SARS-CoV-2 Neurotropism and Single Cell Responses in Brain Organoids Containing Innately Developing Microglia

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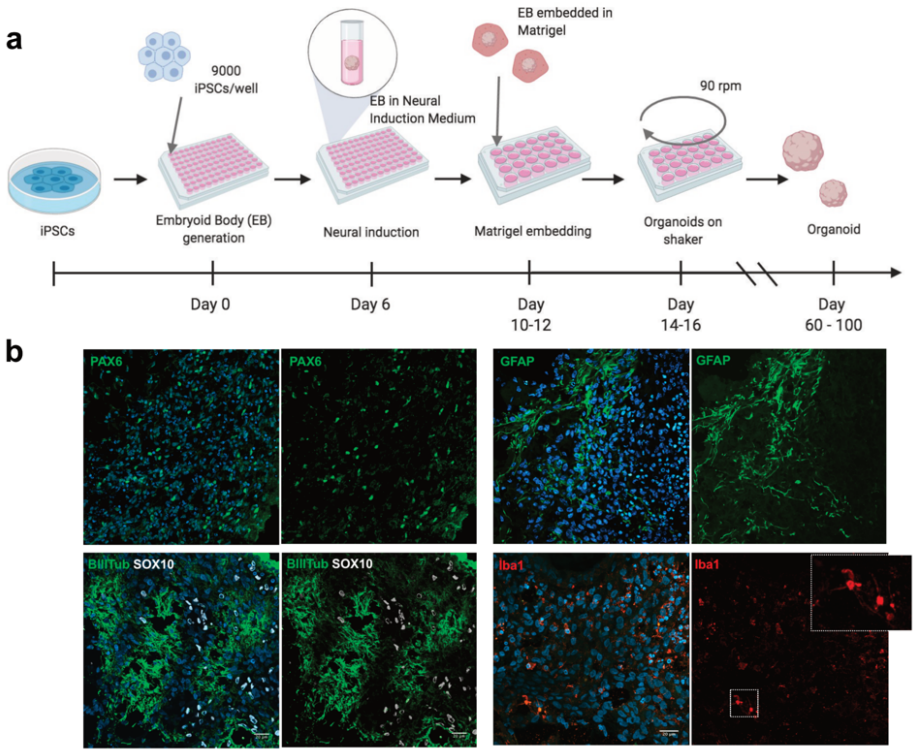
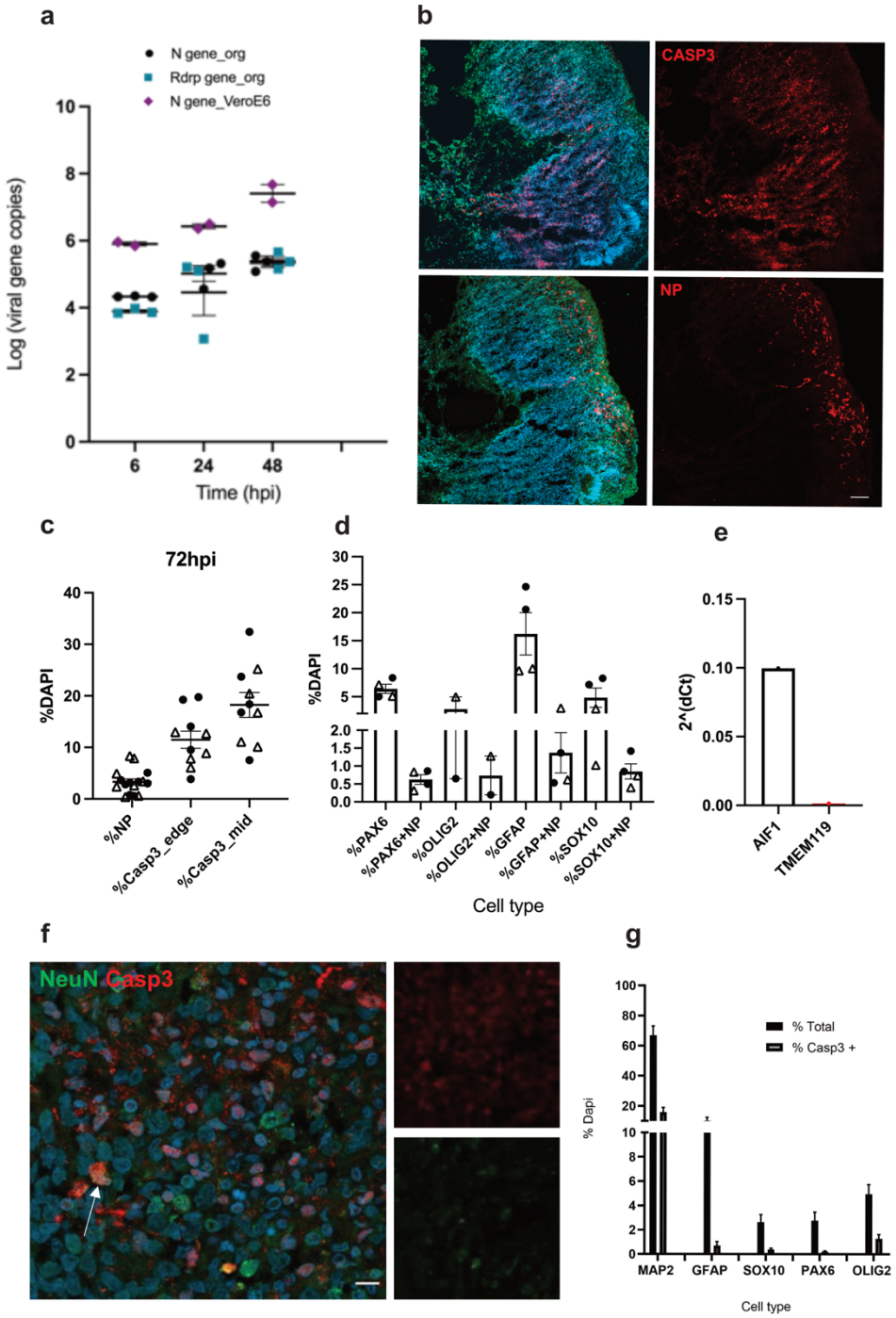
Supplementary Figure 1



Supplementary Figure 1

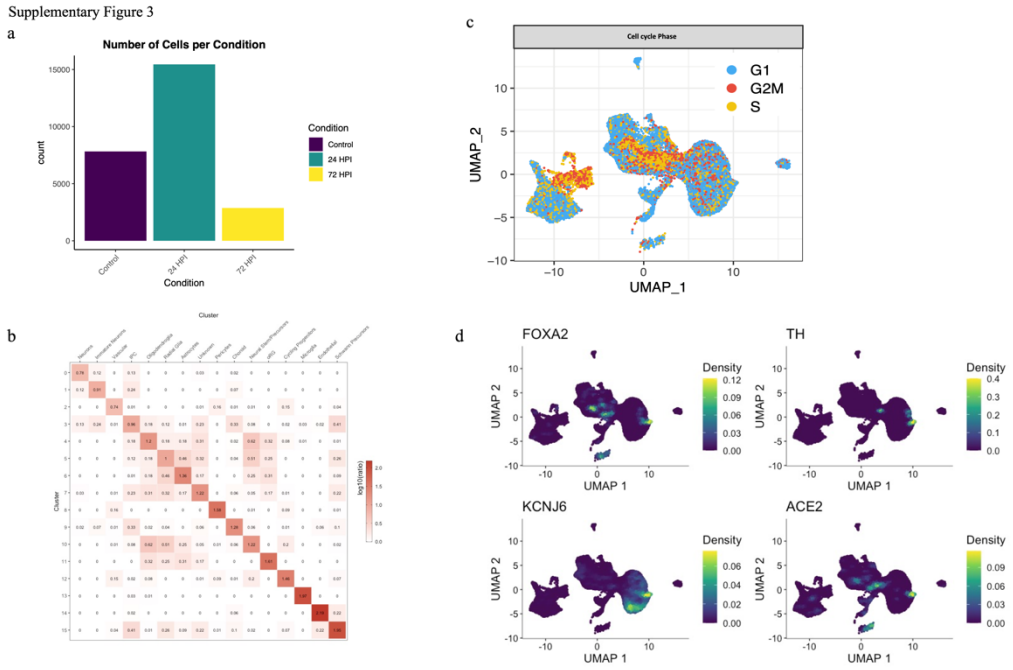
(a) Schematic of cerebral organoid generation using unguided protocol to facilitate development of innate microglia. **(b)** IHC staining of different cell-type markers (PAX6, GFAP, BIII-Tubulin, SOX10 and IBA1) in D66 organoid.



Supplementary Figure 2

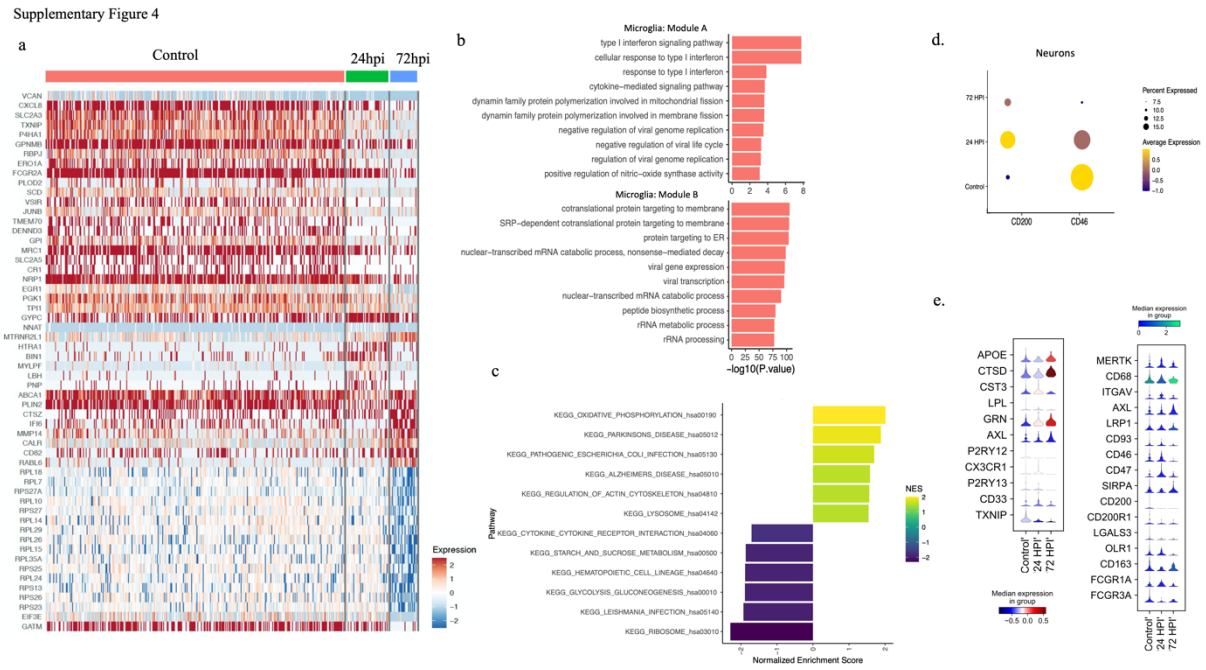
(a) qPCR analysis on supernatants of SARS-CoV-2 infected cerebral organoids at D66 (n=3) showing log fold change in viral transcripts corresponding to nucleocapsid (*N*, black circle) and RNA-dependent RNA polymerase (*Rdrp*, turquoise square) genes. Also shown qPCR analysis on supernatants of infected VeroE6 cells for nucleocapsid (*N*, magenta diamond) gene

(b) IHC staining of viral nucleocapsid protein (NP) and Caspase3 (CASP3) on sequential sections capturing the same organoid area on D66 infected organoid (72hpi). **(c)** NP+ and Casp+ cells expressed as percentage of DAPI from two D66 infected organoids (corresponding data points indicated by circles and triangles). Each data point is an average of 2-3 FOVs per section. Error bars indicate SEM. (**d)** Distribution of NP+ cells amongst different cell-type markers, expressed as percentage of DAPI from two D66 infected organoids (corresponding data points indicated by circles and triangles). Each data point is an average of 4 FOVs representing different areas on the same section. Error bars indicate SEM. (**e)** qRT-PCR on cell lysate fraction obtained following MACS with CD11b beads, showing expression levels of two microglial specific genes (*AIF1*, *TMEM119).* **(f)** 40X confocal images showing colocalization of CASP3 with mature neuronal marker NEUN **(g)** Bar plot showing distribution of CASP3+ cells amongst different cell-type markers, expressed as percentage of DAPI from two D66 infected organoids. Mean represents an average of 4 FOVs representing different areas on the same section. Error bars indicate SEM.



Supplementary Figure 3

(a) Bar plot with final number of cells obtained after quality control from each condition. **(b)** Heatmap displaying cluster similarity by showing the difference between observed and expected edge weights between each cluster pair. **(c)** UMAP plot of integrated dataset from all three conditions colored by its cell cycle phase upon assigning a cell cycle phase to each individual cell (see Methods). **(d)** UMAP plots showing expression of midbrain dopaminergic markers and *ACE2*.



Supplementary Figure 4

(a) Heatmap of differentially expressed genes in microglia upon SARS-CoV-2 infection using MAST (see Methods). **(b)** Bar plots showing top significant pathways enriched for the two modules of genes found in microglia by over-representation analysis (Adjusted P<0.05). **(c)** Top significant upregulated and down-regulated KEGG pathways between SARS-CoV-2 exposed microglia (24hpi+72hpi) versus control microglia obtained by GSEA (Adjusted P<0.05; see Methods). Color bar shows Normalized expression score (NES). **(d)** Dot plot displaying expression of canonical 'don't-eat-me' signals in neuronal clusters across infected conditions. (Adjusted P<0.05). Size of the dot indicated fraction of cells expressing the gene. Scale bar indicated scaled-average expression values. **(e)** Violin plots showing normalized expression of microglial markers associated with disease and homeostasis (left) and active phagocytic states (right) across conditions.