

November 8-9th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC panels #9 *2 ILT Nov 02*

New CD38 control (baseline)

- Valz -
 - TNF/IFN γ - } stim
 - unstained unstained \rightarrow order a new one.
(really, really old TNF α)
 - NKG2D
 - PD1
 - CD56
- Baseline

∴ CD 161? ← possible, next time

10. $\rightarrow 14$

$$\begin{array}{r} 11 \\ \times 5 \\ \hline 55 \end{array}$$

16 → 54 mRL APC

nedid

$$\begin{array}{r} 11 \\ \times 1.5 \\ \hline 55 \\ 110 \\ \hline 16.5 \end{array}$$

2-13 hcdld

TNF α intake
22.34 \rightarrow 2

22.34

[30 min]

apps...
need to standardize

Basic 37°C @ 8:04 pm
→ 8:34 pm

basic 4°C @ 8:54 \rightarrow
9:24 pm

- 1.5 10x for basic
- 1.2 10x for PMT/ctrl

basic

Rbe lye @ 9:28pm

basic perm @ 9.42 → 52 → 10.02
2 wages

→ PFL @ 10:31 pm
basic ✓

Done @ 1:25 AM

PMA test @ 9:23 pm
→ 10:03 pm

© 378 © 2229-7
22. 59

@4c@ 23:13
rhclysc@23:44

Compare noise ratio for ACID

Fix Perm @ 23:57

Inter @ 00:38

UV3 x \rightarrow % CV: 6.14

5:28pm 11/10/2021

check

* define add neg group for spillover calc *

Dump

#	Filter	Single color (u)	Ref ctrl	Fluorochrome	Marker	During stimulation	Live/Dead RT for 15min	Tetramer (RT) for 40 min	37°C (RT) for 30min	Surface staining x4	Surface staining x4	Lysing, then CytoChk/Perm	RT for 40min Universal Intra mix (10x diluted)	x4
1	UV2	1.25	Cells	C	BUV395	CD62L (DREG-56)								
2	UV7	1.25	Cells	C	BUV496	CD8 (RPA-T8)				1.2	4.8			
3	UV9	1.25	Cells	C	BUV563	CD69 (FN50)				1.0	4.0			10
4	UV10	1.25	Cells	C	BUV615	CD4 (1G1)				1.0	4.0			10
5	UV11	1.25	Cells	C	BUV661	CD4 (1G1)				1.0	4.0			10
6	UV14	1.25	Cells	C	BUV737	CD4 (1G1)				1.0	4.0			10
7	UV16	1.25	Cells	C	BUV805	CD4 (1G1)				1.0	4.0			10
8	UV1	1.25	Cells	C	BUV421	CD127 (A01905)				1.5	6.0			10
9	UV3	1.25	Cells	C	Pacific Blue	CD14 (M5E2)				1.0	4.0			
10	UV5	1.25	Cells	C	Pacific Blue	CD19 (H1B19)				2.0	8.0			
11	UV7	1.25	Cells	C	BUV480	CD161 (REA63)				2.0	8.0			
12	UV10	1.25	Cells	C	BUV510 (dim)	CD45RA (H1100)				1.0	4.0			
13	UV11	1.25	Cells	C	BUV605	CD56				1.0	4.0			
14	UV13	1.25	Cells	C	BUV711	HLA-DR (0.8)				1.0	4.0			
15	UV15	1.25	Cells	C	BUV750	IFN- γ (B27)				1.0	4.0			
16	UV16	1.25	Cells	C	BUV786	CCR6 (1A9)				1.0	4.0			
17	UV18	1.25	Cells	C	Alexa488/IFN- γ	hCD107a/241a18				1.8	7.2			
18	UV23	1.25	Cells	C	Speck-Biotin550	CD3 (SK7)				1.2	4.8			
19	UV23	1.25	Cells	C	PE	MLG2D				1.2	4.8			
20	UV23	1.25	Cells	C	PE-CH594	CD26 (MAA261)				1.2	4.8			
21	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
22	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
23	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
24	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
25	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
26	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
27	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
28	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
29	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
30	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
31	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
32	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
33	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
34	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
35	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
36	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
37	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
38	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
39	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
40	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
41	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
42	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
43	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
44	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
45	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
46	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
47	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
48	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
49	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
50	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
51	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
52	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
53	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
54	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
55	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
56	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
57	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
58	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
59	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
60	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
61	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
62	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
63	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
64	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
65	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
66	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
67	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
68	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
69	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
70	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
71	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
72	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
73	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
74	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
75	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
76	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
77	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
78	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
79	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
80	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
81	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
82	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
83	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
84	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
85	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
86	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
87	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
88	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
89	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
90	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
91	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
92	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
93	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
94	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
95	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
96	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
97	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
98	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
99	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			
100	UV23	1.25	Cells	C	PE-Cy5	CD25 (MAA251)				1.2	4.8			

added =

PCR - eBNC-PD1 - 111021

stim wound FSC at 70 180/230
accepted my Vg2 & TIFA stim controls.

55%
67%
55%
67%

DR

baseline 1.5

In F282 - only Vg2/Mg2 & CD45R2

In F180 - on medium good cells/sec

28 pl/min

10000 - duplicated added, added.

NK62D works in this configuration

In F180 or NK62D less Vg2?

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

Surface mix @ 40c 30min
Lysing solution 300-500ul
PBS-FBS 2ml
CytoChk/perm @ 40c 20min
wash twice PermWash 1 ml
Intra Staining @ RT for 40min
wash once w/ 2ml
Resuspend 100 ul PFA-PBS

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

CCR5
711 CCR7
PE NK62D
PE-Vio PD1
APC14

November 8-10th, 2021

#	Filter	Single color (u)	Ref cct	Fluorochrome	Marker	During stimulation	Live/Dead RT for 15min	Tetramer (RT) for 40 min	37°C (RT) for 30min	Surface staining x6 40C for 30min	Lysing, then Cytochrome/Perm	RT for 40min Universal Intra mix (10x diluted)	x6
1	UV2	1.25	Cells	C	BUV395	CD62L (DREG-56)							
2	UV7	1.25	Cells	C	BUV496	CD8 (RPA-18)				1.2		2.5	15
3	UV9	1.25	Cells	C	BUV563	CD69 (FN50)				1.0		2.5	15
4	UV10	1.25	Cells	C	BUV615	CCR4 (IG1)				1.0		2.5	15
5	UV11	1.25	Cells	C	BUV661	Vα2 (B6)			2.0				
6	UV14	1.25	Cells	C	BUV737	CD38 (1C6/CD38)				1.0			
7	UV16	1.25	Cells	C	BUV805	CD4 (SK3)			2.5				
8	V1	1.25	Cells	C	BUV421	CD127 (A019D5)				1.5		2.5	15
9	V3	1.25	Cells	C	Pacific Blue	CD14 (M5E2)	0.8			1.0			
10	V5	1.25	Cells	C	Pacific Blue	CD19 (H1B19)				2.0			
11	V7	1.25	Cells	C	BUV480	CD161 (HEA631)	0.8		1	2.0			
V10			Cells	C	BUV510 (dim)	CD45RA (H100)						10.5 of 1x	45
V11			Cells	C	BUV605					1.0			
V13			Cells	C	BUV550								
V14			Cells	C	BUV711								
12	V14	1.25	Cells	C	BUV750	IFNγ (B27)							
13	V15	1.25	Cells	C	BUV786	CCR6 (11A9)			1.8			1.5 of 1x	4
14	B2	1.25	Cells	C	Alexa488/IFNγ	hCD14/Na24a18			10.8				
15	B3	1.25	Cells	C	Spark blue 550	CD3 (SK7)		<1.750>					
B4			Cells	C	PE					1.2		2.5	15
16	B6	1.25	Cells	C	PE-CF594	CD26 (M-A261)							
17	B8	1.25	Cells	C	PE-Cy5	CD25 (M-A251)			1.2	7.2			
18	B10	1.5 (<10^4)	Cells	C	PerCP-Cy5.5	TNFα (MAA811)				1.2		2.5	15
B13			Cells	C	PE-w0770							12.5 of 1x	15
19	R1	1.25	Cells	C	APC	IL-4							
20	R2	1.25	Cells	C	Alexa Fluor 647	HMAT1							
21	R4	1.25	Cells	C	APC-R700	CD107a (HA43)		<1.500>					
22	R6	1.2500	Cell	P	Zombie NIR	Viability	6.0						
23	R7	1.3	Cells	P	APC/Fire 750	CD27 (O323)	<1.2500>		2				
24	R8	1.3	Cells	C	APC/Fire 810	CD38 (H17)			12				
HH-cy5(72) to match signal at 10^5										1.6	9.6		
Total volume										12.1	72.6	14.1	84.6
24 color CBMC										50 uL/rxn	50 uL/rxn	50 uL/rxn	50 per rxn
Brilliant Staining Buffer										Each reaction			

0.54 M / 1.50 M

7.5

oocyte

Protocol

Allot cells

PMA-Ionomycin stimulation

Golgi blockers

PBS 2ml

Live/Dead @ RT 15min

PBS-FBS 2ml

Tetramers @ RT 40min

PBS-FBS 2ml

Surface

37°C 30min

PBS-FBS 2ml

Surface mix @ 40C 30min

Lysing solution 300-500ul

PBS-FBS 2ml

Cytochrome/perm @ 40C 20min

wash twice PermWash 1 ml

Intra Staining @ RT for 40min

wash once w/ 2ml

Resuspend 100 ul PPA-PBS

CCRS

Perforin

GrimA

605/650 HLA-DR S6

711 CCR7

PE NKG2D

PE-w0 PD1

APC-IL4

Infected - mix of bone not downy normal Q316

only B cell downy

Back on ed.

+ Uninfected cells for every sample + CD38 (new)

