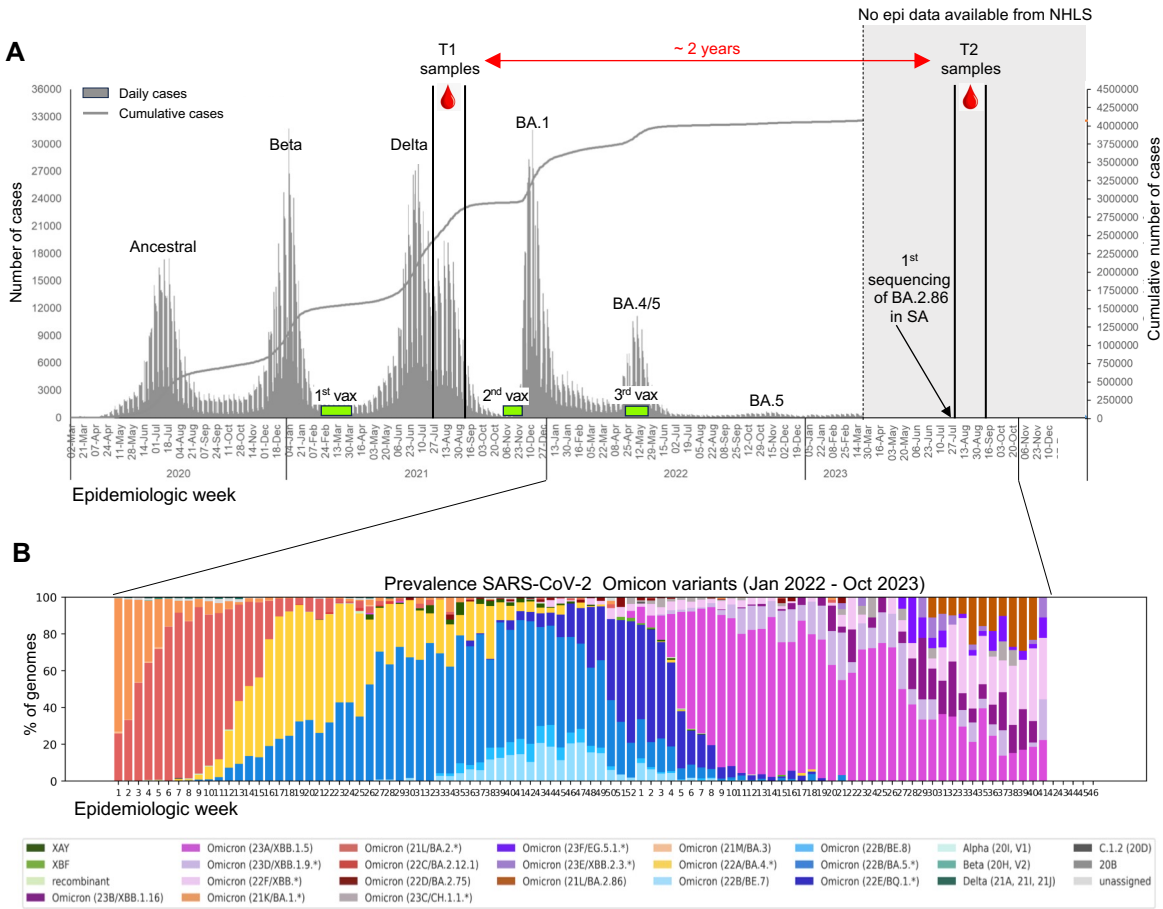
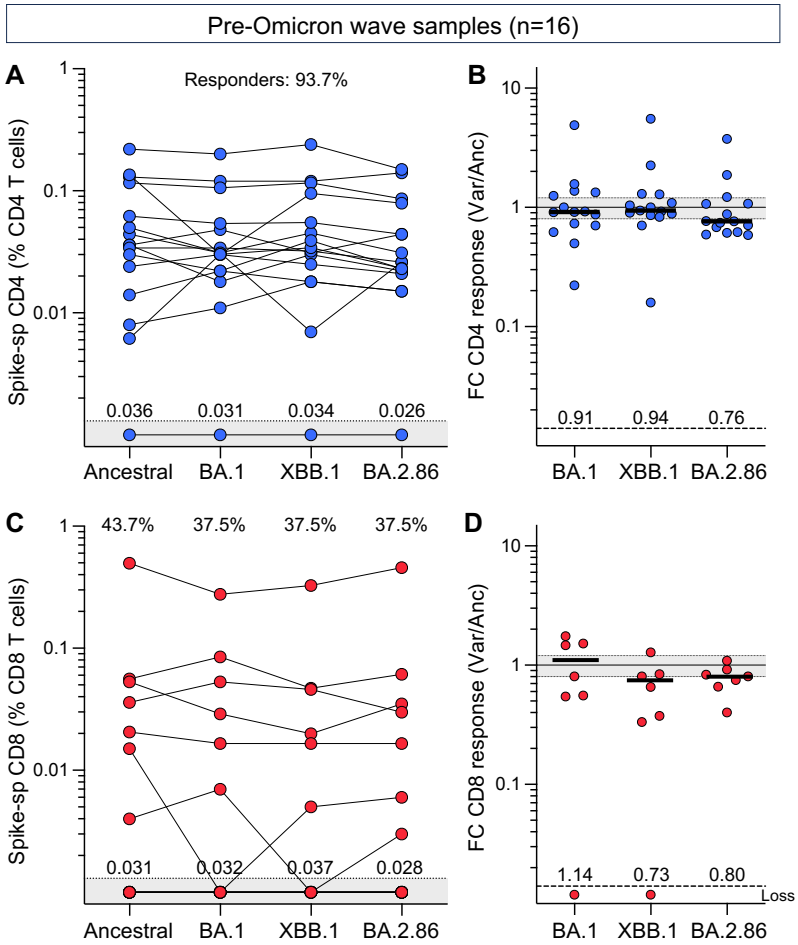


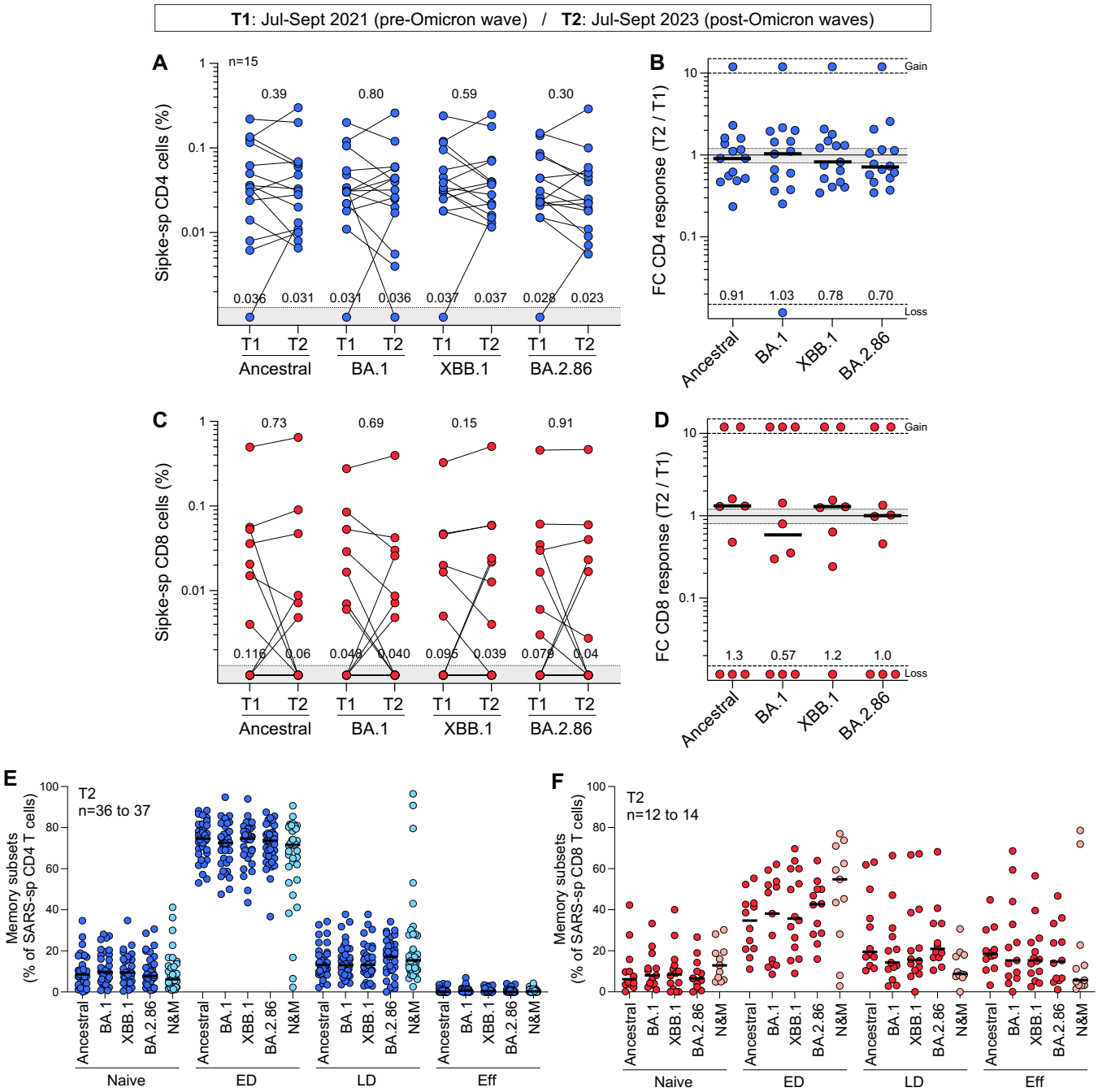
Supplementary Figure S1. Landscape of SARS-CoV-2 waves and vaccination timeline in South Africa with time of sample collection, related to Table 1.
(A) T1 samples were collected between July and September 2021 during the second phase of the Delta wave and after the first vaccination campaign. T2 samples were collected approximately 2 years after T1 between July and September 2023. In the studied cohort, most of the participants (89.7%) were vaccinated with the Ad26.COV2.S vaccine. Three participants received a heterologous vaccination regimen (Ad26.COV2.S and BNT162b2) and one participant received 3 doses of the BNT162b2 vaccine.
(B) Prevalence of Omicron sub-lineages between January 2022 and October 2023 based on 20,014 South African SARS-CoV-2 sequences from GISAID (www.gisaid.org). Epidemiologic and genomic surveillance data were obtained from the Network for Genomic Surveillance in South Africa (NGS-SA), National Institute for Communicable Diseases (NICD) of the National Health Laboratory (NHLS). https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/.



Supplementary Figure S2. CD4+ and CD8+ T cell response to the SARS-CoV-2 ancestral, BA.1, XBB.1 or BA.2.86 spike in pre-Omicron participants, related to Figure 1.

(A and C) Frequency of spike-specific CD4+ T cells (A) and spike-specific CD8+ T cells (C) producing any of the measured cytokines (IFN-γ, IL-2 or TNF-α) in 16 participants who were sampled prior to the emergence of the Omicron wave (July to Sept 2021). The proportion of responders is indicated at the top of the graphs. Median frequencies of spike-specific T cells in responders are indicated at the bottom of the graphs.

(B and D) Fold change in the frequency of spike-specific CD4+ T cells (B) and spike-specific CD8+ T cells (D) between ancestral SARS-CoV-2 and variants. Median fold change in responders is represented by a line and indicated at the bottom of the graphs. Non-cross-reactive responses ("Loss") are depicted at the bottom. No significant differences were observed between variants using a Friedman test with Dunn's multiple comparisons post-test.

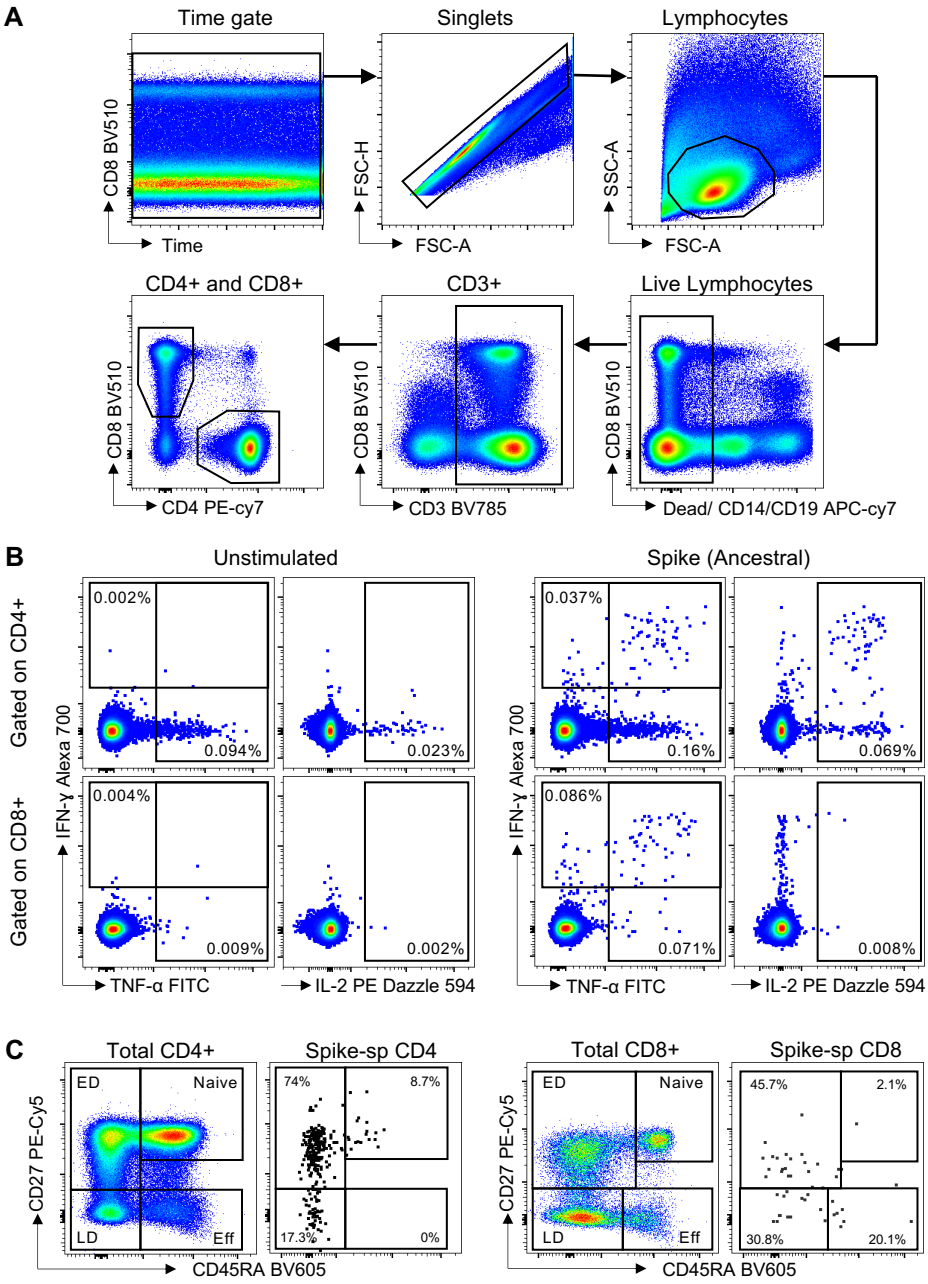


Supplementary Figure S3. Longitudinal assessment of the maintenance and memory profile of SARS-CoV-2 specific T cell responses over 2 years, related to Figure 3.

(A and C) Frequency of ancestral, BA.1, XBB.1 and BA.2.86 spike-specific CD4+ (A) and CD8+ T cells (C) in paired samples (n=15). T1 and T2 samples were collected between July and September 2021 and July and September 2023, respectively. Median frequencies of SARS-CoV-2 spike-specific T cells in responders are indicated at the bottom of the graphs. Statistical comparisons were assessed using a Wilcoxon matched-pairs signed rank test.

(B and D) Fold change in the frequency of SARS-CoV-2 spike-specific CD4+ (B) and CD8+ T cells (D) between T2 and T1. Horizontal bars represent medians, and median fold change is indicated at the bottom of each graph.

(E-F) Comparison of the memory profile of ancestral, BA.1, XBB.1, BA.2.86 spike-specific and N&M-specific CD4+ T cells (E) and CD8+ T cells (F) at T2. To define the memory phenotype of spike-specific T cells, a cut-off of 30 events was used.



Supplementary Figure S4. Gating strategy, related to STAR methods (cell stimulation and flow cytometry staining).

(A) Nested gating strategy to identify CD4+ and CD8+ T cell populations.

(B) Expression of IFN-γ, IL-2 and TNF-α in CD4+ and CD8+ T cells upon stimulation with spike peptide pool.

(C) Identification of memory subsets in the total and SARS-CoV-2-specific CD4+ T cells (left panel) and CD8+ T cells (right panel) based on the expression of CD27 and CD45RA. Naïve: CD45RA+CD27+, Early differentiated (ED): CD45RA-CD27+, Late differentiated (ED): CD45RA-CD27-, and Effector (Eff): CD45RA+CD27-.

Supplementary Table S1: List of spike amino acid mutations in BA.1, XBB.1 and BA.2.86 as compared to Wuhan-1. "-": deletion, related to STAR Methods (SARS-CoV-2 antigens).

Position aa	BA.1	XBB.1	BA.2.86
16			MPLFV
19		I	I
21			T
24		-	-
25		-	-
26		-	-
27		S	S
50			L
67	V		
69	-		-
70	-		-
83		A	
95	I		
127			F
142	D	D	D
143	-		
144	-	Q	-
145	-		
146		-	
152	W		
157			S
158			G
183		E	
211	-		-
212	I		I
213		E	G
214	EPE		
216			F
245			N
252		V	
264			D
332			V
339	D	H	H
346		T	
356			T
368		I	
371	L	F	F
373	P	P	P
375	F	F	F
376		A	A
403			K
405		N	N
408		S	S
417	N	N	N
440	K	K	K
445		P	H
446	S	S	S
450			D
452			W
460		K	K
477	N	N	N
478	K	K	K
481			K
483			-
484	A	A	K
486		S	P
490		S	
493	R		
496	S		
498	R	R	R
501	Y	Y	Y
505	H	H	H
547	K		
554			K
570			V
614	G	G	G
621			S
655	Y	Y	Y
670			V
679	K	K	K
681	H	H	R
764	K	K	K
796	Y	Y	Y
856	K		
939			F
954	H	H	H
969	K	K	K
981	F		
1143			L