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Supplemental information

Epitope diversity of SARS-CoV-2 hyperimmune intravenous human immunoglobulins and neutralization of variants of concern

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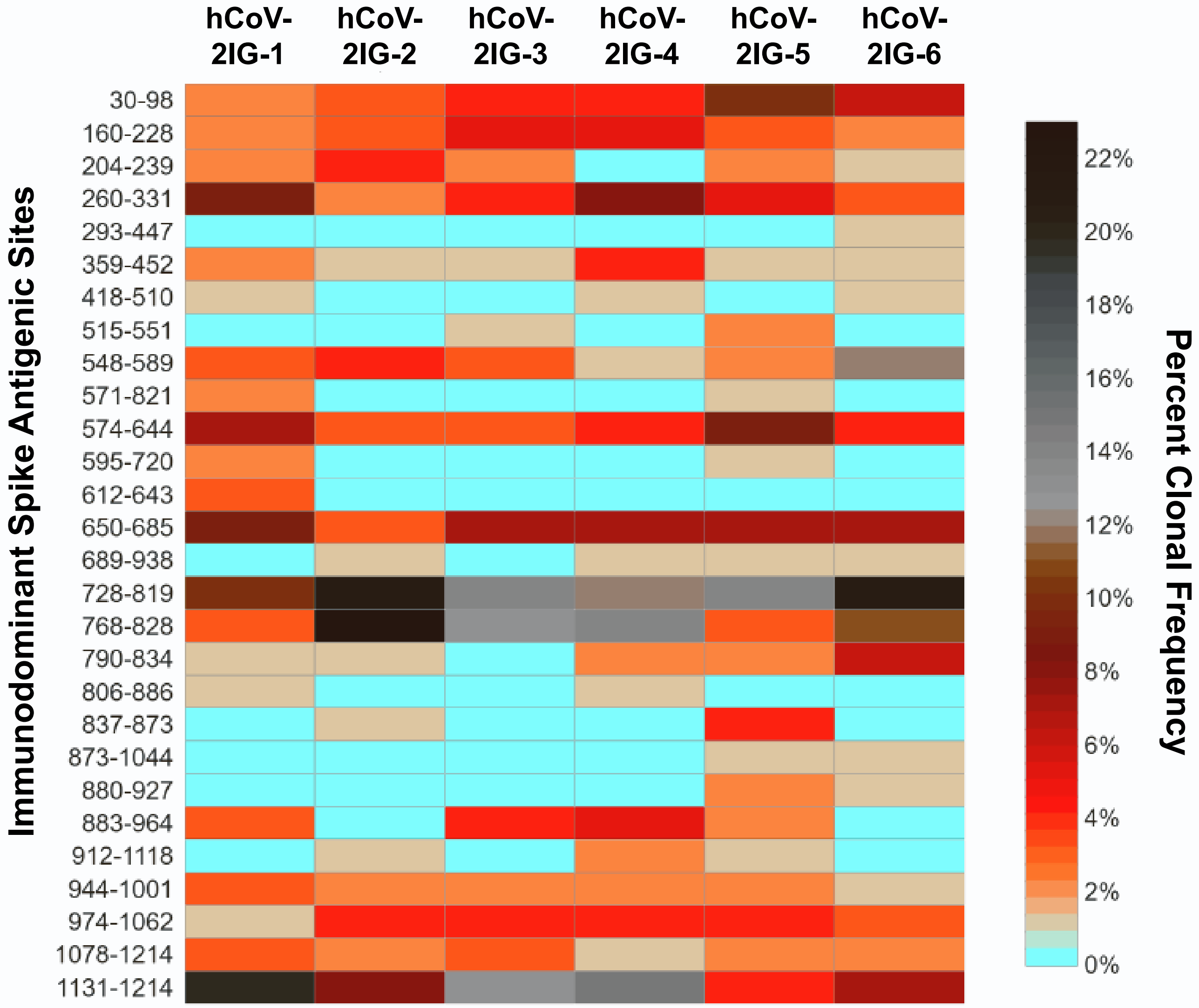

Figure S1. SARS-CoV-2 epitope profile of six hCoV-2IG batches by GFPDL analyses. Related to Figure 1. Heat map of immunodominant sites (≥3 clonal frequency in at least one hCoV-2IG lot) on the SARS-CoV-2 spike recognized by IgG antibodies in six hCoV-2IG lots identified using GFPDL analyses. The immunodominant sites on the left indicate amino acid residue of the antigenic sites in the spike protein. Color scale on the right represents range of percentage of clonal occurrences (frequency) of each site. Heat map was generated using R package.

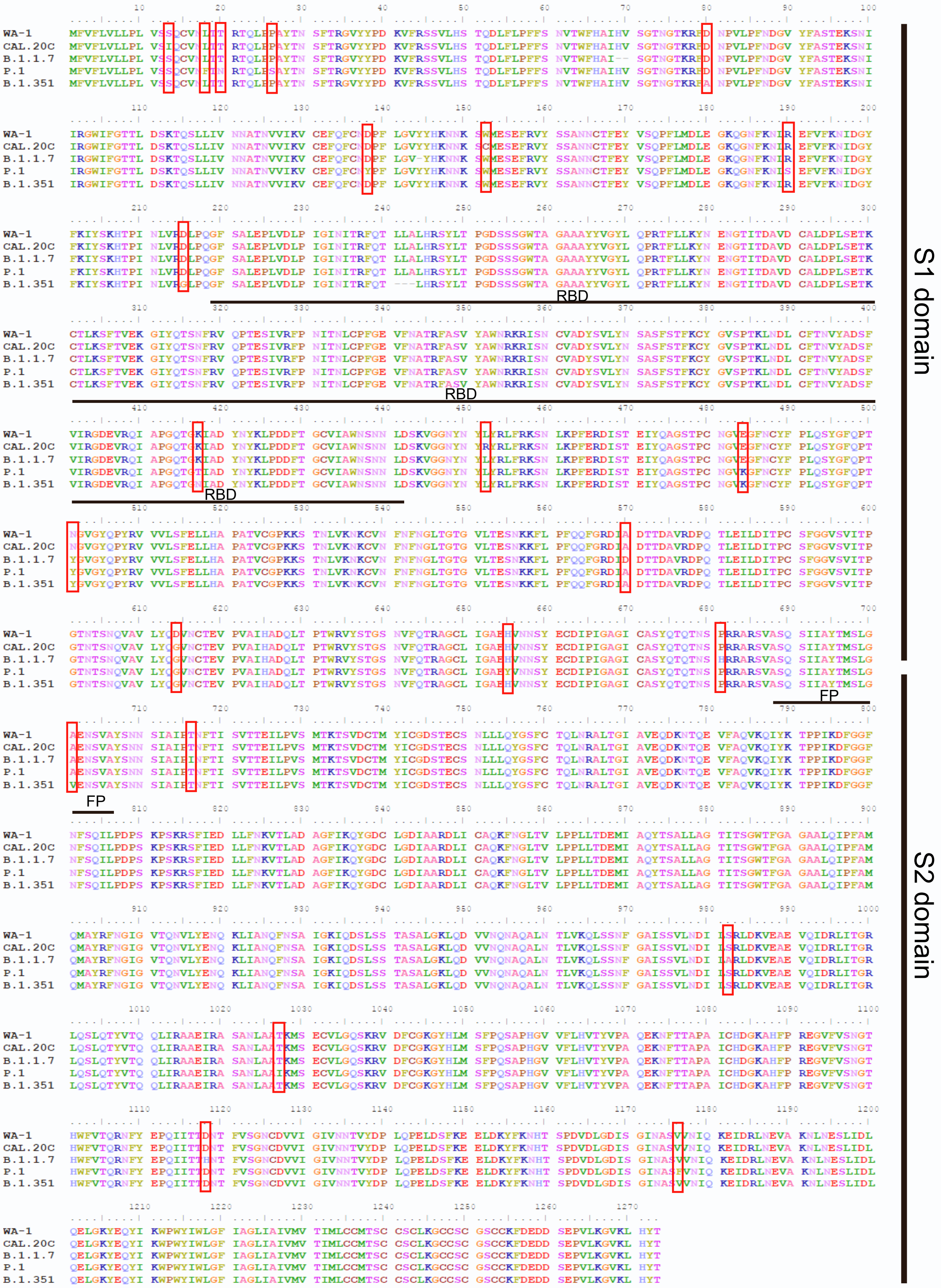

Figure S2. Multiple sequence alignment of Spike protein of SARS-CoV-2 variants. Related to Figure 2. Alignment of spike protein of SARS-CoV-2 strains: WA-1 (QII87782.1), CA (B.1.429, EPI_ISL_648527), UK (B.1.1.7, QQQ47833.1), JP (P.1, QRX39425.1), and SA (B.1.351, EPI_ISL_678597) was performed using MAFFT (https://mafft.cbrc.jp/alignment/software/). Mutations in any or all the variants are indicated with a red outline. Various domains of the spike protein are also indicated namely S1, S2, RBD and FP domains.

Table S1: Sequence conservation of GFPDL-identified antigenic regions/sites among different SARS-CoV-2 VOCs*
Related to Figure 1 and Figure 2.

AA	Sequence	WA-1	CA (B.1.429)	UK (B.1.1.7)	JP (P.1)	SA (B.1.351)
30-98	NSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKS	100	100	97.1	100	98.5
160-228	YSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVD	100	100	100	98.5	98.5
204-239	YSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQ	100	100	100	100	97.2
260-331	AGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPN	100	100	100	100	100
293-447	LDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGG	100	100	100	99.3	99.3
359-452	SNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL	100	98.9	100	98.9	98.9
418-510	IADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV	100	98.9	98.9	97.8	97.8
515-551	FELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGV	100	100	100	100	100
548-589	GTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP	100	100	97.6	100	100
571-821	DTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDL	100	99.6	98.8	99.2	99.2
574-644	DAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQ	100	98.5	98.5	98.5	98.5
595-720	VSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI	100	99.2	97.6	98.4	98.4
612-643	YQDVNCTEVPVAIHADQLTPTWRVYSTGSNVF	100	96.8	96.8	96.8	96.8
650-685	LIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRAR	100	100	97.2	97.2	100
689-938	SQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL	100	100	99.6	100	99.6
728-819	PVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIE	100	100	100	100	100
768-828	TGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTL	100	100	100	100	100
790-834	KTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI	100	100	100	100	100
806-886	KRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGW	100	100	100	100	100
837-873	YGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQY	100	100	100	100	100
873-1044	YTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCG	100	100	100	100	100
880-927	GTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQF	100	100	100	100	100
883-964	TSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVK	100	100	100	100	100
912-1118	TQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTD	100	100	99	99.5	100
944-1001	ALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRL	100	100	98.2	100	100
974-1062	SSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVF	100	100	98.8	98.8	100
1078-1214	APAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPW	100	100	99.2	99.2	100
1131-1214	GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPW	100	100	100	98.8	100

•Percent sequence conservation of GFPDL identified antigenic sites in the spike protein of SARS-CoV-2 variants namely WA-1 strain (QII87782.1), CA variant (B.1.429, EPI_ISL_648527), UK variant (B.1.1.7, QQQ47833.1), JP variant (P.1, QRX39425.1), and SA variant (B.1.351, EPI_ISL_678597) was calculated using Sequence Identity Matrix function in BioEdit.

Table S2: SARS-CoV-2 variants mutations introduced in the spike plasmid for production of SARS-CoV-2 pseudovirions to test them in PsVNA.
Related to Figure 2.

	Mutations constructed in the spike plasmids
CA	S13I, W152C, L452R, D614G
UK	Del69-70, Del145,N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
JP	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, D614G, V1176F
SA	L18F, D80A, D215G, Del242-244, K417N, E484K, N501Y, D614G, A701V

Table S3: Neutralization titers of convalescent plasma, I2019-VIG and hCoV-2IG against SARS-CoV-2 variants* Related to Figure 2.

	WA-1		CA		UK		JP		SA	
	PsVNA50	PsVNA80	PsVNA50	PsVNA80	PsVNA50	PsVNA80	PsVNA50	PsVNA80	PsVNA50	PsVNA80
IVIG batches produced in 2019 prior to COVID-19										
2019-IVIG-1	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-2	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-3	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-4	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-5	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-6	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-7	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-8	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-9	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-10	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-11	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-12	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-13	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-14	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-15	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2019-IVIG-16	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Convalescent plasma batches produced from COVID-19 survivors										
CP-1	243.1	28.0	24.7	<20	53.4	<20	287.0	61.1	107.4	30.6
CP-2	369.2	56.8	122.9	30.0	128.3	23.9	126.0	24.7	25.5	<20
CP-3	204.9	30.1	146.9	37.3	168.4	24.0	112.0	27.3	47.2	<20
CP-4	268.0	31.3	92.8	<20	59.8	<20	53.8	<20	<20	<20
CP-5	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CP-6	211.1	47.2	123.3	26.1	64.8	21.6	<20	<20	<20	<20
CP-7	67.0	<20	98.5	22.2	36.6	<20	24.1	<20	<20	<20
CP-8	119.6	30.2	156.8	33.0	35.4	<20	<20	<20	<20	<20
CP-9	852.7	74.9	191.0	55.6	100.7	35.4	122.6	25.4	51.7	<20
hCoV-2IG batches produced from COVID-19 convalescent plasma donors										
hCoV-2IG-1	2878.8	515.0	2009.3	613.7	1799.4	401.1	672.2	269.1	382.0	154.5
hCoV-2IG-2	2919.7	435.0	1301.5	305.8	1224.9	394.0	681.7	235.8	259.0	119.5
hCoV-2IG-3	3309.1	593.2	2081.3	495.7	1957.1	499.1	833.4	302.2	278.0	124.1
hCoV-2IG-4	1514.5	292.9	1257.8	261.8	935.6	286.8	653.3	176.7	184.5	80.5
hCoV-2IG-5	1590.1	323.0	1295.7	341.2	1288.5	402.4	832.3	280.8	333.6	39.7
hCoV-2IG-6	1239.5	168.3	440.8	144.4	473.4	126.8	298.5	65.2	105.6	33.5

*PsVNA titer Cut-off value: 1:20.

Table S4: PRNT50 and PRNT90 of the six hCoV-2IG batches against SARS-CoV-2 WA-1, UK and SA strains in classical wild-type SARS-CoV-2 virus neutralization assay. Related to Figure 2.

	WA-1		UK		SA	
	PRNT50	**PRNT90**	**PRNT50**	**PRNT90**	**PRNT50**	**PRNT90**
hCoV-2IG-1	5184.7	1723.8	2324.7	730.1	1111.4	296.1
hCoV-2IG-2	2260.6	264.4	1826.4	405.6	553.7	200.3
hCoV-2IG-3	4030	1504	1556.8	463.5	486.4	130.5
hCoV-2IG-4	3580.7	1153.8	1336.9	298.6	361	79.3
hCoV-2IG-5	1929.1	803.8	1674.6	370.8	716	194.3
hCoV-2IG-6	1988.8	663.7	708.1	50.9	96.8	4.3

Table S5: Antibody concentration (in mg/mL) required for each of the six hCoV-2IG batches to achieve 50% (ID50) or 80% (ID80) neutralization of SARS-CoV-2 variants in PsVNA. Related to Figure 2.

	WA-1		CA		UK		JP		SA	
	ID50 (mg/mL)	ID80 (mg/mL)	ID50 (mg/mL)	ID80 (mg/mL)	ID50 (mg/mL)	ID80 (mg/mL)	ID50 (mg/mL)	ID80 (mg/mL)	ID50 (mg/mL)	ID80 (mg/mL)
hCoV-2IG-1	**0.035**	**0.194**	**0.050**	**0.163**	**0.056**	**0.249**	**0.149**	**0.372**	**0.262**	**0.647**
hCoV-2IG-2	**0.034**	**0.230**	**0.077**	**0.327**	**0.082**	**0.254**	**0.147**	**0.424**	**0.386**	**0.837**
hCoV-2IG-3	**0.030**	**0.169**	**0.048**	**0.202**	**0.051**	**0.200**	**0.120**	**0.331**	**0.360**	**0.806**
hCoV-2IG-4	**0.066**	**0.341**	**0.080**	**0.382**	**0.107**	**0.349**	**0.153**	**0.566**	**0.542**	**1.242**
hCoV-2IG-5	**0.063**	**0.310**	**0.077**	**0.293**	**0.078**	**0.249**	**0.120**	**0.356**	**0.300**	**2.519**
hCoV-2IG-6	**0.081**	**0.594**	**0.227**	**0.692**	**0.211**	**0.789**	**0.335**	**1.534**	**0.947**	**2.988**