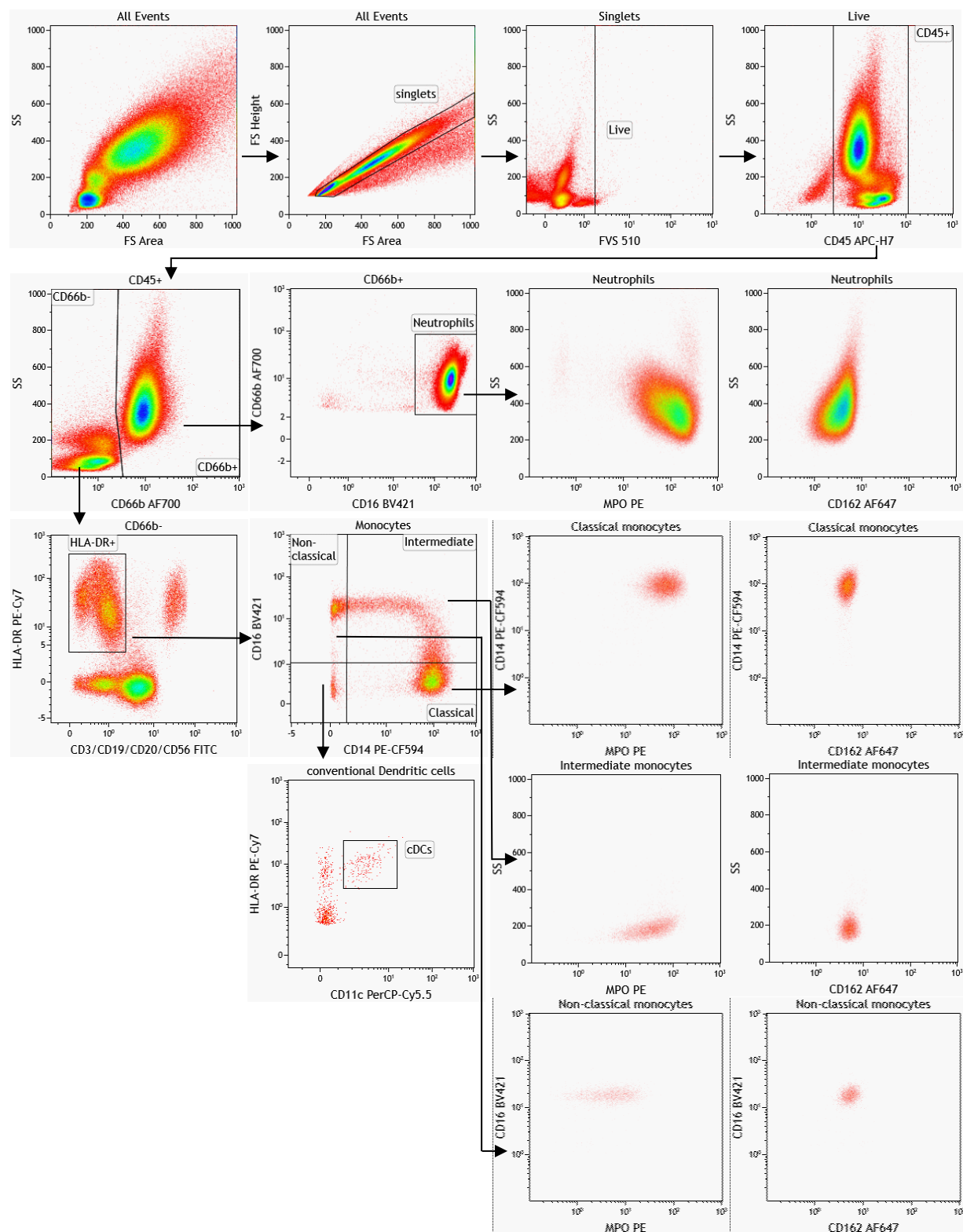


Supplementary Material**1 Supplementary Data****Supplementary Table 1: Overview of the antibodies that were used in this study**

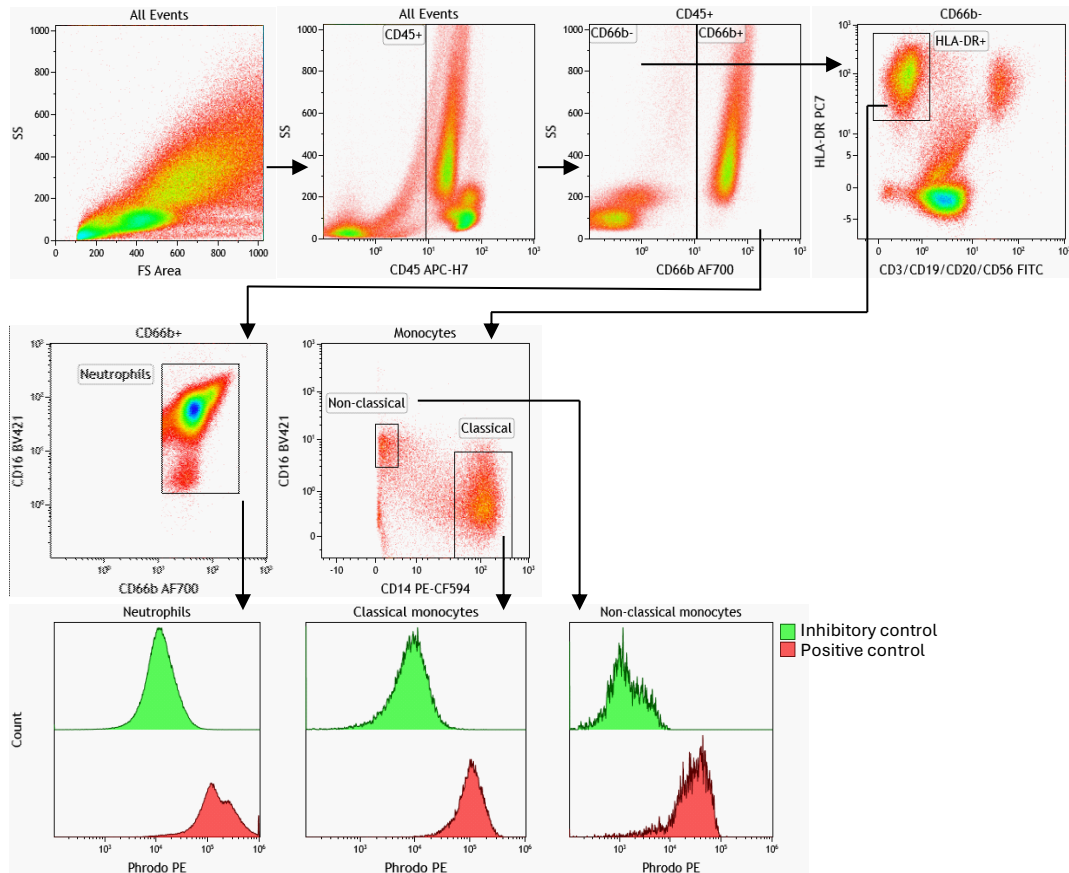
Marker	Fluorochrome	Clone	Catalogue number	Company
CD3	FITC	UCHT1	555332	BD Biosciences
CD19	FITC	HIB19	555412	BD Biosciences
CD20	FITC	2H7	555622	BD Biosciences
CD56	FITC	NCAM16.2	345811	BD Biosciences
MPO	PE	5B8	333139	BD Biosciences
CD14	PE-CF594	MφP9	562335	BD Biosciences
CD11c	PerCP-Cy5.5	B-ly6	565227	BD Biosciences
HLA-DR	PE- Cy7	L243	335830	BD Biosciences
CD162	Alexa Fluor 647	KPL-1	562758	BD Biosciences
CD66b	Alexa Fluor 700	G10F5	305114	Biolegend
CD45	APC- H7	2D1	560178	BD Biosciences
CD16	BV421	3G8	562874	BD Biosciences
Fixable viability stain (FVS)	510		564406	BD Biosciences
CD14	APC-Cy7	MφP-9	557831	BD Biosciences
CD16	PE-CF594	3G8	562293	BD Biosciences
Fixable viability stain (FVS)	520		564407	BD Biosciences
IL-6	PE-CF594	MQ2-13A5	563543	BD Biosciences
TNF-a	PerCP-Cy5.5	MAb11	560679	BD Biosciences
IL-1B	Alexa Fluor 647	AS10	567773	BD Biosciences
CD14	APC- H7	MφP9	560180	BD Biosciences
IL-8	BV510	G265-8	563311	BD Biosciences
Fixable viability stain (FVS)	570		564995	BD Biosciences
CD14	APC	M5E2	555399	BD Biosciences

Figure 1

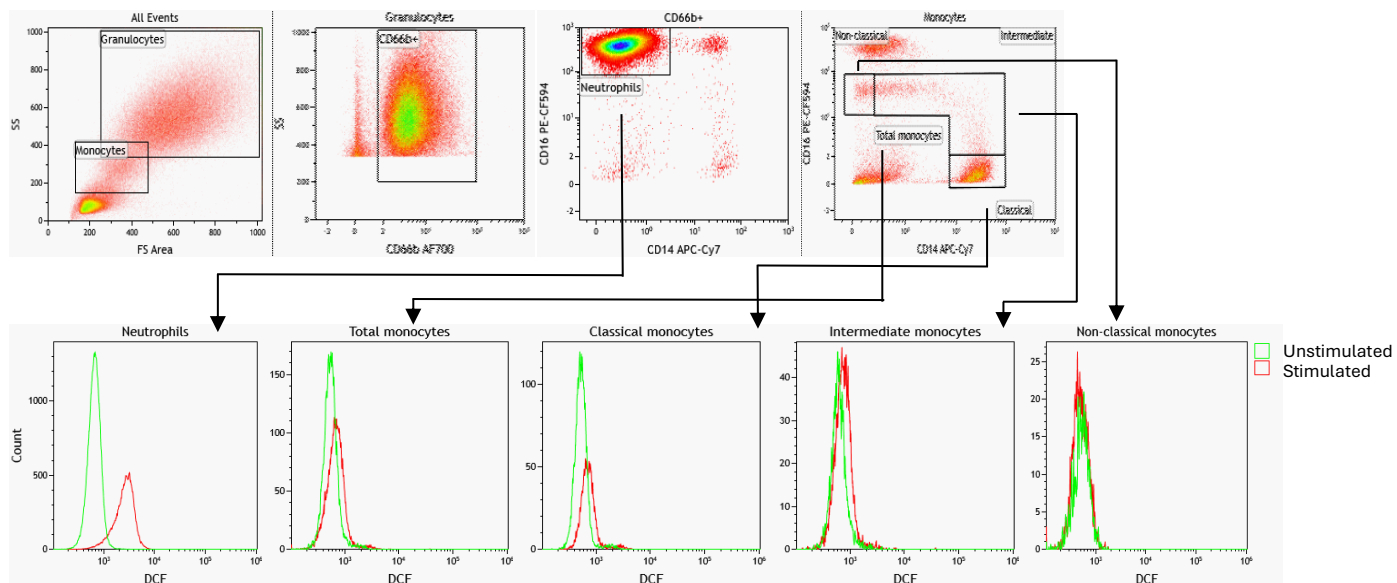
A Immunophenotype of circulating neutrophils, cDCs and monocyte subsets



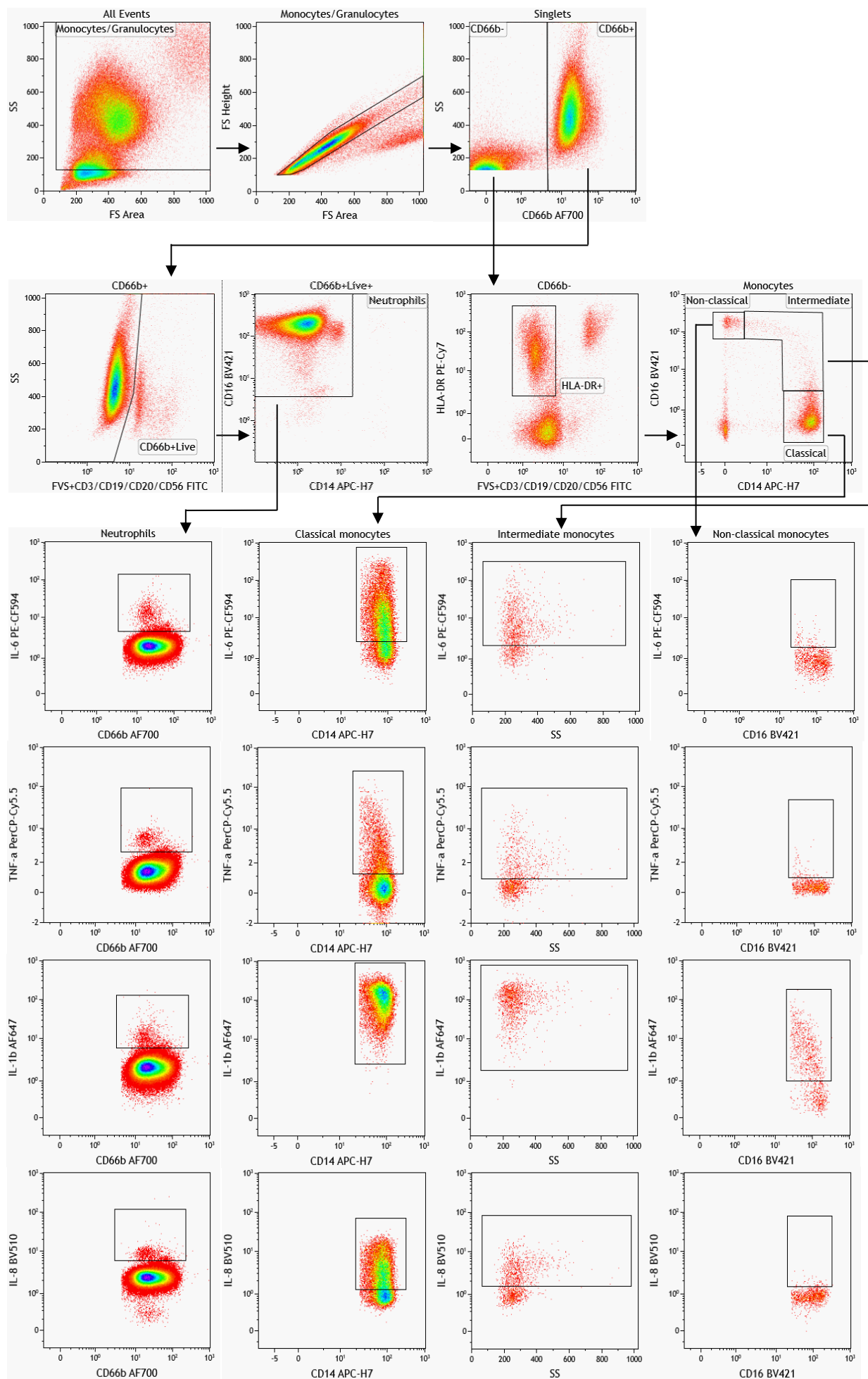
B Phagocytosis assay



C Intracellular ROS



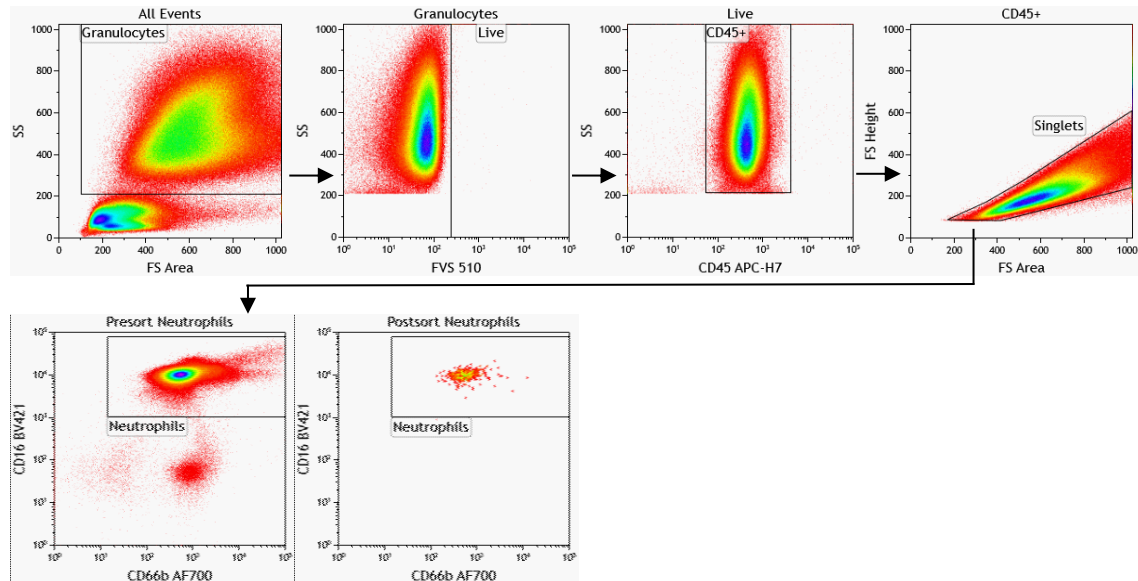
D Cytokine/chemokine staining



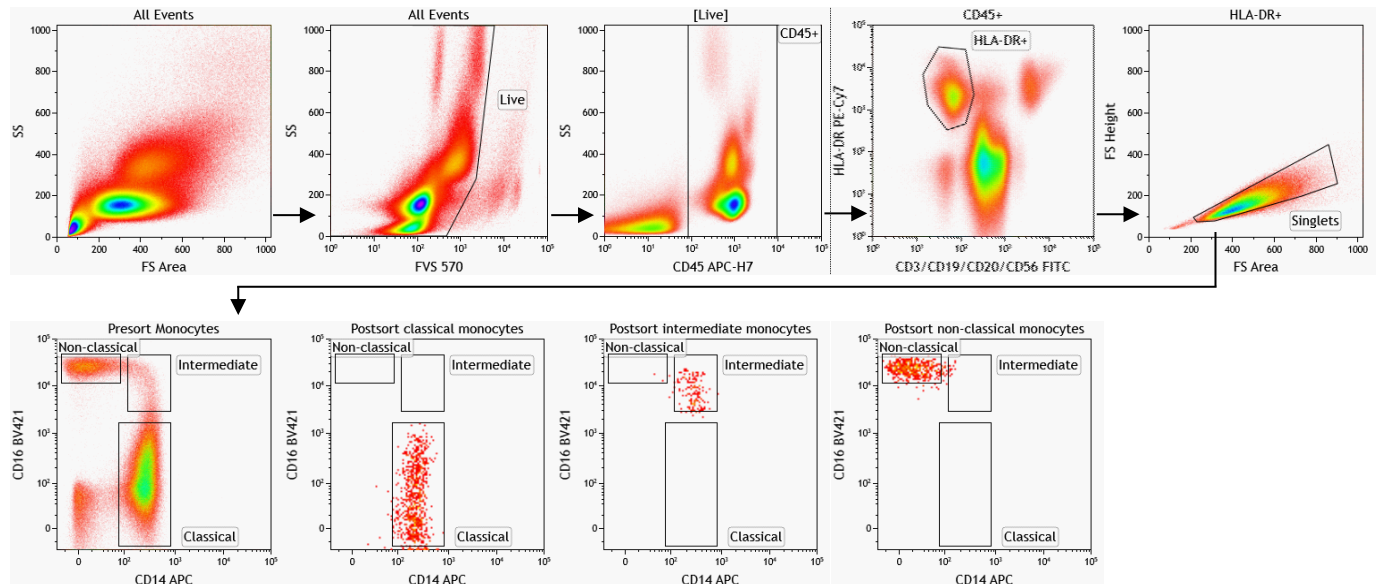
Supplementary figure 1: Gating strategy. (A) Immunophenotype of circulating neutrophils, cDCs and monocyte subsets in whole blood. Forward and side scatter parameters were used to identify lymphocytes, monocytes and granulocytes based on cell size and granularity. Live, single leukocytes (CD45⁺) were selected, and single cells negative for CD66b were subsequently gated on HLA-DR expression and exclusion of CD3, CD19, CD20, CD56. Monocyte subsets were defined based on CD14 and CD16 expression into classical (CD14⁺⁺CD16⁻), intermediate (CD14⁺⁺CD16⁺), non-classical (CD14⁻CD16⁺⁺), and double-negative (CD14⁻CD16⁻) monocytes. The double-negative population was further characterised using CD11c to identify conventional dendritic cells (cDCs). Neutrophils were gated as CD66b⁺CD16⁺CD14⁻. Neutrophils, classical monocytes, intermediate monocytes, and non-classical monocytes were subsequently analysed for MPO and CD162 expression. Gating was based on the unstained negative control and FMO controls. (B) Phagocytosis assay. Neutrophils, classical monocytes, and non-classical monocytes were analysed for PHrodo expression in the presence of cytochalasin D (inhibitory control) and in absence (positive control). (C) Intracellular ROS. Neutrophils, total monocytes and monocyte subsets were analysed for H2-DCFDA expression in unstimulated and stimulated samples. (D) Cytokine/chemokine staining in *in vitro* experiments. Neutrophils, classical monocytes, intermediate monocytes and non-classical monocytes were analysed for IL-6, TNF- α , IL-1 β and IL-8 expression in unstimulated and stimulated samples. Gating was based on the unstained negative control, unstimulated control and FMO controls.

Figure 2

A Neutrophil sorting



B Monocyte subset sorting



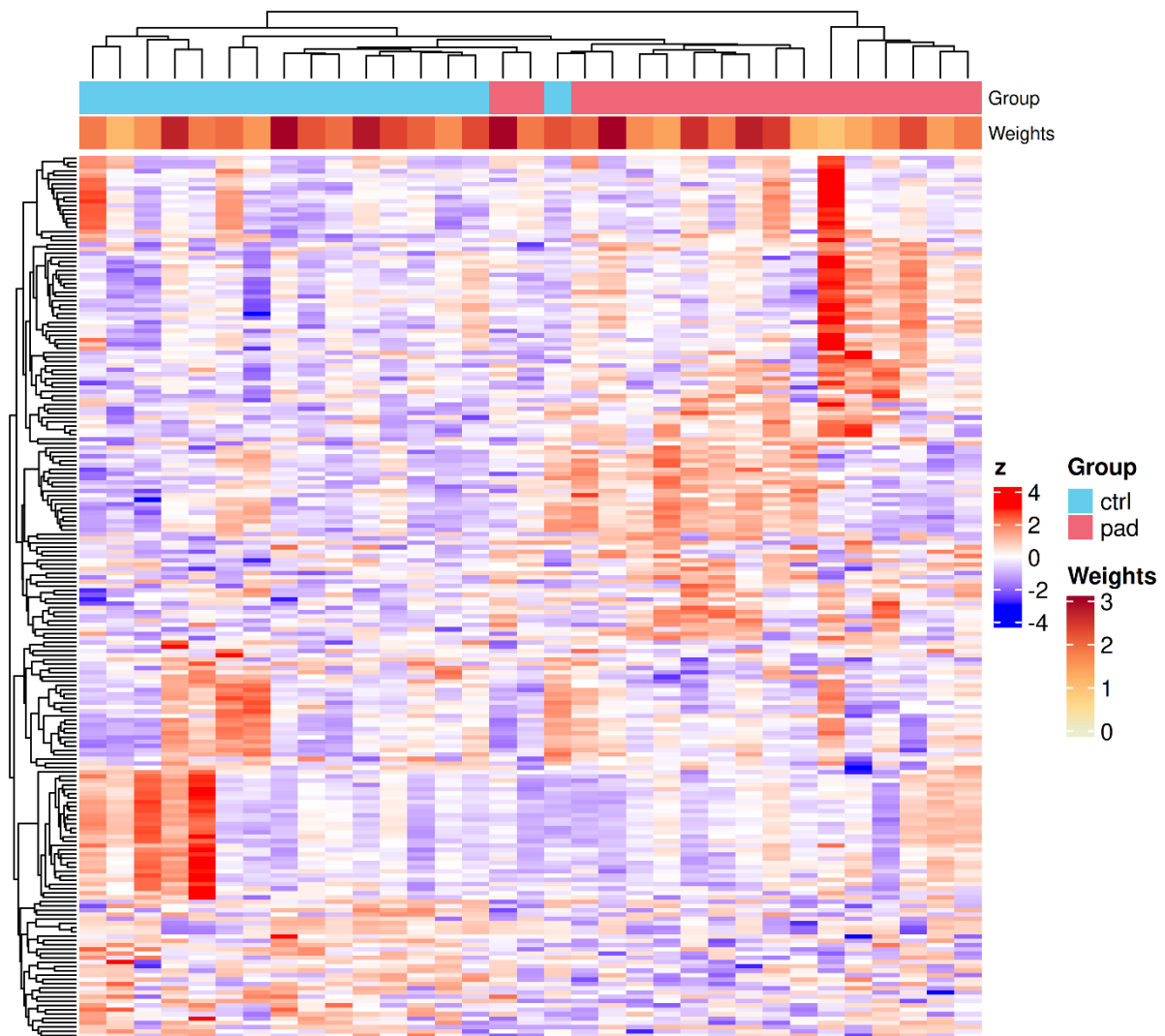
Supplementary figure 2: Gating strategy. (A) Neutrophil sorting from whole blood. Forward and side scatter parameters were used to select granulocytes based on cell size and granularity. Live leukocytes (CD45⁺) were selected, followed by gating on single cells. Neutrophils were subsequently based on CD66b⁺CD16⁺ expression. **(B)** Monocyte subset sorting from PBMCs. Forward and side scatter parameters were used to assess cell size and granularity. Live leukocytes (CD45⁺) were selected and further gated on HLA-DR expression and exclusion of CD3, CD19, CD20, CD56. Single cells were subsequently gated on monocyte subsets based on CD14 and CD16 expression into classical (CD14⁺⁺CD16⁻), intermediate (CD14⁺⁺CD16⁺) and non-classical (CD14⁺CD16⁺⁺) monocytes.

Supplementary Table 2: General linear model univariate analysis adjusted for age, BMI, and smoking status*Adjusted p-values are shown for each immune marker. Significant values are bolded at $p \leq 0.05$.*

Immune marker	Age-adjusted p-value	BMI-adjusted p-value	Smoking-adjusted p-value
Neutrophil proportion	<0.001	<0.001	0.001
Neutrophil cell count	<0.001	<0.001	<0.001
cDCs proportion	0.365	0.102	0.238
cDCs cell count	0.001	<0.001	0.001
Classical monocyte cell count	<0.001	<0.001	<0.001
Intermediate monocyte cell count	<0.001	<0.001	<0.001
Non-classical monocyte cell count	0.028	0.054	0.075
Neutrophil CD162+ cell count	<0.001	<0.001	<0.001
Classical monocyte CD162+ cell count	<0.001	<0.001	<0.001
Intermediate monocyte CD162+ cell count	0.040	0.014	0.078
Non-classical monocyte CD162+ cell count	0.087	0.326	0.192
Neutrophils IL-6+	0.050	0.017	0.106
Neutrophils TNF+	0.078	0.235	0.913
Neutrophils IL-1 β +	0.972	0.822	0.868
Neutrophils IL-8+	0.452	0.807	0.514
Classical monocytes IL-6+	0.181	0.037	0.021
Classical monocytes TNF+	0.925	0.511	0.687
Classical monocytes IL-1 β +	0.057	0.019	0.025
Classical monocytes IL-8+	0.050	0.021	0.014
Intermediate monocytes IL-6+	0.012	0.016	0.045
Intermediate monocytes TNF+	0.329	0.257	0.147
Intermediate monocytes IL-1 β +	0.241	0.228	0.351
Intermediate monocytes IL-8+	0.961	0.744	0.888
Non-classical monocytes IL-6+	0.370	0.526	0.620
Non-classical monocytes TNF+	0.623	0.458	0.371
Non-classical monocytes IL-1 β +	0.840	0.539	0.517
Non-classical monocytes IL-8+	0.302	0.515	0.991
Plasma IL-1 β	0.108	0.172	0.116
Plasma IL-6	0.336	0.936	0.723
Plasma IL-8	0.617	0.945	0.448
PBMC LPS-stimulated TNF	0.900	0.644	0.395
PBMC LPS-stimulated IL-1 β	0.232	0.296	0.511
Neutrophil phagocytosis	0.044	0.044	0.005
Classical monocyte phagocytosis	0.400	0.041	0.015
Non-classical monocyte phagocytosis	0.430	0.326	0.141
Neutrophil intracellular ROS	0.002	0.010	0.046
Neutrophil intracellular MPO cell count	<0.001	<0.001	<0.001
Classical monocyte intracellular MPO cell count	<0.001	<0.001	<0.001
Intermediate monocyte intracellular MPO cell count	0.038	0.012	0.070
Non-classical monocyte intracellular MPO cell count	0.093	0.322	0.282
Plasma MPO	0.011	<0.001	0.003

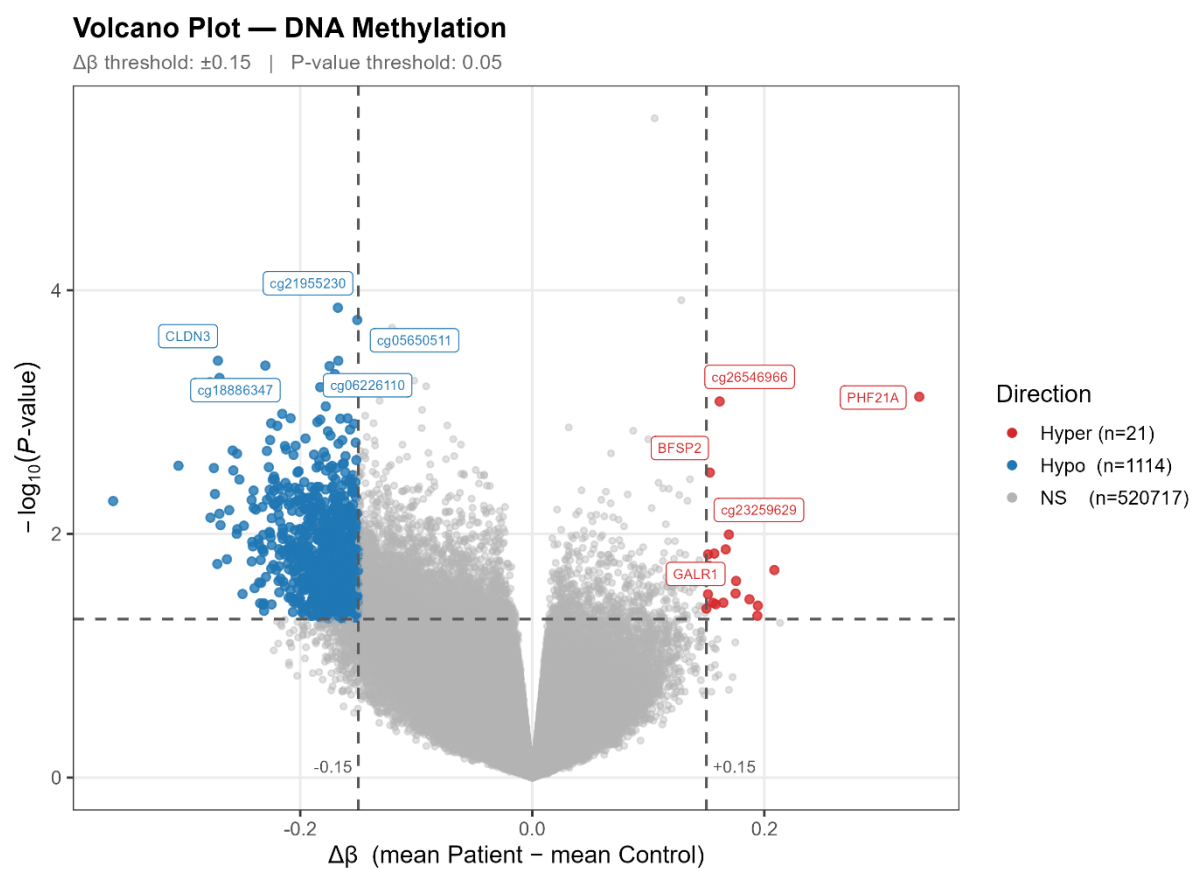
Note. Values are p-values from univariate general linear models. P-values reported as 0.000 in SPSS are presented as $p < 0.001$. Bold values indicate $p \leq 0.05$.

Figure 3



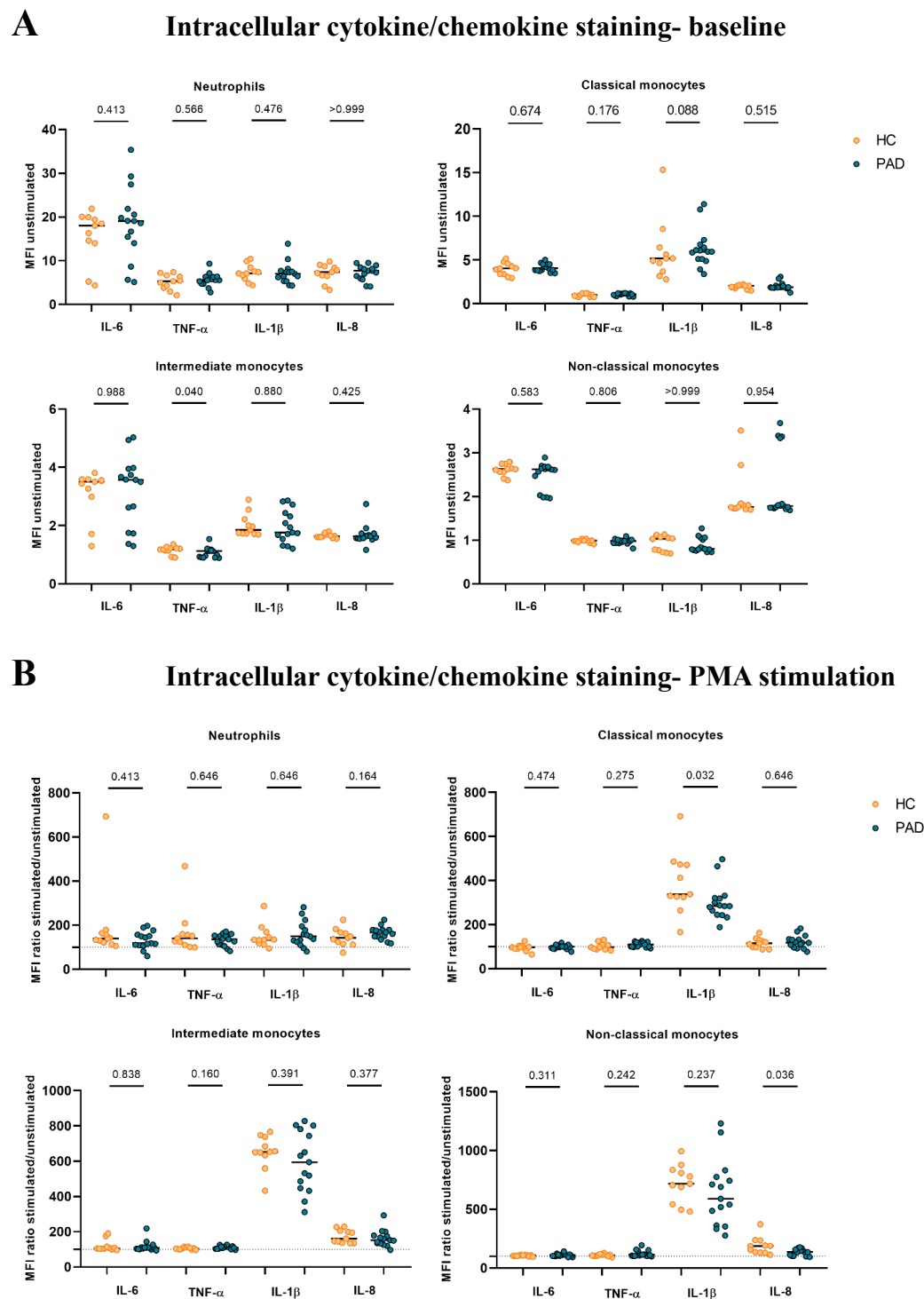
Supplementary figure 3: Monocyte transcriptome remains unchanged in PAD. RNA-sequencing of FACS-sorted classical monocytes from PAD patients (n=20) and controls (n=20). Heat map with unsupervised clustering of classical monocytes transcriptomes.

Figure 4



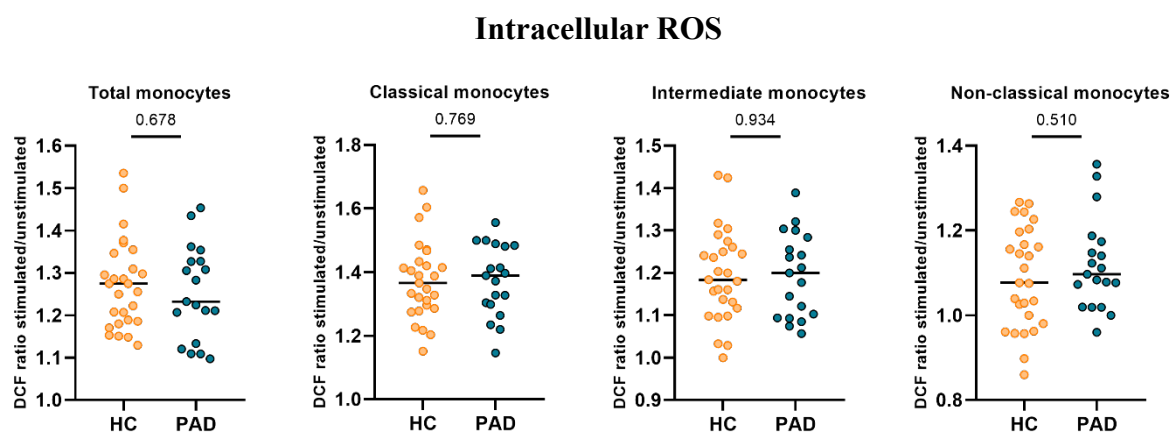
Supplementary figure 4: Volcano plot illustrating differentially methylated CpG sites between patients with peripheral arterial disease (PAD, n=12) and healthy controls (HCs, n=13), identified using a delta beta ($\Delta\beta$) value threshold of 0.15 and an unadjusted p-value of 0.05. Red dots represent hypermethylated CpG sites in PAD (n=21) and blue dots the hypomethylated CpG sites in PAD (n=1114).

Figure 5



Supplementary figure 5: Intracellular cytokine/chemokine staining in whole blood from PAD patients (n=15) and HCs (n=11), analysed by flow cytometry (A) Baseline (B) following PMA stimulation. Data are shown for (A) Neutrophils, (B) classical monocytes, (C) intermediate monocytes and (D) non-classical monocytes. MFI of stimulated cells for each sample was normalized against the unstimulated control.

Figure 6



Supplementary figure 6: Intracellular ROS in total monocytes and monocyte subsets measured by flow cytometry in PAD patients (n=19) and HCs (n=27). MFI of stimulated cells for each sample was normalized against the unstimulated control.