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## Supplemental information

# Prevalent and immunodominant CD8 T cell epitopes are conserved in SARS-CoV-2 variants

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# Supplemental Figures

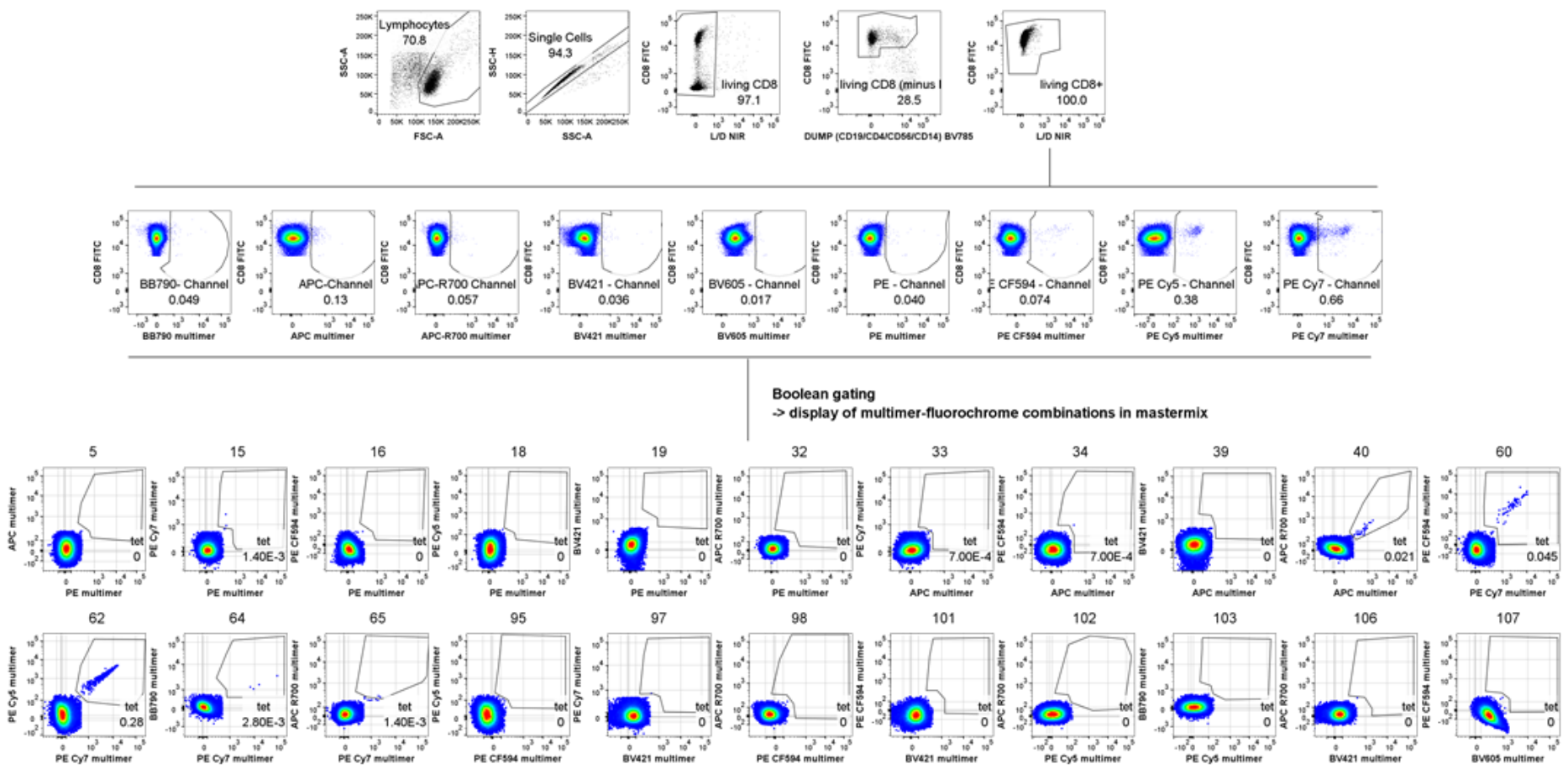


**Figure S1. Gating strategy for combinatorial multimer staining approach (related to Figure 2 and STAR Methods).**

Representative flow plots for a convalescent individual (sample ID 48) stained with multimers for a subset of the tested HLA-A*02:01 peptides.

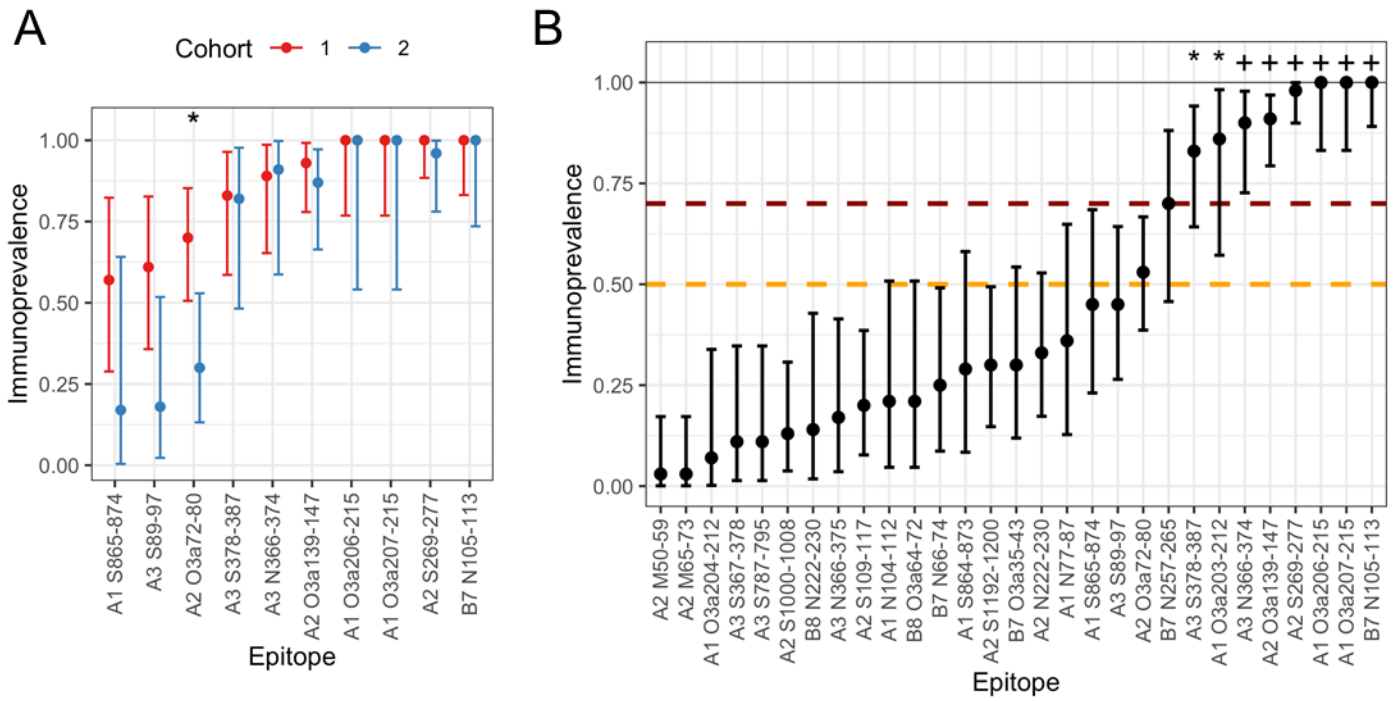


**Figure S2. Immunoprevalence evaluated by binomial tests (related to Figure 3).**

**(A)** Immunoprevalence with exact binomial 95 % confidence intervals estimated separately for the two independent cohorts of convalescent individuals analyzed for multimer responses (Cohort 1 and Cohort 2). Shows all epitopes tested in Cohort 2. Differences in immunoprevalence between the cohorts were assessed using Fisher's exact test (*: $p<0.05$). **(B)** Immunoprevalence with exact binomial 95 % confidence intervals for all immunogenic epitopes identified in our study, estimated based on pooled data from cohorts 1 and 2. Binomial tests were performed to assess whether the immunoprevalence for each epitope was significantly higher than 50% (orange line) and 70% (red line), respectively (*: $p<0.05$ for immunoprevalence > 50%; +: $p<0.05$ for immunoprevalence > 70%).

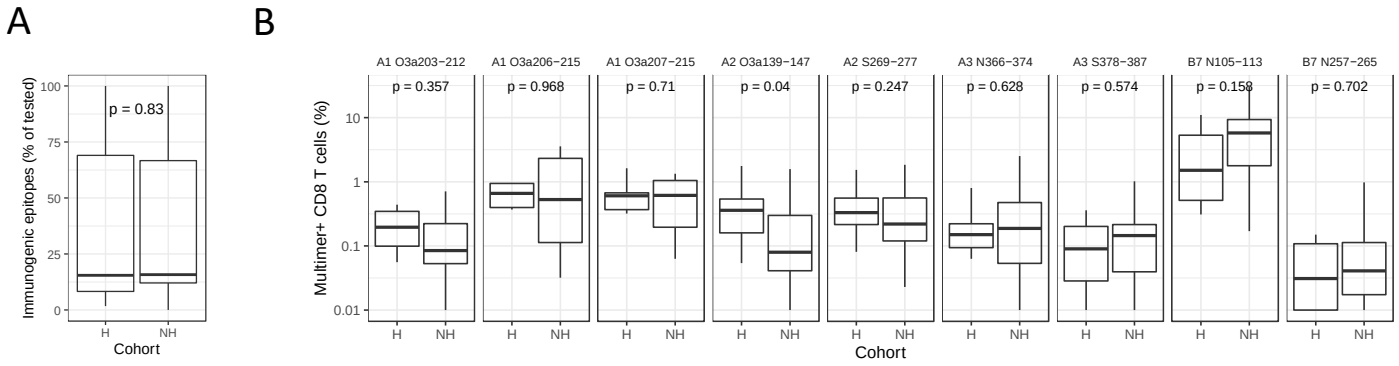


**Figure S3. Association between CD8 T-cell responses and severity of COVID-19 (related to Figure 2).**

**(A)** Proportion of epitopes (peptide/HLA combinations) determined to be immunogenic by MHC multimer assay for each convalescent donor (points) according to the severity of COVID-19 for COVID-19 hospitalized (H, n = 19) versus non-hospitalized (NH, n = 64) individuals. **(B)** Size of the MHC multimer positive population in all convalescent donors tested (points) for the nine most immunoprevalent epitopes according to severity of COVID-19. Negative donors are also included in the comparison, with population size zero substituted by 0.01 % before logarithmic transformation. All p-values were calculated using Wilcoxon rank sum test.

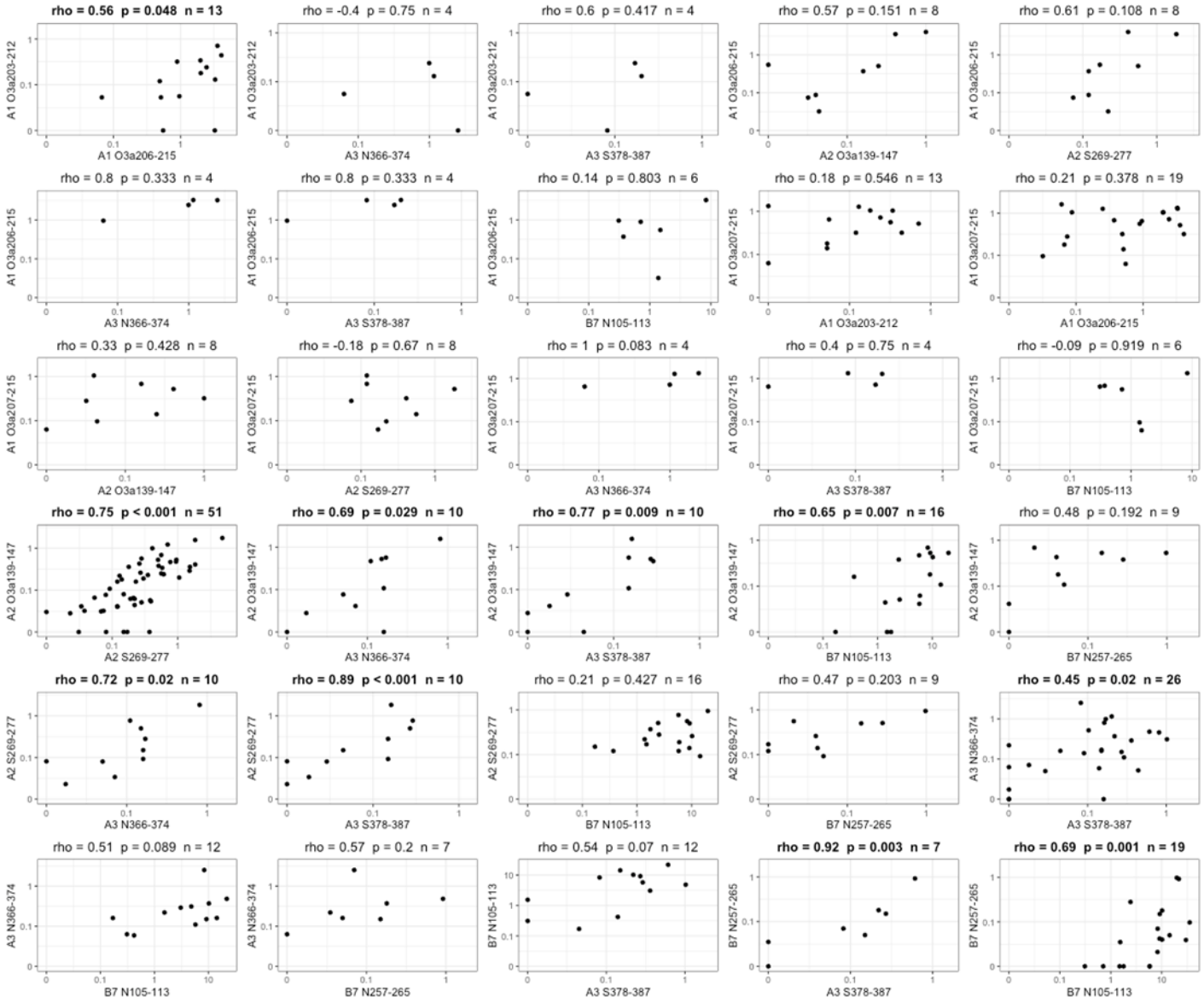


**Figure S4. Pairwise correlation analysis between epitope-specific multimer response magnitudes (related to Figure 4C+D).**

Pairwise correlation between multimer response magnitudes to the nine most immunoprevalent epitopes (Spearman's rank correlation). Each dot represents paired multimer response measurements in the same donor. Epitope pairs with data from at least five donors are shown here. Epitope pairs where multimer response magnitude shows statistically significant correlation (Spearman's rank correlation; $p<0.05$) in bold (same as in Fig. 4D). Sample size (n) is listed for each comparison.

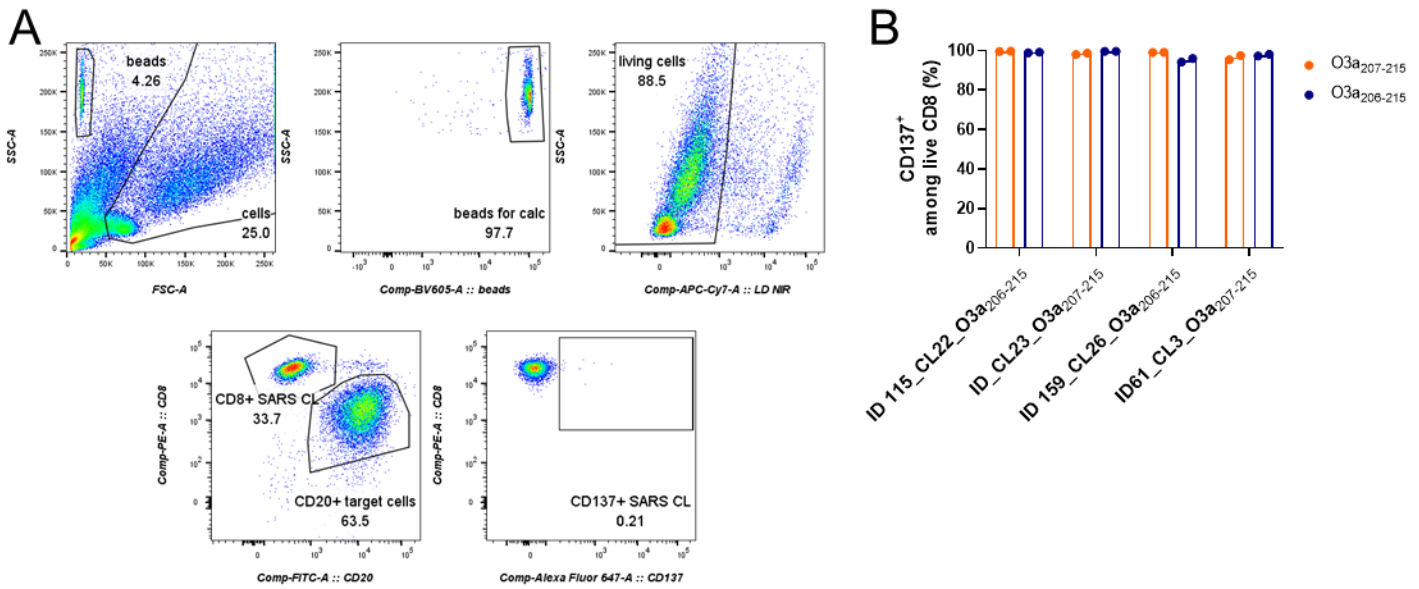


**Figure S5. Gating strategy for functional validation of T-cell lines (A), and cross-recognition of T-cell lines of length variants (related to Figure 5 and STAR Methods).**

**(A)** Gating strategy for functional validation of SARS-CoV-2 specific T-cell lines. Here, flow plots for B721.221-HLA-A*02:01 cells w/o antigen co-cultured with a T-cell line recognizing an HLA-A*02:01 specific peptide are shown. Beads (middle top plot) are added only to the samples used to quantitate absolute numbers of events in the target cell gate (lower left plot) to determine killing by $CD8^+$ T cell lines. In the lower right plot the gate used to determine the fraction of activated $CD137^+$ T cells is shown. **(B)** T-cell lines sorted for binding of multimers complexed with ORF3a-derived peptides $O3a_{207-215}$ (FTSDYYQLY; 9-mer) and $O3a_{206-215}$ (YFTSDYYQLY; 10-mer) from several convalescent individuals recognize the 9- and 10-mer equally well, as determined by activation marker expression (CD137) after 20 h co-culture with peptide-loaded (100 nM) mono-allelic B721.221 cells (technical duplicate).

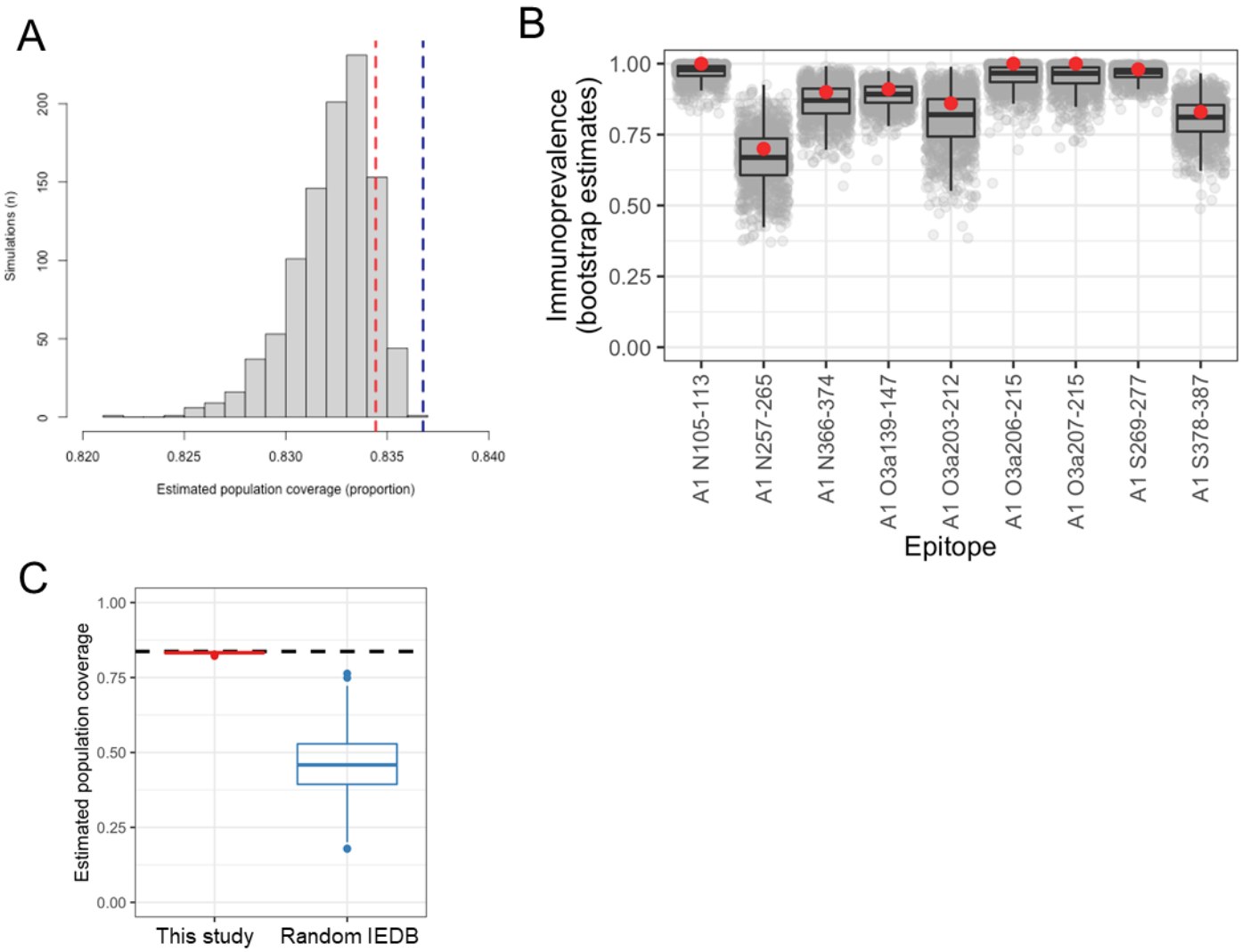


**Figure S6. Uncertainty in immunoprevalence estimates and population coverage (related to STAR Methods).**

**(A)** Coverage in the European Caucasian population for immunoprevalent peptides identified in this study as compared with 1000 random sets of immunogenic peptides from IEDB (dashed black line: population coverage based solely on HLA allele frequencies; red box plot: estimated population coverage with our top 9 epitopes (immunoprevalence 70% or higher) after adjusting for immunoprevalence, using bootstrapping to quantify uncertainty (1000 iterations); blue: results from 1000 random draws of 9 immunogenic epitopes from IEDB, while keeping the HLA allele distribution the same as for our top 9 epitopes). Boxplots show median and inter-quartile range (IQR); whiskers extend to +/- 1.5 times the IQR and more extreme values are drawn as dots. **(B)** Uncertainty in immunoprevalence estimates for our top 9 immunoprevalent epitopes based on bootstrapping from the beta distribution. Each of 1000 iterations per epitope are represented as shaded gray dots. Boxplots show median and inter-quartile range (IQR); whiskers extend to +/- 1.5 times the IQR. The observed immunoprevalence in experimental data is drawn as a red dot. **(C)** Coverage in the European Caucasian population with immunoprevalent peptides identified in this study. The dashed vertical blue line represents population coverage with

peptides recognizing HLA-A*01:01, HLA-A*02:01, HLA-A*03:01 and HLA-B*07:02, based solely on HLA allele frequencies (not considering immunoprevalence). Estimated population coverage with our top 9 epitopes (prevalence 70% or higher) after adjusting for immunoprevalence is shown as a dashed vertical red line. Histogram in gray shows population coverage for the same 9 epitopes when considering uncertainty in immunoprevalence estimates, as represented by bootstrapped data shown in **(A).**