Cell Reports, Volume *39*

Supplemental information

Broadly recognized, cross-reactive SARS-CoV-2 CD4 T cell epitopes are highly conserved across human coronaviruses and presented by common HLA alleles

Aniuska Becerra-Artiles, J. Mauricio Calvo-Calle, Mary Dawn Co, Padma P. Nanaware, John Cruz, Grant C. Weaver, Liying Lu, Catherine Forconi, Robert W. Finberg, Ann M. Moormann, and Lawrence J. Stern

Figure S1

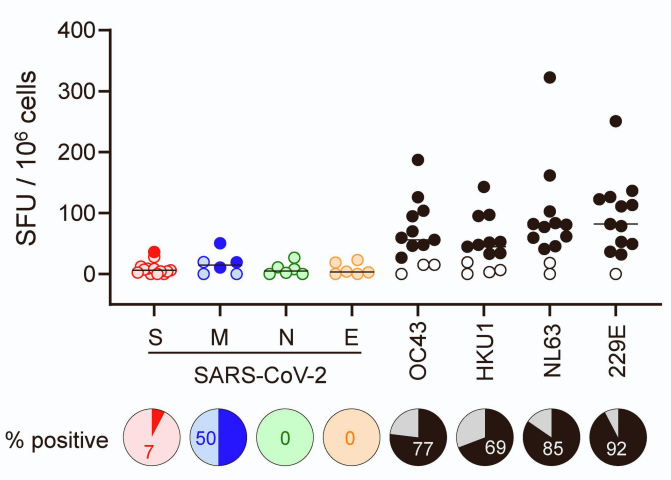


Figure S1 (Related to Figure 1). Ex vivo responses to coronavirus antigens in pre-pandemic donors. Summary of ex vivo IFN-γ responses to pools of overlapping peptides in SARS-CoV-2 S, M, N, and E proteins and S proteins from OC43, HKU1, NL63, and 229E in 13 pre-pandemic donors. Positive responses by DFR test are indicated by dark color circles; pies show the percentage of positive responses (dark color) for each group/condition.

Figure S2

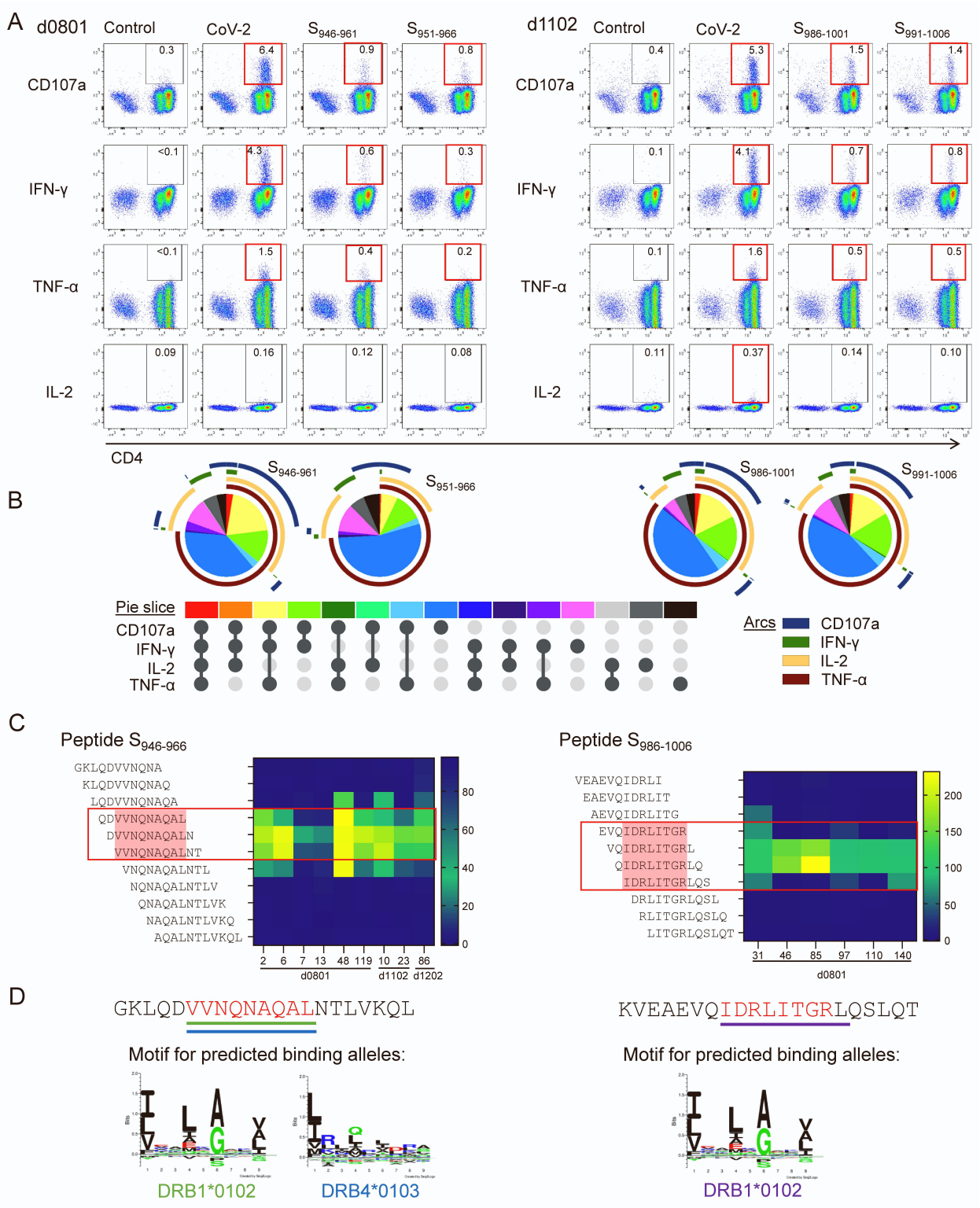
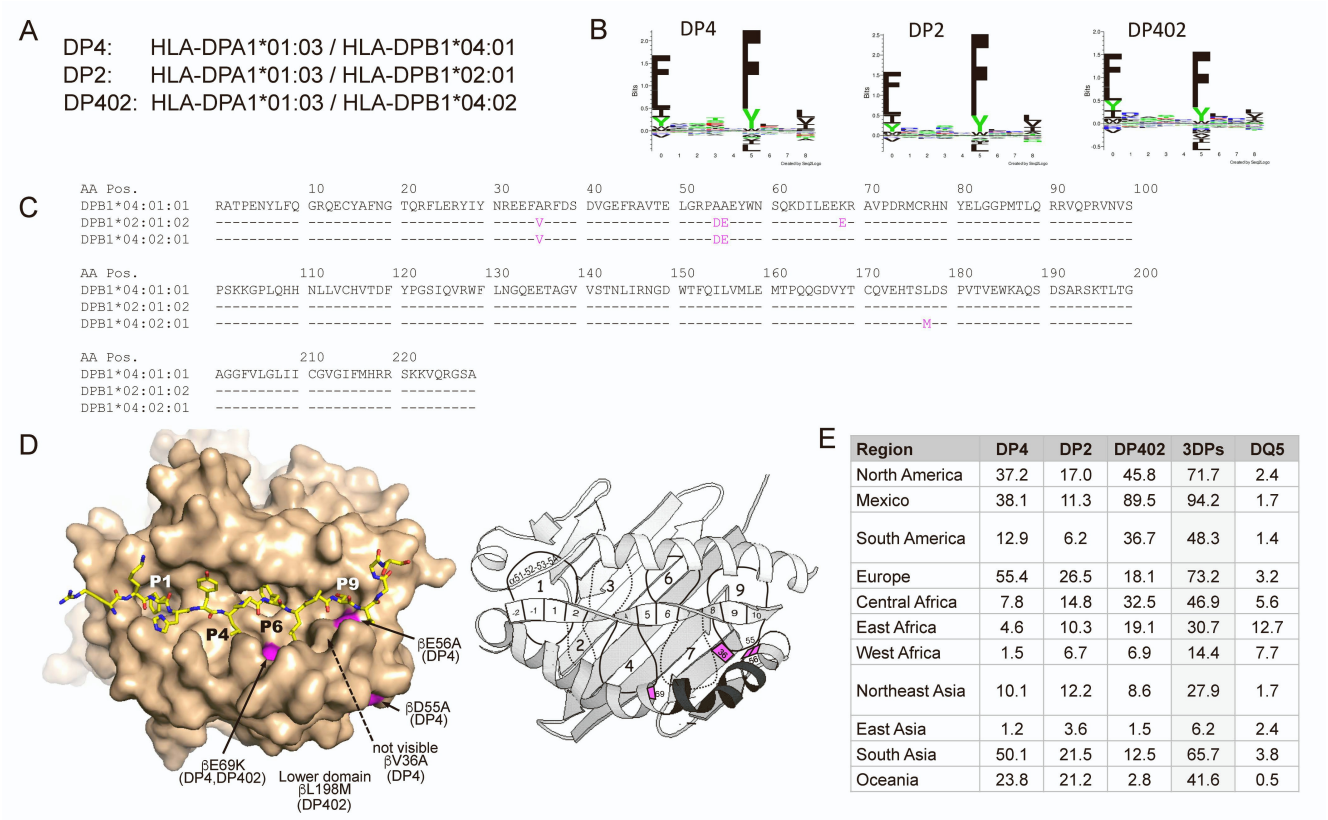


Figure S2 (Related to Figure 3). Functional characterization and epitope mapping of in vitro-expanded cross-reactive cells responding to $S_{946-966}$ and $S_{986-1006}$. A. Representative ICS plots for IFN-γ, TNF-α, and IL-2 production, and CD107a mobilization to surface in the CD3+ population after re-stimulation of cross-reactive in vitro expanded T cells with SARS-CoV-2 S pool (CoV-2 S) or individual peptides $S_{946-961}$ and $S_{951-966}$ (donor d0801), or $S_{946-1001}$ and $S_{991-1006}$ (donor d1102). Positive responses are shown in red boxes (>3-fold background response). B. Visualization of the

polyfunctional response using SPICE (Roederer et al., 2011): pie and arcs graphs showing the combined contribution of each marker. C. Summary of the responses of T cell clones to sets of 11-mer peptides (overlapped by 10) covering the whole $S_{946\text{-}966}$ or $S_{986\text{-}1006}$ sequences, measured as IFN-γ by ELISA. The minimal sequence required to explain reactivity in each case is boxed in red. D. Peptide sequence and mapped minimal sequence (red). For each sequence, predicted binding motifs for relevant HLA alleles are shown as sequence logos (Motif Viewer in NetMHCIIpan 4.0) and the predicted core epitope in the peptide sequence is underlined.

Figure S3



E

Region	DP4	DP2	DP402	3DPs	DQ5
North America	37.2	17.0	45.8	71.7	2.4
Mexico	38.1	11.3	89.5	94.2	1.7
South America	12.9	6.2	36.7	48.3	1.4
Europe	55.4	26.5	18.1	73.2	3.2
Central Africa	7.8	14.8	32.5	46.9	5.6
East Africa	4.6	10.3	19.1	30.7	12.7
West Africa	1.5	6.7	6.9	14.4	7.7
Northeast Asia	10.1	12.2	8.6	27.9	1.7
East Asia	1.2	3.6	1.5	6.2	2.4
South Asia	50.1	21.5	12.5	65.7	3.8
Oceania	23.8	21.2	2.8	41.6	0.5

Figure S3 (Related to Figures 4, 6). Sequences, peptide-binding motifs, and allelic frequencies for DP4, DP2, and DP402. A. Systematic nomenclature for DPα and DPβ subunits of DP4, DP2, and DP402 allelic variants. All carry the same DPα subunit. B. Peptide binding motifs are similar for all three DP proteins. From NetMHCIIpan4.0. C. DPβ subunit sequences, differences from DP4 are indicated in magenta. From IMGT/HLA database D. Locations of allelic differences on shown on DP2 structure (from PDB:3QLZ, (Dai et al., 2010)). Self-peptide bound to DP2 is shown in yellow, with DP4 and DP402 sequence differences shown. Right, schematic diagram of variant DP residues relative to major peptide side-chain binding pockets P1, P4, P6, P7, and P9. Peptide positions P2, P5, and P8 are oriented towards TCR. E. Frequency in various geographic areas of DP4, DP2, and DP402 in various populations, with the combined frequency of at least one of these alleles (DP4/2/402). DQ5 frequencies are shown for comparison. From the IEDB allele frequency tool used to display data in the HLA allele frequency database.

Figure S4

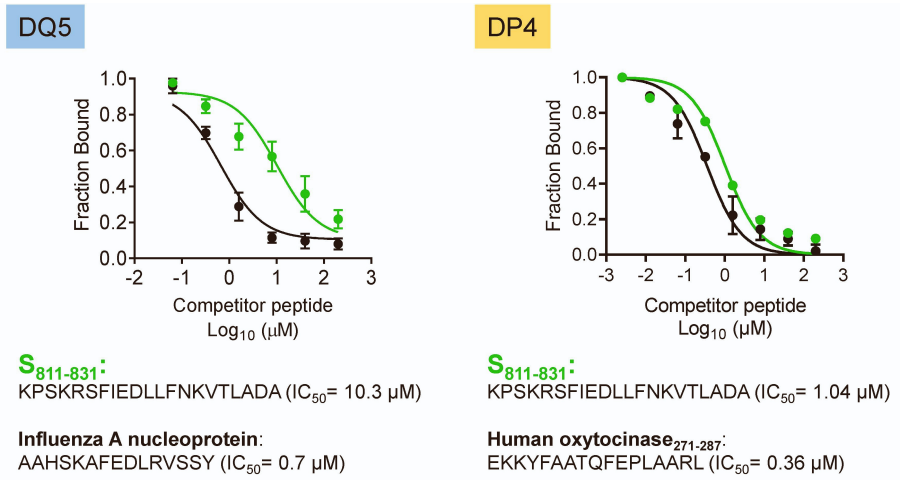


Figure S4 (Related to Figure 4). HLA-DQ5 and HLA-DP4 competition binding assays. Binding of $S_{811-831}$ to recombinant proteins DP4 (DPA1*01:03/DPB1*04:01) and DQ5 (DQA1*01:01/DQB1*05:01). Competitor peptide from Influenza A NP for DQ5 and from human oxytocinase$_{271-287}$ for DP4. IC_{50} (μM) for each peptide in each assay is shown in parenthesis.

Figure S5

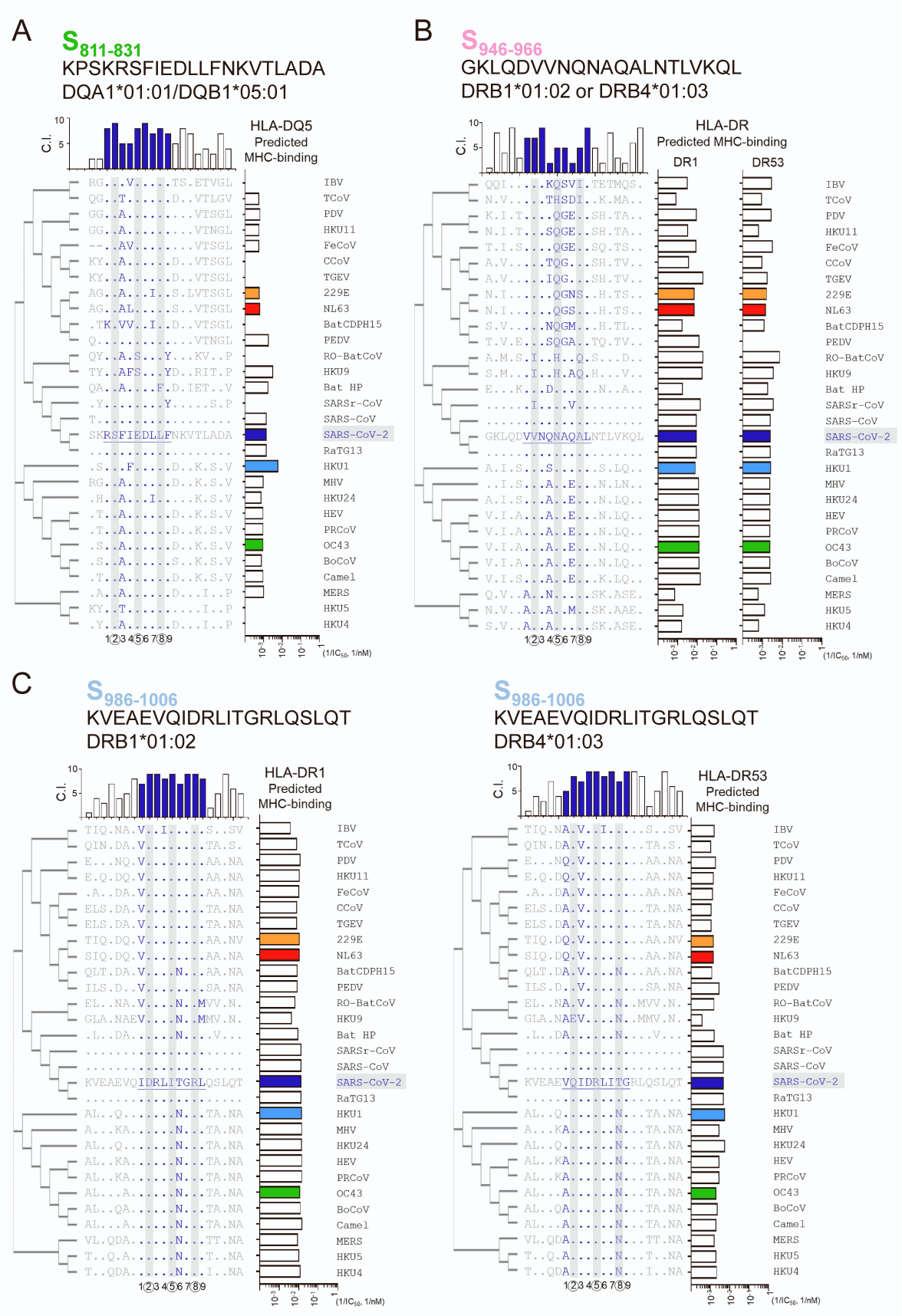

A
S811-831
KPSKRSFIEDLLFNKVTLADA
DQA1*01:01/DQB1*05:01
HLA-DQ5
Predicted
MHC-binding
C.I.
IBV
TCoV
PDV
HKU11
FeCoV
CCoV
TGEV
229E
NL63
BatCDPH15
PEDV
RO-BatCoV
HKU9
Bat HP
SARSr-CoV
SARS-CoV
SARS-CoV-2
RaTG13
HKU1
MHV
HKU24
HEV
PRCoV
OC43
BoCoV
Camel
MERS
HKU5
HKU4
(1/IC50, 1/nM)
B
S946-966
GKLQDVVNQNAQALNTLVKQL
DRB1*01:02 or DRB4*01:03
HLA-DR
Predicted MHC-binding
DR1
DR53
C
S986-1006
KVEAEVQIDRLITGRLQSLQT
DRB1*01:02
HLA-DR1
Predicted
MHC-binding
S986-1006
KVEAEVQIDRLITGRLQSLQT
DRB4*01:03
HLA-DR53
Predicted
MHC-binding

Figure S5 (Related to Figure 5). Sequence alignment of SARS-CoV-2 $S_{811-831}$ (A), $S_{946-966}$ (B), and $S_{986-1006}$ (C), and positional homologs in 28 coronaviruses. For each region, the complete SARS-CoV-2 sequence and differences at every position in the other viruses are shown (**Table S4**). Epitope 9-mers are highlighted in blue. T cell contacts are highlighted with a grey bar. In each panel, a phylogenetic tree of the S proteins is shown on the left; on top, are shown the conservation index (C.I.) for each position; on the right are shown predicted binding affinities for relevant HLA-alleles.

Figure S6

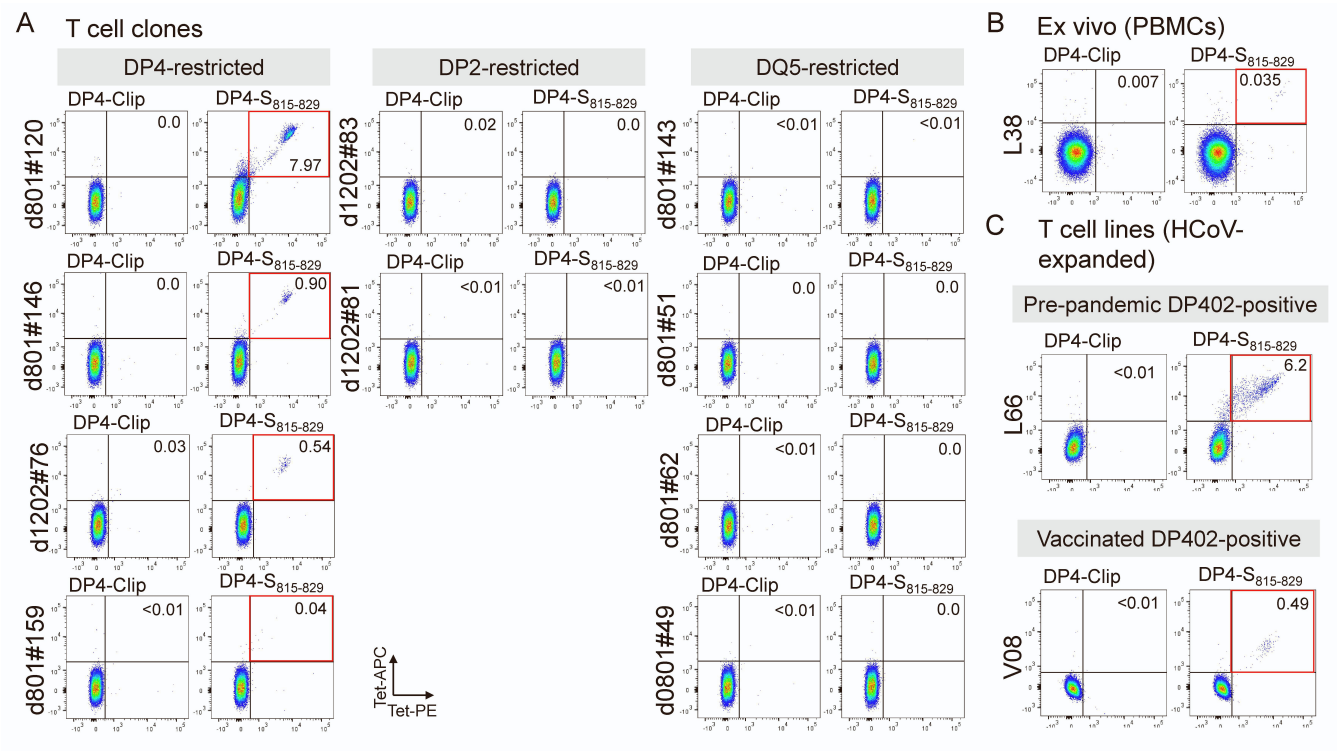


Figure S6 (Related to Figure 6). Additional DP4-$S_{815-829}$ tetramer staining. A. T cell clones: four T cell clones categorized as DP4-restricted (left), two DP2-restricted (middle), and four DQ5-restricted (right) are shown. B. Ex vivo staining of unstimulated PBMC from an unexposed donor. C. Staining of in vitro HCoV S pool-expanded T cells from pre-pandemic and vaccinated donors that expressed DP402 but not DP401. Double-tetramer (PE and APC) staining in the CD4+ population is shown in dot plots. DP4-Clip tetramers were used as controls. Positive responses are shown in red boxes (>3-fold background response).

Figure S7

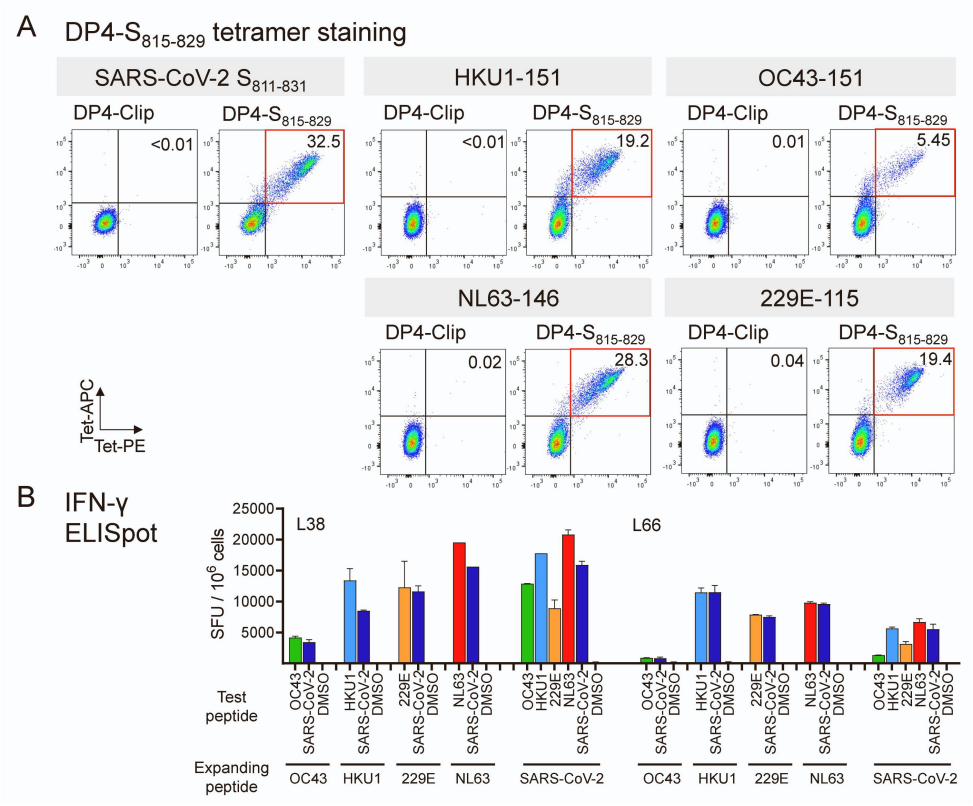


Figure S7 (Related to Figure 6). Cross-reactive response of $S_{811-831}$-expanded T cell lines from pre-pandemic donors. A. DP4-$S_{815-829}$ tetramer staining of cells expanded with SARS-CoV-2 $S_{811-831}$, HKU1-151, OC43-151, NL63-146, or 229E-115. Double-tetramer (PE and APC) staining in the CD4+ population is shown in dot plots; DP4-Clip tetramers were used as controls. Positive responses are shown in red boxes (>3-fold background response). B. IFN-γ ELISpot of same lines responding to the peptide used for expansion (Expanding peptide) or the cross-reactive peptide(s) (Test peptide) in two pre-pandemic donors. DMSO is the negative control.