HLA class I genotypes customize vaccination strategies in immune simulation to combat COVID-19

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Abstract

Memory CD8⁺ T cells are associated with a better outcome in Coronavirus Disease 2019 (COVID-19) and recognized as promising vaccine targets against viral infections. This study determined the efficacy of population-dominant and infection-relevant human leukocyte antigens (HLA) class I proteins to present severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) peptides through calculating binding affinities and simulating CD8⁺ T cell responses. As a result, HLA class I proteins distinguished or shared various viral peptides derived from viruses. HLA class I supertypes clustered viral peptides through recognizing anchor and preferred residues. SARS-CoV-2 peptides overlapped significantly with SARS but minimally with common human coronaviruses. Immune simulation of CD8⁺ T cell activation using predicted SARS-CoV-2 peptide antigens depended on high-affinity peptide binding, anchor residue interaction, and synergistic presentation of HLA class I proteins in individuals. Results demonstrated that multi-epitope vaccination, employing a strong binding affinity, viral adjuvants, and heterozygous HLA class I genes, induced potent immune responses. Therefore, optimal CD8⁺ T cell responses can be achieved and customized contingent on HLA class I genotypes in human populations, supporting a precise vaccination strategy to combat COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19) pandemic has raised more than 55 million cases globally since earlier this year and remains rapid spreading with over a half-million new daily infections worldwide¹. COVID-19 is caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), characterized as pneumonia and lymphopenia, and develops severe respiratory or multiple organ failure in around 5% of patients². Recent vaccine development mainly focused on the induction of neutralizing antibodies. Although multiple vaccination strategies are under clinical trials³, their protection against COVID-19 in large human populations remains unknown. Considering disappointing efficacy or insufficient protection frequently occurred in previous anti-viral vaccine trials using large human populations, alternative approaches to stimulate protective immunity and control infections effectively remain urgently needed.

CD8⁺ T cells mediate essential immune responses against numerous intracellular viral infections to provide durable T cell memory and protection up to many years after infections, such as in immune defense against SARS^{4,6}, HIV^{7,8}, and influenza virus infections^{9,10}. Clinical trials of anti-viral vaccines frequently led to disappointing results with weak CD8⁺ T cell responses^{7,8,11}, supporting the importance of anti-viral CD8⁺ T cell responses in different human populations. Indeed, viral-specific CD8⁺ T cells are essential for viral clearance and long-term protection against HIV¹², Ebola¹³, hepatitis B viruses¹⁴, and cytomegalovirus¹⁵, despite the critical role of neutralizing antibodies for blocking viral entry to human cells^{6,16-18}. In COVID-19, it is likely important to stimulate both neutralizing antibodies and CD8⁺ T cell responses to achieve full protection, as in viral vaccination trials against SARS¹⁹ and influenza virus^{6,20}. SARS-CoV-2 infections induce a dramatic reduction of CD8⁺ T cells associated with severe^{21,22} and elderly patients²³. Multiple recent reports strongly support that viral-specific and memory CD8⁺ T cell responses are associated with milder diseases, younger patients²⁴, and convalescent phases of infections^{25,26}, although HLA genotype²⁷, differentiation kinetics^{28,29}, and adjuvant usage³⁰ impact the protection of CD8⁺ T cells subsets.

Activation of viral-specific CD8⁺ T cells and killing of viral-infected cells rely on antigen-presentation mediated by HLA class I proteins together with co-stimulation and cytokine microenvironment^{31,32}. A fundamental mechanism is that HLA class I proteins bind specific viral peptides as in a key-to-lock model^{33,34}, which interacts with the T cell receptor of CD8⁺ T cells

for activation and viral-infected cell recognition. Similar to the cell entry of SARS-CoV³⁵, SARS-CoV-2 is considered to enter lung epithelial cells and blood cells through interacting with cell surface receptors, such as angiotensin-converting enzyme 2 (ACE2), and follow the endocytic and cytoplasmic compartments³⁶. Intracellular viral proteins in viral assembling and trafficking processes can be degraded in proteasome or endosome to form antigenic peptide ligands that further bind to HLA class I proteins through a secretory pathway or cross-presentation^{37,38}. Currently, computational prediction and experimental identification of peptide antigens for CD8⁺ T cell activation in COVID-19 are ongoing for a purpose of “identifying vaccine candidates”^{26,39-41}. However, the impact of HLA class I genotypes on peptide antigen selection and CD8⁺ T cell activation remains poorly understood.

To date, the HLA-A locus displays 6291 alleles that encode around 3900 proteins, and HLA-B displays 7562 alleles that encode around 4808 proteins in humans⁴². It is unlikely to fully unveil functional CD8⁺ T cell responses to unique peptide sets presented by each HLA class I protein. With a wealth of structural knowledge on HLA-peptide-T cell receptor interaction^{32,43}, computational prediction and immune simulation as a prototypic artificial intelligent tool become possible and essential to facilitate structural identification and functional simulation of peptide antigen presentation for CD8⁺ T cell activation. This study applied affinity binding theory to predict SARS-CoV-2 peptides for binding to different HLA class I proteins and simulate the antigen-presentation process for CD8⁺ T cell activation. Results showed a dramatically different capacity in various HLA class I proteins to bind SARS-CoV-2 peptides. Multiple HLA class I proteins that have been indicated to associate with more severe symptoms or weaker CD8⁺ T cell responses in SARS⁴⁴ were demonstrated in this study for binding a less number of SARS-CoV-2 peptides. In contrast, HLA class I proteins that have been suggested protective or potent stimulators of CD8⁺ T cells in SARS⁴⁵ were predicted to bind a large number of SARS-CoV-2 peptides. Different HLA class I proteins clustered to a supertype share identical sequences and similar binding patterns of SARS-CoV-2 peptides. Across various coronaviruses, a large number of peptides from SARS viruses and a limited number of peptides from “common cold”-causing human coronaviruses are identical to those of SARS-CoV-2. Further, affinity-based immune simulation provided an optimized combination of peptides dependent on HLA class I genotypes to facilitate customizing precise vaccination strategies for individuals and human populations.

Results

Dominant HLA class I proteins sample various numbers of SARS-CoV-2 peptides.

To stimulate anti-COVID-19 CD8⁺ T cell responses, this study used NetMHCpan program to comprehensively determine the binding affinity of SARS-CoV-2 peptides to ten dominant HLA class I A and class I B proteins, which are frequently detected in the United States (US)⁴⁶. HLA-C alleles were not involved since HLA-C proteins mainly induce immune tolerance⁴⁷. The threshold of sufficient binding affinity was defined using a half-maximal inhibitory concentration ($IC_{50} < 500nM$), in which the maximal concentration of a SARS-CoV-2 peptide is required to competing off the binding of a probe peptide to each HLA class I protein. The spike (S), membrane (M), nucleocapsid (N), and multiple ORF-encoded proteins (Fig. S1A) of the SARS-CoV-2 virus are considered immunogenic and are analyzed in this study because representative peptides of these proteins stimulate CD8⁺ T cells isolated from COVID-19 patients^{39,40}. Various numbers of SARS-CoV-2 peptides were predicted with a high binding affinity to ten dominant HLA class I A and B proteins (Fig. S1A). These ten alleles exist in 78.5% of the US population approximately. Upon the further consideration of the earlier processes in antigen presentation, including proteasome degradation (cutoff > 5 potential cutting sites by proteasome enzymes)⁴⁸ and peptide transportation through the transporter associated with antigen processing (TAP, cutoff $IC_{50} < 1nM$)⁴⁹, 80% to 100% peptide ligands for HLA class I proteins remained significant (Fig. 1A).

As a result, striking variation exists in the numbers of SARS-CoV-2 peptides sampled by different dominant HLA class I proteins. Most of the ten dominant HLA-A and B proteins bound high numbers of peptides derived from noted immunogenic SARS-CoV-2 proteins. A*02:01 and A*11:01 bound the highest numbers of peptides (Fig. 1), indicating that a large human population expressing at least one of these dominant HLA class I alleles is able to respond to SARS-CoV-2 infection. The capacity of binding a higher number of viral peptides suggests a potential dominance of viral-induced CD8⁺ T cell responses. These potent peptide binders can compensate with weak peptide binders and balance the overall ability of antigen presentation for CD8⁺ T cell activation, if an HLA class I genotype combines both potent and weak peptide binders in the same individual. Apparently, some SARS-CoV-2 proteins provided a lower number of peptides due to their short chain length of proteins, such as N and ORF8-encoded proteins. Thus, to estimate a critical protein source of viral peptides, peptide numbers were further normalized with the chain length of each SARS-CoV-2 protein (Fig. 1B). As a result,

among different SARS-CoV-2 proteins, fewer percentage of peptides from N and ORF8-encoded proteins are presented by most HLA-A proteins. Among different HLA class I proteins, HLA-A proteins overall displayed a higher peptide loading capacity than HLA-B proteins. For example, HLA-A*02:01, A*11:01, and A*24:02 sampled a higher % of peptides from multiple viral proteins, except for N and ORF8-encoded proteins (Fig. 1B). HLA-B*35:01 showed a high peptide loading capacity than other HLA-B proteins. Although the numbers of peptide ligands do not necessarily reflect the protection of peptide antigens in diseases, predicted peptides binding to HLA proteins raise a critical concern that various human populations with various HLA class I genotypes will respond differently in COVID-19 infections.

To consider whether HLA class I sequence heterogeneity selects various numbers of peptide ligands, $\alpha 1$ and $\alpha 2$ ligand-binding domains of ten HLA class I proteins were aligned (Fig. S1B), using a hierarchical clustering approach⁵⁰. Overall resulted similarity ranged from 75% to 96%, with the highest similarity between A*03:01 and A*11:01 (96.15%) (Fig. S1C). However, the numbers of predicted peptides appear generally unmatched with the degree of similarity between different HLA class I proteins. For example, A*02:01 shows 92.31% similarity to A*03:01, but they have very different numbers of binding peptides for the same proteins (Fig. 1A). Similar non-concordance occurs between A*01:01 and A*11:01 with 93.96% sequence similarity and between A*03:01 and A*11:01 with the highest sequence similarity of 96.15%. These results suggest a need to exam the similarity of peptide-binding domains among HLA class I proteins.

Dominant HLA class I proteins share limited numbers of SARS-CoV-2 peptides. To determine the conservation of viral peptides for CD8⁺ T cell activation in different human populations, we applied Venn diagrams to show the numbers of predicted identical peptides between dominant HLA-A proteins (Fig. 2A) and HLA-B proteins (Fig. 2B). We used the spike and ORF1ab genes, which encode longer protein sequences than others, to provide a higher chance of overlapped peptide sampling by different HLA class I proteins. As a result, identical peptides are infrequently shared by many HLA class I proteins despite high sequence similarity (>90%) between HLA-A or HLA-B proteins (Fig. S1C). For some HLA proteins, vast numbers of shared peptides were observed, for example, between A*03:01 and A*11:01 with an extremely high sequence similarity of 96.15%, across B*40:01 and B*44:03 with a relatively low similarity of 89.56%, or between B*35:01 and B*07:02 with a lower sequence similarity of 88.46%. In particular, almost 80% or 85% of peptides bound to A*03:01 also bind to A*11:01. However,

HLA sequence similarity is overall discordant to the numbers of shared peptides. B*08:01 and B*07:02 (93.41% sequence similarity) shared much fewer numbers of peptides, and B*08:01 and B*40:01 (92.31% sequence similarity) shared no peptide from the S protein or only 1 out of 340 peptides from the ORF1ab-encoded proteins. Therefore, an overall high sequence similarity does not suggest a high number of peptide overlap. As in the sequence alignment (Fig. S1B), potential determinants for the numbers of shared peptides likely originate from hot spots with limited numbers of residues in ligand-binding grooves. The similarity of these key residues and pocket structures assigns HLA class I proteins to multiple supertypes^{51,52}. Indeed, high numbers of identical SARS-CoV-2 peptides were shared between HLA class I proteins of the same supertypes, such as supertypes A03 (A*03:01 and A*11:01), B07 (B*35:01 and B*07:02), and B40 (B*40:01 and B*44:03) (Fig. 2).

Further, peptide-binding motifs of HLA class I proteins interact with anchor and preferred residues of peptide ligands to achieve high binding affinity (Fig. 3). The unique or shared anchor residues validates the binding of predicted SARS-CoV-2 peptides to specific HLA class I proteins or to HLA class I supertypes, respectively. Thus, the GibbsCluster program^{53,54} was applied to determine the residue patterns of predicted SARS-CoV-2 peptides. As a result, SARS-CoV-2 peptides showed conserved residues at positions 2 or 3 and 9, matching the reported anchor residues or preferred residues with corresponding HLA class I proteins (Fig. 3). HLA class I proteins assigned to the same supertypes shared similar anchor and preferred residues, as for supertypes B40 (B*40:01 and B*44:03), and to a less degree for B07 (B*35:01 and B*07:02)⁵⁵⁻⁶⁰. For A*03:01 with undefined anchor and preferred residues, this study showed a similar pattern of peptide binding to A*03:01 as to A*11:01, which display a sequence similarity of 96.15% (Fig. 1C) and belong to A03 supertype⁵². Therefore, predicted SARS-CoV-2 peptides form various patterns of anchor and preferred residues, which are shared within an HLA class I supertype (Fig. 3), as a unique conserved mechanism to implement HLA genotype-customized CD8⁺ T cell activation in various human populations.

HLA class I proteins with weak peptide binding capacity incline to associate with severe infections. Multiple HLA class I proteins have been reported to associate with more severe diseases of viral infections. In SARS infections, B*46:01 was associated with severe cases in a study using a small sample size⁴⁴ and the association of B*07:03 with severe SARS could not pass multiple tests⁶¹. In contrast, peptide binding to the HLA class I proteins of A03 supertype, including A*03:01 and A*11:01, was considered as potent stimulators for CD8⁺ T cells against

SARS⁴⁵, and the potential association of B*15:02 with resistance to SARS is not statistically significant in multiple tests⁶¹. Since individuals usually express two to four heterozygous or homozygous HLA class I A and B paralogous proteins, the potential association of one protein with severe diseases is likely balanced or interfered by other heterozygous HLA class I proteins in human population studies. The numbers of predicted peptide ligands in our study are supportive of a potential association of these HLA class I proteins with severity or protection of infections. For potent peptide binders potentially associated with resistance or protection, A*02:01, A*11:01, and B*15:03 sampled a large number of peptides (Fig. 4A). These results are consistent with a common stimulator of A*11:01 for anti-SARS CD8⁺ T cell responses⁴⁵, a potential association of B*15:02 with the resistance to SARS⁶¹, and needs to further understand the effector phenotypes²⁷ and kinetics²⁹ of A*02-mediated CD8⁺ T cell responses in COVID-19. Belonging to the same supertypes (Fig. 4) and share high sequence homology (Fig. S2), A*02:02 sampled a high number of peptides as for A*02:01 (A02 supertype), and the similar was observed for B*15:01, B*15:02, and B*15:03, although a controversy remains in their assignment to one supertype or two supertypes^{52,62}. Interestingly, SARS-CoV-2 peptides were predicted to be overlapped between A*02 and B*15 proteins or between A*11 and B*15 proteins (Fig. 4B), providing an additional possibility of identical peptides unusually shared by paralogous HLA class I proteins. On the other side, relative weak peptide binders B*07:03 and B*46:01 in this study were suggested potentially associated with severe SARS in some studies⁶¹ or with a small sample size⁴⁴. B*07:02 and B*07:03 alleles (B07 supertype) sampled relatively fewer numbers of peptides in comparison to potent peptide binders such as A*02:02 or B*15:03, consistent with its difficulty to pass multiple tests in severe SARS⁶¹ if co-expressed with strong binders. Surprisingly, B*46:01 is predicted almost not binding to peptides from SARS-CoV-2 (Fig. 4A), suggesting a potential weak CD8⁺ T cell response against COVID-19 and consistent to its association with severe SARS infection⁴⁴. A*25:01 binds a very limited number of SARS-CoV-2 peptides at low binding affinity, indicating a potential concern of weak responses in patients with an A*25:01 allele. These results allow us to predict and prepare to overcome weak peptide presenters in fighting human COVID-19 infections.

Overlapped HCoV and SARS-CoV-2 peptides suggests cross-reactive CD8⁺ T cells.

Recent tests demonstrated that non-infected healthy donors contain CD8⁺ T cells responding to SARS-CoV-2 peptides^{39,40}. Therefore, one can hypothesize that “common cold” coronavirus infection in humans can generate cross-reactive CD8⁺ T cells against SARS-CoV-2 infections. Moreover, cross-reactivity is more prominent and much stronger between SARS and SARS-CoV-

²⁴⁰. Our predicted numbers of overlapped peptides can overall explain this cross-reactivity. SARS and SARS-CoV-2 share various sequence similarity, such as 77.54% for S protein (Fig. S3) and 95.37% for ORF1b-encoded proteins (Fig. S4), contributing to the overlap of 69 peptides from spike protein and 438 peptides from ORF1b-encoded proteins between SARS with SARS-CoV-2 (Fig. 5). Since CD8⁺ T cells exposed to “common cold” coronaviruses are potentially reactive to SARS-CoV-2 peptides, the prediction of human coronavirus peptides contributing to this cross-reactivity will provide bases for cross-virus protection. However, HCoV-OC43 and SARS-CoV-2 share much lower sequence similarity at 33.06% for S protein (Fig. S3) and 62.32% for ORF1b-encoded proteins, contributing to 44 shared peptides (5%) from ORF1b-encoded proteins but nearly zero shared peptide from N, S, and ORF1a-encoded proteins between HCoV-OC43 and SARS-CoV-2 (Fig. 5). This limited number of cross-reactive peptides from HCoV is valuable for further functional investigations for cross protection.

Heterozygous HLA class I genotypes with high affinity peptides enhance CD8⁺ T cell responses in immune simulation. To predict whether high peptide binding affinity, peptide numbers, and HLA class I heterozygosity enhance CD8⁺ T cell responses, we use an agent-based simulator, C-ImmSim, to simulate antigen presentation for CD8⁺ T cell activation⁶³. C-ImmSim mainly incorporated the impact of HLA-peptide binding, innate signaling, memory responses, and adjuvant effect in antigen presentation and CD8⁺ T cell activation, particularly differentiating the effect of heterozygosity or homozygosity of HLA genes⁶³. First, we compared CD8⁺ T cell responses to one versus ten peptides with a high binding affinity (IC₅₀<16nM) without involving innate or adjuvant factors. Results showed that one peptide is insufficient, but a combination of ten peptides, stimulated CD8⁺ T cell responses that became saturated in around two weeks (Fig. 6A). In this simulation, multi-epitopes stimulated more robust CD8⁺ T cell responses than one epitope, possibly due to very strong total binding avidity. Second, to more precisely test the avidity hypothesis, five peptides with the highest IC₅₀ were compared with the other five peptides with the lowest IC₅₀ as loaded to the same A*02:01 protein. Results further supported the conclusion that strong CD8⁺ T cell responses are more likely to be activated by multiple peptides with a high binding affinity (low IC₅₀) to HLA class I proteins (Fig. 6B). Further, to determine whether heterozygous HLA-A or HLA-B proteins show an advantage to enhance CD8⁺ T cell responses than homozygous A*02:01 or B*44:02 alone, results showed peptides with similar or lower binding affinity stimulated stronger CD8⁺ T cell responses by heterozygous HLA-A or B proteins. Moreover, paralogous HLA-A and B proteins showed similar strength for CD8⁺ T cell activation to heterozygous HLA class I A or B proteins

(Fig. 6C). Therefore, high peptide binding affinity, HLA gene heterogeneity, and paralogous HLA-A and B proteins provide advantages to stimulate a robust CD8⁺ T cell response.

Heterozygous HLA class I genotypes with viral adjuvants rescue CD8⁺ T cell responses in immune simulation to weak peptide binders. Although B*46:01 and B*07:02 are potentially relevant to severe SARS^{44,61} and shown as weak peptide binders (Fig. 4), we speculated that CD8⁺ T cell responses in the individuals expressing one of these alleles can still be strongly stimulated by optimizing peptide immunization utilizing HLA class I heterogeneity and viral adjuvant. Strikingly, results showed that only three predicted peptides from multiple SARS-CoV-2 proteins bind to B*46:01 at a sufficient affinity (IC₅₀<500nM) in comparison to hundreds or thousands of peptides sampled by other HLA class I proteins (Fig. 4A). One can imagine that an individual with a B*46:01 allele will be challenging for inducing CD8⁺ T cell activation, as this likely explains why B*46:01 allele is associated with severe SARS in a study using a small sample size⁴⁴. Thus, it is critical to determine whether other HLA-A or B alleles in the same individuals facilitate overcoming this weakness. For example, HLA-A*02:07 and HLA-B*46:01 haplotype is present in around 3.34% of Asian pacific population⁶⁴. Whether the peptides presented by HLA-A*02:07 can rescue or enhance CD8⁺ T cell responses mediated by the weak peptide binder B*46:01 is critical for inducing anti-COVID-19 immunity in this human population. By applying one additional peptide that is presented by a paralogous A*02:07 or by a heterozygous B*07:02 protein, results supported both strategies of antigen presentation overcame the disadvantage of B*46:01 protein for CD8⁺ T cell activation (Fig. 4A). Furthermore, the application of adjuvants such as an attenuated viral strain will help provide a cytokine environment mediated by innate cells and CD4⁺ T cells to facilitate the activation of peptide-specific CD8⁺ T cells (Fig. 7B and 7C). In this immune simulation, the viral adjuvant enhanced the proliferation of peptide-specific memory CD8⁺ T cells and balanced the production of IFN γ and inhibitory cytokines with extended kinetics. Therefore, a precise vaccination strategy considering potent peptide binders, heterozygous and paralogous advantages of HLA class I alleles, multi-epitopes of peptide antigens, and a viral adjuvant will induce strong anti-viral CD8⁺ T cell responses as tailored to individual and populational HLA class I genotypes.

Discussion

More than two, three, and ten thousand HLA-A and B alleles have been detected in Asian, African, and European Americans, respectively⁶⁴. The same viral proteins are expected to induce divergent CD8⁺ T cell responses in different individuals or populations, demanding a precise determination of efficacious peptide antigens presented by individual-specific HLA class I proteins. Remarkably extensive efforts are needed to structurally profile and functionally validate viral peptide pools for various HLA class I genotypes in ethnically diverse human populations. Computational prediction of these tens of thousands of peptides in terms of their binding affinity to each HLA class I proteins from tens of hundreds of HLA class I genotypes provides highly feasible alternatives to predict candidate peptides. Further, CD8⁺ T cell activation using these large numbers of combinations of predicted peptides and HLA class I genotypes can only be possibly performed using immune simulation to provide limited candidate strategies for further experimental and clinical tests. This study performed a computational prediction of peptide ligands and an immune simulation of CD8⁺ T cell activation using representative potent and weak peptide binders, including ten dominant HLA class I alleles and potentially SARS-associated HLA class I alleles. Results demonstrated that multi-epitope high-affinity peptides, tailored to heterozygous and paralogous HLA class I genotypes combined with a viral adjuvant, elicits strong CD8⁺ T cell responses, memory CD8⁺ T cell differentiation, and sustainable cytokine production.

Regardless of the degree of sequence diversity (Fig. S1C), key residues in peptide-binding motifs define the supertypes of HLA class I proteins^{51,52}. HLA class I proteins of the same supertypes share peptide antigens and peptide-binding patterns, suggesting the conservation of anti-COVID-19 antigenic peptides in human populations. Briefly, large numbers of identical peptides overlapped between A*03:01 and A*11:01 (A03 supertype), B*44:03 and B*40:01 (B40) (Fig. 2), A*02:01 and A*02:02 (A02), B*07:02 and B*07:03 (B07), and B*15:01 and B*15:03 (B27) (Fig. 4). In these HLA class I supertypes, A*02:01 and A*02:02 shared more than 50% of identical peptides, and three B*15 proteins shared large numbers of identical peptides (Fig. 4). Overlaps between paralogous HLA-A*02 and B*15 proteins also occurred at a much lower degree, implicating the presence of degenerate epitopes for CD8⁺ T cells⁶⁵. Therefore, by defining HLA class I genotypes in targeted populations, similar or identical peptide antigens shared by HLA class I supertypes can be applied to vaccine design for those populations expressing HLA class I genes of the same supertypes.

Peptides with high affinity and avidity stimulate robust CD8⁺ T cell responses and aid weak peptide binder for T cell activation, as demonstrated by the immune simulation of antigen presentation in this study. One of our selected peptide, “FIAGLIAIV”, presented by HLA-A*02:01 has been validated for the activation of T cell from SARS patients⁶⁶. Evidence was provided with insufficiency for stimulating CD8⁺ T cell responses using a single peptide (Fig. 6A) or five low-affinity peptides (Fig. 6B) in a vaccination setting in comparison to strong responses with ten peptides or five high-affinity peptides, respectively. Therefore, a high total peptide binding affinity (or avidity) is crucial for CD8⁺ T cell activation in vaccination.

Heterozygosity of HLA alleles is considered as a result of balancing selection to shape exceptional polymorphism of HLA proteins and increase host immunocompetence against highly diverse pathogens^{67,68}. In this study, HLA class I heterozygosity was demonstrated to provide an advantage in presenting additional high-affinity peptides complementarily (Figs. 6 and 7). This heterozygous advantage allows the design of optimal peptide vaccination to achieve pronounced CD8⁺ T cell responses. For example, HLA-B*46:01 that was predicted to bind to two low-affinity peptides from ORF1ab-encoded proteins, in comparison to B*15:03 and B*07:02 predicted to bind 1538 and 158 peptides derived from the same SARS-CoV-2 proteins (Fig. 4), likely supports the theory of heterozygous advantage⁶⁸, if these HLA class I alleles express in an individual. This study provided an example of immune simulation that CD8⁺ T cell responses in an individual expressing a weak peptide binder HLA-B*46:01 protein can be enhanced and rescued by the heterozygous expression of B*07:02 protein, which provided complementary capacity in peptide presentation to enhance CD8⁺ T cell activation (Fig. 7A). This heterozygous advantage has also been clinically demonstrated in individuals with heterozygous HLA class I loci A and B for protection against human T-lymphotropic virus type-1 (HTLV-1)^{68,69}. Thus, HLA heterozygosity is an advantageous factor to be critically examined, particularly in the populations or individuals with weak CD8⁺ T cell responses to the virus- or protein-based vaccines, which can be rescued by providing a custom design of peptide antigens for the presentation of heterozygous HLA class I proteins.

Dual antigen presentation mediated by paralogous HLA-A and B proteins, as existing in all HLA class I haplotypes, further enhances CD8⁺ T cell responses. This dual antigen presentation elicits more robust CD8⁺ T cell responses than the antigen presentation mediated by a single HLA-A or B protein for a similar number of peptides (Fig. 6C). Using the weak peptide binder B*46:01 as

an example, peptides presented by HLA-A proteins can be applied to rescue the weak CD8⁺ T cell responses mediated by HLA-B*46:01 protein. For example, in clinical trials of anti-viral vaccination, 3.34% of the Asian Pacific population co-expressing HLA-A*02:07 and the weak peptide binder HLA-B*46:01⁶⁴ can be considered to have additional peptide-based vaccination to elicit optimal CD8⁺ T cell responses, as customized to this specific HLA class I genotype (Fig. 7). Moreover, viral co-vaccination or adjuvant can be applied to boost more robust or stabilized CD8⁺ T cell responses for the haplotype containing B*46:01 in human populations, together with antigen presentation mediated by either heterozygous or paralogous HLA class I A and B proteins (Fig. 7).

In summary, precision vaccination strategies targeting CD8⁺ T cells can be customized on HLA class I genotypes and peptide affinity for human populations to circumvent weak peptide binders in T cell activation. Dependent on HLA class I genotypes from an individual or a population, high-affinity peptides can be matched to heterozygous or paralogous HLA class I A and B alleles together with adjuvant as customized vaccination strategies. This key-to-lock model^{33,34} explains why vaccine efficacy results are usually very different between animal experiments and human trials, because antigen-presenting molecules and peptide antigens for T cell activation are entirely different between animals, including primates, and humans. However, clinical trials to determine the efficacy of a vaccine is time- and labor-consuming, and economically challenging, due to the requirement for double-blind design and the observation of the protection under natural infection conditions in a long-term process. Computational simulation of antigen presentation, using this study as an example, provides valuable candidate strategies for HLA genotype-customized vaccine design using known viral protein sequences. It is reasonable to speculate that the antigen presentation tailored to HLA genotypes of patients is advantageous to overcome weak CD8⁺ T cell reactivity and facilitate achieving a high efficacy of protection in anti-SARS-CoV-2 vaccine trials clinically.

Materials and Methods

1. **Protein sequences were accessed** from GenBank with accession numbers of QHU79204.1 (spike or S protein), QLI50116.1 (membrane or M protein), QHU79211.1 (nucleocapsid or N protein), QJQ39966 (open reading frame 1ab or ORF1ab), QJQ27841 (ORF1a), QJQ39969 (ORF3a), QJQ27861 (ORF8), Protein sequences of human beta coronavirus OC43 isolate include AGT51680.1 (S protein), AIX10719.1 (N protein),

YP_009555238.1 (ORF1ab), and QBP84755.1 (ORF1a). Protein sequences of SARS include AAP51227.1 (S protein), AAP51234.1 (N protein), QJQ39966 (ORF1ab), QJQ27841 (ORF1a). ORF1b fragment is defined by the non-overlapped portions of amino acid sequences between ORF1ab and ORF1a. HLA class I protein sequences for peptide binding prediction were accessed from the website of The European Molecular Biology Laboratory (EMBL)-European Bioinformatics Institute (EBI) (<https://www.ebi.ac.uk/ipd/imgt/hla/allele.html>).

2. **The alignment of protein sequences** was performed using a hierarchical clustering approach (<http://multalin.toulouse.inra.fr/multalin/>)⁵⁰. Neutral consensus residues are labeled in red, low consensus residues are labeled in blue, and high consensus portions are labeled in grey as a background. Upon sequence alignment, percentages of identical sequences are calculated and listed.

3. **Affinity-based prediction of peptide ligands for HLA class I proteins.** The model NetMHCpan was chosen because this model has been widely used to predict the binding affinity of peptide sequences with 8-11 residues to various HLA class I proteins. NetMHCpan calculates a 50% maximal inhibitory concentration (IC₅₀) based on the maximal concentration of test peptides to compete off a probe peptide for binding to the same HLA class I protein⁴⁶. NetMHCpan prediction has been performed accurately in recent antigen peptide prediction to integrate mass spectrometry-eluted ligands and binding affinity of peptides^{70,71}. NetMHCpan is also available for all MHC alleles to analyze dominant and disease-associated HLA class I alleles. This study used the NetMHCpan 4.0 program, which is a default method in the website of Immune Epitope Database (IEDB.org) for predicting peptide ligand binding to HLA class I proteins. This prediction was not limited to the most typical length of peptide sequence (9 amino acids) but expanded to cover a possible length of 8 to 11 residues.

4. **Proteasome degradation** mainly considers the degradation mediated by immune proteasomes, which are induced by Interferon γ to generate antigenic peptides. Immune proteasomes degrade SARS-CoV-2 proteins to polypeptides, which have a high potential to bind to MHC Class I molecules. The prediction was based on *in vitro* proteasomal digestion of enolase and casein proteins as described⁴⁸ to determine the numbers of potential cutting sites at the C-termini induced by various proteasome proteases. In this

prediction, we used the Netchop v3.0 “C-term” model to remove any peptides that were not predicted via proteasomal cleavage of the peptide’s C terminus^{72,73}. A conserved threshold of five was used to select peptide ligands with five or more cutting sites in proteasome degradation to generate peptides for further canonical MHC class I antigen processing.

5. **Transportation by TAP transporters.** Upon proteasome digestion, cytosol peptides are required to be transported by peptide transporters across the endoplasmic reticulum membrane to be loaded to the heavy chain of HLA class I proteins. It has been shown that a high affinity of TAP transporter binding to a peptide translates into high transport rates⁴⁹. Notably, both proteasome and TAP predictions were developed using experimental data for HLA proteins and were highly suitable for predicting viral peptide ligands for HLA class I proteins. Interestingly, TAP ligands with high binding affinity have an increased chance of being cleaved by the proteasome, and TAP specificity has evolved to fit the digestion specificity of proteasomes⁷³. Thus, the prediction of TAP transporter binding to peptides links the processes of proteasome digestion and peptide loading to HLA class I proteins. A TAP score estimates $-\log(\text{IC}_{50})$ values ($\log=\text{base } 10$) of peptides for binding to TAP. The cutoff of IC_{50} at 1 nM was used to remove peptides with higher IC_{50} or lower TAP binding affinity⁴⁹.
6. **Alignment of peptide residues.** We align short peptides using the computational program Gibbs (<http://www.cbs.dtu.dk/services/GibbsCluster/>), which performs two essential tasks simultaneously, alignment and clustering⁵⁴. We used Gibbs to deconvolute binding motifs of SARS-CoV-2 peptide datasets. GibbsCluster simultaneously clusters and aligns peptide data as a powerful tool for unsupervised motif discovery. Results return optimal clusters with characterized sequence motifs for each cluster based on multiple parameters, including adjustable penalties for small clusters, adjustable penalties for overlapping groups, and a trash cluster to remove outliers. The results of cluster peptides are shown with the frequency of specific amino acids. The sequence motifs derived from the best solution are displayed in the form of sequence logos generated with Seq2Logo. Colors highlight different categories of amino acids, including acidic (red), basic (blue), uncharged (green), and other residues (black). The pre-identified anchor and preferred residues of previously defined peptide ligands for HLA class I proteins were obtained from

(<http://www.syfpeithi.de/bin/MHCServer.dll/FindYourMotif.htm>) to compare with the enriched clusters of predicted SARS-CoV-2 peptides.

7. **Venn diagram** was constructed using the website (http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.html) for four-way and five-way Venn diagrams. Sequentially identical or overlapped peptide sequences are defined as identical or overlapped peptides.
8. **Immunity simulation** was performed using C-ImmSim program (<http://150.146.2.1/C-IMMSIM/index.php?page=0>)⁶³. This prediction program implements a Celada-Seiden model as a logical description of the mechanisms making up humoral and cellular immune responses to a genetic antigen by incorporating known principal factors. These core factors include MHC restriction, clonal selection by antigen affinity, antigen presentation in cytosolic or endocytic pathways, cell-cell interactions, T cell differentiation, and T cell memory for T cell responses. Simultaneously, C-ImmSim considers the helper function of CD4⁺ T cell to antibody production and CD8⁺ T cell responses. C-ImmSim has been used to predict immune response in HIV infections, which have been validated with published clinical trials involving antiretroviral therapies⁷⁴. This study utilized C-ImmSim to predict whether peptide binding affinity, peptide numbers, and combined HLA class I alleles impacted CD8⁺ T cell response to SARS-CoV-2 peptides. Input factors included peptide sequences, HLA class I alleles, incorporation of a viral vaccination as an adjuvant for this study. Results are shown with the curve of CD8⁺ T cell proliferation and cytokine production to predict potential effector T cell responses and memory T cell differentiation stimulated by HLA class I-restricted peptides.

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Author Contributions

S.H. developed research workflow, analyzed data, and wrote the manuscript. M.T. provided inputs on viral vaccination and manuscript writing.

Figure legends:

Fig. 1. Dominant HLA class I proteins sampled various numbers of SARS-CoV-2 peptides.

Peptide ligands were predicted based on binding affinity to HLA class I proteins, digestion sites for proteasome enzymes, and binding affinity to TAP transporter. The numbers of predicted peptide ligands from immunogenic SARS-CoV-2 proteins are listed for ten dominant HLA class I proteins **(A)**. % of the numbers of predicted peptides over the amino acid numbers of different SARS-CoV-2 proteins are plotted to reflect the relative frequencies of HLA class I peptide ligands **(B)**.

Fig. 2. Dominant HLA class I proteins shared limited numbers of SARS-CoV-2 peptides.

Numbers of predicted SARS-CoV-2 peptide ligands are shown for HLA-A proteins **(A)** and HLA-B proteins **(B)**.

Fig. 3. Enriched residues in predicted SARS-CoV-2 peptide ligands. Anchor and preferred residues for each HLA class I proteins are compared with enriched residues from predicted SARS-CoV-2 peptide ligands. Peptide ligands from the spike and ORF1ab-encoded proteins are shown for HLA-A proteins **(A)** and HLA-B proteins **(B)**.

Fig. 4. HLA class I proteins with weak peptide binding capacity inclined to associate with severe infections.

Peptide ligands from immunogenic SARS-CoV-2 proteins were predicted for HLA class I proteins that were indicated relevant or irrelevant to the severity or protection of SARS. The numbers of predicted peptide ligands are listed for different dominant HLA class I proteins **(A)**. Venn diagrams show the numbers of predicted peptide ligands from the spike and ORF1ab-encoded proteins for HLA class I proteins **(B)**. White and yellow numbers are for overlaps by two and multiple HLA class I proteins, respectively.

Fig. 5. Overlapped peptides between SARS-CoV-2 and other coronaviruses.

A. Venn diagrams show the numbers of identical predicted peptides shared between SARS-CoV-2 and other coronaviruses for HLA class I proteins. Large numbers show the overlaps between SARS-CoV-2 and other coronaviruses (white). Numbers of identical peptides loaded to both HLA-A and HLA-B proteins are shown in yellow. **B.** % of identical peptides between SARS-CoV-2 and other coronaviruses are listed. Bold % is for overlaps between SARS-CoV-2 and HCoV-OC43.

Fig. 6. Heterozygous HLA class I genotypes with high affinity peptides enhance CD8⁺ T cell responses in immune simulation. Simulations of antigen presentation are compared with one and ten peptide ligands for A*02:01 protein **(A)**. Simulations of antigen presentation are compared between the high and low binding affinity of five peptide ligands for A*02:01 protein **(B)**. Simulations of antigen presentation are compared between homozygous **(i)** and heterozygous **(ii)** HLA-A proteins, homozygous **(iii)** and heterozygous **(iv)** HLA-B proteins, and with paralogous HLA-A and B proteins **(vi)** **(C)**.

Fig. 7. Heterozygous HLA class I genotypes with viral adjuvants rescue CD8⁺ T cell responses in immune simulation to weak peptide binders. Simulations of antigen presentation used B*46:01 only, mixed HLA-A and B*46:01, and heterozygous HLA-B alleles with B*46:01 **(A)**. Simulations of antigen presentation used viral adjuvant and peptide ligands for both HLA-A and B*46:01 proteins **(B)**. Simulations of antigen presentation used viral adjuvant and peptide ligands for heterozygous HLA-B proteins with B*46:01 **(C)**.

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Fig. 1

A. Numbers of predicted SARS-CoV-2 peptide ligands for major HLA class I proteins

SARS-CoV-2 proteins (# residues)	S (1273)	M (222)	N (419)	ORF1ab (6954)	ORF3a (275)	ORF8 (119)
HLA-A*01:01	26	6	4	137	10	2
HLA-A*02:01	91	41	8	656	48	8
HLA-A*03:01	57	9	13	409	14	1
HLA-A*11:01	107	23	19	616	22	1
HLA-A*24:02	53	20	3	286	16	4
HLA-B*07:02	17	6	18	158	6	4
HLA-B*08:01	34	9	8	171	9	5
HLA-B*35:01	67	14	13	334	14	3
HLA-B*40:01	19	5	1	170	5	2
HLA-B*44:03	16	4	3	119	9	0

B. % numbers of predicted peptide ligands for HLA class I proteins over the numbers of amino acid sequence for SARS-CoV-2 proteins

Fig. 2

A. Overlaps of predicted SARS-CoV-2 peptide ligands for HLA-A proteins

B. Overlaps of predicted SARS-CoV-2 peptide ligands for HLA-B proteins

Fig. 3

A. Enriched residues in predicted SARS-CoV-2 peptide ligands for HLA-A proteins

B. Enriched residues in predicted SARS-CoV-2 peptide ligands for HLA-B proteins

Fig. 4

A. Numbers of predicted SARS-CoV-2 peptide ligands for HLA class I proteins based on proteasome digestion, TAP transportation, and peptide binding affinity.

SARS-CoV-2 proteins	S (1273)	M (222)	N (419)	ORF1ab (6954)	ORF3a (275)	ORF8 (119)
HLA-A*02:01	91	41	8	656	48	8
HLA-A*02:02	173	55	12	1104	72	10
HLA-A*11:01	107	23	19	616	22	1
HLA-A*25:01	4	0	1	28	3	0
HLA-B*07:02	17	6	18	158	6	4
HLA-B*07:03	18	7	11	171	5	1
HLA-B*15:01	125	31	19	741	26	11
HLA-B*15:02	65	19	11	393	13	7
HLA-B*15:03	274	47	44	1538	64	29
HLA-B*46:01	0	1	0	2	0	0

B. % numbers of predicted peptide ligands for HLA-A proteins over the numbers of amino acid residues from SARS-CoV-2 proteins

Fig. 5

A. Venn diagrams of overlapped SARS-CoV-2 peptide ligands for major HLA class I A and B proteins

B. % numbers of overlapped peptide ligands predicted from SARS-CoV-2 and other coronavirus proteins for major HLA class I A and B proteins

Ligand binding with	Coronaviruses to compare with SARS-CoV-2	% overlapped SARS-CoV-2 peptides			
		S	N	ORF1a	ORF1b
HLA-A*01:01, A*02:01, A*03:01, A*11:01, and A*24:02	SARS	25.00	58.33	23.03	69.75
	HCoV-OC43	0	0	0.09	4.78
HLA-B*07:02, B*08:01, B*35:01, B*40:01, and B*44:03	SARS	27.14	61.24	25.19	73.58
	HCoV-OC43	0	0	0	5.03

Fig. 6

A. Immune simulation using different numbers of predicted peptide ligands for HLA-A*02:01

B. Immune simulation using peptides with different binding affinity to HLA-A*02:01

C. Immune simulation of antigen presentation mediated by heterozygous and paralogous major HLA class I A and B proteins with predicted peptide ligands from the spike protein as an example

Fig. 7**A. Immune simulation of antigen presentation mediated by a weak peptide binder and a heterozygous or paralogous HLA class I protein.** **B. Immune simulation of antigen presentation with virus adjuvant and heterozygous HLA class I proteins** **C. Immune simulation of antigen presentation with virus adjuvant and paralogous HLA class I proteins**

Supplementary Information

HLA class I genotypes customize vaccination strategies in immune simulation to combat COVID-19

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Keywords: Binding affinity, human leukocyte antigen class I, CD8⁺ T cells, coronavirus disease 2019 (COVID-19), custom vaccination

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Fig. S1

A. Numbers of predicted SARS-CoV-2 peptides for major HLA class I proteins based on peptide binding affinity.

SARS-CoV-2 proteins	S (1273)	M (222)	N (419)	ORF1ab (6954)	ORF3a (275)	ORF8 (119)
HLA-A*01:01	28	6	4	146	13	2
HLA-A*02:01	102	59	8	818	53	13
HLA-A*03:01	63	10	18	481	16	1
HLA-A*11:01	114	25	25	716	24	1
HLA-A*24:02	54	22	3	288	16	4
HLA-B*07:02	28	7	20	189	7	4
HLA-B*08:01	36	11	8	194	11	5
HLA-B*35:01	74	15	13	363	16	3
HLA-B*40:01	19	5	1	186	5	2
HLA-B*44:03	17	4	3	129	9	0

B. Alignment of selected major HLA-A and HLA-B alleles in US populations

HLA-A0101	1	GS	SH	SM	RY	FF	SV	SR	PG	RGE	P	RF	IA	VG	Y	V	D	TQ	FV	R	F	D	S	DA	AS	Q	K	M	E	P	R	A	P	W	I	E	Q	E	G	P	E	Y	D	Q	E	T	R	N	M	K	A	H	S	Q	T	D	R	A	N	L	G	T	L	R	G	Y	Y	N	Q	S	E	D	90																					
HLA-A0301		GS	SH	SM	RY	FF	SV	SR	PG	RGE	P	RF	IA	VG	Y	V	D	TQ	FV	R	F	D	S	DA	AS	Q	R	M	E	P	R	A	P	W	I	E	Q	E	G	P	E	Y	D	Q	E	T	R	N	V	K	A	Q	S	Q	T	D	R	V	D	L	G	T	L	R	G	Y	Y	N	Q	S	E	D																						
HLA-A1101		GS	SH	SM	RY	FF	SV	SR	PG	RGE	P	RF	IA	VG	Y	V	D	TQ	FV	R	F	D	S	DA	AS	Q	R	M	E	P	R	A	P	W	I	E	Q	E	G	P	E	Y	D	Q	E	T	R	N	V	K	A	Q	S	Q	T	D	R	V	D	L	G	T	L	R	G	Y	Y	N	Q	S	E	D																						
HLA-A0201		GS	SH	SM	RY	FF	SV	SR	PG	RGE	P	RF	IA	VG	Y	V	D	TQ	FV	R	F	D	S	DA	AS	Q	R	M	E	P	R	A	P	W	I	E	Q	E	G	P	E	Y	D	G	E	T	R	K	V	K	A	H	S	Q	T	H	R	V	D	L	G	T	L	R	G	Y	Y	N	Q	S	E	A																						
HLA-A2402		GS	SH	SM	RY	FF	SV	SR	PG	RGE	P	RF	IA	VG	Y	V	D	TQ	FV	R	F	D	S	DA	AS	Q	R	M	E	P	R	A	P	W	I	E	Q	E	G	P	E	Y	D	D	E	E	T	G	K	V	K	A	H	S	Q	T	D	R	E	N	L	R	I	A	L	R	Y	Y	N	Q	S	E	A																					
Consensus		GS	SH	SM	RY	FF	SV	SR	PG	RGE	P	RF	IA	VG	Y	V	D	TQ	FV	R	F	D	S	DA	AS	Q	R	M	E	P	R	A	P	W	I	E	Q	E	G	P	E	Y	D	D	E	T	R	K	V	K	A	H	S	Q	T	D	R	V	#	L	G	T	L	R	G	Y	Y	N	Q	S	E	A																						
HLA-A0101	91	GS	SH	T	I	Q	I	M	Y	G		CD	V	G	P	D	G	R	F	L		RG	Y	R	Q	D	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	M	A		Q	I	T	K	R	K	W	E	A	V	H	A	E	Q	R	R	V	Y	L		EG	R	C	V	D	G	L	R		Y	L	E	N	G	K	E	T	L	Q	180					
HLA-A0301		GS	SH	T	I	Q	I	M	Y	G		CD	V	G	P	D	G	R	F	L		RG	Y	R	Q	D	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	M	A		Q	I	T	K	R	K	W	E	A		H	A	E	Q	L	R	A	Y	L		D	G	T	C	V	E	W	L	R		Y	L	E	N	G	K	E	T	L	Q					
HLA-A1101		GS	SH	T	I	Q	I	M	Y	G		CD	V	G	P	D	G	R	F	L		RG	Y	R	Q	D	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	M	A		Q	I	T	K	R	K	W	E	A		H	A	E	Q	R	A	Y	L		E	G	R	C	V	E	W	L	R		Y	L	E	N	G	K	E	T	L	Q						
HLA-A0201		GS	SH	T	V	Q	R	M	Y	G		CD	V	G	P	D	G	R	F	L		RG	Y	H	Q	Y	A	Y	D	G		KD	Y	I	A	L	K	E	D	L		RS	WT	A	A	D	M	A		Q	I	T	K	R	K	W	E	A		H	V	A	E	Q	L	R	A	Y	L		E	G	T	C	V	E	W	L	R		Y	L	E	N	G	K	E	T	L	Q				
HLA-A2402		GS	SH	T	L	Q	M	M	F	G		CD	V	G	P	D	G	R	F	L		RG	Y	H	Q	Y	A	Y	D	G		KD	Y	I	A	L	K	E	D	L		RS	WT	A	A	D	M	A		Q	I	T	K	R	K	W	E	A		H	V	A	E	Q	R	A	Y	L		E	G	T	C	V	D	G	L	R		Y	L	E	N	G	K	E	T	L	Q					
Consensus		GS	SH	T	.	Q	.	M	%	G		CD	V	G	P	D	G	R	F	L		RG	Y	h	Q	Y	A	Y	D	G		KD	Y	I	A	L	k	E	D	L		RS	WT	A	A	D	M	A		Q	i	T	k	R	K	W	E	A		H	v	A	E	Q	.	R	A	Y	L		#	G	t	C	V	#	w	L	R		Y	L	E	N	G	K	E	T	L	Q				
HLA-B0702	1	GS	SH	SM	RY	F	Y	T		SV	SR	P	G	R	G	E	P		RF	I	S	V	G	Y	V	D		TQ	F	V	R	F	D	S	D	A		AS	P	R	E	E	P	R	A	P		W	I	E	Q	E	G	P	E	Y		D	R	N	T	Q	I	Y	K	A	Q		A	Q	T	D	R	E	S	L	R	N		L	R	G	Y	Y	N	Q	S	E	A	90				
HLA-B0801		GS	SH	SM	RY	F	D	T		A	M	S	R	P	G	R	G	E	P		RF	I	S	V	G	Y	V	D		TQ	F	V	R	F	D	S	D	A		AS	P	R	E	E	P	R	A	P		W	I	E	Q	E	G	P	E	Y		D	R	N	T	Q	I	F	K	T	N		T	Q	T	D	R	E	S	L	R	N		L	R	G	Y	Y	N	Q	S	E	A			
HLA-B3501		GS	SH	SM	RY	F	Y	T		A	M	S	R	P	G	R	G	E	P		RF	I	A	V	G	Y	V	D		TQ	F	V	R	F	D	S	D	A		AS	P	R	T	E	P	R	A	P		W	I	E	Q	E	G	P	E	Y		D	R	N	T	Q	I	F	K	T	N		T	Q	T	Y	R	E	S	L	R	N		L	R	G	Y	Y	N	Q	S	E	A			
HLA-B4001		GS	SH	SM	RY	F	H	T		A	M	S	R	P	G	R	G	E	P		RF	I	T	V	G	Y	V	D		T	L	F	V	R	F	D	S	D	A		T	S	P	R	K	E	P	R	A	P		W	I	E	Q	E	G	P	E	Y		D	R	E	T	Q	I	S	K	T	N		T	Q	T	Y	R	E	S	L	R	N		L	R	G	Y	Y	N	Q	S	E	A	
HLA-B4403		GS	SH	SM	RY	F	Y	T		A	M	S	R	P	G	R	G	E	P		RF	I	T	V	G	Y	V	D		T	L	F	V	R	F	D	S	D	A		T	S	P	R	K	E	P	R	A	P		W	I	E	Q	E	G	P	E	Y		D	R	E	T	Q	I	S	K	T	N		T	Q	T	Y	R	E	N	L	R	T		A	L	R	Y	Y	N	Q	S	E	A	
Consensus		GS	SH	SM	RY	F	Y	T		a	m	S	R	P	G	R	G	E	P		RF	I	.	V	G	Y	V	D		T	q	f	v	r	f	d	s	d	a		a	s	p	r	.	e	p	r	a	p		w	i	e	q	e	g	p	e	y		d	r	#	t	q	i	.	k	t	#		t	q	t	y	r	e	s	l	r	n		l	r	g	y	y	n	q	s	e	a	
HLA-B0702	91	GS	SH	T	L	Q	S	M	Y	G		CD	V	G	P	D	G	R	L		RG	H	D	Q	Y	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	T	A		Q	I	T	Q	R	K	W	E	A		R	E	A	E	Q	R	R	A	Y	L		E	G	E	C	V	E	W	L	R		Y	L	E	N	G	K	D	K	L	E	180				
HLA-B0801		GS	SH	T	L	Q	S	M	Y	G		CD	V	G	P	D	G	R	L		RG	H	N	Q	Y	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	T	A		Q	I	T	Q	R	K	W	E	A		R	V	A	E	Q	D	R	A	Y	L		E	G	T	C	V	E	W	L	R		Y	L	E	N	G	K	D	T	L	E					
HLA-B3501		GS	SH	T	I	Q	R	M	Y	G		CD	L	G	P	D	G	R	L		RG	H	D	Q	S	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	T	A		Q	I	T	Q	R	K	W	E	A		R	V	A	E	Q	L	R	A	Y	L		E	G	L	C	V	E	W	L	R		Y	L	E	N	G	K	E	T	L	Q					
HLA-B4001		GS	SH	T	L	Q	R	M	Y	G		CD	V	G	P	D	G	R	L		RG	H	N	Q	Y	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	T	A		Q	I	S	Q	R	K	L	E	A		R	V	A	E	Q	L	R	A	Y	L		E	G	E	C	V	E	W	L	R		Y	L	E	N	G	K	D	K	L	E					
HLA-B4403		GS	SH	T	I	Q	R	M	Y	G		CD	V	G	P	D	G	R	L		RG	Y	D	Q	Y	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		RS	WT	A	A	D	T	A		Q	I	T	Q	R	K	W	E	A		R	V	A	E	Q	L	R	A	Y	L		E	G	L	C	V	E	S	L	R		Y	L	E	N	G	K	E	T	L	Q					
Consensus		GS	SH	T	L	Q	r	M	Y	G		CD	V	G	P	D	G	R	L		RG	h	#	Q	Y	A	Y	D	G		KD	Y	I	A	L	N	E	D	L		r	S	WT	A	A	D	T	A		Q	i	t	Q	R	K	W	E	A		R	v	A	E	Q	L	R	A	Y	L		E	G	.	C	V	E	W	L	R		Y	L	E	N	G	K	#	t	L	#				

C. Sequence similarity of $\alpha 1$ and $\alpha 2$ domains of selected major HLA-A and HLA-B alleles in US populations

	A*01:01	A*02:01	A*03:01	A*11:01	A*24:02	B*07:02	B*08:01	B*35:01	B*40:01	B*44:03
A*01:01	100	86.81	91.76	93.96	86.26	80.22	78.57	78.57	75.82	78.57
A*02:01		100	92.31	90.11	89.01	80.22	80.22	80.22	78.57	78.57
A*03:01			100	96.15	87.36	82.97	81.32	81.32	78.57	80.22
A*11:01				100	86.81	83.52	81.32	81.87	78.57	80.77
A*24:02					100	80.77	80.77	78.57	77.47	80.77
B*07:02						100	93.41	88.46	89.56	83.52
B*08:01							100	91.76	92.31	86.26
B*35:01								100	89.56	91.76
B*40:01									100	89.56
B*44:03										100

Fig. S2

A. Alignment of selected major HLA-A and HLA-B alleles potentially associated with SARS

	HLA-A0201	GSHSMRYFFFT	SVSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASQRMEPRAP	WIEQEGPEYW	DGETRKVKAH	SQTHRVDLTGT	LRGYYNQSEA	90
	HLA-A0202	GSHSMRYFFFT	SVSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASRRMEPRAP	WIEQEGPEYW	DGETRKVKAH	SQTHRVDLTGT	LRGYYNQSEA	
	HLA-A1101	GSHSMRYFYT	SVSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASQRMEPRAP	WIEQEGPEYW	DQETRNVAQA	SQTDRVDLTGT	LRGYYNQSED	
	HLA-A2501	GSHSMRYFST	SVSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASQRMEPRAP	WIEQEGPEYW	DEETGVKKAH	SQTDRENLR	ALRYYNQSEA	
	HLA-B0702	GSHSMRYFYT	SVSRPGRGEP	RFI	SVGYVDD	TQFVRFDSDA	ASPREPRAP	WIEQEGPEYW	DRNTQIYKAQ	AQTDRESLNR	LRGYYNQSEA	
	HLA-B0703	GSHSMRYFYT	SVSRPGRGEP	RFI	SVGYVDD	TQFVRFDSDA	ASPREPRAP	WIEQEGPEYW	DRNTQIYKTN	TQTDRESLNR	LRGYYNQSEA	
	HLA-B1501	GSHSMRYFYT	AMSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASPRMAPRAP	WIEQEGPEYW	DRETQISKTN	TQTYRESLNR	LRGYYNQSEA	
	HLA-B1503	GSHSMRYFYT	AMSRPGRGEP	RFI	SVGYVDD	TQFVRFDSDA	ASPREPRAP	WIEQEGPEYW	DRETQISKTN	TQTYRESLNR	LRGYYNQSEA	
	HLA-B1502	GSHSMRYFYT	AMSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASPRMAPRAP	WIEQEGPEYW	DRNTQISKTN	TQTYRESLNR	LRGYYNQSEA	
	HLA-B4601	GSHSMRYFYT	AMSRPGRGEP	RFI	AVGYVDD	TQFVRFDSDA	ASPRMAPRAP	WIEQEGPEYW	DRETQKYKRQ	AQTDREVSLNR	LRGYYNQSEA	
	Consensus	GSHSMRYFYt	svSRPGRGEP	RFI	avgyvdd	TQFVRFDSDA	ASprmePRAP	WIEQEGPEYW	Dr#Tq..Ka.	.QTDResLrn	lrgYYNQSea	
	HLA-A0201	GSHTVQRMYG	CDVGSDGRFL	RGYHQYAYDG	KDYIALKEDL	RSWTAADMAA	QTTKHKEAA	HVAEQQLRAYL	EGTCVEWLRR	YLENGKETLQ	180	
	HLA-A0202	GSHTLQRMYG	CDVGSDDRFL	RGYHQYAYDG	KDYIALKEDL	RSWTAADMAA	QTTKHKEAA	HVAEQWRAYL	EGTCVEWLRR	YLENGKETLQ		
	HLA-A1101	GSHTIQMIG	CDVGDGGRLL	RGYRQDAYDG	KDYIALNEDL	RSWTAADMAA	QITKKWEAA	HAEQQRAYL	EGRCVEWLRR	YLENGKETLQ		
	HLA-A2501	GSHTLQMMFG	CDVGSDDRFL	RGYHQYAYDG	KDYIALKEDL	RSWTAADMAA	QITKKWEAA	HVAEQQRAYL	EGTCVDGLRR	YLENGKETLQ		
	HLA-B0702	GSHTLQSMYG	CDVGPDGRLL	RGHQYAYDG	KDYIALNEDL	RSWTAADTAA	QITQRKEAA	REAEQRRAYL	EGECVEWLRR	YLENGKD KLE		
	HLA-B0703	GSHTLQSMYG	CDVGPDGRLL	RGHQYAYDG	KDYIALNEDL	RSWTAADTAA	QITQRKEAA	REAEQRRAYL	EGECVEWLRR	YLENGKD KLE		
	HLA-B1501	GSHTLQRMYG	CDVGPDGRLL	RGHQDSAYDG	KDYIALNEDL	SSWTAADTAA	QITQRKEAA	REAEQWRAYL	EGLCVEWLRR	YLENGKETLQ		
	HLA-B1503	GSHTLQRMYG	CDVGPDGRLL	RGHQDSAYDG	KDYIALNEDL	SSWTAADTAA	QITQRKEAA	REAEQLRAYL	EGLCVEWLRR	YLENGKETLQ		
	HLA-B1502	GSHITIORMYG	CDVGPDGRLL	RGYDQSAYDG	KDYIALNEDL	SSWTAADTAA	QITQRKEAA	REAEQLRAYL	EGLCVEWLRR	YLENGKETLQ		
	HLA-B4601	GSHTLQRMYG	CDVGPDGRLL	RGHQDSAYDG	KDYIALNEDL	SSWTAADTAA	QITQRKEAA	REAEQWRAYL	EGLCVEWLRR	YLENGKETLQ		
	Consensus	GSHTLQrM%G	CDVGPdGrLL	RgYdOvAYDG	KDYIALnEDL	rSWTAADtaa	Qitqrkweaa	reABE..RAYL	EG.CV#wLRR	YLENGK#tL#		

B. Sequence similarity of $\alpha 1$ and $\alpha 2$ domains of selected HLA-A and HLA-B alleles associated with SARS

[illegible]

Fig. S2. Alignment and sequence identity of HLA class I proteins relevant or irrelevant to SARS infections. **A.** alignment of $\alpha 1$ and $\alpha 2$ domains for HLA class I proteins that were indicated relevant or irrelevant to the severity or protection of SARS. Residues are colored in grey (identical residues), red (highly diverse), and blue (less diverse). **B.** % identical residues are listed.

	Spike	SARS-CoV-2	SARS	HCoV-OC43
SARS-CoV-2		100	77.54	33.06
SARS			100	33.17
HCoV-OC43				100

SARS-CoV-2	1	MFVFLVLLPL	VSSQCVNLT	RTQ--LPPAY	TN--SFTRGV	YYPDKVFRSS	VLHSTQDLFL	PFFSNVTFWH	AHVSNGTNGT	KRFDNFVLFP	NDGVYFASTE	KSNIIRGWIF	GTTLDKSQTQS	LLIIVNATNV	VIKVCEQFQC	NDPFFGVYH	150
SARS		MFIFLFLFTL	TSGSDLRDCT	TFDDVQAPNY	TQHTSSMRGV	YYPDEIFRSD	TLYLTDQDLFL	PFFSNVTFGH	TINHT-----	--FDNPVIFP	KDGIYFAATE	KSNVVRGWVF	GSTMNNKSQS	VIIINNSTNV	VIRACNFELC	DNPPFAV---	
Consensus		MFIFLlLlPL	tSgqdlrct	rF#..qaPaY	T#...SfmRGV	YYPDEIFRSD	tLhLTQDLFL	PF#SNVTFGH	aInhs.....	..FDNPVIFP	nDG!YFAaTe	KSN!IRGW!F	GsTs#nKsQS	lii!INnATNV	V!raC#F#lC	#PFflav...	
SARS-CoV-2	151	KNKNSWMESE	FRVYSANNC	TFFEYVSQPL	MDLEGKQGNF	KNLREFVFKN	IDGVIKFIYSK	HTPINLVRDL	PQGfSALEPL	VDLPIGINIT	RFQTLALHR	SYLTPGDSSS	GWTAGAAYY	VGYLQPRTF	LKYNENGIT	DAVDCALDPL	300
SARS		--SKPMGTQTH	TMIIDFNAFC	TFFEYISDAFS	LDVSEKSGNF	KHLREFVFKN	KDGLFLVYKG	YQPIIDVVRDL	PSGfLNTKPI	FKLPLGINIT	NFRAILT---	AFLPAQDT--	-WGSAAAYF	VGYLKPTTFM	LKYNENGIT	DAVDCSQNPL	
Consensus		.nnkmgm#se	fr!#dnAnNC	TFFEYIS#aFl	sDleeKqGNF	KNLREFVFKN	iDGs!k!Ykg	hgP!iLVRDL	PqGfna!ePi	fDLPIGINIT	rFraila...	a#lPaqDs..	.WgagAAYF	VGYLqPrTFs	LKY#ENGIT	DAVDCaq#Pl	
SARS-CoV-2	301	SETKCTLKSF	TVEKGIQYTS	NFRVQPTESI	VRFPNITNLC	PFGEVFNATR	FASVIAMNRK	RISNCVADYS	VLYNSAFST	FKCYGVSPTK	LNDLCFTNVY	ADSFVIRGDE	VRQIAPGQTG	KIADYNYKLP	DDFTCCGVIAW	NSNNLDSKVG	450
SARS		AELKCSVKFS	EIDKGIQYTS	NFRVVPsRDV	VRFPNITNLC	PFGEVFNATR	FPSVYAWERK	RISNCVADYS	VLYNSTFFST	FKCYGVSATK	LNDLCFSNVY	ADSFVVKGDD	VRQIAPGQTG	VIADYNYKLP	DDFMGCVLAW	NTRNIDATST	
Consensus		aELKc!KsF	e!#KGIQYTS	NFRVqPsrd!	VRFPNITNLC	PFGEVFNATR	FASVYAW#K	RISNCVADYS	VLYNSaFfST	FKCYGVSaTK	LNDLCFSNVY	ADSFV!IRgD#	VRQIaPGQTG	kIaDYNYKLP	DDFmGcV!aW	NsRnIdaksg	
SARS-CoV-2	451	GNMYNYLYRLF	RKSNLKPFFR	DISTEIIYQAG	STPCNGVEGF	NCYFPLQSYG	FQPTNGVGYQ	PYRVVLSFE	LLHAPATVCG	PKKSTNLVKN	KCVNFNFNGL	TGTGVLTESN	KKFLPFPQQG	RDIADTTDAV	RDPQTEILEID	ITPCSFGGVS	600
SARS		GNMYNYKYRYL	RHGKLRPFER	DISNVPPSDP	GKPCPT-PAL	KCYFWPLNDYG	FYTTTGIIGYQ	PYRVVLSYE	LINAPATVCG	PKLSTDLIKN	QCYNFNFNGL	TGTGVLTPSS	KRFQPFQQFG	RDVSDFTDSV	RDPKTSIEILD	ISPCSFGGVS	
Consensus		GNMYNYLYRl	RhgnLRPFER	Disnei!qad	Gkpcptcng.eal	NCYfPl#dYg	FqPtnG!lGYQ	PYRVVLSFE	LlNAPATVCG	PKLST#lIKN	QCYNFNFNGL	TGTGVLTPSS	KRFQPFQQFG	RdV!aDfTdaV	RDPqT!eIlEID	ISpCSfGGVS	
SARS-CoV-2	601	VITPGTNTSS	QVAVLYQDVN	CTEVPVAIHA	DQLTPTWRYI	STGSNVFOQR	AGCLIGAEHV	NNSYECIDIPI	GAGICASYQT	QTS-----LLRS	VASQSIIAYT	MSLGAENSVIA	YSNNISIAIPT	NFTISITTEI	LPVSMTKTSV	DCTMYICGDS	750
SARS		VITPGTNNASe	EVAVLYQDVN	CTDVPtAIHA	DQLTPAWRIY	STGNNVFOQR	AGCLIGAEHV	AGCLIGAEHV	GAGICASYQT	VNSPRRRARS	VASQSIIAYT	MSLGAENSVIA	YSNNITAIPT	NFSISVITTE	MPVSMAKTSV	DCNMYICGDS	
Consensus		VITPGTNaSn	#VAVLYQDVN	Ct#VpTaIHA	DQLTPAwR!Y	STGnNVFOQR	AGCLIGAEHV	#nSYECIDIPI	GAGICASyQt	qs....rars	tagqSI!AYT	MSLGA#nS!A	YSNNsIAIPT	NfsIS!tTE!l	\$PVSMaKtSV	DcNmYICGDS	
SARS-CoV-2	751	TECSNLLLYQ	GSFCTQLNRA	LTGIAVEQDK	NTOEVEFAQVK	QIYKTPPIKD	FGGFNSQIL	PDPSKPSKRS	FIEDLLFNKV	TLADAGFIKQ	YGDCGLGDIAA	RDLICAQKFN	GLTVLPLLT	DEMIAQYSTA	LLAGTITSGW	TFGAGAAQLI	900
SARS		TECANLLLYQ	GSFCTQLNRA	LSGIAAEQDR	NTRVEFAQVK	QMYKTPTLKD	FGGFNSQIL	PDPLKSTKRS	FIEDLLFNKV	TLADAGFMKQ	YGECGLGDINA	RDLICAQKFN	GLTVLPLLT	DMTAAAYTAA	LVSGTATAGW	TFGAGAAQLI	
Consensus		TEcANLLLYQ	GSFCTQLnRA	LsG!aAeQDr	NtrEvFAQVK	Q!YKtPp!kD	FGGFNSQIL	PdPlKpsKRS	FIEDLLFNKV	TLADAGFIkQ	Yg#CLGD!aA	RDLICAQKFN	GLTVLPLLT	D#MTaAYTaA	LlaGtaTaGw	TfGAGaaLQI	
SARS-CoV-2	901	PFAMQMAyRF	NGIGVTTQNVL	YENQKLIANQ	FNSAIGKIQIE	SLSTASALG	KLQDVVNQNA	QALNTLVKQL	SSNFGAISSV	LNDILSRDLK	VEAEVQIDRL	ITGRLSQSLT	VYTQQLIRAA	EIRASANLAA	TKMSECVLQG	SKRVDFCGKG	1050
SARS		PFAMQMAyRF	NGIGVTTQNVL	YENQKLIANQ	FNSAIGKIQIE	SLSTASALG	KLQDVVNQNA	QALNTLVKQL	SSNFGAISSV	LNDILSRDLK	VEAEVQIDRL	ITGRLSQSLT	VYTQQLIRAA	EIRASANLAA	TKMSECVLQG	SKRVDFCGKG	
Consensus		PFAMQMAyRF	NGIGVTTQNVl	YENqKqIaNQ	FnKa!Gq!Q!E	SLs!sTasALG	KLQDVVNQNA	QALNTLVKQL	SSNFGAISSV	LNDILSRDLK	VEAEVQIDRL	ITGRLSQSLT	VYTQQLIRAA	EIRASANLAA	TKMSECVLQg	SKRVDFCGKG	
SARS-CoV-2	1051	YHLMSFPQSA	PHGVVFLHVT	YVFAQEKNFT	TAPAICHGKG	AHPFREGVVF	SNGTHWFVTQ	RNFYEPIIT	TDNTFVSGNC	DVVGIVNNT	VYDPLQPELD	SFKEELDKYF	KNHTSPDVDL	GDISGINASV	VNIQKEIDRL	NEVAKNLNES	1200
SARS		YHLMSFPQAA	PHGVVFLHVT	YVFSQERNFT	TAPAICHEGK	AHPFREGVVF	FNGTSWFITQ	RNFESPIIT									

1
SARS-CoV-2 MFVFLVLLPL VSSQCNNLT RTQLPPAYTN SFTRGV--YY PDKVFRSSVL HSTQDLFLFP FSNVTWFHAI HVSNGTNGTKR FDNVPLPFND GVYFASTEKS NIIRGWIFGT TLDSTKQSLI IVNNANNVVKI KVCEFCQFND PFLGVYYHKN
HCoV-OC43 MFLIL-LiSL PMAFAVIGLD KCTSDTSYIN DVTGVPPPIIS TDTVDVTNGL GTYYVLDRVY LNTTLFLNGY YPTSGSTYRN MALKGSVLLS TLMFKPPFLS DFING-IFAK VKNTKVIKND VLYSEFPAIT IGSTFVNTSY SVVQPTIN
Consensus MFLiL.LiPl pmaqaVngld rcqlqdaYin dfdrGV..is pDKVdrsnGL gstdqldirp# lnttlflnai hpsggngtrr mangklplnd glwFappels #iIrG.IFaK tI#sKtqkdd !lnnafna! igceFqncnd pflgqprhIn

151
SARS-CoV-2 NKSWMSESEFR VYSSANNCTF EYVSOPFLMD LEGKQGNFKM LREFVFKNID GYFKIYSKHT PINLVRLDLPQ GFSALEPLVD LPIGINITRF QTL-LALHRS YLTPEGSSSG WTAG-AAAYV VGYLQPRTEL LKYNENCTIT DAVDCALDPL
HCoV-OC43 LDNKLQGLLE ISVCQYNMC- EYPHTICHNP LBNHRTHELM LDTGVVSLC KRNFTYDNA DYLFHFYQJ GGTFFAYFDT TGVVTKFLFN YTLGMAISHY YVMPLTCNSK RKDGFTLEAY VTPLTSRQYL LAFNQDQJL NAVDCMSDF
Consensus ndnkS#gclr !sscanNCC- E!phqichmL Lenhrg#lkn PRFVgFknid grnkiYdnk dnifdrfIQ# GgsalaptD lg!ginilrn qtl.\$ALHrs YlmPgdcnS rkaG.aaaYw VgplqrRq#L LaN#G#GiIF #AVDCALDfS

301
SARS-CoV-2 SETKTKLSF TVEKGIIQTS NFRVQPTESI VRFPNITNLC PEGEVFNATR FASVYAWNRR RISNCVADYS VLYNSASFST FKCYGVSPATK LNDLCFTNPK LADSFVIRGE VRQIAPGGQT KIADYKPNRL DDFTFQCVIAW NSNNLDSKVG
HCoV-OC43 SEIKCKTKSI APPTGYVELN GTYVQPTADV YRRKPNLPNC NIEAMLNDKS VSPFLNWERK TFSNCNFMS SLMSFIQADS FTFCNNDIAK IYGMCFSSIT ADKFAIPNGR KVDLQGNLNG YLQSFNYNRL TATFSQCLVY LPLAANVSUS
Consensus SEiKCKlqSi apeKGiY#ln n#rVQPTiad! yRrknlnnC nieawlNakr faSplaW#RK riSNCna#mS Slmfaqads FKcnn!daaK ind\$CFsn! adKFaIrndr kr#ialGlG kiad#Nyrid ddaTFQciaw Nlnaa#skVg

451
SARS-CoV-2 ----GNYNLYL YRLFRKSNLK PFERDISTEI YQAGSTPCNG VEGFNICYFPL QSY-----GF QPTNGVGYQP YRVVLSFEL L--HAPATVC GP----- LVGIGEHCSG LAVKSZYCGG NPCTCQQAQ LGWSADSCIQ GDKCNIFANL
HCoV-OC43 RFNPSTWNKR FGFIEDSVFK PQPAGVFTNH DVVYAQHCFK APKNFCPCCKL NGSLCVSGSP GKNNNGITGCP AGTNYLTCHN LCNPDPTITF GPYKCPQTKS LPVIGEHCSG LAVKSZYCGG NPCTCQQAQ LGWSADSCIQ GDKCNIFANL
ConsensusgnwNkr #e!lridSnIk Pqead!fT#h dqagagCng aeegnCpckL #gs.....GF qknNGlGtst artnyLacen L..haPaTVC GP.....

601
SARS-CoV-2 -----KKST NLVKNKCVMF NFNGLTGTGV LTESNKKFL- PFQQFGRDIA DTTDAVRDPQ TLEILDITPC SFGGVSVITP GTNTSNQVAV LYQDVNCTEV PVAIHADQLT PTWRVYSTGS NVFQTRAGLC IGAEHVN--N
HCoV-OC43 ILHDVNSGLT CSTDLQKANT DIILGVCVNY DLYGSGQGI FVEVNAVYYN SQWNLIDYSN GNLYGRDRI TNRFTMIIRC YSGRVSAAFH AN--SSEPAL LFRNKICNVY FNNSLTRQLQ PI----- NYFDSYLGCV VNAVNSSTAIS
Consensus KanT #i!lnkCVMF #lngISGqG! ltEsNak!l. pfQ#lgrDia dnldafRdpQ TnrildirpC sfGrVSaaah an..Sn#PaI L#r#!nCneV fnaiharQLQ Pi..... NyF#sraGCl !naensn..n

751
SARS-CoV-2 SYECIDIPICA GICASYOTPI TeNPRRARS-V ASQSIAYTWM -SLGAENSVA YSNNSIATPT NFTTSVYTTI LPVSMTKTSV DCTMYICGDS TECSNLLQY GSFCOTLNRH LTGIAVEQDK QTKVSVFAQK Q-IYKTPPIK DFGGNFNSQI
HCoV-OC43 VQTCIDLTVGS GYCVDSKINR RSRRAITTGY RFTNFPEFTV NSVNDSELPV GGLYEIQIPS EFTIGNMEEF IQTSSPKVTI DCAAEVCCGY AACKSQLVEY GSFCNDINAI LTEVNEBLDT TQLQVANSIM NGVTLSTKIK DGVNENVDI
Consensus sqcDEip!Ga GiCadYqk#r rSrRaars.y afqnlea#Tm .s!naenepa ggneia!PS #FTIgmneBi iqtSmpKts! Dcaa#!CGDS aaCknqL!Y GSFCd#iNaA Lte!aeegDk nqq#Vaaqk #.!.tlspkik DggnFnfd#I

901
SARS-CoV-2 LPDP----- ---SKPSKRS FIEDLLFNKV TLADAGFIQY YGDCLGEDIAA RDLICAQKFN GLTVLPPLLT DEMIAQYTTA LLAGIITSGW TFGAGAAQI PFAMQMAHYR NGIGVTONVL YENQKLIANQ FNSAIGKIQD SLSSYASIGH
HCoV-OC43 NFSFVLGCLG SECSKASRS AIEDLLFPKV KLSDBGVFEA YNMCYTGAEI LKIDLVQSYK GIKVLPPLLS ENHISGLTTA ATSAASLPWW TAAAG---V PFYLVNQYRI NGLGVMTQVL SQNQKLIANA FNNALDAIQG GFDAITNSALV
Consensus nfdS.....SKaSKRS AIEDLLF#KV YnClGdaaE RDLICAQ#N RDLICLPPLLS TAAAG....L PFa\$maYRI NGIGVt#QVL S#NQKLIANA FNnaldaiQ# gldatSaIqL

1051
SARS-CoV-2 KIQDVVNQNA QALNTLVQLK SNRFGAISSV LNDILSRLDK VEAEEQIDRL ITGRQLSLQT YVYQQLIRAA EVKASANLAA TKMSECVILQ SKRVDFCGKG YHLMSPQSA PHGVVFLHVT YVPAQEKNFT TAPAICHDG- KAHFPRGEGY
HCoV-OC43 KIQAVVNQNA EALNNLQQL SNNFGAISSV LQBIILSRDLA LEAEAQIDRL INGRLLTALNA YVYQQLIRAA LIRFASAAQAM EKVNECVILQ SKRINFCCNG NHIISLVQNA PYGLYFIHFS YVPTKYVTAT VSPGLCIAGD RGIAPIKSGYF
Consensus KIqAVVNNA #ALnNlLqQL SnrFgaISSV L##ILSRldA lEAeAQIDRL INgrLqal#a YVSQQLIraa eIraSaAqha eKmnECVlGQ sKr!#FCGnG nHiislpQnA PhGlyFIhFS YVPageknak taPaiChaG. rahaFrEGeYF

1201
SARS-CoV-2 VSNQTHFWFT QRNFYEPQII TTDNTFVSGN CDVVGIVVNN TVYDPLQPEL DSFKEELDKY FKNHTSPDVP LGDISGINAS VVNIQKEIRD LNEVAKNLNE SLIDLQELGK YEYQIKWPWY IWLGLFIAGLI AIVMVTIMLC CMTSC-CCSL
HCoV-OC43 VNVNNTWMT GSGYVPEPI TENNVVMVST CAVNYTKAPY VMLNTSTPNL PDFREELDQW FKNQTSVAPD LS-LDYINVT FLNLQVEMNR LQEAATKLNG SYNLKIDGT YEYVKKWPWY WLLIGLAGV AMLVLLFIC CTCGGCSTC
Consensus VNNnnhMytI grnY#eP#iI Te#n#rFVmgn KaVniqiann Lm!pql#K LdFrEELDqW FKNQTSVapD Lg.idqInaL FlNiQkeiR L#EaaKnKNG SYnl!lq#lGk YeYq!KWPWY Allimlilic CctqC.sCsl

1350
SARS-CoV-2 VSNQTHFWFT QRNFYEPQII TTDNTFVSGN CDVVGIVVNN TVYDPLQPEL DSFKEELDKY FKNHTSPDVP LGDISGINAS VVNIQKEIRD LNEVAKNLNE SLIDLQELGK YEYQIKWPWY IWLGLFIAGLI AIVMVTIMLC CMTSC-CCSL
HCoV-OC43 VNVNNTWMT GSGYVPEPI TENNVVMVST CAVNYTKAPY VMLNTSTPNL PDFREELDQW FKNQTSVAPD LS-LDYINVT FLNLQVEMNR LQEAATKLNG SYNLKIDGT YEYVKKWPWY WLLIGLAGV AMLVLLFIC CTCGGCSTC
Consensus VNNnnhMytI grnY#eP#iI Te#n#rFVmgn KaVniqiann Lm!pql#K LdFrEELDqW FKNQTSVapD Lg.idqInaL FlNiQkeiR L#EaaKnKNG SYnl!lq#lGk YeYq!KWPWY Allimlilic CctqC.sCsl

Fig. S3. Alignment and sequence identity of spike protein between SARS-CoV-2 and other coronavirus. A. % sequence identity is listed. **B.** alignment of S protein between SARS-CoV-2 and SARS. **C.** alignment of S protein between SARS-CoV-2 and HCoV-OC43.

Fig. S4. Alignment and sequence identity of ORF1b-encoded proteins between SARS-CoV-2 and other coronaviruses. **A.** % sequence identity is listed. **B.** alignment of ORF1b-encoded proteins between SARS-CoV-2 and SARS. **C.** alignment of ORF1b-encoded proteins between SARS-CoV-2 and HCoV-OC43.

C. Alignment of ORF1b-encoded protein sequences between SARS-CoV-2 and SARS

SARS-CoV-2	RVCGVSA-AR	LTPCGTGTS	DVYVRAPDIY	NKVAGIAFAK	LKTNCCRFQE	KDEDDNLIDS	YFVVKRHFTS	NYHQEETIYN	LLKDCPAVAK	HDFPKFRIDG	DMVPHISRQR	LTKYTMADLV	YALRHFDEGN	CDTLKEILVT	YNCCDDYVNI	150
HCoV-OC43	RVRGTSVDAR	LVPCLASGLST	DVQLRAPDIY	NASVAGIGLH	LKVNCCRFQR	VDENGDKLDQ	FFVVKRDLTL	TYNRMKCYE	RVKDCKEVAE	HDFPFTDVEG	SRVPHIVKRD	LTKYTMLDL	YALRHFDRND	CMLLCDILSI	YACGEQSYET	
Consensus	RvGtSa,AR	LtPcAsGlsT	DvQLRAPDIY	NakVAGIaIh	LkTNCCRFQR	kDE#d#lIdq	#FVVkRhRldS	nY#rEckcy#	rIKDcKaVAe	HDFPKFr!;Idr	drVPHISrqr	LTKYTMADLc	YALRHFDRn#	CdlLe#lIsI	YacC#d#Yfn	
SARS-CoV-2	KKDWYDFVEN	PDILIRVYANL	GERVRQALLK	TVOFCDAMRN	AGIVGVLTLD	NQDLNGNWYD	FGDFIQITPG	SGVPVVDSSY	SLMPLILTLC	RALTAEHVDR	TDLTQPIYKW	DLKLYDFTFE	RKLKFDYFK	YNDQTYHPNC	VNCLDDRCLL	300
HCoV-OC43	KKDWYDFVEN	PDILIRVYANL	GERVRQALLK	TVOFCDAMRN	AGIVGVLTLD	NQDLNGNWYD	FGDFIQITPG	SGVPVVDSSY	SLMPLILTLC	RALTAEHVDR	TDLTQPIYKW	DLKLYDFTFE	RKLKFDYFK	YNDQTYHPNC	VNCLDDRCLL	
Consensus	KKDWYDFVEN	PDILIRVYANL	GERVRQALLK	TVOFCDAMRN	AGIVGVLTLD	NQDLNGNWYD	FGDFIQITPG	SGVPVVDSSY	SLMPLILTLC	RALTAEHVDR	TDLTQPIYKW	DLKLYDFTFE	RKLKFDYFK	YNDQTYHPNC	VNCLDDRCLL	
SARS-CoV-2	HCANFNVLFS	TVFFPLTSFGF	LVRKIFVDGV	PFVVSIGYHF	RELGVVHNQD	VNLHSSRLSF	KELLVIAAADP	AMHAASGNLL	LDRKTRCFVS	AALTNNVAFQ	TVKPGNFNQR	FYDFAVSGKQ	FKEGSSVELK	HHFFAQDQNA	AISDYTYRKY	450
HCoV-OC43	HCANFNVLFS	TVFFPLTSFGF	LVRKIFVDGV	PFVVSIGYHF	RELGVVHNQD	VNLHSSRLSF	KELLVIAAADP	AMHAASGNLL	LDRKTRCFVS	AALTNNVAFQ	TVKPGNFNQR	FYDFAVSGKQ	FKEGSSVELK	HHFFAQDQNA	AISDYTYRKY	
Consensus	HCANFNVLFS	TVFFPLTSFGF	LVRKIFVDGV	PFVVSIGYHF	RELGVVHNQD	VNLHSSRLSF	KELLVIAAADP	AMHAASGNLL	LDRKTRCFVS	AALTNNVAFQ	TVKPGNFNQR	FYDFAVSGKQ	FKEGSSVELK	HHFFAQDQNA	AISDYTYRKY	
SARS-CoV-2	NLPTMCDIRQ	LLFVVEVVDK	YFDCYDGGCI	NANQVIVNNL	DKSAGFFPNK	WGKARLYYDS	MSYEDQDALF	AYTKRNVITP	ITQMNLYKAI	SAKNRARTVA	GVSICSTMTN	RQFHQKLLKS	IAATRGAATV	IGTSKFYGGW	HNML-----	600
HCoV-OC43	NLPTMCDIRQ	LLFVVEVVDK	YFDCYDGGCI	NANQVIVNNL	DKSAGFFPNK	WGKARLYYDS	MSYEDQDALF	AYTKRNVITP	ITQMNLYKAI	SAKNRARTVA	GVSICSTMTN	RQFHQKLLKS	IAATRGAATV	IGTSKFYGGW	HNML-----	
Consensus	NLPTMCDIRQ	LLFVVEVVDK	YFDCYDGGCI	NANQVIVNNL	DKSAGFFPNK	WGKARLYYDS	MSYEDQDALF	AYTKRNVITP	ITQMNLYKAI	SAKNRARTVA	GVSICSTMTN	RQFHQKLLKS	IAATRGAATV	IGTSKFYGGW	HNML-----	
SARS-CoV-2	VDPNVLMGWD	YPKCDRAMPN	LLRIVSSSLV	ARKHETCCSQ	SDRYFLRANE	CAQVLSSEVM	CGGSLYVKPG	GTSSSGDATTA	YANSVFNICQ	AVSANVALLM	SCNCGNIKED	SIRALQHRLY	ECLYVRNDRD	TDVFNEFYAY	LKRHFSSMILL	750
HCoV-OC43	VDPNVLMGWD	YPKCDRAMPN	LLRIVSSSLV	ARKHETCCSQ	SDRYFLRANE	CAQVLSSEVM	CGGSLYVKPG	GTSSSGDATTA	YANSVFNICQ	AVSANVALLM	SCNCGNIKED	SIRALQHRLY	ECLYVRNDRD	TDVFNEFYAY	LKRHFSSMILL	
Consensus	VDPNVLMGWD	YPKCDRAMPN	LLRIVSSSLV	ARKHETCCSQ	SDRYFLRANE	CAQVLSSEVM	CGGSLYVKPG	GTSSSGDATTA	YANSVFNICQ	AVSANVALLM	SCNCGNIKED	SIRALQHRLY	ECLYVRNDRD	TDVFNEFYAY	LKRHFSSMILL	
SARS-CoV-2	SDDAVVCNFS	TYASQGLVAS	IKNFKSLVLY	QNNVFMSSEK	CWTEHIDINN	PHIEFCSQHTM	LVRKQDDDVY	LPYPDPSPRIL	GAGCFVDDIL	KTDGTLMIER	FVSLAIDAYP	LTKHPNQEYA	DVFHLYLQYI	RKLHDELTHG	MLDMYSVILT	900
HCoV-OC43	SDDAVVCNFS	TYASQGLVAS	IKNFKSLVLY	QNNVFMSSEK	CWTEHIDINN	PHIEFCSQHTM	LVRKQDDDVY	LPYPDPSPRIL	GAGCFVDDIL	KTDGTLMIER	FVSLAIDAYP	LTKHPNQEYA	DVFHLYLQYI	RKLHDELTHG	MLDMYSVILT	
Consensus	SDDAVVCNFS	TYASQGLVAS	IKNFKSLVLY	QNNVFMSSEK	CWTEHIDINN	PHIEFCSQHTM	LVRKQDDDVY	LPYPDPSPRIL	GAGCFVDDIL	KTDGTLMIER	FVSLAIDAYP	LTKHPNQEYA	DVFHLYLQYI	RKLHDELTHG	MLDMYSVILT	
SARS-CoV-2	NDNTSRFYDE	EFEYEAHTPH	TVLQAVGACV	LCNSQTSILRC	GACIRPFLIC	GCKCYDHWIS	TSKHLVLSVN	PYYCNAPGCD	VNDVTQLYLG	GMSYCEDEHK	PPISFPLCAN	CGVFGLYKNT	CTGSDNVDTF	NAIATCDWNT	AGDYILANTC	1050
HCoV-OC43	NDNTSRFYDE	EFEYEAHTPH	TVLQAVGACV	LCNSQTSILRC	GACIRPFLIC	GCKCYDHWIS	TSKHLVLSVN	PYYCNAPGCD	VNDVTQLYLG	GMSYCEDEHK	PPISFPLCAN	CGVFGLYKNT	CTGSDNVDTF	NAIATCDWNT	AGDYILANTC	
Consensus	ndfgrqr#t#e	eFyEaHtPh	avSQaVGACV	lCnsQTSILRC	GaCIRPFLIC	GCKCYDHWIS	TSKHLVLSVN	PYYCNAPGCD	VndVtQlYlG	GmsYcEdHk	PqISfKlCaN	GqVFGlYk#s	Ctgsdn!dF	NaIaScDwT#	adDYILANeC	
SARS-CoV-2	TERLKLFAAE	TLKATEETFK	LSYGIATVRE	VLSDRLEHLS	WEVGKPRPPL	NRNYVFTGYR	VTKNKSVQIG	EYTFEKGDYG	DAVVYRGTTT	YKLNVDGYFV	LTSHVMPLS	TPTLVQPEHY	VRITGLYPTL	NISDEFSSNV	ANYQHGMRGK	1200
HCoV-OC43	TERLKLFAAE	TLKATEETFK	LSYGIATVRE	VLSDRLEHLS	WEVGKPRPPL	NRNYVFTGYR	VTKNKSVQIG	EYTFEKGDYG	DAVVYRGTTT	YKLNVDGYFV	LTSHVMPLS	TPTLVQPEHY	VRITGLYPTL	NISDEFSSNV	ANYQHGMRGK	
Consensus																