

Supplementary (S) Figures

Figure S1a

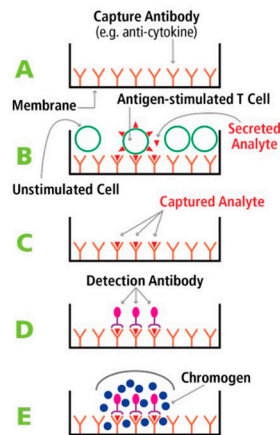


Figure S1b

Typical T cell ImmunoSpot well (IFN γ)

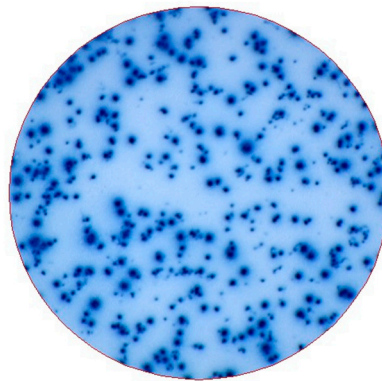


Figure S1c

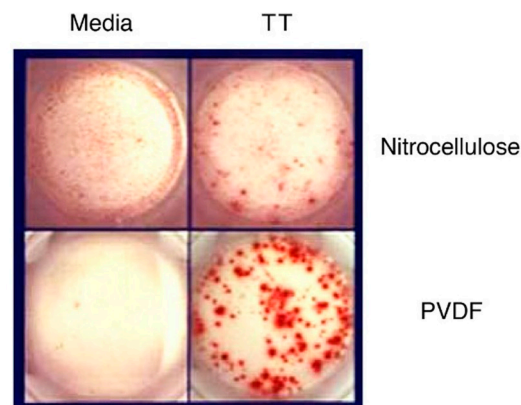


Figure S1. Principle of the T cell ImmunoSpot® assay

a. A schematic of the ELISPOT assay. PVDF-membrane plates are coated with anti-cytokine capture antibody. PBMCs are added with stimuli and incubated for 20 hours. Cells are washed off and captured cytokine is detected with a complementary anti-cytokine antibody conjugated to an enzyme. Excess is washed off and the enzyme caused chromogen precipitation in the location of a secreting cell in the form of a distinct spot.

b. A typical well showing approximately 200 spots representing T cells secreting IFN γ in response to stimulation with CMV peptide pool.

c. A comparison of membranes Nitrocellulose and PVDF, showing distinct spots with the latter (4).

Figure S2a

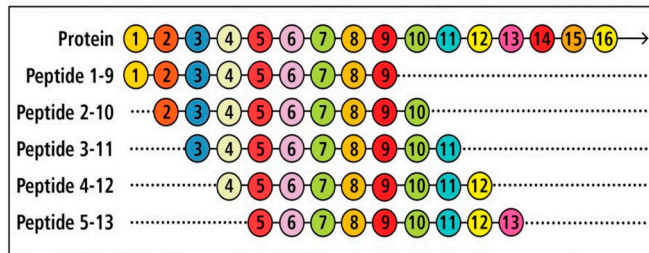


Figure S2b

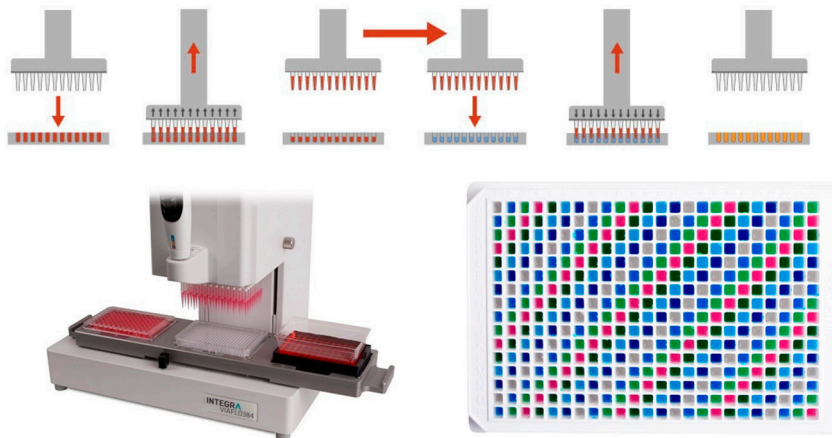


Figure S2. Strategy for comprehensive epitope mapping

a. Peptide design schematic with consecutive peptides moving down the protein sequence one amino acid at a time (10). b. Automated plating of peptides can be carried out where the robot-controlled pipette picks up peptides from a reservoir and then deposits them into a 384-well ELISPOT plate.

Figure S3a

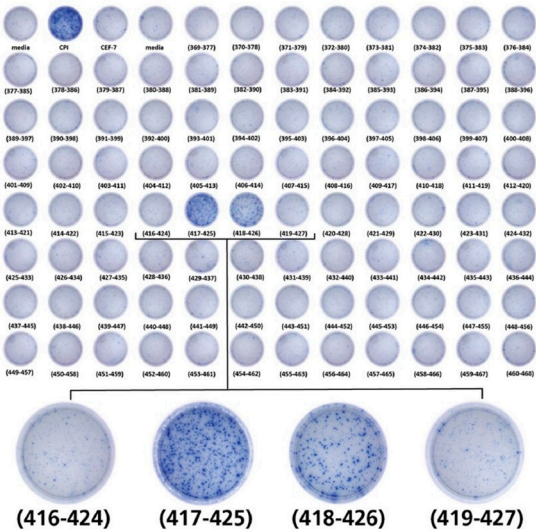


Figure S3b

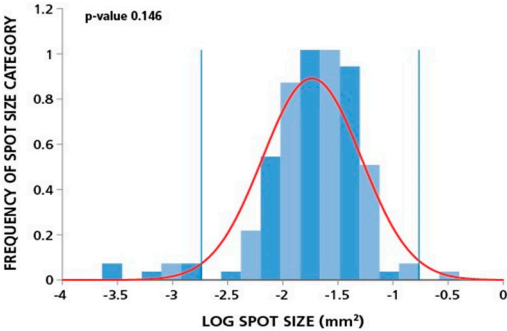


Figure S3c

Negative Controls and Cut Off Values For Response Categories	\bar{x}	n=18	Cryptic epitopes (99.7% conf)
	σ		
	$\bar{x} * 3\sigma$		
	$\bar{x} * 5\sigma$		Subdominant epitopes
	$\bar{x} * 10\sigma$		Dominant epitopes
	>100 SFU		Super-dominant epitopes

Figure S3. Analysis of raw data

a. An example of an image of an entire plate where responses against HCMV peptides are being investigated. 4 wells are zoomed in upon. b. Spot size distribution showing the position of vertical gates for counting spots (11). c. Classification of positive results to peptides based on the number of spots above the negative control (12).

Figure S4a

Peptide Name	Sequence	Individual Subject CDR3 T Cell Response (SFU per 50,000 PBMC)									
		D0-1	D0-2	D0-3	D0-4	D0-5	D0-6	D0-7	D0-8	D0-9	D0-10
pep01-018-026	GGHLLVAV	0	2	2	0	7	1	72	11	0	6
pep01-020-038	GGPVLNNE	0	2	3	1	2	2	5	8	1	12
pep01-065-073	STPCHRSN	16	0	0	44	0	1	1	5	0	1
pep01-070-078	RHCHGCHD	2	0	2	2	0	2	86	22	2	2
pep01-085-103	HNPFERSK	13	0	20	0	0	1	2	10	1	1
pep01-097-105	PTSGSKPS	0	0	41	1	21	0	9	5	0	2
pep01-110-121	CPGSPFAG	15	0	6	2	1	0	5	11	2	7
pep01-106-114	GPMSGVVY	14	0	16	5	0	0	2	13	1	3
pep01-107-108	LPMSGYTA	0	0	17	2	2	0	2	1	1	1
pep01-114-121	VALPMLN	22	1	7	0	3	2	28	14	0	3
pep01-115-123	ALPMLN	11	0	5	7	2	0	6	22	6	2
pep01-116-124	LPMLNAP	71	0	7	14	2	3	5	18	5	2
pep01-119-127	RMALPSPN	1	0	6	10	2	1	21	10	0	0
pep01-119-148	HRALPADA	13	1	1	16	1	1	6	9	5	1
pep01-141-149	MLPNDAM	7	0	1	0	28	0	5	8	0	3
pep01-142-150	LPNDAMN	11	0	2	10	0	0	6	10	1	1
pep01-146-151	VALNDAMN	1	2	5	0	44	1	2	1	1	6
pep01-149-157	HAAGSGMAW	0	0	16	1	2	0	1	2	2	2
pep01-151-158	ASGSGMAW	20	0	2	3	0	1	7	6	1	0
pep01-153-160	SGSGMNGAQ	23	0	9	7	0	1	10	9	3	1
pep01-159-163	QHPGKATV	1	1	7	1	10	2	10	13	0	0
pep01-175-183	WKEPQVYIT	1	0	2	0	10	0	1	7	0	1
pep01-188-196	IFFPQVLA	1	1	5	6	1	1	3	13	1	1
pep01-205-211	ELCHAMET	0	0	0	2	1	1	21	3	7	1
pep01-208-216	MENTATAM	1	1	71	7	5	1	0	14	11	3
pep01-213-219	QGVFVLE	1	1	7	1	76	0	0	10	0	0
pep01-228-236	LESCDVP	0	0	2	6	10	1	1	2	0	0
pep01-250-258	VEELTMTN	3	0	3	6	13	3	2	2	2	1
pep01-251-259	EEELTMTN	0	1	1	2	1	2	10	1	1	2
pep01-262-270	PSMRPHEIN	0	1	10	5	0	2	6	3	9	1
pep01-267-275	HEMGPTA	0	0	3	0	0	0	6	2	0	0
pep01-270-278	NSGFVLPK	0	2	10	0	1	0	5	9	7	10
pep01-273-281	TVLCRNMI	1	0	9	62	0	2	7	2	3	2
pep01-284-292	PIGSGHNL	11	0	0	11	2	7	6	7	10	18
pep01-300-308	LAHNGDPL	14	2	10	17	1	0	21	2	1	21
pep01-324-332	QGVFLVCA	44	0	5	3	3	0	6	5	1	0
pep01-325-333	QFLVGLA	14	1	6	16	5	1	13	0	7	7
pep01-328-336	LEVGNMET	0	0	1	7	1	1	7	10	5	21
pep01-390-398	ISAGAGDSD	0	0	5	56	0	0	7	13	2	6
pep01-395-403	GDQDWMYS	2	0	3	10	0	0	14	8	1	2
pep01-413-425	PRVYSSGA	0	0	3	32	0	1	10	2	10	2
pep01-418-426	PRVYSSAM	1	0	6	6	0	0	6	11	10	0
pep01-430-438	ITAGSRNK	3	1	0	89	1	0	11	18	11	7
pep01-431-439	TSAGSRNS	0	0	8	9	0	1	16	17	1	2
pep01-465-473	EEETDESD	0	1	11	54	1	1	5	6	7	7
pep01-482-490	PTNPFVCAQ	0	21	1	14	1	1	5	1	10	3
pep01-482-500	LARKLVNAV	21	2	5	6	0	0	2	1	5	0
pep01-505-513	LVGRNAVY	40	2	1	10	37	1	1	1	14	10
pep01-510-511	VQGVNAVY	2	1	1	1	0	1	5	3	1	1
pep01-511-519	GFYWDAND	2	1	6	9	0	0	8	17	1	8
pep01-512-520	FFYWDAND	0	28	5	10	1	1	8	13	17	11
pep01-513-521	FFYWDANDY	1	25	5	6	0	1	2	8	8	100
pep01-514-522	FWWDANDN	0	2	1	0	1	10	13	10	10	44
pep01-521-529	WRFALSS	2	1	80	5	0	0	10	7	1	5
pep01-524-532	PAELVYVWL	2	16	6	8	1	7	14	8	11	6
pep01-544-552	QNALPQVQ	2	6	5	3	13	5	13	5	2	2
Negative		2	1.0	0.8	4.2	3.9	3.9	1.8	6.5	8.4	1.2
Controls and Cut		1.0	1.2	1.6	4.4	6.8	2.4	1.8	1.8	1.7	2.7
Off Values For		8% σ	3.9	4.6	14.9	17.1	5.5	8.8	23.3	25.3	11.3
Response		8% σ	5.8	7.2	22.1	25.9	6.5	13.5	34.4	36.3	16.8
Categories		10% σ	10.7	13.7	40.0	47.8	9.1	25.3	62.4	64.2	30.3
		2-100 SFU	>100SFU	>100SFU	>100SFU	>100SFU	>100SFU	>100SFU	>100SFU	>100SFU	>100SFU

Figure S4b

Ref.	Peptides Tested				Individual Subjects' CD8+ T Cell Response (SFU per 300,000 PMAC)									
	Peptide Name	Epitope Sequence	Percentile Binding Score	pp65 Rank	ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10
42,43,44,45	pp65-495-503	NLVPMVATV	0.06	1	60	303	1	100	97	148	287	274	14	318
33	pp65-340-348	RQYDPVAAL	0.06	2	6	7	5	6	0	2	1	2	5	21
30,37	pp65-040-048	RLIGTGHV	0.09	3	0	1	2	7	3	0	5	13	2	1
30,36,37,38,39	pp65-522-530	RFAELGV	0.11	5	5	6	0	9	0	8	5	11	8	10
31,37	pp65-320-328	LMNGQGFL	0.15	4	14	2	10	17	1	0	21	2	1	21
38	pp65-218-226	VIGDQYKV	0.23	7	0	0	10	3	2	1	6	5	17	1
31,32	pp65-155-163	QMWDQRLTV	0.24	6	1	1	7	1	10	2	13	13	5	0
48,41	pp65-014-022	VLGPGSHV	0.24	9	1	1	10	3	5	0	3	24	8	8
30,34,37,48,41,46,47	pp65-120-128	MLNPISNV	0.25	11	8	0	5	2	2	0	9	15	3	8
33	pp65-347-355	ALFFFDL	0.54	12	0	0	8	23	0	0	3	14	1	3
37	pp65-491-499	IAANNVPM	0.74	13	1	0	5	2	1	2	7	2	7	3
30	pp65-425-433	AMAGATSA	0.85	15	2	0	5	2	1	1	8	7	1	2
34	pp65-042-050	LQIGHVRV	0.98	21	1	0	6	18	3	0	5	2	5	10
38	pp65-054-062	SILVSRIT	1.5	23	1	0	1	6	3	1	5	5	0	3
22	pp65-325-333	QIFLEQAI	1.6	27	3	1	6	16	5	1	1	13	0	7
30	pp65-312-320	GLISGNLL	1.9	26	5	0	9	5	0	2	5	6	5	2
30	pp65-110-118	SIYYVALPL	2.2	28	8	0	5	9	0	0	1	18	3	13
38	pp65-227-235	YLSEFQDV	2.6	32	0	0	1	2	1	2	22	7	5	2
34,35	pp65-341-349	QYDPVAALF	3.3	36	1	9	9	28	0	0	1	9	1	2
22	pp65-324-332	QQIFLEVQA	4	43	341	0	5	3	3	0	6	5	1	8
30	pp65-519-527	DTYRIIAEL	4.3	49	1	0	7	0	2	1	14	3	1	1
22	pp65-141-149	HLPVADAVI	5.1	54	7	0	1	0	26	0	5	8	0	3
22	pp65-144-152	VADAVIHAS	11	80	1	2	5	0	44	1	2	3	3	6
38	pp65-509-517	KYGEFFWDA	12	92	0	2	2	2	0	3	11	2	2	7
33	pp65-345-353	VALFFFDI	16	97	0	6	2	23	0	0	8	3	2	5
22	pp65-203-211	ELVCSMENT	23	163	0	0	0	2	1	1	21	3	7	1
22	pp65-221-229	DDYKVYLE	25	229	1	1	7	1	76	0	0	10	6	0
22	pp65-116-124	LPLKMLNP	51	360	71	0	7	14	2	3	5	18	5	2
22	pp65-417-425	TPRYTGGA	63	378	0	0	3	32	0	1	10	2	154	2
22	pp65-418-426	PRVTGGGAM	73	394	1	0	6	6	0	0	6	11	102	0
22	pp65-097-105	PTGRSKIPS	78	510	0	0	41	1	21	0	9	5	0	2

Figure S4c

		Test Subjects' CD8+ T Cells Specific for Epitopes									
		ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10
Cryptic Epitopes											
Number		11	23	32	24	6	2	14	3	8	21
Cum. SFU		12.10	18.67	67.15	92.50	3.04	0.39	58.40	11.53	21.57	56.12
% of total SFU		1.02%	4.41%	3.02%	13.54%	0.73%	0.24%	4.03%	1.04%	2.89%	5.09%
Subdominant Epitopes											
Number		14	8	5	17	6	2	6	0	1	16
Cum. SFU		45	35	63	230	14	21	194	0	7	132
% of total SFU		4%	8%	3%	34%	3%	13%	13%	0%	1%	12%
Dominant Epitopes											
Number		15	4	3	4	9	0	3	1	0	5
Cum. SFU		281	72	147	193	263	0	178	73	0	221
% of total SFU		24%	17%	7%	28%	63%	0%	12%	7%	0%	20%
Super Dominant Epitopes											
Number		3	1	4	2	1	1	3	2	2	3
Cum. SFU		847	298	1948	168	139	139	1019	1027	717	694
% of total SFU		71%	70%	88%	25%	33%	86%	70%	92%	96%	63%
Total Epitopes Recognized		43	36	44	47	22	5	26	6	11	45
Cumulative Spec. SFU		1185	424	2226	683	418	161	1450	1111	746	1103

Figure S4d

Peptide Pools	HCMV-seropositive donors						HCMV-seronegative donors					
	Donor 29	Donor 1	Donor 4	Donor 7	Donor 10	Donor 11	Donor 19	Donor 28	Donor 5	Donor 6	Donor 21	Donor 9
Media	11*	2	8	19	0	1	3	2	3	1	7	0
HE-1(120)	35	46	552	966	278	395	0	0	5	0	0	0
HE-2(143)	6	29	21	37	98	10	14	0	0	0	2	0
pp65(138)	213	565	509	86	437	136	0	2	0	0	5	2
UL28(92)	3	91	6	274	13	208	0	0	0	0	0	2
UL32(260)	6	928	202	32	152	40	3	0	0	0	0	2
UL36(117)	709	77	10	374	139	50	2	0	0	2	0	0
UL40(53)	0	2	10	30	8	3	0	0	0	0	10	2
UL48-sub1(229)	3	3	6	14	2	8	0	0	0	0	0	0
UL48-sub2(229)	40	6	14	45	10	2	2	0	2	0	3	0
UL55(224)	936	42	923	931	651	19	2	0	2	0	2	0
UL82(137)	0	83	81	189	2	2	0	0	2	0	0	0
UL94(84)	89	0	21	997	290	8	0	5	0	0	5	2
UL99(45)	0	0	61	27	6	10	0	0	0	2	0	0
UL103(60)	38	38	21	32	2	10	2	0	0	0	2	0
UL151(82)	0	0	22	14	0	2	0	0	0	0	0	0
UL153(67)	3	14	54	27	10	8	0	0	0	0	2	0
US3(44)	0	587	27	869	269	37	3	0	0	0	0	0
US24(123)	5	0	19	461	5	5	0	0	3	0	0	0
US29(113)	2	0	16	26	0	5	0	0	0	0	5	0
US32(43)	2	0	51	42	5	10	0	0	2	0	0	0
CPI	481	829	243	695	720	262	177	337	322	221	415	266

Figure S4e

	Donor ID	Medium	EBV antigens / peptide pools (No. of peptides)														CPI Pool
			BMF1 (117)	BMRF1 (99)	BRF1F (149)	RZLF1 (59)	EBNA LP (124)	EBNA1 (158)	EBNA2 (119)	EBNA3a (234)	EBNA3b (279)	EBNA3c (265)	GP350 (224)	LMP1 (94)	LMP2 (122)		
EBV seropositive donors	Donor 1	1	1	3	1	1	53	141	8	5	19	161	7	0	3	829	
	Donor 11	0	13	3	128	11	1	23	108	13	13	11	7	3	0	262	
	Donor 15	1	33	189	47	683	72	477	207	65	223	93	24	5	23	874	
	Donor 2	0	1	0	11	207	53	81	17	247	13	3	57	0	0	231	
	Donor 18	1	7	1	0	35	0	3	0	173	3	5	4	0	1	221	
	Donor 25	0	9	25	113	5	8	125	75	7	3	93	15	4	231	419	
	Donor 3	7	15	88	33	73	24	44	80	8	73	19	1	0	12	112	
	Donor 7	7	16	24	51	97	85	80	713	48	21	21	27	5	49	695	
EBV seronegative donors	Donor 9	0	8	24	89	12	11	56	97	80	29	44	12	0	144	266	
	Donor 16	3	0	4	4	0	7	5	0	0	3	1	0	1	3	197	
	Donor 19	16	0	5	5	0	1	9	11	0	0	3	0	1	3	177	
	Donor 20	1	5	4	9	1	3	5	0	0	5	9	1	0	0	601	
	Donor 21	4	5	7	0	1	4	0	5	0	3	0	3	3	3	415	
	Donor 30	1	4	0	0	1	12	1	15	8	1	11	0	0	0	293	

Figure S4f

ID.	ORF3a	N	Nsp12	Nsp5	S (A & B)	S-RBD	M
dC1	10%	12%	13%	0%	40%	7%	16%
dC2	12%	25%	12%	0%	40%	2%	10%
dC3	10%	13%	3%	5%	50%	10%	10%
dC4	3%	21%	6%	3%	47%	7%	13%
dC5	1%	16%	0%	2%	49%	23%	9%
dC6	5%	31%	11%	2%	35%	4%	14%
dC7	12%	10%	9%	1%	51%	3%	15%
dC8	6%	21%	17%	1%	31%	16%	7%
dC9	0%	19%	0%	0%	24%	8%	49%
\bar{x}	6%	18%	8%	2%	40%	9%	16%
σ	4%	6%	6%	3%	9%	6%	12%
# Pept.	66	102	231	74	315	53	53
$\bar{x}/(\# \text{ Pept.})$	0.09%	0.18%	0.03%	0.03%	0.13%	0.17%	0.30%

Figure S4. IFN γ ELISPOT response mapping data for 3 viruses

Responses to peptides spanning antigens for different viruses. Different categories of responses are defined using the scheme in Fig.3c. For HCMV a. overall results for pp65 from 10 healthy individuals. B. Comparison of results to predicted peptide to MHC binders. c. Summary of responses to peptide response categories. d. Summary of responses to peptides representing other HCMV antigens. e. Summary of responses to peptides representing EBV antigens. f. Summary of responses to peptides representing antigens of SARS-CoV-2 (14,15).

Figure S5a

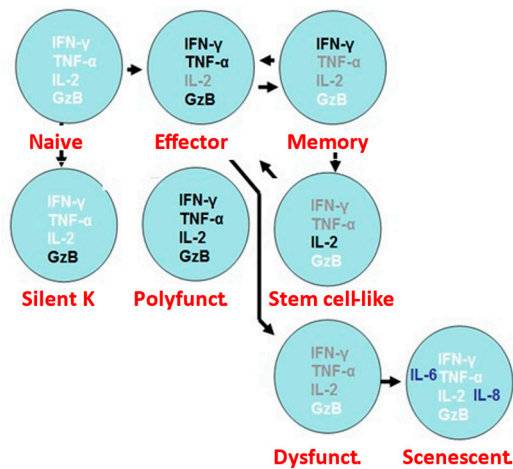


Figure S5. Quality of T cell responses

Schematic of the classification of T cell responses based on their secretion of single or multiple

cytokines in response to antigens using the FluoroSpot assay. T cell response phenotypes are described here as: naïve, effector, memory, silent killer (K), polyfunctional, stem cell-like, dysfunctional and senescent (16).

Figure S6

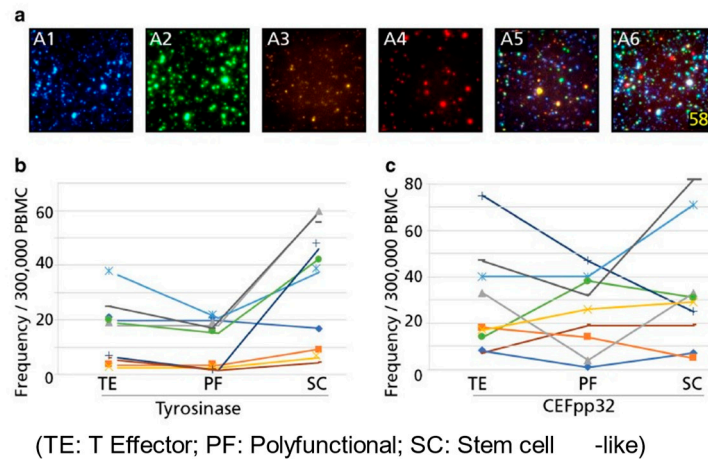


Figure S6. Anti-melanoma T cell qualitative data

a.Examples of FluroSpot responses as single and multi-colour, representing secretion of IFN γ (green A2), IL-2 (red A4), TNF α (yellow A3) and Granzyme B (blue A1), b.The relationship between frequencies of responses against Tyrosinase, c. The relationship between frequencies of responses against positive control peptides (CMV, EBV, 'flu). Each line represents a different individual. (17).