| | Specimen | Status | Location | Conc | Date | Tasks | Volume | Ly | Ly+Mon | Total | 3E+6 | 2E+6 |
|-----|----------|--------|----------|------|----------|---------|----------|----|--------|-------|-------|------|
| | N4062 as | Adult | 4.6. | 20E6 | 08/12/21 | | 3ml | | 6.2BE6 | | | 2110 |
| 204 | N4062 ar | Adult | | 2056 | 06/12/21 | | | | | 13.23 | .,, | |
| | | | | | | | | | 3 B | 26.73 | | |
| | | | | £. | រូក ដ | يدة فعل | . "Kudow | | | J = ~ | 1 - 1 | |
| | | | | | | | . il 454 | 1 | | 1 1 | | |

Biosicion biolle entitel, tree byhts & beeps coul colorstan @ qics Am Very low c/pl ~ Sith of debris in part? (yes)

Everything Tix stimulated? [212 wells]

A Cells into incubation w/ TTX 5pg/ml con @ 9:43 AM (+2 takes RIO alone)

Ab prep start @ 12:51 pm - 3hr (47 hr)

Abs done @ 1:17 pm

+2 unstands + 1 Sc CD19

12 tubes X85m1 10+ 10 10% 60 960 10 2.0

300 pl * 12 ~ 4 ml RBC byse 400H 3600p/ HZO

100 M x 12 -> 1.2 ml B.Q/ 18x-1955

cop(xp => 2.5 ml 40 m 3 pl 7.3 ml Reagants prepred @ 1:37pm.

Spin down @ 1:47 pm [4 horsinchation]

SSC-B > SSC-V for debris removal

> (1-1:30) + CD64

> > (1)

40@2:03 -72:18 LOSpn@2:20pm

+ (0) + (0)

/37° @ 2:38 pm > 3:08 pm out 3:11 37° spin in

+ 00115

, (1)

3:27 4°c in Wall add ons (CDIIS ->04 73157pm instead a6

RBC 15 = @ 3:58 -74:0 pm

Vare@4: Bpm

gating really important NK w jull gotes spreading from of coll into 56.

hemalogones

CDITC negatives ~18 0x30R1 COLF HLX-DR- =1-0038-more we AFS.

CD14 C430R1 56 = CP

CDC's more 86 than the moragles/ HEA-DR, CD163

/ 14 * FCERLa / > Deso running a Packap on 7500 cells Cx3CRI, ID14, 56, 163 88, 86, 144-DR-CO38

CK3CR1, > CO163, > CO14, ×088, CO38

cDCS = equivalent CD18, FERIA, CD119 FD141

COCT'S dart express Cx3CRI or CD14 56 by induidual susets

higher 88 ecpression in 3, but light

ting but more 86 in aDC2's.

- 163 3'5 7 4000

Repeating for Mon/oddballs &

18, 86, 38 (324 culs) rarety un-advated? La subside expresses SF

Man vs aDC3/s

~ CBCRI COILC, 141

~163,40,38

1 co18

TC0114

V FEERLA

7 CO88 1 86

J. HLK-DR

No FEE BLA " CDK

color us addballs y c018

4/- CX3CR1

CO14 - (14-0,3021-) 14-4-3-217-colle J CO 141 Kalso C038

TODIES FAILE no 86

& HLA-DR

no co163

1 5040

No/lov032

oddad relative COINE -CD14 oddball c 088 - 16-1 C088 + blee orange 118 1 18 V 0430R1 7 CL30R1/ J. 11 = 1 de 1 141 b 141 488) ~ 88/ no 86 NO 86 VICTUREDOL F123 100163 1 00003 inkinediake cost No co38

> 38* 28 123 * CD38

| | 50 | 450 | 50 | | | | | | | | | |
|--|---------|------|---------------|----------------|-------------|-------------|---------|---|--------------------|---|-----------|--------------|
| Referitiype crimme Riuva95 Aribon Riuva95 Riuva95 Riuva95 Riuva95 Riuva95 Riuva95 Riuva96 Riuva96 Riuva96 Riuva96 Riuva96 Riuva96 Riuva97 CO19 Riuva97 Riuva9 | | | | | | | | PBS | | | | |
| | | 59.4 | 6.6 | | | | | Antibody Total | | ATROLS !!! | AINED COL | TSNU |
| | RESIDEN | 4.5 | 0.5 | | | | | True Stain FcX | | | | |
| | | 9 | 1.0 | | | (НІТ2) | CD38 | APC/Fire 810 | | | | 2 |
| | _ | | | | | | 14907+ | | | | | |
| Filier (u) Refertivpe criname Fluorochrome Marker Cone | | | | <1:2500> | | | L/D | Zombie NIR | | 1 | | |
| Filiter (u)) Refert type cut name | | 9 | 1.0 | | | (5C3) | CD40 | APC-R700 | | Spin N | | 18 R |
| # Filter (u) Referringe Chrimme Buvass (D18 (57) Vallott Bulfrom 2006 Pluyas (57) Vallott Bulfrom 2 | | | | | | , , , , | | abata integral | | | | R |
| # Filter (u) Ref ctrl type ctrl manue (1) Rivagas (2018 (5.7) (2.00) (2. | | | | | | (GHI/61) | CD163 | Alexa Fluor 647 | | F 6 | 2 | 17 R. |
| # Filter (u) Refctritype ctriname Fluorochrome Marker Clone Vial Lot # 30//2nd Moder Moder | | | | | | (REASUS) | TION-DA | T ATC | | Tell | | R |
| # Filter (u)) Ref ctrit type ctri name (budder) 200C (RT) for (V2) (V2) (V2) (V2) (V2) (V2) (V2) (V2) | | 18 | 2 | | | (/GS) | HI V-DB | PE-Vio770 | | | .3 | 16 B1 |
| # Filter (u)) Ref ctritype ctriname Fluorochrome Warker Cone Vial Lot 300/301 300nin 1 UV2 88UV395 CD18 (6.7) 606 300nin 1 UV2 88UV395 CD18 (6.7) 606 300nin 1 UV10 88UV395 CCR2 (1332.1D9) 606 20.65 20.45 | | | , | | | (707) | CD172 | PerCP-Cy5.5 | | B - 3 | .0 | 15 B: |
| Filter (u) Ref ctrl type ctrl name Buv395 CD18 (6.7) Clone Vial Lot # 30/FM7 30min Diversity 30min Diversity 30min Diversity 30min Diversity | _ | 4.5 | 0.5 | | | (112.2) | CD00 | | | | 9 | В |
| # Filter (ul) Ref ctritype ctriname | | |) | | | (IT7 2) | CD86 | PE-Cy5 | | Ťr. | 00 | 14 B |
| # Filter (u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c(RF) for UV2 BUV395 CD18 (6.7) Uv3 UV4 BUV395 CC182 (5.7) Uv3 UV4 BUV395 CCR2 (5.132.1D9) Uv4 UV4 BUV563 CCR2 (5.132.1D9) Uv4 UV4 BUV395 CC19 UV4 UV4 UV4 BUV395 CC19 UV4 UV4 UV4 BUV395 CC19 UV4 UV4 | | | | - - | | (00) 1 | CD3 | PE-Dazzle594 | | | 6 | 1 |
| # Filter (u) Refetritype ctriname Fluorochrome Marker Clone VialLot# 330c(RF) for UV2 BUV395 CD18 (6.7) VialLot# 341/780 20/780 CD19 VialLot# 341/780 20/780 CD19 VialLot# 341/780 20/880 CCR2 (15132.1D9) 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6 | | | | | | (\$5/1) | CD88 | ₽E | | | 4 | |
| # Filter (u) Ref ctri type ctri name (buvas) (bocker) (buvas) | | | | | | | | Spark blue 550 | | 1.01 | 1 | |
| # Filter (u) Ref ctri type ctri name | | | | | | | CD11b | FITC | | | 52 | |
| Filter (u) Ref ctrl type Ctrl name Buv395 CD18 (E7) Clone Vial Lot # 30c/(RT) For CD18 (E7) CD18 (E7) CD19 C | | | | - - | | | | Strangidal Carios | | | 31 | 6 |
| Filter (u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # Marker UV2 Marker Clone Vial Lot # Vial Lot # Vial Lot # | | ### | <c.t.></c.t.> | | | (207) | CD141 | BV785 | | | 15 | 26 V |
| # Filter (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 370C (RT) for 1 UV2 BUV395 CD18 (6.7) O.6 O | | | 45 | | | (207) | CCR | 08478 | | | 14 | 1 |
| # Filter (u) Ref ctrl type ctrl name | | | | - | | (= 1)=) | | , BV711 | | Y | 13 | 9 |
| Filter (ui) Ref ctrl type Ctrl name Buva95 CD18 (6.7) Clone Vial Lot # 370 C(RT) for UV2 UV3 CD18 (6.7) CD18 (6.7) CD18 (6.7) CD18 CD18 CD18 CD18 CD19 C | | | | + | | (B-lv6) | CD11c | BV650 | | | 11 | 1 |
| Filter (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 370c (RT) for UV2 UV2 BUV395 CD18 (6.7) Uofe 30min | | | | 1 | | (5 1H11) | CD56 | BV605 | | 87 | 10 | |
| Filter (ui) Ref ctrl type Ctrl name Buva95 CD18 (6.7) Clone Vial Lot # 370 C(RT) for UV2 UV3 CD18 (6.7) CD18 (6.7) CD18 (6.7) CD18 CD18 CD18 CD18 CD19 C | | - | | - - | | (200) | | I learned bythe company | | | /10 | |
| Filter (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c/RT) for 37cC (RT) for 20min UV6 AF350 CD18 (6.7) 0.6 30min 30min 0.6 UV7 BUV496 CCR2 (15132,1D9) 0.6 0.6 UV10 BUV563 CCR2 (15132,1D9) 0.6 0.6 UV11 BUV661 CX3CR1 (2A9-1) 0.6 1 UV16 BUV737 CD19 CD14 (M5E2) 0.6 V1 BUV805 CD14 (M5E2) 0.6 1 V2 BV421 FcER1a (M5E2) 0.6 0.6 V3 Pacific Blue CD5 (H111) 0.6 0.6 V3 BV480 CD11a (H1111) 0.6 0.6 | ١, | - | 0 | -+ | | (368) | CD16 | BV570 | | la a | 8 | 6 |
| Filter (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c/RT) for 37cC (RT) for 20min UV6 AR350 CD18 (6.7) 0.6 UV7 BUV496 (6.7) 0.6 UV9 BUV496 CCR2 (15132,1D9) 0.6 UV11 BUV563 CCR2 (15132,1D9) 0.6 UV11 BUV661 CX3CR1 (2A9-1) 1 UV16 BUV395 CD14 (M5E2) 1 V1 BUV395 CD14 (M5E2) 0 V2 BV421 FcER1a (M5E2) 0 V3 Pacific Blue CD5 (H1111) 0 | 1 | | | _ | | (111111) | | BV510 (dim) | * | ile. | V7 | |
| Filter (ul) Ref ctrl type Ctrl name Fluorochrome Marker Clone Vial Lot # 370C (RT) for UV2 BUV395 CD18 (6.7) CD18 (6.7) CD18 CD18 CD18 CD19 | | | | | | (HI111) | CD11a | | # | de. | V5 | 1 |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30cKer 37oC (RT) for BUV395 CD18 (6.7) | | - | | - | | | CD5 | Pacific Blue | | | V3 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c (RT) for 80min 80V395 CD18 (6.7) S0min 80V395 CD18 (6.7) S0min 80V665 CD18 (6.7) S0min 90min | | | | | | | | Finds of the control | | | V2 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c/cRT) for 80min BUV395 CD18 (6.7) SOC/RT) for 30min BUV496 CD18 (6.7) SOC/RT (6 | | | | | | | FCEKIA | TZ+AC | | | V2 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30cKer 37oC (RT) for BUV395 CD18 (6.7) | | | | | | (M5E2) | CD14 | BULLAND | | | V1 | 23 |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c/cRT) for 80v395 CD18 (6.7) Sulfran 30min 80v395 CD18 (6.7) Sulfran 30min 90 Sulfran 30min | 1 | | | | | | Cros | RHV80E | | | JV16 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30c(RT) for 80v395 CD18 (6.7) 30min BUV395 CD18 (6.7) 0.6 AF350 BUV496 CCR2 ((s132.1D9) < 0.6> | 9 | | 1 | - | | (2A9-1) | CD10 | RIIV737 | | | U14 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30/C/RT) for 80/C/RT) for 80/C/RT) for 80/C/RT) for 80/C/RT) for 80/C/RT) for 90/C/RT) for 90/C | | | | | | 10.00 | CVOCA | BUV661 | | - | JV11 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30/C/RT) for 80/495 CD18 (6.7) BUV395 CD18 (6.7) 0.6 80/496 BUV563 CD2 (6.7) BUV563 | # | | <0.6> | | | (18132.109) | CUNZ | 3.19.30 DE | | | JV10 | _ |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 3ul/rxn 30min BUV395 CD18 (6.7) 30/rxn 30min AF350 BUV496 | | | | | | | 2000 | BUV563 | 1 | | UV9 | |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 3ul/rxn 30min BUV395 CD18 (6.7) 467 | 1 | | | - - | | | | BUV496 | | | UV7 | 1 |
| (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 3ul/rxn 30min BUV395 CD18 (6.7) | 15 | | 0.0 | | | | | AF350 | | | 9AA | |
| (u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Int # 3,1/200 200-1 | | | 0.6 | - July IAII | | (6.7) | CD18 | BUV395 | 1 | 100 | UV2 | 1 |
| Juligie Color Unmixing | 9 | | 37oC (RT | blocker | Vial I of # | Clone | Marker | Fluorochrome | tri type ctri name | | | # |

Simplified Protocol

Aliquot cells 3E+6 cells/tube

Wash with 2ml PBS, 1300rpm, 8min
2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT]
Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

x ul of 37oC Ab mix, at 37oC for 30min Remove RBC with 1ml lysing solution for 3min

Wash with 2ml 2%FBS-PBS-2mM EDTA Spin at 1400rpm for 6min

x ul of 4oC Ab mix, at 4oC for 30min

Treat with 600ul of 1x Lysing solution at R.

Treat with 600ul of 1x Lysing solution at RT for 3min

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

Spin at 1400rpm for 6min Resuspend in 0.4%PFA-PBS

No ccas sinds color + backbans

1895 WAF has

5000 cells for int Mon/Ne Mon

HLA-DR/CDIES (CDC) bookspill on FEERIL)

Reproducing had clustering on 2D

C×3CRI × CDI4 > 163

- cNon are 14t × 163 + (addballs fallout)

got 8a

- oddballs coiza us 38

that leading edge that matters

I spit out enough to NE analysis for a paper for breadlfast?

Monocytes SFC Panel

| L | | | 760 | 1 3 | 738 | | | | | | | | | 525 | | 508 | 4/3 | 458 | 443 | 428 | 388 | 373 | Spectrum |
|-----------------|---------------|---------------|--|--------------------|----------|----------------|-----------------|-------------|--------------|------------|-------------------|------------------|--------------|-----|---------------|-----------------|-----------|------------|-----|------------|-------------------|-----|----------|
| | UV16 BUV805 | UV15 | Control of the latest southern | UV14 BUV737 | | UV13 | UV12 | | | N40 BIN615 | COCACC | | N/8 | | UV7 BUV496 | | UV6 AF350 | UV5 | UV4 | UV3 | UV2 BUV395 | UV1 | V |
| 0011 | m CD14 | | STATE OF THE PARTY | [3] CD19 | 5 | 1 | ed | O CASCRI | | 3 | DI CCRA | | | | [2] <af></af> | | | | | .1 | [2] CD18 | | |
| 0 | | BV79F | | V14 BV750 | 0 < 7 | | | V11 BV650 | | | V8 BV570 | 01.9AB | | Vie | | V5 BV480 | V4 | v3 PacBlue | V2 | V1 BV421 | | | Violet |
| | [J] | | STORES. | 7.51 CCP5 | [4] | | | [3.5] CD11c | [3] CD56 | 2_ | CD16 _₹ | [1.5] <af></af> | a name and a | | - | [3] CD11a | | [1] CD5 | | [4] FcER1a | | | |
| B14 | B13 Pe-VIO//0 | 7 | | | B10 | Be PerCP-Cy5.5 | | B7 | B6 PE-Dazzle | B5 | B4 PE | B3 SparkBlue 550 | BZ FIC | | | <u></u> | | | | | | | Blue |
| | [3] HLA-DR | | | | | [2] CD123 | [4.5] CD86 | | [4] CD3 | | [4] CD88 | Ξ | [1.5] CD11b | | | | | | | | | | |
| R8 APC-Fire 810 | APC-Fire 750 | R6 Zombie NIR | | R5 | APC-R700 | R3 | R2 AF647 | R1 APC | | | | | | | | | | | | | | Ven | Dod |
| [3] CD38 | [2] CD64 | [] L/D | | | [3] CD40 | | [3.5] CD163 | [3.5] | | | | | | | | | | | | | | | |

10/14/2022

The two addballs

C 3CR |

28

183

Man 7 c D18 Mt Cx3 c R1
141 186 144-0R 1113 138

oddbil Hen/DSZZ OCS PERMON POCS Tools

DN -> COB, CX3CRI, COHO, CO38,

Acquisition: Unstrind malia 4500 cells levert rote NYOGZ sample loves to be in good health. Sample -> 6000/events rate (the 2 media to bes had less volume) Sample is 9000 events rak 1600 earls for sample / 8300/

NYOGE COR just hickord & duch is my Prosoce. 8500 CCR2

1/ boughtness across samples

AT PacHAP ~ IM~ /12:47 pm/= / 1:20 pm/= 13M 2/ 1:24pm/ 3/ - / 5 fontohod between 3:05 2 4:05pm

BW737 words for CO19 / (easily clears the SSC monogle borrier)

BUV 137 co19 + bashbore =

CO18 negative bleed colle 141

~ CK 30742 colly minorum

N FCERIA

~ 16 1 blowbook * 56

No A between meden & the well for coss

Create a Folder and place all experimental setup Ales withnit

you can eventually export final expressed and de init

tag cleared up 56 split except colla

CD19 worlds well in its new location v

FEERLA moreit.

| Classical | |
|---|-------------------|
| Monogles + oddbolls = col4 00 | |
| FSC-4 | |
| Oddballs accupy Oddballs little bit to ver cois P % CX3CRI - no coit | |
| Des Des Dead Morogres -> interned of Fe | ERIA |
| not all odd balls are dying, Duerlap or healthy Des location, | |
| (this also explains the Mon low AF, its the DCS locate | (00) |
| CN33T us oddballs us FEERLA DCs The only difference in add by The only the sold by On the sold side | ells is kes coas |
| colds on the sold on the side of the dead side | |
| Man I but only bordy | |
| FUCDI41 1 163 is higher cho | |
| 1,88 | |
| 1 that or all the sold of the | |
| 7 CD163 | |
| noticing a tiend here. | |
| | .11.4 |
| Roming a Packap V/ FSC & SSC-A on that monogyk Flush | x 11411celis |
| The CDIH oddballs occupy non-monogle space | |
| Both lich works A mon paps 17 56 7 163 | |
| are to coo | |
| 14 TERM ODES ICHO TIESCA FERNAL | |
| A CD14 - CD88+ POP = No CD38, No CD163, VHLA-DR, TO | 20123 Nalla (200) |
| Do/Low CD141, No CD14, equi CD88 T Cx3CR1 | 3, J co 18 |
| 1 More Charge Hand a not | |
| n more cuscel than a coc1; I 16,56, 7 colle Jeol41 188 | J HLA-DR (|
| Vs coll can no colly | |
| 14-md 4-88 1 C+3CRI 1141 188 | |

| Wonocyte Sr C paner |
|---|
| A lot of the colle- ED123- cells are dying manage gate |
| No discernable +1- to coss or FERIA, |
| COSS or FERIA, |
| FCERIA CO14 * 163 |
| |
| Files double in size once you unaix in Spectra Flo" |
| |
| Backbone + cd19 -> FeERSa on BU421 such spread |
| A badisper enonexing from colla |
| After CD3, basephil removal, Nil, B cell only 3.43 oddballs on gale |
| a consider |
| Markers enrolled in olying |
| 88 Madle |
| 26+ > (1c- markers exclosive to alive |
| radius del 163 -> 1 |
| errally 38-f = 1/ |
| |
| Needa gentl cleanop ahead of basophl, B-A |
| FSC-A SSC-B-A SSC-B-A B-W Inster |
| |
| * \$550-t SSC-W SSC-B-V > oddballs on the side doddds |
| B-A B-A |
| |
| FSC-A * SSC-W -> delans clad |
| SSC-A * SSC-H > a SSC-singlet removes lover edge |
| SSC-A * SSC-H)-> a SSC-singlet removes lover edge * SSC-W)-> Sample had a flatration |
| Peacola catalogo della - |
| Peacoac catches addballs. First FSC-A/WH cleans -> edge |
| FSC-A shows delons, between uncertain LiveDead at |
| |

Debris field is HLA-DR- RBCs R platelets, a afilanele? à most cells? BSSC-H VSSC-H Granulo ytes 3rd ware ? Basophils by Apertider > FEERLA + 141-3, CKBCRI -123 + steth 110-88+ Sheloh HLA-DR renoval Packap 1 -> FEERla Closter 2 -> 182 regative 4 -7 0016/56 4->110 141 -88 FEERIA, CD88, CD123, CD38+ NIN's bit higher see than TB cells FEERIA+ HLA-DR- COB+ 88+ 123+ 38+ any 8 dot deferent 18- for Par HOT 38: Basophils? CDC1s PDC5? 18= These are indeed bughers Brs L, M, 14 / 55 000 7 cols 464 7 FEERIN) ~ CD88) 1 123 high Within the CP3+ gate -> 22,8%, pop 个0038 Subsets small differences but not distinguishable Dying broughis J18, Fee Ria, 20088 6 00123 CDIL a remark. HLA-DR+ 19-1 38 less HLA-DR 1255 0040 19+ comordes exactly up the 40+ 550 low pap oddball HLA-DR+ 38+ (200 cd/s) (probably a precursor pop)