Cell Reports, Volume *40*

Supplemental information

The generation of stem cell-like memory cells early after BNT162b2 vaccination is associated with durability of memory CD8$^+$ T cell responses

Sungmin Jung, Jae Hyung Jung, Ji Yun Noh, Woo-Joong Kim, Soo-Young Yoon, Jongtak Jung, Eu Suk Kim, Hong Bin Kim, Hee Jin Cheong, Woo Joo Kim, Su-Hyung Park, Kyoung-Ho Song, Joon Young Song, and Eui-Cheol Shin

Table S1. Characteristics of the BNT162b2-vaccinated individuals with SARS-CoV-2 MHC class I multimer^{+}CD8^{+} T cells, Related to Figures 1-4.

Donor number	Donor characteristics			Multimer	Time points (days post-vaccination)						
					Pre-vaccination	Post-first		Post-second			
	Sex	Age (yr)	HLA type		0	1 week	3 weeks	1-2 weeks	3 weeks	12 weeks	24 weeks
7	M	44	HLA-A*02	S_{269}, S_{1000}	0	7	18	7	21	84	168
15	F	30	HLA-A*02	S_{269}	0	9	20	8	22	87	170
16	F	28	HLA-A*02	S_{269}, S_{1000}	0	8	19	8	21	86	169
21	M	34	HLA-A*02	S_{269}	0	8	19	8	21	86	173
22	M	34	HLA-A*02	S_{269}	0	7	20	7	25	85	168
24	F	24	HLA-A*02	S_{269}	0	9	20	8	22	84	170
28	F	38	HLA-A*02	S_{269}	0	8	19	8	21	84	168
30	F	56	HLA-A*02	S_{269}	0	9	20	8	22	84	170
32	F	29	HLA-A*02	S_{269}	0	8	19	8	21	84	166
33	F	35	HLA-A*02	S_{269}, S_{1000}	0	8	19	8	21	86	169
45	F	28	HLA-A*02	S_{269}	0	7	18	11	21	86	169
78	F	27	HLA-A*02	S_{269}	0	7	21	8	21	84	169

Yr, year; M, male; F, female.

Table S2. Characteristics of the BNT162b2-vaccinated individuals with SARS-CoV-2 MHC class I multimer^{+}CD8^{+} T cells, Related to Figure 4.

Donor number	Donor characteristics			Multimer	Time points (days post-vaccination)						
					Pre-vaccination	Post-first		Post-second			
	Sex	Age (yr)	HLA type		0	1 week	3 weeks	1-2 weeks	3 weeks	12 weeks	24 weeks
20	F	42	HLA-A*24	S_{448}	0	NA	NA	7	NA	NA	164
25	F	25	HLA-A*24	S_{448}	0	NA	NA	8	NA	NA	170
BH67	F	26	HLA-A*02	S_{269}	0	NA	NA	15	NA	NA	154
BH68	F	26	HLA-A*02	S_{269}	0	NA	NA	13	NA	NA	154
BH70	F	31	HLA-A*02	S_{269}	0	NA	NA	13	NA	NA	153
BH71	F	33	HLA-A*02	S_{269}	0	NA	NA	14	NA	NA	155
BH72	F	30	HLA-A*02	S_{269}	0	NA	NA	13	NA	NA	153
BH73	F	27	HLA-A*02	S_{269}	0	NA	NA	14	NA	NA	159
BH78	F	28	HLA-A*02	S_{269}	0	NA	NA	13	NA	NA	152
BH82	F	26	HLA-A*02	S_{269}	0	NA	NA	14	NA	NA	157

Yr, year; M, male; F, female.

Table S3. Profiling details of the BNT162b2-vaccinated individuals with SARS-CoV-2 MHC class I multimer^{+}CD8^{+} T cells, Related to Figures 1-4.

Data		Frequency of multimer^{+}CD8^{+} T cells (Fig. 1)							CD38/HLA-DR/CD127/KLRG1 expression on multimer^{+}CD8^{+} T cells (Fig. 2)						T-bet/Bcl-2/Ki-67 expression on multimer^{+}CD8^{+} T cells (Fig. 2)						Differentiation of multimer^{+}CD8^{+} T cells (Fig. 3)						Correlation (Fig. 4)	
Donor number	Multimer	Time points (weeks post-vaccination)																											
		Pre-vaccination	Post-first		Post-second				Post-first		Post-second				Post-first		Post-second				Post-first		Post-second				Post-second	
		0	1	3	1	3	12	24	1	3	1	3	12	24	1	3	1	3	12	24	1	3	1	3	12	24	1-2	24
N07	S_{269}	O	O	O	O	O	O	O		O	O	O				O	O	O	O	O	O	O	O	O	O		O	O
N15	S_{269}	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
N16	S_{269}	O	O	O	O	O	O	O		O			O	O		O			O		O	O	O	O	O	O	O	O
N21	S_{269}	O	O	O	O	O	O	O	O		O	O	O	O	O	O	O	O			O		O	O	O	O	O	O
N22	S_{269}			O	O	O	O	O		O		O		O		O		O				O	O	O	O	O	O	O
N24	S_{269}			O	O	O	O	O		O	O		O	O		O	O		O			O	O	O	O	O	O	O
N28	S_{269}	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
N30	S_{269}	O	O	O	O	O	O	O	O	O	O	O	O		O	O	O	O	O		O	O	O	O	O	O	O	O
N32	S_{269}	O	O	O	O	O	O	O	O	O	O	O			O	O	O	O	O		O	O	O	O	O		O	O
N33	S_{269}		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
N45	S_{269}	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
N78	S_{269}	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

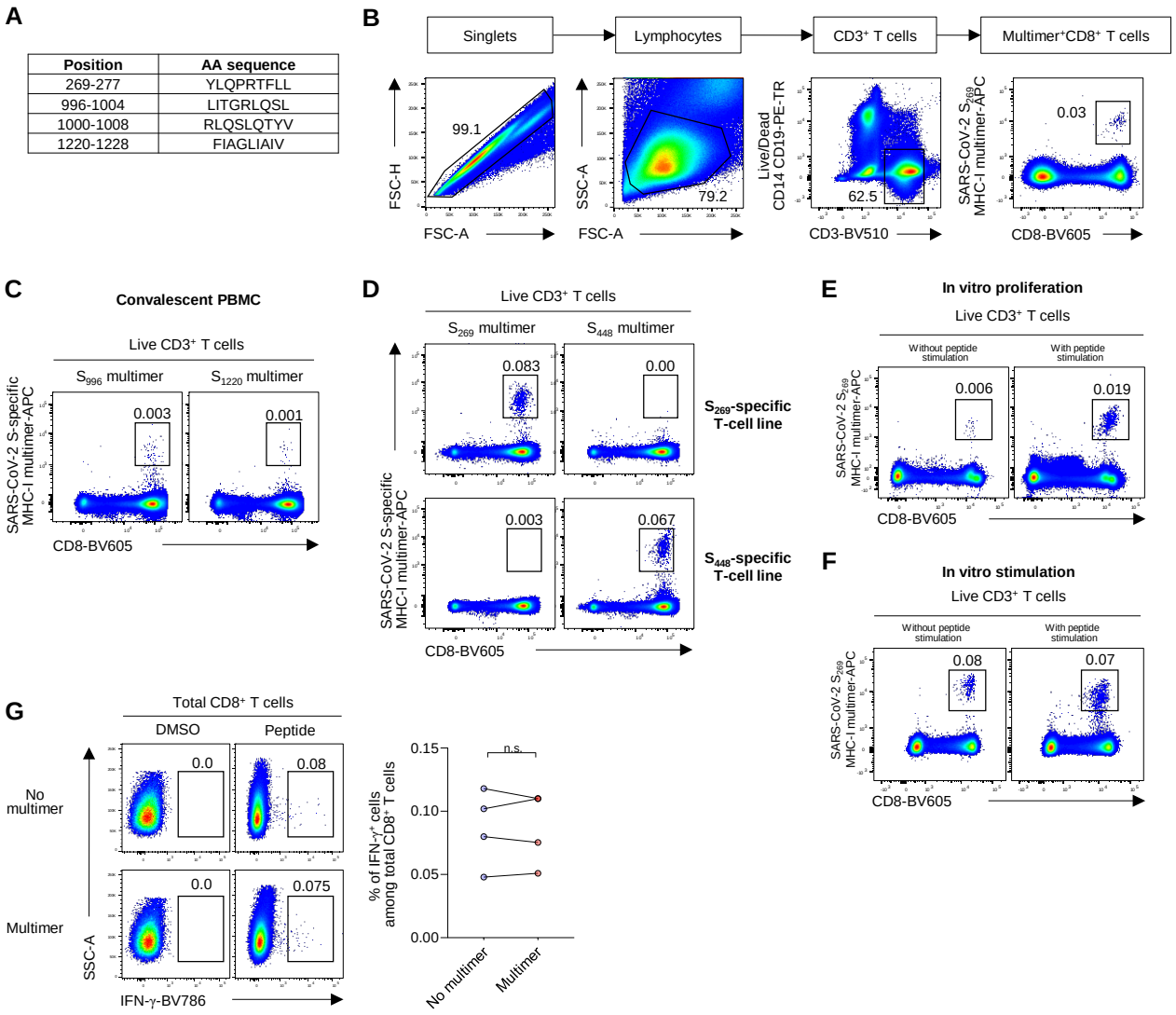

Figure S1. Detection of SARS-CoV-2 S-specific multimer$^+$CD8$^+$ T cells and validation, Related to Figure 1.
(A) Characteristics of SARS-CoV-2 S-specific MHC-I multimers utilized in this study.
(B) Gating strategy used for the detection of SARS-CoV-2 S-specific multimer$^+$CD8$^+$ T cells.
(C) S_{996} and S_{1220}-specific T-cell lines were generated by stimulating PBMCs from HLA-A*02(+) donors who recovered from SARS-CoV-2 infection with S_{996} (LITGRLQSL) or S_{1220} (FIAGLIAIV) peptides and recombinant human IL-2 (200 U/mL) for 2 weeks. Examples of S_{996} or S_{1220} multimer staining are presented.
(D) S_{269}-specific and S_{448}-specific T-cell lines were generated by stimulating PBMCs from HLA-A*02(+)24(-) and HLA-A*02(-)24(+) BNT162b2-vaccinated individuals with S_{269} (YLQPRTFLL) and S_{448} (NYNYLYRLF) peptides and recombinant human IL-2 (200 U/mL), respectively, for 2 weeks. The generated T cells were successfully stained with the corresponding multimer: HLA-A*0201 S_{269} or HLA-A*2402 S_{448}. However, multimer$^+$ cells were not detected in HLA-mismatched cross-staining.
(E-F) Representative flow cytometry plots showing S_{269} multimer$^+$ cells after S_{269} peptide stimulation of CTV-labeled PBMCs for (E) 120 h or (F) 6 h.
(G) Validation of MHC-I multimer-combined IFN-γ intracellular staining (ICS). IFN-γ ICS was performed with or without S_{269} multimer staining using PBMCs from BNT162b2-vaccinated individuals (n=4; PBMCs obtained 6 months post-second vaccination). Left: Representative flow cytometry plots showing PBMCs from one individual. Right: Summary graph showing the frequency of IFN-γ$^+$ cells among CD8$^+$ T cells. Statistical analysis was performed using paired Wilcoxon signed rank test (n.s., not significant).

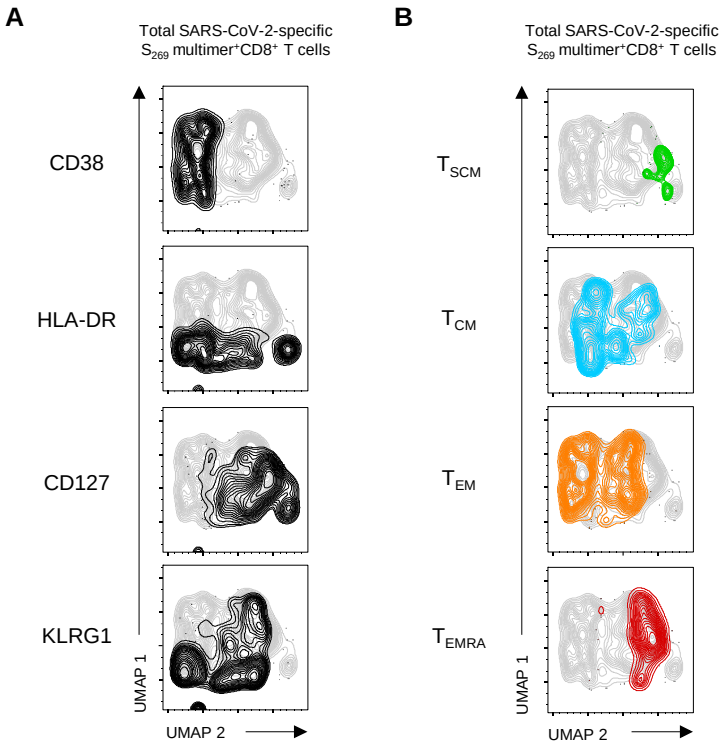

Figure S2. UMAP plot of S_{269} multimer$^+$CD8$^+$ T cells in BNT162b2-vaccinated individuals over time, Related to Figure 2.

Pooled S_{269} multimer$^+$CD8$^+$ T cells from multiple patients. (A) Multimer$^+$ cells expressing the indicated markers. (B) Multimer$^+$ cells exhibiting each memory subset. Each time point consists of 4-5 patients with a total of 650-750 cells.

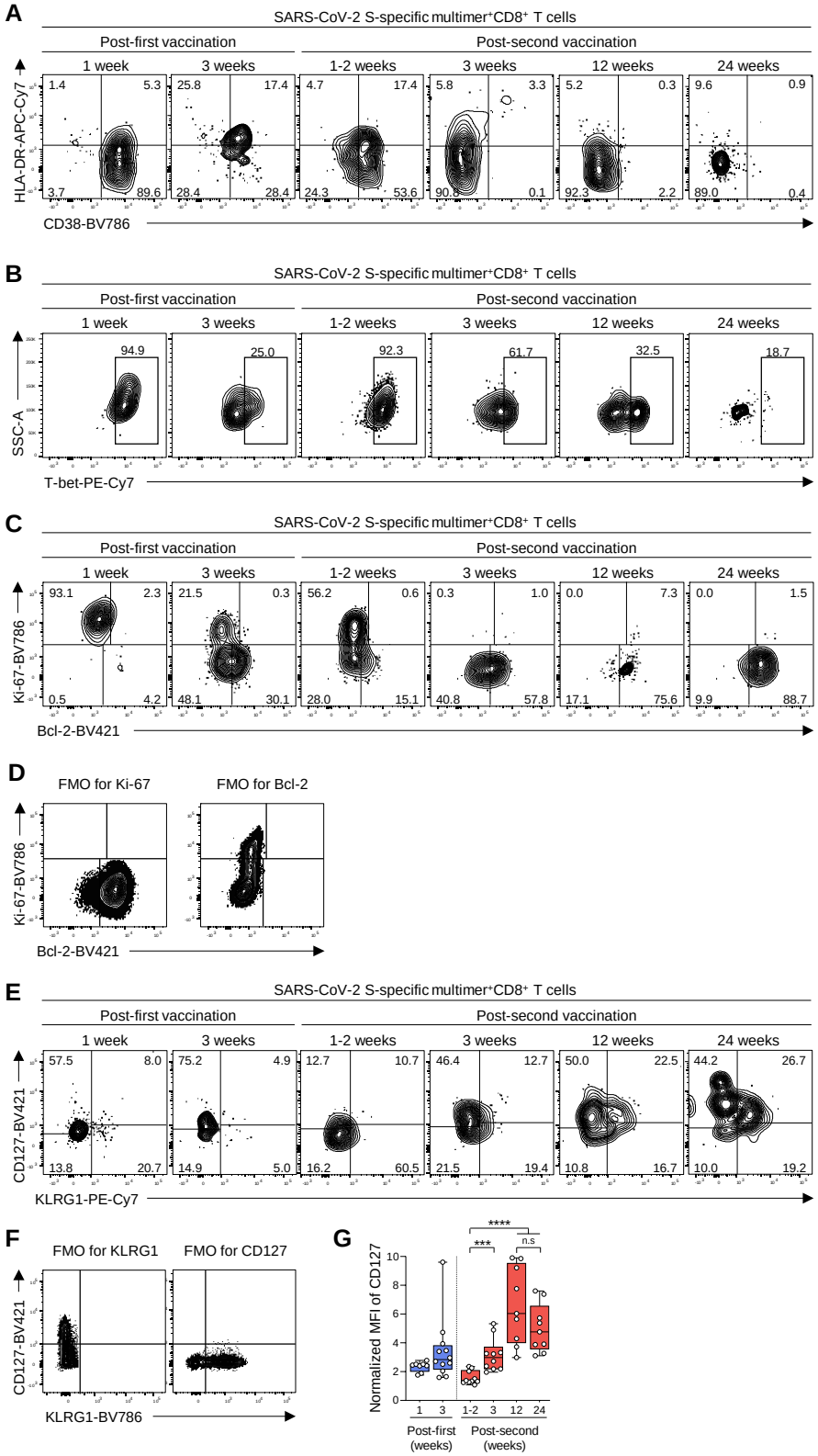

Figure S3. Phenotypes of S_{269} multimer$^+$CD8$^+$ T cells in BNT162b2-vaccinated individuals over time, Related to Figure 2.

(A-C, E) Representative flow cytometry plots showing the percentages of indicated subpopulations among S_{269} multimer$^+$CD8$^+$ T cells.

(D, F) Flow cytometry plots showing the staining of fluorescence-minus-one (FMO) controls for Ki-67, Bcl-2, KLRG1, and CD127.

(G) Normalized MFI of CD127 (MFI of CD127 on S_{269} multimer$^+$CD8$^+$ T cells / MFI of CD127 on CD8$^+$CD127$^-$ T cells) in vaccinated individuals over time summarized in box graphs. Post-first 1 week (n=8) and 3 weeks (n=12); post-second 1-2 weeks (n=10), 3 weeks (n=10), 12 weeks (n=9) and 24 weeks (n=9). Statistical analysis was performed using unpaired Mann-Whitney U test (***$p < 0.001$, ****$p < 0.0001$, n.s., not significant). MFI, median fluorescence intensity.

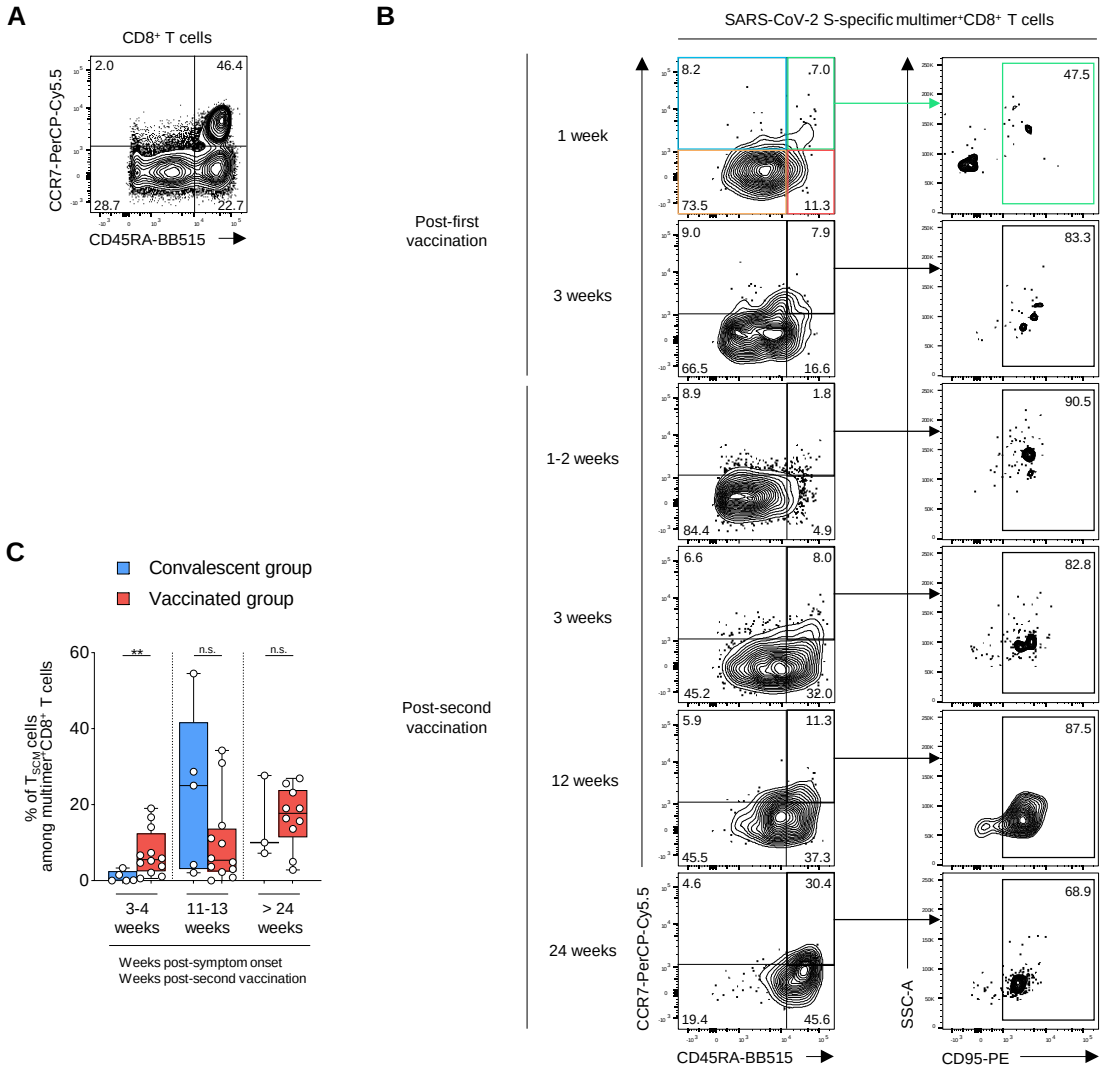

Figure S4. Differentiation kinetics of S_{269} multimer$^+$CD8$^+$ T cells in BNT162b2-vaccinated individuals over time, Related to Figure 3.

(A) Flow cytometry plots showing the expression of CCR7 and CD45RA on total CD8$^+$ T cells.

(B) Representative flow cytometry plots showing the expression of T cell differentiation markers CCR7, CD45RA, and CD95 among S_{269} multimer$^+$CD8$^+$ T cells.

(C) Frequency of T_{SCM} cells among S_{269} multimer$^+$ cells compared between convalescent and vaccinated individuals 3-4 weeks (n=5 vs. n=12), 11-13 weeks (n=5 vs. n=12), and after 24 weeks (n=3 vs. n=10) post-symptom onset or post-second vaccination. The data of convalescent individuals were retrieved from our previous study (Jung et al., 2021). Statistical analysis was performed using unpaired Mann-Whitney U test (**p < 0.01, n.s., not significant).

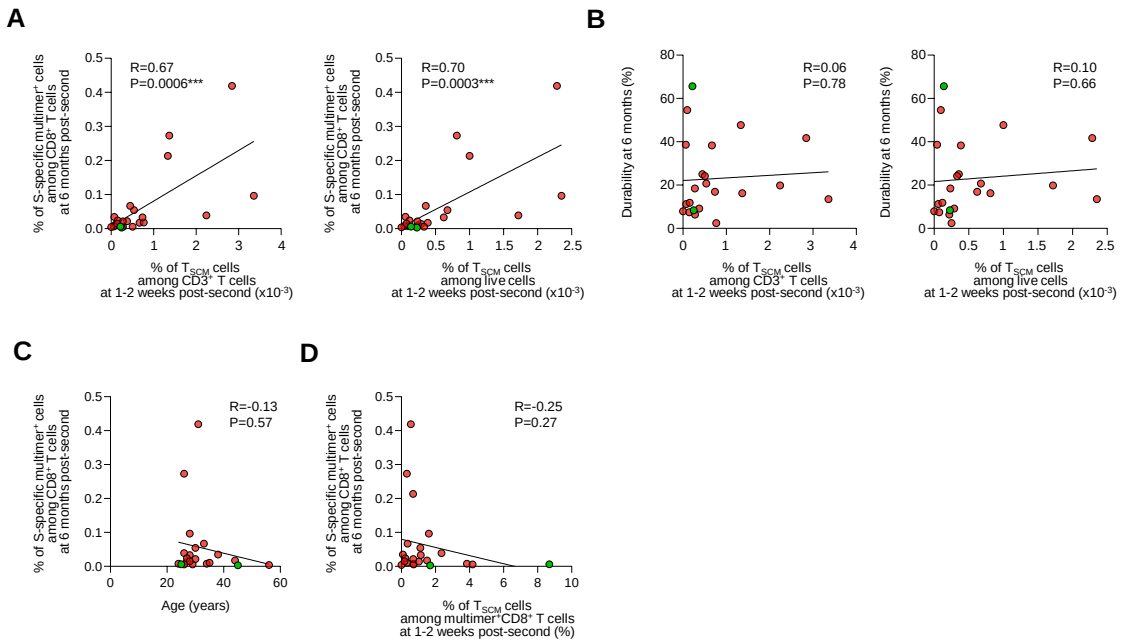

Figure S5. Correlation between the frequency of SARS-CoV-2 S-specific MHC-I multimer$^+$CD8$^+$ T cells, T_{SCM} cells and age, Related to Figure 4.

(A-B) Correlation graphs showing the relationship between the frequency of T_{SCM} cells among CD3$^+$ T cells or live cells 1-2 weeks post-second vaccination and (A) the frequency of SARS-CoV-2 S-specific multimer$^+$CD8$^+$ T cells 6 months post-second vaccination or (B) 6-month durability of S-specific multimer$^+$CD8$^+$ T cells (red = S_{269}, n=20; green = S_{448}, n=2) with black lines representing linear regression.

(C-D) Correlation between the frequency of multimer$^+$ cells among CD8$^+$ T cells 6 months post-second vaccination and (C) donor age or (D) the percentage of T_{SCM} cells among multimer$^+$ cells 1-2 weeks post-second vaccination (red = S_{269}, n=20; green = S_{448}, n=2) with black lines representing linear regression.

Statistical analysis was performed using Spearman correlation test with linear regression analysis (***$p < 0.001$).