Supplementary Figures and Figure legend

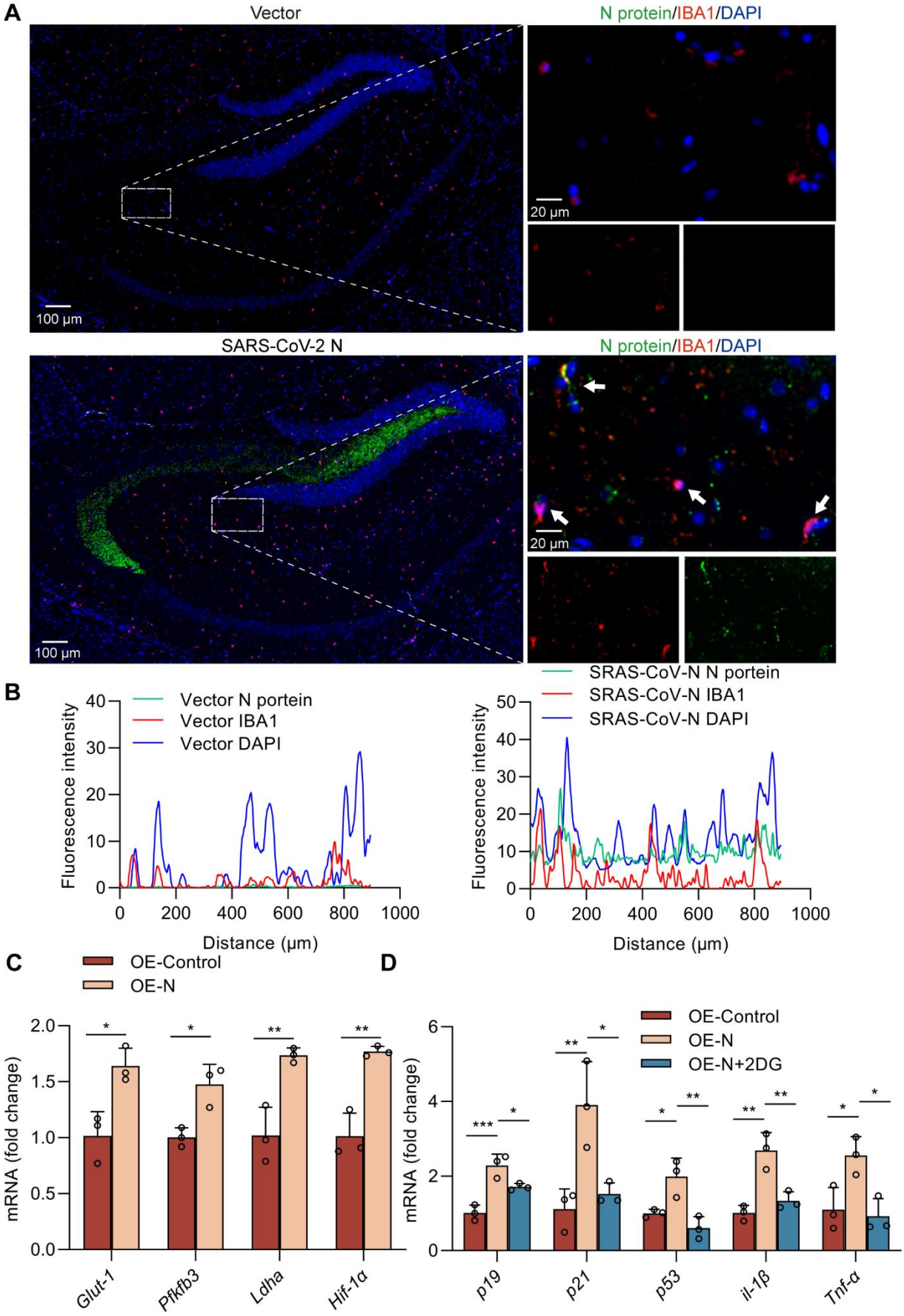

Figure S1. **The expression of the SARS-CoV-2 N protein increased in the hippocampus of mice. A-B** Immunofluorescence staining was used to determine the localization of IBA1 and SARS-CoV-2 N protein (bar=100 μm). Red fluorescence represents microglia activation marker IBA1, green fluorescence represents

SARS-CoV-2 N protein, and DAPI blue fluorescence represents the nucleus. **C** The expression levels of *Glut-1*, *Pfkfb3*, *Ldha,* and *Hif-1α* mRNA in BV2 microglial cells detected by real-time PCR (*n*=3). **D** The expression levels of *p19*, *p21*, *p53*, *Il-1β,* and *Tnf-α* mRNA in BV2 microglial cells detected by real-time PCR (*n*=3). Data are expressed as the mean ± SD. Comparisons between the two groups were made with an unpaired *t*-test. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

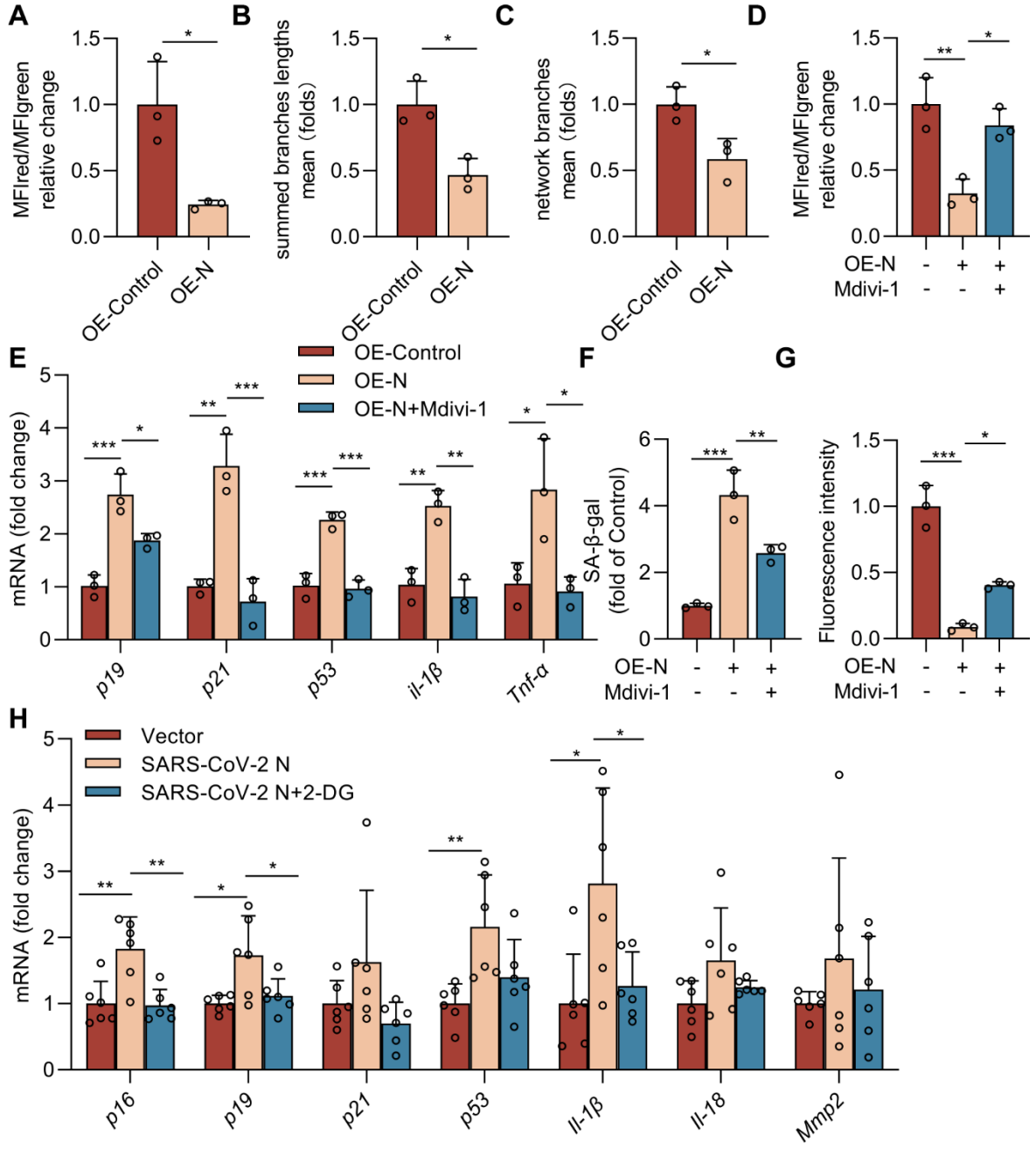

Figure S2. SARS-CoV-2 N protein induces senescence in BV2 microglial cells through mitochondrial dysfunction. BV2 microglial cells were treated with Mdivi-1 (100 nM) 30 min before Codon-optimized pLJM1-SARS-CoV-2 N-FLAG transfection. **A** Representative image of BV2 cells loaded with the mitochondrial membrane potential indicator JC-1. **B-C** The morphological skeleton and quantification of mitochondrial morphology were detected in BV2 cells. **D** Representative image of BV2 cells loaded with the

mitochondrial membrane potential indicator JC-1. **E** The expression levels of *p19*, *p21*, *p53*, *Il-1β,* and *Tnf-α* mRNA in BV2 microglial cells detected by real-time PCR (n=3). **F** SA-β-gal staining was performed after transfection of Codon-optimized pLJM1-SARS-CoV-2 N-FLAG and treatment with Mdivi-1 for 36 h **G** The fluorescence intensity of Ki67 (green) was detected by immunofluorescence. **H** The expression levels of *p16, p19, p21, p53, Il-1β, Il-18*, and *Mmp2* mRNA in the hippocampal tissues of the mice were detected by real-time PCR (n=6). Data are expressed as the Mean ± SD. Comparisons between the two groups were made with an unpaired t-test. Differences among multiple groups were performed using ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

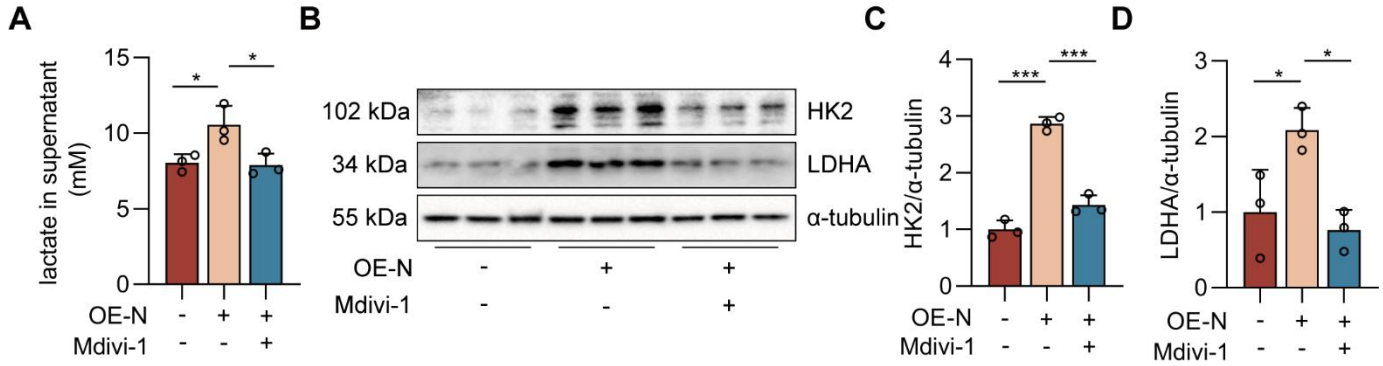

Figure S3. Inhibition of mitochondrial fission attenuates glycolysis in SARS-CoV-2 N protein-induced microglial senescence. BV2 microglial cells were treated with Mdivi-1 (100 nM) 30 min before Codon-optimized pLJM1-SARS-CoV-2 N-FLAG transfection. **A** The lactate in the supernatant was assayed. **B-D** The expression levels of HK2 and LDHA proteins in BV2 microglial cells detected by Western blot (n=3). Bar graph data represent the mean ± SD of the target protein intensity (normalized to α-tubulin) in the experimental group relative to that in the control group. Differences among multiple groups were performed using ANOVA. * $P < 0.05$ and *** $P < 0.001$.