2023 ILT 15

150	Specime	n Status	Location	Con	0 D-4				2023	_ILT_15	
810	7	11. 7		40,45 215M	c Dat	e	otes	Volume	Lym	Lym+Mon	Total
54	INF179-	3 59LA	5		1			1		January	Total
		(6).	1 6	74:00				Die oli	9.16	11.5	1
19:40	c					-			1 1	9 = (4 )	
1840 H106	- (F) (I - A -	. HU 9		ar G NA							
bort	Inf149-1	4	(6)	~9M <11.87 46.827					1 1 1		
1660			(8)	26.02		-			6.31	9,41	
50 11	1	4 57 W3		\$14.187							
6600				194.107				1	5,45	9.10	
HOY	11nt0/1-2	RWM6	(A)	411.067	,			0 =	7011		
6100			<u> </u>	111.00	-	-		0.5	7.94	12.7	
HOY	ce-2	RWMT	Tu	< 9.397				OT			
	Inf 187-6	HEU-hi 3						0.5	9.46	15.4	
50 yl	2111101-6 a-3	5A10	(2)	~15 M							
7 1	0.7	1)1110		(8.017			L C-2.23.3 )		9.11	15.8	
-											
21000	Inf 251-8	HEU-hi q				-	. 19	. 7- 2			
HON	a-1	5FPØ		(12.115		175	eA!				
		5516		(12,47				1	7.63	10.7	
1830C.	2-2	5FPI	(8)								
		HEU-high	(15)	13.987					6.84	9,08	T,
1100	24313-1	SNFF	(1)	.0 017		rin5	ingide?	1	2 1 1		
193C.		tas alig	iof? (3)4	18.017		L. Het e	يرا الحيرا عليه	4	3,63	7.72	
ocil	0.3	SNFE		el		MY	2 Msidel		2 24	0	
	1nf627-5	TSRW2 O	100	(17.317					3.80	8.09	1
1	Inf627-5	HEU-hi 7	(3)			LNZ	snapped, not evenerasy. He controls appeal & rig zvicits				1
4.0	07		9	-		1-1	12-50 apport riggines				
501	Inf 568-9	HEV-hig TFDE	2	14.047	ebilii V	-		».	( 00		
3 (	a-1		19 c	17.07				41 /44	6.00	10.1	
800E	ND050	Adult 8	01	5E6 1	/13/23			1	2.	12.0	* *
800 F			7	11.02				2.1 4.3	8.61	445	3.
1110 c.	N0006	Adulf 7	6	1586	5/12/23			1	/··· ^	1/ 2	
44/1			8	6.82				· ·	14.0	16.9	

Start @ 9.57 am Spin @ 10:12 10:36 drase resus 10:55 Spin @ 11:13 11:39 am DWAsc 10:56 un

12:04 stan for cant

1:02 fishing extra 313 viul

2:02 all quot some

2:15 Eprai

2:41 midegas

3:01 pm Inabation Start

-> 9:07 pm AI Abs done @ 3:42

reagents mostly done @ 9:050m

Cells out @ q:13pm

< 9:22 pm cells spin 7

1400 Inf 568-9 HEU-4: 9 (12) Iml SOM. TFDD a2 (13.2M7) L13.247 6.39 10.8

500 9:39 pm

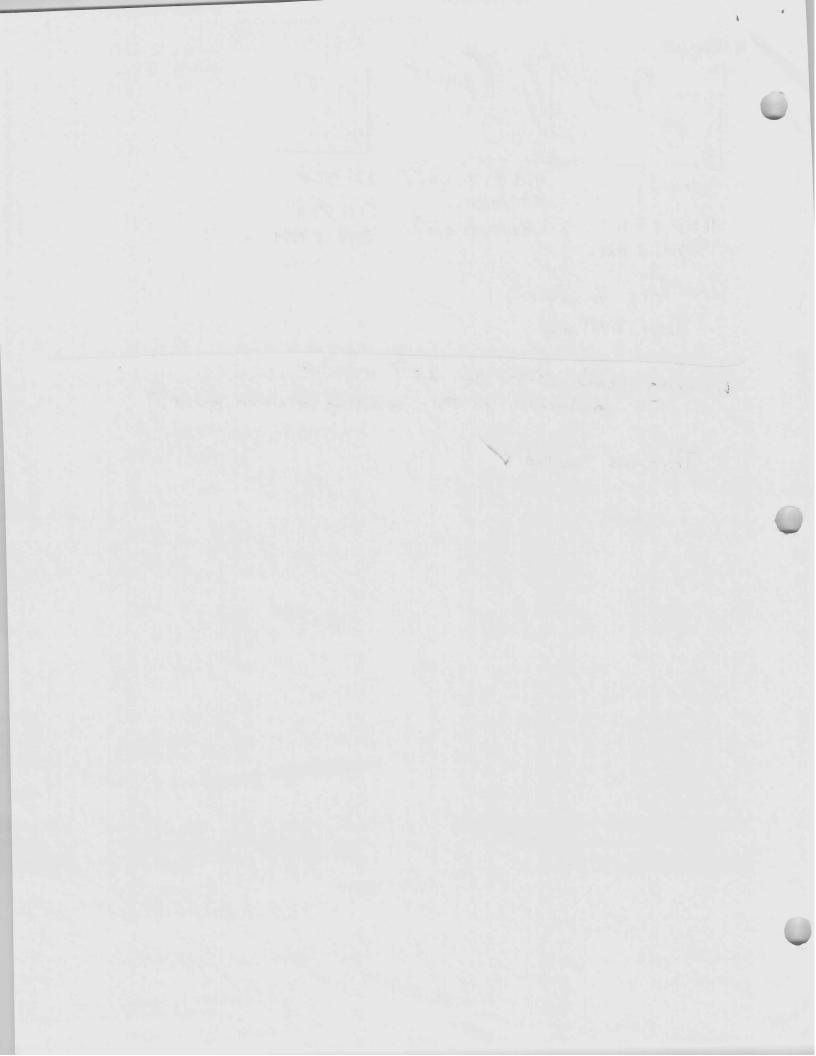
40 Samples @ a:43 -> 58 pm C:49 for scis Hot scis@ 9:57 pm -> 27 pm Spin @ 27:22 Cold cold 2 William -> 24 pm Spin @ 27:22 cold so's @ 10:14 pm 744 pm nash @ 499

Hot samples @ 10:30pm -> 11:00 Tab made before 10:49 pm spin @11:09 SEFIX PENCILOZ-12-> 32 PR

tets @11:32 pm > 12:12 am  Nose11:43 pm > 12:13 am  Nash@ 00:20 cm  2nd se penerast@ 11:39 am  Sc intrae @ 23:53 > 00:33 am > 5pn@ 00:36 an  sc's in 15pl @ 00:03 am  Cold samples @ 00:40 am > 1:10 am
Scis don e @ 00:45 an  [RBC 1/5e @ 1:14 am ] 5An @ 1:22 am
Fixfum Samples @ 1:37 am > 47 -> 57  First washe 2:04 am  2nd @ 2:19 am  [2:36 am Intracellular's ] -> 3:16 am
2:42 am unstained's my 15pl @ 2:43am  (Done @ 3:40AM)
Autorn QC @ 4:02 AM II event rate 7100 , pl/m.n > 19.69  & @ 4:09 am Cord SC moneyle dyng relatively healthy SSC-1.  [AF647 CD3+Va7.2. And 2 instanced partial got T dose chil gdg. block]  Thefore I ferget to write down experiment notes.  Sis don @ 5:04 am  Silb  O  O  Info71  Inf 187  NO056  Inf179  Inf 149  Inf 15N/In  ISN/In  ISN/In  Gopl 19 m  70/1 2.6m  70/1 2.5m  Topl 2m  GSpl 7.5m

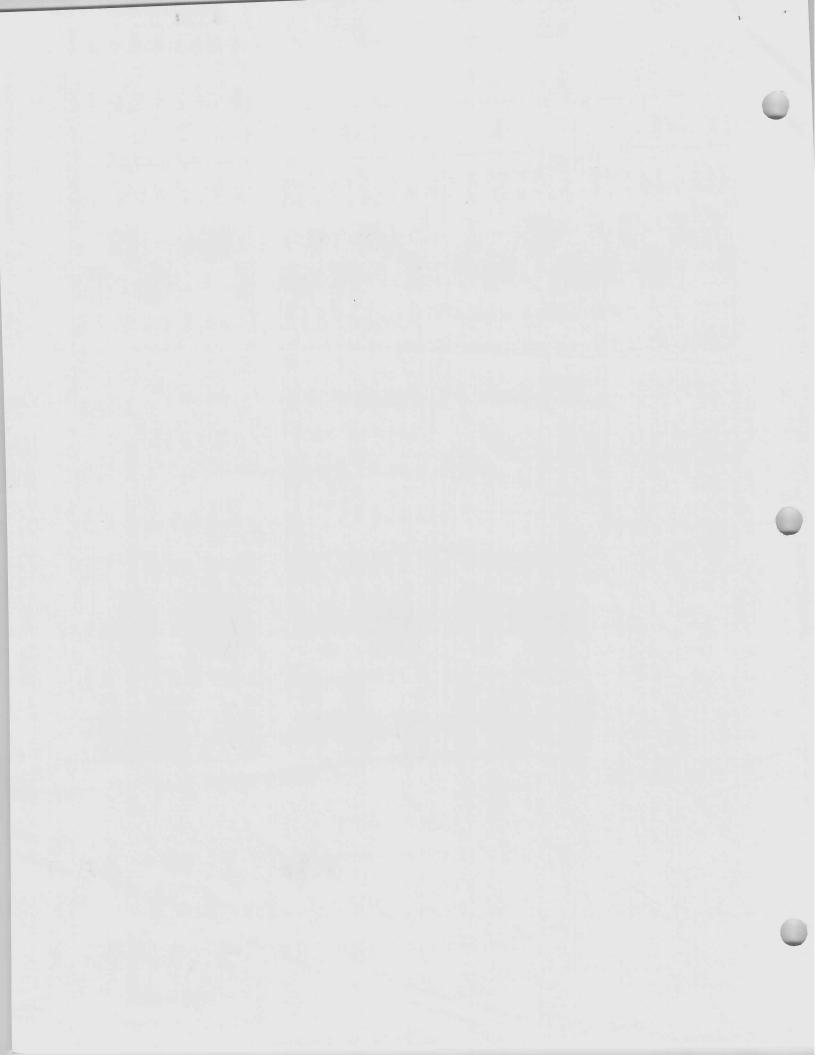
Vamiking Cord's CD8 1 AF? signature similar-ish on PDI, something specimen specific?

Tetrances worked



					CDIO/a												PMA										į	R10				
ND050	INF568	INF313	2 (	INF251	INF187	INFU/1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	NF149	INF179	Sample		ND050	INF568	INF313	INF251	IN+187	INFOZI	INF149	, i	INF179	Sample		ND050	INF568	INF313	INF251	INF187	INFUZI	INITO ZA	INIE140	INE170	Sample
0	6	6	C	'n	6	6	C	ת	6	3M		J	2	2	2	2	2	2	,	ر د	3M	67.9	0.65	0.52	043	9:50	652	0.66				
л	5.5		0.0	י	5.5	5.5	5.5	י ר י ה	5.5	2.75M	1.03	1 00	1 83	1.83	1.83	1.83	1.83	1.83	1.03	1 00	2.75M			0.472	0.49 0.175					10		1
7	5	ū	U	1	Сī	G	U	) ا	л	2.5M	1.00	1.00	1 66	1.66	1.66	1.66	1.66	1.66	1.66	4 00	1 2.5M	7				5 0.487	0.6145 140.559	0,600 50.546	0.408	(	M 2.5M	
	4.5	4.5	4.5	i	4.5	4.5	4.5	i.	_	2.25M	1.5	C.1	n (	7	1.5	1.5	1.5	1.5	1.5		2.25M						9 0.50	6 0.49	8 0.37		/ 2.25M	
	4	4	4	J	Δ	4	4	4	1.014	2 OM	1.32	1.32	1.32	1 2 7	1.32	1.32	1.32	1.32	1.32	1	2.0M	0.10.	0 434	0.343	0 127	0.390	0.446	0.436	0.326	0.448	2.0M	
ر:	o (	ω ω	3.5	0.0	υ (i	ν J	3.5	ω. 5.5	T./SIVI	1 7500	1.16	1.16	1.16	, i	1 16	1.16	1.16	1.16	1.16	TIVIST	1 7EM	0.500	100:0	0.11.2	0.341	0 2 4 1	0.391	0.382	0.285	0.392	1.75M	
u	) ر	ىد 	ω	u	ى ر	υ —	ω	ω	1.5M		1	ь	щ	·  -	4 د	_	↦	ъ	ш	IAIC:T		0.33	0.20	0.1.0	0.29	) i	0.34	0.33	0.24	0.34	1.5M	
											0.83	0.83	0.83	0.03	0.00	0 83	0.83	0.83	0.83	1.25M	4 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	0.271	0.214	0.0/9	0.243	0212	0.270	0 272	0.203	0.280	1.25M	
					F	۵.	0.5	Volume			0.66	0.66	0.66	U.66	0.00	0.66	0.66	0.66	0.66	1.0M		0.217	N. A.	0.064	0.195 /	0.223	0.22.0	0 218	0.163/15	0.224	1.0M	
					7.43	1	7.43	Lym			0.5	0.5	0.5	0.5	) (		0.5	0.5	0.5	0.75M		0.16	0.13	0.05	0.15	V.T.	0.10	0 16	S 0.12	0.17	0.75M	
				11.145	1.43	5	3.715	Total			0.33	0.33	0.33	0.33	0.33		0.33	0.33	0.33	0.5M		0.108	0.086	0.032	0.098	0. XX 5	S DIA'D		0.082	0.1476	0.5M	
					5.573			Average		ı											ן נ	D	Č			10	, (	3		10		

Camala														
	iorai	volume	Concentration	3M	2.75M	2.5M 2.25M	2.25M	2.0M	1 75M	1 1 1	2 2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7			
INF179	9.16	ь	9.16	0.33	0.30	0.27	0.25	0.22	0.10	TINE:	IAIC7.T		0.75M	0.5M
INF149	11 76	J					į		67.0	0.16	0.14	0.11	0.08	0.05
		,	 	0.51	0.47	0.43	0.38	0.34	0.30	0.26	0.21	0.17	0 13	0 09
INF071	8.7	<b>⊢</b>	8.70	0 34	0 27	20	2					()±/	2	0.00
NF187	0 11	٠.			0.52	0.29	0.26	0.23	0.20	0.17	0.14	0.11	0.09	0.06
	į	۰	9.11	0.33	0.30	0.27	0.25	0.22	0.19	0.16	0 14	0 11	000	2
INF251	14.47	2	7 24	0 44							4	0.11	0.00	0.05
INF313	1 1 1 1 1 1 1 1	د		1.	0.38	0.35	0.31	0.28	0.24	0.21	0.17	0.14	0.10	0.07
	11.14	u	3.71	0.81	0.74	0.67	0.61	0.54	0 47	2		}		
INF568	12.39	2	6 20	200					71.0	0.40	0.34	0.27	0.20	0.13
NDOED	2		Marie	0.48	0.44	0.40	0.36	0.32	0.28	0.24	0.20	1	0 13	
140000	8.61	Ь	8.61	0.35	0.32	0.29	0.26	2	)			1	, i	0.00
					No. of the last of	1	0.10	0.23	0.20	0.17	0.15	0.12	0.09	0.06
				0.21									2000	
	7													



Af-882   O.5   O.5     Af-687   NMR1.6 FP   Z   2.4	Tetzmer Mis	FITC			And UNSTAINED CONTROLS !!!		78	R7	27 R6	20.			888	21 86	0.1	83				13 VII				10 V5		9 V3	7 0V16			11An 5	3 1749			# Filter Single-color (ui) Ref ctrl Unmiking ctrl name
	,					The same	APC/Fire 750	Zombie NIR	APC-R700	AlexaFluor647	APC APC	PE-Cy5	PE-Dazzie594	PE	PerCP-Cy5.5	Spark blue 550	FITC/AE4ee	BV750	BV711	BV650	BV605	01CA9	BV480	Pacific Blue	Pacific Blue	BV421	BUV805	BUV737	BUV661	BUV615	BUV563	BUV496	AF	Fluorochrome
Tet:PBS Tet:PBS	Tet:PBS	Zombie NIR 0.8				CD38	CD27	1/0	CD107a	Va7.2/hMR1	PD1	CD25	TNFa	NKG2D	CD26	vaz4/hcD1d	CCR6	IFNy	GD7	CCR7	CDS6	CD45RA	CD161	CD19	CD14	CD127	CD4	CXCR3	V82	CCR4	CD69	AF-UV6	CD62L	
1 <u>9</u>	10 1.9	21.6 8.64		Pippette draw volume /sample	Antibody Total	НТ2	0323	N/A	H4A3	3010	PD1.3.1.3	M-A251	MAB11	1011	SK7	6811	11A9	827	M-T701	G043H7		H1100	HP-3G10	\$J25C1	MSE2	A019D5	SVS	1C6/CXCR3	86	161	RPA-T8		SK11	Clone
20 2_18 2_18	20 2_18 2_18	22.5 PBS 9 Zombie		1e /sample																						1								Vial Lot #
30 3_27 3_27	30 3 27 3 27	985 ombie	10:0	14.5	6.0			0.0																1										During stim!!!
П	Z	N N N		362.5	150			T.OC.	100																1			1	-					25
PEA PBS	Number Intracellular SCs Tota FBS PBS	Number Samples Number Unstained Number Surface SCs (29)	rippette draw volume /sample	Brilliant Stain	Antibody Total		1.63007	<1.2500								1																		L/D 15 min (RT)
0:18 1:62	Total  140 140	(29)	ume /sample	Stain	Total				<2:10/1.2>							<:10/1.5>																	STATE OF STA	Tetramer 40 min @ RT
		Total F 25 11	65	1	17.8	1.6	2				1.2		1.5			1	1 1	1	1	1.0		7			1.5	1.5			2.0					HotStain 30min @37C
RBC Water	280 280 252 200 Perm	FBS RBC 200 A4		1250	445	4 6	5			1	30	3	37.5			37.3	37 5	25	25	25		50			37.5	37.5			50					c 25
1.08 9.72	10.8 20.6 185	RBC Lyse Fix	59.5	50.0	12.5					4	10				1.2						0.7	2	2.0	2.0			1.3	0.7		0.5	0.7	1.2	10	ColdStain 30min @4C
	12	7.5		1250	312.5					17.5					30.0						17.5		50.0	50.0			32.5	17.5		12.5	17.5	30.0	300	25
	206 185.4	Perm 100 P																																RBC Lyse, then
	0.12	0FA	19.5	11	9.5					0.5	0.5	1.5	0.5	2.5	01		1.5		0.7	0.1						0.5		0.1		1				Spiked 40 min @RT
				275	237.5					12.5	12.5	37.5	12.5	62.5	25		37.5		17.5	2.5						12.5		2.5		25.0				25

## Simplified Protocol

Thaw cells, DNAss, count.

Collect, count, aliquot cells 2-3.0 E+6 Cells R10 / 5ml polystyrene tube Bring volume up to "x" mL R10, add "y" µL PMA/Ctrl and "z" µL C0107a Cap and incubate at 37°C for 8 hours

Wash with 2 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1:2600) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min Add HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS.FBS 1400 rpm, 6 min

Add Tetramers, incubate @RT for 10 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ut 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 ul BD FixPerm, incubate @ 4C for 20min

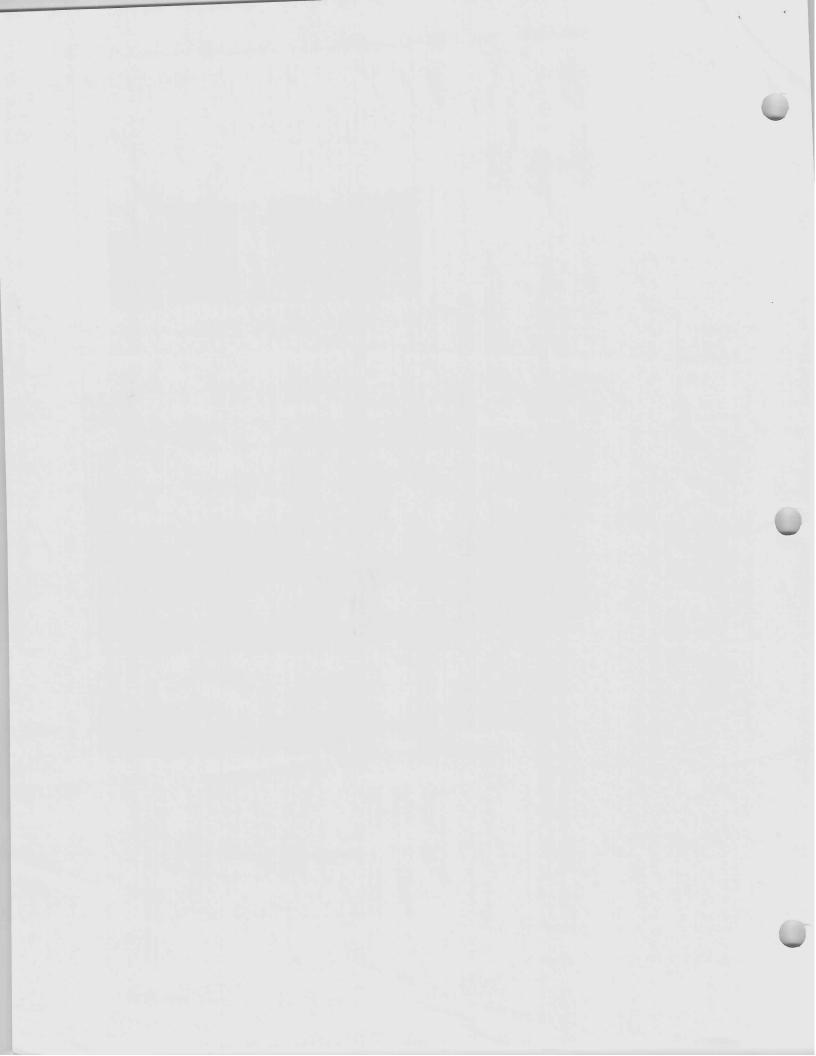
(vortex every 10 minutes)

First PermWash: Second Perm Wash: 1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS

Cap tubes, wrap rack in foil, store at 4°C



Abs done @ 20.42

	210		760						598			514 525					373	Spectrum	
					UV13	UV12	UV11			6/7	5	UV7	OV6	UV5	UV4	UV2	UV1		F
	S08ADB		DUV/3/				BUV661	BUV615		BUV563		BUV496	AF			BUV395		V	ILI & NK SFC Panel
	ŝ		[40]					145	35	200		[55]				[180]	1	7	anel
	CD4		CXCR3				V62		0	CDS9		CD8				CD62L			
ł	V16	V15	V14		V13	V12	V11	V10	5 6	<b>\$ \$</b>	6	5	≨ ≲	<b>%</b>	<u> </u>		†	1	
		BV786	BV750		BV711		BV650	BV605	0/6/0	BV510		BV480	PacBlue	!	BV421		ATOIGE	Violat	
		1501	100		55		3					[55]	[55]		[45]		t	1	
		COBR	IFNγ		CD7		CCR7	005		CD45RA		CD161	CD14/19		CD127				
4	0 0	B12	-	B 0	B9	B8	B6 B7	B5	B4	B3 P	3	В1					t	l	
					PerCP-Cy5.5					SparkBlue 550	EITO AT 400						Blue		
					(45)					1881									
					CD26					Va24/hCD1d CD3									
	YG9	- 500-				YG5	YG3		ĭĞ1										
	Pe-Vio770					PE-Cy5	PE-Dazzle594	ļ	PE								Yellow-Green		
	1501					B	[60]		B								ı		
	PD1					CD25	TNFα	1417020	NKG2D										
R8	R7	R6	R5	R4	R3	R2 R3						-				+	1		
	_	Zombie NIR		APC-R700		AF647	;										Red		
CD38	CD27	Viability		CD107a		CD16 Vα7.2/hMR1	)												

