag
HA H1N1(2009)	E	Protein Sciences corporation	BIC		

[a]Antigen was used in ELISA (E) or protein microarray (P)

[b]Antigen was produced in Escheria coli (E.coli), Drosophila S2 cells (DS2), Baculovirus infected insect cells (BIC), human embryonic kidney cells clone 293 transformed with SV40 T antigen (HEK293T) or Freestyle HEK293 (HEK293F)

[c]Antigen contains a transcription factor GCN4 trimerization motif (GCN4), T4 fibritin trimerization motif (T4T), mutated S1/S2 furin cleavage site (mFCS), Histidine-tag (His-tag), Streptavidin-tag (Strep-tag)

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