Methods

Study subjects

Three pairs of monozygotic twins from the Twin Research Registry at SRI International and three unrelated individuals were included. The protocol was approved by the Stanford University Institutional Review Board, and all participants gave written informed consent. Demographic information is provided in Table 1. All individuals were healthy. None of the individuals had zoster reactivation in the five years prior enrollment, one individual (E) had reactivation more than 5 years ago. All individuals were vaccinated with live attenuated VZV (Zostavax^{R}, Merck). Peripheral blood was obtained before vaccination and on days 8$\pm$1, 14$\pm$1 and 28$\pm$3 days after vaccination. Blood at similar intervals was also obtained from a control individual who was not vaccinated. Serum samples were obtained before vaccination and on days 1 and 28±3 days after vaccination. Demographic data including HSV and CMV status and humoral and cellular vaccine response is given in Table S1. HSV-1, HSV-2 and CMV status were measured using Focus Diagnostics HerpeSelect 1 ELISA IgG and HerpeSelect 2 ELISA IgG test kits on an Inova Diagnostics Quanta-Lyser at Stanford Health Care Clinical Laboratories. Antibody responses to VZV were determined using the VZV glycoprotein-based Serion ELISA Classic VZV IgG test (Institut Virion\Serion GmbH). Serum samples in duplicate were run according to manufacturer's instructions. All individuals had >100 mIU/ml VZV IgG on prevaccination serum samples, which is considered as seropositive. ELISpot assays were performed using serial dilutions of PBMCs in duplicate on precoated IFN-γ-specific ELISpot plates (Mabtech) and stimulated with 3 μg/ml VZV lysate for 16 hours. Plates were analyzed using Immunospot software (Cellular Technology Limited). VZV viral lysate from pOKa-infected melanoma MEL39 cells and mock lysate from uninfected MEL39 cells were a gift from Dr. A. Arvin, Stanford. Serum cytokine concentrations were measured using Human 63-plex fluorescent bead assay at Stanford Human Immune Monitoring Center.

Generation of VZV-antigen reactive cells

To obtain VZV-specific CD4 T cells, PBMCs were labeled with CFSE, and ten million cells per culture were stimulated with 3μg/ml VZV viral lysate. Control cultures were stimulated with 3μg/ml lysate of non-infected cells. In pilot studies, we determined that proliferating T cells were first detected on days 3 and 4 after stimulation and optimal separation of proliferating and non-proliferating cells were obtain after 7-8 days of culture.

To confirm that proliferating cells are VZV reactive, CFSE-labeled PBMCs were stimulated with 3μg/ml VZV viral lysate. On day 8, cultured cells were harvested; non-T cells from fresh PBMCs of the same individuals were isolated by negative selection using anti-CD3 magnetic microbeads (Miltenyi Biotec) and labeled with CellTrace Violet (CTV, ThermoFisher Scientific). 1 million of the harvested PBMCs were restimulated with 0.4 million non-T cells and 3μg/ml fresh VZV viral lysate for 16 hours. In parallel, harvested PBMCs were restimulated with 50 ng/ml PMA and 500 nM ionomycin. After incubation, cells were stained with AmCyan-anti-CD4 (BD Biosciences), AlexaFluor700-anti-CD8 (BioLegend), APC-Cy7-anti-CD3 (BioLegend) antibodies, and then fixed and permeablized (BD Cytofix/Cytoperm kit), followed by staining with APC-anti-TNFα (BD Biosciences), PerCP-Cy5.5-anti-IL2 (BD Biosciences) and PE-anti-IFNγ (eBioscience) antibodies.

To assess VZV-reactivity of naïve T cells, T cells were negatively purified from 10 million PBMCs using EasySep human T cell enrichment kit (StemCell Technologies), stained with PerCP-Cy5.5-anti-CCR7 (BioLegend) and APC-anti-CD45RA (BD Biosciences), followed by cell sorting using a BD Aria 3 cell sorter to obtain naïve T cells ($CCR7^{+}CD45RA^{high}$). Naïve T cells were labeled with CFSE and cultured with 10 million CTV-labeled PBMCs from the same individual and 3μg/ml VZV viral lysate. On day 8, cells were stained with anti-CD4, anti-CD8 and anti-CD3 antibodies. Flow cytometric data were collected with a Fortessa flow cytometer and analyzed with Flowjo software (Flowjo, LLC).

For repertoire studies of VZV-reactive T cells, cells were stained on day 8 of culture with Live/Dead Fixable Aqua Dead cell stain dye (Life Technologies), APC-Cy7-anti-CD3 (BioLegend), V450-anti-CD4 (Affymetrix eBioscience) and PerCP-Cy5.5-anti-CD8 antibodies, followed by cell sorting using a BD Aria 3 cell sorter to obtain a minimum of 5,000 and up to 10,000 CD4 T cells ($CFSE^{low}CD4^{+}CD8^{-}CD3^{+}$) that had divided in response to VZV lysate stimulation. Two to three replicates of PBMC were cultured independently for each prevaccination and postvaccination time point. RNA was prepared using RNeasy micro kit (Qiagen).

For repertoire studies of unstimulated T cells, total CD4 T cells were purified from PBMC using CD4 microbeads and the autoMACS Pro Separator (Miltenyi Biotec). Two replicates of $1x10^{6}$ CD4 T cells were

collected at each time point. Naïve CD4 T cells ($CD3^+CD4^+CD8^-CCR7^+CD45RA^{high}CD28^+$) and memory CD4 T cells ($CD3^+CD4^+CD45RA^{med/low}$) were collected by cell sorting using FITC-anti-CD3 (BioLegend), V450-anti-CD4 (Affymetrix eBioscience), PE-Cy7-anti-CD8 (BD Biosciences), PerCP-Cy5.5-anti-CCR7 (BioLegend), PE-antiCD28 (BD Biosciences) and APC-anti-CD45RA (BD Biosciences) antibodies. RNA of each T cell population was extracted using AllPrep DNA/RNA mini kit (Qiagen).

Generation of TRB gene library and sequence data analysis

cDNA were prepared from RNA using SuperScript VILO master mix (Invitrogen). The preparation and sequencing of TCRB gene libraries were performed as described (8). Sequencing reads were first filtered by read quality, then assembled and mapped to human TRB reference sequences downloaded from international ImMunoGeneTics (IMGT) information system (http://imgt.org/). V, J gene annotation, CDR3 definition and sequencing error correction were performed as described (8). CDR3 out-of-frame sequences were excluded from further analysis.

Identical TCRβ chains (clones) were defined as sequences that have the same BV and BJ gene segment and identical CDR3 amino acid sequences. For analysis of TRBV usage, TRBJ usage and CDR3 feature, replicate sequences were collapsed to a single sequence. Sequences that contain ambiguous V genes or J genes were excluded for V and J usage analysis. .

Identification of VZV antigen-reactive TRB sequences

To call a TCR sequence to be derived from a VZV-specific T cell and to exclude contaminating sequences from non-specifically proliferating T cells, we required that (1) the identical sequence was found in at least two independent replicate libraries from the same time point; (2) the frequency of the sequence in the proliferating population was enriched by at least a factor of 2 compared to total CD4 T cells and the enrichment was significant (one-sided Fisher exact test, $q<0.05$).

Estimation of the genetic influence

TRBV-TRBJ combination usage frequencies were determined after collapsing replicate sequences into a single sequence. Expression patterns between any two individuals were compared using Pearson correlation. Hierarchical clustering was performed based on correlation coefficients. The within twin-pair similarities in TRBV or TRBJ gene usage were determined from the ratio of the average absolute difference in gene frequencies of two unrelated individuals and those within twin-pairs. The nonparametric permutation test was used to test if the average difference within twin-pairs is less than that between unrelated individuals. The overall repertoire similarities of a given cell type between two individuals were expressed as the number of shared TCRβ chain sequences divided by the product of the total number of distinct TCRβ chain sequences from these two individuals. Similarities between twin-pairs and unrelated individuals were then compared using permutation test.

Estimation of vaccination-induced T cell expansion

To estimate the proportion of clones expanded after the VZV vaccination, we assume a negative binomial model for the count of each clone. Specifically, we denote the count of the j-th clone in i-th replicate at the baseline by n_{ij0}. Similarly, we let n_{ij1} be the count of the j-th clone in i-th replicate at day 8 after vaccination. We assume that

$$n_{ijk} \sim Poisson\left(N_{ik}\lambda_{jk}\theta_{ik}\right)$$
$$\theta_{ijk} \sim Gamma\left(\frac{1}{\sigma_k^2}, \frac{1}{\sigma_k^2}\right)$$

where $i = 1,2;\ j = 1,\cdots,J, k = 0,1$ and N_{ik} is the sequencing depth in the i-th replicate. Our goal is to test the null hypothesis $H_0: \lambda_{j0} = \lambda_{j1}$ and estimate the proportion of clones with $\lambda_{j1} > \lambda_{j0}$. To this end, we consider the test statistics $n_{1j0} + n_{2j0}$. Under the null hypothesis, the conditional distribution

$$n_{1j0} + n_{2j0} | (n_{1j0} + n_{2j0} + n_{1j1} + n_{2j1} = n_j) \sim Binom\left(n_j, \frac{N_{10}\theta_{1j0} + N_{20}\theta_{2j0}}{N_{10}\theta_{1j0} + N_{20}\theta_{2j0} + N_{11}\theta_{1j1} + N_{21}\theta_{2j1}}\right),$$
$$\theta_{ijk} \sim Gamma\left(\frac{1}{\sigma_k^2}, \frac{1}{\sigma_k^2}\right).$$

Let $\widetilde{N}_{j0}$ denote the null distribution above, which can be easily simulated. Therefore, we may obtain the one-sided mid p-value testing $H_0: \lambda_{j0} = \lambda_{j1}$ vs $H_1: \lambda_{j0} < \lambda_{j1}$ by

$$p_j = Prob\left(n_{1j0} + n_{2j0} < \widetilde{N}_{j0}\right) + 0.5 \times Prob\left(n_{1j0} + n_{2j0} = \widetilde{N}_{j0}\right).$$

Since we know that under the null distribution the one-sided p-values approximately follow a uniform distribution, we can estimate the proportion of true alternatives as

$$1 - \frac{\sum_j I\left(p_j > \pi_0\right)}{(1-\pi_0)J},$$

where the tuning parameter $\pi_0 \in (0,1)$ is set as 0.2. To conduct the analysis, one needs to know the value of σ_k^2, which can be estimated by the moment estimator

$$\hat{\sigma}_k^2 = \frac{\sum_j \left(n_{1jk}N_{2k} - n_{2jk}N_{1k}\right)^2 - n_{1jk} - n_{2jk}}{2\sum_j n_{1jk}n_{2jk}N_{1k}N_{2k}}, k = 0, 1.$$

Diversity analysis

The diversity of repertoire was calculated as the number of distinct clones, ordered by decreasing clonal sizes that comprise 80% of repertoire. This metric is unchanged for inferential purposes as the depth of sequencing varies. To study the association of relative change of clones and their sizes, we considered the following regression model:

$$\frac{\lambda_{28jk}}{\lambda_{0jk}} = \alpha_k e^{\beta_k \lambda_{0jk}},$$

$$Y_{28jk} \sim Poisson\left(N_{28k}\lambda_{28jk}\right), \qquad Y_{0jk} \sim Poisson\left(N_{0k}\lambda_{0jk}\right),$$

$$\text{and } \beta_k \sim N(\beta_0, \tau^2),$$

where (Y_{0jk}, Y_{28jk}) are observed frequencies for clone j of the k[th] subject at baseline and day 28, (N_{0k}, N_{28k}) are the observed total frequencies for all clones of the k[th] subject at baseline and day 28 and $(\lambda_{0jk}, \lambda_{28jk})$ are underlying sizes for clone j of the k[th] subject at baseline and day 28. Therefore, the parameter β_k represents the association between the relative changes in the clone sizes measured by the ratio $\lambda_{28jk}/\lambda_{0jk}$ and the baseline clone size λ_{0jk} for the k[th] subject. For example, if β_k is negative, then small clones at the baseline tend to expand less than big clones. β_0 is the population counterpart of β_k and the parameter summarizing the association in the population. To fit this model, we first estimate β_k for each individual and the corresponding standard error is obtained by a parametric bootstrap. We then employ the random effects model used in meta-analysis to obtain the point and interval estimates of β_0. Due to the small sample size and model-dependent nature of the approach, the results may be sensitive to few influential observations.

Descriptive statistics

The Spearman correlation coefficients between the number of distinct VZV-reactive CD4 TCRβ chains and the space taken up by the most abundant clones as well as between the frequencies of VZV antigen-reactive T cells and the richness of their TCRβ chain repertoires were estimated. The similarity of TRBV and TRBJ genes frequencies within twin pair and between unrelated individuals were compared using a nonparametric permutation. The frequencies of VZV antigen-reactive TCRβ chain sequences in total CD4 T cells and the number of unique VZV-specific TCRβ chains before and on days 8, 14, and 28 after vaccination were compared with the paired Wilcoxon-Mann-Whitney test. A p value of 0.05 or less was considered to be statistically significant. All statistical analyses were performed using R3.2.2. (The R Foundation for Statistical Computing, 2015).

Table S1. Demographics of study population.

Subject ID	Gender	Age	Twin_status	Twin_Sibling	Zoster	HSV1	HSV2	CMV	Antibody (D28/D0)	ELISPOT (D28/D0)
A1	Male	52	Identical twin	A2	No	negative	negative	negative	5.66	4.57
A2	Male	52	Identical twin	A1	No	positive	negative	negative	1.04	3.89
B1	Female	58	Identical twin	B2	No	negative	negative	positive	1.48	N.D.
B2	Female	58	Identical twin	B1	no	negative	negative	negative	3.2	2.40
C1	Female	57	Identical twin	C2	no	negative	positive	negative	4.92	5.99
C2	Female	57	Identical twin	C1	no	negative	negative	positive	>10	6.85
D	Male	55	Non-twin		no	positive	negative	positive	2.23	5.61
E	Male	75	Non-twin		>5 years	positive	negative	negative	>10	1.68
F	Male	60	Non-twin		no	negative	negative	positive	3.37	5.69

Table S2. Number of TRB sequence reads obtained for individual replicates.

VZV-specific CD4 T cells	Subject ID	Day 0	Day 0	Day 0	Day 8	Day 8	Day 8	Day 14	Day 14	Day 14	Day 28	Day 28	Day 28
		L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
	A1	48695	57633	48716	59732	53081	42513	53294	50904	51965	62741	64280	62849
	A2	58076	59575	54089	60881	60012	64411	60677	55149	58241	65310	60867	60069
	B1	47391	59401	52956	43023	47453	50177	49698	47818	44815	43212	39879	41288
	B2	46941	45472	47035	44366	45691	-	45487	48536	52138	47767	-	-
	C1	57353	61798	60348	59435	51922	56616	67634	68780	68230	63041	63572	58650
	C2	62122	67081	66718	76838	71034	69057	69088	56647	65190	62144	68341	-
	D	47208	46804	47735	52346	54221	50207	39540	49646	51417	51643	54449	58415
	E	78715	88679	-	90600	75731	71436	74851	70089	80839	80449	75447	87440
	F	43190	56208	53529	59691	51390	54116	66700	60754	67888	48977	59206	48032

Total CD4 T cells	Subject ID	Day 0	Day 0	Day 8	Day 8	Day 14	Day 14	Day 28	Day 28
	A1	222406	205464	234247	235525	250567	253631	274945	269052
	A2	210203	229380	279221	274866	285546	291564	249733	326067
	B1	431852	398666	398520	414006	429836	416636	381954	385298
	B2	380704	368087	417171	417837	396486	410511	412817	406971
	C1	333912	384750	477405	401121	416192	354103	412915	443915
	C2	376250	436517	391237	429679	388229	386030	420384	403174
	D	388874	432311	415786	427977	464124	459797	443345	403249
	E	376809	399194	378732	438010	442264	483192	393922	374185
	F	361072	392749	398108	458999	370432	401485	335422	278238
CD4 T cell subsets day 0	Subject ID	Naïve	Naïve	Memory	Memory				
	A1	630202		647555					
	A2	306913	287861	152222	163975				
	B1	417332		420268					
	B2	431890		461370					
	C1	319468		287179					
	C2	271646	304954	291309	296227				
	D	406796		397015					
	E	262911		356331					
	F	650645		632600					

Table S3. BV and BJ gene segment usage in VZV-reactive CD4 T cells compared to naïve and memory CD4 T cells (FDR≤0.1).

Increase	Naïve CD4 T cells	Memory CD4 T cells
TRBV5-4	0.004*	Non-significant
TRBV5-5	0.02	Non-significant
TRBV5-8	0.027	0.008
TRBV6-1	0.02	Non-significant
TRBV7-3	0.02	0.02
TRBV7-4	0.012	0.02
TRBV12-4	0.004	0.004
Decrease	Naïve CD4 T cells	Memory CD4 T cells
TRBV10-3	0.008	0.012
TRBV14	0.008	0.008
TRBV15	0.012	0.004
TRBV19	0.004	0.012
TRBV20-1	0.004	0.004
TRBV4-3	0.004	0.004
TRBV5-3	0.022	0.004
TRBJ1-4	Non-significant	0.008
TRBJ1-5	Non-significant	0.02
TRBJ2-1	0.004	0.004

* Only comparisons that were significantly different are shown. Data are shown as p-values with FDR≤0.1 based on Benjamini Hochberg adjustment.

Table S4. Serum cytokine levels before and one day after vaccination (mean fluorescence intensity)

Subject ID	B1	B1	B2	B2	C1	C1	C2	C2	D	D	E	E	F	F
Visit	D0	D1	D0	D1	D0	D1	D0	D1	D0	D1	D0	D1	D0	D1
IL17F	88	91	112	114	226	260	371	391	56	57	55	58	207	201
FASL	46	46	52	54	38	42	105	108	33	33	37	35	92	89
TGFA	26	24	28	25	59	68	53	64	21	22	24	23	35	31
MIP1A	103	100	171	184	159	178	690	779	62	57	72	67	371	364
SDF1A	218	209	191	199	310	337	223	272	175	178	180	169	202	195
IL27	29	31	36	35	27	28	56	63	27	26	25	26	55	52
LIF	46	51	60	60	39	41	113	125	41	42	45	45	61	71
IL1B	15	18	22	22	17	18	26	31	17	18	20	19	23	22
IL2	21	20	25	24	17	17	56	58	16	18	19	20	40	38
IL4	89	90	86	91	89	91	117	129	96	91	126	116	116	114
IL5	30	34	31	32	23	23	82	91	26	26	25	26	57	59
IP10	91	124	125	371	49	78	51	937	63	72	170	196	91	92
IL6	33	35	39	40	31	32	65	72	29	31	29	31	46	44
IL7	271	295	238	245	491	502	445	515	544	586	905	946	715	609
IL8	50	48	66	67	52	59	57	62	48	49	48	45	78	66
IL10	35	34	30	29	16	16	88	112	16	16	17	19	42	41
PIGF1	224	216	202	204	338	355	289	290	158	160	208	201	244	243
IFNB	98	102	117	125	305	348	404	469	78	77	80	79	233	227
EOTAXIN	76	63	161	185	174	231	320	364	239	196	149	107	379	365
IL12P70	27	25	36	40	33	34	61	108	30	30	34	34	63	60
IL13	30	30	32	36	29	32	39	44	33	32	38	36	37	36
IL17A	62	64	55	56	35	38	50	55	40	40	35	34	84	80
IL31	27	27	30	32	35	36	36	33	27	24	28	25	43	42
IL1RA	28	30	34	34	25	26	37	108	21	24	33	35	32	31
SCF	12	12	77	75	11	11	363	417	16	16	27	26	20	19
RANTES	1999	1904	2435	2354	2635	2695	2358	2009	1550	1545	1794	1590	2813	2876
IFNG	37	38	61	67	46	55	69	200	54	51	68	69	63	59
GMCSF	6220	6167	3299	3792	2091	2631	1497	2436	4198	3689	5659	5147	3785	4123
TNFA	322	314	312	323	317	317	311	347	325	316	368	351	316	314
HGF	178	172	112	119	179	182	174	253	194	187	331	270	295	279
MIP1B	107	95	570	609	151	170	142	215	74	74	73	71	77	75
IFNA	166	158	189	188	178	187	213	227	182	177	178	176	223	212
TGFB	68	70	120	118	106	102	170	189	120	104	114	114	94	92
MCP1	606	632	578	749	429	554	405	2332	1116	985	751	739	1611	1540
IL9	76	77	97	99	68	71	101	104	70	72	72	69	117	113
VEGFD	56	61	42	43	39	38	65	74	48	51	61	59	64	60
TNFB	78	80	97	100	98	102	162	160	90	93	96	91	161	154
NGF	20	24	27	28	18	17	26	31	21	22	18	20	22	22
EGF	136	98	78	115	237	486	1391	901	1079	509	1527	672	91	117
BDNF	3485	3359	3747	4017	4404	5203	4861	4152	6740	6523	7960	7081	2494	2563
TRAIL	163	153	147	143	254	290	110	130	71	73	76	74	76	74
GCSF	88	90	88	102	84	90	148	208	103	101	100	97	138	133
GROA	32	33	40	41	36	39	42	45	32	30	32	30	44	41
IL1A	110	103	98	100	195	211	122	123	72	70	95	90	112	112
IL23	25	23	26	25	25	27	31	34	23	25	24	22	32	32
IL12P40	444	436	517	551	678	735	492	606	566	532	796	745	664	650

IL15	43	41	38	38	32	35	79	84	33	36	39	39	67	63
IL18	211	207	222	230	308	321	347	378	201	187	378	374	234	232
MCSF	37	39	45	45	39	40	52	52	33	34	35	32	53	50
MCP3	50	57	53	61	56	59	54	65	57	58	48	52	58	63
MIG	76	82	63	72	138	160	93	583	48	49	105	106	63	63
RESISTIN	4024	3576	2662	3014	2275	2280	3374	4572	2387	2505	4969	4280	3709	3060
IL21	48	47	40	37	118	126	56	65	28	29	30	30	33	34
ICAM1	1002	973	935	1019	1996	1956	942	1104	1959	1857	3049	2882	1907	1835
VCAM1	17279	17216	16949	17093	16881	16878	16501	16621	17114	16952	17708	17570	17212	17398
FGFB	39	37	47	50	29	31	77	81	34	33	36	34	72	71
IL22	44	83	66	64	54	59	61	96	105	78	425	119	51	86
PDGFBB	54	60	42	42	147	145	140	145	192	230	730	864	231	173
VEGF	222	212	152	156	281	331	210	309	415	399	293	270	404	380
LEPTIN	3601	3417	1401	1676	961	1272	584	1207	2776	2598	3426	3202	2431	2769
PAI1	10933	10775	11385	11538	11608	11636	11287	11009	11690	11656	12171	11947	11361	11897
CD40L	197	167	239	278	239	286	992	1037	156	126	251	163	694	711
ENA78	595	551	550	569	355	339	123	95	263	230	169	162	530	459
CHEX1	11335	11816	11569	11375	11304	11996	11199	11660	11625	11604	11667	11737	11457	11606
CHEX2	1003	915	1184	1082	1249	1333	1232	1314	1171	1173	1174	1075	449	1341
CHEX3	607	591	555	579	625	629	663	677	578	609	623	588	584	663
CHEX4	32	46	49	45	23	23	22	27	29	31	27	31	14	16

Table S5. Frequencies of VZV antigen-reactive TCRβ chain sequences in total CD4 T cells (%)

Subject ID	A1		A2		B1		B2		C1		C2		D		E		F	
Day 0	0.25	0.24	0.19	0.18	0.26	0.27	0.12	0.09	0.34	0.35	0.47	0.58	0.30	0.33	0.68	0.67	0.55	0.52
Day 8	1.49	1.49	0.50	0.57	1.41	1.37	0.48	0.48	0.76	0.79	3.08	3.37	1.54	1.60	1.17	1.09	1.01	1.08
Day 14	1.18	1.21	0.58	0.66	1.01	1.04	0.87	0.82	0.73	0.71	2.07	2.13	1.00	0.98	0.97	0.91	1.11	1.11
Day 28	0.69	0.66	0.49	0.51	0.67	0.74	0.64	0.68	0.70	0.73	1.07	1.07	0.54	0.52	0.86	0.84	0.82	0.98

Two replicates of one million ex vivo total CD4 T cells were sequenced for each individual at each time point and examine for the presence and frequencies of TCRβ chain sequences from VZV-reactive T cells. Summary metrics is shown in Figure 3A.

Table S6. Clonal sizes of VZV antigen-reactive CD4 T cells derived from naïve or memory cells (#reads/million reads)

Naïve derived	Subject ID	A1	A2	B1	B2	C1	C2	D	E	F
	Day 0	0.00	2.77	13.71	0.00	5.95	8.44	4.07	4.93	2.93
	Day 8	2.57	9.46	20.18	7.74	9.36	16.32	19.12	6.48	3.67
	Day 14	4.83	9.68	22.68	24.10	4.92	12.61	9.74	6.27	3.98
	Day 28	6.71	9.41	13.86	44.11	7.28	5.91	8.78	5.44	7.72
Memory derived	Subject ID	A1	A2	B1	B2	C1	C2	D	E	F
	Day 0	12.79	12.23	9.39	11.84	12.65	10.19	9.34	18.33	13.23
	Day 8	49.32	20.53	33.56	37.03	20.53	47.55	28.71	26.14	15.28
	Day 14	39.06	22.52	23.61	52.44	18.34	31.72	17.08	21.58	15.45
	Day 28	23.36	19.47	20.38	43.10	18.08	16.08	10.94	19.68	14.92

Summary metrics is shown in Figure 4C.

Table S7. Diversity of VZV-specific CD4 TCR repertoire

Number of clones	Subject ID	Day 0	Day 28
	A1	83	96
	A2	68	65
	B1	166	219
	B2	50	78
	C1	150	220
	C2	230	260
	D	155	206
	E	120	140
	F	194	365
Percent of clones	Subject ID	Day 0	Day 28
	A1	0.05	0.06
	A2	0.07	0.07
	B1	0.07	0.09
	B2	0.07	0.11
	C1	0.06	0.08
	C2	0.09	0.11
	D	0.06	0.08
	E	0.10	0.11
	F	0.06	0.11

The diversity index was calculated as described in Figure 5A and is expressed as either the number or the percent of the largest clones that take up 80% of the repertoire space. Summary metrics are shown in Figures 5B and C.

Supplementary Figures

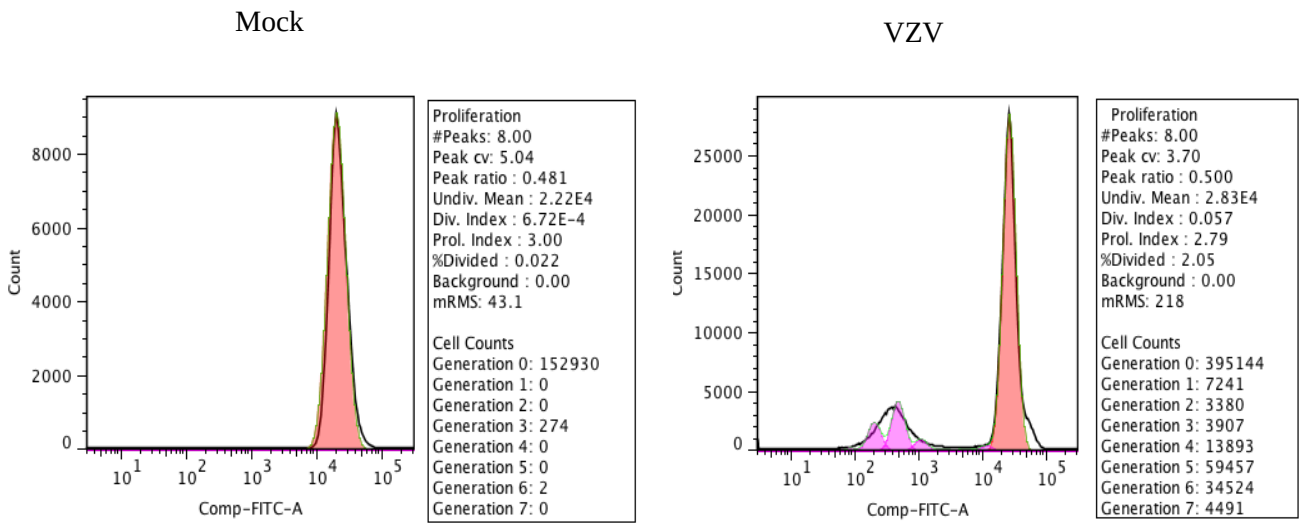

Fig. S1. VZV lysate-induced T cell proliferation. CFSE-labeled PBMCs were stimulated with VZV lysate or mock lysate for 8 days as described in Fig. 1a. The figure of gated CD4 T cells shows the data analysis using the FlowJo Proliferation Platform shows with number of cell division and cell numbers in each generation.

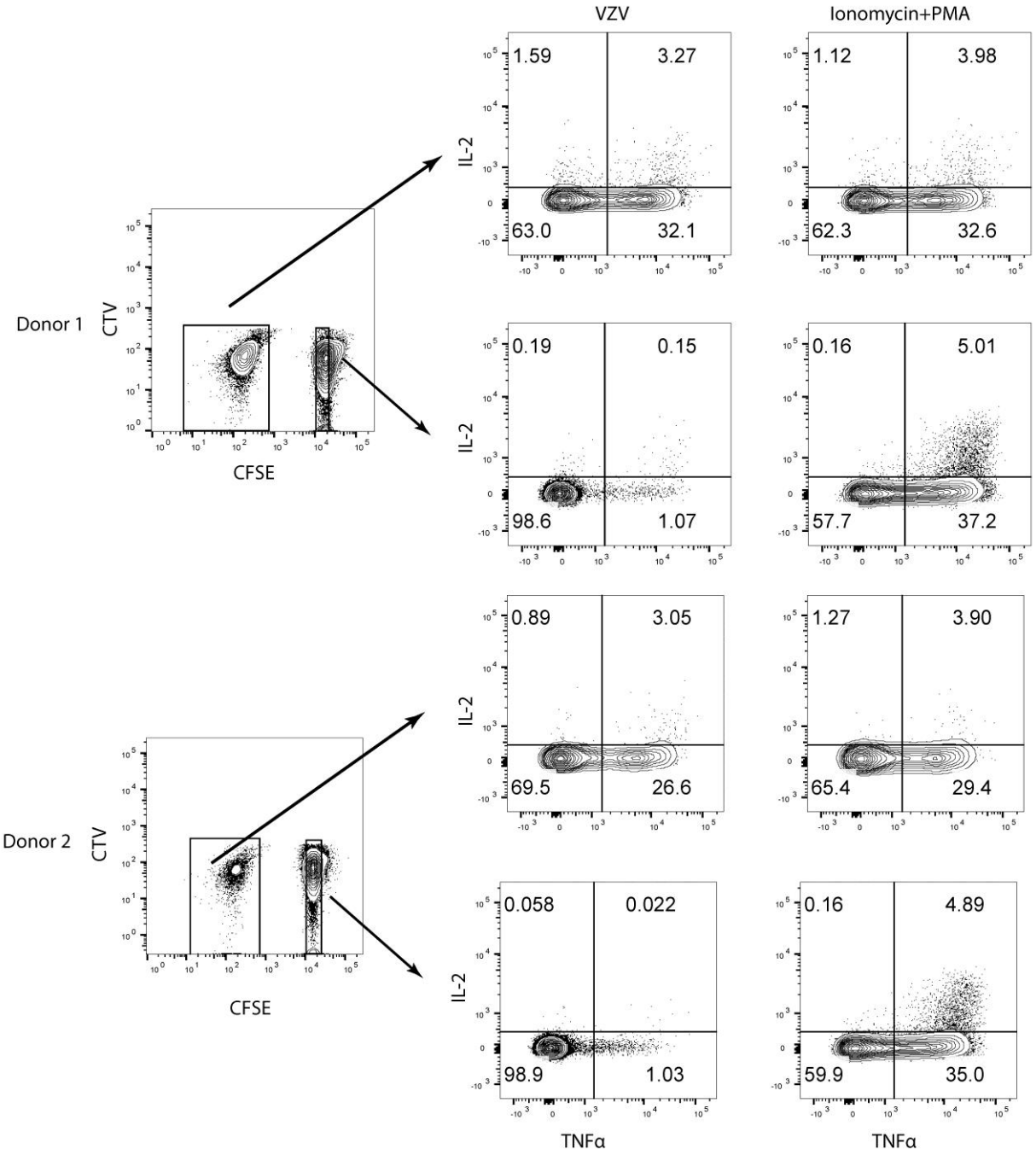

Fig. S2. Cytokine production of VZV-antigen reactive CD4 T cells. 8-day VZV lysate-activated CSFE-labeled PBMCs were restimulated by either PMA and ionomycin or VZV lysate together with fresh CTV-T cell-depleted PBMCs. CFSE low and CFSE high CTV-negative CD4 T cells were gated and plotted for TNFα and IL-2. Cytokine production in VZV antigen- and ionomycin/PMA-stimulated cells were compared and the fraction of cytokine-producing cells stimulated by VZV lysate after normalization for the number of cytokine-producing cells after stimulation with PMA and ionomycin is shown in Figure 1b.

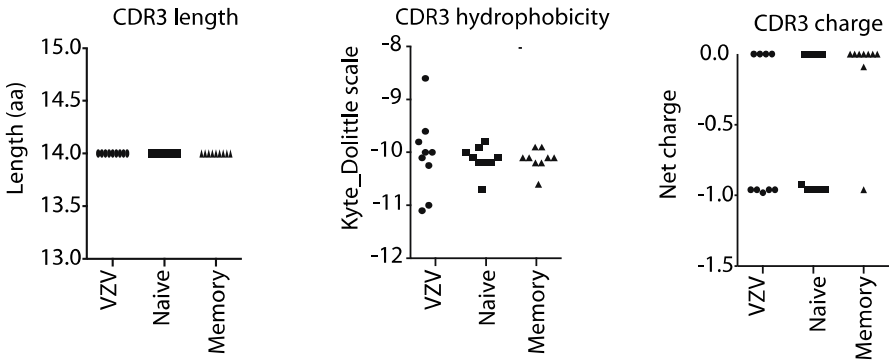

Fig. S3. CDR3 features of VZV antigen-reactive CD4 T cells. The median length (left panel), hydrophobicity (middle panel) and net charge (right panel) of CDR3 amino acid sequences in VZV-reactive, naïve and memory CD4 TCRβ repertoires are compared using paired Wilcoxon-Mann-Whitney test.