Supplemental information

SARS-CoV-2 infection increases

the gene expression profile

for Alzheimer's disease risk

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Supplementary Methods and Figures

Immune cell profiling with CIBERSORT

To estimate the relative percentage of different immune cell types in COVID-19 brains compared to control, we used CIBERSORTx. CIBERSORTx is an analytical tool that provides an estimation of the abundances of member cell types in a mixed cell population using gene expression data. Mapped read counts for each patient sample were input into CIBERSORT along with the standard "LM22" gene signature file. CIBERSORT output is displayed as fractional proportions of each immune cell sub-population as well as the overall proportion of immune cells within each sample.

Table S1 Differentially expressed genes depicted in figure 1C.

Please see supplementary spreadsheet file for table S1.

Table S2 Fold change and p value of ADRD pathology genes in COVID-19 brains

Gene	log(fold change)	Adj p value
CTCFL	1.239	2.43E-03
CXCL8	1.129	7.31E-03
IFITM3	1.075	1.85E-03
LGALS3	1.071	3.66E-04
IFI16	1.026	5.21E-04
C4A/C4B	0.963	2.36E-02
FKBP5	0.938	1.89E-03
C5AR1	0.913	2.91E-02
IL18	0.864	4.41E-03
GFAP	0.832	1.61E-02
PLAT	0.806	1.39E-02
C4B_2	0.79	4.93E-02
IL6R	0.786	2.52E-02
CAV1	0.733	3.84E-02
KLF4	0.706	4.93E-02
STAT3	0.611	3.89E-02
EGFR	0.521	2.51E-02
HSP90AB1	-0.41	2.68E-02
HSP90AA1	-0.431	2.09E-02
APP	-0.468	4.31E-03
CALB1	-0.572	3.20E-02
HSPA8	-0.579	3.07E-03
CCKBR	-0.77	1.59E-02
BDNF	-0.824	3.14E-02
CAMKK2	-0.858	2.99E-04
TAC1	-1.424	5.14E-07

Table S3 Hub genes identified in protein interaction network of COVID brain samples using STRING database and CytoHubba.

Matching Algorithms	Gene
3	CCL2
3	CXCL8
3	NFKBIA
3	PTPRC
3	STAT1
3	STAT3
3	TLR2
2	APP
2	CAV1
2	CXCL2
2	EGFR
2	EZH2
2	HRAS
2	HSP90AA1
2	HSP90AB1
2	ITGB1
2	LRRK2
2	MYC
2	SMARCA4

Table S4. qPCR Primer Sequences

	Gene	Forward Primer	Reverse primer
1	SARS-CoV-2 N protein	CACATTGGCACCCGCAATC	GAGGAACGAGAAGAGGCTTG
2	IL-6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
3	IL-1β	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
4	TNFα	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
5	CCL20	ATGGCCTGCGGTGGCAAGCGTCTG	TAGGCTGAGGAGGTTCACAGCCCT
6	NLRP3	TCACAACTCGCCCAAGGAGGAA	AAGAGACCACGGCAGAAGCTAG
7	IFITM3	TTCTGCTGCCTGGGCTTCATAG	ACCAAGGTGCTGATGTTCAGGC
8	CR1	ATGAAAGGAGCCAGCAGTGTGC	GGAATCCACTCATCTCCTGAGG
9	FKBP5	GATTGCCGAGATGTGGTGTTCG	GGCTTCTCCAAAACCATAGCGTG
10	C5AR1	CCATTAGTGCCGACCGTTTCCT	CACGAAGGATGGAATGGTGAGG
11	IFI 204	CCAGTCACCAATACTCCACAGC	CTCTGAGTGGAGAACAGCACCT

Table S5. Primary antibodies, corresponding secondary antibodies, and associated information

| Primary antibody	Source	Catalogue number	Dilution	Secondary antibody	Source	Catalogue number	Dilution	Development
Anti-Tau (phospho T231) antibody	Abcam, Cambridge, MA	ab151559	1-100	Biotinylated goat antirabbit/ Alexafluor 488 anti rabbit	Vector Laboratories Inc., / Abcam, Cambridge, MA	BA-1000/ /AB150077	1:400/ 1-1000	DAB/ Fluorescence
Anti-Tau (T22), oligomeric Antibody	Millipoe, Temecula, California	ABN454	1-200	Biotinylated goat antirabbit	Vector Laboratories Inc.,	BA-1000	1:400	DAB
Anti-FKBP5 antibody	R&D systems	AF4094	1-300	Biotinylated swine antigoat	Southern Biotech	Biotech 4050-08	1-500	DAB
Anti-CD31 antibody	Invitrogen	14-0311-82	1-500	Goat Anti-Rat IgG H&L (Alexa Fluor® 594)	Abcam, Cambridge, MA	ab150160	1-1000	Fluorescence
Caspase 1 p20 (Cleaved Asp296)	Thermo Scientific	PA599390	1-250	Alexafluor 594 anti rabbit	Abcam, Cambridge, MA	A32740	1:400/ 1-1000	Fluorescence

Figure S1
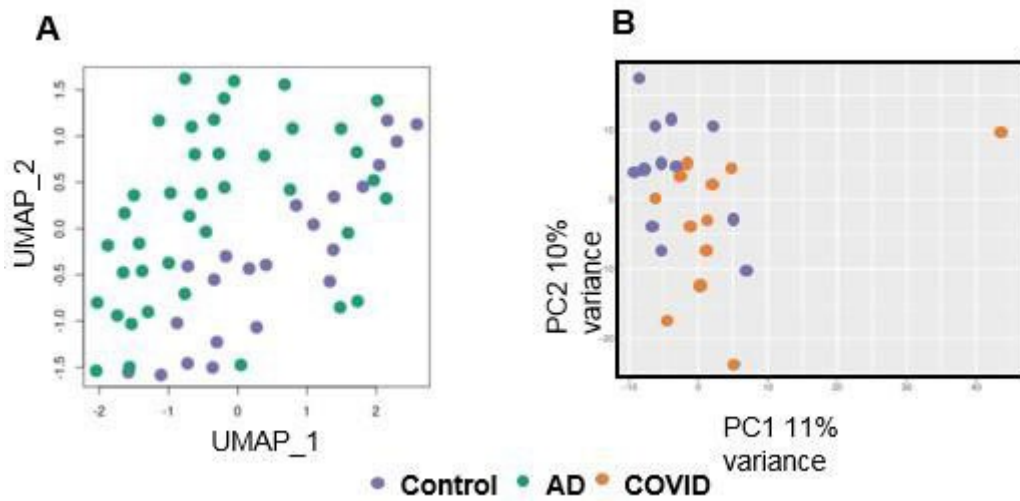
 Figure S1. Dimensionality reduction and clustering of individual patient samples.
A. Uniform Manifold Approximation and Projection (UMAP) clustering of individual AD vs
control sample. **B.** Principle component (PC) clustering of individual COVID-19 vs
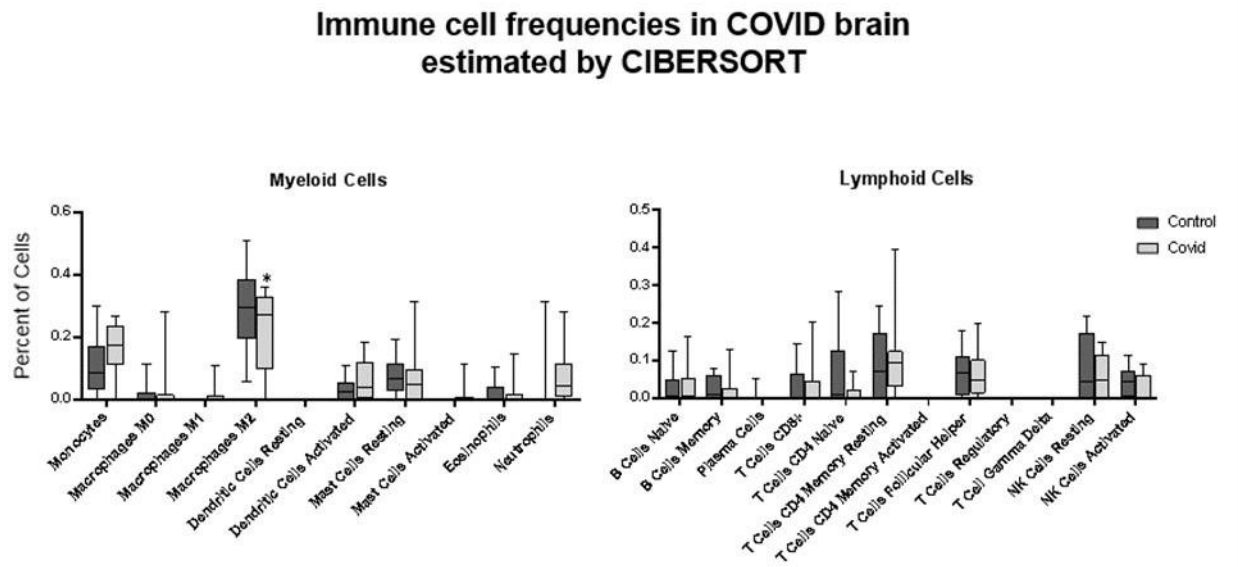control samples.

Figure S2.

Figure S2. Immune cell types present in COVID and control brains as estimated from sequencing data with CIBERSORT.
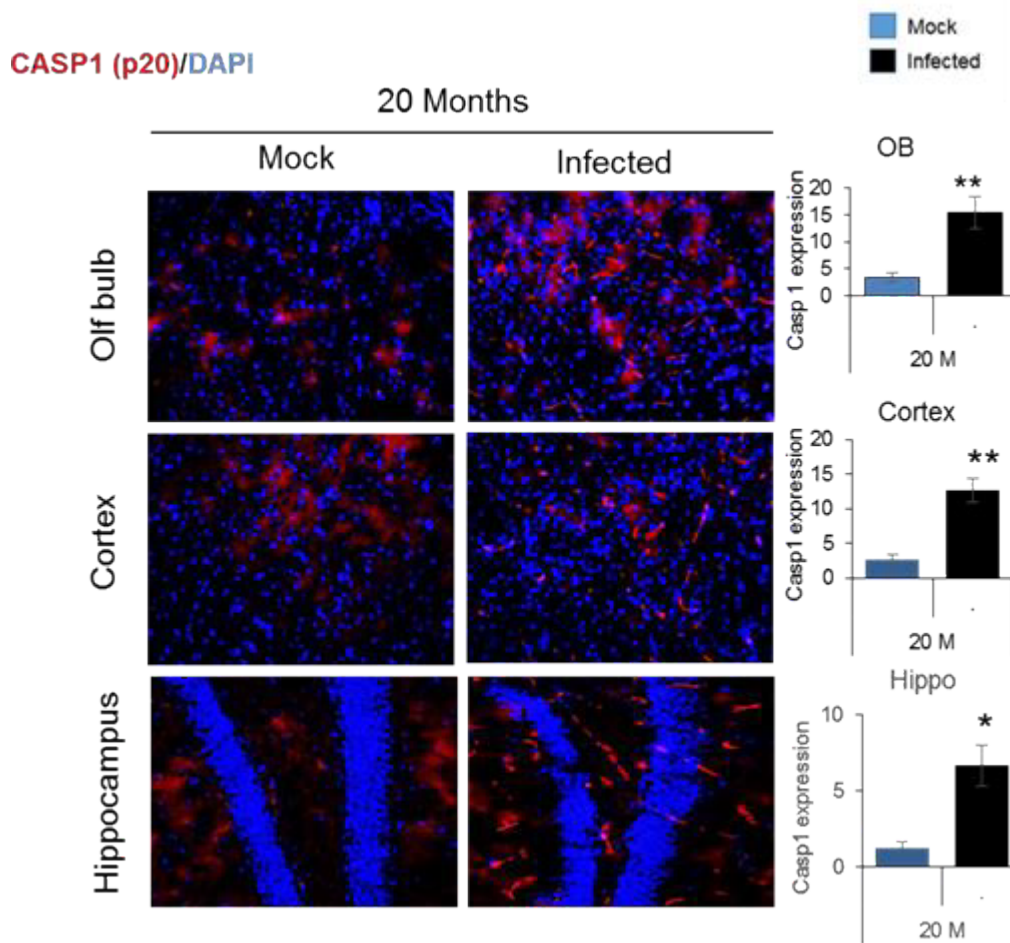
Figure S3

Figure S3. SARS-CoV-2 infection induces Casp1(p20) expression in Aged Mice.
Immunostaining and Histogram showing Caspase1 expression in infected brain –olfactory bulb, cortex , hippocampus, n=3/ group, data expressed as mean ± SEM,* Compared to respective mock, *p<0.05,**p<0.005
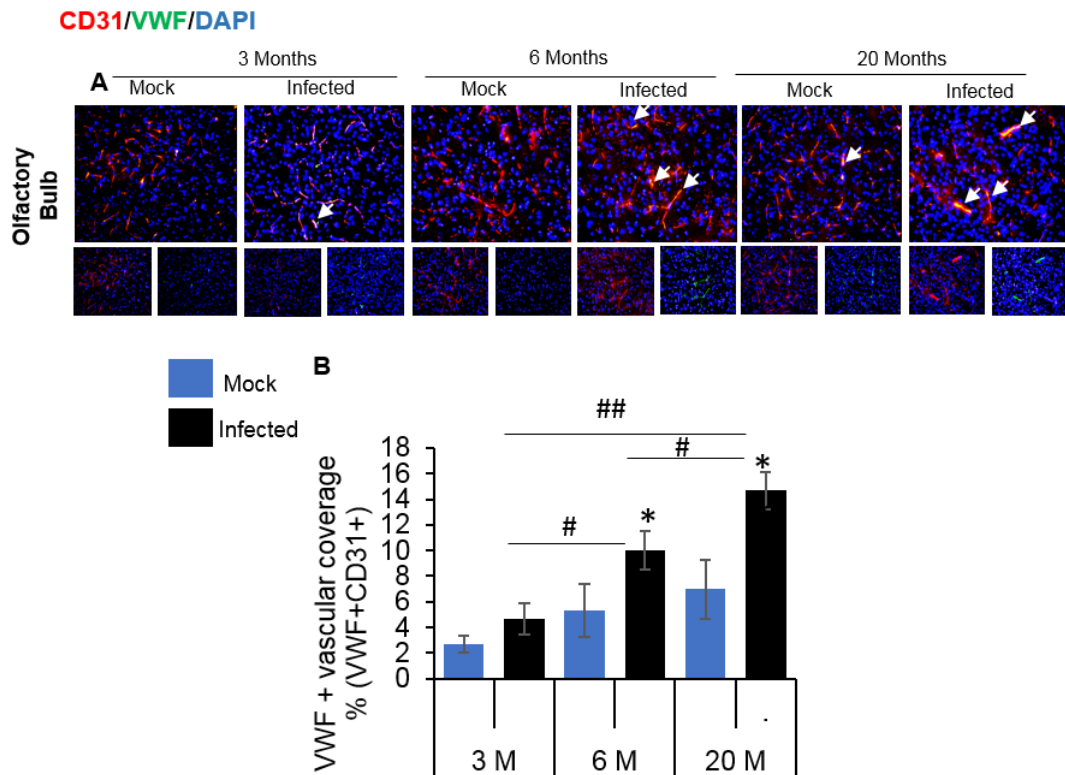
Figure S4

Figure S4. CD31 and VWF staining of MA10 infected mouse brains: C57Bl/6 3, 6 and 20 month old mice were infected with SARS CoV2 MA10 virus- 100k PFU. A. Representative images showing the expression of CD31 (marker for endothelial cells) and VWF (**von Willebrand factor** marker for vascular damage) in olfactory bulb. Upper panel: co localization of CD31 (RFP) and VWF (GFP) depicting vascular damage shown by arrows, lower panel: CD31 (RFP) and VWF (GFP). **B.** Histogram representing the quantification of VWF+ vascular coverage (% VWF expression with respect to CD31 expression). $n = 3$, Data expressed as mean ± SEM, * Compared to control ,*p<0.05, ##, **p<0.005,
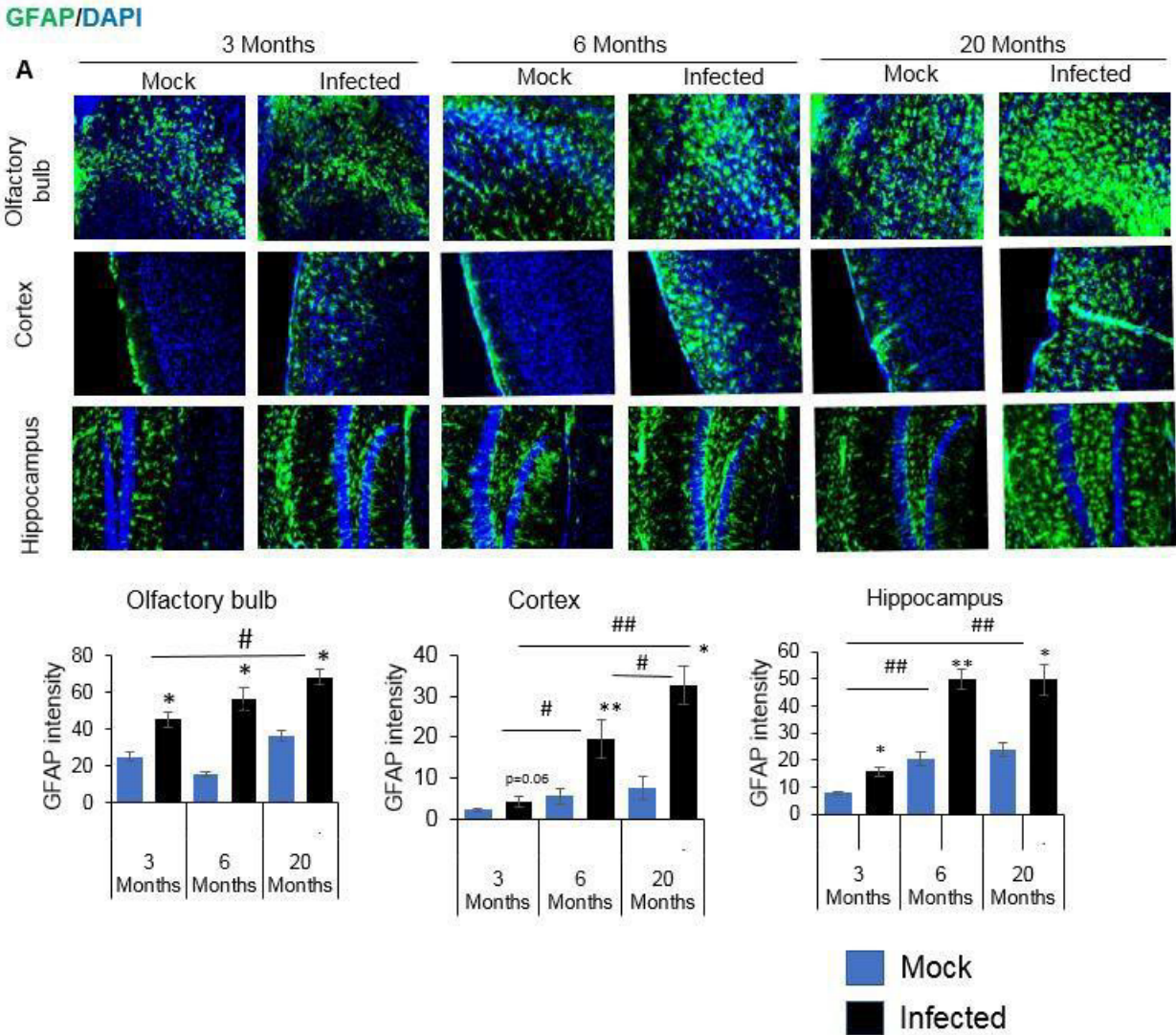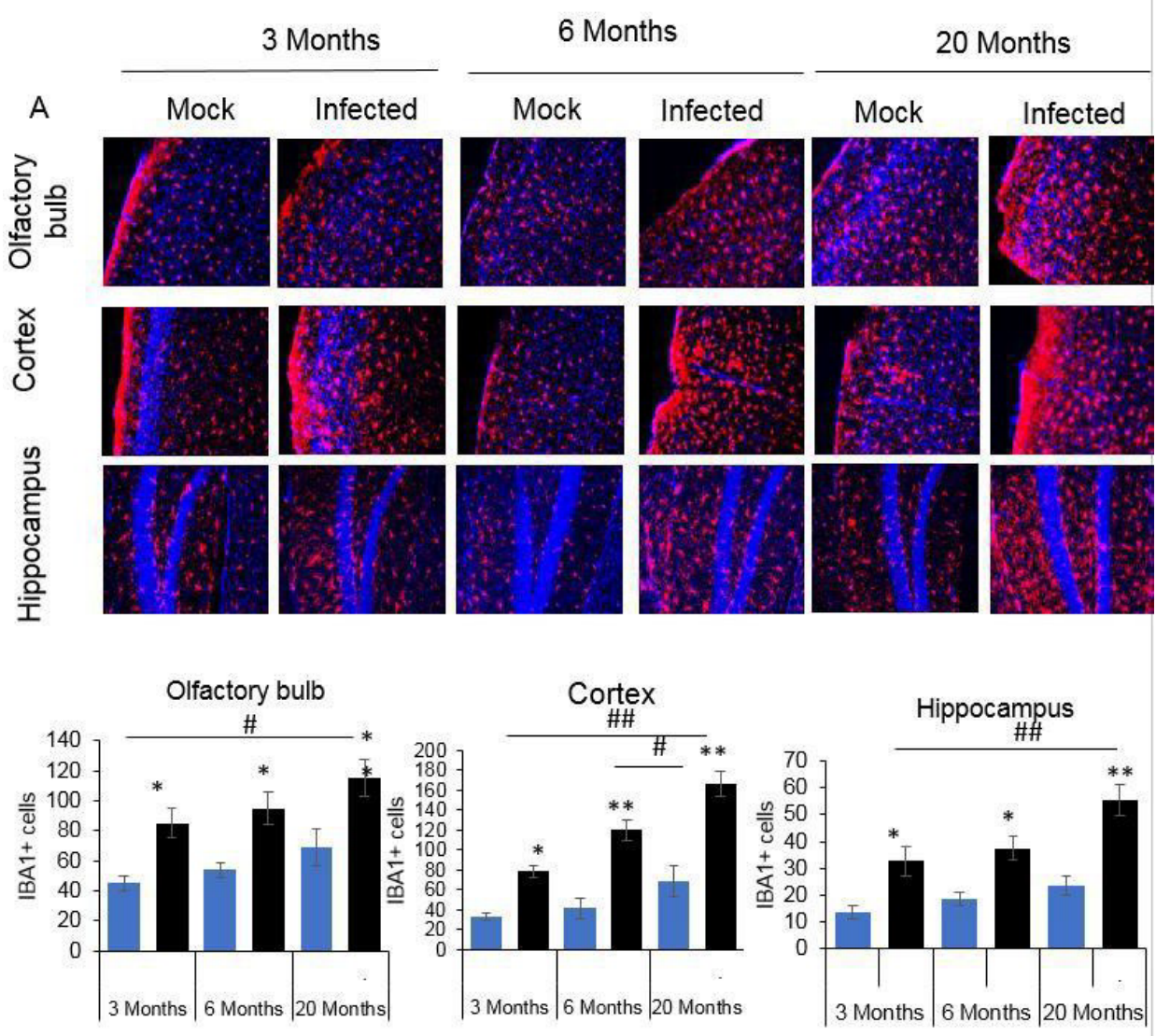
Figure S5

Figure S5. SARS-CoV-2 MA 10 infection induces gliosis in olfactory bulb, cortex and hippocampus of 6 and 20 Months aged mice. A. Immunostaining showing GFAP/DAPI staining in infected brain – olfactory bulb (upper panel), cortex (middle panel) and hippocampus (lower panel) at 18 DPI, B. histogram representing quantification of GFAP immunoreactivity (Intden/unit area), n=3/ group, data expressed as mean ± SEM,* Compared to respective mock, # Compared to infection *,#p<0.05,**,## p<0.005

Figure S6

Figure S6. SARS-CoV-2 MA 10 infection induces gliosis in olfactory bulb, cortex and hippocampus of 6 and 20 Months aged mice. **A.** Immunostaining showing IBA1 (red)/DAPI staining in infected brain – olfactory bulb (upper panel), cortex (middle panel) and hippocampus (lower panel), at 18 DPI, **B.** histogram representing quantification of IBA1+ cells , n=3/ group, data expressed as mean ± SEM,* Compared to respective mock, # Compared to infection *, $p<0.05$,**, ## $p<0.005$,***,
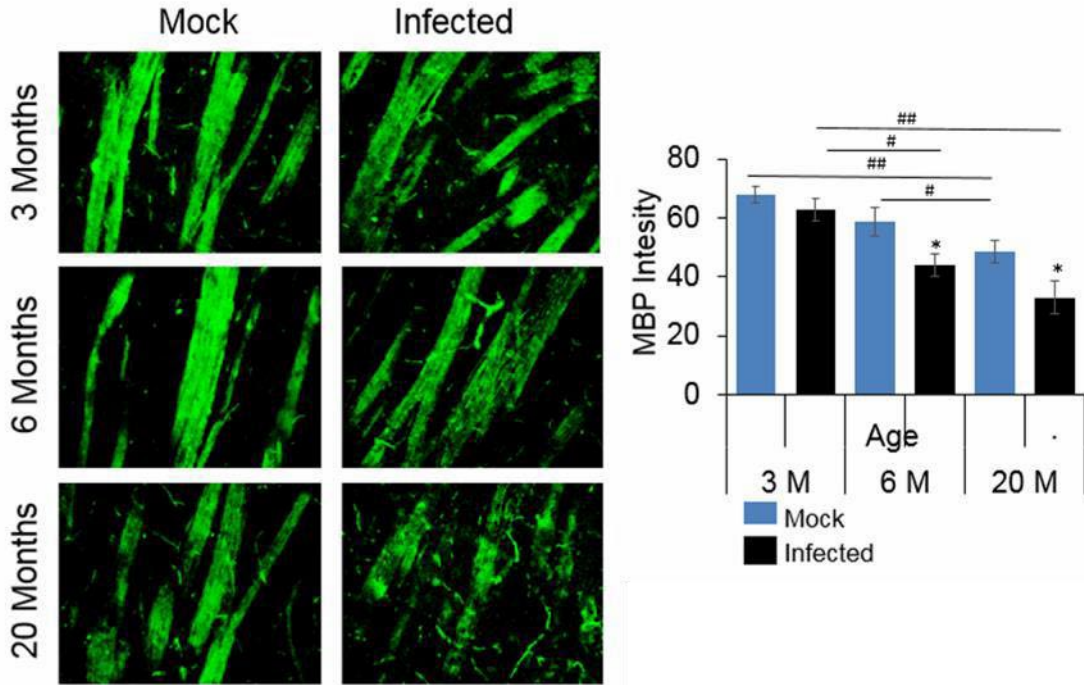
Figure S7

Figure S7. SARS-CoV-2 infection induces demylination in Aged Mice. 3, 6 and 20 months aged C57Bl/6 mice were infected with SARS CoV2 MA10 virus- 100k PFU. The infected mice were sacrificed 18 days post infection. **A.** Immunostaining showing MBP staining in infected brain – Striatum, **B.** quantification of immunostaining (MBP intensity), n=3/ group, data expressed as mean ± SEM,* Compared to respective mock, # Compared to infection *, #p<0.05,**, ## p<0.005
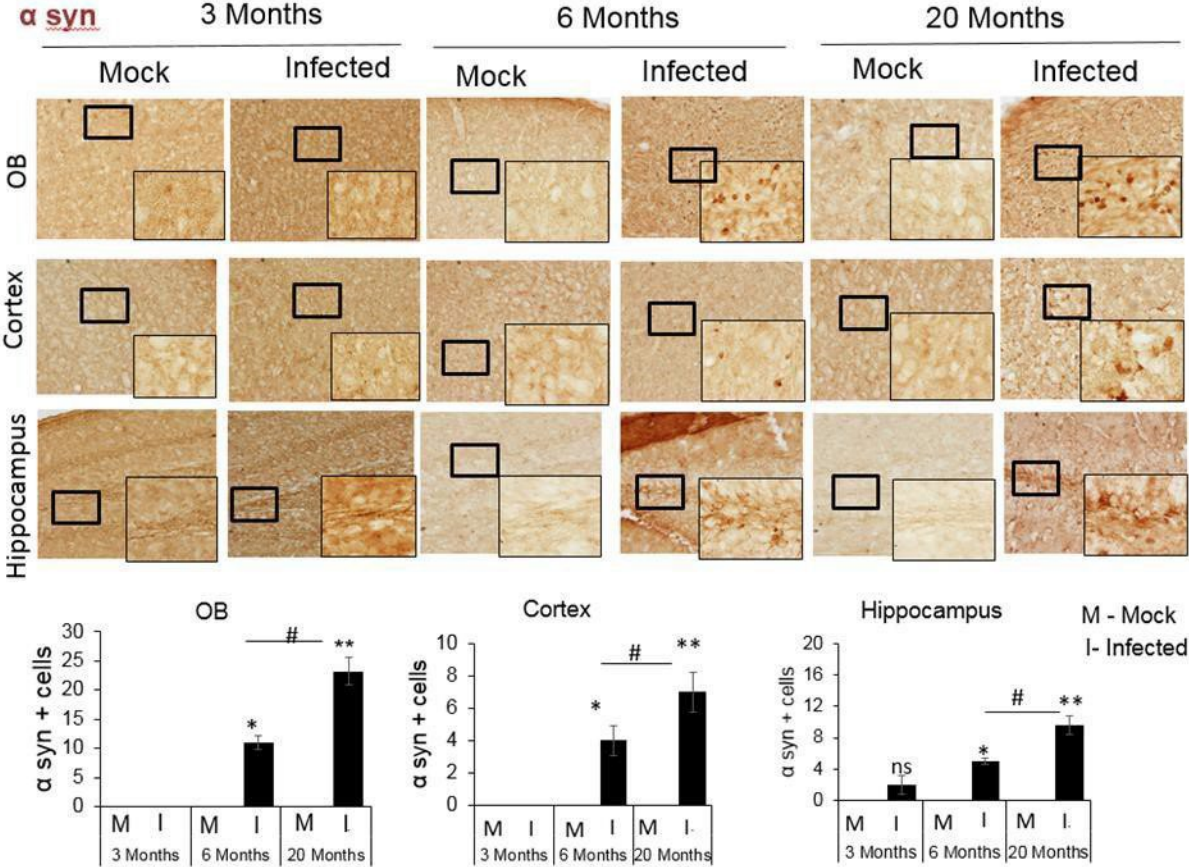
Figure S8

Figure S8. SARS-CoV-2 infection induces α-synuclein expression in Aged Mice.
3, 6 and 20 month old C57Bl/6 mice were infected with SARS CoV2 MA10 virus,
Immunostaining showing aSyn staining in infected brain – olfactory bulb, cortex and
hippocampus, quantification of immunostaining at 18 DPI, n=3/ group, data expressed
as mean ± SEM,* Compared to respective mock, # Compared to infection *, p<0.05