

Shared Antigen-specific CD8+ T cell Responses Against the SARS-COV-2 Spike Protein in HLA A*02:01 COVID-19 Patients

Supplemental Materials

Table S1. Top HLA A*02.01 9-mer to 11-mer epitopes as predicted by NetMHC4.0 algorithm.

Table S2. Selected bacterial and viral A*02:01 peptides used for SCT expression.

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Fig. S1. SCT library expression of reported A*02:01 bacterial and viral peptides.

Fig. S2. SCT design and optimization.

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Fig. S5. Supplemental clinical data for participant InCov005.

Fig. S6. Immunogenicity of spike protein peptides compared to predicted binding affinity.

ID	peptide	domain	span	length	predicted nM affinity	yield	ID	ID	peptide	domain	span	length	predicted nM affinity	yield	ID
1	YLQPRTFLL	NTD	269-277	9	5.4	0.33	1	49	VLYENQKLI	HR1	915-923	9	194.4	0.16	49
2	FQFCNDPFL	NTD	133-141	9	9.2	1.19	2	50	KLIANQFNNSA	HR1	921-930	10	195.6	1.09	50
3	FIAGLIAIV	TM	1220-1228	9	10.3	0.09	3	51	VTWFHAIHV	NTD	62-70	9	202	0.17	51
4	FVSNGTHWFV	CD	1095-1104	10	13.1	0.53	4	52	DLFLFNKVTLA	FP	820-829	10	202	0.08	52
5	SIAYTMSL	S2	691-699	9	13.5	0.88	5	53	QSIIAYTMSL	S2	690-699	10	207.4	0.00	53
6	KLNDLCFTNV	RBD	386-395	10	15.3	0.21	6	54	SRLDKVVEAEV	HR1/CH	982-991	10	221.2	0.08	54
7	RLQSLQTYV	CH	1000-1008	9	16.7	0.18	7	55	QYIKWPWYIWL	S2/TM	1208-1218	11	223.4	0.00	55
8	YHLMSFPQSA	S2	1047-1056	10	17.9	0.08	8	56	LLALHRSYL	NTD	240-249	10	227.8	0.33	56
9	YIKWPWYIWL	S2/TM	1209-1218	10	18.8	0.00	9	57	MFVFLVLLPLV	S	1-11	11	229	0.00	57
10	YIWLGFIAGL	TM	1215-1224	10	20.5	0.09	10	58	GLIAIVMVT	TM	1223-1231	9	242	0.06	58
11	FELLHAPATV	RBD/S1	515-524	10	21	0.16	11	59	YTSFTRGV	NTD	28-36	9	245.1	0.18	59
12	FTISVTEI	S2	718-726	9	25.4	1.74	12	60	ITSGWTFGA	S2	882-890	9	265.1	0.11	60
13	LLFNKVTLA	FP	821-829	9	25.4	1.74	13	61	KNLNESLIDL	HR2	1191-1200	10	268.8	0.07	61
14	HLMSPQSA	S2	1048-1056	9	26.4	1.24	14	62	GVFLVHTYV	S2	1059-1068	10	272.4	0.12	62
15	FVFLVLLPLV	S	2-11	10	32.6	0.00	15	63	LLVINNATNV	NTD	117-126	10	272.8	0.16	63
16	VLNDILSRL	HR1	976-984	9	33.6	0.11	16	64	VLSSTASAL	HR1	937-945	9	273.3	0.22	64
17	FIEDLLFNKV	FP	817-826	10	36	0.11	17	65	KIYSKHTPI	NTD	202-210	9	288.5	0.36	65
18	GYLQPRTFLL	NTD	268-277	10	36.1	0.13	18	66	FVSGNCDV	CD	1121-1129	9	290.4	0.12	66
19	KIADYNYKL	RBD	417-425	9	36.1	0.50	19	67	FQFCNDPFLG	NTD	133-142	10	296.2	1.17	67
20	VFLHVTYV	S2	1060-1068	9	36.6	0.08	20	68	KLNDLCFTNVY	RBD	386-396	11	302.6	0.16	68
21	RLDKVEAEV	HR1/CH	983-991	9	39	0.23	21	69	VLSFELLHA	RBD	512-520	9	302.6	0.11	69
22	FVFLVLLPL	S	2-10	9	41.7	0.00	22	70	AIPTNFTISV	S2	713-722	10	324.5	0.11	70
23	FGIAGLIAIV	TM	1219-1228	10	56.9	0.15	23	71	VLLPLVSSQCV	S/NTD	6-16	11	325.3	0.49	71
24	YQDVNCTEV	S1	612-620	9	57.8	0.83	24	72	LLALHRSYL	NTD	241-249	9	329.1	0.23	72
25	FLHVTYVPA	S2	1062-1070	9	65.2	0.73	25	73	IITDNTVF	CD	1114-1122	9	338.8	0.10	73
26	MIAQYTSAL	S2	869-877	9	69	0.92	26	74	IMLCCMTSC	TM/CT	1232-1240	9	349	0.13	74
27	KLPDDFTGCV	RBD	424-433	10	77.1	0.26	27	75	VFLHVTYVPA	S2	1061-1070	10	377.3	0.00	75
28	GLIAIVMVTI	TM	1223-1232	10	80.8	0.00	28	76	ELLHAPATV	RBD/S1	516-524	9	380.7	0.99	76
29	KQYKTPPI	S2	786-794	9	85.6	0.61	29	77	VLHSTQDLFL	NTD	47-56	10	382.2	0.00	77
30	EFQFCNDPFL	NTD	132-141	10	87.8	0.00	30	78	VLYNSASFST	RBD	367-376	10	388.9	0.29	78
31	TKLNDLCFTNV	RBD	385-395	11	91.2	0.00	31	79	YRVVLSFEL	RBD	508-517	10	391.5	0.00	79
32	SVTTEILPV	S2	721-729	9	113.1	0.90	32	80	SPIEDLLFNKV	FP	816-826	11	394.6	0.05	80
33	YLQPRTFLLK	NTD	269-278	10	118.8	0.54	33	81	FLPFFSNVT	NTD	55-63	9	398.9	0.15	81
34	MQMAYRFNGI	S2/HR1	900-909	10	120.9	0.39	34	82	SVLNDILSRL	HR1	975-984	10	399.3	0.22	82
35	FQFCNDPFLGV	NTD	133-143	11	126.8	1.09	35	83	ALEPLVDLPI	NTD	222-231	10	405.6	0.07	83
36	GRQLSLQTYV	CH	999-1008	10	128.4	0.13	36	84	ALQIPFAMQM	S2	893-902	10	415.7	1.76	84
37	GLTVLPPLL	S2	857-865	9	130.4	0.13	37	85	RVVVLSFEL	RBD	509-517	9	419.8	0.12	85
38	FIAGLIAIVMV	TM	1220-1230	11	136.3	0.09	38	86	FTISVTEIL	S2	718-727	10	419.8	1.66	86
39	FVSNGTHWFV	CD	1094-1104	11	137.7	0.00	39	87	FELLHAPATVC	RBD/S1	515-525	11	435.6	0.09	87
40	WYIWLGFIAGL	TM	1214-1224	11	140.1	0.00	40	88	YIWLGFIAGLI	TM	1215-1225	11	436.3	0.00	88
41	SFELLHAPATV	RBD/S1	514-524	11	140.8	0.10	41	89	KTSVDCTMYI	S2	733-742	10	438.1	0.00	89
42	KQLSSNFGA	HR1	964-972	9	141.2	0.22	42	90	GIYQTSNFRV	S1	311-320	10	449	0.09	90
43	FIAGLIAIVM	TM	1220-1229	10	143	0.10	43	91	MIAQYTSALL	S2	869-878	10	453	1.10	91
44	TLDSKTQSL	NTD	109-117	9	153.6	0.12	44	92	LGFIAGLIAIV	TM	1218-1228	11	457.3	0.08	92
45	MFVFLVLLPL	S	1-10	10	157.8	0.00	45	93	VGYLQPRTFLL	NTD	267-277	11	461.4	0.06	93
46	RLITGRQLSL	CH	995-1004	10	167	1.06	46	94	WTAGAAAYV	NTD	258-267	10	463.3	0.08	94
47	GYHLMSFPQSA	S2	1046-1056	11	172.6	0.09	47	95	SWMESEFRV	NTD	151-159	9	475.3	0.09	95
48	NLNESLIDL	HR2	1192-1200	9	177.3	0.24	48	96	NFTISVTEI	S2	717-726	10	501.6	0.13	96

Table S1. Top HLA-A*02:01 9-mer to 11-mer epitopes as predicted by NetMHC4.0 algorithm. The peptides selected for expression as SCTs are listed in this table, indexed according to binding affinity. ‘Yield’ column denotes the relative expression of each SCT based on ratio of protein gel band intensity to positive control band (see Fig. S3). Relative yield is also shaded in grayscale in the column to the right.

ID	peptide	antigen	organism	length	predicted nM affinity
1	LLFGYPVYV	Protein Tax-1	HTLV-1	9	2.72
2	KLVALGINAV	Genome polyprotein	Hepatitis C virus	10	40.72
3	YLDHLIVV	Replication & transcription activator	Epstein Barr virus	9	4.13
4	FLLSLGIHL	Protein P	Hepatitis B virus	9	9.41
5	SLFNTVATL	Gag polyprotein	HIV-1	9	39.13
6	YLLFEVFDV	Ad11 Hexon	Adenovirus	9	4.00
7	FLDKGTYTL	Envelope glycoprotein B	Epstein Barr virus	9	3.79
8	YLQQNWWTL	Latent membrane protein 1	Epstein Barr virus	9	9.46
9	AIMDKNIIL	Non-structural protein 1	Influenza A virus	9	53.78
10	KLIANNTRV	Ag85A protein	M. tuberculosis	9	25.06
11	NLVPMVATV	65 kDa phosphoprotein	Human CMV	9	25.85
12	FMYSDFHFI	Polymerase acidic protein	Influenza A virus	9	2.66

Table S2. Selected bacterial and viral A*02:01 peptides used for SCT expression. High affinity epitopes as predicted by NetMHC4.0 and experimentally reported from IEDB are (www.iedb.org) were selected for initial testing of SCT library expression platform.

Patient: Time frame:	InCoV002			InCoV005		
	Admit:	Time 1:	Time 2:	Admit:	Time 1:	Time 2:
Study Blood Draw Hospital Day & Time:	N/A	HD3 09:30	HD8 10:05	N/A	Day 2 13:00	Day 6 06:20
Vital Signs & Clinical Status (most recent, range in 24 hour prior):						
Temp (°C):	38.9	37.3	36.8	37.1	36.8	36.6
HR (/min):	101	59	80	120	116	105
BP (mmHg):	139/88	96/57	125/74	104/60	126/105	130/79
RR (/min):	18	20	20	20	26	31
SpO2 (%):	90	96	91	93	93	91
O2 delivery device:	NC	HFNC	VENT	NC	NC	HFNC
WHO Ordinal Scale:	4	5	6	4	4	5
Laboratory Data (closest to time frame):						
WBC (10 ³ /mm ³):	6.9	6.0	8.9	11.5	8.0	5.0
ANC (10 ³ /mm ³):	5.38	4.56	5.34	10.46	6.88	3.8
ALC (10 ³ /mm ³):	1.10	1.08	2.40	0.58	0.56	0.60
Cr (mg/dL):	0.79	0.76	0.73	1.23	0.83	0.86
ALT (U/L):	16	16	1291	-	39	31
AST (U/L):	26	27	1194	-	33	39
PCT (ng/mL):	0.07	0.29	0.07	0.22	0.17	-
CRP (mg/L):	129	260	10.0	203.1	178	40
Treatments (number of doses, units per dose, during the interval prior to blood draw time frame, and subsequent to previous draw).						
RDV, # x dose:	0	0	1x200mg 3x100mg	0	0	1x200mg 3x100mg
RDV, cumulative:	0	0	500 mg	0	0	500 mg
HCQ, # x dose:	0	1x400mg	0	0	1x400mg	0
HCQ, cumulative:	0	400mg	400 mg	0	400 mg	400 mg
AZM, # x dose:	0	3x500mg	0	0	1x500mg	0
AZM, cumulative:	0	1500 mg	1500 mg	0	500 mg	500 mg
Steroid, # x dose:	0	0	0	*	0	0
Steroid, cumulative:	0	0	0	*	*	*
Toci, # x dose:	0	1x8mg/kg	2x8mg/kg	0	0	3x8mg/kg
Toci, cumulative:	0	8 mg/kg	24 mg/kg	0	0	24 mg/kg

* Steroid taper finished 8 days prior to admission, see Fig S5.

Table S3. Metadata on participants InCov002 and InCov005.

Abbreviations: Temp = temperature, HR = heart rate, BP = blood pressure, RR = respiratory rate, SpO2 = peripheral capillary oxygen saturation, O2 = oxygen, NC = nasal cannula, HFNC = high flow nasal cannula, VENT = mechanical ventilation, WHO = World Health Organization, WBCs = white blood cells, ANC = absolute neutrophil count, ALC = absolute lymphocyte count, ALT = alanine aminotransferase, AST = aspartate aminotransferase, PCT = procalcitonin, CRP = C-reactive protein, RDV = Remdesivir, HCQ = hydroxychloroquine, AZM = azithromycin, Toci = tocilizumab.

Sample ID	Locus	Allele 1	Allele 2	Diploid Ambiguities	Allele 1 Ambiguities	Allele 2 Ambiguities
InCov002	A	A*01:01:01	A*02:01:01			A*02:01:130, A*02:01:159, A*02:844
	B	B*15:17:01	B*44:02:01			
	C	C*05:01:01	C*07:01:02			
	DPA1	DPA1*01:03:01	DPA1*01:03:01			
	DPB1	DPB1*02:01:02	DPB1*20:01:01	DPB1*352:01:02 + DPB1*905:01	DPB1*02:01:19	
	DQA1	DQA1*01:02:01	DQA1*03:03:01			
	DQB1	DQB1*03:01:01	DQB1*06:04:01			
	DRB1	DRB1*04:01:01	DRB1*13:02:01			
	DRB345	DRB3*03:01:01	DRB4*01:03:01			
InCov005	A	A*01:01:01	A*02:01:01			A*02:01:130, A*02:01:159, A*02:844
	B	B*08:01:01	B*18:01:01			
	C	C*07:01:01	C*07:01:01			
	DPA1	DPA1*01:03:01	DPA1*01:03:01			
	DPB1	DPB1*03:01:01	DPB1*04:02:01	DPB1*351:01 + DPB1*463:01:01		
	DQA1	DQA1*05:01:01	DQA1*05:05:01			
	DQB1	DQB1*02:01:01	DQB1*03:01:01			
	DRB1	DRB1*03:01:01	DRB1*11:04:01	DRB1*03:01:08 + DRB1*11:04:20	DRB1*03:147	
	DRB345	DRB3*01:01:02	DRB3*02:02:01			
healthy donor	A	A*02:01	A*24:02			
	B	B*39:05	B*51:01			
	C	C*02:02	C*07:02			
	DPA1	DPA1*01:03	DPA1*01:03			
	DPB1	DPB1*04:01G	DPB1*04:02G			
	DQA1	n/a	n/a			
	DQB1	DQB1*03:02	DQB1*03:04			
	DRB1	DRB1*04:03	DRB1*04:07			
	DRB345	DRB4*01:03	DRB4*01:03			

Table S4. HLA haplotypes of COVID-19 participants (InCov002 and InCov005) and healthy donor sample.

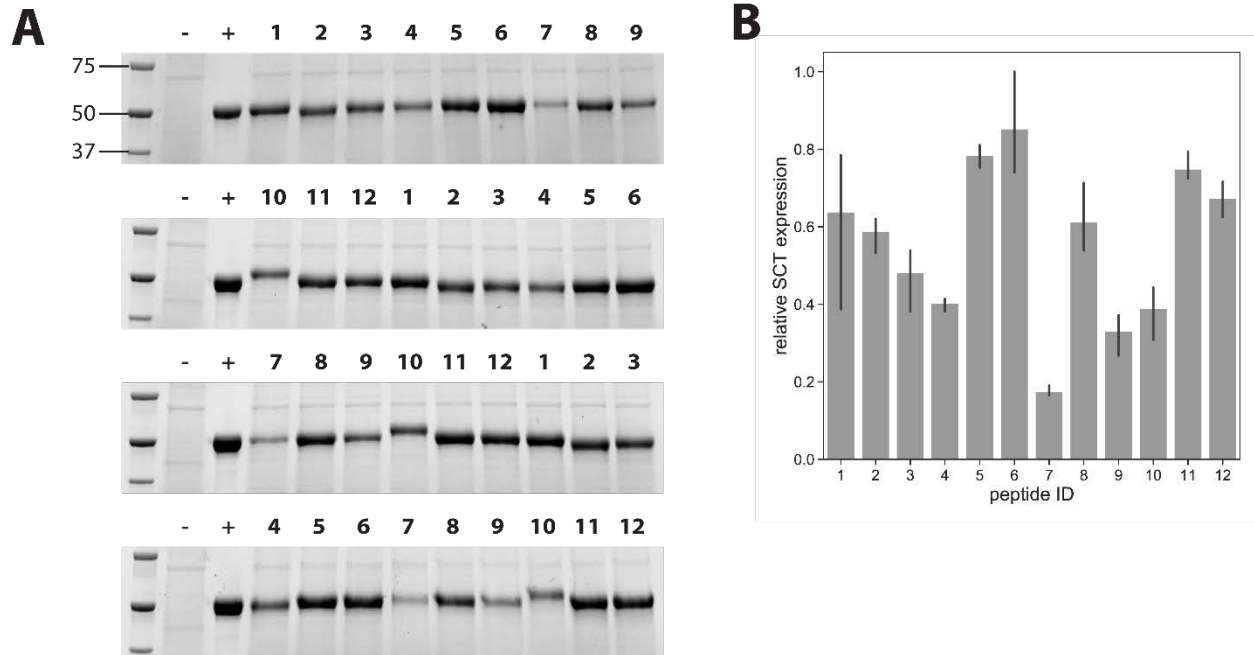


Fig. S1. SCT library expression of reported A*02:01 bacterial and viral peptides. **A.** Reduced SDS-PAGE of SCT library. Transfection was conducted as a biological triplicate. Each unique SCT library is numbered according to peptide ID from Table S2. Leftmost lane of each gel is protein ladder (kDa masses indicated). -, supernatant from mammalian cells transfected without plasmid; +, purified WT1 SCT as mass reference and positive control. **B.** Quantified SCT expression. Average protein band intensity of each SCT library element relative to WT1 control band is plotted to demonstrate reproducibility of antigen-dependent SCT expression. Peptide IDs correspond to those of Table S2 and to numbered lanes in (A).

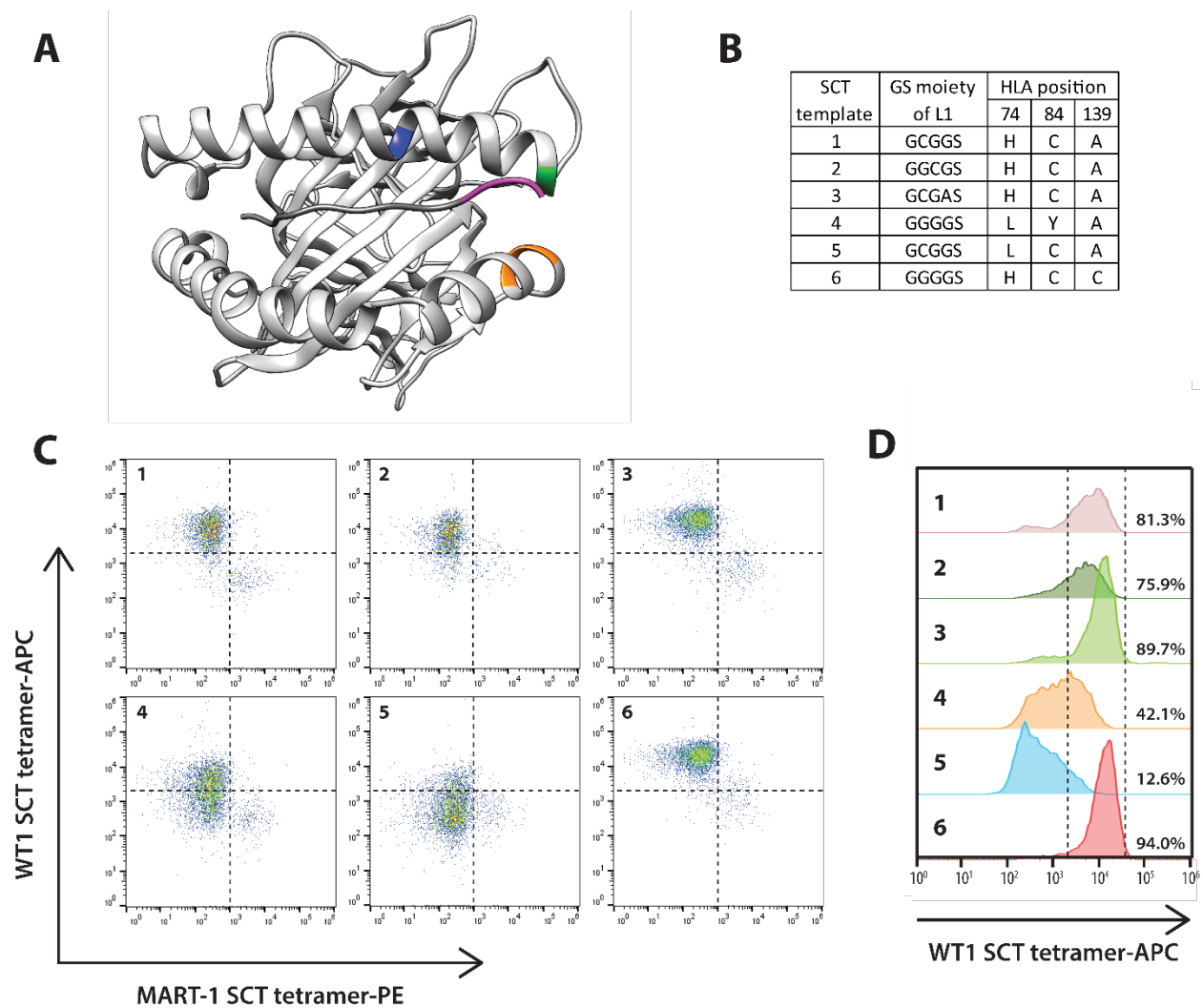


Fig. S2. SCT design and optimization. **A.** Axial view of crystal structure of HLA-A*02:01 SCT.⁴¹ Highlighted regions of interest: H74 (blue), Y84 (green), A139 (orange), first three amino acids of L1 linker (purple). Peptide is loaded into pocket in N-to-C direction (left-to-right). **B.** Summary of L1 and HLA amino acid modifications for each of the 6 SCT templates tested. **C.** Flow cytometry assay to identify optimized SCT-TCR capture. WT1 (RMFPNAPYL) SCTs constructed according to each of the 6 designs in (B) were paired with a MART-1 (ELAGIGILTV) SCT (constructed with design 1) to identify their cognate TCR-transduced cells in a 95/5 mixture of C4 TCR-transduced primary T cells and MART-1 Jurkat T cells. Number at top left of each plot indicates the SCT template used for WT1 SCT in the flow assay. **D.** Histogram of WT1 SCT tetramer-APC signal from flow assay. Percentages indicate the proportion of total cell population captured between the dashed lines by each of the six WT1 SCT designs.

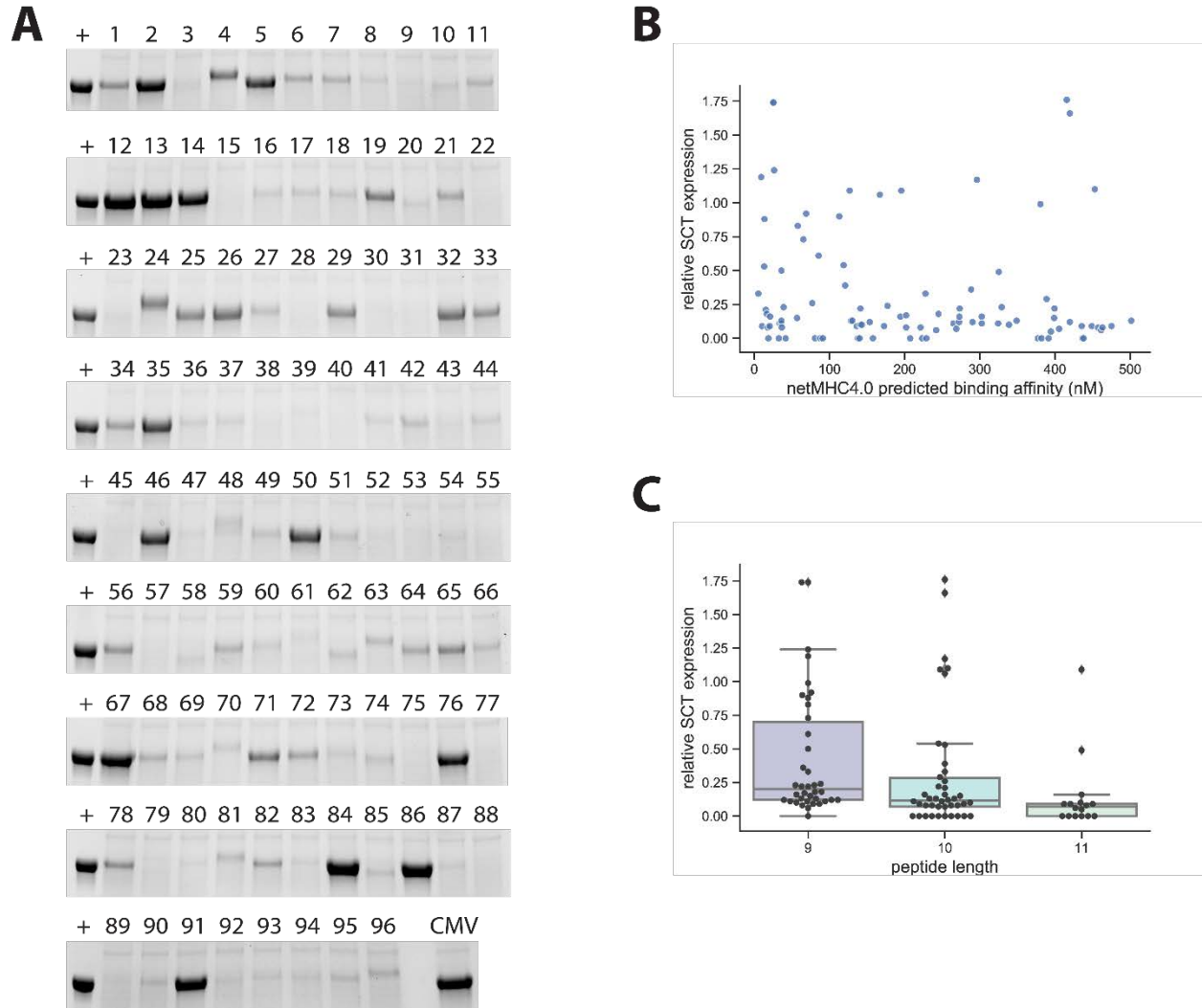
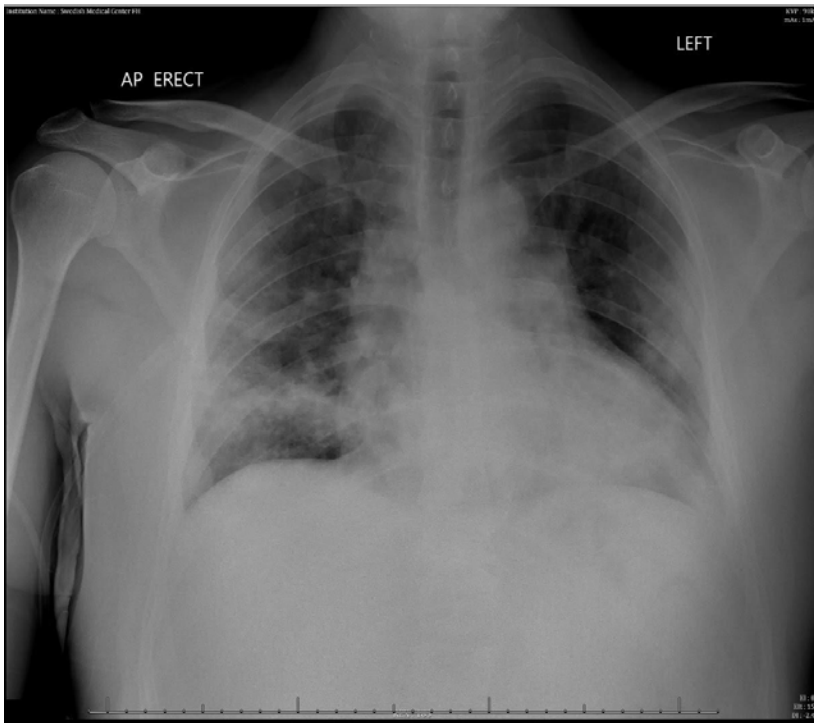


Fig. S3. Expression of HLA-A*02:01 SCTs containing SARS-CoV-2 spike protein-derived epitopes. **A.** Reduced SDS-PAGE of each transfected library element. Numbers above lanes correspond to peptide ID (Table S1). +, purified WT1 SCT as mass reference and positive control. See Table S1 for quantified relative intensities of spike protein SCTs against WT1 SCT. **B.** Scatterplot comparing relative SCT expression of spike protein SCTs against NetMHC4.0 predicted binding affinity for each peptide. **C.** Boxplot comparing relative SCT expression of spike protein SCTs against the length of each peptide.

A. Chest Radiograph.



B. Cytokine Profile:

Name:	Result (pg/mL):	Ref Range:
IL-2	<5	≤5
IL-2R	836	≤1033
IL-12	<5	≤6
INF-γ	<5	≤5
IL-4	<5	≤5
IL-5	<5	≤5
IL-10	<5	≤18
IL-13	<5	≤5
IL-17	<5	≤5
IL-1β	<5	≤5
IL-6	7	≤5
IL-8	<5	≤5

Cytokine profile (ARUP), on hospital day #2 at 14:00, prior to tocilizumab showing mildly elevated IL-6 levels.

C. Time course with relevant clinical biomarkers

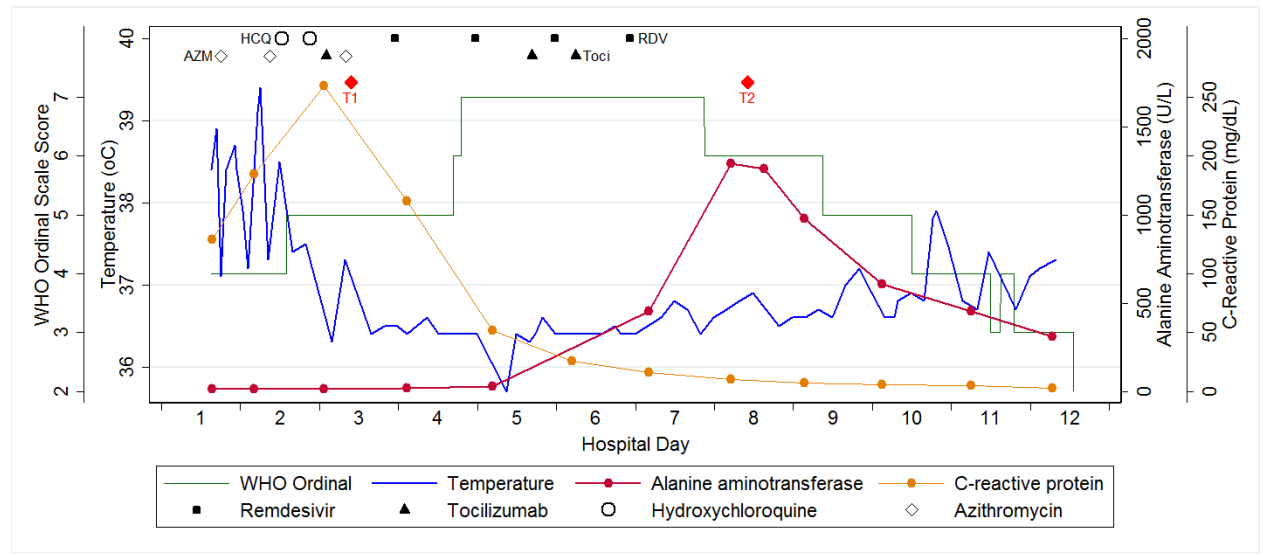


Fig. S4. Supplemental clinical data for participant InCov002. **A.** Chest radiograph at arrival in the emergency room showing patchy bilaterally pulmonary infiltrates. **B.** Cytokine profile prior to tocilizumab. **C.** Clinical time course with relevant clinical biomarkers. T1 and T2 refer to the two time points of study blood collection. Abbreviations: RDV = remdesivir, Toci = tocilizumab, HCQ = hydroxychloroquine, AZM = azithromycin.


A. CT angiography on arrival to the Emergency Room:		B. Cytokine Profile:			
		Name:	Result (pg/mL):	Ref Range:	
		IL-2	<5	≤12	
		IL-2R	203	≤1033	
		IL-12	<5	≤6	
		INF-γ	<5	≤5	
		IL-4	<5	≤5	
		IL-5	<5	≤5	
		IL-10	<5	≤18	
		IL-13	<5	≤5	
		IL-17	<5	≤13	
		IL-1β	<5	≤36	
		IL-6	74	≤5	
		IL-8	<5	≤5	
		Cytokine profile (ARUP), on hospital day #7 at 15:32 after tocilizumab, showing persistent elevation in IL-6 levels.			
C. Pulmonary Function Testing:		Baseline	%Pred	Post-BD	%Pred
FVC (L)		3.25	77%	3.42	81%
FEV ₁ (L)		1.98	63%	2.05	65%
FEV ₁ /FVC		61	81%	60	79%
FEF 25-75% (L/sec)		0.88	38%	1.27	55%
TLC (Pleth) (L)		7.24	100%		
DLCOunc (mL/min/mmHg)		21.4	82%		
D. Partial Medication List: albuterol 90 mcg/puff 2 puffs inhaled q 4-6 hours prn; albuterol 1.25 mg/3 mL nebulizer inhaled q 6 hours prn; azithromycin 250 mg PO 3 times weekly; fluticasone-umeclidinium-vilanterol 100-62.5-25 mcg/puff, 1 puff inhaled daily; predniSONE 40 mg daily X 3 days, 30 mg daily X 3 days, 20 mg daily X 3 days, 10 mg daily X 3 days starting 3/4/20; losartan 50 mg PO daily.					

Fig. S5. Supplemental clinical data for participant InCov005. **A.** Cut from computed tomography with angiography at arrival in the emergency room showing patchy geographic well-circumscribed regions of ground glass opacities predominantly throughout the periphery bilaterally with superimposed moderate to severe emphysematous changes. **B.** Cytokine profile. **C.** Pulmonary function testing 24 days prior to arrival at the emergency room with spirometry, lung volumes and diffusion capacity. **D.** Relevant home medications prior to hospitalization.

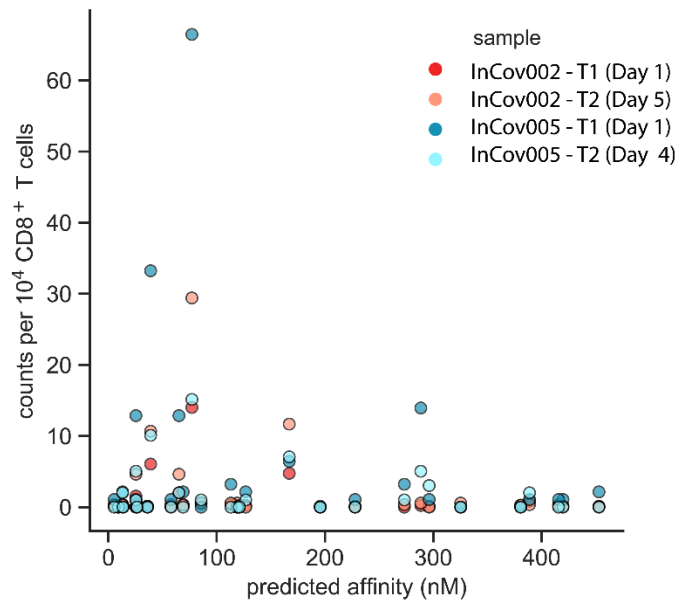


Fig. S6. Immunogenicity of spike protein peptides compared to predicted binding affinity. Scatterplot of counts per 10⁴ CD8⁺ T cells as quantified by NP-NACS for each participant sample against each peptide's NetMHC4.0 predicted binding affinity to HLA-A*02:01.