

## **Supplemental information**

### **Identification of TCR repertoires in functionally competent cytotoxic T cells cross-reactive to SARS-CoV-2**

Shimizu K et al.

#### **Inventory:**

#### **-Methods**

#### **-References**

#### **-Extended Data**

**Extended Data Fig. 1** Data on HLA-A\*02:01 positive population worldwide and homology of amino acid sequences in coronaviruses

**Extended Data Fig. 2** Memory T cell response for CMV or EBV in HLA-A\*24:02 positive UHD

**Extended Data Fig. 3** Cross-reactivity of SARS-CoV-2-specific CD8<sup>+</sup> T cells to other coronaviruses

**Extended Data Fig. 4** Homology of amino acid sequences in various regions of SARS-CoV-2 Spike protein

#### **-Supplementary Tables**

**Table S1.** Characteristics of HLA-A24<sup>+</sup> healthy volunteers

**Table S2.** Characteristics of HLA-A24<sup>+</sup> patients with hematological malignancies

**Table S3.** Data collection and refinement statistics of crystal structure

**Table S4.** List of peptides

**Table S5.** List of antibodies

**Table S6.** Single cell TCR sequence and cloning primer list

## **Methods**

### **Human samples and preparation**

PBMCs were obtained from healthy blood donors at our Institute or buffy coats from healthy blood donors (2004-2010) (Tokyo Red Cross, Tokyo, Japan) and separated by Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) density centrifugation.

Peripheral blood samples from 28 patients with hematological malignancies (HM) were obtained from the National Hospital Organization Kumamoto Medical Center (Kumamoto, Japan). The PBMCs were washed twice with phosphate-buffered saline (PBS) and stored in liquid nitrogen until further use. Plasma samples from healthy donors and HM-patients were assessed by anti-SARS-CoV-2 IgG ELISA kit (EUROIMMUN) and confirmed negative. Information on the healthy blood donors and characteristics of the HM patients is listed in **Tables S1 and S2**, respectively. Written informed consent was obtained from all patients according to the Declaration of Helsinki. All experiments were performed with authorization from the Institutional Review Board for Human Research at RIKEN IMS. HLA-A24<sup>+</sup> donors were selected using flow cytometry for T-cell analysis.

### **HLA allele frequency**

Data related to HLA-A\*24:02 and HLA-A\*02:01 alleles from 27 different countries were obtained from the Allele Frequency Net Database (AFND) ([www.allelefrequencies.net](http://www.allelefrequencies.net))<sup>1</sup>. The allele frequency of each country was determined by averaging the allele frequencies of that country.

### **Information related to COVID-19**

Data Availability Statement: Publicly available datasets were analyzed in this study.

This data can be found at: [https://www.who.int/emergencies/diseases/novel-coronavirus-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactivetimeline?gclid=CjwKCAiAl4WABhAJEiwATUnEF5RRrhA4IGUZEpKYWv7jCsg3jdx1A_i1IROWObN_C3YIL7JkYNP5yRoC8cMQAvD_BwE#event-115)

2019/interactivetimeline?gclid=CjwKCAiAl4WABhAJEiwATUnEF5RRrhA4IGUZEp

KYWv7jCsg3jdx1A\_i1IROWObN\_C3YIL7JkYNP5yRoC8cMQAvD\_BwE#event-115

(accessed on 16 February 2021).

### **Cell lines**

CIR cells expressing HLA-A\*24:02 (A24/CIR) were kindly provided by Dr. Masafumi Takiguchi (Kumamoto University). The SKW-3 (T-ALL) line and TCR  $\alpha/\beta$ <sup>-/-</sup> mice were procured from RIKEN BRC (Japan).

## Epitope prediction and peptide selection

The spike protein CD8<sup>+</sup> T epitopes of SARS-CoV-2 and other HCoV for HLA-A\*24:02 were predicted using the Immune Epitope Database and Analysis Resource (IEDB) (<https://www.iedb.org/>)<sup>2</sup> and NetMHC 4.0

(<http://www.cbs.dtu.dk/services/NetMHC/>)<sup>3, 4</sup> The corresponding protein accession identification numbers are: SARS-CoV-2 isolate, Wuhan-Hu-1 NCBI:

YP\_009724390.1, SARS-CoV-1 NCBI: NP\_828852.2, MERS NCBI:

YP\_009047204.1, HCoV(HKU1) NCBI: YP\_173238.1, HCoV(OC43) NCBI:

YP\_009555241.1, HCoV(NL63) NCBI: YP\_003767.1, HCoV(229E) NCBI:

NP\_073551.1. NFKDQVILL in the nucleocapsid phosphoprotein has been previously reported as a SARS-CoV-2 dominant T cell epitope<sup>5</sup>

## Reagents

All peptides were synthesized by GeneScript (summarized in **Table S4**). The peptides were solubilized in a high-concentration solution of DMSO. Human IL-2 was purchased from Shionogi Co., Ltd..

## Flow cytometry

Antibodies were purchased from BD Bioscience, Biolegend, or e-Bioscience (summarized in **Table S5**). A Fixable Aqua or Violet Dead Cell Stain Kit (Invitrogen) was used to eliminate the dead cells. Intracellular staining for cytokines was performed using a BD Intracellular Cytokine Staining Kit (BD). The LSR Fortessa X-20 instrument and FACSDiva (v 8.0.1) (BD Biosciences) or FlowJo software (v10.3B2) were used for data analyses.

### **In vitro expansion of SARS-CoV-2 specific T cells**

PBMCs ( $3\text{--}5 \times 10^6$  cells) were cultured with the indicated peptide or peptide mixtures (each peptide  $10 \mu\text{g/mL}$ ) in the culture medium in the presence of IL-2 ( $100 \text{ U/mL}$ ) and restimulated with irradiated autologous PBMCs pulsed with each peptide on days 7 and 14. In some experiments,  $\text{CD8}^+$  T cells ( $0.5\text{--}1 \times 10^6$  cells) were isolated from PBMCs using CD8 MACS beads (Miltenyi Biotec), and the isolated cells were co-cultured with irradiated peptide-pulsed autologous PBMCs in the presence of IL-2 ( $100 \text{ U/mL}$ ) and further restimulated with irradiated autologous PBMCs pulsed with each peptide on Day 7. The harvested cells were restimulated with or without the indicated peptide in the presence of brefeldin A ( $50 \mu\text{g/mL}$ ) and monensin ( $750 \text{ ng/mL}$ ) for 16 h and analyzed using flow cytometry. Generation of peptide-specific  $\text{CD8}^+$  T cells was determined by a

1.5-fold increase in +pep vs. –pep as a responder.

### **Single-cell TCR sequencing and construction of retroviral TCR vector**

Single-cell TCR sequencing was performed as described previously<sup>6</sup>, with some modifications. All primers used are listed in **Table S6**. CoV-2 pep#3-specific CD8<sup>+</sup> T cell lines were restimulated with anti-human CD107a-BV421 in the presence or absence of 10  $\mu$ M CoV-2 pep#3 for 6 h and then stained with anti-human CD8-PE and Aqua. The peptide-specific CD8<sup>+</sup>CD107a<sup>+</sup> cells were sorted as single cells into 5  $\mu$ L of RT-PCR mix in 96 wells plate, using FACS Aria III (BD). The RT-PCR mix comprised 1  $\mu$ L 5 $\times$  Prime STAR GXL Buffer, 0.45  $\mu$ L RT-PCR primer mix, 0.4  $\mu$ L of 2.5 mM dNTP, 0.05  $\mu$ L of 40 U/ $\mu$ L RNase Inhibitor, 200 U/ $\mu$ L PrimeScript II Reverse Transcriptase (Takara Bio Inc.), 0.05  $\mu$ L of 1.25 U/ $\mu$ L PrimeSTAR GXL DNA Polymerase (Takara Bio Inc.), and 2.95  $\mu$ L nuclease-free water. The program used for the one-step RT-PCR was as follows: 45 °C for 40 min, 98 °C for 1 min, and 35 cycles of 98 °C for 10 s, 55 °C for 15 s, and 68 °C for 1 min. The resultant PCR products were diluted 10-fold with nuclease-free water and used as templates for the 2nd-PCR. 2nd-PCR was performed using PrimeSTAR GXL with pMXs-BamHI-InFusion primer and CA-rev2 primer for TCR $\alpha$  or CB-rev2 primer for TCR $\beta$  in 10  $\mu$ L reaction volume. The program

for the 2nd-PCR was as follows: 98 °C for 1 min and 44 cycles of 98 °C for 10 s, 55 °C for 15 s, and 68 °C for 1 min. The 2nd-PCR products were treated with ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific K.K.) and sequenced using the CA-rev3 primer for TCR $\alpha$  or CB-rev3 primer for TCR $\beta$ . Sequencing results were analyzed using the V-QUEST tool of the IMGT database ([http://www.imgt.org/IMGT\\_vquest/input](http://www.imgt.org/IMGT_vquest/input))<sup>7</sup>. For cloning the TCR, VDJ and C regions were amplified from the 2nd-PCR products and PBMC-derived cDNAs of the donors, respectively. PrimeSTAR Max was used for amplification with the pMXs-BamHI-InFusion and CA-rev3 primers or CB-rev3 primers. Each fragment was cloned into BamHI-digested linearized pMXs-IRES-GFP for TCR $\alpha$  or -TdTomato for TCR $\beta$  by the InFusion HD reaction.

### **Establishment of SKW-3-CD8AB expressing cells**

SKW-3 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, 55 mM 2-ME, and 1% penicillin/streptomycin. hCD8A-IRES-Puro and hCD8B-IRES-Puro VSV-G retroviruses were transduced into the SKW-3 cells in the presence of 5  $\mu$ g/mL polybrene (Millipore) by centrifugation at 2300 rpm for 90 min at 35 °C. CD8AB expressing cells were stained with PE-CD8A (RPA-T8) and BV421-CD8B (2ST8.5H7)

and sorted twice using FACS Aria III. SKW-3-CD8AB expressing cells were maintained with 3 µg/mL puromycin (InvivoGen).

### **TCR clonotyping and $\alpha\beta$ TCR cell line generation**

The concentrated TCR V $\alpha$  and TCR V $\beta$  viruses were used to infect  $2 \times 10^6$  SKW-3-CD8AB cells with 5 µg/mL polybrene (Millipore) by centrifugation for 1 h at 1500 xg at 35 °C. The virus was removed, and the media for cell culture was replaced. TCR-transduced SKW-3-CD8AB cells were enriched by sorting the GFP<sup>+</sup> tdTOMATO<sup>+</sup> cells.

### **Functional assay of TCR-transduced SKW-3-CD8AB cells**

The transduced SKW-3-CD8AB cells were co-cultured with A24/CIR pulsed with the indicated peptide in a 1:1 ratio for 16 h. CD69 was measured to detect early T cell activation by flow cytometry.

### **Cytotoxicity assays**

CD8<sup>+</sup> T-cell lines were cultured for three weeks and then harvested as effector cells. T-cell cytotoxicity assays were performed using the following methodology: CFSE (Molecular Probe)-labeled A24-CIR cells were pulsed with the indicated peptide or



DMSO for 2 h and washed twice. Effector cells were cultured with  $1 \times 10^4$  target cells at an effector/target cell ratio of 12.5, 25, and 50 for 6 h and then stained with TO-PRO3 (Molecular Probe) immediately prior to their analysis to identify dead cells.

Spontaneous target cell death (SD) was determined by labeling the target cells that were cultured alone. As a positive control for total cytotoxicity (TD), the labeled target cells were permeabilized with BD Cytotfix/Cytoperm reagent (BD Pharmingen). Specific lysis was calculated using the following formula:  $(\text{Sample-SD}/\text{TD-SD}) \times 100$ . The cells were analyzed using flow cytometry.

### **Protein expression and purification**

HLA-A24 alpha chain and Beta-2-microglobulin with an N-terminal histidine tag and a TEV protease cleavage site were co-expressed in a cell-free expression system<sup>8, 9</sup>. The cell-free reaction mixture was prepared with 0.15 mg/mL of each peptide (CoV2, 229E, and HKU1). The HLA-A\*24:02•peptide complexes were purified by chromatography using a HisTrap column (Cytiva) and subjected to TEV protease digestion. Protein solutions were then applied to the HisTrap column to remove the histidine-tag. The flow-through fractions were subsequently purified using HiTrap Q and Superdex200 gel filtration chromatography (Cytiva). The HLA-A\*24:02•peptide complexes were

concentrated in 20 mM Tris-HCl buffer (pH 8.0) containing 50 mM NaCl.

### **Crystallization and data collection**

Crystallization was performed using the sitting-drop vapor-diffusion method at 293 K.

The HLA-A\*24:02•CoV2 peptide complex crystals were grown in 1.0 M Na/K

phosphate (pH 8.2) at a concentration of 6.6 mg/mL. The HLA-\*24:02•229E peptide

complex crystals were grown in 0.2 sodium citrate and 20 % PEG3350 at a

concentration of 6.9 mg/mL. The HLA-\*24:02•HKU1 peptide complex crystals were

grown in 0.1 M Tris-HCl (pH 8.5) and 25 % PEG3350 at a concentration of 2.4 mg/mL.

Data collection was carried out at 100 K with 10% glycerol as a cryoprotectant. Data

were collected at BL26B2<sup>10-13</sup> and BL32XU<sup>14</sup> of the SPring-8 synchrotron beamlines.

The diffraction data were processed and scaled using the ZOO/KAMO: Automated Data

Processing System for Microcrystals<sup>15, 16</sup> and the CCP4 software suite<sup>17</sup>.

### **Structure determination and refinement**

The structures were determined using the molecular replacement method (MR) with

Phaser<sup>18</sup>. The coordinates of the HLA-A\*24:02 complex with the newly identified

peptide (PDB ID: 4F7M) were used as the search model. The model was corrected and

further refined using Phenix<sup>19</sup> and Coot<sup>20</sup>. The data collection and refinement statistics are summarized in Table 1. The ribbon models in the figures are depicted using PyMOL software (<http://www.pymol.org>).

### **Accession numbers**

The structures of HLA-A\*24:02•peptide (CoV2, 224E and HKU1) complexes were deposited in the RCSB Protein DataBank (PDB) under the accession codes 7EJL, 7EJM and 7EJN respectively.

### **Statistical analysis**

Statistical analysis was performed StatMate V (Nihon 3B Scientific Inc.). Fisher's/Chi-square tests were used to compare ratio difference between two groups. Data were analyzed using Turkey or Neuman-Keuls test for non-parametric multiple comparisons;

\*\*\*P < 0.001; \*\*P < 0.01, \*P < 0.05.

### **Data availability**

Data related to the findings of this study are available from the corresponding author upon reasonable request. Source data are provided in this article.

## References

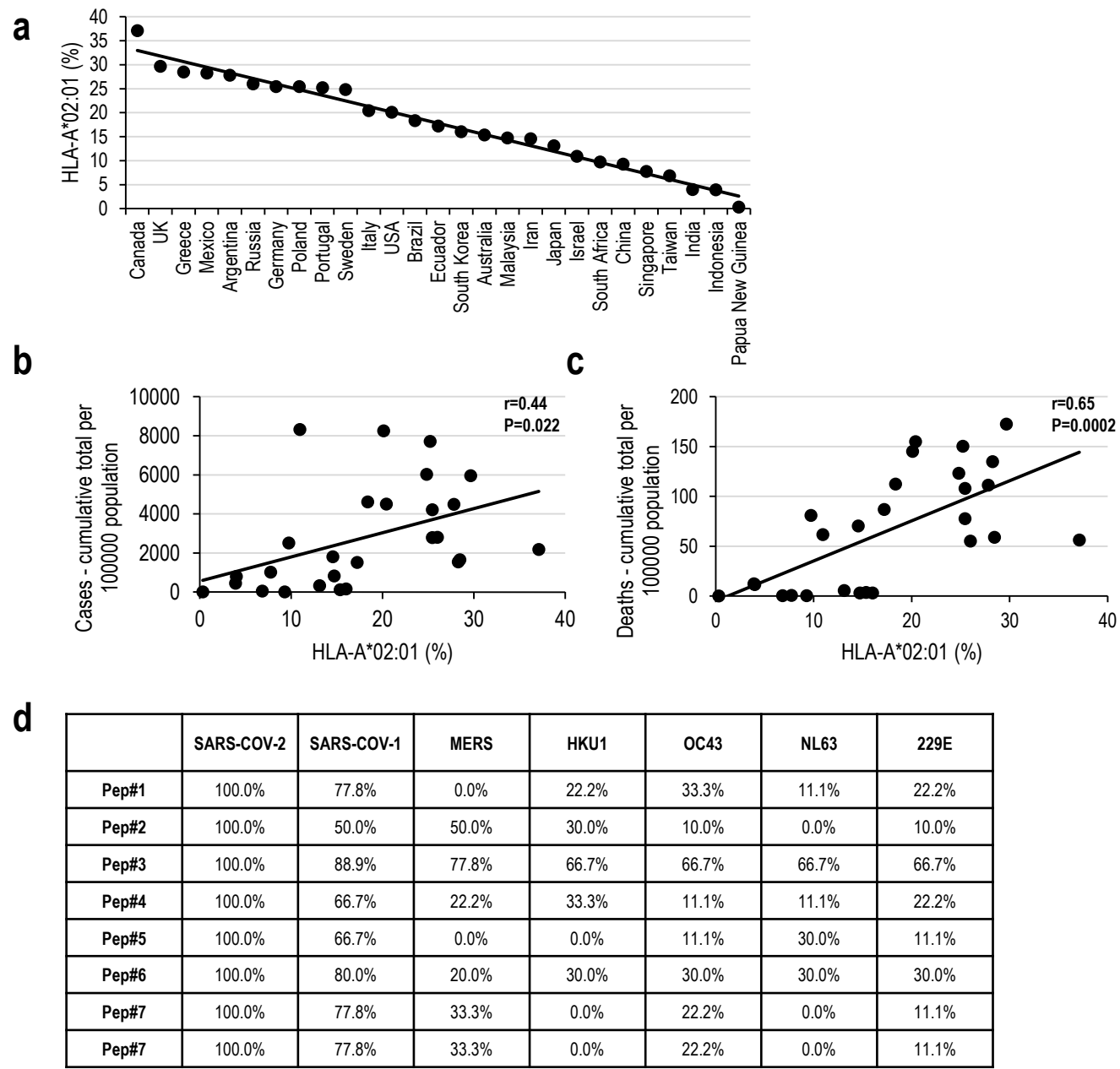
1. Gonzalez-Galarza, F.F. *et al.* Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acids Res* **48**, D783-D788 (2020).
2. Vita, R. *et al.* The Immune Epitope Database (IEDB): 2018 update. *Nucleic Acids Res* **47**, D339-D343 (2019).
3. Andreatta, M. & Nielsen, M. Gapped sequence alignment using artificial neural networks: application to the MHC class I system. *Bioinformatics* **32**, 511-517 (2016).
4. Nielsen, M. *et al.* Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. *Protein Sci* **12**, 1007-1017 (2003).
5. Grifoni, A. *et al.* A Sequence Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune Responses to SARS-CoV-2. *Cell Host Microbe* **27**, 671-680 e672 (2020).
6. Hamana, H., Shitaoka, K., Kishi, H., Ozawa, T. & Muraguchi, A. A novel, rapid and efficient method of cloning functional antigen-specific T-cell receptors from single human and mouse T-cells. *Biochem Biophys Res Commun* **474**, 709-714 (2016).
7. Giudicelli, V., Brochet, X. & Lefranc, M.P. IMGT/V-QUEST: IMGT standardized analysis of the immunoglobulin (IG) and T cell receptor (TR) nucleotide sequences. *Cold Spring Harb Protoc* **2011**, 695-715 (2011).
8. Katsura, K. *et al.* A reproducible and scalable procedure for preparing bacterial extracts for cell-free protein synthesis. *J Biochem* **162**, 357-389 (2017).
9. Matsuda, T. *et al.* Cell-free synthesis of functional antibody fragments to provide a structural basis for antibody-antigen interaction. *PLoS One* **13**, e0193158 (2018).
10. Ueno, G. *et al.* RIKEN structural genomics beamlines at the SPring-8; high

throughput protein crystallography with automated beamline operation. *J Struct Funct Genomics* **7**, 15-22 (2006).

11. Ito, S., Ueno, G. & Yamamoto, M. DeepCentering: fully automated crystal centering using deep learning for macromolecular crystallography. *J Synchrotron Radiat* **26**, 1361-1366 (2019).
12. Okazaki, N. *et al.* Mail-in data collection at SPring-8 protein crystallography beamlines. *J Synchrotron Radiat* **15**, 288-291 (2008).
13. Murakami, H., Ueno, G., Shimizu, N., Kumasaka, T. & Yamamoto, M. Upgrade of automated sample exchanger SPACE. *J Appl Crystallogr* **45**, 234-238 (2012).
14. Hirata, K. *et al.* Achievement of protein micro-crystallography at SPring-8 beamline BL32XU. *J Physics* **425**, 012002 (2013).
15. Hirata, K. *et al.* ZOO: an automatic data-collection system for high-throughput structure analysis in protein microcrystallography. *Acta Crystallogr D Struct Biol* **75**, 138-150 (2019).
16. Yamashita, K., Hirata, K. & Yamamoto, M. KAMO: towards automated data processing for microcrystals. *Acta Crystallogr D Struct Biol* **74**, 441-449 (2018).
17. Winn, M.D. *et al.* Overview of the CCP4 suite and current developments. *Acta Crystallogr D Biol Crystallogr* **67**, 235-242 (2011).
18. McCoy, A.J. *et al.* Phaser crystallographic software. *J Appl Crystallogr* **40**, 658-674 (2007).
19. Adams, P.D. *et al.* PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* **66**, 213-221 (2010).
20. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **60**, 2126-2132 (2004).



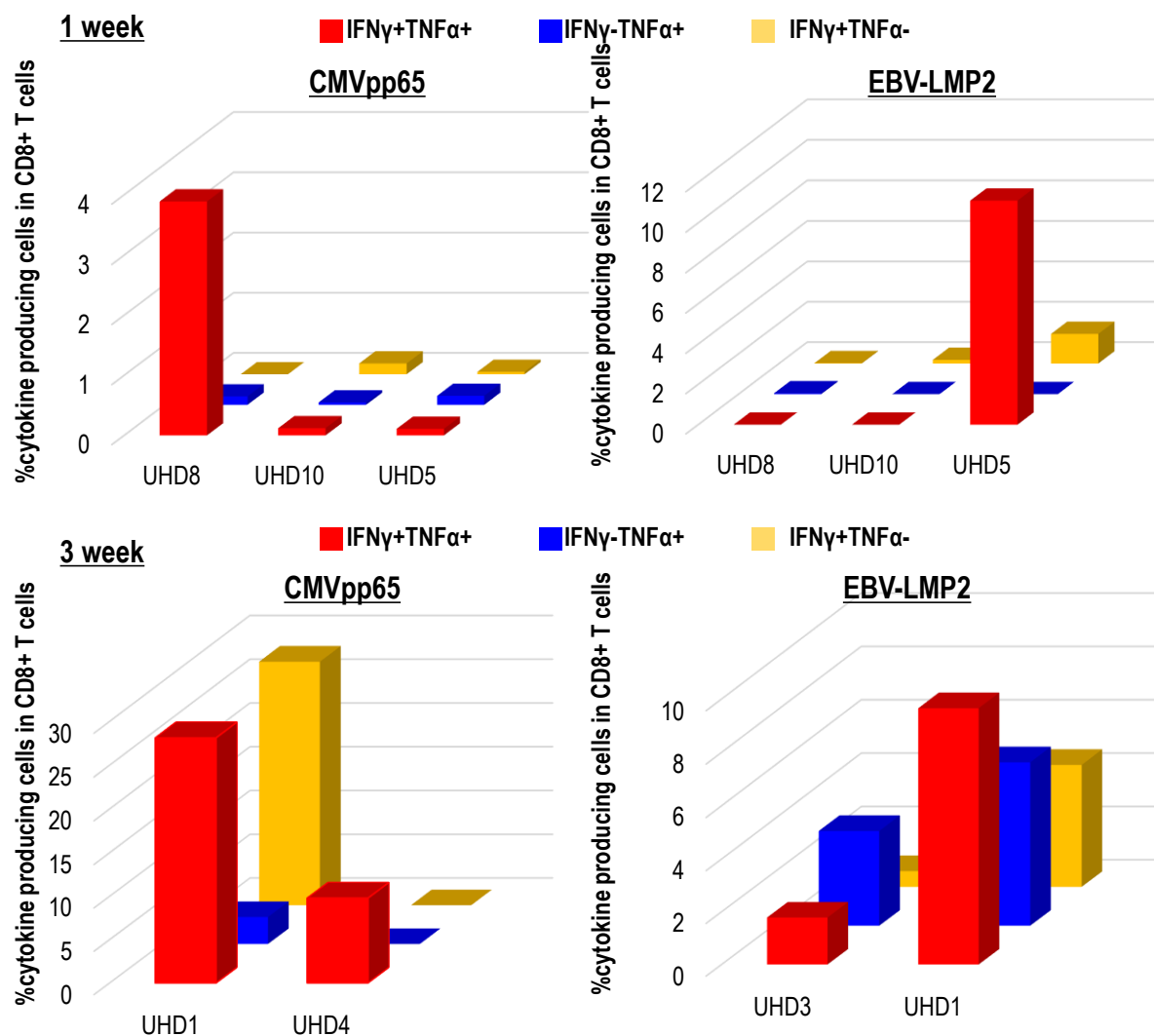
Extended Data Fig. 1



**Extended Data Fig. 1 Data on HLA-A\*02:01 positive population worldwide and homology of amino acid sequences in coronaviruses (related to Fig. 1)**

**a.** Data on HLA-A\*02:01 positive population in the indicated countries. **b, c.** Correlation of HLA-A\*02:01 and COVID-19 cases- cumulative total per 10<sup>5</sup> population (**b**) and COVID-19 deaths- cumulative total per 10<sup>5</sup> population (**c**). Pearson’s correlation coefficients (Pearson’s *r*) are shown. **d.** Homology of amino acid sequences in relevant peptide epitopes of other coronaviruses compared to that of SARS-CoV-2.

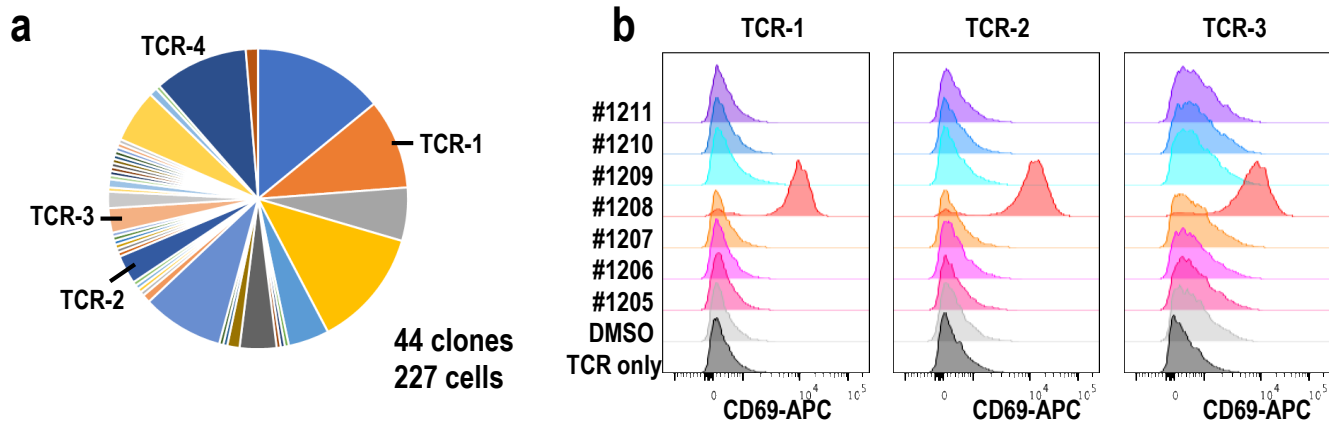
## Extended Data Fig. 2



**Extended Data Fig. 2 Memory T cell response for CMV or EBV in HLA-A\*24:02 positive UHD (related to Fig. 2)** PBMCs from HLA-A\*24:02 positive UHD were cultured with peptides of CMVpp65 or EBV-LMP2 having high affinity to HLA-A\*24:02 individually, and restimulated with the same peptides weekly. The polyfunctionality of peptide-specific CD8<sup>+</sup> T cells was determined by the frequency of IFN- $\gamma$ <sup>+</sup>TNF- $\alpha$ <sup>+</sup> CD8<sup>+</sup> T cells by intracellular cytokine staining on days 7 (upper panel) and 21 (lower panel).



## Extended Data Fig. 3



**Extended Data Fig. 3 Cross-reactivity of SARS-CoV-2-specific CD8<sup>+</sup> T cells to other coronaviruses** **a.** As described in Fig. 4, the single-cell TCR sequences in CoV-2 pep#3-specific CD8<sup>+</sup> T cells were analyzed from 227 CD107a<sup>+</sup>CD8<sup>+</sup> T cells, and 44 clones were detected. **b.** TCR-transduced SKW3-CD8AB response for the peptide, when each peptide was shifted back and forth in CoV-2 pep#3(#1208). To assess the differences in peptides, TCR-transduced SKW3-CD8AB were co-cultured with the indicated peptide-pulsed A24/CIR for 16 h, and the upregulation of CD69 was measured by FACS.

Extended Data Fig. 4

|                    | SARS-COV-2 | SARS-COV-1 | MERS  | HKU1  | OC43  | NL63  | 229E  |
|--------------------|------------|------------|-------|-------|-------|-------|-------|
| S1                 | 100.0%     | 63.2%      | 17.7% | 15.7% | 16.4% | 12.3% | 15.0% |
| RBD                | 100.0%     | 73.0%      | 18.1% | 19.2% | 18.3% | 14.8% | 13.1% |
| S2                 | 100.0%     | 88.5%      | 38.8% | 37.6% | 40.2% | 31.7% | 31.3% |
| Spike<br>1200-1226 | 100.0%     | 96.3%      | 77.7% | 48.1% | 48.1% | 40.7% | 48.1% |

**Extended Data Fig. 4 Homology of amino acid sequences in various regions of SARS-CoV-2 Spike protein** % Homology of amino acid sequences in S1, RBD, total S2, and the region covered by the 15mer peptide library (Spike 1200-1226) of SARS-CoV-2 compared to other coronaviruses.

**Table S1. Characteristics of HLA-A24+ healthy volunteers**

| Sampling time | HLA-A24+ healthy Donor | Sex                               | Age | anti-SARS-CoV-2 IgG |
|---------------|------------------------|-----------------------------------|-----|---------------------|
| 2020          | UHD1                   | Male                              | 57  | negative            |
|               | UHD2                   | Male                              | 34  | negative            |
|               | UHD3                   | Male                              | 39  | negative            |
|               | UHD4                   | Male                              | 45  | negative            |
|               | UHD5                   | Male                              | 35  | negative            |
|               | UHD6                   | Male                              | 50  | negative            |
|               | UHD7                   | Male                              | 50  | negative            |
|               | UHD8                   | Female                            | 37  | negative            |
|               | UHD9                   | Female                            | 20  | negative            |
|               | UHD10                  | Female                            | 42  | N.D.                |
| 2004-2010     | UHD11                  | no information but from Red cross |     | N.D.                |
|               | UHD12                  | no information but from Red cross |     |                     |
|               | UHD13                  | no information but from Red cross |     |                     |
|               | UHD14                  | no information but from Red cross |     |                     |
|               | UHD15                  | no information but from Red cross |     |                     |
|               | UHD16                  | no information but from Red cross |     |                     |
|               | UHD17                  | no information but from Red cross |     |                     |
|               | UHD18                  | no information but from Red cross |     |                     |
|               | UHD19                  | no information but from Red cross |     |                     |
|               | UHD20                  | no information but from Red cross |     |                     |
|               | UHD21                  | no information but from Red cross |     |                     |

N.D. not done

**Table S2. Characteristics of HLA-A24+ patients with hematological malignancies**

| Patient ID | F/M | Age | Disease     | Status | Treatment within 1 year     | Treatment over a year ago              | WBC(x10 <sup>3</sup> / μL) | Hb (g/dL) | Plts (x10 <sup>4</sup> /μL) | anti-SARS CoV-2 IgG |
|------------|-----|-----|-------------|--------|-----------------------------|----------------------------------------|----------------------------|-----------|-----------------------------|---------------------|
| HM-1       | M   | 50  | AML NOS     | non CR | IDA AraC induction          |                                        | 25.7                       | 14        | 16.5                        | negative            |
| HM-2       | M   | 78  | MM IgG      | CR     | Bd                          |                                        | 77                         | 12        | 16.4                        | negative            |
| HM-3       | F   | 48  | MM IgG      | PR     | Rd                          |                                        | 28.4                       | 12.5      | 16.8                        | negative            |
| HM-4       | M   | 53  | AML inv16   | CR     | IDA AraC induction          |                                        | 53.2                       | 14.2      | 15.9                        | negative            |
| HM-5       | M   | 73  | MM IgG      | PR     | Rd                          |                                        | 38.7                       | 10.3      | 11.8                        | negative            |
| HM-6       | M   | 76  | MM IgA      | non CR | IRd                         |                                        | 69.8                       | 14.1      | 12.6                        | negative            |
| HM-7       | F   | 78  | AML NOS     | CR     | CAG                         |                                        | 66.4                       | 7.6       | 3.9                         | negative            |
| HM-8       | F   | 84  | CML         | DMR    |                             | Imatinib                               | 50.8                       | 12.4      | 26.9                        | negative            |
| HM-9       | F   | 63  | MM IgG      | VGPR   | Rd                          | high dose L-PAM+ autologous PBSCT      | 43.5                       | 13.3      | 20.9                        | negative            |
| HM-10      | F   | 90  | MM IgA      | non CR | MP                          |                                        | 15                         | 10.2      | 16.2                        | negative            |
| HM-11      | M   | 66  | AML t(8;21) | CR     | IDA AraC induction, HD-AraC |                                        | 59.2                       | 10.5      | 11.3                        | negative            |
| HM-12      | F   | 74  | MM IgA      | non CR | Ird                         | Rd , Bd                                | 33                         | 9.1       | 16.9                        | negative            |
| HM-13      | F   | 68  | IgG         | CR     | Rd                          | high dose L-PAM+ autologous PBSCT      | 33.8                       | 10.7      | 24.8                        | negative            |
| HM-14      | F   | 37  | AML M3      | CR     | Tamibarotene                | IDA/AraC, MIT/AraC, DNR/AraC, IDA/AraC | 43.9                       | 13.2      | 22.7                        | negative            |
| HM-15      | M   | 83  | AML         | CR     | CAG                         |                                        | 56                         | 14.5      | 12.7                        | negative            |
| HM-16      | F   | 50  | AML inv16   | CR     |                             | IDA/AraC, HD-AraC x3                   | 55.4                       | 10        | 32                          | negative            |
| HM-17      | M   | 71  | MM IgG      | VGPR   | Bd                          |                                        | 83.8                       | 13.7      | 16.3                        | negative            |
| HM-18      | M   | 69  | MM IgA      | non CR | Pd                          | BD, Rd, ERd, DRd, IRd                  | 28.6                       | 7.2       | 17.8                        | negative            |
| HM-19      | M   | 64  | MM IgG      | VGPR   | DBd                         | high dose L-PAM+ autologous PBSCT      | 34.1                       | 14.5      | 13.7                        | negative            |
| HM-20      | M   | 41  | AML M3      | CR     | DNR/AraC, ATRA              |                                        | 39.8                       | 9.2       | 27.1                        | negative            |
| HM-21      | M   | 59  | DLBCL       | CR     |                             | R-CHOP x6                              | 57.9                       | 15.8      | 18.9                        | negative            |
| HM-22      | F   | 67  | MM IgG      | non CR | IRd                         | Rd, BD, Pd, ERd, DRd, Kd               | 21.89                      | 9.4       | 13.9                        | negative            |
| HM-23      | F   | 79  | CML         | DMR    | Imatinib                    |                                        | 42.3                       | 10.6      | 22.6                        | negative            |
| HM-24      | M   | 44  | AML M3      | CR     | Tamibarotene                | IDA/AraC, MIT/AraC, DNR/AraC, IDA/AraC | 56.5                       | 14.4      | 22.1                        | negative            |
| HM-25      | F   | 78  | CML         | MMR    | Imatinib                    | Dasatinib                              | 56.5                       | 10.8      | 21.1                        | negative            |
| HM-26      | M   | 85  | AML         | CR     |                             | CAG                                    | 63.1                       | 14.3      | 16.1                        | negative            |
| HM-27      | M   | 61  | AML t(8;21) | CR     |                             | IDA/AraC, MIT/AraC, DNR/AraC           | 43.1                       | 13.3      | 11.2                        | negative            |
| HM-28      | F   | 76  | CML         | DMR    |                             | Dasatinib                              | 54.5                       | 13.7      | 26.7                        | negative            |

AML; Acute myelogenous leukemia, MM; Multiple myeloma, CML; Chronic myelogenous leukemia, DLBCL; Diffuse large B cell lymphoma, NOS; not otherwise specified

CR: complete response, PR; partial response, DMR; deep molecular response, VGPR; very good PR, MMR; major molecular response

Bd (bortezomib +dexamethasone), Rd (lenalidomide+dexamethasone), Ird (izazomib +lenalidomide+dexamethasone), Pd (pomalidomide+dexamethasone), DBd (daratumumab+bortezomib +dexamethasone)

CAG; cytarabine+aclerubicin+G-CSF, PBSCT; peripheral blood stem cell transplantation

**Table S3. Data collection and refinement statistics of crystal structure**

| Data collection                            | HLA•CoV2                                      |        |        | HLA•229E                                      |        |        | HLA•HKU1                                      |        |        |
|--------------------------------------------|-----------------------------------------------|--------|--------|-----------------------------------------------|--------|--------|-----------------------------------------------|--------|--------|
| Space group                                | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> |        |        | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> |        |        | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> |        |        |
| Resolution range <sup>a</sup>              | 45.89 - 1.89                                  |        |        | 45.95 - 1.71                                  |        |        | 43.87 - 2.10                                  |        |        |
| Unit Cell                                  |                                               |        |        |                                               |        |        |                                               |        |        |
| Length (Å )                                | 67.717                                        | 85.465 | 91.373 | 67.946                                        | 85.586 | 91.091 | 66.702                                        | 77.716 | 87.948 |
| Angle (°)                                  | 90.000                                        | 90.000 | 90.000 | 90.000                                        | 90.000 | 90.000 | 90.000                                        | 90.000 | 90.000 |
| Total no. of reflections                   | 629450                                        |        |        | 859537                                        |        |        | 365379                                        |        |        |
| No. of unique reflections                  | 80351                                         |        |        | 111576                                        |        |        | 51282                                         |        |        |
| Completeness (%) <sup>a</sup>              | 98.73 (97.72)                                 |        |        | 99.99 (99.97)                                 |        |        | 99.96 (99.90)                                 |        |        |
| Redundancy <sup>a</sup>                    | 7.8 (7.8)                                     |        |        | 7.7 (7.6)                                     |        |        | 7.1 (7.3)                                     |        |        |
| <i>I</i> /σ( <i>I</i> ) <sup>a</sup>       | 7.7 (0.7)                                     |        |        | 13.5 (0.8)                                    |        |        | 7.7 (0.8)                                     |        |        |
| CC <sub>1/2</sub>                          | 0.996 (0.483)                                 |        |        | 0.999 (0.492)                                 |        |        | 0.996 (0.395)                                 |        |        |
| <i>R</i> <sub>merge</sub> <sup>a,b</sup>   | 0.220 (2.797)                                 |        |        | 0.096 (2.250)                                 |        |        | 0.136 (2.179)                                 |        |        |
| Refinement statistics                      |                                               |        |        |                                               |        |        |                                               |        |        |
| Resolution (Å )                            | 1.89                                          |        |        | 1.71                                          |        |        | 2.11                                          |        |        |
| <i>R</i> <sub>work</sub> <sup>c</sup>      | 0.1995                                        |        |        | 0.1915                                        |        |        | 0.2027                                        |        |        |
| <i>R</i> <sub>free</sub> <sup>c</sup>      | 0.2550                                        |        |        | 0.2210                                        |        |        | 0.2644                                        |        |        |
| R.m.s. deviations                          |                                               |        |        |                                               |        |        |                                               |        |        |
| Bond length (Å )                           | 0.007                                         |        |        | 0.007                                         |        |        | 0.008                                         |        |        |
| Bond angles (°)                            | 0.86                                          |        |        | 0.83                                          |        |        | 0.93                                          |        |        |
| Average <i>B</i> factors (Å <sup>2</sup> ) | 33.1                                          |        |        | 31.6                                          |        |        | 53.3                                          |        |        |
| Ramachandran plot (%)                      |                                               |        |        |                                               |        |        |                                               |        |        |
| Favored                                    | 97.37                                         |        |        | 97.37                                         |        |        | 97.65                                         |        |        |
| Outliers                                   | 0.00                                          |        |        | 0.00                                          |        |        | 0.00                                          |        |        |

<sup>a</sup> Numbers in parentheses refer to the highest resolution shell.

<sup>b</sup>  $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$ , where  $I_i(hkl)$  is the observed intensity and  $\langle I(hkl) \rangle$  is the average intensity.

<sup>c</sup>  $R_{\text{work}} = \frac{\sum |F_{\text{obs}}| - |F_{\text{cal}}|}{\sum |F_{\text{obs}}|}$ , where  $F_{\text{obs}}$  and  $F_{\text{cal}}$  are observed and calculated structure factors, respectively.  $R_{\text{free}}$  is calculated for a randomly selected test data set of reflections excluded from refinement.

**Table S4. List of peptides**

| Peptide size          | Peptide sequence | Peptide name                          |
|-----------------------|------------------|---------------------------------------|
| <b>9-mer peptides</b> | VYSTGSNVF        | Pep #1 (Spike protein 635-643)        |
|                       | VYSSANNCTF       | Pep #2 (Spike protein 159-168)        |
|                       | QYIKWPWYI        | Pep #3 (Spike protein 1208-1216)      |
|                       | NYNLYRLF         | Pep #4 (Spike protein 448-456)        |
|                       | YFPLQSYGF        | Pep #5 (Spike protein 489-497)        |
|                       | TYVPAQEKNF       | Pep #6 (Spike protein 1066-1075)      |
|                       | NFKDQVILL        | Pep #7 (Nucleocapsid protein 345-353) |
|                       | QYIKWPWYV        | SARS-CoV-1                            |
|                       | YYNKWPWYI        | MERS                                  |
|                       | MYVKWPWYV        | HKU1                                  |
|                       | YYVKWPWYV        | OC43                                  |
|                       | NYIKWPWWV        | NL63                                  |
|                       | TYIKWPWWV        | 229E                                  |
|                       | QYDPVAALF        | CMVpp65                               |
|                       | TYGPVFMSL        | EBV-LMP2                              |
| <b>15-mer library</b> | LQELGKYEQYIKWPW  | 15 mer #1                             |
|                       | GKYEQYIKWPWYIWL  | 15 mer #2                             |
|                       | QYIKWPWYIWLGFIA  | 15 mer #3                             |
|                       | WPWYIWLGFIAGLIA  | 15 mer #4                             |
| <b>9-mer library</b>  | LQELGKYEQ        | S2-pep #1200                          |
|                       | QELGKYEQY        | S2-pep #1201                          |
|                       | ELGKYEQYI        | S2-pe #p1202                          |
|                       | LGKYEQYIK        | S2-pep #1203                          |
|                       | GKYEQYIKW        | S2-pep #1204                          |
|                       | KYEQYIKWP        | S2-pep #1205                          |
|                       | YEQYIKWPW        | S2-pep #1206                          |
|                       | EQYIKWPWY        | S2-pep #1207                          |
|                       | QYIKWPWYI        | S2-pep #1208                          |
|                       | YIKWPWYIW        | S2-pep #1209                          |
|                       | IKWPWYIWL        | S2-pep #1210                          |
|                       | KWPWYIWL         | S2-pep #1211                          |
|                       | WPWYIWLGF        | S2-pep #1212                          |
|                       | PWYIWLGFI        | S2-pep #1213                          |
|                       | WYIWLGFIA        | S2-pep #1214                          |
|                       | YIWLGFIA         | S2-pep #1215                          |
|                       | IWLGFIA          | S2-pep #1216                          |
|                       | WLGFIA           | S2-pep #1217                          |
|                       | LGFIAGLIA        | S2-pep #1218                          |

**Table S5. List of antibodies**

| <b>Antibodies</b>                              | <b>Source</b> | <b>Identifier</b>            | <b>Lot#</b> |
|------------------------------------------------|---------------|------------------------------|-------------|
| Mouse anti-human CD3 (UCHT1) PE/Cyanine7       | Biolegend     | Cat#300420; RRID:AB_439781   | B311571     |
| Mouse anti-human CD4 (OKT4) PerCP/Cy5.5        | Biolegend     | Cat#317428; RRID:AB_1186122  | B278611     |
| Mouse anti-human CD4 (RPA-T4) PerCP/Cyanine5.5 | Biolegend     | Cat#300530; RRID:AB_893322   | B313463     |
| Mouse anti-human CD8a (RPA-T8) FITC            | Biolegend     | Cat#301006; RRID:AB_314124   | B279938     |
| Mouse Anti-Human CD8 (SK1) BUV737              | BD            | Cat#564629                   | 7061723     |
| Mouse anti-human CD69 (FN50) APC               | Biolegend     | Cat#310904; RRID:AB_314839   | B268175     |
| Mouse anti-human CD107a (H4A3) BV421           | Biolegend     | Cat#328626; RRID:AB_11203537 | B310468     |
| Rat anti-human IL-2 (MQ1-17H12) BV785          | Biolegend     | Cat#500348; RRID:AB_2566471  | B308975     |
| Mouse anti-human TNF- $\alpha$ (MAb11) PE      | Biolegend     | Cat#502909; RRID:AB_315261   | B250947     |
| Mouse anti-human IFN- $\gamma$ (B27) APC       | Biolegend     | Cat#506510; RRID:AB_315443   | B314290     |
| Mouse Anti-Human HLA-A24 (17A10) FITC          | MBL           | Cat#K0208-4; RRID:AB_592231  | 016         |

**Table S6. Single cell TCR sequence and cloning primer list**

| Primer Name         | Primer Sequence                              | PCR reaction   |
|---------------------|----------------------------------------------|----------------|
| TRAC-inf-for        | GTCTGCCTATTCACCGATTTTG                       | TRAC InFusion  |
| TRAC-inf-revcom     | CACCACACTGG GATC c TCAGCTGGACCACAGCCGCAG     | TRAC InFusion  |
| AV1-1               | AGTTAATTAAG GATC c gcc atgtggggagcttctctct   | RT-PCR 0.44 µM |
| AV2                 | AGTTAATTAAG GATC c gcc atggcttgcagagcactct   | RT-PCR 0.44 µM |
| AV3                 | AGTTAATTAAG GATC c gcc atggcctctgcacccatctc  | RT-PCR 0.44 µM |
| AV4                 | AGTTAATTAAG GATC c gcc atgaggcaagtggcgagagt  | RT-PCR 0.44 µM |
| AV5                 | AGTTAATTAAG GATC c gcc atgaagacatttgctggatt  | RT-PCR 0.44 µM |
| AV6                 | AGTTAATTAAG GATC c gcc atggagtcattcctgggagg  | RT-PCR 0.44 µM |
| AV7                 | AGTTAATTAAG GATC c gcc atggagaagatgcggagacc  | RT-PCR 0.44 µM |
| AV8-1               | AGTTAATTAAG GATC c gcc atgctcctgttgctcatacc  | RT-PCR 0.44 µM |
| AV8-2               | AGTTAATTAAG GATC c gcc atgctcctgtctgctgctccc | RT-PCR 0.44 µM |
| AV8-3               | AGTTAATTAAG GATC c gcc atgctcctggagcttatccc  | RT-PCR 0.44 µM |
| AV9-1               | AGTTAATTAAG GATC c gcc atgaattcttccaggacc    | RT-PCR 0.44 µM |
| AV9-2               | AGTTAATTAAG GATC c gcc atgaactatttccaggctt   | RT-PCR 0.44 µM |
| AV10                | AGTTAATTAAG GATC c gcc atgaaaaagcatctgacgac  | RT-PCR 0.44 µM |
| AV12-1              | AGTTAATTAAG GATC c gcc atgatatccttgagagtttt  | RT-PCR 0.44 µM |
| AV12-3              | AGTTAATTAAG GATC c gcc atgatgaaatccttgagagt  | RT-PCR 0.44 µM |
| AV13-1              | AGTTAATTAAG GATC c gcc atgacatccattcgagctgt  | RT-PCR 0.44 µM |
| AV13-2              | AGTTAATTAAG GATC c gcc atggcaggcattcgagcttt  | RT-PCR 0.44 µM |
| AV14-DV4            | AGTTAATTAAG GATC c gcc atgtcactttctagcctgct  | RT-PCR 0.44 µM |
| AV16                | AGTTAATTAAG GATC c gcc atgaagcccaccctcatctc  | RT-PCR 0.44 µM |
| AV17                | AGTTAATTAAG GATC c gcc atggaaactctcctgggagt  | RT-PCR 0.44 µM |
| AV18                | AGTTAATTAAG GATC c gcc atgctgtctgcttctctgctc | RT-PCR 0.44 µM |
| AV19                | AGTTAATTAAG GATC c gcc atgctgactgccagcctgtt  | RT-PCR 0.44 µM |
| AV20                | AGTTAATTAAG GATC c gcc atggagaaaatgttgagtg   | RT-PCR 0.44 µM |
| AV21                | AGTTAATTAAG GATC c gcc atggagaccctcttgggcct  | RT-PCR 0.44 µM |
| AV22                | AGTTAATTAAG GATC c gcc atgaagaggatattgggagc  | RT-PCR 0.44 µM |
| AV23-DV6            | AGTTAATTAAG GATC c gcc atggacaagatcttaggagc  | RT-PCR 0.44 µM |
| AV24                | AGTTAATTAAG GATC c gcc atggagaagaatcctttggc  | RT-PCR 0.44 µM |
| AV25                | AGTTAATTAAG GATC c gcc atgctactcatcacatcaat  | RT-PCR 0.44 µM |
| AV26-1              | AGTTAATTAAG GATC c gcc atgaggctggtggcaagagt  | RT-PCR 0.44 µM |
| AV26-2              | AGTTAATTAAG GATC c gcc atgaagtgggtgacaagcat  | RT-PCR 0.44 µM |
| AV27                | AGTTAATTAAG GATC c gcc atggtcctgaaattctccgt  | RT-PCR 0.44 µM |
| AV29-DV5            | AGTTAATTAAG GATC c gcc atggccatgctcctgggggc  | RT-PCR 0.44 µM |
| AV30                | AGTTAATTAAG GATC c gcc atggagactctcctgaaagt  | RT-PCR 0.44 µM |
| AV34                | AGTTAATTAAG GATC c gcc atggagactgttctgcaagt  | RT-PCR 0.44 µM |
| AV35                | AGTTAATTAAG GATC c gcc atgctccttgaacatttatt  | RT-PCR 0.44 µM |
| AV36-DV7            | AGTTAATTAAG GATC c gcc atgatgaagtgtccacaggc  | RT-PCR 0.44 µM |
| AV38-1              | AGTTAATTAAG GATC c gcc atgacacgagttagcttgct  | RT-PCR 0.44 µM |
| AV38-2-DV8          | AGTTAATTAAG GATC c gcc atggcatgccctggcttct   | RT-PCR 0.44 µM |
| AV39                | AGTTAATTAAG GATC c gcc atgaagaagctactagcaat  | RT-PCR 0.44 µM |
| AV40                | AGTTAATTAAG GATC c gcc atgaactcctctctggactt  | RT-PCR 0.44 µM |
| AV41                | AGTTAATTAAG GATC c gcc atggtgaagatccggcaatt  | RT-PCR 0.44 µM |
| CA-rev1             | AGGTTCGTATCTGTTTCAAAGCTT                     | RT-PCR 2.22 µM |
| pMXs-BamHI-InFusion | AGTTAATTAAG GATC c gcc                       | 2nd/3rd PCR    |
| CA-rev2             | TGTGACACATTTGTTTGAGAA                        | 2nd PCR        |



|                     |                                              |                  |
|---------------------|----------------------------------------------|------------------|
| CA-rev3             | GGTGAATAGGCAGACAGACTT                        | Sequence/3rd PCR |
| TRBC-inf-for        | GGTGTGCCTGGCCACAGGCTTC                       | TRBC1/2 InFusion |
| TRBC1-inf-revcom    | CACCACACTGG GATC c TCAGAAATCCTTTCTCTTGAC     | TRBC1 InFusion   |
| TRBC2-inf-revcom    | CACCACACTGG GATC c CTAGCCTCTGGAATCCTTTC      | TRBC2 InFusion   |
| BV2                 | AGTTAATTAAG GATC c gcc atggatacctggctcgtatg  | RT-PCR 0.44 µM   |
| BV3-1               | AGTTAATTAAG GATC c gcc atgggctgcaggctcctctg  | RT-PCR 0.44 µM   |
| BV4-1               | AGTTAATTAAG GATC c gcc atgggctgcaggctcctctg  | RT-PCR 0.44 µM   |
| BV5-1               | AGTTAATTAAG GATC c gcc atgggctccaggctcctctg  | RT-PCR 0.44 µM   |
| BV5-4               | AGTTAATTAAG GATC c gcc atgggccctgggctcctctg  | RT-PCR 0.44 µM   |
| BV5-6               | AGTTAATTAAG GATC c gcc atgggccccgggctcctctg  | RT-PCR 0.44 µM   |
| BV5-8               | AGTTAATTAAG GATC c gcc atgggaccaggctcctott   | RT-PCR 0.44 µM   |
| BV6-1               | AGTTAATTAAG GATC c gcc atgagcatcgggctcctctg  | RT-PCR 0.44 µM   |
| BV6-2               | AGTTAATTAAG GATC c gcc atgagcctcgggctcctctg  | RT-PCR 0.44 µM   |
| BV6-4               | AGTTAATTAAG GATC c gcc atgagaatcaggctcctctg  | RT-PCR 0.44 µM   |
| BV6-5               | AGTTAATTAAG GATC c gcc atgagcatcggcctcctctg  | RT-PCR 0.44 µM   |
| BV6-6               | AGTTAATTAAG GATC c gcc atgagcatcaggctcctctg  | RT-PCR 0.44 µM   |
| BV7-2               | AGTTAATTAAG GATC c gcc atgggcaccaggctcctott  | RT-PCR 0.44 µM   |
| BV7-3               | AGTTAATTAAG GATC c gcc atgggcaccaggctcctctg  | RT-PCR 0.44 µM   |
| BV7-6               | AGTTAATTAAG GATC c gcc atgggcaccagctcctatg   | RT-PCR 0.44 µM   |
| BV7-7               | AGTTAATTAAG GATC c gcc atgggtaccagctcctatg   | RT-PCR 0.44 µM   |
| BV7-9               | AGTTAATTAAG GATC c gcc atgggcaccagcctcctctg  | RT-PCR 0.44 µM   |
| BV9                 | AGTTAATTAAG GATC c gcc atgggcttcaggctcctctg  | RT-PCR 0.44 µM   |
| BV10-1              | AGTTAATTAAG GATC c gcc atgggcacgaggctcttctt  | RT-PCR 0.44 µM   |
| BV10-2              | AGTTAATTAAG GATC c gcc atgggcaccaggctcttctt  | RT-PCR 0.44 µM   |
| BV10-3              | AGTTAATTAAG GATC c gcc atgggcacaaaggtgttctt  | RT-PCR 0.44 µM   |
| BV11-1              | AGTTAATTAAG GATC c gcc atgagcaccaggcttctctg  | RT-PCR 0.44 µM   |
| BV11-3              | AGTTAATTAAG GATC c gcc atgggtaccaggctcctctg  | RT-PCR 0.44 µM   |
| BV12-3              | AGTTAATTAAG GATC c gcc atggactcctggacctctg   | RT-PCR 0.44 µM   |
| BV12-4              | AGTTAATTAAG GATC c gcc atgggctcctggacctctg   | RT-PCR 0.44 µM   |
| BV12-5              | AGTTAATTAAG GATC c gcc atggccaccaggctcctctg  | RT-PCR 0.44 µM   |
| BV13                | AGTTAATTAAG GATC c gcc atgcttagtctgacctgcc   | RT-PCR 0.44 µM   |
| BV14                | AGTTAATTAAG GATC c gcc atggttccaggcttctcag   | RT-PCR 0.44 µM   |
| BV15                | AGTTAATTAAG GATC c gcc atgggtcctgggcttctcca  | RT-PCR 0.44 µM   |
| BV16                | AGTTAATTAAG GATC c gcc atgagcccaatattcacctg  | RT-PCR 0.44 µM   |
| BV18                | AGTTAATTAAG GATC c gcc atggacaccagagtactctg  | RT-PCR 0.44 µM   |
| BV19                | AGTTAATTAAG GATC c gcc atgagcaaccagggtgctctg | RT-PCR 0.44 µM   |
| BV20-1              | AGTTAATTAAG GATC c gcc atgctgctgcttctgctgct  | RT-PCR 0.44 µM   |
| BV24-1              | AGTTAATTAAG GATC c gcc atggcctccctgctcttctt  | RT-PCR 0.44 µM   |
| BV25-1              | AGTTAATTAAG GATC c gcc atgactatcaggctcctctg  | RT-PCR 0.44 µM   |
| BV27                | AGTTAATTAAG GATC c gcc atgggccccagctccttg    | RT-PCR 0.44 µM   |
| BV28                | AGTTAATTAAG GATC c gcc atgggaatcaggctcctctg  | RT-PCR 0.44 µM   |
| BV29-1              | AGTTAATTAAG GATC c gcc atgctgagtcttctgctcct  | RT-PCR 0.44 µM   |
| BV30                | AGTTAATTAAG GATC c gcc atgctgctctctccttg     | RT-PCR 0.44 µM   |
| CB-rev1             | GGTAAAGCCACAGTCTGCTCTA                       | RT-PCR 2.22 µM   |
| pMXs-BamHI-InFusion | AGTTAATTAAG GATC c gcc                       | 2nd/3rd PCR      |
| CB-rev2             | CTGTGCACCTCCTTCCCATTTC                       | 2nd PCR          |
| CB-rev3             | GTGGCCAGGCACACCAGTGT                         | Sequence/3rd PCR |