Supplementary (S) Figures

Figure S1a
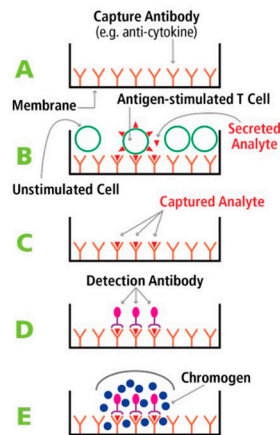
Figure S1b

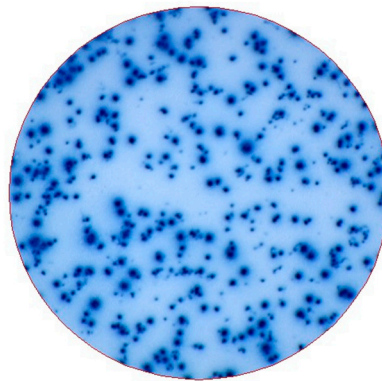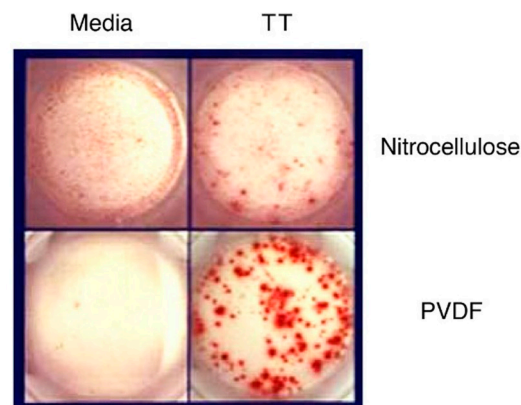**Typical T cell ImmunoSpot well (IFNγ)**

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 sectreting IFNγ in response to stimulation with CMV peptide pool. c.A comparison of membranes Nitrocellulose and PVDF, showing distinct spots with the latter (4).
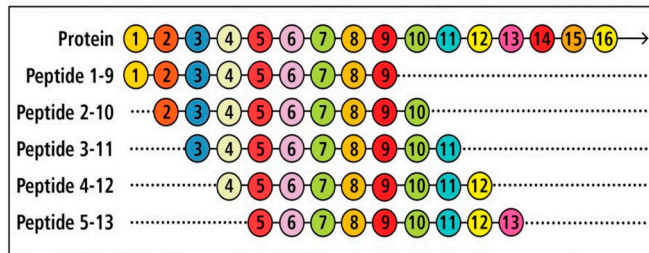
1

Figure S2a
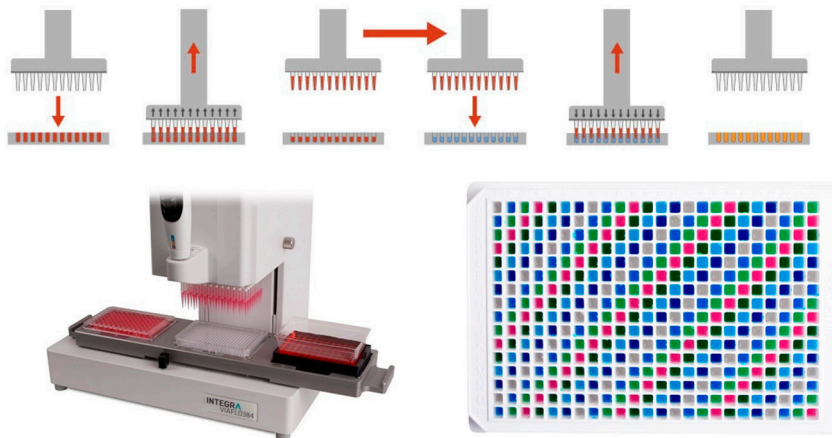

Figure S2b

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.
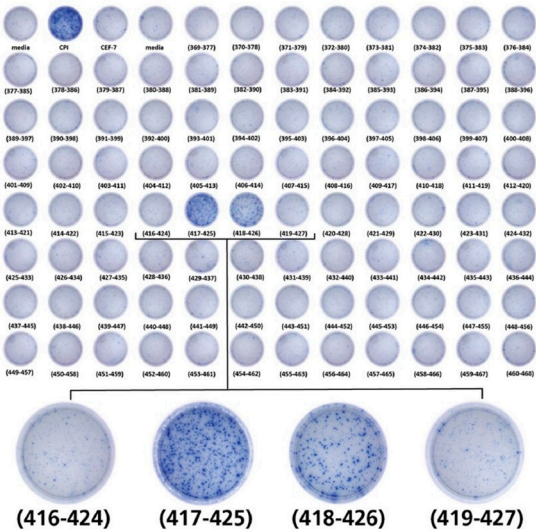
Figure S3a

Figure S3b

Figure S3c

Negative Controls and Cut Off Values For Response Categories	$\bar{x}$	n=18
	σ	
	$\bar{x}*3\sigma$	Cryptic epitopes (99.7% conf)
	$\bar{x}*5\sigma$	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

Figure S4b

Ref.	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	674	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	RLLQTGIHV	0.09	3	0	1	2	7	3	0	5	13	2	1
30,36,37,38,39	pp65:522-530	RIFAELEGV	0.11	5	5	6	0	9	0	8	5	11	8	10
31,37	pp65:320-328	LMNGQQIFL	0.15	4	14	2	10	17	1	0	21	2	1	21
30	pp65:218-226	VIGDQYVKV	0.23	7	0	0	10	3	2	1	6	5	17	1
31,32	pp65:155-163	QMWQARLTV	0.24	6	1	1	7	1	10	2	33	13	5	0
40,41	pp65:014-022	VLGPISGHV	0.24	9	1	1	10	3	5	0	3	24	8	8
30,34,37,40,41,46,47	pp65:120-128	MLNIPSINV	0.25	11	8	0	5	2	2	0	9	15	3	8
33	pp65:347-355	ALFFFDIDL	0.54	12	0	0	8	23	0	0	3	14	1	3
37	pp65:491-499	ILARNLVPM	0.74	13	1	0	5	2	1	2	7	2	7	3
30	pp65:425-433	AMAGASTSA	0.85	15	2	0	5	2	1	1	8	7	1	2
34	pp65:042-050	LQTGIHVRV	0.98	21	1	0	6	18	3	0	5	2	5	10
30	pp65:054-062	SLILVSQYT	1.5	23	1	0	1	6	3	1	5	5	0	3
22	pp65:325-333	QIFLEVQAI	1.6	27	398	1	6	16	5	1	1	13	0	7
30	pp65:312-320	GLSISGNLL	1.9	26	5	0	9	5	0	2	5	6	5	2
30	pp65:110-118	SIYVYALPL	2.2	28	8	0	5	9	0	0	1	18	3	13
30	pp65:227-235	YLESFCEDV	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	DIYRIFAEL	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
30	pp65:509-517	KYQEFFWDA	12	92	0	2	2	2	0	3	11	2	2	7
33	pp65:345-353	VAALFFFDI	16	97	0	6	2	23	0	0	8	3	2	5
22	pp65:203-211	ELVCSMENT	23	163	118	0	0	2	1	1	21	3	7	1
22	pp65:221-229	DQYVKVYLE	25	229	1	1	7	1	76	0	0	10	6	0
22	pp65:116-124	LPLKMLNIP	51	360	71	0	7	14	2	3	5	18	5	2
22	pp65:417-425	TPRVTGGGA	63	378	0	0	3	32	0	1	10	2	558	2
22	pp65:418-426	PRVTGGGAM	73	394	1	0	6	6	0	0	6	11	192	0
22	pp65:097-105	PTGRSICPS	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
IE-1/(120)	35	46	552	966	278	395	0	0	5	0	0	0
IE-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	53	51	189	2	2	0	0	2	0	0	0
UL94/(84)	59	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	0	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
			BMLF1 (117)	BMRF1 (99)	BRLF1 (149)	BZLF1 (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	33	81	17	347	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
	Donor 9	0	8	24	89	12	11	56	97	80	29	44	12	0	144	266
EBV seronegative donors	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

ID.	ORF3a	N	Nsp12	Nsp5	S (A & B)	S-RBD	M
dC1	10%	12%	13%	0%	40%	7%	18%
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%
x̄	6%	18%	8%	2%	40%	9%	16%
σ	4%	6%	6%	3%	9%	6%	12%
# Pept.	66	102	231	74	315	53	53
x̄/(# Pept.)	0.09%	0.18%	0.03%	0.03%	0.13%	0.17%	0.30%

Figure S4. IFNγ 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).
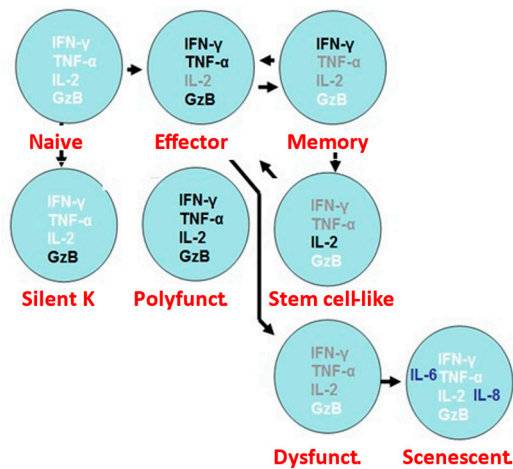
Figure S5a

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).
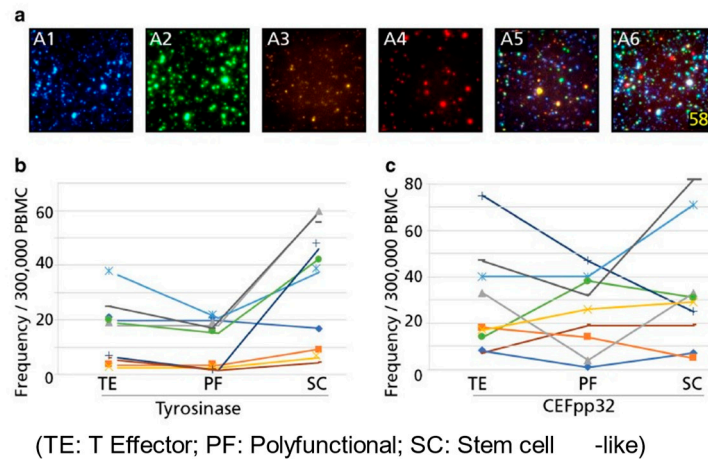
Figure S6

Figure S6. Anti-melanoma T cell qualitative data

a.Examples of FluroSpot responses as single and multi-colour, representing secretion of IFNγ (green A2), IL-2 (red A4), TNFα (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).