

2023\_AB\_TNFa

[illegible]

PM @ 11:30

SC bull in 5/1 @ 1:20 //

Intra  $\leftarrow$  02:13  $\rightarrow$  43 pm  
under start  $\leftarrow$  32  $\rightarrow$  22  $\rightarrow$  32  
50 cm

Old ND050 ... couldn't recall if CD26 had been added @ hot  
(unlucky, only recall biotin) so added 0.5  $\mu$ l PerCF594 eD26  
@ intake

$\downarrow$  2.5  $\rightarrow$  1.5 PerCP-Cy5.5 ID26 (suspect works) (done...)

TNFA PerCP-Cy5.5  $\rightarrow$  2.5  $\mu$ l

TNFA PerCF594  $\rightarrow$  1.2  $\mu$ l

] will see how extra 40 minutes  
 $\Delta$  things.

$\rightarrow$  out around 2:43pm

resus in 25  $\mu$ l

Done @ 3:04 am

July 11th, 2023

CD107a came from ND050 dcl

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FSC 140 SSC 380 SSC-B 392

Sc start @ 4:43pm:

[HLA-DR activation]

SC's @ 5400 events/120 <sup>reject</sup> sec

< need to adjust SSC-B in the future

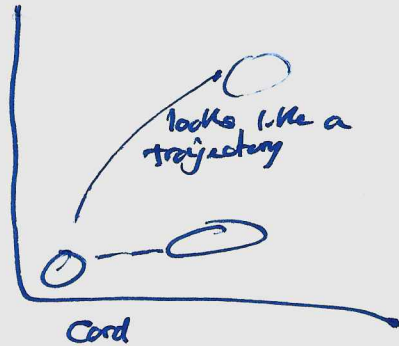
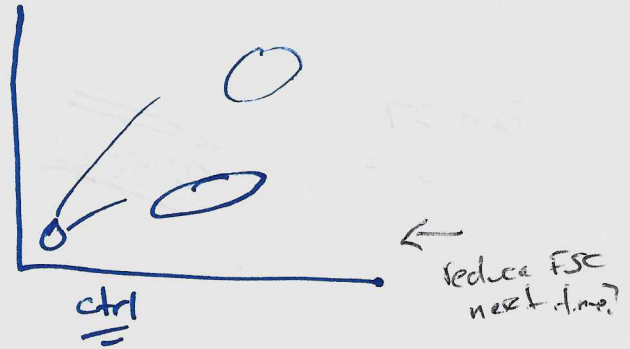
Lot fewer doublets w/ this processing.

" FoxP3, VD2 & Va7.2 are ND050 unstained..."

sc CD161 stained ✓

HLA-DR fine

Sc done @ 5:33 pm:



250M sc done

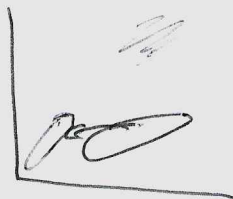


CA1106

16K / 1.2M

60p / 1.9M

residual  
44p / 53p



CA PBMC

11.4K / 0.53M

48p / 1.1M

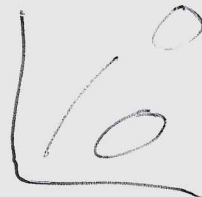


C6022

12K / .7M

1.1M

←  
HLA/ctrl switched?



ND050

12K / .7M

45p / 1M

ctrl old ↔ ctrl unstained.

Done @ 6:25pm

Initial unmix 7/12/23 @ 7:48pm

→ old unmix @ 7:53pm

Bed 27

7L2

2:48 PM

2:55



Sample	Total	Volume	Concentration	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF 8375	4.48	0.5	8.96	0.33	0.31	0.28	0.25	0.22	0.20	0.17	0.14	0.11	0.08	0.06
INF 8640	16.66	2	8.33	0.36	0.33	0.30	0.27	0.24	0.21	0.18	0.15	0.12	0.09	0.06
CVD 6022	5.01	0.5	10.02	0.30	0.27	0.25	0.22	0.20	0.17	0.15	0.12	0.10	0.07	0.05
ND050	14.3	1	14.30	0.21	0.19	0.17	0.16	0.14	0.12	0.10	0.09	0.07	0.05	0.03
INF	2	2	1.00	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50
INF	2	2	1.00	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50
ND	2	2	1.00	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50
ND	2	2	1.00	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50

Sample	R10													
	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M			
INF 8375	0.67	0.609	0.554	0.50	0.443	0.388	0.33	0.276	0.221	0.17	0.111			
INF 8640	0.64	0.586	0.533	0.48	0.426	0.373	0.32	0.266	0.213	0.16	0.107			
CVD 6022	0.70	0.642	0.583	0.53	0.466	0.408	0.35	0.291	0.233	0.18	0.117			
ND050	0.79	0.724	0.658	0.59	0.526	0.461	0.40	0.329	0.263	0.20	0.132			
INF	-2.00	-1.834	-1.667	-1.50	-1.334	-1.167	-1.00	-0.834	-0.667	-0.50	-0.333			
INF	-2.00	-1.834	-1.667	-1.50	-1.334	-1.167	-1.00	-0.834	-0.667	-0.50	-0.333			
ND	-2.00	-1.834	-1.667	-1.50	-1.334	-1.167	-1.00	-0.834	-0.667	-0.50	-0.333			
ND	-2.00	-1.834	-1.667	-1.50	-1.334	-1.167	-1.00	-0.834	-0.667	-0.50	-0.333			

Sample	PMA													
	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M			
INF 8375	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
INF 8640	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
CVD 6022	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
ND050	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
INF	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
INF	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
ND	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			
ND	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33			

Sample	CD107a													
	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M			
INF 8375	6	5.5	5	4.5	4	3.5	3							
INF 8640	6	5.5	5	4.5	4	3.5	3							
CVD 6022	6	5.5	5	4.5	4	3.5	3							
ND050	6	5.5	5	4.5	4	3.5	3							
INF	6	5.5	5	4.5	4	3.5	3							
INF	6	5.5	5	4.5	4	3.5	3							
ND	6	5.5	5	4.5	4	3.5	3							
ND	6	5.5	5	4.5	4	3.5	3							
ND	6	5.5	5	4.5	4	3.5	3							

Volume  
Lym  
Total  
Average

2  
8.33  
16.66  
5.1  
10.88  
21.76



#	Filter	Single color (u)	Ref ctrl type	Unmask ctrl	Fluorescence	Marker	Clone	Value #	During stain	10	U/D 15 min	RT for 10 min	Hoechst 30min @37C	10	Costain 30min @4C	10	4°C for 15 min Fixation	RBC Lysed Stain 40 min @RT	10
1	UV7				AF	CD62L	(DRG-56)												
2	UV7				BUV496	CD8	(RPA-18)												
3	UV9				BUV661	CD89	(7H50)												
4	UV10				BUV661	CD89	(7H50)												
5	UV11				BUV661	CD89	(7H50)												
6	UV16				BUV661	CD89	(7H50)												
7	UV16				BUV661	CD89	(7H50)												
8	UV1				BUV661	CD89	(7H50)												
9	UV3				BUV661	CD89	(7H50)												
10	UV5				BUV661	CD89	(7H50)												
11	UV7				BUV661	CD89	(7H50)												
12	UV10				BUV661	CD89	(7H50)												
13	UV11				BUV661	CD89	(7H50)												
14	UV16				BUV661	CD89	(7H50)												
15	UV16				BUV661	CD89	(7H50)												
16	UV16				BUV661	CD89	(7H50)												
17	UV16				BUV661	CD89	(7H50)												
18	UV16				BUV661	CD89	(7H50)												
19	UV16				BUV661	CD89	(7H50)												
20	UV16				BUV661	CD89	(7H50)												
21	UV16				BUV661	CD89	(7H50)												
22	UV16				BUV661	CD89	(7H50)												
23	UV16				BUV661	CD89	(7H50)												
24	UV16				BUV661	CD89	(7H50)												
25	UV16				BUV661	CD89	(7H50)												
26	UV16				BUV661	CD89	(7H50)												
27	UV16				BUV661	CD89	(7H50)												
28	UV16				BUV661	CD89	(7H50)												
29	UV16				BUV661	CD89	(7H50)												
30	UV16				BUV661	CD89	(7H50)												
31	UV16				BUV661	CD89	(7H50)												
32	UV16				BUV661	CD89	(7H50)												
33	UV16				BUV661	CD89	(7H50)												
34	UV16				BUV661	CD89	(7H50)												
35	UV16				BUV661	CD89	(7H50)												
36	UV16				BUV661	CD89	(7H50)												
37	UV16				BUV661	CD89	(7H50)												
38	UV16				BUV661	CD89	(7H50)												
39	UV16				BUV661	CD89	(7H50)												
40	UV16				BUV661	CD89	(7H50)												
41	UV16				BUV661	CD89	(7H50)												
42	UV16				BUV661	CD89	(7H50)												
43	UV16				BUV661	CD89	(7H50)												
44	UV16				BUV661	CD89	(7H50)												
45	UV16				BUV661	CD89	(7H50)												
46	UV16				BUV661	CD89	(7H50)												
47	UV16				BUV661	CD89	(7H50)												
48	UV16				BUV661	CD89	(7H50)												
49	UV16				BUV661	CD89	(7H50)												
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51	UV16				BUV661	CD89	(7H50)												
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62	UV16				BUV661	CD89	(7H50)												
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67	UV16				BUV661	CD89	(7H50)												
68	UV16				BUV661	CD89	(7H50)												
69	UV16				BUV661	CD89	(7H50)												
70	UV16				BUV661	CD89	(7H50)												
71	UV16				BUV661	CD89	(7H50)												
72	UV16				BUV661	CD89	(7H50)												
73	UV16				BUV661	CD89	(7H50)												
74	UV16				BUV661	CD89	(7H50)												
75	UV16				BUV661	CD89	(7H50)												
76	UV16				BUV661	CD89	(7H50)												
77	UV16				BUV661	CD89	(7H50)												
78	UV16				BUV661	CD89	(7H50)												
79	UV16				BUV661	CD89	(7H50)												
80	UV16				BUV661	CD89	(7H50)												
81	UV16				BUV661	CD89	(7H50)												
82	UV16				BUV661	CD89	(7H50)												
83	UV16				BUV661	CD89	(7H50)												
84	UV16				BUV661	CD89	(7H50)												
85	UV16				BUV661	CD89	(7H50)												
86	UV16				BUV661	CD89	(7H50)												
87	UV16				BUV661	CD89	(7H50)												
88	UV16				BUV661	CD89	(7H50)												
89	UV16				BUV661	CD89	(7H50)												
90	UV16				BUV661	CD89	(7H50)												
91	UV16				BUV661	CD89	(7H50)												
92	UV16				BUV661	CD89	(7H50)												
93	UV16				BUV661	CD89	(7H50)												
94	UV16				BUV661	CD89	(7H50)												
95	UV16				BUV661	CD89	(7H50)												
96	UV16				BUV661	CD89	(7H50)												
97	UV16				BUV661	CD89	(7H50)												
98	UV16				BUV661	CD89	(7H50)												
99	UV16				BUV661	CD89	(7H50)												
100	UV16				BUV661	CD89	(7H50)												

## Simplified Protocol

Thin cells, DNase count.

&lt;1000 cells at 1E6/ml of RT, rest cells for &lt;12 hrs in a 6 well plate.

Collect, count, aliquot cells 2.0E+6 cells/ml polypropylene tube

Add antigens, stain antibodies, and DAPI/DAPI antibodies in 500 ul RT

Cap and incubate at 37°C for 2 hours

Add PBS back and continue to incubate for &lt;2 hours

Wash with 2 ml PBS, spin down 1000gpm 5min

800 ul of Livehead mix (1:200) @RT for 15min

Wash 2 ml 5% PBS-FBS, spin 1000 rpm, 5min

Add CD161 biotin antibody for 10 minutes at RT

Add to RT biotin mix, incubate @37C for 30 min

Wash 2 ml 5% PBS-FBS 1400 rpm, 5min

Add CD161 biotin antibody @ 4C for 30min

Add 300-500 ul 1% RBC lysis for 5 minutes

Wash 2 ml 5% PBS-FBS 1400 rpm, 5min

Add Streptavidin-APC, incubate @ 4C for 15min

Wash 2 ml 5% PBS-FBS 1400 rpm, 5min

1 ml Nuclear FixPerm, incubate @ 4C for 30min

(vortex every 10 minutes)

First PermWash: 2 ml NuclearWash 1500 rpm 5 min

Second Perm Wash: 2 ml NuclearWash 1500 rpm 5 min

Add Intracellular Stain, incubate @ RT for 40min

First PermWash: 2 ml NuclearWash 1500 rpm 5 min

Second Perm Wash: 2 ml NuclearWash 1500 rpm 5 min

Resuspend in 100 ul 0.4% PFA-FBS

Cap tubes, wrap rack in foil, store at

1215

-



Spectrum		UV		Violet		Blue		Yellow-Green		Red	
373	UV1										
388	UV2	BUV395	CD62L	BV421	CD127						
428	UV3										
443	UV4			PacBlue	CD14/19						
458	UV5										
473	UV6	AF									
508	UV7			BV480	CD161						
514	UV8	BUV496	CD8								
525	UV9										
542	UV10			BV510	CD45RA						
582	UV11	BUV563	CD69	BV570							
598	UV12										
613	UV13	BUV615	CCR4	BV605	CXCR5						
664	UV14	BUV661	V62	BV650	CCR7						
679	UV15										
697	UV16										
717	UV17			BV711	Va7.2						
738	UV18										
750	UV19	BUV737	CXCR3	BV750	IFN $\gamma$						
760	UV20										
783	UV21										
812	UV22	BUV805	CD4	BV786	CCR6						

**Viability**  
**CD27**  
**CD38**



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