2023 ILT 03

	Specimen	Status	T4*		F			2023_I	L1_03	
10c.			Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
8U t	Inf018-4	HU Z	Box 1A	<10.57			1	6.17	9.07	1000
4.30c. 3011	a-5		(10)	×11.67			1	6.31	9.32	
00%	Influor	HEU-1000 RY9 L	BCX4A (2)	<20.77		death spiral	1	3.65	7.99	
011		Ryam				drathspiral merceytes	1	4.98	9.67	
)~	Inf 134-8 a-6	HEU-67 57 11	Box5 A	(11.7 >			ı	5.84	9,58	
30c.	a-5	5710		(12.77			1	5.53	8.83	
6c.	Inf314-8	HEU-hi3 SNHF	BOX8B	<7.6>			(8.44	17.70	
0/1	a-7	SNAG		(6.1)			1	10.00	14.10	
1 . oc		Adult		2 2	1/13/23		1			
A	ND 006	Adult	(2)		120/23		1	8.38	13.90	

1834 1stack cord thow start Spin @ 11:4 pare DNASE@ 12.08 -11:48 2nd set cord than start 7 Pin @ 12:03 ON Nose @ 12:00 12:40 cell stand 1:32 pm aliquot start 2:20 NDOOEpma/preps made

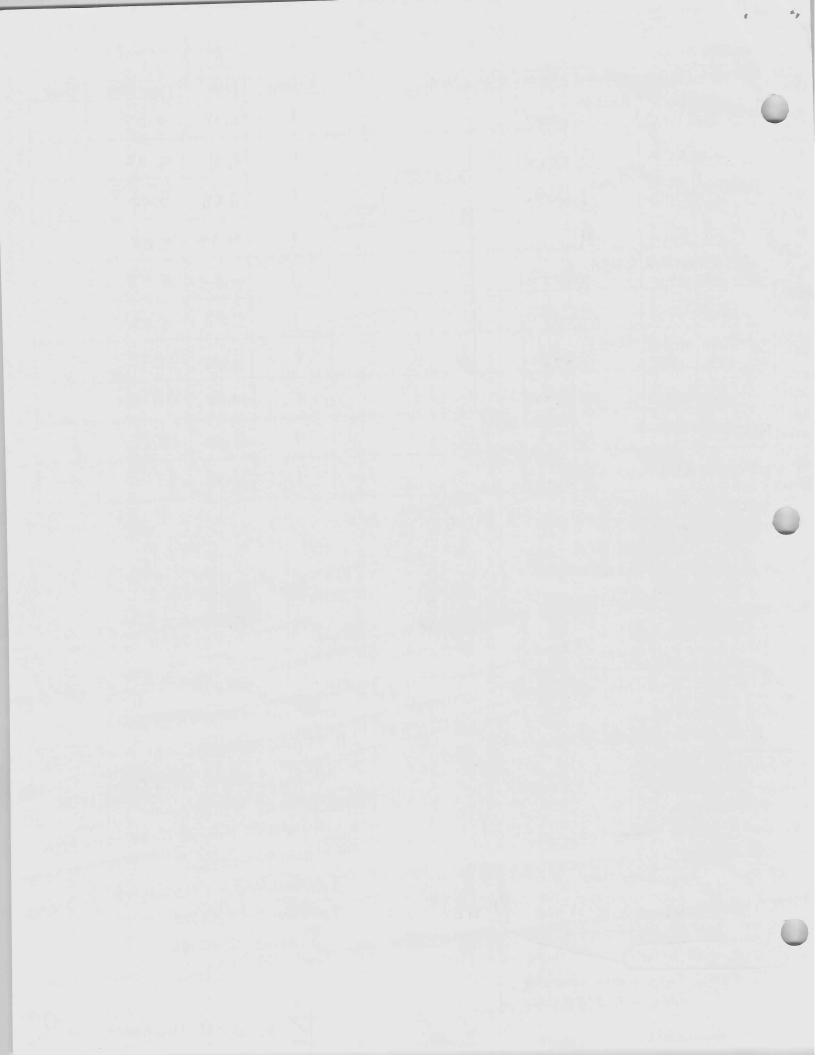
3M (or close) (2.5) 2.5 0

2:35pm Inabation stourt 5 5 resusponded 201/ Abs @ TH3pm gamples @ 30pl 8:03 pm reagents prepped 8:46pm spin Laca; oupm Sc spin 9:11 pm Sam 9:28 5pm 9:33 5c 40 -> :48 0:41 hot ses -> 10:11 pm 6:50 hot samples > 10:20 pm (22:02 cold 50's) 5500 @ 10:27 pm

10:45pm Tets -> 11:25 Ahs -> 10:58 -> 11:28 pm

FIXPRIM Sc15 @ 23:05-715-725 Combined spin @ 11:38 pm 11:55 pm samples cold - 100:25/ RBC lyse @ 12:27 am wash @ 12:36 Sc intra @ 12:31 -> 1:11am Sample Fix Pean @ 12:50->00->10 Xist wash @ 1:17am Intracellular @ 1:45 am > 2:25 am Final spin @ 2:33 am Pone @ 2:45 am

Mark of the freezer log !



Aurora 5 on @ 4:30 pm. running 420 @ 4:49 pm. Qce 5:02 pm

) N [2023

5 sec mart abort, aiming 30pt (added 20pt se's) 1504 cells about a dop left.

4 coffees

6:15 pm restert

6.24 NO050 -> 12,400 events, about 614. 30/1 -> 55// collection. 9600 events about 389 1,350,000 0 0015

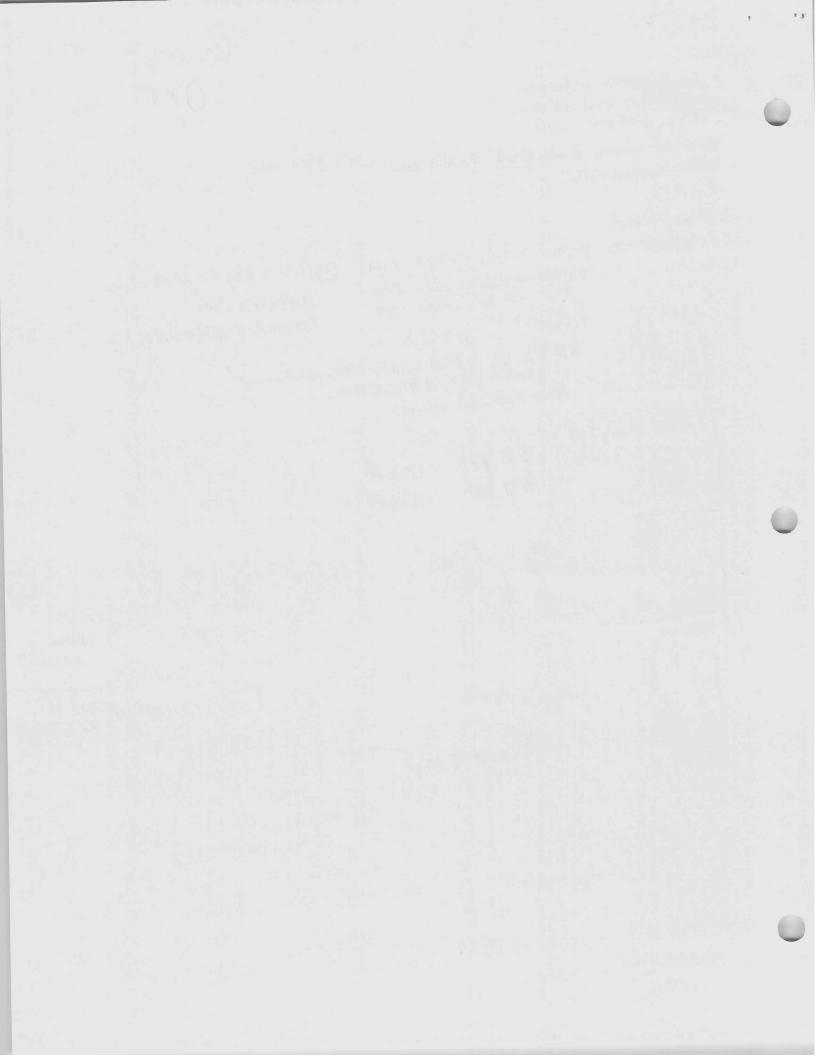
(probably 2 drops left...) 15,200 tet accident, added a drop PBS, ran to end, rescued: 1.7 Marche cells. abord 1000 PMA notable effect.

Infroo mostly dead 24,600 3300 27,00 4800 19000/1900

chilus Actorited)

20,000 /2000

Done @ 7:39 pm



				_													
				ADOOR		ND050	1	INE314	TOT	INE12/	TOO	NE100		INFO18		sample	
-			7	N.01	2 6	۵ ۵ ۵	10.44	10 //	11.57	11 27	0.03	3		12.48		Total	
				Р	. +	۷	^	J	^	J	~	,		2		Volume	
INFULO		Sample		10.80	8.38	0 0	9.22)	5.69	1	4.32			6.24		Concentration	
0.52	2 2	3M		0.28	0.36		0.33		0.53		0.70			0 48		3M	
0.52 0.475 0.432	2 141	2 75M		0.25	0.33	•	0.30		0.48		0.64		. 1	0 44	- 1	2.75M	
	IAIC7.7 IAIC.7	2 7 2		0.23	0.30		0.27		0.44		0.58		. 1	0 40	1.0141	2.5M	
0.39	IAIC7.7	3 2EM		0.21	0.27		0.24		0.40		0.52		0.50	0 0	14107.7	2 25M	
0.345	Z.UIVI			0.19	0.24		0.22		0.35		0.46		0.32	2	Z.01VI	3 OM	
0.303	1.75M		0	0.16	0.21		0.19	i	0.31		0.41		0.28)	T./5IVI	4	
0.26	1.5M		0.1	0 14	0.18		0.16	1	96.0		75.0		0.24		1.5M		
0.216	1.25M		0.12	0 1 3	0.15	i	0.14	77.0	0 22	0.22	0.29		0.20		1.25M		
0.173	1.0M		0.09		0.12	(0 11	0.10	0 10	0.23	0 13		0.16		1.0M		
0.13	0.75M		0.07)))	0.09	0.00	0 08	0.13	2	0.17	0 11		0.12		0.75M		
0.087	0.5M		0.05		0.06	0.00	0 00	0.09		71.0))		0.08		0.5M		

	Г			Transition .							, ,					
						PMA										į
	12000	INF314 ND050 ND006		INF134	INF100	INFOLO	INEO10	sample			ND006	ND050	INF314	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	INF13/	
	_	٦ ٢)	2	2	2) /	ر	3M		1	0 73	0.64	0.6/	0.47	0 47
	1.83	1.00	1 &2	1.83	1.83	1.83	1.83	4 02	2.75M		0.001	0 661	0.588	0.618	0.432	
	1.66	1.00	1 66	1.66	1.66	1.66	1.66	,	2.5M		0.002	0 600	0.535	0.562	0.393	
	1.5	. L.5	1.5		1.5	1.5	1.5		2.25M		0.54	0 0	0 48	0.51	0.35)) !
	1.32	1.32	, ,	1.32	1.32	1.32	1.32		2.0M		0.481	0.427	0 427	0.449	0.314	
	1.16	1.16		1 16	1.16	1.16	1.16		1.75M		0.421	0.3/4	777	0.393	0.275	;
	Ъ	Ь	+	ح	1	1	1		1.5M		0.36	0.32		0.34	0.24	0.10
	0.83	0.83	0.83		0.83	0.83	0.83		1.25M		0.300	0.267	0 10	0.280	0.196	0.120
	0.66	0.66	0.66) (0.66	0.66	0.66	1.0141	1.0M		0.240	0.214	0.22.0	0 225	0.157	0.101
	0.5	0.5	0.5		0.5	0.5	0.5	0.70141	0 75M		0.18	0.16	0.1/	0 17	0.12	0.08
	0.33	0.33	0.33	0	0 33	0.33	0.33	0.5141	0 AM		0.121	0.107	0.113	0 113	0.079	0.051
Ī										L	_			THE REAL PROPERTY.		chial.

R10

INF100

0.30

0.279

0.254

0.23

0.203 0.345

0.177 0.303

0.15

0.126 0.216

0.101 0.173

0.08 0.13

0.051

0.087

0.26

CD107a

INF134

6

5.5 5.5 5.5

4.5 4.5 4.5

3.5

ω ω ω

3.5 3.5

INF100

6 6

5 G

Sample INF018

2.75M

2.5M

2.25M 4.5

2.0M

1.75M

1.5M

w

Volume

Lym

Average

8.44 10

> 8.44 Total

18.44

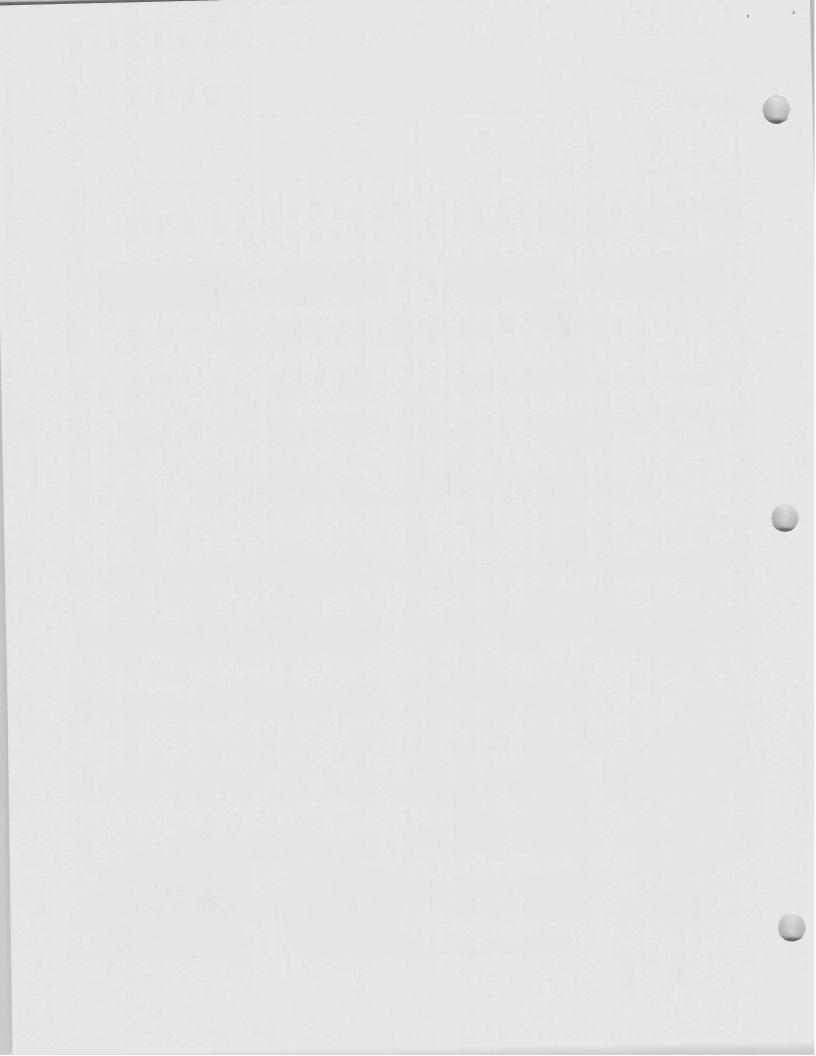
10

9.22

ND006 ND050 INF314

3.5 3.5

ω



2 12	Tetramer Mix	Va7 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2	Va Mix 10 FITC Va24/a18 1.5 15		111	And UNSTAINED CONTROLS III		- NO	29 ES CO.	Ro Coody ONA	26 R4 0747 0750	RZ			88	B6 9			180 B2			V13		10	- 5	m w Grd		V3		60 V1		-			UVO CCFO		1 002		
			T z					APC/Fire 810	APC/Fire 750	Zombie NIR	APC-P700	APC	PE-vio770	PE-Cy5	PE-Dazzie594	PE	PerCP-Cy5.5	Spark blue 550	FITC/AF488	BV786	BV750	BV711	BV650	BV605	OTCAG	00440	Pacinc Blue	Pacinc Blue	BV421	BUV805	ВИУ737	BUV661	BUV615	BUV563	BUV496	ĄF	BUV395	Fluorochrome	
Tet:PBS	Tet:PBS	C	Zombie NIR					CD38	CD27	T/D	Val. 2/nMK1	CD16	PD1	CD25	TNFa	NKG2D	CD26	CD3	Va24/hcD1d	CCR6	IFN+	CD7	CCR7	CD56	CD45RA	19103	CD19	CD14	CD127	CD4	CXCR3	V82	CCR4	CD69	CD8	AF-UV6	CD62L	Marker	
1.9	1 0	5.76	18	rippette draw volume /sample	NTO Media	Antibody lotal		HIT2	0323	N/A	3C10	3G8	PD1.3.1.3	M-A251	MAB11	1D11	BASb	SK7	6811	11A9	B27	M-T701	GOASH7	5 1411	HI100	HP-3G10	SJ25C1	MSE2	A019D5	SK3	1C6/CXCR3	B6	161	FN50	RPA-T8		SK11	Clone	
2_18	20	15 PBS 6 Zombie																																				Vial Lot #	
3.27	8	0		19.5		9 15	60	-		6.0			1			1			1		1																	During stim!!!	
	Wumb	Numb	7	Pipp	232	0	96	+	41:	96				1	1	-	1	-	-		-					-				-				-				16	
FBS PBS	Total	Number Samples Number Unstained Number Surface SCs (34)		Pippette draw volume /sample	Brilliant Stain	Cumpody Total	Antihoda Tatal		<1:2500>		<2:10/1.2>			1		<u> </u>		<:10,														1						L/D 15 min Tets	
146				ample							/1.2>	1						<:10/1.5>									1											Tetramer 40 min @ RT	
≥ ¬		16 12 28	1	65	50	17.8		12	*	7			1.2		1.5				1.5		1	1	1.0		4	3	\$	(1.5	1			2.0	2				(HotStain 30min @37C	
Perm Water	12 292	128 RBC Lyse 96 56			800	285	25.6	32					19.2		24				24		16	16	16		26	23		47	24			32	3					16	
16.8 151	8.4	3.6		59.5	50.0	12.5					0.1	15	;				1.2							((200	2.0			1.3	0.7		6.6	0.7) \$	(i))	ColdStain	
	1.2	4.8 2.4 5.6			800	200.0					7.17	24.0					19.2							77.7	113	32.0	32.0			20.8	11.2		8.0	11.2		19.2	1	16	
	24	Perm 64 24																																			Fix/Perm	RBC Lyse,	
	0.15 1.87	0.72 0.3		19.5	11	9.5						0.5	0.5	1.5	0.5	2.5	0.1		-	1.5		0.7	01						0.5		0.1		1					Spiked 40 min	
					176	152						8.0	8.0	24.0	8.0	40.0	1.6			24.0		11.2	16						8.0		1.6		16.0					16	
	Cart	A CD27	\$ C) 50		cap cubes, widp rack in foll, Store at 4 °C	Can tubes wran rack in fail store at Av	Resuspend in 70 ul 0.4% PFA-PBS	4 III F GI	Add intracellular Stain, incubate @ RT First PermWash:		ž	First PermWash: 1 ml Pen			(vortex	ove at DD rixreitt, incubate (# 40 lor	300 ul BD EivBorn incubata @ 40 fa-	the state of the s	Wash 2 ml 5% PRS-FRS 1400 rpm 6mir	Add ColdStain mix, incubate @ 4C for Amin		Wash 2 ml 6% PBS-FBS 1400 rpm, 6 mi	Add Tetramers, incubate @RT for 10 m		Wash 2 ml 5% PBS-FBS 1400 rpm, 6 mi	Add HotStain mix, incubate @37C for :	3	Wash 2 ml 5% PBS-FBS, spin 1300 rpm	800 ul of LiveDead mix (1:2500) @RT fr	Wash with 2 ml PBS, spin down 1300n		Cap and incubate at 37°C for 6 hours	Bring volume up to "x" mL R10, add "	Collect, count, aliquot cells 2-3 0F+6 0	Thaw colls DNAse count			Simplified Protocol	

Thave cells, DNAse, count.

Collect, count., aliquois cells 2.3.0E-6 Cells R10 / Sml polystyrene lube.
Bring volume up to "x" mL R10, add "y" j.L PMA/Ctri and "z" j.L CD107a
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1:2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add Tetramers, incubate @RT for 10 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min Add HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS.FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 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)

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

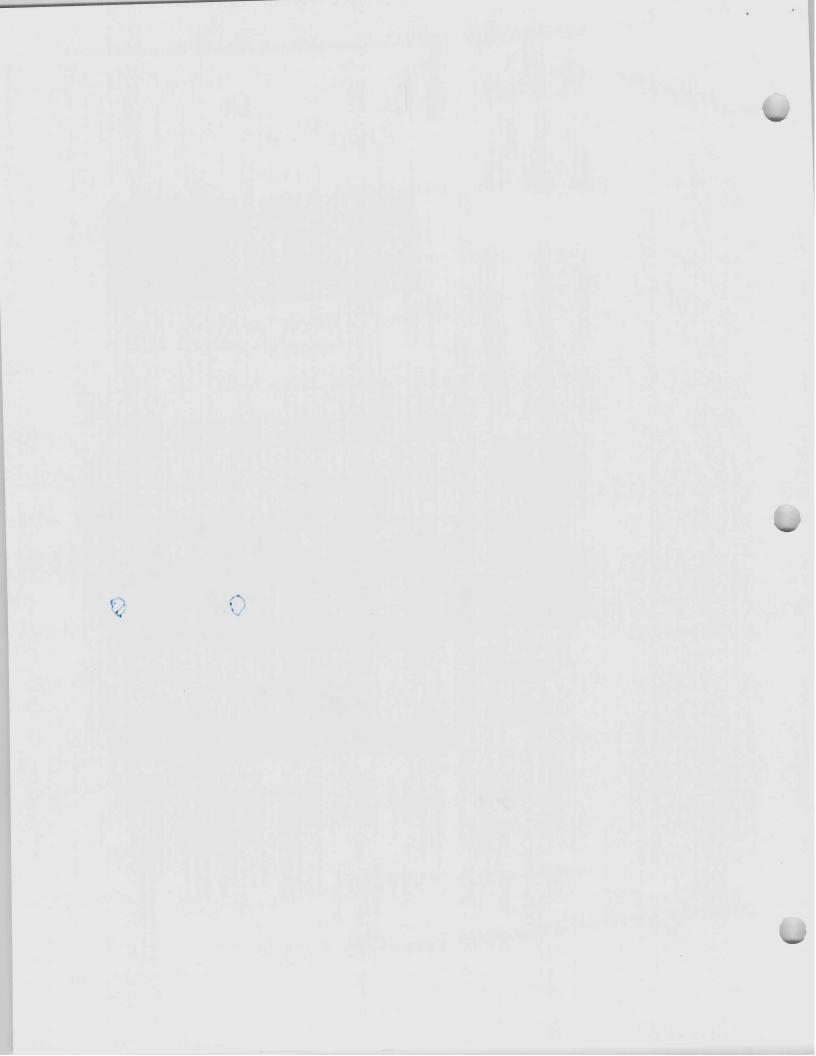
Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

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

(net c038) 351050

 PFA
 0.187

 PBS
 1.683



ILT & NK SFC Panel

783 812	738 750 760	697 717	664 679	582 598	514 525 542	473 508	443 443	388	373
UV15 UV16	UV14	UV12	UV10		UV8	UV6			UV1
BUV805	BUV737		BUV615 BUV661		BUV496	AF	ω 44 ω	2 BUV395	
[50]	[40]		[50]	[55]	[65]			[66]	+
CD4	CXCR3		CCR4 V82	CD69	CD8			CD62L	
V15	V14	V12	V10 V11	§ & <	i 6	\$ \$ \$	≤ ≤		\dagger
BV786	BV750	BV711	BV605 BV650	BV570		PacBlue BV480	BV421		Violet
[50]	Post 3	5	[50]	[88]		55]	[45]		t
CCR6		CD7	CD56 CCR7	CD45RA	3	CD14/19	CD127		
B12 B13 B14	B11	B B8	B6 B7	B3	B2 B	?			L
		PerCP-Cy5.5		SparkBlue 550	FITC/AF488				Blue
		[45] CD26		[50] CD3	™ Vα24/hCD1d				
YG9		YG5	YG3	YG1					
Pe-Vio770		PE-Cy5	PE-Dazzle594	PE					Yellow-Green
[50]		晝	[60]	1501					1
PD1		CD25	TNFα	NKG2D					
R6 R7	R R R R R R R R R R R R R R R R R R R	Z Z Z	2						
Zombie NIR APC-Fire 750 APC-Fire 810	APC-R700	AF647))						Red
Viability CD27 CD38	[55] CD107a	Vα7.2/hMR1							

