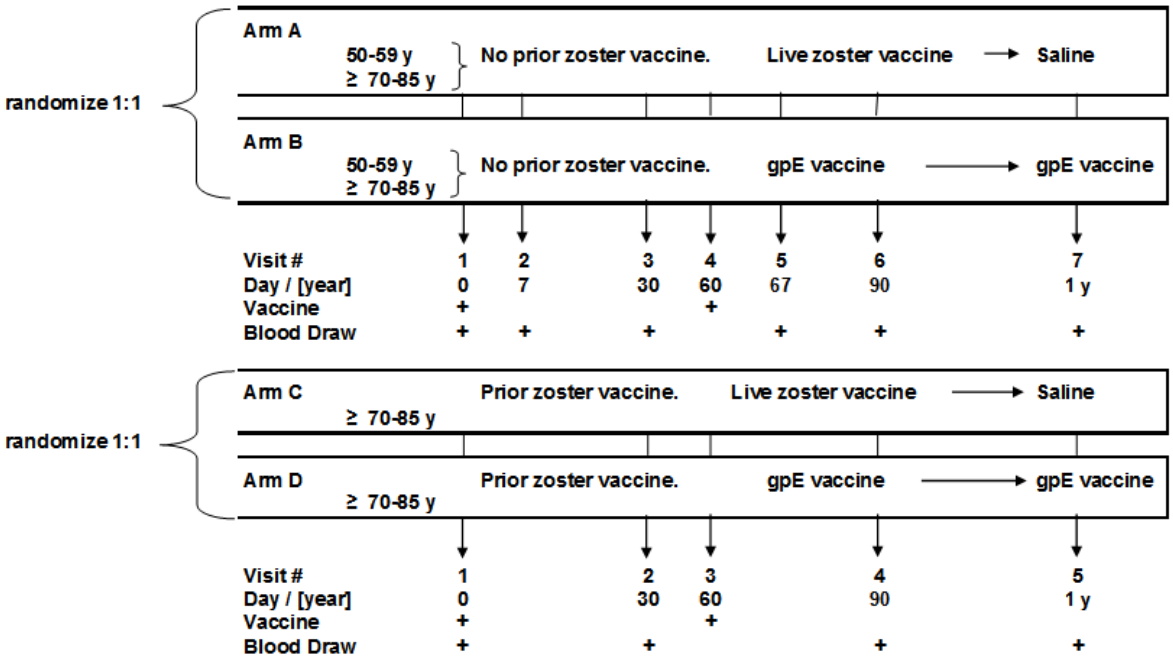
A


randomize 1:1
Arm A
50-59 y
≥ 70-85 y
No prior zoster vaccine.
Live zoster vaccine
Saline
Arm B
50-59 y
≥ 70-85 y
No prior zoster vaccine.
gpE vaccine
gpE vaccine
Visit # 1 2 3 4 5 6 7
Day / [year] 0 7 30 60 67 90 1 y
Vaccine + +
Blood Draw + + + + + + +
randomize 1:1
Arm C
≥ 70-85 y
Prior zoster vaccine.
Live zoster vaccine
Saline
Arm D
≥ 70-85 y
Prior zoster vaccine.
gpE vaccine
gpE vaccine
Visit # 1 2 3 4 5
Day / [year] 0 30 60 90 1 y
Vaccine + +
Blood Draw + + + + +

B

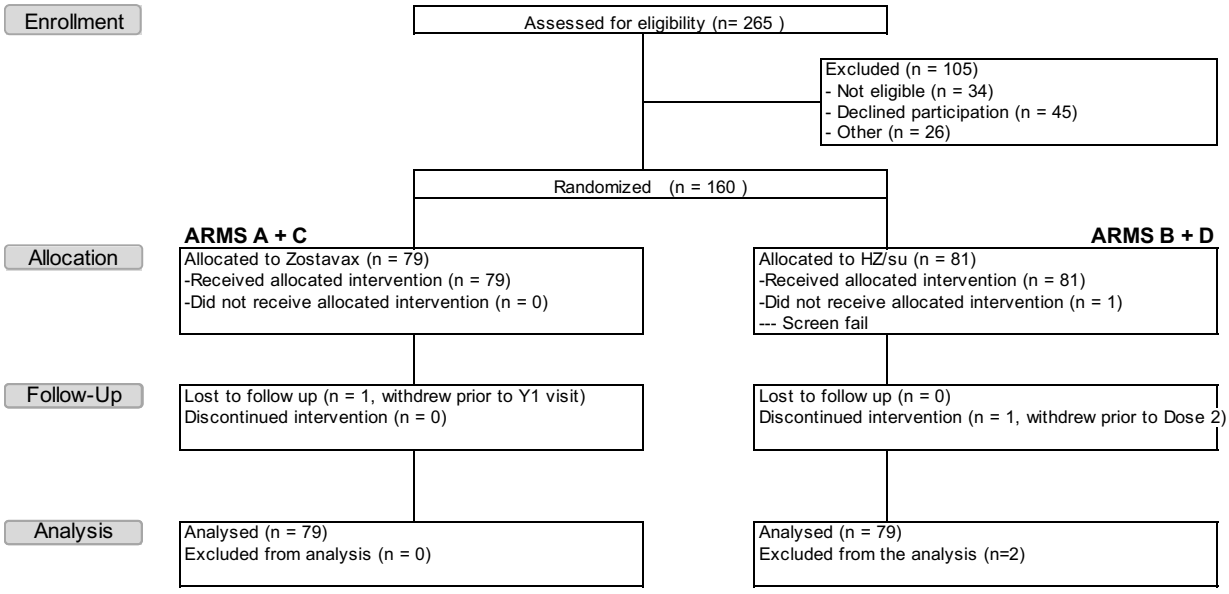

Enrollment
Assessed for eligibility (n= 265)
Excluded (n = 105)
- Not eligible (n = 34)
- Declined participation (n = 45)
- Other (n = 26)
Randomized (n = 160)
ARMS A + C
ARMS B + D
Allocation
Allocated to Zostavax (n = 79)
-Received allocated intervention (n = 79)
-Did not receive allocated intervention (n = 0)
Allocated to HZ/su (n = 81)
-Received allocated intervention (n = 81)
-Did not receive allocated intervention (n = 1)
--- Screen fail
Follow-Up
Lost to follow up (n = 1, withdrew prior to Y1 visit)
Discontinued intervention (n = 0)
Lost to follow up (n = 0)
Discontinued intervention (n = 1, withdrew prior to Dose 2)
Analysis
Analysed (n = 79)
Excluded from analysis (n = 0)
Analysed (n = 79)
Excluded from the analysis (n=2)

Figure S1. Schematic representation of the study design (A) and consort diagram (B).

Subjects were recruited through posters, flyers, broadcast emails to the campus community, mailings to the surrounding community, advertisements in local publications, and contact with former subjects who expressed interest. Approximately 265 individuals were screened from 4/22/14 through 3/3/15 after calling in to the study site for information in response to the above materials. Subjects were screened based on the following criteria: A stratified randomization list was generated by statistician. Investigators were not blinded, though laboratorians were. As subjects were enrolled they were placed into the Arm that was next on the list for their age and vaccine history. Subjects were notified of their allocation assignment at the Year 2 visit.

Table S1. Enrolled Subjects

Arm	Age (yrs)	Prior Zoster Vaccine	1st Dose	2nd Dose	Sample size (after 10% drop-out)	Sample Size
A	50-59	0	Live	placebo	20	22
A	≥70-85	0	Live	placebo	20	23
B	50-59	0	HZ/su	HZ/su	20	22
B	≥70-85	0	HZ/su	HZ/su	20	23
C	≥70-85	+	Live	placebo	31	35
D	≥70-85	+	HZ/su	HZ/su	31	35

Table S2: Demographic Characteristics of the Population Tested by Flow Cytometry at Peak Response

		HZ/su	ZV
		N/mean	N/mean
		N = 30	N = 30
age	years	68	69
race	B	N = 2	N = 2
	W	N = 28	N = 28
ethnicity	H	N = 1	N = 1
	NH	N = 29	N = 29
group	1ary	N = 20	N = 20
	boost	N = 10	N = 10

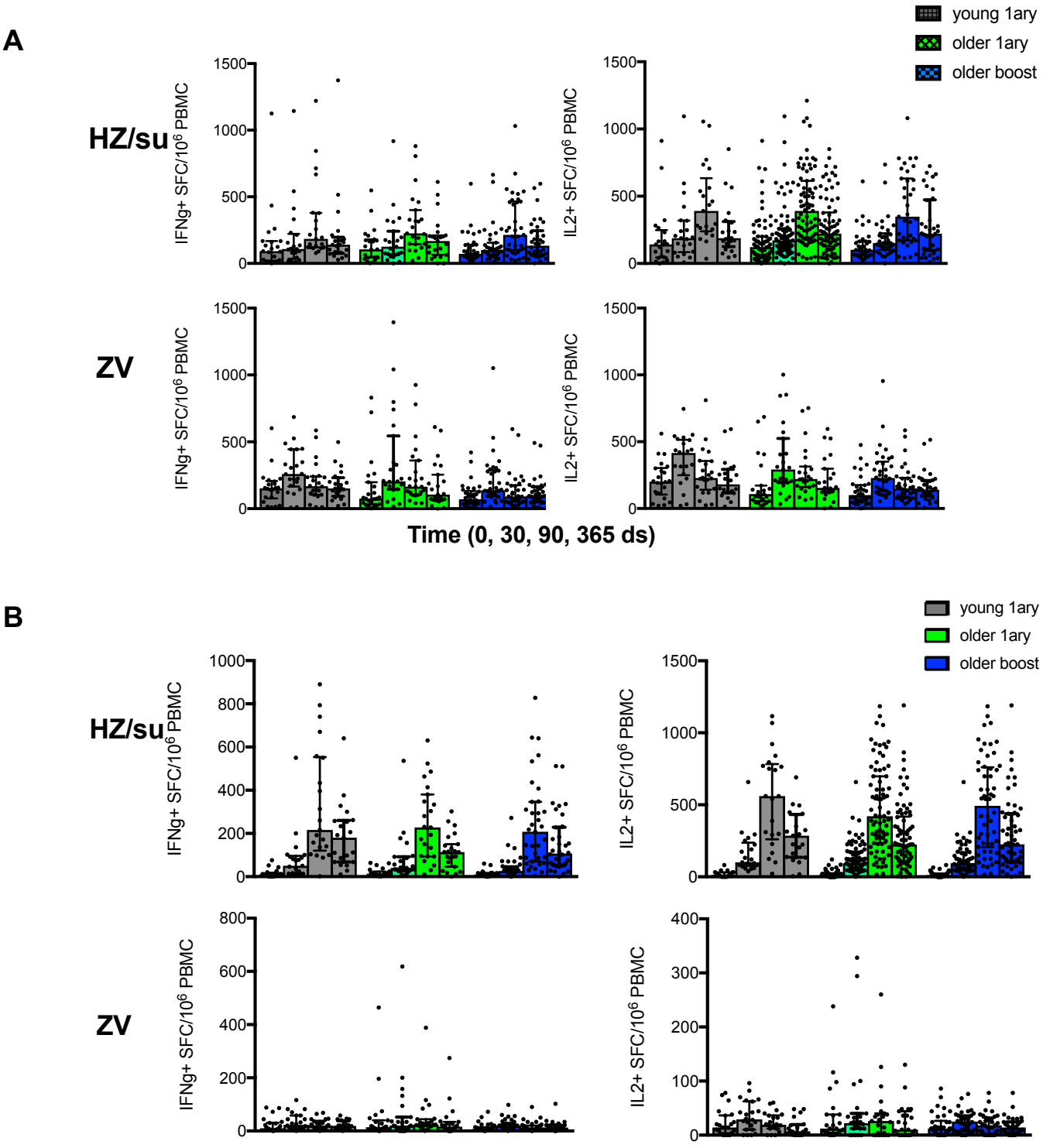
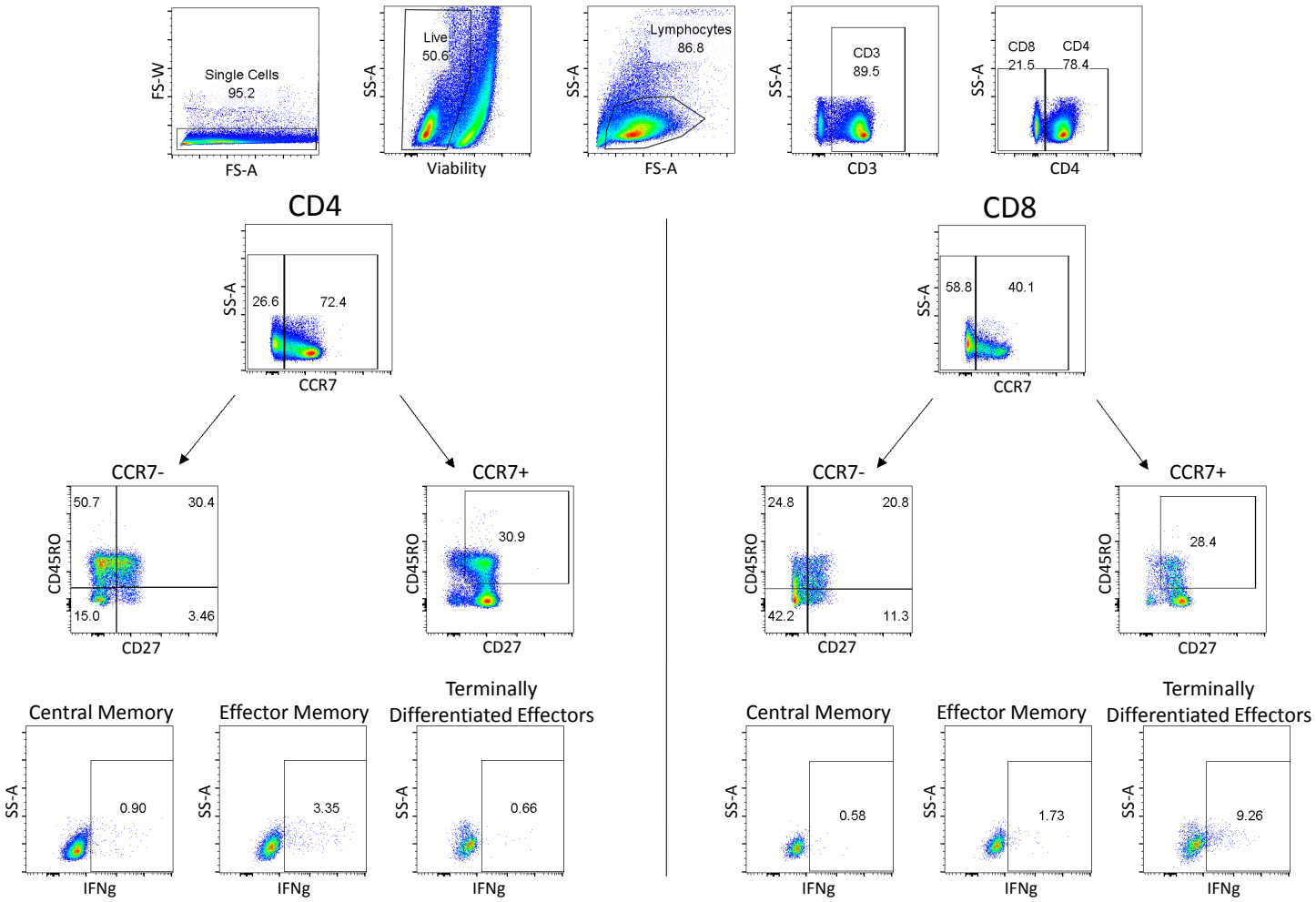
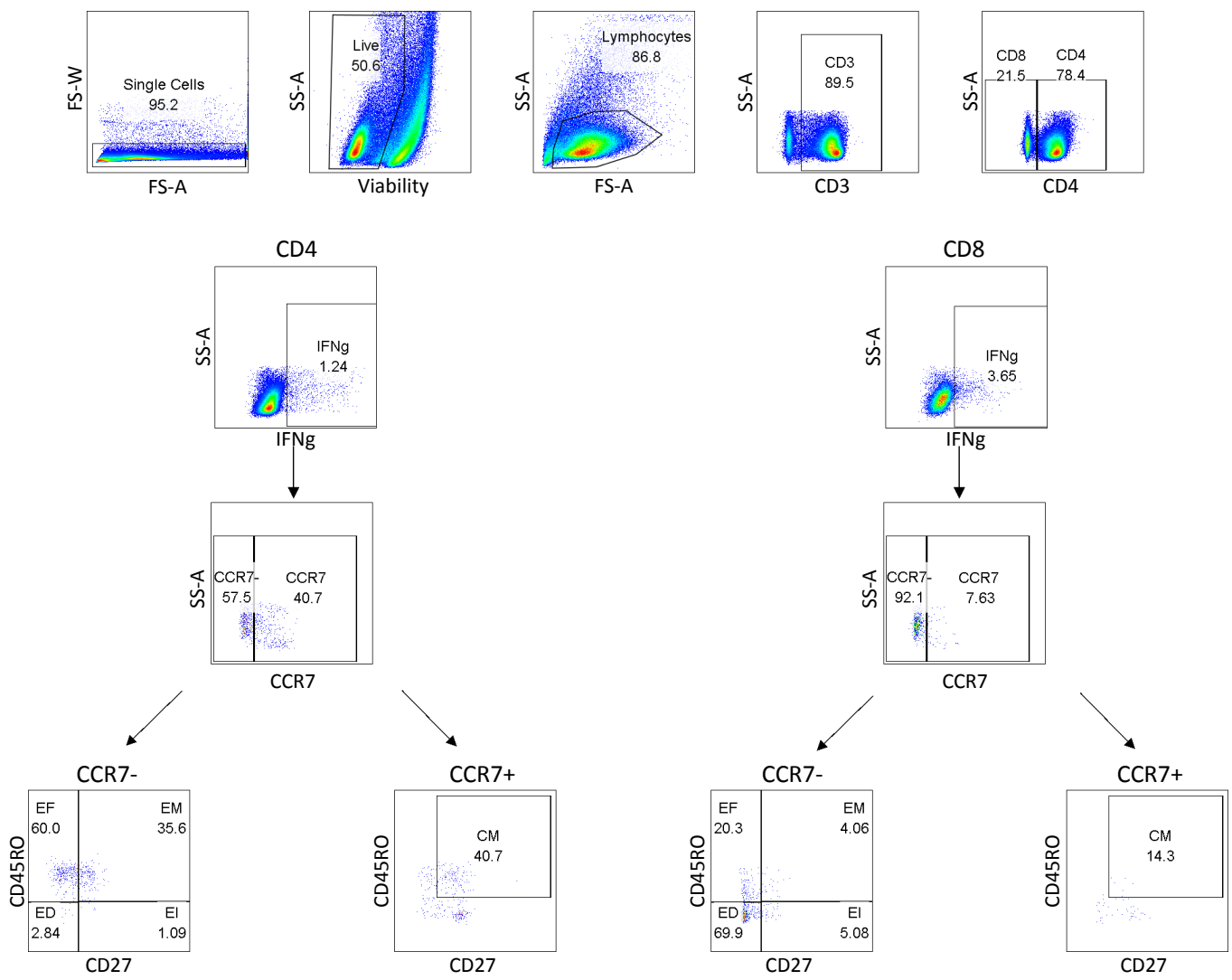


Figure S2. ELISPOT responses to HZ/su and ZV by age and treatment group. Data were derived from 158 participants. Bars represent geometric mean SFC/10^6 PBMC and 95% CI at the time points indicated at the bottom of each graph. Effector indicates IFNγ and Memory IL2 responses. **Panel A** shows responses to VZV inactivated antigen ex vivo restimulation. **Panel B** shows responses to gE peptide restimulation.

A


Single Cells
95.2
FS-W
FS-A
Live
50.6
SS-A
Viability
Lymphocytes
86.8
SS-A
FS-A
CD3
89.5
SS-A
CD3
CD8
21.5
CD4
78.4
SS-A
CD4
CD4
SS-A
26.6
72.4
CCR7
CD8
SS-A
58.8
40.1
CCR7
CCR7-
CCR7+
CCR7-
CCR7+
50.7
30.4
15.0
3.46
CD45RO
CD27
30.9
CD45RO
CD27
24.8
20.8
42.2
11.3
CD45RO
CD27
28.4
CD45RO
CD27
Central Memory
Effector Memory
Terminally Differentiated Effectors
Central Memory
Effector Memory
Terminally Differentiated Effectors
SS-A
0.90
IFNg
SS-A
3.35
IFNg
SS-A
0.66
IFNg
SS-A
0.58
IFNg
SS-A
1.73
IFNg
SS-A
9.26
IFNg

B



C

Differentiation stage	Effect Estimate	SE	p value	FDR p value
Intermediate Effector CD8	0.68	0.21	0.001	0.01
Effector CD4	0.52	0.20	0.009	0.04
Intermediate Effector CD4	0.23	0.10	0.01	0.06

Figure S3. T cell responses to the HZ vaccines have distinct differentiation profiles. The data were derived from 60 participants equally distributed across vaccination and age groups. **A** shows the gating strategy described in the main manuscript. **B** shows the alternative gating strategy. **C** shows the results of the regression analysis adjusted for baseline comparing the two vaccines for the alternative gating strategy (panel B). The effect estimates indicate the magnitude of the difference between the two vaccines on log scale. In this analysis effect estimates < 1 indicate higher proportions of the T cell subset in ZV recipients.

Table S3: Demographics Proliferation Population

		HZ/su	ZV
		N/mean	N/mean
		N = 47	N = 47
age	years	68	69
race	AmInd	N = 0	N = 1
	B	N = 2	N = 2
	W	N = 45	N = 44
ethnicity	H	N = 1	N = 1
	NH	N = 46	N = 46
group	1ary	N = 30	N = 30
	boost	N = 17	N = 17

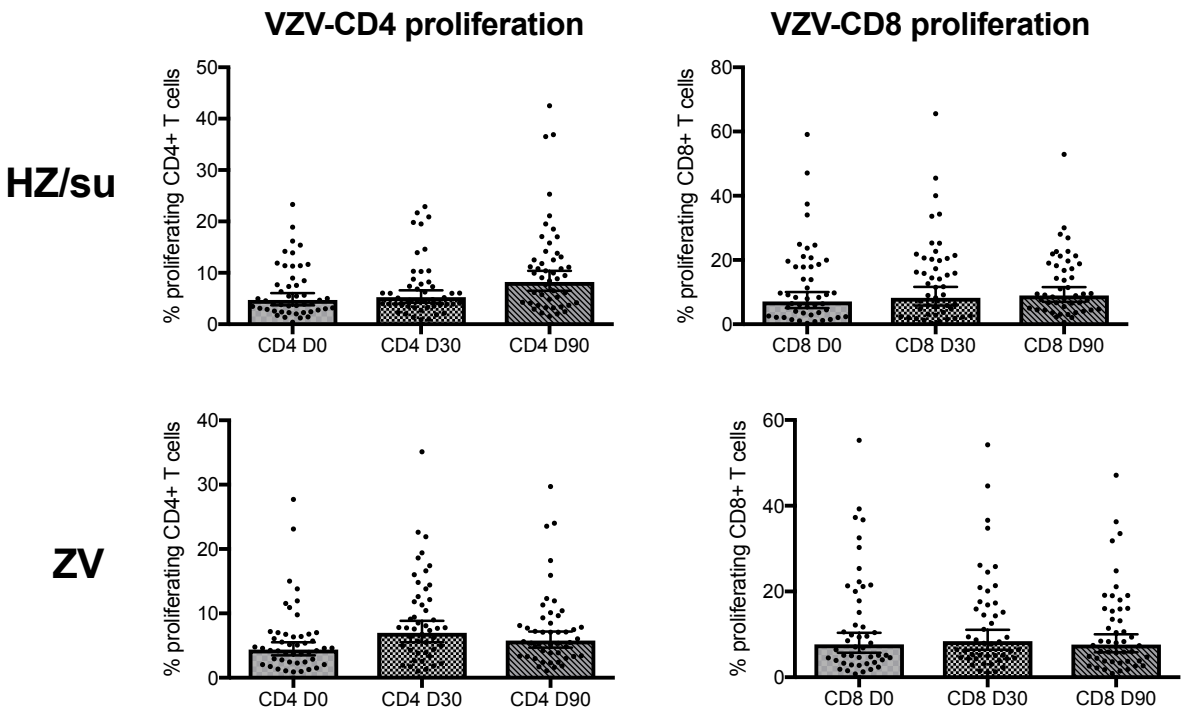


Figure S4. VZV-specific memory CD4+ and CD8+ proliferative peak responses. Data were derived from 94 participants equally distributed between the two vaccines. Bars represent geometric means and 95% CI.

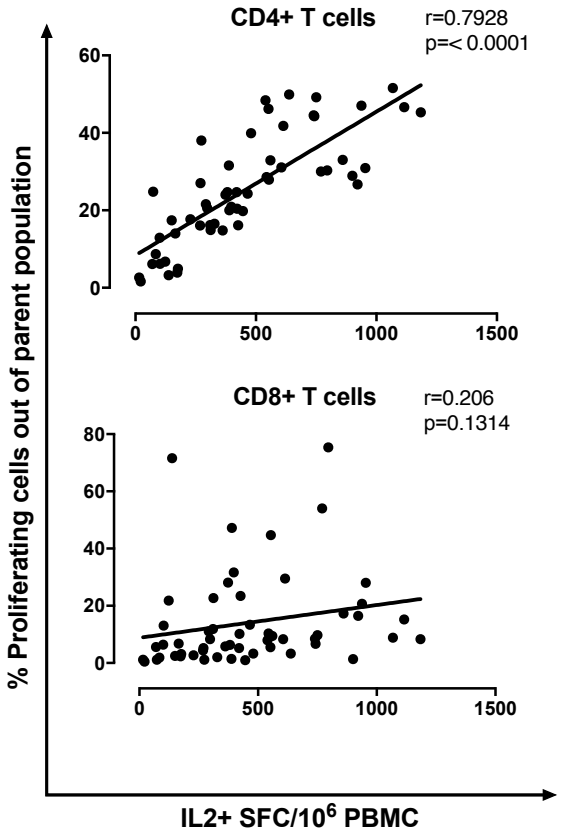


Figure S5. Correlation analysis of gE-specific CD4+ and CD8+ T cell proliferation at PMR with IL2 FluoroSpot results. Data were derived from 55 HZ/su recipients. Results of the gE FluroSpot and proliferation assays at day 90 (PMR) were used in two-tailed Pearson correlation and linear regression analyses.

Table S4. T-cell phenotypic profiles studied at PMR

Panel 1	CD4+TNFα+
	CD4+IFNγ+
	CD4+CD107a+
	CD4+CD103+
	CD4+IFNγ+TNFα+
	CD4+CD107a+IFNγ+
	CD4+CD107a+TNFα+
	CD4+CD107a+IFNγ+TNFα+
Panel 2	CD4+CD25+
	CD4+CD57+
	CD4+CD127-
	CD4+FoxP3+
	CD4+IL10+
	CD4+PD1+
	CD4+TGFβ+
	CD4+CD25+FoxP3+
	CD4+CD127-CD25+
Panel 3	CD4+CD39+
	CD4+CLA+
	CD4+CTLA4+
	CD4+CXCR3+
	CD4+KLRG1+
	CD4+LAG3+
	CD4+TIM3+
	CD4+CD39+TIM3+
	CD4+LAG3+TIM3+
	CD4+CXCR3+CD39+
	CD4+CXCR3+CLA+
	CD4+CXCR3+CTLA4+
	CD4+CXCR3+KLRG1+
	CD4+CXCR3+LAG3+
	CD4+CXCR3+TIM3+

Table S5. Adjusted effect of booster over primary Iimmunization on peak responses to HZ/su

Specificity	Subset % of parent	Effect Estimate	95% CI	p-value	FDR p-value
gE	CD8+CXCR3+LAG3+	0.55	0.38, 0.81	0.004	0.01
	CD8+LAG3+TIM3+	0.54	0.36, 0.82	0.01	0.02
	CD8+LAG3+	0.51	0.33, 0.80	0.01	0.02
	CD8+TIM3+	0.82	0.71, 0.95	0.01	0.04
VZV	CD4+CXCR3+CTLA4+	0.35	0.23, 0.54	<0.001	<0.0001
	CD4+CD107a+IFNγ+	0.50	0.33, 0.76	0.002	0.007
	CD4+CD39+TIM3+	0.59	0.44, 0.80	<0.001	0.006
	CD4+LAG3+TIM3+	0.42	0.25, 0.70	0.002	0.007
	CD8+LAG3+TIM3+	0.41	0.25, 0.68	<0.001	0.004
	CD8+CXCR3+TIM3+	0.72	0.58, 0.89	0.004	0.01
	CD8+TIM3+	0.70	0.56, 0.88	0.003	0.01

Data were derived from 60 participants equally distributed across vaccination and age groups. The effect estimates indicate the magnitude of the difference between the two vaccines on log scale. Effect estimates <1 indicate higher responses in the primary group.

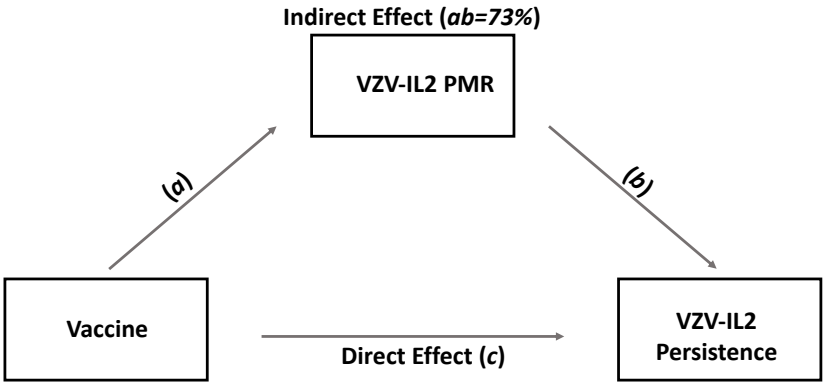


Figure S6. Diagram of the mediation analysis.

(a) represents the effect of vaccine on IL2 PMR, (b) the effect of IL2 PMR on IL2 persistence and (c) the effect of vaccine on IL2 persistence that is not mediated by the IL2 PMR.

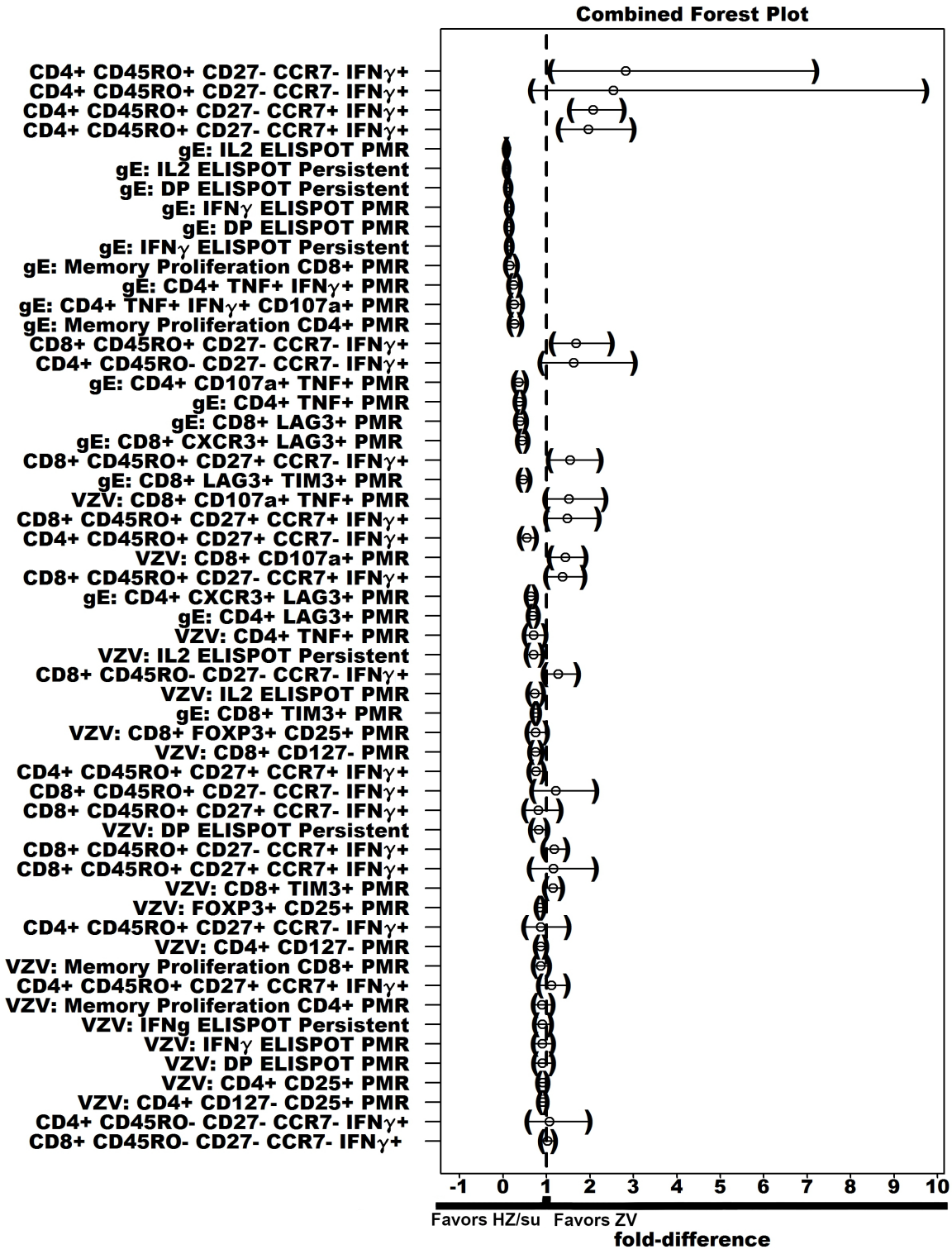
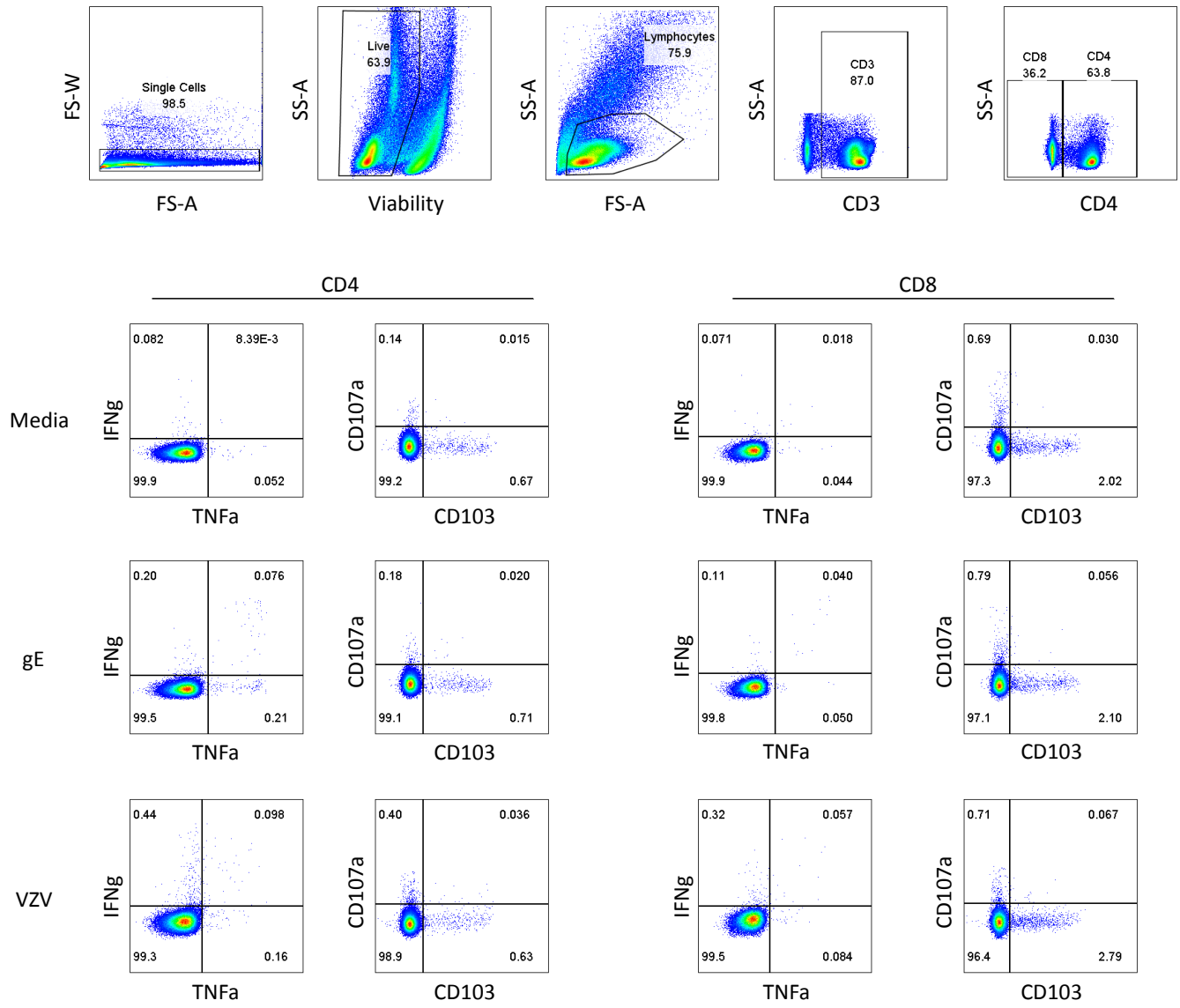


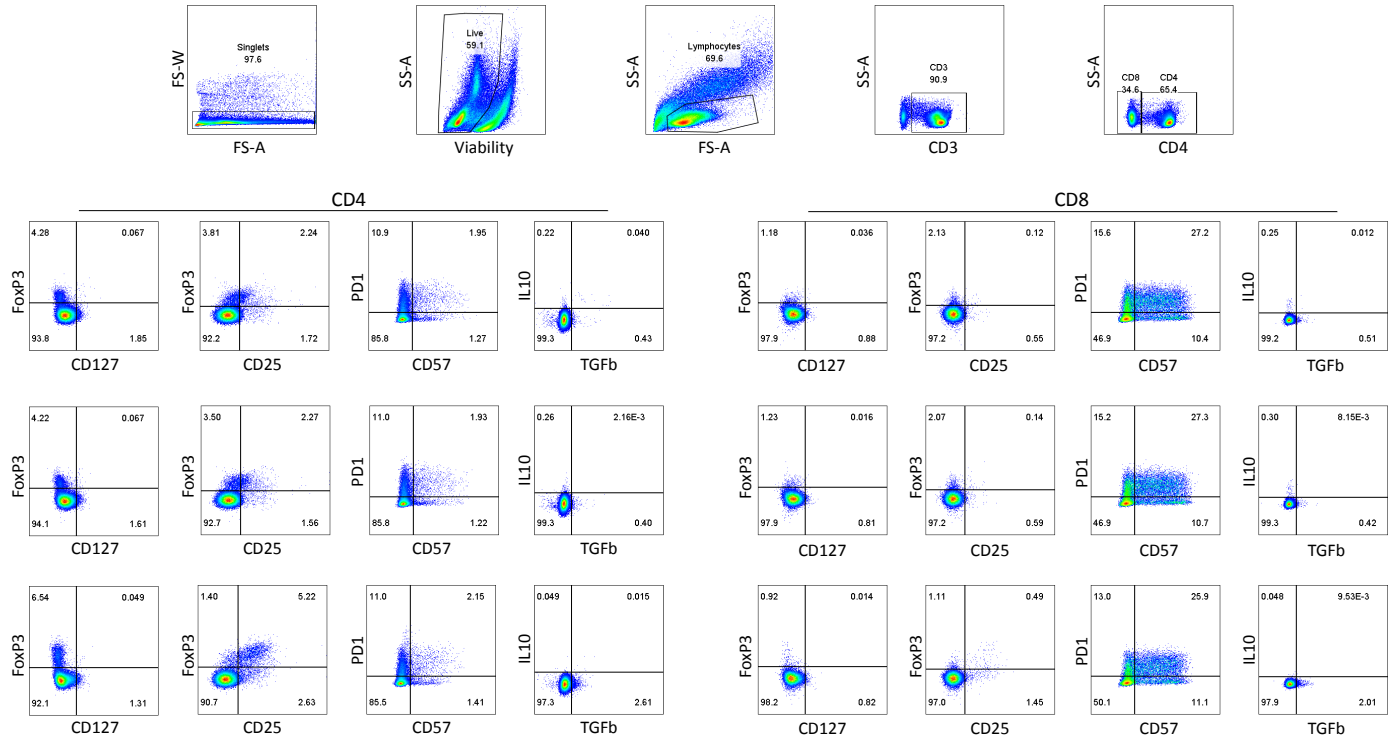
Figure S7. Hierarchical presentation of T cell responses analyzed and how they differentiate the two vaccines. Data were derived from 158 participants for ELISPOT, 94 for proliferation, 60 for T cell differentiation and functional PMR. The plot shows means estimated for the fold-differences of ZV/HZ/su results and 95% CI for significantly different parameters (95% CI does not overlap the null effect, i.e. equivalence, indicated by the vertical dotted). All other parameters are shown in Figure S7. The stimulant and T cell responses are indicated on the coordinate. Means <1 indicate higher responses in the HZ/su group and >1 indicate higher responses in the ZV group.

Figure S8. Gating strategies for each of the 3 panels

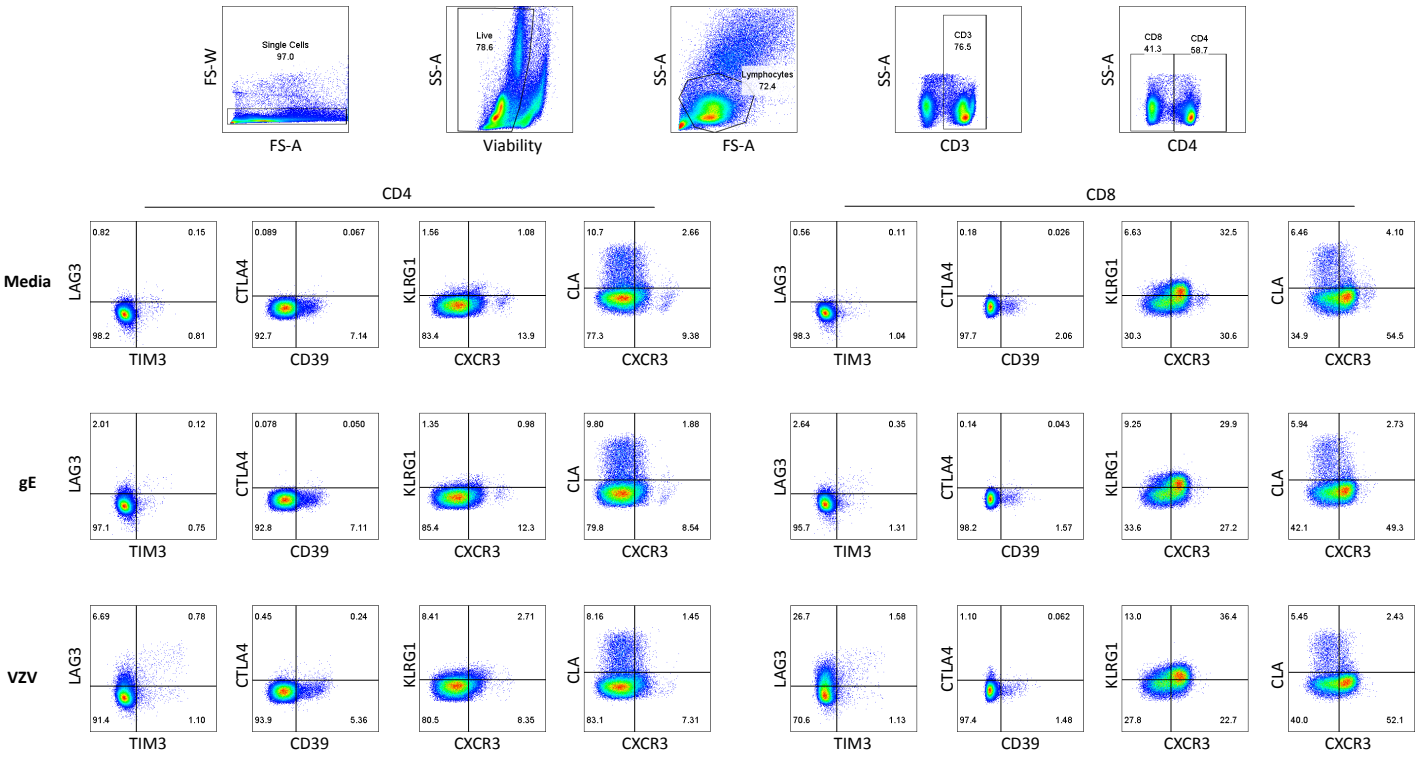
Panel 1



Panel 2



Panel 3



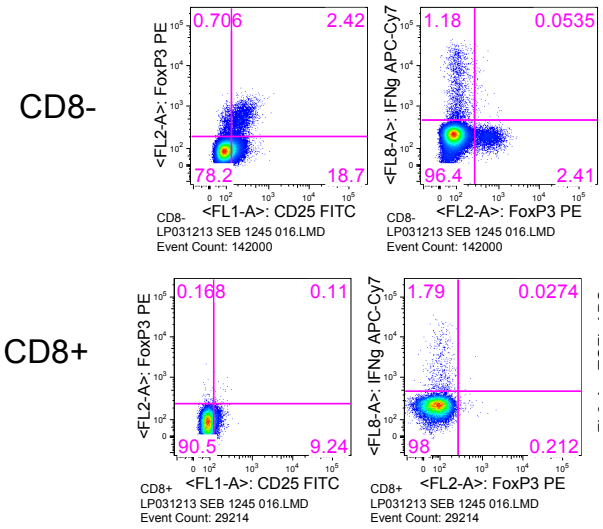


Figure S9. Specificity of FOXP3+CD25+ marker combination for Treg.

Data were generated by stimulating PBMC with SEB for 48 h. The scatterplots show 2.42% CD4+(CD8-) CD25+FOXP3+ and 0.11% CD8+CD25+FOXP3+ Treg; 1.18% CD4+IFNg+ and 1.79% CD8+IFNg+ activated T cells; and insignificant 0.05% CD4+FOXP3+IFNg+ and 0.02 CD8+FOXP3+IFNg+ FOXP3-expressing activated T cells. These data show that FOXP3 expression was specific for Treg.