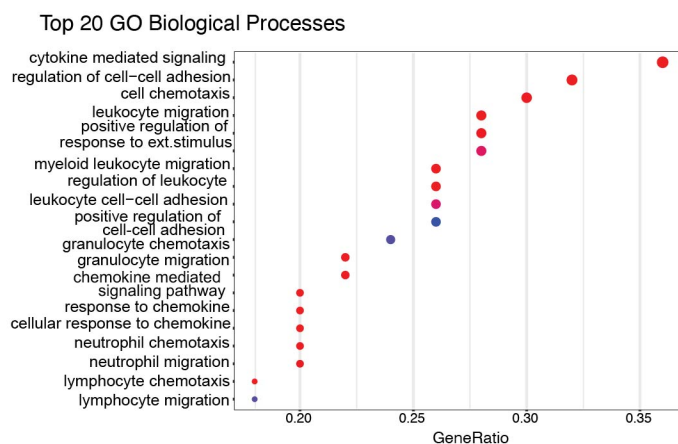


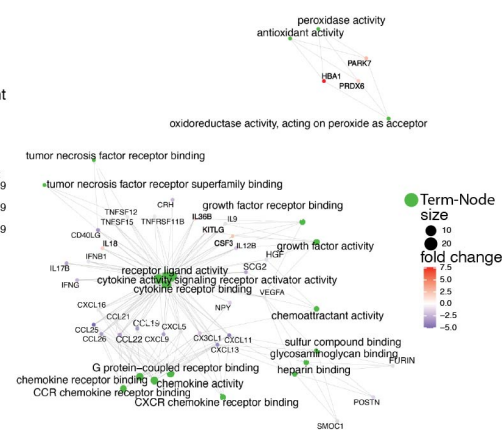
eFigures

A

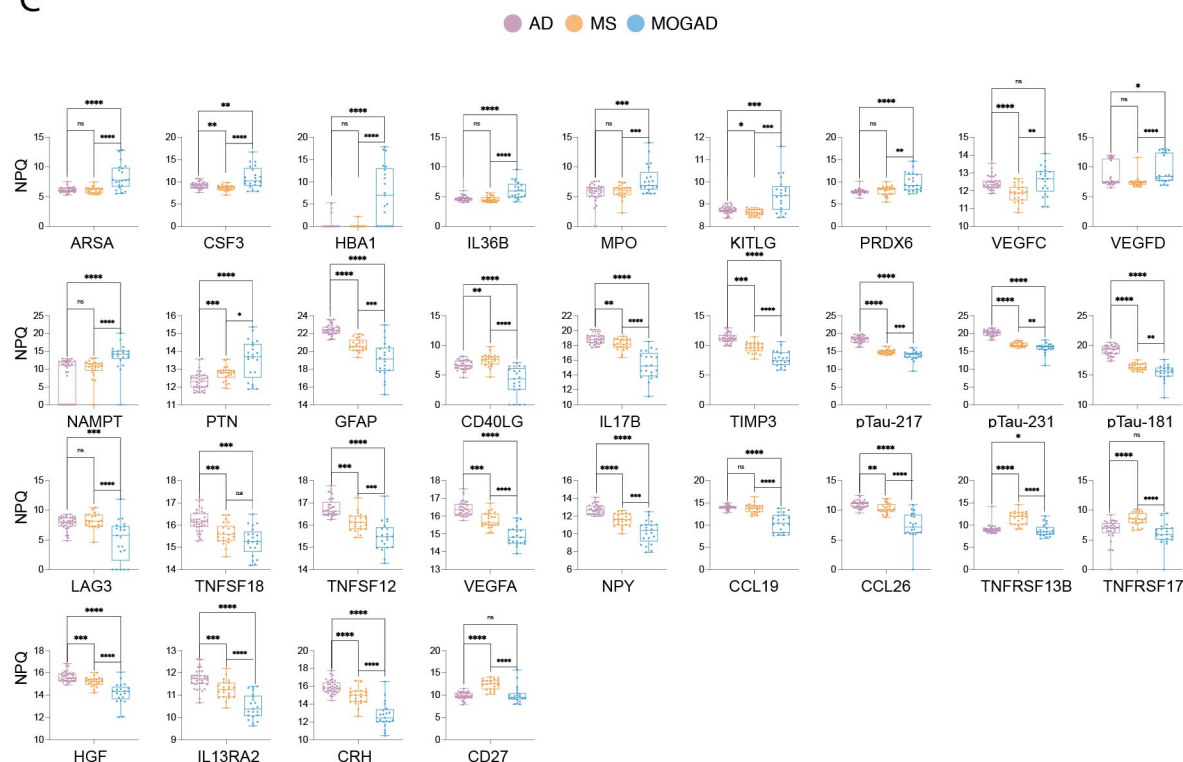


B

Top 20 GO Terms-Molecular Functions

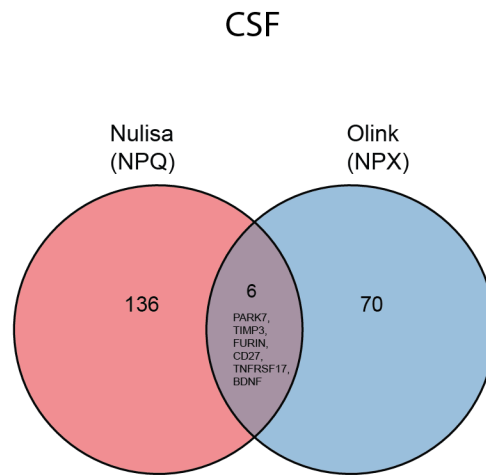


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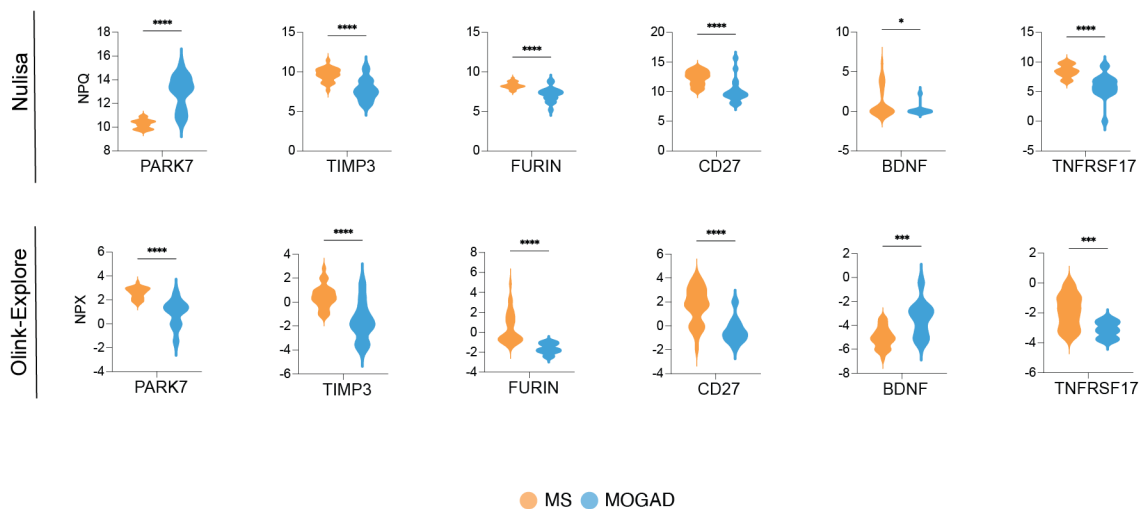


eFigure 1. CSF AD vs MS vs MOGAD. (A) Gene Ontology (GO) enrichment analysis of DEPs using the *clusterProfiler* package. The bubble plot shows the 20 top enriched Biological Process (BP) terms, with the x-axis representing the gene ratio and the y-axis the GO terms. Bubble color corresponds to adjusted p -values, and bubble size indicates the number of proteins contributing to each term. (B) The network plot illustrates the Molecular Function (MF) enrichment results, where green nodes represent GO categories and connected proteins are color-coded according to their adjusted p -values. (C) Comparison of NPQ values across disease group AD($n=36$), MS($n=22$) and MOGAD ($n=21$) from CSF samples. Box plots display the NPQ values for proteins identified as DEPs. Statistical analysis was conducted using mixed-effects models followed by Tukey's multiple comparisons test. Each dot represents an individual samples; boxes indicate the interquartile range, and whiskers denote the minimum and the maximum values. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$.

A



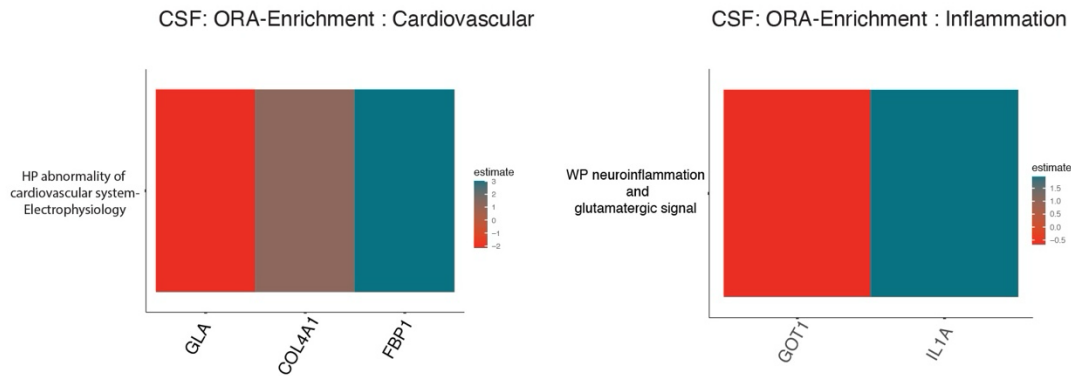
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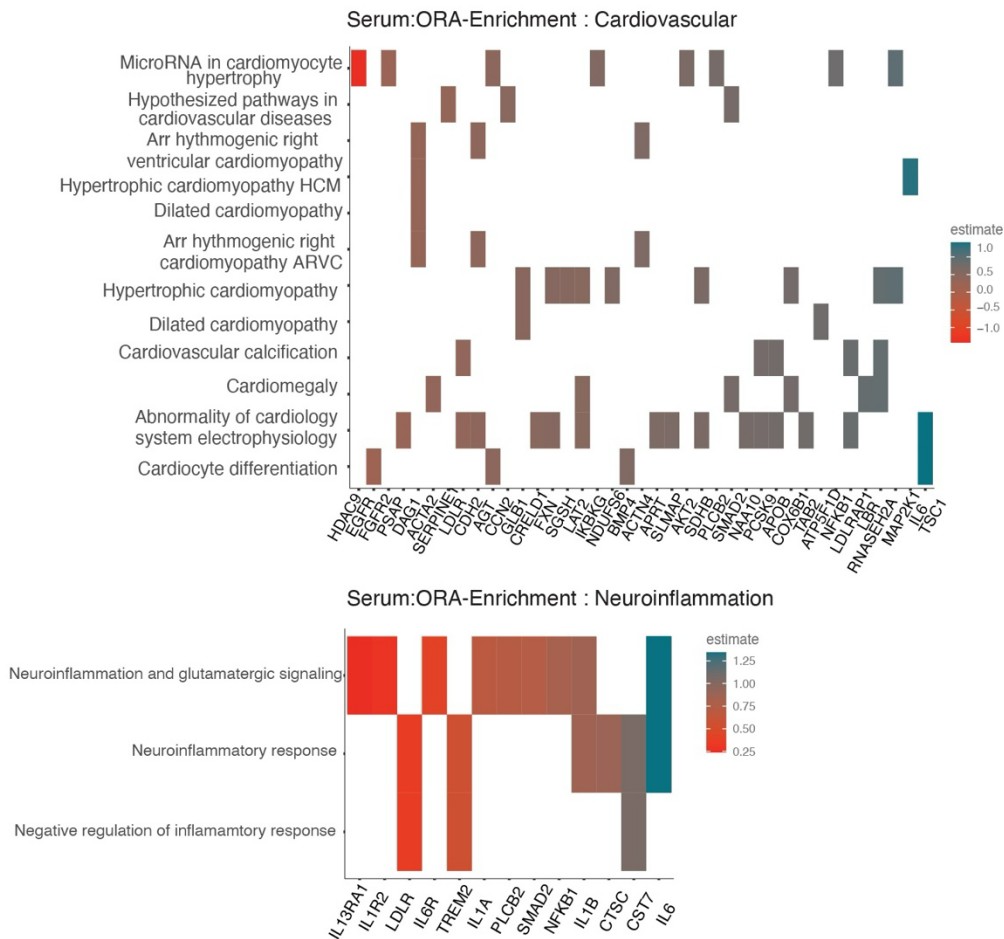
eFigure 2. Overlap of DEPs quantified in CSF using NULISA and Olink platforms.

(A) Venn diagram illustrating the overlap between DEPs identified by NULISA (NPQ values) and Olink Explore (NPX values) in CSF samples. Six proteins were identified as differentially expressed by both platforms. (B) Violin plots showing the expression levels of the six overlapping DEPs measured by NULISA (NPQ) and Olink (NPX). Statistical significance was assessed using the Mann–Whitney U test with multiple comparison correction according to the Benjamini, Krieger, and Yekutieli method. $p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

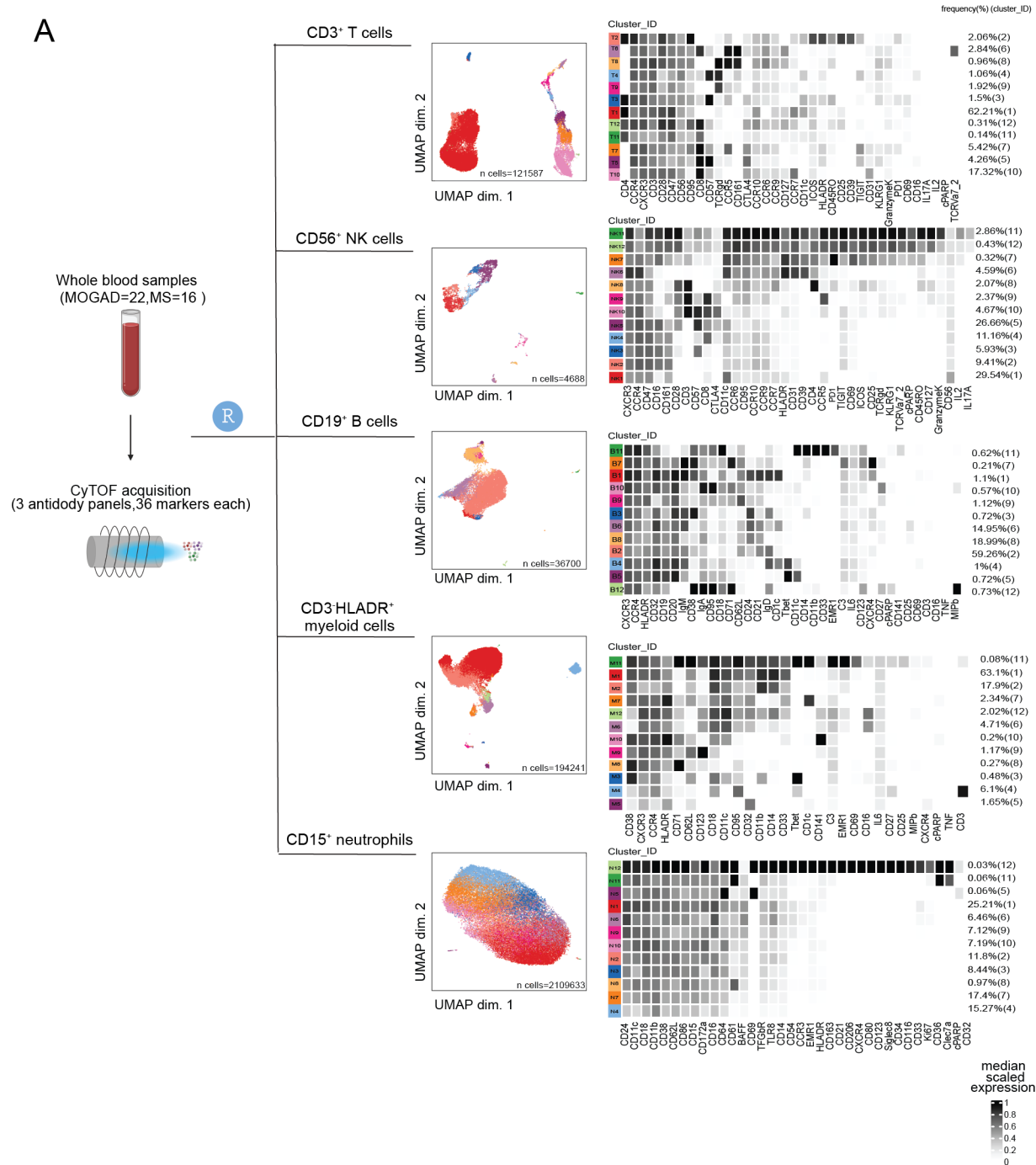
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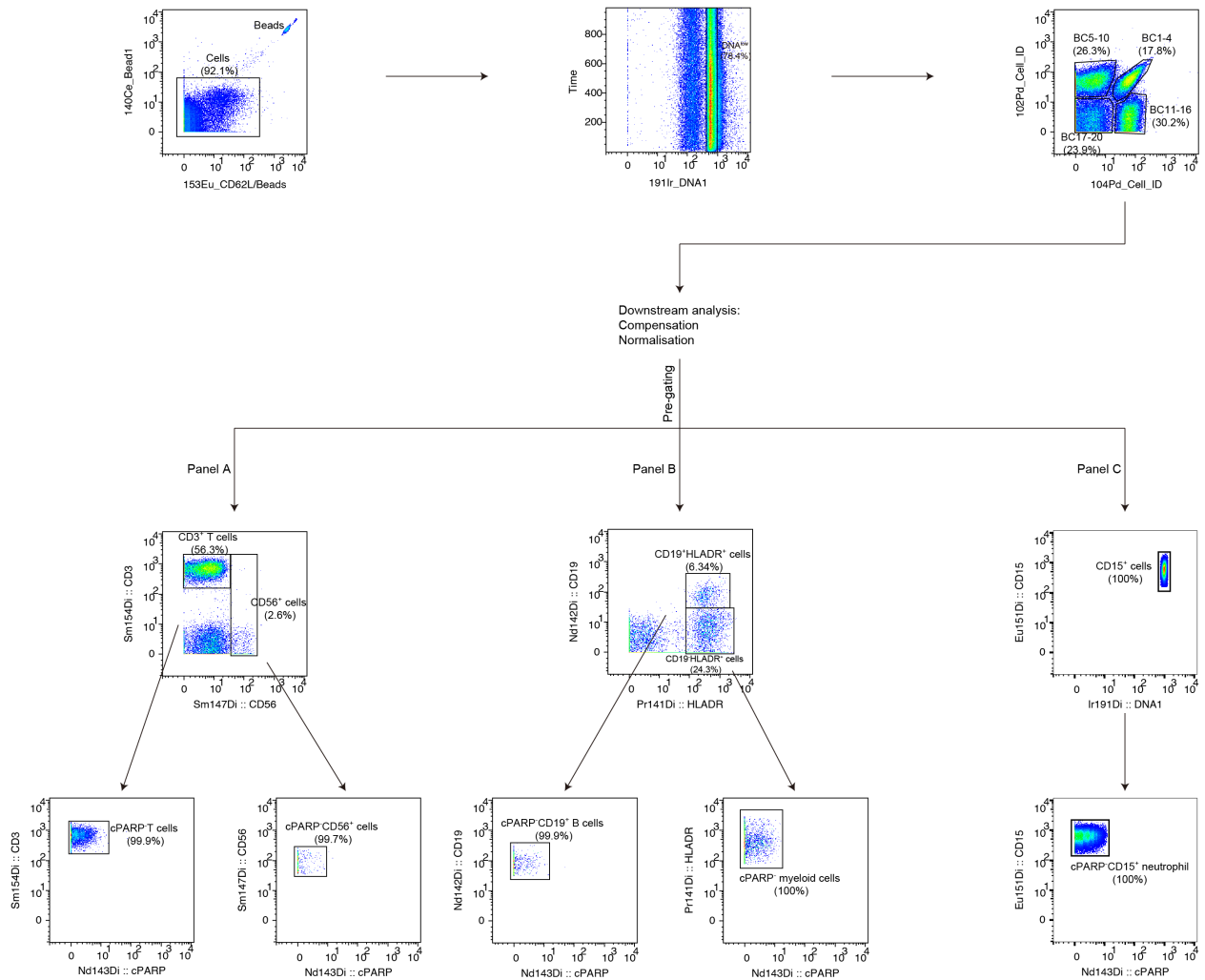


eFigure 3. Enrichment pathway analysis in CSF and Serum. The over-representation analysis (ORA) of proteins in CSF samples (A) and Serum samples (B). The analysis was performed using the OlinkExplore Analyze package by selecting keyword-associated pathways related to “Cardiovascular”, “Inflammation” and “Neuroinflammation” panels. Enrichment was assessed using ORA test based on the DEPs (significant p.value 0.05). The color scale represents the estimated effect size: blue indicates higher enrichment, brown intermediate levels and red represents negative enrichment estimates.



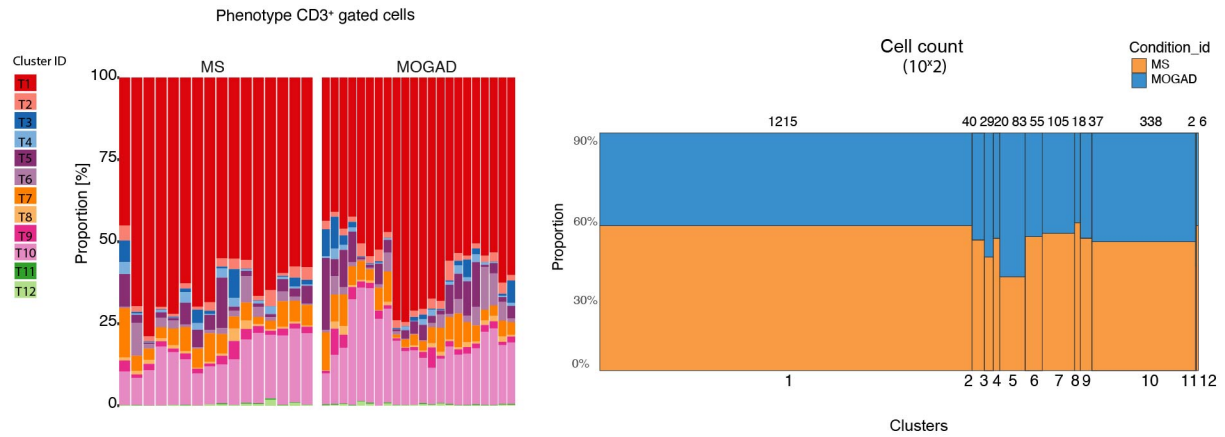
eFigure4. Whole blood immune cell profiling. (A) Immune cell populations in whole blood were profiled using CyTOF with three antibody panels. Unsupervised clustering was performed using FlowSOM, and clusters were visualized using UMAP dimensionality reduction. UMAP plots (right) display the distribution of annotated immune cell phenotypes, while the corresponding FlowSOM heatmap (left) shows median marker expression across clusters.

Pre-gating strategy for whole blood cells

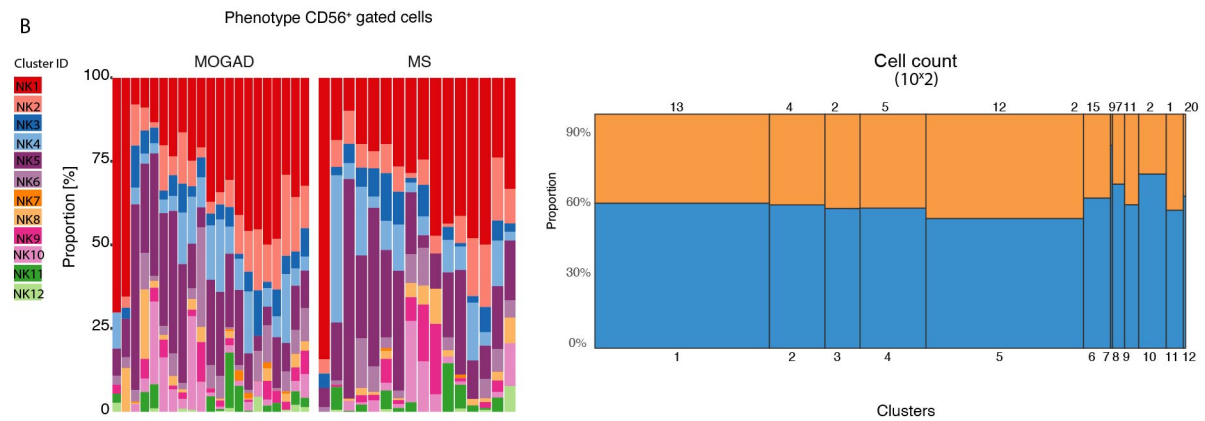


eFigure5. Gating strategy for mass cytometry. Whole blood samples were collected from AD.MS and MOGAD patients. Whole blood samples were barcoded and pooled prior to staining with three panels of metal-conjugated antibodies were used (Panel A, B and C). Samples were acquired on a CyTOF instrument. Data were debarcoded, compensated and normalized prior to pre-gating. In Panel A (targeting T and NK cell populations), the gating strategy included selection of CD3⁺ cells and the CD56⁺ cells. Panel B (targeting B and myeloid cell populations) included gating for CD19⁺HLADR⁺ B cells and CD19⁺HLADR⁺ myeloid cells. Panel C (targeting neutrophils) characterized pre-gated CD15⁺ cells.

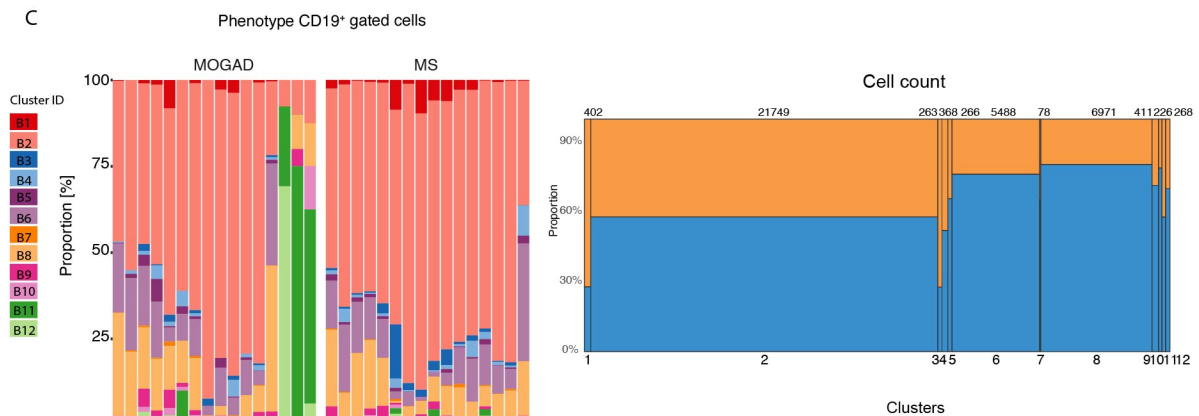
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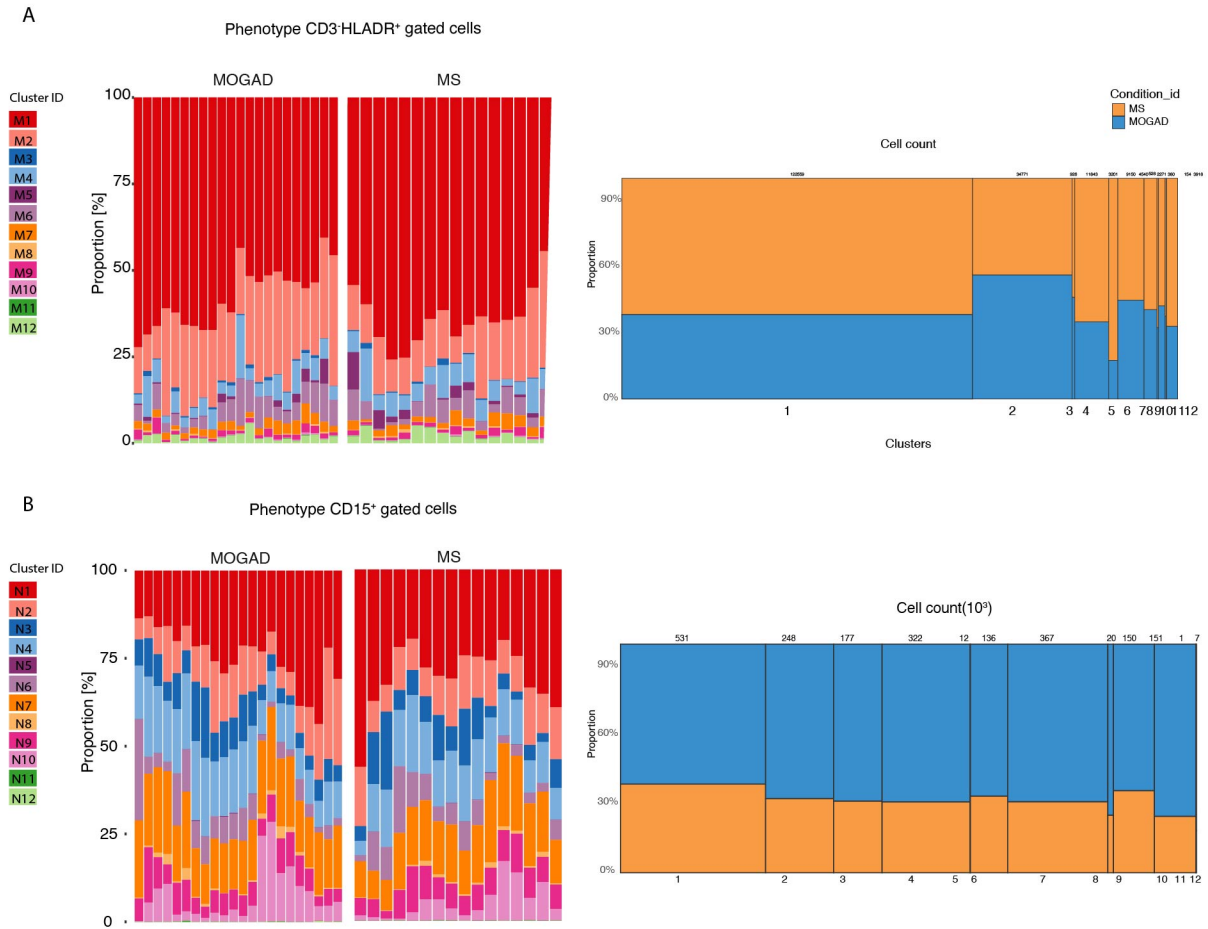


C



eFigure 6. FlowSOM-based phenotypic analysis of immune cells populations in whole blood (Panel A and B)

(A) CD3⁺ T cells, (B) CD56⁺ natural killer (NK) (C) CD19⁺ B cells, and cells were analyzed using FlowSOM clustering and *ConsensusClusterplus* algorithm. On the right the average frequency distributions of cell populations between MOGAD and MS. Corresponding mosaic plots to the left of each heatmap show the relative abundance (cluster proportions) and absolute cell counts per cluster in MS and MOGAD groups.



eFigure 7. FlowSOM-based phenotypic analysis of immune cells populations in whole blood (Panel B and C)
 (A) HLADR⁺ myeloid cells, (B) CD15⁺ neutrophils cells (N), were analyzed using FlowSOM clustering and *ConsensusClusterplus* algorithm. On the right side the cell population distributions between MOGAD and MS. Corresponding mosaic plots to the left of each heatmap show the relative abundance (cluster proportions) and absolute cell counts per cluster in MS and MOGAD groups.