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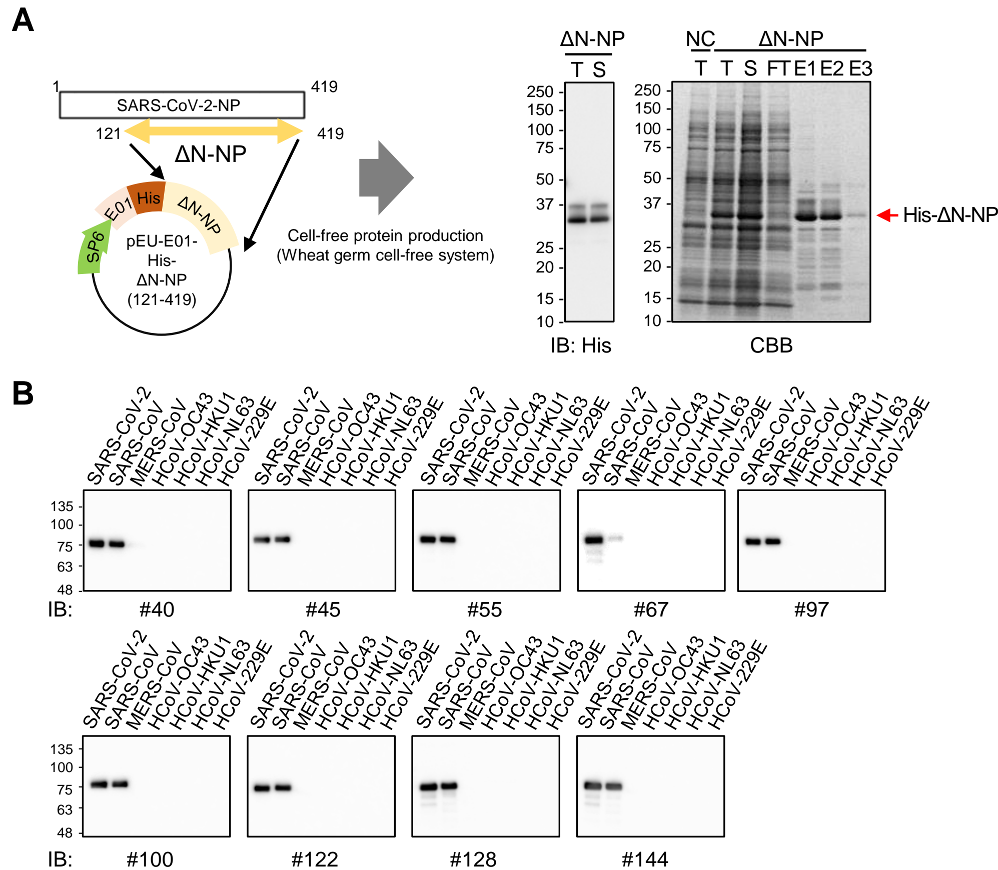
Supplemental information

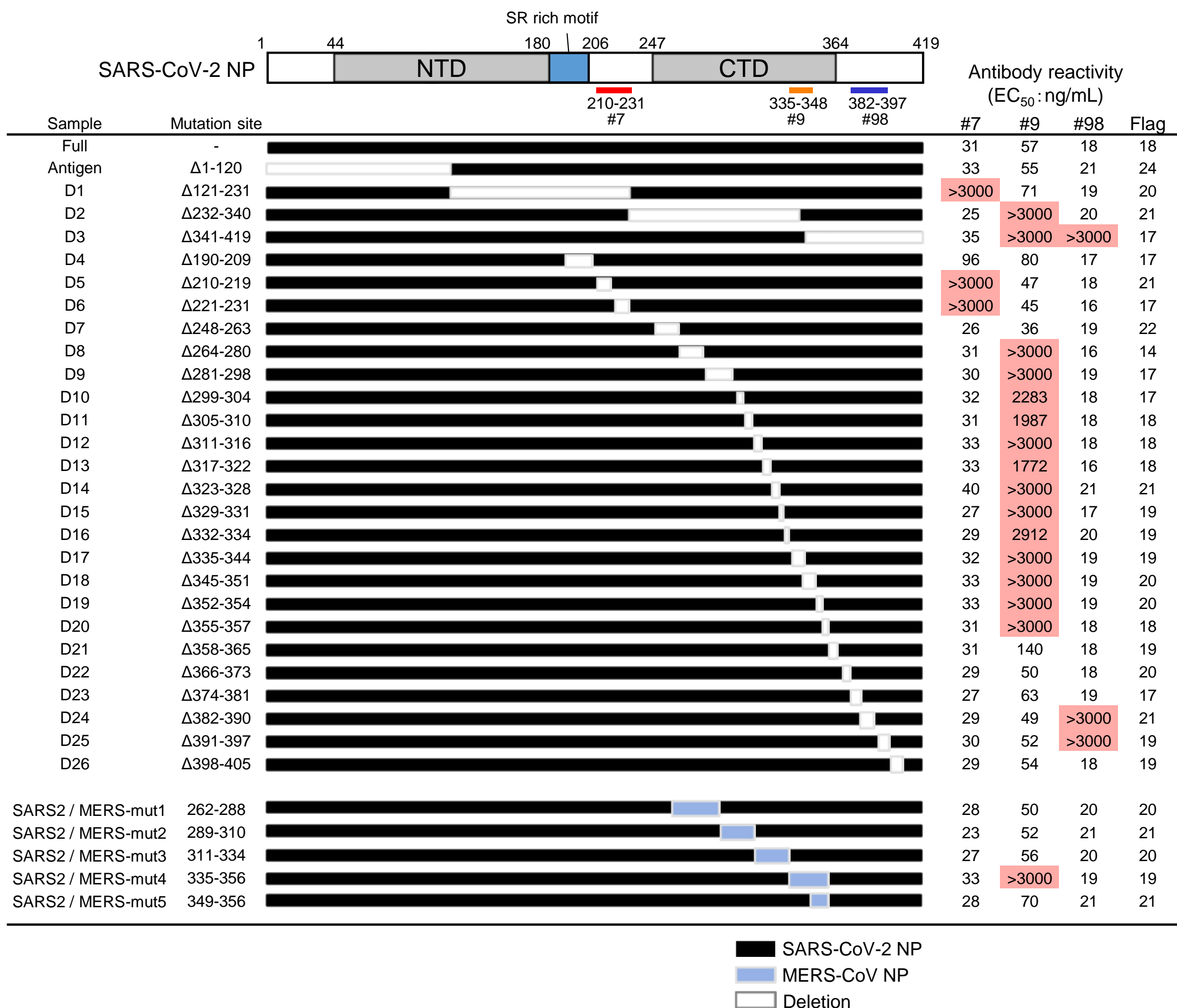
Highly specific monoclonal antibodies and epitope identification against SARS-CoV-2 nucleocapsid protein for antigen detection tests

Yutaro Yamaoka, Kei Miyakawa, Sundararaj Stanleyraj Jeremiah, Rikako Funabashi, Koji Okudela, Sayaka Kikuchi, Junichi Katada, Atsuhiko Wada, Toshiki Takei, Mayuko Nishi, Kohei Shimizu, Hiroki Ozawa, Shuzo Usuku, Chiharu Kawakami, Nobuko Tanaka, Takeshi Morita, Hiroyuki Hayashi, Hideaki Mitsui, Keita Suzuki, Daisuke Aizawa, Yukihiro Yoshimura, Tomoyuki Miyazaki, Etsuko Yamazaki, Tadaki Suzuki, Hirokazu Kimura, Hideaki Shimizu, Nobuhiko Okabe, Hideki Hasegawa, and Akihide Ryo



Supplementary Figure 1. (Related to Figure 1) Antigen protein production and specificity screening of developed mAbs.

(A) Schematic representation of antigen protein production. Recombinant Histidine (His)-tagged N-terminally truncated SARS-CoV-2-NP (121-419: ΔN-NP) was produced in a wheat germ cell-free system, and then purified using nickel-chelated Sepharose beads. Each protein fraction was analyzed by SDS-PAGE and visualized by CBB staining. Red arrow indicate the target protein. NC, Negative Control; T, Total fraction; S, Supernatant; P, Precipitate; FT, Flow-Through; E1–3, Elution fractions 1-3. (B) Flag-GST-tagged NPs derived from several human coronaviruses were produced in the wheat germ cell-free system. Reactivity of generated mAbs was validated by immunoblot analysis using the indicated antibodies.



Supplementary Figure 2. (Related to Figure 1) Epitope mapping of mAbs.

Schematic diagram of SARS-CoV-2-NP domain architecture, mutants, and region of determined epitope of each monoclonal antibody. For epitope mapping, 26 deletion mutants (D1-D26) and 5 substitution mutants (SARS2/MERS-mut1-5) were produced as FLAG-GST-tagged proteins in the wheat germ cell-free system. NTD, N-terminal domain; LKR, flexible linker region; CTD, C-terminal domain. Reactivity of each mAb to deletion mutants was evaluated by indirect-ELISA. EC_{50} was calculated using six different antibody concentrations, and samples with an over 5-fold increase in EC_{50} compared to full-length NP were defined as negative (Red).

A

#7 : 210-231 aa

SARS-CoV-2	210	M A G N G G D A A - - - - - - - - - L A L L L L D R L N Q L E	231
SARS-CoV	211	M A S G G G E T A - - - - - - - - - L A L L L L D R L N Q L E	232
MERS-CoV	206	I G A V G G D - - - - - - - - - - - - - L L Y L D L L N R L Q	223
HCoV-OC43	216	- A N S G N R T P - - - - - - - - - T S G V T P D M A D Q I A	237
HCoV-HKU1	215	- S N S N F R H S - - - - - - - - - D S I V K P D M A D E I A	236
HCoV-NL63	193	L K N L G F D - - - - - - - - - - - - - - - - - N Q S K S P S	206
HCoV-229E	190	L K S L G F D K P Q E K D K K S A K T G T P K P S R N Q S P A	220

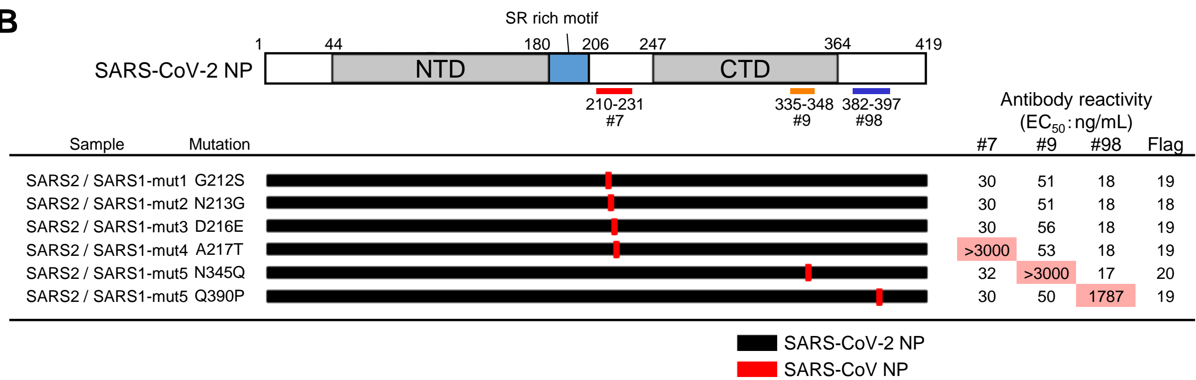
#9 : 335-348 aa

SARS-CoV-2	335	G A I K L D D K D P N F K D	348
SARS-CoV	336	G A I K L D D K D P Q F K D	349
MERS-CoV	333	G A I K L D P K N P N Y N K	346
HCoV-OC43	355	G A I R F D S T L S G F E T	368
HCoV-HKU1	350	G S I R F D S T L P G F E T	363
HCoV-NL63	308	Y K M L V A K D N K N L P K	321
HCoV-229E	323	T R V T V P K D H P H L G K	336

#98 : 382-397 aa

SARS-CoV-2	382	L P Q R Q K K Q - - - - Q T V T L L P A	397
SARS-CoV	383	L P Q R Q K K Q - - - - P T V T L L P A	398
MERS-CoV	382	P P K E Q R V Q - - - - G S I T - - - -	393
HCoV-OC43	412	A V P K S R V Q Q N K S R E L T - - - -	427
HCoV-HKU1	410	S A G T Q H I S - - - - N D F T - - - -	421
HCoV-NL63	354	S I P E S - - - - - - - K P L A - - - -	362
HCoV-229E	367	S P A T A - - - - - - - E P V R - - - -	375

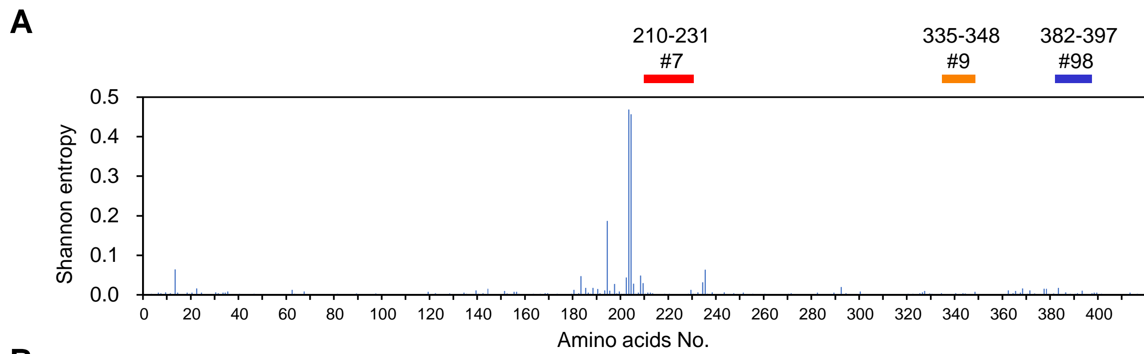
B



Supplementary Figure 3. (Related to Figure 1) Critical residues for desecting SARS-CoV and SARS-CoV-2 in epitope of developed mAbs.

(A) Multiple sequence alignments of NP of human coronaviruses in the epitope region using MUSCLE software. Gray shaded positions represent conserved residues among the sequences. (B) Determination of critical residues for specific detection of not SARS-CoV but SARS-CoV-2. Five point substitution mutants (SARS2/SARS1-mut1-5) were produced as FLAG-GST-tagged proteins in the wheat germ cell-free system. NTD, N-terminal domain; LKR, flexible linker region; CTD, C-terminal domain. Reactivity of each mAb to deletion mutants was evaluated by indirect-ELISA. EC_{50} was calculated using six different antibody concentrations. The samples with an over 5-fold increase in EC_{50} compared to full-length NP were defined as negative (Red).

A



B

#7 : 210-231 aa

PANGO lineage of SARS-CoV-2		Amino acid sequence of epitope	
A (Prototype)	210	M A G N G G D A A L A L L L L D R L N Q L E	231
B.1.1.7 (501Y.V1)	210	M A G N G G D A A L A L L L L D R L N Q L E	231
B.1.351 (501Y.V2)	210	M A G N G G D A A L A L L L L D R L N Q L E	231
B.1.427	210	M A G N G G D A A L A L L L L D R L N Q L E	231
B.1.429	210	M A G N G G D A A L A L L L L D R L N Q L E	231
B.1.525	210	M A G N G G D A A L A L L L L D R L N Q L E	231
B.1.526	210	M A G N G G D A A L A L L L L D R L N Q L E	231
P.1 (501Y.V3)	210	M A G N G G D A A L A L L L L D R L N Q L E	231
P.2	210	M A G N G G D A A L A L L L L D R L N Q L E	231

#9 : 335-348 aa

PANGO lineage of SARS-CoV-2		Amino acid sequence of epitope	
A (Prototype)	335	G A I K L D D K D P N F K D	348
B.1.1.7 (501Y.V1)	335	G A I K L D D K D P N F K D	348
B.1.351 (501Y.V2)	335	G A I K L D D K D P N F K D	348
B.1.427	335	G A I K L D D K D P N F K D	348
B.1.429	335	G A I K L D D K D P N F K D	348
B.1.525	335	G A I K L D D K D P N F K D	348
B.1.526	335	G A I K L D D K D P N F K D	348
P.1 (501Y.V3)	335	G A I K L D D K D P N F K D	348
P.2	335	G A I K L D D K D P N F K D	348

#98 : 382-397 aa

PANGO lineage of SARS-CoV-2		Amino acid sequence of epitope	
A (Prototype)	382	L P Q R Q K K Q Q T V T L L P A	397
B.1.1.7 (501Y.V1)	382	L P Q R Q K K Q Q T V T L L P A	397
B.1.351 (501Y.V2)	382	L P Q R Q K K Q Q T V T L L P A	397
B.1.427	382	L P Q R Q K K Q Q T V T L L P A	397
B.1.429	382	L P Q R Q K K Q Q T V T L L P A	397
B.1.525	382	L P Q R Q K K Q Q T V T L L P A	397
B.1.526	382	L P Q R Q K K Q Q T V T L L P A	397
P.1 (501Y.V3)	382	L P Q R Q K K Q Q T V T L L P A	397
P.2	382	L P Q R Q K K Q Q T V T L L P A	397

Supplementary Figure 4. (Related to Figure 1) Shannon entropy analysis of nucleocapsid proteins and comparison of epitope sequences between prototype and current variant of SARS-CoV-2.

(A) Shannon entropy, as a quantitative measure of variation, was calculated for each amino-acid residue of SARS-CoV-2 NP derived from 8,127 strains. Binding sites of each mAb are indicated in different colors. **(B)** Amino acid sequence in the antigenic epitope of reference strain against each of the currently reported epidemic variant of SARS-CoV-2 [11]. Gray shaded positions represent conserved residues among the sequences.

A

#7 : 210-231 aa

ACCESSION ID	Name of strain	Number of strains	Relative abundance	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231
NC_045512	Reference genome	8091	99.56%	M	A	G	N	G	G	D	A	A	L	A	L	L	L	L	D	R	L	N	Q	L	E
MT847220	BGD/BCSIR NILMRC 281/2020	3	0.04%	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT706376	USA/WI-UW-249/2020	1	0.01%	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT831837	USA/WA-S2367/2020	1	0.01%	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT358640	DEU/FFM4/2020	2	0.02%	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F	.
MT873396	USA/MA-MGH-01121/2020	1	0.01%	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT628169	USA/CA-CZB-1484/2020	2	0.02%	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.
MT472626	USA/IA-CDC-8200/2020	2	0.02%	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
MT601287	BGD/BCSIR NILMRC 75/2020	13	0.16%	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.	.
MT535490	USA/UT-01568/2020	2	0.02%	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT614522	USA/WA-QDX-62/2020	2	0.02%	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT252734	USA/WA-UW154/2020	4	0.05%	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT451476	AUS/VIC680/2020	2	0.02%	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Y	.	.	.
MT806787	USA/IN-QDX-251/2020	1	0.01%	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

#9 : 335-348 aa

ACCESSION ID	Name of strain	Number of strains	Relative abundance	335	336	337	338	339	340	341	342	343	344	345	346	347	348
NC_045512	Reference genome	8107	99.75%	G	A	I	K	L	D	D	K	D	P	N	F	K	D
MT873324	USA/MA-MGH-01405/2020	1	0.01%	.	S	.	.	.	.	.	.	.	.	.	.	.	.
MT706363	USA/WI-UW-235/2020	1	0.01%	.	.	F	.	.	.	.	.	.	.	.	.	.	.
MT326140	USA/WA-UW-1680/2020	2	0.02%	.	.	.	.	.	N	.	.	.	.	.	.	.	.
MT831745	USA/WA-S2426/2020	1	0.01%	.	.	.	.	.	.	.	.	.	.	T	.	.	.
LR877181	SWE/STO-KI/2020	1	0.01%	.	.	.	N	.	.	.	.	.	.	.	.	.	.
MT683418	USA/WA-UW-10138/2020	1	0.01%	.	.	.	.	.	.	.	N	.	.	.	.	.	.
LC529905	JP/TKYE6182/2020	3	0.04%	.	.	.	.	.	.	.	.	.	S	.	.	.	.
MT358737	USA/WA-UW-3994/2020	1	0.01%	.	.	.	.	.	G	.	.	.	.	.	.	.	.
MT509498	IND/GBRC107/2020	4	0.05%	.	.	.	.	.	.	.	.	.	.	.	.	.	Y
MT731292	MAR/RMPS02/2020	2	0.02%	.	.	.	.	.	.	.	.	.	.	.	.	.	H
MT135043	CHN/233/2020	1	0.01%	.	.	.	.	.	.	.	.	V	.	.	.	.	.
MT811401	USA/SEARCH-0834-IPL/2020	2	0.02%	.	.	.	.	.	.	.	.	Y	.	.	.	.	.

#98 : 382-397 aa

ACCESSION ID	Name of strain	Number of strains	Relative abundance	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397
NC_045512	Reference genome	8079	99.41%	L	P	Q	R	Q	K	K	Q	Q	T	V	T	L	L	P	A
MT811458	USA/SEARCH-0997-IPL/2020	1	0.01%	.	.	.	.	.	.	R	.	.	.	.	.	.	.	.	.
MT252716	USA/WA-UW180/2020	2	0.02%	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S
MT614463	USA/CA-QDX-40/2020	1	0.01%	.	.	.	.	K	.	.	.	.	.	.	.	.	.	.	.
LC571012	JP/DP0654/2020	1	0.01%	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V
MT831158	USA/WA-S2249/2020	5	0.06%	.	.	.	.	H	.	.	.	.	.	.	.	.	.	.	.
MT252737	USA/WA-UW146/2020	2	0.02%	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.
MT358637	IND/GBRC1/2020	11	0.14%	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.
MT757020	USA/FL-BPHL-0351/2020	1	0.01%	.	.	H	.	.	.	.	.	.	.	.	.	.	.	.	.
MT451616	AUS/VIC879/2020	9	0.11%	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT646100	USA/MD-HP00086/2020	9	0.11%	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT831179	USA/WA-S2105/2020	1	0.01%	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT825091	IRN/COVID19-IRVSH1/2020	1	0.01%	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.
MT811500	USA/SEARCH-1065-SAN/2020	1	0.01%	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.
MT757121	USA/FL-BPHL-0313/2020	1	0.01%	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.
MT810952	USA/SEARCH-0240-SAN/2020	1	0.01%	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.
MT434815	USA/NY-CDC-SURV0175NYC/2020	1	0.01%	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.

B

#7 : 210-231 aa

Name	Mutation site	Antibody reactivity (EC_{50} : ng/mL) #7	#9	#98	Flag
Full	-	31	57	18	18
M1	A211S	28	51	17	20
M2	M210I	31	54	18	20
M3	M210V	30	55	18	20
M4	L230F	25	54	19	20
M5	A211V	31	57	18	20
M6	A218V	40	79	19	20
M7	A220T	29	58	18	20
M8	Q229H	27	56	19	21
M9	G212V	36	64	18	16
M10	G212C	153	77	18	17
M11	N213Y	51	74	18	17
M12	N228Y	56	89	17	18
M13	G214C	185	70	17	18

#9 : 335-348 aa

Name	Mutation site	Antibody reactivity (EC_{50} : ng/mL) #7	#9	#98	Flag
Full	-	31	57	18	18
M13	A336S	29	57	18	18
M14	I337F	29	62	18	19
M15	D340N	30	56	18	18
M16	N345T	30	63	17	19
M17	K338N	30	57	18	20
M18	K342N	30	52	18	18
M19	P344S	31	2658	16	19
M20	D340G	32	77	17	19
M21	D348Y	32	2348	16	18
M22	D348H	31	1755	17	18
M23	D343V	30	>3000	17	18
M24	D343Y	31	2316	16	17

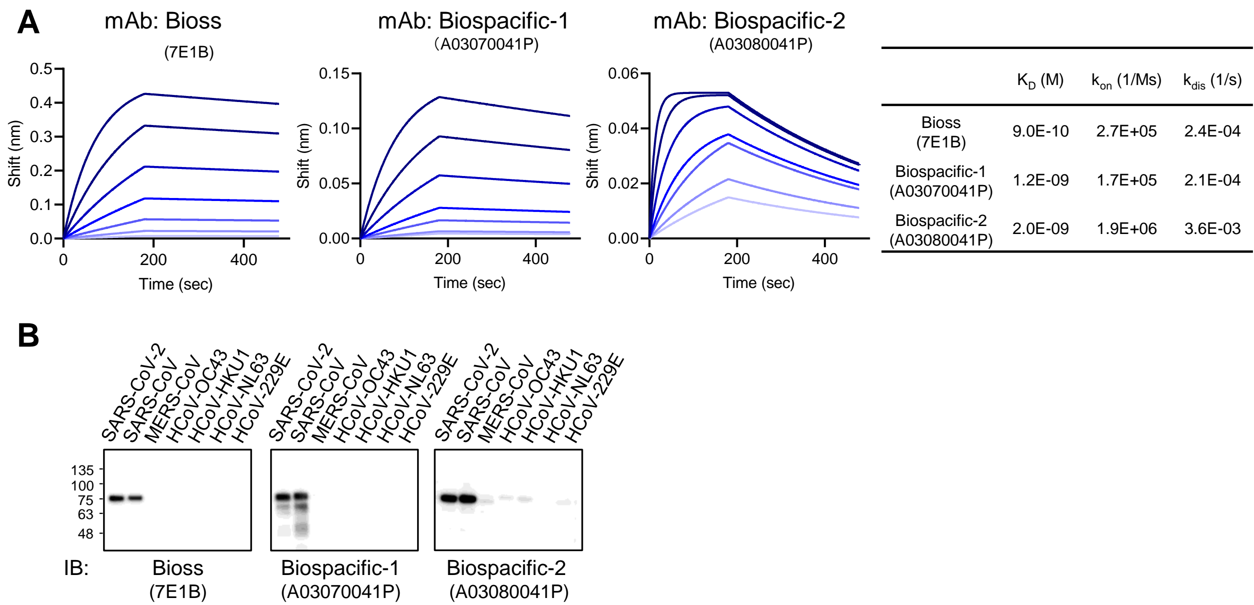
#98 : 382-397 aa

Name	Mutation site	Antibody reactivity (EC_{50} : ng/mL) #7	#9	#98	Flag
Full	-	31	57	18	18
M25	K388R	30	52	16	18
M26	A397S	28	53	16	18
M27	Q386K	29	56	16	18
M28	A397V	29	54	15	19
M29	Q386H	28	54	17	18
M30	T391I	30	52	16	19
M31	T393I	28	50	>3000	19
M32	Q384H	29	56	18	19
M33	P383S	31	63	17	20
M34	P383L	31	58	18	21
M35	L382S	30	60	18	21
M36	Q390L	31	60	98	20
M37	K388I	30	60	19	21
M38	R385I	31	58	18	22
M39	P396H	30	58	15	20
M40	Δ390-392	29	53	>3000	21

Supplementary Figure 5. (Related to Figure 1) Reactivity of developed monoclonal antibodies against recombinant protein from divergent strains.

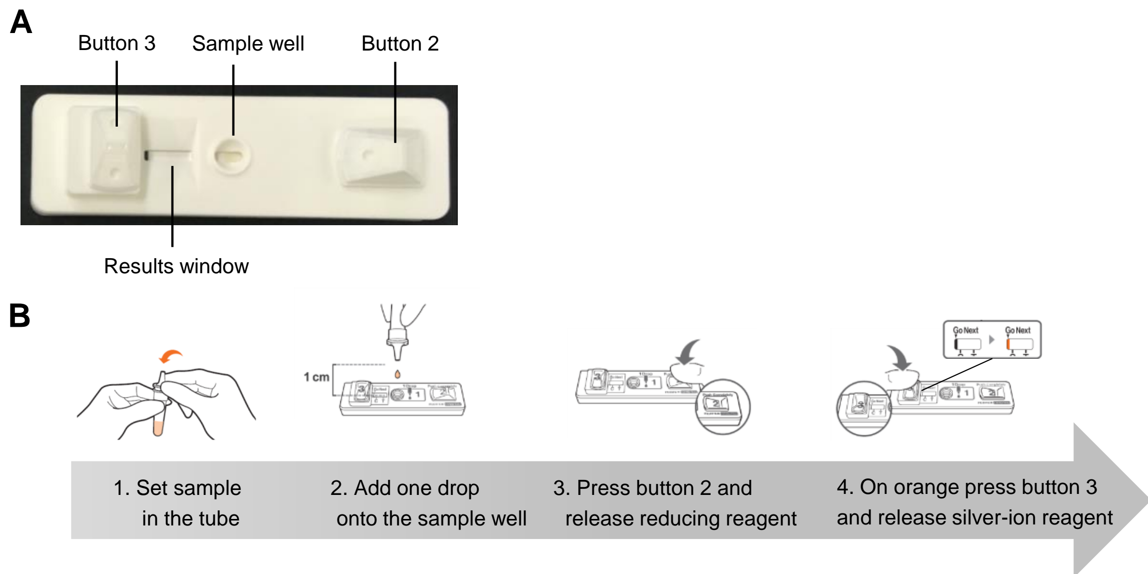
(A) Identification of amino-acid substitution in the antigenic epitope of each identical strain from 8,127 clinical strains compared with the reference strain. Dots indicate sequence identity relative to the reference strain. Gray shaded

positions represent conserved residues among the sequences. (B) Reactivity of mAbs to recombinant NP from strains possessing amino-acid substitution in the epitope regions. Forty point substitution mutants identified in (A) were produced as FLAG-GST-tagged proteins in the wheat germ cell-free system. Reactivity of each mAb to mutants was evaluated by indirect-ELISA. EC_{50} was calculated using six different antibody concentrations. The samples with an over 5-fold increase in EC_{50} compared to full-length NP were defined as negative (Red).



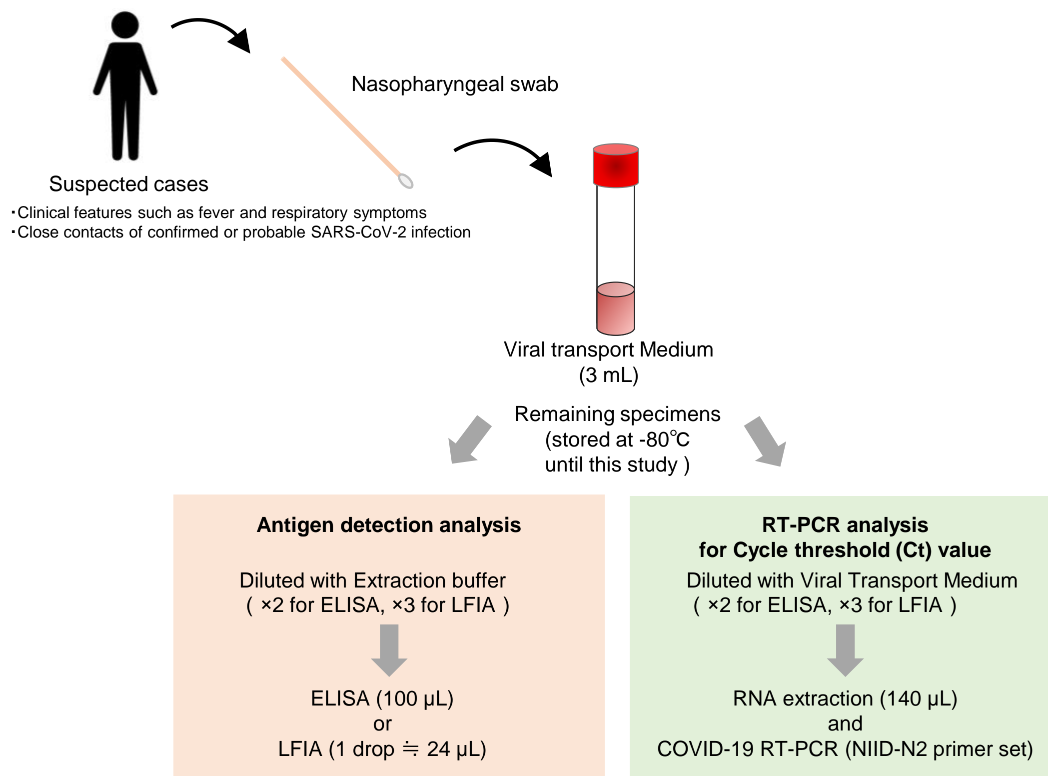
Supplementary Figure 6. (Related to Figure 2) Comparison of commercially available existing mAbs against SARS-CoV-2 NP.

(A) Affinity measurement of commercially available monoclonal antibodies on the octetRED96 instrument. Association and dissociation of each mAbs to full-length NP at various concentrations (50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 nM) was evaluated using AMC sensor. (B) Specificity of existing mAbs were examined by immunoblot analysis using the indicated recombinant proteins from human coronaviruses.



Supplementary Figure 7. (Related to Figure 4) Overview and procedure of LFIA.

(A) Image of LFIA Cartridge. (B) Samples were diluted with an extraction buffer (Tris buffer containing 0.1% non-ionic surfactant) in the tube and one drop of the sample was added onto the sample well of LFIA cartridge. Following this, button 2 was immediately pressed to release a reducing reagent for silver amplification. After the color indicator mark turned orange (about 10 min), button 3 was pressed to release a silver-ion reagent to activate the silver amplification reaction.



Supplementary Figure 8. (Related to STAR Methods) Evaluation of clinical specimens for antigen detection assay or RT-PCR.

Clinical specimens of nasopharyngeal swabs from cases with suspected SARS-CoV-2 infection were retrieved for this study. Swabs were collected in 3 mL of viral transport medium. RNA extraction and RT-PCR using NIID-N2 set primer were carried out as an administrative inspection. The remaining clinical samples were centrifuged, and the supernatant was used for this study. For antigen detection assays, samples were diluted 2-fold (ELISA) or 3-fold (LFIA) in extraction buffer and 100 µL (ELISA) or 1 drop (LFIA) was subjected to analysis. For RT-PCR analysis to quantify Ct value, samples were diluted to same concentration used in antigen detection assay (2-fold for ELISA, 3-fold for LFIA) with viral transport medium. RNA extraction and RT-PCR analysis were carried out using 140 µL of diluted samples.