New

## Tetramer Titrations and Monocytes

|          | Specimen | Status        | Location | C .    | 15       |                     |        | 1:100 |  |       |        | •            |
|----------|----------|---------------|----------|--------|----------|---------------------|--------|-------|--|-------|--------|--------------|
| Less.    |          |               | Location | Conc   | Date     | Tasks               | Volume | Ly    | Ly+Mon   | Total | 3E+6   | 2E+6         |
| ud Spl.  | ND006    | 500pl<br>rest |          | 7.5 E6 | 11-14-22 | 1 clumpy<br>1 ccelb | 4      | 3.11  | 4.20   | 3.11  |        | <u>≱E</u> +0 |
| my godf  | NDOOG    | 1m(           |          | 10E6   | 11-14-23 | Sal.                | 2      | 11.10 | and the same of th |       | 0.964  |              |
| ofCh,    | A . 2    |               |          |        |          | @-86'e              | 2      | 4.14  | 5.46   | 8.28  | 0.724  |              |
|          | NOOCE    | Im            |          | IDES   | 11-14-22 |                     | ) 2    | 5,23  | 6.50   | 10.46 | 0.573  |              |
| मिया पार | NDacq    | Ini           |          | late   | 11 (1.0) |                     | 7      |       |  | 70.76 | 0.4 (3 |              |
|          |          |               |          | IOEG   | 11-14-22 |                     | 2      | 4.83  | 6.17   | 9.66  | 0.621  |              |
|          |          |               |          | (6)    |          | T                   |        |       | er a   |       |        |              |
| L        |          |               |          |        |          |                     |        |       | 1  | 31.51 |        |              |
|          | -to      |               |          |        |          |                     |        |       |  | ·Gn   | 10/    |              |

A check 500pl for viability subsequently 2:16pm stain cont

Abs prepede 4:16 pm

No Mis stim condition this go around 4:53 pm 7 cell goil 101 LO -> 22 5:13 Hot sets 7 43

Spin @ 5:26 pm

Cold Scis @ 5.36pm -76:06pm (

37°C Samples @ 5:44, 39°C sts out. 7/6, 14 pm

1. 1

Tive RBClyse -> 5/52

AM I forgot to Block w LD AN

06:00 pm 372 + 3pl to block (temontes (ak!)
re-added to the inches or 3/6:20/pm) 4° Scis 06:00-06:00 Rbc 480 -> 6:10pm 27° & PFAcd-7 ficts. ] Spire 6:13pm

[37 % & PFAcd-> ficty.]

samples spine 6:24 pm

[ 50's done @ 6:29 pm /

6:41 Se's 42 in -> 7:11pm

Done @ 7:38 pm ( bichazerd & careage tips also to actodare

CO64 CD123 CDGG FERCH 8 6 5 5

+ SCS + unstained

23+3= 26 scis

7 8 M 21.0 M

28.8 Million Cells

SOX4=200/ 1 EAA

| Marker   Clone   Vial Lot #   Bocker   370c (RT) for   A   A   A   A   A   A   A   A   A   | 100       |             |      | 2000          |         |            |             |           | Pippette draw volume/sample | Pippe | 111     |           |         |       |
|--|-----------|-------------|------|---------------|---------|------------|-------------|-----------|-----------------------------|-------|---------|-----------|---------|-------|
| # Eller (ull) Controller (ull) Controlle | 400       | 50          | 400  | 50            |         |            |             |           |                             | 2     | OLS III | D CONTRC  | NSTAINE | And U |
| # Biller (b) Department (b) Unniving (b) Department | 85.6      | 10.7        | 36.8 |               |         |            |             |           | Brilliant Stain             |       |         |           |         |       |
| # Either (III) Obor Unmining Uniformating Enurochrome (III)  | 4         | 0.5         |      |               |         | -          |             |           | Antibody Total              |       |         |           |         |       |
| Filler   Charles   Control   Contr   |           |             | 4    | 0.5           |         |            |             |           | True Stain FcX              |       | nreo    | 0 3 0 3 0 |         |       |
| # filter   |           |             | ∞    | 1.0           |         | -          | (HIT2)      | CD38      | oro chine oro               |       | 5       | : -       | 20      | j.    |
| Brillet   Color   Co   | #VALUE! « | <1.2>       |      |               | _       |            | CD64        | CDIZ3     | APC/Fire 810                |       | Cells   | 5         | R8      | 18    |
| ## Filter   Cult   Dumbing   Fluorochrome   Marker   Clone   Val Cat   Ducker   300C (R3) for   8   48   48   48   48   48   48   48   |           |             |      |               | 1:2500> | _          |             | 1/0       | APC/Fire 750                |       | 200     | 17        | R7      | 22    |
| # Filter (ul) Referrit type crit name   Buyass   Cobe   Vali (crit   Marker   Cobe   Marker    |           |             | 8    | 1.0           |         |            | (503)       | CD40      | Zomhie NIR                  |       | (EII    | 200       | R6      | 17    |
| # Filter   Unmixing   Endorothrome   Marker   Clone   Vial Lot #   Morker   370C (RF) for 8   Act for 30min   Marker   Clone   Vial Lot #   Morker   370C (RF) for 8   Act for 30min   Marker   Clone   Vial Lot #   Morker   370C (RF) for 8   Act for 30min   Marker   Clone   Vial Lot #   Morker   370C (RF) for 8   Act for 30min   Marker   Clone   Vial Lot #   Morker   370C (RF) for 8   Act for 30min   Marker   Clone   Vial Lot #   Morker   370C (RF) for 8   Act for 30min   Marker   Clone   Vial Lot #   Morker   Marker   Clone   Clone   Marker   Cl | 8         | 1.0         |      |               | -       |            | (AUI/OT)    |           | APC-R700                    |       | Cells   | 03        | R4      | 16    |
| # Filter (u)) Refethlype ethname   Fluorechrome   Marker   Clone   Vial Lot #   Moker   330c (Rit) to   8   46 for 30min   1   1   1   1   1   2   4   40   40   40   40   40   40   |           |             |      |               | -       | <u>.</u>   | CHICA       |           | Alexa Fluor 647             |       | (ell)   | 10        | R2      | 15    |
| # Filter (u) Referritype citriname   Fluorochrome   Marker   Cione   Vialicit   Cione   C | 2.4       | 0.3         |      |               | +       |            |             |           | APC                         |       | Cells   |           | 2       |       |
| # Filter (u) Refettitype ctriname Fluorochrome Narker Clone Vialicit # 300c/RFI for 8  |           |             |      |               |         |            | (REA805)    |           | PE-Vio770                   |       | SHIS    | 02.1      | D1      |       |
| # Filter (u) Refettitype ctriname Fluorochrome Narker (clone Vialicit # 300cker 370C (RT) for UVS (). () Refettitype ctriname Bluy395 (D13 (6.7))   Diocker 370C (RT) for UVS (0.6)   A.8   A.5   A.5  |           |             |      | \$            |         | _          |             | CD123     | PerCP-Cy5.5                 |       | 1       | 200       | B13     | 14    |
| Filter   | 4.8       | 0.0         | 4    | 0.5           |         |            | (IT2.2)     | CD86      | PE-Cy5                      |       |         | 0         | B10     |       |
| Filter   Color   Continame   Fluorochrome   Marker   Colone   Via Lot #   30C (RT) for   8   40C for 30min   1 UV2   C. C.   6   1   | 10        | 0.6         |      |               |         |            |             | CD3       | PE-Dazzie594                |       | SHED    | C         | B8      | 13    |
| Filter   Color   Continuing     | 3.7       | 0.4         |      |               |         |            | (\$5/1)     | CD88      | DE-Davidoroa                |       | Cells   | 0         |         | 12    |
| Filter   Cumining    |           |             |      |               | -       |            |             |           | DE .                        |       | Celts   | 0.4       |         | 11    |
| # Filter (u) Referritype ctri name   Filter (u) Referritype   Filter (u) Referritype ctri name   Filter (u) Referritype   Filter (u) Referrit | 3.2       | 0.4         |      |               | ļ<br>-  |            |             |           | Spark blue 550              |       | (ells   |           | 1       | T     |
| Filter   Cum   Name   Filter   Cione   Vial Lot #   Blocker   Strolle color   Cione    | 9.6       | 1.2         |      |               | _       |            |             | CD11h     | 圆                           |       | (ells   | 0,0       |         | į     |
| Filter   Cum   Name   Fluorochrome   Marker   Clone   Vial Lot #   Blucker   370C (RT) for 30min   Marker   Clone   Vial Lot #   Blucker   370C (RT) for 30min   Marker   Clone   Vial Lot #   Blucker   370C (RT) for 30min   Marker   Clone   Vial Lot #   Blucker   370C (RT) for 30min   Marker   Clone   Vial Lot #   Marker   Clone      |           |             |      |               | .       |            |             | CD141     | BV785                       |       | Siles & | -         |         | 10    |
| # Filter (u) Ref ctri type ctri name   Fluorochrome   Marker   Clone   Vial Lot ##   Molectri   Mol | #VALUE!   | 71.47       |      |               | -+      |            |             |           | BV750                       |       | *       | 3         | V15     | 9     |
| Filter   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Unmixing   Bluv395   CD18   (6.7)   Undixer   370C (RT) for 8   Marker   Clone   Vial Lot #   Single color   CD18      | o         | 61.35       |      |               | _       |            |             | FcER1a    | 87/11                       |       |         | 20        | V14     | 1     |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   8   4co (200min   Vial Lot #   3u// Price   3co (RT) for   3c   | 0 0       | 1.0         |      |               |         |            | (B-ly6)     | CD11c     | BVb5U                       |       | 7 8     | 7         | - 4     | 21    |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot   Biocker   30c/RT   for   Mocker   4.8   for   Mocker   | ×         | 1.0         |      |               |         |            | (5.1H11)    | CD56      | DYCEO                       |       | 2 Cells | 11        |         | 00    |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Blocker   370 (RT) for   8   40 (for 30min   10 th      | 12        | 1.5         |      |               | _       |            | (3G8)       | CD16      | RVIENE                      |       | Cells   | 1.0       |         | 7     |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Bluva95   Marker   Clone   Vial Lot #   Marker   Clone   Close   Marker   Clone   Vial Lot #   Marker   Clone   Close   Close   Clone   Close   Close   Clone   Close   C   | #VALUE!   | <1.2?       |      |               | ļ.      |            |             | CD04      | BV570                       |       | OH OH   | 125       |         | 6     |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Blocker   370C (RT) for   Blocker   310/frxn   30min   40C for 30min    |           |             |      |               | -       |            |             | CBCA      | BV510                       |       | 7 Cells | 1.5       |         | 12    |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Unmixing   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   Unmixing   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   Single color   Union   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color     | #VALUE    | <150        |      |               |         |            |             |           | BV480                       |       | Cells   | 1.25      |         | : [   |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Unmixing   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   Unmixing   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   Unit   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   Single color   Unit   Single color   Child   Single color   Clone   Vial Lot #   Single color   Single co   |           | <1>         |      |               | Į       |            |             | CD66h     | Pacific Blue                |       | N A     | 7.7       |         | T     |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Unmixing   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   UV7   Medicine   Single color   Clone   Vial Lot #   Single color   Single color   Single color   Uv7   Single color   Single color   Clone   Vial Lot #   Single color   Single color   Single color   Uv7   Single color   Single color   Clone   Vial Lot #   Single color   Uv7   Single color   Single   | 00        | 1.0         |      |               | ]-      |            | (INICIA)    | FcER1a    | BV421                       |       | 0 0     |           |         |       |
| Single color   Unmixing   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Unmixing   Ref ctrl type   ctrl name   Fluorochrome   Marker   Clone   Vial Lot #   Single color   Single color   U/D +FC   Single color   U/D +FC   Single color   U/D +FC   Single color     | 9.6       | 1.2         |      |               |         |            | (Casha)     | CD14      | BUV805                      |       | O Cells | 4         |         | Τ.,   |
| Single color  Unmixing  Filter  (u)  Ref ctrl type  ctrl name  BUV395  DV6  AF  DV7  BUV496  UV7  BUV496  UV9  UV10  BUV661  CX3CR1  C |           |             | 0    |               |         |            |             | CD19      | BUV737                      |       |         | .1        |         | Τ.    |
| Single color Unmixing Filter (u) Ref ctrl type ctrl name BUV395 CD18 (6.7)  UV6 AF AF-UV6  UV7 BUV496  UV9 C C BUV496  UV10 C C C C C CRT type CTrl name BUV496  UV9 C C C C CRT type CTrl name BUV496  UV9 C CRT CONE Vial Lot # 3ul/rxm 30min 30min 40c for 30min 4 |           |             | •    | •             |         |            | (2A9-1)     | CX3CR1    | BUV661                      |       | j (ells |           |         | Τ.    |
| Single color Unmixing Filter (u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 3ul/rxn 30min  UV2 O.C. BUV395 CD18 (6.7)  | 4.0       | 0.0         |      |               |         |            |             |           | ST9AAB                      |       |         |           |         | Т     |
| Single color Unmixing Filter (ul) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 30min 4oc for 30min  UV2 OC BU395 CD18 (6.7) 0.6 4.8  UV7 BUV496 BUV496   | 40        | 0.6         |      |               |         |            | (ls132.1D9) | CCR2      | BUV563                      |       | · Const |           |         | Т     |
| Single color   Unmixing   L/D + FC   Single color   Unmixing   U/D + FC   Single color   U/D + FC   U/D + FC   Single color   U/D + FC   U/D + U   |           |             |      |               |         |            |             |           | BUV496                      |       | 1       |           |         | Т     |
| Single color Unmixing  Filter (u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 310/Lyan 30min  UV2 OCC 80 BUV395 CD18 (6.7) 40C for 30min  |           |             |      |               |         |            |             | AF-UV6    | Ar                          |       |         |           | UV7     |       |
| Single color  Unmixing  Filter (u) Ref ctrl type ctrl name Fluorochrome Marker Clone Vial Lot # 3ul/rxn 30min  BIV/395  BIV/395  BIV/395   |           |             | 4.8  | 0.6           |         |            | (6.7)       | CDIS      | VE.                         |       |         |           | 9VU     |       |
| Single color Unmixing  Filter (ul) Refettl type ctrl name Fluorochrome Marker Cl.   Dlocker 37oC (RT) for 8  | 5         | 4oC for 30m |      | 30min         | 3ul/rxn | Vial Lot # | cione       | - Widikel | BUV395                      | - 0   |         |           | 1       | Т     |
|  | 00        |             |      | 37oC (RT) for | blocker |            | 2           | No.       | Fluorochrome                |       |         | 1         | 1       | Т     |
|  |           |             |      |               |         |            |             |           |                             |       | Color C | Single 8  |         |       |

Simplified Protocol
Aliquot cells 3E+6 cells/tube

Wash with 2ml PBS, 1300rpm, 8min

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

Spin at 1300rpm for 8min

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

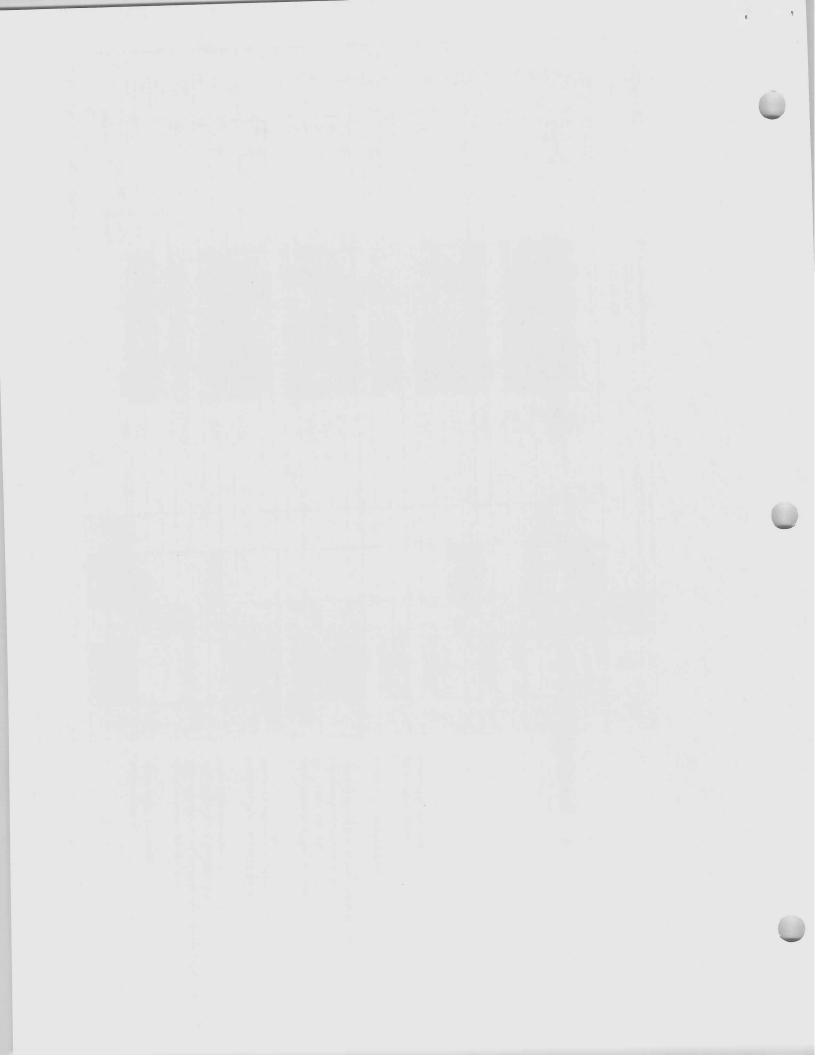
2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT for 15min

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 RT for 3min

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

Spin at 1400rpm for 6min

Resuspend in 0.4%PFA-PBS



Monocytes SFC Panel

|              | 812 UV16     | 783 UV15   | /60   | 750 UV14   | 7,00     |        |                |        | 679         |             |       |    |       |               |         | 525      |           |                 |                |         |            |          |     | 388        |      | Special all |
|--------------|--------------|------------|---|------------|----------|--------|----------------|--------|-------------|-------------|-------|----|-------|---------------|---------|----------|-----------|-----------------|----------------|---------|------------|----------|-----|------------|------|-------------|
|              | 16 BUV805    | 15         |   | 114 BUV737 |          |        | /13            | UV12   |             | UV11 BUV661 |       |    |       | UV9 BUV563    | UV8     |          | BUV496    |                 | A7-046         |         | IIV6       | IIVA :   | UV3 | UV2 BUV395 | UV1  | 0           |
| ŀ            | Ξ            |            |   | [3]        |          |        |                |        |             | [3]         | [3]   |    | 3     | <u></u>       |         |          | [2]       |                 |                |         |            |          |     | [2]        |      |             |
|              | CD14         |            |   | CD194 V14  |          |        |                |        |             | CX3CR1      |       |    | 00176 | CCB3          |         |          | <af></af> |                 | A              | i       |            |          |     | CD18       |      |             |
| -            | < 10         | <15<br>—   |   | V14        |          | V13    | 7 1 2          | 3      |             | V11         | V10   | 8  | 8     | <             | 1       | <b>6</b> |           | <b>V</b> 5      | < <sub>4</sub> | -≲      | <b>\</b> 2 | <u> </u> |     |            | 1    |             |
|              |              | RV785      |   | BV750      |          | BV711  | Į.             |        |             | BV650       | BV605 |    | BV5/0 | DVSTO         | DIVERO  |          |           | BV480           |                | PacBlue |            | BV421    |     |            |      | Violet      |
|              | 3            |            |   | [2.5]      |          | [4]    |                |        | [6:6]       | 3 5         | ယ     |    |       | [7.5]         |         |          |           | <u></u> <u></u> |                | Ξ       |            | 143      |     |            | t    |             |
|              |              | 4          | SANCE SERVICE |            |          | FcER1a | ∌              |        |             | CD116       | CD56  |    | CD16  | CD64          |         |          |           |                 |                | CD66b   |            | FcER1a   |     |            |      |             |
| B14          | B13          | B12        |   | _          | D        | B10    | B9             | B8     | 87          | 2 0         | D     | B5 | В4    | ВЗ            | 20      | 3        |           | B.1             | -              | 1       | (          | R        |     |            | †    |             |
|              | Pe-Vio770    |            |   |            |          |        | PerCP-Cy5.5    | PE-Cy5 | )<br>1<br>) | re-Dazzie   |       |    | PE    | SparkBlue 550 | ור<br>כ | OFIG     |           |                 |                |         |            |          |     |            | Biue | ol          |
|              | <u> </u>     |            |   |            |          |        | [2]            | [4.5]  |             | [4          | }     |    | 4     | Ξ             | [1.5]   |          | _         |                 |                |         |            |          |     |            |      |             |
|              | HLA-DR       |            |   |            |          |        | CD123.△        | CD86   |             | CD3         | )     |    | CD88  |               | CD11b   |          |           |                 |                |         |            |          |     |            |      |             |
| D<br>XX      | R7           | R6         |   | R5         | 4        | ]      | R <sub>3</sub> | R2     | R1          |             |       |    |       |               |         |          |           |                 |                |         |            |          |     |            | _    |             |
| APC-Fire 810 | APC-Fire 750 | Zombie NIR |   |            | AFC-K/OO | 700    |                | AF647  | APC         |             |       |    |       |               |         |          |           |                 |                |         |            |          |     |            | Red  |             |
| 51           | [2]          | =          |   |            | 3        |        |                | [3.5]  | [3.5]       |             |       |    |       |               |         |          |           |                 |                |         |            |          |     |            |      | İ           |
| CD38         | CD123/64     | L/D        |   |            | CD40     |        |                | CD163  |             |             |       |    |       |               |         |          |           |                 |                |         |            |          |     |            |      |             |

to extra se's

November 17th, 2022

Tetramer Titrations and Monocytes

10:30 sc agrestous
1200 entrisec for 50,000 40ses
C0123 @ 200,000 events.
FERRA #

Ab amont by volume or?

by cell? bitliothy

TUN Scis

141 gradient 163) > added volume, collecting to the last drop

Done @ 12:30 pm

Including doublet AT uniquess

Unmixing's COHO needs instanced supped to bym

affer 1st A swapped out APC-Fic 750 for co64

Perchages 123 > THE PBMCa Clibery

AF correction?

3 signatures in monocytes

green 2 red elep in V, not Ws

Grange (18311)

(2741)

CD666 commiss + (crey dom marker)

(carect Ludead -> Vimbility

(Lost screenshots (deshit save)
but swapping to library controls
1.5 Me pand)

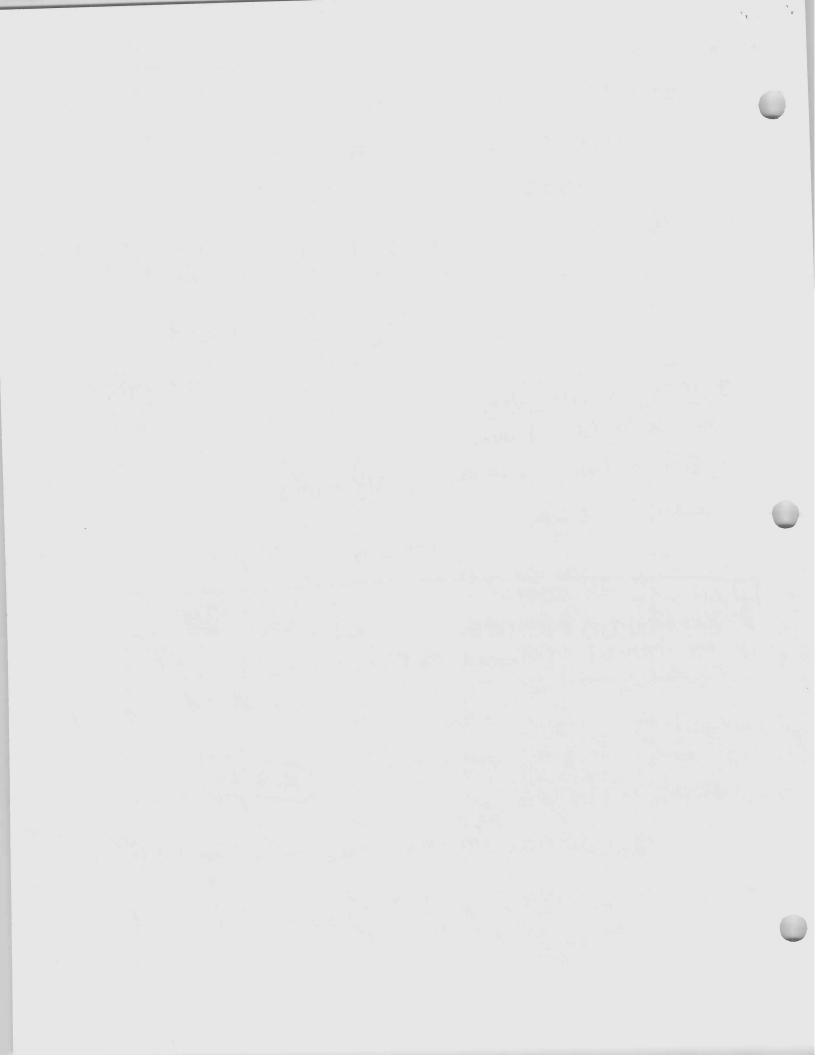
Calls 6664/ PESULOF not
Fe Blodling To
Some COB correct To
- core to
Some COB CX3CRI

Teme COK
32me COK
32me COHO



## Tetramer Titrations and Monocytes

|          | 123 Juffers more spread                     | ling on APC Fire 750 Subjected to spreading       |
|----------|---|---|
|          | 123+ 1 in-vitro                             | (4%) for the CD110+ 867                           |
|          |   | Julier more stretched                             |
|          | Pdeb 180 low 1                              | CD123 on APC FIC 750 > Per CP-Cy5. 5 (despike nog |
|          | ¿ 16 mas brighter mold?                     | My Laveal spread upon outwater                    |
|          |   | FER12 V   |
|          |   | Improved COGH marginelly improved                 |
|          | 3 AFS for Monogles.                         | novergenent) > a quivalent                        |
|          | Main ~ Violet, V UVs.                       | but now al<br>mercy At-mency                      |
|          | Eide ~ 1 Ws fad 64 Us                       |   |
|          | dead/dying? I both.                         | · V3 * UV3  |
|          |   | UV5 + V7 (UV3 + VIO)                              |
| of L     | IAPC-Fa750 CD64                             |   |
| 0/       | HH - SUT(4) CD123 PEREP-Cy5.5               | Went loid   |
| 0 0      | ) DTR PBNC6 - CD19-2020                     | Pac Blue Ono af 000 000                           |
|          | · Trimbles                                  | Oyes af 0 0 0                                     |
| dy f     | ys are not "pure" per                       |   |
| <i>y</i> | No AF UN3 tag                               | [88 * 64]   |
|          | الكر  |   |
|          | doesn't look like                           | This is the tag we are looking for,               |
|          | So FEERJa or<br>CD123 PercRCy5.5<br>CD64 on | Detter I see what happens in combination          |
|          |   |   |



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