

SUPPLEMENTAL MATERIAL

Supplemental Tables

Table S1. Product details of cytokines and chemokines measured with ELISA.

	Item	Manufacturer	Category number
24 hour stimulation	Human TNF α duoset	R&D	DY210
	Human IL-1 β duoset	R&D	DY201
	Human IL-6 duoset	R&D	DY206
	Human Pelipair IL-8	Sanquin	M9318
	Human Pelipair IL-10	Sanquin	M9310
7 day stimulation	Human PelipairIFN γ	Sanquin	M9333
	Human IL-17 duoset	R&D	DY317
	Human IL-22 duoset	R&D	DY782
Plasma	Human VCAM-1 duoset	R&D	DY809
	Human E-selectin duoset	R&D	DY724
	Human MMP-2 duoset	R&D	DY902
	Human Leptin duoset	R&D	DY398
	Human Adiponectin duoset	R&D	DY1065

MMP-2 indicates matrix metalloproteinase-2; VCAM-1, vascular cell adhesion molecule 1.

Supplemental Tables S2-S4 show data of group1.0.

Table S2. Circulating cytokines in group1.0 (n=8).

Circulating cytokines	Pre	Post
IL-1RA (pg/mL)	263 [215-377]	260 [199-371]
IL-1 β (pg/mL)	0.15 [0.09-0.17]	0.15 [0.08-0.27]
IL-6 (pg/mL)	2.66 [1.90-4.26]	3.43 [1.82-5.62]
IL-18 (pg/mL)	172 [112-193]	173 [117-226]

* $P < 0.05$, ** $P < 0.01$. Wilcoxon signed rank test, median, IQR. IL: interleukin.

Table S3. Cytokine production capacity in group1.0 (n=8). **A.** Innate cytokine production after 24 hour stimulation, **B.** Adaptive cytokine production after 7 day stimulation.

A. Innate cytokine production	Pre	Post
Unstimulated		
IL-1 β (pg/mL)	78 [78-78]	78 [78-78]
IL-6 (ng/mL)	0.5 [0.5-0.5]	0.5 [0.5-0.5]
IL-8 (ng/mL)	1.6 [1.6-1.6]	1.6 [1.6-1.6]
IL-10 (pg/mL)	47 [47-47]	47 [47-47]
TNF α (pg/mL)	78 [78-127]	78 [78-110]
LPS stimulated		
IL-1 β (pg/mL)	3478 [1689-6317]	12187 [6393-16901]*
IL-6 (pg/mL)	12.6 [7.0-27.7]	23.4 [21.6-25.9]*
IL-8 (ng/mL)	55.6 [27.7-87.7]	88.1 [67.7-123.2]*
IL-10 (pg/mL)	930 [804-1920]	1606 [974-1926]
TNF α (pg/mL)	447 [182-1117]	787 [489-1590]*
P3C stimulated		
IL-1 β (pg/mL)	2637 [1234-4333]	3552 [2617-5441]
IL-6 (ng/mL)	16.4 [10.9-22.6]	20.1 [17.1-26.7]*
IL-8 (ng/mL)	84.4 [70.7-114.6]	123.3 [89.4-157.6]*
IL-10 (pg/mL)	170 [106-768]	200 [128-440]
TNF α (pg/mL)	135 [115-297]	493 [110-923]
R848 stimulated		
IL-1 β (pg/mL)	OFR	OFR
IL-6 (ng/mL)	24.5 [18.7-40.6]	34.3 [26.9-50.1]
IL-8 (ng/mL)	37.7 [24.4-51.5]	67.7 [44.7-85.1]*
IL-10 (pg/mL)	1249 [1050-2642]	1299 [784-3837]
TNF α (pg/mL)	6093 [2811-7898]	5148 [2871-7435]
C16.0 stimulated		
IL-1 β (pg/mL)	39 [39-145]	176 [39-372]*
IL-6 (pg/mL)	94 [94-225]	252 [94-1061]*
IL-8 (ng/mL)	2.1 [1.6-4.5]	6.3 [1.6-20.1]*
TNF α (pg/mL)	272 [168-363]	322 [183-555]
C16.0-MSU stimulated		
IL-1 β (pg/mL)	39 [39-374]	299 [46-798]
IL-6 (pg/mL)	94 [94-1542]	882 [166-1723]
IL-8 (ng/mL)	3.0 [1.6-20.6]	22.9 [1.7-31.2]
TNF α (pg/mL)	98 [78-119]	124 [78-730]

B. Adaptive cytokine production	Pre	Post
Unstimulated		
IFN γ (pg/mL)	12 [12-12]	12 [12-12]
IL-22 (pg/mL)	78 [78-78]	78 [78-78]
IL-17 (pg/mL)	39 [39-39]	39 [39-39]
Candida stimulated		
IFN γ (pg/mL)	448 [179-1032]	293 [110-553]
IL-22 (pg/mL)	4396 [803-9046]	2755 [1132-7182]
IL-17 (pg/mL)	556 [350-819]	444 [373-897]
St. aureus stimulated		
IFN γ (pg/mL)	44 [18-150]	75 [61-1161]*
IL-22 (pg/mL)	158 [78-464]	101 [78-754]
IL-17 (pg/mL)	147 [39-810]	63 [39-262]

* $P < 0.05$, ** $P < 0.01$. Wilcoxon signed rank test, median, IQR. OFR indicates out of range. LPS, lipopolysaccharide; P3C, Pam3CysK4; R848, Resiquimod; C16.0+MSU, C16.0 with monosodium urate crystals.

Table S4. Metabolism: Lactate and oxygen consumption in group1.0 (n=8).

Oxygen consumption (O ₂ /10 ⁶ cells)	Pre	Post
Basal (pmol/sec)	2.5 \pm 0.2	1.9 \pm 0.5
Leak (pmol/sec)	0.6 \pm 0.2	0.6 \pm 0.2
Max (pmol/sec)	9.0 \pm 1.0	7.1 \pm 1.7
ROX (pmol/sec)	0.1 \pm 0.1	0.1 \pm 0.1
Absolute reserve (pmol/sec)	6.5 \pm 0.9	5.2 \pm 1.3
Relative reserve (%)	72 \pm 2	73 \pm 3
Lactate production (pg/mL)	854 [708-986]	1321 [1046-1339]

* $P < 0.05$, ** $P < 0.01$. Wilcoxon signed rank test. Basal respiration (basal), proton leak after inhibition of ATP synthase (leak), maximal oxygen consumption (max), residual oxygen consumption or non-mitochondrial oxygen consumption (ROX).

Table S5. Circulating markers of fat tissue, inflammation, oxidative stress and endothelial dysfunction (n=8).

Circulating markers	Pre	Post
Adiponectin (pg/mL)	4.17 [3.78-4.99]	4.18 [2.75-5.50]
Leptin (µg/mL)	16.2 [10.2-20.0]	14.4 [12.3-31.0]
Leptin adiponectin ratio	5.1 [2.1-7.8]	5.6 [2.3-12.0]
CRP (pg/mL)	2.72 [1.62-3.69]	3.07 [2.22-3.54]
TBAR (nmol/L)	1.38 [1.33-1.72]	1.41 [1.34-1.94]
MMP-2 (ng/mL)	377 [374-421]	383 [360-404]
VCAM-1 (ng/mL)	908 [828-978]	938 [781-978]
E-selectin (ng/mL)	10.3 [7.8-12.8]	10.8 [7.7-12.8]

* $P < 0.05$, ** $P < 0.01$. Wilcoxon signed rank test, median, IQR. MMP-2 indicates matrix metalloproteinase-2; VCAM-1, vascular cell adhesion molecule 1.

Table S6. Cell types in PBMC fraction (n=8).

Cell types in PBMC fraction	Pre	Post
Neutrophils(%)	1.1 [0.7-1.4]	0.9 [0.6-1.3]
Lymphocytes (%)	71 [69-74]	73 [67-81]*
Monocytes(%)	28 [24-30]	26 [18-30]*

* $P < 0.05$, ** $P < 0.01$. Wilcoxon signed rank test, median, IQR.

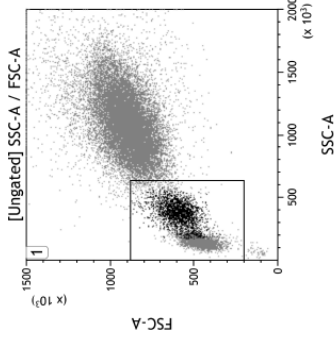
Table S7. Power analysis of cytokine production capacity.

Stimulus	Cytokine	Difference \pm SD	r	Power (1- β)	Sample size needed for $\beta=0.80$
LPS	IL-6 (ng/mL)	-8.1 \pm 7.3	0.46	0.68	10
	IL-10 (pg/mL)	-611 \pm 468	0.55	0.81	8
	IL-8 (ng/mL)	-27 \pm 41	0.21	0.31	24
R848	IL-6 (ng/mL)	-22.4 \pm 5.9	0.93	0.99	4
	IL-10 (pg/mL)	-959 \pm 337	0.92	0.99	4
	IL-8 (ng/mL)	-16.9 \pm 18.2	0.40	0.53	13

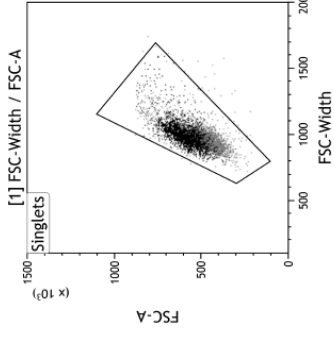
Power calculations of cytokine production capacity using Wilcoxon-signed ranked test. Difference \pm SD indicates the difference in cytokine production capacity before and after intervention. R indicates correlation coefficient, LPS, lipopolysaccharide; R848, Resiquimod.

Supplemental Figure

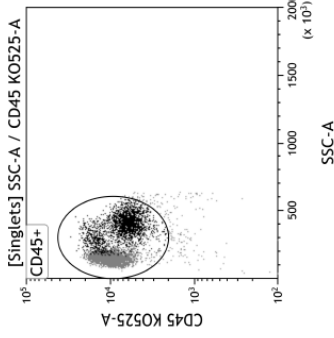
Figure S1. Gating strategy of monocyte subsets and expression markers. Monocytes were selected based on CD45⁺ HLA-DR⁺ and monocyte scatter properties, then CD3⁺ lymphocytes and CD56⁺ NK-cells were excluded, and monocyte subsets were identified in the CD14/CD16 plot as percentage of gated.



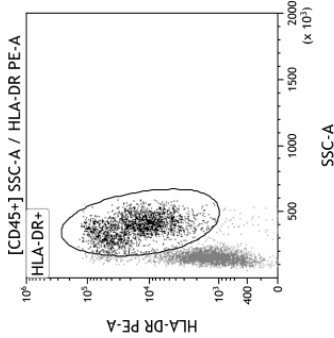
SSC-A	
Gate	%Gated
All	26.859
1	100.00
	5.614
	20.90



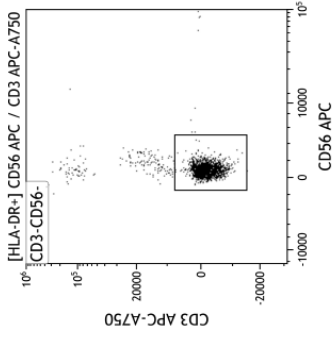
FSC-Width	
Gate	%Gated
All	100.00
Singlets	99.54



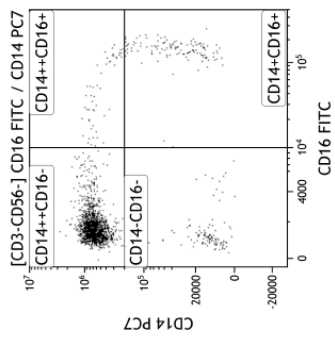
SSC-A	
Gate	%Gated
All	100.00
CD45+	96.66



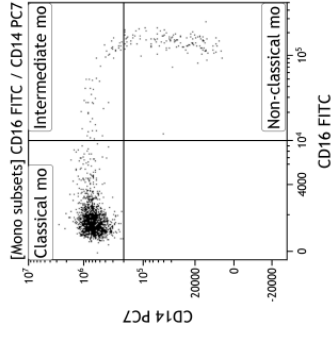
SSC-A	
Gate	%Gated
All	100.00
HLA-DR+	39.94



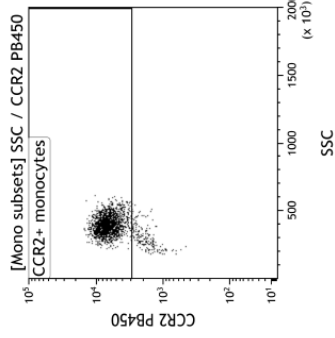
CD56 APC	
Gate	%Gated
All	100.00
CD3-CD56-	91.32



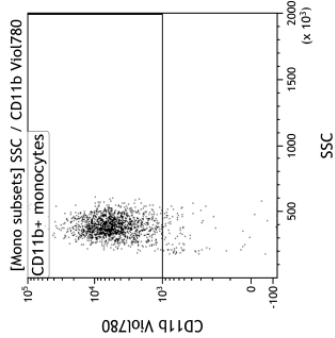
CD16 FITC		
Gate	Number	%Gated
All	1.842	100.00
CD14-CD16-	117	6.35
CD14+-CD16-	1.545	83.88
CD14++CD16+	53	2.88
CD14+CD16+	127	6.89



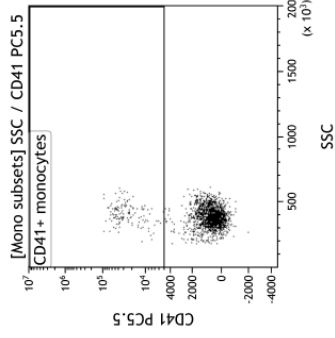
CD16 FITC		
Gate	Number	%Gated
All	1.725	100.00
Classical mo	1.545	89.57
Intermediate mo	53	3.07
J--	0	0.00
Non-classical mo	127	7.36



SSC		
Gate	Number	%Gated
All	1.725	100.00
CCR2+ monocytes	1.541	89.33
	5.644	326.22
	6.481	42.42



SSC		
Gate	Number	%Gated
All	1.725	100.00
CD11b+ monocytes	1.595	92.46
	4.260	246.12
	5.536	318.82



SSC		
Gate	Number	%Gated
All	1.725	100.00
CD41+ monocytes	1.31	7.59
	1.147	66.16
	24.909	1442.29