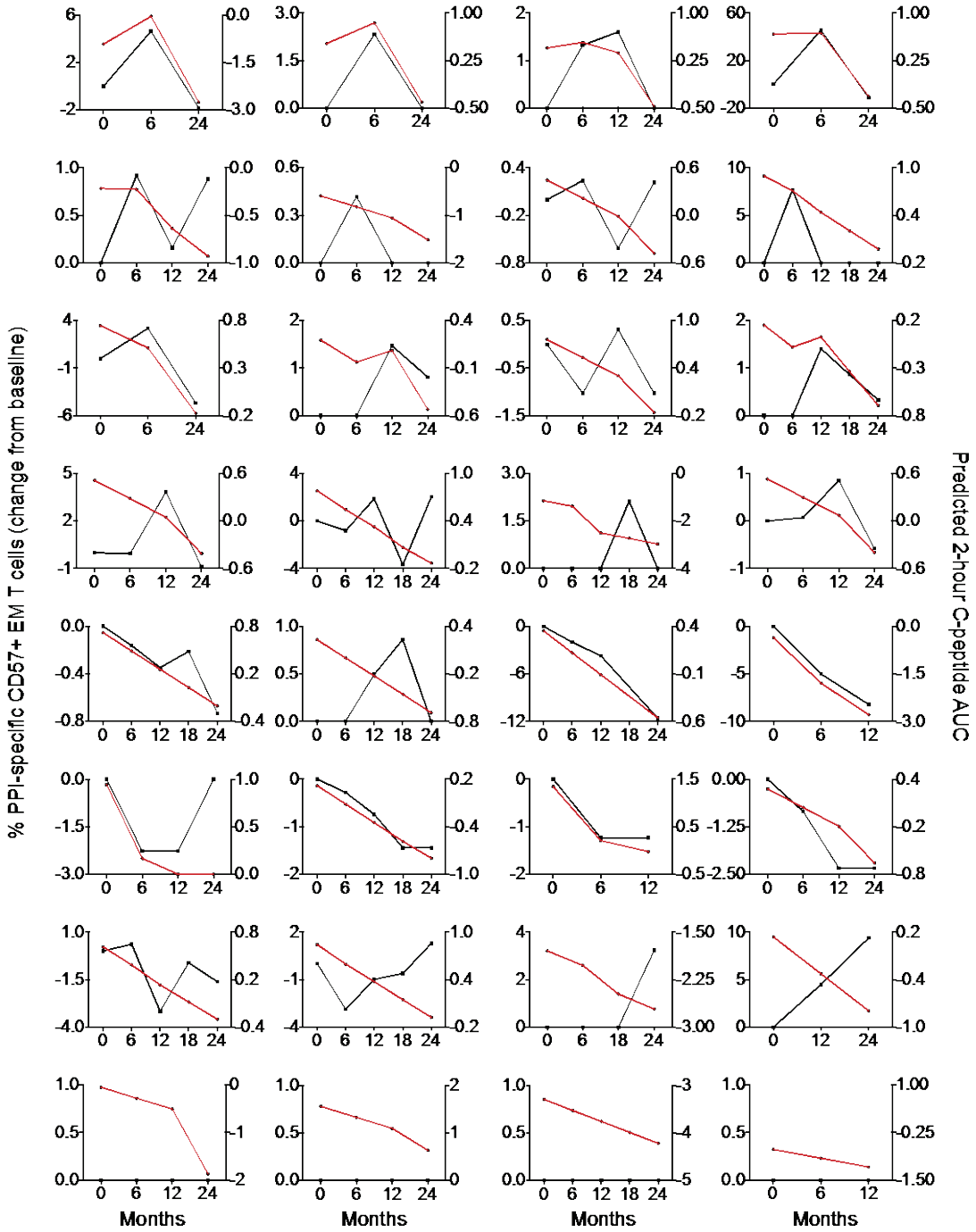
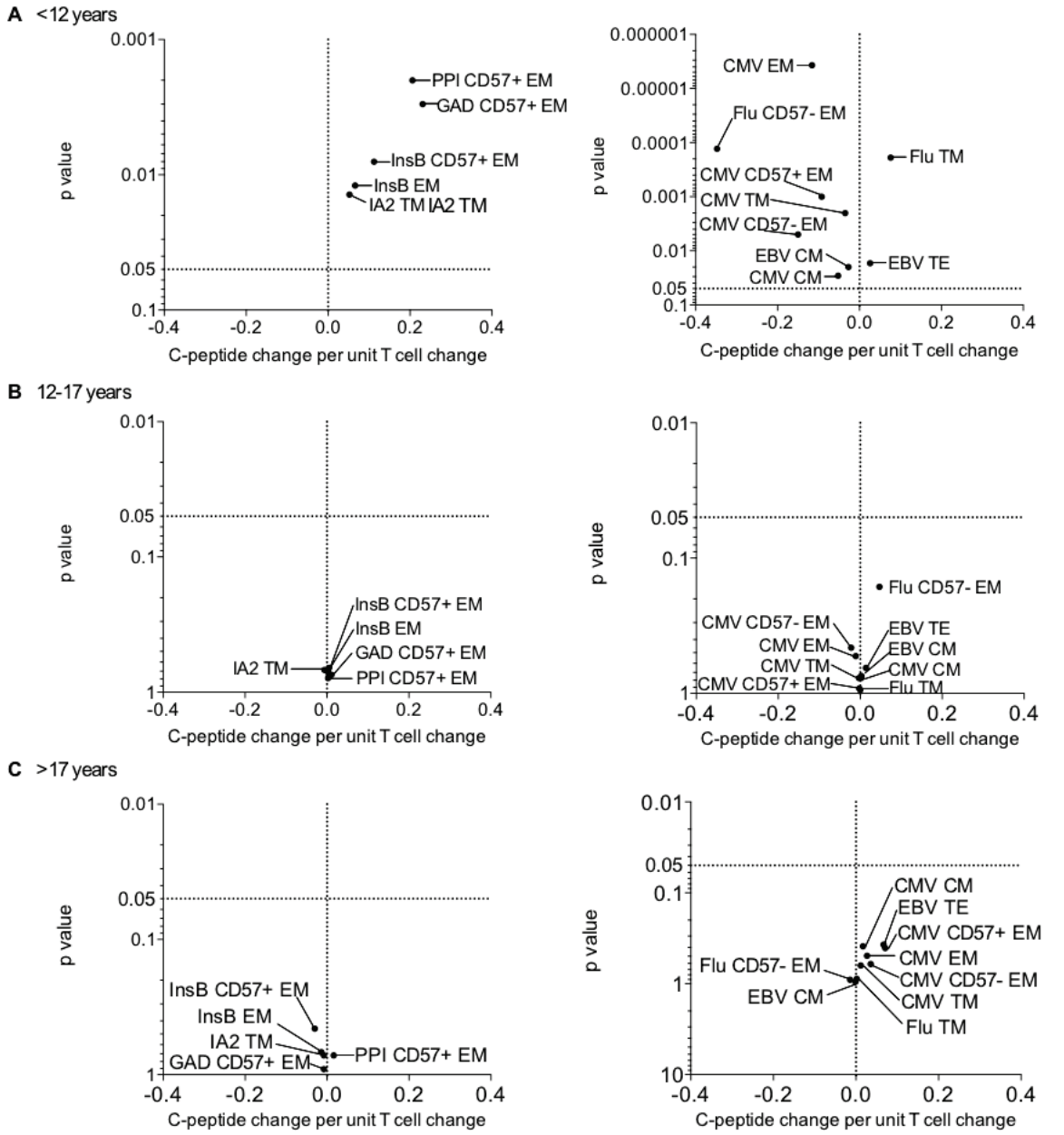
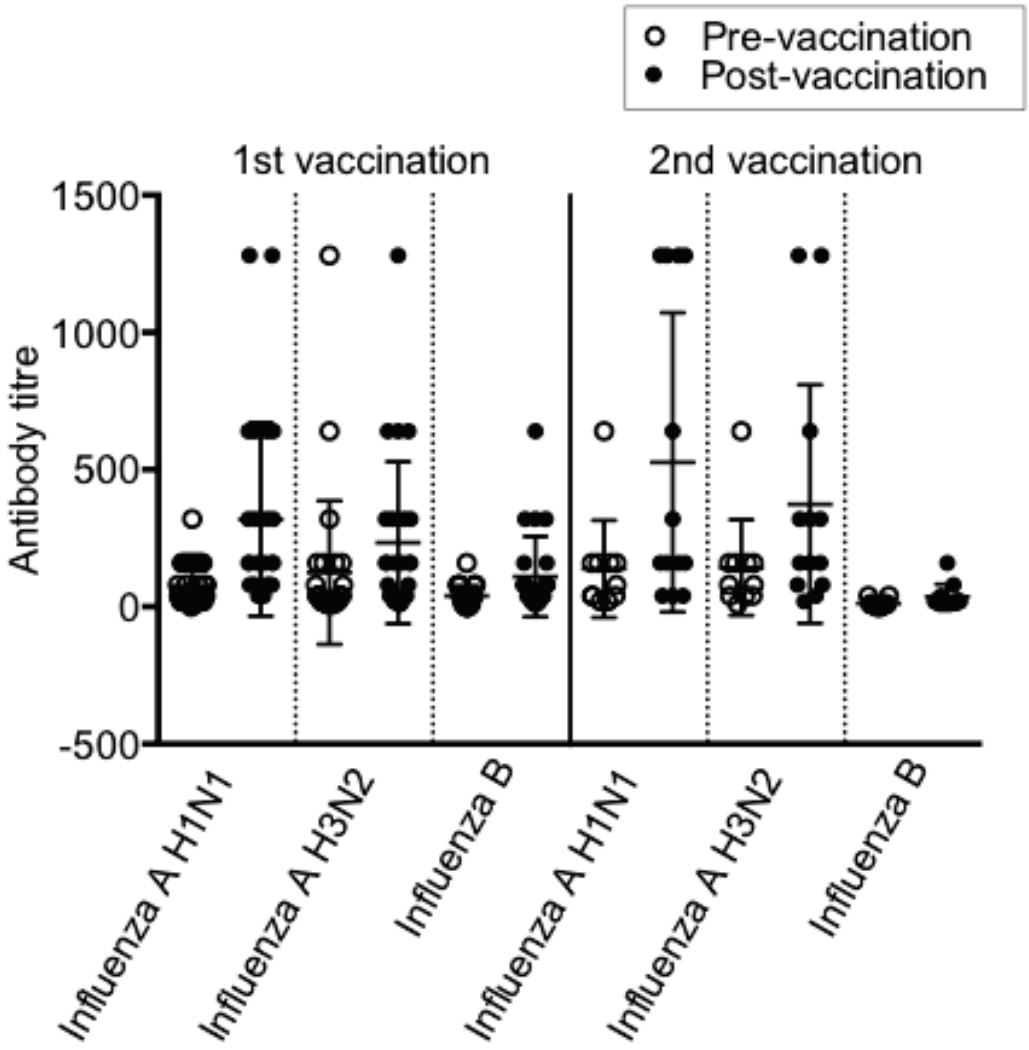
Supplemental data



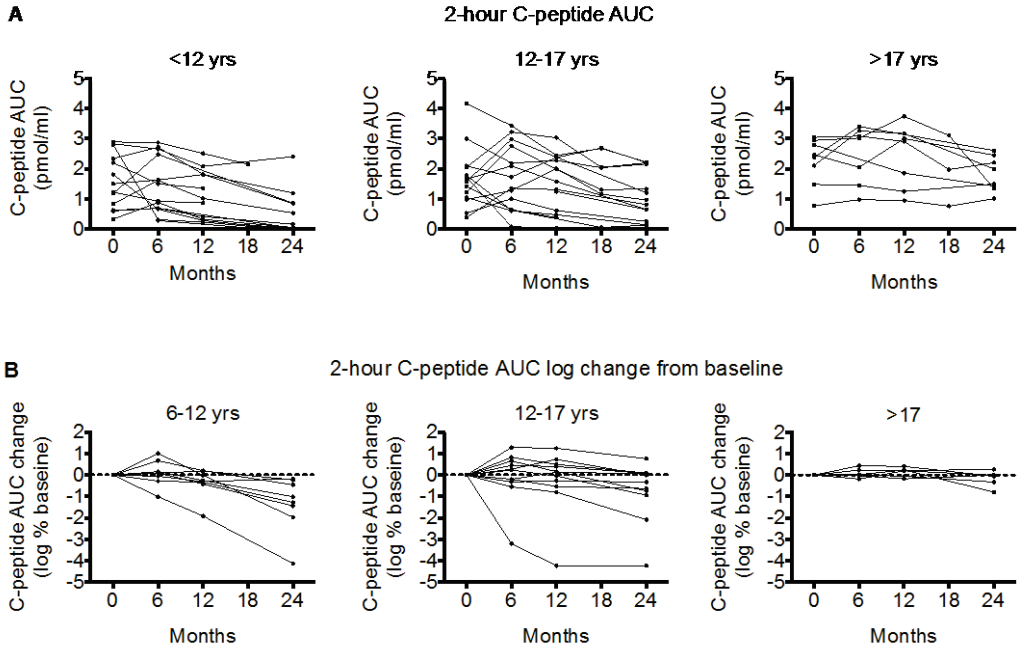
Supplementary Figure 1. Individual data plots. Trajectories of the change in percentage of PPI-specific CD57+ effector memory of total PPI-specific CD8 T cells from baseline (black lines; left y-axis) and predicted 2-hour C-peptide AUC (red line; right y-axis) over time.



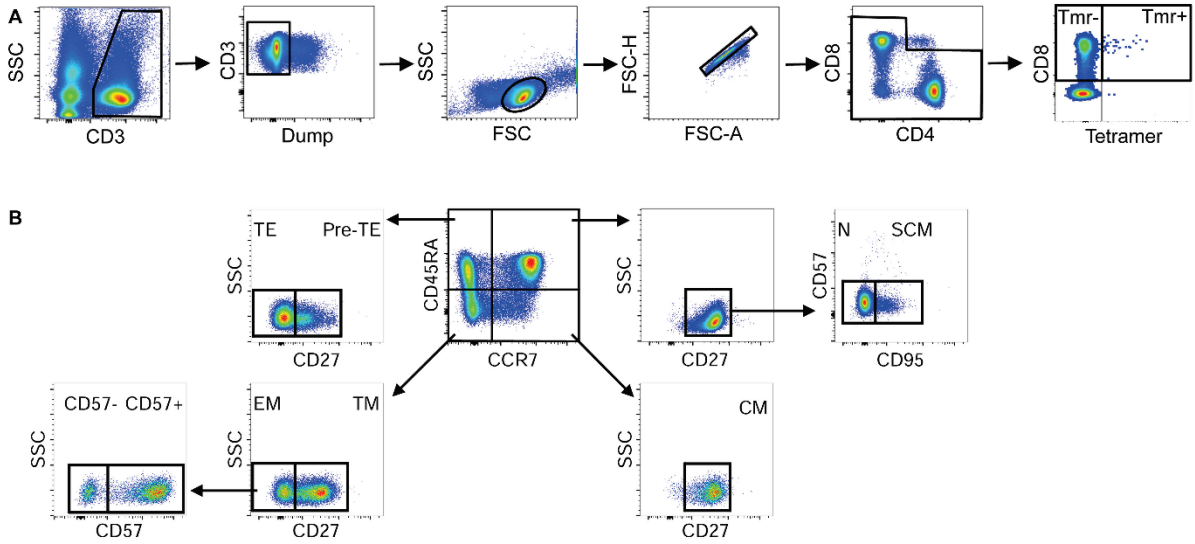
Supplementary Figure 2. β cell-specific and virus-specific CD8 T cell subsets are significantly correlated with longitudinal C-peptide change in subjects <12 years. (A) Antigen-specific CD8 T cell subsets are significantly correlated with C-peptide change in subjects <12 years. (**B)** Correlations between the CD8 T cell subsets identified as significant when all subjects were included in the model and C-peptide change are not significant in subjects 12-17 years. **(C)** Correlations between the CD8 T cell subsets and C-peptide change are not significant in subjects >17 years. The predicted log C-peptide change from baseline per 1% increase in each T cell subset is plotted. Abbreviations: central memory (CM), transitional memory (TM), effector memory (EM), terminal effector (TE). Relationships between T cell and C-peptide changes from baseline across time were analyzed with mixed-effects general linear models. $p<0.05$ (indicated by dotted horizontal line) was regarded as significant.



Supplementary Figure 2 Influenza antibody titres before and after killed influenza vaccination. Over the 2 year monitoring period of the study, some subjects received one vaccination while others received two vaccinations. Mean + SD is shown (n=28).



Supplementary Figure 3 2-hour C-peptide AUC. (A) Individual trajectories of 2-hour C-peptide AUC over 2 years in subjects grouped by age. **(B)** Individual trajectories of log change in 2-hour C-peptide AUC from baseline time in subjects grouped by age.



Supplementary Figure 4 Flow cytometry gating strategy for determination of antigen-specific CD8 T cell subsets. (A) Lymphocytes were defined as CD3 positive, "Dump" (dead cell stain, CD14, CD16, CD19) negative gated on FSC and SSC characteristics. Doublets and CD4+CD8+ double-positive cells were excluded. Cells were gated as CD8+Tetramer+ or CD8+Tetramer-. **(B)** Gating of CD8+ T cell subsets: Naïve (N;CCR7+CD45RA+CD27+CD57-CD95-), stem-cell memory-like (SCM; CCR7+CD45RA+CD27+CD57-CD95+), central memory (CM; CCR7+CD45RA-CD27+), transitional memory (TM; CCR7-CD45RA-CD27+), effector memory (EM; CCR7-CD45RA-CD27-), effector memory populations defined by presence or absence of the activation marker CD57 (CD57+ EM/CD57- EM), pre-terminal effector (pre-TE; CCR7-CD45RA+CD27+) and terminal effector (TE; CCR7-CD45RA+CD27).

Supplementary Table 1. Summary of demographic and clinical characteristics of study participants used for longitudinal assessment of CD8 T cells and C-peptide

	n	Mean (SD) or frequency (%)
Duration of T1D (days)	38	77 (20)
Age (years)	38	14 (6)
Age (categorical)		
≤12	15	39%
12-17	15	39%
>17	8	21%
Race		
White	37	97%
Other	1	3%
Ethnicity		
Hispanic or Latino	7	18%
Not Hispanic or Latino	31	82%
Sex		
Male	26	68%
Female	12	32%
HLA		
A*02	30	79%
A*2402	8	21%
BMI	38	20.4 (3.3)
C-peptide (pmol/ml) 2-h AUC mean	35	1.8 (0.9)
HbA1c %	32	6.8 (1.0)
Islet cell autoantibodies		
mIAA (titre)	24	0.25 (0.34)
GAD65 (titre)	22	0.17 (0.20)
ICA512 (titre)	22	0.44 (0.40)
Virus antibodies		
CMV positive	13	34%
EBV positive	22	58%
Pre-immunization:		
FLUA H1N1 (titre)	28	77 (76)
FLUA H3N2 (titre)	25	124 (261)
FLUB (titre)	28	39 (38)
Post-immunization:		
FLUA H1N1 (titre)	25	318 (353)
FLUB H3N2 (titre)	28	234 (295)
FLUB (titre)	25	110 (147)

Abbreviations: Micro-insulin autoantibodies (mIAA); Glutamic acid decarboxylase 65 (GAD65); Insulinoma-associated antigen-2 (ICA512); Influenza A (FLUA); Influenza B (FLUB).

Supplementary Table 2. Baseline clinical characteristics of individual subjects used for longitudinal assessment of CD8 T cells and C-peptide

Subject	T1D duration (days)	Age (years)	Sex	HLA	BMI	C-peptide (pmol/ml) 2-h AUC mean	HbA1c	mIAA (titre)	GAD65 (titre)	ICA512 (titre)
1	43	2	F	A2402+	42	2.8	6.8	0.004	0.216	0.691
2	67	6	M	A2+	24	0.8	6.9	NA	NA	NA
3	68	7	F	A2+	21	0.6	8.2	NA	NA	NA
4	66	8	M	A2+	22	NA	8.5	0.085	0.008	0.447
5	73	8	F	A2+	27	2.9	NA	0.646	0.137	0.008
6	97	8	M	A2+	23	2.8	6.8	0.004	0.216	0.691
7	98	9	F	A2+	26	1.2	4.8	NA	NA	NA
8	100	9	M	A2+	24	NA	7.2	0.094	0.014	0.301
9	80	9	F	A2402+	28	1.2	7.7	NA	NA	NA
10	96	9	M	A2+	29	2.8	5.8	NA	NA	NA
11	98	10	F	A2+	24	0.6	7.4	NA	NA	NA
12	55	10	M	A2+	36	0.3	7.9	NA	NA	NA
13	35	10	F	A2402+	25	1.4	7.9	0.029	0.243	0.490
14	76	11	F	A2402+	36	2.3	5.7	0.759	0.362	0.060
15	97	11	M	A2402+	31	1.8	5.9	0.113	NA	NA
16	89	11	M	A2+	29	1.5	6.9	NA	NA	NA
17	66	12	M	A2+	22	1.0	8.2	0.581	0.609	-0.007
18	107	12	M	A2+	26	1.6	6.9	0.372	0.001	1.078
19	90	12	M	A2+	35	1.7	6	NA	NA	NA
20	46	12	F	A2402+	29	0.8	7.4	-0.005	0.037	0.473
21	78	13	M	A2402+	35	1.2	6.4	0.088	0.648	-0.001
22	48	13	M	A2+	28	2.1	7	0.275	0.094	-0.007
23	98	14	M	A2+	39	2.0	5.8	0.468	0.001	0.995
24	59	14	F	A2+	32	3.0	NA	0.046	0.003	0.873
25	62	14	M	A2402+	34	0.5	7.8	NA	NA	NA
26	99	14	M	A2+	27	0.4	6.1	1.050	0.147	0.573
27	55	15	F	A2+	36	4.2	NA	0.019	0.206	0.564
28	100	15	M	A2+	44	1.0	7.2	0.049	NA	NA
29	28	15	M	A2+	93	2.1	NA	0.002	0.012	0.938
30	84	16	M	A2+	39	1.8	NA	0.005	0.063	1.184
31	87	16	F	A2+	29	NA	7.4		NA	NA
32	81	17	M	A2+	46	3.0	5.7	0.244	0.123	0.197
33	60	17	M	A2+	36	2.4	NA	0.041	0.532	-0.010
34	80	20	M	A2+	39	1.5	6.5			
35	89	25	M	A2+	32	2.2	7.7	0.027	-0.009	0.208
36	99	26	M	A2+	45	0.0	5.1	NA	NA	NA
37	98	31	M	A2+	45	2.5	6.1	NA	NA	NA
38	77	34	M	A2+	51	2.9	4.9	1.071	0.086	0.001

Supplementary Table 3. CD8 T cell subsets significantly correlated with both absolute C-peptide levels and log C-peptide change from baseline

CD8 T cell subset	***p***	**Pearson *r***
InsB CD57+ Effector memory	0.0060	0.2380
InsB Effector memory	0.0045	0.2459
CMV Effector memory	0.0205	-0.2217
CMV Transitional memory	0.0186	-0.2251

Supplementary Table 4. Total TCRβ sequences obtained from sorted CD57+ and CD57− effector memory CD8 T cell populations

Sample	Total no TCRβ clones
G540 EM CD57-	1984
G540 EM CD57+	3009
G551 EM CD57-	1737
G551 EM CD57+	4750
G662 EM CD57-	3572
G662 EM CD57+	4503
B091 EM CD57-	2649
B091 EM CD57+	650
B115 EM CD57-	4611