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Supplemental information

Divergent and self-reactive immune responses in the CNS of COVID-19 patients with neurological symptoms

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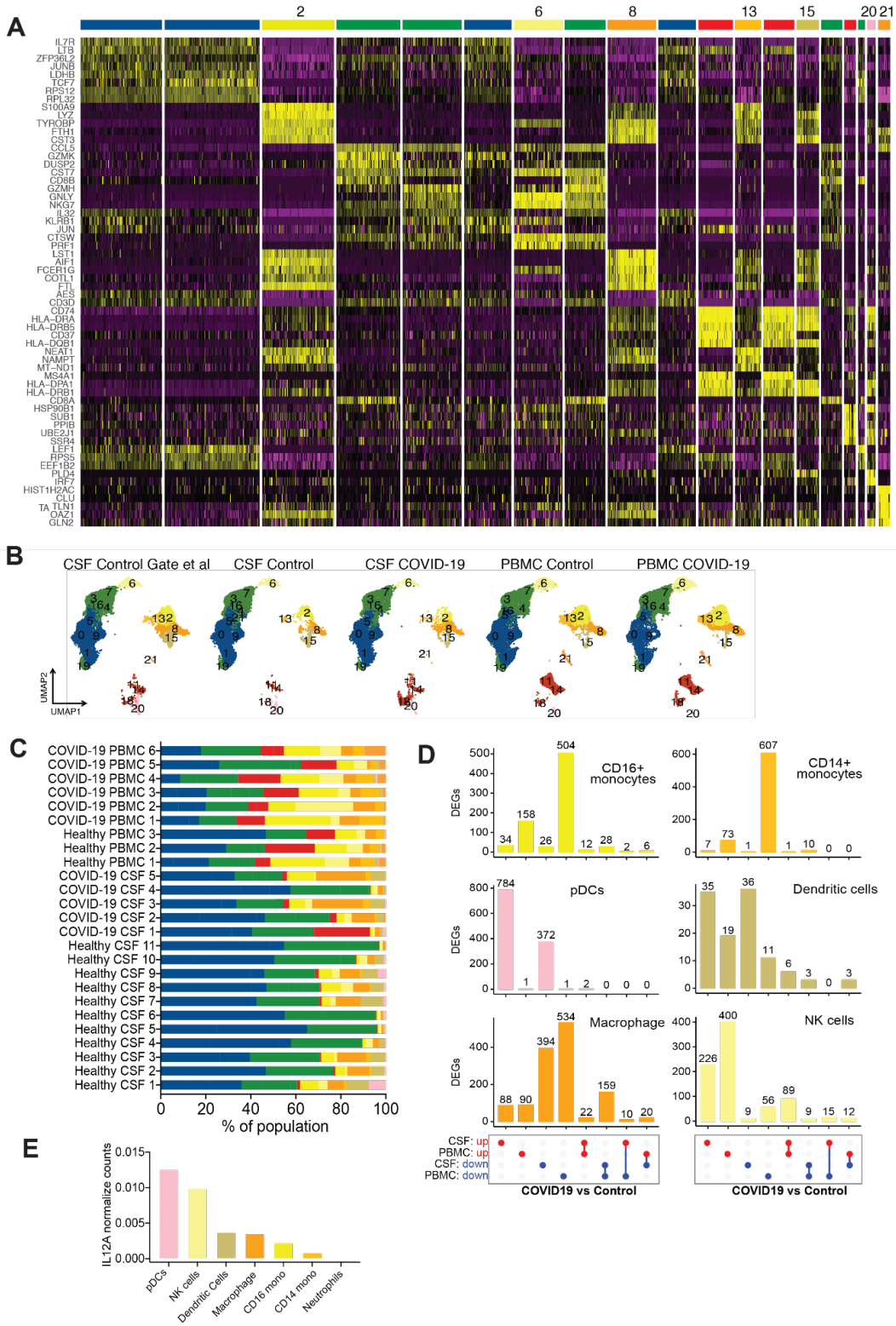
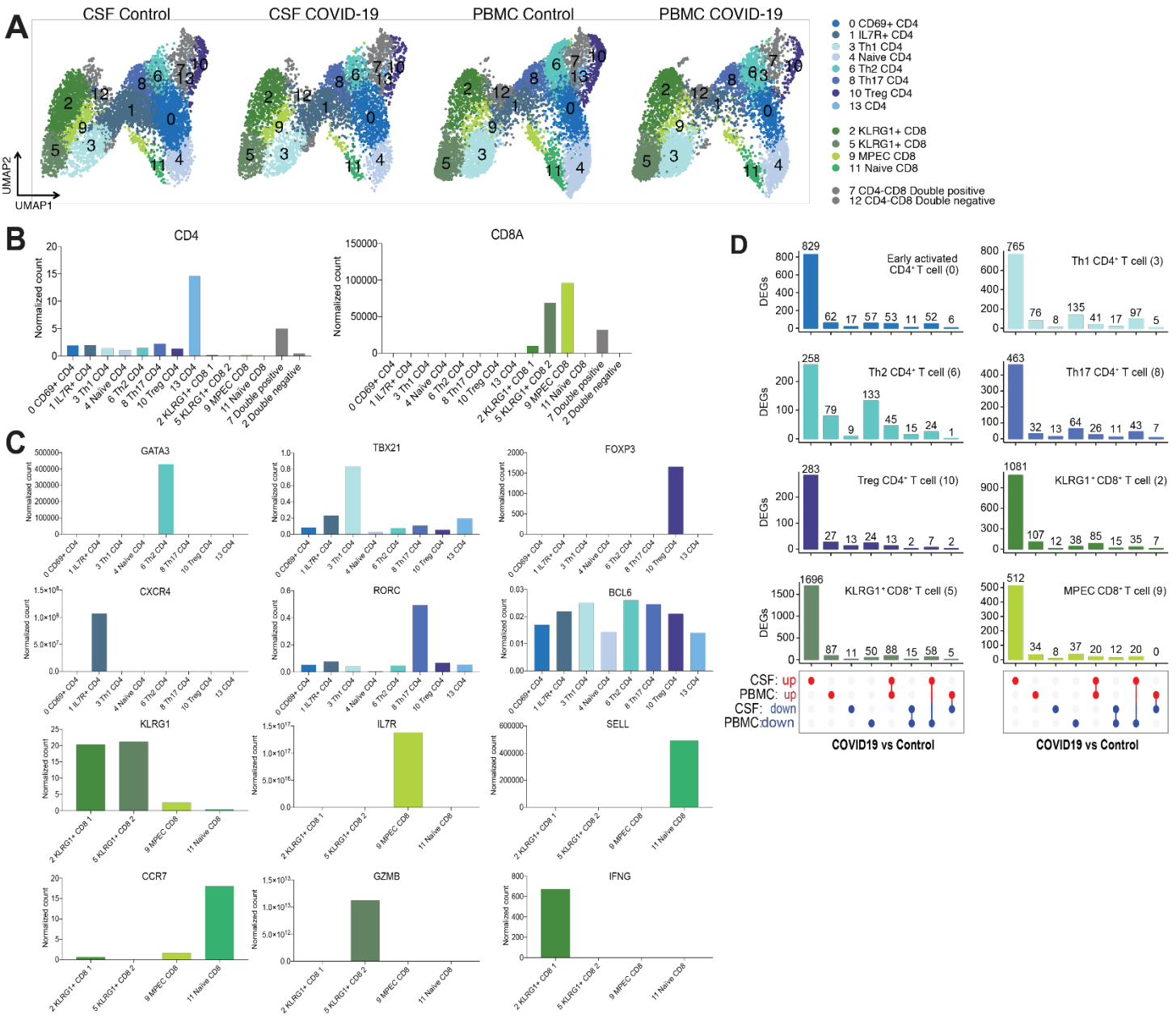
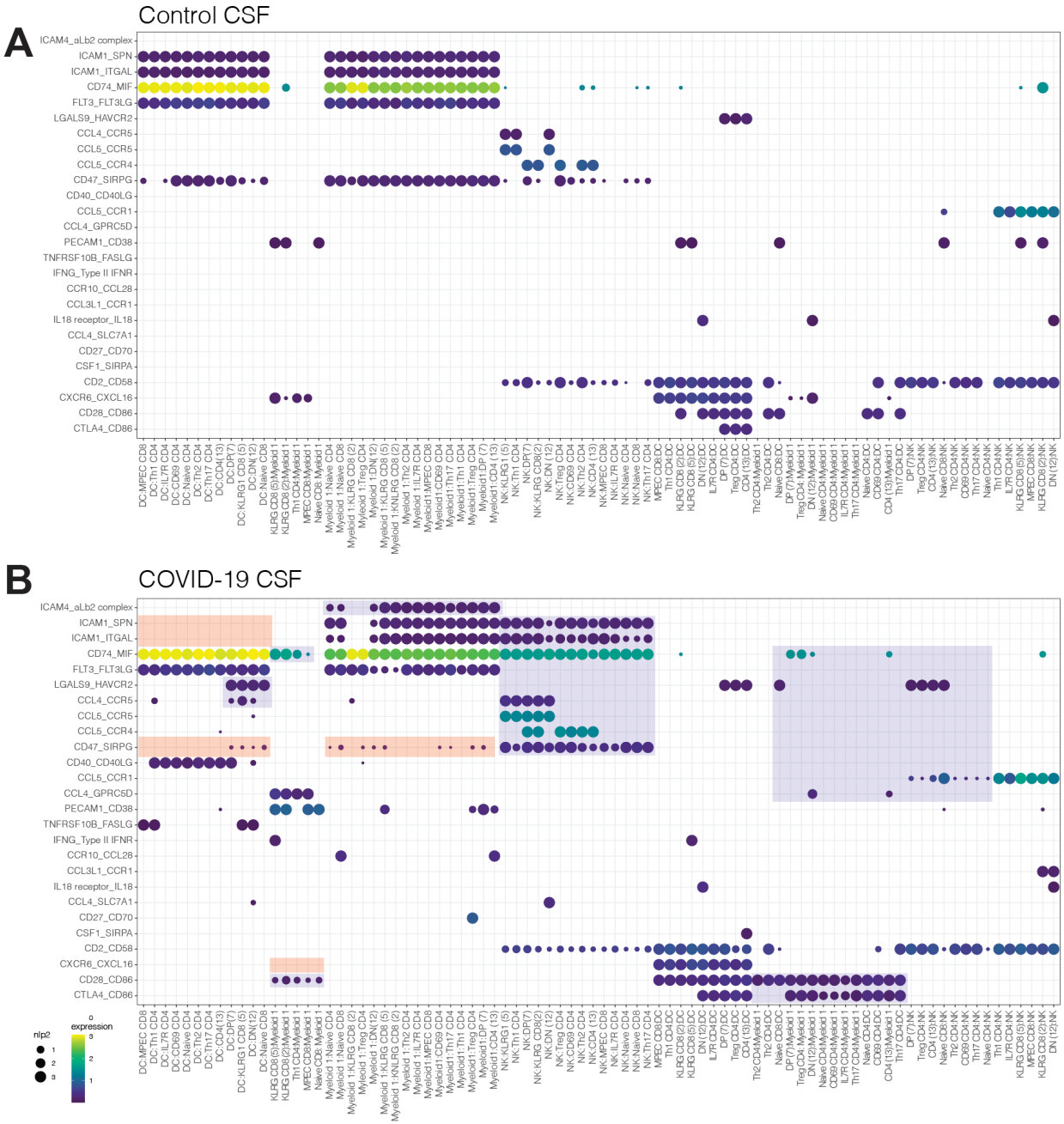


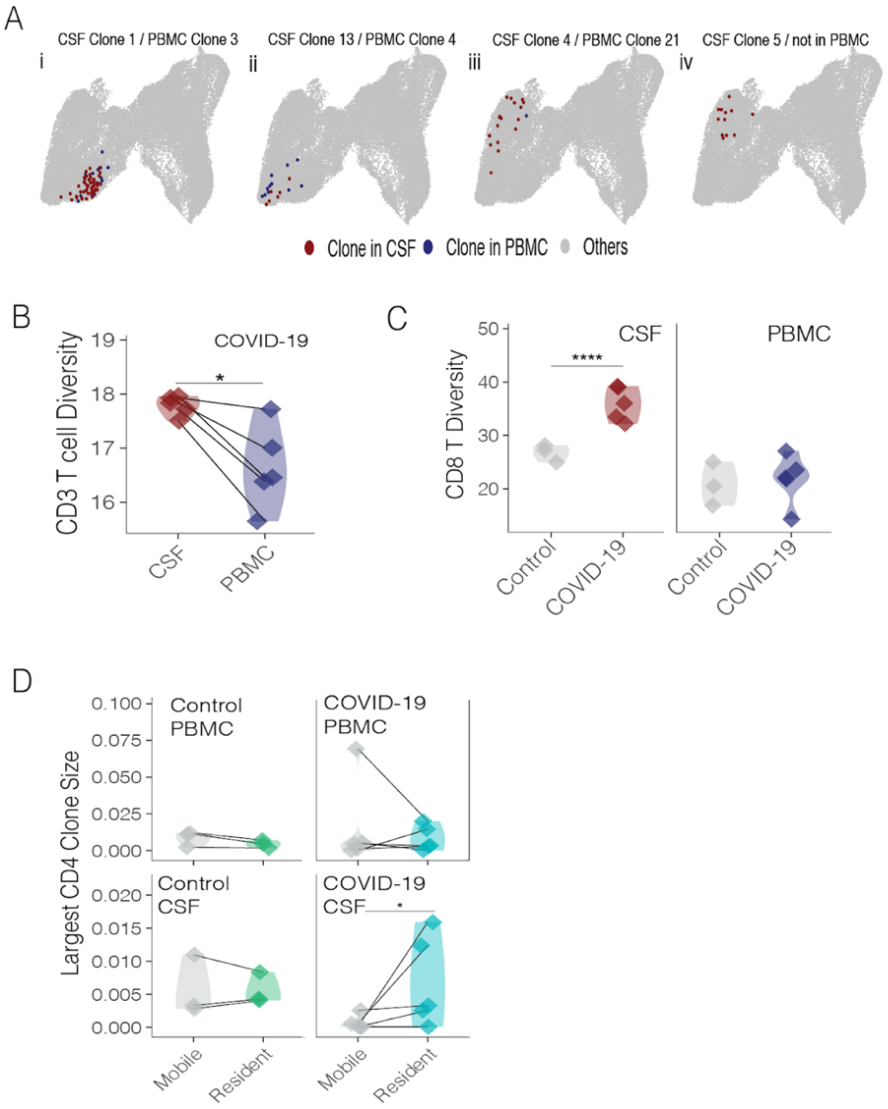
Supplementary Figure 1. Distinct immunological landscape of COVID-19 patient CSF versus PBMC. Related to Figure 1. (**A**) Top DEGs distinguishing clusters found in Figure 1. (**B**) UMAP projection of cell types in CSF and PBMC of COVID-19 cases and healthy controls, separated by tissue of origin and disease status. CSF libraries were made from prospectively enrolled health controls ("CSF control") and from publicly available data ("CSF Control Gate et al"). (**C**) Relative proportion of cell types found in biological samples for the study. (**D**) UpSet plot showing differentially expressed genes (DEGs) in innate immune cells of COVID-19 cases versus controls. The legend for each column is indicated in the bottom panel. Each column denotes the number of genes that are significantly (adjusted p value <0.05) up (red) or down (blue) regulated in COVID-19 tissue when compared to control. Connecting dots indicate the intersection of DEGs in CSF and PBMC. (**E**) Normalized counts of IL12A transcripts on a population level for innate immune cells.



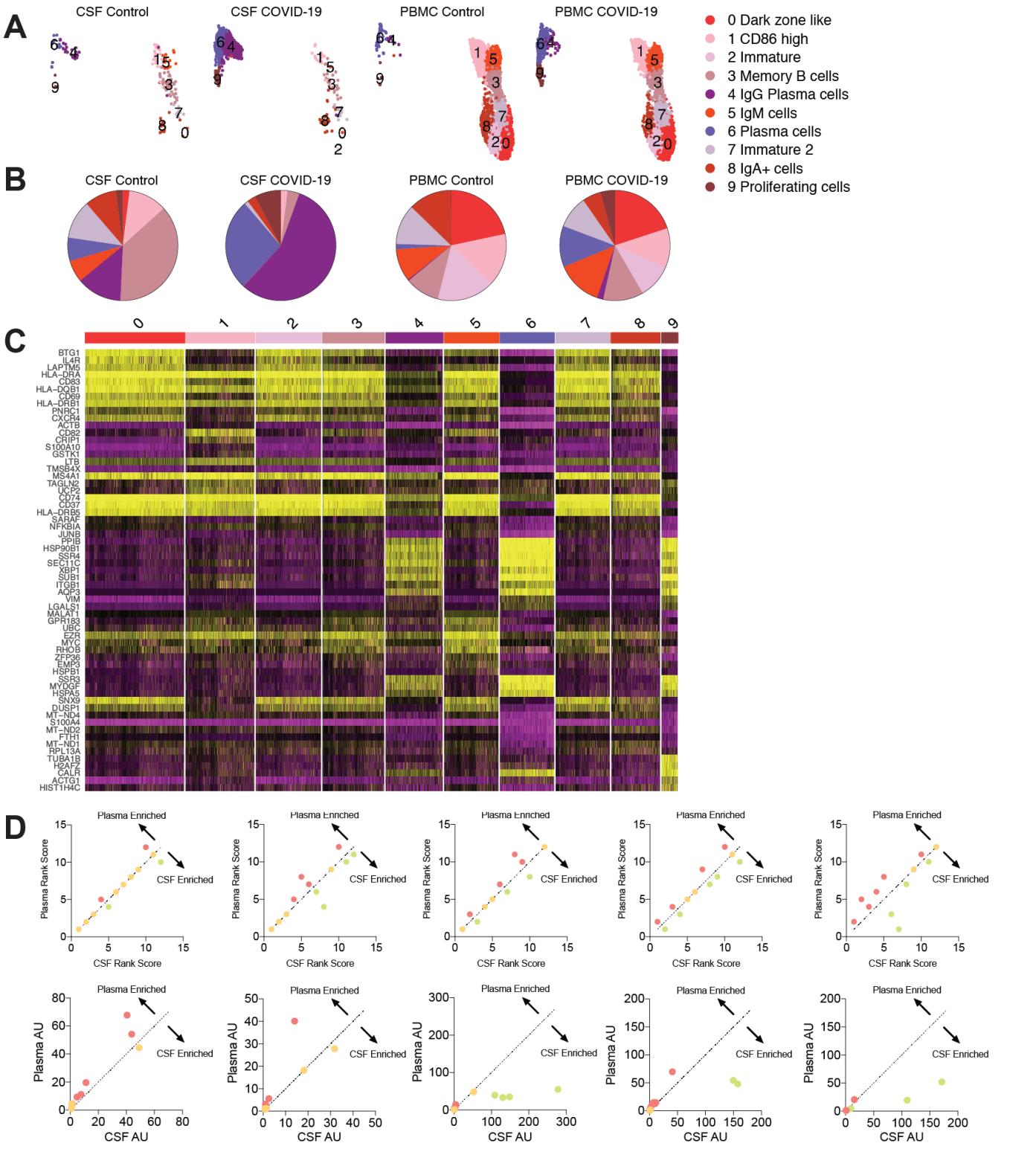
Supplementary Figure 2. Characterization of T cell subsets found in CSF and PBMC. Related to Figure 2. (A) UMAP projection of T cell types in CSF and PBMC of COVID-19 patients and control patients. (**B, C**) Normalized gene expression data of T cell identification genes in T cells. **(D)** UpSet plot showing differentially expressed genes (DEGs) in T cell subsets of COVID-19 cases versus healthy controls in CSF and PBMC. Each column denotes the number of genes that are up or down-regulated in CSF and/or PBMC as indicated in the bottom panel. Numbers in parenthesis refer to cluster in subpanel A.



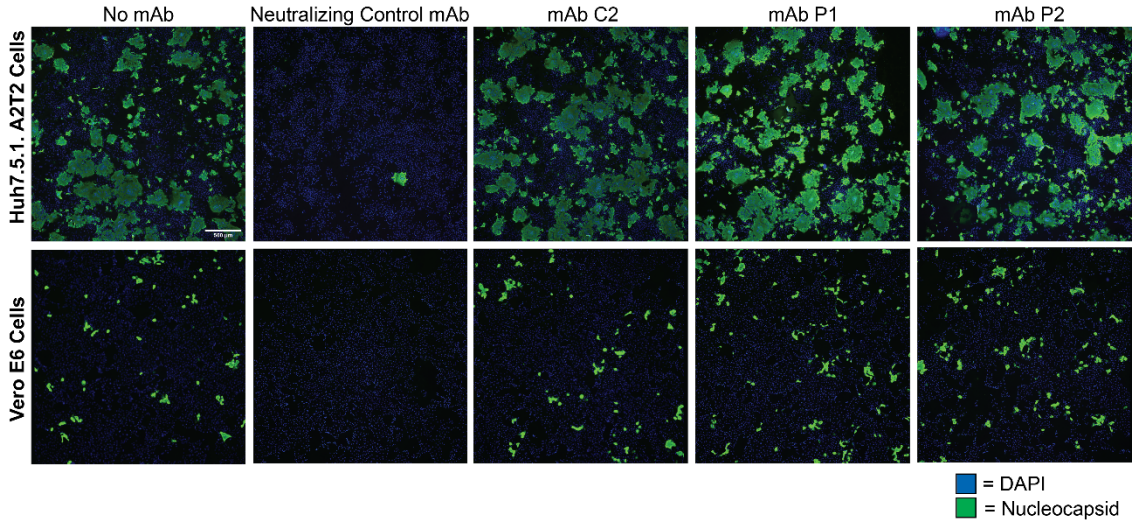
Supplementary Figure 3. Predicted interactions enriched in CSF of COVID-19 patients using CellPhoneDB. Reltaed to Figure 1. CellPhoneDB interaction map between innate immune cells identified in Fig 1A and T cell subtypes identified in Fig 2A of COVID-19 patient CSF. (**A**) Depicts control patient CSF interactions. (**B**) Depicts interactions in CSF of COVID-19 cases. Red box depicts interactions disappearing compared to control CSF and blue box depicts interactions enriched compared to control CSF.



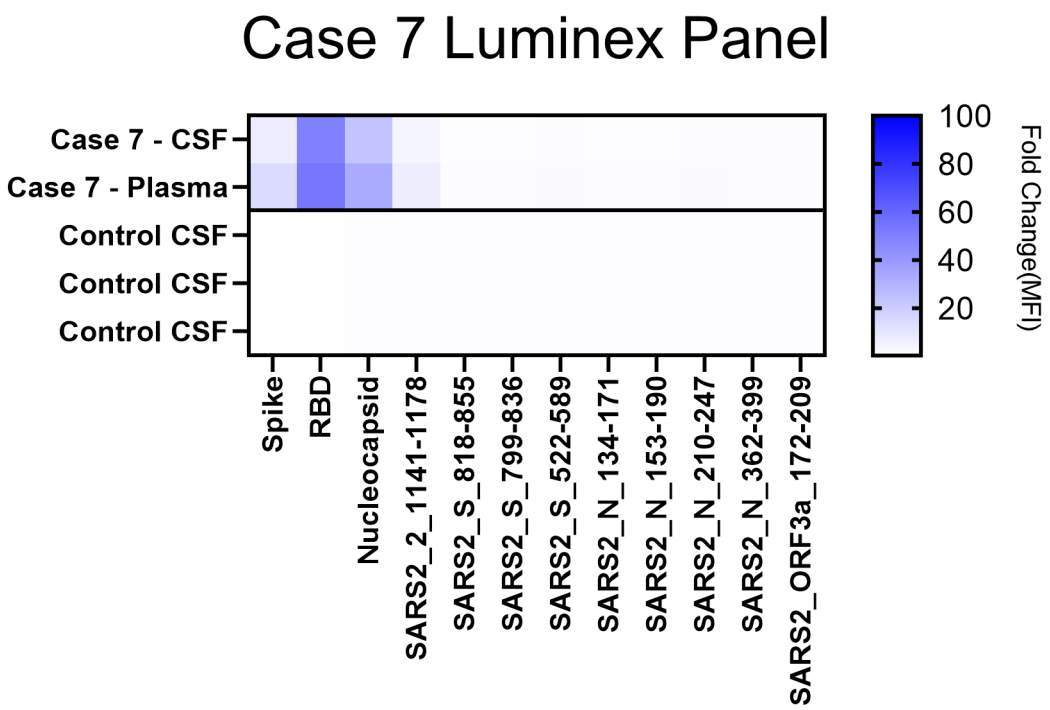
Supplementary Figure 4. TCR clonotype analysis. Related to Figure 2. T cell clones that were prevalent in the CSF were also expanded in matching PBMC **(A, i)**. However, we also identified clones that were abundant in CSF but rare in the PBMC **(A, iii)**, and *vice versa* **(A, ii).** In some cases, clones that were highly represented in the CSF (e.g. clone 5) were not found at all in the PBMC **(A, iv).** The top expanded T cell clones in CSF were characterized by transcriptional profiles of Th1-like CD4 T cells and effector CD8 T cells. To further dissect the dynamics of T cell expansions in the CNS, we assigned T cells as either unique or shared based on whether their TCRB V(D)Js were exclusive to one compartment or shared between compartments. Overall few differences were found when comparing the diversity of mobile T cell repertoires in CSF versus PBMC, but there was greater diversity of the unique T cell repertoire in the CSF of COVID-19 patients when compared to the unique T cells in the PBMC **(B).** To identify the source of this difference, we examined CD4 and CD8 T cell repertoires separately. The higher diversity of unique T cells in CSF was largely explained by an increase in the diversity of CD8 T cells in the CSF **(C).** This may reflect activation and expansion of bystander CD8 T cells in the setting of viral infection. In contrast, we observed clonal expansion of unique but not shared CD4 T cell clones in the CSF of COVID-19 patients **(D).**



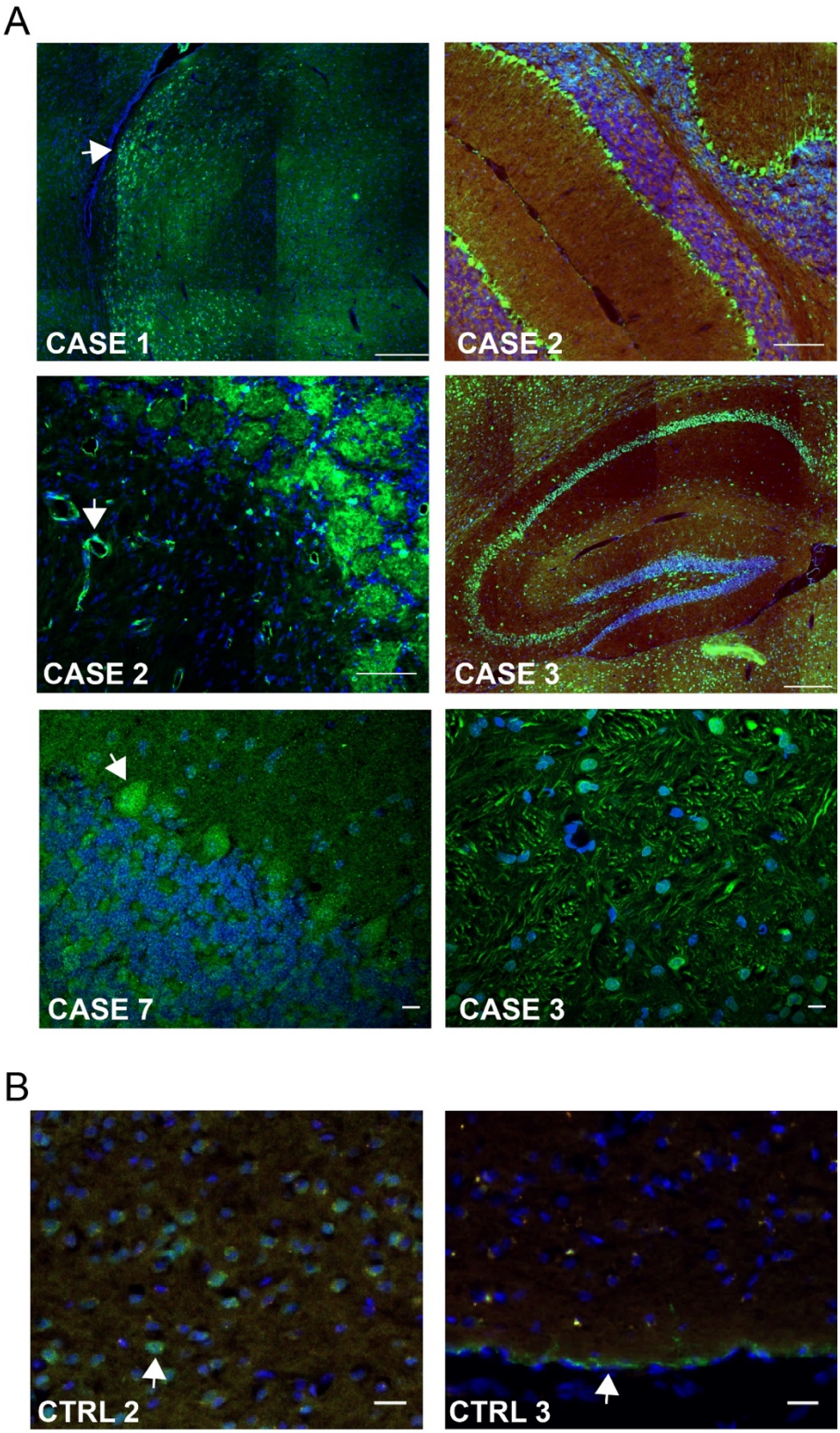
Supplementary Figure 5. **CNS-resident B cell responses in COVID-19 patients. Related to Figure 3. (A)** UMAP projection of B cell types in CSF and PBMC of COVID-19 cases and controls. (**B**) Pie charts depicting relative population frequency of different B cell subtypes found in CSF and PBMC of COVID-19 cases and controls. (**C**) Top DEGs distinguishing clusters found in Figure 3. D (**D, top row**) SARS-CoV-2 epitope binding antibody frequency from Figure 3c was ranked and correlation between CSF and plasma was performed (higher rank score depicts more frequent epitope. (**D, bottom row**) Similar to top row, but normalized arbitrary unit was used to derive a correlation between plasma and CSF antibodies. Each panel represents individual patient samples. Each dot represents different epitopes. Green represents epitopes enriched in CSF, Red represents epitopes enriched in Plasma, Orange represents epitopes that are not enriched in either compartment.



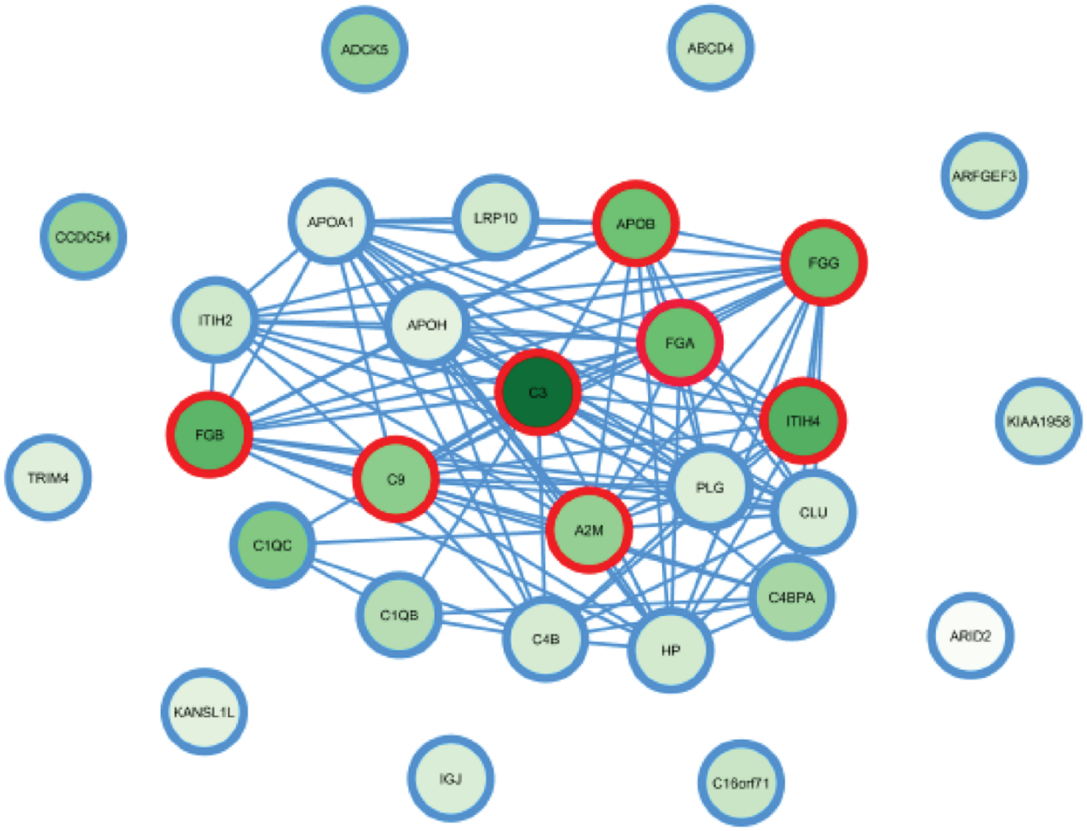
Supplementary Figure 6. Neutralization assays of CSF and peripheral blood derived monoclonal antibodies from COVID-19 case 1. Related to Figure 5. SARS-CoV-2 (SARS-CoV-2/human/USA/CA-UCSF-0001H/2020) was incubated without or with monoclonal antibodies for 1 hour. Mouse-anti-spike (Sino Biological #40591-MM43) was used as a positive neutralizing control. The positive control and mAbs C2 and P2 were tested at 2.5 and 25µg/mL on Huh7.5.1 A2T2 cells and Vero E6 cells. mAb P1 was tested at 5 and 2.5µg/mL on Huh7.5.1 A2T2 and Vero E6 cells respectively. Images were acquired at 4x magnification, scale bar = 500 µm. **Top row, Huh7.5.1 A2T2 cells:** control mAb effectively neutralizes SARS-CoV-2 but mAbs C2 (25 µg/mL), P1 (5 µg/mL), and P2 (25 µg/mL) do not. **Bottom row Vero E6 cells:** control mAb effectively neutralizes SARS-CoV-2 but mAbs C2 (25 µg/mL), P1 (2.5 µg/mL), and P2 (25 µg/mL) do not.



Supplementary Figure 7. Luminex panel demonstrating SARS-CoV-2 antibodies in post-COVID-19 case 7. Related to Figure 3. Heatmap of anti-SARS-CoV-2 antibody specificity of CSF and plasma of case 7. CSF and plasma were run in technical replicate. Heatmap values represent the mean fold change of median fluorescence intensity (MFI) for each biospecimen.



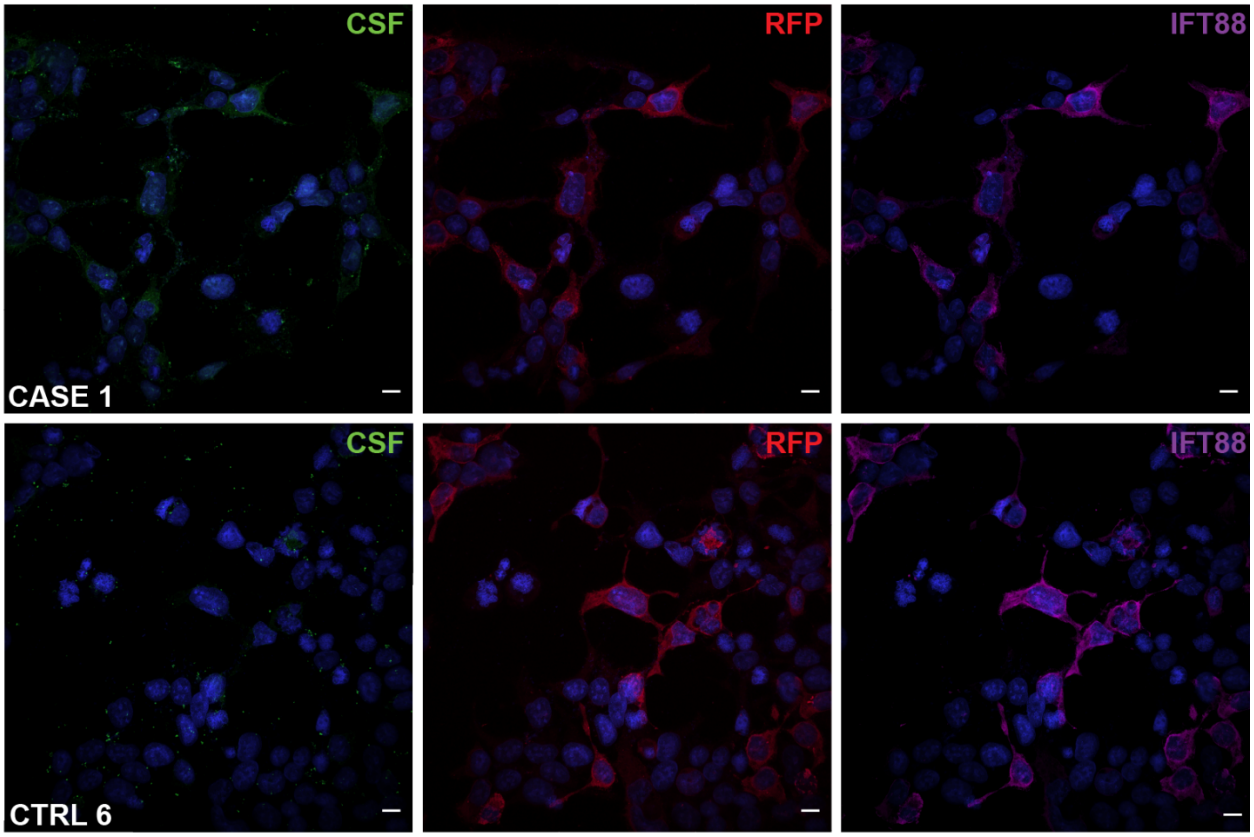
Supplementary Figure 8. Additional anatomic regions immunostained by COVID-19 CSF and controls. Related to Figure 6. **(A)** COVID-19 CSF clockwise from top left: (1) Case 1, anterior paraventricular nucleus (arrow) of the thalamus, (2) Case 3, Purkinje cells and glial like processes in the cerebellum, (3) Case 3, hilus, CA fields of the hippocampus, subiculum, and overlying cortical cells, (4) Case 3, anterior pons of the brain stem, (5) Case 7, Purkinje cell bodies (arrow) and synaptic-like puncta in the overlying molecular layer of the cerebellum, (6) Case 2, endothelial-like cells (arrow) in the olfactory bulb. **(B)** Two of six control CSF samples were immunoreactive to mouse brain tissue at a 1:10 dilution but not at higher dilutions. CTRL 2 (left) immunostained nuclei (arrow) throughout the brain. CTRL 3 was primarily immunoreactive to subpial glial (arrow).



Rank	Pathway	p-value	Bonferroni-corrected p-value	Proteins
1	Complement and coagulation cascades	$1.0x10^{-21}$	2.4x10-19	A2M, FGA, FGB, C1QB, C1QC, C3, C4B, C4BPA, FGG, PLG, C9, CLU
2	Platelet degranulation	$5.5x10^{-13}$	1.3x10-10	A2M, FGA, FGB, APOA1, FGG, PLG, APOH, CLU ITIH4
3	Classical complement pathway	$3.1x10^{-11}$	7.3x10-9	C1QB, C1QC, C3, C4B, C9

Supplementary Figure 9. Network analysis and gene ontology pathway analysis of IP-MS identified human plasma proteins from COVID-19 patients. Related to Figure 6. Top network: COVID-19 plasma immunoprecipitations were compared as a group to control plasma immunoprecipitations. Human proteins that were elevated 1.5-fold or greater by MS1 peak area (Quandenser) were analyzed by string-db.org. String-db network connection data were imported into cytoscape along with IP-MS q-values (a q-vlaue is an FDR-adjusted p-value). The protein nodes are shaded (green) according to their log2 fold enrichment. Red borders indicate those proteins with a q-value < 0.05, and blue borders indicate q-values > 0.05. Bottom table: ToppGene gene ontology pathway anaylsis of

the set of proteins (28/29, IGJ was excluded) that were elevated 1.5-fold or greater in the plasma IgG IP-MS fractions of COVID-19 patients.



Supplementary Figure 10. HEK 293 overexpression cell based assay for IFT88. Related to Figure 6. Confocal images of IFT-88 CBA. Top row: Case 1 CSF (green) immunoreacts to overexpressed RFP-IFT88 (red) and colocalizes with a commercial anti-IFT88 antibody (magenta). Bottom row: Control CSF (green) is not immunoreactive RFP-IFT88 (red) and does not colocalize with a commercial anti-IFT88 antibody (magenta). Scale bars = 10 μm.

Table S1. COVID-19 Patient Characteristics. Related to STAR Methods *Human Subjects*

Case:	***CASE 1***	***CASE 2***	***CASE 3***	***CASE 4***	***CASE 5***	***CASE 6***	***CASE 7****
Age/Gender	43M	84F	60M	55F	64M	67M	64M
Hospital Status	ICU	Hospitalized	ICU	Hospitalized	ICU	ICU	Hospitalized
Ventilation	No	No	Yes	No	Yes	Yes	No
Neurologic Symptoms (NeuroSx)	Encephalopathy	Intractable headache, delirium	Seizures	Intractable headache, blurred vision	Impaired consciousness	Encephalopathy, Neuromuscular weakness	Confusion, auditory hallucinations, language impairment, apraxia
Est. time from:							
SARS-CoV-2 infection to NeuroSx	14 days (Acute COVID-19)	7 – 14 days (Acute COVID-19)	7 - 14 days (Acute COVID-19)	7 – 14 days (Acute COVID-19)	14 – 21 days (Acute COVID-19)	7 – 14 days (Acute COVID-19)	50 – 70 days (post-COVID-19)
Neurologic Outcome	Recovered	Recovered	Deceased	Recovered	Unknown	Partially Recovered	Recovered
Comorbidities	Diabetes mellitus	Dementia, hypertension, diabetes mellitus, cerebral aneurysm	Metastatic (brain) melanoma, hypertension, Diabetes mellitus, chronic kidney disease	None known	renal transplant, diabetes mellitus, DVT, brain micro-hemorrhages	Obesity	Hypertension
Neuroimaging	MRI: N.D. CT: Unremarkable	N.D.	MRI: Unremarkable	MRI/MRV: Unremarkable	MRI/MRA: scattered acute infarctions	MRI: Unremarkable	MRI: unremarkable
EEG	Generalized slowing	N.D.	Ictal activity	N.D.	Diffuse theta slowing	Generalized slowing	Fronto-central seizures
CSF Profile:							
WBC (cell/uL)	3	2	2	1	0	0	16
Protein (mg/dL)	57.4	29.3	66	20.5	43.8	85.6	61.6
Oligoclonal Bands	0	N.D.	Mirrored	0	0	Mirrored	0
IgG Index	0.52	0	0.51	N.D.	0.38	0.4	0.6
SARS-CoV-2 RT-PCR (CT value, N1 gene) Nasopharyngeal swab*	23	25	31	38[s]	40	31[s]	Not detected
SARS-CoV-2 RT-PCR of CSF	Not detected	Not detected	Not detected	Not detected	Not detected	N.D.	Not detected

SARS-CoV-2 PCR of Plasma	Not detected	Not detected	39	Not detected	Not detected	Not detected	N.D.
SARS-CoV-2 IgG: Plasma/CSF	Detected/Detected	Detected/Detected	Detected/Detected	Detected/Detected	Detected/Detected	Detected/Detected	Detected/Detected
Screened by:							
Immunostaining	Yes	Yes	Yes	Yes	Yes	No	Yes
IP-MS	Yes	Yes	Yes	Yes	Yes	No	Yes
PhIP-Seq	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CSF Autoantigens:							
Validated	IFT88		THAP3				Ro52
Select Candidates‡	BATF2, DBN1, DLG5, HLA-A, HLA-F, INA, INO80E, KIF13A, NEFH, OLFR668, PCMT1, PRDM11, RBM28, RNF8, SLC25A12, SPTAN1, TIF1α, TIF1 TUB, UBASH3B, USHA2A	**APOB**, BAZ1B, CAST, CRB2, EMILIN2, EMX1, FAM161B, IQCA1L, IQCC, MCM9, MECP2, POMC, RGS14, RYR3, SYNJ2, TRDN, **UHRF1BP1**, VPS13D, XAF1, ZDHHC16	ADAMTS16, BEST1, CNKSR2, CRH, DCAF12L2, DDX25, DLG4, IQSEC2, MINDY4, LMTK3, MAP3K19, NRG3, **NUAK1**, OLFR1413, PCLO, SEMA6C, SEMA6D, SHANK1/2/3, THAP3, SRCIN1	AP3B2, CCDC67, HSPA12A, INA, NEFM, NEFH, NAIP, PCDHB13, RBM25, USP14	C5orf49, CCN3, DPYSL2, FMR1, KCNC2, MAP6, TPPP	NAIP	ATP1A3, ARHGAP25, BAIAP3, BSN, CBX2, CCDC177, DBN1, **DBN1**, DNAH7A, ENTHD2, GFAP, IQCH, IQUB, MAP1B, MYH9, MYO5A, MYRIP, NPAS1, NSF, PSRC1, RAB3A, RYR1, SBSN, SLC1A3, SSB, SYN2, TBC1D32,

N,D, = Not Done, GRDA = generalize rhythmic delta activity, IgG index = ((IgG (CSF) x Albumin (serum)) / (IgG (serum) x Albumin (CSF))) x 100 *The patient was negative for SARS-CoV-2 by NP swab RT-qPCR, but was included based on positive serum anti-SARS-CoV-2 IgG; close exposure to several household members with confirmed COVID-19; and recent respiratory illness consistent with COVID-19. **All subjects were clinically diagnosed by nasopharyngeal swab at the time of presentation. The provided cycle threshold values (CT) were derived from NP PCRs performed at the time of lumbar puncture. [s]In two cases, CT values were obtained from saliva ‡bolded proteins were detected by PhIP-Seq and IP-MS. Ro52 was detected in the serum of Case 7 during the course of his clinical evaluation for new-onset neuropsychiatric impairment.

Table S2. Control Characteristics. Related to STAR Methods *Human Subjects*
**pre-pandemic control

Control ID	***C1***	***C2***	***C3***	***C4***	***C5***	***C6***	***C7***	***C8***	***C9***	***C10***	***C11***	***C12***	***C13***
Age/Gender	75M	54M	54M	58M	45F	49M	23M	32F	49M	52M	33M	52M	56M
Neurologic Symptoms	Lower Extremity polyneuropathy	No	No	No	No	No	No	No	No	No	No	No	No
Comorbidities	Alcohol use disorder	None	None	None	None	None	None	None	None	None	None	None	None
CSF Profile:													
WBC (cell/uL)	12	2	1	0	3	1	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,
Protein (mg/dL)	40,7	37	48,7	43,5	38,3	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,
OCBs	0	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,
SARS-CoV-2:													
PCR: NP/CSF	-/N,D,	-/N,D,	-/N,D,	N,D,/N,D,	N,D,/N,D,	N,D,/N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,
IgG: Plasma/CSF	-/-	-/-	-/-	-/-	-/-	-/-	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,	N,D,
Used as control for:													
Immunostaining	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO
IP-MS	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
PhIP-Seq	YES	YES	YES	YES	YES	YES	NO	YES	YES	NO	NO	NO	NO

N,D, = Not Done