Introducing the significance proportion of patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) display acute neurological and psychiatric symptoms [1], and a subset displays widespread disruptions to micro-structural and functional brain integrity in the recovery stages [2]. Several studies have also reported increased incidence of neurological or psychiatric symptoms after a COVID-19 infection [3–5]. Long-lasting neuro-behavioral and cognitive impairments are then common features of other neuro-invasive RNA viruses, with the CNS-damaging processes also continuing after virus elimination [6]. So far, the mechanisms causing such neurocognitive sequelae are poorly understood, but recent studies in rodents suggest that interferon-responses in microglia, the innate immune cells of the brain parenchyma, can lead to excessive microglial synapse elimination and disruption of neuronal circuit integrity [7, 8]. To some extent, this could explain why the developmental period of infection influences the extent of neurocognitive sequelae [9], as microglial functions involving circuit refinement exhibit a clear temporal pattern during neurodevelopment.

Human brain organoids have been successfully employed to study neurotropism and neurotoxic effects of viruses such as the Zika virus [10, 11], and now more recently SARS-CoV-2 [12–16]. However, as microglia are of non-ectodermal origin [17, 18], brain organoids typically lack developing microglia. In this study, we address this limitation by using a newly established protocol for generating undirected brain organoids with innately developing microglia [19], and study cellular responses to SARS-CoV-2 by using single cell RNA sequencing (scRNA-seq) combined with functional characterizations.

Materials and Methods

Ethics

All individuals signed a written informed consent before participating in the study, as approved by the Institutional Review Board of Partners HealthCare (Boston, MA, USA) and the Regional Ethical Review Boards in Stockholm, Sweden.

iPSC reprogramming and brain organoid cultures

Two healthy human iPSC lines (males) were used and iPSC mRNA reprogramming was performed as described previously [20]. Undirected brain organoids were prepared from single cell suspension of human iPSCs as previously described [19] but with some modifications (Supplementary Methods).

Induced microglia-like cells

iMGs were derived from monocytes (donated from one healthy male) using established methods previously described in detail [20, 21].

Virus isolate, infections, and plaque-forming unit assay

We used original live SARS-CoV-2 (GenBank: MT093571.1). 3 × 10^5 PFU/ml of SARS-CoV-2 was used to infect organoids for 2 h (the estimated MOI was...
of infected cells either have an inefficient assembly and shedding of viral particles, or that cell death decreases the availability of viable cells to sustain the production of fully assembled viral particles.

Based on previous literature [12–16], we decided to fix infected and mock-treated organoids (56 DIV) for immunohistochemical (IHC) analyses at two-time points (24 and 72hpi). At 24hpi, a strong signal for the viral nucleocapsid protein (NP) was observed mainly in the periphery of the infected organoids, whereas at 72hpi, we observed NP staining in cellular cytoplasm or processes of infected cells (Fig. 2c; Supplementary Fig. 2b). Cleaved caspase3 (CASP3) staining indicated a pronounced increase in early cell death-related events in infected organoids as compared to mock-treated organoids (Fig. 2d). CASP3" cells were not limited to superficial layers and exceeded the number of NP" cells at both time points (Supplementary Fig. 2b). Given previous reports [7, 8] that other RNA viruses can induce long-lasting interferon-responses in microglia that lead to excessive microglial synapse elimination and disruption of neuronal circuit integrity post infection, we also decided to evaluate synapse density at 72hpi. Infected organoids then displayed a significant decrease in postsynaptic density (PSD-95) as compared to the mock-treated organoids (Fig. 2e, f).

Next, we investigated infectivity and cell death in the context of cellular identity. IHC staining on infected organoids at 72hpi showed viral NP or dsRNA overlapping with PAX6" MAP2", GFAP", SOX10", OLIG2", and Iba1" cells (Fig. 3a–f). Both NP" and CASP3" staining was foremost observed in neurons, but also included other cell types (Fig. 3g–i and Supplementary Fig. 2c, d). Additionally, we enriched microglial cells from two infected organoids. Consistent with our previous microglial characterization (IHC), qRT-PCR on cell lysates suggested the capture of more immature microglia (Supplementary Fig. 2e) and included viral N gene copies (Fig. 3j). To exclude those microglia that only stained positive for viral RNA (dsRNA, N gene), and/or CASP3, due to engulfment of infected cells within the organoid, we derived induced human microglia-like cells (iMGs) in 2D-culture [20] from one donor and exposed these cells to live SARS-CoV-2 virus (using a lower MOI of 0.01 given monoculture). The proportion of IBA1" cells positive for dsRNA and CASP3 then increased with time (Supplementary Fig. 2f, g).

Increased microglial engulfment of postsynaptic termini in infected organoids

To determine if SARS-CoV-2 has the capability to induce microglial phagocytosis of synaptic structures and contribute to the observed decrease in postsynaptic density observed in infected organoids, we first investigated to what extent microglia contributed to synapse remodeling in mock-treated organoids. In line with our previous findings in 2D models [20], this revealed a baseline uptake of postsynaptic structures (indicated by PSD-95) in microglia residing in brain organoids (Fig. 4a–c). In infected organoids (MOI 0.3, 72hpi), the total numbers of Iba1" cells were slightly increased but did not reach significance (Supplementary Fig. 3a) while we observed a clear increase in CD68" cells (Supplementary Fig. 3b) and a threefold increase in uptake of synaptic structures in microglia (Fig. 4d, e). After viral exposure, microglia cells also displayed a subtle but significant structural change that indicated retraction of fine processes and a transformation to a less ramified morphology (Supplementary Fig. 3c–g).

Single-cell characterization of brain organoids containing resident immune cells

Droplet-based encapsulation of single cells allowed us to profile and compare transcriptomes of individual cells isolated from SARS-CoV-2 infected brain organoids as compared to mock-treated organoids. Given the extent of cell death in the organoids...
exposed to an estimated MOI of 0.3, we then decreased the MOI to 0.1, and to also capture more mature cells we used organoids cultured up to 130 DIV and enriched for CD11b+ cells (MACS). In this way we were able to obtain good-quality transcriptomic data from fresh cells isolated in three experimental conditions: mock-treated organoids (7257 cells), 24hpi organoids (15254 cells), and 72hpi organoids (2825 cells), respectively (Supplementary Fig. 4a–d). Then, we integrated pre-processed data from each condition and performed unsupervised graph-based clustering to obtain cellular clusters with similar transcriptomic identities across conditions (Fig. 5a, b, Supplementary Fig. 4e–i). Supervised inspection of top differentially expressed genes (DEGs) per cluster combined with cell type-specific gene signatures and cluster correlations to other developing human brain [23–29], as well as organoid [30–32], datasets confirmed 16 clusters (Fig. 5c, d, Supplementary Table 1, Supplementary Fig. 5 and Supplementary Fig. 6a–e). To address if organoid-grown microglia at 130 DIV more resembled adult or fetal primary microglia, we performed integration of single-cell RNA sequencing data from our microglia cluster with two primary fetal microglia datasets [23, 27], as well as a primary adult dataset [28], and concluded that the overall transcriptomic profile was closest to fetal microglia (Supplementary Fig. 6f).

We then assessed the expression of previously identified entry factors for SARS-CoV-2 and observed basal RNA expression for most factors although the relative expression was rather low (Supplementary Fig. 6g–i, and Supplementary Results for further details). Next, we identified infected cells by aligning cellular viral transcripts to the whole SARS-CoV-2 genome in the infected conditions. The percentage of infected cells was low (0.1–0.2% of sequenced cells), which was expected given the lower MOI and to some extent the removal of non-viable infected cells, although we were able to qualitatively identify infected neurons, radial glia, astrocytes, and choroid plexus cell types (Supplementary Table 2). Amongst infected cells, we observed a preferential infection of neurons (p = 0.014) as well as radial glia (p = 0.002). For further

Fig. 1 Cellular composition of DIV 56 brain organoids containing innately developing microglia. a Schematic of brain organoid generation using an undirected protocol adapted from Ormel et al. [19], b–e Representative confocal images (40×) of different cell type markers in organoid cryosections showing the presence of NPCs (PAX6), immature and mature neurons (Beta III Tubulin and MAP2), neural crest cells (SOX10), astrocytic lineage cells (GFAP, S100B) and f microglia (IBA1, CD68). Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). Scale bar for representative images in the panel: 20 μM.
downstream analyses, we then did not make any distinction between infected and non-infected cells, instead focusing on overall cellular responses in the infected conditions.

**Interferon-responsive microglia with upregulation of genes promoting synapse elimination**

To define microglial responses to SARS-CoV-2 exposure, we first performed pseudo-bulk differential gene expression testing across the three experimental conditions. An unbiased hierarchical clustering approach generated two unique groups of DEGs (labeled modules A and B). Module A comprised of genes whose expression on average decreased at 24hpi and then at 72hpi displayed a more pronounced increase, while module B genes on average displayed reduced expression at 24hpi and a more dramatic decrease at 72hpi (Fig. 6a). While module B was largely comprised of genes encoding for cytosolic ribosomal proteins (Supplementary Fig. 7a, b), we detected several interferon-stimulated genes (ISGs) in module A (Fig. 6b, Supplementary Table 3). Accordingly, IHC validation of ISG15 protein showed a general increase in interferon response in organoids at 72hpi as well as a specific upregulation in IBA1+ cells, compared to control mock organoids (Fig. 6c, Supplementary Fig. 7c). Nonetheless, we detected no clear upregulation of pro-inflammatory cytokines in microglia or microglial subclusters (Supplementary Fig. 7d, e), at either 24hpi or at 72hpi, although several pathogen sensors and upstream effectors of interferon signaling were upregulated.
including nuclear factor kappa B (NFKB1) (Fig. 6d). At 24hpi, we detected upregulation of pathways (Supplementary Table 4) that can serve as activating stimuli for primed inflamasome components, and are required for the production and maturation of pro-inflammatory cytokines (Fig. 6d) [33]. Taken together, this suggests that SARS-CoV-2 activates interferon signaling in microglia although the expression of pro-inflammatory cytokines are at normal levels already at 24hpi.

In the infected conditions, unbiased analyses revealed a significant upregulation of pathways related to neurodegenerative diseases, including Alzheimer’s disease (AD) and Parkinson disease (PD) (Supplementary Fig. 7f). Notably, both these disorders are
characterized by a microglia-mediated early synapse loss [34], and epidemiological register studies have indicated an increased risk of dementia and Parkinsonism after COVID-19 infection [4]. This led us to examine the microglial transcriptional state more closely after exposure to SARS-CoV-2 in relation to previously described disease-associated microglial activation states. First, we compared SARS-CoV-2-exposed microglia to such activation states observed in experimental murine models. Injury-responsive microglia (IRM) have been described in mice and are to a large extent defined by upregulation of ISGs [35, 36], while the disease-associated microglia (DAM) described in mouse models of AD and ALS are thought to result in neurodegeneration and disease development [37]. SARS-CoV-2-exposed microglia at 72hpi (although not at 24hpi; data not shown) then displayed a significant enrichment for genes associated with the IRM activation state (p = 6.9 x 10^{-24}; Fig. 6e, Supplementary Fig. 7g), and less so for the unique DAM signature genes (p = 0.11), and the significant IRM-intersecting signature enriched for genes found in the “Coronavirus disease” pathway (Supplementary Fig. 7g). However, microglia displayed upregulation of genes implicated in AD with a non-significant decrease of core microglial homeostasis genes (e.g., P2RY12 and C3CR1; Supplementary Fig. 7h). In fact, several of these genes were observed to be commonly dysregulated across all three signatures (IRM, DAM and SARS-CoV-2 exposed microglia), suggesting unique as well as shared activation mechanisms across these microglial states. Further, we compared the SARS-CoV-2 microglial transcriptomic signature to previously identified human microglial populations identified in autopsy and surgical brain tissues obtained in the Memory and Aging Project (MAP) [38], and found an enrichment with genes in so-called interferon responsive microglia (cluster 4 in this dataset) (Fig. 6f), defined by ISG15 and with increased expression of multiple sclerosis (MS) and AD susceptibility genes. To exclude effects of tissue processing, we also compared microglia from the organoids with a cluster defined by general cellular distress due to tissue processing (cluster 3) but observed no enrichment (Fig. 6f).

Actin-cytoskeletal remodeling pathways, essential for promoting migration and phagocytosis [39], were also upregulated in microglia exposed to SARS-CoV-2 (Fig. 6g, h, Supplementary Fig. 7f). Consistent with this upregulation in microglia, and the extensive initiation of neuronal apoptosis and decreased post-synaptic density in infected organoids, neurons at 72hpi downregulated the expression of ‘don’t-eat-me’ signals such as CD46 and CD200 (Supplementary Fig. 7i) [40]. Consequently, we observed increased microglial expression of genes associated with microglia-mediated phagocytosis [41–44], such as CD68 (in line with the observed increase of CD68^+ cells), TREM2, ITGB5, CD47, MSR1, CALR, as well as genes previously identified to be directly involved in synapse elimination in the aftermath of viral encephalitis, such as C3, C3AR1 and FCGR3A [6] (Fig. 6g).

**Astrocytic subclusters with enrichment for genes implicated in neurodegenerative diseases**

In response to SARS-CoV-2, astrocytes displayed DEGs enriched for mechanisms involved in cell cycle, carbon metabolism and disorders such as Huntington’s disease (HD) and PD (Fig. 7a). Already at 24hpi, three subclusters of astrocytes could be identified: AS-0, AS-3 and AS-4 (Fig. 7b, Supplementary Fig. 8a, b). AS-0 cells expressed relatively higher levels of anti-viral ISGs, while AS-3 and AS-4 cells had a proliferative profile and exhibited lower expression levels of GFAP (Fig. 7b, c, Supplementary Fig. 8c). Subcluster AS-2, comprising of cells sampled at 72hpi, showed elevated levels of GFAP and STAT3, indicative of reactive astrogliosis [45], along with phagocytosis related genes such as MEGF8 and ABCA1 (Fig. 7c, Supplementary Fig. 8d). We also observed upregulation of metal ion (zinc, copper, iron) homeostasis pathways involving metallothioneins in AS-2 astrocytes at 72hpi (Fig. 7c). Notably, proliferative reactive astrocytes (AS-3 and AS-4) appeared mainly at 24hpi but were largely replaced with non-proliferative reactive astrocytes at 72hpi, then in line with the microglial signature suggesting an early switch to a more chronic reactive state [46].

In neurodegenerative diseases, astrocytes have been shown to express markers of a putative reactive neurotoxic state commonly referred to as A1 [46]. We then integrated scRNA-seq data from A1 astrocytes (stimulated with IL-1A, TNF-α, and C1q) [47], and SARS-CoV-2 exposed astrocytes via canonical correlation analysis (CCA). Although the majority of SARS-CoV-2 exposed astrocytes (AS-0, AS-1, AS-2) clustered separately from A1 astrocytes, the proliferative clusters (AS-3 and AS-4), as well as a subset of AS-0 astrocytes, showed clustering similarities with a portion of A1 astrocytes (Fig. 7d, Supplementary Fig. 8e, f). This is in line with a decreased phagocytic capacity of A1 astrocytes [48], and an upregulation of phagocytosis-related genes in the AS-2 cluster. Furthermore, higher expression levels of metallothioneins, as in AS-2 cluster (72hpi), have been observed in astrocytes from HD patients [49].

**Secretome alterations indicate a compromised capacity to keep BBB integrity**

Potential entry routes to the CNS for SARS-CoV-2 include the blood–brain barrier (BBB) or the blood–cerebrospinal–fluid-barrier, involve cell types such as endothelial cells, pericytes, astrocytes and choroid plexus-epithelium. Upon encountering pathogens, these barrier cells act in concert to activate and regulate signal transduction pathways that aid invasion of peripheral immune cells and restoring of CNS homeostasis [50]. Correspondingly, at 24hpi we found increased ligand-receptor communication between astrocytes, neurons, microglia, perivascular, and endothelial cells, whereas at 72hpi, both microglia and neurons significantly reduced their communication with most other cell types, while choroid plexus cell types gained interactions (Fig. 7e). Sub-clustering of choroid plexus related cells (Fig. 7f, Supplementary Fig. 9a, b) showed two control enriched clusters (CP-0 and CP-2), consisting of genes involved in maintaining solute homeostasis, barrier, or extracellular matrix (ECM) integrity, and amyloid clearan amongst others (Supplementary Fig. 9c). At 24hpi, we observed an enrichment of metallothioneins, similar to what we observed in astrocytes at 72hpi, whereas genes involved in viral defense response, regulation of reactive oxygen species, and ECM
organization, were enriched at 72hpi (CP-5) (Supplementary Fig. 9c). In addition to choroid plexus, a differential expression analysis showed significant increase in expression of matrix metalloproteases and ECM-regulatory enzymes at 72hpi in microglia, astrocytes and perivascular cells, whereas endothelial cells reduced expression levels of solute carrier SLC2A1 (Fig. 7g), all in agreement with signaling that promotes compromised CNS barriers. Accordingly, by IHC we validated increased MMP14 protein expression by PDGFRB+ (pericytes) as well as GFAP+ cells within 72hpi organoids (Fig. 7h, i). Further, astrocytes, pericytes
and choroid plexus-related cells were observed to downregulate VEGF signaling, a potent inducer of BBB permeability, at 24hpi but then at 72hpi instead display an upregulation, coinciding with increased signaling for leukocyte chemotaxis and activation in astrocytes, as well as antigen processing and presentation in choroid plexus (Fig. 7g).

Metabolic dysregulation across cell types

Protein accumulation in the endoplasmic reticulum (ER) following viral infection or excessive production of secretory proteins can induce ER stress and initiate countermeasures in the form of reduced translation and export to ER along with unfolded protein responses (UPRs) [51]. In exposed organoids, we detected an upregulation of pathways related to UPRs, ER stress, proteasomal degradation, and autophagy, among cell types such as microglia, astrocytes, choroid plexus, and perivascular cells. Further, a partial translational inhibition could be observed in microglia (module B), as well as most of the other cell types. We also observed downregulation of the ROS metabolism across cell types, which can lead to dysregulation of the antioxidant cellular systems and result in oxidative stress [52]. See also Supplementary Results.

Fig. 5  Single-cell transcriptome profiling of brain organoids with innately developing microglia. DIV 130 brain organoids (n = 3, MOI 0.1) per experimental condition (Mock-treated, 24 and 72hpi) were dissociated into whole-cell suspensions and single-cell RNA sequencing libraries were generated using the droplet-based 10X chromium platform. a UMAP plot of integrated dataset containing 25336 single cells from all three conditions depicting the presence of key neurodevelopmental cell types. Individual dots representing single cells are colored by the identified cell type. b UMAP plot of overall embedding of cells colored by the experimental condition (top) and cell cycle phase (bottom) c Heatmap showing expression of top-differentially expressed markers (rows) across all clusters (columns) with a two-sided Benjamini–Hochberg corrected p value < 0.05. Colored column bars (right) highlight the identified cellular groups. A list of all differentially expressed genes conserved across conditions for each cluster is provided in Supplementary Table 1. d Cell type classification obtained by scoring cells by their expression of known individual cell type signatures (Supplementary table 1) plotted as estimated joint density on a UMAP.
Expression of SARS-CoV-2 entry factors in brain organoids and microglial responses to SARS-CoV-2. 

a. Modules of genes with similar expression behavior across conditions in microglia identified by pseudo-bulk differential gene expression analysis (using LRT implemented in DESeq2).

b. Protein-protein interaction network of genes belonging to module A using STRING database with the top Gene Ontology term identified as ‘Type I interferon response’ (two-sided Benjamini-Hochberg corrected p-value < 0.05).

c. Confocal images (40×) validating ISG15 expression in control and 72hpi organoid at DIV 130. Quantifications provided in supplementary Fig. 6c. Scale bars for representative images: 20μM.

d. Dot plot showing expression levels of neuro-immune related genes such as cytokines & chemokines, interferon-stimulated genes, and their upstream effector molecules across infected conditions for each cell type [C- mock-treated; 24–24 hpi; 72–72 hpi]. Color scale represents average log-scaled expression values across all cells, in each cluster. Size of the dot represents the percentage of cells in each cluster, expressing the gene.

e. Venn diagram of genes overlapping between gene signatures of Disease-associated microglia (DAM), Injury-associated microglia (IRM), and SARS-CoV-2 infected microglia at 72hpi (* two-sided Benjamini-Hochberg corrected p-value < 0.005). No significant overlap was observed at 24hpi.

f. Heatmap of comparisons between SARS-CoV-2-associated microglia at 24hpi and 72hpi along with 9 defined human microglial clusters [38], colored by odds ratio (with two-sided Bonferroni corrected p-values shown in red) from hypergeometric gene overlap testing (Fisher’s exact test). Transcriptomic gene signatures were defined as differentially upregulated genes with log2FC > 0.25 and FDR < 5%.

g. Violin plot showing normalized expression of microglial genes associated with active phagocytic states across conditions.

h. Heatmap showing gene set enrichment analyses (MSigDB GO:BP gene sets) of differentially expressed genes (DEGs) from 24hpi versus mock-treated (left) and 72hpi versus mock-treated (right) for each cell type. Pathways commonly dysregulated across most cell types were selected. All significantly altered pathways for individual cell types are listed in Supplementary Table 4 (FDR < 5%; Benjamini–Hochberg correction, NES= Normalized Enrichment Score).
sequelae overlap with post-infection syndromes described in patients that have recovered from infection with more uncommon neuro-invasive RNA viruses [6]. In murine models for encephalitis caused by such viruses, CNS damage continues after virus elimination [6], and can be largely attributed to interferon-responsive microglia that excessively eliminate synaptic termini as well as induce neuronal apoptosis [7, 8].

Here we infect brain organoids containing developing microglia with live SARS-CoV-2 virus and observe an increase in early cell-death related events. Further, we observe a striking reduction in post-synaptic density and show that microglia in the infected organoids increase the engulfment of postsynaptic termini with a concomitant upregulation of interferon-responsive genes as well as genes promoting phagocytosis and synapse elimination, while neurons down-regulate “don’t-eat-me” signals. While several postmortem studies have observed neuronal cell death [53], none have, to the best of our knowledge, evaluated synapse density and microglial synapse engulfment. However, a recent longitudinal and observational brain imaging study, reported a reduction of grey matter thickness also in non-hospitalized patients post COVID-19 [54], then suggesting that Covid-19 infection causes a reduction in synapse density.

Single-cell transcriptomics revealed that the glial gene signatures in infected condition early adopt a transcriptomic profile overlapping with those observed in neurodegenerative conditions. In line with this, a recent study also observed ApoE-isiform-dependent decreases in neurite length and synaptic loss in SARS-CoV-2 infected neuron-astrocyte co-cultures [55], while gene expression analyses of postmortem material also indicate a similar immune activation overlapping with profiles observed in neurodegenerative disorders [56]. Accordingly, these disorders are characterized by an early synapse loss [34], and the incidence of
symptoms overlapping with these disorders is increased after COVID-19 [4]. Similar microglia-mediated synapse elimination has also been observed in schizophrenia models [20], another disorder that displays an increased incident risk after COVID-19 infection [4].

We also observed secretome alterations in astrocytes, pericytes, and choroid plexus-related cells indicating a compromised capacity to keep BBB integrity, as well as an upregulation of pathways related to signaling with peripheral immune cells. Murine models of brain infection with other RNA viruses have revealed a role of infiltrating CD8+ T-cells to activate interferon signaling in microglia [8], then suggesting that a similar mechanism could also exacerbate microglial synapse elimination secondary to SARS-CoV-2 exposure.

Existing protocols for generating brain organoids have several constraints that also limit our interpretations. Brain organoids most closely resemble the developing fetal brain rather than the mature adult brain. Thus, we cannot exclude important differences exist in the cellular responses and tropism between immature and more mature brain cells, as our model primarily recapitulates the responses to SARS-CoV-2 of the developing brain. By only including male lines, as well as original SARS-CoV-2 virus, we cannot exclude that using female lines as well as using other SARS-CoV-2 variants could have influenced our results. That said, to the best of our knowledge, there is currently no data suggesting that CNS-related symptoms differ between sexes or SARS-CoV-2 variants. Using a larger number of lines could also have revealed subject-specific differences regarding infectivity and microglial synapse engulfment. Finally, as our cellular characterizations were performed at later time points, we may have missed functional changes directly related to active viral release.

In summary, we here provide an experimental approach for evaluating viral effects on the brain that includes tightly orchestrated responses of microglia and astrocytes in the context of neuronal circuits. Challenging this model with modest live SARS-CoV-2 virus titers, we observe neuronal cell death and microglia-mediated synapse loss. A key next step will be to determine the clinical importance of such molecular processes, and specifically whether they may contribute to synapse loss and the neurocognitive and neuropsychiatric symptoms observed in a subset of COVID-19 patients across different developmental stages. If so, these microglia-containing organoid models may provide an opportunity to evaluate microglia-targeted therapeutics aimed at minimizing or preventing COVID-19 sequelae.

DATA AVAILABILITY

Processing of data and downstream analysis was performed in R (version 4.0.3). Figures used in this manuscript were generated in Python (version 3.6.12). Raw single-cell RNA sequencing data is deposited into GEO database (GSE181422). Source code to reproduce the findings are available in our repository on Github (https://github.com/SellgrenLab/organoid-Covid19). All other data are available from the corresponding authors upon request.

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ACKNOWLEDGEMENTS
We thank the study participants. We are also grateful to the participant core facilities at Karolinska Institutet; The BioSafety Level (BSL)-3 laboratory at Biomedicum, BIC, and SciLife Lab. We are thankful to Judit Ozsvár, an intern at the Selgren laboratory at the time of the experiments, for her help with cell culture work, as well as to Gretchen Majkwitz for valuable feedback on the manuscript. This work was supported by grants from Hjärnfonden postdoktoral stipendier (S: PS2019-0040, FO: PS2018-0058), the Swedish Society for Medical Research (FO: P18-0120), the Swedish Research Council (C.M.S.: 2017-02559), Karolinska Institutet (C.M.S.: KID), regional agreement on medical training and clinical research between Stockholm County Council (A.L.F., C.M.S.), One Mind Foundation/Kaiser Permanente (C.M.S.), and Marianne and Marcus Wallenberg Foundation (C.M.S.).

AUTHOR CONTRIBUTIONS
CMS, S, AOO, SKG, and SM conceived the project. RHP and SDS provided iPSCs. AOO derived the organoids. NRS and S performed infections, dissociations and related assays, supervised by AR, with help from LS. AOO, S, SM optimized and performed iHCs, imaging and quantifications, with help from JGL. SKG optimized the qPCR assays and performed them with SM. FO derived microglia for 2D assays. SM designed the scRNA-seq experiments with inputs from S and CMS. SM analyzed scRNA-seq data. S and SM interpreted the data. S, SM, MS, and CMS wrote the manuscript with inputs from the other co-authors.

FUNDING
Open access funding provided by Karolinska Institute.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41380-022-01786-2.
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