October 26-27th, 2021

HEU: Ex-vivo γδ CBMC panels #7/ILT novo 1

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf070											
Inf161						2.5					
Inf023											
NY062											

PMA/Golgi experiment

basic PMA stim ctrl stim
○ ○ ○

R10

2M Inf070 → 398 μl + 302 + 300
Inf161 → 398 μl + 302
(Inf 23 → ~~882~~ μl ~~+~~ ~~+200~~
Cntyl.5m PMA 670 + 30 +300 ← (not 300 μl PMA either ~ 280)
~1 ctrl 500 + 200

basic cust LD CD3
○ ○ ○

Δ concentration
○ ○ ○ ○ ○ | basic 5 new SCs
| ○ ○ ○ ○ ○ ○ |
basic +2

PMA wells in @ 3:09 pm   PMA controls Aliquot # 350 μl
PMA controls in @ 3:57 pm

(Aurora Ab mix done around ~7:40 pm)

15 × .8 = 12.0 mls   6 - 15 ml

4:30 pm

basic 6 + 3 = 9
stim 6

Basic/AllVols
L/D @ 5:07 → 5:22
Tetramers @ 7:51 pm → 8:31 pm
(SCs spin down @ 8:25 pm)

PMA wells → 9:09 → 9:57 respectively

(6 μl → 15 mls)
15

15 specimens × 5 = 75 μl   1:200
15 × 2 = 30 μl   1:250
OPRU 8 → 72   HEDW 34 → 36

@ 9:03 Basic + AllVol 37°
→ 9:33 pm 3 AllVol tubes got 1.3x 37°c (including + CCR7)
All basic got normal (one got bit both leftovers, so ab cross? Thu-pm
2 remaining allvol got 1x & little to 1x
→ 4 (→) 5 1 allvol discarded due # ab.

22:34 → PMA SCs spin down
Rbc lyse allvol 22:37
stimNIR sc in @ 22:47 +15 →
AllVol fix Perm @ 22:52 → 11:02 → 11:12
PMA 37°c @ 11:10 ✓ → 11:40 pm
last permwash spin allvol @ 23:14 pm
Allvol/basic done @ 23:25
PMA 4°c @ 23:54 → 00:24

9:31 SCs fixPermed w/ 300 μl
37°c out of inc @ 9:33
PMA ~~FP~~ @ 9:35 → 9:50
37°c wash @ 9:39
4°c allvol/basic @ 9:56 pm → 26 (34)
PMA tetramers @ 10:16 → 56 pm late
(2 μl instead of 2.5 μl) Redo

Friday!! unstained aurora ack
+2 sep Permwash after
stay FBS after FixPerm

SCs basics done @ 22:22

October 26-27th, 2021

#	Filter	Single color (ul)	Ref ctrl		Fluorochrome	Marker	During stimulation	Live/Dead RT for 15min	Tetramer (RT) for 40 min	37oC (RT) for 30min	x3	Surface staining 4oC for 30min	x3	Lysing, then CytoFix/Perm	RT for 40min Universal Intra mix (10x diluted)	x3
1	UV2	1.25	Cells	C	BUV395	CD62L (DREG-56)						1.2	3.6			0
2	UV7	~~1.25~~ 3	Cells	C	BUV496	CD8 (RPA-T8)						1.0	3.0		2.5	7.5
3	UV9	1.25	Cells	C	BUV563	CD69 (FN50)						1.0	3.0		2.5	7.5
4	UV10	1.25	Cells	C	BUV615	CCR4 (1G1)				2.0	6					
5	UV11	1.25	Cells	C	BUV661	Vd2 (B6)						1.0	3.0			
6	U14	1.25	Cells	C	BUV737	CXCR3 (1C6/CXCR3)				2.5	7.5					
7	UV16	1.25	Cells	C	BUV805	CD4 (SK3)						1.5	4.5		2.5	7.5
8	V1	1.25	Cells	C	BV421	CD127 (A019D5)	~~0.8~~ 1					1.0	3.0			
	V3	1.25	Cells	C	Pacific Blue	CD14 (M5E2)						2.0	6.0			
9	V3	1.25	Cells	C	Pacific Blue	CD19 (HIB19)						2.0	6.0			
10	V5	1.25	Cells	C	BV480	CD161 (REA631)				1	3				[0.5 of 1x]	
11	V7	1.25	Cells	C	BV510 (dim)	CD45RA (HI100)						1.0	3.0			
	V10	~~1.25~~ 1	Cells	C	BV605		CD56					1				
	V11	1	Cells	C	BV650		HLA-DR {0.8}					1 ~~1.0~~ 1.5	3.0			
	V13	~~1.25~~ 1	Cells	C	BV711		CCR7			1						
	V14	1.25	Cells	C	BV750	IFNg (B27)									[1.5 of 1x]	
12	V15	1.25	Cells	C	BV786	CCR6 (11A9)				1.8	5.4					
13	B2	1.25	Cells	C	Alexa488/FITC	hCD1d/Va24Ja18			<1:750>							
14	B3	1.25	Cells	C	Spark blue 550	CD3 (SK7)						1.2	3.6		2.5	7.5
	B4	~~1.25~~ 1	Cells	C	PE	-	NKG2D					12	3.6		2.5	7.5
15	B6	1.25	Cells	C	PE-CF594	CD26 (M-A261)				1.2	3.6					
16	B8	1.25	Cells	C	PE-Cy5	CD25 (M-A251)						1.2	3.6		2.5	7.5
	B10	1.5 (<=10^4)	Cells	C	PerCP-Cy5.5	TNFa (MAB11)									[2.5 of 1x]	
	B13	~~0.5~~ 1	Cells	C	PE-vio770		PD1					~~0.5~~ ~~1.35~~	1.5			
	R1	1.25	Cells	C	APC		IL-4									
17	R2	1.25	Cells	C	Alexa Fluor 647	hMR1			<1:500>						[1.2 of 1x]	
	R4	1.25	Cells	C	APC-R700	CD107a (H4A3)	6.0									
18	R6	1:2500	Cell	P	Zombie NIR	Viability		<1:2500>								
19	R7	1.3	Cells	P	APC/Fire 750	CD27 (O323)				2	6					
20	R8	1.3	Cells	C	APC/Fire 810	CD38 (HIT2)				1.6	4.8					
	HH-cytoFP(2) to match signal at 10^5					Total volume	6.8			12.1	36.3	16.8	50.4			
	unstained Cells					Brillian Staining Buffer	50ul			50 ul/rxn		50 ul/rxn			50 ul/rxn	
	20 color CBMC					Each reaction										

Protocol

Aliquot cells		
PMA-Ionomycin stimulation		
PBS 2ml	1400rpm, 6min	
Live/Dead @RT 15min		
PBS-FBS 2ml	1400rpm, 6min	✓
Tetramers @RT 40min		✓
PBS-FBS 2ml	1400rpm, 6min	✓
Surface 37oC 30min	CCR4, CXCR3, CD161, CXCR5	CCR6, CD26, CD27, CD38
PBS-FBS 2ml	1400rpm, 6min	
Surface mix @ 4oC 30min		✓
Lysing solution 300-500ul		
PBS-FBS 2ml	1400rpm, 6min	
Cytofix/perm @ 4oC 20min		
wash twice PermWash 1 ml	1500rpm, 6min	
Intra Staining @ RT for 40min		
wash once w/ 2ml	1500rpm, 6min	
Resuspend 100 ul PFA-PBS		

CCR5	605/650 HLA-DR 56
Perforin	711 CCR7
GrzmA	PE NKG2D
	PE-Vio PD1
	APC Il-4

Leave a 10-20 sec gap between cells first appearing before collecting

Abs!! ~~(x3)~~ (x2)!

Name: Boucher BU605 CD56
DTR 10-27-21 CD56
1.3x 37°C 1x 4°C CD56

CD56 well CIf701

L/D Dump as nomenclature going forward

Using DR-NKT run#1 as instrument settings
didn't move my FSC/SSC much

not in culture media...

62 (58)

~~82~~ Nope

opps... just repeated for all vol/basic

3 wells (CD8/L/D/unstained)

CD19 old basic new others
CD45RA old b/av pmn new (2nd well)

All Vol sample experiments

☆ CBMC takes all library controls (old ones) except new exp BVeO5 · of
☆ run mix on CD56 bright   oohh... switching to your all stained might help.

VD2 & hMRI issues. (the non-specific junk.

basic   adj FSC 50 SSC 200.

-> for L/D, gated on the dead adjacent pop (not the lymph) -> moved detector from R5 to R6
-> for CD8, @ >3 µl, clean peak (w/ 25,000 events)   What effect this has

w/ old CD3   CD45RA CD161 CD26

Unmixed w/ CD8 3µl control, then appended on the rest of Inf070

-> on average a little better   dont use CD8 2021 b !!

-> Noticeably vs CD45RA comp eff.   @ 5:45 pm start

☆ record upon rate stabilizing ☆

DTR-α CBMC - CD62L - V1

October 26-27th, 2021

#	Filter	Single color (ul)	Ref ctrl		Fluorochrome	Marker	During stimulation	Live/Dead RT for 15min	Tetramer (RT) for 40 min	37oC (RT) for 30min	x3	Surface staining 4oC for 30min	x3	Lysing, then CytoFix/Perm	RT for 40min Universal Intra mix (10x diluted)	x3
1	UV2	1.25	Cells	C	BUV395	CD62L (DREG-56)						1.2	3.6			
2	UV7	1.25 (2)	Cells	C	BUV496	CD8 (RPA-T8)						1.0	3.0		2.5	7.5
3	UV9	1.25	Cells	C	BUV563	CD69 (FN50)						1.0	3.0		2.5	7.5
4	UV10	1.25	Cells	C	BUV615	CCR4 (1G1)				2.0	6					
5	UV11	1.25	Cells	C	BUV661	Vd2 (B6)						1.0	3.0			
6	U14	1.25	Cells	C	BUV737	CXCR3 (1C6/CXCR3)				2.5	7.5					
7	UV16	1.25	Cells	C	BUV805	CD4 (SK3)						1.5	4.5		2.5	7.5
8	V1	1.25	Cells	C	BV421	CD127 (A019D5)	0.8					1.0	3.0			
	V3	1.25	Cells	C	Pacific Blue	CD14 (M5E2)						2.0	6.0			
9	V3 (V3?)	1.25	Cells	C	Pacific Blue	CD19 (HIB19)						2.0	6.0			
10	V5 (V4)	1.25	Cells	C	BV480	CD161 (REA631)				1	3				[0.5 of 1x]	
11	V7 (V5)	1.25	Cells	C	BV510 (dim)	CD45RA (HI100)						1.0	3.0			
	V10	-	Cells	C	BV605											
	V11		Cells	C	BV650											
	V13		Cells	C	BV711											
12	V14	1.25	Cells	C	BV750	IFNg (B27)									[1.5 of 1x]	
13	V15	1.25	Cells	C	BV786	CCR6 (11A9)				1.8	5.4					
14	B2	1.25	Cells	C	Alexa488/FITC	hCD1d/Va24Ja18			<1:750>							
15	B3	1.25	Cells	C	Spark blue 550	CD3 (SK7)						1.2	3.6		2.5	7.5
	B4		Cells	C	PE (in tube)											
16	B6	1.25	Cells	C	PE-CF594	CD26 (M-A261)				1.2	3.6					
17	B8 (B7)	1.25	Cells	C	PE-Cy5	CD25 (M-A251)						1.2	3.6		2.5	7.5
18	B10	1.5 (<=10^4)	Cells	C	PerCP-Cy5.5	TNFa (MAB11)									[2.5 of 1x]	
	B13	-	Cells	C	PE-vio770											
19	R1	1.25	Cells	C	APC	IL-4									1.25?	
20	R2 (R1)	1.25	Cells	C	Alexa Fluor 647	hMR1			<1:500>							
21	R4 (R3)	1.25	Cells	C	APC-R700	CD107a (H4A3)	6.0									
22	R6 (R5)	1:2500	Cell	P	Zombie NIR	Viability		<1:2500>								
23	R7	1.3	Cells	P	APC/Fire 750	CD27 (O323)				2	6					
24	R8	1.3	Cells	C	APC/Fire 810	CD38 (HIT2)				1.6	4.8					
	HH-cytoFP(2) to match signal at 10^5					Total volume	6.8			12.1	36.3	14.1	42.3			38
	unstained Cells					Brillian Staining Buffer Each reaction	50 ul/rxn			50 ul/rxn		50 ul/rxn			50 per rxn	
	24 color CBMC															

x2! x2! x2!

10x measurements 50... .25?

Redshift →

Protocol

Aliquot cells	
PMA-Ionomycin stimulation	
Golgi blockers	
PBS 2ml	**1400rpm, 6min**
Live/Dead @RT 15min	
PBS-FBS 2ml	**1400rpm, 6min**
Tetramers @RT 40min	
PBS-FBS 2ml	**1400rpm, 6min**
Surface 37oC 30min	**CCR4, CXCR3, CD161, CCR6, CD26, CD27, CD38**
PBS-FBS 2ml	**1400rpm, 6min**
Surface mix @ 4oC 30min	
Lysing solution 300-500ul	
PBS-FBS 2ml	**1400rpm, 6min**
Cytofix/perm @ 4oC 20min	
wash twice PermWash 1 ml	**1500rpm, 6min**
Intra Staining @ RT for 40min	
wash once w/ 2ml	**1500rpm, 6min**
Resuspend 100 ul PFA-PBS	

CCR5	605/650 HLA-DR 56
Perforin	711 CCR7
GrzmA	PE NKG2D
	PE-Vio PD1
	APC Il-4

(55)

25
x .2
5.0 million cells

You can copy past names @ → 20,000 evts

Mandarin orange

Activated reference controls: (20,000 events

W/ each append FSC/SSC may shift.

- BUV395 CD62L is struggling - (vs on monocytes?) ← should I?
- ↑ 50,000 events - visible staining for CD8 stopped early

CD69 is a waterfall (grab highest

- CCR4 low hugging axis.

Not adjust the preselect unless needed $10^{3\frac{1}{2}}$ $10^{4\frac{1}{2}}$

Where to place CD62L + CCR4?

→ $10^{4\frac{1}{2}}$ → $10^{5\frac{1}{2}}$

VD2 (50K events) + appending (50K events)

→ $10^{4 \, 1"}$ → $10^{4 \, 7"}$ (brightest reads)

vs. 69 was obvious

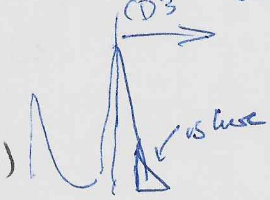
ditto CD4 Not CD4

ditto CD26

give just a little red stripe for high flyers

(does position of the peak matter?)

↑ re titration question



CxCR3 similar boat.

CD127 (IL-7Rα) expression is M ← focusing on.

We want the low %, high expressors (think iNKT, etc's)

Cytokines: IFNγ ✓ no issue

how all this a tetramer flow vs CD3 flow?

remember ≠ what's the individual cell, just all & brightest, how clean

PeCy5 & PerCPCy5 swapped in Aurora order

CD25 rare (vs CD26 similar to others $10^{4\,3"}$ → 10^5 (ghost red)

TNFα... ✓ no issue (in this state)

how is this showing? gate

IL-4... issue append more events...

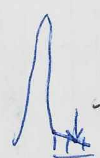
↳ getting enough events challenging. I have $10^{4\,1"}$ to 10^5, just a bit read.

Zombie NIR was placeable on ~~CD3~~ Lym pop ✓ not an issue.

downregulated

↓

CD62L got flagged not enough + ↑ ab amount



not happy about it but okay

CCR4 as well

~~And VD2~~

↑ expanded decade out to 10^4 - 10^5

↑ still not enough

→ let me squeeze $10^{3\,5"}$ ← not happy with it, replace next go. re VD2

And CCCR3

gate $10^{3\frac{1}{2}}$ → $10^{4\frac{1}{2}}$

And CD25 10^4

~~And IL-4~~ another footpad. ← not happy with, replace

8:06 AM rec library!

SCs @ 00:08 for 20 min @ respective temps
@ 00:10 for fixperm intracellular SCs

[@00:24] next step for PMA samples
RBC lyse @ 27

@ 00:36 pm → everything into centrifuges

→ fixperm up next
@ 00:51 ⇒ 1:01 → 1:11
↳ 1st spin @ 1:16 am
2nd spin @ 1:30 am

2nd wash
→ intracellular stains @ 00:57
→ 1:37 am

SCs done @ 01:57

Intake @ 1:43pm
+ 40
2:23

Last QC: Oct 25, 2021: UV3 7.64 % rCV (spread)

"SOM restart your program pop-ups" ... this is bullshit!
→ turning on your cytometer might help. 4:10 pm

Won't let you on an unsaved worksheet proceed.

all acBMC unmixings
and both unstained for AF...

Unstained

Using my good UD2 significant improvement
and my CD39 APC
worlds [illegible]

ran second [illegible]
medium

Exporting cytokine file before I mess w/ controls (duplicated version hopefully)

ctrl does seem cleaner on its own old comp

62L
CD127

≠ 56 ✓ (copy & paste/duplicate & edit are soo useful)

need toast med [18/11] +

≠ PDI ✓ & append

CCR7 looks coolio

≠ NRG2D ? AF488 → Fitc ?

HLA-DR @ 10:17 pm.... yuuuppees, bleb on CO3 vs dump.