

Appendices

A.1 MIFlowCyt

1 Overview

- 1.1 **Purpose:** Identify the markers expressed by THP-1 monocytes and macrophages.
- 1.2 **Keywords:** Macrophages Monocytes
- 1.3 **Experimental Variables:** We assessed the expression of markers on untreated THP-1 cell line monocytes, THP-1 cells differentiated with PMA to macrophages and THP-1 PMA macrophages with phenotypes altered with LPS, LPS and IFN- γ , IL-4, Immune complex and IL-1 β , IL-10 and hemoglobin:haptoglobin. N=4.
- 1.4 **Organisation:**
 - 1.4.1 **Name:** Immunity, Infection and Inflammation, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen
 - 1.4.2 **Address:** Institute of Medical Sciences, Foresterhill, Aberdeen, UK, AB25 2ZD
- 1.5 **Primary Contact:**
 - 1.5.1 **Name:** Dr Megan Forrester
 - 1.5.2 **Email Address:** m.forrester@abdn.ac.uk
- 1.6 **Date:** Experiments were performed 23/11/16 – 23/12/17; Data were analyzed 1/2017-8/2017.
- 1.7 **Conclusions:** Of the 49 markers tested: THP-1 monocytes express 15 (CD169, CD33, CD170, Siglec 10, CD32, CD64, TIM-1, CD299, CLEC10A, CLEC13A, CD205, SIRP α , SIRP β , CD13, HLA-DR), THP-1 macrophages express 28 (CD169, CD33, CD170, CD329, Siglec 10, CD35, CD55, CD59, CD36, CD163, CD204, CD32, CD64, TIM-1, TIM-3, CLEC4A, CD299, CLEC10A, CLEC13A, CD205, KLRG1, SIRP α , SIRP β , CD13, CD11b, CD11c, CD14, Mertk). Of those 28 markers, 12 markers change their expression with phenotype altering stimuli (CD169, CD33, CD329, CD36, CD64, TIM-3, CLEC4A, CLEC10A, CD205, CD11b, CD14, Mertk), along with 4 markers which show expression only with phenotype alteration (CD209, CD80, CD86, HLA-DR).
- 1.8 **Quality Control Measures:** Single stain controls by staining 60 ul of Anti-Mouse or Anti-Rat or Anti-REA Ig CompBeads (BD#552843) and 60 ul anti-FBS negative control beads with 5 ul of each antibody used were used for compensation. Non-specific binding controlled for with manufacturer and conjugate matched isotype controls. Unstained samples of monocytes and macrophages were used for each experiment. N = 4.
- 1.9 **Other Relevant Experiment Information**

2 Flow Sample/Specimen Details

- 2.1 **Sample/Specimen Material Description:**
 - 2.1.1 **Biological Samples**
 - 2.1.1.1 **Biological Sample Description:** THP-1 cell line
 - 2.1.1.2 **Biological Sample Source Description:** human peripheral blood
 - 2.1.1.3 **Biological Sample Source Organism Description:** 1 year old human male with acute monocytic leukemia.
 - 2.1.2 **Environmental Samples:** Not Applicable
 - 2.1.3 **Other Samples:** Compensation beads – Mouse (BD Biosciences 552843); Rat (BD Biosciences 552844); REA (Miltenyi Biotec 130-104-693)

2.2 Sample Characteristics: Monocytes and macrophages

2.3 Sample Treatment Description: THP-1 monocytes were kept in culture conditions before staining and analysis (RPMI 1640 Gibco, 20% heat inactivated FCS, penicillin, streptomycin and L-glutamine). THP-1 macrophages produced by PMA 1 ng/mL (Sigma Aldrich P1585) for four days followed by 48 hours without PMA for macrophages or 24 hours before stimulation for polarisation study. They were seeded at 5×10^5 cells/mL in T80cm³ flasks, 5 mL/flask two flasks per condition. To alter phenotype by polarising macrophages they were stimulated for 24 hours with LPS (10 ng/mL; Sigma Aldrich L6529), LPS and IFN- γ (20 ng/mL; Miltenyi Biotec 130-096-872), IL-4 (20 ng/mL; Miltenyi Biotec 130-095-373), IL-1 β (20 ng/mL; Miltenyi Biotec 130-093-895) and immune complex (rabbit anti-OVA 100 μ g/mL bound to OVA 10 μ g/mL, incubated together for 30 min at 37°C; Sigma Aldrich AB1225, A7641), IL-10 (20 ng/mL; Miltenyi Biotec 130-093-948) or hemoglobin:haptoglobin complex (1:1, 100 nM; Sigma Aldrich H0267, H0138).

Monocytes were transferred to 50 mL falcon tubes. Flasks containing macrophages were placed on ice, the contents transferred into 50 mL falcon tubes and replaced with 5 mL ice-cold HBSS, 2 mM EDTA (Sigma Aldrich 03690) and 5% heat inactivated FCS. After 10 minutes on ice the cells were gently scraped then transferred into the 50 mL falcon tube. The two flasks for each condition were combined. Cells were then spun down and Fc block added (Human TruStain FcX; BioLegend 422302) for 10 minutes at room temperature. Cells were then split between mastermixes of antibody panels. The untreated macrophages and monocytes were also added to isotype control mastermixes and kept for unstained controls. After 30 min incubation with antibodies at 4°C in the dark cells were washed with cell staining buffer (BioLegend 420201). Cells were then incubated for 30 min at 4°C in fixation buffer (Cytofix; BD Biosciences 554655). Cells were then washed and resuspended in cell staining buffer and analyzed within 48 hours.

2.4 Fluorescence Reagent(s) Description: Antibodies were split into seven panels to measure cell surface proteins as follows:-

Target (aka)	Fluorochrome	Antibody Clone (Isotype)	Vendor	Cat#	Compensation	Isotype Control Cat#
Dectin1 (CLEC7A, CD369)	VioBright FITC	REA515 (REA)	Miltenyi Biotec	130-107-728 130-107-693	Anti-REA Ig CompBeads	130-104-576
CD69 (CLEC2C)	BV650	FN50 (mIgG1)	BioLegend	310933 310934	Anti-Mouse CompBeads	400164
CD207 (CLEC4K, Langerin)	VioBlue	MB22-9F5 (mIgG1)	Miltenyi Biotec	130-106-147 130-106-096	Anti-Mouse CompBeads	130-094-670
CLEC12A (MICL, CLL-1)	PerCP-Cy5.5	50C1 (mIgG2a)	BioLegend	353611 353612	Anti-Mouse CompBeads	400251
CLEC4A (DCIR, CD367)	PE-Vio770	REA329 (REA)	Miltenyi Biotec	130-105-032 130-104-980	Anti-REA Ig CompBeads	130-104-616
KLRG1 (CLEC15A, MAFA)	PE-Vio615	REA261 (REA)	Miltenyi Biotec	130-108-395 130-108-366	Anti-REA Ig CompBeads	130-107-146
Dectin 2 (CLEC6A)	PE	545943 (mIgG1)	RnD Systems	FAB3114P	Anti-Mouse CompBeads	IC002P
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages	

Appendix Table A.1 – Panel 1 Antibodies

Target (aka)	Fluorochrome	Antibody Clone (Isotype)	Vendor	Cat#	Compensation	Isotype Control Cat#
ASGPR1 (CLEC4H1)	VioBright FITC	REA608 (REA)	Miltenyi Biotec	130-109-411	Anti-REA Ig CompBeads	130-104-576
HLA-DR	BV650	L243 (mIgG2a)	BioLegend	307649 307650	Anti-Mouse CompBeads	400265
CLEC9A (DNGR-1)	VioBlue	8F9 (mIgG2a)	Miltenyi Biotec	130-099-906 130-097-406	Anti-Mouse CompBeads	130-098-898
CD1a (HTA1, Leu-6)	PerCP/Cy5.5	HI149 (mIgG1)	BioLegend	300129 300130	Anti-Mouse CompBeads	400149
CLEC13A (CD302)	PE-Vio770	REA509 (REA)	Miltenyi Biotec	130-107-851 130-107-798	Anti-REA Ig CompBeads	130-104-616
CD303 (BDCA-2)	PE/Dazzle 594	201A (mIgG2a)	BioLegend	354225 354226	Anti-Mouse CompBeads	400275
CLEC10A (CD301, MGL)	PE	REA586 (REA)	Miltenyi Biotec	130-109-641 130-109-582	Anti-REA Ig CompBeads	130-104-612
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages	

Appendix Table A.2 – Panel 2 Antibodies

Target (aka)	Fluorochoime	Antibody Clone (Isotype)	Vendor	Cat#	Compensation	Isotype Control Cat#
CD329 (Siglec 9)	VioBright FITC	REA492 (REA)	Miltenyi Biotec	130-107-651 130-107-607	Anti-REA Ig CompBeads	130-104-576
CD14	BV650	M5E2 (mIgG2a)	BioLegend	301835 301836	Anti-Mouse CompBeads	400265
CD328 (Siglec 7)	VioBlue	REA214 (REA)	Miltenyi Biotec	130-100-969 130-100-971	Anti-REA Ig CompBeads	130-104-609
Siglec 8	PerCP	837535 (mIgG1)	RnD Systems	FAB7975C	Anti-Mouse CompBeads	IC002C
CD169 (Siglec 1)	PE-Vio770	7-239 (mIgG1)	Miltenyi Biotec	130-098-638 130-098-640	Anti-Mouse CompBeads	130-096-654
CD33 (Siglec 3)	PE-CF594	WM53 (mIgG1)	BD Biosciences	562492	Anti-Mouse CompBeads	562292
Siglec 10	PE	5G6 (mIgG1)	Miltenyi Biotec	130-103-730 130-103-665	Anti-Mouse CompBeads	130-092-212
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages	

Appendix Table A.3 – Panel 3 Antibodies

Target (aka)	Fluorochoime	Antibody Clone (Isotype)	Vendor	Cat#	Compensation	Isotype Control Cat#
CD327 (Siglec 6)	AF488	767329 (mIgG1)	RnD Systems	FAB2859G	Anti-Mouse CompBeads	IC002G
CD11b (CR3A, Mac-1)	VioBlue	M1/70.15.11.5 (rat IgG2b)	Miltenyi Biotec	130-098-086 130-097-336	Anti-Rat CompBeads	130-103-083
CD16 (FcγRIII)	PerCP-Vio770	REA423 (REA)	Miltenyi Biotec	130-106-766 130-106-708	Anti-REA Ig CompBeads	130-104-620
CD32 (FcγRII)	PE-Cy7	FUN-2 (mIgG2b)	BioLegend	303213 303214	Anti-Mouse CompBeads	400325
CD64 (FcγRI)	PE/Dazzle594	10.1 (mIgG1)	BioLegend	305031 305032	Anti-Mouse CompBeads	400176
CLEC4D (CD368, Dectin 3)	PE	9B9 (mIgG2b)	BioLegend	360203 360204	Anti-Mouse CompBeads	400313
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages	

Appendix Table A.4 – Panel 4 Antibody Controls

Target (aka)	Fluorochoime	Antibody Clone (Isotype)	Vendor	Cat#	Compensation	Isotype Control Cat#
CD204 (MSR1)	VioBright FITC	REA460 (REA)	Miltenyi Biotec	130-107-063 130-107-037	Anti-REA Ig CompBeads	130-104-576
TIM-3 (CD366, HAVCR2)	BV650	F38-2E2 (mIgG1)	BioLegend	345027 345028	Anti-Mouse CompBeads	400164
CD59 (MIRL)	BV421	P282 (H19) (mIgG2a)	BD Biosciences	565982 564329	Anti-Mouse CompBeads	562439
CD36 (GP4)	PerCP	AC106 (mIgG2a)	Miltenyi Biotec	130-095-480	Anti-Mouse CompBeads	130-099-190
CD55 (CR, DAF)	PerCP/Cy5.5	JS11 (mIgG1)	BioLegend	311315 311316	Anti-Mouse CompBeads	400149
TIM-1 (CD365, HAVCR)	PE-Vio770	REA692 (REA)	Miltenyi Biotec	130-106-075 130-106-026	Anti-REA Ig CompBeads	130-104-616
CD163	PE/Dazzle594	GHI/61 (mIgG1)	BioLegend	333623 333624	Anti-Mouse CompBeads	400176
TIM-4	PE	9F4 (mIgG1)	BioLegend	354004	Anti-Mouse CompBeads	400113
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages	

Appendix Table A.5 – Panel 5 Antibodies

Target (aka)	Fluorochoime	Antibody Clone (Isotype)	Vendor	Cat#	Compensation
SIRPα (CD172a)	FITC	REA490 (REA)	Miltenyi Biotec	130-099-897 130-099-896	Anti-REA Ig CompBeads
CD86 (B7.2)	BV650	IT2.2 (mIgG2b)	BioLegend	305427 305428	Anti-Mouse CompBeads
CD80 (B7.1)	BV421	L307.4 (mIgG1)	BD Biosciences	566263 564160	Anti-Mouse CompBeads
CD209 (DC-SIGN)	PerCP-Vio770	REA617 (REA)	Miltenyi Biotec	130-109-652 130-109-593	Anti-REA Ig CompBeads
SIRPβ (CD172b)	PE-Vio770	B4B6 (mIgG1)	Miltenyi Biotec	130-105-311 130-105-269	Anti-Mouse CompBeads
CD206 (MRC1, CLEC13D)	PE/Dazzle594	15-2 (mIgG1)	BioLegend	321129 321130	Anti-Mouse CompBeads
CD205 (CLEC13B, DEC-205)	PE	HD30 (mIgG1)	Miltenyi Biotec	130-096-369	Anti-Mouse CompBeads
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages

Appendix Table A.6 – Panel 6 Antibodies

Target (aka)	Fluorochrome	Antibody Clone (Isotype)	Vendor	Cat#	Compensation	Isotype Control Cat#
CD93 (C1QR1)	FITC	VIMD2 (mIgG1)	Miltenyi Biotec	130-098-431	Anti-Mouse CompBeads	130-092-213
CD11c (CR4)	BV650	3.9 (mIgG1)	BioLegend	301637 301638	Anti-Mouse CompBeads	400164
Mertk	BV421	590H11G1E3 (mIgG1)	BioLegend	367603 367604	Anti-Mouse CompBeads	400157
CD35 (CR1)	PerCP-Vio770	E11 (mIgG1)	Miltenyi Biotec	130-101-618 130-101-612	Anti-Mouse CompBeads	130-097-561
CD170 (Siglec 5)	PE-Vio770	1A5 (mIgG1)	Miltenyi Biotec	130-101-794 130-101-772	Anti-Mouse CompBeads	130-096-654
CD13 (gp150, LAP1)	PE/Dazzle594	WM15 (mIgG1)	BioLegend	301719 301720	Anti-Mouse CompBeads	400176
CD299 (L-SIGN, CLEC4M)	PE	REA587 (REA)	Miltenyi Biotec	130-109-299 130-109-221	Anti-REA Ig CompBeads	130-104-612
CD45	VioGreen	5B1 (mIgG2a)	Miltenyi Biotec	130-096-906	THP-1 Macrophages	

Appendix Table A.7 – Panel 7 Antibodies

3 Instrument Details:

3.1 Instrument Manufacturer: Becton Dickinson

3.2 Instrument Model: LSR Fortessa 5 Laser Blue, Red, Yellow/Green, Violet, UV

3.3 Instrument Configuration and Settings

Laser	BP Filter	LP Mirror	Parameter Detected	Detector Voltage
UV (355nm)	530/30	505	-	-
	450/50	-	-	-
Violet (405nm)	655/8	630	Brilliant Violet 650	405
	605/12	595	-	-
	585/15	575	-	-
	525/50	505	VioGreen	250
	450/50	-	VioBlue; Brilliant Violet 421	215
Blue (488nm)	695/40	685	PerCP-Cy5.5; PerCP-Vio700	405
	670/14	635	PerCP	405
	530/30	505	FITC; Alexa Fluor 488; VioBright FITC	330
	488/10	-	SSC	170
Yellow /Green (561nm)	780/60	750	PE-Cy7; PE-Vio770	555
	710/50	685	-	-
	660/20	635	-	-
	610/20	600	PE-CF594; PE-Dazzle594; PE-Vio615	455
	585/15	570	PE	435
Red (640nm)	780/60	755	-	-
	730/45	685	-	-
	670/14	-	Cell Trace Far Red – Not Used In This Study	385

Appendix Table A.8 – Cytometer Laser and Filter Settings

4 Data Analysis Details

4.1 List-mode Data File

4.2 Compensation Details

4.2.1 Compensation Description: Compensation matrices for each panel were created using single stained compensation beads and THP-1 macrophages stained for CD45 VioGreen and unstained macrophages acquired and generated by the Diva software. Post-acquisition compensation matrices were adjusted using FlowJoX. The matrices for each panel are as follows:-

Panel 01	VioBright FITC	PerCP-Cy5.5	APC	VioBlue	VioGreen	BV650	PE	PE-Vio615	PE-Vio770
VioBright FITC - Dectin 1	-	2.7763	0	0	0.0765	0	0	0	0
PerCP-Cy5.5 - CLEC122A	0	-	7.8074	0	0	0	0	0	35.9332
APC - CTFR RBC	0.5199	1.3549	-	0.2299	0.3679	19.6969	0.0085	1.0134	10.9597
VioBue - CD207	0	0	0	-	0	0	0	0	0
VioGreen - CD45	0	0	0	6.3492	-	413.1746	0	0	0
BV650 - CD69	0	0	6.5302	0	0	-	0	0	0
PE - Dectin 2	0	2.2546	0	0	0	0	-	34.1136	0.9557
PE-Vio615 - KLRG1	0	28.6593	0.1612	0	0	5.9552	21.3943	-	23.909
PE-Vio770 - CLEC4A	0	0	0	0	0	0	0.5654	0.1489	-

Appendix Table A.9 - Panel 01 Compensation Matrix

Panel 02	VioBright FITC	PerCP-Cy5.5	APC	VioBlue	VioGreen	BV650	PE	PE-Dazzle 594	PE-Vio770
VioBright FITC - ASGPR1	-	0	0	0	0	0	0	0	0
PerCP-Cy5.5 - CD1a	0	-	7.7963	0	0	0	0	0	35.882
APC - CTFR RBC	0.3665	1.703	-	0.2517	0.2307	17.3457	0	1.8644	9.788
VioBlue - CLEC9A	0	0	0	-	0	0	0	0	0
VioGreen - CD45	0	0	0.4884	6.8017	-	43.1259	0	0	0
BV650 - HLA-DR	0	0	6.6389	0	0	-	0	0	0
PE - CLEC10A	0.013	3.1762	0	0	0	0.644	-	34.1131	1.882
PE-Dazzle 594 - CD303	0	14.1603	0	0	0	0.6112	21.4063	-	9.8896
PE-Vio770 - CLEC13A	0	0	0	0	0	0	0.4712	0.1104	-

Appendix Table A.10 - Panel 02 Compensation Matrix

Panel 03	VioBright FITC	PerCP	APC	VioBlue	VioGreen	BV650	PE	PE-CF594	PE-Vio770
VioBright FITC - CD329	-	6.0342	0	0	0	0	4.0953	0	0
PerCP - Siglec 8	0	-	8.3246	0	0	0	0	0	0.6997
APC - CTFR RBC	0.6069	2.4276	-	0.5558	0.7642	17.7674	0.3474	13.122	9.726
VioBlue - CD328	0	0	0	-	18.4556	0	0	0	0
VioGreen - CD45	0	0	0.7315	5.0715	-	41.3524	0	0	0
BV650 - CD14	0	0	6.8805	0	0	-	0	0	1.191
PE - Siglec 10	0	4.6714	0	0	0	0	-	34.1258	1.3566
PE-CF594 - CD33	0	27.6293	0	0	0	0.9175	19.0975	-	13.4022
PE-Vio770 - CD169	0	0	0	0	0	0	0.5984	0.0361	-

Appendix Table A.11 - Panel 03 Compensation Matrix

Panel 04	AF488	APC	VioBlue	VioGreen	PE	PE-Dazzle594	PE-Cy7	PerCP-Vio770
AF488 - CD327	-	0	0	0.1494	5.6821	0.5535	0	2.901
APC - CTFR RBC	1.1908	-	0.5444	1.2563	0.791	2.5901	11.5082	2.8262
VioBlue - CD11b	0	0	-	0	0	0	0	0
VioGreen - CD45	1.5403	0.3065	7.7657	-	1.3624	3.0276	0.9083	1.5403
PE - CLEC4D	0	0	0	0	-	32.5272	1.4754	2.6705
PE-Dazzle 594 - CD64	0	0	0	0	26.7822	-	10.1196	14.3176
PE-Cy7 - CD32	0	0	0	0	0.3235	0	-	0
PerCP-Vio770 - CD16	0	4.3536	0	0	0	0	46.6628	-

Appendix Table A.12 - Panel 04 Compensation Matrix

Panel 05	VioBright FITC	PerCP	PerCP-Cy5.5	APC	BV421	VioGreen	BV650	PE	PE-Dazzle 594	PE-Vio770
VioBright FITC - CD204	-	10.8299	4.0478	0	0	0.9456	0	0	0	0
PerCP - CD36	0	-	17.7778	7.1453	0	0	0	0	0	0
PerCP-Cy5.5 - CD55	0	75.6955	-	7.5323	0	0	0	0	0	34.6642
APC - CTFR RBC	0.4298	3.2457	1.464	-	0.3516	0.7092	18.3294	0.4984	0.9967	9.8664
BV421 - CD59	0	0	0	0	-	1.9565	0	0	0	0
VioGreen - CD45	0.2728	2.7962	1.4322	0	8.4273	-	43.5966	1.2534	0	0.8848
BV650 - TIM-3	0	0	0	6.8528	0	0	-	0	0	0.6392
PE - TIM-4	0	5.6278	2.6001	0	0	0	0	-	33.0307	1.3133
PE-Dazzle 594 - CD163	0	29.9739	14.2005	0	0	0	0.8963	21.789	-	10.1111
PE-Vio770 - TIM-1	0	0	0	0	0	0	0	0.8326	0.2109	-

Appendix Table A.13 - Panel 05 Compensation Matrix

Panel 06	FITC	PerCP-Vio770	APC	BV421	VioGreen	BV650	PE	PE-Dazzle 594	PE-Vio770
FITC - SIRPa		0	0	0	0.7664	0	0	0	0
PerCP-Vio770 - CD209	0		1.1694	0	0	0	0	0	41.118
APC - CTFR RBC	0.7367	1.9189		0.6069	1.0809	19.3754	0.6808	1.1047	10.7706
BV421 - CD80	0	0	0		0.6891	0	0	0	0
VioGreen - CD45	1.963	2.0751	0.5344	13.5392		49.0499	1.0557	0	1.0557
BV650 - CD86	0	0	6.0436	0	0		0	0	0
PE - CD205	0	2.3872	0	0	0	0.1066		33.9494	1.4026
PE-Dazzle 594 - CD206	0	14.1075	0	0	0	1.0176	20.8554		10.0037
PE-Vio770 - SIRPb	0	0	0	0	0	0	0.4053	0	

Appendix Table A.14 - Panel 06 Compensation Matrix

Panel 07	FITC	PerCP-Vio770	APC	BV421	VioGreen	BV650	PE	PE-Dazzle 594	PE-Vio770
FITC - CD93		0	0	0	0	0	0	0	0
PerCP-Vio770 - CD35	0		1.8533	0	0	0	0	0	41.4128
APC - CTFR RBC	0.8421	2.3468		0.2418	0.9805	19.2875	0.5223	0.7014	11.2674
BV421 - Merck	0	0	0		2.3082	0	0	0	0
VioGreen - CD45	1.7246	0.8277	0	11.0067		45.9732	0	0	0
BV650 - CD11c	0	0.4637	7.0781	0.7212	0		0	0.5661	3.6011
PE - CD299	0	2.6319	0	0	0	0.2336		34.3699	1.192
PE-Dazzle 594 - CD13	0	14.342	0	0	0	1.1192	20.3993		10.1312
PE-Vio770 - CD170	0	0	0	0	0	0	0.8492	0	

Appendix Table A.15 - Panel 07 Compensation Matrix

4.2.2 Compensation Information

4.3 Data Transformation Details:

4.3.1 Purpose of Data Transformation: To visualise events against the axis better and to account for non-specific binding and autofluorescence.

4.3.2 Data Transformation Description: Bi-exponential transformation was used.

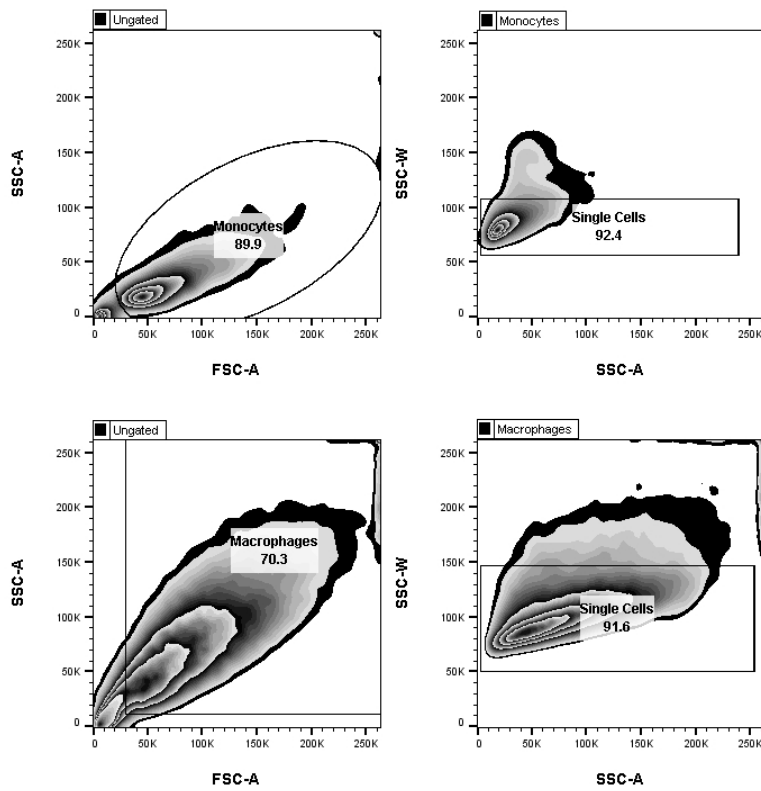
4.3.3 Other Relevant Data Transformation Details: GeoMean was normalised by subtracting the matched isotype control value. For binding GeoMean was normalised by subtracting the unstained value from the isotype control.

4.4 Gating Details

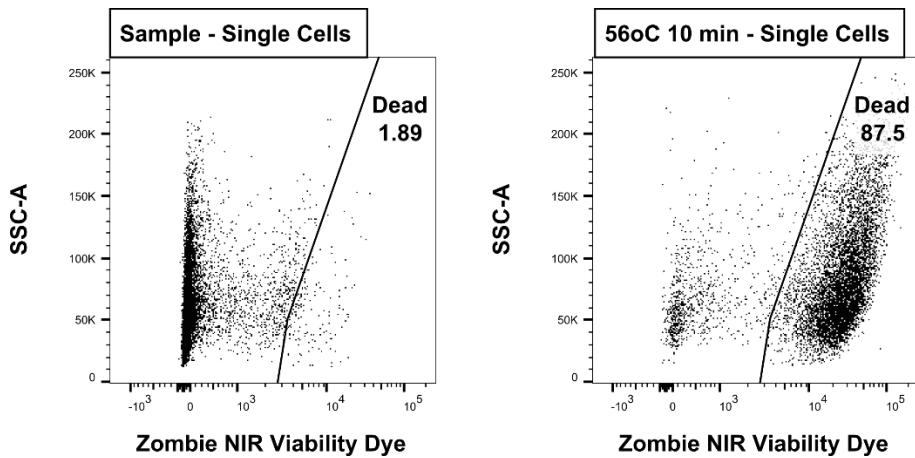
4.4.1 Gate Description: Cells were first gated to eliminate debris (FSC-A vs SSC-A) then gated for single cells (SSC-A vs SSC-W).

4.4.2 Gate Statistics: Monocytes: Cells average 87.4% single cells: 93.6%.
Macrophages: Cells average 67.1% single cells average 85.4%

4.4.3 Gate Boundaries:



4.4.4 Other Relevant Gate Information



A viability dye was not used in these experiments but it was confirmed that the culturing conditions, staining protocol and gating strategy resulted in dead cells comprising less than 2% of the cells analyzed.

