Figure S1A

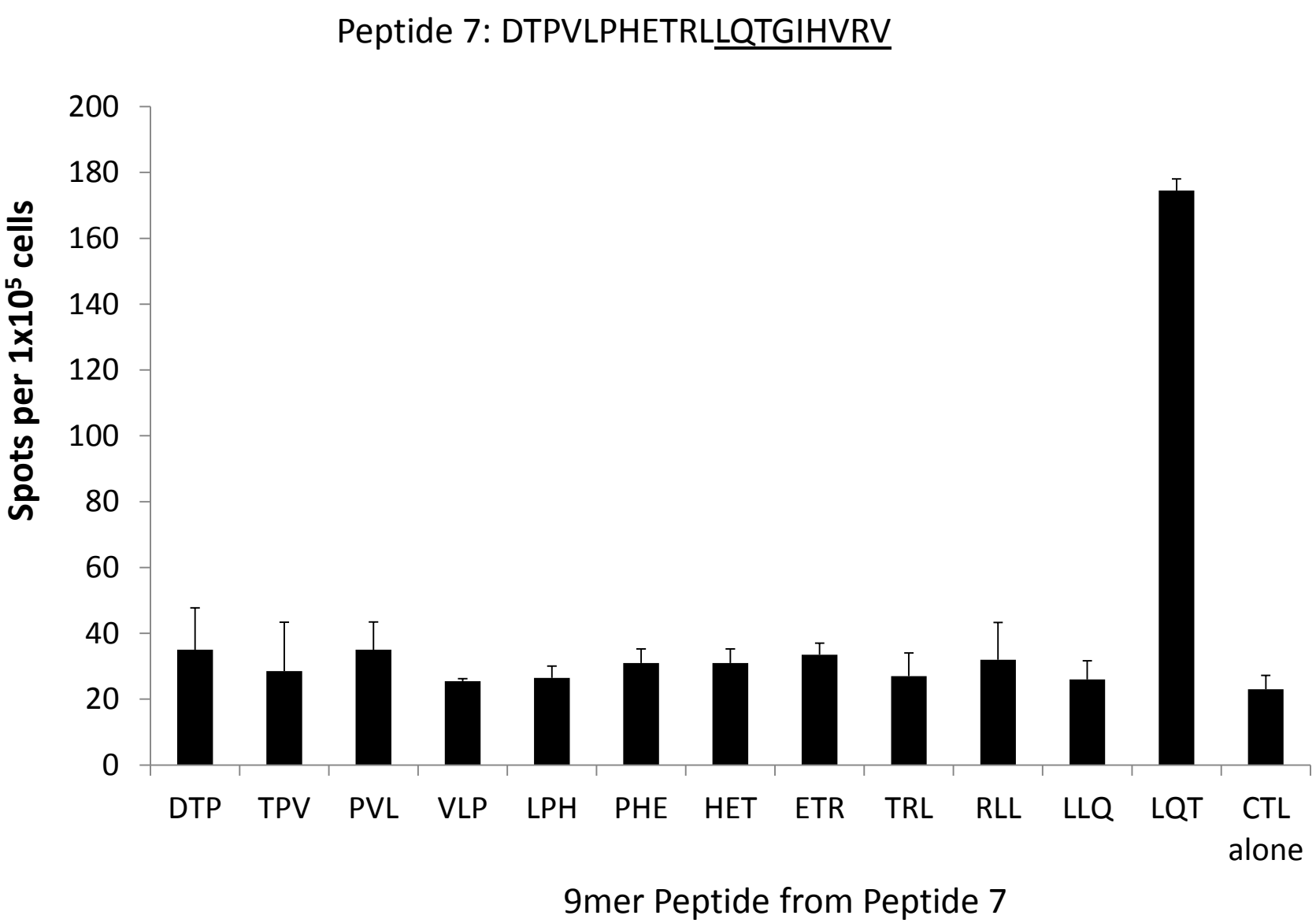

Peptide 7: DTPVLPHETRLLQTGIHVRV
Spots per 1x10^5 cells
200
180
160
140
120
100
80
60
40
20
0
DTP
TPV
PVL
VLP
LPH
PHE
HET
ETR
TRL
RLL
LLQ
LQT
CTL alone
9mer Peptide from Peptide 7

Figure S1B

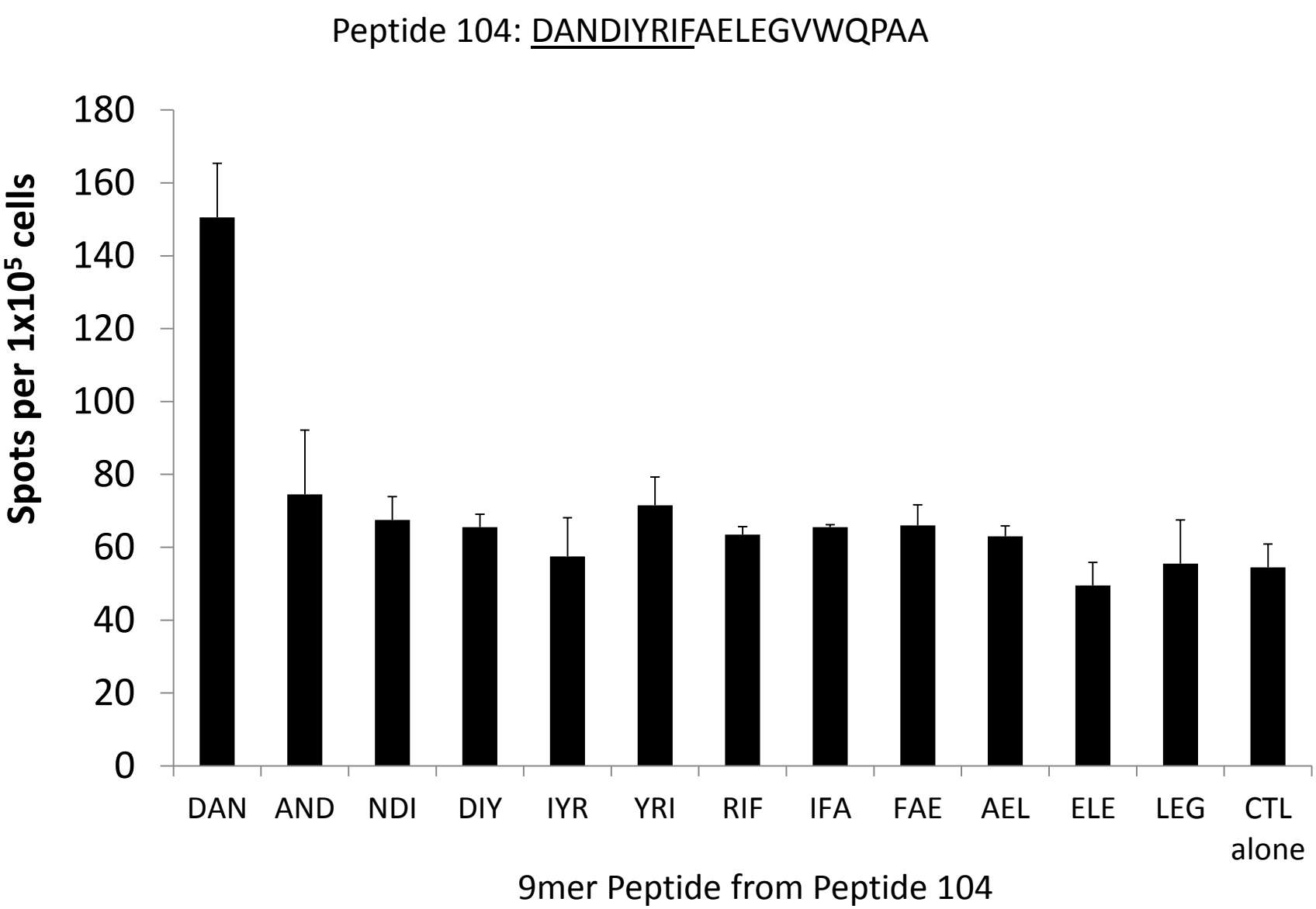
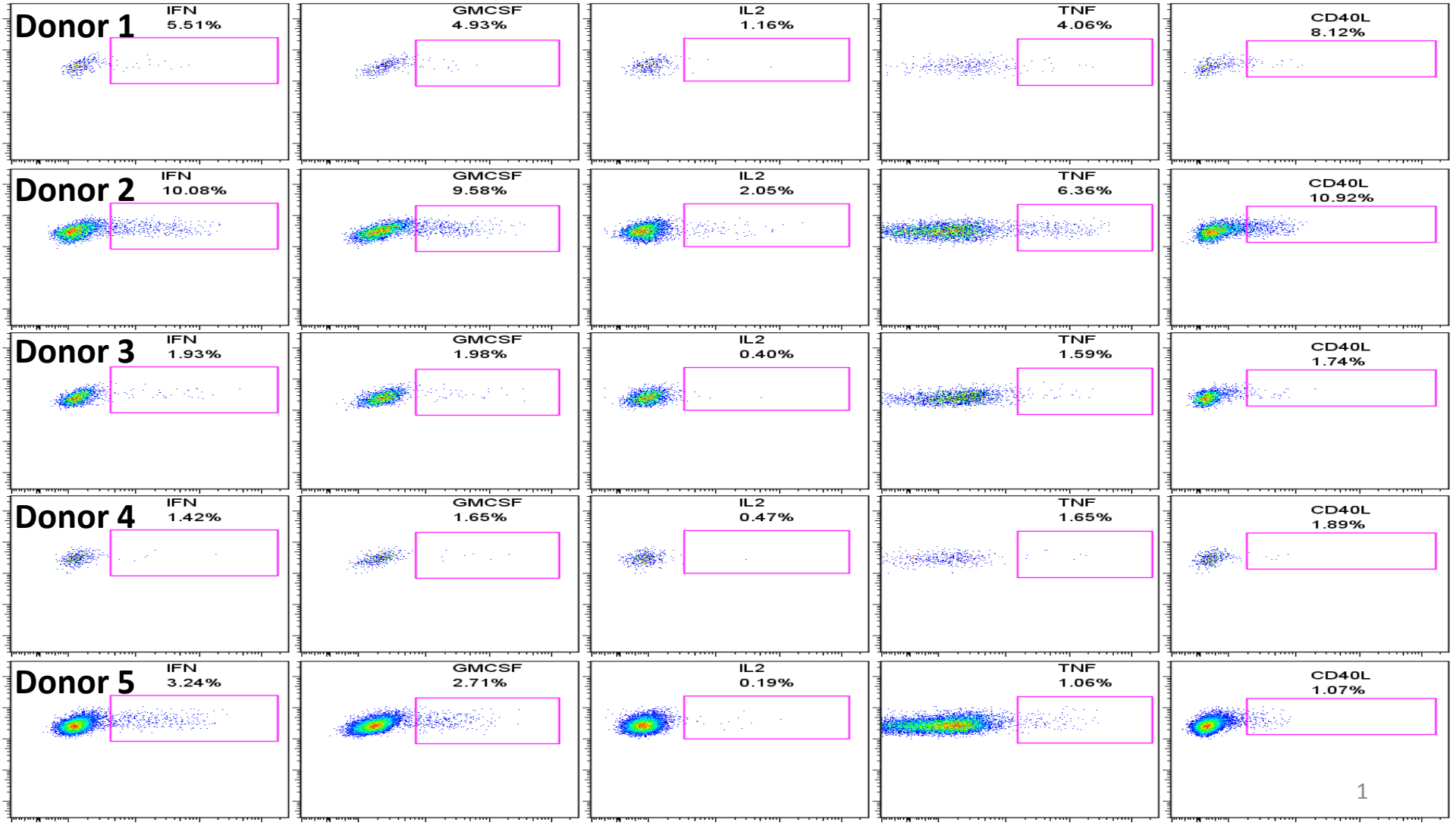
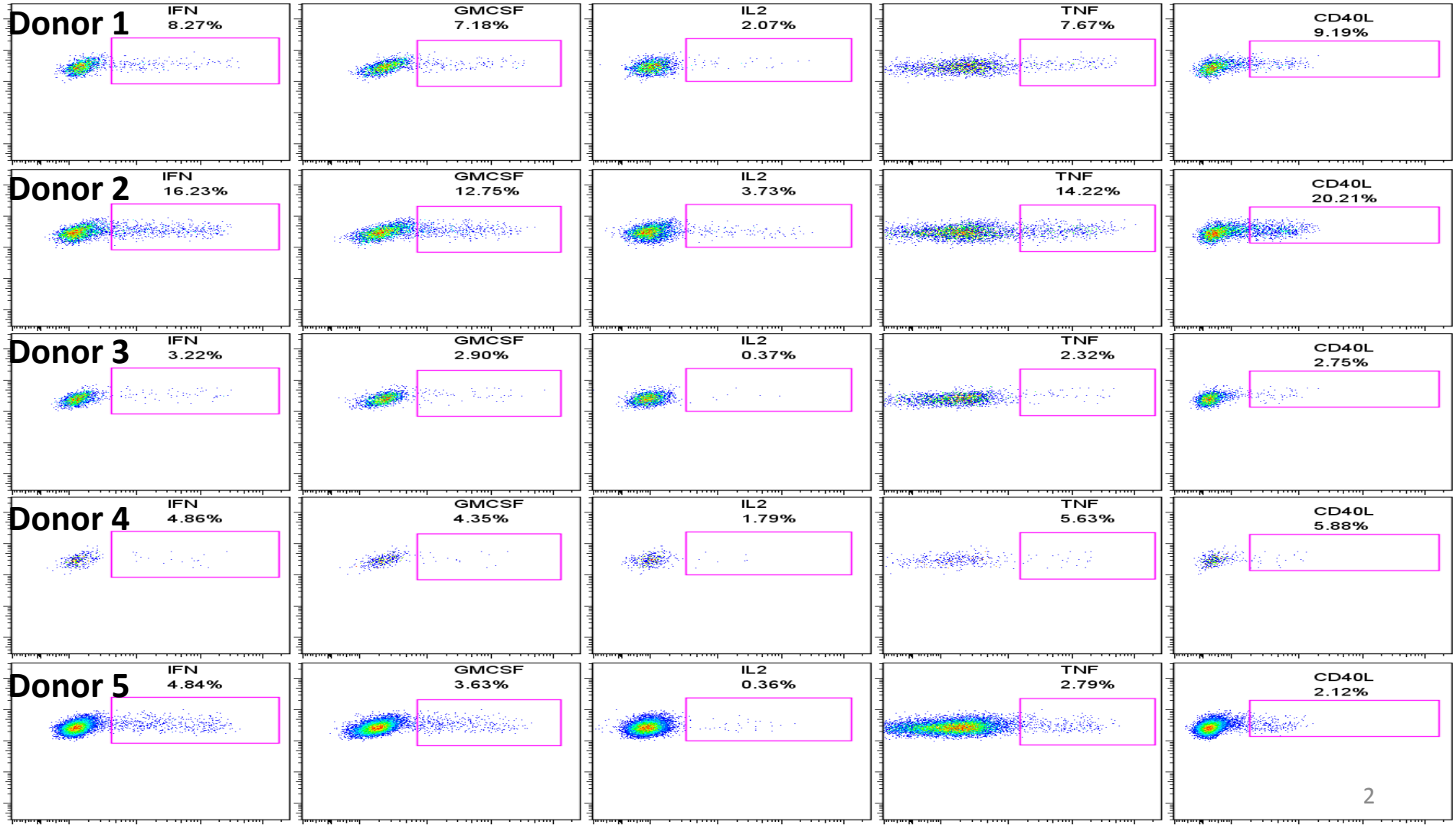

Peptide 104: DANDIYRIFAELEGVWQPAA
Spots per 1x10^5 cells
180
160
140
120
100
80
60
40
20
0
DAN
AND
NDI
DIY
IYR
YRI
RIF
IFA
FAE
AEL
ELE
LEG
CTL alone
9mer Peptide from Peptide 104

Figure S1A,B. Identifying Atypical epitopes from CMVpp65. To determine the minimal epitope, T-cells specific for the peptide DTPVLPHETRLLQTGIHVRV (S1A) and DANDIYRIFAELEGVWQPAA (S1B) were divided into 9 amino acid peptides spanning the entire 20mer and used to stimulate T-cells in an IFN-γ ELISPOT assay. Error bars indicate standard deviation from the mean.

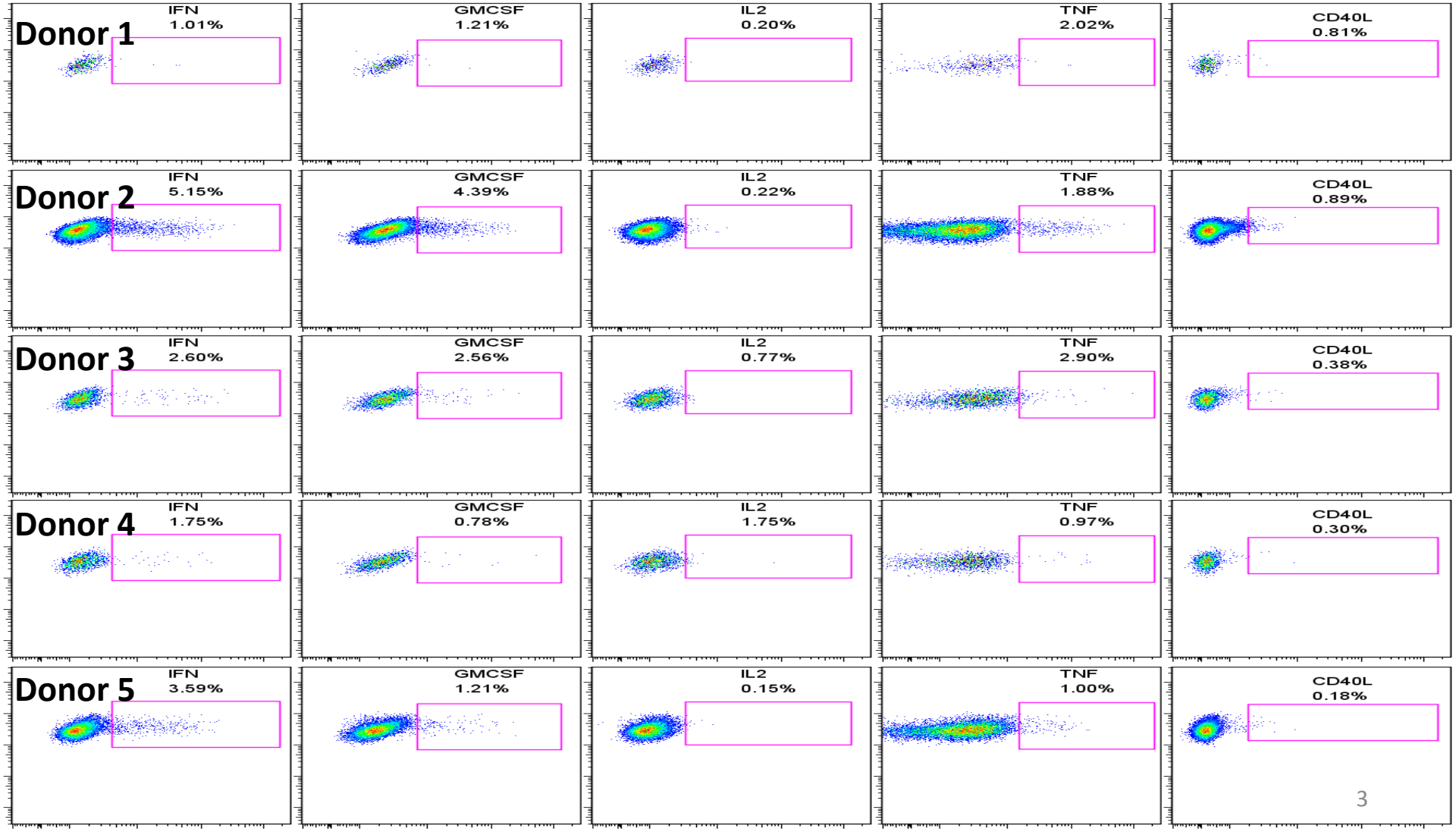
S2A


Donor 1
IFN 5.51%
GMCSF 4.93%
IL2 1.16%
TNF 4.06%
CD40L 8.12%
Donor 2
IFN 10.08%
GMCSF 9.58%
IL2 2.05%
TNF 6.36%
CD40L 10.92%
Donor 3
IFN 1.93%
GMCSF 1.98%
IL2 0.40%
TNF 1.59%
CD40L 1.74%
Donor 4
IFN 1.42%
GMCSF 1.65%
IL2 0.47%
TNF 1.65%
CD40L 1.89%
Donor 5
IFN 3.24%
GMCSF 2.71%
IL2 0.19%
TNF 1.06%
CD40L 1.07%

S2B


Donor 1
IFN 8.27%
GMCSF 7.18%
IL2 2.07%
TNF 7.67%
CD40L 9.19%
Donor 2
IFN 16.23%
GMCSF 12.75%
IL2 3.73%
TNF 14.22%
CD40L 20.21%
Donor 3
IFN 3.22%
GMCSF 2.90%
IL2 0.37%
TNF 2.32%
CD40L 2.75%
Donor 4
IFN 4.86%
GMCSF 4.35%
IL2 1.79%
TNF 5.63%
CD40L 5.88%
Donor 5
IFN 4.84%
GMCSF 3.63%
IL2 0.36%
TNF 2.79%
CD40L 2.12%

S2C


Donor 1
IFN
1.01%
GMCSF
1.21%
IL2
0.20%
TNF
2.02%
CD40L
0.81%
Donor 2
IFN
5.15%
GMCSF
4.39%
IL2
0.22%
TNF
1.88%
CD40L
0.89%
Donor 3
IFN
2.60%
GMCSF
2.56%
IL2
0.77%
TNF
2.90%
CD40L
0.38%
Donor 4
IFN
1.75%
GMCSF
0.78%
IL2
1.75%
TNF
0.97%
CD40L
0.30%
Donor 5
IFN
3.59%
GMCSF
1.21%
IL2
0.15%
TNF
1.00%
CD40L
0.18%

S2D

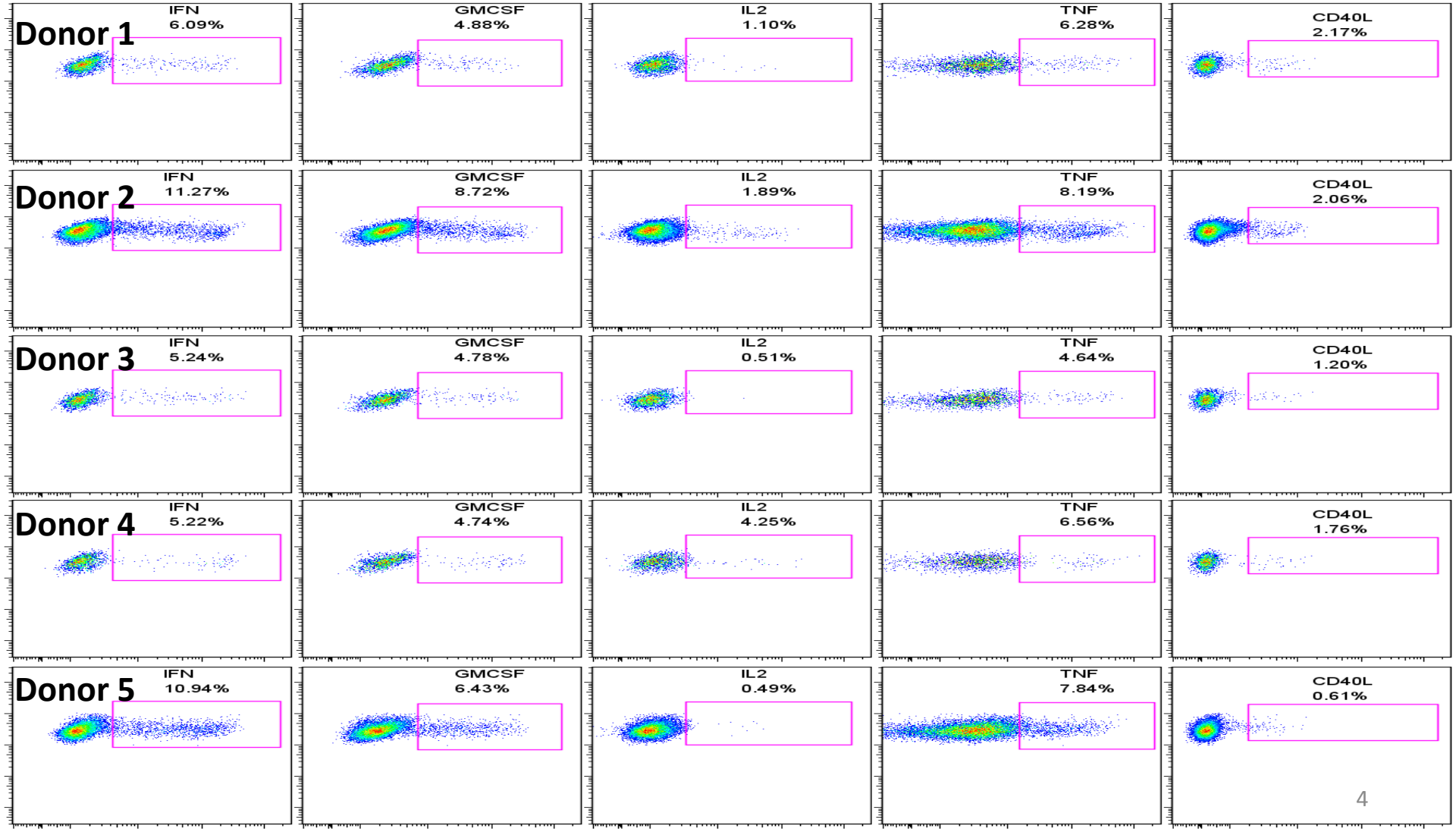

Donor 1
IFN
6.09%
GMCSF
4.88%
IL2
1.10%
TNF
6.28%
CD40L
2.17%
Donor 2
IFN
11.27%
GMCSF
8.72%
IL2
1.89%
TNF
8.19%
CD40L
2.06%
Donor 3
IFN
5.24%
GMCSF
4.78%
IL2
0.51%
TNF
4.64%
CD40L
1.20%
Donor 4
IFN
5.22%
GMCSF
4.74%
IL2
4.25%
TNF
6.56%
CD40L
1.76%
Donor 5
IFN
10.94%
GMCSF
6.43%
IL2
0.49%
TNF
7.84%
CD40L
0.61%

Figure S2. Polyfunctionality of pp65-specific T cells derived from naïve T-cells. Shown are five CMVpp65-specific T cell lines expanded from naïve donors. Lines were stimulated with CMVpp65 Pepmix for 6 hours and stained for IFN-γ, GM-CSF, IL-2, TNF-α, and CD40L in the presence of Brefeldin A. A) Unstimulated CD4+ T cells B) CD4+ T cells stimulated for 6 hours with CMVpp65, C) Unstimulated CD8+ T cells, D) CD8+ T cells stimulated for 6 hours with CMVpp65.

Supplementary Figure 3

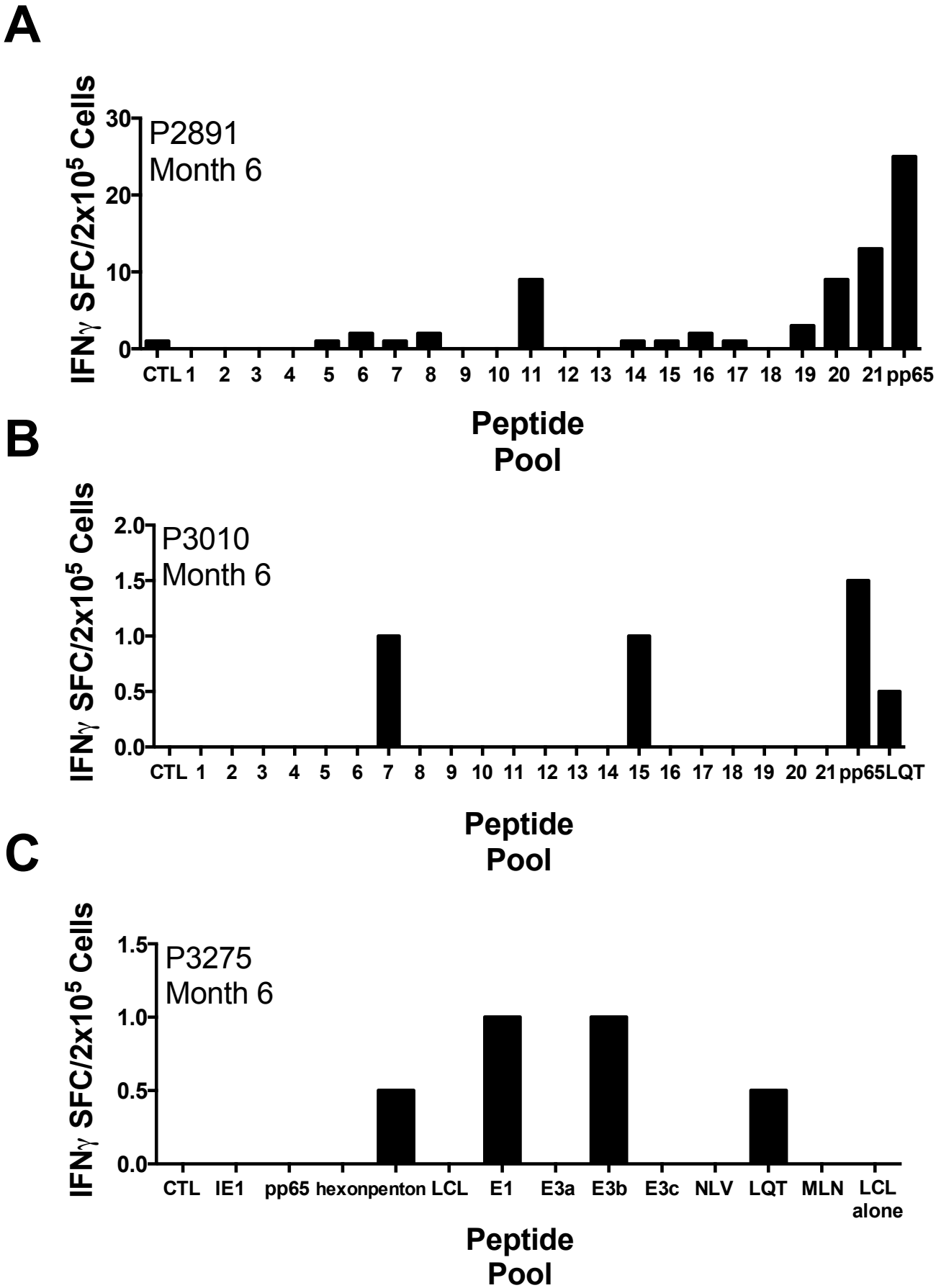


Figure S3. Atypical epitope recognition in patient receiving cord blood-derived virus-specific T- cells. Shown is the virus-specific response from patient P3010, an HLA-A2+ cord blood transplant recipient who received CB-derived virus-specific T-cells. S3A) After two stimulations the expanded CB T-cells were tested for specificity in an IFN-γ ELISPOT assay. Error bars indicate standard deviation from the mean. S3B) One year post-T-cell infusion, peripheral blood mononuclear cells were isolated from the recipient and expanded using EBV-LCL transduced with the Ad5f35pp65 vector in the presence of IL-4 and IL-7. After one week the expanded cells were tested for activity against CMV, EBV, and adenovirus, as well as the CMVpp65 epitopes MLN and NLV. Data represent one well.

Figure S4

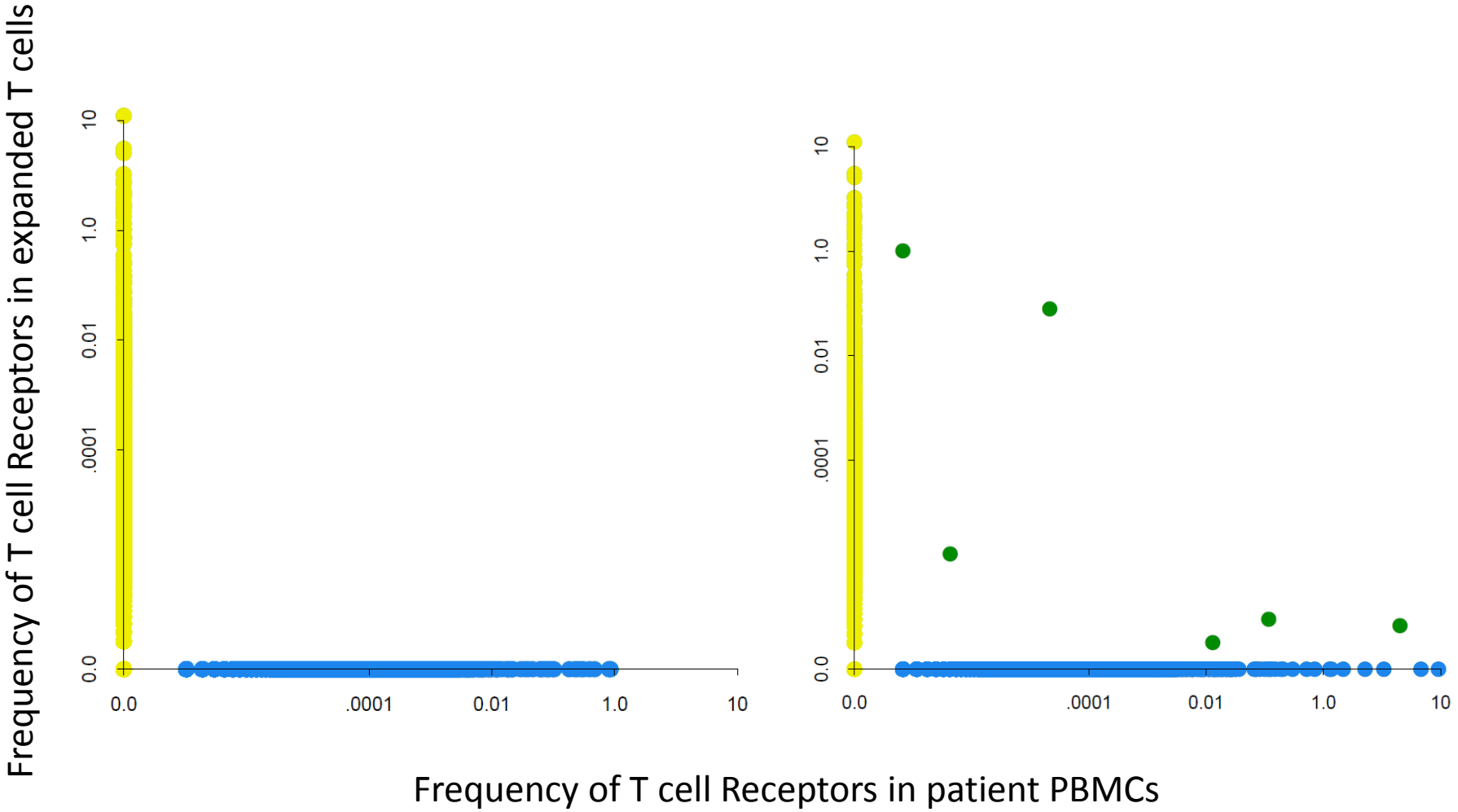

Frequency of T cell Receptors in expanded T cells
10
1.0
0.01
.0001
0.0
0.0
.0001
0.01
1.0
10
Frequency of T cell Receptors in patient PBMCs
Pre T-cell infusion
Month 6

Figure S4. Shared T-cell receptors in the CB-derived virus-specific T-cells and Patient P2891 6 months post-T-cell infusion. TCRs were sequenced from both the expanded T cell product and the patient pre-infusion and 6 months post-T-cell infusion. Blue dots indicate T cell receptors found in patient PBMC samples pre-infusion or at 6 months post-T cell infusion, and yellow dots indicate TCRs found in the expanded T cell product. Green dots indicate TCRs found in both the expanded T cell product and the patient PBMCs.

Table S1. Epitope recognition by CMV-seronegative donors

Donor	HLA type	Predicted Epitope(s)	Actual Epitope or peptide	HLA restriction
CMVneg A	A2,24;B15,35	NLV, QYD	DANDIYRIF, MLNIPSINV	HLA-B35, HLA-A2
CMVneg B	A2; B7,40	NLV, RPH, TPR	LQTGIHVRV	HLA-A2
CMVneg C	A2; B40	NLV	LQTGIHVRV, MLNIPSINV	HLA-A2
CMVneg D	A3,24;B8,35	QYD	PHETRLLQTGIHVRVSQPSL	Not Identified
CMVneg E	A2;B7,40	NLV, TPR, RPH	LQTGIHVRV	HLA-A2
CMVneg F	A1,2; B8,51	YSE, NLV	LQTGIHVRV, MLNIPSINV	HLA-A2
CMVneg G	A24; B15,40	QYD	IYVYALPLKMLNIPSINVHH	Not Identified
CMVneg H	A11,32;B27,37	ATVQGQNLK	IHVRVSQPSLILVSQYTPDS	Not Identified

CMVneg, CMV-seronegative donor; HLA, Human Leukocyte Antigen; NLV, NLVPMVATV; QYD, QYDPVAALF; TPR, TPRVTGGGAM; YSE, YSEHPTFTSQY; RPH, RPHERNGFTVL

Table S2. Polyclonality of T cells from CMVnegs and CMVpos donors that recognize typical and atypical epitopes

Source	**Epitope**	**TCRBV**	**CDR3**	**TCRBJ**	**Freq (%)**
CMVpos D	NLV	27	CASSLSAGAPGAEAFF	1.1	35
		12.4	CASSPGSLYYGYTF	1.2	15
		6.5	CASSPITGTGIYGYTF	1.2	13
		12.4	CASSSTNGYTF	1.2	10
		6.5	CASSYATGTAYGYTF	1.2	8
		27	CASSLDRVTGELFF	2.2	8
		6.5	CASSLVTGTGWGYTF	1.2	5
		6.5	CAGSLVTGTGWGYTF	1.2	3
		12.4	CASSSVGEWHF	1.5	3
		27	CASSLDGVTGELFF	2.2	3
CMVpos C	NLV	6.5	CASRLQTGTIYGYTF	1.2	91
		6.5	CASSFSTGTAGGYTF	1.2	6
		6.5	CASRPQTGTIYGYTF	1.2	3
CMVPos D	MLN	12.3	CASSFTGIAETQYF	2.5	21
		5.1	CASSLRGPGLYNEQFF	2.1	19
		12.3	CASRSPRAGETQYF	2.5	16
		12.4	CASSFRGDTEAFF	1.1	5
		5.1	CASSLETGSNEQFF	2.1	5
		5.6	CASSWDRRAPSYNEQFF	2.1	5
		5.1	CASSSGGGEQYF	2.7	5
		4.3	CASSQGTSGGLSTDTQYF	2.3	2
		5.1	CASSSGTGDKETQYF	2.5	2
		6.1	CASNKGRYSGANVLTF	2.6	2
		6.2	CASSRGFGLRAGQRNQPQHI	1.5	2
		5.1	CASSLGPGSEAFF	1.1	2

		6.3	CASSYGTGGQETQYF	2.5	2
		7.9	CASRGRTQETQYF	2.5	2
		13	CASSNTGTSGGYEQFF	2.1	2
		12.3	CASSSYLTAPTDTQYF	2.3	2
		12.4	CASSFAYGYTF	1.2	2
		30	CASLKGGGTEAFF	1.1	2
CMVpos C	MLN	28	CASSLGLNYEQYF	2.7	28
		24.1	CATHSGNTGELFF	2.2	18
		27	CASSLSTSGDNEQFF	2.1	10
		6.1	CASSDFRDRGHNEKLFF	1.4	8
		24.1	CATFDGNTGELFF	2.2	8
		4.3	CASSQVGPGRANTEAFF	1.1	3
		2	CASSAAGGTGSGELFF	2.2	3
		5.6	CASSRNWVDTEAFF	1.1	3
		5.6	CASSSLWPELFF	2.2	3
		7.9	CASSPGDRRYSPLHF	1.6	3
		10.2	CASSSEGSGVQGEQYF	2.7	3
		19	CATENRGYSYNEQFF	2.1	3
		24.1	CATFSGNTGELFF	2.2	3
		25.1	CASNYYNEQFF	2.1	3
		28	CASRREGRVNEKLFF	1.4	3
		28	CASSLGLNHEQYF	2.7	3
		28	CASSSRGLPYEQYF	2.7	3
CMVpos A	MLN	12.4	CASRDRGLGQPQHF	1.5	50
		9	CASSARTPRPGSSYNEQFF	2.1	16
		28	CASSPPGGSGNTIYF	1.3	13
		28	CASSFPTGGLWPSDGQYF	2.7	8
		30	CAWSVASTGLNYEQYF	2.7	8
		5.4	CASSLAPSTEQYF	2.7	3
		9	CASSARTLRPGSSYNEQFF	2.1	3
CMVneg H	MLN	28	CASSLGPHDSTNYGYTF	1.2	17
		12.3	CASSFGVNTEAFF	1.1	8
		5.4	CASRPKSPLHF	1.6	6
		7.9	CASSLARRVNPRTDTQYF	2.3	6
		6.5	CASSLQGRGNQPQHF	1.5	6
		5.6	CASSPTGTGEAFF	1.1	6
		12.4	CASSQDRELAFF	1.1	6
		10.3	CAISEDSLHNSPLHF	1.6	3
		7.9	CASRAVSTDTQYF	2.3	3
		7.8	CASSFLRRQTQYF	2.5	3
		7.9	CASSLAGFGNTIYF	1.3	3
		11.1	CASSLDLGASTDTQYF	2.3	3
		28	CASSLGSHDSTNYGYTF	1.2	3
		27	CASSLLPPGQGRNQPQHF	1.5	3

		28	CASSLWPKLRYEQYF	2.7	3
		12.4	CASSPPTLEDSGANVLTF	2.6	3
		4.1	CASSQDWAGGLEQFF	2.1	3
		4.1	CASSQFGRNYEQYF	2.7	3
		6.2	CASSQGSAGNQPQHF	1.5	3
		4.3	CASSQVLAGEGTDTQYF	2.3	3
		15	CATSGRTGELFF	2.2	3
		15	CATSPTANTEAFF	1.1	3
		30	CAWGRNTEAFF	1.1	3
		30	CAWTVGNTEAFF	1.1	3
CMVneg A	MLN	5.1	CASSLAVQSDEQYF	2.7	76
		5.1	CASSPSGYNEQFF	2.1	7
		9	CASSPSGGVGTGELFF	2.2	7
		5.1	CASSLTVQSDEQYF	2.7	2
		7.9	CASSGRDNYNEQFF	2.1	2
		18	CASSTRDRGSEQFF	2.1	2
		20.1	CSARDRDRGYEQYF	2.7	2

CMVpos, CMV-seropositive donor; CMVneg, CMV-seronegative donor; NLV, NLVPMVATV; MLN, MLNIPSINV

Table S3. Precursor frequencies of TCRs recognizing NLV and MLN from CB

Peptide	CDR3 Sequence	CB #1 (%)	CB #2 (%)	CB #3 (%)	CB#4 (%)	CB #5 (%)	**Total frequency (%)**
NLV	CASSYNSAGYNEQF	$4.12X10^{-6}$					**$4.12x10^{-6}$**
MLN	CASSFGVNTEAFF			$1.89x10^{-5}$			**$1.89x10^{-5}$**
MLN	CASSFRGDTEAFF	$2.78X10^{-5}$	$1.33X10^{-6}$	$1.61X10^{-5}$			**$4.52X10^{-5}$**
MLN	CAWGRNTEAFF			$7.32X10^{-5}$			**$7.32X10^{-5}$**
MLN	CSARDRDRGYEQYF	$4.12X10^{-6}$					**$4.12X10^{-6}$**
LQT	CASSITDTQY			$1.42X10^{-6}$	$7.12X10^{-5}$	$3.14X10^{-5}$	**$1.04X10^{-4}$**

CDR3, Complimentarity determining region; CB, Cord Blood Unit

Table S4. Inhibition of CMV dissemination by T-cells recognizing typical and atypical epitopes

Condition	CMV CPE?
CMV supernatant + fibroblasts	Yes
CMV lysate + fibroblasts	Yes
CMV + fibroblasts + atypical (MLN)-specific T cells	No
CMV + fibroblasts + typical (NLV)-specific T cells	No

T cells were expanded against either typical (NLV) or atypical epitopes from a CMV-seropositive donor. These T cells were then co-cultured with CMV-infected fibroblasts for three days, after which the supernatant or lysate was added to new fibroblasts to measure the CMV CPE.

Source Data
Figure 1A.

	T cells alone	**Irrelevant peptide**	**CMVpp65**
Neg1	**22.8**	**26.4**	**27.6**
Neg2	**4**	**11.9**	**39.3**
Neg3	**34.4**	**37.3**	**42.2**
Neg4	**25**	**26.2**	**32.3**
Neg5	**8.3**	**7.4**	**13.6**
Neg6	**31.3**	**49.7**	**33.3**
Net7	**4**	**5.7**	**6**
Neg8	**11.8**	**9.4**	**13.1**
Neg9	**66**	**83.5**	**122**

Figure 1B.

	T cells alone	Irrelevant peptide	CMVpp65
Neg1	5	3.3	464.33
Neg2	21.33	19.33	71
Neg3	7.33	10	329.67
Neg4	7.33	4.66	172.33
Neg5	73.67	96	102
Neg6	1.33	0.67	0.67
Neg8	1.5	4.33	38
Neg9	11	16.33	18.33
Neg10	2	3	611
Neg11	0.33	0.33	26.33

Figure 1C

	CD3/CD8	CD3/CD4	CD16/CD56	CD45RO+/CD62L+	CD45RO+/CD62L-
Neg2	97.86	11.25	3.06	10.97	59.23
Neg3	67.01	34.49	2.35	14.97	84.23
Neg1	88.67	14.91	5.86	29.28	7.88
Neg9	43.41	67.79	2.52	71.42	26.67
Neg4	57.78	36.68	6.28	34.37	4.65
Neg8	92.56	4.74	0.54	89.55	6.94
Neg10	69.1	32.15	0.52	72.65	7.2
Neg11	7.54	94.98	0.71	49.93	72.95

Figure 1E

	Cells alone	IE-1	pp65	LCL
Naïve	2	3	611	0
Non-naive	12.67	8.33	12.67	98.5

Figure 2A

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21/22
CMVposA	1.05	27.26	5.38	1.277	1.55	1.78	4.78	9.49	7.83	0.78	2.83	2.39	1.11	1.78	0.44	0.5	1	7.72	0.56	0.39	20.1
CMVposB	0	54.36	5.52	0	0	0	0.29	0.29	0	0	0	0	0	0	0	0	0	0	0	0	39.53
CMVposC	1.47	8.1	2.04	0.45	4.85	7.86	5.7	1.43	0.86	6.6	17.32	0.98	0.33	3.5	18.17	5.9	1.92	2.04	0.45	0.16	9.86

Figure 2B

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21/22
Neg1	2.83	2.88	2.63	3.23	3.28	6.95	10.38	18.52	3.48	3.03	2.14	1.54	2.23	2.23	1.99	2.73	3.03	3.23	2.58	2.58	18.52
Neg2	0.87	0.55	0.46	0.73	0.69	0.46	25.38	15.1	20.16	0.41	0.5	0.6	30.92	0.55	0.18	0.41	0.32	0.5	0.46	0.46	0.32
Neg3	6.6	9.74	8.53	8.16	7.19	7.53	7.91	8.71	7.41	7.91	6.82	6.08	0.56	0.9	0.68	0.4	1.15	0.5	0.74	1.55	0.93
Neg9	0	0	2.04	4.08	2.04	0	4.08	18.37	0	4.08	2.04	16.33	0	0	2.04	0	4.08	28.57	0	2.04	10.2

Figure 3A

	Cells alone	CMVpp65	NLV	LQT	MLN
Donor 1	10	756	661.5	14.5	2

Donor 2	5	708.5	823	1.5	1
Donor 3	208	382.5	339	327	458.5
Donor 4	33	289	372	76.5	48
Donor 5	82	577	912.5	130.5	159

Figure 3C

	CTL alone	LQT
1	39	310
2	4	508
3	22.5	48.67

Figure 3D

	CTL alone	MLN
1	5	276
2	28.5	195.83
3	11	526.5

Figure 3E

	CTL alone	Irrelevant	NLV	Pos Control
CB1	26.5	23.667	20.33	183
CB2	8	7	6.33	182
CB3	12	20	4	16
CMVneg 1	0	1.667	2.667	425
CMVneg 2	2.5	2	0.33	436
CMVneg 3	0	0.667	0.667	400
CB4	0.5	0.5	2	568
CB5	23	22	19	945

Figure 3F

ng/ml	CMVpos NLV	CMVpos MLN	CB MLN
100	875.33	224.33	276
10	888.33	199	265.53
1	512.33	149.33	241.67
0.1	254	45	123.33
0.01	38.67	24.67	57.67
0.001	7.67	26.33	42.33
0.0001	6.33	33.33	40.33
CTL alone	5.5	28.5	44.5

Figure 4A

	Cells alone	CMVpp65
CMVpos	1.67	248
CMVneg	9	120.33
CB	4	58

Figure 4E

	Cells alone	LQT
CB	15.67	280
CMVneg	46.33	187

Figure 5A

	CD3/CD4	CD3/CD8	CD3/CD45RA-/CD62L+	CD3/CD45RA-/CD62L-	CD3-/CD56+	CD3-/CD19+
P2891	875.33	224.33	22	74.05	0	0
P3010	888.33	199	39	46.52	2.14	0
P3275	512.33	149.33	44	36.89	1	0

Figure 5B

	T cells alone	IE-1	pp65	Hexon	Penton	Auto LCL	LCL alone	NLV	MLN	LQT	SEB
P3275	875.33	224.33	22	74.05	0	0	0	12	10.5	143.5	331

Figure 5C

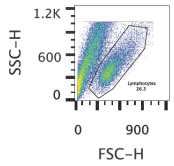
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21/22	CTL alone
P3275	10	9	11	17	25	18	164	21	58	13	8	72	122	10	9	11	21	132	94	11	12	9

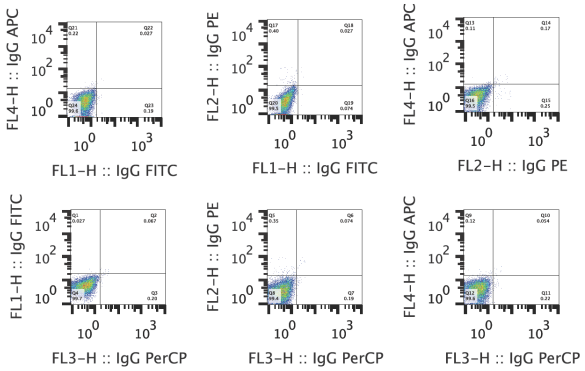
Figure 5D

Day	-49	-26	-13	13	15	17	20	22	24	27	29	31	34	38	41	43	47	49	50	54	58	61	68	77	79	83	93	98	101	105	107	111	112	114
CMV copies	0	0	0	1500	1700	1200	1600	1100	2600	32000	1200	900	4000	1000	500	300	200	100	100	100	0	100	100	0	0	0	400	1200	800	19000	20000	300	300	200
CMV SFC																					6	10.5	24.5						1064.5			188		
T cell infusion																						Y (day 63)								Y				
Antivirals										Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	Y			Y

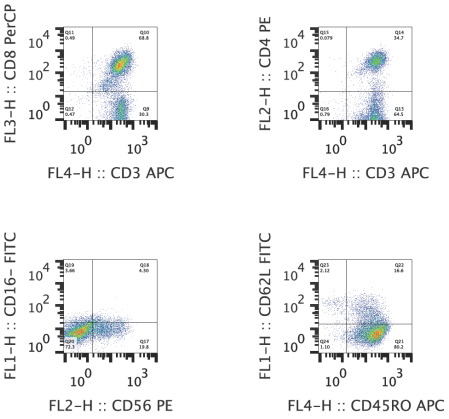
118	121	126	132	135	142	143	149	153	156	160	163	171	178	185	192	197	199	206	213	218	227	231	234	239	241	247	255
200	0	0	0	0	0	0	0	0	0	0	0	0	0	100	900	400	3000	100	0	200	300	400	300	200	200	200	100
												38					8.5						25				
																	Y										
Y	Y	Y	Y		Y		Y		Y																		

Gating Strategy & Isotype Control (Figure 1C)

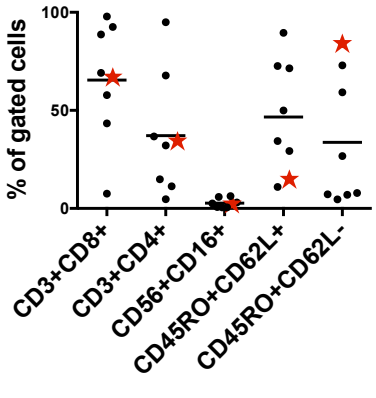




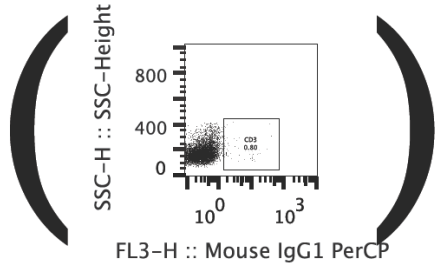
Representative Flow Graph

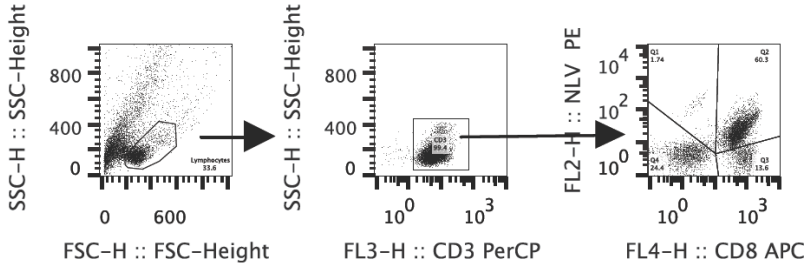


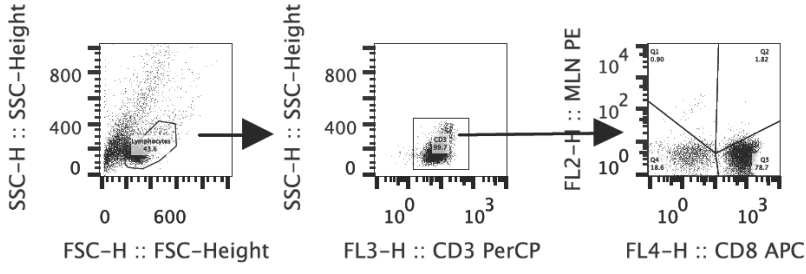
Data Point Location of Representative Flow Above

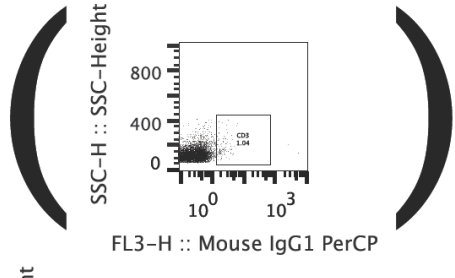


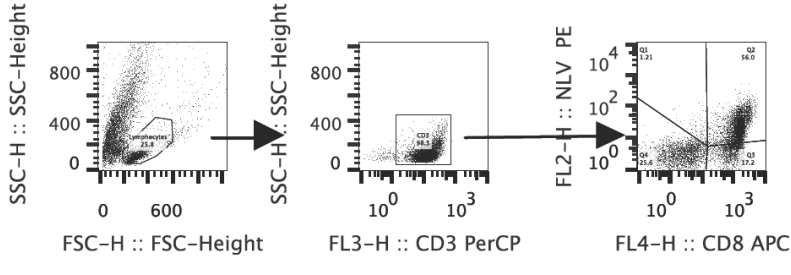
Gating Strategy & Isotype Control (Figure 3C)

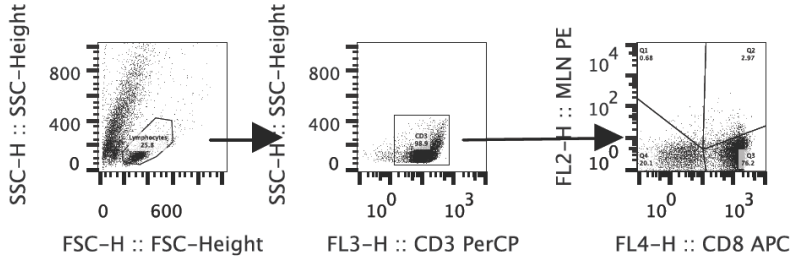




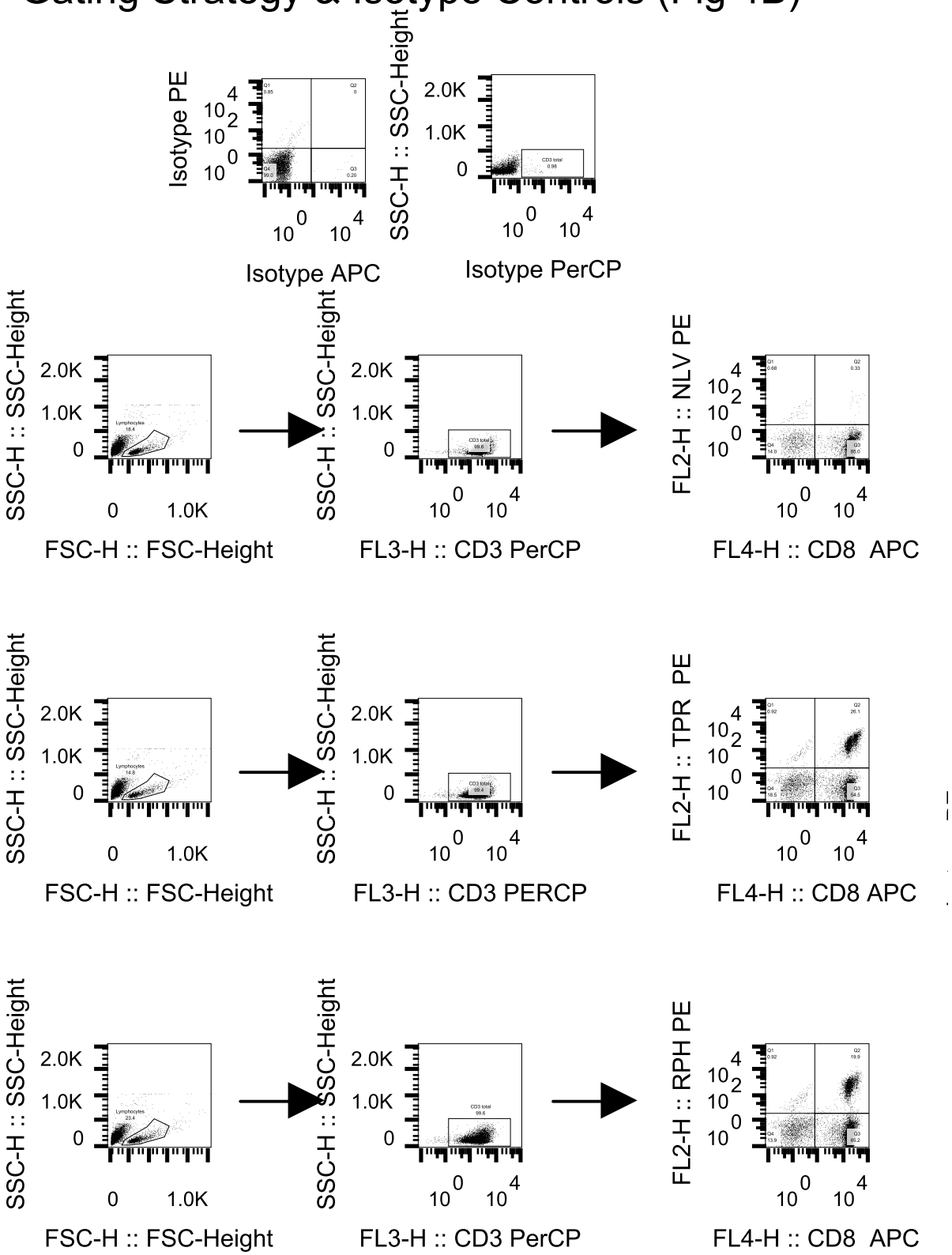




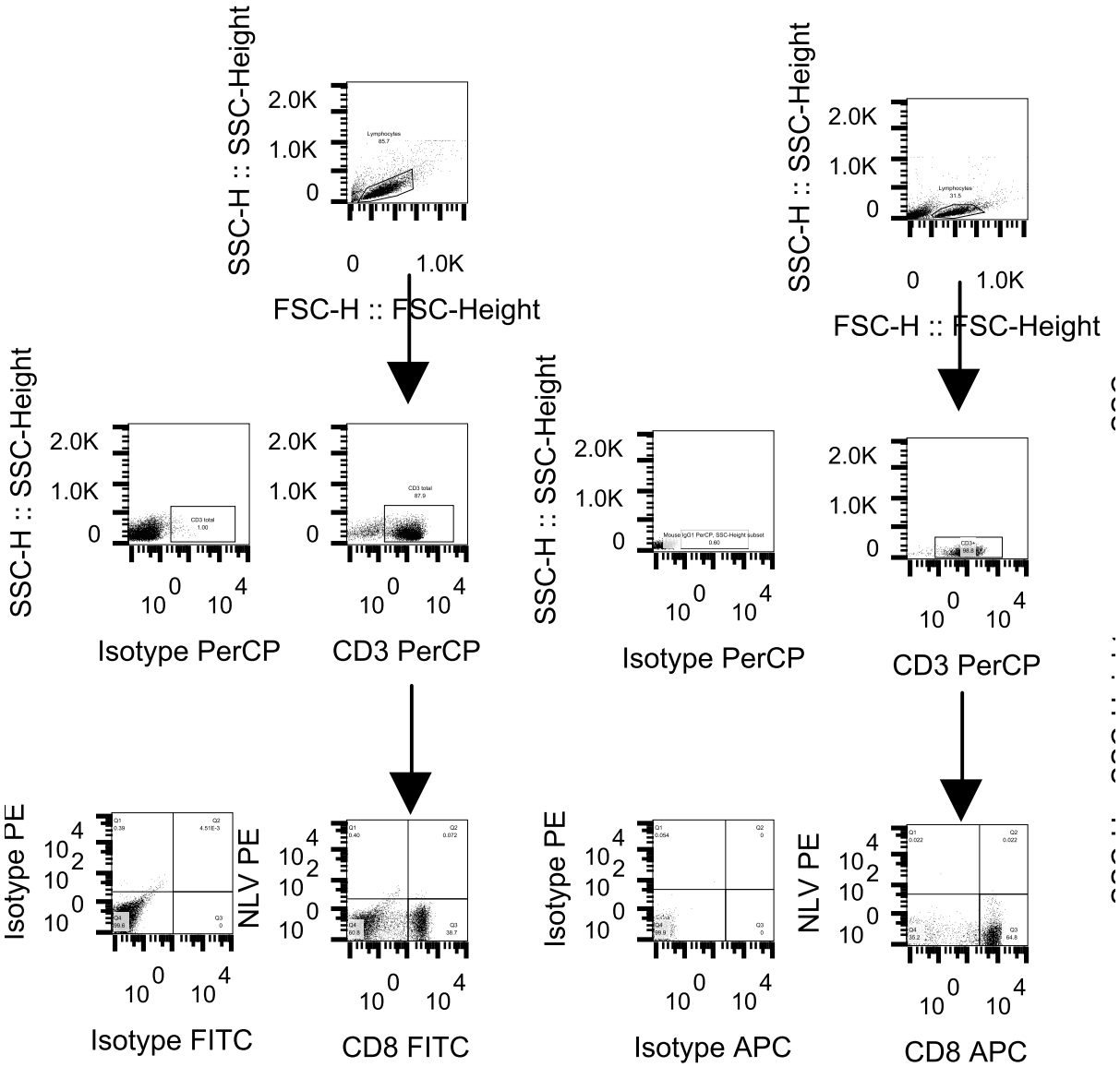




Gating Strategy & Isotype Controls (Fig 4B)



Gating Strategy & Isotype Controls (Fig 4C)



Gating Strategy & Isotype Controls (Fig 4D)