

September 26th, 2022

Monocyte SFC panel #2

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	2E+6
NY062 #1			20E6	08/12/21		2	5.89	7.93		510pl	51pl
NY062 #2			20E6	08/12/21		2	6.21	8.32		483pl	48pl
NY062 #3			20E6	08/12/21		2	6.83	9.11		439pl	44pl
NY062 #4			20E6	08/12/21		2	7.34	9.76		409pl	41pl
							52.5				

↑ relative density clean bone?

ctrl Backbone ○
ctrl sc ○ unstained

PPD stim backbone ○
ctrl stim sc ○ unstained

TT stim backbone ○
TT stim sc ○ unstained

PPD stim backbone
+ CD19 ○
+ CD20 ○
+ FcER1a ○
+ CD5 ○
+ CD141 ○

PPD stim backbone
+ CD19
+ FcER1a ○
+ CD5 ○
+ CD141 ○

PPD stim backbone
+ CD19
+ FcER1a
+ CD5 ○
+ CD141 ○

PPD stim backbone
+ CD19
+ FcER1a
+ CD141
+ CD5 ○

14 stained backbone samples

+ 26 sc controls (stim) ~ 300k
+ 34 unstained controls (1 unstained 2-3 stim)

~ 3 M cells/tube
42 million + 9.0 million = 51 million cells

+ 26 sc bead stained wells (0.5pl, 1pl)

52 sc bead stained wells

+ 1 unstained bead well

10 ml media
x 3-4 tubes
resuspended 2-3mls

↑ 500pl

0.5M
4pl/ml extra

2% FBS
5 2.0%
4 2.5%
3 3.3%
2 5%
1 10%

10%
25ml
125
x 4
500pl

Hot scs @ 4:45pm → 5:15pm

Cold scs @ 4:57pm → 5:27pm

40 sc @ 4:58pm → 5:13pm

Hot sample spin @ 5:05pm ✓

Washed @ 5:14pm

Hot scs @ 5:17-5:20pm
wash w/ Arg 10% FBS → 2% EDTA

15
x 800
12,000 ml

4/0
2pl 5ml
6pl 15ml

Thaw 1050 #2 +13 melted

#1 pellet

#4 hang on

10:50 pm thaw spin

11:16 pm stain count

Count @ 11:30 am

Stim @ 12:14 pm ✓

Abx prepped @ 2:18 pm

Reagents prepped @ 2:51pm

44 44
x.1 <.3

13.2 ml RBC lysis

4.4 ml 1.5 + 13.5 ml #20

0.4% PFA-PBS

4/27 28

3:17 cell out

3:25pm cells spin down

15:42 4/0

→ 16:00

4/0 spin @ 4:25pm

Vortexed sorted @ 4:24

4:30 hobs in → 5:00 pm

21 hotwash

5:34 hot out.

36 → 39/40

Hot PFAed @ 5:36 pm

Cold spin @ 5:42.

return adding test fines FDS

5:14 2: 5:44 all indiv combos added

(best estimate
20 minutes above
sit for CD141, FeERI_n, CD19)
pre add CD5 (indiv) & 4°C mix

cold 4°C samples @ 5:49 pm → 6:19 pm (rheolse)

sds organized @ 5:58 pm

samples rheolse 6:20

6:44 pm

Dave moved w/ fridge to emergency power

#	Filter	Single color (u)	Ref ctrl type	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	L/D + FC blocker 30ul/pxn	170C (RT) for 30min	14 40C for 30min	14
1	UV2				BUV395	CD18	(6.7)		0.8	11.2		
2	UV7				BUV496	CD19						
2	UV9				BUV563	CCR2	(15132.1D9)		0.8	11.2		0
3	UV11				BUV661	CD3CR1	(2A9-1)		1	14		
4	UV16				BUV805	CD14	(M5E2)				1.0	14
23	V1				BV421	FcER1a						0
V2												
24	V3				Pacific Blue	CD5						0
5	V7				BUV480	CD11a	(H1111)				0.6	8.4
25	V7				BUV510 (dim)	CD11a	(2H7)		0			
6	V8				BUV570	CD16	(3G8)				1.5	21
V10												
7	V10				BUV605	CD36	(5.1H11)				1.0	14
8	V11				BUV650	CD11c	(B-ly6)				1.0	14
9	V13				BUV711	CD80	(1307.4)					
10	V14				BUV750	CCR5	(2D7)		1.5	21		
26	V15				BUV785	CD141						0
B1												
11	B2				FITC	CD11b					0.8	11.2
B3					Spark Blue 550							
12	B4				PE	CD88	(SS/1)		0.6	8.4		
13	B6				PE-Dazzle594	CD3					0.6	8.4
14	B8				PE-Cy5	CD86	(TT2.2)		0.5	7		
B9												
15	B10				PerCP-Cy5.5	CD123	(7G3)		2	28		
16	B13				PE-Vio770	HLA-DR	(REA805)				0.5	7
R1												
17	R2				Alexa Fluor 647	CD163	(GH1/61)				1.0	14
R3												
18	R4				APC-R700	CD40	(5C3)		1.0	14		
19	R6				Zombie NIR	L/D			<1.2500>			
20	R7				APC/Fire 750	CD64	(10.1)		1	14		
21	R8				APC/Fire 810	CD38	(H1T2)		1.0	14		
					True Stain FCX				0.5	7	0.5	7
And UNSTAINED CONTROLS !!!												
Antibody Total												
PBS												
Pipette draw volume/sample												
50												12.2
700												17.1
50												8.5
700												11.9
61.2												57.5

Notes: TrueStain FCX (BioLegend 422301)
InvivoGen TrF-3releps

final 0.1ug/ml for 24hr
LPS stock 5mg/ml, 10ul, take one aliquot out and add 490ul R10 to make 100ug/ml working solution

Lipopolysaccharides from Escherichia coli O111:84
Wash with 2mM EDTA

SA not quite

500 uL media ~ 2.5 x 10⁶ cells ~ 15 x 10⁶ cells

Simplified Protocol 3EC cells 4h

Aliquot cells 1.25 x 10⁶ cells/tube
Wash with 2mL PBS, 1300 rpm, 8min
2500x diluted Zombie NIR, 1E+6/1mL + 3ul FC blocker at RT for 15min
Wash with 2mL 2% FBS-PBS 2mM EDTA
Spin at 1300 rpm for 8min

x ul of 370C Ab mix, at 370C for 30min
Remove RBC with 1mL lysing solution for 3min

Wash with 2mL 2% FBS-PBS 2mM EDTA
Spin at 1300 rpm for 8min
x ul of 40C Ab mix, at 40C for 30min
Treat with 600ul of 1x lysing solution at RT for 3min

Wash with 2mL 2% FBS-PBS 2mM EDTA

Spin at 1300 rpm for 8min
Resuspend in 0.4% PFA-PBS

APCs: ?

15:42

46

CD19 run @ 40C not yet

Monocytes SFC Panel

9/26/2022

Spectrum	UV		Violet		Blue		Red	
373	UV1							
388	UV2	BUV395	[2]	CD18				
428	UV3							
443	UV4							
458	UV5							
473	UV6							
508	UV7	BUV496	[2]	CD19				
514	UV8							
525	UV9	BUV563	[3]	CCR2				
542	UV10	BUV615	[3]					
582	UV11	BUV661	[3]	CX3CR1				
598	UV12							
613	UV13							
664	UV14	BUV737	[3]					
679	UV15							
697	UV16	BUV805	[1]	CD14				
717								
738								
750								
760								
783								
812								

AF Monocyte
 Lymphocytes ~ 0.95
 0.29 → 0.48
 dead dying signals
 less intense
 extra-extra extra-extra
 concentration

0.72

Not good for a PDC marker

Date		Time		Location		Remarks	
1941	10-10	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-11	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-12	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-13	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-14	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-15	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-16	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-17	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-18	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-19	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-20	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-21	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-22	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-23	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-24	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-25	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-26	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-27	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-28	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-29	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-30	10:00	10:15	10:30	10:45	11:00	11:15
1941	10-31	10:00	10:15	10:30	10:45	11:00	11:15

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September 26th, 2022

Monocyte SFC panel

Aurora-UV early AM edition: button ran on @ 5:12 AM
shifted time 6:30-9:30 AM, will adjust accordingly.

Template set up done @ 5:40 AM, start @ 5:45 AM (3hr)
Sc's → 30000, medium event rate ~ 700 ~ 1 minute (lymphocytes)

remembers monocytes today love. (similar)

PE CD88

↑ in Pdc's → due to decoupling?

Pe - dazzle

Pe - cys

Pe - Vio770

test via export se as fcs and unmix in ExtraTools.

Samples start @ 6:30 am ~ 5m each

Playing short-term gains paying long term consequences / #Covid #vaccines

CD19, CD3, CD56 unmixed w/ Lym AF, everything else monocyte.

HLA-DR Pe-Vio770 almost off scale!!

CD19 AF signal ~ brightest B cell on Buv495

BV570 CD16 brightest might be on Nils ~ monocytes ~ 10^5 (few 16+ monocytes in this donor)
unmixing w/ monocyte AF

BUT11 CD80 ≠ @ 3 hrs stim positive leading edge negative ≠ great control

CCRS dribbles 10^5

exaggerated



HLA-DR high expression ($10^5 \sim 10^6$)
38 $10^4 \sim 10^5$

CD18 ✓

CD80 minimal 10^4

fCER1a marginal

~ CCR5 spotty?

Pos Blue most CDS 10^4 edge

~ CD141

~ CD86

~ CD123

G4/46

Monocyte vs Dc/PDC express on the G4/40 expression array

May need to draw new AF gate for 4/6 unmixing

Swap out CD16 AF for CD3

Did 16 get added? Jordan's specific issue?

1. The first part of the report is a general introduction to the project. It describes the purpose of the study and the objectives that were set at the beginning.

2. The second part of the report is a detailed description of the methodology used in the study. This includes information about the data collection methods, the sample size, and the statistical tests that were used to analyze the data.

3. The third part of the report is a discussion of the results of the study. This section presents the findings of the research and compares them to the expectations that were set at the beginning of the project.

4. The fourth part of the report is a conclusion that summarizes the main findings of the study and provides some suggestions for future research. This section also discusses the limitations of the study and the strengths of the methodology.

5. The fifth part of the report is a list of references that includes all of the sources that were used in the study. This section is important for providing credit to the original authors of the work that was cited.

6. The sixth part of the report is an appendix that contains additional information that is not included in the main body of the report. This may include raw data, detailed calculations, or other supporting materials.

7. The seventh part of the report is a glossary that defines the key terms and concepts that are used in the study. This is helpful for readers who may not be familiar with the terminology.

8. The eighth part of the report is a list of figures and tables that are included in the study. This section provides a brief description of each figure or table and explains how it relates to the overall findings of the research.

9. The ninth part of the report is a list of acknowledgments that thanks the individuals and organizations that provided support and assistance during the course of the study. This is a personal and important section of the report.

10. The final part of the report is a list of appendices that includes all of the additional materials that are included in the study. This section provides a detailed description of each appendix and explains how it relates to the overall findings of the research.

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Monocyte SFC panel

Nd CD3s:

CD3 + CD11c
CD11b

CCR2 CD11b
CD64
CD38

CX3CR1 CD11b / 84
CD64
CD38

CD14 . CD11b
CD84
64
38

CD11a . CD11c
CD11b
64
38

CD16 . CX3CR1
CD11c
CD11b
CD38

