October 4-6th, 2021

HEU: Ex-vivo γδ CBMC #4 – Aurora ILT #1

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf029				2018-07-28							
NDO50				2021-08-24							

Thawed ~ 10:30 AM. Aliquoted ~ 3pm @ 6000 cells (10/04/2021), 4°c

@ 6:27 Sc's addition BUVs start

Sc's setup

@ 7:05 4°c → fridge
37°c → incubator for 30 min @ 7:35

* New FBS aliquot (PMA side process)

@ 7:44pm → 1ml FBS-PBS wash, spin @ 1300/rpm 8 min

@ 20:09 pm – fix perm first vortex
first wash @ 20:23 pm
2nd wash @ 20:37 pm

PFAed (bulk tubes) @ 8:56
Intracellular @ 8:58pm → 9:38 pm

Done @ 9:50 pm

Tuesday – 1 cord Inf 29
– 1 adult NDO50

still need to make 1 L/D well
1 CD27 sc

L/D @ 2:18 pm

~~Incubate~~ @ 38°c stash - 4:00 pm

Fix Perm @ 5:23 pm

Done @ 6:07pm

3

Aug-18-21 training

everyone @ 1.25 µl

[x4 · x4

#	Filter	Single color (ul)		Ref ctrl	Fluorochrome	Marker	Druing stimulation [Skip this time]	Live/Dead RT for 15min	RT for 10min	37oC (RT) for 30min	Surface staining 4oC for 30min	4oC for 15min	Lysing, then CytoFix/Perm	RT for 40min Universal Intra mix (10x diluted) [Skip this time]
1	UV2	0.5		Cells	BUV395	CD62L (DREG-56)					1.2 4.8			
2	UV7	0.5		Cells	BUV496	CD8 (RPA-T8)					1.0 4			2.5
	UV9	0.2		Cells	BUV563	CD69 (FN50)					1.0 4			
	UV10	0.6	1.25	Cells	BUV615	CCR4 (1G1)				2 8				
	UV11	0.5		Cells	BUV661	Vd2 (B6)					1.0 4			
3	U14	0.5		Cells	BUV737	CXCR3 (1C6/CXCR3)				2.5 10				
4	UV16	1.0	1.25	Cells	BUV805	CD4 (SK3)					1.5 6			2.5
5	V1	0.8	1.25	Cells	BV421	CD127 (A019D5)	0.8				1.0 4			
	V3	0.2		Cells	Pacific Blue	CD14 (M5E2)					2 8			
	V3	0.2	1.25	Cells	Pacific Blue	CD19 (HIB19)					2 8			
6	V5	0.2		Cells	BV480	CD161 (REA631) (direct)			0.8 1		0.5 2	Step 1.0ul		[0.5 of 1x]
7	V7	1.0	1.25	Cells	BV510 (dim)	CD45RA (HI100)					1.0 4			
8	V10	0.5	1.25	Cells	BV605	CXCR5 (J252D4)				2.5 10				
9	V11	0.2	1.25	Cells	BV650	CCR7 (G043H7)	0.8				1.0 4			
10	V13	0.2		Cells	BV711									
	V14	0.2		Cells	BV750	IFNg (B27)					2.5			2.5
11	V15	0.8	1.25	Cells	BV786	CCR6 (11A9)								[1.5 of 1x]
	B2	0.2	1.25	Cells	Alexa488/FITC	hCD1d/Va24Ja18				1.8 7.2				
12	B3	0.5	1.25	Cells	Spark blue 550	CD3 (SK7)								
13	B4	0.2	1.25	Cells	PE	PD-1 (EH12.2H7)					1.2 4.8			2.5
14	B6	0.2	1.25	Cells	PE-CF594	CD26 (M-A261)					1.2 4.8			2.5
15	B8	0.2		Cells	PE-Cy5	CD25 (M-A251) (FERRY-1)				1.2 4.8				
	B10	1.5 (<=10^4)		Cells	PerCPVio700	TNFa (MAB11) (PerCP-Cy5.5)					1.2 4.8			2.5
16	B13	0.8	0.5	Cells	PE-vio770	HLA-DR (REA805) (MD)								[2.5 of 1x]
17	R1	0.8	1.25	Cells	APC	CD39 (A1)					0.5 2			
	R2	0.2	1.25	Cells	Alexa Fluor 647	hMR1				1.2 4.8				
	R4	0.2	1.25	Cells	APC-R700	CD107a (H4A3)								[1.2 of 1x]
18	R6	1:2500		Cell	Zombie NIR	Viability tomorrow	0.8	<1:2500>						
	R7	1.0		Cells	APC/Fire 750	CD27 (O323) tomorrow								
19	R8	1.0	1.25	Cells	APC/Fire 810	CD38 (HIT2)				1.6 6.4				
	HH-cytoFP(2) to match signal at 10^5					Total volume	7.6	0.8	10.8		12.5			
20	unstained Beads					Brillian Staining Buffer			50 per rxn		50 per rxn			
21	unstained Cells					Each reaction	50ul	locak	60.5ul		61.5			50 per rxn
22	18 color PBMC													

Protocol

- Aliquot cells
- ~~SEB stimulation~~
- ~~Golgi blockers~~
- PBS 2ml 1400rpm, 6min
- Live/Dead
- PBS-FBS 2ml 1400rpm, 6min
- CD161-biotin @RT 15min

CXCR3	CD26
CXCR5	CD39
CCR6	CD27
CCR4	CD38

- PBS-FBS 2ml 1400rpm, 6min
- Surface mix @ 4oC 30min
- PBS-FBS 2ml 1400rpm, 6min
- ~~Strep BV480~~
- Lysing solution 300-500ul
- ~~PBS-FBS 2ml 1400rpm, 6min~~
- Cytofix/perm @ 4oC 20min
- wash twice 1500rpm, 6min
- ~~Intra Staining @ RT for 40min~~
- ~~wash Once w/ 2ml 1500rpm, 6min~~

CCR5	GrzmA
IL-4	NKG2D
CD161	Perforin
CD56	
PD1	

1:2500 VD NIR 5ml 2µl 1ml

26 colors

2 1 1 1 5 = x2 (10 + 14) = 25

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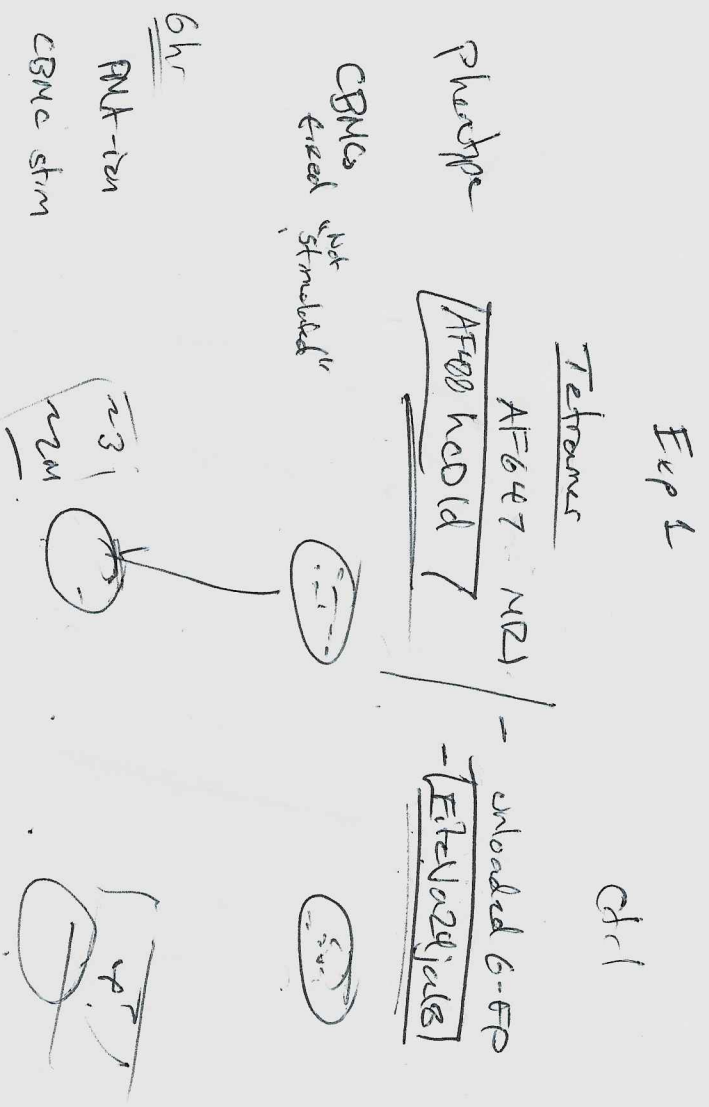

Inf 029

Evt rate ~ 2500 : flowrate low ~13 μl/min

Ran new Ref controls, appended 100,000 evts for VD2 (+15000 original), everything else run ~ ok.

☐ tube

Acquisition

Save sources edit

☐ tube ☐ Group

run initial, run unstained, run remainder all in the default raw worksheet.

- refgroup unstained
- Folder
  - cells #1
  - cells #2

Live unmixing

CD26 × CD62L → 2 guard filter

extra small on CCR6 * CD4

CD8 ≤ CD4 bit backcorner spill * CD26

CD8 & CD45RA blob balance

AF647 → stripe CCR7 / CCR6 CD39

CD38 & 26 bizzare

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Cytometer lab

Fluidics shutdown.

Bleach water contrad bleach

NKT

Aug 18-21 training

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						[Skip this time]							[Skip this time]
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2	UV7	0.5	Beads	BUV496	CD8 (RPA-T8)					1.0 2			2.5
	UV9	0.2	Beads	BUV563	CD69 (FN50)					1 0 2			
	UV10	0.6	Beads	BUV615	CCR4 (1G1)				2 4				
	UV11	0.5	Beads	BUV661	Vd2 (B6)					1 0 2			
3	U14	0.5	Beads	BUV737	CXCR3 (1C6/CXCR3)				2.5 5				
4	UV16	1.0	Beads	BUV805	CD4 (SK3)					1.5 3			2.5
5	V1	0.8	Beads	BV421	CD127 (A019D5)					1.0 2			
	V3	0.2	Beads	Pacific Blue	CD14 (M5E2)					2 4			
	V3	0.2	Beads	Pacific Blue	CD19 (HIB19)					2 4			
6	V5	0.2	Beads	BV480	CD161 (REA631)-Biotin			0.8	1 2	0.5	Step 1.0ul		[0.5 of 1x]
7	V7	1.0	Beads	BV510 (dim)	CD45RA (HI100)					1.0 2			
8	V10	0.5	Beads	BV605	CXCR5 (J252D4)				2.5 5				
9	V11	0.2	Beads	BV650	CCR7 (G043H7)					1.0 2			
10	V13	0.2	Beads	BV711	Va7.2 (3C10)					1.0 2			
	V14	0.2	Beads	BV750	IFNg (B27)					0.5			2.5
11	V15	0.8	Beads	BV786	CCR6 (11A9)								[1.5 of 1x]
	B2	0.2	Beads	Alexa488/FITC	hCD1d/Va24Ja18				1.8 3.6				
12	B3	0.5	Beads	Spark blue 550	CD3 (SK7)					Tef			
13	B4	0.2	Beads	PE	PD-1 (EH12.2H7)					1.2 2.4			2.5
14	B6	0.2	Beads	PE-CF594	CD26 (M-A261)					1.2 2.4			2.5
15	B8	0.2	Beads	PE-Cy5	CD25 (M-A251)				1.2 2.4				
	B10	1.5 (<=10^4)	Beads	PerCPVio700	TNFa (MAB11)					1.2 2.4			2.5
16	B13	0.8	Beads	PE-vio770	HLA-DR (REA805)								[2.5 of 1x]
17	R1	0.8	Beads	APC	CD39 (A1)					0.5 1			
	R2	0.2	Beads	Alexa Fluor 647	hMR1				1.2 2.4				
	R4	0.2	Beads	APC-R700	CD107a (H4A3)					Tef			
18	R6	1:2500	Cell	Zombie NIR	Viability		<1:2500>						[1.2 of 1x]
	R7	1.0	Beads	APC/Fire 750	CD27 (O323)								
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	HH-cytoFP(2) to match signal at 10^5				Total volume	7.6		0.8	10.8 9.8	12.5 10.8			
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21	unstained Cells				Each reaction	50ul			60.5ul 58.8	61.5 56.8			
22	18 color PBMC								56.8				

Protocol

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SEB stimulation	
Golgi blockers	
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CD161-biotin @RT 15min	
CXCR3 CD26	
CXCR5 CD39	
CCR6 CD27	
CCR4 CD38	
PBS-FBS 2ml	1400rpm, 6min
Surface mix @ 4oC 30min	
PBS-FBS 2ml	1400rpm, 6min
Strep BV480	
Lysing solution 300-500ul	
PBS-FBS 2ml	1400rpm, 6min
Cytofix/perm @ 4oC 20min	
wash twice	1500rpm, 6min
Intra Staining @ RT for 40min	
wash Once w/ 2ml	1500rpm, 6min

CCR5 GrzmA
IL-4 NKG2D
~~CD161~~ Perforin
CD56
~~PD1~~

Tuesday

IL-4/TNF-IFNγ

26 colors

CCR4 CD26
CCR3 CD25
CXCR5 CD27