

Supplementary Materials

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A Blister fluid (prophylactic)

Cytokine	4 hr Blister Untreated	4 hr Blister Treated	24 hr Blister Untreated	24 hr Blister Treated
TNFA	5770.61 +/- 6043.72	6623.62 +/- 2687.49	1753.68 +/- 3606.08	249.60 +/- 97.58
IL-10	4462.07 +/- 1619.61	4614.98 +/- 2970.57	2476.65 +/- 1252.78	2686.17 +/- 1255.25
IL-1B	929.51 +/- 632.77	983.84 +/- 512.10	828.24 +/- 1666.27	98.08 +/- 50.02
IL-8	4850.06 +/- 1935.69	3697.13 +/- 2285.54	1916.58 +/- 4296.83	172.83 +/- 104.44
CCL2	9784.31 +/- 2964.26	11325.21 +/- 5783.37	5479.48 +/- 3539.92	3630.42 +/- 1350.96
CX3CL1	2415.38 +/- 568.15	2951.71 +/- 919.69	1641.72 +/- 618.15	1525.01 +/- 289.61
CXCL10	6910.95 +/- 493.70	8328.63 +/- 4285.09	6783.12 +/- 464.07	6725.70 +/- 372.77
IL-17E	85.73 +/- 98.18	169.82 +/- 195.92	72.36 +/- 59.37	176.82 +/- 121.40
VEGF	944.98 +/- 513.37	1757.22 +/- 632.37	334.53 +/- 625.51	102.36 +/- 29.20
CCL5	1605.39 +/- 835.08	2465.39 +/- 603.47		

A Blister fluid (therapeutic)

Cytokine	24 hr Blister Untreated	24 hr Blister Treated	48 hr Blister Untreated	48 hr Blister Treated
TNFA	302.66 +/- 105.54	363.11 +/- 89.65	134.66 +/- 66.06	157.85 +/- 106.79
IL-10	2861.99 +/- 509.05	2938.51 +/- 773.86	1922.51 +/- 682.28	2394.15 +/- 942.87
IL-1B	172.56 +/- 103.91	153.91 +/- 71.24	33.56 +/- 27.72	46.45 +/- 47.96
IL-8	212.55 +/- 77.75	341.49 +/- 215.95	189.27 +/- 118.30	1554.25 +/- 4427.92
CCL2	3646.41 +/- 1561.72	4321.93 +/- 1418.11	2432.15 +/- 933.10	2593.43 +/- 812.96
CX3CL1	1855.90 +/- 391.97	2103.13 +/- 385.96	954.59 +/- 408.35	1212.09 +/- 695.53
CXCL10	8526.37 +/- 348.10	8726.21 +/- 740.57	9579.92 +/- 3230.12	10514.04 +/- 3195.06
IL-17E	315.89 +/- 244.45	152.18 +/- 137.59	446.14 +/- 210.68	531.29 +/- 438.59
VEGF	185.33 +/- 93.64	186.44 +/- 129.34	91.78 +/- 43.05	127.53 +/- 103.52

Supplementary Table 1 – Cytokines and chemokine levels in blister fluid with prophylactic and therapeutic sEH inhibition.

Fluorophore	Marker	Category Code	Concentration	Target
FITC	CD3	300306	1:100	T cell
FITC	CD19	302206	1:100	B cell
FITC	CD20	302304	1:100	B cell
FITC	CD56	392414	1:100	NK cell
FITC	CD66b	305104	1:100	Neutrophil
PerCP-Cy5.5	CD56	318322	1:100	NK cell
PerCP-Cy5.5	CD141	344112	1:100	DC
PerCP-Cy5.5	CD11b	301328	1:100	
BV421	CD11c	301628	2.5:100	Mononuclear Phagocyte
BV421	CD62L	304828	1:50	
BV510	CCR2	357218	2.5:100	Monocyte
BV510	CD11b	301628	1:100	Mononuclear phagocyte
BV510	HLA-DR	307646	1:100	Mononuclear phagocyte
BV605	CD86	374214	1:100	Monocyte/DC
BV605	CD25	302632	1:50	T cell
BV711	CD16	302044	1:100	Monocyte/ Granulocyte
BV785	CD206	321142	1:50	Monocyte/Macrophage
BV785	CD163	333632	1:100	Mononuclear phagocyte
BV786	CD27	563327	1:100	T cell
BUV395	CD123	564195	1:100	pDC
BUV395	CD4	564724	1:100	T cell
BUV395	CD1a	756536	1:100	DC/Langerhan
APC	CD163	333610	3:100	Monocyte/Macrophage
APC	CD207	352205	1:100	Langerhan
AF700	HLA-DR	307626	2.5:100	Mononuclear Phagocyte
AF700	CD66b	305114	1:50	Neutrophil
APC-Cy7	CD1c	331520	2.5:100	DC
APC-Cy7	CD8	301016	1:100	T cell
APC-Cy7	CD206	321120	1:100	Mononuclear phagocyte
PE	CD205	359204	2.5:100	Monocyte/DC
PE	FOXP3	320107	1:10	T cell
PE	MerTK	367608	1:20	Mononuclear Phagocyte
PE	CCR7	535204	1:100	
PE	CD25	302605	1:100	T cell
PE-Dazzle	CD19	302252	1:100	B cell
PE-Dazzle	CD20	302348	1:100	B cell
PE-Dazzle	CD3	300336	1:100	T cell
PE-Dazzle	CD56	392410	1:100	NK cell
PE-Dazzle	CD66b	305122	1:100	Neutrophil
PE-Dazzle	CD64	305032	1:100	Monocyte
PE-Cy7	TIM-4	354006	1:100	Monocyte/Macrophage
PE-Cy7	CD62L	304822	1:100	
PE-Cy7	CD45RA	304125	1:100	T cell
BUV805	CD14	612902	1:100	Monocyte
BUV805	CD69	748763	1:100	Activation
Zombie UV	Live/Dead	423107	1:1000	
AF647	P-p38	526066	1:10	

Supplementary Table 2 - Table of antibodies used for Flow Cytometry

Fluorophore	Marker	Category Code	Concentration	Target
Starbright UV 665	CD45RO	MCA461SBUV665	1:50	T cell
BUV615	HLA-DR	751142	1:100	
BUV737	CD103	748502	1:50	T cell
BV421	CCR6	562515	1:20	
BV480	CD45RA	566155	1:100	T cell
BV650	CD16	563692	1:100	Monocyte
BV711	CTLA4/CD152	369632	1:50	
FITC	CLA	321306	1:100	
PercPFluor710	TIGIT	46-9500-42	1:100	
RB780	CD39	570123	1:100	
AF700	CD14	325614	1:100	Monocyte
APC-H7	CD3	560275	1:100	T cell
PE	FoxP3	12-4777-43	1:100	T reg cell
PE-Dazzle	Ki67	350533	1:100	
Zombie NIR	Live/Dead	423106	1:1000	

Supplementary Table 2 - Table of antibodies used for Flow Cytometry cont.

Primary Antibody	Clone	Host	Reactivity	Dilution	Supplier
CYP2C8,9,18,19	Polyclonal	Rabbit	Human	1:150	Biorbyt
CYP2J2	Polyclonal	Rabbit	Human	1:300	Abcam
mEH	Polyclonal	Rabbit	Human	1:200	Biorbyt
sEH	Polyclonal	Rabbit	Human	1:150	Biorbyt
EH3	Polyclonal	Rabbit	Human	1:200	Biorbyt

Supplementary Table 3 - Table of antibodies used for immunohistochemistry

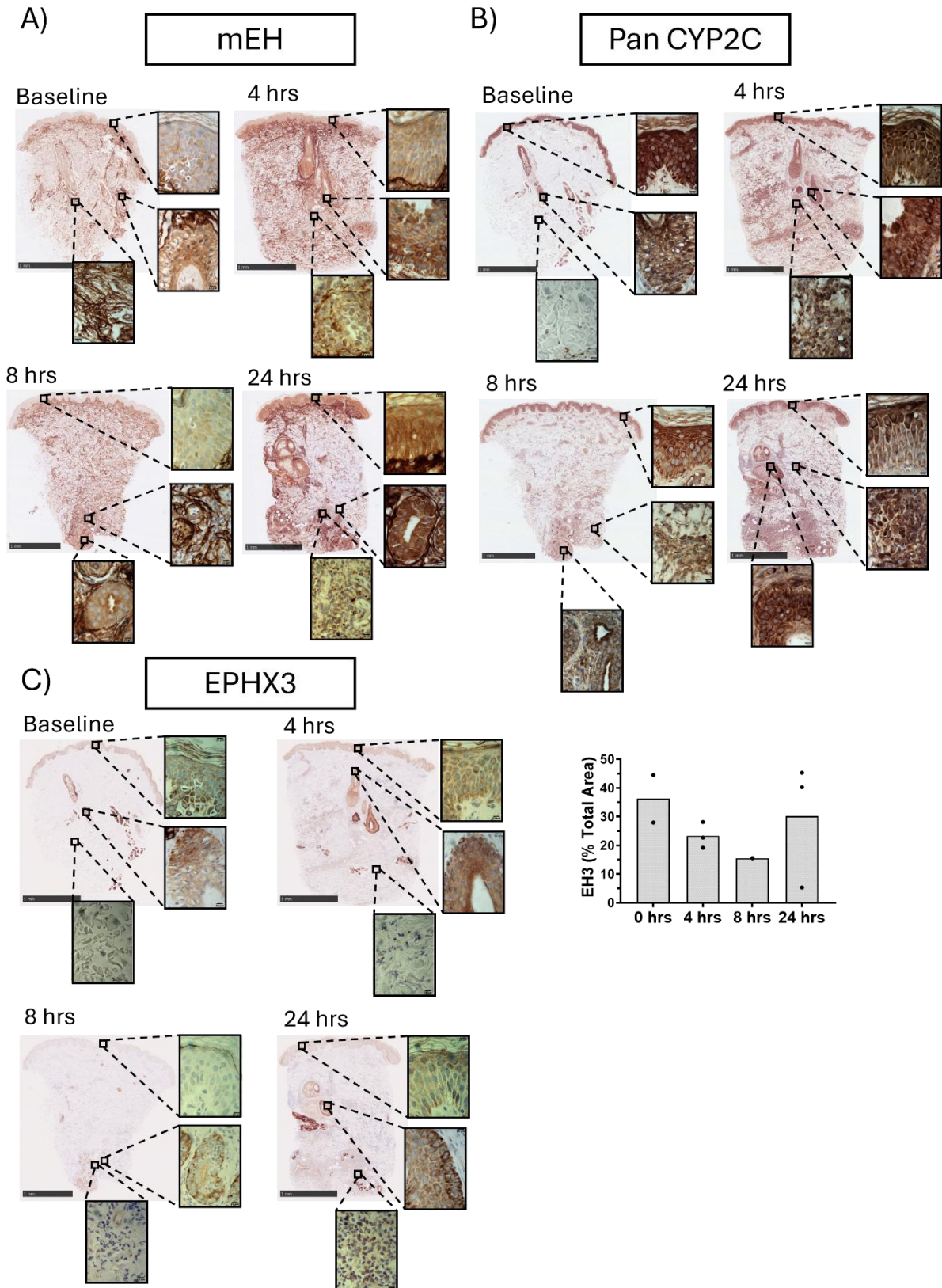
	Precursor	Product	RT		Low	High	
Analyte	(m/z)	(m/z)	(min)	ISTD	Standard	Standard	r ²
11,12-DHET	337.2	167.2	7.23	11,12-DHET-d11	0.1	120	0.995
11,12-DiHETE	335.2	167.1	5.99	11,12-DHET-d11	0.1	120	0.99
11,12-EET	319.2	179.2	11.99	11,12-EET-d11	0.2	240	0.996
11-HETE	319.2	167.2	10.04	15-HETE-d8	0.1	120	0.994
12,13-DHOME	313.2	183.2	6.08	11,12-DHET-d11	1	1200	0.985
12,13-EpOME	295.2	195.2	11.2	11,12-EET-d11	0.5	600	0.992
12-HETE	319.2	179.2	10.32	15-HETE-d8	1.25	1500	0.995
13,14-DiHDP	361.2	193.1	7.36	11,12-DHET-d11	0.1	120	0.994
13-HODE	295.2	195.2	9.04	15-HETE-d8	2.5	3000	0.993
14,15-DHET	337.2	207.2	6.69	11,12-DHET-d11	0.1	120	0.992
14,15-DiHETE	335.2	207.1	5.8	11,12-DHET-d11	0.1	120	0.993
14,15-EET	319.2	219.3	11.39	11,12-EET-d11	0.2	240	0.994
15-HETE	319.2	219.1	9.62	15-HETE-d8	0.1	120	0.994
16,17-DiHDP	361.2	233.1	7.12	11,12-DHET-d11	0.1	120	0.994
16,17-EpDPA	343.2	233.1	11.77	11,12-EET-d11	0.2	240	0.995
17,18-DiHETE	335.2	247.1	5.57	11,12-DHET-d11	0.1	120	0.991
17,18-EpETE	317.2	259.1	9.65	11,12-EET-d11	0.1	120	0.995
17-HDHA	343.2	281.2	9.91	15-HETE-d8	0.025	30	0.992
19,20-DiHDP	361.2	273.2	6.76	11,12-DHET-d11	0.1	120	0.994
19,20-EpDPE	343.2	281.1	11.35	11,12-EET-d11	0.2	240	0.994
19-HETE	319.2	231.1	8.06	15-HETE-d8	0.5	600	0.993
20-HETE	319.2	245.1	8.23	15-HETE-d8	0.5	600	0.991
22-HDOHE	343.2	269.1	8.99	15-HETE-d8	0.025	30	0.994
5,6-DHET	337.2	145.1	12.3	11,12-DHET-d11	0.1	120	0.994
5-HETE	319.2	257.3	10.86	15-HETE-d8	0.1	120	0.993
8,9-DHET	337.2	127.3	7.66	11,12-DHET-d11	0.1	120	0.994
8,9-EET	319.2	151.1	12.2	11,12-EET-d11	0.2	240	0.993
9,10-DHOME	313.2	201.1	6.35	11,12-DHET-d11	1	1200	0.972
9,10-EpOME	295.2	277.3	11.42	11,12-EET-d11	0.5	600	0.998
9-HODE	295.2	171.2	9.19	15-HETE-d8	2.5	3000	0.994

Supplementary Table 4 - Details of oxylipin analysis by LCMS.

Ionization masses (Precursor and Product) in mass/charge ratio (m/z), retention time (RT), internal standard (ISTD) used, and the minimum and maximum concentrations used in the standard curve (pg/μl injected) and r² of the linear standard curves is indicated. Analyte quantification was determined using standard curves determined based on peak area analyte/peak area of indicated ISTDs. The lower limit of quantification LLOQ was at or above the lowest standard used for each curve (S/N>10).

SUPPLEMENTARY FIGURES

Supplementary, Figure 1



Supplementary Figure 1 – Expression of CYP2C, mEH and EH3 during inflammation in human tissue

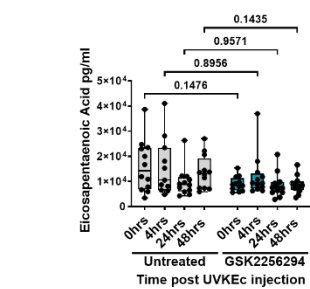
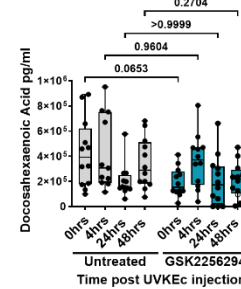
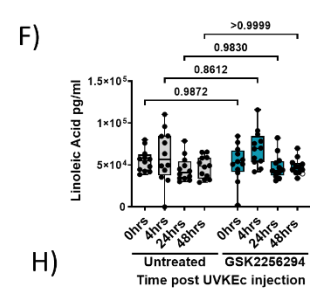
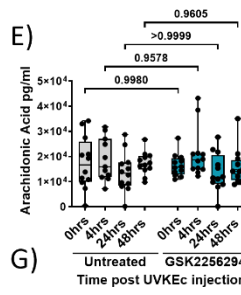
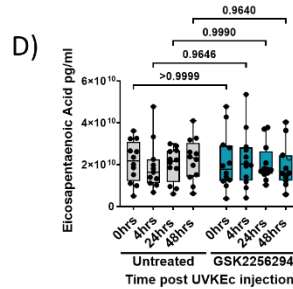
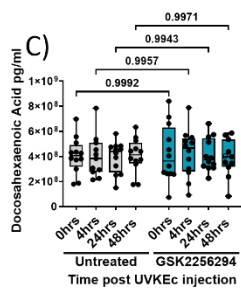
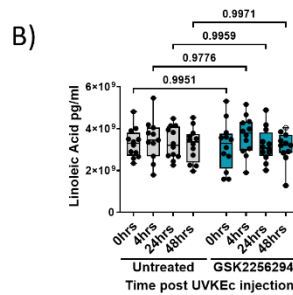
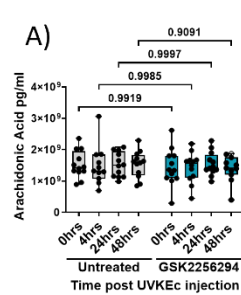
Paraffin-embedded skin punch biopsies were obtained at baseline, 4, 8 and 24 hrs post UVKEc injection.

Sections were incubated with (A) mEH, (B) Pan CYP2C8/9/18/19 and (C) EPHX3 primary antibodies and visualized by avidin-biotin complex-based detection methods. The sections were subsequently counterstained with haematoxylin.

(C) Skin biopsies at baseline, 4, 8 and 24 hrs were stained and the % area of staining quantified for EPHX3 (n =1-3; biologically independent samples).

Supplementary, Figure 2

Therapeutic



Supplementary Figure 2 – Levels of PUFAs in peripheral blood with prophylactic and therapeutic dosing of GSK2256294 during inflammation.

The forearms of participants were intradermally injected with UV-killed *E. coli* (UVKEc) resulting in a local and peripheral inflammatory response. 2 hrs prior to (prophylactic) or 4 hrs after (therapeutic) UVKEc injection, participants were dosed with 15 mg of GSK2256294. Plasma was collected and subject to lipidomic analysis at all timepoints. Levels of (A) Arachidonic Acid (AA), (B) Linoleic Acid, (C) Docosahexaenoic Acid (DHA), and (D) Eicosapentaenoic Acid (EPA) in the therapeutic arm of the study (untreated: n = 11-12; GSK2256294: n = 11-12; biologically independent samples). Levels of (E) Arachidonic Acid (AA), (F) Linoleic Acid, (G) Docosahexaenoic Acid (DHA), and (H) Eicosapentaenoic Acid (EPA) in the prophylactic arm of the study (untreated: n = 11-12; GSK2256294: n = 12; biologically independent samples). All data are expressed in pg/ml. Box-and-whisker plots show the median (center line), the interquartile range (25th–75th percentiles; box), and the full data range (whiskers, minimum to maximum). Normality was assessed using the D'Agostino & Pearson test, Shapiro-Wilk test and visualised using a QQ plot. Data were analysed using two-way-ANOVA mixed effect analysis with Šidák's multiple comparison test. Source data are provided as a Source Data file.

Supplementary, Figure 3

Prophylactic

Lipid	0hr Untreated	4hr Untreated	24hr Untreated	48hr Untreated	0hr Treated	4hr Treated	24hr Treated	48hr Treated
11,12-DHET	136.67 +/- 50.45	118.83 +/- 50.70	114.96 +/- 32.86	132.27 +/- 38.77	131.65 +/- 72.56	150.79 +/- 46.09	114.75 +/- 31.40	114.73 +/- 35.40
11,12-EET	20.85 +/- 10.86	20.65 +/- 12.95	13.77 +/- 6.03	17.94 +/- 7.64	15.94 +/- 5.55	27.46 +/- 12.95	17.02 +/- 5.99	17.85 +/- 7.20
11-HETE	221.90 +/- 148.48	123.63 +/- 56.16	277.23 +/- 117.22	449.48 +/- 229.83	136.52 +/- 32.71	153.15 +/- 55.66	256.58 +/- 112.66	384.10 +/- 218.91
12,13-DHOME	2901.48 +/- 1284.35	4283.38 +/- 3067.55	2290.17 +/- 1096.67	2376.21 +/- 2033.26	1465.33 +/- 1043.09	1916.15 +/- 3085.74	1173.13 +/- 1158.00	1530.13 +/- 2051.44
12,13-EpOME	1297.31 +/- 1065.40	2225.63 +/- 1586.25	1635.79 +/- 2652.96	1243.02 +/- 1588.07	5259.10 +/- 6758.25	6289.48 +/- 2777.48	3161.10 +/- 2646.04	3386.48 +/- 1560.72
12-HETE	2174.42 +/- 2444.80	1041.44 +/- 1419.34	1232.44 +/- 980.04	1567.75 +/- 776.62	1534.08 +/- 1562.50	1444.25 +/- 1407.83	1690.23 +/- 996.33	1346.60 +/- 779.58
13,14-DiHDPa	52.88 +/- 28.24	49.25 +/- 27.53	43.10 +/- 13.85	49.58 +/- 23.17	36.69 +/- 11.05	43.21 +/- 24.95	34.58 +/- 13.95	34.92 +/- 23.04
13-HODE	9396.33 +/- 11192.94	7344.69 +/- 7111.88	14138.52 +/- 13143.27	13564.10 +/- 11342.64	4522.35 +/- 4511.11	5071.58 +/- 7637.86	8499.75 +/- 13463.04	12321.46 +/- 11063.73
14,15-DHET	157.90 +/- 60.79	60.63 +/- 66.96	122.38 +/- 30.42	141.27 +/- 38.17	102.94 +/- 50.92	84.83 +/- 67.34	110.83 +/- 45.10	100.40 +/- 38.57
14,15-DiHETE	88.38 +/- 33.06	87.56 +/- 49.18	73.19 +/- 17.78	74.75 +/- 26.10	61.67 +/- 14.83	75.67 +/- 49.26	59.65 +/- 17.40	50.75 +/- 27.84
14,15-EET	32.63 +/- 18.34	29.00 +/- 16.26	23.90 +/- 6.07	29.98 +/- 11.17	41.69 +/- 22.41	61.77 +/- 17.44	42.40 +/- 6.81	43.00 +/- 13.69
15-HETE	215.17 +/- 92.09	164.06 +/- 65.67	232.67 +/- 90.45	360.85 +/- 158.20	183.19 +/- 46.23	199.33 +/- 65.96	250.15 +/- 86.55	327.94 +/- 141.83
16,17-DiHDPa	54.08 +/- 34.37	48.75 +/- 27.71	40.40 +/- 12.07	46.48 +/- 21.59	35.35 +/- 11.07	39.75 +/- 23.37	33.17 +/- 11.61	31.79 +/- 21.90
17,18-DiHETE	1284.46 +/- 469.70	1311.35 +/- 858.31	1054.88 +/- 298.91	1023.98 +/- 391.05	856.90 +/- 226.68	1012.21 +/- 858.12	829.58 +/- 317.74	712.40 +/- 360.78
17-HDHA	776.08 +/- 467.80	478.90 +/- 193.58	900.96 +/- 357.89	1703.56 +/- 1090.71	414.67 +/- 199.04	449.50 +/- 195.83	652.48 +/- 380.05	949.90 +/- 1107.07
18-HEPE	309.79 +/- 160.43	284.71 +/- 128.89	356.73 +/- 135.71	470.88 +/- 273.44	206.98 +/- 57.38	231.79 +/- 136.03	270.21 +/- 132.29	325.27 +/- 270.35
19,20-DiHDPa	767.96 +/- 495.38	713.92 +/- 475.09	642.38 +/- 279.46	679.48 +/- 297.72	466.15 +/- 167.04	601.08 +/- 385.97	520.15 +/- 284.72	458.56 +/- 298.37
19,20-EpDPE	526.06 +/- 334.74	269.31 +/- 129.49	651.31 +/- 311.70	1213.85 +/- 686.70	227.75 +/- 93.93	301.69 +/- 128.67	429.85 +/- 320.92	690.23 +/- 685.59
19-HETE	87.96 +/- 46.14	67.08 +/- 36.35	63.90 +/- 26.54	75.46 +/- 23.36	70.13 +/- 17.79	90.13 +/- 30.59	66.85 +/- 27.44	64.94 +/- 23.31
20-carboxy-AA	227.83 +/- 91.78	134.77 +/- 62.08	332.10 +/- 93.93	424.52 +/- 141.23	222.02 +/- 126.93	207.48 +/- 68.46	347.63 +/- 81.83	436.17 +/- 130.85
20-HETE	202.08 +/- 146.06	181.27 +/- 163.93	153.75 +/- 75.21	175.88 +/- 102.57	115.88 +/- 25.82	171.98 +/- 161.80	113.17 +/- 75.41	130.67 +/- 100.96
22-HDoHE	272.56 +/- 206.82	233.44 +/- 201.91	171.23 +/- 75.34	229.23 +/- 135.97	159.19 +/- 62.35	214.81 +/- 187.11	138.60 +/- 75.36	152.13 +/- 132.19
5,6-DHET	106.56 +/- 29.78	95.44 +/- 34.25	89.96 +/- 23.66	100.67 +/- 26.44	99.90 +/- 20.10	113.25 +/- 34.17	99.56 +/- 24.83	114.00 +/- 28.86
5-HETE	388.33 +/- 207.22	264.29 +/- 111.96	458.21 +/- 179.47	680.04 +/- 280.40	252.75 +/- 61.18	304.54 +/- 112.03	410.29 +/- 171.78	622.15 +/- 254.77
8,9-DHET	58.54 +/- 19.93	54.35 +/- 21.63	49.75 +/- 14.75	48.44 +/- 12.60	46.38 +/- 17.14	59.08 +/- 19.64	48.13 +/- 15.13	48.08 +/- 11.62
8-HETE	2485.00 +/- 1293.98	1511.13 +/- 646.25	3305.71 +/- 1277.48	5074.42 +/- 2287.69	1691.13 +/- 498.98	1969.79 +/- 644.57	3182.60 +/- 1236.72	4552.27 +/- 2141.61
9,10-DHOME	2544.92 +/- 2271.53	7103.46 +/- 11540.68	1781.40 +/- 1308.87	1601.92 +/- 1527.04	1288.10 +/- 1352.51	2263.46 +/- 11554.31	944.31 +/- 1346.41	1130.63 +/- 1532.52
9,10-EpOME	498.31 +/- 636.28	1105.19 +/- 1558.48	467.96 +/- 610.74	417.19 +/- 363.04	686.63 +/- 1163.24	1022.56 +/- 1629.76	554.65 +/- 606.36	603.50 +/- 352.33
9-HODE	11929.02 +/- 9291.57	20016.40 +/- 19000.39	13426.79 +/- 10465.73	15972.58 +/- 8331.88	11000.88 +/- 9249.59	13110.92 +/- 18891.89	9258.58 +/- 10401.76	13408.52 +/- 7802.38

Therapeutic

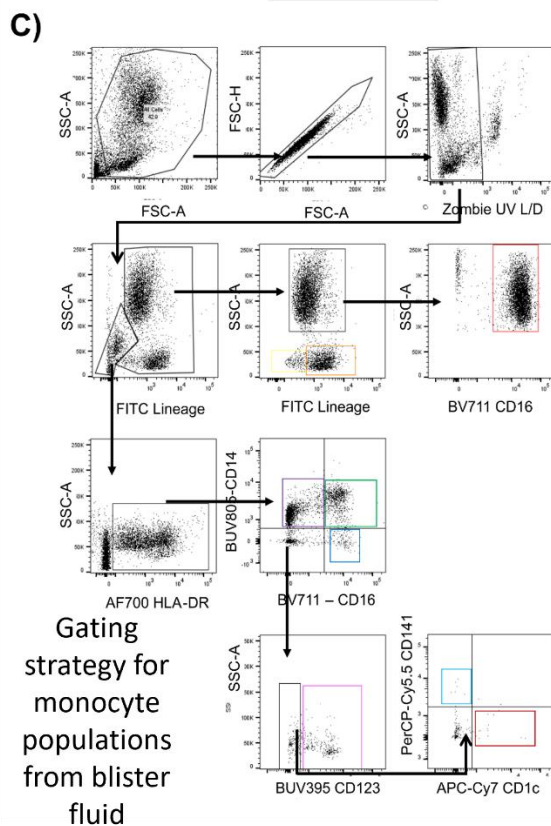
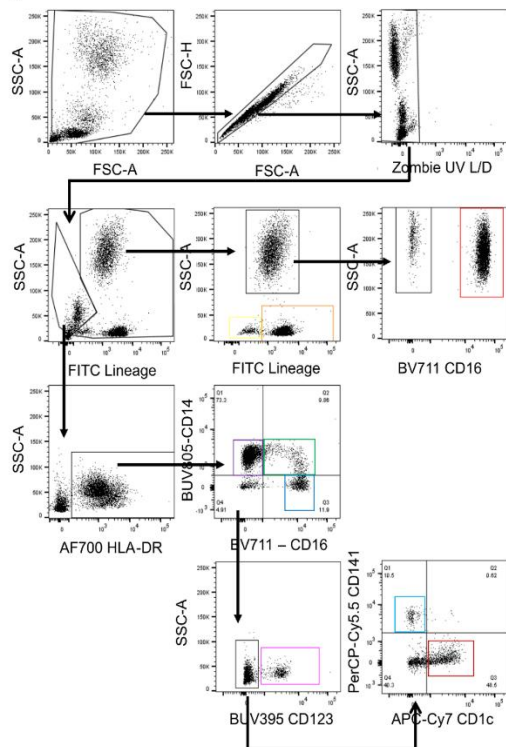
Lipid	0hr Untreated	4hr Untreated	24hr Untreated	48hr Untreated	0hr Treated	4hr Treated	24hr Treated	48hr Treated
11,12-DHET	297.92 +/- 66.43	289.70 +/- 95.21	263.69 +/- 51.31	267.52 +/- 67.83	275.77 +/- 86.13	362.16 +/- 249.03	231.72 +/- 55.87	240.86 +/- 67.05
11,12-EET	54.63 +/- 5.54	56.40 +/- 10.41	51.96 +/- 8.55	53.95 +/- 12.32	52.47 +/- 6.54	58.80 +/- 24.27	55.40 +/- 13.29	59.88 +/- 10.88
11-HETE	298.38 +/- 103.63	313.87 +/- 182.64	269.60 +/- 95.82	285.03 +/- 111.59	304.93 +/- 123.62	331.51 +/- 179.49	255.87 +/- 101.77	258.40 +/- 87.48
12,13-DHOME	7199.11 +/- 8750.16	5674.35 +/- 2871.51	9405.65 +/- 10893.17	8047.75 +/- 8005.57	2697.58 +/- 2019.86	7905.87 +/- 4753.78	1900.64 +/- 1449.39	2489.24 +/- 2061.88
12,13-EpOME	3498.30 +/- 4288.44	4094.06 +/- 2999.58	4496.60 +/- 5290.38	5055.14 +/- 9416.43	1626.15 +/- 1609.07	4210.08 +/- 2448.30	6807.48 +/- 7261.40	12471.06 +/- 16713.12
12-HETE	2115.60 +/- 761.97	2399.77 +/- 1774.33	2746.39 +/- 2018.25	2179.21 +/- 1538.53	2139.50 +/- 1569.73	6627.51 +/- 11563.62	2178.49 +/- 1154.09	1789.47 +/- 1070.10
13,14-DiHDPa	113.05 +/- 31.39	105.06 +/- 29.25	96.00 +/- 29.37	95.07 +/- 30.94	120.54 +/- 36.84	143.54 +/- 67.78	104.63 +/- 32.64	108.49 +/- 40.54
13-HODE	71167.01 +/- 93504.61	64382.37 +/- 53850.63	85876.06 +/- 113347.24	77908.46 +/- 114331.03	20331.29 +/- 14636.91	55645.92 +/- 37910.68	26619.01 +/- 32674.45	36418.74 +/- 40385.22
14,15-DHET	720.54 +/- 126.94	736.60 +/- 180.07	647.96 +/- 118.49	643.07 +/- 140.10	730.19 +/- 193.78	847.96 +/- 485.69	621.22 +/- 149.76	626.07 +/- 181.95
14,15-DiHETE	484.31 +/- 195.02	487.94 +/- 162.16	424.40 +/- 172.90	430.10 +/- 154.98	525.04 +/- 197.75	552.76 +/- 208.32	518.58 +/- 139.70	486.87 +/- 192.32
14,15-EET	135.46 +/- 24.65	137.80 +/- 33.50	132.31 +/- 22.39	136.73 +/- 18.81	142.01 +/- 47.14	159.14 +/- 71.36	189.80 +/- 30.27	194.48 +/- 33.61
15-HETE	630.36 +/- 201.86	608.58 +/- 224.90	560.42 +/- 112.20	582.06 +/- 177.47	673.98 +/- 205.09	729.46 +/- 265.38	582.40 +/- 177.82	557.70 +/- 200.69
16,17-DiHDPa	214.50 +/- 86.15	186.22 +/- 98.47	205.65 +/- 57.06	197.58 +/- 49.73	265.22 +/- 80.64	310.76 +/- 178.81	251.74 +/- 95.05	212.24 +/- 100.63
16,17-EpDPA	8.76 +/- 6.59	6.77 +/- 5.92	6.42 +/- 4.49	7.53 +/- 5.81	6.76 +/- 4.48	12.16 +/- 10.26	10.12 +/- 4.70	10.62 +/- 6.26
17,18-DiHETE	5327.85 +/- 1863.43	4890.58 +/- 1528.35	4586.01 +/- 1850.24	4670.83 +/- 1933.60	5428.40 +/- 1790.72	5968.92 +/- 2390.01	5650.09 +/- 1982.79	5579.31 +/- 2320.80
17,18-EpETE	26.66 +/- 15.79	26.90 +/- 15.27	21.73 +/- 10.58	25.51 +/- 12.01	38.95 +/- 46.74	43.45 +/- 41.39	27.17 +/- 17.81	32.93 +/- 16.13
17-HDHA	614.84 +/- 271.40	568.00 +/- 266.54	502.25 +/- 192.94	604.93 +/- 294.30	753.53 +/- 288.22	784.31 +/- 286.74	542.13 +/- 154.64	569.20 +/- 218.91
19,20-DiHDPa	1333.80 +/- 434.45	1246.47 +/- 352.98	1145.03 +/- 357.58	1111.86 +/- 343.01	1436.90 +/- 572.54	1799.11 +/- 1115.39	1453.02 +/- 546.38	1386.41 +/- 562.86
19,20-EpDPE	286.65 +/- 140.44	295.41 +/- 138.80	234.74 +/- 112.37	259.86 +/- 110.35	335.37 +/- 168.41	375.81 +/- 228.45	230.91 +/- 91.57	268.88 +/- 104.98
19-HETE	347.53 +/- 117.81	334.88 +/- 154.36	290.43 +/- 85.84	304.21 +/- 128.49	342.39 +/- 113.74	427.68 +/- 347.58	312.88 +/- 64.71	283.81 +/- 79.17
20-HETE	613.01 +/- 267.54	622.08 +/- 250.64	515.99 +/- 250.31	556.25 +/- 258.06	621.05 +/- 286.64	932.23 +/- 967.64	539.20 +/- 175.81	581.59 +/- 195.99
22-HDoHE	692.81 +/- 472.69	641.65 +/- 344.65	588.10 +/- 401.03	578.95 +/- 434.97	705.36 +/- 298.96	1006.04 +/- 747.54	554.12 +/- 175.66	621.94 +/- 221.45
5,6-DHET	194.94 +/- 65.10	183.81 +/- 58.63	161.05 +/- 45.31	165.99 +/- 42.69	217.60 +/- 64.76	260.42 +/- 161.43	227.68 +/- 64.04	236.83 +/- 91.76
5-HETE	664.41 +/- 201.19	681.22 +/- 345.93	634.91 +/- 277.15	626.83 +/- 286.91	726.02 +/- 229.62	849.57 +/- 357.25	685.16 +/- 334.29	599.58 +/- 208.14
8,9-DHET	286.27 +/- 81.70	273.01 +/- 84.98	254.34 +/- 64.99	250.00 +/- 57.53	281.59 +/- 61.67	370.24 +/- 257.67	264.29 +/- 48.77	262.20 +/- 73.30
8,9-EET	163.31 +/- 62.06	151.44 +/- 83.53	153.91 +/- 65.30	142.60 +/- 52.38	130.78 +/- 48.71	167.52 +/- 134.29	124.91 +/- 53.33	148.75 +/- 55.21
8-HETE	4491.92 +/- 1358.56	4415.49 +/- 2278.26	4089.46 +/- 1163.26	4163.35 +/- 1389.19	4321.26 +/- 1488.35	4832.95 +/- 2401.41	3750.38 +/- 965.47	3947.17 +/- 1076.53
9,10-DHOME	7702.51 +/- 10872.12	6537.89 +/- 5167.69	8667.30 +/- 11896.70	8639.50 +/- 12239.70	1829.34 +/- 1792.33	5999.53 +/- 4616.08	1811.01 +/- 2032.68	2425.65 +/- 2457.76
9,10-EpOME	1128.83 +/- 1227.68	1200.93 +/- 875.35	1302.30 +/- 1483.98	1723.68 +/- 3191.92	496.00 +/- 462.63	1002.85 +/- 561.32	1150.26 +/- 938.12	1765.72 +/- 1680.72
9-HODE	52812.76 +/- 62837.09	58807.70 +/- 40635.86	59976.53 +/- 74225.56	61541.24 +/- 103677.64	24826.79 +/- 27809.08	59733.08 +/- 42784.63	19618.96 +/- 20114.75	35214.75 +/- 39553.89

Supplementary Figure 3 - Changes to epoxy-oxylin levels in peripheral blood with prophylactic and therapeutic dosing with GSK2256294 during inflammation.

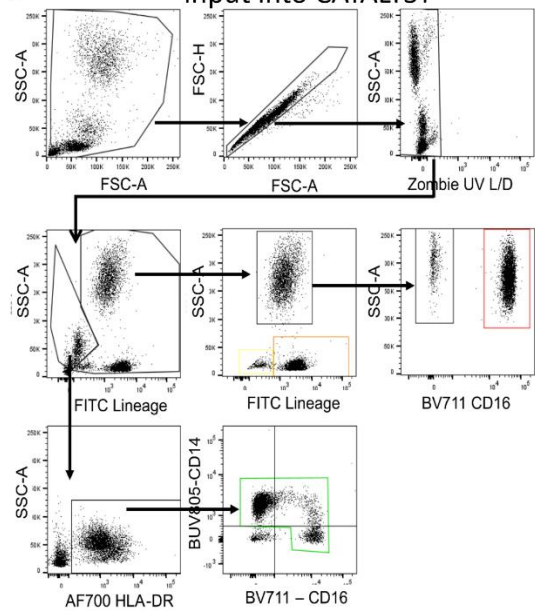
The forearms of participants were intradermally injected with UV-killed *E. coli* (UVKEc) resulting in a local and peripheral inflammatory response. 2 hrs prior to (prophylactic) or 4 hrs after (therapeutic) UVKEc injection, participants were dosed with 15 mg of GSK2256294. Plasma was collected and subject to lipidomic analysis at all timepoints. Data are presented as mean \pm SD. In the therapeutic arm of the study each data point is representative of: (untreated: n = 11-12; GSK2256294: n = 11-12; biologically independent samples). In the prophylactic arm of the study each data point is representative of: (untreated: n = 11-12; GSK2256294: n = 12; biologically independent samples).

Supplementary, Figure 4

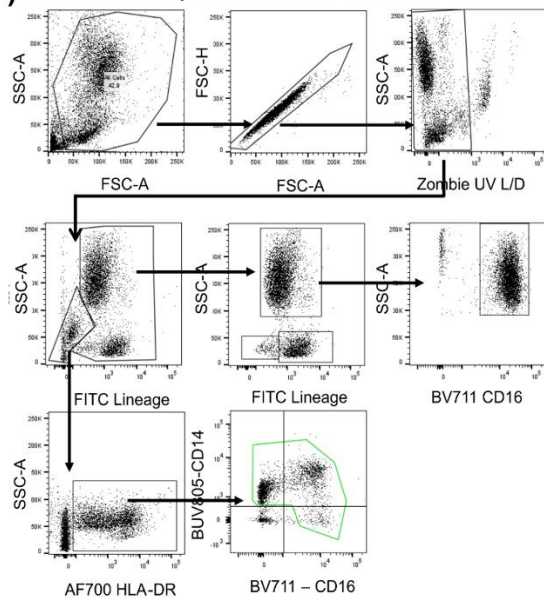
A) Gating strategy for monocyte populations from peripheral blood



B) Gating strategy for monocyte populations from peripheral blood for input into CATALYST



D) Gating strategy for monocyte populations from blister fluid for input into CATALYST



Supplementary Figure 4 - Flow cytometry gating strategies for identification of monocyte subsets in peripheral blood and blister.

(A) Identification of monocyte subsets from peripheral blood for analysis in FlowJo. An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets and dead cells. NK cells, T cells, B cells and neutrophils are removed from the sample by gating for the FITC positive cells, which includes markers for CD3, CD56, CD19, CD20 and CD66b. T cells and B cells are gated in orange and NK cells in yellow. Neutrophils are assigned based on high SSC and high CD16 expression. The FITC negative cells are taken forward and gated for AF700 HLA-DR positive cells. Classical (CD14+CD16-), Intermediate (CD14+CD16+) and Non-Classical (CD14-CD16+) subsets are assigned according to CD14 and CD16 expression. pDCs are taken from the CD14-CD16- and assigned as CD123+. cDCs are taken from the CD123- cells and assigned according to expression of CD141 (cDC1) and CD1c (cDC2).

(B) Identification of HLA-DR+CD14+CD16+ monocytes from peripheral blood for input into CATALYST for supervised clustering. An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets and dead cells. NK cells, T cells, B cells and neutrophils are removed from the sample by gating for the FITC positive cells, which includes markers for CD3, CD56, CD19, CD20 and CD66b. T cells and B cells are gated in orange and NK cells in yellow. Neutrophils are assigned based on high SSC and high CD16 expression. The FITC negative cells are taken forward and gated for AF700 HLA-DR positive cells. Monocytes that are used in the supervised clustering are gated in green.

(C) Identification of monocyte subsets from the blister for analysis in FlowJo. An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets and dead cells. NK cells, T cells, B cells and neutrophils are removed from the sample by gating for the FITC positive cells, which includes markers for CD3, CD56, CD19, CD20 and CD66b. T cells and B cells are gated in orange and NK cells in yellow. Neutrophils are assigned based on high SSC and high CD16 expression. The FITC negative cells are taken forward and gated for AF700 HLA-DR positive cells. Classical (CD14+CD16-), Intermediate (CD14+CD16+) and Non-Classical (CD14-CD16+) subsets are assigned according to CD14 and CD16 expression. pDCs are taken from the CD14-CD16- and assigned as CD123+. cDCs are taken from the CD123- cells and assigned according to expression of CD141 (cDC1) and CD1c (cDC2).

(D) Identification of HLA-DR+CD14+CD16+ monocytes from the blister for input into CATALYST for supervised clustering. An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets and dead cells. NK cells, T cells, B cells and neutrophils are removed from the sample by gating for the FITC positive cells, which includes markers for CD3, CD56, CD19, CD20 and CD66b. T cells and B cells are gated in orange and NK cells in yellow. Neutrophils are assigned based on high SSC and high CD16 expression. The FITC negative cells are taken forward and gated for AF700 HLA-DR positive cells. Monocytes that are used in the supervised clustering are gated in green.

Supplementary, Figure 5

Prophylactic

Cell Type	0hr Untreated	4hr Untreated	24hr Untreated	48hr Untreated	0hr Treated	4hr Treated	24hr Treated	48hr Treated
Neutrophil	2547074.75 +/- 836542.25	5738180.89 +/- 2641353.55	3866420.34 +/- 802199.04	3128995.31 +/- 2214850.99 +/- 1735260.66	840392.58	5259655.24 +/- 1361479.42	2944666.50 +/- 1005366.72	2269832.09 +/- 1084821.33
Eosinophil	195722.60 +/- 119931.06	100279.27 +/- 64292.90	94212.17 +/- 55836.45	66594.42	88841.50 +/- 27403.35	57496.25 +/- 28396.90	48451.18 +/- 21655.40	62653.72 +/- 26124.36
B cells	115535.59 +/- 57976.33	77568.19 +/- 41584.63	89312.18 +/- 42852.84	70005.19	96165.82 +/- 35613.06	77709.90 +/- 69723.13	76061.29 +/- 45727.35	95072.84 +/- 53696.03

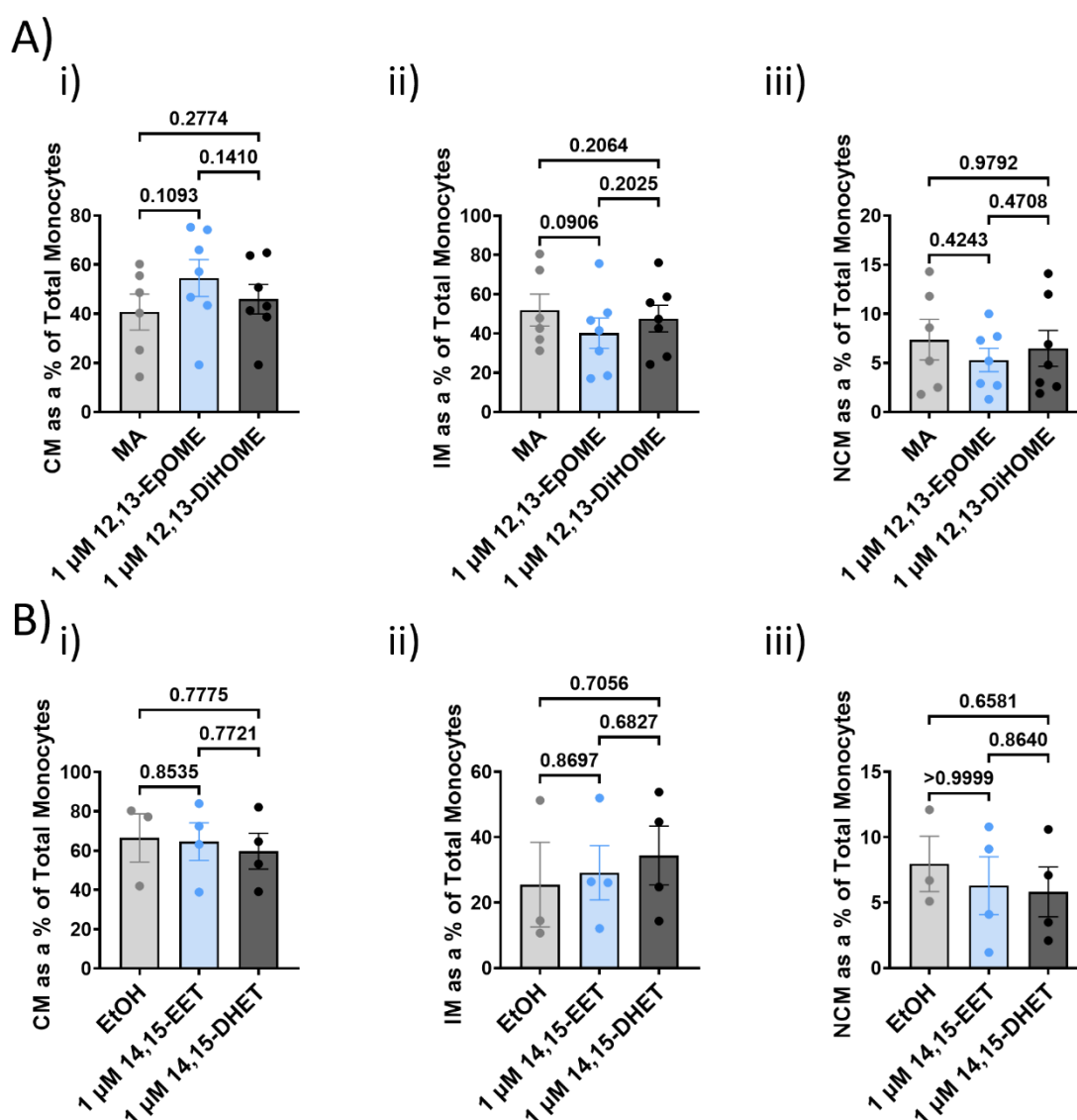
Therapeutic

Cell Type	0hr Untreated	4hr Untreated	24hr Untreated	48hr Untreated	0hr Treated	4hr Treated	24hr Treated	48hr Treated
Neutrophil	2003764.00 +/- 1195907.70	6646634.71 +/- 1793999.42	3858131.55 +/- 1043502.81	2650358.28 +/- 1253299.05	2279707.91 +/- 924942.65	8137764.90 +/- 3071801.72	4380888.53 +/- 1861149.24	2619628.43 +/- 1116279.46
Eosinophil	65604.46 +/- 77356.56	59161.46 +/- 80259.56	82048.13 +/- 117597.84	144594.90	89266.36 +/- 80901.99	76746.01 +/- 53832.06	80196.66 +/- 75411.85	129348.80 +/- 142997.66
CD4 T cell	476038.31 +/- 180692.74	290370.53 +/- 86552.49	285085.76 +/- 97977.00	238595.92	521125.26 +/- 348115.54	358384.85 +/- 182923.81	407767.11 +/- 157857.74	365355.63 +/- 98722.24
CD8 T cell	240006.56 +/- 147139.03	136819.56 +/- 71972.94	163149.86 +/- 75851.58	155009.88	275976.86 +/- 109223.61	195028.18 +/- 74738.32	242629.40 +/- 67854.79	233306.20 +/- 96027.19
Natural Killer cell	132858.95 +/- 67786.86	121055.06 +/- 106192.30	143642.44 +/- 93844.28	94191.90	88427.61 +/- 65205.10	77126.10 +/- 45311.90	90308.68 +/- 50585.20	109124.95 +/- 100744.03
Plasmacytoid Dendritic Cell	9314.52 +/- 5928.65	9247.36 +/- 4896.58	11529.55 +/- 4899.29	5446.37	4856.87 +/- 1573.46	6782.25 +/- 2560.22	6283.16 +/- 2848.51	7508.95 +/- 5517.02
Classical Dendritic Cell 1	1056.78 +/- 623.26	1000.09 +/- 632.66	1510.58 +/- 1005.48	1321.34	722.02 +/- 387.20	508.25 +/- 216.11	553.92 +/- 495.30	1101.13 +/- 956.49
Classical Dendritic Cell 2	5112.44 +/- 2681.07	6573.38 +/- 2870.89	5654.89 +/- 3834.42	11447.18 +/- 11652.67	4064.48 +/- 1991.11	4814.88 +/- 1941.82	3178.69 +/- 1428.56	3516.10 +/- 1863.77
B cells	133645.00 +/- 71925.39	94552.58 +/- 49289.85	73979.17 +/- 27958.70	67536.89	115666.52 +/- 43817.93	100827.27 +/- 42160.17	108533.73 +/- 28391.22	118430.57 +/- 29700.16
FOXP3 T cells	1125.30 +/- 1170.50	798.18 +/- 1257.25	847.48 +/- 792.27	771.51	113.41 +/- 133.98	65.15 +/- 44.07	250.94 +/- 402.36	1008.33 +/- 1854.95

Supplementary Figure 5 – Changes to leukocyte numbers in peripheral blood with prophylactic and therapeutic dosing with GSK2256294 during inflammation.

The forearms of participants were intradermally injected with UV-killed *E. coli* (UVKEc) resulting in a local and peripheral inflammatory response. 2 hrs prior to (prophylactic) or 4 hrs after (therapeutic) UVKEc injection, participants were dosed with 15 mg of GSK2256294, a soluble epoxide hydrolase inhibitor. Peripheral blood was collected, and leukocytes analysed at baseline and 4 hrs, 24 hrs and 48 hrs post UVKEc injection using multiparameter flow cytometry. Data are presented as mean cell number per ml \pm SD.

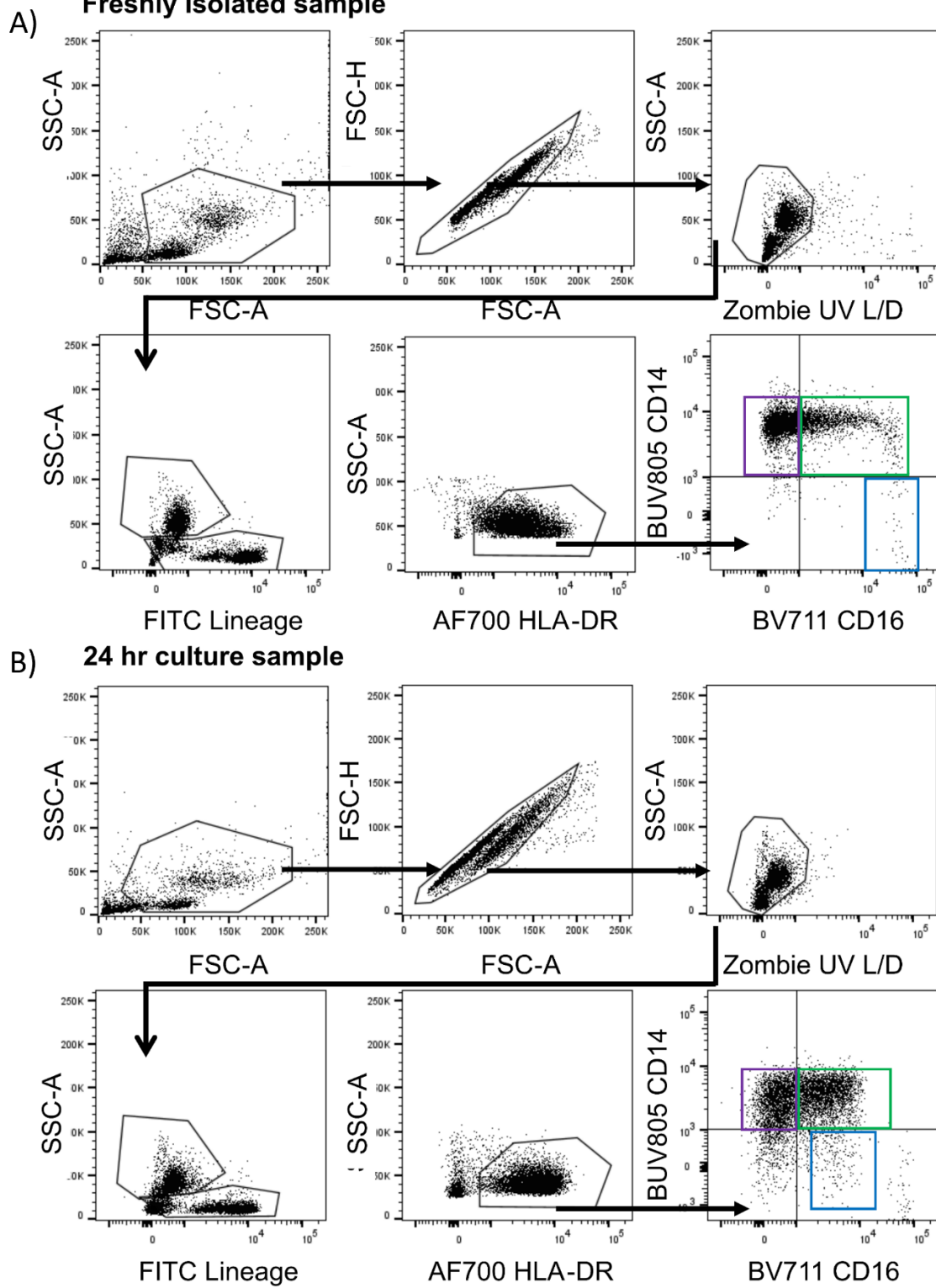
Supplementary, Figure 6



Supplementary Figure 6 – Effect of 12,13-EpOME and 12,13-DiHOME on monocyte differentiation *in vitro*.

PBMCs (2×10^6 /well) were seeded and treated with 1 μ M epoxy-oxylipin or vehicle for 24 h. Cells were phenotyped by flow cytometry. (A) (i) % classical monocytes (CM), (ii) % intermediate monocytes (IM), (iii) % non-classical monocytes (NCM) with 1 μ M 12,13-EpOME, 1 μ M 12,13-DiHOME or MA (MA: n = 6; 12,13-EpOME and 12,13-DiHOME: n = 7; biologically independent samples). (B) (i) % CM, (ii) IM, and (iii) NCM with 1 μ M 14,15-EET, 1 μ M 14,15-DHET or EtOH (EtOH: n = 3; 14,15-EET and 14,15-DHET: n = 4; biologically independent samples). Data were assessed for normalisation using the D'Agostino & Pearson test, Shapiro-Wilk test and visualised using a QQ plot. Parametric data are presented as mean \pm SD. Data were analysed using a one-way-ANOVA. Source data are provided as a Source Data file.

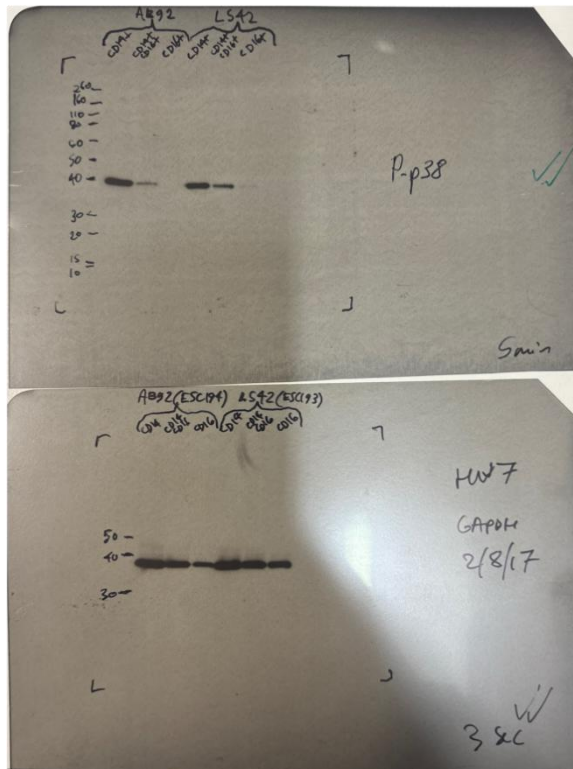
Supplementary, Figure 7



Supplementary Figure 7 - Flow cytometry gating strategies for identification of monocyte subsets in (A) freshly isolated peripheral blood and (B) after 24 hrs in culture.

An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets and dead cells using Zombie UV. NK cells, T cells and B cells are removed from the sample by gating for the FITC positive cells, which includes markers for CD3, CD56 and CD19. The FITC negative cells are taken forward and gated for AF700. Classical (CD14+CD16-), Intermediate (CD14+CD16+) and Non-Classical (CD14-CD16+) subsets are assigned according to CD14 and CD16 expression.

A)



ES 201 ES 202

201p 201b 201c 201d 201e 201f 201g 201h 201i 201j 201k 201l 201m 201n 201o 201p 201q 201r 201s

200
180
160
140
120
100
80
60
50
40
30
20
15
10

✓✓

HW8

10p38

4/8/17

5 min

200
180
160
140
120
100
80
60
50
40
30
20
15
10

← COX2

← GAPDH

HW8 ✓✓

GAPDH

20x2

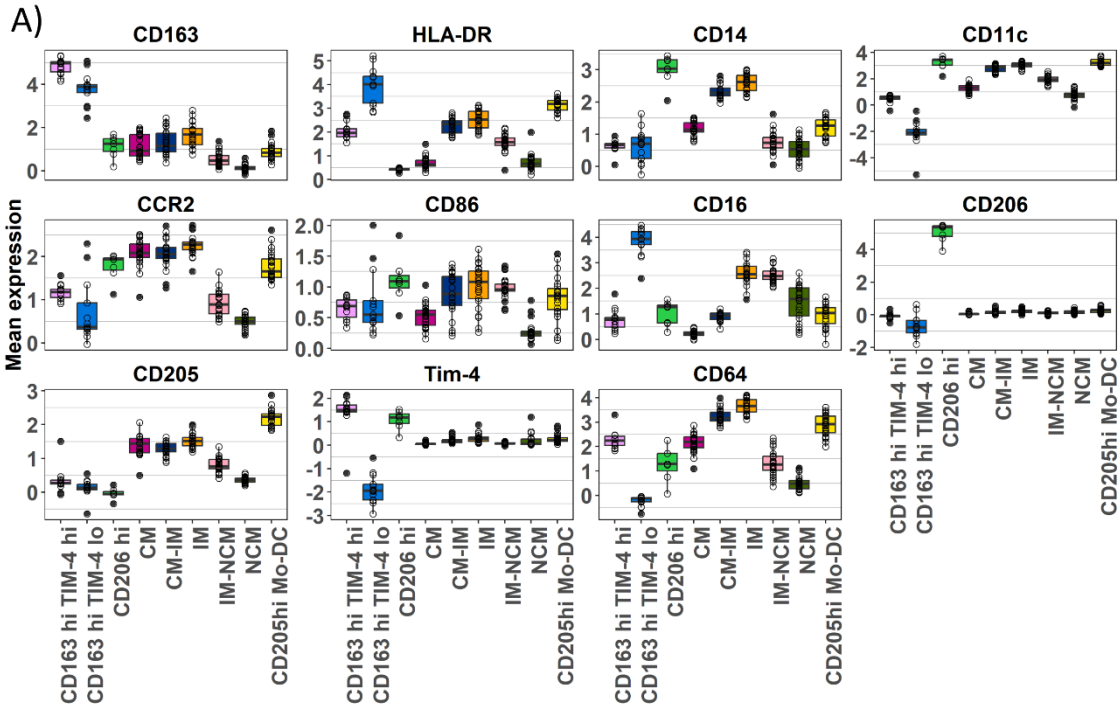
16/08/17

HT

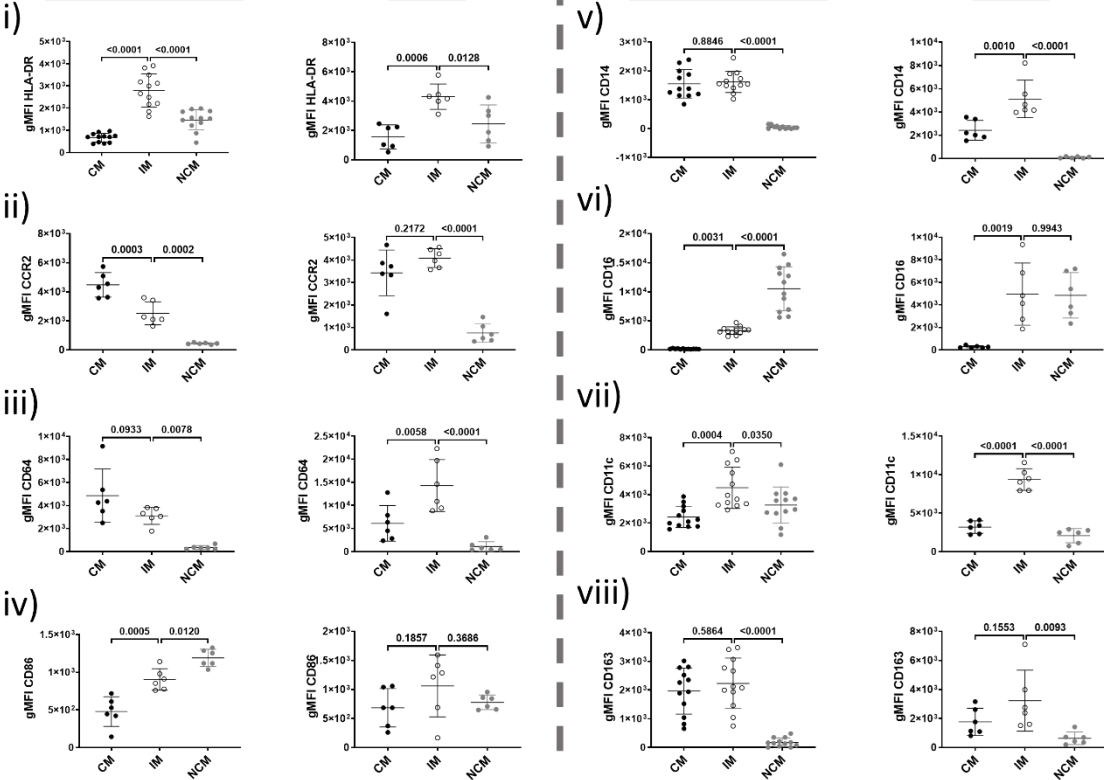
(A) Expression of P-p38 and GAPDH in freshly isolated monocyte subsets for donors AB92 and L542.

(B) Expression of P-p38 and GAPDH in freshly isolated monocyte subsets for donors ESC201 and ESC202.

Supplementary, Figure 9



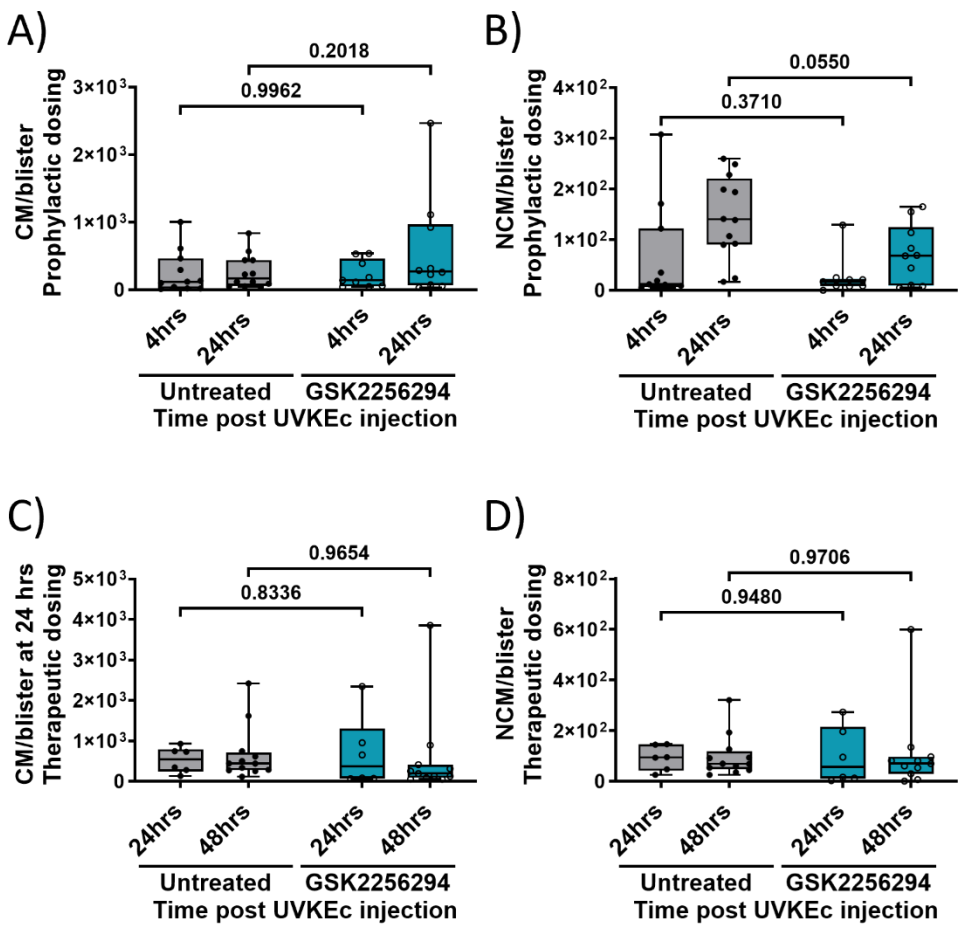
B) Peripheral blood



Supplementary Figure 9 – Marker expression in mononuclear phagocyte clusters in the blister

(A) Boxplots of marker expression in mononuclear phagocyte populations in the blister. Total monocytes were extracted from FCS files in FlowJo and then subsequently clustered using the package CATALYST based on the expression of CD163, HLA-DR, CD14, CD16, CD11c, CCR2, CD86, CD16, CD206, CD205, Tim-4 and CD64. Eight monocyte populations were identified and labelled (n = 6; biologically independent samples). (B) Monocyte phenotype in blood vs. blister 24 hrs after inflammatory onset. The forearms of participants were intradermally injected with UV-killed *E. coli* (UVKEc) resulting in a local and peripheral inflammatory response. Peripheral bloods and local inflammatory exudate were collected and analysed at baseline and 4 hrs, 24 hrs and 48 hrs post UVKEc injection. Data shows monocyte expression for (i) HLA-DR, (ii) CCR2, (iii) CD64, (iv) CD86, (v) CD14, (vi) CD16, (vii) CD11c and (viii) CD163 on peripheral blood or blister monocyte subsets at 24 hrs post UVKEc. Data are presented as mean \pm SD. Peripheral blood HLA-DR, CD14, CD16, CD11c and CD163 n = 12 (biologically independent samples). Peripheral blood CCR2, CD64, CD86 n = 6 (biologically independent samples). Blister samples n = 6 (biologically independent samples). Data were analysed using a one-way-ANOVA. Source data are provided as a Source Data file.

Supplementary, Figure 10

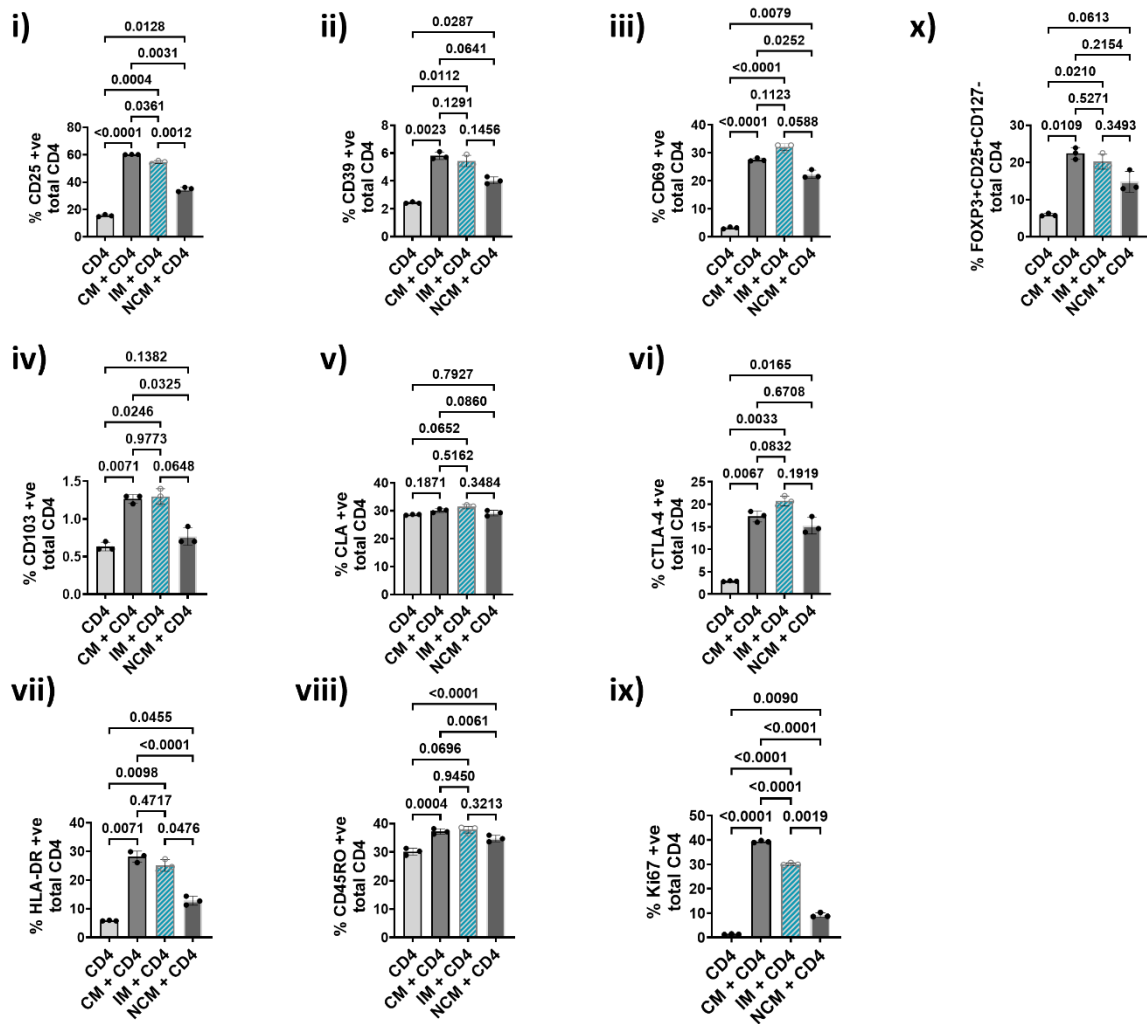


Supplementary Figure 10 – Classical and Non-classical monocytes per blister in the prophylactic and therapeutic arms of the study.

The forearms of participants were intradermally injected with UV-killed *E. coli* (UVKEc) resulting in a local inflammatory response. 2 hrs prior to (prophylactic) or 4 hrs after (therapeutic) UVKEc injection, participants were dosed with 15 mg of GSK2256294. Local inflammatory exudate was collected and subject to lipidomic and flow cytometric analysis at 4 and 24 hrs (prophylactic) and 24 and 48 hrs (therapeutic).

Classical monocytes (CD14+CD16-), intermediate monocytes (CD14+CD16+) and non-classical monocytes (CD14-CD16+) were quantified using manual gating in FlowJo. (A) Classical monocytes/blister at 4 and 24 hrs (prophylactic) (untreated: n = 12; GSK2256294: n = 10; biologically independent samples). (B) Non-classical monocytes/blister at 4 and 24 hrs (prophylactic) (untreated: n = 12; GSK2256294: n = 10; biologically independent samples). (C) Classical monocytes/blister at 24 and 48 hrs (therapeutic) (untreated: n = 6-12; GSK2256294: n = 6-11; biologically independent samples). (D) Non-classical monocytes/blister at 24 and 48 hrs (therapeutic) (untreated: n = 6-12; GSK2256294: n = 6-11; biologically independent samples). Normality was assessed using the D'Agostino & Pearson test, Shapiro-Wilk test and visualised using a QQ plot. Box-and-whisker plots show the median (center line), the interquartile range (25th–75th percentiles; box), and the full data range (whiskers, minimum to maximum). Data were analysed using two-way-ANOVA mixed effect analysis with Uncorrected Fisher's LSD. Source data are provided as a Source Data file.

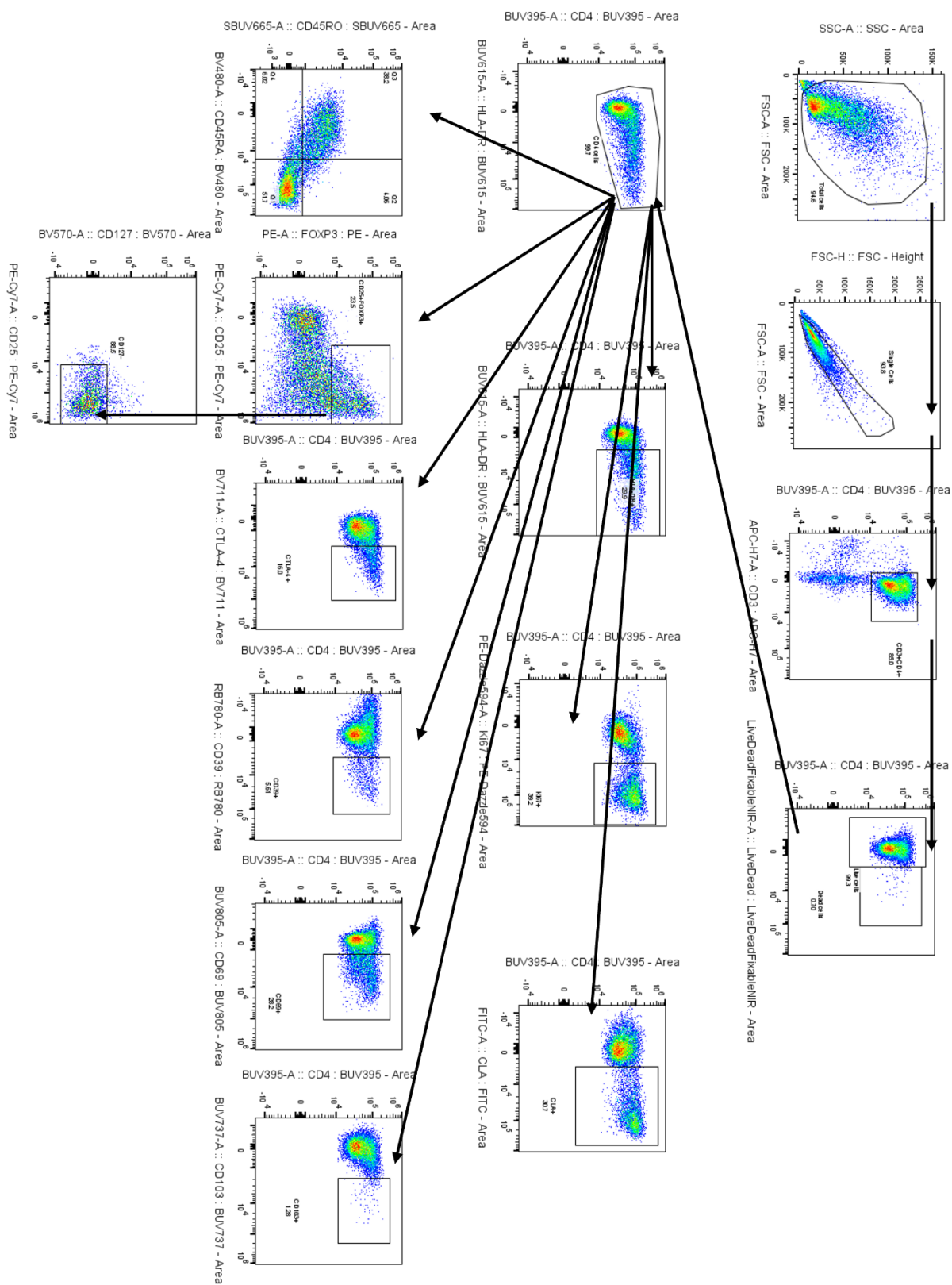
Supplementary, Figure 11



Supplementary Figure 11 – CD4 T cell phenotype after co-culture with monocyte subsets

Classical, intermediate and non-classical monocytes were sorted using FACS and co-cultured with CD4 T cells at a 5:1 (T cell:monocyte) ratio for 48 hours and subsequently analysed by multi-parameter spectral flow cytometry. The % expression on total CD4 cells was visualised for (i) CD25, (ii) CD39, (iii) CD69, (iv) CD103, (v) CLA, (vi) CTLA-4, (vii) HLA-DR, (viii) CD45RO, (ix) Ki67 and (x) FOXP3+CD25+CD127- (n = 3; technical repeat). Parametric data are presented as mean \pm SD. Data were analysed using a one-way-ANOVA. Source data are provided as a Source Data file.

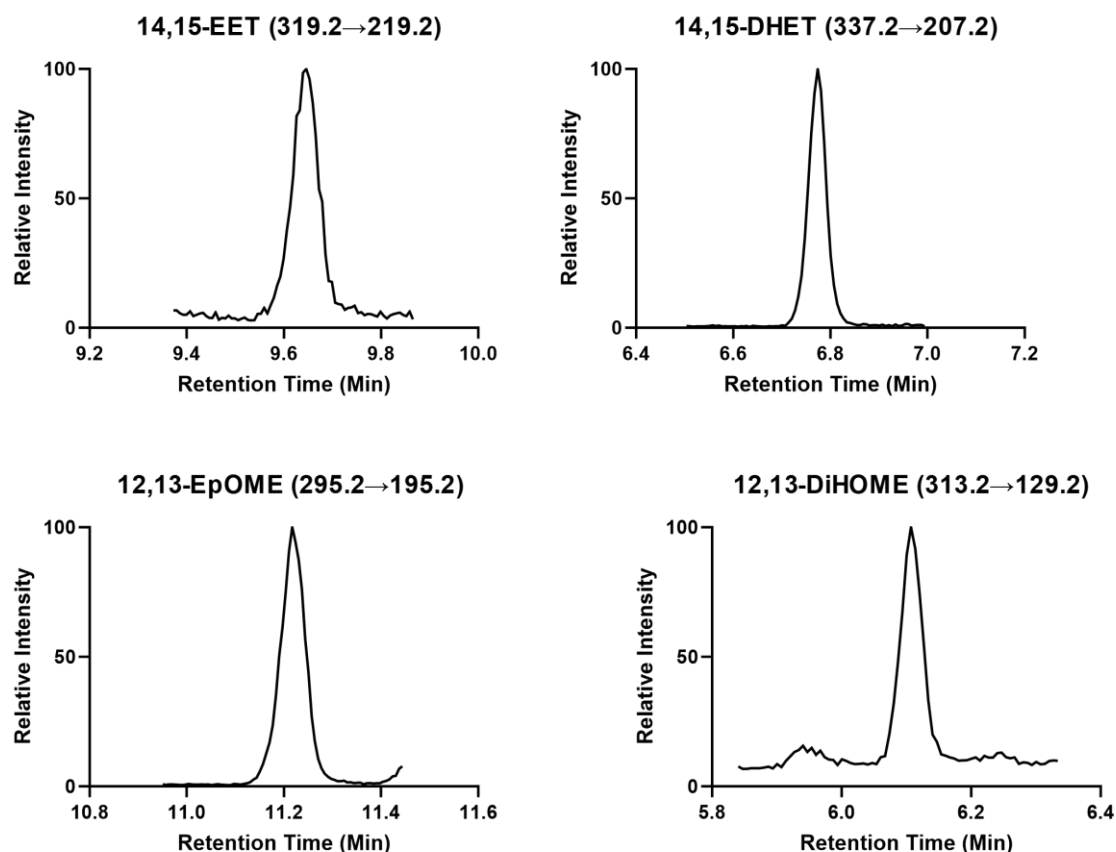
Supplementary, Figure 12



Supplementary Figure 12 – Gating strategy for CD4 phenotyping flow cytometry experiments

Plot representative of a PBMC sample. An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets. CD4⁺ T cells were gating based on the expression of CD4 and CD3. Dead cells were excluded using Zombie NIR. Representative plots of expression on total CD4 cells are shown for HLA-DR, Ki67, CLA, CD45RO, CTLA-4, CD39, CD69, CD103. CD25⁺FOXP3⁺ cells were gated and these cells were taken forward and a gate on the CD127 cells was assigned. These cells were designated CD25⁺FOXP3⁺CD127⁻ T-regulatory cells.

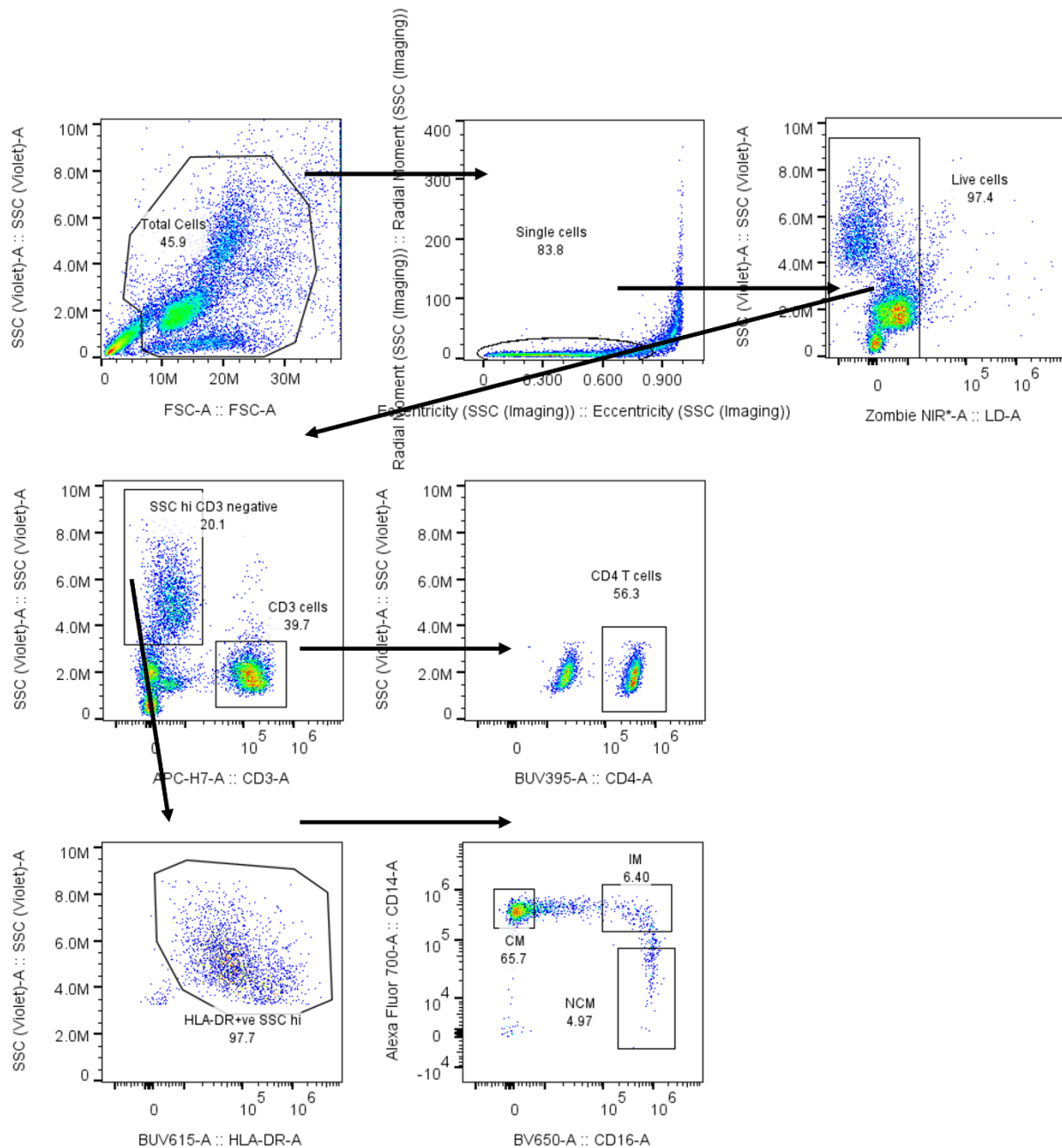
Supplementary, Figure 13



Supplementary Figure 13 -Chromatograms of select plasma LCMS analytes.

LCMS analysis of plasma oxylipins was performed as described in methods. Representative chromatograms of an extracted plasma sample for 14,15-EET (m/z transition 319.2→219.2), 14,15-DHET (m/z transition 337.2→207.2), 12,13-EpOME (m/z transition 295.2→195.2) and 12,13-DiHOME (m/z transition 313.2→129.2) are shown as relative intensity (max value for each peak set to 100).

Supplementary, Figure 14



Supplementary Figure 14 - Gating strategy to FACS sort monocytes and CD4 T cells from PBMCs.

Plot representative of a PBMC sample. An initial gate around all leukocytes assigned. These cells are taken forward to exclude doublets and dead cells using Zombie NIR. CD3+CD4+ positive cells are gated for using CD3 on APC-H7 and CD4 on BUV395. Monocytes are gated as CD3-ve SSC hi HLA-DR+ve and then into the three monocyte subsets based on the expression of CD14 on AF700 and CD16 on BV650. Classical monocytes (CM) are defined as CD14+CD16-, intermediate monocytes are defined as CD14+CD16+ and non-classical monocytes (NCM) are defined as CD14-CD16+.