Supplemental Information

Neurological Manifestations of COVID-19 Feature

T Cell Exhaustion and Dedifferentiated Monocytes

in Cerebrospinal Fluid

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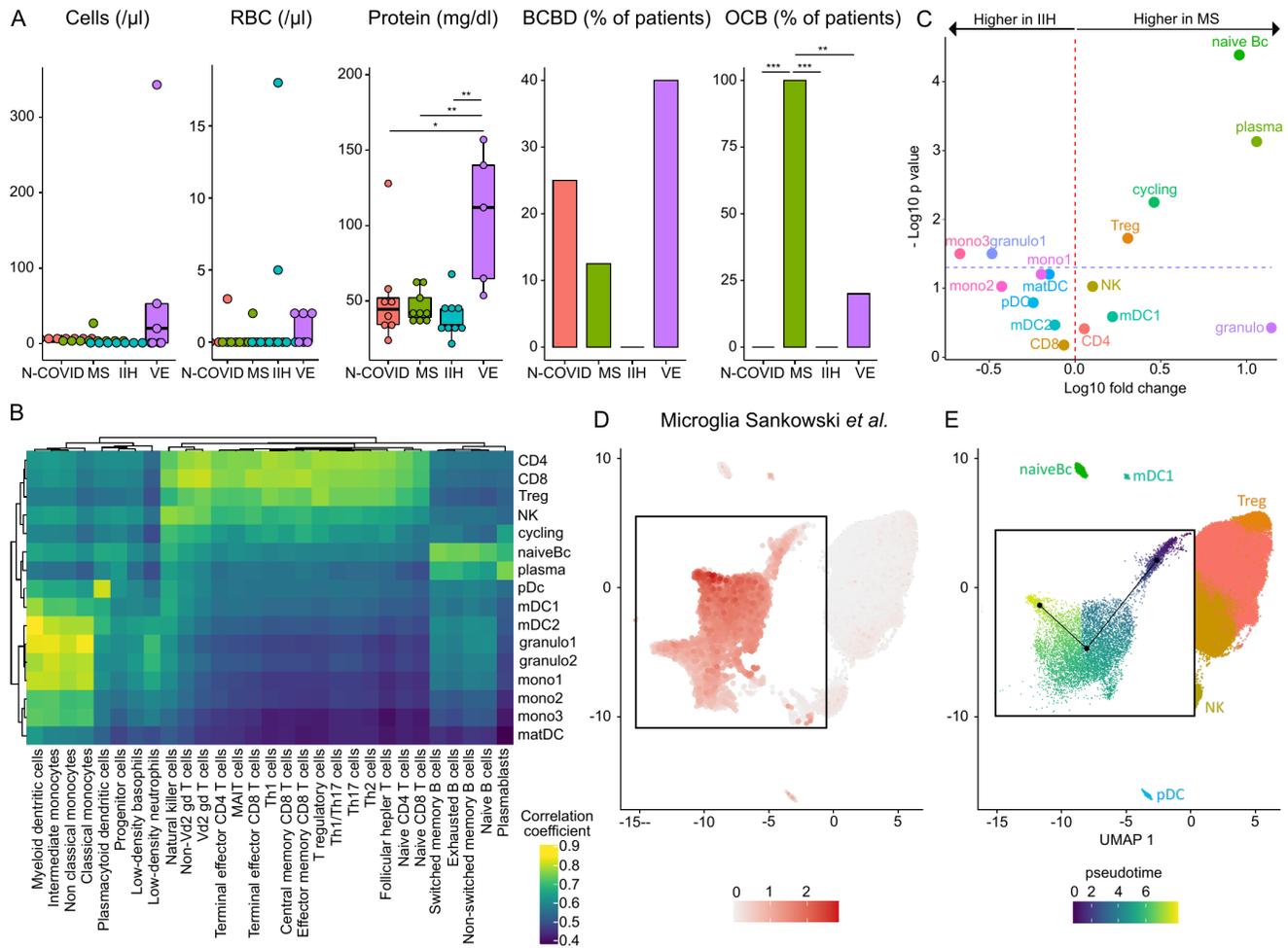
Figure S1: Known Disease-Associated CSF Alterations Are Replicated, Related to Figure 1.

(A) Basic CSF parameters of 8 Neuro-COVID (N-COVID), 9 idiopathic intracranial hypertension (IIH), and 9 relapsing-remitting multiple sclerosis (MS), and 5 viral encephalitis (VE) patients. Oligoclonal band (OCB) information was unavailable in 2 Neuro-COVID and 3 IIH patients. Boxes show the median, the lower and upper quartile and whiskers include 1.5 times the interquartile range of the box, further outliers are marked as dots. Dot plots are overlaid. Significance was tested with Kruskal-Wallis with Dunn post-hoc test (cells, red blood cells (RBC), protein) or the Freeman-Halton extension of Fisher's exact test (two-sided) with post-hoc pairwise comparisons adjusted with Benjamini-Hochberg's method.

(B) Correlation coefficients between clusters from this study and Monaco et al.

(C) Changes of cluster abundances in MS (n = 9) vs. IIH (n = 9) patients. Logarithmic fold change of cluster abundance is plotted against negative logarithmic p value (two-sided Wilcoxon's rank-sum test). The horizontal dashed line represents the significance threshold (p = 0.05).

(D) Gene score feature plot of a microglia gene set from Sankowski et al. Markers that were used for this plot are provided in Table S2.

(E) Pseudotime time analysis performed with Slingshot of mono1, mono2 and mono3 clusters.

Abbreviations: RBC - red blood cells; BCBD - blood-CSF-barrier disruption; OCB - oligoclonal bands; CD4 - CD4+ T cells; Treg - regulatory T cells; CD8 - CD8+ T cells; NK - NK cells; plasma - plasma cells; naiveBc - naive B cells; mDC - myeloid dendritic cells; pDC - plasmacytoid dendritic cells; matDC - mature dendritic cells; granulo - granulocytes; mono – monocytes.
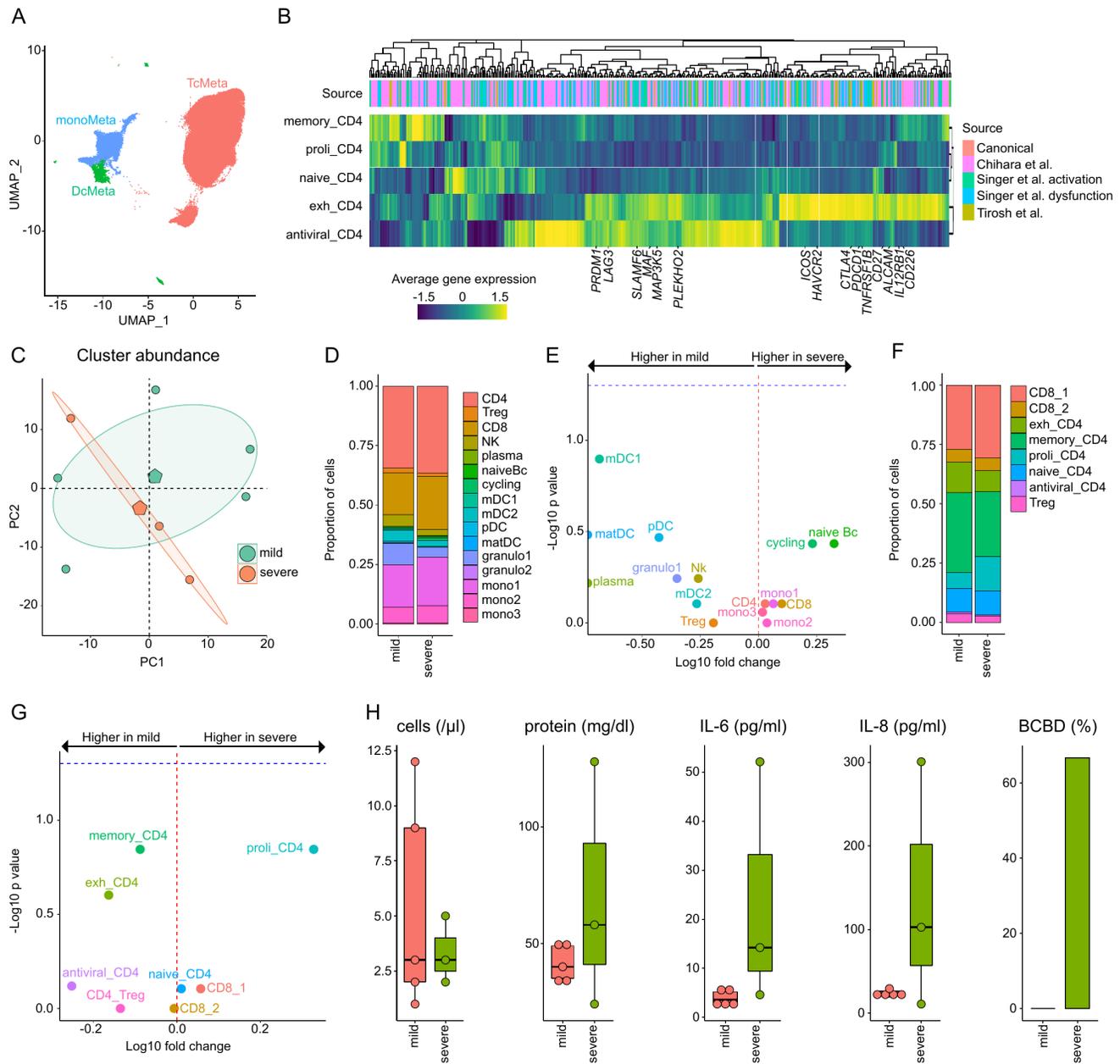
Figure S2: Further Severity-Associated Observations in Neuro-COVID, Related To Figure 4.
(A) UMAP plot showing the merged meta clusters that were used for differentially expression (DE) analysis across conditions.
(B) Average gene expression of T cell exhaustion markers from Singer et al., Tirosh et al., Chihara et al. and canonical markers in CD4+ clusters. Selected transcripts are labeled. The complete gene sets are listed in Table S4.
(C) Principal component analysis (PCA) of cluster abundances in Neuro-COVID patients categorized by neurological severity (mild n = 5; severe n = 3). Each circle represents one patient, the polygons represent the group means. The ellipses around the group means show the confidence regions.
(D) Proportions of all cells in Neuro-COVID split by neurological disease severity (mild n = 5; severe n = 3).
(E) Changes of cluster abundances in severely (n = 3) vs. mildly (n = 5) affected Neuro-COVID patients. Logarithmic fold change of cluster abundance is plotted against negative logarithmic p value (two-sided Wilcoxon's rank-sum test). The horizontal dashed line represents the significance threshold (p = 0.05).
(F) Proportions of T cells in Neuro-COVID split by neurological disease severity (mild n = 5; severe n = 3).

(G) Changes of cluster abundances in severely (n = 3) vs. mildly (n = 5) affected Neuro-COVID patients. Logarithmic fold change of cluster abundance is plotted against negative logarithmic p value (two-sided Wilcoxon's rank-sum test). The horizontal dashed line represents the significance threshold (p = 0.05).

(H) Basic CSF parameters and interleukin (IL)-6 and IL-8 were quantified in neurologically mildly (n =3) and severely (n = 5) affected COVID-19 patients. Boxes show the median, the lower and upper quartile and whiskers include 1.5 times the interquartile range of the box, further outliers are marked as dots. Dot plots are overlaid. Significance was tested using two-sided Wilcoxon's rank sum-test or Fisher's exact test.

Abbreviations: proli_CD4 - proliferating CD4+ T cells; memory_CD4 - memory-like CD4+ T cells; exh_CD4: exhausted CD4+ T cells; CD8 - CD8+ T cells; CD4_Treg - regulatory CD4+ T cells; BCBD - blood-CSF-barrier disruption.
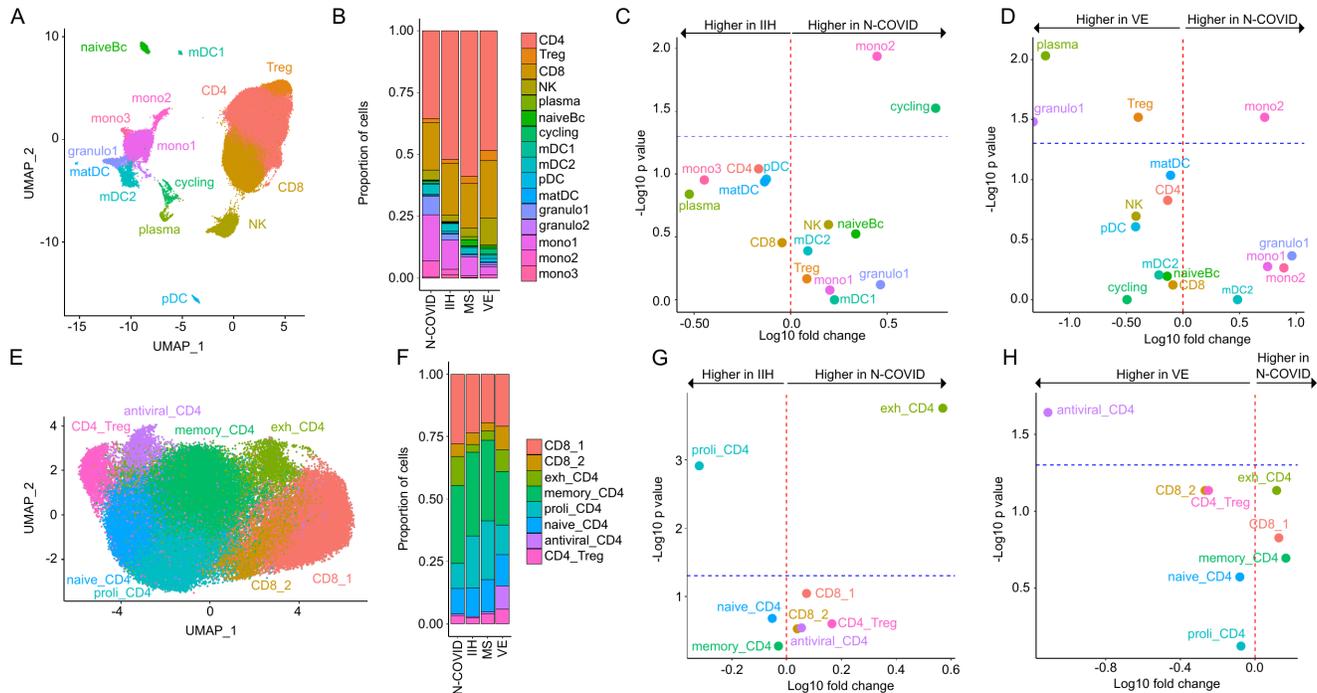
Figure S3: Core Findings Are Replicated After Removal of One Neuro-COVID With Comorbid Multiple Sclerosis, Related to Figure 1 and Figure 3.

(A) UMAP plot showing 16 color-coded cell clusters of 80,820 raw single cell transcriptomes from CSF cells from Neuro-COVID (N-COVID; n = 7) after removal of patient with pseudonym C24 , IIH (n = 9), MS (n = 9) and VE (n = 5) patients.

(B) Proportions of cells split by diagnosis.

(C-D) Changes of cluster abundances in Neuro-COVID (n = 7) vs. IIH (n = 9) (C) and Neuro-COVID (n = 7) vs. VE (n = 5) (D). Logarithmic fold change of cluster size is plotted against negative logarithmic p value (two-sided Wilcoxon's rank-sum test). The horizontal dashed line represents the significance threshold (p = 0.05).

(E) UMAP plot displaying 8 color-coded cell clusters of 61,584 raw single cell transcriptomes from CSF cells from Neuro-COVID (n = 7), IIH (n = 9), MS (n = 9) and VE (n = 5) patients.

(F) Proportions of T cells split by diagnosis.

(G-H) Changes of T cell subcluster abundance in Neuro-COVID (n = 7) vs. IIH (n = 9) (G) and Neuro-COVID (n = 7) vs. VE (n = 5) (H). Logarithmic fold change of cluster size is plotted against negative logarithmic p value (two-sided Wilcoxon's rank-sum test). The horizontal dashed line represents the significance threshold (p = 0.05).

Abbreviations: CD4 - CD4$^+$ T cells; Treg - regulatory T cells; CD8 - CD8$^+$ T cells; NK - NK cells; plasma - plasma cells; naiveBc - naive B cells; mDC - myeloid dendritic cells; pDC - plasmacytoid dendritic cells; matDC - mature dendritic cells; granulo - granulocytes; mono - monocytes; proli_CD4 - proliferating CD4$^+$ T cells; memory_CD4 - memory-like CD4$^+$ T cells; exh_CD4: exhausted CD4$^+$ T cells; CD4_Treg - regulatory CD4$^+$ T cells.