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Functional Analysis of Immunocompromised Patients' Leucocytes by Single-cell Mass Cytometry

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APPENDIX

Lausanne, 31.01.2020

List of Figures

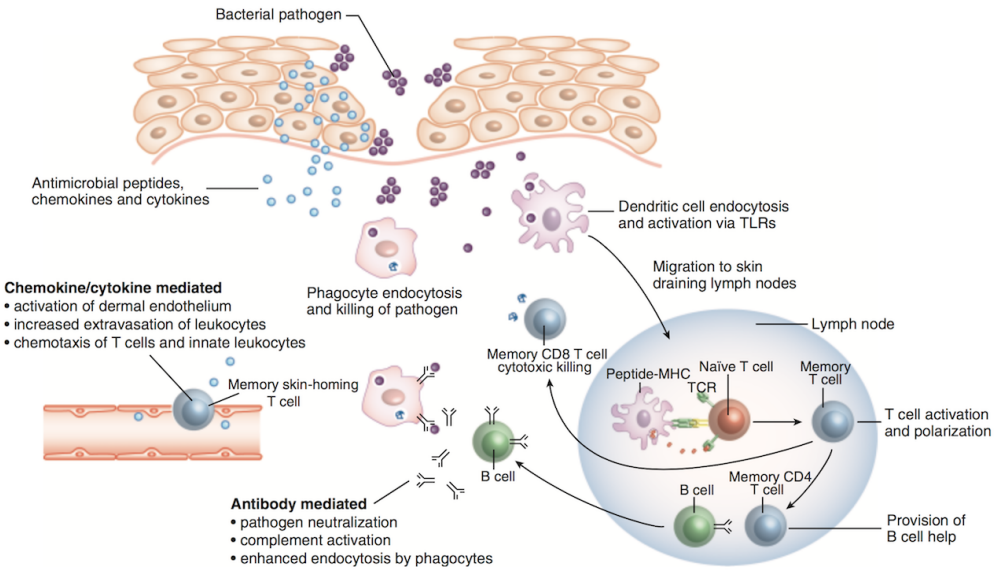

Figure 1: **General overview of the interactions between the innate and the adaptive immune systems in response to a bacterial skin infection** - This figure highlights the essential role of dendritic cells in order to assure the proper interaction between the two parts of the immune system, by presenting the bacterial antigen at its surface to T lymphocytes after its migration to the skin lymphnodes. This triggers the downstream activation of the adaptive system in order to fight against the microorganism invasion of the skin by T and B lymphocytes. It also illustrates the importance of the different mediators of the immune response, among others cytokines. Modified from (61).

Actors of the immune system	
Family	**Actors**
Physical and Chemical Barriers	Epithelia (skin) Mucosa (gut, lungs, nose, eyes) Antimicrobial products Enzymes (pepsine, lysozyme) Fatty acid Low pH Endogenous microbial flora
Hematopoietic Stem Cell-Derived Immune Cells	*Innate immunity* *Phagocytic cells* Neutrophils Macrophages Monocytes Eosinophils Basophils Dendritic cells (DCs) Mast cells Natural killer (NK cells) NKT cells Innate lymphoid cells (ILC) *Adaptive immunity* B cells T cells
Blood Molecules	Complement system Mediators of inflammation Cytokines Chemokines Proteases Lipid mediators Peptides, amines Nitric oxyde Adhesion molecules Acute-phase proteins (CRP) Antibodies
Lymphoid Tissues	*Primary* Thymus Bone marrow *Secondary* Lymph nodes Tonsils Spleen Mucosa-Associated Lymphoid Tissue (MALT) *Tertiary* Bronchus-Associated Lymphoid Tissues (BALTs) Inducible Lymphoid Follicles (ILFs)

Figure 2: **Overview of the main actors of the immune system** - Principal actors of different nature involved in the immune system.

Cytokines characteristics and functions				
Cytokine and Subunits		**Principal Cell Source**	**Cytokine Receptor and Subunits**	**Principal Cellular Targets and Biologic Effects**
Type I Cytokine Familiy Members				
IL-2		T cells	CD25 (IL-2Ra) CD122 (IL-2Rß) CD132 (yc)	**T cells** - Proliferation and differentiation into effector and memory cells - Promotes regulatory T cell development, survival and function **NK cells** - Proliferation - Activation **B cells** - Proliferation - Ab synthesis (in vitro)
IL-4		CD4+ T cells (Th2, Tfh) Mast cells Macrophages M2	CD124 (IL-4Ra) CD132 (yc)	**B cells** - Isotype switching to IgE and IgG4 **T cells** - Th2 differentiation - Proliferation (growth factor) **Macrophages** - Alternative activation (M2) - Inhibition of IFNy-mediated classical activation **GI tract** - Stimulate peristalsis **Endothelium** - Promotes the expression of adhesion molecules and secretion of chemokines
IL-5		CD4+ T cells (Th2) ILCs group 2	CD125 (IL-5Rα) CD131 (βc)	**Eosinophils** - Activation - Increased growth and differentiation
IL-6		Macropages Endothelial cells T cells	CD126 (IL-6Ra) CD130 (gp130)	**Liver** - Synthesis of acute-phase protein **B cells** - Proliferation of antibody-producing cells **T cells** - Th17 differentiation
IL-9		CD4+ T cells	CD129 (IL-9R) CD132 (yc)	**Mast cells, B cells, T cells and tissue cells:** - Survival and activation
IL-10		T cells Monocytes Macrophages M2 DC B cells Eosinophils Mast cells Keratinocytes Epithelial cells	IL10R1 IL10R2 Stimulation is dose-dependent	**DC and Macrophages** - Potent inhibitor of Ag presentation - Inhibits MHC class II and upregulation of CD80 and CD86 - Inhibits production of proinflammatory cytokines and mediators (IL-1, -6, -12, TNF) - Inhbition of CC and CXC **DC** - Inhibits differentiation from Monocytes to DC - Inhibits DC maturation **Macrophages** - Inhibition of macrophage matrix metalloproteases - Increase secretion of IL-1RA **Immature T cells** - Inhibition of CD28 pathway **B cells** - Costimulation of B cells activation - Prolonged B survival - Class switching **NK** - Co-stimulation of NK cells proliferation and cytokines production **T cells** - Growth factor to stimulate the proliferation of certain subsets of CD8+ T cells
IL-12	IL-12A (p35) IL-12B (p40	Macrophage Dendritic cells	CD212 (IL-12Rß1) IL-12Rß2	**T cells** - Th1 differentiation **T and NK cells** - IFN-y synthesis - Increased cytotoxic activity
IL-13		CD4+ T cells (Th2) NKT cells ILCs group 2 Mast cells Macrophages M2	CD213a1 (IL-13Ra1) CD213a2 (IL-13Ra2) CD132 (yc)	**B cells** - Isotype switching to IgE and IgG4 isotypes **Macrophages** - Alternative activation (M2) **Epithelial cells** - Increased mucus production from airway and gut epithelial cells **GI tract** - Stimulates peristalsis **Endothelium** - Promotes the expression of adhesion molecules and secretion of chemokines
IL-15		Macrophages Other cell types	IL-15Rα CD122 (IL-2Rβ) CD132 (yc)	**NK cells** - Proliferation **T cells** - Survival and proliferation of memory CD8+ cells
IL-17	IL-17A IL-17F	CD4+ T cells (Th17) ILCs group 3 γδ T cells CD8+ T cells	CD217 (IL-17RA) IL-17RC	**Epithelial cells, macrophages, and other cell types** - Increased chemokine and cytokine production to recruit neutrophils and monocytes - GM-CSF and G-CSF production to enhance neutrophils production
IL-21		Th2 cells Th17 cells Tfh cells	CD360 (IL-21R) CD132 (yc)	**B cells** - Activation, proliferation, differentiation in germinal centers **Tfh cells** - Development **Th17** - Increased generation (differentiation) (amplification) **NK cells** - Increased proliferation, differentiation and effector function **CD8+ T cells** - Increased proliferation, differentiation and effector function
IL-23	IL-23A (p19) IL-12B (p40)	Macrophages DCs	IL-23R CD212 (IL-12Rβ1)	**T cells** - Differentiation and expansion of Th17

Figure 3: **I- Cytokines characteristics and functions** - Type I cytokine familiy members: cell sources, related receptors and biological effects of the principal members. Modified from (7).

Cytokines characteristics and functions (following)			
Cytokine and Subunits	**Principal Cell Source**	**Cytokine Receptor and Subunits**	**Principal Cellular Targets and Biologic Effects**
Type I Cytokine Familiy Members (following)			
c-Kit Ligand (Stem cell factor)	Bone marrow stromal cells	CD117 (KIT)	**Pluripotent hematopoietic stem cells** - Maturation of all hematopoietic linages
GM-CSF (Granulocyte-monocyte CSF)	T cells Macrophages Endothelial cells Fibroblasts	CD116 (GM-CSFRa) CD131 (ßc)	**Immature and committed progenitors, mature macrophages** - Maturation of granulocytes and monocytes - Macrophage activation
M-CSF, CSF1 (Monocyte CSF)	Macrophages Endothelial cells Bone marrow cells Fibroblasts	CD115 (CSF1R)	**Committed hematopoietic progenitors** - Maturation of monocytes
G-CSF (Granulocyte CSF)	Macrophages Fibroblasts Endothelial cells	CD114 (CSF3R)	**Committed hematopoietic progenitors** - Maturation of granulocytes
TSLP (Thymic stromal lymphopoietin)	Keratinocytes Bronchial epithelial cells Fibroblasts Smooth muscle cells Endothelial cells Mast cells Macrophages Granulocytes Dendritic cells	TSLP-receptor CD127 (IL-7R)	**DC** - Activation **Eosinophils** - Activation **Mast cells** - Cytokine production **T cells** - Th2 differentiation
Type II Cytokine Familiy Members			
IFN-a *Type I interferon*	pDC Macrophages	IFNAR1 CD118 (IFNAR2)	**All cells** - Antiviral state - Increased class I MHC expression **NK cells** - Activation
IFN-ß *Type I interferon*	pDC Fibroblasts	IFNAR1 CD118 (IFNAR2)	**All cells** - Antiviral state - Increased class I MHC expression **NK cells** - Activation
IFN-y *Type II interferon*	T cells (Th1, CD8+ T cells) NK cells	CD119 (IFNGR1) IFNGR2	**Macrophages** - Classical activation - Increased microbicidal functions **B cells** - Isotype switching to opsonizing and complement-fixing IgG subclasses **T cells** - Th1 differentiation; inhibition of Th2 and Th17 differentiation **APCs** - Increased expression of B7 costimulators at their surface **Various cells** - Immunoproteasome production and activation - Increased expression of class I and II MHC - Increased antigen processing and presentation to T cells
IFN-λ1-3 *Type III interferon*	DCs	IFNLR1 (IL-28Rα) CD210B (IL-10Rβ2)	**Epithelial cells** - Antiviral state
IL-10	Macrophages T cells (mainly Treg)	CD210 (IL-10Ra) IL-10Rß	**Macrophages, DC** - Inhibition of expression of IL-12, costimulators and class II MHC
IL-22	Th17 cells NK cells ILCs group 3	IL-22Ra1 IL-22Ra2 IL-10Rß2	**Epithelial cells** - Production of defensins (antimicrobial peptides) - Increased barrier function (repair reactions) - Increased production of chemokines **Hepatocytes** - Survival

Figure 3: **II- Cytokines characteristics and functions** - Type I and II cytokine familiy members: cell sources, related receptors and biological effects of the principal members. Modified from (7).

Cytokines characteristics and functions (following)				
Cytokine and Subunits		**Principal Cell Source**	**Cytokine Receptor and Subunits**	**Principal Cellular Targets and Biologic Effects**
TNF Superfamily Cytokines				
TNFa		Macrophages NK cells T cells Neutrophils	CD120a (TNFRSF1) CD120b (TNFRSF2)	**Endothelial cells** - Activation (inflammation, coagulation) **Neutrophils** - Activation **Hypothalamus** - Fever **Muscle, fat** - Catabolism (cachexia)
Lymphotoxin-a	LTa TNFSF1	T cells B cells	CD120a (TNFRSF1) CD120b (TNFRSF2)	Same as TNF
Lymphotoxin-aß	LTaß	T cells NK cells Follicula B cells Lymphoid inducer cells	LTßR	**Lymphoid tissue stromal cells and follicular dendritic cells (FDC)** - Chemokine expression - Lymphoid organogenesis
BAFF	CD257 TNFSF13B	DC Monocytes Follicular dendritic cells B cells	BAFF-R (TNFRSF13C) TACI (TNFRSF13B) BCMA (TNFRSF17)	**B cells** - Survival - Proliferation
APRIL	CD256 TNFSF13	T cells DC Monocytes Follicular dendritic cells	TACI (TNFRSF13B) BCMA (TNFRSF17)	**B cells** - Survival - Proliferation
Osteoprotegerin	OPG TNFRSF11B	Osteoblasts	RANKL	**Osteoclast precursor cells** - Inhibits osteoclast differentiation
IL-1 Family Cytokines				
IL-1a		Macrophages DC Fibroblasts Endothelial cells Keratinocytes Hepatocytes Neutrophils	CD121a (IL-1R1) IL-1RAP CD121b (IL-1R2)	**Endothelial cells** - Activation (inflammation, coagulation) **Hypothalamus** - Fever
IL-1b		Macrophages DC Fibroblasts Endothelial cells Keratinocytes Neutrophils	CD121a (IL-1R1) IL-1RAP CD121b (IL-1R2)	**Endothelial cells** - Activation (inflammation, coagulation) **Hypothalamus** - Fever **Liver** - Synthesis of acute-phase proteins **T cells** - Th17 differentiation
IL-1RA		Macrophages	CD121a (IL-1R1) IL-1RAP	**Various cells** - Competitive antagonist of IL-1
IL-18		Monocytes Macrophages DC Kupffer cells Keratinocytes Chondrocytes Synovial fibroblasts Osteoblasts	CD218a (IL-18Ra) CD218b (IL-18Rb)	**NK cells, T cells** - IFN-γ synthesis **Monocytes** - Expression of GM-CSF, TNF, IL-1b **Neutrophils** - Activation - Cytokine release
IL-33		Endothelial cells Smooth muscle cells Keratinocytes Fibroblasts	ST2 (IL1RL1) IL-1RAP	**T cells** - Th2 development **ILCs** - Activation of group 2 ILCs
Other Cytokines				
TGF-ß		T cells (Tregs) Macrophages Other cell types	TGF-ß R1 TGF-ß R2 TGF-ß R3	**T cells** - Inhibition of proliferation and effector functions - Differentiation of Th17 and Treg **B cells** - Inhibition of proliferation - IgA production **Macrophages** - Inhibition of activation - Stimulation of angiogenic factors **Fibroblasts** - Increased collagen synthesis and matrix-modifying enzymes **Neutrophils** - Inhibition of activation

Figure 3: **III- Cytokines characteristics and functions** - TNF superfamily cytokines, IL-1 family cytokines and other cytokines: cell sources, related receptors and biological effects of the principal members. Modified from (7).

General overview of the principal Pattern Recognition Receptors (PRR) with their characteristics								
PRR class	**Subtypes**	**Molecular structure**	**Associated molecules**	**Distribution**	**Location**	**Ligands (PAMPs or DAMPs)**	**Transcription factors associated**	**Effects**
Cell-associated								
TLRs *(TIR superfamily)*	**TLRs 1-9**	Transmembrane proteins EC: **Leucin-rich modules** IC: **TIR domain**	**TLR 2, TLR6** CD36 **TLR 4** MD2 CD14	DCs Phagocytes B cells Endothelial cells Many other cells	Plasma membrane Endosomal membrane	Various microbial molecules - LPS - Peptidoglycans - Nucleic acids - Proteins	NF-κB AP-1 IRF3 IRF7	**Inflammatory response** Cytokines: TNF, IL-1 Chemokines: CCL2, CXCL8 Adhesion molecules: E-selectin **Antiviral response** Cytokines: IFN-α, IFN-β
NLRs	**NLRA** **NLRB** **NLRC** NOD1 NOD2 NLRC4 **NLRP** (inflammasomes) NLRPs 1-10	**Leucin-rich repeat domain** -> Binds to ligands **NOD domain** -> Forms oligomers **Effector domain** NLRA: Transactivating domain NLRB: BIR NLRC: CARDs NLRP: Pyrin domains -> Recruit other proteins -> Signalling complexes		Phagocytes Epithelial cells Other cells	Cytosol	**NLRA** IFN-γ **NLRB** Flagellin **NLRC** *NOD1* Peptidoglycan (DAP) *NOD2* Peptidoglycan (MDP) *NLRC4* Flagellin **NLRP** *NLRP1* Anthrax lethal toxin *NLRP3* ATP release from mitochondria Extracellular ATP Alum Asbestos Bacterial products and toxins Intracellular crystals Aluminum hydroxide crystals Urate Silica ROS Reduced cytosolic K+ *NLRP7* Lipopeptides	NF-κB	**NLRA** Class II MHC expression **NLRB** Control of Legionella pneumophila infection **NLRC** *NOD1* NF-κB activation *NOD2* NF-κB activation Autophagy Type I interferons production *NLRC4* Caspase 1 activation Cell death **NLRP** *NLRP3* Caspase 1 activation Inflammasome activation Cytokines: IL-1, IL-18, IL-33 *NLRP7* Caspase 1 activation
RLRs	RIG-1 MDA-5	**Caspase recruitment domains** -> Interact with signalling proteins **RNA-helicase domain** -> RNA recognition **C-terminal domain** -> RNA recognition	MAVS	Phagocytes Other cells	Cytosol	RNA (virus) dsRNA RNA-DNA heteroduplexes	IRF3 IRF7 NF-κB	**Cytokines production**: IFN-α, IFN-β **Direct viral replication inhibition**
CDSs	**STING-independent** AIM2 RNA polymerase 3 **STING-pathway** cGAS DAI IFI16	**STING** Transmembrane adaptor protein on ER membrane	**STING pathway** *cGAS* cGAMP TBK1 kinase **STING-independent** *RNA polymerase 3* RIG-I pathway *AIM2* Inflammasome	Phagocytes	Cytosol	dsDNA (bacteria and virus)	**STING pathway** *cGAS* IRF3	**STING-pathway** **Cytokines production**: IFN-α, IFN-β **Autophagy** **STING-independent** *AIM2* **Inflammasome** Cytokines: IL-1, IL-18, IL-33

Figure 4: **I- Overview of the principal pathogen recognition receptors of the innate immune system.** - Cell-associated PRRs (1).

General overview of the principal Pattern Recognition Receptors (PRR) with their characteristics (following)								
PRR class	**Subtypes**	**Molecular structure**	**Associated molecules**	**Distribution**	**Location**	**Ligands (PAMPs or DAMPs)**	**Transcription factors associated**	**Effects**
Cell-associated (following)								
CLRs	Mannose receptor (CD206) Dectins Dectin-1 (CD369) Dectin-2 Mincle Langerin (CD207) DC-SIGN (CD209)	Transmembrane receptor **Dectins** *Dectin-1* Cytoplasmic tail: ITAM *Dectin-2* Cytoplasmic tail: ITAM Relies on FcRγ	**Dectins** ITAM SYK CARD9	Macrophages DCs Somme tissue cells Blood EC fluid	Plasma membrane	**Mannose receptor** (CD206) D-mannose L-fucose N-acetyl-D-glucosamine **Dectins** *Dectin-1* β-glucans (bacteria and fungi) *Dectin-2* High-mannose oligosaccharides (fungi, bacteria) **Langerin** Mannose **DC-SIGN** Mannose Fucose	**Dectins** NF-κB	**Mannose receptor** (CD206) **Phagocytosis** of microbes **Antifungal immunity** **Dectins** **Antifungal** immunity **Mycobacterial** immunity **Inflammatory** response **Antigen presentation** **Th17** cell induction **Langerin** **Phagocytosis** **Antigen presentation** **DC-SIGN** **Adhesion** **Pathogenic role** in disseminating infections HBV HIV-1
Scavenger receptors	SR-A CD36		**CD36** TLR2, TLR 6	Phagocytes	Plasma membrane	Diacylglycerides Oxidized lipoproteins Lipoteichoic acid LPS Nucleic acids β-glucans Proteins		**SR-A** Mediate **phagocytosis** of microorganisms **CD36** Mediate **phagocytosis** of microorganisms **Coreceptor** in **TLR2/6** pathway
FPRs	FPR1 FPRL1	GPCR	G proteins	Phagocytes	Plasma membrane	Peptides with N-formylmethionyl residues -> Act as chemoattractants		Increased **cell motility** **Phagocytosis**

Figure 4: **II- Overview of the principal pathogen recognition receptors of the innate immune system.** - Cell-associated PRRs (2).

General overview of the principal Pattern Recognition Receptors (PRR) with their characteristics (following)								
PRR class	**Subtypes**	**Molecular structure**	**Associated molecules**	**Distribution**	**Location**	**Ligands (PAMPs or DAMPs)**	**Transcription factors associated**	**Effects**
Soluble								
Pentraxins	*Short pentraxins* CRP SAP *Long pentraxins* PTX3	Pentameric proteins		*Produced by* **CRP, SAP** Liver **PTX3** DCs Macrophages Endothelium Neutrophils	Plasma	**CRP, SAP** Phosphorylcholine Phosphatidylethanolamine Amyloid fibrils **PTX3** Bacteria Virus Fungi (e.g. *Aspergillus fumigatus*) Apoptotic cells		**Complement activation** by binding C1q -> Classical pathway initiation **Pathogenic responses** Autoimmunity Amyloidosis
Collectins	Mannose-binding lectin Surfactant proteins SP-A SP-D	Trimeric or hexameric proteins Collagen-like tail Calcium-dependent (C-type) lectin head	**MBL** C1q receptor -> Internalization MASP1/2		**MBL** Plasma **SP-A, SP-D** Alveoli	**MBL** Carbohydrates with terminal mannose or fucose **SP-A, SP-D** Various microbial structures		**MBL** **Complement activation** by binding MASP1/2 -> Lectin pathway initiation **Opsonization** -> Enhancing phagocytosis **SP-A, SP-D** Reduction of **alveoli fluid surface tension** in lungs -> Ability of alveoly to expand upon inhalation **Opsonization** -> Ingestion by alveolar macrophages
Ficolins	Ficolin	Collagen-like domain Fibrinogen-tpe carbohydrate recognition domain	MASP1/2		Plasma	N-acetylglucosamine (Gram+ bacteria) Lipoteichoic acid (Gram+ bacteria)		**Opsonization** -> Enhancing phagocytosis **Complement activation** by binding MASP1/2 -> Lectin pathway initiation
Complement	Various complement proteins C1q, C1r, C1s C2, C3, C4 C5, C6, C7, C8, C9 B protein P protein	Serine proteases	*Inhibitors* C1 inhibitor C4-binding protein DAF CR1 I factor MCP H factor *Activators* B protein P protein MBL, MASP1/2 Pentraxins		Plasma	Microbial surfaces		**Opsonization** **Killing** of microorganisms Eliciting **inflammatory response** Increased vessels permeability Increased expression of adhesion. Molecules Phagocytes activation Mast cell degranulation **Chemoattractants**

Figure 4: **III- Overview of the principal pathogen recognition receptors of the innate immune system.** - Soluble PRRs. PAMP, pathogen-associated molecular pattern; DAMP, Damaged-associated molecular pattern; PRR, Pattern recognition receptor; DC, Dendritic cell; TLR, Toll-like receptor; TIR, Toll/interleukin-1 receptor; NF-κB, Nuclear factor-κB; NLR, NOD-like receptors; NOD, Nucleotide oligomerization domain; NLRP, NACHT, NALP, LRR and PYD domains-containing protein 3; RLR, RIG-like receptors; MDA, Melanoma differentiation-associated protein; CDSs, Cytosolic DNA sensors; CLRs, C-type lectin-like receptors; AIM, Absent in melanoma; SP, Surfactant protein; STING, Stimulator of IFN genes; MD2, Myeloid differenitation protein 2; EC, Extracellular; IC, Intracellular; NF-κB, Nuclear factor κB; AP-1, Activation protein 1; IRF, Interferon response factor; BIR, Baclovirus inhibition of apoptosis protein repeat; CARDs, Caspase recruitment and activation domains; DAP, Diaminopimelic acid; MDP, Muramyl dipeptide; K+, Potassium ion; STING, Stimulator of IFN genes; cGAS, Cyclic GMP-AMP synthase; cGAMP, Cyclic GMP-AMP; DAI, DNA-dependent activator of IFN-regulatory factors; IFI16, Interferon inducible protein 16; ER, Endoplasmic reticulum; MAVS, Mitochondrial antiviral-signalling; ITAM, Immunoreceptor ryrosine-based activation motif; DC-SIGN, Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin; Mincle, Macrophage inducible Ca2+ dependent lectin; SRA, Scavenger receptor A; FPRs, Formyl-peptide receptor; FPRL, Formyl-peptide receptor-like; GPCR, GTP-binding G protein-coupled receptor; CRP, C-reactive protein; SAP, Serum amyloid P; MBL, Mannose-binding lectin; MASP, Mannose/mannan-associated serine proteases; DAF, Decay accelerating factor; MCP, Membrane co-factor proteolysis. Sources: (7), (62) and (63).

Pathogens structures recognized by PRR			
Type	**Structure**	**Source**	**PRR recognition**
Pathogen-Associated Molecular Patterns (PAMPs)			
Nucleic acids	ssRNA dsRNA CpG	Virus Virus Virus, bacteria	TLR 7, 8 TLR 3, RNA polymerase 3, AIM2, cGAS, DAI, IFI16, RIG-I, MDA-5 TLR 9
Proteins	Pilin Flagellin F protein N-formylmethionine	Bacteria Bacteria Virus (RSV) Bacteria	TLR 2 TLR 5, NLRB, NLRC4 TLR 4 FPRs
Cell wall lipids	LPS Lipoteichoic acid Phosphorylcholine Phosphatidylethanolamine	Gram- bacteria Gram+ bacteria Bacteria Bacteria	TLR 4, NLRP7, SRs TLR 2, SRs, Ficolin CRP, SAP CRP, SAP
Cell wall polymers	Peptidoglycan	Bacteria	TLR 2, TLR 6, NOD 1, NOD 2
Carbohydrates	Mannan Fucose N-acetyl-D-glucosamine Glucans	Fungi, bacteria Bacteria Bacteria Fungi	CD206, Dectin-2, Langerin, DC-SIGN, MBL CD206, DC-SIGN, MBL CD206, Ficolin TLR 2, TLR 6, Dectin-1, SRs
Damage-Associated Molecuar Patterns (DAMPs)			
Stress-induced proteins	HSPs	Damaged cells	TLR 2, TLR 4
Crystals	Monosodium urate Silica	Damaged cells	NLRP3 NLRP3
Proteolytically cleaved extracellular matrix	Proteoglycan peptides	Damaged cells	TLR 2, TLR 4, NLRP3
Cell wall lipid	Phosphorylcholine Phosphatidylethanolamine	Apoptotic cells	CRP, SAP
Mitochondria and mitochondrial components	Formylated peptides and ATP	Damaged cells	NLRP3
Nuclear proteins	HMGB1 Histones	Damaged cells	TLR 2, TLR 4
Nucleic acids	Extracellular ATP	Damaged cells	NLRP3
Lysosomal damage	ROS	Damaged cells	NLRP3
Toxic	Alum Asbestos	Exogenous	NLRP3 NLRP3
Therapeutic agents			
R848 (Resiquimod)	Imidazoquinolinamine	Synthetic drug	TLR 7, TLR 8

Figure 5: **Overview of the principal PAMPs and DAMPs.** - Type, subtype and origin of the principal molecular patterns recognized by the innate immunity. PAMP, Pathogen-associated molecular pattern; DAMP, Damaged-associated molecular pattern; ATP, Adenosine triphosphate; CpG, Cytosine-guanine-rich oligonucleotide; dsRNA, Double-stranded RNA; HMGB1, High-mobility group box 1; HSP, Heat shock protein; LPS, Lipopolysaccharide; ssRNA, Single-stranded RNA; TLR, Toll-like receptor; RSV, Respiratory syncytial virus; AIM2, Absent in melanoma-2; cGAS, Cyclic GMP-AMP synthase; DAI, DNA-dependent activator of IFN-regulatory factors; IFI16, Interferon inducible protein 16; NLR, NOD-like receptor; NOD, Nucleotide oligomerization domain; DC-SIGN, Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin; SRs, Scavenger receptors; FPRs, Formyl peptide receptors; CRP, C-reactive protein; SAP, Serum amyloid P; MBL, Mannose-binding lectin. Sources: (7), (62), (63) and (64).

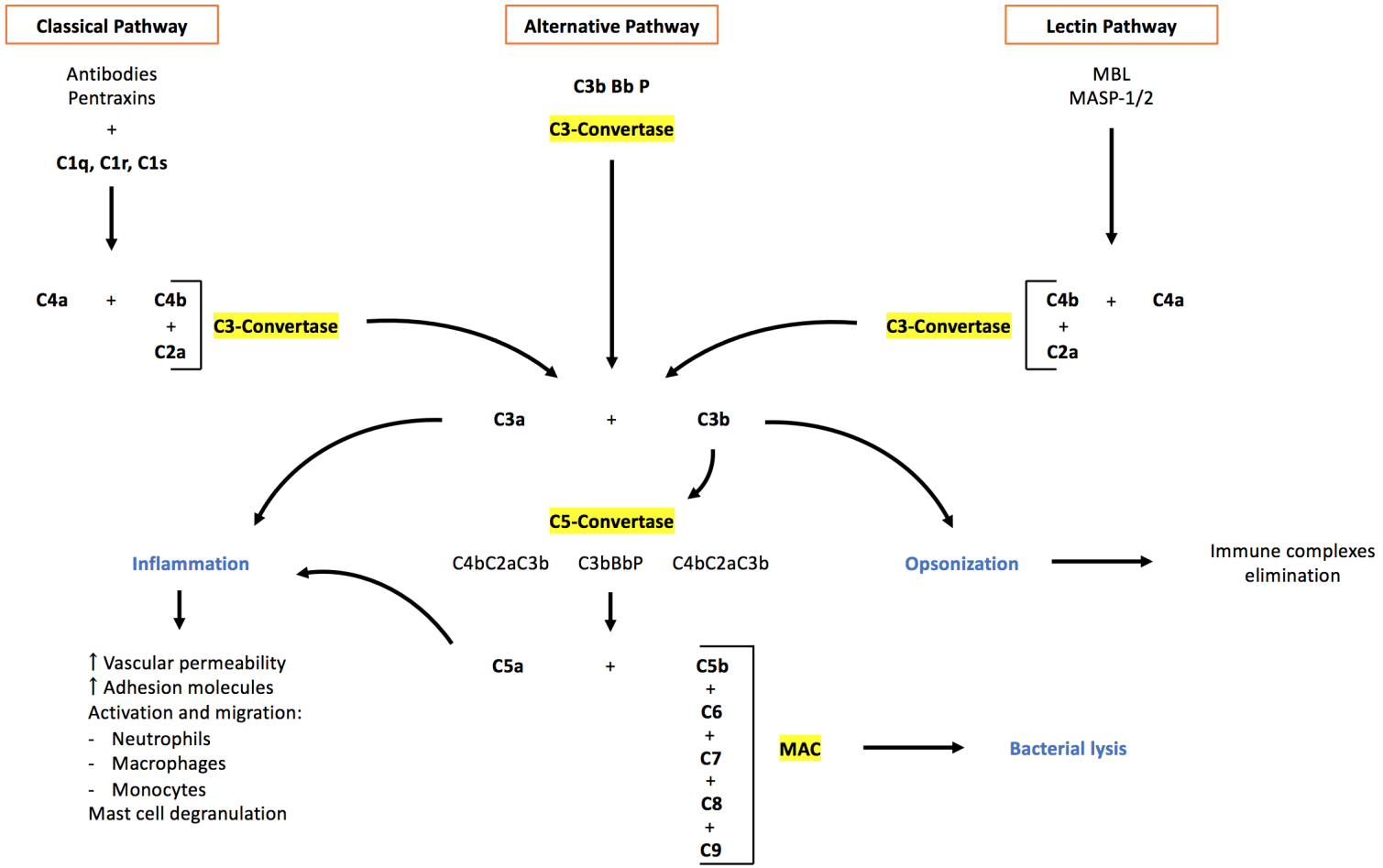

Figure 6: **Schematical overview of the different complement activation pathways** - The classical, alternative and lectin pathways are illustrated. Their activation converges to the formation of the C3-convertase, afterwards having the same effector mechanisms. MAC, Membrane attack complex; MCP, Membrane cofactor proteolysis; DAF, Decay accelerating factor; MBL, Mannose-binding lectin; MASP, Mannose/mannan-associated serine protease. Sources: (7), (65), (62) and (66).

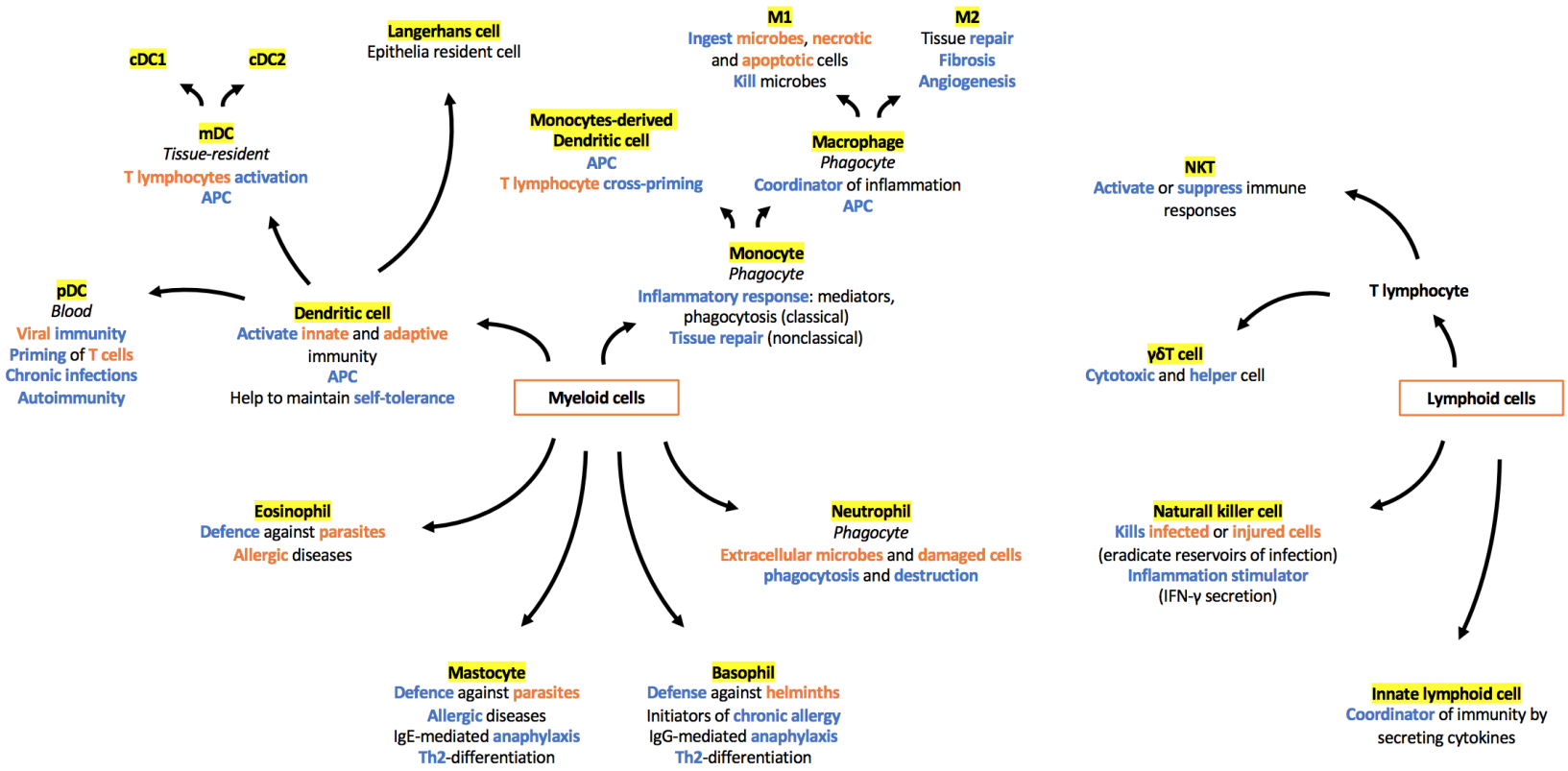

Figure 7: **Overview of the different hematopoietic stem cell-derived innate immune cells** - Innate immune cells are the products of differentiation from two different haematopoietic lineages: the myeloid and lymphoid progenitors. Sources: (7), (65), (67), (68) and (69).

CD Molecules			
CD Number (Other Names)	**Molecular Structure, Family**	**Main Cellular Expression**	**Known or Proposed Function(s)**
CD1a-d	Class I MHC-like Ig superfamily ß2-microglobulin associated	Thymocytes DC (including Langerhans cells)	- Presentaion of nonpeptid (lipid and gylcolipid) antigens to some T cells
CD3yy	Associated with CD3d and CD3e in TCR complex Ig superfamily ITAM in cytoplasmic tail	T cells	- Cell surface expression of and signal transduction by the T cell antigen receptor
CD3d	Associated with CD3y and CD3e in TCR complex Ig superfamily ITAM in cytoplasmic tail	T cells	- Cell surface expression of and signal transduction by the T cell antigen receptor
CD3e	Associated with CD3d and CD3y in TCR complex Ig superfamily ITAM in cytoplasmic tail	T cells	- Cell surface expression of and signal transduction by the T cell antigen receptor
CD4	Ig superfamily	Class II MHC-restricted T cells Some macrophages	- Coreceptor in class II MHC-restricted antigen-induced T cell activation (binds to class II MHC molecules) - Thymocyte development - Receptor for HIV
CD7 gp40 TP41	Associated to PI3-Kinase	T cells NK cells Stem cell/Precursor	- T cell interactions
CD8a	Expressed as a homodimer or heterodimer with CD8ß	Class I MHC-restricted T cells Subset of DC	- Coreceptor in class I MHC-restricted antigen-induced T cell activation (binds to class I MHC molecules) - Thymocyte development
CD8ßß	Expressed as a heterodimer with CD8a Ig superfamily	Class I MHC-restricted T cells	- Coreceptor in class I MHC-restricted antigen-induced T cell activation (binds to class I MHC molecules) - Thymocyte development
CD11a LFA-1 a chain	Noncovalently linked to CD18 to form LFA-1 integrin	Leukocytes	- Cell-cell adhesion - Binds to ICAM-1 (CD54), ICAM-2 (CD102), ICAM-3(CD50)
CD11b Mac-1 CR3	Noncovalently linked to CD18 to form Mac-1 integrin	Granulocytes Monocytes Macrophages DC NK cells	- Phagocytosis of iC3b-coated particles - Neutrophil and monocyte adhesion to endothelium (binds CD54) and extracellular matrix proteins
CD11c p150, 95 CR4aa chain	Noncovalently linked to CD18 to form p150, 95 integrin	Monocytes Macrophages Granulocytes NK cells	- Phagocytosis of iC3b-coated particles - Neutrophil and monocyte adhesion to endothelium (binds CD54) and extracellular matrix proteins
CD14	GPI linked	DC Monocytes Macrophages Granulocytes	- Binds complex of LPS and LPS-binding protein and displays LPS to TLR4 - Required for LPS-induced macrophage activation
CD15	Carbohydrate largely used for Dx of Hodgkin Lymphoma	Stem cell/Precursor Macrophages Monocytes Granulocytes	- Adhesion - Granulocyte activation
CD16a FcγγRIIIA	Transmembrane protein Ig superfamily	NK cells Macrophages	- Binds FC region of IgG - Phagocytosis and Ab-dependent cellular cytotoxicity
CD16b FcγγRIIIB	GPI linked Ig superfamily	Neutrophils	- Binds Fc region of IgG - Synergy with FcγγRII in immune complex-mediated neutrophil activation
CD19	Ig superfamily	Most B cells	- B cell activation - Forms a coreceptor complex with CD21 and CD81 that delivers signals that synergize with signals from B cell antigen receptor complex
CD20	Tetraspan (TM4SF) family	B cells	- Possible role in B cell activation or regulation - Calcium ion channel
CD21 CR2 C3d receptor	Regulators of complement activation	Mature B cells Follicular DCs	- Receptor for complement fragment C3d - Forms a coreceptor complex with CD19 and CD81 that delivers activating signals in B cells - Receptor for Epstein-Barr virus

Figure 8: **I- CD molecules characteristics and functions** - CD molecules characteristics: structure, main cellular expression and functions. Modified from (7), (70) and (71).

CD Molecules (following)			
CD Number (Other Names)	**Molecular Structure, Family**	**Main Cellular Expression**	**Known or Proposed Function(s)**
CD23 FceRIIB	C-type lectin	Activated B cells Monocytes Macrophages	- Low-affinity Fce receptor, induced by IL-4 - Function is not clear
CD25 IL-2 receptor a chain	Noncovalently associated with IL-2Rßß (CD122) and IL-2Ry (CD132) chains to form a high-affinity IL-2 receptor	Activated T cells Activated B cells Regulatory T cells (Treg)	- Binds IL-2 and promotes responses to low concentrations of IL-2
CD26 DPP4	Dipeptidyl peptidase	T cells B cells NK cells Macrophages Epithlial cells	- Exopeptidase - Régulation immunitaire - Transduction signal - Apoptose - Métabolisme glucose (augmente GLP-1 et GIP)
CD27	TNF receptor superfamily	T cells B cells NK cells	- Generation and long term maintenance of T cell immunity - Regulation of B cell activation and Ig synthesis
CD28	Ig superfamily	CD4+ T cells >50% of CD8+ cells	- T cell receptor for costimulatory molecues CD80 (B7.1) and CD86 (B7.2)
CD30 TNFRSF8	TNFR superfamily	Activated T and B cells NK cells Monocytes Reed-Sternberg cells in HL	- Not established
CD31 PECAM-1	Ig superfamily	Platelets Monocytes Granulocytes B cells Endothelial cells	- Adhesion molecule involved in leukocyte transmigration through endothelium
CD33	Sialoahesin Ig superfamily	DC Macrophages Monocytes Granulocytes Stem cell/Precursor Mast cells	- Cell adhesion - Cell-cell signaling - Inhibitory receptor - Apoptosis - Granulocytes: decreasing expression with maturation
CD38	Type II transmembrane glycoprotein Synthesizes and hydrolyzes ADP (IC Ca2+ messenger)	T cells B cells DC NK cells Macrophages Monocytes Stem cell/Precursors	- Cell adhesion - Signal transduction
CD40	TNFR superfamily	B cells Macrophages DC Endothelial cells	- Binds CD154 (CD40L) - Role in T cell-mediated activation of B cells, macrophages and DC
CD45 LCA	Protein tyrosine phosphatase receptor family Fibronectin type III family	Hematopoietic cells	- Tyrosine phosphatase that regulates T and B cells activation
CD56 NCAM	Ig superfamily	T cells NK cells DC	- Cell adhesion *NK activation: upregulation* - Neural plasticity *NK suppression: downregulation*
CD62L L-Selectin	Selectin family	B cells T cells Monocytes Granulocytes Some NK cells	- Leukocyte-endothelial adhesion - Homing of naive T cells to peripheral lymph nodes
CD66b	Carcinoembryonic antigen family	Granulocytes	- Cell adhesion - Cellular migration - Pathogen binding and activation of signaling pathways
CD69	C-type lectin	Activated B cells T cells NK cells Neutrophils	- Binds to and impairs surface expression of S1PR1, thereby promoting retention of recently activated lymphocytes in lymphoid tissues (Transient marker)

Figure 8: **II- CD molecules characteristics and functions** - CD molecules characteristics (following): structure, main cellular expression and functions. Modified from (7), (70) and (71).

CD Molecules (following)				
CD Number (Other Names)		**Molecular Structure, Family**	**Main Cellular Expression**	**Known or Proposed Function(s)**
CD86	B7-2	Ig superfamily	B cells Monocytes DC Some T cells	- Costimulator for T lymphocyte activation - Ligand for CD28 and CD152 (CTLA-4)
CD94		C-type lectin On NK cells covalently assembles with other C-type lectin molecules (NKG2)	NK cells Subset of CD8+ T cells	- CD94/NKG2 complex functions as an NK cell inhibitory receptor - Binds HLA-E class I MHC molecules
CD123	IL-3RA	Beta common (ßc) family of cytokines	DC Granulocytes Stem cell/Precursor Endothelial cells	- Hematopoietic progenitor cell growth and differentiation
CD141	BDCA-3 CLEC9A Thrombomodulin	EGF-like domains	Cross-presenting DC Monocytes Endothelial cells	- Binds thrombin and prevents blood coagulation
CD154	CD40L	TNFR superfamily	Activated CD4+ T cells	- Activation of B cells, macrophages, and endothelial cells - Ligand for CD40
CD159a	NKG2A	C-type lectin Forms heterodimer with CD94	NK cells T cell subset	- Inhibition or activation of NK cells on interation with class I HLA molecules
CD159c	NKG2C	C-type lectin Forms heterodimer with CD94	NK cells	- Activation of NK cells on interaction with the appropriate class I HLA molecules
CD314	NKG2D	C-type lectin	NK cells Activated CD8+ T cells NK-T cells Some myeloid cells	- Binds MHC class I, and the class I-like molecules MIC-A, MIC-B, Rae1, and ULBP4 - Role in NK cell and CTL activation
CD337	NKp30	I-type Ig-like fold	NK cells	- Immune surveillance in anti-tumor immunity

Figure 8: **III- CD molecules characteristics and functions** - CD molecules characteristics (following): structure, main cellular expression and functions. Modified from (7), (70) and (71).

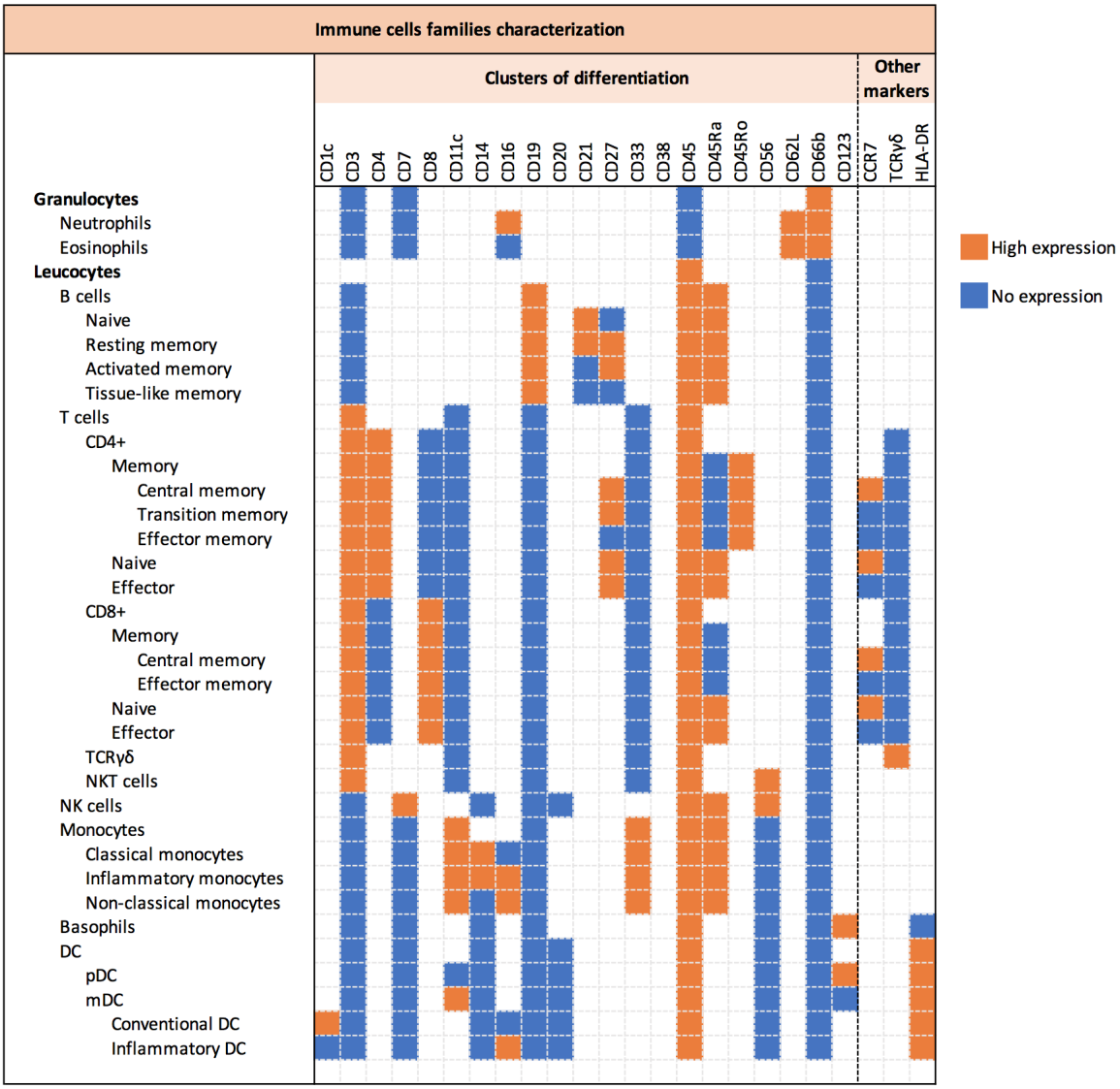

Figure 9: **Major immune cells molecular characterization** - This non-exhaustive table illustrates the phenotypic molecular signatures characterizing the major known human immune cells subsets. Blue means an absence of expression, whereas orange means that the molecule is expressed. Sources: (2) and (7).

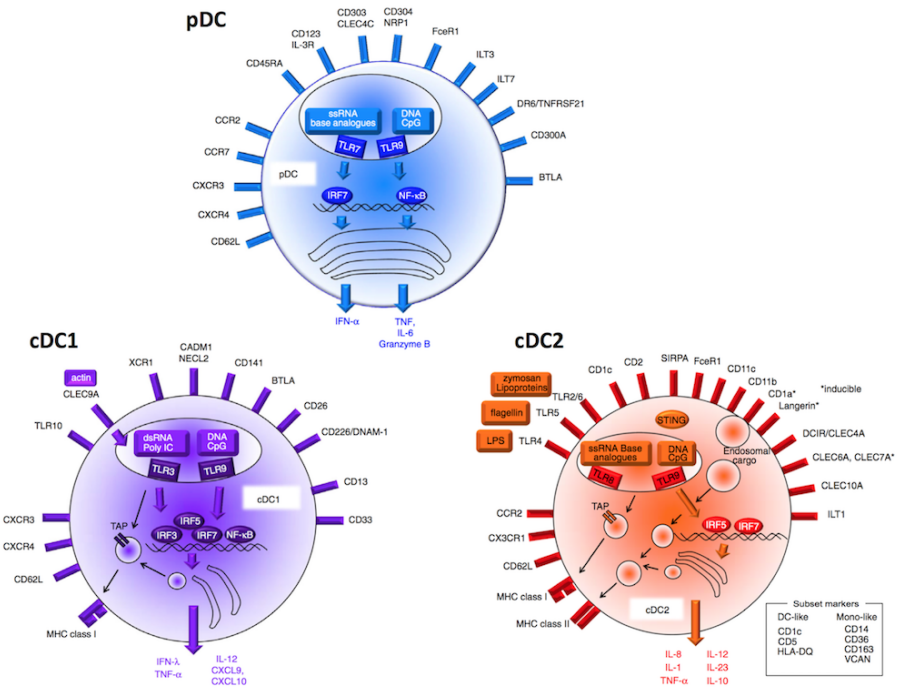

Figure 10: **Main DCs subsets molecular characterization** - Surface molecules, intracellular PRRs, expressed genes and released cytokines by plasmacytoid DCs, type 1 and type 2 conventional DCs. Sources: (5).

Classification of primary immune deficiency diseases	
Deficiencies of the innate immune system	
Phagocytic cells	Impaired production: severe congenital neutropenia (SCN) Asplenia Impaired adhesion: leukocyte adhesion deficiency (LAD) Impaired killing: chronic granulomatous disease (CGD)
Innate immunity receptors and signal transduction	Defects in Toll-like receptor signalling Mendelian susceptibility to mycobacterial disease
Complement deficiencies	Classical, alternative, and lectin pathways Lytic phase
Deficiencies of the adaptive immune system	
T lymphocytes	
Impaired development	Severe combined immune deficiencies (SCIDs) DiGeorge syndrome
Impaired survival, migration, function	Combined immunodeficiencies Hper-IgE syndrome (Job syndrome) DOCK8 deficiency CD40 ligand deficiency Wiskott-Aldrich syndrome Ataxia-telangiectasia and other DNA repair deficiencies
B lymphocytes	
Impaired development	XL and AR agammaglobulinemia
Impaired function	Hyper-IgM syndrome Common variable immunodeficiency (CVID) IgA deficiency
Regulatory defects	
Innate immunity	Autoinflammatory syndromes Severe colitis
Adaptive immunity	Hemophagocytic lymphohistiocytosis (HLH) Autoimmune lymphoproliferation syndrome (ALPS) Autoimmunity and inflammatory diseases (IPEX, APECED)

Figure 11: **Primary immunodeficiencies classification** - Classification of PID based on the side of immunity that is disturbed, and the mechanism responsible for this defect. Sources: (12).

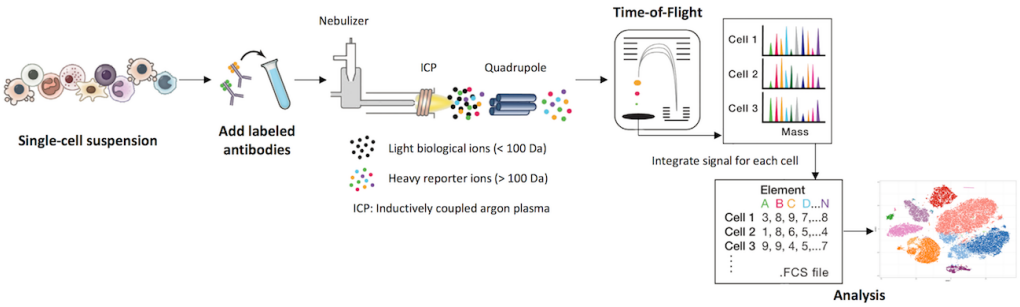

Figure 12: **Mass Cytometry Workflow** - General outlook of mass cytometry workflow, from single-cell suspension to data analysis. Modified from (33).

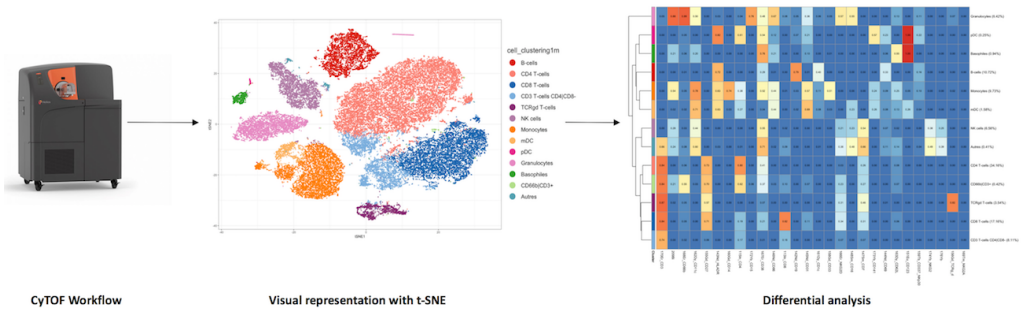

Figure 13: **General Analysis Pipeline** - General data processing pipeline starting with data acquisition through a mass cytometer, then cluster identifications and finally intra-clusters evaluation.

Immunodeficiency panel used in this project				
Isotope	**Marker**	**Clone**	**Source**	**Titration [µL]**
Extracellular markers				
89 Y	CD45 #1	HI30	DVS	0.50
113 In	CD8	RPA-T8	CHUV	0.25
115 In	CD4	RPA-T4	CHUV	0.30
141 Pr	CD45 #2	HI30	DVS	0.70
142 Nd	CD19	HIB19	DVS	0.80
143 Nd	HLADR	L243	DVS	0.75
144 Nd	CD69	FN50	DVS	0.50
145 Nd	CD31	WM59	DVS	0.60
146 Nd	CD86	IT2.2	CHUV	0.25
147 Sm	CD7	CD7-6B7	DVS	0.45
148 Nd	CD16	3G8	DVS	0.72
151 Eu	CD123	6H6	DVS	0.80
155 Gd	CD27	L128	DVS	0.50
156 Gd	TCRγδ	B1	CHUV	0.80
158 Gd	CD33	WM53	DVS	0.50
159 Tb	CD337/NKp30	Z25	DVS	0.50
160 Gd	CD14	M5E2	DVS	0.97
161 Dy	CD1c	L161	CHUV	0.61
162 Dy	CD11c	Bu15	DVS	0.96
163 Dy	CD62L	DREG-56	CHUV	0.25
166 Er	CD314/NKG2D	ON72	DVS	0.50
167 Er	CD38	HIT2	DVS	0.30
168 Er	CD66b	G10F5	CHUV	0.80
169 Er	CD159a/NKG2A	Z199	DVS	0.50
170 Er	CD3	UCHT1	DVS	0.40
172 Yb	CD15	W6D3	DVS	0.50
173 Yb	CD141	1A4	DVS	0.75
174 Yb	CD94/NKG2	HP-3D9	DVS	0.50
176 Yb	CD56	HCD56	DVS	0.96
209 Bi	CD11b	ICRF44	DVS	0.40
Intracellular markers				
149 Sm	IL12p40	C11.5	CHUV	0.50
150 Nd	IFNα	LT27:295	CHUV	0.60
152 Sm	TNFα	Mab11	DVS	0.50
153 Eu	IL1b	AS10	CHUV	0.50
154 Sm	IL6	MQ2-13A5	DVS	0.80
164 Dy	IL17a	N49-653	DVS	0.80
165 Ho	IFNγ	B27	DVS	0.50
171 Yb	Granzyme B	GB11	DVS	0.50
175 Lu	Perforin	B-D48	DVS	0.60

Figure 14: **Innate immune functional panel** - Panel of antibodies used in this project, with the corresponding heavy metal isotope, marker, clone and source. In, Indium; Pr, Praseodymium; Nd, Neodymium; Sm, Samarium; Eu, Europium; Gd, Gadolinium; Tb, Terbium; Dy, Dysprosium; Er, Erbium; Yb, Ytterbium; Bi, Bismuth; Ho, Holmium; Lu, Lutetium.

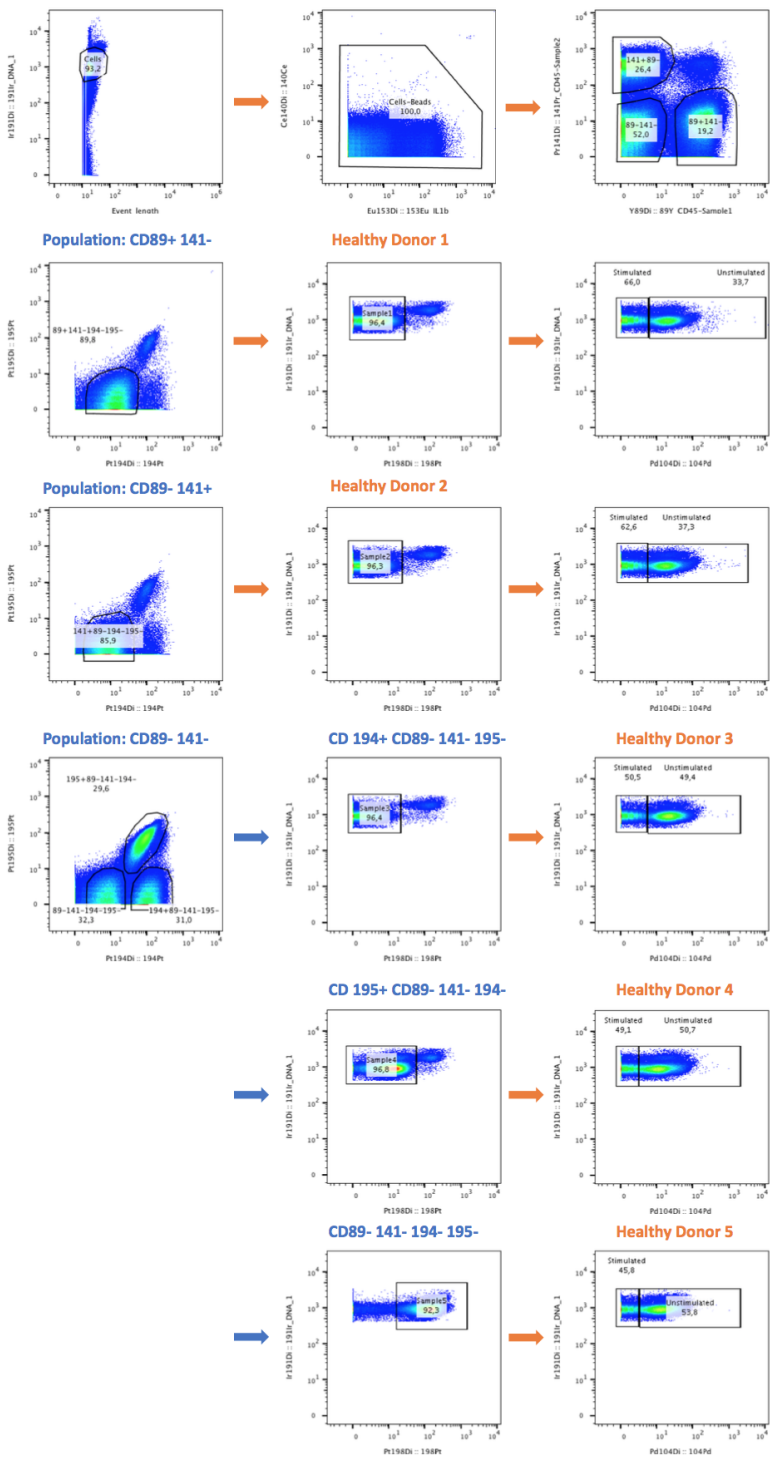

Figure 15: **Initial gating strategy to individualize each condition for each patient and healthy donor** - Gating strategy using FlowJo, allowing to get individualized FCS files for each samples condition, illustrated for the batched data acquired containing the healthy donors 1 to 5. At the end, this means that 60 FCS files will be created (15 patients, 15 healthy donors, with each of them having 2 conditions).

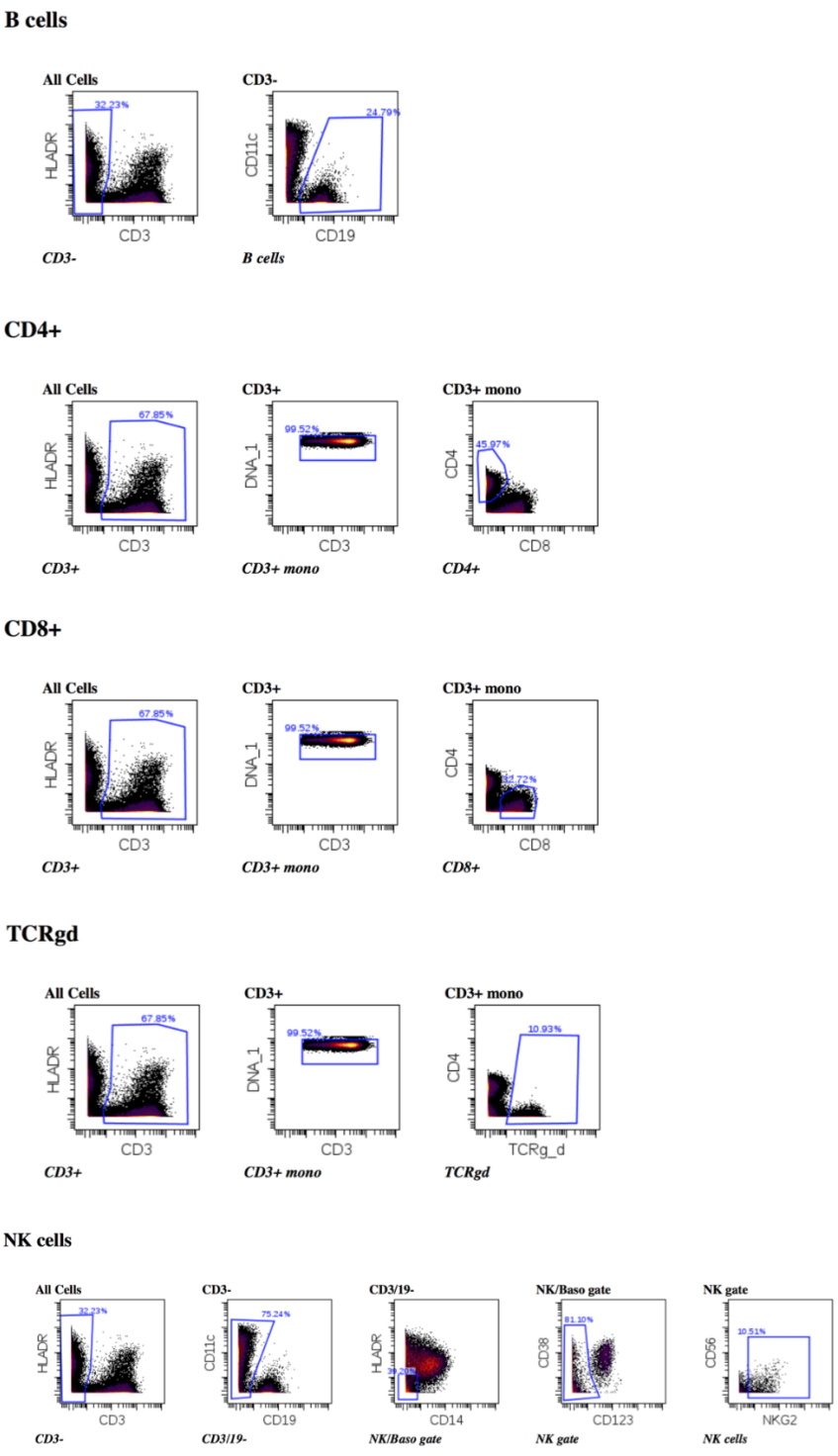

Figure 16: **I- Manual gating strategy for the major known immune cells populations** - Manual gating using Cytobank, plotting two-dimension scatterplots to iteratively cluster cells groups according to their surface markers. It can be seen as an iterative selection process of different markers expressions which at the end will define the corresponding cells populations.

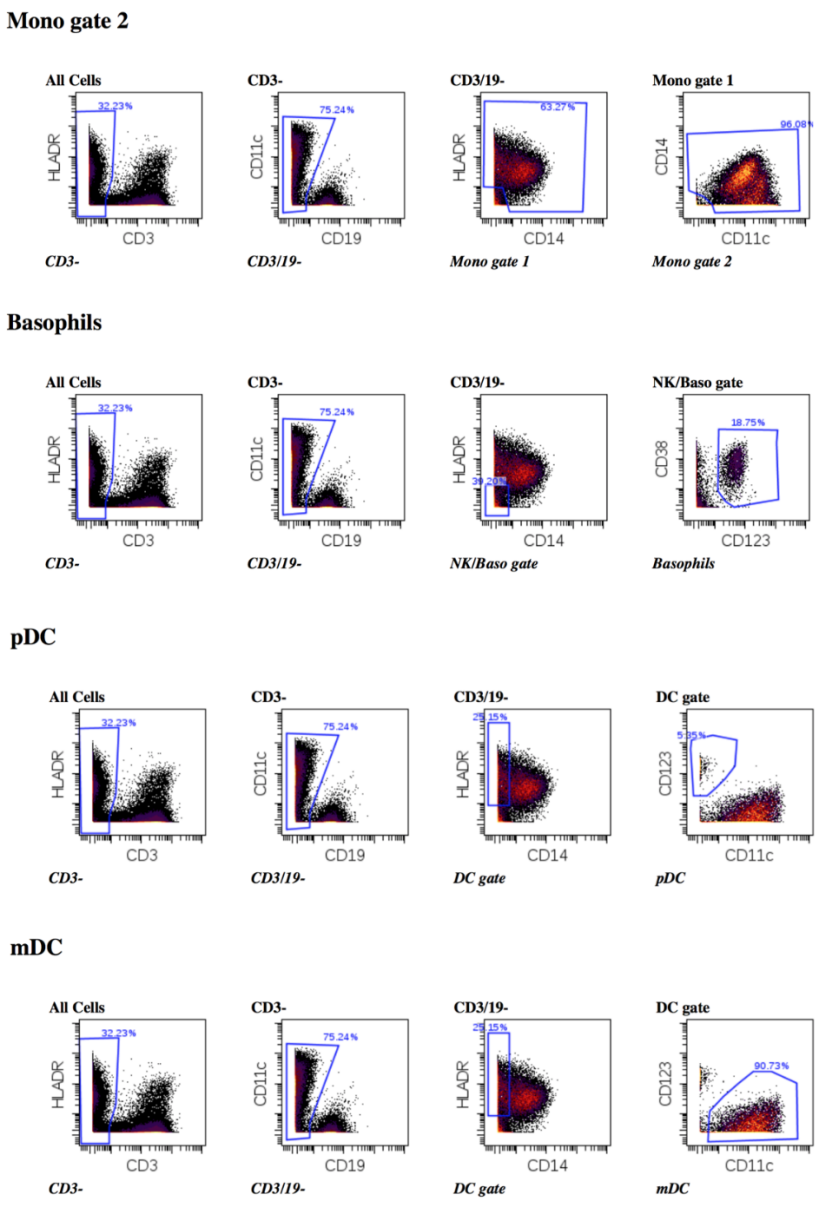

Figure 16: **II- Manual gating strategy for the major known immune cells populations** - Following.

Clustering Suface Markers	
All cells	**mDCs**
CD1c	CD1c
CD3	CD11b
CD4	CD11c
CD7	CD16
CD8	CD31
CD11c	CD38
CD14	CD62L
CD16	CD69
CD19	CD86
CD56	CD123
CD66b	CD141
CD123	HLA-DR
HLA-DR	
TCRγ	

Figure 17: **Clustering surface markers** - Surface markers used to discriminate cells sub-populations when automatically clustering them, for all cells and mDC population.

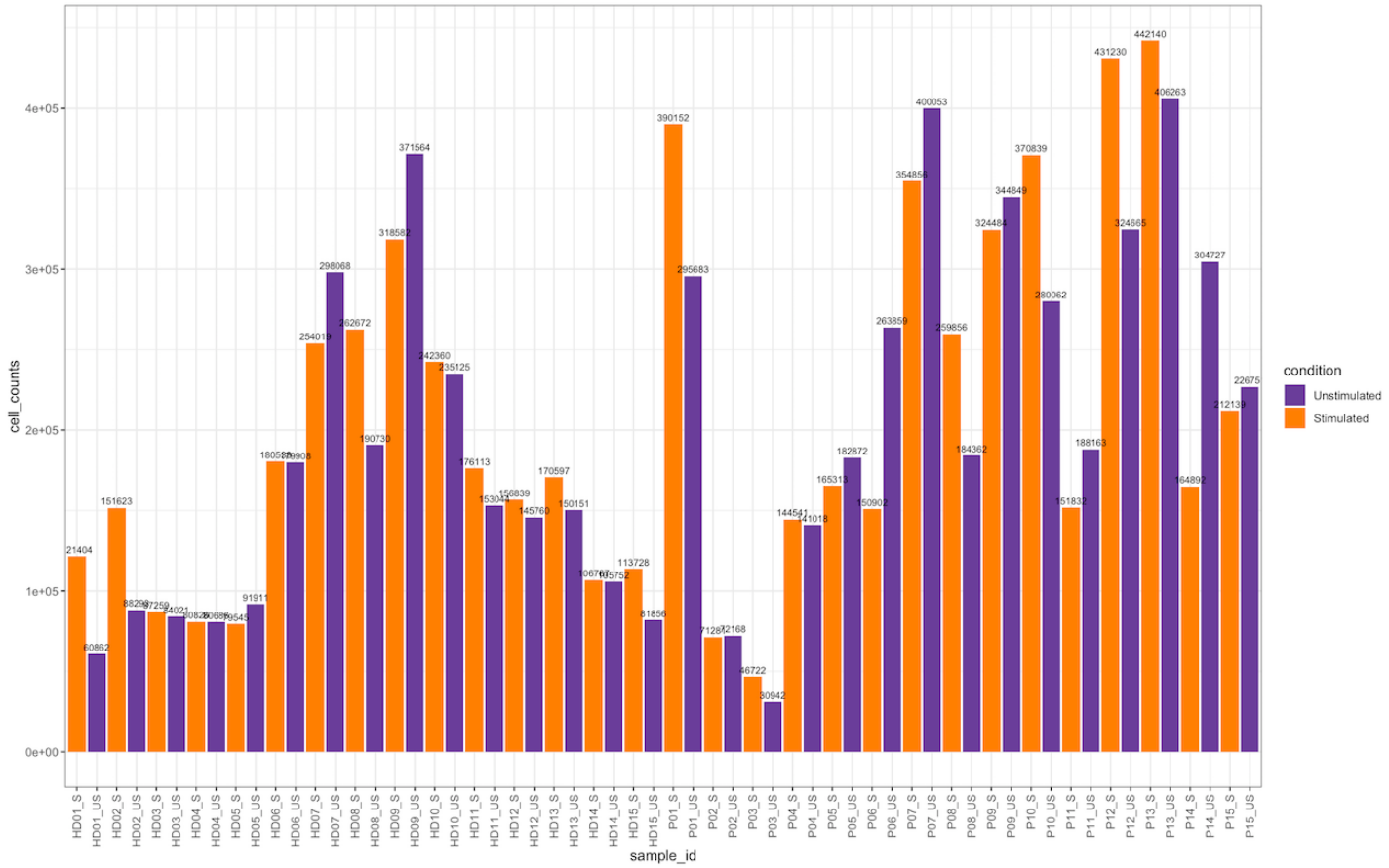

Figure 18: **Barplot displaying the number of acquired events for each sample and condition** - The x-axis represents the different samples and conditions, distinguished by color. The y-axis indicates the cell counts for each of the sample, and is written on the top of each barplot. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

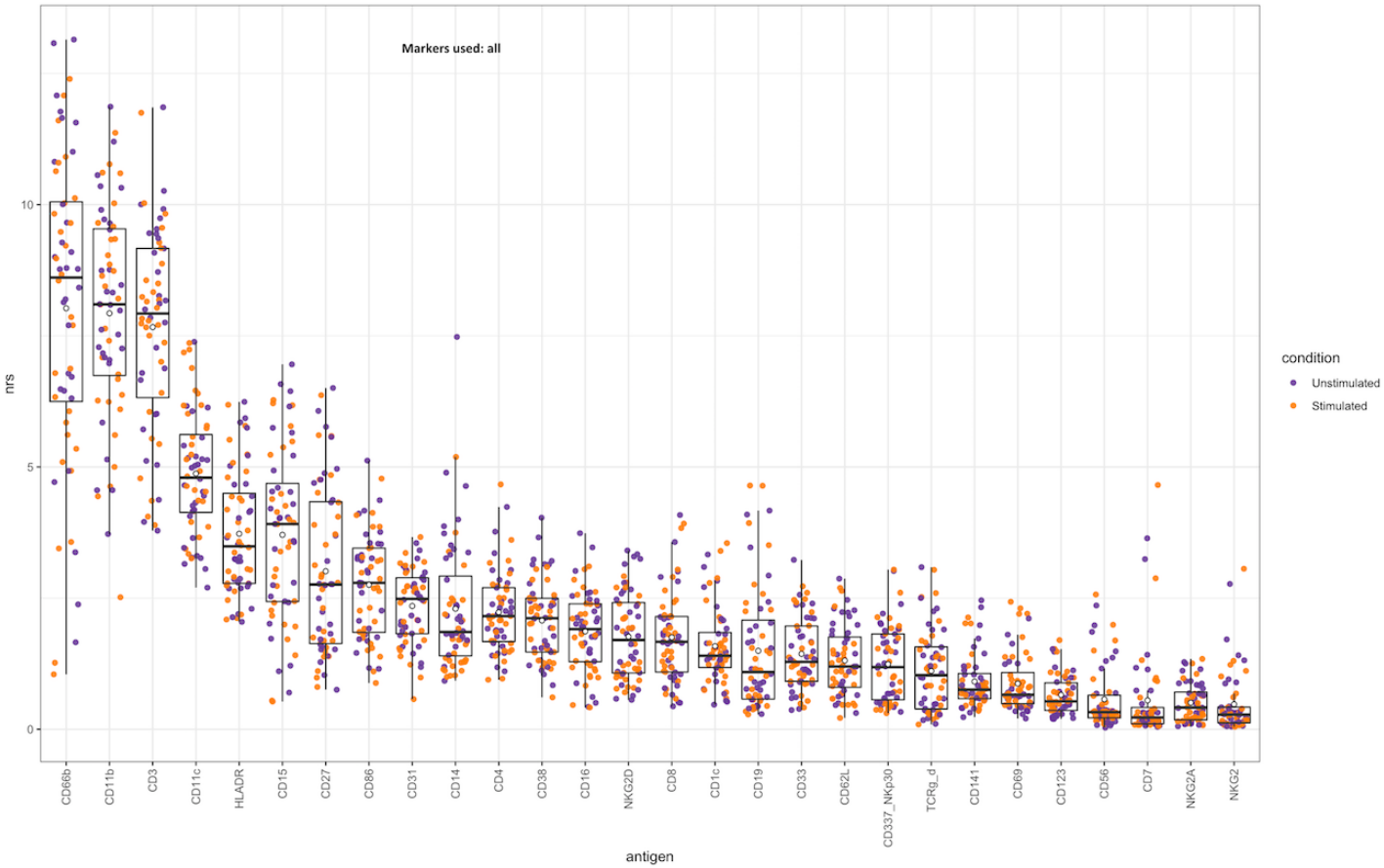

Figure 19: **Non-redundancy scores for all samples and all surface markers** - Surface markers are arranged according to their NRS from the statistically most to the least discriminative, for all samples. This should be used as a help to identify the markers that will be used for the downstream clustering, but should not be taken as an absolute verity as it does not take into account biological meanings.

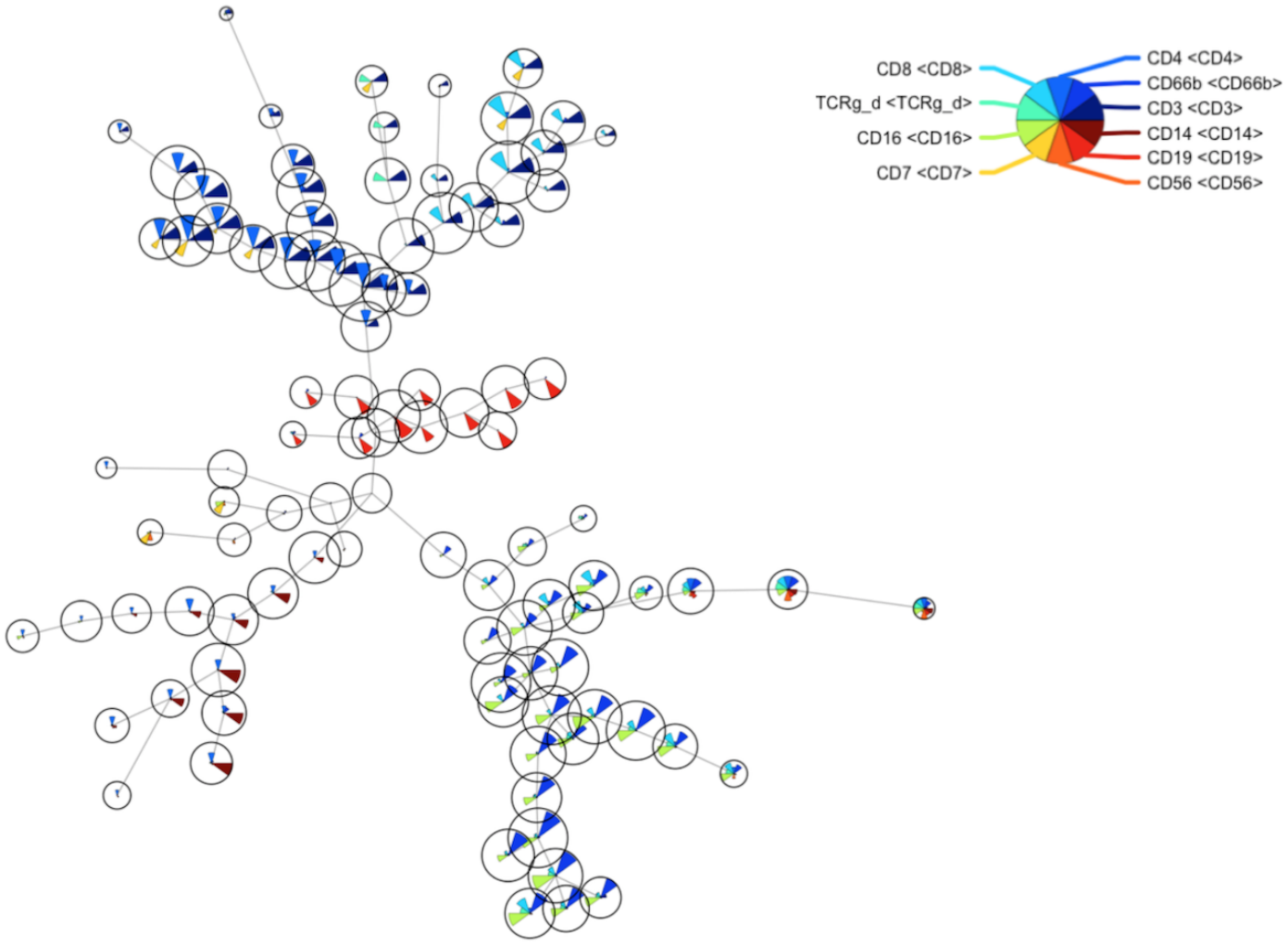

Figure 20: **FlowSOM minimal spanning tree** - Minimal spanning tree representing 100 clusters, or nodes, resulting from a FlowSOM algorithm. 10 markers intensities are presented for each node.

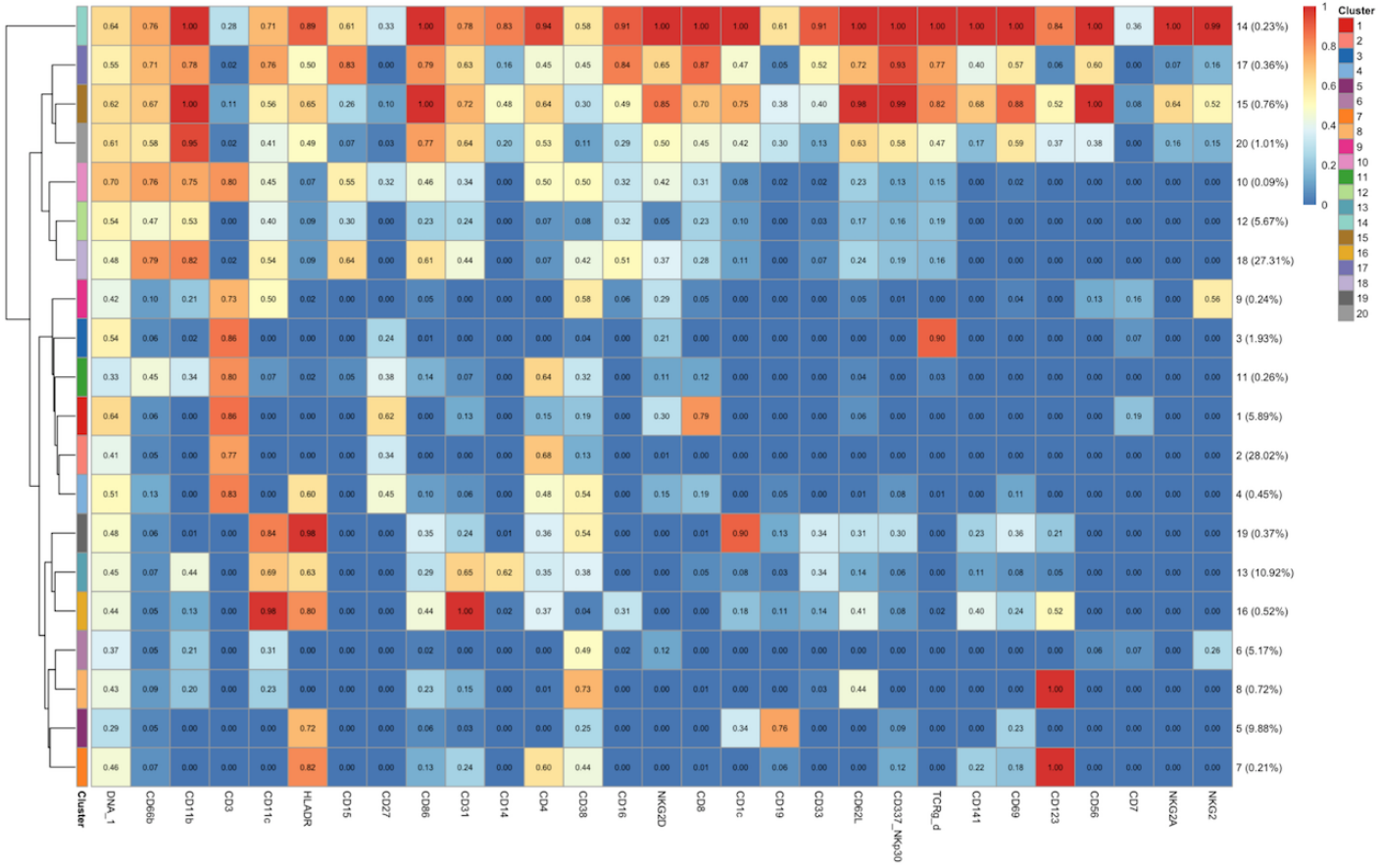

Figure 21: **Heatmap of the median intensities of each surface markers in each population for all samples** - Median intensities for each cluster have been calculated and are represented with the corresponding color on this heatmap.

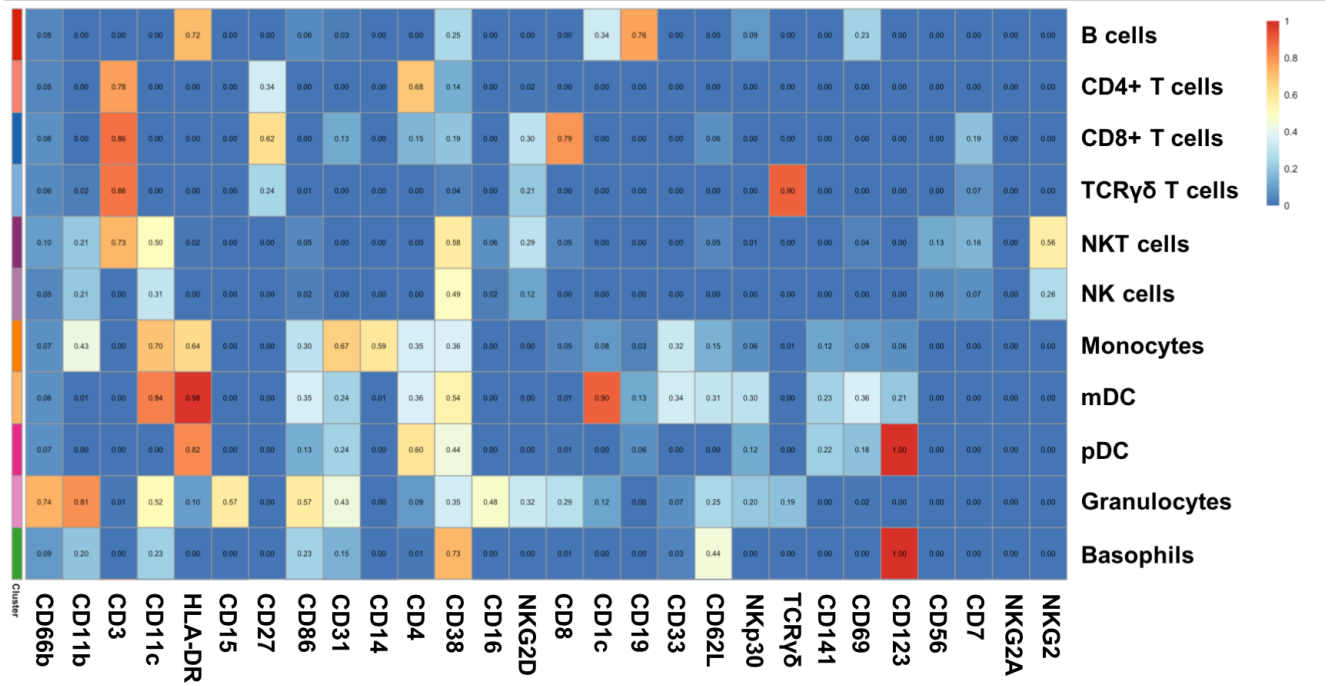

Figure 22: **Heatmap of the median intensities of each surface markers the major known immune cells populations** - Each known identified immune cell population is represented with its signature of surface molecular expression.

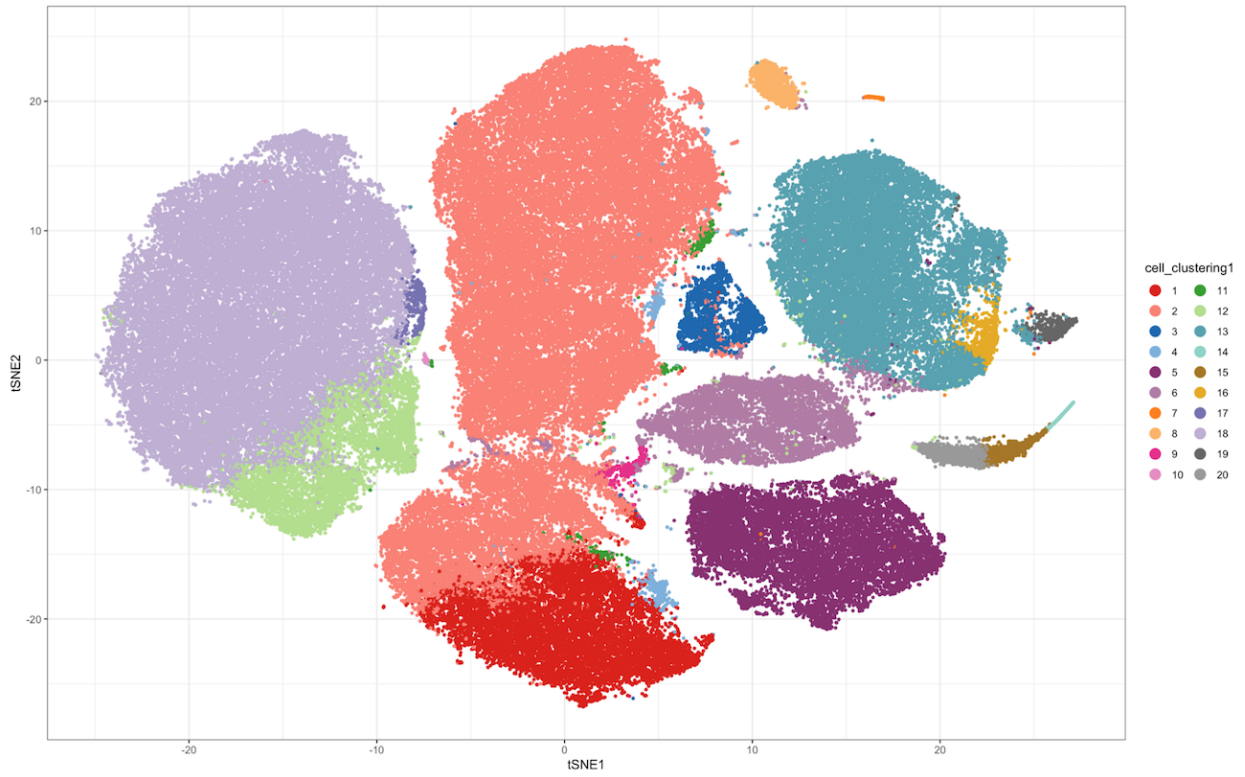

Figure 23: **tSNE plot of all cells** - Each cell is coloured according to the cluster it has been assigned to.

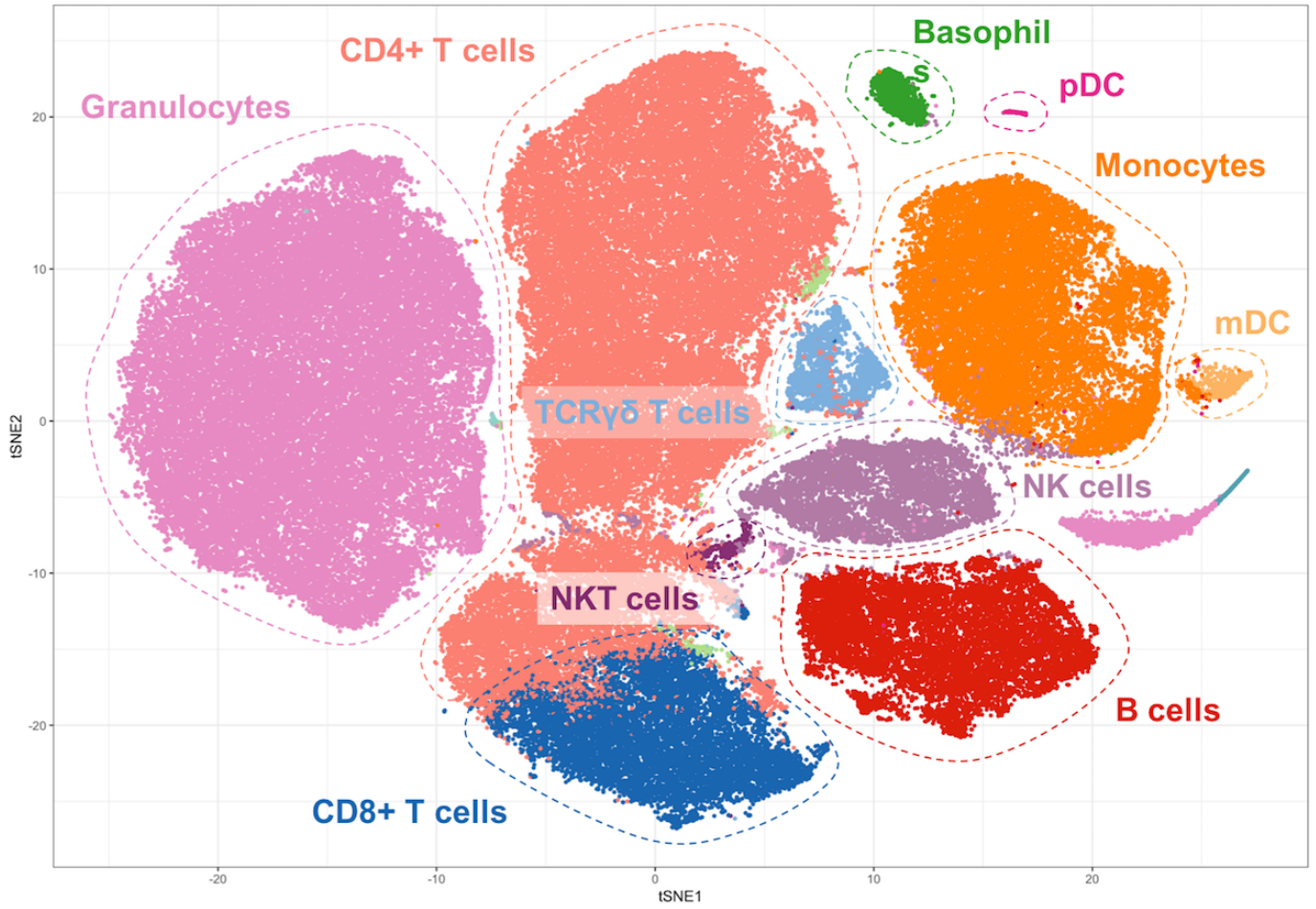

Figure 24: **tSNE plot of known major immune cells populations** - tSNE plot displaying the final result of clustering after the manual step, highlighting the different immune cells populations that could be isolated and identified.

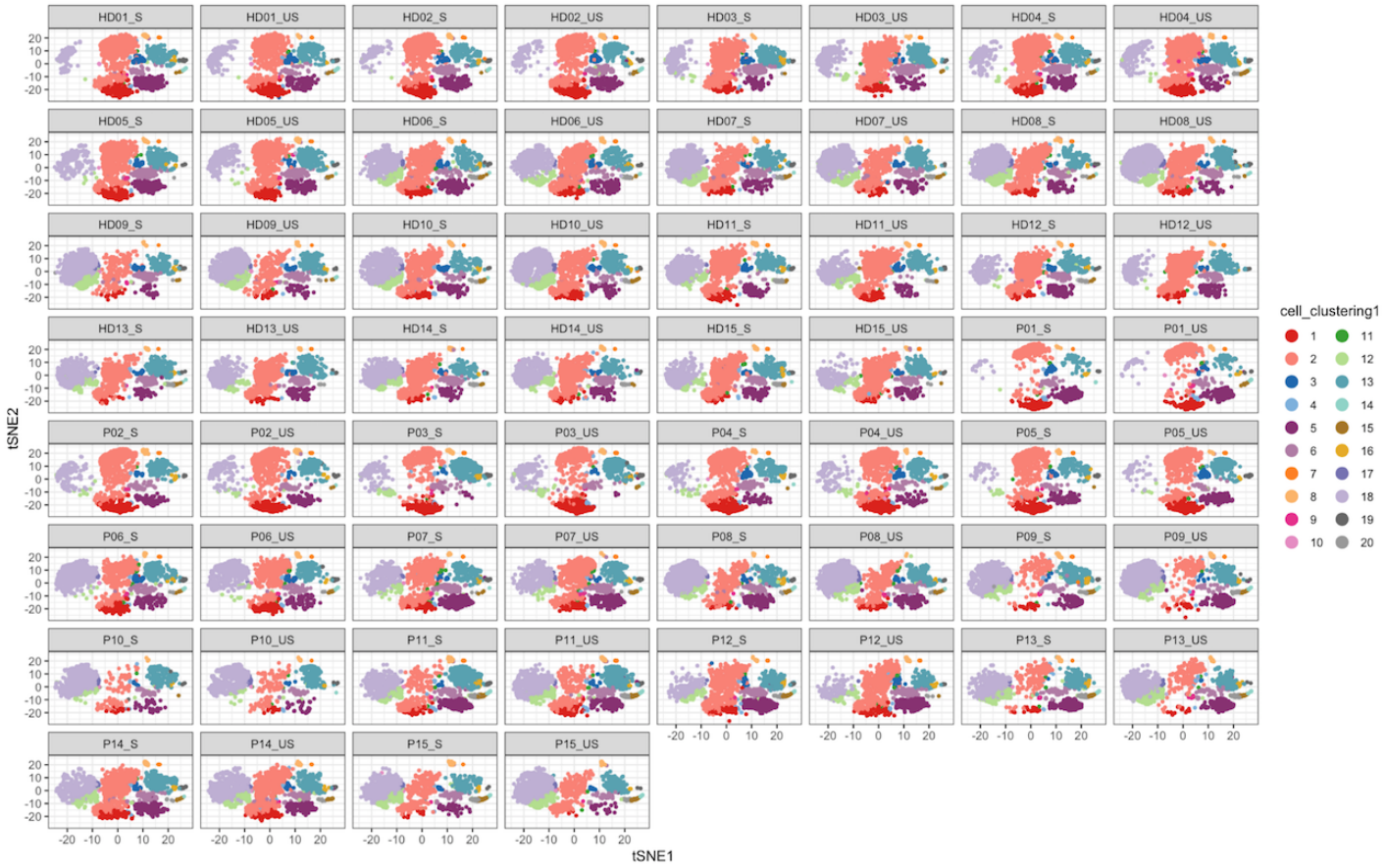

Figure 25: **tSNE plot stratified by samples and conditions** - tSNE plot displaying the meta-clustering for each of the samples and conditions, allowing a quick overview of the presence or absence of each immune cell population. US, unstimulated; S, stimulated.

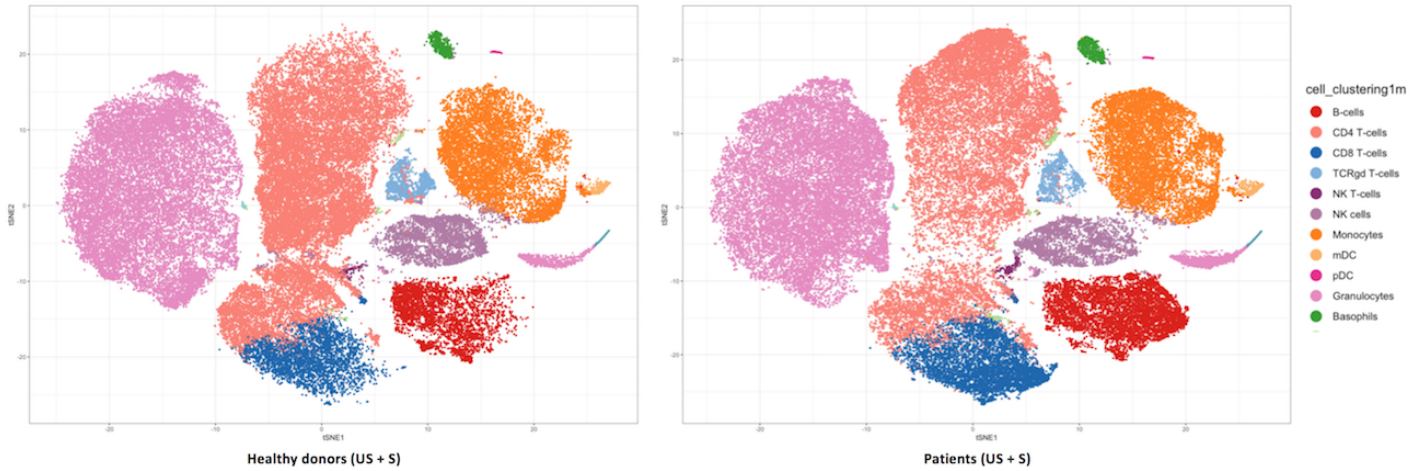

Figure 26: **tSNE plot comparison between healthy donors and patients** - tSNE plot displaying the identified immune cells populations for the healthy donors group, compared to the one obtained for the patients group. US, unstimulated; S, stimulated.

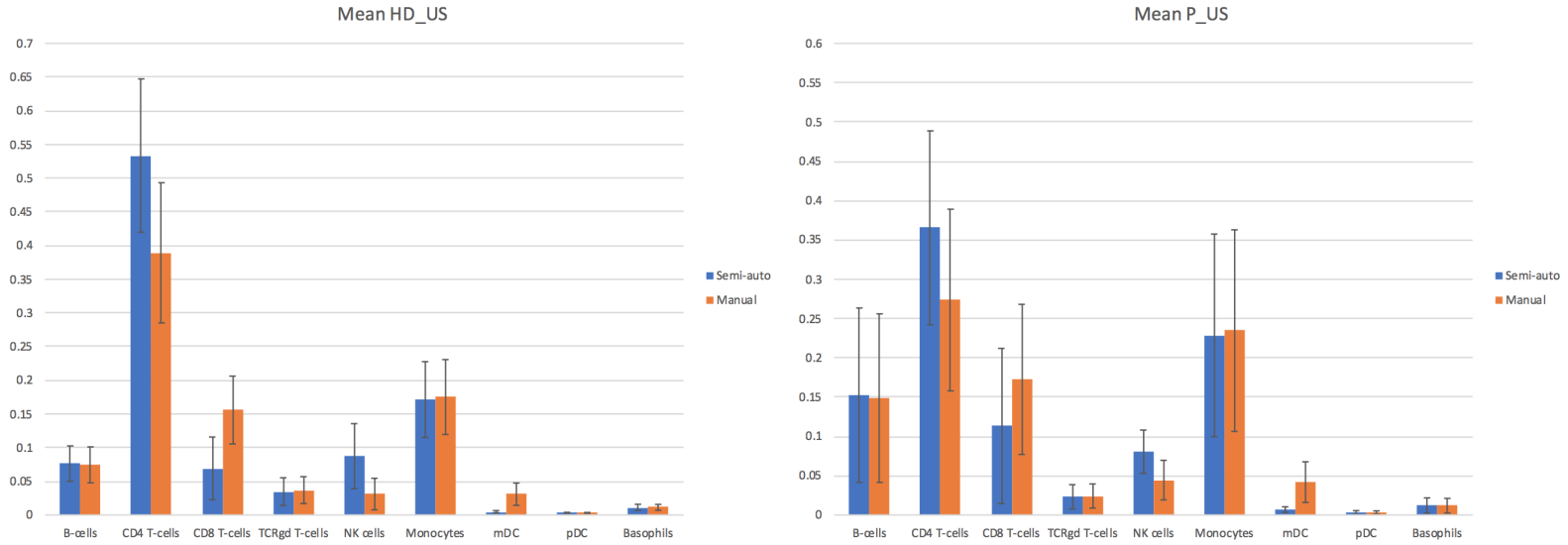

Figure 27: **Immune cells populations concordance between manual and semi-automatic clustering methods** - The mean manual and semi-automatic obtained proportion of each cell population is represented for healthy donors and patients in the unstimulated condition. HD, healthy donor; P, patient; US, unstimulated.

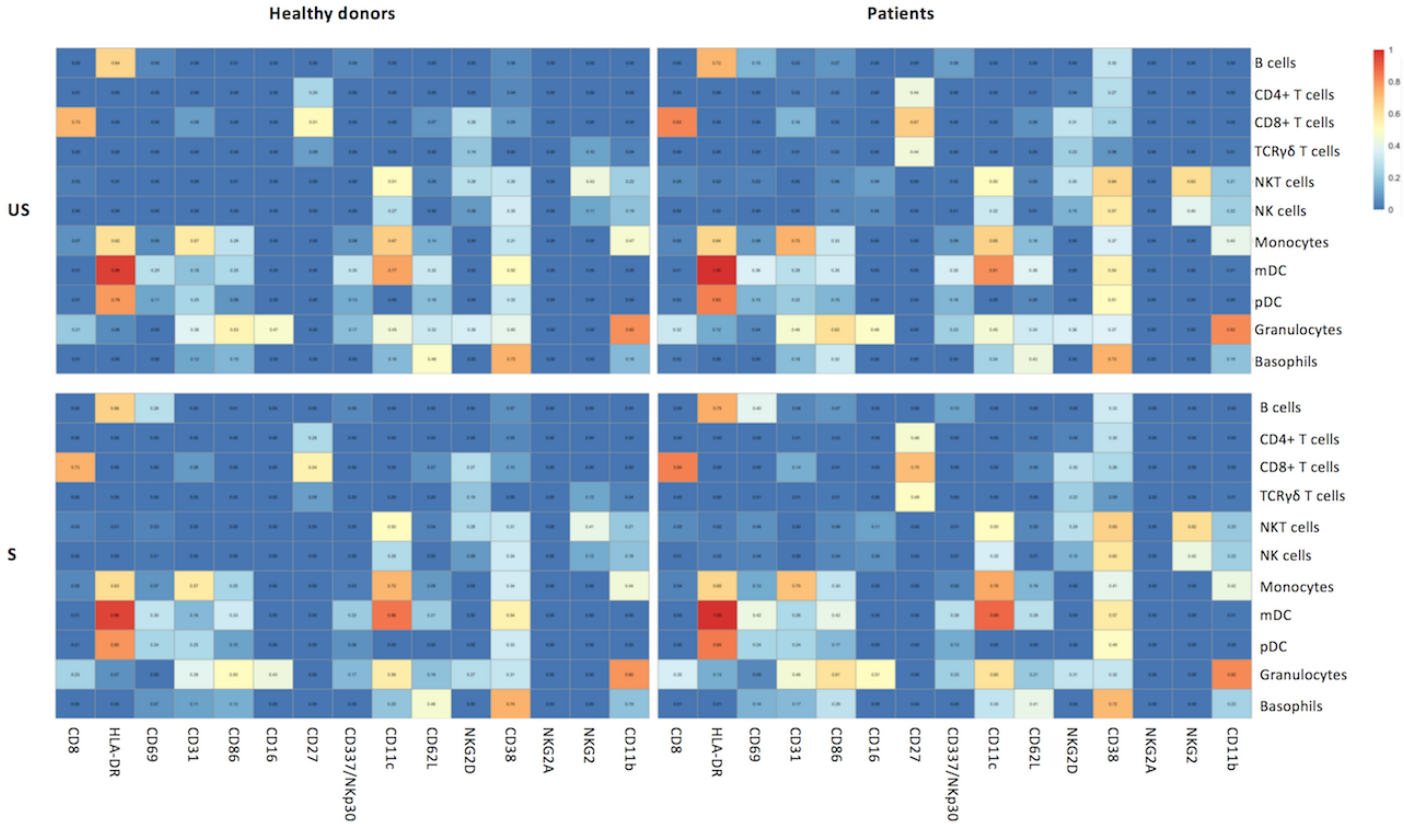

Figure 28: **Median activation markers intensities for each of the groups and conditions** - Visual representation of the activation markers intensities in each of the populations for the healthy donors and patients groups, compared by conditions. US, unstimulated; S, stimulated.

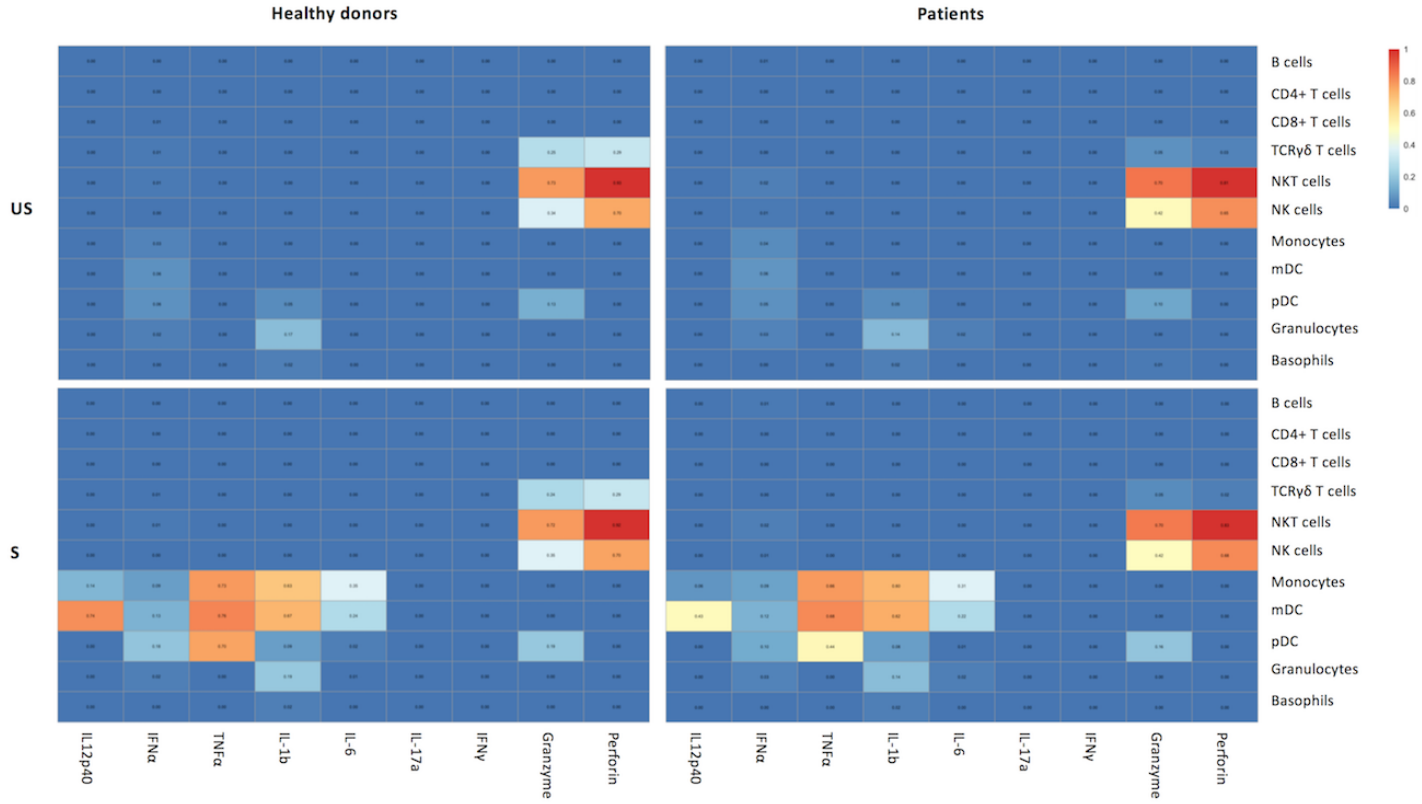

Figure 29: **Median cytokines expressions intensities for each of the groups and conditions** - Visual representation of the cytokines expressions in each of the populations for the healthy donors and patients groups, compared by conditions. US, unstimulated; S, stimulated.

Figure 30: **Boxplots with jittered points representing the immune cells relative abundance between healthy donors and patients in the unstimulated condition** - General overview of the immune cells relative abundance distribution between patients and healthy donors. HD, healthy donor.

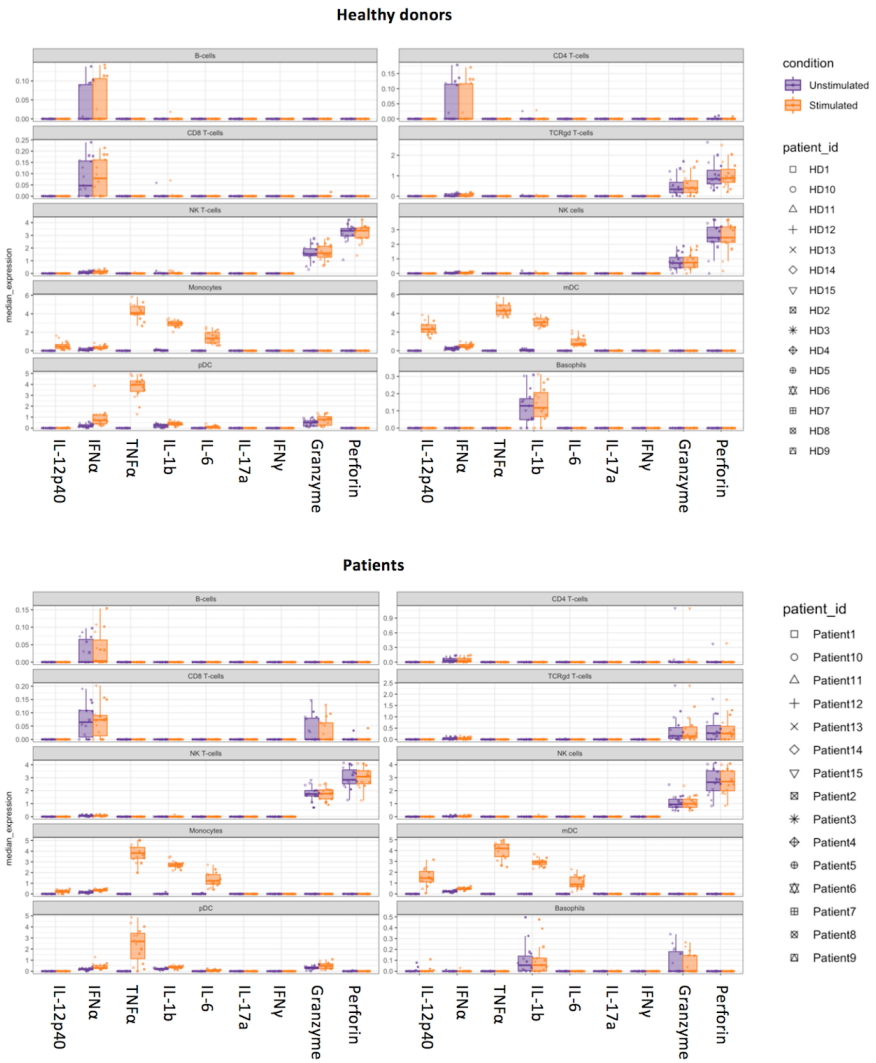

Figure 31: **Boxplots with jittered points representing the cytokines expression in the different immune cells populations** - Comparison for each of the populations the difference in cytokines expression between the unstimulated and stimulated conditions. HD, healthy donor.

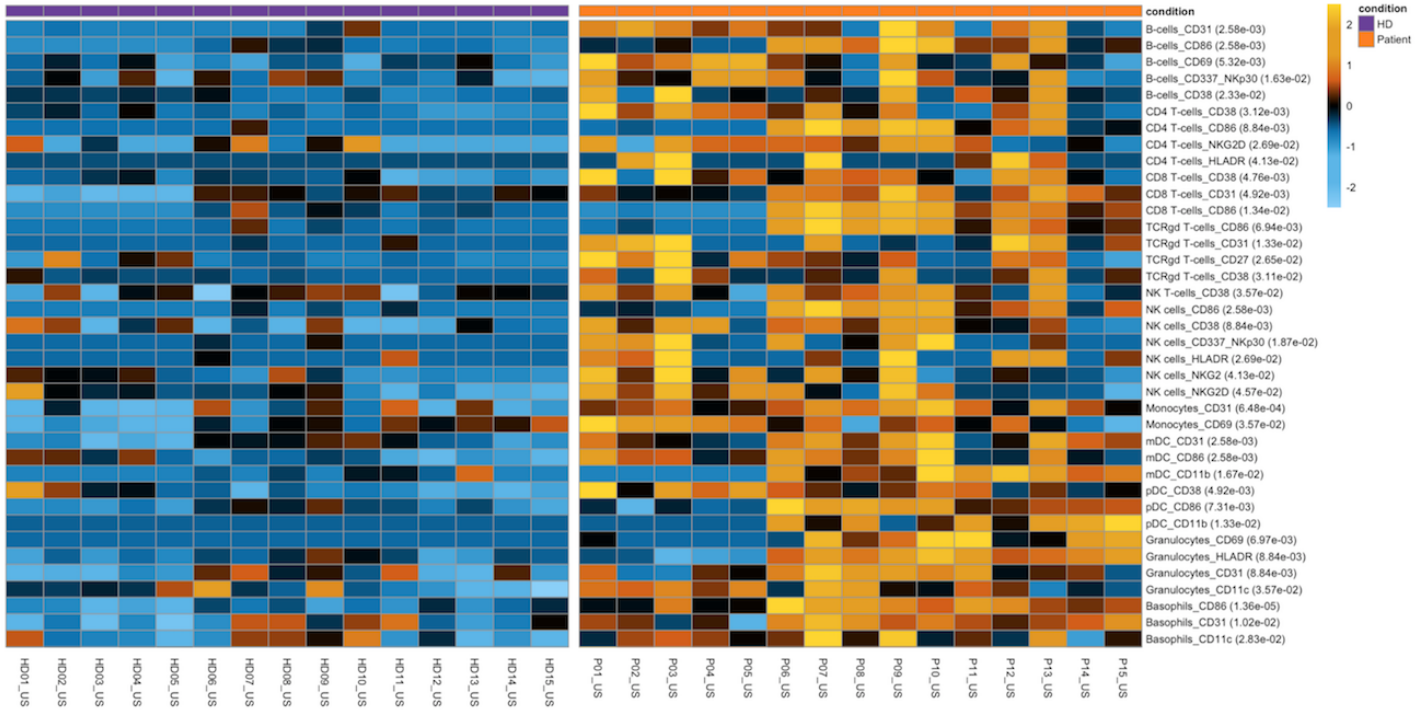

Figure 32: **Normalized activation markers expression comparison between the healthy donors and patients group in the unstimulated condition** - Heatmap illustrating the differences in the activation markers expressions that are statistically significant in the identified immune cells populations between the patients and healthy donors groups. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

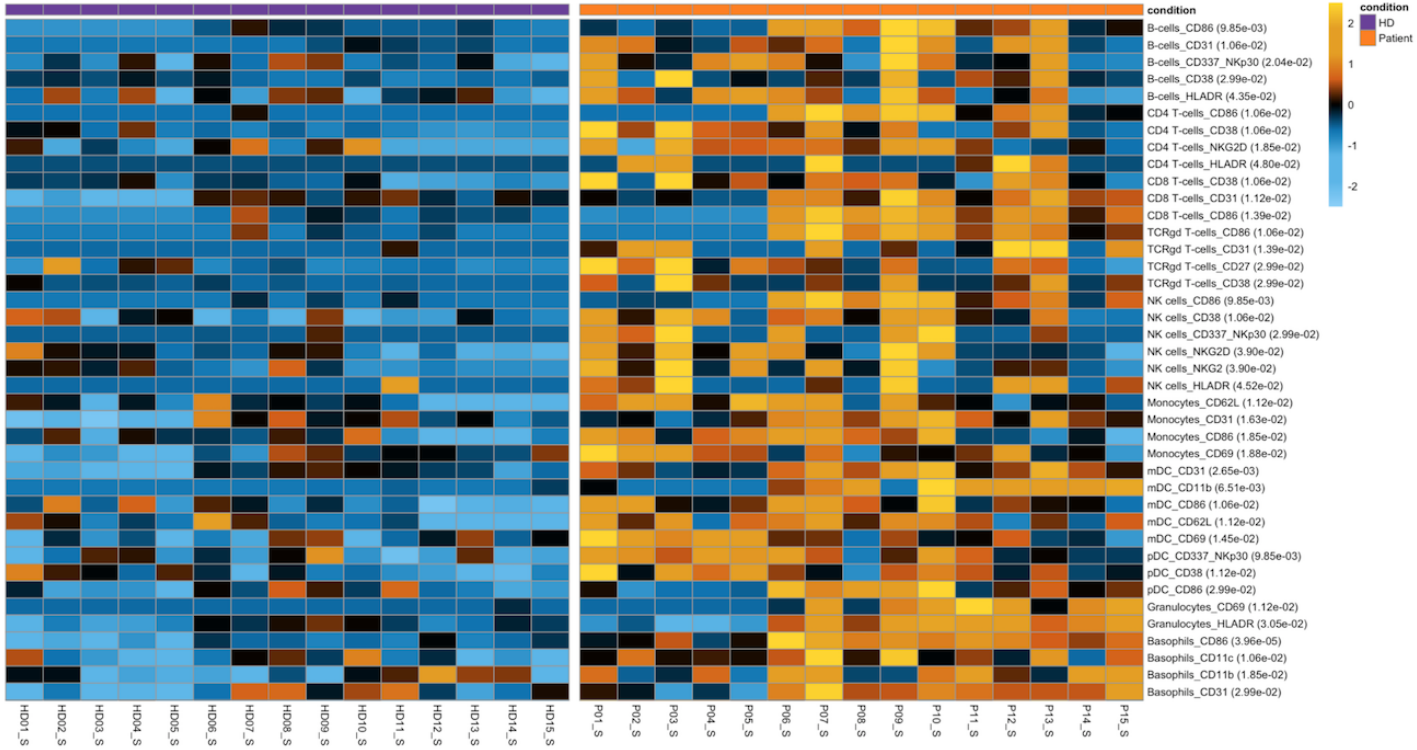

Figure 33: **Normalized activation markers expression comparison between the healthy donors and patients group in the stimulated condition** - Heatmap illustrating the differences in the activation markers expressions that are statistically significant in the identified immune cells populations between the patients and healthy donors groups. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

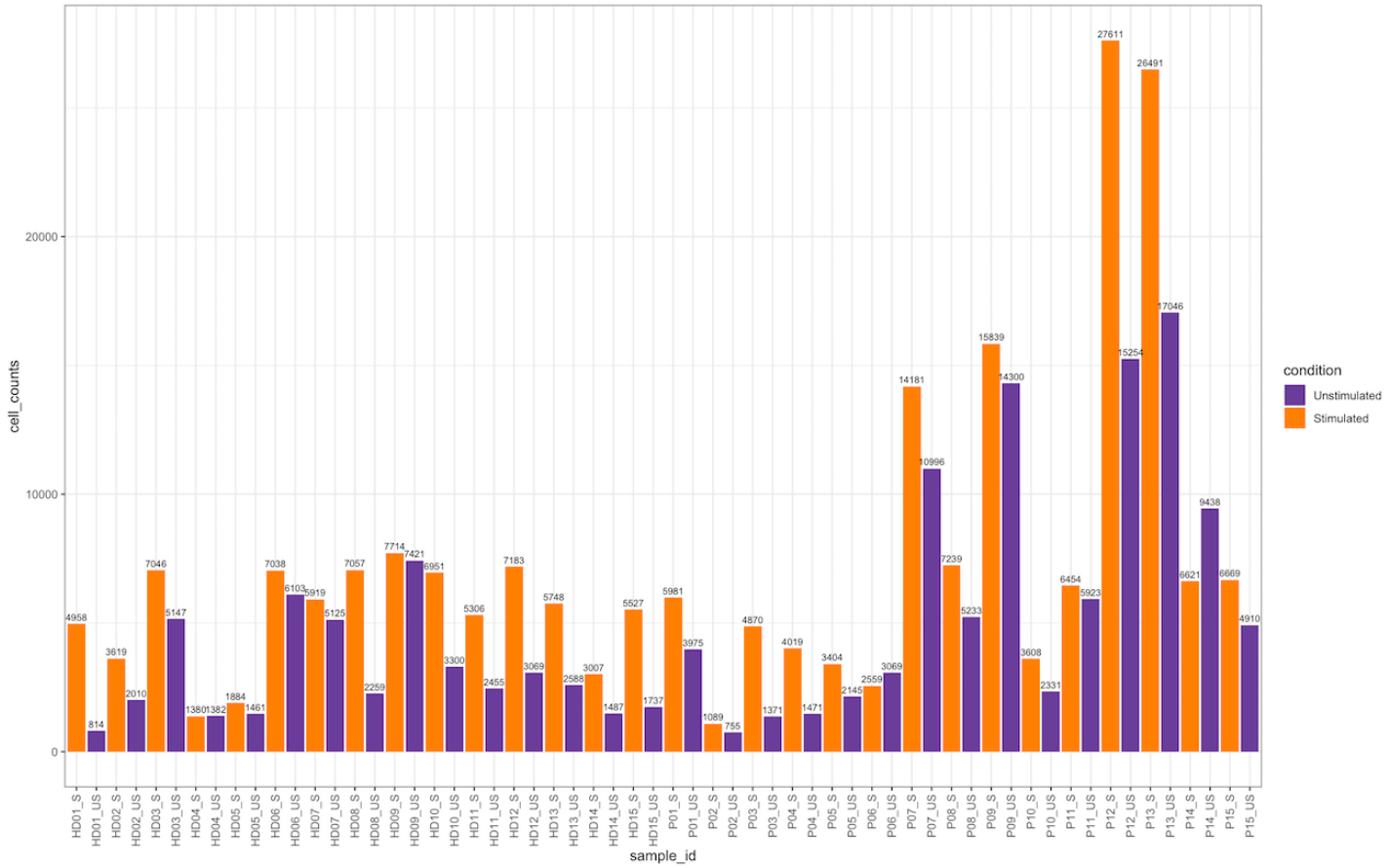

Figure 34: **Barplot displaying the number of acquired events for each samples and conditions in the mDC population** - The x-axis represents the different samples and conditions, distinguished by color. The y-axis indicates the cell counts for each of the sample, and is written on the top of each barplot. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

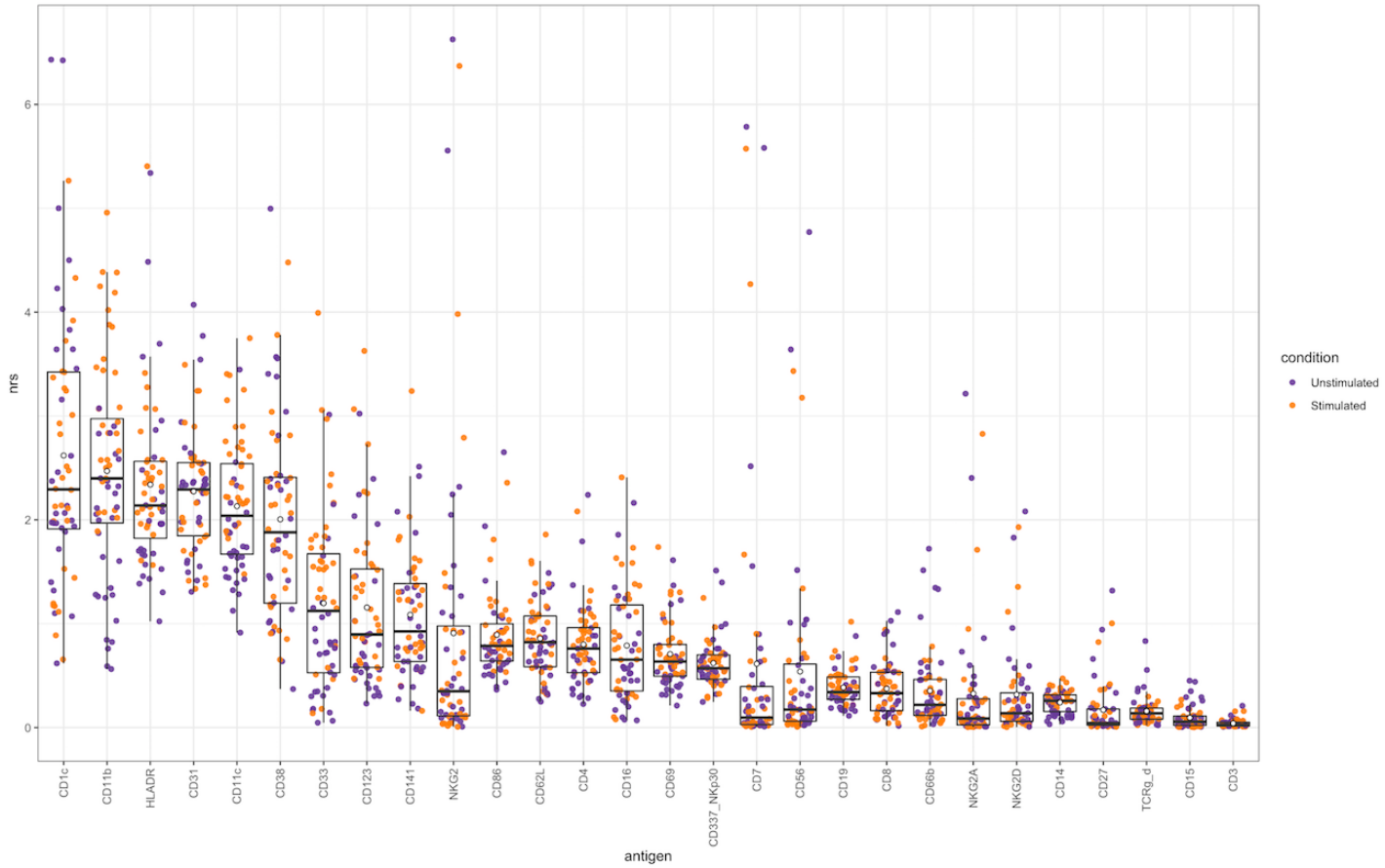

Figure 35: **Non-redundancy scores for all samples and all surface markers in the mDC population** - Surface markers are arranged according to their NRS from the statistically most to the least discriminative, for all samples. This should be used as a help to identify the markers that will be used for the downstream clustering, but should not be taken as an absolute verity as it does not take into account biological meanings.

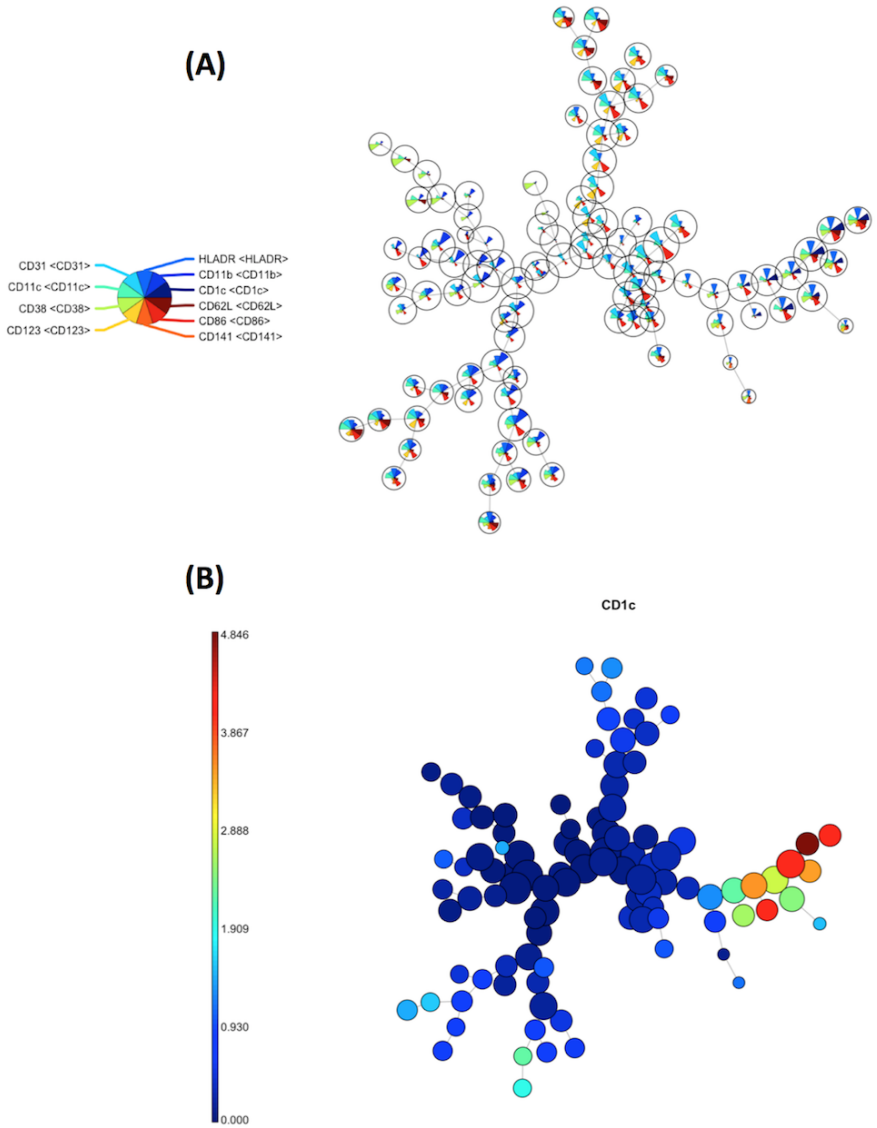

Figure 36: **Minimal spanning tree obtained with FlowSOM on the mDC population** - (A) Minimal spanning tree displaying the intensity in some marker expressions of the 100 identified clusters. (B) CD1c expression intensities among the 100 clusters.

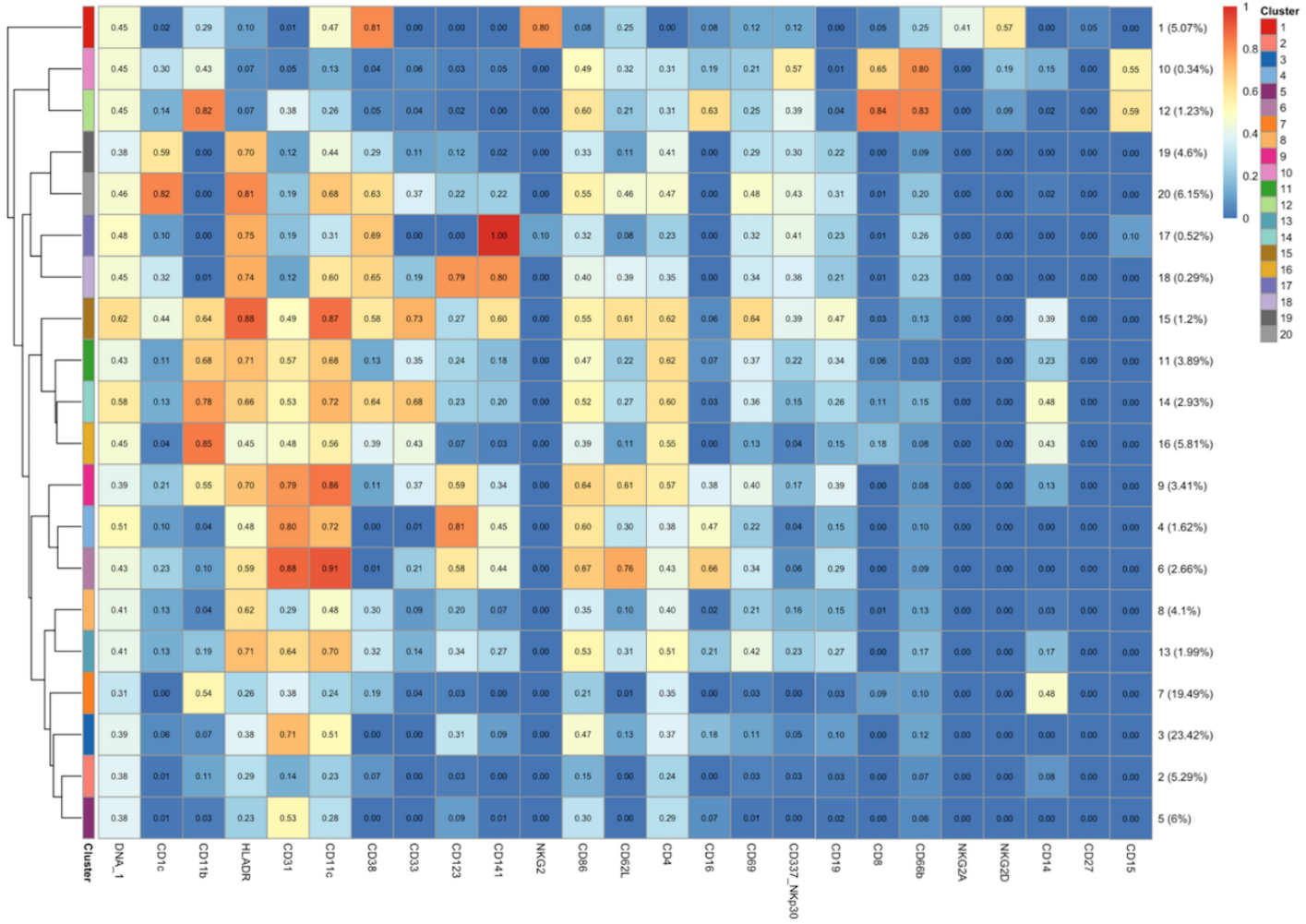

Figure 37: **Heatmap of the median intensities of each surface markers in each population for all samples in the mDC population** - Median intensities for each cluster have been calculated and are represented with the corresponding color on this heatmap.

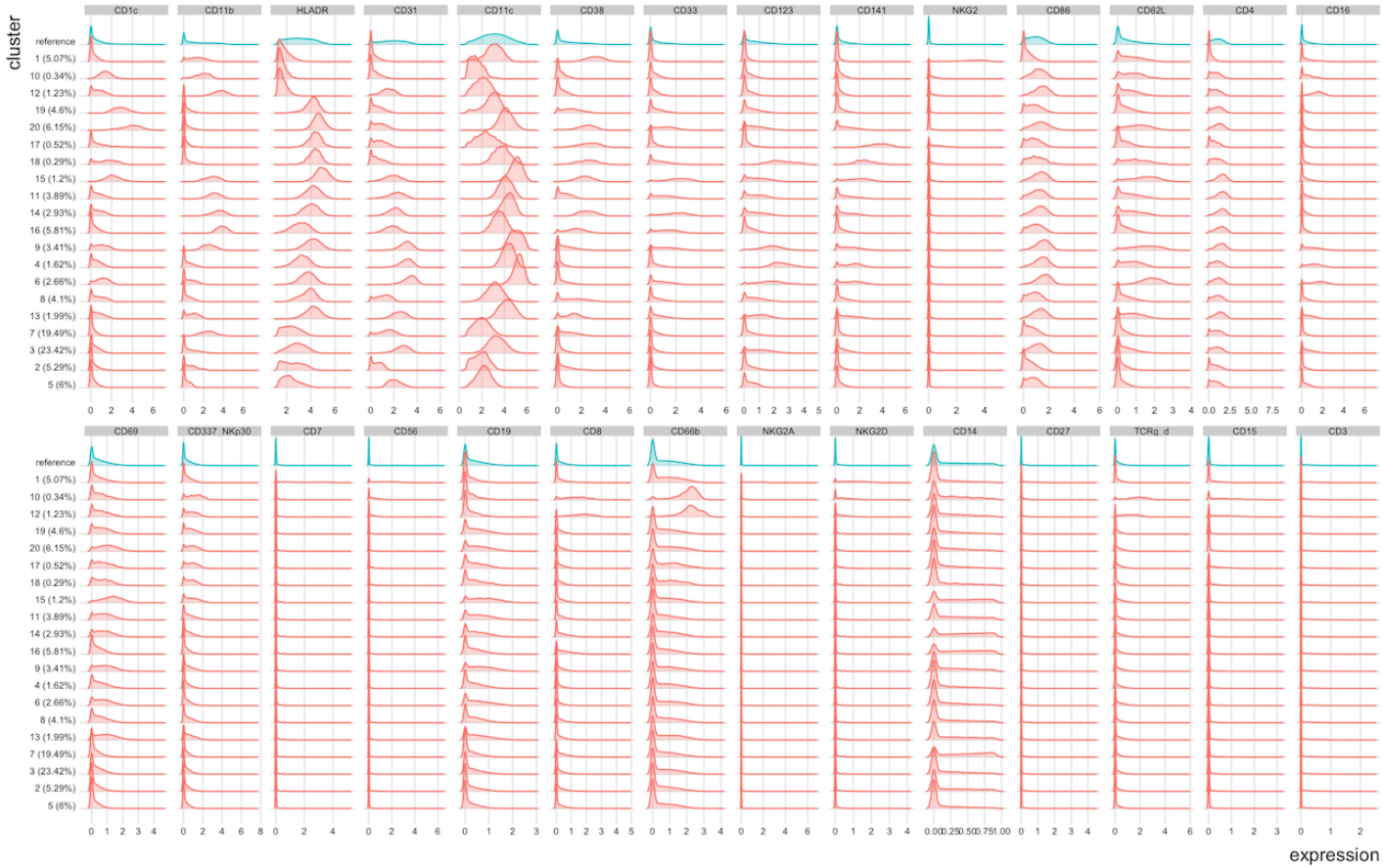

Figure 38: **Median markers intensities of the mDC family 20 meta-clusters** - Surface markers intensities are represented for each of the clusters obtained with FlowSOM and ConsensusClusterPlus metaclustering.

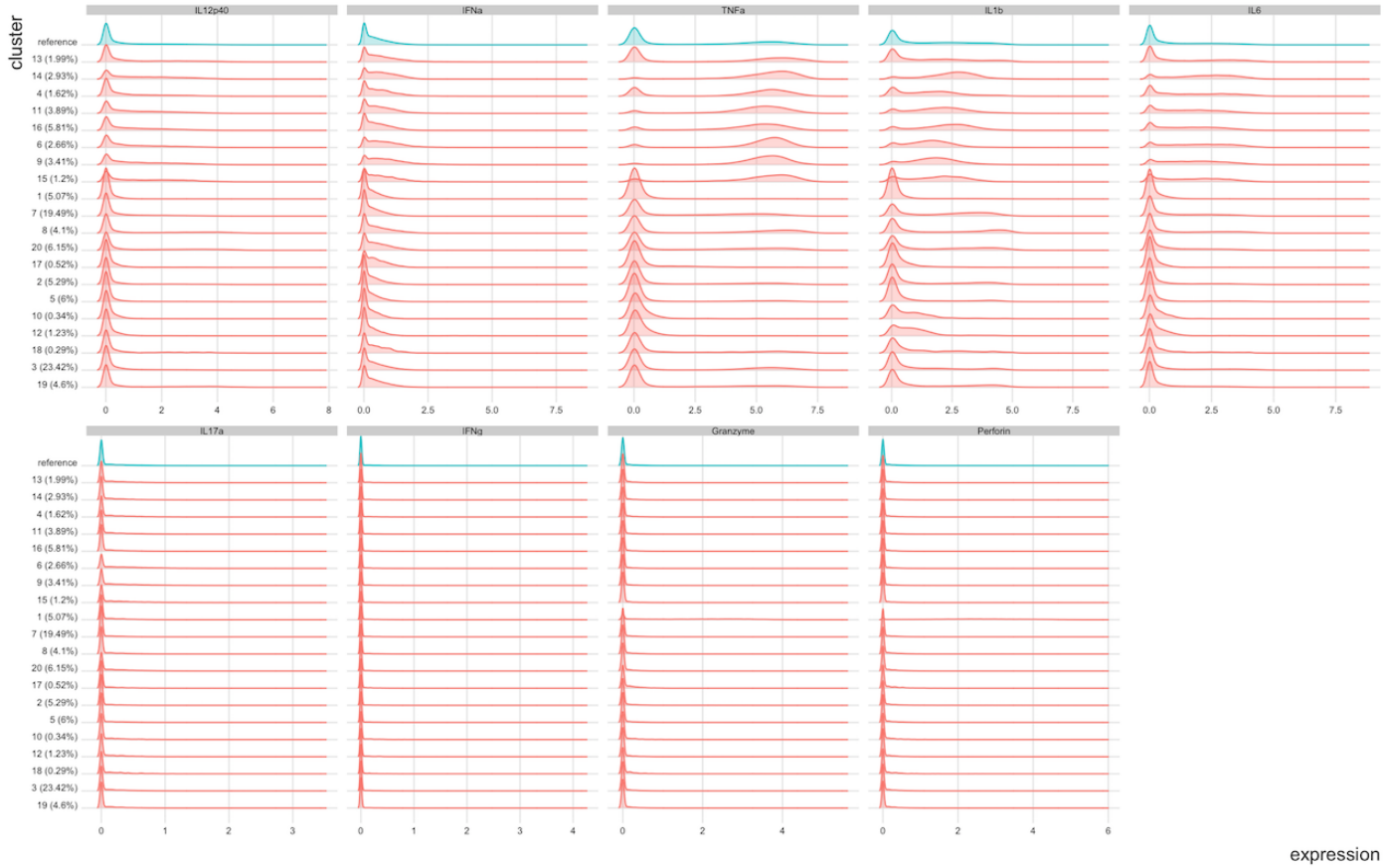

Figure 39: **Median cytokines intensities of the mDC family 20 meta-clusters** - Cytokines intensities are represented for each of the clusters obtained with FlowSOM and ConsensusClusterPlus metaclustering.

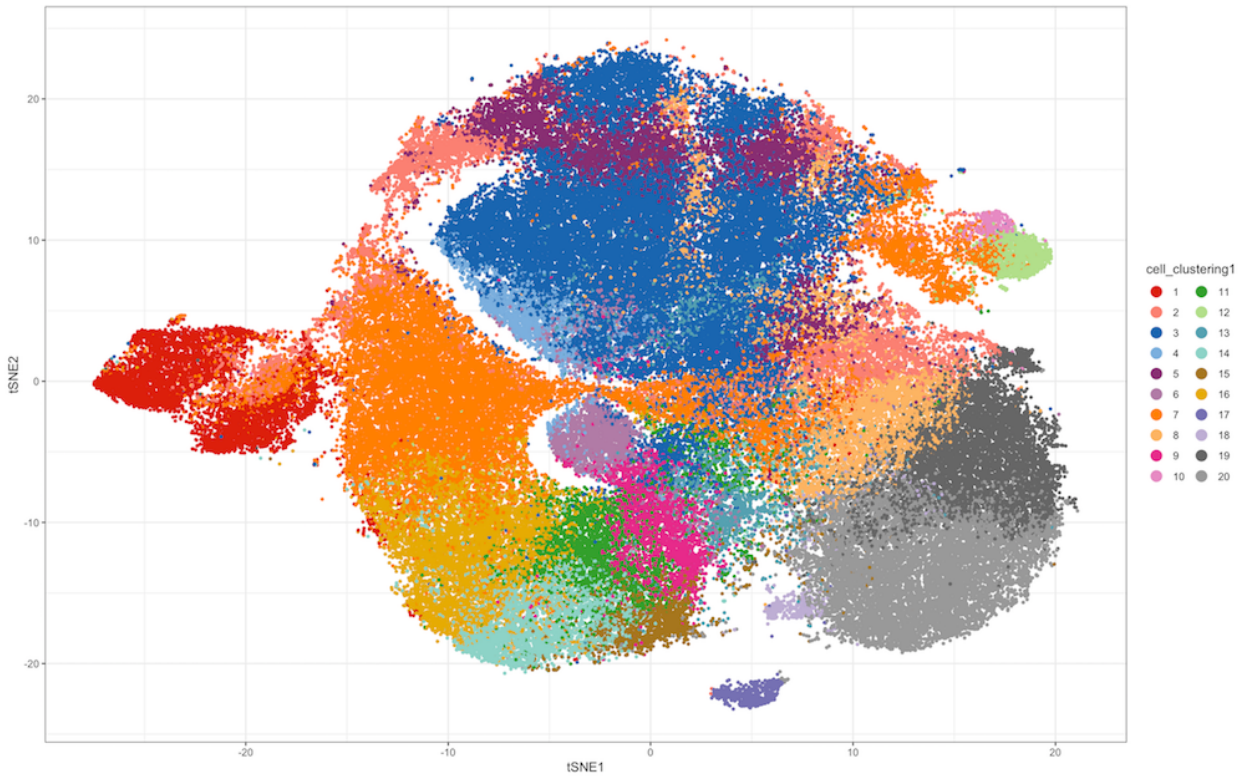

Figure 40: **tSNE plot of all myeloid dendritic cells** - Each cell is coloured according to the cluster it has been assigned to.

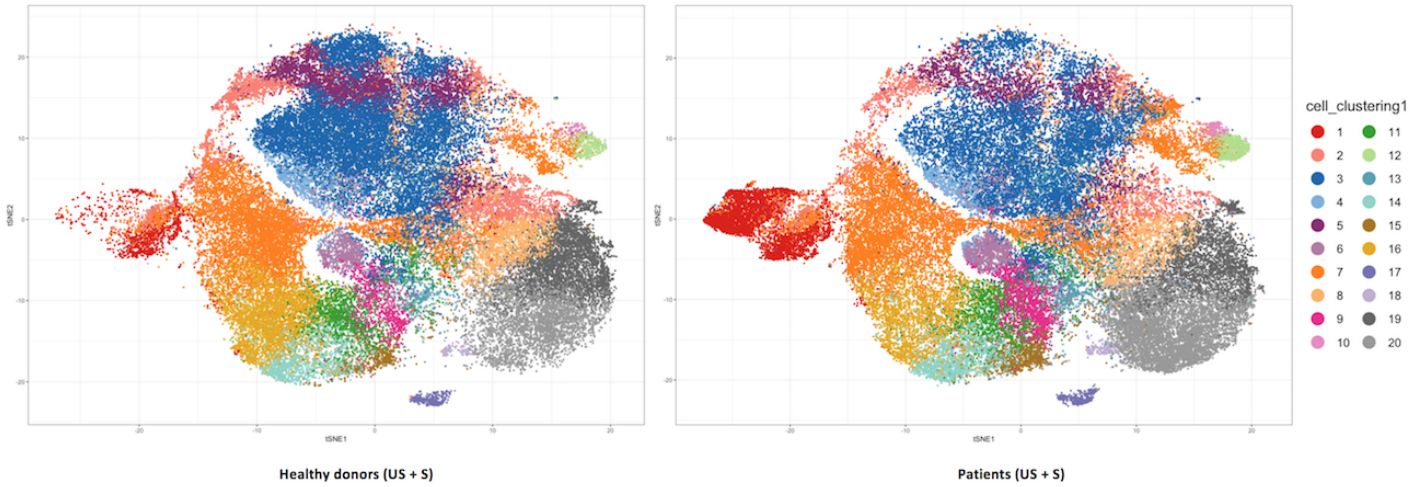

Figure 41: **tSNE plot comparison between healthy donors and patients in the mDC population** - tSNE plot displaying the identified immune cells populations for the healthy donors group, compared to the one obtained for the patients group. US, unstimulated; S, stimulated.

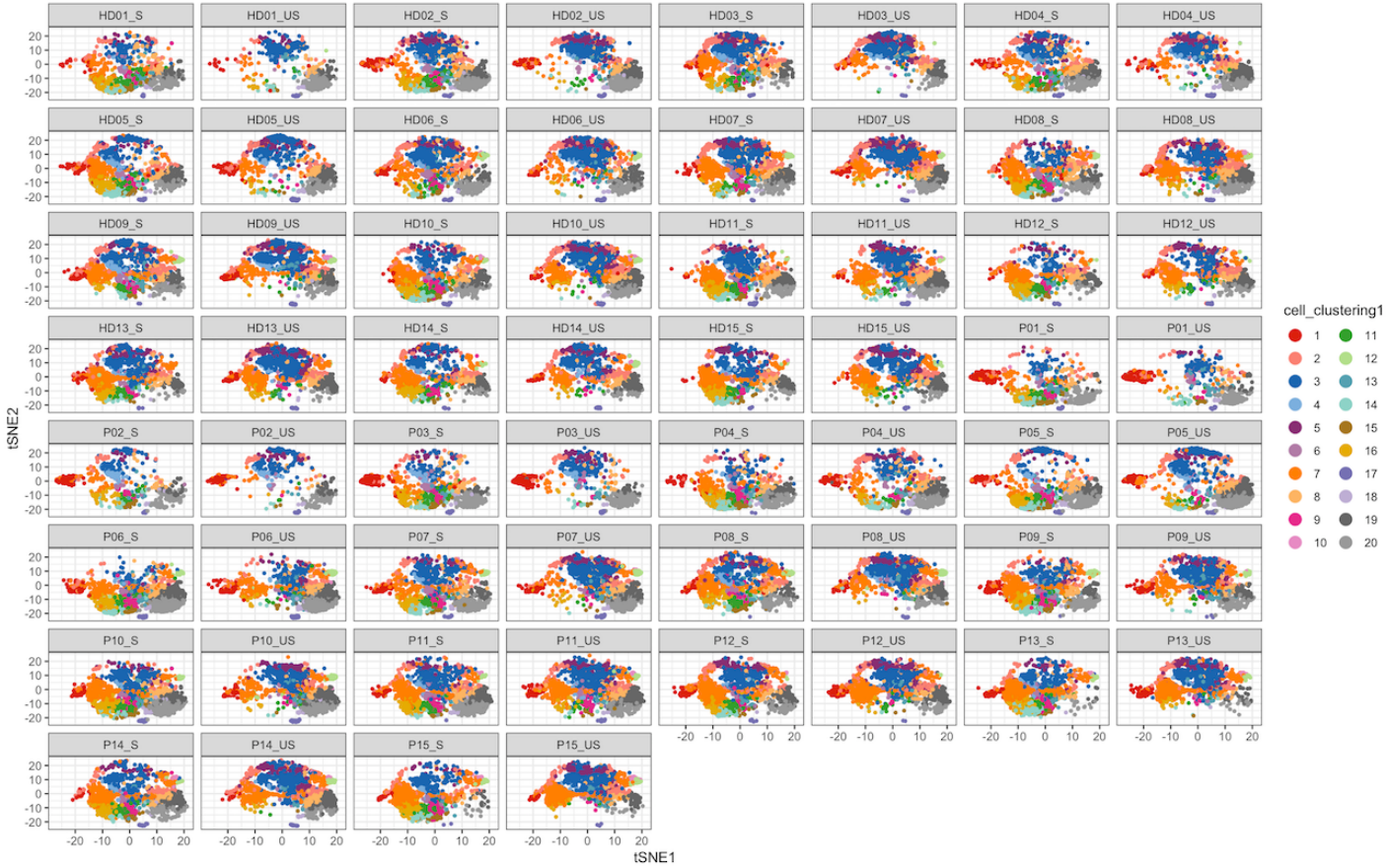

Figure 42: **tSNE plot stratified by samples and conditions in the mDC population** - tSNE plot displaying the meta-clustering for each of the samples and conditions, allowing a quick overview of the presence or absence of each clustered mDC subpopulation. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

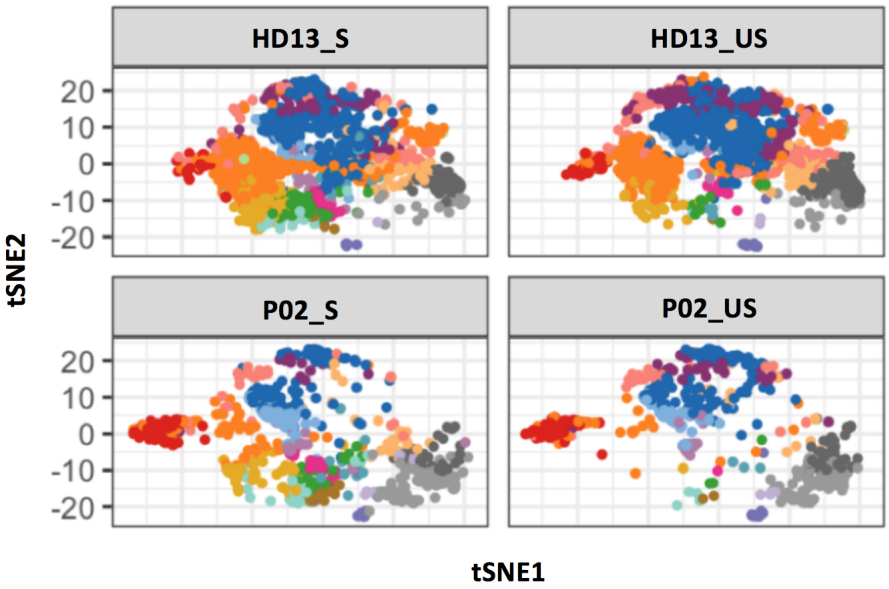

Figure 43: **mDC tSNE graph of patient 02 and healthy donor 13** - tSNE graphs focusing on patient 02 and healthy donor 13. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

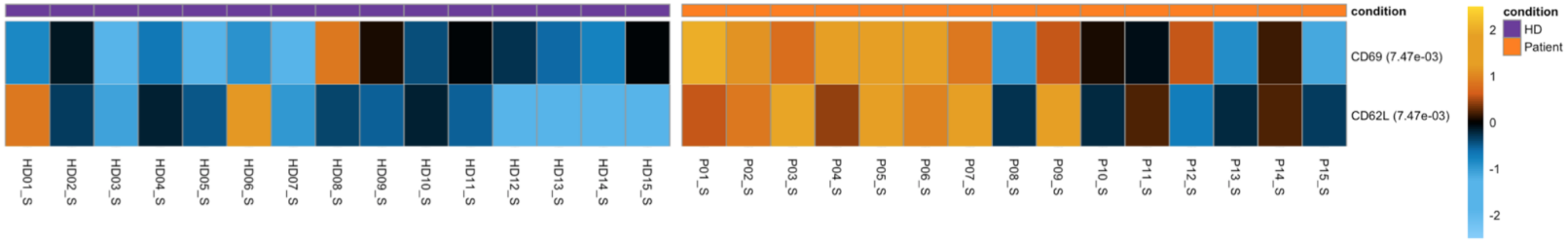

Figure 44: **Normalized activation markers expression comparison between the healthy donors and patients group in the stimulated condition of all mDCs** - Heatmap illustrating the differences in the activation markers expressions that are statistically significant in the overall mDCs between the patients and healthy donors groups. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

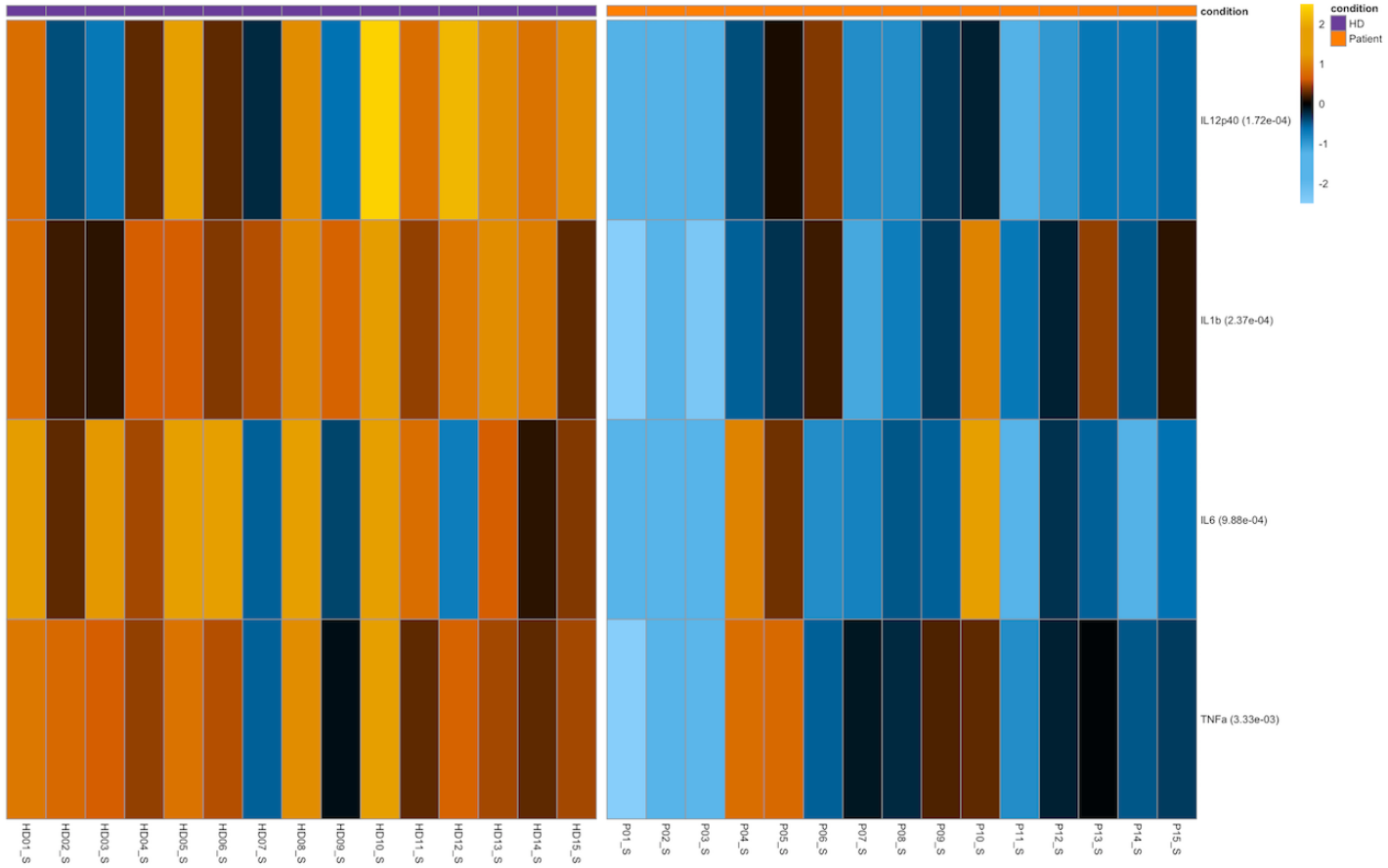

Figure 45: **Normalized cytokines expression comparison between the healthy donors and patients group in the stimulated condition of all mDCs** - Heatmap illustrating the differences in the cytokines expressions that are statistically significant in the overall mDCs between the patients and healthy donors groups. HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

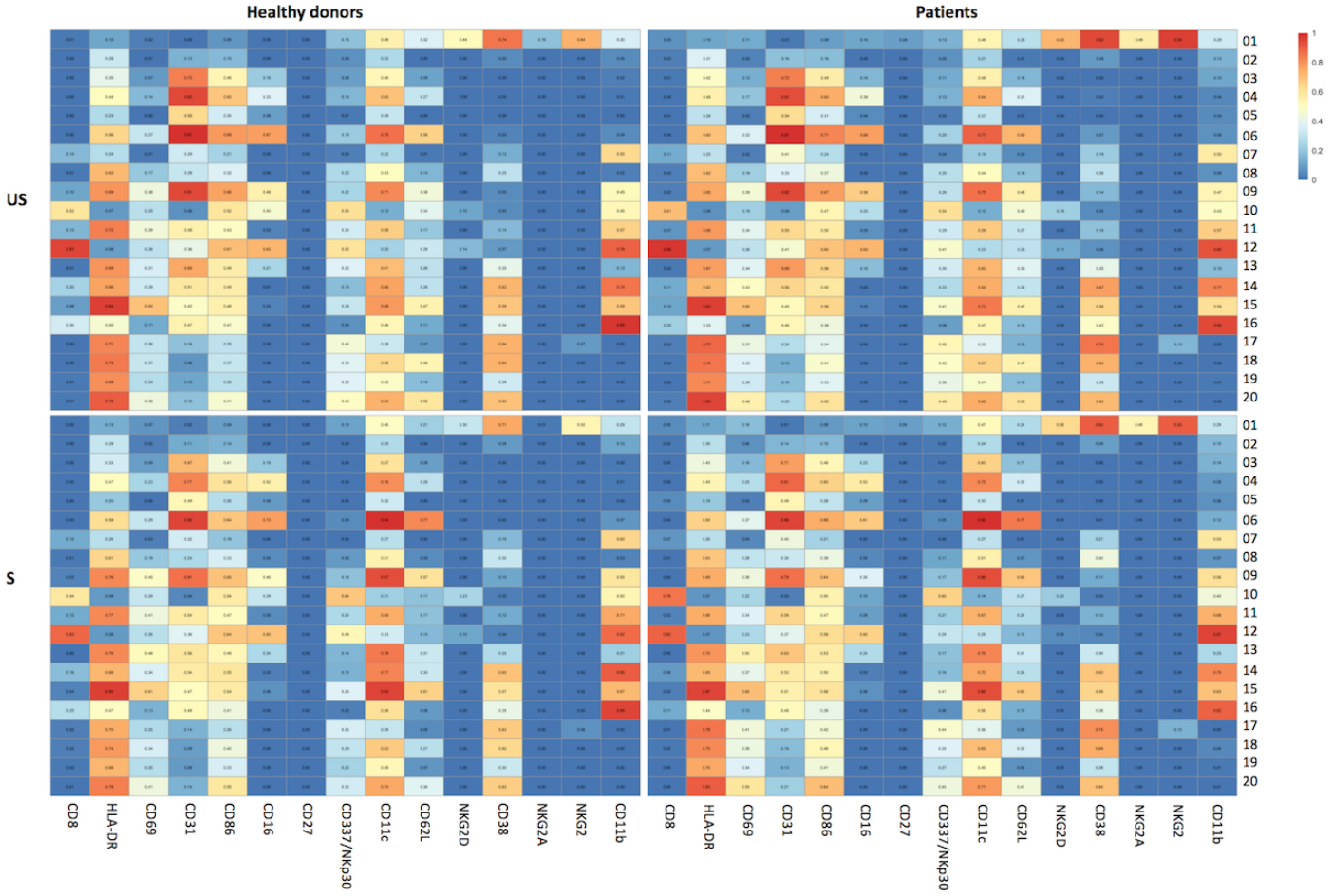

Figure 46: **Median activation markers expressions intensities for each of the clusters and conditions among mDCs -** Visual representation of the activation markers expressions in each of the mDCs clusters for the healthy donors and patients groups, compared by conditions. US, unstimulated; S, stimulated.

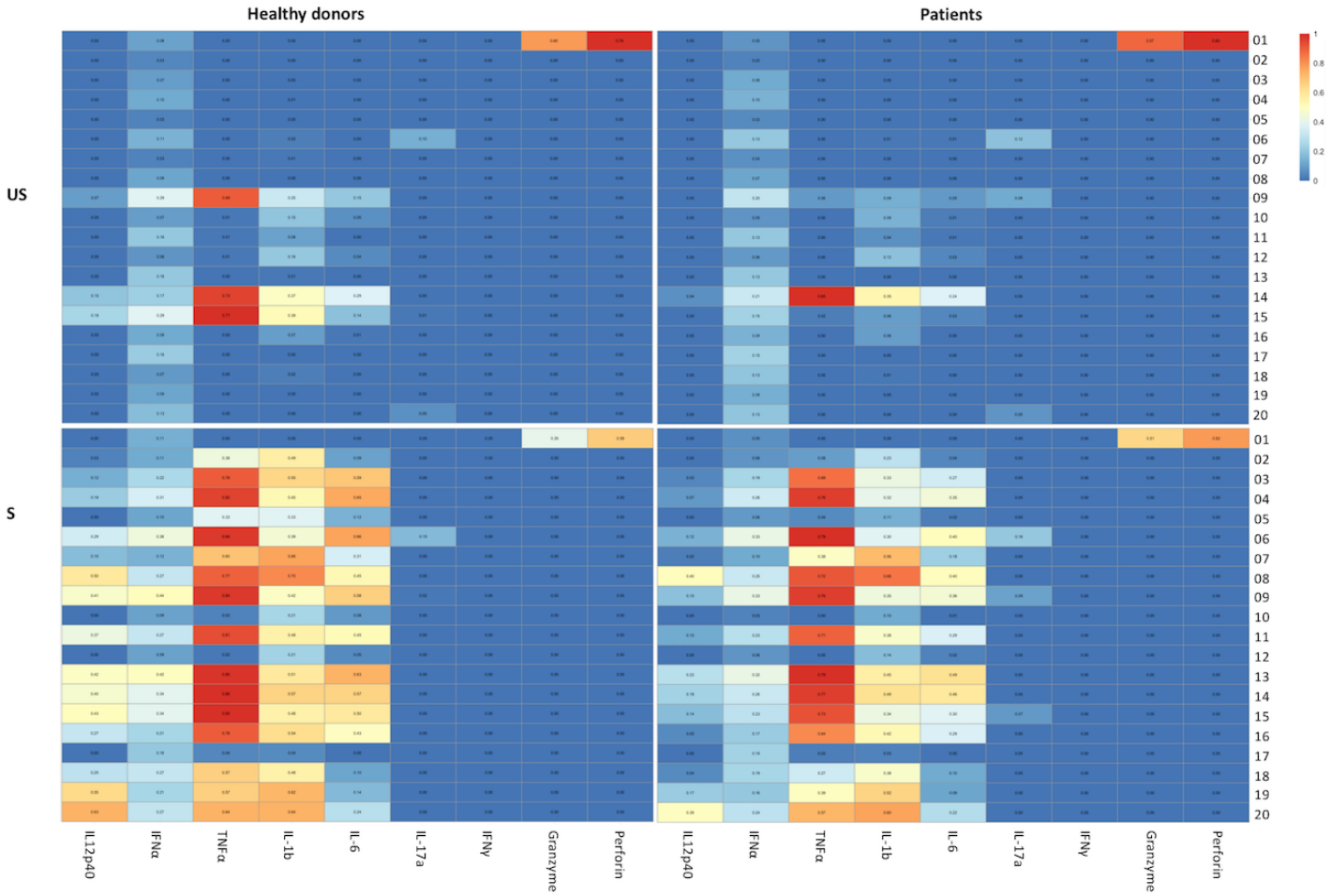

Figure 47: **Median cytokines expressions intensities for each of the clusters and conditions among mDCs** - Visual representation of the cytokines expressions in each of the mDCs clusters for the healthy donors and patients groups, compared by conditions. US, unstimulated; S, stimulated.

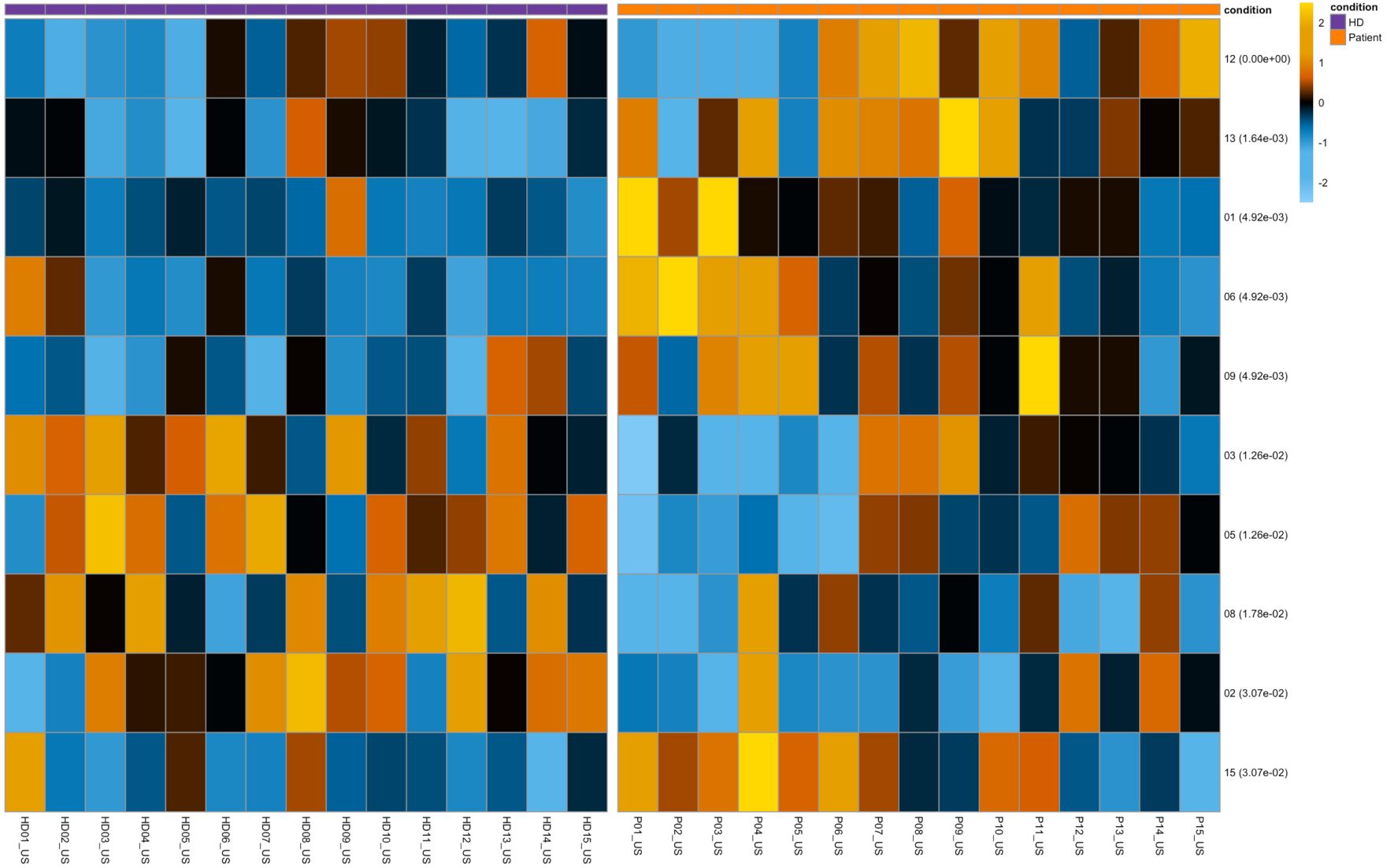

Figure 48: **Normalized proportions of mDC subpopulations that are significantly different between the healthy donors and patients groups in the unstimulated condition** - HD, healthy donor; P, patient; US, unstimulated; S, stimulated.

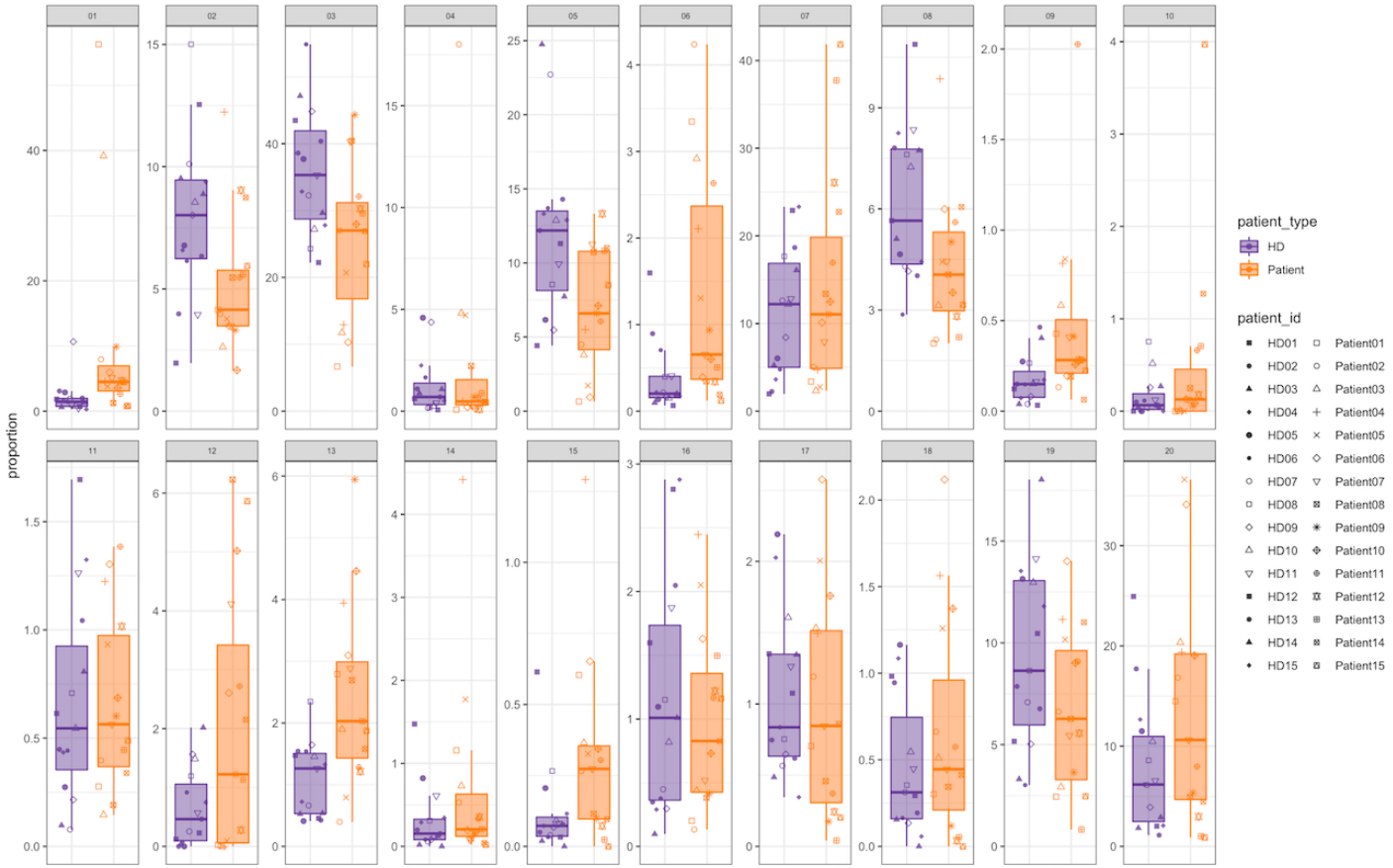

Figure 49: **Boxplots with jittered points representing the mDC subpopulations relative abundance between healthy donors and patients in the unstimulated condition** - General overview of the mDC subpopulations relative abundance distribution between patients and healthy donors in the unstimulated condition. HD, healthy donor; P, patient.

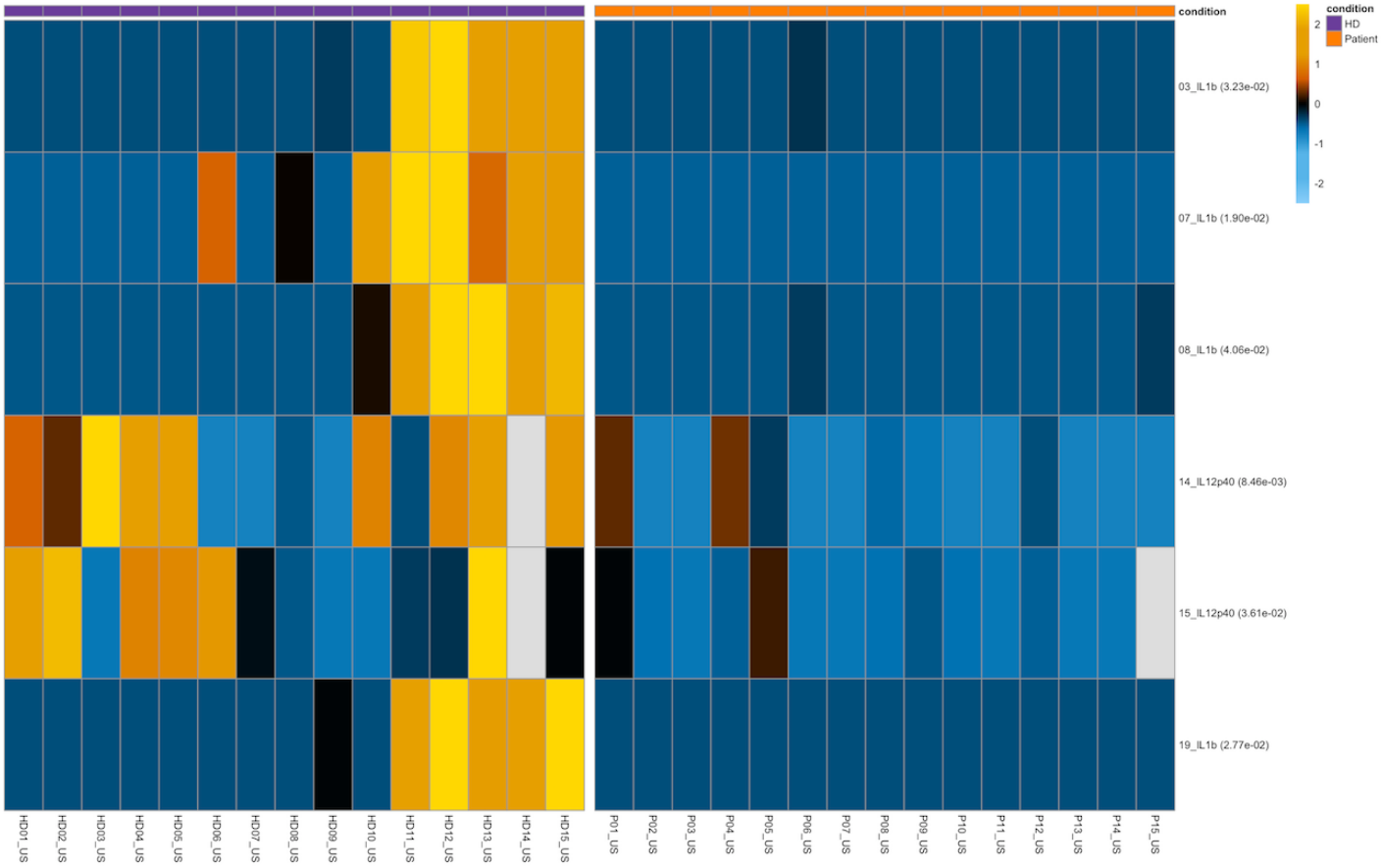

Figure 50: **Normalized cytokines expression comparison between the healthy donors and patients group in the unstimulated condition of mDCs subpopulations** - Heatmap illustrating the differences in the cytokines expressions that are statistically significant in the identified mDC subpopulations between the patients and healthy donors groups. HD, healthy donor; P, patient; US, unstimulated.

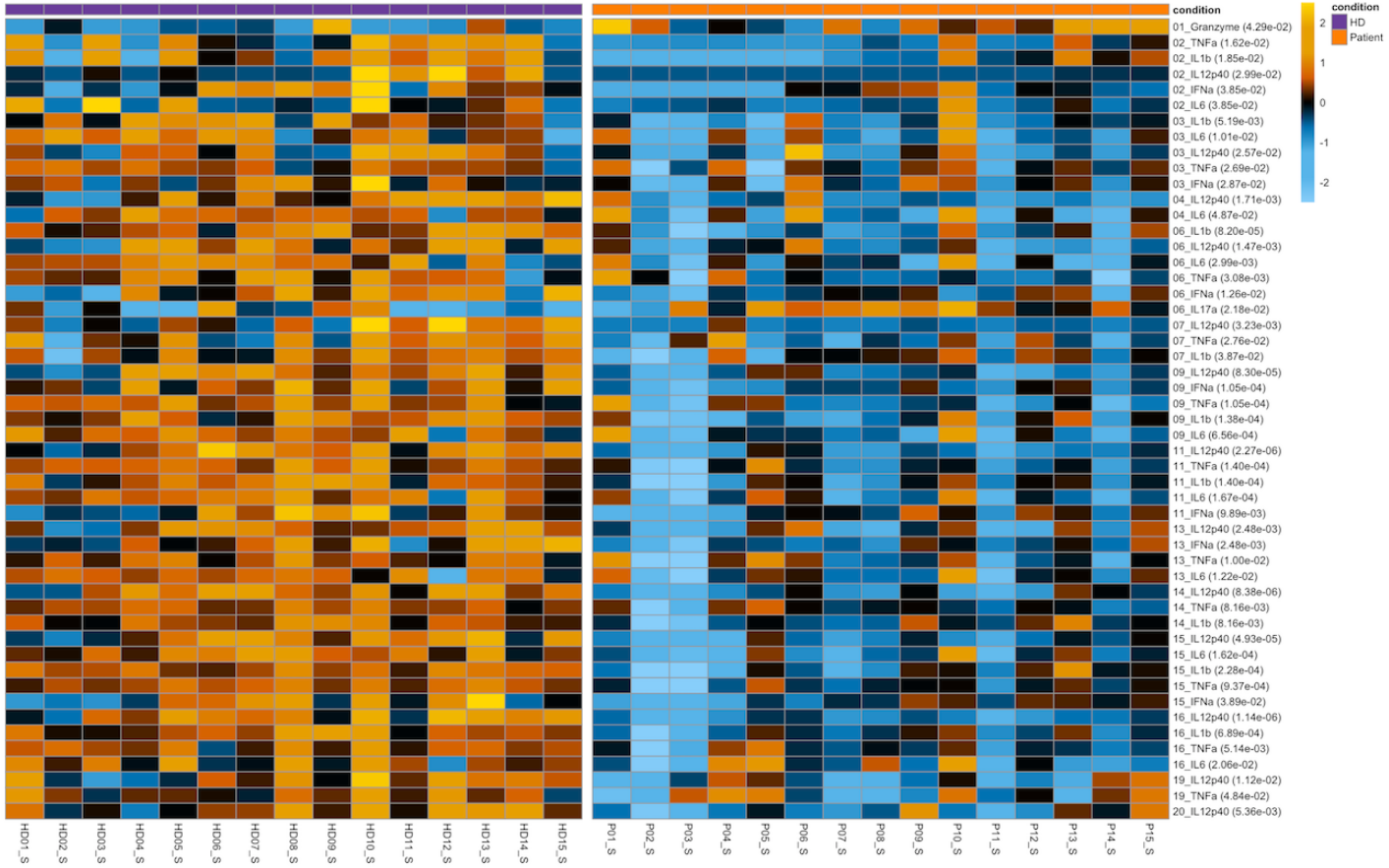

Figure 51: **Normalized cytokines expression comparison between the healthy donors and patients group in the stimulated condition of mDCs subpopulations** - Heatmap illustrating the differences in the cytokines expressions that are statistically significant in the identified mDC subpopulations between the patients and healthy donors groups. HD, healthy donor; P, patient; S, stimulated.

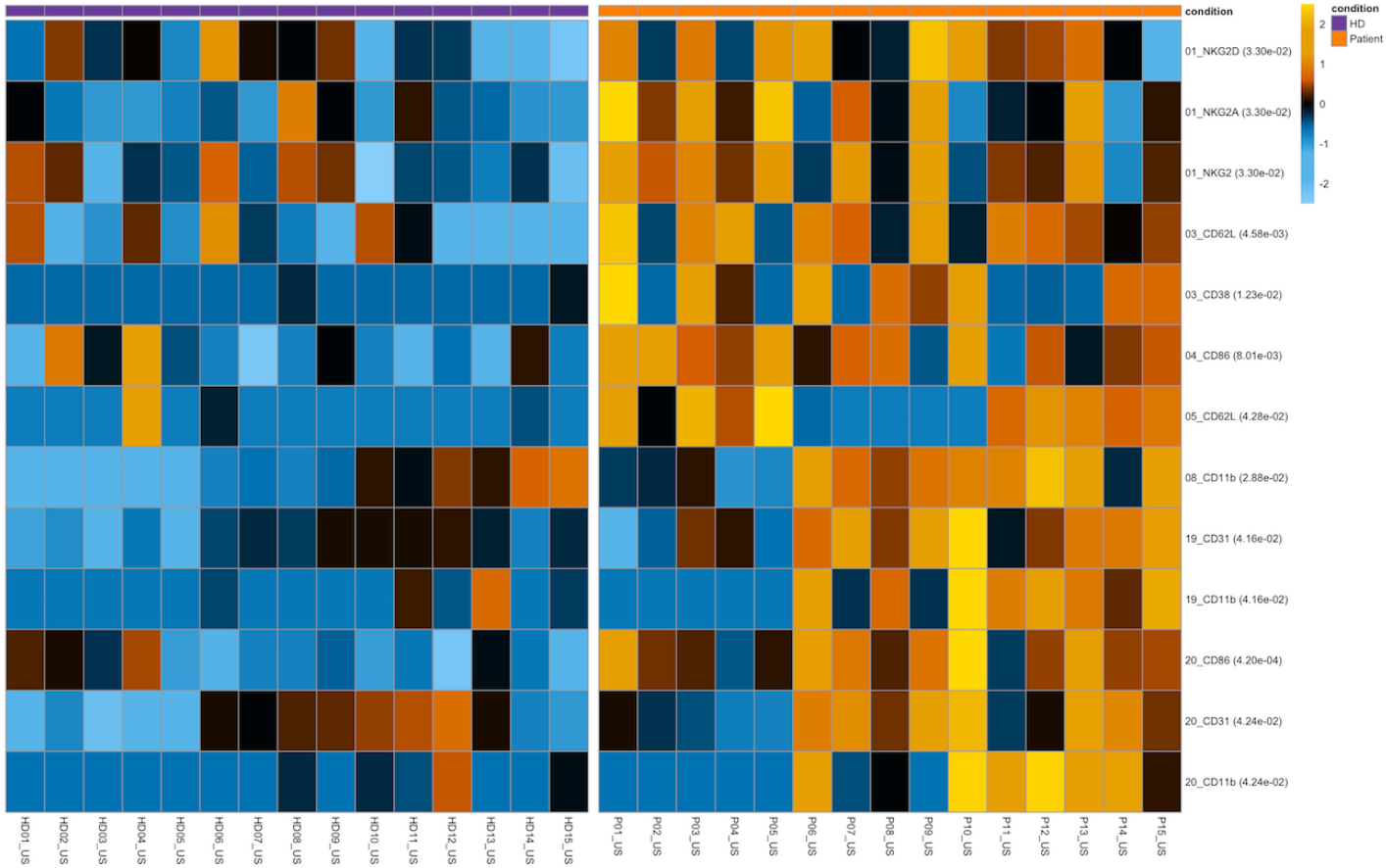

Figure 52: **Normalized activation markers expression comparison between the healthy donors and patients group in the unstimulated condition of mDCs subpopulations** - Heatmap illustrating the differences in the activation markers expressions that are statistically significant in the identified mDCs subpopulations between the patients and healthy donors groups. HD, healthy donor; P, patient; US, unstimulated.

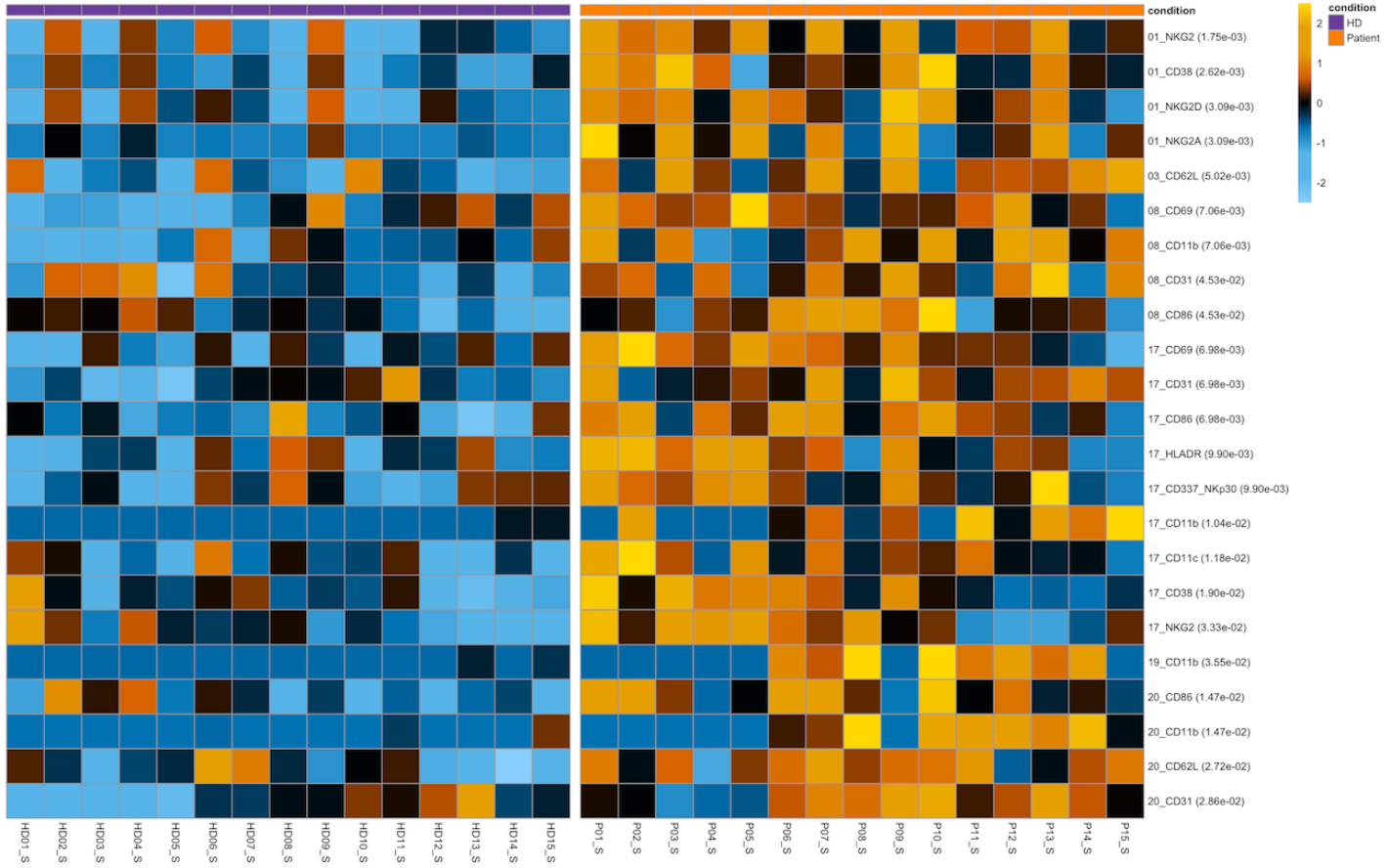

Figure 53: **Normalized activation markers expression comparison between the healthy donors and patients group in the stimulated condition of mDCs subpopulations** - Heatmap illustrating the differences in the activation markers expressions that are statistically significant in the identified mDCs subpopulations between the patients and healthy donors groups. HD, healthy donor; P, patient; S, stimulated.