Supplemental information

Spheromers reveal robust T cell responses to the

Pfizer/BioNTech vaccine and attenuated peripheral

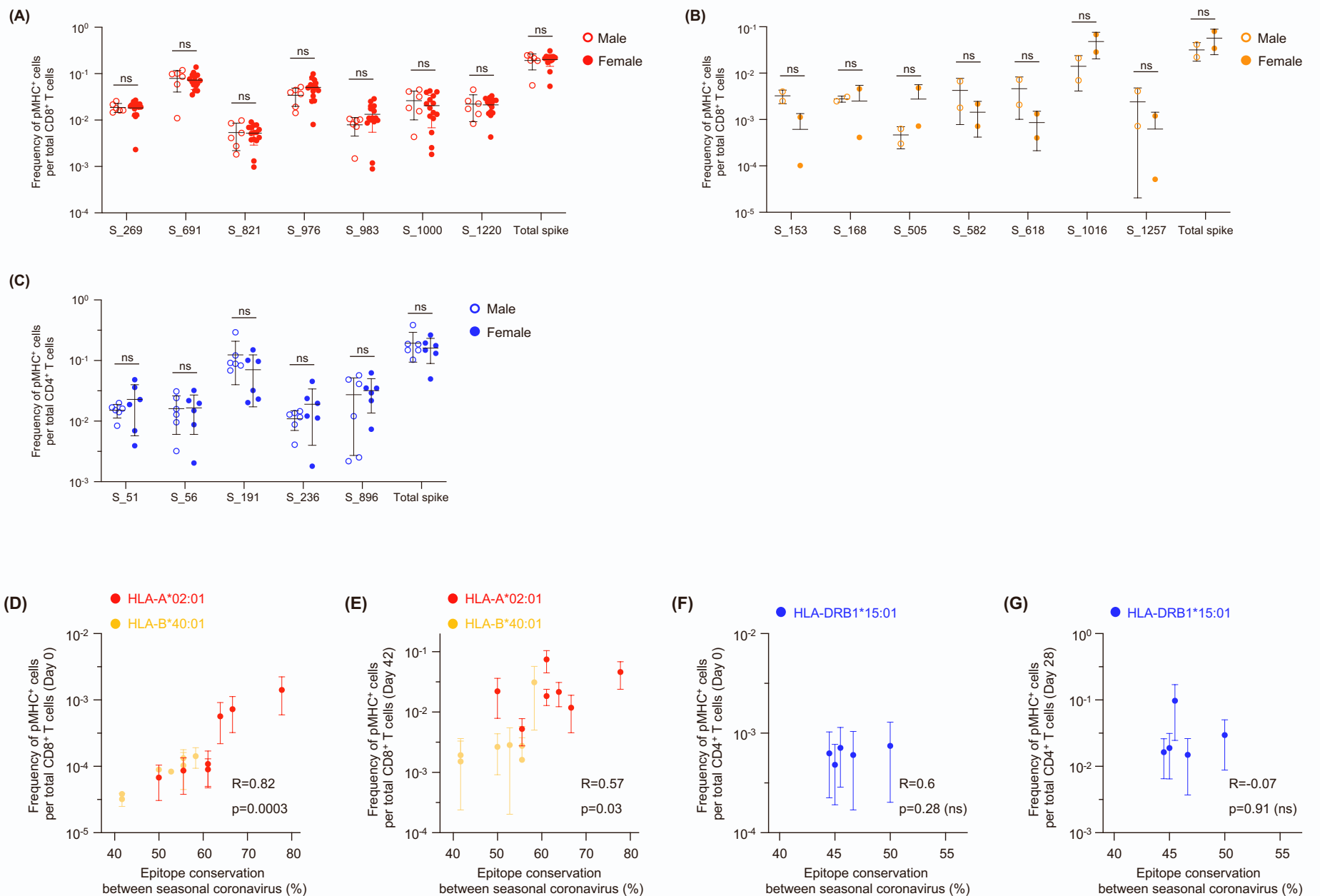# CD8+ T cell responses post SARS-CoV-2 infection

Fei Gao, Vamsee Mallajoysula, Prabhu S. Arunachalam, Kattria van der Ploeg, Monali Manohar, Katharina Röltgen, Fan Yang, Oliver Wirz, Ramona Hoh, Emily Haraguchi, Ji-Yeun Lee, Richard Willis, Vasanthi Ramachandiran, Jiefu Li, Karan Raj Kathuria, Chunfeng Li, Alexandra S. Lee, Mihir M. Shah, Sayantani B. Sindher, Joseph Gonzalez, John D. Altman, Taia T. Wang, Scott D. Boyd, Bali Pulendran, Prasanna Jagannathan, Kari C. Nadeau, and Mark.M. Davis

Supplementary table 1

HLA-restriction	Protein	ID	SARS-CoV-2 sequence
HLA-A*02:01	M	M_15	KLLEQWNLV
		M_26	FLFLTWICL
		M_61	TLACFVLAA
		M_89	GLMWLSYFI
	N	N_222	LLLDRLNQL
		N_316	GMSRIGMEV
	ORF1ab	ORF1ab_84	VMVELVAEL
		ORF1ab_1675	YLATALLTL
		ORF1ab_3013	SLPGVFCGV
		ORF1ab_3467	VLAWLYAAV
		ORF1ab_3482	FLNRFTTTL
		ORF1ab_3710	TLMNVLTLV
		ORF1ab_3732	SMWALIISV
		ORF1ab_3871	VLLSVLQQL
		ORF1ab_4032	MLFTMLRKL
		ORF1ab_4094	ALWEIQQVV
		ORF1ab_4515	TMADLVYAL
		ORF1ab_4725	IFVDGVPFV
	S	S_269	YLQPRTFLL
		S_691	SIIAYTMSL
		S_821	LLFNKVTLA
		S_976	VLNDILSRL
		S_983	RLDKVEAEV
		S_1000	RLQSLQTYV
		S_1220	FIAGLIAIV
HLA-B*40:01	N	N_322	MEVTPSGTWL
	ORF1ab	ORF1ab_1705	GEAANFCAL
		ORF1ab_2325	AEWFLAYIL
		ORF1ab_744	GETLPTEVL
		ORF1ab_1548	GEVITFDNL
		ORF1ab_2069	TEVVGDIIL
	S	S_153	MESEFRVYS
		S_168	FEYVSQPFL
		S_505	YQPYRVVVL
		S_582	LEILDITPC
		S_618	TEVPVAIHA
		S_1016	AEIRASANL
		S_1257	DEDDSEPVL
HLA-DRB1*15:01	M	M_91	MWLSYFIASFRLFAR
		M_166	KEITVATSRTLSYYK
	N	N_301	WPQIAQFAPSASAFF
		N_346	FKDQVILLNKHIDAY
	ORF1ab	ORF1ab_471	EEIAIILASFSASTS
		ORF1ab_5016	RAMPNMLRIMASLVL
	S	S_51	TQDLFLPFFSNVTWF
		S_56	LPFFSNVTWFHAIHV
		S_191	EFVFKNIDGYFKIYS
		S_236	TRFQTLLALHRSYLT
		S_896	IPFAMQMAYRFNGIG

Supplementary Table 1. List of SARS-CoV-2 epitopes evaluated in this study. Spheromers displaying these peptides in context of the indicated MHC-I/II proteins were used to study the CD8^{+} and CD4^{+} T cell responses against the SARS-CoV-2 proteins (Wuhan-1 strain). The table shows the HLA-restriction, source protein, epitope start number and peptide sequence. Related to STAR Methods.
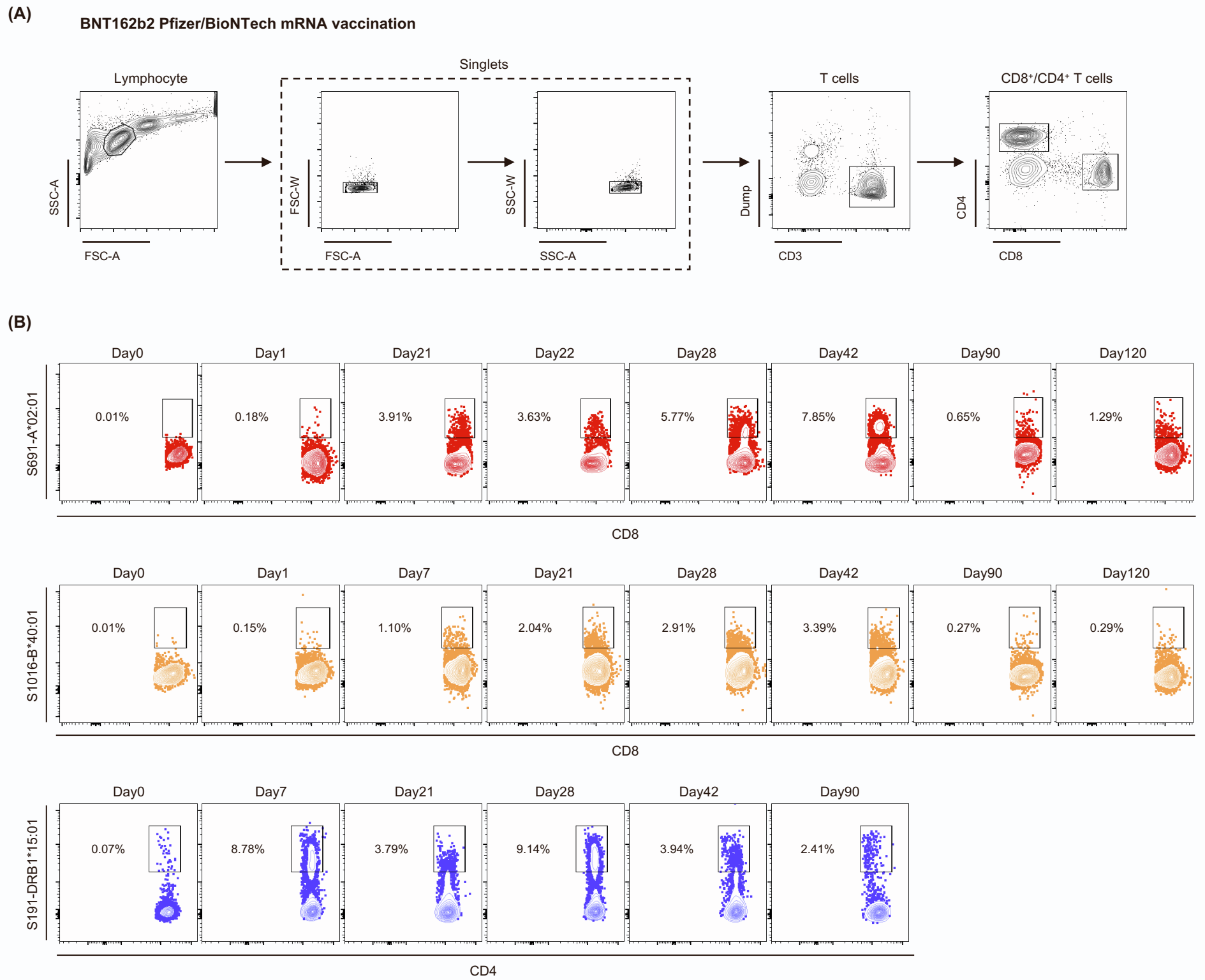
Supplementary figure 1

Supplementary figure 1. Correlation between spike-specific T cell response, sex and epitope conservation. (A and B) Correlation between epitope specific CD8+ T cell response (day 42) and sex in (A) HLA-A*02:01 and (B) HLA-B*40:01 restricted vaccinees. (C) Correlation between epitope specific CD4+ T cell response (day 28) and gender in HLA-DRB*15:01 restricted vaccinees. (D) Correlation between baseline spike-specific CD8+ T cell response (day 0) and the conservation of tested epitope. (E) Correlation between peak spike specific CD8+ T cell response (day 42) and the conservation of tested epitope. (F) Correlation between baseline spike specific CD4+ T cell response (day 0) and the conservation of tested epitope. (G) Correlation between peak spike specific CD4+ T cell response (day 28) and the conservation of tested epitope. Data are presented as mean ± SD. P values were determined by Mann–Whitney test with Holm–Šídák method. Related to Figure 1 and 2.
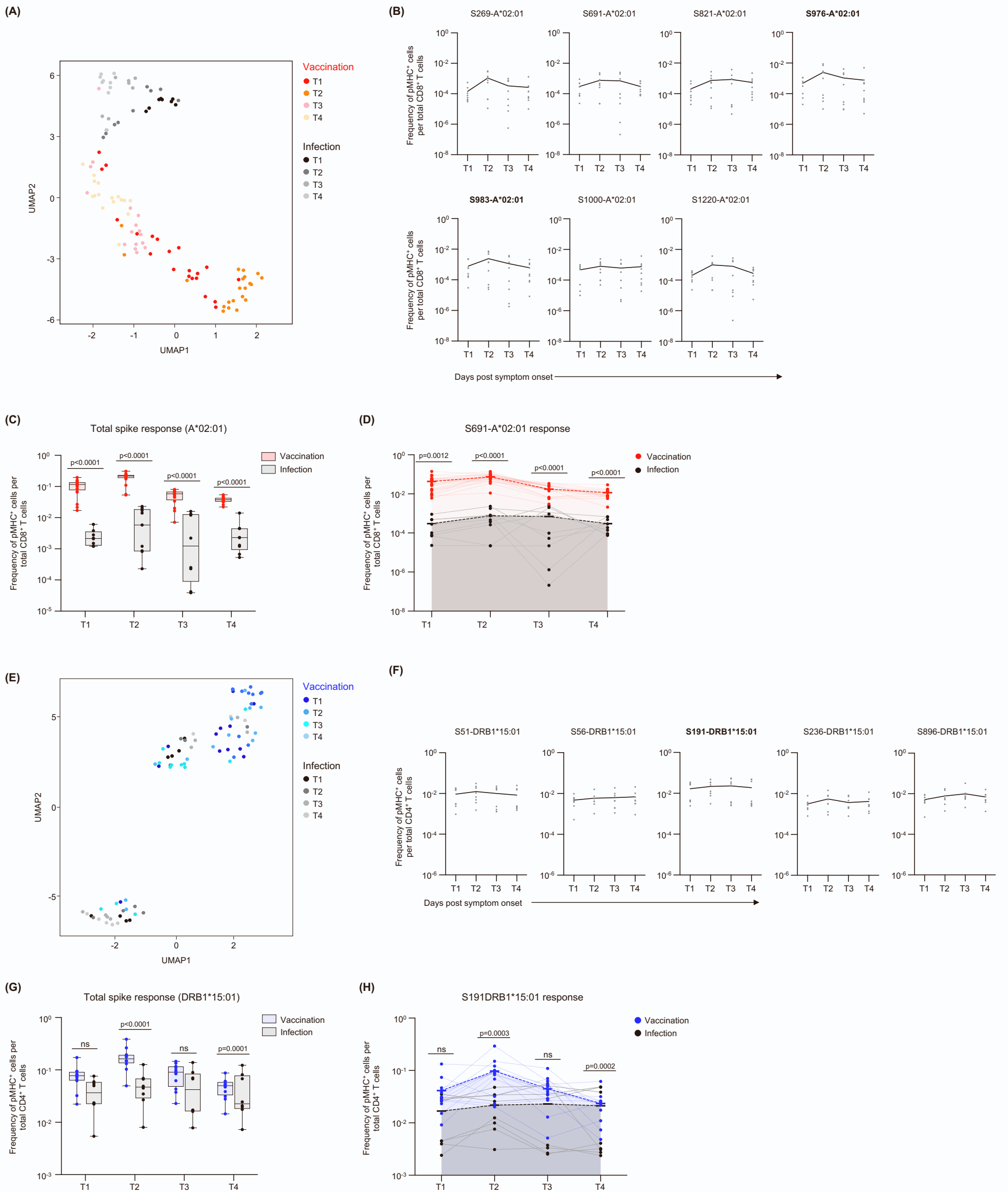
Supplementary figure 2

(A)

BNT162b2 Pfizer/BioNTech mRNA vaccination

(B)

Supplementary figure 2. Representative FACS plots of epitope-specific CD8+ and CD4+ T cells after spheromer staining.
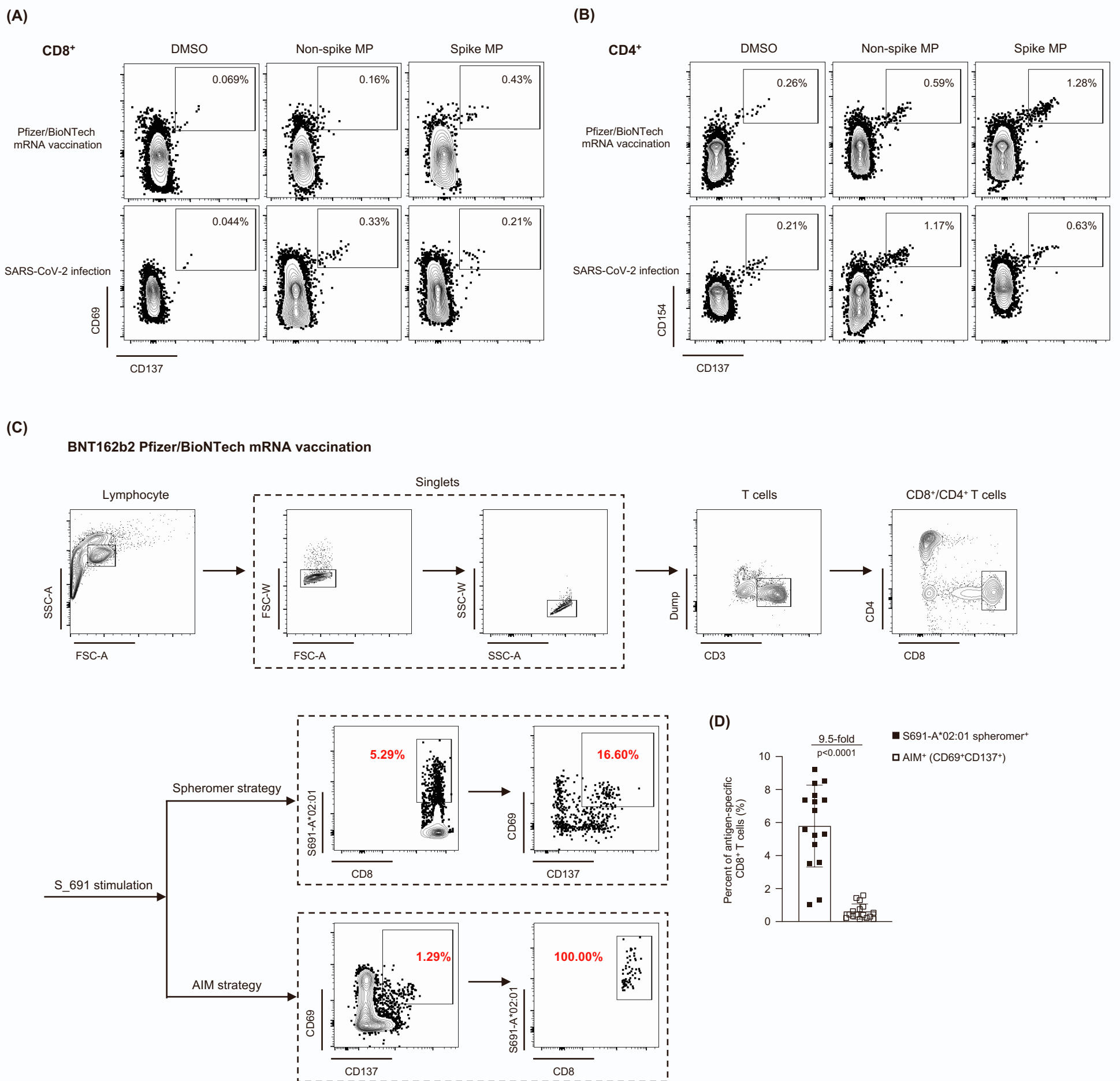
(A) Gating strategy and **(B)** representative FACS plots of dominant epitopes HLA-A*02:01/S691 (upper panel) HLA-B*40:01/S1016 (middle panel) and HLA-DRB1*15:01/S191 (lower panel) from SARS-CoV-2 naïve vaccinees. Related to Figure 1 and 2.
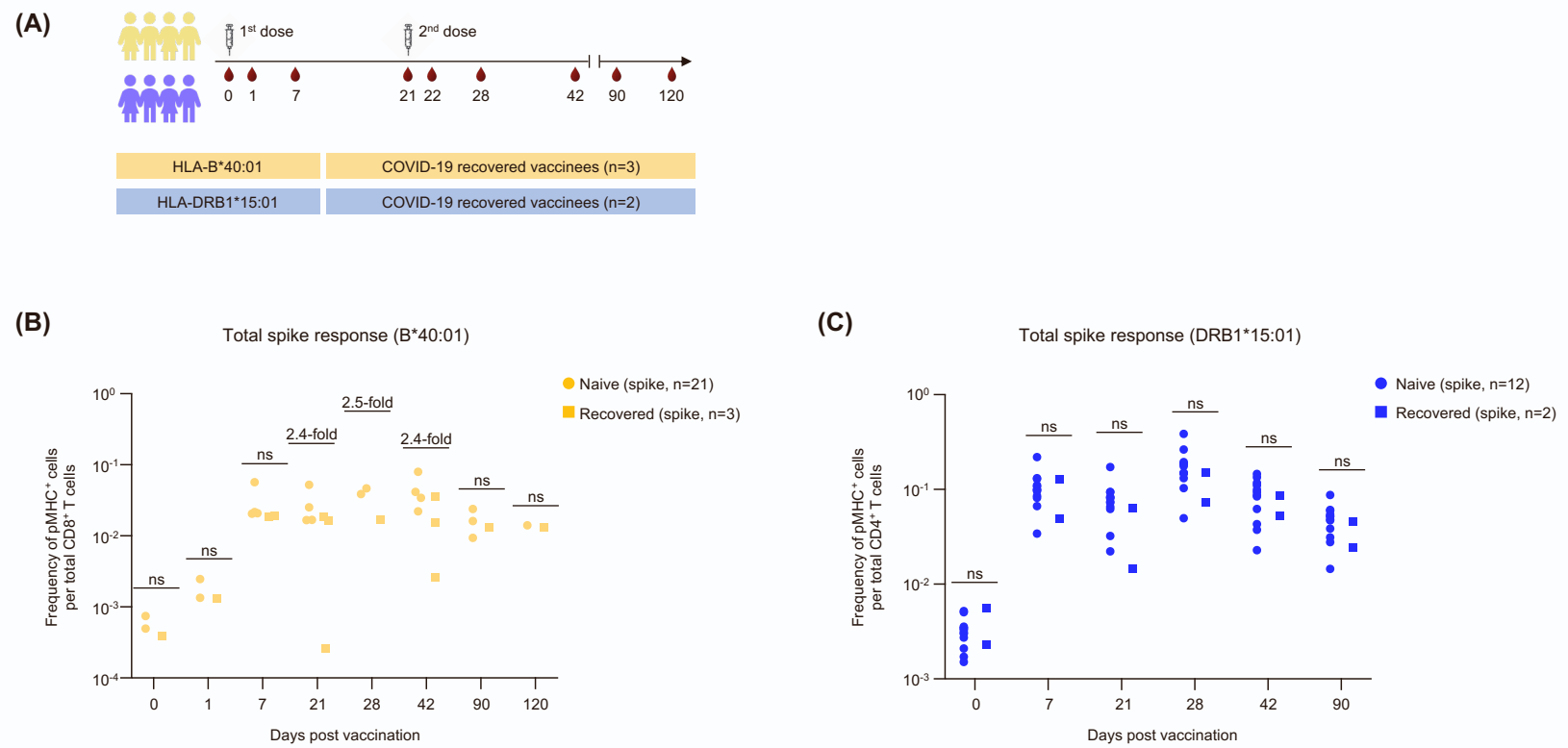
Supplementary figure 3

Supplementary figure 3. The comparison of BNT162b2 vaccination and SARS-CoV-2 infection induced spike-specific T cell response. (A) UMAP representation of flow cytometry data, depicting the trajectory of spike-specific CD8^{+} T cell profiles after vaccination and natural infection in HLA-A*02:01 restricted individuals. Each dot represents one individual. The color code indicates the time points of sample collection. **(B)** The magnitude of CD8^{+} T cell response to spike epitopes in COVID-19 patients. The comparison of CD8^{+} T cell response to **(D)** the spike protein and to **(E)** the dominant epitope (S691) between vaccinees and COVID-19 patients. **(E)** UMAP representation of flow cytometry data, depicting the trajectory of spike-specific CD4^{+} T cell profiles after vaccination and natural infection in HLA-DRB1*15:01 restricted individuals. Each dot represents one individual. The color code indicates the time points of sample collection. **(F)** The magnitude of CD4^{+} T cell response to spike epitopes in COVID-19 patients. The comparison of CD4^{+} T cell response to **(G)** the spike protein and to **(H)** the dominant epitope (1691) between vaccinees and COVID-19 patients. Related to Figure 3 and 4.

(A)

(B)

(C)

BNT162b2 Pfizer/BioNTech mRNA vaccination

(D)

Supplementary figure 4. Antigen-specific CD8⁺ T cell measured by AIM assay. (A) and **(B)** Representative FACS plots of spike and non-spike specific CD8⁺ and CD4⁺ T cells after peptide mega pools (MPs) stimulation. PBMCs of SARS-CoV-2 naïve vaccinees (day 42) and COVID-19 patients (T2) were stimulated with the spike MP, non-spike MP or DMSO. The gating strategy for the AIM assay is illustrated by representative plots defining spike-specific and non-spike-specific **(A)** CD8⁺ and **(B)** CD4⁺ T cells by expression of CD69⁺CD137⁺ and CD154⁺CD137⁺, respectively. **(C)** and **(D)** The comparison of antigen-specific CD8⁺ T cell captured by spheromer staining and AIM assay. **(C)** Gating strategy of spheromer staining (upper panel) and AIM assay (lower panel) after peptide (S691) stimulation of day 42 PBMCs samples in HLA-A*02:01 restricted naïve vaccinees. **(D)** The frequency of HLA-A*02:01/S691 specific CD8⁺ T cells captured by spheromer staining and AIM assay. Data are presented as mean ± SD. Related to Figure 3 and 4.

Supplementary figure 5. The magnitude of vaccine-elicited CD8+ and CD4+ T cell responses to spike epitopes in recovered COVID-19 patients. (A) Experimental design to study the longitudinal epitope-specific CD8+ and CD4+ T cell responses to BNT162b2 vaccine in vaccinees recovered from previous COVID-19 infection. Timeline indicating the collection of sequential blood samples from HLA-B*40:01 (day 0, 1, 7, 21, 28, 42, 90 and 120) and HLA-DRB1*15:01 (day 0, 7, 21, 28, 42 and 90) restricted recovered vaccinees. The number of donors (n) is indicated. The comparison of (B) CD8+ and (C) CD4+ T cell responses to spike protein in naïve and recovered vaccinees. Data are presented as mean ± range. Related to Figure 5.