1  **Identification of Cross-Reactive CD8^{+} T Cell Receptors with High Functional**

2  **Avidity to a SARS-CoV-2 Immunodominant Epitope and Its Natural Mutant**

3  **Variants**

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**ABSTRACT**

Despite the growing knowledge of T cell responses and their epitopes in COVID-19 patients, there is a lack of detailed characterizations for T cell-antigen interactions and T cell functions. Using a peptide library predicted with HLA class I-restriction, specific CD8$^+$ T cell responses were identified in over 75% of COVID-19 convalescent patients. Among the 15 SARS-CoV-2 epitopes identified from the S and N proteins, N$^{361-369}$ (KTFPPTEPK) was the most dominant epitope. Importantly, we discovered 2 N$^{361-369}$-specific T cell receptors (TCRs) with high functional avidity, and they exhibited complementary cross-reactivity to reported N$^{361-369}$ mutant variants. In dendritic cells (DCs) and the lung organoid model, we found that the N$^{361-369}$ epitope could be processed and endogenously presented to elicit the activation and cytotoxicity of CD8$^+$ T cells *ex vivo*. Our study evidenced potential mechanisms of cellular immunity to SARS-CoV-2, illuminating natural ways of viral clearance with high relevancy in the vaccine development.

**KEYWORDS**

SARS-CoV-2, CD8$^+$ T cell, T cell epitope, TCR, Lung organoid, HLA class I

## INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results in the global pandemic of COVID-19 (Dong et al., 2020; Zhou et al., 2020). So far, over 40 million positive cases of SARS-CoV-2 infection have been reported with one million deaths worldwide (*https://covid19.who.int/*). Despite the fact that most patients can recover spontaneously with supportive care, COVID-19 mortality rate is still a worrying social concern. A better understanding of the adaptive immunity to SARS-CoV-2 infection is important to assist vaccine development and evaluation.

SARS-CoV-2 contains four structural proteins, namely nucleoprotein (N), membrane protein (M), envelope protein (E) and spike glycoprotein (S) (Zhou et al., 2020). SARS-CoV-2 S protein is responsible for the virus-target cell recognition (Hoffmann et al., 2020; Walls et al., 2020; Zhou et al., 2020). It has been demonstrated that the S protein was capable of inducing not only humoral immunity but also cellular immunity (Cao et al., 2020; Chen et al., 2020; Grifoni et al., 2020b; Ju et al., 2020; Le Bert et al., 2020a; Ni et al., 2020; Shi et al., 2020; Weiskopf et al., 2020). And the S protein has always been the primary consideration for SARS-CoV-2 vaccine development (Corbett et al., 2020a; Mercado et al., 2020b; Yu et al., 2020). Besides the S protein, the N protein can also elicit high levels of T cell responses in COVID-19 patients, highlighting the possibility that S and N are both predominant targets for T cell responses in SARS-CoV-2 (Grifoni et al., 2020b; Le Bert et al., 2020b; Peng et al., 2020). It is still unknown whether SARS-CoV-2 is effective to induce long-lasting protective immunity due to the limited time window post-outbreak.

Pandemic outbreaks of infectious diseases resulted from pathogenic human coronaviruses, such as SARS-CoV and middle eastern respiratory syndrome coronavirus (MERS-CoV), have caused high mortality in the past two decades. For SARS-CoV, evidences supporting T cell immunity to its N protein has been reported, with some still observed over 17 years later in SARS-CoV infected individuals (Le Bert et al., 2020b). Because SARS-CoV N protein exhibits more than 90% homology

88  of amino acid sequence to that of SARS-CoV-2 (Grifoni et al., 2020a), it is natural to

89  suspect that T cell responses to the N protein of SARS-CoV-2 may also provide

90  cellular immune protection, which may last for a long time. Recently, using predicted

91  or established peptide libraries of the S and N proteins, a number of SARS-CoV-2 T

92  cell epitopes were identified in COVID-19 convalescent patients across independent

93  studies, providing important clues for vaccine development (Chour et al., 2020;

94  Ferretti et al., 2020; Le Bert et al., 2020b; Nelde et al., 2020; Peng et al., 2020; Poran

95  et al., 2020; Shomuradova et al., 2020; Snyder et al., 2020). However, detailed

96  information of how SARS-CoV-2 induces cellular immune responses is currently

97  limited.

98  Cellular immunity against viral infections is mediated by cytotoxic T cells, which

99  eliminate the viruses by killing infected cells. To trigger optimal T cells responses,

100  viral antigens must be processed before binding to the major histocompatibility

101  complex (MHC) molecules to form the peptide-MHC complex (pMHC), which is

102  then presented on the cell membrane. When TCRs identify the cognate pMHC,

103  together with matching CD8 co-receptor binding to the MHC, the T cells will be

104  activated. Such activation is dependent on the binding kinetics of the TCR-pMHC,

105  which is influenced by the density of processed epitopes on the membrane of the

106  virus-infected cells or antigen presenting cells (APC) (Campillo-Davo et al., 2020;

107  Gonzalez et al., 2005). Particularly, CD8 co-receptor is indispensable for enhanced

108  activation of T cells with low-affinity TCRs, but not for T cells with high-affinity

109  TCRs. (Campillo-Davo et al., 2020; Lyons et al., 2006; Tan et al., 2017). Activation of

110  T cells independent of the CD8 co-receptor reflects the high functional avidity of their

111  TCRs. Hence, the functional avidity of TCRs is a critical parameter to be evaluated

112  for determining the pMHC recognition and posterior T-cell activation, which is

113  essential for the killing of virus-infected cells and effective viral clearance

114  (Campillo-Davo et al., 2020).On the other hand, RNA viruses have a tendency to

115  accumulate mutations over time (Duffy, 2018; Villa et al., 2020), which present

116  challenges for T cell-based vaccine development. Therefore, it is important to identify

117  predominant epitopes together with diverse TCRs carrying broader cross-reactivity to

118  SARS-CoV-2 and its mutant variants, which can provide detailed information to

119  understand the mechanisms of T cell-mediated viral clearance in COVID-19 patients.

120  In this study, we evaluated SARS-CoV-2 specific CD8$^+$ T cell responses in

121  COVID-19 convalescent patients with HLA-A2, HLA-A24 or HLA-A11 allele, the 3

122  most prominent HLA-A alleles in the Asian population (He et al., 2018). A total of 15

123  epitopes derived from SARS-CoV-2 S and N were identified. The most dominant

124  epitope N$^{361-369}$ (KTFPPTEPK) were characterized in detail, including the wild type

125  and clinically presented mutant variants of N$^{361-369}$ and the functional avidity of

126  N$^{361-369}$-specific TCRs. Our study is, to our knowledge, the first to reveal that an

127  immunodominant CD8$^+$ T cell epitope derived from SARS-CoV-2 N can be

128  intracellularly processed and presented by DCs and lung organoids, leading to robust

129  T cell activation and cytotoxicity. Our findings illustrated potential mechanisms of

130  CD8$^+$ T cell immunity against SARS-CoV-2 infection, which could be of great use to

131  understand the viral control process and to facilitate the vaccine development for

132  COVID-19.

133

134  **RESULTS**

135  **Identification of SARS-CoV-2 Specific CD8$^+$ T Cell Epitopes**

136  It has been reported that SARS-CoV-2 S and N proteins were the predominant T cell

137  targets in COVID-19 patients (Grifoni et al., 2020b). In order to predict SARS-CoV-2

138  specific CD8$^+$ T cell epitopes, the NetMHCpan 4.0 algorithm was used to analyse the

139  binding of 9-mer peptides derived from the full lengths of SARS-CoV-2 S and N

140  proteins, with HLA-A*02:01, HLA-A*24:02 and HLA-A*11:01, the 3 most frequent

141  HLA class I A alleles in the Asian population. We synthesized a peptide library of 72

142  predicted SARS-CoV-2 epitopes, which overlapped 29 SARS-CoV epitopes, together

143  with additional 6 confirmed SARS-CoV epitopes (Table S1) (Ahmed et al., 2020;

144  Grifoni et al., 2020a). Among these 78 peptides, 62 were from the S protein and 16

145  were from the N protein, with 3 different HLA-A restrictions (Table S2).

146    In parallel, we collected a total of 37 COVID-19 convalescent PBMC samples and

147    subjected them for the HLA typing analysis. Among the 20 HLA-A2$^+$ samples and the

148    12 HLA-A24$^+$ samples revealed by flow cytometric assay, we selected 8 samples from

149    each group. A matching 8 samples with HLA-A*11:01$^+$ genotype was identified by

150    RT-PCR, which included 2 overlapping with the HLA-A2$^+$ group and 2 with the

151    HLA-A24$^+$ group (Table S3). These PBMCs were stimulated for 10 days in the

152    presence of individual peptide mixtures and IL-2, and the induction of IFN-γ secretion

153    was determined by the enzyme-linked immunospot (ELISPOT)assay. We found that

154    75% COVID-19 convalescent PBMCs demonstrated positive T cell responses when

155    stimulated by corresponding peptide mixtures. Particularly, the HLA-A2$^+$ group

156    showed dominant responses to Mixture (M) 1/3/5, the HLA-A24$^+$ group to M6/7 and

157    the HLA-A*11:01$^+$ group to M14 (Figures 1A-1C and S1). Next, individual peptides

158    from each positive mixture were applied to identify specific T cell epitopes, and 15

159    SARS-CoV-2 specific peptides were found to be recognized by the convalescent

160    PBMCs (Figures 1A-1C, S2 and S3; Tables 1 and S5). Among the 15 positive

161    peptides, 5 peptides were derived from the N protein, and the rest were derived from

162    the S protein (Table 1). Four dominant peptides were identified, with at least one

163    peptide from each HLA group (Table 1). Two of these peptides, P63 (N$^{338-346}$) and P77

164    (N$^{219-227}$), were both HLA-A*02:01-restricted, and they could induce IFN-γ release in

165    4/8 and 2/8 samples, respectively (Figures 1A and S2; Table 1). The

166    HLA-A*24:02-restricted P45 (S$^{448-456}$) was located at the receptor binding domain of

167    the S protein and could be recognized by 2/8 samples tested (Figure 1B; Table 1).

168    Importantly, 5 out of 8 samples showed positive responses to the

169    HLA-A*11:01-restricted P64 (N$^{361-369}$). Hence we termed P64, or N$^{361-369}$, as the most

170    dominant epitope in this study (Figure 1C; Table 1). Of note, IFN-γ induction was not

171    observed when these 4 dominant peptides were used to stimulate PBMCs of healthy

172    individuals (Figure S4; Table S4). Overall, these data demonstrated that 15 predicted

173    peptides derived from the S and the N protein sequences could induce

174    SARS-CoV-2-specific CD8$^+$ T cell responses in about 75% of COVID-19

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175    convalescent patient samples, and at least 4 peptides were identified to be dominant

176    CD8^{+} T cell epitopes.

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178    **Identification of Specific CD8^{+} TCRs to SARS-CoV-2 Dominant Epitopes**

179    To identify the dominant epitope-specific TCRs, we selected P64, P45 and P63, the

180    most dominant epitopes from the 3 HLA-A alleles. After stimulated with these

181    epitopes, the populations of IFN-γ secreting T cells from the corresponding

182    COVID-19 convalescent patient samples were sorted at single cell levels (Figure 2A).

183    The TCRs of sorted cells were amplified (Hamana et al., 2016), and the sequences of

184    the variable regions of α- and β-chains were analysed. In the P64-specific TCR

185    repertoire, we found 7 dominant TCR clones (TCR 1-7), with the most dominant

186    clone 1 (TCR 1) accounted for 17.82% (Figure 2B). Similarly, 3 dominant P63 TCR

187    clones and 5 dominant P45 TCR clones were obtained, with the most dominant clone

188    accounted for 10.34% and 33.34% respectively (Figure 2B). These results showed

189    that COVID-19 convalescent patients with these three HLA-A restrictions could

190    produce diverse T cell clones for the recognition of corresponding dominant epitopes.

191    Furthermore, the specificities of the P64 reactive TCRs were verified in CD8^{+}

192    Jurkat cells, which were transduced with the top 4 dominant TCR clones (TCR 1-4).

193    Using the P64-HLA-A*11:01 tetramer, we confirmed that only TCR 1 and TCR 4

194    were P64-specific (Figure 2C; Table S6). Also, with co-culture of the

195    HLA-A*11:01-expressing COS-7 cells loaded with P64, TCR 1- or TCR 4-transduced

196    CD8^{+} Jurkat cells could be activated, indicated by elevated CD69 expression (Figure

197    2D). These observations were not found with TCR 2 or TCR 3 (Figures 2C and 2D).

198    Altogether, we successfully identified 2 different CD8^{+} TCRs targeting the

199    SARS-CoV-2 dominant epitope P64 ($N^{361-369}$) from the COVID-19 convalescent

200    PBMCs.

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202    **Characterization of the Functional Avidity of the $N^{361-369}$-Specific TCRs**

203     To further evaluate the functional activities of the $N^{361-369}$-specific TCRs, TCR 1 and

204     TCR 4 were individually transduced into the primary $CD3^+$ T cells isolated from the

205     PBMCs of healthy individuals. These cells were expanded for 12 days following the

206     rapid expansion protocol (REP) (Jin et al., 2012), before the evaluation of their

207     transfection efficiency by $N^{361-369}$-HLA-A*11:01 tetramer. We found that 9.86%

208     $CD8^+$ T cells expressed TCR 1 and 6.34% expressed TCR 4 (Figure 3A).

209     Unexpectedly, we observed higher percentages of TCR-transduced $CD4^+$ T cells, with

210     38.4% for TCR 1 and 11.7% for TCR 4 (Figure 3A). These results indicated that

211     TCR-transduced $CD4^+$ T cells were able to recognize the $N^{361-369}$-HLA complex with

212     the absence of CD8 co-receptor. Also, the similar fluorescence intensities detected in

213     the $CD4^+$ T cells and in the $CD8^+$ T cells implied that TCR 1 and TCR 4 might both

214     have high avidity for the $N^{361-369}$-HLA-A*11:01 complex (Figure 3A). To confirm the

215     binding of these TCRs to $N^{361-369}$-HLA-A*11:01 was independent of CD8 co-receptor,

216     TCR 1 and TCR 4 were separately transduced to $CD8^-$ and $CD8^+$ Jurkat cells. Both

217     TCR 1- and TCR 4-transduced $CD8^-$ Jurkat cells showed positive tetramer staining,

218     and comparable fluorescent signal levels were found between $CD8^-$ and $CD8^+$ Jurkat

219     cells (Figure 3B). These results were in line with those of the $CD4^+$ and $CD8^+$ T cells

220     (Figures 3A and 3B).

221      Next, we evaluated the functionality of TCR 1 and TCR 4 in $CD8^+$ T cells and

222     $CD4^+$ T cells from expanded $CD3^+$ T cells expressing TCR 1 or TCR 4. After

223     co-cultured with HLA-A*11:01$^+$ K-562 cells pulsed with $N^{361-369}$, remarkable levels

224     of T cell activation were detected in TCR 1- and TCR 4-$CD8^+$ T cells, indicated by

225     the upregulation of T cell activation marker CD137 expression, compared to the

226     DMSO controls (Figure 3C). Similar results were obtained in TCR-transduced $CD4^+$

227     T cells stimulated with $N^{361-369}$ (Figure 3C). Moreover, we evaluated the cytotoxicity

228     of TCR 1- and TCR 4-transduced $CD8^+$ T cells against HLA-A*11:01$^+$ K-562 cells,

229     using Calcein-AM release assay. Significant levels of target cell lysis were detected in

230     the presence of $N^{361-369}$, relative to the DMSO controls (Figure 3D). Increasing

231     cytotoxic efficiency was observed at the effector to target (E:T) cell ratios of 20:1 or

232  higher, and approximately 70% target cell lysis was achieved at E:T cell ratio of 40:1

233  (Figure 3D). The functional activity of TCR 1- and TCR 4-transduced CD4$^+$ T cells

234  was analysed by the production of IFN-γ, IL-2 and TNF-α via cytokine secretion

235  assay. We found that N$^{361-369}$ could elicit significant induction of all three cytokines in

236  these CD4$^+$ T cells, comparing to an equimolar amount of DMSO (Figure 3E). These

237  results proved that the N$^{361-369}$-specific TCR 1 and TCR 4 could mediate the

238  cytotoxicity of CD8$^+$ T cells and the effector cytokine secretion of CD4$^+$ T cells.

239

240  **Evaluation of the Affinity and the Cross-Reactivity of the N$^{361-369}$-Specific TCRs**

241  We addressed the functional avidity of TCR 1 and TCR 4 to the N$^{361-369}$-HLA

242  complex with the peptide concentrations required to induce a half-maximal activation

243  (EC50) per IFN-γ production by the TCR-transduced T cells. The IFN-γ release assay

244  revealed that the EC50 concentrations of TCR 1 and TCR 4 in CD8$^+$ T cells were

245  46.83 nM and 47.17 nM respectively, when stimulated by HLA-A*11:01$^+$ K-562 cells

246  loaded with sequential ten-fold dilutions of the N$^{361-369}$ peptide (Figure 4A). Notably,

247  TCR 1 and TCR 4 in CD4$^+$ T cells also demonstrated high functional avidity, with

248  EC50s of 118 nM and 380.9 nM respectively, in a co-receptor independent manner

249  (Figure 4A). These data confirmed that TCR 1 and TCR 4 both had high affinity to

250  the N$^{361-369}$-HLA complex in CD8$^+$ and CD4$^+$ T cells.

251  Recently, SARS-CoV-2 mutation strains have been reported continuously. To

252  evaluate whether TCR 1 and TCR 4 could recognize known N$^{361-369}$ mutations, we

253  synthesized a total of 9 mutant peptides with clinically presented point mutations of

254  residues between 361 to 369 amino acids of the N protein (Figure 4B; Table S7)

255  (Zhao et al., 2020). The HLA binding assay revealed that 7 different point mutations

256  (T362I, F363L, F363S, P364L, P364R, T366I and E367K) did not affect the binding

257  of N$^{361-369}$ to HLA*A11:01, whereas T362K markedly diminished the ability of the

258  N$^{T362K}$ peptide to bind to HLA*A11:01. These results, particularly the different

259  reactivity of N$^{T362I}$ and N$^{T362K}$, implied that the charge of amino acid side chain, at

260  least, might influence the binding affinity of N$^{361-369}$ to HLA*A11:01. We also found

261      that the $N^{361-369}$ homologous peptide from MERS-CoV ($N^{MERS}$), which exhibited 3

262      amino acids in difference to $N^{361-369}$, bound to HLA*A11:01 weakly (Figure 4B).

263      Meanwhile, IFN-γ production was evaluated for the reactivity of TCR 1- and TCR

264      4-CD8$^+$ T cells, after co-culturing TCR-transduced cells with HLA-A*11:01$^+$ COS-7

265      cells loaded with each mutant peptide. We observed that $N^{361-369}$-specific TCR-T cells

266      responded differently to these mutant peptides. The T366I, F363L or T362I mutations

267      of $N^{361-369}$ could still induce high levels of IFN-γ production, but not other point

268      mutations (Figure 4B). Interestingly, we found that $N^{T366I}$ preferably led to an

269      activation of the TCR 1-T cells similar to that of the wild type $N^{361-369}$, and the TCR

270      4-T cells were mainly activated by $N^{T362I}$ or $N^{F363L}$ (Figure 4B). These findings

271      indicated that $N^{361-369}$-specific TCRs derived from the COVID-19 convalescent

272      patients had cross-reactivity to certain $N^{361-369}$ variants. Altogether, the $N^{361-369}$ is

273      shown to be a T cell epitope with strong immunogenicity that can be recognized by a

274      diversity of TCRs with high affinity. Such diverse TCRs may play critical protective

275      roles in SASR-CoV-2 infection and decrease the mutation driven immune escape.

276

**Confirmation of the Endogenous Presentation of the $N^{361-369}$ Epitope**

278      Furthermore, we studied whether the bioinformatically predicted $N^{361-369}$ epitope

279      could be naturally processed and presented from SARS-CoV-2 N protein. First, as

280      dendritic cells (DCs) can ingest virus-infected cells or cellular fragments and

281      cross-present antigens to activate CD8$^+$ T cells, we tested whether the $N^{361-369}$

282      specific-TCR-T cells could be activated by the N protein loaded mature DCs that

283      were induced from the HLA-A*11:01$^+$ healthy donors' monocytes. Substantial levels

284      of T cell activation were detected in TCR 1- and TCR 4-CD8$^+$ T cells, indicated by

285      elevated CD137 expression, comparing to the S protein loaded groups (Figure 5A).

286      Also, we found significantly higher levels of IFN-γ secretion in the N protein loaded

287      groups, relative to the S protein controls (Figure 5B).

288      Next, we verified whether $N^{361-369}$ could be endogenously presented by lung

289      epithelial cells that were infected by SASR-CoV-2. To this end, we established a

290    series of lung tissue organoids, and HLA-A*11:01$^+$ lung organoids, referred as LO,

291    were selected for the subsequent experiments (Figures S5A-5C). The expression of N

292    protein in lung organoid (N-LO) was achieved by ectopically expressing the N protein

293    via the lentiviral EGFP system, with the transfection efficiency confirmed by

294    fluorescent detection (Figure S5D). The lung organoids expressing S protein (S-LO)

295    were used as control. After N$^{361-369}$-specific TCR-T cells were co-cultured with N-LO

296    or S-LO for 24 hours, flow cytometric analysis demonstrated that only N-LO could

297    induce an activation of these TCR-CD8$^+$ T cells, indicated by enhanced CD137

298    expression and significantly elevated IFN-γ production (Figures 5C and 5D). These

299    results demonstrated that the N$^{361-369}$ epitope could be naturally processed from the

300    full length N protein, and presented on the surface of APCs by the intracellular HLA

301    machinery, leading to the activation of N$^{361-369}$ specific CD8$^+$ T cells.

302    To evaluate the N$^{361-369}$ activated CD8$^+$ T cells could kill lung organoids, we

303    co-cultured HLA-A*11:01$^+$ LO loaded with the N$^{361-369}$ peptide with TCR 1-CD8$^+$ T

304    cells. After 24 h, we observed apparent organoid lysis, together with the recruitment

305    of TCR 1-T cells, only with the presence of N$^{361-369}$ (Figure 5E). We found a

306    significantly higher level of target cell lysis in the N$^{361-369}$ loaded group, when these

307    effects were quantified by Calcein-AM release assay (Figure 5E). To determine

308    whether the cytotoxic CD8$^+$ T cells could eliminate infected lung organoids, we

309    co-cultured HLA-A*11:01$^+$ N-LO or S-LO with TCR 1-CD8$^+$ T cells. We found that

310    lung organoids with endogenous expression of the N protein became more susceptible

311    to the cytotoxicity of specific T cells, comparing to the S-LO group (Figure 5F). Such

312    difference of the cytotoxic effect was statistically significant, analysed by LDH

313    release assay (Figure 5F). Taken together, we identified a dominant T cell epitope,

314    N$^{361-369}$, that could activate specific CD8$^+$ T cells, and the cytotoxicity of these T cells

315    would result in the lysis of virus infected cells, executively managing the virus

316    clearance in COVID-19 patients.

317

318    **DISCUSSION**

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319    Currently, limited information is available for the mechanisms of the cellular

320    immunity against SARS-CoV-2. Better understanding of the T cell responses and

321    their protective effects are essential for the vaccine development for COVID-19. Here,

322    we have identified immunodominant epitopes from SARS-CoV-2 S and N proteins

323    that can induce specific CD8$^+$ T cell responses, in the majority of COVID-19

324    convalescent patients with HLA-A*02:01, HLA-A*24:02 or HLA-A*11:01.

325    Importantly, we have discovered $N^{361-369}$, a dominant epitope from the N protein, and

326    2 different CD8$^+$ TCRs recognizing $N^{361-369}$. These 2 TCRs displayed high functional

327    avidity for the wild-type and the mutant variants of $N^{361-369}$, which provided

328    complementary protections for each other against SASR-CoV-2. We are the first to

329    present evidence that $N^{361-369}$ can be endogenously presented by HLA-A*11:01 on

330    APCs, which, in turn, can activate specific CD8$^+$ T cells that may contribute to

331    effective virus clearance in COVID-19 convalescent patients.

332    Both the S and N proteins of SARS-CoV-2 have been reported to effectively

333    eliciting CD8$^+$ T cell responses (Grifoni et al., 2020b). Indeed, when stimulated by

334    synthesized peptides corresponding to SARS-CoV-2 S and N, robust CD8$^+$ T cell

335    responses could be detected in nearly 75% of COVID-19 convalescent PBMCs

336    (Figure 1; Table S5). Strikingly, we found that 3 out of 4 dominant peptides were

337    derived from the N protein, and accounted for approximately 50% of tested CD8$^+$ T

338    cell reactivity. These data suggested that the N protein might be a dominant target for

339    CD8$^+$ T cell recognition. Similar observations have been made (Grifoni et al., 2020b;

340    Weiskopf et al., 2020). Of note, T cell responses to the 4 dominant CD8$^+$ T cell

341    epitopes identified in our study were not observed in uninfected individuals (Figure

342    S4), while cross-reactivities with other common coronaviruses in unexposed healthy

343    donors had been reported. This difference might due to the refined sample size of

344    healthy donors with corresponding HLA restrictions in our study. Contrast to the

345    rather limited HLA-A phenotype information associated with available T cell epitopes

346    from COVID-19 samples, we provided 15 SARS-CoV-2 epitopes for the 3 most

347    prominent HLA class I A alleles in the Asian population (Table 1), offering more

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348  accurate information for vaccine designs. Of these dominant epitopes, the

349  HLA-A*11:01-restricted $N^{361-369}$ (KTFPPTEPK) was found in 5/8 COVID-19

350  PBMCs (Figure 1C; Table 1). Independently, this epitope has also been identified in

351  multiple individuals in the British COVID-19 convalescent patients (Peng et al.,

352  2020). These findings strongly suggested that $N^{361-369}$ was highly immunogenic and

353  capable to induce robust CD8$^+$ T cell responses to SARS-CoV-2.

354      Presently, reports for SARS-CoV-2 inducing cellular immune responses were

355  largely focused on the T cell-antigen reactivity. Detailed mechanisms regarding the

356  cellular immunity in COVID-19 convalescent patients were needed for advancing the

357  vaccine development and evaluation. In our study, we found 2 $N^{361-369}$-specific TCRs

358  with distinct functions. When ectopically transduced, they could elicit CD8$^+$ T cell

359  cytotoxicity upon target cells (Figure 3D), and mediate the activation of T helper type

360  1 CD4$^+$ T cells to secret cytokines in a CD8-independent manner (Figure 3E). It has

361  been reported that such independency could only be achieved when the TCR

362  exhibited high-affinity binding to the cognate pMHC (Campillo-Davo et al., 2020;

363  Lyons et al., 2006; Tan et al., 2017). In the affinity test of the $N^{361-369}$-specific TCRs in

364  the transduced CD8$^+$ T or CD4$^+$ T cells, we found that their EC50 concentrations

365  ranged from 47.17 nM to 380.9 nM (Figure 4A). These values are remarkably smaller

366  than most of the reported thresholds of affinity for maximal T-cell activity to peptide

367  epitopes, which were around 5-10 µM (Oren et al., 2014; Schmid et al., 2010; Tan et

368  al., 2015; Zhong et al., 2013). Hence, our data evidenced that the $N^{361-369}$-specific

369  TCRs had high affinity and functional avidity, both of which were key features to

370  induce sufficient levels of T cell activation for antivirus responses. As a newly

371  emerged single stranded RNA virus belonging to the highly contagious coronavirus

372  subfamily, SARS-CoV-2 has a tendency to accumulate mutations over time (Duffy,

373  2018; Villa et al., 2020). Here, another innovative piece of our finding was that the 2

374  distinct $N^{361-369}$-specific TCRs could recognize multiple $N^{361-369}$ mutations in

375  complementary (Figure 4B). Thus, our findings have revealed the high functional

376  avidity of the 2 TCRs for $N^{361-369}$, with compatible characteristics for clinical mutants

13

377 of this epitope, providing practical tools to interpret potential mechanisms of $CD8^+$ T

378 cell responses against SARS-CoV-2, which are highly relevant in vaccine design and

379 evaluation.

380 Viral antigens can be phagocytosed and cross-presented by DCs to prime $CD8^+$ T

381 cells, or directly processed and presented by infected cells to activate cellular immune

382 responses. Having proven the strong immunogenicity of $N^{361-369}$ *in vitro*, it is of

383 physiological importance to confirm that the N protein can be processed and

384 presented naturally. We proved that $N^{361-369}$ could be presented by DC cells and

385 elicited robust activation of specific $CD8^+$ T cells (Figures 5A and 5B). Similarly,

386 marked $CD8^+$ T cell cytotoxicity were observed with lung organoids either

387 exogenously loaded or endogenously expressing the $N^{361-369}$ epitope (Figures 5C-5F).

388 The significance of $CD8^+$ T cell response for viral clearance has been demonstrated in

389 SARS-CoV infection (Channappanavar et al., 2014; Zhao et al., 2010). Therefore, our

390 findings from DCs and the lung organoid model have, for the first time, revealed

391 potential mechanisms of cellular immunity to eliminate SARS-CoV-2 infected lung

392 cells.

393 At present, majority of COVID-19 vaccine candidates under development focus on

394 SARS-CoV-2 S protein as the antigen, due to its location and functional significance

395 in virus entry (Chandrashekar et al., 2020; Corbett et al., 2020b; Keech et al., 2020;

396 Mercado et al., 2020a; Yang et al., 2020; Yu et al., 2020; Zhu et al., 2020). Hereby, we

397 have presented evidences of 4 dominant SARS-CoV-2 epitopes, most of which were

398 derived from the N protein, highlighting the potential benefit of SARS-CoV-2 N in

399 vaccine optimization. It should be mentioned that the sequence of the $N^{361-369}$ epitope

400 is identical to a fragment of the SARS-CoV N protein, as this region of the two

401 coronaviruses share more than 90% homology (Grifoni et al., 2020a). In fact, of the

402 total 15 SARS-CoV-2 T cell epitopes presented in our study, 8 were independently

403 confirmed with amino acid sequences identical to SARS-CoV (Tables 1 and S1),

404 indicating probable cross-protective effects of vaccines developed according to these

405 $CD8^+$ T cell epitopes.

406 A larger sample size with subgrouping of COVID-19 patients based on the
407 intensities of their diseases may better represent epitope diversities and be
408 advantageous to understand the correlations between various T cell immune responses
409 and the disease severities. In addition to bioinformatics predicted peptide pools, the
410 application of peptide libraries covering the entire SARS-CoV-2 protein sequences
411 can add detailed resolution of the SARS-CoV-2-specific T cell responses and is
412 currently under investigation. The magnitude and breadth of the observed cytotoxic
413 effect of CD8$^+$ T cells may be subjected for debate, and potential undesired
414 pathological damage resulted from the T-cell immunity should be addressed in future
415 studies.

416 In conclusion, this study has demonstrated potent and broad SARS-CoV-2-specific
417 CD8$^+$ T cell responses in the majority of COVID-19 convalescent patients. These
418 confirmed immunodominant epitopes and the identified TCRs provide critical
419 knowledge to understand the cellular immune response to SARS-CoV-2 and the
420 mechanisms for eliminating infected cells, and revealed valuable candidates for
421 vaccine designs.

422

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431

**AUTHOR CONTRIBUTIONS**

433 A.J., and D.P. conceived and designed the study. Q.C., F.G., Y.W., T.L., S.L., J.H., and
434 J.W. were responsible for collecting samples. C.H., M.S., X.H., Q.C., L.L., S.C., J.Z.,

435 J.Z., D.C., and Q.W. performed the experiments. C.H., L.L., and X.H. performed the

436 single cell TCR cloning. M.S., and J.Z. established the lung organoids model. M.S.,

437 X.H., C.H., W.W., and K.T. performed data analysis and incorporation. C.H., A.J., and

438 W.W. wrote the manuscript.

439

440 **DECLARATION OF INTERESTS**

441 Patent has been filed for some of the epitopes presented here.

442

443 **MATERIALS AND METHODS**

444 **Ethics Statement**

445 This project "Screening of SARS-CoV-2-specific T cell epitopes" was approved by

446 the ethics committee of Chongqing Medical University. Informed consents were

447 obtained from all participants.

448

449 **Cell Culture**

450 Lenti-X 293T cells was purchased from Takara Biomedical Technology, COS-7 and

451 K-562 cells were purchased from American Type Culture Collection (ATCC). Lenti-X

452 293T and COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM,

453 Thermo Fisher Scientific, USA) supplemented with 10% Fetal Bovine Serum (FBS,

454 Thermo Fisher Scientific, USA), 100 IU/ml penicillin and 100 μg/ml streptomycin

455 (Gibco, USA). K-562 cells were cultured in RPMI-1640 (Thermo Fisher Scientific,

456 USA) with 10% FBS, 100 IU/ml penicillin and 100 μg/ml streptomycin. All cells

457 were maintained at 37°C in an incubator with 5% $CO_2$.

458

459 **PBMCs Isolation**

460 Blood samples of COVID-19 convalescent patients (Table S3) were obtained from

461 Yongchuan Hospital of Chongqing Medical University and the Third Affiliated

462 Hospital of Chongqing Medical University of Chongqing Medical University.

463 Additionally, blood samples of healthy individuals were obtained from Chongqing

16

464  Medical University (Table S4). Samples were collected and processed as described

465  before (Weiskopf et al., 2013). All blood was collected in ethylene diamine tetra

466  acetic acid (EDTA) tubes and stored at room temperature prior to processing for

467  PBMCs isolation and plasma collection. The whole blood was centrifuged for 15 min

468  at 1850 rpm to separate the cellular fraction and plasma. The plasma was then

469  carefully separated from the cell pellets and stored at -20°C. The PBMCs were

470  isolated via density gradient centrifugation (Lymphoprep, STEMCELL Technologies,

471  Canada). Isolated PBMC were cryopreserved and stored in liquid nitrogen until used

472  in the assays.

473

474  **Preparation and Maturation of DCs**

475  Monocytes were enriched from the PBMCs of healthy individuals by using the

476  EasySep CD14 positive selection kit according to the manufacturer's instructions

477  (STEMCELL Technologies, Canada). Monocytes were cultured in the DC medium for

478  6 days, which was made with RPMI-1640 medium supplemented with 10% FBS, 100

479  IU/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Gibco, USA), 800

480  IU/ml GM-CSF (PeproTech, USA) and 800 IU/ml IL-4 (PeproTech, USA). On day 3,

481  fresh DC media were added to the cultures. On day 6, cells were cryopreserved and

482  stored in liquid nitrogen until used in the assays.

483

484  **Lung Organoid Culture**

485  All lung tissues used in this study were obtained after surgical resections from

486  volunteers, and processed for organoid culture within 4 hours. Normal lung tissue

487  organoids were established as reported (Sachs et al., 2019). Briefly, a piece of lung

488  tissue was minced, washed with 10 ml Advanced DMEM/F12 (Thermo Fisher

489  Scientific, USA) containing 1× Glutamax (Thermo Fisher Scientific, USA), 10 mM

490  HEPES (Thermo Fisher Scientific, USA) and antibiotics, termed as AdDF+++, and

491  digested in 10 ml AO medium (Table S8) containing 1 μg/ml collagenase

492  (Sigma-Aldrich, USA) on a shaker at 37°C for 1 hour. The digested lung tissue

17

493    suspension was sheared using 5 ml plastic Pasteur pipettes before straining over a 100

494    μm filter. The retained tissue pieces were resuspended in 10 ml AdDF+++ following

495    another shearing step. Strained suspensions were combined and centrifuged, and the

496    pellet was resuspended in 10 ml AdDF+++ and washed once. Lung cell pellets were

497    resuspended in 10 mg/ml cold Cultrex growth factor reduced basement membrane

498    extract (BME), type 2 (Trevigen, USA). Drops of 40 μl BME-cell suspension were

499    added into 24-well plates and solidified at 37°C for 10-20 min. Then 400 μl of AO

500    medium was added to each well and culture in the 37°C incubator with 5% $CO_2$. The

501    medium was changed every 4 days and the organoids were passaged every 14-18

502    days.

**Peptide Library**

505    To define the SARS-CoV-2-specific CD8^{+} T cell epitopes of the S and N proteins

506    derived from SARS-CoV-2 reference sequences (GenBank: MN908947). The

507    NetMHCpan 4.0 algorithm (Jurtz et al., 2017) was used to analyze the binding of

508    9-mer peptides derived from the full lengths of SARS-CoV-2 S and N proteins, with

509    HLA-A*02:01, HLA-A*24:02 or HLA-A*11:01. For each allele, binding peptides

510    with high strength (Rank Threshold < 0.5) were selected, and a total of 72 predicted

511    epitopes were generated. Additional 6 SARS-CoV epitopes were selected (Ahmed et

512    al., 2020; Grifoni et al., 2020a) (Table S1). Individual peptide's HLA-A specificity

513    was listed in Table S2. All 78 epitopes were synthesized (GenScript) and dissolved in

514    the corresponding solvents for the subsequent experiments. Detailed information for

515    peptide grouping was summarized in Table S2.

**HLA-A Typing for COVID-19 Convalescent Patients and Healthy Individuals**

518    To screen the HLA-A*11:01 phenotypes of the COVID-19 convalescent patients and

519    health individuals, we used Dynabeads™ mRNA DIRECT™ Purification kit (Thermo

520    Fisher Scientific, USA) to extract RNAs from the PBMC samples. Then, reverse

521    transcription and PCR were performed to amplify HLA-A molecules, with available

18

522　　PCR primer information (Simon et al., 2014). The PCR products were ligated into the

523　　T vector (TransGen Biotech, China) and then transformed into the Trans5α competent

524　　cells (TransGen Biotech, China). Single bacterial clones of each sample were selected

525　　for PCR verification and sequencing. The sequencing results were analyzed in the

526　　IMGT/HLA (https://www.ebi.ac.uk/ipd/imgt/hla/) website.

527　　　The HLA-A2 and HLA-A24 typing of the COVID-19 convalescent patients and

528　　health individuals were analyzed using flow cytometry. For staining, $5×10^5$ PBMCs

529　　were resuspended in PBS with 2% FBS (FACS buffer), and stained with the

530　　HLA-A2-PE (MBL, Clone BB7.2, K0186-5, Japan) or HLA-A24-PE (MBL, Clone

531　　17A10, K0208-5, Japan) for 30 min at room temperature in the dark. Samples were

532　　washed twice with FACS buffer following staining. After the final wash, cells were

533　　resuspended in 200 µl FACS buffer. All samples were acquired on a FACSCelesta

534　　cytometer (BD Biosciences, USA). The data were analyzed using FlowJo software

535　　(TreeStar Inc, USA).

536

537　　***In vitro* Stimulation of IFN-γ-producing CD8+ T Cells with Selected Peptides**

538　　PBMCs were plated in 24-well plates at $2×10^6$ cells per well with 100 IU/ml IL-2

539　　(PeproTech, USA) in the complete medium, which was RPMI-1640 supplemented

540　　with 10% FBS, 2 mM L-glutamine, 25 mM HEPES and 10 μg/ml gentamicin. The

541　　cells were subsequently stimulated with indicated pools of predicted SARS-CoV-2

542　　peptides, at 5 μM for each peptide. Cells were cultured for 10 days to expand specific

543　　T cell populations to corresponding stimulations. Half of the media were refreshed

544　　every 3 days, or as required. At day 10, cells were harvested and tested for

545　　peptide-specific CD8+ T cells by the IFN-γ release ELISPOT assay. The remaining

546　　cells were cryopreserved and stored in liquid nitrogen until used in assays.

547

548　　**IFN-γ ELISPOT Assays**

549　　IFN-γ ELISPOT assays were performed as reported and with minor modification (Le

550　　Bert et al., 2020b). Briefly, ELISPOT plates (Millipore, USA) were coated with

551    human IFN-γ antibody (Clone 1-D1K, 2 μg/ml, Mabtech, Sweden) overnight at 4°C.

552    T cells were rested in cytokine-free media overnight. Then $2×10^4$ T cells were seeded

553    per well in ELISPOT plates and stimulated for 24 h with pools of predicted

554    SARS-CoV-2 peptides (5 μM each). Stimulation with an equimolar amount of DMSO

555    was performed as the negative control, and Phorbol 12-myristate 13-acetate

556    (PMA)/Ionomycin (P/I) (Sigma-Aldrich, USA) was used as the positive control.

557    Subsequently, the plates were developed with human biotinylated IFN-γ detection

558    antibody (Clone 7-B6-1, 1 μg/ml, Mabtech, Sweden), followed by incubation with

559    Streptavidin-AP (1:1000, Mabtech, Sweden) and BCIP/NBT-plus substrate (Mabtech,

560    Sweden). IFN-γ spots were quantified with the AID ELISPOT Reader (AID,

561    Germany). To quantify positive peptide-specific responses, with 2 × mean spots of the

562    unstimulated wells subtracted from the peptide-stimulated wells, and the results

563    expressed as IFN-γ spots/$2×10^4$ PBMCs.

564

565    **Flow Cytometry Analysis**

566    For staining, $5×10^5$ cells were re-suspended in FACS buffer, and stained with the

567    anti-human antibody cocktail for 30 min at room temperature in the dark.

568    CD3-BV510 (Clone SK7), CD4-PerCP-Cy5.5 (Clone RPA-T4), CD8-FITC (Clone

569    HIT8a) and CD137-BV421 (Clone 4B4-1) were used for the activation induced T cell

570    marker assay. The P64-tetramer-PE used for the identification of antigen-specific

571    CD8$^+$ T lymphocytes was generated using the PE QuickSwitch™ Quant

572    HLA-A*11:01 Tetramer Kit (MBL, tb-7305-k1, Japan). The IFN-γ Secretion Assay

573    Detection Kit (MACS, 130-090-762, Germany) was used for labeling IFN-γ secreting

574    T cells, which were sorted into DNase and RNase free 96-well PCR plates (Bio-Rad,

575    USA) at a single cell level by FACSAria III (BD Biosciences, USA).

576    Cytokine production was assessed by intracellular cytokine staining (ICS). Briefly,

577    after target cells and effector cells were co-cultured in the well of a 96-well plate for 2

578    h, both Monensin (1:1000, Biolegend, USA) and Brefeldin A (1:1000, Biolegend,

579    USA) were added to the culture. After 6 h stimulation, cells were washed with FACS

580 buffer, and stained for cell surface markers (described above). Cells were then washed

581 with FACS buffer twice following fixation and permeabilization. Next, cells were

582 washed with Perm/Wash buffer (Biolegend, USA) twice and stained for 25 min at

583 room temperature with anti-human antibody against IFN-γ (BV421, Clone 4S.B3).

584 Cells were washed twice with Perm/Wash buffer and resuspended in FACS buffer,

585 then data was acquired via the FACSCelesta cytometer and analyzed using the FlowJo

586 software.

587

588 **HLA Binding Assay**

589 The HLA binding assay was performed using the PE QuickSwitch™ Quant

590 HLA-A*11:01 Tetramer kit following the manufacturer's instructions. Each

591 lyophilized peptide was dissolved in 1 mM recommended solution. In each well of a

592 96-well plate, the following materials were added sequentially and gently mixed via

593 pipetting: 25 µl QuickSwitch™ Tetramer, 0.5 µl Peptide Exchange Factor and 0.5 µl

594 of 1 mM peptide. Then, the plate was incubated for 4 h at room temperature while

595 protected from light. The tetramer's exchange rates were measured by the BD

596 FACSCelesta and calculated by QuickSwitch™ Calculator

597 (https://www.mblintl.com/quickswitch-peptide- exchange -calculator/).

598

599 **T Cell Receptor Sequencing and Analysis**

600 For single T cell PCR, the TCRα and TCRβ chain genes were amplified as previously

601 described (Hamana et al., 2016), with minor modifications. Briefly, the reverse

602 transcription (RT) reaction was performed in a two-step method following the

603 PrimeScript™ II Reverse Transcriptase kit protocol (Takara, Japan). For the first step,

604 single-cell sorted plates were thawed on ice and added with 5 µl mix containing dNTP

605 mixture and the primers for TCRα and TCRβ, then incubated at 65°C for 5 min and

606 put on ice immediately. For the second step, the above plates were added with the

607 RNase inhibitor and PrimeScript II Reverse Transcriptase (Takara, Japan) to a total

608 volume of 10 µl. The RT program was 45°C for 45 min and 70°C for 15 min. The RT

21

609 products were amplified by nested PCR following the PrimeSTAR® HS DNA

610 Polymerase kit protocol (Takara, Japan), with primers for TCRα and TCRβ. Here, the

611 5×PrimeSTAR® Buffer (Mg$^{2+}$ plus) in the kit was replaced with the 2 × PrimeSTAR®

612 GC Buffer (Mg$^{2+}$ plus) (Takara, Japan). The first PCR program was as follows: 98℃

613 for 1 min, 98℃ for 10 sec for 30 cycles, 52℃ for 5 sec and 72℃ for 40 sec. The

614 resultant PCR mixtures were diluted 50-fold with water, and 2 µl of the diluted PCR

615 mixtures were added to 18 µl of the nested PCR mixture as the template DNA. The

616 nested PCR program was as follows: 98℃ for 1 min, 98℃ for 10 sec for 35 cycles,

617 52℃ for 5 sec and 72℃ for 30 sec. The PCR products were then analyzed with the

618 α-nest or β-nest primers by direct sequencing. The TCR repertoire was analyzed with

619 the IMGT/V-Quest tool (http://www.imgt.org/) (Brochet et al., 2008).

620

621 **Enrichment of CD3$^+$, CD4$^+$ and CD8$^+$ T Cells**

622 CD3$^+$, CD4$^+$ or CD8$^+$ T cells were selected using the EasySep human CD3 positive

623 selection kit II, EasySep human CD4$^+$ T cell enrichment kit and EasySep human CD8$^+$

624 T cell enrichment kit (STEMCELL Technologies, Canada), respectively, according to

625 the manufacturer's instructions.

626

627 **Construction of Lentivirus Vectors and Transduction of CD3$^+$ T Cells**

628 For the construction of TCR lentivirus vectors, TCRα or TCRβ chains were

629 amplificated from the corresponding nested PCR mixtures and cloned into the

630 lentivirus vector pWPXL (Addgene Plasmid #12257). The constant regions were

631 replaced by mouse counterparts with improved TCR pairing and TCR/CD3 stability

632 that were convenient for the detection of TCR-T cells (Cohen et al., 2006; Jin et al.,

633 2018). The coding sequence of the S or N gene of SARS-CoV-2 and the human

634 *HLA-A*11:01* gene were synthesized, and cloned into the pWPXL vector.

635 Lentiviruses were generated by co-transfecting Lenti-X 293T cells with the

636 lenti-vector and the packaging plasmids psPAX2 (Addgene Plasmid #12260) and

637 pMD2.G (Addgene Plasmid #12259) at a ratio of 5:2:1, using the Xfect Transfection

638    Reagent (Takara, Japan). The lentiviral supernatants were harvested in 48 h.

639    Transduction of CD3$^+$ T cells was conducted as delineated before (Jin et al., 2018),

640    with minor modifications. CD3$^+$ T cells were stimulated in T cell media with 1 µg/ml

641    anti-CD3 antibody (Clone OKT3) and 1 µg/ml anti-CD28 antibody (Clone 15E8) for

642    2 days. Then, the transduction efficiency was assessed by flow cytometry using

643    anti-mouse TCR-β chain constant region antibody (Clone H57-597).

644

645    **TCR Transduced-T Cell Rapid Expansion Protocol**

646    TCR transduced-T cells were rapidly expanded with the method as described before

647    (Jin et al., 2012). Briefly, in indicated experiments, sorted T cells were expanded

648    using excess irradiated (50Gy) allogeneic PBMC feeder cells (a pool from 3-5

649    different donors) at a ratio of 1 to 100 with 30 ng/ml anti-CD3 antibody (Clone OKT3)

650    and 3000 IU/ml IL-2, in the 50/50 media consisted of a 1:1 mixture of the complete

651    media and the AIM-V media (Gibco, USA). All cells were cultured at 37°C with 5%

652    $CO_2$. Media were exchanged 3 days post-stimulation and then every other day. The

653    cells were rapidly expanded in this way for two weeks, before use.

654

655    **Antigen Presentation in Human DCs**

656    DCs were incubated in AIM-V for 1 h and then 5 µg/ml recombinant N or S proteins

657    of SARS-CoV-2 were added. After 4 h, the media were replaced to DC media

658    supplemented with DC maturation cytokines including 10 ng/ml Lipopolysaccharide

659    (LPS) (Sigma-Aldrich, USA) and 100 IU/ml IFN-γ (PeproTech, USA). After

660    incubated overnight, DCs were washed and co-cultured with TCR-T cells for 24 h.

661    The supernatants were used for measuring IFN-γ concentrations by ELISA.

662

663    **TCR-T Cytotoxicity Assay**

664    The ability of T cells to lyse target cells was measured using a Calcein-AM release

665    assay. Target cells were labelled for 15 min at 37°C with 10 µM Calcein-AM

666    (Dojindo, Japan), and then co-cultured with T cells at indicated ratios for 24 h in the

23

black-walled, 96-well flat-bottom plates (Corning, USA). The controls of these labelled target cells were separated into the spontaneous death group and the maximal killing group, the latter were treated with 0.1% Triton-X (Sigma-Aldrich, USA). After 24 hours, 50 μl/well supernatant was removed, and the relative fluorescence units (RFUs) was measured by Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, USA). The percentage of lysed cells was calculated as follows: specific lysis = 100% × (Test RFUs - Spontaneous death RFUs) / (Maximal killing RFUs - Spontaneous death RFUs).

The ability of T cells to lyse organoids was also measured using LDH-Glo™ Cytotoxicity Assay Kit (Promega, USA) according to the manufacturer's instructions. The organoids were dissociated to single cells and counted, which was used to infer the number of organoids to allow co-cultured with T cells at a 4:1 effector:target ratio. After co-cultured for 24 h in the 96-well flat-bottom plates, the relative luminescent units (RLUs) of the co-culture and the control groups were measured by the Varioskan LUX Multimode Microplate Reader. The percentage of lysed organoids was calculated as follows: specific lysis = 100% × (Test RLUs - Spontaneous death RLUs) / (Maximal killing RLUs - Spontaneous death RLUs) .

**Cytokine Release ELISA**

The antibody pairs used for cytokine detection are as follows: IFN-γ capture antibody (Clone MD-1, 2 μg/ml), IFN-γ detection antibody (Clone 4S.B3, 1 μg/ml), IL-2 capture antibody (Clone MQ1-17H12, 4 μg/ml), IL-2 detection antibody (Clone Poly5176, 1 μg/ml), TNF-α capture antibody (Clone MAb1, 1 μg/ml) and TNF-α detection antibody (Clone MAb11, 1μg/ml). The ELISA plate was coated with individual anti-cytokine capture antibodies for 12 h, then washed with phosphate-buffered saline with Tween (PBST) buffer for 3 times, followed by blocking with 3% BSA for at least 1 h at room temperature. The supernatants of T cell and target cell co-cultures were added into the plate at 50 μl per well. After incubation at room temperature for 1 h, the plate was washed 3 times with PBST. Biotinylated

696    capture antibodies (50 μl/well) were added and the plate was incubated for 1 h at

697    room temperature, then washed for 5 times. Streptavidin-ALP (1:1000) was added at

698    50 μl per well, with, and incubated for 1 h at room temperature in the dark. Then, the

699    plate was washed 6 times and the pNPP solution (Mabtech, Sweden) was added at 50

700    μl per well. After 20 min incubation at room temperature in the dark, 50 μl stop

701    solution was added per well. The plate was immediately analyzed by the Varioskan

702    LUX Multimode Microplate Reader for the OD405 value.

703

704    **Statistical Analysis**

705    Statistical analyses of the data were performed using GraphPad Prism version 8.0

706    (GraphPad Software, Inc. La Jolla, CA, USA) software. Quantitative data in

707    histograms and line charts were presented as mean ± standard deviation (mean ± SD).

708    Statistical significance was determined using ANOVA for multiple comparisons.

709    Student's *t*-tests were applied to compare two groups. A value of $P < 0.05$ was

710    considered statistically significant.

711

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**Table 1 Identification of CD8^{+} T Cell Epitopes of SARS-CoV-2 in COVID-19 Convalescent Patients**

| Numb. | Epitope ID | Protein | Peptide Position | Epitope sequence | HLA restriction | responded /tested donors |
|---|---|---|---|---|---|---|
| 1 | P64 | N | 361-369 | KTFPPTEPK | A*11:01 | 5/8 |
| 2 | P63 | N | 338-346 | KLDDKDPNF | A*02:01 | 4/8 |
| 3 | P77 | N | 219-227 | LALLLLDRL | A*02:01 | 2/8 |
| 4 | P62 | N | 316-324 | GMSRIGMEV | A*02:01 | 1/8 |
| 5 | P61 | N | 222-230 | LLLDRLNQL | A*02:01 | 1/8 |
| 9 | P45 | S | 448-456 | NYNYLYRLF | A*24:02 | 2/8 |
| 6 | P74 | S | 958-966 | ALNTLVKQL | A*02:01 | 1/8 |
| 7 | P16 | S | 718-726 | FTISVTTEI | A*02:01 | 1/8 |
| 8 | P4 | S | 983-991 | RLDKVEAEV | A*02:01 | 1/8 |
| 10 | P52 | S | 1137-1145 | VYDPLQPEL | A*24:02 | 1/8 |
| 11 | P49 | S | 169-177 | EYVSQPFLM | A*24:02 | 1/8 |
| 12 | P53 | S | 312-320 | IYQTSNFRV | A*24:02 | 1/8 |
| 13 | P54 | S | 193-201 | VFKNIDGYF | A*24:02 | 1/8 |
| 14 | P55 | S | 1101-1109 | HWFVTQRNF | A*24:02 | 1/8 |
| 15 | P57 | S | 268-276 | GYLQPRTFL | A*24:02 | 1/8 |

**FIGURE LEGENDS**

**Figure 1. CD8^{+} T Cell Responses of SARS-CoV-2 Peptides.**

IFN-γ ELISPOT assay results for the PBMCs of COVID-19 convalescent patients with (A) HLA-A2^{+}, (B) HLA-A24[+] or (C) HLA-A*11:01[+] allele, stimulated with mixed peptides (top) and single reactive peptide (middle and bottom). A stimulation with an equimolar amount of DMSO was performed as negative control, and Phorbol 12-myristate 13-acetate (PMA)/Ionomycin (P/I) was performed as positive control. Results are expressed as number of peptide-specific IFN-γ spots/$2\times10^4$ PBMCs of each patient, substracted those from the corresponding DMSO groups. N = 8. For peptide grouping: 26 HLA-A*02:01-restricted peptides were pooled into 5 mixtures (M1-M5), 22 HLA-A*24:02-restricted peptides were pooled into 4 mixtures (M6-M9), and 30 HLA-A*11:01-restricted peptides were pooled into 5 mixtures (M10-M14). Each mixture contained 3-6 peptides. Positive peptide mixture and single peptide with the most dominant reactivity were marked in bold. For the single dominant peptide, P63 was from M3, P45 was from M6 and P64 was from M14. C8-C41: COVID-19

33

946     convalescent patients. Data were respresentative of two independent experiments.

947

948     **Figure 2. The Isolation and Validation of the SARS-CoV-2 Specific CD8$^+$ TCRs.**

949     (A) After stimulated by peptide mixtures and expanded for 10 days, single IFN-γ

950     secreting T cell from three COVID-19 convalescent PBMC samples (C40:

951     HLA-A*11:01$^+$, C33: HLA-A24$^+$ and C27: HLA-A2$^+$) were individually stimulated

952     by P64, P45 and P63, from three corresponding HLA subtypes, and isolated by flow

953     cytometric sorter. (B) The TCRs of sorted cells were amplified by RT-PCR and the

954     TCR repertoire was analyzed with the IMGT/V-Quest tool (http://www.imgt.org/) to

955     obtain dominant TCR clones. Unique clones were marked in white. Different colors

956     represented relative dominant clones (copy≧2). (C-D) Specificity verificaiton for the

957     top 4 P64-TCR clones in CD8$^+$ Jurkat cells. P64-HLA-A*11:01 tetramer (Tetramer)

958     and anti-mouse TCR (mTCR) antibody were applied to evaluate the specific binding

959     of P64-TCRs (C). CD69 was used to determine the specific activation of

960     TCR-transduced CD8$^+$ Jurkat cells, after co-cultured with HLA-A*11:01 expressing

961     COS-7 cells pulsed with P64 (D). Grey represented DMSO-stimulated samples and

962     pink represented P64-stimulated samples. Data were respresentative of two

963     independent experiments.

964

965     **Figure 3. Functional Characterization of the N$^{361-369}$-Specific TCR 1 and TCR 4.**

966     (A) Flow cytometric analysis of the transfection efficiency of TCR 1 and TCR 4 in

967     CD4$^+$ and CD8$^+$ T cells, by N$^{361-369}$-HLA-A*11:01 tetramer. (B) Binding assay of

968     TCR-transduced CD8$^-$ and CD8$^+$ Jurkat cells with N$^{361-369}$-HLA-A*11:01 tetramer. (C)

969     Flow cytometric analysis of T cell activation evaluated by CD137 expression.

970     TCR-transduced CD8$^+$ T cells and CD4$^+$ T cells were individually co-cultured with

971     HLA-A*11$^+$ K-562 cells pulsed with N$^{361-369}$, or an equimolar amount of DMSO, for

972     24 hours. Expression of CD137 on CD8$^+$ T cells (left) and CD4$^+$ T cells (right) was

973     evaluated. (D) N$^{361-369}$ loaded HLA-A*11:01$^+$ K-562 cells lysis by TCR 1-CD8$^+$ T

974     cells after 8 h at different E:T ratios. DMSO was used as negative control. Reported

975 values are the mean of triplicates, with error bars indicating. E, Effector T cells. T,

976 Target cells. (E) Detection of IFN-γ, IL-2 and TNF-α secretion from TCR 1 and TCR

977 4-transduced CD4$^+$ T cells by ELISA, after co-cultured with HLA-A*11:01$^+$ K-562

978 cells loaded with N$^{361-369}$ for 24 h. Data were presented as mean ± SD, n = 3, **p <

979 0.01, ***p < 0.001. Respresentative data of two independent experiments were

980 shown.

981

982 **Figure 4. Evaluation of the Affinity and the Cross-Reactivity of TCR 1 and TCR**

983 **4 to the N$^{361-369}$ Mutants.**

984 (A) EC$_{50}$ analysis with IFN-γ secretion assessed by ELISA. HLA-A*11$^+$ K-562 cells

985 loaded with titrating concentrations of the N$^{361-369}$ peptide were co-cultured with the

986 TCR-CD4$^+$ T cells or the TCR-CD8$^+$ T cells for 24 h. Individual EC$_{50}$ of N$^{361-369}$ was

987 calculated using nonlinear regression analysis. (B) Nine N$^{361-369}$ variants were aligned

988 with the wild type peptide and the homologous MERS peptide (left). Individual

989 mutations were marked in red. HLA-A*11:01 binding affinity of each peptide was

990 determined by the peptide exchange rates and shown by grey bars (n = 2). The

991 reactivity of TCR 1- and TCR 4-CD8$^+$ T cells to the N$^{361-369}$ mutant peptides were

992 tested by IFN-γ ELISA assay and shown in connecting lines (n = 3). Data were

993 presented as mean + SD. Respresentative data of two independent experiments were

994 shown.

995

996 **Figure 5. The Reactivity of TCR-Transduced CD8$^+$ T Cells to the Endogenously**

997 **Presented N$^{361-369}$ Epitope on APCs.**

998 The reactivity assay of TCR 1- and TCR 4-transduced CD8$^+$ T cells following

999 co-culturing with (A and B) HLA-A*11:01$^+$ DCs loaded with N or (C and D)

1000 HLA-A*11:01$^+$ lung organoids endogenously expressing N protein. (A and C) CD137

1001 expression was evaluated by flow cytometry. (B and D) IFN-γ secretion was

1002 determind by ELISA. (E) Cytotoxicity assay of TCR 1-CD8$^+$ T cells against

1003 HLA-A*11:01$^+$ lung organoids exogenously pulsed with N$^{361-369}$ peptide (left).

DMSO was used as negative control. T cells were stained with blue dye and lung organoids were stained in green. Target cell lysis was quantified using Calcein-AM release assay (right). (F) Cytotoxicity assay of TCR 1-CD8$^+$ T cells against HLA-A*11:01$^+$ lung organoids endogenously expressing the N protein (left). Organoids expressing the S protein was applied as negative control. T cells were stained in red and lung organoids were stained in green.Target cell lysis was quantified by LDH release assay (right). Correspoding microphotographs of brightfield displayed all cells in view. White arrows show examples of organoids that are efficiently lysed. Bar: 200 μm. Data were presented as the mean + SD, n = 3, *p < 0.05, ***p < 0.001. Respresentative data of two independent experiments were shown.

**Figure S1. T Cell Responses after Stimulation with Predicted SARS-CoV-2 Epitope Pools.**

Related to Figure 1. IFN-γ ELISPOT assay results for the PBMCs of COVID-19 convalescent patients with (A) HLA-A2$^+$ allele, (B) HLA-A24$^+$ allele and (C) HLA-A11:01$^+$ allele, stimulated with peptide mixtures. Results are expressed as number of peptide-specific IFN-γ spots/2×10$^4$ PBMCs of each patient, substracted those from the corresponding DMSO groups. For peptide grouping: 26 HLA-A*02:01-restricted peptides were pooled into 5 mixtures (M1-M5), 22 HLA-A*24:02-restricted peptides were pooled into 4 mixtures (M6-M9), and 30 HLA-A*11:01-restricted peptides were pooled into 5 mixtures (M10-M14). Each mixture contained 3-6 peptides. Positive peptide mixtures were marked in bold. A stimulation with an equimolar amount of DMSO was performed as the negative control, and phorbol 12-myristate 13-acetate (PMA)/Ionomycin (P/I) was used as the positive control. Data were representative of two independent experiments.

**Figure S2. Identification of T Cell Epitopes in HLA-A2$^+$ Patients.**

Related to Figure 1. Individual image summarized (top) and directly showed (bottom)

1033   IFN-γ ELISPOT assay results for PBMCs of COVID-19 convalescent patients with

1034   HLA-A2$^+$ allele, which were stimulated with a positive peptide mixture and the

1035   corresponding individual peptides from the mixture. Results are expressed as number

1036   of peptide-specific IFN-γ spots/2×10$^4$ PBMCs of each patient, substracted those from

1037   the corresponding DMSO groups. DMSO and P/I were used as the negative and

1038   positive control respectively. Positive single peoptide was marked in bold. Data were

1039   representative of two independent experiments.

1040

1041   **Figure S3. Identification of T Cell Epitopes in HLA-A24$^+$ Patients.**

1042   Related to Figure 1. Individual image summarized (top) and directly showed (bottom)

1043   IFN-γ ELISPOT assay results for PBMCs of COVID-19 convalescent patients with

1044   HLA-A24$^+$ allele, which were stimulated with the positive peptide mixture and the

1045   corresponding 6 individual peptides from the mixture. Results are expressed as

1046   number of peptide-specific IFN-γ spots/2×10$^4$ PBMCs of each patient, substracted

1047   those from the corresponding DMSO groups. DMSO and P/I were used as the

1048   negative and positive control respectively. Positive single peoptide was marked in

1049   bold. Data were representative of two independent experiments.

1050

1051   **Figure S4. Assessment of SARS-CoV-2 Dominant Epitopes Reactivity in Healthy**

1052   **Individuals.**

1053   Related to Figure 1. Individual image summarized (top) and directly showed (bottom)

1054   IFN-γ ELISPOT assay results for the PBMCs of healthy individuals with (A)

1055   HLA-A2$^+$ allele, (B) HLA-A24$^+$ allele and (C) HLA-A11:01$^+$ allele, stimulated with

1056   corresponding dominant epitope(s). Results are expressed as number of

1057   peptide-specific IFN-γ spots/2×10$^4$ PBMCs of each patient, substracted those from the

1058   corresponding DMSO group. DMSO and P/I were used as the negative and positive

1059   control respectively. Data were representative of two independent experiments.

1060

1061   **Figure S5. Establishment of the Lung Tissue Organoid Model for the Assessment**

1062 **of SARS-CoV-2 N Antigen Presentation.**

1063 Related to Figure 5. (A and B) Bright-field microscopy images and (C) H&E staining

1064 image of lung tissue organoids cultured for one month. Bar indicates 100 μm. (D) A

1065 schematic diagram represents the open reading frame (ORF) of the N protein

1066 lentivirus expression vector (left). The coding sequences of N protein, 2A and EGFP

1067 were connected in this vector. The transfection efficiency of N protein lentiviruses in

1068 lung organiods was examined. EGFP expression of the positively transfected organoid

1069 was shown in the fluorescent microphotograph (right).

Figure.1
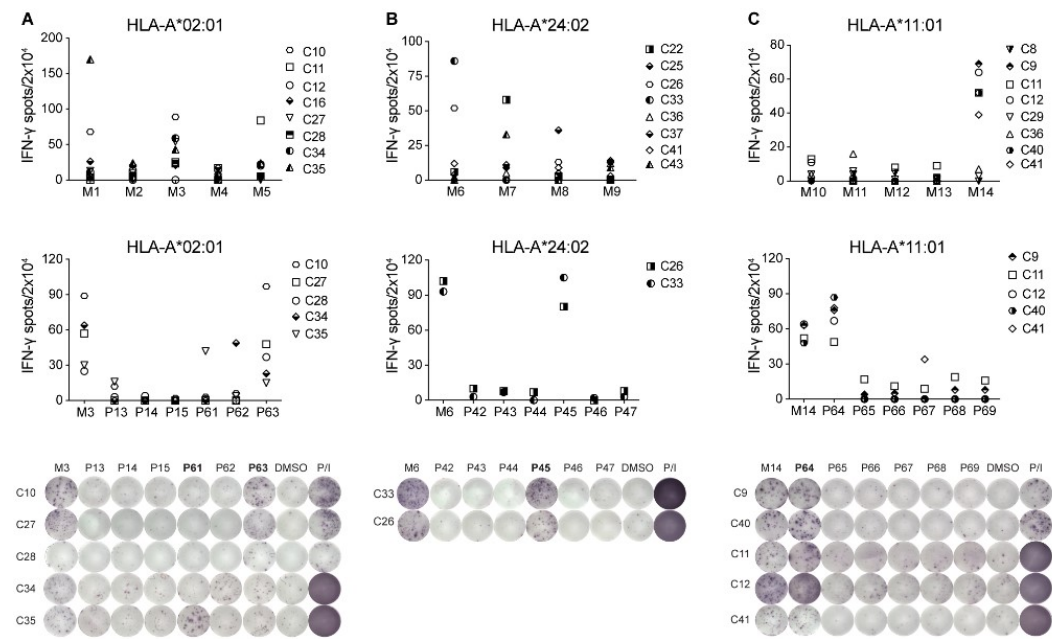
**Figure.2**

**Figure.3**

**Figure.4**

A



B

| | |
|---|---|
| Wild-type | KTFPPTEPK |
| T362K | KKFPPTEPK |
| T362I | KIFPPTEPK |
| F363L | KTLPPTEPK |
| F363S | KTSPPTEPK |
| P364L | KTFLPTEPK |
| P364R | KTFRPTEPK |
| P365S | KTFPSTEPK |
| T366I | KTFPPIEPK |
| E367K | KTFPPTKPK |
| MERS | KTFPKKEKK |
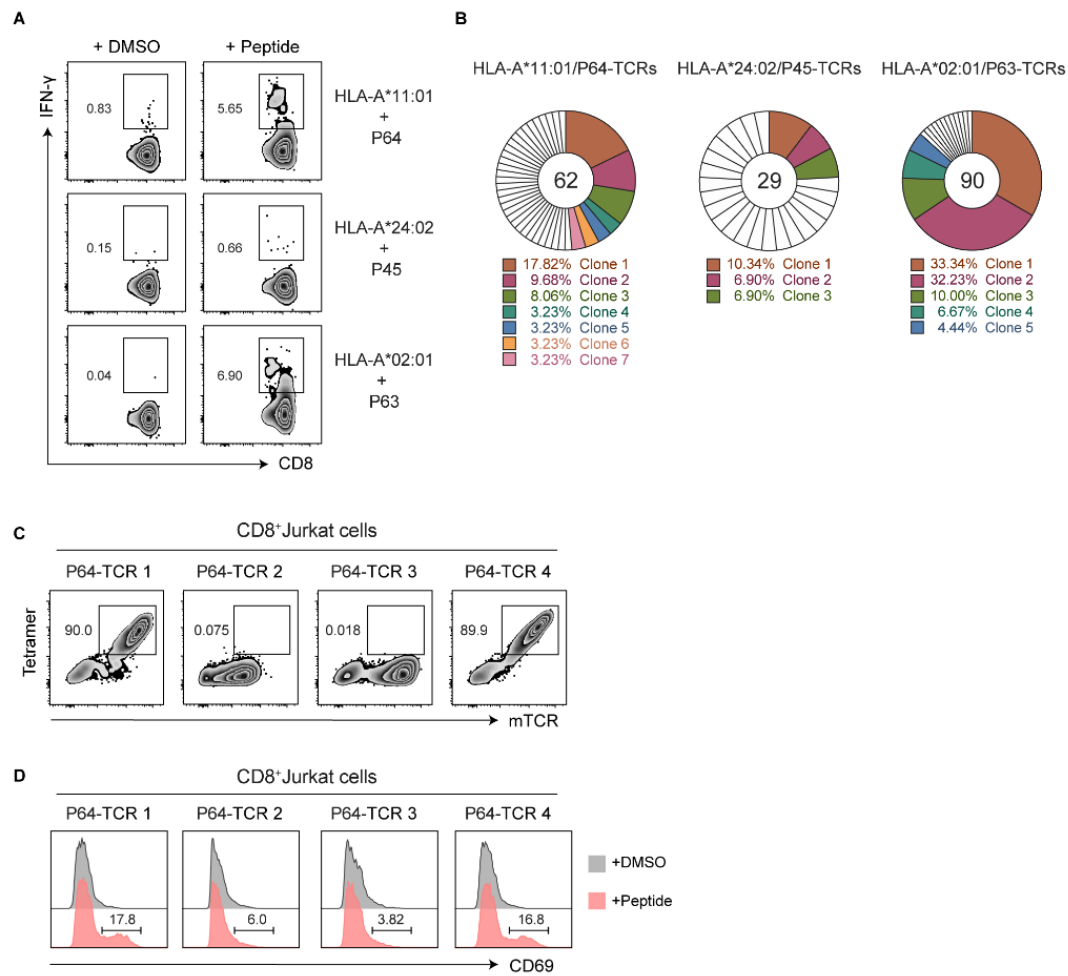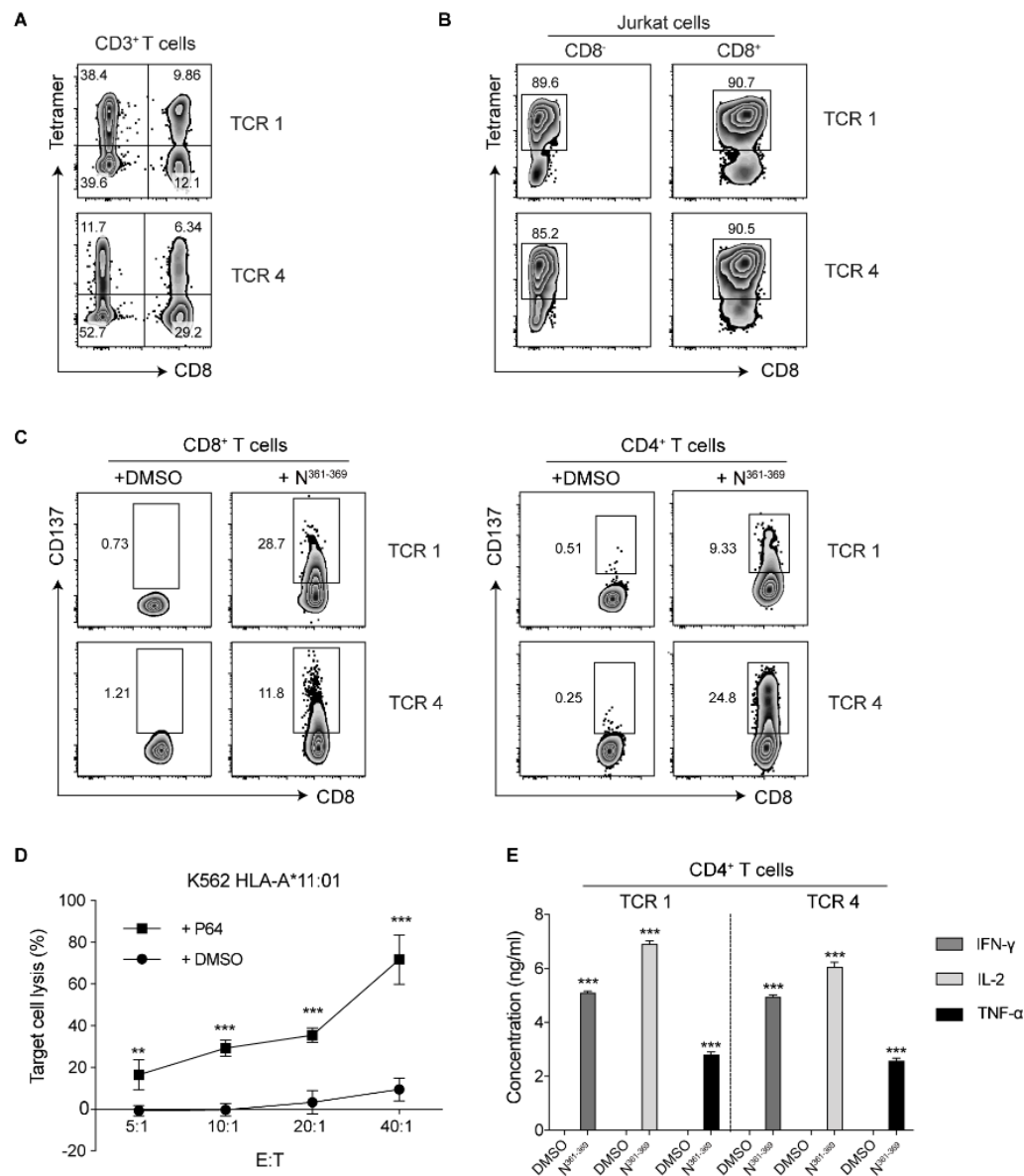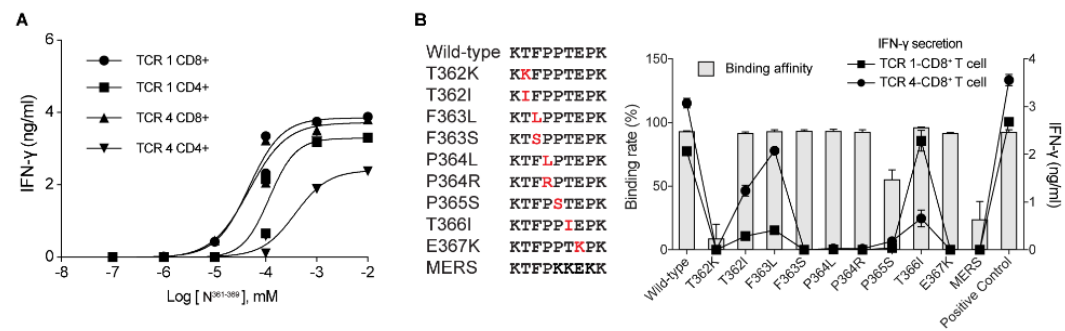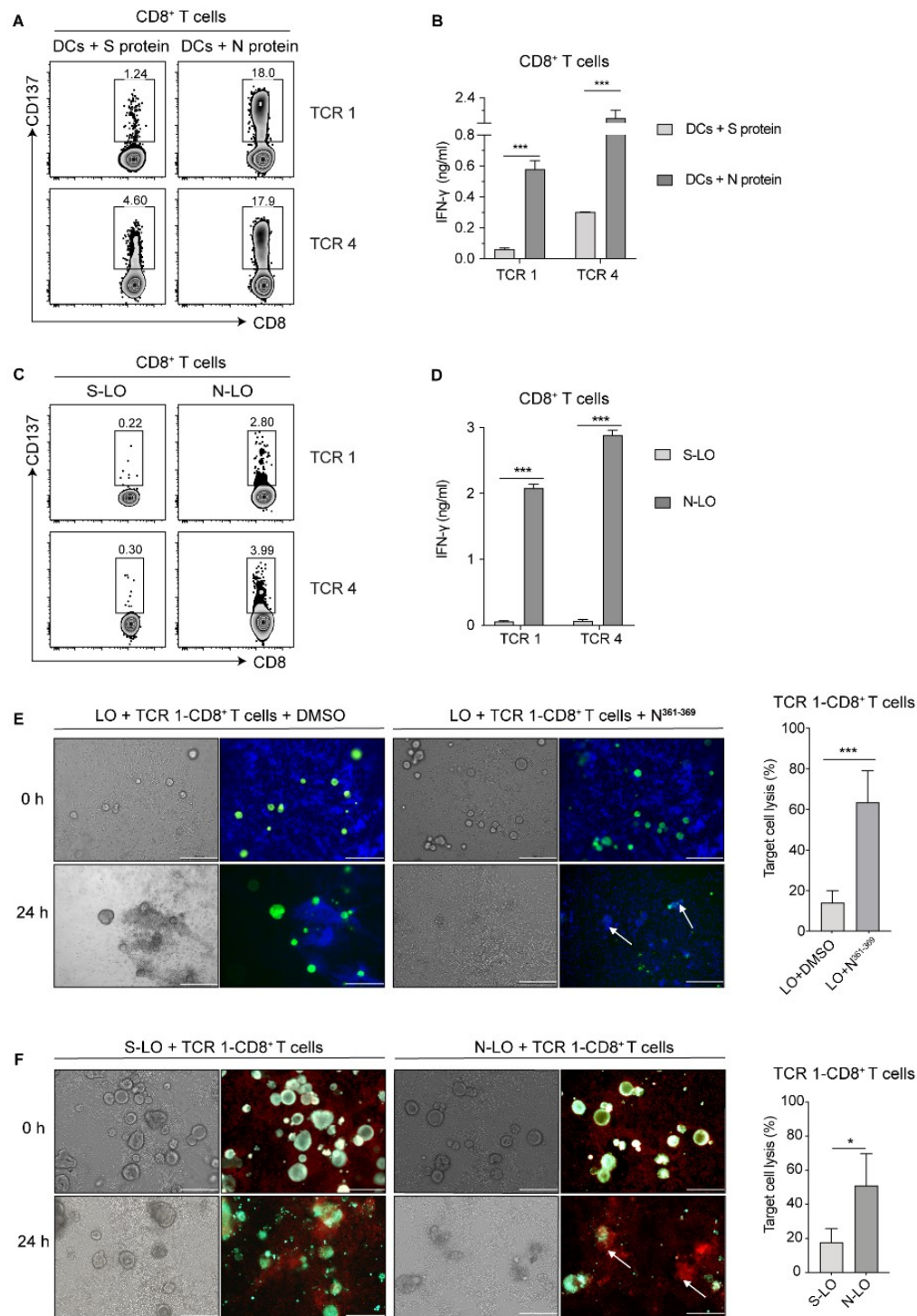
**Figure.5**

**Supplementary Figure. 1**

**Supplementary Figure. 2**

**Supplementary Figure. 3**

**Supplementary Figure. 4**

**Supplementary Figure. 5**

A

B

C

D

Supplementary Table 1. Prediction and Selection of T Cell Epitopes from SARS-CoV-2 S and N Proteins

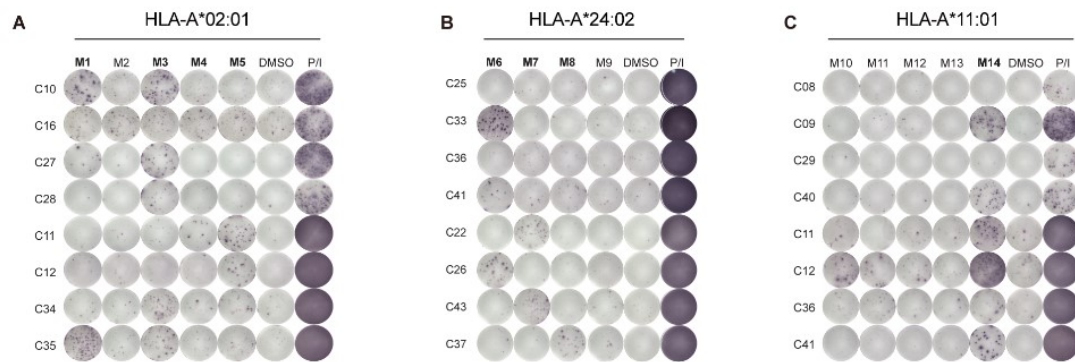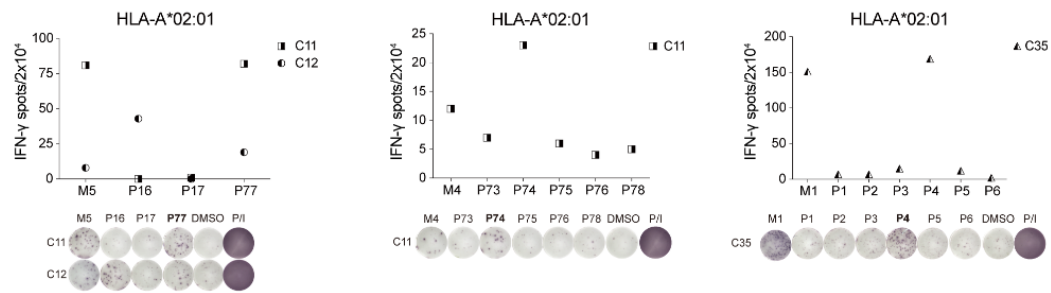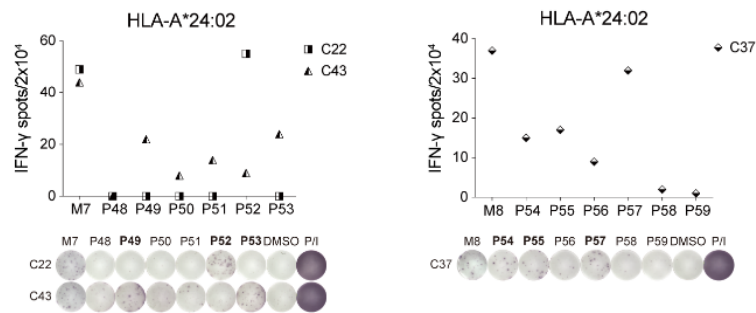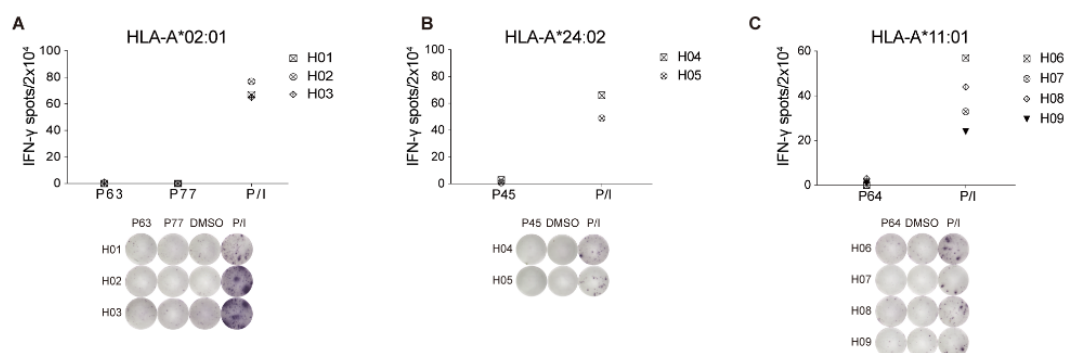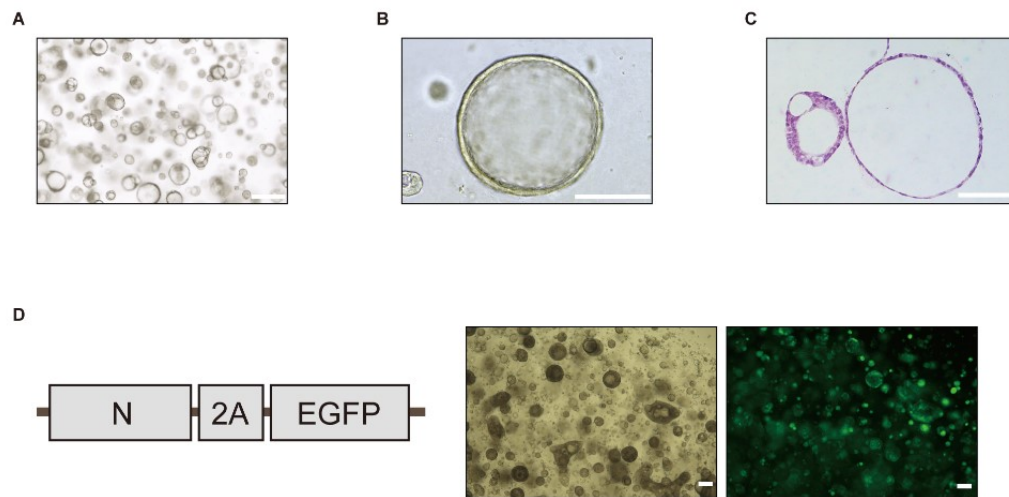| Peptide ID | Peptide | HLA-Type | Source | Rank | Methods of obtaining peptides | 100% homology to SARS-CoV | Reported SARS-CoV epitope |
|---|---|---|---|---|---|---|---|
| P1 | YLQPRTFLL | HLA-A02:01 | S protein | 0.015 | predicted | N.R. | N.R. |
| P2 | VLNDILSRL | HLA-A02:01 | S protein | 0.0356 | predicted | Yes | Yes |
| P3 | KIADYNYKL | HLA-A02:01 | S protein | 0.0583 | predicted | N.R. | N.R. |
| P4 | RLDKVEAEV | HLA-A02:01 | S protein | 0.0962 | predicted | Yes | N.R. |
| P5 | FIAGLIAIV | HLA-A02:01 | S protein | 0.1288 | predicted | Yes | Yes |
| P6 | SIIAYTMSL | HLA-A02:01 | S protein | 0.1479 | predicted | N.R. | N.R. |
| P7 | LLFNKVTLA | HLA-A02:01 | S protein | 0.1599 | predicted | Yes | N.R. |
| P8 | RLQSLQTYV | HLA-A02:01 | S protein | 0.199 | predicted | Yes | N.R. |
| P9 | HLMSFPQSA | HLA-A02:01 | S protein | 0.2033 | predicted | N.R. | N.R. |
| P10 | NLNESLIDL | HLA-A02:01 | S protein | 0.2411 | predicted | Yes | Yes |
| P11 | VVFLHVTYV | HLA-A02:01 | S protein | 0.2433 | predicted | Yes | Yes |
| P12 | GLTVLPPLL | HLA-A02:01 | S protein | 0.2595 | predicted | Yes | N.R. |
| P13 | TLDSKTQSL | HLA-A02:01 | S protein | 0.2599 | predicted | N.R. | N.R. |
| P14 | RLNEVAKNL | HLA-A02:01 | S protein | 0.3295 | predicted | Yes | Yes |
| P15 | VLYENQKLI | HLA-A02:01 | S protein | 0.4563 | predicted | N.R. | N.R. |
| P16 | FTISVTTEI | HLA-A02:01 | S protein | 0.4789 | predicted | N.R. | N.R. |
| P17 | FQFCNDPFL | HLA-A02:01 | S protein | 0.493 | predicted | N.R. | N.R. |
| P18 | GVYFASTEK | HLA-A11:01 | S protein | 0.0129 | predicted | N.R. | N.R. |
| P19 | VTYVPAQEK | HLA-A11:01 | S protein | 0.0225 | predicted | N.R. | N.R. |
| P20 | ASANLAATK | HLA-A11:01 | S protein | 0.0254 | predicted | Yes | N.R. |
| P21 | SVLNDILSR | HLA-A11:01 | S protein | 0.0583 | predicted | Yes | N.R. |
| P22 | TLADAGFIK | HLA-A11:01 | S protein | 0.0713 | predicted | N.R. | N.R. |
| P23 | TLKSFTVEK | HLA-A11:01 | S protein | 0.0798 | predicted | N.R. | N.R. |
| P24 | NSASFSTFK | HLA-A11:01 | S protein | 0.0861 | predicted | N.R. | N.R. |
| P25 | SSTASALGK | HLA-A11:01 | S protein | 0.0959 | predicted | N.R. | N.R. |
| P26 | GTHWFVTQR | HLA-A11:01 | S protein | 0.0973 | predicted | N.R. | N.R. |
| P27 | EILPVSMTK | HLA-A11:01 | S protein | 0.0989 | predicted | N.R. | N.R. |
| P28 | QIYKTPPIK | HLA-A11:01 | S protein | 0.1272 | predicted | N.R. | N.R. |
| P29 | GVLTESNKK | HLA-A11:01 | S protein | 0.1326 | predicted | N.R. | N.R. |
| P30 | GVYYHKNNK | HLA-A11:01 | S protein | 0.1395 | predicted | N.R. | N.R. |
| P31 | ALDPLSETK | HLA-A11:01 | S protein | 0.1813 | predicted | N.R. | N.R. |
| P32 | RLFRKSNLK | HLA-A11:01 | S protein | 0.1833 | predicted | N.R. | N.R. |
| P33 | QIAPGQTGK | HLA-A11:01 | S protein | 0.1852 | predicted | N.R. | N.R. |
| P34 | ASVYAWNRK | HLA-A11:01 | S protein | 0.2025 | predicted | N.R. | N.R. |
| P35 | GIYQTSNFR | HLA-A11:01 | S protein | 0.2914 | predicted | Yes | N.R. |
| P36 | SVYAWNRKR | HLA-A11:01 | S protein | 0.3119 | predicted | N.R. | N.R. |
| P37 | NQFNSAIGK | HLA-A11:01 | S protein | 0.3979 | predicted | N.R. | N.R. |
| P38 | AQALNTLVK | HLA-A11:01 | S protein | 0.4 | predicted | Yes | N.R. |
| P39 | FAQVKQIYK | HLA-A11:01 | S protein | 0.4445 | predicted | N.R. | N.R. |
| P40 | KSTNLVKNK | HLA-A11:01 | S protein | 0.4473 | predicted | N.R. | N.R. |
| P41 | FIEDLLFNK | HLA-A11:01 | S protein | 0.4699 | predicted | Yes | N.R. |
| P42 | VYSTGSNVF | HLA-A24:02 | S protein | 0.0188 | predicted | N.R. | N.R. |
| P43 | QYIKWPWYI | HLA-A24:02 | S protein | 0.0273 | predicted | N.R. | N.R. |
| P44 | YFPLQSYGF | HLA-A24:02 | S protein | 0.0465 | predicted | N.R. | N.R. |
| P45 | NYNYLYRLF | HLA-A24:02 | S protein | 0.0572 | predicted | N.R. | N.R. |
| P46 | PFFSNVTWF | HLA-A24:02 | S protein | 0.1216 | predicted | N.R. | N.R. |
| P47 | VFVSNGTHW | HLA-A24:02 | S protein | 0.1288 | predicted | N.R. | N.R. |
| P48 | RFDNPVLPF | HLA-A24:02 | S protein | 0.1429 | predicted | N.R. | N.R. |
| P49 | EYVSQPFLM | HLA-A24:02 | S protein | 0.1493 | predicted | N.R. | N.R. |

| P50 | YYHKNNKSW | HLA-A24:02 | S protein | 0.1568 | predicted | N.R. | N.R. |
|-----|-----------|-----------|-----------|--------|-----------|------|------|
| P51 | PYRVVVLSF | HLA-A24:02 | S protein | 0.1858 | predicted | Yes | N.R. |
| P52 | VYDPLQPEL | HLA-A24:02 | S protein | 0.194 | predicted | Yes | N.R. |
| P53 | IYQTSNFRV | HLA-A24:02 | S protein | 0.2183 | predicted | Yes | N.R. |
| P54 | VFKNIDGYF | HLA-A24:02 | S protein | 0.2438 | predicted | N.R. | N.R. |
| P55 | HWFVTQRNF | HLA-A24:02 | S protein | 0.2459 | predicted | N.R. | N.R. |
| P56 | AYSNNSIAI | HLA-A24:02 | S protein | 0.2847 | predicted | N.R. | N.R. |
| P57 | GYLQPRTFL | HLA-A24:02 | S protein | 0.296 | predicted | N.R. | N.R. |
| P58 | GYQPYRVVV | HLA-A24:02 | S protein | 0.4562 | predicted | Yes | N.R. |
| P59 | QYGSFCTQL | HLA-A24:02 | S protein | 0.4724 | predicted | Yes | N.R. |
| P60 | VYAWNRKRI | HLA-A24:02 | S protein | 0.4958 | predicted | N.R. | N.R. |
| P61 | LLLDRLNQL | HLA-A02:01 | N protein | 0.0423 | predicted | Yes | Yes |
| P62 | GMSRIGMEV | HLA-A02:01 | N protein | 0.2558 | predicted | Yes | Yes |
| P63 | KLDDKDPNF | HLA-A02:01 | N protein | 0.6589 | predicted | N.R. | N.R. |
| P64 | KTFPPTEPK | HLA-A11:01 | N protein | 0.0061 | predicted | Yes | N.R. |
| P65 | ASAFFGMSR | HLA-A11:01 | N protein | 0.0616 | predicted | Yes | N.R. |
| P66 | KSAAEASKK | HLA-A11:01 | N protein | 0.1885 | predicted | Yes | N.R. |
| P67 | FTALTQHGK | HLA-A11:01 | N protein | 0.2058 | predicted | Yes | N.R. |
| P68 | AGLPYGANK | HLA-A11:01 | N protein | 0.3526 | predicted | N.R. | N.R. |
| P69 | LIRQGTDYK | HLA-A11:01 | N protein | 0.5124 | predicted | Yes | N.R. |
| P70 | QFAPSASAF | HLA-A24:02 | N protein | 0.189 | predicted | Yes | N.R. |
| P71 | KHWPQIAQF | HLA-A24:02 | N protein | 0.3775 | predicted | Yes | N.R. |
| P72 | KHIDAYKTF | HLA-A24:02 | N protein | 0.4148 | predicted | Yes | N.R. |
| P73 | LQLPQGTTL | HLA-A02:01 | N protein | N.D. | Reported | Yes | Yes |
| P74 | ALNTLVKQL | HLA-A02:01 | S protein | N.D. | Reported | Yes | Yes |
| P75 | ALNTPKDHI | HLA-A02:01 | N protein | N.D. | Reported | Yes | Yes |
| P76 | ILLNKHIDA | HLA-A02:01 | N protein | N.D. | Reported | Yes | Yes |
| P77 | LALLLLDRL | HLA-A02:01 | N protein | N.D. | Reported | Yes | Yes |
| P78 | LITGRLQSL | HLA-A02:01 | S protein | N.D. | Reported | Yes | Yes |

Note: Prediction of T cell epitope was conducted by NetMHCpan 4.0 Tools. Strong binding peptide ( Rank Threshold < 0.5) were synthesized for use in downstream experiments. N.R., Not reported.

Supplementary Table 2. The Grouping Information of Synthetic Peptides

| Peptide ID | Peptide | HLA-Type | Source | Mixture ID |
|---|---|---|---|---|
| P1 | YLQPRTFLL | HLA-A02:01 | S protein | M1 |
| P2 | VLNDILSRL | | S protein | |
| P3 | KIADYNYKL | | S protein | |
| P4 | RLDKVEAEV | | S protein | |
| P5 | FIAGLIAIV | | S protein | |
| P6 | SIIAYTMSL | | S protein | |
| P7 | LLFNKVTLA | | S protein | M2 |
| P8 | RLQSLQTYV | | S protein | |
| P9 | HLMSFPQSA | | S protein | |
| P10 | NLNESLIDL | | S protein | |
| P11 | VVFLHVTYV | | S protein | |
| P12 | GLTVLPPLL | | S protein | |
| P13 | TLDSKTQSL | | S protein | M3 |
| P14 | RLNEVAKNL | | S protein | |
| P15 | VLYENQKLI | | S protein | |
| P61 | LLLDRLNQL | | N protein | |
| P62 | GMSRIGMEV | | N protein | |
| P63 | KLDDKDPNF | | N protein | |
| P73 | LQLPQGTTL | | N protein | M4 |
| P74 | ALNTLVKQL | | S protein | |
| P75 | ALNTPKDHI | | N protein | |
| P76 | ILLNKHIDA | | N protein | |
| P78 | LITGRLQSL | | S protein | |
| P16 | FTISVTTEI | | S protein | M5 |
| P17 | FQFCNDPFL | | S protein | |
| P77 | LALLLLDRL | | N protein | |
| P42 | VYSTGSNVF | HLA-A24:02 | S protein | M6 |
| P43 | QYIKWPWYI | | S protein | |
| P44 | YFPLQSYGF | | S protein | |
| P45 | NYNYLYRLF | | S protein | |
| P46 | PFFSNVTWF | | S protein | |
| P47 | VFVSNGTHW | | S protein | |
| P48 | RFDNPVLPF | | S protein | M7 |
| P49 | EYVSQPFLM | | S protein | |
| P50 | YYHKNNKSW | | S protein | |
| P51 | PYRVVVLSF | | S protein | |
| P52 | VYDPLQPEL | | S protein | |
| P53 | IYQTSNFRV | | S protein | |
| P54 | VFKNIDGYF | | S protein | M8 |
| P55 | HWFVTQRNF | | S protein | |
| P56 | AYSNNSIAI | | S protein | |
| P57 | GYLQPRTFL | | S protein | |
| P58 | GYQPYRVVV | | S protein | |
| P59 | QYGSFCTQL | | S protein | |
| P60 | VYAWNRKRI | | S protein | M9 |
| P70 | QFAPSASAF | | N protein | |
| P71 | KHWPQIAQF | | N protein | |
| P72 | KHIDAYKTF | | N protein | |
| P18 | GVYFASTEK | | S protein | M10 |
| P19 | VTYVPAQEK | | S protein | |
| P20 | ASANLAATK | | S protein | |
| P21 | SVLNDILSR | | S protein | |

| P22 | TLADAGFIK | | S protein | |
|-----|-----------|---|-----------|---|
| P23 | TLKSFTVEK | | S protein | |
| P24 | NSASFSTFK | | S protein | M11 |
| P25 | SSTASALGK | | S protein | |
| P26 | GTHWFVTQR | | S protein | |
| P27 | EILPVSMTK | | S protein | |
| P28 | QIYKTPPIK | | S protein | |
| P29 | GVLTESNKK | | S protein | |
| P30 | GVYYHKNNK | | S protein | M12 |
| P31 | ALDPLSETK | | S protein | |
| P32 | RLFRKSNLK | HLA-A11:01 | S protein | |
| P33 | QIAPGQTGK | | S protein | |
| P34 | ASVYAWNRK | | S protein | |
| P35 | GIYQTSNFR | | S protein | |
| P36 | SVYAWNRKR | | S protein | M13 |
| P37 | NQFNSAIGK | | S protein | |
| P38 | AQALNTLVK | | S protein | |
| P39 | FAQVKQIYK | | S protein | |
| P40 | KSTNLVKNK | | S protein | |
| P41 | FIEDLLFNK | | S protein | |
| P64 | KTFPPTEPK | | N protein | M14 |
| P65 | ASAFFGMSR | | N protein | |
| P66 | KSAAEASKK | | N protein | |
| P67 | FTALTQHGK | | N protein | |
| P68 | AGLPYGANK | | N protein | |
| P69 | LIRQGTDYK | | N protein | |

Supplementary Table 3. SARS-CoV-2 Convalescent Patient Information

| Patient ID | Age (years) | Sex | Subject type | Citywhere infected | Clinical classification (mild:0, severe:1) | Date of admission | Date of discharge | HLA-A type | | | Usage for the epitopes screening |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | A2 | A24 | A11:01 | |
| C08 | 51 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/31 | 2020/2/17 | - | - | + | A11:01 epitope screening |
| C09 | 48 | M | SARS-CoV-2 convalescent patient | Chongqing | 1 | 2020/1/30 | 2020/2/18 | - | - | + | A11:01 epitope screening |
| C10 | 32 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/29 | 2020/2/18 | + | - | N.D. | A02:01 epitope screening |
| C11 | 54 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/2 | 2020/2/18 | + | - | + | A02:01 epitope screening A11:01 epitope screening |
| C12 | 43 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/31 | 2020/2/25 | + | - | + | A02:01 epitope screening A11:01 epitope screening |
| C13 | 47 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/31 | 2020/2/10 | + | - | N.D. | No |
| C14 | 38 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/10 | 2020/2/19 | + | - | N.D. | No |
| C15 | 33 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/10 | 2020/3/9 | + | - | N.D. | No |
| C16 | 44 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/5 | 2020/2/14 | + | - | N.D. | A02:01 epitope screening |
| C17 | 60 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/11 | 2020/3/5 | N.D. | N.D. | N.D. | No |
| C18 | 48 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/12 | 2020/3/6 | N.D. | N.D. | N.D. | No |
| C19 | 53 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/16 | 2020/2/28 | - | - | N.D. | No |
| C20 | 57 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/29 | 2020/2/14 | + | - | N.D. | No |
| C21 | 44 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/6 | 2020/2/29 | - | - | N.D. | No |
| C22 | 49 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/4 | 2020/3/2 | - | + | N.D. | A24:02 epitope screening |
| C23 | 23 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/6 | 2020/3/9 | - | + | N.D. | No |
| C24 | 44 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/13 | 2020/3/11 | + | - | N.D. | No |
| C25 | 59 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/1 | 2020/2/18 | - | + | N.D. | A24:02 epitope screening |
| C26 | 20 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/15 | 2020/2/25 | + | + | N.D. | A24:02 epitope screening |
| C27 | 37 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/2 | 2020/3/9 | + | - | N.D. | A02:01 epitope screening |
| C28 | 44 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/3 | 2020/2/28 | + | - | N.D. | A02:01 epitope screening |
| C29 | 41 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/31 | 2020/2/16 | - | - | + | A11:01 epitope screening |
| C30 | 66 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/3 | 2020/2/16 | + | + | N.D. | No |
| C31 | 67 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/4 | 2020/2/16 | - | + | N.D. | No |
| C32 | 50 | M | SARS-CoV-2 convalescent patient | Chongqing | 1 | 2020/2/1 | 2020/2/29 | - | - | N.D. | No |
| C33 | 50 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/28 | 2020/2/29 | + | + | N.D. | A24:02 epitope screening |
| C34 | 49 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/8 | 2020/3/6 | + | - | N.D. | A02:01 epitope screening |
| C35 | 56 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/1 | 2020/2/9 | + | - | N.D. | A02:01 epitope screening |
| C36 | 55 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/14 | 2020/2/21 | + | + | + | A11:01 epitope screening A24:02 epitope screening |
| C37 | 45 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/1 | 2020/2/15 | + | + | N.D. | A24:02 epitope screening |
| C38 | 47 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/6 | 2020/2/17 | + | + | N.D. | No |
| C39 | 34 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/28 | 2020/2/7 | - | - | N.D. | No |
| C40 | 47 | M | SARS-CoV-2 convalescent patient | Chongqing | 1 | 2020/1/27 | 2020/2/23 | - | - | + | A11:01 epitope screening |
| C41 | 56 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/30 | 2020/2/7 | - | + | + | A11:01 epitope screening A24:02 epitope screening |
| C42 | 63 | F | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/2/1 | 2020/2/25 | + | - | N.D. | No |
| C43 | 41 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/30 | 2020/2/18 | - | + | N.D. | A24:02 epitope screening |
| C44 | 37 | M | SARS-CoV-2 convalescent patient | Chongqing | 0 | 2020/1/27 | 2020/2/14 | - | - | N.D. | No |

Note: F, Female. M, Male. N.D., Not detected.

Supplementary Table 4. HLA-A Types of Healthy Donors

| Donor ID | HLA-A Type | | | Usage for the epitopes screening |
|---|---|---|---|---|
| | A2 | A24 | A11:01 | |
| H01 | + | - | - | A02:01 epitope screening |
| H02 | + | - | - | A02:01 epitope screening |
| H03 | + | - | - | A02:01 epitope screening |
| H04 | - | + | - | A24:02 epitope screening |
| H05 | - | + | - | A24:02 epitope screening |
| H06 | - | - | + | A11:01 epitope screening |
| H07 | - | - | + | A11:01 epitope screening |
| H08 | - | - | + | A11:01 epitope screening |
| H09 | - | - | + | A11:01 epitope screening |

Note: H, Healthy Donor.

Supplementary Table 5. The Reactivity of Identified T Cell Epitopes in COVID-19 Convalescent Patients

| HLA restriction | Number | Patient ID | Reactive peptide |
|---|---|---|---|
| HLA-A*02:01 | 1 | C10 | P63 |
| | 2 | C11* | P74, P77 |
| | 3 | C12* | P16, P77 |
| | 4 | C16 | N.D. |
| | 5 | C27 | P63 |
| | 6 | C28 | P63 |
| | 7 | C34 | P62 |
| | 8 | C35 | P4, P61, P63 |
| HLA-A*24:02 | 1 | C22 | P52 |
| | 2 | C25 | N.D. |
| | 3 | C26 | P45 |
| | 4 | C33 | P45 |
| | 5 | C36* | N.D. |
| | 6 | C37 | P54, P55, P57 |
| | 7 | C41* | N.D. |
| | 8 | C43 | P49, P53 |
| HLA-A*11:01 | 1 | C8 | N.D. |
| | 2 | C9 | P64 |
| | 3 | C11* | P64 |
| | 4 | C12* | P64 |
| | 5 | C29 | N.D. |
| | 6 | C36* | N.D. |
| | 7 | C40 | P64 |
| | 8 | C41* | P64 |

Note: C, COVID-19 convalescent patient. N.D., Not detected. *indicates the repeatly used patient samples.

Supplementary Table 6. The Sequences of P64 Reactive TCRs

| TCR Name | V-D-J nucleotide sequence (CDR3 highlighted) | V-D-J amino acid sequence (CDR3 highlighted) |
|---|---|---|
| P64_TCR 1 alpha chain | gctcagacagtcactcagtctcaaccagagatgtctgtgcaggaggcagagaccgtg accctgagctgcacatatgacaccagtgagagtgattattatttattctggtacaagcag cctcccagcaggcagatgattctcgttattcgccaagaagcttataagcaacagaatg caacagagaatcgtttctctgtgaacttccagaaagcagccaaatccttcagtctcaag atctcagactcacagctggggggatgccgcgatgtatttctgtgcttataggagcgtccc actgaacagagatgacaagatcatctttggaaaagggacacgacttcatattctcccc | AQTVTQSQPEMSVQEAETVTLSCT YDTSESDYYLFWYKQPPSRQMILVI RQEAYKQQNATENRFSVNFQKAAK SFSLKISDSQLGDAAMYFCAYRSVP LNRDDKIIFGKGTRLHILP |
| P64_TCR 1 beta chain | gatggtggaatcactcagtccccaaagtacctgttcagaaaggaaggacagaatgtg accctgagttgtgaacagaatttgaaccacgatgccatgtactggtaccgacaggacc cagggcaagggctgagattgatctactactcacagatagtaaatgactttcagaaagg agatatagctgaaggggtacagcgtctctcgggagaagaaggaatcctttcctctcact gtgacatcggcccaaaagaacccgacagctttctatctctgtgccagtagtagagccg ggacagggtacaatgagcagttcttcgggccagggacacggctcaccgtgctag | DGGITQSPKYLFRKEGQNVTLSCEQ NLNHDAMYWYRQDPGQGLRLIYY SQIVNDFQKGDIAEGYSVSREKKES FPLTVTSAQKNPTAFYLCASSRAGT GYNEQFFGPGTRLTVL |
| P64_TCR 4 alpha chain | gaccagcaagttaagcaaaattcaccatccctgagcgtccaggaaggaagaatttcta ttctgaactgtgactatactaacagcatgtttgattatttcctatggtacaaaaaatacct gctgaaggtcctacattcctgatatctataagttccattaaggataaaaatgaagatgga agattcactgttttcttaaacaaaagtgccaagcacctctctctgcacattgtgccctccc agcctggagactctgcagtgtacttctgtgcagcaagcggagaatacaataacaatga |gtgacg|tttggagcaggaacccaagacaccacccaaccacccc | DQQVKQNSPSLSVQEGRISILNCDY TNSMFDYFLWYKKYPAEGPTFLISI SSIKDKNEDGRFTVFLNKSAKHLSL HIVPSQPGDSAVYFCAASGEYNNN DMRFGAGTRLTVKP |
| P64_TCR 4 beta chain | aatgccggcgtcatgcagaaccaagacacctggtcaggaggagggggacaggag gcaagactgagatgcagcccaatgaaaggacacagtcatgtttactggtatcggcag ctcccagaggaaggtctgaaattcatggtttatctccagaaagaaaatatcatagatga gtcaggaatgccaaaggaacgatttctgctgaatttcccaaagagggccccagcatc ctgaggatccagcaggtagtgcgaggagattcggcagcttatttctgtgccagctcac catcagtgtacttcgaggtctctggggccaacgtcctgactttcgggggccggcagcag | NAGVMQNPRHLVRRRGQEARLRCS PMKGHSHVYWYRQLPEEGLKFMV YLQKENIIDESGMPKERFSAEFPKE GPSILRIQQVVRGDSAAYFCASSPSV YFEVSGANVLTFGAGSRLTVL |

Supplementary Table 7. Mutant Variants and Homologous Peptides of $N^{361-369}$

| Peptide name | Sequence | Number of mutant strain (note-01) | Note |
|---|---|---|---|
| Wild-type | KTFPPTEPK | N.D. | Derived from SARS-CoV, SARS-CoV-2 |
| T362K | KKFPPTEPK | 3 | |
| T362I | KIFPPTEPK | 53 | |
| F363L | KTLPPTEPK | 2 | |
| F363S | KTSPPTEPK | 4 | Derived from SARS-CoV-2 mutation strains |
| P364L | KTFLPTEPK | 28 | |
| P364R | KTFRPTEPK | 2 | |
| P365S | KTFPSTEPK | 10 | |
| T366I | KTFPPIEPK | 28 | |
| E367K | KTFPPTKPK | 5 | |
| MERS | KTFPKKEKK | N.D. | Derived from MERS-CoV |

Note: Data for mutant variants is from 2019 Novel Coronavirus Resource (https://bigd.big.ac.cn/ncov).

Data were updated to 2020/09/08. N.D., Not detected.

Supplementary Table 8. Lung Organoid Medium Recipe

| Number | Media component | Supplier | Catalogue number | Final concentration |
|---|---|---|---|---|
| 1 | B27 supplement | Gibco | 17504-44 | 1x |
| 2 | R-Spondin 1 | Peprotech | 120-38 | 500 ng·ml$^{-1}$ |
| 3 | FGF 7 | Peprotech | 100-19 | 25 ng·ml$^{-1}$ |
| 4 | FGF 10 | Peprotech | 100-26 | 100 ng·ml$^{-1}$ |
| 5 | Noggin | Peprotech | 120-10C | 100 ng·ml$^{-1}$ |
| 6 | Y-27632 | Abmole | Y-27632 | 5 mM |
| 7 | N- | Sigma | A9165-5g | 1.25 mM |
| 8 | Nicotinamide | Sigma | N0636 | 5 mM |
| 9 | Primocin | Invivogen | Ant-pm-1 | 50 mg·ml$^{-1}$ |
| 10 | A83-01 | Tocris | 2939 | 500 nM |
| 11 | SB202190 | Sigma | S7067 | 500 nM |

Note: the factors were added into Advanced DMEM/F12, then was used as the complete medium.