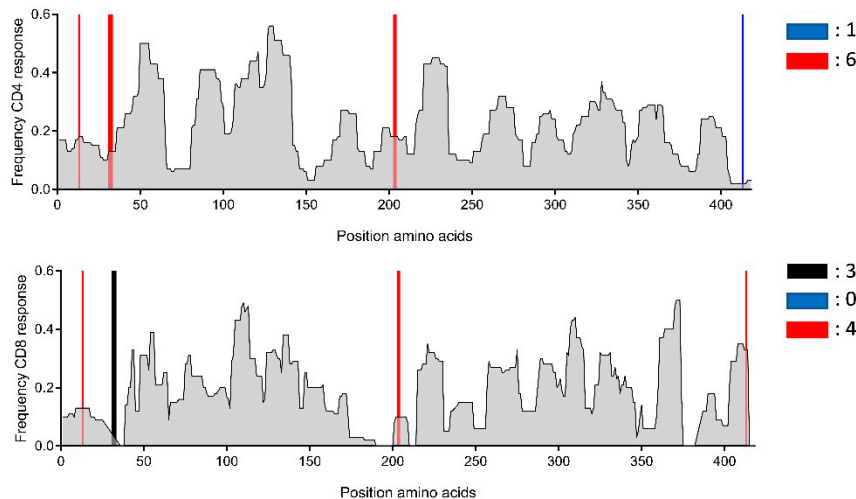


Supplementary Material

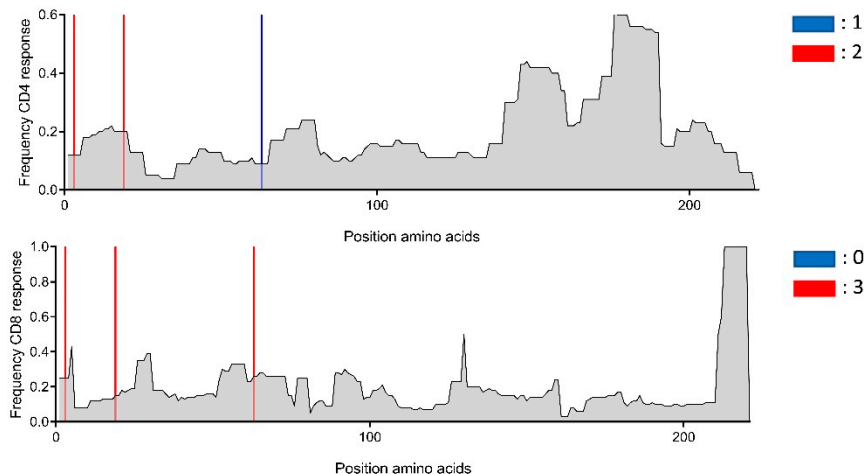
SARS-CoV-2-Specific T-Cell as a Potent Therapeutic Strategy against Immune Evasion of Emerging COVID-19 Variants

S1.1. Supplementary Figures

- A. SARS-CoV-2 Omicron variant (BA.5)**
- 7 mutations in the nucleocapsid
- *Red line: Ancestral SARS-CoV-2 frequency of recognition > 10%
 - *Blue line: Ancestral SARS-CoV-2 frequency of recognition < 10%
 - *Black line: Ancestral SARS-CoV-2 frequency of recognition unknown

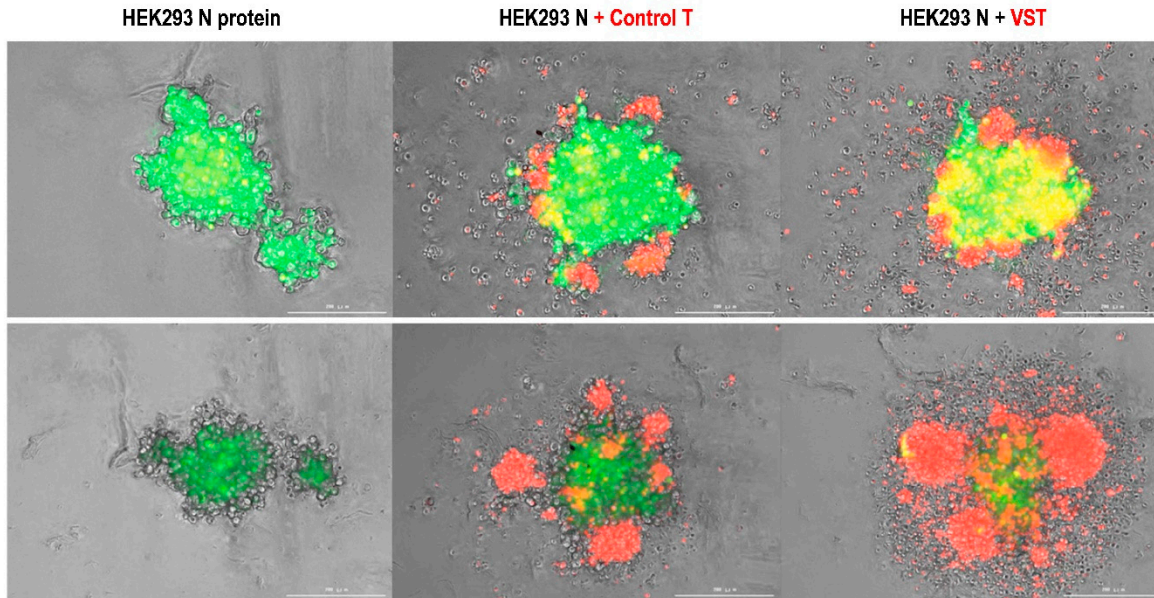


- B. SARS-CoV-2 Omicron variant (BA.5)**
- 3 mutations in the membrane
- *Red line: Ancestral SARS-CoV-2 frequency of recognition > 10%
 - *Blue line: Ancestral SARS-CoV-2 frequency of recognition < 10%

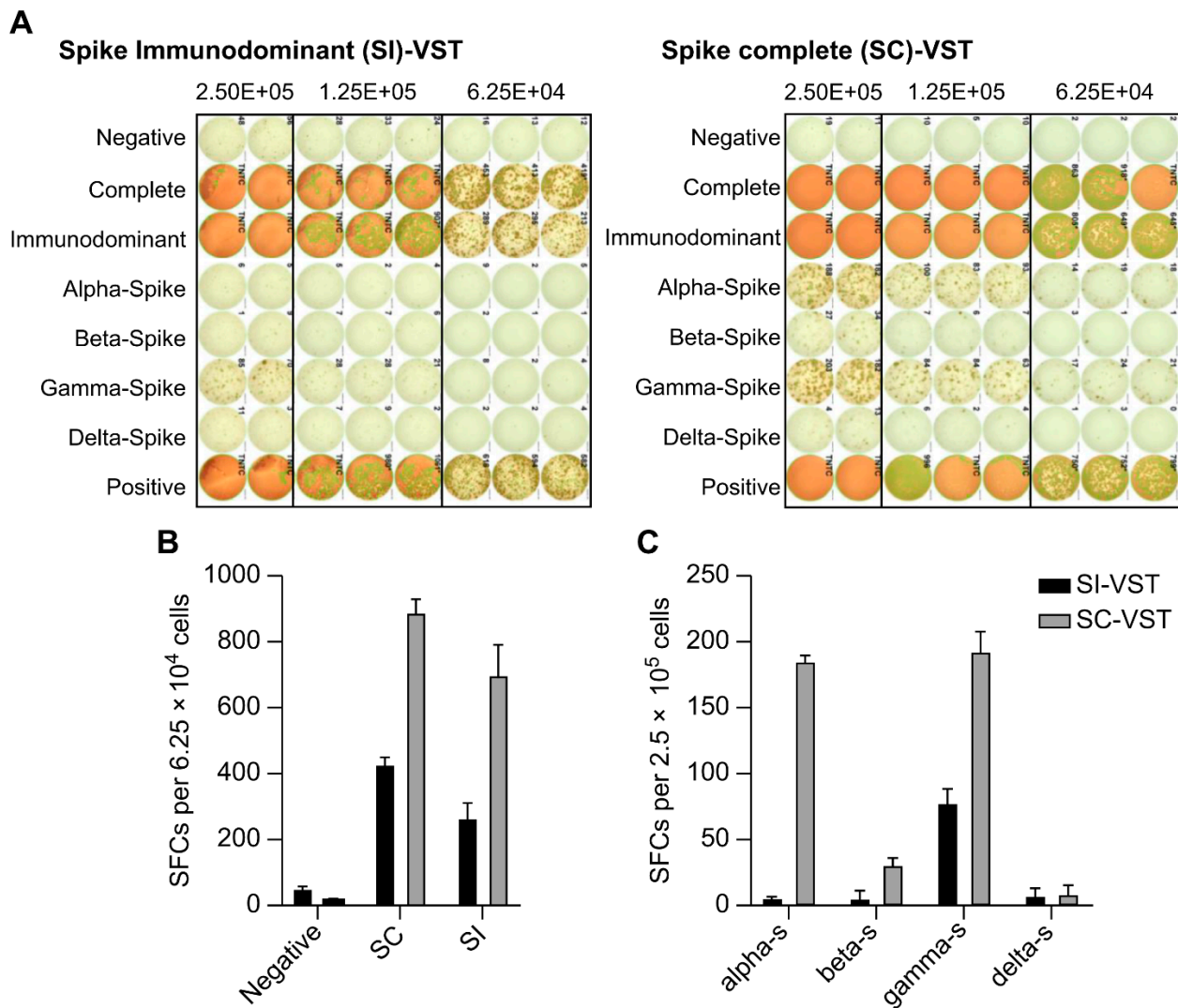


Supplementary Figure S1. VSTs educated by the ancestral SARS-CoV-2 strain recognize conserved nucleocapsid and membrane epitopes in the Omicron variant. The expected response frequency for the nucleocapsid and membrane-mutation domain of Omicron BA.5. (A) Analysis of nucleocapsid-protein epitopes in the Omicron sub-variant (BA.5) targeted by CD4⁺ T cells (conservation rate: 82%) and recognized by CD8⁺ T cells (conservation rate: 85%). (B) Analysis of membrane-protein epitopes in the Omicron sub-variant (BA.5) targeted by CD4⁺ T cells (conservation rate: 96%) and recognized by CD8⁺ T cells (conservation rate: 96%). SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; VST: virus-specific T cell.

- ✓ Target Cells: HEK293 (N) (CFSE / Ex: 488nm)
- ✓ Effector Cells: VST (PKH26 Red / Ex: 551nm, Em: 567nm)



Supplementary Figure S2. Evaluation of VST cytotoxicity against SARS-CoV-2 N protein-expressing cells. Live cell imaging of VSTs on HEK293 N cells expressing the SARS-CoV-2 N protein. CFSE-labelled HEK293 N cell spheroids were co-cultured with PKH26 Red-labeled control T cells (Control T) and PKH26 Red-labeled SARS-CoV-2-specific T cells (VSTs) for 24 h. CFSE: carboxyfluorescein succinimidyl ester; N: nucleocapsid; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VST: virus-specific T cell.



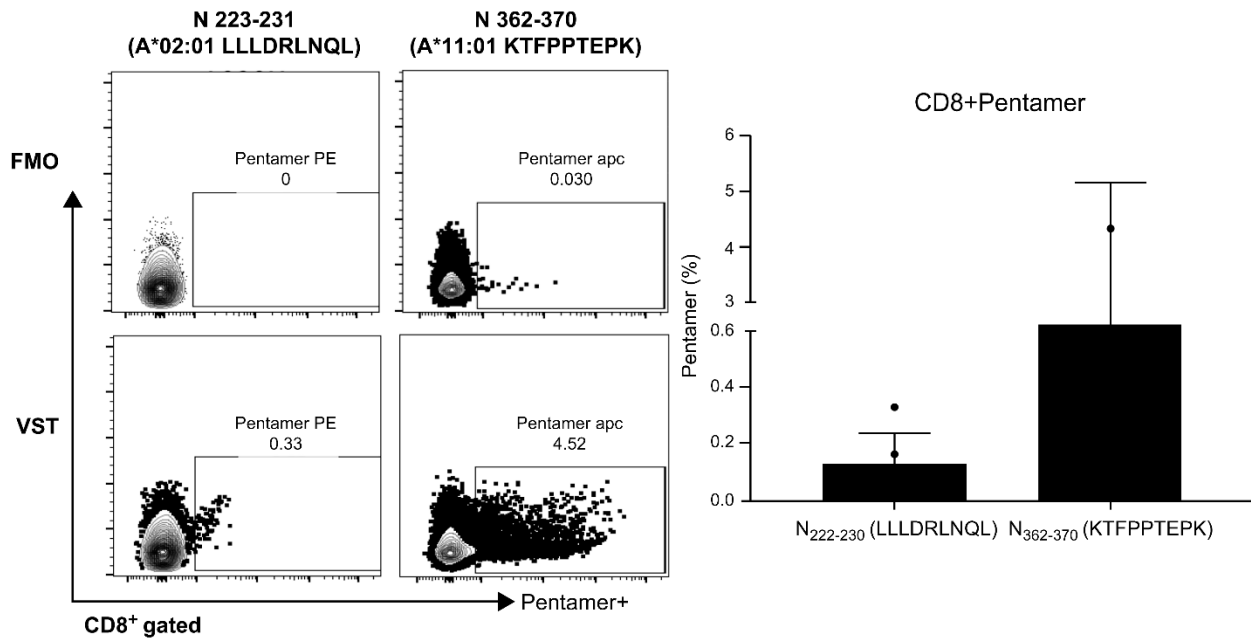
Supplementary Figure S3. Comparison of multiple epitope recognition abilities between VSTs stimulated by spike immunodominant peptide and VSTs stimulated by spike complete peptide. Based on ELISPOT analysis, the multiple epitope recognition abilities were compared between VSTs stimulated by spike immunodominant (SI) peptide and VSTs stimulated by spike complete (SC) peptide. (A) VST response against SARS-CoV-2 antigens. Data in box plots show spot-forming cells (SFCs). (B) Summarized results from 6.25×10^4 cells and (C) 2.5×10^5 cells. SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; VST: virus-specific T cell.

Spike Mutation (SARS-CoV-2)		1	130
Wuhan-Hu-1		MEVFLVLLPL VSSQCVNLTIT RTQLPPAYTN SFRGYYVYPD KVFHSSVLHS TQDLFLPFFS NVTFHAIHV SGTNGTKRFD NPVLFPNDGV YFASTEKSN I IRGWIFGTLT DSKTQSLIY NNATNWIKV	
Alpha		MEVFLVLLPL VSSQCVNLTIT RTQLPPSYTN SFRGYYVYPD KVFHSSVLHS TQDLFLPFFS NVTFHAIHV SGTNGTKRFA NPVLFPNDGV YFASTEKSN I IRGWIFGTLT DSKTQSLIY NNATNWIKV	
Beta		MEVFLVLLPL VSSQCVNLTIT RTQLPPAYTN SFRGYYVYPD KVFHSSVLHS TQDLFLPFFS NVTFHAIHV SGTNGTKRFA NPVLFPNDGV YFASTEKSN I IRGWIFGTLT DSKTQSLIY NNATNWIKV	
Gamma		MEVFLVLLPL VSSQCVNLTIT RTQLPPAYTN SFRGYYVYPD KVFHSSVLHS TQDLFLPFFS NVTFHAIHV SGTNGTKRFD NPVLFPNDGV YFASTEKSN I IRGWIFGTLT DSKTQSLIY NNATNWIKV	
Delta		MEVFLVLLPL VSSQCVNLTIT RTQLPPAYTN SFRGYYVYPD KVFHSSVLHS TQDLFLPFFS NVTFHAIHV SGTNGTKRFD NPVLFPNDGV YFASXESKN I IRGWIFGTLT DSKTQSLIY NNATNWIKV	
Omicron		MEVFLVLLPL VSSQCVNLTIT RTQLPPAYTN SFRGYYVYPD KVFHSSVLHS TQDLFLPFFS NVTFHAIHV SGTNGTKRFD NPVLFPNDGV YFASXESKN I IRGWIFGTLT DSKTQSLIY NNATNWIKV	
		131	260
Wuhan-Hu-1		CEFGQNDPF LGVYYKNNK SIMESEFRVY SSANNGTFEY VSQFFLMOLE GQGQNFKNLR EFVFNIDGY FKLYSKHTPI NLVRQLPGGF SALEPLVDLP IGINITRFQT LLALHFSYLT PGDSSSQNTA	
Alpha		CEFGQNDPF LGVYYKNNK SIMESEFRVY SSANNGTFEY VSQFFLMOLE GQGQNFKNLR EFVFNIDGY FKLYSKHTPI NLVRQLPGGF SALEPLVDLP IGINITRFQT L---HFSYLT PGDSSSQNTA	
Beta		CEFGQNDPF LGVYYKNNK SIMESEFRVY SSANNGTFEY VSQFFLMOLE GQGQNFKNLR EFVFNIDGY FKLYSKHTPI NLVRQLPGGF SALEPLVDLP IGINITRFQT L---HFSYLT PGDSSSQNTA	
Gamma		CEFGQNDPF LGVYYKNNK SIMESEFRVY SSANNGTFEY VSQFFLMOLE GQGQNFKNLR EFVFNIDGY FKLYSKHTPI NLVRQLPGGF SALEPLVDLP IGINITRFQT LLALHFSYLT PGDSSSQNTA	
Delta		CEFGQNDPF LDVYYKNNK SIMESEFRVY SSANNGTFEY VSQFFLMOLE GQGQNFKNLR EFVFNIDGY FKLYSKHTPI NLVRQLPGGF SALEPLVDLP IGINITRFQT LLALHFSYLT PGDSSSQNTA	
Omicron		CEFGQNDPF LD---NNK SIMESEFRVY SSANNGTFEY VSQFFLMOLE GQGQNFKNLR EFVFNIDGY FKLYSKHTPI -IVRQLPGGF SALEPLVDLP IGINITRFQT LLALHFSYLT PGDSSSQNTA	
		261	390
Wuhan-Hu-1		GAAYYYVQL QPTIFLLKYN ENGITDAVD CALDPLSEIK CTLKSFTEY GIYQTSNFRY QPTESIVRFP NITNLCPFGF VFNATRFASV YAMNKRISN CVADYSVLN SASSTFKCY GVSPTKLNDL	
Alpha		GAAYYYVQL QPTIFLLKYN ENGITDAVD CALDPLSEIK CTLKSFTEY GIYQTSNFRY QPTESIVRFP NITNLCPFGF VFNATRFASV YAMNKRISN CVADYSVLN SASSTFKCY GVSPTKLNDL	
Beta		GAAYYYVQL QPTIFLLKYN ENGITDAVD CALDPLSEIK CTLKSFTEY GIYQTSNFRY QPTESIVRFP NITNLCPFGF VFNATRFASV YAMNKRISN CVADYSVLN SASSTFKCY GVSPTKLNDL	
Gamma		GAAYYYVQL QPTIFLLKYN ENGITDAVD CALDPLSEIK CTLKSFTEY GIYQTSNFRY QPTESIVRFP NITNLCPFGF VFNATRFASV YAMNKRISN CVADYSVLN SASSTFKCY GVSPTKLNDL	
Delta		GAAYYYVQL QPTIFLLKYN ENGITDAVD CALDPLSEIK CTLKSFTEY GIYQTSNFRY QPTESIVRFP NITNLCPFGF VFNATRFASV YAMNKRISN CVADYSVLN SASSTFKCY GVSPTKLNDL	
Omicron		GAAYYYVQL QPTIFLLKYN ENGITDAVD CALDPLSEIK CTLKSFTEY GIYQTSNFRY QPTESIVRFP NITNLCPFGF VFNATRFASV YAMNKRISN CVADYSVLN LAPPTFKCY GVSPTKLNDL	
		391	520
Wuhan-Hu-1		CTNYYADSF VIRGDEVQI APQQTQKID YNNKLPODT GOVIAMNSIN LDSKVGQYNI YLYLFRKSN LKPFERDIST EIVQAGSTPC NGVEGFNCYF PLQSYGFOPT NGVGYQPYRV VLSFELLHA	
Alpha		CTNYYADSF VIRGDEVQI APQQTQKID YNNKLPODT GOVIAMNSIN LDSKVGQYNI YLYLFRKSN LKPFERDIST EIVQAGSTPC NGVGFNCYF PLQSYGFOPT YGVGYQPYRV VLSFELLHA	
Beta		CTNYYADSF VIRGDEVQI APQQTQKID YNNKLPODT GOVIAMNSIN LDSKVGQYNI YLYLFRKSN LKPFERDIST EIVQAGSTPC NGVGFNCYF PLQSYGFOPT YGVGYQPYRV VLSFELLHA	
Gamma		CTNYYADSF VIRGDEVQI APQQTQKID YNNKLPODT GOVIAMNSIN LDSKVGQYNI YLYLFRKSN LKPFERDIST EIVQAGSTPC NGVEGFNCYF PLQSYGFOPT NGVGYQPYRV VLSFELLHA	
Delta		CTNYYADSF VIRGDEVQI APQQTQKID YNNKLPODT GOVIAMNSIN LDSKVGQYNI YLYLFRKSN LKPFERDIST EIVQAGSKPC NGVEGFNCYF PLQSYGFOPT NGVGYQPYRV VLSFELLHA	
Omicron		XXXNYYADSF VIRGDEVQI APQQTQKID YNNKLPODT GOVIAMNSIN LDSKVGQYNI YLYLFRKSN LKPFERDIST EIVQAGNPF NGVGFNCYF PLRSYSFPT YGVGYQPYRV VLSFELLHA	
		521	650
Wuhan-Hu-1		PATVGGPKS TNLNWKQVN FNFNLGTGT VLTESKNKFL PFQDQGRDIA DTTDAVRDQ TLEILDITPC SFGGVSITP GTNSNQAV LYQGVNCTEV PVAIHADQL PTNRVYSTGS NVFQTRAGCL	
Alpha		PATVGGPKS TNLNWKQVN FNFNLGTGT VLTESKNKFL PFQDQGRDIA DTTDAVRDQ TLEILDITPC SFGGVSITP GTNSNQAV LYQGVNCTEV PVAIHADQL PTNRVYSTGS NVFQTRAGCL	
Beta		PATVGGPKS TNLNWKQVN FNFNLGTGT VLTESKNKFL PFQDQGRDIA DTTDAVRDQ TLEILDITPC SFGGVSITP GTNSNQAV LYQGVNCTEV PVAIHADQL PTNRVYSTGS NVFQTRAGCL	
Gamma		PATVGGPKS TNLNWKQVN FNFNLGTGT VLTESKNKFL PFQDQGRDIA DTTDAVRDQ TLEILDITPC SFGGVSITP GTNSNQAV LYQGVNCTEV PVAIHADQL PTNRVYSTGS NVFQTRAGCL	
Delta		PATVGGPKS TNLNWKQVN FNFNLGTGT VLTESKNKFL PFQDQGRDIA DTTDAVRDQ TLEILDITPC SFGGVSITP GTNSNQAV LYQGVNCTEV PVAIHADQL PTNRVYSTGS NVFQTRAGCL	
Omicron		PATVGGPKS TNLNWKQVN FNFNLGTGT VLTESKNKFL PFQDQGRDIA DTTDAVRDQ TLEILDITPC SFGGVSITP GTNSNQAV LYQGVNCTEV PVAIHADQL PTNRVYSTGS NVFQTRAGCL	
		651	780
Wuhan-Hu-1		IGAEHNSY ECDIPIGAGI CASYQTQNS PFRARSVAS SIIAYTMSLG AENSAYSNN SIAIPTNFTI SVTTEILPVS MKTSVDOCTM YIGDSTEC NLLQYGSFC TQNLNALTGI AVEGDKNITGE	
Alpha		IGAEHNSY ECDIPIGAGI CASYQTQNS PFRARSVAS SIIAYTMSLG VENSAYSNN SIAIPTNFTI SVTTEILPVS MKTSVDOCTM YIGDSTEC NLLQYGSFC TQNLNALTGI AVEGDKNITGE	
Beta		IGAEHNSY ECDIPIGAGI CASYQTQNS PFRARSVAS SIIAYTMSLG VENSAYSNN SIAIPTNFTI SVTTEILPVS MKTSVDOCTM YIGDSTEC NLLQYGSFC TQNLNALTGI AVEGDKNITGE	
Gamma		IGAEHNSY ECDIPIGAGI CASYQTQNS PFRARSVAS SIIAYTMSLG AENSAYSNN SIAIPTNFTI SVTTEILPVS MKTSVDOCTM YIGDSTEC NLLQYGSFC TQNLNALTGI AVEGDKNITGE	
Delta		IGAEHNSY ECDIPIGAGI CASYQTQNS PFRARSVAS SIIAYTMSLG AENSAYSNN SIAIPTNFTI SVTTEILPVS MKTSVDOCTM YIGDSTEC NLLQYGSFC TQNLNALTGI AVEGDKNITGE	
Omicron		IGAEHNSY ECDIPIGAGI CASYQTQNS PFRARSVAS SIIAYTMSLG AENSAYSNN SIAIPTNFTI SVTTEILPVS MKTSVDOCTM YIGDSTEC NLLQYGSFC TQNLNALTGI AVEGDKNITGE	
		781	910
Wuhan-Hu-1		VFAQKQIYK TPIIKDFGQF NFSQILPOPS KPSKRSFIED LLFNKVTLD AGFIKQYDGC LGDIAARDLI CAQKFNGLTV LPRLLTDEMI AQYTSALLAG TITTSWTFGA GAALQIPFAM QMAYRFNGIG	
Alpha		VFAQKQIYK TPIIKDFGQF NFSQILPOPS KPSKRSFIED LLFNKVTLD AGFIKQYDGC LGDIAARDLI CAQKFNGLTV LPRLLTDEMI AQYTSALLAG TITTSWTFGA GAALQIPFAM QMAYRFNGIG	
Beta		VFAQKQIYK TPIIKDFGQF NFSQILPOPS KPSKRSFIED LLFNKVTLD AGFIKQYDGC LGDIAARDLI CAQKFNGLTV LPRLLTDEMI AQYTSALLAG TITTSWTFGA GAALQIPFAM QMAYRFNGIG	
Gamma		VFAQKQIYK TPIIKDFGQF NFSQILPOPS KPSKRSFIED LLFNKVTLD AGFIKQYDGC LGDIAARDLI CAQKFNGLTV LPRLLTDEMI AQYTSALLAG TITTSWTFGA GAALQIPFAM QMAYRFNGIG	
Delta		VFAQKQIYK TPIIKDFGQF NFSQILPOPS KPSKRSFIED LLFNKVTLD AGFIKQYDGC LGDIAARDLI CAQKFNGLTV LPRLLTDEMI AQYTSALLAG TITTSWTFGA GAALQIPFAM QMAYRFNGIG	
Omicron		VFAQKQIYK TPIIKDFGQF NFSQILPOPS KPSKRSFIED LLFNKVTLD AGFIKQYDGC LGDIAARDLI CAQKFNGLTV LPRLLTDEMI AQYTSALLAG TITTSWTFGA GAALQIPFAM QMAYRFNGIG	
		911 S 884-899	1040
Wuhan-Hu-1		VTQMLVYENQ KLIANQNSA IGIQDQSLSS TASALQKLD VVQVQALN TLVKQSSNF GAISSVLNDI LSRIDKVEAE VQIDRLITGR LOSQTYTQ TQIRAAEIRA SANLAATHMS ECVLQSKRV	
Alpha		VTQMLVYENQ KLIANQNSA IGIQDQSLSS TASALQKLD VVQVQALN TLVKQSSNF GAISSVLNDI LSRIDKVEAE VQIDRLITGR LOSQTYTQ TQIRAAEIRA SANLAATHMS ECVLQSKRV	
Beta		VTQMLVYENQ KLIANQNSA IGIQDQSLSS TASALQKLD VVQVQALN TLVKQSSNF GAISSVLNDI LSRIDKVEAE VQIDRLITGR LOSQTYTQ TQIRAAEIRA SANLAATHMS ECVLQSKRV	
Gamma		VTQMLVYENQ KLIANQNSA IGIQDQSLSS TASALQKLD VVQVQALN TLVKQSSNF GAISSVLNDI LSRIDKVEAE VQIDRLITGR LOSQTYTQ TQIRAAEIRA SANLAATHMS ECVLQSKRV	
Delta		VTQMLVYENQ KLIANQNSA IGIQDQSLSS TASALQKLD VVQVQALN TLVKQSSNF GAISSVLNDI LSRIDKVEAE VQIDRLITGR LOSQTYTQ TQIRAAEIRA SANLAATHMS ECVLQSKRV	
Omicron		VTQMLVYENQ KLIANQNSA IGIQDQSLSS TASALQKLD VVQVQALN TLVKQSSNF GAISSVLNDI LSRIDKVEAE VQIDRLITGR LOSQTYTQ TQIRAAEIRA SANLAATHMS ECVLQSKRV	
		1041	1170
Wuhan-Hu-1		DFCGKGYHLM SFQGSAPHGV VFUHVITYPA QENIFTTAPA IQHDKAHEP REGVFSNGT HMFVYQRNFY EPQIITNT FVSGNCDVVI GIVNINTYDP LQPELDSFKE ELDKYFKNHT SPOVDLGDIS	
Alpha		DFCGKGYHLM SFQGSAPHGV VFUHVITYPA QENIFTTAPA IQHDKAHEP REGVFSNGT HMFVYQRNFY EPQIITNT FVSGNCDVVI GIVNINTYDP LQPELDSFKE ELDKYFKNHT SPOVDLGDIS	
Beta		DFCGKGYHLM SFQGSAPHGV VFUHVITYPA QENIFTTAPA IQHDKAHEP REGVFSNGT HMFVYQRNFY EPQIITNT FVSGNCDVVI GIVNINTYDP LQPELDSFKE ELDKYFKNHT SPOVDLGDIS	
Gamma		DFCGKGYHLM SFQGSAPHGV VFUHVITYPA QENIFTTAPA IQHDKAHEP REGVFSNGT HMFVYQRNFY EPQIITNT FVSGNCDVVI GIVNINTYDP LQPELDSFKE ELDKYFKNHT SPOVDLGDIS	
Delta		DFCGKGYHLM SFQGSAPHGV VFUHVITYPA QENIFTTAPA IQHDKAHEP REGVFSNGT HMFVYQRNFY EPQIITNT FVSGNCDVVI GIVNINTYDP LQPELDSFKE ELDKYFKNHT SPOVDLGDIS	
Omicron		DFCGKGYHLM SFQGSAPHGV VFUHVITYPA QENIFTTAPA IQHDKAHEP REGVFSNGT HMFVYQRNFY EPQIITNT FVSGNCDVVI GIVNINTYDP LQPELDSFKE ELDKYFKNHT SPOVDLGDIS	
		1171	1273
Wuhan-Hu-1		GINASVNIQ KEIDRLNEVA HILNESLIDL DELQKYEQYI KWPWYIWLGF IAGLIAIHWY TMLCOMTSC CSCLKGCCSC GSCOKFDEDD SEPVLKGKVL HYT	
Alpha		GINASVNIQ KEIDRLNEVA HILNESLIDL DELQKYEQYI KWPWYIWLGF IAGLIAIHWY TMLCOMTSC CSCLKGCCSC GSCOKFDEDD SEPVLKGKVL HYT	
Beta		GINASVNIQ KEIDRLNEVA HILNESLIDL DELQKYEQYI KWPWYIWLGF IAGLIAIHWY TMLCOMTSC CSCLKGCCSC GSCOKFDEDD SEPVLKGKVL HYT	
Gamma		GINASVNIQ KEIDRLNEVA HILNESLIDL DELQKYEQYI KWPWYIWLGF IAGLIAIHWY TMLCOMTSC CSCLKGCCSC GSCOKFDEDD SEPVLKGKVL HYT	
Delta		GINASVNIQ KEIDRLNEVA HILNESLIDL DELQKYEQYI KWPWYIWLGF IAGLIAIHWY TMLCOMTSC CSCLKGCCSC GSCOKFDEDD SEPVLKGKVL HYT	
Omicron		GINASVNIQ KEIDRLNEVA HILNESLIDL DELQKYEQYI KWPWYIWLGF IAGLIAIHWY TMLCOMTSC CSCLKGCCSC GSCOKFDEDD SEPVLKGKVL HYT	

Supplementary Figure S4. SARS-CoV-2 spike mutation bioinformatic analysis. Changes in the subvariant (Alpha, Beta, Gamma, Delta, Omicron) spike sequences are shown compared to the reference ancestral SARS-CoV-2-1 spike sequence. The MHC class I and MHC class II pentamer sequences used in the pentamer assay are indicated with green and purple boxes, respectively. MHC: major histocompatibility complex; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Nucleocapsid Mutation (SARS-CoV -2)																
	1												N 105-113		130	
Wuhan-Hu-1	MSDNGPQNGR	NAPRI	TFGGP	SDSTGSNQNG	ERSGARSQQR	RPQGLPNNTA	SWIFTALTQHG	KEDLKFRPGQ	GVPINTNSSP	DDQIGYYRRA	TRRI	RGGDGK	MKDLSPRWYF	YY	GTGPEAG	LPYGANKDGI
Alpha	MSDNGPQNGR	NAPRI	TFGGP	SDSTGSNQNG	ERSGARSQQR	RPQGLPNNTA	SWIFTALTQHG	KEDLKFRPGQ	GVPINTNSSP	DDQIGYYRRA	TRRI	RGGDGK	MKDLSPRWYF	YY	GTGPEAG	LPYGANKDGI
Beta	MSDNGPQNGR	NAPRI	TFGGP	SDSTGSNQNG	ERSGARSQQR	RPQGLPNNTA	SWIFTALTQHG	KEDLKFRPGQ	GVPINTNSSP	DDQIGYYRRA	TRRI	RGGDGK	MKDLSPRWYF	YY	GTGPEAG	LPYGANKDGI
Gamma	MSDNGPQNGR	NAPRI	TFGGP	SDSTGSNQNG	ERSGARSQQR	RPQGLPNNTA	SWIFTALTQHG	KEDLKFRPGQ	GVPINTNSSP	DDQIGYYRRA	TRRI	RGGDGK	MKDLSPRWYF	YY	GTGPEAG	LPYGANKDGI
Delta	MSDNGPQNGR	NAPRI	TFGGP	SDSTGSNQNG	ERSGARSQQR	RPQGLPNNTA	SWIFTALTQHG	KEDLKFRPGQ	GVPINTNSSP	DDQIGYYRRA	TRRI	RGGDGK	MKDLSPRWYF	YY	GTGPEAG	LPYGANKDGI
Omicron	MSDNGPQNGR	NALRI	TFGGP	SDSTGSNQNG	XXXGARSQQR	RPQGLPNNTA	SWIFTALTQHG	KEDLKFRPGQ	GVPINTNSSP	DDQIGYYRRA	TRRI	RGGDGK	MKDLSPRWYF	YY	GTGPEAG	LPYGANKDGI
	131												N 223-231		260	
Wuhan-Hu-1	IWVATEGALN	TPKDHIGTRN	PANNAAI	VLQ	LPQGTTLPGK	FYAEGSRGGS	QASSRSSRS	RNSSRNSTPG	SSRGTSPARM	AGNGDAALA	LLLLDLNLQ	ESKMSGKGQ	QQGQTVTKKS	AAEASKKPRQ		
Alpha	IWVATEGALN	TPKDHIGTRN	PANNAAI	VLQ	LPQGTTLPGK	FYAEGSRGGS	QASSRSSRS	RNSSRNSTPG	SSRGTSPARM	AGNGDAALA	LLLLDLNLQ	ESKMSGKGQ	QQGQTVTKKS	AAEASKKPRQ		
Beta	IWVATEGALN	TPKDHIGTRN	PANNAAI	VLQ	LPQGTTLPGK	FYAEGSRGGS	QASSRSSRS	RNSSRNSTPG	SSRGTSPARM	AGNGDAALA	LLLLDLNLQ	ESKMSGKGQ	QQGQTVTKKS	AAEASKKPRQ		
Gamma	IWVATEGALN	TPKDHIGTRN	PANNAAI	VLQ	LPQGTTLPGK	FYAEGSRGGS	QASSRSSRS	RNSSRNSTPG	SSKRTSPARM	AGNGDAALA	LLLLDLNLQ	ESKMSGKGQ	QQGQTVTKKS	AAEASKKPRQ		
Delta	IWVATEGALN	TPKDHIGTRN	PANNAAI	VLQ	LPQGTTLPGK	FYAEGSRGGS	QASSRSSRS	RNSSRNSTPG	SSMGTSPARM	AGNGDAALA	LLLLDLNLQ	ESKMSGKGQ	QQGQTVTKKS	AAEASKKPRQ		
Omicron	IWVATEGALN	TPKDHIGTRN	PANNAAI	VLQ	LPQGTTLPGK	FYAEGSRGGS	QASSRSSRS	RNSSRNSTPG	SSKRTSPARM	AGNGDAALA	LLLLDLNLQ	ESKMSGKGQ	QQGQTVTKKS	AAEASKKPRQ		
	261															390
Wuhan-Hu-1	KRTATKAYNV	TQAFGRRGPE	QTQGNFGDQE	LIRQGTDYKH	WPQIAQFAPS	ASAFFGMSRI	GMEVTPSGTW	LYTGAIKLD	DKDPNFKDQV	ILLNKHIDAY	KTFPPTPEKK	DKKKKADETQ	ALPQRQKKQQ			
Alpha	KRTATKAYNV	TQAFGRRGPE	QTQGNFGDQE	LIRQGTDYKH	WPQIAQFAPS	ASAFFGMSRI	GMEVTPSGTW	LYTGAIKLD	DKDPNFKDQV	ILLNKHIDAY	KTFPPTPEKK	DKKKKADETQ	ALPQRQKKQQ			
Beta	KRTATKAYNV	TQAFGRRGPE	QTQGNFGDQE	LIRQGTDYKH	WPQIAQFAPS	ASAFFGMSRI	GMEVTPSGTW	LYTGAIKLD	DKDPNFKDQV	ILLNKHIDAY	KTFPPTPEKK	DKKKKADETQ	ALPQRQKKQQ			
Gamma	KRTATKAYNV	TQAFGRRGPE	QTQGNFGDQE	LIRQGTDYKH	WPQIAQFAPS	ASAFFGMSRI	GMEVTPSGTW	LYTGAIKLD	DKDPNFKDQV	ILLNKHIDAY	KTFPPTPEKK	DKKKKADETQ	ALPQRQKKQQ			
Delta	KRTATKAYNV	TQAFGRRGPE	QTQGNFGDQE	LIRQGTDYKH	WPQIAQFAPS	ASAFFGMSRI	GMEVTPSGTW	LYTGAIKLD	DKDPNFKDQV	ILLNKHIDAY	KTFPPTPEKK	DKKKKADETQ	ALPQRQKKQQ			
Omicron	KRTATKAYNV	TQAFGRRGPE	QTQGNFGDQE	LIRQGTDYKH	WPQIAQFAPS	ASAFFGMSRI	GMEVTPSGTW	LYTGAIKLD	DKDPNFKDQV	ILLNKHIDAY	KTFPPTPEKK	DKKKKADETQ	ALPQRQKKQQ			
	391						419									
Wuhan-Hu-1	TVTLLPAADL	DDFSKQLQQS	MSSADSTQA													
Alpha	TVTLLPAADL	DDFSKQLQQS	MSSADSTQA													
Beta	TVTLLPAADL	DDFSKQLQQS	MSSADSTQA													
Gamma	TVTLLPAADL	DDFSKQLQQS	MSSADSTQA													
Delta	TVTLLPAADL	DDFSKQLQQS	MSSADSTQA													
Omicron	TVTLLPAADL	DDFSKQLQQS	MSSADSTQA													
Membrane Mutation (SARS-CoV-2)																
	1															130
Wuhan-Hu-1	MADSGNTITV	EELKKLLEQW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLACFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
Alpha	MADSGNTITV	EELKKLLEQW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLACFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
Beta	MADSGNTITV	EELKKLLEQW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLACFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
Gamma	MADSGNTITV	EELKKLLEQW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLACFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
Delta	MADSGNTITV	EELKKLLEQW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLACFVLA	AV	YRINWITGGI	AXAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
Omicron	MADSGNTITV	EELKKLLEEW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLTCFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
BA.2.12.1	MADSGNTITV	EELKKLLEEW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLTCFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
BA.5	MADSGNTITV	EELKKLLEEW	NLVIGFLFLT	WICLLQFAYA	NNRFLYIIK	LIFLWLLWPV	TLTCFVLA	AV	YRINWITGGI	AIAMACLVLG	MLLSYF	IASF	RLFARTRSMW	SFNPETNILL	NVPLHGTILT	
	131															222
Wuhan-Hu-1	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
Alpha	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
Beta	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
Gamma	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
Delta	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
Omicron	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
BA.2.12.1	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			
BA.5	RPLLESELVI	GAVILRGHLR	IAQHILGRCD	IKDLPKEITV	ATSRTL	SYK	LGASQ	RVAGD	SGFAAYSRYR	IGNYKLNTDH	SSSSDN	IALL	VQ			

Supplementary Figure S5. SARS-CoV-2 nucleocapsid and membrane mutation bioinformatic analysis. Changes in the subvariant (Alpha, Beta, Gamma, Delta, Omicron) nucleocapsid and membrane sequences are shown compared to the reference ancestral SARS-CoV-2-1 nucleocapsid and membrane sequence. The MHC class I pentamer sequence used in the pentamer assay is indicated by a green box. MHC: major histocompatibility complex; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.



Supplementary Figure S6. VSTs educated by the ancestral SARS-CoV-2 strain recognize conserved nucleocapsid epitopes in the Omicron variant. Representative data showcasing the pronounced reactivity of VSTs against two specific Omicron BA.5 nucleocapsid-protein immunogenic epitopes, determined by Pentamer analysis. A bar graph consolidates the results from 10 donors. APC: allophycocyanin; FMO: fluorescence minus one; N: nucleocapsid; PE: phycoerythrin; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; VST: virus-specific T cell.

S1.2. Supplementary Table

Supplementary Table S1. HLA types of donors

Donor	HLA-A		HLA-B		HLA-C		HLA-DRB	
1	*02:01	*33:03	*15:07	*58:01	*03:02	*03:03	*04:03	*13:02
2	*24:02	*30:01	*07:02	*40:06	*07:02	*08:01	*01:01	*08:03
3	*24:02	*30:01	*13:02	*40:02	*03:04	*06:02	*07:01	*11:01
4	*24:02	*26:02	*51:01	*54:01	*07:02	*14:02	*11:01	*14:05
5	*02:01	*02:01	*07:02	*15:11	*03:03	*07:02	*09:01	*15:01
6	*24:02	*33:03	*40:06	*44:03	*01:02	*14:03	*09:01	*13:02
7	*02:06	*33:03	*44:03	*54:01	*01:02	*14:03	*13:02	*15:01
8	*02:07	*24:02	*15:11	*46:01	*01:02	*03:03	*09:01	*09:01
9	*02:01	*24:02	*27:05	*40:06	*01:02	*02:02	*14:05	*15:02
10	*24:02	*24:02	*15:07	*56:01	*01:02	*03:03	*04:03	*14:54
11	*02:06	*30:01	*13:02	*51:01	*06:02	*14:02	*04:05	*11:01
12	*24:02	*24:01	*51:01	*54:01	*01:02	*14:02	*04:05	*12:01
13	*24:02	*33:03	*44:03	*51:01	*07:06	*14:02	*07:01	*12:01
14	*02:06	*24:02	*07:02	*54:01	*01:02	*07:02	*01:01	*04:05
15	*02:01	*11:01	*40:02	*56:01	*01:02	*03:04	*04:05	*09:01
16	*02:01	*24:02	*13:01	*51:01	*03:04	*14:02	*09:01	*14:54
17	*24:02	*26:01	*51:01	*54:01	*07:02	*14:02	*11:01	*14:05
18	*02:01	*02:06	*37:01	*38:02	*06:02	*07:02	*08:03	*10:01
19	*02:01	*31:01	*07:02	*51:02	*07:02	*15:02	*01:01	*08:02
20	*02:01	*31:01	*15:01	*51:01	*04:01	*14:02	*04:04	*04:06
21	*02:01	*24:02	*15:18	*40:06	*07:04	*08:01	*04:05	*04:10
22	*24:02	*33:03	*51:01	*58:01	*03:02	*14:02	*13:02	*14:05
23	*11:01	*24:02	*15:01	*51:01	*04:01	*14:02	*04:06	*14:05
24	*02:01	*02:07	*15:01	*15:01	*04:01	*04:01	*04:06	*14:54
25	*01:01	*24:02	*35:01	*35:01	*03:03	*04:01	*04:07	*11:01
26	*02:01	*33:03	*55:02	*58:01	*03:02	*03:03	*12:01	*13:02
27	*24:02	*26:02	*15:07	*51:02	*03:03	*15:02	*04:03	*09:01
28	*11:01	*24:02	*54:01	*54:01	*01:02	*01:02	*04:05	*08:03
29	*02:01	*11:01	*51:01	*15:11	*03:03	*15:02	*04:05	*14:05
30	*02:01	*30:01	*40:06	*44:03	*03:03	*07:06	*07:01	*14:03
31	*24:02	*33:03	*35:01	*44:03	*03:04	*07:06	*04:03	*07:01
32	*02:01	*03:01	*13:01	*27:05	*02:02	*03:04	*04:05	*15:02
33	*24:02	*33:03	*40:06	*58:01	*03:02	*08:01	*03:01	*04:10
34	*02:01	*33:03	*44:03	*54:01	*01:02	*14:03	*08:03	*13:02
35	*02:01	*24:04	*15:01	*40:03	*01:02	*03:01	*09:01	*15:01
36	*02:01	*33:03	*15:18	*58:01	*03:02	*07:04	*04:03	*13:02
37	*26:02	*31:01	*15:18	*40:06	*07:04	*08:01	*04:01	*09:01
38	*24:02	*26:03	*15:01	*35:01	*03:03	*03:03	*09:01	*15:01
39	*11:01	*31:01	*15:18	*40:02	*03:04	*08:01	*04:51	*08:02
40	*24:02	*30:01	*52:01	*55:04	*03:03	*12:02	*04:05	*15:02

HLA: human leukocyte antigen; RBD: receptor-binding domain.