Science Advances
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Supplementary Materials for

High-resolution profiling of MHC II peptide presentation capacity reveals SARS-CoV-2 CD4 T cell targets and mechanisms of immune escape

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Published 29 April 2022, *Sci. Adv.* **8**, eabl5394 (2022)
DOI: 10.1126/sciadv.abl5394

The PDF file includes:

Figs. S1 to S7
Table S1
Legends for Data files S1 and S2

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S2

SUPPLEMENTARY MATERIALS

Supplementary Figures and Tables

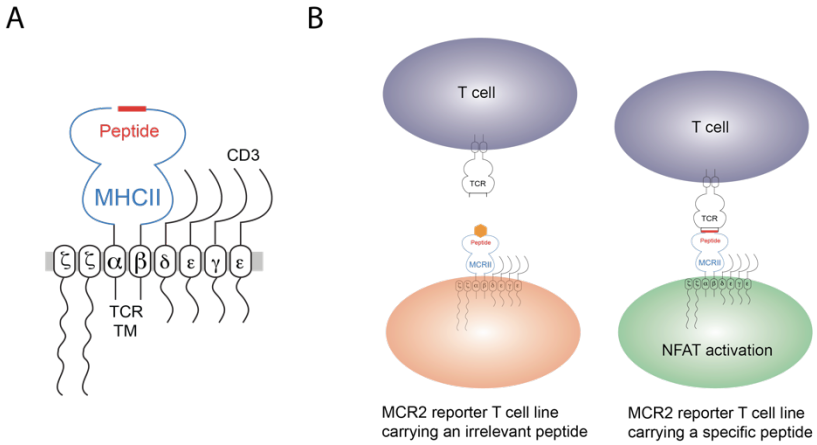

Fig. S1. The MCR system. The MCR2 is a chimeric molecule composed of the extracellular domains of the MHC class II grafted on the transmembrane regions of the TCR. The beta chain carries a covalently attached peptide. Such a chimera associates with the CD3 components of the TCR complex and can signal like a TCR. Expressed in a TCR-negative reporter T cell line it can transduce a signal when a TCR binds to it.

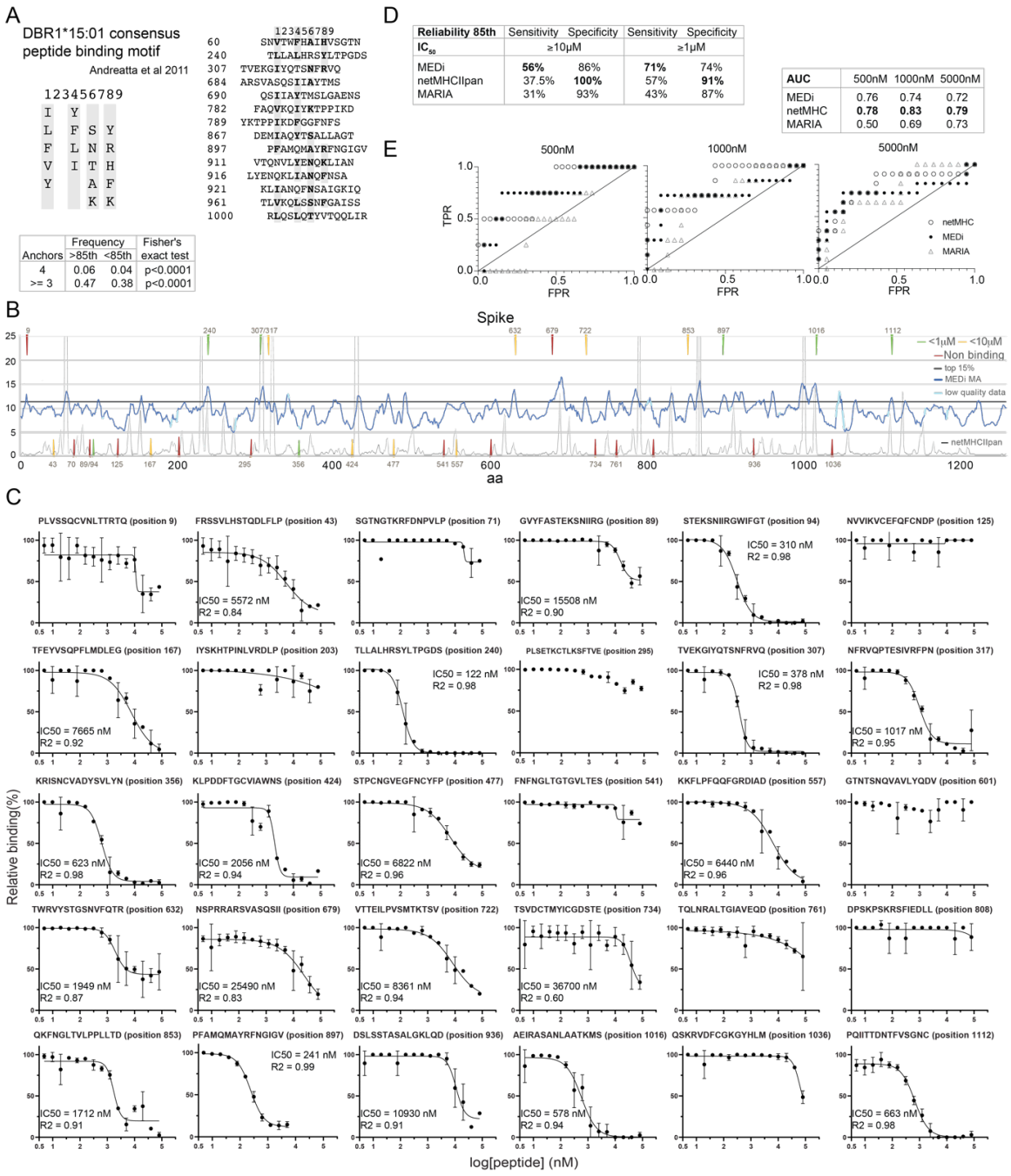

Fig. S2. MEDi analysis of Spike peptide presentability by DRB1*15:01 compared to netMHCIIpan and MHC binding IC_{50}. **A**, Sequence comparison of Spike peptides representative for the major peaks above the 85th percentile containing at least 6 peptides. Residues matching the HLA binding consensus are highlighted in grey. Table shown fraction of peptides with 4, or at least 3, correct anchor residues among peptides above the 85th percentile cut-off, or below. **B**, MEDi-MA score graphs (dark blue line) for all Spike-derived peptides presented by DRB1*07:01. The horizontal black line indicates the 85th percentile threshold and the light blue color datapoints on the MEDi-MA graphs below the quality threshold (see M&M). Arrows indicate peptides chosen for HLA-binding IC_{50} calculation by the fluorescence polarization assay, color-coded dependent on the result of the binding assay._Thin black line indicates netMHCIIpan prediction scores scaled to fit on the same plot (20/Rank_EL, threshold for weak presentation > 2). **C**, Results of the competitive peptide binding fluorescence polarization assay for individual peptides. IC_{50} and R^2 values are shown. **D**, Reliability of the assay was tested by calculating the sensitivity and specificity. **E**, ROC curves of the MEDi-MA, netMHCIIpan and MARIA scores qualifying peptides as HLA-binders. Calculations were done for peptides analyzed in C, positive binding thresholds at IC_{50} of 500nM, 1mM or 5mM.

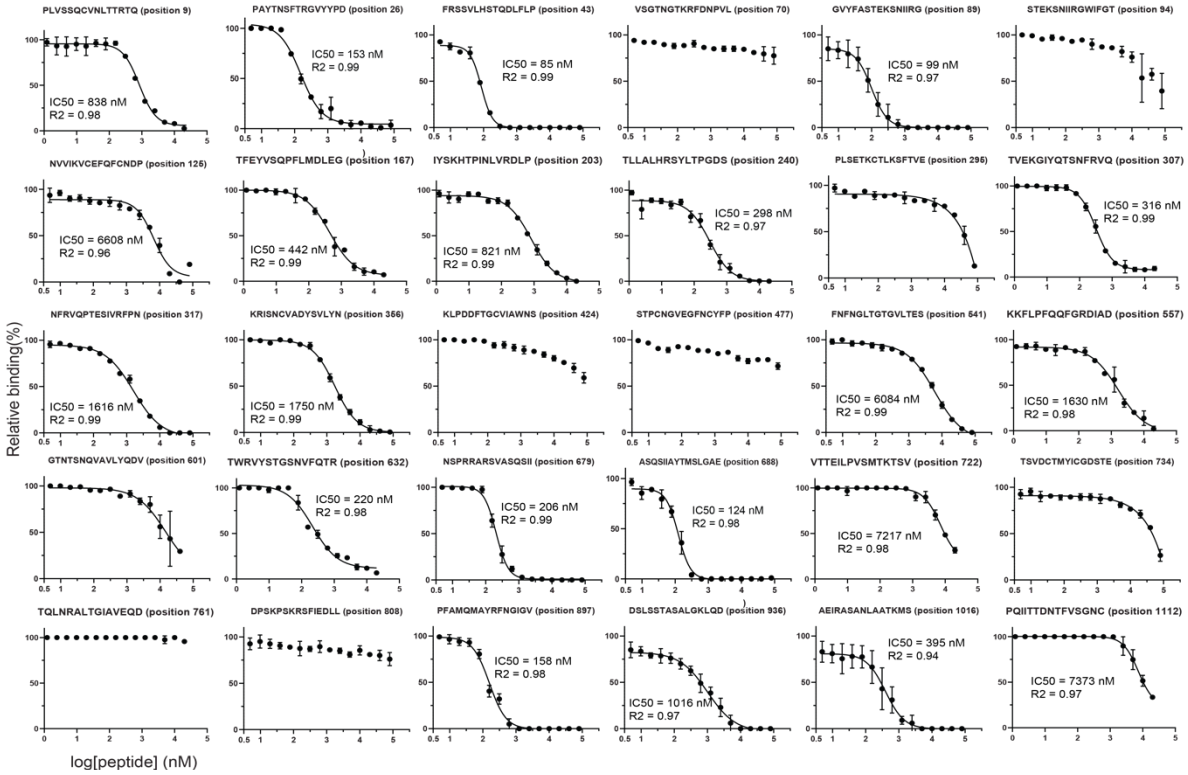

Fig. S3. Competitive peptide binding assay results for DRB1*07:01.

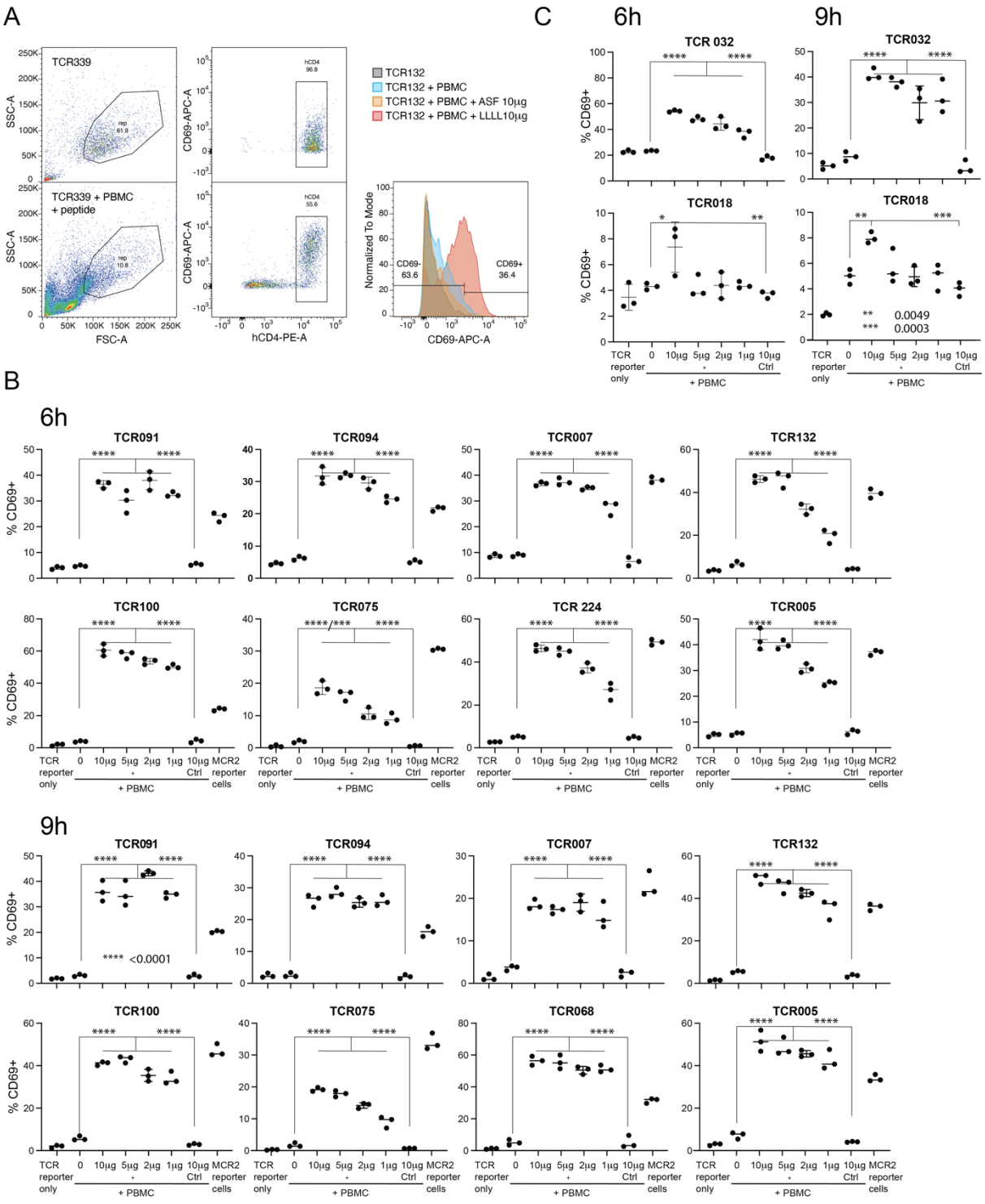

Fig. S4. Validation of TCR reactivity. TCR carrying reporter cell lines (hCD4^{+}) were cocultured with PBMCs pulsed with the identified peptides (Fig.4D, purity >95%). Peptide concentrations of 10, 5, 2 and 1µg/ml were used. **A**. Example gating and analysis strategy are shown for reporters alone and PBMC cocultures. **B**. Cocultures with MHC class II restricted peptides for 6h and 9h. **C.** Cocultures with MHC class I restricted peptides for 6h and 9h.

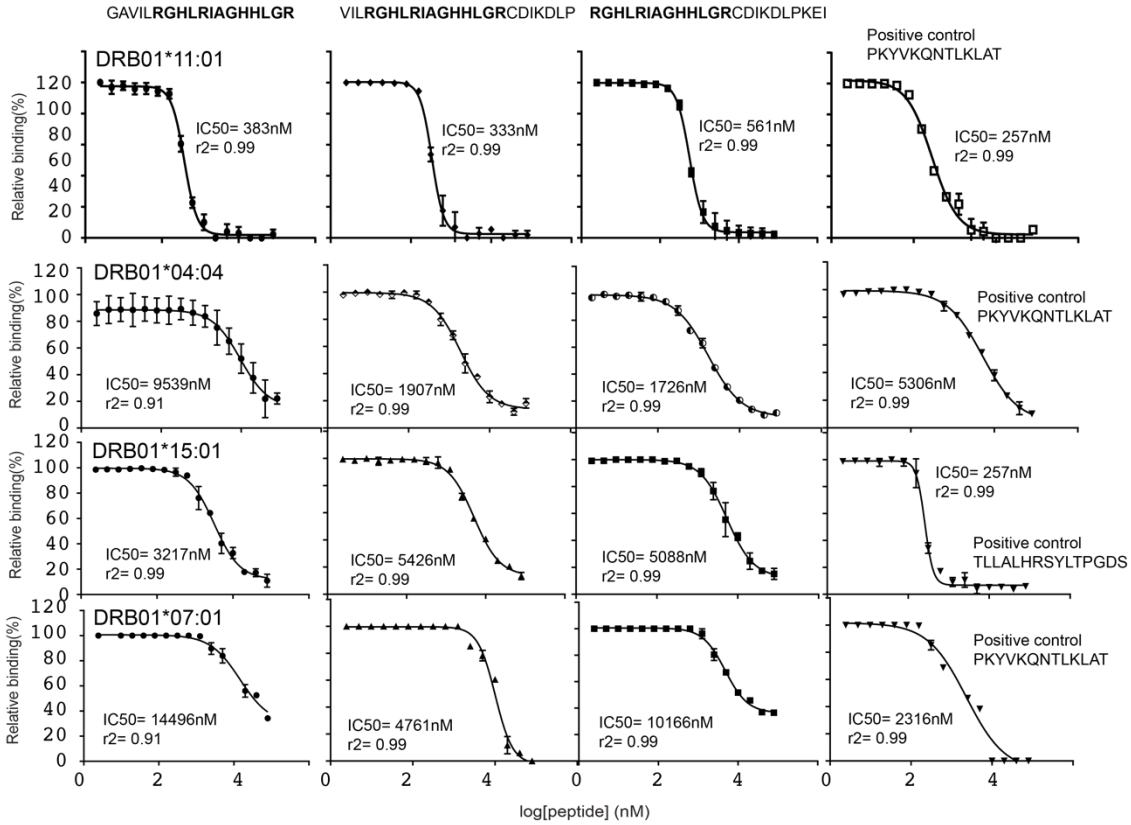

Fig. S5. Competitive peptide binding assay confirming the MEDi results.

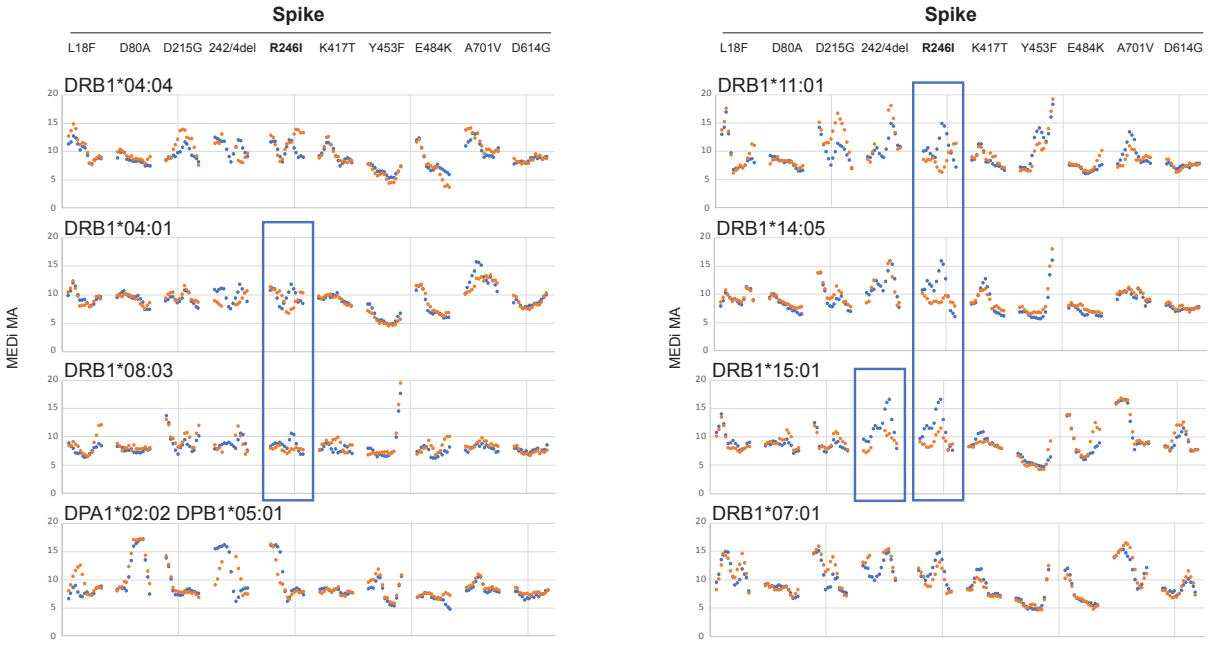

Fig. S6. Supplementary MEDi analysis of mutated peptides present in arising SARS-CoV-2 variants.

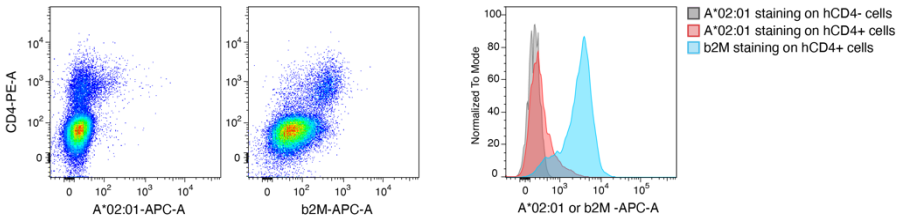

Fig. S7. Expression of SCTz on the surface of reporter cells. Flow cytometric analysis of reporter cells transduced with the SCTz-A*02:01 peptide library and stained for hCD4, A*02:01 and b2M. All transduced (hCD4+) cells express the SCTz on the surface, as shown by the staining with the b2M antibody. However, not all are stained by the A*02:01 antibody, indicating misfolding of the A*02:01 part of the SCTz on a substantial fraction of the cells.

Table ST1. Patient HLAs.

Patient	C141	C142	C143	C144	C146	C148	C149
CD4 TCRs	10	5	8	4	5	10	5
CD8 TCRs	7	9	8	15	10	8	5
All class I alleles carry 10aa peptides across the whole SARS-CoV-2 genome (1aa shifts)							
HLA class I	A*30:01	A*02:06	A*32:01	A*11:303	A*03:218	A*02:06	A*11:01
	A*02:07	A*26:01	A*26:01	A*11:03	A*02:03	A*11:274	A*11:303
	B*13:02	B*46:01	B*44:128	B*51:01	B*07:02	B*50:01	B*48:01
	B*46:01	B*40:06	B*40:06	B*52:01	B*37:01	B*51:01	B*51:01
	C*01:02	C*03:37	C*04:01	C*07:118	C*06:02	C*14:02	C*14:02
	C*06:02	C*01:126			C*07:68	C*06:02	C*08:01
All class II alleles carry 15 or 23aa peptides across the whole SARS-CoV-2 genome (1aa shifts)							
HLA class II	DPA1*02:01 DPB1*02:01	DPA1*02:02 DPB1*02:02	DPA1*02:02 DPB1*02:02	DPA1*01:03 DPB1*04:01	DPA1*02:02 DPB1*04:01	DPA1*02:01 DPB1*02:01	DPA1*02:02 DPB1*02:01
	DPA1*01:03 DPB1*02:01	DPA1*02:02 DPB1*05:01	DPA1*02:02 DPB1*05:01	DPA1*01:03 DPB1*04:02	DPA1*01:03 DPB1*04:01	DPA1*01:03 DPB1*02:01	DPA1*02:01 DPB1*02:01
	DPA1*02:01 DPB1*14:01				DPA1*02:02 DPB1*05:01	DPA1*02:01 DPB1*10:01	DPA1*02:02 DPB1*13:01
	DPA1*01:03 DPB1*14:01	DQA1*06:01 DQB1*03:01	DQA1*06:01 DQB1*02:12	DQA1*01:04 DQB1*05:56	DPA1*01:03 DPB1*05:01	DPA1*01:03 DPB1*10:01	DPA1*02:01 DPB1*13:01
		DQA1*01:03 DQB1*03:01	DQA1*02:01 DQB1*02:12	DQA1*01:02 DQB1*05:56			
	DQA1*03:01 DQB1*03:01	DQA1*06:01 DQB1*06:01	DQA1*06:01 DQB1*03:01	DQA1*01:04 DQB1*05:02	DQA1*03:02 DQB1*03:03	DQA1*01:04 DQB1*02:02	DQA1*01:04 DQB1*05:56
	DQA1*05:05 DQB1*03:01	DQA1*01:03 DQB1*06:01	DQA1*02:01 DQB1*03:01	DQA1*01:02 DQB1*05:02	DQA1*01:02 DQB1*03:03	DQA1*02:01 DQB1*02:02	DQA1*01:04 DQB1*05:03
	DQA1*05:05 DQB1*03:02				DQA1*03:02 DQB1*06:03	DQA1*01:04 DQB1*05:03	
	DQA1*03:01 DQB1*03:02	DRA1*01:02 DRB1*12:02	DRA1*01:01 DRB1*12:02	DRA1*01:01 DRB1*16:02	DQA1*01:02 DQB1*06:03	DQA1*02:01 DQB1*05:03	DRA1*01:02 DRB1*14:05
		DRA1*01:02 DRB1*08:03	DRA1*01:02 DRB1*12:02	DRA1*01:02 DRB1*16:02			DRA1*01:02 DRB1*14:04
	DRA1*01:01 DRB1*04:03	DRA1*01:02 DRB3*03:01	DRA1*01:01 DRB1*07:01	DRA1*01:01 DRB1*14:04	DRA1*01:01 DRB1*09:01	DRA1*01:01 DRB1*07:01	DRA1*01:02 DRB3*02:02
	DRA1*01:01 DRB1*11:01	DRA1*01:02 DRB5*01:01	DRA1*01:02 DRB1*07:01	DRA1*01:02 DRB1*14:04	DRA1*01:02 DRB1*09:01	DRA1*01:02 DRB1*07:01	
	DRA1*01:01 DRB3*02:02	DRA1*01:02 DRB5*02:02	DRA1*01:01 DRB3*03:01	DRA1*01:01 DRB3*01:01	DRA1*01:01 DRB1*15:01	DRA1*01:01 DRB1*14:04	
	DRA1*01:01 DRB5*01:01		DRA1*01:02 DRB5*02:02	DRA1*01:02 DRB3*01:01	DRA1*01:02 DRB1*15:01	DRA1*01:02 DRB1*14:04	
	DRA1*01:01 DRB5*02:02			DRA1*01:01 DRB5*02:02	DRA1*01:01 DRB5*01:01	DRA1*01:01 DRB3*02:02	
				DRA1*01:02 DRB5*02:02	DRA1*01:02 DRB5*01:01	DRA1*01:02 DRB3*02:02	

Supplementary Files

excel file S1: Numerical MEDi MA5 data and presentable peptides derived from the SARS-CoV-2 genome by MEDi. 15aa peptides from the center of peaks having at least 5 consecutive peptides with MEDi-MA scores above the 85th percentile are selected.
excel file S2: TCR screening data.