

DECIPHERING THE ROLE OF THE PERIPHERAL IMMUNE SYSTEM IN THE PATHOLOGY OF FRONTOTEMPORAL DEMENTIA

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Introduction

Translational and clinical evidence support the contribution of chronic systemic inflammation in the etiopathology and progression of neurodegenerative diseases, including frontotemporal dementia (FTD). However, a comprehensive understanding of the intricate functional and mechanistic implications of how the peripheral immune system participates in neurodegeneration remains elusive. Here, we investigate the status of peripheral immune cells and the activation of inflammatory cascades in FTD individuals.

Methods

Participants. 22 FTD and 11 age-matched healthy control (HC) individuals were recruited by the Houston Methodist Nantz National Alzheimer's Center. Written informed consent was obtained following ethics approval from the Institutional Review Board.

To evaluate changes in **Treg immunosuppressive function**, we isolated CD4⁺CD25^{high}Tregs and CD4⁺CD25^{low} T responders (Tresp) in a 2-step procedure, using a Treg isolation kit (Miltenyi Biotec). Isolated Tregs and Tresp were co-cultured at 1:1 and 1:2 ratio (Treg:Tresp). Proliferation of Tresp was measured via tritium incorporation and calculated as suppression of Tresp proliferation in the presence of Tregs. Lymphocyte phenotype was evaluated by flow cytometry analysis.

Monocyte transcript analysis. From the same blood samples, monocytes were isolated, and the mRNA was extracted. For human neuroinflammation panel analysis, 770 transcripts were quantified with the NanoString nCounter multiplexed target platform. nCounts of mRNA transcripts were normalized using the geometric means of 10 housekeeping genes.

Plasma inflammatory chemokine and cytokine levels were analyzed with the Olink® Target 48 Cytokine panel (Olink Proteomics, Uppsala, Sweden).

Peripheral Blood Mononuclear Cells (PBMCs) were submitted to single-cell mass cytometry (CyTOF) analysis. A validated comprehensive panel of 36 immune cell markers, including myeloid, lymphoid, and the CXCL9-11/CXCR3 cascade along with other inflammatory and exhaustive markers were applied.

Statistical analysis were performed in GraphPad Prism 10 software. Graphs were displayed as the mean ± SEM (standard error of the mean). Changes were considered significant if p-value was <0.05.

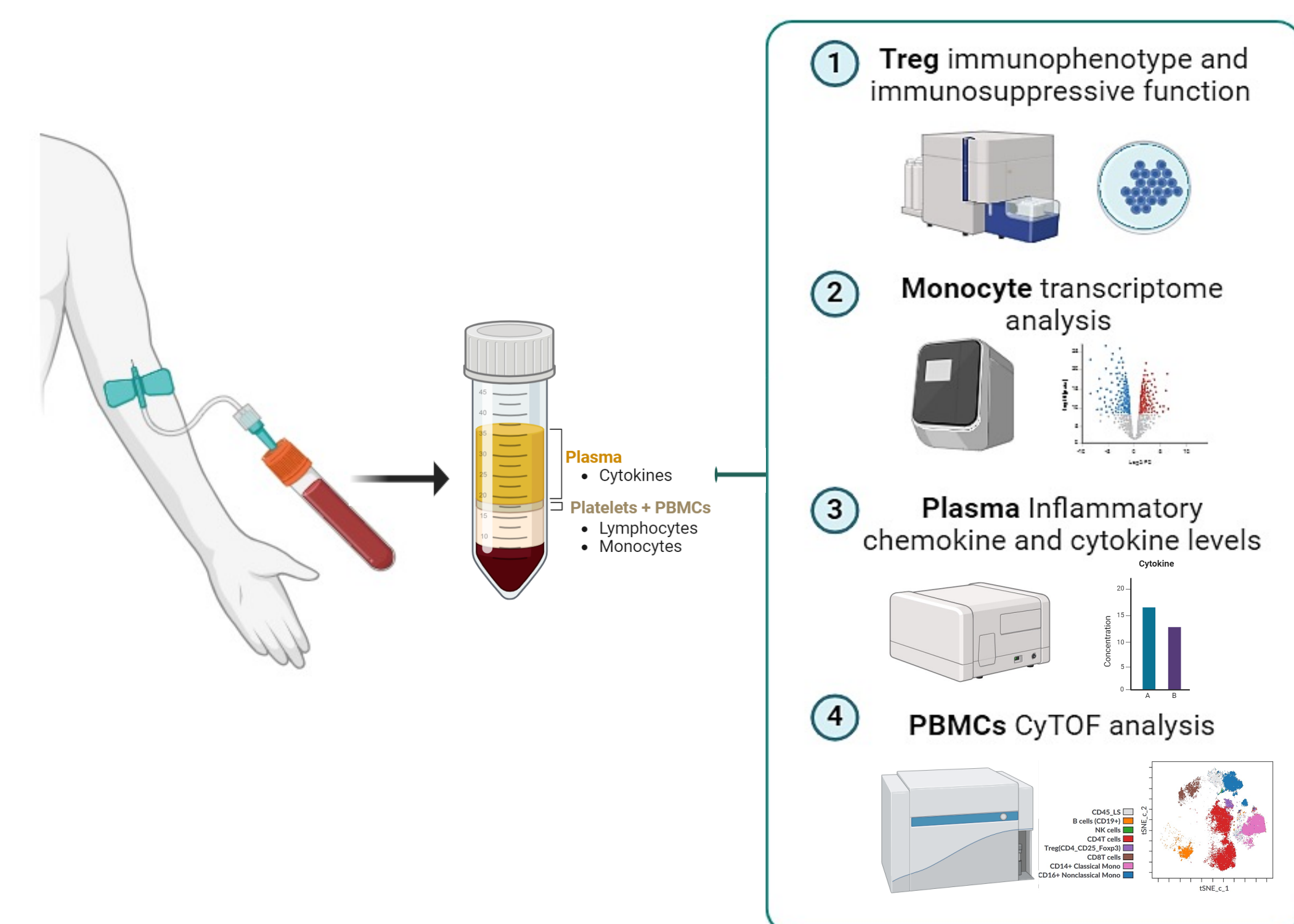


Figure 1. Study design. Human blood was collected, and plasma and PBMCs were isolated. T cells phenotype and Treg immunosuppressive function, as well as monocyte transcriptome were analyzed. Plasma samples were utilized to determine the concentration of inflammatory cytokines and chemokines. PBMCs were evaluated for inflammatory markers by single-cell CyTOF analysis.

Results

Diagnosis	Number	Age, yr (Mean ± SD)	Sex (M, F)	CDR
FTD	22	68.09 ± 8.223	8, 4	0.5
			1, 4	1
			1, 0	2
			1, 3	3
HC	11	67.09 ± 8.166	4, 7	N/A

Table 1. Patient demographics.

The number of Frontotemporal dementia (FTD) and healthy control (HC) individuals, as well as the age (mean ± SD), and sex distribution (M, F) are shown. Clinical evaluations for prodromal (CDR0.5), mild dementia (CDR1), moderate/severe dementia (CDR2/3) was based on the Clinical Dementia Rating (CDR) scale.

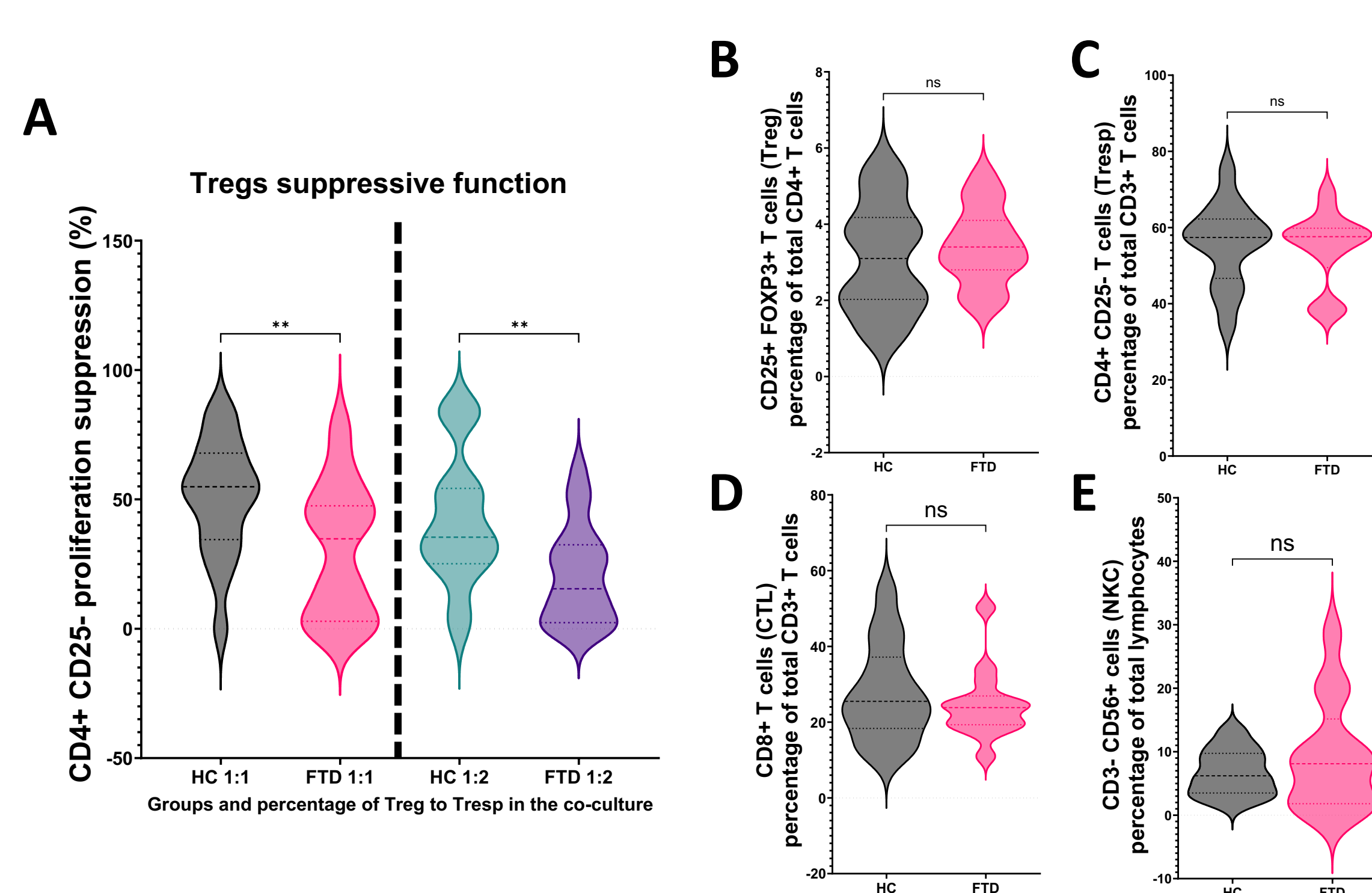


Figure 2. Treg immunomodulatory function is compromised in FTD. The Treg suppression of Tresp proliferation was significantly reduced in FTD compared to HC when co-cultured at a Treg:Tresp ratio of either 1:1 or 1:2, respectively (A). No changes with respect to the number of Tregs (B), Tresp (C), CD8 T cell (D), or natural killer (NK) cells (E) population was observed among FTD and HC individuals. Graphs depicting mean ± SEM; T-student **p<0.01, ns = non-significant.

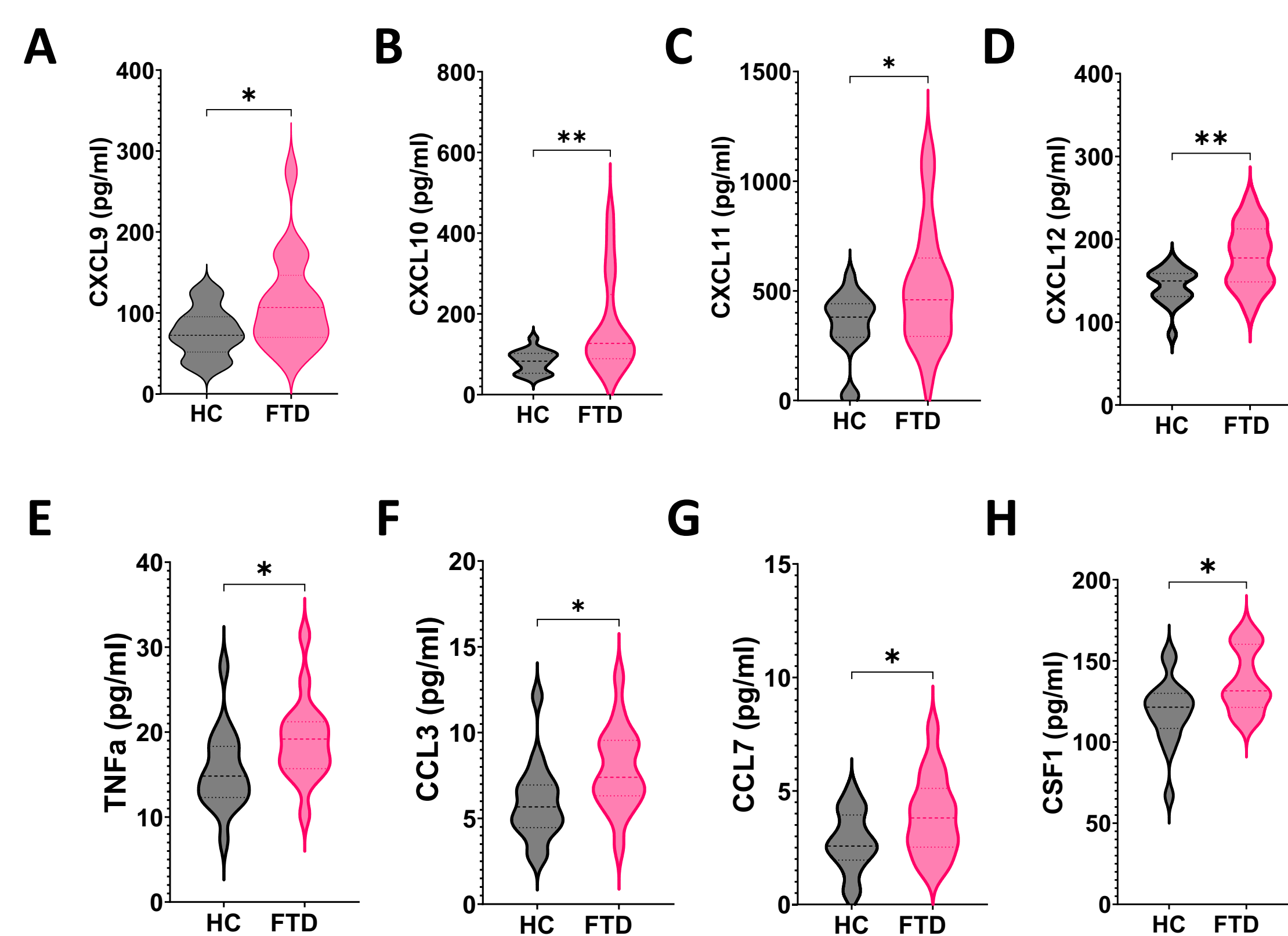


Figure 4. Increased levels of CXCL9-11 and other pro-inflammatory cytokines in the plasma of FTD individuals. The concentration of 46 inflammatory cytokines and chemokines was measured in the plasma of HC and FTD individuals. Increased levels of Interferon gamma-induced CXCL9, 10, and 11(A-C), CXCL12 (D), TNF (E) and 3 other pro-inflammatory mediators (F-H) were noted in the serum of FTD compared to HC individuals. Graphs depicting mean ± SEM; T-student *p<0.05, **p<0.01

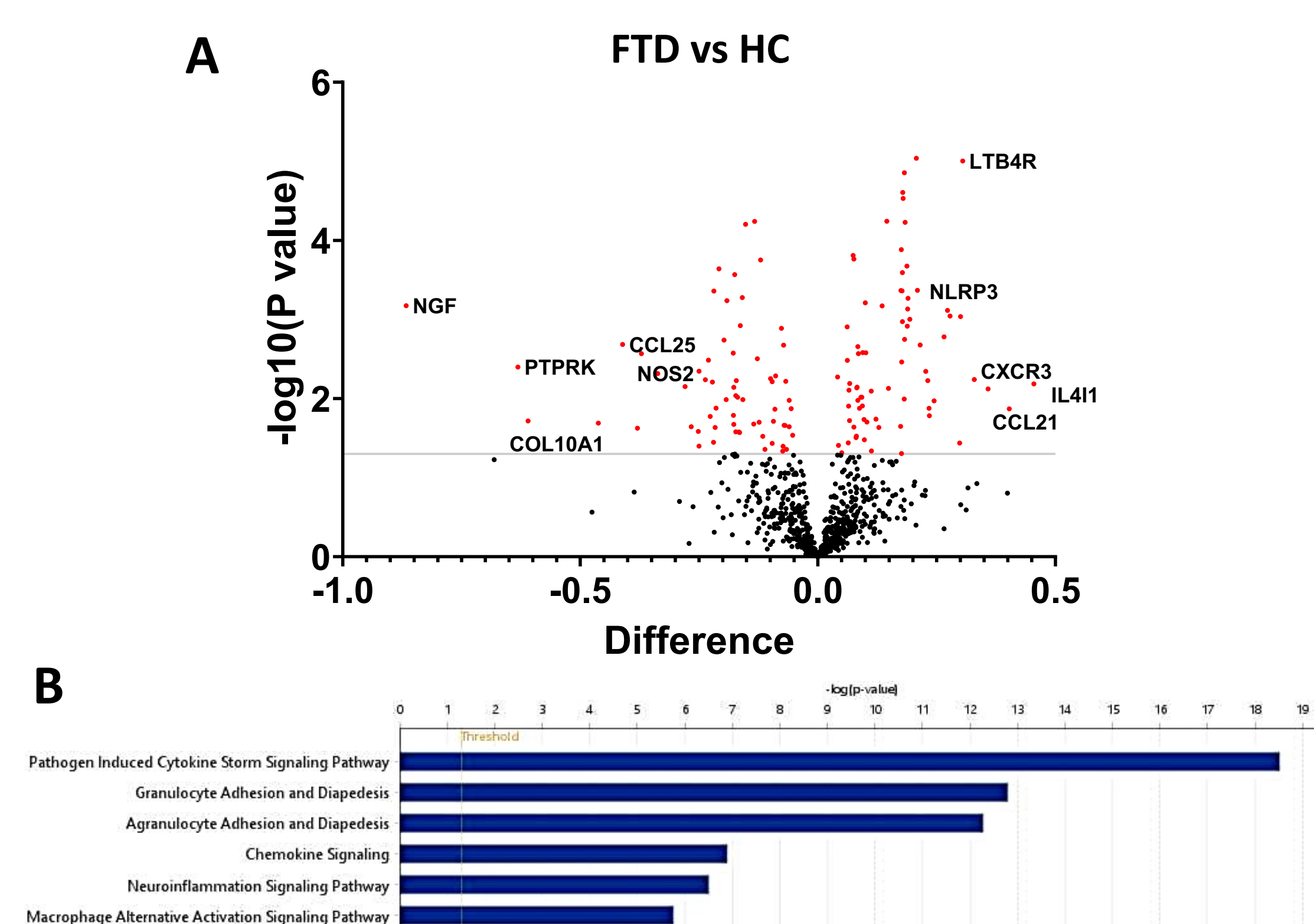


Figure 3. Dysregulation of monocyte's pro-inflammatory mediators in FTD. Volcano plot showing log10 expression ratios vs fold change of nCounter human inflammation panel for FTD vs HC individuals. Out of all 770 analyzed inflammation transcripts, 72 were upregulated and 61 were downregulated in FTD individuals vs HC (A) Ingenuity's pathway analysis displayed that the main dysregulated monocyte's genes are involved in cytokine storm, chemokine, and neuroinflammation signaling pathways (B).

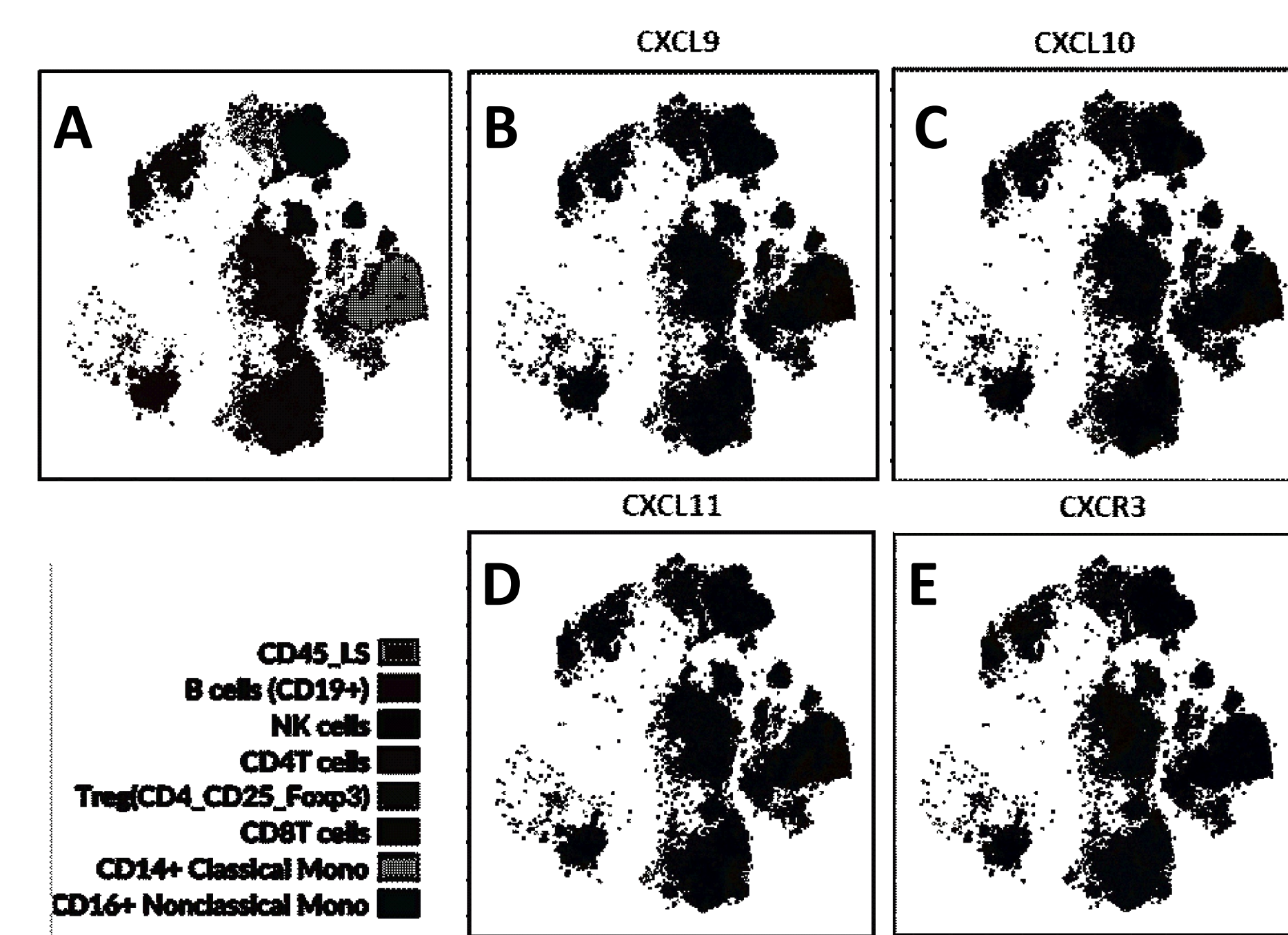


Figure 5. CXCL9-11/CXCR3 protein expression in peripheral immune cells by CyTOF analysis. A) tSNE graph depicting immune cell clusters. B-E) tSNE graphs show that CD14+classical monocytes highly express CXCL9-11 (pink cluster in A), whereas the receptor, CXCR3, is mainly expressed in CD4 and CD8 T cells (red and brown clusters in A).

Conclusions

FTD individuals exhibit a compromised immunosuppressive function of Tregs along with dysregulation of peripheral monocyte's inflammation transcriptome and systemic activation of the CXCL9-11/CXCR3 inflammatory cascade, supporting the role of systemic inflammation in FTD-associated pathology.

Our findings demonstrate the activation of a novel inflammatory cascade in the clinical setting of FTD that merits further research as a potential therapeutic target.

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