

Supplementary Information

Identification and characterization of a SARS-CoV-2 specific CD8 T cell response with immunodominant features

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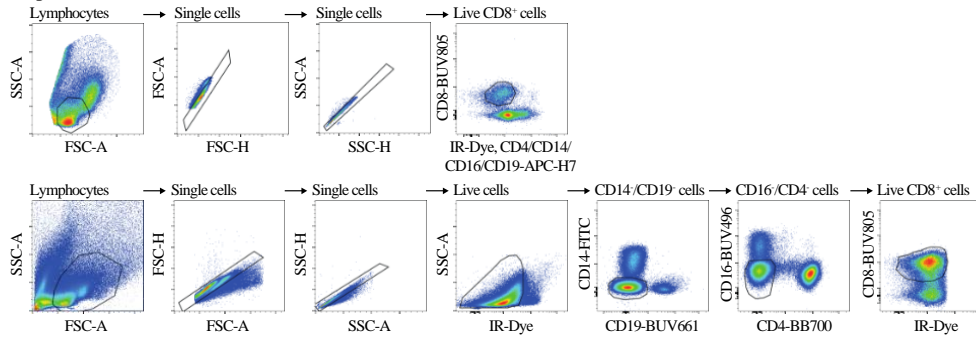
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These authors contributed equally to this work: Anastasia Gangaev, Steven L. C. Ketelaars

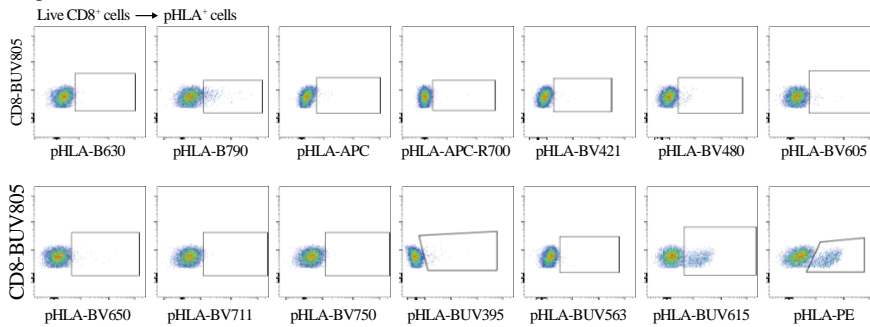
These authors jointly supervised this work: Andrea Cossarizza, Pia Kvistborg

28 Supplementary Figures

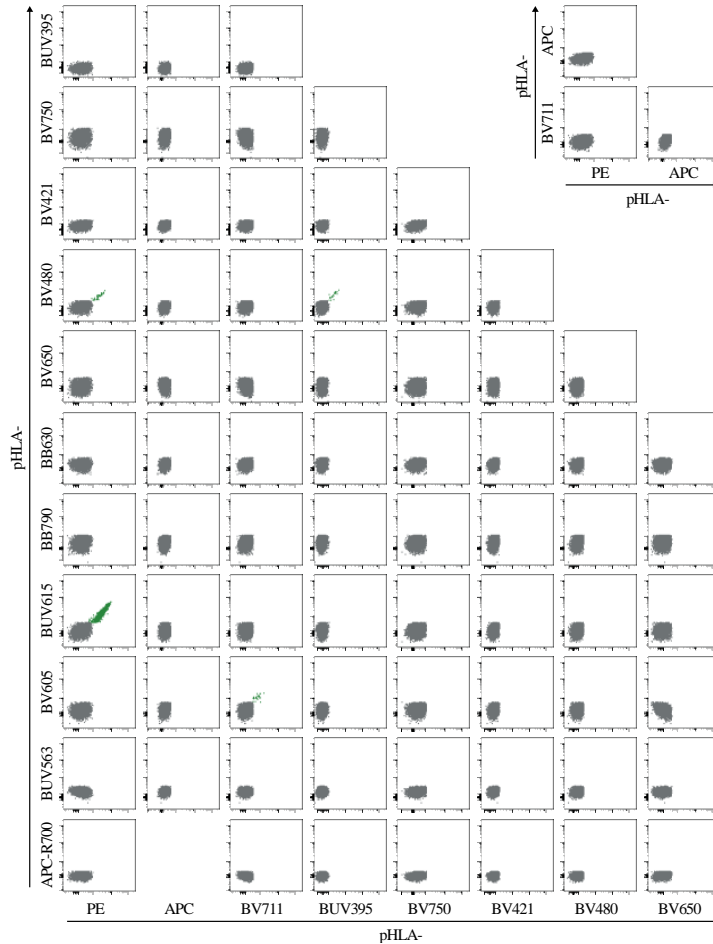
Step 1:



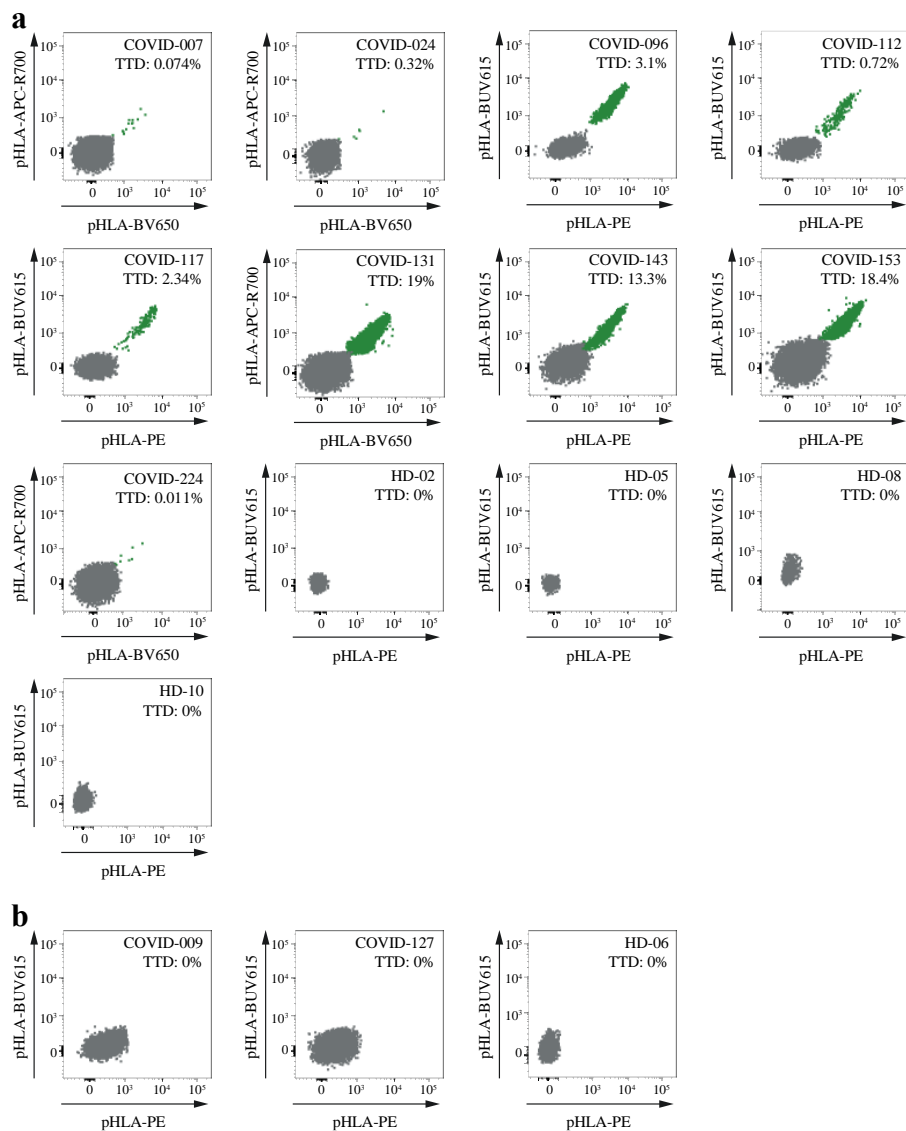
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Step 3:

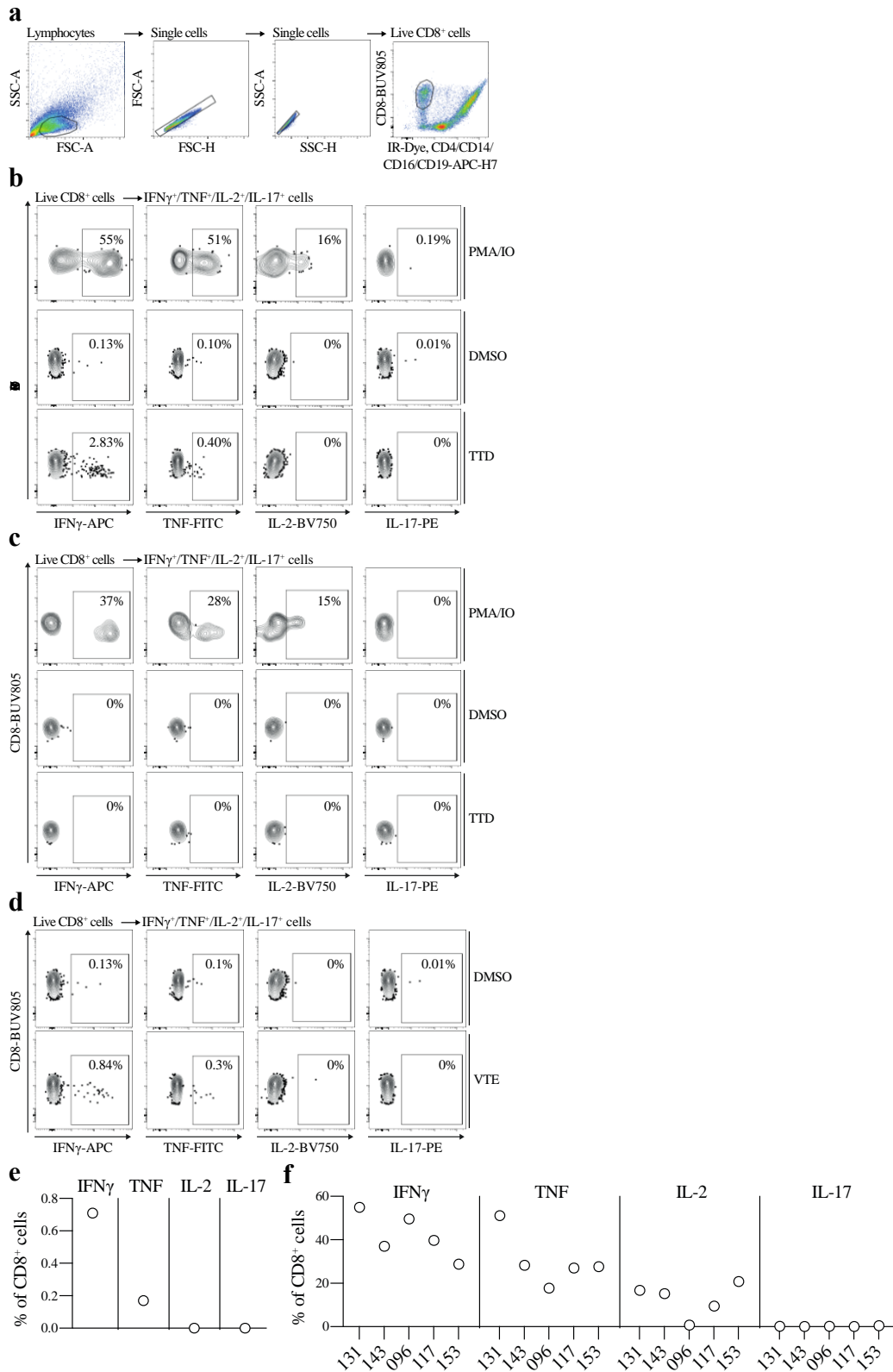


30 **Supplementary Fig. 1:** Representative gating strategy to identify SARS-CoV-2-specific CD8
31 T cell responses presented in Fig. 1c-e and Fig. 5a and b. Step 1: Gating strategy used to identify
32 live CD8⁺ cells. Step 2: Gating strategy used to identify pHLA⁺ cells within the live CD8⁺ cell
33 population. Step 3: Representative overview of all 75 pHLA dual color code combinations.
34 Boolean gating. Was used to identify SARS-CoV-2-specific CD8 T cells (double-positive
35 pHLA⁺ CD8⁺ cells, green) and bulk CD8 T cells (pHLA⁻ CD8⁺ cells, grey). pHLA: peptide-
36 human leukocyte antigen.
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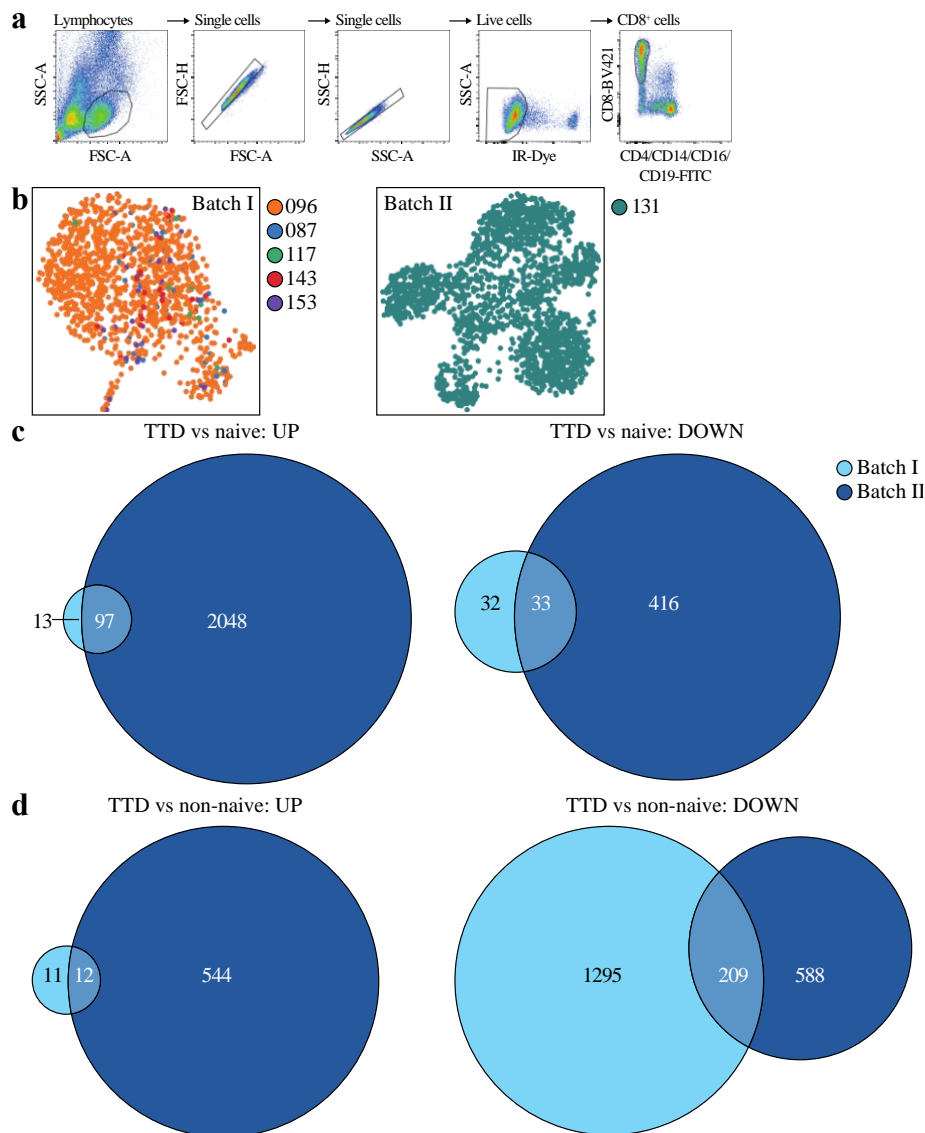
39 **Supplementary Fig. 2:** Flow cytometry plots of the TTD-specific CD8 T cell responses
 40 presented in Fig. 2a-c. **a** Dot plots of TTD-specific CD8 T cell responses detected in HLA-
 41 A*01:01-positive donors. Magnitude of TTD-specific CD8 T cell responses is represented as
 42 the percentage of double-positive pHLA⁺ cells (green) of total CD8⁺ cells (grey).
 43 Representative gating strategy is provided in Supplementary Fig. 1. **b** Dot plots representing
 44 the negative control for the detected TTD-specific CD8 T cell responses in HLA-A*01:01-
 45 negative donors. The magnitude of the TTD-specific CD8 T cell responses is represented as
 46 the percentage of double-positive pHLA⁺ cells (green) of total CD8⁺ cells (grey).
 47 Representative gating strategy is provided in Supplementary Fig. 1. pHLA: peptide-human
 48 leukocyte antigen, TTD: TTDPSFLGRY, HD: healthy donor.



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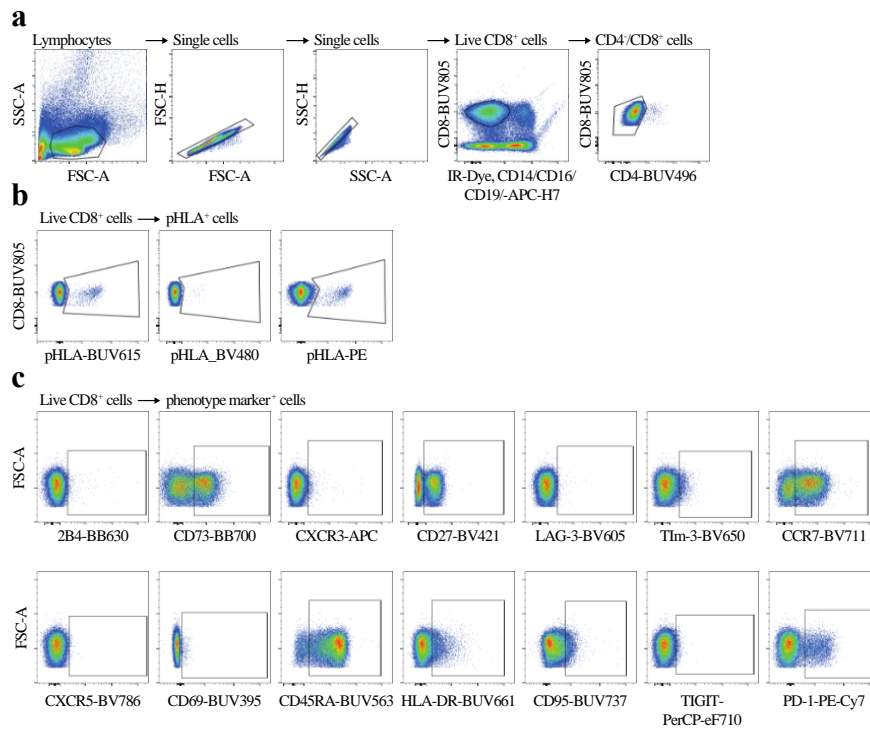
50 **Supplementary Fig. 3:** Representative gating strategy used for the functional assessment of
 51 SARS-CoV-2-specific CD8 T cells presented in Fig. 3a and b and Fig. 5g and h. **a** Gating
 52 strategy used to identify live CD8⁺ cells. **b** Representative gating strategy used to assess the
 53 expression of IFN γ , TNF, IL-2 and IL-17 in CD8 T cells from patient COVID-131 after

54 stimulation with PMA/IO (technical control, top row), DMSO (negative control, middle row)
55 and the SARS-CoV-2 epitope TTD (bottom row). Percentages represent the frequency of
56 cytokine producing cells. The gates were set relative to the DMSO and adjusted for the
57 PMA/IO control if needed. **c** Representative flow cytometry plots illustrating the gating
58 strategy used to assess the expression of IFN γ , TNF, IL-2 and IL-17 production in CD8 T cells
59 from COVID-143 after stimulation with PMA/IO (technical control, top row), DMSO
60 (negative control, middle row) and the SARS-CoV-2 epitope TTD (bottom row). Percentages
61 represent the frequency of cytokine producing cells. The gates are based on the DMSO control
62 and adjusted for the PMA/IO control if needed. **d** Representative gating strategy used to assess
63 the expression of IFN γ , TNF, IL-2 and IL-17 in CD8 T cells from patient COVID-131 after
64 stimulation with VTE (HLA-A*01:01-restricted CMV epitope, bottom row), DMSO (negative
65 control, top row). Percentages represent the frequency of cytokine producing cells. The gates
66 were set relative to the DMSO and adjusted for the PMA/IO control if needed. **e** Expression of
67 IFN γ , TNF, IL-2 and IL-17 in CD8 T cells from COVID-131 with acute disease after
68 stimulation with VTEHDTLLY (HLA-A*01:01-restricted CMV epitope) for 12h. Percentages
69 represent the frequency of cytokine producing cells after subtracting the percentages of the
70 DMSO control. **f** Expression of IFN γ , TNF, IL-2 and IL-17 in CD8 T cells from COVID-19
71 patients (n=5) with acute disease after stimulation with PMA/IO (technical control) for 12h.
72 Percentages represent the frequency of cytokine producing cells after subtracting the
73 percentages of the DMSO control. TTD: TTDPSFLGRY, VTE: VTEHDTLLY.



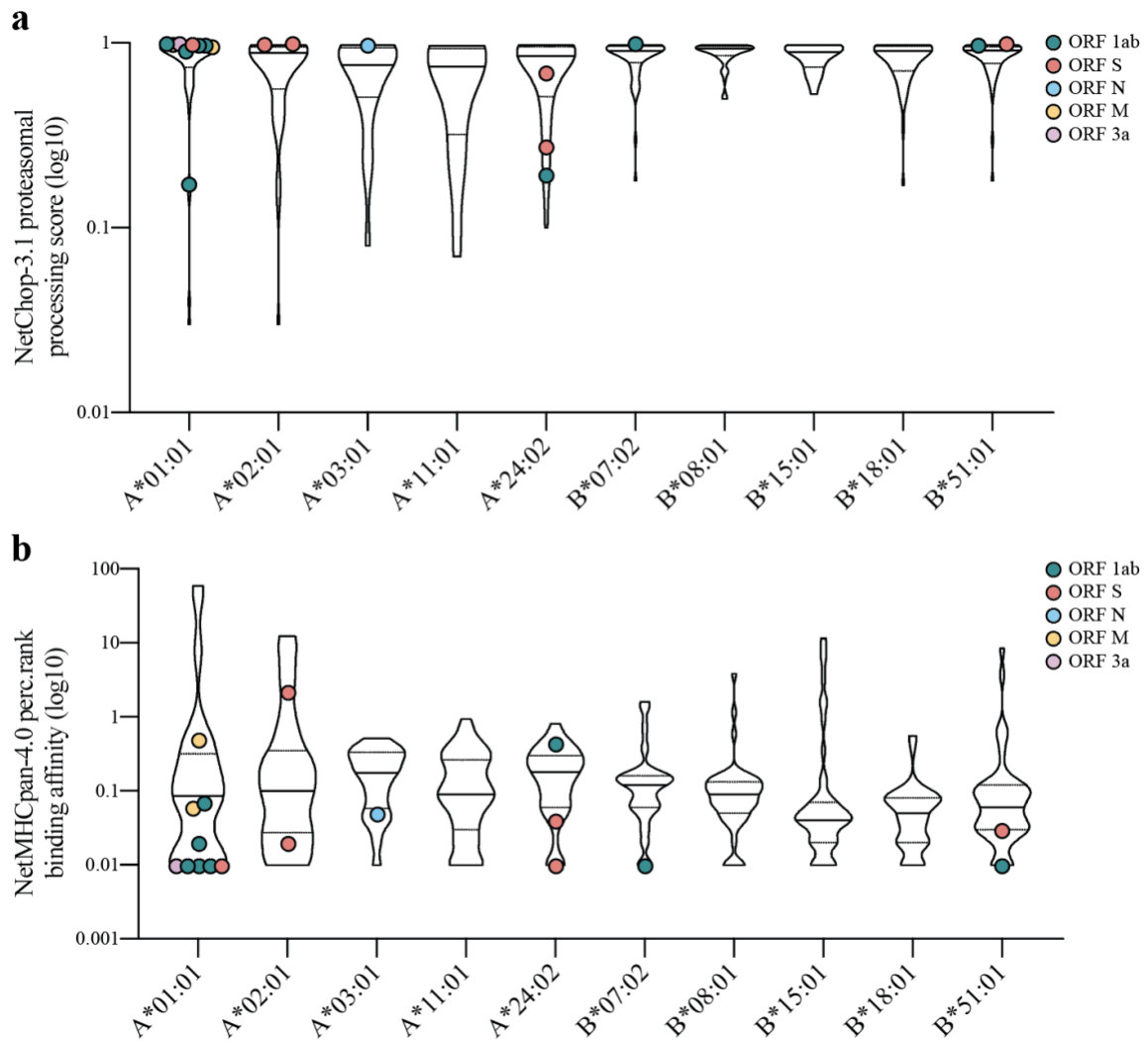
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Supplementary Fig. 4: Gene expression analysis of CD8 T cells from COVID-19 patients presented in Fig. 4. **a** Representative gating strategy used to sort on live CD8⁺ cells for single-cell RNA and TCR sequencing. **b** UMAP representations of single-cell gene expression data of CD8 T cells (batch I: n=1180 and batch II: n=1884) isolated from COVID-19 patients (batch I: n=5 and batch II: n=1). Detailed information about the number of patients and cells for each individual patient/batch is provided in Table 2. **c** Number of differentially expressed genes in TTD-specific CD8 T cells compared to bulk naïve CD8 T cells. Proportional Venn diagrams illustrate the overlap and differences in the number of genes that were found to be differentially expressed in batch I (light blue) compared to batch II (dark blue). **d** Number of differentially expressed genes in TTD-specific CD8 T cells compared to bulk non-naïve CD8 T cells. Proportional Venn diagram illustrate the overlap and differences in the number of genes that were found to be differentially expressed batch I (light blue) compared to batch II (dark blue).



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89 **Supplementary Fig. 5:** Representative gating strategy used for phenotypic characterization
 90 presented in Fig. 5c-f. **a** Gating strategy used to identify live CD8⁺ cells. **b** Gating of pHLA⁺
 91 cells within the live CD8⁺ cell population to identify SARS-CoV-2-specific CD8 T cells as
 92 shown in Supplementary Fig. 1. **c** Representative gating on live CD8⁺ cells that were used to
 93 assess expression levels of phenotypic markers on SARS-CoV-2-specific CD8 T cells.



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95 **Supplementary Fig. 6:** Prediction quality of selected SARS-CoV-2 epitopes. **a** Violin plots
 96 representing the distribution of the proteasomal processing scores predicted by Netchop-3.1
 97 for the 50 selected SARS-CoV-2 epitopes per HLA allele included in this study. Colored dots
 98 represent CD8 T cell recognized epitopes (n=18) and provide information about their protein
 99 origin. **b** Violin plots representing the distribution of the percentile rank binding affinity as
 100 predicted by NetMHCpan-4.0 for the 50 selected SARS-CoV-2 epitopes per HLA allele
 101 included in this study. Colored dots represent CD8 T cell recognized epitopes (n=18) and
 102 provide information about their protein origin. ORF: open reading frame, S: spike, N:
 103 nucleoprotein, M: membrane.

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105 **Supplementary Tables**

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107 **Supplementary Table 1:** Characteristics of COVID-19 patients and healthy donors included in the study. Covered HLA alleles are indicated (*).
 108 Patients were excluded from the analysis of SARS-CoV-2 CD8 T cells if: (1) none of the HLA alleles were covered, (2) <1000 CD8 T cells were
 109 acquired, and (3) HLA typing failed.

| Patient | Disease Status | Gender | Age range | Treatment | Hospitalization (weeks) | Outcome | Treatment prior to sampling (days) | Hospitalization prior to sampling (days) | HLA-A | HLA-A | HLA-B | HLA-B | Analysis of SARS-CoV-2 CD8 T cell responses |
|-----------|----------------|--------|-----------|-------------|-------------------------|-------------|------------------------------------|------------------------------------------|--------|--------|--------|--------|---------------------------------------------|
| COVID-024 | Critical | Male | 70-80 | Anakinra | 4 | Discharged | 2 | 2 | 01:01* | 30:02 | 18:01* | 57:02 | Yes |
| COVID-040 | Critical | Male | 60-70 | N/A | 6 | Deceased | N/A | 3 | 02:01* | 66:01 | 18:01* | 41:02 | No (2) |
| COVID-042 | Critical | Female | 50-60 | Anakinra | 11 | Transferred | 2 | -2 | 03:01* | 24:02* | 35:02 | - | Yes |
| COVID-084 | Critical | Male | 60-70 | Tocilizumab | 3 | Discharged | 18 | 17 | 02:01* | 03:01* | 08:01* | 35:02 | Yes |
| COVID-094 | Critical | Female | 70-80 | Tocilizumab | 2 | Deceased | 13 | 17 | 02:01* | 32:01 | 51:08 | 56:01 | Yes |
| COVID-112 | Critical | Male | 70-80 | Tocilizumab | 5 | Discharged | 12 | 20 | 01:01* | 26:01 | 35:02 | 35:03 | Yes |
| COVID-121 | Critical | Male | 30-40 | Tocilizumab | 3 | Discharged | 8 | 9 | 03:01* | 74:01 | 07:02* | 42:01 | Yes |
| COVID-123 | Critical | Male | 70-80 | Tocilizumab | 6 | Deceased | 13 | 14 | 03:01* | 32:01 | 27:05 | 51:01* | Yes |
| COVID-127 | Critical | Male | 70-80 | Tocilizumab | 3 | Deceased | 2 | 2 | 29:02 | 31:01 | 35:01 | 44:03 | No (1) |
| COVID-129 | Critical | Male | 70-80 | Tocilizumab | 20 | Transferred | 15 | 18 | 02:01* | 29:02 | 15:01* | 44:03 | Yes |
| COVID-131 | Critical | Male | 40-50 | Tocilizumab | 3 | Discharged | 7 | 10 | 01:01* | 68:01 | 15:17 | 41:01 | Yes |
| COVID-140 | Critical | Male | 50-60 | Tocilizumab | 5 | Deceased | 6 | 24 | 11:01* | 68:02 | 35:01 | 53:01 | Yes |
| COVID-141 | Critical | Male | 70-80 | Tocilizumab | 28 | Discharged | 6 | 11 | 11:01* | 68:01 | 35:03 | 50:01 | Yes |
| COVID-143 | Critical | Male | 70-80 | Tocilizumab | 2 | Discharged | 10 | 12 | 01:01* | 02:01* | 44:03 | 51:01* | Yes |
| COVID-147 | Critical | Male | 70-80 | Tocilizumab | 18 | Discharged | 6 | 10 | 02:01* | 26:01 | 38:01 | 44:02 | Yes |
| COVID-150 | Critical | Male | 70-80 | Anakinra | 13 | Deceased | 14 | 14 | 24:02* | 29:01 | 13:02 | 35:03 | Yes |
| COVID-152 | Critical | Male | 60-70 | Tocilizumab | 2 | Deceased | 1 | 22 | 01:01* | 30:01 | 13:02 | 53:01 | No (2) |
| COVID-180 | Critical | Male | 40-50 | Tocilizumab | 4 | Deceased | 5 | 8 | 24:07 | 24:07 | 35:05 | 35:05 | No (1) |
| COVID-007 | Severe | Female | 30-40 | Tocilizumab | 4 | Deceased | 1 | 5 | 01:01* | 32:01 | 39:01 | 57:01 | Yes |
| COVID-009 | Severe | Male | 60-70 | Tocilizumab | 3 | Discharged | 3 | 6 | 02:05 | 32:01 | 13:02 | 14:02 | No (1) |
| COVID-015 | Severe | Male | 50-60 | Tocilizumab | 14 | Discharged | 2 | 1 | 02:01* | 24:02* | 18:01* | 35:03 | Yes |
| COVID-033 | Severe | Female | 70-80 | Tocilizumab | 3 | Discharged | 0 | 1 | 02:01* | 68:01 | 35:01 | 49:01 | Yes |
| COVID-087 | Severe | Female | 50-60 | Tocilizumab | 12 | Discharged | 8 | 8 | 02:01* | 03:02 | 39:01 | 50:01 | Yes |
| COVID-096 | Severe | Male | 40-50 | Tocilizumab | 4 | Discharged | 7 | 7 | 01:01* | 23:01 | 49:01 | 57:01 | Yes |
| COVID-111 | Severe | Male | 60-70 | Tocilizumab | 9 | Discharged | 7 | 9 | 02:01* | 30:02 | 18:01* | 51:01* | Yes |
| COVID-116 | Severe | Male | 80-90 | Tocilizumab | 4 | Discharged | 1 | 5 | 01:01* | 26:01 | 13:02 | 44:02 | No (2) |
| COVID-117 | Severe | Male | 60-70 | Tocilizumab | 2 | Discharged | 1 | 2 | 01:01* | 31:01 | 15:17 | 38:01 | Yes |
| COVID-153 | Severe | Male | 50-60 | Tocilizumab | 3 | Discharged | 7 | 9 | 01:01* | 02:01* | 35:01 | 35:02 | Yes |
| COVID-166 | Severe | Male | 70-80 | Tocilizumab | 1 | Discharged | 15 | 29 | 02:01* | 32:01 | 27:05 | 35:02 | Yes |
| COVID-174 | Severe | Male | 80-90 | Tocilizumab | 16 | Discharged | 8 | 15 | 02:01* | 24:02* | 08:01* | 44:02 | Yes |
| COVID-002 | Moderate | Male | 80-90 | None | 3 | Deceased | 0 | 2 | 03:02 | 32:01 | 18:01* | 44:02 | Yes |
| COVID-004 | Moderate | Male | 30-40 | None | 1 | Discharged | 0 | 3 | 03:01* | 24:02* | 07:02* | 35:01 | Yes |
| COVID-218 | Asymptomatic | Male | 30-40 | None | N/A | N/A | N/A | N/A | 03:01* | 26:01 | 18:01* | 38:01 | Yes |
| COVID-219 | Asymptomatic | Male | 20-30 | None | N/A | N/A | N/A | N/A | 02:01* | 03:01* | 18:01* | 41:01 | Yes |
| COVID-220 | Asymptomatic | Male | 20-30 | None | N/A | N/A | N/A | N/A | - | - | - | - | No (3) |
| COVID-221 | Asymptomatic | Male | 30-40 | None | N/A | N/A | N/A | N/A | 30:01 | 74:01 | 18:01* | 51:01* | Yes |
| COVID-222 | Asymptomatic | Male | 30-40 | None | N/A | N/A | N/A | N/A | 24:02* | 26:01 | 07:02* | 38:01 | Yes |
| COVID-223 | Asymptomatic | Male | 50-60 | None | N/A | N/A | N/A | N/A | 32:01 | 32:01 | 15:17 | 49:01 | No (1) |
| COVID-224 | Asymptomatic | Male | 30-40 | None | N/A | N/A | N/A | N/A | 01:01* | 30:01 | 08:01* | 39:01 | Yes |
| HD-01 | Healthy | Male | 70-80 | None | N/A | N/A | N/A | N/A | 02:01* | 03:01* | 15:01* | 35:01 | Yes |
| HD-02 | Healthy | Male | 70-80 | None | N/A | N/A | N/A | N/A | 01:01* | 03:01* | 07:02* | 40:01 | Yes |
| HD-03 | Healthy | Female | 70-80 | None | N/A | N/A | N/A | N/A | 03:01* | - | 35:01 | - | Yes |
| HD-05 | Healthy | Male | 50-60 | None | N/A | N/A | N/A | N/A | 01:01* | 11:01* | 08:01* | 38:01 | Yes |
| HD-06 | Healthy | Male | 60-70 | None | N/A | N/A | N/A | N/A | 02:05 | 11:01* | 40:01 | 50:01 | Yes |
| HD-08 | Healthy | Female | 60-70 | None | N/A | N/A | N/A | N/A | 01:01* | 23:01 | - | 41:02 | Yes |
| HD-10 | Healthy | Female | 60-70 | None | N/A | N/A | N/A | N/A | 01:01* | - | 35:01 | 44:03 | Yes |

111 **Supplementary Table 2:** Detected SARS-CoV-2-specific CD8 T cell responses. Magnitude
112 represents the percentage of pHLA⁺ cells of total CD8 T cells. ORF: open reading frame, S:
113 spike, N: nucleoprotein, M: membrane.

| Patient | Disease status | Epitope | Epitope origin | HLA restriction | Magnitude (% of pHLA ⁺ cells of total CD8 ⁺ cells) |
|-----------|----------------|-------------|----------------|-----------------|--------------------------------------------------------------------------|
| COVID-024 | Critical | TTDPSFLGRY | ORF 1ab | A*01:01 | 0.320 |
| COVID-042 | Critical | QYIKWPWYI | ORF S | A*24:02 | 0.006 |
| COVID-094 | Critical | YLQPRTFLL | ORF S | A*02:01 | 0.085 |
| COVID-112 | Critical | TTDPSFLGRY | ORF 1ab | A*01:01 | 0.720 |
| COVID-131 | Critical | ATSRTLSTYY | ORF M | A*01:01 | 0.021 |
| COVID-131 | Critical | CTDDNALAYY | ORF 1ab | A*01:01 | 0.017 |
| COVID-131 | Critical | FTSDYYQLY | ORF 3a | A*01:01 | 0.377 |
| COVID-131 | Critical | LTDEMIAQY | ORF S | A*01:01 | 0.020 |
| COVID-131 | Critical | PTDNYITTY | ORF 1ab | A*01:01 | 0.390 |
| COVID-131 | Critical | TTDPSFLGRY | ORF 1ab | A*01:01 | 19.000 |
| COVID-143 | Critical | CTDDNALAYY | ORF 1ab | A*01:01 | 0.210 |
| COVID-143 | Critical | DTDFVNEFY | ORF 1ab | A*01:01 | 0.270 |
| COVID-143 | Critical | PTDNYITTY | ORF 1ab | A*01:01 | 0.580 |
| COVID-143 | Critical | TTDPSFLGRY | ORF 1ab | A*01:01 | 13.300 |
| COVID-007 | Severe | FTSDYYQLY | ORF 3a | A*01:01 | 0.062 |
| COVID-007 | Severe | TTDPSFLGRY | ORF 1ab | A*01:01 | 0.074 |
| COVID-087 | Severe | RLNEVAKNL | ORF S | A*02:01 | 0.017 |
| COVID-087 | Severe | YLQPRTFLL | ORF S | A*02:01 | 0.038 |
| COVID-096 | Severe | PTDNYITTY | ORF 1ab | A*01:01 | 0.057 |
| COVID-096 | Severe | TTDPSFLGRY | ORF 1ab | A*01:01 | 3.100 |
| COVID-096 | Severe | TVATSRTLSTY | ORF M | A*01:01 | 0.100 |
| COVID-111 | Severe | LPYDPDSRI | ORF 1ab | B*51:01 | 0.220 |
| COVID-111 | Severe | YLQPRTFLL | ORF S | A*02:01 | 0.210 |
| COVID-117 | Severe | TTDPSFLGRY | ORF 1ab | A*01:01 | 2.340 |
| COVID-153 | Severe | PTDNYITTY | ORF 1ab | A*01:01 | 0.085 |
| COVID-153 | Severe | TTDPSFLGRY | ORF 1ab | A*01:01 | 18.400 |
| COVID-004 | Moderate | KTFPPTEPK | ORF N | A*03:01 | 0.038 |
| COVID-219 | Asymptomatic | KTFPPTEPK | ORF N | A*03:01 | 0.125 |
| COVID-221 | Asymptomatic | IPTNFTISV | ORF S | B*51:01 | 0.018 |
| COVID-222 | Asymptomatic | IPRRNVATL | ORF 1ab | B*07:02 | 0.009 |
| COVID-222 | Asymptomatic | NYNLYRLF | ORF S | A*24:02 | 0.009 |
| COVID-222 | Asymptomatic | QYIKWPWYI | ORF S | A*24:02 | 0.011 |
| COVID-222 | Asymptomatic | VQSTQWSLF | ORF 1ab | A*24:02 | 0.005 |
| COVID-224 | Asymptomatic | LTGHMLDMY | ORF 1ab | A*01:01 | 0.010 |
| COVID-224 | Asymptomatic | TTDPSFLGRY | ORF 1ab | A*01:01 | 0.011 |
| HD-01 | Healthy | ILMTARTVY | ORF 1ab | B*15:01 | 0.008 |

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115 **Supplementary Table 3.** Characteristics of CD8 T cell recognized SARS-CoV-2 epitopes. Epitopes with reported hotspot mutations (SNV
 116 frequency cutoff: 2.5%) are indicated (*). AA: amino acid, NT: nucleotide, ORF: open reading frame, S: spike, N: nucleoprotein, M: membrane.

| Epitope | AA length | Peptide origin | HLA restriction | Patients with detectable CD8 T cell responses (n, %) | SARS-CoV-1 | HCoV-NL63 | HCoV-229E | HCoV-OC43 | HCoV-HKU1 | AA location | NT location | Mutation | Alternative epitope | SNV frequency |
|------------|-----------|----------------|-----------------|------------------------------------------------------|------------|-----------|-----------|-----------|-----------|-------------|-------------|----------|---------------------|---------------|
| ATSRTLSYY | 9 | ORF M | A*01:01 | 1/9 (11%) | Yes | No | No | No | No | 171-179 | 27033-27059 | T175M | ATSRMLSY | 0.332 |
| CTDDNALAYY | 10 | ORF1ab | A*01:01 | 2/9 (22%) | Yes | No | No | No | No | 4163-4172 | 12752-12781 | T4164I | CIDDNALAYY | 0.051 |
| | | | | | | | | | | | | T4164A | CADDNALAYY | 0.017 |
| | | | | | | | | | | | | D4165G | CTGDDNALAYY | 0.052 |
| DTDFVNEFY | 9 | ORF1ab | A*01:01 | 1/9 (11%) | No | No | No | No | No | 5130-5138 | 15652-15678 | D5130Y | YTDVNEFY | 0.267 |
| | | | | | | | | | | | | T5131I | DIDVNEFY | 0.398 |
| | | | | | | | | | | | | E5136D | DTDFVNDY | 0.103 |
| | | | | | | | | | | | | F207L | LTSDYYQLY | 0.032 |
| FTSDYYQLY | 9 | ORF3a | A*01:01 | 2/9 (22%) | No | No | No | No | No | 207-215 | 26011-26037 | D210Y | FTSYYQLY | 0.022 |
| | | | | | | | | | | | | Q213K | FTSDYYKLY | 0.080 |
| | | | | | | | | | | | | Q213H | FTSDYYHLY | 0.010 |
| | | | | | | | | | | | | A5922S | IPRRNVSTL | 1.730 |
| IPRRNVATL | 9 | ORF1ab | B*07:02 | 1/3 (33%) | Yes | No | No | No | No | 5916-5924 | 18010-18036 | A5922V | IPRRNVVTL | 0.100 |
| | | | | | | | | | | | | T5923I | IPRRNVAIL | 0.182 |
| | | | | | | | | | | | | T716I | IPINFTISV | 8.439* |
| KTFPPTEPK | 9 | ORF N | A*03:01 | 2/7 (28%) | Yes | No | No | No | No | 361-369 | 29354-29380 | T719I | IPINFTISV | 0.022 |
| | | | | | | | | | | | | T362K | KKFPPEPK | 0.015 |
| | | | | | | | | | | | | T362I | KIFPPEPK | 0.114 |
| | | | | | | | | | | | | P364S | KTFSPTEPK | 0.031 |
| | | | | | | | | | | | | P364L | KTFPLTEPK | 0.063 |
| | | | | | | | | | | | | P365L | KTFPLTEPK | 0.062 |
| | | | | | | | | | | | | P365S | KTFPSTPEPK | 2.051 |
| | | | | | | | | | | | | T366R | KTFPPREPK | 0.014 |
| | | | | | | | | | | | | T366I | KTFPPEPK | 0.087 |
| | | | | | | | | | | | | E367D | KTFPPTEPK | 0.022 |
| | | | | | | | | | | | | P368L | KTFPPEPK | 0.012 |
| | | | | | | | | | | | | P368S | KTFPPEPK | 0.028 |
| | | | | | | | | | | | | L5221F | FPYPDPRI | 0.041 |
| | | | | | | | | | | | | P5222S | LSYPDPRI | 0.021 |
| LTDEMIAQY | 9 | ORF S | A*01:01 | 1/9 (11%) | No | No | No | No | No | 865-873 | 24155-24181 | I870V | LTDEMVAQY | 0.029 |
| | | | | | | | | | | | | A871V | LTDEMIVQY | 0.014 |
| | | | | | | | | | | | | A871S | LTDEMISQY | 0.026 |
| LTGHMLDMY | 9 | ORF1ab | A*01:01 | 1/9 (11%) | Yes | No | No | No | No | 5287-5295 | 16123-16149 | - | - | - |
| NYYLYRLF | 9 | ORF S | A*24:02 | 1/6 (16%) | No | No | No | No | No | 448-456 | 22904-22930 | L452M | NYYMYRLF | 0.024 |
| | | | | | | | | | | | | L452R | NYYRYRLF | 0.327 |
| | | | | | | | | | | | | Y453F | NYYLFRLF | 0.342 |
| | | | | | | | | | | | | L455F | NYYLYRFF | 0.014 |
| PTDNYITTY | 9 | ORF | A*01:01 | 4/9 (44%) | No | No | No | No | No | 1321-1329 | 4226-4252 | P1321S | STDNYITTY | 0.170 |
| | | | | | | | | | | | | T1322I | PIDNYITTY | 0.013 |
| | | | | | | | | | | | | T1322P | PPDNYITTY | 0.010 |
| | | | | | | | | | | | | D1323Y | PTYNYITTY | 0.040 |
| | | | | | | | | | | | | N1324S | PTDSYITTY | 0.014 |
| QYIKWPWYI | 9 | ORF S | A*24:02 | 2/6 (33%) | No | No | No | No | No | 1208-1216 | 25184-25210 | Q1208H | HYKWPWYI | 0.037 |
| RLNEVAKNL | 9 | ORF S | A*02:01 | 1/13 (7%) | Yes | No | No | No | No | 1185-1193 | 25115-25141 | N1187Y | RLYEVAKNL | 0.036 |
| | | | | | | | | | | | | K1191N | RLNEVANL | 0.095 |
| | | | | | | | | | | | | T1637I | ITDPSFLGRY | 0.140 |
| TTDPSFLGRY | 10 | ORF1ab | A*01:01 | 9/9 (100%) | No | No | No | No | No | 1637-1646 | 5174-5203 | T1638I | TIDPSFLGRY | 0.020 |
| | | | | | | | | | | | | P1640L | TTDLSFLGRY | 0.105 |
| | | | | | | | | | | | | P1640S | TTDSSFLGRY | 0.229 |
| | | | | | | | | | | | | P1640H | TTDHSFLGRY | 0.090 |
| TVATSRTLSY | 10 | ORF M | A*01:01 | 1/9 (11%) | Yes | No | No | No | No | 169-178 | 27027-27056 | T175M | TVATSRMLSY | 0.332 |
| VQSTQWSLF | 9 | ORF1ab | A*24:02 | 1/6 (16%) | Yes | No | No | No | No | 3595-3603 | 11048-11074 | V3595G | GQSTQWSLF | 0.010 |
| | | | | | | | | | | | | L3602F | VQSTQWSFF | 0.016 |
| YLQPRTFLL | 9 | ORF S | A*02:01 | 3/13 (23%) | No | No | No | No | No | 269-277 | 22367-22393 | P272L | YLQPRTFLL | 0.820 |

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Supplementary Table 4: Overview of UV-sensitive peptides used for generating the corresponding UV-sensitive pHLA monomers. ‘J’ indicates the UV-sensitive amino acid.

| HLA allele | Conditional ligand |
|------------|--------------------------|
| A*01:01 | STAPG-J-LEY ¹ |
| A*02:01 | KILGFVF-J-V ² |
| A*03:01 | RIYR-J-GATR ¹ |
| A*11:01 | AIFQSS-J-TK ¹ |
| A*24:01 | VYG-J-VRACL ³ |
| B*07:02 | AARG-J-TLAM ¹ |
| B*08:01 | FLRGRA-J-GL ² |
| B*15:01 | ILGP-J-GSVY ² |
| B*18:01 | SELE-J-KRY |
| B*51:01 | IPT-J-FSISI |

Supplementary Table 5: Streptavidin reagents used to generate fluorescent pHLA multimers.

| Fluorochrome / DNA oligo | Manufacturer | Cat. No. | Lot. No. | Dilution |
|--------------------------|--------------|----------|----------|----------|
| APC | Invitrogen | S868 | 1876191 | 1/16 |
| APC-R700 | BD | 565144 | 9023546 | 1/10 |
| BB630 | BD | custom | 0091407 | 1/10 |
| BB790 | BD | custom | 0091415 | 1/10 |
| BUV395 | BD | 564176 | 9078721 | 1/5 |
| BUV563 | BD | 567655 | 9227337 | 1/6 |
| BUV615 | BD | 613013 | 9193969 | 1/10 |
| BV421 | BD | 563259 | 9197684 | 1/5 |
| BV480 | BD | 564876 | 9115527 | 1/5 |
| BV605 | BD | 563260 | 9119807 | 1/5 |
| BV650 | BD | 563855 | 9197600 | 1/5 |
| BV711 | BD | 563262 | 0007729 | 1/8 |
| BV750 | BD | custom | 0091421 | 1/10 |
| PE | Invitrogen | S866 | 1736956 | 1/9 |
| Total-Seq-C0971 | Biolegend | 405271 | B295732 | 1/50 |
| Total-Seq-C0972 | Biolegend | 405273 | B295731 | 1/50 |
| Total-Seq-C0973 | Biolegend | 405275 | B304487 | 1/50 |
| Total-Seq-C0974 | Biolegend | 405277 | B304481 | 1/50 |

Supplementary Table 6: Antibodies used in this study. pHLA: peptide HLA multimer assay, PC: phenotypic characterization by flow cytometry, PS: peptide stimulation assay, RNA-seq: sc-RNA- and TCR-seq assay, N/A: not applicable.

| Antibody | Fluorochrome / DNA oligo | Company | Cat. No. | Clone | Lot. No. | Dilution | Assay |
|---------------------|--------------------------|-------------|------------|-------------|----------|----------|-----------------------|
| CCR7 | BV711 | BD | 563712 | 150503 | 9022605 | 1/133 | PC |
| CD14 | APC-H7 | BD | 560180 | M ϕ P9 | 85600 | 1/100 | pHLA, PS, PC, RNA-seq |
| CD14 | FITC | BD | 345784 | M ϕ P9 | 156613 | 1/100 | pHLA, RNA-seq |
| CD16 | APC-H7 | BD | 560715 | 3G8 | 9309425 | 1/100 | pHLA, PS, PC, RNA-seq |
| CD16 | BUV496 | BD | 612944 | 3G8 | 9213597 | 1/100 | pHLA |
| CD16 | FITC | BD | 335035 | NKP15 | 7003932 | 1/100 | RNA-seq |
| CD19 | APC-H7 | BD | 560177 | SJ25C1 | 140610 | 1/100 | pHLA, PS, PC, RNA-seq |
| CD19 | BUV661 | BD | 750536 | SJ25C1 | 288444 | 1/100 | pHLA |
| CD27 | BV421 | BD | 562514 | M-T271 | 5051571 | 1/100 | PC |
| CD28 and β 2m | Total-Seq-C0251 | BioLegend | 394661 | N/A | B282243 | 1/100 | RNA-seq |
| CD28 and β 2m | Total-Seq-C0253 | BioLegend | 394665 | N/A | B282244 | 1/100 | RNA-seq |
| CD28 and β 2m | Total-Seq-C0254 | BioLegend | 394667 | N/A | B282246 | 1/100 | RNA-seq |
| CD28 and β 2m | Total-Seq-C0255 | BioLegend | 394669 | N/A | B282245 | 1/100 | RNA-seq |
| CD28 and β 2m | Total-Seq-C0257 | BioLegend | 394673 | N/A | B306454 | 1/100 | RNA-seq |
| CD4 | APC-H7 | BD | 641398 | SK3 | 35560 | 1/100 | pHLA, PS, PC, RNA-seq |
| CD4 | BB700 | BD | 566393 | SK3 | 8248607 | 1/100 | pHLA |
| CD4 | FITC | BD | 345768 | SK3 | 6313547 | 1/100 | RNA-seq |
| CD45RA | BUV563 | BD | 612926 | HI100 | 7219651 | 1/400 | PC |
| CD69 | BUV395 | BD | 564364 | FN50 | 7108931 | 1/50 | PC |
| CD73 | BB700 | BD | 746000 | AD2 | 8229890 | 1/100 | PC |
| CD8 | BUV805 | BD | 612889 | SK1 | 86704 | 1/50 | pHLA, PS, PC |
| CD8 | BV421 | BD | 562428 | RPA-T8 | 9254861 | 1/50 | RNA-seq |
| CD95 | BUV737 | BD | 612790 | DX2 | 7032582 | 1/50 | PC |
| CXCR3 | APC | BioLegend | 353708 | G025H7 | 7121923 | 1/100 | PS |
| CXCR5 | BV786 | BioLegend | 356936 | J252D4 | B245355 | 1/200 | PC |
| HLA-DR | BUV661 | BD | 612980 | G46-6 | 7249926 | 1/400 | PC |
| IFN γ | APC | BD | 554702 | B27 | 31187 | 1/50 | PS |
| IL-17 | PE | Biolegend | 512306 | BL168 | B206920 | 1/100 | PS |
| IL-2 | BV750 | BD | 566361 | MQ1-17H12 | 64351 | 1/200 | PS |
| LAG-3 | BV605 | BD | 745160 | T47-530 | 8233745 | 1/100 | PC |
| NKG2A | PE-Cy7 | Beckman | B10246 | Z199 | 200051 | 1/100 | pHLA |
| PD-1 | BUV737 | BD | 612791 | EH12.1 | 9212298 | 1/100 | pHLA |
| PD-1 | PE-Cy7 | BD | 561272 | EH12.1 | 0022949 | 1/100 | PC |
| pHLA | APC | N/A | N/A | N/A | N/A | 1/50 | pHLA |
| pHLA | APC-R700 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BB630 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BB790 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BUV395 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BUV563 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BUV615 | N/A | N/A | N/A | N/A | 1/100 | pHLA, PC |
| pHLA | BV421 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BV480 | N/A | N/A | N/A | N/A | 1/100 | pHLA, PC |
| pHLA | BV605 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BV650 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BV711 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | BV750 | N/A | N/A | N/A | N/A | 1/100 | pHLA |
| pHLA | PE | N/A | N/A | N/A | N/A | 1/100 | pHLA, PC |
| pHLA | Total-Seq-C0971 | N/A | N/A | N/A | N/A | 1/100 | RNA-seq |
| pHLA | Total-Seq-C0972 | N/A | N/A | N/A | N/A | 1/100 | RNA-seq |
| pHLA | Total-Seq-C0973 | N/A | N/A | N/A | N/A | 1/100 | RNA-seq |
| pHLA | Total-Seq-C0974 | N/A | N/A | N/A | N/A | 1/100 | RNA-seq |
| TIGIT | PerCP-eF710 | eBioscience | 46-9500-42 | MBSA43 | 4318928 | 1/100 | PC |
| TIM3 | BV650 | BD | 565564 | 7D3 | 7241582 | 1/200 | PC |
| TNF | FITC | BD | 554512 | MAb11 | 15360 | 1/50 | PS |

Supplementary References

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